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University of Alberta

Synthesis of Peptide Analogs with Replacement of Sulfur by Carbon: Effects on the Biological Activity and Stability

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Department of Chemistry

Edmonton, Alberta

Spring 2005

Dedicated to my parents for their encouragement and continued support

ABSTRACT

Among other effects, the neuropeptide oxytocin (**11**) controls uterine smooth muscle contraction leading to child birth and in some cases pre-term labor. The synthesis of analogs of **11**, its antagonist atosiban (**13**) and L-tyrosinamide, N-(3-vinyl-3,3-cyclohexyl-1-oxopropyl)-D-thienyl-L-isoleucyl-L-threonyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-ornithyl-, cyclic (1→5)-disulfide (**15**), in which the disulfide bridge was replaced with a carbon-carbon linker (saturated or unsaturated) was explored. Replacement of cysteine (**1**) residues 1 and 6 in **11** with amino acids such as L-allylglycine (**10**) afforded a bis-olefin containing linear peptide precursor, which could be cyclized using a ring-closing metathesis (RCM) reaction to afford *cis* and *trans* peptide olefin analogs of **11**. An analogous approach was used to generate dicarba olefin analogs of atosiban (**13**). Reduction of the olefin carbocyclic peptides was also done to afford the conformationally less restricted saturated derivatives by simple hydrogenation with Pd/C. A dicarba analog of **15**, which was not available by RCM, was achieved by incorporation of a cyclohexyl-containing derivative **60** of α-L-aminosuberic acid into a linear peptide precursor by solid phase peptide synthesis. Cyclization using standard amide bond forming techniques afforded the desired carbocycle [(1,6-α-L-amino-α'-deamino-β',β'-cyclohexylsuberic acid)-2-D-thienyl-9-L-tyrosyl]-atosiban (**29**). Biological testing on rat uterine muscle tissure showed that [α,α'-L,L-diaminosuberic acid]-oxytocin **17** (EC_{50} = 348 nM), and *cis/trans* analogs [α,α'-L,L-diamino-γ,γ'-dehydrosuberic acid]-oxytocin **18a** (EC_{50} = 38 nM) and **18b** (EC_{50} = 250 nM) and larger ring analog [1,6-α,α'-L,L-diamino-γ,δ'-dehydroadipic acid]-oxytocin **19** (EC_{50} = 1.4 ± 0.4 x 10^3 nM) exhibit agonistic activity in comparison to **11** (7 ± 2 nM). Biological testing for antagonistic

activity revealed that 1:4 *cis/trans* mixture [1,6-α-L-amino-α'-deamino-γ,γ'-dehydrosuberic acid]-atosiban **26** (pA_2 = 7.8 ± 0.1) and **29** (pA_2 = 6.1 ± 0.1) exhibit antagonistic activity with **26** and **27** approaching the activities of **13** (pA_2 = 9.9 ± 0.3) and **15** (pA_2 = 8.8 ± 0.5). Testing for increased rat placental tissue stability showed that agonist **19** has a longer half-life (11-18 min) than **11**. The atosiban analog **27** also exhibited an extended half-life (> 2x) compared to atosiban (**13**).

Using similar RCM methodology, a 9,14-dicarba analog of the type II bacteriocin leucocin A **75** was synthesized by native chemical ligation of a 20-residue, N-terminal, peptide thioester (**84**) to a 17-residue N-terminal cysteine-containing peptide (**85**) to afford [9,14-α,α'-L,L-diamino-γ,γ'-dehydrosuberic acid, 20-Cys]-LeuA (**88**). Desulfurization using $NiBH_4$ afforded small quantities of [9,14-α,α'-L,L-diaminosuberic acid]-LeuA (**76**). A hexa-carba analog of 14 C-terminal residues of the lantibiotic, lacticin 3147 A2, [16,20-22,25-26,29-α,α'-D,L-diamino-γ,γ'-dehydrosuberic acid] (**95**) was also constructed using three sequential RCM reactions on resin-bound linear precursors.

The large scale synthesis of poly(ethylene glycol) bis-(6(-methylsulfinyl)hexanoate (**103**) and its use to oxidize cholesterol (**106**) to cholest-5-ene-3-one (**105**) was also explored. This afforded the oxidized product (**105**) in both high yield (ca 96%) and quantity (≥10 g). The procedure greatly facilitates the study of enzymes such as cholesterol oxidase[187] and 3-oxo-Δ^5-steroid isomerase[188] by making substrates readily available in an efficient one-pot reaction.

Acknowledgements

I would like to acknowledge the outstanding support and encouragement of my research supervisor, Professor John. C. Vederas. The time spent in his research group has been rewarding and has no doubt prepared me for future endeavors. The members of the Vederas research group, both past and present, deserve acknowledgement for making time spent in the lab both an enjoyable and unique experience. A special thanks to Drs. Christopher Diaper and Hanna Pettersson for their valuable insight and assistance in proofreading this manuscript. I also would like to send out a deep appreciation for the dedicated support staff in the Chemistry Department, especially those in spectral services who have provided valuable assistance with this project. I would like to acknowledge both Dr. Bryan Mitchell and Susan Wong of Department of Obstetrics and Gynecology at the University of Alberta for both their expertise and use of the laboratory equipment required for the testing of all the analogs related to oxytocin.

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Finally, I send a great deal of gratitude and appreciation to Maggie Wilcox who has been both extremely patient and understanding during this time. You are the person I come home to every night, and the shoulder I lean on when times are rough. Thank you.

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LIST OF ABBREVIATIONS

$[\alpha]_D$	specific rotation
Abu	α-aminobutyric acid
Ac	acetyl
AcO	acetate
Ac_2O	acetic anhydride
AcOH	acetic acid
Ala	alanine
AllGly	allylglycine
Anal.	analysis
ap.	apparent
Arg	arginine
aq	aqueous
Ar	aryl
Asn	asparagine
Asp	aspartic acid
AT	atosiban
atm	atomsphere
AVP	arginine[8]-vasopressin
Bn	benzyl
Boc	butoxycarbonyl
Boc_2O	Boc anhydride
br	broad

t-Bu	*tertiary* butyl
c	concentration g/mL (optical rotation)
calcd.	calculated
Cbz	carboxybenzyl
Cys	cysteine
δ	chemical shift in parts per million downfield from TMS
d	doublet (in NMR)
DAP	diaminopimelic acid
DAS	diaminosuberic acid
DCC	1,3-dicyclohexylcarbodiimide
Dha	dehydroalanine
Dhb	dehydrobutyrine
DIPCDI	1,3-diisopropylcyclohexylcarbodiimide
DIPEA	diisopropylethylamine
DMAc	*N*,*N*-dimethylacetamide
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone
DMSO	dimethylsulfoxide
DTT	dithiothreitol
EC_{50}	effective concentration causing 50% activity
EI	electron impact
ES	electrospray

Et	ethyl
Et_2O	diethyl ether
EtOH	ethanol
eq	equivalents
Fmoc	9*H*-fluorenylmethoxycarbonyl
Fmoc-Cl	9*H*-fluorenylmethoxycarbonyl chloroformate
Fmoc-Osu	9*H*-fluorenylmethoxycarbonyl succinamide
FTIR	Fourier transform infrared spectroscopy
gCOSY	gradient correlation spectroscopy
gHMBC	gradient heteronuclear multiple bond correlation spectroscopy
gHMQC	gradient heteronuclear multiple quantum correlation spectroscopy
IC_{50}	concentration causing 50% inhibition
g	gram
Gln	glutamine
Glu	glutamic acid
Gly	glycine
GPCR	G-protein coupled receptor
h	hour
Hag	homoallylglycine
HCCA	4-hydroxy-2-cyano-cinnamic acid
Hcy	homocysteine
His	histidine
HOBt	hydroxybenzotriazole

HPLC	high performance liquid chromatography
HRMS	high resolution mass spectroscopy
Hz	Hertz (s^{-1})
Ile	isoleucine
IR	infrared
J	coupling constant in Hertz
L	liter
LAB	lactic acid bacteria
Leu	leucine
Leu A	leucocin A
Lys	lysine
m	multiplet (in NMR)
M	molar concentration
MALDI	matrix assisted laser desorption/ionization
MeCN	acetonitrile
MeOH	methanol
min	minutes
mL	milliliter
mol	mole
mp	melting point
Mpa	3-mercaptopropionic acid
MHz	megahertz (10^6 s^{-1})
min	minutes

MW	molecular weight
m/z	mass-to-charge ratio
μ	micro
n	nano
NCL	native chemical ligation
NMM	*N*-methylmorpholine
NMP	*N*-methylpyrollidinone
NMR	nuclear magnetic resonance
Orn	ornithine
OT	oxytocin
OTR	oxytocin receptor
PEG	polyethylene glycol
Ph	phenyl
Phe	phenylalanine
Pmc	2,2,5,7,8-pentamethylchroman-6-sulfonyl
Pro	proline
PS	polystyrene
PVP	poly-(*N*-vinyl-2-pyrrolidone)
PyBOP	benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
q	quartet (in NMR)
RCM	ring-closing metathesis
ROMP	ring-opening polymerization

RP	reverse phase
rt	room temperature
s	singlet (in NMR)
SA	sinapinnic acid
Ser	serine
SPPS	solid phase peptide synthesis
t	triplet (in NMR)
TES	triethylsilane
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Thi	thienylalanine
Thr	threonine
TIPSH	triisopropylsilane
TLC	thin layer chromatography
TMS	tetramethylsilyl
TOF	time of flight
TPSH	2,4,6-triisopropylbenzenesulfonyl hydrazide
t_R	retention time
Trt	trityl
Trp	tryptophan
Tyr	tyrosine
UV	ultra violet
Val	valine

Vca	1-(vinylcyclohexyl)-1-acetic acid
VPR	vasopressin receptor

1. INTRODUCTION

1.1 100 Years of Peptide Synthesis

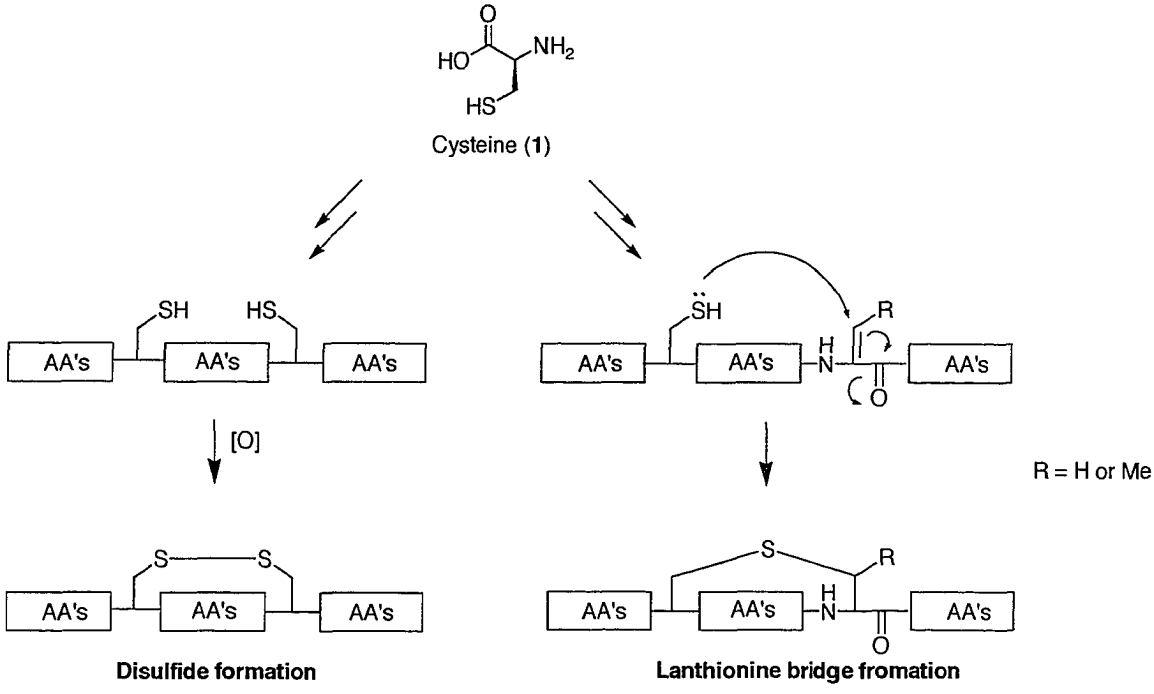
When Emil Fischer[1] prepared the first unprotected synthetic peptide, glycylglycine, he gave birth to peptide chemistry. From this initiation, the field has grown enormously over the last century. By the 1930's Bergmann and Zervas addressed the problems of peptide chain elongation at the amino terminus with the development of the benzyloxycarbonyl (Cbz) protecting group,[2] which could be cleaved by standard hydrogenolysis. This led to a new era in peptide chemistry where multifunctional amino acids could be incorporated into peptide chains. During the 1950's much work was done on the development of new techniques for peptide bond formation, including the use of mixed anhydrides,[3,4] active esters[5-7] and carbodiimides.[8,9] In parallel to these contributions was the development of orthogonally protected amino acids that could be more readily incorporated and manipulated within a growing peptide chain. The structural determination[10,11] and synthesis of oxytocin[12] by du Vigneaud in 1953, an achievement for which he was awarded the Nobel prize in 1955, was truly a milestone in peptide chemistry. This started a new era in which biologically active peptides were synthesized and studied for their structure-activity relationships. With the need for improved orthogonal amino acid protection came the advent of the *tert*-butoxycarbonyl (Boc) group[13,14] for N-terminal amino acid protection. This group is quickly and completely removed from amino functions using dilute trifluoroacetic acid (TFA), and is stable to base and hydrogenolysis. Up to this point, the synthesis of peptides containing more than a few residues was a huge undertaking. Synthesis of peptides > 20 residues required teams of chemists and many months or years for completion. The invention of solid

phase peptide synthesis (SPPS) by Merrifield in the early 1960's,[15] a feat which won him the Nobel Prize in 1984, was one of the most fundamental advances in peptide chemistry. This method of peptide synthesis involved the immobilization of a growing polypeptide on styrene-divinylbenzene (DVB) co-polymers, allowing for easy purification by simple filtration. This allowed for the use of excess reagent, without the worry of product contamination and separation.[16] This methodology led to the synthesis of larger more complex peptides like ribonuclease A.[17] As these peptides grew in size there were concerns about peptide purification, especially in SPPS, due to the presence of truncated impurities that accumulate on the resin. The advent of high performance liquid chromatography (HPLC) in the 1970's helped circumnavigate this problem and allowed for the purification of large peptides in multi-gram quantities. It thereby solidified SPPS as the method of choice for peptide synthesis. The 1980's saw the invention of the 9-fluorenylmethoxycarbonyl (Fmoc) amino protecting group by Carpino.[18] This allowed for the orthogonal protection of amino acids with both base and acid labile groups. The advantage of this methodology over Boc protection is the regioselective coupling of amino acids under basic conditions with the acid labile side chain protecting groups remaining completely intact. This also lead to the development of resins by Wang,[19] Rink[20] and Sieber,[21] from which peptides could be cleaved under very mild acidic conditions using TFA. Recently, extensive efforts have focused on the development of new techniques for the synthesis of large peptides or proteins using recombinant DNA or chemical ligation procedures. Key advancements include native chemical ligation (NCL) by Kent and Dawson[22,23] and traceless Staudinger ligation by Raines[24,25] and Bertozzi.[26] The chemoselectivty of these techniques has allowed for the synthesis of peptides and

proteins of > 200 residues in length by the coupling of two (or more) unprotected peptide fragments. These advances in peptide chemistry, as well as traditional synthetic organic chemistry, has opened the door for the generation of a multitude of peptides with unnatural amino acids and peptidomimetics of structural or biological interest.

