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Semi-synthesis and structure-activity relationship studies of Stemona alkaloids and related analogues

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Semi-Synthesis and Structure-Activity Relationship Studies of *Stemona* Alkaloids and Related Analogues

A thesis submitted in fulfilment of the requirements for the award of the degree of

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Kwankamol Sastraruji

B.Sc. Chemistry (Hons.)

Supervisors: Professor Stephen G. Pyne and Associate Professor Alison T. Ung

School of Chemistry

July, 2011
DECLARATION

I, Kwankamol Sastraruji, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Chemistry, University of Wollongong, is wholly my own work unless reference is provided. This document has not been submitted for qualifications at any other academic institution.

Kwankamol Sastraruji
July, 2011
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The *Stemona* species are monocotyledonous plants that belong to the *Stemonaceae* family. These plants are widely spread throughout South East Asia and northern Australia. The crude extracts from the roots of *Stemona sp.* have been used in China and South East Asia for agricultural and medicinal purposes. *Stemona* alkaloids have been reported for their insecticidal activities which may be associated with their ability to inhibit the enzyme acetylcholinesterase (AChE). Inhibitors of this enzyme are currently used to treat patients with Alzheimer’s disease and therefore the discovery of new AChE inhibitors is of medical importance. During the course of this study stemofoline was reported to increase sensitivity of anticancer drugs in treatment of multidrug resistance (MDR) cervical cancer cells.

The aims of the project were to use the known stemofoline-type alkaloid, 11(Z)-1',2'-didehydrostemofoline, as a template to prepare rare *Stemona* alkaloids and their analogues for testing as AChE inhibitors and structure-activity relationship (SAR) studies and to test their abilities to increase the sensitivity of anticancer drugs to MDR cancer cells.

11(Z)-1',2'-Didehydrostemofoline was isolated in grams quantities from the root extracts of unknown *Stemona sp.* which had been collected in Amphur Mae Moh, Lampang, Thailand.

From this starting material, four *Stemona* alkaloids, included stemoburkilline, oxystemofoline, methoxystemofoline and (1'R)-hydroxystemofoline, were prepared along with many analogues that incorporated hydroxyl and amino groups at the C-3 side chain position on the stemofoline back bone. The semi-synthesis of stemoburkilline was reported in Chapter 3. Using 11(Z)-1',2'-didehydrostemofoline as the starting material, stemoburkilline was prepared in four steps, these included the hydrogenation of the side chain alkene to produce stemofoline. Hydrogenation of the C-11-C-12 alkene moiety of stemofoline followed by a base catalysed ring-opening reaction of 11,12-dihydrostemofoline in the presence of TMSCl to give the TMS protected version of stemoburkilline. Mild TMS deprotection then gave stemoburkilline. The ring-opening process was proposed to occur through an E1cB mechanism. The NMR spectroscopic data of the synthesised stemoburkilline, which indicated the formation of the Z-isomer, was identical to those of the natural product.
This study led to the revision of the stemoburkilline structure from an $E$-isomer to a $Z$-isomer.

Chapter 4 of this thesis reports the preparation of the key aldehyde intermediate which was used to prepare several stemofoline derivatives that contained a hydroxyl group in the C-3 butyl side chain. These included the natural products, oxystemofoline, methoxystemofoline and (1'R)-hydroxystemofoline. The key aldehyde was prepared in two steps from 11(Z)-1',2'-didehydrostemofoline via an asymmetric dihydroxylation reaction of the C-3 1-buteryl side chain and then oxidative cleavage of the subsequence diol. A modified Julia olefination reaction on the aldehyde was employed as a key step in the preparation of oxystemofoline and methoxystemofoline, using sulfone reagents which had 4-hydroxybutyl and 4-methoxybutyl side chains, respectively. The synthesis of oxystemofoline and methoxystemofoline allowed reassignment of the $^{13}$C NMR signals for C-6 and C-1' from those reported for the natural products. Allylation of the aldehyde under indium-mediated conditions or using chiral allylborane reagents provided (1'R) and (1'S)-homoallylic alcohol products that lead to (1'R)-hydroxystemofoline and its (S)-epimer, respectively. Surprisingly, the synthesised (1'R)-hydroxystemofoline proved to be identical to a natural product that was later isolated from the root extracts of *Stemona aphylla*. The Wittig reaction of the aldehyde with (triphenylphosphoranylidene)acetaldehyde provided a mixture of three aldehyde products formed from consecutive Wittig reactions. These aldehyde products were later reduced to three different alcohols, included the enol, the dienol and the trienol. The A, B, C ring core structure of stemofoline was also provided in two steps via an Upjohn dihydroxylation reaction of stemofoline followed by an oxidative cleavage of the corresponding C-11, C-12 diol. The oxidation reaction of 11(Z)-1',2'-didehydrostemofoline using Na$_2$WO$_4$·2H$_2$O as a catalyst provided its N-oxide product in a shorter reaction time than the formerly reported method.

In Chapter 5 of this thesis, the reductive amination reactions of the aldehyde were reported to prepare 17 secondary amine derivatives in yields ranging from 25-93%. Some secondary amines were used in further methylation and carbamylation reactions to prepare tertiary amine and carbamate derivatives, respectively. A guanidine derivative was also prepared as its HCl salt.
In Chapter 6 of this thesis, an examination of Cu\textsuperscript{I}-catalysed click reactions was studied around a C-3 ethyne stemofoline analogue. The alkyne was prepared in a one step reaction from the key aldehyde and the Bestmann-Ohira reagent. Four triazoles were prepared using two different methods, a classical method using azides and a microwave-assisted method in which the azides were generated \textit{in situ}. While two isoxazoles were prepared using chlorooximes as precursors to the corresponding nitrile oxides. The Sonogashira coupling of the alkyne gave the phenylalkyne product and the alkyne dimer which was more efficiently and directly prepared under Eglington coupling conditions. A hydrogenation reaction of the phenylalkyne product provided a compound with a flexible C-3 2-phenylethyl side chain.

In Chapter 7 the AChE inhibitory activities of the synthesised stemofoline alkaloids and analogues are reported using a TLC bioautographic method which measured the activity as a minimum inhibitory requirement (MIR) in ng or nmol against electric eel AChE (eeAChE). Galanthamine was used as a positive control having a MIR of 1 ng (0.003 nmol). The four \textit{Stemona} alkaloids synthesised from this study, stemoburkilline, oxystemofoline, methoxystemofoline and (1'R)-hydroxystemofoline, showed MIRs of 50, 50, 50 and 5 ng, respectively. (1'R)-Hydroxystemofoline also showed the highest activity among the alcohol derivatives. Compared to 11(Z)-1',2'-didehydrostemofoline (MIR = 5 ng), its \textit{N}-oxide had similar activity (MIR = 5 ng) while that of stemofoline, which lacked the side chain alkene functionality, showed less activity with a MIR of 10 ng. Lacking the lactone ring of the stemofoline, the A, B, C ring core structure derivative was 10 times less active than stemofoline (MIR = 100 ng). The cyclopentyl amino carbamate, the dimethylamine and the alkyne derivatives had the highest activities in the group with MIR values of 1 ng. In general it was found that most of the amine derivatives were more active than the alcohol derivatives. While the click products showed moderate activities in the range of 50-100 ng except for the benzyl triazole derivative which had a higher activity with a MIR value of 5 ng. Compared to the alkyne (MIR = 1 ng), the phenylalkyne and the alkyne dimer were less active (MIRs = 50 and 100 ng, respectively). However, the compound with a flexible 2-phenylethyl side chain was 10 times more active than the phenylalkyne with a MIR of 5 ng. Some compounds were tested for their IC\textsubscript{50} values against eeAChE and human AChE (hAChE) using a colorimetric assay (known as Ellman’s method). 11(Z)-1',2'-Didehydrostemofoline
and an isopropylamine analogue showed good activities against eeAChE (IC$_{50}$ values = 19.2 and 12.9 μM, respectively) and hAChE (IC$_{50}$ values = 25.0 and 19.9 μM, respectively) but were not as potent as galanthamine (IC$_{50}$ values = 0.9 and 0.6 μM for eeAChE and hAChE, respectively). While other *Stemona* derivatives showed lower activities against eeAChE and hAChE with IC$_{50}$ values in the range of 52.5-302.3 μM and 28.7-52.4 μM, respectively. The MDR-reversing properties of some *Stemona* compounds were performed using the colorimetric MTT assay. Among the tested compounds, stemofoline showed the highest modulating effect on resistant KB-V1 cells by decreasing the IC$_{50}$ of paclitaxel from 10.06 ± 1.56 μM to 1.4 ± 0.45 μM and that of vinblastine from 0.61 ± 0.05 μM to 0.09 ± 0.01 μM. Stemofoline had the highest modulating effect on the resistant KB-V1 cells.

In Chapter 8 of this thesis, SAR studies are described using pharmacophore generation and molecular docking. The best seven different pharmacophore models were generated in order to search for the binding mode of the *Stemona* compounds. Unfortunately, based on the results from pharmacophore mapping alone we could not confirm the exact binding site. Thus, protein-ligand docking was performed using three different AChEs. The results from molecular docking suggested that the *Stemona* compounds were more likely to fit vertically in the active-site gorge of AChEs and bind between the active site and the PAS of AChEs. These computational studies showed that *Stemona* compounds may inhibit AChEs by allosterically binding at the PAS and blocking acetylcholine from reaching the active site.
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LIST OF ABBREVIATIONS

δ  Chemical shift (NMR)
λ  Wavelength
Δ  Difference
ν_{max}  Maximum absorbance
[α]_D  Specific rotation
[M]^{+}  molecular ion
[M+H]^{+}  Protonated molecular ion
μL  microlitre
μM  micromolar
3D QSAR  Three dimensional quantitative structure-activity relationship
(CD_{3})_{2}CO  Deuterated acetone
ABq  AB quartet (NMR)
Ac_{2}O  Acetic anhydride
ACH  Acetylcholine
ACHE  Acetylcholinesterase
AD  Alzheimer’s disease
AD  Asymmetric dihydroxylation
APT  Attached proton test
AR  Analytical reagent
Ar  Aromatic
ASB  Active site binding
ATCh  Acetylthiocholine
ATChI  Acetylthiocholine iodide
BFB  Bis-functional binding
Boc  tert-butyloxycarbonyl
br  broad
c  Concentration in g/mL
cia.  Circa (about)
calcd  calculated
CD_{3}OD  Deuterated methanol
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>CDCl₃</td>
<td>Deuterated chloroform</td>
</tr>
<tr>
<td>CYPI</td>
<td>Cyclic π-interaction group (pharmacophore feature)</td>
</tr>
<tr>
<td>d</td>
<td>Doublet (NMR)</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-Diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DCE</td>
<td>1,2-Dichloroethane</td>
</tr>
<tr>
<td>dd</td>
<td>Doublet of doublets (NMR)</td>
</tr>
<tr>
<td>DEPT</td>
<td>Distortionless enhancement by polarization transfer</td>
</tr>
<tr>
<td>DIAD</td>
<td>Diisopropylazodicarboxylate</td>
</tr>
<tr>
<td>DME</td>
<td>1,2-Dimethoxyethane</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s modified Eagle’s medium</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>dq</td>
<td>Doublet of quartets (NMR)</td>
</tr>
<tr>
<td>dr</td>
<td>Diastereomeric ratio</td>
</tr>
<tr>
<td>dt</td>
<td>Doublet of triplets (NMR)</td>
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<tr>
<td>DTNB</td>
<td>Dithiobisnitrobenzoate</td>
</tr>
<tr>
<td>E1cB</td>
<td>Elimination unimolecular conjugate base</td>
</tr>
<tr>
<td>eeAChE</td>
<td>Electric eel acetylcholinesterase</td>
</tr>
<tr>
<td>EI</td>
<td>Electron impact ionization</td>
</tr>
<tr>
<td>equiv</td>
<td>equivalents</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionization</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
</tr>
<tr>
<td>FR</td>
<td>Fold-reversal resistance</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>GA</td>
<td>Genetic algorithm</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>gCOSY</td>
<td>Correlated spectroscopy</td>
</tr>
<tr>
<td>gHMBC</td>
<td>Heteronuclear multiple bond correlation</td>
</tr>
<tr>
<td>gHSQC</td>
<td>Heteronuclear single quantum correlation</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>hv</td>
<td>Light</td>
</tr>
<tr>
<td>hAChE</td>
<td>Human acetylcholinesterase</td>
</tr>
<tr>
<td>Hal</td>
<td>Hydrophobic aliphatic group (pharmacophore feature)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>Har</td>
<td>Hydrophobic aromatic group (pharmacophore feature)</td>
</tr>
<tr>
<td>Hb_ext</td>
<td>Protein-ligand hydrogen bond energy</td>
</tr>
<tr>
<td>HBA</td>
<td>Hydrogen bond acceptor (pharmacophore feature)</td>
</tr>
<tr>
<td>HBD</td>
<td>Hydrogen bond donor (pharmacophore feature)</td>
</tr>
<tr>
<td>HR</td>
<td>High resolution</td>
</tr>
<tr>
<td>HUX</td>
<td>Huprine X</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>The half maximal inhibitory concentration</td>
</tr>
<tr>
<td>Int</td>
<td>Single internal energy</td>
</tr>
<tr>
<td>Ipc₂Ball</td>
<td>B-Allyldiisopinocampheylborane</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant (NMR)</td>
</tr>
<tr>
<td>LDA</td>
<td>Lithium diisopropylamide</td>
</tr>
<tr>
<td>LiHMDS</td>
<td>Lithium hexamethyldisilazide</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet (NMR)</td>
</tr>
<tr>
<td>m/z</td>
<td>mass/charge ratio</td>
</tr>
<tr>
<td>mCPBA</td>
<td>meta-Chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>MDR</td>
<td>Multidrug resistance</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
</tr>
<tr>
<td>MIR</td>
<td>Minimum inhibitory requirement</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre</td>
</tr>
<tr>
<td>mp</td>
<td>Melting point</td>
</tr>
<tr>
<td>MTT</td>
<td>3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide</td>
</tr>
<tr>
<td>MW</td>
<td>Microwave</td>
</tr>
<tr>
<td>nAChR</td>
<td>Nicotinic acetylcholine receptor</td>
</tr>
<tr>
<td>NCS</td>
<td>N-Chlorosuccinimide</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram</td>
</tr>
<tr>
<td>NMO</td>
<td>N-Methylmorpholine-N-oxide</td>
</tr>
<tr>
<td>nmol</td>
<td>Nanomole</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NOE</td>
<td>Nuclear overhauser effect</td>
</tr>
<tr>
<td>PAS</td>
<td>Peripheral anionic site</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>PDB</td>
<td>Protein databank</td>
</tr>
<tr>
<td>petrol</td>
<td>Petroleum spirit bp 40-60 °C</td>
</tr>
<tr>
<td>P-gp</td>
<td>Permeability glycoprotein</td>
</tr>
<tr>
<td>Ph</td>
<td>Phenyl</td>
</tr>
<tr>
<td>PI</td>
<td>Positive ionisable group (pharmacophore feature)</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>PTLC</td>
<td>Preparative thin-layer chromatography</td>
</tr>
<tr>
<td>q</td>
<td>Quartet (NMR)</td>
</tr>
<tr>
<td>$R_f$</td>
<td>Relative mobility</td>
</tr>
<tr>
<td>RR</td>
<td>Relative resistance</td>
</tr>
<tr>
<td>rt</td>
<td>Room temperature</td>
</tr>
<tr>
<td>s</td>
<td>Singlet (NMR)</td>
</tr>
<tr>
<td>SAR</td>
<td>Structure-activity relationship</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>sec</td>
<td>second</td>
</tr>
<tr>
<td>sp.</td>
<td>species</td>
</tr>
<tr>
<td>t</td>
<td>Triplet (NMR)</td>
</tr>
<tr>
<td>TBS</td>
<td><em>tert</em>-Butyldimethylsilyl</td>
</tr>
<tr>
<td>TeAChE</td>
<td><em>Torpedo californica</em> (electric ray) acetylcholinesterase</td>
</tr>
<tr>
<td>td</td>
<td>Triplet of doublets (NMR)</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin-layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilyl or tetramethylsilane</td>
</tr>
<tr>
<td>TMSCl</td>
<td>Trimethylsilyl chloride</td>
</tr>
<tr>
<td>TNB</td>
<td>5-Thio-2-nitrobenzoic acid</td>
</tr>
<tr>
<td>Ts</td>
<td>$p$-Toluenesulfonyl</td>
</tr>
<tr>
<td>$vdw$</td>
<td><em>van der Waals</em></td>
</tr>
<tr>
<td>$V_{dw_ext}$</td>
<td>Protein-ligand <em>van der Waals</em> energy</td>
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</table>
CHAPTER 1 INTRODUCTION

1.1 *Stemona* alkaloids

The *Stemonaceae* family of monocotelydonous plants consists of three genera, these are *Croomia*, *Stemona* and *Stichoneuron* (Figure 1.1).\(^1\) *Stemona* plants are distributed throughout South East Asia and northern Australia and have been named as 'Bai Bu' in China, 'Bach Bo' in Vietnam and 'Non Tai Yak' in Thailand. The main constituents isolated from the roots of *Stemona* plants are alkaloids with complex polycyclic structures. At present, over 139 *Stemona* alkaloids have been reported.\(^2\)

![Croomia pauciflora](image1) \(^3\)  \[Stemona sessifolia](image2) \(^4\)  \[Stichoneuron caudatum](image3) \(^5\)

Figure 1.1 *Stemonaceae* family.

1.2 Structural classification

*Stemona* alkaloids have been classified into a number of structural types by Pilli.\(^1\)-\(^2\) The main core structure is a pyrrolo[1,2-\(a\)]azepine nucleus. Pilli has classified the *Stemona* alkaloids into eight groups according to the different characteristic structures. Six groups, stenine, stemoamide, tuberostemospironine, stemonamide, parvistemoline and stemofoline contain a pyrrolo[1,2-\(a\)]azepine nucleus (Figure 1.2). One group with a pyrrolo[1,2-\(a\)]azepine was named the stemocurtisine group after its first member, stemocurtisine.\(^6\) The last structural group comprises compounds that either lack the pyrrolo[1,2-\(a\)]azepine or the pyrrolo[1,2-\(a\)]azepine base structure or do not fit into the above mentioned six groups. This group has thus been classified as the miscellaneous group. A large number of
alkaloids in this group however, are derived from a pyrrolo[1,2-\(a\)]azepine alkaloid precursor by a ring-opening reaction.

More recently, two different core structures were isolated. In 2010 two *Stemona* alkaloids with a pyrido[1,2-\(a\)]azonine core structure (150 and 151, Figure 1.10) were isolated from the roots of *Stemona sessilifolia*\(^7\) and in 2008 an indolizidine based alkaloid (152, Figure 1.10) was reported from the roots of *Stemona sessilifolia*.\(^8\) The former two compounds have been put into the miscellaneous group in this thesis. While Pilli classified the latter alkaloid as a member of the miscellaneous group.

![figure 1.2](image)

**Figure 1.2** *Stemona* alkaloid groups.\(^2,6-8\)

### 1.2.1 Stenine group

The alkaloids in the stenine group display the tetracyclic furo[2,3-\(h\)]pyrrolo[3,2,1-\(jk\)][1]benzazepin-10-(2\(H\))-one skeleton (Figure 1.2). The first alkaloid in this group, named stenine (1), was first isolated from the roots of *Stemona tuberosa*. Currently, there are 26 alkaloids reported in this group: stenine (1), 2-oxostenine (2), isostenine (3), sessilifoline B (4), tuberostemonine (5), tuberostemonine A (6), tuberostemonine J (7), tuberostemonine H (8), tuberostemonine N (9), tuberostemonine K (10), neotuberostemonine (11), tuberostemonine L (12), tuberostemonine M (13), neotuberostemonol (14),

2
tuberostemonol (15), didehydrotuberostemonine (16), bisdehydroneotuberostemonine (17), *epi*-bisdehydroneotuberostemonine J (18), 9α-bisdehydroneotuberostemonine (19), 9α-bisdehydroneotuberostemonine A (20), sessilifoline A (21), tridehydrotuberostemonine (22), oxotuberostemonine (23), stemoxazolidinone D (24), stemoxazolidinone E (25) and stemoxazolidinone F (26) (Figure 1.3). The majority of these compounds have the basic stenine structure with an appended γ-lactone ring at C-3.

1.2.2 Stemonamide group

Alkaloids in the stemonamide group have the tricyclic 2*H*-furo[3,2-c]pyrrolo[1,2-a]azepine skeleton (Figure 1.2). Currently, 35 alkaloids have been reported in this group: stemoamide (27), stemonine (28), neostemonine (29), bisdehydroneostemonine (30), protostemonine (31), 1-hydroxyprotostemonine (32), dehydroprotostemonine (33), oxyprotostemonine (34), didehydroprotostemonine (35), isoprotostemonine (36), stemocochinin (37), tuberostemoamide (38), sessilifoliamide A (39), stemoninine (40), protostemonamide (41), saxorumamide (42), isosaxorumamide (43), neostemocochinine (44), isoneostemocochinine (45), 13-demethoxy-(11S*,12R*)-dihydroprotostemonine (46), bisdehydrostemocochinin (47), isobisdehydrostemocochinin (48), neostemofoline (49), bisdehydroneostemoninine (50), bisdehydrostemoninine A (51), bisdehydrostemoninin B (52), bisdehydrostemoninine (53), isobisdehydrostemonanine (54), stemoninine A (55), stemoninine B (56), dihydrostemoninine (57), oxystemoninine (58), oxystemononine (59), stemoenonine (60) and 9α-O-methylstemoenonine (61) (Figure 1.4). The majority of these compounds have a γ-lactone ring attached to the A or C rings of stemonamide or γ-lactone rings attached to both A and C rings.
Figure 1.3 *Stemona* alkaloids of the stenine group.1-2,9-10
Figure 1.4 Stemona alkaloids of the stemoamide group.\textsuperscript{1-2,11}
1.2.3 Tuberospironine group

The tuberospironine group is characterized by the presence of a spiro[furan-2-(5H),9'[9H]pyrrolo[1,2-a]azepin]-5-one nucleus (Figure 1.2). This group comprises of 12 members: tuberospironine (62), croomine (63), stemospironine (64), stemotinine (65), istemotinine (66), stemonidine (67), didehydrocroomine (68), 10-hydroxycroomine (69), 6-hydroxycroomine (70), dehydrocroomine (71), tuberospironine (72) and sessilifoliamine A (73) (Figure 1.5).

Figure 1.5 Stemona alkaloids of the tuberospironine group.1-2

1.2.4 Stemonamine group

This group is characterized by the presence of the tetracyclic spiro[1H-cyclopenta[b]pyrrolo[1,2-a]azepine-11(10H),2′(5′H)-furan]-5′,10-dione skeleton. There are 11 alkaloids included in this group: stemonamine (74), istemonamine (75), stemonamide (76), istemonamide (77), maistemonine (78), oxymaistemonine (79), isoxygenistemonine (80), isomaistemonine (81), sessilistemamonine A (82), sessilistemamonine B (83) and sessilistemamonine C (84) (Figure 1.6).
1.2.5 Parvistemoline group

The parvistemoline alkaloids are characterized by the lack of a B-C ring fusion and the presence of a substituent attached to C-9 of the pyrrolo[1,2-α]azepine nucleus. This group comprises 12 alkaloids: parvistemoline (85), parvistemonine (86), didehydroparvistemonine (87), sessilifoliamide B (88), sessilifoliamide C (89), sessilifoliamide D (90), neostemodiol (91), stemaphylline (92), stemaphylline-N-oxide (93), stichoneurine A (94), stichoneurine B (95) and protostemodiol (96) (Figure 1.7).
1.2.6 Stemofoline group

Alkaloids in this group typically have a complex tetracyclic moiety which has an oxygen bridge between C-2 and C-8 and a carbon-carbon bond between C-3 and C-7. The number of alkaloids in this group has increased rapidly in the past five years to 22 compounds and consists of the following alkaloids: stemofoline (97), (1′R)-hydroxystemofoline (98), (2′S)-hydroxystemofoline (99), (2′R)-hydroxystemofoline (100), (3′R)-hydroxystemofolenol (101), (3′S)-hydroxystemofolenol (102), stemofolinoside (103), 16,17-didehydro-16(E)-stemofoline or (11Z)-1′,2′-didehydrostemofoline (104), 1′,2′-didehydrostemofoline-N-oxide (105), 16,17-didehydro-4(E)-16(E)-stemofoline (106), methylstemofoline (107), stemoburkilline (108), isostemofoline (109), (3′S)-hydroxystemofoline (110), (11S,12R)-dihydrostemofoline (111), (2′S)-hydroxy-(11S,12R)-dihydrostemofoline (112), 6β-hydroxystemofoline (113), 16-hydroxystemofoline (114), oxystemofoline (115), methoxystemofoline (116), parvistemoninine (117) and parvistemoninol (118) (Figure 1.8).

Figure 1.8 Stema alkaloids of the stemofoline group.1-2,9,12
1.2.7 Stemocurtisine group

The characteristic structure of this group is a pyrido[1,2-α]azepine nucleus (Figure 1.2) instead of the common pyrrolo[1,2-α]azepine nucleus as seen in the previous groups. This group was named after the first pyrido[1,2-α]azepine alkaloid, stemocurtisine, that was isolated from the root extract of *Stemona curtisii* in 2003. Members of this group of alkaloids have also been found in other plant species for example, *Stemona cochinichinensis, Stemona saxorum, Stemona kerrii* and *Stemona sessilifolia.* Currently, members of this group include 13 alkaloids: stemocurtisine (119), stemocurtisine-\(N\)-oxide (120), stemocochinamine (121), oxystemokerrilactone (122), cochinistemonine (123), cochinistemoninone (124), stemokerrin (125), stemokerrin-\(N\)-oxide (126), methoxystemokerrin-\(N\)-oxide (127), stemocurtisinol (128), oxystemokerrin (129), oxystemokerrin-\(N\)-oxide (130) and stemosessifoine (131) (Figure 1.9).

![Figure 1.9 Stemona alkaloids of the stemocurtisine group.](image-url)
1.2.8 Miscellaneous group

The miscellaneous group comprises alkaloids that do not fit in any of the general structural classifications shown in Figure 1.2. This group displays a polycyclic skeleton but neither a pyrrolo[1,2-\(a\)]azepine nor a pyrido[1,2-\(a\)]azepine system except for tuberostemoenone (145), sessilistemonamine D (147) and stemoxazolidinone A-C (153-155). Members of this group are parvistemoamide (132), tuberostemoninol (133), neotuberostemoninol (134), maireistemoninol (135), sessilifoliamide E (136), sessilifoliamide F (137), tuberostemoline (138), sessilifoliamide I (139), sessilifoliamide G (140), sessilifoliamide H (141), tuberostemonone (142), neotuberostemonone (143), epoxytuberostemonone (144), tuberocrooline (146), 1,9-a-seco-stemoenonine (148) and parvineostemonine (149) (Figure 1.10). However, other bicyclic systems were also found for example a pyrrolo[1,2-\(a\)]azonine nucleus (Figure 1.2) in sessilifoliamide K (150) and sessilifoliamide L (151)\(^7\) and an indolizidine nucleus (Figure 1.2) in sessilifoliamide J (152).\(^8\) Overall this group comprises 24 members.
Figure 1.10 *Stemona* alkaloids of the miscellaneous group.\(^{1-2,7-8,10}\)
1.3  **Biosynthetic pathway of Stemona alkaloids**

In 2003, Greger proposed a biosynthetic connection between the pyrrolo- and pyrido[1,2-\(a\)]azepine alkaloids.\(^{13}\) This study investigated the co-occurrence of alkaloids of both skeleton types from the root extracts of the same plant species. It was proposed that hydrolysis of the lactone ring of protostemonine (31) followed by decarboxylation might result in the C-3 1'-hydroxybutyl side chain, which has been found in some stemofoline alkaloids (Scheme 1.1). Furthermore, it was proposed that ring expansion of the pyrrolidine A ring occurred to form the six-membered pyridine ring of the pyrido[1,2-\(a\)]azepines (Scheme 1.1).

![Scheme 1.1 Proposed biosynthetic connections between pyrrolo- and pyridoazepines.\(^{13}\)](image)

In 2004, Seger suggested another possible biosynthetic pathway for the pyrrolo[1,2-\(a\)]azepine nucleus.\(^{14}\) A number of Stemona alkaloids with a pyrrolo[1,2-\(a\)]azepine core were analysed (Figure 1.11). The C- and D-ring carbons were proposed to be of terpenoid origin while the A-ring of these alkaloids was suggested to arise from spermidine (Scheme 1.2 and 1.3).
Figure 1.11 Structure comparison of different *Stemona* alkaloids. The spermidine part of the pyrrolo[1,2-α]azepine core is depicted with grey bold bonds and the terpenoid units with black bold bonds.\textsuperscript{14}

Scheme 1.2 Biosynthesis of spermidine.\textsuperscript{14}
Scheme 1.3 Proposed biosynthetic pathway of stemofoline.¹⁴
In 2005, a proposed biosynthesis of the pyrido[1,2-\(\alpha\)]azepine alkaloids was suggested by Mungkornasawakul.\(^{15}\) The alternative pathway to A-ring formation was based on the biosynthesis of the hemlock alkaloid (+)-conhydrine (Scheme 1.4).

Scheme 1.4 Proposed biosynthesis pathway of pyrido[1,2-\(\alpha\)]azepine alkaloids.\(^{15}\)

Based on various biosynthesis considerations, Greger has suggested a new classification of *Stemona* alkaloids into three skeleton types: Stichoneurine-type, Protostemonine-type and Croomine-type (Scheme 1.5).\(^{16}\)

Scheme 1.5 Classification of *Stemona* alkaloids into three skeleton types based on different carbon chains attached to C-9 of the pyrroloazepine core.\(^{16}\)
In 2009, Greger and his co-workers suggested a possible biosynthetic pathway to *Stemona* alkaloids based on earlier published phytochemical studies on *Stemonaceae* and *Pandanaceae* plants.\(^\text{17}\) The discovery of pandanamine (159) from *Stemonaceae* plants convinced Greger of the biosynthetic relationship between *Pandanus* and *Stemona* alkaloids. Pandanamine (159) was known to be a precursor of the pyrrolidine-type alkaloid pandamarilactonine (156) and the spiro-piperidine alkaloid 158 isolated from *Pandanus amaryllifolius* Roxb.\(^\text{18}\) From the isolation of 159 from *Stichoneuron calcicola*, Greger has proposed 159 as a biogenetic precursor of the *Stemona* alkaloids, including croomine (63) (Scheme 1.6). Pandanamine (159) was proposed to form through a decarboxylation, cyclization, reduction and dehydration process of the intermediate 157 which was produced by condensation of two units of 4-hydroxy-4-methylglutamic acid and a C-4-N-C-4 dicarboxylic acid.\(^\text{19}\)

\[\text{Scheme 1.6 Hypothetical biosynthetic pathway of pandanamide and the probable artificial cyclization to the pyrrolidine and pyrroloazepine alkaloids.}\]
1.4 Biological activities of the *Stemona* alkaloids

The root extracts from *Stemona* plants are used in Chinese and Southeast Asian traditional medicines for the treatment of respiratory diseases and as antihelmintics. The aqueous and alkaloid extracts from *Stemona tuberosa* showed strong antitussive activities in guinea pigs. A further study on the isolated individual alkaloids revealed that isostenine (3), neotuberostemonine (11), bisdehydrostemoninine (53), stemoninine (40) and stemonamide (76) were potent antitussive agents.\(^{20-22}\)

The stemofoline-typed alkaloids including, stemofoline (97) and (11Z)-1′,2′-didehydrostemofoline (104) showed strong insecticidal and antifeedant activities.\(^{23}\)

The activities of these alkaloids were also determined on the nicotinic acetylcholine receptors (nAChRs) of insects. These receptors are cholinergic receptors which are triggered by the binding of the neurotransmitter acetylcholine (ACh).\(^{24}\) This study indicated that 6β-hydroxystemofoline (113), 16-hydroxystemofoline (114) and neostemofoline (49) showed agonist effects on the pest insect nAChR while protostemodiol (96) and 13-demethoxy-(11S*,12R*)-dihydroprotostemonine (46) acted as antagonists.\(^{25}\)

Acetylcholine (ACh) receptors in humans are located at the top end of dendrites and are mainly present in the central nervous system. The acetylcholinesterase (AChE) enzyme controls the level and duration of action of ACh. This enzyme catalyses the hydrolysis of ACh to inactive choline and acetate (Scheme 1.7).\(^{26-27}\) The inhibition of AChE prolongs the duration of action of ACh which is useful for the treatment of Alzheimer's disease (AD). The cholinergic hypothesis states that AD patients have an abnormal level of ACh. Many alkaloids have been reported to be AChE inhibitors. The well known AD drugs are galanthamine, rivastigmine, donepezil and memantine (Figure 1.12).\(^{28}\) Moreover, the *Stemona* alkaloids, sessilistemonamine A (82) and B (83), were observed to act as AChE inhibitors. These and other *Stemona* alkaloids may also have therapeutic applications in the treatment of AD.\(^{29}\)

![Scheme 1.7 Hydrolysis of acetylcholine (ACh)].(27)
In 2011, stemofoline (97) was reported as a potential multidrug resistance (MDR) chemosensitizer for the treatment of MDR cancers. This alkaloid inhibits P-glycoprotein-mediated drug efflux and increased the efficiency of the chemotherapeutic drugs vinblastine, paclitaxel and doxorubicin.30

1.5 Aims of Project

As described above, the Stemona alkaloids have a wide range of biological activities that are useful with potential applications in medicine and agriculture. Unfortunately, the limited supply of many of these alkaloids prevented further biological investigation and development. However, we have discovered that the roots of an unidentified Stemona species31 can supply a major alkaloid, (11Z)-1′,2′-didehydrostemofoline (104), in grams quantities which could potentially allow us to prepare rare Stemona alkaloids and analogues for biological testing and structure-activity relationship (SAR) studies. The AChE inhibition was of particular interest for this study as it may allow us to discover new derivatives that can be used as a treatment of Alzheimer’s disease. In collaboration with the Department of Biochemistry, Faculty of Medicine, Chiang Mai University, we also planned to test our synthesised compounds as P-glycoprotein (P-gp) inhibitors.

The aims of this project are:

1) To prepare stemoburkilline (108) by semisynthesis and determine the actual stereochemistry at C-11;
2) To prepare rare Stemona alkaloids in sufficient amounts for SAR studies against AChE;
3) To prepare stemofoline analogues A-D by modification of the C-3 side chain;
4) To test the prepared alkaloids and analogues as AChE inhibitors and against P-gp; and
5) To study the binding mode of *Stemona* alkaloids and analogues on AChE inhibition using computer-aided docking and pharmacophore modelling.

**Scheme 1.8** Synthetic plan for this project.

The overall synthetic plan for this project is summarized in Scheme 1.8. The readily available (11Z)-1′,2′-didehydrostemofoline (104) will be converted to stemoburkilline (108) to verify its structure. (11Z)-1′,2′-Didehydrostemofoline (104) will be converted to the key scaffolds, the aldehyde 160 and the alkyne 161, from which a library of compounds based on the general structures A-D can be prepared.
CHAPTER 2 ISOLATION OF (11Z)-1′,2′-DIDEHYDROSTEMOFOLINE

2.1 Plant material

The plant material was first identified by Mr. James Maxwell as an unknown *Stemona* species which was collected from Phitsanulok, Thailand, in October 2004. A voucher specimen, number 25375, was deposited at the Herbarium of the Department of Biology, Chiang Mai University. Extraction of the roots of this plant and separation of its major phytochemical identified six new stemofoline alkaloids, (2′R)-hydroxystemofoline (100), (3′R)-stemofolenol (101), (3′S)-stemofolenol (102), (11Z)-1′,2′-didehydrostemofoline-N-oxide (105), the first C_{19} stemofoline alkaloid, methylstemofoline (107) and the first glycosidated *Stemona* alkaloid, stemofolininoside (103). There were also three known alkaloids isolated namely, (2′S)-hydroxystemofoline (99), (11Z)-1′,2′-didehydrostemofoline (104) and (11E)-1′,2′-didehydrostemofoline (106). The major alkaloid was found to be (11Z)-1′,2′-didehydrostemofoline (104).

![Chemical structures](image)

For this project, the roots of the same unknown species used in the above mentioned study were collected from Amphur Mae Moh, Lampang, Thailand, in November 2007 (Figure 2.1). For the purpose of this study, only (11Z)-1′,2′-didehydrostemofoline (104) was isolated from the roots extracts.
2.2 Extraction and isolation

The dry ground root of the *Stemona* species (935 g) was soaked in 95% EtOH over 4 days at rt to extract the organic components from the plant material. Evaporation of the ethanol gave a dark brown crude extract (148 g) which was partitioned between MeOH/H$_2$O (1:1) and CH$_2$Cl$_2$. The organic extract was dried and then concentrated *in vacuo* to give a dark brown residue (20 g). This material was purified in ~ 2 g batches by column chromatography to give 6 g in total of pure (11Z)-1’,2’-didehydrostemofoline (104) as a yellow-brown gum (Figure 2.2).
The $^1$H NMR spectroscopic data of 104 were in close agreement with those reported (Table 2.1, Figure 2.3). The EIMS showed a peak at $m/z$ 385 (50%) for the $M^+$ ion. Its optical rotation ($[\alpha]_D^{24} = +245$ (c 0.74, MeOH)) was similar to that of the reported value ($[\alpha]_D^{20} = +210$ (c 0.5, MeOH)).

**Figure 2.2** Extraction and isolation outline.
Table 2.1 $^1$H NMR spectroscopic data comparison between our isolated (11Z)-1',2'-didehydrostemofoline (104) with that from the literature.$^{14}$

<table>
<thead>
<tr>
<th>Position</th>
<th>Isolated $\delta_H$ (500 MHz, CDCl$_3$)</th>
<th>Literature $\delta_H$ (400 MHz, CDCl$_3$)</th>
</tr>
</thead>
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<tr>
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<td>1.95 (d, $J$ 12.4 Hz)</td>
</tr>
<tr>
<td></td>
<td>1.84 – 1.80 (m)</td>
<td>1.79 (dt, $J$ 12.4 Hz, 3.0 Hz)</td>
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<td>2</td>
<td>4.21 (br s)</td>
<td>4.21 (br s)</td>
</tr>
<tr>
<td>5</td>
<td>3.13 – 3.07 (m)</td>
<td>3.10 (ddd, $J$ 13.2 Hz, 8.4 Hz, 4.8 Hz)</td>
</tr>
<tr>
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<td>6</td>
<td>1.90 – 1.86 (m)</td>
<td>1.82 (m)</td>
</tr>
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<td>7</td>
<td>2.86 (d, $J$ 6.0 Hz)</td>
<td>2.86 (d, $J$ 6.1 Hz)</td>
</tr>
<tr>
<td>9</td>
<td>1.79 – 1.77 (m)</td>
<td>1.78 (m)</td>
</tr>
<tr>
<td>9a</td>
<td>3.50 (br s)</td>
<td>3.51 (br s)</td>
</tr>
<tr>
<td>10</td>
<td>3.13 – 3.07 (m)</td>
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</tr>
<tr>
<td>16</td>
<td>2.07 (s)</td>
<td>2.07 (s)</td>
</tr>
<tr>
<td>17</td>
<td>1.37 (d, $J$ 6.0 Hz)</td>
<td>1.37 (d, $J$ 6.6 Hz)</td>
</tr>
<tr>
<td>1'</td>
<td>5.50 (d, $J$ 15.0 Hz)</td>
<td>5.50 (dt, $J$ 15.2 Hz, 1.3 Hz)</td>
</tr>
<tr>
<td>2'</td>
<td>5.78 (dt, $J$ 15.5 Hz, 6.0 Hz)</td>
<td>5.78 (dt, $J$ 15.2 Hz, 6.3 Hz)</td>
</tr>
<tr>
<td>3'</td>
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<td>2.09 (dq, $J$ 15.2 Hz, 7.6 Hz, 1.3 Hz)</td>
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<tr>
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<td>0.99 (t, $J$ 7.0 Hz)</td>
<td>0.99 (t, $J$ 7.6 Hz)</td>
</tr>
<tr>
<td>OMe</td>
<td>4.13 (s)</td>
<td>4.13 (s)</td>
</tr>
</tbody>
</table>

Figure 2.3 $^1$H NMR spectrum (500 MHz, CDCl$_3$) of (11Z)-1',2'-didehydrostemofoline (104) used in this study.
CHAPTER 3 SEMI-SYNTHESIS AND STRUCTURAL PROOF OF STEMOBURKILLINE

3.1 Background

In 2004, stemoburkilline was first isolated from a root extract of *Stemona burkillii* Prain, along with another new stemofoline alkaloid, 11(S),12(R)-dihydrostemofoline (111), and two known alkaloids, stemofoline (97) and (2′S)-hydroxystemofoline (99). The structure and relative configuration of stemoburkilline was proposed as 162 based on an interpretation of its spectroscopic data. The configuration of the *exo*-cyclic alkene group in stemoburkilline was tentively assigned as (E) based upon mechanistic considerations. It was speculated that 162 arose from 111 via a base catalysed ring-opening reaction involving a *trans*-elimination (E2) process. As part of this project, we planned to determine the exact stereochemistry of stemoburkilline at C-11 and the mechanism of its formation. This required the synthesis of (E)- and (Z)-stemoburkilline (162 and 108, respectively) using (11Z)-1’,2’-didehydrostemofoline (104) as the starting material.

![Figure 3.1 Structures of alkaloids from *Stemona burkillii* Prain.](image-url)
3.2 Synthetic plan

The overall synthetic plan is shown in Scheme 3.1. (11Z)-1’,2’-Didehydrostemofoline (104) will be converted to stemofoline (97) by the selective hydrogenation of the less hindered butenyl side chain double bond of 97 over Pd/C under a H₂ atmosphere. Then photolysis of 97 will be conducted to provide isostemofoline (109) by a C-11-C-12 alkene photoisomerization reaction. Further hydrogenation of stemofoline (97) and its isomer 109 should give different stereoisomeric 11,12-dihydrostemofolines, 163 and 111, respectively. On exposure of these compounds to base, a trans-elimination process would be expected to give the ring-opened products 162 and 108, respectively as illustrated in Scheme 3.2. The NMR spectroscopic data of the resulting compounds will then be compared with the natural product stemoburkilline.

Scheme 3.1 Proposed synthetic plan.
3.3 Synthesis of stemoburkilline

The starting material 104 was hydrogenated over Pd/C for 1 h to provide stemofoline (97) in 97% yield (Scheme 3.3). A similar hydrogenation procedure for stemofoline analogues was reported by Baird in 2009. The disappearance of the $^1$H NMR signals at $\delta$ 5.50 (d, $J$ 15.0 Hz, H-1') and $\delta$ 5.78 (dt, $J$ 15.5 Hz, 6.0 Hz, H-2') confirmed the formation of 97 by the selective reduction of side chain double bond of 104. The $^1$H NMR spectroscopic data of 97 agreed well with those reported for the natural product (Table 3.1). The EIMS of 97 showed a peak at $m/z$ 387 (50%) for the M$^+$ ion. Its optical rotation ($[\alpha]_D^{24}$ = +245 (c 0.74, MeOH)) compared favourably with that reported ($[\alpha]_D^{20}$ = +270 (c 0.8, MeOH)).

Scheme 3.3 Synthesis of stemofoline 97 and its isomer 109.
Table 3.1 $^1$H NMR spectroscopic data comparison between the synthesised stemofoline (97) and the natural product.23

<table>
<thead>
<tr>
<th>Position</th>
<th>Synthesised $\delta_H$ (500 MHz, CDCl$_3$)</th>
<th>Literature $\delta_H$ (400 MHz, CDCl$_3$)</th>
</tr>
</thead>
<tbody>
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<td>1.73 – 1.69 (m)</td>
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<td>4.25 (br s)</td>
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<tr>
<td>5</td>
<td>3.16 – 3.07 (m)</td>
<td>~ 3.0 – 3.1 (m)</td>
</tr>
<tr>
<td></td>
<td>3.02 – 2.97 (m)</td>
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</tr>
<tr>
<td>6</td>
<td>1.93 – 1.88 (m)</td>
<td>1.7 – 2.0 (m)</td>
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<tr>
<td></td>
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<td>7</td>
<td>2.70 (d, $J$ 6.0 Hz)</td>
<td>2.7 (d, $J$ 6.4 Hz)</td>
</tr>
<tr>
<td>9</td>
<td>1.81 (dd, $J$ 10.0 Hz, 4.0 Hz)</td>
<td>1.7 – 2.0 (m)</td>
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<tr>
<td>9a</td>
<td>3.46 (br s)</td>
<td>3.49 (br s)</td>
</tr>
<tr>
<td>10</td>
<td>3.16 – 3.07 (m)</td>
<td>~ 3.1 (m)</td>
</tr>
<tr>
<td>16</td>
<td>2.07 (s)</td>
<td>2.07 (s)</td>
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<td>17</td>
<td>1.37 (d, $J$ 6.5 Hz)</td>
<td>1.36 (d, $J$ 6.4 Hz)</td>
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<tr>
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<td>1.46 – 1.40 (m)</td>
<td>1.5 – 1.6 (m)</td>
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<td>1.29 – 1.22 (m)</td>
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<tr>
<td>2'</td>
<td>1.63 – 1.52 (m)</td>
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<td>1.5 – 1.6 (m)</td>
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</tr>
<tr>
<td>OMe</td>
<td>4.14 (s)</td>
<td>4.13 (s)</td>
</tr>
</tbody>
</table>

The photoisomerization of stemofoline alkaloids was reported in 2001.23 This involved the photoisomerization of a mixture of (11$^{\text{Z}}$)-1',2'-didehydrostemofoline (104) and (11$^{\text{E}}$)-1',2'-didehydrostemofoline (106) (Scheme 3.4). The percentage amount of 104 was observed to increase slowly with the irradiated time.

Scheme 3.4 Photoisomerization of 106 to 104.

A similar procedure was later reported for the photolysis of (11$^{\text{Z}}$)-1',2'-didehydrostemofoline (104) to give a mixture of 104 and its isomer 106 (Scheme 3.5).31

Scheme 3.5 Photoisomerization of 104 to 106.
The latter procedure was applied to stemofoline (97) to prepare its isomer 109 (Scheme 3.3). Photoisomerization of 97, in the presence of acetophenone as a photosensitizer for 7 h, gave a mixture of 97 and 109 in a ratio of 9:11, respectively (Scheme 3.3). Separation of the mixture by column chromatography gave 109 in 41% yield and recovered 97 in a yield of 33%. The $^1$H NMR chemical shift of the C-17 methyl group for 97 and 109 clearly indicated a difference between these isomers. The C-17 methyl group of 109 resonated at $\delta$ 1.46 (d, $J$ 6.5 Hz, H-17) while that of 97 was at $\delta$ 1.37 (d, $J$ 6.4 Hz, H-17). The $^1$H NMR spectroscopic data of 109 were in good agreement with those reported.$^{35}$

Table 3.2 $^1$H NMR spectroscopic data comparison between the synthesised isostemofoline (109) and the natural product.$^{35}$

<table>
<thead>
<tr>
<th>Position</th>
<th>Synthesised $\delta_H$ (500 MHz, CDCl₃)</th>
<th>Literature $\delta_H$ (400 MHz, CDCl₃)</th>
</tr>
</thead>
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<td>2.00 (m)</td>
</tr>
<tr>
<td>2</td>
<td>4.27 (s)</td>
<td>4.29 (s)</td>
</tr>
<tr>
<td>5</td>
<td>3.23 – 3.16 (m) 3.03 -2.98 (m)</td>
<td>3.20 (m, 2H)</td>
</tr>
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<td>1.94 – 1.88 (m) 1.85 – 1.79 (m)</td>
<td>1.74 (dd, 2H, $J$ 10.8 Hz, 3.6 Hz)</td>
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<td>2.69 (d, $J$ 6.0 Hz)</td>
<td>2.73 (d, $J$ 5.2 Hz)</td>
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<td>1.84 (m)</td>
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<td>3.49 (m)</td>
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<td>10</td>
<td>3.15 – 3.11 (m)</td>
<td>3.03 (m)</td>
</tr>
<tr>
<td>16</td>
<td>2.04 (s)</td>
<td>2.02 (s)</td>
</tr>
<tr>
<td>17</td>
<td>1.45 (d, $J$ 6.5 Hz)</td>
<td>1.46 (d, $J$ 6.4 Hz)</td>
</tr>
<tr>
<td>1’</td>
<td>1.43 – 1.39 (m)</td>
<td>1.58 (m)</td>
</tr>
<tr>
<td>2’</td>
<td>1.62 – 1.52 (m)</td>
<td>1.58 (m)</td>
</tr>
<tr>
<td>3’</td>
<td>1.36 (q, $J$ 7.5 Hz)</td>
<td>1.36 (q, $J$ 6.8 Hz)</td>
</tr>
<tr>
<td>4’</td>
<td>0.92 (t, $J$ 7.0 Hz)</td>
<td>0.92 (t, $J$ 6.8 Hz)</td>
</tr>
<tr>
<td>OMe</td>
<td>4.11 (s)</td>
<td>4.12 (s)</td>
</tr>
</tbody>
</table>
Scheme 3.6 Synthesis of dihydrostemofoline 163 and 111.

The controlled hydrogenation of 97 and 109 proved difficult. Compounds 97 and 109 gave mixtures of the desired compounds 163 and 111, respectively, plus the known over-reduced compound 164 which was a mixture of diastereomers (Scheme 3.6). The ring-opened product 164 was also reported by Ye as an unexpected byproduct from the hydrogenation reaction of stemofoline 97 (Scheme 3.7). It was thought that 164 arises from ring-opening of 163 or 111 to give 108 or 162 (Figure 3.1), respectively, which undergoes further hydrogenation of the exo-cyclic alkene group.

Scheme 3.7 Hydrogenation of stemofoline 97.

Compounds 111, 163 and 164 were successfully separated by column chromatography. Differences between the 11,12-dihydrostemofoline isomers 111 and 163 could be observed in their $^1$H NMR spectra (Table 3.3, Table 3.4, Figure 3.2 and Figure 3.3). The chemical shift of H-11 in 163 was at $\delta$ 3.66 (t, $J$ 7.5 Hz, H-11) while that of 111 was at $\delta$ 3.76 (dd, $J_{10,11}$ 8.5 Hz, $J_{11,12}$ 3.0 Hz, H-11). The chemical shift of H-12 of 163 was at $\delta$ 4.73 (d, $J_{11,12}$ 6.5 Hz, H-12) while that of 111 was at $\delta$ 4.58 (br s, H-12). The $^1$H NMR spectral data of both isomers matched closely to those previous reported.
Table 3.3 $^1$H NMR spectroscopic data comparison between synthetic 11(S),12(R)-dihydrostemofoline (111) and the natural product.\(^{33}\)

<table>
<thead>
<tr>
<th>Position</th>
<th>Synthesised $\delta_H$ (500 MHz, CDCl$_3$)</th>
<th>Literature $\delta_H$ (500 MHz, CDCl$_3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.96 (d, $J_{10.0}$ Hz)</td>
<td>1.99 (m)</td>
</tr>
<tr>
<td>2</td>
<td>1.62 – 1.56 (m)</td>
<td>1.63 (d, $J_{7.5}$ Hz)</td>
</tr>
<tr>
<td>5</td>
<td>3.12 - 3.06 (m)</td>
<td>3.14 (m)</td>
</tr>
<tr>
<td>6</td>
<td>1.89 – 1.79 (m)</td>
<td>1.82 (m)</td>
</tr>
<tr>
<td>7</td>
<td>2.43 (d, $J_{6.5}$ Hz)</td>
<td>2.45 (d, $J_{6.0}$ Hz)</td>
</tr>
<tr>
<td>9</td>
<td>1.62 – 1.56 (m)</td>
<td>1.64 (dd, $J_{12.0}$ Hz, 3.0 Hz)</td>
</tr>
<tr>
<td>9a</td>
<td>3.37 (br s)</td>
<td>3.44 (br s)</td>
</tr>
<tr>
<td>10</td>
<td>2.63 – 2.55 (m)</td>
<td>2.61 (m)</td>
</tr>
<tr>
<td>11</td>
<td>3.76 (dd, $J_{10.11,11,12}$ 8.5 Hz, $J_{11.12}$ 3.0 Hz)</td>
<td>3.79 (dd, $J_{10.11,11}$ 9.0 Hz, $J_{11.12}$ 3.0 Hz)</td>
</tr>
<tr>
<td>12</td>
<td>4.58 (br s)</td>
<td>4.60 (br s)</td>
</tr>
<tr>
<td>16</td>
<td>2.00 (s)</td>
<td>2.01 (br s)</td>
</tr>
<tr>
<td>17</td>
<td>1.08 (d, $J_{6.5}$ Hz)</td>
<td>1.08 (d, $J_{6.5}$ Hz)</td>
</tr>
<tr>
<td>1′</td>
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</tr>
<tr>
<td>2′</td>
<td>1.55 – 1.49 (m)</td>
<td>1.40 (m)</td>
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<tr>
<td>3′</td>
<td>1.31 (quartet, $J_{7.0}$ Hz)</td>
<td>1.33 (m)</td>
</tr>
<tr>
<td>4′</td>
<td>0.89 (t, $J_{7.5}$ Hz)</td>
<td>0.87 (t, $J_{7.0}$ Hz)</td>
</tr>
<tr>
<td>OMe</td>
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<td>4.11 (s)</td>
</tr>
</tbody>
</table>

Table 3.4 $^1$H NMR spectroscopic data comparison between synthetic 11(S),12(S)-dihydrostemofoline (163) and reference.\(^{33}\)

<table>
<thead>
<tr>
<th>Position</th>
<th>Synthesised $\delta_H$ (500 MHz, CDCl$_3$)</th>
<th>Literature $\delta_H$ (500 MHz, CDCl$_3$)</th>
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</thead>
<tbody>
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<td>1.97 (m)</td>
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<tr>
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<td>1.61 – 1.57 (m)</td>
<td>1.62 (m)</td>
</tr>
<tr>
<td>5</td>
<td>3.10 – 3.04 (m)</td>
<td>3.09 (m)</td>
</tr>
<tr>
<td>6</td>
<td>1.86 – 1.79 (m)</td>
<td>1.85 (m)</td>
</tr>
<tr>
<td>7</td>
<td>2.45 (d, $J_{6.5}$ Hz)</td>
<td>2.47 (d, $J_{6.0}$ Hz)</td>
</tr>
<tr>
<td>9</td>
<td>1.61 – 1.57 (m)</td>
<td>1.60 (m)</td>
</tr>
<tr>
<td>9a</td>
<td>3.35 (br s)</td>
<td>3.38 (br s)</td>
</tr>
<tr>
<td>10</td>
<td>2.50 – 2.46 (m)</td>
<td>2.48 (m)</td>
</tr>
<tr>
<td>11</td>
<td>3.66 (t, $J_{7.5}$ Hz)</td>
<td>3.69 (t, $J_{7.0}$ Hz)</td>
</tr>
<tr>
<td>12</td>
<td>4.73 (d, $J_{11.12}$ 6.5 Hz)</td>
<td>4.75 (d, $J_{11.12}$ 6.5 Hz)</td>
</tr>
<tr>
<td>16</td>
<td>1.94 (s)</td>
<td>1.95 (br s)</td>
</tr>
<tr>
<td>17</td>
<td>1.10 (d, $J_{7.0}$ Hz)</td>
<td>1.12 (d, $J_{6.3}$ Hz)</td>
</tr>
<tr>
<td>1′</td>
<td>1.54 – 1.49 (m)</td>
<td>1.54 (m)</td>
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<tr>
<td>2′</td>
<td>1.43 – 1.36 (m)</td>
<td>1.40 (m)</td>
</tr>
<tr>
<td>3′</td>
<td>1.62 (q, $J_{7.5}$ Hz)</td>
<td>1.35 (q, $J_{6.8}$ Hz)</td>
</tr>
<tr>
<td>4′</td>
<td>0.89 (t, $J_{7.0}$ Hz)</td>
<td>0.92 (t, $J_{6.8}$ Hz)</td>
</tr>
<tr>
<td>OMe</td>
<td>4.08 (s)</td>
<td>4.10 (s)</td>
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</table>
Figure 3.2 $^1$H NMR spectrum (500 MHz, CDCl$_3$) of 11(S),12(R)-dihydrostemofoline (111).

Figure 3.3 $^1$H NMR spectrum (500 MHz, CDCl$_3$) of 11(S),12(S)-dihydrostemofoline (163).
Treatment of a solution of 163 in CH₂Cl₂ with DBU (2 equiv) resulted in a 37:39:24 mixture of 111, 163 and 108, respectively, that was difficult to separate (Scheme 3.8 (a)). These products most likely arise from interconversion reactions via a reversible base-catalysed ring-opening of 163 to give intermediate A and a reversible Michael addition reaction of A to the desired product 108 and/or base-catalysed epimerization reactions at C-12 between 111 and 163 (Scheme 3.8 (b)).

Scheme 3.8 Ring-opening reaction of 163.

To circumvent this problem, the ring-opening reaction was repeated on 163 in the presence of TMSCl (2 equiv) to trap the intermediate ring-opened alkoxide product A (Scheme 3.9). Under this condition clean formation of the TMS ether 165 was realized from MS (EIMS m/z 461 (100 %) for the M⁺ ion) and NMR analysis of the crude reaction mixture. In the ¹H NMR spectrum of 165, the signal of H-12 was absence while the signal of H-11 was present at δ 5.48 (d, J 10.0 Hz, 1H, H-11). Removal of the TMS ether of 165 under acidic conditions then provided a pure sample of 108 in 61% yield after purification by column chromatography. Under similar reaction conditions compound 111 was also converted to 108 in 69% yield (Scheme 3.9 (a)).
The $^1$H NMR spectrum of 108 was similar to that of stemoburkilline that was isolated and reported earlier, but was not identical (Table 3.5, Figure 3.4). A marked difference was observed for the chemical shift at $\delta$ 3.28 which is indicative of H-9a in 108 and which was reported to be at $\delta$ 3.60 in the natural product.\textsuperscript{33}

An examination of the original $^1$H NMR spectra of the original partially purified extracts of Stemona burkillii showed compounds 111, 163 and 108 to be present in a ratio of 42:11:47, respectively (Figure 3.6). In this mixture a signal at $\delta$ 3.32 was observed along with other resonances (e.g., $\delta$ 5.48 (d, $J$ 10.0 Hz, 1H, H-11) and $\delta$ 4.30 (br s, 1H, H-2)) that were consistent with those of compound 108; however no signal was seen at $\delta$ 3.60. Unfortunately there was no original sample of pure stemoburkilline to re-run its $^1$H NMR spectrum under identical conditions to that of 108. With pure synthetic compound 108 in hand, the determination that it was the Z-isomer was made on the basis of a NOE cross-peak between the furanone methoxy group and the alkene proton (Scheme 3.9 (b)).
Figure 3.4 $^1$H NMR spectrum (500 MHz, CDCl$_3$) of synthesised stemoburkilline (108).

