THE DESIGN AND SYNTHETIC STUDIES OF
N\textsuperscript{\alpha}-[1-(CARBOXY)-9,11,21-(TRIOXO)-HENEICOSYL]-L-ASPARTYL-L-LYSYL-(TERT-BUTYL)-L-ASPARAGINATE,
A TRIPEPTIDE ANALOGUE OF IONOMYCIN

BY

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(Chemistry)

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
SEPTEMBER 1990

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Department of Chemistry

The University of British Columbia
Vancouver, Canada

Date Oct. 5, 1990
Ionomycin is a stereochemically complex calcium ionophore. An analogue (34) of ionomycin has been designed and its synthesis initiated. Regions of the analogue which parallel portions of ionomycin do not incorporate any of the chiral centers found in the natural product. The analogue 34 also incorporates a highly conserved tripeptide found in the calcium binding site of troponin C proteins.
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LIST OF ABBREVIATIONS

Å  angstrom
ATP  adenosine triphosphate
br. dd.  broad doublet of doublets
br. s.  broad singlet
°C  degrees celcius
calcd  calculated
cAMP  cyclic adenosine monophosphate
Cbz  carbobenzoxy
CI  chemical ionization (with methane or ammonia)
C-terminal  carboxy terminal
d  doublet
DCC  1,3-dicyclohexylcarbodiimide
dd  doublet of doublets
DMAP  4-dimethylaminopyridine
DMPU  1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
EI  electron impact
g  gram
^1H NMR  proton nuclear magnetic resonance
HRMS  high resolution mass spectrum
Hz  Hertz
IR  infrared
KBr  potassium bromide pellet
LD50  lethal dose to kill 50% of test subjects
LDA  lithium diisopropylamide
<table>
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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>LRMS</td>
<td>low resolution mass spectrum</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>M⁺</td>
<td>parent ion</td>
</tr>
<tr>
<td>MHz</td>
<td>megahertz</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre</td>
</tr>
<tr>
<td>mmol</td>
<td>millimole</td>
</tr>
<tr>
<td>m/z</td>
<td>mass to charge ratio</td>
</tr>
<tr>
<td>N-terminal</td>
<td>amino-terminal</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>rbf</td>
<td>round bottom flask</td>
</tr>
<tr>
<td>s</td>
<td>singlet</td>
</tr>
<tr>
<td>SRS</td>
<td>slow reacting substance</td>
</tr>
<tr>
<td>t</td>
<td>triplet</td>
</tr>
<tr>
<td>TBDMS</td>
<td>tertiary-butyl dimethylsilyl</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
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This thesis is dedicated to
my family and friends.
INTRODUCTION

GENERAL:

Recently, the efforts of several research groups have been directed to the total synthesis of the polyether antibiotic ionomycin (1) which is produced by Streptomyces conglobatus. Due to the complex stereochemical nature of this compound, its synthesis has not been a trivial task.

The goal of this research is to develop a simple ionomycin analogue which might mimic the activity of the antibiotic. The analogue would incorporate a tripeptide moiety found to be conserved in the calcium ion (Ca$^{2+}$) binding site of the troponin C family of proteins.

HISTORY OF THE POLYETHER ANTIBIOTICS:

In 1951, two groups reported the isolation of three novel antibiotics which are now known to be polyether antibiotics.$^{1,2}$ Berger and coworkers reported the isolation of antibiotics X-206, X-464 (later known as nigericin) and X-537A (later known as lasalocid A). Harned and coworkers also reported the isolation of nigericin. These three
antibiotics had common characteristics which distinguished them from other known antibiotics and suggested they constituted a new class. For example, all were acidic and very soluble in organic solvents. An unusual characteristic was the fact that when organic solutions of the antibiotics were exposed to aqueous base, there was no extraction of the antibiotic anion into the aqueous phase. In fact, when sodium carbonate was used as the base, sodium ions (Na\(^+\)) were extracted into the organic phase. Evaporation of the solvent yielded a crystalline sodium salt of the antibiotic. The three new antibiotics showed antimicrobial activity against Gram positive bacteria and mycobacteria \textit{in vitro}. Due to their relatively high parenteral toxicity (X-206 has an LD\(_{50}\) of 1.2 mg/kg of mouse), little interest was shown in them for the following sixteen years.\(^3\)

In 1958, a fourth polyether antibiotic called dianemycin (A16183) was reported.\(^4\) Lardy and coworkers used dianemycin, nigericin, and a number of other antibiotics as tools for metabolic studies. They reported that dianemycin and nigericin were powerful inhibitors of the oxidation of most pyridine-nucleoside linked substrates.

Nine years elapsed before the fifth polyether antibiotic (monensin) was reported in 1967.\(^5\) The structure of monensin was elucidated and the antibiotic was stated to have potent anticoccidial activity. In 1976, control of coccidiosis in the poultry industry was estimated to have
cost $100 million worldwide. This activity, as well as an ability to improve feed conversion in ruminant animals sparked much of the recent interest in the polyether antibiotics.

In 1968, monensin's biological activity was attributed to its direct interaction with alkali metal cations and the carrier mechanisms which regulate transport of potassium ions (K⁺) across mitochondrial membranes.

In the ensuing years, many additional polyether antibiotics have been reported. The structure and absolute configuration have been fully characterized for most compounds. The polyether antibiotics are produced primarily by members of the Streptomyces genus. However, some are produced by species of the Actinomadura and Dactylosporangium genera. Many are harvested through large scale fermentations.

CHARACTERISTICS OF THE POLYETHER ANTIBIOTICS:

The polyether antibiotics are members of a large group of compounds known as ionophores. All polyether antibiotics possess a carboxylic acid moiety introduced during the polyketide biosynthesis of the antibiotic precursor. The term "polyether" refers to the tetrahydrofuran and tetrahydropyran rings found in these compounds. Describing these compounds as ionophores refers to their ability to form organic soluble complexes with
alkali earth cations, and mediate their transport across lipid barriers.\(^9\)

The polyether antibiotics may be divided into two groups depending on their ability to transport monovalent or divalent cations.\(^8\) Most of the divalent cation transporters do so as a dimeric complex (2:1 ratio of antibiotic : cation). Ionomycin is unique because it transports divalent calcium ions as a monomeric (1:1) complex.

**MODE OF ACTION:**

The polyether antibiotics act as catalysts to facilitate the transfer of cations through a non-aqueous barrier. In the antibiotic, the energy required for total ion desolvation is minimized by having oxygen or nitrogen atoms replace the solvent molecules around the ion. The ionophore-cation complex is quite stable in the non-aqueous phase while a dehydrated (naked) cation is not.\(^10\)

The formation of a structurally distinct complex between the cation and the ionophore, coupled with movement of the complex across a membrane, distinguishes the polyether antibiotics from other types of antibiotics and other agents which increase the permeability of the membranes to cations by different mechanisms. The polyethers possess multiple ether linkages usually in the form of substituted tetrahydrofuran and tetrahydropyran rings. All ionophores of this class have an internal
ligating site with the outer surface of the complex being composed largely of nonpolar hydrocarbon chains or heterocyclic and aromatic residues (Figure 1). In each structure the polar ligating portion of the complex is shielded from the environment by the lipophilic side chains of the antibiotic molecule. The potassium complex is in a 1:1 ratio while the barium complex has a 2:1 stoichiometry of antibiotic to cation.

\[ \text{MONENSIN} \quad \text{LASALOCID A} \]

FIGURE 1: Crystal structures of the potassium salt of the monovalent antibiotic monensin and the barium salt of the divalent antibiotic lasalocid A.\(^{11,12}\)

The antibiotic’s carbon backbone is capable of assuming conformations that focus strategically placed electron donors about a cavity in which a cation may fit. Polyether
ionophores possess flexible carbon backbones and may open to permit the entry of a cation. The binding energy depends upon the conformational constraints on the flexibility imparted by the specific substitutional and stereochemical arrays along the molecule. The nonligating substituents and their stereochemistry are important and exert their effect by preorganizing the structure to favour the binding conformation. Antibiotics with smaller carbon backbones will usually have a very rigid conformation, while longer backbones will permit more flexibility and folding in the complexed state.

The ligating oxygen atoms are incorporated in a variety of functional groups such as ethers, alcohols, carboxylates, and amides. The neutral oxygen atoms chelate the cation via ion-dipole interactions analogous to those between the ion and high-dielectric solvents. It is important to note that the polyether antibiotics will only form cation complexes when their carboxylic acid moiety is deprotonated. Thus, the ionophores will only carry ions as electrically neutral, ionic complexes. However, the polyether may cross the membrane to pick up another cation in its neutral state. The antibiotic acts as an exchange diffusion carrier by transporting hydrogen or alkali metal ions down their concentration gradients across membranes. The polyether antibiotics will usually form a 1:1 complex with an appropriate monovalent cation and a 2:1 complex of ionophore
to divalent cation. One exception is ionomycin which has an ionizable β-diketone functionality, as well as the carboxylic acid moiety. This allows the antibiotic to become doubly ionized so it may transport divalent cations as a neutral 1:1 complex. The number of ligating oxygens or nitrogens for a given cation often varies with the ionophore-cation complex. In some cases, water may take up some of the ligating sites on the ion. The lipid solubility of the complex may be partly explained by effective shielding of the polar interior which stabilizes the cation charge. The compatibility of the exterior of the complex with low-dielectric solvents also improves lipid solubility.

Polyether antibiotics serve as the carrier in a counter-current cation transport system. The ligating cavity is similar to an enzyme active site, due to its ability to discriminate between very similar substrates (cations). Each antibiotic has its own stable conformation for complexing with cations which may be maintained by hydrogen bonds. The bulky hydrophobic groups are arranged strategically to help maintain an active conformation.

