Synthesis of Unnatural Analogues of Pancratistatin and Narciclasine

Sergey Vshyvenko

Department of Chemistry

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Faculty of Mathematics and Science, Brock University
St. Catharines, Ontario

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ABSTRACT

Described herein is the chemoenzymatic synthesis of several different types of unnatural analogues of Amaryllidaceae constituents. Development and refinement of existing and design and execution of new approaches towards the synthesis of C-1 analogues of pancratistatin and A-ring heterocyclic analogues of narciclasine are discussed. Evaluation of the new analogues as cancer growth inhibitory agents is also described.
ACKNOWLEDGEMENTS

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<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,2-DMP</td>
<td>2,2-dimethoxypropane</td>
</tr>
<tr>
<td>AcOH</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>AD-mix</td>
<td>Asymmetric dihydroxylation mixture</td>
</tr>
<tr>
<td>AIBN</td>
<td>2,2'-Azobis(isobutyronitrile)</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>BOP</td>
<td>(Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>BTMSA</td>
<td>bis-Trimethylsilyl acetylene</td>
</tr>
<tr>
<td>CDI</td>
<td>1,1'-Carbonyldimidazole</td>
</tr>
<tr>
<td>Cp</td>
<td>Cyclopentadienyl</td>
</tr>
<tr>
<td>Cp*</td>
<td>Pentamethylcyclopentadienyl</td>
</tr>
<tr>
<td>CpCo(CO)₂</td>
<td>Cyclopentadienylcobalt dicarbonyl</td>
</tr>
<tr>
<td>CyJohnphos</td>
<td>2-(Dicyclohexylphosphino)biphenyl</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-Diazabicycloundec-7-ene</td>
</tr>
<tr>
<td>DCC</td>
<td>N,N'-Dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCN</td>
<td>1,4-dicyanonaphthalene</td>
</tr>
<tr>
<td>DEAD</td>
<td>Diethyl azodicarboxylate</td>
</tr>
<tr>
<td>DHP</td>
<td>2,3-dihydropyran</td>
</tr>
<tr>
<td>DIBAL</td>
<td>Diisobutylaluminium hydride</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-Diisopropylethylamine</td>
</tr>
<tr>
<td>DMIPS</td>
<td>Dimethylisopropylsilyl</td>
</tr>
<tr>
<td>DMP</td>
<td>Dess-Martin periodinane</td>
</tr>
<tr>
<td>DPPA</td>
<td>Diphenylphosphoryl azide</td>
</tr>
<tr>
<td>EDCI</td>
<td>1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>EE</td>
<td>Ethoxyethyl</td>
</tr>
<tr>
<td>Ee</td>
<td>Enantiomeric excess</td>
</tr>
<tr>
<td>Er</td>
<td>Enantiomeric ratio</td>
</tr>
<tr>
<td>EVE</td>
<td>Ethoxyvinyl ether</td>
</tr>
<tr>
<td>HBTU</td>
<td>O-Benzotriazole-(N,N,N',N')-tetramethyluronium hexafluorophosphate</td>
</tr>
<tr>
<td>HOBr</td>
<td>Hydroxybenzotriazole</td>
</tr>
<tr>
<td>LHMDS</td>
<td>Lithium bis(trimethylsilyl)amide</td>
</tr>
<tr>
<td>LDA</td>
<td>Lithium diisopropylamide</td>
</tr>
<tr>
<td>LTMP</td>
<td>Lithium 2,2,6,6-tetramethylpiperidide</td>
</tr>
<tr>
<td>mCPBA</td>
<td>(m)-Chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
</tbody>
</table>
MOM  Methoxymethyl
Ms   Methanesulfonate
NaHMDS Sodium bis(trimethylsilyl)amide
nBu  n-Butyl
NMO  N-Methylmorpholine N-oxide
nPr  n-Propyl
oDCB o-Dichlorobenzene
P(o-Tol)3 tris(ortho-tolyl) phosphine
Ph   Phenyl
PhMe Toluene
PivCl Pivaloyl chloride
PMB  p-Methoxylbenzyl
pMBDMA p-Methoxybenzaldehyde dimethylacetal
PMP  p-Methoxyphenyl
PPTS Pyridinium p-toluene sulfonate
SEM  2-(Trimethylsilyl)ethoxymethyl
SMEAH Sodium bis(2-methoxyethoxy)aluminum hydride
TBAF Tetra-n-butylammonium fluoride
TBAT Tetra-n-butylammonium triphenyldifluorosilicate
TCDI 1,1'-Thiocarbonyldiimidazole
TDO Toluene dioxygenase enzyme
TES Triethysilyl
Tf   Trifluoromethylsulfonate
TFA  Trifluoroacetic acid
TFAA Trifluoroacetic acid anhydride
TIPS Triisopropylsilyl
TMEDA N,N,N',N'-Tetramethylethylenediamine
Ts   p-Toluenesulfonyl
UHP  Urea-hydrogen peroxide complex
1. Introduction

Since the discovery and isolation of narciclasine (1) in 1968 by Ceriotti\textsuperscript{1} and pancratistatin (2), Figure 1, by Pettit\textsuperscript{2} in 1984 the scientific community has been drawn towards these molecules because of their potent antineoplastic activity. The highly selective cytotoxicity towards malignant cells ensured the interest of the community in developing potential drugs based on their molecular pattern of Amaryllidaceae constituents. However, their preclinical development is significantly hampered by the relatively low abundance of 1 and 2 in natural sources as well as their poor water solubility.

![Figure 1](image1.png)

**Figure 1.** Structure of narciclasine and pancratistatin.

Synthetic organic chemistry is an indispensable tool which can address both of these issues by developing efficient ways to synthesize of the different analogues of 1 and 2 which may also help to establish the biological mechanism of action of these fascinating molecules. The main goal of this thesis will be to devise new ways and refine existing approaches towards synthesis of C-1 analogues of pancratistatin such as 5 a-c and A-ring heterocyclic analogues of narciclasine of type 6 and 7, Figure 2. Biological
evaluation of these synthetic compounds will be performed in order to provide us with new insight towards mechanism of action and help to refine minimal pharmacophore requirements.

**Figure 2.** General strategy for synthesis of analogues of pancratistatin and narciclasine.

The unifying theme of all these approaches will be the use of a highly versatile chiral building block – cis-cyclohexadiene diol 4. The utilisation of such a chiral building block for the synthesis of complex organic targets has been a common theme in the Hudlický group for many years. This compound is obtained by biooxidation of bromobenzene 3 by the whole cell fermentation with strain of *E. Coli* JM109 (pDTG601A). Diol 4 will be used in the synthesis of unnatural derivatives 5 a-c, 6, 7. When completed these compounds will be evaluated by screening against cancer cell lines.
2. Historical

2.1. Amaryllidaceae alkaloids

2.1.1. Discovery and biosynthesis

Amaryllidaceae is a large family of flowering perennial plants. There have been used for a long time in folk medicine for treatment of various ailments because of the high amounts of various bioactive alkaloids. In the ancient Greek and Roman medicine the plants of the \textit{Narcissus} genus and their essences were used for the treatment of tumours and cancer–like diseases.\textsuperscript{3}

In 1877 the first alkaloid from this family was isolated from \textit{N. pseudonarcicuss} and named lycorine (8), Figure 3.\textsuperscript{4} It is one of the most abundant alkaloids in the family and possesses some antitumoral activity. More than 100 members of this family of alkaloids were subsequently isolated and despite their shared isoquinoline structures they belong to structurally different groups. Here our attention will be focused on the group of these alkaloids which share the isocarbostyril structural motif, namely (9).

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{lycorine_isocarbostyril.png}
\caption{Structure of lycorine and isocarbostyril.}
\end{figure}
Compounds with the highest anticancer activity were isolated and characterised in the late 20th century. All of these congeners are highly oxygenated compounds and share the same structural isocarbostyril pattern (9). The first of this class of naturals products, narciclasine (1), was isolated in 1968, closely followed by isolation of 7-deoxynarcilasine or lycoricidine, (10). Two decades later pancratistatin (2) was isolated by Pettit et al. in 1984, and 7-deoxypancratistatin (11) was isolated by Ghosal in 1989. trans-Dihydronarciclasine (12) was produced first semi-synthetically in 1975 from narciclasine and only in 1990 was isolated from a natural source by Pettit. The latest of the active compounds was 7-deoxy-trans-dihydronarciclasine (13) isolated in 1993 by the same group, Figure 4.

![Figure 4](image)

**Figure 4.** Three major isocarbostyril congeners of Amaryllidaceae family and their respective 7-deoxy versions.

While the exact biosynthetic pathway towards these compounds is unknown, it is reasonable to assume that they all share common biosynthetic origin. Two major starting materials for biogenesis of all alkaloids of Amaryllidaceae family are phenylalanine (14) and tyrosine (17). They undergo transformation into protocatechuic aldehyde (15) and tyramine (18) respectively. Coupling of those two compounds followed by reduction and
methylation leads to the central intermediate in the biosynthesis - O-methylnorbelladine (19), Figure 5.\textsuperscript{10}

\textbf{Figure 5.} Biosynthetic pathway to a common intermediate O-methylnorbelladine (19).

The general pathway to phenanthridine type alkaloids proceeds via oxidative cyclization of 19. Different types of regioselective oxidative cyclization lead to a wide variety of products. The main question is whether a para-para or para-ortho regioselective coupling is involved in the particular case of narciclasine (1). Studies of the biosynthetic pathway were performed with labeled compounds to show that narciclasine is most likely produced via a para-para phenol oxidative process.\textsuperscript{10} Oxidation of O-methylnorbelladine (19) followed by Michael addition yields noroxomaritidine (20). Oxidation of this intermediate is thought to produce narciclasine in series of oxidation steps,\textsuperscript{10} Figure 6.
**Figure 6.** Transformation of *O*-methylnorbelladine to narciclasine.

The exact biosynthetic route has not yet been established for other congeners of narciclasine such as 2, 10-13. But as all of these compounds share a similar structure, it is reasonable to assume that they all share the same biosynthetic pathway.

2.1.2. Selected total syntheses.

2.1.2.1. Total syntheses of narciclasine.

The goal of this section is not to present all existing syntheses of narciclasine and pancratistatin-type molecules, which have been the topic of a number of reviews.4, 10-22 First racemic and first enantioselective synthesis will be presented in detail. A few other selected syntheses, especially those published after 2008, will be shown in an abbreviated form.

Despite being the earliest of all isocarbostyril congeners discovered and displaying the most potent anticancer properties, narciclasine (1) has received the least attention from a synthetic standpoint. Indeed, only four total syntheses of this compound are known to date. The first synthesis of narciclasine (1) was reported by Rigby and Matteo23 in 1997. Their synthetic strategy involved the transformation of the commercially available methyl
ester of the cyclohexene carboxylate acid 21 to epoxyalcohol 22, which was converted to a chiral isocyananate 23 by lipase resolution, Scheme 1. Reaction of the lithium derivative of 25 with this isocyanate led to amide 26, which upon photocyclization conditions furnished the full framework of 1, Scheme 1. A similar strategy was utilised by this group three years later in the synthesis of pancratistatin.24

Scheme 1. Rigby’s first enantioselective synthesis of narciclasine.
In the second reported synthesis of Hudlický\textsuperscript{25, 26} used chiral cyclohexadiene diol 29 as a C-ring fragment for synthesis of narciclasine. This diene was submitted to a hetero-Diels-Alder reaction to provide the key bicyclic intermediate 30. Suzuki coupling of this compound with arylboronic acid 31 and reduction led to enone 32, which, after installation of the correct stereochemistry in position 2 and cyclization was followed by deprotection, furnishing narciclasine (1) in twelve steps.

![Chemical structure diagram]

**Figure 7.** Hudlický’s synthesis of narciclasine.

The third synthesis of narciclasine was reported by Keck et al.\textsuperscript{27} In 1999 their group published a general approach towards both natural narciclasine and ent-lycoricidine. The pivotal point of their synthesis is an intramolecular 6-\textit{exo} radical cyclization between a radical generated from alkyne and oxime 35, Figure 8. Chirality in this synthesis was achieved by using a chiral pool starting material, namely D-gulonolactone 33, as a precursor to alkyne 34.
Figure 8. Keck’s synthesis of narciclasine.

The most recent synthesis of 1 was reported in 2002 by Elango and Yan. Their approach to the construction of narciclasine was similar to that previously reported by Hudlický for the synthesis of 10-epi-7-deoxypancratistatin and consists of intramolecular opening of epoxide 41 to form phenanthridine 42, Scheme 2. The starting material used in their synthesis was the achiral cyclohexadiene diol 39 produced by the whole-cell oxidation of benzene. In order to transform it to the chiral conduramine 40 an enantioselective hetero-Diels-Alder reaction with chiral nitroso compound 37 was performed. In a few steps, that included bromohydrine formation and alkylation, the key epoxide 41 was prepared. Intramolecular Lewis acid-catalyzed epoxide opening led to the tricyclic product 42, which upon benzylic oxidation and elimination provided protected narciclasine 42. Finally, deprotection gave enantiomerically pure narciclasine (1) in ten steps.
Scheme 2. Yan’s synthesis of narciclasine.

2.1.2.2. Total syntheses of lycoricidine.

Lycoricidine (10) has attracted much more attention as a synthetic target and constitutes the earliest example of an isocarbostyrl Amaryllidaceae alkaloid made by synthesis. The first racemic synthesis was performed by Ohta and Kimoto. Incidentally, on the way to this compound they had also prepared the protected version (49) of another related natural product, then as yet undiscovered 7-deoxypancratistatin (11).

The synthesis began with the allylic alcohol 44, a precursor for a diene whose Diels-Alder reaction with ethyl acrylate provided, after hydrolysis, acid 45, Scheme 3. After Curtius rearrangement and electrophilic cyclization tetracycle 46 was further
functionalised to establish the four hydroxyl stereocenters of 7-deoxypancratistatin.

Finally, elimination of the unprotected C-1 alcohol moiety in 49 led to lycoricidine (10).

![Chemical structures and reaction schemes]

Reaction conditions: (a) ethyl acrylate, TsOH, 56%; (b) NaOEt, EtOH, (ii) H₂O, 74%; (c) ClCO₂Et, Et₃N, acetone, H₂O; 42%; (d) (i) NaN₃, H₂O; (ii) toluene, reflux 40%; (e) BF₃·Et₂O, 89% for 3 steps; (f) Ac₂O, pyridine, 83%; (g) KOH, EtOH, 68%; (h) NBS, THF, 96%; (j) DBU, pyridine, 98%; (k) NaOH, EtOH, 90%; (l) DHP, TsOH, 75%; (m) mCPBA, CHCl₃, 85%; (n) (i) (PhSe)₂, NaBH₄; (ii) H₂O₂, 63%; (o) Ac₂O, pyridine, 97%; (p) TsOH, AcOH, MeOH, 59%; (q) OsO₄, pyridine, 87%; (q) 2,2-DMP, TsOH, DMF, 84%, (s) SOCl₂, pyridine, 58%; (t) TsOH, CHCl₃, CH₃OH, H₂O.

**Scheme 3.** First racemic synthesis of lycoricidine.

The first enantioselective synthesis of (+)-lycoricidine was performed by Paulsen and Stubbe. Enantioselectivity was achieved by utilising a chiral pool starting material, namely a derivative of glucose, aldehyde 50, Scheme 4. This aldehyde was transformed into nitroolefin 51 and reacted with the lithium derivative of piperonylic ester 52 to produce advanced intermediate 53. Upon acidic deprotection it was cyclized to cyclohexane 54, whereupon reduction and recyclization led to 7-deoxypancratistatin (11).
This natural product was submitted to selective reprotection followed by elimination to produce lycoricidine (10).

![Chemical structure](image)

**Scheme 4.** First enantioselective synthesis of lycoricidine.

Other syntheses of lycoricidine were reported by Chida in 1991,³²,³³ Hudlický in 1992,³⁴ by Martin in 1993,³⁵ by Keck in 1996,²⁷,³⁷ and Yan in 2002.³⁸

The latest synthesis of lycoricidine was performed by Yadav in 2009.³⁹ In this chiral pool approach D-(+)-mannose was transformed to ω-iodo glycoside 57 in five steps, as shown in Scheme 5. Iodoether 57 was opened with allyl bromide in presence of zinc and the product of this transformation was cyclized with Grubbs 1st generation catalyst to furnish the cyclohexenol acetate 59. This product was subjected to two different sets of
aziridination conditions in order to produce the key aziridine 59. Coupling of 6-iodopiperonylic acid 56 with compound 59 led to N-acylated aziridine 60. Deprotection and oxidation was followed by silica gel-catalyzed rearrangement of the aziridine moiety to allyl amide product 61. The tertiary amide 62 was subjected to an intramolecular Heck cyclization. This cyclization has been featured in many approaches of lycoricidinum, namely those by Ogawa, Hudlický, Martin, and Weinreb. Isocarbostyril 63 was deprotected with formic acid to provide lycoricidinum (10).

Scheme 5. Yadav’s synthesis of lycoricidinum.
2.1.2.3. Pancratistatin total syntheses.

Pancratistatin (2) has attracted much more attention from the synthetic community than other members of the Amaryllidaceae family. The first total synthesis of 2 was reported by Danishefsky and Lee\textsuperscript{41} in 1989 only five years after its isolation. The synthesis began with pyrogallol 64 as the precursor for the A-ring of 2, Scheme 6. Further functionalization provided amide 65 with all carbons required for the skeleton of pancratistatin. Iodolactonization of cyclohexene ring was performed followed by key installation of the nitrogen atom \textit{via} Overmann rearrangement of compound 68, lactone 69 was produced. This compound was submitted to dihydroxylation, followed by rearrangement of lactone to amide, and finally, upon the removal of protecting groups, pancratistatin (2) was attained.
Scheme 6. Danishefsky’s synthesis of pancratistatin.

The first enantioselective synthesis was reported by Hudlický in 1995. Chirality in this synthesis was provided by the microbial metabolite cis-diol 4, Scheme 7. It was transformed into vinyl aziridine 70, which was subjected to nucleophilic opening with the cuprate derived from ortho-lithiation of amide 71. This sequence provided compound 72, containing all carbons of (2). Cyclization of 72 upon detosylation should have provided short access to the phenanthridone skeleton of the final target, but because of atropoisomerism the cyclization of amide did not take place and no desired phenanthridone was obtained. Therefore the expected short access to the major skeleton
was somewhat hampered by lengthy functionalization steps in order to provide the desired phenanthridone. The endgame was achieved by heating epoxide 74 in water, providing pancratistatin in 13 steps.

Scheme 7. Hudlický’s enantioselective synthesis of pancratistatin.

This report was closely followed by that of Trost who reported another synthesis of (2) in the same year. The next formal synthesis was performed by Haseltine in 1997, and Magnus and Sebhat completed their total synthesis in 1998. Rigby utilized his previously used narciclasine strategy for the synthesis of pancratistatin in 2000. A relay synthesis of pancratistatin from narciclasine was published in 2001 by Pettit and will be discussed in detail in section 2.1.3.4. This approach was followed by Kim’s synthesis in
2002. In 2006 Li completed one of the shortest synthesis of pancratistatin. All of these early syntheses of (2) up to 2008 have been covered in an excellent review by Kornienko and Manpadi along with the estimation of economic viability of each route.

In 2009 Madsen applied a chiral pool strategy for the synthesis of pancratistatin (2). The chief strategy rests on the reaction of methyl ω-iodoglycoside 82 with allyl bromide 78 in the presence of zinc, followed by ring-closing metathesis,

**Figure 9.** Allyl bromide 78 was produced from piperonal 75 in 10 steps. Lactone 80 was obtained from 79 by ring-closing metathesis. At this point, a formal total synthesis of pancratistatin was achieved by intercepting the intermediate reported by Danishefsky. Nevertheless, the endgame of the synthesis was improved in comparison with the original sequence by utilisation of milder conditions.
The most recent enantioselective synthesis of pancratistatin was published by Alonso.\textsuperscript{53} The main strategy of Alonso’s approach lies in the organocatalytic condensation of \(\beta\)-aryl-\(\alpha\)-nitro-\(\alpha,\beta\)-enals such as 85 with protected dihydroxyacetone 83 in the presence of pyrrolidine catalysts, Figure 10. Enal 85 was obtained from 5-methoxypiperonal 84 in two steps. Formal [3+3] annulation reaction of 85 with protected dihydroxyketone 83 provided the key intermediate 86 with five contiguous chiral centres identical to those of final target. The optical purity of 86 was improved up to 99:1 \(er\) by a single recrystallization and 86 was then subjected to subsequent reduction, reprotection and Bischler-Napieralski cyclization to provide phenanthridone 89. Deprotection of 89 furnished (2), accessed in nine one-pot operations. This strategy was proven to be
versatile and was applied to the synthesis of 7-deoxypancratistatin and a few analogues that will be discussed in the Section 2.1.3.3.

![Chemical Structures and Reactions](image)

**Figure 10.** Alonso’s synthesis of pancratistatin.

The most recent racemic synthesis of pancratistatin has been performed by Cho *et al.*[^54] This synthesis followed they previously described strategy, which was already utilized once for the synthesis of pancratistatin (2) in 2011[^55]. It employed a Diels-Alder reaction between 3,5-dibromo-2-pyrone (92) and styrene 91, Scheme 8. Since those two syntheses are strategically similar, only the latest one will be discussed. The synthesis began with *trans*-borylation of alkyne 90, thus affording alkene 91, which underwent the required [4+2] cycloaddition with 92. Oxidation of boron yielded bicyclic product 93 which was reduced and ring-opened to provide diol 94, selective protection and dihydroxylation of which provided the skeleton of 1-*epi*-pancratistatin 95. Inversion of the C-1 center provided tetraol 96, which was submitted to a sequence previously developed by this
group, that included Curtius rearrangement and Bischler-Napieralski cyclization to yield pancratistatin (2), Scheme 8.

![Chemical structure](image)

Reaction and conditions: (a) B$_2$(Pin)$_2$, Cp$_2$ZrHCl, CH$_2$Cl$_2$, 82%; (b) 92, PhMe, 86%; (c) NaBO$_3$, THF, H$_2$O, 81%; (d) (i) Zn, NH$_4$Cl, H$_2$O; (ii) TsOH, MeOH, 52%; (e) TBDPSCl, imidazole, CH$_2$Cl$_2$, 88%;(f) OsO$_4$, NMO, THF, H$_2$O, 100%; (g) 2,2-DMP, CSA, 93%; (h) DMP, CH$_2$Cl$_2$, 95%; (i) NaBH$_4$, MeOH, 100%; (k) AcOH, H$_2$O, 100%; (l) TBAF, THF, 100%; (m) LiOH, THF, 100%; (n) (i) DPPA, Et$_3$N, PhMe; (ii)NaOMe, MeOH, 86%; (o) Ac$_2$O, pyridine; (p) Tf$_2$O, CH$_2$Cl$_2$, DMAP, 60 % for 2 steps; (q) BBr$_3$,CH$_2$Cl$_2$, 70%; (r) NaOMe,THF, 90%.

**Scheme 8.** Cho’s synthesis of pancratistatin.

2.1.2.4. Total syntheses of 7-deoxypancratistatin.

Since the first enantioselective total synthesis of 11 was performed even before its isolation from a natural source in 1989, as part of the synthesis of lycoricidine, Scheme 4, it will not be reproduced in this section. A list of syntheses performed after its isolation consists of enantioselective synthesis by Hudlický in 1995,\textsuperscript{56} which was realised through a strategy similar to that for pancratistatin, described by this group previously.\textsuperscript{42} This synthesis was closely followed by a radical approach by Keck in 1995.\textsuperscript{57} One year later
Chida\textsuperscript{58} utilised a chiral pool approach, followed by Plumet in 2000,\textsuperscript{59} who made use of the nucleophilic opening of vinylsulfones. A combination of ring closing metathesis and allyl coupling was utilised by Madsen in 2006,\textsuperscript{60} and Padwa reported a racemic synthesis in 2007\textsuperscript{61} making use of an intramolecular Diels-Alder reaction.

The latest formal total synthesis of racemic 7-deoxy pancratistatin (11) was performed by DeShong and Shukla\textsuperscript{62} in 2012. Crucial connectivity in 100 was established by coupling of a $\pi$-allyl complex formed from protected conduramine 97 with siloxane 98, Scheme 9. Preparation of the C-ring fragment ensued from the protected diol 39, previously used in the synthesis of narciclasine (1) by Yan.\textsuperscript{28} A hetero-Diels-Alder reaction was performed on 39, followed by reduction and protection to provide the key intermediate 97 which was then submitted to Hiyama coupling with silane 98 in the presence of palladium catalyst 99, which yielded product 100 with moderate yield. Further cyclization and oxidation, described previously by Hudlický,\textsuperscript{56} led to epoxide 101, an intermediate in the aforementioned synthesis.
2.1.3. Synthesis of analogues of Amaryllidaceae alkaloids

Promising anticancer activity and the as-yet unresolved mechanism of the biological action of isocarbostyril congeners of Amaryllidaceae family has attracted the interest of the synthetic organic and biological community alike. One of the major obstacles on the way to a marketable drug is the poor aqueous solubility of isocarbostyril compounds. This problem can be somewhat compensated for by the preparation of water–soluble prodrugs such as phosphates, however, the relatively low availability of Amaryllidaceae congeners from natural sources has ensured that significant efforts have been directed towards total synthesis. Unfortunately, because of the complexity of targets, existing approaches are somewhat lengthy (12-18 steps) and suffer from impracticality for large scale preparation, given the overall yields of 2-7%. The few syntheses with the higher yields do not provide the necessary flexibility to perform variation in different parts of

Scheme 9. DeShong’s formal synthesis of 7-deoxypancreatistatin.

Reaction and conditions: (a) NH(OH)CO₂Me, nBu₄O₂, CHCl₃/DMF, 31%; (b) Mo(CO)₆, MeCN, H₂O, 79%; (c) ClCO₂Et, pyridine, CH₂Cl₂, 86%; (d) 98, 99, TBAF, THF, 35%; (e) AcOH, THF, H₂O, 94%; (f) iBuOOH, VO(acac)₂, MeCN, 85%.
the target compounds in order to generate libraries of analogues. Therefore there are three goals that the syntheses of analogues of the aforementioned compounds need to target: (i) to establish the minimal pharmacophore required for anticancer activity and elucidate the mode of action, (ii) to produce water-soluble version of these compounds, and (iii) to devise short and practical routes to biologically active compounds.

There are currently two general approaches to the analogues: the first one is the modification of the most abundant natural congener of this family – narciclasine (1). This approach was extensively developed by Pettit, the discoverer of pancratistatin. The attractiveness of such a route to study the pharmacophore lies in the relatively fast access to the desired compounds. This approach will be discussed in a Section 2.1.3.4.

The second approach, which is somewhat related to total synthesis effort, is to produce fully synthetic analogues of Amaryllidaceae congeners from commercially available starting materials. This route allows an approach to analogues that cannot be produced by modification of natural congeners and does not rely on limited natural sources. The analogues can be divided into three main groups depending on where the major structural differences to the natural products are located. In the Section 2.1.3.1, synthetic analogues with modification in C-ring will be discussed, followed by synthetic analogues of the B-ring, Section 2.1.3.2, and A-rings analogues, Section 2.1.3.3.
2.1.3.1. C-ring analogues

The most explored class of analogues possess variations in the ring C. Because of the inherent complexity, namely four contiguous hydroxylated stereogenic centers, it only seems logical to explore deoxy analogues as well as compounds with different relative stereochemistry of the hydroxyl groups in order to establish a minimum pharmacophore.

One of the first synthetic analogues of 2 with a completely deoxygenated C-ring was synthesized by Heathcock.\textsuperscript{63} The main strategy involved the Michael-type 1,4-addition of ortho-metalated amide 103 to the 1-nitrocyclohexene 107 to produce aryl nitrocyclohexane 104, Scheme 10. Piperonilic acid 102 was converted into its diethylamide, selectively ortho-lithiated, borylated, oxidised, and protected to produce amide 103. The latter was in turn ortho-metalated and the resulting aryl lithium was added to 107 to produce the mixture of cis- and trans- stereoisomers 104 which was epimerised to the trans-isomer and reduced to the corresponding amine 105. It was cyclized by treatment with s-BuLi and deprotected to produce 106 in seven steps. Unfortunately, this model compound was not subjected to any biological studies. This approach was supposed to be a model for the actual synthesis of 2, but it is worth to note
that such strategy failed to produce the desirable outcome, transamidation of arylamide with the amino group, when it was later applied to the synthesis of pancratistatin.42

\[
\begin{align*}
&\text{O} &\text{O} &\text{N} &\text{Et}_2 \\
&\text{O} &\text{O} &\text{N} &\text{Et}_2 \\
&\text{O} &\text{O} &\text{N} &\text{Et}_2
\end{align*}
\]

**Scheme 10.** Heathcock’s synthesis of a pancratistatin model.

Banwell64 developed a new way to analogues of narciclasine and pancratistatin while exploring approaches to the synthesis of the skeleton of narciclasine itself. The key strategy in his synthesis lies in a Suzuki coupling between boronic acid 113 and functionalised 1-bromocyclohexenes 110a and 110b, Scheme 11. The synthetic sequence started with dihydroxylation of cyclopentadiene 108 with Pb(OAc)₄ followed by protection of the diol, and addition of dibromocarbene led to the formation of 109. This dibromocyclopropane was rearranged upon reaction with silver isocyanate and trapped with methanol to produce a mixture of isomeric bromocyclohexenes 110a and 110b (in 28% and 40% yield respectively), both of which could be elaborated to natural product
analogue. These compounds were separated and subjected to Suzuki coupling with boronic acid 113. Carbamate 111 was obtained by acetate re-protection after Suzuki coupling of 110a and was subjected to Bischler-Napieralski cyclization with triflic anhydride and further deprotected by sodium methoxide to produce 2,7-dideoxynarcilasine (112) in six steps. Vinylbromide 110b was subjected to the same sequence to produce alkene 114. This isomer was stereoselectively borylated (as a result of steric hindrance of ketal group), oxidised, and reprotected as a triacetate to produce carbamate 115. This compound was submitted to the Bischler-Napieralski reaction to furnish triacetate of 1,2-epi-4,7 dideoxypancratistatin (116). Banwell’s strategy thus allowed for the synthesis of analogues of narcilasine and pancratistatin utilizing the same sequence and starting materials.
Scheme 11. Banwell’s synthesis of 2-deoxynarciclasine and 1-epi-4-deoxypancratistatin.

Significant efforts toward the refinement of the C-ring pharmacophore have been reported by McNulty,\textsuperscript{65-67} whose systematic study led to the recognition of nitrostyrene 122 as a common intermediate for the synthesis of several different racemic deoxy analogues. The first approach was based on a stereoselective intramolecular aldol condensation of nitroaldehyde 118,\textsuperscript{67} Scheme 12. Addition of an organometallic reagent generated from ethyl 4-bromobutyrate 117 to nitrostyrene 122 which upon reduction led
to aldehyde 118. Submission of this intermediate to solid-phase condensation in the presence of neutral alumina yielded nitrocyclohexanol 119 with good stereoselectivity (95:5). A series of protection and reduction transformations afforded carbamate 120 which, after Bischler-Napieralski cyclization and deprotection, afforded 2,3-dideoxy \textit{trans}-dihydrolycoricidine (121) in 8 steps.

\begin{center}
\begin{tikzpicture}
\node[draw,rectangle] (117) at (0,0) {\(\text{Br} \quad \text{CO}_2\text{Et}\)};
\node[draw,rectangle] (118) at (2,0) {\(\text{CHO}\)};
\node[draw,rectangle] (119) at (2,1) {\(\text{NO}_2\)};
\node[draw,rectangle] (120) at (4,1) {\(\text{NHCO}_2\text{Me}\)};
\node[draw,rectangle] (121) at (4,0) {\(\text{O}\)};
\node[draw,rectangle] (122) at (2,-1) {\(\text{NO}_2\)};
\node[draw,rectangle] (123) at (4,-1) {\(\text{O}\)};
\node[draw,rectangle] (124) at (2,-2) {\(\text{NH}\)};
\node[draw,rectangle] (125) at (4,-2) {\(\text{OH}\)};
\node[draw,rectangle] (126) at (2,-3) {\(\text{O}\)};
\node[draw,rectangle] (127) at (4,-3) {\(\text{O}\)};
\draw (117) -- (118) -- (119) -- (120) -- (121) -- (122) -- (123) -- (124) -- (125) -- (126) -- (127);
\end{tikzpicture}
\end{center}

Reactions conditions: (a) (i) Zn, LiI, DMF; (ii) CuCN, LiCl, THF; (iii) 122, 81%; (b) DIBAL, CH\(_2\)Cl\(_2\); (c) Al\(_2\)O\(_3\), 48h, 71% for 2 steps; (d) TBSCI, imidazole, DMF, 96%; (e) H\(_2\), 43 atm, Raney Ni, MeOH, 100%; (f) CICO\(_2\)Me, Et\(_3\)N, CH\(_2\)Cl\(_2\), 96%; (g) Ac\(_2\)O, FeCl\(_3\), 85%; (h) (i) Tf\(_2\)O, DMAP, CH\(_2\)Cl\(_2\); (ii) HCl, THF, (iii) Ac\(_2\)O, DMAP, Et\(_3\)N, 85%; (j) NaOMe, THF, MeOH, 96%.

**Scheme 12.** McNulty’s synthesis of 2,3-dideoxy \textit{trans}-dihydrolycoricidine.

Latter approaches by McNulty towards the construction of deoxy analogues were based on a Diels-Alder reaction between the aforementioned nitrostyrene 122 and different dienes.\(^{66,65}\) It was shown that this approach can lead to the formation of the essential \textit{trans}-stereochemical relationship between the aryl ring and nitro group. Independently, the same stereoselectivity was reported by Iglesias\(^ {68}\) on the similar systems. Two different dienes were utilised for the synthesis of two analogues of \textit{trans}-dihydrolycoricidine.
For the synthesis of 4-deoxy \textit{trans}-dihydrolycoricidine (126), 2,5-dihydrosulfolane (127) as a diene precursor was used in a Diels-Alder reaction with nitrostyrene 122 in the presence of Lewis acidic ZnCl$_2$,\textsuperscript{66} Scheme 13. The cycloadduct was reduced with aluminum amalgam and transformed to carbamate 123, which was further epoxidised to a mixture of $\alpha$- and $\beta$-epoxides 124. Nucleophilic \textit{trans}-dixial opening of both isomers, followed by acetylation led to the same stereochemical relationship in diacetate 125. The same result for selective ring opening of similar system was reported three years earlier by Toke.\textsuperscript{69} Carbamate 125 was submitted to modified cyclization conditions described by Banwell,\textsuperscript{70} and deacetylated to provide 4-deoxy \textit{trans}-dihydrolycoricidine (126) in seven steps.

\begin{center}
\begin{tikzpicture}
\node[draw=black,fill=white,align=center] (a) {\textbf{122}}; \node[draw=black,fill=white,align=center] (b) at (1.5,0) {\textbf{123}}; \node[draw=black,fill=white,align=center] (c) at (3,0) {\textbf{124}}; \node[draw=black,fill=white,align=center] (d) at (4.5,0) {\textbf{125}}; \draw[->] (a) -- node[above] {a-c} (b); \draw[->] (b) -- node[above] {d} (c); \node[draw=black,fill=white,align=center] (e) at (6,0) {\textbf{126}}; \node[draw=black,fill=white,align=center] (f) at (7.5,0) {\textbf{127}}; \node[draw=black,fill=white,align=center] (g) at (9,0) {\textbf{128}}; \draw[->] (c) -- node[above] {e,f} (d); \draw[->] (d) -- node[above] {g,h} (e); \draw[->] (f) -- node[above] {} (g);
\end{tikzpicture}
\end{center}

Reaction conditions: (a) 127, ZnCl$_2$, PhMe, 85%; (b) Al/Hg, THF, H$_2$O; (c) ClCO$_2$Me, Et$_3$N, CH$_2$Cl$_2$, 96% for 2 steps; (d) mCPBA, CH$_2$Cl$_2$, 93%; (e) PhCO$_2$Na, H$_2$O; (f) Ac$_2$O, pyridine, 62% for 2 steps; (g) (i) Tf$_2$O, DMAP, CH$_2$Cl$_2$; (ii) HCl, dioxane, 65%; (iii) Ac$_2$O, pyridine, 85%; (h) NaOMe, THF, MeOH, 96%.

\textbf{Scheme 13.} McNulty’s synthesis of 4-deoxy \textit{trans}-dihydrolycoricidine.

In order to make the last analogue 133, nitrostyrene 122 was subjected to the cycloaddition with Danishefsky’s diene 128 in refluxing toluene to provide
cyclohexanone 129 in good yield, Scheme 14. The ketone was reduced to alcohol with sodium borohydride and converted via Mitsunobu reaction to nitrocyclohexane 130. The nitrogroup was then reduced to carbamate 131, which was subjected to the Bischler-Napieralski reaction and after demethylation and removal of the ester provided 3-deoxy trans-dihydrolycoricidine (133).66

![Chemical reactions and products](image)

Reaction conditions: (a) PhMe; (b) NaBH₄, EtOH; (c) MesCOOH, Bu₃P=C(CO₂Me)₂, PhCH₃, 70% for 3 steps; (d) (i)Al/Hg, EtOH, H₂O; (ii) ClCO₂Me, pyridine, CH₂Cl₂, 70%; (e) Tf₂O, DMAP, 65%; (f) I₂, CH₂Cl₂, 15%; (g) LiAlH₄, THF.

