Probes of tocopherol biochemistry: fluorophores, imaging agents, and fake antioxidants

by

Mikel Ghelfi

A thesis submitted to the Department of Chemistry
In partial fulfillment of the requirements for the degree of Doctorate of Philosophy

Supervised by
Professor Jeffrey K. Atkinson

Brock University
St. Catharines, Ontario
2017

© Mikel D. Ghelfi 2017
Abstract

The body has many defence systems against reactive radical species, but none are as crucial in the protection of lipid membranes as vitamin E. As a result of a selection process mediated by the α-tocopherol transfer protein (α-TTP), α-tocopherol is the only form of vitamin E retained in the body. This chaperon protein has been well studied because of its role in vitamin E transport. Furthermore, malfunctions of α-TTP cause vitamin E deficiency leading to ataxia and other neurodegenerative disease. Protection of neuronal tissue is critical and is reflected in the high retention of α-tocopherol in the central nervous system. Neuronal tissues receive α-tocopherol from astrocytes, cells that are linked to hepatic tissue and able to express α-TTP, however the exact path of delivery between these cells is still unclear.

A technique called fluorescent microscopy allows the tracking of fluorescent molecules in cells to find their location and interactions with other parts of the cell. The focus of this study is the synthesis of a fluorescent tocopherol analogue with a long absorption wavelength, high photostability, and that binds selectively to α-TTP with high affinity.

Most health benefits associated with vitamin E consumption are based on its capability to inhibit lipid peroxidation in cell membranes by scavenging reactive oxygen species (ROS). Oxidative damage in membranes puts cells in a “stressful” state, activating signalling events that trigger apoptosis. Vitamin E down-regulates apoptotic functions like inflammation, macrophage activation and cell arrest in a stressed state, returning the cell back to normal functioning. At the same time, vitamin E has a preventive effect for atherosclerosis, Alzheimer’s and cancer.
With the deeper understanding of cell signalling processes associated with vitamin E the question arose whether protein interactions or the ROS scavenging is responsible for cell survival. To test this hypothesis, a non-antioxidant but α-TTP binding tocopherol analogue was synthesized and administered into oxidatively stressed, α-TTP deficient cells. If the cells were unable to restore homeostasis and stop apoptosis with the new molecule, this would suggest that the antioxidant function of α-tocopherol is the reason for survival.

Cancer is regarded as one of the most detrimental diseases with a high mortality rate. One key aspect in medical research is the increased drug specificity towards targeting cancer. Chemotherapy applies cytotoxic compounds, which weaken the immune system because both malignant and healthy cells are destroyed. The specificity of the anti-cancer drugs are enhanced when encapsulated into liposomes that bear target-directing molecules such as antibodies which recognize cancer cell specific antigens on the cell membrane. The question remains if the encapsulated drug reaches the cancer or not.

Magnetic resonance imaging (MRI) and computed tomography (CT) are used to find malignant tissue in the body. CT imaging uses highly charged X-ray particles to scan the patient, possibly having damaging cytotoxic effects. Obtaining MRI results require the use of contrast agents to enhance the quality of images. These agents are based on transition metals, which potentially have chronic toxicity when retained in the body. Alternatively short-lived radiotracers that emit a γ-photon upon positron decay are used through a process called positron emission tomography (PET). Rapid decay times make the use of PET a less toxic alternative, however the decay products might be toxic to the cell.

For this reason a vitamin E based PET agent was created, which produces naturally safe decay products based on known metabolites of vitamin E, useful to track liposomal delivery of chemotherapeutic agents. This work describes the non-radioactive synthetic procedures towards
a variety of vitamin E PET analogues. The cytotoxicity of the most promising vitamin E PET tracer was evaluated along with its synthetic byproducts.
I would like to thank my supervisor Dr. Jeffrey Akinson for allowing me to pursue my graduate studies in his laboratory. I am grateful for his unmeasurable support and guidance form the beginning of my journey towards my degree. Dr Atkinson truly inspired my passion for science with his knowledge and enthusiasm, something I value deeply and will to carry on for the rest of my life.

I further extend my gratitude to my thesis committee members Dr. Tony Yan and Dr. Melanie Pilkington for all their great input into my work. I thank you for always being helpful throughout all these years.

I am thankful for the tremendous contributions towards this work from our collaborators. Mr. Lucas Maddalena and Dr. Jeff Stuart for the help in the toxicological analysis and fluorescence microscopy work on the imaging agents. My thanks are extended to Dr. Drew Marquardt and Dr. Thad Harroun for their insight in the creation of the imaging agents.

I thank Dr. Theocharis Stamatatos for referring me to his colleague, Dr. Kyriakos Bourikas, helping me to solve a crucial part of my work.

I would like to thank Mr. Razvan Simionescu, Mr. Tim Jones and Mrs. Liquin Qiu for all their help and assistance in the analysis of spectra.

I especially thank our collaborators at Case Western University, Dr. Danny Manor and Dr. Lynn Ulatowski, for their contributions on the study towards a fluorescent tocopherol and fake-antioxidant.

My sincere gratitude goes towards all my past and present group members and co-workers who contributed towards this work with all their help in the lab and the fruitful discussions. Special
thanks to: Candace Panagabko, Matilda Baptist, Parthajit Mukherjee, Andrew Hildering, Nick Kruger, Venkata Krishna Garapati Roa, Ryan West, Stephan Ohnmacht.

Finally, I would like to thank both NSERC and Hoffmann-La Roche, whose funding made this work possible.
Table of contents

Fluorescent Tocopherol 1-4

1 Introduction .................................................................................................................. 29
  1.1 Discovery and Structure .............................................................................................. 29
  1.2 Natural sources and tocopherol synthesis .................................................................. 30
  1.3 Tocopherol chemical synthesis .................................................................................. 32
  1.4 Biosynthesis ............................................................................................................... 35
    1.4.1 Shikimate pathway ............................................................................................... 35
    1.4.2 Non-mevalonate pathway .................................................................................... 37
  1.5 Biological absorption & transport .............................................................................. 38
    1.5.1 Tocopherol metabolism ....................................................................................... 41
  1.6 Structure of the α-Tocopherol transfer protein ......................................................... 43
    1.6.1 AVED .................................................................................................................... 49
    1.6.2 Vitamin E and neurological health ...................................................................... 51
  1.7 Biological tracing ....................................................................................................... 60
    1.7.1 Principles of fluorescence .................................................................................... 63
    1.7.2 Light scattering ..................................................................................................... 65
    1.7.3 Vitamin E labeling: Advantages of fluorophores .................................................. 71
    1.7.4 Fluorescent labeling: Fluorophores ...................................................................... 71
    1.7.5 Tocopherol labeled fluorophores .......................................................................... 74

2 Project overview .......................................................................................................... 75
  2.1 Structure design ........................................................................................................ 75
  2.2 Synthesis plan ........................................................................................................... 81

3 Results and Discussion ............................................................................................... 85
  3.1 Synthesis .................................................................................................................. 85
  3.2 Photophysical studies ............................................................................................... 104
    3.2.1 Absorption spectra ............................................................................................... 104
    3.2.2 Quantum yield ..................................................................................................... 105
  3.3 Binding Studies ......................................................................................................... 106
    3.3.1 α-TTP expression & purification .......................................................................... 106
    3.3.2 Binding study to α-TTP in SET-Buffer ................................................................ 107
    3.3.3 Binding study to α-TTP in TKE-Buffer ................................................................ 118
  3.4 Cell studies ............................................................................................................. 130
    3.4.1 Cell assay ........................................................................................................... 130

4 Conclusion .................................................................................................................. 135

Design and synthesis of a biomimetic non-antioxidant tocopherol 5-8

5 Introduction .................................................................................................................. 136
  5.1 Antioxidant function ................................................................................................. 136
    5.1.1 Lipid peroxidation ............................................................................................... 136
    5.1.2 Kinetics of hydroperoxyl radical quenching by tocopherol ................................ 138
    5.1.3 Quenching of vitamin E ....................................................................................... 143
    5.1.4 Hydrophobic antioxidants; carotene and ubiquinol ........................................... 145
5.1.5 Vitamin E function and movement in membranes ........................................... 146
5.1.6 Position and dynamics of vitamin E in membranes ........................................ 147
5.1.7 Vitamin E membrane curvature and flip-flop .............................................. 149
5.2 Cellular role of vitamin E .................................................................................. 150
5.2.1 Protein kinase C and Phospholipase A2 ....................................................... 150
5.2.2 Atherosclerosis ......................................................................................... 151

6 Project overview .................................................................................................. 153
6.1 Structural design ............................................................................................. 154
6.1.1 Halogen tocopherol .................................................................................. 154
6.1.2 Hydroxymethyl tocopherol ...................................................................... 158
6.2 Synthesis ........................................................................................................ 161

7 Results and Discussion ....................................................................................... 163
7.1 Synthesis ......................................................................................................... 163
7.2 Spectroscopic and chemical properties of HM-Toc ........................................ 165
7.3 Binding studies .............................................................................................. 166
7.4 Oxidation assay .............................................................................................. 170
7.4.1 Cyclic voltammetry studies ................................................................... 171
7.4.2 Lipid peroxidation .................................................................................. 173
7.4.3 Cellular oxidation studies ...................................................................... 174
7.5 Cell studies and Animal trials ....................................................................... 175

8 Conclusion .......................................................................................................... 176

PET-tocopherol 9-12

9 Introduction ......................................................................................................... 177
9.1 Positron emission tomography (PET) ............................................................. 179
9.1.1 Clinical PET Tracer ................................................................................ 183
9.1.2 Single-photon emission computed tomography SPECT ........................... 185
9.1.3 18F radio-nuclide synthesis .................................................................. 187
9.1.4 18F nuclide synthesis ........................................................................... 189
9.2 18F-Fluorination .......................................................................................... 190
9.2.1 Nucleophilic fluorination ................................................................... 190
9.2.2 Aromatic 18F-fluorination .................................................................. 193
9.2.3 Electrophilic / radical fluorination .......................................................... 199

10 Project overview ................................................................................................ 203
10.1 Structure design ........................................................................................... 204
10.1.1 Tocopherol based PET tracer ............................................................... 204
10.1.2 Tocopherol dual label: PET tracer and fluorophore ............................... 206

11 Results and Discussion ....................................................................................... 207
11.1 6-F-Tocopherol synthesis .............................................................................. 207
11.2 Synthesis ....................................................................................................... 209
11.2.1 Nucleophilic F-Toc synthesis ................................................................ 209
11.2.2 Synthesis of 6-F-tocopherol by electrophilic fluorination ..................... 235
11.2.3 γ-Tocopherol fluorination to 5-fluoro-γ-tocopherol ................................ 245
11.2.4 5-F-methyl-α-tocopherol synthesis ....................................................... 251
11.2.5 6-F-methyl-α-tocopherol synthesis ......................................................... 261
11.2.6 13-HO-α-tocopherol fluorination to 13-F-α-tocopherol ......................... 266
11.3 BODIPY fluorination .................................................................................... 269
11.4 Cell cytotoxicity of 6-F-α-tocopherol, thienyl-ene-BODIPY and hydroxymethyl tocopherol ................................................................. 277
11.1.4 Cell cytotoxicity of the liposomal delivery 6-F-α-tocopherol

11.5 Cellular uptake of in liposome incorporated thieryl-ene-BODIPY

12 Conclusion

13 General procedures

13.1 Reagents

13.2 Ligand binding and competitive binding assays with α-TTP

13.3 Cyclic voltammetry

13.4 AMVN lipid peroxidation

13.5 HM-Toc stability

13.6 Cytotoxicity study

13.7 Lipid-BODIPY extrusion

13.8 Imaging of cellular uptake of thienyl-ene-BODIPY liposomes

14 Synthetic procedures and NMR & MS

14.1 Fluorescent Tocopherol

14.1.1 Synthesis of (S)-methyl 6-((tert-butyldimethylsilyl)oxy)-20(7,8-tetramethylchroman-2-carboxylate (5)

14.1.2 Synthesis of (S)-6-((tert-butyldimethylsilyl)oxy)-20(7,8-tetramethylchroman-2-cyraldehyde (6)

14.1.3 Synthesis of (3-hydroxypropyl)triphenylphosphonium bromide (7)

14.1.4 Synthesis of (Z)-4-((tert-butyldimethylsilyl)oxy)-20(7,8-tetramethylchroman-2-but-3-en-1-ol (8)

14.1.5 Synthesis of (R)-4-((tert-butyldimethylsilyl)oxy)-20(7,8-tetramethylchroman-2-butanol-1-ol (9)

14.1.6 Synthesis of (R)-4-((tert-butyldimethylsilyl)oxy)-20(7,8-tetramethylchroman-2-butanol (10)

14.1.7 Byproduct (R)-tert-butyl((2-butyl-20(7,8-tetramethylchroman-6-yloxy)methyldimethylsilane (9.2)

14.1.8 Synthesis of 1-(1-benzyl-1H-pyrrol-2-yl)-N,N-dimethylaniline (11)

14.1.9 Synthesis of N,N-dimethyl-1-(1H-pyrrol-2-yl) methanamine (10)

14.1.10 Synthesis of N,N-dimethyl-1-(1H-pyrrol-2-yl) methanamine (11)

14.1.11 Synthesis of (1H-pyrrol-2-yl) methyltriphosphonium iodide (10)

14.1.12 Synthesis of (3-bromopropyl)triphenylphosphonium bromide (14)

14.1.13 Synthesis of (R)-4-((tert-butyldimethylsilyl)oxy)-20(7,8-tetramethylchroman-2-butyl methanesulfonate (9-OMs)

14.1.14 Synthesis of (R)-((2-4-bromo-butyl)-20(7,8-tetramethylchroman-6-yloxy)((tert-butyl)methyldimethylsilane (16)

14.1.15 Synthesis of (R)-((2-4-bromo-butyl)-20(7,8-tetramethylchroman-2-yloxy)((tert-butyl)methanesulfonate (18)

14.1.16 Synthesis of (R)-((2-4-iodo-butyl)-20(7,8-tetramethylchroman-6-yloxy)methyldimethylsilane (17)

14.1.17 Synthesis of (R)-((2-4-iodo-butyl)-20(7,8-tetramethylchroman-6-yloxy)methyldimethylsilane (18)

14.1.18 Synthesis of (R)-((2-4-bromo-butyl)-20(7,8-tetramethylchroman-2-yloxy)methanesulfonate (19)

14.1.19 Synthesis of (R)-((2-4-bromo-butyl)-20(7,8-tetramethylchroman-2-yloxy)methyl methanesulfonate (22-OMs)

14.1.20 Synthesis of (R)-((2-4-bromo-butyl)-20(7,8-tetramethylchroman-6-yloxy)((tert-butyl)methyldimethylsilane (23)

14.1.21 Synthesis of (R)-((2-4-bromo-butyl)-20(7,8-tetramethylchroman-2-yloxy)methyl methanesulfonate (24)

14.1.22 Synthesis of (R)-((2-4-bromo-butyl)-20(7,8-tetramethylchroman-6-yloxy)((tert-butyl)methyldimethylsilane (26)
14.1.23 Synthesis of (S)-methyl 6-(benzoxyl)-2,5,7,8-tetramethylchroman-2-carboxylate (27)
315
14.1.24 Synthesis of (S)-6-(benzoxyl)-2,5,7,8-tetramethylchroman-2-carbaldehyde (21)
316
14.1.25 Synthesis of (R)-((S)-6-(benzoxyl)-2,5,7,8-tetramethylchroman-2-yl)(5-bromothiophen-2-yl)methanol (28) .......................................................... 317
14.1.26 Synthesis of (S)-6-(benzoxyl)-2-((5-bromothiophen-2-yl)methyl)-2,5,7,8-tetramethylchroman (29) .......................................................... 318
14.1.27 Synthesis of (S)-2-[(6-((tert-butyldimethylsilyl)oxy)-2,5,7,8-tetramethylchroman-2-yl)methyl]thiophen-2-yl)-1H-pyrrrole (30) .................................................. 319
14.1.28 Synthesis of (S)-2-[(6-(benzoxyl)-2,5,7,8-tetramethylchroman-2-yl)methyl]thiophen-2-yl)-1H-pyrrrole (32) .................................................. 321
14.1.29 Synthesis of (S)-7-((S)-6-(benzoxyl)-2,5,7,8-tetramethylchroman-2-yl)methyl]thiophen-2-yl)-5,5-difluoro-1,3-dimethyl-5H-dipyrrolo[1,2-c:2,1'-f][1,3,2]diazaborinin-4-iium-5-uide (33) .................................................. 322
14.1.30 Synthesis of (S)-5-((6-((tert-butyldimethylsilyl)oxy)-2,5,7,8-tetramethylchroman-2-yl)methyl]thiophene-2-carbaldehyde (34) .................................................. 323
14.1.31 Synthesis of byproduct (S)-2,5,7,8-tetramethyl-2-((thiophen-2-ylmethyl)chroman-6-yl)formate (36) .................................................. 324
14.1.32 Synthesis of 5,5-difluoro-7,9-dimethyl-5H-dipyrrolo[1,2-c:2,1'-f][1,3,2]diazaborinin-4-iium-5-uide (37) .................................................. 325
14.1.33 Synthesis of (S,E)-7-((2-5-((6-((tert-butyldimethylsilyl)oxy)-2,5,7,8-tetramethylchroman-2-yl)methyl]thiophen-2-yl)vinyl)-5,5-difluoro-9-methyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-iium-5-uide (39) .................................................. 327
14.1.34 Synthesis of (S,E)-5,5-difluoro-7-((2-5-((6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)methyl]thiophen-2-yl)vinyl)-9-methyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-iium-5-uide (3) .................................................. 328
14.1.35 Synthesis of (S,E)-5,5-difluoro-7-((2-5-((6-methoxy-2,5,7,8-tetramethylchroman-2-yl)methyl]thiophen-2-yl)vinyl)-9-methyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-iium-5-uide (40) .................................................. 332

14.2 Non-antioxidant-Tocopherol .................................................................................. 333
14.2.1 Synthesis of (2R)-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl trifluoromethanesulfonate (41) .......................................................... 333
14.2.2 Synthesis of (2R)-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman (42) .......................................................... 333
14.2.3 Synthesis of (2R)-6-chloro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman (43) .......................................................... 334
14.2.4 Synthesis of (2R)-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-carbaldehyde (46) .......................................................... 335
14.2.5 Synthesis of ((2R)-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl)methanol (44) .......................................................... 335

14.3 PET-Tocopherol ................................................................................................. 338
14.3.1 Synthesis of (2R)-6-fluoro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman (47) .......................................................... 338
14.3.2 Synthesis of 2,2,5,7,8-pentamethylchroman-6-yl 4-methylbenzenesulfonate (52) .......................................................... 341
14.3.3 Synthesis of naphthalen-2-yl 4-methylbenzenesulfonate (54) .......................................................... 341
14.3.4 Synthesis of 4,4,5,5-tetramethyl-2-((2R)-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl)-1,3,2-dioxaborolane (56) .......................................................... 342
14.3.5 Synthesis of 1-iodopyridin-1-iium chloride (71) .......................................................... 343
14.3.6 Synthesis of (2R)-6-iodo-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman (70) .......................................................... 344
14.3.7 Synthesis of phenyl((2R)-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl)iodonium 4-methylbenzenesulfonate (69) .......................................................... 345
14.3.8 Synthesis of (2R)-5-bromo-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol (76) .......................................................... 346
14.3.9 Synthesis of (2R)-5-fluoro-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol (48) ......................................................... 346
14.3.10 Synthesis of (2,5-dihydroxy-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis(dimethylsulfonium) acetate (S-Ylide) .................................................................................................................. 348
14.3.11 Synthesis of (2R)-5-(fluoromethyl)-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl acetate (78) .................................................................................................................. 349

References
List of Figures

Figure 1. Conditions used in the industrial synthesis of α-tocopherol by condensation of TMHQ and isophytol.36,37,42,43 ......................................................................................................................... 33
Figure 2. Structure of Tocominol & marine derived Tocopherol (MDT).56 ................................................................. 34
Figure 3. Shikimate pathway. Synthesis of Chlorismate from D-erythrose-4-phosphate (E4P) and phosphoenolpyruvlate (PEP).63,64 ........................................................................................................... 36
Figure 4. Biosynthetic conversion of chorismate to 4-HPPA.66 ......................................................................................... 36
Figure 5. Tocopherol biosynthesis.69 ............................................................................................................................ 38
Figure 6. Tocopherol intestinal absorption & distribution. .............................................................................................. 40
Figure 7. Vitamin E metabolites. .................................................................................................................................... 42
Figure 8. Protein interactions of the α-TTP active side with α-tocopherol ........................................................................ 45
Figure 9. Open and closed lid crystal structure. ............................................................................................................. 47
Figure 10. Calculated interactions between the lipid-binding domains of α-TTP with the hydrocarbon core of the lipid bilayer: (A) α-TTP in "open" (1O1Z) and (B) "closed" (1R5L) conformations. ................................................................................................................................. 48
Figure 11. α-TTP structure highlighting the amino acid residues in the P(4,5)P2 binding cavity and lid helix 9-11 α-TTP structure enclosing α-Toc. ........................................................................................................... 49
Figure 12. α-TTP mutations across the ttpa gene. ................................................................................................................ 50
Figure 13. Structural differences in Purkinje neurons between ttpa+/+ and ttpa+/-VE (vitamin E) mice.111 ....................................................................................................................................................... 52
Figure 14. Inhibition of lipid peroxidation with α-tocopherol and NMDA. .............................................................................. 54
Figure 15. Localization of vitamin E in neurons by TOF-MS. ................................................................................................. 58
Figure 16. Localization of α-TTP expression in neuronal tissue. .......................................................................................... 59
Figure 17. Proposed model of vitamin E delivery from astrocytes to neurons.111 .............................................................. 60
Figure 18. Fluorescent stained actin filaments of gastrula-stage Drosophila embryo. ......................................................... 62
Figure 19. Jablonski diagram. .............................................................................................................................................. 63
Figure 20. Quantum yield (Φ) and fluorescent lifetime (τ) of Eosin & Erythrosin B.161 ...................................................... 65
Figure 21. Interaction of visible light with particles. ........................................................................................................... 67
Figure 22. Extinction coefficient (k) of water for the 200 – 1000nm spectral region as a summary from CJ: Clark and Jelly, LSG: Lenoble, Saint-Guily and TSW: Tyler.171,172,173 ........................................................................................................ 68
Figure 23. Total absorption coefficient μa (cm⁻¹), as water is added (volume fraction f_{water} = 0.1 by 0.1 to 0.9), blood at 75 oxygen saturation is added (average f_{blood} = 10⁻⁴ by 10⁻⁴ to 2 x 10⁻³), bilirubin is added (1 by 1 to 20 mg dL⁻¹, where 20 mg dL⁻¹ = 342 μM is a bilirubin concentration in the blood of a jaundiced neonate), fat is added (f_{fat} = 0.3 by 0.3 to 0.9), and melanin is added (f_{melanosome} = 0.01 by 0.01 to 0.10).176 .................................................................................................................................................................. 69
Figure 24. Reduced scattering coefficient for different tissues based on literature values for the seven groups of tissues (red circles, data found in reference). .......................................................................................................................... 70
Figure 25. Structures of frequently applied fluorescent microscopy dyes.190-197 .................................................................. 73
Figure 26. Nummerring system of the BODIPY core structure and example structures of differently substituted BODIPYs.217-220 ....................................................................................................................................... 77
Figure 27. X-ray diffraction analysis of AO-α-toc (1a & 1b) and α-tocopherol (2a & 2b) bond to α-TTP. ......................... 79
Figure 28. Novel designed fluorescent α-Toc ligands compared to BODIPY-Toc and α-tocopherol .......................................................... 81
Figure 29. (a) BODIPY formation from oct-7-ene-pyrrole and 2,4-dimethylpyrrole aldehyde. Formation of byproduct 1,3,5,7-tetramethyl-BODIPY. (b) Olefin metathesis reaction between vinyl TBSO-Trolox-ene and oct-7-ene-BODIPY. Formation of dimer bis-oct-7-ene-BODIPY. 206 .................................................. 83
Figure 30. Synthetic strategy towards product 1 .............................................................................. 84
Figure 31. Synthetic strategy towards product 2 .............................................................................. 84
Figure 32. Synthetic strategy towards product 3 .............................................................................. 85
Figure 33. Synthesis of TBSO trolox aldehyde from Trolox® by esterification, TBS protection of the chroman phenol and reduction of the methylester to the aldehyde. .............................................................................. 86
Figure 34. Synthesis of phosphonium salt, 7, from 3-bromopropan-1-ol235 and Wittig reaction of 7 with TBSO Trolox aldehyde to create product 8.206 .................................................. 86
Figure 35. Synthesis of product 9 by hydrogenation of 8 with 10% palladium on carbon. 87
Figure 36. (a) Double bond hydrogenation of compound 8 ......................................................... 88
Figure 37. Griffin’s proposed alcohol oxidation mechanism with water and palladium / platinum metal [M] .............................................................................. 89
Figure 38. Synthesis of product 10 by Dess-Martin oxidation of 9.246 ........................................... 89
Figure 39. Synthesis of product 13 by Wittig reaction of aldehyde 10 with phosphonium salt 11 and 12 .............................................................................. 90
Figure 40. Synthesis of product 20 by Wittig reaction of aldehyde 16 with phosphonium salt 7 and lithium hexamethyldisilazide as a base.250 .................................................. 90
Figure 41. Conversion of alcohol 9 to the bromine 16 and iodine 17 by mesylation, followed by nucleophilic bromide and iodide substitution of the mesylate with lithium bromide (LiBr) and potassium iodide (KI).254 .................................................. 91
Figure 42. Synthesis of phosphonium salts 18 and 19 by reaction of 16 and 17 with triphenylphosphine (PPh₃) in a microwave oven (MV).255 .................................................. 91
Figure 43. Synthesis of product 20 by Wittig reaction of N-benzylpyrrole-2-carboxaldehyde with phosphonium salt 7 and lithium hexamethyldisilazide as a base.201 .................................................. 92
Figure 44. Synthesis of product 13 by Wittig reaction of N-benzylpyrrole-2-carboxaldehyde with phosphonium salt 18 and 19 and lithium hexamethyldisilazide as a base.201,249 .................................................. 92
Figure 45. Decrease in chain length from target product 1 to 2 ................................................... 93
Figure 46. 2-Bromothiophene addition to Trolox by displacement of an electrophile on position 1 .............................................................................. 93
Figure 47. Synthetic routes chosen to synthesize 26 from TBS trolox aldehyde 6 .................... 94
Figure 48. (a) Synthesis of product 23 by mesylation of TBS trolox alcohol 22, followed by nucleophilic bromide displacement of the mesyl group with lithium bromide.254 (b) Appel reaction of 22 yielded TBS deprotected product 24.259 .................................................. 95
Figure 49. Synthesis of product 26 by reaction of 23 with 2-bromothiophene.261,262 .......... 95
Figure 50. Synthesis of product 25 by reaction of 6 with 2-bromothiophene.262 ............. 96
Figure 51. Synthesis of benzyl protected trolox aldehyde 21 ................................................... 96
Figure 52. Synthesis of product 28 by reaction of 21 with 2-bromothiophene.262 ............. 97
Figure 53. Reduction of 28 to product 29 and 25 to product 26 with triethylsilane and boron trifluoride.274 .................................................. 98
Figure 54. Negishi coupling of 26 with pyrrole to create product 30. Synthesis of dimer byproduct, 31.275 .................................................. 98
Figure 55. Negishi coupling of 29 with pyrrole to create product 32 and trace amounts of 29-H.275 .................................................................................................................. 99
Figure 56. Synthesis of 33 by condensation of 32 with 3,5-dimethylpyrrole-2-carbaldehyde.206 .................................................................................................................. 99
Figure 57. Attempts to debenzylate 33 to form product 2 ............................................... 100
Figure 58. Synthesis of product 34 by formylation of 26 by lithium halogen exchanged and quenching with DMP.291 ......................................................................................... 101
Figure 59. (a) Synthesis of 36 from 35 by Vilsmeier-Haack formylation. (b) Proposed reaction mechanism of the aryl O-formate protection of TBSO protected 2-thiophenemethyl α-chromane. .................................................................................. 102
Figure 60. Synthesis of dimethyl-BODIPY building block, 37, by condensation of 2,4-dimethyl pyrrole and 2-pyrrrole carboxaldehyde.206,295 ......................................................................... 103
Figure 61. Synthesis of 39 by Knovenagel condensation of 34 with dimethyl BODIPY 37.296 ................................................................. ................................. 103
Figure 62. Synthesis of target product 3 by acetic phenol TBSO-deprotection of 39 with 8% HCl MeOH.206 ........................................................................................................... 104
Figure 63. Thienyl-ene-BODIPY absorption and emission curve .................................. 104
Figure 64. Fluorescein and thienyl-ene-BODIPY gradients for quantum yield determination. .............................................................................................. 106
Figure 65. Direct binding of [ligand] BODIPY-α-Toc C6, C7 and C8 to 0.2 μM α-TTP in SET buffer ............................................................ 108
Figure 66. BODIPY-α-Toc in EtOH direct binding to 0.2 μM α-TTP in SET buffer repeated with freshly expressed protein ......................................................................................... 109
Figure 67. Direct binding of thienyl-ene-BODIPY (3) in EtOH to 0.5 μM α-TTP in SET buffer using West’s et al. conditions ......................................................... 110
Figure 68. Competition assay of 0.2 μM α-TTP, saturated with 1.0 μM BODIPY-α-Toc in SET buffer, with [competitor] α-Toc & cholesterol in EtOH ........................................... 111
Figure 69. Competition assay of 0.2 μM α-TTP, saturated with 0.25 μM thienyl-ene-BODIPY (3) in SET buffer, with α-tocopherol (α-toc) in EtOH ........................................... 112
Figure 70. Direct binding of C9-NBD-α-Toc in EtOH to 0.2 μM α-TTP in SET buffer. λ_ex = 495 nm λ_em = 535 nm ......................................................................................... 112
Figure 71. Competition assay of 0.2 μM α-TTP, saturated with 1.0 μM NBD-Toc in SET buffer and 100 μM TX100, with [competitor] α-Toc & cholesterol in EtOH........ 112
Figure 72. Competition assay of 0.5 μM α-TTP, saturated with 1.0 μM thienyl-ene-BODIPY (3) in SET buffer, with α-Toc & 100μM TritonX100 .................................................................. 114
Figure 73. Direct binding of thienyl-ene-BODIPY (3) in EtOH to 0.2 μM α-TTP in SET buffer with 100μM TX100 ......................................................................................... 114
Figure 74. Direct binding of thienyl-ene-BODIPY (3) in EtOH to 0.2 μM α-TTP in SET buffer with 10μM TX100 ......................................................................................... 115
Figure 75. Titration of 0.5 μM α-TTP, saturated with 1.0 μM thienyl-ene-BODIPY (3) in SET buffer, test with increasing TX100 amounts by 10μM .......................................... 116
Figure 76. Direct binding of thienyl-ene-BODIPY (3) in EtOH to 0.5 μM α-TTP & BSA .......... 117
Figure 77. Thienyl-ene-BODIPY (3) solubility test with DMSO in TKE buffer .................... 118
Figure 78. Direct binding of thienyl-ene-BODIPY (3) in EtOH to 0.2μM α-TTP in TKE buffer .................................................................................................................. 119
Figure 79. Direct binding of thienyl-ene-BODIPY (3) in EtOH to 0.5μM α-TTP in TKE buffer .................................................................................................................. 119
Figure 80. Direct binding of thienyl-ene-BODIPY (3) in EtOH to 0.5 µM α-TTP & BSA in TKE buffer........................................................................................................120
Figure 81. Test of ligand over addition. Direct binding of thienyl-ene-BODIPY (3) in EtOH to 0.4µM α-TTP in TKE buffer.................................................................121
Figure 82. Competition assay of 0.1 µM α-TTP, saturated with 0.2 µM thienyl-ene-BODIPY (3) in TKE buffer, with α-Toc in EtOH....................................................122
Figure 83. Competition assay of 0.1 µM α-TTP, saturated with 0.5 µM thienyl-ene-BODIPY (3) in EtOH in TKE buffer, with cholesterol.................................122
Figure 84. Thienyl-ene-BODIPY (3) test competition assay of 0.4 µM α-TTP, saturated with 0.8 µM (black), 0.4 µM (red and pink) thienyl-ene-BODIPY (3) in TKE buffer, with cholesterol (as EtOH solutions, red & black) and EtOH only (pink).........................................123
Figure 85. Direct binding of thienyl-ene-BODIPY (3) in DMF to 0.4 µM α-TTP in TKE buffer........................................................................................................124
Figure 86. Competition assay of 0.4 µM α-TTP, saturated with 0.4 µM thienyl-ene-BODIPY (3) in TKE buffer, with α-Toc solution in DMF and DMF alone........................124
Figure 87. Competition assay of 0.4 µM α-TTP, saturated with 0.4 µM thienyl-ene-BODIPY (3) in TKE buffer, with cholesterol (in DMF) and DMF..........................125
Figure 88. Competition assay of 0.4 µM α-TTP, saturated with 0.4 µM thienyl-ene-BODIPY (3) in TKE buffer, with αToc (DMF), EtOH and DMF.................................126
Figure 89. Synthesis of MeO-thienyl-ene-BODIPY (40).................................................................................................................................126
Figure 90. Direct binding of MeO-Thienyl-ene-BODIPY (40) in DMF to 0.4 µM α-TTP in TKE buffer........................................................................................................127
Figure 91. Direct binding of thienyl-ene-BODIPY (3) in dioxane 0.4 µM to α-TTP in TKE buffer........................................................................................................128
Figure 92. Competition assay of 0.4 µM α-TTP, saturated with 0.4 µM thienyl-ene-BODIPY (3) in TKE buffer, with α-Toc (in dioxane) and dioxane..............................128
Figure 93. Competition assay of 0.4 µM α-TTP, saturated with 0.4 µM thienyl-ene-BODIPY (3) in TKE buffer, with cholesterol (in dioxane) and dioxane....................129
Figure 94. α-TTP facilitated secretion of thienyl-ene-BODIPY 3, from cultured hepatocytes. ..................................................................................................................131
Figure 95. Averages and standard deviations of 5 images from three independent experiments ........................................................................................................132
Figure 96. α-TTP-induced α-tocopherol secretion in HepG2-TetOn-TTP cells. ..................133
Figure 97. Quantification of intracellular NBD-α-tocopherol fluorescence over time in cells that do (white bars) or do not (black bars) express TTP..................................................134
Figure 98. PUFA oxidation: Initiation, propagation and termination.319.............................137
Figure 99. Product distribution for the peroxidation of methyl linolenate peroxide product.322................................................................................................................138
Figure 100. Radical reactions in lipids................................................................................139
Figure 101. Oxidation products of methyl linolate and α-tocopherol.327.............................140
Figure 102. The decay of 5,7-diisopropyl-tocopherol radical reacted with ethyl stearate, linoleate, linolenate and arachidonate observed at 417 nm in benzene at 25.0°C [Toc 0.17 mM and [LH]t = 75.0 mM].333........................................................................142
Figure 103. Orbital alignments in chroman and benzoferane.334.................................143
Figure 104. Disappearance of vitamin E and vitamin C in the oxidation of methyl linolate at 37°C in tert-butyl alcohol/methanol (3:1 by volume)..................................144
Figure 105. β-carotene reaction with singlet oxygen (1O2) and RO*, ROO*.347..................145
Figure 106. Effect of oxygen concentration in peroxynuclear formation in methyl linolate 18:3 (200mM) initiated by AMVN (1mM) at 37°C. ........................................146
Figure 107. Position of tocopherol to the phospholipid head group.363,364.............148
Figure 108. Location of tocopherol in membranes.363 ........................................149
Figure 109. Vitamin E inhibition of LDL and oxLDL receptor mediated atherosclerosis.390
..................................................................................................................152
Figure 110. Basic principle of halogen and hydrogen bonding to a Lewis base........155
Figure 111. Calculated charge distribution of alkyl halides (top) and halogenated nucleobases (bottom).403 .................................................................156
Figure 112. Halogen bonding interaction of a substituted indomethacin ethanolamide analogue to COX-1.405 .................................................................157
Figure 113. Cartoon representation of possible halogen bonding of a halo-α-tocopherol interaction in α-TTP. .................................................................158
Figure 114. Radical abstractions bond dissociation energies of HM-Toc & α-Toc.409 ........159
Figure 115. Calculated hydrophilic interaction of α-tocopherol (A, top) and γ-tocopherol (B, bottom) in the wild-type α-TTP binding pocket ....................................160
Figure 116. Cartoon representation of possible HM-Toc position in α-TTP. ...............161
Figure 117 Synthetic strategies to Cl-Toc, 43, and HM-Toc, 44, from α-tocopherol.423,428,429 ...........................................................................................................162
Figure 118. Synthetic strategies to racemic Cl-Toc, 43, and racemic HM-Toc, 44, from 2,3,5-trimethylphenol and 45.423,428 .........................................................163
Figure 119. AcO-α-Toc deprotection to α-tocopherol. ...............................................163
Figure 120. α-Toc conversion to α-tocopherol triflate 41, followed by reduction to H-Toc,
42,423 ...........................................................................................................164
Figure 121. Chlorination of H-Toc 42 to Cl-Toc 43.429 .............................................164
Figure 122. Hydroxymethylation of H-Toc 42 to HM-Toc 44 ....................................164
Figure 123. Rieche formylation of H-Toc 42 to product 46.428 ...................................165
Figure 124. Reduction of 46 to HM-Toc 44. .............................................................165
Figure 125. Absorption spectra HM-Toc in EtOH ......................................................166
Figure 126. Competition assay of 0.4 μM α-TTP, saturated with 1.6 μM NBD-C9-Toc in SET buffer, with α-Toc and Cl-Toc in EtOH. .........................................167
Figure 127. Competition assay of 0.4 μM α-TTP, saturated with 1.6 μM NBD-C9-Toc in SET buffer, with α-Toc and HM-Toc in EtOH. .....................................168
Figure 128. Micelle test 1.6 μM NBD-C9-Toc with HM-Toc and α-Toc in SET buffer ..........169
Figure 129. Competition assay of 0.4 μM α-TTP, saturated with 1.6 μM NBD-C9-Toc in SET buffer, with α-Toc and HM-Toc in EtOH adjusted with micelle data (Figure 137) .............................169
Figure 130. Expected UV absorption (230nm) of the non-antioxidant tocopherol. ........170
Figure 131. Radical initiators and peroxidation systems used to oxidize PC.432-434 ........171
Figure 132. CV (BASI-Epsilon) oxidation of α-tocopherol (2.5 mM in dry dichloromethane) at 200 mV/s with 80 mM tetrabutylammonium hexafluorophosphate. ........171
Figure 133. CV (BASI-Epsilon) oxidation of HM-Toc (2.5 mM in dry dichloromethane) at 200 mV/s with 80 mM tetrabutylammonium hexafluorophosphate. ........172
Figure 134. CV (BASI-Epsilon) oxidation of H-Toc (2.5 mM in dry dichloromethane) at 200 mV/s with 80 mM tetrabutylammonium hexafluorophosphate. ........172
Figure 135. AMVN initiated oxidation of multilamellar SoyPC vesicle with HM-Toc & α-
Toc................................................................................................................173
Figure 136. Products observed during the assessment of HM-Toc stability in acetic media. ........................................................................................................175
Figure 167. Synthesis of \( \alpha \)-tocopherolpinacolboronic ester 56, \( \delta \)-6'-tocopherolpinacolboronic ester from \( \alpha \)-tocopherol trflate.\(^{528}\)

Figure 168. (a) Mazzini's \( \alpha \)-tocopherol amine synthesis,\(^{529}\) (b) \( \alpha \)-Tocopherol trflate borylation with Mazzini's conditions. (c) \( \alpha \)-Tocopherol trflate borylation with PdCl\(_2\)(dpf).\(^{530}\)

Figure 169 Palladium catalyzed borylation of sterically demanding, electron-rich aromatics.\(^{619}\)

Figure 170. Tetrakispyridine copper(II)trflate catalyzed fluorination of 56,\(^{533}\)

Figure 171. Synthesis of Phenofluor\(^\circledR\) precursor Cl-NHC,\(^{534}\)

Figure 172. Attempted Phenofluor\(^\circledR\) fluorination of \( \alpha \)-tocopherol,\(^{534}\)

Figure 173 Phenofluor\(^\circledR\) intermediate 59 formation with phenols.\(^{522}\)

Figure 174. \(^{18}\)F-fluorination of NHC-bound phenols.\(^{517}\)

Figure 175. Ruthenium catalysed Phenofluor\(^\circledR\) fluorination of \( \delta \)-tocopherol.\(^{537}\)

Figure 176. Nucleophilic fluorination of diaryliodonium salts.\(^{224}\)

Figure 177. Designed electron rich 6-\( \alpha \)-tocopherol iodonium salts with different auxiliaries: Thiophene 60 OTs, 61 OTf, 62 BF\(_4\) and p-methoxbenzene 63 OTs, 64 OTf, 65 BF\(_4\).\(^{224}\)

Figure 178. Synthesis of iodonium salts via insitu oxidation of aryl iodides with aromatics (Y) (top), with pre-oxidized hypervalent iodines / aromatics (middle) and boronic acids / esters or stannylates (bottom).\(^{540,541,489}\)

Figure 179. Fluorination of Meldrum's acid iodonium ylides.\(^{542}\)

Figure 180. (Aryl)-\( \alpha \)-tocopherol iodonium salt synthesis from H-Toc or 6-I-\( \alpha \)-Toc.\(^{226}\)

Figure 181. Synthesis of (thiophene)-\( \alpha \)-tocopherol iodonium trifluoroacetate, 61,\(^{544}\)

Figure 182. 2-(Diacetoxy)thiophene synthesis by Wu,\(^{545}\) followed by addition to H-Toc.\(^{227}\)

Figure 183. Synthesis of (R)-(2-hexadecyl-2,5,7,8-tetramethylchroman-6-yl)(phenyl)iodonium bromide 67 from H-Toc,\(^{538}\)

Figure 184. Synthesis of (R)-(2-hexadecyl-2,5,7,8-tetramethylchroman-6-yl)(phenyl)iodonium trifluoroacetate 68 from H-Toc.\(^{544}\)

Figure 185. Synthesis of (R)-(2-hexadecyl-2,5,7,8-tetramethylchroman-6-yl)(phenyl)iodonium tosylate 69 from H-Toc.\(^{546}\)

Figure 186. Carboxylation of 6-I-\( \alpha \)-Toc via lithium-halogen and Grignard reaction.\(^{231}\)

Figure 187. Carboxylation of 6-I-\( \alpha \)-Toc 71 with magnesium (top),\(^{553}\) lithium-halogen exchange (middle) and palladium dichloro catalyzed carbonylation (bottom).\(^{554}\)

Figure 188. Photoborylation of aryl iodides with B\(_2\)OH\(_2\) by Chen.\(^{555}\)

Figure 189. Fluorination of spirocyclic Meldrum's acid iodonium ylides.\(^{556}\)

Figure 190. Meldrum's acid-\( \alpha \)-tocopherol iodonium ylide synthesis 73 from 6-I-\( \alpha \)-Toc.\(^{542}\)

Figure 191. Synthesis of (diacetoxy)iodo-\( \alpha \)-tocopherol 74 from 70.\(^{540}\)

Figure 192. Iodination of H-Toc. (a) with ICl,\(^{558}\) (b) ICl, DDQ,\(^{558}\) (c) I\(_2\), AgOTf\(^\circledR\),\(^{559}\) and (d) 1-iodopyridinium chloride 71.\(^{560}\)

Figure 193. Synthesis of F-Toc from H-Toc with electrophilic fluorinating reagents F-Py BF\(_4\), Selectfluor\(^\circledR\) and NFSI.\(^{567,505}\)

Figure 194. HPLC separation of \( \alpha \)-toc, F-Toc, H-Toc, 6-I-\( \alpha \)-toc, 6-Cl-\( \alpha \)-toc.\(^{241}\)

Figure 195. HPLC separation of F-Toc and H-Toc.\(^{243}\)

Figure 196. HPLC separation of 6-I-\( \alpha \)-Toc, 6-Cl-\( \alpha \)-Toc and H-Toc.\(^{244}\)

Figure 197. Separation of benzenes with increasing fluorine substitution.\(^{245}\)

Figure 198. Synthetic strategies towards 5-F-\( \gamma \)-tocopherol by electrophilic fluorination.\(^{246}\)
Figure 199. Electrophilic $^{18}$F-fluorination of phenols ........................................247
Figure 200. Phenol fluorination with electrophilic fluorinating reagents (a) NFSI, (b) Selectfluor and (c) 1-Fluoropyridinium triflate.$^{584,586,587}$ ........................................248
Figure 201. Byproducts 76 formed during the fluorination of γ-tocopherol sodium phenolate with NFSI. ..................................................249
Figure 202 Byproduct 76 formation from γ-tocopherol sodium phenolate fluorination with NFSI.$^{561}$ ..................................................250
Figure 203 Poon's 5,5′-δ-telluro-bis-tocopherol synthesis.$^{577}$ ..................................................250
Figure 204. Bromination of γ-tocopherol with TBAB.$^{577}$ ..................................................250
Figure 205 Fluorination of 5-Br-γ-tocopherol via lithium-halogen exchange reaction ..................................................251
Figure 206 γ-Tocopherol fluorination with acetyl hypofluorite. ..................................................251
Figure 207 Synthetic strategies towards 5-F-Me-α-tocopherol by nucleophilic fluorination. ..................................................252
Figure 208 Benzylically $^{18}$F-labeled biologically active compounds.$^{591}$ ...............................................252
Figure 209 Manganese(salen) catalyzed fluorination of δ-tocopherol.$^{591}$ ...............................................253
Figure 210. Benzylc $^{18}$F-fluorination by nucleophilic substitution.$^{592,593,594}$ ...............................................253
Figure 211 Substitution of 5-Br-Me-α-toc with nucleophiles.$^{595}$ $N$-protection of aminoacids with α-tocopherol $^{596}$ ..................................................254
Figure 212 Thermally induced formation of α-tocopherol spiro-dimer formation from 5-Br-Me-α-toc.$^{588}$ ..................................................254
Figure 213 AcO-5′Br-Me-α-toc 77 synthesis from α-tocopherol.$^{588}$ ..................................................255
Figure 214. Fluorination of 78 with CsF and t-BuOH. Formation of product 78 and byproduct 79.$^{597}$ ..................................................256
Figure 215 Deprotection of 78 in basic media with KOH in EtOH. Formation of ethoxyether 80.$^{279}$ ..................................................256
Figure 216. Attempted deprotection of AcO-5-F-Me-α-toc 78 in acetic media with TFA and formation of byproduct 5-trifluoracetoxyl alpha-tocopherol acetate 81. ..................................................257
Figure 217. α-Tocopherol ortho-quinone methide (oQM) dimerization to the α-tocopherol-spirodimer and stabilization of the α-tocopherol oQM by NMMO and S-ylide.$^{599,600,601}$ ..................................................258
Figure 218. Synthesis of (O-acetylsalicyl)saligenin from o-cresol.$^{601}$ ..................................................258
Figure 219. Synthetic strategies towards 6'-F-Me-α-tocopherol 50 ..................................................261
Figure 220. Synthesis of benzylic-$^{18}$F-fluoromethyl-spiroperone and 1-phenylpiperazine,$^{605}$ ..................................................261
Figure 221. $^{18}$F-Fluorination of methyl 4-(bromomethyl)-2-chlorobenzoate.$^{606}$ ..................................................262
Figure 222. Deoxyfluorination of benzylic alcohols with DAST, Xtalfluor-E® and Xtalfluor-M®.$^{607-608}$ ..................................................262
Figure 223. HM-Toc fluorination with CsF and t-BuOH. Synthesis of byproduct 46 and 81.$^{597}$ ..................................................264
Figure 224. Synthesis of sulfonate esters, 82 and 83, from HM-Toc, formation of dimer $^{46,603,610}$ ..................................................265
Figure 225. Iodination of HM-Toc with I$_2$, PPh$_3$ and DMAP to product 84 ..................................................265
Figure 226. Alternative benzylic iodination / bromination of HM-Toc.$^{613,616,617}$ ..................................................266
Figure 227. Synthesis of 13-F-α-Toc, 51, from α-tocotrienol ..................................................267
Figure 228. Taylor’s synthesis of 13-HO-α-Toc, 86, from α-tocotrienol ..................................................268
Figure 229. Synthesis of 13-HO-α-Toc, 86, from garcinoic acid.$^{619}$ ..................................................269
Figure 230. BODIPY $^{19}$F.$^{18}$F exchange with (a) TMSOTf $^{621}$ and (b) Lewis acid.$^{622}$ ..................................................270
Figure 231. 1,3-Dimethyl BODIPY fluoride exchange via mono-OTf BODIPY intermediate 87,621

Figure 232. Direct TBAF addition to 1,3-Dimethyl BODIPY. .................................................270
Figure 233. Direct TBAF addition to 2,8-diethyl-1,3,5,7,9-pentamethyl BODIPY. ....................272
Figure 234. Fluorine exchange of 1,3-dimethyl-BODIPY, 3, followed by UV/VIS. ...............273
Figure 235. 1,3-Dimethyl-BODIPY. Left: standard reference 0.32 μM. Right cuvette: 0.32 μM. Blue line in Figure 234. ..........................................................273
Figure 236. 1,3-Dimethyl-BODIPY. Left cuvette: standard reference 0.32 μM. Right cuvette: Tf₂O addition. Red line in Figure 234. ........................................274
Figure 237. 1,3-Dimethyl-BODIPY. Left cuvette: standard reference 0.32 μM. Right cuvette: 1) t-BuOH 2) Lutidine 3) TBAF addition. Green line in Figure 234. ..................274
Figure 238. Fluorine exchange of thienyl-ene-BODIPY, 3, followed by UV/VIS. ....................275
Figure 239. Thienyl-ene-BODIPY. Left cuvette: standard reference 0.16 μM. Right cuvette: 0.32 μM. Blue line in Figure 238. ..........................................................276
Figure 240. Thienyl-ene-BODIPY. Left cuvette: standard reference 0.16 μM. Right cuvette: Tf₂O addition. Red line in Figure 238. ........................................276
Figure 241. Thienyl-ene-BODIPY. Left cuvette: standard reference 0.16 μM. Right cuvette: 1) t-BuOH 2) Lutidine 3) TBAF addition. Green line in Figure 238. ...............277
Figure 251. Viability of mouse cells cultured in the presence of α-, F-, and HM-tocopherol derivatives at concentrations ranging from 1 nM to 1 mM. ........................................279
Figure 252. Viability of mouse cells cultured in the presence of BODIPY-tocopherol at concentrations ranging from 1 nM to 0.1 mM. .........................................................280
Figure 253. Viability of mouse cells cultured in the presence of H-tocopherol (H-toc) at concentrations ranging from 1 nM to 1 mM. .................................................281
Figure 254. Viability of mouse cells cultured in the presence of I-tocopherol (I-toc) at concentrations ranging from 1 nM to 1 mM. .................................................282
Figure 255. Viability of mouse cells cultured in the presence of varying concentrations of large unilamellar vesicles (LUVs). .........................................................283
Figure 256. Viability of mouse cells cultured in the presence of varying concentrations of large unilamellar vesicles (LUVs). .........................................................284
Figure 257. C2C12 cell uptake of thienyl-ene-BODIPY-tocopherol (BODIPY-tocopherol) delivered in LUVs. ..........................................................285
Figure 258. Uptake of thienyl-ene-BODIPY-tocopherol (BODIPY-tocopherol) in C2C12 cells at 30 min after delivery via DMSO or LUVs. ..............................................286
Figure 259. Mouse embryonic fibroblast uptake of thienyl-ene-BODIPY-tocopherol (BODIPY-tocopherol) delivered in LUVs. .........................................................287
Figure 260. Mouse embryonic fibroblast uptake of BODIPY-tocopherol delivered in DMSO. .............................................................................................................288
Figure 261. Fluorine exchange (F₃-F₆) on thienyl-ene-BODIPY. ............................................290
Table 1. Tocopherol and tocotrienol structures.  
Table 2. Vitamin E amount in unsaponifiable palm oil.  
Table 3. Ligand binding to CRAL-domain proteins; Comparison of dissociation constants of α-TTP, S. cerevisiae Sec14p, SPF, and CRALBP for various hydrophobic ligands.  
Table 4. Antioxidant inhibition of α-tocopherol, melatonin & N-acetyl-serotonin in mitochondria and microsomes as IC50 values based on 50% inhibition of light emission.  
Table 5. Maximum wavelength, absorption and fluorescence of aromatic amino acid.  
Table 6. Exitation and emission wavelength of commercial dyes for fluorescent microscopy.  
Table 7. Comparison of BODIPY-Toc to α-Toc-C9-NBD.  
Table 8. Influence of chain length linker on the α-TTP dissociation constants (Kd) of NBD-α-Toc and BODIPY (BDP)-α-Toc.  
Table 9. Conjugation effects to BODIPY.  
Table 10. Inhibition rates (kinh) of vitamin E by different techniques.  
Table 11. Content of α-tocopherol (mmol/mol lipid) in subcellular membranes of rats fed a standard laboratory diet.  
Table 12. Halogen bond and the influence of bonding angle α on bond length and strength.  
Table 13. Stability assessment of HM-Toc.  
Table 14. Radiation weight factor (Wr) of different radiation types.  
Table 15. Tissue weigh factor (Wr).  
Table 16. Nucleophilic and electrophilic 18F radio nucleotide synthesis.  
Table 17. Solubility of alkali halides in methanol.  
Table 19. Anatase percentage (%A) in Degussa P25 TiO2 at increasing temperature, after 24 h.  
Table 20. TiO2 mediated fluorination of TsO-PMC.  
Table 21. TiO2 mediated fluorination of TsO-Naphthol.  
Table 22. TiO2 mediated fluorination of with EPRUI® 200 nm TiO2 NP.  
Table 23 Fluorination of (R)-(2-hexadecyl-2,5,7,8-tetramethylchroman-6-yl)(phenyl)iodonium tosylate 69.  
Table 24. Photoborylation of 6-1-α-Toc to form 72.  
Table 25. Fluorination of 6-I-α-toc with BuLi and NFSI.  
Table 26. Fluorination of H-Toc with electrophilic fluorinating reagents.  
Table 27. Fluorination of γ-tocopherol with electrophilic fluorinating reagents.  
Table 28. Fluorination of Ac0-5-Br-Me-α-toc.  
Table 29. 78 was stirred in a µ-wave oven or for 10-20 min.  
Table 30 α-Tocopherol fluorination with Ag2O.  
Table 31. α-Tocopherol fluorination with Ag2O and stabilizing agents.  
Table 32. Deoxyfluorination of HM-Toc.  
Table 33. Nucleophilic fluorination of HM-Toc.  
Table 34. Contents for 10 mg/mL of 7:3:4-POPC:POPG:Cholesterol.
Table 39. Contents for 2 mg/mL of 7:3:4:0.1-POPC:POPG:Cholesterol:BODIPY .................295
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>-CH₂-</td>
<td>Methylene (-CH₂-) bridge</td>
</tr>
<tr>
<td>4-HPPA</td>
<td>4-Hydroxypyruvate</td>
</tr>
<tr>
<td>5-LOX</td>
<td>5-Lipoxygenase</td>
</tr>
<tr>
<td>9-CDMOHC</td>
<td>9-Carboxymethyloctylhydroxychromanol</td>
</tr>
<tr>
<td>11-CDMDHC</td>
<td>11-Carboxydimethyldecylhydroxychromanol</td>
</tr>
<tr>
<td>17-β-E₂</td>
<td>17-β-estradiol</td>
</tr>
<tr>
<td>[³T]</td>
<td>Tritium</td>
</tr>
<tr>
<td>α</td>
<td>Alpha</td>
</tr>
<tr>
<td>β</td>
<td>Beta</td>
</tr>
<tr>
<td>γ</td>
<td>Gamma</td>
</tr>
<tr>
<td>δ</td>
<td>Delta</td>
</tr>
<tr>
<td>ω</td>
<td>Omega</td>
</tr>
<tr>
<td>ε</td>
<td>Extinction coefficient</td>
</tr>
<tr>
<td>Φₚ</td>
<td>Fluorescence quantum yield</td>
</tr>
<tr>
<td>τ</td>
<td>Fluorescence lifetime</td>
</tr>
<tr>
<td>λ</td>
<td>Wavelength</td>
</tr>
<tr>
<td>α-CEHC</td>
<td>Alpha-carboxyethylhydroxychroman</td>
</tr>
<tr>
<td>α-TL</td>
<td>Alpha-tocopheronelactoneα-tocopheronelactone</td>
</tr>
<tr>
<td>α-Toc</td>
<td>Alpha-tocopherol</td>
</tr>
<tr>
<td>α-TocO’</td>
<td>Alpha-tocopherol radical</td>
</tr>
<tr>
<td>α-TTP</td>
<td>Alpha-tocopherol transfer protein</td>
</tr>
<tr>
<td>γ-NGT</td>
<td>5’-NO₂ Tocopherol</td>
</tr>
<tr>
<td>Å</td>
<td>Ångström</td>
</tr>
<tr>
<td>AA</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>ABCA1</td>
<td>ATP-binding cascade A1 transporters</td>
</tr>
<tr>
<td>Aβ</td>
<td>β-amyloid protein</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor</td>
</tr>
<tr>
<td>AO-α-Toc</td>
<td>ω-anthroyloxy-α-tocopherol</td>
</tr>
</tbody>
</table>
Apo  Apolipoprotein
APP  Amyloid precursor protein
ATP  Adenosine triphosphate
AVED Ataxia with Vitamin E Deficiency
BHT  Butylated hydroxyl toluene / 2,6-di-tert-butyl-4-methylphenol
BODIPY 4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene
Bq  Bequerel
BSA  Bovine serum albumin
BuLi  Butyllithium
CCl₄  Carbon tetrachloride
CEHC  Carboxyethyl hydroxychromans
CLSM  Confocal light scanning microscope
CNS  Central nervous system
CoQ(10) Coenzyme Q 10 / Ubiquinone
CoQH₂  Ubiquinonol
COX-2  Cyclooxygenase-2
CRALBP  Cellular retinaldehyde binding protein 1
DABCO / TEDA 1,4-Diazabicyclo[2.2.2]octan / Triethylendiamin
DAG  Diacylglycerol
DAHP  3-Dehydroxy-d-arbino-heptalose-7-phosphate
DCM  Dichloromethane
DFOA  Desferrioxamine
DHQ  3-Dehydroquinate
DHQD  Dehydroquinase dehydratase
Di-i-pro-TocO•  Di-i-propyl tocopheroxy radicals
DIBAL  Diisobutylaluminium hydride
DLPC  1,2-Dilauroyl-sn-glycero-3-phosphorylcholine
DMAPP  Dimethylallyl pyrophosphate
DMF  Dimethylformamide
DMSO  Dimethylsulfoxide
DOPE  Dioleoylphosphatidylethanolamine
DOPS  1,2-Dioleoyl-sn-glycero-3-phospho-L-serine
DPPH  2,2-Diphenyl-1-picrylhydrazyl
DSSC  Dye sensitized solar cell
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>E4P</td>
<td>D-erythrose-4-phosphate</td>
</tr>
<tr>
<td>EAATS</td>
<td>Excitatory amino acid transporters</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediamine tetraacetic acid</td>
</tr>
<tr>
<td>EPR</td>
<td>Electron spin resonance</td>
</tr>
<tr>
<td>EPSP</td>
<td>5-Enolpyruvylshikimate-3-phosphate</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic reticulum</td>
</tr>
<tr>
<td>FA</td>
<td>Fatty acid</td>
</tr>
<tr>
<td>Fluorazophore-L</td>
<td>2,3-Diazabicyclo[2.2.2]-2-octene palmate (DBO-palmitate)</td>
</tr>
<tr>
<td>FRET</td>
<td>Förster resonance energy transfer</td>
</tr>
<tr>
<td>GGDP</td>
<td>Geranylgeranyldiphosphate</td>
</tr>
<tr>
<td>GGPP</td>
<td>Geranylgeranylpyrophosphate</td>
</tr>
<tr>
<td>GGR</td>
<td>Geranylgeranyl reductase</td>
</tr>
<tr>
<td>GOLD</td>
<td>Golgi dynamics</td>
</tr>
<tr>
<td>GPCR</td>
<td>G-protein coupled receptors</td>
</tr>
<tr>
<td>GPP</td>
<td>Geranyl pyrophosphate</td>
</tr>
<tr>
<td>GS</td>
<td>Glutamine synthase</td>
</tr>
<tr>
<td>GSSG</td>
<td>Glutathione disulfide</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>Hc</td>
<td>Hectare</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrogen chloride</td>
</tr>
<tr>
<td>HDL</td>
<td>High-dense lipoproteins</td>
</tr>
<tr>
<td>HGA</td>
<td>Homogentistic acid</td>
</tr>
<tr>
<td>HO’</td>
<td>Hydroxyl radical</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest occupied molecular orbital</td>
</tr>
<tr>
<td>IAS</td>
<td>Inhibitory auto-oxidation assay with the styrene</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>Inhibitory concentration 50%</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intracellular adhesion molecule-1</td>
</tr>
<tr>
<td>ICM</td>
<td>Intermediate chain metabolites</td>
</tr>
<tr>
<td>IP$_3$</td>
<td>Inositol trisphosphate</td>
</tr>
<tr>
<td>IPP</td>
<td>Isopentenyl pyrophosphate</td>
</tr>
<tr>
<td>IRS-1</td>
<td>Insulin receptor substrate 1</td>
</tr>
<tr>
<td>$k_d$</td>
<td>Dissociation constant</td>
</tr>
<tr>
<td>$k_{inh}$</td>
<td>Inhibition rate</td>
</tr>
<tr>
<td>$k_p$</td>
<td>Propagation rate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>$k_q$</td>
<td>Antioxidant efficiency</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilo dalton</td>
</tr>
<tr>
<td>KIE</td>
<td>Kinetic isotope effect</td>
</tr>
<tr>
<td>LCM</td>
<td>Long chain metabolites</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>LHMDS</td>
<td>Lithium hexamethyldisilazide</td>
</tr>
<tr>
<td>LOO’</td>
<td>Lipid peroxyl radical</td>
</tr>
<tr>
<td>LPA</td>
<td>Lysophosphatic acid</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
</tr>
<tr>
<td>LRP</td>
<td>Low-density receptor related protein receptors</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest unoccupied molecular orbital</td>
</tr>
<tr>
<td>MAO-B</td>
<td>Monoamine oxidase B</td>
</tr>
<tr>
<td>MAP</td>
<td>Mitogen-activated protein</td>
</tr>
<tr>
<td>MD</td>
<td>Molecular dynamic</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>MDT</td>
<td>Marine-derived tocopherol</td>
</tr>
<tr>
<td>mGluR</td>
<td>Metabotropic glutamate receptor</td>
</tr>
<tr>
<td>MMT</td>
<td>L-Methionine s-methyl transferase</td>
</tr>
<tr>
<td>MPP+</td>
<td>1-Methyl-4-phenylpyridinium</td>
</tr>
<tr>
<td>MPTP</td>
<td>1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridin</td>
</tr>
<tr>
<td>mtNOS</td>
<td>Mitochondrial nitric oxide synthase</td>
</tr>
<tr>
<td>NAS</td>
<td>N-acetyl-serotonin</td>
</tr>
<tr>
<td>NBD</td>
<td>Nitrobenzoxadiazole</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear factor-κB</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-Methyl-D-aspartate</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>NPC1L1</td>
<td>Niemann-Pick C1-like protein 1 receptors</td>
</tr>
<tr>
<td>$O_2^{•−}$</td>
<td>Superoxide</td>
</tr>
<tr>
<td>$O_2^{1}$</td>
<td>Singlet oxygen</td>
</tr>
<tr>
<td>PA</td>
<td>Phosphatidic acid</td>
</tr>
<tr>
<td>PC</td>
<td>Phosphatidylcholine</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>Pd/C</td>
<td>Palladium on carbon</td>
</tr>
<tr>
<td>PDP</td>
<td>Phytidiphosphate</td>
</tr>
<tr>
<td>PEP</td>
<td>Phosphoenolpyruvate</td>
</tr>
<tr>
<td>PE</td>
<td>Phosphatidylethanolamine</td>
</tr>
<tr>
<td>PI</td>
<td>Phosphatidylinositol</td>
</tr>
<tr>
<td>PI(4,5)P₂</td>
<td>Phosphatidylinositol(4,5)bisphosphate</td>
</tr>
<tr>
<td>PI(3,4,5)P₃</td>
<td>Phosphatidylinositol(3,4,5)triphosphate</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein kinase C</td>
</tr>
<tr>
<td>PLA₂</td>
<td>Phospholipase A₂</td>
</tr>
<tr>
<td>PLC</td>
<td>Phospholipase C</td>
</tr>
<tr>
<td>PMC</td>
<td>Pentamethylchromanol</td>
</tr>
<tr>
<td>POPC</td>
<td>1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine</td>
</tr>
<tr>
<td>PP₂A</td>
<td>Protein phosphatase 2A</td>
</tr>
<tr>
<td>PP₃</td>
<td>Triphenylphosphine</td>
</tr>
<tr>
<td>PTP</td>
<td>Protein tyrosine phosphate</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
</tr>
<tr>
<td>RAF</td>
<td>Rapidly accelerated fibrosarcoma protein kinase</td>
</tr>
<tr>
<td>Rᵢ</td>
<td>Initiation rate</td>
</tr>
<tr>
<td>RKIP</td>
<td>RAF-1 kinase inhibitor protein</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SCM</td>
<td>Short-chain metabolites</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>Sec14p</td>
<td><em>Saccharomyces cerevisiae</em> phosphatidylinositol transfer protein</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of mean</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>SPF</td>
<td>Supernatant protein factor</td>
</tr>
<tr>
<td>SR-B1</td>
<td>Class B type 1 scavenger receptor</td>
</tr>
<tr>
<td>STED</td>
<td>Stimulated emission depletion microscopy</td>
</tr>
<tr>
<td>Sv</td>
<td>Sievert</td>
</tr>
<tr>
<td>TAP</td>
<td>Tocopherol associated protein</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid reactive substances</td>
</tr>
<tr>
<td>TBS</td>
<td>t-Butyldimethylsilane</td>
</tr>
<tr>
<td>TAP/SPF</td>
<td>Tocopherol alike protein / Supernatant protein factor</td>
</tr>
<tr>
<td>TH</td>
<td>Tyrosine hydroxylase</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TMHQ</td>
<td>2,3,6-Trimethylhydroquinone</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid reactive substance</td>
</tr>
<tr>
<td>Tocored</td>
<td>2,2,7,8-tetramethyl-5,6-chromanquinone</td>
</tr>
<tr>
<td>TOF-MS</td>
<td>Time-of-flight mass spectrometry</td>
</tr>
<tr>
<td>TRIO</td>
<td>TRIO guanine exchange factor</td>
</tr>
<tr>
<td>Trolox®</td>
<td>6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid</td>
</tr>
<tr>
<td>TS</td>
<td>Tocopherol synthase</td>
</tr>
<tr>
<td>tta</td>
<td>Total tocopherol amount</td>
</tr>
<tr>
<td>ttc</td>
<td>Total tocopherol content</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Vascular cell adhesion molecule-1</td>
</tr>
<tr>
<td>VE</td>
<td>Vitamin E</td>
</tr>
<tr>
<td>VE-RBA</td>
<td>Vitamin E relative biological activity</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very-low-density lipoprotein</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Ascorbate</td>
</tr>
</tbody>
</table>
1 Introduction

1.1 Discovery and Structure

The discovery of Vitamin E started in 1920 when Henry A. Mattill investigated the effect of a milk diet on the reproductive success of female rats. The female rats, given a strict diet of only fresh milk, were healthy and grew normally but were not able to reproduce.¹ Later tests with other diets, containing different amounts of dried milk and various additives like starch, lard and salts, also could not complete live births. Mattill proposed that the female rats did not reproduce because they lacked a compound found in their normal diet.² He based this idea on the paper “Oestrous cycle in the Rat” published by Evans in 1922 and the associative effects on health seen by the recently discovered vitamins A, B and C.³ In the same year Evans and Bishop provided evidence that the assumption of Mattill was correct, that female rats did not reproduce on a purified diet of casein, cornstarch, lard, butterfat, salts and Vitamin A, B and C. Successful reproduction was recovered when rats were fed lettuce leaves.⁴

Barnett Sure named the missing substrate Vitamin E however the actual structure and biochemical mechanism were unknown until Evans isolated and characterised Vitamin E in 1936 from concentrated wheat germ oil.⁵⁶⁷ The isolated compounds were named “tocopherol”, derived by the Greek words “tocos”, which means childbirth and “phero”, meaning to bring. The ending “of” refers to the hydroxyl group in the molecule.⁸
Vitamin E represents a collective of eight molecules (vitamers) split into two groups called tocopherols and tocotrienols, both with a chromanol “head” substituted with a 16-carbon “tail” on the 2′ position as a basic skeleton.