Conformational constraints in the polyether antibiotics can limit the strength with which cations of different sizes may be ligated. The dimensions of the ligating cavity are reasonably selective for a cation of specific size and charge. The ion selectivity of ionophores is a function of the energy required for ion desolvation and the binding
energy obtained on complexation. For polyether antibiotics with a relatively unconstrained backbone, the free energy of ionophore complexation is governed by the same considerations that determine the free energy of solvation. That is, the charge density of the ion is an important factor. Ionophores with highly constrained backbones will select for a cation of critical ionic radius. Thus, form-fit becomes very determinative in the net free-energy difference between desolvation and complexation. As a result of this selectivity, one cation in a series (Table 1) is usually accommodated more efficiently than the others which are smaller or larger than the optimal size.

TABLE 1: Some metals and their ionic radii.

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<tr>
<th>ELEMENT</th>
<th>CHARGE</th>
<th>ATOMIC NUMBER</th>
<th>RADIUS (Å)</th>
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<tr>
<td>Li</td>
<td>+1</td>
<td>3</td>
<td>0.68</td>
</tr>
<tr>
<td>Na</td>
<td>+1</td>
<td>11</td>
<td>0.97</td>
</tr>
<tr>
<td>K</td>
<td>+1</td>
<td>19</td>
<td>1.33</td>
</tr>
<tr>
<td>Rb</td>
<td>+1</td>
<td>37</td>
<td>1.47</td>
</tr>
<tr>
<td>Cs</td>
<td>+1</td>
<td>55</td>
<td>1.67</td>
</tr>
<tr>
<td>Mg</td>
<td>+2</td>
<td>12</td>
<td>0.66</td>
</tr>
<tr>
<td>Ca</td>
<td>+2</td>
<td>20</td>
<td>0.99</td>
</tr>
<tr>
<td>Ba</td>
<td>+2</td>
<td>56</td>
<td>1.34</td>
</tr>
</tbody>
</table>

A balance between the energy required for desolvation (which need not be complete in all cases), the energy of association with the ionophore, and the energy necessary to convert the ionophore from its uncomplexed conformation to that in the complex all influence the complexation process.

In general, movement of ionophores through membranes may be viewed as a problem of lipid-ionophore interactions while the complexation-decomplexation reactions are related
to interfacial chemistry. Electroneutrality of total charge movements is maintained in the overall transport cycle. The net charge of species which traverse the membrane are neutral. Consecutive reactions by which the transporting species form and dissociate (together with diffusion reactions) constitute transport cycles. Each reaction could be described in terms of an equilibrium constant and rate constants for forward and reverse directions.\textsuperscript{3}

The direction and extent of cation transport by polyether antibiotics are determined by metal and hydrogen ion concentration gradients. The transport of cations across cell membranes may be efficient if certain kinetic criteria are fulfilled. At the high-dielectric region of the membrane interface, complexation and decomplexation reactions must be fast. The exchange of the ion’s solvation shell for the ionophore’s ligating system must be a concerted reaction in order that the energy of activation for transport remain low. When the complex enters the low-dielectric region of the membrane interior, where it is immune to solvent attack, it may then attain high stability.\textsuperscript{15}

Ion transport by the polyethers may be measured by filling a U-tube with antibiotic-doped chloroform to just above each bend (Figure 2). Each side arm of the apparatus is then filled with an appropriate aqueous solution. The chloroform layer is then gently stirred. If one arm of the
U-tube is filled with a salt solution and the other arm is filled with a salt free solution, the pH of the salt free side rises while that on the saline side decreases (Figure 3). This result might be explained by the transport sequence that follows (Figure 4). The anionic ionophore on the high salt side of the "membrane" associates with an appropriate cation. The zwitterionic complex diffuses down the cation gradient across the membrane. The complexed cation is released on the low salt side of the membrane. The ionophore then picks up a proton from the solvent and diffuses back across the membrane to the high salt side. Release of the proton results in a decrease in pH on the high salt side while the pH on the low salt side increases.\textsuperscript{10}

\textbf{FIGURE 2:} The U-tube used to measure ion transport by polyether antibiotics.\textsuperscript{10}

\textbf{FIGURE 3:} Changes in pH on the two sides of a U-tube as a result of the counter current flow of metal ions with
hydrogen ions results in a decreasing pH in the high salt side of the tube.\textsuperscript{10}

\[
\begin{align*}
\text{H}^+ & \quad \text{HI} \\
\text{(H}_2\text{O)}_n:\text{M}^+ & \quad \text{I}^- \\
n\text{H}_2\text{O} & \quad \text{M}^+ \quad \text{I}^- \quad \text{M}^+\text{I}^- \quad \text{I}^- \quad \text{M}^+ \quad n\text{H}_2\text{O} \\
\text{HI} & \quad \text{IH}
\end{align*}
\]

**FIGURE 4:** Transport mode for carboxylic acid ionophores. I represents an ionophore, M represents an alkali metal, and H represents hydrogen.\textsuperscript{15}

This simple measurement is a good method of illustrating the counter current transport catalyzed by polyether antibiotics. However, protons are not always the counter cation and the ionophore may be mono- or divalent. Counter transport of a mono- or divalent cation may provide the zwitterionic species that will cross the membrane to complete the transport cycle (**Figure 5**).
FIGURE 5: The overall transport reactions (left) and individual component reactions (right) required to complete a single transport cycle for monovalent (A) and divalent (B) monocarboxylic ionophores. Pairs of vertical lines represent the membrane across which transport occurs. Subscripts i and o denote inside and outside of a membrane respectively. Component reactions where diffusion occurs across the unstirred aqueous layer at the interface are indicated with an asterisk.³
Ion transport specificities of the ionophores can be determined in the above system. By placing different cations in the solution in donor arm, the rate of appearance of cations in the acceptor arm can be measured by atomic absorption spectrometry for example. On the other hand, the change in pH of the saline solution can also be measured with time (Figure 6). Ions whose specificity is high with a given ionophore would show a more rapid decrease in pH in the donor arm of the U-tube.

![Figure 6](image)

**FIGURE 6:** Changes in pH of the donor phase of the U-tube using various cations at 1.0 M. (a) Monensin. (b) Nigericin. (c) Dianemycin.\(^{10}\)

It has been postulated that the ionophore conformation required for the complexation-decomplexation reactions may differ from that required for diffusion of the complex across the membrane. Molecular modeling has been used to predict the likely conformations of ionomycin in its lipophilic and lipid-water interface states.\(^ {17}\) Molecular modeling is a technique to calculate the most likely conformation of a molecule in a solution of a given dielectric constant. The more stable conformations would
have minimal steric interactions between substituents. The dielectric constant discontinuity existing at the lipid-water interface mediates transformation of one conformation into another (Figure 7). It was found that rotation about three of the bonds in ionomycin can provide the change from one conformer to the other. Calcium ions leaving the cryptic cavity within the ionophore would be liberated into the aqueous phase as the ionophore changed conformations due to changing solvent dielectric at the lipid-water interface. The degree of penetration of the ionophore into the membrane could provide the driving force for the conformational change (Figure 8). An important facet of these conformational changes is that the molecule would only undergo a low energy transition (few bonds rotated) otherwise the barrier to ion complexation would be too large for efficient transport across the membrane.17

FIGURE 7: Conformation of the ionomycin-Ca ion complex. A: The hydrophobic complex. B: Interfacial complex. The
calcium ion is shown as a closed circle. Open circles refer to carbon atoms and dotted circles to oxygen atoms. The dotted line delineates the hydrophobic (dielectric=3) and the hydrophilic medium (dielectric=30).17

FIGURE 8: Transition between the ionomycin-calcium interfacial complex and the hydrophobic complex. The dotted line denotes the surface of the membrane.17

INTEREST IN IONOMYCIN:

Cells of multicellular organisms communicate with each other via the passage of chemical messengers between cells. These chemicals coordinate metabolic activity of various tissues, allow an organism to adapt to environmental changes, and prepare it for reproduction. One major class of chemical messengers is liberated by neurons in response to stimulation. If these compounds diffuse to neighboring cells to activate specific responses, they are referred to as neurotransmitters. Another major class of chemical messengers is known as hormones.
All hormones act by binding to macromolecular receptors that are located either on the cell membrane or inside responsive cells. Binding of the hormone induces a change in the conformation of the receptor; this change in conformation perturbs other molecules and elicits a chain of events that leads to a vast array of cellular changes ranging from alterations in enzyme activity to changes in gene expression. These effects may lead to profound alterations in cell growth, morphology, and function. It is important to note that different cell types may respond quite differently to the same hormone.

Two major signal transduction mechanisms are known to transmit signals across the cell membrane. One mechanism involves the activation of membrane-bound adenylate cyclase to convert adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). Cyclic AMP then serves to influence enzymes within the cell. The second mechanism used in signal transduction utilizes several messengers such as calcium ions, inositol triphosphate, and diacylglycerol. It is the influence of Ca\(^{2+}\) on the cell which may be controlled by a transmembrane calcium transporter such as ionomycin.

Intracellular Ca\(^{2+}\) concentration has been found to influence several cellular processes while bound to the calcium binding protein calmodulin. The calcium-calmodulin complex activates calcium stimulated ATPase, several kinases, adenylate cyclase, phospholipase A\(_2\), and
phosphodiesterase. Since these enzymes are activated by intracellular calcium, cellular processes such as protein phosphorylation, arachidonic acid oxygenation, glycogenolysis, muscle contraction, and cAMP levels are all influenced by calcium ion concentration. The role of calcium ions in the oxygenation of arachidonic acid is of particular interest in the treatment of asthma.