**Scheme 14.** McNulty’s approach to 3-deoxy analogues of trans-dihydrolycoricidine.

Pandey71 reported an enantioselective photochemical approach towards pancratistatin analogues. His synthesis started from D-quinic acid 134, which was transformed into ketal 135 and subjected to several protecting group manipulations, followed by sodium periodate cleavage of unprotected diol moiety to produce hydroxy ketone 136, Scheme 15. A mesylation-elimination sequence of the free hydroxyl produced enone 137, which served as the C-ring component in this synthesis. Michael addition of carbamate 138 to this enone followed by silyl protection of the enol led to key intermediate 139, which in
turn was submitted to photocyclization in the presence of 1,4-dicyanonaphthalene (DCN). This transformation established the necessary trans ring juncture in tricycle 140. Steric hindrance of the β-face resulted in selective reduction of the carbonyl to establish the correct stereochemistry in the C-ring of the product. Further protection and ruthenium-catalyzed oxidation of the benzylic position afforded amide 141, which after protection yielded the final product 2,7-dideoxypancratistatin (142).

Scheme 15. Pandey’s synthesis of 2,7-dideoxypancratistatin.

In order to perform the synthesis of a further deoxygenated analogue of 7-deoxypancratistatin,72 Hudlický utilized the route previously developed in his group towards 7-deoxypancratistatin (11),56 Scheme 16. The synthesis started with whole-cell
oxidation of bromobenzene 3 followed by acetonide protection of the resulting diol, which was converted to aziridine 144 followed by dehalogenation. Nucleophilic opening of this aziridine with cuprate 143 in the presence of a Lewis acid led to amide 100, which in turn was deprotected to diol 145. Hydrogenation of alkene moiety with hydrogen on palladium on carbon was followed by Bischler-Napieralski cyclization and base-catalyzed deprotection to provide 1,2,7-trideoxypancratistatin (147). Synthesis and biological studies of this compound along with those synthesized by McNulty, (Scheme 13, Scheme 14), completed all possible deoxy models of the C-ring of Amaryllidaceae compounds. Biological results will be discussed in section 2.1.4.

Scheme 16. Hudlický’s synthesis of 1,2,7-trideoxypanratistatin.

Since lycorine (8) showed some activity in anticancer assays and it possesses relative stereochemistry in positions 10b, 1, and 2 (narciclasine numbering) opposite to those of pancratistatin, it seemed reasonable to explore biological activity of the enantiomer of 7-deoxypancratistatin in anticancer assays. In order to synthesize such compounds and
study their activity, Hudlický and Akgun\textsuperscript{39} utilised the toluene dioxygenase (TDO) enzyme, which can discriminate between the dihydroxylation of aromatic compounds based on relative size of their substituents. \textit{p}-Bromoidoobenzene $\text{148}$ was submitted to the whole-cell oxidation and after selective radical reduction provided a mixture of bromocyclohexadienediols \textbf{4} and \textit{ent}-\textbf{4}, Scheme 17. Submission of this mixture to a nitroso hetero-Diels-Alder reaction and reduction led to a mixture of conduramines $\text{149}$, which upon acylation and enzymatic resolution provided enantiomerically pure $\text{149}$, Scheme 17. This conduramine was transformed to aziridine $\textit{ent}$-$\text{144}$, the enantiomer of which was successfully utilised before for the synthesis of 7-deoxypancratistatin (11).\textsuperscript{56} The same conditions were applied in case of this compound to provide \textit{ent}-\textbf{11} and enabled the study of its biological activity, which turned out to be one order of magnitude less active than natural congener.
Scheme 17. Hudlický’s synthesis of ent-7-deoxypancratistatin.

DeShong\textsuperscript{73} developed a palladium-catalyzed racemic approach towards pancratistatin-type compounds. It consists in coupling between aryl fragments and the Pd \( \pi \)-allyl complex formed from allyl carbonate of type 152, Scheme 18. The synthesis began with a hetero-Diels Alder reaction between cyclohexadiene 150 and an acynitroso compound formed \textit{in situ} to provide bicyclic oxazine 151, which was reduced with molybdenum hexacarbonyl and protected with ethyl chloroformate to give allylic alcohol 152. This compound underwent Hiyama coupling with silane 98 to furnish intermediate 46 and its regioisomer (not shown) in a 1:1.6 ratio. After isolation the desired isomer was submitted to a Bischler-Napieralski cyclization followed by installation of the \textit{trans}-diol moiety by treatment peacid epoxidation and ring opening to yield compound 154.
Scheme 18. DeShong’s synthesis of 3,4,7-dideoxypancratistatin.

Banwell\textsuperscript{74, 75} used chiral diol 4 to secure access to a few unnatural enantiomers of narciclasine-type compounds. The strategy utilised for the formation of the core of the compound was similar to that used by Hudlický\textsuperscript{25, 26} and relies on tandem Suzuki coupling and lactam formation between arylboronic ester 165 and different chiral vinyl bromides to provide access to narciclasine type compounds in a short sequence, Scheme 19. Diol 4 was protected as \textit{p}-methoxybenzyl acetal 155, which was selectively dihydroxylated. Protection of the diol with methoxymethyl groups and reductive ring opening of the PMB-acetal gave vinylbromide 156. Further manipulation of protective groups secured access to the key intermediate 157. Subjection of this alcohol to Overmann rearrangement conditions produced amine 159, which served as building block for \textit{ent}-narciclasine (\textit{ent}-1), Scheme 20. Mesylation of alcohol 157 followed by azidation and hydrolytic Staudinger reaction provided building block 162 for synthesis of \textit{ent}-3-\textit{epi}-lycoricidine (167), Figure 12.

The aromatic building block 165 for coupling was produced in seven steps starting from piperonal (75), which was efficiently transformed to diethylamide 103 via Corey–Gilman–Ganem oxidation, Scheme 20. It was subjected to a typical metallation/borylation/oxidation sequence and protection conditions, further ortho-metallation, halogenation and deprotection with Meerwein’s reagent provided phenol ester 164. After protection with methoxymethyl group it was borylated with
pinacolborane in presence of a palladium catalyst. Boronate 165 was submitted to an one-pot Suzuki coupling and amide formation to form the skeleton of the final compound, which upon acidic deprotection provided ent-narciclasine (1). A similar strategy was applied to the synthesis of several other analogues, Figure 12.

**Scheme 20.** Banwell’s synthesis of ent-narciclasine.

**Figure 12.** Different analogues of ent-narciclasine.

Kornienko\textsuperscript{76} designed a synthesis of truncated analogues that lacked the cyclohexane structure of the C-ring. This approach began with Sharpless asymmetric dihydroxylation
of ethyl sorbate 168 followed by acetonide protection to produce a chiral acrylic acid ester 169, Scheme 21. Stereoselective conjugate addition of aryl cuprates led to compounds 170a and 170b, enolates of which were subjected to azidation reaction, which unfortunately led to a mixture of diastereomers. After reduction of the mixture of azides 171 with lithium aluminium hydride and protection as carbamate, the required trans-isomer of 172 was purified. Standard reprotection with acetates (before the key Bischler-Napieralski cyclization) led to triacetate 173. The procedure for the cyclization, originally designed by Banwell,64 was modified and studied in some detail. Changing the base to 2-chloropyridine led to a clean conversion to imidate 174. Subsequent demethylation with trimethylsilyl iodide and basic hydrolysis provided compounds 175a and 175b - open C-ring analogues of 11 and 2 respectively. 7-Deoxy analogue 175a was carried further to periodate assisted cleavage of diol to produce truncated cyclic analogue 176.

Kim has been studying the effect of complete aromatisation of C-ring on the biological activity of narciclasine and pancreaticatin. Because of the lack of any chiral centers it was possible to develop a short scalable route towards these compounds. Synthesis began with complete benzylation of 2,3,4-trihydroxybenzaldehyde 179, followed by Pinnick oxidation of the intermediate to the carboxylic acid, which underwent a Curtius rearrangement to aniline 178, Scheme 22. Coupling of this product with acyl chloride
made in situ from 6-iodopiperonylic acid (56) provided amide 179. In order to undergo intramolecular coupling the secondary amide 179 needed to be transformed to the tertiary amide 180, which was achieved by alkylation of 179 with SEMCl. Direct arylation was achieved in high yield in the presence of palladium acetate, tris(o-tolyl)phosphine and silver salt as a base. One-pot global deprotection of the coupled product 181 was achieved by hydrogenation and treatment with trifluoroacetic acid to furnish the final trihydroxy product 182 in low yield. Kim also described an analogous synthesis of three aromatic dihydroxy analogues (not shown), neither of these compounds displayed anticancer activity.

Scheme 22. Kim’s approach to C-aromatic analogues.

Hudlický78, 79 designed a synthetic pathway towards a variety of compounds, which are homologues of 7-deoxypancratistatin at position C-1. The syntheses originated from
chiral diol 4, which upon protection was immediately submitted to an aziridination reaction following the procedure developed by Yamada\textsuperscript{80} and Evans\textsuperscript{81} to provide aziridine 183 in moderate yield, Scheme 23. Radical dehalogenation in the presence of tributyltin hydride led to vinylaziridine 70, which upon epoxidation with m-chloroperbenzoic acid in refluxing 1,2-dichloroethane yielded a mixture of diastereomeric epoxides in a 3:1 ratio (major diastereomer 184 shown). After fractional recrystallization, the major isomer was submitted to epoxide opening reaction with the alkynylalane, formed from alkyne 191. The product of epoxide opening was protected with tert-butylidimethyl triflate without isolation to provide 185 in good yield. Selective reduction of the alkyne moiety to the cis-alkene was achieved upon treatment with borane and after isolation, alkene 186 was submitted to solid phase silica gel-catalyzed aziridine opening.\textsuperscript{82, 83} The product of this opening, namely phenantrene 187, was isolated in moderate yield and submitted to oxidative cleavage in order to produce dialdehyde 188. This transformation was achieved by sequential treatment with osmium tetroxide, reduction of the ketoalcohol with sodium borohydride, and cleavage of diol with sodium periodate. Transient dialdehyde 188 was not isolated but instead was allowed to undergo the cyclization to hemiaminal 189. The next step involved the oxidation of the hemiaminal to amide 190 in order to furnish the complete skeleton of the final product. This intermediate 190 was used as a point of divergence to produce, via oxidation and deprotection, carboxylic acid 193a and methyl ester 193b. Reduction of aldehyde 181 with sodium borohydride and acetylation led to alcohol 192b, and acetate 192a respectively, following deprotection.
Scheme 23. Hudlický’s approach towards C-1 homologues of pancratistatin.
Alonso\textsuperscript{84, 85} developed yet another general strategy towards different analogues of pancratistatin and applied it towards the synthesis of 2-\textit{epi}-7-deoxypancratistatin (197) and 2,4-die\textit{epi}-7-deoxypancratistatin (198), Scheme 24. The strategy was identical to the one described previously for pancratistatin, (see Figure 10). The [3+3] annulation was performed with a derivative of piperonal (75), and dihydroxyacetone (83) to produce protected cyclohexanone 194. A divergence point between synthesis of pancratistatin and its analogues lies in the nature of the stereoselective reduction of the carbonyl function in 194. Because of steric hindrance from the acetonide protecting group the reduction gave the opposite stereoselectivity than that observed with a similar reduction,\textsuperscript{53} which led to the synthesis of the natural product. Reduction of the nitro group, deprotection and reprotection afforded intermediate 195, which was submitted to cyclization under the standard Banwell procedure\textsuperscript{64} followed by deprotection to provide to 2-\textit{epi}-7-deoxypancratistatin 197 in racemic form. A similar strategy was applied to the synthesis of racemic 2,4-die\textit{epi}-7-deoxypancratistatin (190): intermediate 194 was submitted to a base-induced epimerization of the C-4 free hydroxyl group, followed by the same reduction-deprotection-cyclization sequence as described above. These compounds can also be prepared in an enantiopure form by utilisation of previously developed enantioselective protocol.\textsuperscript{53}

2.1.3.2. B-ring analogues

Chapleur\(^{86}\) was one of the first to systematically study the pharmacophore of pancratistatin. He utilized the chiral pool approach towards open B-ring analogues or seco-analogues. Methyl D-glucopiranoside (199) was subjected to protection, azidation and regioselective oxidative opening to produce bromide 200, Scheme 25. Reprotection and sequential elimination led to intermediate 201. The key step of this sequence was the transformation of pyranoside 201 via Ferrier rearrangement to provide cyclohexane core, which upon Luche reduction provided azido alcohol 202.
Scheme 25. Chapleur’s synthesis of open B-ring analogues.

In order to establish the correct stereochemical relation of hydroxyl groups in 202, the C-2 hydroxyl (pancratistatin numbering) was subjected to a Mitsunobu reaction, followed by the reduction of azide, which led to the protected conduramine 203, which was coupled to piperonylic acid (102) and deprotected to provide open-chain analogue 204. This compound was shown to be completely inactive.

Hudlický\textsuperscript{87} synthesized the previously unknown 10-\textit{epi}-7-deoxypancratistatin (210) in order to compare the effect of \textit{cis}-junction of the B-ring on anticancer activity, Scheme 26. Synthesis started from the common intermediate aziridine 70, used before in few total syntheses of Amaryllidaceae alkaloids. It was transformed to cyclic sulfate 206 which was opened with ammonium benzoate, to provide upon protection and hydrolysis \textit{trans}-
dial 207. Formation of the lithium salt of this compound was followed by immediate aza-
Payne rearrangement and, after alkylation with piperonyl bromide (205), provided
epoxide 208. Intramolecular opening of the epoxide catalyzed by Lewis acid provided the
skeleton of the final compound 209 with the required cis-stereochemistry. Finally
oxidation of the benzylic position and deprotection provided 10-epi-7-deoxypancrastatin
(210). Anticancer assays of this analogue showed complete lack of activity/

Scheme 26. Hudlický’s synthesis of 10-epi-7-deoxypancrastatin.

Chapleur studied the effect of replacement of nitrogen in the lactam moiety by oxygen
on biological activity. Ortho-metallated amide 211 was reacted with protected D-
glucuronolactone (212) followed by selective acid-catalyzed acetonide deprotection led to
polyol 213, which was oxidised with sodium periodate and was subjected to DBU-
induced Knoevenagel condensation and acetate protection, Scheme 27. The resulting enone 214 was subjected to Luche reduction, which unfortunately did not appear to be stereoselective. Removal of acetate and exposure to acid conditions led to a smooth formation of the lactone and deprotection of acetonide group. Two lactones, 216 and 217, were separated and subjected to biological testing. Although this is one the shortest syntheses of the pancratistatin analogues, it did not lead to lactone 216 stereoselectively, and both of these compounds showed lack of any anticancer activity.

**Scheme 27.** Chapleur’s synthesis of lactone analogues.

Hudlický used the strategy previously developed in his group for the synthesis of 7-deoxypancratistatin (11)56 for the synthesis of analogues with open C and B-rings.26 The key step was oxidation of intermediate tetraols 219 in order to produce open ring
analogues of 11, Scheme 28. Oxidative cleavage was followed by reduction to produce diols 220, then depending on a protecting compound was either deprotected completely to provide an amine 221 or protected with t-butoxycarbonyl group to produce 222. Both of these compounds were subjected to biological studies, which showed that only 222 was slightly active, section 2.1.4.

Scheme 28. Hudlický’s synthesis of truncated ring-opened analogues.

Fessner\(^9\) used a biocatalytical approach towards synthesis of sugar-based heterocyclic analogue 228, Scheme 29. He utilised whole-cell oxidation of naphtalene (223) with recombinant E.Coli JM109 (pDTG121) as a first step. The product of oxidation, diol 225, was isolated as a single enantiomer in good yield and subjected to ozonolysis. Dialdehyde 226 was, without isolation, carried through the sequential enzymatic aldol reaction with dihydroxyacetone phosphate 224, dephosphorylation, and oxidation to produce a mixture of desired pyranoside 228 and the by-product furanoside 229 in 4 steps. Unfortunately, despite enzyme screening, no selective way to form 228 were found.
Complementary to the efforts of Chapleur, Kornienko\textsuperscript{90,91} described an approach to the analogues of pancratistatin that completely lack the amide moiety but retain stereochemical relation between A- and C-ring. The synthesis started by selective benzylation of D-xylose \textsuperscript{230} to a protected hemiacetal \textsuperscript{231}, which was submitted to a Wittig reaction, and oxidation followed by the second Wittig reaction, Scheme 30. Conjugate addition of aryl cuprate \textsuperscript{239} to the acrylic ester \textsuperscript{232} led to the stereoselective formation (ratio >50:1) of product \textsuperscript{233}. In order to cyclize C-ring of this compound, the ester moiety was reduced and transformed to the alkene by means of Grieco elimination.\textsuperscript{92} Ring-closing methathesis of two terminal alkenes was achieved by 1\textsuperscript{st} generation Grubbs catalyst (\textsuperscript{240}) and led to the general precursor \textsuperscript{235}, which can be selectively transformed to alkene analogue \textsuperscript{237}, alkane \textsuperscript{236}, or diol \textsuperscript{238} depending on

\textbf{Scheme 29.} Fessner’s biocatalytic approach towards analogues of 7-deoxypancratistatin.
conditions. By varying arylcuprates different analogues were obtained and all were assayed for anticancer activity with complete lack of any activity.

**Scheme 30.** Kornienko’s approach to an open B-ring analogues.

Further studies towards refining of pharmacophore were performed by McNulty. The key step of his approach consisted of Evans aldol reaction, which allowed the establishment, in one step, of the correct stereochemistry at C10b and C1 carbons. Synthesis started by the installation of Evans auxiliary onto phenylacetic acid 243,
Scheme 31. Product of this reaction 244 was submitted to aldol reaction in presence of magnesium chloride with chiral aldehyde 242, produced from tartaric acid, to provide product 245 with 95:5 selectivity. Reductive removal of the auxiliary group and reprotuction with benzylidene acetal led to acetal 246. Regiolselective oxidative cleavage of the acetal with N-bromosuccinimide provided a convenient leaving group which was replaced by azide and followed by reduction and concomitant O to N benzoyl migration, thus ensuring the installation of the amide fragment in product 247. Two different deprotection conditions led to product 248a and 248b respectively. These open-chaing analogues displayed no anticancer activity.

2.1.3.3. A-ring analogues

Analogues of A-ring modifications comprise the most underrepresented group of the analogues of isocarbostyril Amaryllidaceae congeners. The main reason is that most synthetic efforts were focused on solving the complexity of B- and C-rings therefore less challenging A-ring modifications received little attention.

Only a few compounds are known that represent A-ring modification of pancratistatin. One of the first examples is the 7,8-dideoxypancratistatin (254) synthesized by Hudlický,\(^72\) Scheme 32. General strategy was similar to the synthesis of actual 4,\(^56\) but
different aryl cuprate 255 was used. Bischler-Napieralski cyclization of tetraacetate 251 would expect to form of two regioisomers 252 and 253. But in fact only one isomer, namely 252, was formed in the course of reaction and after deprotection it afforded 254. Activity of this compound was shown to be two orders of magnitude lower than pancratistatin.

Scheme 32. Hudlický’s synthesis of 7,8-dideoxypancratistatin.

The effect of complete removal of methylenedioxy fragment from A-ring was later studied by the same group. It was reasoned that indole moiety has similar steric and to some extent electronic properties of original arylmethylenedioxy fragment. In order to produce such analogue 259 vinylaziridine 70 was subjected to silica gel-catalyzed
nucleophilic opening by methyl 2-indolecarboxylate (260) to produce cyclohexene 256, Scheme 33. Iodolactonization of this alkene followed by basic hydrolysis with lithium methoxide led to formation of epoxide. In order to remove the tosyl protecting group amide was transformed into t-butoxycarbamate 258 and submitted to one-electron reduction to remove tosyl group, followed by prolonged exposure to wet silica-gel upon heating furnished the final indole β-carboline 249. Indole substitution rendered compound completely inactive.

Reactions and conditions: (a) 260, SiO₂, 68%; (b) LiOH, H₂O, 95%; (c) I₂, NaHCO₃, THF, 71%; (d) LiOMe, MeOH, 85%; (e) Boc₂O, 88%; (f) Na, naphthalene, DME; (g) SiO₂, H₂O, 31%.

**Scheme 33.** Hudlický’s synthesis of indole analogue of pancratistatin.

Further development of modification of A-ring was based on a [2+2+2] cycloaddition strategy.⁹⁴,⁹⁵ This time the effect of replacement of methylenedioxy moiety with silyl groups was studied. Synthesis was based on nucleophilic opening of vinylaziridine 70
with alane generated from TMS acetylene (268), Scheme 34. The resulting enyne 261 was then selectively dihydroxylated with osmium tetroxide and transformed to a cyclic sulfate 262. Nucleophilic opening of sulfate with ammonium benzoate established the required trans-diol stereochemistry followed by selective desilylation of terminal alkyne and alcohol protection to provide 263. Alkylation of amide moiety with propargyl bromide led to the key dialkyne 264, which was submitted to [2+2+2] cycloaddition with bis(TMS)acetylene (269) catalyzed by cyclopentadienyl cobalt dicarbonyl. This reaction furnished skeleton of the desired product 265 in good yield.
Scheme 34. Hudlický’s synthesis of disilyl pancratistatin analogue.

In order to oxidise the benzylic position by high-valent ruthenium reagents, protecting group exchange was performed and, after oxidation, phenanthridone 266 was obtained. Detosylation and sequential basic and acidic hydrolysis led to bis-trimethylsilyl-7-deoxypancratistatin 267, completely inactive in anticancer assays.

Alonso\textsuperscript{85} developed general organocatalytic [3+3] route towards synthesis of A-ring analogues. The early stages of synthesis followed his general approach towards
nitrocyclohexanones such as 271 from furfural (270), Scheme 35. Protection and reduction of nitro group in 271 was followed by acylation with acryloyl chloride in order to furnish starting material 272 for the intramolecular Diels-Alder reaction. Chlorination of furan with N-chlorosuccinimide helped to facilitate formation of Diels-Alder product 273 as well as subsequent aromatisation. Deprotection of the acetonide was followed by reduction at C-2 to ensure correct relative stereochemistry, and further reprotection with acetates. Finally, aromatisation was achieved by treatment with sodium methoxide and led simultaneously to the complete deprotection of the ester groups. Analogue 274, with a free phenolic group was subsequently transformed to protected version 275 and both of these compound were submitted to anticancer testing and showed no activity.
Scheme 35. Alonso’s synthesis of 7,9-dideoxypancratistatin analogues.

2.1.3.4. Semi-synthesis from natural products.

Because of the relatively high abundance of narciclasine in common perennials of temperate climate such as *Narcissus pseudonarcissus* and *Narcissus poeticus* in comparison with other congeners, it has always been considered an attractive route to utilize 1 as a starting material for synthesis of analogues. One of the first effort in this field was reported by Mondon and Krohn, who studied hydrogenation and other transformations of narciclasine to different derivatives. Synthesis of these compounds and biological studies performed on them constited one of the first examples of refining the
pharmacophore of Amaryllidaceae congeners. Several of the compounds produced in this study showed activity comparable with narciclasine itself in assays against cancer cell lines, especially trans-dihydronarciclasine (9), other derivatives such as cis-dihydronarciclasine (276), iso-narciclasine (277), and 7-methylnarciclasine (278), showed significantly reduced activity in comparison with natural compounds, Figure 13.

![Chemical structures](image)

**Figure 13.** First synthetic derivatives of narciclasine.

During their studies Mondon and Krohn first observed selective protection of the syn-diol to its ketal to form compounds of type 279, Scheme 36. Selective oxidation of the allylic alcohol in position C-2 lead to unstable ketone (not shown) which upon reduction provided mixture of protected 2-epi-narciclasine 280 and 279.

Further development in the area of hydrogenation of narciclasine and 7-deoxynarciclasine was later provided by Pettit\textsuperscript{96, 97} in order to improve the selectivity of hydrogenation towards the most active congener, namely trans-dihydronarciclasine (12). Despite screening of different catalysts and conditions,\textsuperscript{97} the best selectivity was obtained with palladium on carbon, but is still remains low (9:276:277=51:47:2) on milligram scale. Upon scaling the reaction, the ratio of 12:276 drops to 30:62.

Systematic studies on the conversion of narciclasine to different analogues were performed by Pettit.\textsuperscript{9, 48, 97-102} His major goal was to convert narciclasine (1) to pancratistatin (2), since his group has been largely involved in the discovery and the modification of pancratistatin skeleton. This task seems to be trivial, as the only difference is the presence of a hydroxyl group at C-1 and the absence of a double bond. In theory, this transformation can be achieved by stereoselective hydration of narciclasine, Figure 14.
Figure 14. Theoretical transformation of narciclasine to pancratistatin.

In order to study this transformation Pettit performed studies on face-selective dihydroxylation of narciclasine. Depending on protection groups pattern, selectivity between α- and β-face dihydroxylation can be achieved. His studies showed\textsuperscript{102} that unprotected narciclasine (1) as well as acetate protected \textbf{281a} upon different dihydroxylation conditions, including Upjohn and Sharpless asymmetric procedures, provided selective β-face dihydroxylation and yielded 10b-(R)-hydroxypancratistatin (285), Scheme 37. Different attempts to reduce the benzylic hydroxyl upon hydrogenolysis conditions in order to produce 2 did not provide the desired product. Later Pettit\textsuperscript{98} studied radical approaches to reduce thiocarbonate derivative \textbf{283} but instead 10b-(S) \textit{epi}-pancratistatin (284) was produced.
Scheme 37. Selective dihydroxylation and synthesis of 10b-(S) epi-pancratistatin.

Introduction of a more sterically demanding protecting group, such as an acetonide, can alter the selectivity of dihydroxylation preferentially to α-face. Pettit\textsuperscript{101} showed that upon exposure of narciclasine acetonide 279 to Sharpless asymmetric dihydroxylation conditions led to product 286 and its β-diastereomer in the ratio of 2:1, Scheme 38. Deprotection of the major product under acidic conditions led to another hydroxylated analogue -10b-(S)-1-epi-pancratistatin (287). In order to achieve the inversion of the C-1 position in this structure the cyclic sulfate 288 was formed by treatment of 286 with thionyl chloride and subsequent oxidation. Unfortunately, nucleophilic opening of the cyclic sulfate 288 with cesium benzoate at position C-2, afforded only 10b-(S)-1-epi-2-epi-pancratistatin (289).
Scheme 38. Selective $\alpha$-dihydroxylation of narciclasine.

In 2001 Pettit developed a relay synthesis of pancratistatin (2) form narciclasine (1). The first steps of which consisted in stepwise protection of narciclasine by acetonide and acetates to form the protected intermediate 290, Scheme 39. Protected narciclasine was submitted to the epoxidation with $m$CPBA in phosphate buffer. Face selective epoxidation led to epoxide 291, which was in turn submitted to hydrogenation on Pd/C. Unfortunately, this step turned to be a bottleneck of the synthesis and provided a mixture of products, amongst which the desired diol 292 was produced in only 28% yield. This diol was transformed to a cyclic sulfate 293, and then was followed by nucleophilic opening with cesium benzoate and acid deprotection to produce C-1 benzoate of pancratistatin 294. Upon basic hydrolysis the ester was hydrolyzed to produce

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Reaction and conditions: (a) 2,2-DMP, DMF, TsOH, 97%; (b) OsO$_4$, NMO, (DHQD)$_2$PHAL, DMF, H$_2$O 54%; (c) H$_2$SO$_4$, THF, H$_2$O, 52%; (d) (i) SOCl$_2$, Et$_3$N, THF; (ii) RuCl$_3$·3H$_2$O, NaI, MeCN/CCl$_4$/H$_2$O; 63%; (e) (i) PhCO$_2$H, Cs$_2$CO$_3$, DMF; (ii) H$_2$SO$_4$, THF, H$_2$O; (iii) K$_2$CO$_3$, MeOH, 33%.
pancratistatin (2) in 10 steps from narciclasine (1). Despite quite a low yield this study provided essential data about biological activity, since all of the intermediates were subjected to anticancer cell assays. C-1 benzoate ester (294) in particular has shown the highest activity of all known analogues and congeners of Amaryllidaceae alkaloids, see 2.1.4.2.

Scheme 39. Relay synthesis of pancratistatin form narciclasine.

Later the same strategy of nucleophilic opening of sulfate 293 was utilised by Marion from Pierre Fabre Laboratories\textsuperscript{103} to generate a library of nitrogenated derivatives of pancratistatin. Cyclic sulfate 291 was produced the way described by Pettit,\textsuperscript{48} and was subjected to nucleophilic opening by sodium azide, and deprotected to provide 1-azido-
pancratistatin 295 which can, in turn, be reduced to amine 296, Scheme 40. Library of different amides and amines, Figure 15, was generated from 296. Some of these compounds have shown significant biological activity exceeding that of narciclasine itself. Most pronounced activity was shown by compounds with flat lypophylc substituents at position C-1, such as benzamide, see 2.1.4.2.

![Scheme 40. Amino C-1 analogues.](image)

Reaction conditions: (a) (i) NaN₃, DMF, 80°C; (ii) H₂SO₄, THF, 72% for 2 steps; (b) Pd/C, H₂, 52%;

**Figure 15.** Library of amino C-1 derivatives.

Synthesis of two more analogues stemmed from a study on the separation of lycoricidine (10) and trans-dihydrolycoricidine (13) from bulbs of *P. Littorale* by Pettit.⁹⁹ Protection of the chromatographically inseparable mixture of these compounds as acetonides led to the mixture of 301 and 302, Scheme 41. Further protection with *t*-butyldimethylsilyl
group and reduction of amide moiety with lithium alumohydride produced separable mixture of amines 303 and 304. Upon deprotection, the two new analogues without amide moiety 305 and 306 were isolated as their respective hydrochloride salts and subjected to biological studies.

Scheme 41. Pettit’s synthesis of amine analogues.

Kiss104 extensively studied various structural modifications of narciclasine (1) to produce series of 28 diverse analogues. Some of the compounds which have shown anticancer activity are presented on Figure 16. He utilized a previously described strategy in order to generate selectively protected C-2 and C-7 substituted esters 307-309, 4-substituted amides 310, and 2-epi-amines 311, 312. Stability toward hydrolysis as well as anticancer activity of all of these derivatives were studied.

All of the studies presented above show the wealth of methods developed toward the modification and synthesis of different analogues of Amaryllidaceae congeners. Some of
these syntheses demonstrated to be very efficient in terms of the step count and yield, but synthetic efficiency cannot be a single criterion for the estimation of success in the field of synthesis of bioactive molecules. Therefore the next part will be dedicated to discussion of biological activity of produced analogues as well as possible ventures in this field.

Figure 16. Narciclasine semisynthetic derivatives.

2.1.4. Biological studies and search for pharmacophore.

2.1.4.1. Biological effects and mechanism of action.

Members of Amaryllidaceae family have been recognized for a long time in folk medicine for the treatment of cancer related illnesses. Narciclasine was reported by Ceriotti\textsuperscript{105} to have antimitotic activity on a murine sarcoma cell line (Sarcoma 180), as
well as inhibition of growth of wheat grain radicles. Isolation of isocarbostyril family of Amaryllidaceae alkaloids was guided by anticancer assays, mainly by murine P-388 lymphocytic leukemia. These congeners have shown to have ED$_{50}$ values as low as 0.01 µg/ml, they also exhibit activity against a wide panel of different cancer cell lines in vitro, as well as in vivo, particularly against the melanoma sub-family.$^9$

The natural Amaryllidaceae isocarbostyril compounds 1, 2, and 10-13 displayed promising antiviral activity in vitro against flaviviruses such as Japanese encephalitis, Yellow fever and Dengue fever viruses, and bunyaviruses such as Punta Toro and Rift Valley.$^{96,106}$ Furthermore, pancratistatin, narciclasine and 7-deoxypanratistatin have also shown some effect in vivo against mice infected with Japanese encephalitis, which constitutes one of the rare examples of non-immunomodulatory treatment of Japanese encephalitis.$^{96}$ Narciclasine was shown by McNulty$^{107}$ to be an inhibitor of CYP3A4 human cytochrome, while structurally similar trans-dihydonarciclasine does not exhibit inhibitory activity towards several types of human cytochromes. This study can be important for future preclinical development of drugs based on the isocarbostyril pharmacophore. Some antifungal activity of narciclasine$^{101}$ and antiparasitic activity of pancratistatin and 7-deoxypanracilasine$^{108}$ has been observed in vitro, unfortunately all activity in assays had a narrow therapeutic window in comparison with activity of these compounds against cancerous cells. Further discussion will be focused solely on anticancer properties.

Narciclasine and its congeners display several different mechanisms of action against cancerous and leukemia cells studies including apoptosis (programmed cell death), impaired cell proliferation, and decreased cell migration activity.$^{109,110}$ Simultaneously,
these compounds show little or no activity towards healthy cell lines, therefore presenting the possibility that these compounds could be developed into selective anticancer agents and prospective drugs. Naturally, the mechanism of action of these molecules attracted significant attention from the chemical and biological communities, which can serve to deepen our knowledge about cell proliferation and differentiation between healthy and cancerous cells.

Early studies of the mechanism of action of narciclasine performed by Carrasco demonstrated inhibition of eukaryotic ribosomal protein biosynthesis. These studies have been performed on Saccharomyces cerevisiae and rabbit reticulocytes (immature red blood cells). Experiments with $^{14}$C and $^3$H labeled peptides have shown, that narciclasine inhibits poly U-directed phenylalanine synthesis in ribosomes of rabbit reticulocytes as well as polypeptide synthesis in yeast polysomes, without affecting prokaryotic E.Coli cells. Furthermore, narciclasine may interact with peptidyl transferase in an eukaryotic large ribosomal subunit (60S) in the area known as the “anisomycin” area. It competitively binds to the same site as anisomycin (300) and to a lesser extent with trichodermin (301) – another potent protein synthesis inhibitor, Figure 17. Further evidence of a similar mode of action is the fact that a mutant yeast strain with resistance for 300 and 301, also exhibits cross-resistance for narciclasine. Studies of the interaction of narciclasine and its semisynthetic derivatives were performed by Baez and Vasquez with tritium-labeled compounds and further supported specific interaction of narciclasine with the 60-S ribosome subunit.
One of the most interesting effects is the pronounced selective cytotoxicity of isocarbostyril congeners. It has been shown in the independent studies of Pandey and Van Goietsenoven that narciclasine and pancratistatin have a pronounced cytotoxicity and selectively induce apoptosis in cancer cell lines but are 200 times less toxic against normal cell lines. According to Pandey, pancratistatin causes disruption of the mitochondria. He therefore reasoned that pancratistatin targets parts of the mitochondria, and their disruption activates apoptosis. Pandey postulated that the selectivity was caused by the ability of pancratistatin to differentiate between mitochondria of cancerous cells and normal ones.

More recently, Van Goietsenoven proposed that narciclasine (1) targets the ribosomal translation elongation factor eEF1A. This particular factor is responsible for the transport of aminoacyl tRNAs and the nuclear export of proteins. The disruption of eEF1A can cause irreversible damage and promote cell death. This hypothesis was supported by molecular docking studies, as well as binding studies on isolated human recombinant eEF1A and similar yeast elongation eEF2. Also, the fact that cancerous cells overexpress this particular factor can explain selectivity of narciclasine towards cancerous cells.
2.1.4.2. Anticancer activity of diverse analogues of Amaryllidaceae congeners

Initial studies towards refinement of cytotoxic pharmacophore of the Amaryllidaceae alkaloids relied on the comparison between compounds isolated from natural sources and those produced by semisynthesis. Seminal work on the refinement of pharmacophore was performed by Mondon and Krohn\(^7\) which revealed that trans B/C ring juncture is essential for activity; *cis*-dihydronarciclasine (276) is less active by an order of magnitude then *trans*-dihydronarciclasine (12), whilst isonarciclasine (277) was inactive. The methylation of the hydroxyl at position C-7 reduces the activity of narciclasine. Unfortunately, no inhibition concentrations were reported in the article, however these values were presented later,\(^9\) see Table 1, Table 3.

The evaluation of anticancer and antineoplastic activity of natural compounds, semisynthetic and synthetic derivatives were performed by different groups on various cell lines, and the assays were reported with different units of measurement. It was decided to include only compounds, which constitute actual structural analogues, rather than prodrugs which undergo *in vivo* transformation to an active form. One of the most prominent examples are cyclic phosphates synthesized by Pettit,\(^100\) which have shown relatively low inhibitory activity *in vitro* but were much more efficient on live models.\(^116\)

In order to make direct comparison of the results of assays, different values of inhibition from a variety of sources were compiled in a few tables, based on which part of pharmacophore was being altered. Data will be presented in the tables as mean values of all *in vitro* IC\(_{50}^\text{ED}_{50}/\text{GI}_{50}\) values reported for the compound and normalised as \(\mu\text{M}\).
Table 1. Activity of compounds isolated from natural sources.