There are a plethora of naturally occurring peptides that contain post-translational modifications essential for their biological properties. One common post-translational modification is cyclization within the peptide backbone. Such peptides are abundant as cyclization confers rigidity to the peptide backbone and fixes the secondary and tertiary structure necessary for activity.[27] Aside from N to C cyclization, most cyclic peptides result from the modification of cysteine residues within the backbone. The most common is a disulfide bond formed by oxidation (Figure 1). These motifs can be found in a wide variety of naturally occurring peptides and proteins, including hormones,[12] neurotoxins,[28] and non-lantibiotic bacteriocins.[29] A less common type of a sulfur-containing ring is the thio-ether bridge (Figure 1). These motifs are most commonly found in lantibiotic peptides produced by certain strains of lactic acid bacteria.[30] These thio-ether cycles, often called lanthionine bridges,[30] arise from the Michael addition of the free thiol of cysteine onto α,β unsaturated amino acids (Figure 1) such as dehydroalanine (Dha) or dehydrobutyrine (Dbh).[30]

Figure 1 Post-translational formation of disulfide and thio-ether bridges in peptides.



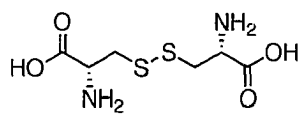
The sulfur bridges that conformationally restrict peptides in nature are potentially labile, both chemically and biologically. Disulfide bridges are prone to reduction in the metabolically reducing environment of the mammalian body, which can render the peptides inactive.[27] Lanthionine bridges are susceptible to oxidation to sulfoxides and sulfones, which has also been shown to abolish biological activity in lantibiotics such as nisin A[31] and lacticin 3147.[30,32,33] Hence, various groups have investigated peptidomimetics in which the sulfur linkage has been replaced with a more chemically robust carbon functionality.[27,34-45] This structural change can give rise to analogs with comparable biological activity[34-37,44] and possibly increased stability. Initially this may seem surprising as the polarity of the sulfurs and their preference for a close to 90° dihedral angle is clearly different from two methylene (CH_2) or methine (CH) units.

However, if the interaction of the peptide with a receptor molecule occurs at a surface remote from the modified bridge, the correct conformational arrangement can potentially be maintained at the binding site. To our knowledge, a complete study of the biological stability of such modified peptides, in comparison to their sulfur containing parents has not been done. Comparison of olefinic bridges (*cis* vs. *trans*) in peptides has also not been examined prior to our work.

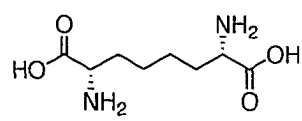
1.2 Incorporation of DAP and DAS analogs into peptidomimetics

Cystine (**2**) (Figure 2) in disulfide containing peptides or lanthionine bridges in lantibiotics can be replaced with orthogonally protected diaminosuberic acid (DAS) (**3**) or diaminopimelic acid (DAP) (**4**) analogs (Figure 2).

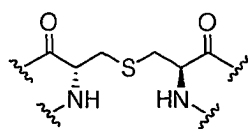
Figure 2 LL-Cystine (**2**), LL-DAS (**3**), lanthionine bridge, LL-DAP (**4**).



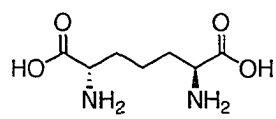
LL-Cystine; **2**



LL-DAS **3**



Lanthionine bridge



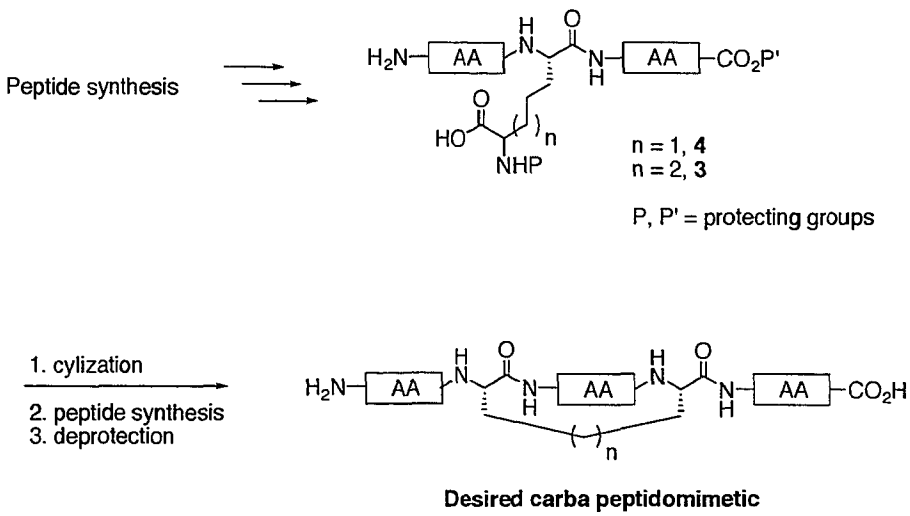
LL-DAP **4**

Two methods for the replacement of these natural sulfur-containing amino acids with **3** or **4** would be either the incorporation of preformed carbon bridges during peptide synthesis, or the formation of these bridges by a ring closing metathesis (RCM) reaction on olefin containing amino acids incorporated into the backbone.

1.2.1 Preformed Carbon Bridges via Incorporation of Analogs of 3 and 4

The integration of preformed carbon bridges into peptides dates back over three decades.[42] Orthogonally protected derivatives of **3** and **4** can be incorporated into a peptide backbone using standard peptide chemistry. Sequential deprotection and ring closure to afford the desired carba analogs can be readily accomplished using standard peptide coupling methods[46] (Scheme 1).

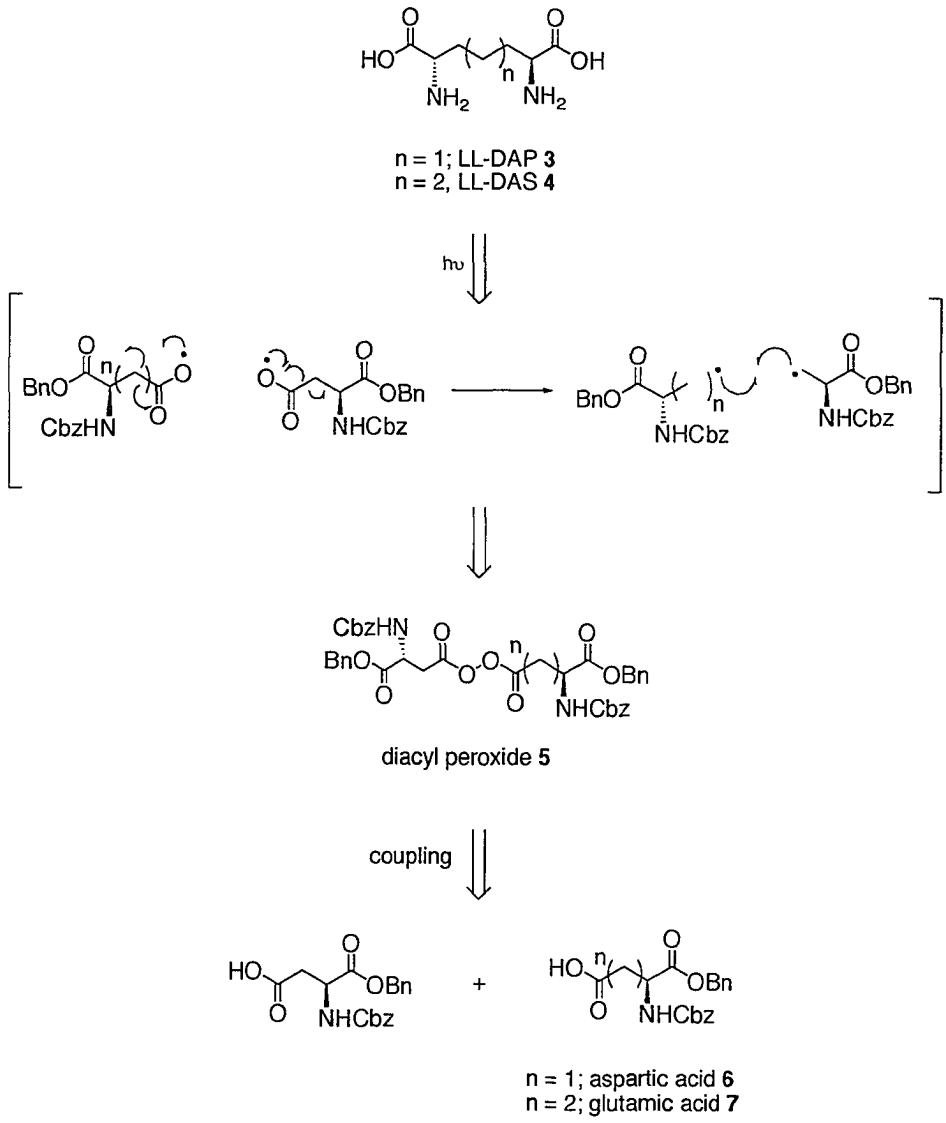
Scheme 1 Incorporation and cyclization of DAS (**3**) or DAP (**4**) into the peptide backbone.



A major drawback of this methodology is the required synthesis of enantiopure protected forms of DAP and DAS. Many syntheses of optically pure analogs of **3** and **4** have been reported.[27,47-53] One approach involves the use of selectively protected amino acid subunits (e.g., Asn or Gln) that are coupled together via their carboxylic acid side chains by a low yielding (ca. 9%) Kolbe electrolysis reaction.[47,48] Many others involve multi-step sequences relying on the use of cumbersome chiral auxiliaries to introduce the stereochemistry for the enantiopure DAP and DAS derivatives.[49-52] Recently, a new approach was reported by Vederas and co-workers.[54,55] This methodology is based on the photolysis of diacyl peroxides in the absence of solvent to give a carboxylate radical formation, followed by decarboxylation and recombination of the resultant radicals to give a new carbon-carbon bond.

This coupling procedure has many advantages over the previous methodologies. The required diacyl peroxides (e.g., **5**) are readily obtained from the coupling of two, commercially available, asymmetrically protected amino acids such as aspartate **6** or glutamate **7** (Scheme 2). If the photolysis reaction is done on the diacyl peroxide at low temperature, in the absence of solvent, coupling of the resultant radicals can be achieved without crossover to afford DAP and DAS analogs. The method allows for the generation of compounds with varying chain lengths and structural features in good yield.[54,55]

Scheme 2 Retrosynthesis of DAS (**3**) and DAP (**3**) via diacyl peroxides.

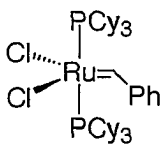


1.2.2 Ring Closing Metathesis (RCM)

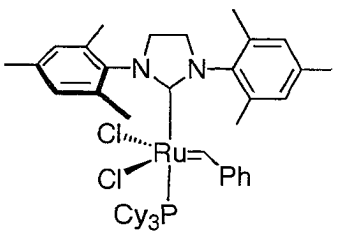
The discovery by Karl Ziegler that catalysts formed in situ from certain transition metal salts and main group alkylating agents promote the polymerization of olefins under unprecedented mild conditions has had a tremendous impact on modern synthetic chemistry.[56] The preparation of highly reactive molybdenum and tungsten based olefin

metathesis catalysts by Schrock[56,57] has led to useful synthetic applications of RCM in organic synthesis. However, it was not until the advent of catalysts such as **8**[58,59] and **9**[60] (Figure 3) by Grubbs and co-workers that the true power of facile and functionally tolerant olefin metathesis catalysts was fully realized.[56] Since the discovery of **8** and **9**, many variations of these catalysts have been synthesized and shown to exhibit improved reactivity in various solvent systems[61,62] as well as catalyst regeneration.[63] Immobilization of recyclable derivatives of **8** and **9** onto poly-divinylbenzene (DVB) solid support has also been reported.[64]

Figure 3 Grubbs first **8** and second **9** generation catalysts.



Grubbs 1st Generation Catalyst (**8**)

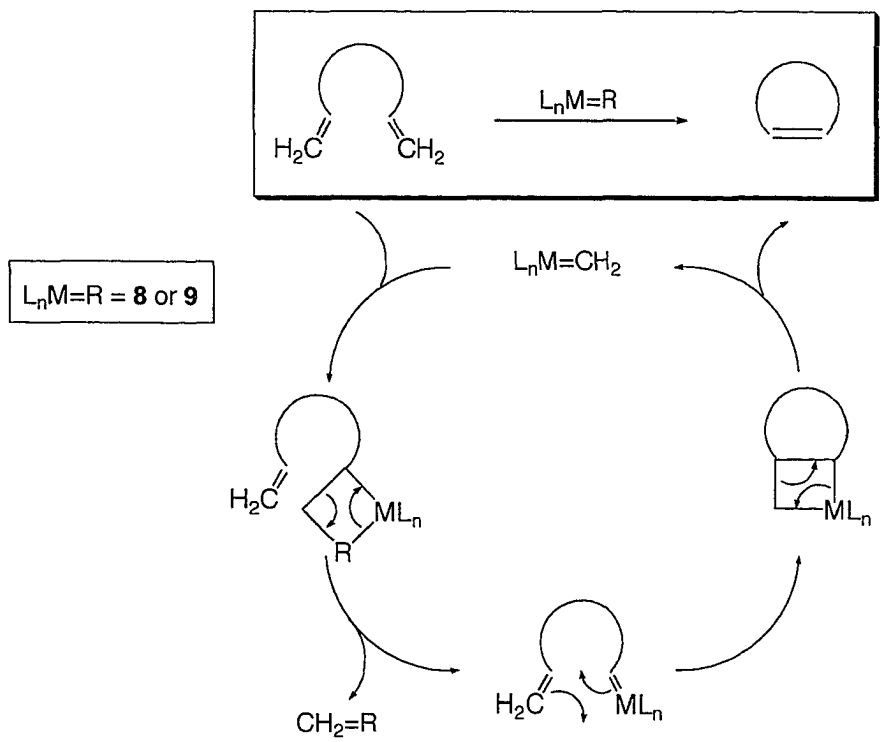


Grubbs 2nd Generation Catalyst (**9**)

The generally accepted mechanism for the RCM reaction by **8** and **9** consists of a sequence of formal [2+2] cycloadditions/cycloreversions involving alkenes, metal carbenes and metallocyclobutane intermediates (Scheme 3).[56] Each individual step is reversible, and thus an equilibrium mixture of olefins is produced. With **8** and **9**, the reaction is entropically driven because the RCM reaction cuts one substrate molecule into two compounds,[56] namely the desired cyclized product and a small alkene (Scheme 3). As

the latter is volatile, the equilibrium is driven toward the desired cycloalkene.[56] It is important to note that RCM reactions are done under dilute conditions to promote ring closure and avoid polymerization.