Figure 3.5 $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of synthesised stemoburkilline (108).
Table 3.5 $^1$H NMR spectroscopic data comparison between synthetic stemoburkilline (108) and the natural product.\textsuperscript{33}

<table>
<thead>
<tr>
<th>Position</th>
<th>Synthesised 108 $\delta_H$ (500 MHz, CDCl\textsubscript{3})</th>
<th>Natural Product $\delta_H$ (500 MHz, CDCl\textsubscript{3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.91 (m)</td>
<td>1.99 (m)</td>
</tr>
<tr>
<td></td>
<td>1.59 (m)</td>
<td>1.80 (m)</td>
</tr>
<tr>
<td>2</td>
<td>4.30 (br s)</td>
<td>4.38 (br s)</td>
</tr>
<tr>
<td>5</td>
<td>3.05 (m)</td>
<td>3.27 (m)</td>
</tr>
<tr>
<td></td>
<td>2.94 (m)</td>
<td>3.09 (m)</td>
</tr>
<tr>
<td>6</td>
<td>1.83 (m)</td>
<td>1.93 (m)</td>
</tr>
<tr>
<td>7</td>
<td>2.15 (d, $J$ 5.0 Hz)</td>
<td>2.34 (d, $J$ 5.9 Hz)</td>
</tr>
<tr>
<td>9</td>
<td>1.74 (m)</td>
<td>1.85 (m)</td>
</tr>
<tr>
<td>9a</td>
<td>3.28 (br s)</td>
<td>3.60 (br s)</td>
</tr>
<tr>
<td>10</td>
<td>3.13 (m)</td>
<td>3.18 (m)</td>
</tr>
<tr>
<td>11</td>
<td>5.48 (d, $J$ 10.0 Hz)</td>
<td>5.50 (d, $J$ 10.0 Hz)</td>
</tr>
<tr>
<td>16</td>
<td>2.05 (s)</td>
<td>2.07 (s)</td>
</tr>
<tr>
<td>17</td>
<td>1.05 (d, $J$ 6.5 Hz)</td>
<td>1.08 (d, $J$ 6.8 Hz)</td>
</tr>
<tr>
<td>1’</td>
<td>1.48 (m)</td>
<td>1.67 (m)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.60 (m)</td>
</tr>
<tr>
<td>2’</td>
<td>1.38 (m)</td>
<td>1.36 (m)</td>
</tr>
<tr>
<td></td>
<td>1.24 (m)</td>
<td>1.29 (m)</td>
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<tr>
<td>3’</td>
<td>1.32 (m)</td>
<td>1.36 (m)</td>
</tr>
<tr>
<td>4’</td>
<td>0.90 (t, $J$ 7.5 Hz)</td>
<td>0.92 (t, $J$ 6.8 Hz)</td>
</tr>
<tr>
<td>OMe</td>
<td>4.10 (s)</td>
<td>4.13 (s)</td>
</tr>
</tbody>
</table>

A re-examination of the original crude extracts of *Stemona burkillii* was made. Partial purification of this extract by column chromatography showed compounds 111, 163 and 108 to be present from $^1$H NMR analysis (in a ratio of 36:22:42, respectively, Figure 3.7). In this mixture a signal was observed at $\delta$ 3.27, along with those also corresponding to compound 108. The original NMR sample of stemoburkilline was suspected to have traces of HCl in the solution (the CDCl\textsubscript{3} had not been base treated), resulting in a downfield shift of H-9a, although other protons near the protonated nitrogen atom would have also been expected to be observed significantly more downfield if this were the case. On the basis of these considerations and from the results of the ring-opening reactions, the reassignment of the structure of stemoburkilline from the $E$-configuration to the $Z$-configuration was made. It is quite possible that some of compounds 111, 163 and 108 are artefacts, which have interconverted under non-enzymatic catalysed reactions (compare to Scheme 3.8 (b)) either in the plant or during the extraction and purification process. It was difficult, however, to analyse the crude roots extracts by NMR analysis to determine the ratio of these products due to their relative low abundances.
11(S),12(R)-Dihydrostemofoline (111)
11(S),12(S)-Dihydrostemofoline (163)
(Z)-stemoburkilline (108)

Figure 3.6 $^1$H NMR spectrum (500 MHz, CDCl$_3$) of the partially purified fraction from the original crude extract of *Stemona burkillii* (from Chiang Mai, Thailand, re-examined on 22$^{nd}$ October 2008).

11(S),12(R)-Dihydrostemofoline (111)
11(S),12(S)-Dihydrostemofoline (163)
(Z)-stemoburkilline (108)

Figure 3.7 $^1$H NMR spectrum (300 MHz, CDCl$_3$) of the original partially purified extract of *Stemona burkillii* (from Chiang Mai, Thailand, examined on 3$^{rd}$ February 2004).
The stereochemical outcome of the base/TMScI-initiated ring-opening reaction of 111 and 163 can be rationalized as occurring through an E1cB mechanism,\textsuperscript{37} as shown in Scheme 3.9 (b). Deprotonation of 111 or 163 by DBU at the acidic $\gamma$-position of the lactone ring would result in the anionic intermediate C. TMScI-assisted ring-opening would then give the (Z)-isomer 108. Ring-opening via the anionic intermediate D, which would lead to (E)-stemoburkilline, is less likely due to an unfavourable steric interaction between the methoxy and methyl groups in this intermediate (Scheme 3.9 (c)). This intermediate is expected to protonate back to 111 or 163.

3.4 Conclusions

Stemoburkilline was reassigned to be the (Z)-isomer 108 rather than the (E)-isomer as original proposed. This assignment was based on the semi-synthesis of 108 from a base catalysed ring-opening reaction of 111 or 163 in the presence of TMScI. An E1cB mechanism has been proposed for this process. An NOE experiment on compound 108 also confirmed its (Z)-stereochemistry at C-11.
CHAPTER 4 SEMI-SYNTHESIS OF OXYSTEMOFOLINE,
METHOXYSYSTEMOFOLINE AND (1′R)-HYDROXYSYSTEMOFOLINE
AND RELATED ANALOGUES

4.1 Background

At the start of this project, (2′R) and (2′S)-hydroxy- (99 and 100), (3′S)-hydroxy- (110) and 4′-hydroxystemofoline (115, named as oxystemofoline) were known natural products. During the period of this project, (1′R)-hydroxystemofoline (98) was synthesised and was later identified to be a natural product.

A previous study from our group resulted in the semi-synthesis of (3′R)-stemofolenol (101), (3′S)-stemofolenol (102), methylstemofoline (107) and (3′S)-hydroxystemofoline (110) and the unnatural analogue (3′R)-hydroxystemofoline (167) starting from (11Z)-1′,2′-didehydrostemofoline (104). This study also included the testing of the AChE inhibitory activities of these compounds. With a sufficient quantity of (11Z)-1′,2′-didehydrostemofoline (104) in hand, we planned to prepare some rare stemofoline alkaloids and their analogues for SAR studies as inhibitors of AChE. Two alkaloids from the stemofoline group, oxystemofoline (115) and methoxystemofoline (116) were chosen as interesting synthetic targets. The alkaloids were isolated from roots of Stemona parviflora Wright C. H. by Chinese scientists in 1991. However, no biological activity studies were reported on these alkaloids. Other interesting targets were (1′R)-hydroxystemofoline (98) and (1′S)-hydroxystemofoline (166) since neither of these compounds were known natural products at the start of this study.
4.2 Proposed semi-synthetic plan for the natural products 115 and 116 and the analogues (1′R)- and (1′S)-hydroxystemofoline (98 and 166)

The proposed semi-synthetic plans to prepare oxystemofoline (115), methoxystemofoline (116), (1′R)-hydroxystemofoline (98) and (1′S)-hydroxystemofoline (172) are shown in Scheme 4.1(a-c). (11Z)-1′,2′-Didehydrostemofoline (104) will be converted to the aldehyde 160 which is the key scaffold for further reactions. In an earlier study by our group, the Sharpless asymmetric dihydroxylation reaction of the 3-(1′-butenyl) side chain of 104 proved to be more efficient in providing the diol 168. The diol 168 will be treated with NaIO₄ to give the aldehyde 160 (Scheme 4.1 (a)). The preparation of 115 or 116 can be conducted via a modified Julia olefination reaction using heteroarylsulfones to provide the olefin derivatives 169 and 170 as precursors towards oxystemofoline (115) and methoxystemofoline (116), respectively (Scheme 4.1 (b)). Compound 160 can also be used to prepare the (1′R)- and (1′S)- isomers 98 and 166 in two synthetic steps via an allylation reaction and then a regioselective hydrogenation reaction (Scheme 4.1 (c)). Furthermore, aldehyde 160 can also provide other derivatives via the Wittig reaction which will be described later in this chapter.

Scheme 4.1 Proposed semi-synthetic plans.
4.3 Preparation of the aldehyde 160

The aldehyde 160 was prepared using the method reported by our group in 2009 (Scheme 4.2). In this previous study, the diol 168, of undefined stereochemistry, was reported to be obtained as a single diastereomer from the reaction of 104 with AD-mix-α.

Scheme 4.2 Synthesis of the aldehyde 160.

A similar procedure was carried out in this study to prepare the aldehyde 160 (Scheme 4.3). This procedure gave a mixture of diastereomeric diols 173 and 174. These were obtained in yields of 65% and 2%, respectively, after separation by column chromatography. The diol 173 was identical spectroscopically to the compound 168, obtained previously (Table 4.1). In some cases, this diol mixture was taken through to the next step without separation. Although the stereochemistry of the diols 173 and 174 was not important for the synthesis of the aldehyde 160, we tentatively assigned their stereochemistries as (1′S,2′S) and (1′R,2′R), respectively, as shown in Scheme 4.3 based upon the Sharpless mnemonic (Figure 4.1). In the case of the side chain alkene group of 104, the caged heterocyclic moiety would be the large substituent (Rₐ) and the ethyl substituent would be the medium sized group (Rₘ). When AD-mix-β was employed, the diol 173 and 174 were obtained in 7% and 48% yields, respectively (Scheme 4.3).
Scheme 4.3 Modified procedure for preparation of the aldehyde 160.

The major differences in the $^1$H NMR spectra of 173 and 174 were the chemical shifts of H-1’ and H-2’. The signals for these protons for 173 were at $\delta$ 3.73 (s, H-1’) and $\delta$ 3.55 (t, $J$ 7.0 Hz, H-2’) while those of 174 were at $\delta$ 3.52 (s, H-1’) and $\delta$ 3.75 (t, $J$ 7.5 Hz, H-2’). Large differences were also noted in the chemical shifts of protons located close to the nitrogen, including those of H-2, H-5, H-6 and H-7. The signals for 173 were at $\delta$ 4.23 (s, H-2), 3.26 – 3.20 (m, H-5), 2.33 – 2.27 (m, H-6) and 2.95 (d, $J$ 6.5 Hz, H-7) while the signals for 174 were at $\delta$ 4.68 (s, H-2), 3.03 – 2.94 (m, H-5), 2.02 – 1.95 (m, H-6) and 3.10 (d, $J$ 6.5 Hz, H-7). These significant differences in chemical shifts may indicate the formation of a H-bond between the 1’-hydroxyl or 2’-hydroxyl group and the nitrogen as shown in the possible 5-membered or 6-membered ring H-bonded structures in Figure 4.2. The $^1$H NMR signals of 173 and 174 and those of the 1’-hydroxystemofolines 98 and 166, which could only form a 5-membered ring H-bonded structure, did not show any similarity. This may suggest that if H-bonded structures are formed in 173 and 174 then the 6-membered ring intermediates are more favourable.
Earlier our group reported the preparation of the aldehyde 160 from the diol 168 using NaIO₄ on silica gel (Scheme 4.2). In this study, we used NaIO₄ for the oxidative cleavage of 173 without silica gel and this proved a convenient method to prepare 160 of sufficient purity to take through to the next step without the need for purification. The ¹H NMR spectroscopic data of 160 matched closely to those reported.³⁴ The aldehyde 160 was crystalized as its hydrate form from a CH₂Cl₂/H₂O (1:1) mixture. The X-ray structure of the hydrated form of 160 is shown in Figure 4.3.

![Figure 4.1 Predictive model for the Sharpless asymmetric dihydroxylation reaction, where R₁, R₉ and R₅ represent the large, medium and small substituents, respectively.³⁹](image)

Table 4.1 ¹H NMR spectroscopic data comparison between synthetic diols 173 and 174 and reference 168.³⁴

<table>
<thead>
<tr>
<th>Position</th>
<th>Synthesised 173 δₜH (500 MHz, CDCl₃)</th>
<th>Synthesised 174 δₜH (500 MHz, CDCl₃)</th>
<th>Literature 168 δₜH (500 MHz, CDCl₃)</th>
</tr>
</thead>
<tbody>
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<td>1.78 (m)</td>
</tr>
<tr>
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<td>4.23 (br s)</td>
<td>4.68 (s)</td>
<td>4.24 (s)</td>
</tr>
<tr>
<td>4</td>
<td>3.26 – 3.20 (m)</td>
<td>3.03 – 2.94 (m, 2H)</td>
<td>3.24 (m)</td>
</tr>
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<td>3.03 (m)</td>
<td>3.02 (m)</td>
</tr>
<tr>
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<td>2.33 – 2.27 (m)</td>
<td>2.02 – 1.95 (m)</td>
<td>2.32 (m)</td>
</tr>
<tr>
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<td>1.88 – 1.84 (m)</td>
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</tr>
<tr>
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</tr>
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<td>3.52 (br s)</td>
<td>3.49 (br s)</td>
<td>3.53 (br s)</td>
</tr>
<tr>
<td>10</td>
<td>3.11 – 3.05 (m)</td>
<td>3.12 – 3.07 (m)</td>
<td>3.09 (m)</td>
</tr>
<tr>
<td>11</td>
<td>2.06 (s)</td>
<td>2.05 (s)</td>
<td>2.08 (s)</td>
</tr>
<tr>
<td>12</td>
<td>1.37 (d, J 6.5 Hz)</td>
<td>1.36 (d, J 6.5 Hz)</td>
<td>1.38 (d, J 6.5 Hz)</td>
</tr>
<tr>
<td>13</td>
<td>3.73 (s)</td>
<td>3.52 (s)</td>
<td>3.74 (s)</td>
</tr>
<tr>
<td>14</td>
<td>3.55 (t, J 7.0 Hz)</td>
<td>3.75 (t, J 7.5 Hz)</td>
<td>3.56 (t, J 6.5 Hz)</td>
</tr>
<tr>
<td>15</td>
<td>1.64 (sextet, J 7.5 Hz)</td>
<td>1.67 – 1.50 (m, 2H)</td>
<td>1.64 (sextet, J 7.0 Hz)</td>
</tr>
<tr>
<td>16</td>
<td>1.51 (quintet, J 6.5 Hz)</td>
<td>1.54 (quintet, J 7.0 Hz)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>0.96 (t, J 7.5 Hz)</td>
<td>0.95 (t, J 7.5 Hz)</td>
<td>0.97 (t, J 7.0 Hz)</td>
</tr>
<tr>
<td>OMe</td>
<td>4.14 (s)</td>
<td>4.13 (s)</td>
<td>4.14 (s)</td>
</tr>
</tbody>
</table>
Figure 4.2 Possible H-bonded structures for compounds 173 and 174.

Figure 4.3 The single crystal X-ray structure of the hydrated form of 160.
4.4 Synthesis of oxystemofoline and methoxystemofoline

4.4.1 Synthesis of the sulfone reagents for the modified Julia olefination reaction

To examine the modified Julia olefination reaction of aldehyde 160, the sulfone reagent 176 was prepared in two steps as shown in Scheme 4.4 (a). Sulfone 176, which included a TBS-protected propyl alcohol, was especially designed for the synthesis of oxystemofoline. Compound 174a was prepared from 3-bromopropanol using the procedure reported by Thompson (Scheme 4.4 (b)). Unlike Thompson, we found that this procedure gave not pure 174a but a 2:3 mixture of the bromide 174a and the chloride 174b which would not be separated. Both halides were identified from GC-MS analysis (m/z 195 (90%) and 197 (90%) for the [M[Br79]-C4H9]+ and [M[Br81]-C4H9]+ ions of 174a and m/z 151 (85%) and 153 (50%) for the [M[Cl35]-C4H9]+ and [M[Cl37]-C4H9]+ ions of 174b, Figure 4.3). This halide exchange problem was not mentioned in Thompson’s publication. The mixture of the TBS protected alcohols 174a/b was carried through to the next step to give thiazole 175 in 47% yield. Compound 175 was confirmed by ESIMS (m/z 340 (3%) for the [M+H]+ ion) and NMR analysis. The less reactive chloride 174b was also recovered from this reaction after column chromatography. This allowed for its complete characterization by NMR and MS (Table 4.3 and Table 4.4). Thiazole 175 was finally converted to sulfone 176 by oxidation with mCPBA. The 1H and 13C NMR signals due to H-1’ and C-1’ of 176 (δH 3.65 – 3.60 (m, H-1’) and δC 52.1) shifted significantly downfield compared to those in 175 (δH 3.43 (t, J 6.9 Hz, H-1’) and δC 30.4), consistent with the presence of the more electron withdrawing sulfonyl group. The ESIMS of 176 showed a peak at m/z 372 (50%) for the [M+H]+ ion.
Scheme 4.4 Synthesis of sulfone 176.

Table 4.2 $^1$H NMR spectroscopic data comparison between the synthesised bromide 174a, chloride 174b and the literature data for 174a.$^{40}$

<table>
<thead>
<tr>
<th>Position</th>
<th>Synthesised 174a $\delta_H$ (300 MHz, CD$_3$OD)</th>
<th>Synthesised 174b $\delta_H$ (300 MHz, CD$_3$OD)</th>
<th>Literature for 174a $\delta_H$ (500 MHz, CD$_3$OD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.76 (t, $J$ 6.0 Hz, 2H)</td>
<td>3.77 (t, $J$ 6.0 Hz, 2H)</td>
<td>3.75 (t, $J$ 5.8 Hz, 2H)</td>
</tr>
<tr>
<td>2</td>
<td>2.01 (quintet, $J$ 6.3 Hz, 2H)</td>
<td>1.93 (quintet, $J$ 6.0 Hz, 2H)</td>
<td>2.01 (m, 2H)</td>
</tr>
<tr>
<td>3</td>
<td>3.53 (t, $J$ 6.3 Hz, 2H)</td>
<td>3.65 (t, $J$ 6.3 Hz, 2H)</td>
<td>3.50 (t, $J$ 6.5 Hz, 2H)</td>
</tr>
<tr>
<td>OTBS</td>
<td>0.91 (s, 9H)</td>
<td>0.91 (s, 9H)</td>
<td>0.91 (s, 9H)</td>
</tr>
<tr>
<td></td>
<td>0.08 (s, 6H)</td>
<td>0.08 (s, 6H)</td>
<td>0.05 (s, 6H)</td>
</tr>
</tbody>
</table>

Table 4.3 $^{13}$C NMR spectroscopic data comparison between the synthesised bromide 174a, chloride 174b and the literature data for 174a.$^{40}$

<table>
<thead>
<tr>
<th>Position</th>
<th>Synthesised 174a $\delta_C$ (75 MHz, CD$_3$OD)</th>
<th>Synthesised 174b $\delta_C$ (75 MHz, CD$_3$OD)</th>
<th>Literature for 174a $\delta_C$ (125 MHz, CDCl$_3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61.5</td>
<td>60.5</td>
<td>60.4</td>
</tr>
<tr>
<td>2</td>
<td>36.7</td>
<td>36.6</td>
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</tr>
<tr>
<td>3</td>
<td>31.0</td>
<td>42.3</td>
<td>30.7</td>
</tr>
<tr>
<td>OTBS</td>
<td>26.4</td>
<td>26.4</td>
<td>25.9</td>
</tr>
<tr>
<td></td>
<td>19.1</td>
<td>19.1</td>
<td>18.3</td>
</tr>
<tr>
<td></td>
<td>-5.3</td>
<td>-5.3</td>
<td>-5.4</td>
</tr>
</tbody>
</table>
Sulfone 178 was chosen to be used as a reagent in the synthesis of methoxystemofoline (Scheme 4.5). Benzothiazole was selected as the heterocyclic part of a reagent and then was treated with 3-methoxy-1-propanol under Mitsunobu reaction conditions\(^{41}\) to give thiazole 177 in 84% yield after purification by column chromatography. The ESIMS analysis of 177 showed a peak at \(m/z\) 340 (100%) for the \([M+H]^+\) ion. The oxidation of the thiazole 177 to the sulfone 178 was conducted under similar reaction conditions used in the synthesis of sulfone 176. The sulfone 178 was obtained in 94% yield. Its structure was confirmed from NMR and MS analysis. The \(^1\)H and \(^{13}\)C NMR signals for H-1’ and C-1’ were, as expected, significantly downfield of those of the thiazole 177.

**Figure 4.4 GC spectrum of the mixture of 174a and 174b.**
Unfortunately, the modified Julia olefination reaction between the sulfone 178 and 160 proved unsuccessful (Section 4.4.3). An alternative sulfone 181 was then designed by the replacement of the benzothiazole moiety with a tetrazole ring (Scheme 4.6 (a)). Sulfone 181 was prepared using a similar procedure for the preparation of the sulfone 176 in Scheme 4.4. Compound 179 was prepared from tosylation of 3-methoxy-1-propanol following a procedure reported by Dale (Scheme 4.6 (b)). The ESIMS analysis showed a peak at \( m/z \) 245 (100%) for the \([M+H]^+\) ion and the \(^1\)H NMR spectroscopic data was in good agreement with those reported.\(^43\) Tosylate 179 was treated with the sodium salt of 1-phenyl-1\(H\)-tetrazole-5-thiol to give the thiazole 180 in 51% yield. The structure of 180 was confirmed by ESIMS (\(m/z\) 251 (100%) for the \([M+H]^+\) ion) and NMR analysis. Oxidation of the thiazole 180 with \(m\)CPBA resulted in the sulfone 181 which was also detected by ESIMS (\(m/z\) 283 (100%) for the \([M+H]^+\) ion). The \(^1\)H and \(^{13}\)C NMR signals due to H-1’ and C-1’ of 181 (\(\delta_H\) 3.82 (t, \(J\) 7.5 Hz, H-1’) and \(\delta_C\) 53.5) shifted significantly downfield compared to those in 180 (\(\delta_H\) 3.47 (t, \(J\) 7.5 Hz, H-1’) and \(\delta_C\) 30.4), consistent with the more electron withdrawing nature of the sulfonyl group.

Scheme 4.5 Synthesis of sulfone 178.

![Scheme 4.5](image-url)
4.4.2 Synthesis of oxystemofoline via a modified Julia olefination reaction

In order to form the trans-alkene 182, a modified Julia olefination reaction was employed using the sulfone 176 (Scheme 4.7) and a literature procedure. The sulfone 176 was treated with LiHMDS in DMF at -60 °C for 2 h to form the α-lithiated sulfone. This was then treated with a solution of 160 in DMF at -60 °C. The reaction mixture was slowly warmed to rt and then stirred for a further 20 h. The desired alkene 182 was isolated as the E-isomer (E:Z = >99 : <1) in 33% yield after purification by column chromatography. We suspect that the yield was low due to the highly sensitivity of the aldehyde 160 to the strongly basic conditions, however no other major side products could be detected or isolated. The 1H NMR spectroscopic data of 182 showed signals at δ 5.74 (dt, \( J_{1',2'} = 15.5 \) Hz, \( J_{2',3'} = 7.0 \) Hz, H-2') and δ 5.58 (d, \( J_{1',2'} = 15.5 \) Hz, H-1') which confirmed the trans-alkene structure by the relatively large coupling constant between H-1' and H-2'.

The proposed mechanism for the modified Julia olefination has been reviewed by Blakemore (Scheme 4.8). Firstly, the benzothiazoyl sulfone is converted to the metallated intermediate B by treatment with a strong base such as

Scheme 4.6 Synthesis of sulfone 181.

Scheme 4.7 Semi-synthesis of the TBS protected alkene 182.
LDA or LiHMDS. The coupling of B with an aldehyde gives the β-alkoxysulfone (C) which undergoes a Smiles rearrangement via reaction with the electrophilic imine-like moiety within the heterocycle to give the spirocyclic intermediate (D). This intermediate then converts to the Smiles rearrangement product, the sulfinate salt (E). Spontaneous elimination of sulfur dioxide and lithium benzothiazolone then yields the alkene product.

Scheme 4.8 Mechanism of the modified Julia olefination.45

The stereochemical outcome of the modified Julia olefination was found to be dependent upon the nature of the coupling partners and the polarity of the solvent. DME, THF and DMF are moderate to highly trans selective solvents whereas toluene and diethyl ether are not. The anti β-alkoxysulfone diastereoisomer Ca yields a trans-alkene while the syn β-alkoxysulfone diastereoisomer Cb yields a cis-alkene (Scheme 4.9). From the proposed mechanism in Scheme 4.9, a cis-alkene is expected to be more likely than a trans-alkene due to the unfavourable eclipsed arrangement of R1 and R2 in the spirocycle intermediate Da. Therefore, an alternative mechanism has been suggested that involves the zwitterionic intermediates Fa or Fb formed from the spirocycle ring opening of the intermediates Da or Db (Scheme 4.10). The equilibration between these conformers would shift to the more stable intermediate Fb to give a trans-alkene product prior to the loss of sulfur dioxide. As described previously, the stemofoline moiety of aldehyde 160 is bulky which preferentially would favour formation of the intermediate Fb and give the trans-alkene product.
Scheme 4.9 Proposed mechanism for the modified Julia olefination.\textsuperscript{45}

Scheme 4.10 Alternative mechanism for \textit{trans} selective reaction.\textsuperscript{45}
TBS-deprotection of compound 182 under acidic conditions gave the homoallylic alcohol 169 (87% yield) as evident from ESIMS analysis (m/z 402 (100%) for [M+H]^+ ion) (Scheme 4.11). The ^1H NMR resonances at δ 0.88 (s) and δ 0.04 (s) for the TBS group of 182 were now absent in the ^1H NMR spectrum of 169. Alcohol 169 was then regioselectively hydrogenated over PdCl$_2$ under a H$_2$ atmosphere to give oxystemofoline 115 in 60% yield (Scheme 4.11). The specific optical rotation of 115 ([α]$_D$$^{22}$ +298 (c 0.52, CH$_3$OH)) was of the same sign but significantly larger in magnitude than that reported for the natural product ([α]$_D$$^{20}$ +106.0 (c 0.1, CH$_3$OH)). This difference might be because we had a relatively larger amount of the pure compound that we could more accurately weigh. The ^1H and ^13C NMR spectroscopic data of 115 were in a good agreement with those reported for the natural product (Table 4.4 and Table 4.5) except for the assignment of the ^13C NMR signals for C-6 and C-1’ which were originally incorrectly assigned from our analysis of the COSY and HMBC spectra of 115. The HMBC correlations were seen between δ 32.4 (C-1’) and δ 1.55 – 1.54 (m, 2H, H-2’, H-3’) and also between δ 27.2 (C-6) and δ 2.53 (d, J 6.0 Hz, H-7). Thus this semi-synthesis confirmed the proposed structure of the natural product and established its absolute configuration since that of stemofoline.HBr salt has been established by X-ray crystallographic analysis.46

Scheme 4.11 Semi-synthesis of oxystemofoline (115).
Table 4.4 $^1$H NMR spectroscopic data comparison between the synthesised oystemofoline (115) and the natural product.38

<table>
<thead>
<tr>
<th>Position</th>
<th>Synthesised $\delta_H$ (500 MHz, (CD$_3$)$_2$CO)</th>
<th>Natural product $\delta_H$ (300 MHz, (CD$_3$)$_2$CO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.93 – 1.88 (m)</td>
<td>1.98 (m)</td>
</tr>
<tr>
<td>2</td>
<td>4.22 (br s)</td>
<td>4.23 (m)</td>
</tr>
<tr>
<td>5</td>
<td>3.08 – 3.04 (m)</td>
<td>3.01 (m)</td>
</tr>
<tr>
<td>6</td>
<td>1.93 – 1.88 (m)</td>
<td>1.96 (m)</td>
</tr>
<tr>
<td>7</td>
<td>2.53 (d, J 6.0 Hz)</td>
<td>2.68 (dd, J 6.1 Hz, 2.5 Hz)</td>
</tr>
<tr>
<td>9</td>
<td>1.93 – 1.88 (m)</td>
<td>1.81 (m)</td>
</tr>
<tr>
<td>9a</td>
<td>3.47 (br s)</td>
<td>3.47 (m)</td>
</tr>
<tr>
<td>10</td>
<td>3.08 – 3.04 (m)</td>
<td>3.10 (dq, J 7.5 Hz, 6.5 Hz)</td>
</tr>
<tr>
<td>16</td>
<td>2.04 (s)</td>
<td>2.06 (s)</td>
</tr>
<tr>
<td>17</td>
<td>1.38 (d, J 6.3 Hz)</td>
<td>1.36 (d, J 6.5 Hz)</td>
</tr>
<tr>
<td>1’</td>
<td>1.62 – 1.58 (m)</td>
<td>1.72 – 1.52 (m)</td>
</tr>
<tr>
<td>2’</td>
<td>1.55 – 1.54 (m)</td>
<td>1.57 (m)</td>
</tr>
<tr>
<td>3’</td>
<td>1.55 – 1.54 (m)</td>
<td>1.61 (m)</td>
</tr>
<tr>
<td>4’</td>
<td>3.55 (t, J 6.0 Hz)</td>
<td>3.56 (t, J 6.2 Hz)</td>
</tr>
<tr>
<td>4’-OH</td>
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<td>2.85 (br)</td>
</tr>
<tr>
<td>OMe</td>
<td>4.24 (s)</td>
<td>4.13 (s)</td>
</tr>
</tbody>
</table>

Table 4.5 $^{13}$C NMR spectroscopic data comparison between the synthesised oystemofoline (115) and the natural product.38

<table>
<thead>
<tr>
<th>Position</th>
<th>Synthesised $\delta_C$ (125 MHz, (CD$_3$)$_2$CO)</th>
<th>Natural product $\delta_C$ (75 MHz, (CD$_3$)$_2$CO)</th>
<th>Δ$\delta_C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33.8</td>
<td>33.7</td>
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</tr>
<tr>
<td>2</td>
<td>79.3</td>
<td>79.1</td>
<td>+ 0.2</td>
</tr>
<tr>
<td>3</td>
<td>83.6</td>
<td>82.9</td>
<td>+ 0.7</td>
</tr>
<tr>
<td>5</td>
<td>48.1</td>
<td>48.0</td>
<td>+ 0.1</td>
</tr>
<tr>
<td>6</td>
<td>27.2</td>
<td>33.9*</td>
<td>+ 0.1</td>
</tr>
<tr>
<td>7</td>
<td>51.2</td>
<td>51.0</td>
<td>+ 0.2</td>
</tr>
<tr>
<td>8</td>
<td>113.6</td>
<td>112.7</td>
<td>+ 0.9</td>
</tr>
<tr>
<td>9</td>
<td>48.3</td>
<td>48.1</td>
<td>+ 0.2</td>
</tr>
<tr>
<td>9a</td>
<td>61.6</td>
<td>61.5</td>
<td>+ 0.1</td>
</tr>
<tr>
<td>10</td>
<td>35.5</td>
<td>35.4</td>
<td>+ 0.1</td>
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<td>148.4</td>
<td>+ 1.4</td>
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<td>164.0</td>
<td>163.1</td>
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</tr>
<tr>
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<td>- 0.3</td>
</tr>
<tr>
<td>15</td>
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<td>169.5</td>
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</tr>
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<td>9.0</td>
<td>+ 0.1</td>
</tr>
<tr>
<td>17</td>
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<td>18.3</td>
<td>+ 0.1</td>
</tr>
<tr>
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<td>- 1.5</td>
</tr>
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<td>2’</td>
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<tr>
<td>OMe</td>
<td>59.7</td>
<td>59.6</td>
<td>+ 0.1</td>
</tr>
</tbody>
</table>

* These signals were missassigned in the original publication. The differences between some of these chemical shifts could be from errors in the original reported data.38
Figure 4.5 $^1$H NMR spectrum (500 MHz, (CD$_3$)$_2$CO) of semi-synthetic oxystemofoline (115).

Figure 4.6 $^{13}$C NMR spectrum (125 MHz, (CD$_3$)$_2$CO) of semi-synthetic oxystemofoline (115).
4.4.3 Synthesis of methoxystemofoline via a modified Julia olefination reaction

The modified Julia olefination reaction between the sulfone 178 and the aldehyde 160, using identical reaction conditions to those described in Scheme 4.7 for the synthesis of alkene 182, were unsuccessful (Scheme 4.12). There was no other product detected or isolated from this reaction (including the aldehyde 160) except for the recovered sulfone 178.

**Scheme 4.12** Synthesis of compound 170.

The semi-synthesis of methoxystemofoline (116) however (Scheme 4.13) was achieved using sulfone 181 under similar reaction conditions to that used for the synthesis of oxystemofoline (115). Eventhough the stereoselectivity was good ($E:Z > 99 : < 1$), the yield of the $E$-alkene 170 was very low (15%). Alkene 170 was characterized as the trans isomer from the $^1$H NMR signals at $\delta$ 5.75 (dt, $J_{1',2'} 15.5$ Hz, $J_{2',3'} 7.0$ Hz, H-2') and $\delta$ 5.60 (d, $J_{1',2'} 16.0$ Hz, H-1'). The selective hydrogenation of 170 over PdCl$_2$ under a H$_2$ atmosphere yielded methoxystemofoline (116) in 43% (Scheme 4.13). The specific optical rotation of synthesised 116 ($[\alpha]^{25}_D +247$ (c 0.29, MeOH)) was of the same sign but much larger in magnitude to that reported for the natural product ($[\alpha]^{6.21}_D +75.6$ (c 0.037, MeOH)). The $^1$H and $^{13}$C NMR data of 116 agreed closely to those of the natural product except for the incorrect assignment of the $^{13}$C NMR signals for C-6 and C-1' (Table 4.6). The full $^1$H NMR spectroscopic data for the natural product was not reported.
Scheme 4.13 Semi-synthesis of methoxystemofoline (116).

Table 4.6 \(^1\)H and \(^{13}\)C NMR spectroscopic data comparison between the synthesised methoxystemofoline (116) and the natural product.\(^{38}\)

<table>
<thead>
<tr>
<th>Position</th>
<th>Synthesised 116 (\delta_H) (500 MHz, CDCl(_3))</th>
<th>Natural product (\delta_H) (300 MHz, CDCl(_3))</th>
<th>Synthesised 116 (\delta_C) (125 MHz, CDCl(_3))</th>
<th>Natural product (\delta_C) (75 MHz, CDCl(_3))</th>
<th>(\Delta\delta_C)</th>
</tr>
</thead>
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</tr>
<tr>
<td></td>
<td>1.70 (d, (J 12.0) Hz)</td>
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</tr>
<tr>
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<td>4.26 (br s)</td>
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<td>78.6</td>
<td>+ 0.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>82.9</td>
<td>47.7</td>
<td>46.0</td>
<td>+ 1.7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.15 – 3.06 (m)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.01 – 2.96 (m)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>6</td>
<td>1.91 – 1.87 (m)</td>
<td>26.8</td>
<td>31.5*</td>
<td>+ 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.84 – 1.79 (m)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2.69 (d, (J 6.0) Hz)</td>
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<td>50.1</td>
<td>+ 0.1</td>
<td></td>
</tr>
<tr>
<td>8</td>
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<td>112.8</td>
<td>113.5</td>
<td>- 0.7</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1.84 – 1.79 (m)</td>
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<td></td>
</tr>
<tr>
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<td>61.0</td>
<td>+ 0.1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.15 – 3.06 (m)</td>
<td>34.7</td>
<td>36.2</td>
<td>- 1.5</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>148.6</td>
<td>149.8</td>
<td>- 1.2</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>128.0</td>
<td>128.3</td>
<td>- 0.3</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>163.0</td>
<td>163.5</td>
<td>- 0.5</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>98.7</td>
<td>98.8</td>
<td>- 0.1</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>169.8</td>
<td>170.5</td>
<td>- 0.7</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>2.07 (s, 3H)</td>
<td>9.3</td>
<td>8.7</td>
<td>+ 0.6</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>1.37 (d, (J 6.0) Hz, 3H)</td>
<td>1.44 (d, (J 6.7) Hz, 3H)</td>
<td>18.5</td>
<td>16.2</td>
<td>+ 2.3</td>
</tr>
<tr>
<td>1’</td>
<td>1.62 – 1.56 (m, 2H)</td>
<td>30.2</td>
<td>26.5*</td>
<td>- 1.3</td>
<td></td>
</tr>
<tr>
<td>2’</td>
<td>1.35 – 1.32 (m, 2H)</td>
<td>22.0</td>
<td>21.9</td>
<td>+ 0.1</td>
<td></td>
</tr>
<tr>
<td>3’</td>
<td>1.62 – 1.56 (m, 2H)</td>
<td>31.9</td>
<td>30.0</td>
<td>+ 1.9</td>
<td></td>
</tr>
<tr>
<td>4’</td>
<td>3.37 (t, (J 6.0) Hz, 2H)</td>
<td>72.7</td>
<td>72.4</td>
<td>+ 0.3</td>
<td></td>
</tr>
<tr>
<td>4’-OMe</td>
<td>3.32 (s, 3H)</td>
<td>3.31 (s, 3H)</td>
<td>58.8</td>
<td>58.4</td>
<td>+ 0.4</td>
</tr>
<tr>
<td>OMe</td>
<td>4.13 (s, 3H)</td>
<td>4.15 (s, 3H)</td>
<td>59.7</td>
<td>59.4</td>
<td>+ 0.3</td>
</tr>
</tbody>
</table>

* These signals were missassigned in the original publication.\(^{38}\)
Figure 4.7 $^1$H NMR spectrum (500 MHz, CDCl$_3$) of semi-synthetic methoxystemofoline (116).

Figure 4.8 $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of semi-synthetic methoxystemofoline (116).
4.5 Semi-synthesis of (1′R)- and (1′S)-hydroxystemofoline

The allylation of 160 was achieved using indium powder and allyl bromide with sonication following the procedure reported by Kong. This reaction gave an inseparable diastereomeric mixture of the alcohols 183 and 184 in a ratio of 65:35 in 68% yield (Scheme 4.14 (i)). These compounds were evident from ESIMS analysis (m/z 402 (100%) for [M+H]+ ion) and their NMR spectroscopic data. The 1H NMR spectrum showed signals for two diastereomeric compounds having terminal alkene resonances (δH ~5.98 – 5.82 (m) and ~5.19 – 5.16 (m)) and two resolved peaks corresponding to signals for H-2 (δH 4.48 (br s) and 4.40 (s)).

Scheme 4.14 Synthesis of alcohols 183 and 184.

<table>
<thead>
<tr>
<th>Reagents and conditions</th>
<th>183 : 184</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Indium powder, allyl bromide THF/ac. NH4Cl (52) sonication, 3 h</td>
<td>65 : 35</td>
<td>68 %</td>
</tr>
<tr>
<td>(ii) 1Ipc2Ball, THF, 0°C, 2 h</td>
<td>9 : 1</td>
<td>77 %</td>
</tr>
<tr>
<td>(iii) dIpc2Ball, THF, 0°C, 2 h</td>
<td>14 : 86</td>
<td>69 %</td>
</tr>
</tbody>
</table>

The configuration at C-1′ of 183 and 184 were assigned from their synthesis from 160 using the chiral borane reagents 1Ipc2Ball or 1Ipc2Ball. The 1Ipc2Ball reagent is generally stereoselective for the (R)-homoallylic alcohol product while the dIpc2Ball reagent gives the (S)-homoallylic alcohol product (Scheme 4.15). The reaction of 160 with 1Ipc2Ball gave a mixture of 183 and 184 in a ratio of 9:1 in a yield of 77% (Scheme 4.14 (ii)). When dIpc2Ball was employed a mixture of 183 and 184 was obtained in a ratio of 14:86 in a yield of 69% (Scheme 4.14 (iii)). These relative diastereoselectivities are understandable in terms of substrate and reagent controlled reactions. The substrate 160 slightly favours formation of the (1′R) diastereomer (183) (Scheme 4.14 (i)) and this preference is enhanced using 1Ipc2Ball. The use of dIpc2Ball is the mismatched case in which the substrate and reagent favour different diastereomers, (183 and 184, respectively). In this case, the overall diastereoselectivity is more modest.
While we could not separate compounds 183 and 184, we discovered that their acetate derivatives 185 and 186 could be separated by column chromatography. Acetylation of a 65:35 mixture of 183/184 gave, after separation, the acetates 185 and 186 in yields of 48% and 30%, respectively (Scheme 4.16). The acetate derivatives 185 and 186 were confirmed by ESIMS analysis (m/z 444 (100%) for [M+H]⁺ ion). The acetate groups of 185 and 186 were then removed under transesterification conditions using MeOH/K₂CO₃. However, methanol Michael addition products at C-11-C-12 were also formed which were detected by EIMS analysis (m/z 433 (100%) for M⁺ ion). Hydrolysis using LiOH in aqueous THF proved more successful in providing the desired pure alcohols 183 and 184 (Scheme 4.16). The ¹H NMR spectra of 183 and 184 were identical to the major products obtained from ¹Ipc₂Ball and ⁴Ipc₂Ball, respectively.

Scheme 4.16 Acetylation and hydrogenation to give alcohols 183 and 184.
The pure alcohols 183 and 184 were hydrogenated over Pd/C under a H₂ atmosphere to give (1’R)-hydroxyystemofoline (98) and (1’S)-hydroxyystemofoline (166), respectively (Scheme 4.17). The yields were relatively low (35-40%) due to the formation of side products arising from reduction of the C-11-C-12 double bond. Compounds 98 and 166 were purified by column chromatography and were identified by ESIMS analysis (m/z 404 (100%) for [M+H]+ ion) and from their NMR spectroscopic data. The ¹H NMR signals for H-2, H-7 and H-1’ of compound 98 (δH 4.47 (br s, H-2), 2.80 (br s, H-7) and 3.64 – 3.59 (m, H-1’)) distinguished it from compound 166 (δH 4.36 (br s, H-2), 3.00 (d, J 6.0 Hz, H-7) and 3.73 (d, J 9.5 Hz, H-1’)). Surprisingly, the NMR spectroscopic data of (1’R)-hydroxyystemofoline (98) (Table 4.6) matched closely to those of the alkaloid that our group had isolated previously from the root extracts of Stemona aphylla and which was incorrectly reported as (2’S)-hydroxyystemofoline. While a consistent difference of about 0.06 – 0.07 ppm was noted between the ¹H NMR data, the ¹³C NMR chemical shifts matched very closely (δ 0 – 0.1 ppm). We suspect that different referencing of the ¹H NMR spectra was responsible for these differences. This synthesis established (1’R)-hydroxyystemofoline (98) as a natural product and indicated that the earlier paper from our group required correction. The specific optical rotation of synthesised 98 ([α]²³D +299 (c 0.34, CHCl₃)) was of the same sign but much larger in magnitude to that of the natural product ([α]²⁵D +176 (c 0.07, CHCl₃)) while the specific optical rotation of synthesised 166 was [α]²³D +219 (c 0.58, CHCl₃).

Scheme 4.17 Hydrogenation of compounds 183 and 184.
Table 4.7 $^1$H and $^{13}$C NMR spectroscopic data comparison between the synthesised (1’R)-hydroxystemofoline (98) and the natural product.

<table>
<thead>
<tr>
<th>Position</th>
<th>Synthesised 98 $\delta$ (300 MHz, CDCl$_3$)</th>
<th>Natural product $\delta$ (500 MHz, CDCl$_3$)</th>
<th>Synthesised 98 $\delta$ (75 MHz, CDCl$_3$)</th>
<th>Natural product $\delta$ (125 MHz, CDCl$_3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.98 (d, $J$ 12.3 Hz)</td>
<td>1.92 (d, $J$ 12.5 Hz)</td>
<td>34.0</td>
<td>33.9</td>
</tr>
<tr>
<td></td>
<td>1.70 – 1.62 (m)</td>
<td>1.63 – 1.58 (m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4.47 (br s)</td>
<td>4.41 (br s)</td>
<td>75.6</td>
<td>75.6</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td>87.4</td>
<td>87.4</td>
</tr>
<tr>
<td>5</td>
<td>3.23 – 3.15 (m)</td>
<td>3.14 – 3.06 (m)</td>
<td>47.5</td>
<td>47.5</td>
</tr>
<tr>
<td></td>
<td>3.07 – 2.99 (m)</td>
<td>3.00 – 2.95 (m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.91 – 1.86 (m, 2H)</td>
<td>1.87 – 1.79 (m, 2H)</td>
<td>27.4</td>
<td>27.3</td>
</tr>
<tr>
<td>7</td>
<td>2.80 (br s)</td>
<td>2.73 (d, $J$ 4.5 Hz)</td>
<td>48.1</td>
<td>48.1</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td>112.6</td>
<td>112.6</td>
</tr>
<tr>
<td>9</td>
<td>1.91 – 1.86 (m)</td>
<td>1.87 – 1.79 (m)</td>
<td>48.2</td>
<td>48.1</td>
</tr>
<tr>
<td>9a</td>
<td>3.54 (br s)</td>
<td>3.48 (br s)</td>
<td>61.1</td>
<td>61.1</td>
</tr>
<tr>
<td>10</td>
<td>3.15 – 3.07 (m)</td>
<td>3.05 – 3.00 (m)</td>
<td>34.5</td>
<td>34.5</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td>148.2</td>
<td>148.2</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td>128.2</td>
<td>128.1</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td>162.9</td>
<td>162.8</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td>98.8</td>
<td>98.8</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td>169.8</td>
<td>169.8</td>
</tr>
<tr>
<td>16</td>
<td>2.07 (s, 3H)</td>
<td>2.00 (s, 3H)</td>
<td>9.3</td>
<td>9.3</td>
</tr>
<tr>
<td>17</td>
<td>1.38 (d, $J$ 6.6 Hz, 3H)</td>
<td>1.31 (d, $J$ 6.6 Hz, 3H)</td>
<td>18.5</td>
<td>18.4</td>
</tr>
<tr>
<td>1’</td>
<td>3.64 – 3.59 (m)</td>
<td>3.56 – 3.53 (m)</td>
<td>67.9</td>
<td>67.9</td>
</tr>
<tr>
<td>2’</td>
<td>1.55 – 1.47 (m, 2H)</td>
<td>1.46 – 1.42 (m, 2H)</td>
<td>34.2</td>
<td>34.2</td>
</tr>
<tr>
<td>3’</td>
<td>1.70 – 1.62 (m)</td>
<td>1.63 – 1.58 (m)</td>
<td>20.2</td>
<td>20.1</td>
</tr>
<tr>
<td></td>
<td>1.44 – 1.42 (m)</td>
<td>1.40 – 1.34 (m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4’</td>
<td>0.97 (t, $J$ 7.2 Hz, 3H)</td>
<td>0.90 (t, $J$ 7.5 Hz, 3H)</td>
<td>14.3</td>
<td>14.2</td>
</tr>
<tr>
<td>OMe</td>
<td>4.14 (s, 3H)</td>
<td>4.08 (s, 3H)</td>
<td>59.0</td>
<td>59.0</td>
</tr>
</tbody>
</table>
Figure 4.9 $^1$H NMR spectrum (300 MHz, CDCl$_3$) of semi-synthetic (1'$_R$)-hydroxystemofoline (98).

Figure 4.10 $^{13}$C NMR spectrum (75 MHz, CDCl$_3$) of semi-synthetic (1'$_R$)-hydroxystemofoline (98).
4.6 Synthesis of other derivatives

4.6.1 Synthesis of the A, B, C ring core structure

For SAR studies, the synthesis of the lactone 188 having only the A, B, C ring core structure of stemofoline was desirable. Dihydroxylation of stemofoline (97) using catalytic K$_2$OsO$_4$.2H$_2$O and stoichiometric NMO (Upjohn conditions), gave the diol 187 in 56% yield (Scheme 4.18). Compound 187 was detected by ESIMS analysis ($m/z$ 422 (100%) for [M+H]$^+$ ion) and was confirmed as a single diastereomer by NMR analysis. The $^{13}$C NMR spectrum of 187 showed signals for the new tertiary carbinol carbons at C-11 and C-12 at $\delta$ 109.1 and 104.1 in CD$_3$OD, respectively. The reaction scheme for an Upjohn dihydroxylation reaction which uses NMO as a catalytic co-oxidant is shown in Scheme 4.19.\textsuperscript{53}

![Scheme 4.18 Synthesis of the A, B, C ring core structure (188).](image)

![Scheme 4.19 Reaction scheme for an Upjohn dihydroxylation reaction.\textsuperscript{53}](image)
Oxidative cleavage of the diol 187 followed a procedure reported by Baird\textsuperscript{34} using freshly prepared NaIO\textsubscript{4} on silica gel. This reaction gave the lactone 188 having the A, B, C ring core structure of stemofoline (Scheme 4.18). Compound 188 was confirmed by ESIMS analysis (\textit{m/z} 278 (100\%) for \([\text{M}+\text{H}]^+\) ion). The \textsuperscript{1}H NMR spectrum of 188 showed a signal for the C-12 methyl group at \(\delta_H 1.26\) (d, \(J 7.0\) Hz, 3H) which corresponded to a signal of the C-17 methyl in diol 187 (\(\delta_H 1.03\) (d, \(J 6.0\) Hz, 3H)). As expected, signals for the methoxy group and C-16 methyl group were not observed in the NMR spectrum of 188 (Table 4.8). The \textsuperscript{13}C NMR signal of C-11 in 188 shifted significantly downfield to \(\delta 178.5\) compared to that of the corresponding carbon in 187 at \(\delta 101.9\) (Figure 4.12).

**Table 4.8** \textsuperscript{1}H NMR spectroscopic data comparison between the synthesised diol 187 and the A, B, C ring core structure 188.

<table>
<thead>
<tr>
<th>Position</th>
<th>Diol 187 (\delta_H (500\text{ MHz, CDCl}_3))</th>
<th>Core structure 188 (\delta_H (500\text{ MHz, CDCl}_3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.98 (d, (J 12.5) Hz)</td>
<td>1.98 (d, (J 12.5) Hz)</td>
</tr>
<tr>
<td>1</td>
<td>1.70 (d, (J 12.5) Hz)</td>
<td>1.75 (dt, (J_{1,1} 12.5) Hz, (J_{1,2} 3.5) Hz)</td>
</tr>
<tr>
<td>2</td>
<td>4.25 (br s)</td>
<td>4.32 (br s)</td>
</tr>
<tr>
<td>5</td>
<td>3.14 – 3.08 (m)</td>
<td>3.19 – 3.12 (m)</td>
</tr>
<tr>
<td>6</td>
<td>3.02 – 2.96 (m)</td>
<td>3.05 – 2.98 (m)</td>
</tr>
<tr>
<td>7</td>
<td>1.86 -1.81 (m)</td>
<td>1.92 – 1.88 (m)</td>
</tr>
<tr>
<td>8</td>
<td>1.79 – 1.73 (m)</td>
<td>1.84 – 1.78 (m)</td>
</tr>
<tr>
<td>10</td>
<td>2.51 (d, (J 6.0) Hz)</td>
<td>2.65 (d, (J 6.0) Hz)</td>
</tr>
<tr>
<td>9</td>
<td>1.91 (d, (J 10.0) Hz)</td>
<td>1.96 – 1.92 (m)</td>
</tr>
<tr>
<td>9a</td>
<td>3.45 (br s)</td>
<td>3.41 (br s)</td>
</tr>
<tr>
<td>10</td>
<td>2.73 – 2.66 (m)</td>
<td>2.77 (dq, (J_{9,10} 11.5) Hz, (J_{10,17} 7.5) Hz)</td>
</tr>
<tr>
<td>12</td>
<td>1.26 (d, (J 7.0) Hz, 3H)</td>
<td>1.28 – 1.20 (m)</td>
</tr>
<tr>
<td>16</td>
<td>2.01 (s, 3H)</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>1.03 (d, (J 6.5) Hz, 3H)</td>
<td>1.62 – 1.50 (m, 2H)</td>
</tr>
<tr>
<td>1'</td>
<td>1.54 (d, (J 9.5) Hz, 2H)</td>
<td>1.46 – 1.38 (m)</td>
</tr>
<tr>
<td>2'</td>
<td>1.42 -1.36 (m)</td>
<td>1.35 (q, (J 7.0) Hz)</td>
</tr>
<tr>
<td>3'</td>
<td>1.28 – 1.20 (m)</td>
<td>0.92 (t, (J 7.0) Hz, 3H)</td>
</tr>
<tr>
<td>4'</td>
<td>0.90 (t, (J 7.0) Hz, 3H)</td>
<td>1.13 (s, 2H)</td>
</tr>
<tr>
<td>OH</td>
<td>4.12 (s, 3H)</td>
<td>-</td>
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<tr>
<td>OMe</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 4.11 $^1$H NMR spectrum (500 MHz, CDCl$_3$) of the A, B, C ring core structure (188).

Figure 4.12 $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of the A, B, C ring core structure (188).
4.6.2 Synthesis of alcohol derivatives via the Wittig reaction

The Wittig reaction was utilized for the synthesis of enal 189a from aldehyde 160 using (triphenylphosphoranylidene)acetaldehyde (2 equiv.) based on a modification of the method reported by Izquierdo54 (Scheme 4.20). This reaction produced a mixture of the expected enal 189a, the dienal 189b and the trienal 189c as a result of consecutive Wittig reactions (Scheme 4.21). These aldehydes were indicated from analysis of their 1H NMR spectra which indicated their terminal aldehyde groups at δ ~ 9.63 – 9.57 ppm and their alkene groups at δ ~ 7.17 – 6.15 ppm. The aldehydes 189a-c were difficult to separate from each other and the triphenylphosphine oxide by-product. Thus, the mixture was reduced with NaBH₄/MeOH to give a mixture of the corresponding alcohols 190a-c. This mixture was then separated by PTLC to give pure samples of 190a-c which confirmed the formation of the aldehydes 189a-c in the previous step. The 1H NMR spectroscopic data of each alcohol product showed characteristic alkene group resonances (Table 4.9). These compounds were also specified by ESIMS analysis (m/z 388 (100%) for [M+H]⁺ ion of 190a, m/z 414 (100%) for [M+H]⁺ ion of 190b, m/z 440 (100%) for [M+H]⁺ ion of 190c).

![Scheme 4.20 Synthesis of alcohols 190a, 190b and 190c.](attachment:scheme_4.20.png)
**Scheme 4.21** Proposed mechanism for the Wittig reaction.

The enol 190a was identified as a *trans*-alkene from the coupling constant between H-1’ and H-2’ (*J*<sub>1’,2’</sub> 15.3 Hz). The dienol 190b was characterized as a *trans-trans*-diene from the coupling constants for *J*<sub>1’,2’</sub> 15.0 Hz and *J*<sub>3’,4’</sub> 15.0 Hz. For trienol 190c, we could only measure *J*<sub>3’,6’</sub> = 14.5 Hz and not *J*<sub>1’,2’</sub> and *J*<sub>3’,4’</sub> due to peak overlap. However because 189c must have arisen from dienal 189b, we have assigned the *trans-trans-trans*-triene structures to 189c and 190c.

**Table 4.9** ¹H NMR spectroscopic data comparison between alcohols 190a-c.

<table>
<thead>
<tr>
<th>Position</th>
<th>Enol 190a &lt;br&gt;δ&lt;sub&gt;H&lt;/sub&gt; (500 MHz, CDCl₃)</th>
<th>Dienol 190b &lt;br&gt;δ&lt;sub&gt;H&lt;/sub&gt; (500 MHz, CDCl₃)</th>
<th>Trienol 190c &lt;br&gt;δ&lt;sub&gt;H&lt;/sub&gt; (500 MHz, CDCl₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.96 (d, <em>J</em> 12.0 Hz)</td>
<td>1.96 (d, <em>J</em> 12.5 Hz)</td>
<td>1.96 (d, <em>J</em> 12.0 Hz)</td>
</tr>
<tr>
<td>2</td>
<td>1.78 (d, <em>J</em> 12.0 Hz)</td>
<td>1.78 (d, <em>J</em> 12.5 Hz)</td>
<td>1.78 (d, <em>J</em> 12.5 Hz)</td>
</tr>
<tr>
<td>3</td>
<td>3.13 – 3.07 (m)</td>
<td>3.14 – 3.06 (m)</td>
<td>3.14 – 3.06 (m)</td>
</tr>
<tr>
<td>4</td>
<td>3.02 – 2.97 (m)</td>
<td>3.03 – 2.97 (m)</td>
<td>3.04 – 2.88 (m)</td>
</tr>
<tr>
<td>5</td>
<td>1.92 – 1.86 (m, 2H)</td>
<td>1.90 – 1.82 (m, 2H)</td>
<td>1.86 – 1.77 (m, 2H)</td>
</tr>
<tr>
<td>6</td>
<td>2.88 (d, <em>J</em> 5.5 Hz)</td>
<td>2.88 (d, <em>J</em> 5.5 Hz)</td>
<td>2.88 (d, <em>J</em> 5.5 Hz)</td>
</tr>
<tr>
<td>7</td>
<td>1.86 – 1.82 (m)</td>
<td>1.90 – 1.82 (m)</td>
<td>1.91 – 1.86 (m)</td>
</tr>
<tr>
<td>8</td>
<td>3.51 (br s)</td>
<td>3.52 (br s)</td>
<td>3.52 (br s)</td>
</tr>
<tr>
<td>9</td>
<td>3.13 – 3.07 (m)</td>
<td>3.14 – 3.06 (m)</td>
<td>3.14 – 3.06 (m)</td>
</tr>
<tr>
<td>10</td>
<td>2.07 (s, 3H)</td>
<td>2.08 (s, 3H)</td>
<td>2.07 (s, 3H)</td>
</tr>
<tr>
<td>11</td>
<td>1.38 (d, <em>J</em> 6.5 Hz, 3H)</td>
<td>1.38 (d, <em>J</em> 6.5 Hz, 3H)</td>
<td>1.38 (d, <em>J</em> 6.5 Hz, 3H)</td>
</tr>
<tr>
<td>12</td>
<td>5.81 (d, <em>J</em> 15.3 Hz)</td>
<td>5.76 (d, <em>J</em> 14.5 Hz)</td>
<td>5.77 (d, <em>J</em> 15.0 Hz)</td>
</tr>
<tr>
<td>13</td>
<td>5.94 (dt, <em>J</em> 15.3 Hz, 5.0 Hz)</td>
<td>6.30 (dd, <em>J</em> 15.0 Hz, 10.5 Hz)</td>
<td>6.38 – 6.33 (m)</td>
</tr>
<tr>
<td>14</td>
<td>4.19 (br s, 2H)</td>
<td>6.34 (dd, <em>J</em> 15.0 Hz, 10.5 Hz)</td>
<td>6.26 – 6.23 (m)</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>5.87 (dt, <em>J</em> 14.5 Hz, 5.5 Hz)</td>
<td>6.26 – 6.23 (m)</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
<td>4.20 (d, <em>J</em> 6.0 Hz, 2H)</td>
<td>6.31 – 6.26 (m)</td>
</tr>
<tr>
<td>17</td>
<td>-</td>
<td>5.87 (dt, <em>J</em> 14.5 Hz, 5.5 Hz)</td>
<td>6.21 (d, <em>J</em> 5.5 Hz, 2H)</td>
</tr>
<tr>
<td>18</td>
<td>1.70 (br s)</td>
<td>1.59 (br s)</td>
<td>1.75 (br s)</td>
</tr>
<tr>
<td>OMe</td>
<td>4.14 (s, 3H)</td>
<td>4.14 (s, 3H)</td>
<td>4.14 (s, 3H)</td>
</tr>
</tbody>
</table>
Figure 4.13 $^1$H NMR spectrum (500 MHz, CDCl$_3$) of the enol 190a.

Figure 4.14 $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of the enol 190a.
Figure 4.15 $^1$H NMR spectrum (500 MHz, CDCl$_3$) of the dienol 190b.