<table>
<thead>
<tr>
<th>Name of compound</th>
<th>Mean value of IC50/ED50/GI50 (µM)</th>
<th>Difference from parent structure</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Narciclasine (1)</td>
<td>0.0155</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Pancratistatin (2)</td>
<td>0.0955</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td><em>trans</em>-dihydronarciclasine (12)</td>
<td>0.0126</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Lycoricidine (10)</td>
<td>0.145</td>
<td>Lacks 7-hydroxyl</td>
<td>9</td>
</tr>
<tr>
<td>7-deoxypancratistatin (11)</td>
<td>0.100</td>
<td>Lacks 7-hydroxyl</td>
<td>26</td>
</tr>
<tr>
<td><em>trans</em>-dihydrolycoricidine (13)</td>
<td>0.0676</td>
<td>Lacks 7-hydroxyl</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>[313]</td>
<td>0.91 3-hydroxybutric acid on C-1</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>[314]</td>
<td>5.8 3(β-Glucopyranoside)-hydroxybutric acid on C-1</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>[315]</td>
<td>4.3 Epimer on C-3 position</td>
<td>118</td>
</tr>
</tbody>
</table>

The removal of 7-hydroxyl consistently lowers the activity of all congeners by one order of magnitude. Hydroxyl on the position C-1 in pancratistatin is also deleterious to activity in about 5-10 times in comparison with narciclasine and *trans*-dihydronarciclasine. Relatively polar substituents on position 1 such as 3-hydroxybutyryl also lower activity, as well as the alteration of stereochemistry of C-3 hydroxyl.
Table 2. C-ring analogues.

<table>
<thead>
<tr>
<th>Name of compound</th>
<th>Mean value of $\text{IC}<em>{50}/\text{ED}</em>{50}/\text{GI}_{50}$ ($\mu$M)</th>
<th>Difference from parent structure</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="image" />  (\text{(316)})</td>
<td>0.72</td>
<td>Acetate ester on position 2</td>
<td>97</td>
</tr>
<tr>
<td><img src="image2" alt="image" />  (\text{(317)})</td>
<td>1.01</td>
<td>Acetate ester on position 2, 3, 4</td>
<td>97</td>
</tr>
<tr>
<td><img src="image3" alt="image" />  (\text{(318)})</td>
<td>1.93</td>
<td>Acetate ester on position 2, 3, 4</td>
<td>97</td>
</tr>
<tr>
<td>307a</td>
<td>0.51</td>
<td>Acetate ester on position 2</td>
<td>104</td>
</tr>
<tr>
<td>307b</td>
<td>0.17</td>
<td>Benzoate ester on position 2</td>
<td>104</td>
</tr>
<tr>
<td>307c</td>
<td>0.04</td>
<td>Propanoate ester on position 2</td>
<td>104</td>
</tr>
<tr>
<td>307d</td>
<td>0.07</td>
<td>isobutiric ester on position 2</td>
<td>104</td>
</tr>
<tr>
<td>308a</td>
<td>0.75</td>
<td>Acetate ester on position 2, 7</td>
<td>104</td>
</tr>
<tr>
<td>308b</td>
<td>3.4</td>
<td>Benzoate ester on position 2, 7</td>
<td>104</td>
</tr>
<tr>
<td>309a</td>
<td>3.0</td>
<td>$\beta$-Glucoside on position 7</td>
<td>104</td>
</tr>
<tr>
<td>309b</td>
<td>Inactive</td>
<td>$\alpha$-Glucoside on position 7</td>
<td>104</td>
</tr>
<tr>
<td>309c</td>
<td>16.7</td>
<td>Methyl acetate on position 7</td>
<td>104</td>
</tr>
<tr>
<td>309d</td>
<td>15.0</td>
<td>Acetic acid on position 7</td>
<td>104</td>
</tr>
<tr>
<td>309e</td>
<td>7.4</td>
<td>2-methylenenapthalene on position 7</td>
<td>104</td>
</tr>
<tr>
<td>310a</td>
<td>Inactive</td>
<td>Lacks amide</td>
<td>104</td>
</tr>
<tr>
<td>310b</td>
<td>Inactive</td>
<td>Lacks amide, acetate on nitrogen</td>
<td>104</td>
</tr>
<tr>
<td><strong>310c</strong></td>
<td>Inactive</td>
<td>Lacks amide, urea on nitrogen</td>
<td>104</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>-----------------------------</td>
<td>-----</td>
</tr>
<tr>
<td>311</td>
<td>Inactive</td>
<td>2-epi ethylamine</td>
<td>104</td>
</tr>
<tr>
<td>311</td>
<td>Inactive</td>
<td>2-epi ethylamine, cis-B/C junction</td>
<td>104</td>
</tr>
<tr>
<td>294</td>
<td>&lt;0.0023</td>
<td>C-1 benzoate ester</td>
<td>48</td>
</tr>
<tr>
<td>193a</td>
<td>2.3</td>
<td>C-1 homologue alcohol, lacks 7-hydroxyl</td>
<td>79</td>
</tr>
<tr>
<td>192b</td>
<td>0.56</td>
<td>C-1 homologue acetate ester, lacks 7-hydroxyl</td>
<td>79</td>
</tr>
<tr>
<td>193a</td>
<td>Inactive</td>
<td>C-1 carboxylic acid methyl ester, lacks 7-hydroxyl</td>
<td>79</td>
</tr>
<tr>
<td>193b</td>
<td>Inactive</td>
<td>C-1 carboxylic acid, lacks 7-hydroxyl</td>
<td>79</td>
</tr>
<tr>
<td>297</td>
<td>0.018</td>
<td>isobutylamine on C-1</td>
<td>103</td>
</tr>
<tr>
<td>298</td>
<td>0.017</td>
<td>Benzylamine on C-1</td>
<td>103</td>
</tr>
<tr>
<td>299</td>
<td>0.03</td>
<td>Cyclohexylcarboxylic acid amide on C-1</td>
<td>103</td>
</tr>
<tr>
<td>300</td>
<td>0.007</td>
<td>benzoic acid amide on C-1</td>
<td>103</td>
</tr>
<tr>
<td>175a,b</td>
<td>Inactive</td>
<td>open C-ring, lacks C-3 hydroxyls</td>
<td>76</td>
</tr>
<tr>
<td>176</td>
<td>Inactive</td>
<td>Pyranoside replaced C-ring, lacks C-2, C-3, C-4 hydroxyls, C-1 epi</td>
<td>76</td>
</tr>
<tr>
<td>147</td>
<td>Inactive</td>
<td>Lacks C-1,C-2, C-7 hydroxyls</td>
<td>72</td>
</tr>
<tr>
<td>197, 198</td>
<td>Inactive</td>
<td>197 epi C-2, 198 epi C-2, C-4, both lack C-7 hydroxyl</td>
<td>84</td>
</tr>
<tr>
<td>133</td>
<td>Inactive</td>
<td>Lacks C-1,C-3, C-7 hydroxyls</td>
<td>65</td>
</tr>
<tr>
<td>126</td>
<td>2.3b</td>
<td>Lacks C-1,C-4, C-7 hydroxyls</td>
<td>66</td>
</tr>
</tbody>
</table>

a For all tested cell lines except, ED$_{50}$ = 5.0 μM for murine leukemia P388.

b Average for 4 cell lines, results against NCI 60 panel was 2-3 magnitudes lower than 2.
Significant information on the pharmacophore was obtained during biological studies of C-ring analogues. Esterification of hydroxyls at positions C-2, -3, -4 decreases activity by one or two orders of magnitude, depending on substituents. Changes of stereochemistry at positions C-2, -3, and -4 of hydroxyl or absence of any of these groups significantly deteriorates activity, therefore confirming the importance of all three groups in the molecule in a specific stereochemical array. The installation of lipophilic groups at C-1 does not deteriorate activity, whilst polar groups, such as carboxylic group render compounds inactive. The notable example of the beneficial effect of lipophilic groups is C-1 benzoate (294). It is unclear whether ester 294 serves as a prodrug and just delivers pancretistatin to the active site, but the later discovery\textsuperscript{103} of benzamide 300 and benzyl amine 298 with comparable activity shows that this aromatic ring might be a part of the pharmacophore.

Table 3. B-ring analogues.

<table>
<thead>
<tr>
<th>Name of compound</th>
<th>Mean value of IC\textsubscript{50}/ED\textsubscript{50}/GI\textsubscript{50}</th>
<th>Difference from parent structure</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td>Inactive</td>
<td>Aromatic C-ring</td>
<td>77</td>
</tr>
<tr>
<td>\textit{ent-1, ent-10, 167},</td>
<td>Inactive\textsuperscript{d}</td>
<td></td>
<td>119</td>
</tr>
<tr>
<td>\textit{ent-11}</td>
<td>10-fold less active than 11</td>
<td></td>
<td>72</td>
</tr>
<tr>
<td>121</td>
<td>Inactive\textsuperscript{c}</td>
<td>Lacks C-1, C-2, C-3, C-7 hydroxyls</td>
<td>66</td>
</tr>
</tbody>
</table>

\textsuperscript{c} ED\textsubscript{50}>153 \textmu M for murine leukemia P388.

\textsuperscript{d} 0-15\% inhibition at 2\textmu M for 7 cell lines.
<table>
<thead>
<tr>
<th></th>
<th>(µM)</th>
<th>Description</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>292</td>
<td>Inactive&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Lacks amide oxygen</td>
<td>97</td>
</tr>
<tr>
<td>292</td>
<td>13.0</td>
<td>Lacks amide oxygen</td>
<td>97</td>
</tr>
<tr>
<td>293</td>
<td>5.7</td>
<td>Lacks amide oxygen</td>
<td>97</td>
</tr>
<tr>
<td>293</td>
<td>7.7</td>
<td>Lacks amide oxygen</td>
<td>97</td>
</tr>
<tr>
<td>216</td>
<td>Inactive</td>
<td>Nitrogen replaced with oxygen</td>
<td>88</td>
</tr>
<tr>
<td>217</td>
<td>Inactive</td>
<td>Nitrogen replaced with oxygen, epi-stereochemistry of 4a</td>
<td>88</td>
</tr>
<tr>
<td>204</td>
<td>Inactive</td>
<td>No bond 10a-10b</td>
<td>86</td>
</tr>
<tr>
<td>236-238</td>
<td>Inactive</td>
<td>No amide fragment, open B-ring</td>
<td>91</td>
</tr>
<tr>
<td>210</td>
<td>Inactive</td>
<td>10b-epi configuration</td>
<td>26</td>
</tr>
<tr>
<td>222</td>
<td>12.0</td>
<td>Truncated B and C-ring</td>
<td>26</td>
</tr>
<tr>
<td>248a, 248b</td>
<td>Inactive</td>
<td>Truncated B and C-ring</td>
<td>93</td>
</tr>
<tr>
<td>iso-narciclasine (277)</td>
<td>11.8</td>
<td>Double bond 10b-4a</td>
<td>9</td>
</tr>
<tr>
<td>cis-dihydrolycoricidine</td>
<td>95.5</td>
<td>Cis B/C junction, lacks C-7-hydroxyl</td>
<td>9</td>
</tr>
<tr>
<td>cis-dihydronarciclasine(276)</td>
<td>3.8</td>
<td>Cis B/C junction,</td>
<td>9</td>
</tr>
</tbody>
</table>

In order to retain biological activity, the B-ring must remain unchanged. Any alteration in functional groups, such as replacement of amide moiety or truncation of rings led to complete disappearance of anticancer activity. Conversion of stereochemical relation of B/C ring from trans to cis-junction significantly reduces activity and unexpectedly

<sup>e</sup> IC<sub>50</sub> > 36 µM.
isomerisation of position of double bond to 10b-4a in isonarciclasine (277) renders compound inactive.

**Table 4. C-ring analogues.**

<table>
<thead>
<tr>
<th>Name of compound</th>
<th>Mean value of IC&lt;sub&gt;50&lt;/sub&gt;/ED&lt;sub&gt;50&lt;/sub&gt;/GI&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Difference from parent structure</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>254</td>
<td>12.5</td>
<td>Lacks C-7 hydroxyl, oxygen on position C-8</td>
<td>72</td>
</tr>
<tr>
<td>259</td>
<td>60.1</td>
<td>Indole replacement of A-ring</td>
<td>82</td>
</tr>
<tr>
<td>267</td>
<td>Inactive</td>
<td>TMS on position C-8,C-9</td>
<td>95</td>
</tr>
<tr>
<td>274</td>
<td>Inactive</td>
<td>Lacks C-7 hydroxyl, methylene dioxy bridge, hydroxyl on C-8</td>
<td>85</td>
</tr>
<tr>
<td>275</td>
<td>Inactive</td>
<td>Lacks C-7 hydroxyl, methylene dioxy, Carboxybenzyl on C-8</td>
<td>85</td>
</tr>
</tbody>
</table>

These results, however limited, nevertheless show that the significant changes in A-ring lead to decrease or complete disappearance of anticancer activity. Replacement of methylenedioxyarene fragment with bioisostere indole fragment<sup>82</sup> led to three orders of magnitude drop in activity. Removal of any of oxygenated substituents on position C-8/C-9 or replacement with substantially different functional groups also lowers activity. In fact, the only untested position for replacement or introduction of the new functional groups is C-10.

General information on the pharmacophore of the isocarbostyril group of Amaryllidaceae alkaloids with all these studies started to take shape. Two sites for possible modification,
without risk of significantly reducing activity, are positions C-1 and C-10. Therefore our goal is to design new efficient and general ways towards these two types on analogues.

![Figure 18. Model of pharmacophore presented on pancratistatin.]

2.2. Aromatic dioxygenases

2.2.1. Discovery of aromatic dioxygenases.

The majority of animal and fungi organisms are capable of metabolizing aromatic compounds and transforming them to benign compounds *via* trans-diol 320 *via* transient intermediate epoxide 319. It was believed that the bacterial degradation pathway involved the same intermediates, Figure 19. Gibson¹²⁰ was the first to show that some bacterial strains, namely *Pseudomonas Putida* F1, have an alternate aromatic degradation pathway. This pathway involves *cis*-dihydroxylation of the aromatic compounds for example toluene to the *cis*-cyclohexadiene diol (316), followed by oxidation by catecholde hydrogenase to catechol 317 and further degradation to non-aromatic compounds. This bacterial strain is able to utilize aromatic compounds as the sole source of carbon and energy.
Figure 19. Possible oxidation pathways in eukaryotic and prokaryotic organisms.

Several years later, Gibson\textsuperscript{121} created a mutant strain \textit{Pseudomonas Putida} 39/D (\textit{Pp} 39/D), which lacks the enzymes necessary for the oxidative transformation of \textit{cis}-diol \textbf{321} to catechol \textbf{322} and therefore accumulates larger quantities of the transient metabolite \textbf{321}. This unusual type of 3,5-cyclohexadiene 1,2-diols (cyclohexadiene diols) is produced by bacteria with high enantioselectivity (\textgreater{} 98\% \textit{er}). The absolute configuration of \textbf{321} was proven by its oxidative degradation of to the known compounds.\textsuperscript{122} In 1989 Gibson\textsuperscript{123} identified the gene sequence responsible for the production of toluene dioxygenase enzyme (TDO) complex and transferred them into appropriate plasmid for functional expression in \textit{E. Coli}. By designing this engineered strain, few major obstacles of biotransformation were avoided. First, the requirement for presence of certain aromatic molecules such as toluene or chlorobenzene as inducers of TDO expression was removed. The use of inducers with \textit{Pp} 39/D inevitably led to contamination of the desired metabolite with cyclohexadiene diols, derived from inducers. Natural host of enzyme, \textit{Pp} 39/D, has a relatively slow growth and expression rate of required enzyme, which decreases the space/time yield. The recombinant strain \textit{E. Coli} JM109 (pDTG601), was created to overexpress the TDO enzyme upon treatment with sugar β-
isopropylthiogalactopyranoside (IPTG). Creation of this strain allows to streamline the production of cell mass and enzyme complex for biotransformation and significantly increases space/time yield of production of cyclohexadiene diols.

The structure of toluene dioxygenase (TDO) enzyme active site is unknown, even though X-ray crystal structure of the enzyme from the same family naphthalene dioxygenase (NDO) was solved. Nevertheless, because of the large number of metabolites characterized, some empirical rules were established and the stereochemical outcome of dihydroxylation of unsymmetrically substituted arenes can be predicted, Figure 20.

**Figure 20.** Boyd’s model for prediction of regioselectivity of dihydroxylation.

2.2.2. Utilization of diols in the natural product synthesis.

In a little over forty years since the first isolation and identification of stable cis-cyclohexadiene diols, more than 400 substrates produced by the dioxygenase family of enzymes were characterized. Only a small fraction of this library of richly functionalized compounds has been utilized in organic synthesis. All known metabolites and their applications for synthesis have been amply reviewed.

Despite the acknowledgment by the biological community, chemists has been reluctant to include these new fascinating molecules into their manifold of chiral building blocks. The first example of application of benzene cyclohexadiene diol (323) in synthesis was that reported by Taylor from ICI in 1983. He exploited radical polymerization of diene 323,
followed by elimination of diol moiety towards synthesis of a polyphenylene polymer. The same year the synthesis of indigo from indole was reported by Gibson.\textsuperscript{133} This synthesis also proceeded through the intermediacy of arene diol, but it was produced with the help of naphthalene dioxygenase-expressing organism. It is important to note that in both of these syntheses the diol functionality was destroyed in the process of forming product.

The first example of incorporation and exploitation of the diol functionality in synthesis was presented by Ley\textsuperscript{134} almost nine years later after Gibson’s discovery in a short synthesis of pinitol (325). He used the steric hindrance of the protecting groups on the diol for facial selectivity in epoxidation reaction to produce epoxide 324, Figure 21. Selective opening of the epoxide led to the protected tetraol 325, which upon further functionalization provided racemic pinitol (326). The use of the arene diol functionality for steric guidance of further modifications as well as steric and electronic differentiation of alkene would prove to be standard strategies in the applications of these arene diols in organic synthesis.

**Figure 21.** Ley’s synthesis of pinitol.

The next milestone in the application of the arene diols for organic synthesis occurred in 1988. The first utilization of asymmetric arene diol (321) in enantioselective synthesis was performed by Hudlický\textsuperscript{135} in a formal total synthesis of prostaglandin E2
(PGE$_2$, 329), Figure 22. The key step in the synthesis consists of the oxidative cleavage of both double bonds of protected diol 321 by means of ozonolysis. Ketoldehyde 327 was subjected to an intramolecular aldol reaction and provided enone 328, a formal intermediate of prostaglandine E2 (329) synthesis, in only four steps.

![Chemical Structure](image)

**Figure 22.** Hudlický's formal synthesis of prostaglandine E2.

Rich functionality of cyclohexadiene diols can be exploited in many ways toward the synthesis of different classes of complex organic molecules. Key steps of some total syntheses will be presented with the emphasis on the diversity of approaches rather than diversity of targets.

One of the earliest and most explored transformations applied to the cyclohexadiene diols is the use of the diene moiety in the cycloaddition reactions. Gibson$^{121}$ was the first to report cyclohexadiene diols serve as diene components in the Diels-Alder reaction. Hudlický applied this approach extensively to the total synthesis of a variety of natural products such as zeylena,$^{136}$ conduramine A,$^{137}$ lycorcidine,$^{34, 35}$ and narciclasine.$^{26, 127}$ Last three syntheses share common steps, namely the tendency of diol 4 protected with the sterically demanding ketal group, to undergo facial and regioselective [4+2] cycloaddition with nitrosyl dienophiles. When applied to protected diol 330, bicyclic oxazine 331 was formed, reductive cleavage of N-O bond with sodium amalgam of
which was followed by dehalogenation and retention of stereochemistry to provide protected conduramine 332, Figure 23.

\[
\begin{align*}
4 & \overset{\text{acetonide protection}}{\rightarrow} 330 \\
330 & \overset{\text{Hetero Diels-Alder reaction}}{\rightarrow} 331 \\
331 & \overset{\text{amalgam reduction}}{\rightarrow} 332
\end{align*}
\]

Figure 23. Hudlický’s approach towards conduramine-type compounds.

Alternative face selectivity of Diels-Alder cycloaddition reaction can be achieved upon performing reaction on unprotected cyclohexadiene diol. This strategy was utilized by Banwell\textsuperscript{138} in the synthesis of (+)-armillavirin. Diol 321, produced by dihydroxylation of toluene, was exposed to 19 kbar pressure in the presence of cyclopentenone (333) to provide tricyclic adduct 334, Figure 24. Further lengthy modifications provided tricyclic ketone 335, which upon subjection to photochemical conditions yielded the product of [1,3] acyl rearrangement, namely ketone 336, as the major product. Few more transformations yielded natural sesquiterpenoid armillarivin (337).
Figure 24. Banwell’s synthesis of armillarivin.

In addition to Diels-Alder type reactions, cyclohexadiene diols have also been employed in a variety of sigmatropic rearrangements and thermal transpositions, as well facilitated by metals. Two prominent examples of the latter transformations include reductive and oxidative allylic transpositions performed by Micalizio\textsuperscript{139} and Hudlický\textsuperscript{140}.

Micalizio utilized reductive titanium-mediated coupling of alkynes with allylic alcohol towards total synthesis of phorbasin C. The synthetic sequence started from cyclohexadiene diol 4 derived by oxidation of bromobenzene with TDO. Dihydroxylation of the less sterically hindered double bond was followed by radical dehalogenation to provide diol 339, Figure 25. The key step involved the formation of metallacycle 338 from TMS-propyne followed by coordination with allylic hydroxyl in 339 and reductive transposition to provide 1,4-diene 340, which was then transformed into final product (+)-phorbacin C (341).
Oxidative allylic transposition was utilized by Hudlický in a short synthesis of the antiviral drug Oseltamivir. The starting material for the synthesis was diol 342 produced from ethyl benzoate with moderate yield ca. 1g/L. Diol 342 was protected, submitted to cycloaddition to provide upon reduction of the N-O bond the key intermediate allylic alcohol 343, which upon submission to oxidative transposition (Dauben-Michno reaction) mediated by chromium trioxide in acetic anhydride yielded enone 344, Figure 26. Further transformations provided access to the Oseltamivir (345) in only seven one-pot operations.
Cross–coupling was also recognized and utilized as an efficient way to introduce new functionality to the halogen-containing cyclohexadiene diols. One of the most prominent examples is the utilization of iodo cyclohexadiene diol $346$ by Banwell$^{141}$ towards a family of epoxyquinol-based natural products, Figure 27. In order to produce biosynthetic precursors of two complex natural products panepophenatrin ($349$) and hexacyclinol ($350$), iodo cyclohexadiene diol $346$ was submitted to the sequential halobromination, Payne rearrangement, and Mitsunobu reaction to provide the key intermediate $347$. Stille coupling of enone $347$ with different vinylstannanes provided two intermediates $348a$ and $348b$ which upon selective deprotection spontaneously underwent dimerization in the course intermolecular Diels-Alder reaction to form panepophenatrin ($345$) and hexacyclinol ($350$) respectively.

Figure 26. Hudlický’s synthesis of Oseltamivir.
Figure 27. Banwell’s synthesis of panepophenantrin and hexacyclinol.

These examples of the synthetic utility of cyclohexadiene diols only represent a fraction of known syntheses. Cyclohexadiene diols also have been effectively utilized in the synthesis of carbasugars,\textsuperscript{142} morphinan\textsuperscript{143-145} type alkaloids and many other classes of natural products.\textsuperscript{126-131} Many targets attained from cyclohexadiene diols contain richly oxygenated amino cyclitol motif similar to those present in Amaryllidaceae alkaloids and therefore present an ideal starting point to develop a general and divergent strategy towards synthesis of different analogues of narciclasine and pancratistatin.

2.3. Polysubstituted pyridines

Pyridine ring systems are rarely observed in nature with only a limited number of complex natural structures containing a pyridine ring such as vitamin B\textsubscript{6} and nicotin.\textsuperscript{146}
The pyridine scaffold, however is widespread in the pharmaceutical industry.\textsuperscript{147-149} The biological activity of and wide range of applications for pyridine-containing compounds ensures synthetic interest and has led to the development of many approaches towards these fascinating molecules.

General approaches towards \textit{de novo} synthesis of pyridines can be classified into three major categories: (i) cycloaddition, (ii) cyclocondensations, and (iii) rearrangements of different types of heterocycles, as shown in Figure 28. In this chapter the objective is not to give a comprehensive overview of all existing approaches, but the rather attention will be given to the application of more developed approaches to the synthesis of diversely substituted pyridines with the particular emphasis on polyoxygenated pyridines, because of their resemblance to the aromatic A-ring of the Amaryllidaceae alkaloids and hence the relevance to the topic of this dissertation. Some examples of syntheses of natural products containing polyoxygenated pyridine fragments will be presented in Section 2.3.4.

**Figure 28.** General routes towards pyridine ring scaffolds.
2.3.1. Cycloaddition reaction for the synthesis of polysubstituted pyridines

Cycloaddition methods offer many advantages in the synthesis of polysubstituted pyridine systems, since these transformations can be performed with high atom economy and their convergence allows, in principle, the regioselective preparation of pentasubstituted pyridines. One of the most widely used and prominent examples of a cycloaddition reaction is the [4+2] cycloaddition process also known as the Diels-Alder reaction. Nitrogen can be present in either the diene or the dienophile fragment of the ring to be formed and therefore three different retrosynthetic disconnections of the same hetero-Diels-Alder approach to pyridines are shown on the Figure 29. The first and one of the most developed approaches is the utilization of 2-azadiene with different dienophiles. Due to electron-withdrawing nature of nitrogen this reaction usually proceeds as an inverse electron demand Diels-Alder reaction. This reaction can be performed with isolated 2-azadiene but much more common is the application of different heterocycles bearing a 2-azadiene fragment and a leaving group such as 1,2,4-triazine (352) or oxazinone (354). Other versions of hetero-Diels-Alder reactions include the reaction between 1-azadienes (eneimines) and alkene fragments, and diene fragment with azadienophile such as imine. Imine and 1-azadiene fragments can be easily produced in situ from nitrogen nucleophiles and aldehydes, thus rendering these Diels-Alder approaches similar to condensation reactions.
Figure 29. Three different variants of hetero-Diels-Alder approach to pyridine.

The hetero-Diels-Alder reaction was one of the earliest examples of a cycloaddition reaction, and its utility has long been recognized and extensively explored for the synthesis of natural products. One of the first examples of such a transformation was the use of oxazoles as azadiene fragments in what has become known as Kondrat’yeva reaction,\textsuperscript{150} which was extensively used in the synthesis of vitamin B\textsubscript{6}. Researchers from Merck developed their route\textsuperscript{151} starting from 4-methyl-5-ethoxyoxazole (355) which was coupled with maleic anhydride (356), to produce tricyclic anhydride 357, which upon treatment with anhydrous hydrogen chloride underwent ring opening and elimination yielded diester 358 in moderate yield, Scheme 42. Reduction of this pyridine yielded pyridoxine (359).
Scheme 42. Example of pyridine ring construction from the synthesis of vitamin B₆.

A general way of production of such highly functionalised pyridines was used to generate libraries of polysubstituted pyridines by coupling a range of oxazoles with a variety of dienophiles. However, one of the major limitations of this approach are the strict requirements on the substitution pattern of the oxazole ring. This reaction only proceeds successfully if the oxazole contains an electron donating group in the 5-position (usually an alkoxy group) and an alkyl substituent in position 4, therefore severely limiting the substitution pattern of the final pyridine.

Hetero-Diels-Alder reactions based on the use of 1,2,4-triazines as heterodiene fragments constitutes another well-studied approach towards polysubstituted pyridines. Early work in this field was performed by Neunhoeffer, and extensively studied by Boger, and, as a result, this particular transformation is sometimes known as Boger reaction. The major difference in this particular transformation is that the presence of the three nitrogen atoms that make the diene component very electron deficient; as such, this process can be described as an inverse electron-demand Diels-Alder reaction and can be performed with electron-rich dienophiles such as enamines, enol ethers and strained alkenes. The cycloaddition usually occurs regioselectively between the C-3 and C-6 positions of the triazine. An example of such a transformation was utilized by Boger towards the
synthesis of phomazarin (365). The inverse electron-demand hetero-Diels-Alder reaction between the electron poor 1,2,4-triazine 360 and 1,1,2-trimethoxyethene (361) forms the transient bicycle 362, which upon retro Diels-Alder elimination formed azadiene 363 and finally, upon elimination of methanol yielded pyridine ester 364 with good yield, Scheme 43. A major limitation of this approach, however, is the requirement of generating appropriately substituted triazines, which usually requires several steps.156

Scheme 43. Hetero Diels Alder approach towards the key intermediate in the synthesis of phomazarin.

Alternative [4+2] approaches to a pyridine core have also been extensively studied and reviewed.157 It must be noted that unsubstituted 1-azadienes (eneimines) are unstable and require stabilizing groups in order to prevent their tautomerization to the more stable enamines. Therefore, it is usually not eneimines, but eneoximes158 and enehydrazones159 that are used as diene fragments. Other eneimines equivalents have been described160 such as dimethylaminoazadienium iodide 366, which can undergo cycloadditions with
different types of dienophiles, including ketene 367, followed by a elimination of the amine moiety and aromatization to produce substituted pyridone 369, Scheme 44.

Scheme 44. 1-azadienes as cycloaddition partners in the synthesis of heterocycles.

Potent inhibitors of electron-transport mitochondrial chain piericidins A1 (375a) and B1 (375b) and, subsequently, their analogues have been synthesized by Boger\textsuperscript{161, 162} by applying the same strategy of an inverse-demand Diels-Alder cycloaddition of N-sulfonylimine 371, Scheme 45. In order to perform this key transformation, the azadiene fragment was produced from α-carbonylester 370 by mesylation of the corresponding oxime. Reaction of azadiene 371 with tetramethoxyethene (376) led to tetrahydropyridine 372, which upon treatment with Lewis acid eliminated methanol and yielded dimethoxypyridine 373. Further transformation involved a directed ortho-metallation (DoM)/borylation/oxidation sequence to the install hydroxyl moiety, followed by reduction of the ester group and an Appel reaction to form pyridine 374. Coupling this building block to the alkyl side chain and deprotection provided both natural products. The same building block was used towards the synthesis of analogues of these natural products.\textsuperscript{157}
Scheme 45. Boger’s synthesis of piericidins.

Pyridines can also be synthesized by a \([4+2]\) cycloaddition between azadienophiles such as imines and nitriles and dienes.\textsuperscript{163, 164} Additional electronic activation of imines is necessary in order to undergo a Diels-Alder reaction and this is usually achieved by treatment with Lewis acids such as Yb(OTf)$_3$, SnCl$_4$, TiCl$_4$, Et$_2$AlCl, \textit{etc.}\textsuperscript{164} Other possibilities, such as the use of oximes as imine substitutes, were successfully employed by Weinreb\textsuperscript{165} for the synthesis of pyridylacetic acids. Malonoacyloxime 377 was shown to undergo thermal cycloaddition under high dilution to form bicycle 378, Scheme 46.

Base promoted elimination of cyanide from oxime 378 led to aromatization and formation of pyridylacetic acid 379 good yield.
Scheme 46. Weinreb’s synthesis of pyridylacetic acids.

Another attractive and atom-economical approach towards polysubstituted pyridines is their production via [2+2+2] cycloaddition of alkynes and nitriles. In principle, this process is symmetry allowed but negative entropy barriers of approximation of three different fragments and the activation energy makes purely thermal cycloaddition of alkynes and nitriles a rare case. In fact, there is only one example of a metal-free transformation of this type, and it is not an actual [2+2+2] cycloaddition, but rather a case of an intramolecular sequential ene/Diels-Alder reaction. The vast majority of these cycloadditions are performed with a metal catalyst, which assists the pre-coordination of unsaturated fragment, and lowers entropic and enthalpic barriers. The general mechanism of such heterocyclic [2+2+2] cycloaddition is similar to their benzene counterparts. A significant number of reviews on the topic of pyridine formation via [2+2+2] cycloaddition have been published.

The first reported case of a cycloaddition for the synthesis of pyridine was described in 1876 by William Ramsay by passing the mixture of acetylene and hydrogen cyanide through a red-hot iron tube. However modern metal-catalyzed methods allow for this transformation to occur under significantly milder conditions. Rediscovery of this cycloaddition was observed with complexes of cyclopentadienylcobalt by Wakatsaki and Yamazaki, and therefore these complexes are explored often for promoting such
cycloadditions and cobalt is the catalyst of choice because of its low cost, wide scope and substrate tolerance.

The general mechanism of the cobalt-catalyzed cycloaddition was studied by Bönneman\textsuperscript{172,173} and is displayed on Figure 30. Mechanistic details are similar to those in the formation of a benzene ring. The first step generates the catalytically active species \textsuperscript{380} from stable precatalyst CpCoL\textsubscript{2} which coordinates with two alkyne fragments. The coordinated alkynes undergo reversible oxidative coupling with formation of metallocyclopentadiene \textsuperscript{381}. In the last step the unsaturated metallocycle reacts with the nitrile or other alkynes (unproductive pathway). After an insertion (\textsuperscript{383}) or cycloaddition (\textsuperscript{384}) reaction with nitrile, these intermediates undergo a reductive elimination of the reactive metal species and yield the pyridine product \textsuperscript{385}. There are a few other mechanisms that have been proposed, but this particular one is supported by the isolation of reactive intermediates and their independent reaction with alkynes.
Figure 30. Catalytic cycle of Co-catalyzed [2+2+2] cycloaddition.

Although many examples of the cobalt-catalyzed reaction have been reported, the intermolecular regioselective assembly of two unsymmetrical alkynes with a nitrile to yield a single pyridine isomer remains an unsolved synthetic challenge. Major competing side-reactions are the oligomerization and trimerization of the alkynes. This obstacle can be overcome by performing the reaction in the presence of an excess of the nitrile component and using non-terminal alkynes with bulky protective groups, such as trimethylsilyl. A significant problem is the competitive formation of two pyridine regioisomers. Upon the addition of the monosubstituted alkyne to the nitrile it is common to see formation of both 2,4,6-(389) and 2,3,6-substituted pyridines (390), Figure 31. An investigation of different catalysts has been conducted,\textsuperscript{174,175} and it appears that electron-rich ligands provide moderate regioselectivity and poor yields, while electron-poor ligands provide good yields low regioselectivity.
Figure 31. Regioselectivity in the reaction between propyne and propionitrile.

The best way to circumvent this regioselectivity issue is to perform the coupling on tethered α,ω-diynes (391)\textsuperscript{176} or ω-cyanoalkynes (394),\textsuperscript{177} with a third building block (nitrile or alkyne respectively). Therefore the bicyclic products such as 393 and 396 can be produced in a regioselective manner, Scheme 47. The reaction proceeded well with electron-rich alkyl and aryl nitriles, with 17:1 regioselectivity for 387, but unfortunately, the reaction scope is somewhat limited, and electron poor nitriles do not undergo cycloaddition in good yields. In the case of cyanoalkyne 394 only one isomer was formed. The regioselectivity of the pyridine ring formation can be predicted, based on the formation of metallocyclopentadiene intermediate between two alkynes, followed by such addition of nitrile, the nitrogen of the pyridine ring will form a bond with the most hindered substituent of alkynes.
Scheme 47. Regioselectivity of the cycloaddition on tethered systems.

One of the most recent and elegant application of the cobalt-catalyzed cycloaddition was utilised by Siegel\textsuperscript{178} for the synthesis of complanadine A (401). The challenge of this pseudo-dimeric structure was addressed by utilizing a strategy of sequential regioselective additions of TMS-diyne 398 to α,ω-cyanoalkyne 397a, Scheme 48. The first [2+2+2] cycloaddition proceeded under the standard conditions in the presence of one equivalent of cyclopentadienyl cobalt dicarbonyl (CpCo(CO)\textsubscript{2}) in high yield and with excellent regioselectivity (25:1) to provide pyridyl alkyne 399. After completing the first cycloaddition, desilylation of the pyridine ring was performed and the second cycloaddition was observed to have a different regioselectivity, due to the addition of PPh\textsubscript{3} and utilization of a different building block 397b. After further deprotection steps, the synthesis of the final bipyridyl natural product 401 was completed.
Scheme 48. Siegel’s synthesis of complanadine A.

Other building instead of nitriles blocks that can be successfully utilized for the synthesis of pyridines are heteroallenes such as isocyanates. Earl and Vollhardt\textsuperscript{179} applied this particular transformation to the total synthesis of the cytotoxic quinoline alkaloid camptothecin 405. In order to complete the formation of the pyridone ring, protected \(\alpha,\omega\)-isocyanatoalkyne 402 was submitted to a cycloaddition with the silylated pentyne 403 using \(\text{CpCo(CO)}_2\), Figure 32. Regioselective formation of a single pyridone 404 was observed, which upon further modification provided for two formal approaches towards racemic camptothecin (405).
Figure 32. Vollhardt’s synthesis of camptothecin.

The original conditions of the cobalt-catalyzed cycloaddition were somewhat harsh, and consisted of refluxing catalyst CpCo(CO)₂ and the substrate in xylenes.¹⁷¹ Prolonged exposure to heat and the thermal instability of this catalyst was somewhat overcome by making use of excess of the catalyst (up to 100 mol. %) and/or syringe pump addition of a mixture of the substrate and the catalyst to the boiling solvent. For example, Vollhardt’s synthesis¹⁸⁰ of lysergic acid derivatives utilised the cycloaddition of silypropyonamide 407 with indolonitrile 406 as a key strategy, Figure 33. In order for this reaction to proceed successfully 1 equivalent of catalyst was required, prolonged exposure to heat and irradiation led to desilylation and low yield of cycloaddition product, pyridine 408.

Figure 33. Vollhardt’s cycloaddition approach towards lysergic acid derivatives.