Tocopherols have a saturated carbon tail, based on the structure of phytlyldiphosphate (PDP), whereas tocotrienols have an unsaturated tail with unsaturation on the 3′, 7′, and 11′ positions, and which is derived from geranylgeranyl diphosphate (GGDP). Tocopherols and tocotrienols are further differentiated by their methyl substitution pattern on the aromatic side of the chroman ring (α, β, γ, and δ).9,10 All Vitamin E molecules have a chiral centre at the 2′ position, the tocopherols have two additional ones at the 4′ and 8′ position. The main natural form is the (RRR) isomer (Table 1).11

<table>
<thead>
<tr>
<th>Tocopherol</th>
<th>Tocotrienol</th>
<th>R₁</th>
<th>R₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tocopherol</td>
<td>α-Tocotrienol</td>
<td>Me</td>
<td>Me</td>
</tr>
<tr>
<td>β-Tocopherol</td>
<td>β-Tocotrienol</td>
<td>Me</td>
<td>H</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>γ-Tocotrienol</td>
<td>H</td>
<td>Me</td>
</tr>
<tr>
<td>δ-Tocopherol</td>
<td>δ-Tocotrienol</td>
<td>H</td>
<td>H</td>
</tr>
</tbody>
</table>

Table 1. Tocopherol and tocotrienol structures.10

1.2 Natural sources and tocopherol synthesis

All eight commonly known vitamin E vitamers are synthesized primarily in plants and are distributed throughout the plant organs in different amounts. The largest amounts are found in seeds, where most plants store predominantly α- or γ-tocopherol. In humans, dietary differences across populations change intake of the different forms. The American diet uses predominantly corn oil (~ 70% γ-, ~ 20% α- in 1000 µg/g total tocopherol amount (tta))10 and soybean oil (~ 70% γ-, ~ 7% α- in 1200 µg tta)10 and partially sesame seed oil (~ 98% γ, ~ 2% α in 530 µg
tta), all containing mainly γ-tocopherol. European diets, on the other hand, contain mostly α-tocopherol enriched oils. High amounts of α-tocopherol are found in sunflower seed oil (~ 96% α- , ~ 3% γ- in 700µg tta)\textsuperscript{10}, safflower seed oil (~ 94% α-, ~ 3% γ- in 609 µg tta) and olive oil (~ 94% α-, ~ 1% γ- in 191 µg tta). Rape seed (canola oil) (~ 45% α-, ~ 55% γ- in µg tta) contains both tocopherols in equal amounts.\textsuperscript{12}

Oil palm fruits are known as rich sources of hydrophobic natural products and are therefore annually harvested in extraordinary amounts (4-5t per Hec per year). Other oils mentioned yield around 0.2-1.0t per Hec per year.\textsuperscript{13} Despite being a major plant oil source the overall vitamin E content is low (500-800 µg/g tta, 350-630 µg/g when refined) (Table 3).\textsuperscript{14-15}

The highest overall amount in vitamin E content is found in wheat germ oil (2.15 mg/g).\textsuperscript{16}

<table>
<thead>
<tr>
<th>Component</th>
<th>%</th>
<th>Mg/kg (in palm oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotenoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-carotene</td>
<td>36.2</td>
<td>500 - 700</td>
</tr>
<tr>
<td>β-carotene</td>
<td>54.4</td>
<td></td>
</tr>
<tr>
<td>γ-carotene</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>Lycopene</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Xanthophylls</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>28</td>
<td>500 - 800</td>
</tr>
<tr>
<td>α-tocotrienol</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>γ-tocotrienol</td>
<td>28</td>
<td>500 - 800</td>
</tr>
<tr>
<td>δ-tocotrienol</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Sterols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>4</td>
<td>~300</td>
</tr>
<tr>
<td>Campesterol</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Stigmasterols</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>β-sitosterol</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>Phosphatides</td>
<td></td>
<td>500 - 1000</td>
</tr>
<tr>
<td>Total alcohols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triterpenic alcohol</td>
<td>80</td>
<td>~800</td>
</tr>
<tr>
<td>Aliphatic alcohol</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Vitamin E amount in unsaponifiable palm oil.\textsuperscript{12}

More information on the vitamin E oil composition can be found in DellaPenna (2005)\textsuperscript{10}, Hess (1993)\textsuperscript{17}, Schreurs (1985)\textsuperscript{18}, Taylor and Barnes (1980)\textsuperscript{19} & (1981)\textsuperscript{20} and their amounts in foods\textsuperscript{21}.
1.3 Tocopherol chemical synthesis

RRR-α-tocopherol is the most biologically potent antioxidant of all the vitamers.\(^{22}\) Since only low amounts of α-tocopherol are isolated from plants, industrial synthetic processes have been developed to satisfy demand, which was 100,000T/year (synthetic) in 2015 (22,000T/Yr in 2001 with 2,000T from natural sources). Its main use is in animal stock feeds, dietary supplementation, and pharmaceuticals. The current market value for mixed tocopherols lies around $ 2 B/Yr in 2015 (~100 M/Yr in 1998).\(^{23}\) The vitamin E business used to be monopolised by F. Hoffmann-La Roche Ltd., but is currently shared by several companies. The main manufacturers are Dutch States Mines “DSM” (who bought the vitamin division from Roche in 2003) (25,000T/Yr, 50% of the world wide demand in 2010),\(^{24}\) BASF, Sanofi-Aventis (ex Rhone-Poulenc Animal Nutrition) and Chinese Chemical and Pharmaceutical Industry association “cpia” (12’000T/Yr, 30% of the worldwide demand in 2010).\(^{25}\)

Synthetic α-tocopherol is industrially produced as a racemate by an acid catalyzed condensation of 2,3,6-trimethylhydroquinone (TMHQ)\(^{26}\) and isophytol.\(^{27}\) Much research effort has been put into the synthetic optimization in production of the two starting materials as outlined by Mercier & Chabardes (1994).\(^{28}\) Oxidative assays have verified the potency of the racemic mixture and shown an antioxidant power of (1 : 1.49) to the RRR-α-tocopherol isomer.\(^{29}\)

To further increase the yield of α-tocopherol isolation from natural oil sources was a semisynthetic route developed by methylating residual γ- and δ-tocopherol.\(^{30}\) Diverse synthetic strategies towards optically pure tocopherols have been described in literature. The key step in all approaches is a cyclization reaction mimicking the tocopherol cyclase (Figure 1).\(^{31,32,33,34,35,36,37,38,39,40,41,42,43}\) Further synthetic effort lies in the synthesis of the phytol / geranylgeranyl side chain.\(^{44,45,46,47}\)
The vitamin E family has in recent years been extended by new derivatives found in nature. Studies on different plant and vegetable oil extracts in 1995-1996 discovered partially reduced form of α-tocotrienol, tocomonoenol. Palm oil fibre is a rich source of tocotrienols with high concentrations of α-tocomonoenol (430 ± 6 ppm, α-tocopherol; 1810 ± 10 µg/g). Higher quantities of γ-tocomonoenol (118.7 ± 1.0 µg/g) and γ-tocopherol; 586.0 ± 4.6 µg/g) were found alongside α–tocomonoenol (17.6 ± 0.6 µg/g) in pumpkin parts of Slovenska golica. δ-Tocomonoenol (23.0 ± 0.1 µg/g, δ-tocopherol; 30.7 ± 0.1 µg/g) was successfully isolated from kiwi fruits, Actinidia chinensis, by Fiorentino. His group further assessed δ-tocomonoenol antioxidant properties by a thiobarbituric acid reactive substance (TBARS) assay and its radical scavenging activity against DPPH and superoxide. The results showed lower activities compared to natural α-tocopherol, but higher activities than its δ-tocopherol brethren.

In 2001, Yamamoto isolated from chum salmon eggs a new α-tocomonoenol, dubbed as marine derived tocopherol (MDT). The vitamin E concentrations of diverse salmon, cod, and herring fish tissues were determined and it was concluded that MDTs are common among cold water fish. MDT is not a fish metabolite but are obtained from the fish’s diet. Cold water phyto- and
zooplankton keep 10-20% MDT in their vitamin E stock. Interestingly, no tocotrienols were found in any of the samples (Figure 2).

![Structure of Tocomononol & marine derived Tocopherol (MDT)](image)

MDTs have similar antioxidant values to tocomonoenols. When equimolar cholestereol amounts were added to enhance the liposomal membrane microviscosity showed MDT superior antioxidant activity to α tocopherol at 0°C. The increase antioxidant ability is based on the increased unsaturation in the phytol chain, which leads to enhanced membrane permeability at lower temperature, as Serbinova showed for tocotrienols. The vitamin E relative biological activity (VE-RBA) of both mono-unsaturated derivatives showed a higher bioavailability than β- & γ-tocopherol when fed to mice over a 28-day period. The majority of MDT and tocomonoenol VE-RBA were found in the liver, none in the lungs & spleen. The brain, known for its long lasting vitamin E stock, only accumulated minimal amounts of monounsaturated-tocopherols. This is explained by having already residual α-tocopherol stockpiled. Between marine derived tocopherol (MDTs) and tocomonoenols have MDTs a higher VE-RBA. The small difference of olefin position on the phytol-chain influences the biophysical nature of the cell membrane. Furthermore, both monoenols have different lateral diffusion coefficients in membranes, affecting the capability to reach sites of oxidative stress. The anti-oxidant function does not change since it is conserved in the chroman ring portion.
1.4 Biosynthesis

All vitamin E vitamers are produced in photosynthetic plants by the combination of the shikimic acid\textsuperscript{62,63} and non-mevalonate pathway.

1.4.1 Shikimiate pathway

The shikimic acid pathway starts by combing phosphoenolpyruvate (PEP) with D-erythrose-4-phosphate (E4P). 3-Dehydroxy-d-arbino-heptalose-7-phosphate (DAHP) is the product of the two starting synthons. DAHP is rearranged to form a cyclohexanone in 3-dehydroquinate (DHQ). In the third and forth step dehydroquinase dehydratase (DHQD) and shikimate dehydrogenase DHQ catalyze the formation of shikimate. After phosphorylation of the third hydroxyl group, pyruvate is added to the C-5 position by PEP. Dehydration of the obtained 5-enolpyruvylshikimate-3-phosphate (EPSP) yields the final intermediate of the pathway, chorismate. Chorismate is a key precursor for aromatic compounds in plant biosynthesis (Figure 3)\textsuperscript{64,63}.
Figure 3. Shikimate pathway. Synthesis of Chlorismate from D-erythrose-4-phosphate (E4P) and phosphoenolpyruvate (PEP).\textsuperscript{63,64}

For tocopherol biosynthesis, chorismate is turned into 4-HPPA by a two-step process; first, a Claisen rearrangement produces prephenate, which is aromatized by NAD\textsuperscript{+} mediated decarboxylation to the 4-hydroxy-pyruvate (4-HPPA) (Figure 4).\textsuperscript{65-66}

Figure 4. Biosynthetic conversion of chorismate to 4-HPPA.\textsuperscript{66}
The next part of the biosynthesis is a rearrangement of 4-HPPA by 4-HPPA-dioxygenase. In one conserved step 4-HPPA is oxidized, undergoes a rearrangement and is decarboxylated to form Homogentistic acid (HGA). The CH₂ of 4-HPPA is now in the meta-position with respect to 4-hydroxy phenol and resembles the 8′-methyl group in vitamin E.

1.4.2 Non-mevalonate pathway

The second part of the tocopherol biosynthesis prepares the phytol/geranyl sidechain. The chains are synthesised from the isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) monomers, the final products from the non-mevalonate pathway (MEP/DOXP pathway).⁶⁷⁶⁸

IPP and DMAPP are combined to form geranyl pyrophosphate (GPP), which is driven by the loss of diphosphate. Two more IPP units are attached to form geranylgeranyl pyrophosphate (GGPP). GGPP is reduced by geranylgeranyl reductase (GGR) to phytolpyrophosphate (PPP) and combined with HGA. The resulting 2-methyl-6-phytyl-1,4-benzoquinol is directly cyclized by tocopherol synthase (TS) to δ-tocopherol or methylated by a L-methionine s-methyl transferase (MMT) to access the γ-quinol. β-Tocopherol is obtained by methylation of δ-tocopherol, α-tocopherol by cyclization of γ-tocopherol followed by a methylation. The same methylation / cyclization pathway is used when GGPP is cyclized with HGA to obtain the tocotrienols (Figure 5).⁶⁹
1.5 Biological absorption & transport

The human body is not able to synthesize vitamin E and therefore is a requirement in our diet; 15-30 milligrams daily intake is suggested. Of the consumed vitamin, 20-40% passes through the intestinal membrane. Tocopherol is absorbed across the intestinal membrane as micelles.
with fatty acid esters and cholesterol (and bile salts) by passive diffusion or via class B type 1 scavenger receptor (SR-B1) and Niemann-Pick C1-like protein 1 receptors (NPC1L1) on the gut lumen wall. Once transported across the luminal site all vitamers are packed into chylomicrons at the ER. Chylomicrons are 75-600 nm in size and composed of ~90% triglycerides, ~10% phospholipids, ~2% cholesterol. Chylomicrons bind the intestinal apolipoprotein ApoB-48 (non-HDL) and ATP-binding cascade A1 transporters (ABCA1) at the apical side of the lumen, which sends the remnants into the lymphatic system and eventually to the bloodstream.\textsuperscript{70,71,72}

High-density lipoproteins (HDL) place ApoE and ApoC2 onto the now mature chylomicron remnants. The chylomicron ApoC-2 activates lipoprotein lipase (LPL) which turns chylomicrons into smaller vesicles called chylomicron remnants (30-50 nm). Extra hepatic cells absorb the released fatty acids and triglycerides (and some tocopherol) as part of muscle stimulation and fat storage in adipose cells. The Apo proteins attached on the LPL are transferred back to free flowing HDLs. Remnant specific low-density receptor related protein receptors (remnant-LRP) on the liver cell endocytose these small packages. The resulting endosomes contain all possible vitamers, which at this stage are not distinguished from each other (Figure 6).\textsuperscript{73}
Most of the β−, γ−, and δ-tocopherols /trienols are degraded to carboxyethylhydroxychromans (CEHC), which are excreted via the urine.⁷⁴ Patients that obtained deuterium labeled vitamin E mixtures have shown higher blood plasma concentrations of the RRR-α-tocopherol after 24h over any other form.⁷⁵ The liver contains one third of all the α-tocopherol in the body, making it the largest storage pool of α-tocopherol.⁷⁶ Endosomal α-tocopherol is bound and transported to the cell membrane by the α-tocopherol transfer protein (α-TTP). α-TTP is expressed from the ttpa gene and has a calculated mass of 31.7kD and consists of 278 amino acids and is expressed by the ttpa gene.

Recent studies describe that α-TTP is transported to the hepatocyte membrane in recycling endosomes, bearing the GTPase Rab8, transferrin and the transferrin receptor CD71.⁷⁷ α-TTP is transferred from the endosome to the hepatocyte cell membrane, where α-tocopherol release is mediated by PI(4,5)P₂ binding to α-TTP.⁷⁸ On the membrane, ABCA1 participates in the secretion of very-low-density lipoproteins (VLDLs) as a transport system for α-tocopherol in
the blood. VLDL binds ApoE and ApoC, and interacts during the transport in the blood with LPLs, creating low-density lipoproteins (LDL), which are transformed to HDLs. LDL or HDL-receptors (LDLR/SR-BI) on extrahepatic cells incorporate the α-tocopherol by endocytosis. Excess tocopherol is reintegrated into the liver by SR-BI mediated uptake of HDL particles and is either recycled or metabolized.\textsuperscript{79,80}

1.5.1 Tocopherol metabolism

Two groups of tocol metabolites can be extracted from urine. The first group consists of three metabolites called Simon products, α-tocopheronic acid, α-tocopheronelactone (α-TL), and α-tocopheryl quinone. Ring opening of tocopheryl quinone creates Simon products. The second group are chain-shortened products, ultimately resulting in α-carboxyethylhydroxychroman (α-CEHC). It is still questioned whether Simon products are oxidized prior to or during the chain breakdown. The metabolites still bearing the chroman ring are excreted as sulfonated and glycosylated adducts.\textsuperscript{81,82}

Tocopherol breakdown starts in the ER, where the cytochrome P450 enzymes CYP4F2 and 3A4 hydroxylates tocopherol at the ω-position of the phytyl chain. CYP4F2 and 3A4 oxidation is the rate-limiting step of tocol metabolism (blue colored part, top panel, in Figure 7).\textsuperscript{83}

Dehydrogenases oxidize α-tocopherol-13-OH to the carboxylic acid and afterwards undergo rounds of β-oxidation (β-scission) producing 3-carbon products, forming first carboxydimethyldecyhydroxychromanol (11-CDMDHC, green colored part, middle panel, Figure 7). The chain shortens by one more β-scission cleaving to carboxymethyloctylhydroxychromanol (9-CDMOHC). All oxidized tocopherol products to this point are termed long-chain metabolites (LCM) and are not found in urine. Two more β-oxidations in the peroxisome form the 5 and 7-COOH tocopherol metabolites. Both belong to the category of intermediate-chain metabolites (ICM), and are found in small percentages in
urine (mainly the 5-COOH). α-CEHC is formed in mitochondria after a final β-oxidation.

Because of its short remaining chain length of three carbons, α-CEHC forms the group of short-chain metabolites (SCM, light brown part, lower panel, in Figure 7).73

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Tocopherols</th>
<th>Tocotrienols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endoplasmic reticulum</td>
<td><img src="image1.png" alt="image" /></td>
<td><img src="image2.png" alt="image" /></td>
</tr>
<tr>
<td>Peroxisome</td>
<td><img src="image3.png" alt="image" /></td>
<td><img src="image4.png" alt="image" /></td>
</tr>
<tr>
<td>Mitochondrium</td>
<td><img src="image5.png" alt="image" /></td>
<td><img src="image6.png" alt="image" /></td>
</tr>
</tbody>
</table>

Figure 7. Vitamin E metabolites.

Blue part: hydroxylation by CYP4F2 and 3A4 ω-oxidation, formation of long-chain metabolite (LCM) 13'-hydroxychromanol. Green & light brown part: sequential β-scission to the phytol chain, formation of LCM 11-CDMDHC and 9-CDMDHC, intermediate-chain metabolites (ICM) 7-CDMDHC and 5-CDMDHC, and short chain metabolite (SCM) α-CEHC.73

All tocopherols and tocotrienols follow the same path of sidechain degradation despite their methylation pattern. Serum half-life of tocopherols differ and at least partially reflects the trend of binding affinity to α-TTP. α-Tocopherol is retained in plasma and tissues by TTP more successfully than other vitamers. In a recent study, people were treated with a mix of α-, γ- and δ-tocopherol; (2400 mg γ-Toc, 1596 mg α-Toc, 936 mg δ-Toc) and examined after 24h. The average plasma concentrations found in humans were α-, γ-, δ-tocopherol, 21.1, 6.19, 0.5
µmol/L. The plasma level amounts of α-, γ-, δ-CEHC levels after the same 24h were 0.02, 0.35, 0.09 µmol/L. Analysis of the metabolic profile of tocopherol, specifically the α-CEHC metabolite, is suggested to be an adequate way to monitor the daily α-tocopherol intake. Concentrations of tocopherol metabolites were found to be an indication of cellular levels of oxidative stress. Deuterated α-CEHC was used as a biomarker to detect oxidative stress of smokers. After supplementation with deuterated α-tocopherol for 6 days, concentrations of deuterated α-CEHC found in urinal and serum samples of smokers showed a lower concentration of α-tocopherol, suggesting an increased use of α-tocopherol in protection against oxidative damage associated with smoking. Increased oxidative stress is also seen in early onsets of type I and II diabetes in children. An increase in all tocopherol metabolites was observed in the test group. The most significant difference found was the high ratio (~14:1) between α-tocopheronelactone and its conjugates to the control group.

1.6 Structure of the α–Tocopherol transfer protein

In early studies, incubation of [³H]-α-tocopherol with rat liver cytoplasm allowed the purification and identification a ~30 kDa protein. Studies with α-, β-, γ-, δ-tocopherols showed a high specificity for protein binding to α-tocopherol and the ability to catalyze tocopherol transfer between membranes, resulting in the protein being called the α-tocopherol transfer protein. α-TTP is most abundant in liver tissue, but α-TTP mRNA expression has also been found in kidney heart, spleen, and brain tissue. The tocopherol transfer protein genes have high sequence homology among other mammals, as seen between human and rat α-TPP (94%). α-TTP gene expression is increased upon tocopherol depletion, which is caused by oxidative stress, liver diseases and neurological pathologies like Alzheimer’s disease.
TPP belongs to the SEC14 lipid binding protein family. The Sec14 domain (Smart entry: smart00516) binds small hydrophobic molecules and is named based on the archetypal protein from Sec14 from Saccharomyces cerevisiae (Sec14p). Almost all of these transporters contain another set of domains named after their similarity to cellular retinaldehyde binding protein 1 (CRALBP) and a TRIO guanine exchange factor (TRIO) binding domain (CRAL_TRIO Pfam entry: PF00650) in the active side. Sec14 domains differ in their ligand specificity. Yeast Sec14p was found to bind both phosphatidylinositol and phosphatidylcholine. Additional domains like Golgi dynamics (GOLD), are found in SEC14L1 – SEC14L5. The GOLD domain on the C-terminus of SEC14L consisted of eight β-sheets (jelly-roll) and are thought to interact with other protein domains in the Golgi such as Fab1p/YotB/Vac1p/ EEA1 (FYVE domain), regulating cell signalling and lipid transport. Tocopherol associated protein (TAP), also known as supernatant protein factor (SPF) by its original discoverers, bears a SEC14L like lipid binding domain. Five TAP (1-5) proteins have been found so far. TAP1 (also known as SEC14L2) was found to bind squalene, all tocopherols, α-tocopheryl quinone, PI and PC. The binding site of TAP1 has a similar folding to SEC14p and α-TTP, but is slightly larger. TAP2 (SEC14L-3) differs from TAP1 by having a more preferred binding to PI(3,4,5)P3, despite being 86% identical to TAP1. The structure of the active side of α-TPP explains the preferential binding to RRR-α-tocopherol. Crystal structures of the TTP active site describe a predominantly hydrophobic environment. Ser136, Ser140 and three water molecules form a hydrophilic pocket deep in the active site where they and Tyr 117 form H-bonds to the 6-hydroxyl of the chroman ring. One water molecule is H-bonded to Val182 and Leu189 for further stabilisation of the bound substrate (Figure 8).
Figure 8. Protein interactions of the α-TTP active side with α-tocopherol.

W3 and W7 are water molecules.⁹⁴

The specific binding of α-TPP to α-tocopherol comes from the additional interaction of the 5-methyl group with Leu183 Ile154 and Ile194. Binding studies by Hosomi et al. showed the binding preference of α-TPP to the RRR-forms of tocopherols have the order $\alpha > \beta > \gamma > \delta$ tocopherol.⁹⁷ RRR-α-Toc has a stronger affinity than the SRR form. Leth & Sondergraad determined biological activity of the tocols in a rat resorption gestation assays (relative to $D,L$-tocopheryl acetate = 100%). α-TTP binding in vivo follows the same trend of protein affinity as direct enzyme titration with the $\alpha > \beta > \gamma > \delta$ tocopherols.⁹⁸ More recent values by Panagabko et al. compared the tocopherols against other binding and transfer proteins containing the CRAL-TRIO motif. α-TTP was found to have a higher affinity to α-tocopherol than Sec14p, SPF/TAP, or CRALBP (Table 3).⁹⁹
Table 3. Ligand binding to CRAL-domain proteins; Comparison of dissociation constants of α-TTP, S. cerevisiae Sec14p, SPF, and CRALBP for various hydrophobic ligands.

(a) All data are expressed as the average ± SEM. (b) Ref 102 (Hosomi et al.) (c) Ref 103 (Leth & Sondergaard) (d) nd: not determined. ic: incomplete competition; competition was not achieved at 20µM [ligand].

The phytyl tail interacts with hydrophobic residues in the binding pocket, but the effect is stronger for RRR-α-tocopherol form due to interactions with Phe133, Val182, and Ile179. The tail bends into a U form to fill the cavity.

X-ray crystal structures of α-TTP with and without bound ligand show that the sequence 198-211 (helix A10) is a mobile lipid-exchange loop or “lid”, that is used to close the active side. The lid side facing inside the cavity has a hydrophobic nature, interacting with the phytyl chain of bound ligand through Ile202, Phe203, Val206, Ile210, Leu214. The other side of the lid has a more polar surface. The lid undergoes conformational changes that include an 80° rotation from the open to the closed form. The lid closes towards the hydrophobic phenylalanine residues of the helix A8 (F165, F169), which resides at the lower end of residues 165-185 (A9) (Figure 9). [Protein Data Bank (PDB) ID: 1OIP open, 1R5L closed state. 1OIZ, with bound Triton-X-100 open lid]
Figure 9. Open and closed lid crystal structure.

Yellow: hydrophobic residues (Ala, Val, Cys, Leu, Ile, Phe, Tyr, Trp, Pro), blue: basic residues (Arg and Lys), red: acidic amino acids (Glu and Asp), cyan: polar residues (Asn, Gln, Ser, Thr, Gly). Light gray: "lid" (residues 198–221).94

Helices A8 and A10 are the key motifs for α-TTP binding to the plasma membrane, transferring α-Toc into the membrane upon contact. Zhang et al. found that the hydrophobic lid binds to the membrane and opens upon binding to the membrane.100 The A8 helix (Phe F165 & F169) is thereby anchored at a fixed position in the membrane in the apo (open, 7.9 ± 0.1Å) and holo (closed, 4.5 ± 0.1Å) form. A205, V206, M209, I210, P212, F213 of helix A10 reside in the membrane during the tocopherol transfer as the second, movable anchor (purple, Figure 10). The importance of this residue was tested by mutation of F165 & F169 to aspartate (F165D, F169D) which lead to a large decrease in the rate of tocopherol transfer, 90% & 88% respectively. Mutation of residues I202D & M209D in A10 affected the transfer rates less (78% & 63% reduction). More conserved mutations of hydrophobic residues to alanines rather than charged aspartates such as F165A (71%) and F169A (47%) reduced the rate of transfer only partially. Exchange of the basic K211 and K217 residues on the A10 lid to alanine hand no impact on α-TPP binding, proving that hydrophobic and not electrostatic interactions are the mayor driving force of α-
TPP binding to the membrane.\textsuperscript{100}

Figure 10. Calculated interactions between the lipid-binding domains of α-TTP with the hydrocarbon core of the lipid bilayer: (A) α-TTP in "open" (1OIZ) and (B) "closed" (1R5L) conformations.

Protein backbone is shown in a cartoon representation with helices colored in green, N-terminal α-helical domain is colored in yellow, and lid helices (fixed and mobile) enclosing the binding cavity are colored in pink. Residues that penetrate into the acyl chain region of the lipid bilayer are colored purple. Molecules of detergents bound to "open" conformations (Triton X-100 in 1oiz, located in protein binding pocket and in lipid bilayer in dark green), hydrophobic ligands bound to "closed" conformations (α-tocopherol in 1r5l, located in protein binding pocket in dark green).\textsuperscript{100}

Binding of phosphatidylinositolbisphosphate (PI(4,5)P\textsubscript{2} and PI(3,4)P\textsubscript{2}) may initiate opening of the lid by electrostatic interactions to a positively charged groove of lysine (K190, K217) and arginine (R59, R68, R192, R221) residing on the sequence 184-192. The lysine K190 and arginine R192 are part of sequence connecting helices A9-A11. Helix A11 contains residues K217, R221 and helix A2 R59, R68) (Figure 11).\textsuperscript{101} PIP(4,5)\textsubscript{2} was found bind more favourably to α-TTP than other phosphatidylinositol diphosphates.\textsuperscript{100}
Figure 11. $\alpha$-TTP structure highlighting the amino acid residues in the PI(4,5)P2 binding cavity and lid helix 9-11 $\alpha$-TTP structure enclosing $\alpha$-Toc.

Electrostatic potential surfaces: blue area represents electropositive sites, red electronegative.$^{78}$

Other proteins besides $\alpha$-TTP have been found to act as transporters of tocopherols. Afamin is a human plasma protein encoded from the AFM-gene, which binds $\alpha$- and $\gamma$-tocopherol with low affinity ($K_d = 18\mu M$). Afamin may transport tocopherols between cells through human plasma and follicular fluids. The intercellular transportation by afamin is suggested to be more predominant when tocopherol transport by lipoproteins is impaired.$^{102,103}$

1.6.1 AVED
Low cellular plasma vitamin E concentrations ($3 \leq \mu\text{M}$) are associated with certain pathologies especially neurodegeneration causing ataxia and other symptoms like dysarthria hyporeflexia and vibratory sensation. Ataxia based on vitamin E deficiency (AVED, Friedrich’s ataxia) is caused by an inherited mutation of the $ttpa$ gene. The defect lowers the incorporation of tocopherol into VLDLs and thus vitamin E is not retained in the plasma. Over 20 different mutations have been characterized so far that results in varying degrees of neuropathology. Cavalier, Schuelke, Mariotti, Hentati, Arai & more (see full list in Manor & Morley) analysed the key amino acid residues affected and studied the effects of the mutated proteins.

Cavalier, Schuelke, Mariotti, Hentati, Arai & more (see full list in Manor & Morley) analysed the key amino acid residues affected and studied the effects of the mutated proteins.

**Figure 12. $\alpha$-TTP mutations across the $ttpa$ gene.**

**Blue box:** CRAL_TRIO_N domain and green box Sec14 domain. On top: insertions, deletions, and splicing mutations. **Below:** Missense mutations. Insertion, deletion, and splicing errors raise problems during gene transcription and protein expression. Premature terminations, disrupted initiations and frame shifts create shortened and mis-folded protein structures. Missense mutations of single amino acid affected tocopherol / PIP$_2$ binding and overall stability of the protein.

Arai studied the missense mutations of arginine residues R59W, R192H, and R221W (orange in Figure 12) in the PIP$_2$ binding region. R59W and R221W have a severe effect on the structure, incapacitating the ability to differentiate between RRS and RRR isomers. A ~20% decrease in tocopherol secretion was shown in assays using McA-RH7777 hepatic cells having the R59W
mutation in the TTP gene. Usuki and Maruyama described another missense mutation on the CRAL-TRIO part on α-TTP where Asp64 turned to glycine (D64G).\textsuperscript{109}

Current regulations on healthy dietary intake generally suggest a regular uptake of the necessary \( \approx 15 \text{ mg} \leq \text{ of vitamin E per day.}^{11} \) In the case of AVED, excess amounts of vitamin E need to be ingested to combat the low uptake.

\subsection*{1.6.2 Vitamin E and neurological health}

The poor physiological outcomes of AVED make it clear that vitamin E plays an important role in the protection of neurological tissue. The brain and nervous system have a high potential for oxidative damage. Increased oxygen use, increased number of mitochondria, higher amounts of polyunsaturated acids, and lower amounts of antioxidants present an increased risk for generation of reactive oxygen species (ROS). Several studies on neurological damage induced by oxidative stress indicated vitamin E as the main source of protection against ROS. The study of the neurological protection by vitamin E is of special interest, as a better understanding benefits the treatment of neurological diseases.

Vitamin E depleted rats (around 50\% of normal values) that were subjected to a 100\% oxygen atmosphere over 48h had changes in synaptic morphology such as decreased membrane fluidity, swollen astrocytes, mitochondria, nerve terminals and nuclei deformation. Vitamin E addition to normal rats 48h prior to the assay (1.3x to normal values) protected synaptic tissue. In both cases vitamin E was depleted in the hyperoxic environment by 60\% of the starting value. Endothelial cells didn’t show these symptoms, confirming that neurological tissue is more susceptible to this form of oxidative stress.\textsuperscript{110} Ulatowski studied the structural integrity and motor coordination function of Purkinje neurons in vitamin E deficient \textit{ttpa}^{-/} mice,
mimicking AVED conditions. Ttpα−/− mice had shorter dendrites (70 µm compared to 130 µm) and 30% less connections than wild type ttpα+/+ mice (Figure 13). Oxidative stress monitored by increased 3-nitrotyrosine concentrations was 3x higher in ttpα−/− mice, but was reduced upon vitamin E supplementation and seen by partially regained cognitive functions and motor coordination. Vitamin E is therefore crucial in maintaining structural integrity and function of neuronal tissue.

Figure 13. Structural differences in Purkinje neurons between ttpα+/+ and ttpα−/−VE (vitamin E) mice.111

1.6.2.1 Vitamin E, Parkinson’s and Alzheimer’s disease: influence of metal ions on ROS production, inhibition of glutamin synthase

Diseases like Parkinson’s and Alzheimer’s affect millions of people worldwide and are associated with increased ROS levels. An accumulation of iron as a part of aging was observed in rat brains as a consequence of leakage in ferritin iron storage or degradation of iron-dependent proteins like haemoglobin, cytochrome oxidase, aconitase and proline hydroxylase. Ferric iron (Fe³⁺) is reduced to ferrous (Fe²⁺) by ascorbate or superoxide (Fenton reaction). The ferrous species then acts as a reducing agent with hydrogen peroxide (H₂O₂) to form a hydroxyl radical (HO’). Hydrogen peroxide is produced by SOD dismutation of superoxide. The overall conversion of O₂− to HO’ is known as the Haber-Weiss reaction.112 Hydroxyl radicals can react with virtually anything, including polyunsaturated lipids, DNA bases and ribose.113 Direct
injection of iron(III) chloride into rat brains showed increased malondialdehyde (MDA) and decreased superoxide dismutase levels (SOD) in younger rats.\textsuperscript{114}

ROS generation by reaction of 6-hydroxydopamine with oxygen in neonatal rat pups has been shown to inhibit glutamine synthase (GS). Glutamate is a neuronal transmitter synthesized in the brain and assists in the initiation of an action potential by interacting with ionotropic receptors $N$-methyl-$D$-aspartate (NMDA, Ca\textsuperscript{2+}), $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA, Na\textsuperscript{+}, K\textsuperscript{+}), or kainate (Na\textsuperscript{+}, K\textsuperscript{+}) receptor, and the regulatory metabotropic glutamate receptor (mGluR). After the ion release, glutamate is rapidly transported from the synaptic cleft to astrocytes by excitatory amino acid transporters (EAATS). Glutamine synthase in the astrocytes turn glutamate to glutamine by using ammonia and ATP. Glutamine is then redistributed into neurons, where it is converted back to glutamate. This process is known as the glutamate-glutamine cycle, that protects neurons from excitotoxicity by metabolizing excess glutamate and ammonia. The oxidative species damaging glutamine synthase is not known. A test reaction with hydroxy radical quencher DMSO didn’t stop the inhibition.\textsuperscript{115} Vitamin E injection has been found to inhibit damage to astrocyte GS by ROS, preventing excess Ca\textsuperscript{2+} initiated cellular necrosis that forms oxidized lipids upon membrane degradation. FeCl\textsubscript{2} was used to initiate oxidative stress. FeCl\textsubscript{2} + vitamin E reduced malondialdehyde (MDA) levels back to the normal (control) state (Figure 14). Increasing Ca\textsuperscript{2+} levels by adding NMDA was cancelled by increased GS activity.\textsuperscript{116}
Decrease of MDA levels with α-tocopherol + iron(II) chloride and NMDA. The effect of NMDA (320 mM) on lipid peroxidation as expressed by the concentration of malondialdehyde (MDA)/mg protein. Data are the mean ± SEM for more than three experiments. *P < 0.05 compared with controls; †P < 0.05 as compared with FeCl₂ alone (Student t-test).

1.6.2.2 Vitamin E and Alzheimer’s disease: β-amyloid aggregation as an result of increased ROS levels

Besides impaired motor systems in Parkinson’s disease, iron- or copper catalyzed oxidative products are partially responsible for the memory loss and neuro-degradation in Alzheimer’s disease.

Alzheimer’s disease is linked to β-amyloid protein (Aβ) aggregation with consequent increase of H₂O₂. Aβ is formed upon degradation of the neuronal membrane stabilizer amyloid precursor protein (APP) by β- and γ-secretases (β-secretase cleaves APP outside the membrane, γ-secretase inside the membrane). The reduction of ROS by vitamin E has been recognized as a beneficial nutritional supplement in efforts to treat Aβ associated diseases.

1.6.2.3 Vitamin E in neuronal mitochondria protection
ROS damage in the electron transport chain of mitochondria has been found to be associated with Parkinson’s disease. NADH-dehydrogenase (or ubiquinone oxidoreductase, complex I) oxidizes NADH to NAD⁺, thereby reducing ubiquinone (CoQ10) to ubiquinol. This is the first step in the mitochondrial electron transport and, when inhibited, reduces or halts ATP production potentially leading to apoptosis. Neurons have a high demand for ATP and thus severe pathological issues can result upon mitochondrial damage.

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridin (MPTP) is used to induce oxidative stress in mitochondria of dopaminergic neurons in the substantia nigra of the brain. MPTP easily passes the blood brain barrier where it is oxidized by monoamine oxidase B (MAO-B) to MPP⁺, which incorporates into complex I to inhibit CoQ10 reduction. MPP⁺ further reduces dopamine synthesis by inhibiting tyrosine hydroxylase (TH), which catalyzes the oxidation of tyrosine to L-dopamine with O₂. Mitochondria and TH use oxygen in their catalytic processes, making them susceptible to oxidative damage. A study by Lan and Jiang looked at iron and MPP⁺ damage to dopaminergic neurons with regards to vitamin E and the iron chelator desferrioxamine (DFO(A)). The assay showed increased glutathione disulfide (GSSG) and MDA production as oxidative stress increased. Increased free iron levels were found to accumulate in all tissues from aged Parkinson’s patients. The importance of the studies of Lan and Jiang was that dopamine synthesis was reduced as a result of oxidative damage, and returned to normal when treated with vitamin E.

Studies of oxidative damage in hippocampal mitochondria were followed by monitoring complex I activity. Complex I activity decreased during ageing 53% (hippocampus) and 35% (frontal cortex) over an 8-month period. Supplementation with vitamin E restored enzymatic
activity back to 95% (hippocampus) and 92% (frontal cortex). Similar results were observed in cytochrome oxidase (complex IV) and mitochondrial nitric oxide synthase (mtNOS).\textsuperscript{121}

1.6.2.4 *Vitamin E antioxidant efficiency compared to other neuro-protective antioxidants: melatonin, N-acetyl-serotonin (NAS) and 17-β-estradiol*

Neuro-protective antioxidants like melatonin, \( N \)-acetyl-serotonin (NAS) and 17-β-estradiol were found to decrease oxidative stress and reduced apoptosis initiated by ROS-sensitive nuclear factor-κB (NF-κB).\textsuperscript{122} Addition of vitamin E to bovine retinal homogenates revealed that copper(I) ion-promoted lipid peroxidation was 29% more effectively quenched by α-tocopherol than \( N \)-acetyl-serotonin (NAS).\textsuperscript{123} Inhibition of ascorbate/\( \text{Fe}^{2+} \)-induced lipid peroxidation in rat testicular microsomes and mitochondria were tested with melatonin, NAS and vitamin E. Chemiluminescence increased following ascorbate-\( \text{Fe}^{2+} \) addition as a direct response to oxidative stress in the form of malondialdehyde formation.\textsuperscript{124} Equimolar amounts of each antioxidant were tested and revealed better protection by α-tocopherol over the other antioxidants tested (Table 4).\textsuperscript{125}

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Mitochondria IC\textsubscript{50}</th>
<th>Microsomes IC\textsubscript{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tocopherol</td>
<td>0.078</td>
<td>0.144</td>
</tr>
<tr>
<td>Melatonin</td>
<td>0.67</td>
<td>4.98</td>
</tr>
<tr>
<td>( N )-acetyl-serotonin</td>
<td>0.25</td>
<td>4.50</td>
</tr>
</tbody>
</table>

Table 4. Antioxidant inhibition of \( \alpha \)-tocopherol, melatonin & \( N \)-acetyl-serotonin in mitochondria and microsomes as IC\textsubscript{50} valuus based on 50% inhibition of light emission.\textsuperscript{125}

Neuronal protection by 17-β-estradiol (17-β-\( \text{E}_2 \)) by ROS reduction and glutamate reduction by NMDA inhibition, has been described for spinal motor neurons\textsuperscript{126} and dopaminic neurons.\textsuperscript{127} Other steroids like testosterone, corticosterone, and cholesterol did not show these effects. Protection of ROS by estrogen was compared to tocopherol in cerebellular rat neurons as an effect of β-amyloid protein induced \( \text{H}_2\text{O}_2 \) and glutamate increase. The results included a
maximum of 20% increased cell survival with preincubated natural (65% \( \alpha \)-tocopherol in soybean oil) and synthetic \( \alpha \)-tocopherol upon peroxide addition, which was matched by 17-\( \beta \)-E\(_2\) at higher concentrations (10\(^{-6}\)M). Cell survival tests with increased glutamate concentration showed a 90-100% survival at the lowest concentration tested with both tocopherols. 17-\( \beta \)-E\(_2\) on the other hand was able reach 80% at increased concentrations (10\(^{-6}\)M). An increase in NF-\( \kappa \)B induced apoptosis was also observed by matching luciferase activity increase with higher amounts of tocopherols.\(^{128}\)

### 1.6.2.5 Vitamin E localization in neuronal tissue

Vitamin E distribution in a single neuron was analyzed by TOF-MS. Lipid abundance was visualized using choline (Panel a in Figure 15) and acetyl (Panel b) fragments from PC and sphingolipids. Tocopherol fragmented on both sides of the chroman 2’-position (165 m/z, 205 m/z) (Panel c). The relative intensity of vitamin E (Panel d) revealed high vitamin E presence in the junction (between soma and axon) > soma > neurite (= axon with myelin-sheets).\(^{129}\) The lack of tocopherol in axons explains why axonal swelling is one of the first issues observed in AVED patients.\(^{130}\)
Figure 15. Localization of vitamin E in neurons by TOF-MS.

Lipid abundance visualization by MS fragments (a) choline, (b) acetyl from sphingolipids. (c) Visualization of tocopherol fragments. (d) Relative intensity of vitamin E and choline in neurite, junction and soma.129

Vitamin E / α-tocopherol has a crucial role in neuronal protection and is therefore one to two orders of magnitude better retained in the CNS than in any other tissue.131 The ttpa gene expression was localized in rat brains and CNS when oxidative stress was applied.91,132 Antibody staining of cerebellar astrocytes with glial fibrillary acidic protein (GFAP) and β-tubulin-III marked neurons localized α-TTP in GFAP-stained astrocytes (yellow, Figure 16).111
Neurons normally receive other lipophilic molecules from astrocytes and this seems to be also the case for vitamin E.\textsuperscript{133} This finding led to the model proposed by Manor and Ulatowski, that HDL-loaded vitamin E is shuttled across the blood brain barrier (BBB) by SR-BI receptors, where ApoE lipoproteins guide the particles to the astrocytes (Figure 17).\textsuperscript{134} Oxidative stress stimulates \textit{tpa} gene expression and stored vitamin E is transported by \textalpha-TTP to the cell membrane, packaged into ApoE liposome constructs by ABCA-1 and delivered through the cerebrospinal fluid to neurons.\textsuperscript{135} The proposed model raises the question of how oxidative stress activates \textit{tpa} and how neurons receive the vitamin E packages from astrocytes.
1.7 Biological tracing

How do molecules move and interact in living organisms? Distinguishing single molecules from each other without any specific label in real time in a living system is not yet possible. Larger molecular structures or networks in the cell are visible by microscopy. Optical microscopy has been used for biological research since the late 17th century.\textsuperscript{136} Despite major improvements over the last centuries optical microscopy (bright field microscopy) cannot overcome certain physical limits. The technique depends on a difference in contrast on the surface observed. The high permeability of cells lets conventional optical microscopy only visualize larger, stronger absorbing or reflecting structures like nuclei or cell membranes.\textsuperscript{137}

The diffraction limit (d) for light microscopy is around 200 nm according to Abbe \( d = \frac{\lambda}{2NA} \) and Rayleigh resolution \( d = \frac{0.61\lambda}{NA} \) (NA stands for the numerical aperture and is defined by the refractive index, \( n \), times \( \sin \theta \). \( \lambda \) stands for the wavelength).\textsuperscript{138, 139} More powerful microscopy techniques use x-ray radiation. X-rays penetrate tissue better and diffract with all tissue
allowing resolution down to 10-100 nm.\textsuperscript{140} Since this radiation is high in energy the samples are often destroyed during the experiment.\textsuperscript{141}

To increase the visibility of specific structures, cells are stained with light sensitive compounds.\textsuperscript{136} However, addition of external stains often changes the cell physiologies, inhibits cellular processes, or are cytotoxic.\textsuperscript{142} Staining of larger areas normally requires fixed cells and is commonly used in histology to visualize diseased or damaged tissue.\textsuperscript{143} Such staining techniques are sufficient to create static pictures of organelles, but are impractical when biological processes are to be followed over time. Instead of light, electron beams can be used to enhance the resolution. Scanning electron microscopy and transmission electron microscopy allow a 200,000x – 500,000x magnification down to 1nm to observe organelles like mitochondria. The samples used are fixed since the sample is in a vacuum environment, then bombarded with high energetic radiation.\textsuperscript{144} Currently the technique with the highest resolution / selectivity and that is non-invasive\textsuperscript{145} uses optical microscopy and fluorescent reporter molecules.

The main difference between fluorescence microscopy and light microscopy is the use of monochromatic light to illuminate the incorporated fluorophore in the cells.\textsuperscript{137} The light source used to create monochromatic light are Xe-arc lamps / Hg-vapour arc lamps and lasers more recently light emitting diodes LED. Most microscopes use noble gas lasers (Xe, Ne).\textsuperscript{146,147} Lasers are more intense and allow deeper penetration into tissues. Point scanning microscopy techniques like confocal laser scanning microscopy (CLSM) need high powered energy light sources to create better, more resolved pictures and videos.\textsuperscript{148} CLSM revolutionized the field of microscopy as a technique able to overcome background fluorescence interference of deeper layers. To differentiate the axial depth of labelled structures unwanted reflected light is discarded by filters and resolved to create three-dimensional pictures. A newer type of
microscopy called $4\pi$ microscopy has superior axial differentiation over CLSM and has 6-7 times stronger resolution down to ~100 nm.$^{149,150,151}$

$4\pi$ microscopy is used for new fluorescence techniques like RESOLFT based on stimulated emission depletion microscopy (STED). In STED is a secondary light beam used to eliminate a section of stimulated light, thereby enhancing the resolution of the residual stimulated section. STED pushed the boundaries of diffraction limit for live imaging down to 20 nm, giving clear images of small particles like vesicles or proteins (Figure 18).$^{152,150}$

![Image](image.png)

Figure 18. Fluorescent stained actin filaments of gastrula-stage Drosophila embryo.

Conventional (A) to confocal (B) microscopy.$^{136}$

Live cell imaging captures fluorophore emission as transient light (bright field microscopy), scattered light (dark field microscopy) and interference pattern of two different combining light waves (differential interference microscopy).$^{145}$ The short lifetime of fluorescence (usually nanoseconds) requires a constant flux of light onto the fluorophore for prolonged observation. These so called steady-state conditions are great to observe particle movement in cells.$^{153}$ Unfortunately, the lifetime of the fluorophores under such intense light can be brief, as photobleaching and destructive radical formation increases. Hence, the fluorophores should have high photochemical and chemical stability.
1.7.1 Principles of fluorescence

The principle of fluorescence describes the absorption of a photon ($h\nu_{ex}$) by the ground state of the fluorophore ($S_0$) and release of a lower energetic photon ($h\nu_{em}$) after relaxation from the first singlet exited state ($S_1$) to the ground state by loss in vibrational energy (heat). A bathochromic (red-shift) shift in the wavelength is consequently observed (1)\textsuperscript{154}

\[ S_0 + h\nu_{ex} \rightarrow S_1 \]

\[ S_1 \rightarrow S_0 + h\nu_{em} + \text{heat} \]

The process is explained by the Frank-Condon principle, stating that the energy states of the excited state after internal conversion (IC) from higher exited state $S_2$ to $S_{1\text{vib}(x)}$ favours the same vibrational energy state in the ground state ($S_{0\text{vib}(x)}$) before reaching $S_{1\text{vib}(0)}$\textsuperscript{155} Other energy transitions compete with fluorescence; energy transfer into nearby located orbitals (spin coupling / crossover), IC of the singlet exited state $S_1$ to the triplet exited state $T_1$ (intersystem crossing, ISC) and release of the energy as phosphorescence, and other IC by complete nonradiative decay via heat release or chemical reactions. The Jablonski diagram is used to portray these different types of energy relaxation (Figure 19)\textsuperscript{156-158}.
The fluorescence efficiency of an atom or molecule is described by the fluorescence quantum yield \( \Phi_F \), which is the ratio of how much of the total excitation energy is converted to fluorescence, divided by how many of the absorbed photos are emitted back as photons. \( \Phi_F \) is calculated from the emission rate of the fluorophore (\( \Gamma \)) minus the rate of all other nonradiative pathways combined (\( k_{nr} \)) (2).

\[
\Phi_F = \frac{\Gamma}{\Gamma + k_{nr}}
\]

The rates of these processes vary. Fluorescence lifetimes are around \( 10^{-9} - 10^{-7} \) s. Light absorption is extremely fast \( 10^{-15} \) s, as is internal conversion \( 10^{-14} - 10^{-12} \) s. Phosphorescence on the other hand is several orders of magnitude slower (\( \sim 10^{-3} \) s or longer). The time spent in the \( S_1 \) excited state before decay to \( S_0 \) ground state is known as the fluorescence lifetime (\( \tau \)) and depends on the nonradiative decay rate \( k_{nr} \) (3).

\[
\tau = \frac{1}{\Gamma + k_{nr}}
\]

The natural fluorescence lifetime (\( \tau_n \)) excludes nonradiative decay, giving the actual time of fluorescence and is on average \( \sim 1 - 10 \) ns (4).

\[
\tau_n = \frac{1}{\Gamma}
\]

To calculate \( \tau_n \), \( \tau \) is divided by the quantum yield \( \Phi_F \) (5).

\[
\tau_n = \frac{\tau}{\Phi_F}
\]

The addition of nitro groups and heavy heteroaromatic groups like halogens reduces the lifetime and quantum yield. Eosin (bromine) and Erythrosine B (iodine) have the same structure, but differ between their halogen substitutions. Despite similar extinction coefficients and radiative
decay rates has Erythrosine B has a lower quantum yield ($\Phi$) and fluorescence lifetime $\tau_n$ (Figure 20). \textsuperscript{161,154}

![Eosin and Erythrosine B structures](image)

<table>
<thead>
<tr>
<th></th>
<th>$\tau$ (ns)</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosin</td>
<td>3.1</td>
<td>0.65</td>
</tr>
<tr>
<td>Erythrosin</td>
<td>0.61</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*Figure 20. Quantum yield ($\Phi$) and fluorescent lifetime ($\tau$) of Eosin & Erythrosine B.* \textsuperscript{161}

The surrounding environment also effects fluorophore emission. 1-Hydroxypyrene-3,6,8-trisulfonate emits at ~400 nm (pH 1), at ~510 nm (pH 7) and at ~450 nm (pH 13) in water. \textsuperscript{162}

Individual solvents create blue and red shifts in the maximum emission or in / decrease the fluorescence intensity. Some solvents can quench the fluorescence completely. \textsuperscript{154,163,164,165}

### 1.7.2 Light scattering

Fluorescent microscopy uses monochromatic light to excite the fluorophore in use. An excitation filter selects the wavelength necessary and is reflected by a dichronic mirror towards the sample. Refractive light is captured on the same side as emitted light and selected when passing through the dichronic mirror. This detection method is known as epifluorescent microscopy, and replaced transparent fluorescent microscopy. The higher the quantum yield and extinction coefficient of the fluorophore, the better is the targeted fluorophore visible. \textsuperscript{166}

Obtaining good microscopic images depends on the amount of light reaching the detector besides fluorophore absorption / emission properties. Light particles interact with any molecular structure of a certain size in the tissue. \textsuperscript{167} The interaction puts the energy into the particle and is released in all directions. This scattering is the effect of the relaxation of the induced dipole
moment upon light absorption. The interaction is more prone with blue light (400nm) as higher frequency light results in more collisions in the same distance traveled as red light (700nm).\textsuperscript{154}

Hence, the smaller the propensity the molecule is to the wavelength ($\lambda$), the stronger the scattering. Different types of scattering are possible with visible light. Rayleigh, Mie and optical scattering:\textsuperscript{168-169,170}

Rayleigh described the scattering by particles below one-tenth the size of the photons wavelength. The scattering coefficient ($\alpha$) is determined by the particle size ($d$) and the wavelength of the incident light ($\lambda$) ($\alpha = 1:10$ for Rayleigh scattering) (6).

$$\alpha = \frac{\pi d}{\lambda}$$

For visible light (400-700nm) the particles are around $0.1 - \sim 70$nm. The intensity of the scattered light ($I$) depends on the amount of photons ($N$) and its distance to the spherical ($8\pi$) particle ($R$), scattering angle ($\theta$), the polarizability of the molecule ($\alpha$) and the photon influx intensity ($I_0$) (7).

$$I = I_0 \frac{8\pi^4 N \alpha^2}{\lambda^4 R^2} (1 + \cos^2 \theta)$$

Mie scattering describes the interaction for particles between $\alpha = 1/10 < \lambda$ to the size of $\lambda$. Assuming a wavelength of 500nm, particles of the size of 50-500nm would experience Mie scattering, ranging from larger protein structures to viruses.

Optical (geometrical) scattering occurs on particles the same size or bigger than the wavelength of the photons ($\alpha = \text{particle} > \lambda$). It starts around 1-50\(\mu\)m for wavelengths in the visible region. Particles of such size always scatter light in any direction with regards to its surface pattern (Figure 21).\textsuperscript{168,169}
Increasing particle size (0.1nm<1µm) favors scattering of visible light into the direction of the incident light. Larger particles (1µm<) scatter light into all directions.\textsuperscript{168,169}

The bigger the particle, the less scattering depends on the wavelength.

The cross section of the particle, referring to the amount of back-scattering (reflection) of the particle ($\sigma$), is the same or bigger (1\leq) to the size of the particle (8).

\[ 1 \leq \frac{\sigma}{\pi r^2} \]

Tissue and blood have a broad range of UV / VIS absorbance, with most biological molecules having absorptions between 200-400nm. Water as the most abundant molecule in the body absorbs light most efficiently shorter than 180nm and longer than 1000nm. Extinction coefficients (k) of water was measured by several groups in the past and summarized by Hale and Querry (Figure 22).\textsuperscript{171-174}
Besides water, tissues consist of fats and other absorbing/scattering molecules. Proteins, for example, have aromatic amino acids like tryptophan, tyrosine, and phenylalanine, absorbing in the UV light (Table 5).\textsuperscript{175}

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Wavelength (nm)</th>
<th>Absorptivity</th>
<th>Wavelength (nm)</th>
<th>Quantum yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td>280</td>
<td>5,600</td>
<td>348</td>
<td>0.20</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>274</td>
<td>1,400</td>
<td>303</td>
<td>0.14</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>257</td>
<td>200</td>
<td>282</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Table 5. Maximum wavelength, absorption and fluorescence of aromatic amino acid.\textsuperscript{175}

To reproduce the visible light absorption of tissue, water (W), fat (F), melanin (M), bilirubin ($C_{bil}$), β-carotene ($C_{βC}$), haemoglobin oxygen saturated mixed with arterio-venous vasculature (BS) and deoxygenated blood as an average blood volume fraction (B) were combined (Figure 23). Total absorbance coefficient ($\mu_a$) measurement by Rayleigh and Mie scattering is given by the transmission of the residual scattered light ($T$) and the path length traveled in cm ($L$) (9).\textsuperscript{176}

$$\mu_a = -\frac{1}{T \frac{\partial T}{\partial L}}$$
Transmission of all components was measured from 300-1200nm, with decreasing $\mu_a$ from $10^2$ to $10^0$. This trend is based on an experimentally made tissue with the chosen concentrations. In the same review article by Jacques is a reference list of 47 different tissues listed with their B, S, W, F, and M concentrations to calculate their individual absorptivity profile.\(^\text{176}\)

10 \[ \mu_a = BS\mu_{a,\text{oxy}} + B(1 - S)\mu_{a,\text{deoxy}} + W\mu_{a,\text{water}} + F\mu_{a,\text{fat}} + M\mu_{a,\text{melanosome}} + 2.3C\beta \text{bili}\delta \text{bili} + 2.3\text{C}\beta \text{C}. \]

Figure 23. Total absorption coefficient $\mu a$ (cm\(^{-1}\)), as water is added (volume fraction $f_v,\text{water} = 0.1$ by 0.1 to 0.9), blood at 75 oxygen saturation is added (average $f_v,\text{blood} = 10^{-4}$ by 10\(^{-4}\) to 2 $\times$ 10\(^{-3}\)), bilirubin is added (1 by 1 to 20 mg dL\(^{-1}\), where 20 mg dL\(^{-1}\) = 342 $\mu$M is a bilirubin concentration in the blood of a jaundiced neonate), fat is added ($f_v,\text{fat} = 0.3$ by 0.3 to 0.9), and melanin is added ($f_v,\text{melanosome} = 0.01$ by 0.01 to 0.10).\(^\text{176}\)

The decrease in scattering with increasing wavelength is found among all tissue. The review compared the scattering coefficient ($u'_s$) of skin, brain, breast, bone, soft tissues (liver, muscle, heart, stomach wall etc.), fibrous tissues (tumor, prostate etc.) and fatty tissues (red dots) (Figure 24). The experimental values were plotted as a function to the wavelength. Two functions were used: 11 determines ($u'_s$) by normalizing the wavelength ($\lambda$) with a reference wavelength of 500nm. The normalized value is then raised by the “scattering power” $b$, which shows the dependents to the wavelength used in $u'_s$. The term is scaled by the factor $a$, which represents the value $u'_s(\lambda = 500nm)$. Equation 12 takes in count the separate fractions for
Rayleigh ($f_{\text{Ray}} (\lambda / 500\text{nm})^{-4}$) and Mie scattering \((1 - f_{\text{Ray}}) (\lambda / 500\text{nm})^{-b_{\text{Mie}}})\), seen as the dashed black lines in the graphs. Scaling factor $a'$ is the same as $a' (u' (\lambda = 500\text{nm}) )$ in equation 11.\(^{176}\)

\[
\mu'_s = a \left( \frac{\lambda}{500(\text{nm})} \right)^{-b}
\]

\[
\mu'_s (\lambda) = a' \left( f_{\text{Ray}} \left( \frac{\lambda}{500(\text{nm})} \right)^{-4} + (1 - f_{\text{Ray}}) \left( \frac{\lambda}{500(\text{nm})} \right)^{-b_{\text{Mie}}} \right)
\]

---

**Figure 24.** Reduced scattering coefficient for different tissues based on literature values for the seven groups of tissues (red circles, data found in reference).

The green line is the fit using equation (11). The black solid line is the fit using equation (12), with the black dashed lines showing the Rayleigh and Mie components of the fit.\(^{176}\)

When measuring scattering coefficients, it is best to have tissues samples at a thickness of 100µm or less to avoid loss of the signal by multiple scattering. Mie scattering is more prone in
all tissues than Rayleigh scattering. In average have tissues with higher overall lipid composition like fibrous, skin and brain tissue a high scattering coefficient. As a general trend does tissue scatter and absorb light with lower wavelength much better. In fluorescent microscopy, therefore, fluorophores with higher absorption wavelengths are more visible.

1.7.3 Vitamin E labeling: Advantages of fluorophores

The transport of α-tocopherol has been studied previously with radio labeled \[^3\text{TI}\] and fluorescent derivatives. Radio labeled molecules expose the organism to radiation, which lowers the overall radiation tolerance per year (20 Bq/L, 0.0003 mSv in Canadian drinking water) and can cause cell damage. Fluorescence labeling offers a non-invasive way to track the cellular pathway of α-tocopherol. Fluorescence microscopy has been used to observe the transport of the vitamin by following intensity changes of the fluorescence emission in cultured cells, or Förster resonance energy transfer (FRET) in assays to study in vitro the interactions with proteins or and phospholipid membranes. Fluorescence bleaching is commonly used to determine diffusion rates in phospholipid membranes.

1.7.4 Fluorescent labeling: Fluorophores

When talking about fluorophores in fluorescent microscopy imaging, a line has to be drawn between staining of organelles and labeled molecules / proteins. Stains as discussed earlier are for specific organelles. They are based on chemical interactions to specific sites as in the case of Höechst stains or 4',6-diamidino-2-phenylindole (DAPI), that specifically bind to A-T, A-U regions in the minor groove of the DNA / RNA and thus easily visualize nuclei. Others are based on polarity like Nile red that selectively stains non-polar regions of cells such as lipid bodies. Immunofluorescence describes the use of fluorescent anti-bodies to targeted
to antigen expressing cells, or expression of fluorescent proteins in the targeted cells. The reactive fluorescent linkers in antibody staining are often isothiocyanates (FITC), which create stable thiourea linkages. The chosen dye should not interfere with protein function. Staining multiple cellular compartments requires different excitation / emission wavelengths for each dye. For this purpose new dyes have been developed and sold by various manufacturers (Table 6).¹⁸²,¹⁸³,¹⁸⁴,¹⁸⁵,¹⁸⁶,¹⁸⁷,¹⁸⁸

<table>
<thead>
<tr>
<th>Name</th>
<th>Target organelle</th>
<th>Exitation λ</th>
<th>Emission λ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Höchst stains, DAPI¹⁷⁹,¹⁸⁰</td>
<td>DNA-minor groove, mycoplasma</td>
<td>455nm</td>
<td>510-540nm</td>
</tr>
<tr>
<td>Nile red</td>
<td>Lipid membranes</td>
<td>450-500nm</td>
<td>500-600nm</td>
</tr>
<tr>
<td>3,3'-dihexyloxycarbocyanine iodide (DIOC₆)¹⁸²,¹⁸⁵</td>
<td>Endoplasmatic reticulum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhodamine dyes</td>
<td>Antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acridine orange¹⁸²</td>
<td>RNA / DNA differentiation</td>
<td>460nm RNA</td>
<td>650nm RNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>502nm DNA</td>
<td>525nm DNA</td>
</tr>
<tr>
<td>Propidium iodide¹⁸⁷</td>
<td>Perforated cells</td>
<td>488nm</td>
<td>535-610nm</td>
</tr>
</tbody>
</table>

Table 6. Exitation and emission wavelength of commercial dyes for fluorescent microscopy.¹⁸²-¹⁸⁸

The most prominent dyes in microscopy are based on coumarin, xanthine and cyanine structures.¹⁸⁹ Coumarin commonly emits in the blue-green region between 440-520 nm, but can reach 560 nm in some derivatives. They offer a high Stokes shift of 50-100 nm, making them useful in microscopy with energy transfer like FRET.¹⁸²,¹⁹⁰

Dyes based on xanthenes include fluorescein and rhodamine.¹⁹¹ They are known to have high absorption coefficients, high absorption / emission wavelength, quantum yields and chemical stability. At the same time these properties are easily modified by substitution on the aromatic rings and change of the phenol / ketone to amines / imines or sulfonates as in the Alexa Fluor® series of dyes. Fluorescein and rhodamine have attached at the 9′-position an aromatic group, red-shifting the wavelength of the dyes more into the yellow-red region. When exchanging the 9′-carbon with a nitrogen atom an oxazine is created, which also exhibits a red shift in absorption / emission.¹⁹¹
Cyanine dyes are based on alkyl / aryl nitrogen capped polymethines. Increasing the size of the aromatic group or the number of double bonds in the polyene chain increases the wavelength into the near to far IR region.\textsuperscript{192}

Other dyes in the visible region used in microscopy are naphthalimides like brilliant sulfaflavin\textsuperscript{193}, phenyl-conjugated dyes like PQP\textsuperscript{194} or antracenes (PMAA),\textsuperscript{195,196,197} BODIPYs (Figure 25).

\textbf{Figure 25. Sturctures of frequently applied fluorescent microscopy dyes.}\textsuperscript{190-197}

Instead of synthetic fluorescent small molecules, one can use naturally fluorescent proteins expressed as a fusion with a targeted protein. The green fluorescent protein (GFP) from \textit{Aequorea victoria} jellyfish was the first such naturally fluorescent protein to be discovered and used as a tool in cell biology. This protein converts blue light (395 nm) to green light (509 nm).\textsuperscript{198} The GFP sequence can easily be expressed in \textit{C. elegans} and \textit{E. coli} and several similar proteins have been engineered with a variety of emission wavelengths.\textsuperscript{199-200}
1.7.5 Tocopherol labeled fluorophores

Over the last decade our lab has synthesised several fluorescent α-tocopherol tracers. Most successful were the tocopherol analogues that incorporated nitrobenzoxadiazole (NBD) and dipyrrometheneborono difluoride (BODIPY) dyes.\textsuperscript{201,202,203,204} Both dyes are have a small molecular size and are non-polar enough, which benefits the binding affinity to the α-TTP. However, the NBD dye is linked to the vitamin E skeleton through a nitrogen atom that increases the polarity and consequently shows a lowered binding to α-TTP compared to α-tocopherol.\textsuperscript{205} The chain terminal tocopherol-BODIPY analogues are connected by a carbon-carbon bond to the C2-position and good mimics of the non-polar tocopherol tail. Other advantages of the BODIPY structure are a three times higher quantum yield than NBD, a five times higher extinction coefficient ($\varepsilon$), longer chemical stability in-vivo / in-vitro and, most importantly, longer wavelengths of absorption and emission (Table 7).\textsuperscript{206}

<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>BODIPY-Toc</th>
<th>α-Toc-C9-NBD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structure</strong></td>
<td><img src="image" alt="BODIPY-Toc" /></td>
<td><img src="image" alt="α-Toc-C9-NBD" /></td>
</tr>
<tr>
<td>Quantum yield $\Phi_F$</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>$\varepsilon$ (M$^{-1}$cm$^{-1}$)</td>
<td>90'000 M$^{-1}$cm$^{-1}$</td>
<td>20'000 M$^{-1}$cm$^{-1}$</td>
</tr>
<tr>
<td>Wavelength (abs.-em. λ max.)</td>
<td>507-511nm</td>
<td>460-480nm</td>
</tr>
<tr>
<td>Photostability</td>
<td>High</td>
<td>Low (&lt;5min)</td>
</tr>
<tr>
<td>Use</td>
<td>Microscopy</td>
<td>Microscopy &amp; FRET</td>
</tr>
<tr>
<td>$K_d$ (α-TTP) (nM)</td>
<td>94nM</td>
<td>54nM</td>
</tr>
</tbody>
</table>

Table 7. Comparison of BODIPY-Toc to α-Toc-C9-NBD.\textsuperscript{201,206}

One drawback of BODIPY is the small Stokes shift of only ~ 4 nm, which does not allow easy FRET-based assays. The overlap of the maximum absorption and emission band wavelength
causes self-quenching and decreases the fluorescence intensity, however, the high extinction coefficient compensates this problem. When looking at the binding to \( \alpha \)-TTP, NBD-tocopherol has slightly higher affinity.

Both molecules have been successfully used in several biological studies.

## 2 Project overview

The goal of this research project is the synthesis of a new BODIPY fluorophore containing \( \alpha \)-tocopherol analogue with a longer excitation wavelength than previous molecules, potentially a larger Stokes shift, and with a similar or higher affinity to \( \alpha \)-TTP. Such a molecule would make the tracking of intracellular tocopherol transport easier and lead to a better understanding of the biological role of Vitamin E.

Fluorescent conjugates for biomolecular research should have absorption and emission wavelengths that allow for efficient cell and tissue penetration of both the incident and fluorescent light. Better penetration of light through tissues occurs at a wavelength around 500-700 nm, and can yield brighter and more detailed images in fluorescence microscopy. To achieve this goal for a fluorescent tocopherol, we envisioned an additional aromatic functional group added to the BODIPY to increase the absorption and emission maximum up to 550-580 nm. Additionally, this structural change must not alter the molecule’s ability to bind with high affinity to the tocopherol transport protein.

### 2.1 Structure design
Two key aspects need to be considered in the structural design. First, the molecule needs to have a longer excitation wavelength than our previously synthesized molecule. Second, the molecule needs to bind to α-TTP with high affinity.

One way to increase the wavelength of the BODIPY fluorophore is additional conjugation of aromatic functional groups. The overlap of the π-orbitals lowers the HOMO, decreases the overall energy of absorptive electronic transition, thus shifting light absorption to less energetic light. Several examples in the literature describe BODIPYs with increased wavelengths into the red and even the near-IR region as potential sensitizers for solar cell applications. Conjugation of aromatic groups to the 1, 2, 3, 5, 6, 7 and 8 (meso) position have proven to increase the wavelength. For instance, fusing BODIPY with other ring systems like benzothienophene increase the absorption maximum to 670nm. Substitution of the boron with alkyl (C-BODIPY) or ethynyl (E-BODIPY) also increases the Stokes shift (Figure 26).
Figure 26. Nummering system of the BODIPY core structure and example structures of differently substituted BODIPYs.

The second issue of ligand design is the binding to the hydrophobic pocket of α-TTP. To ensure specific binding the chroman ring should remain intact. This means that changes can only be made to the phytol chain. Natural tocopherol has a chain length of thirteen carbons. Our previously prepared BODIPY-α-tocopherol ligands revealed that binding affinities differed significantly with chain lengths (Table 8). The best results were obtained with an eight-carbon linker ($K_d = 94 \pm 3$ nM). Similarly, NBD-tocopherols with a nine-carbon spacer (C9) had the highest affinity ($K_d = 56 \pm 15$ nM). Longer chains than C9 decreased the binding affinity. A
higher $K_d$ for the BODIPY can be rationalized by having a larger size than NBD, interfering with protein binding.

<table>
<thead>
<tr>
<th>NBD-α-Toc</th>
<th>$K_d$ (nM)</th>
<th>BDP-α-Toc</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6</td>
<td>299 ± 37</td>
<td>C6</td>
<td>232 ± 10</td>
</tr>
<tr>
<td>C7</td>
<td>106 ± 21</td>
<td>C7</td>
<td>130 ± 4</td>
</tr>
<tr>
<td>C8</td>
<td>142 ± 35</td>
<td>C8</td>
<td>94 ± 3</td>
</tr>
<tr>
<td>C9</td>
<td>56 ± 15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8. Influence of chain length linker on the α-TTP dissociation constants ($K_d$) of NBD-α-Toc and BODIPY (BDP)-α-Toc.\(^{206}\)

The smallest groups that can be added to the BODIPY to extend conjugation are a simple double bond, or the aromatic thiophene and benzene groups. Arroyo described the synthesis and optical properties when functional groups are conjugated at the 8-positions. Double bond conjugation followed, to a certain extent, the Woodward-Fieser rules, increasing the absorption maxima by 5-20 nm.\(^{221,222}\) In addition, an increase of the Stokes shift upon double bond conjugation occurs, in some cases up to 40 nm, which would be useful for FRET studies. However, the quantum yields ($\Phi_F$) reported were 15-110 fold lower. Alkyl groups on C8 have a slight inductive effect into the BODIPY conjugate, decreasing the wavelength by < 5 nm.\(^{223}\) Substitution of the BODIPY meso position by alkyl groups stabilizes the BODIPY structure when treated in acetic conditions and during fluorine ligand exchange. A close resemblance is seen when comparing our anthroyloxy-α-tocopherol (AO-α-Toc) to a BODIPY linked at the meso position. Computational studies of the AO-α-Toc - α-TTP complex suggested that the large fluorophore fits into the hydrophobic binding pocket. Tocopherols phytol chain and AO-α-Toc are slightly curved in α-TTP (Figure 27). Experimental results however revealed that the 9-position linked anthracene created too much steric bulk in the hydrophobic binding pocket ($K_d = 279±124$nM for the C9-chain-α-Toc).\(^{201}\)
Figure 27. X-ray diffraction analysis of AO-α-toc (1a & 1b) and α-tocopherol (2a & 2b) bond to α-TTP.

Only amino acid residues 99-225, resembling the mayor part of the active side, are shown in two ways: 1a and 2a projected for looking down the long axis of the binding site. 1b and 2b from above.201

Previous BODIPY-α-Tocs had the fluorophore attached at the 3 position, which proved to be superior in binding to α-TTP ($K_a = 94 \pm 3$ nM). Wavelength extension by an aromatic group must therefore be at the 3- or 5-position of the BODIPY. A BODIPY conjugated at the 3-, 5-, and 8-position have been reported in the literature with anisole, para-toluene, and thiethyl groups providing increased wavelengths of absorption and emission for all compounds (Table 9).223,224,225,226,227,228,229
### Functional Group

<table>
<thead>
<tr>
<th>No substitution</th>
<th>Absorption ($\lambda_{\text{max}}$)</th>
<th>Emission ($\lambda_{\text{max}}$)</th>
<th>$\varepsilon$ (M$^{-1}$cm$^{-1}$)</th>
<th>$\Phi_F(%)$</th>
<th>Solvent</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>500nm</td>
<td>516nm</td>
<td>dnr</td>
<td>77%$^a$</td>
<td>THF</td>
<td>223</td>
<td></td>
</tr>
<tr>
<td>496nm</td>
<td>511nm</td>
<td>dnr</td>
<td>78%$^a$</td>
<td>THF</td>
<td>223</td>
<td></td>
</tr>
<tr>
<td>507nm</td>
<td>554nm</td>
<td>dnr</td>
<td>5%$^a$</td>
<td>THF</td>
<td>223</td>
<td></td>
</tr>
<tr>
<td>540nm</td>
<td>568nm</td>
<td>dnr</td>
<td>dnr</td>
<td>EtOH</td>
<td>224</td>
<td></td>
</tr>
<tr>
<td>525nm</td>
<td>549nm</td>
<td>dnr</td>
<td>0.04$^b$</td>
<td>MeOH</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>564nm</td>
<td>579nm</td>
<td>dnr</td>
<td>0.55$^b$</td>
<td>MeOH</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>581nm</td>
<td>591nm</td>
<td>139,444</td>
<td>dnr</td>
<td>ACN</td>
<td>226, 227</td>
<td></td>
</tr>
<tr>
<td>556nm</td>
<td>569nm</td>
<td>dnr</td>
<td>0.98$^b$</td>
<td>MeOH</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>570nm</td>
<td>587nm</td>
<td>71,500</td>
<td>0.40</td>
<td>EtOH</td>
<td>228</td>
<td></td>
</tr>
<tr>
<td>606nm</td>
<td>649nm</td>
<td>58,000</td>
<td>0.81</td>
<td>Tol.</td>
<td>229</td>
<td></td>
</tr>
<tr>
<td>622nm</td>
<td>643nm</td>
<td>69,700</td>
<td>0.82</td>
<td>EtOH</td>
<td>228</td>
<td></td>
</tr>
<tr>
<td>580nm</td>
<td>593nm</td>
<td>98,400</td>
<td>0.82</td>
<td>EtOH</td>
<td>228</td>
<td></td>
</tr>
<tr>
<td>562nm</td>
<td>584nm</td>
<td>dnr</td>
<td>0.24%</td>
<td>Cyclohexane</td>
<td>217</td>
<td></td>
</tr>
</tbody>
</table>

| Table 9. Conjugation effects to BODIPY. |

| a: In paper ($\Phi_F$ compared to fluorescein ($\Phi_F$ 79% in 0.1M NaOH) b: $\Phi_F$ Standards; rhodamine 6G in water ($\Phi_F = 0.78$) or cresyl violet acetate in methanol ($\Phi_F = 0.55$) c: sulfur is connected to the 3 position (β, 2 position = α) d: "Absolute quantum yields determined by a calibrated integrating sphere system" ref 217. Data not recorded in publication (dnr). |

Thienyl- and thienyl-ene- structures were chosen since they are somewhat smaller in size than phenyl groups.