Figure 4.16 $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of the dienol 190b.
Figure 4.17 $^1$H NMR spectrum (500 MHz, CDCl$_3$) of the trienol 190c.

Figure 4.18 $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of the trienol 190c.
4.6.3 Synthesis of (11Z)-1′,2′-didehydrostemofoline-N-oxide

In order to test the AChE inhibitory activity of the natural product, (11Z)-1′,2′-didehydrostemofoline-N-oxide (105), we prepared it from oxidation of (11Z)-1′,2′-didehydrostemofoline (104). A synthesis of (11Z)-1′,2′-didehydrostemofoline-N-oxide (105) from (11Z)-1′,2′-didehydrostemofoline (104) was reported earlier by our group (Scheme 4.22 (a)).31 Because of the long reaction time, an attempt to improve this reaction was made. Murahashi reported that Na$_2$WO$_4$·2H$_2$O could be used to catalyze the oxidation of secondary amines with hydrogen peroxide.55 Thus, the oxidation reaction of 104 was repeated in the presence of Na$_2$WO$_4$·2H$_2$O which proved a more efficient method with a better yield (75%) and shortened reaction time (16 h versus 7 days) (Scheme 4.22 (b)). The $^1$H and $^{13}$C NMR of 105 were in good agreement to those of the natural product.31

![Scheme 4.22](image)

Scheme 4.22 Synthesis of (11Z)-1′,2′-didehydrostemofoline-N-oxide (105).

4.7 Conclusions

The aldehyde (160) was synthesised from (11Z)-1′,2′-didehydrostemofoline (104) as a key scaffold for various reactions to prepare rare Stemona alkaloids and derivatives. A modified Julia olefination reaction on the aldehyde 160 was a key step for preparation of oxystemofoline (115) and methoxystemofoline (116) using the sulfones 176 and 181, respectively. The synthesis of compounds 115 and 116 allowed reassignment of the $^{13}$C NMR signals for C-6 and C-1′ from those reported for the natural products. Allylation of 160 under indium-mediated conditions or using chiral allylborane reagents provided (1′R) and (1′S)-homoallylic alcohol
products that lead to (1'R)-hydroxystemofoline (98) and (1'S)-hydroxystemofoline (166), respectively. Surprisingly, the synthesised compound 98 proved to be identical to a natural product that was later isolated from the root extracts of *Stemona aphylla*.\(^{51}\) The Wittig reaction of 160 with (triphenylphosphoranylidene)acetaldehyde was observed to be uncontrollable as this reaction provided a mixture of three aldehyde products formed from consecutive Wittig reactions. The A, B, C ring core structure 188 was also prepared in two steps via an Upjohn dihydroxylation reaction of stemofoline (97) followed by an oxidative cleavage of the corresponding C-11, C-12 diol. Also the oxidation reaction of 104 to its N-oxide 105 was improved by using Na\(_2\)WO\(_4\)·2H\(_2\)O as a catalyst.

The synthesised compounds in this chapter were prepared in sufficient amounts for biological testing as AChE inhibitors. Some compounds were selected for testing as P-gp inhibitors for their potential applications with the anti-cancer drugs, paclitaxel and vinblastine, for the treatment of multi-drug resistant (MDR) cancers. These biological studies will be described in Chapter 7.
CHAPTER 5 SEMI-SYNTHESIS OF C-3 AMINOMETHYL STEMOFOLINE ANALOGUES

5.1 Proposed synthetic plans

To allow further SAR studies on our compounds as AChE inhibitors, a small library of amine derivatives was prepared using the general procedure shown in Scheme 5.1 (a-c). Our synthetic plan was to use the aldehyde 160 as a starting material and perform reductive amination reactions to introduce various amino side chains at C-3 of the stemofoline skeleton to give the amines A (Scheme 5.1 (a)). Secondary amines A ($R^2 = H$) could further undergo methylation (Scheme 5.1 (b)) to prepare the tertiary amines B or may undergo a carbamylation reaction (Scheme 5.1 (c)) to provide the carbamate protected amines C. The synthesis of a guanidine derivative will also be described in this chapter.

Scheme 5.1 Proposed synthetic plans.
5.2 Reductive amination reactions

The reductive amination reactions of the aldehyde 160 were conducted using reaction conditions similar to those reported by Abdel-Magid. Sodium triacetoxyborohydride (NaBH(OAc)₃) was employed as a mild reducing agent which selectively reduces iminium ions over aldehydes and ketones. The reaction scheme is shown in Scheme 5.2. This reaction involves initial formation of a carbinol amine followed by dehydration and protonation, respectively, to give an iminium ion. The reducing agent then reduces the iminium ion to the alkylated amine product.

![Scheme 5.2 Reaction scheme for reductive amination reaction.](image)

In our first attempt to prepare an amine derivative, methylamine was selected as the reacting amine in order to give the desired secondary amine product 191 (Scheme 5.3). This reaction was performed as a one-pot reaction. A solution of the aldehyde 160 was treated with methylamine in methanol solution, NaBH(OAc)₃ and 0.1% AcOH in DCE at rt for 24 h. After purification by column chromatography, the amine product 191 was obtained in 60% yield. The structure 191 was confirmed from its ESIMS (m/z 375 (100%) for the [M+H]⁺ ion) and NMR spectroscopic data. The characteristic ¹H NMR signals of compound 191 were at δ 2.70 (s, 2H) for H-1’ and δ 2.45 (s, 3H) for the N-methyl group (Figure 5.1). While the ¹³C NMR spectrum showed signals for C-1’ at δ 52.6 and the N-methyl group at δ 37.2.

![Scheme 5.3 Synthesis of the amine 191.](image)
Figure 5.1 $^1$H NMR spectrum (500 MHz, CDCl$_3$) of the amine 191.

Figure 5.2 $^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of the amine 191.
The general reductive amination reaction procedure was also applied to 160 using different amines to provide the desired products 192 – 207 in yields ranging from 25 – 93 % (Table 5.1). The less nucleophilic amine, aniline, gave the lowest yield (Table 5.1, entry 9, 25% of amine product 200). The reactions involving dimethylamine (Table 5.1, entry 1) and 2-(methylamino)ethylamine (Table 5.1, entry 4) were also low yielding (39% and 28%, respectively). Both primary and secondary amines were generally effective. The known alcohol 208 was also obtained from some of these reactions including those reactions in entries 4 and 9 of Table 5.1, but not entry 1. In an earlier report, the alcohol 208 was prepared by reducing the aldehyde 160 with NaBH₄ (Scheme 5.4). The amines 192 – 207 were characterized by their NMR spectroscopic data and MS analysis. The ¹H NMR resonances for H-1’ were observed between δ 2.87 – 2.35 ppm except for those of 200 which were more downfield at δ 3.45 – 3.16 ppm. In some cases the H-1’ protons resonated as a singlet while in others these protons were diastereotopic and resonated as two mutually coupled doublets. The ¹³C NMR resonances for C-1’ for the secondary amine products were observed between δ 44.2 – 52.6 ppm while those for tertiary amine products, the amines 192, 197, 198 and 199, were further downfield between δ 58.4 – 59.9 ppm.

Scheme 5.4 Reduction reaction of the aldehyde 160.³⁴
Table 5.1 Reductive amination reactions of the aldehyde 160.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amine component</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dimethylamine</td>
<td>192; R = N(_2); Me</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>Isopropylamine</td>
<td>193; R = N(_2); Me</td>
<td>78</td>
</tr>
<tr>
<td>3</td>
<td>Allylamine</td>
<td>194; R = N(_2); Me</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>2-(Dimethylamino)ethylamine</td>
<td>195; R = N(_2); Me</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>Ethanolamine</td>
<td>196; R = N(_2); Me</td>
<td>48</td>
</tr>
<tr>
<td>6</td>
<td>Morpholine</td>
<td>197; R = N(_2); Me</td>
<td>63</td>
</tr>
<tr>
<td>7</td>
<td>Ethyl-1-piperazinecarboxylate</td>
<td>198; R = N(_2); Me</td>
<td>78</td>
</tr>
<tr>
<td>8</td>
<td>1-Methylpiperazine</td>
<td>199; R = N(_2); Me</td>
<td>64</td>
</tr>
<tr>
<td>9</td>
<td>Aniline</td>
<td>200; R = N(_2); Me</td>
<td>25</td>
</tr>
<tr>
<td>10</td>
<td>N-Benzylamine</td>
<td>201; R = N(_2); Me</td>
<td>86</td>
</tr>
<tr>
<td>11</td>
<td>(Aminomethyl)cyclopropane</td>
<td>202; R = N(_2); Me</td>
<td>60</td>
</tr>
<tr>
<td>12</td>
<td>Cyclopropylamine</td>
<td>203; R = N(_2); Me</td>
<td>93</td>
</tr>
<tr>
<td>13</td>
<td>Cyclopentylamine</td>
<td>204; R = N(_2); Me</td>
<td>90</td>
</tr>
<tr>
<td>14</td>
<td>Cyclohexylamine</td>
<td>205; R = N(_2); Me</td>
<td>86</td>
</tr>
<tr>
<td>15</td>
<td>(S)-1-Aminoethylbenzene</td>
<td>206; R = N(S); Me</td>
<td>81</td>
</tr>
<tr>
<td>16</td>
<td>(R)-1-Aminoethylbenzene</td>
<td>207; R = N(R); Me</td>
<td>62</td>
</tr>
</tbody>
</table>
5.3 Methylation reactions

To prepare the tertiary amine product 209, a procedure similar to the previous reductive amination reaction was used (Scheme 5.5). Using formaldehyde as the aldehyde component and the secondary amine 194, the tertiary amine 209 was obtained in 94% yield. The amine 209 was confirmed from its NMR spectroscopic data. The $^1$H and $^{13}$C NMR signals corresponding to the N-methyl group were observed at $\delta_H$ 2.30 (s, 3H) and $\delta_C$ 44.2 ppm, respectively. The $^1$H NMR resonance of H-1’ in 209 shifted to $\delta$ 2.53 (d, $J$ 13.8 Hz) and 2.42 (d, $J$ 13.8 Hz) while these protons in 194 resonated at $\delta$ 2.71 (ABq, $J$ 10.0 Hz). The $^{13}$C NMR signal for C-1’ of 209 was at $\delta$ 56.9 ppm while that of 194 was at $\delta$ 49.5 ppm. The ESIMS showed a molecular ion at $m/z$ 415 (100%) for the [M+H]$^+$ ion.

![Scheme 5.5](image)

Scheme 5.5 Synthesis of the tertiary amine 209.

The amine 191 was also treated under similar reaction conditions to provide the tertiary amine 192 (Scheme 5.6), however the isolated yield was low (26%) and recovered starting material 191 was also isolated (22%). The amine 192 was confirmed from its ESIMS analysis ($m/z$ 389 (100%) for the [M+H]$^+$ ion) and its NMR spectroscopic data.

![Scheme 5.6](image)

Scheme 5.6 Synthesis of the tertiary amine 192.
5.4 Carbamylation reactions

The carbamate derivatives of the amines 203 – 205 were prepared by treating these amines with ethyl chloroformate in the presence of the base NaHCO₃ in THF solution at 0 °C for 3 h (Scheme 5.7). The yields of the carbamates 210 – 212 ranged from 56 – 80%. The carbamate 210 was obtained in 76% yield after purification on column chromatography. Compound 210 was evident from ESIMS analysis (m/z 472.6 (100%) for the [M+H]+ ion) and was confirmed by NMR spectroscopic analysis. The characteristic ¹H NMR signals corresponding to the ethoxy group were observed at δ 4.16 (q, J 7.5 Hz, 2H) and 1.26 (t, J 7.5 Hz, 3H). The ¹H NMR signals for H-1’ of 210 shifted significantly downfield (δ 3.62 (d, J 13.0 Hz) and 3.29 (d, J 13.0 Hz)) compared to those of the amine 203 (δ 2.81 (d, J 12.0 Hz) and 2.75 (d, J 12.0 Hz)). The carbamates 211 and 212 were also fully characterized by NMR and MS analysis.

Scheme 5.7 Synthesis of the amines 210, 211 and 212.
5.5 Synthesis of a guanidine derivative

The guanidine derivative 214 was prepared in two steps as shown in Scheme 5.8. The amine 191 was condensed with guanidine triflate to give the Boc-protected product 213 in 54% yield. The ESIMS showed a peak at \( m/z \) 617 (100%) for the [M+H]⁺ ion while the \(^1\)H NMR showed signals corresponding to the two equivalent Boc groups at \( \delta \) 1.47 (s, 18H). The \(^1\)H NMR signals for H-1’ in 213 were observed more downfield at \( \delta \) 3.73 (apparent broad d, \( J \) 13.5 Hz, 2H). To deprotect the Boc groups, compound 213 was treated with TFA in CH₂Cl₂ at rt for 3 h and then was treated with HCl in ether to form the HCl salt 214. Compound 214 was confirmed by ESIMS (\( m/z \) 417(100%) for the [M+H]⁺ ion) and NMR analysis. The \(^1\)H NMR spectrum was very broad and difficult to analyse but the \(^{13}\)C NMR spectrum showed a characteristic peak at \( \delta \) 160.0 which corresponded to the sp² carbon of the guanidine group.
5.6 Conclusions

The reductive amination reaction of the aldehyde 160 gave 17 amine derivatives 191 – 207 in yields ranging from 25 – 93%. Some secondary amines were selected to prepare their tertiary amine and carbamate derivatives. The guanidine derivative 214 was also prepared as its HCl salt for AChE inhibitory testing (Chapter 7).
CHAPTER 6 SEMI-SYNTHESIS OF TRIAZOLE AND 3,5-ISOXAZOLE SIDE CHAIN STEMOFOLINE ANALOGUES

6.1 Proposed synthetic plans

In this study, our aims were to prepare C-3 triazole and oxazole stemofoline derivatives for SAR studies using 1,3-dipolar cycloaddition reactions or click chemistry. This reaction was chosen as it allows the rapid synthesis of a diverse set of compounds for biological testing. The synthetic plans are shown in Scheme 6.1 (a-c). The alkyne 215 that was required for the click chemistry was expected to be prepared from the aldehyde 160 (Scheme (a)) using the Bestmann-Ohira reagent. The click reaction of the alkyne 215 was expected to provide the triazoles A or the oxazoles B depending on the 1,3-dipoles used (Scheme 6.1 (b)). Moreover, Sonogashira coupling of the alkyne 213 will provide the phenyl alkyne analogue 216 for biological testing (Scheme 6.1 (c)).

![Scheme 6.1 Proposed synthetic plans.](image-url)
6.2 Preparation of the alkyne 215

6.2.1 Synthesis of the Bestmann-Ohira reagent 217

The Bestmann-Ohira reagent, dimethyl-1-diazo-2-oxopropylphosphonate (217), was prepared in a one step process by a diazo transfer reaction between tosyl azide and dimethyl-2-oxopropylphosphonate using the reported procedure by Ghosh (Scheme 6.2 (a)). Tosyl azide was prepared from tosyl chloride using a procedure from the same literature (Scheme 6.2 (b)). The identity of compound 217 was confirmed by ESIMS (m/z 193 (100%) for the [M+H]+ ion) and NMR analysis.

6.2.2 Synthesis of the alkyne 215

A general procedure to synthesise alkynes from aldehydes were reported by Ohira and Bestmann. This procedure required the Bestmann-Ohira reagent 217 which was not commercially available. An improved one-pot procedure which generates the Bestmann-Ohira reagent in situ followed by addition of the aldehyde, has been reported. The one-pot procedure is more convenient but not as effective as the original procedure in which the Bestmann-Ohira reagent is prepared separately. The mechanism of this reaction is similar to the Seyferth-Gilbert homologation (via intermediates D – F) except for the formation of the dimethyl(diazomethyl)phosphonate anion D (Scheme 6.3).
The aldehyde 160 was initially treated with the reagent 217 in the presence of K₂CO₃ in methanol but this method was unsuccessful in yielding the alkyne 215. This reaction resulted in a number of unidentified products.

We then used a mixture of methanol and acetonitrile (1:3) as the solvent following the procedure reported by Jørgensen. This procedure (Scheme 6.4) provided the alkyne 215 in good yield (76%). The alkyne 215 was identified by ESIMS (m/z 356 (100%) for the [M+H]⁺ ion) and NMR analysis. The ¹H NMR signal of the characteristic terminal alkyne proton was seen at δ 2.54 (s, H-2') while that of the aldehyde was absent (Figure 6.1). The ¹³C NMR spectrum showed alkyne resonances at δ 75.2 (C-1') and 74.6 (C-2'). The IR spectra of the alkyne 215 showed νmax 3249 cm⁻¹, for the –C≡C–H stretch and 2366 cm⁻¹, for the –C≡C– stretch. The alkyne 215 was crystallized from CH₂Cl₂. Its single crystal X-ray structure is shown in Figure 6.2.
Figure 6.1 $^1$H NMR spectrum (500 MHz, CDCl$_3$) of the alkyne 215.

Figure 6.2 The single crystal X-ray structure of the alkyne 215.
6.3 Click chemistry of the alkyne 215

The term ‘click chemistry’ was coined by Sharpless to describe reactions which generate substances by joining small units together with heteroatom links (C-X-C).\textsuperscript{63} Sharpless stated that reactions that fit the criteria must be modular, wide in scope, high yielding, generate only inoffensive by-products, require no chromatographic isolation, stereospecific, simple to perform and using easily removed solvents. Click chemistry comprises a number of carbon-heteroatom bond forming reactions for example, 1,3-dipolar cycloaddition reactions, Diels-Alder cycloaddition reactions, nucleophilic ring-opening reactions, non-aldol carbonyl chemistry and carbon-carbon multiple bond additions. The most common click reaction is the Huisgen 1,3-dipolar cycloaddition of alkynes and azides to yield 1,2,3-triazoles.\textsuperscript{64} The kinetic stability of alkynes and azides is directly responsible for their slow rates of cycloaddition which usually gives a mixture of the 1,4- and 1,5-regioisomers (Scheme 6.5).\textsuperscript{65} Under Cu\textsuperscript{1}-catalyzed reaction conditions, the reactivity and regioselectivity of these reactions using terminal alkynes is greatly improved affording the 1,4-regioisomer exclusively.

\begin{center}
\includegraphics[width=0.5\textwidth]{_scheme_6_5.png}
\end{center}

\textbf{Scheme 6.5} Products of thermal 1,3-cycloadditions.\textsuperscript{65}
The proposed mechanistic scheme for the Cu\(^{1}\)-catalyzed terminal alkyne alkyne-azide coupling was reported by van Maarseveen as shown in Scheme 6.6.\(^{65}\) In the first step, an insertion of Cu\(^{1}\) into the terminal alkyne gives a Cu\(^{1}\) acetylide species (I and J) via the \(\pi\) complex H. The active copper acetylide species J replaces one of its ligands by azide to give a copper acetylide-azide complex K followed by the cyclization to a metallocycle L. The azide functionality as shown in the complex K is believed to be activated by the second copper atom while the \(\pi\) complexation of the acetylide to another copper atom may occur. Binding of the Cu to N\(^{1}\) of the azide component rather than at N\(^{3}\) is favoured sterically by avoiding adverse steric interactions between R\(^{1}\) and R\(^{2}\). This ensures formation of the 1,4-regioisomeric triazole product. Then the ring contraction of L leads to the alkyne-azide coupling product M and N, respectively. After protonation of the triazole-copper derivative N, the 1,4-regioisomeric product is obtained.

Scheme 6.6 Proposed outline of species involved in the catalytic cycle.\(^{65}\)

To examine the click reaction of the alkyne 215, it was treated under one-pot click reaction conditions with microwave assisted heating using the procedure reported by Van der Eycken\(^{66}\) (Scheme 6.5). An azide species was generated \textit{in situ} by using sodium azide and benzyl bromide. After purification by column chromatography, the triazole 218 was obtained in a yield of 23%. The yield was low
possibly due to the high temperature which may have caused the decomposition of both starting materials and product. The structure of triazole 218 was confirmed from its ESIMS (m/z 489 (100%) for the [M+H]⁺ ion) and NMR spectra. The ¹H NMR resonance for the proton of the 1,2,3-triazole ring was observed as a singlet at δ 7.34 (s, H-5′) while signals for the benzyl group were at δ 7.32-7.28 (m, 3H, Ph), 7.22-7.20 (m, 2H, Ph) and 5.44 (ABq, J 2.5 Hz, 2H, H-1′′) (Figure 6.3). The ¹³C NMR resonances of C-4′ and C-5′ of the 1,2,3-triazole were observed at δ 134.5 and 120.9, respectively.

**Scheme 6.7** Synthesis of the triazole 218.

**Figure 6.3** ¹H NMR spectrum (500 MHz, CDCl₃) of the 1,2,3-triazole 218.
A classical reaction procedure which required phenyl azide\(^6\) was also performed to prepare the triazole 219 (Scheme 6.8). Sodium ascorbate was added to reduce Cu\(^{II}\) to Cu\(^I\). After purification by column chromatography, the triazole 219 was obtained in 33% yield. Its structure was confirmed from ESIMS (\(m/z\) 475 (100%) for the [M+H]\(^+\) ion) and NMR analysis. The characteristic signal of H-5\(^{'}\) of the 1,2,3-triazole ring was at \(\delta\) 7.95 (s, H-5\(^{'}\)) which was found shifted downfield compare to that of benzyl triazole derivative 218. The \(^{13}\)C NMR signals for C-4\(^{'}\) and C-5\(^{'}\) were at \(\delta\) 128.2 and 119.4, respectively.

Scheme 6.8 Synthesis of the triazole 217.

A similar Cu\(^I\)-catalysed reaction procedure was employed using in situ prepared nitrile oxides instead of azides to prepare 3,5-disubstituted isoxazoles.\(^6\) The alkyne 215 was treated with the chlorooxime, N-hydroxybenzimidoyl chloride (see Chapter 10 for synthetic details),\(^6\) in a presence of Cu(OAc)\(_2\), sodium ascorbate and sodium hydroxide at rt for 4 h to give the isoxazole 220 in 59% yield (Scheme 6.9). Compound 220 was confirmed from ESIMS (\(m/z\) 475 (100%) for the [M+H]\(^+\) ion) and NMR analysis. The \(^1\)H NMR signal corresponding to H-4\(^{'}\) was observed at \(\delta\) 6.56 (s, H-4\(^{'}\)) while those of the phenyl group were at \(\delta\) 7.82-7.79 (m, 2H) and 7.45 (br s, 3H). The \(^{13}\)C NMR signals of C-3\(^{'}\), C-4\(^{'}\) and C-5\(^{'}\) were at \(\delta\) 162.6, 100.2 and 171.2, respectively.

Scheme 6.9 Synthesis of the isoxazole 220.
Moreover, compounds 221 – 223 were also prepared using similar reaction conditions to those described above (Table 6.1, entries 4 – 6). Compounds 221 – 223 were fully characterized by MS and NMR analysis. Using microwave assisted heating to generate the azide in situ at high temperature proved to be the most convenient with the shortest reaction time (10 min) however this method gave the product 221 in very low yield (18%, Table 6.1, entry 4), a similar low yield as found for compound 218 (23% yield). While the more classical reaction conditions which required the initial preparation of the azides, showed moderate yields of 33% and 46% for compounds 219 and 222, respectively. The coupling of alkyne 215 and nitrile oxides, which were generated in situ from base assisted elimination of HCl from chlorooximes, proved to be more efficient and gave yields of 59% for both isoxazoles 220 and 223 (Table 6.1, entries 3 and 6, respectively).
Table 6.1 Click reactions of the alkyne 215.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction condition</th>
<th>Coupling component</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NaN₃, Cu(0), CuSO₄, tBuOH:H₂O (1:1) MW 125 °C, 10 min</td>
<td>218; R = N=N-N=H</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Cu(OAc)₂ Na ascorbate MeOH:H₂O (1:1) rt, 4 h</td>
<td>219; R = N=N-N=H</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Cu(OAc)₂ Na ascorbate MeOH:H₂O (1:1) NaOH rt, 4 h</td>
<td>220; R = O-N=N=H</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>NaN₃, Cu(0), CuSO₄ tBuOH:H₂O (1:1) MW 125 °C, 10 min</td>
<td>221; R = N=N-N=H</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Cu(OAc)₂ Na ascorbate MeOH:H₂O (1:1) rt, 4 h</td>
<td>222; R = O-N=N-O=H</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Cu(OAc)₂ Na ascorbate MeOH:H₂O (1:1) NaOH rt, 4 h</td>
<td>223; R = O-N=H</td>
<td>59</td>
<td></td>
</tr>
</tbody>
</table>

*aSee Chapter 10 for synthetic details.*
6.4 Sonogashira coupling of the alkyne 215

The Sonogashira reaction of the alkyne 215 was also examined (Scheme 6.10). A reaction procedure reported by Hong was employed. Iodobenzene was used as the coupling component to introduce a phenyl group onto a terminal alkyne. The phenyl alkyne 216 was obtained in a yield of 62%, however the self-condensation of the alkyne 215 was also observed from the formation of its dimer 224 in 4% yield. The phenyl alkyne 216 was confirmed from ESIMS (m/z 432 (100%) for the [M+H]^+ ion) and NMR spectroscopic data. The ^1H NMR signal corresponding to the terminal alkyne proton of the alkyne was absent while those of phenyl group were seen at δ 7.45-7.44 (m, 2H) and 7.31-7.30 (m, 3H). The ^13C NMR signals for C-1′ and C-2′ were at δ 86.1 and 86.3, respectively. The dimer 224 was also fully characterized by ESIMS (m/z 709 (100%) for the [M+H]^+ ion) and NMR spectroscopic data. The ^1H NMR spectroscopic data of the dimer 224 was found to be similar to the alkyne 215 except for the signal corresponding to H-2′ that was missing. The formation of dialkynes from these types of reaction in the presence oxygen is called the Glaser reaction.53

![Scheme 6.10 Sonogashira reaction of the alkyne 215.](image)

The mechanistic catalytic cycles for the copper and palladium catalysts was proposed by Chinchilla and Nájera (Scheme 6.11). In the copper catalytic cycle, Cu^I is inserted into the alkyne to form a Cu^I acetylide which then reacts with the R^I PdL_2X intermediate to give a R^I PdL_2(C≡CR^2) complex which upon reductive elimination generates the Sonogashira product.
6.5 Eglington coupling of the alkyne 215

To specifically prepare the dimer 224, Eglington reaction conditions were employed using a modification of the procedure reported by Vögtle. The alkyne 215 was treated with Cu(OAc)$_2$ (5 equiv.) in acetonitrile under an argon atmosphere at 40 °C for 4 h (Scheme 6.12). The desired product 224 was then obtained in 89% yield after purification by column chromatography. Its spectroscopic data were identical to those of the by-product obtained from the Sonogashira coupling reaction (Scheme 6.10).
6.6 Hydrogenation of the Sonogashira product 216

The Sonogashira product 216 was hydrogenated over Pd/C under a H₂ atmosphere for 24 h to give the reduced product 225 in 74% yield (Scheme 6.13). Compound 225 was confirmed from ESIMS (m/z 436 (100%) for the [M+H]⁺ ion) and NMR analysis. The ¹H NMR signals corresponding of H-1’ and H-2’ were observed at δ 2.72 (td, J 11.5 Hz, 5.0 Hz, 1H, H-1’), 2.50 (td, J 13.5 Hz, 5.5 Hz, 1H, H-1’), 1.90-1.79 (m, 1H, H-2’) and 1.78-1.74 (m, 1H, H-2’) while those of a phenyl group were at δ 7.20 (d, J 7.0 Hz, 2H) and 7.13-7.10 (m, 3H). The ¹³C NMR signals for C-1’ and C-2’ of 225 were at significantly highfield, δ 31.4 and 33.7, respectively, compared to those of the corresponding carbons of the phenyl alkyne 216.

Scheme 6.13 Hydrogenation reaction of the Sonogashira product 216.

6.7 Conclusions

The alkyne 215 was prepared from the aldehyde 160 in one step using the Bestmann-Ohira reagent 217. Cu¹-catalysed click reactions were conducted on the alkyne 215 to prepare four triazoles, 218, 219, 221 and 222, and two isoxazoles, 220 and 223. The Sonogashira coupling of the alkyne 215 gave the phenyl alkyne product 216 and also the dimer 224 which was more efficiency and directly prepared under Eglington coupling conditions. The hydrogenation of 216 provided the flexible 2-phenylethyl side chain of compound 225. The biological activities of these compounds will be discussed in Chapter 7.
7.1 Acetylcholinesterase (AChE) inhibitory activity

The enzyme assay was performed in our laboratory on thin-layer chromatography (TLC) plates using a modification of a rapid TLC bioautographic method developed by Hostettmann.73 A mixture of Fast Blue B salt and naphthyl acetate were used as indicators. An outline of this assay is shown in Scheme 7.1. The AChE enzyme hydrolyses naphthyl acetate into α-naphthol which further reacts with Fast Blue B salt to give a purple diazonium dye. The inhibition of AChE prevents the formation of the azo dye and reveals a white region on the TLC plates as shown in Figure 7.1. The tested compounds were applied to TLC plates in a range of concentrations using a Camag Nanomat 4 TLC spotter with 0.5 μL capillaries. The plate was then sprayed with the AChE enzyme stock solution, incubated in a covered water bath at 37 °C for 20 min and then sprayed with the indicator mixture. The results were interpreted as a minimum inhibitory requirement (MIR) in ng or nmol. For our studies, the known AChE inhibitor, galanthamine, was used as a positive control (MIR = 1 ng or 0.003 nmol).

Scheme 7.1 Reaction of acetylcholinesterase with naphthyl acetate and the subsequent formation of the purple dye in the TLC bioassay.73
Figure 7.1 Bioautograph showing the inhibition of acetylcholinesterase activity by (11Z)-1′,2′-didehydrostemofoline (104) (1 – 1000 ng applied). MIR estimated as 5 ng.

The assay using AChE from electric eel (eeAChE), was used earlier by our group to determine the AChE inhibitory activities of stemofoline alkaloids.\textsuperscript{34} In this study, (11Z)-1′,2′-didehydrostemofoline (104) was reported to have the highest activity among the tested alkaloids as an AChE inhibitor with a MIR of 5 ng or 0.013 nmol (results are included in Table 7.3). Later, the activities of the stemocurtisine alkaloids from \textit{Stemona curtisii} were reported using the same assay however they exhibited low activities against AChE with MIRs in the range of 100 - >1000 ng or 0.24 - >2.88 nmol (Table 7.1).\textsuperscript{11} In 2011, a new stemona alkaloid from \textit{Stemona aphylla}, (2′-S)-hydroxy-(11S,12R)-dihydrostemofoline (112), and its related analogues 99, 227 and 228 were reported to have MIRs of 50, 10, 10 and 100 ng, respectively (Table 7.2).\textsuperscript{12}

Compounds described in Chapters 3-6 of this thesis were tested for their AChE inhibitory activities. Their MIRs are shown in Tables 7.3-7.6, arranged according to the C-3 side chain type, and comprehensively in Table 7.7. The inhibitory activities of the previously tested compounds, 99, 101, 102, 104, 107, 110, 112, 167 and 230 are also included in these Tables.\textsuperscript{12,34}

In the group of alcohol derivatives (Table 7.3), (1′R)-hydroxystemofoline (98) showed the highest inhibitory activity with a MIR of 5 ng (0.012 nmol, Table 7.3, entry 1), while its 3′,4′-didehydro derivative 183 (Table 7.3, entry 11) and its (1′S)-epimer 166 (Table 7.3, entry 10) showed MIRs of 10 ng. The acetate derivatives 185 and 186 (Table 7.3, entries 14 and 15, respectively), of 98 and 166, along with their 3′,4′-didehydro derivative 184 (Table 7.3, entry 20) were 10 times less active than 98 (MIR = 50 ng). While (11Z)-1′,2′-didehydrostemofoline (104) (Table 7.3, entry 3) and its N-oxide 105 (Table 7.3, entry 2) were reported to have MIRs of 5 ng (0.013 nmol), stemofoline (97) was slightly less active (MIR = 10 ng or 0.026 nmol, Table 7.3, entry 12). Its 11,12-dihydroxy derivative 187 (Table 7.3, entry 30) and the tricyclic derivative 188 (Table 7.3, entry 29), that is missing the γ-
butyrolactone ring found in 97, were 50 and 10 times less active, respectively, with MIRs of 500 ng and 100 ng, respectively. The 11,12-dihydrostembranoles 111 and 163 (MIR = 50 ng, Table 7.3, entries 22 and 23, respectively) were less active than 97 and had the same activity as (Z)-stemoburkilline (108, Table 7.3, entry 21). The other compounds of similar activity to stemofoline (97) (which all had a MIR of 10 ng) included the dien-ol 190b (Table 7.3, entry 6), the trien-ol 190c (Table 7.3, entry 4), the C-1’ alcohols 166 and 183 (Table 7.3, entries 10 and 11, respectively), the C-2’ alcohols 99 and 100 (Table 7.3, entries 7 and 8, respectively) and the 1’,2’-diol 173 (Table 7.3, entry 5), which was five times more active than its epimer 174 (MIR = 50 ng, Table 7.3, entry 16). Compared to the 11,12-dihydrostembranoles 111 and 163, the 11,12-dihydrostembranol derivatives 112 and 230 (Table 7.2, entries 3 and 4, respectively) were less active than 99 (Table 7.2, entry 2 or Table 7.3, entry 7) with MIRs of 50 and 100 ng, respectively. The C-3’ hydroxy analogues, (3’S)-hydroxystemofoline (110, Table 7.3, entry 9) and (3’R)-hydroxystemofoline (167, Table 7.3, entry 26), showed a 10 fold difference in activities with MIRs of 10 ng and 100 ng, respectively. (3’R)-stemofolinol 101 (Table 7.3, entry 31, MIR = 500 ng) was five times less active than its (3’S)-epimer 102 (Table 7.3, entry 27, MIR = 100 ng). The C-4’ hydroxy and methoxy substituted stemofoline derivatives 115 (oxystemofoline), 116 (methoxystemofoline), 169 and 170 (Table 7.3, entries 18, 17, 19 and 25, respectively) showed relatively weak activities (MIRs = 50 – 100 ng). Of the truncated side chain derivatives, the hydroxymethyl derivative 208 (Table 7.3, entry 13) showed a relatively high activity (MIR = 10 ng) while the methyl derivative 107 (methylstemofoline) and the 3’-hydroxyl-1-propenyl derivative 190a (Table 7.3, entries 28 and 24, respectively) had much reduced activities (MIRs = 100 ng and 50 ng, respectively).

Of the group of amine derivatives (Table 7.4), the carbamate 211 (Table 7.4, entry 1) showed the highest inhibitory activities with a MIR of 1 ng (0.002 nmol) while the other carbamates 210 and 212 (Table 7.4, entries 8 and 7, respectively) and their secondary amines 203, 204 and 205 (Table 7.4, entries 17, 12 and 13, respectively) were 10 times less active than 211 with MIRs of 10 ng. The tertiary amine 192 (Table 7.4, entry 2) had a MIR of 1 ng, while that of the corresponding second amine 191 (Table 7.4, entry 6) was slightly less active (MIR = 5 ng) which had similar activity to the amines 193 and 207 and the guanidine derivative 214.
(Table 7.4, entries 4, 3 and 5, respectively). For the amine \textbf{207}, its (S)-epimer \textbf{206} (Table 7.4, entry 10) and the benzyl amine derivative \textbf{201} (Table 7.4, entry 9) were less active with MIRs of 10 ng. The other compounds of similar activity to the amine \textbf{201} (which all had a MIR of 10 ng) included the morpholine derivative \textbf{197}, the allyl amine \textbf{194} and its methylated derivative \textbf{209} and the ethanolamine derivative \textbf{196} (Table 7.4, entries 11, 15, 14 and 16, respectively). The amine \textbf{195} (Table 7.4, entry 22), which had a N,N-dimethyl group instead of the hydroxyl group of \textbf{196}, showed a 10 folded difference in activity (MIR = 100 ng) while that of the methyl piperazine derivative \textbf{199} (Table 7.4, entry 21) was two times less active than the ethoxy carbonyl piperazine derivative \textbf{198} (Table 7.4, entry 18) with MIRs of 100 ng and 50 ng, respectively. The other compounds having MIRs of 50 ng included the amine \textbf{202} and the phenyl amine \textbf{200} (Table 7.4, entries 20 and 19, respectively). This latter amine derivative was 5 - 10 times less active than its homologues, the benzyl amine derivatives \textbf{201}, \textbf{206} and \textbf{207}. This difference may be due to the more flexible and the one carbon longer side chain in the benzyl derivatives.

Of the group of click products (Table 7.5), the benzyl triazole \textbf{218} (Table 7.5, entry 1) showed the highest activity among the group with a MIR of 10 ng. The triazoles \textbf{221} and \textbf{222} (Table 7.5, entries 3 and 2, respectively) having a longer side chain linker were less active than \textbf{218} (with MIRs of 50 ng). While the less flexible triazole \textbf{219} (Table 7.5, entry 6) was the least active of the group with a MIR of 100 ng. The isoxazoles \textbf{220} and \textbf{223} (Table 7.5, entries 5 and 4, respectively) showed similar activities to the triazoles \textbf{221} and \textbf{222} with MIRs of 50 ng.

The alkyne \textbf{215} (Table 7.6, entry 1) showed a high activity with a MIR of 1 ng while its dimer \textbf{224} (Table 7.6, entry 4) was 100 times less active (MIR = 100 ng, Table 7.6). The Sonogashira product \textbf{216} (Table 7.6, entry 3) was 10 times less active than its reduced product \textbf{225} (Table 7.6, entry 2) which had a more flexible side chain with MIRs of 50 ng and 5 ng, respectively.

Compared to the previous reported results,\textsuperscript{11} the pyrrolo[1,2-\textit{a}]azepine alkaloids (Table 7.7) established significantly higher activities than those of the pyrido[1,2-\textit{a}]azepine alkaloids in Table 7.1. The overall results showed that in general the amine derivatives were more active than the alcohol derivatives. The flexibility and length of the C-3 side chain and the stereochemistry of its substituents often had an effect on the activity. The \(\gamma\)-butyrolactone ring of stemofoline (\textbf{97}) was
clearly shown to have an important role in activity as the activity drop about 10 times in the case of its tricyclic analogue 188.

Table 7.1 AChE inhibitory activities of alkaloids from *Stemona curtisii* and derivatives.\(^{11}\)

<table>
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<tr>
<th>Entry</th>
<th>Compound</th>
<th>Minimum inhibitory requirements</th>
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<th>nmol</th>
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<td>Galanthamine</td>
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<td></td>
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</tr>
<tr>
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<td>Oxyproscleremonine (34)</td>
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<td>Stemcurtisine (119)</td>
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<td>&gt;2.88</td>
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Table 7.2 AChE inhibitory activities of alkaloids from *Stemona aphylla* and related analogues.\(^1\)

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Table 7.3 AChE inhibitory activities of stemofoline alkaloids and the alcohol derivatives.

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Table 7.4 AChE inhibitory activities of the amine derivatives.

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### Table 7.5 AChE inhibitory activities of the click products.

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<tr>
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<td>50 0.085</td>
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<td>50 0.097</td>
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<td>4</td>
<td>223</td>
<td><img src="image4" alt="Image" /></td>
<td>50 0.102</td>
</tr>
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<td>5</td>
<td>220</td>
<td><img src="image5" alt="Image" /></td>
<td>50 0.105</td>
</tr>
<tr>
<td>6</td>
<td>219</td>
<td><img src="image6" alt="Image" /></td>
<td>100 0.211</td>
</tr>
</tbody>
</table>

### Table 7.6 AChE inhibitory activities of miscellaneous derivatives.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Side chain</th>
<th>Minimum inhibitory requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ng nmol</td>
</tr>
<tr>
<td>1</td>
<td>215</td>
<td><img src="image7" alt="Image" /></td>
<td>1 0.003</td>
</tr>
<tr>
<td>2</td>
<td>225</td>
<td><img src="image8" alt="Image" /></td>
<td>5 0.011</td>
</tr>
<tr>
<td>3</td>
<td>216</td>
<td><img src="image9" alt="Image" /></td>
<td>50 0.116</td>
</tr>
<tr>
<td>4</td>
<td>224</td>
<td><img src="image10" alt="Image" /></td>
<td>100 0.141</td>
</tr>
</tbody>
</table>
Table 7.7 Minimum inhibitory requirements for stemofoline alkaloids and derivatives, the C-3 substituent (R) is shown in the boxes.
Compounds 104, 183, 193, 196, 197, and 208 were taken to test their IC\(_{50}\) values against AChE using a modification of the spectrometric-based assay reported by Bastida.\(^{74}\) The original methodology and principle were described by Ellman\(^{75}\) which is now the most widely used assay. This assay uses the thiol ester acetylthiocholine (ATCh) which is hydrolysed by AChE to produce thiocholine (Scheme 7.2 (a)). The enzyme activity is determined by the amount of the yellow coloured 5-thio-2-nitrobenzoic acid (TNB) that is produced from the reaction between thiocholine and dithiobisnitrobenzoate (DTNB) (Scheme 7.2 (b)). The colour intensity (absorbance) of TNB is measured at \(\lambda 412\) nm at 25 °C every 15 sec for 30 min. This intensity is proportional to the enzyme activity. The inhibition of AChE prevents the hydrolysis of ATCh to thiocholine which terminates the formation of yellow coloured TNB. The AChE inhibitory activities were analysed with the software package GraphPad Prism\(^{86}\) and were determined as IC\(_{50}\) values or the concentration of tested compounds that inhibited AChE by 50%.

\[
\text{(a)} \quad (\text{CH}_3)_2\text{N}^+\text{CH}_2\text{CH}_2\text{S}\text{CO} \cdot \text{CH}_3 \quad + \quad \text{H}_2\text{O} \quad \xrightarrow{\text{AChE}} \quad (\text{CH}_3)_2\text{N}^+\text{CH}_2\text{CH}_2\text{SH} \quad + \quad \text{CH}_3\text{COOH} \\
\text{ATCh} \quad \text{Thiocholine} \quad \text{Acetic acid}
\]

\[
\text{(b)} \quad (\text{CH}_3)_2\text{N}^+\text{CH}_2\text{CH}_2\text{SH} \quad + \quad \text{DTNB} \quad \xrightarrow{\text{Mixed disulphide}} \quad (\text{CH}_3)_2\text{N}^+\text{CH}_2\text{CH}_2\text{S} \quad + \quad \text{TNB (yellow)}
\]

**Scheme 7.2** Principle of the Ellman method.

This assay was performed using enzymes from two different sources, electric eel AChE (eeAChE) and human AChE (hAChE) (Table 7.8). Galanthamine was used as a reference compound and had, in our assay, IC\(_{50}\) values of 0.9 μM and 0.6 μM against eeAChE and hAChE, respectively (Table 7.8, entry 1). Many of the compounds that we tried to assay were not soluble in the assay DMSO/buffer mixture and therefore reliable IC\(_{50}\) values could not be determined, including some of the most active ones from Table 7.7, for example, the alkyne 215, the carbamate 210, the amine 206, the Sonogashira product 216 and the triazole 219. Of the soluble
compounds tested, the amine 193 showed the highest activities of the tested compounds with IC$_{50}$ values of 12.9 μM and 19.9 μM against eeAChE and hAChE, respectively (Table 7.8, entry 3). Thus while (11Z)-1’,2’-didehydrostemofoline (104) showed higher activity than the amine 193, by the TLC autographic method, it showed slightly less activities than 193 by Ellman’s assay (IC$_{50}$ values of 19.2 μM and 25.0 μM against eeAChE and hAChE, respectively, Table 7.8, entry 2). Among the compounds with minimum inhibitory requirements of 10 ng, it was observed that the amines 196 and 197 exhibited better IC$_{50}$ values (a range between 28.7 – 77.2 μM, Table 7.8, entry 3) than the alcohols 183 and 208 (a range between 41.2 – 302.3 μM) for both eeAChE and hAChE. According to the results, the inhibitory activities against hAChE was observed to be slightly better than against eeAChE, except for compounds 183, 197 and 208 which showed about 2 – 8 times differences in IC$_{50}$ values between eeAChE and hAChE. The advantage of this assay is the accuracy of the results, however, the preparation procedure is less straightforward than the TLC bioautographic method.
Table 7.8 Acetylcholinesterase inhibitory activity of stemofoline derivatives.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Minimum inhibitory requirements</th>
<th>IC₅₀ values μM (R²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ng</td>
<td>nmol</td>
</tr>
<tr>
<td>1</td>
<td>Galanthamine</td>
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<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(11Z)-1′,2′-didehydrostemofoline (104)</td>
<td>5</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>193</td>
<td>10</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>183</td>
<td>10</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
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<tr>
<td>5</td>
<td>196</td>
<td>10</td>
<td>0.025</td>
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<td></td>
</tr>
<tr>
<td>7</td>
<td>208</td>
<td>10</td>
<td>0.028</td>
</tr>
<tr>
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</tr>
</tbody>
</table>
7.2 Modulation of resistance to anticancer drugs

In collaboration with the Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Thailand, some compounds prepared in our laboratory were selected to be tested for inhibitory activity against P-glycoprotein (P-gp). P-gp is a member of the highly conserved superfamily of ATP-binding cassette (ABC) transporter proteins which acts as an ATP-driven efflux pump that decreases intracellular drug accumulation. The overexpression of P-gp is a major cause of the failure of chemotherapeutic drugs in the treatment of multidrug resistance (MDR) cancer cells. The calcium channel blocker, verapamil, was the first compound able to enhance intracellular accumulation of many anticancer drugs, however, it suffered as a therapeutic drug due to its intrinsic toxicity at the doses required to inhibit P-gp function. The agents used to enhance drug accumulation and cause no potentiation of drug cytotoxicity in sensitive cells are called chemozensitizers or resistance modifiers. In 2011, stemofoline (97) was the first Stemona alkaloid to be reported as a chemosensitizer for the anticancer drugs, vinblastine, paclitaxel and doxorubicin, in a dose- and time-dependent manner in KB-V1 cells, which are MDR human cervical carcinoma with P-gp expression.

The cytotoxicity of stemofoline and its derivatives were first estimated against KB-V1 and KB-3-1 cell lines using the tetrazolium-based colorimetric MTT assay. It was found that at 5 μM concentrations of these tested compounds, there was >80% cell survival after 24 h. These cell lines were then treated with stemofoline and or its derivatives at 5 μM and various concentrations of paclitaxel or vinblastine (0-20 μM paclitaxel for KB-V1 and 0-5 nM for KB-3-1) or (0-1 μM vinblastine for KB-V1 and 0-1 nM for KB-3-1). The cells were incubated for 48 h at 37 °C, and then cell growth was assessed by means of an MTT colorimetric assay. Verapamil (20 μM) was used as a positive control. The relative resistance (RR) was calculated as the ratio of the IC50 value of the KB-V1 cells to the IC50 value of the KB-3-1 cells. The fold-reversal activity (FR) was calculated as the ratio of the RR for cells with the anticancer agent but without stemofoline or its derivatives to the RR for cells with the anticancer agent and the alkaloid compounds. The results for modulation of resistance to paclitaxel and vinblastine in KB cells after 48 h treatment are shown in Tables 7.9 and 7.10, respectively.
### Table 7.9 Modulation of resistance to paclitaxel in KB cells treated with stemofoline (97) and its derivatives (5μM) after 48 h treatment.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cell line</th>
<th>Modulator</th>
<th>IC$_{50}$ value of paclitaxel (μM)</th>
<th>Relative resistance (RR)</th>
<th>Fold reversal activity (FR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KB-3-1</td>
<td></td>
<td>2.13 ± 0.15 nM</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>KB-V1</td>
<td></td>
<td>10.06 ± 1.56 μM</td>
<td>4723</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>KB-V1</td>
<td>+ 5 μM Stemofoline (97) R=  [\text{OH} ]</td>
<td>1.4 ± 0.45</td>
<td>657</td>
<td>7.19</td>
</tr>
<tr>
<td>4</td>
<td>KB-V1</td>
<td>+ 5 μM 104 R=  [\text{CH} ]</td>
<td>3.5 ± 0.87</td>
<td>1643</td>
<td>2.87</td>
</tr>
<tr>
<td>5</td>
<td>KB-V1</td>
<td>+ 5 μM 173 R=  [\text{OH} ]</td>
<td>13 ± 3.18</td>
<td>6103</td>
<td>0.77</td>
</tr>
<tr>
<td>6</td>
<td>KB-V1</td>
<td>+ 5 μM 183 R=  [\text{OH} ]</td>
<td>11 ± 3.18</td>
<td>5164</td>
<td>1.00</td>
</tr>
<tr>
<td>7</td>
<td>KB-V1</td>
<td>+ 5 μM 190a R=  [\text{CH} ]</td>
<td>10 ± 1.77</td>
<td>4695</td>
<td>1.01</td>
</tr>
<tr>
<td>8</td>
<td>KB-V1</td>
<td>+ 5 μM 190b R=  [\text{OH} ]</td>
<td>12 ± 3.28</td>
<td>5634</td>
<td>0.84</td>
</tr>
<tr>
<td>9</td>
<td>KB-V1</td>
<td>+ 5 μM 208 R=  [\text{OH} ]</td>
<td>8 ± 2.26</td>
<td>3756</td>
<td>1.26</td>
</tr>
<tr>
<td>10</td>
<td>KB-V1</td>
<td>+ 5 μM 193 R=  [\text{Me} ]</td>
<td>9.75 ± 2.47</td>
<td>4577</td>
<td>1.03</td>
</tr>
<tr>
<td>11</td>
<td>KB-V1</td>
<td>+ 5 μM 197 R=  [\text{Me} ]</td>
<td>3.78 ± 1.45</td>
<td>1775</td>
<td>2.66</td>
</tr>
<tr>
<td>12</td>
<td>KB-V1</td>
<td>+ 5 μM 199 R=  [\text{Me} ]</td>
<td>10.25 ± 3.18</td>
<td>4812</td>
<td>0.98</td>
</tr>
<tr>
<td>13</td>
<td>KB-V1</td>
<td>+ 5 μM 206 R=  [\text{Me} ]</td>
<td>3.27 ± 1.57</td>
<td>1535</td>
<td>3.08</td>
</tr>
<tr>
<td>14</td>
<td>KB-V1</td>
<td>+ 5 μM 210 R=  [\text{Me} ]</td>
<td>4.63 ± 0.18</td>
<td>2174</td>
<td>2.17</td>
</tr>
<tr>
<td>15</td>
<td>KB-V1</td>
<td>+ 5 μM 218 R=  [\text{Me} ]</td>
<td>7.5 ± 1.41</td>
<td>3521</td>
<td>1.34</td>
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</table>
Table 7.10 Modulation of resistance to vinblastine in KB cells treated with stemofoline (97) and its derivatives (5μM) after 48 h treatment.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cell line</th>
<th>Modulator</th>
<th>IC₅₀ value of vinblastine (μM)</th>
<th>Relative resistance (RR)</th>
<th>Fold reversal activity (FR)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>KB-3-1</td>
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<td>0.55 ± 0.06 nM</td>
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<td>1</td>
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<tr>
<td>2</td>
<td>KB-V1</td>
<td></td>
<td>0.61 ± 0.05 μM</td>
<td>1109</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>KB-V1</td>
<td>+ 5 μM Stemofoline (97) R=</td>
<td>0.09 ± 0.01</td>
<td>164</td>
<td>6.8</td>
</tr>
<tr>
<td>4</td>
<td>KB-V1</td>
<td>+ 5 μM 104 R=</td>
<td>0.11 ± 0.04</td>
<td>200</td>
<td>5.5</td>
</tr>
<tr>
<td>5</td>
<td>KB-V1</td>
<td>+ 5 μM 173 R=</td>
<td>0.72 ± 1.13</td>
<td>1309</td>
<td>0.8</td>
</tr>
<tr>
<td>6</td>
<td>KB-V1</td>
<td>+ 5 μM 183 R=</td>
<td>0.71 ± 0.08</td>
<td>1291</td>
<td>0.9</td>
</tr>
<tr>
<td>7</td>
<td>KB-V1</td>
<td>+ 5 μM 190a R=</td>
<td>0.71 ± 0.01</td>
<td>1291</td>
<td>0.9</td>
</tr>
<tr>
<td>8</td>
<td>KB-V1</td>
<td>+ 5 μM 190b R=</td>
<td>0.75 ± 0.02</td>
<td>1364</td>
<td>0.8</td>
</tr>
<tr>
<td>9</td>
<td>KB-V1</td>
<td>+ 5 μM 208 R=</td>
<td>0.8 ± 0.14</td>
<td>1455</td>
<td>0.8</td>
</tr>
<tr>
<td>10</td>
<td>KB-V1</td>
<td>+ 5 μM 193 R=</td>
<td>0.59 ± 0.10</td>
<td>1073</td>
<td>1.0</td>
</tr>
<tr>
<td>11</td>
<td>KB-V1</td>
<td>+ 5 μM 197 R=</td>
<td>0.67 ± 0.01</td>
<td>1218</td>
<td>0.9</td>
</tr>
<tr>
<td>12</td>
<td>KB-V1</td>
<td>+ 5 μM 199 R=</td>
<td>0.75 ± 0.07</td>
<td>1364</td>
<td>0.8</td>
</tr>
<tr>
<td>13</td>
<td>KB-V1</td>
<td>+ 5 μM 206 R=</td>
<td>0.17 ± 0.01</td>
<td>309</td>
<td>3.6</td>
</tr>
<tr>
<td>14</td>
<td>KB-V1</td>
<td>+ 5 μM 210 R=</td>
<td>0.25 ± 0.02</td>
<td>455</td>
<td>2.4</td>
</tr>
<tr>
<td>15</td>
<td>KB-V1</td>
<td>+ 5 μM 218 R=</td>
<td>0.67 ± 0.01</td>
<td>1218</td>
<td>0.9</td>
</tr>
</tbody>
</table>
The MDR-reversing properties of stemofoline (97) and its derivatives on paclitaxel cytotoxicity are shown in Table 7.9. The efficiency of this modulating effect is indicated by a lowering of the RR value and an increase in the FR value. Among the tested compounds, stemofoline (97) showed the highest modulating effect on the resistant KB-V1 cells by decreasing the IC$_{50}$ of paclitaxel from 10.06 ± 1.56 μM (Table 7.9, entry 2) to 1.4 ± 0.45 μM (Table 7.9, entry 3). It showed the lowest RR of 657 and the highest FR of 7.19 (Table 7.9, entry 3). The other compounds that showed MDR-reversing properties were 206, 104, 197 and 210. In the presence of these compounds, paclitaxel had IC$_{50}$ values of 3.27 ± 1.57, 3.5 ± 0.87, 3.78 ± 1.45 and 4.63 ± 0.18 μM, respectively (Table 7.9, entries 13, 4, 11 and 14, respectively). Compounds 206, 104, 197 and 210 increased sensitivity of KB-V1 cells to paclitaxel as measured by their FR values of 3.08, 2.87, 2.66 and 2.16 fold, respectively, while other compounds showed no modulating effect.

The MDR-reversing properties of stemofoline (97) and its derivatives on vinblastine cytotoxicity is shown in Table 7.10. Among the tested compounds, stemofoline (97) showed the highest modulating effect on the resistant KB-V1 cells by decreasing the IC$_{50}$ of vinblastine from 0.61 ± 0.05 μM (Table 7.10, entry 2) to 0.09 ± 0.01 μM (Table 7.10, entry 3). It showed the lowest RR of 164 and the highest FR of 6.8 (Table 7.10, entry 3). The other compounds that showed MDR-reversing properties were 104, 206 and 210. In the presence of these compounds, vinblastine had IC$_{50}$ values of 0.11 ± 0.04, 0.17 ± 0.01 and 0.25 ± 0.02 μM, respectively (Table 7.10, entries 4, 13 and 14, respectively). Compounds 104, 206 and 210 increased sensitivity of KB-V1 cells to vinblastine as measured by their FR values of 5.5, 3.6 and 2.4 fold, respectively, while other compounds showed no modulating effect.

In comparison, compounds 97 and 104, which had a non-polar side chain were observed to be good modulators for both paclitaxel and vinblastine on KB-V1 cells. The amines 197 and 206 and the carbamate 210 showed moderate modulation activities for paclitaxel cytotoxicity while the amine 206 and the carbamate 210 showed high modulating activities for vinblastine. In contrast, none of the alcohol derivatives showed a modulating effect.
7.3 Conclusions

The AChE inhibitory activities of stemofoline derivatives were performed using two different enzyme assays, a TLC bioautographic method and a spectroscopic-based method known as Ellman’s method. In comparison, these two assays were used for different purposes as the former method was easier and only gave less precise results while the latter method was more time consuming but gave more accurate quantitative results. The overall results from both methods showed that most of the amine derivatives were more active than the alcohol derivatives. The mode of inhibition of AChE is unknown and further SAR studies using computer aided modelling may help to better understand the binding mode of these compounds which would be useful in future drug discovery. The results of our studies in this area will be discussed in Chapter 8.

Surprisingly, the amine derivatives also exhibited better activities than the alcohol derivatives as modulators of resistance to anticancer drugs. The highest modulating activity was observed with stemofoline (97) which had a simple butyl side chain.
8.1 Background

As indicated in Chapter 7, the mode of inhibition of acetylcholinesterase (AChE) by the stemofoline alkaloids and their derivatives is unknown. Computer aided pharmacophore modelling and molecular docking were therefore performed to provide a better understanding of the potential mode of inhibition of AChE.

To inhibit AChE, the inhibitors must bind to one or more sites of the enzyme. AChE has been reported to have at least two binding sites, the active site and the peripheral anionic site (PAS). The first X-ray crystal structure of *Torpedo californica* AChE (TcAChE) revealed that the active site is buried at the bottom of a 20 Å deep narrow gorge lined with conserved aromatic residues. The active site includes four catalytic subsites, the esteratic site (which contains Ser200, His440 and Glu327), the oxyanion hole (which contains Gly118, Gly119 and Ala201), the acyl pocket (which contains Phe288 and Phe290) and the anionic subsite (which contains Trp84, Phe330 and Glu199). These sites are cooperatively responsible for the hydrolysis of acetylcholine (ACh) into acetate and choline. In contrast, the PAS which consists of Tyr70, Asp72, Tyr121, Tyr334 and Trp279, is located at the entrance of the active-site gorge. The binding of ligands to the PAS may block the passage of ACh or change the conformation of the active site allosterically and inhibit its function.

From X-ray crystal structures of AChE-ligand complexes, the binding sites have been observed in atomic detail. In cases where it has been difficult to obtain a single crystal of an AChE-ligand complex, molecular modelling has proven a useful method to predict the binding mode of ligands for use in future drug development and discovery. For example, the AChE inhibitor, galanthamine, was studied and demonstrated to bind at the active site of TcAChE from the X-ray structure of the TcAChE-galanthamine complex (PDB code 1W6R). Based on the binding mode of galanthamine to AChE, many galanthamine derivatives were designed by generating pharmacophores and using protein-ligand docking techniques.
8.2 Pharmacophore generation

The term ‘pharmacophore’ was described by IUPAC as ‘the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response’. All pharmacophore models in this study were generated using the 3D QSAR (quantitative structure-activity relationship) Pharmacophore Generation tool from the Discovery Studio 2.5.5 (DS 2.5.5) software package (Accelrys Inc., San Diego, CA, USA). Pharmacophores were derived from a set of compounds (Table 8.1), which had their inhibitory activities against electric eel AChE (eeAChE) measured as IC$_{50}$ values using Ellman’s method. These data were compiled from the published literature. Only IC$_{50}$ values were included that employed galanthamine and tacrine as standard controls in their assays (Table 8.1, entries 1 and 9, respectively). These compounds then were specifically selected based on the similarity of their structures to the stemofoline alkaloids (Table 8.4) and were grouped into five training sets according to their known binding modes. Five training sets included, ‘Active site binding’ (ASB, Table 8.1), ‘Bis-functional binding’ (BFB, Table 8.2), ‘Tacripyrine’ (Table 8.3), ‘Stemona’ (Table 8.4, entries 2-43) and ‘Unknown’ which was a combination set of ‘Tacripyrine’ and ‘Stemona’.