As can be seen, a prolonged exposure to the boiling solvent led to a significant decomposition of thermally unstable products, especially silylated pyridines, and
therefore milder ways of activation of the catalyst were sought. Activation of more weakly-bound complexes such as 414 and 415 can be achieved in milder conditions, thus the cycloadditions may be performed in more environmentally benign solvents such as water.181 This approach has been used by Eaton182 to create library of polysubstituted pyridines such as 412, by means of a cycloaddition between nitriles and 1,4-butynediol (410) in water-methanol mixture in the presence of water-soluble catalyst 413, Figure 34.

Figure 34. Cycloaddition reactions in water using new cobalt catalysts.

CpCo(COD) (414) also was shown to be a competent catalyst at room temperature upon irradiation with UV-visible light (350-500 nm).169 Sensitive chiral nitriles183 underwent cycloaddition under these mild conditions without significant racemization. Another approach to improve the yield and reduce the decomposition of sensitive substrates is to shorten the reaction time, which can be achieved by applying microwave conditions.184 Recently, a more stable and efficient catalyst was developed by Malacria and Gandon.185, 186 Most of the cobalt (0) catalytic systems are air-sensitive and therefore require special handling techniques as well as degassed solvents; however, it was discovered that the
complex of cobalt cyclopentadienyl with electron deficient alkenes, such as dimethyl fumarate, namely CpCo(CO)dmfu (415), can efficiently catalyze the transformation under mild thermal conditions and even be recycled after the reaction.\textsuperscript{178}

Other transition metals can also catalyze [2+2+2] cycloadditions and might represent viable alternatives to the widely used cobalt method. These metals, especially ruthenium and rhodium, despite their cost, offer a distinctively different electronic preference for substituents and therefore offer complementary approach to design of reaction. For example, ruthenium (II) catalysts such as Cp*Ru(COD)Cl,\textsuperscript{187-189} and [Cp*Ru(MeCN)\textsubscript{3}]PF\textsubscript{6}\textsuperscript{190} can catalyze the reaction between 1,6-diynes and electron poor nitriles such as cyanoesters, and \(\alpha\)-halonitriles, something which cannot be attained cobalt catalysis. Low valent nickel\textsuperscript{191} and iron\textsuperscript{192} catalysts showed opposite regioselectivity of cycloaddition to that expected from the common cobalt-catalyzed cycloadditions described above. Recent discoveries in the field of cationic rhodium complexes\textsuperscript{193} have shown that the [2+2+2] cycloaddition can be performed at room temperature in the presence of rhodium(I) and BINAP-type ligands. For instance, the regioselective cycloaddition of terminal electron-rich alkynes such as 416 with electron deficient nitriles as well as electron-rich nitriles was discovered by Tanaka,\textsuperscript{194} Figure 35.
One distinctive case of the metal-facilitated [2+2+2] cycloaddition is the use of complexes of low-valent titanium and zirconium for the construction of the pyridine ring. The main difference lies in fact that it is not an actual catalytic process but rather the nucleophilic addition of organometallic reagents to unsaturated carbon-carbon and carbon-nitrogen bonds. Because of different mechanism, this particular type of cycloaddition can result in a stepwise regioselective coupling of three unsymmetrical fragments, something which cannot be achieved selectively with any other transition metal.

Sato\textsuperscript{195} utilized divalent titanium species generated \textit{in situ} and similar to intermediates in the Kulinkovich reaction. Due to high oxophilicity of titanium, it coordinates regioselectively to the alkylpropiolic amide 420 and phenylacetylene 421 to form adduct 422, Scheme 49. Addition of tosylcyanide (423) to the metallo-cyclopentadiene intermediate 422 proceeds smoothly and after series of rearrangements the titanium intermediate 425 can be quenched with a variety of electrophiles, including proton, halogens, or alkyl halides, providing tetrasubstituted pyridines in one step in good yields.
Scheme 49. Sato’s synthesis of polybubstitued pyridine.

A similar case was observed for zirconium facilitated reaction. Takahashi\textsuperscript{196} studied the formation of azametallocyclopentadienes 429 upon treatment of internal alkynes and nitriles with low-valent zirconium species. The formed intermediates undergo coupling with different alkynes in the presence of nickel dichlorodiphosphine to produce pentasubstituted pyridines, Scheme 50. Unfortunately, this reaction was observed only with alkyl- and aryl-substituted alkynes and nitriles.

Scheme 50. Takahashi’s synthesis of pentasubstituted pyridines.
All of these improvements extended the scope of the reaction and allowed for the formation of the pyridine core under much milder conditions and with improved regioselectivity, but nevertheless, cobalt remains the first catalytic metal of choice for the [2+2+2] cycloaddition in the synthesis of complex natural products.

2.3.2. Condensation reaction for the synthesis of polysubstituted pyridines

Historically, a condensation route is one of the earliest to be discovered and the most developed route towards polysubstituted pyridines. Generally it consists of the reaction between carbonyl compounds of different degrees of unsaturation and a nitrogen source such as a primary amine or ammonia. An overview of different retrosynthetic routes is presented in Figure 36. It is not to any extent a comprehensive representation of all existing condensation methods, which can be found elsewhere, but rather examples of syntheses which lead to differently substituted hydroxypyridines.

![Chemical Structures]

**Figure 36.** General condensation strategies towards pyridine core.
The Hantzsch reaction is one of the oldest multicomponent reactions and it consists of a condensation between two 1,3-dicarbonyl fragments 433, ammonia and aldehydes 435, Figure 37. This reaction provide access to a wide range of 1,4-dihydropyridine compounds 435, which can be oxidised to the corresponding pyridines 436. The scope of the classical Hantzsch reaction is somewhat limited by the fact that the substitution pattern of the product will always bear carbonyl/ester substituents at the positions C-3 and C-5.

\[
\begin{align*}
\text{EtO}_2\text{C} & \quad \text{EtO}_2\text{C} \\
\text{CHO} & \quad \text{CO}_2\text{Et} \\
\text{NH}_3 & \quad \Delta \\
433 & \quad 432 \\
& \quad \rightarrow \\
\text{EtO}_2\text{C} & \quad \text{EtO}_2\text{C} \\
\text{CO}_2\text{Et} & \quad \text{CO}_2\text{Et} \\
\text{NH} & \quad [\text{O}] \\
435 & \quad 436 \\
R - \text{alkyl, aryl} & \quad 
\end{align*}
\]

**Figure 37.** Hantzsch synthesis of pyridines.

New modifications of this transformation have been developed to overcome the problem in the synthesis of unsymmetrical pyridines. One way to approach it is to utilize preformed enamines such as β-aminocrotonate 439 and its reaction with ketoester 438 and aldehyde 437 in order to produce dihydropyridine 440 with high yield, Figure 38.
Figure 38. Modified Hantzsch synthesis.

The related Guareschi-Thorpe synthesis is a convenient way to generate polysubstituted 2-pyridones. It is conceptually similar to the Hantzsch pyridine synthesis with the difference that cyanoacetic derivatives are used as one of the building blocks instead of one of the 1,3-dicarbonyls. For example, in order to assemble 2,6-dihydroxypyridine 443, cyanoacetamide (442) was subjected to reflux in the presence of methylacetoacetate(441) and the basic amine in methanol to provide 2,6-dihydroxypyridine 443 in good yield, Figure 39.

Figure 39. Guareschi-Thorpe condensation.

The Friedlander condensation is one of the most common and reliable methods for the preparation of 2-hydroxypyridines. It consists of the condensation between β-aminocrotonate 444 and 1,3-dicarbonyl compounds, Scheme 51. Deshong199 applied this method towards the synthesis of 2,4-dihydroxypyridine 445. The synthesis started from methylacetoacetate 441, which was converted to aminocrotonate 444 upon treatment with
ammonia. The formed enamine 444 was submitted to the reaction with diethylmalonate in basic conditions led to desired dihydroxypyridine ester 445.

![Reaction scheme](image)

**Scheme 51.** Friedlander condensation.

2.3.3. Isomerisation of different heterocycles and acyclic compounds

Oxygen-containing five and six-membered heterocycles such as furans, pyrilium salts and 2-/4-pyrones have been successfully used for the synthesis of pyridines and especially hydroxypyridines/pyridones. Aminolysis of readily available 2-furan carbonyl derivatives of type 446 leads to nucleophilic ring-opening/ring-closure sequence and yields unstable intermediate 449, which upon treatment with acid,200 or prolonged heating201 undergoes by aromatization to 3-hydroxypyridines 450 in moderate yields, Figure 40.
**Figure 40.** Aminolysis of furan derivatives to hydroxypiridines.

An unusual and a little known example of the oxidative rearrangement of furfural (270) to dihydroxypyridine 451 was published by Dallacker,202 Figure 41. Sequential treatment of furfural with one equivalent of bromine, hydrochloric acid, and then one more equivalent of bromine followed by heating with sulfamic acid led to 5-bromo-2,3-dihydroxypyridine (451) in good yield in a one pot-operation.

![Reaction scheme](image)

R\textsuperscript{1}=alkyl, H; R\textsuperscript{2}=aryl, heteroaryl, alkyl

yield 39% for R\textsuperscript{1}=(CH\textsubscript{2})\textsubscript{2}COOH, R\textsuperscript{2}=CH\textsubscript{3}

---

**Figure 41.** Synthesis of 5-bromo-2,3-dihydroxypyridine from furfural.

Six-membered oxygen containing heterocycles such as pyrylium cations, 2- and 4-pyrones also have been successfully used for synthesis of pyridine scaffolds. Different sources of nitrogen can be used for such transformations. Standard aminolysis with methylamine in a sealed tube with methoxy derivative of comenic acid (452) led to 4-
pyridone carboxylic acid 453 in excellent yield. Figure 42. Much milder conditions for nitrogen replacement were developed by Kvita, who observed that upon treatment of 2-pyrone 454 with hexamethyldisilazane (HMDS) as the nitrogen source in the presence of a base at room temperature 2-pyridone 455 can be isolated in good yield without the aminolysis of ester group.

![Diagram of chemical transformations]

**Figure 42.** Transformation of 4- and 2-pyrone.

2.3.4. Selected syntheses of pyridine-containing natural products and biologically active compounds.

Natural products containing polyoxygenated pyridine rings constitute a relatively small group of compounds especially in comparison with isoquinoline and indole alkaloids. In the previous chapters quite a large number of diverse and creative methodologies for pyridine synthesis were presented but the ultimate test for every methodology is its application to total synthesis. However, most of the syntheses of polyoxygenated pyridines do not start with the creation of the pyridine, rather a modification of a simple pyridine building block is utilised instead. This occurs due to a large selection of available pyridine building blocks and the widespread application of simple
commercially available pyridines in medicinal chemistry, fine chemistry, and material science. These demands which renders the *de novo* synthesis of pyridine building blocks impractical.

In this chapter the key steps of syntheses of several pyridine-containing natural compounds will be reviewed, which can serve as an inspiration and a guide for the synthesis of analogues of narcilasine. One family of such compounds is the piericidines antibiotics, potent inhibitors of the mitochondrial respiratory chain in eukaryotic and prokaryotic organisms. One approach towards this family was already described in previous chapter, Scheme 45. A completely different strategy was utilized by Keaton and Phillips,\(^206\) in order to provide access to the key pyridine 460, Scheme 52. 2,3-Dimethoxypiridine (456) was submitted to a DoM/borylation/oxidation sequence, followed by protection to provide trialkoxypyridine 457. Further DoM provided bromopyridine 458, which upon treatment with the hindered base lithium 2,2,6,6-tetramethylpiperidide (LTMP) underwent migration of the halogen atom also known as a halogen-dance.\(^207\) Transient aryl lithium intermediate 459 was quenched with methyl iodide to provide 460 which after further metallation and stannylation yielded key fragment 461. Installation of alkyl side chain by Stille coupling and deprotection yielded 7-desmethylpiericidine A1 (462).
Another family of natural compounds with polyoxygenated pyridines are the atpenins. These compounds exhibit their major biological activity as inhibitors of the mitochondrial complex II of the respiratory chain. The syntheses of this family of natural products by different groups share some similar features, especially the halogen dance, so the seminal paper on synthesis of atpenin B by Queginer\textsuperscript{208} will be omitted and the most recent synthesis of atpenin A5 by Nakamitsu\textsuperscript{209} is described instead. The starting material for this sequence, commercially available 2-chloro-3-hydroxypyridine (463) was methylated and treated with sodium methoxide, followed by standard DoM/borylation/oxydation sequence for the introduction of a hydroxyl, Scheme 53. Iodination of 4-hydroxypyridine 464 was followed by protection to attain iodopyridine 465, which was subjected to a halogen dance reaction in presence of LDA. Product of rearrangement, iodopyridine 466 was treated with the standard \textit{trans}-metallation/borylation/oxydation sequence. It is

\textbf{Scheme 52.} Phillip’s synthesis of 7-desmethylpiericidin A1.
interesting to note, that during the oxidation step the by-product, iodine, performed in situ halogenation of 2-pyridone intermediate to provide iodopyridone 467, which was further protected and functionalised by means of trans-metallation and coupling with aldehyde 469. Final oxidation and deprotection yielded atpenin A5 (470) in a relatively short sequence.

Scheme 53. Nagamitsu`s synthesis of atpenin A5.

As can be seen from the two previous examples and some other syntheses of related compounds\textsuperscript{210, 211} the halogen dance reaction is a strategic reaction for selective functionalization of pyridine rings and a key step in many total syntheses of natural compounds with polyhydroxypyridine fragments. Together the diverse approaches towards de novo synthesis of pyridine core combined with different functionalization tactics present a powerful tool in the design of complex pyridine-containing molecules.
The preceding chapter described previously known approaches towards the synthesis of different *Amaryllidaceae* alkaloids, their structural analogues, the excursion into the field of enzymatic dihydroxylation of arenes, as well as diverse approaches towards syntheses of polysubstituted pyridines. It is hoped that it provided a sufficient overview of the current state of strategies as well as tactics in the field of synthesis of analogues of narciclasine and pacrastistatin and provide the background for the importance of research presented in the next section.
3. Discussion

3.1. Introduction

Natural products have always been an indispensable source and inspiration for new drugs and have produced significant insight into molecular interactions of different biological processes. The Amaryllidaceae alkaloid congeners, in particular the isocarbostyril family, present a good example of a family of bioactive compounds with high activity, a largely unexplored mechanism of action, and a potential as viable drug candidates. Research in this area has been hampered by scarce availability of most of these compounds from natural sources and their poor solubility profiles. While the issue of solubility can be addressed by the synthesis of soluble phosphate prodrugs, the generation of these derivatives still relies on the supply of natural products. Therefore a major goal is to develop a general and divergent approach towards analogues of pancratistatin and narciclasine. In order to perform this task, a few major issues must be addressed. First of all, the synthesis has to provide access to a range of compounds starting from a common starting material. Second, the design of the synthesis needs to be efficient in order to deliver the desired product in the minimum number of steps.

A major theme of the discussion will be focused on our efforts to develop and test a few distinctly different and general approaches to produce analogues of pancratistatin (2) and narciclasine (1) utilising cyclohexadienediol (4) as a common chiral building block. Therefore the discussion is separated into three parts. The first one describes our approach to C-1 homologues of pancratistatin and synthesis of three new compounds (5a-
c), Figure 43. This approach is based on a route previously developed in our group towards C-1 analogues of 7-deoxypancratistatin. Application of a similar strategy, differences and modifications on the way to the three novel analogues and their anticancer activity are described.

Figure 43. Route towards C-1 homologues of pancratistatin.

The second part is focused on the approach towards pyridine analogues of pancratistatin (472) based on the cobalt-catalyzed [2+2+2] cycloaddition reaction. Our investigation of this reaction and attempts to produce and further functionalize analogues based on the skeleton of 472 is discussed.
Figure 44. [2+2+2] cycloaddition approach towards A-pyridine ring analogues.

In the last section a convergent synthesis of different aza-analogues (6, 476, 7) of narciclasine is described, based on the intramolecular Heck coupling of amides 475 and 478. These amides were in turn produced from halopyridine carboxylic acids 474 and 475 and the protected conduramine 473. The main focus of this section will be an development of conditions for the Heck reaction and the synthesis of pyridine building blocks.
Figure 45. Intramolecular Heck approach to heterocyclic narciclasine analogues.

The biological activity of these derivatives as well as new information on the minimal pharmacophore requirements will be discussed as well as the beneficial effect of certain functional groups and new directions for analogues development.

3.2. Synthesis of C-1 analogues of pancratistatin

The Hudlický group has dedicated significant efforts towards the synthesis of Amaryllidaceae constituents and their unnatural analogues for the past 20 years. Recently the group published a series of papers on the synthesis and biological activity of the C-1 homologues of 7-deoxypancratistatin.\textsuperscript{78, 79} Two of these compounds, namely the C-1 hydroxyl (\textbf{183a}) and C-1 acetate (\textbf{183b}) have shown activity against a variety of cancer cell lines comparable to those of the natural congener. It is known that the 7-hydroxy group plays an important role in the cytotoxicity as Amaryllidaceae isocarbostyril congeners with this substituent present are 10-100 times more active towards different
cancer cell lines. The goal of our project therefore was to explore and utilize a similar synthetic strategy for the synthesis of a few new analogues of pancratistatin and study the influence of different substituents in the position C-1 on the anti-cancer activity.

**Figure 46.** General retrosynthetic strategy towards C-1 homologues.

The general strategy of the synthesis is outlined in Figure 46. In order to study different substituents on the C-1 position, alcohol 479 would present an ideal common intermediate. It could be generated from phenanthrene 480 via an oxidative cleavage/oxidative recyclization sequence. Intramolecular opening of aziridine 481 would serve as a good route towards functionalized phenanthrene 479. Generation of this aziridine 481 was envisioned through selective opening of oxirane 175 by alkyne 482. The chiral cyclohexadiene diol 4 which bears all necessary functionality to produce previously reported oxirane 175\textsuperscript{78} and was envisioned as a good starting material for the synthesis.
The synthesis of intermediate 481 began with the production of the first key intermediate, alkyne 482. The shortest and the most convenient way to approach this compound was to start from readily available piperonal (75). Introduction of the hydroxyl group was envisioned via DoM/borylation/oxidation sequence. Several of the total syntheses of pancratistatin and narciclasine have relied on this strategy to introduce the required 7-hydroxyl group.²⁷, ⁴², ⁵² Most of these transformations were performed with tertiary amides of piperonylic acid, which were proven to be cumbersome to reduce to the corresponding aldehydes. In order to shorten the synthetic sequence a directing group was sought, which could be deprotected directly to produce aldehyde under mild conditions. Two different directing group have been described in the literature for direct ortho-metalation of piperonal: 1,3-dimethylimidazolidine 483²¹² and cyclohexylimine 485²¹³.

![Chemical structure](image)

**Reaction conditions:** (a) N,N’-Dimethyl-ethylenediamine, toluene, reflux, 79%; (b) (i) RLi, additive, solvent; (ii) B(OMe)₃, (iii) AcOH, H₂O₂.

**Scheme 54.** Studies of DoM of 1,3-dimethylimidazolidine.

In our hands, intermediate 483 failed to perform the transformation in the previously reported yields, Scheme 54. Different conditions and organometallic reagents for the metallation were tried and these attempts are summarized in Table 5. Aside from the fact that the yields of the desired transformation were low, also the synthesis of intermediate 483 itself was proven to be cumbersome. The original paper reported distillation of
product at low pressure (108 °C/5*10^{-3} Torr); we performed column chromatography, which proved to be impractical on a large scale.

**Table 5. Ortho-metallation conditions.**

<table>
<thead>
<tr>
<th>Conditions,solvent</th>
<th>RLi; eq.; (additive)</th>
<th>Yield,%</th>
</tr>
</thead>
<tbody>
<tr>
<td>−78 °C→r.t. → −78 °C, Et₂O (lit. procedure)</td>
<td>t-BuLi; 2;</td>
<td>30</td>
</tr>
<tr>
<td>−78 °C→−10 °C, THF</td>
<td>t-BuLi; 2</td>
<td>22</td>
</tr>
<tr>
<td>−78 °C→0 °C→−78 °C, Et₂O</td>
<td>t-BuLi; 1.2</td>
<td>20</td>
</tr>
<tr>
<td>−78 °C→r.t., Et₂O</td>
<td>n-BuLi; 1.2 (TMEDA)</td>
<td>26</td>
</tr>
<tr>
<td>−78 °C→r.t., Et₂O</td>
<td>n-BuLi; 2 (TMEDA)</td>
<td>34</td>
</tr>
<tr>
<td>−78 °C→r.t., THF</td>
<td>n-BuLi; 1.2(TMEDA)</td>
<td>30</td>
</tr>
<tr>
<td>−78 °C→r.t., THF</td>
<td>n-BuLi; 2(TMEDA)</td>
<td>39</td>
</tr>
</tbody>
</table>

We tested therefore a second approach with the introduction of a cyclohexylimine auxiliary 485, Scheme 55. Isolation of intermediate 485 did not pose any problems, since it was a solid and was easily recrystallized from methanol. *ortho*-Metallation was performed at -78 °C, and after borylation, oxidation and hydrolysis provided the desired hydroxyldehyde 484.
Scheme 55. Ortho-metallation of cyclohexylimine 485.

Methylation of phenol 484 was performed with dimethyl sulfate in the presence of potassium carbonate, yielding methoxyaldehyde 486, Scheme 56. This compound was allowed to react with carbon tetrabromide in the presence of triphenylphosphine to provide dibromoalkene 487, which after treatment with nBuLi provided desired alkyne 482 in 5 steps from piperonal in good overall yield. The largest scale, this sequence was performed on, provided 40 g of alkyne in a single run.

Scheme 56. Synthesis of alkyne 482.

The chiral epoxyaziridine 175 was prepared by a previously described route developed in the Hudlický group. The product of enzymatic dihydroxylation of bromobenzene (3),
the chiral diol 4, was protected as acetonide 183 and immediately subjected to aziridination protocol under the Yamada-Evans conditions, Scheme 57. Complete facial selectivity was achieved as a result of the steric hindrance of the ketal. The bromine atom was removed under radical reduction conditions with tri-\textit{n}-butyl tin hydride to provide \textit{N}-tosyl aziridine 70 in good yield. Epoxidation of the alkene moiety was performed by treatment with \textit{m}-chloroperbenzoic acid in refluxing dichloroethane. This epoxidation did not provide complete facial selectivity, but instead led to a 3:1 mixture of diastereomers in favour of the desired isomer 184. This ratio was increased by three successive fractional recrystallizations from isopropanol to 6:1-7:1 and produced 10 g of epoxyaziridine 184 in a single run.

\begin{center}
\begin{tikzpicture}
  \node (a) at (0,0) {3};
  \node (b) at (1,0) {4};
  \node (c) at (2,0) {183};
  \node (d) at (3,0) {70};
  \node (e) at (4,0) {184};
  \node (f) at (5,0) {489};
  \node (g) at (6,0) {488};

  \begin{scope}[thick]
    \draw[->] (a) -- node[above] {Br} (b);
    \draw[->] (b) -- node[above] {OH} (c);
    \draw[->] (c) -- node[above] {TsN} (d);
    \draw[->] (d) -- node[above] {O} (e);
    \draw[->] (e) -- node[above] {TsN} (f);
    \draw[->] (f) -- node[above] {O} (g);
  \end{scope}

\end{tikzpicture}
\end{center}

Reaction conditions: (a) \textit{E. coli} JM109 (pDTG601A), 10-15 g/L; (b) (i) 2,2-DMP, \textit{p}-TsOH, acetone; (ii) PhI=NTs, Cu(acac)$_2$, 0 \textdegree C to r.t., 52%; (c) \textit{n}Bu$_3$SnH,THF, AIBN, reflux, 76%; (d) mCPBA, 1,2-DCE, reflux, 95\% \textit{dr} 3:1; (e) fractional recrystallisation from iPrOH.

\textbf{Scheme 57}. Synthesis of chiral epoxiaziridine 184.

The next step consisted of selective nucleophilic opening of oxirane 184 by the alkynylalane formed \textit{in situ} from the anion dervided from alkyne 482 and
dimethylaluminum chloride, Scheme 58. Stringent reaction conditions and carefully controlled temperature had to be used to ensure selective opening of oxirane moiety in the presence of the aziridine ring. The alkyne 490 was somewhat unstable and the yield was significantly increased (from 42 to 70%) if the crude reaction mixture is directly submitted to the next protection step with t-butyldimethylsilyl triflate.

The next step was to perform selective reduction of alkyne 490 to the Z-alkene 481. The original conditions for the reduction of 7-deoxyanalogues78 consisted of reduction with boranes. Unfortunately, reaction of dicyclohexyl borane with alkyne 491 did not lead to the desired product, probably because of the steric hindrance of the methoxy group. Catalytic hydrogenation is one of the most common ways to generate Z-alkenes;214 we discovered that this particular reaction was sensitive to the steric environment of the triple bond, i.e. reduction of the TBS-protected alcohol 491 did not lead to reproducible yields. Much more successful results were observed during the reduction of alcohol 492. Nevertheless, over-reduction of the alkyne posed a significant problem and screening of conditions and solvents for selective reduction was performed.
Scheme 58. Selective opening of epoxide and further reduction.

Lindlar catalyst and palladium on carbon in methanol led to significant over-reduction. Reduction of substrate in the ethyl acetate did not proceed well, but it was discovered that addition of 20 mol. % of quinoline to 20 mol. % Lindlar catalyst and reduction in methanol allowed for selective reduction and nearly quantitative conversion with negligible amounts of by-products was observed. After protection with a tert-butyldimethylsilyl group the key intermediate 481 was submitted to silica-gel catalysed closure, Figure 47. This step consisted of adsorption of compound on pre-dried silica-gel and heating at 120 °C for 24-36 h. It was discovered that traces of quinoline (ca. 5%) from the reduction step led to a significant increase in yield, albeit with a prolonged reaction time. Our hypothesis for this phenomenon is that quinoline neutralises inherent acidity of silica-gel and therefore reduces unwanted decomposition of the acid-labile groups in compound 481.
Figure 47. Solid phase silica-gel-catalyzed closure.

After securing access to phenanthrene 480, our next goal was to perform oxidative cleavage of the olefin bond which upon oxidative cyclization, was expected to form hemiaminal 494, Figure 48. The original conditions developed for 7-deoxypancratistatin analogues for cleavage of this type of bonds (OsO₄/IO⁴⁻) did not lead to a clean transformation and therefore different conditions were screened.

Figure 48. Oxidative cleavage and recyclization of phenathrene 480.

Ozonolysis is one of the most convenient ways to cleave double bonds. But an important issue is the selectivity of double bond oxidation using ozone. Electron–rich aromatic rings are prone to oxidation by ozone and therefore standard conditions of ozonolysis, i.e. the appearance of a blue colour of excess of ozone in solution, are too destructive for the selective transformation. Therefore, selective indicator such as Sudan Red 7b²¹⁵ was
introduced as an indicator to ensure selective cleavage of the alkene. Reductive quenching was performed with sodium borohydride to provide a mixture of diol 495 and hemiaminal 496 in 2:1 ratio, Scheme 59. It was found that this particular reaction turned out to be sensitive to the solvent; upon performing the reaction in methanol significant decomposition to polar by-products was observed. Transformation of diol 495 to hemiaminal 496 was investigated next. Screening of different conditions for selective benzylic oxidation was performed. The only conditions that led to good conversion to hemiaminal 496 were the use manganese dioxide in dichloromethane. The next goal was to oxidize the hemiaminal moiety to an amide to complete the skeleton of the desired target. It required selective protection of the primary alcohol in 496 in the presence of the hemiaminal, which was achieved by reaction with acetic anhydride with pyridine in dichloromethane and yielded a mixture of acetate protected anomeric hemiaminals 497 in 87% yield. Different oxidation conditions were tried, including IBX, Dess-Marin periodinane, PDC, but the best yield was obtained with the milder oxidation conditions of tetrapropyl ammonium perruthenate and N-morpholine oxide, also known as the Ley-Griffith oxidation. Desired amide 498, the common intermediate for the synthesis of all C-1 homologues of pancratistatin, was isolated with 84% yield.
Reaction conditions: (a) (i) O₃, Sudan red 7b; (ii) NaBH₄, 60% of 495, 31% of 496; (b) MnO₂, CH₂Cl₂, 87%; (c) Ac₂O, pyridine, CH₂Cl₂, 87%; (d) TPAP, NMO, CH₂Cl₂, 84%.

**Scheme 59.** Conversion of phenanthrene 480 to amide 498.

Having secured access to this fully protected C-1 acetoxymethyl pancratistatin, deprotection steps were studied. Detosylation of 498 was performed by means of reductive cleavage with sodium napthalenide to provide amide 499 in 62% yield, alcohol by-product 479 was also isolated in small quantities from this reaction, Scheme 60. Screening of standard demethylation conditions were performed, including sodium thiolate, boron tribromide and lithium chloride; only the latter conditions provided the desired phenol 500 successfully. Deprotection of the acetate group in 499 was also studied in order to provide access to a different ester substituent on position C-1. Removal of acetate group proceeded smoothly upon treatment of 499 with a concentrated aqueous solution of sodium hydroxide in methanol.
Removal of the silyl group did not pose any problems and was performed under standard conditions with tetrabutyl ammonium fluoride (TBAF). The last step, however, posed some difficulties, since the previously reported conditions for selective deprotection of acetonide led to deprotection of the acetate as well and formation of C-1 pancratistatin homologue 5a, Scheme 61. Treatment of acetonide 501 with trifluoroacetic acid at 0 °C provided C-1 acetate 5b in excellent yield.

**Scheme 60.** Production of common intermediate 479.
Scheme 61. Deprotection sequence towards C-1 pancratistatin homologue 5a and acetate 5b.

The next goal was to produce the benzoate ester of C-1 pancratistatin homologue, since it has been known that this particular group plays a beneficial role in anticancer activity of Amaryllidaceae congeners. Benzylation of alcohol 479 was performed with benzoyl chloride and provided ester 503, which was submitted to deprotection conditions, similar to those employed before for C-1 acetate 5a, to yield C-1 benzoate 5c in three steps, Scheme 62. Synthesis of the three analogues was performed with 17 steps in the longest linear sequence. Biological studies of these three analogues 5a, 5b, 5c were performed on a panel of cancer cell lines and will be discussed in Section 3.5.

Reaction conditions: (a) TBAF, THF, 0 °C, 95%; (b) 3% HCl, MeOH, 92%; (c) TFA, CH₂Cl₂, 90%.
After performing the synthesis and anticancer evaluation of the C-1 analogues, our attention was drawn towards the synthesis of much less explored analogues of the A-ring of pancratistatin. Since it is common practice to utilise a pyridine fragment as a bioisostere of a benzene ring for improving pharmacokinetics, we decided to design a general route towards pyridine analogues of pancratistatin. Our attention was drawn to a [2+2+2] cycloaddition strategy of pyridine ring formation, since it offers general...
versatility and has been shown to work successfully in the synthesis of similar bis-silylated analogues of pancratistatin.\textsuperscript{94,95}

The general retrosynthetic scheme is outlined on Figure 49. \textit{bis}-Silylated pyridine 472 was considered the most convenient intermediate to provide access to polyoxygenated pyridine analogue 506 through oxidation reactions. Synthesis of the key heterocyclic intermediate 472 was envisioned via a cobalt-catalyzed [2+2+2] intermolecular cycloaddition between \textit{bis}(trimethylsilyl)acetylene (269) and nitrile 471. This cyanoalkyne 471 in turn can be attained by functionalization of aziridine 70 by means of nucleophilic opening, selective dihydroxylation of the double bond, and alkylation of the amide with the nitrile fragment. Aziridine 70 is a common intermediate in the synthesis of Amaryllidaceae alkaloids in the Hudlický group and was generated in three steps from cyclohexadiene diol 4.

\textbf{Figure 49.} Retrosynthetic scheme for the [2+2+2] strategy of pyridine ring analogues.
Synthesis of aziridine 70 was discussed in the previous Section 3.2, Scheme 57. Aziridine 70 was submitted to a protocol previously developed in the Hudlický group\textsuperscript{92} of nucleophilic opening of the aziridine moiety with a large excess of alkynylalane generated \textit{in situ} from lithium trimethylsilyl acetylide and aluminium chloride, Scheme 63. Simultaneously, the acetonide was hydrolyzed during this reaction, which required reprotction to provide tosylamide 508 in good yield. Selective dihydroxylation of the alkene fragment in the presence of the alkyne was performed by treatment with osmium tetroxide in presence of N-methylmorpholine N-oxide. This reaction was performed in dichloromethane, unlike the usual conditions,\textsuperscript{220} which involve polar solvent system. These were the only conditions that provided a reasonable conversion without significant decomposition of the product 509. Benzoylation of diol 509 provided protected alkyne 510 in good yield.

\begin{center}
\includegraphics[width=\textwidth]{Scheme63.png}
\end{center}

\textbf{Scheme 63.} Synthesis of alkyne 510.
Our next goal was to study the alkylation of the amide fragment in tosylamide 510 to yield α,ω-cyanoalkyne 471, Scheme 64. Conditions originally developed on the Hudlický group\textsuperscript{94, 221} for the synthesis of dialkyne 264 consisted of desilylation with a non-basic reagent, tetrabutyl ammonium triphenyldifluorosilicate (TBAT), followed by alkylation. In this particular case two different routes were studied: (i) desilylation of amide 510 followed by alkylation with chloroacetonitrile in the presence of sodium bis(trimethylsilyl)amide or (ii) alkylation of amide 510 with chloroacetonitrile followed by desilylation of formed α,ω-cyanoalkyne 512. The second route was shown to be more efficient in terms of cleaner conversion, and overall yield.

Scheme 64. Synthesis of key intermediate 471.

Having secured the access to the key intermediate α,ω-cyanoalkyne 471, the cobalt-catalyzed cycloaddition with bis-trimethylsilylacetylene (269) was studied, Scheme 65. In order to prevent side-reactions, such as the unproductive cycloaddition of two molecules
of 471 and decomposition of the labile precatalyst CpCo(CO)$_2$ the reaction was performed by slow addition of the solution of 269, 471 and CpCo(CO)$_2$ to the refluxed solution of 269 and CpCo(CO)$_2$ in xylenes under irradiation with visible light. Syringe pump addition over long periods of time (36 h), as was used in the previous approaches,$^{94,221}$ did not lead to better results.


Figure 50. Two required oxidation transformations of 472.

In order to install the functional groups required to mimic pancratistatin (2) in the tricyclic pyridine 472 two different oxidations were required to occur. First of all, replacement of the silyl groups by hydroxyls was envisioned. This approach was based on the fact that silicon group can serve as a masked oxygen and can be replaced in the well-known Tamao-Fleming oxidation.$^{222}$ A variety of different conditions was attempted, see Table 6, but unfortunately these conditions did not lead to the formation of
the desired product 513, the only isolated products were either remaining unreactive starting material or formation of protodesilylated pyridine 515 (by $^1\text{H}$ NMR), Figure 51.

![Chemical structure of 472 and 515](image.png)

**Figure 51.** Tamao-Fleming oxidation of bis-silylated pyridine 472.

It has also been observed that upon prolonged exposure to air of sample pyridine 472 the same by-product 515 started to form. All of these observation and some precedents from the literature\(^ \text{223} \) on silylated pyridines led to the conclusion that the trimethylsilyl group is not a viable group for performing the transformation to the hydroxyl functionality.

**Table 6.** Conditions for the oxidations of silyl groups (Tamao-Fleming reaction).

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Solvent</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>KF, $\text{H}_2\text{O}_2$, NaHCO$_3$</td>
<td>THF</td>
<td>472 (SM)</td>
</tr>
<tr>
<td>KF, $\text{H}_2\text{O}_2$, NaHCO$_3$</td>
<td>THF/MeOH</td>
<td>515</td>
</tr>
<tr>
<td>KF*HF, $\text{H}_2\text{O}_2$</td>
<td>MeOH</td>
<td>515</td>
</tr>
<tr>
<td>KF*HF, $\text{H}_2\text{O}_2$, NaHCO$_3$</td>
<td>MeOH</td>
<td>515</td>
</tr>
</tbody>
</table>

Further studies on the model pyridine 472 were performed in order to establish general conditions for benzylic oxidation. The first conditions attempted were those previously
established in the Hudlický group for a similar system\textsuperscript{93, 216} with catalytic ruthenium trichloride and sodium periodate in biphasic solvent mixture, Figure 52. Unfortunately no reaction occurred under this conditions. The second approach was based on well-known\textsuperscript{224} selenium dioxide oxidation of benzylic positions. But upon prolonged reflux with SeO\textsubscript{2} in ethyl acetate only monodesilated pyridine \textbf{515} was isolated.

\begin{center}
\begin{tikzpicture}
\node (1) at (0,0) {472};
\node (2) at (2,0) {514};
\draw[->,dashed] (1) -- (2);
\end{tikzpicture}
\end{center}

Reaction conditions: (a) RuCl\textsubscript{3}, NaIO\textsubscript{4}, CCl\textsubscript{4}/MeCN/H\textsubscript{2}O; (b) SeO\textsubscript{2}, EtOAc, reflux.

\textbf{Figure 52}. Attempts to oxidise benzylic position in \textbf{472}.

Our next attempt was driven towards oxidation of anionic intermediate \textbf{516}, which was envisioned to form upon treatment of pyridine \textbf{472} with a strong base such as LDA, Figure 53. Different electrophiles were used and the scope of them is presented in Table 7.
Figure 53. Deprotonation and functionalization of pyridine 472.

The first attempt at oxidation was performed by molecular oxygen, however, no reaction was observed. The same results were observed with oxaziridine 517. In order to observe some incorporation of deuterium, anion 516 was quenched with deuteromethanol, but the only isolated product was the debenzoylated pyridine 519. Finally, in order to prove the formation of the transient anion 516, the reaction was performed with TMSCl, the reagent known to trap anions in situ, but once again only starting material was recovered.

