Synthesis of 4-Hydroxycinnamic Amides of Di-, Tri-, and Tetraamines: Potential Insect Toxins

by

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THE ROLE OF GOLD IN ECONOMIC GROWTH AND DEVELOPMENT

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Last but the most important, I want to express my gratitude to God, my creator- “In whom I live and move and have my being”.

Solomon N.K. Fixon-Owoo
The development of a comprehensive strategy for the next fiscal year involves several key considerations. First, an assessment of current financial conditions and resource allocation is essential. This includes reviewing budgetary constraints, identifying areas of potential cost savings, and prioritizing investment opportunities. Secondly, external factors such as market trends, regulatory changes, and economic forecasts should be taken into account. A thorough analysis of these external influences helps in aligning the strategy with broader industry dynamics. Thirdly, internal capabilities and capacities must be evaluated, focusing on strengths and areas for improvement. This involves reviewing current processes, identifying inefficiencies, and planning for capacity expansion as necessary. Lastly, stakeholder engagement is crucial. Regular feedback from employees, customers, and other key stakeholders can provide valuable insights and help tailor the strategy to meet diverse needs and expectations. Overall, a well-rounded strategy should balance financial sustainability, market competitiveness, and organizational growth.
Dedication

With much love to my extraordinary wife Christie and to my wonderful daughter Karol-Denise (and siblings yet to come), all of whom bring so much joy to my life.
Abstract

The monoconjugates of phenolic acids (i.e. coumaric acid) with polyamines such as spermidine and spermine are strikingly similar to some toxins from spiders and predatory wasps. Many plants contain phenolic acid polyamine conjugates and there is some reliable information supporting their roles as plant defense chemicals. Eleven monoacylated compounds of diamines, triamines, tetraamines and oxa-polyamine amines were prepared in three to seven steps: 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32. The synthesis proceeds through stepwise construction of the polyamine backbone (as in 62 and 72), followed by protection and deprotection steps of the amino functions. Desymmetrization of readily available and prepared symmetrical polyamines is a key step in the synthesis. The protecting groups employed were tert-butoxycarbonyl (BOC) and trifluoroacetyl (TFA) group which were removed under different conditions: acid and base respectively. Deprotection and refunctionalization of the polyamine reagent demonstrated the versatility of these systems for N-acylation.
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<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>aq</td>
<td>aqueous</td>
</tr>
<tr>
<td>arom.</td>
<td>aromatic</td>
</tr>
<tr>
<td>(BOC)₂</td>
<td>di-tertbutyldicarbonate</td>
</tr>
<tr>
<td>Bu</td>
<td>butyl</td>
</tr>
<tr>
<td>CDCl₃</td>
<td>deuterated chloroform</td>
</tr>
<tr>
<td>¹³C-NMR</td>
<td>carbon nuclear magnetic resonance</td>
</tr>
<tr>
<td>cum</td>
<td>coumaroyl</td>
</tr>
<tr>
<td>DCC</td>
<td>N, N-dicyclohexycarbodiimide</td>
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<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N, N’-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionisation</td>
</tr>
<tr>
<td>ETFA</td>
<td>ethyl trifluoroacetate</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>Et₂O</td>
<td>diethyl ether</td>
</tr>
<tr>
<td>FAB-MS</td>
<td>fast atom bombardment mass spectroscopy</td>
</tr>
<tr>
<td>FT-NMR</td>
<td>Fourier transform nuclear magnetic resonance</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>HOBT</td>
<td>1-hydroxybenzotriazole hydrate</td>
</tr>
<tr>
<td>Rf</td>
<td>receding factor</td>
</tr>
<tr>
<td>Rt</td>
<td>retention time</td>
</tr>
<tr>
<td>R₂t</td>
<td>room temperature</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetyl</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TMG</td>
<td>1,1,3,3-tetramethylguanine</td>
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INTRODUCTION

1.1 History and Discovery of Polyamines

Polyamines (PA’s) are biologically ubiquitous aliphatic nitrogen containing compounds of low molecular weight and polycationic in nature. They are water soluble, and at physiological pH all the amino groups will be positively charged; hence these compounds are organic bases, their basicity increasing with the number of amino groups. Unlike inorganic molecules or ions, the positive charges on the PA’s are spaced out at intervals and although the hydrocarbon chains are flexible, they can introduce steric properties into the molecules.¹

Although PA’s have received little attention in biochemical and physiological texts they have a long history and there has amassed considerable literature. In 1678 Antoni van Leeuwenhoek ² described crystals that formed in samples of human semen that had been left to cool. The phenomenon was rediscovered several times during the next 200 years, in each case the discoverer apparently unaware of what had gone before. By the end of the 19th century there were ten different names for these crystals ². These crystals were later described as the salt, spermine phosphate.
1.2 Occurrence and Distribution of Polyamines.

The PA's putrescine 2 (butane-1, 4-diamine), spermidine 4 (N- (3-aminopropyl) butane-1, 4-diamine), spermine 5 (N, N'-bis (3-aminopropyl)-butane-1, 4-diamine), and further biogenic amines form the basic polyamine backbone of a group of naturally occurring compounds, the polyamine alkaloids. One or more of these compounds are present in every living cell. All have been found in eukaryotes, but spermine rarely occurs in prokaryotes. In addition to the three most abundant PA's (putrescine, spermidine and spermine), a large number of other linear and some branched-chain PA's have been detected in mammalian tissues and excreta, plants, bacteria and microorganisms. In general prokaryotes have a higher concentration of putrescine than spermidine and lack spermine. Eukaryotes on the other hand have a low concentration of putrescine but a high concentration of spermidine and spermine. Cadaverine 3, which normally arises from decarboxylation of lysine, has a limited occurrence and its distribution has not been thoroughly studied.

\[
\begin{align*}
\text{Diaminopropane} & \quad (1) \\
\text{Putrescine} & \quad (2) \\
\text{Cadaverine} & \quad (3) \\
\text{Spermidine} & \quad (4) \\
\text{Spermine} & \quad (5)
\end{align*}
\]
Many unusual PA's also exist in nature but most of them are highly restricted in their distribution. Moreover, PA's can occur not only as free bases but also as amides when covalently bound to macromolecules such as proteins, and to soluble cellular components such as organic acids, forming $N$-acetyl putrescine in animal cells and the widespread $N$-cinnamoyl type amide conjugates in the plant kingdom.

In plants putrescine has been identified in both prokaryotes and eukaryotic algae $^6$. Spermidine is widely distributed in prokaryotic and eukaryotic algae, while spermine occurs in appreciable amounts only in some eukaryotic species of red algae. Spermidine and spermine are commonly distributed in fungi, lichens, bryophytes, and pteridophytes as well as in higher plants. In higher plants high levels of PA's have been associated with meristematic and rapidly dividing tissues whereas low levels of PA's are characteristic of senescent tissues $^8$.

Diaminopropane 1, (DAP), an oxidation product of spermidine and spermine occurs widely in most unicellular eukaryotic algae and lichens $^7$. In fungi DAP is irregularly distributed and in pteridophytes it has not been detected. PA's appear to be constituents of many compounds found in plants and insects. $N$-carbamoylputrescine,
conjugates of hydroxycinnamoyl putrescine and caffeoyl putrescine are among polyamine conjugates that have been isolated from plant tissues. N-Methylputrescine is a precursor of the tropane alkaloids and putrescine-containing alkaloids have also been isolated from the marine gastropod mollusk Monodonta labio. Palustrine, kukoamine and cannabisativine (from marijuana) are examples of the many spermidine-containing alkaloids found in plants (Fig.1). Aphelandrine is a spermidine containing alkaloid from the flowering shrub Aphelandrine tetragona.

Low molecular weight spider and wasp toxins are selective inhibitors of glutamate receptors of the central nervous systems (CNS) and consist of a polyamine backbone to which are linked one or several carboxylic acids and/or amino acids. Hydroxylamine containing amines have been isolated from the venom of the funnel web spider Agelenopsis aperta. However in many cases it is not clear whether the polyamines are precursors in the biosynthesis of these compounds. A glutathione-spermidine conjugate, N-monoglutathionylspermidine is found in Escherichia coli; trypanothione, a glutathione spermidine conjugate is unique to trypanosomes where it appears to substitute for glutathione.
...
Fig. 1 Examples of polyamine containing natural compounds: N-carbamoylputrescine (A), 4-coumaroylputrescine (B), caffeoylputrescine (C); the spermidine alkaloids cannabisativine (D), palustrine (E), and kukoamine (F); the spider toxin NSTX-3 contains both putrescine and cadaverine residues (G). The emphasized bonds indicate the polyamine moieties.

The difficulties experienced in attempting to identify and measure the amount of PA's in biological materials follow from three characteristics: their size, the low concentration in which they are present and their only reactive centers are in the amino groups. Thus methods for polyamine analysis include procedures to extract and perhaps concentrate the polyamines; procedures to separate them from other
amino containing compounds (such as amino acids) and from each other; procedures to convert them into colored or fluorescent derivatives; procedures to identify them; and then to make quantitative measurements\textsuperscript{17,18}.

1.3 Polyamine Biosynthesis

With the exception of laboratory bred mutant cell lines, all cells have the ability to synthesize putrescine and spermidine. Spermine synthesis appears to be confined to eukaryotic systems. There are differences of detail between the pathways in different cell types and a generalized pathway of polyamine biosynthesis is shown in Fig 2. In mammalian cells and in fungi the initial and rate limiting step is the decarboxylation of ornithine (Scheme 1) to form putrescine catalyzed by ornithine decarboxylase (EC 4.1.1.17). In mammalian cells and fungi there is only one pathway for putrescine synthesis but many microorganisms\textsuperscript{19} and higher plants\textsuperscript{20,21} possess a second constitutive pathway via agmatine, formed by the decarboxylation of arginine by arginine decarboxylase. Agmatine is then hydrolyzed by agmatinase to form putrescine with the elimination of urea. Recently agmatine has been found in the rat tissue\textsuperscript{22}. In a step common to most organisms, spermidine is formed from putrescine by addition of an aminopropyl group donated by decarboxylated S-adenosylmethionine, a reaction catalyzed by spermidine synthase and aminopropyltransferase.
Fig. 2. The main pathways of polyamine biosynthesis in animals, plants, and microorganisms. The enzymes involved are (1) arginase, (2) ornithine decarboxylase, (3) arginine decarboxylase, (4) agmatinase, (5) agmatine deiminase, (6) N-carbamoylputrescine, (7) spermidine synthase, (8) S-adenosylmethionine decarboxylase, (9) Spermine synthase, (10) spermidine/spermine N1-acetyltransferase, (11) polyamine oxidase.
Scheme 1 Reaction sequence involved in the decarboxylation of ornithine

Addition of a second aminopropyl moiety to spermidine catalyzed by a different aminopropyltransferase, spermine synthase, forms spermine (Scheme 2). The source of the aminopropyl group is a second molecule of decarboxylated S-adenosylmethionine. The synthesis of spermidine and spermine is dependent on the availability of aminopropyl donor, hence S-adenosylmethionine decarboxylase is also rate limiting in the biosynthesis of spermine. The three key enzymes that regulate polyamine biosynthesis are ornithine decarboxylase, S-adenosylmethionine decarboxylase, and the enzyme $N^\beta$-acetyl transferase that initiates polyamine catabolism.$^{23}$
Scheme 2 Reaction sequence involved in the decarboxylation of S-adenosylmethionine and synthesis of spermidine and spermine.
1.4 Functions of Polyamines

It has been shown that polyamines play a vital role in cellular growth processes. Adequate intracellular levels of polyamines are necessary for optimal growth and replication of plants, bacteria, fungi and all types of cells examined so far. The first unequivocally established function for polyamines at the molecular level is the donation of a 4-aminobutyl moiety by spermidine to the eukaryotic initiation factor 5a precursor protein to form the amino acid hypusine.

Polyamines promote DNA replication, RNA transcription and translational stages of protein synthesis, stabilize membranes, alter intracellular free calcium levels, and also have important messenger functions. Polyamines are involved in various plant growth and development processes: cell division, embryogenesis, rooting, flower initiation, senescence and fruit ripening. Polyamines also have important neurophysiological functions. It has been discussed that polyamines may influence the integrity of the blood-brain barrier.

1.5 Polyamine Conjugates in Plants

Although the identification of conjugates formed between amines and cinnamic acids in various plants have been reported sporadically over many years, it is only recently that the widespread nature and potential significance of these amides have been recognized. Hydroxycinnamic acid (HCA) amides such as the conjugates of
putrescine, spermidine, and tyramine have been found in several plant families. HCA amides were detected in flowering parts of various plants and were linked with pollen fertility. It was also reported that they might play a role as stress substances accumulating during viral, bacterial and fugal infections and as protectants against ozone damage.

In tobacco, HCA amides were first isolated from callus tissue culture by Mizusaki and co-workers who were working on nicotine biosynthesis. Although at that time these researchers could not find HCA amides in normal tobacco tissue, caffeoylputrescine was later isolated from the flowers and vegetative apex of tobacco plants. Coumaric, caffeic, and ferulic acids are found conjugated with putrescine, spermidine, and tyramine in the tobacco plant in large concentrations (2 \( \mu \text{mol/ g} \)) and in a variety of combinations (Fig 3).

* Nicotinia tabaccum* forms large amounts of mono- and di-feruloylputrescine after infection with tobacco mosaic virus (TMV). Similarly, *N. sylvestris* forms di-feruloylputrescine on infection with the TMV strain Aucuba. These amides are at their greatest concentration when synthesis of the virus has ended.
Fig. 3 Structures of HCA amides isolated from plants

\[ \begin{align*}
\text{R} = \text{H} & \quad \text{Coumaroylputrescine} \\
\text{R} = \text{OH} & \quad \text{Caffeoylputrescine} \\
\text{R} = \text{OCH}_3 & \quad \text{Feruloylputrescine}
\end{align*} \]

\[ \begin{align*}
\text{NH}_2 & \quad \text{N}^9\text{-Caffeoylspermidine} \\
\text{OH} & \quad \text{dicoumaroylputrescine} \\
\text{R} & \quad \text{diferuloylputrescine}
\end{align*} \]

\[ \begin{align*}
\text{dicoumaroylspermidine}
\end{align*} \]

Scheme 3 Biosynthesis of Coumaroylagmatine

\[ \begin{align*}
\text{Coumaroyl-CoA} & + \text{Agmatine} \\
& \xrightarrow{\text{CoASH}} \text{Coumaroylagmatine}
\end{align*} \]
Dimers of coumaroylagmatine known as the hordatines are found in barley seedlings and have been shown to inhibit fungal spore germination at $10^{-5}$ M and probably contribute significantly to the fungal resistance of seedlings up to five days old. The dimerization of coumaroylagmatine can be effected in vitro by a peroxidase system from barley, but the product is optically inactive unlike the natural hordatines. The enzyme was absent from the seed, and it showed maximum activity three to four days after germination. No activity was found five days after germination.

Polyamines also occur conjugated with sugars, steroids, phospholipids and peptides and also as substructural units within numerous families of plant alkaloids. For many years the organic chemist has regarded these latter substances as secondary metabolic products offering little in the way of interesting pharmacological activity. However quite recently polyamine derivatives have been found to possess remarkably diverse biochemical profiles. Among the most fascinating are the series of new analogues of 15-deoxyspergualin (DSG), an immunosuppressive agent commercialized in Japan and tested in a graft-versus-host disease (GVHD) model in mice.

\[
\begin{align*}
&\text{HN} \quad \text{NH}_2 \\
&\quad \text{NH} \quad \text{N} \\
&\quad \text{HN} \quad \text{O} \quad \text{OH} \quad \text{H} \\
&\quad \text{NH} \quad \text{N} \quad \text{R} \\
&\quad \text{HN} \quad \text{NH}_2
\end{align*}
\]

\((+/\text{-})\text{-15-Deoxyspergualin}\)
1.6 Polyamine Conjugates in Arthropods.

Natural products provide an important source of lead compounds for both pharmaceutical and agrochemical research projects. The potential role of arthropod toxins from spiders, wasps and scorpions in the development of selective pharmacological tools has been significantly advanced by the discovery of the low molecular weight, nonproteinaceous toxins from the solitary digger-wasp Philantus triangulum.\textsuperscript{40}

![Fig. 4 Backbone of a polyamine conjugate](image)

These spider and wasp toxins block neuromuscular junctions\textsuperscript{41}. In this way they are used by predators to paralyze or kill their prey. The polyamine amide toxins also block mammalian ion channels and therefore have potential as selective pharmacological tools and ultimately as lead compounds for pharmaceutical products. In mammals and other vertebrates polyamine amides are selective, noncompetitive antagonists of excitatory ionotopic glutamate receptors (GLU-R), but they also interact with other ionotopic receptors, e.g. nicotinic acetylcholine receptors (nACh-
and with voltage sensitive cation channels. Thus they are channel blockers which are selective at low concentrations, for cation channels. However, they also block gamma-aminobutyric acid (GABA) gated Cl⁻ channels and Ca²⁺ activated Cl⁻ channels.

Polyamine amides derived from arthropod toxins (spider, wasp and scorpions) have been isolated, purified and characterized in many laboratories. Usherwood et al. first reported such toxins were low molecular weight compounds. Eventually, polyamine amides toxins were isolated and characterized from venoms of a wide variety of spiders: argiootoxins (ArgTX) isolated from the venom of orb-weaver spiders *Argiope* and *Araneus*; Joro spider (*Nephilia maculata*) toxins (JSTX), *N. clavata* spider toxins (NPTX), agatoxins from *Agelenopsis aperta* and hettoxins from *Hebestatis theveniti*, *Nephelia* peptide-like spider toxins (NPTX), argiopinins, and pseudoargiopinins. (see Figure 5)

Eldefrawi et al. and Piek et al. independently reported a polyamine amide toxin in the venom of the predatory wasp *P. triangulum* (Sphecidae), which paralyses honeybees. PhTX-4.3.3 (δ-philanthotoxin) has been synthesized together with its regioisomeric analogues PhTX-3.4.3 and PhTX-3.3.4. (Figure 5). There is a close structural relationship between the polyamine amides from spiders (ArgTXs) and from wasps (PhTX). These can also be compared with synthetic polyamine amides.
synthesized by Blagbrough and coworkers\textsuperscript{56,57} (Table 1). These low molecular weight toxins are generally composed of an aromatic chromophore, a polyamine backbone, one or more amino acid residues, and a spacer group (Fig 4). The spacer group is known to have a significant effect on activity\textsuperscript{58}. The toxins have in common a terminal primary amine or guanidino functional group, which may be important for gaining entry to the membrane spanning ion channel.