Table 8.1 Compounds used in the active site binding (ASB) training and test sets.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Code</th>
<th>Compound</th>
<th>R, X</th>
<th>IC$_{50}$ values$^a$ (μM)</th>
<th>Reference number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Galanthamine</td>
<td></td>
<td>0.36</td>
<td>82</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Narwedine</td>
<td></td>
<td>30</td>
<td>82</td>
</tr>
<tr>
<td>3</td>
<td>1-2</td>
<td></td>
<td></td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>1-3</td>
<td></td>
<td>R = H</td>
<td>0.03</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>1-4</td>
<td></td>
<td>R = Ph</td>
<td>3.15</td>
<td>80</td>
</tr>
<tr>
<td>Entry</td>
<td>Code</td>
<td>Compound</td>
<td>R, X</td>
<td>IC50 values(^a) (µM)</td>
<td>Reference number</td>
</tr>
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<td>-------</td>
<td>------</td>
<td>----------</td>
<td>------</td>
<td>--------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>7</td>
<td>5-N-Allylnorgalanthamine</td>
<td><img src="https://example.com/structure1.png" alt="Chemical Structure" /></td>
<td>R = <img src="https://example.com/structure2.png" alt="Chemical Structure" /></td>
<td>0.18</td>
<td>74</td>
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<tr>
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\(^a\)IC50 values were determined by Ellman’s method against eeAChE.
Table 8.2 Compounds used in the bis-functional binding (BFB) training and test sets.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Code</th>
<th>Compound</th>
<th>n, R, X</th>
<th>IC₅₀ values (µM)</th>
<th>Reference number</th>
</tr>
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<tbody>
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<tr>
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<tr>
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<td><img src="image" alt="Compound 2-3d" /></td>
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<tr>
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Table 8.2 (continued)

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Table 8.2 (continued)

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Table 8.2 (continued)

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<th>Entry</th>
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<th>Compound</th>
<th>n, R, X</th>
<th>IC₅₀ values</th>
<th>Reference number</th>
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*IC₅₀ values were determined by Ellman’s method against eeAChE.*

Table 8.3 Compounds used in the Tacripyrine training and test sets.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Code</th>
<th>Compound</th>
<th>X, Y, Z</th>
<th>IC₅₀ values</th>
<th>Reference number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6-1</td>
<td></td>
<td>X = H</td>
<td>Y, Z = CH</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>6-2</td>
<td></td>
<td>X = 4’,F</td>
<td>Y = C, Z = CH</td>
<td>0.052</td>
</tr>
<tr>
<td>3</td>
<td>6-3</td>
<td></td>
<td>X = 2’,CF₃</td>
<td>Y, Z = CH</td>
<td>0.21</td>
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<tr>
<td>4</td>
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<td></td>
<td>X = 2’,NO₂</td>
<td>Y, Z = CH</td>
<td>0.304</td>
</tr>
<tr>
<td>5</td>
<td>6-5</td>
<td></td>
<td>X = 3’,NO₂</td>
<td>Y, Z = CH</td>
<td>0.11</td>
</tr>
<tr>
<td>6</td>
<td>6-6</td>
<td></td>
<td>X = 4’,NO₂</td>
<td>Y = C, Z = CH</td>
<td>0.6</td>
</tr>
<tr>
<td>7</td>
<td>6-7</td>
<td></td>
<td>X = 4’,Me</td>
<td>Y = C, Z = CH</td>
<td>0.091</td>
</tr>
<tr>
<td>8</td>
<td>6-8</td>
<td></td>
<td>X = 4’,C₆H₅</td>
<td>Y = C, Z = CH</td>
<td>0.09</td>
</tr>
<tr>
<td>9</td>
<td>6-9</td>
<td></td>
<td>X = 2’,OMe</td>
<td>Y, Z = CH</td>
<td>0.16</td>
</tr>
<tr>
<td>10</td>
<td>6-10</td>
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<td>X = 3’,OMe</td>
<td>Y, Z = CH</td>
<td>0.061</td>
</tr>
<tr>
<td>11</td>
<td>6-11</td>
<td></td>
<td>X = 4’,OMe</td>
<td>Y, Z = CH</td>
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</tr>
<tr>
<td>12</td>
<td>6-12</td>
<td></td>
<td>X = 3’,4’,di-OMe</td>
<td>Y, Z = CH</td>
<td>0.103</td>
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<tr>
<td>13</td>
<td>6-13</td>
<td></td>
<td>X = H</td>
<td>Y = CH, Z = N</td>
<td>0.047</td>
</tr>
<tr>
<td>14</td>
<td>6-14</td>
<td></td>
<td>X = H</td>
<td>Y = N, Z = CH</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*IC₅₀ values were determined by Ellman’s method against eeAChE.*
### Table 8.4 Stemofoline alkaloids and derivatives used in training and test sets.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Code</th>
<th>Compound</th>
<th>R</th>
<th>Minimum inhibitory requirements (nmol)</th>
<th>Estimated IC(_{50}) values (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Galanthamine</td>
<td><img src="image" alt="Compound" /></td>
<td>0.003</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>OH-1</td>
<td><img src="image" alt="Compound" /></td>
<td>1.247</td>
<td>124.7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>OH-2</td>
<td><img src="image" alt="Compound" /></td>
<td>0.249</td>
<td>24.9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>OH-3</td>
<td><img src="image" alt="Compound" /></td>
<td>0.290</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>OH-4</td>
<td><img src="image" alt="Compound" /></td>
<td>0.025</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>OH-5</td>
<td><img src="image" alt="Compound" /></td>
<td>0.248</td>
<td>24.8</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>OH-6</td>
<td><img src="image" alt="Compound" /></td>
<td>0.013</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>OH-7</td>
<td><img src="image" alt="Compound" /></td>
<td>0.124</td>
<td>12.4</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>OH-8</td>
<td><img src="image" alt="Compound" /></td>
<td>0.120</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>OH-9</td>
<td><img src="image" alt="Compound" /></td>
<td>0.012</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>OH-10</td>
<td><img src="image" alt="Compound" /></td>
<td>0.025</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>OH-11</td>
<td><img src="image" alt="Compound" /></td>
<td>0.026</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>OH-12</td>
<td><img src="image" alt="Compound" /></td>
<td>0.025</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>OH-13</td>
<td><img src="image" alt="Compound" /></td>
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<td></td>
</tr>
<tr>
<td>15</td>
<td>OH-14</td>
<td><img src="image" alt="Compound" /></td>
<td>0.028</td>
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<td></td>
</tr>
<tr>
<td>16</td>
<td>OH-15</td>
<td><img src="image" alt="Compound" /></td>
<td>0.024</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>OH-16</td>
<td><img src="image" alt="Compound" /></td>
<td>0.119</td>
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<td></td>
</tr>
<tr>
<td>18</td>
<td>OH-19</td>
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<td>12.5</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>OH-26</td>
<td><img src="image" alt="Compound" /></td>
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<td>24.1</td>
<td></td>
</tr>
<tr>
<td>Entry</td>
<td>Code</td>
<td>Compound</td>
<td>R</td>
<td>Minimum inhibitory requirements (nmol)(^a)</td>
<td>Estimated IC(_{50}) values ((\mu M))(^b)</td>
</tr>
<tr>
<td>-------</td>
<td>-------</td>
<td>----------</td>
<td>------------</td>
<td>---------------------------------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>20</td>
<td>OH-27</td>
<td><img src="image" alt="Compound 183" /></td>
<td><img src="image" alt="R 183" /></td>
<td>0.025</td>
<td>2.5</td>
</tr>
<tr>
<td>21</td>
<td>OH-28</td>
<td><img src="image" alt="Compound 184" /></td>
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<td>0.125</td>
<td>12.5</td>
</tr>
<tr>
<td>22</td>
<td>OH-29</td>
<td><img src="image" alt="Compound 185" /></td>
<td><img src="image" alt="R 185" /></td>
<td>0.113</td>
<td>11.3</td>
</tr>
<tr>
<td>23</td>
<td>OH-30</td>
<td><img src="image" alt="Compound 186" /></td>
<td><img src="image" alt="R 186" /></td>
<td>0.113</td>
<td>11.3</td>
</tr>
<tr>
<td>24</td>
<td>OH-31</td>
<td><img src="image" alt="Compound 188" /></td>
<td><img src="image" alt="R 188" /></td>
<td>1.188</td>
<td>118.8</td>
</tr>
<tr>
<td>25</td>
<td>OH-32</td>
<td><img src="image" alt="Compound 187" /></td>
<td><img src="image" alt="R 187" /></td>
<td>0.361</td>
<td>36.1</td>
</tr>
<tr>
<td>26</td>
<td>OH-34a</td>
<td><img src="image" alt="Compound 190a" /></td>
<td><img src="image" alt="R 190a" /></td>
<td>0.129</td>
<td>12.9</td>
</tr>
<tr>
<td>27</td>
<td>OH-34b</td>
<td><img src="image" alt="Compound 190b" /></td>
<td><img src="image" alt="R 190b" /></td>
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<td>2.4</td>
</tr>
<tr>
<td>28</td>
<td>OH-34c</td>
<td><img src="image" alt="Compound 190c" /></td>
<td><img src="image" alt="R 190c" /></td>
<td>0.023</td>
<td>2.3</td>
</tr>
<tr>
<td>29</td>
<td>NH-1</td>
<td><img src="image" alt="Compound 193" /></td>
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<td>0.012</td>
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<td>30</td>
<td>NH-2</td>
<td><img src="image" alt="Compound 191" /></td>
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<td>0.013</td>
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</tr>
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<td>31</td>
<td>NH-3</td>
<td><img src="image" alt="Compound 201" /></td>
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<tr>
<td>32</td>
<td>NH-4</td>
<td><img src="image" alt="Compound 197" /></td>
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<td>0.023</td>
<td>2.3</td>
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<tr>
<td>33</td>
<td>NH-5</td>
<td><img src="image" alt="Compound 209" /></td>
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<td>34</td>
<td>NH-6</td>
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<td>35</td>
<td>NH-7</td>
<td><img src="image" alt="Compound 196" /></td>
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<td>0.025</td>
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<tr>
<td>36</td>
<td>NH-8</td>
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<td><img src="image" alt="R 198" /></td>
<td>0.100</td>
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<td>37</td>
<td>NH-9</td>
<td><img src="image" alt="Compound 200" /></td>
<td><img src="image" alt="R 200" /></td>
<td>0.115</td>
<td>11.5</td>
</tr>
</tbody>
</table>
Table 8.4 (continued)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Code</th>
<th>Compound</th>
<th>R</th>
<th>Minimum inhibitory requirements (nmol)</th>
<th>Estimated IC$_{50}$ values (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>NH-10</td>
<td>![Compound 202]</td>
<td>$X$</td>
<td>0.121</td>
<td>12.1</td>
</tr>
<tr>
<td>39</td>
<td>NH-11</td>
<td>![Compound 199]</td>
<td>$X$</td>
<td>0.226</td>
<td>22.6</td>
</tr>
<tr>
<td>40</td>
<td>NH-12</td>
<td>![Compound 195]</td>
<td>$X$</td>
<td>0.232</td>
<td>23.2</td>
</tr>
<tr>
<td>41</td>
<td>NH-15</td>
<td>![Compound 205]</td>
<td>$X$</td>
<td>0.023</td>
<td>2.3</td>
</tr>
<tr>
<td>42</td>
<td>NH-16</td>
<td>![Compound 206]</td>
<td>$X$</td>
<td>0.022</td>
<td>2.2</td>
</tr>
<tr>
<td>43</td>
<td>NH-17</td>
<td>![Compound 207]</td>
<td>$X$</td>
<td>0.011</td>
<td>1.1</td>
</tr>
</tbody>
</table>

$^a$Minimum inhibitory requirements (MIRs) were determined by the TLC bioautographic method against eeAChE. $^b$Estimated IC$_{50}$ values were calculated by multiplying their MIRs by 100.

The IC$_{50}$ value of galanthamine (0.36 µM, Table 8.4, entry 1) that was used is from the literature obtained from an assay using Ellman’s method (reference number 82) while its minimum inhibitory requirement (MIR), which was obtained from the TLC bioautographic method (which was performed in our laboratory), was 0.003 nmol. At the time, none of the stemofoline alkaloids or their derivatives had been tested for their IC$_{50}$ values against AChE. Therefore, based on the relationship between the IC$_{50}$ value and the MIR of galanthamine, we have estimated the IC$_{50}$ values of our test compounds by multiplying their MIR values by 100 and expressing these values in µM.
Pharmacophores, also called hypotheses, are created in three phases, constructive, subtractive and optimization phases. The constructive phase is to generate hypotheses with chemical features that are common to the active compounds of the training set (leads) while the subtractive phase is to remove common chemical features in the inactive compounds of the training set. If the excluded volumes option is used, the subtractive phase is not applied. Rather, inactive compounds are used to place excluded volumes. A maximum of five general chemical features can be selected for building hypotheses. The chemical features used were supported by DS 2.5.5 including hydrogen-bond acceptor (HBA), hydrogen-bond donor (HBD), positive ionisable groups (PI) which represent atoms that are or could be protonated at physiological pH and become positively charged (e.g. amino groups), hydrophobic aliphatic groups (Hal) and hydrophobic aromatic groups (Har), and one user-defined feature, cyclic π-interaction (CYPI), which maps all five- or six-membered rings capable of π-interactions. Excluded volumes represent regions where none of the parts of the molecule can appear.

In the optimization phase, all hypotheses are validated by measured parameters. Two theoretical costs are calculated and measured in units of bits, ‘fixed cost’ is the minimum cost and ‘null cost’ is the maximum cost. The greater the difference between these two cost values, the higher the probability for finding useful models. The optimized hypothesis cost (total cost) should be different to the null cost by >60 bits which indicates a >90% chance of representing a true correlation in the data. The correlation value ($R^2$) is calculated between real and estimated affinity values in which 1.0 is a perfect correlation. The configuration value should be less than 17 to ensure all possibilities have been considered. Variable weights and tolerances are allowed in order to fully explore the possibilities of the training set. In these calculations, the rescale activity function was sometimes used. This function inflates the spread of activities and sometimes provides better results. Sometimes an editing of the training set was required particularly if the configuration cost was high. This could be achieved by choosing one compound only, from a group of compounds having the same activities and similar structures, to keep in the training set while excluding the others.
8.2.1 The active site binding pharmacophore model (ASB)

The training set for the ASB model contained 28 compounds (Table 8.1) which were assumed to bind at the active site of AChE due to their galanthamine-like or compact structures. The initial hypotheses (Table 8.5) were generated using HBA, HBD, Hal, PI and CYPI features. The parameters were varied without excluded volumes or with a maximum of 20 excluded volumes (Table 8.5, trials 1 and 2, respectively). Rescale activity was enabled since the range of input activities was quite small. Even though the configuration values were less than 17 in both hypotheses and the addition of excluded volumes (Table 8.5, trial 2) gave much better correlation value of 0.93, both cost differences were less than 60 bits. To improve the cost difference, variable weights and tolerances were applied to these two trials (Table 8.5, trials 3 and 4). However, both trials gave inferior results than the previous experiments. The experiment was then repeated with the same features, a maximum of 20 excluded volumes, without rescale activity and variable weights and tolerances and the activity uncertainty value was changed to 2.5 instead of the default value of 3 (Table 8.5, trial 5). The uncertainty value determines how many compounds are used as leads (in the constructive phase). A significant improvement was observed as the cost difference was improved to 60.27 bits, the configuration value was 8.48 and the correlation value was close to 1.0 with a value of 0.93. In each trial, 10 hypotheses were generated (default option) and the first hypothesis of the best trial (Table 8.5, trial 5) was chosen to be the final ASB model. This model contained 1x Hal, 1x HBD, 1x CYPI features and 8 excluded volumes as shown in Figure 8.1. The most active compound in this training set (Table 8.1, entry 8) had the hydroxyl group, the methoxy group and the aromatic ring mapped to the HBD, Hal and CYPI pharmacophore features, respectively, while no part of the molecule appeared in any area occupied by the excluded volumes.
Figure 8.1 The final ASB model with the most active compound in the training set mapped. 3D representation on the left with the pharmacophore features represented as colour coded mesh spheres for the location constraints placed on each feature. For the HBD and CYPI features, an arrow indicates the directionality of the interaction with the second mesh sphere indicating the proposed location of the interacting feature on the protein. For clarity, a 2D representation is shown on the right.
Table 8.5 Pharmacophore generation history for the ASB pharmacophore model. See text for more detailed explanation.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Training set</th>
<th>Chemical features</th>
<th>Maximum excluded volumes</th>
<th>Activity uncertainty</th>
<th>Rescale activity</th>
<th>Variable weights &amp; tolerances</th>
<th>Results for the top-scored hypothesis in each trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Table 8.1 with total conformers of 1,565.</td>
<td>HBA, HBD, Hal, PI, CYPI</td>
<td>0</td>
<td>3</td>
<td>Y</td>
<td>N</td>
<td>Five leads (Entries 4, 6, 8, 7 and 9) Four inactive compounds (Entries 10, 15, 16 and 17) Total cost = 147.013 Null cost = 167.128 Fixed cost = 103.243 Cost difference = 20.115 Correlation value = 0.64 Configuration value = 7.94</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>20</td>
<td>3</td>
<td>Y</td>
<td>N</td>
<td>Five leads (Entries 4, 6, 8, 7 and 9) Total cost = 114.986 Null cost = 167.128 Fixed cost = 103.243 Cost difference = 52.142 Correlation value = 0.93 Configuration value = 8.48</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td>0</td>
<td>3</td>
<td>Y</td>
<td>Y</td>
<td>Five leads (Entries 4, 6, 8, 7 and 9) Four inactive compounds (Entries 10, 15, 16 and 17) Total cost = 157.472 Null cost = 167.128 Fixed cost = 127.610 Cost difference = 9.656 Correlation value = 0.78 Configuration value = 31.16</td>
</tr>
</tbody>
</table>
The results highlighted in green are considered as good results while those in red are considered as poor and require further optimization. The final pharmacophore models are highlighted in orange.
8.2.2 The bis-functional binding pharmacophore model (BFB)

The initial training set for the BFB model contained 74 compounds (Table 8.2) which were assumed to bind at both the active site and the PAS of AChE based on an X-ray crystal structure of TcAChE-compound 2-3d complex (PDB code 1W4L). The hypotheses (Table 8.6) were generated using the HBA, HBD, Hal, PI and CYPI features. The parameters were varied without excluded volumes or with a maximum of 20 excluded volumes (Table 8.6, trials 1 and 2, respectively). Rescale activity was enabled since the range of input activities was quite small. The cost differences were >60 bits, the correlation values were slightly low (0.74 and 0.76) and the configuration values were more than 17. This may be because there were too many inactive compounds in the training set (Table 8.6, trial 1). Thus the training set was edited with the removal of compounds 9-18, 9-21a-c (Table 8.2, entries 67-70) and the experiment was repeated with the same features, a maximum of 20 excluded volumes, without rescale activity and variable weights and tolerances (Table 8.6, trials 3 and 4). The cost difference and the correlation value were better with excluded volumes application (Table 8.6, trial 4), however the configuration value was slightly higher than the limitation of 17. Since there were no HBA or HBD features included in any generated hypothesis, a similar experiment to that of trial 3 was performed that excluded these features but included the Hal, PI and CYPI features. However, this modification provided no improvement in the quality of the results. The training set was then reduced to a smaller set (Table 8.6, trial 6). With excluded volumes application, the correlation value was improved to 0.92 but the configuration value was more than 17 which was similar to that in trial 5. Compound 9-22c (Table 8.2, entry 73) was excluded from the training set and the experiment was repeated using similar features and parameters to those of the previous experiment (Table 8.6, trial 7). Even though the cost difference was lower than 60 bits, the configuration and correlation values were good (16.49 and 0.93, respectively). The experiment was repeated again using HBA, HBD, Hal, PI and CYPI features (Table 8.6, trial 8). The cost difference and the correlation value were only slightly better than those of trial 7. Two final pharmacophore models were selected from the first hypotheses of trials 7 and 8 in which both hypotheses included 4x Hals, 1x PI pharmacophore features and 10 excluded volumes (Figure 8.2).
Compound 9-22b (Table 8.2, entry 72) was the most active compound in the BFB training set. In both models, the positively charged nitrogen mapped onto the PI feature while the uncharged aromatic rings and the aliphatic linkers mapped to the Hal features (Figure 8.2).

**Figure 8.2** The final BFB pharmacophore models with the most active compound in the training set (Table 8.2, entry 72). See Figure 8.1 for further explanation.
Table 8.6 Pharmacophore generation history for the BFB pharmacophore model. See text for more detailed explanation.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Training set</th>
<th>Chemical features</th>
<th>Maximum excluded volumes</th>
<th>Activity uncertainty</th>
<th>Rescale activity</th>
<th>Variable weights &amp; tolerances</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Table 8.2 with total conformers of 8,616.</td>
<td>HBA, HBD, Hal, PI, CYPI</td>
<td>0</td>
<td>3</td>
<td>Y</td>
<td>N</td>
<td>Two leads (Entries 72 and 73) Eight inactive compounds (Entries 7, 12, 23, 62, 67, 68, 69 and 70) Total cost = 334.976 Null cost = 396.505 Fixed cost = 268.050 Cost difference = 61.525 Correlation value = 0.74 Configuration value = 18.02</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>3</td>
<td>Y</td>
<td>N</td>
<td></td>
<td></td>
<td>Two leads (Entries 72 and 73) Total cost = 329.775 Null cost = 396.505 Fixed cost = 268.140 Cost difference = 66.730 Correlation value = 0.76 Configuration value = 18.11</td>
</tr>
<tr>
<td>3</td>
<td>Excluded Entries 67-70. Total conformers is 7,622.</td>
<td>0</td>
<td>3</td>
<td>Y</td>
<td>N</td>
<td></td>
<td>Two leads (Entries 72 and 73) Eight inactive compounds (Entries 7, 12, 16, 23, 32, 50 and 62) Total cost = 339.836 Null cost = 367.785 Fixed cost = 254.673 Cost difference = 27.949 Correlation value = 0.60 Configuration value = 18.10</td>
</tr>
</tbody>
</table>
Table 8.6 (continued)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Training set</th>
<th>Chemical features</th>
<th>Maximum excluded volumes</th>
<th>Activity uncertainty</th>
<th>Rescale activity</th>
<th>Variable weights &amp; tolerances</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Excluded entries 67-70. Total conformers is 7,622.</td>
<td>HBA, HBD, Hal, PI, CYPI</td>
<td>20</td>
<td>3</td>
<td>Y</td>
<td>N</td>
<td>Two leads (Entries 72 and 73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cost difference = 68.062</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Hal, Pi, CYPI</td>
<td>0</td>
<td>3</td>
<td>Y</td>
<td>N</td>
<td>Two leads (Entries 72 and 73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cost difference = 38.916</td>
</tr>
<tr>
<td>6</td>
<td>Compounds excluded.*</td>
<td>Total conformers is 3,717.</td>
<td>20</td>
<td>3</td>
<td>N</td>
<td>N</td>
<td>Two leads (Entries 72 and 73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cost difference = 44.884</td>
</tr>
</tbody>
</table>

*Compounds from Table 8.2, entries 2, 5-7, 13, 15, 17-27, 33, 35, 37-45, 48, 51, 54, 56, 58, 59, 61, 62, 65, 66 and 71 were excluded from the previous training set used in trial 5.
Table 8.4 (continued)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Training set</th>
<th>Chemical features</th>
<th>Maximum excluded volumes</th>
<th>Activity uncertainty</th>
<th>Rescale activity</th>
<th>Variable weights &amp; tolerances</th>
<th>Results</th>
</tr>
</thead>
</table>
| 7     | Excluded entry 73. Total conformers is 3,474. | Hal, Pi, CYPI | 20 | 3 | N | N | One lead (Entry 72)  
Total cost = 127.040  
Null cost = 163.537  
Fixed cost = 118.523  
Cost difference = 36.497  
Correlation value = 0.93  
Configuration value = 16.49 |
| 8     | HBA, HBD, Hal, PI, CYPI | 20 | 3 | N | N | One lead (Entry 72)  
Total cost = 125.452  
Null cost = 163.537  
Fixed cost = 118.523  
Cost difference = 38.085  
Correlation value = 0.95  
Configuration value = 16.49 |

The results highlighted in green are considered as good results while those in red are considered as poor and require further optimization. When compounds were excluded from the training set and were used in the previous trial, the new training set was indicated in yellow. The final pharmacophore models are highlighted in orange.
8.2.3 The unknown pharmacophore model (Unknown)

The Unknown training set comprised 56 compounds from the Tacripyrine set (Table 8.3) and the *Stemona* set (Table 8.4, entries 2-43). While the binding mode of *Stemona* compounds was unknown, this model assumed these compounds bind at the PAS of AChE. Previous molecular modelling studies on tacripyrine and its derivatives suggested that these compounds bind at the PAS of AChE. The initial hypotheses were generated using HBA, HBD, Hal, PI and CYPI features with a maximum of 20 excluded volumes (Table 8.7, trial 1). The cost difference was >60 bits and the correlation and configuration values were good (0.88 and 10.91, respectively). The final model from trial 1 contained 2x Hals and 1x CYPI features (Figure 8.3). A similar experiment was repeated using HBA, HBD, Hal, Har and PI features with a maximum of 20 excluded volumes (Table 8.7, trial 2). The results of the first hypothesis of trial 2 were similar to those of trial 1, however, the model included 3x Hals and 1x HBA features (Figure 8.3).

Compound 6-5 (Table 8.3, entry 5) was the most active compound in the training set and was mapped onto the Unknown pharmacophore model from trial 1. The aromatic ring mapped to the CYPI feature, while the methyl group and the ethoxy group mapped onto the Hal features (Figure 8.3). The model generated from trial 2 had compound 6-10 (Table 8.3, entry 10) as the most active compound in the training set. Its nitrogen in the aromatic ring mapped to the HBA feature while the methyl group, the ethoxy group and the aliphatic ring mapped to the Hal features (Figure 8.3).
**Figure 8.3** The final Unknown pharmacophore models with the most active compounds in the training set. See Figure 8.1 for further explanation.
Table 8.7 Pharmacophore generation history for the Unknown pharmacophore model. See text for more detailed explanation.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Training set</th>
<th>Chemical features</th>
<th>Maximum excluded volumes</th>
<th>Activity uncertainty</th>
<th>Rescale activity</th>
<th>Variable weights &amp; tolerances</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Table 8.3 and Table 8.4, entries 2-43 with total conformers of 641.</td>
<td>HBA, HBD, Hal, PI, CYPI</td>
<td>20</td>
<td>3</td>
<td>N</td>
<td>N</td>
<td>Eight leads (Table 8.3, entries 1, 2, 7, 8, 10, 11 and 13) Total cost = 220.083 Null cost = 283.414 Fixed cost = 197.035 Cost difference = 63.331 Correlation value = 0.88 Configuration value = 10.91</td>
</tr>
<tr>
<td>2</td>
<td>HBA, HBD, Hal, Har, PI</td>
<td>20</td>
<td>3</td>
<td>N</td>
<td>N</td>
<td>Eight leads (Table 8.3, entries 1, 2, 7, 8, 10, 11 and 13) Total cost = 220.011 Null cost = 283.414 Fixed cost = 196.201 Cost difference = 63.403 Correlation value = 0.86 Configuration value = 10.08</td>
<td></td>
</tr>
</tbody>
</table>

The results highlighted in green are considered as good results and the final pharmacophore models are highlighted in orange.
8.2.4 The Tacripyrine pharmacophore model (Tacripyrine)

A set of compounds with a tacripyrine skeleton (Table 8.3) was used as a training set. The initial hypotheses were generated using HBA, HBD, Hal, PI and CYPI features with a maximum of 20 excluded volumes (Table 8.8, trial 1). Despite the good configuration value of 10.79, the total cost was not in the range between the fixed and null costs which indicated poor statistical significance of the data in this training set. A similar experiment was repeated with replacement of the CYPI feature with Har and PI features (Table 8.8, trial 2). This experiment resulted in similar results to the previous trial. As this set of compounds was small and their activities were highly similar, the activity rescaling was applied (Table 8.8, trial 3). As a result, the cost difference was found to be in the range between the fixed and null costs but still less than 60. The correlation value was significantly improved to 0.96 with an acceptable configuration value as 10.34. The final model (Figure 8.4) was constructed with 2x Hals, 1x Har, 1x HBD features and 7 excluded volumes. Compound 6-2 (Table 8.3, entry 2) was the most active compound in the training set. The primary amine, the aromatic ring, the methyl group and the aliphatic ring mapped to the HBD, Har, Hal and Hal pharmacophore features, respectively (Figure 8.4).

![Figure 8.4](image.png)

**Figure 8.4** The final Tacripyrine pharmacophore model with the most active compound in the training set. See Figure 8.1 for further explanation.
Table 8.8 Pharmacophore generation history for the Tacipryrine pharmacophore model. See text for more detailed explanation.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Training set</th>
<th>Chemical features</th>
<th>Maximum excluded volumes</th>
<th>Activity uncertainty</th>
<th>Rescale activity</th>
<th>Variable weights &amp; tolerances</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Table 8.3 with total conformers of 453.</td>
<td>HBA, HBD, Hal, PI, CYPI</td>
<td>20</td>
<td>3</td>
<td>N N</td>
<td>Eight leads (Table 8.3, entries 1, 2, 7, 8, 10, 11 and 13) Total cost = 61.11 Null cost = 50.21 Fixed cost = 59.01 Cost difference = 10.90 Correlation value = 0.57 Configuration value = 10.79</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>HBA, HBD, Hal, Har, PI</td>
<td></td>
<td>20</td>
<td>3</td>
<td>N N</td>
<td>Eight leads (Table 8.3, entries 1, 2, 7, 8, 10, 11 and 13) Total cost = 59.86 Null cost = 50.21 Fixed cost = 58.16 Cost difference = 9.65 Correlation value = 0.68 Configuration value = 9.95</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td>20</td>
<td>3</td>
<td>Y N</td>
<td>Five leads (Table 8.3, entries 1, 2, 10, 11 and 13) Total cost = 61.43 Null cost = 86.58 Fixed cost = 58.56 Cost difference = 25.15 Correlation value = 0.96 Configuration value = 10.34</td>
<td></td>
</tr>
</tbody>
</table>

The results highlighted in green are considered as good results while those in red are considered as poor and require further optimization. The final pharmacophore model is highlighted in orange.
8.2.5 The *Stemona* pharmacophore model (*Stemona*)

A number of *Stemona* alkaloids and derivatives (Table 8.4, entries 2-43) was used as a training set. The first hypotheses were generated using HBA, HBD, Hal, Har and PI features with a maximum of 20 excluded volumes (Table 8.9, trial 1). Only nine hypotheses were generated which indicated a lack of significant variability in the training set. Since the range of input IC₅₀ values was very narrow, the experiment was then repeated with the activity rescaling application (Table 8.9, trial 2) and resulted in 10 hypotheses. The first hypothesis showed a good configuration value of 13.49 but its cost difference and correlation values were low (30.753 and 0.67, respectively). The best hypothesis from trial 2 used 2x HBAs, 1x HBD, 1x PI features and 2 excluded volumes (Figure 8.5). Compound NH-1 (Table 8.4, entry 29) was the most active compound in the training set. The oxygen of the carbonyl group, the oxygen bridge, the secondary amine at the side chain and the nitrogen of the core structure mapped onto the HBA, HBA, HBD and PI features, respectively (Figure 8.5).

When using a different set of features (HBA, HBD, Hal, PI and CYPI), again only nine hypotheses were generated (Table 8.9, trial 3). This problem was also solved with the activity rescaling application (Table 8.9, trial 4). The best hypothesis from trial 4 was chosen as the *Stemona* model. It contained 1x HBA, 1x HBD, 1x PI and 1x CYPI feature (Figure 8.5). Compound OH-27 (Table 8.4, entry 20) was the most active compound in the training set. The oxygen bridge, the hydroxyl group, the nitrogen of the core structure and the lactone ring mapped onto the HBA, HBD, PI and CYPI features, respectively (Figure 8.5). Therefore, only the final model from trial 4 was selected for use in the ligand-pharmacophore mapping because it had a better correlation value than the model from trial 2.
Table 8.9 Pharmacophore generation history for the Stemona pharmacophore model. See text for more detailed explanation.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Training set</th>
<th>Chemical features</th>
<th>Maximum excluded volumes</th>
<th>Activity uncertainty</th>
<th>Rescale activity</th>
<th>Variable weights &amp; tolerances</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Table 8.4, entries 2-43 with total conformers of 574.</td>
<td>HBA, HBD, Hal, Har, PI</td>
<td>20</td>
<td>3</td>
<td>N</td>
<td>N</td>
<td>Only nine hypotheses were generated.</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>3</td>
<td>Y</td>
<td>N</td>
<td>Eight leads (Table 8.4, entries 7, 10, 29, 30, 31, 32, 41 and 42) Total cost = 208.500 Null cost = 239.253 Fixed cost = 152.519 Cost difference = 30.753 Correlation value = 0.67 Configuration value = 13.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>HBA, HBD, Hal, PI, CYPI</td>
<td>20</td>
<td>3</td>
<td>N</td>
<td>N</td>
<td>Only nine hypotheses were generated.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>3</td>
<td>Y</td>
<td>N</td>
<td>Eight leads (Table 8.4, entries 7, 10, 29, 30, 31, 32, 41 and 42) Total cost = 202.865 Null cost = 239.253 Fixed cost = 152.847 Cost difference = 36.388 Correlation value = 0.71 Configuration value = 13.81</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results highlighted in green are considered as good results while those in red are considered as poor and require further optimization. The final pharmacophore model is highlighted in orange.
8.2.6 Ligand-pharmacophore mapping

To examine the pharmacophore models, the training sets previously used in pharmacophore generation were called the test sets for the ligand-pharmacophore mapping. Seven pharmacophore models were selected from Sections 8.2.1 – 8.2.5 including the ASB pharmacophore model (Table 8.5, trial 5), the BFB pharmacophore models (Table 8.6, trials 7 and 8), the Unknown pharmacophore model (Table 8.7, trial 1 and 2), the Tacipyrine pharmacophore model (Table 8.8, trial 3) and the Stemona pharmacophore model (Table 8.9, trial 4). Four test sets included the ASB set (Table 8.1), the BFB set (Table 8.2), the Unknown set (Table 8.3 and Table 8.4, entries 2-43) and the Stemona set (Table 8.4, entries 2-43).

The Stemona set was mapped onto the ASB (active site) pharmacophore model and only two compounds from this set, NH-3 and NH-9, were able to map (Table 8.8). The nitrogen in the side chain, the aromatic ring and the C-C bond between C-3 and C-7 mapped onto the HBD, CYPI and Hal features, respectively (Figure 8.6). The A,B,C,D ring structure did not map onto a pharmacophoric region.
**Figure 8.6** *Stemona* compounds mapped onto the ASB pharmacophore model. See Figure 8.1 for further explanation.

**Table 8.10** Number of compounds from each test set that were able to map to the final pharmacophore models.*

<table>
<thead>
<tr>
<th>Test sets</th>
<th>Pharmacophore models</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASB Trial 5</td>
</tr>
<tr>
<td>ASB</td>
<td>23</td>
</tr>
<tr>
<td>BFB</td>
<td>20</td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
</tr>
<tr>
<td><em>Stemona</em></td>
<td>2</td>
</tr>
</tbody>
</table>

*Red numbers are a number of *Stemona* compounds that mapped to a pharmacophore model.

Twenty compounds (1-5, 2-3c, 2-3d, 2-5c, 2-5d, 2-5e, 2-5f, 2-7b, 2-7c, 2-9d, 4-30, 9-11a, 9-11b, 9-11d, 9-11e, 9-11g, 9-11i, 9-12, 9-15 and 9-16a) of the BFB test set mapped onto the ASB pharmacophore model (Table 8.10). The galanthamine moiety of the *N* and *O*-substituted galanthamine derivatives 1-5 to 2-9d mapped onto the ASB pharmacophore model as shown in Figure 8.7. While for the bis-carbazole like compounds, 9-11a – 9-16a, the carbazole moiety mapped onto the ASB model as shown in Figure 8.7. These results lead us to the hypothesis that some parts of the molecules in the BFB (bis-functional) set, especially their core heterocyclic structures, may bind to the active site of AChE.
Figure 8.7 Galanthamine derivatives and carbazole like derivatives mapped onto the ABS pharmacophore model.

When we tried to map the Stemona set onto both BFB pharmacophore models, none of compounds fitted to the pharmacophore model (Table 8.10). This result showed that the BFB model simulated a particular part of the binding site either the active site or the PAS which did not fit to any Stemona compound. It showed a small number of compounds from the ASB and BFB sets mapped to the BFB model which indicated that the BFB model was unreliable.

According to Table 8.10, the Stemona test set was able to map onto both Unknown pharmacophore models. Mapping to the Unknown trial 1 pharmacophore model (Table 8.11), most of the compounds had their lactone rings mapped to the CYPI pharmacophore feature while the methyl group and the aliphatic carbons C-5 and C-6 mapped onto two of the Hal features as shown in the 2D representation of compound OH-27 (Figure 8.8). The exceptions, NH-3 and OH-29, showed the aromatic ring of NH-3 mapped to the CYPI feature and the alkene of OH-29 mapped to the Hal feature (Figure 8.8).

When mapping the Stemona set to the Unknown trial 2 model, Stemona compounds could fit in various orientations. The compounds with good estimated activities (Table 8.12, entries 1-9) fitted to the model with the hydroxyl groups on the side chain mapping close to the HBA feature (Figure 8.9). On the other hand, compounds with lowest estimated activities (Table 8.10, entries 21-36) had a different orientation according to the absence of a hydroxyl group in the side chain. This resulted in the oxygen on the furan ring to map onto the HBA feature and forced 1x Hal feature to be missed (Figure 8.9).
**Figure 8.8** *Stemona* compounds mapped onto the Unknown trial 1 pharmacophore model. See Figure 8.1 for further explanation.

**Figure 8.9** *Stemona* compounds mapped onto the Unknown trial 2 pharmacophore model.

When the two Unknown models were compared, the error range between actual and estimated activities for the Unknown trial 1 pharmacophore model (Table 8.11) was better than that for the Unknown trial 2 pharmacophore model (Table 8.12). Moreover, the number of *Stemona* compounds that fit to the Unknown trial 1 pharmacophore model was slightly higher than that for the Unknown trial 1 pharmacophore model (Table 8.10). The orientation was limited when using the Unknown trial 1 pharmacophore model which contained the CYPI feature while a number of orientations occurred when using the Unknown trial 2 pharmacophore model. The Unknown trial 1 model proved to be reliable and suitable for the *Stemona* compounds in prediction of their activities.
Table 8.11 Activity prediction (MIRs) of *Stemona* derivatives mapped to the Unknown trial 1 pharmacophore model by Discovery Studio. The error ratios were calculated between actual activities and estimated activities.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound code (Compound number)</th>
<th>Estimated activities* (MIRs, x100 nmol)</th>
<th>Actual activities (MIRs, x100 nmol)</th>
<th>Error ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OH-27 (183)</td>
<td>1.62</td>
<td>2.50</td>
<td>1.54</td>
</tr>
<tr>
<td>2</td>
<td>NH-1 (193)</td>
<td>2.28</td>
<td>1.20</td>
<td>1.90</td>
</tr>
<tr>
<td>3</td>
<td>OH-34c (190c)</td>
<td>2.29</td>
<td>2.30</td>
<td>1.01</td>
</tr>
<tr>
<td>4</td>
<td>OH-26 (170)</td>
<td>2.38</td>
<td>24.10</td>
<td>10.14</td>
</tr>
<tr>
<td>5</td>
<td>NH-4 (197)</td>
<td>2.49</td>
<td>2.30</td>
<td>1.08</td>
</tr>
<tr>
<td>6</td>
<td>OH-14 (208)</td>
<td>2.85</td>
<td>2.80</td>
<td>1.02</td>
</tr>
<tr>
<td>7</td>
<td>NH-3 (201)</td>
<td>3.49</td>
<td>2.20</td>
<td>1.59</td>
</tr>
<tr>
<td>8</td>
<td>OH-11 (97)</td>
<td>4.15</td>
<td>2.60</td>
<td>1.60</td>
</tr>
<tr>
<td>9</td>
<td>OH-15 (173)</td>
<td>4.15</td>
<td>2.40</td>
<td>1.73</td>
</tr>
<tr>
<td>10</td>
<td>OH-7 (115)</td>
<td>4.18</td>
<td>12.40</td>
<td>2.97</td>
</tr>
<tr>
<td>11</td>
<td>NH-11 (199)</td>
<td>4.23</td>
<td>22.60</td>
<td>5.35</td>
</tr>
<tr>
<td>12</td>
<td>OH-4 (110)</td>
<td>4.31</td>
<td>2.50</td>
<td>1.72</td>
</tr>
<tr>
<td>13</td>
<td>NH-8 (198)</td>
<td>4.38</td>
<td>10.00</td>
<td>2.28</td>
</tr>
<tr>
<td>14</td>
<td>OH-9 (98)</td>
<td>4.40</td>
<td>1.20</td>
<td>3.66</td>
</tr>
<tr>
<td>15</td>
<td>NH-16 (206)</td>
<td>4.82</td>
<td>2.20</td>
<td>2.19</td>
</tr>
<tr>
<td>16</td>
<td>NH-5 (209)</td>
<td>5.41</td>
<td>2.40</td>
<td>2.25</td>
</tr>
<tr>
<td>17</td>
<td>OH-8 (116)</td>
<td>5.41</td>
<td>12.00</td>
<td>2.22</td>
</tr>
<tr>
<td>18</td>
<td>OH-29 (185)</td>
<td>5.54</td>
<td>11.30</td>
<td>2.04</td>
</tr>
<tr>
<td>19</td>
<td>OH-6 (104)</td>
<td>5.77</td>
<td>1.30</td>
<td>4.44</td>
</tr>
<tr>
<td>20</td>
<td>OH-12 (100)</td>
<td>5.78</td>
<td>2.50</td>
<td>2.31</td>
</tr>
<tr>
<td>21</td>
<td>OH-30 (186)</td>
<td>5.87</td>
<td>11.30</td>
<td>1.93</td>
</tr>
<tr>
<td>22</td>
<td>NH-6 (194)</td>
<td>6.62</td>
<td>2.50</td>
<td>2.65</td>
</tr>
<tr>
<td>23</td>
<td>OH-13 (99)</td>
<td>6.67</td>
<td>2.50</td>
<td>2.67</td>
</tr>
<tr>
<td>24</td>
<td>OH-28 (184)</td>
<td>6.67</td>
<td>12.50</td>
<td>1.87</td>
</tr>
<tr>
<td>25</td>
<td>NH-15 (205)</td>
<td>7.03</td>
<td>2.30</td>
<td>3.06</td>
</tr>
<tr>
<td>26</td>
<td>OH-34a (190a)</td>
<td>7.28</td>
<td>12.90</td>
<td>1.77</td>
</tr>
<tr>
<td>27</td>
<td>NH-34b (190b)</td>
<td>7.35</td>
<td>2.40</td>
<td>3.06</td>
</tr>
<tr>
<td>28</td>
<td>OH-19 (169)</td>
<td>7.51</td>
<td>12.50</td>
<td>1.66</td>
</tr>
<tr>
<td>29</td>
<td>NH-7 (196)</td>
<td>7.56</td>
<td>2.50</td>
<td>3.02</td>
</tr>
<tr>
<td>30</td>
<td>NH-9 (200)</td>
<td>7.60</td>
<td>11.50</td>
<td>1.51</td>
</tr>
<tr>
<td>31</td>
<td>OH-16 (174)</td>
<td>7.79</td>
<td>11.90</td>
<td>1.53</td>
</tr>
<tr>
<td>32</td>
<td>NH-10 (202)</td>
<td>8.46</td>
<td>12.10</td>
<td>1.43</td>
</tr>
<tr>
<td>33</td>
<td>OH-10 (166)</td>
<td>9.59</td>
<td>2.50</td>
<td>3.84</td>
</tr>
<tr>
<td>34</td>
<td>OH-2 (102)</td>
<td>11.63</td>
<td>24.90</td>
<td>2.14</td>
</tr>
<tr>
<td>35</td>
<td>NH-12 (195)</td>
<td>11.68</td>
<td>23.20</td>
<td>1.99</td>
</tr>
<tr>
<td>36</td>
<td>OH-1 (101)</td>
<td>12.57</td>
<td>124.70</td>
<td>9.92</td>
</tr>
<tr>
<td>37</td>
<td>OH-3 (107)</td>
<td>12.85</td>
<td>29.00</td>
<td>2.26</td>
</tr>
<tr>
<td>38</td>
<td>OH-5 (167)</td>
<td>12.86</td>
<td>24.80</td>
<td>1.93</td>
</tr>
<tr>
<td>39</td>
<td>NH-2 (191)</td>
<td>16.19</td>
<td>1.30</td>
<td>12.45</td>
</tr>
</tbody>
</table>

* Calculated from the estimated IC<sub>50</sub> by dividing by 100.
Table 8.12 Activity prediction (MIRs) of *Stemona* derivatives mapped to the Unknown trial 2 pharmacophore model by Discovery Studio. The error ratios were calculated between actual activities and estimated activities.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound code (Compound number)</th>
<th>Estimated activities* (MIRs, x100 nmol)</th>
<th>Actual activities (MIRs, x100 nmol)</th>
<th>Error ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OH-28 (184)</td>
<td>3.78</td>
<td>12.50</td>
<td>3.31</td>
</tr>
<tr>
<td>2</td>
<td>OH-10 (166)</td>
<td>4.19</td>
<td>2.50</td>
<td>1.68</td>
</tr>
<tr>
<td>3</td>
<td>OH-9 (98)</td>
<td>4.20</td>
<td>1.20</td>
<td>3.5</td>
</tr>
<tr>
<td>4</td>
<td>OH-15 (173)</td>
<td>5.60</td>
<td>2.40</td>
<td>2.33</td>
</tr>
<tr>
<td>5</td>
<td>OH-16 (174)</td>
<td>10.09</td>
<td>11.90</td>
<td>1.18</td>
</tr>
<tr>
<td>6</td>
<td>OH-27 (183)</td>
<td>12.29</td>
<td>2.50</td>
<td>4.92</td>
</tr>
<tr>
<td>7</td>
<td>OH-12 (100)</td>
<td>23.44</td>
<td>2.50</td>
<td>9.38</td>
</tr>
<tr>
<td>8</td>
<td>OH-13 (99)</td>
<td>23.94</td>
<td>2.50</td>
<td>9.58</td>
</tr>
<tr>
<td>9</td>
<td>OH-29 (185)</td>
<td>25.29</td>
<td>11.30</td>
<td>2.24</td>
</tr>
<tr>
<td>10</td>
<td>OH-30 (186)</td>
<td>31.29</td>
<td>11.30</td>
<td>2.77</td>
</tr>
<tr>
<td>11</td>
<td>NH-16 (206)</td>
<td>63.48</td>
<td>2.20</td>
<td>28.85</td>
</tr>
<tr>
<td>12</td>
<td>OH-11 (97)</td>
<td>99.34</td>
<td>2.60</td>
<td>38.21</td>
</tr>
<tr>
<td>13</td>
<td>OH-31 (188)</td>
<td>101.09</td>
<td>118.80</td>
<td>1.18</td>
</tr>
<tr>
<td>14</td>
<td>NH-5 (209)</td>
<td>172.10</td>
<td>2.40</td>
<td>71.71</td>
</tr>
<tr>
<td>15</td>
<td>NH-8 (198)</td>
<td>853.39</td>
<td>10.00</td>
<td>85.34</td>
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<tr>
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<td>OH-8 (116)</td>
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<td>12.00</td>
<td>107.52</td>
</tr>
<tr>
<td>17</td>
<td>OH-32 (197)</td>
<td>1861.58</td>
<td>36.10</td>
<td>51.57</td>
</tr>
<tr>
<td>18</td>
<td>NH-6 (194)</td>
<td>3186.94</td>
<td>2.50</td>
<td>1274.78</td>
</tr>
<tr>
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<td>OH-6 (104)</td>
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<td>2623.52</td>
</tr>
<tr>
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<td>NH-3 (201)</td>
<td>15478.40</td>
<td>2.20</td>
<td>7035.64</td>
</tr>
<tr>
<td>21</td>
<td>NH-1 (193)</td>
<td>16236.30</td>
<td>1.20</td>
<td>13530.25</td>
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<tr>
<td>22</td>
<td>NH-4 (197)</td>
<td>16288.60</td>
<td>2.30</td>
<td>7082.00</td>
</tr>
<tr>
<td>23</td>
<td>OH-7 (115)</td>
<td>20786.80</td>
<td>12.40</td>
<td>1676.35</td>
</tr>
<tr>
<td>24</td>
<td>OH-34a (190b)</td>
<td>25205.50</td>
<td>2.40</td>
<td>10502.29</td>
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<tr>
<td>25</td>
<td>OH-14 (208)</td>
<td>27216.70</td>
<td>2.80</td>
<td>9720.25</td>
</tr>
<tr>
<td>26</td>
<td>NH-15 (205)</td>
<td>28048.70</td>
<td>2.30</td>
<td>12195.09</td>
</tr>
<tr>
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<td>NH-9 (200)</td>
<td>28672.80</td>
<td>11.50</td>
<td>2493.29</td>
</tr>
<tr>
<td>28</td>
<td>OH-34a (190a)</td>
<td>30303.80</td>
<td>12.90</td>
<td>2349.13</td>
</tr>
<tr>
<td>29</td>
<td>NH-11 (199)</td>
<td>30618.20</td>
<td>22.60</td>
<td>1354.79</td>
</tr>
<tr>
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<td>OH-4 (110)</td>
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<td>2.50</td>
<td>13081.12</td>
</tr>
<tr>
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<td>OH-3 (107)</td>
<td>36957.50</td>
<td>29.00</td>
<td>1274.40</td>
</tr>
<tr>
<td>32</td>
<td>NH-12 (195)</td>
<td>53714.00</td>
<td>23.20</td>
<td>2315.26</td>
</tr>
<tr>
<td>33</td>
<td>OH-1 (101)</td>
<td>55366.30</td>
<td>124.70</td>
<td>444.00</td>
</tr>
<tr>
<td>34</td>
<td>OH-2 (102)</td>
<td>58177.10</td>
<td>24.90</td>
<td>2336.43</td>
</tr>
<tr>
<td>35</td>
<td>NH-2 (191)</td>
<td>64171.20</td>
<td>1.30</td>
<td>49362.46</td>
</tr>
<tr>
<td>36</td>
<td>OH-5 (167)</td>
<td>86012.70</td>
<td>24.80</td>
<td>3468.25</td>
</tr>
</tbody>
</table>

* Calculated from the estimated IC_{50} by dividing by 100.

For the ligand-pharmacophore mapping of the BFB set on both Unknown models, the output was found to be similar showing high numbers of compounds mapped. This is caused by the high flexibility and foldable structures of compounds in the BFB set plus the small number of features included in the models. However, the core structures of compounds in the BFB (bis-functional) set consisting of galanthamine and β-carboline (Figure 8.7) preferred to fit on the CYPI feature of the Unknown trial 1 pharmacophore model.
As shown in Table 8.10, none of compounds in the *Stemona* set was able to map onto the Tacrypyrine pharmacophore model. However, some compounds from the ASB, BFB and Unknown sets fitted to this model (Tables 8.13-8.15), but the errors were large. The activity prediction using the Tacrypyrine pharmacophore model was thus very specific to its own training set structures.

**Table 8.13** Activity prediction (MIRs) of the ASB set mapped to the Tacrypyrine pharmacophore model by Discovery Studio. The error ratios were calculated between actual activities and estimated activities.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound code</th>
<th>Estimated activities* (MIRs, x100 nmol)</th>
<th>Actual activities (MIRs, x100 nmol)</th>
<th>Error ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7-17</td>
<td>1.50</td>
<td>7.85</td>
<td>5.23</td>
</tr>
<tr>
<td>2</td>
<td>1-4</td>
<td>31.16</td>
<td>3.15</td>
<td>9.89</td>
</tr>
<tr>
<td>3</td>
<td>7-6</td>
<td>225.97</td>
<td>4.86</td>
<td>46.50</td>
</tr>
<tr>
<td>4</td>
<td>7-2</td>
<td>305.73</td>
<td>10.00</td>
<td>30.57</td>
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<td>5</td>
<td>7-18</td>
<td>940.95</td>
<td>8.22</td>
<td>114.47</td>
</tr>
<tr>
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<td>7-19</td>
<td>1915.26</td>
<td>1.55</td>
<td>1235.65</td>
</tr>
<tr>
<td>7</td>
<td>Galanthamine</td>
<td>5889.17</td>
<td>0.36</td>
<td>16359.81</td>
</tr>
<tr>
<td>8</td>
<td>7-15</td>
<td>145176.00</td>
<td>27.86</td>
<td>5210.91</td>
</tr>
<tr>
<td>9</td>
<td>7-4</td>
<td>179719.00</td>
<td>10.00</td>
<td>17971.90</td>
</tr>
</tbody>
</table>

* Calculated from the estimated IC$_{50}$ by dividing by 100.

**Table 8.14** Activity prediction (MIRs) of the BFB set mapped to the Tacrypyrine pharmacophore model by Discovery Studio. The error ratios were calculated between actual activities and estimated activities.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound code</th>
<th>Estimated activities* (MIRs, x100 nmol)</th>
<th>Actual activities (MIRs, x100 nmol)</th>
<th>Error ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-7e</td>
<td>0.76</td>
<td>4.00</td>
<td>5.26</td>
</tr>
<tr>
<td>2</td>
<td>2-3c</td>
<td>7.38</td>
<td>0.99</td>
<td>7.46</td>
</tr>
<tr>
<td>3</td>
<td>9-11i</td>
<td>12.28</td>
<td>0.06</td>
<td>194.87</td>
</tr>
<tr>
<td>4</td>
<td>2-9d</td>
<td>19.02</td>
<td>9.70</td>
<td>1.96</td>
</tr>
<tr>
<td>5</td>
<td>2-3d</td>
<td>57.31</td>
<td>0.28</td>
<td>204.70</td>
</tr>
<tr>
<td>6</td>
<td>9-11e</td>
<td>903.93</td>
<td>0.25</td>
<td>3644.88</td>
</tr>
<tr>
<td>7</td>
<td>2-7b</td>
<td>1068.16</td>
<td>16.10</td>
<td>66.35</td>
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<td>3366.21</td>
<td>4.26</td>
<td>790.00</td>
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<td>4031.48</td>
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</tr>
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<td>15395.60</td>
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<td>2009.61</td>
</tr>
<tr>
<td>12</td>
<td>1-5</td>
<td>18399.50</td>
<td>0.03</td>
<td>613316.67</td>
</tr>
<tr>
<td>13</td>
<td>2-5d</td>
<td>50496.50</td>
<td>0.31</td>
<td>162891.94</td>
</tr>
<tr>
<td>14</td>
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<td>62033.30</td>
<td>0.12</td>
<td>516944.17</td>
</tr>
<tr>
<td>15</td>
<td>9-12</td>
<td>75905.40</td>
<td>3.14</td>
<td>24166.00</td>
</tr>
<tr>
<td>16</td>
<td>9-11d</td>
<td>121842.00</td>
<td>0.60</td>
<td>204776.47</td>
</tr>
<tr>
<td>17</td>
<td>2-5c</td>
<td>193887.00</td>
<td>1.70</td>
<td>114051.18</td>
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</tbody>
</table>

* Calculated from the estimated IC$_{50}$ by dividing by 100.
Table 8.15 Activity prediction (MIRs) of the Unknown set mapped to the Tacipryline pharmacophore model by Discovery Studio. The error ratios were calculated between actual activities and estimated activities.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound code</th>
<th>Estimated activities* (MIRs, x100 nmol)</th>
<th>Actual activities (MIRs, x100 nmol)</th>
<th>Error ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6-12</td>
<td>0.19</td>
<td>0.10</td>
<td>1.82</td>
</tr>
<tr>
<td>2</td>
<td>6-14</td>
<td>1.42</td>
<td>0.22</td>
<td>6.47</td>
</tr>
<tr>
<td>3</td>
<td>6-7</td>
<td>1.53</td>
<td>0.09</td>
<td>16.86</td>
</tr>
<tr>
<td>4</td>
<td>6-11</td>
<td>1.89</td>
<td>0.05</td>
<td>41.99</td>
</tr>
<tr>
<td>5</td>
<td>6-8</td>
<td>2.03</td>
<td>0.09</td>
<td>22.59</td>
</tr>
<tr>
<td>6</td>
<td>6-1</td>
<td>89.20</td>
<td>0.08</td>
<td>1114.99</td>
</tr>
<tr>
<td>7</td>
<td>6-3</td>
<td>161.10</td>
<td>0.21</td>
<td>767.13</td>
</tr>
<tr>
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<td>6-10</td>
<td>255.54</td>
<td>0.06</td>
<td>4189.23</td>
</tr>
<tr>
<td>9</td>
<td>6-5</td>
<td>260.29</td>
<td>0.11</td>
<td>2366.26</td>
</tr>
<tr>
<td>10</td>
<td>6-13</td>
<td>417.82</td>
<td>0.05</td>
<td>8889.77</td>
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<tr>
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<td>6-9</td>
<td>2512.05</td>
<td>0.16</td>
<td>15700.31</td>
</tr>
<tr>
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<td>6-2</td>
<td>39406.60</td>
<td>0.05</td>
<td>757819.23</td>
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<tr>
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<td>6-4</td>
<td>2144415.00</td>
<td>0.30</td>
<td>705312.50</td>
</tr>
</tbody>
</table>

* Calculated from the estimated IC<sub>50</sub> by dividing by 100.

The Stemona set was mapped onto the Stemona pharmacophore model and only 25 compounds fitted (Table 8.10). In general, the nitrogen atom of the core structure, the hydroxyl group or the amine of the side chain and the oxygen bridge mapped onto the PI, HBD and HBA features, respectively, as shown in Figure 8.10. The error range of prediction was acceptable and the estimated activities were close to the actual results (Table 8.16).

Figure 8.10 Stemona compounds mapped onto the Stemona model.

An analysis of the overall results from the ligand-pharmacophore mapping indicated that four out of seven final pharmacophore models fitted the Stemona set including the ABS, the Unknown trial 1, the Unknown trial 2 and the Stemona pharmacophore models. The Unknown trial 1 and Stemona pharmacophore models were the best as they predicted the activities with low error ratios. The lactone ring of the Stemona compounds mapped on the CYPI feature of both pharmacophore models while the other moieties of these compounds mapped to the other features as shown in Figure 8.8 and 8.10 for the Unknown trial 1 and the Stemona pharmacophore.
model, respectively. Our pharmacophore results suggested that the *Stemona* compounds did not strongly bind to the active site as most of compounds in the *Stemona* set mapped poorly onto the ASB model. The BFB model may represent the area around the active site since none of compounds in the Unknown set, which were assumed to bind at the PAS, mapped to this model. Further, it was not clear if the Unknown trial 1 model, even though it fitted to the *Stemona* set quite well, represented the area around the PAS. From the pharmacophore results, it appeared most likely that *Stemona* derivatives had their own unique binding mode, which was different from the binding modes observed for the known compounds in the other training sets. To shed further light onto potential binding modes, AChE-ligand docking was next performed.