Target 1 has the thienyl-group at the 5′-position, with a five-carbon chain. Compound 2 has the thienyl-group as a part of the carbon chain and is directly linked to the BODIPY core.
Compound 3 uses the thienyl-ene extension with the design of 2 (Figure 28). Rohand found during his cross-coupling studies that phenyl groups directly attached to the 2-position have a low quantum yield.\textsuperscript{225} This is not the case with thienyl or thienyl-ene groups as seen in Rihn’s paper.\textsuperscript{228} The only decrease in $\Phi_F$ was observed with chlorine substituted in the 5-position.

![α-Tocopherol](image)

**Figure 28.** Novel designed fluorescent α-Toc ligands compared to BODIPY-Toc and α-tocopherol.

Target 1 and 2 have a thiophene group directly linked at the 2-position to the 5-position of the BODIPY. Target 3 resembles target 2, but has an ethylene bridge between the thiophene and the BODIPY.\textsuperscript{206,216}

### 2.2 Synthesis plan

Target 1 resembles the structure of previously prepared BODIPY-α-Tocs, having an additional terminal thienyl-group on the BODIPY. The synthesis had certain issues, which have to be considered in the design of the targeted BODIPY tocopherols. The formation of the BODPY
framework from condensation of 2,4-dimethylpyrrole aldehyde and oct-7-ene-2-pyrrole produced the self-condensed 1,3,5,7-tetramethyl-BODIPY as a side product, tricky to isolate and purify (a, Figure 29). In the next step, the oct-7-ene-BODIPY was reacted in a cross metathesis reaction with (S)-tert-butyl(dimethyl)((2,5,7,8-tetramethyl-2-vinylchroman-6-yl)oxy)silane (TBSO-Trolox-ene). Hoveyda-Grubbs second generation catalysts produced only moderate yields and might form small amounts smaller chain length isomers as byproducts that require separation by preparative HPLC. Further, the oct-7-ene-BODIPY favoured self-condensation over cross metathesis (b, Figure 29). Therefore, another scheme was devised that followed synthetic plans similar to long chain N-heterocyclic tocopherols and NBD-tocopherols.
Figure 29. (a) BODIPY formation from oct-7-ene-2-pyrrole and 2,4-dimethylpyrrole aldehyde. Formation of byproduct 1,3,5,7-tetramethyl-BODIPY. (b) Olefin metathesis reaction between vinyl TBSO-Trolox-ene and oct-7-ene-BODIPY. Formation of dimer bis-oct-7-ene-BODIPY.²⁰⁶

Trolox® is a chroman acid commonly used as an antioxidant reference to measure the total antioxidant capacity of fruits and vegetables.²³⁰ Successful use in prior projects made Trolox® the choice of starting material for the new BODIPY-tocopherols as well. Trolox® is converted over three steps to 6 by methyl ester formation, TBS-protection of the phenol and reduction of the ester to an aldehyde (Trolox aldehyde). A Wittig reaction with 4-hydroxypropyltriphenylphosphonium bromide produced 8, which was converted to the aldehyde 10. Pyrrole was added by a Wittig reaction with 2-methylpyrrolephosphonium
bromide to 13 and condensation to the BODIPY core with thiophene-pyrrole aldehyde followed by deprotection yielded target 1 (Figure 30).

![Chemical structures](image1)

Figure 30. Synthetic strategy towards product 1.

TBSO trolox aldehyde, 6, is converted to product 8 by a Wittig reaction. Reduction of the olefin and oxidation of the terminal hydroxide creates product 10, which is turned into product 10 by a second Wittig reaction. BODIPY product 1 is formed by reacting 13 with 5-(thiophen-2-yl)-1H-pyrrole-2-carbaldehyde.

For target 2, Trolox aldehyde was reacted with thiophene bromide. The resulting product 26 was cross-coupled with pyrrole to product 30 and condensation with pyrrole aldehyde forms product 2 (Figure 31).

![Chemical structures](image2)

Figure 31. Synthetic strategy towards product 2.
TBSO trolox aldehyde, 6, is reacted with 2-bromothiophene to product 26. Negishi cross coupling reaction of 26 with pyrrole creates product 30. BODIPY product 2 is formed by reacting 30 with 3,5-dimethylpyrrole-2-carbaldehyde.

Formylated product 34 was used as a starting material in a Knoevenagel condensation with 1,3-dimethyl-BODIPY to form target 3 (Figure 32).

Formylated product 34 was used as a starting material in a Knoevenagel condensation with 1,3-dimethyl-BODIPY to form target 3 (Figure 32).

Figure 32. Synthetic strategy towards product 3.

TBSO trolox aldehyde, 6, is reacted with 2-bromothiophene to product 26. Formylation of 26 creates product 34. Knoevenagel condensation of 34 with BODIPY, 37, creates product 3.

3 Results and Discussion

3.1 Synthesis

The first target was compound 1 by the linear synthesis proposed, starting from S-Trolox®.

Synthesis of R-Trolox aldehyde from S-Trolox® has been described by our prior group members Lei, Nava, Ohnmach, Wang, West, and Hildering (Figure 33).

Esterification of S-Trolox® to the methylester 4 was achieved with methanol and p-toluenesulfonic acid in yields of around 90%. Yields for the TBS-protection to 5 are in the +90%. Diisobutylaluminium hydride reduction in earlier attempts reported various yields in different solvents; (THF) 55%-78%, (DCM) 83% and (toluene) 86%. DCM and THF have been reported to over reduce S-Trolox® to the alcohol, even at lower temperature. Toluene was the most reliable solvent to reproducibly give good yields.
Figure 33. Synthesis of TBSO trolox aldehyde from Trolox® by esterification, TBS protection of the chroman phenol and reduction of the methylester to the aldehyde.

A saturated three-carbon linker was added using hydroxy phosphonium salt 7, itself synthesised from 3-bromo-1-propanol. Two equivalents of lithium hexamethyldisilazide (LHMDS) were used to create the active ylide at room temperature. Addition of 6 in dry THF yielded 76% of product 8 (Figure 34).

Figure 34. Synthesis of phosphonium salt, 7, from 3-bromopropan-1-ol and Wittig reaction of 7 with TBSO Trolox aldehyde to create product 8.

Following the Wittig condensation the olefin 8 was reduced with H2 over Pd/C 10%. This reaction, however, gave a mixture of products upon hydrogenation. Ethyl acetate was chosen as a solvent based on a report by Sajiki, which mentioned desilylation in EtOH, MeOH, water, hexane, DMF and THF. Besides the expected product 9 (40%) also obtained were the saturated aldehyde 10 (31%) and the fully reduced C4 alkyl product 9.2 (8.6%). Reduction of 8
to the 9 was accomplished specifically and completely when the solvent was thoroughly dried prior before use (Figure 35).

![Figure 35. Synthesis of product 9 by hydrogenation of 8 with 10% palladium on carbon.](image)

In dry solvent conditions was the product obtained in high yields. Trace amounts water influences the reaction, forming aldehyde 10 and alkyl product 9.2. Such side reactions have not been reported in prior Wittig reactions with the Trolox aldehyde. Linkers had a minimum length of five carbons following the efforts of Lei et al. After the discovery of these side-reactions the same results were observed with the Trolox allylic alcohol (12) in Hildering’s attempts to synthesize tocopentaenol. Using new palladium catalyst did not change the outcome. It is known that primary alcohols can oxidize to the corresponding aldehyde in the presence of palladium as a catalyst. To understand this phenomenon the unsaturated C4 alcohol 8 was reacted with palladium acetate and 10% Pd/C in ethyl acetate with air as an atmosphere for several days at elevated temperature. No product aldehyde was formed in both cases. Migrations of double bonds by metal complexes have been described in the literature. Isomerization of allyl ethers to aldehydes by platinum(II)hydrido complexes was reported by Clark in 1972. Metal mediated isomerization with 10% Pd/C is more prone with allylic alcohols and allylic ethers and less with more distant double bonds. However, no reaction occurred without any hydrogen gas present.
Most likely two separate reactions were taking place when water was present. The hydrogenation of the double bound occurs with and without water (a, Figure 36), as it is missing in all isolated products, 9, 9.1, 10. Any water present in the reaction is turned into an active nucleophile by the hydrogen present on the palladium surface (b, Figure 36).

![Figure 36. (a) Double bond hydrogenation of compound 8.](image)

The cartoon represents the addition of the palladium-hydride from the catalyst surface to the double bond. (b) Model of hydroxide oxidation under reductive conditions. The hydroxide is interacting with the metal surface, making the hydroxide more delta-positive, and thereby weakens the carbon-hydrogen bond (blue dashed line and arrow). The water acts as a nucleophile and abstracts the alcohol carbon-hydrogen. The newly formed hydronium ion is instantly quenched on the metal surface by metal-hydrides (Pd-H), reforming the metal catalyst and water. The abstraction of the alcohol carbon-hydrogen and hydronium quenching are in this picture combined in one step (red arrows).

Griffin described the oxidation of 1-, 2- and 3-pentanol to the corresponding aldehyde and acid (Figure 37). He used 1-5% water and water / base mixtures on palladium and platinum catalysts in his study. Full conversion of geraniol to citral was seen after 6h. The metal catalyst is in this case solely acting as a Lewis acid. Pentanol binds to the metal surface and water acts as a base by abstracting the hydrogen on the alcohol carbon, releasing the aldehyde / ketone and a metal hydride species (M-H), which reacts with water to reform the metal catalyst. Strong basic conditions increased the reaction rate. Oxidation of to the carboxylic acid occurs in the presents oxygen in the solvent.245
Pentanol is activated by binding to the metal, allowing C-H hydrogen abstraction by water to occur to form the aldehyde (ketone if 2-, 3-pentanol). The metal hydride (M-H) formed in the process reacts with water to reform the metal catalyst. Oxidation of the aldehyde to the carboxylic acid was observed when oxygen was present in the solvent.\textsuperscript{245}

The reaction of 8 has not been tested with catalytic amounts of hydroxide. The proximity of the Trolox ring system may have influenced the reactivity of longer carbon chain linkers as the latter did not show any aldehyde sideproducts. The details of palladium involvement is unknown.

The oxidation of 8 under Swern conditions (Oxalylchloride, DMSO, TEA in dry DCM) gave a complex mixture of inseparable products, but \textsuperscript{1}H-NMR indicated the absence of the TBS group.\textsuperscript{246} The TBS-C4 aldehyde 10 was successfully synthesised in a 22\% yield with Dess-Martin periodinate (DMP) as the oxidizing reagent. The yield was improved to 67\% by stirring the reaction for 16 h instead of 2 h (Figure 38).
Wittig reaction of 10 with 2-methyl pyrrole triphenylphosphonium bromide 11 did not work as described in the literature.\textsuperscript{247} To increase reactivity the pyrrole was protected at nitrogen with a benzyl group\textsuperscript{248} despite this adding one more step in the synthesis.\textsuperscript{249} An acid labile group like \(t\)-butyloxycarbonyl (Boc) cannot be used since the phenol TBS would be simultaneously cleaved under such conditions. \(N\)-Benzyl protection 12 was chosen because of inert deprotection conditions by catalytic hydrogenation. Unfortunately, reaction of 10 with 2-methyl pyrrole triphenylphosphonium bromide did not yield any of product 13, only starting material was recovered (Figure 39). Testing different bases like sodium hydride (NaH), \(n\)-butyl lithium (\(n\)-BuLi), or potassium \(t\)-butoxide (\(t\)-BuOK) to deprotonate the phosphonium salt followed by stirring the ylide with the aldehyde for over 16h did not work either.

Figure 39. Synthesis of product 13 by Wittig reaction of aldehyde 10 with phosphonium salt 11 and 12.

The reaction was run with sodium hydride (NaH), \(n\)-BuLi, \(t\)-BuOK as a base.\textsuperscript{247,249} To solve this problem the functional groups were reversed on the pyrrole and chroman.

Synthesis of the chroman phosphonium bromide was achieved by converting alcohol 9 to the bromide 16 via the mesylate. A more direct path was tried following reports in the literature using Trolox aldehyde and a 3-bromopropyl phosphonium bromide 14 to access the bromide 15 directly (Figure 40).\textsuperscript{250,251}

Figure 40. Synthesis of product 20 by Wittig reaction of aldehyde 16 with phosphonium salt 7 and lithium hexamethyldisilazide as a base.\textsuperscript{250}
The reaction did not yield any product 15, but mixtures of cyclopropane based byproducts along with starting material were suspected in the crude $^1$H-NMR, could not be isolated.

Cyclopropane formation by internal ylide cyclization is known in the literature.\textsuperscript{252,253} The conversion of alcohol 9 to the bromide 16 and iodine 17 was achieved in 57\% / 54\% yield via formation of the intermediate mesylate of 9 (Figure 41).\textsuperscript{254}

Both halides were converted to the triphenylphosphonium salts 18 (bromide) and 19 (iodide) (Figure 42).\textsuperscript{255}

Deprotonation of the phosphonium salts to the ylides was achieved at -10 to 0\(^\circ\)C with LiHMDS and NaH in THF, leading to a colour change to orange/red.
Before these Trolox linked phosphonium ylides were reacted with the protected pyrrole 6, the C3 hydroxy phosphonium salt 7 was tested with \(N\)-benzylpyrrole aldehyde, using the same conditions when forming 8 (Figure 43).\(^{201}\) A yield of 14-19% was obtained for product 20.

\[
\text{Bn} \quad \text{OH} \\
\text{Ph} \quad \text{Br}^- \\
\text{Ph} \quad \text{P} \\
\text{Ph} \quad \text{OH} \\
\text{1. 7, LiHMDS 2eq., THF, N}_2, \text{rt, 1.5h} \\
\text{2. N-benzyl-pyrrole-2-carboxaldehyde THF, rt, 3h} \\
\text{14-17%}
\]

**Figure 43. Synthesis of product 20 by Wittig reaction of \(N\)-benzylpyrrole-2-carboxaldehyde with phosphonium salt 7 and lithium hexamethyldisilazide as a base.\(^{201}\)**

Similar reactions reported by Soares with methyl or ethyl triphenylphosphonium bromide on \(N\)-benzylpyrrole aldehyde.\(^{249,256}\) Unfortunately, the reaction was unsuccessful with ylides 18 and 19. This was peculiar, given the success of the other Wittig reaction to form product 20. (Figure 44) At this point in the project, the synthesis of 2 was started. The Wittig reaction was re-attempted in dry conditions with \(n\)-BuLi as a base at 0°C, but still didn’t yield any product.

\[
\text{N-benzyl-pyrrole-2-carboxaldehyde} \\
\text{18 / 19, LiHMDS, THF, N}_2, 0^\circ\text{C-rt, 1.5h} \\
\text{16, THF, rt, 3h} \\
\text{13}
\]

**Figure 44. Synthesis of product 13 by Wittig reaction of \(N\)-benzylpyrrole-2-carboxaldehyde with phosphonium salt 18 and 19 and lithium hexamethyldisilazide as a base.\(^{201,249}\)**

During the work to synthesis compound 1, we considered putting the thiophene extension between the chroman and the BODIPY group. This new target, 2, design has a shorter carbon chain length than target \(\alpha\)-tocopherol and 1 (Figure 45). The curvature of the phytol chain in the active site seen in model studies suggests that there is enough space to accommodate the thiophene. Evidence for ligands with shortened chainlengths being better as \(\alpha\)-TTP ligands is seen with NBD-Toe, which is shorter in overall length and less bulky than BODIPY-\(\alpha\)-Toc.

92
Figure 45. Decrease in chain length from target product 1 to 2.

The innermost chain was used to compare the overall chain length. The blue numbers represent the first and last atom of the chain.

Placing the thiophene group closer to the chroman ring has certain synthetic advantages. Thiophenes are known to have a higher reactivity than benzene and react regioselectively with electrophiles under mild conditions. Addition of the thiophene to the chroman can be performed by nucleophilic addition to the electrophilic center connected to the 1'-position on the chroman ring (Figure 46).

Figure 46. 2-Bromothiophene addition to Trolox by displacement of an electrophile on position 1.

Synthesis of compound 2 started with the TBS-protected aldehyde 6 or benzyl-protected 21. The benzyl group was chosen because of its high stability to most reaction conditions and the low yields observed during the TBS-deprotection with HCl / MeOH and TBAF in our prior BODIPY synthesis by West. Deprotection of the chroman by hydrogenation was expected to be high yielding and gentle on the final structure.

Two ways were chosen to attach the thiophene group: reduction to the Trolox alcohol 22, followed by conversion to alkylbromide 23 and displacement of the halogen by thiophene, or by thiophene addition to the aldehyde followed by reduction of the alcohol 25 to the alkane 26 (Figure 47).
Figure 47. Synthetic routes chosen to synthesize 26 from TBS trolox aldehyde 6.

(a) Reduction of the aldehyde and bromination of the hydroxide created TBS Trolox bromide 23. Displacement of the bromine with 2-bromothiophene created product 26. (b) Nuclophilic addition of 2-bromothiophene to 6 creates 25, which by reduction of the secondary alcohol is turned into product 26. Racemic benzyl protected Trolox aldehyde was used because of higher stability towards most reaction conditions and the low yields observed during TBS deprotection.\(^{206}\)

Wang described the protected Trolox alcohol 22 by reduction of the methyl ester or acid with lithium aluminium hydride.\(^{254,258}\) It can also be obtained as an over-reduced byproduct during the DIBAL reduction to the Trolox aldehyde 6, more so when the reaction is run at 0°C.\(^{233}\)

Swern oxidation of alcohol 22 can be used to regain the aldehyde.\(^{246}\) Conversion of 22 to the bromide 23 was successful by syntheisis of the mesylate 22-OMs (73\%) followed by addition of excess lithium bromide (42\%).\(^{254}\) Appel reaction conditions (CBr\(_4\), PPh\(_3\) in toluene) were tested as described in the literature, but this deprotected the phenol TBS group (24) (Figure 48).\(^{259}\)
Hoye described a thiophene addition to a bromo alkene in the presence of an alkyl chloride. The reaction was conducted at 0°C to room temperature, with prior formation of the 2-lithiothiophene from 2-bromothiophene. Unfortunately, the arylbromide functional group is lost in the product, which would then have to be re-installed in the next step for the Negishi cross coupling to pyrrole. Rebromination on the 5-position has been described, but would involve an additional synthetic step. Lithiation of 2-bromothiophene at -78°C produces the anion opposite to the bromine at the 5-position. The reaction of 5-bromo-2-lithiothiophene with 23 at low temperature was attempted, but did not yield any product (Figure 49).

Figure 49. Synthesis of product 26 by reaction of 23 with 2-bromothiophene.

Peyron described this reaction with alkyl aldehydes as electrophiles. Alternatively, β-(5-bromo)-2-thienyl nucleotides have been synthesized by Friedel-Crafts type reaction from Lewis acid activated C-methyl glycosides. Following Peyron’s reaction protocol 6 was reacted with 2-bromothiophene, providing a stereochemical mixture of secondary alcohols 25 (75:25) with a 53-87% yield (Figure 50).
Figure 50. Synthesis of product 25 by reaction of 6 with 2-bromothiophene.262

Based on these reasonable yields and the previously known chemistry to synthesize the Trolox aldehyde 6 in high yield, the alkyl bromide pathway (Figure 47, a) was abandoned. To maintain high yields and not waste the expensive enantiomerically pure R-TBS protected product 25 for the dehydroxylation step a racemic chroman was synthesized. The protecting group on the phenol changed to a benzyl group (Bn), since acidic conditions might be used to assist the reduction of the secondary alcohol.264

The racemic chroman building block for the trial benzyl protection was synthesized by an improved oxa-Diels-Alder reaction of 2-methylmethacrylate and trimethylhydroquinone described by Hildering.234 Product yields were in the high 90% range and obtained in a short amount of time. The racemic product was benzyl protected 27 and reduced to the aldehyde (21) in very good yields. Trace amounts of over reduced alcohol 21.2 were observed (Figure 51).265

Figure 51. Synthesis of benzyl protected trolox aldehyde 21.
Oxa-Diels Alder reaction of trimethylhydroquinone with methylmethacrylate to racemic \((\text{rac})\) product 4. Benzylic protection of 4 created 27, which was reduced to aldehyde 21. Overreduced product, 21.2, was isolated in trace amounts.\(^{234,232}\)

A chemoenzymatic synthesis was described by Chenevert using *Candida antarctica* lipase B lipase catalyzed esterification to obtain the benzylated chroman aldehyde.\(^{266}\) A choice was made to use the racemic material to test the next steps in the reaction scheme, despite not being the pure \(R\)-enantiomer. Synthesis of the benzylated thiophene alcohol 28 followed the same procedure as described in Figure 50 (Figure 52).

![Chemical structure](image)

**Figure 52. Synthesis of product 28 by reaction of 21 with 2-bromothiophene.\(^{262}\)**

To dehydroxylate 28 different hydrides were added with acids and Lewis acids for activation of the benzylic alcohols.\(^{267,268}\) Protonation of the alcohol with trifluoroacetic acid, followed by addition of triethylsilane (TES) as a hydride source did not yield product.\(^{264}\) Zinc chloride or iodide with sodium cyanoborohydride yielded <1%-11% of product.\(^{269}\) LiAlH\(_4\) with AlCl\(_3\) did not react at all.\(^{270,271}\) Activation of the alcohol using mesyl or tosyl groups followed by reduction have been described in the literature.\(^{272,273}\) The mesyl group was successfully synthesised, but the crude product did not yield any of the reduced deoxygenated product when treated with excess LiAlH\(_4\).

Finally, the product 29 was successfully synthesized in a short reaction time and high yield by reduction with TES, but instead of TFA using BF\(_3\) as a Lewis acid catalyst. A similar system, using perfluorotriphenylborane with TES has been used to fully reduce carboxylic acids, esters and aldehydes to the alkanes.\(^{274}\) Similar yields (80-90%) were observed with the TBS protected alcohol 25 to product 26 (Figure 53).
To attach the pyrrole moiety that is needed for the BODIPY formation the TBSO-thiophene bromide 26 was reacted with a pyrrole zinicate in a Negishi coupling. Two fractions were isolated, one as an oil and the other as a solid, together yielding 10.8\% of products (Figure 54). Product 30 was formed in traces along with the dimer (31). NMR spectroscopy of the oil showed noise peaks in the aromatic region whereas the solid showed clear peaks indicating a mixture of two products. This seemed to be the effect of π-stacking between the molecules. To verify the products an EI+ mass spectrum was recorded, but unfortunately did not give any molecular ion. The dimer was confirmed by MALDI mass spectroscopy.
Separation of the dimer and 31 was not possible by column chromatography and was used as a 1:1 mixture in the next step. The Negishi chemistry was tried with the BnO-protected bromothiophene 29. Product was isolated in similar yield (10.3% 32 only) as a mixture of products. Purification by chromatography afforded the debrominated byproduct (29-H, 1%), but the dimer was inseparable. 32 was used as a 1:1 mixture in the next step (Figure 55).

Figure 55. Negishi coupling of 29 with pyrrole to create product 32 and trace amounts of 29-H.²⁷⁵

BODIPY formation was carried out with 3,5-dimethylpyrrole-2-carboxaldehyde, following the conditions used for the synthesis of BODIPY-α-Toc.²⁰⁶ Product 33 was isolated in 13% yield (Figure 56).

Figure 56. Synthesis of 33 by condensation of 32 with 3,5-dimethylpyrrole-2-carbaldehyde.²⁰⁶

At this point there were 23 mg of 33 available for the deprotection of the chroman ring to product 2. Hydrogolysis with Wilkinson’s catalyst in EtOH was chosen from our experience with BODIPY-α-Toc synthesis, but did not yield product.²⁰⁶ The reaction was rerun in EtOAc for the same time, but no product was found. The starting material was then reacted with Pd/C 10% on carbon in dry EtOAc overnight.²⁷⁷ A new fluorescent spot was seen by TLC and
isolated, giving 1 mg of seemingly de-benzylated product by NMR, but had too much contaminating grease to be verified and used further. Hydrogenation with H\textsubscript{2} under prolonged stirring with Pd/C 10\% at elevated pressure can decompose BODIPYs.\textsuperscript{206} To increase hydrogen absorption a transfer hydrogenation with excess trimethylsilane and Pd/C 10\% was attempted at room temperature, but also did not yield any product.\textsuperscript{278} Birch reduction conditions are known to de-benzylate alpha-tocopherol\textsuperscript{266} thus, 5 mg of 33 were treated with Li metal and ethylamine/ether at -78\degree C, but no product was observed after 1h (Figure 57).

![Chemical structure](image)

**Figure 57. Attempts to debenzylate 33 to form product 2.**

Conditions tried were hydrogenation with H\textsubscript{2}, using 10\% palladium on carbon (Pd/C 10\%) as a catalyst with ethyl acetate and methanol as a solvent\textsuperscript{277}, and with Wilkinson's catalyst (Rh(PPh\textsubscript{3})\textsubscript{3}Cl) in EtOH.\textsuperscript{206} Pd/C 10\% was further used in a transfer hydrogenation with triethylsilane (TES) in DCM.\textsuperscript{278} Alternatively was lithium metal with ethylamine applied as a reductive condition.\textsuperscript{266}

The recovered benzylated chroman BODIPY was stored as a reference. The remaining quantity, even with a successful debenzylation, was not enough material to conduct binding studies with \(\alpha\)-TTP or trials in hepatic cell culture.

With more material in hand, different oxidative and reductive debenzylations would be tested. Transfer hydrogenation with different hydrogen sources like formamide, cyclohexadiene and palladium catalyst.\textsuperscript{279,280} Lewis acids like BX\textsubscript{3}, TiCl\textsubscript{4} have also shown promising results as de-benzyla-tion additives in the literature, but might be problematic because BODIPY B-F bonds are activated by the same molecules with subsequent halogen exchange or addition of any nucleophile present in solution.\textsuperscript{281,282,283}

100
With the pyrrole coupling and BODIPY formation reaction not yielding enough product an alternative approach was necessary to reach 2. To avoid the Negishi pyrrole coupling a cross coupling with bromothiophene 29 and a N-protected pyrrole with a boronic acid / pinacol ester or alkylstannylate at the 2-position is required. The leaving groups on thiophene and N-protected pyrrole can be reversed in case of low reactivity. 284,285,286,287,288

While searching through the literature on how to connect the pyrrole to the thiophene bromide an alternative route was considered to link the BODIPY framework to the thiophene. Cross coupling reactions of aryl groups with BODIPY halides have been described in the literature. 289

A common way to C-C couple BODIPYs to aryl groups is by Knoevenagel reaction of aryl carbonyls with a methyl group at the 2’-position on the BODIPY. 290 As mentioned earlier, target 2 has a shorter length than the phytyl chain in tocopherol. The Knoevenagel reaction bridges a trans-double bond of the aryl aldehyde and BODIPY, which brings the overall sidechain length back to the length of the tocopherol phytyl chain. Furthermore, the wavelength would be extended by an additional ~10-20 nm. Therefore, we shifted focus to target 3.

Formylation of thiophenes are well described in literature. 290,291 Lithium halogen exchange at -78°C followed by DMF addition formed aldehyde 34 in 88% yield (Figure 58).

![Figure 58. Synthesis of product 34 by formylation of 26 by lithium halogen exchanged and quenching with DMF. 291](image-url)
A debrominated byproduct 35 was formed due to residual water in the reaction mixture. Attempts to recycle the byproduct by Vilsmeier-Haack conditions with DMF and POCl₃ did not work, as the TBS protection group was cleaved, followed by in situ protection of the phenol to the aryl formate 36 (37%) (Figure 59).²⁹²,²⁹³,²⁹⁴,²⁹⁵ The formate protection was a rather unexpected result. It is most likely that in the increasingly acidic environment the TBS-group was cleaved to the phenol, which then reacted with the chloroimminium ion reagent.

Figure 59. (a) Synthesis of 36 from 35 by Vilsmeier-Haack formylation. (b) Proposed reaction mechanism of the aryl O-formate protection of TBSO protected 2-thiophenemethyl α-chromane.

In acetic environment is the TBSO protonated and deprotected by phosphorodichloridate. DMF is turned into the Vilsmeier-Haack reagent (VMHR), which reacts with the phenol to form product 36.²⁹⁵
BODIPY building block 37 was synthesized by using 2,4-dimethyl pyrrole and 2-pyrrole carboxaldehyde (Figure 60). Pyrrole was first reacted with 3,5-dimethyl pyrrole carboxaldehyde, however this reaction yielded more of the tetramethyl-BODIPY byproduct (38).\(^\text{206}\)

![Figure 60. Synthesis of dimethyl-BODIPY building block, 37, by condensation of 2,4-dimethyl pyrrole and 2-pyrrole carboxaldehyde.\(^\text{206,295}\)](image)

Knovenagel reaction yielded BODIPY 39 in rather low yield (24\%) (Figure 61).\(^\text{296}\) Such aryl carbonyl condensations with methylated BODIPYs often vary in yield, and usually do not exceed a median yield of 50\%.\(^\text{297,290,298}\)

![Figure 61. Synthesis of 39 by Knovenagel condensation of 34 with dimethyl BODIPY 37.\(^\text{296}\)](image)
Yields seem to drop with prolonged stirring, a condition that would have to be investigated in future scale up reactions. Deprotection to compound 39 followed by West’s protocol yielded product 3 in moderate yield (Figure 62).

![Chemical structures](image)

Figure 62. Synthesis of target product 3 by acetic phenol TBSO-deprotection of 39 with 8% HCl MeOH.

### 3.2 Photophysical studies

#### 3.2.1 Absorption spectra

The UV absorption maximum ($\lambda_{\text{abs}}$) of product 3 was determined to be 571 nm in absolute EtOH, with a secondary absorption maximum at 536 nm (Figure 63). The emission maxima ($\lambda_{\text{em}}$) was at 583 nm, giving a Stokes shift of 12 nm.

![Absorption Emission Spectra](image)

Figure 63. Thienyl-ene-BODIPY absorption and emission curve.
The molar extinction coefficient ($\varepsilon$) was determined, being 125,000 M$^{-1}$ cm$^{-1}$ at 571 nm (abs. EtOH, diam. 1 cm).

### 3.2.2 Quantum yield

The quantum yield was determined by using Williams method, comparing the fluorescence intensity to standard fluorescein (0.1M NaOH). Fluorescein is used as a standard to compounds with emission maxima from 500-600 nm.

Plotting UV/VIS absorbance (A) vs fluorescence intensity (FI) at a set concentration created a gradient for the standard and compound 3 (3-BODIPY). The determined gradient from the plotted line 3 ($Grad_3$) is divided by the gradient of the standard ($Grad_{fluorescein}$), multiplied by the ratio of the refractive index ($\eta_3^2 / \eta_{fluorescein}^2$) of the solvents (EtOH $\eta = 1.33$, 0.1M NaOH $\eta = 1.33$) used and multiplied by the relative quantum yield of fluorescein ($\Phi_{fluorescein} = 0.79$).

$$
\Phi_3 = \Phi_{fluorescein} \left( \frac{FI_{3-BODIPY}}{FI_{fluorescein}} \right) \left( \frac{A_{fluorescein}}{A_{3-BODIPY}} \right) \left( \frac{\eta_3^2 - BODIPY}{\eta_{fluorescein}^2} \right)
$$

$$
\Phi_3 = \Phi_{fluorescein} \left( \frac{Grad_{3-BODIPY}}{Grad_{fluorescein}} \right) \left( \frac{\eta_3^2 - BODIPY}{\eta_{fluorescein}^2} \right)
$$

The relative quantum yield was determined to be 0.98.
Figure 64. Fluorescein and thienyl-ene-BODIPY gradients for quantum yield determination.

Conc.: 90nM, 180nM, 270nM, 360nM, 450nM. \( \eta = 1.33 \) (for EtOH and 0.1M NaOH). \( \Phi_{\text{Fluorescein}} = 0.95 \).

### 3.3 Binding Studies

Binding affinities will be determined by direct binding towards \( \alpha \)-TTP. The ligand will be tested as a competitive inhibitor against natural tocopherol and cholesterol. Cholesterol does not bind to \( \alpha \)-TTP, and therefore shouldn’t decrease the bound ligand. To access binding specificity towards the \( \alpha \)-TTP protein the ligand will be tested with bovine serum albumin (BSA).

#### 3.3.1 \( \alpha \)-TTP expression & purification

Human \( \alpha \)-TTP was expressed following a protocol we have described previously.\(^{207}\) Frozen cultures of BL21(DE3) \( E. \ coli \) cells containing a pGEX 4T-3/ \( \alpha \)-TTP construct were induced with ampicillin at 37°C overnight. The preculture was then inoculated in 1 L of lysogeny broth (LB) (1 : 100 LB broth). After an optical density (OD\(_{600}\)) of 0.4-0.6 was achieved, the culture was treated with isopropyl-\( \beta \)-D-thiogalactopyranosid (IPTG) overnight, after which centrifugation yielded the TTP cells.
The cells were treated with lysozyme for 30 min at 0°C. The solution was then treated with MgCl₂, Triton-X-100, DNase, RNase and incubated for 30 min at 0°C. After sonication (3x for 15 s at 500 W) cell debris was removed by centrifugation (17500 rpm for 25 min at 4°C). The supernatant was purified by affinity chromatography over a pre-washed glutathione-Sepharose column. After flow through of supernatant, column was washed again and incubated with thrombin and stored at 4°C overnight. The protein was collected the next day. The purified protein concentration was quantified by using the Bradford assay. A standard curve was prepared, plotting absorbance at 595 nm against bovine serum albumin (BSA) concentration from 0-1 mg/ml. The concentration of the purified protein stock solution used for the binding assays was 130 µM.

3.3.2 Binding study to α-TTP in SET-Buffer

We tested how well the new ligand, 3, would bind to the apo-form of α-TTP by performing a fluorescence titration. The increase in fluorescence intensity was measured as increasing amounts of ligand were titrated into a buffered solution containing α-TTP. The dissociation constant (K_d) is measured by fitting the data points to a one site specific binding model using Prism. Equation 1 was used to calculate the K_d.

\[ Y = \frac{B_{max}X}{(K_d + X)} \]

To obtain the dissociation constant (K_d) of 3 the methods reported by West et al. were repeated. In the event, to 0.2 µM α-TTP in SET buffer (50 mM Tris-HCl, 100 mM KCl, 1 mM EDTA, 150 mM sucrose pH 7.4), was added 0-5 µM ligand (final concentration 3.5µM) in small aliquots of absolute EtOH. The total amount of EtOH did not exceed 0.1% of the final solution. The sample was stirred for 25-40 min at room temperature for equilibrium to be
established and fluorescence measured at 514 nm at an excitation wavelength of 506 nm and 5 nm slit widths (Figure 65).

![Graph showing fluorescence data for different ligands]

**Figure 65.** Direct binding of [ligand] BODIPY-α-Toc C6, C7 and C8 to 0.2 µM α-TTP in SET buffer.

Final concentration reached was 3.5 µM. Data was measured and triplicate and error bars represent standard deviations from the mean. Data from literature.\(^{206}\)
The excitation wavelength was adjusted to \( \lambda = 492 \text{ nm} \), with an emission from 512 - 528 nm, to not reach the fluorometers maximum fluorescent count (FC) of \( 4 \times 10^6 \) (Figure 66). Titration with C8-BODIPY-Toc was repeated with fresh \( \alpha \)-TTP. The fluorescent counts were half as recorded prior. The obtained \( K_d \) of 90 nM is in the close range of the recorded literature value (94 ± 3 nM).\(^{206}\)

![Chemical structure of BODIPY-\( \alpha \)-Toc](image)

**Figure 66.** BODIPY-\( \alpha \)-Toc in EtOH direct binding to 0.2 \( \mu \text{M} \) \( \alpha \)-TTP in SET buffer repeated with freshly expressed protein.

Data was measured as a singular titration. A \( K_d \) of 90 nM was determined, which matches literature values 94 ± 3 nM.

Ligand 3 was also tested in triplicate under these same conditions. A blank sample, containing no protein, was also measured to see how the fluorescence intensity increased due to free ligand in buffer. The excitation wavelength was 564 nm, and the emission was measured from 584 - 588 nm at a slit width of 5 nm (Figure 67).
The data was collected in triplicate and error bars represent standard deviations from the mean. The $K_d$ of 4.2 ± 0.8 nM obtained from these tests was surprisingly low compared to C8-BODIPY-$\alpha$-Toc. However, the fluorescent counts (FC) were also ten times lower.

This phenomenon most likely is due to the interaction of 3 with the binding site of $\alpha$-TTP being different than for C8-BODIPY-$\alpha$-Toc. It would appear the fluorophore of 3 is more solvent exposed and thus has its fluorescence intensity suppressed.

The difference between the no-protein control and the ligand 3 is rather small, about 10000 fluorescence counts at 15 $\mu$M of ligand. The increasing amounts of EtOH added to the SET buffer increases the solvent hydrophobicity, likely explaining the increase in fluorescence intensity in the blank.

Ligand 3 was also tested in a competition assay with $\alpha$-tocopherol and cholesterol, following previous protocols. To assure full ligand saturation, $\alpha$-TTP was preincubated with 5x more 3 prior to addition of tocopherol or cholesterol. In case of C8-BODIPY-$\alpha$-Toc, 0.2 $\mu$M protein was mixed with 1.0 $\mu$M ligand, however, for the thienyl-ene-BODIPY 3 only 1.25-times excess ligand was used for 0.25 $\mu$M protein since the $K_d$ was so much lower (Figure 68).
Addition of the natural α-tocopherol to a sample of α-TTP saturated with 3 will lower the starting fluorescence should the added ligand compete with the 3 for access to the binding site. α-Tocopherol nearly completely reduced the original fluorescence intensity from bound C8-BODIPY-α-Toc, but cholesterol had almost no effect.

![Graph](image)

**Figure 68.** Competition assay of 0.2 μM α-TTP, saturated with 1.0 μM BODIPY-α-Toc in SET buffer, with [competitor] α-Toc & cholesterol in EtOH.

In the graph were the collected data points normalized to 1.0. Data was measured and triplicate and error bars represent standard deviations from the mean. Data from literature.\(^{206}\)

The fluorescence intensity of α-TTP bound 3 decreased, but not as much as with C8-BODIPY-α-Toc. The addition of tocopherol was ~30% successful in competing the ligand off the protein (Figure 69). A control titration was used to obtain a value of fully out-competed ligand 3. To a solution of thienyl-ene-BODIPY without α-TTP was tocopherol added. No change in absorption was observed. After 0.6 μM α-tocopherol was added to the solution with α-TTP was a plateau reached.
Figure 69. Competition assay of 0.2 μM α-TTP, saturated with 0.25 μM thienyl-ene-BODIPY (3) in SET buffer, with α-tocopherol (α-toc) in EtOH.

In the graph were the collected data points normalized to 1.0. Data was measured and triplicate and error bars represent standard deviations from the mean.

To get higher fluorescence intensities with a larger differentiation to the no-protein control, the competitive assay was run in the presence of detergent, as had been used in the assessment of NBD-Toc binding (Figure 70). Detergents are used to make it more thermodynamically favourable for poorly soluble hydrophobic ligands to leave the active site during a competition experiment.

Figure 70. Direct binding of C9-NBD-α-Toc in EtOH to 0.2 μM α-TTP in SET buffer. λ_{ex} = 495nm λ_{em} = 535nm.
Data was measured and triplicate and error bars represent standard deviations from the mean. Data from literature.\textsuperscript{201}

Competition assay with NBD-Toc (Figure 71): 0.2 \(\mu\)M \(\alpha\)-TTP in 3 ml SET buffer and 100 \(\mu\)M Triton-X-100 (TX100), adding 1 \(\mu\)M NBD-Toc (5x excess). After each ligand addition the cuvette was equilibrated for 15 min.\textsuperscript{201} The same time was applied for the thienyl-ene-BODIPY 3 ligand.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure71.png}
\caption{Competition assay of 0.2 \(\mu\)M \(\alpha\)-TTP, saturated with 1.0 \(\mu\)M NBD-Toc in SET buffer and 100 \(\mu\)M TX100, with [competitor] \(\alpha\)-Toc & cholesterol in EtOH.}
\end{figure}

In the graph were the collected data points normalized to 1.0. Data was measured and triplicate and error bars represent standard deviations from the mean. Data from literature.\textsuperscript{201}

0.5 \(\mu\)M \(\alpha\)-TTP, 1.0 \(\mu\)M thienyl-ene-BODIPY, 3, with 100 \(\mu\)M TX100 were titrated with \(\alpha\)-tocopherol (Figure 72). Adding a detergent makes the media more hydrophobic, which makes the ligand less likely to bind to \(\alpha\)-TTP. Therefore, in the competition experiment with TX100 was twice the amount of thienyl-ene-BODIPY to \(\alpha\)-TTP used to have more ligand-bond protein. Increasing hydrophobicity raised the background fluorescent to 67000 FC in the no-protein control. The gap between no protein control and ligand was increased from 1500 FC to 20000 FC, which would suffice to measure the competition qualitatively. Unfortunately, the background fluorescence was too high to accurately detect any changes in the competition experiment.
Figure 72. Competition assay of 0.5 μM α-TTP, saturated with 1.0 μM thienyl-ene-BODIPY (3) in SET buffer, with α-Toc & 100μM TritonX100.

Data was measured and triplicate and error bars represent standard deviations from the mean.

A direct binding assay with 100 μM detergent gave no difference to the no-protein control (Figure 73).

Figure 73. Direct binding of thienyl-ene-BODIPY (3) in EtOH to 0.2 μM α-TTP in SET buffer with 100μM TX100.

The same test was repeated with 10 μM detergent, which showed an increase of about 1000 fluorescence counts to the direct binding curve having no detergent present (Figure 74).
Figure 74. Direct binding of thienyl-ene-BODIPY (3) in EtOH to 0.2 μM α-TTP in SET buffer with 10 μM TX100.

To find the optimal amount of detergent a competition assay with saturated protein (0.5 μM α-TTP, 1.0 μM thienyl-ene-BODIPY) and a no-protein control were treated with increasing increments of TX100 (10 μM) (Figure 75).

The difference between the control and protein sample at 100 μM TX100 was again of 20000 FC. At 10 μM a value of 15000 FC was observed. Compared to the first binding assay (Figure 67) without TX100 and using the same amount of protein (0.5 μM), is an increase of 5000 FC was observed. The direct binding with TX100 (Figure 74) used less protein (0.2 μM) and has therefore a smaller difference of 3000 FC.
Figure 75. Titration of 0.5 µM α-TTP, saturated with 1.0 µM thienyl-ene-BODIPY (3) in SET buffer, test with increasing TX100 amounts by 10µM.

The difference in fluorescence between a no-protein control and α-TTP with 100µM TX100 diverges somewhere between 70 and 100 uM. Since a larger observable window with low fluorescence background (30% <) is needed to make a qualitative judgement, we concluded that TX100 not beneficial for the study of thienyl-ene-BODIPY 3.

As a further control, titration of 3 to BSA solutions verified ligand specificity to α-TTP. BSA is known to be a lipid carrier and does bind tocopherol non-specifically with low affinity. The shape of the titration curve would suggest that BSA does bind 3, however the no-protein control shows higher fluorescence than the BSA curve, so the signal for a BSA titration may not reflect real protein-ligand association (Figure 76).
To further explore the effect of additives in binding assays we tested a new buffer TKE (50 mM Tris-HCl, 100 mM KCl, 1 mM EDTA) and used DMSO as supportive organic solvent. Fluorimeter slits were set to 6 nm to better observe changes at low fluorescence counts. DMSO is, like EtOH, a standard solvent used in biological assays. This was tested by first adding to 0.2 µM α-TTP 0.5 µM solutions of 3 prepared with different percentages of DMSO (0%, 5%, 10% & 15%). After 1 min, 10 min and 15 min of equilibration time fluorescence intensity was measured (Figure 77).

The highest increase in fluorescence intensity was observed at 10 min in the cuvette without any DMSO. 2. After 15 min another 0.5 µM of 3 was added to the non-DMSO cuvette to see if there would be any further increase in fluorescent intensity. No increase was observed. The FC plateaued at 30000.

Figure 76. Direct binding of thiencyl-ene-BODIPY (3) in EtOH to 0.5 µM α-TTP & BSA.
To four different 0.2 µM α-TTP solutions in TKE buffer, three of them containing either 5% (orange), 10% (blue) and 15% (pink) DMSO in the overall 3ml volume, was 0.5 µM thienyl-ene-BODIPY 3 in EtOH added. (1.) The fluorescence of all four solutions was measured after 1 min, 10 min and 15 min. To the solution without DMSO (yellow) was after the 15min (2.) scan an additional 0.5 µM thienyl-ene-BODIPY 3 in EtOH added.

### 3.3.3 Binding study to α-TTP in TKE-Buffer

Thienyl-ene-BODIPY 3 was tested in a direct binding assay against 0.2 µM and 0.5 µM α-TTP in TKE buffer (Figure 78 and 79). The binding curves in both concentrations had a higher fluorescence intensity and a greater difference from the non-protein control than the assay performed in SET buffer.
Figure 78. Direct binding of thienyl-ene-BODIPY (3) in EtOH to 0.2µM α·TTP in TKE buffer. The data was collected in triplicate and error bars represent standard deviations from the mean.

Figure 79. Direct binding of thienyl-ene-BODIPY (3) in EtOH to 0.5µM α·TTP in TKE buffer. The data was collected in triplicate and error bars represent standard deviations from the mean.

Direct binding to BSA in TKE was giving the expected result. nPC is lower than the BSA in the TKE buffer (Figure 80).
In all the direct binding assays with ligand 3 a slight decrease in fluorescence was observed after saturation (around 120 nM). Therefore, we tested if this trend would continue when increasing amounts of 3 were added (Figure 81). After a ~120 nM there was a steady decrease in fluorescence signal that plateaued at approximately 10 µM. Such concentrations are not realistic in terms of measuring the binding affinity and are unlikely to ever be used in cells. However, it indicates that self-quenching occurs (inner filter effect) or aggregation of the fluorophore occurs as the concentration is increased.303

Future competition assays will take this effect into account by not oversaturating the protein with the ligand. A maximum ratio of 2:1 ligand to protein seems to be still acceptable.
Figure 81. Test of ligand over addition. Direct binding of thienyl-ene-BODIPY (3) in EtOH to 0.4µM α-TTP in TKE buffer.

Now that the best conditions have been determined to perform binding assays, a competition assay with α-tocopherol was conducted (Figure 82). Less thienyl-ene-BODIPY was added in this assay since from prior binding assays it was clear that protein saturation under these conditions occurs at ~20 nM of 3.

Fluorescence intensity dropped by 50% (16000 fluorescence counts) at a concentration 180 nM of α-tocopherol. The non-protein control increased 50% (4000 FC) at the same concentration. When taking the increased fluorescence intensity in non-protein controls into account, the fluorescence decrease by α-tocopherol addition clearly shows that competition has occurred. After 200 nM the increase in EtOH content increases the fluorescence of both curves in a similar fashion, negating the observation of more complete competition at higher tocopherol concentrations.
Figure 82. Competition assay of 0.1 µM α-TTP, saturated with 0.2 µM thienyl-ene-BODIPY (3) in TKE buffer, with α-Toc in EtOH.

The data was collected in triplicate and error bars represent standard deviations from the mean.

For the competition assay with cholesterol α-TTP was precinubated with a 5-fold excess of ligand 3. Surprisingly, cholesterol successfully reduced the observed fluorescence by almost 65%. On the other hand the no protein control (but still containing 0.4 µM of 3) had extremely low fluorescent counts that did not increase over the course of cholesterol addition (Figure 83).

Figure 83. Competition assay of 0.1 µM α-TTP, saturated with 0.5 µM thienyl-ene-BODIPY (3) in EtOH in TKE buffer, with cholesterol.

The data was collected in triplicate and error bars represent standard deviations from the mean.
The experiment was repeated with a 1:1 eq and 2:1 mole ratio of 3 to α-TTP (Figure 84). Both concentrations were titrated with cholesterol, but one additional cuvette with 0.4 µM 3 was titrated with only EtOH.

The 2:1 eq cuvette (black) was a higher FC, but had the same decrease in fluorescent intensity by 30% at 250 nM (2:1 = 8000 FC 1:1 = 6000 FC). More importantly is the observation that EtOH (square pink points) addition creates the same curve as 1:1 eq cholesterol addition, undoubtly prove that the solvent is causing the decrease in fluorescents.

To see if the effect of EtOH as the supporting solvent could be avoided we tried DMF as an alternative solvent. The fluorescence titration curve with DMF as the supporting organic solvent (Figure 85) was similar to the experiment with EtOH in TKE buffer (Figure 78 & 79).
Figure 85. Direct binding of thienyl-ene-BODIPY (3) in DMF to 0.4 μM α-TTP in TKE buffer.

The data was collected in triplicate and error bars represent standard deviations from the mean.

When competition assays were performed using DMF solutions of α-toc to displace 3 from α-TTP, we observed competition regardless of whether the DMF stock solution contained tocopherol or not (Figure 86). As in the case of cholesterol in EtOH (Figure 86) the solvent was displacing 3 from the protein. In fact, DMF alone was a better competitor than α-tocopherol up to about 200 nM DMF. The solvent may interfere with the protein binding by interfering with interactions of the ligand in the binding site or by partially denaturing the protein causing ligand release.

Figure 86. Competition assay of 0.4 μM α-TTP, saturated with 0.4 μM thienyl-ene-BODIPY (3) in TKE buffer, with α-Toc solution in DMF and DMF alone.
The data was collected in duplicate (for α-toc in DMF) and error bars represent standard deviations from the mean.

Competitive displacement assays with solutions of cholesterol dissolved in DMF followed the same trend as α-toc (Figure 87).

![Graph](image)

**Figure 87.** Competition assay of 0.4 μM α-TTP, saturated with 0.4 μM thienyl-ene-BODIPY (3) in TKE buffer, with cholesterol (in DMF) and DMF.

The data was collected in duplicate (for cholesterol in DMF) and error bars represent standard deviations from the mean.

Comparing EtOH to DMF in a competition assay with tocopherol showed that EtOH initially outcompetes most of the ligand, DMF itself and α-toc in DMF have similar decays up to 200 nM. After 200 nM decreases the fluorescence of α-toc in DMF the most, DMF by itself has the least decrease overall (Figure 88).
Thus, we showed that neither DMF nor EtOH are appropriate organic co-solvents for ligand binding and competition assays of 3 with α-TTP. The solvent seems to strongly interact with the protein interfering with ligand binding. Concerned that maybe 3 did not actually exhibit specific binding to α-TTP but maybe only bound non-specifically on the protein surface we modified the phenol of the chroman ring with a methyl group, as non-phenolic ligands (such as α-tocopheryl acetate) have much lower affinity to α-TTP (Figure 89).
The addition of methoxy (MeO) thienyl-ene-BODIPY 40 to solutions of α-TTP showed very small increases of fluorescence intensity, even lower than the non-protein controls. At high added ligand concentrations, 40 increased the fluorescence and plateaued around 500 nM perhaps as a result of ligand self-quenching (Figure 90).

Since the the free phenol 3 showed a markedly higher fluorescence intensity than the ether 40, it is very likely that compound 3 binds specifically in the α-TTP binding site.

![Figure 90. Direct binding of MeO-Thienyl-ene-BODIPY (40) in DMF to 0.4 µM α-TTP in TKE buffer.](image)

The data was collected in triplicate and error bars represent standard deviations from the mean.

Finally, dioxane was chosen as a solvent to solve the competition assay problem. Dioxane has a lower dielectric constant (ε = 2.25) than DMF (ε = 36.7) and EtOH (ε = 24.5), which should lower the solvent interactions with the polar residues on the protein.305-306 Fluorescence quenching in buffer has been found to be lower in dioxane compared to DMF and other solvents.307 The fluorescence titration binding curve using dioxane as the supporting organic co-solvent shows the same intensity of fluorescent as in DMF (see Figure 91, Figure 85 describes the binding with DMF).
The data was collected in triplicate and error bars represent standard deviations from the mean.

Importantly, the use of dioxane allows a distinct difference to be seen between α-tocopherol and dioxane solvent, about 4000-5000 fluorescence counts. A plateau was reached at around 3-4 µM. Around 57% of the fluorescence of 3 was reduced by competition at 2 µM concentration of tocopherol (Figure 92).

Figure 92. Competition assay of 0.4 µM α-TTP, saturated with 0.4 µM thienyl-ene-BODIPY (3) in TKE buffer, with α-Toc (in dioxane) and dioxane.

The data was collected in duplicate (for α-Toc in dioxane) and error bars represent standard deviations from the mean.
No fluorescence change was observed when cholesterol, dissolved in dioxane, was used as a competing ligand (Figure 93). Dioxane alone competed for ligand 3 and reduced the fluorescence by only 8% at 2 nM.

![Graph](image-url)

**Figure 93.** Competition assay of 0.4 μM α-TTP, saturated with 0.4 μM thienyl-ene-BODIPY (3) in TKE buffer, with cholesterol (in dioxane) and dioxane.

The data was collected in duplicate (for cholesterol in dioxane) and error bars represent standard deviations from the mean.

The titration of α-TTP bound 3 with dioxane solutions of α-tocopherol in TKE buffer showed that thienyl-ene-ligand 3 exhibits specific and reversible binding to α-TTP. Cholesterol, which does not bind to α-TTP, did not displace 3 from α-TTP, emphasizing the specific nature of binding.

Certain points have to be addressed at the end of the binding study. Testing fluorescent ligand affinities to proteins by measuring fluorescence intensity is a valid method, but needs to be well-screened for solvent effects and the affects of additives.

On advise by the comittee was the solubility of ligand 3 tested. The ligands solubility was tested in water and aqueous solutions with EtOH and dioxane (1%, 5% and 10%), but no result was obtained.\textsuperscript{308}
In comparison with C8-BODIPY-α-Toc, 3 has a 5x lower fluorescence intensity at 100 nM ligand concentration in buffer, but has a higher extinction coefficient and similar quantum yield.\(^{206}\) However, binding to α-TTP was confirmed to be of higher affinity for 3.

In conclusion, 3 binds to α-TTP in high affinity in both buffer systems (SET and TKE) and all solvents tested (EtOH, DMF and dioxane). The calculated dissociation constant of \(K_d = 8.7 \pm 1.1\) nM (dioxane, TKE) is lower than that of natural tocopherol \(K_d = 25\) nM determined in a radiolabeled binding assay.\(^{99}\) Thus, compound 3 binds to α-TTP as well as or better natural α-tocopherol. The specificity of the ligand to α-TTP was verified by competition assay using cholesterol, which failed to compete, and by comparing the binding to BSA. The ligand binding is reversible since α-tocopherol competes for α-TTP binding with the fluorescent ligand. Specific binding requires the free phenol, as seen by the lack of binding of the methoxy analogues 40.

3.4 Cell studies

The ligand with the highest binding affinity will be accessed in a doxycycline-induced secretion assay in Morris hepatoma 7777 (McA-RH7777) rat hepatoma cells. The decrease in the area of fluorescence will be used to determine the amount of secretion. Our prior fluorophores were tested in the same cell lines.

3.4.1 Cell assay

Thienyl-ene-BODIPY-α-tocopherol was sent to our collaborator at Case Western University, Dr. Danny Manor to test the suitability of the ligand in cell-based assays of intracellular tocopherol transfer and secretion. The transport was followed by observing the location and
duration of fluorescence in cultured cells, in what is known as a secretion assay. It is understood that α-TTP is responsible for the specific retention of α-tocopherol in the liver and its secretion into plasma lipoproteins such as VLDL. In fact, cultured hepatotcytes are shown to transport compound 3 out of the cell as observed by an overall fluorescence decrease. The test was carried out as described in the following section:

For secretion assays, previously described doxycycline-inducible α-TTP-expressing McA-RH7777 rat hepatoma cells were used. Forty-eight hours after treating cells with 1 µg/ml doxycycline or vehicle control, all cells were loaded with 15 µM of fetal bovine serum complexed thienyl-ene-BODIPY 3, for 18 h. Media was removed and the cells were washed four times prior to a four-hour secretion / incubation into serum-containing normal growth media. Following α-TTP-facilitated secretion, cells were washed three times in PBS and live cell imaging was performed using a Leica DM 4000B inverted fluorescence microscope. For each experiment the accumulated fluorescence of 3 was captured from 10 equally confluent microscopic fields at 20x magnification (Figure 94). Fluorescence intensities were quantitated and normalized using Image J software (http://rsbweb.nih.gov/ij/index.html) as previously described.

![Figure 94. α-TTP facilitated secretion of thienyl-ene-BODIPY 3, from cultured hepatocytes.](image-url)
(A) McA-RH7777-TetOn-TTP cells\textsuperscript{210,212} were cultured as described. Where indicated, expression of α-TTP was induced by addition of doxycycline (1 mg/ml). Doxycycline activates the Tet-On gene, starting the transcription of tppa.\textsuperscript{312} Twenty-four hours post induction, serum-complexed 3 was added to the culture media (final concentration 15 µM) for 18 h. The thienyl-ene-BODIPY 3 was then washed with normal media, and after 4 h ('secretion' phase) the cells were imaged by epifluorescence microscopy. Fluorescence in images were quantified in multiple fields and normalized to cell protein content.\textsuperscript{311}

The decrease in the total area of fluorescence is around 30\% after 4 h (Figure 95). The results are not directly relatable to other tocopherol analogues like \textsuperscript{[14]}C-Toc and NBD-tocopherol because of the difference in structure. However, similar secretion assay have been performed with both ligands.\textsuperscript{309,77}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig95}
\caption{Averages and standard deviations of 5 images from three independent experiments. Asterisks denote significant difference ($P > 0.05$) between the α-TTP-expressing and nonexpressing cells, as determined by Student’s $t$-test.\textsuperscript{311}}
\end{figure}
Secretion of $[^{14}\text{C}]-\text{Toc}$ in HepG2-TetOn-TTP cell lines was around 20% after 48 h when $\alpha$-TTP was induced with doxycycline (Figure 96). The value was obtained by measuring radioactivity by scintillation counting. The authors found that HepG2-TetOn-TTP cell line $[^{14}\text{C}]-\text{Toc}$ secretion is identical in McA-RH7777-TetOn-TTP cells.

![Figure 96. $\alpha$-TTP-induced $\alpha$-tocopherol secretion in HepG2-TetOn-TTP cells.](image)

Cells were loaded with $[^{14}\text{C}]\text{RRR-}\alpha$-tocopherol for 36 h and washed, and the appearance of radioactivity in the media was assayed at the indicated times by scintillation counting. Shown are averages and standard deviation of quadruplicate wells. Data are representative of 10 independent experiments. When those data are fitted to linear functions (dashed lines), their slopes differ by 3.3-fold.

NBD-Tocopherol was used to study the intracellular trafficking in liver cells and the influence of PIP$_2$. Fluorescence decrease after $\alpha$-TTP expression (TTP +) decreased by 50% after 1h, 60% after 2h respectively (Figure 97). The concentration of NBD-Toc in these cells was 10$\mu$M. The higher decrease of fluorescence is partly caused by photobleaching of the NBD-tocopherol.
Figure 97. Quantification of intracellular NBD-α-tocopherol fluorescence over time in cells that do (white bars) or do not (black bars) express TTP.

Shown are averages and standard errors of NBD-α-tocopherol fluorescence quantified in confocal images of ~50 cells of each group from 3 independent experiments. Asterisks denote statistical significance with \( p < 0.01 \), calculated by Student's \( t \)-test. Fluorescence intensities were normalized to the values of non-TTP expressing cells.⁷⁷

NBD-tocopherol loses around 50% of its fluorescence intensity after 20 minutes of observation due to photobleaching by the excitation laser light. No fluorescent decay was observed with thienyl-ene-BODIPY in the same time span.

Our collaborators mentioned that while testing label 3 an overall similar loss of fluorescence was observed as in NBD-tocopherol secretion. It was stated that the use of higher wavelengths allowed the capturing of microscopic pictures much more easily with smaller quantities of ligand than used before.
4 Conclusion

The fluorescent tocopherol 3 has shown similar results in cell assays as the previously prepared NBD-tocopherol. No photobleaching was observed with 3 and the high quantum yield, extinction coefficient and longer absorption wavelength produced qualitatively better fluorescence micrographs, which can be seen in even low amounts of applied ligand. The synthesis of 3 was successful with a 14% overall yield from Trolox®. Currently the compound is being used for studies of tocopherol location and transport in neuronal cells and brain tissue slices, and to visualize lipid bodies in a study that uses tocopherol to treat fatty liver disease.
5 Introduction

5.1 Antioxidant function

The most studied and understood action of vitamin E is its function as an antioxidant to protect the cell membrane from oxidation, which would lead to the death of the cell. The components protected in the cell membrane are lipids that are susceptible to attack from highly reactive species such as radicals.

5.1.1 Lipid peroxidation

Molecules bearing unpaired electrons, or free radicals, are generated in the body by metabolic processes in the mitochondria or by external sources such as X-rays, pesticides (DDT), smoke, and ozone. When any type of electron rich non-radical species donates an electron to oxygen (O₂), superoxide (O₂⁻) is formed. The O₂⁻ is a short lived species and can abstract hydrogen atoms from labile sites like bis-allylic C-H bonds to form a hydrogen peroxide anion. Superoxide is one of the so-called reactive oxygen species (ROS). Superoxide is technically able to oxidize lipids, but is too polar to enter the hydrophobic membrane environment unless protonated to form HOO⁻ (pKₐ ~4.7). The reduced polarity of HOO⁻ allows diffusion into the hydrophobic parts of the membrane. Cell membranes consist of different types of lipids, of which the majority are made up of glycerophospholipids. The phospholipid structure comprises a glycerol esterified to two hydrophobic fatty acids and a negatively charged, polar, phosphate-containing head group. The mono-esterified phospholipid structure is doubly negatively charged and referred to as phosphatidic acid (PA). Other diacylglycerol phosphates are found substituted with ethanolamine, choline, serine, or inositol. The acyl chains may be either saturated or unsaturated. HOO⁻ radicals can react with polyunsaturated fatty acids (PUFAs),
containing varying numbers of *cis*-double bonds with a methylene (–CH₂–) bridges.\textsuperscript{317}  

Hydroperoxyl radicals are reactive enough to abstract the hydrogen at the methylene bridge (pKₐ: 4-5, bond-dissociation energy bis-allylic hydrogen: ~73 kcal/mol) and form a carbon centred radical, which rearranges to a stable conjugated dienyl radical (Figure 98).\textsuperscript{318} The remaining allylic radical reacts extremely quickly with oxygen (k = 10⁹M⁻¹s⁻¹) to form a peroxyl radical on the lipid chain (LOO').\textsuperscript{319} Several reactions occur after the formation of the diene peroxyl radical.\textsuperscript{320,321} Propagation of the radical chain occurs by lipid peroxyl radical abstracting a neighbouring –CH₂– bridge hydrogen atom. The LOOH rearranges after β-scission to form alkenals, hydroxyalkenals and malondialdehyde (MDA). Two dienyl radicals terminate, forming a dimer.

![Figure 98. PUFA oxidation: Initiation, propagation and termination.\textsuperscript{320}](image)

Cells contain a wide variety of unsaturated lipids. The unsaturated FA varies based on the chain length, the position, and degree of unsaturation.
PUFAs can have up to six double bonds, and the greater the degree of unsaturation the more prone the lipid is to hydrogen atom abstraction. As shown with methyl linoleate (Figure 99) several peroxide products are obtained upon autooxidation which can rearrange to different metabolites. The 9-cis, trans and 16-cis, trans isomers are the favoured products. When 5 mole % α-tocopherol was present, an almost equal distribution of all products was observed.322,323

![Figure 99. Product distribution for the peroxidation of methyl linolenate peroxide product.](image)

Vitamin E is a chain-breaking antioxidant and prevents membrane peroxidation by donating the hydrogen atom from the chroman ring phenol to the lipid peroxyl radical, forming a stable tocopheroxyl radical.

5.1.2 Kinetics of hydroperoxyl radical quenching by tocopherol

The activity of tocopherol as an antioxidant is dependent on its ability to neutralize lipid peroxyl radicals.

Antioxidant strength is often given as an inhibition constant by measurement of oxygen ($d[O_2]$) consumption during the peroxidation reaction of lipids in the presence of tocopherol, thereby
taking into account initiation, propagation and termination rates (1). The equation to determine the inhibition constant $k_{inh}$ is represented by $n$, the number of radical chains inhibited by antioxidant, $R_i$ the initiation and $k_p$ the propagation rate constant of the radical in a certain time (t).

$$\frac{d[O_2]}{dt} = \frac{k_p[RH]R_i}{(n[AH]k_{inh})}$$

Electron spin resonance (ESR) has been used to follow the different types of radicals formed during auto-oxidation of lipids. A more lab friendly method measures the UV absorption of conjugated dienes (233-235 nm) to follow reaction progress. The essential steps of tocopherol inhibition of lipid peroxidation are shown in Figure 100. The rate constant $k_1$ describes the H-atom donation (quenching) from tocopherol to the peroxyl radical. The deuterium kinetic isotope effect in an inhibitory auto-oxidation assay (KIE $\alpha$-toc: $k_1^H / k_1^D$ 5.4 ± 0.4) indicated that this was the rate-determining step.

$$k_1 \quad \text{describes the quenching of lipid peroxy radicals with tocopherol, } k_{-1} \text{ the reverse reaction, } k_2 \text{ the termination reaction of lipid peroxy- and tocopheroyl radicals and } k_3 \text{ the radical abstraction of a lipid bis-allyl hydrogen.}$$

The reverse reaction $k_{-1}$ and initiation of a new lipid radical $k_3$ were studied to determine the oxidative power of tocopherol. Radical terminations in $k_2$ are often neglected since they do not contribute to any pro-antioxidant activity (2). Various products are formed depending on which
Radicals react with each other. LOO’ / LO’ radicals (L = methyl linolate) terminate with α-tocopheroxyl radicals (TocO’) at the C-8 position of tocopherol to a lipid-peroxy / lipid oxy tocopherol (α-Toc-8a-OOL / α-Toc-8a-OL). Also observed were a phenol ether with L’. α-Toc-8a-OOL / α-Toc-8a-OL react further to form the α-Toc-para-quinone, which can even further oxidize with O₂ to epoxy tocopherols (Figure 101).³²⁷,³²⁸

![Figure 101. Oxidation products of methyl linolate and α-tocopherol.³²⁸](image)

Many researchers in the 1980s determined \( k_1 \) of tocopherol in various medias, using pulse radiolysis, chemoluminescence, or oxygen consumption assays (Table 10).³²⁹ Results depend heavily on the concentration, reaction media, pH, temperature and technique applied.³²³,³²⁴

<table>
<thead>
<tr>
<th>Peroxy radical</th>
<th>Technique</th>
<th>Temperature °C</th>
<th>( k_{\text{inh}} ) ( \text{M}^{-1} \text{s}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃COO</td>
<td>Pulse radiolysis</td>
<td></td>
<td>( 5.0 \times 10^6 )</td>
</tr>
<tr>
<td>cyclo-C₆H₅OO</td>
<td>Pulse radiolysis</td>
<td></td>
<td>( 2.3 \times 10^7 )</td>
</tr>
<tr>
<td>C₆H₆CH(CH₃)OO</td>
<td>Chemiluminescence</td>
<td>60</td>
<td>( 3.3 \times 10^6 )</td>
</tr>
<tr>
<td>X-(CH₂CH(C₆H₅)OO)ₙ</td>
<td>Oxygen consumption</td>
<td>30</td>
<td>( 2.35 \times 10^6 )</td>
</tr>
<tr>
<td>C₆H₆CH(CH₃)OO</td>
<td>Chemiluminescence</td>
<td>37</td>
<td>( 1.8 \times 10^6 )</td>
</tr>
<tr>
<td>Methyl linoleoylperoxy</td>
<td>( k_{\text{inh}} ) and ( t_{\text{inh}} )</td>
<td>37</td>
<td>( 5.1 \times 10^6 )</td>
</tr>
<tr>
<td>C₆H₆C(CH₃)₂OO</td>
<td>Oxygen consumption</td>
<td>60</td>
<td>( 2.0 \times 10^6 )</td>
</tr>
<tr>
<td>C₆H₆CH(CH₃)₂OO</td>
<td>Chemiluminescence</td>
<td>37</td>
<td>( 1.5 \times 10^6 )</td>
</tr>
<tr>
<td>Methyl linoleoylperoxy</td>
<td>Pulse radiolysis</td>
<td></td>
<td>( 8 \times 10^4 )</td>
</tr>
</tbody>
</table>

Table 10. Inhibition rates \( (k_{\text{inh}}) \) of vitamin E by different techniques.³²⁹
A reliable \( k_1 (2.35 \times 10^6 \text{ M}^{-1}\text{s}^{-1}) \) for \( \alpha \)-tocopherol was determined by Burton et al. using an inhibitory auto-oxidation assay with styrene (IAS) (3.95 M styrene in chlorobenzene at 1 atm \( \text{O}_2 \), AIBN as initiator). \(^{330}\) Styrene is not effected by \( k_3 \) or \( k_1 \) because there is no hydrogen abstraction taking place to form a \( \text{LOO}^- \), but instead polyperoxy styrene products (LOO-styrene). The insignificance of \( k_3 / k_1 \) was determined when \( \alpha \text{TocO}^- \) was reacted with the same styrene concentration, yielding a rate eight orders of magnitude lower than \( k_1 (5.04 \times 10^{-4} \text{ M}^{-1}\text{s}^{-1}) \) to \( k_1 2.35 \times 10^6 \text{ M}^{-1}\text{s}^{-1}) \).

Tocopherol can act as a pro-oxidant when TocO\(^-\) reacts with LH, assisting the overall oxidative damage of the lipid. This prooxidant activity occurs under mild oxidative conditions and when co-antioxidants vitamin C or ubiquinone are lacking. \(^{331}\)

To determine an accurate \( k_1 \) of \( \alpha \)-tocopherol are the \( k_1 \) and \( k_3 \) values are necessary.

Mukai determined with 5,7-diisopropyl-tocopherol the \( k_1 \) and \( k_3 \) on PC-OO\(^-\) and linoleate peroxyl radicals. Di-\( i \)-propyltocopheroxyl radicals do not as easily decompose (natural decay \( k_0 \) 3.2 \( \times \) \( 10^{-5} \text{ M}^{-1}\text{s}^{-1} \) in benzene at 25°C) as \( \alpha \)-tocopheroxyl radicals and allow a more accurate rate determination (1\(^{\text{st}}\) order). The \( k_1 \) was determined with alkyl hydroperoxides (\( n \)-, \( s \)-, \( t \)-butyl) against di-\( i \)-propyl-TocO\(^-\) (1.34, 2.42, 3.65 \( \times \) \( 10^{-1} \text{ M}^{-1}\text{s}^{-1} \)).

Methyl linoleate peroxide inhibiton gave a 2\(^{\text{nd}}\) order rate constant for \( \alpha \)-toc \( k_1 \) of \( 5.0 \times 10^{-1} \text{ M}^{-1}\text{s}^{-1} \). This value is a decrease in seven orders of magnitude from Burton’s result (Burtons \( k_1 \) 3.2 \( \times \) \( 10^{-1} \text{ M}^{-1}\text{s}^{-1} \)). Di-\( i \)-pro-TocO\(^-\) is not quite as reactive (\( k_1 \) \( 1.34 \times 10^{-1} \text{ M}^{-1}\text{s}^{-1} \)), but still in the same order of magnitude as \( \alpha \)-tocopherol. \(^{333}\) The second order abstraction rate \( k_3 \) of ethyl stearate, oleate (1.04 \( \times \) \( 10^{-5} \text{ M}^{-1}\text{s}^{-1} \)), linoleate (1.82 \( \times \) \( 10^{-2} \text{ M}^{-1}\text{s}^{-1} \)), linolenate (3.84 \( \times \) \( 10^{-2} \text{ M}^{-1}\text{s}^{-1} \)) and arachidonate (4.84 \( \times \) \( 10^{-2} \text{ M}^{-1}\text{s}^{-1} \)) was calculated with the diisopropyl substituted radical (Figure 105). Di-\( i \)-propyl-TocO\(^-\) decay measurements showed faster reactions with increased unsaturation of the substrate (Figure 102). Increased fatty acid concentration (LH: 25 mM, 50 mM, 75 mM 100 mM) verified a pseudo-first order dependence (\( k_{\text{obsd}} \)). \(^{334}\)
Figure 102. The decay of 5,7-diisopropyl-tocopheroxyl radical reacted with ethyl stearate, linoleate, linolenate and arachidonate observed at 417 nm in benzene at 25.0°C [Toc 0.17 mM and [LH]_o = 75.0 mM].