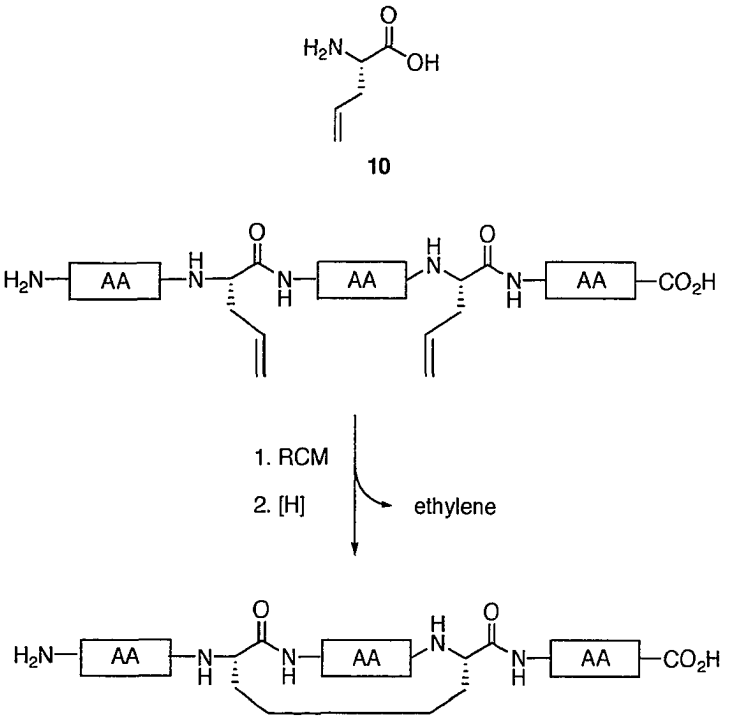
Scheme 3 Basic catalytic cycle for the RCM reaction[56]



The RCM reaction has been employed in the preparation of both DAP and DAS analogs.[27,53] These syntheses require the use of tethers between two selectively protected amino acid subunits to allow the intramolecular RCM reaction to occur.[27,53] The use of catalysts **8** and **9** in the RCM reaction to synthesize "carba" analogs of cyclic peptides has been achieved on an assortment of peptidic diene systems, in both solution[34,35,38,40,65-68] and on solid-support.[36,37,39,44,45,69,70] The general approach involves the substitution of

cysteine residues in the natural peptide backbone with an L-allylglycine **10**, followed by RCM resulting in the replacement of LL-cystine with LL-diaminosuberic acid (DAS)[27,41,53] (Scheme 4).

Scheme 4 Replacement by Cys (**1**) for L-allylglycine (**10**) followed by RCM.

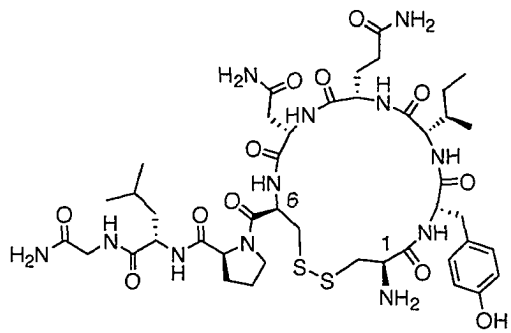


1.3 Oxytocin

To investigate the ramifications of replacing sulfur bridges with carbon moieties, the synthesis of dicarba analogs of biologically important peptides, which contain one or more of these cyclic structural motifs, has been of interest. Oxytocin (**11**) (Figure 4) is a small mammalian nonapeptide hormone synthesized by the magnocellular neurons of the hypothalamus. It has a wide spectrum of physiological activities, which include the

control of mammary and uterine smooth muscle contraction,[71] neurotransmission in the central nervous system, autocrine/paracrine functions in the ovaries and testes.[72,73]

Figure 4 Oxytocin (**11**).



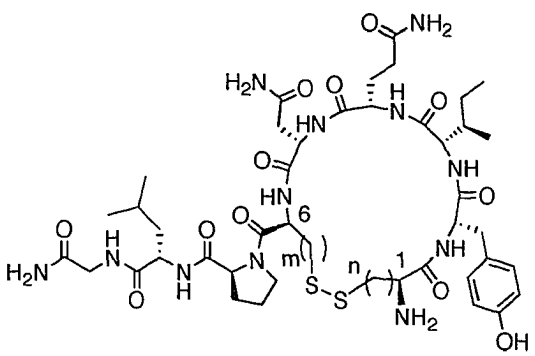
Oxytocin (**11**)

These physiological effects of oxytocin are mediated through a specific oxytocin receptor (OTR), which is a member of the G-protein coupled receptor (GPCR) family. These receptors are characterized by seven putative trans-membrane domains.[74-79] Oxytocin (**11**) is the most potent uteronic substance known and is clinically used to induce labour in many mammalian species.[71] However, due to its short half-life *in vivo* (2-5 min) in many cased must be administered to patients intravenously.[80] It is speculated that the presence of a metabolically unstable disulfide bridge in **11** is a contributing factor to the observed short half-life of this hormone. In 1953, oxytocin, became the first peptide hormone to be synthesized[12] and since then has been extensively studied. A large number of analogs have been reported with varying degrees of activity and stability.[43,76,81-98]

1.3.1 Oxytocin Antagonists

Antagonists of oxytocin are of interest as tocolytic agents that inhibit pre-term labour and delay premature birth.[93,97] There is an estimate of 13 million premature births worldwide per annum[93] that account for 66% of all neonatal mortality[87] and contribute to serious complications and infant morbidity. In the US, about 470,000 infants (ca 12%) were born prematurely in 2002, and their hospital stays cost in excess of $13.6 billion.[99] In 1976 Smith and Ferger reported that increasing the ring size of oxytocin (**11**) by two methylene units (substitution of homocysteine for cysteine residue) results in an analog **12** (Figure 5) with antagonistic activity (pA_2 = 6.0) and greatly reduced agonistic activity.[82]

Figure 5 Antagonist **12**.



Antagonist **12**, m, n = 2

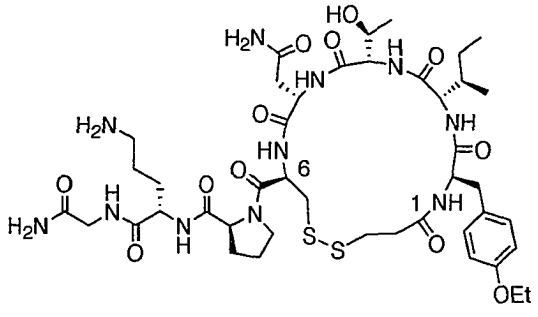
This was attributed to an increase in steric bulk within the cyclic portion of the peptide.[82] The pA_2 values for oxytocin antagonists are defined as the negative logarithm of the concentration of antagonist required to reduce the response of a double dose of oxytocin (**11**) to that of a single dose.[100]

Other studies showed that the removal of the N-terminal amino group at position 1 leads to analogs of oxytocin (**11**) with increased duration of activity *in vivo*.[43,81,101] The increase in half-life of these analogs results from the lack of recognition by aminopeptidases, which require the presence of a free N-terminal amino group.[102] It was later demonstrated that the replacement of tyrosine (Tyr) at position 2 in oxytocin (**11**) with unnatural "D" configured amino acids generates analogs of oxytocin with potent anti-uteronic activity.[86,89,93,95,98,103,104] Atosiban (Tractocile™) (**13**)[86,105] (Figure 6) is a competitive antagonist of OTRs that is approved in Europe for treatment of preterm labour. Atosiban has been studied extensively in both rats and humans and has proven to be a potent tocolytic agent ($pA_2 = 7.7$)[97,106-110] However, **13** has a short metabolic half-life (ca 16-18 min), is usually administered intravenously, and can have unwanted side effects due to lack of selectivity for the OTR over the vasopressin receptors (VPs).[97,105] Arginine[8]-vasopressin (AVP) (**14**) (Figure 4), a hormone synthesized in the posterior pituitary that plays a pivotal role in osmotic regulation and vasoconstriction,[97] is structurally very similar to atosiban (**13**) and oxytocin (**11**). Considerable effort over the past decade has been devoted to the synthesis of non-peptidic OTR antagonists in an effort to increase oral availability and longevity.[85,87,91,92,94,111,112] However, drug approval and liability issues have kept such compounds from clinical use.[112-114]

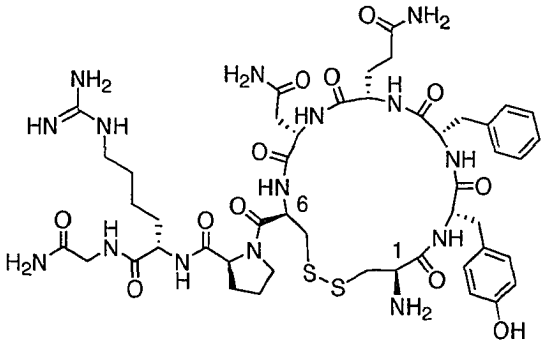
To address the selectivity problem with peptide analogs, Manning and co-workers synthesized a series of compounds including antagonist **15** (Figure 6), which differs from atosiban (**13**) by the presence of a bulky cyclohexyl moiety at position 1 and the introduction of a tyrosine residue at position 9 as well as a D-thienylalanine residue at position 2.[97] Nonapeptide **15** has comparable potency to **13**, but the selectivity for the

OTR over the VP receptor is greatly enhanced,[97] probably due to the increased steric bulk of the cyclohexyl group.

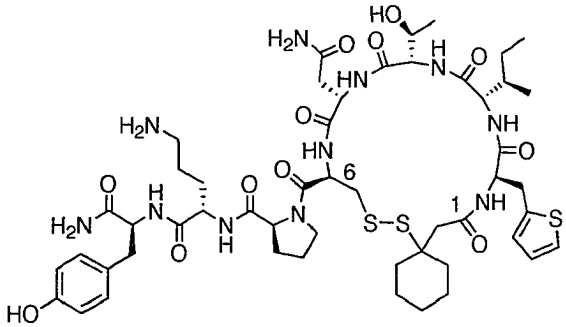
Figure 6 Atosiban (**13**) AVP (**14**) and antagonist **15**.



Atosiban (**13**)



AVP (**14**)



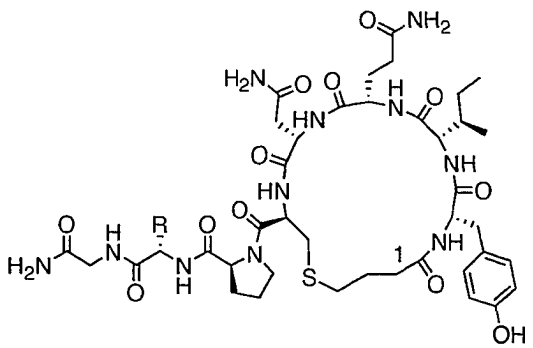
Antagonist **15**

1.3.2 The Disulfide Bridge: Structure and Function

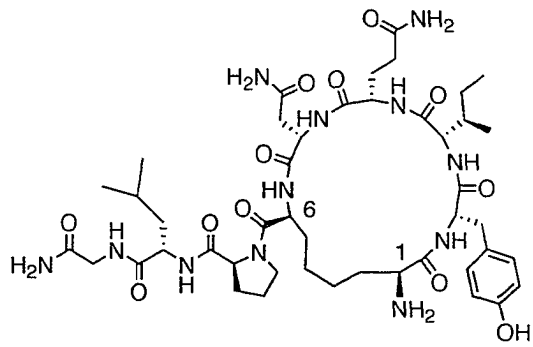
The common structural theme shared between oxytocin (**11**) and many of its analogs is the disulfide bridge, which is potentially a site of metabolism (e.g., reduction). The discovery of potent analogs, both agonists and antagonists, that have a longer duration of *in vivo* activity and greater bio-availability than **11**, **13** and **14** is highly desirable for

development of tocolytic drugs. The possible sensitivity of the disulfide moiety in such peptides to biochemical reduction, and its requirement for restriction of conformational mobility, may potentially be overcome by replacement with carbon-carbon linkers. Over 40 years ago, Rudinger and co-workers showed that the disulfide bridge in oxytocin can be modified.[43,81] They synthesized a carba analog of oxytocin, desaminooxytocin (**16**) (Figure 7), in which one of the sulfurs in the disulfide bridge was replaced with a methylene unit.

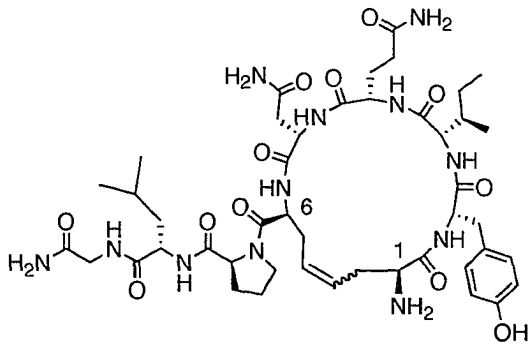
Figure 7 Various "carba" analogs of oxytocin (**11**).



16



17

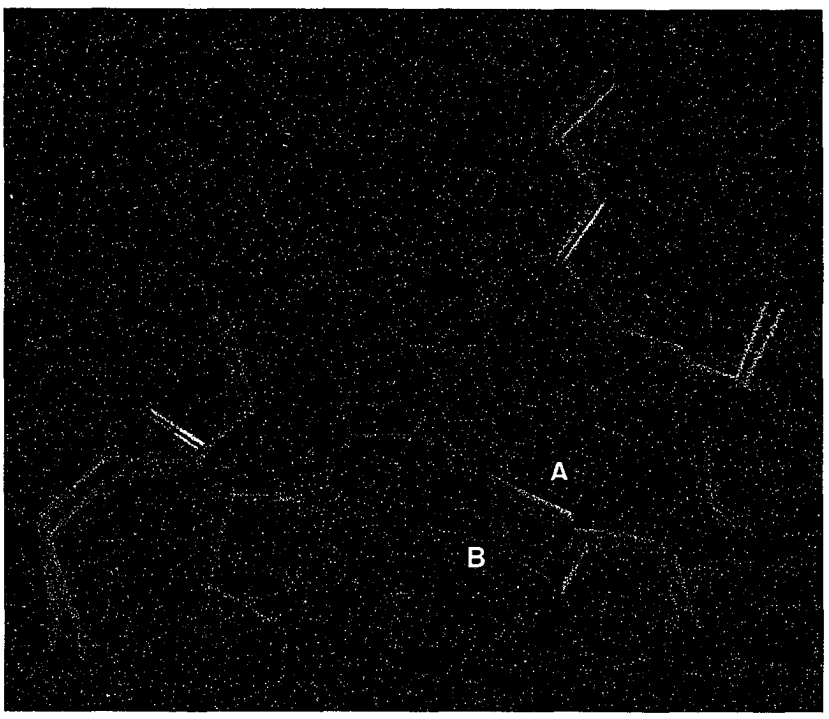


18a *cis*
18b *trans*

Compound **16** was shown to have very potent agonistic activity. Many mono-carba analogs of oxytocin have since been synthesized and their activities fully characterized.[101] In 1974 the synthesis of a fully saturated dicarba analog, **17** (Figure 7) of oxytocin was reported.[42] The approach involved a cumbersome multi-step procedure to incorporate an orthogonally protected DAS moiety into the peptide chain. The analog **17** was observed as being less active than oxytocin (**11**) and mono-carba analog **16**. The reduced activity was attributed to the greater conformational flexibility at the bis-methylene unit.[44]

Studies using NMR, laser Raman and circular dichroism spectroscopy on oxytocin (**11**) and X-ray crystallography on **16** show inherent conformational flexibility about the disulfide bridge.[115] In contrast, crystal structures of **11** bound to a neurophyseal carrier protein show that the C-S-S-C angle is clearly fixed in a single orientation.[116,117] Energy minimized structures of oxytocin (**11**) and its saturated **17** and unsaturated analogs **18a** and **18b** were compared to delineate the possible conformational changes resulting from modification of the disulfide bridge. Overlaying the energy minimized tertiary structures of oxytocin (**11**) and the *cis*-olefin containing 1,6-dicarba analog **18a** showed the two peptides were very similar in structure except for a small kink imposed by the preference of the S-S bond for a close to 90° dihedral angle (Figure 8). This suggests that an unsaturated, *cis* oriented ethylene linker would be a viable biostere for the disulfide bridge.

Figure 8 Overlaid MacSpartan Pro™ energy minimized structures of oxytocin (**11**) (A) and dicarba analog **18a** (B) (done by Dr. Kamaljit Kaur)*.



*Side chains on Tyr(2), Ile(3), Gln(4), Asn(5) and Leu(8) omitted for clarity.