Calcium ions may come from the extracellular fluid and their transport is usually associated with potassium or hydrogen ion countertransport mediated by a membrane bound ion pump. Intracellular stores of calcium ions are usually associated with the cytoplasmic side of the plasma membrane or sequestered within the mitochondria or reticulum. A calcium gradient exists across the cell membrane where the extracellular concentration is approximately 100 times that found within the cell. Cellular processes are influenced by a change in the intracellular calcium concentration which can be perturbed quite easily as a result of the transmembrane gradient.

Chemicals released from mast cells and immunological reactions can alter the calibre of an airway. Mast cells located in the connective tissue underlying smooth muscle and in airway walls release histamine and slow reacting substance (SRS). For many years, scientists have been interested in the biochemical processes involved with asthma. Possibly involved in this disease is SRS, an
extremely potent muscle contractant that can severely constrict small airways in the lung. SRS, now known to be leukotriene C, is a metabolite of arachidonic acid (Figure 9) whose synthesis is stimulated by ionomycin mediated calcium transport. It has been shown that ionomycin can stimulate the activation of phospholipase A₂ which is involved in the liberation of arachidonic acid from diacylglycerol. However, prostaglandin synthesis from arachidonic acid is not stimulated by ionomycin. The prostaglandins are a group of compounds important to many physiological processes including constriction and dilation of the bronchioles of the lung. As a result, the effects of SRS may be assessed without the interference of potent chemicals such as the prostaglandins. It is hoped that this probe would give a better understanding of the biochemical basis of asthma and possibly lead to better treatments of the disease.

CALCIUM BINDING PROTEINS:

The intracellular concentration of Ca^{2+} must be kept at a low level due to the high intracellular concentration of phosphate esters which form insoluble calcium salts. As a result, a calcium gradient is established across a cell membrane where the external concentration of calcium is orders of magnitude higher than that found within the cell. This gradient has evolved into a signal mechanism where cytosolic Ca^{2+} concentration may be increased suddenly by
opening channels to calcium located in the plasma or reticular membrane.

Calcium ions are also capable of binding to proteins. The negatively charged oxygens found on the side chains of aspartate and glutamate residues as well as uncharged oxygens from main-chain carbonyls bind to Ca\(^{2+}\). Since Ca\(^{2+}\) can facilitate multiple coordination with six to eight oxygen atoms, it may cross link various regions of a protein and thus induce major conformational changes in the protein. Also, the binding of Ca\(^{2+}\) to a protein is quite selective.

**FIGURE 9:** Leukotriene biosynthesis from arachidonic acid.\(^{19}\)
due to the ion's high affinity for uncharged oxygen atoms. Magnesium ions (Mg$^{2+}$) can serve as a competitor for protein binding sites. However, it turns out that Mg$^{2+}$ is not very effective as a competitor for Ca$^{2+}$. Mg$^{2+}$ has a low affinity for uncharged oxygen atoms, and it prefers to form small, symmetric coordination shells. Whereas Ca$^{2+}$ forms asymmetric shells of larger radius. As a result, Ca$^{2+}$ can bind to irregularly shaped sites in proteins and can be selected over Mg$^{2+}$ even if the latter ion is much more abundant.$^{22}$

X-ray crystallographic studies of calcium-binding proteins have suggested how calcium binding may occur. Parvalbumin, a carp muscle protein with two similar calcium binding sites, has been studied. The binding site consists of a helix, a loop, and another helix. Each calcium ion is coordinated by eight oxygen atoms: three aspartate and three glutamate carboxylate oxygens, a main chain carbonyl oxygen, and one oxygen from a complexed water molecule. It has been suggested that the E and F helices of the binding site resemble the forefinger and thumb of the right hand (Figure 10). The Ca$^{2+}$ binding site is formed by the loop found between these helices. The calcium binding site of parvalbumin and troponin C (involved in muscle contraction) are believed to have evolved from the duplication of a primordial gene coding for the calcium-binding loop. The amino acid sequences of the binding sites show some
similarity and the EF hand has been found to recur in the Ca\(^{2+}\) binding sites of other proteins.\(^{22}\) Calmodulin is a calcium binding protein found in nearly all eukaryotic cells. This protein has been constructed from repeating modules with the EF hand motif. Thus, certain amino acid sequences should be conserved throughout the evolution of the proteins in order for the binding site conformation to be conserved. This, in fact, has been found to be the case within the troponin C family of proteins.\(^{23}\)

FIGURE 10: The EF Hand model for the calcium binding site of various proteins.\(^{22}\)

OBJECTIVES OF THIS WORK:

It is our objective to design a simple ionomycin analogue which would be capable of mimicking the activity of
ionomycin without having the stereochemical complexity associated with the natural product. The analogue would incorporate part of the carbon skeleton of ionomycin, including the terminal carboxylate and the β-diketone moieties, but without the alkyl substituents which create the numerous stereochemical centers and complicate a synthesis. We wished to substitute a short peptide in place of the bistetrahydrofuranyl portion of the natural product. A tripeptide found in the calcium binding site of troponin C was chosen for this purpose. It was deemed important to maintain the potential membrane solubility of the analogue and retain the charge balance which could be found in an ionomycin-calcium complex.

The aim was to design a simple calcium transporter which could not only be synthesized relatively easily but might also provide information on the relationship between Ca²⁺ affinity and transport, as well as the toxicity of such compounds.
RESULTS AND DISCUSSION

DESIGNING THE ANALOGUE:

The first stage of this project involved the design of a novel molecule which would exhibit an affinity for calcium ions and an ability to transport those ions across a cell membrane. In short, we wanted to design a calcium ionophore based partially on the information we had gathered about the mode of action of the polyether antibiotic, ionomycin. The analogue would be a molecule which would only possess those functional groups thought to be vital to the function of the natural product. The target would also be simplified by not incorporating the numerous stereochemical centers found along the carbon backbone of ionomycin.

Initially, we studied the crystal structure of the calcium salt of ionomycin (Figure 11). In this complex, it was possible to determine which functional groups within ionomycin were important for the binding of a calcium ion. Ionomycin binds Ca\(^{2+}\) as an electrically neutral complex. Thus, it was important to ensure that the analogue be capable of binding Ca\(^{2+}\) as an electrically neutral complex.

The natural product possesses two ionizable hydrogens from the carboxylic acid and the β-diketone units. The β-diketone exists primarily in its keto-enol form with the enolic proton being lost upon the chelation of Ca\(^{2+}\) (Scheme 1). Since both of these functional groups were pivotal in
controlling the charge balance of the calcium salt of ionomycin, it was deemed important that those two functional groups be retained in the design of an analogue.

Scheme 1: The enolization and ionization of a \( \beta \)-diketone.
Location of the chelating functional groups along the carbon backbone of ionomycin allows the electron donating atoms to rotate into one of the octahedral chelation sites on the calcium ion. In other words, the ability of the molecule to wrap around the ion and bind snugly to it would be an important consideration in the design of the analogue. As a result, our analogue would incorporate potential ligating functional groups at intervals along the carbon backbone approximating those found in ionomycin.

The X-ray structure of calcium salt of ionomycin reveals which atoms in the molecule are important in chelating the calcium ion. One of the oxygens found in the carboxylic acid binds to the cation as expected, due to electrostatic attractions. Both oxygen atoms in the β-diketone moiety are involved in the ligation site, likely due to electrostatic attractions as well. The alcoholic oxygens six and nine are also found to coordinate to the centrally complexed calcium ion. Finally, oxygen seven, found in a tetrahydrofuran moiety, is also involved in cation chelation. Thus, there are six donor atoms located octahedrally around the central calcium ion. The analogue should attempt to incorporate six potential ligating atoms at distances approximating those found in ionomycin.

As mentioned previously, the analogue should also incorporate a tripeptide found in the Ca$^{2+}$ binding site of the troponin C proteins. The sequence of amino acids in a
polypeptide confer a specific conformation upon that polypeptide, allowing it to function in a specific manner. Since the binding of calcium ions is very important in the regulation of many cellular functions, it is possible that the amino acid sequence of a calcium binding site is unlikely to undergo radical changes during evolution. This has been demonstrated for troponin C which is an important protein for muscle contraction. The calcium binding loop found in Troponin C has been isolated from 149 binding sites and the sequence of amino acids determined. The binding site consists of an α-helix, a loop, and a second α-helix. The loop of amino acids runs from residues 13 to 24 in a polypeptide that consists of 32 amino acids for the E and F helices and loop. It is the loop of the binding site which harbors the ability to chelate calcium ions even after it has been excised from the polypeptide. We arbitrarily chose to limit composition of our analogue to three amino acids in order to maximize its calcium binding site similarity as well as minimizing synthetic demands.

We chose three consecutive amino acids that are highly conserved in the binding site loop (residues 13 to 15) of troponin C. The N-terminal of the tripeptide always consists of an L-aspartic acid residue (100% conservation). The higher the degree of conservation of a particular residue, the more crucial that amino acid is thought to be in the structure and function of a protein. The second
amino acid of our tripeptide (derived from residue 14) was chosen to be L-lysine which was the most highly conserved (29%) amino acid in that position. Another useful feature of using lysine as the second amino acid was the fact that it has a basic side chain which may neutralize any charge on the acidic side chain of the aspartic acid. The C-terminal amino acid of our tripeptide would preferably be a neutral molecule to maintain charge neutrality required for calcium transport. Unfortunately, the most highly conserved amino acid in that position (residue 15) was L-aspartic acid (70%). The acidic nature of this molecule's side chain would make it an undesirable choice for our analogue. The second most highly conserved amino acid was L-asparagine (24%), a molecule with a neutral side chain functional group.