Tocopherol is a much better antioxidant than other phenols. Butylated hydroxy toluene (2,6-di-tert-butyl-4-methylphenol, BHT), a commercially available phenol used as a preservative in foods, cosmetics, and fuels, is often used as a lipophilic standard to compare antioxidant properties. BHT is 250-times less reactive than tocopherol and 300-times more reactive than PMC in a styrene auto-peroxidation assay. However, tetramethyl-<i>p</i>-methoxyphenol is only 30-times more active than BHT. The presence of a substituted <i>para</i>-oxygen atom greatly stabilizes the phenoxy radical. The fused ring system helps by favouring the overlap of the <i>p</i>-orbitals of both oxygen atoms with each other through the \( \pi \)-system of the ring. Geometrical analysis by X-ray diffraction revealed a 16° offset of the <i>para</i>-oxygen <i>p</i>-orbitals (Figure 103). An almost perfect alignment was achieved by reducing the ring size to a benzofuran. 2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-ol (B) is 445-times more potent than BHT.
Hydrogen abstraction measurements in various media showed that \(\alpha\)-tocopherol has the highest rate of all tocols.\(^{338}\) Decreasing the methyl substitution on the chroman ring lowers the radical stability. Reduction potentials of \(\alpha\)-, \(\beta\)-, \(\gamma\)- and \(\delta\)-tocopherol measured by Wacks indicated the higher reduction potential of \(\alpha\)-tocopherol: \((< \alpha)> +0.273, +0.343, +0.348, \) and +0.405 volts.\(^{339}\) Burton’s styrene auto-oxidation assay showed a similar trend (23.5, 16.6, 15.9, and \(6.5 \times 10^5 \text{ M}^{-1}\text{s}^{-1}\)).\(^{330}\) Membrane ratios of tocopherol to phospholipid are relatively low (1:500-3000), which makes ROS lipid peroxidation more likely than direct quenching of ROS by tocopherol. In terms of lipid oxidation, \(\text{HOO}^+\) is the only species found to damage the membrane substantially.\(^{340}\)

### 5.1.3 Quenching of vitamin E

Water-soluble reductants like vitamin C (ascorbic acid) reduce the tocopheroxyl radical back to tocopherol once it reaches the membrane surface.\(^{11}\)

Peroxyl radical quenching rates for each form of tocopherol were tested against methyl linoleate in protic solvent (Figure 104). The inhibition rate determined for \(\alpha\)-tocopherol was \(5.1 \times 10^5 \text{ M}^{-1}\text{s}^{-1}\).\(^{341}\) Vitamin C alone showed antioxidant properties in the same assay one order of magnitude slower than vitamin E \(3.2 \times 10^4 \text{ M}^{-1}\text{s}^{-1}\). Both antioxidants combined had a rate of lipid peroxide inhibition of \(4.0 \times 10^5 \text{ M}^{-1}\text{s}^{-1}\), meaning that vitamin E abstracts the peroxyl radical and vitamin C works synergistically to reduce the tocopheroxy radical. In the case of in vivo cytosolic
radicals, other antioxidants are involved in radical scavenging, enhancing the antioxidant effect of tocopherols.  

![Figure 104. Disappearance of vitamin E and vitamin C in the oxidation of methyl linoleate at 37°C in tert-butyl alcohol/methanol (3:1 by volume).](image)

\[ [LH] = 0.60 \text{ M}, [AMVN] = 0.010 \text{ M}, \text{[vitamin E]} = 0.595 \text{ mM}, \text{[vitamin C]} = 0.620 \text{ mM} \] (A) (Niki et al.).  

Ascorbate quenches the tocopheroxy radical relatively fast \(1.55 \pm 0.26 \text{ M}^{-1}\text{s}^{-1}\).  

144
5.1.4 Hydrophobic antioxidants; carotene and ubiquinol

Other antioxidants like carotenes and ubiquinol are also present in membranes and have important synergistic effects with vitamin E and ascorbic acid.\textsuperscript{343} Carotene is a tremendous quencher of singlet oxygen (O$_2^\cdot$)\textsuperscript{344}, a ROS detected as products from oxygen-consuming enzymes like myeloperoxidase \textsuperscript{345} and xanthine oxidase.\textsuperscript{346,347} O$_2^\cdot$-based lipid oxidation and its inhibition by carotene follows a 4+2 cycloaddition. Alkoxy and peroxy radicals are quenched by free radical reactions (Figure 105).\textsuperscript{348}

\textbf{Figure 105. β-carotene reaction with singlet oxygen (1O$_2$) and RO*, ROO*}.\textsuperscript{348}
Comparison of chain-breaking antioxidant functions between vitamin E and carotenes show the dependence on partial oxygen pressure (Figure 106). Carotene only exhibits similar inhibitory results at lower partial oxygen pressure (at 2% oxygen concentration; 20% as standard).\textsuperscript{349,343}

![Figure 106. Effect of oxygen concentration in peroxyradical formation in methyl linolate 18:3 (200mM) initiated by AMVN (1mM) at 37°C.

Clear symbols represent absence of β-carotene, full with (5.36µM β-carotene). Squares represent the reaction in air (20% O₂), circles 2% O₂.\textsuperscript{343}

Mitochondrial ubiquinol (CoQH\textsubscript{2}) and ubiquinone (CoQ) transport electrons from complex I and II to III as part of the respiratory chain.\textsuperscript{350} Ubiquinone is the only hydrophobic antioxidant produced in mammalian cells.\textsuperscript{351,352} To assure a continuous electron flow in mitochondria protection from ROS, CoQ is reduced by NADPH to reform CoQH\textsubscript{2}. The Constantinescu group proved that CoQH\textsubscript{2} reforms vitamin E by quenching tocopheryloxyl radicals in cells. Experimental evidence by Villalba shows that ubiquinyl radicals are quenched by ascorbic acid.\textsuperscript{353,354} The combination of vitamin E, ascorbate and CoQ assure a maximal protection against ROS, especially in oxygen rich environments like the mitochondria.

\subsection*{5.1.5 Vitamin E function and movement in membranes}
The membrane protection capability of vitamin E in living cells is heavily dependent on the membrane composition. Membranes with a low vitamin E : phospholipid ratio of 1:1000-3000 need higher recycling rate of vitamin E (Table 11).  

<table>
<thead>
<tr>
<th>Subcellular fraction</th>
<th>α-Tocopherol (mmol/mol lipid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear fraction</td>
<td>0.72 ± 0.16</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>1.07 ± 0.27</td>
</tr>
<tr>
<td>Inner membrane</td>
<td>1.27 ± 0.32</td>
</tr>
<tr>
<td>Outer membrane</td>
<td>1.14 ± 0.35</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>14.60 ± 2.33</td>
</tr>
<tr>
<td>Microsomes</td>
<td>0.52 ± 0.08</td>
</tr>
</tbody>
</table>

Table 11. Content of α-tocopherol (mmol/mol lipid) in subcellular membranes of rats fed a standard laboratory diet.

Vitamin E stabilizes lipid bilayers via Van der Waals interactions with phospholipids. Comparison between the known phospholipids showed that vitamin E has a strong association towards phosphatidylethanolamine (PE).

After phospholipids, cholesterol is the major lipid component of cell membranes (around 20-30% in plasma membranes, less in intracellular membranes) and helps to maintain its stiffness. Membrane proteins are fixed in specific places by cholesterol and moved via lipid rafts to induce protein-protein interactions and initiate cell signaling processes. Higher cholesterol content in membranes makes them more viscous / stiffer by ordering the crystalline phase, which decreases lateral diffusion and membrane permeability of electrolytes and small molecules like acetate or propionate by disordering the gel phase. Nau showed that vitamin E diffusion and mobility is affected by temperature and cholesterol percentage by monitoring fluorescence quenching in POPC membranes.

5.1.6 Position and dynamics of vitamin E in membranes

The efficiency of tocopherol quenching of free radicals depends on the location of each molecule in the membrane. Quenching is less prone the deeper the radical lies in the interior of the membrane, and more prone on the cell surface. Solid state NMR, neutron scattering, spin...
labeling, and MD-simulation experiments provide strong evidence of the location and movement of vitamin E in cell membranes. The chromanol portion lies between the hydrophilic-hydrophobic interphase around 5-10 Å deep into the membrane. This is approximately at the position of the first few methylenes from the acyl chain carboxyl end. Neutron diffraction, NMR, and UV spectroscopy experiments with all types of PUFA have concluded that in this interphase all LOO’ chain length types with an unsaturated position between carbons 5-15, and fast diffusing cytosolic HOO’ radicals are quenched.\textsuperscript{364} Hydrogen bonding between the chroman phenol and the phospholipid head groups dictates the depth, and can vary by around 5Å. This ∼5Å difference is based on the three different possibilities of how the chroman phenol hydrogen bonds to the phosphate headgroup; closer to the membrane surface by bonding only to the phospholipid oxygens (A, Figure 107), deeper when H-bonds are shared between phospholipid oxygen and ester carbonyl (B, Figure 107) and buried deepest into the bilayer when only bonding to the ester (C, Figure 107).\textsuperscript{365}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{tocopherol.png}
\caption{Position of tocopherol to the phospholipid head group.\textsuperscript{364,365}}
\end{figure}

The transverse motion (bobbing) is affected by the degree of unsaturation of the fatty acid chains. Increased unsaturation on the phospholipid fatty acids puts vitamin E deeper into the
membrane. Headgroups in membranes are rather static, whereas the carbon chains are bending in all directions. Hence, interactions between the double bonds of polyunsaturated lipids and the chroman occur, dragging the head group deeper into the membrane to the active zone where peroxyl radical quenching can occur (Figure 108). Oxidized lipids increase in polarity and move their tails towards the bilayer surface into the active zone.\(^{364}\)

![Figure 108. Location of tocopherol in membranes.\(^{364}\)](image)

\section*{5.1.7 Vitamin E membrane curvature and flip-flop}

Membrane curvature describes the geometrical stresses and shape of the membrane and is a function of the chemical composition of the membrane and associated proteins. A biological membrane consists of many distinct regions acting out specific functions, like protein anchoring. In addition, some lipids take part in signal cascades and are therefore enriched in certain cell regions. An example of the importance of membrane curvature is the BAR (Bin, Amphiphysin, Rvs) domain, binding only onto highly curved membrane parts and changing the membranes curvature.\(^{207,366}\) A more closely related example was the finding that \(\alpha\)-TTP transports NBD-tocopherol much faster to vesicles with more curvature like small unilamellar vesicles (SUV).\(^{207}\)
Distribution of lipids across the whole membrane is important for maintenance of a constant ratio of lipids on each side of the bilayer. Lipid movement across membranes in a so-called flip-flop motion is generally extremely slow and restricted in the case of phospholipids. Enzymes called flipases (inside to outside) and flopases (outside to inside) are membrane proteins responsible for a faster distribution of phospholipids. However, α-tocopherol flip-flops in 50-75 ns intervals, therefore, it easily reaches any radical on either side of the membrane (membrane thickness = ~60Å). Fast inter-leaflet transfer is necessary as oxidized membranes disturb the membrane surface, ultimately leading to perforation that allows biomolecules to leak from the cell. Research on lipid membrane restoration has shown that tocopherol is capable of assisting the repair of damaged membranes. The antioxidant action was found to be crucial for membrane restoration.

5.2 Cellular role of vitamin E

Tocopherols and tocotrienols have been found to participate several biological functions that effect cell proliferation. α-Tocopherol has been the focus of most studies because of its preferential retention in the body. Most cellular effects are traced back to the radical scavenging, but not all functions have been fully determined yet.

5.2.1 Protein kinase C and Phospholipase A₂

α-Tocopherol interacts with certain isoforms of protein kinase C (PKC). cPKCs are part of a signal transduction pathway that regulates cell growth and proliferation by phosphorylating serine and threonine residues of several proteins like rapidly accelerated fibrosarcoma protein kinase (RAF) in the RAF / RAF-1 kinase inhibitor protein (RKIP) complex (part of the mitogen-activated protein (MAP)), insulin receptor substrate 1 (IRS-1) thyrosine and nuclear factor-κB (NF-κB) (part of B-cell immune response).
Vitamin E was found to have a stimulatory effect on protein phosphatase 2A (PP2A) in endothelial cells, which leads to higher dephosphorylation of PKC and therefore inhibits growth in vascular muscle cells.\textsuperscript{374,375} PKC inhibition was confirmed in several varieties of cell types, such as monocytes\textsuperscript{376}, fibroblasts\textsuperscript{377}, neutrophils\textsuperscript{378}, and mesangial cells.\textsuperscript{379,380} Tocopherol stimulation of PP2A is not based on ligand-protein binding. Inhibitory effects of vitamin E are largely based on secondary interaction / stimulatory effects as in the case of PP2A / PKC inhibition.\textsuperscript{381}

\textbf{5.2.2 Atherosclerosis}

Vitamin E has been shown to prevent atherosclerosis in early stages. In early stages of the disease, oxidized low-density lipoproteins (LDLs) are removed by macrophages, which turn into lipid-laden foam cells that accumulate within the cell wall. Studies have revealed that Vitamin E acts as an inhibitor / regulator of several post-transcriptional processes to prevent the early stages of atherosclerosis.\textsuperscript{382,383}

1) Monocyte (macrophage) adhesion to arterial walls is prevented by downregulation of intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). These two molecules regulate the amount of monocytes produced in the body.\textsuperscript{384,385}

2) Platelet adhesion and aggregation was reduced with vitamin E in a clinical trial and the findings were that $\gamma$-tocopherol is more potent than $\alpha$-tocopherol.\textsuperscript{386} Further studies have shown that the reduction is an effect of PKC inhibition rather than its anti-oxidant function towards LDLs\textsuperscript{387}

3) Cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) are inflammatory mediators and when inhibited by tocopherols showed less monocyte activation.\textsuperscript{388}
4) Inhibition of scavenger receptor SR-BI and cluster of differentiation 36 (CD36) scavenger receptors on monocytes stops the uptake of oxidized low-density lipoproteins (oxLDL), hindering the formation of foam cells. Inhibition occurs in two ways: decrease of oxLDL levels by radical scavenging ROS that create oxLDL from LDLs and inhibition of oxLDL activated protein kinase B (PKB) / peroxisome proliferator-activated receptor gamma (PPARγ) stops the upregulation of SR-BI, SR-AI and CD36 (Figure 109).³⁸⁹,³⁹⁰

![Diagram](image)

**Figure 109. Vitamin E inhibition of LDL and oxLDL receptor mediated atherosclerosis.**³⁹¹

A detailed update on the effects of vitamin E on signal transduction is found in Zingg’s review.³⁹¹
6 Project overview

Over the last 60 years, vitamin E research has been primarily devoted to its antioxidant properties and corresponding biological functions. Vitamin E is the most potent chain breaking antioxidant that protects membrane structure, and is involved in the inhibition of inflammation, cell proliferation, and prevention of atherosclerosis.

A question has arisen in recent years whether tocopherol might protect cells through other mechanisms unrelated to its antioxidant capability. A role has been suggested for α-tocopherol affecting the transcription of genes responsible for cellular survival when under stress.392,393 Such ideas were based on research where tocopherol was linked to elevated or inhibited enzyme functions, as in the case of PKC, PP2A and COX-2.391

The idea that vitamin E has biological activities independent of antioxidant action gained more attention as the other tocopherols (β, γ, δ) and antioxidants like N-acetyl cysteine (NAC) were shown to lack the activity of α-tocopherol, despite being similar radical scavengers. Preliminary results from our collaborators have shown that transcription of the TTPA gene is upregulated by α-tocopherol but not by NAC in astrocytes under oxidative stress. Tocopherol analogues like tocopherol succinate394, tocopheryloxy acetic acid395 and tocopheryloxybutyric acid (TOB)396,397, which are not capable of acting as antioxidants, can induce apoptosis in cancer cells.

Furthermore, these tocopherol analogues cannot bind to α-TTP, meaning they are not reliable substrates to study the function of α-tocopherol in gene activation and enzyme upregulation. To shine light on this additional function we designed a tocopherol that maintains most of its
structure and interaction to \( \alpha \)-TTP, but lacks antioxidant properties. Ultimately, this molecule will be studied in dietary trials with normal and TTP-knockout mice. Knockout mice lacking TTP have chronically low concentrations of plasma and tissue vitamin E, so they eventually develop the neurological symptoms of deficiency. However, they can be recovered by large dietary doses of vitamin E. A recovery in deficient mice with a “fake” non-antioxidant form of tocopherol would mean that tocopherol’s activation of other ROS scavenging mechanisms is an important function for cell survival, even more than its antioxidant function towards lipidperoxyl radicals.\(^{398}\)

6.1 Structural design

The tocopherol structure has two parts, the chromanol and the phytol chain. The antioxidant chemistry is associated with the chromanol by H-atom donation to a lipid peroxyl radical to form the kinetically more stable tocopheroxyl radical. Any analogue that is to be a non-functional antioxidant must remove the phenol but leave the phytol chain untouched. However, certain properties must be maintained besides the lack of radical abstraction. The new molecule must be transported to cell membranes by \( \alpha \)-TTP. The molecule should also be able to mimic the position of tocopherol in a membrane. The chosen functional group cannot be too big in size, as it would not bind to \( \alpha \)-TTP. The easiest way would be to turn the phenol into the methyl ether, but binding studies with 40 (MeO-thienyl-ene-BODIPY, See Figure 90) have shown no affinity for TTP. An alternative would be a functional group with similar high polarity and hydrogen bonding ability.

6.1.1 Halogen tocopherol
One possibility is to substitute the phenol oxygen with a halogen atom. A halogen would maintain a single bond to the aromatic ring, and a similar electronegativity as an oxygen atom (Figure 110).

Halogens can act as Lewis acids (and make H-bonds) by accepting a lone pair of electrons into an electron deficient region opposite to the covalent bond of halogen, called a sigma hole. Halogen bonding distance and strength is similar to weaker H-O bonding.

Figure 110. Basic principle of halogen and hydrogen bonding to a Lewis base.

Indication of the optimal bonding angle $\alpha$. \( ^{400} \)

However, steric hindrance and binding angle ($\alpha$) have a larger influence on the bonding strength.\(^ {400} \) A perfect angle (C-X$^-$O) for a halogen bond is 180°. Calculations have shown a decrease in bond length the further the C-X$^-$O angle is away from 180° (Table 12).\(^ {401} \)

<table>
<thead>
<tr>
<th>Halogen – Oxygen (X-OH)</th>
<th>Mean: C-X$^-$O angle $\alpha$ (°) / Distance X$^-$O (Å)</th>
<th>Degrees away to 180° = % decrease in bond strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine</td>
<td>157.4° / 3.08Å</td>
<td>20° - 20-30%</td>
</tr>
<tr>
<td>Bromine</td>
<td>159.9° / 3.13Å</td>
<td>30° - 45-60%</td>
</tr>
<tr>
<td>Iodine</td>
<td>160.4° / 3.27Å</td>
<td></td>
</tr>
<tr>
<td>O-H$^-$O(H)-C</td>
<td>160.4° / 2.2-3.2Å</td>
<td></td>
</tr>
</tbody>
</table>

Table 12. Halogen bond and the influence of bonding angle $\alpha$ on bond length and strength.\(^ {400,403} \)

In recent years, researchers have recognized that halogen bonding plays an important role in ligand-protein interaction and drug design.\(^ {402} \) Amino acids having an oxygen (serine, threonine, tyrosine), sulfur (cysteine), amine (lysine) or aromatic groups (phenylalanine, trypthophan) act
as Lewis bases.\textsuperscript{403} Iodine has the largest sigma hole of all halogens because it has the least electron density distributed over the largest volume (Figure 111).\textsuperscript{404}

\[ \text{Figure 111. Calculated charge distribution of alkyl halides (top) and halogenated nucleobases (bottom).} \textsuperscript{404} \]

The sigma hole is diminishingly small in alkyl chlorides (i.e. CH\textsubscript{3}Cl) and is non-existent in the case of alkyl or aryl fluorides. However, when electrons are pulled from the halogen atom as in CF\textsubscript{3}Cl, both chlorides and fluorides are able to create significant electropositive $\sigma$-holes.\textsuperscript{405,403}

In biological systems, a halogen bonding interaction with aryl chlorides was found while studying the active site of S-$\alpha$-substituted indomethacin ethanolamides to COX-1. The inhibitors place the aryl chloride close to the Ser530, partially helping to stabilize the drug in the active side. The calculated bond distance was 3.23-3.18Å with C-Cl-O bond angles of 147.5°-140.7° (Figure 112).\textsuperscript{406}
6.1.1.1 Halogen tocopherol in α-TTP

All halide derivative of α-tocopherol would be able to fit into the active site of α-TTP, based on the covalent radius of the halides; however, a rearrangement of the static water molecules and the decreased distance to serine with increased covalent diameter will lower the overall binding (Figure 113). Hydrophobic interactions of the phytyl chain with the hydrophobic pocket might add additional stability, but generally does not contribute much to the overall binding strength. Chlorine seems to be the best choice of all halogens, since it balances the steric repulsion in the binding area with a moderate halogen bonding acceptor and donor capability. Furthermore, chlorine is less prone to oxidation by cellular ROS or iron-porphyrins.407,408,409
6.1.2 Hydroxymethyl tocopherol

Since α-tocopherol is the major lipid-soluble antioxidant in mammals, the analogue will need to maintain the same three methyl groups on the chroman ring. Maintaining a hydroxyl group would be the best solution to keep the hydrogen bonding interactions. Placing a one-carbon spacer between the oxygen and the aromatic structure forms a hydroxymethyl (HM), small in size and no longer overlapping with the π-system of the aromatic group. If H-atom donation were to occur in a manner similar to α-tocopherol, then an H-atom could be removed from either the OH of the hydroxyl group or either of the two benzylic methylenes. Hydroxyl H-O bond dissociation energies (BDE) for benzylic hydroxides are ~107.0 kcal/mol. Lipid peroxide radical abstraction by tocopherol is much lower in energy (~78.23 kcal/mol as measured for pentamethylhydroxychroman (PMHC)). The BDE of the benzylic C-H bonds are stabilized by resonance stabilization, but were calculated to be less prone to H-atom abstraction than tocopherol (~90.3 kcal/mol) (Figure 114).
Helbling’s studies of α- and γ-tocopherol binding in wild type human α-TTP showed changes in the hydrogen-bonding network between Tyr117 and Ser140 surrounding the phenol (Figure 115). A small change, such as the missing methyl group in γ-tocopherol shortens the distance between Tyr117 and Ser140 from 6.1 Å to 5.1 Å, allowing only one water molecule to bridge the two amino acid residues. The change in conformation leads to a 10-fold decrease in binding affinity from 25 nM (α-toc) to 266 nM (γ-toc).
Figure 115. Calculated hydrophilic interaction of α-tocopherol (A, top) and γ-tocopherol (B, bottom) in the wild-type α-TTP binding pocket.

The residues involved in the H-bond network (Ser140 and Tyr117) are shown as licorice representation.413

The hydroxymethyl group will most likely have the same or a slightly decreased binding affinity. This assumption is based on the shifted chroman framework, which is around 1.3-1.5 Å away from the original position (Figure 116).
6.2 Synthesis

The main structural difference between \( \alpha \)-tocopherol and the targeted 6-hydroxymethyl analogue is the oxygen at the 6-position on the chroman ring. These 6H-chromanes are commonly synthesized by cyclization reactions of phenols. Tocopherol is industrially synthesised by a cyclization reaction between iso-phytol and hydroquinones. Natural \( RRR-\alpha \)-tocopherol would be a compelling starting material, since the phytyl chain is already present with the correct \( R \)-stereochemistry at the 2\(^\prime\)-position on the chroman. Direct nucleophilic substitution at the ipso-position with chloride or a carbon nucleophile is possible when the phenol is turned into a leaving group. To enhance the reactivity phenols are bonded to an oxophylic functional group with a strong electron withdrawing effect. Bay has chlorinated diverse phenols with phenylphosphorus tetrachloride at 160°C. Schnabel turned phenols into the chloroformate esters and reacted them at 100-200°C with PPh\(_3\) in an autoclave to obtain the chlorobenzenes. Carbon-carbon bond formation to the hydroxymethyl product is not possible with the free phenol. Turning the phenol into the aryl triflate allows metal mediated cross-coupling reactions with carbonyl functional groups.
The best way to introduce the chlorine and HM at C6 of tocopherol is by electrophilic aromatic substitution from \((R)-2,5,7,8\text{-tetramethyl}-2-((4R,8R)-4,8,12\text{-trimethyltridecyl})\text{chroman (6H-tocopherol or H-Toc)}\).\textsuperscript{422,423} To do so, the phenol of \(\alpha\)-tocopherol must first be deoxygenated. Mahdavian described this exact transformation of \(\alpha\)-tocopherol H-Toc by converting the phenol to the triflate \textsuperscript{41} followed by reduction by hydrogenation with \(\text{Pd/C}\).\textsuperscript{424} Wang, Sajiki,\textsuperscript{426} and Mori\textsuperscript{427} described similar chemistry, with different sulfonates and hydride sources. After the deoxygenation chemistry H-Toc may be directly hydroxymethylated with formaldehyde or formylated by methods of Gattermann, Vielsmeyer-Hack, or Rieche reactions with subsequent reduction.\textsuperscript{428,292,429} Chlorination of H-Toc to \textsuperscript{43} could then be achieved with a number of diverse electrophilic chlorinating agents (Figure 117).\textsuperscript{430}

An alternative design uses an oxa-Diels-Alder reaction of \(4\)-hydroxymethyl-2,3,5-trimethylphenol \textsuperscript{45} with isophytol to obtain HM-Toc. Isophytol is commercially available and the aryl co-reactant can by synthesized from 2,3,5-trimethylphenol using the same formylation chemistry as described for H-Toc. The cyclization with isophytol has been described several times in the literature with 2,3,5-trimethylhydroquinone.\textsuperscript{37} Both reactions described would, however, form racemic products (Figure 118).
In the case that either of these two routes did not work, 41 could be turned into 44 by transition metal mediated carbonylation or esterification followed by reduction with LiAlH$_4$.\textsuperscript{431,421}

7 Results and Discussion

7.1 Synthesis

The first goal was the synthesis of H-Toc 42 by following Mahdavian’s route. The Diels-Alder cyclization between isophytol and 2,3,5-trimethylphenol would create H-Toc in one step, but does not maintain the $R$ stereochemistry at the 2'-position, which would decrease specific binding to $\alpha$-TTP. Therefore, the synthesis started with deprotection of acetylated $\alpha$-tocopherol (AcO-Toc) under basic conditions (Figure 119). AcO-Toc is an inexpensive, stable, non-oxidizable source of tocopherol, which can be stored long-term in a cool environment.
α-Tocopherol was then reacted with trifluoromethanesulfonic anhydride to generate the triflate 41.424 Hydrogenation of 41 with Pd/C 10% and H₂ was conducted in a Paar-shaker (15 psi). A faster reaction time is achieved when a pressurized autoclave system (Paar/Ashcroft) was used, which is capable of reaching pressures up to (60-70 psi). Running the reaction for 2-5 days ensured a high product yield (Figure 120).

Chlorination of 42 with N-chlorosuccinimide (NCS) generated product 43 in 56% yield (Figure 121).430

Hydroxymethylation of 42 was first attempted with two equivalents of paraformaldehyde and p-TsOH in EtOH. Some small new spots were observed on TLC, but even after addition of an extra nine equivalents of paraformaldehyde and refluxing at 70° for several hours, the starting material was still the major component of the reaction. Dioxane was then chosen as the solvent to increase the solubility of the starting material. After refluxing for 30 h there several new polar spots were observed on TLC. The new spots were isolated, giving product 44 in a 14% yield (Figure 122).

---

**Figure 120.** α-Toc conversion to α-tocopherol triflate 41, followed by reduction to H-Toc, 42.424

**Figure 121.** Chlorination of H-Toc 42 to Cl-Toc 43.430

**Figure 122.** Hydroxymethylation of H-Toc 42 to HM-Toc 44.
An alternative method was attempted by first formylating at the 6-position, followed by reduction. Rieche’s procedure was used, reacting H-Toe 42 with $\alpha,\alpha'$-dichloromethylmethyl ether ($\alpha,\alpha'$-Cl$_2$OMe) and titanium tetrachloride (TiCl$_4$), which produced 46 in a 95% yield.\textsuperscript{429} Scaling up the reaction (5× to a mass of 8g) lowered the yield slightly to 77% (Figure 123).

![Figure 123. Rieche formylation of H-Toe 42 to product 46.\textsuperscript{429}](image)

Reduction of aldehyde 46 was successful with NaBH$_4$, creating product 43 in a 52% yield. Reduction with LiAlH$_4$ increased the yield to 74-98% (Figure 124).

![Figure 124. Reduction of 46 to HM-Toe 44.](image)

### 7.2 Spectroscopic and chemical properties of HM-Toe

The maximum absorption of HM-Toe 44 measured by UV spectroscopy was at 278 nm & 287 nm (in EtOH), with a calculated extinction coefficient ($\varepsilon$) of 1080 cm$^{-1}$M$^{-1}$, significantly less than that for $\alpha$-tocopherol 3056 cm$^{-1}$M$^{-1}$ (Figure 125).\textsuperscript{432}
7.3 Binding studies

The new molecules were tested to see if they were capable of binding to α-TTP. A competitive binding assay against NBD-tocopherol was used to evaluate binding strength.\textsuperscript{201} α-TTP was expressed the same way as described in previously (Section 1, Binding studies to α-TTP).

In a first assay, Cl-Toc 43 was titrated to TTP following Nava’s protocol.\textsuperscript{201} To determine the ability of chlorochroman 43 to bind to α-TTP, it was tested in a competitive assay with the well-characterized NBD-Toc. In the assay, 0.4 μM α-TTP was saturated with 1.6 μM NBD-C9-Toc in SET buffer and compound 43 was added in increments as a solution in EtOH (Figure 126). No decay in fluorescence (λ\textsubscript{em} 535 nm) was observed even when 2 mM of 43 was added. α-Tocopherol addition to NBD-C9-Toc loaded α-TTP was used as a control experiment, which showed about 30% competition of the fluorophore after addition of 2 mM α-tocopherol. The

Figure 125. Absorption spectra HM-Toc in EtOH.
same extent of competition was seen in Nava’s α-tocopherol competitive assay, which used 0.2 µM α-TTP saturated with 1.0 µM NBD-C9-Toc.\textsuperscript{201}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure126.png}
\caption{Competition assay of 0.4 µM α-TTP, saturated with 1.6 µM NBD-C9-Toc in SET buffer, with α-Toc and Cl-Toc in EtOH.}
\end{figure}

The graph was normalized to 1.0. The data was collected in triplicate (Cl-Toc) and error bars represent standard deviations from the mean.

This result proved that Cl-Toc (43) does not bind to α-TTP, even at high concentrations. Therefore, no further tests were conducted with Cl-Toc.

Titration with HM-Toc 44 showed a positive result, seemingly competing for bound NBD-Toc with a similar efficiency to α-toc. HM-Toc plateaued at 1.5 mM and started to increase at higher concentration while the residual fluorescence following addition of tocopherol continued to drop (Figure 127).
Figure 127. Competition assay of 0.4 µM α-TTP, saturated with 1.6 µM NBD-C9-Toc in SET buffer, with α-Toc and HM-Toc in EtOH.

The graph was normalized to 1.0. The data was collected in triplicate (Cl-Toc) and error bars represent standard deviations from the mean.

To understand why fluorescence increase for 6-HM-Toc trials at higher concentration α-Toc was added to a solution of NBD-C9-Toc in SET buffer that lacked the α-TTP protein (Figure 128). HM-Toc increased the observed fluorescence by almost 50% at 1mM, whereas α-Toc had no such effect over the course of the titration. The increase observed is likely based on the formation of micellar structures between displaced NBD-C9-Toc and 6-HM-Toc. These structures provide a more hydrophobic environment which enhances the fluorescence of the NBD-fluorophore in aqueous media.
Figure 128. Micelle test 1.6 µM NBD-C9-Toc with HM-Toc and α-Toc in SET buffer.

The graph was normalized to 1.0. The data was collected in triplicate and error bars represent standard deviations from the mean.

A more reliable competition curve was obtained by subtracting the micellar data from the competition assay. α-Tocopherol follows the same decay trend as seen before in the Cl-Toc titration (Figure 126). HM-Toc thus appears to be an equal or marginally better competitor for α-TTP bound NBD-C9-Toc than natural α-tocopherol when the micelle data is removed from to the graph (Figure 129).

Figure 129. Competition assay of 0.4 µM α-TTP, saturated with 1.6 µM NBD-C9-Toc in SET buffer, with α-Toc and HM-Toc in EtOH adjusted with micelle data (Figure 137).

The graph was normalized to 1.0.
This result assures that the new HM-Toe binds to \( \alpha \)-TTP.

### 7.4 Oxidation assay

Lipid peroxidation inhibition is tested by measuring the formation of conjugated dienes in liposomes in the presence of a postulated inhibitor. An immediate response is expected as seen by an increase of diene absorption in a UV/VIS spectrometer, observable at 230 nm. With \( \alpha \)-tocopherol, there is a several minute delay, before the absorption at 230 nm increases (Figure 130).

![Figure 130. Expected UV absorption (230 nm) of the non-antioxidant tocopherol.](image)

Different conditions can be used to start the peroxidation. The hydrophilic radical initiator 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) and the hydrophobic initiators azobis(isobutylnitrile) (AIBN), and 2'-azobis(2,4-dimethylvaleronitrile) (AMVN), and iron(II)chloride with \( \text{H}_2\text{O}_2 \) or copper(II)chloride with or without ascorbate.\textsuperscript{433,434,435} The lipids chosen were soyPC (Figure 131) that contains a rich supply of PUFA.
7.4.1 Cyclic voltammetry studies

Cyclic voltammetry (CV) measurements on HM-Toc 44 were compared to the oxidation potential of α-tocopherol and H-Toc. Tocopherol showed two electron oxidation potentials around 1100 mV (Figure 132). The reversible wave has two potentials at 800 mV and 550 mV.

Figure 132. CV (BASI-Epsilon) oxidation of α-tocopherol (2.5 mM in dry dichloromethane) at 200 mV/s with 80 mM tetrabutylammonium hexafluorophosphate.

Ag wire was used as a reference electrode.

For HM-tocopherol the oxidation potential shifts to 1300 mV (Figure 143 HM-Toc). Similarly, the oxidation potential of H-Toc shows maxima at 1300 mV and 1700 mV (Figure 134 H-Toc).
Figure 133. CV (BASI-Epsilon) oxidation of HM-Toc (2.5 mM in dry dichloromethane) at 200 mV/s with 80 mM tetrabutylammonium hexafluorophosphate.

Ag wire was used as a reference electrode.

Figure 134. CV (BASI-Epsilon) oxidation of H-Toc (2.5 mM in dry dichloromethane) at 200 mV/s with 80 mM tetrabutylammonium hexafluorophosphate.

Ag wire was used as a reference electrode

These results indicate that, not surprisingly, the more electron rich phenols are more readily oxidized than the hydroxymethyl and unsubstituted analogues. CV studies on γ-, δ-tocopherols and α-, γ-, δ-tocotrienols by Kruger\(^1\) showed a similar oxidation potential for α-tocopherol around 1100-1200 mV. Since the oxidation is conducted solely in dichloromethane, no direct

\(^1\) Data by Nick Nick Krueger, MSc thesis, 2017
connection can be drawn to its antioxidant capability in cells, as the lipid environment influences the oxidation potential and rate. The antioxidant ability is based on the capability of donating a hydrogen atom to reactive radicals, and so the phenol should be a far better antioxidant than HM-Toc and H-Toc.\textsuperscript{436}

The higher oxidation potential for HM-toc, was expected to lower the overall antioxidant capability in a cellular environment.

### 7.4.2 Lipid peroxidation

Oxidation of soy PC lipids with the radical initiator 2,2'-azobis(2,4-dimethylvaleronitrile), AMVN, indicated that the HM-Toc 44 is not capable of inhibiting lipid peroxidation (Figure 145). α-Tocopherol efficiently inhibits the formation of conjugated dienes as observed by an increase in UV absorption at 234 nm for about the first 75 minutes of the assay, at which point it has been fully consumed and a steady increase in absorbance was observed with a slope similar to both the assay with no antioxidant and that containing 6-HM-α-Toc. The data clearly indicates that HM-Toc cannot act as an antioxidant in a simple lipid peroxidation assay.

![AMVN initiated peroxidation of soy PC phospholipids in MLVs.](image)

**Figure 135.** AMVN initiated oxidation of multilamellar SoyPC vesicle with HM-Toc & α-Toc.
7.4.3 Cellular oxidation studies

Knowing that HM-Toc can bind to α-TTP and that it is not an effective antioxidant in vitro, the compound was sent to our collaborators at Case Western Reserve University to study whether HM-Toc could rescue cells and animals (mice) that were subjected to oxidative stress. Six grams of HM-Toc were produced for this purpose. The overall yield was 50% over five steps starting from AcO-Toc.

The stability of the product was assessed, after our collaborators observed impurities in ethanol stock solutions. The compound was tested in biologically relevant solvents and the effect of different pH conditions for certain periods of time. Acidic conditions were mimicked by addition of hydrochloric acid, basic conditions with potassium carbonate (Table 13). $^1$H-NMR spectroscopy was used to follow the decay.

<table>
<thead>
<tr>
<th>Solubility</th>
<th>Acidic</th>
<th>Neutral</th>
<th>Basic</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH</td>
<td>1.2%</td>
<td>-</td>
<td>-</td>
<td>1h</td>
</tr>
<tr>
<td>EtOH</td>
<td>20%</td>
<td>-</td>
<td>-</td>
<td>30h</td>
</tr>
<tr>
<td>EtOH</td>
<td>25%</td>
<td>-</td>
<td>-</td>
<td>40h</td>
</tr>
<tr>
<td>Dioxane</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9 days</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10 days</td>
</tr>
<tr>
<td>EtOH + TKE 1:1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>30h</td>
</tr>
</tbody>
</table>

Table 13. Stability assessment of HM-Toc.

Aqueous HCl or potassium carbonate was added to the HM solutions and stored for the described time. The solvents were evaporated and extracted with water and hexane, the organic phase was then dried over sodium sulphate. $^1$H-NMR in CDCl$_3$ was used to monitor the decay. Green (-): No change was observed by $^1$H-NMR. Yellow: low quantities of byproduct (% of 47 and 48 combined) were detected. Red: large amounts off byproduct (% of 47 and 48 combined) were detected. White: conditions not tested.

Decomposition under acidic conditions was first recognized during the analysis of the synthetic HM-Toc. After reduction of aldehyde 45 with LiAlH$_4$, analysis by $^1$H-NMR spectroscopy...
indicated impurities in the product. It was discovered that the older CDCl$_3$ solvent used for NMR sample preparation was the problem. When the CDCl$_3$ was washed through a small pad of basic alumina, no byproduct was observed. An experiment was conducted whereby the HM-Toc was monitored over time. The new compound formed was the self-condensed HM-Toc ether 47. Benzylic ethyl ether 48 was formed using protic solvents like EtOH form under slightly acetic condition (Figure 136). No decay was observed when the compound was stored in basic or neutral environment.

![Figure 136. Products observed during the assessment of HM-Toc stability in acetic media.](image)

Dimer 47 in old CDCl$_3$ and 48 in EtOH.

### 7.5 Cell studies and Animal trials

As mentioned, the new ligand will be tested for their ability to rescue +/- $\alpha$-TTP knockout mice.$^{111}$ A concentration dependent cytotoxicity screen of the new ligand will be conducted in cell lines that have not been previously stressed. Any possible toxic effects by the new molecule would have to be considered in the study with deficient mice. The ligand’s ability to reinstate normal conditions will be tested first in oxidatively stressed astrocytes. In these stressed cells it will also be determined if the $ttpa$ gene has been upregulated.

$\sim$ $\alpha$-TTP knockout mice will be used to mimic the vitamin-deficient state. These mice show the first signs of ataxia after 10-12 days after birth. Three groups of $\sim$ $\alpha$-TTP knockout mice will be tested: with the new ligand in the diet (800mg ligand / kg diet), with $\alpha$-tocopherol (800mg $\alpha$-
tocopherol / kg diet) and with a α-tocopherol depleted diet. The hypothesis is that tocopherol supplementation will prevent the development of ataxia, whereas supplementation with 6-HM-Toc will not.

Preliminary work in cells has been finished, but the study is currently ongoing.

8 Conclusion

The designed non-antioxidant tocopherols Cl- and HM-Toc were successfully synthesized without major issues in moderate to high yields in each step. HM-Toc showed a similar affinity to α-TTP as natural α-tocopherol, but Cl-Toc did not bind at all to α-TTP. Cl-Toc would have been better in terms of oxidative resistance, but the binding affinity had to be maintained. Despite this, a high oxidation potential was observed for HM-Toc by cyclic voltammetry. Lipid peroxidation assays with AMVN showed that HM-Toc is not an antioxidant.

Cell and animal studies are ongoing. With the result of the current biological studies on HM-Toc activity as an antioxidant will the ongoing debate about the importance of the antioxidant function of tocopherols to protect membranes be solved.
9 Introduction

Medical research scientists have had great success in treating or even curing most life-threatening diseases, most often without inducing additional harm to the patient. But despite this tremendous costly research effort, no broad applicable cure been found for the king of all diseases, cancer. Cancer is described as a malfunctioning of cells associated with uncontrolled cell reproduction that spreads through the vascular and lymph system, leading potentially to organ and system failure, and death.437,438

The causes of cancer are diverse and challenging to uncover, and equally more so to prevent. The disease is frequently traced back to a chronic exposure to low levels of a toxin over a prolonged time. External sources like intense radiation (α, β, γ rays) or chemicals used in cigarettes (formaldehyde) or produced during combustion, pesticides (dioxines), industrial solvents (benzene) and mining (heavy metals like chromium) have all been shown in many studies to induce cancer. DNA and RNA viruses, specifically Epstein-Baar, hepatitis B, human papilloma viruses (DNA) and hepatitis C, human T lymphotrophic virus type 1 (RNA), have been shown to induce cancer.439 Lowering the possible exposure to harmful sources can reduce the risk, but nobody is guaranteed a cancer-free life. Life with cancer is like a game of roulette. You can be lucky by having a non-malignant tumor, which is operable or is diagnosed early enough to be treatable without major side effects. People with prostate cancer can live up to several decades without any returned symptoms (in the USA) after successful treatment. And yet other cancers guarantee a > 90% mortality, as it is for pancreatic cancer (Table 137).438,440
Figure 137. Relative survival of cancer over a 5-year span in the USA.

Comparison between data collected from 1975-1977 to 2006-2012 with a high confidence interval. The change over time between the collected data is given as an absolute and proportional percentage. NOS: not otherwise specified. 440

In cancer your own cells start to work against you. The close similarity of cancerous cells to normal cells in the early stages of the disease lowers the chance of detection. Further, cancer cells differ from patient to patient, as the cause of uncontrolled growth varies. 441

To kill malignant cells cytotoxic drugs or radiation are frequently used and almost all cancer types have shown susceptibility to a certain degree. The drawback with these treatments is the uniform damage across all tissue, especially in the near environment around the cancer. It is often the case that the patient suffers worse health effects as a direct effect to the treatment. Side effects include weakness, fatigue, loss of appetite, loss of hair and a weakened immune system that leaves the patient vulnerable to infection. 442 A guaranteed cure is rarely possible. Even if the cancerous solid tumour has been reduced in size, or blood-borne tumours reduced to undetectable levels, there remains the possibility that surviving cancer cells will continue to grow. It is often the case that such relapsed cases show a higher resistance to the chemotherapeutic agent used in the first round of treatment. 443 More indepth research to
increase cancer cell specific treatment is crucial to patient survival and well-being. Treatment of malignant tissue is first and foremost dependent on its localization in the body.

9.1 Positron emission tomography (PET)

Nuclear magnetic imaging techniques are used to monitor and visualize the physiological functioning of tissues and cells with low risk to the patient. Positron emission tomography (PET) is a nuclear imaging technique which relies on the decay of radionuclides. The decay of the nuclei chosen results in the emission of a positively charged electron ($e^+$, $\beta^+$), called positron, and a neutrino ($\nu$), which both are produced during the conversion of a proton into a neutron (1). Energy of at least 1.02 MeV between parent and daughter nuclei is necessary for a nucleus to undergo $\beta^+$ decay. If the energy is below 1.02 MeV, $\beta^+$ decay is forbidden and an electron of the inner shells ($k$ or $l_1$ shell) reacting with the excess proton in a process called electron capture (2). Thereby an electron-hole is formed in the $k(l_1)$ shell after neutron formation. Two specific types of electronic transitions can occur to fill the empty electron-hole (Figure 138). An electron from an outer shell fills the electron-hole of the inner shell ($l_1$ to $k$ transition), with emission of an x-ray photon, leaving a positively charged atom behind. The x-ray emitted has a distinct wavelength, depending on which shell the electron has transitioned from. This process is better known as x-ray fluorescence. Alternatively the transition energy is transferred to an electron in a higher shell ($l_x,m,n$ etc.), which is then ejected from an atom. This specific decay process is also known as Auger-Meitner effect and the particles emitted are so-called Auger electrons. An example of 100% electron capture is the decay of $^{83}$Rb to $^{83}$Kr, emitting an energy of 0.9MeV.\footnote{1}

\[
\frac{4}{2}X \overset{>1.02\text{MeV}}{\longrightarrow} A+\frac{1}{2}Y + \frac{0}{1}e + \nu
\]
A positron (e\(^+\)) travels some millimeters in tissue losing most of its energy before reacting with an electron (e\(^-\)) in an event called electron–positron annihilation, which results in the perpendicular emission of two gamma ray photons. These two photons travel with an energy of 551 keV \((1.02 \text{ MeV} : 2 = 511 \text{ keV})\) and are recognized by a circular detector around the patient (Figure 139). This type of data collection is defined as coincidence counting and allows for an
emitted pair of $\gamma$-photons to record the position of positron annihilation. Because the annihilation radiation is high in energy are most of the photons are detected. Despite the small offset in electron–positron annihilation from the original decay, scattering effects of photons and photon annihilation on tissue provides on average a resolution of 3-7 mm with PET.\textsuperscript{445}

**Figure 139. Positron annihilation, formation of two $\gamma$-photons each having an energy of 511keV.\textsuperscript{450}**

The PET marker can be an analog of a molecule of interest bearing within its structure radionuclides such as $^{15}$O, $^{13}$N, $^{11}$C and $^{18}$F, suitable for acquiring in vivo biochemical and physiological information about a tissue or an organ.\textsuperscript{445} The nuclei vary in their half lives ($t_\frac{1}{2}$), from 2 min up to 109 min (3-6). These PET agents have a high $\beta^+$ transition probability all above 97\% (C, O and N have a 100\% probability).\textsuperscript{445}

\begin{align*}
\text{3} & \quad t_{\frac{1}{2}} 20.3 \text{ min} \\
^{11}C \rightarrow & \quad ^{11}B + ^0_{+1}e \\
\text{4} & \quad t_{\frac{1}{2}} 2 \text{ min} \\
^5_{^{15}}O \rightarrow & \quad ^7_{^{15}}N + ^0_{+1}e \\
\text{5} & \quad t_{\frac{1}{2}} 10 \text{ min} \\
^7_{^{13}}N \rightarrow & \quad ^6_{^{13}}C + ^0_{+1}e \\
\text{6} & \quad t_{\frac{1}{2}} 109.7 \text{ min} \\
^9_{^{18}}F \rightarrow & \quad ^8_{^{18}}O + ^0_{+1}e
\end{align*}
The half-life states describes the loss of half a radionuclides initial activity in a certain time and creates for each radionuclei its own decay constant ($\lambda$) (7).

$$\lambda = \frac{0.693}{\frac{t_1}{2}}$$

The rate of decay, called activity ($A$), is needed to calculate the numbers of nuclei ($N$) left after the initial time (8). The Si-unit is the Bequerel (Bq) and describes the number of decays per second.

$$A = \lambda N$$

To determine the amounts of radionuclei and radioactivity the activity after a certain time ($A_t$) is calculated by plotting the initial activity ($A_0$) against time ($t$) (9).

$$A_t = A_0e^{-\lambda t}$$

Radioactive exposure has to be kept to a minimum in a patient to minimize radiation damage in cells. When looking at living organisms radiation is measured as a response to a biological effect. The SI-unit gray (Gy) describes the deposition of a joule of radiation in a kilogram (1 J/kg) of matter (tissue). When an imminent response to large amounts of radiation occurs the gray unit is used to describe the health effects from the absorbed dose. In cases of small amounts of radiation, as it is with PET scans, a so-called radiation weighting factor ($W_r$) is used to scale the different radiation ($\alpha$-, $\beta$-, $\gamma$-, p-, n-particles) in terms of their impact on the biological tissue and is described by the SI-unit Sievert (Table 14).

<table>
<thead>
<tr>
<th>Type of radiation</th>
<th>$W_r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-rays, $\gamma$ rays, $\beta$ particles</td>
<td>1.0</td>
</tr>
<tr>
<td>Neutrons and protons</td>
<td>10.0</td>
</tr>
<tr>
<td>$\alpha$ particles</td>
<td>20.0</td>
</tr>
<tr>
<td>Heavy ions</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Table 14. Radiation weight factor ($W_r$) of different radiation types.
Sieverts describes the equivalent dose absorbed by a tissue (\(H_T\) in Sv) and therefore depends on the type of the radiation weighting factor \(W_R\) and the amounts of a specific radiation a certain tissue can absorb (\(D_{T,r}\) in grays) \(^{10}\).\(^{446,447}

\[
H_T = \sum_R W_R D_{T,r}
\]

Since PET scans are directly injected into the body the long-term exposure of the effective dose per organ / body is calculated (\(H_E\)), as certain organs cover a larger area in the body. The tissue weigh factor (\(W_T\)) determines the biological susceptibility of the tissue to a certain type of radiation (Table 15).\(^{446,447}

\[
H_E = \sum_T W_T H_T
\]

\begin{tabular}{|l|c|}
\hline
Tissue & \(W_T^a\) \\
\hline
Gonads & 0.25 \\
Breast & 0.15 \\
Red bone marrow & 0.12 \\
Lungs & 0.12 \\
Thyroid & 0.03 \\
Bone surfaces & 0.03 \\
Remainder & 0.30 \\
Total body & 1.00 \\
\hline
\end{tabular}

Table 15. Tissue weigh factor (\(W_T\)).

(a) \(^{10}\text{th Code of federal regulation page 20.}\)^{447}

Radiation exposure is tightly regulated, as high doses induce chronic health problems like cancer. In the US each person receives a dose of 3.1 mSv as background radiation, from radioactive gases like radon (\(^{222}\text{Ra}\)) and cosmic / terrestrial radiation. Doses of \(> 500\) mSv are known to induce cancer.\(^{448}

9.1.1 Clinical PET Tracer
Most PET markers have $^{18}\text{F}$ incorporated in their structure because it has the longest half-life of all relevant second period $\beta^+$ emitters. In a recent computer tomography (CT)/PET scan study the effective dose of $^{18}\text{F}$-FDG and $^{18}\text{F}$-L-DOPA were tested.\textsuperscript{449} CT scans are run with PET scans to create a background image of the patient, which is overlayed with the PET image to identify metabolic hotspots with higher precision. $^{18}\text{F}$-FDG is a glucose analog containing a $^{18}\text{F}$ at the C-2 position and is used as a standard metabolic tracker to detect cancer.\textsuperscript{450,451} $^{18}\text{F}$ L-DOPA is used to study neurological diseases like Parkinson’s disease.\textsuperscript{452}

The study focused on the dose in the whole body (60kg) and the brain in 300 patients. PET scans apply around 1.6 MBq/kg body weight, 2x for CT/PET. An $^{18}\text{F}$-FDG dose of 370 MBq was the effective dose 6.5-18 mSv (PET only, tube current 100-350 mA). CT scans add around 50% of mSv to the PET imaging. The overall exposure was around 12-36 mSv per scan and 4-12 times the natural dose exposed per year. However, the fast clearance of the PET agents form the body lowers the risk of cancer development to a minimum.\textsuperscript{453}

Many different $^{18}\text{F}$ based PET agents like $^{18}\text{F}$-Flucicolovine and $^{18}\text{F}$-Fluoroethyltyrosine are used to detect various cancer types. $^{18}\text{F}$-containing molecules like $^{18}\text{F}$-Flutemetamol or $^{18}\text{F}$-Cyclofox have clinical significance in the study of neurological diseases like addictions and Alzheimer’s and Parkinson’s disease (Figure 140).\textsuperscript{454,455,456,457}
9.1.2 Single-photon emission computed tomography SPECT

The closely related technique called single-photon emission computed tomography (SPECT) has in comparison to PET a lower resolution of 7-12 mm. SPECT’s disadvantage is the annihilation of only one, weaker photon upon nucleide decay. To detect the photon one or several large scintillation cameras (0.5 m-0.5 m diameter) are moved 180° around the patient in an orbital or body-contour orbit around the patient. Movement of the detector is often restricted to 180° as 360° lowers the resolution, which compared to PET offers less sides of detection. The main problem is the use of collimators in the scintillation cameras, lowering the total amount of radiation detected to a minimum.
The nuclei commonly used in SPECT are $^{123}$I and $^{99m}$Tc for myocardial perfusion, brain or bone scans (Figure 141). Both nuclei have energetically lower $\gamma$-photons emitted, which leads to increased small-angle photon scattering and therefore a smaller signal to noise ratio ($^{123}$I = 159 keV, $^{99m}$Tc = 98.6% 140.5 keV, 1.4% 142.6 keV). A larger amount of SPECT agent is required to obtain a qualitatively good image. Patients with thyroid cancer obtain doses of 5.5-7.4 GBq of $^{123}$I-imaging agents and up to 29.5 GBq in repeated treatments. SPECT imaging is more often used to study longer lasting events in the brain and heart.\footnote{445}

\begin{itemize}
  \item \textbf{123-iodine}
  \begin{align*}
    \text{Decay:} \quad & ^{123}_{53}\text{I} \quad \rightarrow \quad ^{123}_{52}\text{Te} + \quad \text{E.C.} \\
    \text{Iobenguane - Neuroblastoma detection}
  \end{align*}

  \begin{align*}
    \text{Decay:} \quad & ^{99}_{43}\text{Tc} \quad \rightarrow \quad ^{99}_{43}\text{Tc} + \quad \text{$\gamma$-photons: 141keV} \\
    \text{Sestamibi - Myocardium imaging (heart muscle)}
  \end{align*}

  \begin{align*}
    \text{Decay:} \quad & ^{99}_{44}\text{Ru} \quad \rightarrow \quad \beta^{-} \quad \text{249keV} \\
    \text{Ceretec - Inflammation - Stroke}
  \end{align*}

\end{itemize}

Figure 141. SPECT imaging agents based on $^{123}$I (Iobenguane, Ioflupane) and $^{99m}$Tc (Sestamibi, Ceretec) and the nuclei decay times and emitted particle energies.
9.1.3 $^{18}$F radio-nuclide synthesis

Radionuclides for positron emission tomography are synthesised in cyclotrons. Cyclotrons accelerate charged particles like protons, deuterons and alpha-particles in an outwards spiralling trajectory. Two circular electromagnets create a perpendicular magnetic field holding the particles in a planar space. The charged particles are added in the middle of a metal vacuum chamber and the magnetic field induces circular motion perpendicular to the magnetic field. To accelerate the particles two semi-circular, hollow, D-shaped, oscillating high-frequency generating electrodes (dees) are used, which surround the particles. In between the semi-circles is a small gap, which is needed for particles to break the circular motion in an outwards spiralling motion (acceleration gap). In each turn passing the acceleration gap the electrodes are switched to the same pole as the particle, creating static repulsion. By increasing the frequency of the electrode, each passing turn the charged particle is accelerated. At the furthest point of the circle the particle has the necessary energy to induce a nuclear reaction and is ejected into a reaction chamber (Figure 142).

Figure 142. Particle acceleration in a cyclotron.
Particles increase in velocity in a spiral motion perpendicular to an electromagnetic field with the use of static repulsion induced by oscillation of two D-shaped electrodes.\(^{459}\)

The centripetal force \(F_C\) required to maintain a particle in the spiral path depends on the mass of the particle \(m\), its velocity \(v\) and the radius \(r\) from the center of the chamber \((12)^{445,447}\):

\[
F_C = \frac{mv^2}{r}
\]

The magnetic field strength \(F_B\) sets the limit for the maximum possible energy reached. The Lorentz force describes the force of a moving \(v\) charged particle \(q\) in a magnetic field \(B\) \((13)^{14}\):

\[
F_B = qvB
\]

The energy of the particle depends on the mass \(m\) and charge \(q\) of the particle, the particles velocity \(v\) upon exit of the circle, the radius \(R\) of the circle before exiting the acceleration chamber and the magnetic field \(B\) \((14)^{445,447}\):

\[
E = \frac{1}{2}mv^2 = \frac{q^2B^2R^2}{2m}
\]

A median energy difference of > 1.02 MeV is necessary for a nuclei to undergo \(\beta^+\) decay. The more energy is inserted into the nucleus the more likely is positron decay. In the case of \(^{18}\)F the maximum energy possible in the nuclei is 1.655 MeV, resulting in an energy loss upon \(\beta^+\) transition of 0.634 MeV. On average the \(\beta^+\) particle has an energy of 250 keV.\(^{460}\) The activity \((A)\) of a daughter nuclei can be increased by longer bombardment or increasing the energy of the charged particle and the intensity \((I)\) of irradiation \((15)\). A quantitative calculation can be used to figure out the activity achieved, by knowing the intensity \((I)\) of the particle beam (nr of particles/cm\(^2\) s), the number of targeted nuclei \((n)\), formation cross section in cm\(^2\) \((\sigma\), given in barn, were 1 barn = \(10^{-12}\) cm\(^2\)), decay constant \((\lambda)\) and the bombardment time \((t)\). The unit of
activity is given in MBq/mAh, were mAh describes the intensity used of the beam (1 Ampere (A) = 1 Coulumb (C/s). 1 C = $6.25 \times 10^{18}$ protons). \textsuperscript{447}

$$A = In\sigma (1 - e^{-\lambda t})$$

The activity is more commonly expressed as the specific activity (SA), which describes the radioactivity per mass (m) of a radioactive nucleotide (MBq/mg) (16). Often the unit is expressed as MBq/mole or mCi/µmol.

$$SA = \frac{A}{m} = \frac{\lambda N}{m}$$

9.1.4 \textsuperscript{18}F nuclide synthesis

Different ways are used to produce radioactive fluorine, either as fluoride ions \textsuperscript{18}F\textsuperscript{-} or as fluorine gas \textsuperscript{18}F\textsubscript{2}. \textsuperscript{18}F\textsuperscript{-} is either synthesized by bombardment of H\textsubscript{2}\textsuperscript{18}O with a proton (p) or alternatively by H\textsubscript{2}\textsuperscript{16}O reaction with helium-3 ion (\textsuperscript{3}He) (Table 16).\textsuperscript{447}

Of all these reactions is the synthesis of \textsuperscript{18}F\textsuperscript{-} fluoride ions by \textsuperscript{18}O bombardment with protons yielding the highest activity. Cyclotrons fire protons with energies of 11-20 MeV with a current of 20-60 mA, yielding in 1-2 h of 37-370 GBq (1-10 Ci) of \textsuperscript{18}F\textsuperscript{-}. The reaction with \textsuperscript{16}H\textsubscript{2}O yields around 70 mCi in 30 min (25 µA) when irradiated with 22 MeV \textsuperscript{3}He ions.\textsuperscript{461} Synthesized aqueous fluoride ions are bound to metal ions like caesium (CsF) or potassium (KF) present in the reactor for nucleophilic fluorinations in non-aqueous media.\textsuperscript{447,462}

Fluorine gas is produced by bombardment of neon-20 with a deuteron, splitting off a \textsuperscript{3}He or reaction of \textsuperscript{18}O\textsubscript{2} with a proton.\textsuperscript{445,447} Synthetic protocols for \textsuperscript{18}F\textsubscript{2} production by \textsuperscript{20}Ne bombardment with deuterons (60 MeV) yield after 2.5 h an activity of 0.125 GBq (3.4 mCi), around half the dose normally applied for PET scans.\textsuperscript{463} Synthesis from \textsuperscript{18}O\textsubscript{2} (10 MeV, 30 mA)
yields 0.117 GBq (0.7 Ci) of F₂ gas. The isolated ¹⁸F gas is used further as a 1% gas mixture in neon or krypton, having a 0.1-1% of ¹⁸F₂ to ¹⁹F₂ present.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Nucleophilic fluoride (F)</th>
<th>Electrophilic fluorine (F₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target</td>
<td>¹⁸O(p,n) ¹⁸F</td>
<td>¹⁰O(³He,p) ¹⁸F</td>
</tr>
<tr>
<td></td>
<td>¹⁸H₂O</td>
<td>¹⁶H₂O</td>
</tr>
<tr>
<td>Products</td>
<td>¹⁸F₂₀ (CsF, KF)</td>
<td>[¹⁸F]F₂</td>
</tr>
<tr>
<td>Activity</td>
<td>1-10Ci</td>
<td>70mCi</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6mCi/µAh)⁴⁶¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.4mCi</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7Ci</td>
</tr>
</tbody>
</table>

Table 16. Nucleophilic and electrophilic ¹⁸F radio nucleotide synthesis.⁴⁶¹-⁴⁶⁴

Production of PET imaging agents like ¹⁸F is a so-called carrier free process, which means that the newly synthezised daughter nuclei (¹⁸F) differs chemically from the parent nuclei (¹⁸O).⁴⁴⁵,⁴⁶⁵

9.2 ¹⁸F-Fluorination

Introduction of ¹⁸F fluorine into a molecule is possible in three ways; nucleophilic, electrophilic, and radical fluorination.

9.2.1 Nucleophilic fluorination

9.2.1.1 Fluoride properties in organic reactions

Fluoride is found naturally in minerals, the most important source being calciumfluoride (CaF₂). In terms of a fluorination chemistry alkali metal fluorides are the preferred fluoride salts because of their increased reactivity due to a lower lattice energy than CaF₂ (CsF>RbF>KF>>NaF>>LiF). Caesium and rubidium fluoride are the most reactive of these alkali metal salts, but because of their lower abundance are more expensive. Sodium fluoride is the cheapest of all, but barely reactive as a nucleophile. On an industrial scale potassium fluoride is the most suitable salt, as it balances reactivity with expense in the best way.⁴⁶⁶ A large difference is seen between sodium and potassium fluoride reactivity in fluorination...
Sodium fluoride has a higher lattice energy and lower solvation energy. Consequently, sodium fluoride has a lower solubility. Compared to the other halogens the lattice energies of alkali fluorides are much higher. The solubility of fluoride salts increases with increasing atomic radius of the alkali metal, because of higher solvation energy. The other halogens (chloride, bromide, iodide) differ in terms of solubility as no linear increase is seen with increasing atomic size of the alkali metal (Table 17). The solvation of fluoride salts decreases the more hydrophobic the solvent. Reaction with organic substrates are generally run in polar aprotic solvents like acetonitrile, dimethylformamide and dimethylsulfoxide to balance the solubility between reactant and salt. Fluorides have a high tendency to react as a base, which has been applied in aldol-, alkylation-, elimination-, cyclization- and oxidation reactions.

<table>
<thead>
<tr>
<th>Solubility in MeOH (g/100ml)</th>
<th>Fluoride</th>
<th>Chloride</th>
<th>Bromide</th>
<th>Iodine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium</td>
<td>0.0024</td>
<td>41.8</td>
<td>120</td>
<td>298</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.020</td>
<td>1.38</td>
<td>16.8</td>
<td>79.4</td>
</tr>
<tr>
<td>Potassium</td>
<td>10.3</td>
<td>0.54</td>
<td>2.15</td>
<td>16</td>
</tr>
<tr>
<td>Rubidium</td>
<td>69.7</td>
<td>1.36</td>
<td>2.48</td>
<td>10.8</td>
</tr>
<tr>
<td>Caesium</td>
<td>152</td>
<td>3.26</td>
<td>2.12</td>
<td>3.45</td>
</tr>
</tbody>
</table>

Table 17. Solubility of alkali halides in methanol.

To increase the nucleophilicity of fluorides, phase transfer catalysts are used to break the strong ionic fluoride-metal interactions and distribute them better into the organic media. Tetralkylammonium salts are able to disrupt the metal salts lattice and bind the fluoride ion to the positively charged nitrogen center. The alkyl chain lengths of these salts vary, but ethyl and butyl have been shown to be the most effective in radiochemical fluorinations. To bind the fluoride to these alkyl ammonium salts another weakly basic ion must first be displaced, commonly bicarbonates are the ion of choice. The drawback of ammonium salts is their high
hygroscopic nature and low thermal stability (100°C <). Another way to increase fluoride nucleophilicity is the cageing of the metal ion in between cyclic polyethers, leaving the fluoride ion “naked” in solution with. Such cyclic polyethers are called crown ethers and are available in different ring sizes. 18-Crown-6 is the most commonly used crown ether and has a strong affinity for potassium and caesium metal ions. Wynn tested the effect of crown ethers capable of solvating fluoride ions in different organic media. The solubility of KF and CsF increased by at least 10-fold in acetonitrile and dimethylformamide (DMF) (Table 18). Polycyclic diaza polyether analogues of crown ethers, known as cryptands, are better in caging metal ions. The most popular analogue used in radiochemistry is cryptand 2.2.2 (Kryptofix® 2.2.2), having a high affinity for potassium over the other ions. The only drawback of crown ethers and cryptands is their toxicity, as they are able to assist the diffusion of various cations across lipid membranes (ionotropes), disrupting membrane potentials in cells.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Solvent</th>
<th>18-Crown-6</th>
<th>Solubility 25°C (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium fluoride</td>
<td>Acetonitrile</td>
<td>yes</td>
<td>0.029</td>
</tr>
<tr>
<td>Dimethylformamide</td>
<td>yes</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td>Potassium fluoride</td>
<td>Acetonitrile</td>
<td>yes</td>
<td>0.031</td>
</tr>
<tr>
<td>Dimethylformamide</td>
<td></td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>

| 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane |

Cryptand 2.2.2
Table 18. Solubility of sodium-, potassium- and caesium fluoride in acetonitrile and dimethylformamide with and without 18-Crown-6.471

<table>
<thead>
<tr>
<th></th>
<th>yes</th>
<th>1.57</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caesium fluoride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>yes</td>
<td>0.25</td>
</tr>
<tr>
<td>Dimethylformamide</td>
<td>yes</td>
<td>3.50</td>
</tr>
<tr>
<td></td>
<td>yes</td>
<td>5.59</td>
</tr>
</tbody>
</table>

9.2.2 Aromatic 18F-fluorination

9.2.2.1 Halex reaction

Historically the fluorination of aromatic compounds was restricted to electron deficient aromatic compounds via nucleophilic aromatic fluorination. The reaction precedes thereby through a Meisenheimer complex (MHC) (Figure 143).475

Figure 143. Halex reaction, formation of a Meisenheimer complex (MHC).475

This process is better known as the Halex (halogen exchange) reaction, because the main functional groups exchanged were other halides.476 However, quartery ammonium salts,477 nitro-478 and t-butyl groups have been used as leaving groups. Heteroaromatics are fluorinated in good yields, which allows the easy labeling of derivatized nucleotides or natural building blocks like nicotinamide.479,480 The Halex process is still used in 18F chemistry, especially when 18F is not introduced in late state fluorination, but rather to synthesize aromatic linkers. Synthesis of 18F-fluoro-aldehydes, esters or nitriles use the Halex process, which are then linked to other molecules like amines or alcohols (Figure 144).481,482
Figure 144. Halex fluorination of electron deficient aromatics.

(a) Halogen exchange on aryl cyanides. (b) Exchange of nitro groups on aromatic aldehydes (c) Exchange of quartery ammonium salts on aromatic aldehydes.477 Halogen exchange on heterocycles: (d) fluorination of 3-iodotriazole-5-methyluridine and (e) methyl-2,6-dichloronicotinate.476-479

9.2.2.2 Balz-Schiemann and Wallach reaction

The earliest nucleophilic fluorinations on electron rich aromatic were possible by using diazonium piperidines (Wallach reaction 1886) and diazonium salts (Balz-Schiemann 1927). Heating induced decomposition of the diazonium species and creates a positive charge on the aromatic ring, which is quenched by a fluoride ion.483

The Balz-Schiemann reaction is a variation of the Sandmeyer reaction, using boron tetrafluoride (BF₄⁻) as a counter ion. Only one fluoride of the BF₄⁻ is added to the aromatic ring, hence the chance of ¹⁸F incorporation is a maximum of 25%. Molecules like ¹⁸F-L-DOPA, ¹⁸F-tryptophan and ¹⁸F-peridone, that are used to study dopamine receptors in the brain, were synthesized in
way (Figure 145).\textsuperscript{484,485,486}

(a) Balz-Schiemann reaction of benzene,\textsuperscript{483} (b) 5-\textsuperscript{18}F-L-DOPA\textsuperscript{484} and (c) \textsuperscript{18}F-Haloperidol synthesis.\textsuperscript{485}

The Wallach reaction uses triazenes with a piperidine as a terminal group and, as with the Balz-Schiemann reaction, is a positive charge formed upon triazene decomposition (Figure 146). Despite having an equimolar ratio of fluorine to starting material the isolated products of radiochemical Wallach reactions have low radiochemical yields.\textsuperscript{487}

9.2.2.3 Fluorination of diaryliodonium salts

As PET agents gained more attention in medical imaging there has been an increased demand for new, high yielding radiofluorination methods of electron rich aromatic compounds. A way to lower the electron density on the aromatic ring is by bonding to a positively charged atom.
However, prior work with diazonium salts has shown this approach to be impractical for $^{18}$F fluorination, as generally only small amounts of radiochemical product was obtained. Instead, hypervalent iodonium and sulphur salts were used in the form of diaryliodonium salts and sulphur triaryl iodonium salts (Figure 147). Both have been successfully used in radiochemical fluorinations.\textsuperscript{488,489}

![Figure 147. Radio-fluorination of diaryliodonium- and triarylsulphonium salts.\textsuperscript{488,489}]

Iodonium salts are synthesized by the reaction of oxygen or halide hypervalent iodine species with aromatics. Reactions are mediated in acetic media, which also provides the counter ion of the iodonium salt formed (Figure 148).\textsuperscript{489}

![Figure 148. Formation of diaryliodonium salts by reaction of aromatics with aromatic hypervalent iodines.\textsuperscript{489}]

Fluorination of aryl iodonium salt occurs on both aryl groups. Electron poor aromatics are more prone to be fluorinated over electron rich aromatics. This electronic effect allows even products with a more electron rich nature to be fluorinated, by having a strong electron donating counter aromatic group (auxiliary) also substituted on the ring. Radiotracers like $^{18}$F-flumazenil were successfully synthezised by nucleophilic fluorination with this method (Figure 149).\textsuperscript{489,490}
Electron rich aromatic groups (auxiliary aromatic) forces the fluorination onto of the other aromatic.\textsuperscript{489}

(b) Synthesis of $^{18}$F-flumazenil from a diaryliodonium salt.\textsuperscript{490}

9.2.2.4 Transition metal mediated $^{18}$F fluorination

Transition metal mediated reactions have revolutionized the functionalization of aromatics and allowed the synthesis of many previously inaccessible compounds. Fluorination of aromatics with transition metals has been used to incorporate $^{19}$F-fluoride with palladium,\textsuperscript{491} ruthenium,\textsuperscript{492} and copper.\textsuperscript{493} However, the use in radiofluorination with transition metals has been limited because of long reaction times. In recent years, researchers like Ritter\textsuperscript{494,495} and Sanfort\textsuperscript{496} have improved the fluorination reactions with palladium, nickel, and copper and demonstrated the use with $^{18}$F. The benefit of metal catalyzed $^{18}$F-fluorination is the use of simple aryl starting materials compared to diaryliodonium salts (Figure 150).
9.2.2.5 Deoxy fluorination of phenols

Functional groups like nitro, halogens, t-butyl, ammonium and diazonium salts on electron deficient aromatic rings are exchanged with fluorine by nucleophilic fluorination. However, phenols are often neglected as addition of fluoride creates a non-exchangeable leaving group in the form of a phenolate. Phenols are common starting materials found in high abundance in nature, making them attractive building blocks for many synthetic applications. To enhance leaving group characteristics of phenols electron withdrawing sulfonates have been linked to the
phenol. Heating in the presence of fluoride makes deoxygenative fluorination of aromatic sulfonates possible. This method has so far not been used in radiochemical late-stage fluorinations as they have long reaction times and are low yielding (Figure 151).