\textit{Fig 5 Structures of polyamine amide toxins}

\begin{center}
\begin{tikzpicture}
  \node (PhTX-343) at (0,0) {\includegraphics[width=0.5\textwidth]{PhTX-343.png}};
  \node (PhTX-433) at (0,-2) {\includegraphics[width=0.5\textwidth]{PhTX-433.png}};
  \node (JSTX-1) at (0,-4) {\includegraphics[width=0.5\textwidth]{JSTX-1.png}};
  \node (ArgTX-636) at (0,-6) {\includegraphics[width=0.5\textwidth]{ArgTX-636.png}};
  \node (NPTX-1) at (0,-8) {\includegraphics[width=0.5\textwidth]{NPTX-1.png}};
\end{tikzpicture}
\end{center}
Table 1 Natural and synthetic polyamine containing toxins and IC₅₀ values obtained on the locust nerve-muscle preparation⁵⁶,⁵⁷.

### Synthetic polyamine structure

<table>
<thead>
<tr>
<th>Synthetic polyamine structure</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ArgTX-636</td>
<td>3.2</td>
</tr>
<tr>
<td>Spermine</td>
<td>400</td>
</tr>
<tr>
<td>PhTX-433</td>
<td>18</td>
</tr>
<tr>
<td>PhTX-343</td>
<td>23</td>
</tr>
<tr>
<td>4HOPP-343</td>
<td>6</td>
</tr>
<tr>
<td>4HOPA-343</td>
<td>8.7</td>
</tr>
<tr>
<td>4HOCinn-343 (N¹-coumaroyl spermine)</td>
<td>62</td>
</tr>
</tbody>
</table>
1.7 Neuropharmacological Properties of Polyamine Amide Toxins.

Glutamate is a major excitatory transmitter in the central nervous system (CNS). There are several varieties of glutamate receptors that can be grouped into two broad classes, NMDA (N-methyl-D-aspartic acid) and non-NMDA, based on their sensitivity to the glutamate agonist N-methyl-D-aspartate.\textsuperscript{59,60} Excitatory synaptic potentials in neurons from a variety of CNS regions have been found to consist of a fast, rapidly decaying non-NMDA receptor-mediated component and a slower NMDA receptor-mediated component. Non-NMDA receptors, which include kainate and quisqualate-AMPA (2-amino-3-[3-hydroxy-5-methyl-isoxazoyl] propanoic acid) receptors, are typically directly coupled receptors that allow cations, predominantly sodium and potassium to flow down their electrochemical gradients. The NMDA-R is believed to be important in learning and memory. Excessive stimulation of GLU-R in general is neurotoxic and it has been suggested that GLU-R mediated toxicity may contribute to neurodegeneration following ischaemia (hypoxia or stroke), epilepsy, Alzheimer’s disease and Huntington’s chorea. The distinctive patterns of seizures and neurodegeneration (excitotoxicity) can be prevented by administering an appropriate antagonist for the excitatory amino acid agonist.

Polyamine amides are novel, low molecular weight, polar compounds, which are lead structures for the further advancement of drug design and discovery. Their
high polarity may present some significant difficulties in selective drug delivery and targeting, but this may be offset by their high potency. The possibilities for the development of pesticides with novel modes of action also exist. The polyamine amides are noncompetitive antagonists of ligand–gated ion channels and although they are not selective between GLU-R and nACh-R, and even block activated Cl⁻ ion currents, they are channel blockers which form an exciting new class of compounds with important roles to play in neuropharmacology.

1.8 Electrophysiology of Polyamines Amides.

The pharmacological properties of polyamine amides are complex. In general they both potentiate and antagonize transmitter receptors which gate cation selective ion channels, and both phenomena may involve a number of distinct, possibly interacting, mechanisms. Ligand binding studies have shown that low concentrations of endogenous spermine and spermidine potentiate NMDA-R function, whereas high concentrations of these compounds antagonize this type of receptor. These conclusions were drawn from studies of the effects of polyamines on ³H-labeled (+)-5-methyl-10,11-dihydro-5 H-dibenzox(a,d) cyclohepten-5,10 imine maleate ([³H]MK-801) binding to NMDA-R; that is although spermine and spermidine increase the binding of the noncompetitive antagonist [³H]MK-801,
presumably through their allosteric interactions with a polyamine binding site\textsuperscript{65}, high concentrations (~ millimolar) of these polyamines inhibit binding of [H]MK-801. The electrophysiological studies on spermine showed that low concentrations of spermine potentiate NMDA induced currents whilst with millimolar concentrations the currents are antagonised \textsuperscript{66}. It seems therefore, that there are at least two polyamine binding sites on NMDA-R. It has been shown that polyamine amides are potentiators and antagonists of NMDA-R but are more potent than polyamines in both respects \textsuperscript{67}.

1.9 Synthetic Efforts Towards Selective Protection of Polyamines.

Naturally occurring polyamines such as putrescine, spermidine, and spermine have been widely studied. Many researchers have reviewed some novel aspects of the chemistry of these compounds. All three amines, after suitable transformations, provide valuable starting materials for synthetic work. Owing to the presence of two non-equivalent primary amines in addition to a secondary one, spermidine constitutes a particular challenge in this context. Monoacylation even of diamines, in high yield, poses considerable difficulties \textsuperscript{68}. To accomplish specific synthesis, it is very important to protect selectively the various amine functions in a polyamine. From a synthetic point of view selective protection allows the synthesis of longer and
branched polyamines. For example, without protecting groups, acylation reactions of the polyamines with acid chlorides or anhydrides gives mixtures of products. There are examples of acylation of primary amines in the presence of secondary amines by means of bulky or aromatic acylating agents, but one is not always assured of selective acylation. Thus many different procedures have been studied for selective protection of various polyamines.

Below are described some synthetic efforts geared towards selective functionalization of polyamines.

a) tert-butoxycarbonylation of primary amines.

A large number of protecting groups for amino functions are available these days for synthetic manipulation. However, there seems to exist no satisfactory method of general applicability for the specific blocking of primary and secondary amine moieties present in the same molecule.

Ragnarsson and co-workers were able to selectively protect a model of mixed primary-secondary amines based on acylation followed by exhaustive tert-butoxy-carbonylation (Scheme 4). This protection strategy was also applicable to spermidine. Introduction of two benzyloxy carbonyl (Z) groups onto 6a and 6b with conventional methods afforded 7a and 7b, which were smoothly converted into 8a and 8b in high yields (90 and 92% respectively) using a slight excess of di-tert-butyl dicarbonate in
acetonitrile in the presence of catalytic amounts of 4-dimethylaminopyridine (DMAP).

![Chemical Reaction Diagram]

Scheme 4  
Z = PhCH₂OCO, BOC = Bu′ OCO, a : R = Et, X = CH₂CH₂, b : R = Me, X = p-C₆H₄

Reagents : (I) Z-Cl (pyridine or aq. Na₂CO₃); (II) BOC₂O; DMAP (MeCN, RT, 15 hours); (III) 8a, HCO₂NH₄, Pd/C (80% aq. HOAc, RT, 2 hours) 8b, H₂/Pd/C(MeOH); (IV) TMG (MeOH, room temp., 15 h).

The removal of the auxiliary Z groups offered two possibilities. First, the catalytic hydrogenolysis of both Z functions furnished the selectively protected amines 9a and 9b in 94 and 98% yields respectively. On the other hand, treatment of 8a and 8b with 1.5 equiv. of 1,1,3,3-tetramethylguanine (TMG) in methanol cleaved only the Z group residing on the originally primary amino function, thus giving the protected amine 10a and 10b in 93 and 99% yields respectively.
b) Protecting a secondary amine with tert-Butyloxy carbonyl (BOC) in the presence of a primary amine.

Prugh et al. \textsuperscript{71} (1992) devised a simple method for chemically differentiating primary and secondary amines, in which the primary amine is condensed with benzaldehyde to form an imine leaving the secondary amine available to be protected with BOC. The imine is then hydrolyzed to provide the free primary amine. Differentiation of primary and secondary amines by using low temperature to form the amide with the secondary amine using butyloxy carbonyl (BOC) was problematic and at best involved chromatography and a low yield. An alternate method was adopted in which the parent diamine was condensed with benzaldehyde to form the imine of the primary amine, leaving the secondary amine untouched (Scheme 5). The secondary amine was then treated with BOC anhydride to form the \textit{N}-BOC derivative. The solvent was then removed in vacuo and the residue hydrolysed with 1N KHSO\textsubscript{4} at room temperature. When this reaction was complete, the mixture was extracted with ether to remove benzaldehyde and other unwanted materials from the BOC reaction. Basification with sodium hydroxide while protecting with argon or nitrogen and saturation with sodium chloride followed by extraction gave the desired amine in good yields. A similar transformation was carried out by a colleague in our
lab to synthesize $N$-BOCmethylenespermidine (4-[3-(2,2-dimethylpropanoyl)tetrahydropyrimidin-1-(2H)-yl]butylamine), 11\textsuperscript{72}.

Scheme 5 Protection of secondary amine \textsuperscript{71}.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Product</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>HN__CH_2NH_2</td>
<td>BOCN__NH_2</td>
<td>92</td>
</tr>
<tr>
<td>H_3C_N___CH_2NH_2</td>
<td>H_3C_N___BOC __NH_2</td>
<td>61</td>
</tr>
<tr>
<td>H_3C_N___CH_2NH_2</td>
<td>H_3C_N___BOC __NH_2</td>
<td>72</td>
</tr>
</tbody>
</table>
c) Selective Functionalization of Spermidine

Haemers et al.\textsuperscript{73} (1993) described a method for the synthesis of selectively protected \textit{tert}-butoxycarbonyl (BOC), \textit{9}-fluoromethoxycarbonyl (F-MOC), or benzylxycarbonyl (Z)- spermidine derivatives. They used the starting materials N\textsuperscript{1} amino-N\textsuperscript{4}-benzylxycarbonylaminobutane (NBCAB) and diethylacetal of 3-aminopropanal. The latter was BOC protected with \textit{di-tert}-butyldicarbonate,

\begin{itemize}
  \item \textbf{Scheme 6}
  \item \begin{align*}
    &\text{OEt} &\text{OEt} &\text{a) BOC}_2\text{O/Dioxane/NEt}_3 &\text{b) AcOH-H}_2\text{O} \\
    &\text{H}_2\text{N} &\text{H}_2\text{N} &\text{c) ZNH(CH}_2)_4\text{NH}_2 (NBCAB) / THF/NaBH}_3(CN)/TsOH(pH 6-7) \\
    &\text{BOCH} &\text{BOCH} &\text{d) F-mocCl/CH}_2\text{Cl}_2/\text{Net}_3 (16); \text{BOC}_2\text{O/CH}_2\text{Cl}_2/\text{Net}_3 (17); \text{ZCl/dioxane/Net}_3 (18). \\
    &\text{14} &\text{15} &\text{e) TFA/2N HCl in EtOAc (19); H}_2\text{-Pd/C (20); 2N HCl in EtOAc (21).}
  \end{align*}
\end{itemize}

16 \text{ R =Fmoc (82\% yield); 17 R=BOC (89\% yield); 18 R=Z (85\% yield)}
19 \text{ R=Fmoc, R1=H, R2=Z (84\% yield as HCl)}
20 \text{ R=BOC, R1=BOC, R2=H (92\% yield)}
21 \text{ R=Z, R1=H, R2=Z (73\% yield as HCl)}

a) BOC\textsubscript{2}O/Dioxane/NEt\textsubscript{3} b) AcOH-H\textsubscript{2}O

c) ZNH(CH\textsubscript{2})\textsubscript{4}NH\textsubscript{2} (NBCAB) / THF/NaBH\textsubscript{3}(CN)/TsOH(pH 6-7)
d) F-mocCl/CH\textsubscript{2}Cl\textsubscript{2}/Net\textsubscript{3} (16); BOC\textsubscript{2}O/CH\textsubscript{2}Cl\textsubscript{2}/Net\textsubscript{3} (17); ZCl/dioxane/Net\textsubscript{3} (18).
e) TFA/2N HCl in EtOAc (19); H\textsubscript{2}-Pd/C (20); 2N HCl in EtOAc (21).
and the acetal was hydrolyzed with diluted acetic acid (Scheme 6). Reductive amination of 3-tert-butoxy-carbonylaminopropanal with aminobutane and sodium cyanoborohydride afforded $N^\text{B}$-benzylxycarbonyl-$N'^\text{B}$-tert-butoxycarbonylspermidine, which can be a starting material for selectively substituted spermidines. One of the set-backs of this method is that the aldehyde derivative, 14, decomposes slowly and must therefore be used immediately.

\[ \text{Reductive amination} \]

\[ \text{Scheme 6} \]

\[ \text{Reductive amination} \]

\[ \text{Scheme 7} \]

\[ \begin{align*}
\text{Diampines} \\
A & \text{ } x = \text{C-NH}_2; \text{ } R_1 = \text{H or alkyl; } R_2 = \text{alkyl} \\
B & \text{ } x = \text{N; } R_1 = \text{H; } R_2 = \text{alkyl, alkyl-OH}
\end{align*} \]

Although there are literature examples describing the synthesis of selectively protected polyamines, these methods employ reagents which initially afford mixtures that require extensive purification to give the protected polyamine or cumbersome stepwise synthesis from protected small amine-containing building blocks. Adamczyk et al. reported the chemoselective protection of amino groups on primary carbons in various linear polyamines using $O$-alkyl-$O'$-($N$-succinimidyl) carbonates and application of this method for the facile synthesis of polyamine
derivatives (Scheme 7). In the event, 0.97 eq of the succinimidyl carbonate per amine in methylene chloride was added to a solution of polyamine in methylene chloride at –40 °C under nitrogen. After reaction was complete, the product was isolated by aqueous workup to give the desired product in good yield without further purification. Formation of the mono-protected diamine from unsymmetrical diamines was highly chemoselective and economical, using only 1.03 eq of the diamine. Additionally, a terminal alcohol group is unaffected during the selective protection of a primary amine in polyamino alcohol.

e) Mono-protection of polyamines via ethyl trifluoroacetate

Scheme 8

Osullivan and Dalrymple used trifluoroacetyl as a protecting group which allows monofunctionalized polyamine amides to be easily prepared on a gram scale (Scheme 8). In this protocol, spermine was selectively protected on a primary amino functional group by reaction with ethyl trifluoroacetate (1.0 eq, MeOH, -78 °C for 1 hour, then to 0 °C over 1 hour) to afford predominantly mono-trifluoroacetamide.
The ratio of the primary amine to protecting group reagent is very critical in order to avoid diprotection (of primary amines) and polyprotection (of secondary amines). It is, however, presumed that corresponding steric effects\textsuperscript{63} mask the nucleophilicity of the secondary amines.

\textit{f) Mono-protection of alkanediamines via di-tert-butyldicarbonate.}

Krapcho and Kuell\textsuperscript{77} reported that the treatment of \( \alpha, \omega \) alkanediamines with di-\textit{tert}-butyldicarbonate in dioxane as solvent (6-7: 1 molar excess of diamine to di-\textit{tert}-butyldicarbonate) leads to high yields of the desired mono-protected \( N- (BOC)-\alpha, \omega \)-alkanediamines, along with the diBOC-protected diamines (Scheme 9). These bis adducts can be readily removed by taking advantage of their water insolubility.

\textit{Scheme 9}

\[ \begin{align*} \text{H}_2\text{N} & \quad (\text{BOC})_x \quad \text{NH}_2 \\ \text{H}_2\text{N} & \quad (\text{BOC})_2\text{O} \\ \text{BOCHN} & \quad (\text{NHBOC})_x \quad \text{NHBOC} \end{align*} \]

\textit{g) Protection of the internal secondary amine of spermidine}

The internal secondary amine of spermidine was successfully protected with formaldehyde to give a 95\% yield of the hexahydropyrimidine. This compound was
acylated or alkylated by a variety of reagents and then reduced to form the desired polyamine.\(^7\) An example of this route is shown in Scheme 10.

**Scheme 10**

The 1,3-diamine functionality is important for this type of protection.

Polyamines that have successive 1,3-diamine units react with formaldehyde to form mixtures of the diazacyclohexanes.\(^7\) Dutasta and co-workers prepared 4,8-diaza-1,11-undecandiamine with protected internal amines by reaction of 1,3-propanediamine with two moles of acrylonitrile followed by reaction with formaldehyde and reduction as shown in scheme 11.
Scheme 11

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{CH}_2\text{O} \quad \text{NC} \quad \text{N} \\
\text{H} & \quad \text{CN} \quad \text{CN} \quad \text{NH}_2 \\
\text{H}_2 & \quad \text{Raney Ni} \\
\text{H}_2\text{N} & \quad \text{N} \quad \text{N} \quad \text{NH}_2
\end{align*}
\]

1.10 Aims and Objectives

The recent production of pesticides that have resulted from studies of natural products obtained from plants and microorganisms has awakened interest in the potential of natural products (toxins) from other living organisms such as spiders, wasps and scorpions as lead structures for pesticide discovery. This is the reason for the recent wave of interest in the study of these arthropods. This is manifested in the chemical and pharmacological literature in the past decade, coinciding with the realization that the venoms from these arthropods contain many neuroactive components, of low (<1000 amu) molecular weight, which may have important uses.

Various studies have shown that sodium, potassium and calcium channels as well as glutamate and gamma-aminobutyric acid (GABA) receptors are the biological targets.
null
This thesis explores the scope of the synthesis of hydroxycinnamic acid polyamine conjugates of diamines, triamines, tetraamines and oxo-analogues which bear some structural relationship to the toxins found in the spider and wasp toxins. We intend to synthesize new analogues that change substructure in the amine tail and maintain the hydroxycinnamoyl moiety. The analogues were designed to reflect a) different chain lengths, b) absence of inner nitrogen atoms, c) increase in hydrophobic characteristics and d) replacement of inner nitrogens with oxygen atoms.

The biological activity of these conjugates in the neuromuscular junctions of invertebrates and vertebrates will be investigated and this will be reported in another work.
1.11 Design of Natural Plant Polyamine Conjugates

1.11.1 Requirements for Biological Activity of Analogues of Wasp and Spider Toxins.

The venoms of spiders are complex composites of free amino acids, large proteinaceous toxins (>3000 Da), and relatively small polyamine toxins (<1000 Da). For a long time the interest in such venoms primarily focussed on the high molecular weight components, particularly those extracted from tropical spiders. Recently, with the development of improved analytical techniques, combined with the interesting neurotoxic activities of those compounds, the polyamine venom components of common spider species, as well as wasps, has attracted more attention.

Polyamine toxins of spiders and wasps share striking peculiarities: all possess a linear α, ω- diamino polyazaalkane backbone modified at one end with a more or less lipophilic unit, in most cases an aromatic acyl group. From studies conducted on some of these venoms some portions of the toxins are required for biological activity whereas some portions are not (Fig. 6).