1.3.3 Replacing Sulfur for Carbon Using the RCM Reaction

The synthesis of analogs **18a**, **18b** and **17** appears easily accessible by replacement of cysteine (**1**) residues 1 and 6 in **11** with L-allylglycine (**10**) followed by an RCM reaction.[44] Similarily, carba analogs of antagonists **12**, **13** and **15** could also be made using RCM (Figure 9). The sulfur-containing amino acid residues in these compounds could be substituted by the corresponding olefin analogs to provide the bis-olefins required for the RCM cyclization to yield analogs **19-29** (Figure 9 and Table 1).

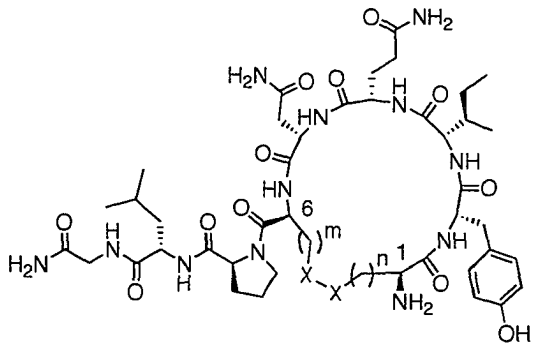
The development of RCM in SPPS can open the door for the synthesis of a variety of stabilized carba analogs of peptides with different structural features and biological

activities. This facile reaction can simplify the replacement of disulfide bridges with ethylene linkers in peptides. It also has the potential to give three separate analogs, namely *cis*, *trans* and saturated, by one reaction sequence. Testing of such compounds for biological activity would help delineate the possible receptor-bound conformations of neuropeptide hormones or antimicrobial peptides.

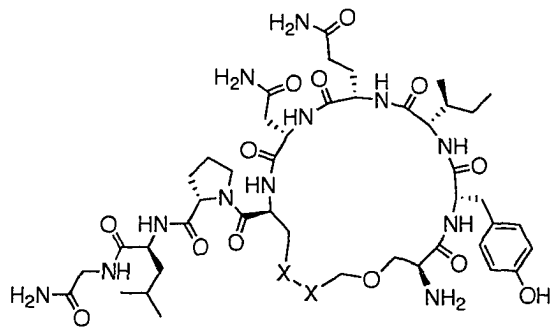
1.4 Project Goals: Design, Synthesis and Testing of a Variety of Carba Analogs of Known, Biologically Active Hormones

The objective of the first part of this thesis is the design, synthesis and examination of the biological activities and stabilities of a variety of peptidomimetics, **17**-**29** (Figure 9) in which the metabolically unstable sulfur bridges have been exchanged for more robust carbon linkers. Analogs of oxytocin (**11**), nonapeptide **12**, atosiban (**13**) and antagonist **15** can be synthesized using the RCM reaction on resin-bound peptides containing olefin side chains (Table 1). Peptide analogs in which RCM is problematic can be synthesized by the incorporation of a DAS analog, which is prepared via photolysis of the correct diacyl peroxide.

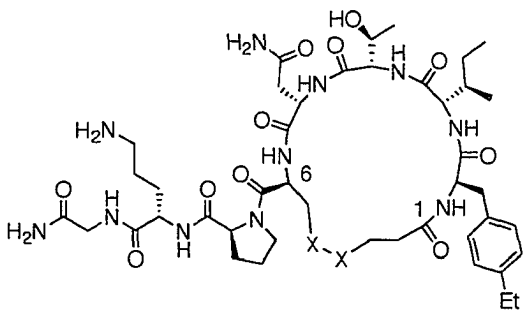
Figure 9 Possible peptide carbocycles available using olefin-containing amino acid residues and RCM reactions.



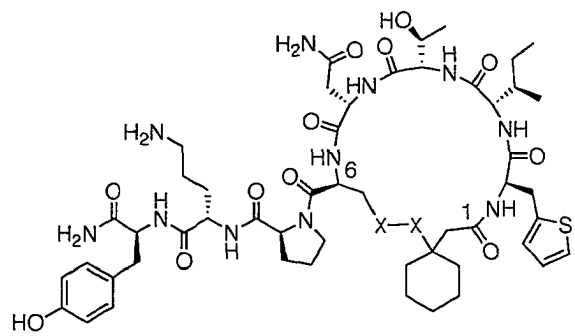
11 m, n = 1, X-X = S-S
18a m, n = 1, X-X = CH=CH (*cis*)
18b m, n = 1, X-X = CH=CH (*trans*)
19a m = 1, n = 2, X-X = CH=CH (*cis*)
19b m = 1, n = 2, X-X = CH=CH (*trans*)
20 m = 1, n = 2, X-X = CH_2-CH_2
21a m = 2, n = 1, X-X = CH=CH (*cis*)
21b m = 2, n = 1, X-X = CH=CH (*trans*)
22a m = 2, n = 2, X-X = CH=CH (*cis*)
22b m = 2, n = 2, X-X = CH=CH (*trans*)
23 m = 2, n = 2, X-X = CH_2-CH_2



24a X-X = CH=CH (*cis*)
24b X-X = CH=CH (*trans*)
25 X-X = CH_2-CH_2

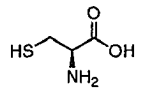
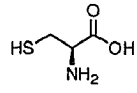
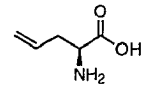
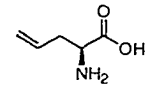
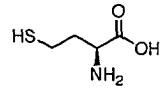
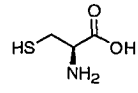
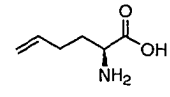
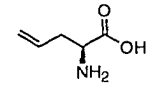
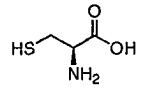
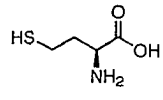
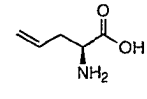
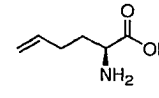
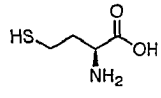
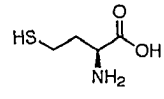
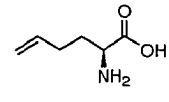
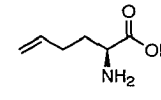
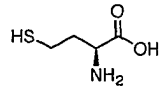
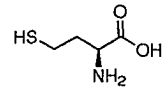
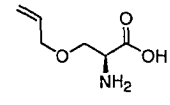
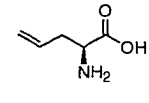
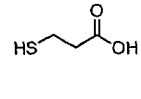
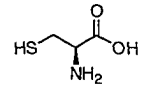
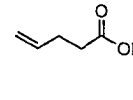
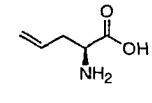
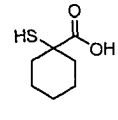
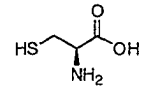
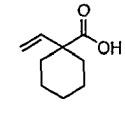
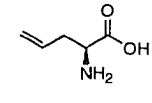


13 X-X = S-S
26a X-X = CH=CH (*cis*)
26b X-X = CH=CH (*trans*)
27 X-X = CH_2-CH_2



15 X-X = S-S
28a X-X = CH=CH (*cis*)
28b X-X = CH=CH (*trans*)
29 X-X = CH_2-CH_2

Table 1 Olefin substitutes for sulfur-containing amino acid derivatives (Figure 9).

Structure	Analog	Parent 1-residue	Parent 6-residue	Modified 1-residue	Modified 6-residue	Ring-size
11	**17** **18a** **18b**			 **10**		20
12	**19a** **19b** **20**			 **30**		21
	21a **21b** **22**					21
	23a **23b** **24**					22
	24a **24b** **25**			 **31**		22
13	**26a** **26b** **27**					20
15	**28a** **28b** **29**			 **32**		20

These compounds can then be tested for biological activity and stability in comparison to their parent peptides. This will provide a good model system to determine the effects of carbon bridges of varying degrees of saturation and conformation on biological activity. It can also stabilize these relatively short-lived compounds *in vivo*. If successful, this will be the first comprehensive study of the biological activity and stability of olefin-containing analogs of a peptide hormone in comparison to their saturated and disulfide containing congerners.

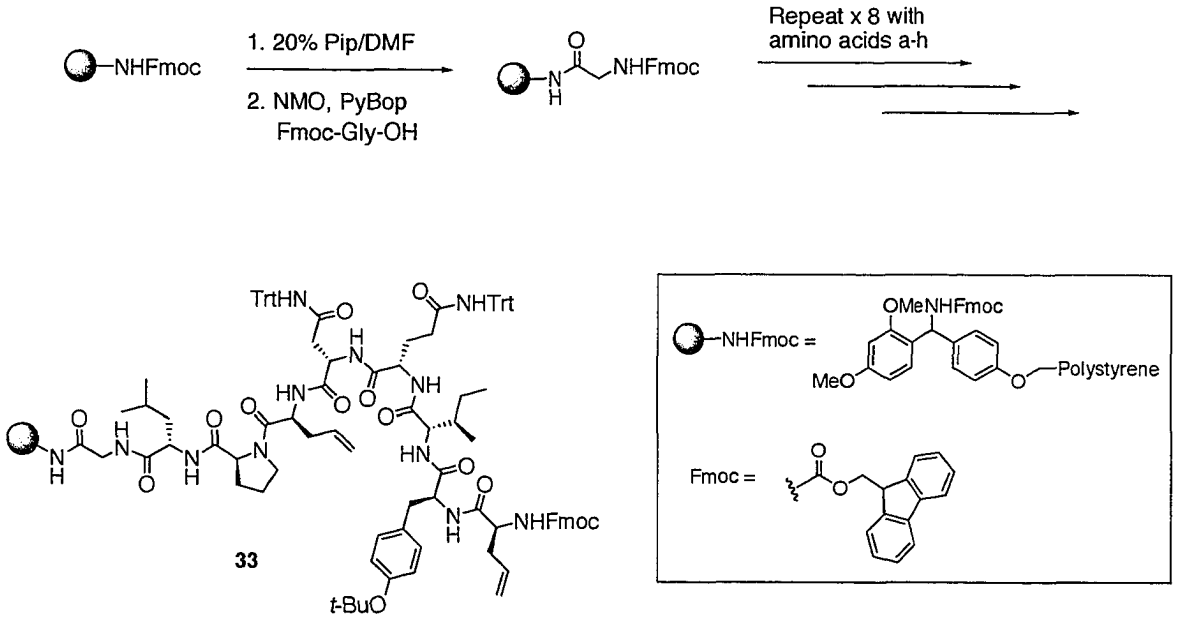
2. RESULTS & DISCUSSION (OXYTOCIN ANALOGS)

2.1 Synthesis of 1,6-Dicarba Analogs of Oxytocin (11)

Initial studies focused on the synthesis of 1,6-dicarba analogs of oxytocin using SPPS and an RCM reaction on bis-olefin containing linear precursors to probe the effects of replacement of the disulfide bridge with ethylene linkers (saturated or unsaturated) on biological activity. The synthesis (Scheme 5) involves the construction of the linear peptide backbone **33** using L-allylglycine (**10**) in place of the cysteine (**1**) residues at positions 1 and 6. Rink amide resin is used in conjunction with standard Fmoc chemistry for the coupling of the amino acid residues.[21,118] This resin was chosen so that the C-terminal carboxamide functionality is revealed on acidic cleavage. Protection of the side chains of Tyr(*O*-*t*-Bu), Asn(*N*-Trt), and Gln(*N*-Trt) is essential. The trityl (Trt) groups ensure optimal coupling by preventing tandem cyclization and dehydration of the primary amide side chains.[16] The *tert*-butyl (*t*-Bu) protection of tyrosine is necessary to avoid interference of the phenol in the RCM reaction.[44]

The resin-bound linear precursor **33** is readily cyclized using 10 mol% of Grubbs first generation catalyst **8** to give a mixture of olefinic products **34a** and **34b**.[44] Initial attempts to form the cyclic peptides **34a** and **34b** were problematic due to difficulties encountered with separating the peptides from ruthenium contaminants formed during the RCM reaction.[44] This problem is resolved by the addition of DMSO (50 eq relative to **8**) to the resin bound peptide after cyclization via RCM. This is an adaptation of a literature procedure[119] wherein DMSO was added to non-peptidic solution-phase RCM reactions to facilitate purification.

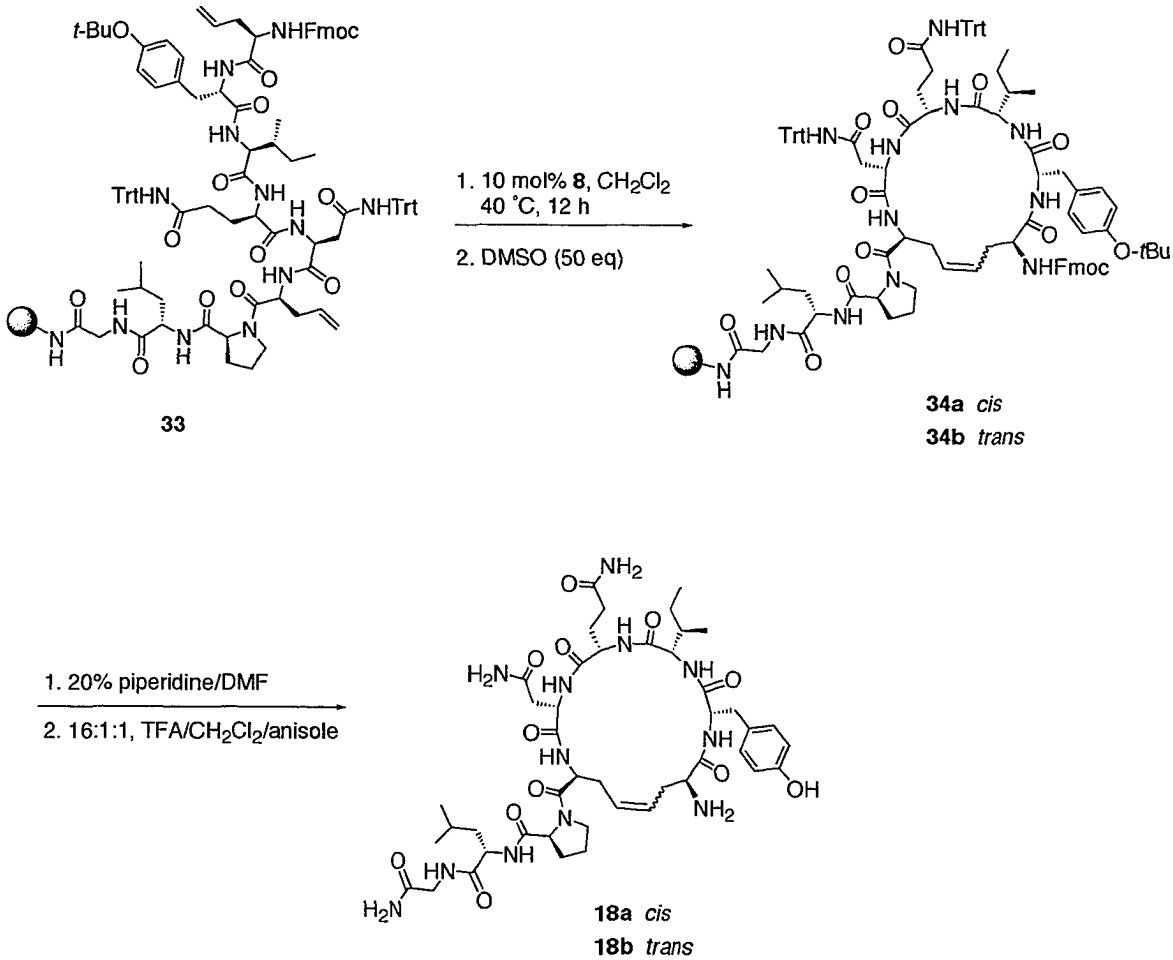
Scheme 5 Synthesis of the linear peptide backbone **33**.[a]



[a] Amino acids: (a) Fmoc-Leu-OH, (b) Fmoc-Pro-OH, (c) Fmoc-AllGly-OH (**10a**), (d) Fmoc-Asn(*N*-Trt)-OH, (e) Fmoc-Gln(*N*-Trt)-OH, (f) Fmoc-Ile-OH, (g) Fmoc-Tyr(*O*-*t*-Bu)-OH, (h) Fmoc-AllGly-OH (**10a**).