Thus, we planned to fuse a carbon chain analogous to the C-1 to C-21 unit of ionomycin (Fragment A) to a tripeptide consisting of L-aspartic acid, L-lysine, and L-asparagine. We were left with one more consideration. The terminus of the tripeptide fragment would be a free carboxylic acid. Leaving the acid in its free form was thought to be undesirable since it introduced a charge imbalance which we had been careful to avoid. Another possible problem with the free acid was the fact that it might decrease lipid solubility for the analogue by increasing the polar nature of the compound. Keeping these considerations in mind, we decided to make a tert-butyl
ester at the C-terminus of the tripeptide fragment. Not only would this prevent formation of a charge imbalance, but it could also improve the lipid solubility of the analogue. The tripeptide ester would constitute what will be referred to as Fragment B. Thus, our complete analogue, $N^\alpha$-(1-carboxy-9,11,21-trioxoheneicosyl)-L-aspargyl-L-lysyl-(tert-butyl)-L-asparagine (34) could be divided into two main fragments (Scheme 2). The structure of ionomycin is compared to the analogue 34 to illustrate that certain functional and structural features have been retained in the proposed analogue (Figure 12).

**FIGURE 12:** Comparison of ionomycin (1) with the analogue 34.
RETROSYNTHETIC ANALYSIS OF THE ANALOGUE (34):

Our synthetic plan was based on the retrosynthetic analysis of the desired compound 34 shown in Scheme 2. The desired product 34 was divided into fragments A and B. Fragment A could be broken into three units: 1,7-heptanediol (7), acetylacetone (5), and 1,9-nonanediol (2). Fragment B could be broken into three units as well: L-aspartic acid (19), L-lysine (23), and L-asparagine (27). A great advantage to using these six simple starting materials was that they are commercially available and all were inexpensive. Using other simple starting materials would allow substitution of different amino acids and/or different diols to make other analogues of ionomycin.

Scheme 2: Retrosynthesis of the analogue 34.
TOWARDS THE SYNTHESIS OF ANALOGUE 34:

The proposed synthesis of our tripeptide analogue was broken into three phases: the synthesis of fragment A (Scheme 3), the synthesis of fragment B (Scheme 4), and the coupling of fragments A and B with their subsequent deprotection (Scheme 5). A dotted line across the schemes denotes the point up to which synthesis was performed in the course of this project.

It is important to note that in the convergent synthesis of the analogue 34, the functional groups were protected in a manner which would allow complete deprotection in the final step of the synthesis.

**FRAGMENT A SYNTHESIS:** Both 1,9-nonanediol (2) and 1,7-heptanediol (7) were monobrominated to give 9-bromo-1-nonanol (3) and 7-bromo-1-heptanol (8) respectively in high yield.\(^2^5\) Alcohol 3 was then protected using tert-butylidemethylysilyl chloride to give 9-bromo-1-[(tert-butylidemethylysilyl)oxy]-nonane (4) in 99% yield.\(^2^6\) The dianion of acetylacetone (5) was prepared using 2.1 equivalents of lithium diisopropylamide at 0°C in THF and this was monoalkylated on a primary carbon by slow addition of 0.9 equivalents of the bromo compound 4.\(^2^7\) The alkylation reaction produced the desired 14-[(tert-butylidemethylysilyl)oxy]-2,4-tetradecadione (6) in 61% yield. Alkylation can be shown to have occurred on a primary carbon
Scheme 3: The synthesis of fragment A.
**Scheme 4:** The synthesis of fragment B.
Scheme 5: Fragment coupling and deprotection.
by the presence of a methyl signal in the 2.0 ppm region of the NMR spectrum integrating to only three protons. If alkylation had occurred at the central carbon, these methyl signals would integrate to six protons. The singlet at 5.49 ppm would not be present if the α-alkylated product had been produced.

Alcohol 8 was protected as its methoxymethyl ether to give 7-bromoheptyl-1-methoxymethyl ether (9) in 67% yield.\textsuperscript{28} The kinetically deprotonated dianion of 6 (Scheme 6) was generated using 3.0 equivalents of lithium diisopropylamide in a 2:1 mixture of THF:DMPU.\textsuperscript{29} DMPU was necessary to get dianion formation. To this dianion was added 1.5 equivalents of bromo compound 9 and the alkylation was allowed to take place over 12 hours at -78°C. The reaction produced 21-[(tert-butyl dimethylsilyl)oxy]-heneicosa-9,11-dioxo-1-methoxymethyl ether (10) in good yield (72%). Introduction of the methoxymethyl group was indicated by the two singlets at 4.61 ppm (2H) and 3.37 ppm (3H) in the NMR spectrum of 10. Once again it was important to verify that the alkylation had occurred at the primary rather than a secondary anionic center. Alkylation at the methyl carbon derived from ketone 6 was supported by the presence of a singlet at 5.49 ppm and the disappearance of the methyl signal at 2.0 ppm in the NMR spectrum. Mass spectral data also shows no significant ion which would correspond to the loss of an acetyl. The acetyl ion would be lost quite
easily if the isolated product had been alkylated on the \( \alpha \)-carbon.

Scheme 6: Formation of the dianion of 6.

The methoxymethyl ether of compound 10 was removed using bromodimethyl borane in methylene chloride.\(^{30}\) Unfortunately, even using freshly distilled boron reagent, the reaction only gave selective deprotection to yield 50% of the expected 21-[(tert-butyldimethylsilyl)oxy]-9,11-dioxoheneicosanol (11). The liberation of the alcohol was verified by spectral data. The appearance of an alcohol stretch (3600 to 3200 cm\(^{-1}\)) in the IR accompanied by disappearance of the methoxymethyl singlets in the NMR suggest that deprotection did occur. It is believed that the reagent was generating HBr which caused cleavage of the silyl ether as well. The alcohol 11 was subjected to Moffatt oxidation conditions to yield the corresponding
aldehyde 12 in greater than 90% yield. The crude 21-[(tert-butyldimethylsilyl)oxy]-9,11-dioxoheneicosanal (12), which was difficult to purify, was oxidized using an excess of silver nitrate and sodium hydroxide with Celite to give 21-[(tert-butyldimethylsilyl)oxy]-9,11-dioxoheneicosanoic acid (13) in 25% yield. Difficulties arose in extracting the desired acid from the residues produced in the reaction. The impure acid 13 was then esterified using an excess of DCC and benzyl alcohol to give the desired ester, benzyl-21-[(tert-butyldimethylsilyl)oxy]-9,11-dioxoheneicosanate (14) in low yield. The appearance of aromatic protons at 7.35 ppm (5H) and methylene protons at 5.10 ppm in the NMR spectrum of the product indicate that the esterification was successful. The mass spectrum for this compound has a signal at 338 corresponding to loss of silyloxy and benzyloxy fragments.

The silyl ether 14 was cleaved using tetrabutylammonium fluoride to give the corresponding alcohol 15 in 75% yield. Infrared verified the loss of the silyl protecting group by appearance of the alcohol stretch in the 3600 cm⁻¹ region. Benzyl 21-hydroxy-9,11-dioxoheneicosanate (15) was the last compound characterized in the synthesis toward Fragment A. It is proposed that 15 could be subjected to the Moffatt oxidation followed by another silver nitrate oxidation to give aldehyde 16 and carboxylic acid 17 respectively. Finally, the preparation of Fragment A
could be completed by forming the active ester 18 using an excess of DCC and p-nitrophenol.\textsuperscript{33} The preparation of the activated ester, benzyl-20-(p-nitrophenyloxy carbonyl)-9,11-dioxoeicosanate (18) would leave Fragment A ready for coupling with the free amine of the tripeptide.\textsuperscript{35} As proposed, the completion of the synthesis of fragment A would involve repetition of basic reaction conditions used earlier in the preparation of the compound.

**FRAGMENT B SYNTHESIS:** The synthesis of this portion of the analogue primarily involved protecting various functional groups of the amino acids in order to allow their logical assembly into the desired tripeptide. We chose to assemble our tripeptide using activated ester methodology since this technique suited our choice of protecting groups and facilitated the introduction of the \textit{tert}-butyl ester of L-asparagine.

L-Aspartic acid (19) was esterified with excess benzyl alcohol under acidic conditions to selectively protect the side chain carboxylic acid and yield $\beta$-benzyl-L-aspartic acid (20) in 27\% yield.\textsuperscript{36} Deprotection of this functional group could later be achieved by hydrogenolysis. The next stage of preparing this amino acid for peptide synthesis was proposed to be the protection of the free amine by subjecting the compound to 2,2,2-trichloroethyl chloroformate in pyridine.\textsuperscript{37} This should give rise to N-(2,2,2-trichloroethylformyl)-$\beta$-benzyl-L-aspartic acid (21).
This amine protecting group was chosen because it could be cleaved in the presence of the benzyl protecting groups. The next step involved forming the active ester of the free acid 21 in preparation for its coupling to the next amino acid chosen for the peptide. Compound 21 would be subjected to DCC and an excess of p-nitrophenol in order to form the desired active p-nitrophenyl ester of aspartic acid (22).

The second amino acid in our tripeptide was L-lysine. This amino acid would require protection of its side chain amino group prior to attempting a coupling with the aspartic acid derivative. L-Lysine monohydrochloride (23) was treated with a basic solution of copper (II) to form a dimeric complex (Scheme 7) involving the α-amine as well as electrostatic complexation with the α-carboxyl group. Thus, the side chain amine could be selectively protected with benzyl chloroformate. The copper complex was subsequently destroyed, liberating N'-benzyloxycarbonyl-L-lysine (24) in 57% yield, by passing H₂S through a solution of the dimer and resulting in the formation of copper (II) sulfide.³⁸ It was proposed that mixing of the activated ester of aspartic acid 22 with the free amine of the protected lysine (24) would lead to the formation of a dipeptide 25 whose side chains were protected with groups that could be removed by hydrogenolysis. The free carboxyl of the lysyl residue could then be esterified with p-nitrophenol under previously described conditions to give the active ester 26.
Scheme 7: The selective protection of the side chain amine of L-lysine.