The low molecular weight polyamine toxins are generally composed of an aromatic chromophore, a polyamine backbone, one or more amino acid residues and a
spacer group, which has significant effect on activity. The extent of protonation of polyamine toxins in aqueous solution may be unrelated to their activities at ionotropic receptors, although it is interesting to note that several analogues, which are predicted to be more easily deprotonated (i.e. less basic) at the secondary amino groups than

Fig. 6 Structures of PhTX-4.3.3 and PhTX-3.4.3

PhTX- 3.4.3, exhibited higher antagonistic activity. This is exemplified when considering the relative potencies of philanthotoxins which have polyamine chains of the same length, but in which separation of the secondary amino groups is changed from four to three methylene groups, resulting in reduced protonation. Thus PhTX-
4.3.3 is less protonated but more potent than PhTX-3.4.3. It was also observed that analogues of PhTX- 4.3.3 and PhTX- 3.3.4 lacking the phenolic hydroxyl group exhibited higher potencies. Jaroszewski and co-workers \(^{83}\) investigated the acid-base properties (pKa values and proton distribution patterns) of PhTX-3.4.3 by \(^1\)H and \(^{13}\)C NMR titration. They observed that the first protonation mainly takes place at the inner amino group and the phenol group is deprotonated either in the second or third deprotonation steps. The preferential deprotonation of the inner amino group is apparent also in the diprotonated form. The monoprotonated form carries a practically fully ionized phenol group and the proton shared between the three amino groups.

The terminal, primary amino group, which has been shown to be essential for biological activity, remains practically fully protonated at biologically relevant pH values, and this charge is likely to participate in receptor-binding event. Protonation of the central amino group, however, does not appear to be relevant for biological activity.

**Table 2 Proton distribution patterns for PhTX-3.4.3 at various stages of protonation\(^{83}\)**

<table>
<thead>
<tr>
<th>Protonation stage</th>
<th>% protonation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
</tr>
<tr>
<td>monoprotonated</td>
<td>0.35</td>
</tr>
<tr>
<td>diprotonated</td>
<td>0.66</td>
</tr>
<tr>
<td>triprotonated</td>
<td>0.89</td>
</tr>
</tbody>
</table>
Bergeron and co-workers\textsuperscript{84} synthesized a series of analogues and homologues of $N'^{1},N'^{2}$-diethylsperrmine (DESPM) and their biological properties were evaluated. The analogues $N'^{1},N'^{2}$-bis(2,2,2-trifluorethyl) spermine (FDESPM), $N,N'$-bis(4-piperidinylmethyl)-1,4-diaminobutane (PIP 4,4,4), and $N,N'$-bis-(4-pyridylmethyl)-1,4-diaminobutane (PYR 4,4,4) have distances between their nitrogen almost identical to DESPM. The longer analogues, $N,N'$-bis[2-(4-piperidinyl) ethyl]-1,4-diaminobutane (PIP 5,4,5) and $N,N'$-bis[2-(4-pyridyl) ethyl]-1,4-diaminobutane (PYR 5,4,5) are very similar in the spacing of their amino groups. However, the characteristics among the biological properties of these compounds clearly demonstrate that the tetraamines must be charged to be "recognized" by the cell.

\begin{align*}
\text{DESPM (3,4,3)} & \quad \text{IC}_{50} = 0.2 \\
\text{FDESPM (3,4,3)} & \quad \text{IC}_{50} = >100 \\
\text{PIP (4,4,4)} & \quad \text{IC}_{50} = 0.1 \\
\text{PYR (4,4,4)} & \quad \text{IC}_{50} = 60 \\
\text{PIP (5,4,5)} & \quad \text{IC}_{50} = 0.3 \\
\text{PYR (5,4,5)} & \quad \text{IC}_{50} = >100
\end{align*}

Analogues with low nitrogen pKa's such that the nitrogens are poorly protonated at physiological pH do not compete well with spermine for uptake and
have high 96 h IC_{50} values (i.e. the concentration of compound necessary to reduce cell growth to 50% of control growth in 96 hours) and have little effect on S-adenosylmethionine decarboxylase, ornithine decarboxylase, and spermidine/spermine N'-acetyltransferase activities and on intracellular polyamine pools. For example, DESPM has a 96 h IC_{50} of 0.2\mu M, while the corresponding terminal hexafluoro derivative has an IC_{50} in excess of 100\mu M. The trifluorinated compound is only protonated at the two central nitrogens at physiological pH.

These observations prompted us to synthesize analogues of the plant conjugates with the aromatic chromophore intact but with altered polyamine tail. We decided also to synthesize analogues in which the secondary amino groups are replaced with an oxygen atom, resulting in analogues lacking inner basic nitrogens. Although it may be true that the phenolic hydroxyl group is not essential for biological activity, we decided to maintain its presence because the natural plant analogues are found with a phenolic hydroxyl group attached to the cinnamoyl moiety.

1.11.2 Polyamine Conjugates and Oxo-analogues.

Taking into consideration the necessary portions needed for biological activity, we targeted to synthesize the compounds 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, and 32. The structures are as shown in Table 3. Compounds 22, 23 and 24 were designed to
investigate the effect of chain length on bioactivity. With the core structure of spermidine in mind, conjugate 23 was also designed to find out if the inner basic nitrogens were necessary for activity. If there is the requirement for a heteroatom but not a basic amine, then that would be compensated for by compounds 25 and 26 for spermidine and spermine respectively. See Table 3 for comparison.

Another interesting class of compounds that we designed were the 1,4-diaminocyclohexane and 1,2-diaminocyclohexane derivatives 30,31 and 32. These were considered based on the core structure of spermine but in this case the four inner methylene groups were replaced with the cyclohexyl group, which will increase the hydrophobicity of the conjugates and may increase their rigidity based on the conformations they assume. The synthesis of the compound 27 was also based on a similar reasoning.
Table 3 Structures of coumaric acid conjugates

<table>
<thead>
<tr>
<th>Compound or Modification(s)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N'^{1})-coumaroylspermidine ((N'^{1})-couspd)</td>
<td>![Structure 1]</td>
</tr>
<tr>
<td>(N'^{1})-coumaroylspermine ((N'^{1})-couspm)</td>
<td>![Structure 2]</td>
</tr>
</tbody>
</table>

Remove inner amine function and shorten chain length (compare with \(N'^{1}\)-couspd)

Remove inner amine function (compare with \(N'^{1}\)-couspd)

Remove inner amine functions (compare with \(N'^{1}\)-couspm)

Replace inner amino functions with oxygen + shorten chain length (compare with \(N'^{1}\)-couspm)

Replace inner amino functions with oxygen (compare with \(N'^{1}\)-couspm)

Same number of nitrogen atoms, different spacing and increasing hydrophobicity (compare with \(N'^{1}\)-couspm)

Same number of nitrogen atoms but shorter chain length (compare with \(N'^{1}\)-couspd)
Table 3 (continued) Structures of coumaric acid conjugates

<table>
<thead>
<tr>
<th>Compound or Modification(s)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N^1$-coumaroylspermidine ($N^1$-couspd)</td>
<td><img src="structure1.png" alt="Structure" /></td>
</tr>
<tr>
<td>$N^1$-coumaroylspermine ($N^1$-couspm)</td>
<td><img src="structure2.png" alt="Structure" /></td>
</tr>
<tr>
<td>Same number of nitrogen atoms but shorter chain length (compare with $N^1$-couspd)</td>
<td><img src="structure3.png" alt="Structure" /></td>
</tr>
<tr>
<td>Same number of nitrogen atoms but different spacing and increasing hydrophobicity (compare with $N^1$-couspm)</td>
<td><img src="structure4.png" alt="Structure" /></td>
</tr>
<tr>
<td>Same number of nitrogen atoms but shorter chain length (compare with $N^1$-couspd)</td>
<td><img src="structure5.png" alt="Structure" /></td>
</tr>
<tr>
<td>Same number of nitrogen atoms but different spacing and increasing hydrophobicity (compare with $N^1$-couspm)</td>
<td><img src="structure6.png" alt="Structure" /></td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

2.1 Synthesis of (2E)-N-(6-aminohexyl)-3-(4-hydroxyphenyl)-2-propenamide (22)

In the chemical synthesis of polyamine amides, which are physiologically interesting derivatives, polyamines in their terminally \( N \)-protected form represent the starting material of choice.\(^\text{85}\) Preparation of these compounds, however, is not facile, not even in the simplest cases of the synthesis of \( N \)-monoderivatized alkane-\( \alpha,\omega \)-diamines.

Scheme 12

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{H}_2\text{N} \\
\text{NH}_2 & \quad \text{NH}_2 \\
\text{O} & \quad \text{O} \\
\text{Cl} & \quad \text{Cl} \\
\text{Et}_3\text{N} & \quad \text{Et}_3\text{N} \\
\text{MeOH} & \quad \text{MeOH} \\
\text{HCl(g), 0°C} & \quad 	ext{HCl(g), 0°C} \\
\text{36} & \quad \text{36} \\
\text{33} & \quad \text{33} \\
\text{35} & \quad \text{35} \\
\text{34} & \quad \text{34} \\
\text{32} & \quad \text{32} \\
\text{31} & \quad \text{31} \\
\text{30} & \quad \text{30} \\
\text{29} & \quad \text{29} \\
\text{28} & \quad \text{28} \\
\text{27} & \quad \text{27} \\
\text{26} & \quad \text{26} \\
\text{25} & \quad \text{25} \\
\text{24} & \quad \text{24} \\
\text{23} & \quad \text{23} \\
\text{22} & \quad \text{22}
\end{align*}
\]
2.2 Synthesis of (2E)-(8-aminooctyl)-3-(4-hydroxyphenyl)-2-propenamide (23)

Scheme 13
(a) Syntheses of compounds 33 and 37

Preparation of 22 and 23 began with the selective protection of one of the primary amine functions of the required diamine with ethyl trifluoroacetate (ETFA)\textsuperscript{76} using methanol as solvent at $-78^\circ$C to give 33 and 37 respectively. The reaction temperature plays a very significant role in determining the ratio of the monoprotected and the unwanted diprotected amine. It was, however, not possible to prevent diprotection of the amine functions. The mixture was purified by flash chromatography by taking advantage of the wide difference in the $R_f$ values (~ 0.4) of the two products. This afforded 33 and 37 in 95\% and 94\% yields respectively.

(b) Syntheses of compounds 34 and 38
In the next step of the synthesis, 33 and 37 were reacted with (BOC)$_2$O using THF as solvent. The reaction was done at room temperature. Both 33 and 37 were turned smoothly into 34 and 38 respectively in 99% yield.

\( c) \) Syntheses of compounds 35 and 39

The TFA protecting group was removed by reacting 34 in MeOH with concentrated aqueous NH$_4$OH. This step turned out to be very problematic. In certain attempts it took about 6 hours for the reaction to go to completion and on other occasions it took not less than 48 hours. On the whole no definite time was determined within which the reaction will be complete. The average yield for the synthesis of 35 was found to be 70%.

For the removal of the TFA protecting group other reagents were also explored. We examined the use of saturated NH$_3$ (g) in MeOH and NaOH in MeOH. These reagents did not improve the results obtained by using NH$_4$OH. Rather we observed the removal of about 20% of the BOC group, which gave a mixture of 33 and 35. We therefore reverted to the use of conc NH$_4$OH as the standard reagent for
the removal of the TFA protecting group. Reaction of 38 with \( \text{NH}_2\text{OH} \) for two days gave 39 in 99% crude yield.

\[ \text{Reaction of 38 with \( \text{NH}_2\text{OH} \) for two days gave 39 in 99\% crude yield.} \]

\[ d) \text{Syntheses of 36 and 40} \]

\[ \text{The conversion of 35 into 36 was the key step in the synthesis of the conjugate.} \]

\[ \text{Because the phenol group in the commercially available acid is highly reactive, it must be protected during the synthetic procedure. This was achieved by using acetic anhydride/\( \text{Et}_3\text{N} \) and dichloromethane as reaction solvent to afford } p\text{-acetoxy cinnamic acid in 82\% yield. To increase the reactivity of the acid it was converted into the corresponding acyl chloride by reaction with oxalyl chloride in the presence of catalytic DMF.} \]

\[ \text{The main problem encountered at this step was incomplete acylation. This was realized from the excess acid left in the reaction mixture. Increasing the reaction} \]
time, and refluxing did not improve the yields significantly. The yields obtained for 36 and 40 were 40% and 60% respectively.

The acylation was also tried using dicyclohexyl-carbodiimide (DCC) as a coupling agent in the presence of catalytic hydroxy-benzotriazole (HOBT). The setback in this procedure was that the dicyclohexyl urea formed as a by-product of the reaction could not be easily separated from the target molecule. This made the final product contaminated with dicyclohexyl urea. The acylation protocol using the acyl chloride was therefore adopted for all future acylation reactions.

N-acylation of 39 also was done with p-acetoxyccinnamoyl chloride in CH₂Cl₂. This reaction gave a mixture of two products, one was a conjugate with the BOC group removed. It could be that there was an acid build up in the reaction, which caused the removal of the acid sensitive BOC protecting group. In the light of this we used 2.4 eq of the Et₃N but this did not give any improvement. In the event 40 was isolated in 60% yield with significant amount of acid in the reaction mixture. This confirmed our previous suggestion that the acylation reaction was never driven to completion. The excess coumaric acid was removed by washing the mixture with 0.25M Na₂CO₃. We observed an increase in the yield with increase in chain length of two methylene units.
(e) Syntheses of compounds 22 and 23

The BOC and O-acetyl protecting groups which are acid sensitive were cleaved by bubbling HCl (g) into a solution of 36 in methanol at 0 °C, then warming to room temperature. Initially a mixture was recovered comprising of one conjugate with both groups cleaved and another conjugate with either one of the protecting groups still attached. This was overcome by warming the reaction mixture at 40 °C for 1.5 hours, to afford 22 in 68% yield. The HPLC chromatogram of 22 gave a retention time of 11.7 minutes. The same protocol was followed for 40, and 23 was obtained as fine yellow crystals in 88% yield, which again was higher than the yield obtained in the shorter chain polyamine. The HPLC chromatogram of 23 gave a retention time of 15.1 minutes. The increase in retention time was expected since the longer alkyl chain is more hydrophobic and would have a higher affinity for the reverse phase column.
2.3 Synthesis of (2E)-N-(12-aminododecyl)-3-(4-hydroxyphenyl)-2-
propenamide (24)

Several attempts to monoprotect one of the amino functions of 1,12-dodecanediamine
with ETFA did not give good yields of the desired product. At best the
monoprotected derivative was obtained in 20% yield. A new protocol (Scheme
14) was adopted from Krapcho and co-workers\textsuperscript{71} to synthesize the carbamate, 41.

Although this method is efficient it is beneficial if the starting material is

\textit{Scheme 14}
cheap and readily available. This is because this procedure requires a ratio of 6-8:1 of the diamine to the protecting agent. One obvious advantage about this method is that it reduces the synthetic procedure by two steps, bearing in mind also that we avoided the tedious removal of the TFA protecting group.

The preparation of 41 proceeded smoothly by reacting the diamine with (BOC)₂O for 6.5 hours. After distillation the derivative was obtained as a yellow oil in 74% yield. In fact, this good yield was obtained when the reactants were more efficiently mixed using a 'high-dilution technique': the slow addition of a highly diluted solution of (BOC)₂O to a well stirred solution of the diamine. It was detected that during the acylation using the acyl chloride prepared by standard conditions some of the acetoxy protecting groups were cleaved. We assumed that this was due to the fact that there was insufficient Et₃N used in the reaction to scavenge the HCl gas produced during the reaction. Purification of the residue on silica gel gave three fractions which were labelled A, B and C (with C being the most polar component). Mass spectra of fraction A gave an M+1 of 447 which corresponds to that of 42. Interestingly, although B reveals two spots on TLC, the mass spectra gave an M+1 of 447. The NMR of fraction A revealed that the fraction was predominantly the trans-isomer. However, after 10 days a repeat of the NMR showed that the fraction was a
1:1 ratio of the *cis* and *trans*-isomers. This could arise as a result of photoisomerization. Fraction B was determined to be predominantly the *trans*-isomer. Because of the cinnamoyl chromophores, the hydroxycinnamic amides undergo (*E*) to (*Z*) isomerization under normal daylight conditions.\(^6\) In a quantitative structure-activity relationship study of the antifungal properties of some cinnamic-acid derivatives, it was shown that only (*E*)-isomers are biologically active\(^7\). Furthermore, the position of the C=C bond is a key factor for the antifungal property, since the reduction of the C=C bond or interconversion into the (*Z*)-isomer by UV light results in total loss of biological activity. It is also known that silica gel can influence (*E*)/*(Z*) interconversion and as such the (*E*)/*(Z*) equilibrium.

**Scheme 15 Products obtained from acylation of 41**
Fraction C on the other hand gave an M+1 of 493 which corresponds to the mass of the dicoumaroyl conjugate. This supports the suggestion that excess HCl gas was still produced despite 2.4 eq of Et₃N used and caused the removal of the acid sensitive BOC protecting group to regenerate the diamine (Scheme 15).

Removal of the protecting groups was not very successful. Compound 24 was obtained in only 32% yield after deprotection of 42 with HCl (g) in MeOH and washing with Et₂O.

\[
\begin{align*}
\text{CONH} & \quad \text{NH}_2 \\
\text{HO} & \quad \text{24}
\end{align*}
\]

2.4 Synthesis of (2E)-N-(2,2'-ethylenedioxy) bis (ethylamine)-3-(4-hydroxyphenyl) prop-2-enamide (25)

Following the protocol described earlier for the synthesis of 33 and 37, the monoprotected TFA derivatives 43 and 47 were obtained in 54% and 82% yield (Scheme 16). Once again we observed a higher yield with the longer chain polyamine. In the synthesis of both 43 and 47 the crude product which contained some diprotected amine was purified on silica gel. The silica gel reacted with the

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{O} \quad \text{O} \quad \text{N} \quad \text{CF}_3 \\
\text{43} & \quad \text{H}_2\text{N} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{N} \quad \text{CF}_3 \\
\text{47}
\end{align*}
\]
Scheme 16

Crude 47 and a pink-red band was formed during the purification process. This was not observed in the purification of 43. It was later found that the mixture containing the mono- and diprotected derivatives can be easily separated by suspending the residue in water and extracting with dichloromethane. In this instance the mono-derivatized compound distributes itself in the aqueous phase and the diprotected derivative was extracted into the organic phase.
2.4.1 Synthesis of (2E)-N-(4,9-dioxa-1,12-dodecanediamine)-3-(4-hydroxyphenyl) prop-2-enamide (26)

Scheme 17

\[
\begin{align*}
&\text{H}_2\text{N} - \text{O} - \text{O} - \text{NH}_2 \\
&\text{ETFA, MeOH} \quad -78 \degree \text{C} \\
&\text{H}_2\text{N} - \text{O} - \text{O} - \text{N} - \text{CF}_3 \\
&(\text{BOC})_2\text{O} \text{ THF, RT} \\
&\text{MeOH} \quad \text{NH}_4\text{OH, pH 11, } \Delta \\
&\text{Et}_3\text{N, DMF, } \Delta \\
&\text{Cl}_2\text{C} - \text{O} - \text{Cl} \quad \text{CH}_2\text{Cl}_2 \\
&\text{MeOH, HCl(g)} \quad 0 \degree \text{C} \\
&\text{HO} - \text{N} - \text{O} - \text{O} - \text{O} - \text{NH}_2
\end{align*}
\]
Reacting 43 and 47 with di-tert-butyl dicarbonate using THF as the reaction solvent gave both 44 and 48 in 97% yield (Scheme 17). The removal of the TFA groups from 44 and 48 to give 45 and 49 respectively, was complete between 20-24 hours. This reflected a significant reduction in the reaction time as compared to the reaction time for the synthesis of 35 and 39. The yields for 45 and 49 were excellent, 99% and 97% respectively.

\[ \text{45} \]

\[ \text{49} \]

The problem of incomplete acylation of 45 in the presence of an excess of the acylating agent still prevailed. We attempted to purify 46 on silica gel. Although we were able to isolate 46 in high purity we found this process to be very time consuming. After a tedious purification procedure 46 was isolated in 46% yield. A similar problem of incomplete acylation was observed in the synthesis of 50. In this
case, however, the reaction was much cleaner and therefore we avoided purification by silica gel. After work up with Na$_2$CO$_3$ to extract the excess coumaric acid, 50 was obtained in 62% yield. Removal of the protecting groups in 46 and 50 using HCl (g) at zero degrees afforded 25 and 26 in 65% and 91% yields respectively. These conjugates were obtained as oils due to their hygroscopic nature, which is accounted for by the presence of the oxygen atoms sandwiched between the methylenes.