Table 7. Attempts to functionalize benzylic position in pyridine 472.

<table>
<thead>
<tr>
<th>Electrophile</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂</td>
<td>No reaction</td>
</tr>
<tr>
<td>TsN&lt;sub&gt;Ph&lt;/sub&gt;</td>
<td>No reaction</td>
</tr>
</tbody>
</table>
The last attempt to transform the benzylic position was envisioned via base-induced elimination of \( p \)-toluenesulfinate to form imine \( 518 \), which was expected to serve as a precursor for the formation of amide group, Figure 54.

![Chemical structures](image)

**Figure 54.** Base-induced formation of imine \( 518 \).

The reaction was expected to proceed at high temperatures, so the base of choice had to be thermally stable and easy to remove upon purification. We decided to use KO\( \text{tBu} \), which can be easily sublimed and has the basicity sufficient to facilitate such rearrangement. Since the benzoates protecting groups in the pyridine \( 472 \) were incompatible with strong base such as KO\( \text{tBu} \), exchange of protection group was performed in a short sequence, Scheme 66. Deprotection by K\( _2 \)CO\( _3 \) in methanol afforded diol \( 519 \), followed by reprotection with the acetonide group provided \( \text{bis}-\text{acetonide pyridine 520} \), Scheme 66. Treatment of this compound with KO\( \text{tBu} \) did not lead to formation of the imine but rather desilylation and elimination of the acetonide group was observed.

In order to suppress these unwanted reactions, desilylation of 520 was performed and isolated pyridine 521 was submitted to an NMR scale reaction in the presence of CD3ONa. Formation of a new peak was observed in the alkene region, characteristic for the C-1 position, therefore we concluded that elimination of the acetonide occurred before elimination of p-toluene sulfinate on the NMR time scale. At this point it became clear that the cycloaddition route we pursued, requires major revisiting. Tricyclic alkene 522, which was observed in minute quantities shares a similar structural pattern to narciclasine (1), so we decided to focus our attention on the synthesis of A-ring aza-analogues of narciclasine following a different route.
Figure 55. Base-induced elimination of acetonide in pyridine 521.

3.4. Synthesis of pyridine analogues of narciclasine via intramolecular Heck approach

Our plan was to gain access to narciclasine aza-analogues in a convergent and short way, to produce several analogues having different position of nitrogen in the aromatic A-ring, and to study their biological activity. Our first goal was to design and perform the synthesis of 7-aza-8,9-dideoxynarciclasine 6 and its N-oxide 476, which was of particular interest, since we thought, that N-O moiety can serve as a bioisostere for the hydroxyl in position C-7 of narciclasine and improve water solubility. After testing the validity of this approach, the synthesis of the more complex analogue 10-azanarciclasine (7) would be performed.

Figure 56. Aza-analogues of narciclasine.
3.4.1. Synthesis of 7-aza-8,9-dideoxynericlasine.

In order to synthesize the desired analogues, we proposed to use an intramolecular Heck approach as one of the shortest and the most general approaches towards the narciclasine skeleton. Synthesis of both pyridine 5 and N-oxide 476 was envisioned via intramolecular coupling of tertiary amide 475, which in turn was expected to be produced by coupling of 3-iodopicolinic acid 474 and protected conduramine 473, Figure 57. Cyclohexadiene diol 4 was envisioned to be an ideal starting material for synthesis of conduramines such as 473.

Figure 57. Retrosynthetic scheme towards 7-aza-8,9-dideoxynarciclasine 6.

One of the most convenient ways to access halopyridinecarboxylic acid derivatives is to produce them via directed ortho-metallation (DoM).\(^{225}\) In general, it requires the formation and hydrolysis of a strong directing group, such as a tertiary amide, which lengthens the synthesis. Nevertheless, it was observed by Queginer\(^{226}\) that in some cases unprotected pyridinecarboxylic acid can serve as a suitable substrate in DoM reaction with lithium tetramethylpiperidide (LTMP) as a base. This particular ortho-metallation
proceeds in good yield, but transformation of lithium 3-iodopicolinate (524) to the corresponding acid was reported to be surprisingly low-yielding, Scheme 67. This low yield can be attributed to the inherent instability of the free acid; in our hands samples of acid 474 produced by this procedure discoloured and started to decompose significantly upon standing at room temperature for a few days. Carson\textsuperscript{227} modified this procedure in order to isolate pure lithium salt 524 in high yield. Since carboxylates can serve as a partners in amide coupling reactions\textsuperscript{228} and salt 524 was significantly more stable than acid 474, we decided to use it as a precursor for the coupling.

\begin{equation}
\begin{array}{c}
\text{O} \\
\text{=}
\end{array}
\begin{array}{c}
\text{N} \\
\text{H}
\end{array}
\begin{array}{c}
\text{H}
\end{array}
\begin{array}{c}
\text{O}
\end{array}
\begin{array}{c}
\text{H}
\end{array}
\begin{array}{c}
\text{I}
\end{array}
\begin{array}{c}
\text{Li}^+
\end{array}
\begin{array}{c}
\text{O}
\end{array}
\begin{array}{c}
\text{H}
\end{array}
\begin{array}{c}
\text{N}
\end{array}
\begin{array}{c}
\text{H}
\end{array}
\begin{array}{c}
\text{O}
\end{array}
\begin{array}{c}
\text{H}
\end{array}
\begin{array}{c}
\text{I}
\end{array}
\end{equation}

\text{Reaction conditions: (a) (i) 2 eq. LTMP, 1 eq. nBuLi, -10 °C; (ii) I}_2, 90%; (b) Amberlyst-15, MeOH, 35%.

\textbf{Scheme 67.} Directed ortho-metallation of picolinic acid.

Our next goal was to produce the amine partner for coupling. Hudlický and Olivo\textsuperscript{137} developed an efficient route towards this type of compounds with effective utilisation of hetero-Diels-Alder reaction of dienes with nitroso compounds formed \textit{in situ}. The synthesis started with the formation of the acetonide protected cyclohexadiene diol 525 which, without purification, was submitted to Diels-Alder reaction with the nitroso compound generated from \textit{t-}butyloxycarboxhydroxamic acid (BocNHOH)\textsuperscript{229} to yield oxazine 526 with good yield, Scheme 68. Reduction of bicyclic oxazine 526 with sodium amalgam provided alcohol 527 with all stereocenters in a configuration identical to narciclasine. Further protection of the alcohol group was performed with TBSCI and imidazole in CH\textsubscript{2}Cl\textsubscript{2} to provide completely protected conduramine 528. Unfortunately
several conditions for the amide coupling including CDI, DCC/HOBT and SOCl₂/Et₃N did not lead to coupling of amide 528 to 3-iodopicolinic acid 474. Therefore selective deprotection of the tert-butyloxycarbamate group was performed with trifluoroacetic acid to yield the free amine 473.

**Scheme 68.** Synthesis of protected conduramine 473.

Coupling of amine 473 with lithium carboxylate 524 was achieved under standard conditions with HBTU and diisopropylethylamine (DIPEA) in dry DMF, Scheme 69. The moderate yield of this reaction can be attributed to the steric hindrance of the iodo group and the generally lower reactivity of salts in coupling reactions. The next step consisted of transforming the secondary amide 529 to the protected amide 475 and this reaction proceeded in high yield.

In all published intramolecular Heck approaches to the narciclasine or lycorcidine skeleton\(^{32, 34, 36, 39, 40}\) the actual transformation is performed on a tertiary amide or imide and salts of silver or thallium are used as a base in order to facilitate the cationic pathway.\(^{230}\) We decided to focus our attention on silver salts, since thallium salts are extremely toxic and less desirable for production of pharmaceuticals. Screening of different conditions for Heck coupling of amide 475 to naphthyridinone 530 was performed, Figure 58.

Figure 58. Heck intramolecular coupling.

Different phosphines, solvents, and bases were tried and the best yield was obtained in toluene in presence of dppe, Table 8. This reaction seems to be especially sensitive to the phosphine ligands, which is in accordance literature precendent that indicates bidentate phosphines tend to perform well in cationic pathway.\(^{230}\)
Table 8. Screening of Heck conditions.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd(dppf)₂Cl₂, dppf, Ag₃PO₄ dioxane, 60°C, 24 h</td>
<td>No reaction</td>
</tr>
<tr>
<td>Pd(OAc)₂, dppe, Ag₃PO₄, Et₃N toluene, 80°C, 18 h</td>
<td>Traces of 530</td>
</tr>
<tr>
<td>Pd(OAc)₂, PPh₃, Et₃N, AgNO₃, MeCN, 24 h, r.t to 80°C</td>
<td>No reaction</td>
</tr>
<tr>
<td>Pd(OAc)₂, dppe, AgNO₃, Cs₂CO₃, toluene, 24 h, 110°C</td>
<td>530, 35% (45 % brsm)</td>
</tr>
<tr>
<td>Pd(OAc)₂, BINAP, Ag₃PO₄, Et₃N toluene, 80°C, 18 h</td>
<td>No reaction</td>
</tr>
</tbody>
</table>

After developing the conditions for the key cyclization, our attention was driven towards deprotection of naphthyridinone 530 to desired compound 7-azanarcyclasine 6, Scheme 70. Desilylation was performed under standard conditions with TBAF, followed by acidic deprotection of the acetonide and t-butyloxycarbamate group to attain the hydrochloride of the desired 7-aza-8,9-dideoxynarciclasine 6·HCl. Upon reaction neutralization with concentrated ammonia and column chromatography was isolated free base 6.

![Reaction conditions: (a) TBAF, THF, 0°C, 77%, (b) HCl, MeOH, quant, (c) NH₃](image)

**Scheme 70.** Deprotection of naphthyridinone 530.
Study of the formation of N-oxide 476 from pyridine 6 was performed as shown Figure 59. Various conditions were tried\textsuperscript{231} and the reaction turned out to be sensitive not just to the oxidant but also for the solvent in which reaction was performed, Table 9. The best conditions for transformation were found to be in use of recrystallized \textit{m}CPBA as an oxidant in a mixture of dichloromethane and methanol. Conversion was slow and the reaction was low-yielding in general. Surprisingly enough, the solubility of pyridine 6 in water was observed to be much higher than corresponding N-oxide 476. Biological studies were performed on 7-azanarciclasine 6, the corresponding hydrochloride, and N-oxide 476, and will be discussed in Section 3.5.

![Chemical Structures](image)

**Figure 59.** Selective oxidation of pyridine 6.

**Table 9.** Conditions screening for formation of N-oxide 476.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{m}CPBA, CH\textsubscript{2}Cl\textsubscript{2}</td>
<td>decomposition</td>
</tr>
<tr>
<td>\textit{m}CPBA, CH\textsubscript{2}Cl\textsubscript{2}:MeOH=5:1</td>
<td>Traces of 476 and inseparable impurities</td>
</tr>
<tr>
<td>\textit{m}CPBA, CH\textsubscript{2}Cl\textsubscript{2}:MeOH=10:1</td>
<td>Very slow conversion</td>
</tr>
<tr>
<td>\textit{m}CPBA, CH\textsubscript{2}Cl\textsubscript{2}:MeOH=8:1</td>
<td>20% conversion to 476 in 48 h, 25% in a 120 h</td>
</tr>
<tr>
<td>TFA, H\textsubscript{2}O\textsubscript{2} 30% aq.</td>
<td>Decomposition</td>
</tr>
<tr>
<td>Reagents</td>
<td>Reaction Outcome</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>TFA, UHP (urea*H$_2$O$_2$), CH$_2$Cl$_2$</td>
<td>No reaction</td>
</tr>
<tr>
<td>UHP, CH$_2$Cl$_2$</td>
<td>No reaction</td>
</tr>
<tr>
<td>TFAA, UHP, CH$_2$Cl$_2$:MeOH</td>
<td>Traces of 476 and inseparable impurities</td>
</tr>
</tbody>
</table>

3.4.2. Synthesis of 10-azanarciclasine

After developing a short route to heterocyclic analogues of narciclasine we decided to test this approach on the synthesis of more complex target, namely 10-azanarciclasine (7). For the synthesis of 7 the convergence of our previous approach allowed to use of the same intramolecular Heck approach from amide 478, and use of the same conduramine 473 as a precursor for the C-ring, Figure 60. The only challenge was to develop a short and efficient synthesis of bromonicotinic acid 477. We envisioned that this acid could be obtained by submission of bromopyridine 532 to the condition of the halogen dance or halogen scrambling with strong base, followed by quenching of the anionic intermediate with CO$_2$. Directed ortho-metallation of dioxolopyridine 533 followed by a borylation/oxidation/methylation sequence should provide access to bromopyridine 532. In turn, the synthesis of pyridine 533 from furfural in two step was already published. 202
Figure 60. Retrosynthetic analysis for the synthesis of 10-azanarciclasine (7).

The key intermediate for this synthesis had to meet two requirements: (i) the presence of 2,3-dihydroxyl moiety in the pyridine ring, (ii) to be accessible in a short synthetic route from a common chemical. Synthesis of 6-bromo[1,3]dioxolo[4,5-b]pyridine (533) was published by Dallacker\textsuperscript{202} complied with both of these conditions. In our hands, the intermediate 2,3-dihydroxy-5-bromopyridine (534) was obtained by treatment of furfural (270) with bromine and sulfamic acid under acidic conditions in a 65\% yield, slightly higher than in the original publication (48\%), and after treatment with CH\textsubscript{2}Br\textsubscript{2} a methylenedioxy bridge was installed in low yield. This yield, while low, is in accordance with limited examples of similar reactions in the literature.\textsuperscript{232}
Scheme 71. Synthesis of intermediate 533.

Our next goal was to synthesize 6-bromo-7-methoxy[1,3]dioxolo[4,5-b]pyridine (532) from pyridine 533, Scheme 72. The most logical way was to perform the sequence DoM with LTMP, followed by borylation with B(OMe)_3 and in situ oxidation with the urea-hydrogen peroxide complex. It is worth mentioning, that this oxidation is sensitive to moisture and in the presence of usual oxidants for such transformation, such as 30% aq. H_2O_2, led to complete protiodeborylation and only starting material 533 was isolated. Transient 4-hydroxypyridine 536 was not isolated but instead submitted directly to the methylation step without purification. In order to perform selective O-alkylation, diazomethane was used, since reaction did not proceed selectively with dimethylsulfate, methyl iodide, and Meerwein salt. The desired methoxypyridine 532 was isolated in a moderate yield and despite the persisting presence of unidentified impurity was successfully employed in the next steps.

Scheme 72. Synthesis of intermediate 532.
Having secured access to the key intermediate 532, the next goal was to study the halogen dance reaction of this bromopyridine upon treatment with LTMP, Figure 61. In order to provide evidence of formation of aryllithium intermediate 537 it was quenched with two electrophiles, methanol and DMF to provide isomeric halopyridines 538 and aldehyde 539 respectively. Spectroscopic NMR characteristics of 538 and 539, were sufficient to provide the definitive proof of structure of regioselective formation of aldehyde 539.

![Figure 61. Halogen dance reaction.](image)

The halogen dance reaction on similar systems has some precedence in the literature, especially in the field of total synthesis of polyoxygenated pyridine natural products (see section 2.3.4). However, it is worth mentioning that all published procedures report the direct addition of the substrate to the organometallic reagent (LTMP). In our hands these conditions did not provide the desired product, but at low temperature (~90 – ~80°C) led to simple deprotonation of α-position, and at higher temperatures (~75 – ~70°C) decomposition was observed. Reverse slow addition of LTMP to the substrate 532 led to conversion to the intermediate aryllithium intermediate 537, which is in accordance with the current hypotheses of the mechanism of this reaction.233 In order to prove this observation we decided to match products of electrophile quench, obtained by halogen dance reaction of 532, with the product of the direct addition of base to 2-bromopyridine
To our satisfaction both of these reaction proceeded well and yielded upon quench with CO$_2$ the same lithium carboxylate 540, Figure 62.

**Figure 62.** Formation of carboxylate 540 upon different conditions.

In order to attain the key intermediate for the Heck reaction, namely amide 478, we decided to proceed to the coupling of amine 473 without isolation of the carboxylic acid salt 540, Scheme 73. Bromopyridine 532 was used as the starting material, since it provided shorter access to the desired compound. Compound 532 was submitted to the halogen dance reaction in the presence of LTMP, followed by electrophilic quench with solid CO$_2$. The reaction proceeded well, and was immediately followed by transformation to the amide 478 under conditions of HBTU coupling. For the intramolecular Heck cyclization, conditions similar to those described before were used. The only difference was that the reaction was performed at lower temperature and therefore a longer reaction time was required. Due to this modification the fully protected 10-azanarciclasine (542) was isolated in higher yield than the corresponding 7-azacompound.
Scheme 73. Synthesis of the fully functionalized skeleton of 10-azanarciclasine.

Deprotection of naphthyridinone 542 was performed under the same conditions as C-1 analogues, see Scheme 62. Demethylation of 542 was achieved by treatment with fused lithium chloride in DMF at 100 °C, Scheme 74. Product 543 was isolated and submitted to desilylation conditions in the presence of TBAF, which led to a clean conversion to alcohol 544, which in turn was submitted to acid-catalyzed deprotection conditions in order to remove the acetonide and the t-butyloxy carbonyl group. Different conditions for deprotection were tried: strong acid such as HCl in methanol led to a significant decomposition of the starting material, and weaker acids such as formic or trifluoroacetic did not lead to deprotection. The best results were obtained with excess of wet (H₂O 5% v/v) trifluoroacetic acid, yielding 7 in good yield. The synthesis of this analogue was achieved in 11 steps of longest linear sequence (9 one-pot reactions).
Scheme 74. Deprotection of 10-azanarcicasine.

3.5. Biological activities of unnatural analogues

The strategies described above provided access to the three new analogues of pancratistatin with substituent on C-1 and three new analogues of narciclasine with a nitrogen placement in the A-ring. These new Amaryllidaceae constituents were tested in vitro against a panel of cancer cell lines through collaboration with the groups of Dr. Kornienko and Dr. Rodelj from New Mexico Institute of Mining and Technology, NM, Dr. Pandey from University of Windsor, ON, and the group in the Center for Research and Drug Development, BC.
Figure 63. Unnatural analogues of pancratistatin and narciclasine.

The C-1 analogues of pancratistatin were expected to have high activity since our group has previously reported\(^9\) that C-1 analogues of 7-deoxypancratistatin (11), namely the alcohol 192a and acetate 192b, displayed antiproliferative activity equal to that of the parent compound 11. Since the parent compound 2 of our analogues displays activity 10-100 times higher than 11, we had reasonable expectations that our C-1 compounds and especially benzoate 5c would display activity similar to the benzoate ester 294 because of their very similar structures. The results of the antiproliferative activity are presented in Table 10.

Table 10. Activity of C-1 analogues with narciclasine as standard (IC\(_{50}\), EC\(_{50}\), \(\mu\)M)

<table>
<thead>
<tr>
<th>Cell line (type of cancer)</th>
<th>Compound 5a</th>
<th>Compound 5b</th>
<th>Compound 5c</th>
<th>Narciclasine (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BxPC-3</td>
<td>0.22 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td><strong>0.01 ± 0.00</strong></td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>(Pancreatic)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>---------</td>
</tr>
<tr>
<td>DU-145 (Prostate)</td>
<td>0.09 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td><strong>0.01 ± 0.00</strong></td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>NCI-H460 (Lung)</td>
<td>0.09 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td><strong>0.03 ± 0.01</strong></td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>MCF-7 (Breast)</td>
<td>0.24 ± 0.10</td>
<td>0.52 ± 0.47</td>
<td><strong>0.08 ± 0.01</strong></td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>HCC1954 (Breast)</td>
<td>0.163</td>
<td>0.086</td>
<td><strong>0.011</strong></td>
<td></td>
</tr>
<tr>
<td>Jurkat (Leukemia)</td>
<td>0.266</td>
<td>0.211</td>
<td><strong>0.007</strong></td>
<td></td>
</tr>
</tbody>
</table>

As it can be seen from the table, C-1 benzoate homologue 5c displays particularly strong antiproliferative activity, exceeding even the natural congener narciclasine (1), which is amongst the most active natural products in this family. It further underlines the previously observed\textsuperscript{48,103} increased beneficial effect of large lipophilic groups, especially benzoates, on the position C-1, on activity. At the same time, despite the pronounced activity, our pancratistatin homologue compound 5c did not quite reach activity of C-1 benzoate pancratistatin ester (294). These results show that there are some limitations on the length and size of the lipophilic group on the position C-1.

Parallel studies have also been performed on different cancer lines, namely colorectal cancer HCT 116 and osteosarcoma Saos-2 cell lines and are presented in the following Figure 64. The difference between 7-deoxypancratistatin C-1 homologue alcohol 192a and acetate 192b, and their respective pancratistatin counterparts 5a and 5b can be clearly seen, with the latter being significantly more active. This fact once again shows the
importance of the presence of the 7-hydroxyl group in order to display high antiproliferative potency.

**Figure 64.** Cytotoxicity of C-1 analogues in dose dependant manner.

Next goal was to test the heterocyclic analogues of narciclasine we prepared. Three compounds were tested against a panel of cell lines and the results are presented in Table 11. It can be seen from the table, that 7-aza compounds show no activity (IC$_{50}$>100 μM) against two different cancer cell lines. This result might be explained by the belief that requirement of the C-8 and C-9 alkoxy groups that may be necessary to retain activity. A second reason might be that these compounds have very different physicochemical properties from parent narciclasine, such as high aqueous solubility and low lipophilicity.

**Table 11.** Activity of 7-aza analogues of narciclasine (IC$_{50}$, μM, extrapolated)

<table>
<thead>
<tr>
<th>Cell line (type of cancer)</th>
<th>Compound 6</th>
<th>Compound 6*HCl</th>
<th>Compound 476</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa (Cervical)</td>
<td>452</td>
<td>152</td>
<td>300</td>
</tr>
</tbody>
</table>
Testing performed by CDRD on a two different cancer cell of all four azaanallogues confirmed our previous observations, Table 12. 7-aza compounds displayed no cytotoxicity at low micromolar concentrations and, in turn, 10-azanarciclasine showed only reduced activity in comparison with the natural congener narciclasine. These results further support our conclusion about requirements of fully oxygenated A-ring for retaining anticancer activity and that nitrogen in A-ring plays deleterious role in pharmacophore.

**Table 12. Activity of 7-aza and 10-aza analogues of narciclasine (IC$_{50}$, μM)**

<table>
<thead>
<tr>
<th>Cell line (type of cancer)</th>
<th>Compound 6</th>
<th>Compound 6*HCl</th>
<th>Compound 476</th>
<th>Compound 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jurkat (Leukemia)</td>
<td>Inactive$^f$</td>
<td>Inactive</td>
<td>Inactive</td>
<td>0.5</td>
</tr>
<tr>
<td>HCC1954 (Breast)</td>
<td>Inactive</td>
<td>Inactive</td>
<td>Inactive</td>
<td>0.5</td>
</tr>
</tbody>
</table>

$^f$ Showed no cytotoxicity at 2.7 μM concentration.
4. Conclusions and Future work

The Amaryllidaceae constituents narciclasine (1) and pancratistatin (2) are isocarbostyril natural products which possess strong anticancer activity. In the course of the present study three different approaches towards analogues of these two natural products based on chemoenzymatic methods were studied. Two of these of approaches resulted in efficient synthetic routes and led to the synthesis of several structurally diverse C- and A-ring analogues of pancratistatin and narciclasine respectively.

The synthesis of six new analogues was accomplished and two of these analogues, namely C-1 acetate 5b and C-1 benzoate 5c have shown significant activity against a panel of cancer cell lines. The two truncated heterocyclic analogues, 7-aza-8,9-dideoxynarciclasine 6 and its N-oxide 476 displayed no activity, and one heterocyclic analogue 7 showed only low activity.

Derivatization at the C-1 position was shown to be beneficial in increasing the potency of natural compounds. Also it was shown that truncated 7-aza analogues do not possess any antiproliferative activity and therefore the alkoxy groups, are likely a requirement for the minimal pharmacophore. Bioisosteric replacement with nitrogen apparently plays a deleterious role in the anticancer activity of compound, but in the same time azanarciclasine analogues exhibit higher aqueous solubility than natural compounds 1 and 2.

Future studies are necessary in order to further probe the minimal pharmacophore requirements of the aromatic A-ring and to refine the synthesis of the C-ring analogues in
terms of step count and overall efficiency. New route to the C-1 analogues will be required, in order to refine existing synthesis, to avoid toxic reagents, and shorten step count to meet strict demands of pharmaceutical industry. Also, the C-1 pancreaticatin analogues could be obtained through efficient borylation and hydroboration transformation of narciclasine-type compounds. The reason for that, is that narciclasine compounds can be synthesised in much more efficient ways and can also be isolated from natural sources.
5. Experimental section

5.1. General Experimental section.

Reactions were carried out under inert atmosphere in oven-dried or flame-dried glassware unless stated otherwise. LiCl was fused under vacuum immediately before use. Solvents were distilled prior to use: CH$_2$Cl$_2$, DMF, iPr$_2$NEt, and pyridine from CaH$_2$, MeOH from magnesium methoxide, THF and DME from Na/benzophenone, toluene from Na, quinoline from Zn. Qualitative TLC was done with precoated silica gel aluminum sheets (EMD silica gel 60 F$_{254}$), detection by UV or by spraying with “CAM” solution (5 g of (NH$_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O, 1 g of Ce(SO$_4$)$_2$, 100 mL of 10% H$_2$SO$_4$) or 0.5% aqueous KMnO$_4$ solution followed by heating. Melting points are uncorrected. Flash chromatography was performed using silica gel SiliaFlash P60 from Silicycle (40–66 μm). Optical rotation was measured in a 1-dm cell at 20-25 °C and 589 nm, concentration c in g/100 mL, specific rotation measurements are given in deg cm$^3$g$^{-1}$dm$^{-1}$ and were recorded on a Perkin-Elmer 341 polarimeter, IR spectra were recorded on Perkin-Elmer FT-IR 1600 Series Spectrum One instrument in KBr pellets or as thin films. $^1$H NMR and $^{13}$C NMR spectra were recorded at 300 or 600 MHz and 75 or 150 MHz, respectively, and were calibrated on the solvent residual peak or TMS signal (CDCl$_3$ - 7.28 ppm; DMSO - d$_6$ - 2.51), the chemical shifts are reported in ppm. Data are reported as (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad); coupling constants(s) in Hz, integration.

5.2. Detailed experimental section.

4-methoxy-1,3-benzodioxole-5-carbaldehyde (486)
Dimethyl sulfate (22.7 mL, 240 mmol) was added to a mixture of K$_2$CO$_3$ (55.2 g, 400 mmol) and phenol 484 (33.2 g, 200 mmol) in acetone (260 mL). The reaction mixture was stirred under reflux until consumption of starting material (TLC, approximately 4 h). Then reaction mixture was cooled and inorganic salts were removed by filtration and rinsed with acetone (2×100 mL). The solution was evaporated, redissolved in CH$_2$Cl$_2$, washed sequentially with 10 % solution of NaOH, water, and saturated solution of NaCl, then organic solution dried over anhydrous Na$_2$SO$_4$ and evaporated to obtain compound 486 (30.2 g, 84%) as pale brown crystals, which was used without further purification.

$R_f$ 0.65 (Hexanes/EtOAc 9:1); mp 102-104 °C (EtOH); [Lit. value$^{234}$ 103-105 °C (EtOH)]; $^1$H NMR (CDCl$_3$, 300MHz): δ 10.24 (s, 1H); 7.49 (d, $J = 8.3$ Hz, 1H), 6.62 (d, $J = 8.3$ Hz, 1H), 6.05 (s, 2H), 4.14 (s, 3H).

5-(2,2-dibromovinyl)-4-methoxy-1,3-benzodioxole (487)

Triphenylphosphine (64.0 g, 244 mmol) in CH$_2$Cl$_2$ (100 mL) was added dropwise to a stirring solution of CBr$_4$ (40.5 g, 122 mmol) in CH$_2$Cl$_2$ (150 mL) at 0 °C (ice bath). After 15 min of stirring, a solution of aldehyde 486 (11.0 g, 61.0 mmol) in CH$_2$Cl$_2$ (50 mL) was added dropwise. Upon completion the reaction was reduced in volume to 100 mL
and slowly poured into vigorously stirred hexanes (1400 mL). Then mixture was filtered through short plug of silica, washed with a mixture of hexanes:EtOAc (10:1, 200 mL) and evaporated. Subjection of this material to flash column chromatography (Eluent hexanes/EtOAc 9:1) and concentration of the relevant fractions gave 487 (16.11 g, 78.6%) as a white solid.

\[ R_f \text{ 0.9 (Hexanes/EtOAc 9:1); mp } 38 - 40 \degree \text{C (pentane); IR (KBr, cm}^{-1}) \nu \text{ 3448, 2981, 2948, 2934, 2900, 2876, 2838, 2770, 1625, 1605, 1471, 1427, 1384, 1350, 1265, 1213, 1126, 1072, 1045, 979, 960, 939, 929, 848, 829, 788, 767, 729, 644; } \]

\[ ^1\text{H NMR (CDCl}_3, 300\text{MHz)} \delta \text{ 7.49 (s, 1H), 7.24 (d, } J = 8.3 \text{ Hz, 1H), 6.57 (d, } J = 8.3 \text{ Hz, 1H), 5.96 (s, 2H), 4.02 (s, 3H); } \]

\[ ^1\text{C NMR (CDCl}_3, 75\text{MHz)} \delta \text{ 149.7, 141.1, 136.1, 132.3, 122.5, 121.4, 102.3, 101.2, 89.0, 59.9; MS (+EI) } m/z \text{ (%): 338 (49) }[^{81}\text{Br}^{+81}\text{Br, M}]^{+}, 336 (100)[]^{81}\text{Br}^{+79}\text{Br, M}]^{+}, 334 (51) []^{79}\text{Br}^{+79}\text{Br, M}]^{-}, 242 (55), 240 (57), 176 (53), 175 (42), 131 (29); HRMS (+EI) calcd for } C_{10}H_{8}Br_2O_3: 333.8820; \text{ found 333.8845; Anal. Calcd for } C_{10}H_{8}Br_2O_3: C, 35.75; H, 2.40. \text{ Found C, 35.99; H, 2.41.} \]

**5-ethynyl-4-methoxy-1,3-benzodioxole (482)**

![Structural diagram]

To a solution of 487 (19.38 g, 57.68 mmol) in THF (350 mL) was added solution of \( n\text{BuLi (52.9 mL, 2.5 M, 130 mmol) at -78 \degree \text{C. After 20 min of stirring at -78 \degree \text{C, the reaction mixture was warmed to room temperature over a period of 2 h. A saturated solution of NH}_4\text{Cl (40 mL) was poured into reaction mixture, which was later extracted} \]

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by CH₂Cl₂ (3 × 50 mL). The combined organic phases were dried over Na₂SO₄, filtered and evaporated. The crude compound was purified by flash chromatography (hexanes:EtOAc 2:1) and 482 was obtained as white crystals (8.5 g, 81.7%).

Rᵣ 0.9 (Hex:EtOAc 2:1); mp 77-78 °C (pentane); IR (KBr, cm⁻¹) ν 3278, 3254, 3000, 2945, 2901, 2846, 2794, 1620, 1600, 1469, 1433, 1336, 1267, 1229, 1077, 1043, 979, 950, 930, 797; ¹H NMR (CDCl₃, 300MHz) δ 7.01 (d, J = 7.9 Hz, 1H), 6.50 (d, J = 8.29 Hz, 1H), 5.98 (s, 2H), 4.11 (s, 3H), 3.20 (s, 1H); ¹³C NMR (CDCl₃, 75MHz) δ 150.1, 144.8, 136.2, 128.0, 108.1, 102.9, 101.3, 79.9, 79.4, 60.0; MS (+EI) m/z (%): 176 [M]⁺ (100), 175 (29), 131 (16), 53 (18); HRMS (+EI) calcd for C₁₀H₈O₃: 176.0473; found, 176.0475; Anal. calcd for C₁₀H₈O₃: C, 68.18; H, 4.58. Found C, 68.27; H, 4.55.

(3aS,4R,5R,6R,7S,7aR)-6-[(4-methoxy-1,3-benzodioxol-5-yl)ethynyl]-2,2-dimethyl-8-[(4-methylphenyl)sulfonyl]hexahydro-4,5-epimino-1,3-benzodioxol-7-ol (490)

To a solution of alkyne 482 (1.580 g, 8.95 mmol) in toluene (30 mL) at -50 °C n-BuLi (3.80 mL, 2.35 M, 8.95 mmol) was added dropwise. After 15 min of stirring Me₂AlCl (9.0 mL, 1.0 M, 8.95 mmol) was added dropwise. The reaction mixture was warmed to 0 °C within 1 h and stirred for an additional 40 min at 0 °C. The reaction mixture allowed to warm room to temperature and stirred for 40 min. The reaction mixture was cooled to -30 °C and a solution of epoxide 184 (1.510 g, 4.47 mmol) in toluene (20 mL) was added dropwise. The reaction mixture was stirred for 1 h and was allowed to warm to room
temperature overnight. The reaction mixture was cooled to 0 °C by ice bath and quenched with 1 N HCl (1 mL), followed by ice-cold water (1 mL) and 1 N HCl (2 mL). Reaction mixture was filtered through a plug of Celite® and extracted with EtOAc (3 × 20 mL). The combined organic phases were dried over Na2SO4, filtered and evaporated. The crude product was purified by column chromatography (gradient hexanes/EtOAc 5:1->3:1) to give product as colorless oil (1.02g, 44%).

\[ R_f \ 0.3 \ (\text{Hex/EtOAc} \ 2:1); [\alpha]^{24}_D + 78.4 \ (c = 1.0, \text{CHCl}_3); \] IR (film, cm\(^{-1}\)) ν 3482, 3093, 2986, 2935, 2900, 1620, 1599, 1470, 1434, 1404, 1383, 1332, 1307, 1260, 1225, 1186, 1183, 1071, 985; \(^1\)H NMR (CDCl3, 300 MHz) δ 7.83 (d, \(J = 8.3\) Hz, 2H), 7.39 (d, \(J = 8.3\) Hz, 2H), 6.88 (d, \(J = 8.1\) Hz, 1H), 6.47 (d, \(J = 8.3\) Hz, 1H), 5.96 (s, 2H), 4.48 (d, \(J = 6.2\) Hz, 1H), 4.19 (t, \(J = 5.7\) Hz, 1H), 4.06 (s, 3H), 3.98-3.95 (m, 1H), 3.44-3.41 (m, 1H), 3.26 (d, \(J = 6.8\) Hz, 1H), 3.24 (dd, \(J = 5.0, 2.1\) Hz, 1H), 3.08 (d, \(J = 8.7\) Hz, 1H), 2.48 (s, 3H), 1.51 (s, 3H), 1.34 (s, 3H); \(^{13}\)C NMR (CDCl3, 75 MHz) δ 149.7, 145.4, 144.4, 136.3, 134.1, 130.1, 127.9, 127.2, 110.1, 108.8, 102.8, 101.3, 87.6, 80.5, 75.5, 70.3, 69.0, 60.0, 42.3, 40.2, 31.9, 27.3, 25.1, 21.7; MS (+FAB) \(m/\epsilon\) (%): 514 (24) [M+H]+, 513 (13) [M]+, 258 (12), 238 (11), 230 (14), 179 (26), 155 (28), 149 (23), 43 (100); HRMS (+FAB) calcd for C\(_{26}\)H\(_{28}\)NO\(_8\)S\(^+\) [M+1]+: 514.1436; found, 514.1502.

\((3aS,4R,5R,6R,7S,7aR)-6-[(Z)-2-(4-methoxy-1,3-benzodioxol-5-yl)vinyl]-2,2-dimethyl-8-[(4-methylphenyl)sulfonyl]hexahydro-4,5-epimino-1,3-benzodioxol-7-ol\) (492)
To a solution of compound 490 (427 mg, 0.83 mmol) in MeOH (20 mL) was added quinoline (11 mg, 0.09 mmol). The solution was charged with Lindlar catalyst (5%, 100 mg) and allowed to stir under H₂ (1 atm) for 45 min. After consumption of starting material (NMR), the reaction mixture was filtered through a pad of Celite®, washed with CH₃OH (3 × 30 mL), evaporated, and used without further purification. Analytical sample was purified by column chromatography (hexanes/EtOAc 3:1).