Our task with L-asparagine (27) would involve formation of the tert-butyl ester without affecting the free amine of the molecule. As a result, the amine was protected by mixing with benzyl chloroformate under alkaline conditions to give N°-benzyloxycarbonyl-L-asparagine (28) in 50% yield. This derivative was then treated with isobutylene under acidic conditions to form the desired tert-butyl ester (29) in 97% yield. Subsequently, the benzyloxycarbonyl protecting group was selectively removed by hydrogenolysis to give tert-butyl-L-asparaginate (30) in 30% yield. Finally, it has been proposed that the free amine of the asparagine derivative 30 would be coupled with the active ester of the dipeptide 26 to yield the protected tripeptide 31. The α-amino protecting group found on the aspartic acid
residue could finally be removed by a zinc reduction to liberate the amine in preparation for coupling with Fragment A.\textsuperscript{37}

**COUPLING THE FRAGMENTS AND COMPLETION OF THE SYNTHESIS:**
Mixing of the active ester of Fragment A 18 with the free amine of Fragment B 32 should result in the formation of an amide bond forming the protected analogue 33. Finally, subjecting 33 to hydrogenolysis conditions would result in three simultaneous deprotections liberating: the carboxyl group of Fragment A, the side chain carboxyl of the aspartyl residue, and the side chain amine of the lysyl residue, to yield the analogue 34.
CONCLUSION

Although completion of the synthesis was not achieved, a great deal has been accomplished in this early research. The logic behind designing a peptide analogue of ionomycin has been explored. Further exploration of this analogue, and others, may reveal some very interesting information on factors important to the binding and transport of calcium ions.
EXPERIMENTAL

GENERAL:

Unless otherwise stated, all reactions were performed under $N_2$ in flame-dried glassware. The cold temperature baths used were: dry ice-acetone (-78°C), and ice-water (0°C).

Anhydrous reagents and solvents were prepared according to the procedure given in the literature.\(^4\) \(n\)-Butyllithium (Aldrich Chemical Co.) was standardized against diphenylacetic acid in THF, a faint yellow color being indicative of the end point.

Silica gel 60, 230-400 mesh, supplied by E. Merck Co. was used for preparative flash column chromatography.\(^4\)

Melting points were performed on a Fischer-Johns hot stage melting point apparatus and are corrected.

Infrared (IR) spectra were recorded on a BOMEM FT-IR Michaelson-100 connected to an IBM compatible computer. IR spectra were taken neat using NaCl plates, in a chloroform solution using NaCl cells of 0.2 mm thickness, or in the form of a KBr pellet. IR spectra are uncalibrated.

Proton nuclear magnetic resonance ($^1$H NMR) spectra were recorded in CDCl$_3$ or DMSO-d$^6$ solutions on a Bruker AC-200 (200 MHz) and Bruker WH-400 (400 MHz) instruments. Chemical shifts are given in parts per million (ppm) on the $\delta$ scale.
versus tetramethylsilane (δ 0 ppm) or chloroform (δ 7.27 ppm) as internal standards. Signal multiplicity, coupling constants, and integration ratios are given in parentheses.

Low resolution mass spectra (LRMS) were recorded on a Kratos-AEI model MS 50 or MS 9 spectrometer. Only peaks with greater than 20% relative intensity or those which were analytically useful are reported. High resolution mass spectra (HRMS) were obtained from a Kratos-AEI model MS 50 spectrometer. An ionization potential of 70 eV was used in all measurements.
9-Bromo-1-nonanol (3):

A liquid-liquid extractor was charged with 100 mL of n-heptane followed by crushed 1,9-nonanediol (2) (10.00 g, 62.4 mmol) and 48% hydrobromic acid (16.0 mL, 141 mmol). A 500-mL rbf was filled with 300 mL n-heptane and attached to the side arm of the extractor. The aqueous phase in the extractor was warmed to 80°C in an oil bath. The solvent reservoir was heated to reflux and n-heptane allowed to percolate through the aqueous layer for 24 hours. The organic and aqueous layers were separated and the pH of the aqueous layer was made alkaline by the slow addition of concentrated ammonium hydroxide. The aqueous solution was extracted with diethyl ether (3 x 20 mL). The combined organic phases were washed with saturated sodium bicarbonate solution (2 x 20 mL) followed by brine (2 x 20 mL). The organic extracts were dried over anhydrous magnesium sulfate, filtered, then concentrated under reduced pressure to yield a yellow oil. The crude product was purified by flash chromatography using petroleum ether/ethyl acetate (4:1) as eluent. The reaction produced 3 (11.84 g, 53.0 mmol, 85% yield) as a colourless oil.
IR (CHCl₃, cm⁻¹): 3624, 3535-3350, 2932, 2857, 1461, 1400, 1275, 1043.

¹H NMR (CDCl₃, 400 MHz) δ: 3.65 (t, J=6.5 Hz, 2H), 3.41 (t, J=6.8 Hz, 2H), 1.86 (m, 2H), 1.57 (m, 2H), 1.43 (m, 2H), 1.38 (s, 1H exchangeable in D₂O), 1.32 (br. s, 8H).

LRMS (m/z) from CI: 242 (⁸¹Br, M⁺+18, 96), 240 (⁷⁹Br, M⁺+18, 100).

LRMS (m/z) from EI ionization: 206(0.1), 204(0.1), 150(28), 148(30), 137(45), 135(46), 97(64), 83(62), 82(40), 81(31), 70(33), 69(87), 68(46), 67(41), 57(39), 56(45), 55(100), 54(35), 43(63), 42(51), 41(91), 39(45), 31(54), 29(67), 28(32).

9-Bromo-1-[(tert-butyldimethylsilyl)oxy]-nonane (4):

![Chemical Structure](image)

A dry 500-mL rbf was charged with methylene chloride (300 mL) and cooled to 0°C under an atmosphere of nitrogen. Compound 3 (11.84 g, 53.0 mmol) was dissolved in the solvent prior to adding triethyl amine (15.5 mL, 111 mmol) and a catalytic amount of 4-dimethylamino pyridine (100 mg). A solution of tert-butyldimethylsilyl chloride (11.19 g, 74.3 mmol) in methylene chloride (50 mL) was slowly added to the reaction mixture which was slowly warmed to room temperature while stirring for 24 hours. The mixture was poured into ice cold 0.5 M hydrochloric acid (150 mL) and the organic and aqueous layers were quickly separated. The aqueous phase was extracted with diethyl ether (3 x 20 mL) and the combined organic phases were subsequently washed with saturated sodium bicarbonate solution (2 x 20 mL) followed by brine (2 x 20 mL). The organic extracts were dried over anhydrous magnesium sulfate, filtered, then concentrated under reduced pressure to yield a yellow oil. The crude product was purified by flash chromatography using hexanes as eluent. The reaction produced 4 (17.72 g, 52.5 mmol, 99% yield) as a colorless oil.
**IR (neat, cm⁻¹):** 2905, 1465, 1387, 1361, 1252, 1100, 1006, 938, 840, 776, 721.

**¹H NMR (CDCl₃, 400 MHz) δ:** 3.62 (t, J=6.5 Hz, 2H), 3.42 (t, J=7.0 Hz, 2H), 1.86 (m, 2H), 1.51 (m, 2H), 1.43 (m, 2H), 1.31 (br. s, 8H), 0.90 (s, 9H), 0.05 (s, 6H).

**LRMS (m/z) from CI:** 339 (⁸¹Br, M⁺+1, 89), 337 (⁷⁹Br, M⁺+1, 100).

**LRMS (m/z) from EI ionization:** 281(6.2), 279(6.2), 169(21), 167(21), 125(30), 83(45), 75(100), 73(43), 70(26), 69(24), 57(75), 55(56), 43(22), 41(41).

**HRMS:** exact mass calcd for C₁₁H₂₄OSi⁸¹Br (M⁺-C₄H₉): 281.0173; found: 281.0766. Calcd for C₁₁H₂₄OSi⁷⁹Br (M⁺-C₄H₉): 279.0193; found: 279.0784.
14-[(tert-Butyldimethylsilyl)oxy]-2,4-tetradecadione (6):

A dry 500-mL, 2-neck rbf was equipped with an addition funnel and charged with anhydrous tetrahydrofuran (300 mL) which was subsequently cooled to 0°C under an atmosphere of nitrogen. Diisopropylamine (17.7 mL, 126 mmol) was added to the solvent prior to the slow, dropwise addition of 1.6 M n-butyllithium in hexanes (75.5 mL, 120.77 mmol). The reaction was stirred for 45 minutes in order to ensure formation of lithium diisopropylamine. Acetylacetone (5) (5.9 mL, 58 mmol) was slowly syringed into the LDA mixture over a period of one hour and the reaction was stirred for a further 45 minutes to ensure formation of the dianion. Compound 4 (17.72 g, 52.5 mmol) was dissolved in THF (20 mL) and this solution was added dropwise to the LDA solution over a period of 30 minutes by an addition funnel. The reaction was kept at 0°C and stirred overnight. The reaction was quenched by pouring it into ice cold 0.5 M hydrochloric acid (50 mL) and quickly separating the aqueous and organic phases. The organic layer was washed with saturated sodium bicarbonate solution (2 x 20 mL) followed by brine (2 x 20 mL). The organic phase was dried over anhydrous magnesium sulfate, filtered, then concentrated
under reduced pressure to yield a yellow oil. The crude product was purified by flash chromatography using petroleum ether/ethyl acetate (25:1) as eluent. The reaction produced 6 (11.42 g, 32.0 mmol, 61% yield) as a yellow oil.

IR (neat, cm⁻¹): 2906, 2853, 1742, 1713, 1613, 1463, 1390, 1361, 1250, 1100, 1006, 939, 840, 776.