![Figure 151. Nucleophilic fluorination of aromatic sulfonates.](image)

In recent years several groups have looked into the deoxygenative fluorination, most notably Buchwalds, and Ritters. Buchwald described a palladium-mediated fluorination of aryl triflates with phosphine ligands, which has been tested with $^{18}$F-fluoride. Ritter found that N-heterocyclic carbenes could be used to exchange phenols with fluorines in good yields. The reagent developed, called Phenofluor®, has proven to be tolerant towards many functional groups, fluorinating electron rich phenols and was found to work well as a radiofluorinating agent (Figure 152).

![Figure 152. (a) Palladium mediated fluorination of aryl triflates. (b) Deoxyfluorination with Phenofluor®.](image)

9.2.3 Electrophilic / radical fluorination

199
9.2.3.1 Electrophilic fluoride reagents

Because of its high electronegativity, fluorine is not prone to react as an electrophile and therefore no electrophilic fluorine source is found in nature. Fluorine gas (F₂) was the first chemical used as an electrophilic fluorinating agent, synthesized by electrolysis of potassium bifluoride KHF₂ in HF. However, the applications were limited in organic chemistry since F₂ is a strong oxidizer, tending to oxidize most functional groups. Milder reagents were soon developed by binding fluorine to a strongly electron withdrawing leaving groups. Halogen and oxygen based withdrawing groups were the first reagents produced in the form of peroxychlorides, fluoroxysulfates and hypofluorites.¹⁰⁰

These reagents were easier to handle, but still strong oxidizers, extremely sensitive to moisture and needed to be used after in situ creation. Xenon difluoride was developed as a mild, stable alternative, but is still a strong oxidizer and expensive to create.¹⁰¹ Binding fluorine to electron withdrawing nitrogen-based leaving groups created bench stable, crystalline electrophilic fluorinating agents (N⁺-F). Electrophilic fluorination in current organic chemistry is almost completely based on N⁺-F agents, with the most prominent agents being Selectfluor®, N-fluoropyridines and N-fluoro-N-(phenylsulfonyl)benzenesulfonamide (Figure 153).¹⁰² These reagents are soluble in most organic solvents and do not need of carrier additives compared to inorganic fluorides.
9.2.3.2 Electrophilic aromatic fluorination

The application of electrophilic radiofluorination is usually avoided when possible because of the low $^{18}$F$_2$ activity obtained from the cyclotron synthesis. However, before the use of iodonium ylides as starting materials electrophilic fluorination with F$_2$ and acetyl hypofluorite was the best option to introduce $^{18}$F into electron-rich molecules. Reactions were conducted on electron-rich arenes like phenols and aryl stannanes, synthesizing molecules like $^{18}$F-2b-carboxethoxy-3b-(4-fluoro)tropanefluorococaine (CFT) and 2,4,5-trifluororezazurin (2,4,5-TFRA). (Figure 154).$^{503,504,505}$
Figure 154. Electrophilic aromatic radiofluorination ($^{18}$F$_2$) with aryl stannanes and electron-rich phenols.

(a) Synthesis of $^{18}$F-CFT$^{504}$ and (b) $^{18}$F-2,4,5-TFRA.$^{505}$

Because of the weak bond strength, two different types of reactivities are possible with N-F reagents. Nucleophiles react by a simple electrophilic aromatic substitution reaction ($S_{EA}$) or by a process involving a single-electron transfer (SET) mechanism (Figure 155).$^{506}$

![Chemical Structures](image)

Figure 155. Electrophilic aromatic fluorination reaction mechanisms.

(Top) Electrophilic aromatic substitution $S_{EA}$ or (bottom) single electron transfer SET.$^{506}$

9.2.3.3 Radical fluorination

The ability of electrophilic fluorine reagents to react in a single electron transfer mechanism offers the possibility to fluorinate by a strictly radical reaction. The Hunsdieker reaction is based on radical fluorination, whereby an aromatic carboxylic acid acts as a leaving group.$^{501}$

Metals like silver and manganese have been used in radical fluorination. Fluorination
generally takes place on the metal and N\textsuperscript{-}-F reagents are commonly used to act as fluorine transfer agents.\textsuperscript{507} Silver catalyzed fluorinations have been described on aryl stannanes and borylates (Figure 156).\textsuperscript{508,509}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure156.png}
\caption{(a) Hunsdieker reaction of benzoic acid with xenon difluoride,\textsuperscript{501} Silver mediated fluorination of (b) aryl stannane\textsuperscript{508} and (c) aryl boronic acids.\textsuperscript{509}}
\end{figure}

10 Project overview

The goal of this project was to create a \textsuperscript{18}F-containing vitamin E (\(\alpha\)-tocopherol) analogue as an \textit{in vivo} tracker of a liposomal drug delivery system. Any decay products of such tracers resemble \(\alpha\)-tocopherol and \(\alpha\)-tocopherol metabolites and are not toxic to the body, guaranteeing their safe use. Our liposomal constructs in use are sensitive to degradation by radicals. Reformation of tocopherols helps protecting the liposomal constructs from radical based damage on the phospholipid fatty acid chains and maintains at the same time the liposomal integrity better than other liposomal trackers, leading to a longer survival time during admission.
and circulation in the blood stream. This new $^{18}$F-containing molecule will be incorporated into liposomes targeting cancer cells (Figure 157). The delivery technology is the intellectual property of the company Exact Delivery, Inc.

Figure 157. $^{18}$F-containing $\alpha$-tocopherol radiotracers as a tool to follow cancer-targeting liposomes.$^{510}$

10.1 Structure design

10.1.1 Tocopherol based PET tracer

The most important aspect of the design of a $^{18}$F-containing vitamin E is to maintain the structure of $\alpha$-tocopherol after the $^{18}$F has decayed to $^{18}$O. The C6 position of the phenol oxygen is the most suitable place to fluorinate as $\alpha$-tocopherol is reformed after decay (F-Toe 47, Figure 158). Tocopherol has been found in some cases to prevent cancer, to lower cancer growth and to reduce side effects occurring in chemotherapy.$^{511,512}$ Hence, the main goal of this project is the establishment of a rapid synthetic process towards F-Toe, 47. Other positions were considered for fluorination, but would not form natural $\alpha$-tocopherol after $^{18}$F decay.
Insertion of the fluorine at the 5-position of γ-tocopherol (5-F-γ-Toc 48, Figure 158) will form a 5,6-catechol-tocopherol (5,6-catechol-Tox) decay product, which oxidizes to the metabolite tocored. 323 A metabolite observed in studies of α-tocopherol is the 5-hydroxymethyl-α-tocopherol (5-HO-Me-α-Tox).323 Fluorination of the benzylic positions on the chroman ring may be possible by nucleophilic fluorination, creating the 5-F-Me-α-Tox 49 (Figure 158). Further, HM-Tox was chosen as a substrate for benzylic fluorination (6-F-Me-α-Tox 50, Figure 158) because of its resemblance to α-tocopherol (see Chapter 2, non-antioxidant-tocopherol). Insertion of 18F into the phytol chain is possible by nucleophilic fluorination using tocotrienols as starting materials. Fluorination at the terminal methyl position (C13 or C13’) creates 13-F-α-Tox 51. The decay product would in this case be 13-HO-α-Tox, which is the first product of the tocopherol phytol chain oxidative metabolism.73
Figure 158. Designed $^{18}$F-tocopherol tracers, 47-51, and their products formed after radioactive decay.

10.1.2 Tocopherol dual label: PET tracer and fluorophore

BODIPY groups have two fluorines attached to the boron center. Several fluorine exchange reactions on BODIPYs have been described in the literature. An exchange of the BODIPY fluorines with $^{18}$F in compound 3 would create a PET and fluorescent lable ($^{18}$F-3) that binds to $\alpha$-TTP (Figure 159). Dual labels have the advantage to act as PET agents to highlight a specific site in vivo and as an optical probe, showing the location of the tracer in in vitro.
Two possible products are formed, mono- or di-substituted $^{18}$F-3.

Certain time limits were set for the fluorination chemistry in considering the follow up work that would be required to make liposomes. We decided that a maximum reaction time of 30 min should not be exceeded. Reaction work-up and purification should be rapid and clean with as few by-products as possible. Analysis by HPLC has to be highly reproducible and fast. If possible, the starting material for the fluorination should be stable for a prolonged time before use. Also, hazardous / toxic chemicals like metal catalysts and moisture sensitive reagents should be avoided in the synthetic procedure if possible, to lower the risk for any possible danger to patient and worker.

The creation of a tocopherol PET tracer is part of the intellectual properties of Exact Delivery, Inc. In regards to use of a $^{18}$F-tocopherol PET tracer in the future, it would be more convenient if its preparation avoided currently patented methodologies.

11 Results and Discussion

11.1 6-F-Tocopherol synthesis

6-Fluorotocopherol (F-Toc) structurally resembles the previously synthezised molecule Cl-Toc$^{41}$. The radioactive decay of the $^{18}$F nuclide is limiting the reaction time of the chemistry and therefore demands a rapid synthesis. Fluorination chemistry could proceed either by electrophilic or nucleophilic fluorination.$^{515,516}$ Similar to Cl-Toc, F-Toc can be synthesized by
Electrophilic fluorination, but higher $^{18}\text{F}$ incorporation is possible with nucleophilic radiofluorination. Electrophilic fluorinating agents are synthesized from $^{18}\text{F}_2$ gas and have a maximum $^{18}\text{F}$ incorporation of 50%. Nucleophilic fluorination with fluoride incorporates 100% $^{18}\text{F}$ and is, therefore, the preferred method of radiofluorination. Unfortunately, nucleophilic fluorinations of electron-rich, sterically hindered aromatics like tocopherol are not high yielding (Figure 160).

![Figure 160. Problems associated with electrophilic- and nucleophilic fluorination.](image)

Electrophilic fluorination uses [$^{18}\text{F}$]-F$_2$ gas as a source of $^{18}\text{F}$. Only one of the atoms in F$_2$ is a $^{18}\text{F}$, which leads to maximum possible radiochemical yield (RCY) of 50%. Direct nucleophilic fluorination with $^{18}\text{F}$-fluoride favours aromatics with electron withdrawing groups (EWG) like carbonyls or nitro groups and are less effective (or not possible) with aromatic substrates bearing electron donating groups (EDG) like methoxy or amines.

$\alpha$-Tocopherol is a good starting material because of its availability and low cost. Ritter has described the direct fluorination of phenols with the deoxyfluorination reagent Phenofluor®. Recently, Phenofluor® has been used to fluorinate phenols with radioactive $^{18}\text{F}$. However, the Phenofluor methodology is patented and will only be used here to create a 6-F-Toc reference sample. Figure 161 highlights our chemical approaches: (a) nucleophilic aromatic $^{18}\text{F}$-fluorination chemistry to obtain 6-F-$\alpha$-tocopherol: (b) titanium dioxide (TiO$_2$) catalyzed fluorination of aryl tosylates (c) reaction with aryl iodonium salts (d) electrophilic fluorination by electrophilic aromatic substitution with H-Toc and (e) lithium-halogen exchanged of 6-iodotocopherol.
Leaving groups like aryl stannanes or borylates are used to incorporate $^{18}\text{F}$ $\delta$-Tocopherol has been fluorinated at the 6-position via the stannylated 6-$\delta$-tocopherol. Attempts will be made to fluorinate $\alpha$-tocopherol with borylates and stannylates as leaving groups (f, Figure 161).

**Figure 161. Synthetic strategies towards 6'$\text{F}$-$\alpha$-tocopherol by nucleophilic and electrophilic fluorination$^{515,516}$**

11.2 Synthesis

11.2.1 Nucleophilic F-Toc synthesis:

11.2.1.1 TiO2 catalysed $\alpha$-tocopherol tosylate fluorination
The chemistry discovered by Sergeev and co-workers describes the fluorination of aryl tosylates with H$_2$O solvated $^{18}$F fluoride in ACN / thexyl alcohol (Thex-OH 1:1 v/v) with TiO$_2$ nanoparticles (NP) comprising 55:45 anatase / rutile (<200nm particle size) and tetrabutylammonium bicarbonate (Bu$_2$N$^+$HCO$_3^-$) at 110°C for 5-15 min (Figure 162). Benefits of this reaction are; the use of aqueous $^{18}$F fluoride saves time by avoiding the full azeotropic drying of the $^{18}$F with ACN and resuspension in another reaction solvent. Good yields were recorded when the reaction was diluted with organic solvents to the point where water made up 25% of the total reaction volume. Additionally, aryl tosylates are simple starting materials and the p-toluenesulfonic acid byproduct is easily separated by extraction.

Figure 162. Synthetic scheme for TiO$_2$ mediated fluorination of aryl tosylates.$^{519}$

To test the reaction we used pentamethylchromanol (PMC), which allows a more critical analysis of the products formed by NMR and mass spectroscopy. PMC was tosylated with tosylchloride (TsCl) and pyridine in a 51% yield (Figure 163).

Figure 163. Tosylation of pentamethylchromanol.

No description was given for the synthesis of the TiO$_2$ NP or which vendor sold them. An 80:20 mixture of anatase / rutile is commercially available, called Degussa P25, having a 150 nm
To obtain a 55:45 anatase / rutile TiO₂ mixture, a synthesis was attempted according to a procedure by Xiong.Titanium trichloride (TiCl₃) was oxidized with a specific amount of graphene oxide (GO) (However, the reaction did not yield a 55:45 ratio, only 100% anatase TiO₂ was isolated.

While trying to find a way to synthesize the TiO₂ nanoparticles mentioned above, Brock University chemistry professor Dr. Stamatatos told me that his friend at Hellenic Open University of Patras, Dr. Bourikas, was studying the phase transitions and surface changes of TiO₂. In Dr. Bourikas’ study, the TiO₂ mixture Degussa P25 anatase/rutile (80:20) was calcinated at different temperatures ranging over 500-800°C for up to 24 h, which increased the amount of rutile phase (Figure 164 and Table 19). Translating Dr. Bourikas’ research results to the procedure used by Sergeev would mean that heating the 55:45 mixture of anatase / rutile at 550°C for 12 h will increase the overall percentage of rutile phase. Unsure of the actual TiO₂ phase ratio responsible for the chemistry, it was decided to test the Degussa P25 TiO₂, each TiO₂ anatase and rutile phase separately, and in the correct 55:45 ratio.

![Figure 164. Change of TiO₂ anatase percentage dependend on the calcination time at 600°C.](image)

Preliminary results sent by Dr Bourikas.
The values of the anatase percentage (%A) in P25 determined by temperature programed furnace (TP)-XRD analysis.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>%A</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>81.5</td>
</tr>
<tr>
<td>550</td>
<td>84.1</td>
</tr>
<tr>
<td>600</td>
<td>83.3</td>
</tr>
<tr>
<td>650</td>
<td>80.4</td>
</tr>
<tr>
<td>700</td>
<td>73.1</td>
</tr>
<tr>
<td>750</td>
<td>47.8</td>
</tr>
<tr>
<td>800</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Table 19. Anatase percentage (%A) in Degussa P25 TiO₂ at increasing temperature, after 24 h.

The reaction was conducted in temperature programed furnace with an X-ray powder diffraction (TP-XRD). Preliminary results sent by Dr Bourikas.⁵²⁴

By calcination at 700°C for 16h, part of the isolated anatase material was converted to the rutile TiO₂.

Fluorination of PMC-OTs, ⁴⁷, was conducted according to the optimized procedure found in the supporting information of Sergeev’s paper, with adjustments for fluorination with the “cold” ¹⁹F isotope.

At this point it is important to mention the synthetic difference between ¹⁸F and ¹⁹F fluorination in terms of the actual equivalency of reagent used. Synchrotron synthesized ¹⁸F in Sergeev’s procedure had an activity of 1.5-4 mCi, what corresponds to 0.3-0.8 nmol (¹⁸F-Fallypride specific activity of 5±2 Ci/µmol⁵¹⁹). TiO₂ was used in a 165000:1 ratio (10 mg / 0.125 mmol of
TiO$_2$) to the $^{18}$F fluoride. Commonly, the ratio of synchrotron isolated $^{18}$F fluoride to $^{19}$F fluoride is approximately 1:1000, which makes the actual ratio to all fluoride present in the reaction to TiO$_2$ 1:165. $^{19}$F-fluorination mimicking radioactive fluorination normally uses equimolar amounts of reagents or excess fluorine. To monitor the formation of 6-F-PMC, $^{19}$F-NMR spectroscopy was used. The test reactions were run with 10-20 mg of TsO-PMC, 52, to keep the amount of TiO$_2$ low (130-260 mg) (Table 20).

![Reaction Scheme](image)

<table>
<thead>
<tr>
<th>KF (eq)</th>
<th>TBAB (eq)</th>
<th>Thex-OH : ACN</th>
<th>TiO$_2$ phase (eq)</th>
<th>Temperature (°C)</th>
<th>Time</th>
<th>Yield$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>14</td>
<td>1:1</td>
<td>P25 80:20, 60 eq</td>
<td>110</td>
<td>10min</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>1:1</td>
<td>P25 80:20, 60 eq</td>
<td>130</td>
<td>15min</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>1:1</td>
<td>P25 80:20, 60 eq</td>
<td>110</td>
<td>10min</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>1:1</td>
<td>rutile 100%, 60 eq</td>
<td>130</td>
<td>8min, +16h</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>1:1</td>
<td>Anatas 100%, 60 eq</td>
<td>130</td>
<td>8min, +16h</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>1:2</td>
<td>Anatas 55% / rutile 45%, 60 eq</td>
<td>130°C</td>
<td>8min, +16h</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 20. TiO$_2$ mediated fluorination of TsO-PMC.

TiO$_2$ was dried in the oven at 500°C for 6-12 h and crushed in a mortar to a fine powder before use. TsO-PMC 47 (20 mg, 67 µM) was added with thexyl alcohol (0.35 ml) and dry ACN (0.35 ml) to the TiO$_2$ NP (260 mg) in a closable vial under N$_2$. TBAB (227 mg) was incubated with KF (3.1 mg) in 25% v/v water (0.23 ml) for 1h at 60°C in a separate vial. The KF-TBAB was transferred to the TiO$_2$ and heated at 110°C for 8-15 min (a) Yield $^{19}$F-NMR.

Only starting material was observed in the spectra of crude products; there was no trace of product in the $^{19}$F-NMR (Table 20). Stirring the reaction for a longer time (16 h) did not change the outcome. To understand the problem with this reaction a reference compound in the original
The publication was chosen as a test substrate. Tosylation of 2-naphthol with TsCl, \( \text{K}_2\text{CO}_3 \) in THF/H\(_2\)O led to product 54 in a 93.2\% yield.\(^{526}\) In the reaction with 2-tosylnaphthol, 54, the TiO\(_2\) was only dried for 30-60 minutes at 550°C (Table 21).

\[ \text{HO} \quad \text{TsO} \quad \text{2-Naphthol} \quad \text{TiO}_2 \quad \text{2-TsO-Naphthol 54} \]

<table>
<thead>
<tr>
<th>KF (eq)</th>
<th>TBAB (eq)</th>
<th>Thex-OH : ACN 1:1(ul)</th>
<th>TiO(_2) phase (eq)</th>
<th>Temperature (°C)</th>
<th>Time (min)</th>
<th>Yield (%)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1eq</td>
<td>7(^b)</td>
<td>0.476ml</td>
<td>rutile 100%, 60 eq</td>
<td>110</td>
<td>12min</td>
<td>-</td>
</tr>
<tr>
<td>1eq</td>
<td>7</td>
<td>1:1</td>
<td>Anatase 55% / rutile 45%, 60 eq</td>
<td>110</td>
<td>12min</td>
<td>-</td>
</tr>
<tr>
<td>1eq</td>
<td>14</td>
<td>1:1</td>
<td>Anatase 55% / rutile 45%, 60 eq</td>
<td>110</td>
<td>12min</td>
<td>-</td>
</tr>
<tr>
<td>1eq</td>
<td>14(^c)</td>
<td>1:1</td>
<td>H(_2)O 0%</td>
<td>Anatase 55% / rutile 45%, 60 eq</td>
<td>110</td>
<td>15min</td>
</tr>
</tbody>
</table>

Table 21. TiO\(_2\) mediated fluorination of TsO-Naphthol.

\( a \) Yield \(^{19}\text{F-NMR}. \( b \) KF & TBAB incubation for 20 min at rt. \( c \) A SepPak® cartridge was washed with water and EtOH. KF in water was loaded onto the cartridge (1ml), and washed with TBAB in ACN (1ml), followed by ACN (3ml). The solution was dried at 80°C and resuspended in ACN.

To enhance the activity of the phase transfer catalyst the water was eliminated from the reaction. The TiO\(_2\) used to this point was produced in our lab, and had an unknown particle size which might have caused the low yield. Anatase and rutile TiO\(_2\) NP with a ~200 nm size were ordered from Eprui® (Table 22). No pre-heating of the TiO\(_2\) was required.

\[ \text{TsO} \quad \text{52} \quad \text{TiO}_2 \quad \text{rutile 200nm Epuri®}, \quad \text{KF, TBAB, (TBAF 1M THF)} \quad \text{Thexyralcohol 1:1 ACN, 110°C, 12-15min} \quad \text{F} \quad \text{6-F-PMC 53} \]
<table>
<thead>
<tr>
<th>KF (eq)</th>
<th>TBAB (eq)</th>
<th>Thex-OH : ACN</th>
<th>TiO₂ phase (eq)</th>
<th>Temperature (°C)</th>
<th>Time</th>
<th>Yield(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1eq</td>
<td>7eq(^b)</td>
<td>1:1(^c)</td>
<td>rutile 100%, 60 eq</td>
<td>110</td>
<td>12min</td>
<td>-</td>
</tr>
<tr>
<td>2eq(^d)</td>
<td>-</td>
<td>1:1</td>
<td>rutile 100%, 60 eq</td>
<td>110</td>
<td>10min</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 22. TiO₂ mediated fluorination of with EPRUI® 200 nm TiO₂ NP.

(a) Yield \(^{19}\)F-NMR. (b) SepPak® elution as prior described. (c) TsO-PMC, Thex-OH and ACN preincubated for 1h at rt. (d) 1M TBAF in THF (53 µl) was used instead of KF with TBAB.

Again, even using the commercial TiO₂ sample, no product was observed by \(^{19}\)F-NMR, and thus we decided to contact the author for advice. The author stated that the TiO₂ catalysed reaction had not been done with \(^{19}\)F in their laboratory, only with \(^{18}\)F. In the original paper a reaction was possible in acetonitrile and thexyl alcohol with a 25% v/v water present, but the author’s replied that their best results occurred when water was absent. The reaction in table 22 were run without water, but no product was observed. Sergeev mentioned that when they analysed their 55:45 anatase / rutile TiO₂, a composition of anatase / rutile of 97:3 was found. The influence of the TiO₂ phase composition is still unknown, but smaller particle sizes were confirmed in his group to yield more product. It remains possible that TiO₂-mediated fluorination creates only trace amounts of product, not detectable by standard laboratory techniques like NMR. The author mentioned also that their follow up studies with electron rich and bulky halides have shown zero conversion. Thus, tosylated tocopherol is not suitable for this type of chemistry.

11.2.1.2 Aryl borane and stannylated tocopherol fluorination

\(^{18}\)F-fluorination of aromatics by de-stannylation and de-borylation has been used to create biologically relevant molecules.\(^{521,527}\) \(^{18}\)F-3-fluoro-5-[(pyridin-3-yl)ethynyl]benzonitrile (\(^{18}\)F FPEB) is used to quantify metabotropic glutamate receptors 5 (mGluR5) and has been synthesized by copper-catalyzed \(^{18}\)F-fluorination of boronic acids / esters.\(^{496}\) 2-\(^{18}\)F-Fluoro-\(L-\)
tyrosine was synthesized by fluoro-destannylation and δ-tocopherol has been fluorinated at the 6-position by Ritter via a silver mediated stannyl fluoride exchange reaction (Figure 165).

![Chemical Structures]

Figure 165. (a) Copper mediated fluorination of boronic esters and synthesis of [18F]FPEB. (b) 2-[18F]-fluoro-L-tyrosine synthesis via Fluoro-destannylation and silver mediated fluorination of stannyl-δ-tocopherol.

Tocopherol stannylates and borylates have so far only been synthezised on δ-tocopheryl triflates. Stannylation of α-tocopherol triflate to 55 using dibutyltinhydride and (Bu₃Sn₂) and tetrakis(triphenylphosphine)palladium gave no discernible fluorination products despite longer reaction times (5 days) (Figure 166).
The yield of the δ-stannyl tocopherol in Miller’s case was moderate, because the purification process by chromatography leads to protodesilylation. In their case the crude stannyl product was directly iodinated in a 93% yield. α-Tocopherol triflate seems to be too sterically hindered to attach the stannylate. It is also expected to have a higher tendency towards protodesilylation with 6-stannyl-α-tocopherol, which would not allow purification and would create further purification problems during the $^{18}$F fluorination. Therefore, this idea was discarded.

In the same paper, δ-tocopherol triflate was Myaura-borylated in high yields with Pd$_2$(dba)$_3$ and bispinacolborane over 12 h. However, attempted borylation of α-tocopherol triflate to 56 of did not occur after 5 days (Figure 167).
Figure 167. Synthesis of α-tocopherolpinacolboronic ester 56, δ-6’-tocopherolpinacolboronic ester from α-tocopherol triflate.\textsuperscript{529}

Since Miller’s stannylation and borylation did not work new reaction conditions were needed to borylate the α-tocopherol triflate. The catalyst was changed to palladium acetate (Pd(OAc)\textsubscript{2}) and BINAP, a version that worked for Mazzini in α-tocopherol triflate cross coupling reaction with amines.\textsuperscript{530} After 5 days the pinacole boronic acid was isolated in 6\% yield. Changing the catalyst to 1,1’-bis(diphenylphosphino)ferrocene (dpff) was tested, as it had been reported to lead to high yields with aryl triflates, but it did not provide the expected product after 16 h (Figure 168).\textsuperscript{531}
Figure 168. (a) Mazzini’s α-tocopherol amine synthesis.\textsuperscript{530} (b) α-Tocopherol triflate borylation with Mazzini’s conditions. (c) α-Tocopherol triflate borylation with PdCl\textsubscript{2}(dppf).\textsuperscript{531}

Diemer found that ferrocene based ligands like 1,1′-bis(diphenylphosphino)ferrocene (dppf) are able to borylate sterically demanding, electron-rich iodoarenes. Recently, the reaction with 6-I-α-Toc yielded the α-tocopherol pinacole borylane ester 56 in low yield (Figure 169).\textsuperscript{532,533}

Figure 169 Palladium catalyzed borylation of sterically demanding, electron-rich aromatics.

(a) Diemer's borylation of 2,3,5,6-tetramethyliodoanisol. (b) Borylation of 6-I-α-Toc.\textsuperscript{532}
Fluorination of 56 with tetrakispyridine copper(II)triflate fully converted the starting material, yielding H-Toc as the main product with trace amounts of 47 (Figure 170). Formation of H-Toc was caused by the presence of water in the reaction mixture.

![Figure 170. Tetrakispyridine copper(II)triflate catalyzed fluorination of 56.]

### 11.2.1.3 Deoxyfluorination

Ritter’s deoxyfluorination reaction uses the difluorinated N-heterocyclic carbene (NHC) Phenofluor® at elevated temperature in organic solvents to fluorinate phenols. An updated version of the original procedure was used for the fluorination of tocopherol. Instead of using the very hygroscopic phenofluor directly the precursor chloro-NHC salt, 58, reacted with excess cesium fluoride (CsF) to form the active reagent in situ. 58 was produced according to the literature by chlorination of 57 with hexachloroethane (Figure 171).

![Figure 171. Synthesis of Phenofluor® precursor Cl-NHC.]

220
α-Tocopherol was reacted with 58 and 10 eq of CsF in dry toluene at 110°C for several days, but no fluorination product was observed. Testing the reaction on γ- and δ-tocopherol also did not yield a fluorinated product (Figure 172).

The starting material was re-isolated with trace amounts of unidentifiable products of high molecular mass (ESI: 600-1000 m/z). The crude product did not show any $^{19}$F NMR peaks of the expected 6-F-α-Toc at 131.49 ppm. No NHC substituted phenol intermediate 59 was seen by NMR spectroscopy with α-, γ-, or δ-tocopherol, an intermediate isolated with other aromatic phenols when studying the Phenofluor® reactivity (Figure 173).
No NHC-tocopherol intermediate was observed.\textsuperscript{535}

Since Phenofluor has been shown to yield product on a variety of phenols, this result was rather unexpected. However, in almost all literature examples no ortho-substituents are present. The two methyl groups ortho to the phenol of α-tocopherol seem to provide enough steric repulsion to inhibit the fluorination by Phenofluor\textsuperscript{®}. δ-Tocopherol, on the other hand, has no ortho-methyl groups and still did not produce fluorinated product. Phenofluor has been recently used as a way to introduce \(^{18}\text{F}\) fluoride. The method described in Neumann’s paper attaches the chloro-NHC salt to the phenol with silver carbonate (\(\text{Ag}_2\text{CO}_3\)), hence assisting fluorination (Figure 174).\textsuperscript{518}
In the case of tocopherols, silver salts are known to act as oxidizers, possibly forming the quinone or the quinone methide.\textsuperscript{536,537} Ritter’s group published a new way to substitute phenols with fluorine by using Phenofluor\textsuperscript{®} with ruthenium complex CpRu(COD)Cl. The group was able to \textsuperscript{18}F-lable δ-tocopherol with a 62% RCY in a short overall reaction time (Figure 175).\textsuperscript{538}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure174.png}
\caption{\textsuperscript{18}F-fluorination of NHC-bound phenols.\textsuperscript{518}}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure175.png}
\caption{Ruthenium catalysed Phenofluor\textsuperscript{®} fluorination of δ-tocopherol.\textsuperscript{538}}
\end{figure}

\textbf{11.2.1.4 Fluorination of α-Tocopherol Iodonium Salts}

Nucleophilic fluorination of electron rich aromatic substrates has been achieved in high yields with diaryl iodonium salts. Fluorination depends on the electronic nature of the substituents and the aromatic groups involved. Electron-poor aromatics have higher fluoride incorporation. Substituents on the \textit{ortho}-position have been shown to effect the transition state equilibrium during the fluoride addition, \( T_{s1} \) vs \( T_{s2} \), partially favouring the substituted aromatic transition state, \( T_{s1} \). (Figure 176).\textsuperscript{539,540}
Electronic effects of the aromatic ring: electron deficient aromatics are more prone to fluorination. Steric effects of the aromatic ring: aromatics with ortho-substitution are more prone to fluorination, represented in $T_{s1} > T_{s2}$. Yields represent observed radiochemical yields (RCY).$^{539,540}$

Tocopherol has two methyl groups at the ortho-position and is electron rich in nature. Therefore, the aromatic group opposite to tocopherol (auxiliary) should be as electron-rich as possible. Two functional groups suitable for this purpose are $p$-methoxybenzene and thiophene (Figure 177).$^{539,540}$

*Observed RCYs

**Figure 176. Nucleophilic fluorination of diaryliodonium salts.**

**Electronic effect**

Br\(\text{Cl}^-\) + F\(^-\), DMF

$68\%$ $25\%$

O

**Steric effect**

Br\(\text{Cl}^-\) + F\(^-\), DMF

$6.5\%$ $60\%$

$63\%$ $19\%$

**Figure 177. Designed electron rich 6-α-tocopherol iodonium salts with different auxiliaries:**

Thiophene 60-OTs, 61-OTf, 62-BF\(_4\)

(p-methoxybenzene)-α-tocopherol iodonium salt

OTs: 60, OTf: 61, BF\(_4\): 62

OTs: 63, OTf: 64, BF\(_4\): 65

Diaryliodonium salts are synthesized by coupling hypervalent iodines with an aromatic group. The hypervalent iodine is thereby either created *in situ* by oxidation of the aryliodide with the
second aromatic group present or in a prior reaction. Boronic esters and stannanes have been used with hypervalent iodines to form the diaryliodonium salts (Figure 178).

\[
\begin{align*}
\text{I} & \quad \text{[O], } X^- \\
\text{I} & \quad X^- \\
\text{I} & \quad X^- \\
\text{O} & \quad \text{Bu} \quad \text{Bu} \quad \text{Sn} & \quad \text{R = H, Me, Pin} \\
\end{align*}
\]

Figure 178. Synthesis of iodonium salts via in situ oxidation of aryl iodides with aromatics (Y) (top), with pre-oxidized hypervalent iodines / aromatics (middle) and boronic acids / esters or stannylates (bottom).

Recently, iodonium ylides with Meldrum’s acid have been used as the counter auxiliary with good fluoride incorporation (Figure 188).

\[
\begin{align*}
\text{Meldrum’s acid} & \quad \text{I} & \quad \text{O} & \quad \text{F} \\
\end{align*}
\]

Figure 179. Fluorination of Meldrum’s acid iodonium ylides.

The synthesis of 6-diaryliodonium salts of tocopherol can be achieved by reacting H-Toc with hypervalent iodine or by oxidation of 6-I-Toc in reaction with another aryl group (Figure 180).
In a first attempt to create the 6-(thiophene)iodo-α-tocopherol, trifluoroacetate salt, 61, was prepared by reacting H-Toc with 2-iodothiophene, meta-chloroperbenzoic acid (mCPBA) and trifluoroacetic acid at -78°C.\textsuperscript{545} The reaction did not yield the desired product, but rather α-tocopheryl quinone and 6,6'-bis-tocopherol, 66, besides the unreacted starting material (Figure 181).

Byproducts α-tocopherol quinone and 6,6'-bis-α-tocopherol 66 were formed as products.

Oxidizing agents like m-CPBA react with H-Toc to form oxidized tocopherol products after prolonged stirring. To avoid this problem the auxiliary ligand was created in a prior reaction is created before being mixed with H-Toc (Figure 182).\textsuperscript{546}
Before trying the reaction with (diacetoxy)iodothiophene the (phenyl)tocopherol iodonium salt was created with (diacetoxy)iodobenzene. Electron rich aromatics like (diacetoxy)iodothiophenes are more reactive when forming the diaryliodonium salt and form less stable diaryliodonium salts that are prone to faster decomposition. The (phenyl)tocopherol iodonium salt offers a more stable, easier to synthesize alternative to study the fluorination reaction. The reaction of H-Toc with (diacetoxy)iodobenzene and acetic acid with potassium bromide should yield 67 or with trifluoroacetic acid 68, but neither product was obtained, only dimer 66 (Figure 183 and 184).539
By using a procedure described by Chun (diacetoxy)iodobenzene was reacted with H-Toc in the presence of 7-toluenesulfonic acid, yielding product 69 in low yield (Figure 185).

In a first attempt, 69 was fluorinated using cesium fluoride (CsF) without any fluoride carrier additives like cryptand[2.2.2] or 18-crown-6 (non-carrier additive, NCA) in acetonitrile, which yielded 47 in low yields after 1-2 days. Fluorination was then conducted in the presence of 18-crown-6, cryptand[2.2.2] as carrier additive (CA) and potassium fluoride (KF), and CsF as fluoride sources at 80°C. Reactions were run for a maximum of 30 min and one third isolated after 5 min and 10 min. Acetonitrile was chosen as the solvent of choice because it is easier to remove from the reaction mixture. Fluorination with CA tetrabutylammonium fluoride (TBAF) was conducted in THF and DMF to see the how different solvents effect the outcome of the fluorination (Table 23).
<table>
<thead>
<tr>
<th>F-source</th>
<th>Fluoride carrier additive</th>
<th>Solvent (^{2+})</th>
<th>Temp (^{0}(\degree))</th>
<th>Time</th>
<th>Yield F-Toc(^{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>CsF (2eq)</td>
<td>-</td>
<td>Dry ACN 2.5mM</td>
<td>rt</td>
<td>2days</td>
<td>9.1%</td>
</tr>
<tr>
<td>CsF (2eq)</td>
<td>-</td>
<td>Dry ACN 2.5mM</td>
<td>80</td>
<td>1day</td>
<td>3.6%</td>
</tr>
<tr>
<td>CsF (1eq)</td>
<td>18-Crown-6 (1eq)</td>
<td>Dry ACN 5mM</td>
<td>80</td>
<td>5min(^{b})</td>
<td>-</td>
</tr>
<tr>
<td>CsF (5eq)</td>
<td>18-Crown-6 (5eq)</td>
<td>Dry ACN 5mM</td>
<td>80</td>
<td>5min(^{b})</td>
<td>-</td>
</tr>
<tr>
<td>KF (1eq)</td>
<td>Cryptand[2.2.2] (1eq)</td>
<td>Dry ACN 5mM</td>
<td>80</td>
<td>5min(^{b})</td>
<td>-</td>
</tr>
<tr>
<td>KF (5eq)</td>
<td>Cryptand[2.2.2] (5eq)</td>
<td>Dry ACN 5mM</td>
<td>80</td>
<td>5min(^{b})</td>
<td>-</td>
</tr>
<tr>
<td>KF (1eq)</td>
<td>18-Crown-6 (1eq)</td>
<td>Dry ACN 5mM</td>
<td>80</td>
<td>30min</td>
<td>6.6%</td>
</tr>
<tr>
<td>TBAF 1M in THF (1eq)</td>
<td></td>
<td>Dry ACN 5mM</td>
<td>80</td>
<td>30min</td>
<td>5.7%</td>
</tr>
<tr>
<td>TBAF 1M in THF (1eq)</td>
<td></td>
<td>Dry THF 5mM</td>
<td>80</td>
<td>30min</td>
<td>2.3%</td>
</tr>
<tr>
<td>TBAF 1M in THF (1eq)</td>
<td></td>
<td>Dry DMF 5mM</td>
<td>80</td>
<td>30min</td>
<td>10.1%</td>
</tr>
<tr>
<td>KF (1eq)</td>
<td>Cryptant[2.2.2] (1eq)</td>
<td>Dry DMF 5mM</td>
<td>150</td>
<td>15min</td>
<td>25.6%</td>
</tr>
<tr>
<td>KF (1eq)</td>
<td>Cryptant[2.2.2] (1eq)</td>
<td>Dry DMF 5mM</td>
<td>150</td>
<td>15min</td>
<td>23.4%</td>
</tr>
<tr>
<td>TBAF 1M in THF (1eq)</td>
<td></td>
<td>Dry DMF 5mM</td>
<td>150</td>
<td>15min</td>
<td>24.4%</td>
</tr>
<tr>
<td>TBAF 1M in THF (1eq)</td>
<td>TEMPO (0.2eq)</td>
<td>Dry DMF 5mM</td>
<td>150</td>
<td>15min</td>
<td>22.3%</td>
</tr>
</tbody>
</table>

Table 23 Fluorination of \((R)-(2\text{-}hexadecyl\text{-}2,5,7,8\text{-}tetramethylchroman\text{-}6\text{-}yl)(phenyl)iodonium tosylate\) 69.
69 (20 mg, 25.3 µmol) was dissolved in ACN, THF or DMF. Fluoride was added with an additive and stirred for 5-30 min. The solvent was evaporated and the residual mixture partitioned between with hexane and water. The organic phase was dried with Na₂SO₄, filtrated and purified over a small SiO₂ column with hexane. (a) Yield by ¹H-NMR (b) After 5 min was 1/3 of the reaction mixture worked up. The reaction yield is calculated to 1/3 of the starting material.

It was expected that the (phenyl)tocopherol iodonium salt, 69, would lead to low fluorine incorporation into the tocopherol. NCA fluorination without carrier additives like 18-crown-6, cryptand[2.2.2] and tetraalkylammonium in ACN does not yield product in a short time period. A reaction time of at least 30 minutes is needed to obtain product in a 10% yield without CA. 549

6-Iodotocopherol was the major by-product obtained. DMF was the best solvent for the fluorination with TBAF when the temperature was kept at 80°C. Running the reaction at 150°C in DMF for 20 min increased the yield to 22-25%. 541 The best reaction conditions discovered so far (1eq KF/Cryptand[2.2.2] and TBAF in DMF, 150°C) need to be tested in the future with different counter ions. 550 Reports by Ross and Hamnett showed a higher reactivity with boron tetrafluoride 551 and trifluoroacetic acid 552 as counter ions. Radical scavengers like TEMPO and BHT have been found to increase the yield, but showed no effect when run with KF/Cryptand[2.2.2] or TBAF in DMF. 553

The reaction also forms the 6-iodo-α-tocopherol (6-I-α-Toc, 70) as a by-product. Purification of the F-Toc by column chromatography is possible, but the retention time of the 6-I-α-Toc is quite similar and this makes the purification less efficient. A better separation is achieved when the 6-I-α-Toc is converted to a more polar functional group, as the retention time difference on the SiO₂ column is increased. Two possibilities were the conversion of 6-I-α-Toc into a carboxylic acid 71 by quenching the respective Grignard species or organolithium from lithium-
halogen exchange of 6-I-α-Toc, with CO₂ (Figure 186).

![Chemical reaction diagram]

**Figure 186. Carboxylation of 6-I-α-Toc via lithium-halogen and Grignard reaction.**

Formation of the Grignard species with 6-I-α-Toc in short reaction times did not work with excess magnesium or methylmagnesium chloride (MgMeCl). Lithium-halogen exchange was conducted at room temperature for an immediate reaction to reduce the reaction time to 1-5 min. However, rapid bubbling with dried CO₂ gas did not yield any carboxylic acid. Fast carbonylation chemistry was used by Al-Qahtani to create \(^{11}\)C-acetophenone adducts. When the reaction was conducted in water instead, a carboxylic acid formed as the main product. Using catalytic amounts of palladium chloride (PdCl₂) did not yield any product with CO gas. Using equimolar amounts of PdCl₂ to simulate one catalytic turnover did not yield product either (Figure 187).
Changing the functional group from a carboxylate to a boronic acid would have the same effect of enhancing the retention time difference to ease purification. Chen published a procedure for a fast photoborylation of aryl halides in continuous flow conditions. Bispinacol borane and tetrahydroxydiborane (B$_2$OH$_4$) were used as the borylating agent. Reaction times were in the range of 15 min to obtain products in yields around 90% (Figure 188).

6-I-α-Toc 70 and B$_2$OH$_4$ were passed in different solvent mixtures through a photoreactor with a 300W Hg lamp having an emission maximum around 350 nm (Luzchem, UVA lamp). Initial problems with starting material solubility were overcome by dilution and the use of more non-polar solvents such as acetonitrile and THF. Increasing the reaction time led to almost full
consumption of the starting material, however only H-Toc was isolated, and $^{11}$B-NMR has shown no signs of any aryl borylate or boronic acid (around +30 ppm) (Table 24).

![Reaction Diagram]

**Table 24. Photoborylation of 6-I-α-Toc to form 72.**

<table>
<thead>
<tr>
<th>Borylating agent (eq)</th>
<th>Solvent</th>
<th>Time</th>
<th>Yield (HO)$_2$B-Toc (%)$^a$</th>
<th>Yield H-Toc (%)</th>
<th>Yield I-Toc recovered (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{B}_2\text{OH}_4$ 2eq</td>
<td>MeOH:H$_2$O 4:1 92mM</td>
<td>10min</td>
<td>-%</td>
<td>40%</td>
<td>60%</td>
</tr>
<tr>
<td>$\text{B}_2\text{OH}_4$ 2eq</td>
<td>ACN:THF 1:1 + H$_2$O 5% 25mM</td>
<td>3h</td>
<td>-%</td>
<td>65%</td>
<td>3%</td>
</tr>
</tbody>
</table>

6-I-α-Toc was mixed with $\text{B}_2\text{OH}_2$ in the solvents and pressed through a 70 cm, diameter fluorinated ethylene propylene (FEP) tubing by an injection pump. The solvent was evaporated and the crude mixture columned over a SiO$_2$ column. (a) Verified by $^{11}$B-NMR.$^{556}$

Spirocyclic Meldrum’s acid iodonium ylides were reported to be stable precursors and good for the incorporation of non-reactive, sterically demanding aromatics (Figure 189).$^{557}$

![Spirocyclic Meldrum's Acid Iodonium Ylide Reaction]

**Figure 189. Fluorination of spirocyclic Meldrum’s acid iodonium ylides.$^{557}$**

An attempt was made to create the 6'(Meldrum’s acid)-α-tocopherol iodonium ylide 73, following the synthesis of Cardinale.$^{543}$ Compound 70 (for the synthesis of 6-I-α-Toc 70, see section 16.2.2 fluorination of 6-iodo-α-tocopherol) was treated first with $m$-CPBA, followed by
addition of Meldrum’s acid and excess KOH (7eq). No reaction occurred after the time suggested in the literature. When the reaction was stirred for a longer time after the addition of mCPBA and Meldrum’s acid, α-tocopherol quinone was formed (Figure 190).

![Figure 190. Meldrum’s acid-α-tocopherol iodonium ylide synthesis 73 from 6-I-α-Toc.](image)

The oxidation of iodotocopherol was tried with peracetic acid in acetic acid with the hope of forming the (diacetoxy)iodo-α-tocopherol, 74. After 8h reaction time no product could be isolated, and only starting material and α-tocopherol quinone were obtained (Figure 191).

![Figure 191. Synthesis of (diacetoxy)iodo-α-tocopherol 74 from 70.](image)

Fluorination of iodonium salts or ylides are a promising methods to synthesize F-Toc. Future work with more electron donating auxiliaries like thiophenes will be necessary to improve the reaction yield. So far, no conditions have been found to oxidize iodotocopherol into a hypervalent iodine species, which makes the addition of more electron rich auxiliaries like thiophene and p-methoxybenzene more difficult and prohibits the synthesis of Meldrum’s acid derivatives.
**11.2.2 Synthesis of 6-F-tocopherol by electrophilic fluorination:**

**11.2.2.1 Fluorination of 6-Iodo-α-tocopherol**

Miller described the synthesis of 6-iodo-δ-tocopherol from the δ-tocopherol triflate via the 6-stannane-δ-tocopherol. But as described previously, attempts to obtain these compounds were not successful with α-tocopherol (see section stannylation of aryl stannane tocopherol fluorination). Hence, the same strategy was used when synthesizing Cl-Toc, 43, by iodination of H-Toc. The starting material H-Toc, 42, was synthesized from α-tocopherol by reduction of its triflate 41.

Synthesis of 70 with iodine monochloride (ICl) in DCM did not yield any product 42. In the same literature DDQ was used to quench radical intermediates, but no product were observed when the reaction was run with DDQ. Iodination with iodine (I2) and silvertriflate (AgOTf) did not produce the iodinated product, but the dimerized product 66. I-Toc 70 was synthesized with 1-iodopyridinium chloride 71 in a 71% yield (Figure 192).
Nagaki developed an aryl fluorination of lithiated electron-rich and poor aromatic halides (Br, I) in a microreactor flow system.\(^5^{20}\) Fluorination occurred at temperatures from -40°C to 0°C with N-fluorobenzenesulfonimide (NFSI) and N-fluorosulfam yielding fluoroaryls in 30-85% yields. Reaction times from the injection to the isolation of the product were 15 s.

The electrophilic fluorinating reagent chosen for the fluorination of 6-I-\(\alpha\)-Toc was N-fluorobenzenesulfonimide (NFSI), based on reports by Barnette, Satyamurthy and Teare.\(^5^{62,563,564}\) 70 (80-150 mg) was lithiated and fluorinated in batch reactions, first with 1eq of NFSI at 0°C and \(n\)-BuLi as a test reaction (Table 25). The reaction time of \(n\)-BuLi was kept short (5 min), which led to a 5.3% yield of F-Toc and 49% of H-Toc. Deprotonation (lithium halogen exchange) of aryl halides are normally done with 2eq of \(t\)-BuLi, the first for lithium - halogen exchange, the second to quench the \(t\)-butyl halide to isoprene and lithium iodide.\(^5^{65,566}\) NFSI was dried azeotropically with ACN prior to the next reaction and 2eq of NFSI were used to
achieve a higher yield. A one minute reaction time after t-BuLi addition yielded 15% F-Toc (19% H-Toc). Decreasing the reaction time to 30s for each step was attempted with excess n- and t-BuLi (fresh t-BuLi was used) and excess NFSI. Short reaction times with excess base resemble the flow conditions used by Nagaki. In the same paper it was mentioned that the reaction temperature does not influence the reaction yield and so the temperature was changed to room temperature to ease the addition conditions. However, the decreased reaction time did not improve the yield, and even when an 8eq excess of NFSI was used only a small amount of F-Toc was produced. Increasing the lithiation time of 6-I-α-Toc to 50 min at -78°C yielded 8% product after isolation. Lithium-exchange up to 2.75 h did not increase the yield of F-Toc.

![Chemical structure of F-Toc](image)

<table>
<thead>
<tr>
<th>Reagent (eq)</th>
<th>NFSI (eq)</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Stirring time BuLi</th>
<th>Stirring time rxn</th>
<th>Yield F-Toc</th>
<th>Yield H-Toc</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-BuLi (1.3eq)</td>
<td>1.3eq</td>
<td>THF</td>
<td>0°C</td>
<td>5min</td>
<td>30min</td>
<td>5.3%</td>
<td>49%</td>
</tr>
<tr>
<td>t-BuLi (2eq)</td>
<td>2eq</td>
<td>THF</td>
<td>0°C</td>
<td>1min</td>
<td>1min</td>
<td>15%</td>
<td>19%</td>
</tr>
<tr>
<td>n-BuLi (2eq)</td>
<td>4eq</td>
<td>THF</td>
<td>rt</td>
<td>30s</td>
<td>30s</td>
<td>1.1%</td>
<td>4.6%</td>
</tr>
<tr>
<td>t-BuLi (2eq)</td>
<td>4eq</td>
<td>THF</td>
<td>rt</td>
<td>30s</td>
<td>30s</td>
<td>3.1%</td>
<td>30%</td>
</tr>
<tr>
<td>t-BuLi (4eq)</td>
<td>8eq</td>
<td>THF</td>
<td>rt</td>
<td>30s</td>
<td>30s</td>
<td>4.2%</td>
<td>21%</td>
</tr>
<tr>
<td>t-BuLi (2eq)</td>
<td>2eq</td>
<td>THF</td>
<td>-78°C</td>
<td>50min</td>
<td>30min</td>
<td>8.3%</td>
<td>16.4%</td>
</tr>
<tr>
<td>t-BuLi (2eq)</td>
<td>2eq</td>
<td>THF</td>
<td>-78°C</td>
<td>2.5h</td>
<td>15min</td>
<td>9.6%</td>
<td>53.0%</td>
</tr>
</tbody>
</table>

Table 25. Fluorination of 6-I-α-toc with BuLi and NFSI.

Reactions were run on a 0.16-0.27 mmol scale as 0.85 M solutions.

All the conditions tried were not as successful as expected. F-Toc was synthesized in a 15% yield in a 2 min reaction time. This is a promising result for this chemistry, but optimization is necessary. Running the reaction in a flow microreactor should be the next step in future attempts. Automated processes based on flow chemistry are already used in hospitals to produce radioactive tracers like $^{18}$F-FDG. Advantages of the flow microreactor are the fast reaction times, superior control of the addition speed and better mixing.\textsuperscript{567}
11.2.2.2 H-Toc fluorination to 6-F-Toc

Tocopherol analogue H-Toc was fluorinated with nitrogen based fluorinating agents like N-fluorobenzenesulffimide (NFSI), Selectfluor® and 1-fluoropyridinium tetrafluoroborate (F-Py BF₄) (Figure 193).⁵⁶⁸, ⁵⁰⁶

![Figure 193. Synthesis of F-Toc from H-Toc with electrophilic fluorinating reagents F-Py BF₄, Selectfluor® and NFSI.⁵⁶⁸,⁵⁰⁶](image)

Reaction of H-Toc, 42, with Selectfluor® did not yield any product in 0.25 M acetonitrile or THF. NFSI yielded product after several days of stirring in THF. NFSI fluorination has been done in neat conditions with mono- to pentamethylated benzene, yielding fluorinated polysubstituted benzenes with yields up to 70%.⁵⁶⁹ H-Toc reaction with neat NFSI yielded with H-Toc in 10-15 min reaction time up to 34% product (Table 26). Increasing the temperature to 150°C slightly increased the yield with NFSI and additionally gave product with neat Selectfluor®. To increase reactivity, the reaction was tested as a concentrated solution (1M solutions) in different solvents. The solvent should help to increase contact between the electrophilic fluorinating reagents and H-Toc. When THF was the solvent product was recovered with NFSI, and DMSO did not yield any product in any case. Fortunately, when the solvent was DMF all fluorinating reagents gave product. The highest yields were obtained with
acetonitrile and NFSI. Using excess (4 eq) NFSI or radical scavenger (2,2,6,6-
tetramethylpiperidin)-1-oxyl (TEMPO) did decrease product yield. The addition of TEMPO
was though to lower radical byproduct formation and increase the yield by an electrophilic
aromatic fluorination mechansim.\textsuperscript{570} However, the decreased yield with TEMPO verifies that
reaction mechanism is based on a single-electron transfer (SET).\textsuperscript{571,572}

<table>
<thead>
<tr>
<th>F\textsuperscript{+}-Source</th>
<th>Solvent\textsuperscript{a}</th>
<th>Temp (°C)</th>
<th>Time</th>
<th>Yield\textsuperscript{b} 6-F-Toc (%)</th>
<th>Re-isolated 6-H-Toc (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Select F</td>
<td>1eq 0.25M ACN</td>
<td>120</td>
<td>20min-</td>
<td>days 0%</td>
<td>0%</td>
</tr>
<tr>
<td>Select F</td>
<td>1eq 0.25M THF</td>
<td>80</td>
<td>20min</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>NFSI</td>
<td>2eq 0.12M THF</td>
<td>40</td>
<td>4days</td>
<td>25%</td>
<td>26%</td>
</tr>
<tr>
<td>NFSI</td>
<td>2eq 0.12M THF</td>
<td>60</td>
<td>4days</td>
<td>20%</td>
<td>0%</td>
</tr>
<tr>
<td>NFSI</td>
<td>1eq Neat</td>
<td>100</td>
<td>13min</td>
<td>34%</td>
<td>26%</td>
</tr>
<tr>
<td>Py-F BF\textsubscript{4}</td>
<td>1eq Neat</td>
<td>100</td>
<td>13min</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Py-F BF\textsubscript{4}</td>
<td>1eq Neat</td>
<td>150</td>
<td>13min</td>
<td>38%</td>
<td>11%</td>
</tr>
<tr>
<td>Py-F BF\textsubscript{4}</td>
<td>1eq 1.0M THF</td>
<td>80</td>
<td>13min</td>
<td>37%</td>
<td>60%</td>
</tr>
<tr>
<td>Py-F BF\textsubscript{4}</td>
<td>1eq 1.0M THF</td>
<td>80</td>
<td>13min</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Py-F BF\textsubscript{4}</td>
<td>1eq 1.0M ACN</td>
<td>80</td>
<td>13min</td>
<td>43%</td>
<td>34%</td>
</tr>
<tr>
<td>NFSI</td>
<td>1eq 1.0M DMSO</td>
<td>150</td>
<td>13min</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>NFSI</td>
<td>1eq 1.0M DMSO</td>
<td>150</td>
<td>13min</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Py-F BF\textsubscript{4}</td>
<td>1eq 1.0M DMSO</td>
<td>150</td>
<td>13min</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>NFSI</td>
<td>1eq 1.0M DMF</td>
<td>150</td>
<td>13min</td>
<td>15%</td>
<td>30%</td>
</tr>
<tr>
<td>NFSI</td>
<td>1eq 1.0M DMF</td>
<td>150</td>
<td>13min</td>
<td>12%</td>
<td>43%</td>
</tr>
<tr>
<td>Py-F BF\textsubscript{4}</td>
<td>1eq 1.0M DMF</td>
<td>150</td>
<td>13min</td>
<td>2.3%</td>
<td>97%</td>
</tr>
<tr>
<td>NFSI</td>
<td>4eq Neat</td>
<td>100</td>
<td>13min</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>NFSI</td>
<td>0.5eq Neat</td>
<td>100</td>
<td>13min</td>
<td>12%</td>
<td>52%</td>
</tr>
<tr>
<td>NFSI\textsuperscript{c}</td>
<td>1eq Neat</td>
<td>100</td>
<td>13min</td>
<td>3.3%</td>
<td>38%</td>
</tr>
</tbody>
</table>

Table 26. Fluorination of H-Toc with electrophilic fluorinating reagents.

General procedure: In an open vial, 6H-Toc (20-60 mg, 0.480-1.45 mmol) was stirred with the chosen
fluorinating agent for 10-15 min at elevated temperature neat or in a solvent. The reaction mixture
was extracted with DCM and water, and the organic phase evaporated. The crude mixture was
washed over a small SiO\textsubscript{2} column with hexane and was analysed by \textsuperscript{1}H and \textsuperscript{19}F NMR. (a) dry solvents
were used. (b) \textsuperscript{1}H NMR yields (c) 1 eq TEMPO added.
11.2.2.3 HPLC profile of 6-F-tocopherol

In PET imaging facilities the radioactive product is analysed by HPLC. A radioactive detector is used to see if the radionuclide has been incorporated into the molecule and if any radioactive by-products have been produced. Radionuclide detection by HPLC is also needed to verify the specific activity of the ¹⁸F-product prior to injection into the patient. A chromatographic method has to be created with “cold” ¹⁹F-product to have a reference for the PET imaging facilities. A short-time method is preferred to save time in the overall process of producing and analysing the imaging agent.⁵⁷³

Halogenated tocopherols and H-Toc are not soluble in water, and barely in acetonitrile and methanol (< 1mg/ml). Thus, dichloromethane was chosen as a solvent to solubilize the product for injection into the HPLC.

HPLC separation on a standard reverse phase C18 column with polar solvents turned out to be not applicable here, as no chromatographic separation was seen between H-Toc and F-Toc at retention times up to 40-50 minutes, with ACN : water (60:40). In the hope of a better separation, the mobile phase was changed to non-polar solvents. Non-polar reverse phase (NARP) chromatography has been used in the past to separate non-polar molecules like carotenes with great success.⁵⁷⁴ Using a Zorbax ODS column, as in the examples with carotenes, however, did not enhance the separation of H-Toc and F-Toc. A polystyrene based XDB-phenyl column was then chosen, since it promises a better separation of differently substituted aromatic compounds.⁵⁷⁵ The NARP was tested first with a DCM : MeOH or DCM : ACN (95:5 - 80:20). In short elution times of 2-5 minutes separation of the halogenated tocopherols was achieved, but no separation of F-Toc and H-Toc was observed (Figure 194).
Figure 194. HPLC separation of α-toc, F-Toc, H-Toc, 6-I-α-toc, 6-Cl-α-toc.
Mobile phase gradient ACN:DCM 80:20, Flow rate: 1.0 ml/min. Stationary phase Zorbax XBD Phenyl 30 cm. The sample was dissolved in 10 µl DCM and 40 µl MeOH, injection volume 2 µl. 25°C oven temperature, UV detection at 280 nm.

Switching the mobile phase back to MeOH : H2O led to the separation of H-Toc (26.21 min) and F-Toc (26.52 min) (Figure 195). Separation between H-Toc (26.46 min), Cl-Toc (28.80 min) and I-Toc (31.65 min) using the same mobile phase was effective to the point were no overlap occurs between the peaks (Figure 196).
Figure 195. HPLC separation of F-Toc and H-Toc.

Overlay of two separate 6'F-Toc and 6'H-Toc injections to show the spectral overlap. Mobile phase gradient MeOH:H₂O 80:20, 10 min 85:15, 15 min 90:10, 20 min 95:5, Flow rate: 1.0 ml/min.
Stationary phase Zorbax XBD Phenyl 30cm. The sample was dissolved in 10 µl DCM and 40 µl MeOH, injection volume 2 µl. 25°C oven temperature, UV detection at 280nm. Y-axis shows a normalized mAU.
Mobile phase gradient MeOH:H₂O 80:20, 10 min 85:15, 15 min 90:10, 20 min 95:5. Flow rate: 1.0 ml/min. Stationary phase Zorbax XBD Phenyl 30 cm. The sample was dissolved in 10 µl DCM and 40 µl MeOH, injection volume 2 µl. 25°C oven temperature, UV detection at 280 nm. Y-axis shows a normalized mAU.

To obtain a faster and better separation, a polyfluorinated C8 silica column will be used in the future. Differentially fluorinated benzenes have been separated on this stationary phase in less than 10 min (Figure 197). ⁵⁷⁶
Figure 197. Separation of benzenes with increasing fluorine substitution.

Top spectra: 18C-column, bottom spectra: C_{8}F_{17}-column.\textsuperscript{576}

11.2.3 \(\gamma\)-Tocopherol fluorination to 5-fluoro-\(\gamma\)-tocopherol

Electrophilic fluorination of \(\gamma\)-tocopherol was attempted using similar reagents as for F-Toc.\textsuperscript{577}

In a recent paper by Poon \textit{et al.} \(\gamma\)-tocopherol was lithiated at the 5-position and reacted with tellurium tetrachloride (TeCl\(_{4}\)) to create a telluro-\textit{bis}-tocopherol antioxidant.\textsuperscript{578} Instead of tellurium we used fluorinating reagents to quench the lithiated \(\gamma\)-tocopherol (Figure 198).
Electrophilic $^{18}$F-fluorination of phenols has been used to create $^{18}$F-labeled L-DOPA / tyrosine for neurological studies or $^{18}$F-phenolphthalein as in situ pH sensors.$^{579,580,581,582}$ Fluorine gas and AcOF have been used to fluorinate phenols, but the high reactivity of these reagents does require careful handling and tends to produce unwanted oxidized or polyfluorinated sideproducts (Figure 199).$^{583}$
Figure 199. Electrophilic $^{18}$F-fluorination of phenols.

(a) 3-$^{18}$F-tyrosine, (b) L-$^{18}$F-DOPA and (c) $^{18}$F-phenolphthalein.$^{579-582}$

Alternatively, nitrogen based electrophilic fluorinating reagents have been used because they are easier to handle and bench stable (Figure 200).$^{584,516}$
Figure 200. Phenol fluorination with electrophilic fluorinating reagents (a) NFSI, (b) Selectfluor and (c) 1-Fluoropyridinium triflate,^585,587,588^ Bench stable, commercially available nitrogen-based fluorinating reagents N-fluorobenzenesulfonyl fluoride (NFSI),^585^ 1-fluoropyridinium tetrafluoroborate (F-Py BF₄ or 1-Fluoropyridinium triflate F-Py OTf)^586,587^ and Selectfluor®^588^ have been used to fluorinate phenols (Figure 200) and were chosen for the fluorination of γ-tocopherol (Table 27)^516^.
<table>
<thead>
<tr>
<th>Reaction</th>
<th>Fluorinating Agent</th>
<th>Concentration</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selectfluor®</td>
<td>1eq</td>
<td>0.25M ACN</td>
<td>80</td>
<td>15min-2h</td>
</tr>
<tr>
<td>Selectfluor®</td>
<td>1eq</td>
<td>0.25M ACN</td>
<td>rt</td>
<td>80min</td>
</tr>
<tr>
<td>NFSI</td>
<td>1eq</td>
<td>0.25M THF</td>
<td>rt</td>
<td>1h</td>
</tr>
<tr>
<td>NFSI</td>
<td>3eq</td>
<td>0.25M THF</td>
<td>0°C</td>
<td>1h</td>
</tr>
</tbody>
</table>

Table 27. Fluorination of $\gamma$-tocopherol with electrophilic fluorinating reagents.

To a solution of $\gamma$-tocopherol was added the fluorinating reagent added under $N_2$ atmosphere. Reactions with Py BF$_4$ and Selectfluor® were conducted at room temperature and 80°C. NFSI was reated at room temperature and at lower temperature with increased amounts of NFSI. The solvent was evaporated and the residual mixture was extracted with water and DCM. NFSI reactions were purified by SiO$_2$ chromatography. (a) dry solvents (b) added 1 eq of NEt$_3$.

None of the reactions with F-Py-BF$_4$ produced any product, 48, even at elevated temperature and extended reaction time. Selectfluor® produced traces of unidentifiable byproducts. None of these byproducts showed any signal in the $^{19}$F-NMR. Reaction with NFSI created byproducts 5-NFSI-tocopherol, 74, bis-5,5'-tocopherol, 75, and $\gamma$-tocopherol quinone (Figure 201).

![Figure 201. Byproducts 76 formed during the fluorination of $\gamma$-tocopherol sodium phenolate with NFSI.](image)

Radical reactions are common with electrophilic fluorinating agents. To counteract the radical reaction the nucleophilicity of the phenol was increased by turning it into the sodium phenoxide, described by Barnette with 2-naphthol.562 However, no fluorination occurred, but the phenol was turned into the benzenesulfonate 75 (Figure 202).
Fluorination was then attempted via lithium-halogen exchange of 5-bromo-δ-tocopherol. Poon was able to create a 5,5′-δ-telluro-bis-bistocopherol this way. Stirring the reaction at -78°C overnight was necessary to install the tellurium (Figure 203).

To obtain 5-Br-α-tocopherol 76 γ-tocopherol was reacted with tetrabutylammonium bromide (TBAB) (Figure 204).

To rapidly fluorinate γ-tocopherol, after the lithium-bromide exchange at -78°C for 2 h, the fluorinating agent was added and warmed to room temperature. Despite increasing the temperature to 50°C the reaction only yielded γ-tocopherol with F-Py BF₄ and Selectfluor®. After 5 minutes reaction time with NFSI, 77 yielded only the benzosulfonate byproduct 76 (Figure 205).
Attemps to fluorinate γ-tocopherol by electrophilic fluorination did not work with any of the chosen fluorinating reagents. In the future, instead of nitrogen-based reagents, acetylhypofluorite in acidic media will be used to fluorinate γ-tocopherol (Figure 206).

11.2.4 5-F-methyl-α-tocopherol synthesis

Benzylic fluorination at the 5-methyl was tested on the acetyl protected α-tocopherol 5-bromomethyl (AcO-5-Br-Me-α-toc) as a precursor, with nucleophilic fluorinating agents like TBAF, KF-cryptand[2.2.2] and CsF. After deprotection of the acetyl group the targeted 5-F-methyl-α-tocopherol was formed. Oxidation of α-tocopherol with silver oxide in non-polar solvents forms the highly reactive ortho-quinone methide (oQM), which is susceptible to nucleophilic addition at the 5-position. Formation of 5-Br-Me-α-toc goes through the same oQM mechanism when brominated with bromine. One part of the bromine is used to abstract
the benzylic hydrogen at the 5-position, the second bromine atom acts as a nucleophile at the electrophilic benzylic position. Thus, fluoride addition might occur in the same manner as in the second step of the bromination (Figure 207).\textsuperscript{589} Since the oQM is such a reactive intermediate additives like N-methylmorpholine-N-oxide (NMMO) and sulfur-ylids (S-ylide) can be used to stabilize the intermediate oQM.\textsuperscript{590,591}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure207.png}
\caption{Synthetic strategies towards 5-F-Me-\(\alpha\)-tocopherol by nucleophilic fluorination.}
\end{figure}

Fluorination at the benzylic position of an aromatic group has been used to create \(^{18}\text{F}\)-labeled biologically active compounds like nabumetone, celestolide or papaverine (Figure 208).\textsuperscript{592}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure208.png}
\caption{Benzylically \(^{18}\text{F}\)-labeled biologically active compounds.\textsuperscript{592}}
\end{figure}

Synthetically, benzylic fluorinations are much easier than aromatic fluorinations, because nucleophilic substitution reactions can be used. More recently, metal catalyzed benzylic C-H
fluorinations have been used for PET ligand chemistry with great success. δ-Tocopherol was fluorinated by C-H activation with a manganese(salen) catalyst (Figure 209).\(^{592}\)

![Figure 209 Manganese(salen) catalyzed fluorination of δ-tocopherol.](image)

11.2.4.1 Synthesis of 5'-F-methyl-α-tocopherol by nucleophilic substitution:

Nucleophilic substitution with fluoride is done by replacing leaving groups like bromine, iodine or tosylates. Substitution at benzylic positions is seen in late stage fluorination to create \(^{18}\)F-trifluoromethyl groups and in the creation of \(^{18}\)F-precursors used in a multistep synthesis (Figure 210).\(^{593,594,595}\)

![Figure 210. Benzylic \(^{18}\)F-fluorination by nucleophilic substitution.](image)
To fluorinate α-tocopherol at the 5-methyl position O-acetyl protected 5-bromomethyl-α-tocopherol, 77, was chosen. The bromine has been replaced previously with other nucleophiles like alcohols, acids, phosphines, and amines.596 The 5'-methyl position of tocopherol was used as a benzylic protecting group of the nitrogen in amino acids (Figure 211).597

Because 18F-fluorination reactions are run at elevated temperature (80-250°C) protection of the tocopherol phenol is necessary. At temperatures above > 40°C the 5-Br-α-tocopherol spontaneously turns into the spiro-dimer (Figure 212).589

AcO-5-Br-α-tocopherol 77 is obtained by bromination of α-tocopherol, followed by reaction with acetic anhydride (Figure 213).589
Compound 77 was exposed to potential fluorination conditions using potassium fluoride in acetonitrile or DMF and carrier additives 18-crown-6, cryptand[2.2.2], or tetrabutylammonium (Table 28).

Table 28. Fluorination of AcO-5-Br-Me-α-toc.\textsuperscript{77}

<table>
<thead>
<tr>
<th>F-Source</th>
<th>Equiv.</th>
<th>Carrier</th>
<th>Solvent\textsuperscript{a}</th>
<th>Temp (°C)</th>
<th>Time</th>
<th>Prod 5-F</th>
<th>Prod 5-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>KF\textsuperscript{b}</td>
<td>1eq</td>
<td>-</td>
<td>50mM ACN</td>
<td>70</td>
<td>16h</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KF\textsuperscript{b}</td>
<td>1eq</td>
<td>18-Crown-6</td>
<td>50mM ACN</td>
<td>70</td>
<td>16h</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KF\textsuperscript{c}</td>
<td>1eq</td>
<td>Cryptand 2.2.2</td>
<td>50mM ACN</td>
<td>100</td>
<td>5min (16h)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KF</td>
<td>1eq</td>
<td>-</td>
<td>50mM DMF</td>
<td>70</td>
<td>16h</td>
<td>-</td>
<td>23%</td>
</tr>
<tr>
<td>KF</td>
<td>2eq</td>
<td>-</td>
<td>250mM DMF</td>
<td>100</td>
<td>1h</td>
<td>6%</td>
<td>13%</td>
</tr>
<tr>
<td>KF\textsuperscript{c}</td>
<td>2eq</td>
<td>-</td>
<td>160mM DMF</td>
<td>100</td>
<td>1h</td>
<td>-</td>
<td>16%</td>
</tr>
<tr>
<td>KF</td>
<td>2eq</td>
<td>-</td>
<td>100mM DMF</td>
<td>100</td>
<td>16h</td>
<td>6%</td>
<td>11%</td>
</tr>
<tr>
<td>TBAF</td>
<td>3eq</td>
<td>-</td>
<td>40mM THF\textsuperscript{c}</td>
<td>60</td>
<td>20min</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

All reactants were stirred for 20 min and analysed. (a) dry solvent (b) No product was observed in 20 min-1 h. Continued the reaction up to 16 h. (c) Microwave reactor 200 W.