Rᵣ 0.4 (hexanes/EtOAc 2:1); [α]²₀° + 2.7 (c = 1.78, CHCl₃); IR (film, cm⁻¹) ʋ 3482, 2987, 2934, 2900, 1621, 1598, 1470, 1434, 1404, 1382, 1332, 1260, 1218, 1162, 1070, 1043; ¹H NMR (CDCl₃, 300 MHz) δ 7.74 (d, J = 8.3 Hz, 2H), 7.32 (d, J = 8.3 Hz, 2H), 6.60 (d, J = 11.4 Hz, 1H), 6.55 (d, J = 8.1 Hz, 1H), 6.46 (d, J = 8.0 Hz, 1H), 5.98 (d, J = 1.2 Hz, 1H), 5.96 (d, J = 1.2 Hz, 1H), 5.73 (t, J = 11.2 Hz, 1H), 4.43 (d, J = 6.2 Hz, 1H), 4.16-4.13 (m, 1H), 3.96 (s, 3H), 3.72-3.68 (m, 1H), 3.18 (d, J = 6.4 Hz, 1H), 3.15-3.10 (m, 1H), 3.08 (d, J = 6.4 Hz, 1H), 2.84 (d, J = 9.2 Hz, 2H), 2.43 (s, 3H), 1.49 (s, 3H), 1.30 (s, 3H). ¹³C NMR (CDCl₃, 75MHz) δ 148.9, 145.2, 141.3, 136.6, 134.3, 130.0, 128.1, 127.9, 127.8, 122.5, 122.2, 109.7, 102.6, 101.0, 75.8, 70.1, 69.3, 59.7, 43.1, 40.5, 37.8, 27.1, 24.7, 21.7. MS (+FAB) m/z (%) [M]+: 517 (16), 516 (42), 515 (29), 514 (15), 386 (25), 285 (14), 284 (15), 269 (15), 203 (30), 165 (61), 91 (100); HRMS (+FAB) calcd for
(3aS,4R,5R,6R,7S,7aR)-7-\{tert-butyl[dimethylsilyl]oxy\}-6-\{(Z)-2-(4-methoxy-1,3-benzodioxol-5-yl)vinyl\}-2,2-dimethyl-8-\{(4-methylphenyl)sulfonyl\}hexahydro-4,5-epimino-1,3-benzodioxol (481)

To a solution of alcohol 492 (1.07 g, 2.07 mmol) in 30 mL of CH₂Cl₂ was added Et₃N (0.58 mL, 4.15 mmol) at 0 °C and t-butyldimethylsilyl triflate (0.53 mL, 2.29 mmol) was added dropwise. After complete consumption of starting material (TLC) reaction mixture was quenched by water (10 mL), and extracted with CH₂Cl₂ (3 × 20 mL). The combined organic phases were washed with 10 % citric acid (5 mL), brine (5 mL), dried over Na₂SO₄, and concentrated to afford 481 as pale yellow oil (1.26 g, 97%). The Compound was used without further purification. Analytical sample was purified by silica gel chromatography (hexanes/EtOAc 4:1).

Rᵣ 0.85 (hexanes/EtOAc 2:1); [α]²⁴D − 18.5 (c = 2.0, CHCl₃); IR (KBr, cm⁻¹) ν 3446, 2986, 2954, 2931, 2887, 2856, 1622, 1600, 1470, 1435, 1382, 1332, 1257, 1218, 1163, 1071, 1043; ¹H NMR (CDCl₃, 300MHz): δ 7.78 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.2 Hz, 2H), 6.67 (d, J = 8.0 Hz, 1H), 6.60 (d, J = 11.5 Hz, 1H), 6.39 (d, J = 8.0 Hz, 1H), 5.96-5.93 (m, 2H), 5.65 (t, J = 11.5 Hz, 1H), 4.39 (d, J = 5.9 Hz, 1H), 3.98 (s, 3H), 3.89 (t, J = 6.03 Hz, 1H), 3.67 (t, J = 6.3 Hz, 1H), 3.12 (d, J = 6.6 Hz, 1H), 2.98-2.91 (m, 2H), 2.45
(s, 3H), 1.52 (s, 3H), 1.34 (s, 3H), 0.78 (s, 9H), 0.00(s, 3H), −0.08 (s, 3H); $^{13}$C NMR (CDCl$_3$, 75 MHz): δ 148.7, 144.4, 141.4, 136.3, 135.0, 129.7, 129.6, 127.8, 127.6, 122.9, 122.4, 109.3, 102.3, 100.8, 71.8, 71.4, 59.6, 43.4, 39.7, 39.2, 27.7, 25.7, 25.5, 21.6, 18.0, -4.6, -4.8; MS (+FAB) m/z (%)[M]$^+$: 628 (8), 514 (13), 343 (17), 256 (10), 228 (10), 215 (19), 165 (36), 73(100); HRMS (+FAB) calcd for C$_{32}$H$_{44}$NO$_8$SSi [M+1]$^+$: 630.2557, found 630.2492; Anal. calcd for C$_{32}$H$_{43}$NO$_8$SSi: C, 61.02; H, 6.88. Found C, 61.28, H, 7.02.

$N$-((1R,2S,3S,4S,4aR,11bR)-4-{{[tert-butyl(dimethyl)silyl]oxy}-3,3-dimethoxy-7-methoxy-1,2,3,4,4a,11b-hexahydrophenanthro[2,3-d][1,3]dioxol-1-yl}benzenesulfonamide. (480)

Olefin 481 (0.100 g, 0.561 mmol), quinoline (15 mg, 0.12 mmol) and silica gel (500 mg), which has been activated in advance by heating under vacuum for 24 h at 150 °C, was charged into flask and suspended in CH$_2$Cl$_2$ (10 mL), The solvent was removed in vacuo and the flask containing silica gel supporting the adsorbed reactants was heated at 120 °C under nitrogen atmosphere and stirred for 36 h. After this time the reaction mixture was purified directly by column chromatography (hexanes/EtOAc 4:1) to give olefin 480 as a clear and colorless oil (0.074 g, 74%).
$R_f$ 0.45 (hexanes: EtOAc 2:1); $[\alpha]^D_0 = -25.1 \ (c = 1.0, \ \text{CHCl}_3)$; IR (KBr, cm$^{-1}$) ν 3275, 2983, 2953, 2932, 2889, 2857, 1633, 1614, 1599, 1479, 1384, 1361, 1331, 1221, 1158, 1092, 841; $^1$H NMR (CDCl$_3$, 300MHz) δ 7.43 (d, $J = 8.1$ Hz, 2H), 7.13 (d, $J = 8.1$ Hz, 2H), 6.65 (dd, $J = 9.8$, 3.4 Hz, 1H), 6.22 (s, 1H), 5.92 (d, $J = 1.5$Hz, 1H), 5.82 (d, $J = 1.5$Hz, 1H), 5.74 (dd, $J = 9.8$, 1.5 Hz, 1H), 4.59 (d, $J = 8.8$Hz, 1H), 4.28 (m, 1H), 4.11 (s, 1H), 4.02-3.99 (m, 1H) 3.80-3.70 (m, 1H), 2.79-2.78 (m, 1H), 2.61-2.56(m, 1H), 2.41 (s, 3H), 1.73 (s, 1H), 1.45 (s, 3H), 1.34 (s, 3H), 0.88 (s, 9H), 0.10 (s, 3H), 0.05 (s, 3H); $^{13}$C NMR (CDCl$_3$, 75MHz) δ 147.5, 142.1, 139.5, 138.6, 135.5, 129.4, 129.8, 126.8, 125.3, 120.5, 119.7, 109.2, 104.8, 100.7, 79.1, 78.3, 70.3, 59.7, 53.7, 42.4, 39.1, 27.8, 26.3, 25.7, 21.5, 18.0, −5.03, −5.06. MS (+FAB) m/z (%): [M]$^+$ 629 (3), 129 (13), 111 (12), 99 (13), 57 (100); HRMS (+FAB) calcd for C$_{32}$H$_{43}$NO$_8$SSi [M]$^+$: 629.2479; found, 629.2472.

**N-[(3aS,4R,5R,6S,7S,7aS)-7-[(tert-butyl(dimethyl)silyl)oxy]-6,6'-bis(hydroxymethyl)-7'-methoxy-2,2-dimethyl-3,4,5,6,7,7a-hexahydro-5,5'-bi-1,3-benzodioxol-4-yl]-4-methylbenzenesulfonamide (495)**

\[\text{MeO} \quad \text{OH} \quad \text{OTBS} \quad \text{NHTs} \]

To a solution of 480 (0.254 g, 0.404 mmol) in MeOH (50 mL) a few crystals of Sudan Red 7B were added. The solution was cooled down to −80 °C and oxygen-ozone mixture was bubbled through until the disappearance of the pink color. The consumption of starting material was also checked by TLC. A stream of nitrogen was bubbled through
reaction mixture for 5 min. sodium borohydride (0.250 g, 6.67 mmol) was slowly added and reaction mixture was gradually warmed from −80 °C to room temperature. The solvent was removed in vacuo and the residue was redissolved in CH$_2$Cl$_2$ (50mL), neutralized by 10% citric acid and washed with water (50 mL). The organic phase was separated, dried over Na$_2$SO$_4$, filtered and evaporated. The crude product was subjected to column chromatography (hexanes/EtOAc 1:1) to yield 495 as white crystalline solid (0.1643 g, 61%) and 496 as mixture of anomers (80 mg, 30%).

$R_f$ 0.45 (hexanes/EtOAc 1:1); mp 121-123 °C (CHCl$_3$); $[\alpha]^{20}_D$ −30.8 (c = 1.09, CHCl$_3$); IR (KBr, cm$^{-1}$) ν 3472, 3386, 3172, 2927, 2855, 1622, 1482, 1385, 1332, 1255, 1220, 1158, 1095, 1057, 837; $^1$H NMR (CDCl$_3$, 600MHz) δ 7.51 (d, $J$ = 7.8 Hz, 2H), 7.13 (d, $J$ = 7.8 Hz, 2H), 6.57 (s, 1H), 5.96 (s, 1H), 5.90 (s, 1H), 5.44 (d, $J$ = 7.0 Hz, 1H), 4.77 (d, $J$ = 11.8 Hz, 1H), 4.42 (d, $J$ = 11.9 Hz, 1H), 4.27-4.24 (m, 1H) 4.17-4.10 (m, 2H), 4.00 (s, 3H), 3.95-3.86 (m, 1H), 3.70 (dd, $J$ =11.9, 6.1 Hz, 1H), 3.59 (dd, $J$ =11.8, 6.3 Hz, 1H), 3.37 (dd, $J$ = 11.8, 3.9 Hz, 1H), 2.92 (br s, 2H), 2.39 (s, 3H), 2.00-1.96 (m, 1H), 1.56 (s, 3H), 1.32 (s, 3H), 0.92 (s, 9H), 0.14 (s, 3H), 0.11 (s, 3H); $^{13}$C NMR (CDCl$_3$, 150MHz): δ 149.1, 142.1, 141.6, 139.2, 135.1, 132.4, 128.8, 126.8, 124.6, 109.7, 103.5, 101.0, 79.9, 79.0, 71.5, 61.3, 60.0, 57.0, 55.2, 47.3, 38.2, 27.4, 25.9, 25.8, 21.5, 18.0, 21.6, 21.0, 18.0, −4.8, −5.0; MS (+FAB) m/z (%): 664 [M-H]$^+$ (6), 648 (7), 372 (11), 302 (11), 254 (21), 248 (12), 73 (100), HRMS(+EI) calcd for C$_{32}$H$_{47}$NO$_{10}$SSi [M]$^+$: 665.2690; found 665.2803; Anal. calcd for C$_{32}$H$_{47}$NO$_{10}$SSi: C, 57.72; H, 7.11. Found C, 57.76; H, 6.99.

(3a$S$,3b$R$,10b$R$,11$S$,12$S$,12a$S$)-12-[[tert-butyl(dimethyl)silyl]oxy]-11-(hydroxymethyl)-6-methoxy-2,2-dimethyl-4-[(4-methylphenyl)sulfonyl]-3,3b,4,5,10b,11,12,12a-octahydonaphthalene-1,3(1H,3aH)-dione (496)
To a solution of alcohol 495 (0.100 g, 0.15 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (100 mL) was added MnO\textsubscript{2} (268 mg, 3 mmol). The reaction mixture was vigorously stirred until total consumption of starting material (TLC). The reaction mixture was filtered through a plug of Celite\textsuperscript{®} and washed with CH\textsubscript{2}Cl\textsubscript{2} (3 × 100 mL). Solvent was removed in vacuo, affording 496 as white solid (87mg, 87 %, mixture of anomers).

\[ R_f \] 0.5 and 0.65 (hexanes:EtOAc 1:1); mp 106-116 °C (CHCl\textsubscript{3}); IR (KBr, cm\textsuperscript{-1}) ν 3452, 2985, 2954, 2931, 2894, 2857, 1624, 1483, 1384, 1341, 1251, 1163, 1077, 839; (NMR of the major anomer) \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300MHz) δ 7.55 (d, \( J = 8.1 \) Hz, 2H), 7.03 (d, \( J = 8.1 \) Hz, 2H), 6.65 (s, 1H), 6.08 (s, 1H), 5.92-5.91 (m, 2H), 5.26 (dd, \( J = 9.9, 5.4 \) Hz,1H), 4.38 (t, \( J = 3.0 \) Hz, 1H) 4.25-4.21 (m, 2H), 4.11 (s, 3H), 3.74 (dd, \( J = 11.0, 7.9 \) Hz, 1H), 3.37 (dd, \( J = 11.0, 3.6 \) Hz, 1H) 2.87 (dd, \( J = 12.9, 5.1 \) Hz, 1H), 2.33 (s, 3H), 2.16 (br s, 1H), 2.15 (br s, 1H), 2.06 (s, 1H), 1.44 (s, 3H), 1.37 (s, 3H), 0.92 (s, 9H), 0.15 (s, 3H), 0.10 (s, 3H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 150MHz) δ 150.0, 142.9, 140.5, 138.3, 134.0, 130.0, 128.9, 127.2, 121.3, 109.4, 101.0, 100.2, 78.9, 76.4, 73.5, 59.9, 59.3, 53.1, 48.1, 34.4, 27.9, 26.2, 25.7, 21.4, 17.9, −4.8, −5.1; MS (±EI) m/z (%): [M-Ts-H\textsubscript{2}O]\textsuperscript{+} 491 (15), 432 (13), 302 (22.2), 207 (10.5), 247 (31.1), 246 (19.9), 43 (100); HRMS (+EI) calcd for C\textsubscript{32}H\textsubscript{45}NO\textsubscript{10}SSi [M]\textsuperscript{+}: 663.2533; found 663.2549; Anal. Calcd for C\textsubscript{32}H\textsubscript{45}NO\textsubscript{10}SSi: C, 57.90; H, 6.83. Found C, 58.02; H, 7.03.
{(3aS,3bR,10bR,11S,12S,12aS)-12-[(tert-butyl(dimethyl)silyl)oxy]-5-hydroxy-6-methoxy-2,2-dimethyl-4-[(4-methylphenyl)sulfonyl]-3,3b,4,5,10b,11,12,12a-octahydropis[1,3]dioxolo[4,5-c:4',5'-j]phenanthridin-11-yl}methyl acetate (497)

Pyridine (0.050 g, 0.63 mmol) was added to a solution of hemiacetal 496 (0.048 g, 0.072 mmol) in CH$_2$Cl$_2$ (3 mL), followed by addition of acetic anhydride (0.0295 g, 0.29 mmol). The reaction mixture was stirred until consumption of starting material (TLC). The reaction mixture was quenched with water (5 mL) and extracted by CH$_2$Cl$_2$ (3 × 4 mL). The combined organic phases were dried over Na$_2$SO$_4$, filtered and evaporated. The crude product was subjected to column chromatography (eluent hexanes/EtOAc 4:1) affording 497 as colourless oil (0.043 g, 87 % mixture of anomers).

$R_t$ 0.9 and 0.8 (Hexanes/EtOAc 1:1); IR (KBr, cm$^{-1}$) ν 3462, 2984, 2953, 2931, 2896, 2857, 1743, 1624, 1481, 1371, 1342, 1330, 1250, 1222, 1164, 1077, 840; (NMR of the major anomer) $^1$H NMR (CDCl$_3$, 600MHz) δ 7.56 (d, $J = 8.2$ Hz, 2H), 7.08 (d, $J = 8.0$ Hz, 2H), 6.66 (s, 1H), 6.012 (s, 1H), 5.94 (m, 2H), 5.31 (dd, $J = 10.0$, 5.1 Hz, 1H), 4.37 (m, 1H) 4.22-4.21 (m, 1H), 4.08 (s, 3H), 3.96 (s, 1H), 3.91 (dd, $J = 11.4$, 4.4 Hz, 1H), 3.05 (s, 1H), 2.91 (dd, $J = 13.2$, 4.5 Hz, 1H), 2.35 (s, 3H), 2.15 (s, 1H), 1.93 (s, 3H), 1.47 (s, 3H), 1.38 (s, 3H), 0.93 (s, 9H), 0.15 (s, 3H), 0.11 (s, 3H); $^{13}$C NMR (CDCl$_3$, 150MHz): δ 170.8 150.0, 143.0, 140.6, 138.5, 134.1, 129.8, 128.9, 127.2, 121.3, 109.3, 101.0, 100.1, 78.9, 76.3, 73.3, 67.2, 61.2, 59.9, 53.2, 45.6, 33.8, 29.7, 26.3, 25.7, 25.5,
21.4, 20.8, 17.9 −5.0, −5.2; MS (+FAB) m/z (%): [M−H₂O]^+ 688 (47), 230 (12), 117 (17), 302 (11), 247 (31), 117 (17), 73 (100); HRMS (+FAB) calcd for C₃₄H₄₆NO₁₀SSi [M−H₂O]^+: 688.2612; found 688.2642; Anal. calcd for C₃₄H₄₇NO₁₁SSi: C, 57.85; H, 6.71. Found C, 57.80; H, 6.67.

{(3aS,3bR,10bR,11S,12S,12aS)-12-{{[tert-butyl(dimethyl)silyl]oxy}-6-methoxy-2,2-dimethyl-4-[(4-methylphenyl)sulfonyl]-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydropis[1,3]dioxolo[4,5-c:4′,5′-j]phenanthridin-11-yl}methyl acetate (498)

![Chemical structure](Image)

A pre-dried solution of N-methylmorpholine-N-oxide (0.100 g, 0.85 mmol) in CH₂Cl₂ (20 mL) was added to hemiaminal 497 (0.030 g, 0.042 mmol) in CH₂Cl₂ (10 mL), followed by activated crushed molecular sieves (0.5 g, 4 Å). After stirring for 15 min, a few crystals of tetrapropylammonium perruthenate were added and reaction was stirred until consumption of starting material (TLC). The reaction mixture was filtered through a plug of Celite® and washed with CH₂Cl₂ (3 × 50 mL). The combined organic phases were evaporated and subjected to column chromatography (hexanes/EtOAc 2:1) affording 498 as colorless oil (0.025 g, 84 %)
$R_f$ 0.65 (eluent hexanes/EtOAc 1:1); $[\alpha]^{24}_D + 41.4$ ($c = 0.9$, CHCl$_3$); IR (KBr, cm$^{-1}$): ν 3450, 2984, 2953, 2930, 2857, 1742, 1710, 1614, 1484, 1360, 1253, 1169, 1090, 1021, 839; $^1$H NMR (CDCl$_3$, 300MHz) δ 8.20 (d, $J = 8.3$ Hz, 2H), 7.29 (d, $J = 8.3$ Hz, 2H), 6.73 (s, 1H), 6.06 (s, 1H), 6.02 (s, 1H), 4.83 (dd, $J = 7.9$, 5.5 Hz, 1H), 4.66 (s, 1H), 4.26 (t, $J = 11.1$, 1H), 4.17-4.13 (m, 1H), 4.12-4.08 (m, 1H), 4.04 (s, 3H), 4.00-3.95 (m, 1H), 3.47 (dd, $J = 13.1$, 3.3 Hz, 1H), 2.66-2.63 (m, 1H), 2.43 (s, 3H), 2.08 (s, 1H), 1.48 (s, 3H), 1.33 (s, 3H), 0.89 (s, 9H), 0.15 (s, 3H), 0.13 (s, 3H); $^{13}$C NMR (CDCl$_3$, 150MHz) δ 170.8, 163.7, 153.4, 144.8, 143.4, 138.8, 136.7, 136.1, 128.9, 128.7, 126.8, 117.8, 109.1, 102.0, 100.1, 79.0, 76.3, 66.9, 60.9, 60.7, 60.4, 59.7, 43.4, 36.5, 27.9, 26.1, 25.7, 21.6, 20.9, 18.0, −4.9, −5.1; MS (+EI) $m/z$ (%): 639 (3.1), 549 (6.6), 492 (3.4), 434 (2.9), 374 (3.9), 43 (100); HRMS (+EI) Calcd for C$_{33}$H$_{42}$NO$_{11}$SSi$^+ [M−$CH$_3]^+$: 688.2248; found 688.2436.

$((3a$S,3b$R,10b$R,11$S,12$S,12a$S)-12-{{tert-butyl(dimethyl)silyl}oxy}-6-methoxy-2,2-dimethyl-5-oxo-3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-$c$:4',5'-$j$]phenanthridin-11-yl)methyl acetate (499)

![Chemical Structure](image)

To a solution of 498 (0.064 mg, 0.09 mmol) in dry DME (7 mL) at $−50$ °C was added dropwise a solution of Na/naphthalene in DME (0.5 M), until a light green color persisted and total consumption of starting material was observed (TLC). The solution was stirred for 10 minutes before the reaction was quenched with NH$_4$Cl (satd. aq., 1 mL). The
reaction was warmed to room temperature and extracted with CH₂Cl₂ (6 × 15 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated. The products 499 and 479 were isolated by column chromatography (gradient hexanes/EtOAc 2:1 → 1:1) as clear and colorless oil 499 (0.030 g, 62%) and white crystalline solid 479 (0.002 g; 5%).

Rf 0.28 (hexanes: EtOAc 1:1); [α]²⁰°D + 32.4 (c = 1.0, CHCl₃); IR (KBr, cm⁻¹) ν 3417, 3228, 3109, 2987, 2953, 2932, 2897, 2858, 1743, 1676, 1617, 1481, 1385, 1366, 1339, 1250, 1222, 1169, 1088, 1071, 1057, 1033, 840; ¹H NMR (CDCl₃, 600MHz) δ 6.69 (s, 1H), 6.06 (s, 1 H), 6.01 (s, 1H), 5.92 (s, 1H), 4.57 (s, 1H), 4.23 (d, J = 7.5 Hz, 1H), 4.19-4.18 (m, 1H), 4.16-4.14 (m, 1H), 4.07 (s, 3H), 3.42 (dd, J =13.8, 8.3 Hz, 1H), 3.31 (dd, J =13.8, 3.6 Hz, 1H), 2.66-2.65 (m, 1H), 2.11 (s, 3H), 1.45 (s, 3H), 1.39 (s, 3H), 0.91 (s, 9H), 0.16 (s, 3H), 0.15 (s, 3H); ¹³C NMR (CDCl₃, 150MHz) δ 170.9, 163.5, 152.4, 145.4, 137.5, 135.5, 116.2, 109.9, 101.8, 99.9, 78.3, 77.8, 67.0, 61.1, 60.9, 52.5, 41.9, 35.1, 28.2, 26.1, 25.69, 25.65, 20.9, 18.0, −5.0, −5.1; MS (+FAB) m/z (%): 552 (12), 551 (36), 550 [M+1]⁺ (100), 246 (10), 220 (11), 117 (101); HRMS (+FAB) calcd for C₂₇H₄₀NO₉Si [M+1]⁺: 550.2472; found 550.2459.

((3aS,3bR,10bR,11S,12S,12aS)-12-{[tert-butyl(dimethyl)silyl]oxy}-6-hydroxy-2,2-dimethyl-5-oxo-3,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-c:4',5'-j]phenanthridin-11-yl)methyl acetate (500)
To a solution of 499 (0.039 mg, 0.071 mmol) in DMF (5 mL) was added LiCl (0.05 mg, 1.2 mmol), followed by three cycles freeze-pump-thaw. The reaction mixture was heated to 120 °C for 2.5 h. The reaction mixture was cooled to room temperature, diluted with water (100 ml) and extracted with diethyl ether (10 × 15 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated. The product was isolated by column chromatography (hexanes:EtOAc, 3:1) as a clear and colorless oil which solidifies after drying (25.7 mg, 68%).

Rf 0.8 (hexanes: EtOAc 1:1); mp 68-69° C (CHCl₃); [α]₂₀°D – 35.2 (c = 1.0, CHCl₃); IR (KBr, cm⁻¹) v 3402, 3348, 3285, 3212, 3087, 2987, 2953, 2933, 2899, 2859, 2795, 1743, 1627, 1601, 1464, 1389, 1366, 1353, 1341, 1301, 1250, 1226, 1171, 1081, 1032, 940, 838, 778; ¹H NMR (CDCl₃, 600MHz) δ 12.68 (s, 1H), 6.53 (s, 1H), 6.26 (s, 2H), 4.57 (s, 1H), 4.30 (dd, J = 11.1, 3.3 Hz, 1H), 4.22 (t, J = 11.0 Hz, 1H) 4.21-4.18 (m, 2H), 3.50 (dd, J = 14.4, 7.8 Hz, 1H), 3.36 (dd, J = 14.4, 3.7 Hz, 1H), 2.70-2.69 (m, 1H), 2.11 (s, 3H), 1.49 (s, 3H), 1.41 (s, 3H), 0.91 (s, 9H), 0.17 (s, 3H), 0.16 (s, 3H); ¹³C NMR (CDCl₃, 150MHz) δ 170.8, 169.9, 153.1, 146.9, 133.9, 133.0, 110.0, 107.3, 102.3, 97.3, 78.4, 77.6, 67.0, 61.10, 53.2, 41.7, 33.9, 28.3, 26.0, 25.7, 20.9, 18.0, −5.0, −5.1; MS (+EI) m/z (%): 536 [M+1]⁺(9), 535 (25), 360 (17), 256 (11), 231 (10), 218 (19), 205 (21), 149 (25), 43 (100); HRMS (+EI) calcd for C₂₆H₃₇NO₉Si [M]⁺: 535.2238; found 535.2248.

[(3aS,3bR,10bR,11S,12S,12aR)-6,12-dihydroxy-2,2-dimethyl-5-oxo-3,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5-c:4',5'-j]phenanthridin-11-yl]methyl acetate (501)
The compound \textbf{500} \((0.052 \text{ g}, 0.097 \text{ mmol})\) was taken up in THF \((2.5 \text{ mL})\) and cooled to \(0\) °C. A solution of TBAF in THF \((1 \text{ M}, 0.107 \text{ mL}, 0.107 \text{ mmol})\) was added dropwise over 1 min. The reaction mixture was stirred until total consumption of starting material was observed (TLC). The reaction mixture was quenched with water \((5 \text{ ml})\) and extracted with \(\text{CH}_2\text{Cl}_2 \,(6 \times 15 \text{ mL})\). The combined organic phases were dried over \(\text{Na}_2\text{SO}_4\), filtered and evaporated. The final product was isolated by column chromatography (hexanes/EtOAc 1:1) as a white crystalline solid \((38 \text{ mg}, 95\%)\).

\(R_f\) 0.3 (hexanes/EtOAc 1:1); \(\text{mp} > 200\) °C \((\text{CH}_2\text{Cl}_2 - \text{CH}_3\text{OH})\); \([\alpha]^{24}_D + 6.2 \,(c = 0.48, \text{DMSO})\); IR \((\text{KBr, cm}^{-1})\) \(\nu = 3449, 3270, 2988, 2911, 1743, 1672, 1625, 1600, 1466, 1443, 1357, 1307, 1245, 1231, 1166, 1087, 1071, 1032, 844\); \(^1\text{H NMR (DMSO-d}_6, 600\text{MHz})\) \(\delta\)

13.35 (s, 1H), 8.55 (s, 1H), 6.61 (s, 1H) 6.08 (s, 1H), 6.06 (s, 1H), 5.50 (d, \(J = 3.6 \text{ Hz, 1H}\), 4.34 (br. s, 1H), 4.25 (d, \(J = 4.8 \text{ Hz, 1H}\), 4.20 (dd, \(J = 11.4, 3.6 \text{ Hz, 1H}\)), 4.16 - 4.15 (m, 1H), 4.13 (d, \(J = 11.4 \text{ Hz, 1H}\)) 3.51 (dd, \(J = 15.0, 8.4 \text{ Hz, 1H}\)), 3.19 (dd, \(J = 14.4, 3.6 \text{ Hz, 1H}\)), 2.03 (s, 3H), 1.41 (s, 3H), 1.31 (s, 3H); \(^{13}\text{C NMR (DMSO-d}_6, 150\text{MHz})\) \(\delta\)

170.9, 169.8, 152.7, 146.3, 135.0, 132.6, 109.0, 107.7, 102.4, 97.6, 77.9, 76.9, 65.2, 61.2, 53.4, 34.3, 28.3, 26.4, 21.2; MS (+EI) \(m/z\) (%): 422 (24) \([\text{M+1}], 421 \,(100) \,[\text{M}^+], 248 \,(34), 247 \,(60), 232 \,(27), 231 \,(32), 218 \,(11), 206 \,(17), 145 \,(14); \text{HRMS}\) Calcd for \(\text{C}_{20}\text{H}_{23}\text{NO}_9 [\text{M}^+]\): 421.13728; found 421.13792; Anal. calcd for \(\text{C}_{20}\text{H}_{23}\text{NO}_9\): C, 57.00; H, 5.50. Found C, 57.18, H, 5.48.
[(1S,2S,3R,4S,4aR,11bR)-2,3,4,7-tetrahydroxy-6-oxo-1,2,3,4,4a,5,6,11b-octahydro[1,3]dioxolo[4,5-j]phenanthridin-1-yl]methyl acetate (5b)

The compound 501 (0.038 g, 0.09 mmol) was taken up in CH₂Cl₂:CH₃OH mixture (1:1, 4 mL) and cooled to 0 °C. Trifluoroacetic acid (2 mL) was added dropwise and the reaction mixture was stirred until consumption of starting material was observed (TLC). The reaction mixture was dried in vacuo, triturated with CH₂Cl₂ (3 × 15 mL) and finally dried under high-vac. The final product was isolated by column chromatography on silica gel (deactivated by 10% w/w of water, eluent CH₂Cl₂:CH₃OH 10:1) as a white crystalline solid (0.031 g, 90%).

Rf 0.3 (CH₂Cl₂:CH₃OH 10:1); mp > 200 °C (CH₂Cl₂ - CH₃OH); [α]²⁴D + 36.8 (c = 0.2,THF); IR (KBr, cm⁻¹) ν 3459, 3287, 3214, 2991, 2923, 1750, 1709, 1670, 1628, 1595, 1466, 1436, 1384, 1342, 1264, 1227, 1196, 1090, 1070, 1034; ¹H NMR (DMSO-d₆, 600MHz) δ 13.26 (s, 1H), 7.40 (s, 1H), 6.59 (s, 1H), 6.08 (s, 1H), 6.06 (s, 1H), 5.17 (m, 1H), 5.10-5.09 (m, 2H), 4.40 (m, 1H), 4.16 (dd, J = 10.9, 3.5 Hz, 1H), 4.11 (s, 1H), 3.84 (m, 1H), 3.71 (m, 1H), 3.52 (dd, J = 13.6, 9.8 Hz, 1H), 3.26 (m, 1H), 2.66 (m, 1H), 2.03 (s, 3H); ¹³C NMR (DMSO-d₆, 150MHz) δ 171.0, 169.9, 152.8, 146.3, 135.7, 132.5, 107.5, 102.4, 97.8, 73.1, 71.2, 68.9, 61.8, 51.6, 40.5, 36.5, 21.3; MS (+EI) m/z (%): 381 [M⁺] (12), 321 (16), 279 (12), 277 (99), 276 (11), 256 (12), 247 (11), 205 (13), 201 (16), 179
199 (12), 185 (13), 183 (11), 179 (21), 167 (14), 149 (37), 129 (12), 123 (11), 69 (100); HRMS (+EI) calcd for C_{17}H_{19}NO_9: 381.1060; found 381.1055;

(1S,2S,3R,4S,4aR,11bR)-2,3,4,7-tetrahydroxy-1-(hydroxymethyl)-1,3,4,4a,5,11b-hexahydro[1,3]dioxolo[4,5-j]phenanthridin-6(2H)-one (5a)

The compound **501** (48 mg, 0.11 mmol) was taken up in CH_2Cl_2-CH_3OH mixture (1:1, 4 mL) and 2 drops of concentrated HCl were added. The reaction was stirred until total consumption of starting material was observed (TLC). The reaction mixture was neutralized by dropwise addition of saturated solution of NaHCO_3 and evaporated to dryness. The final product was isolated by column chromatography on silica gel (deactivated by 10% of water, eluent CH_2Cl_2:CH_3OH 5:1) as white crystalline compound (36 mg, 92%).

mp >200 °C (CH_2Cl_2-CH_3OH); R_f 0.4 (CH_2Cl_2: CH_3OH 5:1); [α]^{20}_{D} = +48.0 (c = 0.5, abs DMSO); IR (KBr, cm^{-1}) ν 3386, 2917, 1670, 162, 1598, 1466, 1439, 1384, 1352, 1304, 1228, , 1089, 1077, 1064, 1032; ^1H NMR (DMSO-d6, 300MHz) δ 13.25 (s, 1H), 7.31 (s, 1H), 6.55 (s, 1H), 6.07 (s, 1H), 5.02 (m, 3H), 4.47 (dd, J =6.4, 4.0 Hz, 1H), 4.18 (m, 1H), 3.91 (m, 1H), 3.82 (m, 1H), 3.67 (m, 1H), 3.43 (m, 1H), 3.14 (m, 1H), 2.37
(3aS,3bR,10bR,11S,12S,12aS)-12-\{[\textit{tert}-\textit{butyl}(dimethyl)silyl]oxy\}-11-(hydroxymethyl)-6-methoxy-2,2-dimethyl-3b,4,10b,11,12,12a-hexahydrobis[1,3]dioxolo[4,5-c:4',5'-j]phenanthridin-5(3aH)-one (479)

To a solution of 499 (0.170 g, 0.309 mmol) in methanol (5 mL) was added solution of NaOH (aq. 40%, 0.5 mL) and stirred until total consumption of starting material was observed (TLC). The reaction was quenched with solution of NH₄Cl (sat. aq., 1 mL). The mixture was evaporated and the residue was extracted with CH₂Cl₂ (6 × 15 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated. The final product was isolated by column chromatography (gradient hexanes:EtOAc 2:1 to 1:1) as a white crystalline solid (0.125 mg; 80%).

R_f 0.2 (hexanes/EtOAc 1:1); mp 148-149 °C (CHCl₃); [\(\alpha\)]D\textsubscript{20} +32.6 (c = 0.63, CHCl₃); IR (KBr, cm\(^{-1}\)) ν 3416, 2987, 2952, 2932, 2893, 2857, 1660, 1618, 1501, 1481, 1439, 1384, 1350, 1295, 1250, 1220, 1169, 1083, 1056, 841; \(^1\)H NMR (CDCl₃, 600MHz) δ 6.61 (s,
1H) 6.05 (s, 1H), 6.00 (s, 1H), 5.86 (s, 1H), 4.68 (s, 1H), 4.20 (d, $J = 4.5$ Hz, 1H), 4.14 (dd, $J = 8.2$, 5.0 Hz, 1H), 4.08 (s, 3H), 3.95 (dt, $J = 10.1$, 6.0, 1H), 3.67-3.66 (m, 1H), 3.49 (dd, $J = 13.8$, 8.4 Hz, 1H), 3.28 (dd, $J = 13.8$, 3.7 Hz, 1H), 2.53-2.52 (m, 1H), 1.91 (s, 1H), 1.46 (s, 3H), 1.40 (s, 3H), 0.91 (s, 9H), 0.18 (s, 6H); $^{13}$C NMR (CDCl$_3$, 150MHz) δ 163.6, 152.2, 145.4, 137.3, 136.3, 116.3, 101.7, 99.8, 78.3, 78.0, 67.4, 60.9, 58.8, 52.7, 45.2, 35.3, 28.1, 26.2, 25.7, 17.9, −4.92, −4.94; MS (+FAB) m/z (%): 508 (14) [M+1], 507 (37) [M$^+$], 450 (16), 449 (10), 434 (17), 433 (16), 392 (22), 374 (12), 345 (12), 261(100); HRMS (+FAB) Calc for C$_{25}$H$_{37}$NO$_8$Si [M$^+$]: 507.2289; found: 507.2287.

((3a$S$,3b$R$,10b$R$,11$S$,12$S$,12a$S$)-12-{{tert-butyl(dimethyl)silyl}oxy}-6-methoxy-2,2-dimethyl-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5- c:4',5'-j]phenanthridin-11-yl)methyl benzoate (503)

To a solution of 479 (0.121 g, 0.24 mmol) in CH$_2$Cl$_2$ (25 mL) was added triethylamine (0.04 mL, 0.48 mmol) at 0 °C followed by benzoyl chloride (0.03 mL, 0.26 mmol) and crystal of DMAP. The reaction mixture was stirred at 0 °C until total consumption of starting material was observed (TLC). The reaction mixture was quenched with distilled water (10 mL) and extracted with CH$_2$Cl$_2$ (3 × 15 mL). The combined organic phases were washed with solution of citric acid (10%, 10 mL), dried over sodium sulfate,
filtered, and concentrated. The final product was isolated by column chromatography (gradient hexanes/EtOAc 2:1→1:1) as white crystalline powder (0.094 g, 64%).