2.5 Synthesis of (2E)-N-[3-[4-(3-aminopropyl)-1-piperazinyl]propyl]-3-(4-hydroxyphenyl)2-propenamide (27)

We attempted to synthesize compound 27 beginning with the synthesis of monoprotected-1,4-bis (3-aminopropyl) piperazine, 51a, using ETFA by standard conditions. Initial attempts to purify a mixture of 51a and the diprotected amine by flash column chromatography were not successful. Surprisingly, rinsing of the
...
column with 1N HCl solution flushed out the mono-TFA derivative. This was, however, obtained in a very low yield of 7%. This very low yield coupled with the difficulty associated with the purification procedure made us revert to Krapcho’s protocol to mono protect one of the primary amine groups using (BOC)₂O as the protecting agent. This procedure afforded 51 in 89% yield (Scheme 18).

Scheme 18
After acylating 51 with \textit{p}-acetoxycinnamoyl chloride, followed by purification by flash chromatography compound 52 was isolated as a yellow voluminous spongy solid. This reaction was selective because the inner nitrogens were tertiary. Removal of the protecting groups with HCl (g) in MeOH gave 27 as a pale yellow solid. The HPLC retention time for 27 was 9.2 minutes.

![Chemical structures of 52 and 27]

\textit{2.6 Synthesis of (2E)-N-\{2-aminoethyl\}amino\{ethyl\}-3-(4-hydroxyphenyl) prop-2-enamide (28)}

Starting the synthesis of 28 by monofunctionalization of one of the primary amines of diethylenetriamine using ETFA to give 53b (Scheme 20) was not successful. In the reaction with ETFA it was observed that only the diTFA protected derivative was isolated. This observation suggests that 53b remains an equally good or better nucleophile than the starting material. In any case, after the reaction was thought to be complete, the reaction solvent was removed and the residue suspended in water.
The aqueous phase was then extracted with dichloromethane and compound 53a was partitioned into the organic phase. Analysis of the aqueous phase which was thought to contain compound 53b revealed that it was the unreacted starting material. The $^1$H-
NMR looked different from that of the starting material and we believe a carbonate salt of the amine was rather produced in this reaction. The mass spectra also did not produce an M+1 peak of 220 as expected. To further convince ourselves that the product obtained was not 53b we reacted the aqueous phase product with (BOC)$_2$O. The reaction yielded the triBOC-3,3-iminobispropylamine as the only product, which confirmed our earlier disappointment that the product was the unreacted triamine.

Once again we returned to the monoprotection of the primary amine with (BOC)$_2$O and 53 was obtained in an excellent yield of 89% (Scheme 19). Since we were not successful in the synthesis of 53b we could not synthesize 53c and subsequently 53d. If we had succeeded in the preparation of 53d there would be only one primary amine group in the compound to acylate. With 53 in hand we decided to attempt selective acylation of the primary amine. This reaction yielded two products, 54 and 55. Again we see that there is no selectivity in the acylation of the primary amine over secondary amine. We were of the opinion that the monoacylated conjugate 55 is readily converted into the diacylated conjugate 54 but in this case at a slower rate giving rise to a 2.5:1 ratio of the products. Removal of the protecting groups in 54 and 55 gave 56 (50% yield) and 28 (73% yield) respectively.
We encountered similar problems in our attempt to synthesize compound 29.

We isolated the diTFA derivative, 57a, but the not the monoderivative, 57b, which was our target molecule (Scheme 22). 57a was reacted with (BOC)$_2$O to protect the secondary amine to give 57c. Reaction of 57c with conc. aqueous NH$_4$OH to remove the TFA groups gave 57d. These reactions were done prior to the acylation of 53. But with our experience with the non-selectivity of acylation of primary over secondary amines, we decided not to proceed with the acylation of 57d which will give the diconjugate without a free primary amine.
2.6.1 Synthesis of (2E)-N-{3-[(3-aminopropyl)amino]propyl}-3-(4-hydroxyphenyl)prop-2-enamide (29).

Scheme 21

![Scheme 21 Image](image-url)
Scheme 22

Under the same reaction conditions as described for the preparation of 53, compound 57 was obtained in 63% yield by reacting 3,3-iminobispropylamine with (BOC)$_2$O (Scheme 21). N-acylation of 57 with $p$-acetoxycinnamoyl chloride in the presence of DMF gave 58 and 59. No selectivity was observed in this reaction. The ratio of the diconjugate, 58, to the monoconjugate, 59, was 1:1.

Removal of the protecting groups with HCl/MeOH of 58 and 59 gave 60 (100 mg, 72%) and 29 (105 mg, 79%) respectively.
2.7 Synthesis of (2E)-N-[3-([2-[(3-aminopropyl)amino]cyclohexyl]amino) propyl]-3-(4-hydroxyphenyl)prop-2-enamide (30)

Scheme 23
a) Syntheses of Compounds 61, 62, 71 and 72

61 and 71 were both readily obtained in 99% yield by reacting 1,2-trans-diaminocyclohexane and 1,4-trans-diaminocyclohexane with acrylonitrile (Schemes 23 and 24). To ensure complete cyanoethylation we used 2.5 eq of the acrylonitrile.

Reduction of 71 to give 72 with 1.5 g of Raney-Ni at 50 psi of hydrogen in EtOH/1N NaOH gave a mixture of products. Separation of the mixture by chromatography gave 72, 72a and 72b. The structure of 72a was thought to be quite unique and we decided to add it to our list of compounds to be tested for bioactivity. We propose that 72a was formed via the hydrolysis of 72b.
Scheme 24 Products obtained from reduction of 71

2.7.1 Synthesis of (2E)-N-[3-[(4-[(3-aminopropyl)amino]cyclohexyl]amino)propyl]-3-(4-hydroxyphenyl)prop-2-enamide (32)

Scheme 25
To account for the incomplete reduction it was possible that insufficient Ra-Ni was provided such that reduction occurred slowly giving time for nitrile hydrolysis.

In the light of this the reduction of 71 was repeated and 3 g of Ra-Ni was used instead of 1 g. We observed in this instance 90% reduction as opposed to 30% in the previous reduction. Hydrogenation of 19 g 61 in the presence of 10 g of Raney-Ni catalyst gave 62 in 75% yield. No amide was isolated as side product in this reaction.

*b) Syntheses of 63 and 73*

Monoprotecting one of the primary amines of 62 with ETFA under standard conditions gave 63 in 46% yield. However, treatment of 72 with ETFA under the same conditions converted 72 into 73 in 98% yield.

c) *Syntheses of 65 and 75*

Attempts to react 63 and 73 with (BOC)₂O and then isolate 64 and 74 respectively were not successful. We always obtained a mixture of the 64 or 74 and the tetraBOC derivative. Since both the target compounds and the tetraBOC derivatives have the same Rf value we were not able to separate them by column chromatography. We
therefore added conc NH$_2$OH to the mixture to give the triBOC derivatives 65 and 75, which were easily separable from the tetraBOC derivatives.

**Scheme 26** Reaction of 63 and 73 with (BOC)$_2$O and removal of TFA from 64 and 74

d) Syntheses of 66 and 76
Acylation of 65 and 75 was done with p-acetoxycinnamoyl chloride in the presence of Et₃N and DMF to give 66 and 76 in 69% and 87% yield respectively. In view of the problem of premature deprotection of the BOC groups and incomplete acylations that we encountered in our previous syntheses, we decided to purge the acyl chloride with argon first, then mix with Et₃N to scavenge any residual HCl evolved during the formation of the acyl chloride. The mixture was then added to the triBOC derivatives 65 and 75. This change in procedure led to a significant increase (~30%) in the yield, especially in 76.

e) Syntheses of 30 and 32
The BOC and O-acetoxy groups of 66 and 76 were removed by bubbling HCl (g) in MeOH to give compounds 30 and 32 respectively. The yields obtained for 30 and 32 after column chromatography were 53% and 35% respectively, which were rather disappointing.
2.8 Synthesis of (2E)-N-{3-[(4-aminocyclohexyl)amino]propyl}-3-(4-hydroxyphenyl)prop-2-enamide (31)

Scheme 27
a) Synthesis of 67 and 68

Reaction of 1,4-diaminocyclohexane with 1 eq acrylonitrile did not provide a good yield of the monocyanoethylated derivative. Only 9% of the desired product was obtained and the rest was the dicyanoethylated derivative. We therefore protected one of the primary amines with (BOC)$_2$O to obtain 67 in 74% yield. Subsequent Michael addition of acrylonitrile to 67 in MeOH gave 68 in 94% yield.

b) Synthesis of 69

We attempted to protect the secondary amine of 68 with (BOC)$_2$O but we did not succeed. This could be due to the fact that the secondary amine is sterically hindered by the cyclohexyl moiety. With 68 in hand the only option we had was to reduce the nitrile and then selectively acylate the primary amine over the secondary amine. We thought this will be quite feasible because the cyclohexyl group would hinder the acylating agent to access the secondary amine.
Hydrogenation of 68 in 95% EtOH/1N NaOH and 1 g Ra-Ni at 60 psi of hydrogen gave 69 in 77% yield.

c) Synthesis of 70

In the acylation of 69 we used a milder acylating agent to functionalize the primary amine. Compound 69 was reacted with p-acetoxy cinnamic acid mediated by DCC and catalytic amount of HOBt.

Although DCU was obtained as a side reaction product, it fell out of solution as nice crystals. The residual DCU was removed by column chromatography and 70 was obtained as a pale yellow solid in 63% yield.

d) Synthesis of 31

Removal of the protecting groups did not proceed as smoothly as always. The BOC groups in particular were not cleaved readily. To overcome this the usual reaction
temperature was increased to 32 °C and the solution was allowed to stir for 18 hours.

Evaporation of the reaction solvent and recrystallization of the residue in EtOH gave 31 in 77% yield as a very pale yellow solid.
CONCLUSIONS

We were successful in synthesizing all the 11 target compounds depicted in Table 3.

It was observed that monoprotection of the primary amine with ETFA was not feasible for 1,4-bis(3-aminopropyl)piperazine, diethylenetriamine and 3,3-iminobispropylamine. In such instances we monofunctionalized the primary amine with (BOC)₂O. Although this method was efficient, it required about eight equivalents of the starting amine. This limits its use to readily available and cheap starting materials.

Removal of the TFA protecting group using concentrated NH₄OH was not as facile as we anticipated. It required between 24-48 hours for the reaction to be driven to completion. Alternate routes neither decreased the reaction time nor increased the percent yields of the desired products.

Acylation of the primary amine was achieved with varying degrees of success (30%-90% yields). In most cases removal of the BOC and O-acetyl groups, and recrystallization resulted in significant decrease in the yield of the final product. Percent yields ranged between 25% to 88%.
FUTURE WORK

Although we were successful in synthesizing all eleven target compounds, yields should be optimized by exploring reaction condition times, the equivalents of Et₃N added during acylation, and the order of addition of the solutions. The phenolic group causes a decrease in yields and since the phenyl group is bioactive future work should aim at preparing analogues which lack the hydroxyl group. The hydroxyl group attached to the cinnamoyl moiety could also be replaced with a methoxy group. Indole derivatives of the acylating agent could also be investigated in the future. In the instances where the monofunctionalization of the primary amine with ETFA failed, I suggest that future work directed towards the synthesis of such analogues should consider stepwise synthesis from protected small-amine containing building blocks. For example: the synthesis of 29 can begin with monoprotection of 1,3-diaminopropane with (BOC)₂O, followed by reaction of the monoprotected derivative with 3-chloropropanenitrile. A second protection of the nitrile with (BOC)₂O followed by reduction will give the diprotected triamine which could be a good handle for acylation. In order to support the notion that monoacylated conjugates of polyamines function as plant defense chemicals, we intend to investigate the biological activity of these novel conjugates (22-32) in the neuromuscular junctions of invertebrates and vertebrates. This work would be done in collaboration with Dr. Keith Williams (SUNY, Brooklyn).
3.1 General Experimental Procedures

3.1.1 General Procedures

Unless otherwise stated, all starting materials were obtained from commercial suppliers and were used without further purification. All non-aqueous reactions were conducted in flame dried or oven dried (120 °C) apparatus under an argon atmosphere. All reactions were magnetically stirred unless otherwise noted. Air sensitive reagents and solution were transferred via syringe or cannula and were introduced under positive pressure of argon. Reactions requiring heating were immersed in a temperature controlled oil bath. The low temperature baths were acetone/liquid nitrogen (-78 °C) and water/ice (0 °C). Removal of solvents was normally accomplished by using a Buchi rotary evaporator connected to an aspirator vacuum. Final solvent removal was accomplished by a high vacuum pump.

3.1.2 Chromatography

Chromatography was carried out using Aldrich silica gel (70-230 mesh). Analytical thin layer chromatography (TLC) was performed on silica gel 60 F_{254} on aluminium backed plates from EM Science. Visualization was effected either by short-wavelength UV lamp, exposure to iodine vapor, or by immersion in a solution of ninhydrin in ethanol (0.2 g in 100 ml) followed by heating with hot air from a heat gun.
3.1.3 Reagents and Solvents

Reagent grade solvents were used for all extraction and workup procedures without further purification. Distilled water was used for all aqueous extractions and obtaining solutions. Reaction solvents were dried and purified according to standard procedures. THF was dried over Na metal in the presence of benzophenone. Dichloromethane was distilled from calcium hydride. Methanol was distilled from magnesium turnings. N, N- dimethylformamide (DMF) was refluxed and subsequently distilled from BaO.

3.1.4 Physical Data

Melting point ranges were determined on a Kofler hot stage apparatus and are uncorrected. In most cases, the melting point of the final compounds could not be determined with accuracy because of their hygroscopic nature. $^1$H (at 273 K and 300 MHz) and $^{13}$C (at 75.6 MHz) spectra were obtained on a Bruker Advanced DPX-300 digital FT NMR spectrometer with CDCl$_3$ and $d_6$-DMSO as the solvents. The chemical shifts, $\delta$, for the $^1$H were recorded in ppm relative to CDCl$_3$ ($\delta=7.26$ ppm) and that for $^{13}$C was relative to CDCl$_3$ ($\delta=77.0$ ppm) or DMSO ($\delta=39.5$ ppm). The coupling constants, $J$, were recorded in Hz. Coupling patterns are abbreviated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). FAB-MS
(fast atom bombardment) was obtained using a Carlo Erba/Kratos HR GC/MS concept 1S double focussing mass spectrometer interfaced to a kratos DART acquisition system and a SUN SPARC workstation. Data were recorded as m/z values for the molecular ion and the major fragments. The matrix material used was 3-Nitrobenzyl alcohol (NBA).

3.2 Synthesis of \((2E)\)-\(N\)-(6-aminohexyl)-3-(4-hydroxyphenyl)prop-2-enamide (22).

a) Synthesis of \(N\)-(6-aminohexyl)-2,2,2-trifluoroacetamide (33)

\[
\text{H}_2\text{N}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CF}_3
\]

9.75 g (83.9 mmol) of 1, 6-hexanediame was dissolved in MeOH (50 ml). A solution of ethyl trifluoroacetate, ETFA, (1 eq, 11.9 g, 9.98 ml) was added dropwise to the diamine with a gas tight syringe at \(-78 \, ^\circ C\) and the reaction temperature maintained for 1 hr 30 mins. The reaction mixture was then warmed to \(0 \, ^\circ C\) over the next 1 hr. The MeOH solvent was evaporated under reduced pressure to afford 33 as a clear viscous pale yellow oil (16.9 g, 95%).

TLC \(R_f = 0.47\) \((\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH} 30\% = 10:4:1)\)

\(^1\text{H-NMR} \ (\text{CDCl}_3) \delta \ 8.27(\text{s br.}, 1\text{H}), \ 3.28(\text{t}, 2\text{H}), \ 2.65-2.61 \ (\text{t}, 2\text{H}), \ 1.53 \ (2\text{H}), \ 1.40-1.38 \ (\text{m}, 4\text{H}), \ 1.30-1.28(\text{m}, 4\text{H}).\)
13C-NMR (CDCl$_3$) 157.79 (q, 36 Hz), 116.43 (q, 286 Hz), 42.36, 40.04, 33.92, 33.62, 29.15, 29.04

FAB-MS (CDCl$_3$) m/z 213 ([M+1]$^+$, 100%), 117 (10%)

b) *Synthesis of tert-butyl 6-[(trifluoroacetyl)amino]hexylcarbamate (34)*

![Structural formula of 34](image)

A solution of (BOC)$_2$O (1 eq., 16.5 g, 75.5 mmol) in THF was added dropwise to 16.0 g (75.5 mmol) of 33 in THF (35 ml) and mixture stirred overnight at RT. The reaction was monitored by TLC. The solvent was removed under reduced pressure to give 34 as a clear yellow viscous oil (23.3 g, 99%) which solidified at RT.

TLC $R_f = 0.85$ (CH$_2$Cl$_2$/MeOH/NH$_4$OH 30% = 10:4:1)

$^1$H-NMR (CDCl$_3$) $\delta$ 6.5 (s br., 1H), 4.52 (s, br., 1H), 3.39 (t, 2H), 3.11 (s br., 2H), 1.45 (s, 9H, t-Bu), 1.39-1.28 (m, 8H).

$^{13}$C-NMR (CDCl$_3$) 160.0, 39.87, 30.43, 29.17, 29.07, 28.78, 26.74, 26.16, 25.90

FAB-MS m/z 313 ([M+1]$^+$, 8%), 256 (46%), 239 (29%), 161 (65%).

c) *Synthesis of tert-butyl 6-aminohexylcarbamate (35)*

23.0 g of 34 was suspended in MeOH (50 ml). Aqueous conc. NH$_4$OH was added to
the solution and the pH adjusted to 11. The reaction mixture was stirred and refluxed for 48 hours. The reaction mixture was then evaporated to dryness. Water (60 ml) was added and the aqueous phase extracted with CH$_2$Cl$_2$. Compound 35 remained in the aqueous phase and was concentrated under reduced pressure to give a colorless oil (12.0 g, 75%).