**Table 8.16** Activity prediction (MIRs) of *Stemona* derivatives mapped to the *Stemona* pharmacophore model by Discovery Studio. The error ratios were calculated between actual activities and estimated activities.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound code (Compound number)</th>
<th>Estimated activities* (MIRs, 100 nmol)</th>
<th>Actual activities (MIRs, 100 nmol)</th>
<th>Error ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OH-27 (183)</td>
<td>1.40</td>
<td>2.50</td>
<td>1.78</td>
</tr>
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<td>2</td>
<td>NH-1 (193)</td>
<td>2.36</td>
<td>1.20</td>
<td>1.96</td>
</tr>
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<td>NH-6 (194)</td>
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<td>2.50</td>
<td>1.04</td>
</tr>
<tr>
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<td>OH-13 (99)</td>
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<td>2.50</td>
<td>1.16</td>
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<td>OH-9 (98)</td>
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<td>1.20</td>
<td>2.54</td>
</tr>
<tr>
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<td>NH-15 (205)</td>
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<td>2.30</td>
<td>1.90</td>
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<tr>
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<td>NH-10 (202)</td>
<td>4.70</td>
<td>12.10</td>
<td>2.57</td>
</tr>
<tr>
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<td>NH-3 (201)</td>
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<td>OH-15 (173)</td>
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<td>2.40</td>
<td>2.52</td>
</tr>
<tr>
<td>10</td>
<td>NH-7 (196)</td>
<td>7.21</td>
<td>2.50</td>
<td>2.88</td>
</tr>
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<td>OH-10 (166)</td>
<td>7.27</td>
<td>2.50</td>
<td>2.91</td>
</tr>
<tr>
<td>12</td>
<td>NH-16 (206)</td>
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<td>2.20</td>
<td>3.40</td>
</tr>
<tr>
<td>13</td>
<td>NH-2 (191)</td>
<td>8.28</td>
<td>1.30</td>
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</tr>
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<td>14</td>
<td>OH-14 (208)</td>
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<td>OH-16 (174)</td>
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<td>11.90</td>
<td>1.18</td>
</tr>
<tr>
<td>16</td>
<td>OH-12 (100)</td>
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<td>2.50</td>
<td>4.71</td>
</tr>
<tr>
<td>17</td>
<td>OH-4 (110)</td>
<td>19.22</td>
<td>2.50</td>
<td>7.69</td>
</tr>
<tr>
<td>18</td>
<td>OH-19 (169)</td>
<td>35.21</td>
<td>12.50</td>
<td>2.82</td>
</tr>
<tr>
<td>19</td>
<td>OH-28 (184)</td>
<td>36.69</td>
<td>12.50</td>
<td>2.94</td>
</tr>
<tr>
<td>20</td>
<td>OH-34a (190a)</td>
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<td>OH-7 (115)</td>
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<td>24</td>
<td>OH-5 (167)</td>
<td>127.29</td>
<td>24.80</td>
<td>5.13</td>
</tr>
<tr>
<td>25</td>
<td>OH-1 (101)</td>
<td>1843.71</td>
<td>124.70</td>
<td>14.79</td>
</tr>
</tbody>
</table>

* Calculated from the estimated IC₅₀ by dividing by 100.
8.3 Protein-ligand docking

Protein-ligand docking is a method to predict the preferred orientation and conformation of a small molecule (also called a ligand) binding to a protein while forming a stable complex. The protein used in the docking was obtained from an X-ray crystal structure of TcAChE bound with huprine X (HUX) at the active site (PDB code 1E66). The HUX-TcAChE structure was chosen over the galanthamine-TcAChE complex because of the lower resolution of the latter crystal structure. While other ligand-TcAChE crystal structures are available, the HUX ligand more closely resembles the *Stemona* alkaloids than the others because of its rigid and bridged ring structure. This X-ray structure was chosen to provide a conformer of AChE when binding to a ligand. Since there were only X-ray structures of native electric eel AChE (eeAChE) and native human AChE (hAChE), these two enzymes were modelled in the SWISS-MODEL workspace\textsuperscript{91} using TcAChE (PDB code 1E66) as a template. All docking runs were performed using GOLD (CCDC, Cambridge, UK) on a Windows-based platform. Starting the docking with the GOLD setup wizard, all ligands and water molecules were removed from the X-ray structure and hydrogen atoms were added to the protein. The protein was set to be rigid while the ligands were set to be flexible in order to find the best ligand conformation that fitted to the binding site of the protein. The binding site was defined by the centre coordinates of huprine X ($X = 5.480$ Å, $Y = 66.057$ Å and $Z = 64.850$ Å), including all atoms within 20 Å from this point. This region included the active site and the PAS. The cavity detection was set as on. All input ligands were docked with 100 genetic algorithm (GA) runs and the GoldScore was used to calculate the fitness score whereby the higher the score the more favourable the protein-ligand binding interactions. GA settings were as preset with 100,000 operations. The clustering of docking results was performed within 2.0 Å (root-mean-square-deviations of ligand heavy atoms). The output results were analysed and processed in DS 2.5.5 to monitor the protein-ligand interactions.

8.3.1 Protein modelling and validation

The X-ray structure of TcAChE (PDB code 1E66) was selected as a template for modelling eeAChE and hAChE. The sequences of all three AChEs were aligned
in MAFFT using a ClustalW format. The edited alignment file was submitted as an input alignment for modelling in SWISS-MODEL.

The sequence alignment between AChEs from different organisms is shown in Figure 8.11. All amino residues at the binding sites are highlighted in colour and were highly conserved. The alignments of TcAChE and hAChE were quite similar which allowed the use of these alignments for modelling hAChE without any modification. On the other hand, eeAChE had a longer sequence than the TcAChE template which led to the modification of those sequences. The sequences in grey (Figure 8.11) were excluded from the alignment for modelling of the eeAChE model. The resulting models and the template enzyme were validated by protein structure validation tools implemented in DS 2.5.5. The Ramachandran plot analysis indicated >95% allowed regions and <0.5% disallowed regions. There were no missing side chain atoms in either model. All amino acid residues at the binding sites of all three AChEs were compared in Table 8.17. The sequence numbers were based on the number in the X-ray crystal structure of TcAChE and are not the same sequence of numbers as shown in Figure 8.11. The structures of all three AChEs with the overlay of all amino residues at the binding sites are shown in Figure 8.12.

<table>
<thead>
<tr>
<th>Binding site</th>
<th>TcAChE</th>
<th>hAChE model</th>
<th>eeAChE model</th>
</tr>
</thead>
<tbody>
<tr>
<td>The esteratic site</td>
<td>Ser200 (S)</td>
<td>Ser234 (S)</td>
<td>Ser225 (S)</td>
</tr>
<tr>
<td></td>
<td>Glu327 (E)</td>
<td>Glu365 (E)</td>
<td>Glu352 (E)</td>
</tr>
<tr>
<td></td>
<td>His440 (H)</td>
<td>His478 (H)</td>
<td>His465 (H)</td>
</tr>
<tr>
<td>The oxyanion hole</td>
<td>Gly118 (G)</td>
<td>Gly152 (G)</td>
<td>Gly143 (G)</td>
</tr>
<tr>
<td></td>
<td>Gly119 (G)</td>
<td>Gly153 (G)</td>
<td>Gly144 (G)</td>
</tr>
<tr>
<td></td>
<td>Ala201 (A)</td>
<td>Ala235 (A)</td>
<td>Ala226 (A)</td>
</tr>
<tr>
<td>The acyl pocket</td>
<td>Phe288 (F)</td>
<td>Phe326 (F)</td>
<td>Phe313 (F)</td>
</tr>
<tr>
<td></td>
<td>Phe290 (F)</td>
<td>Phe328 (F)</td>
<td>Phe315 (F)</td>
</tr>
<tr>
<td>The anionic subsite</td>
<td>Glu199 (E)</td>
<td>Glu233 (E)</td>
<td>Glu224 (E)</td>
</tr>
<tr>
<td></td>
<td>Trp279 (W)</td>
<td>Trp317 (W)</td>
<td>Trp304 (W)</td>
</tr>
<tr>
<td></td>
<td>Phe330 (F)</td>
<td>Phe368 (Y)</td>
<td>Tyr359 (Y)</td>
</tr>
</tbody>
</table>

Table 8.17 Sequence number comparison between TcAChE, hAChE model and eeAChE model. One letter amino acid codes are shown in brackets.
An * (asterisk) indicates positions which have a single, fully conserved residue. A : (colon) indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix. A . (period) indicates conservation between groups of weakly similar properties - scoring < 0.5 in the Gonnet PAM 250 matrix. The amino acid residues at the esteratic site are highlighted in yellow, those at the oxyanion hole in green, those at the acyl pocket in red, those at the anionic subsite in pink and those at the PAS in turquoise. The sequences of eeAChE in grey were excluded from the alignment for modelling of the eeAChE model.

Figure 8.11 Sequence alignment between AChEs from different organisms.
Figure 8.12 The structures of TcAChE and the hAChE and eeAChE models and the overlay of amino acid residues at the binding sites of all three AChEs.

8.3.2 Docking results

To validate the reliability of the docking program, huprine X (HUX) which was originally bound to TcAChE in the X-ray crystal structure (PDB code 1E66) was re-introduced into the defined active site of TcAChE. The result showed very close correspondence between HUX in the crystal and the one from the docking program as shown in Figure 8.13. Other compounds used in the docking included galanthamine and four of our active compounds, NH-1, NH-2, OH-6 and OH-9 (Figure 8.14). These were docked into TcAChE and the hAChE and eeAChE models and the results are shown in Table 8.18.
Figure 8.13 Overlay of structures of huprine X in the X-ray crystal of TcAChE (green) and after docking (red).

Figure 8.14 Ligands used in docking experiments.

The docking between HUX and TcAChE showed the highest fitness score of 72.76 as it was similar to the original X-ray structure. The number of clusters shown in Table 8.18 represents the number of possible ligand orientations in binding to a protein, also called poses. In the docking of HUX to hAChE and eeAChE models, HUX also showed the highest fitness score even though the number of clusters increased from one in TcAChE to two in both the hAChE and eeAChE models. This may be a result of their slightly different protein structures. Docking with galanthamine to these proteins resulted in higher fitness scores (in a range of 51.81 – 55.50) than those of the Stemona compounds, but were still lower than those for HUX. All Stemona compounds resulted in many poses, suggesting many possible ligand-protein complexes of similar energies and their highest fitness scores were similar in a range of 34.94 – 48.93. Therefore, only the poses with the highest fitness score were analysed for their interactions and their score breakdowns are shown in Table 8.19. The GOLD fitness score is calculated from four components, protein-ligand hydrogen bond energy (Hb_ext), protein-ligand van der Waals (vdw) energy (vdw_ext), ligand torsional strain energy (internal torsion) and ligand internal vdw
energy (internal vdw) and the sum of internal torsion and internal vdw terms is a single internal energy term (Int). The vdw_ext score is multiplied by a factor of 1.375 as an empirical correction to encourage protein-ligand hydrophobic contact. The fitness score is taken as the negative of the sum of the component energy terms, so that larger fitness scores indicate stronger ligand binding.

Table 8.18 Docking results.

<table>
<thead>
<tr>
<th>Ligand (Compound number)</th>
<th>TcAChE</th>
<th>hAChE model</th>
<th>eeAChE model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of clusters</td>
<td>Highest fitness score (run number)</td>
<td>Number of clusters</td>
</tr>
<tr>
<td>HUX</td>
<td>1</td>
<td>72.76 (51)</td>
<td>2</td>
</tr>
<tr>
<td>Galanthamine</td>
<td>1</td>
<td>52.96 (72)</td>
<td>3</td>
</tr>
<tr>
<td>NH-1 (193)</td>
<td>9</td>
<td>48.93 (26) 36.49 (61) 26.12 (63) 23.85 (99) 23.19 (49) 22.72 (12) 14.93 (66) 8.16 (55) 8.13 (80)</td>
<td>7</td>
</tr>
<tr>
<td>NH-2 (191)</td>
<td>4</td>
<td>34.94 (16) 28.21 (13) 26.63 (44) 25.91 (59)</td>
<td>3</td>
</tr>
<tr>
<td>OH-6 (104)</td>
<td>7</td>
<td>49.77 (87) 33.09 (27) 26.96 (35) 22.39 (38) 21.18 (34) 17.92 (37) 0.71 (67)</td>
<td>6</td>
</tr>
<tr>
<td>OH-9 (98)</td>
<td>8</td>
<td>45.92 (91) 30.73 (95) 30.16 (39) 25.66 (20) 25.47 (51) 1.02 (96) -6.26 (44) -11.62 (70)</td>
<td>5</td>
</tr>
</tbody>
</table>
From Table 8.19, HUX had the highest fitness score as it had the highest vdw energy and the lowest internal energy. Galanthamine and Stemona compounds had similar vdw and internal energies in the range of 34.91 – 48.53 and (-11.93) - (-4.80), respectively. However, galanthamine had the highest hydrogen bond energy while those for compounds NH-1, NH-2 and OH-9 were low, or zero in the case of compound OH-6 lacking a side chain hydroxyl or amine group which could be involved in H-bonding. Thus, the final fitness scores for the Stemona compounds were lower than that for galanthamine. The docking results are consistent with the higher IC50 values (Chapter 7, Table 7.8, entry 1) and lower inhibitory activities of the Stemona alkaloids compared with galanthamine.

### Table 8.19 Breakdown for the best fitness score achieved for different protein-ligand docking experiments.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Ligand (Compound number)</th>
<th>Highest fitness score (run number)</th>
<th>Score breakdowna</th>
<th>Hb_ext</th>
<th>Vdw_ext</th>
<th>Int</th>
</tr>
</thead>
<tbody>
<tr>
<td>TcAChE</td>
<td>HUX</td>
<td>72.76 (51)</td>
<td></td>
<td>0.00</td>
<td>54.12</td>
<td>-1.66</td>
</tr>
<tr>
<td></td>
<td>Galanthamine</td>
<td>52.96 (72)</td>
<td></td>
<td>5.85</td>
<td>39.79</td>
<td>-7.60</td>
</tr>
<tr>
<td></td>
<td>NH-1 (193)</td>
<td>48.93 (26)</td>
<td></td>
<td>0.13</td>
<td>41.38</td>
<td>-8.10</td>
</tr>
<tr>
<td></td>
<td>NH-2 (191)</td>
<td>34.94 (16)</td>
<td></td>
<td>1.51</td>
<td>32.99</td>
<td>-11.93</td>
</tr>
<tr>
<td></td>
<td>OH-6 (104)</td>
<td>49.77 (87)</td>
<td></td>
<td>0.00</td>
<td>40.14</td>
<td>-5.42</td>
</tr>
<tr>
<td></td>
<td>OH-9 (98)</td>
<td>45.92 (91)</td>
<td></td>
<td>0.14</td>
<td>37.34</td>
<td>-5.57</td>
</tr>
<tr>
<td>hAChE model</td>
<td>HUX</td>
<td>65.02 (91)</td>
<td></td>
<td>0.00</td>
<td>48.53</td>
<td>-1.71</td>
</tr>
<tr>
<td></td>
<td>Galanthamine</td>
<td>55.50 (62)</td>
<td></td>
<td>5.98</td>
<td>41.60</td>
<td>-7.69</td>
</tr>
<tr>
<td></td>
<td>NH-1 (193)</td>
<td>44.60 (82)</td>
<td></td>
<td>0.00</td>
<td>39.95</td>
<td>-10.32</td>
</tr>
<tr>
<td></td>
<td>NH-2 (191)</td>
<td>45.82 (43)</td>
<td></td>
<td>0.00</td>
<td>39.46</td>
<td>-8.44</td>
</tr>
<tr>
<td></td>
<td>OH-6 (104)</td>
<td>45.42 (38)</td>
<td></td>
<td>0.00</td>
<td>36.52</td>
<td>-4.80</td>
</tr>
<tr>
<td></td>
<td>OH-9 (98)</td>
<td>41.41 (47)</td>
<td></td>
<td>0.40</td>
<td>34.91</td>
<td>-6.98</td>
</tr>
<tr>
<td>eeAChE model</td>
<td>HUX</td>
<td>64.72 (57)</td>
<td></td>
<td>0.00</td>
<td>48.34</td>
<td>-1.76</td>
</tr>
<tr>
<td></td>
<td>Galanthamine</td>
<td>53.61 (82)</td>
<td></td>
<td>5.25</td>
<td>40.53</td>
<td>-7.37</td>
</tr>
<tr>
<td></td>
<td>NH-1 (193)</td>
<td>45.86 (67)</td>
<td></td>
<td>0.00</td>
<td>39.19</td>
<td>-8.03</td>
</tr>
<tr>
<td></td>
<td>NH-2 (191)</td>
<td>44.16 (94)</td>
<td></td>
<td>0.00</td>
<td>37.78</td>
<td>-7.79</td>
</tr>
<tr>
<td></td>
<td>OH-6 (104)</td>
<td>47.41 (96)</td>
<td></td>
<td>0.00</td>
<td>38.71</td>
<td>-5.81</td>
</tr>
<tr>
<td></td>
<td>OH-9 (98)</td>
<td>42.20 (87)</td>
<td></td>
<td>0.00</td>
<td>35.81</td>
<td>-7.03</td>
</tr>
</tbody>
</table>

aSee text for details.

The interactions between the ligands with the highest fitness score and TcAChE are shown in Table 8.20 and Figure 8.16. All interactions were monitored within 5 Å distance from the ligands. HUX formed six \(\pi-\pi\) interactions between its aromatic rings and Trp84 and Phe330. Galanthamine formed three \(\pi-\pi\) interactions between its aromatic ring and Trp84 and Phe330 and one H-bond between the hydroxyl group and Glu199. All Stemona compounds had two H-bonds and compound OH-9 had an extra \(\pi-\sigma\) interaction between H-9a and Tyr334. Compounds NH-1, OH-6 and OH-9 had two H-bonds between the oxygen bridge of the core
structure and Tyr121 and between the oxygen of the furan ring and Tyr121. While those of compound NH-2 were between the carbonyl group of the lactone ring and Tyr334 and between the amine at the side chain and Trp84. The binding mode of galanthamine and those of compounds NH-2 and OH-9 are compared in Figure 8.15.

From an analysis of the amino acids at the binding sites of TcAChE in Table 8.17, HUX and galanthamine bound at the anionic subsite of the active site while the Stemona compounds vertically bound (blue structure in Figure 8.15) to the PAS of TcAChE except for compound NH-2 (red structure in Figure 8.15) which bound horizontally in between the anionic subsite and the PAS of TcAChE.

Table 8.20 The interactions between ligand poses with the highest fitness scores and TcAChE.

<table>
<thead>
<tr>
<th>Ligand (run number)</th>
<th>Protein-ligand interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>HUX (51)</td>
<td>6x π-π 4x between two aromatic rings and Trp84 2x between two aromatic rings and Phe330</td>
</tr>
<tr>
<td>Galanthamine (72)</td>
<td>3x π-π 2x between the aromatic ring and Trp84 1x between the aromatic ring and Phe330 1x H-bond 1x between the hydroxyl group and Glu199</td>
</tr>
<tr>
<td>NH-1&lt;sup&gt;a&lt;/sup&gt; (26)</td>
<td>2x H-bond 1x between the oxygen bridge of the core structure and Tyr121 1x between the oxygen of the furan ring and Tyr121</td>
</tr>
<tr>
<td>NH-2&lt;sup&gt;b&lt;/sup&gt; (16)</td>
<td>2x H-bond 1x between the carbonyl group of the lactone ring and Tyr334 1x the amine at the side chain and Trp84</td>
</tr>
<tr>
<td>OH-6&lt;sup&gt;c&lt;/sup&gt; (87)</td>
<td>2x H-bond 1x between the oxygen bridge of the core structure and Tyr121 1x between the oxygen of the furan ring and Tyr121</td>
</tr>
<tr>
<td>OH-9&lt;sup&gt;d&lt;/sup&gt; (91)</td>
<td>2x H-bond 1x between the oxygen bridge of the core structure and Tyr121 1x between the oxygen of the furan ring and Tyr121</td>
</tr>
<tr>
<td></td>
<td>1x π-σ 1x between H-9α of the A,B,C ring core structure and Tyr334</td>
</tr>
</tbody>
</table>

<sup>a</sup>Compound 193. <sup>b</sup>Compound 191. <sup>c</sup>Compound 104. <sup>d</sup>Compound 98.

Figure 8.15 The overlay of galanthamine (green), compounds NH-2 (red) and OH-9 (blue) and the amino acid residues (yellow) at the binding site of TcAChE.
The interactions between the ligands with the highest fitness score and the hAChE model are shown in Table 8.21 and Figure 8.18. HUX had four \( \pi-\pi \) interactions between its aromatic rings and Trp117. Galanthamine had two \( \pi-\pi \) interactions between its aromatic ring and Trp117 and one H-bond between the hydroxyl group and Glu233. Compound NH-1 had two H-bonds between the oxygen bridge of the core structure and Tyr155, one H-bond between the methoxyl group

Figure 8.16 The interactions between poses with the highest fitness scores and TeAChE.
and Tyr368 and one H-bond between the carbonyl group of the lactone ring and Asp105. Compound OH-6 was similar to compound NH-1 except for not displaying a H-bonding interaction with Tyr368. Compound NH-2 had one H-bond between the oxygen of the lactone ring and Tyr155. Compound OH-9 had no H-bonds but had a \( \pi-\sigma \) interaction between H-5 and Trp117. The binding modes of galanthamine and compounds NH-1 and OH-9 are compared in Figure 8.17. From an examination of the amino acids at the binding sites of hAChE in Table 8.17, HUX and galanthamine bound at the anionic subsite of the active site while the *Stemona* compounds bound to a region between the anionic subsite and the PAS of the hAChE model.

**Table 8.21** The interactions between ligand poses with the highest fitness scores and the hAChE model.

<table>
<thead>
<tr>
<th>Ligand (run number)</th>
<th>Protein-ligand interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>HUX (91)</td>
<td>4x ( \pi-\pi ) 4x between two aromatic rings and Trp117</td>
</tr>
<tr>
<td>Galanthamine (62)</td>
<td>2x ( \pi-\pi ) 1x H-bond 2x between the aromatic ring and Trp117 1x between the hydroxyl group and Glu233</td>
</tr>
<tr>
<td>NH-1(^a) (82)</td>
<td>4x H-bond 2x between the oxygen bridge of the core structure and Tyr155 1x between the carbonyl group of the lactone ring and Asp105 1x between the methoxyl group and Tyr368</td>
</tr>
<tr>
<td>NH-2(^b) (43)</td>
<td>1x H-bond 1x between the oxygen of the lactone ring and Tyr155</td>
</tr>
<tr>
<td>OH-6(^c) (38)</td>
<td>3x H-bond 2x between the oxygen bridge of the core structure and Tyr155 1x between the carbonyl group of the lactone ring and Asp105</td>
</tr>
<tr>
<td>OH-9(^d) (47)</td>
<td>1x ( \pi-\sigma ) 1x between H-5 and Trp117</td>
</tr>
</tbody>
</table>

\(^a\)Compound 193. \(^b\)Compound 191. \(^c\)Compound 104. \(^d\)Compound 98.

**Figure 8.17** The overlay of galanthamine (green), compounds NH-1 (red) and OH-9 (blue) and the amino acid residues (yellow) at the binding site of the hAChE model.
The interactions between the ligands with the highest fitness score and the eeAChE model are shown in Table 8.22 and Figure 8.20. HUX had four $\pi-\pi$ interactions between its aromatic rings and Trp108. Galanthamine had two $\pi-\sigma$ interactions between the aliphatic ring and Trp108 and one H-bond between the oxygen of the methoxyl group and Ser225. Compound NH-1 had one H-bond between the oxygen bridge of the core structure and Tyr146 and one H-bond between
the oxygen of the furan ring and Tyr146. Compounds NH-2, OH-6 and OH-9 had one H-bond between the oxygen atom of their lactone ring and Tyr146 while compounds OH-6 and OH-9 had another H-bond between the oxygen bridge of their core structures and Tyr146. The binding modes of galanthamine and compounds NH-1 and OH-9 are compared in Figure 8.19. From an examination of the amino acids at the binding sites of eeAChE in Table 8.17, HUX bound at the anionic subsite of the active site and galanthamine bound between the anionic subsite and the esteratic site of the active site. \textit{Stemona} compounds bound at the PAS of the eeAChE model.

**Table 8.22** The interactions between ligand poses with the highest fitness scores and the eeAChE model.

<table>
<thead>
<tr>
<th>Ligand (run number)</th>
<th>Protein-ligand interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>HUX (57)</td>
<td>4x ( \pi-\pi ) 4x between two aromatic rings and Trp108</td>
</tr>
<tr>
<td>Galanthamine (82)</td>
<td>2x ( \pi-\sigma ) 2x between two protons of the aliphatic ring and Trp108 1x H-bond 1x between the oxygen of the methoxyl group and Ser225</td>
</tr>
<tr>
<td>NH-1(^a) (67)</td>
<td>2x H-bond 1x between the oxygen bridge of the core structure and Tyr146 1x between the oxygen of the furan ring and Tyr146</td>
</tr>
<tr>
<td>NH-2(^b) (94)</td>
<td>1x H-bond 1x between the oxygen of the lactone ring and Tyr146</td>
</tr>
<tr>
<td>OH-6(^c) (96)</td>
<td>2x H-bond 1x between the oxygen bridge of the core structure and Tyr146 1x between the oxygen of the lactone ring and Tyr146</td>
</tr>
<tr>
<td>OH-9(^d) (87)</td>
<td>2x H-bond 1x between the oxygen bridge of the core structure and Tyr146 1x between the oxygen of the lactone ring and Tyr146</td>
</tr>
</tbody>
</table>

\(^a\)Compound 193. \(^b\)Compound 191. \(^c\)Compound 104. \(^d\)Compound 98.

**Figure 8.19** The overlay of galanthamine (green), compounds NH-1 (red) and NH-2 (blue) and the amino acid residues (yellow) at the binding site of the eeAChE model.
Figure 8.20 The interactions between ligand poses with the highest fitness scores and the eeAChE model.

An analysis of the docking experiments to the three different AChEs showed similar ligand-binding results. HUX and galanthamine which had compact structures could bind at the anionic subsite or the esteractic site of the active site of AChE. While the *Stemona* compounds having a more bulky pyrrolo[1,2-α]azepine core structure were more likely bind at the PAS of AChE rather than the active site. However, some *Stemona* compounds could reach the anionic subsite of the active site.
as they fitted vertically into the gorge, for example the methoxy group of NH-1 (the most active derivative on hAChE) had a H-bond interaction with the amino acid residue (Tyr 368) which was located at the anionic subsite of hAChE.

8.4 Conclusions

Seven final pharmacophore models were chosen for ligand-pharmacophore mapping including the ASB, BFB trial 7, BFB trial 8, Unknown trail 1, Unknown trial 2, Tacripyrine and *Stemona* pharmacophore models. We tested the ligand-pharmacophore mapping of the *Stemona* test set and found this set mapped onto the ASB, Unknown trial 1, Unknown trial 2 and *Stemona* pharmacophore models. The results showed that *Stemona* compounds were less likely to bind at the active site of AChE. However, based on pharmocophore results alone we could not confirm that *Stemona* compounds bind at the PAS of AChE.

Protein-ligand docking was performed to predict the binding mode of *Stemona* compounds. The results showed that the *Stemona* compounds had two possible binding orientations, vertical or horizontal, to fit into the active-site gorge. The former orientation was more preferable. An analysis of interactions between the ligands and AChEs showed that small ligands such as HUX and galanthamine bound at the active site while the bulky *Stemona* compounds were more likely to bind at the PAS of AChEs rather than the active site.

The results from the molecular docking supported the hypothesis raised from the pharmacophore generation, that *Stemona* compounds may bind at the PAS or in the gorge between the active site and the PAS of AChE. These computational studies showed that *Stemona* compounds may inhibit AChEs by allosterically binding at the PAS and blocking acetylcholine from reaching the active site.
CHAPTER 9 CONCLUSIONS

The discovery of an unknown *Stemona* sp. from Thailand provided gram quantities of the major *Stemona* alkaloid, 11(Z)-1',2'-didehydrostemofoline (104), after extraction of the roots and chromatographic purification (Chapter 2). This alkaloid allowed the possibility of preparing some rare *Stemona* alkaloids using semi-synthesis and extending the structure-activity relationship (SAR) studies of stemofoline alkaloids.

From the study reported in this thesis, four *Stemona* alkaloids, stemoburkilline (108), oxystemofoline (115), methoxystemofoline (116) and (1'R)-hydroxystemofoline (98), were prepared (Chapters 3 and 4). The semi-synthesis of stemoburkilline (108) allowed the reassignment of the absolute configuration of the C-11-C-12 alkene from an *E*-alkene to a *Z*-alkene (Chapter 3). The key aldehyde 160 was prepared from 11(Z)-1',2'-didehydrostemofoline (104) in two steps via, an asymmetric dihydroxylation reaction of the C-3 1-butenyl side chain and then an oxidative cleavage of the subsequence diol 173 (Chapter 4). The aldehyde 160 was used as a key scaffold for further reactions. Oxystemofoline (115) and methoxystemofoline (116) were prepared via modified Julia olefination reactions, as a key step, between the aldehyde 160 and sulfones 176 and 181, respectively. The $^{13}$C NMR spectroscopic assignments for C-6 and C-1' of both compounds were reassigned. The allylation reaction of 160 was a key step towards the semi-synthesis of (1'R)-hydroxystemofoline (98) and its epimer (1'S)-hydroxystemofoline 166. (1'R)-Hydroxystemofoline (98) was later found to be identical to a new *Stemona* alkaloid isolated from the root extracts of *Stemona aphylla*.\(^{51}\) The lactone 188, having the A, B, C ring core structure of stemofoline, was prepared from a dihydroxylation reaction and then an oxidative cleavage of the C-11-C-12 double bond of stemofoline (97).

In Chapter 5, reductive amination reactions on the aldehyde 160 were used to introduce a C-3 aminomethyl side chain and provided 13 secondary amine (191, 193-196, 200-207) and four tertiary amine derivatives (192, 197-199). Another five derivatives, the N-methyl allylamine 209, the three carbamates 210-212 and one guanidine derivative 214, were obtained from further methylation, carbamylation and
guanidination reactions of the amine products prepared from the reductive amination reactions.

Another key scaffold, the alkyne 215, was prepared from the aldehyde 160 in one step using the Bestmann-Ohira reagent 217. As was described in Chapter 6, the alkyne 215 was a starting material for the preparation of triazole and isoxazole derivatives using click reactions. The Sonogashira and Eglington coupling reactions of 215 were also performed to prepare the phenylalkyne product 216 and the alkyne dimer 224, respectively.

The biological activities of the Stemona alkaloids and analogues were presented in Chapter 7. The AChE inhibitory activities of the stemofoline alkaloids and analogues were reported using a TLC bioautographic method which measured the activity as a minimum inhibitory requirement (MIR) in ng or nmol against electric eel AChE (eeAChE). Galanthamine was used as a positive control having a MIR of 1 ng (0.003 nmol). The four Stemona alkaloids synthesised from this study, stemoburkilline (108), oxystemofoline (115), methoxystemofoline (116) and (1′R)-hydroxystemofoline (98), showed MIRs of 50, 50, 50 and 5 ng, respectively. (1′R)-Hydroxystemofoline (98) also showed the highest activity among the alcohol derivatives. Compared to 11(Z)-1′,2′-didehydrostemofoline (104) (MIR = 5 ng), its N-oxide 105 had similar activity (MIR = 5 ng) while that of stemofoline (97), which lacked a side chain alkene, showed less activity with a MIR of 10 ng. Lacking the lactone ring, the A, B, C ring core structure derivative 188 was 10 times less active than 97 (MIR = 100 ng). The cyclopentyl amino carbamate 211, the dimethyl amine 192 and the alkyne 215 had the highest activities in the group with MIR values of 1 ng. While the click products 219-223 showed moderate activities in the range of 50-100 ng except for the benzyl triazole derivative 218 which has a higher activity with a MIR value of 5 ng. Compared to the alkyne 215 (MIR = 1 ng), the phenylalkyne 216 and the alkyne dimer 224 were less active (MIRs = 50 and 100 ng, respectively). However, compound 225 with a flexible 2-phenylethyl side chain was 10 times more active than 216 with a MIR of 5 ng. Compounds 104, 183, 193, 196, 197 and 208 were tested for their IC₅₀ values against eeAChE and human AChE (hAChE) using a colorimetric assay (known as Ellman’s method). 11(Z)-1′,2′-Didehydrostemofoline (104) and an isopropylamine analogue (193) showed good activities against eeAChE (IC₅₀ values = 19.2 and 12.9 µM, respectively) and hAChE (IC₅₀ values = 25.0 and
19.9 μM, respectively) but were not as potent as galanthamine (IC₅₀ values = 0.9 and 0.6 μM for eeAChE and hAChE, respectively). While other *Stemona* derivatives (183, 196, 197 and 208) showed lower activities against eeAChE and hAChE in the range of 52.5-302.3 μM and 28.7-52.4 μM, respectively. The MDR-reversing properties of some *Stemona* compounds (97, 104, 173, 183, 190a, 190b, 193, 197, 199, 206, 208, 210 and 218) were performed using the colorimetric MTT assay. Among the tested compounds, stemofoline (97) showed the highest modulating effect on the resistant KB-V1 cells by decreasing the IC₅₀ of paclitaxel from 10.06 ± 1.56 μM to 1.4 ± 0.45 μM and that of vinblastine from 0.61 ± 0.05 μM to 0.09 ± 0.01 μM. In comparison to the results from the AChE inhibitory study, in which most of the amine derivatives (193, 197, 199, 206, 210 and 218) were more active than the alcohol derivatives (173, 183, 190a, 190b and 208), stemofoline (97) had the highest modulating effect on the resistant KB-V1 cells.

In Chapter 8 of this thesis, SAR studies were reported using pharmacophore generation and molecular docking. The best seven different pharmacophore models were generated in order to search for the potential binding mode of the *Stemona* compounds. Unfortunately, based on the results from pharmacophore mapping alone we could not confirm the exact binding site. Thus, protein-ligand docking experiments were performed using three different AChEs, including TcAChE and eeAChE and hAChE models. The results from molecular docking suggested that the *Stemona* compounds were more likely to fit vertically in the active-site gorge of AChEs and bind between the active site and the PAS of AChEs. These computational studies showed that *Stemona* compounds may inhibit AChEs by allosterically binding at the PAS and blocking acetylcholine from reaching the active site.
10.1 General experimental

10.1.1 General reaction conditions

In general, all reactions, unless otherwise stated, were performed in oven dried, single-necked round bottom flasks under an atmosphere of dry nitrogen. Progress of reactions was monitored by thin-layer chromatographic (TLC) analysis. Solvents were purchased as Analytical Reagent (AR) grade. Petrol refers to the hydrocarbon fraction of bp 40 – 60 °C. THF was stored over KOH pellet until needed, then distilled over sodium wire under nitrogen, using benzophenone as an indicator. Anhydrous CH₂Cl₂ and MeOH were purchased from Aldrich.

Where 'dried' is specified, this refer to the drying of the organic extract over MgSO₄, unless otherwise indicated, followed by filtration. Where 'evaporation' is specified, this refers to the evaporation of solvent under reduced pressure using a rotary evaporator. Purified compounds were dried thoroughly under high vacuum. All reaction yields were obtained only after this drying process.

10.1.2 Chromatography

TLC analysis was performed using aluminium backed Merck F₂₅⁴ sorbent silica gel. Compounds were detected under a 254 nm ultraviolet lamp, or by staining with the Dragendorff reagent or acidified, aqueous solution of ammonium molybdate and cerium(IV) sulphate, followed by development with a 1400 Watt heat gun.

The Dragendorff reagent was prepared from a mixture of two stock solutions. Stock solution A contained H₂O (10 mL), concentrated HCl (2 mL) and bismuth subnitrate (0.6 g). Stock solution B contained H₂O (10 mL) and KI (6 g). These two stock solutions were combined and diluted with H₂O (15 mL) and concentrated HCl (7 mL). The mixture was then made up to 400 mL with H₂O.

One litre of the molybdate dip contained H₂O (950 mL), concentrated H₂SO₄ (50 mL), (NH₄)₆MoO₂₄ (50 g) and Ce(SO₄)₂ (2 g).
Purification of compounds by column chromatography was achieved using Merck flash silica gel (40 – 63 µm) and the technique reported by Still.94

10.1.3 Polarimetry

Optical rotations were measured using a 1 cm cell, in a Jasco DIP-370 digital polarimeter or a 10 cm cell, in a Jasco P-2000 polarimeter. Ten measurements were taken and the average was used to calculate the specific rotation.

10.1.4 Mass spectrometry

Low resolution mass spectra were obtained either on a Shimadzu GC mass spectrometer (EI and CI) or a Waters LCZ single quadropole (ESI). High-resolution mass spectra were obtained either on a VG Autospec mass spectrometer (EI and CI) or a Waters QTOF (ESI). HRMS (exact masses) were used in lieu of elemental analysis and TLC analysis and $^1$H and $^{13}$C NMR spectroscopy were used as criteria for purity.

10.1.5 Infrared spectrometry

Infrared spectra were obtained as neat samples on a Smart Omni-Sampler Avator ESP Nicolet spectrometer.

10.1.6 Nuclear magnetic resonance spectroscopy

$^1$H and $^{13}$C NMR spectra were recorded on a Varian Inova-500 spectrometer (500 MHz $^1$H, 125 MHz $^{13}$C) or a Varian Unity-300 spectrometer (300 MHz $^1$H, 75 MHz $^{13}$C) in deuterochloroform (CDCl$_3$), unless otherwise specified. NMR assignments were based on gCOSY, gHSQC, gHMBC and DEPT or APT experiments. NMR solvents used in the experimental and their associated referencing data are displayed in Table 9.1. Unless otherwise stated, the applied NMR frequency was 500 MHz for $^1$H NMR experiments and 125 MHz for $^{13}$C NMR experiments, with samples dissolved in CDCl$_3$. $^1$H and $^{13}$C NMR assignments are based on the numbering system used for stemofoline (97) and not on the systematic name and
numbering used in the naming of many stemofoline derivatives and analogues in the experimental section.

Table 10.1 The references used for $^1$H and $^{13}$C NMR spectroscopy.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$^1$H NMR</th>
<th>$^{13}$C NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Internal standard</td>
<td>Other</td>
</tr>
<tr>
<td>CDCl$_3$</td>
<td>TMS, s, 0.00 ppm</td>
<td>Residual CHCl$_3$, s, 7.26 ppm</td>
</tr>
<tr>
<td>CD$_3$OD</td>
<td>TMS, s, 0.00 ppm</td>
<td>Residual MeOH, s, 3.31 ppm</td>
</tr>
<tr>
<td>(CD$_3$)$_2$CO</td>
<td>TMS, s, 0.00 ppm</td>
<td>Residual (CH$_3$)$_2$CO, s, 2.05 ppm</td>
</tr>
</tbody>
</table>

10.2 Experimental for Chapter 2

**Plant material.** The roots of an unknown *Stemona* species were collected at Amphur Mae Moh, Lampang, Thailand, in November 2007. The plant material was identified by Mr. James Maxwell as the same species as we had previously studied.$^{31}$ A voucher specimen, number 25375, was deposited at the Herbarium of the Department of Biology, Chiang Mai University.

**Extraction and isolation of** (11$^Z$)-1$'$,2$'$-didehydrostemofoline (104).

The fresh roots were cleaned with H$_2$O and sliced into small pieces. The cut roots were air dried for 2 days and oven dried at 50 - 60 °C for 24 h. The dry roots of the *Stemona* species (935 g) were macerated in 95% EtOH (4 x 3000 mL) over 4 days at rt. The ethanolic solution was evaporated to give a dark brown crude extract (148 g). The crude was partitioned between MeOH/H$_2$O (1:1) and CH$_2$Cl$_2$. The organic extract was dried (MgSO$_4$) and then concentrated in vacuo to give a dark brown residue (20 g). A portion of this material (2.50 g) was purified by column chromatography on silica gel (100 mL) with gradient elution from CH$_2$Cl$_2$ to CH$_2$Cl$_2$/MeOH/NH$_4$OH (95:5:1) to give pure (11$^Z$)-1$'$,2$'$-didehydrostemofoline (104) as a yellow-brown gum (1.48 g, 59% w/w).

$R_f$ = 0.50 in MeOH/CH$_2$Cl$_2$ (1:9).

$[\alpha]_D^{24} = +245$ (c 0.74, MeOH) (Lit.$^{14}$ $[\alpha]_D^{20} = +210$ (c 0.5, MeOH))
EIMS m/z 385 (50%) [M]$^+$.

$^1$H NMR $\delta$ 5.78 (dt, $J$ 15.5 Hz, 6.0 Hz, 1H, H-2$'$), 5.50 (d, $J$ 15.0 Hz, 1H, H-1$'$), 4.21 (br s, 1H, H-2), 4.13 (s, 3H, O-CH$_3$), 3.50 (br s, 1H, H-9a), 3.13 – 3.07 (m, 2H, H-5a, H-10), 3.00 – 2.95 (m, 1H, H-5b), 2.86 (d, $J$ 6.0 Hz, 1H, H-7), 2.09 – 2.06 (m, 2H, H-3$''$), 2.07 (s, 3H, H-16), 1.95 (d, $J$ 12.5 Hz, 1H, H-1b), 1.90 – 1.86 (m, 2H, H-6), 1.84 – 1.80 (m, 1H, H-1a), 1.79 – 1.77 (m, 1H, H-9), 1.37 (d, $J$ 6.0 Hz, 3H, H-16), 0.99 (t, $J$ 7.0 Hz, 3H, H-4$'$).

$^1$H NMR data agreed with that of the natural product.$^{14}$

10.3 Experimental for Chapter 3

Stemofoline (97).

To a solution of 11(Z)-1$'$,2$'$-didehydrostemofoline (104) (51.4 mg, 0.133 mmol) in EtOAc (2.0 mL) at rt was added Pd/C (5.1 mg, 10% w/w) and the flask was flushed with N$_2$ for 10 min before left to stir under a H$_2$ atmosphere (balloon) for 1 h. The flask was flushed with N$_2$ and the solution was filtered through a pad of Celite and washed with EtOAc. The filtrate was dried (MgSO$_4$) and concentrated in vacuo. After purification by column chromatography using gradient elution from CH$_2$Cl$_2$ to CH$_2$Cl$_2$/MeOH/NH$_4$OH (95:5:1), stemofoline (97) was obtained as a yellow-brown gum (50.0 mg, 0.129 mmol, 97% yield).

$R_f$ = 0.43 in MeOH/EtOAc (2:8).

$[\alpha]_D^{24} = +245$ (c 0.74, MeOH) (Lit.$^{14}$ $[\alpha]_D^{20} = +270$ (c 0.8, MeOH))

EIMS m/z 387 (50%) [M]$^+$.  

$^1$H NMR $\delta$ 4.26 (br s, 1H, H-2), 4.14 (s, 3H, O-CH$_3$), 3.46 (br s, 1H, H-9a), 3.16-3.07 (m, 2H, H-5a, H-10), 3.02-2.97 (m, 1H, H-5b), 2.70 (d, $J$ 6.0 Hz, 1H, H-7), 2.07 (s, 3H, H-16), 1.96 (d, $J$ 12.0 Hz, 1H, H-1b), 1.93-1.88 (m, 1H, H-6a), 1.85-1.82 (m, 1H, H-6b), 1.81 (dd, $J$ 10.0 Hz, 4.0 Hz, 1H, H-9), 1.73-1.69 (m, 1H, H-1a), 1.63-1.52 (m, 2H, H-2$'$), 1.46-1.40 (m, 1H, H-1$'$), 1.37 (d, $J$ 6.5 Hz, 1H, H-17), 1.35-1.32 (m, 2H, H-3$'$), 1.29-1.22 9M, 1H, H-1$'$), 0.92 (t, $J$ 7.0 Hz, 3H, H-4$'$).

$^1$H NMR data agreed with that of the natural product.$^{23}$
Isostemofoline (109).

To a large NMR tube (5 mm diameter) containing a solution of stemofoline (97) (48.3 mg, 0.125 mmol) in CHCl₃ (2 mL) at rt was added acetophenone (50 μL). The mixture was irradiated with a 500 W lamp for 7 h to give a mixture of 97 and 109 (ca. 9:11). The mixture was separated by column chromatography using gradient elution from CH₂Cl₂ to CH₂Cl₂/MeOH/NH₃ (95:5:1) as eluent to give 109 as a white, amorphous solid (20 mg, 0.052 mmol, 41% yield) and 97 (15.8 mg, 0.041 mmol, 33% yield).

\[ R_f = 0.58 \text{ in MeOH/CH}_2\text{Cl}_2 (1:9). \]

EIMS \( m/z 387 \) (50%) [M⁺].

\( ^1H \) NMR δ 4.27 (s, 1H, H-2), 4.11 (s, 3H, O-CH₃), 3.47 (br s, 1H, H-9a), 3.23-3.16 (m, 1H, H-5a), 3.15-3.11 (m, 1H, H-10), 3.03-2.98 (m, 1H, H-5b), 2.69 (d, \( J = 6.0 \) Hz, 1H, H-7), 2.04 (s, 3H, H-16), 1.97 (d, \( J = 12.5 \) Hz, 1H, H-1b), 1.94-1.88 (m, 1H, H-6a), 1.85-1.79 (m, 1H, H-6b), 1.75-1.72 (m, 1H, H-9), 1.73-1.69 (m, 1H, H-1a), 1.62-1.52 (m, 2H, H-2'), 1.45 (d, \( J = 6.5 \) Hz, 1H, H-17), 1.43-1.39 (m, 1H, H-1'), 1.36 (q, \( J = 7.5 \) Hz, 2H, H-3'), 1.30-1.22 (m, 1H, H-1'), 0.92 (t, \( J = 7.0 \) Hz, 3H, H-4').

\( ^1H \) NMR data agreed with that of the natural product.35

11(S),12(S)-Dihydrostemofoline (163).

Preparation based on the general method for hydrogenation above, using stemofoline (97) (83.1 mg, 0.214 mmol) and Pd/C (8.3 mg, 10% w/w), except that the reaction was stirred in EtOH (3.0 mL) for 24 h. The product 163 was obtained as a colourless gum (20.9 mg, 0.054 mmol, 25% yield) and the ring-open product 164 (13.5 mg, 0.035 mmol, 16% yield, \( \text{dr} = 72:28 \)) as a brown gum after separation by column chromatography. NMR data of 163 agreed with that reported.33 The \( ^1H \) NMR data of the major diastereomer of 164 agreed with those supplied to us by Dr. Velton.36

\[ R_f = 0.22 \text{ in MeOH/CH}_2\text{Cl}_2 (1:9). \]

ESIMS \( m/z 390 \) (100%) [M+H]⁺.

\( ^1H \) NMR δ 4.73 (d, \( J_{11,12} = 6.5 \) Hz, 1H, H-12), 4.20 (br s, 1H, H-2), 4.08 (s, 3H, O-CH₃), 3.66 (t, \( J = 7.5 \) Hz, H-11), 3.35 (br s, 1H, H-9a), 3.10-3.04 (m, 1H, H-5a), 2.97-
2.92 (m, 1H, H-5b), 2.50-2.46 (m, 1H, H-10), 1.96 (s, 3H, H-16), 1.95 (s, 1H, H-1), 1.86-1.79 (m, 1H, H-6a), 1.73-1.67 (m, 1H, H-6b), 1.62 (q, J 7.5 Hz, 2H, H-3'), 1.61-1.57 (m, 2H, H-1, H-9), 1.54-1.49 (m, 2H, H-1'), 1.43-1.36 (m, 1H, H-2'), 1.26-1.19 (m, 1H, H-2'), 1.10 (d, J 7.0 Hz, H-17), 0.89 (t, J 7.0 Hz, H-4').

13C NMR δ 174.5 (C-15), 173.3 (C-13), 113.0 (C-8), 99.0 (C-14), 88.0 (C-11), 82.2 (C-3), 78.9 (C-2, C-12), 61.2 (C-9a), 59.4 (O-CH3), 50.7 (C-7), 47.7 (C-5), 47.5 (C-9), 35.3 (C-10), 33.7 (C-5), 32.0 (C-2'), 27.5 (C-1'), 27.0 (C-6), 23.4 (C-3'), 16.7 (C-17), 14.2 (C-4'), 8.4 (C-16).

**Compound 164:**

![164](image)

\[ R_f = 0.10 \text{ in MeOH/EtOAc (1:4).} \]

[\[\alpha\]24D] = +6.3 (c 0.65, CHCl3).

ESIMS m/z 392 (100%) [M+H]+.

HRESIMS m/z 392.2432 [M+H]+, calcd for C22H34NO5 392.2437.

1H NMR (major diastereomer) δ 4.72 (d, J 8.0 Hz, 1H, H-12), 4.28 (br s, 1H, H-2), 4.05 (s, 3H, O-CH3), 3.24 (br s, 1H, H-9a), 3.02 (m, 1H, H-5a), 2.93 (m, 1H, H-5b), 2.48 (m, 1H, H-11a), 2.13 (m, 1H, H-10), 2.04 (d, J 3.5 Hz, 1H, H-7), 1.94 (s, 3H, H-16), 1.88 (m, 1H, H-1a), 1.85 (m, 2H, H-6a, H-6b), 1.66 (m, 1H, H-9), 1.62 (m, 1H, H-1b), 1.49 (m, 2H, H-1'a, H-1'b), 1.44 (m, 1H, H-11b), 1.40 (m, 1H, H-2'a), 1.33 (m, 2H, H-3'a, H-3'b), 1.22 (m, 1H, H-2'b), 1.00 (d, J 6.5 Hz, 3H, H-18), 0.91 (t, J 7.5 Hz, 3H, H-4').

13C NMR δ 175.0 (C-15), 174.6 (C-13), 107.0 (C-8), 97.6 (C-14), 82.1 (C-3), 80.1 (C-2), 78.1 (C-12), 63.5 (C-9a), 58.8 (O-CH3), 57.2 (C-7), 47.5 (C-5), 43.6 (C-9), 40.8 (C-11), 34.3 (C-1'), 31.8 (C-1'), 28.4 (C-10), 27.5 (C-2'), 26.6 (C-6), 23.3 (C-3'), 18.5 (C-17), 14.2 (C-4'), 8.3 (C-16).

Minor diastereomer: 13C NMR δ 175.0 (C-15), 174.3 (C-13), 106.7 (C-8), 97.4 (C-14), 82.1 (C-3), 80.2 (C-2), 76.6 (C-12), 63.7 (C-9a), 58.9 (O-CH3), 57.0 (C-7), 47.5 (C-5), 43.3 (C-9), 40.2 (C-11), 34.4 (C-1), 31.7 (C-1'), 28.4 (C-10), 27.2 (C-2'), 26.7 (C-6), 23.3 (C-3'), 17.8 (C-17), 14.2 (C-4'), 8.5 (C-16).
**11(S),12(R)-dihydrostemofoline (111)**

To a solution of isostemofoline (109) (29.6 mg, 0.076 mmol) in EtOH (3.0 mL) at rt was added Pd/C (3.0 mg, 10% w/w), and the flask was flushed with N₂ for 10 min before the solution was left to stir under a H₂ atmosphere (balloon) for 24 h. The flask was flushed with N₂, and the solution was filtered through Celite and washed with MeOH. The filtrate was concentrated *in vacuo*, and the residue was purified by column chromatography using gradient elution from CH₂Cl₂ to CH₂Cl₂/MeOH/NH₄OH (95:5:1) to give 111 as a colorless gum (6.2 mg, 0.016 mmol, 21% yield) and the ring-open product 164 (8.9 mg, 0.023 mmol, 30% yield, dr = 68:32) as a brown gum. 

\[ R_f = 0.19 \text{ in MeOH/CH}_2\text{Cl}_2 (1:9). \]

**ESIMS**

\[ m/z = 390 \text{ (100%)} [M+H]^+. \]

**1H NMR**

\[ \delta = 4.58 \text{ (br s, 1H, H-12)}, 4.20 \text{ (br s, 1H, H-2)}, 4.11 \text{ (s, 3H, O-CH}_3\text{)}, 3.76 \text{ (dd, J}_{10,11} = 8.5 \text{ Hz, J}_{11,12} = 3.0 \text{ Hz, 1H, H-11)}, 3.37 \text{ (br s, 1H, H-9a)}, 3.12-3.06 \text{ (m, 1H, H-5a)}, 2.98-2.93 \text{ (m, 1H, H-5b)}, 2.63-2.55 \text{ (m, 1H, H-10)}, 2.00 \text{ (s, 3H, H-16)}, 1.96 \text{ (d, J = 10.0 Hz, 1H, H-1)}, 1.89-1.79 \text{ (m, 1H, H-6a)}, 1.71-1.66 \text{ (m, 1H, H-6b)}, 1.62-1.56 \text{ (m, 2H, H-1, H-9)}, 1.55-1.49 \text{ (m, 2H, H-2')}, 1.41-1.35 \text{ (m, 1H, H-1')}, 1.31 \text{ (q, J = 7.0 Hz, 2H, H-3')}, 1.25-1.18 \text{ (m, 1H, H-1')}, 1.08 \text{ (d, J = 6.5 Hz, H-17)}, 0.89 \text{ (t, J = 7.5 Hz, H-4').} \]

**13C NMR**

\[ \delta = 174.8 \text{ (C-15)}, 170.5 \text{ (C-13)}, 112.2 \text{ (C-8)}, 98.8 \text{ (C-14)}, 86.4 \text{ (C-11)}, 82.3 \text{ (C-3)}, 78.6 \text{ (C-2)}, 76.8 \text{ (C-12)}, 61.2 \text{ (C-9a)}, 59.1 \text{ (O-CH}_3\text{)}, 50.8 \text{ (C-7)}, 47.7 \text{ (C-5, C-9)}, 33.8 \text{ (C-1)}, 33.3 \text{ (C-10)}, 32.0 \text{ (C-2'), 27.5 (C-1'), 27.0 (C-6), 23.4 (C-3'), 15.1 (C-17), 14.1 (C-4'), 8.9 (C-16).} \]

NMR data of 111 agreed with that of the natural product.\(^{33}\)

**\((Z)-\text{Stemoburkilline (108).}\)**

To a solution of 163 (20.9 mg, 0.054 mmol) in dry CH₂Cl₂ (0.5 mL) at rt under a N₂ atmosphere were added DBU (16.0 μL, 0.107 mmol, 2.0 equiv) and TMSCl (13.7 μL, 0.107 mmol, 2.0 equiv), and the reaction mixture was left to stir at rt for 15 h until the reaction was complete by TLC analysis. The mixture was then diluted with CH₂Cl₂ (10 mL) and the solution was washed with brine, dried...
over MgSO₄, and concentrated in vacuo to give the TMS-protected product 165 as a dark brown gum (EIMS m/z 461 (M⁺, 100%)). The residue was dissolved in MeOH (1.5 mL), and then 10% w/v HCl (1.0 mL) was added and the solution was left to stir at rt under a N₂ atmosphere for 45 min. The solution was then evaporated under vacuum to give a white residue. A saturated NaHCO₃ solution (7 mL) was then added, and the mixture was extracted with CH₂Cl₂ (3 × 20 mL). The combined organic extracts were washed with brine and dried over MgSO₄ before being concentrated in vacuo. The crude product was purified by column chromatography using gradient elution from CH₂Cl₂ to CH₂Cl₂/MeOH/NH₄OH (95:5:1) to give (Z)-stemburkilline (108) as a pale yellow, amorphous solid (12.9 mg, 0.033 mmol, 61% yield for the 2 steps).

\[ R_f = 0.20 \text{ in MeOH/CH}_2\text{Cl}_2 (1:9). \]
\[ [\alpha]_D^{24} = +7.6 (c 0.64, \text{CHCl}_3). \]

IR \( \nu_{\text{max}} \) 3751, 2933, 1750, 1635, 974, 756 cm\(^{-1}\).

EIMS m/z 389 (33%) [M]+.

HREIMS m/z 389.2213 [M]+, calcd for C\(_{22}\)H\(_{31}\)NO\(_5\) 389.2202.

\(^1\)H NMR \( \delta \) 5.48 (d, \( J 10.0 \text{ Hz}, 1\text{H, }H-11\)), 4.30 (br s, 1H, H-2), 4.10 (s, 3H, O-Me), 3.28 (br s, 1H, H-9a), 3.13 (m, 1H, H-10), 3.05 (m, 1H, H-5a), 2.94 (m, 1H, H-5b), 2.15 (d, \( J 5.0 \text{ Hz}, 1\text{H, }H-7\)), 2.05 (s, 3H, H-16), 1.91 (m, 1H, H-1a), 1.83 (m, 2H, H-6a, H-6b), 1.74 (m, 1H, H-9), 1.59 (m, 1H, H-1b), 1.48 (m, 2H, H-1’a, H-1’b), 1.38 (m, 1H, H-2’a), 1.32 (m, 2H, H-3’), 1.24 (m, 1H, H-2’b), 1.05 (d, \( J 6.5 \text{ Hz}, 3\text{H, }H-17\)), 0.90 (t, \( J 7.5 \text{ Hz}, 3\text{H, }H-4’\)).

\(^{13}\)C NMR \( \delta \) 170.8 (C-15), 162.1 (C-13), 142.1 (C-12), 115.2 (C-11), 106.2 (C-8), 99.5 (C-14), 82.1 (C-3), 80.6 (C-2), 63.8 (C-9a), 59.1 (O-CH₃), 55.9 (C-7), 47.5 (C-5), 45.0 (C-9), 33.7(C-1), 31.7 (C-1’), 28.6 (C-10), 27.5 (C-2’), 26.7 (C-6), 23.4 (C-3’), 18.8 (C-17), 14.2 (C-4’), 8.8 (C-16).

The title compound 108 was also prepared from 111 (18.7 mg, 0.048 mmol) using the above procedure. The product was isolated as a pale yellow, amorphous solid (12.8 mg, 0.033 mmol, 69% yield for the 2 steps).
10.4 Experimental for Chapter 4

10.4.1 Preparation of the aldehyde 160

(11Z)-1′α,2′α- and (11Z)-1′β,2′β-Dihydroxystemofoline (173 and 174).

To a solution of AD-mix-α (4.55 g, Aldrich) and methanesulfonamide (617 mg, 6.49 mmol) in tBuOH/H₂O (1:1, 15.0 mL) at 0 ºC was added a solution of (11Z)-1′,2′-didehydrostemofoline (104) (1.25 g, 3.25 mmol) in tBuOH/H₂O (1:1, 5.0 mL). The reaction was left to stir at rt for 45 h. Sodium sulphite (ca 500 mg) was added and left to stir further 1 h. The mixture was extracted with CH₂Cl₂ (3 x 20 mL) and combined organic extracts were washed with 2 M KOH, dried (MgSO₄) and evaporated to give a crude mixture. The crude product was purified by column chromatography with gradient elution from CH₂Cl₂ to CH₂Cl₂/MeOH (9:1) to give 173 (889 mg, 2.12 mmol, 65% yield) as a white solid and 174 (28 mg, 0.07 mmol, 2% yield) as a colourless gum.

These compounds were also prepared from a similar method using AD-mix-β (474 mg, Aldrich), methanesulfonamide (64 mg, 0.68 mmol), and 104 (130 g, 0.339 mmol) to give 173 (9.9 mg, 0.024 mmol, 7% yield) and 174 (68.9 mg, 0.164 mmol, 48% yield). The ¹H and ¹³C NMR spectra of 173 from both methods agreed with those previously reported.³⁴

Rᵡ = 0.43 in MeOH/CH₂Cl₂ (1:9).