Conducting the reaction in acetonitrile did not yield any product, even with carrier addition. Only small amounts of product, 78, were observed with DMF as the solvent with 2 eq of KF along with the hydro-debrominated product (α-tocopherol acetate) as a byproduct. Heating the
reaction with microwave irradiation was not successful, but decreasing the amount of solvent (250 mM) yield some product in 1h.

A higher yield of product 78 was obtained when the reaction was run in t-butanol and cesium fluoride (CsF). Along with product, 78, 5’-t-butoxyl substituted byproduct, 79, was also formed (Figure 214).

![Figure 214. Fluorination of 78 with CsF and t-BuOH. Formation of product 78 and byproduct 79.](image_url)

The CsF / t-BuOH methods has an advantage over the KF / DMF fluorination because the t-BuO-containing byproduct, 79, is more easily removed by chromatographic purification than the α-tocopheryl acetate.

Acetyl protecting groups of phenols are normally removed in basic media. Deprotection with KOH in EtOH did cleave the acetoxy group, but also substituted the fluorine to create the ethoxyether, 80 (Figure 215).

![Figure 215 Deprotection of 78 in basic media with KOH in EtOH. Formation of ethoxyether 80.](image_url)

Substitution was not expected, since fluorine-carbon bonds are stronger than bromine-carbon bonds (BDE $\Delta H^\circ_{298} = \text{CH}_3\text{F}: (95.9 \text{ kcal/mol}) - \text{CH}_3\text{Br}: (56.2 \text{ kcal/mol})$. Instead of basic deprotection we tried the hydrolysis with water and catalytic amounts of trifluoroacetic acid (TFA) in acetonitrile. Deprotection did not work and instead the TFA ester, 81, was formed by TFA anion substitution of the 5-fluoromethyl of 78 (Figure 216).
Figure 216. Attempted deprotection of AcO-5-F-Me-α-toc 78 in acetic media with TFA and formation of byproduct 5-trifluoracetoxyl alpha-tocopherol acetate 81.

Perchloric acid (HClO₄) was chosen as an acid, because the perchlorate (ClO₄) counter ion is less nucleophilic than the trifluoroacetate (Table 29).

![Chemical reaction diagram]

**Table 29.** 78 was stirred in a μ-wave oven or for 10-20 min.

(a) When no product was observed was the reaction continued stirring for 16 h with conventional heating. (b) AcO-α-tocopherol.

None of the applied conditions yielded any de-acetylated product, 49. Stirring the reaction overnight formed a unidentifiable spot on TLC, which did not contain any fluorine peak in the ¹⁹F-NMR. The same unidentifiable spot was observed when acetyl protected-α-tocopherol (last entry (b) ) was stirred over night.
11.2.4.2 5-F-methyl-α-tocopherol synthesis by orthoquinone methide formation:

Silveroxide (Ag₂O) is used in non-polar solvent to form the α-tocopherol ortho-quinone methide (oQM) from α-tocopherol. Without stabilization of the oQM the tocopherol-spirodimer is formed instantly at any temperature. Rosenau reported the stabilization of the oQM with NMNO at -78°C.⁶⁰⁰,⁶⁰¹ A later paper by the same author described the used of 2,5-bis(dimethylsulfonio)-3,6-dioxocyclohexa-1,4-diene-1,4-bis(olate) (S-ylide) to stabilize the oQM intermediate (Figure 217). The S-ylide stabilizes oQM for a longer time at temperatures up to 40°C.⁶⁰²

Figure 217. α-Tocopherol ortho-quinone methide (oQM) dimerization to the α-tocopherol-spirodimer and stabilization of the α-tocopherol oQM by NMNO and S-ylide.⁶⁰⁰,⁶⁰¹,⁶⁰²

The sulfur-ylide has been applied to stabilize the ortho-quinone methide of ortho-cresol, which was quenched with acetylsalicylic acid to form (O-acetylsalicyl)saligenin (Figure 218).⁶⁰²

Figure 218. Synthesis of (O-acetylsalicyl)saligenin from o-cresol.⁶⁰²
The reaction was first tested without any stabilizing agent with excess cesium fluoride in CHCl₃ and DMSO at -78°C and room temperature, but no product was obtained. Tetrabutylammonium fluoride was chosen as the carrier additive in hexane and THF, without success (Table 30).

<table>
<thead>
<tr>
<th>F-Source</th>
<th>Equiv.</th>
<th>Stabilizer</th>
<th>Solvent</th>
<th>Temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CsF</td>
<td>5eq</td>
<td>-</td>
<td>5mM CHCl₃</td>
<td>-78</td>
</tr>
<tr>
<td>CsF</td>
<td>10eq</td>
<td>-</td>
<td>10mM DMSO</td>
<td>rt</td>
</tr>
<tr>
<td>CsF</td>
<td>6eq</td>
<td>-</td>
<td>15mM DMSO / hexane 1:2</td>
<td>rt</td>
</tr>
<tr>
<td>TBAF 1M THF</td>
<td>10eq</td>
<td>hexane</td>
<td></td>
<td>rt</td>
</tr>
<tr>
<td>TBAF 1M THF</td>
<td>10eq</td>
<td>THF</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

Table 30 α-Tocopherol fluorination with Ag₂O.

To silver oxide (Ag₂O) and fluoride (CsF or TBAF) in the chosen solvent was α-tocopherol added at the given temperature. (a) dry solvent.

All attempts to quench α-tocopherol oQM with fluoride did not result in fluorination but rather formed the spirodimer byproduct. Stabilizing the reaction with NMMO in CHCl₃ was reported to occur at -78°C, however CHCl₃ solidifies at this temperature, preventing any reaction.

Having the temperature above -78°C to melt the solvent initiated spirodimer formation. The solvent was therefore changed to hexane, but did not yield any product. The reaction with the S-ylide was followed according to procedure, but did not yield any product either. No fluorine incorporation was observed by ¹⁹F-NMR in any attempt and the only products observed were the α-tocopherol-spirodimer and α-tocopherol-quinone after prolonged stirring (Table 31).
α-Tocopherol fluorination at the γ-methyl position was successful by nucleophilic substitution of the acetyl protected 5-bromomethyl-α-tocopherol, but deprotection of the phenol ester was not possible without defluorination. Nucleophilic fluorination on α-tocopherol ortho-quinone methide did not produce any product, despite the use of stabilizing agents.

Table 31. α-Tocopherol fluorination with Ag₂O and stabilizing agents.

<table>
<thead>
<tr>
<th>F⁻-Source</th>
<th>Equiv.</th>
<th>Stabilizer</th>
<th>Stirring time ylide</th>
<th>Solvent a</th>
<th>Temp F⁻-addition(°C)</th>
<th>Stirring time</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBAF</td>
<td>2 eq</td>
<td>NMMO</td>
<td>1 min</td>
<td>10 mM CHCl₃</td>
<td>-78</td>
<td>5 min (rt)</td>
</tr>
<tr>
<td>TBAF</td>
<td>2 eq</td>
<td>NMMO</td>
<td>1 min</td>
<td>10 mM hexane</td>
<td>-78</td>
<td>5 min (rt)</td>
</tr>
<tr>
<td>CsF / cryptant 2.2.2</td>
<td>2 eq</td>
<td>NMMO</td>
<td>1 min</td>
<td>10 mM hexane</td>
<td>-78</td>
<td>5 min (rt)</td>
</tr>
<tr>
<td>TBAF</td>
<td>1 eq</td>
<td>S-ylide</td>
<td></td>
<td>160 mM CHCl₃/DMSO</td>
<td>-10</td>
<td>15 min</td>
</tr>
<tr>
<td>TBAF</td>
<td>2 eq</td>
<td>S-ylide</td>
<td>20 min</td>
<td>160 mM CHCl₃/DMSO</td>
<td>40</td>
<td>1h</td>
</tr>
<tr>
<td>CsF / cryptant 2.2.2</td>
<td>2 eq</td>
<td>S-ylide</td>
<td>30 min</td>
<td>160 mM CHCl₃/DMSO</td>
<td>rt</td>
<td>2h</td>
</tr>
<tr>
<td>TBAF</td>
<td>1 eq</td>
<td>S-ylide</td>
<td>30 min</td>
<td>1M CHCl₃/DMSO</td>
<td>rt</td>
<td>16h</td>
</tr>
</tbody>
</table>

Procedure NMMO: Silver oxide in CHCl₃ or hexane was cooled to -78°C. NMMO was added, followed by addition of NMMO. α-Tocopherol was added directly followed by F⁻. Procedure S-ylide: Silver oxide (5 eq) was cooled in dry CHCl₃ (70 ml) to -40°C and α-tocopherol (100 mg) added. S-ylide (0.55 eq) was added with DMSO (2 ml). After 30 min was F⁻ added to the α-tocopherol mixture and the reaction warmed up to -10-40°C in 5 min. Work up: After the given time both reactions were quenched with water and the organic phase separated and washed several times with water. Note: The silver oxide reactivity was tested by adding silver oxide directly into a solution of α-tocopherol in CHCl₃, which directly formed the spiro-dimer.
11.2.5 6-F-methyl-\(\alpha\)-tocopherol synthesis

HM-toc 44 was used as the starting material to create 50 by displace the benzyl hydroxyl group with sulfur based deoxyfluorination reagents diethylaminosulphurtrifluoride (DAST) and Xtalfluor E & M\textsuperscript{603}; and by nucleophilic fluorination of hydroxyl activated sulfonates\textsuperscript{604} or activation by protic / Lewis acids (Figure 219).\textsuperscript{605}

\[
\begin{align*}
\text{HM-Toc} & \xrightarrow{- \text{deoxyfluorination}} \text{6'-F-Me-\(\alpha\)-Toc} \\
R = \text{OTs, OMs} & \xrightarrow{\text{F}^{-}, \text{Lewis acid or H}^{+}} \text{6'-F-Me-\(\alpha\)-Toc}
\end{align*}
\]

Figure 219. Synthetic strategies towards 6'-F-Me-\(\alpha\)-tocopherol 50.

Fluorination of HM-Toc with deoxyfluorination reagents and by Lewis acid / H\(^+\) activation.\textsuperscript{603}

Alternatively by fluorination of the benzylic sulfonates.\textsuperscript{604,605}

Radiofluorination of benzylic hydroxides has been described by Chen to create a \(^{18}\text{F}\)-fluoromethyl linker, which was used to attach to spiperone and 1-phenylpiperazine (Figure 220).\textsuperscript{606}

\[
\begin{align*}
\text{HO} & \xrightarrow{\text{XCl}} \text{XO} \\
X = \text{Ms, Ts} & \xrightarrow{^{18}\text{F}^{-}} \text{XO} \text{C}^{18}\text{F}
\end{align*}
\]

\[
\begin{align*}
N-(\text{benzyl-}p-{^{18}\text{F}}\text{methyl})\text{ spiperone} & \xrightarrow{\text{n-Bu}_4\text{NOH}} \text{N-(benzyl-}p-{^{18}\text{F}}\text{methyl)-1-phenylpiperazine}
\end{align*}
\]

Figure 220. Synthesis of benzylic-\(^{18}\text{F}\)-fluoromethyl-spiperone and 1-phenylpiperazine.\textsuperscript{606}
Instead of having sulfonates as leaving groups halides like bromine and iodine have been successfully used, as seen in the example of the fluorination \(^{18}\)F-fluorination of methyl 4-(bromomethyl)-2-chlorobenzoate (Figure 221). \(^{607}\)

\[
\begin{align*}
\text{Br} & \quad \text{Cl} & \quad \text{O} & \quad \text{Cl} & \quad \text{O} \\
\text{n-Bu}_4\text{NOH} & \quad \text{THF} & \quad 90^\circ\text{C}, 5\text{min} & \quad \text{Br} & \quad \text{Cl} & \quad \text{O} & \quad \text{Cl} & \quad \text{O} \\
& & & & & & & & & & & & & & & & \text{18F-} & \quad \text{OF} \\

\text{18F-fluorination of methyl 4-(bromomethyl)-2-chlorobenzoate.}^{607}
\end{align*}
\]

Xtal-fluor M\(^\text{®}\), Xtalfluor E\(^\text{®}\) and DAST are reagents used to displace hydroxyl groups by forming a reactive sulfonate intermediate, which is quenched in situ with a fluorine from the reagent. \(^{608,609}\) A \(^{18}\)F version of DAST has been synthesized, but has not yet been used in any relevant synthetic procedure (Figure 222). \(^{610}\)

\[
\begin{align*}
\text{HO} & \quad \text{Deoxyfluorination} & \quad \text{F} & \quad \text{HO} \\
\text{DAST} & \quad \text{Xtalfluor-E\textsuperscript{®}} & \quad \text{Xtalfluor-M\textsuperscript{®}} \\
\text{F} & \quad \text{F} & \quad \text{F} & \quad \text{F} & \quad \text{F} & \quad \text{F} & \quad \text{F} & \quad \text{F} & \quad \text{F} & \quad \text{F} \\
\text{N} & \quad \text{S} & \quad \text{F} & \quad \text{N} & \quad \text{S} & \quad \text{F} & \quad \text{N} & \quad \text{S} & \quad \text{F} & \quad \text{N} & \quad \text{S} & \quad \text{F} \\
\text{R}_2\text{N} & \quad \text{S} & \quad \text{O} & \quad \text{F} & \quad \text{R}_2\text{N} & \quad \text{S} & \quad \text{O} & \quad \text{F} \\

\text{Figure 222. Deoxyfluorination of benzylic alcohols with DAST, Xtalfluor-E\textsuperscript{®} and Xtalfluor-M\textsuperscript{®}.}^{608-609}
\end{align*}
\]

Reaction with of Xtalfluor M, E and DAST with hydroxymethyl-tocopherol (HM-Toc) instantaneously formed ether 46, as the only isolated product in all attempts (Table 32). \(^{608}\)
Table 32. Deoxyfluorination of HM-Toc.

<table>
<thead>
<tr>
<th>F⁻-Source</th>
<th>Reaction time: N⁺-F</th>
<th>Base</th>
<th>Reaction time: base</th>
<th>Solvent</th>
<th>Temp (°C)</th>
<th>Byproduct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xtalfluor E</td>
<td>2min</td>
<td>DBU (1.5eq)</td>
<td>5min</td>
<td>DCM (130µM)</td>
<td>-78</td>
<td>20%</td>
</tr>
<tr>
<td>(1.5eq)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xtalfluor M</td>
<td>5min</td>
<td>DBU (1.5eq)</td>
<td>5min</td>
<td>DCM (75µM)</td>
<td>0</td>
<td>80%</td>
</tr>
<tr>
<td>(1.5eq)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xtalfluor M</td>
<td>1min</td>
<td>DBU (1.0eq)</td>
<td>2min</td>
<td>DCM (75µM)</td>
<td>0</td>
<td>88%</td>
</tr>
<tr>
<td>(1.0eq)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAST</td>
<td></td>
<td>Morpholine</td>
<td>15min</td>
<td>DCM (75µM)</td>
<td>0</td>
<td>94%</td>
</tr>
<tr>
<td>(1.0eq)</td>
<td></td>
<td>(0.1eq)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xtalfluor M</td>
<td>2min</td>
<td>DBU (1.0eq)</td>
<td>2min</td>
<td>DCM (37µM)</td>
<td>-78</td>
<td>35%</td>
</tr>
<tr>
<td>(1.0eq)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xtalfluor M</td>
<td>2min</td>
<td>DBU (1.0eq)</td>
<td>5min</td>
<td>DCM 15ml (7.6µM)</td>
<td>-78</td>
<td>22%</td>
</tr>
<tr>
<td>(1.0eq)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xtalfluor E</td>
<td></td>
<td>DBU</td>
<td>3h</td>
<td>DCM (38µM)</td>
<td>-78</td>
<td>6%</td>
</tr>
<tr>
<td>(1.0eq)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAST</td>
<td></td>
<td>-</td>
<td>3h</td>
<td>DCM (18µM)</td>
<td>-78</td>
<td>43%</td>
</tr>
<tr>
<td>(1.0eq)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To a solution of HM-Toc in dry DCM was Xtalfluor E, -M or DAST added at the given temperature under N₂. After 1-5 min was the chosen base added and stirred for a given time. The reaction mixture was concentrated and directly purified over a SiO₂ column. (a) Reverse addition: HM-Toc was added to the deoxyfluorinating agent and DBU at -78°C temperature.

Nucleophilic substitution with activated F⁻ in the form of CsF in t-BuOH has worked to replace the 5'-bromomethyl-α-tocopherol to form the 5-fluoromethyl-α-tocopherol (see section 5-F-
methyl-α-tocopherol synthesis). Repeating the same reaction with HM-Toc, using catalytic amounts of hydrogen iodide (HI) to activate the benzylic hydroxide yielded instead the byproduct 6′-t-BuO-α-Toc, 81, alongside dimer, 46, as a crude mixture. Acidic activation in the presents of excess TBAF did not yield any product (Figure 223).

Figure 223. HM-Toc fluorination with CsF and t-BuOH. Synthesis of byproduct 46 and 81.598

Makino described a way to directly fluorinate benzyl alcohols using KF activated with a phase transfer catalyst like 18-crown-6 and tosyl fluoride (TsF) as a Lewis acid. Attempts to fluorinate HM-Toc with KF-16-crown-6 and CsF-cryptand[2.2.2] with TsF did not produce any product (Table 33).605

<table>
<thead>
<tr>
<th>Source</th>
<th>Carrier additive</th>
<th>Lewis acid</th>
<th>Solvent</th>
<th>Time</th>
<th>Temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KF (4eq)b</td>
<td>[2.2.2] cryptant (1eq)</td>
<td>TsF (2eq)</td>
<td>THF (80mM)</td>
<td>3h</td>
<td>rt</td>
</tr>
<tr>
<td>CsF (4eq)a</td>
<td>18-crown-6 (1eq)</td>
<td>TsF (2eq)</td>
<td>THF (80mM)</td>
<td>3h</td>
<td>rt</td>
</tr>
</tbody>
</table>

Table 33. Nucleophilic fluorination of HM-Toc.
44, nucleophilic fluoride reagent, carrier additive, TsF and 4Å molecular sieves (MS) were stirred in THF for up to 3 h. Extracted with water. (a) dry solvents (b) 4Å Molecular sieves 4.5 mg per 1 mg HM-Toc.605

Trying to convert the HM-Toc to the tosylate 82 with triethylamine and tosylchloride only formed small amounts of dimer 46 and starting material HM-Toc. Reports by Kochi mentioned the instability of electron rich tosylates and the difficulty to synthesise them.604 Trying instead to mesylate 83 HM-tocopherol produced only dimer 46 (Figure 224).611

Since HM-Toc is more nucleophilic than activated fluoride the plan was to convert the hydroxyl group into the benzyl iodide, 84. Iodides are good leaving groups and weak nucleophiles in their ionic form.612 However, the reaction of HM-Toc with iodine (I2), triphenylphosphine (PPh3) and 4-dimethylaminopyridine (DMAP) did only yield dimer 46 (Figure 225).613

All the attempts to fluorinate HM-Toc did not work and formed dimer 46 as the main product. Future work will therefore be focusing on converting the benzylic alcohol into the halide leaving group. Attempts to convert HM-Toc in the tosylate / mesitylate failed so far, but
different reagents will be used to convert the benzylic hydroxide to the benzylic iodide \(84\) or benzylic bromide \(85\) (Figure 226).

**Figure 226. Alternative benzylic iodination / bromination of HM-Toc.**

### 11.2.6 13-HO-\(\alpha\)-tocopherol fluorination to 13-F-\(\alpha\)-tocopherol

Synthesis of the 13-F-\(\alpha\)-tocopherol requires the installment of a functional group at the terminal end (13\textsuperscript{th} carbon) of the phytly chain. \(\alpha\)-Tocotrienol has three double bonds and serves as the starting material for the synthesis. Allylic oxidation of the terminal double bond of acetyl-protected tocotrienol with selenium dioxide (SeO\(_2\)) produces 13-hydroxy-\(\alpha\)-tocotrienol. Reduction of the olefins turns the hydroxylated trienol into the acetyl protected 13-hydroxy-\(\alpha\)-tocopherol. Deoxyfluorination or nucleophilic fluorination with subsequent deprotection of the phenol would produce the 13-F-\(\alpha\)-tocopherol (Figure 227).
During the synthesis of the other targeted compounds (47-50) BSc Honours student Luke Taylor was working on a project which had the 13-HO-α-tocopherol as an intermediate. He succeeded in synthesizing the 13-HO-α-tocopherol over a three-step synthesis from α-tocotrienol however, the allylic hydroxylation with selenium dioxide, tert-butylhydroperoxide (TBHP) and salicylic acid turned out to be not selectivte to the terminal olefin, decreasing the overall yield drastically. Despite several attempts was AcO-13′-HO-α-tocotrienol, 86, produced in maximum 10% yield (Figure 228).
High yielding reactions are required to compensate for the high cost of pure α-tocotrienol (100 mg = $1,260 CAD Sigma Alderich). Isolation of α-tocotrienol from oil mixtures called Tocomin®50 is cheaper, yielding around 100 mg per 1 g mixture (1 g mixture = $3.8 CAD). However, a tedious isolation by chromatography is required using large amounts of solvent.

As an alternative starting material the antioxidant garcinoic acid can be used, which is advantageous because of the already installed carboxylic acid at the 13-position. However, the high price of garcinoic acid (90% pure garcinoic acid, 1 mg = $256 CAD Sigma Alderich) and a necessary three step synthesis to form 13-HO-α-Toc, 86, would not be profitable (Figure 229).
Figure 229. Synthesis of 13-HO-α-Toc, 86, from garcinoic acid.620

11.3 BODIPY fluorination

Hendricks and Liu have described the fluorine exchange reaction of a BODIPY boron-fluorine with a radioactive $^{18}$F-fluoride.622,623 Hendricks’ approach used in the first step trimethylsilyltrifluoroacetic acid (TMSOTf) to exchange the BODIPY boron-fluorine with a triflate group (OTf), followed by $^{18}$F-fluoride addition to replace the OTf with $^{18}$F.622 Liu instead activated the boron-fluorine bond with Lewis acids like AlCl$_3$, TiCl$_4$, SnCl$_4$ and ZnCl$_2$ to achieve fluorine exchange.623 Radiochemical yields up to 95% were described with equimolar amounts of SnCl$_4$ (Figure 230).
Figure 230. BOIDPY $^{19}$F-$^{18}$F exchange with (a) TMSOTf$^{622}$ and (b) Lewis acid$^{623}$

The same $^{19}$F-$^{18}$F reaction was attempted with thienyl-ene-BODIPY, 3. The reaction was first tested on 1,3-dimethyl-BODIPY, 37. Hendricks method was chosen for the exchange, with the modifications of adding $t$-butanol and lutidine, which act as quenchers of excess TMSOTf and help to stabilize the BODIPY triflate intermediate, 87.$^{622}$ It is not known if fluorine exchange might take place at both boron-fluorine bonds. TBAF was used as the fluoride source and dry ACN / DCM 2:1 as a solvent (Figure 231).

Figure 231. 1,3-Dimethyl BODIPY fluoride exchange via mono-OTf BODIPY intermediate 87.$^{622}$

To monitor the exchange the reaction was run in a fluorimeter and UV/VIS spectrometer. A change in the absorption wavelength was observed when TMSOTf is added, accompanied by a visible color change. Addition of TBAF returned the absorption to the original wavelength.

Compared to Liu’s and Hendrick’s BODIPYs our substrates, thienyl-ene-BODIPY 3 and 1,3-
dimethyl-BODIPY 37, had no substituent on the meso-position. The wavelength of BODIPY 37 had a higher wavelength when tested in the fluorimeter with the maximum at 542 nm when exited at 480 nm. A first test reaction in the fluorimeter showed that addition of solid TBAF hydrate to TfO-exchanged dimethyl-BODIPY (586 nm) induces rapid decomposition, seen by a colour change to dark brown and by having no remaining fluorescence at 542 nm. When TBAF was added directly to 37, the sample decomposed the sample in 5 min (Figure 232).

![Fast decomposition diagram](attachment:Fast_decomposition_diagram.png)

**Figure 232. Direct TBAF addition to 1,3-Dimethyl BODIPY.**

1,3-Dimethyl BODIPY, 3, (5.6 mg, 25.4 µmol) in dry ACN:DCM 2:1 (3ml)(blue line) was treated with TBAF hydrate (3.3 mg, 12.7 µmol), shaken and directly measured (green line) with the excitation set at 480 nm (λ max 542 nm). After 5 min the absorbance was measured again (red line).

Comparing the the decay to the fully substituted 2,8-ethyl-1,3,7,9,10-pentamethyl-BODIPY, 88, revealed a slowed decay on increased substitution (Figure 233).^624^
Figure 233. Direct TBAF addition to 2,8-diethyl-1,3,5,7,9-pentamethyl BODIPY.

2,8-Diethyl-1,3,5,7,9-pentamethyl BODIPY (2.8 mg, 8.8 µmol) in dry ACN:DCM 2:1 (3 ml)(blue line) was treated with TBAF hydrate (2.3 mg, 8.8 µmol), shaken and directly measured (green line) with the excitation set at 480 nm (λ max 562 nm). The absorbance was measured at 10 min intervals.

The addition of t-BuOH and lutidine helped to stop the decomposition. To better monitor the conversion the reaction was conducted in a UV/Vis spectrometer. UV absorption before the reaction showed a lambda maximum at 503 nm. Addition of the TMSOTf led to a color change from green to orange, with a wavelength of 543 nm. After the addition of t-BuOH, lutidine, followed by TBAF hydrate turned the color back to green. A lower absorption was observed at 503 nm (Figure 234).
Figure 234. Fluorine exchange of 1,3-dimethyl-BODIPY, 37, followed by UV / VIS.

In a cuvette 37 (32 µM, dry ACN:DCM 2:1) was scanned from 200-700 nm. TMSOTf (4 eq) was added to the cuvette, shaken and measured. t-BuOH (4 eq), Lutidine (4 eq) and TBAF hydrate (4 eq) were added, shaken and measured. λ max: BODIPY-F 503 nm, BODIPY-OTf 543 nm.

Figure 235. 1,3-Dimethyl-BODIPY. Left: standard reference 0.32 µM. Right cuvette: 0.32 µM. Blue line in Figure 234
1,3-Dimethyl BODIPY successfully exchanged the boron-fluorine with a triflate and was resubstituted with fluoride. The same reaction was repeated with thienyl-ene-BODIPY, 3. The
maximum absorption of 571 nm shifted after Tf₂O addition to 639 nm (Figure 238). A color change from pink to blue was observed when the triflate was formed.

Figure 238. Fluorine exchange of thienyl-ene-BODIPY, 3, followed by UV/Vis.

In a cuvette 3 (32 μM, dry ACN:DCM 2:1) was scanned from 200-700 nm. TMSOTf (4 eq) was added to the cuvette, shaken and measured. t-BuOH (4 eq), Lutidine (4 eq) and TBAF hydrate (4 eq) were added, shaken and UV/Vis absorption measured. λ max: BODIPY-F 571 nm, BODIPY-OTf 639 nm.
Figure 239. Thienyl-ene-BODIPY. Left cuvette: standard reference 0.16 μM. Right cuvette: 0.32 μM. Blue line in Figure 238.

Figure 240. Thienyl-ene-BODIPY. Left cuvette: standard reference 0.16 μM. Right cuvette: Tf₂O addition. Red line in Figure 238.
Fluorine exchange was successful with thienyl-ene-BODIPY, 3. In conclusion, both BODIPYs, 37 and 3, were able to undergo fluorine exchange after the boron center was exchanged with a triflate. The addition of t-BuOH and lutidine was helpful to stabilize the BODIPY triflate, as described by Hendricks. The next step of the project is to exchange thienyl-ene-BODIPY, 3, with $^{18}$F-fluoride.

11.4 Cell cytotoxicity of 6-F-α-tocopherol, thienyl-ene-BODIPY and hydroxymethyl tocopherol

Before the newly created 6-F-α-tocopherol (F-Toc) and thienyl-ene-BODIPY can be tested in a living system with $^{18}$F a cytotoxic analysis of the non-radioactive $^{19}$F has to be performed to see if any of these compounds are toxic. Injections of $^{18}$F radiolabels into patients are normally in the pico- to nanomolar (ng-mg) amounts. Everaert found that with $^{18}$F-fluoroglucose good PET images were obtained when a dose of at least 8 MBq/kg (0.21 Ci/kg) bodymass was achieved, which are around 40 picomoles/kg bodymass (calculated assuming the specific activity of $^{18}$F is 5,283,333 Ci/mol). Images are generally taken after 30-40 min after
injection. FDG is injected as a 0.740-7.4 GBq solution, which would mean that a maximum of 40 nanomols/kg bodymass are used. A concentration range from 1 nM to 1 mM of F-Toc was used to ensure that even at higher concentration no toxicity occurs. A methyl tetrazolium (MTT) assay was used to assess the cytotoxicity to cultured C2C12 myoblasts and and mouse embryonic fibroblast cells. The assay looks at the mitochondrial metabolic activity of cells, which corresponds directly to the cell viability. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is thereby converted by mitochondrial NAD(P)H-dependent reductase enzymes to formazan \((E,Z)-5-(4,5\text{-dimethylthiazol-2-yl})-1,3\text{-diphenyltetrazolium bromide}\), which has an absorption around 570 nm. The compounds were tested in a C2C12 myoblastoma and mouse embryonic fibroblast cells, grown by our collaborator Dr. Jeff Stuart, Brock University. Cytotoxicity experiments and data processing were conducted with the help of Lucus Maddalena. \(\alpha\)-Tocopherol was used as a non-toxic standard.

Hydroxymethyl tocopherol (HM-Toe) was also tested using the same concentration range in the same cytotoxic assay. This result was used for future studies in the non-antioxidant project (see Chapter 2) (Figure 251).
Figure 242. Viability of mouse cells cultured in the presence of α-, F-, and HM-tocopherol derivatives at concentrations ranging from 1 nM to 1 mM.

(A) C2C12 mouse myoblasts and (B) mouse embryonic fibroblasts (MEFs) were treated with α-, F-, or HM-tocopherol for 24h prior to assessing viability via spectrophotometric measurement of formazan (absorbance at 570 nm) produced from the live-cell-catalyzed reduction of MTT tetrazolium. There were no significant differences between absorbance values of all tocopherol groups compared to the corresponding vehicle control (0.1% DMSO; Tukey’s post-hoc test). Data points represent means ± SEM, with n = 4 for all conditions except for F-toc in MEFs (n = 3). Note: absorbance values for vehicle control groups in C2C12 cells and MEFs were 0.4343 ± 0.0299 and 0.2984 ± 0.0154, respectively.

No increase in absorption was observed with 6-F-α-tocopherol, HM-Toc and tocopherol at all concentrations, indicating that all compounds tested are non-toxic.

The MTT assay cannot be used to assess thienyl-ene-BODIPY’s toxicity, because the absorbance of formazane is at the same wavelength and has a much lower extinction coefficient than thienyl-ene-BODIPY. (formazan: 570 nm / 13,000 M⁻¹ cm⁻¹, thienyl-ene-BODIPY: 571 nm / 120'000 M⁻¹ cm⁻¹). Instead cell counting was used to determine the cell viability (Figure 252).
Figure 243. Viability of mouse cells cultured in the presence of BODIPY-tocopherol at concentrations ranging from 1 nM to 0.1 mM.

C2C12 mouse myoblasts and MEFs were treated with up to 0.1 mM BODIPY-tocopherol for 24h before determining the number of cells excluding Trypan blue dye. The mean number of viable cells in all BODIPY-tocopherol groups were not significantly different than that of the corresponding vehicle control (0.1% DMSO; Tukey’s post-hoc test). Data points represent means ± SEM (n = 2). Note: 0.1 mM BODIPY-toc partially precipitated out of solution when added to culture media; it completely precipitated out of solution at 1 mM and was therefore not tested at this concentration. The number of viable cells in the vehicle control groups for C2C12 cells and MEFs were 424,875 ± 31,125 and 249,000 ± 3,000.

F-Toc, HM-Toc and α-tocopherol turned out to be non-toxic up to millimolar concentrations in both, C2C12 cells and MEFs. Thienyl-ene-BODIPY showed some toxicity at higher micromolar concentrations in MEFs.

To ensure the safe use of the new F-Toc label the cytotoxicity of the starting material from the electrophilic fluorination, H-Toc (Figure 253), and byproduct from the nucleophilic fluorination, I-Toc (Figure 254), were tested in MEF and C2C12 cells.
Figure 2.44. Viability of mouse cells cultured in the presence of H-tocopherol (H-toc) at concentrations ranging from 1 nM to 1 mM.

C2C12 mouse myoblasts (red) and mouse embryonic fibroblasts (MEFs, blue) were treated with H-tocopherol for 24h prior to assessing viability via spectrophotometric measurement of formazan (absorbance at 570 nm) produced from the live-cell-catalyzed reduction of MTT tetrazolium. There were no significant differences between absorbance values of all tocopherol groups compared to the corresponding vehicle control (0.1% DMSO; Tukey’s post-hoc test). Data points represent means ± SEM, with n = 5 for all conditions. Note: absorbance values for vehicle control groups in C2C12 cells and MEFs were 0.303 ± 0.002 and 0.151 ± 0.004, respectively.
Figure 245. Viability of mouse cells cultured in the presence of I-tocopherol (I-toc) at concentrations ranging from 1 nM to 1 mM.

C2C12 mouse myoblasts (red) and mouse embryonic fibroblasts (MEFs, blue) were treated with I-tocopherol for 24h prior to assessing viability via spectrophotometric measurement of formazan (absorbance at 570 nm) produced from the live-cell-catalyzed reduction of MTT tetrazolium. There were no significant differences between absorbance values of all tocopherol groups compared to the corresponding vehicle control (0.1% DMSO; Tukey's post-hoc test). Data points represent means ± SEM, with n = 5 for all conditions. Note: absorbance values for vehicle control groups in C2C12 cells and MEFs were 0.303 ± 0.002 and 0.151 ± 0.004, respectively.

No toxic effect was observed in the MTT assay in both cell lines at all concentrations tested. Unexpectedly, in C2C12 cells an increase in cell survival was seen. Since H-Toc and I-Toc have shown no evidence of toxicity in mammalian cell culture is the injection of both materials into test animals may proceed with caution. Purification of F-Toc from these compounds is therefore not necessary, saving valuable time in the overall PET-label production process.

11.4.1 Cell cytotoxicity of the liposomal delivery 6-F-α-tocopherol
The designed PET labels, F-Toc and thienyl-ene-BODIPY, will be distributed *in vivo* with the help of a specific liposomal construct, consisting of POPC, POPG and cholesterol in a 7:3:4 ratio. Toxicity of the liposomes was tested in C2C12 (Figure 255) and MEF (Figure 256) cell lines to assure safe use in upcoming animal trials.

**Figure 246. Viability of mouse cells cultured in the presence of varying concentrations of large unilamellar vesicles (LUVs).**

*C2C12* mouse myoblasts and were treated with LUVs for 24h prior to assessing viability via spectrophotometric measurement of formazan (absorbance at 570 nm) produced from the live-cell-catalyzed reduction of MTT tetrazolium. Data points represent means ± SEM (n = 8). *Note:* LUVs consisted of POPC:POPG:Cholesterol at a molar ratio of 7:3:4.
Figure 247. Viability of mouse cells cultured in the presence of varying concentrations of large unilamellar vesicles (LUVs).

Mouse embryonic fibroblasts (MEFs) were treated with LUVs for 24 h prior to assessing viability via spectrophotometric measurement of formazan (absorbance at 570 nm) produced from the live-cell-catalyzed reduction of MTT tetrazolium. Data points represent means ± SEM (n = 8). Note: LUVs consisted of POPC:POPG:Cholesterol at a molar ratio of 7:3:4.

In both cell lines, C2C12 and MEF, the liposomes showed no toxicity until a concentration 1mg/ml was exposed to cultured cells. The reason for the toxicity at higher concentrations is the oversaturation of the cells with lipids.631

In conclusion tested compounds showed no overt signs of cytotoxicity at concentrations used in PET imaging (5-740 mBq = pM-n) and above and can be further assessed in animal trials.632

11.5 Cellular uptake of in liposome incorporated thienyl-ene-BODIPY

Uptake of thienyl-ene-BODIPY POPC:POPG:cholesterol liposomes into cells was tested in MEF and C2C12 cells and monitored by fluorescence microscopy. The size of the prepared liposomes was 50 nm, this size being the smallest used in a patent describing this drug delivery technology.630 Images were reported as stacked images in fluorescent (ex. 587 nm - em. 610 nm) and brightfield mode.
The C2C12 cells were imaged, first without any label present, then 5 min, 10 min, 20 min and 30 min after addition of the fluorophore (Figure 257). After 30 min the increase in fluorescence was marginal and not included here. The effect of liposome uptake was compared to directly added thienyl-ene-BODIPY in DMSO.

![Figure 248. C2C12 cell uptake of thienyl-ene-BODIPY-tocopherol (BODIPY-tocopherol) delivered in LUVs.](image)

Images are maximum projections of z-stacks taken at 0.32 nm intervals.

Fluorescence was observed in the cytosol of the cells. Almost no difference in fluorescence intensity was seen between 5-10 min. After 20 min, a larger increase in intensity was observed. Addition of thienyl-ene-BODIPY was compared after 30 min to the liposome delivery (Figure 258).
Figure 249. Uptake of thienyl-ene-BODIPY-tocopherol (BODIPY-tocopherol) in C2C12 cells at 30 min after delivery via DMSO or LUVs.

Images are maximum projections of z-stacks taken at 0.32 nm intervals.

The cells showed a higher fluorescence intensity after direct addition of the fluorophore (DMSO) than after 30 min exposure to fluorophore containing liposomes. Addition of the DMSO solution into the surrounding media offered excess thienyl-ene-BODIPY to diffuse into the cells, leading to an increased uptake compared to the liposomes, which first had to find the cells before incorporation by fusion with the cell membrane.
Tests in MEF cells were more closely monitored in terms of differences between liposomal (Figure 259) and direct delivery (Figure 260). Images were taken after 5 min, 20 min and 30 min.

![Figure 250. Mouse embryonic fibroblast uptake of thienyl-ene-BODIPY-tocopherol (BODIPY-tocopherol) delivered in LUVs.](image)

Images are maximum projections of z-stacks taken at 0.32 nm intervals.
Figure 251. Mouse embryonic fibroblast uptake of BODIPY-tocopherol delivered in DMSO.

Images are maximum projections of z-stacks taken at 0.32 nm intervals.

As with MEF cells was the fluorescence intensity was higher with direct thienyl-ene-BODIPY addition in DMSO solution. However, both addition methods seem to incorporate the fluorescent label in sufficient amounts after 20-30 min. Also, MEF and C2C12 cells incorporated most of the liposomes in a 20-30 min period. PET agents have different distribution times depending on the target location. Generally are PET images are taken after a maximum time of 45-90 min. This depends on the distribution of the radiolabel in the body. Cellular uptake of our liposomes may thus be sufficient in this given time frame to obtain a PET image.

12 Conclusion

The main target 6-fluoro-α-tocopherol (F-Toc) was synthesized in short reaction times by nucleophilic and electrophilic fluorinations. The synthetic process most suitable for future
studies with $^{18}$F-fluoride is the electrophilic fluorination of 6-hydrogen-α-tocopherol (H-Toc) with NFSI, yielding F-Toc in a 40-45% yield. NFSI has been prepared and used successfully as an $^{18}$F-reagent in literature. H-Toc is a stable against light, heat and oxidation and produced in high yields (> 90%) over a two-step synthesis from α-tocopherol (Figure 263).

![Chemical Structures](image)

Figure 263. Synthesis of F-Toc from α-tocopherol.

In cell viability assays it was found that F-Toc and H-Toc showed no signs of overt cytotoxicity, potentially allowing the administration of F-Toc into a living organism as a crude mixture with H-Toc present. Furthermore, we showed that the liposomal construct used to deliver F-Toc displayed cytotoxicity only at very high concentrations of applied lipid. Both compounds were found to be partially separable by HPLC chromatography with a XBD-phenyl column. However, the retention time of both compounds was past 20 min and the separation of both compounds was not well-resolved. Future effort should be made towards the development of a more rapid and cleaner separation between F-Toc and H-Toc which might be achieved by the use of a poly-fluorinated reverse phase HPLC column.

Unfortunately, all attempts to synthesize alternative tocopherol based compounds, bearing fluorine on the 5-aromatic position or the 5- or 6-benzylic position on the chroman ring were unsuccessful. Synthetic efforts towards these secondary targeted compounds will be abandoned,
as future work will focus on the main compound, 6-F-Toc, specifically on its further performance as a PET-label in animal studies.

Fluorine exchange reaction of thienyl-ene-BODIPY boron-fluorine was succesfull and can in the future be used to create a dual label for \textit{in vivo} ($^{18}$F) and \textit{in vitro} (fluorescence) applications (Figure 261). The cellular uptake of the designed PET-labels when encapsulated in specific liposome constructs was tested with thienyl-ene-BODIPY and proved to successful occupy and allow the imaging of internal cellular membranes in a short amount of time.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fluorine_exchange.png}
\caption{Fluorine exchange ($F_a$-$F_b$) on thienyl-ene-BODIPY.}
\end{figure}
13 General procedures

13.1 Reagents
All reagents were purchased from Sigma-Aldrich Chemical Co., Oakville, Ontario. Glassware was flame dried before used. Air and moisture sensitive reactions were conducted under N₂ atmosphere. Solvents were dried according to standard procedures; THF was distilled over sodium and benzophenone; hexane and dichloromethane were refluxed for 3 h with calcium hydride and then distilled under protective atmosphere. Solvents for oxygen sensitive reactions were purged for 2 h with nitrogen. Reactions requiring hydrogenation were purged 3x with hydrogen gas (vacuum, then H₂). The Celite® used to filter the catalysts was washed with hexane followed by the solvent used in the reaction before use to remove any impurities. Bu₄N⁺HCO₃⁻ was synthezised by Culbert’s method by bubbling CO₂ to a solution of tetrabutylammoniumhydroxide in water, till a pH of 7.4 was reached. The solution was concentrated to a colourless oil, resuspended in ACN and the suspension filtrated and evaporated. The 200 nm sized TiO₂ nanoparticles (rutile: EPRUI-T200, anatase: EPRUI-TA200) were purchased from EPRUI Nanoparticles & Microspheres Co. Ltd.

13.2 Ligand binding and competitive binding assays with α-TTP
Fluorescence measurements were recorded with a QuantaMaster2000 fluorometer (Photon Technologies International PTI, London, Ontario) FeliX-32, using a 150 W Hg-Xe vapor arc lamp with a LPS-220B lamp supply cooled to 5-8°C via chilled water circulation, MD-50 20 motor drive, SC-500 shutter control and a Brytebox™. All the data points were stored in Excel files and analyzed with GraphPad Prism 5. Slit-width was consistent for all measurements at 5
nm. OS high precision cells, 10 x 10 mm quartz glass cuvettes from Hellma Analytics were used for all assays. Human α-TTP was expressed and purified as described previously (Zhang). α-TTP stock solution concentrations were measured via Bradford assay. Fluorescence binding assays were performed essentially as previously described. (Nava, West) The ligand 2 was added from stock solutions in EtOH, DMF or dioxane, to 3.0 mL of a 0.2-0.5 μM solution of α-TTP in TKE buffer (50 mM Tris-HCl, 100 mM KCl, 1 mM EDTA, pH 7.4). The total amount of added organic solvent was not allowed to exceed 1% of the total volume. After each addition of ligand, fluorescence intensity was monitored for 10-15 min to assure equilibrium had been reached and a maximum signal obtained. The excitation wavelength was 564 nm (slightly less than the absorption maximum) and emission was recorded at 584 nm.

For competitive binding assays α-TTP (0.2 μM in TKE buffer) was pre-incubated with 0.4 μM 2 for 1 hr. To this complex was added increments of stock solutions of α-tocopherol or cholesterol, the mixture allowed to reach equilibrium for 15 minutes, and the fluorescence intensity recorded.

13.3 Cyclic voltammetry

Cyclic voltammetry experiments were conducted on a Bioanalytical System Inc. (BASI) Epsilon electrochemical workstation. Compounds were dissolved in dry DCM (2.5 mM) and purged for 5-10min with N₂. Tetrabutylammonium hexafluorophosphate (n-Bu₄NPF₆, 80 mM) was used as the electrolyte and a silver wire as a reference electrode. Samples were run at 200 mV/s.

13.4 AMVN lipid peroxidation
Lipid samples were prepared containing 10.7 mM soyPC, 2.53 mM AMVN, and 45.0 µM of either α-Toc or HM-Toc. A fourth sample was prepared that contained only soyPC and was used as a blank. All necessary reagents were combined in chloroform solution then evaporated under high vacuum for one hour and re-suspended in 2.0 mL of 25 mM Tris (pH 7). Samples were vortexed for 1 min yielding cloudy suspensions of multilamellar liposomes. Reaction vials were incubated at 60°C exposed to air in a water bath. 50 µL aliquots were taken approximately every 20 minutes, diluted with 950 µL of acetonitrile and the absorbance measured at 234 nm.

13.5 HM-Toc stability

HM-Toc (10 mg) was dissolved in EtOH, DMSO or Dioxane (2 ml). Solid K₂CO₃ (2 mg) was used to keep the solution basic. To acidify the solution 10 drops of aqueous HCl solution (1 M) were added. The mixtures were capped and stored in the dark. After the described times the solvents were evaporated with a N₂ stream (DMSO: high vacum at 60°C), extracted with 1 M NaOH (0.5 ml) and 2x EtOAc. The organic layers were decanted and evaporated with an N₂ stream and analyzed by ¹H-NMR with CDCl₃ as a solvent.

13.6 Cytotoxicity study

Materials and Methods:

Materials:

C2C12 mouse myoblasts, Dulbecco’s Modified Eagle Medium (DMEM; with high glucose, L-glutamine, sodium pyruvate, and sodium bicarbonate), Eagle’s minimum essential medium (MEM) non-essential amino acids solution, penicillin/streptomycin solution, fetal bovine serum (FBS), and thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO). Mouse embryonic fibroblasts (MEFs) were purchased from ATCC (Manassas, VA). 96-well cell culture microplates (polystyrene, clear flat-bottom) were purchased from
Greiner Bio-One (Frickenhausen, Germany). 6-well cell culture plates were purchased from Sarstedt (Newton, SC). Dimethyl sulfoxide (DMSO) was purchased from Bio-Shop (Burlington, ON, Canada). Unless otherwise indicated, all other materials were purchased from Sigma-Aldrich (St. Louis, MO, USA), BioShop (Burlington, ON, Canada), or Fisher Scientific (Mississauga, ON, Canada).

**Cell culture:**

C2C12 mouse myoblasts and mouse embryonic fibroblasts (MEFs) were cultured in DMEM supplemented with 10% (v/v) FBS, 4500 mg/L glucose, 4 mM L-glutamine, 1 mM sodium pyruvate, 2% (v/v) MEM nonessential amino acid solution, and penicillin (50 I.U./mL) / streptomycin (50 µg/mL) solution (complete media). Cells were cultured in a humidified 5% CO₂ atmosphere within a Thermo Forma Series II water-jacketed CO₂ incubator maintained at 37°C. Cells were transferred to fresh 96- or 6-well plates (2,000 cells/well & 60,000 cells/well, respectively) in the evening prior to commencing tocopherol treatments.

**Tocopherol stock solutions and cell treatments:**

Each tocopherol was dissolved in sterile 100% DMSO to yield a 1 M stock solution. Less concentrated stock solutions were subsequently prepared using ten-fold serial dilutions. All tocopherol solutions were stored at -20°C. To treat cultured cells, media was replaced with complete media containing freshly-added tocopherol; the final amount of vehicle (DMSO) for all concentrations tested was 0.1% (v/v).

**MTT tetrazolium reduction assay:**

Media was discarded, wells were washed once with phenol red-free complete culture media, and 100 µL/well phenol red-free complete culture media containing 0.45 mg/mL MTT was added. Two hours later, solubilization solution [40% (v/v) dimethylformamide, 2% (v/v) glacial acetic acid, 16% (w/v) sodium dodecyl sulfate, pH 4.7] was added (100 µL/well) and well contents were gently mixed by re-suspension to dissolve the formazan precipitate. Plates were
incubated at room temperature in darkness for 2 h before recording absorbance at 570 nm using a Bio-Tek PowerWave Microplate UV-Vis spectrophotometer (Winooski, VT, USA). For each plate, background signal averaged from cell-free wells containing vehicle treatments was subtracted.

**Trypan blue exclusion assay:**

Media was discarded, wells were washed once with phosphate-buffered saline, and cells were harvested via trypsinization. After centrifugation (240 g, 3 min), cell pellets were re-suspended in complete culture media and subsequently diluted in 0.4% (w/v) Trypan Blue solution. Three minutes later, the numbers of viable (non-stained) cells were counted using a hemocytometer (Hausser Scientific, Horsham, PA) viewed under a Hund Wetzlar Wilovert Inverted Phase-Contrast light microscope (Fisher Scientific, Mississauga, ON, Canada).

**Statistical Analyses:**

Data sets were analyzed in GraphPad Prism 5 using one-way ANOVA and Tukey’s post-hoc test, with a p-value of less than 0.05 considered significant.

### 13.7 Lipid-BODIPY extrusion

<table>
<thead>
<tr>
<th></th>
<th>Molar Weight (g/mol)</th>
<th>Molar Ratio</th>
<th>Weight needed (mg)</th>
<th>Concentration of stock lipid (mg/mL)</th>
<th>Volume needed (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POPC</td>
<td>760.076</td>
<td>7</td>
<td>5.80</td>
<td>10</td>
<td>580.2</td>
</tr>
<tr>
<td>POPG</td>
<td>770.989</td>
<td>3</td>
<td>2.52</td>
<td>10</td>
<td>252.2</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>386.654</td>
<td>4</td>
<td>1.69</td>
<td>1</td>
<td>1686.6</td>
</tr>
</tbody>
</table>

*Table 34. Contents for 10 mg/mL of 7:3:4-POPC:POPG:Cholesterol.*

<table>
<thead>
<tr>
<th></th>
<th>Molar Weight (g/mol)</th>
<th>Molar Ratio</th>
<th>Weight needed (mg)</th>
<th>Concentration of stock lipid (mg/mL)</th>
<th>Volume needed (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POPC</td>
<td>760.076</td>
<td>7</td>
<td>1.15</td>
<td>10</td>
<td>115.2</td>
</tr>
<tr>
<td>POPG</td>
<td>770.989</td>
<td>3</td>
<td>0.50</td>
<td>10</td>
<td>50.1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>386.654</td>
<td>4</td>
<td>0.33</td>
<td>1</td>
<td>334.9</td>
</tr>
<tr>
<td>BODIPY, 3</td>
<td>532.2169</td>
<td>0.1</td>
<td>0.011</td>
<td>8.898*10^-3 (16.72µM)</td>
<td>1295.2</td>
</tr>
</tbody>
</table>

*Table 35. Contents for 2 mg/mL of 7:3:4:0.1-POPC:POPG:Cholesterol:BODIPY.*
Contents for the desired liposomes were mixed in a glass vial in a biosafety cabinet (BSC) according to Table 34 and 35. Solvents of the lipid composition (Ethanol and Chloroform) were evaporated by blowing nitrogen in the vials near a flame. The vials were placed under vacuum for 1.5 hours. In a BSC, 1000 µL of 1X autoclaved phosphate buffered saline (1XPBS) at pH 7.4 was added to each vial. The vials containing the lipids and 1XPBS were vortexed to resuspend the lipids. Lipids were extruded with a 50 nm sterile extruder (already sterilized with ethylene oxide) in a BSC.

Extrusion apparatus (LiposoFast 9AVestin, Ottawa, ON) was set up as follows:

- The needles were attached to their syringes. The 50nm filter along with the filter support and the scaffolds were already packaged in a plastic extrusion casing.
- A syringe was attached to one side of the extrusion casing. The other syringe was filled with approximately 0.5 mL of the lipids and then attached to the other side of the casing. The lipids in the syringe were passed through the filter to the other syringe 20 times.
- The lipids after extrusion were transferred to a fresh vial.
- The extruded lipids were stored in 4°C fridge overnight.

13.8 Imaging of cellular uptake of thienyl-ene-BODIPY liposomes

Fluorescent imaging was conducted on a Carl Zeiss Axio Observer.Z1 inverted light/epifluorescence microscope equipped with a Apotome.2 optical sectioning and a Hamamatsu Orca-Flash4.0 V2 digital camera. The excitation and emission wavelength were set for 587 / 610 nm with a X-Cite 120LED and image intensity was kept at 10%. Z-stack were taken at 0.32 nm intervals and stacked into a 2D image using the “extended depth of focus” processing tool. A Zen 2 pro microscopy software was used.

14 Synthetic procedures and NMR & MS
All experiments (\(^1\text{H}, \ ^{11}\text{B}, \ ^{13}\text{C}, \ ^{19}\text{F}, \ ^{31}\text{P}\)) were performed on either a Bruker Advanced DPX-300 MHz, 400 MHz or 600 MHz. Bruker TOPSPIN 3.5 PL2 (400 MHz) and Bruker TOPSPIN 2.1 PL6 software (300 MHz) were used to analysed FID data. Deuterated chloroform (CDCl\(_3\)) was used as the standard solvent if not otherwise noted. All deuterted solvents were purchased by Cambridge Isotopes Laboratories\(^\circledast\). Internal standards were tetramethylsilane for \(^1\text{H}\)-NMR and trichlorofluoromethane (CFCl\(_3\)) for \(^{19}\text{F}\)-NMR used. Chemical shifts were reported as δ values and coupling constants as J-values and reported in Hertz (Hz). Following abbreviations were used for splitting patterns: s = singlet, d = doublet, dd = doublet of doublet, dt = doublet of triplet, t = triplet, q = quartet, p = pentet, sep = septet m = multiplet, br = broad, enant = enantiotopic.

Electron impact (EI) and fast atomic bombardment (FAB) mass spectras were recorded on a ThermoFisher high resolution double focusing magnetic sector mass spectrometer system, electrospray ionization (ES) on a Bruker HCT Plus Proteineer LC-MS. High performance liquid chromatography (HPLC) analysis was conducted on an Agilent 1100 with autosampler and diode array detector, ODS-2 spherisorb column and XBD-Phenyl column were used. Powder X-ray diffraction (pXRD) measurements of TiO\(_2\) samples was conducted on a Rigaku SmartLab X-ray diffraction system (XRD).

14.1 Fluorescent Tocopherol

Methyl 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate (4) was synthesized following Hildering’s procedure.\(^{234}\)
1H-NMR (400MHz, CDCl₃) δ 3.68 (s, 3H, OMe), δ 2.58 (m, 2H, ArCH₂CH₂), δ 2.43 (m, 1H (ArCH₂CH₂)), δ 2.16 (s, 3H, ArCH₃), δ 2.12 (s, 3H, ArCH₃), δ 2.03 (s, 3H, ArCH₃), δ 1.90-1.80 (m, 1H, ArCH₂), δ 1.61 (s, 3H, 2-R-CH₃)

13C-NMR (300MHz, CDCl₃) 174.5, 145.5, 121.2, 118.4, 116.9, 52.35, 30.61, 25.42, 20.95, 20.83, 12.20, 11.81, 12.24

MS [EI+] m/z 378 (M+1, 8.9%), 189 (25%), 147 (100%)

14.1.1 Synthesis of (S)-methyl 6-((tert-butyldimethylsilyl)oxy)-2,5,7,8-tetramethylchroman-2-carboxylate (5)

4 (3.7g, 0.014mol), tert-butyldimethylsilyl chloride (3.17g, 0.021mol) and imidazole (3.81g, 0.056mol) were stirred in DMF (40ml) at 85°C for 16h. The reaction was cooled to room temperature and most of the solvent evaporated. The residual mixture was resuspended in diethyl ether and washed 5x with ice-water. The organic phase was dried over Na₂SO₄ and evaporated down to dryness. Silica column chromatography (gradient Hex/CH₂Cl₂, 4:1 to 1:1 to CH₂Cl₂) afforded 5 (4.94g, 93.4%) as a clear oil.

TLC: Rᵣ = 0.75 (CH₂Cl₂)

1H-NMR (400MHz, CDCl₃) δ 3.68 (s, 3H, OMe), δ 2.58 (m, 2H, ArCH₂CH₂), δ 2.43 (m, 1H (ArCH₂CH₂)), δ 2.16 (s, 3H, ArCH₃), δ 2.12 (s, 3H, ArCH₃), δ 2.03 (s, 3H, ArCH₃), δ 1.90-1.80 (m, 1H, ArCH₂), δ 1.61 (s, 3H, 2-R-CH₃), δ 1.06 (s, 9H, Si-tBu), δ 0.137 (s, 6H, 2 x Si-CH₃)

13C-NMR (400MHz, CDCl₃) 174.6, 144.0, 122.7, 124.1, 123.0, 117.9, 52.27, 30.56, 26.09, 25.38, 21.05, 18.62, 14.32, 13.86, 11.89, -3.30

MS [EI+] m/z 264 (M⁺1, 77%), 205 (100%), 164 (75%)

14.1.2 Synthesis of (S)-6-((tert-butyldimethylsilyl)oxy)-2,5,7,8-tetramethylchroman-2-carbaldehyde (6)
5 (4.94g, 0.013mol) was dissolved in dry toluene (40ml) and cooled to -78°C under an N₂ atmosphere. A 1M solution of diisobutylaluminiumhydride in toluene (DIBAL 1M Tol. 23.52ml, 0.0235mol) was added in 10min and stirred for 1.5h at -78°C. The reaction quenched with methanol (20ml), warmed to room temperature followed by slow addition of first water (70ml), then 2M HCl. The phases were stirred for 10min, then separated. The water phase was washed with diethyl ether 4x volume. The organic phases were dried over Na₂SO₄, combined and evaporated down to dryness. Silica column chromatography (gradient Hex/CH₂Cl₂, 1:1 to DCM) afforded 6 (3.95g, 86.8%) as a clear oil.

TLC: \( R_f = 0.53 \) (Hex/EtOAc, 4:1)

\(^1\)H-NMR (400MHz, CDCl₃) \( \delta \) 9.65 (s, 1H, CHO), \( \delta \) 2.56 (m, 2H, ArCH₂CH₂), \( \delta \) 2.30 (m, 1H (ArCH₂CH₂) \( \delta \) 2.19 (s, 3H, ArCH₃), \( \delta \) 2.14 (s, 3H, ArCH₃), \( \delta \) 2.04 (s, 3H, ArCH₃), \( \delta \) 1.80-1.89 (m, 1H, ArCH₂CH₂), \( \delta \) 1.41 (s, 3H, 2-R-CH₃), \( \delta \) 1.07 (s, 9H, Si-tBu), \( \delta \) 0.137 (s, 6H, 2 x Si-CH₃),

\(^{13}\)C-NMR (400MHz, CDCl₃) 205.2, 146.0, 145.6, 126.9, 124.3, 123.2, 118.0, 80.67, 28.34, 26.49, 22.00, 20.90, 19.01, 14.76, 13.78, 12.47, -2.92

MS [EI+] \( m/z \) 348 (100%), 319 (56%), 293 (12%), 221 (29%)

14.1.3 Synthesis of (3-hydroxypropyl)triphenylphosphonium bromide (7)

3-Bromopropanol (3.2g, 0.023mol) and triphenylphosphine (4.01g, 0.0153mol) were stirred in toluene (15ml) at 110°C under a nitrogen atmosphere for 16h. The reaction was cooled to room temperature and the white precipitate was filtrated and washed with cold toluene. The solid was dried, which yielded compound 7 (5.89g, 64.1%) as a white solid.

Mp: 226-228°C

TLC: \( R_f = 0.16 \) (Hex/DCM, 3:1)

\(^1\)H-NMR (300MHz, CDCl₃) \( \delta \) 7.85-7.71 (m, 15H, P⁺-Ph), \( \delta \) 4.97 (brs, 1H, P⁺CH₂CH₂CH₂-OH), \( \delta \) 3.89 (m, 4H, R- P⁺CH₂CH₂CH₂OH), \( \delta \) 1.86 (dd, 2H, P⁺CH₂CH₂CH₂-OH)

\(^{13}\)C-NMR (400MHz, CDCl₃) 135.1, 133.6, 133.5, 130.6, 130.5, 60.41, 25.90, 20.56

\(^{32}\)P-NMR (400MHz, CDCl₃) 24.54

MS [EI+] \( m/z \) 321.2 (M⁺H, 100%), 385.1 (4%)

MS Calculated for C₂₄H₃₈O₃Si 320.259
14.1.4 Synthesis of \((S,Z)-4-(6-((\text{tert-butyldimethylsilyl})oxy)-2,5,7,8\text{-tetramethylchroman-2-yl})\text{but-3-en-1-ol} (8)\)

To a suspension of 7 (576mg, 1.436mmol) in dry THF (30ml) was added dropwise LiHMDS 0.8M sol. in dry THF (7.58ml, 4.31mmol) at room temperature under a nitrogen atmosphere. After 1.5h, a solution of 6 (500mg, 1.436mmol) in dry THF (2ml) was added dropwise and stirred for 3h. The solvent was evaporated until only small quantities of THF were left. The residual mixture was then quenched with a 1:1 NH\(_4\)Cl : H\(_2\)O (20ml) solution and stirred with EtOAc for 5min. The phases were separated, the water phase washed 2x with EtOAc. The combined organic phases were dried over Na\(_2\)SO\(_4\) and evaporated down to dryness. Silica column chromatography (gradient Hex/CH\(_2\)Cl\(_2\), 4:1 to 1:1 to 2:3) afforded 8 (426mg, 76%) as a clear oil.

TLC: \(R_f = 0.2\) (Hex/DCM, 2:3)

\(^1\)H-NMR (300MHz, CDCl\(_3\)) 5.63 (d, \(J = 15.60\) Hz, 1H, -RCH=CHCH\(_2\)), 5.50 (q, \(J = 15.60\) Hz, 1H, -RCH=CHCH\(_2\)), 3.55 (m, \(J = 6.00\) Hz, 2H, -RCH=CHCH\(_2\)), \(\delta\) 2.62 -2.42 (m, 2H, Ar-CH\(_2\)-CH\(_2\)), \(\delta\) 2.50 (q, \(J = 6.30\) Hz, 2H, RCH=CHCH\(_2\)), \(\delta\) 2.14 (s, 3H, ArCH\(_3\)), \(\delta\) 2.05 (s, 3H, ArCH\(_3\)), \(\delta\) 2.07 (s, 3H, ArCH\(_3\)), \(\delta\) 2.03-1.76 (m, 2H, ArCH\(_2\)CH\(_2\)), \(\delta\) 1.40 (s, 3H, 2’R-CH\(_3\)), \(\delta\) 1.06 (s, 9H, Si-tBu), \(\delta\) 0.130 (s, 6H, Si-CH\(_3\))

\(^{13}\)C-NMR (300MHz, CDCl\(_3\)) 137.4, 125.9, 124.8 123.6, 122.3, 117.9, 117.1, 74.79, 62.51, 61.78, 60.39, 35.63, 32.30, 27.27, 26.10, 21.19, 18.60, 14.34, 13.39, 11.99, -3.40

MS [EI+] \(m/z\) 390 (M\(^+\), 100%), 278 (60%), 221 (24%), 73 (52%)

MS Calculated for C\(_{24}\)H\(_{38}\)O\(_3\)Si 390.259

14.1.5 Synthesis of \((R)-4-(6-((\text{tert-butyldimethylsilyl})oxy)-2,5,7,8\text{-tetramethylchroman-2-yl})\text{butan-1-ol} (9)\)

The ethyl acetate used in the reaction was dried prior to the reaction by stirring 25ml in Na\(_2\)SO\(_4\) for 3h under an N\(_2\) atmosphere.

To a suspension of \(\text{TBSO} \quad \text{O} \quad \text{OH}\) in dry THF (30ml) was added dropwise LiHMDS 0.8M sol. in dry THF (7.58ml, 4.31mmol) at room temperature under a nitrogen atmosphere. After 1.5h, a solution of \(\text{TBSO} \quad \text{O} \quad \text{OH}\) in dry THF (2ml) was added dropwise and stirred for 3h. The solvent was evaporated until only small quantities of THF were left. The residual mixture was then quenched with a 1:1 NH\(_4\)Cl : H\(_2\)O (20ml) solution and stirred with EtOAc for 5min. The phases were separated, the water phase washed 2x with EtOAc. The combined organic phases were dried over Na\(_2\)SO\(_4\) and evaporated down to dryness. Silica column chromatography (gradient Hex/CH\(_2\)Cl\(_2\), 4:1 to 1:1 to 2:3) afforded 8 (426mg, 76%) as a clear oil.

TLC: \(R_f = 0.2\) (Hex/DCM, 2:3)

\(^1\)H-NMR (300MHz, CDCl\(_3\)) 5.63 (d, \(J = 15.60\) Hz, 1H, -RCH=CHCH\(_2\)), 5.50 (q, \(J = 15.60\) Hz, 1H, -RCH=CHCH\(_2\)), 3.55 (m, \(J = 6.00\) Hz, 2H, -RCH=CHCH\(_2\)), \(\delta\) 2.62 -2.42 (m, 2H, Ar-CH\(_2\)-CH\(_2\)), \(\delta\) 2.50 (q, \(J = 6.30\) Hz, 2H, RCH=CHCH\(_2\)), \(\delta\) 2.14 (s, 3H, ArCH\(_3\)), \(\delta\) 2.05 (s, 3H, ArCH\(_3\)), \(\delta\) 2.07 (s, 3H, ArCH\(_3\)), \(\delta\) 2.03-1.76 (m, 2H, ArCH\(_2\)CH\(_2\)), \(\delta\) 1.40 (s, 3H, 2’R-CH\(_3\)), \(\delta\) 1.06 (s, 9H, Si-tBu), \(\delta\) 0.130 (s, 6H, Si-CH\(_3\))

\(^{13}\)C-NMR (300MHz, CDCl\(_3\)) 137.4, 125.9, 124.8 123.6, 122.3, 117.9, 117.1, 74.79, 62.51, 61.78, 60.39, 35.63, 32.30, 27.27, 26.10, 21.19, 18.60, 14.34, 13.39, 11.99, -3.40

MS [EI+] \(m/z\) 390 (M\(^+\), 100%), 278 (60%), 221 (24%), 73 (52%)

MS Calculated for C\(_{24}\)H\(_{38}\)O\(_3\)Si 390.259
To 8 (550mg, 1.409mmol) was Pd/C 10% (147mg) added and dried EtOAc (25ml). The system was purged 3x with H<sub>2</sub> gas and stirred overnight with an H<sub>2</sub> balloon. The reaction mixture was filtrated over purified celite, washed with EtOAc and evaporated down to dryness that afforded pure product 9 (530mg, 96.4%) as a clear oil.

**TLC:** \( R_f = 0.23 \) (DCM)

**<sup>1</sup>H-NMR** (300MHz, CDCl<sub>3</sub>) \( \delta \) 2.57 (t, \( J = 6.90 \) Hz, 2H, ArCH<sub>2</sub>), \( \delta \) 2.11 (s, 3H, ArCH<sub>3</sub>), \( \delta \) 2.09 (s, 3H, ArCH<sub>3</sub>), \( \delta \) 2.07 (s, 3H, ArCH<sub>3</sub>), \( \delta \) 1.78 (sex, \( J = 6.90 \) Hz, 2H, ArCH<sub>2</sub>CH<sub>2</sub>), \( \delta \) 1.69-1.52 (m, 8H, 2'R-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OH), \( \delta \) 1.06 (s, 9H, Si-tBu), \( \delta \) 0.145 (s, 3H, Si-CH<sub>3</sub>)

**<sup>13</sup>C-NMR** (400MHz, CDCl<sub>3</sub>) 145.8, 144.1, 125.9, 123.6, 122.1, 117.4, 74.32, 62.51, 39.33, 33.20, 31.57, 26.10, 23.73, 20.87, 19.86, 18.60, 14.32, 13.39, 11.85, -3.34

**MS [EI+]** \( m/z \) 392 (M<sup>+</sup>, 100%), 279 (43%), 73 (61%)

MS Calculated for C<sub>23</sub>H<sub>40</sub>O<sub>3</sub>Si 392.275

### 14.1.6 Synthesis of (R)-4-(6-((tert-butyldimethylsilyl)oxy)-2,5,7,8-tetramethylchroman-2-yl)butanal (10)

![structure of 10]

To a suspension of Dess Martin periodinate (110.8mg, 0.2614mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (1ml) was added a solution of 9 (60mg, 0.1538mol) in CH<sub>2</sub>Cl<sub>2</sub> (1ml) and the solution stirred for 16h at room temperature. The reaction was diluted with CH<sub>2</sub>Cl<sub>2</sub> and quenched with 1M NaOH. After extraction the water phase was washed an additional two times with CH<sub>2</sub>Cl<sub>2</sub>, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated down to dryness. Silica column chromatography (gradient Hex/CH<sub>2</sub>Cl<sub>2</sub>, 3:1 to 1:1 to 1:3) afforded 10 (40mg 67.0%) as a clear oil.

**TLC:** \( R_f = 0.73 \) (Hex/CH<sub>2</sub>Cl<sub>2</sub>, 3:1)

**<sup>1</sup>H-NMR** (300MHz, CDCl<sub>3</sub>) 9.79 (t, \( J = 1.80 \) Hz, 1H, CHO), \( \delta \) 2.59 (t, \( J = 6.90 \) Hz, 2H, Ar-CH<sub>2</sub>-CH<sub>2</sub>), \( \delta \) 2.59 (tt, \( J = 9.60 \) Hz, \( J = 1.50 \) Hz, 2H, CHO-CH<sub>2</sub>), \( \delta \) 2.12 (s, 3H, ArCH<sub>3</sub>), \( \delta \) 2.09 (s, 3H, ArCH<sub>3</sub>), \( \delta \) 2.07 (s, 3H, ArCH<sub>3</sub>), \( \delta \) 1.80 (m, 4H, ArCH<sub>2</sub>CH<sub>2</sub> + CH<sub>2</sub>CH<sub>2</sub>CHO), \( \delta \) 1.62 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CHO), \( \delta \) 1.27 (s, 3H, 2'R-CH<sub>3</sub>), \( \delta \) 1.07 (s, 9H, Si-tBu), \( \delta \) 0.140 (s, 3H, Si-CH<sub>3</sub>)

**<sup>13</sup>C-NMR** (300MHz, CDCl<sub>3</sub>) 202.6, 145.6, 144.2, 126.0, 123.6, 122.7, 117.3, 74.10, 44.12, 38.96, 31.59, 26.10, 23.64, 20.79, 18.60, 16.37, 14.32, 13.40, 11.98, -3.33
MS [EI+]  
\( m/z \) 390 (M\(^+\), 100%), 279 (35%), 73 (49%)  
MS Calculated for C\(_{24}\)H\(_{38}\)O\(_3\)Si 390.259

14.1.7 Byproduct (\(R\)-tert-butyl((2-butyl-2,5,7,8-tetramethylchroman-6-yl)oxy)dimethylsilane (9.2)

TLC:  
\( R_f = 0.79 \) (Hex/CH\(_2\)Cl\(_2\), 3:1)

\(^1\)H-NMR  
(300MHz, CDCl\(_3\)) \( \delta \) 2.58 (\( t, J = 6.90 \) Hz, 2H, Ar-CH\(_2\)-CH\(_2\)), \( \delta \) 2.13 (\( s, 3H, \) ArCH\(_3\)), \( \delta \) 2.11 (\( s, 3H, \) ArCH\(_3\)), \( \delta \) 1.8 (sex, 2H, CH\(_2\)CH\(_2\)CH\(_3\)\(_2\)), \( \delta \) 1.69-1.25 (\( m, 6H, \) ArCH\(_2\)CH\(_2\) + CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\) + Ar-CH\(_2\)-CH\(_2\)), \( \delta \) 1.26 (\( s, 3H, \) 2’-R-CH\(_3\)), \( \delta \) 1.3 (\( t, J = 7.20 \) Hz, 2H, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_3\)), \( \delta \) 1.07 (\( s, 9H, \) Si-tBu), \( \delta \) 0.140 (\( s, 3H, \) Si-CH\(_3\))

\(^{13}\)C-NMR  
(400MHz, CDCl\(_3\)) 145.9, 144.1, 125.8, 123.5, 122.7, 117.5, 74.47, 39.36, 31.54, 26.12, 25.91, 23.82, 23.27, 20.91, 18.62, 14.33, 14.15, 13.41, 11.94, -3.34

MS [ESI+]  
\( m/z \) 377.3 (M\(^+\), 100%)
MS Calculated for C\(_{23}\)H\(_{40}\)BrO\(_2\)Si 376.280

14.1.8 Synthesis of 1-(1-benzyl-1\(H\)-pyrrol-2-yl)-\(N,N\)-dimethylmethanamine (11x)

To a solution of formalin 37% (0.368ml, 5.01mmol) and dimethylamine HCl (408mg, 5.01mmol) was added \(N\)-benzylpyrrole (788mg, 5.01mmol) dissolved in CH\(_2\)Cl\(_2\) (2ml) and stirred for 4h at room temperature. The reaction was quenched with a NaOH solution (1g in 10ml H\(_2\)O), which was extracted 3x with ether. The combined organic phases were dried over Na\(_2\)SO\(_4\) and evaporated down to dryness. Silica column chromatography (Hex/EE, 6:1) afforded 11x (200mg 18.7%) as a light yellow oil.