R$_f = 0.57$ (CH$_2$Cl$_2$/MeOH/NH$_4$OH 30% = 10:4:1)

$^1$H-NMR (CDCl$_3$) $\delta$ 4.63 (s br., 1H), 3.09 (t, 2H), 2.70 (m, 2H), 2.25 (2H, -NH$_2$), 1.42 (s, 9H, t-Bu), 1.40 (m, 4H), 1.29-1.20 (m, 4H)

$^{13}$C-NMR (CDCl$_3$) 156.4, 79.4, 42.2, 40.8, 33.4, 30.4, 28.8, 26.8.

FAB-MS m/z 433 ([2M+1]$^+$, 7%), 217 ([M+1]$^+$, 100%), 161 (56%), 117 (19%)

d) Synthesis of 4-[(E)-3-({6-[((tert-butoxycarbonyl)amino]hexyl}amino)3-oxoprop-1-enyl]phenyl acetate (36)

2.50 g (11.6 mmol) of 35 was suspended in CH$_2$Cl$_2$ (30 ml) and Et$_3$N (1.20 eq, 1.41 g, 1.94 ml) was added to the solution and stirred. Under an argon atmosphere (E)-3-(4-acetoxyphenyl) prop-2-enoic acid (0.90 eq, 2.15 g, 10.4 mmol) was dissolved in 20 ml of CH$_2$Cl$_2$ and a solution of oxalyl chloride (1.20 eq, 1.76 g, 1.21 ml) in CH$_2$Cl$_2$ (20 ml) added dropwise in the presence of ~ 70 µL DMF to form the (E)-3-(4-
acetoxyphenyl) prop-2-enoyl chloride. The reaction mixture was refluxed for 1 hr. The corresponding acid chloride was added dropwise to 35 and Et$_3$N mixture at RT over 10 minutes. The reaction mixture was refluxed overnight. The solvent was removed under reduced pressure, and the residue dissolved in distilled water. The aqueous phase was then extracted with CH$_2$Cl$_2$. The combined organic fractions were washed with 0.25 M Na$_2$CO$_3$ to remove the excess coumaric acid in solution. The organic phase was dried with anhydrous Na$_2$SO$_4$ and evaporated to dryness to afford 1.74 g of a yellow spongy voluminous product. The crude product was purified on silica gel with CH$_2$Cl$_2$/MeOH = 20:1, followed by CH$_2$Cl$_2$/MeOH = 10:1 as eluent to give 1.63 g of 36. Yield was 40%.

TLC $R_f = 0.50$ (CH$_2$Cl$_2$/MeOH = 10:1)

$^1$H-NMR (CDCl$_3$) δ 7.64 (d, J=16 Hz), 7.54 (d, 2H, J=8 Hz), 7.12 (d, 2H, J=8 Hz), 6.4 (d, 1H, J=16 Hz) 5.90 (s br., 1H), 4.55 (s br., 1H) 3.42 (m, 2H), 3.1 (m, 2H), 2.34 (s, 3H, OCH$_3$), 1.61 (m, 2H), 1.46 (s, 9H, t-Bu), 1.39 (m, 4H)

$^{13}$C-NMR (CDCl$_3$) 169.4, 162.7, 153.3, 147.9, 131.8, 130.2, 129.2, 122.4, 121.5, 117.2, 42.2, 40.8, 33.4, 30.4, 28.8, 21.5.

FAB-MS m/z 809 ([2M+1]$^+$, 6%), 405 ([M+1]$^+$, 22%) 349 (24%), 305 (63%), 189 (49%), 147 (100%).
e) Synthesis of (2E)-N-(6-aminohexyl)-3-(4-hydroxyphenyl)prop-2-enamide (22)

1.63 g (4.50 mmol) of 36 was suspended in 40 ml dry MeOH. Dry HCl(g) was bubbled into the solution at 0 °C to give compound 22 as yellow crystals after solvent has been removed on the rotary evaporator, recrystallized with EtOH and finally dried on the high vacuum (800 mg, 68%).

TLC R_f = 0.30 (CH_2Cl_2/MeOH = 10:1)

HPLC Rt = 11.7 mins

^1H-NMR (CDCl_3) δ 8.02 (s br., 1H), 7.37 (d, 2H, J=8 Hz), 7.31 (d, 1H, J=16 Hz), 3.94 (s br.,1H), 3.14-3.13 (m, 2H), 2.74 (m, 2H), 1.53-1.08 (m, 8H).

^13C-NMR (CDCl_3) 165.15, 159.65, 139.27, 129.93, 126.81, 119.74, 116.59, 31.51, 29.88, 27.76, 26.78, 26.35

FAB- MS m/z 525 ([2M+1]^+, 40%), 263 ([M+1]^+, 100%), 147 (78%)
3.3 Synthesis of 2(E)-(8-aminooctyl)-3-(4-hydroxyphenyl)prop-2-enamide (23)

a) Synthesis of N-(8-aminooctyl)-2,2,2-trifluoroacetamide (37)

\[ \text{H}_2\text{N} - \text{CF}_3 \]

1,8-octanediamine (7.60 g, 52.7 mmol) was dissolved in MeOH (50 ml). A solution of ETFA (1 eq., 7.49 g, 6.27 ml) was added to the diamine solution with a gas tight syringe within 10 mins at -78 °C and temperature maintained for 1 hr 30 mins. The reaction mixture was warmed to 0 °C for the next hour. The solvent was evaporated under reduced pressure to afford 37 as a pale yellow oil. This was purified on silica gel (MeOH/CH\textsubscript{2}Cl\textsubscript{2}/AcOH = 60:40:2) to afford 11.9 g (94%) of a pale yellow oil containing some fine crystals.

\[ \text{TLC } R_f = 0.48 \quad (\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH }30\% = 10:4:1) \]

\[ ^1\text{H-NMR} \ (\text{CDCl}_3) \delta \ 7.68 \ (s \ br., \ 1H) \ 3.29 \ (t, \ 2H), \ 2.65 \ (t, \ 2H), \ 1.73 \ (2H), \ 1.55 \ (2H), \ 1.42 \ (2H), \ 1.28 \ (m, \ 8H) \]

\[ ^13\text{C-NMR} \ (\text{CDCl}_3) \ 157.79 \ (q, \ J=36 \text{ Hz}), \ 116.38 \ (q, \ J=286 \text{ Hz}), \ 50.38, \ 42.45, \ 40.2, \ 33.96, \ 29.78, \ 29.43, \ 27.17, \ 27.06 \]

\[ \text{FAB-MS m/z} \ 241 [(M+1]^+, \ 100\%), \ 185 (7\%), \ 145 (34\%), \ 126 (10\%), \ 69 (22\%), \ 56 (21\%), \ 30 (52\%). \]
b) Synthesis of tert-butyl 8-[(trifluoroacetyl)amino]octylcarbamate (38)

A solution of 1 eq (BOC)$_2$O (10.85 g) in THF (30 ml) was added to a solution of 37 (11.94 g, 49.75 mmol) in THF (30 ml) with syringe at RT. The reaction was allowed to proceed overnight. The solvent was evaporated in vacuo to give a yellow oil which was purified on silica gel (CH$_2$Cl$_2$/MeOH/ammonia water 30% = 10:4:1) to afford 38 (16.71 g, 99%) as a yellow viscous oil.

TLC $R_f = 0.75$ (CH$_2$Cl$_2$/MeOH/NH$_4$OH = 10:4:1)

$^1$H-NMR (CDCl$_3$) $\delta$ 6.48 (s, br., 1 H), 4.53 (s br., 1 H), 3.38 – 3.33 (m, 2H), 3.13 - 3.07 (m, 2H), 1.66 – 1.57 (m, 2 H), 1.45 (s, 9H, t-Bu), 1.43 – 1.32 (10H).

$^{13}$C-NMR (CDCl$_3$) 175.4,157.64 (q, $J=36$ Hz),116.32 (q, $J=287$ Hz), 40.9, 40.3, 30.4, 29.6, 29.3, 29.2, 28.8, 27.0, 26.9, 26.8.

FAB-MS m/z 363 ([M+Na]$^+$, 82%), 341 ([M+1]$^+$, 17%), 285 (74%), 241 (100%), 57 (96%)

c) tert- butyl 8-aminooctylcarbamate(39)

16.6 g of 38 was suspended in MeOH (50 ml). Conc. NH$_4$OH was added and pH
adjusted to 11. The reaction mixture was stirred and refluxed for 48 hrs. A white precipitate was formed and filtered off. The solvent was evaporated. Water (60 ml) was added to the residue and the aqueous phase was washed with CH₂Cl₂. The aqueous phase which contained 39 was concentrated under reduced pressure to afford 11.78 g (99%) of yellow viscous oil, which solidifies at RT.

TLC Rₜ = 0.50 (CH₂Cl₂/MeOH/NH₄OH 30% = 10:4:1)

¹H-NMR (CDCl₃) δ 7.79 (s, br., 2H), 4.6 (s, br., 1H), 3.08 (t, 2H) 2.93 (t, 2H), 1.67 (t, 2H), 1.45 (s, 9H, t-Bu), 1.40-1.30 (10H).

¹³C-NMR (CDCl₃) 175.4, 50.6, 40.9, 40.3, 30.2, 29.0, 28.8, 27.6, 26.7, 26.4.

FAB-MS m/z 489 ([2M+1]⁺, 7%), 245 ([M+1]⁺, 100%), 189 (93%), 145 (18%), 57 (83%).


4.00 g of 39 (16.4 mmol) was dissolved in 15 ml of CH₂Cl₂ and Et₃N (1.20 eq, 1.99 g, 2.74 ml) was added to the solution and stirred. In a separate flask under argon atmosphere, (E)-3-(4-acetoxylphenyl) prop-2-enoic acid (0.90 eq, 3.04 g, 14.7 mmol)
was suspended in 25 ml of CH$_2$Cl$_2$ plus 70 µL of DMF and a solution of oxalyl chloride (1.20 eq. 2.08 g, 1.72 ml) in CH$_2$Cl$_2$ (15 ml) added dropwise to form the (E)-3-(4-acetoxyphenyl) prop-2-enoyl chloride, and solution refluxed for 1hr. The acid chloride so produced was added dropwise to 39/E$_3$N mixture over 10 minutes and the reaction mixture was refluxed for 24 hrs. The solvent was removed under reduced pressure, the residue was suspended in water, and extracted with CH$_2$Cl$_2$. The organic phase was then washed with 0.25 M Na$_2$CO$_3$ and extracted with CH$_2$Cl$_2$. The combined organic fractions were dried with Na$_2$SO$_4$. Evaporation of the dried organic phase afforded 40 (4.28 g, 60%) as a yellow spongy voluminous solid.

TLC $R_f = 0.37\quad$ (CH$_2$Cl$_2$/MeOH = 10:1)

$^1$H-NMR (CDCl$_3$) $\delta$ 7.63 (d, 1H, J= 16 Hz), 7.53 (d, 2H, J= 8 Hz), 7.11 (d, 2H, J= 8 Hz), 6.39 (d, 1H, J=16 Hz), 5.78 (s, br, 1H), 4.54 (s, br, 1H) 3.41-3.32 (m, 2H), 3.14 (m, 2H), 2.34 (s, 3H, OCH$_3$), 1.58 (m, 2H), 1.45 (s, 9H), 1.32 (10H).

$^{13}$C-NMR (CDCl$_3$) 166.3 159.8, 139.7, 130.0, 129.9, 126.9, 119.5, 116.6, 70.1, 69.9, 30.1, 29.0, 28.8, 27.6, 26.9

FAB-MS m/z 865([2M+1]$^+$, 5%),433([M+1]$^+$, 17%), 377(14%), 359(5%)

333(31%), 189(20%), 147(40%).
e) Synthesis of (2E)-N-(8-aminooctyl)-3-(4-hydroxyphenyl)prop-2-enamide (23)

1.10 g of 40 (2.54 mmol) was suspended in MeOH (30 ml). Dry HCl (g) was bubbled into the solution at 0°C. Fine yellow crystals of 23 were isolated by filtration (650 mg, 88%).

TLC \( R_f = 0.36 \) (CH\(_2\)Cl\(_2\)/MeOH=10:1)

HPLC \( R_t = 15.1 \) mins.

\(^{1}\)H-NMR (DMSO) \( \delta \) 7.37 (d, 2H, J=8 Hz), 7.31 (d, 1H, J=16 Hz), 6.81 (d, 2H, J=8 Hz), 6.45 (d, 1H, J=16 Hz), 3.16 (2H) 2.88 (2H), 1.54-1.43 (4H), 1.26 (8H).

\(^{13}\)C-NMR (DMSO) 166.1, 159.7, 139.2, 129.9, 119.7, 116.6, 30.0, 29.3, 29.1, 27.7, 27.2, 26.5

FAB-MS m/z 581 ([2M+1]\(^{+}\), 5%), 291 ([M+1]\(^{+}\), 100%), 176 (26%), 147 (89%) 145 (41%).
3.4 Synthesis of (2E)-N-(12-aminododecyl)-3-(4-hydroxyphenyl)prop-2-enamide (24)

a) Synthesis of tert-butyl 12-aminododecylcarbamate (41)

10.0 g of 1,12-dodecanediamine (50.0 mmol) was dissolved in dioxane (50 ml). A solution of 0.125 equivalent (BOC)$_2$O (1.36 g, 6.25 mmol) in 25 ml of dioxane was added to the diamine solution dropwise at RT and stirred for 6.5 hrs. Reaction mixture turned milky with significant amount of white precipitate which was filtered off. The dioxane was evaporated on the rotary evaporator. The residue was suspended in distilled water and extracted with CH$_2$Cl$_2$. The organic phase was dried with Na$_2$SO$_4$ and solvent removed under reduced pressure and the product obtained was distilled to afford 41 (1.36 g, 74%) as yellow oil.

TLC $R_f$ = 0.40 (CH$_2$Cl$_2$/MeOH/NH$_4$OH = 10:4:1)

$^1$H-NMR (CDCl$_3$) $\delta$ 4.52 (s br., 1H), 2.71 (t, 2H), 1.45 (s, 9H, t-Bu), 1.28 (22H).

$^{13}$C-NMR (CDCl$_3$) 176.5, 79.1, 42.9, 40.4, 34.3, 30.1, 29.9, 29.8, 29.7, 29.3, 29.0, 26.9, 26.5, 26.1

FAB-MS m/z 601 ([2M+1]$^+$, 2%), 301 ([M + 1]$^+$, 100%), 245 (79%), 201 (36%).
b) Synthesis of 4-[(1E)-3-((12-[(tert-butoxycarbonyl)amino]dodecyl)amino)-3-oxoprop-1-enyl]phenyl acetate(42)

0.95 g of (E)-3-(4-acetoxyphenyl) prop-2-enoic acid was suspended in CH₂Cl₂ (20 ml). A solution of 1 eq oxalyl chloride (0.58 g, 0.40 ml) in CH₂Cl₂ (20 ml) and 70 μLDMF was added. The reaction mixture was heated to reflux for 1 hour. At RT under argon atmosphere a solution of the prepared acyl chloride was added dropwise within 10 minutes to a solution of 41 (1.38 g, 4.60 mmol) and Et₃N (1.20 eq, 0.47 g, 0.64 mmol) in CH₂Cl₂ (30 ml) and stirred for 30 mins. The suspension was then refluxed at 40 °C overnight. The mixture was evaporated to dryness in vacuo to afford 1.85 g of crude product which was purified on silica gel (CH₂Cl₂/MeOH = 50:1, 40:1, 20:1 and 5:1) to give 42 as a yellow spongy solid (540 mg, 22%).

TLC R_f = 0.46 (CH₂Cl₂/MeOH = 10:1)

¹H-NMR (CDCl₃) δ 7.65 (d, 1H, J=16 Hz), 7.54 (d, 2H, J=9 Hz), 7.13 (d, 2H, J=9 Hz), 6.37 (d, 1H, J=16 Hz), 5.62 (s br., 1H), 4.51 (s br., 1H), 3.41 (t, 2H), 3.11 (m, 2H), 2.33 (s, 3H, OCH₃), 1.58 (m, 2H), 1.46 (s, 9 H, t-Bu).

¹³C-NMR (CDCl₃) 169.8, 166.5, 156.7, 152.1, 139.7, 133.3, 129.7, 129.1, 122.6, 121.9, 79.3, 71.4, 42.9, 40.6, 34.5, 30.2, 29.8, 29.7, 29.6, 29.4, 29.1, 28.9, 27.0, 26.8
FAB-MS m/z 489 ([M + 1]*, 7%), 433 (5%), 389 (31%), 245 (48%), 189 (20%), 147 (60%), 57 (100%).

c) Synthesis of (2E)-N-(12-aminododecyl)-3-(4-hydroxyphenyl)prop-2-enamide (24)

\[
\begin{align*}
\text{HO} & \quad \text{N} \quad \text{CH} \quad \text{NH}_2 \\
\text{24}
\end{align*}
\]

Into a solution of 42 (520 mg, 0.107 mmol) in 40 ml of dry MeOH was bubbled dry HCl (g) at 0 °C. After stirring at RT for 1.5 hours, and evaporation to dryness the residue was washed with Et₂O. Drying of the insoluble precipitate afforded 24 as a yellow solid (120 mg, 32%).

TLC \( R_f = 0.66 \) (CH₂Cl₂/MeOH/NH₄OH = 10:4:1)

HPLC \( R_t = 15.8 \) mins

\(^1\)H-NMR (\( d_6 \)-DMSO) \( \delta \) 7.60 (d, 1H, J=15 Hz), 7.41 (d, 2H, J=9 Hz), 6.87 (d, 2H, J=9 Hz), 6.29 (d, 1H, J=16 Hz), 5.69 (s br., 1H), 3.43 (m, 2H), 3.11 (m, 2H), 1.60-1.57 (m, 4H), 1.27 (16H).

\(^{13}\)C-NMR (\( d_6 \)-DMSO) 172.2, 165.5, 144.2, 134.6, 131.0, 124.2, 121.4, 42.7, 40.4, 34.3, 30.0, 29.9, 29.8, 29.7, 29.5, 29.4, 29.3, 27.3, 27.0

FAB-MS m/z 347 ([M + 1]*, 100%), 330 ([M+1-NH₃, 2%], 371 (2%), 201 (6%), 147 (83%), 86 (4%), 72 (3%).
3.5 Synthesis of (2E)-N-[2-[(2-aminoethoxy)ethoxy]ethyl]-3-(4-hydroxyphenyl)prop-2-enamide (25)

a) Synthesis of N-[2-[(2-aminoethoxy)ethoxy]ethyl]-2,2,2-trifluoroacetamide (43)

30.0 g (29.5 ml, 202 mmol) of 2,2’-(ethylenedioxy) bis (ethyamine) was dissolved in MeOH (180 ml). A solution of ETFA (1 eq, 202 mmol, 28.8 g, 24.1 ml) in MeOH (90 ml) was added to the diamine solution dropwise with a gas tight syringe. The reaction temperature was maintained at -78 °C for 1 hr 30 mins, then warmed to 0 °C for the next hour. The solvent was evaporated in vacuo and poured into 100 ml of water. The aqueous phase was then extracted with CH₂Cl₂. The organic phase was concentrated and purified on silica gel (CH₂Cl₂/MeOH = 20:1 followed by 10:1) to give 43 as a pale yellow oil (26.5 g, 54 %).