¹H NMR (CDCl₃, 400 MHz) δ: 15.51 (br. s, 0.9H exchangeable in D₂O), 5.49 (s, 0.9H), 3.61 (t, J=6.5 Hz), 2H), 3.57 (s, 0.2H), 2.51 (t, J=7.5 Hz, 0.2H), 2.27 (t, J=7.5 Hz, 1.8H), 2.24 (s, 0.3H), 2.06 (s, 2.7H), 1.60 (m, 2H), 1.51 (m, 2H), 1.29 (br. s, 12H), 0.90 (s, 9H), 0.05 (s, 6H).

LRMS (m/z) from CI: 374 (M⁺+18, 8), 357 (M⁺+1, 69).

LRMS (m/z) from EI ionization: 356(0.1), 301(21), 300(48), 299(100), 85(22), 75(57), 73(25), 55(23), 43(41), 41(21).

HRMS: exact mass calcd for C₂₀H₄₀O₃Si (M⁺): 356.2747; found: 356.2753.
7-Bromo-1-heptanol (8):

\[
\text{H}_2\text{C} = \text{C} = \text{C} = \text{C} = \text{C} = \text{C} = \text{C} = \text{Br}
\]

A liquid-liquid extractor was charged with 100 mL of n-heptane followed by 1,7-heptanediol, (7), (10.42 g, 78.8 mmol) and 48% hydrobromic acid (22.4 mL, 197 mmol). A 500-mL rbf was filled with 300 mL n-heptane and attached to the side arm of the extractor. The aqueous phase of the extractor was warmed to 80°C in an oil bath. The solvent reservoir was brought to reflux and n-heptane allowed to percolate through the aqueous layer for 24 hours. The organic and aqueous layers were separated and the pH of the aqueous layer was made alkaline by the slow addition of concentrated ammonium hydroxide and subsequently extracted with diethyl ether (3 x 20 mL). The combined organic phases were washed with saturated sodium bicarbonate solution (2 x 20 mL) followed by brine (2 x 20 mL). The organic extracts were dried over anhydrous magnesium sulfate, filtered, then concentrated under reduced pressure to yield a yellow oil. The crude product was purified by flash chromatography using petroleum ether/ethyl acetate (4:1) as eluent. The reaction produced 8 (13.99 g, 71.7 mmol, 91% yield) as a colourless oil.
IR (neat, cm\(^{-1}\)): 3650-3050, 2903, 2856, 1449, 1255, 1051, 725.

\(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\): 3.64 (t, \(J=6.0\) Hz, 2H), 3.41 (t, \(J=6.8\) Hz, 2H), 1.87 (m, 2H), 1.77 (s, 1H exchangeable in D\(_2\)O), 1.58 (m, 2H), 1.46 (m, 2H), 1.37 (m, 4H).

LRMS (m/z) from CI: 214 (\(^{81}\)Br, \(M^+18\), 100), 212 (\(^{79}\)Br, \(M^+18\), 96).

LRMS (m/z) from EI ionization: 178(1.9), 176(1.9), 150(68), 148(68), 97(66), 81(25), 70(26), 69(91), 68(46), 67(43), 57(23), 56(47), 55(100), 54(26), 43(64), 42(49), 41(84), 39(50), 31(64).

HRMS: exact mass calcd for C\(_7\)H\(_{13}\)\(^{81}\)Br (\(M^+-H_2O\)): 178.0180; found: 178.0195. Calcd for C\(_7\)H\(_{13}\)\(^{79}\)Br (\(M^+-H_2O\)): 176.0200; found: 176.0207.
7-Bromoheptyl-1-methoxymethyl ether (9):

A 2-necked, 500-mL rbf was charged with methylene chloride (300 mL) under an atmosphere of nitrogen and equipped with a mechanical stirrer. Dimethoxymethane (38.1 mL, 430 mmol) and 8 (13.99 g, 71.7 mmol) were slowly added with stirring at room temperature. Phosphorus pentoxide (7.0 g, 49 mmol) was added to the reaction mixture in one gram portions at 15 minute intervals until the reaction was complete as judged from the TLC of the reaction mixture. The reaction mixture was poured into ice cold 1 M sodium carbonate solution (500 mL) and the gummy residue in the rbf was washed with ice cold 1 M sodium carbonate solution (200 mL). The aqueous phase was separated from the organic phase without shaking. The methylene chloride was removed under reduced pressure and the remaining oil was dissolved in diethyl ether (200 mL). The aqueous phase was washed with diethyl ether (2 x 20 mL) and the combined organic phases were washed with brine (2 x 20 mL) then dried over anhydrous magnesium sulfate. The solution was filtered and concentrated under reduced pressure to yield a yellow oil. The crude product was purified by flash chromatography using petroleum ether/ethyl acetate (25:1) as eluent. The
reaction produced 9 (11.49 g, 48.1 mmol, 67% yield) as a yellow oil.

\[ \text{IR (neat, cm}^{-1}\text{): 2909, 2853, 1738, 1453, 1385, 1236,} \]
\[ \text{1200, 1146, 1111, 1046, 919, 725.} \]

\[ \text{\textsuperscript{1}H NMR (CDCl}_3\text{, 400 MHz) } \delta: 4.62 \text{ (s, 2H), 3.53 (t, J=6.5 Hz, 2H), 3.42 (t, J=7.0 Hz, 2H), 3.37 (s, 3H), 1.87} \]
\[ \text{(m, 2H), 1.60 (m, 2H), 1.50 to 1.30 (m, 6H).} \]

\[ \text{LRMS (m/z) from CI: 258 (\textsuperscript{81}Br, M^+18, 84), 256 (\textsuperscript{79}Br,} \]
\[ \text{M^+18, 100).} \]

\[ \text{LRMS (m/z) from EI ionization: 239(1.8), 237(1.9),} \]
\[ \text{177(21), 97(100), 95(24), 75(29), 69(27), 57(21), 55(80),} \]
\[ \text{45(95), 43(21), 41(30).} \]

\[ \text{HRMS: exact mass calcd for C}_{9}\text{H}_{18}\text{O}_{2}\text{\textsuperscript{81}Br (M^+-H\textsuperscript{*})}:} \]
\[ \text{239.0470; found: 239.0470. Calcd for C}_{9}\text{H}_{18}\text{O}_{2}\text{\textsuperscript{79}Br (M^+-H\textsuperscript{*})}:} \]
\[ \text{237.0490; found: 237.0496.} \]
21-[(tert-Butyldimethylsilyl)oxy]-heneicosa-9,11-dioxo-1-methoxymethyl ether (10):

A dry 250-mL, 2-neck rbf was equipped with an addition funnel and charged with anhydrous tetrahydrofuran (50 mL) which was subsequently cooled to 0°C under an atmosphere of nitrogen. Diisopropylamine (14.81 mL, 106 mmol) was dissolved in the solvent prior to the slow, dropwise addition of 1.6 M n-butyllithium in hexanes (60.1 mL, 96.1 mmol). The reaction was stirred for 10 minutes prior to cooling to -78°C. A solution of 6 (11.42 g, 32.0 mmol) in THF (30 mL) was added dropwise from the addition funnel over a period of 30 minutes. The addition funnel was rinsed with DMPU (55 mL) which was also added to the reaction vessel over a 10 minute period. The dianion was to form over an 8 hour period before 9 (11.48 g, 48.0 mmol) was added slowly by syringe. The reaction was stirred at -78°C for 12 hours before quenching with water and a saturated solution of ammonium chloride (100 mL). The organic and aqueous phases were separated and the aqueous phase was extracted with diethyl ether (2 x 20 mL). The combined organic phases were washed with saturated sodium bicarbonate solution (2 x 20 mL) followed by brine (2 x 20 mL). The organic phase was dried over anhydrous magnesium sulfate, filtered, then
concentrated under reduced pressure to yield a red oil. The crude product was purified by flash chromatography using petroleum ether/ethyl acetate (25:1) as eluent. The reaction produced 10 (11.87 g, 23.1 mmol, 72% yield) as a yellow oil.

**IR (neat, cm\(^{-1}\))**: 2927, 2852, 1611, 1461, 1252, 1141, 1115, 1054, 920, 838, 776.

**\(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\)**: 15.55 (br. s, 0.8H exchangeable with D\(_2\)O), 5.49 (s, 0.8H), 4.61 (s, 2H), 3.60 (t, \(J=6.5\) Hz, 2H), 3.56 (s, 0.4H), 3.51 (t, \(J=6.5\) Hz, 2H), 3.37 (s, 3H), 2.50 (t, \(J=7.5\) Hz, 3.2H), 2.28 (t, \(J=7.5\) Hz, 0.8H), 1.60 (m, 4H), 1.50 (m, 4H), 1.30 (m, 20H), 0.90 (s, 9H), 0.05 (s, 6H).