$R_f$ 0.4 (hexanes:EtoAc 1:1); mp 90-92 °C (CHCl$_3$); $[\alpha]_D^{20} - 15.1$ (c = 1, CHCl$_3$); IR (KBr, cm$^{-1}$) ν 3467, 3416, 3387, 3069, 2986, 2952, 2932, 2896, 2857, 1722, 1674, 1616, 1502, 1481, 1453, 1385, 1339, 1273, 1221, 1093, 1071, 1028, 838; $^1$H NMR (CDCl$_3$, 300MHz) δ 8.06 (d, J = 7.2 Hz, 2H), 7.63-7.58 (m, 1H), 7.48 (t, J = 7.5 Hz, 2H), 6.74 (s, 1H), 6.01-6.02 (m, 3H), 4.68 (s, 1H), 4.53-4.45 (m, 2H), 4.20-4.16 (m, 2H), 4.07 (s, 3H), 3.53 (dd, J = 13.8, 7.5 Hz, 1H), 3.40-3.35 (m, 1H), 2.86-2.83 (m, 1H), 1.50 (s, 3H), 1.40 (s, 3H), 0.90 (s, 9H), 0.18 (s, 6H); $^{13}$C NMR (CDCl$_3$, 75MHz) δ 166.4, 163.6,152.4, 145.4, 135.6, 133.2, 129.8, 129.6, 128.4, 116.1, 110.0, 101.7, 99.9, 78.3, 77.9, 67.2, 61.6, 60.9, 52.5, 42.1, 35.2, 28.2, 26.1, 25.6, 17.9, -4.9, -5.0; MS (+FAB) m/z (%): 614 (12) [M+2$^+$], 613 (36), 612 (89), 179 (12), 105 (100); HRMS Calc for C$_{32}$H$_{42}$NO$_9$Si$^+$ [M]$^+$: 612.2629; found: 612.2653.

$((3aS,3bR,10bR,11S,12S,12aS)-12-[[t$ert$]-butyl(dimethyl)silyl]oxy]-6-hydroxy-2,2$-dimethyl-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydrobis[1,3]dioxolo[4,5$-c:4',5'-j]$phenanthridin-11-yl)methyl benzoate (504)
To a solution of 503 (0.0743 g, 0.122 mmol) in dry DMF (5 mL) was added LiCl (0.050 g, 1.2 mmol), followed by three cycles of freeze-pump-thaw. The reaction mixture was heated to 120 °C for 3.5 h. The reaction was then cooled to room temperature, diluted with distilled water (50 ml) and extracted with diethyl ether (6 × 15 mL). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated. The product was isolated by column chromatography (hexanes/EtOAc 2:1) as a white crystalline solid (0.060 g, 83%).

Rf 0.8 (hexanes:EtOAc 1:1); mp 141-145 °C (CHCl₃); [α]²⁰D − 63.0 (c = 1.0, CHCl₃); IR (KBr, cm⁻¹) ν 3449, 2953, 2931, 2858, 1721, 1674, 1655, 1637, 1627, 1603, 1461, 1385, 1351, 1341, 1304, 1271, 1219, 1113, 1081, 837; ¹H NMR (CDCl₃, 600MHz) δ
12.70 (s, 1H), 8.07 (d, J = 8.0 Hz, 2H), 7.62-7.60 (m, 1H), 7.49-7.47 (m, 2H), 6.59 (s, 1H), 6.11 (s, 1H), 6.06 (s, 1H), 6.02 (s,1H), 4.68 (s, 1H), 4.55-4.54 (m, 2H), 4.23-4.21 (m, 2H), 3.63-3.59 (m, 1H), 3.43 (dd, J =14.4, 3.5 Hz, 1H), 2.89 (s br, 1H), 1.53 (s, 3H), 1.42 (s, 3H), 0.91 (s, 9H), 0.16-0.15 (m, 6H); ¹³C NMR (CDCl₃, 150MHz) δ 170.0, 166.4, 153.2, 146.9, 134.0, 133.2, 133.1, 129.8, 129.6, 129.4, 110.1, 107.3, 102.3, 97.3, 78.4, 77.7, 67.2, 61.6, 53.2, 41.8, 34.0, 30.3, 28.4, 26.1, 25.7, 17.9, −4.9, −5.0; MS (+FAB) m/z (%): 599 (11) [M+2]+, 598 (29), 597 (8), 596 (4), 179 (11), 73 (100); HRMS (+FAB) calcd. for C₃₁H₄₀N₁O₉Si⁺ [M+1]+: 598.2472; found 598.2446;

[(3αS,3bR,10bR,11S,12S,12aR)-6,12-dihydroxy-2,2-dimethyl-5-oxo-3a,3b,4,5,10b,11,12,12a-octahydropobis[1,3]dioxolo[4,5-c:4',5'-j]phenanthridin-11-yl]methyl benzoate (505)
The compound 504 (0.042 mg, 0.07 mmol) was taken up in THF (2.5 mL) and cooled to 0 °C. A solution of TBAF in THF (1 M, 0.077 mL, 0.077 mmol) was added dropwise and the reaction mixture was stirred until total consumption of starting material was observed (TLC). The reaction mixture was quenched by distilled water (5 ml) and extracted with CH₂Cl₂ (6 × 15 mL). The combined organic phases were dried over Na₂SO₄, filtered, and evaporated. The final product was isolated by column chromatography (hexanes/EtOAc 1:1) as white crystalline solid (0.0272 g, 80%).

$R_t$ 0.3 (hexanes/EtOAc 1:1); mp > 200 °C (THF); $[\alpha]_{D}^{21}$ $-40.1$ ($c = 1.0$, THF); IR (KBr, cm$^{-1}$) ν 3446, 3255, 2986, 2930, 2905, 2854, 1720, 1672, 1626, 1601, 1466, 1384, 1356, 1343, 1311, 1222, 1166, 1088, 1071, 1027, 713; $^1$H NMR (DMSO-$d_6$, 600 MHz) δ 13.36 (s, 1H), 8.57 (s, 1H), 7.97-7.96 (m, 2H), 7.69-7.67 (m, 1H), 7.56-7.53 (m, 2H), 6.74 (s, 1H), 6.07 (s, 1H), 5.99 (s, 1H), 5.56 (dd, $J = 4.3$ Hz, 1H), 4.53 (dd, $J = 11.3, 4.3$ Hz, 1H), 4.46-4.45 (m, 1H), 4.43-4.39 (m, 1H), 4.29 (d, $J = 5.0$ Hz, 1H), 4.19 (dd, $J = 8.3, 5.3$ Hz, 1H), 3.63 (dd, $J = 14.4, 8.4$ Hz, 1H), 3.26 (dd, $J = 14.4, 3.6$ Hz, 1H), 2.97-2.95 (m, 1H), 1.45 (s, 3H), 1.33 (s, 3H); $^{13}$C NMR (DMSO-$d_6$, 150MHz) δ 169.9, 166.1, 152.7, 152.7, 146.3, 135.0, 133.9, 132.5, 130.0, 129.7, 129.2, 109.0, 107.7, 102.3, 97.9, 78.0, 77.1, 65.5, 62.0, 53.4, 34.3, 30.3, 28.3, 26.4; MS (+FAB) $m/z$ (%): 484 (4) [M+1]$^+$, 483 (26) [M]$^+$, 248
(13), 247 (59), 232 (13), 231 (28), 205 (11), 122 (16), 105 (100); HRMS (+FAB) Calc. for C$_{25}$H$_{25}$NO$_9$ [M]$^+$: 483.1529; found: 483.1532.

[(1S,2S,3R,4S,4aR,11bR)-2,3,4,7-tetrahydroxy-6-oxo-1,2,3,4,4a,5,6,11b-octahydro[1,3]dioxolo[4,5-j]phenanthridin-1-yl]methyl benzoate (5c)

The compound 505 (32 mg, 0.066 mmol) was taken up in a mixture of CH$_2$Cl$_2$:CH$_3$OH (1:1, 4 mL) and cooled to 0 °C. Trifluoroacetic acid (1 mL) was added dropwise and the reaction was stirred until total consumption of starting material was observed (TLC). The reaction mixture was dried in vacuo, triturated with CH$_2$Cl$_2$ (3 × 15 ml) and finally dried under high-vac. The final product was isolated by column chromatography on silica gel (deactivated by 10% of water, gradient CH$_2$Cl$_2$ → CH$_2$Cl$_2$/CH$_3$OH 50:1 → CH$_2$Cl$_2$/CH$_3$OH 25:1) as white crystalline solid (0.025 g, 85%).

$R_f$ 0.6 (CH$_2$Cl$_2$:CH$_3$OH 10:1); mp$> 200$ °C (CH$_2$Cl$_2$); $[\alpha]_D^{20} = -24.9$ ($c = 1.0$, THF); IR (KBr, cm$^{-1}$) ν 3423, 3386, 2956, 2921, 2852, 1716, 1672, 1627, 1600, 1466, 1384, 1363, 1340, 1278, 1095, 1072, 1038, 711; $^1$H NMR (DMSO-d$_6$, 600MHz) δ 13.27 (s, 1H), 8.00 (d, $J = 7.7$ Hz, 2H), 7.68 (t, $J = 7.6$ Hz, 1H), 7.57-7.54 (m, 2H), 7.43 (s, 1H), 6.72 (s, 1H) 6.07 (s, 1H), 6.01 (s, 1H), 5.19 (m, 2H), 5.13 (m, 1H), 4.66 (t, $J = 10.7$ Hz, 1H), 4.48 (dd, $J = 10.9$, 4.0 Hz, 1H), 4.24-4.23 (m, 1H), 3.88-3.87 (m, 1H), 3.75-3.73 (m, 1H), 3.62 (dd, $J = 13.6$, 9.9 Hz, 1H), 3.31 (dd, $J = 13.8$, 4.2 Hz, 1H), 2.85-2.84 (m, 1H); $^{13}$C NMR
(DMSO-d6, 150MHz) δ 170.0, 166.3, 152.8, 146.3, 135.8, 133.8, 132.5, 130.3, 129.7, 129.2, 107.5, 102.4, 98.0, 73.1, 71.2, 69.2, 62.5, 51.6, 36.6; MS (+FAB) m/z (%): 444 (4) [M+1]+, 219 (12), 136 (11), 121 (11), 109 (15), 107 (17), 105 (23), 97 (18), 95 (29), 55 (100); HRMS (+FAB) calcd for C22H22NO9 [M+1]+: 444.1295; found: 444.1262.

(3aR,4R,5R,6S,7S,7aR)-7-[(cyanomethyl)][(4-methylphenyl)sulfonyl]amino]-2,2-dimethyl-6-[(trimethylsilyl)ethynyl]hexahydro-1,3-benzodioxole-4,5-diyl dibenzoate (512)

The solution of acetylene 51092 (0.1713 mg; 0.261 mmol) in THF (40 mL) was added dropwise solution of NaHMDS in toluene (0.725 M, 0.261mmol; 0.36 mL;) at −70 °C. The reaction mixture was allowed to warm up to 0 °C over a period of 20 min and was further stirred for 10 min at this temperature. Chloroacetonitrile (33 μl; 0.522 mmol) and nBu4NI (144.6 mg; 0.391 mmol) were added and solution was allowed to warm to room temperature overnight. The reaction mixture was quenched by addition of NH4Cl (satd. aq., 10 mL) and extracted with EtOAc (3 × 25 mL). The combined organic phases were washed with brine (20 mL), dried over Na2SO4, and the solvent was removed under reduced pressure. Flash column chromatography of the residue (hexanes/EtOAc 9:1) afforded tosylamide 512 (0.088 g; 48%) as colourless oil.
$R_f$ 0.70 (hexanes/EtOAc 2:1); $[\alpha]_D^{20} = 108.1$ (c = 1, CHCl$_3$); IR (KBr, cm$^{-1})$ ν 3440, 3293, 3067, 2990, 2937, 2593, 1729, 1601, 1452, 1375, 1353, 1336, 1316, 1274, 1248, 1221, 1248, 1221, 1160, 1096, 1070, 1040, 926, 890, 853, 815, 710, 666, 545, 564; $^1$H NMR (300 MHz, CDCl$_3$) δ 8.08 (d, $J = 7.5$ Hz, 2H), 7.96 (m, 4H), 7.60 (m, 1H), 7.49 (m, 1H), 7.36 (m, 2H), 7.36 (m, 4H), 6.01 (t, $J = 3.0$ Hz, 1H), 5.69 (m, 1H), 4.93 (br s, 1H), 4.42 (m, 1H), 4.33 (m, 2H), 4.07 (br s, 2H), 2.43 (s, 3H), 1.68 (s, 3H), 1.39 (s, 3H), $-0.02$ (s, 9H); $^{13}$C NMR (150 MHz, CDCl$_3$) δ 165.1, 164.8, 144.6, 136.3, 133.7, 133.2, 129.98, 129.89, 129.88, 129.4, 129.1, 128.7, 128.3, 128.1, 114.7, 110.9, 75.1, 71.2, 68.3, 34.5, 28.3, 26.0, 21.6, $-0.51$; MS (+EI) m/z (%): 686 (2), 685 (4), 149 (12), 105 (100); HRMS (+EI) calcd for C$_{36}$H$_{37}$N$_2$O$_8$SSi$^+$ [M-CH$_3$]$^+$: 685.2040; found: 685.2052.

(3aS,4S,5S,6R,7R,7aS)-7-{{(cyanomethyl)[(4-methylphenyl)sulfonyl]amino}-6-ethynyl-2,2-dimethylhexahydro-1,3-benzodioxole-4,5-diyl dibenzoate (471)
room temperature overnight. The reaction was quenched by NH₄Cl (satd. aq., 40 mL) and extracted with EtOAc (4 × 40 mL). The combined organic phases were washed with brine (20 mL), dried over MgSO₄, and the solvent was removed under reduced pressure. Flash column chromatography of the residue (hexanes/EtOAc 3:2) afforded tosylamide as white crystalline solid (0.044 mg; 44%).

\( R_f \) 0.60 (hexanes/EtOAc 2:1); mp 86 °C (EtOAc/hexanes); [\( \alpha \)]\( _{D}^{20} \) −126.3 (c = 1.35, CHCl₃); IR (KBr, cm\(^{-1}\)) ν 3438, 3291, 3067, 2988, 2937, 2593, 1729, 1601, 1452, 1375, 1353, 1336, 1316, 1274, 1248, 1221, 1248, 1221, 1160, 1096, 1070, 1040, 926, 890, 853, 815, 710, 666, 545, 564; \(^1\)H NMR (600 MHz, CDCl₃) δ 8.06 (d, \( J = 7.5 \) Hz, 2H), 7.93 (m, 4H), 7.60 (m, 1H), 7.52 (m, 1H), 7.47 (m, 2H), 7.36 (m, 4H), 5.98 (t, \( J = 3.0 \) Hz, 1H), 5.69 (m, 1H), 4.92 (br s, 1H), 4.43 (m, 1H), 4.38 (m, 1H), 4.33 (m, 1H), 4.00 (br s, 2H), 2.44 (s, 3H), 2.11 (s, 1H), 1.68 (s, 3H), 1.39 (s, 3H); \(^{13}\)C NMR (150 MHz, CDCl₃) δ 165.0, 164.8, 144.7, 136.2, 133.7, 133.3, 129.97, 129.91, 129.88, 129.3, 129.0, 128.7, 128.4, 128.1, 114.7, 111.0, 75.1, 74.7, 74.0, 71.1, 68.2, 33.5, 28.3, 26.0, 21.6; MS (+EI) \( m/z \) (%) 614 (1), 613 (3), 246 (13), 171 (12), 155 (5), 105 (100); HRMS (+EI) calcd. for C\(_{33}\)H\(_{29}\)N\(_2\)O\(_8\)S: 613.16446; found: 613.16387.

(3a\(S\),3b\(R\),9b\(R\),10\(S\),11\(S\),11a\(S\))-2,2-dimethyl-4-[(4-methylphenyl)sulfonyl]-7,8-bis(trimethylsilyl)-3a,3b,4,5,9b,10,11,11a-octahydro[1,3]benzodioxolo[5,4-f]-1,7-naphthyridine-10,11-diyl dibenzoate (472)
To a solution of CpCo(CO)$_2$ (1 µL; 7.5 µmol) in BTMSA (269) (1.5 mL; 0.012 mmol) solution of α,ω-cyanoalkyne 471 (44 mg; 0.07 mmol), CpCo(CO)$_2$ (1 µL; 7.5 µmol) dissolved in $m$-xylene (2 mL), and 269 (1 mL; 0.008 mmol) was added dropwise under irradiation with visible light at 140 °C over period 2 min. The reaction mixture was heated under inert atmosphere for further 24 h. BTMSA and xylene were removed under high vacuum and the residue was purified by column chromatography (gradient hexanes/EtOAc, 9:1 - 6:1). The product 471 was isolated as slightly yellow crystalline foam (26.0 mg, 46.5%).

$R_f$ 0.34 (hexanes/EtOAc 4:1); mp 110 °C (hexane/EtOAc); $[\alpha]_D^{20} -25.5$ ($c = 1.45$, CHCl$_3$); IR (KBr, cm$^{-1}$) ν 3435, 3291, 3066, 2983, 2957, 2921, 2851, 1725, 1601, 1528, 1493, 1452, 1406, 1383, 1361, 1346, 1317, 1275, 1250, 1223, 1167, 1107, 1096, 1065, 1028, 912, 842, 755, 710, 666, 555, 541; $^1$H NMR (600 MHz, CDCl$_3$) δ 7.93 (d, $J = 7.9$ Hz, 2H), 7.87 (d, $J = 7.5$ Hz, 2H), 7.58 (d, $J = 8.3$ Hz, 2H), 7.54 (m, 2H), 7.37 (m, 5H), 7.05 (d, $J = 7.9$ Hz, 2H), 5.83 (dd, $J = 8.3$, 4.2 Hz, 1H), 5.70 (dd, $J = 6.8$, 4.5 Hz, 1H), 4.78 (d, $J = 17.4$Hz, 1H), 4.65 (m, 2H), 3.81 (dd, $J = 12.5$, 9.4 Hz, 1H), 3.48 (dd, $J = 12.5$, 8.3 Hz, 1H), 2.31 (s, 3H), 1.54 (s, 3H), 1.38 (s, 3H), 0.31 (s, 9H), 0.03 (s, 9H); $^{13}$C NMR (150 MHz, CDCl$_3$) δ 171.4, 165.23, 165.18, 151.8, 142.9, 138.9, 137.6, 133.5, 133.4, 129.83, 129.80, 129.3, 129.1, 128.5, 128.4, 127.5, 126.3, 110.6, 75.1, 74.4, 71.1, 69.8, 60.8, 52.8,
38.5, 29.7, 27.9, 25.5, 21.5, 0.98, 0.86; MS (FAB) m/z (%) 800 (2), 294 (2), 105 (100), 91 (11); HRMS (+FAB) calcd for C_{42}H_{51}N_{2}O_{8}SSi_{2}^{+}: 799.2905; found: 799.2971.

\((3aS,3bR,9bR,10S,11S,11aR)\)-2,2-dimethyl-4-[(4-methylphenyl)sulfonyl]-7,8-bis(trimethylsilyl)-3a,3b,4,5,9b,10,11,11a-octahydropyrido[1,3]benzodioxolo[5,4-f]-1,7-naphthyridine-10,11-diol (519)

![Structural Diagram]

To a solution of 472 (42 mg, 0.052 mmol) in the mixture of CH\(_2\)Cl\(_2\):MeOH (4 mL; 1:1) K\(_2\)CO\(_3\) (22 mg; 0.16 mmol) was added, and reaction mixture was stirred until disappearance of starting material (TLC). Then the reaction mixture was filtered, evaporated and the residue was purified by column chromatography (gradient hexanes:EtOAc, 2:1 to 1:1). The product was isolated as a transparent foam (19.2 mg, 62\%).

\(R_f\) 0.2 (hexanes/EtOAc 2:1); \([\alpha]_D^{21} - 26.5\ (C = 1.0, \text{CHCl}_3)\); IR (KBr, cm\(^{-1}\)) \(\nu\) 3467, 2983, 2954, 2926, 2902, 2856, 1446, 1405, 1384, 1356, 1248, 1219, 1161, 1090, 1070, 1050, 858, 841, 756, 715; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.70, 7.48 (d, \(J = 8.4\) Hz, 2H), 6.99 (d, \(J = 8.1\) Hz, 2H), 5.03 (dd, \(J = 8.7, 6.8\) Hz, 1H), 4.68 (s, 2H), 4.41-4.36 (m, 1H), 4.13 (dd, \(J = 7.9, 5.3\) Hz, 1H), 3.95 (dd, \(J = 7.9, 4.9\) Hz, 1H), 3.67-3.60 (m, 1H), 2.94 (s, 1H), 2.85-2.78 (m, 1H), 2.70 (s, 1H), 2.30 (s, 3H), 1.55 (s, 3H), 1.39 (s, 3H), 0.37 (s, 9H), 0.33 (s, 9H); \(^{13}\)C NMR (150 MHz, CDCl\(_3\)) \(\delta\) 170.7, 152.0, 142.6, 139.7, 138.8, 137.3,
128.9, 127.54, 127.50, 110.2, 75.9, 75.7, 70.3, 70.0, 58.8, 53.4, 51.6, 40.0, 27.9, 27.9,
25.2, 21.5, 1.2, 1.1; MS (+EI) m/z (%) 590 (2), 575 (13), 437 (13), 435 (100); HRMS
(+EI) calcd for C_{28}H_{42}N_{2}O_{6}SSi^{2+}: 590.2302; Found: 590.2299.

(3aS,3bS,6aS,6bR,12bR,12cS)-2,2,5,5-tetramethyl-7-[(4-methylphenyl)sulfonyl]-
10,11-bis(trimethylsilyl)-3a,3b,6a,6b,7,8,12b,12c-octahydropseudo[1,3]dioxolo[3,4:5,6]benzo[1,2-f]-1,7-naphthyridine (520)

To a solution of diol 519 (0.040 g; 0.068 mmol) in 2,2-dimethoxypropane (1.0 mL; 8.16
mmol) was added p-toluenesulfonic acid monohydrate (14 mg, 0.075 mmol). The
reaction mixture was stirred until total consumption of starting material (TLC). The
reaction mixture was quenched by addition of saturated solution of NaHCO_{3} (5 ml),
extracted by EtOAc (3 \times 10 ml). The combined organic phases were dried over Na_{2}SO_{4},
filtered and evaporated. The residue was purified by column chromatography
(hexanes/EtOAc 2:1) and isolated as colourless oil (0.020 g, 47%).

R_{f} 0.8 (hexanes:EtOAc 2:1); [\alpha]^{21}_{D} - 0.4 (c = 1.0, CHCl_{3}); IR (KBr, cm^{-1}) v 2991, 2979,
2936, 2887, 2857, 2852, 1384, 1357, 1244, 1219, 1166, 1106, 1070, 1050, 926, 880, 841,
815, 754; ^{1}H NMR (300 MHz, CDCl_{3}) \delta 7.71, 7.45 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 8.1
Hz, 2H), 4.84 (dd, J = 8.7, 6.8 Hz, 1H), 4.79-4.73 (m, 1H), 4.56-4.50 (m, 1H), 4.45-4.41
(m, 1H), 4.38-4.33 (m, 2H), 3.53 (dd, J = 12.1, 8.7 Hz, 1H), 3.95 (dd, J = 11.7, 8.7 Hz,
1H), 2.30 (s, 3H), 1.60 (s, 3H), 1.50 (s, 3H), 1.42 (s, 3H), 1.39 (s, 3H), 0.36 (s, 9H), 0.33 (s, 9H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 170.8, 152.4, 142.5, 140.0, 138.5, 136.9, 128.9, 127.6, 127.0, 110.2, 110.1, 77.6, 76.36, 76.2, 58.3, 50.3, 39.2, 27.70, 27.66, 25.19, 25.14, 21.5, 1.15, 1.05; MS (+EI) m/z (%) 630 (1), 615 (12), 477 (15),475 (100); HRMS (+EI) calcd for C\(_{31}\)H\(_{46}\)N\(_2\)O\(_8\)SSi\(_2^+\): 630.2615; found: 630.2609.

\((3aS,3bS,6aS,6bR,12bR,12cS)-2,2,5,5-tetramethyl-7-[(4-methylphenyl)sulfonyl]-3a,3b,6a,6b,7,8,12b,12c-octahydrobis[1,3]dioxolo[3,4-f]1,7-naphthyridine (521)\)

![Structure Image]

To a solution of acetonide 520 (0.011 g; 0.017 mmol) in THF (2.0 mL) was added solution TBAF in THF (1 M, 0.05 mL, 0.05 mmol). The reaction mixture was stirred until total consumption of starting material (TLC). The reaction mixture was evaporated and the residue was purified by column chromatography (gradient hexanes/EtOAc 2:1 \(\rightarrow\) 1:1 ) and isolated as colourless oil (0.006 g, 70%).

\(R_f\) 0.6 (hexanes:EtOAc 1:2); \([\alpha]^{21}\)D + 13.0 (c = 0.3, CHCl\(_3\)); IR (CHCl\(_3\), cm\(^{-1}\)) \(\nu\) 2998, 2927, 1456, 1384, 1308, 1272, 1259, 1217, 1164, 1070, 924, 881, 817, 712, 696, 669; \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 8.30 (d, \(J = 4.8\) Hz, 1H), 7.74 (d, \(J = 7.6\) Hz, 1H), 7.59 (d, \(J = 7.8\) Hz, 2H), 7.13 (dd, \(J = 7.6, 4.8\) Hz, 1H), 7.11 (d, \(J = 8.4\) Hz, 2H), 4.70 (dd, \(J = 9.6, 6.6\) Hz, 1H), 4.48-4.42 (m, 2H), 4.38-4.36 (m, 1H), 3.71 (dd, \(J = 12.0, 9.0\) Hz, 1H), 2.67 (t, \(J\)
= 10.8, 1H), 2.36 (s, 3H), 1.61 (s, 3H), 1.50 (s, 3H), 1.45 (s, 3H), 1.38 (s, 3H); \(^{13}\)C NMR
(150 MHz, CDCl\(_3\)) 154.9, 147.1, 143.0, 137.0, 134.1, 130.2, 129.2, 129.0, 127.7, 127.5,
122.8, 110.2, 77.6, 76.43, 76.2, 57.6, 49.3, 39.7, 31.9, 30.91, 29.7, 29.36, 27.7, 27.6,
25.22, 25.19, 21.4; MS (+EI) m/z (%) 486 (6), 279 (10), 167 (25), 150 (11), 149 (79), 123
(12), 43 (100); HRMS (+EI) calcd for C\(_{25}\)H\(_{30}\)N\(_2\)O\(_8\)S\(^+\): 486.1825; found: 486.1821.

**tert-Butyl (3aS,4R,7R,7aS)-4-bromo-2,2-dimethyl-3a,4,7,7a-tetrahydro-4,7-
(epoxyimino)-1,3-benzodioxole-8-carboxylate (526)**

![Chemical Structure]

To a solution of diol 4 (3.8 g, 19.8 mmol) in 2,2-dimethoxypropane (10 mL) was added a
catalytic amount of p-toluenesulfonic acid. After complete consumption of starting
material (vide TLC), the solution was cooled to 0 °C before water (6 mL) was added. On
a preparative scale, the intermediate acetonide 525 was not isolated. NaIO\(_4\) (3.83 g, 17.9
mmol) was added to the reaction vessel before tert-butyl hydroxycarbamate (2.91 g, 21.8
mmol) in 40 mL of methanol was added dropwise. To ensure complete dissolution 40 mL
of CH\(_2\)Cl\(_2\) were added. After addition, the solution was allowed to warm to room
temperature and stirred for 16 h. Upon completion of the reaction (TLC analysis), an
excess of saturated aqueous sodium bisulfite was added carefully until a light straw color
was obtained. The mixture was extracted with Et\(_2\)O (3 × 100 mL), the organic phase was
washed with brine (2 × 15 mL) and dried over Na\(_2\)SO\(_4\), and the solvent was removed in
vacuo. The oxazine 526 was isolated by flash column chromatography (hexanes/EtOAc 4:1) as a colourless solid affording (5.31 g, 74%).

$R_f$ 0.6 (hexanes/EtOAc 3:1); mp 155 °C (EtOH), $[\alpha]^{20}_{D} + 25.3$ (c = 1.0, CHCl$_3$); IR (CHCl$_3$, cm$^{-1}$) v 3072, 2986, 2936, 1752, 1608, 1472, 1456, 1383, 1294, 1273, 1250, 1210, 1155, 1114, 1071, 1035, 1009, 987, 952, 895, 870, 796, 773, 728, 709, 616, 516, 456; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.45 (d, $J$ = 8.1 Hz, 1H), 6.35 (dd, $J$ = 8.4, 5.7 Hz, 1H), 4.94-4.92 (m, 1H), 4.56 (s, 2H), 1.42 (s, 3H), 1.31 (s, 3H), 1.29 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 156.8, 133.8, 131.3, 111.3, 87.4, 83.2, 81.4, 74.2, 53.3, 28.0, 25.6, 25.4; MS (+EI) m/z (%) 348 (2), 173 (10), 124 (22), 100 (11), 96 (11), 94 (12), 85 (14), 83(15) 82(17), 57 (100); HRMS (+EI) calcd for C$_{14}$H$_{20}$BrNO$_5$+: 361.0525; found: 361.0528; Anal. calcd for C$_{14}$H$_{20}$BrNO$_5$: C, 46.42; H, 5.57. Found C, 46.51; H, 5.55.

**tert-Butyl [(3a$S$,4$R$,7$S$,7a$R$)-7-hydroxy-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxol-4-yl]carbamate (527)**

To a solution of 526 (1.02 g, 2.81 mmol) in THF:H$_2$O (70:7 mL) was added an aluminum amalgam prepared by dipping aluminum foil (2.5 g, 92.9 mmol) sequentially to NaOH (1 M), distilled water, HgCl$_2$ (0.5% solution), distilled water, THF. After overnight stirring, the reaction mixture was filtered through diatomaceous earth, washed by methanol (3 $\times$ 50 mL). Filtrate was evaporated, redissolved in toluene and evaporated again. The
reaction product was isolated by flash column chromatography (hexanes/EtOAc 1:1) affording 527 as colourless viscous oil (0.714 g, 89%).

\[ R_f \text{ 0.3 (hexanes/EtOAc 1:1); } \]

\[ ^1\text{H NMR (300 MHz, CDCl}_3\text{) } \delta 5.91 \text{ (ddd, } J = 9.8, 2.2, 2.2 \text{ Hz, 1H), 5.80 \text{ (ddd, } J = 9.8, 2.9, 1.0 \text{ Hz, 1H), 5.07 \text{ (b, 1H), 4.27-16 \text{ (m, 3H), 4.02 \text{ (m, 1H), 2.63(b, 1H), 1.45 (s, 12H), 1.35 (s, 3H).} } ^1\text{H NMR matched with lit. value.}^{235} \]

**tert-Butyl ((3aS,4R,7S,7aS)-7-[[tert-butyl(dimethyl)silyl]oxy]-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxol-4-yl)carbamate (528)**

\[ \text{To a solution of 527 (0.372 g, 1.3 mmol) in CH}_2\text{Cl}_2 \text{ (20 mL) was added imidazole (0.133g, 1.96 mmol) followed by TBSCl (0.215g, 1.43 mmol). After overnight stirring, the reaction mixture quenched by water (10 mL), extracted by CH}_2\text{Cl}_2 \text{ (3 } \times \text{ 10 mL), dried and evaporated. The reaction product was isolated by flash column chromatography (hexanes/EtOAc 8:1) affording 528 as a colourless liquid (0.425 g, 81%).} \]

\[ R_f \text{ 0.9 (hexanes/EtOAc 3:1); } [\alpha]^{20}_D - 0.8 \text{ (c = 1.0, CHCl}_3\text{), IR (CHCl}_3\text{, cm}^{-1}\text{) } \nu 3394, 3055, 2991, 2973, 2962, 2933, 2908, 2860, 1710, 1510, 1463, 1382, 1369, 1325, 1294, 1252, 1211, 1167, 1097, 1059, 1038, 978, 966, 937, 914; ^1\text{H NMR (300 MHz, CDCl}_3\text{) } \delta 5.98-5.93 \text{ (m, , 1H), 5.35 \text{ (d, } J = 8.7 \text{ Hz, 1H), 4.28-4.22 \text{ (m, 3H), 4.15-4.13 \text{ (m, 1H), 1.39 \text{ (s, 9H), 1.33 (s, 3H), 1.27 (s, 3H), 0.88 (s, 9H) 0.10 (s, 3H), 0.08 (s, 3H); } ^13\text{C NMR (75 MHz, CDCl}_3\text{) } \delta 155.2, 131.8, 131.2, 108.1, 79.2, 78.8, 67.1, 47.6, 28.3, 26.4, 25.7, 24.4, 17.9, -4.80, -4.99; MS (+EI) m/z (%) 299 (16), 286 (37), 256 (23), 244 (13), 243 (75),}^{196} \]
238 (16), 228 (54), 225 (17), 215 (24), 212 (17), 210 (38), 199 (28), 196 (11), 184 (14),
182 (12), 168 (24), 167 (100); HRMS (+EI) calcd for C<sub>20</sub>H<sub>37</sub>NO<sub>5</sub>Si<sup>+</sup>: 399.2441; found:
399.2435; Anal. calcd for C<sub>20</sub>H<sub>37</sub>NO<sub>5</sub>Si: C, 60.11; H, 9.33. Found C, 60.36; H, 9.23.

((3aS,4R,7S,7aS)-7-{{tert-Butyl(dimethyl)silyl}oxy}-2,2-dimethyl-3a,4,7,7a-
tetrahydro-1,3-benzodioxol-4-yl)amine (473)

\[
\begin{align*}
\text{QTBS} & \\
\text{NH}_2
\end{align*}
\]

Trifluoroacetic acid (7 mL) was added dropwise to a solution of 528 (6.01 g, 15.04 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (150 mL) at 0 °C. After 15 min reaction has been neutralised by
addition of concentrated ammonia (50 mL). Organic layer was extracted by CH<sub>2</sub>Cl<sub>2</sub> 3 ×
50 mL, washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The free amine 473, 3.86 g (86%), was obtained as colourless oil and of sufficient purity to use in the next step.
Analytical sample of 473 was purified by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH 10:1).

\[R_f 0.8 \text{ (CH}_2\text{Cl}_2/\text{CH}_3\text{OH 10:1); } [\alpha]^{20}_D + 15.5 \text{ (c = 1.0, CHCl}_3); \text{ IR (CHCl}_3, \text{ cm}^{-1}) \nu 3424,
3371, 3323, 3048, 2985, 2955, 2933, 2898, 2858, 1677, 1635, 1600, 1467, 1383, 1255,
1212, 1163, 1117, 1060, 887, 839, 780; \text{ }^1\text{H NMR (300 MHz, CDCl}_3) \delta 5.73 \text{ (dt, } J = 9.8,
2.3 \text{ Hz, 1H), 5.65 \text{ (dt, } J = 9.8, 2.3 \text{ Hz, 1H), 4.19-4.16 \text{ (m, 1H), 4.11 \text{ (dd, } J = 7.8, 4.8 \text{ Hz},
1H), 3.89 \text{ (dd, } J = 7.5, 6.3 \text{ Hz, 1H), 3.28 \text{ (dd, } J = 5.7, 2.1 \text{ Hz, 1H), 1.66 \text{ (s, 2H), 1.44 \text{ (s},
3H), 1.34 \text{ (s, 3H), 0.91 \text{ (s, 9H) 0.12 \text{ (s, 3H), 0.11 \text{ (s, 3H); } ^13\text{C NMR (75 MHz, CDCl}_3) \delta}}
\]
132.3, 131.4, 108.7, 81.0, 80.6, 71.4, 52.0, 27.1, 25.8, 24.7, 18.3, −4.6, −4.9; MS (+EI) m/z (%) 242 (10), 215 (12), 212 (33), 200 (17), 199 (100); HRMS (+EI) calcd for C_{15}H_{29}NO_3Si+: 299.1917; found: 299.1903; Anal. calcd for C_{15}H_{29}NO_3Si: C, 60.16; H, 9.76. Found C, 60.31; H, 9.57.

N-((3aS,4R,7S,7aS)-7-[[tert-Butyl(dimethyl)silyl]oxy]-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxol-4-yl)-3-iodopyridine-2-carboxamide (529)

To a solution of lithium salt of 3-iodopicolinic acid\(^{227}\) (3.23 g, 12.68 mmol) in DMF (60 mL) was added HBTU (3.93 g, 10.35 mmol), stirred for 5 min at 0 °C, followed by solution of 473 (3.67 g, 9.41 mmol) and diisopropylethylamine (2.46 mL, 14.12 mmol) in CH\(_2\)Cl\(_2\) (100 ml). After 1 h stirring, the reaction mixture quenched by water (50 mL), extracted by CH\(_2\)Cl\(_2\) (3 × 100 mL), dried over Na\(_2\)SO\(_4\) and evaporated. The reaction product was isolated by flash column chromatography (hexanes/EtOAc 4:1) affording 529 as a colourless oil (3.27 g, 66%).