TLC R_f = 0.58 (CH₂Cl₂/MeOH/NH₄OH = 10:4:1)

¹H-NMR (CDCl₃) δ 8.75 (s br., 1H), 3.72 (t, 2H), 3.64 (m, 6H), 3.54 (t, 2H), 3.12 (t, 2H).

¹³C-NMR (CDCl₃) 158.16 (q, J=36 Hz), 116.37 (q, J=286 Hz), 70.5, 69.5, 67.4, 40.1, 39.7, 22.3.
b) Synthesis of N-tert butyl 2-(2-[trifluoroacetyl]amino)ethoxy)ethoxy) ethyl carbamate) (44)

\[ \text{Synthesis of N-tert butyl 2-(2-[trifluoroacetyl]amino)ethoxy)ethoxy) ethyl carbamate) (44)} \]

A solution of (BOC)\(_2\)O (1 eq, 21.97 g) in THF (60 ml) was added to 43 (24.56 g, 100.7 mmol) in THF (100 ml) at RT and stirred for 3 hrs. The reaction mixture was evaporated to dryness to afford 44 as a pale yellow oil (33.25 g, 97%) which was used for the next step of the reaction without further purification.

TLC \( R_f = 0.73 \) (CH\(_2\)Cl\(_2\)/MeOH/NH\(_4\)OH = 10:4:1)

\(^1\)H-NMR (CDCl\(_3\)) \( \delta \) 3.69 (m, 6H), 3.54 (m, 4H), 3.31 (t, 2H), 1.43 (s, 9H, t-Bu).

\(^13\)C-NMR (CDCl\(_3\)) 174.98, 157.29 (q, 36 Hz), 116.37 (q, \( J=286 \) Hz), 70.5, 70.4, 69.2, 67.2, 40.0, 39.6, 22.3.

FAB-MS m/z 367 ([M+Na]\(^+\), 100%), 345 ([M+1]\(^+\), 5%), 289 (13%), 245 (42%), 57 (33%).

c) Synthesis of tert-butyl 2-[2-(2-aminoethoxy)ethoxy]ethyl carbamate (45)

\[ \text{Synthesis of tert-butyl 2-[2-(2-aminoethoxy)ethoxy]ethyl carbamate (45)} \]
33.0 g of 44 was suspended in MeOH (100 ml) and conc. NH₄OH was added to the solution. The reaction mixture was stirred and heated to reflux for 24 hours. The reaction mixture was evaporated to dryness in vacuo and water (100 ml) was added. The aqueous phase was then washed with CH₂Cl₂. Evaporation of the aqueous phase gave 45 as yellow oil (23.1 g, 97%).

TLC Rᵢ = 0.53 (CH₂Cl₂/MeOH/NH₄OH = 10:4:1)

¹H-NMR (CDCl₃) δ 5.38 (s br., 1H), 3.72 (t, 2H), 3.69 (m, 4H), 3.54(t, 2H), 3.27(t,2H), 3.14(2H), 1.42(s, 9H, t-Bu).

¹³C-NMR (CDCl₃) 158.5, 114.1, 65.7, 65.6, 65.4, 62.4, 62.1, 61.9, 35.9, 35.1, 23.9, 23.6.

FAB-MS m/z 497 [2M +1]⁺, 5%), 249 ([M+1]⁺, 84%), 193 (30%), 149 (100%), 106 (30%), 57 (40%), 44 (96%).


7.48 g (0.9 eq) of (E)-3-(4-acetoxyphenyl) prop-2-enoic acid was suspended in CH₂Cl₂ (60 ml). A solution of oxalyl chloride (1 eq, 5.12 g, 3.52 ml) in CH₂Cl₂ (20 ml) was added dropwise to the acid in the presence of catalytic DMF to form the acyl
chloride. Under argon atmosphere, a solution of the prepared acyl chloride was added dropwise within 10 mins to a solution of 45 (10.0 g, 40.3 mmol) and Et₃N (1.20 eq, 4.90 g, 6.74 ml) in CH₂Cl₂ (40 ml). The suspension was then refluxed for 20 hours and the mixture was evaporated in vacuo. Water (100 ml) was added and then extracted with CH₂Cl₂. The organic phase was washed with 0.25 M Na₂CO₃ and then dried with Na₂SO₄. After evaporation to dryness, the residue (12.9 g) was purified by flash chromatography on silica gel (CH₂Cl₂/MeOH = 20:1, 10:1). 46 was isolated as a yellow viscous oil (7.90 g, 45%).

TLC R₂f = 0.52 (CH₂Cl₂/MeOH = 10:1)

¹H-NMR (CDCl₃)  δ  7.65 (d, 1H, J=15 Hz), 7.54 (d, 2H, J=8 Hz), 7.12 (d, 2H, J=8 Hz), 6.46 (d, 1 H, J=15 Hz), 3.64 (m, 6H), 3.59 (m, 4H), 3.34 (2H), 2.34 (s, 3H, OCH₃), 1.46 (s, 9H, t-Bu).

¹³C-NMR (CDCl₃)  166.32, 159.70, 139.57, 130.02, 126.71, 119.42, 116.57, 70.33, 70.03, 29.07.

FAB-MS m/z  459 ([M+Na]⁺, 19%), 437 [M+1]⁺, 13%), 339( 6%), 337 (62%), 245 (22%), 189(46%), 147 (100%).
e) Synthesis of (2E)-N-{2-[2-(2-aminoethoxy)ethoxy]ethyl}-3-(4-hydroxyphenyl)prop-2-enamide (25)

To a solution of 46 (2.00 g, 4.60 mmol) in 40 ml of dry MeOH was bubbled dry HCl(g) at 0 °C. Reaction mixture was stirred at RT for 1.5 hrs. After evaporation to dryness the residue was washed with Et₂O, recrystallized in EtOH and then dried by high vacuum drying to afford 25 as a yellow viscous oil (880 mg, 65%).

TLC R<sub>f</sub> = 0.20 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH/NH₄OH = 10:4:0.5)

HPLC Rt = 8.1 mins

<sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 7.37 (d, 2 arom H, J=8.36), 7.31 (d, 1 olef H, J=16), 6.81 (d, 2 arom H, J=8.40), 6.52 (d, 1 olef H, J=16), 3.66 (t, 2H), 3.53 (m, 4 H), 3.45(t, 2H), 3.30 (t, 2H), 2.93 (t, 2H).

<sup>13</sup>C-NMR (CDCl<sub>3</sub>) 171.2, 164.5, 144.3, 134.7, 131.4, 124.2, 121.4, 75.3, 75.1, 74.8, 73.4, 72.2.

FAB-MS m/z 589 ([2M+1]<sup>+</sup>, 5%), 295 [M+1]<sup>+</sup>,96%), 178 (14%), 149 (48%), 147 (7%), 89 (16%), 44 (53%).
3.6 Synthesis of (2E)-N[3-[4-(3-aminopropoxy)butoxy]propyl]-3-(4-hydroxyphenyl)prop-2-enamide (26)

a) Synthesis of N-{3-[4-(3-aminopropoxy)butoxy]propyl})-2,2,2-trifluoroacetamide (47)

![Chemical Structure]

12.0 g (58.8 mmol, 12.5 ml) of 4,9-dioxa-1,12-dodecanediamine was dissolved in MeOH (40 ml). A solution of ETFA (1 eq, 58.8 mmol, 8.40 g, 7.00 ml) in MeOH (40 ml) was added to the diamine dropwise with a gas tight syringe. The reaction temperature was maintained at −78 °C for 1 hr 30 mins, then warmed to 0 °C over the next hour. The solvent was evaporated in vacuo and the residue purified on silica gel (MeOH/CH₂Cl₂/AcOH = 10:20:1, followed by 20:20:1) to afford 47 as a yellow viscous oil (14.6 g, 82%).

TLC R_f = 0.60 (CH₂Cl₂/MeOH/NaOH = 10:4:1)

¹H-NMR (CDCl₃) δ 8.20 (s br., NHCOOCH₃), 3.61-3.51 (m, 4H), 3.40 (t, 4H), 3.06 (m, 2H), 1.94 (M, 6H), 1.85 (m, 2H), 1.61 (4H).

¹³C-NMR (CDCl₃) 157.5 (q, J=36 Hz), 116.4 (q, J=286 Hz), 71.5, 70.2, 69.1, 39.3, 38.6, 28.5, 27.7, 26.7, 23.8.

FAB-MS m/z 301 ([M+1]+, 100%), 205 (18%), 154 (68%), 126 (13%), 58 (26%), 30 (18%).
b) Synthesis of tert-butyl 3-(4-[3-[(trifluoroacetyl)amino]propoxy]butoxy)propylcarbamate (48)

A solution of (BOC)$_2$O (1 eq, 9.13 g) in THF (35 ml) was added to 47 (12.6 g, 41.8 mmol) in THF (45 ml) at RT and the reaction mixture stirred overnight. After evaporation to dryness to afford, 48 was recovered as a pale yellow oil (16.3 g, 97%) which was used for the next step of the synthesis without purification.

TLC $R_f = 0.80$ (CH$_2$Cl$_2$/MeOH/NH$_4$OH = 10:4:1)

$^1$H-NMR (CDCl$_3$) $\delta$ 7.54 (s br., 1H), 4.48 (s br., 1H), 3.61-3.57 (m, 2H), 3.49-3.45 (m, 10H), 3.2 (t, 2H), 1.88-1.81 (m, 4H), 1.72 (2H), 1.43 (s, 9H, t-Bu).

$^{13}$C-NMR (CDCl$_3$) 176.0, 157.3 (q, 36 Hz), 116.4 (q, J=286 Hz), 71.6, 70.7, 69.8, 68.7, 39.8, 31.5, 30.1, 28.8, 28.3, 26.8, 25.9, 21.1.

FAB-MS m/z 423([M+Na]$^+$, 17%), 401([M+1]$^+$, 10%), 345(12%), 301(100%), 263(6%), 249(21%), 126(11%), 102(64%), 74(18%), 57(83%).

c) Synthesis of tert-butyl 3-[4-(3-aminoproxy)butoxy]propyl carbamate (49)
A solution of 16.2 g (40.5 mmol) of 48 in MeOH (100 ml) was reacted with concentrated NH₄OH. The reaction solution was heated to reflux at 65°C for 20 hrs. After evaporation to dryness, the residue was purified on silica gel (CH₂Cl₂/MeOH = 20:1, then 10:1) to give 49 as a yellow oil (12.2 g, 99%).

\[ \text{TLC } R_f = 0.70 \ (\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH} = 10:4:1) \]

\[ ^1\text{H-NMR} \ (\text{CDCl}_3) \delta 5.01 \ (s \ br., 1H), 3.59 \ (t, 2H), 3.49-3.42 \ (m, 6H), 3.20 \ (t, 2H), 3.12(t, 2H), 1.96 \ (m, 2H), 1.92 \ (m, 2H), 1.76 \ (t, 2H), 1.62 \ (m, 4H), 1.43 \ (s, 9H, t-Bu). \]

\[ ^{13}\text{C-NMR} \ (\text{CDCl}_3) \ 176.6, 71.5, 71.1, 70.9, 69.7, 69.3, 39.4, 38.9, 30.1, 28.8, 27.4, 26.8, 26.7. \]

\[ \text{FAB-MS m/z } 327 \ ([M+Na]^+, 14\%), 305 \ ([M+1]^+, 100\%), 249 \ (23\%), 231 \ (6\%), 205(16\%), 146 \ (10\%), 130 \ (6\%), 102 \ (30\%), 74 \ (20\%), 58 \ (84\%), 57(67\%). \]

d) *Synthesis of 4-[(1E)-20,20-dimethyl-3,18-dioxo-8,13,19-trioxa-4,17-diazahenicos-1-en-yl]phenyl acetate (50)*

(E)-3-(4-acetoxyphenyl) prop-2-enoic acid (0.90 eq, 6.10 g) was suspended in CH₂Cl₂ (80 ml). A solution of oxalyl chloride (4.18 g, 2.87 ml) in CH₂Cl₂ (40 ml) was added dropwise to the acid in the presence of catalytic DMF to form (E)-3-(4-
acetoxyphenyl) prop-2-enoyl chloride. The reaction mixture was heated to reflux at 40 °C for 1 hour. At RT under Argon, a solution of the prepared acyl chloride was added within 20 mins to a solution of 49 (10.0 g, 32.9 mmol) and Et₃N (1.20 eq, 3.99 g, 5.50 ml) in CH₂Cl₂ (40 ml) and stirred for 30 mins. The suspension was then heated to reflux for 25 hours. The mixture was evaporated to dryness in vacuo, water (100 ml) was added and the mixture extracted with CH₂Cl₂. The organic phase was then washed with 0.25 M Na₂CO₃ and finally dried with Na₂SO₄. Evaporation to dryness gave 50 as a yellow oil (10.0 g, 62%).

TLC Rf = 0.52 (CH₂Cl₂/MeOH = 10:1)

¹H-NMR (CDCl₃) δ 7.56 (d, 1H, J=16 Hz), 7.46 (d, 2H, J=8 Hz), 7.05 (d, 2H, J=16 Hz), 6.38 (d, 1H, J=8 Hz), 5.03 br., 1H), 3.51-3.47 (t, 2H), 3.45-3.38 (m, 8H), 3.17(m, 2H), 2.26 (s, 3H, OCH₃), 1.81 (m, 2H), 1.78 (m, 2H), 1.64 (m, 4H), 1.40 (s, 9H, t-Bu)

¹³C-NMR (CDCl₃) 169.6, 166.3, 156.5, 151.8, 139.7,133.1, 129.6, 129.1, 122.5, 121.7, 79.3, 71.1, 71.0, 69.9, 69.5, 39.1, 38.6, 30.0, 29.5, 28.8, 26.9, 21.5.

FAB-MS m/z 493 ([M+1]+, 14%), 393 (64%), 305 (15%), 249 (35%), 189 (20%), 147 (55%), 102 (68%), 74 (25%), 57 (100%).
e) Synthesis of (2E)-N-{3-[4-(3-aminoproxy)butoxy]propyl} -3-(4-hydroxyphenyl)prop-2-enamide (26)

\[
\text{To a solution of 50 (8.30 g, 16.9 mmol) in MeOH was bubbled dry HCl(g) at 0°C.}
\]

After stirring at RT for 1.5 hours, the solvent was removed and the residue was washed with Et₂O. The washed product was dissolved in 1N HCl (80 ml) and extracted with CH₂Cl₂. Evaporation of the aqueous phase gave 26 as a bright yellow oil (5.38 g, 91%).

TLC \( R_f = 0.57 \) (CH₂Cl₂/MeOH/NH₄OH = 10:4:1)

HPLC Rt = 10.8 mins

\(^1\)H-NMR \((d_6\text{-DMSO}) \delta 7.38 (d, 2H, J=8 \text{ Hz}), 7.31 (d, 1H, J=16 \text{ Hz}), 6.81 (d, 2H, J=8 \text{ Hz}), 6.46 (d, 1H, J=16 \text{ Hz}), 3.42 (m, 10H), 3.18 (t, 2H), 2.83-2.78 (m, 2H), 1.83 (m, 2H), 1.70 (q, 2H), 1.51 (m, 4H).

\(^{13}\)C-NMR \((d_6\text{-DMSO}) 166.3, 159.7, 139.4, 129.9, 126.7, 119.6, 70.7, 68.5, 67.7, 37.4, 36.8, 30.3, 28.1, 26.8, 23.4.

FAB-MS \( m/z \) 351 ([M+1]*, 100%), 247 (41%), 205 (51%), 147 (64%), 130 (7%), 74 (12%), 58 (79%), 30 (60%).
3.7 Synthesis of (2E)-N-[3-[4-(3-aminopropyl)piperazin-1-yl]propyl]-3-(4-hydroxyphenyl)prop-2-enamide (27)

a) Synthesis of tert-butyl 3-[4-(3-aminopropyl)piperazin-1-yl]propyl carbamate (51)

![Chemical Structure](image)

12.0 g of 1,4-bis (3-aminopropyl) piperazine (60.0 mmol, 12.3 ml) was dissolved in dioxane (120 ml). A solution of 1/7.5 eq (BOC)\_2O (1.75 g, 8 mmol) in dioxane (30 ml) was added to the piperazine solution dropwise at RT and stirred for 8 hours. The solution turned milky and a white precipitate was filtered off. The dioxane solvent was evaporated on the rotary evaporator. The residue obtained was suspended in water (80 ml) and extracted with CH\_2Cl\_2. The organic phase was dried with Na\_2SO\_4 and solvent removed under reduced pressure to afford 51 as colorless oil (2.13 g, 89%) which formed needle-like crystals on the sides of the flask.

TLC R\_f = 0.33 (CH\_2Cl\_2/MeOH/NH\_4OH 30% = 10:4:1)

\(^1\)H-NMR (CDCl\_3) δ 5.52 (s br., 1H, NHBOC), 3.17 (q, 2H), 2.76 (q, 2H), 2.44-2.35 (m, 10H), 1.67-1.57 (m, 6H), 1.42 (s, 9H, t-Bu).

\(^13\)C-NMR (CDCl\_3) 156.5, 67.5, 57.2, 56.9, 53.7, 53.6, 41.3, 40.4, 30.8, 28.8, 26.7.

FAB-MS m/z 301 ([M+1]^+, 92%), 270 (10%), 256 (14%), 242 (11%), 201 (68%), 127 (20%), 97 (39%), 70 (42%), 57 (100%).
b) Synthesis of 4-(1E)-3-[[3-(4-[3-(tert-butoxycarbonyl)amino]propyl)piperazin-1-yl]propyl]amino]-3-oxoprop-1-enyl)phenyl acetate (52)

1.11 g (0.9 eq) of (E)-3-(4-acetoxyphenyl) prop-2-enoic acid was suspended in CH₂Cl₂ (20 ml). A solution of oxalyl chloride (1 eq, 0.76 g, 0.52 ml) in CH₂Cl₂ (20 ml) and catalytic DMF was added slowly to form (E)-3-(4-acetoxyphenyl) prop-2-enoyl chloride. Reaction mixture was heated to reflux at 40 °C for 1 hour. At RT under argon atmosphere a solution of the prepared acyl chloride was added dropwise over 10 minutes to a solution of 51 (1.80 g, 6 mmol) and Et₃N (1.20 eq, 0.73 g, 1.00 ml) in CH₂Cl₂ (20 ml) and stirred for 30 mins. The suspension was then refluxed at 40 °C overnight. After evaporation in vacuo water (60 ml) was added, the residue was extracted with CH₂Cl₂ and the organic phase dried with Na₂SO₄. The extract was purified by chromatography on silica gel (CH₂Cl₂/MeOH/NH₄OH = 10:4:0.5) and 52 was isolated as a yellow spongy solid (1.36 g, 52%).