To our knowledge this is the first example of removal of Ru impurities from resin-bound peptides by treatment of DMSO,[44] and it has since become common practice.[120-123] Deprotection of the N-terminal Fmoc with piperidine followed by acidic cleavage (TFA) and concomitant side chain deprotection affords a 4:1 mixture of *cis* and *trans* isomers **18a** and **18b**, respectively (Scheme 6). In each case, the olefin-containing peptides elute prior to their linear precursors during RP-HPLC analysis.

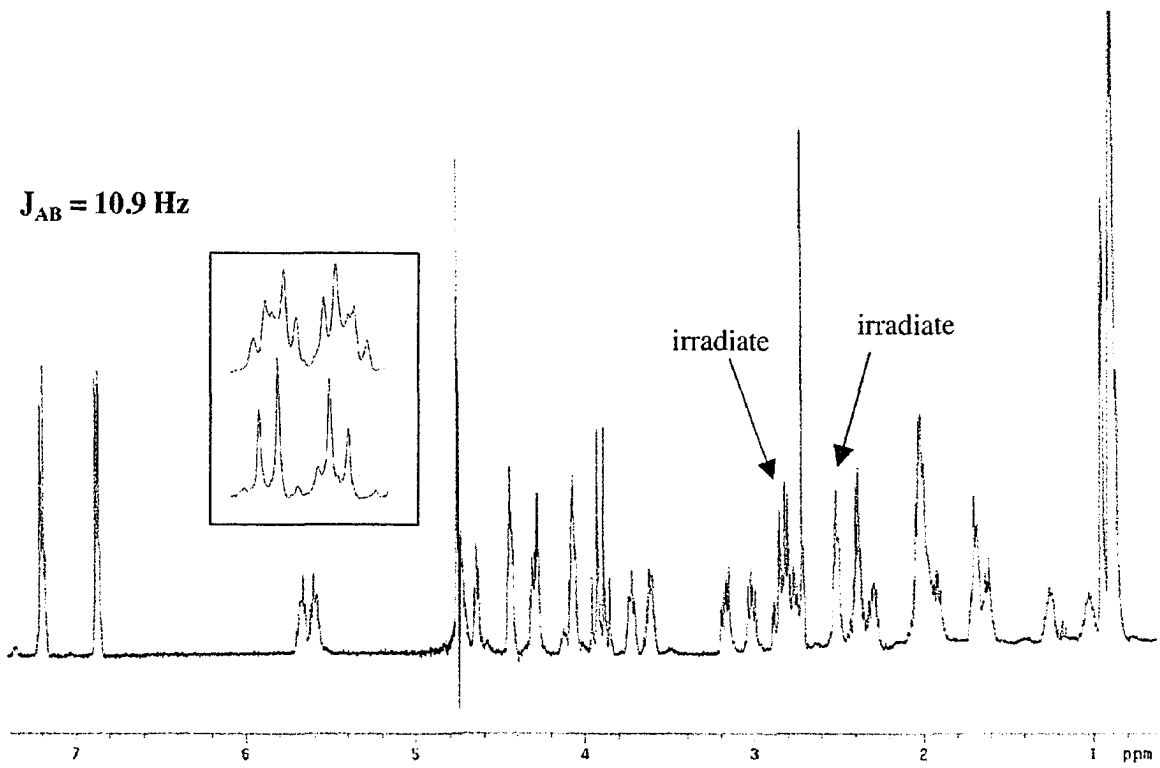
Scheme 6 Cyclization of linear precursor **33** and isolation of isomers **18a** and **18b**.



Separation of **18a** and **18b** could be accomplished using RP-HPLC to give an overall combined yield for the total synthesis of 16% from the starting resin. The double bond geometry is assigned by ^{1}H NMR spectrometry using multiple decoupling experiments in which all methylene protons adjacent to the olefin protons in **18a** and **18b** (assigned by gCOSY) are simultaneously irradiated using two frequencies. This allows for the coupling constants of the AB quartet formed by the two olefin protons in **18a** and **18b** to

be determined. The J_{AB} value for **18a** (*cis* isomer) is 10.9 Hz, where as the J_{AB} for **18b** (*trans* isomer) is 15.9 Hz (Figure 10).

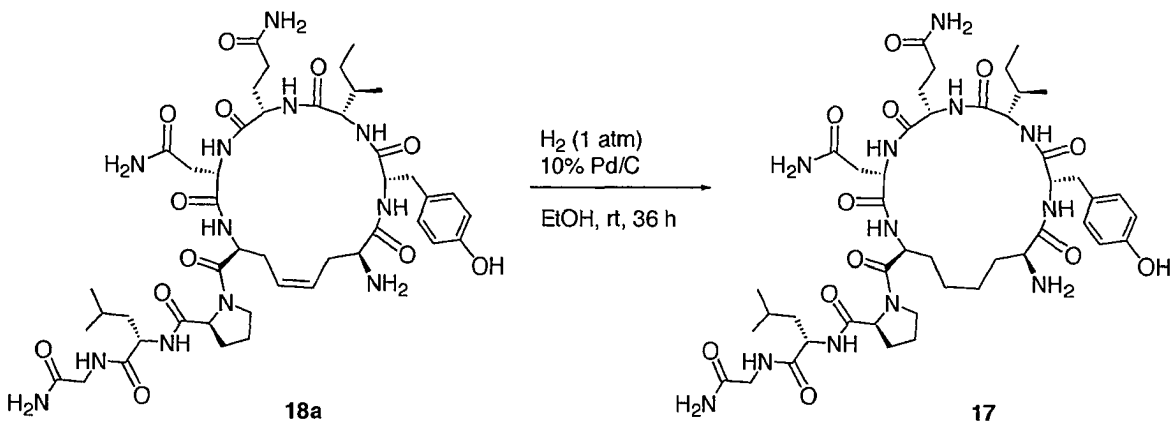
Figure 10 ^{1}H NMR double decoupling of allylic protons in **18a** to observe the AB quartet.



Reduction of the olefin moieties in **18a** and **18b** would provide access to the fully saturated 1,6-dicarba analog **17** previously synthesized in the literature via a lengthy procedure.[42] Initial attempts at reducing a mixture of *N*-Fmoc protected derivatives of **18a** and **18b** to give the reduced analog **17** were done in *N,N*-dimethylacetamide (DMAc) under an H_2 atmosphere (760 torr). However, this procedure yields only starting material.

Attempted reduction using *in situ* diimide production (2,4,6-triisopropylbenzenesulfonyl hydrazide (TPSH) and base)[36,124] yields a mixture of *trans* isomer **18b** and **17**, with selective hydrogenation of **18a** as determined by ^{1}H NMR. It was later found that the *cis* isomer **18a**, which is soluble in EtOH, is easily reduced to **17** with 10% Pd/C under H_2 at 760 torr in quantitative yield (Scheme 7).[44]

Scheme 7 Reduction of cyclic peptide olefin **18a**.

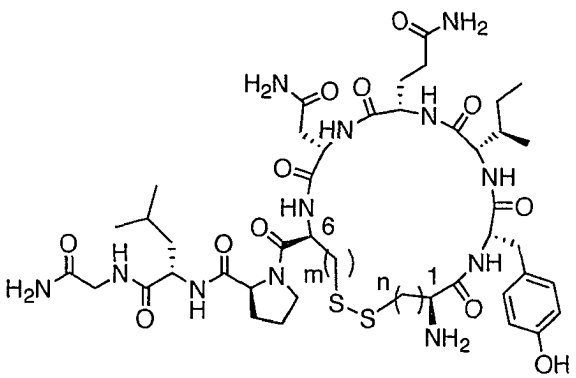


The above RCM and hydrogenation reactions allow for the separation and characterization of analogs **18a**, **18b** and **17** derived from a single cyclization reaction. The peptides are of sufficient purity (> 95% by RP-HPLC) for evaluation of their biological activities (*vide infra*) in comparison to the parent oxytocin (**11**).

2.2 Oxytocin Analogs with Carbocycles of Increased Size

With the successful synthesis of the 1,6-dicarba analogs of oxytocin using the RCM reaction, focus was placed on the synthesis of analogs containing carbocyclic rings larger

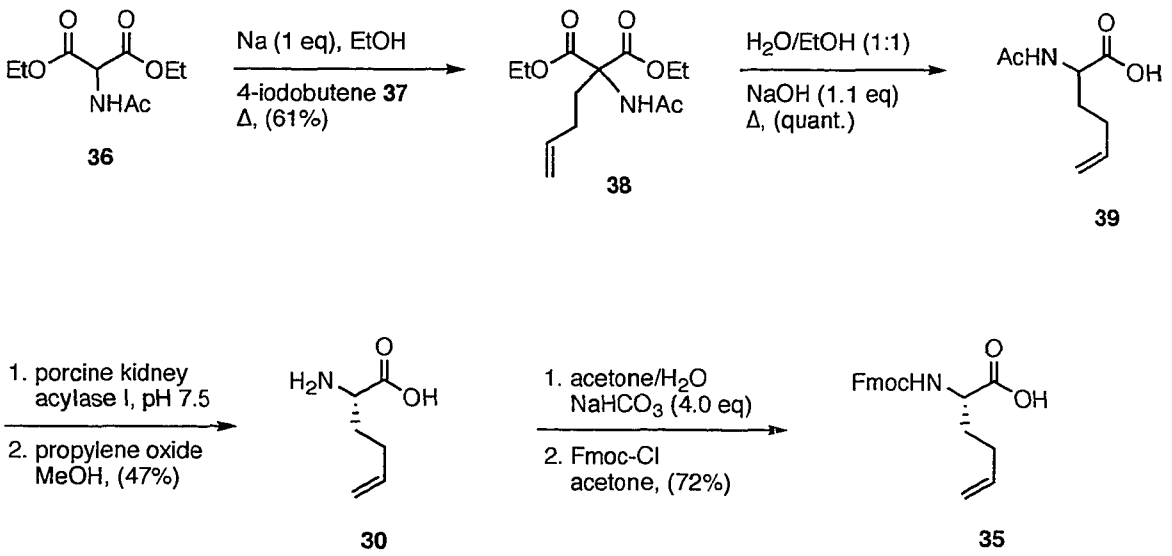
than 20 members. Such compounds are potentially available by RCM reactions of resin-bound linear peptides having two alkenyl side chains of varying length. These larger cyclic analogs would resemble the nonapeptide **12**, synthesized by Smith and Ferger,[82] which exhibited antagonistic activity and greatly reduced agonistic activity.



Antagonist **12**, m, n = 2

As mentioned earlier, the synthesis of antagonist **12** required the replacement of the cysteine (**1**) residue at positions 1 and 6 with homocysteine. As L-allylglycine (**10**) is a viable substitute for cysteine to make cyclic peptides by RCM reactions,[44] L-homoallylglycine (**30**) is a likely choice for the replacement of homocysteine. Depending on whether **30** is incorporated at position 1 or 6, the placement of the double bond in the carbocyclic analogs **19-22** could be manipulated towards either the N or C-terminus and studied for effects on activity. Incorporation of **30** at both positions 1 and 6 could potentially provide 22 membered cyclic analogs **23a**, **23b** and **24** with altered conformation in the remaining peptide portion and thereby lead to increased antagonistic activity.[82] To synthesize carbocyclic analogs of **12** using standard SPPS, known optically pure Fmoc-L-homoallylglycine (**35**) is required (Scheme 8).[125,126]

Scheme 8 Synthesis of Fmoc-L-homoallylglycine (**35**).[125,126]

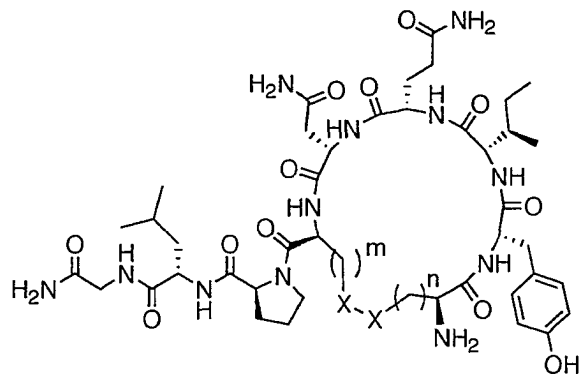


The synthesis of **35** (Scheme 8) commences with the alkylation of diethyl *N*-acetamidomalonate (**36**)[125] with 4-iodobutene (**37**)[127] to afford the olefin adduct (**38**)[125] in 61% yield over two steps. The commercially available 4-bromobutene is not sufficiently reactive for this transformation, but it is easily converted to the more reactive iodide under Finkelstein conditions. Hydrolysis of **38** followed by decarboxylation leads to racemic N-acetylhomoallylglycine (**39**).[125] Enzymatic resolution of this racemic mixture is achieved using porcine kidney acylase I, which selectively deacetylates the natural "L" isomer of **39** to give pure L-homoallylglycine (**30**) in 47% yield.[125,126] The optical rotation of **30** was compared to the known literature value, and shown to match.[125] Fmoc protection of **30** yields Fmoc-L-homoallylglycine (**35**) in 72% for use in Fmoc SPPS. This procedure allows for the synthesis of multi-gram quantities of optically pure **35** in a single reaction sequence.

It was uncertain whether a variety of larger rings would be available via the RCM process because the conformational preferences of the precursors could hinder the cyclization reaction. However, the syntheses of analogs **19a**, **19b**, **21a**, **21b**, **22a** and **22b** (Table 2), containing rings larger than oxytocin (**11**) (>20 members) proceed in the same fashion as for dicarba analogs **17**, **18a** and **18b** (Scheme 6). For 21 membered carbocycles **19-21** (Table 2), the syntheses employed SPPS of linear precursors using Fmoc methodology with incorporation of Fmoc-L-homoallylglycine (**35**) at either positions 1 or 6, with Fmoc-L-allylglycine (**10a**) added to the opposing position. Incorporation of **35** at both residues 1 and 6 generates the linear precursors to analogs **22a**, **22b** and **23** (Table 2). RCM reaction on the linear precursors using **8** or **9** followed by DMSO treatment to remove ruthenium contaminants and acidic cleavage (TFA) from the resin gives the cyclic peptides in yields of 6-23% (after purification by RP-HPLC). The lower yields can be attributed to cyclizations with the less active Grubbs first generation catalyst **8**, which was available commercially prior to the more reactive, second generation catalyst **9**. The *cis/trans* isomers of all but one set of olefin-containing peptides, **19a** and **19b**, are readily separated by HPLC. In each case, the olefinic peptides elute prior to their linear precursors during RP-HPLC analysis.

The assignment of *cis* and *trans* configuration is accomplished using the same simultaneous ^{1}H NMR double decoupling as used for compounds **18a** and **18b**. The coupling constants of the olefin protons for all but the *trans* isomer **22b** are readily measurable, and are 8.5-10.8 Hz for the *cis* isomers and 15.4-15.6 Hz for the *trans* isomers.

Table 2 Carbocyclic analogs with larger ring sizes (>20 members).