TLC:  
\( R_f = 0.16 \) (Hex/EtOAc, 6:1)

\(^1\)H-NMR  
(300MHz, CDCl\(_3\)) 7.42-7.32 (\( m, 3H, \) Bn-), 7.15-7.12 (\( d, J = 7.50 \) Hz, 2H, Bn-), 6.74 (\( d, J = 2.10 \) Hz, 1H, pyrrole-H), \( \delta \) 6.20 (\( t, J = 3.0 \) Hz, 1H, pyrrole-H), \( \delta \) 6.15
(\(d, J = 3.0 \text{ Hz}, 1\text{H, pyrrole-H}\), \(\delta 5.32 (s, 2\text{H, Bn-CH}_2), \delta 3.33 (s, 2\text{H, Ar-pyrrole-CH}_2), \delta 2.27 (s, 6\text{H, 2x-N-CH}_3)\)

\(^{13}\text{C-NMR} \) (300MHz, CDCl\(_3\)) 139.0, 128.7, 127.6, 127.2, 127.0, 126.7, 122.2, 121.2, 109.6, 106.8, 55.72, 50.34, 45.12

MS [ESI+] \(m/z 215.1 (\text{M}^+ , 9\%), 170.1 (\text{M}^+ -(\text{CH}_3)_2\text{N}, 61\%)\)

MS Calculated for C\(_{14}\)H\(_{18}\)N\(_2\) 214.147

14.1.9 Synthesis of \(N,N\)-dimethyl-1-(1\(H\)-pyrrol-2-yl)methanamine (10x)

A solution of formalin 37\% (2.54g, 0.313mol) and dimethyamine HCl (2.54g, 0.313mol) was added over 10min. to pyrrole (2g, 0.0298mol) at room temperature. The reaction solution was stirred for 16h, quenched with 25\% NaOH (10ml) and extracted 3x with ether (10ml). The organic phases were washed with brine, dried over Na\(_2\)SO\(_4\) and evaporated down to dryness. Two fractions were collected by high vacuum distillation at 50°C as a yellow liquid, both being pure product 10x (1.6g + 1.1g = 2.7g, 71.2\%)\(^{634}\)

\(^{1}\text{H-NMR} \) (300MHz, CDCl\(_3\)) 9.08 (\(brs, 1\text{H, pyrrole-NH}\), 6.74 (\(q, J = 3.00 \text{ Hz}, J = 1.50 \text{ Hz}, 1\text{H, pyrrole-H}\), 6.12 (\(q, J = 3.00 \text{ Hz}, 1\text{H, pyrrole-H}\), 6.04 (\(s, 1\text{H, pyrrole-H}\), \(\delta 3.45 (s, 2\text{H, Ar-pyrrole-CH}_2), \delta 2.25 (s, 6\text{H, 2x-N-CH}_3)\)

\(^{13}\text{C-NMR} \) (300MHz, CDCl\(_3\)) 129.2, 117.6, 107.7, 107.4, 56.63, 45.10

MS [ESI+] \(m/z 125.1 (\text{M}^+ 1, 100\%), 80.2 (\text{M}^+ -(\text{CH}_3)_2\text{N}, 34\%)\)

MS Calculated for C\(_7\)H\(_{12}\)N\(_2\) 124.100

14.1.10 Synthesis of \(N,N\)-dimethyl-1-(1\(H\)-pyrrol-2-yl)methanamine (11)

At 5°C, methyl iodide (181mg, 1.277mmol) was added to a clear solution of 11x (111mg, 0.51mmol) in ether : methanol (8ml) at room temperature under N\(_2\) atmosphere and stirred for 1.5h. A white solid formed and the solvent was evaporated. Triphenylphosphine (362mg, 1.38mmol) was added with benzene : methanol (6ml) and stirred at 50°C over night under N\(_2\) atmosphere. The reaction was cooled to room temperature, ether added and sonicated. The
precipitate was filtrated and washed with ether to afford 11 (62mg, 21.7%) as a light yellow solid.

\[ R_t = 0.1 \text{ (EtOAc)} \]

1H-NMR (400MHz, CDCl\textsubscript{3}) 7.87-7.45 (m, 20H, Bn-), 7.20 (d, \( J = 1.60 \text{ Hz}, 1\text{H, pyrrole-H} \)), 6.97 (d, \( J = 1.60 \text{ Hz}, 1\text{H, pyrrole-H} \)), \( \delta \) 6.15 (d, \( J = 3.0 \text{ Hz}, 1\text{H, pyrrole-H} \)), \( \delta \) 4.98 (s, 1H, Bn-\( \text{CH}_2 \)), \( \delta \) 4.95 (s, 1H, Bn-\( \text{CH}_2 \)), \( \delta \) 4.77 (s, 2H, Ar-\( \text{pyrrole-CH}_2 \))

13C-NMR (400MHz, CDCl\textsubscript{3}) 138.0, 135.5, 134.3, 134.2, 130.7, 130.5, 119.4, 119.2, 118.1, 116.1, 115.9, 110.0, 109.9, 109.3, 54.87, 50.39

31P-NMR (400MHz, CDCl\textsubscript{3}) 20.22

MS [ESI+] \( m/z \) 432.1 (M\textsuperscript{+} - I\textsuperscript{-}, 100%), 170.0 (10%)

MS [ESI-] \( m/z \) 126.8 (I\textsuperscript{-}, 100%)

MS Calculated for C\textsubscript{30}H\textsubscript{27}IPN 559.093

14.1.11 Synthesis of ((1H-pyrrol-2-yl)methyl)triphenylphosphonium iodide (10)

\[
\begin{align*}
\text{Ph} & \quad \text{P} & \quad \text{Ph} \\
\text{H} & \quad \text{P} & \quad \text{Ph} \\
& \quad \text{I} \\
\end{align*}
\]

Methyl iodide (4.47g, 0.0315mol) and 10x (1.6g, 0.0126mol) were stirred for 1h in ether / methanol 1:1 (25ml). A white solid instantly formed. After 1.5h, the solvents were evaporated and to the orange residue was added triphenylphosphine (4.96g, 0.0189mol) and stirred in benzene / methanol 1:1 (30ml) for 5h. The solvent was evaporated and the brownish suspension was filtered and washed with cold benzene / methanol 1:1 that afforded 10 (3.81g, 64%).

Mp: 214°C decomp.

TLC: \( R_t = 0.1 \text{ (CH}_2\text{Cl}_2\text{-MeOH, 9:0.2)} \)

1H-NMR (300MHz, DMSO) 10.54 (s, 1H, pyrrole-NH), 7.94-7.89 (t, \( J = 6.60 \text{ Hz}, J = 1.50 \text{ Hz}, 3\text{H, -PPh}_3 \)), 7.77-7.71 (m, \( J = 7.50 \text{ Hz}, J = 3.90 \text{ Hz}, J = 1.20 \text{ Hz}, 6\text{H, -PPh}_3 \)), 7.57-7.50 (m, \( J = 3.90 \text{ Hz}, 6\text{H, -PPh}_3 \)), 6.72 (d, \( J = 1.50 \text{ Hz}, 1\text{H, pyrrole-H} \)), \( \delta \) 6.72 (t, \( J = 3.0 \text{ Hz}, 1\text{H, pyrrole-H} \)), \( \delta \) 5.95 (q, \( J = 2.7 \text{ Hz}, 1\text{H, pyrrole-H} \)), \( \delta \) 5.52 (s, 1H, pyrrole-H), 3.33 (d, \( J = 13.5 \text{ Hz}, 2\text{H, Ar-pyrrole-CH}_2 \)),

13C-NMR (300MHz, DMSO) 135.5, 134.3, 134.2, 130.7, 130.5, 119.4, 119.2, 118.1, 116.1, 115.9, 110.0, 109.9, 109.3, 54.82, 50.31

MS [ESI+] \( m/z \) 342.1 (M\textsuperscript{+} - I\textsuperscript{-}, 100%)

MS Calculated for C\textsubscript{23}H\textsubscript{21}IPN 469.046
14.1.12 Synthesis of (3-bromopropyl)triphenylphosphonium bromide (14)

\[
\begin{array}{c}
\text{Ph} & \text{Br} \\
\text{Ph} & \text{P} & \cdots & \text{Ph} & \text{Br} \\
\end{array}
\]

1,3-Bromopropane (5g / 2.5, 0.0248mol) and triphenylphosphine (6.5g, 0.0248mol) were stirred in toluene (12ml) at 110°C under a nitrogen atmosphere for 16h. The reaction was cooled to room temperature and the white precipitate was filtrated and washed with cold toluene. The solid was dried, and yielded compound 14 (7.61g, 66.5%) as a white solid.

Mp: 226-228°C

TLC: \( R_f = 0.0 \) (Hex/EtOAc, 9:1)

\(^1\)H-NMR (300MHz, CDCl\(_3\)) \( \delta 7.90-7.70 \) (m, 15H, P\(^+\)-Ph), \( \delta 4.15 \) (m, 2H, P\(^+\)CH\(_2\)CH\(_2\)CH\(_2\)-OH), \( \delta 3.88 \) (dt, \( J = 6.40 \) Hz, \( J = 1.20 \) Hz, 2H, R- P\(^+\)CH\(_2\)CH\(_2\)CH\(_2\)Br), \( \delta 2.25 \) (dq, \( J = 6.80 \) Hz, \( J = 1.20 \) Hz, 2H, P\(^+\)CH\(_2\)CH\(_2\)CH\(_2\)-Br)

\(^{13}\)C-NMR (400MHz, CDCl\(_3\)) 135.2, 135.2, 133.8, 133.7, 130.6, 130.5, 118.5, 117.6, 33.69, 33.48, 26.34, 21.90, 21.37

\(^{31}\)P-NMR (400MHz, CDCl\(_3\)) 24.26

MS [EI+] \( m/z \) 383.0 (M\(^+\)H, 100%), 385.0 (98%)

MS Calculated for C\(_{21}\)H\(_{21}\)Br\(_2\)P 461.975 / 383.056 (-Br)

14.1.13 Synthesis of (R)-4-(6-((tert-butyldimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)butyl methanesulfonate (9-OMs)

\[
\begin{array}{c}
\text{TBSO} \\
\text{OMs}
\end{array}
\]

Compound 9 (160mg, 0.408mmol) was dissolved in CH\(_2\)Cl\(_2\) (2ml) and triethylamine (0.126ml, 0.901mmol) was added. The solution was cooled to 5°C and mesyl chloride (49.2\( \mu \)l, 0.633mmol) was added slowly. After 1h, the reaction was quenched with ice. The layers were separated and the water phase was washed two times with CH\(_2\)Cl\(_2\). The organic layers were combined, dried over Na\(_2\)SO\(_4\) and evaporated down to dryness to afford the mesylate 9-OMs (180mg, 93.9%) as a yellow oil. The crude product was used without further purification.

TLC: \( R_f = 0.78 \) (CH\(_2\)Cl\(_2\))
14.1.14 Synthesis of (R)-(2-(4-bromobutyl)-2,5,7,8-tetramethylchroman-6-yl)oxy)(tert-butyl)dimethylsilane (16)

![Chemical Structure](image)

9-OMs (180mg, 0.383mmol) was dissolved in THF (5ml) and lithium bromide (193mg, 2.23mmol) was added and stirred for 3h at reflux. The solvent was evaporated and the remaining was quenched with ice water. The reaction mixture was then extracted three times with CH$_2$Cl$_2$, dried over Na$_2$SO$_4$ and evaporated down to dryness. Silica column chromatography (Hex/CH$_2$Cl$_2$, 2:1) afforded 16 (100mg 57.4%) as a colourless oil.

TLC: $R_f = 0.85$ (Hex/CH$_2$Cl$_2$, 1:2)

$^1$H-NMR (300MHz, CDCl$_3$) $\delta$ 3.43 ($t$, $J = 6.90$ Hz, 2H, CH$_2$Br), $\delta$ 2.58 ($t$, $J = 6.90$, Hz, 2H, ArCH$_2$CH$_2$), $\delta$ 2.09 ($s$, 3H, ArCH$_3$), $\delta$ 2.07 ($s$, 3H, ArCH$_3$), $\delta$ 1.94-1.74 ($m(p)$, $J = 6.90$ Hz, 4H, ArCH$_2$CH$_2$ + CH$_2$CH$_2$Br) $\delta$ 1.65-1.56 ($m$, 4H, CH$_2$CH$_2$CH$_2$CH$_2$Br) $\delta$ 1.26 ($s$, 3H, 2’R-CH$_3$), $\delta$ 1.06 ($s$, 9H, Si-tBu), $\delta$ 0.136 ($s$, 6H, 2x Si-CH$_3$)

$^{13}$C-NMR (300MHz, CDCl$_3$) 145.7, 144.2, 126.0, 123.6, 122.7, 117.4, 74.19, 38.54, 33.83, 33.13, 31.59, 26.10, 23.75, 22.65, 22.32, 20.84, 18.60, 14.32, 13.40, 11.97, -3.34

MS [EI+] $m/z$ 454 (M$^+$, 39%), 279 (42%), 73 (100%)
MS Calculated for C$_{23}$H$_{39}$BrO$_2$Si 454.190

Byproduct (R)-2-(4-bromobutyl)-2,5,7,8-tetramethylchroman-6-ol

![Chemical Structure](image)

TLC: $R_t = 0.52$ (Hex/CH$_2$Cl$_2$, 1:2)

$^1$H-NMR (300MHz, CDCl$_3$) $\delta$ 4.15 ($brs$, 1H, ArOH), $\delta$ 3.44 ($t$, $J = 6.90$ Hz, 2H, CH$_2$Br), $\delta$ 2.63 ($t$, $J = 6.90$, Hz, 2H, ArCH$_2$CH$_2$), $\delta$ 2.18 ($s$, 3H, ArCH$_3$), $\delta$ 2.13 ($s$, 6H, ArCH$_3$), $\delta$ 1.93-1.76 ($m(p)$, $J = 6.90$ Hz, 4H, ArCH$_2$CH$_2$ + CH$_2$CH$_2$Br) $\delta$ 1.65-1.55 ($m$, 4H, CH$_2$CH$_2$CH$_2$CH$_2$Br) $\delta$ 1.26 ($s$, 3H, 2’R-CH$_3$)

$^{13}$C-NMR (300MHz, CDCl$_3$) 145.1, 144.7, 122.9, 121.3, 118.5, 117.0, 74.19, 39.01, 33.13, 31.59, 24.07, 22.65, 20.40, 14.32, 12.22, 11.80, 11.19

MS [EI+] $m/z$ 340 (M$^+$, 23%), 165 (100%)
**14.1.15 Synthesis of (R)-(4-(6-((tert-butyldimethylsilyl)oxy)-2,5,7,8-tetramethylchroman-2-yl)butyl)triphenylphosphonium bromide (18)**

Compound 16 (92mg, 0.203mmol) was dissolved with triphenylphosphine (53mg, 0.203mmol) in dry toluene (1ml). The mixture was heated for 20min at 180°C in a microwave oven. The solvent was evaporated and silica column chromatography (gradient CH₂Cl₂, 9:0.2 to CH₂Cl₂:MeOH) afforded 18 (135mg 34.4%) as a white wax.

**TLC:** \( R_f = 0.12 \) (CH₂Cl₂/MeOH, 9:0.2)

**¹H-NMR** (300MHz, CDCl₃) \( \delta \) 7.90-7.67 (m, 15H, P⁺-Ph), \( \delta \) 4.02 (brs, 1H, P⁺-CH₂CH₂CH₂CH₂), \( \delta \) 3.81 (brs, 1H, P⁺-CH₂CH₂CH₂CH₂), \( \delta \) 2.53 (t, \( J = 6.90 \) Hz, 2H, ArCH₂CH₂), \( \delta \) 2.06 (s, 3H, ArCH₃), \( \delta \) 2.05 (s, 3H, ArCH₃), \( \delta \) 1.94 (s, 3H, ArCH₃), \( \delta \) 1.80-1.59 (m, 8H, ArCH₂CH₂CH₂CH₂), \( \delta \) 1.22 (s, 3H, 2’R-CH₃), \( \delta \) 1.06 (s, 9H, Si-tBu), \( \delta \) 0.125 (s, 6H, 2x Si-CH₃)

**¹³C-NMR** (400MHz, CDCl₃) 144.1, 134.9, 133.8, 133.7, 130.5, 130.4, 125.7, 123.7, 122.4, 119.0, 118.1, 117.7, 74.43, 38.89, 31.46, 26.11, 24.61, 23.85, 23.18, 20.81, 18.60, 14.31, 13.43, 11.98, -3.30, -3.34

**³¹P-NMR** (400MHz, CDCl₃) 24.61

**MS [ESI+]** \( m/z \) 637.4 (M⁺, 100%), 539.3 (10%), 529.3 (M⁺+, - TBS,19%)

**MS [ESI-]** \( m/z \) 78.9/88.9 (Br⁻, 100%)

MS Calculated for C₄₁H₅₄BrO₂PSi 716.281

**14.1.16 Synthesis of (R)-tert-butyl((2-(4-iodobutyl)-2,5,7,8-tetramethylchroman-6-yl)oxy)dimethylsilane (17)**

![Diagram](image-url)
Compound 9-OMs (235mg, 0.500mmol) was dissolved in dry THF (3ml) and acetone (2ml) and potassium iodide (160mg, 1.06mmol) was added and stirred for 16h at reflux. The solvent was evaporated and silica column chromatography (gradient Hex/CH2Cl2, 4:1 to CH2Cl2) afforded 17 (135mg 53.7%) as a colourless oil.

**TLC:**  
$R_f = 0.92$ (CH2Cl2)

**1H-NMR**  
(300MHz, CDCl3) $\delta$ 3.21 ($t, J = 7.2$ Hz, 2H, ArCH2CH2), $\delta$ 2.12 ($s, 3H, ArCH3), $\delta$ 2.09 ($s, 3H, ArCH3), $\delta$ 2.07 ($s, 3H, ArCH3), $\delta$ 1.89-1.73 ($m(p), J = 6.90$ Hz, 4H, ArCH2CH2 + CH2CH2I) $\delta$ 1.29 ($s, 3H, 2'R-CH3), $\delta$ 1.06 ($s, 9H, Si-tBu), $\delta$ 0.135 ($s, 6H, 2x Si-CH3)

**13C-NMR**  
(400MHz, CDCl3) 145.8, 144.2, 125.9, 123.6, 122.7, 117.5, 74.34, 62.97, 39.34, 33.21, 31.58, 26.11, 23.74, 20.87, 19.88, 18.61, 14.33, 13.41, 11.97, -3.34

**MS [EI+]**  
m/z 502 (M+, 100%), 376 (M+ - I-, 72%), 362 (50%), 279 (69%), 221 (50%), 73 (100%)

MS Calculated for C23H39IO2Si 502.176

**14.1.17 Synthesis of (R)-(4-(6-((tert-butyl(dimethyl)silyl)-oxy)-2,5,7,8-tetramethylchroman-2-yl)butyl)triphenylphosphonium iodide (19)**

Compound 17 (67mg, 0.133mmol) and triphenylphosphine (34.8mg, 0.133mmol) were dissolved in toluene (1ml). The mixture was reacted for 45min at 145°C in a microwave oven. The solvent was evaporated and silica column chromatography (gradient CH2Cl2, to CH2Cl2:MeOH 9:0.2) afforded 19 (135mg 43.3%) as a white oil.

**TLC:**  
$R_f = 0.12$ (CH2Cl2/MeOH, 9:0.2)

**1H-NMR**  
(300MHz, CDCl3) $\delta$ 7.82-7.43 ($m, 15H, P'-Ph), $\delta$ 3.69-3.55 ($dm, J = 42.6$ Hz, 2H, P'-CH2CH2CH2CH2), $\delta$ 2.52 ($t, J = 6.83$ Hz, 2H, ArCH2CH2), $\delta$ 2.05 ($s, 3H, ArCH3), $\delta$ 2.03 ($s, 3H, ArCH3), $\delta$ 1.94 ($s, 3H, ArCH3), $\delta$ 1.80-1.62 ($m, 6H, P'-CH2CH2CH2CH2 + ArCH2CH2), $\delta$ 1.62-1.52 ($m, J = 7.60$ Hz, 2H, P'-CH2CH2CH2CH2) $\delta$ 1.21 ($s, 3H, 2'R-CH3), $\delta$ 1.04 ($s, 9H, Si-tBu), $\delta$ 0.105 ($s, 6H, 2x Si-CH3)
\textbf{Synthesis of 1-benzyl-1H-pyrrole-2-carbaldehyde}^{635}

\begin{center}
\includegraphics[width=0.1\textwidth]{benzyl_pyrrole_structure.png}
\end{center}

To a suspension of sodium hydride 60\% (1.00 g, 0.01 mol) in dry DMF (16 ml) was added dropwise a solution of pyrrole-2-carboxaldehyde (1.00 g, 0.01 mol) in dry DMF (8 ml) under N$_2$ atmosphere at 0°C. After 10 min., benzyl bromide (1.5 ml, 0.01 mol) was added dropwise. After 30 min. at room temperature. The solution was diluted with ethyl acetate and extracted four times with ice water, followed by one portion of brine. The reaction was dried over Na$_2$SO$_4$ and evaporated down to dryness. Silica column chromatography (gradient Hex/EtOAc, 9:1 to 1:1) afforded 1-benzyl-1H-pyrrole-2-carbaldehyde (0.42 g 87.4\%) as a brown liquid.

\begin{itemize}
  \item \textbf{TLC: } $R_f = 0.51$ (Hex/EtOAc, 6:1)
  \item \textbf{\textsuperscript{1}H-NMR} (400MHz, CDCl$_3$) 9.59 (s, 1H, CHO), 7.36-7.95 (m, 3H, Bn-), 7.18-7.16 (m, 2H, Bn-), $\delta$ 7.00 (d, $J = 3.20$ Hz, 2H, pyrrole-H), $\delta$ 6.29 (t, $J = 3.20$ Hz, 1H, pyrrole-H), $\delta$ 5.59 (s, 2H, Bn-CH$_2$)
  \item \textbf{\textsuperscript{13}C-NMR} (400MHz, CDCl$_3$) 179.5, 137.5, 131.4, 128.7, 127.7, 127.3, 124.9, 110.1, 51.97
  \item \textbf{MS [ESI+]} m/z 186.1 (M$^+$, 100\%), 208.1 (M+Na, 64\%)
\end{itemize}

\textbf{MS Calculated for C$_{12}$H$_{11}$NO 185.084}

\textbf{Alternative procedure}^{636}:

Oxalylchloride (0.6 ml 6.99 mol) was added under stirring at 0°C to dry DMF (0.539 ml, 6.99 mol) under N$_2$ atmosphere. A white precipitate formed and after 10 min, dry CH$_2$Cl$_2$ (6 ml) was added. A solution of N-benzylpyrrole (1.00 g, 6.36 mol) in CH$_2$Cl$_2$ (6 ml) was added over 15 min. The reaction was stirred for 30 min. and the green solution was quenched with a NaOAc solution (2.6 g in 20 ml H$_2$O). After 30 min. the green water phase was separated and washed 5x with ether. The separate organic phases (CH$_2$Cl$_2$ and ether) were each washed 3x with saturated sodium bicarbonate, followed by 3x with H$_2$O. The organic layers were combined and dried
over Na$_2$SO$_4$ and evaporated down to dryness. Silica column chromatography (Hex/EtOAc, 6:1) afforded 1-benzyl-1H-pyrrole-2-carbaldehyde (450mg 38.2%-45%) as a colourless oil.

Side product: 1-benzyl-1H-pyrrole-3-carbaldehyde (76mg, 6.4%)

TLC: $R_f = 0.13$ (Hex/EE, 6:1)

$^1$H-NMR (400MHz, CDCl$_3$) 9.76 (s, 1H, CHO), 7.42-7.33 (m, 3H, Bn-), δ 6.73 (t, $J = 2.40$ Hz, 1H, pyrrole-H), 7.20-7.18 (m, 2H, Bn-), δ 6.72 (t, $J = 2.40$ Hz, 1H, pyrrole-H), δ 6.68 ($dd$, $J = 2.80$ Hz, $J = 1.60$ Hz, 1H, pyrrole-H), δ 5.12 (s, 2H, Bn-CH$_2$)

$^{13}$C-NMR (400MHz, CDCl$_3$) 185.4, 136.1, 129.2, 129.1, 128.4, 127.4, 126.9, 123.8, 108.7, 54.02

MS [EI+] $m/z$ 185.02 (M, 51%), 91.01 (Bn+ 100%)

MS Calculated for C$_{12}$H$_{11}$NO 185.084

14.1.18 Synthesis of 4-(1-benzyl-1H-pyrrol-2-yl)but-3-en-1-ol (20)

7 (583mg, 1.45mmol) was suspended in dry THF (7ml) under an nitrogen atmosphere at room temperature. A 0.8M solution of LiHMDS in THF (3.8ml, 3.8mmol) was added over 5min. The mixture was stirred for 3h at room temperature. A red solution formed, to which a solution of 1-benzyl-1H-pyrrole-2-carbaldehyde (269mg, 1.45mmol) in dry THF (3ml) was added over 5min at room temperature. The reaction solution was allowed to continue stirring for 4h. The reaction was quenched with an aqueous NH$_4$Cl solution (1:1), extracted with CH$_2$Cl$_2$. The organic phase was dried over Na$_2$SO$_4$ and evaporated down to dryness. Silica column chromatography (gradient, Hex:EtOAc 7:1 to 4:1 to 1:1 to EtOAc) afforded 20 (48mg 14.6%) as a colourless oil.

TLC: $R_f = 0.17$ (Hex/EtOAc, 6:1)

$^1$H-NMR (300MHz, CDCl$_3$) trans ~66% δ 7.37-7.27 (m, 6H, Bn- + pyrrole-H), δ 6.69 (s, 1H, pyrrole-H), δ 6.30 ($d$, $J = 15.6$ Hz, 1H, RCH=CHCH$_2$), δ 6.20 ($t$, $J = 3.30$ Hz, 1H, pyrrole-H), δ 5.95 ($dt$, $J = 15.6$ Hz, $J = 4.20$ Hz, 1H, RCH=CHCH$_2$), δ 5.15 (s, 2H, Bn-CH$_2$), δ 3.64 ($t$, $J = 6.30$ Hz, $J = 5.40$ Hz, 2H,
RCH=CHCH₂CH₂OH), δ 2.37 (dt, J = 6.30 Hz, J = 5.40 Hz, 2H, RCH=CHH₂CH₂OH)
cis ~33% δ 7.37-7.27 (m, 5H, Bn-), δ 6.69 (s, 6H, Bn- + pyrrole-H), δ 6.40 (dd, J = 3.60 Hz, J = 1.20 Hz, 1H, RCH=CHCH₂), δ 6.20 (t, J = 3.00 Hz, 1H, pyrrole-H), δ 6.16 (s, J = 3.30 Hz, 1H, Bn-CH₂), δ 5.35 (d, J = 5.40 Hz, 1H, Bn-CH₂), δ 2.14 (dt, J = 6.30 Hz, 2H, RCH=CHC₃H₂CH₂OH)

13C-NMR (300MHz, CDCl₃) trans 138.3, 131.8, 128.7, 127.3, 126.3, 125.5, 120.5, 108.3, 105.9, 62.43, 50.39, 36.62
cis 138.3, 132.0, 128.8, 127.5, 126.3, 124.6, 122.4, 121.8, 108.3, 106.2, 61.92, 50.39, 36.62

MS [ESI+] m/z 228.1 (M⁺, 1%), 266.1 (M+K, 33%), 244.1 (91%), 208.0 (39%), 186.1 (100%)
MS Calculated for C₁₃H₁₇NO 227.131

Synthesis of (6-((tert-butyldimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)methanol, (22)°

To a solution of lithium aluminium hydride (124mg, 0.00326mol) in dry THF (17ml) was added a solution of 5 (1.23g, 0.00326mol) in dry THF (3ml) at 0°C under N₂ atmosphere. The reaction was stirred at room temperature for 16h, cooled back to 0°C, CH₂Cl₂ added followed by small portions of water (5x 10ml). The water phase was acidified with aqueous HCl solution, stirred for 5min followed by separation of the organic phase. The water phase was washed four times with CH₂Cl₂, the organic phases combined, dried over Na₂SO₄ and evaporated down to dryness which afforded product 22 (1.003g 87.4%) as a clear oil.

TLC: Rf = 0.25 (Hex/CH₂Cl₂ 3:1)

1H-NMR (400MHz, CDCl₃) δ 3.64 (2x d, J = 19.6 Hz, 2H, CH₂OH), δ 2.63 (t, J = 7.6, Hz, 2H, ArCH₂), δ 2.13 (s, 3H, ArCH₃), δ 2.10 (s, 3H, ArCH₃), δ 2.09 (s, 3H, ArCH₃), δ 2.0 (enanti dt, J = 5.6 Hz, 1H, ArCH₂CH₂), δ 1.74 (enanti dt, J = 5.6
Hz, 1H, ArCH₂CH₂), δ 1.24 (s, 3H, 2’R-CH₃), δ 1.07 (s, 9H, Si-tBu), δ 0.14 (s, 6H, 2x Si-CH₃)

**13C-NMR** (300MHz, CDCl₃) 145.3, 144.6, 126.1, 123.8, 122.6, 117.4, 75.05, 69.42, 29.72, 27.85, 26.20, 20.45, 20.39, 18.61, 14.33, 12.02, -3.30, -3.35

**MS [ESI+]** m/z 353.3 (M⁺, 41%), 723.4 (2xM + Na 100%), 373.2 (M + Na 33%), 389.2 (M + K 28%)

MS Calculated for C₂₀H₃₄O₃Si 350.228

**14.1.19 Synthesis of (6-((tert-butyldimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)methyl methanesulfonate (22-OMs)**

![TBSO](image)

![OMs](image)

To a solution of 22 (200mg, 0.571mmol) and triethylamine (159µl, 1.14mmol) in dry CH₂Cl₂ (2ml) was added mesyl chloride (53µl, 0.653mmol) at 0°C under a nitrogen atmosphere. The foggy yellow suspension was stirred for 30min., followed by extraction with water. The water phase was washed with CH₂Cl₂, the organic layers combined, dried over Na₂SO₄ and evaporated down to dryness. Silica column chromatography (Hex/CH₂Cl₂, 1:1) afforded 22-OMs (178mg 72.8%) as a light brown powder.

**TLC:** Rᵣ = 0.73 (CH₂Cl₂)

**¹H-NMR** (300MHz, CDCl₃) δ 4.28 (d, J = 10.2 Hz, 1H, MsCH₂) δ 4.15 (d, J = 10.2 Hz, 1H, MsCH₂), δ 3.04 (s, 3H, SO₂CH₃), δ 2.63 (t, J = 6.9 Hz, 2H, ArCH₂), δ 2.11 (s, 3H, ArCH₃), δ 2.08 (s, 6H, 2x ArCH₃), δ 2.02 (dt, J = 6.9 Hz, 1H, ArCH₂CH₂), δ 1.85 (dt, J = 6.9 Hz, 1H, ArCH₂CH₂), δ 1.34 (s, 3H, 2’R-CH₃), δ 1.07 (s, 9H, Si-tBu), δ 0.14 (s, 6H, 2x Si-CH₃)

**¹³C-NMR** (300MHz, CDCl₃) 144.9, 126.5, 125.8, 125.2, 123.8, 122.7, 116.9, 73.86, 73.12, 37.40, 28.14, 26.08, 21.49, 20.12, 18.60, 14.32, 13.41, 12.01, -3.28, -3.36

**MS [EI+]** m/z 428 (M, 55%), 332 (M-OMs, 100%), 73 (55%)

MS Calculated for C₂₁H₃₆O₃SiS 428.205
14.1.20 Synthesis of ((2-(bromomethyl)-2,5,7,8-tetramethylchroman-6-yl)oxy)(tert-butyl)dimethylsilane (23)

To a solution of 22-OMs (178mg, 0.416mmol) in DMF (2ml) was added lithium bromide (500mg, 5.76mol) and stirred at 70°C for 5 days. The reaction was cooled to room temperature, EtOAc added and extracted five times with water and once with 50% v/v sat. brine / water solution. The organic phase was dried over Na₂SO₄ and evaporated down to dryness. Silica column chromatography (Hex/EtOAc, 4:1 à→ 1:1) afforded 23 (98mg 72.8%) as a colourless oil.

TLC: \( R_f = 0.55 \) (CH₂Cl₂)

\(^1\)H-NMR (300MHz, CDCl₃) \( \delta \) 3.47 (d, \( J = 10.5 \) Hz, 1H, CH₂Br), \( \delta \) 3.42 (d, \( J = 10.5 \) Hz, 1H, CH₂Br), \( \delta \) 2.59 (t, \( J = 6.6 \) Hz, 2H, ArCH₂), \( \delta \) 2.15 (dt, \( J = 21.0 \) Hz, \( J = 7.5 \) Hz, 2H, Ar CH₂CH₂), \( \delta \) 2.14 (s, 3H, ArCH₃), \( \delta \) 2.10 (s, 3H, ArCH₃), \( \delta \) 2.08 (s, 3H, ArCH₃), \( \delta \) 1.88 (dt, \( J = 6.9 \) Hz, 1H, ArCH₂CH₂), \( \delta \) 1.85 (dt, \( J = 21.0 \) Hz, \( J = 7.5 \) Hz, 2H, ArCH₂CH₂), \( \delta \) 1.45 (s, 3H, 2'R-CH₃), \( \delta \) 1.06 (s, 9H, Si-tBu), \( \delta \) 0.14 (s, 6H, 2x Si-CH₃)

\(^{13}\)C-NMR (300MHz, CDCl₃) 144.9, 126.5, 125.8, 123.8, 122.7, 116.9, 73.86, 73.12, 37.40, 28.14, 26.08, 20.12, 14.32, 13.41, 12.01, -3.28, -3.36

MS [EI+] \( m/z \) 412 (M⁺, 10%), 205 (20%), 137 (25%), 97 (39%), 57 (100%)

MS Calculated for C₂₀H₃₃BrO₂Si 412.143

Byproduct 2-(bromomethyl)-2,5,7,8-tetramethylchroman-6-ol (24)

22 (410mg, 0.416mmol), tetrabromomethane (3.10g, 9.14mol), triphenylphosphine (1.50g, 5.72mol) were dissolved in toluene (15ml) and stirred at room temperature for 16h. The solvent was evaporated and extracted with EtOAc and water. The organic phase was dried over Na₂SO₄ and evaporated down to dryness. Silica column chromatography (Hex/EE, 2:1) afforded 24 (261mg 74.8%) as a colourless oil.

TLC: \( R_f = 0.70 \) (Hex/EtOAc, 2:1)
1H-NMR  (300MHz, CDCl₃) δ 3.44 (dd, J = 10.5 Hz, 2H, CH₂Br), δ 2.64 (t, J = 3.6 Hz, 2H, Ar CH₂CH₂CH₂H₂), δ 2.18 (s, 3H, ArCH₃), δ 2.14 (s, 6H, 2x ArCH₃), δ 1.90 (dt, J = 13.5 Hz, J = 7.5 Hz, 1H, ArCH₂CH₂H₂), δ 1.45 (s, 3H, 2'R-CH₃)

13C-NMR  (300MHz, CDCl₃) 145.1, 144.7, 122.9, 121.3, 118.5, 117.0, 73.68, 39.01, 29.59, 24.07, 20.40, 12.22, 11.80, 11.29

MS [EI+]  m/z 298 (M⁺, 100%), 165 (80%)
MS Calculated for C₁₄H₁₉BrO₅ 298.057

14.1.21 Synthesis of (R)-(5-bromothiophen-2-yl)((S)-6-((tert-butyl)dimethylsilyl)oxy)-2,5,7,8-tetramethylchroman-2-yl)methanol (25)

2-Bromothiophene (3.09g, 0.019mol) was dissolved in dry THF (45ml) and cooled under a N₂ atmosphere to -78°C. LDA (9.48ml, 2.0 M in THF) was added dropwise and stirred for 50 min. at -78°C. To the resulting red solution was added 6 (3.00g, 0.0086mol) in dry THF (10ml) slowly at the same temperature. After 1.5 h, the reaction was completed and a mixture of CH₂Cl₂/H₂O (1:1, 100ml) was added and the mixture let warm to room temperature. The layers were separated and the aqueous layer washed with CH₂Cl₂ (3x 20 ml). The combined organic phases were dried over Na₂SO₄ and evaporated down to dryness. Silica column chromatography (Hex/CH₂Cl₂, 1:1) afforded 25 (3.84g 87.4%) as a light brown powder.

TLC:  Rₚ = 0.53 (Hex/CH₂Cl₂, 1:4)

1H-NMR  (300MHz, CDCl₃) diastereomers: majorᵃ 75% / minorᵇ 25%; δ 6.93ᵇ 6.92ᵃ (d, J = 3.78 Hz, 1H, Ar-thiophene-H), δ 6.82ᵇ 6.79ᵃ (d, J = 3.78 Hz 1H, Ar-thiophene-H), δ 4.93ᵇ 4.87ᵃ (d, J = 3.63 Hz, 1H, thiophene-CH), δ 2.95ᵇ 2.93ᵃ (d broad, J = 4.16 Hz, 1H, OH), δ 2.59ᵇ (m, J = 4.78 Hz, 2H, ArCH₃), δ 2.15 2.12ᵇ (split s, 6H, ArCH₃), δ 2.06ᵇ 2.05ᵃ (s, 3H, ArCH₃), δ 1.95ᵇ δ 1.82ᵇ (m, 2H, ArCH₂CH₂), δ 1.71ᵇ δ 1.65ᵇ (m, J = 3.33 Hz (¹⁷Hᵇ), 2H, ArCH₂CH₂), δ 1.23ᵃ 1.19ᵇ (s, 3H, (S)-CH₃), δ 1.05 (s, 9H, Si-tBu), δ 0.128 (s, 6H, 2x Si-CH₃),

¹³C-NMR  (400MHz, CDCl₃)ᵃ 144.9, 144.7, 144.5, 144.4, 141.4, 128.9, 126.4, 126.3, 126.2, 126.0, 123.8, 123.8, 122.8, 122.7, 117.4, 117.4, 112.1, 112.0, 77.27, 77.18, 76.50, 75.98, 34.67, 34.52, 31.59, 30.91, 27.94, 26.92, 26.19, 26.09, 25.29, 22.65, 20.71, 20.31, 20.24, 19.46, 18.60, 18.45, 14.35, 14.12, 13.42, 12.46, 12.27, -3.26, -3.34

314
MS [EI+] \[m/z\] 510 (M⁺, 7%), 348 (20%), 319 (100%), 73 (47%)

HRMS Calculated for C₂₄H₃₅BrO₂SSi 510.1260; found: 510.1250

14.1.22 Synthesis of (S)-(2-((5-bromothiophen-2-yl)methyl)-2,5,7,8-tetramethylchroman-6-yl)oxy)(tert-butyl)dimethylsilane (26)

Thienyl alcohol 25 (2.37g, 0.0046mol) was dissolved in dry CH₂Cl₂ (80ml) and was cooled under an N₂ atmosphere to 0°. Triethylsilane (1.17ml, 0.0093mol) was added via syringe. BF₃·OEt₂ (1.17ml, 0.0093mol) was added drop wise and the solution was stirred for 1h at 0°C. The yellow solution was quenched with H₂O. The water phase was extracted three times with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄ and evaporated to dryness. SiO₂ column chromatography (Hex/CH₂Cl₂, 3:1) afforded 26 (1.983g 86.5%) as a light brown powder.

TLC: \[R_f = 0.79\] (Hexane/CH₂Cl₂, 1:1) mp: 104°C (after chromatography)

¹H-NMR (300MHz, CDCl₃) 6.87 (d, \[J = 3.60\] Hz, 1H, Ar-thiophene-H), δ 6.59 (d, \[J = 3.63\] Hz, 1H, Ar-thiophene-H), δ 3.05 (t, \[J = 11.35\] Hz, 2H, ArCH₂), δ 2.60 (t, \[J = 6.83\] Hz, 2H, ArCH₂), δ 2.068 (t, \[J = 11.35\] Hz, 2H, ArCH₂), δ 2.068 (s, 3H, ArCH₃), δ 2.076 (s, 3H, ArCH₃), δ 1.79 (m, \[J = 6.72\] Hz, 2H, ArCH₂CH₂), δ 1.249 (s, 3H, 2'R-CH₃), δ 1.071 (s, 9H, Si-t-Bu), δ 0.144 (s, 6H, 2x Si-CH₃)

¹³C-NMR (300MHz, CDCl₃) 145.4, 144.4, 141.1, 129.0, 127.3, 126.2, 123.5, 122.9, 116.9, 110.4, 73.97, 41.27, 30.70, 26.08, 23.52, 20.75, 18.58, 14.35, 13.39, 12.37, -3.30, -3.35

MS [EI+] \[m/z\] 494 (M⁺, 9%), 319 (100%), 147 (17%), 73 (18%)

HRMS Calculated for C₂₄H₃₅BrO₂SSi 494.1310; found: 494.1315

14.1.23 Synthesis of (S)-methyl 6-(benzyloxy)-2,5,7,8-tetramethylchroman-2-carboxylate (27)

To a solution of rac 4 (940mg, 3.56mmol) in dry DMF (10ml) was added potassium carbonate (738mg, 5.34mmol) and the suspension stirred for 20min at 0°C. Benzyl bromide (0.5ml) was
added at 0°C and stirring continued for 16h at room temperature. The reaction was quenched with water and extracted with ethyl acetate. The organic phase was washed several times with ice water, dried with Na₂SO₄ and evaporated down to dryness. Silica column chromatography (gradient Hex/CH₂Cl₂, 2:1 to CH₂Cl₂) afforded 27 (1.26g 98.4%) as a white solid.

TLC: \( R_f = 0.57 \) (CH₂Cl₂)

\(^1\)H-NMR (300MHz, CDCl₃) 7.53-7.36 (m, 5H, Bn-), δ 4.71 (s, 2H, Bn-CH₂), δ 3.71 (s, 3H, ArCH₂), δ 2.70-2.43 (m, 3H, ArCH₂+ ArCH₂CH₂), δ 2.25 (s, 3H, ArCH₃), δ 2.21 (s, 3H, ArCH₃), δ 2.15 (s, 3H, ArCH₃), δ 1.96-1.86 (m, 1H, ArCH₂CH₂), δ 1.64 (s, 3H, 2’R-CH₃),

\(^1\)C-NMR (300MHz, CDCl₃) 174.4, 148.9, 147.8, 138.0, 128.5, 128.3, 127.8, 127.7, 125.7, 122.9, 117.2, 74.63, 52.35, 31.60, 30.47, 25.42, 22.66, 20.87, 14.13, 12.89, 11.97, 11.86

MS [EI+] \( m/z \) 354 (M⁺, 11%), 263 (100%), 231 (35%), 203 (33%), 105 (40%), 91 (61%)

MS Calculated for C₂₂H₂₆O₄: 354.183

### 14.1.24 Synthesis of \((S)-6-\)benzyloxy-2,5,7,8-tetramethylchroman-2-carbaldehyde (21)

27 (1.38g, 3.89mol) was dissolved in dry toluene (10ml) and cooled to -78°C under an N₂ atmosphere. A 0.9M solution of diisobutylaluminiumhydride in toluene (DIBAL, 1M in toluene 7.8ml, 7.03mol) was added over 10min and stirred for 1.5h at -78°C. The reaction was quenched with methanol (5ml), warmed to room temperature followed by slow addition of first water (30ml), then 2M HCl. The phases were stirred for 10min, then separated. The water phase was washed with diethyl ether 4x. The organic phases were dried over Na₂SO₄, combined and evaporated down to dryness. Silica column chromatography (gradient Hex/CH₂Cl₂, 1:1 to DCM) afforded 21 (1.18g, 93.6%) as a clear oil.

TLC: \( R_f = 0.76 \) (CH₂Cl₂)

\(^1\)H-NMR (400MHz, CDCl₃) δ 9.66 (d, \( J = 1.20 \) Hz 1H, CHO), δ 7.53-7.51 (m, 2H, Bn-), δ 7.45-7.35 (m, 3H, Bn-), δ 4.71 (s, 2H, Bn-CH₂), δ 2.60 (m, 3H, ArCH₂CH₂), δ 2.30 (m, 1H, ArCH₂CH₂), δ 2.27 (s, 3H, ArCH₃), δ 2.23 (s, 3H, ArCH₃), δ 2.16 (s, 3H, ArCH₃), δ 1.85 (m, 1H, ArCH₂CH₂), δ 1.43 (s, 3H, 2’R-CH₃)
13C-NMR  
(300MHz, CDCl₃) 204.4, 149.2, 147.5, 137.8, 128.6, 128.5, 127.9, 127.7, 126.4, 123.2, 117.8, 80.46, 74.76, 27.76, 21.58, 20.30, 12.91, 11.99, 11.94, 149.2, 147.5, 137.8, 128.6, 128.5, 127.9, 127.7, 126.4, 123.2, 117.8, 80.46, 74.76, 27.76, 21.58, 20.30, 12.91, 11.99, 11.94,

MS [EI+]  
m/z 325.2 (M⁺H, 41%), 379.2 (10%), 363.1 (36%), 347.2 (100%), 284.4 (7%)
MS Calculated for C₂₁H₂₄O₃ 324.173

Byproduct  (6-(benzyloxy)-2,5,7,8-tetramethylchroman-2-yl)methanol (21.2)

TLC:  
Rᵣ = 0.3 (CH₂Cl₂)

1H-NMR  
(400MHz, CDCl₃) δ 7.55-7.37 (m, 5H, Bn-), δ 4.73 (s, 2H, Bn-CH₂), δ 3.61 (q, J = 18.6 Hz, 2H, CH₂OH), δ 2.69 (brt, J = 8.7 Hz, 2H, ArCH₂), δ 2.26 (s, 3H, ArCH₃), δ 2.21 (s, 3H, ArCH₃), δ 2.138 (s, 3H, ArCH₃), δ 2.10-2.00 (m, 1H, ArCH₂CH₂), δ 1.81 (m, 1H, ArCH₂CH₂), δ 1.25 (s, 3H, 2’R-CH₃)

13C-NMR  
(400MHz, CDCl₃) 148.6, 147.3, 137.9, 128.5, 128.3, 127.9, 127.7, 126.3, 122.9, 117.6, 75.37, 74.77, 69.42, 27.67, 20.54, 20.18, 12.88, 12.04, 11.92

MS [EI+]  
m/z 326 (M⁺, 20%), 235 (100%), 205 (32%), 189 (18%), 165 (59%), 91 (52%)
MS Calculated for C₂₁H₂₆O₃ 326.188

14.1.25 Synthesis of (R)-(S)-6-(benzyloxy)-2,5,7,8-tetramethylchroman-2-yl)(5-bromothiophen-2-yl)methanol (28)

2-Bromothiophene (1.18g, 7.24mmol) was dissolved in dry THF (20ml) and was cooled under a N₂ atmosphere to -78°. LDA (3.62ml 2.0 M in THF, 7.24mmol) was added dropwise and stirred for 50 min. at -78°C. To the resulting red solution was added 21 (1.07g, 3.29mmol) in dry THF (3 ml) slowly at the same temperature. After 2.5h, the reaction was completed and a mixture of CH₂Cl₂/H₂O (1:1, 100ml) was added and the mixture let warm to room temperature. The layers were separated and the aqueous layer washed with CH₂Cl₂ (3x 20ml). The combined organic phases were dried over Na₂SO₄ and evaporated down to dryness. Silica column chromatography (gradient Hex/CH₂Cl₂, 2:1 to CH₂Cl₂) afforded 28 (1.18g 73.8%) as a brown gum.

TLC:  
Rᵣ = 0.54 (CH₂Cl₂)
1H-NMR (300MHz, CDCl₃) diastereomers: major a 75% / minor b 25%; 7.57-7.39 (m, 5H, Bn-), δ 6.98b 6.97a (d, J = 3.90 Hz, 1H, Ar-thiophene-H) δ 6.87b 6.83a (d, J = 3.90 Hz, d', J = 3.90 Hz, 1H, Ar-thiophene-H), δ 4.97b 4.92b (d', J = 3.90 Hz, d', J = 3.90 Hz, 1H, thiophene-CH), δ 4.75 (s, 2H, Bn-CH₂), δ 2.96b 2.91a (d, J = 4.20 Hz, d' J = 4.20 Hz, 1H, CH-OH), δ 2.69b (m, 2H, ArCH₂), δ 2.15 2.12b (split s, 6H, ArCH₂), δ 2.06b 2.05a (s, 3H, ArCH₃), δ 2.00b (m, 1H, ArCH₂CH₂), δ 1.87b δ 1.79a (m, J = 3.33 Hz 1.71ab), 1H, ArCH₂CH₂), δ 1.31a 1.27b (s, 3H, (S)-C-CH₃)

13C-NMR (400MHz, CDCl₃) ab 148.9b, 148.6a, 147.4a, 146.9b, 144.34a, 144.30b, 141.0a, 138.0a, 137.9b, 129.02a, 128.99b, 128.52a, 128.50a, 128.3b, 127.9b, 127.8a, 127.7a, 127.4a, 126.33b, 126.31a, 126.11b, 126.09a, 123.2a, 123.1b, 117.6b, 117.1a, 112.3b, 110.6a, 74.77b, 74.74a, 74.31a, 41.46a, 30.45a, 29.73b, 27.79b, 26.07b, 23.66a, 20.58a, 20.06b, 19.60a, 18.54b, 12.94a, 12.39b, 12.30a, 12.18b, 12.05a

**MS [EI+]** m/z 486 (M⁺, -%), 233 (73%), 205 (68%), 91 (100%)

**MS Calculated for C₂₅H₂₇BrO₃S 486.086**

### 14.1.26 Synthesis of (S)-6-(benzylxylo)-2-((5-bromothiophen-2-yl)methyl)-2,5,7,8-tetramethylchroman (29)

Thienyl alcohol 28 (0.72g, 1.48mol) was dissolved in dry CH₂Cl₂ (20ml) and was cooled under a N₂ atmosphere to 0°C. Triethylsilane (0.47ml, 2.94mmol) was added via syringe. BF₃.OEt₂ (0.37ml, 2.94mmol) was added drop wise and the solution was stirred for 1h at 0°C. The yellow solution was quenched with H₂O. The water phase was extracted three times with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄ and evaporated to dryness. SiO₂ column chromatography (Hex/CH₂Cl₂, 1:1) afforded 29 (570mg 82.2%) as a light yellow oil.

**TLC:** Rᵣ = 0.84 (CH₂Cl₂)

1H-NMR (300MHz, CDCl₃) 7.58-7.34 (m, 5H, Bn-), 6.89 (d, J = 3.60 Hz, 1H, Ar-thiophene-H), δ 6.62 (d, J = 3.60 Hz, 1H, Ar-thiophene-H), δ 4.72 (s, 2H, Bn-CH₂), δ 3.09 (s, 2H, thiophene-CH₂), δ 2.66 (t, J = 6.83 Hz, 2H, ArCH₂), δ 2.07 (s, 3H, ArCH₃), δ 2.20 (s, 3H, ArCH₃), δ 2.19 (s, 3H, ArCH₃), δ 1.83 (m, 2H, ArCH₂CH₂), δ 1.27 (s, 3H, 2’R-CH₃)
13C-NMR (300MHz, CDCl₃) 148.5, 141.0, 138.0, 129.0, 128.5, 128.3, 127.8, 127.7, 127.3, 126.1, 123.2, 117.1, 74.72, 74.29, 41.27, 30.43, 22.91, 23.63, 20.55, 12.91, 12.27, 12.02

MS [EI+] m/z 470 (M⁺, -%), 381 (6.7%), 301 (28%), 205 (100%)

MS Calculated for C₂₅H₂₇BrO₂S 470.082

14.1.27 Synthesis of (S)-2-(5-((6-((tert-butyldimethylsilyl)oxy)-2,5,7,8-tetramethylchroman-2-yl)methyl)thiophen-2-yl)-1H-pyrrole (30)

In a flame dried round bottom flask sodium hydride 60% in mineral oil (172mg, 4.30mmol) was washed with dry THF under N₂ atmosphere. The NaH was then suspended with dry THF (15ml) and after 5min, pyrrole (298µl, 4.30mmol) was added slowly at room temperature. After the bubbling stopped the yellow reaction was stirred for an additional 15min. Zinc chloride (586mg, 4.30mmol) was added to the light orange brown solution and after 5min palladium(II)acetate (26.8mg, 0.119mmol) followed by Johnphos® (35.6mg, 0.119mmol). 29 (1.18g, 2.39mmol) in dry THF (5ml) was added and stirred for 6h at 60°C under N₂. The reaction mixture was cooled to room temperature and extracted with CH₂Cl₂/ water. The water phase was washed 3x with CH₂Cl₂, the combined phases with washed with brine, dried over Na₂SO₄ and evaporated to dryness.

SiO₂ column chromatography (gradient, Hex/CH₂Cl₂ 4:1 to CH₂Cl₂) afforded a mix of 31 and some 30 (10mg 10.8%) as a light brown oil. The exact composition of 30/31 is unknown, but by NMR integration is a 50% ratio assumed.

TLC: Rᵣ = 0.05-0.1 (Hex/CH₂Cl₂ 3:1)

1H-NMR (400MHz, CDCl₃) δ 8.27 (brs, 1H, Ar-pyrrole-NH), δ 6.88 (d, J = 3.60 Hz, 1H, Ar-thiophene-H), δ 6.80 (bs, 1H, Ar-pyrrole-H), δ 6.76 (d, J = 3.60 Hz, 1H, Ar-thiophene-H), δ 6.37 (bs, 1H, Ar-pyrrole-H), δ 6.26 (g, J = 3.20 Hz, 1H, Ar-pyrrole-H), δ 3.10 (dd, J₁ = 21.60 Hz, 2H, thiophene-CH₂), δ 2.61 (bm, 2H, ArCH₂), δ 2.18 (s, 3H, ArCH₃), δ 2.14 (s, 3H, ArCH₃), δ 2.08 (s, 3H, ArCH₃), δ 1.82 (m, 2H, ArCH₂CH₂), δ 1.31 (s, 3H, 2’R-CH₃), δ 1.07 (s, 9H, Si-tBu), δ 0.143 (s, 3H, Si-CH₃)
\( \text{\(^{13}\text{C-NMR}\)} \quad (400MHz, CDCl}_3) \quad 145.6, 144.4, 137.2, 136.9, 135.2, 127.8, 126.2, 123.6, 120.4, 118.2, 117.1, 109.9, 107.9, 106.3, 74.37, 40.73, 30.86, 26.17, 23.82, 23.79, 20.90, 19.00, 18.67, 14.44, 13.49, 12.44, -3.22, -3.27

\text{MS [EI+]} \quad m/z \ 481 \ (M^+, -\%), \ 56 \ (100\%)

\text{[MALDI]} \quad (\text{TOF, POS, RP 1000 no matrix}) \quad m/z \ 481.2 \ (M^+, 12\%), \ 319.1 \ (100\%)

MS Calculated for C\textsubscript{28}H\textsubscript{39}NO\textsubscript{2}S\textsubscript{2}Si 481.247

Byproduct 2,5-bis(5-(((S)-6-((tert-butyldimethylsilyl)oxy)-2,5,7,8-tetramethylchroman-2-yl)methyl)thiophen-2-yl)-1H-pyrrole (31)

\[
\begin{align*}
\text{TLC:} & \quad R_f = 0.05-0-1 \ (\text{Hex/CH}_2\text{Cl}_2 \ 3:1) \\
\text{\(^{1}H-NMR\)} & \quad (400MHz, CDCl}_3) \ \delta \ 8.19 \ (brs, 1H, Ar-pyrrole-NH), \ \delta \ 6.92 \ (d, J = 3.20 \ Hz, 2H, Ar-thiophene-H), \ \delta \ 6.77 \ (d, J = 3.20 \ Hz, 2H, Ar-thiophene-H), \ \delta \ 6.37 \ (bs, 2H, Ar-pyrrole-H), \ \delta \ 3.10 \ (dd, J^2 = 21.60 \ Hz, 2H, thiophene-CH}_2), \ \delta \ 2.61 \ (bm, 2H, ArCH}_2), \ \delta \ 2.18 \ (s, 3H, ArCH}_3), \ \delta \ 2.14 \ (s, 3H, ArCH}_3), \ \delta \ 2.08 \ (s, 3H, ArCH}_3), \ \delta \ 1.82 \ (m, 2H, ArCH}_2CH}_2), \ \delta \ 1.31 \ (s, 3H, 2’R-CH}_3), \ \delta \ 1.07 \ (s, 9H, Si-\text{tBu}), \ \delta \ 0.143 \ (s, 3H, Si-CH}_3)
\end{align*}
\]

\( \text{\(^{13}\text{C-NMR}\)} \quad (400MHz, CDCl}_3) \quad 145.6, 144.4, 144.42, 137.2, 136.9, 135.2, 134.74, 127.75, 127.6, 127.5, 127.0, 126.19, 126.17, 123.6, 122.96, 122.94, 120.4, 120.2, 118.2, 117.1, 109.9, 107.9, 106.3, 74.37, 40.73, 30.86, 26.17, 23.82, 23.79, 20.90, 19.00, 18.67, 14.44, 13.49, 12.44, -3.22, -3.27MS

\text{[EI+] } \quad m/z \ 481 \ (M^+, -\%), \ 56 \ (100\%)

\text{[MALDI]} \quad (\text{TOF, POS, RP 1000 no matrix}) \quad m/z \ 895.2 \ (M^+-H, 21\%), \ 765.2 \ (M-TBSOH, 6\%), \ 617.1 \ (M- H+rev. oxa-diels alder, 1\%), \ 576.1 \ (M- H+chroman ring, 3\%), \ 481.2 \ (M^+ = \text{product, 12\%}), \ 343.7 \ (\text{Fragment rev. oxa-diels alder, 8\%}), \ 319.1 \ (\text{Fragment TBSO-chroman+ 100\%})

MS Calculated for C\textsubscript{52}H\textsubscript{73}NO\textsubscript{4}S\textsubscript{2}Si\textsubscript{2} 895.452
14.1.28 Synthesis of (S)-2-(5-((6-(benzyloxy)-2,5,7,8-tetramethylchroman-2-yl)methyl)thiophen-2-yl)-1H-pyrrole (32)

In a flame dried round bottom flask sodium hydride 60% dispersion in mineral oil (15.3mg, 0.383mmol) was washed with dry THF under N₂ atmosphere. The NaH was then suspended with dry THF (0.2ml) and after 5min, pyrrole (26.6µl, 0.383mmol) was added slowly at room temperature. After the bubbling stopped the yellow reaction was stirred for an additional 15min. Zinc chloride (52.2mg, 0.383mmol) and to the light orange-brown solution after 5min was added palladium(II)acetate (2.4mg, 0.0106mmol) followed by Johnphos® (3.2mg, 0.0106mmol). 26 (100mg, 0.2127) in dry THF (1ml) was added and stirred for 16h at 60°C under N₂. The reaction was cooled to r.t. and extracted with CH₂Cl₂/water. The water phase was washed several times with CH₂Cl₂, the combined phases with washed with brine, dried over Na₂SO₄ and evaporated to dryness. SiO₂ column chromatography (gradient 1 CH₂Cl₂ increments increase, Hex/CH₂Cl₂, 4:1 to CH₂Cl₂) afforded 32 (10mg 10.3%) as a light brown oil.

TLC: \( R_f = 0.73 \) (CH₂Cl₂)

\(^1\)H-NMR (300MHz, CDCl₃) δ 8.27 (brs, 1H, Ar-pyrrole-NH), 7.55-7.34 (m, 5H, Bn-), δ 6.88 (d, \( J = 3.60 \) Hz, 1H, Ar-thiophene-H), δ 6.80 (bs, 1H, Ar-pyrrole-H), δ 6.77 (d, \( J = 3.60 \) Hz, 1H, Ar-thiophene-H), δ 6.38 (bs, 1H, Ar-pyrrole-H), δ 6.27 (q, \( J = 3.00 \) Hz, 1H, Ar-pyrrole-H), δ 4.73 (s, 2H, Bn-CH₂), δ 3.12 (s, 2H, thiophene-CH₂), δ 2.67 (t, \( J = 6.00 \) Hz, 2H, ArCH₂), δ 2.27 (s, 3H, ArCH₃), δ 2.22 (s, 3H, ArCH₃), δ 2.20 (s, 3H, ArCH₃), δ 1.86 (m, 2H, ArCH₂CH₂), δ 1.31 (s, 3H, 2’R-CH₃)

\(^{13}\)C-NMR (300MHz, CDCl₃) 148.4, 147.6, 138.0, 136.8, 135.2, 128.5, 128.2, 127.8, 127.7, 127.6, 127.0, 126.1, 123.2, 120.2, 118.2, 117.2, 109.9, 106.2, 74.73, 74.63, 40.84, 30.54, 29.71, 23.84, 20.63, 12.92, 12.25, 12.03

MS [EI⁺] \( m/z \) 457 (M⁺, -%), 392 (6.6%), 301 (64%), 233 (49), 203 (100%), 91 (94%)

MS Calculated for C₂₉H₉₁NO₂S 457.208

Byproduct (S)-6-(benzyloxy)-2,5,7,8-tetramethyl-2-(thiophen-2-ylmethyl)chroman (29-H)

321
TLC: \( R_f = 0.72 \) (Hexane/CH\(_2\)Cl\(_2\), 1:2)

\(^1\)H-NMR (300MHz, CDCl\(_3\)) 7.56-7.35 (m, 5H, Bn-), 7.21 (dd, \( J = 5.10 \) Hz, \( J = 1.2 \) Hz, 1H, Ar-thiophene-H), \( \delta \) 6.62 (dd, \( J = 3.30 \) Hz, \( J = 1.2 \) Hz, 1H, Ar-thiophene-H), \( \delta \) 4.74 (s, 2H, Bn-CH\(_2\)), \( \delta \) 3.18 (s, 2H, thiophene-CH\(_2\)), \( \delta \) 2.70 (m, 2H, ArCH\(_2\)), \( \delta \) 2.28 (s, 3H, ArCH\(_3\)), \( \delta \) 2.22 (s, 3H, ArCH\(_3\)), \( \delta \) 2.21 (s, 3H, ArCH\(_3\)), \( \delta \) 1.82 (m, 2H, ArCH\(_2\)CH\(_2\)), \( \delta \) 1.30 (s, 3H, 2'-R-CH\(_3\))

\(^{13}\)C-NMR (400MHz, CDCl\(_3\)) 148.4, 147.6, 139.1, 138.0, 129.0, 128.5, 128.2, 127.8, 127.7, 127.1, 126.4, 126.0, 124.4, 123.2, 117.2, 74.73, 74.59, 40.61, 30.62, 23.79, 20.67, 12.92, 12.19, 12.03

MS [EI+] \( m/z \) 392 (M\(^+\), 11%), 301 (100%), 263 (56%), 203 (35), 97 (96%)

MS Calculated for C\(_{25}\)H\(_{28}\)O\(_2\)S 392.181

14.1.29 Synthesis of (S)-7-(5-(6-(benzylxy)-2,5,7,8-tetramethylchroman-2-yl)methyl)thiophen-2-yl)-5,5-difluoro-1,3-dimethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (33)

3,5-Dimethylpyrrole carboxaldehyde (70mg, 0.569mmol) and 32 (260mg (as a 1:1 mix with the dimer), 0.569mmol) were dissolved in dry CH\(_2\)Cl\(_2\) (7ml) and cooled to 0°C under an nitrogen atmosphere. POCl\(_3\) (52\( \mu \)l, 0.569mmol) was added dropwise, covered in aluminum foil and stirred for 16h at room temperature, \( \text{N}_2 \). The pink reaction mixture was cooled to 0°C and triethylamine (317\( \mu \)l, 2.28mmol) was added followed by BF\(_3\)OEt (286\( \mu \)l, 2.28mmol). After 16h, the reaction was cooled to 0°C, diluted with CH\(_2\)Cl\(_2\) and quenched with water. After 5min, the water phase was washed twice with CH\(_2\)Cl\(_2\). The combined organic phases were dried over
Na₂SO₄ and evaporated to dryness. SiO₂ column chromatography (gradient Hex/CH₂Cl₂, 3:1 to 1:1) afforded 33 (23mg 6.6% to the mix / 13.2% to assumed pure SM) as a red oil.

TLC: \( R_f = 0.57 \) (CH₂Cl₂)

\(^{1}H\)-NMR (400MHz, CDCl₃) δ 8.00 (d, \( J = 3.6 \) Hz, 1H, ArH), 7.54-7.36 (m, 5H, Bn-), δ 7.07 (s, 1H, ArH), δ 6.94 (d, \( J = 4.0 \) Hz, 2H, ArH), δ 6.71 (d, \( J = 4.40 \) Hz, 1H, ArH), δ 6.14 (s, 1H, ArH), δ 4.73 (s, 2H, Bn-CH₂), δ 3.18 (dd, \( J = 14.88 \) Hz, \( J = 6.8 \) Hz, 2H, thiophene-CH₂), δ 2.68 (m, 2H, ArCH₂), δ 2.63 (s, 3H, ArCH₃), δ 2.28 (s, 3H, ArCH₃), δ 2.27 (s, 3H, ArCH₃), δ 2.24 (s, 3H, ArCH₃), δ 2.20 (s, 3H, ArCH₃), δ 1.84 (m, \( J = 6.80 \) Hz, 2H, ArCH₂CH₂), δ 1.28 (s, 3H, 2′R-CH₃)

\(^{13}C\)-NMR (400MHz, CDCl₃) 159.5, 148.5, 147.5, 142.9, 142.5, 138.0, 135.5, 133.6, 129.8, 129.4, 128.5, 128.3, 127.8, 127.7, 126.1, 123.2, 122.4, 120.4, 118.7, 117.2, 74.73, 74.64, 40.94, 31.94, 30.77, 29.71, 29.38, 23.90, 20.63, 15.05, 12.93, 12.28, 12.04, 11.31

\(^{19}F\)-NMR (400MHz, CDCl₃) δ -143.6, -143.8 (dq, \( J = 36.00 \) Hz, 2F, BF₂)

\(^{11}B\)-NMR (400MHz, CDCl₃) δ 1.30 (t, \( J = 104.00 \) Hz, 1B, BF₂)

MS [EI+] \( m/z \) 610 (M, -%, 149 (25%), 97 (34%), 85 (53), 71 (71), 57 (100%)

MS Calculated for C₃₆H₃₇O₂BF₂S 610.264

14.1.30 Synthesis of (S)-5-((6-((tert-butyldimethylsilyl)oxy)-2,5,7,8-tetramethylchroman-2-yl)methyl)thiophene-2-carbaldehyde (34)

![Chemical structure of compound 34]

Compound 26 (1.74 g, 0.0035 mol) was dissolved in dry THF (15 ml) and cooled under a N₂ atmosphere to -78°. n-BuLi (2.77 ml, 1.4 M in hexane) was added dropwise over 20 min. After 30 min at -78°C, dry DMF (0.3 ml) was added dropwise and the solution stirred for 2.5 h at the same temperature. The orange solution was then warmed to room temperature and extracted with H₂O. The water phase was extracted three times with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄ and evaporated.

SiO₂ column chromatography (Hex/CH₂Cl₂, 4:1 to CH₂Cl₂) afforded 34 (1.57 g 82.9%) as a light yellow, brown powder.

TLC: \( R_f = 0.63 \) (CH₂Cl₂)

\(^{1}H\)-NMR (400MHz, CDCl₃) 9.852 (s, 1H, CHO), δ 7.65 (d, \( J = 3.76 \) Hz, 1H, Ar-thiophene-H), δ 6.99 (d, \( J = 3.76 \) Hz, 1H, Ar-thiophene-H), δ 3.19 (dd, \( J = 16.76 \) Hz, 1H, Ar-thiophene-H)
Hz, 2H, thiophene-CH$_2$), $\delta$ 2.64 ($t, J = 6.84$ Hz, 2H, ArCH$_2$), $\delta$ 2.14 (s, 3H, ArCH$_3$), $\delta$ 2.08 (s, 3H, ArCH$_3$), $\delta$ 2.06 (s, 3H, ArCH$_3$), $\delta$ 1.84 ($m, J = 6.43$ Hz 2H, ArCH$_2$CH$_2$), $\delta$ 1.27 (s, 3H, 2$'$R-CH$_3$), $\delta$ 1.07 (s, 9H, Si-tBu), $\delta$ 0.1444 (s, 3H, Si-CH$_3$), $\delta$ 0.1441 (s, 3H, Si-CH$_3$)

$^{13}$C-NMR (400MHz, CDCl$_3$) 182.8, 151.1, 145.2, 144.6, 143.0, 136.4, 128.7, 126.4, 123.6, 123.0, 116.8, 73.99, 41.27, 31.07, 26.11, 23.80, 20.76, 18.62, 14.38, 13.43, 12.40, -3.28, -3.32

MS [EI+] m/z 444 (M$^+$, 56%), 319 (100%), 73 (23%)

HRMS Calculated for C$_{25}$H$_{36}$O$_3$SSi 444.2154; found: 444.2165

Byproduct (S)-tert-butyldimethyl((2,5,7,8-tetramethyl-2-(thiophen-2-ylmethyl)chroman-6-yl)oxy)silane (35)

TLC: $R_f = 0.96$ (CH$_2$Cl$_2$)

$^1$H-NMR (400MHz, CDCl$_3$) 7.19 ($dd, J = 5.20$ Hz, $J = 1.20$ Hz, 1H, Ar-thiophene-H), $\delta$
6.96 ($dd, J = 5.20$ Hz, $J = 3.20$ Hz, 1H, Ar-thiophene-H), 6.87 ($t, J = 3.20$ Hz, $J$
= 1.20 Hz, 1H, Ar-thiophene-H), $\delta$ 3.14 (2x $d, J = 23.8$ Hz, 2H, thiophene-CH$_2$),
$\delta$ 2.66 ($dt, J = 6.80$, Hz, 2H, ArCH$_2$CH$_2$), $\delta$ 2.07 (s, 3H, ArCH$_3$), $\delta$ 2.04 (s, 3H, ArCH$_3$), $\delta$ 2.08 (s, 3H, ArCH$_3$), $\delta$ 1.81 ($m, 2$H, ArCH$_2$CH$_2$), $\delta$ 1.57 (s, 3H, 2$'$R-
CH$_3$), $\delta$ 1.07 (s, 9H, Si-tBu), $\delta$ 0.141 (s, 3H, Si-CH$_3$)

$^{13}$C-NMR (300MHz, CDCl$_3$) 145.6, 144.4, 139.3, 127.0, 126.3, 126.1, 124.3, 123.6, 122.9,
117.1, 74.29, 40.45, 30.90, 26.12, 23.70, 20.85, 18.62, 14.37, 13.43, 12.32, -3.28, -3.33

MS [EI+] m/z 416 (M$^+$ 26%), 319 (M$^+$ -thienyl-CH$_2$, 100%), 115 (30%), 58 (71%)

MS Calculated for C$_{24}$H$_{36}$O$_2$SSi 416.221

14.1.31 Synthesis of byproduct (S)-2,5,7,8-tetramethyl-2-(thiophen-2-ylmethyl)chroman-6-
yl formate (36)

H
O
O
O
**35** (50mg, 0.12mmol) was dissolved in dry dichloroethane (2ml) at room temperature under a N₂ atmosphere. Dry dimethylformamide (11.1µl, 0.144mmol) was added. POCl₃ (13.2µl, 0.144mmol) was added and the solution was stirred for 16h at room temperature. The yellow solution was quenched with H₂O. 1M NaOH was added until pH 8 was reached. The water phase was extracted three times with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄ and evaporated to dryness. SiO₂ column chromatography (gradient Hex/CH₂Cl₂, 3:1 to CH₂Cl₂/MeOH 9:1) afforded **36** (39.6mg 38%) as a clear oil.