**LRMS (m/z) from CI**: 533 (M\(^+\)18, 6).

**LRMS (m/z) from EI ionization**: 486(0.2), 458(32), 457(100), 425(57), 395(25), 75(38), 69(27), 55(33), 45(35).

**HRMS**: exact mass calcd for C\(_{25}\)H\(_{49}\)SiO\(_5\) (M\(^+\)OCH\(_3\)):
457.3349; found 457.3348.
21-[(tert-Butyldimethylsilyl)oxy]-9,11-dioxoheneicosan-1-ol (11):

A dry 500-mL rbf was charged with anhydrous methylene chloride (250 mL) in which was dissolved 10 (11.87 g, 23.1 mmol). The solution was cooled to -78°C prior to the slow addition of a freshly distilled 0.7 molar solution of bromodimethylborane in methylene chloride (41.0 mL, 28.7 mmol). The reaction was stirred for one hour at -78°C before it was cannulated into a vigorously stirred mixture of tetrahydorfurran (70 mL) and saturated sodium bicarbonate solution (35 mL). The reaction vessel was kept at -78°C and it was rinsed with methylene chloride (2 x 20 mL). The washings were also cannulated into the aqueous mixture. After five minutes, the mixture was diluted with diethyl ether (250 mL), and the aqueous and organic layers were separated. The aqueous phase was extracted with diethyl ether (2 x 20 mL) and then the combined organic phase was washed with water (2 x 20 mL), 10% sodium bisulphate (2 x 20 mL), and finally with brine (2 x 20 mL). The organic phase was dried over anhydrous magnesium sulphate, filtered, and concentrated under reduced pressure to yield a white solid. The crude product was immediately purified by flash chromatography using petroleum ether/ethyl acetate (4:1) as
eluent. The reaction produced 11 (5.43 g, 11.5 mmol, 50% yield) as a yellow oil.

IR (neat, cm$^{-1}$): 3600-3200, 2930, 2856, 1738, 1609, 1464, 1368, 1249, 1098, 838, 777.

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 15.65 (br. s, 0.8H exchangeable with D$_2$O), 5.59 (s, 0.8H), 3.73 (t, J=6.5 Hz, 2H), 3.70 (t, J=6.5 Hz, 2H), 3.64 (s, 0.4H), 2.60 (t, J=7.5 Hz, 0.8H), 2.38 (t, J=7.5 Hz, 3.2H), 1.68 (m, 5H with one being exchangeable with D$_2$O), 1.40 (m, 24H), 0.90 (s, 9H), 0.05 (s, 6H).

LRMS (m/z) from EI ionization: 414(31), 413(100), 111(21), 109(20), 97(46), 95(38), 85(32), 83(44), 81(37), 75(75), 73(26), 71(22), 69(72), 67(27), 57(21), 55(64), 43(24), 41(21).

HRMS: exact mass calcd for C$_{23}$H$_{45}$SiO$_4$ (M$^+$-C$_4$H$_9$): 413.3087; found 413.3068.
21-[(tert-Butyldimethylsilyl)oxy]-9,11-dioxoheneicosanal (12):

A dry 250-mL rbf was charged with methylene chloride (75 mL) and DMSO (75 mL). To this mixture was added alcohol 11 (5.43 g, 11.5 mmol), followed by DCC (14.27 g, 69.2 mmol). After the DCC had fully dissolved, dichloroacetic acid (1.9 mL, 23 mmol) was added slowly by syringe and the reaction was stirred for four hours at 25°C. The mixture was diluted with ethyl acetate (100 mL) and oxalic acid (5.0 g) was added to convert the remaining DCC to the insoluble urea by-product. This mixture was decanted into brine (200 mL) and filtered to remove any precipitate which had formed. The organic and aqueous layers were separated and the aqueous was extracted with ethyl acetate (2 x 20 mL). The combined organic phases were washed with brine (2 x 20 mL), dried over anhydrous magnesium sulphate, filtered, and concentrated under reduced pressure to yield the crude aldehyde, 12, as a yellow oil (5.15 g, 11.0 mmol, 95% yield). Due to difficulties in purifying the product beyond this point, spectra were collected on the crude oil only.

IR (neat, cm\(^{-1}\)): 2932, 2856, 1748, 1608, 1447, 1339, 1318, 1251, 1149, 1098, 960, 914, 837, 814, 776.
LRMS (m/z) from EI ionization: 468(0.1), 412(28), 411(100), 241(20), 83(38), 81(29), 75(34), 67(29), 61(31), 55(79), 43(22), 41(56).

HRMS: exact mass calcd for \(\text{C}_{27}\text{H}_{52}\text{SiO}_{4}(\text{M}^{+}-\text{H})\): 468.3635; found 468.3590.
21-[(tert-Butyldimethylsilyl)oxy]-9,11-dioxoheneicosanoic acid (13):

\[ \text{HO-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-OTBDMS} \]

A 100-mL rbf was charged with tetrahydrofuran (30 mL) and water (30 mL) in which was dissolved the aldehyde 12 (5.15 g, 11.0 mmol). To this solution was added sodium hydroxide (8.76 g, 220 mmol), and Celite (5.00 g), followed by slow addition of silver nitrate (18.62 g, 110 mmol) and the reaction was allowed to stir at 25°C for four hours. The black solution was then filtered and the Celite cake was washed with 1 M HCl (100 mL) and the filtrate was tested to be acidic. The cake was then washed with ethyl acetate (100 mL). The organic and aqueous phases were separated and the aqueous phase was extracted with diethyl ether (2 x 20 mL). The combined organic phases were dried over anhydrous magnesium sulphate, filtered, and concentrated under reduced pressure to yield the crude acid, 13, as a yellow oil (1.33 g, 2.74 mmol, 25% yield). Due to difficulties in purifying the product beyond this point, spectra were collected on the crude oil only.

\[
\text{IR (neat, cm}^{-1}\text{): 2934, 2859, 2350, 1717, 1454, 1378, 1110, 1046, 905.}
\]
Benzyl-21-[(tert-butyl(dimethyl)silyl)oxy]-9,11-dioxoheneicosanate (14):

A dry 250-mL rbf was charged with anhydrous methylene chloride (100 mL) in which was dissolved the crude acid 13 (1.33 g, 2.74 mmol). To this solution was added DCC (0.85 g, 4.1 mmol), benzyl alcohol (0.43 mL, 4.1 mmol), and DMAP (0.03 g, 0.3 mmol). The reaction was stirred over night at 25°C before it was quenched by addition of oxalic acid (1.0 g). The solution was dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to yield a yellow oil. The crude product was purified by flash chromatography using petroleum ether/ethyl acetate (25:1) as eluent. The reaction produced 14 (0.3945 g, 0.685 mmol, 25% yield) as a white solid which melts at 45°C.

IR (chloroform, cm⁻¹): 3418, 2936, 2858, 1691, 1519, 1452, 1346, 1061, 891.

¹H NMR (CDCl₃, 200 MHz) δ: 15.55 (br. s, 1H exchangeable with D₂O), 7.35 (s, 5H), 5.45 (s, 1H), 5.10 (s, 2H), 3.70 (t, J=7.5 Hz, 2H), 2.30 (t, J=7.0 Hz, 2H), 2.22 (t, J=7.0 Hz, 2H), 2.21 (t, J=7.0 Hz, 2H), 1.55 (m, 8H), 1.25 (m, 20H), 0.90 (s, 9H), 0.05 (s, 6H).
LRMS (m/z) from EI ionization: 485(0.1), 445(0.1), 441(0.1), 334(0.6), 322(4), 310(0.8), 278(1.3), 264(8.7), 253(12), 174(11), 147(7), 128(13), 91(100), 83(55), 82(22), 67(22), 55(53), 41(36).
Benzyl-21-hydroxy-9,11-dioxoheneicosanate (15):

A dry 100-mL rbf was charged with anhydrous THF (30 mL) into which the silyl ether 14 (0.3945 g, 0.685 mmol) was dissolved. A 1 M solution of tetrabutylammonium fluoride in THF (1.37 mL, 1.37 mmol) was added dropwise by syringe to the silyl ether. The reaction was stirred at 25°C over night. The solvent was removed under reduced pressure and the residue was taken up in diethyl ether (50 mL) and washed with water (2 x 20 mL) then dried over anhydrous magnesium sulphate. The solution was filtered and solvent removed under reduced pressure to yield a yellow oil. The crude product was purified by flash chromatography using petroleum ether/ethyl acetate (3:1) as eluent. The reaction produced 15 (0.2367 g, 0.514 mmol, 75% yield) as a white solid that melted at 37 to 38°C.

**IR (chloroform, cm⁻¹):** 3622, 2931, 2857, 1726, 1608, 1458, 1374, 1353, 1300, 1165, 1063, 997.

**¹H NMR (CDCl₃, 400 MHz) S:** 15.55 (br. s, 0.8H exchangeable with D₂O), 7.35 (m, 5H), 5.48 (s, 0.4H), 5.12 (s, 2H), 3.63 (t, J=17.5 Hz, 2H), 3.60 (s, 0.8H), 3.55 (s, 1H exchangeable with D₂O), 2.51 (t, J=6.5 Hz, 0.4H), 2.50 (t,
J=6.5 Hz, 0.4H), 2.34 (t, J=6.5 Hz, 2H), 2.26 (t, J=6.5 Hz, 1.6H), 2.25 (t, J=6.5 Hz, 1.6H), 1.60 (m, 8H), 1.25 (m, 18H).

**LRMS (m/z) from EI ionization:** 460 (0.5), 442 (0.5), 427 (0.2), 369 (1), 351 (1.4), 337 (15), 125 (19), 111 (24), 103 (69), 100 (32), 98 (20), 97 (40), 95 (24), 91 (65), 85 (67), 83 (43), 81 (31), 75 (22), 71 (22), 69 (66), 67 (35), 57 (49), 55 (100), 43 (57), 41 (46).