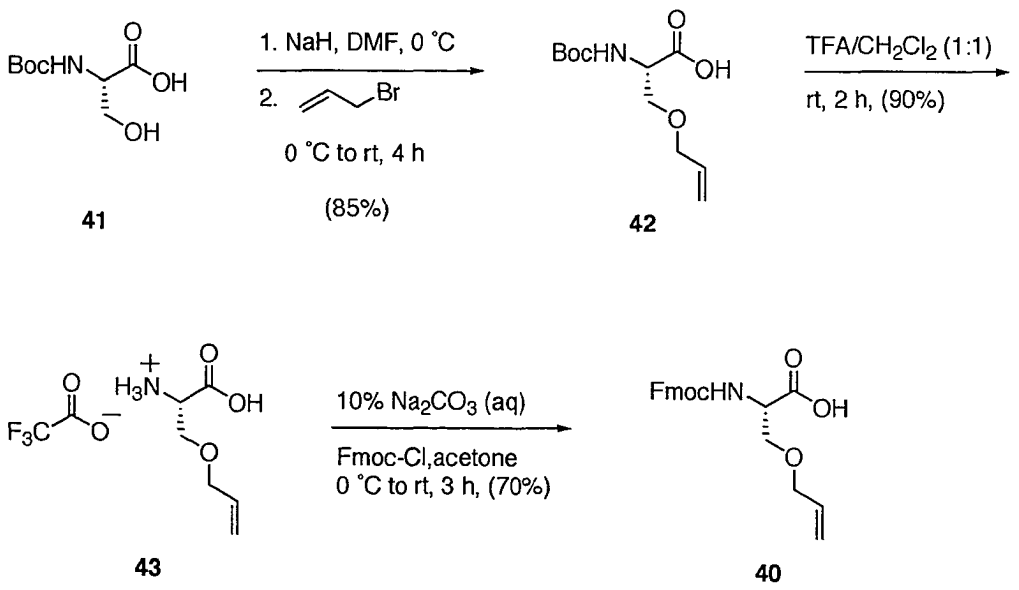
Analog	X-X	m	n	J_{AB} (Hz)	yield (%)	MS ([a]MALDI, [b]ES)
19 (a&b) (2:1 *cis/trans*)	CH=CH	1	2	10.8 (*cis*) 15.4 (*trans*)	8	(M+H) = 984[a]
20	CH_2CH_2	1	2	-	quant.	(M+H) = 986[b]
21a (*cis*)	CH=CH	2	1	8.5	10	(M+Na) = 1006[a]
21b (*trans*)	CH=CH	2	1	15.6	6	(M+Na) = 1006[a]
22a (*cis*)	CH=CH	2	2	9.3	8	(M+Na) = 1020[b]
22b (*trans*)	CH=CH	2	2	-	23	(M+Na) = 1020[b]
23	CH_2CH_2	2	2	-	quant.	(M+H) = 1000[b]

Reduction of **19** and **22a** using the same standard hydrogenation conditions employed for **18a** (Scheme 7) affords the fully saturated analogs **20** and **23**, respectively, in quantitative yield.

Analogs with an oxygen in the ring were investigated to examine its influence on biological activity. Oxygen can alter conformation due to its C-O-C bond lengths (1.41

Å)[128] and angles (111°-124°),[128] and can potentially act as a hydrogen bond acceptor within the OTR site. Linear precursors to analogs of oxytocin (**11**) containing oxygen within the cycle can be prepared by incorporation of L-*O*-allylserine (**30**) at position 1 and L-allylglycine at position 6. To accomplish this by standard Fmoc SPPS the known serine derivative, Fmoc-L-*O*-allylserine (**40**)[129] is required (Scheme 9).

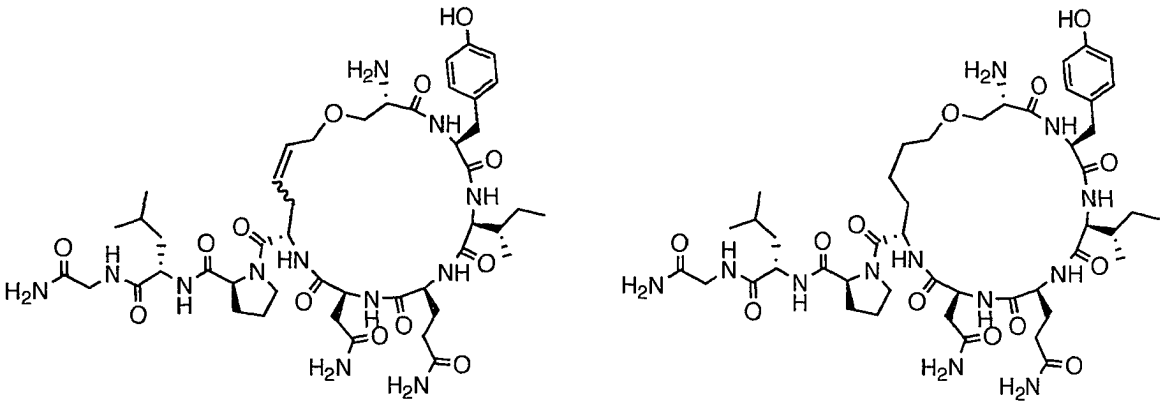
Scheme 9 Synthesis of Fmoc-L-*O*-Allylserine (**40**).[129]



This synthesis (Scheme 9) first entails the alkylation of *tert*-butoxycarbonyl (Boc) protected L-serine (**41**), under basic conditions with allyl bromide to give the *O*-alkylated product **42** in 85% yield. Deprotection of **42** under acidic conditions gives the TFA salt of L-*O*-allylserine (**43**) (90%), which is then protected as its *N*-Fmoc derivative **40** in 70% yield. Incorporation of **40** at position 1 and Fmoc-L-allylglycine at position 6 using standard SPPS methodology affords the linear precursors to oxygen containing carbocycles **24a** and **24b**. Cyclization with Grubbs second generation catalyst **9** followed

by standard DMSO workup[44] yields 22-membered carbocycles in a 1:1 mixture of *cis* **24a** and *trans* **24b** (J_{AB} = 15.9 Hz) isomers (Figure 11). Analogs **24a** and **24b** could be separated as their *N*-Fmoc protected precursors. Deprotection with piperidine then affords the pure olefins **24a** and **24b** (ca 3% overall). Due to this low yielding synthesis, insufficient material was available for their reduction to **25**.

Figure 11 Cyclic peptide analogs **24a** and **24b** and reduced analog **25**.

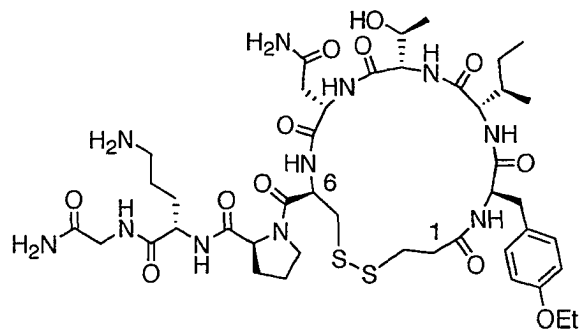


24a *cis*
24b *trans*

25

2.3 1,6-Dicarba Analogs of the Antagonist Atosiban (13)

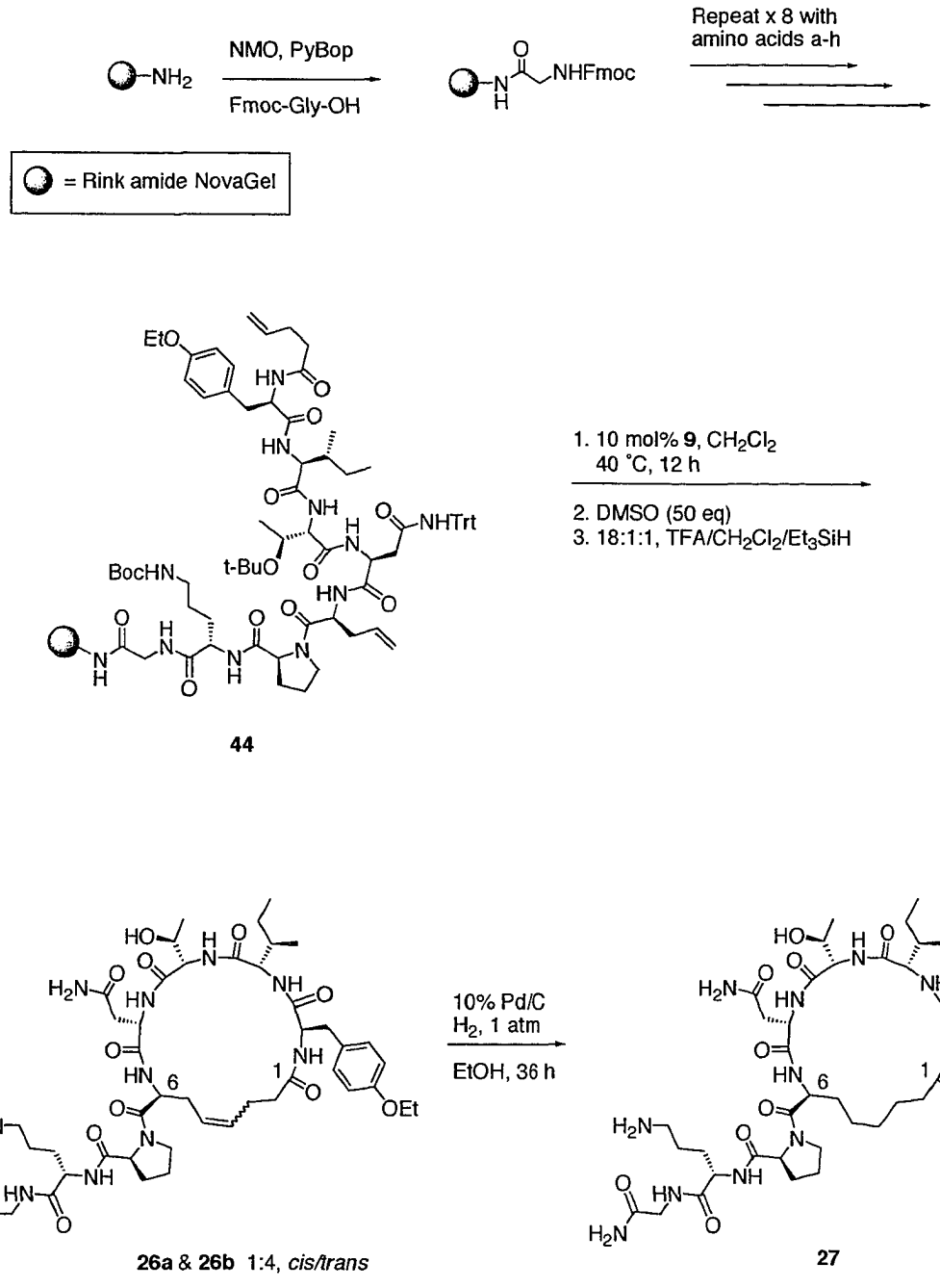
The synthesis of 1,6-dicarba analogs of atosiban (**13**) relies on analogous methods to those discussed for the carbocyclic compounds. The salient features of **13** are that position 2 contains a commercially available D-Tyr(*O*-Et) residue.



Atosiban (**13**)

Position 1 contains 3-mercaptopropionic acid (Mpa), which can be replaced with commercially available 4-pentenoic acid. L-Allylglycine is placed at position 6 to provide the other half of the bis-olefin moiety required for the RCM reaction. Fmoc SPPS yields a linear resin bound precursor **44** that could be cyclized with catalyst **9**. Subsequent cleavage from the resin under acidic conditions (TFA) gives analogs **26a** and **26b** as a 1:4 mixture of *cis/trans* isomers in 21% overall yield (Scheme 10). Various attempts at the separation of these isomers using RP-HPLC proved unfeasible, and the two-component mixture **26** was tested directly. Reduction of mixture **26** gives a fully saturated dicarba analog **27** in 55% isolated yield (quantitative by HPLC and ^{1}H NMR), which was also tested for antagonistic activity.

Scheme 10 Synthesis of 1,6-dicarba atosiban analogs **26a**, **26b** and **27**.[a]



44

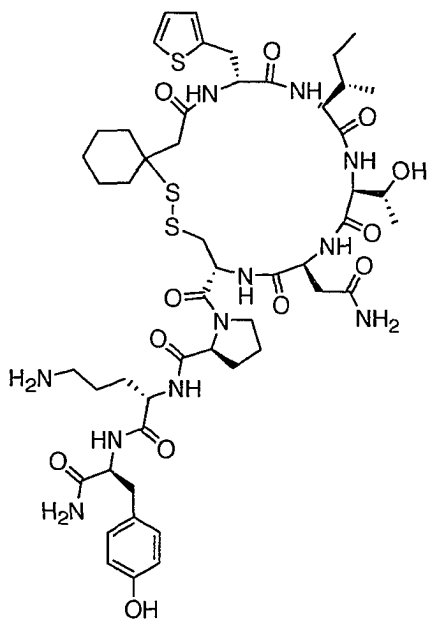
26a & **26b** 1:4, *cis/trans*

27

[a] Amino acids: (a) Fmoc-Orn(*N*-Boc)-OH, (b) Fmoc-Pro-OH, (c) Fmoc-AllGly-OH (**10a**), (d) Fmoc-Asn(*N*-Trt)-OH, (e) Fmoc-Thr(*O*-*t*-Bu)-OH, (f) Fmoc-Ile-OH, (g) Fmoc-D-Tyr(*O*-Et)-OH, (h) 4-Pentenoic acid.

2.4 Synthesis of 1,6-Dicarba Analogs of the Antagonist 15

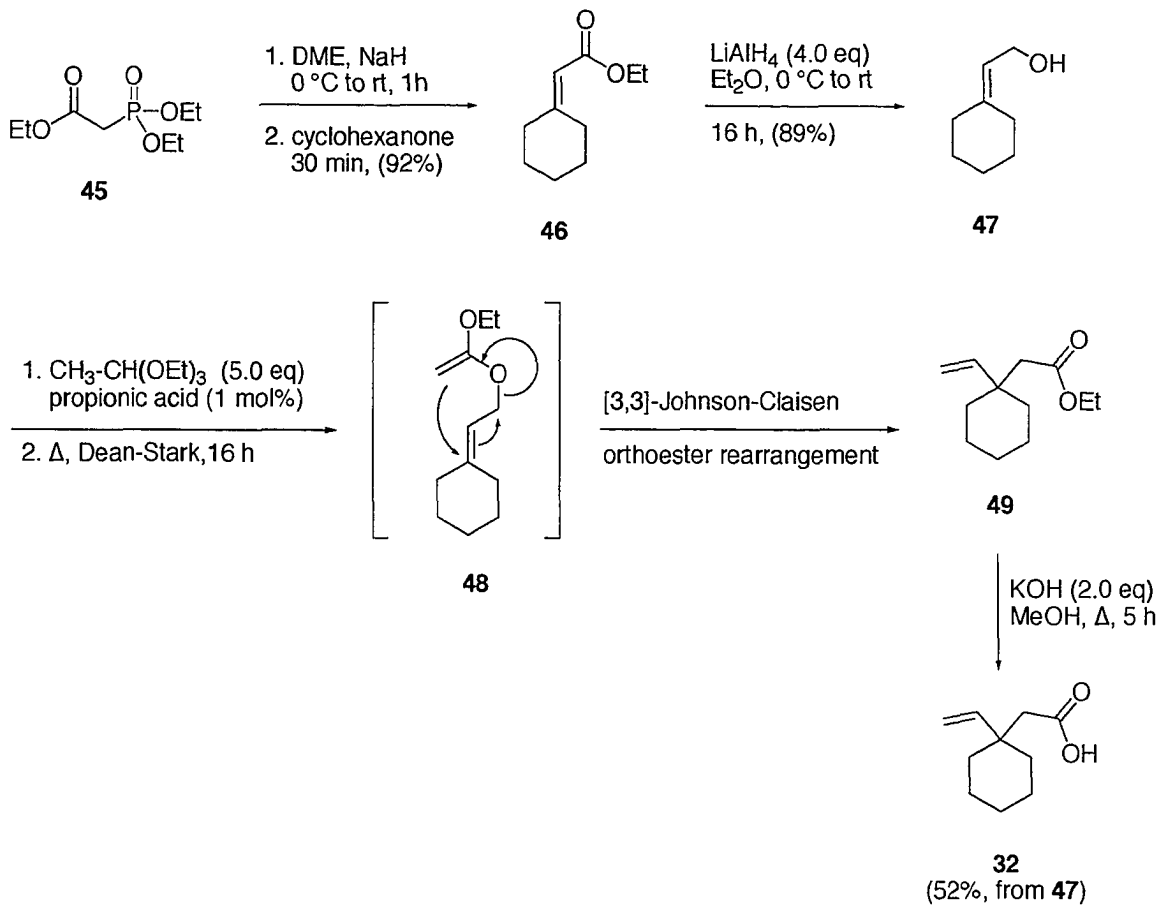
With the successful synthesis of the above carbocyclic peptide analogs using RCM reactions, it seemed that a similar approach would provide access to a 1,6-dicarba analog of antagonist **15**. The salient feature of antagonist **15** is that position 1 contains a bulky 1-(3-mercapto-3,3-cyclopentamethylene)propionic acid moiety, which is thought to invoke superior selectivity for the oxytocin (OTR) versus vasopressin receptors (VPR).[97]