TLC Rₛ = 0.25 (CH₂Cl₂/MeOH = 10:1)

¹H-NMR (CDCl₃) δ 7.57 (d, 1H, J=16 Hz), 7.49 (d, 2H, J= 8 Hz), 7.32 (d, 2H, J=8 Hz), 6.35 (d, 1H, J=16 Hz), 5.43 (s br., 1H), 3.48 (m, 2H), 3.16(m, 2H), 2.51-2.47 (m, 8H), 2.43-2.38 (m, 4H), 2.29 (s, 3H, OCH₃), 1.74 (t, 2H), 1.68 (m ,2H), 1.43 (s, 9H, t-Bu) .
\(^{13}\)C-NMR (CDCl\textsubscript{3}) 169.7, 166.0, 156.5, 151.5, 139.5, 133.2, 129.7, 129.1, 122.4, 121.9, 116.5, 79.2, 57.9, 57.2, 53.8, 46.3, 40.2, 28.8, 26.7, 25.4, 21.5.

FAB-MS m/z 489([M+1]\textsuperscript{+}, 28%), 389(10%), 256(11%), 201(17%), 147(42%), 102(18%), 74(14%), 57(100%).

c) Synthesis of (2E)-\(N\{-3-[4-(3-aminopropyl)piperazin-1-yl]propyl\}-3-(4-hydroxyphenyl)prop-2-enamide\) (27)

![Chemical Structure](image)

A solution of 52 (1.36 g, 2.79 mmol) in MeOH (30 ml) was treated with 20 drops of concentrated HCl. The solution was stirred at RT for 1.5 hours. After evaporation to dryness the residue was dissolved in absolute ethanol and filtered. The ethanolic solution was evaporated giving, 27 (687 mg, 63%) as a pale yellow solid.

TLC \(R_f = 0.20\) (CH\textsubscript{2}Cl\textsubscript{2}/MeOH/\textsubscript{4}OH = 10:4:1)

\(^1\)H-NMR (\(d_6\)-DMSO) \(\delta 7.40 (d, 2H, J=8\text{ Hz}), 7.36 (d, 1H, J=16\text{ Hz}), 6.81 (d, 2H, J=8\text{ Hz}), 6.44 (d, 1H, J=16\text{ Hz}), 3.73 (m, 8H), 3.45 (m, 2H), 3.23-3.09 (4H), 2.91 (2H), 2.02 (m, 4H).

FAB-MS m/z 369 ([M+Na]\textsuperscript{+}, 5%), 347 ([M+1]\textsuperscript{+}, 100%), 204 (17%), 176 (8%), 147 (49%), 144 (6%), 127 (14%), 113 (16%), 86 (13%).
3.8 Synthesis of (2E)-N-{2-[(2-aminoethyl)amino]ethyl}-3-(4-hydroxyphenyl)prop-2-enamide (28)

a) Synthesis of tert-butyl 2-[(2-aminoethyl)amino]ethylcarbamate (53)

\[
\text{H}_2\text{N}\text{NH} - \text{N} - \text{O}\text{NH}_2
\]

10.0 g (97.1 mmol, 10.5 ml) of diethylenetriamine was dissolved in dioxane (90 ml).

A solution of (BOC)O₂ (0.13 eq, 2.83 g, 12.9 mmol) in dioxane (30 ml) was added to the triamine solution at RT and stirred for six hours. After evaporation water (60 ml) was added to the residue and the mixture extracted with CH₂Cl₂. The organic phase was dried with Na₂SO₄ and evaporated to give 53 as a colorless viscous oil (2.30 g, 89%).

TLC Rᵣ = 0.83 (CH₂Cl₂/MeOH/NH₄OH = 10:4:1)

\(^1\)H-NMR (CDCl₃) δ 4.48 (2H), 3.35 (2H), 3.28 (2H), 2.18 (2H), 1.43 (9H)

\(^13\)C-NMR (CDCl₃) 156.6, 79.5, 70.4, 53.2, 49.8, 39.7, 28.8

FAB-MS m/z 204 ([M+1]⁺, 76%), 148 (54%), 131 (16%), 104 (40%), 87 (34%), 73 (35%), 57 (100%).
b) Synthesis of 4-[(1E)-13,13-dimethyl-3,11-dioxo-12-oxa-4,7,10-triazatetradec-1-en-1-yl]phenyl acetate (55)

\[
\begin{array}{c}
\text{O} \\
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\end{array}
\]

(E)-3-(4-acetoxyphenyl) prop-2-enoic acid (0.90 eq, 0.91 g) was suspended in CH$_2$Cl$_2$ (20 ml). A solution of oxalyl chloride (1eq, 0.63 g, 0.43 ml) in CH$_2$Cl$_2$ (15 ml) was added dropwise to the acid in the presence of catalytic DMF to form the acyl chloride. The reaction mixture was heated to reflux for one hour. Under Argon atmosphere a solution of the prepared acyl chloride was added within 10 mins to a solution of 57 (1.00 g, 4.93 mmol) and Et$_3$N (2.40 eq, 1.20 g, 1.65 ml) in CH$_2$Cl$_2$ (15 ml) and stirred for 30 mins. The supension was then refluxed overnight. Removal of solvent under reduced pressure gave 1.31 g of fluffy yellow crude product which was suspended in water (50 ml) and extracted with CH$_2$Cl$_2$. The organic phase was then dried with Na$_2$SO$_4$. Purification on silica gel (CH$_2$Cl$_2$/MeOH = 20:1) gave 54 (0.86 g) and 55 (0.32 g).
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Compound 54

TLC $R_f = 0.66$ (CH$_2$Cl$_2$/MeOH = 10:1)

FAB-MS m/z 580 ([M+1]$^+$, 2%), 480 (10%), 438 (6%), 189 (33%), 147 (100%), 57 (57%).

Compound 55

TLC $R_f = 0.45$ (CH$_2$Cl$_2$/MeOH = 10:1)

$^1$H-NMR (CDCl$_3$) $\delta$ 7.30 (d, 2H, J=8 Hz), 7.10 (d, 1H, J=16 Hz), 6.80 (d, 2H, J=8 Hz), 6.48 (d, 1 olef H, J=15.6), 3.16 (2H), 2.97 (2H), 2.73 (4H), 2.31 (s, 3H, OCH$_3$), 1.43 (s, 9H, t-Bu)

$^{13}$C-NMR (CDCl$_3$) 169.5, 166.8, 158.3, 151.7, 139.8, 133.4, 129.7, 129.0, 127.1, 121.9, 79.3, 70.4, 56.8, 49.9, 39.5, 36.8.
FAB-MS  m/z 391 ([M+1]+, 45%), 335 (20%), 318 (83%), 189 (15%), 147 (45%), 57 (100%).

c) Synthesis of (2E)-N-{2-[((2-aminoethyl)amino)ethyl]-3-(4-hydroxyphenyl)prop-2-enamide (28)

To a solution of 55 (300 mg, 0.77 mmol) in 30 ml MeOH was added 20 drops of concentrated HCl. Reaction mixture was stirred at 35 °C for 1.5 hours. After evaporation of solvent followed by chromatography (CH$_2$Cl$_2$/MeOH/NH$_4$OH = 10:4:0.5), 28 was isolated as a yellow solid (140 mg, 73%).

Similarly, 0.59 g of 54 (1.02 mmol) suspended in MeOH (20 ml) was reacted with 20 drops of concentrated HCl. The reaction mixture was stirred at 35 °C for 1.5 hours. After evaporation of solvent in vacuo, followed by purification by flash chromatography (CH$_2$Cl$_2$/MeOH/NH$_4$OH = 10:4:0.5), 56 was isolated as a very bright yellow solid (200 mg, 50%).

Compound 56

TLC $R_f$ = 0.76 (CH$_2$Cl$_2$/MeOH/NH$_4$OH = 10:4:1)
HPLC Rt = 11.6 mins

FAB-MS  m/z 396 ([M+1]+, 58%), 379 (6%), 250 (40%), 190 (21%), 147 (100%)

Compound 28

TLC R<sub>f</sub> = 0.44  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH/NH<sub>4</sub>OH = 10:4:1)

HPLC Rt = 13 mins

<sup>1</sup>H-NMR  (d<sub>6</sub>-DMSO)  δ 7.30 (d, 2H, J=8 Hz), 7.10 (d, 1H, J=16 Hz), 6.80 (d, 2H, J=8 Hz), 6.48 (d, 1H, J=16 Hz), 3.81 (s br., 1H), 3.60 (1H), 3.41-3.39 (m, 4H), 3.07 (2H).

<sup>13</sup>C-NMR  (d<sub>6</sub>-DMSO)  166.7, 159.9, 139.8, 130.0, 126.5, 119.5, 116.4, 55.8, 47.6, 45.3, 36.9

FAB-MS  m/z 499 ([2M+1]+, 5%), 250 ([M+1]+, 100%), 233 (13%), 219 (5%), 190 (22%), 176 (10%), 147 (94%).

3.9 Synthesis of (2E)-N-{3-[(3-aminopropyl)amino]propyl}-3-(4-hydroxyphenyl)prop-2-enamide (29)

a) 3.9 Synthesis of tert-butyl 3-{(3-aminopropyl)amino}propyl carbamate (57)
10.0 g (76.3 mmol, 10.7 ml) of 3,3'-iminobispropylamine was dissolved in dioxane (90 ml). A solution of (BOC)_2O (0.13 eq, 2.22 g, 10.2 mmol) in 30 ml of dioxane was added slowly to the triamine solution at RT. The reaction mixture was stirred for 6 hours. This was then evaporated to dryness and water (60 ml) was added to the residue and extracted with CH_2Cl_2. The organic phase was dried with Na_2SO_4 and concentrated on the rotary evaporator. The crude product was purified on silica gel (CH_2Cl_2/MeOH/NH_4OH =10:4:1). Compound 57 was obtained as a clear yellow oil (1.48 g, 63%).

TLC R_f = 0.26 (CH_2Cl_2/MeOH/NH_4OH = 10:4:1).

^1^H-NMR (CDCl_3) δ 5.20 (s br., 1H), 3.21 (m, 2H), 2.78 (t, 2H), 2.67-2.62 (m, 4H), 1.69-1.59 (m, 4H), 1.43 (s, 9H, t-Bu).

^13^C-NMR (CDCl_3) 156.54, 79.86, 53.81, 48.10, 40.72, 39.52, 33.99, 30.26, 28.81

FAB-MS m/z 232 ([M+1]^+, 100%), 186 (5%), 132 (36%), 102 (11%), 87 (8%), 74 (9%), 57(80%), 44 (48%).

b) Synthesis of 4-[(1E)-15,15-dimethyl-3,13-dioxo-14-oxa-4,8,12-triazahexadec-1-yl]phenyl acetate(59)
(E)-3-(4-acetoxyphenyl) prop-2-enoic acid (0.90 eq, 0.87 g) was suspended in CH₂Cl₂ (20 ml). A solution of oxalyl chloride (1 eq, 0.60 g, 0.41 ml) in CH₂Cl₂ (15 ml) was added dropwise to the acid in the presence of catalytic DMF to form the acyl chloride. The reaction mixture was heated to reflux for one hour. Under argon atmosphere a solution of the prepared acyl chloride was added over 10 mins to a solution of 57 (1.10 g, 4.72 mmol) and Et₃N (2.40 eq, 1.14 g, 1.58 ml) in CH₂Cl₂ (15 ml) and stirred for 30 mins. The suspension was then refluxed overnight. Removal of solvent under reduced pressure gave 480 mg of fluffy yellow crude product which was suspended in water (50 ml) and extracted with CH₂Cl₂. The organic phase was then dried with Na₂SO₄. Purification on silica gel gave the diconjugate, 58 (0.23 g) and 59 (0.22 g).

Compound 59

TLC R₉ = 0.60 (CH₂Cl₂/MeOH = 10:1)

¹H-NMR (CDCl₃)  δ 7.37 (d, 2H, J=8 Hz), 7.30 (d, 1H, J=16 Hz), 6.98 (d, 2H, J=8 Hz), 6.38 (d, 1H, J=116 Hz), 3.16 (m, 2H), 2.99-2.95(m, 4H), 2.59 (m,2H), 1.89 (s, 3H, OCH₃), 1.56 (2H), 1.50 (2H), 1.43 (s, 9H, t-Bu).

¹³C-NMR (CDCl₃)  169.5, 164.8, 153.3, 147.9, 130.9, 130.2, 129.2, 122.4, 121.5, 117.2, 79.1, 53.9, 48.6, 41.7, 36.7, 34.0, 30.9, 28.6.

FAB-MS m/z 420 ([M+1]⁺, 8%), 320 (15%), 216 (24%), 147 (100%), 129 (18%), 100 (33%), 57 (96%).
c) Synthesis of (2E)-N-{3-[(3-aminopropyl)amino]propyl}-3-(4-hydroxyphenyl)prop-2-enamide (29)

To a methanolic solution (30 ml) of 58 (200 mg, 0.33 mmol,) and 59 (200 mg, 0.48 mmol) in separate flasks was bubbled HCl gas to remove the protecting groups.

Evaporation of the solvent gave 60 (100 mg, 72 %) and 29 (105 mg, 79%) respectively.

Compound 29

TLC \( R_f = 0.39 \) (CH\(_2\)Cl\(_2\)/MeOH/NH\(_4\)OH = 10:4:1)

\(^1\)H-NMR (DMSO) \( \delta 7.37 \text{ (d, 2H, } J=8 \text{ Hz)}, 7.30 \text{ (d, 1H, } J=16 \text{ Hz)}, 6.98 \text{ (d, 2H, } J=8 \text{ Hz)}, 6.38 \text{ (d, 1H, } J16 \text{ Hz)}, 3.10 \text{ (m, 2H), 2.99-2.85(m, 4H), 2.52 (m,2H), 1.56 (2H), 1.49 (2H).} \)

\(^{13}\)C-NMR (d\(_6\)-DMSO) 165.2, 159.7, 139.3, 129.9, 126.8, 119.7, 116.6, 15.6, 47.2, 40.8, 56.0, 33.8, 24.9.

FAB-MS m/z 278 [M+1]^+, 65%), 260 (15%), 190 (21%), 162 (45%), 176 (25%), 147 (80%), 57 (100%).
3.10 Synthesis of (2E)-N-[3-({2-[(3-amnipropyl)amino]cyclohexyl} amino)propyl]-3-(4-hydroxyphenyl)prop-2-enamide (30)

a) Synthesis of N,N'-bis (2-cyanoethyl)cyclohexane-1,2-diamine (61)

10.0 g (87.6 mmol, 6.72 ml) of trans-1,2-diaminocyclohexane was dissolved in MeOH (65 ml) and 2.5 eq of acrylonitrile (14.4 ml, 11.6 g) in MeOH (50 ml) was added dropwise over 30 mins. The reaction mixture was stirred overnight at RT.

Evaporation of the solvent to dryness gave 61 as a yellow oil (19.1 g, 99%).

TLC Rf = 0.88 (CH2Cl2/MeOH/NH4OH = 10:4:1)

1H-NMR (CDCl3) δ 3.04-2.98 (m, 2H), 2.79-2.75 (m, 2H), 2.53-2.48 (t, 4H), 2.17-2.15 (t, 2H), 2.14 (2H), 1.96 (s br., 2H), 1.75-1.72 (m, 2H).

13C-NMR (CDCl3) 119.3, 61.4, 42.8, 32.0, 25.2, 19.7

FAB-MS m/z 221 ([M+1]*, 100%), 194 (4%), 180 (12%), 151 (33%), 109 (14%), 81 (13%)
b) Synthesis of N,N'-bis(3-aminopropyl)cyclohexane-1,2-diamine (62)

![Chemical Structure]

Compound 62 was obtained by reducing 19.0 g (86.4 mmol) of 61 with hydrogen at 50 psi using 95% EtOH/IN NaOH as solvent and Raney-Ni as catalyst. The reduction was complete after 24 hours. The mixture was filtered using Celite @ 521 and concentrated with the rotary evaporator to a volume of about 45 ml. A pale yellow viscous oil settled on top of the mixture and this was separated with the separatory funnel. The oil was then purified by column chromatography on silica gel (CH₂Cl₂/MeOH/NH₄OH = 10:4:0.5) to afford 14.7 g (75%) of 62.

TLC R<sub>f</sub> = 0.25 (CH₂Cl₂/MeOH/NH₄OH=10:4:1)

¹H-NMR (CDCl₃) δ 4.76 (s br., 1H), 2.99 (s br., 1H), 2.75-2.71 (m, 6H), 2.48-2.44 (m, 2H), 2.09-2.05 (m, 4H), 1.70 (2H), 1.67-1.59 (4H), 1.57 (1H), 1.20 (3H), 1.17(2H).

¹³C-NMR (CDCl₃) 62.0, 44.9, 40.5, 34.5, 31.9, 25.5

FAB-MS m/z 229 ([M+1]<sup>+</sup>, 100%), 155 (25%), 110 (10%), 81 (13%), 58 (11%).
c) Synthesis of N-[3-((2-[(3-aminopropyl)amino]cyclohexyl)amino)propyl]-2,2,2-trifluoroacetamide (63)

5.00 g (21.9 mmol) of 62 was dissolved in MeOH (75 ml) and 1 eq of ETFA (3.12 g, 2.61 ml) in 40 ml of MeOH was added slowly to the tetraamine solution at −78 °C.

The mixture obtained after the reaction was complete was purified on silica gel (CH₂Cl₂/MeOH/AcOH =10:20:1) and 3.25 g (46%) of 63 was isolated.

TLC Rf = 0.48 (CH₂Cl₂/MeOH/AcOH=60:40:2)

¹H-NMR (CDCl₃) δ 3.56-3.52 (m, 1H), 3.29-3.27 (m ,1H), 2.82-2.68 (m, 5H), 2.47-2.44 (q, 1H), 1.71-1.62 (t, 2H), 1.19 (2H), 0.93 (2H)

¹³C-NMR (CDCl₃) 157.4 (q, J=36 Hz), 116.5 (q, J=285 Hz), 62.3, 61.7, 45.8, 44.9, 40.7, 40.3, 34.1, 31.8, 31.7, 28.3, 25.5, 25.1

FAB-MS m/z 325 ([M+1]+, 100%), 251 (27%), 155 (37%), 81 (33%), 56 (35%).

d) Synthesis of triBOC-N-[3-((2-[(3-aminopropyl)amino]cyclohexyl)amino)propyl]-2,2,2-trifluoroacetamide (64) and triBOC-N,N'-bis(3-aminopropyl)cyclohexane-1,2-diamine (65)
3.10 g (9.57 mmol) of 63 was suspended in THF (60 ml) and 3 eq of (BOC)\textsubscript{2}O (28.7 mmol, 6.26 g) in THF (40 ml) was added at RT to form compound 64. In the same pot aqueous conc. NH\textsubscript{4}OH was added to remove the TFA protecting group by refluxing at 60 °C for 24 hours to give 3.28 g (60%) of the triBOC conjugate 65 after purification by chromatography.