R\(_f\) 0.85 (hexanes/EtOAc 2:1); [\(\alpha\)]\(_{20}\)D = −38.3 (c = 1.0, CHCl\(_3\)); IR (CHCl\(_3\), cm\(^{-1}\)) ν 3387, 2986, 2953, 2930, 2901, 2856, 1678, 1507, 1462, 1456, 1383, 1254, 1212, 1164, 1120, 1061, 1014; \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 8.50 (dd, \(J = 4.5, 1.5\) Hz, 1H), 8.31 (dd, \(J = 8.2, 1.5\) Hz, 1H), 8.21 (d, \(J = 9.1\) Hz, 1H), 7.08 (dd, \(J = 7.9, 4.5\) Hz, 1H), 6.06 (dd, \(J = 9.8, 4.2\) Hz, 1H), 5.98 (dd, \(J = 9.8, 4.9\) Hz, 1H), 4.78-4.76 (m, 1H), 4.41 (dd, \(J = 7.2, 3.8\) Hz, 1H), 4.15 (dd, \(J = 7.0, 3.8\) Hz, 1H), 3.74-3.71 (m, 1H), 2.58-2.48 (m, 2H), 2.40 (s, 3H), 2.36 (s, 3H), 1.91 (s, 3H), 1.40 (s, 9H), 0.86 (s, 6H).
Hz, 1H), 4.30 (dd, $J = 7.2, 3.4$ Hz, 1H), 4.27 (dd, $J = 4.2, 3.4$ Hz, 1H) 1.42 (s, 3H), 1.33 (s, 3H), 0.9 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H); $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 163.7, 150.4, 148.8, 147.2, 132.9, 129.5, 126.2, 108.5, 89.6, 76.7, 68.2, 47.7, 26.7, 25.8, 25.7, 24.6, 18.2, −4.7, −4.8; MS (+EI) $m/z$ (%) 515 (6), 436 (26), 430 (31), 415 (29), 305 (14), 298 (24), 289 (12), 282 (18) 249 (30), 232 (75), 215 (15), 205 (12), 204 (53), 185 (13), 168 (15), 167 (83), 157 (20), 150 (10), 149 (35), 128 (10), 106 (16), 86 (20), 85 (10), 84 (32), 79 (19), 78 (33), 77 (26), 76 (13), 75 (100); HRMS (+EI) calcd for C$_{20}$H$_{28}$IN$_2$O$_4$Si$^+$: 515.0863; found: 515.0868; Anal. calcd for C$_{21}$H$_{31}$IN$_2$O$_4$Si: C, 47.55; H, 5.89. Found C, 47.84; H, 5.90.

**tert-Butyl ((3aS,4R,7S,7aS)-7-{{tert-butyl(dimethyl)silyl}oxy}-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxol-4-yl){{3-iodopyridin-2-yl}carbonyl}carbamate (475)**

![Chemical structure of 475](image)

To a solution of amide 529 (0.090 g, 0.17 mmol) in acetonitrile (2 mL) was added di-tert-butylidicarbonate (0.083 g, 0.38 mmol), and DMAP (0.047 g, 0.38 mmol). After 2 h stirring, the reaction mixture quenched by water (5 mL), extracted by CH$_2$Cl$_2$ (3 $\times$ 10 mL), dried and evaporated. The reaction product was isolated by flash column chromatography (hexanes/EtOAc 4:1) affording 475 as a colourless oil (0.098 g, 92%). $R_t$ 0.9 (hexanes/EtOAc 2:1); $[\alpha]_{D}^{20} = -11.0$ (c = 1.0, CHCl$_3$); IR (CHCl$_3$, cm$^{-1}$) $\nu$ 3449, 3050, 2983, 2955, 2934, 2901, 2858, 1743, 1684, 1473, 1461, 1430, 1387, 1353, 1343,
1254, 1218, 1154, 1112, 1062, 1010, 888, 839, 777; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 8.55 (dd, \(J = 4.8, 1.2\) Hz, 1H), 8.14 (dd, \(J = 8.1, 1.5\) Hz, 1H), 7.07 (dd, \(J = 8.1, 4.8\) Hz, 1H), 5.71 (s, 2H), 5.26 (br.s, 1H), 4.67 (m, 1H), 4.24 (m, 1H), 4.16 (m, 1H), 1.51 (s, 3H), 1.37 (s, 3H), 1.22 (s, 9H), 0.94 (s, 9H), 0.16 (s, 3H), 0.14 (s, 3H); \(^1^3\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 169.2, 158.7, 151.6, 147.6, 146.6, 131.1, 126.4, 124.8, 108.6, 89.0, 84.4, 80.1, 75.1, 71.2, 27.8, 27.5, 25.9, 25.7, 18.2, −4.5, −4.9; MS (+EI) \(m/z\) (%) 530 (10), 473 (32), 459 (16), 430 (19), 415 (40), 305 (11), 298 (19), 282 (14) 260 (38), 249 (44), 232 (63), 204 (37), 168 (15), 167 (68), 157 (12), 57 (100); HRMS (+EI) calcld for C\(_{26}\)H\(_{39}\)N\(_2\)O\(_6\)Si: 630.1622; found: 630.1615; Anal. calcld for C\(_{26}\)H\(_{39}\)N\(_2\)O\(_6\)Si: C, 49.52; H, 6.23. Found C, 49.66; H, 6.24.

**tert-Butyl (3aS,3bR,11S,11aS)-11-{
[tert-butyl(dimethyl)silyl]oxy}-2,2-dimethyl-5-oxo-3a,5,11,11a-tetrahydro[1,3]benzodioxolo[5,4-f]-1,7-naphthyridine-4(3bH)-carboxylate (530)**

To a solution of 475 (1.30 g, 2.06 mmol) in toluene (45 mL) was added Pd(OAc)\(_2\) (0.093 g, 0.41 mmol) and Ag\(_3\)PO\(_4\) (0.520 g, 1.24 mmol). The reaction mixture was degassed by passing argon for 5 min followed by addition of 1,2-bis(diphenylphosphino)ethane (0.164 g, 0.41 mmol). After addition of dppe the reaction mixture was refluxed for 18 h. Product was isolated by flash column chromatography (hexanes/EtOAc 2:1 → 1:1) affording 0.32
g (31%, 46% based on recovered starting material) of 530 as a colourless oil and 0.440 g of starting material.

$R_f$ 0.2 (hexanes/EtOAc 1:1); $[\alpha]^{20}_D + 18.1$ (c = 1.0, CHCl$_3$); IR (CHCl$_3$, cm$^{-1}$) ν 3450, 3074, 2984, 2955, 2933, 2903, 2858, 1756, 1691, 1634, 1469, 1382, 1254, 1215, 1155, 1125, 1068, 1014, 964, 920, 843, 782; $^1$H NMR (300 MHz, CDCl$_3$) δ 8.73 (dd, $J = 4.2$, 0.9 Hz, 1H), 7.99 (dd, $J = 8.1$, 1.2 Hz, 1H), 7.44 (dd, $J = 8.4$, 4.5 Hz, 1H), 6.51-6.49 (m, 1H), 4.90 (dt, $J = 7.8$, 2.4 Hz 1H), 4.46-4.44 (m, 1H), 4. (dd, $J = 8.1$, 2.7 Hz, 1H), 1.61 (s, 9H), 1.50 (s, 3H), 1.33 (s, 3H), 0.96 (s, 9H), 0.18 (s, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 159.5, 153.2, 150.8, 141.5, 131.2, 130.3, 128.4, 126.9, 125.8, 111.7, 84.3, 79.9, 79.7, 73.6, 57.9, 27.6, 26.9, 25.8, 25.2, 18.2, - 4.5, - 5.0; MS (+FAB) $m/z$ (%) 503 (5), 419 (12), 404 (77), 303 (12), 287 (10), 185 (13), 185 (13), 75 (10), 73 (100); HRMS (+FAB) calcd for C$_{26}$H$_{39}$N$_2$O$_6$Si$^+$: 503.2577; found: 503.2538.

*tert*-Butyl (3aS,3bR,11S,11aR)-11-hydroxy-2,2-dimethyl-5-oxo-3a,5,11,11a-tetrahydro[1,3]benzodioxolo[5,4-f]-1,7-naphthyridine-4(3bH)-carboxylate (531)

Naphthridone 530 (0.121 g, 0.024 mmol) was dissolved in THF (2.5 mL) and cooled to 0 °C. A solution of TBAF in THF (1 M, 0.25 mL, 0.25 mmol) was added dropwise over 1 min. The reaction mixture was stirred until total consumption of starting material was
observed (TLC). The reaction mixture was quenched with solution of NH₄Cl (2 ml, sat. aq. solution) and extracted with EtOAc (6 × 15 mL). The combined organic phases were dried over Na₂SO₄, filtered and evaporated. Compound 531 was isolated by column chromatography (CH₂Cl₂/methanol 100:1) as white crystalline solid (0.072 mg, 77%).

Rᶠ 0.8 (CH₂Cl₂/methanol 10:1); mp > 200 °C (CH₂Cl₂); [α]₂⁰⁰D − 4.1 (c = 0.2, CHCl₃); IR (CHCl₃, cm⁻¹) ν 3431, 2983, 2935, 2877, 1765, 1684, 1627, 1473, 1384, 1248, 1217, 1153, 1115, 1070; ¹H NMR (300 MHz, CDCl₃) δ 8.69 (d, J = 3.9 Hz, 1H), 8.00 (d, J = 8.1 Hz, 1H), 7.42 (dd, J = 8.1, 4.5 Hz, 1H), 6.72 (t, J = 2.7 Hz, 1H), 4.87-4.84 (m, 1H), 4.53-4.52 (m, 1H), 4.38 (br s, 1H), 4.25-4.14 (m, 1H), 1.56 (s, 9H), 1.49 (s, 3H), 1.33 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 159.6, 152.9, 150.6, 141.1, 130.7, 128.7, 127.2, 125.4, 111.9, 84.5, 79.7, 79.2, 72.5, 58.2, 27.4, 26.9, 25.1; MS (+FAB) m/z (%) 389 (5), 290 (12), 289 (60), 243 (14), 242 (74), 186 (44), 185 (13), 184 (15), 142 (22), 81 (12), 77 (10), 73 (18), 69 (24), 67 (12), 57 (100); HRMS (+FAB) calcd for C₂₀H₂₅N₂O₆⁺:389.1713; found: 389.1694.

(6aR,7S,8R,9S)-7,8,9-Trihydroxy-5-oxo-5,6,6a,7,8,9-hexahydrobenzo[f]-1,7-naphthyridin-4-ium chloride (6)
Compound 531 (0.072 g, 0.185 mmol) was taken up in methanol (2.5 mL) and HCl (conc., 0.25 mL) was added dropwise. The reaction mixture was stirred at r.t. until total consumption of starting material was observed (TLC). The reaction mixture was evaporated and dried in vacuo and off-white crystals of hydrochloride of 6 were obtained (53 mg, quantitative). Upon chromatography on silica gel (deactivated with 10% w/w water, eluent CH₂Cl₂/MeOH/NH₃=90:10:1) free base of 6 was isolated (0.040 g, 86 %).

Rf 0.35 (CH₂Cl₂/methanol/ammonia 80:35:4); mp > 200 °C (methanol); [α]⁰²D − 10 (c = 0.5, H₂O); IR (CHCl₃, cm⁻¹) ν 3130, 3037, 2850, 2877, 1655, 1401, 1322, 1087, 1029; for 6·HCl: ¹H NMR (600 MHz, D₂O) δ 8.74-8.72 (m, 2H), 8.07 (s, 1H), 6.55 (t, J = 3.0 Hz, 1H), 4.51 (d, J = 8.4 Hz, 1H), 4.34 (t, J = 2.4 Hz, 1H), 3.98 (dd, J = 9.0, 2.4 Hz, 1H), 3.96 (s, 1H); ¹³C NMR (150 MHz, D₂O) δ 158.8, 142.8, 141.8, 136.4, 134.6, 130.1, 129.3, 126.9, 72.2, 68.6, 68.4, 52.1; MS (+FAB) m/z (%) 389 (5), 290 (12), 289 (60), 243 (14), 242 (74), 186 (44), 185 (13), 184 (15), 142 (22), 81 (12), 77 (10), 73 (18), 69 (24), 67(12), 57 (100); HRMS (+FAB) calcd for C₁₂H₁₃N₂O₄⁺:249.0875; found: 249.0847.

(6aR,7S,8R,9S)-7,8,9-trihydroxy-6a,7,8,9-tetrahydrobenzo[f]-1,7-naphthyridin-5(6H)-one 4-oxide (476)
Triol 6 (0.052 g, 0.21 mmol) was dissolved in a mixture of methanol (2.5 mL) and CHCl₃ (7.5 mL) and 100% m-chloroperbenzoic acid (103 mg, 0.6 mmol) was added in one portion. The reaction mixture was stirred at r.t. until consumption of starting material (TLC). After that period the reaction mixture was evaporated and subjected to a column chromatography on silica gel (deactivated with 10% w/w water, eluent CH₂Cl₂/MeOH/NH₃=80:35:4) to afford off-white crystals of 476 (0.014 g, 25%).

*R* 0.05 (CH₂Cl₂/methanol/ammonia 80:35:4); mp > 200 °C (methanol); [α]°D + 733 (c = 0.1, methanol); IR (CHCl₃, cm⁻¹) ν 3483, 2982, 2930, 2877, 1740, 1670, 1627, 1473, 1400, 1265, 1212, 1155, 1112, 1074; ¹H NMR (600 MHz, CD₃OD) δ 8.35 (d, *J* = 6.6 Hz, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 6.52 (dd, *J* = 4.2, 1.8 Hz, 1H), 6.52 (d, *J* = 2.5 Hz, 1H), 4.36 (d, *J* = 8.4 Hz, 1H), 4.30 (t, *J* = 3.6 Hz 1H), 3.97 (dd, *J* = 8.4, 2.4 Hz, 1H), 3.94 (br s, 1H); ¹³C NMR (600 MHz, CD₃OD) δ 158.4, 141.5, 137.8, 135.4, 129.3, 128.0, 127.5, 123.1, 72.7, 69.3, 69.1, 52.2; MS (+FAB) *m/z* (%) 265 (34), 207 (10), 192 (14), 177 (16), 176 (34), 171 (19), 165 (10), 163 (16), 151 (10), 149 (15), 136 (41), 121 (12), 113 (10), 109(20), 107 (25), 105 (21), 55 (100); HRMS (+FAB) calcd for C₁₂H₁₃N₂O₅⁺:265.0825; found: 265.0826.

**5-bromo pyridine-2,3-diol (534)**

\[
\begin{align*}
\text{HO} & \quad \text{Br} \\
\text{HO} & \quad \text{N}
\end{align*}
\]

Freshly distilled furfural (51 mL, 0.615 mol) was mixed with 700 g of ice and bromine (32 mL, 0.615 mol) was added dropwise to a vigorous stirred reaction mixture while maintaining temperature 0 °C. After 30 min of stirring HCl (conc., 30 mL) was added in
one portion and stirred for an additional 30 min. Bromine (32 mL, 0.615 mol) was added dropwise while maintaining temperature −5 °C in a period of 1 h. The reaction mixture was then filtered and sulfamic acid (60 g, 0.62 mol) was added to a filtrate and vigorously stirred for 1.5 h at 50 °C. The reaction mixture was cooled to 10 °C and filtered. Precipitate was dried for 18 h at 50 °C, then refluxed in glacial acetic acid with charcoal, and recrystallized from acetic acid. Crystals were washed with distilled water till neutral reaction and dried in vacuo to obtain 534 as grey crystals (76 g, 65%).

mp 248-250 °C (AcOH), [lit.202 249 °C (AcOH)].

6-bromo[1,3]dioxolo[4,5-b]pyridine (533)

To a solution of 5-bromo pyridine-2,3-diol 534 (0.700 g, 3.7 mmol) in 30 ml of DMF was added K$_2$CO$_3$(0.50 g, 7.4 mmol), CH$_2$Br$_2$ (0.75 ml, 7.4 mmol) and CuO (0.120 g, 0.74 mmol). Mixture was heated to 95 °C for 6 h, then cooled down, filtered, diluted with H$_2$O (300 mL) and extracted with EtOAc (5 × 10 ml). Organic phases were dried over Na$_2$SO$_4$ and subjected to column chromatography (Hex/EtOAc 4:1). Product was isolated as white needles (0.135 g, 18%).

mp 65-67°C (Hexanes-EtOAc), [Lit.202 69-71°C (EtOH)]; $^1$H NMR (300 MHz, CDCl$_3$) δ 7.71 (d, $J =$ 1.9 Hz, 1 H), 7.12 (d, $J =$ 1.9 Hz, 1 H), 6.10 (s, 2H).

6-bromo-7-methoxy[1,3]dioxolo[4,5-b]pyridine (532)
To a solution of 2,2,6,6-tetramethylpiperidine (6.04 mL, 35.5 mmol) in THF (50 mL) at -50°C nBuLi (14.30 mL, 30.8 mmol) was added dropwise. Reaction mixture was stirred for 15 min at −30 °C, then cooled down to −80 °C and solution of 533 (4.88 g, 23.7 mmol) in THF (20 mL) was added dropwise, while maintaining temperature −80 °C. Reaction mixture was stirred for an additional 30 min, followed by addition of B(OMe)₃ (6.6 mL, 59.1 mmol) in one portion and reaction mixture was warmed up to 0 °C within 30 min. Then reaction mixture was cooled to −50 °C and glacial acetic acid (6.76 mL, 118 mmol) was added, followed by addition of UHP (10 g) and stirred overnight at r.t. Next morning excess of peroxide was quenched by NaHSO₃ (sat. aq.), extracted with EtOAc (3×150 mL). Organic layer was dried over Na₂SO₄, evaporated and redissolved in THF:MeOH mixture (10:1, 200 mL) at 0 °C. Ethereal solution of diazomethane was added dropwise until disappearance of starting material was observed (TLC). Reaction mixture was evaporated and subjected to flash column chromatography (silica gel, hexane/EtOAc 4:1) to yield 532 as a white crystalline compound (2.0 g, 36%).

*R* 0.45 (hexanes/EtOAc 4:1); mp 119-121 °C (CHCl₃); IR (film, cm⁻¹) ν 3092, 3003, 2957, 2924, 2853, 1786, 1614, 1492, 1472, 1431, 1401, 1277, 1268, 1232, 1202, 1146, 1091, 1033, 982; ¹H NMR (600 MHz, CDCl₃) δ 7.74 (s, 0.75 H), 7.62 (s, 0.2 H), 6.05 (s, 2H), 4.21 (s, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 159.6, 145.7, 141.6, 139.1, 127.3, 105.4, 100.5, 100.4, 59.8; MS (+EI) *m/z* (%) 233 (97), 231 (100), 203 (23), 187 (43), 175
(16), 173 (16), 160 (88), 158 (84), 132 (31), 131 (33), 130 (33), 129 (12); HRMS (+EI) calcd for C<sub>7</sub>H<sub>6</sub>BrNO<sub>3</sub>: 232.9512; found: 232.9507.

5-bromo-7-methoxy[1,3]dioxolo[4,5-b]pyridine (538)

To a solution of 2,2,6,6-tetramethylpiperidine (0.320 mL, 1.86 mmol) in THF (5 mL) at -50°C nBuLi (0.770 mL, 1.82 mmol) was added dropwise. Reaction mixture was stirred for 15 min at -30 °C, then cooled down to −80 °C and added dropwise to a solution of 532 (0.206 g, 0.89 mmol) in THF (5 mL) while maintaining temperature −80 °C. Reaction mixture was stirred for 20 min at this temperature, followed by consecutive quenching with methanol (1 mL) and solution of NH<sub>4</sub>Cl (sat. aq., 2 mL). Reaction mixture was extracted with EtOAc (3 × 30 mL). Organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, evaporated subjected to flash column chromatography (silica gel, hexane/EtOAc 4:1) to yield 538 as a white crystalline compound (0.110 g, 54%).

R<sub>f</sub> 0.40 (hexanes/EtOAc 4:1); mp 113-115 °C (CHCl<sub>3</sub>); IR (film, cm<sup>−1</sup>) ν 3107, 3003, 2950, 2924, 1789, 1632, 1591, 1504, 1476, 1453, 1428, 1417, 1334, 1281, 1191, 1129, 1105, 1030, 958, 921, 913, 870, 830 775; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.69 (s, 1H), 6.05 (s, 2H), 3.97 (s, 3H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 158.9, 149.9, 141.6, 130.4, 126.9, 109.2, 100.9, 57.2; MS (+EI) m/z (%) 233 (100), 231 (98), 203 (13), 201 (13), 160
(78), 158 (73), 132 (18), 130 (19), 94 (12); HRMS (+EI) calcd for C_{7}H_{6}BrNO_{3}: 232.9512; found: 232.9516.

5-bromo-7-methoxy[1,3]dioxolo[4,5-b]pyridine-6-carbaldehyde (539)

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      OMe
  O       CHO
     |      |
     N----Br
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To a solution of 2,2,6,6-tetramethylpiperidine (0.137 mL, 0.81 mmol) in THF (5 mL) at −50°C nBuLi (0.324 mL, 0.77 mmol) was added dropwise. Reaction mixture was stirred for 15 min at −30 °C, then cooled down to -80 °C and added dropwise to a solution of 532 (0.170 g, 0.73 mmol) in THF (5 mL) while maintaining temperature −80 °C. The reaction mixture was stirred for 20 min at this temperature, followed by quenching with dry DMF (1 mL), followed by addition of NH_{4}Cl (sat. aq., 2 mL). The reaction mixture was extracted with EtOAc (3 × 20 mL). Organic layer was dried over Na_{2}SO_{4}, evaporated, and subjected to flash column chromatography (silica gel, hexane/EtOAc 4:1 to 2:1) to yield 539 (0.015 g, 8%) as a off-white crystalline compound and 538 as white crystalline compound (0.062 g, 36%).

R_{f} 0.2 (hexanes/EtOAc 2:1); mp 130-135 °C (CH_{2}Cl_{2}); IR (film, cm⁻¹) ν 3110, 3000, 2950, 2825, 2720, 1730, 1630, 1600, 1496, 1466, 1432, 1418, 1411, 1334, 1281, 1191, 1129, 1105, 1040, 960; ¹H NMR (300 MHz, CDCl_{3}) δ 10.22 (s, 1H), 6.13 (s, 2H), 4.23 (s, 3H); ¹³C NMR (150 MHz, CDCl_{3}) δ 188.8, 161.1, 150.2, 137.2, 127.0, 118.0, 101.1, 60.4; MS (+EI) m/z (%) 261 (97), 259 (100), 244 (16), 243 (12), 229 (14), 201 (10), 188
(39), 186 (40), 150 (49), 133 (13), 132 (24), 131 (13), 130 (23), 122 (12), 94 (16); HRMS (+EI) calcd for C₈H₆BrNO₄: 258.9480; found: 258.9470.

5-bromo-N-((3aS,4R,7S,7aS)-7-{{tert-butyl(dimethyl)silyl}oxy}-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxol-4-yl)-7-methoxy[1,3]dioxolo[4,5-b]pyridine-6-carboxamide (541)

A. Synthesis from 532:

To a solution of 2,2,6,6-tetramethylpiperidine (0.240 mL, 1.40 mmol) in THF (30 mL) at −50°C nBuLi (0.640 mL, 1.37 mmol) was added dropwise. The reaction mixture was stirred for 15 min at −30 °C, then cooled down to −85 °C and was added dropwise to a solution of 532 (0.310 g, 1.336 mmol) in THF (20 mL), while maintaining temperature -85°C. The reaction mixture was stirred for 30 min at this temperature, followed by a quick addition to a solid CO₂ and evaporation. Solid residue was redissolved in acetonitrile (50 mL) and HBTU (0.455 g, 1.6 mmol) was added, followed by DIPEA (0.470 mL, 2.67 mmol) and solution of amine 473 (0.480 g, 1.6 mmol) in CH₂Cl₂ (2 mL). Reaction mixture was stirred for five hours, quenched with NH₄Cl (sat. aq., 2 mL) extracted with EtOAc (3 × 10 mL). Combined organic layer was dried over Na₂SO₄, evaporated subjected to flash column chromatography (silica gel, hexane/EtOAc 3:1 to 2:1) to yield 541 as a white crystalline compound (0.224 g, 30% based on 532).
B. Synthesis from 538:

To a solution of 2,2,6,6-tetramethylpiperidine (0.070 mL, 0.41 mmol) in THF (10 mL) at −50°C nBuLi (0.173 mL, 0.41 mmol) was added dropwise. The reaction mixture was stirred for 15 min at −30 °C, then cooled down to −85 °C and solution of 538 (0.091 g, 0.39 mmol) in THF (4 mL) was added dropwise to LTMP, while maintaining temperature at −85 °C. The reaction mixture was stirred for 3 min at this temperature, followed by a quick addition to a solid CO2 and evaporation. Solid residue was redissolved in acetonitrile (5 mL) and HBTU (0.178 g, 0.47 mmol) was added, followed by DIPEA (0.101 g, 0.784 mmol) and solution of amine 473 (0.130g, 0.43 mmol) in CH2Cl2 (2 mL). Reaction mixture was stirred for five hours, quenched with NH4Cl (sat. aq., 2 mL) extracted with EtOAc (3 × 10 mL). Combined organic layer was dried over Na2SO4, evaporated subjected to flash column chromatography (silica gel, hexane/EtOAc 3:1 to 2:1) to yield 541 as a white crystalline compound (0.114 g, 52% based on 538).

RF 0.6 (hexanes/EtOAc 3:2); mp 153-161°C (CHCl3); [α]D20 − 11.9 (c = 1, MeOH); IR (film, cm⁻¹) ν 3641, 3402, 3264, 3054, 2956, 2931, 2858, 2720, 1654, 1624, 1596, 1535, 1502, 1466, 1439, 1413, 1394, 1344, 1324, 1303, 1258, 1212, 1164, 1103, 1061, 999, 841; 1H NMR (300 MHz, CDCl3) δ 6.73 (d, J = 9.5 Hz, 1H), 6.17 (d, J = 2.5 Hz, 2H), 6.02 (s, 2H), 4.88-4.85 (m, 1H), 4.58 – 4.49 (m, 1H), 4.36 (d, J = 6.8 Hz, 1H), 4.21 (s, 1H), 4.09 (s, 3H), 1.36 (s, 3H), 1.31 (s, 3H), 0.72 (s, 9H), 0.06 (s, 3H), 0.01 (s, 3H); 13C NMR (75 MHz, CDCl3) δ 163.3, 159.2, 146.9, 132.7, 130.4, 127.9, 126.3, 122.0, 108.4, 100.6, 78.3, 77.1, 76.4, 66.3, 59.9, 45.9, 26.3, 25.4, 24.4, 17.6, -4.9, -5.1; MS (+EI) m/z (%) 501(10), 499 (10), 458 (10), 284 (14), 282 (13), 260 (32), 258 (33), 246 (26), 243
tert-butyl [(5-bromo-7-methoxy[1,3]dioxolo[4,5-b]pyridin-6-yl)carbonyl][(3aS,4R,7S,7aS)-7-{{tert-butyl(dimethyl)silyl}oxy}-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxol-4-yl]carbamate (478)

To a solution of amide 541 (0.568 g, 1.02 mmol) in acetonitrile (20 mL) was added di-tert-butyl dicarbonate (0.444 g, 2.04 mmol), and DMAP (0.25 g, 2.07 mmol). The reaction was stirred for 12 h and then quenched with water (5 mL), extracted by CH₂Cl₂ (3 × 20 mL), dried and evaporated. The product was isolated by flash column chromatography (hexanes/EtOAc 4:1) affording 478 as a colorless oil (0.470 g, 70%).

$R_f$ 0.8 (hexanes/EtOAc 2:1); [$\alpha$]$_{D}^{17}$ + 19.4 (c = 1, CHCl₃); IR (film, cm$^{-1}$) ν 3431, 2959, 2932, 2858, 1742, 1680, 1625, 1598, 1466, 1438, 1418, 1396, 1371, 1332, 1261, 1235, 1154, 1103, 1061, 1020; $^1$H NMR (600 MHz, Acetone-d₆) δ 6.25 (s, 1H), 6.20 (s, 1H), 5.71 – 5.63 (m, 2H), 5.26 (s, 1H), 4.61 (dd, $J$ = 6.6, 4.8 Hz, 1H), 4.27 – 4.23 (m, 1H), 4.15 (d, $J$ = 1.2 Hz, 3H), 4.09 (dd, $J$ = 12.6, 6.0 Hz, 1H), 4.48 (d, $J$ = 3.0 Hz, 3H), 1.35 (d, $J$ = 4.2 Hz, 9H), 1.34 (d, $J$ = 4.8 Hz, 3H), 0.96 (s, 9H), 0.19 (s, 3H), 0.18 (s, 3H); $^{13}$C NMR (151 MHz, Acetone) δ 165.0, 164.8, 159.2, 159.1, 151.5, 146.3, 145.9, 130.5, 130.4, 127.1, 126.9, 125.7, 124.6, 124.0, 123.9, 108.1, 108.1, 101.3, 84.0, 84.0, 79.8,
79.8, 75.3, 71.3, 71.3, 59.7, 59.7, 27.3, 27.2, 26.9, 25.4, 24.9, 17.8, −5.1, −5.54, HRMS (+EI): [M−CH₃−tBu−CO₂]⁺ calcd for C₂₂H₃₀BrN₂O₇Si: 541.1000; found: 541.1004.

**tert-Butyl (3aS,3bR,12S,12aS)-12-[[tert-butyl(dimethyl)silyl]oxy]-6-methoxy-2,2-dimethyl-5-oxo-3a,5,12,12a-tetrahydro[1,3]benzodioxolo[4,5-h][1,3]dioxolo[4,5-b]-1,6-naphthyridine-4(3bH)-carboxylate (542)**

![Chemical Structure Image]

To a solution of 478 (0.30 g, 0.457 mmol) in toluene (35 mL) was added Pd(OAc)₂ (0.025 g, 0.113 mmol) and Ag₃PO₄ (0.140 g, 0.33 mmol). The reaction mixture was degassed by passing argon for 5 min followed by addition of 1,2-*bis* (diphenylphosphino)ethane (0.036 g, 0.091 mmol). After addition of dppe the reaction mixture was stirred at 95 °C for 20 h. Product was isolated by flash column chromatography (hexanes/EtOAc 2:1 → 1:1) affording 542 as a white solid (0.152 g, 58%, 69% based on recovered starting material) and 0.05 g of starting material.

*R*ₙ 0.3 (hexanes/EtOAc 2:1); mp 86-91 °C (CH₂Cl₂), [α]₁⁷⁻D = 29.5 (c = 1.0, CHCl₃); IR (film, cm⁻¹) ν 2981, 2952, 2929, 2856, 1759, 1681, 1624, 1610, 1579, 1475, 1434, 1399, 1370, 1295, 1246, 1226, 1188, 1157, 1140, 1101, 1054; ¹H NMR (600 MHz, CDCl₃) δ 6.98 (t, *J* = 3.0 Hz, 1H), 6.07 - 6.04 (m, 2H), 4.73 - 4.68 (m, 1H), 4.41 - 4.37 (m, 1H), 4.21 (s, 3H), 4.14 (t, *J* = 8.1 Hz, 1H), 4.05 (dd, *J* = 8.4, 6.0 Hz, 1H), 1.60 (s, 9H), 1.48 (s, 3H), 1.32 (s, 3H), 0.95 (s, 9H), 0.15 (s, 6H). ¹³C NMR (151 MHz, CDCl₃) δ 161.7, 160.5,
tert-Butyl (3aS,3bR,12S,12aS)-12-{{tert-butyl(dimethyl)silyl}oxy}-6-hydroxy-2,2-dimethyl-5-oxo-3a,5,12,12a-tetrahydro[1,3]benzodioxolo[4,5-h][1,3]dioxolo[4,5-b]-1,6-naphthyridine-4(3bH)-carboxylate (543)

To a solution of 542 (0.050 g, 0.086 mmol) in dry DMF (5 mL) was added LiCl (0.010 g), followed by three cycles of freeze-pump-thaw. The reaction mixture was heated to 90 °C for 4.5 h. The reaction was then cooled to room temperature, diluted with distilled water (50 ml) and extracted with EtOAc (6 × 15 mL). The combined organic phases were dried over Na2SO4, filtered, and concentrated. The product was isolated by column chromatography (hexanes/EtOAc, 3:1) as a white crystalline solid (0.027 g, 56%).

mp 169-175 °C (EtOAc); Rf 0.9 (hexanes:EtoAc 2:1); [α]20D + 36.9 (c = 1.0, CHCl3); IR (KBr, cm⁻¹) ν 3401, 2982, 2953, 2930, 2858, 1721, 1681, 1627, 1609, 1579, 1400, 1370, 1247, 1219, 1157, 1054, 835; ¹H NMR (600 MHz, CDCl3) δ 12.70 (s, 1H), 7.02 (t, J = 3.0 Hz, 1H), 6.12 (d, J = 3.3 Hz, 2H), 4.77 - 4.72 (m, 1H), 4.39 (dt, J = 5.3, 2.5 Hz, 1H), 4.19 (t, J = 8.1 Hz, 1H), 4.08 (dd, J = 8.1, 5.8 Hz, 1H), 1.62 (s, 9H), 1.50 (s, 3H), 1.34 (s,
3H), 0.96 (s, 9H), 0.17 (s, 6H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 166.3, 162.2, 151.8, 149.8, 144.4, 133.6, 125.6, 111.6, 103.5, 101.1, 85.3, 79.4, 77.0, 73.6, 58.4, 27.6, 27.0, 25.8, 25.1, 18.2, −4.54, −4.98; HRMS (+EI) calcd for C$_{27}$H$_{38}$N$_2$O$_9$Si: 562.2347; found: 562.2317.

tert-butyl (3aS,3bR,12S,12aR)-6,12-dihydroxy-2,2-dimethyl-5-oxo-3a,5,12,12a-tetrahydro[1,3]benzdioxolo[4,5-h][1,3]dioxolo[4,5-b]-1,6-naphthyridine-4(3bH)-carboxylate (544)

Pyridine 543 (24 mg, 0.043 mmol) was dissolved in THF (2.5 mL) and cooled to 0 °C. A solution of TBAF in THF (1 M, 0.10 mL, 0.10 mmol) was added dropwise over 1 min. The reaction mixture was stirred until total consumption of starting material was observed (TLC). The reaction mixture was quenched with NH$_4$Cl (sat. aq., 2 ml,) and extracted with EtOAc (6 × 15 mL). The combined organic phases were dried over Na$_2$SO$_4$, filtered and evaporated. Compound 544 was isolated by column chromatography (EtOAc) as white crystalline solid (17 mg, 89%).

$R_f$ 0.4 (hexanes:EtOAc 1:1); mp> 200°C (EtOAc); [α]$^D_{20}$ = 32.4 ($c = 0.35$, MeOH); IR (KBr, cm$^{-1}$) ν 3396, 2983, 2933, 1762, 1680, 1609, 1479, 1434, 1400, 1372, 1297, 1227, 1157, 1140, 1104, 1075, 1054; $^1$H NMR (300 MHz, CDCl$_3$) δ 12.59 (s, 1H), 7.02 (t, $J = 3.0$ Hz, 1H), 6.12 (d, $J = 4.8$ Hz, 2H), 4.78 - 4.69 (m, 1H), 4.48 - 4.40 (m, 1H),
4.33 (t, 6 = 8.1 Hz, 1H), 4.21 - 4.12 (m, 1H), 1.61 (s, 9H), 1.52 (s, 3H), 1.38 (s, 3H). 13C NMR (75 MHz, CDCl3) δ 166.1, 162.2, 151.4, 149.6, 144.0, 131.2, 126.2, 125.8, 112.0, 103.3, 101.2, 85.5, 79.2, 79.1, 77.0, 72.9, 58.6, 27.6, 27.0, 25.0; HRMS (+EI) calcd for C22H24N2O9: 448.1482; found: 448.1463.

(2S,3R,4S,4aR)-2,3,4,7-Tetrahydroxy-3,4a,5-tetrahydrobenzo[h][1,3]dioxolo[4,5-b]-1,6-naphthyridin-6(2H)-one (5c)

The Boc-protected amide 544 (0.024 g, 0.054 mmol) was dissolved in CH2Cl2 (4 mL) and cooled to 0 °C. Trifluoroacetic acid (0.2 mL 5% v/v H2O) was added dropwise and the reaction was stirred until total consumption of starting material was observed (TLC). The reaction mixture was neutralized with excess of concetrated NH3, evaporated and finally dried under high-vac. The final product was isolated by column chromatography on silica gel (deactivated by 10% of water, gradient CH2Cl2 - CH2Cl2:CH3OH 50:1 - CH2Cl2:CH3OH 20:1) as off-white crystalline compound (15 mg, 90%).

Rf 0.6 (CH2Cl2:MeOH 5:1); mp> 200°C (MeOH); [α]20D +4.1 (c =1, MeOH); IR (KBr, cm−1) ν 3202, 3054, 2958, 2924, 2857, 1664, 1628, 1593, 1541, 1509, 1458, 1432, 1398, 1258, 1138, 1025, 802; 1H NMR (600 MHz, CD3OD) δ 6.66 (s, 1H), 6.13 (s, 2H), 4.48 (d, 6 = 8.5 Hz, 1H), 4.31 – 4.25 (m, 1H), 3.95 (s, 1H), 3.93 (d, 6 = 9.1 Hz, 1H). 13C NMR (150 MHz, CD3OD) δ 169.1, 161.7, 161.6, 146.6, 130.0, 125.7, 117.8, 115.9, 101.2, 73.0, 69.2, 69.2, 51.9.
6. Selected spectra.
7. References

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8. Vita

Sergey Vshyvenko was born in Artemovsk, Ukraine on October 7th, 1984. He attended high school Liceum at DonNU in Donetsk, Ukraine, before moving onto university studies in Moscow State University, Moscow, Russia. While at Moscow State he studied under supervision of Dr. V. Nenajdenko. In 2009 he moved to St Catharines, Ontario to pursue graduate studies under tutelage of Professor Tomas Hudlický at Brock University. He is presently working towards completion his PhD degree in organic chemistry. His research interest include the synthesis of heterocycles, novel ways to form carbon-carbon and carbon-heteroatom bonds, and total synthesis of natural products.