[α]ᵡ°⁺⁺⁺₂₂₂ (c 1.0, CHCl₃) (Lit.³⁴ [α]ᵡ°⁺⁺⁺₂₂₂ = +252.9 (c 0.89, CHCl₃))

ESIMS m/z 420 (100%) [M+H]⁺.

¹H NMR δ 4.23 (s, 1H, H-2), 4.14 (s, 3H, O-CH₃), 3.73 (s, 1H, H-1'S), 3.55 (t, J 7.0 Hz, 1H, H-2'S), 3.52 (br s, 1H, H-9a), 2.95 (d, J 6.5 Hz, 1H, H-7), 3.26-3.20 (m, 1H, H-5), 3.11-3.05 (m, 1H, H-10), 3.03-2.98 (m, 1H, H-5), 2.06 (s, 3H, H-16), 2.33-2.27 (m, 1H, H-6a), 2.01 (d, J 13.0 Hz, 1H, H-1a), 1.85-1.82 (m, 1H, H-9), 1.82-1.79 (m, 1H, H-6b), 1.77-1.74 (m, 1H, H-1b), 1.64 (sextet, J 7.5 Hz, 1H, H-3'a), 1.51 (quintet, J 6.5 Hz, 1H, H-3'b), 1.37 (d, J 6.5 Hz, 3H, H-17), 0.96 (t, J 7.5 Hz, 3H, H-4').
\(^{13}\)C NMR $\delta$ 169.8 (C-15), 162.8 (C-13), 148.1 (C-11), 128.2 (C-12), 112.7 (C-8), 98.8 (C-14), 86.4 (C-3), 77.6 (C-2), 73.7 (C-2'), 73.1 (C-1'), 61.8 (C-9a), 59.0 (O-CH3), 50.8 (C-7), 50.6 (C-5), 47.7 (C-9), 34.6 (C-10), 32.9 (C-1), 28.6 (C-6), 26.2 (C-3'), 18.4 (C-17), 10.2 (C-4'), 9.3 (C-16).

**Compound 174:**

$R_f = 0.33$ in MeOH/CH\textsubscript{2}Cl\textsubscript{2} (1:9).

[$\alpha$] \textsubscript{D} \textsuperscript{23} +251 (c 1.0, CHCl\textsubscript{3}).

IR $\nu_{\text{max}}$ 3380, 2960, 2919, 2873, 1741, 1680 cm\textsuperscript{-1}.

ESIMS $m/z$ 420 (100%) [M+H]$^+$.  

HRESIMS $m/z$ 420.2008 [M+H]$^+$, calcd for C\textsubscript{22}H\textsubscript{30}NO\textsubscript{7} 420.2022.

\(^1\)H NMR $\delta$ 4.68 (s, 1H, H-2), 4.13 (s, 3H, O-CH\textsubscript{3}), 3.75 (t, $J$ 7.5 Hz, 1H, H-2'R), 3.52 (s, 1H, H-1'R), 3.49 (br s, 1H, H-9a), 3.10 (d, $J$ 6.5 Hz, 1H, H-7), 3.12-3.07 (m, 1H, H-10), 3.03-2.94 (m, 2H, H-5), 2.05 (s, 3H, H-16), 2.02-1.95 (m, 1H, H-6a), 1.93 (d, $J$ 12.5 Hz, 1H, H-1a), 1.88-1.84 (m, 1H, H-6b), 1.82 (dd, $J$ 9.5 Hz, 2.5 Hz, 1H, H-9), 1.78 (d, $J$ 12.5 Hz, 1H, H-1b), 1.67-1.50 (m, 2H, H-3'), 1.36 (d, $J$ 6.5 Hz, 3H, H-17), 0.95 (t, $J$ 7.5 Hz, 3H, H-4').

\(^{13}\)C NMR $\delta$ 170.1 (C-15), 163.1 (C-13), 148.8 (C-11), 128.0 (C-12), 112.5 (C-8), 98.6 (C-14), 85.8 (C-3), 76.4 (C-2), 72.3 (C-1'), 69.9 (C-2'), 61.5 (C-9a), 59.0 (O-CH\textsubscript{3}), 48.9 (C-7), 48.7 (C-5), 47.8 (C-9), 34.7 (C-10), 33.0 (C-1), 28.2 (C-3'), 26.9 (C-6), 18.4 (C-17), 10.4 (C-4'), 9.2 (C-16).

(2\textsubscript{S},2\textsubscript{a}R,6\textsubscript{S},7\textsubscript{a}S,7\textsubscript{b}S,8\textsubscript{R},9\textsubscript{S},10\textsubscript{Z})-Tetrahydro-10-(3-methoxy-4-methyl-5-oxo-2(5\textsubscript{H})-furanylidene)-9-methyl-4\textsubscript{H}-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-\textit{gh}]pyrrolizine-7b(6\textsubscript{H})-carboxaldehyde (160).

To a solution of diol 173 (529.2 mg, 1.263 mmol) in THF (10 mL) at rt was added aqueous solution (10 mL) of NaIO\textsubscript{4} (353.7 mg, 1.6538 mmol) and the reaction was left to stir at rt for 45 min. The mixture was quenched with saturated aqueous NaHCO\textsubscript{3} (10 mL) and was directly extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 x 20 mL). The combined organic extracts were washed with brine and dried over MgSO\textsubscript{4} before being concentrated in vacuo. The crude product as a white gum was obtained in quantitative yield at >95% purity based upon \(^1\)H NMR analysis which agreed to the previous report.\textsuperscript{34
10.4.2 Synthesis of the sulfone reagents for the modified Julia olefination reaction

1-Bromo-3-(tert-butyldimethylsilyloxy)propane (174a) and 1-chloro-3-(tert-butyldimethylsilyloxy)propane (174b)

To a solution of 3-bromo-1-propanol (2.34 g, 16.834 mmol) in DMF (20.0 mL) at rt were added imidazole (1.31 g, 19.235 mmol) and TBSCI (2.90 g, 19.205 mmol) and the mixture was stirred for 17 h. The reaction mixture was quenched with saturated aqueous NH₄Cl (40.0 mL), extracted with diethyl ether (3 x 40 mL) and dried (MgSO₄). The solvent was removed under reduced pressure to give the crude product which was purified by column chromatography using gradient elution of hexane/EtOAc (1:8) to EtOAc to give a 2:3 mixture of 174a and 174b, respectively, as a colourless oil (2.60 g).
NMR data agreed with those previously reported.95

**Compound 174b:**

\( R_f = 0.20 \) in hexane.

EIMS \( m/z \) 151 (85%) \([\text{M}^{\text{Cl}^{35}+}-\text{C}_4\text{H}_9]^+\), 153 (50%) \([\text{M}^{\text{Cl}^{37}+}-\text{C}_4\text{H}_9]^+\).

\(^1\text{H NMR (300 MHz, CD}_3\text{OD)} \delta 3.77 (t, J 6.0 \text{ Hz, 2H, H-1}), 3.65 (t, J 6.3 \text{ Hz, 2H, H-3}), 1.93 (\text{quintet, J 6.0 Hz, 2H, H-2}), 0.91 (s, 9H, OSi(CH}_3)_2C(CH}_3)_3), 0.08 (s, 6H, OSi(CH}_3)_2C(CH}_3)_3).

\(^13\text{C NMR (75 MHz, CD}_3\text{OD)} \delta 60.5 (\text{C-1}), 42.3 (\text{C-3}), 36.6 (\text{C-2}), 26.4 (\text{OSi(CH}_3)_2C(CH}_3)_3), 19.1 (\text{OSi(CH}_3)_2C(CH}_3)_3), -5.3 (\text{OSi(CH}_3)_2C(CH}_3)_3).


![2-[[3\text{][[(1,1-Dimethylethyl)dimethylsilyl]oxy]propyl]thio]benzothiazole (175).](image)

To a solution of 2-mercaptobenzothiazole (179.8 mg, 1.073 mmol) in 3:1 THF/DMF (15 mL) at 0 °C under a \( \text{N}_2 \) atmosphere was added NaH (67.0 mg of 50% assay in mineral oil, 1.395 mmol) and the mixture was left to stirred for 30 min. Then \( 174\text{a/b} \) (1.38 g) was added to the flask and left to warm to rt over 1 h then heated at 50 °C for another 1 h. The reaction mixture was cooled to rt, \( \text{H}_2\text{O} \) was added and the mixture was extracted with diethyl ether (3 x 20 mL). The combined organic extracts were washed with 5% aqueous NaOH and brine and dried (\( \text{MgSO}_4 \)). The concentrated clear oil was purified by column chromatography using gradient elution from petrol to EtOAc/petrol (5:95) to give \( 175 \) (339.9 mg, 1.001 mmol, 47% yield) as a light yellow oil and \( 174\text{b} \) (941.8 mg).

\( R_f = 0.51 \) in EtOAc/petrol (5:95).

IR \( \nu_{\text{max}} \) 2950, 2929, 2858, 1460, 1429, 1100 cm\(^{-1}\).

ESIMS \( m/z \) 339.7 (3 %) \([\text{M}+\text{H}]^+\), 217.5 (100 %).

HRESIMS \( m/z \) 340.1221 \([\text{M}+\text{H}]^+\), calcd for \( \text{C}_{16}\text{H}_{26}\text{NOSiS}_2 \) 340.1225.

\(^1\text{H NMR (300 MHz)} \delta 7.86 (\text{dd, J 7.2 Hz, 0.6 Hz, 1H, ArH}), 7.75 (\text{dquint, J 8.1 Hz, 0.6 Hz, 1H, ArH}), 7.40 (\text{td, J 7.5 Hz, 1.2 Hz, 1H, ArH}), 7.28 (\text{td, J 8.1 Hz, 1.2 Hz, 1H, ArH}), 3.78 (t, J 6.0 Hz, 2H, H-3'), 3.43 (t J 6.9 Hz, 2H, H-1'), 2.05 (\text{quint, J 6.9 Hz, 2H, H-2'}), 0.91 (s, 9H, OSi(CH}_3)_2C(CH}_3)_3), 0.08 (s, 6H, OSi(CH}_3)_2C(CH}_3)_3).

\(^{13}\text{C NMR (75 MHz)} \delta 167.4 (\text{C-2}), 153.5 (\text{ArC-N}), 135.3 (\text{ArC-S}), 126.1 (\text{ArCH}), 124.2 (\text{ArCH}), 121.6 (\text{ArCH}), 121.0 (\text{ArCH}), 61.3 (\text{C-3'}), 32.3 (\text{C-2'}), 30.4 (\text{C-1'}), 26.1 (\text{OSi(CH}_3)_2C(CH}_3)_3), 18.5 (\text{OSi(CH}_3)_2C(CH}_3)_3), -5.2 (\text{OSi(CH}_3)_2C(CH}_3)_3).

To a solution of 175 (176.1 mg, 0.519 mmol) in CH₂Cl₂ (10 mL) at 0 ºC under a N₂ atmosphere was added 3-chloroperbenzoic acid (319.6 mg of 70% assay, 1.2964 mmol) and the solution was left to stir for 14 h. An aqueous solution of sodium sulfite was added and the mixture was extracted with diethyl ether (3 x 20 mL). The combined organic extracts were washed with saturated aqueous NaHCO₃ solution, H₂O, brine and dried (MgSO₄). The concentrated crude product was purified by column chromatography using gradient elution from petrol to EtOAc/petrol (1:9) to give sulfone 176 (138.6 mg, 0.373 mmol, 72% yield) as a white solid.

R_f = 0.31 in EtOAc/petrol (1:9).

IR ν_max 2955, 2929, 2883, 2852, 1470, 1318 cm⁻¹.

ESIMS m/z 371.8 (50 %) [M+H]⁺, 393.7 (40 %) [MNa⁺], 764.2 (100 %).

HRESIMS m/z 372.1141 [M+H]⁺, calcd for C_{16}H_{26}NO_{3}SiS_{2} 372.1123.

¹H NMR (300 MHz) δ 8.21 (d, J 8.1 Hz, 1H, ArH), 8.02 (d, J 7.8 Hz, 1H, ArH), 7.61 (quint, J 8.4 Hz, 2H, ArH), 3.71 (t, J 5.1 Hz, 2H, CH₂CH₂OTBS), 3.65-3.60 (m, 2H, CH₂CH₂OTBS), 2.12-2.03 (m, 2H, CH₂CH₂CH₂OTBS), 0.85 (s, 9H, OSi(CH₃)₂C(CH₃)₃), 0.01 (s, 6H, OSi(CH₃)₂C(CH₃)₃).

¹³C NMR (75 MHz) δ 165.9 (C-2), 152.9 (ArC-N), 136.9 (ArC-S), 128.1 (ArCH), 127.8 (ArCH), 125.6 (ArCH), 122.5 (ArCH), 60.7 (C-3'), 52.1 (C-1'), 26.0 (C-2'), 25.9 (OSi(CH₃)₂C(CH₃)₃), 18.3 (OSi(CH₃)₂C(CH₃)₃), -5.3 (OSi(CH₃)₂C(CH₃)₃).

2-[(3-Methoxypropyl)thio]benzothiazole (177)

To a solution of 3-methoxy-1-propanol (206.3 mg, 2.289 mmol) and Ph₃P (900.6 mg, 3.434 mmol) in THF (10.0 mL) at 0 ºC was added DIAD (694.3 mg, 3.434 mmol) and the reaction mixture was stirred for 20 min at 0 ºC. Then 2-mercaptobenzothiazole (574.3 mg, 3.434 mmol) was added into the reaction and left to stir for 1 h at 0 ºC. The reaction mixture was quenched with H₂O and the THF was removed under reduced pressure. The aqueous phase was extracted with diethyl ether (3 x 20 mL) and the combined organic extracts were washed with brine and dried (MgSO₄). The extract was concentrated in vacuo to give a yellow slurry mixture of
177 and by-product Ph₃PO. This mixture was purified by dissolving in EtOAc in which Ph₃PO formed a white precipitate and was then filtered through a pad of Celite. The filtrate was evaporated to give 177 (463.4 mg, 1.936 mmol, 85% yield) as a clear yellow oil.

\[ R_f = 0.50 \text{ in CH}_2\text{Cl}_2 \].

ESIMS \( m/z \) 240 (100 %) \([\text{M+H}]^+\).

\(^1\text{H NMR} \quad \delta 7.86 (d, J 8.0 \text{ Hz}, 1\text{H, ArH}), 7.74 (d, J 8.0 \text{ Hz}, 1\text{H, ArH}), 7.40 (t, J 8.0 \text{ Hz}, 1\text{H, ArH}), 7.28 (t, J 7.0 \text{ Hz}, 1\text{H, ArH}), 3.53 (t, J 5.5 \text{ Hz}, 2\text{H, C-3'}), 3.43 (t, J 7.0 \text{ Hz}, 2\text{H, C-1'}), 3.36 (s, 3\text{H, O-CH}_3), 2.10 (q, J 7.0 \text{ Hz}, 2\text{H, C-2'}). 

\(^{13}\text{C NMR} \quad \delta 167.1 (C-2), 153.4 (ArC-N), 135.3 (ArC-S), 126.1 (ArC), 124.3 (ArC), 121.6 (ArC), 121.0 (ArC), 70.8 (C-3'), 58.8 (O-CH_3), 30.5 (C-1'), 29.4 (C-2').

2-[(3-Methoxypropyl)sulfonyl]benzothiazole (178)

The title compound was prepared via a similar method to the synthesis of 176, using 177 (245.3 mg, 1.025 mmol) and 3-chloroperbenzoic acid (530.6 mg, 3.074 mmol) to give the sulfone 178 (261.2 mg, 0.962 mmol, 94% yield) as a yellow oil after extraction with saturated aqueous NaHCO₃ solution (3 x 20 mL).

\[ R_f = 0.66 \text{ in EtOAc/petrol (1:1)} \].

ESIMS \( m/z \) 272 (100 %) \([\text{M+H}]^+\), 294 (25 %) \([\text{M+Na}]^+\).

\(^1\text{H NMR} \quad \delta 8.22 (d, J 8.5 \text{ Hz}, 1\text{H, ArH}), 8.02 (d, J 8.0 \text{ Hz}, 1\text{H, ArH}), 7.64 (t, J 7.5 \text{ Hz}, 1\text{H, ArH}), 7.59 (t, J 7.5 \text{ Hz}, 1\text{H, ArH}), 3.62 (t, J 6.0 \text{ Hz}, 2\text{H, C-1'}), 3.49 (t, J 5.0 \text{ Hz}, 2\text{H, C-3'}), 3.29 (s, 3\text{H, O-CH}_3), 2.20-2.12 (m, 2\text{H, C-2'}). 

\(^{13}\text{C NMR} \quad \delta 165.9 (C-2), 152.9 (ArC-N), 136.9 (ArC-S), 128.2 (ArCH), 127.8 (ArCH), 125.6 (ArCH), 122.5 (ArCH), 70.0 (C-3'), 58.7 (O-CH_3), 52.2 (C-1'), 23.0 (C-2').

1-[(3-Methoxypropyl)sulfonyl]-4-methylbenzene (179).

To a solution of 3-methoxy-1-propanol (292.0 mg, 0.003 mol) in pyridine (20.0 mL) at 0 °C was added \( p \)-toluenesulfonyl chloride (1.21 g, 0.006 mol). The reaction mixture was stirred at rt for 16
h. Purification by column chromatography using gradient elution from petrol to EtOAc/petrol (2:8) as eluent gave product 179 (523.3 mg, 0.002 mol, 68% yield) as a colorless oil.

\[ R_f = 0.38 \text{ in EtOAc/petrol (2:8).} \]

IR \( \nu_{\text{max}} \) 2924, 2882, 2835, 1596, 1359, 1175 cm\(^{-1}\).

ESIMS \( m/z \) 245.1 (100 %) [M+H]\(^+\), 246.1 (20 %).

HRESIMS \( m/z \) 245.0848 [M+H]\(^+\), calcd for C\(_{11}\)H\(_{21}\)O\(_4\)S 245.0848.

\(^1\)H NMR (300 MHz) \( \delta \) 7.79 (d, \( J \) 8.1 Hz, 2H, ArH), 7.35 (d, \( J \) 7.8 Hz, 2H, ArH), 4.12 (t, \( J \) 6.0 Hz, 2H, H-1), 3.38 (t, \( J \) 5.7 Hz, 2H, H-3), 3.24 (s, 3H, O-CH\(_3\)), 2.45 (s, 3H, Ar-CH\(_3\)), 1.89 (quint, \( J \) 6.0 Hz, 2H, H-2).

\(^{13}\)C NMR (75 MHz) \( \delta \) 144.8 (ArC-SO\(_2\)), 133.1 (ArC-CH\(_3\)), 129.9 (ArCH), 128.0 (ArCH), 68.0 (C-3), 67.8 (C-1), 58.7 (O-CH\(_3\)), 29.3 (C-2), 21.7 (ArC-CH\(_3\)).

The \(^1\)H NMR data was close to those reported.\(^{43}\)

5-[(3-Methoxypropyl)thio]-1-phenyl-1H-tetrazole (180).

The title compound was prepared via a similar method to the synthesis of 175, using tosylate 179 (301.3 mg, 1.235 mmol), 1-phenyl-1H-tetrazole-5-thiol (264.1 mg, 1.482 mmol) and NaH (71.1 mg of 50% assay in mineral oil, 1.482 mmol) to give 25 (190.3 mg, 0.761 mmol, 51% yield) as a yellow oil after purification by gradient elution column chromatography from CH\(_2\)Cl\(_2\) to EtOAc/CH\(_2\)Cl\(_2\) (5:95).

\[ R_f = 0.43 \text{ in EtOAc/CH}_2\text{Cl}_2 (1:9). \]

IR \( \nu_{\text{max}} \) 2929, 2878, 2826, 1770, 1499, 1116 cm\(^{-1}\).

ESIMS \( m/z \) 251.1 (100 %) [M+H]\(^+\), 252.1 (20 %), 253.1 (10 %).

HRESIMS \( m/z \) 251.0962 [M+H]\(^+\), calcd for C\(_{11}\)H\(_{15}\)N\(_4\)OS 251.0967.

\(^1\)H NMR \( \delta \) 7.59-7.52 (m, 5H, ArH), 3.50 (t, \( J \) 6.0 Hz, 2H, C-3\(^{\prime}\)), 3.47 (t, \( J \) 7.5 Hz, 2H, C-1\(^{\prime}\)), 3.33 (s, 3H, O-CH\(_3\)), 2.11 (quint, \( J \) 6.5 Hz, 2H, C-2\(^{\prime}\)).

\(^{13}\)C NMR \( \delta \) 154.5 (C-5), 133.9 (ArC-N), 130.2 (ArCH), 130.0 (ArCH), 124.0 (ArCH), 70.6 (C-3\(^{\prime}\)), 58.8 (O-CH\(_3\)), 30.4 (C-1\(^{\prime}\)), 29.3 (C-2\(^{\prime}\)).
5-[(3-Methoxypropyl)sulfonyl]-1-phenyl-1H-tetrazole (181).

The title compound was prepared via a similar method to the synthesis of 176, using 180 (176.7 mg, 0.706 mmol) and 3-chloroperbenzoic acid (522.1 mg of 70% assay, 2.1177 mmol) to give the sulfone 181 (147.9 mg, 0.524 mmol, 74% yield) as a yellow oil after purification by gradient elution column chromatography from CH₂Cl₂ to EtOAc/CH₂Cl₂ (1:9).

\[ R_f = 0.61 \] in EtOAc/CH₂Cl₂ (1:9).

ESIMS \( m/z \) 283.1 (100 %) \([M+H]^+\), 284.1 (20 %), 285.1 (10 %).

HRESIMS \( m/z \) 283.0872 \([M+H]^+\), calcd for C₁₁H₁₅N₄O₃S 283.0865.

\(^1\)H NMR (300 MHz) \[ \delta \] 7.68-7.65 (m, 2H, ArH), 7.61-7.56 (m, 3H, ArH), 3.82 (t, \( J \) 7.5 Hz, 2H, C-1'), 3.50 (t, \( J \) 6.0 Hz, 2H, C-3'), 3.29 (s, 3H, O-CH₃), 2.24-2.15 (m, 2H, C-2').

\(^1^3\)C NMR (75 MHz) \[ \delta \] 153.6 (C-5), 133.1 (ArC-N), 131.5 (ArCH), 129.8 (ArCH), 125.2 (ArCH), 69.5 (C-3'), 58.7 (O-C₃H₃), 53.5 (C-1'), 22.8 (C-2').

10.4.3 Synthesis of oxystemofoline (115)


To a solution of the sulfone 176 (157.4 mg, 0.424 mmol) in dry DMF (10 mL) under a N₂ atmosphere at -60 °C was added LiHMDS (0.39 mL of 1 M in THF) and let to stir for 2 h. After this time, the mixture was transferred via a cannula to a flask containing a solution of the aldehyde 160 (126.7 mg, 0.353 mmol) in dry DMF (10 mL) which had been keeping at -60 °C under a N₂ atmosphere. The reaction was left to slowly warm to rt over 20 h before addition of saturated aqueous NaHCO₃ solution and extraction with ether (3 x 20 mL). The combined organic extracts were washed with brine and dried (MgSO₄). The concentrated residue was purified by column chromatography using gradient elution of CH₂Cl₂ to CH₂Cl₂/MeOH (98:2) as
eluent to give the trans-alkene product 182 as a yellow gum (60.5 mg, 0.117 mmol, 33% yield).

\[ R_f = 0.50 \text{ in MeOH/EtOAc (1:4).} \]

\[ [\alpha]_{D}^{22} +179.6 \ (c \ 1.0, \text{CHCl}_3). \]

IR \( \nu_{\text{max}} \) 2955, 2924, 2883, 2852, 1746, 1621 cm\(^{-1}\).

ESIMS \( m/z \) 516.3 (100 %) \([M+H]^+\), 517.3 (30 %), 518.3 (10 %).

HRESIMS \( m/z \) 516.2768 \([M+H]^+\), calcld for C\(_{28}\)H\(_{42}\)NO\(_6\)Si 516.2781.

\(^1\)H NMR \( \delta \) 5.74 (dt, \( J \) 15.5 Hz, 7.0 Hz, 1H, H-2'), 5.58 (d, \( J \) 15.5 Hz, 1H, H-1'), 4.21 (br s, 1H, H-2), 4.14 (s, 3H, O-CH\(_3\)), 3.64 (t, \( J \) 7.0 Hz, 2H, H-4'), 3.50 (br s, 1H, H-9a), 3.10 (m, 2H, H-5a, H-10), 2.98 (m, 1H, H-5b), 2.86 (d, \( J \) 6.0 Hz, 1H, H-7), 2.28 (q, \( J \) 7.0 Hz, 2H, H-3'), 2.07 (s, 3H, H-16), 1.95 (d, \( J \) 12.5 Hz, 1H, H-1a), 1.86 (m, 2H, H-6a, H-9), 1.81 (m, 2H, H-1b, H-6b), 1.38 (d, \( J \) 6.5 Hz, 3H, H-17), 0.88 (s, 9H, O-Si(CH\(_3\))\(_2\)C(CH\(_3\))\(_3\)), 0.04 (s, 6H, O-Si(CH\(_3\))\(_2\)C(CH\(_3\))\(_3\)).

\(^{13}\)C NMR \( \delta \) 169.8 (C-15), 162.9 (C-13), 148.5 (C-11), 129.7 (C-1'), 128.4 (C-2'), 128.0 (C-12), 112.9 (C-8), 98.7 (C-14), 83.2 (C-3), 80.8 (C-2), 62.8 (C-4'), 61.0 (C-9a), 59.0 (O-CH\(_3\)), 51.4 (C-7), 48.2 (C-5), 47.8 (C-9), 36.1 (C-3'), 34.7 (C-10), 33.0 (C-1), 27.1 (C-6), 26.1 (O-Si(CH\(_3\))\(_2\)C(CH\(_3\))\(_3\)), 18.5 (C-17, O-Si(CH\(_3\))\(_2\)C(CH\(_3\))\(_3\)), 9.3 (C-16), -5.1 (O-Si(CH\(_3\))\(_2\)C(CH\(_3\))\(_3\)).


To a solution of 182 (32.7 mg, 0.064 mmol) in MeOH (2.0 mL) at rt was added 10% aqueous HCl solution (1.0 mL) and was left to stir for 30 min. The reaction mixture was evaporated to give a white residue which was re-dissolved in aqueous NaHCO\(_3\) solution and CH\(_2\)Cl\(_2\). The crude solution was extracted with CH\(_2\)Cl\(_2\) (3 x 10 mL). The combined organic extracts were washed with brine and dried (MgSO\(_4\)). The concentrated residue was purified by column chromatography using gradient elution of EtOAc to EtOAc/MeOH (90:10) as eluent to give the alcohol 169 as a cloudy white gum (22.2 mg, 0.055 mmol, 87% yield).

\[ R_f = 0.11 \text{ in MeOH/EtOAc (1:4).} \]
[α]$_D$$^2$ +240.8 (c 1.0, CHCl$_3$).

IR $\nu_{\text{max}}$ 2960, 2919, 2847, 1743, 1683, 1618 cm$^{-1}$.

ESIMS $m/z$ 402.2 (100 %) [M+H]$^+$, 403.2 (20 %), 404.2 (10 %).

HRESIMS $m/z$ 402.1898 [M+H]$^+$, calcd for C$_{22}$H$_{28}$NO$_6$ 402.1917.

$^1$H NMR (300 MHz) δ 5.73 (dt, $J$ 15.3 Hz, 6.6 Hz, 1H, H-2$'$), 5.62 (d, $J$ 15.9 Hz, 1H, H-1$'$), 4.21 (br s, 1H, H-2), 4.13 (s, 3H, O-CH$_3$), 3.64 (t, $J$ 6.6 Hz, 2H, H-4$'$), 3.49 (br s, 1H, H-9a), 3.07 (m, 2H, H-5a, H-10), 3.00 (m, 1H, H-5b), 2.84 (d, $J$ 5.7 Hz, 1H, H-7), 2.32 (q, $J$ 6.3 Hz, 2H, H-3$'$), 2.05 (s, 3H, H-16), 1.94 (d, $J$ 12.0 Hz, 1H, H-1b), 1.86 (m, 2H, H-6a, H-6b), 1.81 (m, 1H, H-9), 1.76 (m, 1H, H-1a), 1.36 (d, $J$ 6.3 Hz, 3H, H-17).

$^{13}$C NMR (75 MHz) δ 169.9 (C-15), 162.9 (C-13), 148.4 (C-11), 130.6 (C-1$'$), 128.0 (C-12), 127.9 (C-2$'$), 112.8 (C-8), 98.7 (C-14), 83.2 (C-3), 80.6 (C-2), 61.9 (C-4$'$), 61.0 (C-9a), 59.0 (O-CH$_3$), 51.4 (C-7), 48.1 (C-5), 47.7 (C-9), 35.9 (C-3$'$), 34.7 (C-10), 33.0 (C-1), 27.0 (C-6), 9.2 (C-16).

Oxystemofoline (115).

To a solution of alcohol 169 (10.9 mg, 0.027 mmol) in dry MeOH (2.0 mL) at rt was added PdCl$_2$ (2.2 mg, 20% w/w) and the flask was flushed with N$_2$ for 10 min before left to stir under a H$_2$ atmosphere (balloon) for 18 h. The flask was flushed with N$_2$ and the solution was filtered through Celite and washed with MeOH. The filtrate was dried (MgSO$_4$) and concentrated in vacuo. Crude product was purified by column chromatography with isocratic elution of EtOAc to give oxystemofoline (115) as a colorless gum (7.3 mg, 0.018 mmol, 66% yield).

$R_f$ = 0.39 in MeOH/CH$_2$Cl$_2$ (1:9).

[α]$_D$$^2$ +297.8 (c 0.52, CH$_3$OH).

IR $\nu_{\text{max}}$ 3281, 2962, 2929, 1743, 1683, 1618 cm$^{-1}$.

ESIMS $m/z$ 403.8 (100 %) [M+H]$^+$, 404.9 (20 %), 405.8 (13 %).

HRESIMS $m/z$ 404.1977 [M+H]$^+$, calcd for C$_{22}$H$_{30}$NO$_6$ 404.2073.

$^1$H NMR (300 MHz, CDCl$_3$) δ 4.26 (br s, 1H, H-2), 4.13 (s, 3H, O-CH$_3$), 3.65 (t, $J$ 6.0 Hz, 2H, H-4$'$), 3.47 (br s, 1H, H-9a), 3.38 (br, 1H, OH), 3.16 (m, 1H, H-5b), 3.09 (m, 1H, H-10), 2.97 (m, 1H, H-5a), 2.68 (d, $J$ 5.7 Hz, 1H, H-7), 2.06 (s, 3H, H-16), 183
1.97 (d, J 3.9 Hz, 1H, H-1a), 1.87 (td, J 10.2 Hz, 3.9 Hz, 2H, H-6), 1.81 (dd, J 9.6 Hz, 3.3 Hz, 1H, H-9), 1.71 (dt, J 12.3 Hz, 3.3 Hz, 1H, H-1b), 1.61 (m, 2H, H-1’), 1.58 (m, 2H, H-3’), 1.48 (m, 2H, H-2’), 1.36 (d, J 6.3 Hz, 3H, H-17).

13C NMR (75 MHz, CDCl3) δ 169.8 (C-15), 162.9 (C-13), 148.5 (C-11), 128.0 (C-12), 112.8 (C-8), 98.7 (C-14), 83.0 (C-3), 78.6 (C-2), 62.4 (C-4’), 61.1 (C-9a), 59.0 (O-CH3), 50.3 (C-7), 47.7 (C-5, C-9), 34.7 (C-10), 33.5 (C-1), 33.0 (C-3’), 31.4 (C-1’), 26.8 (C-6), 21.3 (C-2’), 18.5 (C-17), 9.3 (C-16).

The title compound was also observed NMR data in (CD3)2CO. 1H NMR (500 MHz, (CD3)2CO) δ 4.24 (s, 3H, O-CH3), 4.22 (br s, 1H, H-2), 3.55 (t, J 4.5 Hz, 2H, H-4’), 3.47 (br s, 1H, H-9a), 3.06 (m, 3H, H-5, H-10), 2.85 (br, 1H, OH), 2.53 (d, J 6.5 Hz, 1H, H-7), 2.04 (s, 3H, H-16), 1.91 (m, 3H, H-1a, H-6a, H-9), 1.79 (m, 1H, H-6b), 1.60 (m, 3H, H-1b, H-1’), 1.54 (m, 4H, H-2’, H-3’), 1.38 (d, J 6.5 Hz, 3H, H-17).

13C NMR (125 MHz, (CD3)2CO) δ 170.1 (C-15), 164.0 (C-13), 149.8 (C-11), 128.2 (C-12), 113.6 (C-8), 98.7 (C-14), 83.6 (C-3), 79.3 (C-2), 62.3 (C-4’), 61.6 (C-9a), 59.7 (O-CH3), 51.2 (C-7), 48.3 (C-9), 48.1 (C-5), 35.5 (C-10), 34.1 (C-3’), 33.8 (C-1), 33.4 (C-1’), 27.2 (C-6), 22.2 (C-2’), 18.4 (C-17), 9.1 (C-16).

NMR data agreed to those natural products except 13C NMR at C-6 and C-1’ which were originally misassigned.

10.4.4 Synthesis of methoxystemofoline (116)


The title compound was prepared via a similar method to the synthesis of 182, using the aldehyde 160 (54.0 mg, 0.150 mmol), LiHMDS (0.16 mL of 1 M in THF) and sulfone 181 (50.9 mg, 0.180 mmol) to give 170 (9.2 mg, 0.022 mmol, 15% yield) as a yellow gum after purification by isocratic elution column chromatography with EtOAc.

$R_f = 0.23$ in MeOH/EtOAc (1:4).

$[\alpha]_{D}^{25} +206.2$ (c 0.71, CHCl3).
IR $\nu_{\text{max}}$ 2957, 2929, 2868, 1745, 1621, 1117 cm$^{-1}$.

ESIMS $m/z$ 415.8 (100 %) [M+H]$^+$, 416.9 (20 %), 417.9 (5 %).

HRESIMS $m/z$ 416.2089 [M+H]$^+$, calcd for C$_{23}$H$_{30}$NO$_6$ 416.2073.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 5.75 (dt, $J$ 15.5 Hz, 7.0 Hz, 1H, H-2$'$), 5.60 (d, $J$ 16.0 Hz, 1H, H-1$'$), 4.22 (br s, 1H, H-2), 4.13 (s, 3H, O-CH$_3$), 3.51 (br s, 1H, H-9a), 3.41 (t, $J$ 6.5 Hz, 2H, H-4$'$), 3.33 (s, 3H, 4'-O-CH$_3$), 3.09 (m, 2H, H-5a, H-10), 2.98 (m, 1H, H-5b), 2.86 (d, $J$ 6.0 Hz, 1H, H-7), 2.34 (q, $J$ 7.0 Hz, 2H, H-3$'$), 2.07 (s, 3H, H-16), 1.95 (d, $J$ 12.0 Hz, 1H, H-1a), 1.81 (m, 4H, H-1b, H-6, H-9), 1.37 (d, $J$ 6.5 Hz, 3H, H-17).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 169.8 (C-15), 163.0 (C-13), 148.5 (C-11), 129.6 (C-1$'$), 128.2 (C-2$'$), 128.1 (C-12), 112.9 (C-8), 98.8 (C-14), 83.3 (C-3), 80.7 (C-2), 72.2 (C-4$'$), 61.0 (C-9a), 59.0 (O-CH$_3$), 58.7 (4'-O-CH$_3$), 51.4 (C-7), 48.2 (C-5), 47.8 (C-9), 32.8 (C-3$'$), 34.7 (C-10), 33.0 (C-1), 27.0 (C-6).

Methoxystemofoline (116).

The title compound was prepared via a similar method to the synthesis of 115, using compound 170 (10.2 mg, 0.025 mmol) and PdCl$_2$ (3.1 mg, 30% w/w) and a reaction time of 1 h. The crude product was purified by column chromatography with gradient elution of EtOAc to EtOAc/MeOH (95:5) to give 116 (4.4 mg, 0.010 mmol, 43% yield) as a yellow gum.

$R_f$ = 0.16 in MeOH/EtOAc (1:4).

$[\alpha]_D^{25}$ +247.4 (c 0.29, CH$_3$OH).

IR $\nu_{\text{max}}$ 2955, 2929, 1741, 1618, 1004, 988 cm$^{-1}$.

ESIMS $m/z$ 417.9 (100 %) [M+H]$^+$, 418.9 (25 %), 419.9 (10 %).

HRESIMS $m/z$ 418.2233 [M+H]$^+$, calcd for C$_{23}$H$_{32}$NO$_6$ 418.2230.

$^1$H NMR $\delta$ 4.26 (br s, 1H, H-2), 4.13 (s, 3H, O-CH$_3$), 3.45 (br s, 1H, H-9a), 3.37 (t, $J$ 6.0 Hz, 2H, H-4$'$), 3.32 (s, 3H, 4'-O-CH$_3$), 3.10 (m, 2H, H-5b, H-10), 2.99 (m, 1H, H-5a), 2.69 (d, $J$ 6.0 Hz, 1H, H-7), 2.07 (s, 3H, H-16), 1.94 (d, $J$ 11.5 Hz, 1H, H-1b), 1.89 (m, 1H, H-6b), 1.81 (m, 2H, H-6a, H-9), 1.70 (d, $J$ 12.0 Hz, 1H, H-1a), 1.59 (m, 4H, H-1', H-3$'$), 1.37 (d, $J$ 6.0 Hz, 3H, H-17), 1.33 (m, 2H, H-2$'$).
\[ ^{13}C \text{NMR} \delta 169.8 (C-15), 163.0 (C-13), 148.6 (C-11), 128.0 (C-12), 112.8 (C-8), 98.7 (C-14), 82.9 (C-3), 78.7 (C-2), 72.7 (C-4'), 61.1 (C-9a), 59.0 (O-CH_3), 58.8 (4'-O\text{-CH}_3), 50.2 (C-7), 47.8 (C-9), 47.7 (C-5), 34.7 (C-10), 31.9 (C-3'), 30.2 (C-1'), 26.8 (C-6), 22.0 (C-2'), 18.5 (C-17), 9.3 (C-16). \]

10.4.5 Synthesis of (1´R)- and (1´S)-hydroxystemofoline (98 and 166)


(Scheme 4.14 (i)). To a solution of aldehyde 160 (93.6 mg, 0.261 mmol) in 5:2 THF/saturated aqueous NH_4Cl (6 mL) was added indium powder (59.9 mg, 0.521 mmol) and allylbromide (135 \mu L, 1.564 mmol). The reaction flask was sealed and sonicated for 3 h. The THF was evaporated to give a white residue which then was dissolved in CH_2Cl_2 and aqueous NaHCO_3. The combined organic phase from extraction with CH_2Cl_2 (3 x 10 mL) was washed with brine and dried (MgSO_4). A mixture of 183 and 184 (81.3 mg, 0.184 mmol, 68% yield) was obtained as a white gum.

(Scheme 4.14 (ii)). To a solution of aldehyde 160 (35.4 mg, 0.099 mmol) in dry THF (3.0 mL) at 0 °C under a N_2 atmosphere was added \(^{1}\)Pc_2Ball (0.49 mL of 1 M in pentane, 0.493 mmol) and the solution was left to stir at 0 °C for 2 h. The reaction solution was quenched with MeOH and then 10% aqueous HCl (5.0 mL) and then washed with CH_2Cl_2 (3 x 10 mL). The aqueous phase was basified with aqueous NaOH and then extracted with CH_2Cl_2 (3 x 10 mL). The combined organic extracts were washed with brine and dried (MgSO_4). The concentrated residue was purified
by column chromatography with gradient elution of EtOAc to EtOAc/MeOH (95:5) to give a mixture of 183 and 184 (30.5 mg, 0.076 mmol, 77% yield) as a white gum. (Scheme 4.14 (iii)). The title compound was also prepared from 160 (35.4 mg, 0.099 mmol) using the above procedure except replacing 

\[ ^{1} \text{Ipc}_{2}\text{Ball} \] with \[ ^{4} \text{Ipc}_{2}\text{Ball} \] (0.49 mL of 1 M in pentane, 0.493 mmol). A mixture was obtained as a white gum of 183 and 184 (27.2 mg, 0.068 mmol, 69% yield).


A mixture of 183 and 184 (81.3 mg) was dissolved in pyridine (2.0 mL) and then acetic anhydride (2.0 mL) was added at rt. The reaction mixture was left to stir for 4 h before the addition of a saturated aqueous solution of NaHCO\textsubscript{3} (5.0 mL) and then extraction with CH\textsubscript{2}Cl\textsubscript{2} (3 x 10 mL). The combined organic extracts were washed with brine, dried (MgSO\textsubscript{4}) and concentrated \textit{in vacuo} to give a brown oil. The crude product was purified by column chromatography with gradient elution of petrol/EtOAc (1:1) to EtOAc to give 185 (50.9 mg, 0.115 mmol, 48% yield) a pale yellow gum and 186 (32.4 mg, 0.073 mmol, 30% yield) as a pale yellow gum.

**Compound 185**:

R\textsubscript{f} = 0.46 in MeOH/EtOAc (1:4).

[\textalpha]_{D}^{24} +226.5 (c 1.0, CHCl\textsubscript{3}).

IR \nu\textsubscript{max} 2924, 2858, 2871, 1618, 1372, 1234 cm\textsuperscript{-1}.

ESIMS \textit{m/z} 443.9 (100 \%) [M+H]\textsuperscript{+}, 444.9 (25 \%), 446.0 (5 \%).

HRESIMS \textit{m/z} 444.2011 [M+H]\textsuperscript{+}, calcd for C\textsubscript{24}H\textsubscript{30}NO\textsubscript{7} 444.2022.

\textsuperscript{1}H NMR \delta 5.74 (m, 1H, H-3\textsuperscript{'}), 5.19 (s, 1H, H-1\textsuperscript{'}), 5.14 (s, 1H, H-(4'E)), 5.08 (t, J 12.5 Hz, 1H, H-(4'Z)), 4.45 (br s, 1H, H-2), 4.13 (s, 3H, O-CH\textsubscript{3}), 3.46 (br s, 1H, H-
9a), 3.23 (m, 1H, H-5a), 3.06 (m, 1H, H-10), 2.99 (m, 1H, H-5b), 2.85 (d, $J = 6.0$ Hz, 1H, H-7), 2.40 (m, 1H, H-2'), 2.06 (s, 3H, H-16), 2.03 (s, 3H, 1'-OCOCH$_3$), 1.93 (d, $J = 12.5$ Hz, 1H, H-1a), 1.87 (m, 2H, H-6), 1.83 (dd, $J = 18.0$ Hz, 10.5 Hz, 3.5 Hz, 1H, H-9), 1.63 (d, $J = 12.0$ Hz, 1H, H-1b), 1.36 (d, $J = 6.5$ Hz, 3H, H-17).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 170.5 (1'-OCOCH$_3$), 169.8 (C-15), 162.9 (C-13), 148.2 (C-11), 133.3 (C-3'), 128.1 (C-12), 118.3 (C-4'), 112.7 (C-8), 98.8 (C-14), 85.4 (C-3), 76.6 (C-2), 70.7 (C-1'), 60.9 (C-9a), 59.0 (O-CH$_3$), 49.4 (C-7), 48.2 (C-5), 47.9 (C-9), 35.6 (C-2'), 34.6 (C-10), 33.2 (C-1), 27.4 (C-6), 21.2 (1'-OCOCH$_3$), 18.4 (C-17), 9.3 (C-16).

Compound 186:

$R_f$ = 0.59 in MeOH/EtOAc (1:4).

$[\alpha]_{D}^{24}$ +188.0 (c 1.0, CHCl$_3$).

IR $\nu_{\text{max}}$ 2924, 1740, 1629, 1460, 1362, 1234 cm$^{-1}$.

ESIMS $m/z$ 443.9 (100 %) [M+H]$^+$, 444.9 (25 %), 445.9 (5 %).

HRESIMS $m/z$ 444.2015 [M+H]$^+$, calcd for C$_{24}$H$_{30}$NO$_7$ 444.2022.

$^1$H NMR $\delta$ 5.72 (m, 1H, H-3'), 5.10 (s, 1H, H-1'), 5.05 (dd, $J = 16.5$ Hz, 8.0 Hz, 2H, H-4'), 4.48 (br s, 1H, H-2), 4.13 (s, 3H, O-CH$_3$), 3.48 (br s, 1H, H-9a), 3.15 (m, 1H, H-5a), 3.07 (m, 1H, H-10), 3.01 (m, 1H, H-5b), 2.71 (d, $J = 6.0$ Hz, 1H, H-7), 2.62 (dd, $J = 14.0$ Hz, 3.0 Hz, 1.5 Hz, 1H, H-2'a), 2.11 (m ,1H, H-2'b), 2.08 (s, 3H, 1'-OCOCH$_3$), 2.06 (s, 3H, H-16), 2.02 (m ,1H, H-6b), 1.98 (d, $J = 12.5$ Hz, 1H, H-1a), 1.82 (m, 1H, H-6a), 1.82 (dd, $J = 10.5$ Hz, 4.5 Hz, 1H, H-9), 1.67 (d, $J = 12.0$ Hz, 1H, H-1b), 1.37 (d, $J = 7.5$ Hz, 3H, H-17).

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 170.9 (1'-OCOCH$_3$), 169.7 (C-15), 162.8 (C-13), 148.2 (C-11), 134.0 (C-3'), 128.1 (C-12), 117.9 (C-4'), 112.5 (C-8), 98.8 (C-14), 84.9 (C-3), 75.8 (C-2), 69.7 (C-1'), 61.2 (C-9a), 59.0 (O-CH$_3$), 48.6 (C-7, C-5), 47.8 (C-9), 35.0 (C-2'), 34.6 (C-10), 33.5 (C-1), 26.7 (C-6), 21.0 (1'-OCOCH$_3$), 18.5 (C-17), 9.3 (C-16).

To a solution of the acetate 185 (23.5 mg, 0.053 mmol) in 2:1 THF/H₂O (3.0 mL) was added LiOH (21.0 mg of 53% assay, 0.265 mmol) at rt and then mixture was left to stir for 16 h. H₂O was added and the mixture was extracted with CH₂Cl₂ (3 x 10 mL). The combined organic extracts were washed with saturated aqueous NaHCO₃ solution and brine and dried (MgSO₄). The concentrated residue was purified by column chromatography with gradient elution of EtOAc to EtOAc/MeOH (98:2) to give the alcohol 183 (13.0 mg, 0.032 mmol, 61% yield) as a pale yellow gum. $R_f = 0.23$ in MeOH/EtOAc (1:4).

$[\alpha]^{23}_D +308.0$ (c 1.0, CHCl₃).

IR $\nu_{\text{max}}$ 3446, 2965, 2919, 2847, 1743, 1621 cm⁻¹.

ESIMS $m/z$ 402.2 (100 %) [M+H]+, 403.2 (20 %).

HRESIMS $m/z$ 402.1912 [M+H]+, calcd for C₂₂H₂₈NO₆ 402.1917.

$^1$H NMR (500 MHz, CDCl₃) $\delta$ 5.92 (m, 1H, H-3'), 5.18 (d, $J$ 18.0 Hz, 1H, H-(4'Z)), 5.12 (d, $J$ 10.0 Hz, 1H, H-(4'E)), 4.48 (br s, 1H, H-2), 4.13 (s, 3H, O-CH₃), 3.68 (dd, $J$ 9.5 Hz, 3.5 Hz, 1H, H-1'b), 3.51 (br s, 1H, H-9a), 3.15 (m, 1H, H-5a), 3.07 (m, 1H, H-10), 3.03 (m, 1H, H-5b), 2.82 (d, $J$ 5.0 Hz, 1H, H-7), 2.35 (m ,1H, H-2'b), 2.30 (m, 1H, H-1'a), 2.06 (s, 3H, H-16), 1.97 (d, $J$ 12.5 Hz, 1H, H-1a), 1.87 (m, 2H, H-6), 1.85 (m, 1H, H-9), 1.64 (d, $J$ 13.0 Hz, 1H, H-1b), 1.37 (d, $J$ 6.0 Hz, 3H, H-17).

$^{13}$C NMR (125 MHz, CDCl₃) $\delta$ 169.8 (C-15), 162.9 (C-13), 148.2 (C-11), 135.0 (C-3'), 128.1 (C-12), 117.7 (C-4'), 112.6 (C-8), 98.8 (C-14), 87.0 (C-3), 75.6 (C-2), 67.9 (C-1'), 61.0 (C-9a), 59.0 (O-CH₃), 48.2 (C-7), 48.1 (C-9), 47.6 (C-5), 36.7 (C-2'), 34.5 (C-10), 33.9 (C-1), 27.4 (C-6), 18.4 (C-17), 9.3 (C-16).

The title compound was prepared via a similar method to the synthesis of 183, using the acetate 186 (15.1 mg, 0.034 mmol) and LiOH (13.5 mg of 53% assay, 0.170 mmol) to give the alcohol 184 (10.0 mg, 0.025 mmol, 73% yield) as a white gum.

\[ R_f = 0.36 \text{ in MeOH/EtOAc (1:4).} \]

\[ [\alpha]_{D}^{24} +380.0 \ (c \ 0.41, \text{CHCl}_3). \]

IR \( \nu_{\text{max}} \) 3286, 2957, 2924, 2854, 1744, 1615 cm\(^{-1}\).

ESIMS \( m/z \) 402.2 (100 %) [M+H]+, 403.2 (20 %), 404.2 (10 %).

HRESIMS \( m/z \) 402.1903 [M+H]+, calcd for C\(_{22}\)H\(_{28}\)NO\(_6\) 402.1917.

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 5.86 (m, 1H, H-3‘), 5.19 (d, J 11.5 Hz, 1H, H-(4‘Z)), 5.16 (s, 1H, H-(4‘E)), 4.40 (s, 1H, H-2), 4.13 (s, 3H, O-CH\(_3\)), 3.71 (d, J 10.5 Hz, 1H, H-1’a), 3.49 (br s, 1H, H-9a), 3.15 (m, 1H, H-9), 3.09 (m, 1H, H-10), 3.03 (d, J 5.5 Hz, 1H, H-5a), 3.01 (m, 1H, H-7), 2.53 (dd, J 14.0 Hz, 5.5 Hz, 1H, H-2’a), 2.07 (s, 3H, H-16), 2.02 (d, J 14.5 Hz, 1H, H-2’b), 2.00 (d, J 11.5 Hz, 1H, H-6b), 1.97 (d, J 13.0 Hz, 1H, H-1a), 1.88 (m, 1H, H-6a), 1.85 (d, J 7.5 Hz, 1H, H-5b), 1.69 (d, J 12.5 Hz, 1H, H-1b), 1.37 (d, J 6.0 Hz, 3H, H-17).

\(^13\)C NMR (125 MHz, CDCl\(_3\)) \( \delta \) 169.8 (C-15), 162.9 (C-13), 148.5 (C-11), 135.2 (C-3’), 128.1 (C-12), 118.6 (C-4’), 112.9 (C-8), 98.8 (C-14), 86.0 (C-3), 77.0 (C-2), 69.8 (C-1’), 61.7 (C-9a), 59.0 (O-CH\(_3\)), 49.3 (C-7, C-9), 48.0 (C-5), 37.8 (C-2’), 34.6 (C-1, C-10), 27.3 (C-6), 18.4 (C-17), 9.3 (C-16).

1’(R)-Hydroxystemofoline (98).

To a solution of alcohol 183 (11.7 mg, 0.029 mmol) in dry MeOH (2.0 mL) at rt was added Pd/C (1.2 mg, 10% w/w) and the flask was flushed with N\(_2\) for 10 min before left to stir under a H\(_2\) atmosphere (balloon) for 45 min. The flask was flushed with N\(_2\) and the solution was filtered through Celite and washed with MeOH. The filtrate was dried (MgSO\(_4\)) and concentrated in vacuo. The crude product was purified by column...
chromatography with gradient elution of CH₂Cl₂ to CH₂Cl₂/MeOH (98:2) to give the alcohol 98 as a yellow gum (4.1 mg, 0.010 mmol, 35% yield). 

\( R_f = 0.19 \) in MeOH/EtOAc (1:4).

\([\alpha]_{D}^{23} +298.7 \) (c 0.34, CHCl₃).

IR \( \nu_{\max} \) 3462, 2960, 2919, 2868, 1744, 1620 cm⁻¹.

ESIMS \( m/z \) 404.2 (100 %) [M+H]+, 405.2 (18 %), 406.2 (10 %).

HRESIMS \( m/z \) 404.2069 [M+H]+, calcd for C₂₂H₃₀NO₆ 404.2073.

\(^1\)H NMR (300 MHz, CDCl₃) \( \delta \) 4.47 (br s, 1H, H-2), 4.14 (s, 3H, O-CH₃), 3.60 (m, 1H, H-1’b), 3.54 (br s, 1H, H-9a), 3.15 (m, 1H, H-5a), 3.10 (m, 1H, H-10), 3.07 (m, 1H, H-5b), 2.80 (br s, 1H, H-7), 2.07 (s, 3H, H-16), 1.98 (d, J 12.3 Hz, 1H, H-1a), 1.89 (m, 3H, H-6, H-9), 1.66 (m, 2H, H-1b, H-3’a), 1.52 (m, 2H, H-2’), 1.40 (m, 1H, H-3’b), 1.38 (d, J 6.6 Hz, 3H, H-17), 0.97 (t, J 7.2 Hz, 3H, H-4’).

\(^1\)C NMR (75 MHz, CDCl₃) \( \delta \) 169.8 (C-15), 162.8 (C-13), 148.2 (C-11), 128.2 (C-12), 112.9 (C-8), 98.8 (C-14), 87.4 (C-3), 75.6 (C-2), 67.9 (C-1’), 61.1 (C-9a), 59.0 (O-CH₃), 48.2 (C-9), 48.1 (C-7), 47.5 (C-5), 34.5 (C-10), 34.2 (C-2’), 34.0 (C-1), 27.4 (C-6), 20.2 (C-3’), 18.5 (C-17), 14.3 (C-4’), 9.3 (C-16).

1’(S)-Hydroxystemofoline (166). The title compound was prepared via a similar method to the synthesis of 98, using alcohol 184 (17.0 mg, 0.042 mmol) and Pd/C (1.7 mg, 10% w/w) to give alcohol 166 (6.9 mg, 0.017 mmol, 40% yield) as a white gum.

\( R_f = 0.28 \) in MeOH/EtOAc (1:4).

\([\alpha]_{D}^{23} +219.0 \) (c 0.58, CHCl₃).

IR \( \nu_{\max} \) 3183, 2924, 2854, 1744, 1615, 982 cm⁻¹.

ESIMS \( m/z \) 404.2 (100 %) [M+H]+, 405.2 (20 %), 406.2 (5 %).

HRESIMS \( m/z \) 404.2064 [M+H]+, calcd for C₂₂H₃₀NO₆ 404.2073.

\(^1\)H NMR (500 MHz, CDCl₃) \( \delta \) 4.36 (br s, 1H, H-2), 4.14 (s, 3H, O-CH₃), 3.73 (d, J 9.5 Hz, 1H, H-1’a), 3.58 (br s, 1H, H-9a), 3.23 (br s, 1H, H-5a), 3.10 (m, 1H, H-10), 3.06 (m, 1H, H-5b), 3.00 (d, J 6.0 Hz, 1H, H-7), 2.07 (s, 3H, H-16), 2.04 (m, 1H, H-6a), 1.99 (d, J 12.5 Hz, 1H, H-1a), 1.88 (m, 2H, H-6b, H-9), 1.77 (m, 1H, H-1b),
To a solution of stemofoline (97) (40.2 mg, 0.104 mmol) in 2:1 acetone/H2O (3.0 mL) at rt was added 4-methylmorpholine-N-oxide (22.6 mg, 0.193 mmol) and then K2OsO4·2H2O (1.9 mg, 0.005 mmol). The reaction was left to stir at rt for 16 h and then stirred for 1 h in the presence of sodium sulfite (50 mg). The reaction mixture was filtered, saturated aqueous NaHCO3 solution was added and the mixture was extracted with CH2Cl2 (3 x 10 mL). The combined organic extracts were washed with brine and dried (MgSO4). The concentrated residue was purified by column chromatography with gradient elution of CH2Cl2 to CH2Cl2/MeOH (90:10) to give 187 as a pale yellow gum (24.6 mg, 0.058 mmol, 56% yield).

Rf = 0.40 in MeOH/CH2Cl2 (1:9).

[α]D20 -8.4 (c 1.55, CHCl3).

IR νmax 3282, 2954, 2931, 2871, 1671, 1327, 1022 cm⁻¹.

ESIMS m/z 422.0 (100 %) [M+H]+, 423.1 (20 %), 424.1 (5 %).

HRESIMS m/z 422.2166 [M+H]+, calcd for C22H31NO7 422.2179.

1H NMR (300 MHz, CD3OD) δ 4.25 (br s, 1H, H-2), 4.14 (s, 3H, O-CH3), 3.40 (br s, 1H, H-9a), 3.11 (m, 3H, H-5, H-9), 2.86 (br, 1H, H-10), 2.44 (d, J 5.7 Hz, 1H, H-7), 1.99 (m, 1H, H-1a), 1.96 (s, 3H, H-16), 1.86 (m, 2H, H-6), 1.63 (m, 1H, H-1b), 1.60 (m, 2H, H-1'), 1.37 (m, 4H, H-2', H-3'), 1.08 (d, J 6.9 Hz, 3H, H-17), 0.94 (t, J 11.0 Hz, 3H, H-4').
$^{13}$C NMR (75 MHz, CD$_3$OD) $\delta$ 175.1 (C-15), 171.4 (C-13), 112.3 (C-8), 109.1 (C-11), 104.1 (C-12), 100.4 (C-14), 84.2 (C-3), 79.2 (C-2), 62.5 (C-9a), 59.8 (O-CH$_3$), 51.4 (C-7), 48.3 (C-5), 45.3 (C-9), 37.2 (C-10), 33.6 (C-1), 32.4 (C-1'), 28.3 (C-2'), 26.6 (C-6), 24.2 (C-3'), 14.3 (C-4'), 12.7 (C-17), 8.2 (C-16).

NMR of 185 was also determined in CDCl$_3$. For the $^{13}$C NMR spectrum at C-12 could not be observed.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 4.25 (br s, 1H, H-2), 4.12 (s, 3H, O-CH$_3$), 3.45 (br s, 1H, H-9a), 3.11 (m, 1H, H-5a), 3.00 (m, 1H, H-5b), 2.70 (m, 1H, H-10), 2.51 (d, $J$ 6.0 Hz, 1H, H-7), 2.01 (s, 3H, H-16), 1.98 (d, $J$ 12.5 Hz, 1H, H-1a), 1.91 (d, $J$ 10.0 Hz, 1H, H-9), 1.84 (m, 1H, H-6a), 1.76 (m, 1H, H-6b), 1.70 (d, $J$ 12.5 Hz, 1H, H-1b), 1.54 (d, $J$ 9.5 Hz, 2H, H-1'), 1.38 (m, 1H, H-2'), 1.34 (m, 2H, H-3'), 1.24 (m, 1H, H-2'), 1.13 (br s, 2H, 11-OH, 12-OH), 1.03 (d, $J$ 6.5 Hz, 3H, H-17), 0.90 (t, $J$ 7.0 Hz, 3H, H-4').