Antagonist **15**

Synthesis of a linear precursor to a dicarba analog of **15** would require the incorporation of 1-(vinylcyclohexyl)-1-acetic acid (Vca)[130] (**32**) (Scheme 11) at position 1 and L-allylglycine (**10**) at position 6 during peptide synthesis. A known synthetic pathway[130] was chosen for production of **32**, as Lewis acid mediated reactions of organocopper reagents with **46** yield none of the desired ester product **49**.[131]

Scheme 11 Synthesis of 1-(vinylcyclohexyl)-1-acetic acid (**32**).[130]



45 **46** **47** **48** **49**

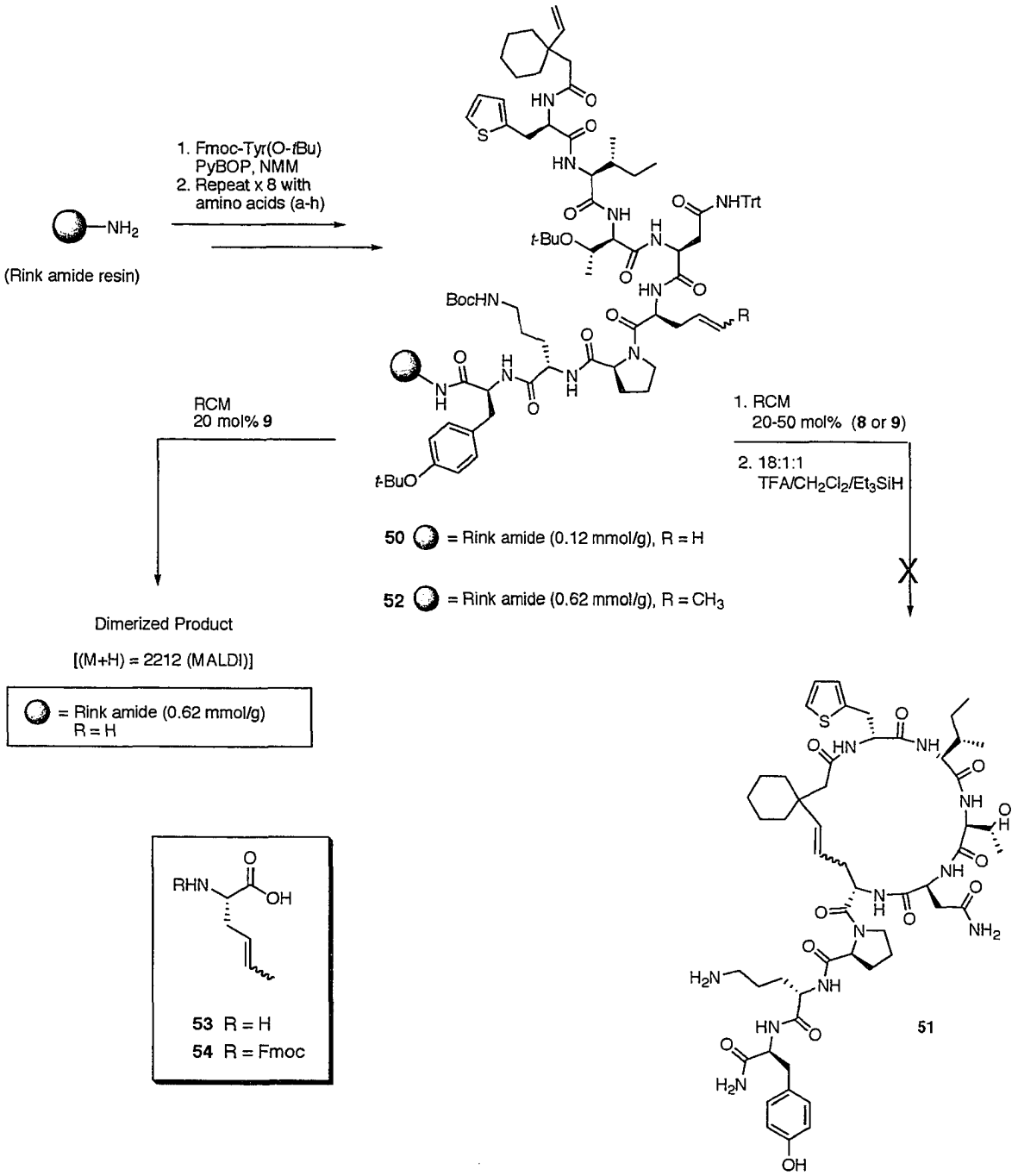
32
(52%, from **47**)

The synthesis of 1-(vinylcyclohexyl)-1-acetic acid (**32**) (Scheme 11)[130] is initiated with a Horner-Emmons-Wadsworth reaction between commercially available phosphonate **45** and cyclohexanone. This reaction yields the known α,β-unsaturated ester **46**[130] in high yield (92%), which is readily reduced to the allylic alcohol **47** with $LiAlH_4$ (89%).[130] Treatment of **47** with triethyl orthoacetate in the presence of propionic acid, with concomitant removal of EtOH using Dean-Stark conditions, affords the ester **49**,[130] via a [3,3] rearrangement of the Johnson-Claisen intermediate **48**. The ester **49** is readily

hydrolyzed to **32** (52% overall from **47**).[130] It is important to note that all precursors to **32** can be used without purification during the reaction sequence.

Synthesis of linear precursor **50** (Scheme 12) with 1-(vinylcyclohexyl)-1-acetic acid (**32**) at position 1 and L-allylglycine (**10**) at position 6 using standard SPPS on Rink amide resin (0.6 mmol/g) proceeds smoothly. However, the RCM reaction of this resin-bound precursor **50** under a variety of conditions generated no cyclized derivative **51**, but instead gave dimerized product (Scheme 12). It appears that this dimerization occurs between L-allylglycine residues of neighboring, resin-bound, linear precursors due to the steric bulk of the 1-(vinylcyclohexyl)-1-acetic acid residue. To counteract this, a linear precursor **52** having L-crotylglycine (**53**) at position 6 was synthesized so as to provide additional steric bulk to the olefin terminus and thus prevent dimerization.[126,132] L-Crotylglycine **53** was incorporated into linear precursor **52** as its N-Fmoc derivative **54** using standard SPPS. Fmoc-L-crotylglycine (**54**) was prepared in an analogous manner to Fmoc-L-homoallylglycine **35** (Scheme 8).[125,133] The enantiomeric purity was not determined for **54** as it was isolated as a 1:4 *cis/trans* mixture of isomers, and the "L" configuration was assumed based on the selectivity of the acylase I enzyme for the 2S geometry.[126] Attempted RCM reaction on linear precursor **52** yielded no detectable metathesis products by MS. The linear precursor **50** was also synthesized on Rink amide resin with a much lower loading (0.12 mmol/g) to achieve greater dilution and thereby retard the dimerization process. Unfortunately, like **52**, this also yielded no metathesis products under RCM conditions (Scheme 12).

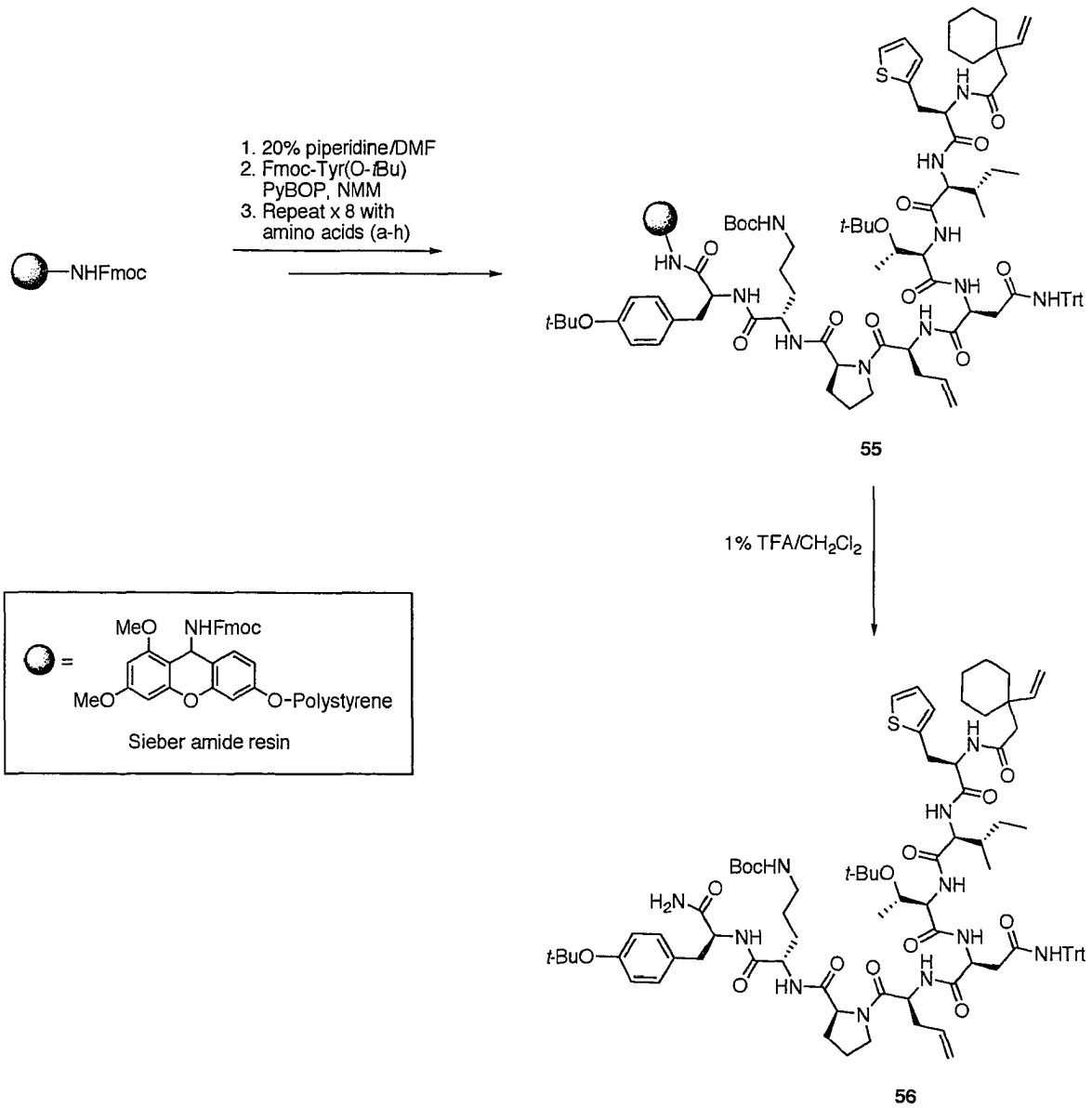
Scheme 12 Attempted synthesis of a 1,6-dicarba analog of **15**.[a]



[a] Amino acids: (a) Fmoc-Orn(*N*-Boc)-OH, (b) Fmoc-Pro-OH, (c) Fmoc-AllGly-OH (**10a**), or Fmoc-L-crotylglycine (**54**) (d) Fmoc-Asn(*N*-Trt)-OH, (e) Fmoc-Thr(*O*-*t*-Bu)-OH, (f) Fmoc-Ile-OH, (g) Fmoc-D-Thi-OH, (h) 1-(Vinylcyclohexyl)-1-acetic acid (**32**).

A linear precursor was also synthesized on Sieber amide resin to permit cleavage from the resin under mild conditions (1% TFA) without side-chain deprotection (Scheme 13).[16]

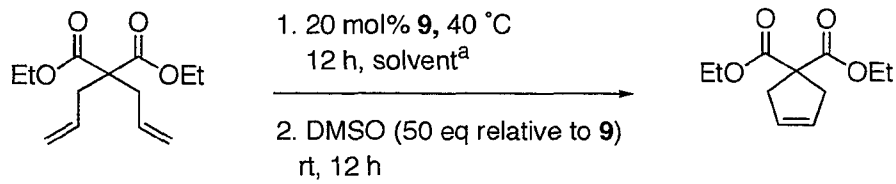
Scheme 13 Synthesis of free linear peptide precursor **56**.[a]



[a] Amino acids: (a) Fmoc-Orn(*N*-Boc)-OH, (b) Fmoc-Pro-OH, (c) Fmoc-AllGly-OH (**10a**), or Fmoc-L-crotylglycine (**54**) (d) Fmoc-Asn(*N*-Trt)-OH, (e) Fmoc-Thr(*O*-*t*-Bu)-OH, (f) Fmoc-Ile-OH, (g) Fmoc-D-Thi-OH, (h) 1-(Vinylcyclohexyl)-1-acetic acid (**32**).

The synthesis of a resin-bound linear precursor **55** (Scheme 13) on Sieber amide resin (0.6 mmol/g) using standard Fmoc SPPS affords the fully protected linear precursor **56** (21%) after cleavage from the resin (1% TFA). Initial attempts to perform an RCM reaction on the linear precursor **56** were hindered by its insolubility in all solvent systems commonly used for such transformations.[134] A model system, diethyl diallylmalonate (**57**)[135] could be used to screen various solvent systems for their compatibility with the RCM methodology. To solubilize **56**, polar organic solvents were studied, such as DMF and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU), in mixtures with CH_2Cl_2 (Table 3). In all cases CH_2Cl_2 was required to solubilize the Grubbs second generation catalyst **9**.

Table 3 Optimization of the RCM reaction using various solvent systems.