**TLC:** \( R_f = 0.19 \) (CH₂Cl₂:MeOH, 1:1)

**¹H-NMR** (400MHz, CDCl₃) 8.34 (s, 1H, CHO), 7.19 (dd, \( J = 5.20 \) Hz, \( J = 1.60 \) Hz, 1H, Ar-thiophene-H), \( \delta \) 6.97 (dd, \( J = 5.20 \) Hz, \( J = 3.20 \) Hz, 1H, Ar-thiophene-H), 6.90 (t, \( J = 3.20 \) Hz, \( J = 1.60 \) Hz, 1H, Ar-thiophene-H), 3.17 (s, \( J = 6.80 \) Hz, 2H, Ar-thiophene-CH₂), \( \delta \) 2.70 (q, \( J = 6.80 \), Hz, 2H, ArCH₂CH₂), \( \delta \) 2.21 (s, 3H, ArCH₃), \( \delta \) 2.11 (s, 3H, ArCH₃), \( \delta \) 2.05 (s, 3H, ArCH₃), \( \delta \) 1.83 (m, 2H, ArCH₂CH₂), \( \delta \) 1.29 (s, 3H, 2'R-CH₃)

**¹³C-NMR** (400MHz, CDCl₃) 160.0, 149.5, 140.0, 138.8, 127.2, 126.9, 126.4, 125.0, 124.5, 123.6, 117.3, 74.95, 40.71, 30.25, 23.78, 20.58, 13.16, 12.29, 12.22

**MS [EI+]** \( m/z \) 331.1 (M⁺ 17%), 369.1 (19%), 348.2 (100%)

**MS Calculated for C₁₉H₂₂O₃S** 330.129

**14.1.32 Synthesis of 5,5-difluoro-7,9-dimethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (37)**

Pyrrole-2-carboxaldehyde (1.0 g, 10.5 mmol) and 2,4-dimethylpyrrole (1.0 g, 10.5 mmol) were dissolved in dry CH₂Cl₂ (40 ml) at room temperature. Phosphoryl chloride (0.98 ml, 10.5 mmol) was added drop wise at 0°C under a N₂ atmosphere. The solution was stirred for 5 h at room temperature. The reaction solution was cooled to 0°C, and triethylamine was added (7.32 ml, 52.6 mmol) followed after 5 min. by BF₃·OEt₂ (6.60 ml, 52.6 mmol). Stirring was continued for 8 h. The reaction mixture was extracted 3x times with a large excess of CH₂Cl₂ (500 ml) and H₂O (500ml). The organic phase was filtered through Celite to get rid of the remaining salts. The combined organic phases were dried over anhydrous Na₂SO₄ and evaporated to dryness.
SiO$_2$ column chromatography (Hex/CH$_2$Cl$_2$, 1:1 to CH$_2$Cl$_2$) afforded 37 (1.8 g 78.3 %) as a shiny green-orange solid.

TLC: $R_f = 0.52$ (CH$_2$Cl$_2$) mp: 144°C

$^1$H-NMR (300MHz, CDCl$_3$) $\delta$ 7.65 (s, 1H, ArH), $\delta$ 7.19 (s, 1H, ArH), $\delta$ 6.92 ($d$, $J = 3.72$ Hz, 1H, ArH), $\delta$ 6.43 (s, 1H, ArH), $\delta$ 6.16 (s, 1H, ArH), $\delta$ 2.59 (s, 3H, Ar-CH$_3$), $\delta$ 2.56 (s, 3H, Ar-CH$_3$)

$^{13}$C-NMR (400MHz, CDCl$_3$) 163.1, 145.9, 139.1, 136.3, 132.7, 126.5, 124.8, 121.3, 116.3, 15.15, 11.35,

$^{19}$F-NMR (400MHz, CDCl$_3$) $\delta$ -146.1 ($q$, $J = 31.16$ Hz, 2F, BF$_2$)

$^{11}$B-NMR (400MHz, CDCl$_3$) $\delta$ 0.58 ($t$, $J = 31.10$ Hz, 1B, BF$_2$)

MS [EI+] $m/z$ 220 (M$^+$, 36%), 219 (52%), 200 (56%), 86 (100%) 43 (72%), HRMS Calculated for C$_{31}$H$_{33}$O$_2$BF$_2$S 220.0977; found: 220.0983

Side product 5,5-difluoro-1,3,7,9-tetramethyl-5H-dipyrrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (38)

TLC: $R_f = 0.50$ (CH$_2$Cl$_2$)

$^1$H-NMR (300MHz, CDCl$_3$) $\delta$ 7.06 (s, 1H, Ar-meso-H), $\delta$ 6.07 (s, 2H, ArH), $\delta$ 2.55 (s, 6H, Ar-CH$_3$), $\delta$ 2.27 (s, 6H, Ar-CH$_3$)

$^{13}$C-NMR (400MHz, CDCl$_3$) 156.8, 141.2, 133.4, 133.4, 120.1, 119.0, 14.65, 11.28

$^{19}$F-NMR (400MHz, CDCl$_3$) $\delta$ -146.6 ($q$, $J = 35.20$ Hz, 2F, BF$_2$)

$^{11}$B-NMR (400MHz, CDCl$_3$) $\delta$ 0.88 ($t$, $J = 104$ Hz, 1B, BF$_2$)

MS [ESI+] $m/z$ 249.1 (M$^+$H, 23%), 431.1 (M-F, 100%) 271.1 (48%), 229.1 (M-F, 30%)

MS Calculated for C$_{13}$H$_{15}$BF$_2$N$_2$ 248.130
14.1.33 Synthesis of (S,E)-7-(2-(5-((tert-butyldimethylsilyl)oxy)-2,5,7,8-tetramethylchroman-2-yl)methyl)thiophen-2-yl)vinyl)-5,5-difluoro-9-methyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (39)

Compound 34 (155 mg, 0.35 mmol) and compound 37 (76.8 mg, 0.35 mmol) were dissolved in benzene (9 ml) at room temperature. Piperidine (0.17 ml, 1.74 mmol) was added followed by acetic acid (87 µl, 1.40 mmol). The red solution was stirred for 11 h at 110°C. Benzene was evaporated by rotary evaporation and the oily residue was partitioned between CH₂Cl₂ and H₂O. The water phase was extracted three times with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄ and evaporated to dryness.

SiO₂ column chromatography (Hex/CH₂Cl₂, 3:1 to CH₂Cl₂) afforded silyl-protected 39 (55 mg 24.5%) as shiny blue, bronze oil.

TLC: \( R_f = 0.87 \) (CH₂Cl₂)

\(^1\)H-NMR (400MHz, CDCl₃) \( \delta 7.68 \) (s, 1H, ArH), \( \delta 7.46 \) (d, \( J = 15.8 \) Hz, 1H, CH=CH), \( \delta 7.36 \) (d, \( J = 16.29 \) Hz, 1H, CH=CH), \( \delta 7.17 \) (d, \( J = 3.68 \) Hz, 1H, ArH), \( \delta 7.15 \) (s, 1H, ArH), \( \delta 6.92 \) (d, \( J = 3.76 \) Hz, 1H, ArH), \( \delta 6.85 \) (d, \( J = 3.68 \) Hz, 1H, ArH), \( \delta 6.72 \) (s, 1H, ArH), \( \delta 6.47 \) (q, \( J = 1.99 \) Hz, 1H, ArH), \( \delta 3.13 \) (q, \( J = 14.88 \) Hz, \( J = 6.8 \) Hz, 2H, thiophene-CH₂), \( \delta 2.65 \) (m, 2H, ArCH₂), \( \delta 2.33 \) (s, 3H, ArCH₃), \( \delta 2.18 \) (s, 3H, ArCH₃), \( \delta 2.15 \) (s, 3H, ArCH₃), \( \delta 2.09 \) (s, 3H, ArCH₃), \( \delta 1.87 \) (m, \( J = 7.20 \) Hz, 2H, ArCH₂CH₂), \( \delta 1.29 \) (s, 3H, 2’R-CH₃), \( \delta 1.07 \) (s, 9H, Si-tBu), \( \delta 0.148 \) (s, 6H, 2x Si-CH₃)

\(^{13}\)C-NMR (400MHz, CDCl₃) 158.8, 145.5, 144.5, 144.2, 140.7, 138.4, 138.1, 133.0, 129.8, 128.7, 126.5, 126.3, 125.3, 123.6, 123.0, 122.2, 117.2, 117.0, 116.7, 116.2, 74.32, 40.87, 31.01, 26.12, 23.97, 20.85, 18.62, 14.37, 13.43, 12.32, 11.46, 11.40, -3.27, -3.31

\(^{19}\)F-NMR (400MHz, CDCl₃) \( \delta -142.6 \) (q, \( J = 31.61 \) Hz, 2F, BF₂)

\(^{11}\)B-NMR (400MHz, CDCl₃) \( \delta 0.74 \) (q, \( J = 31.06 \) Hz, 1B, BF₂)

MS [EI+] \( m/z \) 532 (M-TBS, 7%), 512 (10%), 309 (24%), 205 (100), 55 (66)

HRMS Calculated for C₁₃H₁₄O₂BF₂SSi 646.3032 (M-TBS) 532.2167; found: 532.2177
14.1.34 Synthesis of $(S,E)$-5,5-difluoro-7-(2-(5-((6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)methyl)thiophen-2-yl)vinyl)-9-methyl-5H-dipyrrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (3)

TBS-protected 39 (104 mg, 0.16 mmol) was dissolved in THF (6 ml) at room temperature. 8% HCl in MeOH (4 ml) was added and the violet-coloured solution was stirred under N$_2$ atmosphere for 48 h at room temperature. The solution was partitioned between with CH$_2$Cl$_2$ and H$_2$O. The water phase was extracted three times with CH$_2$Cl$_2$. The combined organic phases were dried over Na$_2$SO$_4$ and evaporated to dryness.

SiO$_2$ column chromatography (Hex/CH$_2$Cl$_2$, 3:1 to CH$_2$Cl$_2$) afforded 3 (36 mg 42%) as a shiny blue, bronze oil.

TLC: $R_f$ = 0.51 (CH$_2$Cl$_2$)

$^1$H-NMR (400MHz, CDCl$_3$) δ 7.68 (s, 1H, ArH), δ 7.45 (d, $J$ = 15.93 Hz, 1H, CH=CH), δ 7.35 (d, $J$ = 15.93 Hz, 1H, CH=CH), δ 7.16 (d, $J$ = 4.0 Hz, 1H, ArH), δ 7.15 (s, 1H, ArH), δ 6.92 (d, $J$ = 3.6 Hz, 1H, ArH), δ 6.85 (d, $J$ = 3.6 Hz, 1H, ArH), δ 6.72 (s, 1H, ArH), δ 6.47 (q, 1H, ArH), δ 4.240 (s broad, 1H, O=H), δ 3.13 (q, 2H, thiophene-CH$_2$), δ 2.70 (m, 2H, ArCH$_2$), δ 2.33 (s, 3H, ArCH$_3$), δ 2.23 (s, 3H, ArCH$_3$), δ 2.22 (s, 3H, ArCH$_3$), δ 2.15 (s, 3H, ArCH$_3$), δ 1.86 (m, $J$ = 6.84 Hz, 2H, ArCH$_2$CH$_2$), δ 1.30 (s, 3H, 2’R-CH$_3$)

$^{13}$C-NMR (400MHz, CDCl$_3$) 158.7, 145.0, 145.0, 144.4, 144.0, 140.7, 138.3, 138.1, 133.1, 133.0, 129.8, 128.7, 125.3, 122.9, 122.2, 121.4, 118.6, 117.2, 116.9, 116.7, 116.2, 74.35, 40.85, 30.92, 23.96, 20.71, 12.27, 12.19, 11.48, 11.34

$^{19}$F-NMR (400MHz, CDCl$_3$) δ -142.6 (s, $J$ = 31.43 Hz, 2F, BF$_2$)

$^{11}$B-NMR (400MHz, CDCl$_3$) δ 0.82 (t, $J$ = 31.70 Hz, 1B, BF$_2$)

MS [EI+] m/z 532 (M$^+$, 3%), 512 (4%), 309 (11%), 205 (100), 190 (7%), 98 (7%), 84 (13) 80 (20) 43 (100)

HRMS Calculated for C$_{30}$H$_{31}$O$_2$BF$_2$S 532.2167; found: 532.2169

[ES$-$] (%) m/z 531 (M$^+$, 100%), 327 (12%),

HRMS Calculated for C$_{30}$H$_{31}$O$_2$BF$_2$S 531.2095; found: 531.3
11B with 1H decoupling

![11B with 1H decoupling](image1)

F19 with 1H decoupling

![F19 with 1H decoupling](image2)
14.1.35 Synthesis of (S,E)-5,5-difluoro-7-(2-(5-((6-methoxy-2,5,7,8-tetramethylchroman-2-yl)methyl)thiophen-2-yl)vinyl)-9-methyl-5H-dipyrrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (40)

Compound 3 (4.6 mg, 4.72 µmol) was dissolved in dry THF (1 ml) at room temperature. Methyl iodide (0.74 µl, 47.2 µmol) and activated NaH (60% dispersion in mineral oil decanted 3x with hexane and dried) (0.2 mg, 4.72 µmol) were added and the violet solution was stirred under N\(_2\) atmosphere for 20 minutes at room temperature. The solvent was evaporated and the residue was purified by SiO\(_2\) column chromatography (Hex/CH\(_2\)Cl\(_2\), 1:1 to Hex/CH\(_2\)Cl\(_2\) 1:4, CH\(_2\)Cl\(_2\)) which afforded 40 (0.49 mg 22.7%) as a shiny blue, bronze oil.

TLC: \(R_f = 0.72\) (CH\(_2\)Cl\(_2\))

\(^1\)H-NMR (600MHz, CDCl\(_3\)) \(\delta\) 7.68 (s, 1H, ArH), \(\delta\) 7.45 (d, \(J = 15.79\) Hz, 1H, \(CH=CH\)), \(\delta\) 7.36 (d, \(J = 15.97\) Hz, 1H, \(CH=CH\)), \(\delta\) 7.16 (d, \(J = 3.66\) Hz, 1H, ArH), \(\delta\) 7.15 (s, 1H, ArH), \(\delta\) 6.93 (d, \(J = 3.72\) Hz, 1H, ArH), \(\delta\) 6.86 (d, \(J = 3.60\) Hz, 1H, ArH), \(\delta\) 6.71 (s, 1H, ArH), \(\delta\) 6.47 (q, \(J = 1.96\) Hz, 1H, ArH), \(\delta\) 3.66 (s, 3H, OCH\(_3\)), \(\delta\) 3.14 (q, \(J = 13.14\) Hz, \(J = 6.8\) Hz 2H, thiophene-CH\(_2\)), \(\delta\) 2.67 (t, \(J = 7.16\) Hz, 2H, ArCH\(_2\)), \(\delta\) 2.323 (s, 3H, ArCH\(_3\)), \(\delta\) 2.24 (s, 3H, ArCH\(_3\)), \(\delta\) 2.20 (s, 3H, ArCH\(_3\)), \(\delta\) 2.18 (s, 3H, ArCH\(_3\)), \(\delta\) 1.86 (m, \(J = 7.33\) Hz, 2H, ArCH\(_2\)CH\(_2\)), \(\delta\) 1.30 (s, 3H, 2-R-CH\(_3\)),

\(^{13}\)C-NMR (from HMBC/HSQC) (600MHz, CDCl\(_3\)) 158.7, 149.5, 147.1, 144.2, 143.9, 140.7, 138.4, 138.0, 133.0, 128.8, 128.7, 128.0, 125.8, 125.4, 132.1, 122.3, 118.5, 117.1, 117.0, 116.7, 116.3, 74.3, 60.3, 41.2, 30.7, 23.9, 20.71, 12.33, 12.12, 11.48, 11.33

\(^{19}\)F-NMR (400MHz, CDCl\(_3\)) \(\delta\) -142.8 (q, \(J = 31.36\) Hz, F, BF\(_2\))

\(^{11}\)B-NMR (400MHz, CDCl\(_3\)) \(\delta\) 0.82 (t, \(J = 31.55\) Hz, 1B, BF\(_2\))

MS [EI+] \(m/z\) 546 (M\(^+\), 13%), 219 (100%), 207 (18%), 43 (13%),

HRMS Calculated for C\(_{31}\)H\(_{33}\)O\(_2\)BF\(_2\)S 546.2324; found: 546.232
14.2 Non-antioxidant-Tocopherol

14.2.1 Synthesis of (2R)-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl trifluoromethanesulfonate (41)

To a solution of α-tocopherol (9.08g, 0.0211mol), pyridine (4.8ml) in CH$_2$Cl$_2$ (100ml) was trifluoromethanesulfonate anhydride (5.96ml) added at 0°C and stirred for 1h at rt. The reaction was quenched with aqueous NaHCO$_3$ and extracted. The water phase was washed with CH$_2$Cl$_2$, the organic phases combined, dried over Na$_2$SO$_4$ and evaporation down to dryness afforded 41 (11.17g, 94.1%) as a clear oil.

TLC: $R_f = 0.62$ (Hex/CH$_2$Cl$_2$, 5:1)

$^1$H-NMR (400MHz, CDCl$_3$) δ 2.62 (t, $J = 6.80$, Hz, 2H, ArCH$_2$CH$_2$), δ 2.25 (s, 3H, ArCH$_3$), δ 2.22 (s, 3H, ArCH$_3$), δ 2.12 (s, 3H, ArCH$_3$), δ 1.84 (enant dt, $J = 6.80$ Hz, 2H, ArCH$_2$CH$_2$), δ 1.67 – 1.09 (m, 21H, phytyl-CH/CH$_2$ + 2’R-CH$_3$) δ 0.88 (m, 12H, phytyl-CH$_3$)

$^{13}$C-NMR (400MHz, CDCl$_3$) 150.9, 139.7, 128.1, 126.7, 124.4, 118.5, 75.68, 39.98, 39.38, 37.29, 32.79, 32.67, 30.86, 27.99, 24.81, 24.44, 23.87, 22.72, 22.63, 21.00, 20.71, 19.75, 19.67, 19.60, 14.02, 13.22, 11.99

$^{19}$F-NMR (400MHz, CDCl$_3$) -73.59

MS [HRMS] HRMS Calculated for C$_{30}$H$_{49}$O$_3$F$_3$S 562.3304; found: 562.3235

14.2.2 Synthesis of (2R)-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman (42)

Compound 41 (11.18g, 0.01987), palladium on carbon 10% (5.1g) and triethylamine (12.2ml, 0.0871mol) were dissolved in MeOH/THF 2:1 (200ml) and stirred for 2 days under H$_2$ (15psi) The reaction mixture was filtered over purified celite, washed with EtOAc and the solvent was evaporated. The residue was extracted with CH$_2$Cl$_2$ and H$_2$O. The water phase was washed with CH$_2$Cl$_2$ five times, the organic phases were combined, dried over Na$_2$SO$_4$ and evaporated down to dryness. Silica column chromatography (Hex/CH$_2$Cl$_2$, 15:1) afforded 42 (8.12g, 98.6%) as a clear oil.
TLC: \( R_f = 0.45 \) (Hexane/\( \text{CH}_2\text{Cl}_2 \), 10:1)

\(^1\)H-NMR (400MHz, CDCl\(_3\)) 6.57 (s, 1H, Ar-H), \( \delta \) 2.60 (t, \( J = 6.80 \text{ Hz} \), 2H, ArCH\(_2\)CH\(_2\)), \( \delta \) 2.22 (s, 3H, ArCH\(_3\)), \( \delta \) 2.18 (s, 3H, ArCH\(_3\)), \( \delta \) 2.09 (s, 3H, ArCH\(_3\)), \( \delta \) 1.82 (enant \( dt \), \( J = 6.72 \text{ Hz} \), 2H, ArCH\(_2\)CH\(_2\)), \( \delta \) 1.63 – 1.09 (m, 21H, phytyl-CH/CH\(_2\) + 2’R-CH\(_3\))

\(^{13}\)C-NMR (400MHz, CDCl\(_3\)) 151.6, 134.6, 133.3, 122.1, 122.0, 116.8, 75.09, 40.14, 40.06, 39.38, 37.56, 37.47, 37.30, 32.79, 32.70, 31.13, 31.07, 27.99, 24.82, 24.46, 24.01, 22.73, 22.64, 21.06, 20.11, 19.76, 19.70, 18.82, 11.35

MS [EI+] \( m/z \) 414 (M, 44%), 189 (13%), 149 (100%)

MS Calculated for C\(_{29}\)H\(_{50}\)O 414.386

14.2.3 Synthesis of (2\( R \))-6-chloro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman (43)

\( 42 \) (100mg, 0.241mmol) was stirred over night with \( N \)-chloro succinimide (33.8mg, 0.253mmol) in dry acetonitrile (2ml) at 60°C under \( N_2 \) atmosphere in the dark (reaction flask covered with aluminum-foil) for 16h. The solvent was evaporated and remaining reaction mixture was extracted with 1M NaOH and CH\(_2\)Cl. The organic phase was washed with water, brine and dried over Na\(_2\)SO\(_4\) and evaporated down to dryness. Silica column chromatography (Hexane/CH\(_2\)Cl\(_2\) 15:1 to 5:1) afforded 43 (61mg, 56.4%) as a light yellow oil.

TLC: \( R_f = 0.45 \) (10:1 Hexan/CH\(_2\)Cl\(_2\))

\(^1\)H-NMR (400MHz, CDCl\(_3\)) \( \delta \) 2.66 (t, \( J = 6.80 \text{ Hz} \), 2H, ArCH\(_2\)CH\(_2\)), \( \delta \) 2.35 (s, 3H, ArCH\(_3\)), \( \delta \) 2.30 (s, 3H, ArCH\(_3\)), \( \delta \) 2.16 (s, 3H, ArCH\(_3\)), \( \delta \) 1.82 (enant \( dt \), \( J = 25.36 \text{ Hz} \), 2H, ArCH\(_2\)CH\(_2\)), \( \delta \) 1.66-1.04 (m, 21H, phytyl-CH/CH\(_2\) + 2’R-CH\(_3\))

\(^{13}\)C-NMR (400MHz, CDCl\(_3\)) 150.0, 132.8, 131.3, 125.7, 123.7, 118.2, 75.19, 39.87, 39.78, 39.39, 37.56, 37.52, 37.47, 37.40, 37.37, 37.33, 37.30, 32.81, 32.79, 32.72, 32.69, 31.35, 31.29, 29.72, 28.00, 24.82, 24.46, 23.81, 22.74, 22.64, 21.35, 21.03, 19.76, 19.70, 19.66, 19.65, 19.60, 17.23, 16.29, 12.43

MS [EI+] \( m/z \) 448 (M\(^+\), 97%), 223 (15%), 183 (100%)

MS Calculated for C\(_{29}\)H\(_{49}\)O 448.347
14.2.4 Synthesis of (2R)-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-carbaldehyde (46)

42 (8.35g, 0.0202mol) and α,α'-dichloromethoxymethane (3.56ml, 0.0403mol) were dissolved in dry CH\textsubscript{2}Cl\textsubscript{2} (85ml) under a nitrogen atmosphere. At room temperature, titanium(IV) chloride 1M in toluene (48.4ml) was added dropwise over 15min. The reaction was stirred for 1.5h, diluted with CH\textsubscript{2}Cl\textsubscript{2} (30ml), then quenched slowly with water and stirred for 10 extra minutes. The phases were separated and the water phase was washed two times with CH\textsubscript{2}Cl\textsubscript{2}. The organic phases were combined and dried over Na\textsubscript{2}SO\textsubscript{4} and evaporated down to dryness. Silica column chromatography (Hex/CH\textsubscript{2}Cl\textsubscript{2}, 1:1) afforded 46 (6.9g 77.3%) as a clear oil.

TLC: \( R_f = 0.55 \) (Hex/CH\textsubscript{2}Cl\textsubscript{2}, 1:1)

\(^1\)H-NMR (400MHz, CDCl\textsubscript{3}) \( \delta \) 10.58 (s, 1H, Ar-CHO), 2.67 (t, \( J = 6.80 \) Hz, 1H, Ar-CH\textsubscript{2}CH\textsubscript{2}), \( \delta \) 2.50 (s, 3H, ArCH\textsubscript{3}), \( \delta \) 2.47 (s, 3H, ArCH\textsubscript{3}), \( \delta \) 2.15 (s, 3H, ArCH\textsubscript{3}), \( \delta \) 1.85 (enant dt, \( J = 6.80 \) Hz, 2H, ArCH\textsubscript{2}CH\textsubscript{2}), \( \delta \) 1.68-1.04 (m, 21H, phytlyl-CH/CH\textsubscript{2} + 2'R-CH\textsubscript{3}) \( \delta \) 8.88 (m, 12H, phytlyl-CH\textsubscript{3})

\(^13\)C-NMR (400MHz, CDCl\textsubscript{3}) 193.9, 155.7, 138.6, 138.2, 126.1, 123.5, 117.8, 77.23, 76.30, 40.04, 39.97, 39.38, 37.50, 37.45, 37.40, 37.36, 37.29, 32.80, 32.79, 32.67, 21.04, 30.99, 27.99, 24.82, 24.44, 23.95, 22.73, 22.64, 21.02, 20.34, 19.76, 19.69, 19.65, 19.59, 15.70, 14.88, 11.40

MS [EI+] \( m/z \) 442 (M\textsuperscript{+}, 41%), 217 (22%), 177 (100%)

MS Calculated for C\textsubscript{30}H\textsubscript{50}O\textsubscript{4} 442.381

14.2.5 Synthesis of ((2R)-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl)methanol (44)

To a solution of 46 (6.9g, 0.0156mol) in dry THF (50ml), lithium aluminiumhydride (1.18g, 0.0312mol) was added in two batches at 0°C under a nitrogen atmosphere. After 3.5h, the reaction mixture was quenched slowly with water and then brought to pH 6-7 with 1M HCl. The phases were separated and the water phase was washed 5x with CH\textsubscript{2}Cl\textsubscript{2}. The organic phases were combined and dried over Na\textsubscript{2}SO\textsubscript{4} and dried down to dryness. Silica column chromatography (CH\textsubscript{2}Cl\textsubscript{2} to CH\textsubscript{2}Cl\textsubscript{2}/MeOH 50:1) afforded 44 (6.9g, 97.7%) as a clear oil.
TLC: $R_f = 0.2$ (CH$_2$Cl$_2$)

$^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 4.77 ($d$, $J = 2.40$ Hz 1H, Ar-CH$_2$OH), 2.65 ($t$, $J = 6.80$ Hz, 1H, Ar-CH$_2$CH$_2$), $\delta$ 2.35 ($s$, 3H, ArCH$_3$), $\delta$ 2.31 ($s$, 3H, ArCH$_3$), $\delta$ 2.15 ($s$, 3H, ArCH$_3$), $\delta$ 1.88 ($enant
dt$, $J = 6.80$ Hz, 2H, ArCH$_2$CH$_2$), $\delta$ 1.66-1.05 ($m$, 21H, phytlyl-CH/CH$_2$ + 2'R-CH$_3$) $\delta$ 8.88 ($m$, 12H, phytlyl-CH$_3$)

$^{13}$C-NMR (400MHz, CDCl$_3$) 151.6, 134.6, 133.4, 127.9, 117.3, 75.10, 59.82, 40.08, 40.02, 39.38, 37.57, 37.46, 37.30, 32.79, 32.72, 31.37, 31.32, 27.99, 24.83, 24.46, 23.91, 22.74, 22.64, 21.06, 20.93, 19.76, 19.70, 19.66, 19.60, 15.65, 14.72, 11.90

MS [EI+] $m/z$ 444 (M$^+$, 37%), 217 (17%), 177 (76%)

MS Calculated for C$_{30}$H$_{52}$O$_2$ 444.397
Byproduct (2R,2′R)-6,6′-(oxybis(methylene))bis(2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman) (47)

TLC:  
R<sub>f</sub> = 0.91 (CH<sub>2</sub>Cl<sub>2</sub>)

<sup>1</sup>H-NMR  
(400MHz, CDCl<sub>3</sub>) δ 4.61 (s, 1H, Ar-CH<sub>2</sub>OH), 2.61 (t, <i>J</i> = 6.80 Hz, 1H, Ar-CH<sub>2</sub>CH<sub>2</sub>), δ 2.31 (s, 3H, ArCH<sub>3</sub>), δ 2.26 (s, 3H, ArCH<sub>3</sub>), δ 2.11 (s, 3H, ArCH<sub>3</sub>), δ 1.79 (enant dt, <i>J</i> = 6.80 Hz, 2H, ArCH<sub>2</sub>CH<sub>2</sub>), δ 1.63-1.03 (m, 21H, phytlyl-CH/CH<sub>2</sub> + 2′R-CH<sub>3</sub>), δ 8.88 (m, 12H, phytlyl-CH<sub>3</sub>)

<sup>13</sup>C-NMR  
(400MHz, CDCl<sub>3</sub>) 151.3, 135.1, 133.8, 125.6, 122.3, 116.9, 74.82, 67.03, 39.85, 39.77, 39.39, 37.55, 37.47, 37.41, 37.30, 32.79, 32.73, 31.94, 31.63, 31.58, 29.72, 29.38, 27.99, 24.46, 23.84, 22.73, 22.64, 21.06, 20.96, 19.76, 19.69, 19.57, 15.68, 14.74, 14.13, 11.85

MS [EI+]  
m/z 853.8 (100%), 739.4 (9%) 429.4 (11%)
MS Calculated for C<sub>60</sub>H<sub>107</sub>O<sub>3</sub> 870.783
14.3 PET-Tocopherol

14.3.1 Synthesis of (2R)-6-fluoro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman (47)

Electrophilic fluorination of H-Toc:
H-Toc 42 (50mg, 1eq) was mixed with N-fluorobenzenesulfonimide (38mg, 1eq) and stirred in dry acetonitrile as a 1M solution for 10-15min at 150°C in a sealed vial. The reaction was cooled to room temperature, extracted with CH$_2$Cl$_2$ and water, the organic phase dried over Na$_2$SO$_4$ and evaporated down to dryness. The crude product was filtered through a silica plug with hexane to remove polar byproducts. Silica column chromatography (gradient hexane to hexane/CH$_2$Cl$_2$ 99:1) afforded 47 (23mg, 44%) as a clear oil.

Electrophilic fluorination of I-Toc:
To a 0.85M solution of I-Toc 70 (100mg, 1eq) in dry THF at 0°C under an N$_2$ atmosphere was a 1.7M t-BuLi solution in pentane (0.217ml, 2eq) added and stirred for 1min. A 0.35M N-fluorobenzenesulfonimide (116mg, 2eq) solution in THF was slowly added and stirred for 1min at 0°C. The reaction was quenched with methanol, the solvents evaporated, extracted with CH$_2$Cl$_2$ and water, the organic phase dried over Na$_2$SO$_4$ and evaporated down to dryness. Silica column chromatography (gradient hexane to hexane/CH$_2$Cl$_2$ 99:1) afforded 47 (12mg, 15%) as a clear oil.

Nucleophilic fluorination (Phenyl)tocopherol iodonium tosylate
(Phenyl)tocopherol iodonium tosylate 69 (10mg, 1eq) was dissolved in DMF as a 5mM solution, 1M tetraethylammonium fluoride in THF (1M TBAF in THF, 1eq) was added and stirred for 15min at 150°C. The solvent was evaporated and the residual mixture partitioned between with hexane and water. The organic phase was dried with Na$_2$SO$_4$, filtrated and purified over a small SiO$_2$ column with hexane. Silica column chromatography (gradient Hexane to Hexane/CH$_2$Cl$_2$ 99:1) afforded 47 (1.33mg, 24%) as a clear oil.

TLC: $R_l = 0.27$ (Hexane)
$^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 2.60 ($t$, $J = 6.80$ Hz, 2H, ArCH$_2$CH$_2$), $\delta$ 2.16 ($d$, $J = 6.80$ Hz, 2H, ArCH$_2$CH$_2$), $\delta$ 2.11 ($s$, 3H, ArCH$_3$), $\delta$ 1.81 (enant. $dt$, $J = 6.80$ Hz, 2H, ArCH$_2$CH$_2$), $\delta$ 154.5, 152.1, 147.1, 123.1, 121.4, 121.2, 119.1, 118.9, 117.6, 74.91, 39.86, 39.77, 39.38, 37.57, 37.53, 37.45, 37.40, 37.33, 37.29, 32.69, 31.26, 31.20, 29.71, 27.99, 24.82, 24.45, 23.81, 22.73, 21.02, 20.36, 20.34, 19.75, 19.69, 19.64, 19.60, 11.51, 11.27, 11.21, 10.40, 10.34

$^{13}$C-NMR (100MHz, CDCl$_3$) 154.5, 152.1, 147.1, 123.1, 121.4, 121.2, 119.1, 118.9, 117.6, 74.91, 39.86, 39.77, 39.38, 37.57, 37.53, 37.45, 37.40, 37.33, 37.29, 32.69, 31.26, 31.20, 29.71, 27.99, 24.82, 24.45, 23.81, 22.73, 21.02, 20.36, 20.34, 19.75, 19.69, 19.64, 19.60, 11.51, 11.27, 11.21, 10.40, 10.34

$^{19}$F-NMR (400MHz, CDCl$_3$) -131.49, -131.5 ($d$, $J = 4$ Hz, 1F, Ar-F)

MS [EI+] m/z 432.49 (M, 10%), m/z 205.18 (100%),
HRMS Calculated for C$_{29}$H$_{51}$NO 432.3767; found: 432.3762
39.86
39.77
39.39
37.57
37.54
37.46
37.40
37.33
37.30
32.81
32.79
32.69
31.26
31.20
27.99
24.83
24.45
23.81
22.73
22.64
21.03
20.36
19.76
19.69
19.64
19.61
11.51
11.27
11.21
10.40
10.34

74.91

123.10
123.06
121.41
121.22
119.08
118.90
117.60
117.56

147.08

154.45
152.14

13C with 1H decoupling

F
O

C16H33

F2 - Acquisition Parameters
Date_
20170308
Time
10.12 h
INSTRUM
spect
PROBHD
Z108618_0716 (
PULPROG
zgpg30
TD
32768
SOLVENT
CDCl3
NS
300
DS
2
SWH
23148.148 Hz
FIDRES
1.412851 Hz
AQ
0.7077888 sec
RG
210.82
DW
21.600 usec
DE
6.50 usec
TE
299.2 K
D1
2.00000000 sec
D11
0.03000000 sec
TD0
1
SFO1
100.6530068 MHz
NUC1
13C
P1
10.00 usec
PLW1
54.00000000 W
SFO2
400.2516010 MHz
NUC2
1H
CPDPRG[2
waltz65
PCPD2
80.00 usec
PLW2
10.71500015 W
PLW12
0.35200000 W
PLW13
0.22528000 W
F2 - Processing parameters
SI
32768
SF
100.6429426 MHz
WDW
EM
SSB
0
LB
1.00 Hz
GB
0
PC
1.40

150

140

130

120

110

100

90

80

70

60

50

40

30

20

10

0

-10

ppm

-131.49
-131.50

F19 with 1H decoupling

F
O

C16H33

F2 - Acquisition Parameters
Date_
20170308
Time
9.56 h
INSTRUM
spect
PROBHD
Z108618_0716 (
PULPROG
zgpg30
TD
131072
SOLVENT
CDCl3
NS
16
DS
4
SWH
89285.711 Hz
FIDRES
1.362392 Hz
AQ
0.7340032 sec
RG
210.82
DW
5.600 usec
DE
6.50 usec
TE
298.7 K
D1
1.00000000 sec
D11
0.03000000 sec
TD0
1
SFO1
376.5736179 MHz
NUC1
19F
P1
15.00 usec
PLW1
19.00000000 W
SFO2
400.2516010 MHz
NUC2
1H
CPDPRG[2
waltz65
PCPD2
80.00 usec
PLW2
10.71500015 W
PLW12
0.35200000 W
PLW13
0.22528000 W
F2 - Processing parameters
SI
65536
SF
376.6112790 MHz
WDW
EM
SSB
0
LB
0.30 Hz
GB
0
PC
1.00

-131.3

-131.4

-131.5

-131.6

-131.7

ppm

100.00

-131.2

	  

340	  
	  


14.3.2 Synthesis of 2,2,5,7,8-pentamethylchroman-6-yl 4-methylbenzenesulfonate (52)

![Chemical structure of 2,2,5,7,8-pentamethylchroman-6-yl 4-methylbenzenesulfonate (52)]

Pentamethylchromanol (800mg, 3.63mmol) was dissolved in dry CH₂Cl₂ (10ml), pyridine (468µl, 5.81mmol) was added under a nitrogen atmosphere and the solution cooled to 0°C. Tosyl chloride (830mg, 4.36mmol) was added in three portions. The reaction solution was stirred for 5h, quenched with water, the phases separated and the water phase washed with CH₂Cl₂. The combined organic phases were dried over Na₂SO₄ and evaporated down to dryness. Silica column chromatography (gradient Hexane/CH₂Cl₂ 7:1 to 1:1 to CH₂Cl₂) afforded 52 (1.36g, 50.3%) as a light yellow oil.

TLC: \( R_f = 0.79 \) (CH₂Cl₂)

\[ ^{1} \text{H-NMR} \]

(400MHz, CDCl₃) 7.84 (d, \( J = 8.00 \) Hz, 1H, Ar-Ts-H), 7.36 (d, \( J = 8.00 \) Hz, 1H, Ar-Ts-H), \( \delta \) 2.58 (t, \( J = 6.80 \) Hz, 2H, ArCH₂CH₂), \( \delta \) 2.49 (s, 3H, Ar-Ts-CH₃), \( \delta \) 2.06 (s, 3H, ArCH₃), \( \delta \) 1.99 (s, 3H, ArCH₃), \( \delta \) 1.96 (s, 3H, ArCH₃), \( \delta \) 1.81 (t, \( J = 6.80 \) Hz, 2H, ArCH₂CH₂), \( \delta \) 1.32 (s, 6H, 2'R-CH₃)

\[ ^{13} \text{C-NMR} \]

(400MHz, CDCl₃) 150.1, 144.8, 140.2, 134.4, 129.7, 128.9, 128.3, 127.5, 123.6, 117.7, 73.25, 32.61, 26.81, 21.72, 20.96, 14.27, 13.54, 11.91

MS [ESI+] \( m/z \) 375.2 (M⁺H, 45%), 392.2 (M⁺NH₃, 100%), 397 (M⁺Na, 67%), 219.2 (M-Ts, 8%)

MS Calculated for C₂₁H₂₆O₄S 374.155

14.3.3 Synthesis of naphthalen-2-yl 4-methylbenzenesulfonate (54)

![Chemical structure of naphthalen-2-yl 4-methylbenzenesulfonate (54)]

To 2-naphthol (300mg, 2.08mmol) and potassium carbonate (517mg, 3.74mmol) in water (5ml) / tetrahydrofuran (2ml) was added tosyl chloride in tetrahydrofuran (4ml) and the mixture stirred for 16h. The THF was evaporated and the residual mixture was extracted with CH₂Cl₂ 3x, the organic phases dried over Na₂SO₄ and evaporated down to dryness. Silica column chromatography (gradient Hexane/CH₂Cl₂ 6:1 to 1:1 to CH₂Cl₂) afforded 54 (620mg, 93.2%) as a light yellow oil.

TLC: \( R_f = 0.74 \) (CH₂Cl₂)
1H-NMR (400MHz, CDCl₃) 7.83 (s, 1H, Ar-H), 7.76 (s, 4H, Ar-H), 7.52 (s, 3H, Ar-H), 7.31 (s, 2H, Ar-H), 7.14 (s, 1H, Ar-H), δ 2.45 (s, 3H, Ar-Ts-CH₃)

13C-NMR (400MHz, CDCl₃) 147.2, 145.4, 133.5, 132.5, 131.9, 129.8, 129.7, 128.6, 127.9, 127.8, 126.8, 124.6, 121.2, 120.0, 21.73

MS [EI+] m/z 337.0 (M⁺K, 32%), 321.1 (M⁺Na, 32%). 316.1 (100%)

MS Calculated for C₁₁H₄O₂S 298.066

14.3.4 Synthesis of 4,4,5,5-tetramethyl-2-((2R)-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl)-1,3,2-dioxaborolane (56)

70 (42mg, 0.078mmol) in dry DMSO (0.46ml) with bis(pinacolato)diborone (43mg, 0.17mmol), PdCl₂(dppf) (6.3mg, 0.0078mmol) and potassium carbonate (32.2mg, 0.233mmol). was heated to 80°C and stirred for 16h. The reaction was cooled to room temperature and extracted with ether and water and the organic phase was washed an additional three times with water. The organic phase was dried over Na₂SO₄ and evaporated down to dryness. The crude product was purified by silica column chromatography (gradient Hex, to Hex/CH₂Cl₂ 1:1) afforded 56 (15mg 35.7%) as a clear oil.

TLC: Rᵣ = 0.34 (Hex/CH₂Cl₂, 1:1)

1H-NMR (400MHz, CDCl₃) δ 2.57 (t, J = 6.80 Hz, 2H, ArCH₂CH₂), δ 2.28 (s, 6H, ArCH₃), δ 2.25 (s, 3H, ArCH₃), δ 2.07 (s, 3H, ArCH₃), δ 1.78 (enant. dt, J = 6.80 Hz, 2H, ArCH₂CH₂), δ 1.64-1.07 (m, 33H, phytyl-CH/CH₂ + 2'R-CH₃ + δ 1.41 pinacole CH₃ 4x) δ 8.88 (m, 12H, phytyl-CH₃)

13C-NMR (400MHz, CDCl₃) 152.5, 138.1, 137.1, 121.9, 121.4, 116.7, 83.41, 74.95, 39.74, 39.66, 39.38, 37.58, 37.56, 37.47, 37.41, 37.38, 37.33, 37.30, 32.79, 32.74, 32.73, 31.50, 31.46, 29.71, 27.99, 25.05, 24.82, 24.46, 23.93, 22.73, 22.64, 21.05, 20.34, 19.76, 19.74, 19.70, 19.68, 19.63, 19.57, 19.51, 18.56, 11.32

MS [ESI+] m/z 541.5 (M⁺1, 100%), 563.4 (M⁺Na, 95%)

MS Calculated for C₃₅H₆₁BO₅ 540.471

Byproduct (2R,2'R)-2,2'-dihexadecyl-2,2',5,5',7,7',8,8'-octamethyl-6,6'-bichroman (66)
TLC: \( R_f = 0.08 \) (Hex/CH\(_2\)Cl\(_2\), 10:1)

\(^1\)H-NMR (400 MHz, CDCl\(_3\)) \( \delta 2.64 \) (\( t, J = 6.80 \) Hz, 4H, ArCH\(_2\)CH\(_2\)), \( \delta 2.15 \) (\( s, 6H, ArCH_3 \)), \( \delta 1.86 \) (\( enan\_ dt, 4H, J = 6.80 \) Hz), \( \delta 1.76 \) (\( s, 3H, ArCH_3 \)), \( \delta 1.75 \) (\( s, 3H, ArCH_3 \)), \( \delta 1.72 \) (\( s, 3H, ArCH_3 \)), \( \delta 1.71 \) (\( s, 3H, ArCH_3 \)), \( \delta 1.67-1.10 \) (\( m, 21H, phytyl-CH/CH_2+2'R-CH_3 \)), \( \delta 0.88 \) (\( t, J = 6.80 \) Hz, 12 H, phytyl-CH\(_3\))

\(^{13}\)C-NMR (400 MHz, CDCl\(_3\)) 149.9, 133.3, 132.7, 131.6, 121.3, 116.5, 74.67, 39.39, 37.44, 37.41, 37.31, 32.82, 32.80, 32.69, 31.62, 27.99, 24.82, 24.45, 23.80, 22.73, 22.64, 21.06, 20.89, 19.77, 19.70, 19.61, 16.64, 15.71, 11.97

MS [ESI+] \( m/z \) 827.8 (M, 100%), 585.4 (54%)

MS Calculated for C\(_{58}\)H\(_{98}\)O\(_2\) 826.757

14.3.5 Synthesis of 1-iodopyridin-1-ium chloride (71)

In an Erlenmeyer flask a solution of acetic acid (45ml) and pyridine (1.49ml, 0.0185mol) was cooled to 0°C and iodochloride (0.92ml, 0.0185) was added dropwise. A yellow precipitate formed, the reaction stirred for 15min at 0°C and was then filtrated and washed with acetic acid (120ml) until most of the red colour disappeared. The yellow crystals were suspended in methanol (35ml), heated until they dissolved, filtered hot and washed with hot methanol (20ml). The red solution was cooled for 30min. The suspension was filtrated at room temperature and washed three times with methanol (3x 20ml). The yellow filamentous crystals were dried. The mother liquor was cooled in the fridge for 20min. filtrated and washed with cold methanol. The second mother liquor was kept in the fridge for 2 days and filtered.

Crystal 1: 977mg, + 2: 647mg, + 3: 844mg = 55.4% of 71)

TLC: \( R_f = 0.2 \) (CH\(_2\)Cl\(_2\))

\(^1\)H-NMR (400MHz, Acetone-D\(_6\)) 8.84 (\( dd, J = 6.40 \) Hz, 1H, Ar-pyridine-H), \( \delta 8.25 \) (\( tt, J = 7.60 \) Hz, 1H, Ar-pyridine-H), 8.01 (\( d, J = 6.40 \) Hz, 2H, Ar-pyridine-H)
\[ ^{13}\text{C-NMR} \quad (400\text{MHz, Aceton-D}_6) \quad 148.6, 140.5, 127.2 \]

\[ \text{MS [ESI+]} \quad m/z \quad 205.9 \text{ (M-Cl, 100\%)} \]

\[ \text{MS Calculated for C}_{14}\text{H}_4\text{NICl} 240.916 \]

**14.3.6 Synthesis of (2R)-6-iodo-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman (70)**

![Chemical Structure](image)

42 (126mg, 0.303mmol) and 1-iodopyridin-1-ium chloride (69mg, 0.303mmol) were stirred in methanol (3ml) for 5h at room temperature. The yellow/white emulsion was evaporated and the residue extracted with CH\(_2\)Cl\(_2\) and water. The phases were separated and the water phase was washed 3x with CH\(_2\)Cl\(_2\). The organic phases were combined and dried over Na\(_2\)SO\(_4\) and dried down to dryness. Silica column chromatography (Hex to Hex/CH\(_2\)Cl\(_2\) 10:1) afforded 70 (131mg 78.1\%) as a clear oil.

**TLC:** \( R_f = 0.48 \) (Hexane)

\[ ^1\text{H-NMR} \quad (400\text{MHz, CDCl}_3) \delta \quad 2.70 (t, J = 6.80 \text{ Hz}, 2\text{H, ArCH}_2\text{CH}_2), \delta \quad 2.50 (s, 3\text{H, ArCH}_3), \delta \quad 2.45 (s, 3\text{H, ArCH}_3), \delta \quad 2.22 (s, 3\text{H, ArCH}_3), \delta \quad 1.80 (\text{enant. dt, } J = 6.80 \text{ Hz}, 2\text{H, ArCH}_2\text{CH}_2), \delta \quad 1.65-1.08 (m, 21\text{H, phytlyl-CH/CH}_2 + 2'R-\text{CH}_3) \delta \quad 8.88 (m, 12\text{H, phytlyl-CH}_3) \]

\[ ^{13}\text{C-NMR} \quad (400\text{MHz, CDCl}_3) \quad 151.8, 137.9, 136.8, 123.5, 118.0, 99.48, 75.67, 39.91, 39.82, 39.38, 37.54, 37.46, 37.41, 37.37, 37.30, 32.81, 32.79, 32.69, 31.51, 31.45, 28.00, 26.97, 26.02, 24.83, 24.45, 23.81, 22.74, 22.64, 22.41, 21.04, 19.76, 19.70, 19.66, 19.60, 13.46 \]

\[ \text{MS [EI+] } \quad m/z \quad 540.4 \text{ (M, 100\%)}, 414.5 \text{ (M, 16\%)}, 275.0 \text{ (M, 68\%)} \]

HRMS Calculated for C\(_{29}\)H\(_{49}\)OI 540.2828; found: 540.2823
14.3.7 Synthesis of phenyl((2R)-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl)iodonium 4-methylbenzenesulfonate (69)

To a suspension of p-toluenesulfonic acid monohydrate (458mg, 2.41 mmol) in acetonitrile (7ml) was added (diacetoxy)iodobenzene (776mg, 2.41mmol) added. Chloroform (70ml) was quickly added and the whole mixture directly transferred to a sealable flask with 42 (1g, 2.41mmol). The remaining mixture was washed into the reaction flask with some chloroform (10ml). The yellow reaction mixture was heated to 50°C and stirred for 16h under a N₂ atmosphere. The solvents were evaporated. No crystallisation occurred upon ether addition. The crude product was purified by silica column chromatography (CH₂Cl₂ to CH₂Cl₂/MeOH, 9:1) afforded 69 (404mg 21.4%) as a light yellow oil.

TLC: \( R_t = 0.2 \) (CH₂Cl₂/MeOH, 9:0.5)

\(^1\)H-NMR

(400MHz, CDCl₃) \( \delta 7.72 \) (d, \( J = 7.60 \) Hz, 2H, Ar-Ts-H), \( \delta 7.69 \) (d, \( J = 7.60 \) Hz, 2H, Benzene-H), \( \delta 7.48 \) (t, \( J = 7.60 \) Hz, 1H, Benzene-H), \( \delta 7.37 \) (t, \( J = 7.60 \) Hz, 2H, Benzene-H) \( \delta 7.11 \) (d, \( J = 7.60 \) Hz, 2H, Ar-Ts-H), \( \delta 2.68 \) (t, \( J = 6.80 \) Hz, 2H, ArCH₂CH₂), \( \delta 2.58 \) (s, 6H, ArCH₃), \( \delta 2.34 \) (s, 3H, Ar-Ts-CH₃), \( \delta 2.19 \) (s, 3H, ArCH₃), \( \delta 1.83 \) (enant. dt, \( J = 6.80 \) Hz, 2H, ArCH₂CH₂), \( \delta 1.64-1.07 \) (m, 21H, phytyl-CH/CH₂ + 2'R-CH₃) \( \delta 8.87 \) (m, 12H, phytyl-CH₃)

\(^{13}\)C-NMR

(400MHz, CDCl₃) 155.9, 142.7, 139.5, 138.9, 138.8, 132.3, 131.8, 131.0, 128.5, 126.0, 125.9, 119.8, 116.6, 114.8, 76.88, 40.18, 39.37, 37.46, 37.39, 37.29, 32.80, 32.70, 27.99, 25.05, 24.81, 24.45, 24.25, 23.86, 22.73, 22.63, 22.29, 21.33, 21.01, 20.77, 19.75, 19.69, 19.63, 19.57, 13.66

MS [EI+] \( m/z 617.3 \) (M⁺ - TsO 100%)

MS [EI-] \( m/z 171.0 \) (TsO⁻, 100%)

MS Calculated for C₄₂H₆₁O₄I₇S 788.334
14.3.8 Synthesis of (2R)-5-bromo-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol (76)

\[ \begin{align*}
\text{HO} & \quad \text{Br} \\
\end{align*} \]

\( \gamma \)-Tocopherol (273mg, 0.655mmol) was added as a solution in \( CH_2Cl_2 \) (20ml) to a stirring solution of tetrabutylammonium bromide (211mg, 0.655mmol) in \( CH_2Cl_2 \) (20ml). Bromine (33\( \mu l \), 0.655mmol) was added and the solution stirred for 1h at room temperature. The solvent was evaporated and the crude mixture was purified by silica column chromatography (Hex/EE, 95:5) and afforded 76 (208mg 64.3%) as a light yellow oil.

TLC: \( R_f = 0.61 \) (Hex/EtOAc, 95:5)

\(^1\)H-NMR (400MHz, CDCl\(_3\)) \( \delta \) 5.21 (s, 1H, Ar-OH), \( \delta \) 2.69 (t, 2H, \( J = 6.80 \) Hz, ArCH\(_2\)CH\(_2\)), \( \delta \) 2.24 (s, 3H, ArCH\(_3\)), \( \delta \) 2.11 (s, 3H, ArCH\(_3\)), \( \delta \) 1.81 (enant dt, 1H, \( J = 17.6 \) Hz, \( J = 6.8 \) Hz, ArCH\(_2\)CH\(_2\)), \( \delta \) 1.62-1.03 (m, 21H, phytyl CH\(_2\)), \( \delta \) 0.88 (m, 12H, phytyl 4-CH\(_3\))

\(^{13}\)C-NMR (400MHz, CDCl\(_3\)) 145.9, 143.4, 125.4, 122.4, 122.4, 117.3, 109.3, 75.42, 39.54, 39.38, 37.45, 37.41, 37.29, 32.81, 32.68, 31.05, 27.99, 24.81, 24.45, 24.08, 23.69, 22.73, 22.64, 20.99, 19.76, 19.66, 12.98, 11.85

MS [ESI+] \( m/z \) 495.3 (M-H, 100%)

MS [ESI-] \( m/z \) 493.2 (M-H, 100%)

MS calculated for C\(_{28}\)H\(_{47}\)O\(_2\)Br 494.276

14.3.9 Synthesis of (2R)-5-fluoro-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol (48)

\[ \begin{align*}
\text{HO} & \quad \text{F} \\
\end{align*} \]

\( \gamma \)-Tocopherol (200mg, 0.48mmol) and N-fluorobenzenesulfonylimide (151mg, 0.48mmol) were stirred in dry THF (2ml) under N\(_2\) at 0°C for 1.5h. The yellow solution was quenched by addition of 1M HCl. CH\(_2\)Cl\(_2\) was added to the red mixture, stirred for 5min and the phases separated. The organic phase was evaporated and purified by silica column chromatography (gradient Hex/CH\(_2\)Cl\(_2\) 2:1, CH\(_2\)Cl\(_2\) to CH\(_2\)Cl\(_2\)/MeOH 9:1). and afforded 74 (10mg, 2.5%) as a brown oil and 75 (78mg, 22.8%) as a brown oil. Product 48 was not produced.
Byproduct (2R,2'R)-2,2'-dihexadecyl-2,2',7,7',8,8'-hexamethyl-[5,5'-bichroman]-6,6'-dial (74)

TLC: \( R_f = 0.7 \) (Hex/\( \text{CH}_2\text{Cl}_2 \) 1:1)

\(^1\)H-NMR (400MHz, CDCl\(_3\)) \( \delta \) 4.44 (s, 1H, Ar-OH), \( \delta \) 2.20 (enant dt, 1H, \( J = 17.6 \) Hz, \( J = 6.8 \) Hz, Ar\( \text{CH}_2\text{CH}_2\)), \( \delta \) 2.22 (s, 3H, Ar\( \text{CH}_3\)), \( \delta \) 2.20 (s, 3H, Ar\( \text{CH}_3\)), \( \delta \) 2.17 (enant dt, 1H, \( J = 17.6 \) Hz, \( J = 6.8 \) Hz, Ar\( \text{CH}_2\text{CH}_2\)), \( \delta \) 1.65 (enant dt, \( J = 17.6 \) Hz, \( J = 6.8 \) Hz, 1H, Ar\( \text{CH}_2\text{CH}_2\)), \( \delta \) 1.61-1.07 (m, 21H, phytlyl \( \text{CH}_2\)), \( \delta \) 0.88 (m, 12H, phytlyl \( \text{CH}_3\))

Byproduct \( N-((2R)-6\text{-hydroxy-2,7,8-trimethyl-2-(4,8,12\text{-trimethyltridecyl})chroman-5-yl})-\(\text{N-}\) (phenylsulfonyl)benzenesulfonamide (75)

TLC: \( R_f = 0.36 \) (Hex/\( \text{CH}_2\text{Cl}_2 \) 1:1)

\(^1\)H-NMR (400MHz, CDCl\(_3\)) \( \delta \) 8.05 (dd, \( J = 7.60 \) Hz, \( J = 1.60 \) Hz, 4H, \( \text{Ph-H} \)), \( \delta \) 8.05 (t, \( J = 7.60 \) Hz, \( J = 1.60 \) Hz, 2H, \( \text{Ph-H} \)), \( \delta \) 7.58 (t, \( J = 7.60 \) Hz, \( J = 1.60 \) Hz, 4H, \( \text{Ph-H} \)), \( \delta \) 5.61 (s, 1H, Ar-OH), \( \delta \) 2.24 (s, 3H, Ar\( \text{CH}_3\)), \( \delta \) 2.20 (t, 2H, \( J = 7.2 \) Hz, Ar\( \text{CH}_2\text{CH}_2\)), \( \delta \) 1.86 (enant dt, 2H, \( J = 6.8 \) Hz, Ar\( \text{CH}_2\text{CH}_2\)), \( \delta \) 1.45-1.09 (m, 21H, phytlyl \( \text{CH}_2\)), \( \delta \) 0.88 (m, 12H, phytlyl \( \text{CH}_3\))

\(^{13}\)C-NMR (400MHz, CDCl\(_3\)) 146.2, 145.8, 139.5, 139.4, 134.22, 129.8, 129.0, 125.6, 120.7, 118.4, 75.65, 40.32, 39.37, 37.47, 37.29, 32.81, 32.70, 30.81, 27.98, 24.81, 24.48, 24.09, 22.73, 22.63, 20.94, 19.75, 19.64, 19.08, 12.73, 12.54

MS [ESI-] \( m/z \) 829.8 (M-H, 100%)

MS calculated for C\(_{55}\)H\(_{94}\)O\(_4\) 830.715

Byproduct \( N-((2R)-6\text{-hydroxy-2,7,8-trimethyl-2-(4,8,12\text{-trimethyltridecyl})chroman-5-yl})-\(\text{N-}\) (phenylsulfonyl)benzenesulfonamide (75)

MS [ESI+] \( m/z \) 734.4 (M\(^+\)Na, 100%), 750.3 (M\(^+\)Na, 46%)

347
Byproduct (2R)-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl benzenesulfonate (77)

γ-Tocopherol (38mg, 91.2µmol) was dissolved in dry THF (2ml) under N\textsubscript{2} and cooled to 0°C. Sodium hydride (3.6mg, 91.2µmol) was added to the solution and stirred for 5min. N-fluorobenzenesulfonimide (58mg, 0.18mmol) was added. After 5min was the reaction quenched with methanol and 1M HCl, the solvents evaporated and extracted with CH\textsubscript{2}Cl\textsubscript{2} and water. The organic phase was dried over Na\textsubscript{2}SO\textsubscript{4} and purified by silica column chromatography (gradient Hex/CH\textsubscript{2}Cl\textsubscript{2} 3:1 to Hex/CH\textsubscript{2}Cl\textsubscript{2} 1:1) and afforded cc (28.7mg, 56.6%) as a clear oil.

TLC: \( R_f = 0.33 \) (Hex/CH\textsubscript{2}Cl\textsubscript{2} 1:1)

\textsuperscript{1}H-NMR (400MHz, CDCl\textsubscript{3}) \( \delta \) 7.89 (\textit{d}, \textit{J} = 6.40 Hz, 2H, SO\textsubscript{2}ArCH), \( \delta \) 7.68 (\textit{t}, \textit{J} = 6.40 Hz, 2H, SO\textsubscript{2}ArCH), \( \delta \) 7.55 (\textit{t}, \textit{J} = 6.40 Hz, 2H, SO\textsubscript{2}ArCH), \( \delta \) 6.61 (\textit{s}, 1H, ArCH), \( \delta \) 2.70 (\textit{m}, \textit{J} = 6.40 Hz, 2H, ArCH\textsubscript{2}CH\textsubscript{2}), 2.05 (\textit{s} \textit{J} = 1.60 Hz, 3H, ArCH\textsubscript{3}), \( \delta \) 1.90 (\textit{s}, 3H, ArCH\textsubscript{3}), \( \delta \) 1.76 (enant. \textit{dt}, \textit{J} = 6.40 Hz, 2H, ArCH\textsubscript{2}CH\textsubscript{2}), \( \delta \) 1.59-1.05 (\textit{m}, 21H, phytyl-CH/CH\textsubscript{2} + 2’R-CH\textsubscript{3}) \( \delta \) 8.88 (\textit{m}, 12H, phytyl-CH\textsubscript{3})

\textsuperscript{13}C-NMR (400MHz, CDCl\textsubscript{3}) 150.3, 140.6, 136.4, 133.9, 129.0, 128.6, 128.5, 126.2, 119.8, 118.5, 76.34, 40.20, 39.38, 37.46, 37.29, 32.81, 32.69, 30.93, 27.99, 24.81, 24.45, 24.12, 22.73, 22.64, 22.21, 20.98, 19.76, 19.66, 12.94, 11.96

MS [ESI+] \( m/z \) 579.3 (M\textsuperscript{+}Na, 100%), \( m/z \) 574.4 (M+NH\textsubscript{3}, 39%),

MS calculated for C\textsubscript{34}H\textsubscript{52}O\textsubscript{4}S 556.36

14.3.10 Synthesis of (2,5-dihydroxy-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bisis(dimethylsulfonium) acetate (S-Ylide)

2,5-Dihydroxy-1,4-benzoquinone (1.4g, 10mmol), dimethylsulfoxide (5ml) and acetic anhydride (20ml) were stirred at 100°C for 1h. A yellow precipitate occurred. The reaction was
cooled to room temperature and stirred for an additional 3h. The suspension was filtered, washed with ethyl acetate, and light brown crystals were obtained.

$^1$H-NMR  (300MHz, H$_2$O) δ 2.98 (s, 12H, S′CH$_3$), δ 1.99 (s, 3H, CH$_3$COOH),

$^{13}$C-NMR  (75MHz, H$_2$O) 176.7, 176.2, 93.52, 24.58, 20.36

MS [EI+]  m/z 261.0 (M-2H, 100%) 283.0 (M-2H + Na$, 18%$)

MS [EI-]  m/z 75.0 (100%), 59.1 (AcO$, 4%$),

MS Calculated for C$_{10}$H$_{14}$O$_4$S$_2$ 262.018

14.3.11 Synthesis of (2R)-5-(fluoromethyl)-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl acetate (78)

(2R)-5-(Bromomethyl)-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol (77) (51mg, 0.363mmol) and caesium fluoride (166mg, 1.09mmol) were stirred in t-butanol (2ml) at 80°C in a sealed vial under a nitrogen atmosphere for 16h. The t-butanol was evaporated and the reaction mixture was purified by silica column chromatography (gradient Hex/CH$_2$Cl$_2$ 4:1 to CH$_2$Cl$_2$) and afforded 78 (26.4mg 30.1%) as a clear oil.

TLC:  $R_f$ = 0.26 (Hex/CH$_2$Cl$_2$, 1:1)

$^1$H-NMR  (600MHz, CDCl$_3$) δ 5.31 (d, $J = 47.2$ Hz, 2H, Ar-CH$_2$F), δ 2.85 (t, $J = 6.6$ Hz, 2H, ArCH$_3$), δ 2.38 (s, 3H, ArCH$_3$), δ 2.16 (d, $J = 3.0$ Hz, 3H, ArCH$_3$), δ 2.06 (s, 3H, AcCH$_3$), δ 1.86 (p, $J = 6.6$ Hz 2H, ArCH$_2$CH$_2$), δ 1.79 (p, $J = 6.6$ Hz 2H, ArCH$_2$CH$_2$), δ 1.66-1.52 (m, 3H, R-CH), δ 1.49-1.09 (m, 21H, phytyl CH$_2$), δ 0.9-0.86 (m, 12H, phytyl 4-CH$_3$)

$^{13}$C-NMR  (600MHz, CDCl$_3$) 170.0, 169.9, 149.8, 141.3, 128.1, 127.6, 122.6, 118.7, 77.79, 75.78, 75.58, 39.40, 38.22, 37.42, 36.63, 33.18, 32.31, 28.42, 27.58, 24.84, 24.44, 23.99, 23.68, 23.17, 23.08, 22.32, 21.84, 20.98, 20.35, 20.12, 19.49, 19.26, 18.64, 14.27, 13.60, 13.43, 12.76, 12.58, 11.91, 11.74, 11.07

$^{19}$F-NMR  (600MHz, CDCl$_3$) 208.6 (t, $J = 54$ Hz, 1F, Ar-CH$_2$F)

MS [ESI+]  m/z 508.2 (M$^+$NH$_4$, %), 513.3 (M$^+$Na, %), 529.2 (M$^+$K, %),

MS calculated for C$_{31}$H$_{51}$O$_3$F 490.382

Side product (2R)-5-(tert-butoxymethyl)-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl acetate (79)

349
Mass amount: (12mg, 18%)

TLC: \( R_f = 0.06 \) (Hex/CH\(_2\)Cl\(_2\), 1:1)

\(^1\)H-NMR (400MHz, CDCl\(_3\)) \( \delta \) 4.26 (s, 2H, Ar-CH\(_2\)OBu), \( \delta \) 2.81 (brs, 2H, ArCH\(_2\)), \( \delta \) 2.33 (s, 3H, AcCH\(_3\)), \( \delta \) 2.10 (s, 3H, ArCH\(_3\)), \( \delta \) 2.02 (s, 3H, ArCH\(_3\)), \( \delta \) 1.80 (enant dt, \( J = 6.8 \) Hz 2H, ArCH\(_2\)CH\(_2\)), \( \delta \) 1.66-1.06 (m, 21H, phytyl-CH/CH\(_2\)+2’R-CH\(_3\)), \( \delta \) 1.26 (s, 9H, -OCCH\(_3\))

\( \delta \) 0.9-0.86 (m, 12H, phytyl 4-CH\(_3\))

\(^{13}\)C-NMR (400MHz, CDCl\(_3\)) 169.9, 149.8, 141.1, 127.6, 125.8, 125.3, 118.7, 75.25, 72.96, 55.98, 39.37, 37.39, 37.29, 32.78, 32.70, 30.93, 29.70, 27.97, 27.47, 24.80, 24.44, 22.72, 22.62, 21.02, 20.61, 19.74, 19.68, 19.58, 19.37, 14.11, 12.99, 12.07

MS [EI+] \( m/z \) 508.4 (M-t-Bu + Na, 45%), 471.4 (M-t-BuO, 100%)

MS calculated for C\(_{35}\)H\(_{60}\)O\(_5\) 544.449

Side product (2R)-5-(ethoxymethyl)-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol (80)

Mass amount: (61mg, 91%)

TLC: \( R_f = 0.58 \) (CH\(_2\)Cl\(_2\), 1:1)

\(^1\)H-NMR (400MHz, CDCl\(_3\)) \( \delta \) 7.73 (s, 1H, Ar-OH), \( \delta \) 4.73 (s, 2H, Ar-CH\(_2\)), \( \delta \) 3.63 (q, \( J = 6.80 \) Hz, 2H, O-CH\(_2\)-CH\(_3\)), \( \delta \) 2.60 (t, 2H, \( J = 6.80 \) Hz, ArCH\(_2\)), \( \delta \) 2.18 (s, 3H, ArCH\(_3\)), \( \delta \) 2.14 (s, 3H, ArCH\(_3\)), \( \delta \) 1.80 (enant dt, \( J = 6.8 \) Hz, 2H, ArCH\(_2\)CH\(_2\)), \( \delta \) 1.65-1.08 (m, 24H, phytyl-CH/CH\(_2\)+2’R-CH\(_3\)+O-CH\(_2\)-CH\(_3\)) \( \delta \) 0.88 (m, 12H, phytyl 4-CH\(_3\))

\(^{13}\)C-NMR (400MHz, CDCl\(_3\)) 147.4, 144.6, 125.6, 123.1, 116.1, 115.2, 74.41, 68.03, 66.19, 39.83, 39.75, 39.37, 37.55, 37.46, 37.39, 32.78, 32.70, 31.39, 29.71, 27.98, 24.81, 24.45, 23.75, 22.75, 22.72, 22.63, 21.03, 19.94, 15.13, 14.13, 11.90, 11.70

MS [EI+] \( m/z \) 445.4 (M-Et, 60%), 391.3 (100%)

MS [ESI-] \( m/z \) 473.3 (M-H, 14%), 459.3 (M-CH\(_3\), 100%), 443.3 (M-Et, 39%)

350
MS calculated for C$_{33}$H$_{54}$O$_{5}$F 474.407

Side product ((2R)-6-acetoxy-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)chroman-5-yl)methyl 2,2,2-trifluoroacetate (81)

Mass amount: (15mg, 23%)

TLC: $R_f = 0.50$ (CH$_2$Cl$_2$, 1:1)

$^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 5.34 (s, 2H, Ar-CH$_2$OTFA), $\delta$ 2.60 (bbrs, 2H, ArCH$_2$CH$_2$), $\delta$ 2.35 (s, 3H, ArCH$_3$), $\delta$ 2.15 (s, 3H, ArCH$_3$), $\delta$ 2.05 (s, 3H, ArCH$_3$), $\delta$ 1.83 (enant $dt$, $J$ = 6.8 Hz, 2H, ArCH$_2$CH$_2$), $\delta$ 1.65-1.08 (m, 21H, phytyl-CH/CH$_2$ + 2’R-CH$_2$) $\delta$ 0.88 (m, 12H, phytyl 4-CH$_3$)

$^{13}$C-NMR (400MHz, CDCl$_3$) 169.7, (q, 158.1, 157.7, 157.2, 156.8), 149.9, 141.5, 128.5, 128.1, 120.3, 118.7, (q, 118.8, 115.9, 113.1, 110.3), 75.68, 61.94, 39.38, 37.52, 37.46, 37.41, 37.40, 37.37, 37.30, 32.79, 32.70, 32.67, 30.61, 29.72, 27.99, 24.83, 24.82, 24.45, 23.96, 22.73, 22.63, 21.00, 20.48, 19.75, 19.69, 19.64, 19.59, 13.08, 12.36

$^{19}$F-NMR (400MHz, CDCl$_3$) -74.80 (s, 3F, COCF$_3$)

MS [ESI+] $m/z$ 524.4 (100%)

MS calculated for C$_{33}$H$_{51}$O$_{5}$F 584.369
References


Mariani, C.; Bellan, G., Presence of tocopherol derivatives in vegetable oils. Rivista Italiana delle Sostanze Grasse 74, 545-552 (1997)


Butinar, B., Bučar-Miklavčič, M., Mariani, C., Raspor, P. New vitamin E isomers (gamma-tococomonoenol and alpha-tococomonoenol) in seeds, roasted seeds and roasted seed oil from the Slovenian pumpkin variety ‘Slovenska golica’. Food Chem. 128, 505–512 (2011).


138 Abbe, E. Beiträge zur Theorie des Mikroskops und der mikroskopischen Wahrnehmung. Archiv für Mikroskopische Anatomie 9, 413–418 (1873)


146 Nutting, P. G. Purity and intensity of MONOmonochromatic light. 439–456 (1906).


Photochemistry.worldpress.com  
https://photochemistry.files.wordpress.com/2009/08/jablonski.png?w=630


Ulatowski, L. M. Regulation of Vitamin E and the Tocopherol Transfer Protein. *Case Western Reserve University* (2012).


Hildering, A. The Synthesis of α-Tocopentaenol (αT5), A fluorescent Analogue of α-Tocopherol *MSC Thesis Brock University* (2016)


305 Wong, P. S. L., Yan, D., Guittard, G. V. Compositions and dosage forms for enhanced absorption of metformin. (2005).


Perrin, J. C. Hodgkin's lymphoma, PET scan C001/7581 *ISM / Science photolibrary*  


Preliminary data received April 27 2015 from Dr. Bourikas via Dr. Stamatatos.


Kuboyama, T., Nakahara, M., Yoshino, M., Cui, Y., Sako, T., Wada, Y., Imanishi, T., Obika, S., Watanabe, Y., Suzuki, M., Doi, H. Stoichiometry-focused 18F-labeling of


Garcinoic acid:  


NDA 21-870: Fludeoxyglucose F 18 Injection. *North Shore / LIJ Research Institute* 4–17