TLC \( R_f = 0.70 \) (CH\textsubscript{2}Cl\textsubscript{2}/MeOH=10:1)

\textsuperscript{1}H-NMR (CDCl\textsubscript{3}) \( \delta \): 5.87 (s br., 1H), 4.90 (4H), 4.59 (2H), 4.03 (4H), 3.82 (1H), 3.03 (6H), 1.75-1.66 (7H), 1.45 (S, 27H).

ESI-MS m/z 1057.8 ([2M+1]\textsuperscript{+}, 8%), 529.4 ([M+1]\textsuperscript{+}, 100%), 429.4 (80%), 329.4 (35%), 229.2 (15%).

e) Synthesis of compound 66

1.06 g (0.90 eq, 5.14 mmol) of (E)-3-(4-acetoxyphenyl)prop-2-enoic acid was suspended in CH\textsubscript{2}Cl\textsubscript{2} (40 ml). A solution of oxalyl chloride (1eq, 0.72 g, 0.50 ml) in CH\textsubscript{2}Cl\textsubscript{2} (20 ml) was added dropwise to the acid in the presence 70 µL DMF to form the acyl chloride. The reaction mixture was refluxed for one hour and then 1.2 eq of Et\textsubscript{3}N (0.69 g, 0.95 ml) was added. The acyl chloride was added to a solution of 65
(3.00 g, 5.68 mmol) in CH₂Cl₂ (40 ml) and the mixture refluxed at 45 °C overnight.

After evaporation of the reaction the residue obtained was dissolved in distilled water and extracted with CH₂Cl₂. The organic phase was washed with 0.25 M Na₂CO₃ and then dried with Na₂SO₄. Evaporation of the solvent gave 66 as a yellow spongy voluminous solid (2.54 g, 69%).

TLC Rₕ = 0.53 (CH₂Cl₂/MeOH=10:1)

¹H-NMR (CDCl₃) δ 7.70 (d, 1H, J=16 Hz), 7.63 (d, 2H, J=8 Hz), 7.13 (d, 2H, J=8 Hz), 6.43 (d, 1H, J=16 Hz), 3.33-3.17 (m, 6H), 2.31 (s, 3H, OCH₃), 1.77 (m, 6H), 1.45-1.43 (27H).

FAB-MS m/z 717 ([M+1]+, 2%), 617 (5%), 529 (65%), 429 (18%), 254 (13%), 147 (13%), 81 (11%), 57 (100%).

f) Synthesis of (2E)-N-[3-{2-[(3-aminopropyl)amino]cyclohexyl}amino]propyl]-3-(4-hydroxyphenyl)prop-2-enamide (30)

3.0 g (4.2 mmol) of compound 54 was dissolved in 50 ml of dry MeOH and dry HCl(g) was bubbled into it at 0 °C. Evaporation of the solvent, followed by washing with diethyl ether and recrystallization in EtOH gave 830 mg (53%) of 30.
TLC $R_f = 0.10$ (CH$_2$Cl$_2$/MeOH=10:1)

$^1$H-NMR ($d_6$-DMSO) $\delta$ 7.40 (d, 2H, J=8 Hz), 7.37 (d, 1H, J=16 Hz), 6.82 (d, 2H, J=8 Hz), 6.48 (d, 1H, J=16 Hz), 3.26 (3H), 2.92 (4H), 2.11(6H), 1.82-1.72 (4H), 1.21 (2H).

FAB-MS m/z 375 ([M+1]$^+$,40%, 325 (92%), 316 (42%), 229 (100%), 155 (68%), 111 (25%), 98 (42%), 81 (60%), 57 (62%).

3.11 Synthesis of (2E)-N-{3-[4-aminocyclohexyl]amino}propyl)-3-(4-hydroxyphenyl)prop-2-enamide (31).

a) Synthesis of tert-butyl 4-aminocyclohexylcarbamate (67)

![NHBOC](image)

30.0 g (263 mmol) of 1,4-diaminocyclohexane was dissolved in dioxane (150 ml) and 0.125 eq of (BOC)$_2$O (32.8 mmol, 7.17 g) in dioxane (100 ml) was added slowly at RT with continuous stirring. The reaction solution turned into a thick slurry. The solvent was evaporated and the residue partitioned between water and dichloromethane. The monoBOC- and diBOC derivatives were extracted into the organic phase leaving the unreacted starting material in the aqueous phase. Separation of the mixture on silica gel with CH$_2$Cl$_2$/MeOH/NH$_4$OH =10:4:1 gave 5.18 g (74%) of 67.

TLC $R_f = 0.58$ (CH$_2$Cl$_2$/MeOH/NH$_4$OH=10:4:1).
1H-NMR (CDCl₃) δ 4.39 (s br., 1 H), 3.44-3.36 (m, 1H), 2.62-2.59 (m, 1H), 2.17 (2H), 1.99 (2H), 1.47 (2H), 1.43 (9H), 1.25-1.06 (4H)

13C-NMR (CDCl₃) 155.6 (C=O), 79.5 (C-O), 50.3, 49.6, 35.7, 32.5, 28.8

FAB-MS m/z 429 ([2M+1]+, 11%), 215 ([M+1]+, 61%), 159 (45%), 142 (100%), 96 (26%), 81 (36%), 57 (65%).

b) Synthesis of tert-butyl 4-[(2-cyanoethyl)amino]cyclohexylcarbamate (68)

5.00 g (23.4 mmol) of 67 was dissolved in MeOH (60 ml) and 1 eq of acrylonitrile (1.24 g, 1.54 ml) in MeOH (40 ml) was added slowly and stirred continuously for six hours. Evaporation of the solvent afforded 5.84 g (94%) of 68.

TLC Rf = 0.63 (CH2Cl2/MeOH/NH4OH=10:4:1)

1H-NMR (CDCl₃) δ 4.38 (s br., 1H), 3.48 (1H), 3.42 (1H), 2.96 (t, 2H), 2.50 (t, 2H), 2.04 (2H), 1.95-1.92 (2H), 1.45 (s, 9H, t-Bu), 1.2-1.07 (m, 5H).

13C-NMR (CDCl₃) 155.6 (C=O), 119.1 (CN), 79.6 (C-O), 55.8, 49.8, 42.7, 32.4, 32.3, 28.8, 19.6.

FAB-MS m/z 535 ([2M+1]+, 5 %), 268 ([M+1]+, 48%), 212 (35%), 171 (17%), 142 (82%), 96 (22%), 81 (44%), 57 (100%).
c) Synthesis of tert-butyl 4-[(3-aminopropyl) amino]cyclohexyl carbamate (69)

\[ \text{H}_2\text{N} - \text{HBOC} \]

5.00 g (18.7 mmol) of 68 was dissolved in 150 ml of 95% EtOH/1N NaOH. About one gram Raney-Ni catalyst was added and the mixture subjected to hydrogenation at 60 psi for 24 hours. After filtering using celite ® 521, the reaction mixture was concentrated to a volume of about 40 ml. After allowing it to stand for some few hours an oil settled at the top which was separated with a separatory funnel.

Purification on silica gel (5:2 = CH\textsubscript{2}Cl\textsubscript{2}/MeOH) gave 69 (3.90 g, 77%) as a white fluffy solid.

TLC R\textsubscript{f} = 0.39  (CH\textsubscript{2}Cl\textsubscript{2}/MeOH/NH\textsubscript{4}OH=10:4:1)

$^1$H-NMR  (CDCl\textsubscript{3}) \( \delta \) 4.44 (s br., 1H), 3.45 (1H), 2.78 (t, 2H), 2.68 (t, 2H), 2.30 (1H), 2.15 (2H), 2.10-1.96 (5H), 1.65-1.60 (m, 2H), 1.43 (s, 9H, t-Bu), 1.19-1.13 (m, 4H).

$^{13}$C-NMR  (CDCl\textsubscript{3}) 155.7 (C=O), 79.5 (C-O), 56.5, 49.9, 45.4, 40.8, 34.1, 32.4, 32.3, 28.8

FAB-MS m/z 272 ([M+1]$^+$, 100%), 216 (30%), 171(7%), 142 (25%), 96 (14%), 81 (30%), 57 (58%).

1.14 g (5.54 mmol) of (E)-3-4-(4-acetoxyphenyl) prop-2-enoic acid was suspended in CH₂Cl₂ (50 ml), 1.10 eq (1.26 g) of DCC in CH₂Cl₂ and 0.25 eq of HOBT was added. The solution was then added to a mixture of 1.5 g of compound 69 in CH₂Cl₂ (80 ml) and 1.20 eq of Et₃N (0.56g, 0.77 ml), and the reaction mixture refluxed for 36 hours. Evaporation of the solvent on the rotary evaporator gave a yellow fluffy crude product which was purified on silica gel (CH₂Cl₂/MeOH =10:1, followed by 100% MeOH) to give 1.60 g (63%) of 70.

TLC R_f =0.1 (CH₂Cl₂/MeOH = 10:1)

^1H-NMR (MeOH) δ 7.51 (d, 1H, J=16 Hz), 7.43 (d, 2H, J=8 Hz), 6.82 (d, 2H, J=8 Hz), 6.45 (d, 1H, J=16 Hz), 3.72 (t, 1H), 2.92-2.85 (m, 4H), 2.07 (2H), 2.03 (s, 3H, OCH₃), 1.96 (1H), 1.92-1.87 (m, 4H), 1.43 (S, 9H, t-Bu), 1.35-1.24 (m,6H).

^13C-NMR (d-MeOH) 168.9, 159.9, 156.8, 141.3, 129.7, 126.4, 124.2, 117.7, 116.8, 115.9, 111.5, 79.0, 67.9, 56.3, 51.1, 43.1, 36.5, 31.0, 29.3, 27.9, 25.5

FAB-MS m/z 460 ([M+1]^+, 100%), 404 (18%), 356 (12%), 147 (49%), 96 (17%), 57 (65%).
e) Synthesis of (2E)-N-[3-[4-aminocyclohexyl]amino]propyl]-3-(4-hydroxyphenyl)prop-2-enamide (31)

1.50 g of 70 was dissolved in MeOH (50 ml) and about 20 drops of conc. HCl was added to the solution. The mixture was stirred at RT for 5 hours. Evaporation of the solvent, followed by recrystallization in EtOH gave 0.75 g (77%) of 31.

TLC R\textsubscript{f} = 0.10 (CH\textsubscript{2}Cl\textsubscript{2}/MeOH = 10:1)

\textsuperscript{1}H-NMR (d\textsubscript{6} DMSO) δ 7.51 (d, 1H, J=16 Hz), 7.42 (d, 2H, J=9 Hz), 6.78 (d, 2H, J=9 Hz), 6.45 (d, 1H, J=16 Hz), 3.62 (t, 2H), 2.96 (m, 4H), 2.70-2.64 (2H), 1.60 (m, 4H), 1.30-1.26 (m, 4H).

\textsuperscript{13}C-NMR (d\textsubscript{6} DMSO) 167.8, 156.8, 141.7, 129.7, 126.5, 119.0, 116.3 57.0, 54.7, 40.8, 40.0, 34.1, 31.4

FAB-MS m/z 318 ([M+1]\textsuperscript{+}, 48%), 214 (59%), 176 (10%), 160 (22%), 147 (17%), 142 (9%), 136 (100%), 113 (6%), 81 (22%).

a) Synthesis of N,N'-bis(2-cyanoethyl)cyclohexane-1,4-diamine (71)

![Chemical Structure](image)

10.0 g (87.6 mmol, 6.72 ml) of trans-1,4-diaminocyclohexane was dissolved in MeOH (65 ml) and 2.5 eq of acrylonitrile (14.4 ml, 11.6 g) in MeOH (50 ml) was added dropwise over 30 minutes with continuous stirring. The reaction mixture was stirred overnight at RT. Evaporation of the solvent to dryness followed by purification gave 71 as off-white crystals (19.1 g, 99%).

TLC $R_f = 0.87$ (CH$_2$Cl$_2$/MeOH/NH$_4$OH = 10:4:1)

$^1$H-NMR (CDCl$_3$) $\delta$ 2.98-2.93 (m, 4H), 2.53-2.49 (m, 6H), 1.97-1.95 (4H), 1.20-1.07 (m, 6H).

$^{13}$C-NMR (CDCl$_3$) 119.1, 56.3, 42.8, 32.3, 19.6

FAB-MS m/z 441 ([2M+1]$^+$, 13%), 221 ([M+1]$^+$, 84%), 180 (12%), 151 (100%), 109 (21%), 81 (34%).
b) Synthesis of N,N’-bis(3-aminopropyl)cyclohexane-1,4-diamine (72)

Compound 72 was obtained by reducing 6.00 g (27.3 mmol) of 71 with hydrogen at 50 psi using 95% EtOH/1N NaOH as solvent and Raney-Ni as catalyst. The reduction was complete after 24 hours. The mixture was filtered using Celite® 521 and concentrated to a volume of about 35 ml. A pale yellow viscous oil settled on top of the mixture and this was separated with the separatory funnel. The oil was then purified by column chromatography on silica gel to afford 5.60 g of 72 (90%).

TLC Rf = 0.20 (CH₂Cl₂/MeOH/NH₄OH=10:4:1)

¹H-NMR (CDCl₃) δ 3.16 (s br., 1H), 2.64-2.50 (m, 8H), 2.30 (m, 2H), 1.84-1.82 (m, 4H), 1.54-1.46 (q, 4H), 1.09-0.96 (m, 6H).

¹³C-NMR (CDCl₃) 56.2, 43.7, 39.1, 32.3, 30.8, 17.2

FAB-MS m/z 229 ([M+1]⁺, 100%), 176 (19%), 155 (22%), 57 (28%).
c) **Synthesis of N-[3-[(4-[(3-aminopropyl)amino]cyclohexyl)amino]propyl]-2,2,2-trifluoroacetamide (73)**

![Chemical Structure](image)

3.70 g (16.2 mmol) of 72 was dissolved in MeOH (60 ml) and 1 eq of ETFA (2.30 g, 1.93 ml) in MeOH (30 ml) was added slowly to the tetraamine solution at −78 °C. The mixture obtained after the reaction was complete was purified on silica gel (CH₂Cl₂/MeOH/AcOH =10:20:1) and 73 (5.16 g, 98%) was isolated.

TLC R_f = 0.48 (CH₂Cl₂/MeOH/AcOH=60:40:2)

\[ ^1H-\text{NMR} \quad (\text{CDCl}_3) \delta 3.16-3.12 \text{ (m, 12H)}, 2.52 \text{ (m, 2H)}, 2.23 \text{ (2H)}, 1.79-1.80 \text{ (4H)}, 1.58 \text{ (1H)}, 1.45-1.39 \text{ (2H)}, 0.92 \text{ (4H)} \]

\[ ^13C-\text{NMR} \quad (\text{CDCl}_3) 57.4, 57.2, 49.4, 45.4, 44.9, 34.7, 32.5, 29.4 \]

FAB-MS m/z 325 ([M]+, 8%), 251 (22%), 191 (13%), 155 (21%), 107 (29%), 96 (21%), 81 (50%), 65 (100%), 56 (42%).

*d) Syntheses of compounds 74 and 75*
5.00 g (15.4 mmol) of 73 was dissolved in MeOH (80 ml) and 3 eq of (BOC)$_2$O (46.3 mmol, 10.1 g) in THF (40 ml) was added at RT to give 74.

In the same pot, concentrated NH$_4$OH was added to remove the TFA protecting group by refluxing for 24 hours to afford 6.20 g (76%) of triBOC conjugate, 75.

\[
\text{TLC } R_f = 0.70 \quad (\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}=10:4:1)
\]

$^1$H-NMR (CDCl$_3$) $\delta$ 4.93 (s br.,1H), 3.35 (8H), 1.76 (4H), 1.65 (8H), 1.48 (13H), 1.45 (27H).

FAB-MS m/z 529 ([M+1]$^+$, 61%), 429 (100%), 329 (3%), 254 (11%), 229 (8%), 199 (15%), 57 (68%).

e) Synthesis of 76

1.06 g (0.9 eq, 5.14 mmol) of (E)-3-(4-acetoxyphenyl)prop-2-enoic acid was suspended in CH$_2$Cl$_2$ (40 ml). A solution of oxalyl chloride (1eq, 0.72 g, 0.50 ml) in
CH₂Cl₂ (20 ml) was added dropwise to the coumaric acid plus 70 µL of DMF to form

the acyl chloride. The mixture was refluxed for one hour and then 1.2 eq of Et₃N

(0.69 g, 0.95 ml) was added. The acyl chloride was added to a solution of compound

75 (3 g, 5.68 mmol) in CH₂Cl₂ (40 ml) and the mixture refluxed at 45 °C overnight.

After evaporation of the reaction, the residue obtained was dissolved in distilled water

and extracted with CH₂Cl₂. The organic phase was washed with 0.25 M Na₂CO₃ and

then dried with Na₂SO₄. Evaporation of the solvent gave 76 as a yellow spongy solid

(3.20 g, 87%).

TLC R_f =0.53 (CH₂Cl₂/MeOH = 10:1)

¹H-NMR (CDCl₃) δ 7.60 (d, 1H, J=16 Hz), 7.46 (d, 2H, J=8 Hz), 7.15 (d, 2H, J=8

Hz), 6.45 (d, 1H, J=16 Hz), 3.40-3.20 (m, 6H), 2.35 (s, 3H, OCH₃), 1.75 (m, 6H),

1.45-1.43 (27H).

FAB-MS m/z 717 ([M+1]+, 2%), 617 (6%), 529 (29%), 471 (8%), 375 (8%), 271

(12%), 133 (23%), 73 (73%), 57 (100%).

f) Synthesis of (2E)-N-{3-[4-aminocyclohexyl]amino}propyl}-3-(4-

hydroxyphenyl)prop-2-enamide (32).

To a solution of 3.0 g (4.2 mmol) of 76 in 50 ml of dry MeOH was bubbled
dry HCl gas at 0 °C. The reaction mixture was stirred for 2 hours. After evaporation
to dryness and recrystallization in EtOH, 32 was obtained as a pale yellow solid (550 mg, 35%).

\begin{center}
\includegraphics[width=0.5\textwidth]{structure32.png}
\end{center}

TLC \( R_f = 0.15 \) (CH\(_2\)Cl\(_2\)/MeOH = 10:1)

\(^1\text{H-NMR}\) (\(d_6\) DMSO) \( \delta \) 7.40 (d, 2H, \( J=9 \) Hz), 7.36 (d, 1H, \( J=16 \) Hz), 6.82 (d, 2H, \( J=9 \) Hz), 6.47 (d, 1H, \( J=16 \) Hz), 4.10 (2H), 2.89 (m, 10H), 2.17 (4H), 1.99 (3H), 1.81 (1H), 1.46 (4H).

FAB-MS m/z 375 ([M+1]+, 57%), 302 (13%), 271 (100%), 229 (54%), 155 (67%), 147 (35%), 57 (72%).
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None


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