$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 172.7 (C-15), 168.0 (C-13), 111.3 (C-8), 101.9 (C-11), 100.0 (C-14), 83.2 (C-3), 78.7 (C-2), 61.0 (C-9a), 59.1 (O-CH$_3$), 49.8 (C-7), 47.5 (C-5), 46.0 (C-9), 36.0 (C-10), 32.9 (C-1), 31.5 (C-1'), 27.4 (C-2'), 26.2 (C-6), 23.2 (C-3'), 14.1 (C-4'), 12.6 (C-17), 8.7 (C-16).


To a suspension of silica gel (166.5 mg) in ether (1.0 mL) at rt was added NaIO$_4$ (16.2 mg, 0.076 mmol) in H$_2$O (1.0 mL). Then a solution of 187 (24.6 mg, 0.058 mmol) in CH$_2$Cl$_2$ (2.0 mL) was added to the mixture and let to stir for 1 h at rt. The reaction mixture was filtered through a plug of cotton wool and the filtrate was dried (MgSO$_4$). The concentrated crude product was purified by column chromatography with gradient elution of CH$_2$Cl$_2$ to CH$_2$Cl$_2$/MeOH (95:5) to give 188 as a clear yellow gum (9.6 mg, 0.035 mmol, 60% yield).

$R_f$ = 0.37 in MeOH/EtOAc (1:4).

$[\alpha]_{D}^{25}$ +26.3 (c 0.21, CHCl$_3$).

IR $\nu_{max}$ 2945, 2921, 2868, 1797, 970 cm$^{-1}$.

ESIMS $m/z$ 278.2 (100 %) [M+H]$^+$, 279.2 (20 %), 280.2 (3 %).
HRESIMS m/z 278.1679 [M+H]^+, calcd for C_{16}H_{24}NO_{6} 278.1756.

$^1$H NMR (500 MHz, CDCl$_3$) δ 4.32 (br s, 1H, H-2), 3.41 (br s, 1H, H-9a), 3.15 (m, 1H, H-5b), 3.02 (m, 1H, H-5a), 2.77 (dq, $J$ 11.5 Hz, 7.5 Hz, 1H, H-10), 2.65 (d, $J$ 6.0 Hz, 1H, H-7), 1.98 (d, $J$ 12.5 Hz, 1H, H-1a), 1.93 (m, 1H, H-9), 1.90 (m, 1H, H-6a), 1.82 (m, 1H, H-6b), 1.75 (dt, $J$ 12.5 Hz, 3.5 Hz, 1H, H-1b), 1.56 (m, 2H, H-1')), 1.43 (m, 1H, H-2'), 1.35 (q, $J$ 7.0 Hz, 2H, H-3'), 1.26 (d, $J$ 7.0 Hz, 3H, 10-CH$_3$), 1.24 (m, 1H, H-2'), 0.92 (t, $J$ 7.0 Hz, 3H, H-4').

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 178.5 (C-11), 109.26 (C-8), 83.2 (C-3), 78.9 (C-2), 61.2 (C-9a), 50.2 (C-7), 47.7 (C-5), 45.7 (C-9), 35.9 (C-10), 32.9 (C-1'), 27.3 (C-2'), 26.6 (C-6), 23.2 (C-3'), 14.1 (C-4'), 13.4 (C-12).

10.4.7 Synthesis of alcohol derivatives via the Wittig reaction


To a solution of the aldehyde 160 (56.3 mg, 0.157 mmol) in dry toluene (3.0 mL) at rt under a N$_2$ atmosphere was added (triphenylphosphoranylidene)acetaldehyde (95.4 mg, 0.314 mmol). Then reaction mixture was heated to 80 °C under a N$_2$ atmosphere for 2 days. The reaction was quenched with saturated aqueous NaHCO$_3$ solution and extracted with CH$_2$Cl$_2$ (3 x 10 mL). The combined organic extracts were washed with brine and dried (MgSO$_4$). After evaporation, the crude product mixture of 189a-c was obtained as a yellow gum (113.6 mg) on the basis of NMR and MS analysis.
\[ R_f = 0.50 \] in MeOH/CH₂Cl₂ (5:95).

ESIMS \( m/z \) 386.0 (30%) [M+H]⁺, 412.0 (100%) [M+H]⁺, 437.0 (20%) [M+H]⁺.


To a solution of \(189a-c\) (113.6 mg) in dry MeOH (2.0 mL) was added NaBH₄ (12.0 mg) at rt. The reaction mixture was left to stir at rt for 45 min. Then the MeOH was evaporated to give a white residue which was extracted with CH₂Cl₂ (3 x 10 mL) and saturated aqueous NaHCO₃ solution. The combined organic extracts were washed with brine and dried over MgSO₄. After evaporation, the crude product mixture of \(190a-c\) was obtained as a yellow gum (111.8 mg). This mixture was separated by PTLC with 10% MeOH/EtOAc as a mobile phase to give \(190a\) (7.1 mg, 0.018 mmol, 12% yield over 2 steps), \(190b\) (17.5 mg, 0.042 mmol, 27% yield over 2 steps) and \(190c\) (3.3 mg, 0.008 mmol, 5% yield over 2 steps), as colourless oils.

\[ R_f = 0.06 \] in MeOH/CH₂Cl₂ (1:9).

\[ \alpha \] \( ^{25}_D \) +280.1 (c 1.14, CHCl₃).

IR \( \nu_{max} \) 3329, 2933, 2921, 2864, 1752, 1621, 1003 cm⁻¹.

ESIMS \( m/z \) 388.0 (100%) [M+H]⁺, 389.1 (20%).

HRESIMS \( m/z \) 388.1762 [M+H]⁺, calcd for C₂₁H₂₅NO₆ 388.1760.

\(^1\)H NMR (500 MHz, CDCl₃) \( \delta \) 5.94 (dt, \( J \) 15.3 Hz, 5.0 Hz, 1H, H-2'), 5.81 (d, \( J \) 15.3 Hz, 1H, H-1'), 4.24 (s, 1H, H-2), 4.19 (br s, 2H, H-3'), 4.14 (s, 3H, O-CH₃), 3.51 (br s, 1H, H-9a), 3.10 (m, 2H, H-5a, H-10), 3.00 (m, 1H, H-5b), 2.88 (d, \( J \) 5.5 Hz, 1H, H-7), 2.07 (s, 3H, H-16), 1.96 (d, \( J \) 12.0 Hz, 1H, H-1a), 1.87 (m, 2H, H-6), 1.83 (m,
1H, H-9), 1.78 (d, J 12.0 Hz, 1H, H-1b), 1.70 (br s, 1H, 3'-OH), 1.38 (d, J 6.5 Hz, 3H, H-17).

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 169.8 (C-15), 162.9 (C-13), 148.4 (C-11), 130.5 (C-2'), 129.1 (C-1'), 128.1 (C-12), 112.9 (C-8), 98.8 (C-14), 83.1 (C-3), 80.6 (C-2), 63.0 (C-3'), 61.1 (C-9a), 59.0 (O-CH$_3$), 51.6 (C-7), 48.3 (C-5), 47.8 (C-9), 34.7 (C-10), 32.9 (C-1), 27.0 (C-6), 18.4 (C-17), 9.3 (C-16).

**Compound 190b:**

$R_f$ = 0.13 in MeOH/CH$_2$Cl$_2$ (1:9).

$[\alpha]_{D}^{25}$ +229.9 (c 0.77, CHCl$_3$).

IR $\nu_{\text{max}}$ 3288, 3007, 2937, 2872, 1726, 1613, 1005 cm$^{-1}$.

ESIMS $m/z$ 414.0 (100 %) [M+H]$^+$, 415.0 (20 %).

HRESIMS $m/z$ 414.1902 [M+H]$^+$, calcd for C$_{23}$H$_{27}$NO$_6$ 414.1917.

$^1$H NMR (500 MHz, CDCl$_3$) δ 6.34 (dd, J 14.5 Hz, 10.5 Hz, 1H, H-3'), 6.30 (dd, J 15.0 Hz, 10.5 Hz, 1H, H-2'), 5.87 (dt, J 14.5 Hz, 5.5 Hz, 1H, H-4'), 5.76 (d, J 15.0 Hz, 1H, H-1'), 4.23 (s, 1H, H-2), 4.20 (d, J 6.0 Hz, 2H, H-5'), 4.14 (s, 3H, O-CH$_3$), 3.52 (br s, 1H, H-9a), 3.10 (m, 2H, H-5a, H-10), 3.00 (m, 1H, H-5b), 2.88 (d, J 5.5 Hz, 1H, H-7), 2.08 (s, 3H, H-16), 1.96 (d, J 12.5 Hz, 1H, H-1a), 1.84 (m, 3H, H-6, H-9), 1.78 (d, J 12.5 Hz, 1H, H-1b), 1.59 (br s, 1H, 5'-OH), 1.38 (d, J 6.5 Hz, 3H, H-17).

$^{13}$C NMR (125 MHz, CDCl$_3$) δ 169.8 (C-15), 162.9 (C-13), 148.4 (C-11), 132.8 (C-4'), 131.7 (C-1'), 130.6 (C-2'), 130.0 (C-3'), 128.1 (C-12), 112.9 (C-8), 98.8 (C-14), 83.5 (C-3), 80.8 (C-2), 63.4 (C-5'), 61.1 (C-9a), 59.0 (O-CH$_3$), 52.1 (C-7), 48.4 (C-5), 47.8 (C-9), 34.7 (C-10), 33.0 (C-1), 27.0 (C-6), 18.5 (C-17), 9.3 (C-16).
**Compound 190c:**

\[ R_f = 0.27 \text{ in MeOH/CH}_2\text{Cl}_2 (1:9). \]
\[ [\alpha]^2_D +174.2 \text{ (c 0.15, CHCl}_3). \]

**IR** \( \nu_{\text{max}} \) 3378, 2962, 2921, 2851, 1742, 1618 cm\(^{-1}\).

**ESIMS** \( m/\text{z} \) 440.1 (100 \%) \([\text{M+H}]^+\), 441.2 (5 \%).

**HRESIMS** \( m/\text{z} \) 440.2049 \([\text{M+H}]^+\), calcd for C\(_{25}\)H\(_{29}\)NO\(_6\) 440.2073.

**\(^1\)H NMR** (500 MHz, CDCl\(_3\)) \( \delta \) 6.38-6.33 (m, 1H, H-2'), 6.31-6.26 (m, 1H, H-5'), 6.26-6.23 (m, 2H, H-3', H-4'), 5.87 (dt, \( J \) 14.5 Hz, 5.5 Hz, 1H, H-6'), 5.77 (d, \( J \) 15.0 Hz, 1H, H-1'), 4.24 (br s, 1H, H-2), 4.21 (d, \( J \) 5.5 Hz, 2H, H-7'), 4.14 (s, 3H, O-CH\(_3\)), 3.52 (br s, 1H, H-9a), 3.10 (m, 2H, H-5b, H-10), 3.01 (m, 1H, H-5a), 2.88 (d, \( J \) 5.5 Hz, 1H, H-7), 2.07 (s, 3H, H-16), 1.96 (d, \( J \) 12.0 Hz, 1H, H-1a), 1.86 (m, 1H, H-9), 1.83 (m, 2H, H-6), 1.78 (d, \( J \) 12.5 Hz, 1H, H-1b), 1.75 (br s, 1H, 7'-OH), 1.38 (d, \( J \) 6.5 Hz, 3H, H-17).

**\(^{13}\)C NMR** (125 MHz, CDCl\(_3\)) \( \delta \) 169.8 (C-15), 162.9 (C-13), 148.4 (C-11), 132.9 (C-6'), 132.5 (C-4'), 132.2 (C-3'), 131.8 (C-1'), 131.2 (C-5'), 130.7 (C-2'), 128.1 (C-12), 112.9 (C-8), 98.8 (C-14), 85.6 (C-3), 80.8 (C-2), 63.5 (C-7'), 61.1 (C-9a), 59.0 (O-CH\(_3\)), 52.1 (C-7), 48.4 (C-5), 47.8 (C-9), 34.7 (C-10), 33.0 (C-1), 27.0 (C-6), 18.5 (C-17), 9.3 (C-16).

**10.4.8 Synthesis of (11Z)-1',2'-didehydrostemofoline-N-oxide (105)**

(11Z)-1',2'-didehydrostemofoline-N-oxide (105).

To a solution mixture of (11Z)-1',2'-didehydrostemofoline (104) (91.9 mg, 0.239 mmol) and Na\(_2\)WO\(_4\)-2H\(_2\)O (47.3 mg, 0.143 mmol, 60 mol%) in MeOH (10.0 mL) at 0 °C, was added 30% H\(_2\)O\(_2\) solution (270 \( \mu \)L, 2.387 mmol). The mixture was stirred at rt for 40 h. Then MnO\(_2\) was added to completely degrade excess H\(_2\)O\(_2\) which was monitored by starch paper. The solvent was removed under reduced pressure to give
the crude product. Purification by column chromatography using gradient elution of CH₂Cl₂ to MeOH/CH₂Cl₂ (5:95) gave 105 (71.8 mg, 0.179 mmol, 75% yield) as a white gum.

\[ R_f = 0.14 \text{ in MeOH/CH}_2\text{Cl}_2 (1:9). \]

ESIMS \( m/z \) 402 (100 %) [M+H]⁺.

\(^1\)H NMR δ 6.15 (d, \( J = 16.0 \text{ Hz, } 1\text{H, } H-1' \)), 5.84 (dt, \( J = 16.0 \text{ Hz, } 6.0 \text{ Hz, } 1\text{H, } H-2' \)), 3.31 (br s, 1H, H-2), 4.13 (s, 3H, O-CH₃), 4.36-3.92 (m, 1H, H-5a), 3.72-3.66 (m, 1H, H-5b), 3.26-3.20 (m, 1H, H-10), 3.16 (d, \( J = 7.0 \text{ Hz, } 1\text{H, } H-7 \)), 3.07 (d, \( J = 13.0 \text{ Hz, } 1\text{H, } H-1a \)), 2.33-2.28 (m, 1H, H-6b), 2.25-2.22 (m, 1H, H-9), 2.19 (quintet, \( J = 7.0 \text{ Hz, } 2\text{H, } H-3' \)), 2.10 (d, \( J = 13.0 \text{ Hz, } 1\text{H, } H-1b \)), 2.05 (s, 3H, H-16), 1.87-1.82 (m, 1H, H-6b), 1.42 (d, \( J = 6.5 \text{ Hz, } 3\text{H, } H-16 \)), 1.03 (t, \( J = 7.5 \text{ Hz, } 3\text{H, } H-4' \)).

\(^{13}\)C NMR δ 169.5 (C-15), 162.4 (C-13), 147.3 (C-11), 137.1 (C-2'), 128.4 (C-12), 120.2 (C-1'), 111.1 (C-8), 99.2 (C-14), 91.6 (C-3), 80.4 (C-2), 77.5 (C-9a), 64.4 (C-5), 59.0 (O-CH₃), 49.0 (C-9), 48.5 (C-7), 34.9 (C-10), 31.9 (C-1), 26.0 (C-3'), 22.1 (C-6), 18.2 (C-17), 13.3 (C-4'), 9.2 (C-16).

10.5 Experimental for Chapter 5

10.5.1 Reductive amination reactions of the aldehyde 160


To a solution of aldehyde 160 (26.4 mg, 0.074 mmol) in dry dichloroethane (2.0 mL) and acetic acid (0.2 mL) at rt was added methylamine (0.147 mmol) and then NaBH(OAc)₃ (46.7 mg, 0.220 mmol). The reaction mixture was left to stir for 24 h. The mixture was quenched with saturated aqueous NaHCO₃ solution (10 mL) and was directly extracted with CH₂Cl₂ (3 x 20 mL). The combined organic extracts were washed with brine and dried (MgSO₄) before being concentrated in vacuo. The concentrated residue was purified by column chromatography using gradient elution from CH₂Cl₂ to CH₂Cl₂/MeOH (9:1) to give the amine 191 (13.8 mg, 0.037 mmol, 60% yield) as a yellow gum.
\( R_f = 0.10 \) in MeOH/CH\(_2\)Cl\(_2\) (1:9).
\([\alpha]^{25}_D +311.1 \) (c 0.92, CHCl\(_3\)).

IR \( \nu_{\text{max}} \) 3350, 2941, 2921, 2880, 2794, 1756, 1623 cm\(^{-1}\).

ESIMS \( m/z \) 375.0 (100\%) [M+H]\(^+\), 376.1 (20%).

HRESIMS \( m/z \) 375.1938 [M+H]\(^+\), calcd for C\(_{20}\)H\(_{27}\)N\(_2\)O\(_5\) 375.1920.

\(^1\)H NMR \( \delta \) 4.42 (s, 1H, H-2), 4.12 (s, 3H, O-CH\(_3\)), 3.44 (br s, 1H, H-9a), 3.12-3.07 (m, 2H, H-5a, H-10), 3.02-2.98 (m, 1H, H-5b), 2.70 (s, 2H, H-1'), 2.67 (d, \( J = 6.5 \) Hz, 1H, H-7), 2.45 (s, 3H, H-3'), 2.06 (s, 3H, H-16), 1.94 (d, \( J = 12.0 \) Hz, 1H, H-1b), 1.92-1.87 (m, 1H, H-6a), 1.85-1.80 (m, 2H, H-6b, H-9), 1.70 (d, \( J = 12.0 \) Hz, 1H, H-1a), 1.58 (br s, 1H, NH), 1.36 (d, J 7.0 Hz, 1H, H-17).

\(^{13}\)C NMR \( \delta \) 169.8 (C-15), 163.0 (C-13), 148.5 (C-12), 128.0 (C-11), 112.6 (C-8), 98.7 (C-14), 82.8 (C-3), 79.0 (C-2), 61.3 (C-9a), 59.0 (O-CH\(_3\)), 52.6 (C-1'), 49.8 (C-7), 48.0 (C-9), 47.8 (C-5), 37.2 (N-CH\(_3\)), 34.7 (C-10), 33.7 (C-1), 27.2 (C-6), 18.4 (C-17), 9.3 (C-16).


Prepared using the general method described above, using the aldehyde 160 (16.6 mg, 0.046 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), dimethylamine (0.092 mmol) and NaBH(OAc)\(_3\) (29.4 mg, 0.139 mmol). The purified product was obtained as a yellow gum (7.0 mg, 0.018 mmol, 39\% yield).

\( R_f = 0.26 \) in MeOH/CH\(_2\)Cl\(_2\) (1:9).
\([\alpha]^{25}_D +281.3 \) (c 0.47, CHCl\(_3\)).

IR \( \nu_{\text{max}} \) 3936, 2936, 2356, 2236, 1743, 1620 cm\(^{-1}\).

ESIMS \( m/z \) 389.0 (100\%) [M+H]\(^+\), 390.2 (20%).

HRESIMS \( m/z \) 389.2059 [M+H]\(^+\), calcd for C\(_{21}\)H\(_{29}\)N\(_2\)O\(_5\) 389.2076.

\(^1\)H NMR \( \delta \) 4.38 (s, 1H, H-2), 4.13 (s, 3H, O-CH\(_3\)), 3.45 (br s, 1H, H-9a), 3.17-3.11 (m, 1H, H-5a), 3.11-3.06 (m, H-10), 3.03-2.97 (m, 1H, H-5b), 2.78 (d, \( J = 6.0 \) Hz, 1H, H-7), 2.48 (d, \( J = 13.5 \) Hz, 1H, H-1'a), 2.35 (d, \( J = 13.5 \) Hz, 1H, H-1'b), 2.30 (s, 6H,
N-CH₃), 2.06 (s, 3H, H-16), 1.95 (d, J 11.5 Hz, 1H, H-1a), 1.91-1.86 (m, 1H, H-6a), 1.84-1.81 (m, 1H, H-6b), 1.79-1.76 (m, 1H, H-9), 1.76-1.74 (m, 1H, H-1b), 1.36 (d, J 7.0 Hz, 3H, H-17).

¹³C NMR δ 169.8 (C-15), 163.0 (C-13), 148.6 (C-12), 128.0 (C-11), 112.7 (C-8), 98.8 (C-14), 83.2 (C-3), 79.0 (C-2), 60.8 (C-9a), 59.9 (C-1′), 59.0 (O-CH₃), 49.7 (C-7), 47.9 (C-5, C-9), 47.3 (N-CH₃), 34.7 (C-10), 33.3 (C-1), 27.1 (C-6), 18.5 (C-17), 9.3 (C-16).


Prepared using the general method described above, using the aldehyde 160 (24.3 mg, 0.068 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), isopropylamine (0.135 mmol) and NaBH(OAc)₃ (43.0 mg, 0.203 mmol). The purified product was obtained as a colourless gum (21.1 mg, 0.052 mmol, 78% yield).

Rᵣ = 0.23 in MeOH/CH₂Cl₂ (1:9).

[α]₂⁵D +246.9 (c 1.26, CHCl₃).

IR νmax 3383, 2961, 2885, 2356, 2337, 1742, 1621 cm⁻¹.

ESIMS m/z 403.0 (100%) [M+H]⁺, 404.1 (20%).

HRESIMS m/z 403.2234 [M+H]⁺, caleld for C₂₂H₃₁N₂O₅ 403.2233.

¹H NMR δ 4.43 (br s, 1H, H-2), 4.13 (s, 3H, O-CH₃), 3.45 (br s, 1H, H-9a), 3.12-3.06 (m, 2H, H-5, H-10), 3.02-2.97 (m, 1H, H-5), 2.77-2.74 (m, 1H, H-1′”), 2.74 (d, J 10.0 Hz, 1H, H-1′), 2.69 (d, J 10.0 Hz, 1H, H-1′), 2.67 (d, J 5.0 Hz, 1H, H-7), 2.06 (s, 3H, H-16), 1.94 (d, J 11.5 Hz, 1H, H-1a), 1.91-1.87 (m, 1H, H-9), 1.85-1.81 (m, 2H, H-6), 1.71 (d, J 12.0 Hz, 1H, H-1b), 1.50 (br s, 1H, NH), 1.37 (d, J 7.0 Hz, 3H, H-17), 1.05 (d, J 6.5 Hz, 3H, H-4′), 1.02 (d, J 6.0 Hz, 3H, H-2′”).

¹³C NMR δ 169.9 (C-15), 163.0 (C-13), 148.6 (C-12), 128.0 (C-11), 112.6 (C-8), 98.7 (C-14), 82.9 (C-3), 79.1 (C-2), 61.3 (C-9a), 59.0 (O-CH₃), 49.9 (C-7), 49.4 (C-1′”), 48.0 (C-1′), 47.8 (C-5), 34.7 (C-10), 33.7 (C-1, C-9), 27.2 (C-6), 23.4 (C-2′”), 22.8 (C-4′), 18.5 (C-17), 9.3 (C-16).

Prepared using the general method described above, using the aldehyde 160 (24.0 mg, 0.067 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), allylamine (0.134 mmol) and NaBH(OAc)₃ (42.5 mg, 0.200 mmol). The purified product was obtained as a yellow gum (18.7 mg, 0.047 mmol, 70% yield).

$R_f = 0.11$ in MeOH/EtOAc (2:8).

$[\alpha]_{D}^{25} +437.6$ ($c$ 1.25, CHCl₃).

IR $\nu_{max}$ 3402, 2952, 2925, 28880, 2847, 1742, 1619 cm⁻¹.

ESIMS $m/z$ 401.0 (100%) [M+H]$^+$, 402.1 (20%).

HRESIMS $m/z$ 401.2073 [M+H]$^+$, calcd for C$_{22}$H$_{29}$N$_2$O$_5$ 401.2076.

$^1$H NMR δ 5.89-5.81 (m, 1H, H-2")', 5.15 (dd, $J$ 16.0 Hz, 1.5 Hz, 1H, H-3"'(E)), 5.08 (dd, $J$ 10.0 Hz, 1.0 Hz, 1H, H-3"'(Z)), 4.44 (br s, 1H, H-2), 4.12 (s, 3H, O-CH$_3$), 3.44 (br s, 1H, H-9a), 3.29-3.22 (m, 2H, H-1"'), 3.11-3.06 (m, 2H, H-5b, H-10), 3.02-2.96 (m, 1H, H-5a), 2.71 (ABq, $J$ 10.0 Hz, 2H, H-1"'), 2.66 (d, $J$ 6.0 Hz, 1H, H-7), 2.05 (s, 3H, H-16), 1.94 (d, $J$ 12.0 Hz, 1H, H-1a), 1.90-1.86 (m, 1H, H-6b), 1.84-1.79 (m, 2H, H-6a, H-9), 1.71 (dt, $J$ 12.0 Hz, 3.0 Hz, 1H, H-1b), 1.63 (br s, 1H, NH), 1.36 (d, $J$ 6.5 Hz, 3H, H-17).

$^{13}$C NMR δ 169.8 (C-15), 163.0 (C-13), 148.5 (C-12), 136.9 (C-2"'), 128.0 (C-11), 116.1 (C-3"'), 112.6 (C-8), 98.7 (C-14), 82.9 (C-3), 79.0 (C-2), 61.3 (C-9a), 59.0 (O-CH$_3$), 52.8 (C-1"'), 49.8 (C-7), 49.5 (C-1"), 48.0 (C-9), 47.8 (C-5), 34.6 (C-10), 33.7 (C-1), 27.2 (C-6), 18.4 (C-17), 9.3 (C-16).

Prepared using the general method described above, using the aldehyde 160 (23.9 mg, 0.067 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), 2-(dimethylamino)ethylamine (0.133 mmol) and NaBH(OAc)₃ (42.3 mg, 0.200 mmol). The purified product was obtained as a colourless gum (8.2 mg, 0.019 mmol, 28% yield).

$R_f = 0.17$ in MeOH/CH₂Cl₂ (4:6).

$[\alpha]_{D}^25 +178.5$ (c 0.55, CHCl₃).

IR νₓᵡₐₐₓ 3384, 2962, 2932, 2360, 1740, 1618 cm⁻¹.

ESIMS $m/z$ 432.0 (100%) [M+H]⁺, 433.2 (20%).

HRESIMS $m/z$ 432.2487 [M+H]⁺, calcd for C₂₃H₃₄N₃O₅ 432.2498.

¹H NMR (300 MHz) δ 4.43 (br s, 1H, H-2), 4.13 (s, 3H, O-CH₃), 3.46 (br s, 1H, H-9a), 3.16-3.06 (m, 2H, H-5, H-10), 3.04-2.95 (m, 1H, H-5), 2.75 (s, 2H, H-1’), 2.74 (t, $\text{J} 6.0 \text{ Hz}$, 2H, H-1’’), 2.69 (d, $\text{J} 5.4 \text{ Hz}$, 1H, H-7), 2.44 (t, $\text{J} 6.0 \text{ Hz}$, 2H, H-2’’), 2.25 (s, 6H, N-CH₃), 2.20 (br s, 1H, NH), 2.06 (s, 3H, H-16), 1.94 (d, $\text{J} 12.0 \text{ Hz}$, 1H, H-1a), 1.90-1.84 (m, 2H, H-6), 1.79 (dd, $\text{J} 10.2 \text{ Hz}$, 3.6 Hz, 1H, H-9), 1.73 (dt, $\text{J} 12.3 \text{ Hz}$, 3.3 Hz, 1H, H-1b), 1.36 (d, $\text{J} 6.6 \text{ Hz}$, 3H, H-17).

¹³C NMR (75 MHz) δ 169.9 (C-15), 163.0 (C-13), 148.5 (C-12), 128.0 (C-11), 112.6 (C-8), 98.7 (C-14), 82.9 (C-3), 78.9 (C-2), 61.2 (C-9a), 59.0 (O-CH₃), 58.9 (C-2’’), 50.3 (C-1’’), 49.8 (C-7), 48.3 (C-9), 47.9 (C-5, C-1’”), 45.6 (N-CH₃), 34.6 (C-10), 33.6 (C-1), 27.1 (C-6), 18.4 (C-17), 9.3 (C-16).

Prepared using the general method described above, using the aldehyde 160 (18.6 mg, 0.052 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), ethanolamine (0.104 mmol) and NaBH(OAc)\(_3\) (32.9 mg, 0.155 mmol). The purified product was obtained as a colourless gum (10.0 mg, 0.025 mmol, 48% yield).

\(R_f = 0.20\) in MeOH/CH\(_2\)Cl\(_2\) (1:9).

\([\alpha]_{D}^{25} +267.7\) (c 0.67, CHCl\(_3\)).

IR \(\nu_{\max}\) 3392, 2961, 2936, 1740, 1618, 1143 cm\(^{-1}\).

ESIMS \(m/z\) 405.0 (100\%) [M+H]\(^+\), 406.1 (15\%).

HRESIMS \(m/z\) 405.2015 [M+H]\(^+\), calcd for C\(_{21}\)H\(_{29}\)N\(_2\)O\(_6\) 405.2026.

\(^1\)H NMR \(\delta\) 4.42 (br s, 1H, H-2), 4.13 (s, 3H, O-CH\(_3\)), 3.66-3.58 (m, 2H, H-2\(''\)), 3.48 (br s, 1H, H-9a), 3.16-3.12 (m, 1H, H-5a), 3.11-3.06 (m, 1H, H-10), 3.04-2.99 (m, 1H, H-5b), 2.87-2.83 (m, 1H, H-1'), 2.84 (d, \(J\ 12.0\ Hz\), 1H, H-1''), 2.81-2.76 (m, 1H, H-1'), 2.76 (d, \(J\ 12.0\ Hz\), 1H, H-1'''), 2.67 (d, \(J\ 6.0\ Hz\), 1H, H-7), 2.11 (br s, 1H, NH), 2.06 (s, 3H, H-16), 1.96 (d, \(J\ 12.0\ Hz\), 1H, H-1a), 1.94-1.88 (m, 1H, H-6a), 1.88-1.85 (m, 1H, H-6b), 1.84-1.81 (m, 1H, H-9), 1.74 (d, \(J\ 12.0\ Hz\), 1H, H-1b), 1.37 (d, \(J\ 6.0\ Hz\), 3H, H-17).

\(^{13}\)C NMR \(\delta\) 169.9 (C-15), 163.0 (C-13), 148.4 (C-12), 128.1 (C-11), 112.5 (C-8), 98.8 (C-14), 83.2 (C-3), 78.9 (C-2), 61.2 (C-9a, C-2'''), 59.0 (O-CH\(_3\)), 51.8 (C-1'''), 49.7 (C-7), 49.6 (C-1'), 48.0 (C-9), 47.8 (C-5), 34.6 (C-10), 33.6 (C-1), 27.1 (C-6), 18.4 (C-17), 9.3 (C-16).

Prepared using the general method described above, using the aldehyde 160 (24.8 mg, 0.069 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), morpholine (0.138 mmol) and NaBH(OAc)₂ (43.9 mg, 0.207 mmol). The purified product was obtained as a colourless gum (18.7 mg, 0.043 mmol, 63% yield).

Rf = 0.28 in MeOH/CH₂Cl₂ (1:9).

[α]D +317.6 (c 0.99, CHCl₃).

IR νmax 2951, 2932, 2359, 2337, 1740, 1620 cm⁻¹.

ESIMS m/z 431.2 (100%) [M+H]+, 432.3 (5%).

HRESIMS m/z 431.2163 [M+H]+, calcd for C₂₃H₃₁N₂O₆ 431.2182.

¹H NMR δ 4.35 (br s, 1H, H-2), 4.13 (s, 3H, O-CH₃), 3.69-3.63 (m, 4H, H-2′′), 3.42 (br s, 1H, H-9a), 3.16-3.11 (m, 1H, H-5b), 3.09-3.05 (m, 1H, H-10), 3.01-2.96 (m, 1H, H-5a), 2.79 (br s, 2H, H-3″), 2.75 (d, J 5.5 Hz, 1H, H-7), 2.49-2.43 (m, 2H, H-1′), 2.40-2.37 (m, 2H, H-3″), 2.06 (s, 3H, H-16), 1.94 (d, J 12.5 Hz, 1H, H-1a), 1.92-1.86 (m, 1H, H-6b), 1.83-1.80 (m, 1H, H-6a), 1.79-1.75 (m, 1H, H-9), 1.71 (br s, 1H, H-1b), 1.36 (d, J 6.5 Hz, 3H, H-17).

¹³C NMR δ 169.8 (C-15), 162.9 (C-13), 148.4 (C-12), 128.0 (C-11), 112.7 (C-8), 98.7 (C-14), 83.2 (C-3), 79.0 (C-2), 67.2 (C-2″), 60.8 (C-9a), 59.0 (O-CH₃), 58.9 (C-1′), 55.2 (C-3″), 49.6 (C-7), 47.8 (C-5, C-9), 34.6 (C-10), 33.3 (C-1), 27.2 (C-6), 18.4 (C-17), 9.3 (C-16).


Prepared using the general method described above, using the aldehyde 160 (26.6 mg, 0.074 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), ethyl-1-piperazinecarboxylate (0.148 mmol) and NaBH(OAc)₃ (47.1 mg, 0.222 mmol).
purified product was obtained as a colourless gum (28.9 mg, 0.058 mmol, 78% yield).

\[ R_f = 0.33 \text{ in MeOH/CH}_2\text{Cl}_2 (1:9). \]

\[ [\alpha]_D^{25} +236.7 \text{ (c } 1.93, \text{ CHCl}_3). \]

IR \( \nu_{\text{max}} \) 2964, 2932, 2356, 2337, 1743, 1694, 1620 cm\(^{-1}\).

ESIMS \( m/z \) 502.0 (100%) [M+H]\(^+\), 503.1 (20%).

HRESIMS \( m/z \) 502.2546 [M+H]\(^+\), calcd for C\(_{26}\)H\(_{36}\)N\(_3\)O\(_7\) 502.2553.

\(^1\)H NMR \( \delta \) 4.34 (br s, 1H, H-2), 4.12 (s, 3H, O-CH\(_3\)), 4.11-4.08 (m, 2H, NCO\(_2\)CH\(_2\)CH\(_3\)), 3.42 (br s, 5H, H-9a, H-3\(''\)), 3.14-3.04 (m, 2H, H-5a, H-10), 3.00-2.95 (m, 1H, H-5b), 2.76 (br s, 2H, H-2\(''\)), 2.72 (d, J 6.0 Hz, 1H, H-7), 2.46 (s, 2H, H-1\(')\), 2.34-2.32 (m, 2H, H-2\(''\)), 2.05 (s, 3H, H-16), 1.93 (d, J 12.5 Hz, 1H, H-1a), 1.89-1.84 (m, 1H, H-6a), 1.82-1.78 (m, 1H, H-6b), 1.75 (dd, J 10.0 Hz, 2.5 Hz, 1H, H-9), 1.69 (d, J 11.5 Hz, 1H, H-1b), 1.35 (d, J 7.0 Hz, 3H, H-17), 1.23 (t, J 7.5 Hz, 3H, NCO\(_2\)CH\(_2\)CH\(_3\)).

\(^{13}\)C NMR \( \delta \) 169.8 (C-15), 162.9 (C-13), 155.6 (NCO\(_2\)CH\(_2\)CH\(_3\)), 148.4 (C-12), 128.0 (C-11), 112.7 (C-8), 98.6 (C-14), 83.2 (C-3), 79.0 (C-2), 61.4 (NCO\(_2\)CH\(_2\)CH\(_3\)), 60.8 (C-9a), 59.0 (O-CH\(_3\)), 58.5 (C-1\(''\)), 54.4 (C-2\(''\)), 49.7 (C-7), 47.8 (C-5, C-9), 44.0 (C-3\(''\)), 34.6 (C-10), 33.3 (C-1), 27.2 (C-6), 18.4 (C-17), 14.8 (NCO\(_2\)CH\(_2\)CH\(_3\)), 9.3 (C-16).


Prepared using the general method described above, using the aldehyde \( 160 \) (26.0 mg, 0.072 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), 1-methylpipperazine (0.145 mmol) and NaBH(OAc)\(_3\) (46.0 mg, 0.217 mmol). The purified product was obtained as a colourless gum (20.7 mg, 0.047 mmol, 64% yield).

\[ R_f = 0.09 \text{ in MeOH/CH}_2\text{Cl}_2 (1:9). \]

\[ [\alpha]_D^{25} +279.4 \text{ (c } 1.38, \text{ CHCl}_3). \]

IR \( \nu_{\text{max}} \) 2961, 2939, 2356, 2325, 1742, 1618 cm\(^{-1}\).

ESIMS \( m/z \) 444.0 (100%) [M+H]\(^+\), 445.2 (10%).
HRESIMS m/z 444.2501 [M+H]^+, calcd for C_{24}H_{34}N_{3}O_{5} 444.2498.

$^1$H NMR δ 4.42 (br s, 1H, H-2), 4.12 (s, 3H, O-CH$_3$), 3.41 (br s, 1H, H-9a), 3.16-3.10 (m, 1H, H-5a), 3.08-3.04 (m, 1H, H-10), 3.00-2.94 (m, 1H, H-5b), 2.80 (br s, 4H, H-2'), 2.75 (d, J 6.0 Hz, 1H, H-7), 2.46 (s, 2H, H-1'), 2.42 (br s, 4H, H-3'), 2.26 (s, 3H, N-CH$_3$), 2.05 (s, 3H, H-16), 1.92 (d, J 12.0 Hz, 1H, H-1a), 1.89-1.85 (m, 1H, H-6a), 1.80-1.77 (m, 1H, H-6b), 1.75 (dd, J 9.0 Hz, 2.5 Hz, 1H, H-9), 1.70 (d, J 12.0 Hz, 1H, H-1b), 1.35 (d, J 6.5 Hz, 3H, H-17).

$^{13}$C NMR δ 169.8 (C-15), 162.9 (C-13), 148.5 (C-12), 128.0 (C-11), 112.8 (C-8), 98.6 (C-14), 83.2 (C-3), 79.0 (C-2), 60.8 (C-9a), 59.0 (O-CH$_3$), 58.4 (C-1'), 55.4 (C-3'), 54.5 (C-2''), 49.5 (C-7), 47.9 (C-5), 47.8 (C-9), 46.0 (N-CH$_3$), 34.6 (C-10), 33.2 (C-1), 27.2 (C-6), 18.4 (C-17), 9.3 (C-16).

(5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-hexahydro-7b-1-anilinomethyl-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (200). Prepared using the general method described above, using the aldehyde 160 (15.9 mg, 0.044 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), aniline (0.089 mmol) and NaBH(OAc)$_3$ (28.2 mg, 0.133 mmol). The purified product was obtained as a colourless gum (3.0 mg, 0.007 mmol, 25% yield).

$R_f$ = 0.74 in MeOH/CH$_2$Cl$_2$ (1:9).

$[\alpha]_{D}^{25} +272.1$ (c 0.51, CHCl$_3$).

IR $\nu_{max}$ 3380, 2964, 2923, 2363, 2331, 1742, 1621 cm$^{-1}$.

ESIMS m/z 437.0 (100%) [M+H]$^+$, 438.1 (20%).

HRESIMS m/z 437.2077 [M+H]$^+$, calcd for C$_{25}$H$_{29}$N$_2$O$_5$ 437.2076.

$^1$H NMR δ 7.18 (t, J 7.5 Hz, 2H, ArH), 6.73 (t, J 7.5 Hz, 1H, ArH), 6.66 (d, J 7.5 Hz, 2H, ArH), 4.42 (s, 1H, H-2'), 4.14 (s, 3H, O-CH$_3$), 3.62 (br s, 1H, H-9a), 3.45 (d, J 10.5 Hz, 1H, H-1'), 3.19-3.16 (m, 2H, H-5a, H-1'), 3.14-3.10 (m, 2H, H-5b, H-10), 2.84 (d, J 6.0 Hz, 1H, H-7), 2.07 (s, 3H, H-16), 2.02 (d, J 12.5 Hz, 1H, H-1a), 2.05-1.98 (m, 1H, H-6a), 1.94-1.90 (m, 1H, H-6b), 1.89 (d, J 3.5 Hz, 1H, H-9), 1.86-1.84 (m, 1H, H-1b), 1.40 (d, J 7.0 Hz, 3H, H-17).
\(^{13}\)C NMR \(\delta\) 169.8 (C-15), 162.8 (C-13), 148.2 (C-12, ArC-N), 129.4 (ArCH), 128.2 (C-11), 118.2 (ArCH), 113.4 (ArCH), 112.4 (C-8), 98.9 (C-14), 83.1 (C-3), 79.0 (C-2), 61.7 (C-9a), 59.0 (O-CH\(_3\)), 49.9 (C-7), 48.0 (C-9), 47.8 (C-5), 44.2 (C-1'), 34.6 (C-10), 33.7 (C-1), 26.9 (C-6), 18.5 (C-17), 9.3 (C-16).


Prepared using the general method described above, using the aldehyde 160 (26.0 mg, 0.072 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), \(N\)-benzylamine (0.145 mmol) and NaBH(OAc)\(_3\) (46.0 mg, 0.217 mmol). The purified product was obtained as a yellow gum (27.9 mg, 0.062 mmol, 86\% yield).

\(R_f\) = 0.46 in MeOH/CH\(_2\)Cl\(_2\) (1:9).

\([\alpha]^{25}_D\) +196.9 (c 1.86, CHCl\(_3\)).

IR \(\nu_{\text{max}}\) 3388, 2958, 2945, 2356, 2337, 1736, 1625 cm\(^{-1}\).

ESIMS \(m/z\) 451.0 (100\% \([M+H]^+\)), 452.3 (20\%).

HRESIMS \(m/z\) 451.2245 \([M+H]^+\), calcd for C\(_{26}\)H\(_{31}\)N\(_2\)O\(_5\) 451.2233.

\(^1\)H NMR (300 MHz) \(\delta\) 7.34-7.29 (m, 4H, ArH), 7.25-7.23 (m, 1H, ArH), 4.43 (br s, 1H, H-2), 4.13 (s, 3H, O-CH\(_3\)), 3.82 (s, 2H, H-1''), 3.45 (br s, 1H, H-9a), 3.11-3.08 (m, 1H, H-10), 3.06-2.95 (m 2H, H-5), 2.71 (q, \(J\) 14.0 Hz, 2H, H-1''), 2.65 (d, \(J\) 6.0 Hz, 1H, H-7), 2.06 (s, 3H, H-16), 1.95 (d, \(J\) 12.5 Hz, 1H, H-1a), 1.82-1.79 (m, 3H, H-6, H-9), 1.74 (dt, \(J\) 12.0 Hz, 3.0 Hz, 1H, H-1b), 1.37 (d, \(J\) 6.0 Hz, 3H, H-17).

\(^{13}\)C NMR (75 MHz) \(\delta\) 169.8 (C-15), 163.0 (C-13), 148.5 (C-12), 140.4 (ArC), 128.5 (ArCH), 128.0 (C-11), 127.0 (ArCH), 112.6 (C-8), 98.6 (C-14), 82.9 (C-3), 79.1 (C-2), 61.3 (C-9a), 59.0 (O-CH\(_3\)), 54.1 (C-1''), 49.7 (C-7), 49.2 (C-1''), 48.0 (C-9), 47.8 (C-5), 34.6 (C-10), 33.7 (C-1), 27.2 (C-6), 18.4 (C-17), 9.2 (C-16).

Prepared using the general method described above, using the aldehyde 160 (24.8 mg, 0.069 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), (aminomethyl)cyclopropane (0.138 mmol) and NaBH(OAc)₃ (43.9 mg, 0.207 mmol). The purified product was obtained as a colourless gum (17.3 mg, 0.042 mmol, 60% yield).

*Rf* = 0.28 in MeOH/CH₂Cl₂ (1:9).

\[ \alpha \] _D_ +276.5 (c 1.15, CHCl₃).

IR ν_max 3347, 2999, 2951, 2359, 2337, 1742, 1619 cm⁻¹.

ESIMS *m/z* 415.1 (100%) [M+H]^+, 416.2 (10%).

HRESIMS *m/z* 415.2252 [M+H]^+, calcd for C₂₃H₃₁N₂O₅ 415.2233.

¹H NMR δ 4.45 (br s, 1H, H-2), 4.12 (s, 3H, O-CH₃), 3.46 (br s, 1H, H-9a), 3.12-3.06 (m, 2H, H-5b, H-10), 3.02-2.97 (m, 1H, H-1′), 2.68 (d, J 6.0 Hz, 1H, H-7), 2.54 (dd, J 12.5 Hz, 6.5 Hz, 1H, H-1″a), 2.44 (dd, J 12.5 Hz, 6.5 Hz, 1H, H-1″b), 2.05 (s, 3H, H-16), 1.99 (br s, 1H, NH), 1.94 (d, J 12.5 Hz, 1H, H-1a), 1.91-1.86 (m, 1H, H-6a), 1.84-1.79 (m, 2H, H-6b, H-9), 1.73 (dt, J 12.5 Hz, 3.5 Hz, 1H, H-1b), 1.36 (d, J 6.5 Hz, 3H, H-17), 0.96-0.89 (m, 1H, H-2″), 0.46-0.45 (m, 2H, H-3″), 0.10-0.08 (m, 2H, H-3″).  

¹³C NMR δ 169.8 (C-15), 163.0 (C-13), 148.5 (C-12), 128.0 (C-11), 112.6 (C-8), 98.7 (C-14), 83.9 (C-3), 79.0 (C-2), 61.3 (C-9a), 59.0 (O-CH₃), 55.5 (C-1″), 49.9 (C-1″), 49.8 (C-7), 48.0 (C-9), 47.8 (C-5), 34.6 (C-10), 33.7 (C-1), 27.2 (C-6), 18.4 (C-17), 11.1 (C-2″), 9.3 (C-16), 3.6 (C-3″), 3.4 (C-3″).


Prepared using the general method described above, using the aldehyde 160 (21.6 mg, 0.060 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), cyclopropylamine (0.120 mmol) and
NaBH(OAc)₃ (38.3 mg, 0.181 mmol). The purified product was obtained as a yellow gum (22.4 mg, 0.056 mmol, 93% yield).

R_f = 0.36 in MeOH/CHCl₃ (1:9).

[α]²⁵ D +281.4 (c 0.25, CHCl₃).

IR νmax 3364, 2961, 2356, 2334, 1742, 1620 cm⁻¹.

ESIMS m/z 400.7 (100%) [M+H]⁺,

HRESIMS m/z 401.2083 [M+H]⁺, calcd for C₂₂H₂₉N₂O₅ 401.2076.

IR νmax 3351, 2951, 2885, 2359, 2337, 1743, 1620 cm⁻¹.

ESIMS m/z 428.9 (100%) [M+H]⁺.

(5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-hexahydro-7b-1-[(N-cyclopentylaminomethyl)-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (204). Prepared using the general method described above, using the aldehyde 160 (22.6 mg, 0.063 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), cyclopentylamine (0.126 mmol) and NaBH(OAc)₃ (40.0 mg, 0.189 mmol). The purified product was obtained as a yellow gum (24.4 mg, 0.057 mmol, 90% yield).

R_f = 0.21 in MeOH/CHCl₃ (1:9).

[α]²⁵ D +206.6 (c 0.35, CHCl₃).

IR νmax 3351, 2951, 2885, 2359, 2337, 1743, 1620 cm⁻¹.

ESIMS m/z 428.9 (100%) [M+H]⁺.
HRESIMS *m/z* 429.2414 [**M+H**]^+^, calcd for C_{24}H_{33}N_{2}O_{5} 429.2389.

$^1$H NMR $\delta$ 4.38 (br s, 1H, H-2), 4.07 (s, 3H, O-CH$_3$), 3.39 (br s, 1H, H-9a), 3.06-3.00 (m, 2H, H-5, H-10), 2.98-2.94 (m, 2H, H-5, H-1'''), 2.67 (s, 2H, H-1'''), 2.61 (d, $J$ 6.0 Hz, 1H, H-7), 2.18 (br s, 1H, NH), 1.99 (s, 3H, H-16), 1.87 (d, $J$ 12.5 Hz, 1H, H-1a), 1.84-1.81 (m, 1H, H-6a), 1.76-1.74 (m, 3H, H-6b, H-2''), 1.65 (d, $J$ 12.0 Hz, 1H, H-1b), 1.60-1.56 (m, 2H, H-3''), 1.48-1.44 (m, 2H, H-3''), 1.30 (d, $J$ 6.5 Hz, 3H, H-17), 1.28-1.21 (m, 2H, H-2 '').

$^{13}$C NMR $\delta$ 169.8 (C-15), 162.9 (C-13), 148.5 (C-12), 127.9 (C-11), 112.5 (C-8), 98.5 (C-14), 82.8 (C-3), 79.0 (C-2), 61.2 (C-9a), 60.2 (C-1''), 58.9 (O-CH$_3$), 49.7 (C-7), 48.7 (C-1''), 47.9 (C-9), 47.7 (C-5), 34.6 (C-10), 33.6 (C-1), 33.1 (C-2''), 33.0 (C-2''), 27.1 (C-6), 24.1 (C-3''), 24.0 (C-3''), 18.4 (C-17), 9.2 (C-16).

$^{(5Z)}$-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-hexahydro-7b-1-((N-cyclohexylaminomethyl)-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (205). Prepared using the general method described above, using the aldehyde 160 (23.2 mg, 0.065 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), cyclohexylamine (0.129 mmol) and NaBH(OAc)$_3$ (41.1 mg, 0.194 mmol). The purified product was obtained as a yellow gum (24.6 mg, 0.056 mmol, 86% yield).

$R_f$ = 0.30 in MeOH/CHCl$_3$ (1:9).

$[\alpha]_{D}^{25}$ +250.9 (c 1.05, CHCl$_3$).

IR $\nu_{\text{max}}$ 3377, 2929, 2856, 2363, 2337, 1743, 1621 cm$^{-1}$.

ESIMS *m/z* 443.0 (100%) [**M+H**]^+^.

HRESIMS *m/z* 443.2555 [**M+H**]^+^, calcd for C$_{25}$H$_{35}$N$_{2}$O$_{5}$ 443.2546.

$^1$H NMR $\delta$ 4.43 (br s, 1H, H-2), 4.12 (s, 3H, O-CH$_3$), 3.45 (br s, 1H, H-9a), 3.11-3.06 (m, 2H, H-5b, H-10), 3.02-2.96 (m, 1H, H-5a), 2.79 (d, $J$ 12.0 Hz, 1H, H-1''), 2.72 (d, $J$ 12.0 Hz, 1H, H-1'''), 2.67 (d, $J$ 6.0 Hz, 1H, H-7), 2.42-2.38 (m, 1H, H-1'''), 2.06 (s, 3H, H-16), 1.93 (d, $J$ 11.5 Hz, 1H, H-1a), 1.90-1.87 (m, 1H, H-6b), 1.84-1.80 (m, 4H, H-6a, H-9, H-2''), 1.73-1.69 (m, 3H, H-1b, H-3''), 1.58 (d, $J$ 12.0 Hz, 1H, H-4'').
1.36 (d, J 6.0 Hz, 3H, H-17), 1.28-1.21 (m, 2H, H-3″), 1.18-1.11 (m, 1H, H-4″), 1.09-1.04 (m, 2H, H-2″).

\[ ^{13}C \text{ NMR} \delta \begin{align*} 
&169.8 \text{ (C-15)}, \\
&163.0 \text{ (C-13)}, \\
&148.5 \text{ (C-12)}, \\
&128.0 \text{ (C-11)}, \\
&112.6 \text{ (C-8)}, \\
&98.7 \text{ (C-14)}, \\
&83.0 \text{ (C-3)}, \\
&79.1 \text{ (C-2)}, \\
&61.3 \text{ (C-9a)}, \\
&59.0 \text{ (O-CH}_3\text{)}, \\
&57.5 \text{ (C-1′)}, \\
&49.8 \text{ (C-7)}, \\
&48.1 \text{ (C-9)}, \\
&47.8 \text{ (C-5)}, \\
&47.3 \text{ (C-1′)}, \\
&34.6 \text{ (C-10)}, \\
&33.6 \text{ (C-1)}, \\
&27.2 \text{ (C-6)}, \\
&26.3 \text{ (C-4″)}, \\
&25.1 \text{ (C-3″)}, \\
&25.0 \text{ (C-3′)}, \\
&18.4 \text{ (C-17)}, \\
&9.3 \text{ (C-16)}. 
\end{align*} \]


Prepared using the general method described above, using the aldehyde 160 (29.7 mg, 0.083 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), \((S)\)-1-aminoethylbenzene (0.165 mmol) and NaBH(OAc)\(_3\) (105.2 mg, 0.496 mmol). The purified product was obtained as a yellow gum (31.1 mg, 0.067 mmol, 81% yield).

\(R_f = 0.38\) in MeOH/CHCl\(_3\) (1:9).

\([\alpha]^{25}_D +166.0\) (c 2.07, CHCl\(_3\)).

IR \(\nu_{max} 3015, 2958, 2359, 2334, 1742, 1621 \text{ cm}^{-1}.\)

ESIMS \(m/z 464.6\) (100%) \([M+H]^+\), 465.2 (25%).

HRESIMS \(m/z 465.2398\) \([M+H]^+\), calcd for C\(_{27}H_{33}N_2O_5\) 465.2389.

\(^1H\) NMR \(\delta \begin{align*} 
&7.34-7.30 \text{ (m, 2H, ArH)}, \\
&7.26 \text{ (s, 2H, ArH)}, \\
&7.25-7.20 \text{ (m, 1H, ArH)}, \\
&4.45 \text{ (br s, 1H, H-2)}, \\
&4.13 \text{ (s, 3H, O-CH}_3\text{)}, \\
&3.71 \text{ (d, J 6.0 Hz, 1H, H-1′′a)}, \\
&3.44 \text{ (br s, 1H, H-9a)}, \\
&3.09 \text{ (br s, 1H, H-10)}, \\
&2.93 \text{ (br s, 2H, H-5)}, \\
&2.64 \text{ (d, J 12.0 Hz, 1H, H-1′)}, \\
&2.62 \text{ (d, J 6.0 Hz, 1H, H-7)}, \\
&2.53 \text{ (d, J 12.0 Hz, 1H, H-1′)}, \\
&2.06 \text{ (s, 3H, H-16)}, \\
&1.98-1.93 \text{ (m, 1H, H-1a)}, \\
&1.80-1.72 \text{ (m, 3H, H-1b, H-6)}, \\
&1.36 \text{ (d, J 6.0 Hz, 3H, H-17)}, \\
&1.33 \text{ (d, J 6.0 Hz, 3H, 1′″-CH}_3\text{)}. 
\end{align*} \)

\(^{13}C\) NMR \(\delta \begin{align*} 
&169.8 \text{ (C-15)}, \\
&162.9 \text{ (C-13)}, \\
&148.5 \text{ (C-12)}, \\
&145.9 \text{ (ArC)}, \\
&128.5 \text{ (ArCH)}, \\
&127.9 \text{ (C-11)}, \\
&126.9 \text{ (ArCH)}, \\
&126.4 \text{ (ArCH)}, \\
&112.5 \text{ (C-8)}, \\
&98.6 \text{ (C-14)}, \\
&82.8 \text{ (C-3)}, \\
&79.1 \text{ (C-2)}, \\
&61.2 \text{ (C-9a)}, \\
&59.0 \text{ (O-CH}_3\text{)}, \\
&58.5 \text{ (C-1′′)}, \\
&49.6 \text{ (C-7)}, \\
&47.8 \text{ (C-5, C-9, C-1′)}, \\
&34.6 \text{ (C-10)}, \\
&33.6 \text{ (C-1)}, \\
&27.1 \text{ (C-6)}, \\
&24.3 \text{ (1′″-CH}_3\text{)}, \\
&18.4 \text{ (C-17)}, \\
&9.2 \text{ (C-16)}. 
\end{align*} \)

Prepared using the general method described above, using the aldehyde 160 (30.9 mg, 0.086 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), (R)-1-aminoethylbenzene (0.172 mmol) and NaBH(OAc)₃ (109.5 mg, 0.517 mmol). The purified product was obtained as a yellow gum (24.7 mg, 0.053 mmol, 62% yield).

Rf = 0.41 in MeOH/CHCl₃ (1:9).

[α]₂⁵ +100.9 (c 1.65, CHCl₃).

IR νmax 3030, 2961, 2936, 2356, 2340, 1742, 1620 cm⁻¹.

ESIMS m/z 464.8 (100%) [M+H]+, 465.9 (25%).

HRESIMS m/z 465.2390 [M+H]+, calcd for C₂₇H₃₃N₂O₅ 465.2389.

¹H NMR δ 7.34-7.28 (m, 2H, H-6′), 7.30 (d, J 2.5 Hz, 2H, ArH), 7.24-7.20 (m, 1H, ArH), 4.38 (br s, 1H, H-2), 4.12 (s, 3H, O-CH₃), 3.74 (q, J 6.5 Hz, 1H, H-1”b), 3.46 (br s, 1H, H-9a), 3.10-3.04 (m, 2H, H-5, H-10), 3.00-2.95 (m, 1H, H-5), 2.62 (d, J 12.0 Hz, 1H, H-1’), 2.55 (d, J 4.5 Hz, 1H, H-7), 2.46 (d, J 12.0 Hz, 1H, H-1’), 2.04 (s, 3H, H-16), 1.95 (d, J 13.0 Hz, 1H, H-1a), 1.79-1.77 (m, 4H, H-1b, H-6, H-9), 1.36 (d, J 6.5 Hz, 3H, H-17), 1.31 (d, J 6.5 Hz, 3H, 1”'-CH₃).

¹³C NMR δ 170.0 (C-15), 163.1 (C-13), 148.7 (C-12), 145.9 (ArC), 128.0 (C-11), 127.2 (ArCH), 126.8 (ArCH), 112.7 (C-8), 98.7 (C-14), 83.1 (C-3), 79.1 (C-2), 61.3 (C-9a), 59.1 (O-CH₃), 59.0 (C-1’”), 49.8 (C-7), 48.1 (C-9, C-1’), 47.9 (C-5), 34.8 (C-10), 33.8 (C-1), 27.2 (C-6), 25.2 (1”’-CH₃), 18.5 (C-17), 9.4 (C-16).
10.5.2 Methylation reaction


Prepared using the general method described above, using formaldehyde (0.245 mmol) as the aldehyde component, dichloroethane (2.0 mL), acetic acid (0.2 mL), the amine 194 (9.8 mg, 0.024 mmol) as the amine component and NaBH(OAc)\(_3\) (31.2 mg, 0.147 mmol). The reaction was left for 16 h. The purified product was obtained as a yellow gum (9.5 mg, 0.023 mmol, 94% yield).

\(R_f = 0.37\) in MeOH/CH\(_2\)Cl\(_2\) (1:9).

\([\alpha]^{25}_D +263.6\) (c 0.63, CHCl\(_3\)).

IR \(\nu_{max}\) 2955, 2920, 2847, 2359, 1743, 1621 cm\(^{-1}\).

ESIMS \(m/z\) 415.2 (100%) [M+H]\(^{+}\), 416.3 (10%).

HRESIMS \(m/z\) 415.2220 [M+H]\(^{+}\), calcd for C\(_{23}\)H\(_{31}\)N\(_2\)O\(_5\) 415.2233.

\(^1\)H NMR (300 MHz) \(\delta\) 5.89-5.75 (m, 1H, H-2\(''\)), 5.15 (d, \(J\) 17.0 Hz, 1H, H-3\(''\)(E)), 5.10 (d, \(J\) 9.0 Hz, 1H, H-3\(''\)(Z)), 4.38 (br s, 1H, H-2), 4.13 (s, 3H, O-CH\(_3\)), 3.44 (br s, 1H, H-9a), 3.23 (dd, \(J\) 13.8 Hz, 6.3 Hz, 1H, H-1\(''\)b), 3.18-3.14 (m, 1H, H-5a), 3.13-3.06 (m, 1H, H-10), 3.03-2.98 (m, 1H, H-5b), 2.92 (dd, \(J\) 13.8 Hz, 6.6 Hz, 1H, H-1\(''\)a), 2.76 (d, \(J\) 5.7 Hz, 1H, H-7), 2.53 (d, \(J\) 13.8 Hz, 1H, H-1'), 2.42 (d, \(J\) 13.8 Hz, 1H, H-1'), 2.30 (s, 3H, N-CH\(_3\)), 2.06 (s, 3H, H-16), 1.94 (d, \(J\) 12.0 Hz, 1H, H-1a), 1.91-1.81 (m, 2H, H-6), 1.79-1.72 (m, 2H, H-1b, H-9), 1.36 (d, \(J\) 6.6 Hz, 3H, H-17).