**HRMS:** exact mass calcd for C_{28}H_{44}O_{5} (M^+): 460.3189; found 460.3195.
**$\beta$-Benzyl-$\text{L}$-aspartic acid (20):**

![Chemical structure](image)

Freshly distilled benzyl alcohol (100 mL, 966 mmol) was added to a mixture of diethyl ether (100 mL) and concentrated sulfuric acid (10.0 mL) in a 500-mL rbf. The diethyl ether was removed under reduced pressure and finely ground $\text{L}$-aspartic acid (19) (13.31 g, 100 mmol) was added in small portions with stirring. The reaction was stirred at 25°C for 24 hours and was then diluted with 95% ethanol (200 mL) and neutralized by dropwise addition of pyridine (50 mL). The mixture was stored in the refrigerator over night and the crystalline product collected by filtration. The white solid was tritutated with diethyl ether prior to recrystallization from hot water containing a few drops of pyridine. The reaction produced 20 (6.03 g, 27 mmol, 27% yield) as a flaky white crystal that decomposed at 215 to 218°C compared with a literature melting point of 218 to 220°C.36

**IR (KBr, cm$^{-1}$):** 3018, 1736, 1620, 1404, 1361, 1309, 1225, 1166, 1022, 962, 862, 783, 738.
$^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$: 8.30 (s, 1H exchangeable with D$_2$O), 7.40 (m, 5H), 5.12 (s, 2H), 3.54 (m, 1H), 2.95 (dd, J=5.0 Hz, J=17.0 Hz, 1H), 2.65 (dd, J=8.0 Hz, J=17.0 Hz, 1H), 1.24 (s, 2H).

LRMS (m/z) from CI: 224 (M$^+\!+1$, 100).

LRMS (m/z) from EI ionization: 223(0.4), 108(44), 107(32), 91(100), 79(62), 77(40), 65(42), 51(21), 44(25), 43(21), 39(20).

HRMS: exact mass calcd for C$_{11}$H$_{13}$NO$_4$ (M$^+$): 223.0844; found 223.0826.
In a 1000-mL rbf, L-lysine monohydrochloride 23 (7.20 g, 39.4 mmol) was dissolved in water (500 mL) and the solution was brought to a boil. Copper (II) carbonate (12.72 g, 53 mmol) was slowly added to the solution and then the solution was filtered through a medium pore fritted glass filter. A 2 M sodium hydroxide solution (20.0 mL) was added and the dark blue solution was cooled in an ice bath. A solution of benzyl chloroformate (8.0 mL, 53 mmol) in 2 M sodium hydroxide (40.0 mL) was added in ten portions over 30 minutes shaking and cooling the reaction over the duration. Care was taken to prevent the solution from becoming too alkaline at any given time. The copper complex which separates as a dark blue precipitate was filtered and washed with water (10 mL) and ethanol (10 mL). The crystalline compound was suspended in water (500 mL) and a steady stream of hydrogen sulfide gas was passed through the solution until the bubbles would no longer decrease in size as they rose to the surface of the solution. The solution was brought to a boil and filtered hot. Cooling of the filtrate
in a refrigerator resulted in crystallization of 24 (6.30 g, 22.5 mmol, 57% yield) as fine white flakes that decomposed from 240 to 242°C compared with 259°C (decomp) in the literature.\cite{38}

**IR (KBr, cm\(^{-1}\))**: 3343, 2931, 2351, 1691, 1546, 1417, 1323, 1269, 1027, 743.

**\(^1\)H NMR (DMSO-\(d^6\), 400 MHz)**

- 8.30 (s, 1H exchangeable with D\(_2\)O), 7.35 (m, 5H), 7.20 (m, 1H), 5.01 (s, 2H), 3.16 (m, 1H), 3.00 (m, 2H), 1.71 (m, 1H), 1.56 (m, 1H), 1.35 (m, 4H), 1.23 (s, 2H).

**LRMS (m/z) from EI ionization**: 280(1.7), 235(37), 174(36), 128(73), 127(68), 108(93), 107(65), 92(33), 91(59), 84(100), 82(27), 79(77), 77(40), 74(36), 72(21), 65(30), 56(40), 43(21), 30(29).

**HRMS**: exact mass calcd for C\(_{14}\)H\(_{20}\)N\(_2\)O\(_4\) (M\(^+\)): 280.1423; found 280.1424.
Nα-Benzyloxycarbonyl-L-asparagine (28):

A 500-mL rbf was charged with water (100 mL), 1 M sodium hydroxide (70.0 mL), and dioxane (100 mL) and the mixture was cooled to 0°C. Finely crushed L-asparagine 27 (8.00 g, 60.55 mmol) was slowly dissolved and to this solution was added benzyl chloroformate (11.2 mL, 78.72 mmol). Over the next 25 minutes, an additional equivalence of 1 M sodium hydroxide (70.0 mL) was added to neutralize any HCl that formed. The reaction was warmed to 25°C and stirred for one hour. After ensuring that the reaction mixture was alkaline, it was extracted with ethyl acetate (2 x 100 mL). The aqueous phase was acidified to pH 2 by adding concentrated HCl. The desired product 28 (8.06 g, 30.3 mmol, 50% yield) precipitated out of solution as white needles which melted at 159 to 160°C compared to 163 to 165°C in the literature.42

IR (KBr, cm\(^{-1}\)): 3412, 3336, 1699, 1644, 1584, 1538, 1427, 1319, 1269, 1230, 1200, 1063, 737.
$^1$H NMR (DMSO-d$_6$, 400 MHz). $\delta$: 12.55 (br. s, 1H), 7.35 (m, 5H), 7.30 (br. s, 2H), 6.86 (br. s, 1H), 5.03 (s, 2H), 4.35 (m, 1H), 2.50 (m, 2H).

LRMS (m/z) from EI ionization: 266(0.1), 113(24), 112(55), 108(100), 107(98), 91(97), 90(21), 79(90), 77(51).

HRMS: exact mass calcd for C$_{12}$H$_{14}$N$_2$O$_5$ (M$^+$): 266.0902; found 266.0902.
**Nα-**(Benzyloxycarbonyl)-**tert-**butyl-**L**-asparaginate (29):**

![Chemical Structure](image)

In a 500-mL pressure bottle was mixed methylene chloride (30 mL) and dioxane (30 mL). The carboxylic acid 28 (5.00 g, 18.8 mmol) was slowly dissolved and this was followed by the addition of concentrated sulfuric acid (3.0 mL). Isobutylene was condensed (30 mL) in a trap at -78°C and poured into the pressure bottle which had been precooled in an ice bath. The pressure bottle was wired closed with a rubber bung equipped with a valve and the valve was closed. The solution was stirred overnight at 25°C. The reaction mixture was poured into ethyl acetate (200 mL) and the organic phase was washed with saturated sodium bicarbonate solution (2 x 20 mL) and brine (2 x 20 mL). The organic solution was then dried over anhydrous magnesium sulphate, filtered, and concentrated under reduced pressure. The ester 29 (5.87 g, 18.21 mmol, 97% yield) was recrystallized from diethyl ether as white flakes that melted from 85 to 86°C.

**IR (chloroform, cm⁻¹):** 3526, 3414, 2968, 1711, 1594, 1499, 1455, 1396, 1356, 1156, 1059, 844.
$^1$H NMR (CDCl$_3$, 400 MHz) δ: 7.35 (m, 5H), 5.91 (br. d, J=6.5 Hz, 1H), 5.61 (br. s, 1H), 5.40 (br. s, 1H), 5.12 (s, 2H), 4.46 (m, 1H), 2.89 (br. dd, J=4.5 Hz, J=16.0 Hz, 1H), 2.73 (br. dd, J=4.0 Hz, J=16.0 Hz, 1H), 1.45 (s, 9H).

LRMS (m/z) from EI ionization: 322(1.2), 305(0.2), 278(2), 277(2), 262(2), 221(4), 214(8), 199(14), 177(11), 159(13), 141(20), 113(23), 108(83), 107(60), 91(56), 79(66), 77(67), 70(34), 59(34), 58(100), 57(45), 56(50), 55(31), 51(35), 44(49), 43(63), 42(38), 41(62), 39(60).

HRMS: exact mass calcd for $C_{16}H_{22}N_2O_5$ (M$^+$): 322.1528; found: 322.1536.
**Tert-Butyl-L-asparaginate (30):**

![Chemical Structure](image)

A solution of the benzyloxycarbonyl compound **29** (5.00 g, 15.5 mmol) in anhydrous methanol (40 mL) was prepared in a dry 100-mL rbf under an atmosphere of nitrogen. A catalytic amount of 10% palladium on activated carbon (0.18 g) was added to the solution and the atmosphere in the reaction vessel was purged with nitrogen for five minutes prior to being connected to a hydrogenation apparatus. The solution was stirred under a positive pressure of hydrogen (500 mL) over night. The reaction mixture was filtered through a Celite cake and the cake was rinsed with methanol (50 mL). The filtrate was concentrated under reduced pressure to give a yellow oil. The crude compound formed pure amine **30** (0.88 g, 4.7 mmol, 30% yield) as white needles with a melting point of 132 to 135°C when recrystallized from diethyl ether.

**IR (chloroform, cm⁻¹):** 3389, 2983, 2351, 1731, 1659, 1394, 1371, 1346, 1278, 1158, 839.
$^1$H NMR (CDCl$_3$, 400 MHz) S: 5.98 (br. s, 2H), 3.85 (m, 1H), 2.61 (dd, $J=5.0$ Hz, $J=17.5$ Hz, 1H), 2.19 (dd, $J=5.0$ Hz, $J=12.0$ Hz), 1.79 (br. d, 2H), 1.46 (s, 9H).

LRMS (m/z) from EI ionization: 188(0.3), 129(54), 128(41), 127(94), 84(100), 70(33), 68(22), 57(35), 56(24), 44(26), 42(22), 41(32).

HRMS: exact mass calcd for C$_8$H$_{16}$N$_2$O$_3$ (M$^+$): 188.1161; found: 188.1168.
REFERENCES


APPENDIX

IR and $^1$H NMR SPECTRA:
13

RELATIVE INTENSITY

0.0526
-0.0089
-0.0705
-0.1320
-0.1935
-0.2550
4000.  3400.  2800.  2200.  1600.  1000.  CM-1
24

400 MHz TMS

RELATIVE INTENSITY

4000.  3400.  2900.  2200.  1600.  1000.  4000.

58.650
400 MHz
TMS