\(^{13}\)C NMR (75 MHz) \(\delta\) 169.9 (C-15), 163.0 (C-13), 148.6 (C-12), 136.0 (C-2\(''\)), 128.0 (C-11), 117.6 (C-3\(''\)), 112.8 (C-8), 98.7 (C-14), 83.4 (C-3), 79.0 (C-2), 62.4 (C-1\(''\)), 60.8 (C-9a), 59.0 (O-CH\(_3\)), 56.9 (C-1'), 49.7 (C-7), 47.9 (C-5, C-9), 44.2 (N-CH\(_3\)), 34.7 (C-10), 33.3 (C-1), 27.1 (C-6), 18.5 (C-17), 9.3 (C-16).
10.5.3 Carbamylation reaction


To a solution of the amine 203 (11.2 mg, 0.028 mmol) in THF/saturated aqueous NaHCO₃ solution (2:1) (3.0 mL) at 0 °C was added ethyl chloroformate (0.056 mmol) and the reaction was left to stir for 3 h. The mixture was quenched with saturated aqueous NaHCO₃ solution (10 mL) and was directly extracted with CH₂Cl₂ (3 x 20 mL). The combined organic extracts were washed with brine and dried (MgSO₄) before being concentrated in vacuo. The concentrated residue was purified by column chromatography using gradient elution from CH₂Cl₂ to CH₂Cl₂/MeOH (9:1) to give 210 as a colourless gum (10.0 mg, 0.021 mmol, 76% yield).

Rf = 0.45 in MeOH/CHCl₃ (1:9).

[α]₂⁵D +164.0 (c 0.91, CHCl₃).

IR νmax 2961, 2932, 2359, 1744, 1690, 1621 cm⁻¹.

ESIMS m/z 472.6 (100%) [M+H]⁺, 473.7 (10%).

HRESIMS m/z 473.2300 [M+H]⁺, calcld for C₂₅H₃₃N₂O₇ 473.2288.

¹H NMR δ 4.63 (br s, 1H, H-2), 4.16 (q, J 7.5 Hz, 2H, NCO₂CH₂CH₃), 4.13 (s, 3H, O-CH₃), 3.62 (d, J 13.0 Hz, 1H, H-1’), 3.44 (br s, 1H, H-9a), 3.29 (d, J 13.0 Hz, 1H, H-1’), 3.28-3.22 (m, 1H, H-5a), 3.10-2.99 (m, 2H, H-5b, H-10), 2.77 (d, J 6.0 Hz, 1H, H-7), 2.64 (br s, 1H, H-1’’), 2.10-2.02 (m, 1H, H-6a), 2.05 (s, 3H, H-16), 1.94 (d, J 12.5 Hz, 1H, H-1a), 1.84-1.80 (m, 2H, H-6b, H-9), 1.72 (d, J 11.5 Hz, 1H, H-1b), 1.36 (d, J 6.5 Hz, 3H, H-17), 1.26 (t, J 7.5 Hz, 3H, NCO₂CH₂CH₃), 0.88-0.82 (m, 1H, H-2’’), 0.78-0.74 (m, 1H, H-2’’), 0.74-0.67 (m, 1H, H-2’’), 0.65-0.56 (m, 1H, H-2’’).

¹³C NMR δ 169.7 (C-15), 162.9 (C-13), 148.3 (C-12), 128.1 (C-11), 112.5 (C-8), 98.8 (C-14), 83.5 (C-3), 78.2 (C-2), 60.7 (C-9a), 61.7 (NCO₂CH₂CH₃), 59.0 (O-CH₃), 49.1 (C-7), 48.2 (C-5), 47.7 (C-9), 47.0 (C-1’’), 34.6 (C-10), 33.0 (C-1), 29.3
(C-1′′), 27.1 (C-6), 18.4 (C-17), 14.7 (NCO₂CH₂CH₃), 10.0 (C-2′′), 9.3 (C-16), 8.8 (C-2′′).

(5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-hexahydro-7b-1-(N-ethoxycarboxyl-N-cyclopentylaminomethyl)-9-methyl-4H-2,2,6-

Prepared using the general method described above, using the amine 204 (11.2 mg, 0.026 mmol) 2:1 THF/saturated aqueous NaHCO₃ solution (3.0 mL) and ethyl chloroformate (0.052 mmol). The purified product was obtained as a yellow gum (7.3 mg, 0.015 mmol, 56% yield).

$R_f = 0.45$ in MeOH/CHCl₃ (1:9).

$[\alpha]_{D}^{25} +205.6$ (c 0.61, CHCl₃).

IR ν$_{max}$ 2958, 2888, 2362, 2337, 1737, 1675, 1616 cm$^{-1}$.

ESIMS m/z 500.7 (100%) [M+H]$^+$, 501.9 (20%).

HRESIMS m/z 501.2622 [M+H]$^+$, calcd for C$_{27}$H$_{37}$N$_{2}$O$_{7}$ 501.2601.

$^1$H NMR δ 4.50 (br s, 1H, H-2), 4.18-4.10 (m, 2H, NCO₂CH₂CH₃), 4.13 (s, 3H, O-CH₃), 3.70-3.63 (m, 2H, H-1′, H-1′′), 3.46 (br s, 1H, H-9a), 3.24 (d, J 15.5 Hz, 1H, H-1′), 3.22-3.16 (m, 1H, H-5), 3.09-3.00 (m, 2H, H-5, H-10), 2.82 (d, J 5.5 Hz, H-7), 2.10-2.02 (m, 1H, H-6a), 2.06 (s, 3H, H-16), 1.93 (d, J 11.5 Hz, 1H, H-1a), 1.84-1.72 (m, 7H, H-1b, H-6b, H-9, H-2′′), 1.48 (br s, 4H, H-3′′), 1.36 (d, J 6.0 Hz, 3H, H-16), 1.26 (t, J 6.0 Hz, 3H, NCO₂CH₂CH₃).

$^{13}$C NMR δ 169.8 (C-15), 162.9 (C-13), 148.4 (C-12), 128.0 (C-11), 112.6 (C-8), 98.8 (C-14), 83.7 (C-3), 77.6 (C-2), 62.6 (C-1′′), 61.4 (NCO₂CH₂CH₃), 60.9 (C-9a), 59.0 (O-CH₃), 49.4 (C-7, C-1′), 48.2 (C-5), 47.8 (C-9), 34.7 (C-10), 33.0 (C-1), 27.1 (C-6), 24.9 (C-2′′), 24.8 (C-3′′), 18.4 (C-17), 14.8 (NCO₂CH₂CH₃), 9.3 (C-16).

(5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-hexahydro-7b-1-(N-ethoxycarboxyl-N-cyclohexylaminomethyl)-9-methyl-4H-2,2,6-
Prepared using the general method described above, using the amine 205 (11.1 mg, 0.025 mmol), 2:1 THF/saturated aqueous NaHCO₃ solution (3.0 mL) and ethyl chloroformate (0.050 mmol). The purified product was obtained as a yellow gum (10.2 mg, 0.020 mmol, 80% yield).

$R_f = 0.45$ in MeOH/CHCl₃ (1:9).

$[\alpha]_{D}^{20} +192.6$ (c 0.78, CHCl₃).

IR $\nu_{max}$ 2929, 2366, 1744, 1684, 1621 cm⁻¹.

ESIMS $m/z$ 514.5 (100%) [M+H]⁺, 515.3 (40%).

HRESIMS $m/z$ 515.2726 [M+H]⁺, cale for C₂₈H₃₉N₂O₇ 515.2757.

$^1$H NMR $\delta$ 4.50 (br s, 1H, H-2), 4.18-4.10 (m, 2H, NCO₂CH₂CH₃), 4.13 (s, 3H, O-CH₃), 3.64 (br s, 1H, H-1’), 3.46 (br s, 1H, H-9a), 3.41 (s, 1H, H-1’’), 3.20 (d, J 15.0 Hz, 1H, H-5), 3.12-3.01 (m, 2H, H-5, H-10), 2.82 (d, J 6.0 Hz, 1H, H-7), 2.07 (s, 3H, H-16), 1.94 (d, J 12.5 Hz, 1H, H-1a), 1.87-1.72 (m, 11H, H-1b, H-6, H-9, H-3’’, H-4’’), 1.61 (apparent d, J 9.0 Hz, 2H, H-2’’), 1.37 (d, J 6.5 Hz, 3H, H-17), 1.27 (t, J 7.0 Hz, 3H, NCO₂CH₂CH₃), 1.22 (apparent d, J 13.0 Hz, 2H, H-3’’), 1.14-1.08 (m, 2H, H-2’’).

$^{13}$C NMR $\delta$ 169.8 (C-15), 162.9 (C-13), 148.4 (C-12), 128.0 (C-11), 112.7 (C-8), 98.8 (C-14), 83.7 (C-3), 77.6 (C-2), 70.8 (C-1’’), 61.4 (C-9a), 61.2 (NCO₂CH₂CH₃), 59.0 (O-CH₃), 48.5 (C-1’), 48.2 (C-5, C-7), 47.8 (C-9), 34.7 (C-10), 33.0 (C-1), 27.2 (C-4’’), 26.6 (C-6, C-3’’), 25.6 (C-2’’), 18.4 (C-17), 14.8 (NCO₂CH₂CH₃), 9.3 (C-16).
10.5.4 Synthesis of a guanidine derivative 214


To a solution of the amine 191 (8.8 mg, 0.024 mmol) in dry CH\(_2\)Cl\(_2\) (2.0 mL) was added 1,3-di-Boc-2-(trifluoromethylsulfonyl)-guanidine (9.2 mg, 0.024 mmol) at rt and the reaction mixture was left to stir for 24 h. The mixture was quenched with saturated aqueous NaHCO\(_3\) solution (10 mL) and then extracted with CH\(_2\)Cl\(_2\) (3 x 20 mL). The combined organic extracts were washed with brine and dried (MgSO\(_4\)) before being concentrated \textit{in vacuo}. The concentrated residue was purified by column chromatography using gradient elution from CH\(_2\)Cl\(_2\) to CH\(_2\)Cl\(_2\)/MeOH (9:1) to give a white gum (7.9 mg, 0.013 mmol, 54% yield).

\(R_f = 0.45\) in MeOH/CH\(_2\)Cl\(_2\) (1:9).

ESIMS \(m/z\) 616.6 (100%) [M+H]+, 617.4 (80%).

HRESIMS \(m/z\) 617.3188 [M+H]+, calcd for C\(_{31}\)H\(_{45}\)N\(_4\)O\(_9\) 617.3187.

\(^1\)H NMR \(\delta\) 4.44 (br s, 1H, H-2), 4.15 (s, 3H, O-CH\(_3\)), 3.73 (d, \(J\ 13.5\ Hz\), 2H, H-1'), 3.54 (br s, 1H, H-9a), 3.28-3.22 (m, 1H, H-5a), 3.12-3.04 (m, 2H, H-5b, H-10), 3.08 (s, 3H, N-CH\(_3\)), 2.74 (d, \(J\ 5.5\ Hz\), 1H, H-7), 2.08 (s, 3H, H-16), 2.01 (d, \(J\ 12.5\ Hz\), 1H, H-1a), 1.99-1.89 (m, 2H, H-6), 1.83 (dd, \(J\ 10.0\ Hz\), 3.5 Hz, 1H, H-9), 1.75 (d, \(J\ 12.0\ Hz\), 1H, H-1b), 1.67 (br s, 1H, NH), 1.47 (s, 18H, CO\(_2\)C(CH\(_3\))\(_3\)), 1.39 (d, \(J\ 6.5\ Hz\), 3H, H-17).

\(^{13}\)C NMR (The signals for C-1’’ and the Boc carbonyls were not observed.) \(\delta\) 169.6 (C-15), 162.6 (C-13), 147.6 (C-12), 128.0 (C-11), 112.0 (C-8), 98.7 (C-14), 83.8 (C-3), 76.5 (C-2), 60.2 (C-9a), 58.9 (O-CH\(_3\)), 52.4 (C-1’), 50.4 (C-7), 47.8 (C-5), 47.5 (C-9), 40.0 (N-CH\(_3\)), 34.2 (C-10), 33.4 (C-1), 28.2 (CO\(_2\)C(CH\(_3\))\(_3\)), 26.4 (C-6), 18.2 (C-17), 9.1 (C-16).
To a solution of 213 (7.9 mg, 0.013 mmol) in dry CH₂Cl₂ (1.0 mL) was added TFA (1.0 mL) at rt and the reaction mixture was left to stir for 3 h. The solvent was removed under vacuum. To the residue was added hydrogen chloride in ether (2.0 mL, 1 M) and concentrated under vacuum. The desired product was isolated as its hydrochloride salt by dissolution in MeOH and precipitation by the addition of diethyl ether as a white solid salt (4.3 mg, 0.010 mmol, 52% yield).

\[ \alpha \]_D \text{ +191.2 (c 0.23, CH}_3\text{OH).} \]

ESIMS m/z 416.7 (100%) [M+H]^+. 
HRESIMS m/z 417.2155 [M+H]^+, calcd for C_{21}H_{29}N_{4}O_{5} 417.2138.

\text{^1H NMR (CD}_3\text{OD, a very broad spectrum was observed, therefore only the methyl signals are reported)} \text{ δ 4.23 (s, 3H, O-CH}_3\text{), 3.25 (s, 3H, N-CH}_3\text{), 2.07 (s, 3H, H-16), 1.47 (d, J 6.0 Hz, 3H, H-17).} 

\text{\textsuperscript{13}C NMR (CD}_3\text{OD) The signals for carbons at C-3, C-5, C-9 and C-9a were not observed. δ 172.0 (C-15), 164.8 (C-13), 160.0 (C-1”\text{), 149.7 (C-12), 129.4 (C-11), 112.5 (C-8), 99.6 (C-14), 76.5 (C-2), 58.9 (O-CH}_3\text{), 51.0 (C-7), 47.3 (C-1”\text{), 38.8 (C-10), 34.3 (N-C}_3\text{), 32.2 (C-1), 24.4 (C-6), 16.8 (C-17), 7.9 (C-16).} \]
10.6 Experimental for Chapter 6

10.6.1 Synthesis of the Bestmann-Ohira reagent 217

Tosyl azide.

To a solution of sodium azide (482.4 mg, 7.422 mmol) in acetone/H$_2$O (1:1, 50.0 mL) at 0 °C was added tosyl chloride (1.4 g, 7.422 mmol) and the reaction mixture was left to stir at 0 °C for 2 h. The mixture was quenched with H$_2$O (20.0 mL) and was extracted with EtOAc (3 x 20 mL). The combined organic extracts were washed with brine and dried (Na$_2$SO$_4$). The azide was obtained as a colourless oil (1.4 g, 7.12 mmol, 96% yield) after removal of the solvent under reduced pressure.

EIMS $m/z$ 197 (13%) [M]$^+$, 155 (100%) [M-N$_3$]$^+$.  
$^1$H NMR $\delta$ 7.84 (d, $J$ 8.0 Hz, 2H, ArH), 7.41 (d, $J$ 8.5 Hz, 2H, ArH), 2.48 (s, 3H, CH$_3$).

Dimethyl-1-diazo-2-oxopropylphosphonate (217).

To a solution of dimethyl-2-oxopropylphosphonate (735.7 mg, 4.432 mmol) in THF (15.0 mL) at 0 °C was added 60% NaH in mineral oil (186.1 mg, 4.654 mmol) and the reaction mixture was left to stir at 0 °C for 1 h. Tosyl azide (917.8 mg, 4.654 mmol) was added and the reaction was left to stir at rt for a further 2 h. The reaction mixture was filtered through a thin pad of Celite. The filtrate was evaporated to give a yellow oil. The oil was purified by column chromatography using gradient elution from EtOAc/petrol (1:1) to EtOAc/petrol (7:3) to give a yellow oil (686.3 mg, 3.574 mmol, 81% yield).

$R_f$ = 0.12 in EtOAc/petrol (1:1).

ESIMS $m/z$ 193 (100%) [M+H]$^+$.  
$^1$H NMR $\delta$ 3.84 (s, 3H, O-CH$_3$), 3.82 (s, 3H, O-CH$_3$), 2.26 (s, 3H, CH$_3$).

$^{13}$C NMR $\delta$ 189.8 (C=N$_2$), 189.7 (C=O), 53.5 (O-CH$_3$), 27.0 (CH$_3$).  
NMR data of 217 agreed with that reported.$^{57}$
10.6.2 Alkynylation reaction of the aldehyde 160

(5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-hexahydro-7b-1-ethynyl-9-methyl-4H-2,2,6-
(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-
methyl-2(5H)-furanone (215).

To a mixture of the aldehyde 160 (150.8 mg, 0.420 mmol) and K$_2$CO$_3$ (69.7 mg, 0.504 mmol) in MeOH:MeCN (1:3) (8.0 mL) at rt was added the Bestmann-Ohira reagent 217 (0.504 mmol) and the reaction mixture was left to stir for 24 h. The mixture was quenched with saturated aqueous NaHCO$_3$ solution (10 mL) and was extracted with diethyl ether (3 x 20 mL). The combined organic extracts were washed with brine and dried over MgSO$_4$ before being concentrated in vacuo. The concentrated residue was purified by column chromatography using gradient elution from CH$_2$Cl$_2$ to CH$_2$Cl$_2$/MeOH (98:2) to give 215 as a white gum (113.2 mg, 0.319 mmol, 76% yield). A small sample was crystallized from CH$_2$Cl$_2$. Mp 174-176 ºC (decomposed).

$R_f$ = 0.30 in MeOH/EtOAc (1:9).

$[\alpha]^{25}_D$ +292.8 (c 0.66, CHCl$_3$).

ESIMS $m/z$ 356.1 (100%) [M+H]$^+$. 

HRESIMS $m/z$ 356.1459 [M+H]$^+$, calculated for C$_{20}$H$_{22}$NO$_5$ 356.1498.

IR $\nu_{\text{max}}$ 3249, 2958, 2917, 2366, 1744, 1627 cm$^{-1}$.

$^1$H NMR (CDCl$_3$) $\delta$ 4.57 (br s, 1H, H-2), 4.13 (s, 3H, O-CH$_3$), 3.51 (br s, 1H, H-9a), 3.40-3.34 (m, 1H, H-5a), 3.11-3.05 (m, 2H, H-5b, H-10), 3.04 (d, J 6.0 Hz, 1H, H-7), 2.54 (s, 1H, H-2'), 2.17-2.10 (m, 1H, H-6a), 2.06 (s, 3H, H-16), 2.00 (d, J 12.5 Hz, 1H, H-1la), 1.91 (dt, J 12.5 Hz, 3.0 Hz, 1H, H-1b), 1.89-1.84 (m, 1H, H-6b), 1.77 (dd, J 10.0 Hz, 3.0 Hz, 1H, H-9), 1.36 (d, J 6.5 Hz, 3H, H-17).

$^{13}$C NMR (CDCl$_3$) $\delta$ 169.7 (C-15), 162.8 (C-13), 147.8 (C-12), 128.2 (C-11), 112.0 (C-8), 98.9 (C-14), 81.2 (C-3), 80.9 (C-2), 75.2 (C-1'), 74.6 (C-2'), 60.7 (C-9a), 59.0 (O-CH$_3$), 54.4 (C-7), 48.6 (C-5), 47.3 (C-9), 34.6 (C-10), 33.5 (C-1), 27.0 (C-6), 18.3 (C-17), 9.3 (C-16).
10.6.3 Preparation of azides and chlorooximes for Click reactions

**Phenyl azide.**

A mixture of phenyl boronic acid (537.7 mg, 4.411 mmol), Cu(OAc)$_2$ (80.1 mg, 0.441 mmol, 10 mol%) and NaN$_3$ (286.7 mg, 4.411 mmol) in MeOH (10.0 mL) was stirred at rt for 16 h. The reaction mixture was diluted with H$_2$O and extracted with CH$_2$Cl$_2$ (3 x 20 mL). The combined organic extracts were washed with brine and dried (MgSO$_4$). The solvent was partially removed under reduced pressure and made to a volume of 50.0 mL. On the basis of 100% conversion, it gave 0.088 M of phenyl azide in MeOH. $R_f = 0.67$ in EtOAc/petrol (2:3).

**N-(3-Azidopropyl)phthalimide.**

To a solution of $N$-(3-bromopropyl)phthalimide (500 mg, 1.865 mmol) in DMF (5.0 mL) was added NaN$_3$ (157.6 mg, 2.424 mmol) at rt. After stirring for 24 h, the reaction mixture was quenched with H$_2$O (2 mL) and extracted with diethyl ether (3 x 20 mL). The combined organic extracts were washed with brine and dried (MgSO$_4$). The solvent was removed under reduced pressure to give a colourless oil (405.5 mg, 1.763 mmol, 94% yield) $R_f = 0.18$ in EtOAc/petrol (1:9).

$^1$H NMR $\delta$ 7.86-7.85 (m, 2H, ArH), 7.74-7.72 (m, 2H, ArH), 3.79 (t, $J$ 7.0 Hz, 2H, H-1), 3.39 (t, $J$ 7.0 Hz, 2H, H-3), 1.96 (quintet, $J$ 6.5 Hz, 2H, H-2).

$^{13}$C NMR $\delta$ 168.3 (C=O), 134.2 (ArC), 134.1 (ArC), 123.4 (ArC), 49.1 (C-3), 35.4 (C-1), 28.1 (C-2).
Scheme 10.1 Synthesis of N-hydroxybenzenecarboximidoyl chloride.

**Benzaldehyde oxime.**

To a solution of hydroxylamine-hydrochloride (360.6 mg, 5.189 mmol) in EtOH/H₂O (1:1, 10.0 mL) at rt was added benzaldehyde (500 µL, 4.717 mmol) and then NaOH (471.7 mg, 11.793 mmol). The reaction mixture was stirred at rt for 1 h and then extracted with diethyl ether (3 x 10 mL). The combined organic extracts were acidified with 10% HCl solution to pH >7 and again extracted with diethyl ether (3 x 10 mL) and dried (Na₂SO₄). The solvent was evaporated *in vacuo* to give a yellow oil (2.24 g). The crude product was of >90% purity from ¹H NMR analysis and was used in the next step without purification.

*Rf* = 0.32 in EtOAc/petrol (1:4).

¹H NMR δ 8.18 (s, 1H, CH), 7.58-7.57 (m, 2H, ArH), 7.39-7.38 (m, 2H, ArH).

**N-Hydroxybenzenecarboximidoyl chloride.**

Benzaldehyde oxime (467.2 mg, 3.861 mmol) was dissolved in DMF (10.0 mL) at rt in the two-neck round bottom flask attached with a thermometer. A portion of *N*-chlorosuccinimide (NCS) about one-tenth of the total of 515.6 mg (3.861 mmol) was slowly added under the temperature control of 30-35 °C. After 10 min, HCl gas (~ 20 mL) was bubbled into the reaction mixture. The remaining of NCS was added and left to stir for a further 1 h at 30 °C. The reaction mixture was quenched with ice/H₂O and extracted with diethyl ether (3 x 20 mL). The combined organic extracts were washed with H₂O and dried (Na₂SO₄). The extracts were concentrated under reduced pressure to give a yellow oil (404.7 mg). The crude product was of >90% purity from ¹H NMR analysis and was used in the next step without purification.

*Rf* = 0.35 in EtOAc/petrol (1:9).

EIMS *m/z* 155 (100%) [M]+.

¹H NMR δ 7.84 (d, *J* 8.0 Hz, 2H, ArH), 7.45-7.40 (m, 3H, ArH).

¹H NMR data agreed with that reported.⁹⁶
**Scheme 10.2** Synthesis of \(N\)-hydroxy-4-fluorobenzenecarboximidoyl chloride.

**4-Fluorobenzaldehyde oxime.**

To a solution of hydroxylamine-hydrochloride (201.6 mg, 2.901 mmol) in EtOH (3.0 mL) at rt was added 4-fluorobenzaldehyde (255 \(\mu\)L, 2.417 mmol) and NaOAc (238.7 mg, 2.901 mmol) and the reaction mixture was stirred at 70 °C for 1 h. The reaction mixture was extracted with EtOAc (3 x 10 mL) and the combined organic extracts were dried (\(\text{Na}_2\text{SO}_4\)). The solvent was evaporated *in vacuo* to give product as a white precipitate (321.4 mg). The crude product was of >90% purity from \(^1\text{H}\) NMR analysis and was used in the next step without purification.

\[ R_f = 0.48 \text{ MeOH/CH}_2\text{Cl}_2 (1:9). \]

\(^1\text{H}\) NMR \(\delta 8.12 \text{ (s, 1H, CH)}, 7.58-7.55 \text{ (m, 2H, ArH), 7.10-7.06 \text{ (m, 2H, ArH).}} \]

\(^1\text{H}\) NMR data agreed with that reported.\(^{97}\)

**\(N\)-hydroxy-4-fluorobenzenecarboximidoyl chloride.**

The compound was prepared using a similar method to that described above for the synthesis of \(N\)-hydroxybenzenecarboximidoyl chloride, using 4-fluorobenzaldehyde oxime (99.0 mg, 0.712 mmol) and NCS (95.1 mg, 0.712 mmol) in DMF (3.0 mL) except the reaction temperature was 70 °C. The crude product (113.6 mg) was of >90% purity from \(^1\text{H}\) NMR analysis and was used in the next step without purification.

\[ R_f = 0.82 \text{ MeOH/CH}_2\text{Cl}_2 (1:9). \]

EIMS \(m/z 173 (100\%) [M]^+\).

\(^1\text{H}\) NMR \(\delta 7.86-7.82 \text{ (m, 2H, ArH), 7.12-7.06 \text{ (m, 2H, ArH).}} \]

\(^1\text{H}\) NMR data agreed with that reported.\(^{97}\)
10.6.4 Click reactions of the alkyne 215


A mixture of the alkyne 215 (12.7 mg, 0.034 mmol), benzylbromide (0.040 mmol), sodium azide (0.040 mmol) and copper powder (2.0 mg) in tBuOH:H2O (1:1) (1.0 mL) was treated with 1M CuSO4 (0.1 mL). The mixture was stirred and heated in microwave reactor at 125 °C for 10 min. The mixture was quenched with saturated aqueous NaHCO3 solution (10 mL) and was directly extracted with diethyl ether (3 x 20 mL). The combined organic extracts were washed with brine and dried (MgSO4) before being concentrated in vacuo. The concentrated residue was purified by column chromatography using gradient elution from CH2Cl2 to CH2Cl2/MeOH (9:1) to give a yellow gum (3.8 mg, 0.008 mmol, 23% yield).

Rf = 0.11 in MeOH/EtOAc (5:95).

[α]25D +207.6 (c 0.25, CHCl3).

ESIMS m/z 489.1 (100%) [M+H]+.

HRESIMS m/z 489.2121 [M+H]+, calcd for C27H29N4O5 489.2138.

1H NMR δ 7.34 (s, 1H, C-5′), 7.32-7.28 (m, 3H, ArH), 7.22-7.21 (m, 2H, ArH), 5.44 (d, J 2.5 Hz, 2H, C-1′′), 4.41 (br s, 1H, H-2), 4.07 (s, 3H, O-CH3), 3.51 (br s, 1H, H-9a), 3.30 (d, J 5.5 Hz, 1H, H-7), 3.09-3.04 (m, 1H, H-10), 3.03-2.99 (m, 2H, H-5), 2.00 (s, 3H, H-16), 1.96 (d, J 12.0 Hz, 1H, H-1a), 1.92-1.86 (m, 3H, H-1b, H-6a), 1.84-1.81 (m, 2H, H-6b), 1.33 (d, J 6.5 Hz, 3H, H-17).

13C NMR δ 169.8 (C-15), 162.9 (C-13), 148.3 (C-12), 147.8 (ArC), 134.5 (C-4′), 129.2 (ArCH), 128.9 (ArCH), 128.3 (ArCH), 128.1 (C-11), 120.9 (C-5′), 112.7 (C-8), 98.7 (C-14), 81.0 (C-2), 79.9 (C-3), 61.1 (C-9a), 59.0 (O-CH3), 54.4 (C-1′′), 52.1 (C-7), 48.6 (C-5), 47.7 (C-9), 34.6 (C-10), 33.3 (C-1), 27.2 (C-6), 18.4 (C-17), 9.2 (C-16),

To a solution of the alkyne 215 (16.2 mg, 0.046 mmol), Cu(OAc)$_3$ (0.005 mmol) and sodium ascorbate (0.068 mmol) in MeOH:H$_2$O (1:1) (1.0 mL) at rt was added 0.09 M phenylazide in MeOH (1.0 mL) and the reaction mixture was left to stir for 3 h. The mixture was quenched with saturated aqueous NaHCO$_3$ solution (10 mL) and was directly extracted with CH$_2$Cl$_2$ (3 x 20 mL). The combined organic extracts were washed with brine and dried (MgSO$_4$) before being concentrated in vacuo. The concentrated residue was purified by column chromatography using gradient elution from CH$_2$Cl$_2$ to CH$_2$Cl$_2$/MeOH (9:1) to give a yellow gum (7.2 mg, 0.015 mmol, 33% yield).

$R_f$ = 0.37 in MeOH/CH$_2$Cl$_2$ (1:9).

$[\alpha]_{D}^{25}$ +229.9 (c 0.48, CHCl$_3$).

ESIMS m/z 475.1 (100%) [M+H]$^+$.  
HRESIMS m/z 475.1972 [M+H]$^+$, calcd for C$_{26}$H$_{27}$N$_4$O$_5$ 475.1981.

$^1$H NMR $\delta$ 7.95 (s, 1H, H-5'), 7.74 (d, $J$ 8.0 Hz, 2H, ArH), 7.54-7.51 (m, 2H, ArH), 7.44 (t, $J$ 7.5 Hz, 1H, ArH), 4.58 (s, 1H, H-2), 4.16 (s, 3H, O-CH$_3$), 3.64 (br s, 1H, H-9a), 3.50 (d, $J$ 5.5 Hz, 1H, H-7), 3.24-3.12 (m, 3H, H-5, H-10), 2.12-2.07 (m, 1H, H-1a), 2.09 (s, 3H, H-16), 2.05-2.02 (m, 2H, H-1b, H-6a), 2.00-1.98 (m, 1H, H-6b), 1.97-1.93 (m, 1H, H-9), 1.43 (d, $J$ 7.0 Hz, 3H, H-17).

$^{13}$C NMR $\delta$ 169.8 (C-15), 163.0 (C-13), 148.3 (C-12), 137.1 (ArC), 129.9 (ArCH), 128.9 (C-11), 128.2 (C-4'), 120.6 (ArCH), 119.4 (C-5’), 112.7 (C-8), 98.9 (C-14), 81.2 (C-2), 80.0 (C-3), 61.3 (C-9a), 59.0 (O-CH$_3$), 52.3 (C-7), 48.7 (C-5), 47.9 (C-9), 34.7 (C-10), 33.4 (C-1), 27.3 (C-6), 18.5 (C-17), 9.3 (C-16).

To a solution of the alkyne 215 (15.1 mg, 0.042 mmol), Cu(OAc)₃ (0.017 mmol) and sodium ascorbate (0.064 mmol) in MeOH:H₂O (1:1) (1.0 mL) at rt was added N-hydroxybenzene-carboximidoyl chloride (0.064 mmol) and sodium hydroxide (0.064 mmol). The reaction was left to stir for 3 h. The mixture was quenched with saturated aqueous NaHCO₃ solution (10 mL) and was directly extracted with CH₂Cl₂ (3 x 20 mL). The combined organic extracts were washed with brine and dried (MgSO₄) before being concentrated in vacuo. The concentrated residue was purified by column chromatography using gradient elution from CH₂Cl₂ to CH₂Cl₂/MeOH (9:1) to give a white gum (11.9 mg, 0.025 mmol, 59% yield).

\[ R_f = 0.37 \text{ in MeOH/}EtOAc (1:9). \]

\[ [\alpha]_{D}^{25} +203.4 \ (c 0.79, \text{CHCl}_3). \]

ESIMS \( m/z \) 475.1 (100%) [M+H]+.

HRESIMS \( m/z \) 475.1871 [M+H]+, calcd for C₂₇H₂₇N₂O₆ 475.1869.

\(^1\)H NMR \( \delta \) 7.82-7.79 (m, 2H, ArH), 7.45 (br s, 3H, ArH), 6.56 (s, 1H, H-4'), 4.62 (s, 1H, H-2), 4.16 (s, 3H, O-CH₃), 3.65 (br s, 1H, H-9a), 3.43 (d, \( J \) 5.5 Hz, 1H, H-7), 3.29-3.24 (m, 1H, H-5b), 3.19-3.14 (m, 2H, H-5a, H-10), 2.11-2.08 (m, 1H, H-1a), 2.09 (s, 3H, H-16), 2.03-1.96 (m, 2H, H-6), 1.93 (dd, \( J \) 9.5 Hz, 2.5 Hz, 1H, H-9), 1.43 (d, \( J \) 6.0 Hz, 3H, H-17).

\(^{13}\)C NMR \( \delta \) 171.2 (C-5'), 169.7 (C-15), 162.8 (C-13), 162.6 (C-3'), 147.8 (C-12), 130.3 (ArC), 128.9 (ArC), 128.8 (ArC), 128.3 (C-11), 127.5 (ArC), 112.5 (C-8), 100.2 (C-4'), 99.0 (C-14), 80.6 (C-3), 80.1 (C-2), 61.3 (C-9a), 59.0 (O-CH₃), 52.3 (C-7), 49.0 (C-5), 47.7 (C-9), 34.6 (C-10), 33.3 (C-1), 27.2 (C-6), 18.4 (C-17), 9.3 (C-16).

Prepared using method A, from the alkyne 215 (15.0 mg, 0.042 mmol), bromopropylbenzene (0.060 mmol), sodium azide (0.060 mmol), copper powder (2.5 mg), BuOH: H2O (1:1) (1.0 mL) and 1M CuSO4 (0.1 mL). The mixture was heated in microwave reactor at 125 °C for 10 min. The product was obtained as a white gum (4.0 mg, 0.008 mmol, 18% yield).

Rf = 0.22 in MeOH/EtOAc (2:8)

[α]D25 +178.4 (c 0.27, CHCl3).

ESIMS m/z 517.4 (100%) [M+H]+.

HRESIMS m/z 517.2439 [M+H]+, calc'd for C29H33N4O5 517.2451.

1H NMR δ 7.40 (s, 1H, H-5), 7.21 (d, J 8.0 Hz, 2H, ArH), 7.13 (t, J 7.5 Hz, 2H, ArH), 7.09 (d, J 7.5 Hz, 1H, ArH), 4.42 (br s, 1H, H-2), 4.27 (t, J 7.0 Hz, 2H, H-1”), 4.08 (s, 3H, O-CH3), 3.53 (br s, 1H, H-9a), 3.30 (d, J 6.0 Hz, 1H, H-7), 3.10-3.06 (m, 1H, H-10), 3.06-3.02 (m, 2H, H-5), 2.60 (t, J 7.5 Hz, 2H, H-3”), 2.18 (quint, J 7.5 Hz, 2H, H-2”), 2.00 (s, 3H, H-16), 1.99 (d, J 14.0 Hz, 1H, H-1a), 1.90-1.87 (m, 2H, H-1b, H-6a), 1.86-1.82 (m, 2H, H-6b, H-9), 1.34 (d, J 6.5 Hz, 3H, H-17).

13C NMR δ 169.8 (C-15), 162.9 (C-13), 148.3 (C-12), 147.3 (C-4’), 140.2 (ArC), 128.6 (ArCH), 128.5 (ArCH), 128.0 (C-11), 126.4 (ArCH), 121.0 (C-5”), 112.6 (C-8), 98.6 (C-14), 81.0 (C-2), 79.8 (C-3), 61.1 (C-9a), 59.0 (O-CH3), 52.1 (C-7), 49.7 (C-1”), 47.7 (C-9), 47.5 (C-5), 34.6 (C-10), 33.3 (C-1), 32.6 (C-3”), 31.6 (C-2”), 27.2 (C-6), 18.3 (C-17), 9.2 (C-16).

Prepared using method B, from the alkyne 215 (12.1 mg, 0.034 mmol), N-propylphthalimide azide (0.068 mmol), sodium ascorbate (0.051 mmol) and Cu(OAc)$_2$ (0.003 mmol). The product was obtained as a colourless gum (9.1 mg, 0.016 mmol, 46% yield).

$R_f = 0.15$ in MeOH/EtOAc (1:9).

$[\alpha]_D^{25} +131.4$ (c 0.61, CHCl$_3$).

ESIMS $m/z$ 586.4 (100%) [M+H]$^+$.  
HRESIMS $m/z$ 586.2318 [M+H]$^+$, calcd for C$_{31}$H$_{32}$N$_5$O$_7$ 586.2302.

$^1$H NMR $\delta$ 7.76-7.74 (m, 2H, ArH), 7.67-7.66 (m, 2H, ArH), 7.65 (s, 1H, H-5'), 4.41 (br s, 1H, H-2), 4.34 (t, $J$ 7.0 Hz, 2H, H-1''), 4.08 (s, 3H, O-CH$_3$), 3.66 (t, $J$ 6.5 Hz, 2H, H-3''), 3.53 (br s, 1H, H-9a), 3.26 (d, $J$ 6.0 Hz, 1H, H-7), 3.10-3.00 (m, 3H, H-5, H-10), 2.27 (quint, $J$ 6.5 Hz, 2H, H-2''), 1.98 (s, 3H, H-16), 1.93-1.89 (m, 1H, H-1b), 1.85-1.80 (m, 3H, H-6, H-9), 1.33 (d, $J$ 6.5 Hz, 3H, H-17).

$^{13}$C NMR $\delta$ 169.5 (C-15), 168.0 (C-1''), 162.7 (C-3''), 162.7 (C-13), 148.1 (C-12), 147.0 (C-4'), 134.0 (ArCH), 131.6 (ArC), 127.6 (C-11), 123.1 (ArCH), 121.6 (C-5'), 112.4 (C-8), 98.2 (C-14), 80.7 (C-2), 79.5 (C-3), 60.8 (C-9a), 58.7 (O-CH$_3$), 51.8 (C-7), 48.2 (C-5), 47.6 (C-1''), 47.3 (C-9), 34.8 (C-3''), 34.4 (C-10), 33.0 (C-1), 29.0 (C-2''), 26.9 (C-6), 18.0 (C-17), 8.9 (C-16).

Prepared using method C, from the alkyne 215 (10.6 mg, 0.030 mmol), N-hydroxy-4-fluorobenzencarboximidoyl chloride (0.045 mmol), sodium ascorbate (0.045 mmol), sodium hydroxide (0.045 mmol) and Cu(OAc)$_2$ (0.012 mmol). The product was obtained as a yellow gum (6.3 mg, 0.013 mmol, 43% yield).

$R_f = 0.62$ in MeOH/EtOAc (2:8).

$[\alpha]_{D}^{25} = +236.1$ (c 0.36, CHCl$_3$).

ESIMS $m/z$ 492.9 (100%) [M+H]$^+$.  
HRESIMS $m/z$ 493.1789 [M+H]$^+$, calcld for C$_{27}$H$_{26}$N$_2$O$_6$F 493.1775.

$^1$H NMR $\delta$ 7.80-7.77 (m, 2H, ArH), 7.15 (t, $J$ 8.5 Hz, 2H, ArH), 6.52 (s, 1H, H-4′), 4.61 (br s, 1H, H-2), 4.16 (s, 3H, O-$\text{CH}_3$), 3.65 (br s, 1H, H-9a), 3.42 (d, $J$ 5.5 Hz, 1H, H-7), 3.29-3.23 (m, 1H, H-5), 3.19-3.12 (m, 2H, H-5, H-10), 2.11 (s, 1H, H-1a), 2.09 (s, 3H, H-16), 2.04-1.96 (m, 3H, H1b, H-6), 1.93 (dd, $J$ 10.0 Hz, 3.0 Hz, 1H, H-9), 1.42 (d, $J$ 6.5 Hz, 3H, H-17).

$^{13}$C NMR $\delta$ 171.5 (C-5′), 169.7 (C-15), 165.0 (ArC-F), 163.0 (ArC-F), 162.8 (C-13), 161.7 (C-3′), 147.8 (C-12), 128.9 (ArCH), 128.8 (ArCH), 128.3 (C-11), 125.1 (ArC), 116.3 (ArCH), 116.1 (ArCH), 112.4 (C-8), 100.1 (C-4′), 99.0 (C-14), 80.6 (C-3), 80.1 (C-2), 61.3 (C-9a), 59.0 (O-$\text{CH}_3$), 52.3 (C-7), 49.0 (C-5), 47.7 (C-9), 34.6 (C-10), 33.3 (C-1), 27.2 (C-6), 18.4 (C-17), 9.3 (C-16).

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10.6.5 Sonogashira coupling of the alkyne 215

\((5Z)-5\{2,5\text{a}R,6\text{S},7\text{a}S,7\text{b}R,8\text{R},9\text{S}\}\text{-hexahydro-7b-1-(2-phenylethyn-1-yl)-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)Furanone (216).}

A solution mixture of the alkyne 215 (13.4 mg, 0.038 mmol), iodobenzene (0.038 mmol), \(\text{PdCl}_2(\text{PPh}_3)_2\) (0.001 mmol), \(\text{CuI}\) (0.002 mmol) and \(\text{Et}_3\text{N}\) (0.189 mmol) in THF (1.0 mL) in a glass tube was bubbled with argon gas and then the tube was sealed. The reaction was left to stir at rt for 24 h. The mixture was extracted with EtOAc (3 x 20 mL). The combined organic phase was dried (\(\text{Na}_2\text{SO}_4\)). The crude residue was purified by PTLC with MeOH/CH\(_2\)Cl\(_2\) (1:99) as a mobile phase to give a phenyl alkyne product 216 as a yellow gum (10.0 mg, 0.023 mmol, 62% yield) and the dimeric by-product 224 (0.6 mg, 0.0001 mmol, 4% yield) which was directly prepared from Eglington coupling.

\(R_f = 0.42\) in MeOH/EtOAc (1:9).

\([\alpha]^{25}_\text{D} +235.9\) (c 0.44, CHCl\(_3\)).

ESIMS \(m/z\) 432.1 (100%) \([\text{M+H}]^+\).

HRESIMS \(m/z\) 432.1821 \([\text{M+H}]^+\), calcd for C\(_{26}\)H\(_{26}\)NO\(_5\) 432.1811.

\(^1\text{H NMR }\delta\) 7.45-7.44 (m, 2H, ArH), 7.31-7.30 (m, 3H, ArH), 4.66 (br s, 1H, H-2), 4.15 (s, 3H, O-CH\(_3\)), 3.56 (br s, 1H, H-9a), 3.48-3.42 (m, 1H, H-5a), 3.14 (d, \(J \) 6.0 Hz, 1H, H-7), 3.11-3.08 (m, 2H, H-5b, H-10), 2.23-2.17 (m, 1H, H-6a), 2.08 (s, 3H, H-16), 2.06 (d, \(J \) 12.5 Hz, 1H, H-1a), 1.98 (d, \(J \) 12.0 Hz, 1H, H-1b), 1.93-1.88 (m, 1H, H-6b), 1.83 (d, \(J \) 10.0 Hz, 1H, H-9), 1.39 (d, \(J \) 6.5 Hz, 3H, H-17).

\(^{13}\text{C NMR }\delta\) 169.8 (C-15), 162.9 (C-13), 148.0 (C-12), 131.9 (ArCH), 128.6 (ArCH), 128.4 (ArCH), 128.2 (C-11), 122.5 (ArC), 112.2 (C-8), 98.9 (C-14), 86.3 (C-2'), 86.1 (C-1'), 81.2 (C-2), 76.0 (C-3), 60.8 (C-9a), 59.0 (O-CH\(_3\)), 54.6 (C-7), 48.7 (C-5), 47.4 (C-9), 34.6 (C-10), 33.6 (C-1), 27.1 (C-6), 18.4 (C-17), 9.3 (C-16).
10.6.6 Eglington coupling of the alkyne 215


A solution of the alkyne 215 (13.5 mg, 0.038 mmol) and Cu(OAc)$_2$ (0.190 mmol) in dry MeCN (1.0 mL) in a sealed tube was bubbled with argon gas and then the tube was sealed. The reaction mixture was heated and stirred at 40 °C for 4 h. The mixture was cooled to rt and filtered through a thin pad of Celite. The filtrate was treated with an aqueous solution of NaHCO$_3$ and extracted with CH$_2$Cl$_2$ (3 x 20 mL). The combined organic phase was washed with brine and dried (MgSO$_4$). The evaporated residue was purified by PTLC with MeOH/CH$_2$Cl$_2$ (2:98) as a mobile phase to give a dimer 224 as a yellow gum (12.0 mg, 0.017 mmol, 89% yield).

$R_f = 0.50$ in MeOH/CH$_2$Cl$_2$ (1:9).

$[\alpha]^{25}_D +286.3$ (c 0.39, CHCl$_3$).

ESIMS $m/z$ 709.5 (100%) [M+H]$^+$. 
HRESIMS $m/z$ 709.2791 [M+H]$^+$, calcd for C$_{40}$H$_{41}$N$_2$O$_{10}$ 709.2761.

$^1$H NMR $\delta$ 4.56 (br s, 1H, H-2), 4.13 (s, 3H, O-CH$_3$), 3.50 (br s, 1H, H-9a), 3.37-3.31 (m, 1H, H-5a), 3.10-3.02 (m, 1H, H-5b, H-10), 3.05 (d, $J$ 6.0 Hz, 1H, H-7), 2.14-2.08 (m, 1H, H-6a), 2.06 (s, 3H, H-16), 2.00 (d, $J$ 12.0 Hz, 1H, H-1a), 1.89-1.86 (m, 1H, H-1b), 1.85-1.83 (m, 1H, H-6b), 1.76 (dd, $J$ 10.5 Hz, 3.5 Hz, 1H, H-9), 1.35 (d, $J$ 6.5 Hz, 3H, H-17).

$^{13}$C NMR $\delta$ 169.7 (C-15), 162.8 (C-13), 147.6 (C-12), 128.2 (C-11), 111.9 (C-8), 99.0 (C-14), 80.9 (C-2), 77.4 (C-3), 75.8 (C-1$'$), 70.4 (C-2$'$), 60.8 (C-9a), 59.0 (O-CH$_3$), 54.8 (C-7), 48.9 (C-5), 47.3 (C-9), 34.6 (C-10), 33.6 (C-1), 27.0 (C-6), 18.3 (C-17), 9.3 (C-16).
10.6.7 Hydrogenation of the Sonogashira product 216


To a mixture of the phenyl alkyne 216 (4.7 mg, 0.011 mmol) and Pd/C (0.002 mmol) in EtOAc (1.0 mL) at rt, a hydrogen gas was bubbled into the solution and the reaction was let to stir for 24 h. The reaction mixture was then filtered through a thin pad of Celite. The concentrated residue was purified by PTLC with MeOH/EtOAc (1:99) as a mobile phase to give a product as a white gum (3.5 mg, 0.008 mmol, 74% yield).

\[ R_f = 0.32 \text{ in MeOH/CH}_2\text{Cl}_2 (1:9). \]

\[ [\alpha]_{25}^D +248.4 \text{ (c 0.23, CHCl}_3). \]

ESIMS $m/z$ 436.1 (100%) [M+H]$^+$. 

HRESIMS $m/z$ 436.2118 [M+H]$^+$, calcd for C$_{26}$H$_{30}$NO$_5$ 436.2124.

$^1$H NMR $\delta$ 7.20 (d, $J$ 7.0 Hz, 2H, ArH), 7.13-7.10 (m, 3H, ArH), 4.24 (br s, 1H, H-2), 4.06 (s, 3H, O-CH$_3$), 3.43 (br s, 1H, H-9a), 3.12-3.06 (m, 1H, H-5), 3.05-3.00 (m, 1H, H-10), 2.99-2.93 (m, 1H, H-5), 2.72 (td, $J$ 11.5 Hz, 5.0 Hz, 1H, H-1’), 2.66 (d, $J$ 6.0 Hz, 1H, H-7), 2.50 (td, $J$ 13.5 Hz, 5.5 Hz, 1H, H-1’), 2.00 (s, 3H, H-16), 1.90 (d, $J$ 12.0 Hz, 1H, H-6a), 1.90-1.79 (m, 2H, H-1a, H-2’), 1.78-1.74 (m, 2H, H-6b, H-2’), 1.67 (dt, $J$ 12.0 Hz, 3.5 Hz, 1H, H-1b), 1.31 (d, $J$ 6.5 Hz, 3H, H-17).

$^{13}$C NMR $\delta$ 169.8 (C-15), 162.9 (C-13), 148.5 (C-12), 141.7 (ArC), 128.6 (ArCH), 128.3 (ArCH), 128.0 (C-11), 126.1 (ArCH), 112.7 (C-8), 98.6 (C-14), 82.7 (C-3), 78.5 (C-2), 61.0 (C-9a), 58.9 (O-CH$_3$), 50.4 (C-7), 47.7 (C-5, C-9), 34.6 (C-10), 33.7 (C-2’), 33.4 (C-1), 31.4 (C-1’), 26.7 (C-6), 18.4 (C-17), 9.2 (C-16).
10.7 Experimental for Chapter 7

10.7.1 TLC Bioautographic method

The AChE used in this assay was extracted from electric eels and purchased from Sigma Aldrich (EC 3.1.1.7). The enzyme stock solution was prepared from a solution of AChE (1000 U) in 150 mL of 0.05 M tris-hydrochloric acid buffer at pH 7.8 to which was added bovine serum albumin (150 mg) to stabilize the enzyme. The stock solution was kept at 4 °C. TLC plates used for the bioautography were washed with acetone and then thoroughly dried. The samples were prepared as solutions in MeOH at concentrations of 1000, 100, 10, 1 and 0.1 ppm. The samples were applied to the TLC plates in varying quantities using Camag Nanomat 4 TLC spotter with 0.5 μL capillaries and sprayed with AChE enzyme stock solution and thoroughly dried again. The plates were laid flat on plastic plugs in a covered water bath (to avoid the plates from contacting the H2O directly) and then incubated in a humid atmosphere at 37 °C for 20 min. The plates were taken out and sprayed with a freshly prepared indicator solution which was a mixture of two solutions, a solution of 1-naphthyl acetate (25 mg) in EtOH (10 mL) and a solution of Fast Blue B salt (40 mg) in H2O (16 mL). After 1-2 min, a purple coloration on the TLC plates appeared and white spots indicated inhibition of AChE by the samples.

10.7.2 Spectroscopic-based method

These assays were performed by Dr. Thanapat Sastraruji at the University of Wollongong. Acetylthiocholine iodide (ATChI) was used as a substrate while 5,5’-dithiobis[2-nitrobenzoic acid] (DTNB) was used as a reagent. Two stock solutions were used in this assay, buffer solution and substrate solution. The pH 7.0 phosphate buffer solution was prepared from a mixture of 37.2 mM NaH2PO4·H2O and 62.7 mM Na2HPO4·2H2O, in Milli Q H2O. The substrate solution was prepared as a 4.73 mM ATChI solution in phosphate buffer pH 7.0. The reagent solution was prepared as a 3.15 mM DTNB solution in phosphate buffer pH 7.0.

The assay was performed in 96-well plates. In each well, 120 μL of phosphate buffer pH 7.0 was mixed with 20 μL of reagent and 20 μL of substrate, then 20 μL of AChE (0.75 U/mL) in phosphate buffer pH 7.0 was added with 20 μL
of the sample (which was prepared in concentrations of 5,000, 1,000, 200, 40, 8, 1.6 and 0.32 μM in DMSO). The final concentrations were 500, 100, 20, 4, 0.8, 0.16 and 0.032, respectively. Then the well plate was directly put into the microplate reader which was thermostated at 25 °C. The absorbances were read using a SPECTRMax® PLUS384 microplate thermostated spectrophotometer (California, USA) at 412 nm, every 15 sec for 30 min continuously. Enzyme activity was calculated as a percentage compared to an assay using a buffer without any inhibitor. The AChE inhibitory data were analyzed with the software package GraphPad Prism® (Graph Pad Inc., San Diego, USA). IC_{50} values are means ± SD of three individual determinations each performed in triplicate.

10.7.3 Cytotoxicity assay and chemosensitivity testing

These assays were performed in Assoc. Prof. Pornngarm Limtrakul’s laboratory at Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Thailand.

Chemicals and reagents

Vinblastin, paclitaxel, verapamil, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Aldrich. Dulbecco’s modified Eagle’s medium (DMEM) was purchased from GibcoBRL. Fetal bovine serum was purchased from HyClone.

Cell lines and culture conditions

The cervical cancer cell lines, KB-V1 (multidrug resistance cervical carcinoma cell line) and KB-3-1 (drug sensitive cervical carcinoma cell line), were generous gifts from Dr. Michael M.Gottesman (National Cancer Institute, Bethesda, MD). Both cell lines were cultured in DMEM with 4.5 g of glucose/L plus 10% fetal bovine serum (FBS), 2 mM L-glutamine, 50 U/mL penicillin and 50 μg/mL streptomycin; 1 μg/mL of vinblastine was added only to the KB-V1 culture medium. These two cell lines were maintained in a humidified incubator with an atmosphere comprising 95% air and 5% CO_2 at 37 °C. When the cells reached confluency, they were harvested and plated either for subsequent passages or for drug treatments.
Cytotoxicity assay and chemosensitivity testing

Cytotoxicity of stemofoline and its derivatives were estimated in KB-V1 and KB-3-1 cell lines using the tetrazolium-based colorimetric MTT assay. KB-V1 and KB-3-1 cells were plated at 1.0×10^3 cells per well in 96-well plates. After 24 h, noncytotoxic concentrations (>80% cell survival) of tested compounds (5 µM) and various concentrations of paclitaxel or vinblastine were added (0-20 µM paclitaxel for KB-V1 and 0-5 nM for KB-3-1) or (0-1 µM vinblastine for KB-V1 and 0-1 nM for KB-3-1). The cells were incubated for 48 h at 37 °C, and then cell growth was assessed by means of an MTT colorimetric assay. Cell viability was assessed by adding 15 µL of MTT reagent (5 mg/mL) for 4 h incubation and the reaction was terminated by adding 200 µL of DMSO. Absorbance was measured using an ELISA microplate autoreader (Biotek Instruments, EL311s, USA) at 540 nm with a reference wavelength of 630 nm. The fractional absorbance was calculated by the following formula: % cell survival = (mean absorbance in test well)/(mean absorbance in control wells)×100.

The reversal effects of tested compounds were then investigated with the same method. The cells seeded in 96-well plates were treated with varying concentrations of paclitaxel or vinblastine in the absence or presence of 5 µM of tested compounds for 48 h. Control cultures included an equivalent amount of DMSO (as the vehicle control), which did not modulate drug sensitivity of these cells of the concentrations used in these studies. Verapamil was used as a positive control. In each experiment, determinations were carried out in triplicate. The relative resistance (RR) was calculated as the ratio of the IC_{50} value of the KB-V1 cells to the IC_{50} value of the KB-3-1 cells. The fold-reversal activity (FR) was calculated as the ratio of the RR for cells with the anticancer without tested compounds to the RR for cells with the anticancer and tested compounds.
10.8 Experimental for Chapter 8

10.8.1 Pharmacophore generation

General methodology

All pharmacophores were generated using the 3D QSAR Pharmacophore Generation tool from the Discovery Studio 2.5.5 (DS 2.5.5) software package (Accelrys Inc., San Diego, CA, USA).

Training set selection

A number of known compounds were compiled from the published data known for their acetylcholinesterase (AChE) inhibitory activity against electric eel AChE. The biological activities from the different sources of data were validated from the common standard controls used in the assay, such as galanthamine and tacrine. These compounds then were specifically selected based on the similarity of their structures to stemofoline alkaloids and were grouped into five training sets according to their known binding modes. Five training sets included the ‘Active site binding’ (ASB), ‘Bis-functional binding’ (BFB), ‘Stemona’, ‘Tacripyrine’ and ‘Unknown’ which was the combination set of Stemona and Tacripyrine sets. All compounds were sketched and their stereochemistry was specified in DS 2.5.5 and conformers of each training set except for the Stemona and Unknown sets were generated using the ‘best’ option with the CHARMm force field, with an energy threshold of 20 kcal/mol and a maximum of 255 conformations. For the Stemona and Unknown sets, the systematic search option was selected because the general method resulted in the wrong conformation of compounds in the Stemona set as the rotation of the lactone ring was observed. Thus, their conformers were generated using torsion increments of \(sp_2-sp_2 = 360.0^\circ\), \(sp_2-sp_3 = 120.0^\circ\), \(sp_3-sp_3 = 120.0^\circ\) with the CHARMm force field and default parameters unless indicated otherwise.

3D QSAR Pharmacophore generation

3D QSAR Pharmacophore Generation tool was used with the feature options of hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), hydrophobic (aliphatic) (Hal), hydrophobic (aromatic) (Har) and positive ionisable (PI). Also the user-defined feature, cyclic \(\pi\)-interaction (CYPI)\(^{89}\), was included. These features
were chosen for hypothesis generation for each training set. The maximum number for each feature was set to 5. All experiments were carried out with the default parameters. To optimise the results, the maximum of 20 excluded volumes was applied, and variable weights and tolerances were used in some cases.

Ligand-pharmacophore mapping

The training sets were used as the test sets and were mapped to seven pharmacophore models using ligand-pharmacophore mapping function in DS 2.5.5. All experiments were performed using the default setting parameters with the mapping energy threshold of 20, fitting method as rigid, best mapping only and the maximum omitted feature was set as zero.

10.8.2 Protein-ligand docking

General methodology

All docking runs were performed with the GOLD suite versions 4.1 and 5.0 (CCDC, Cambridge, UK) on a Windows-based operating system. The X-ray crystal structure of *Torpedo californica* acetylcholinesterase (TcAChE) with huprine X bound (PDB code 1E66) was selected as the protein for docking and also the template for modelling the human AChE (hAChE) and electric eel AChE (eeAChE). The GOLD setup wizard was used. Ligands and water molecules were removed from the X-ray crystal structure and hydrogen atoms were added to TcAChE. The binding site was defined by centre coordinates of huprine X (X = 5.480 Å, Y = 66.057 Å and Z = 64.850 Å), including all atoms within 20 Å from this point. Cavity detection was set to on. All input ligands were docked with 100 genetic algorithm (GA) runs. Under the fitness and search options, GoldScore was selected for docking and early termination was disabled. GA settings were as preset with 100,000 operations. The clustering of docking results was performed within 2.0 Å (root-mean-square-deviations of ligand heavy atoms). This procedure was also performed using hAChE and eeAChE models. All output results were analysed and processed in DS 2.5.5 to monitor the protein-ligand interactions.
Protein modelling and validation

The X-ray structure of TcAChE (PDB code 1E66) was selected as a template for modelling eeAChE and hAChE. The sequences of all three AChEs were retrieved in a FASTA format from UniProtKB. Sequences of template and model AChEs from FASTA files were copied as text to be aligned in MAFFT using a ClustalW format. Output alignments were saved as a text file and were edited in Notepad. The edited alignment file was submitted as an input alignment for modelling in SWISS-MODEL.
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