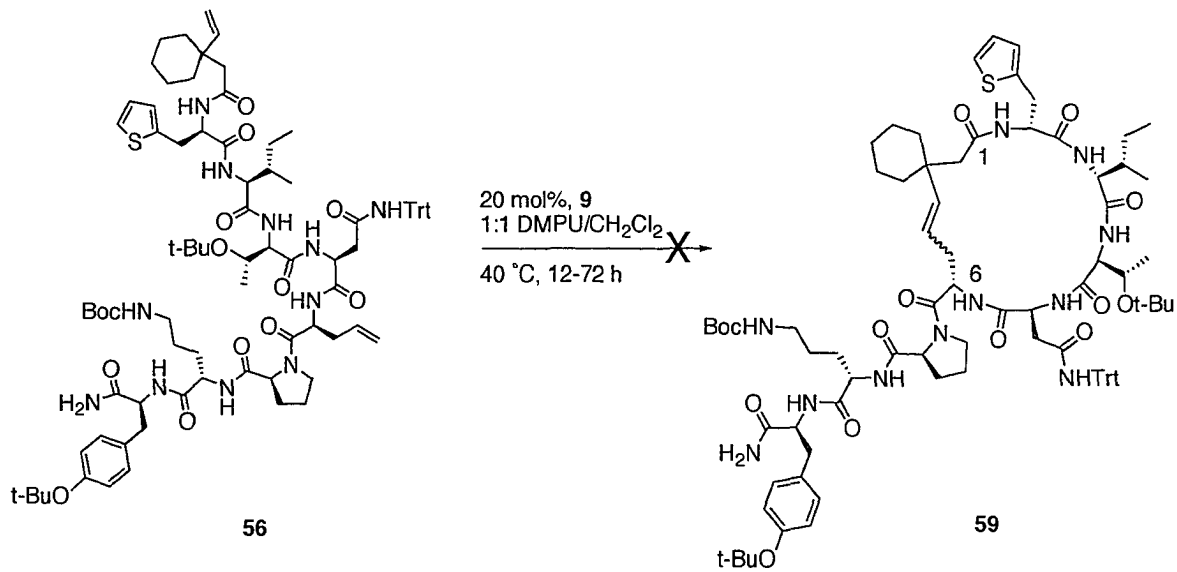
57 **58**

Solvent System	Composition	% Conversion (^{1}H-NMR)	Isolated Yield (%)
A	CH_2Cl_2	100	92
B	DMF/CH_2Cl_2 (1:1)	25	-
C	DMF/CH_2Cl_2 (1:5)	>99	85
D	DMPU/CH_2Cl_2 (1:1)	92	-

Solvent systems C and D are compatible with the RCM reaction. However, protected peptide **56** is not soluble in C, and so solvent system D was employed. The RCM reaction

using solvent system D was attempted on **56** and was monitored by ES/MS every 12 h for 72 h. No metathesis product **59** was detectable by MALDI-TOF (Scheme 14). The failure of the RCM reaction on both the resin-bound and free linear precursors is presumably a result of steric crowding caused by the cyclohexyl group, which inhibits intramolecular cyclization by the bulky organometallic reagent **9**.

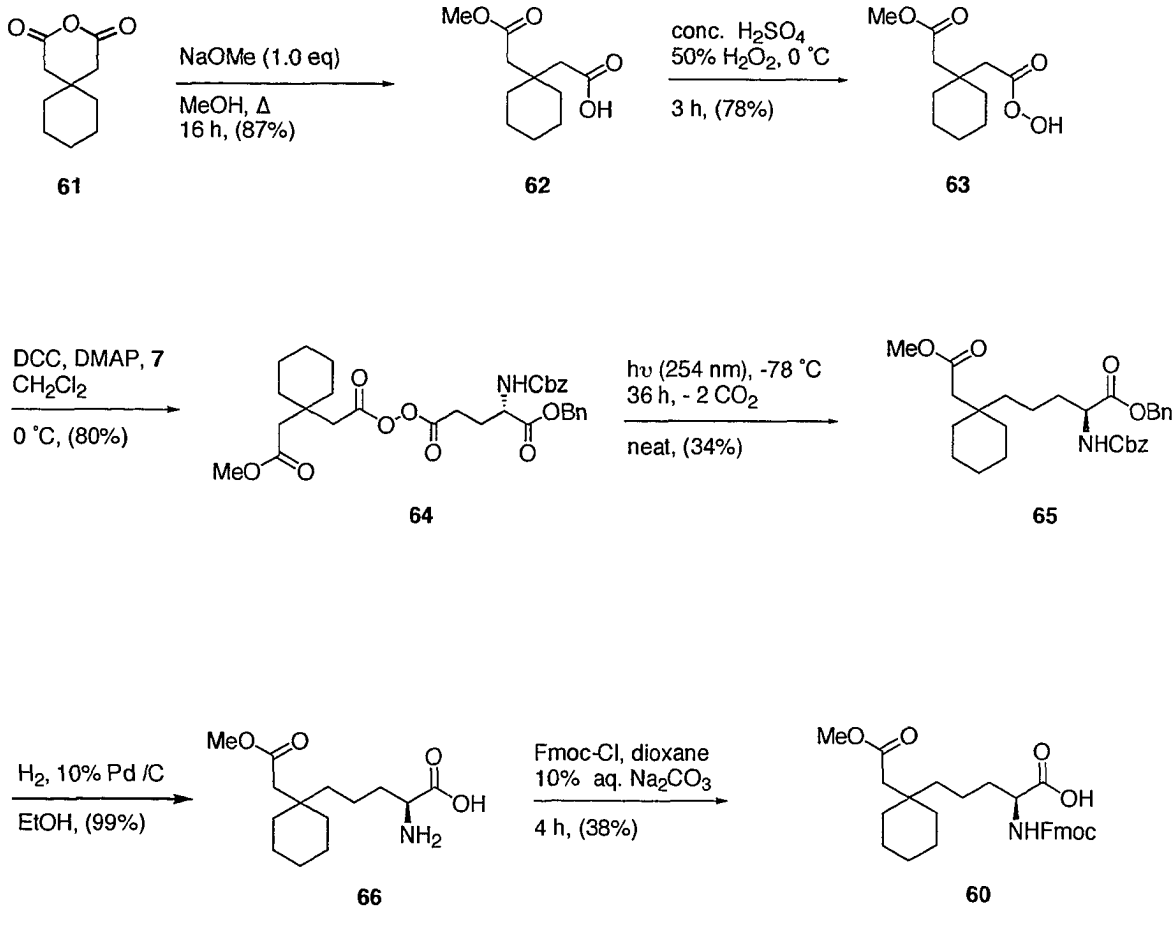
Scheme 14 Attempted solution-phase RCM reaction of linear peptide precursor **56**.



To overcome the difficulty of making a carbocyclic analog of antagonist **15** via RCM, the strategy was altered to a more classical approach. This involves the incorporation of a preformed carbon bridge during SPPS by means of an orthogonally protected, cyclohexyl L-α-aminosuberic acid derivative **60** (Scheme 15). Cyclization using intramolecular amide bond formation would be the ring-closing step. The required L-α-aminosuberic acid analog **60** is readily made as shown in Scheme 15. The key step, photolytic

decomposition of a diacyl peroxide at low temperature (Scheme 2), is based on a novel methodology recently reported by this group.[54,55] This allows for facile synthesis of amino acid derivatives from two carboxylic acids without the production of crossover products.

Scheme 15 Synthesis of α-aminosuberic analog **60**.

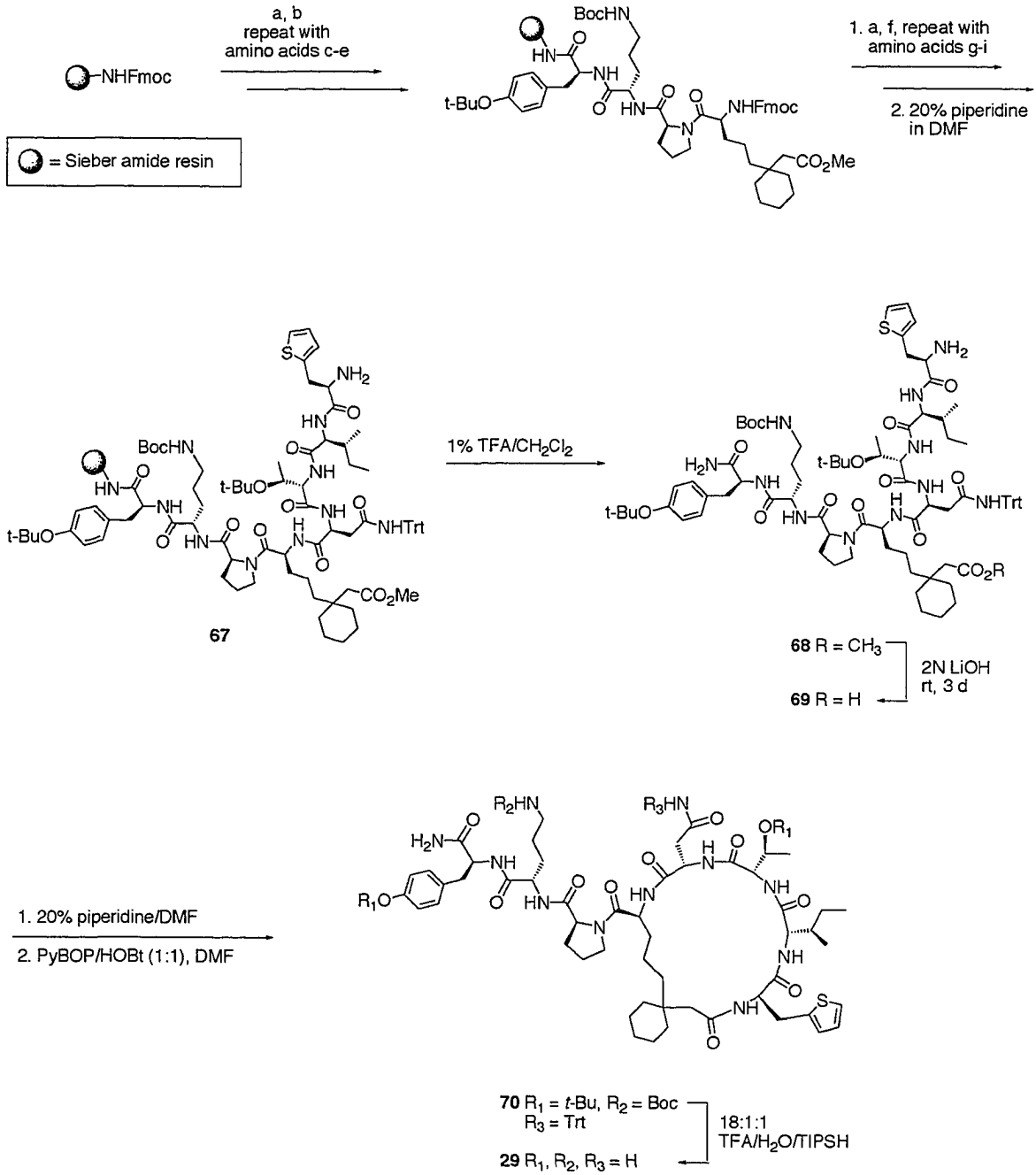


The synthesis of **60** (Scheme 15) is initiated by opening of the known anhydride **61**[136] to the monoester **62** in 87% yield using sodium methoxide in refluxing MeOH. Ester **62** reacts with 50% aqueous H_2O_2 in the presence of strong acid (H_2SO_4) to give the peracid **63** in 78% yield. Coupling of peracid **63** with *N*-carboxybenzyl-L-glutamic acid 1-benzyl

ester (*N*-Cbz-Glu-OBn) (**7**) using DCC produces the corresponding diacyl peroxide **64** in 80% yield. The Cbz and *O*-Bn protecting groups are compatible with the photolysis reaction.[54,55] Previous attempts at photolysis of diacyl peroxides with Fmoc protection has been shown to yield no coupling products.[55] Photolysis of neat **64** at –78 °C with UV light at 254 nm for 48 h yields the desired asymmetrically coupled product **65** in 34% yield, with recovery of 36% of **64**, which could be reacted again. The photolysis of a neat thin film of **64** suppresses both crossover products and unwanted side reactions with solvents, common occurrences in solution phase photolysis of diacyl peroxides.[54,55] However, the low yield of this reaction and recovery of starting material suggests that optimization of this reaction is still required. Quantitative deprotection of **65** by hydrogenolysis gives free amine **66**, which is protected as its *N*-Fmoc derivative **60** for use in SPPS. Unfortunately, the yield of this last step is low, possibly because of the lack of solubility of free amine **66** in dioxane/aqueous $NaHCO_3$. However, recovered **66** can be reacted again to generate additional product. Fmoc protection of **66** using *N*-(9*H*-fluorenylmethoxycarbonyloxy)succinimide (Fmoc-Su) in DMF could potentially solve this solubility problem in future applications.[137]

The orthogonally protected α-aminosuberic analog **60** could then be incorporated in the synthesis of a linear precursor **67** on Sieber amide resin (0.6 mmol/g) using standard SPPS (Scheme 16). Peptide **67** is cleaved from the resin using mild conditions (1% TFA) to yield the fully protected peptide methyl ester **68**.

Scheme 16 Synthesis of 1,6-dicarba analog **29**.[a]

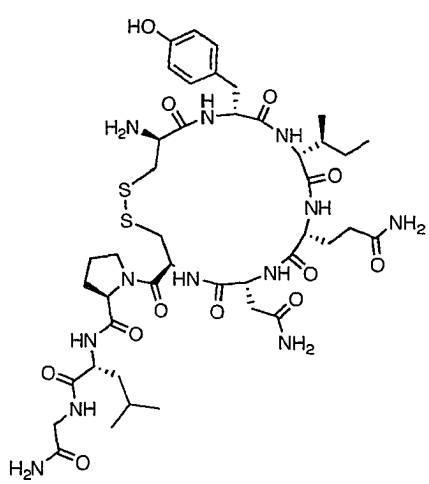


[a] Conditions: (a) PyBOP, NMM, DMF, (b) Fmoc-Tyr(*O*-*t*-Bu)-OH, (c) Fmoc-Orn(*N*-Boc)-OH, (d) Fmoc-Pro-OH, (e) **60**, (f) Fmoc-Asn(*N*-Trt)-OH, (g) Fmoc-Thr(*O*-*t*-Bu)-OH, (h) Fmoc-Ile-OH, (i) Fmoc-D-Thi-OH.

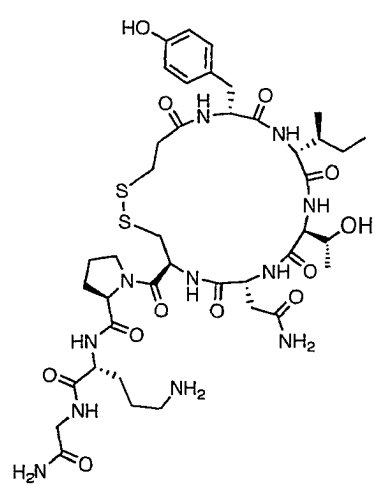
Hydrolysis of **68** is accomplished using 2N LiOH to yield peptide acid **69**. This reaction proceeds to only 59% completion after stirring for 3 d at 20 °C. However, attempts to increase the yield by heating at 50 °C for 3 h leads to decomposition of the peptide (HPLC). Intramolecular cyclization of the peptide acid **69** is done using standard amide bond formation techniques (PyBOP/HOBt/DIPEA).[46] This gives the carbocycle **70** in 29% yield after HPLC. Treatment of **70** with 95% TFA removes all side chain protecting groups and affords the dicarba analog **29** in 76% yield after RP-HPLC purification (Scheme 16).

2.5 Standards for Biological Testing: Oxytocin (11), Atosiban (13) and Antagonist 15

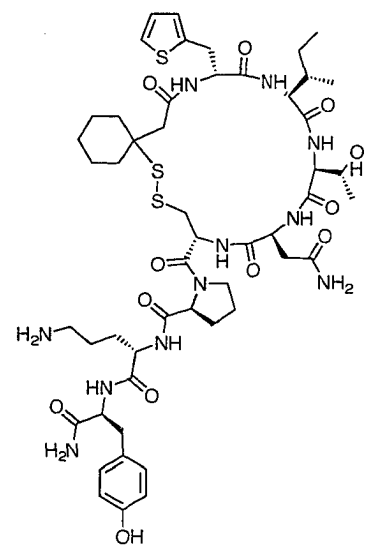
The agonistic behaviors of the synthesized carbocycles can be compared to commercially available oxytocin (**11**). Antagonistic behavior of the analogs was contrasted to either atosiban (**13**) or the antagonist **15**.



Oxytocin (**11**)



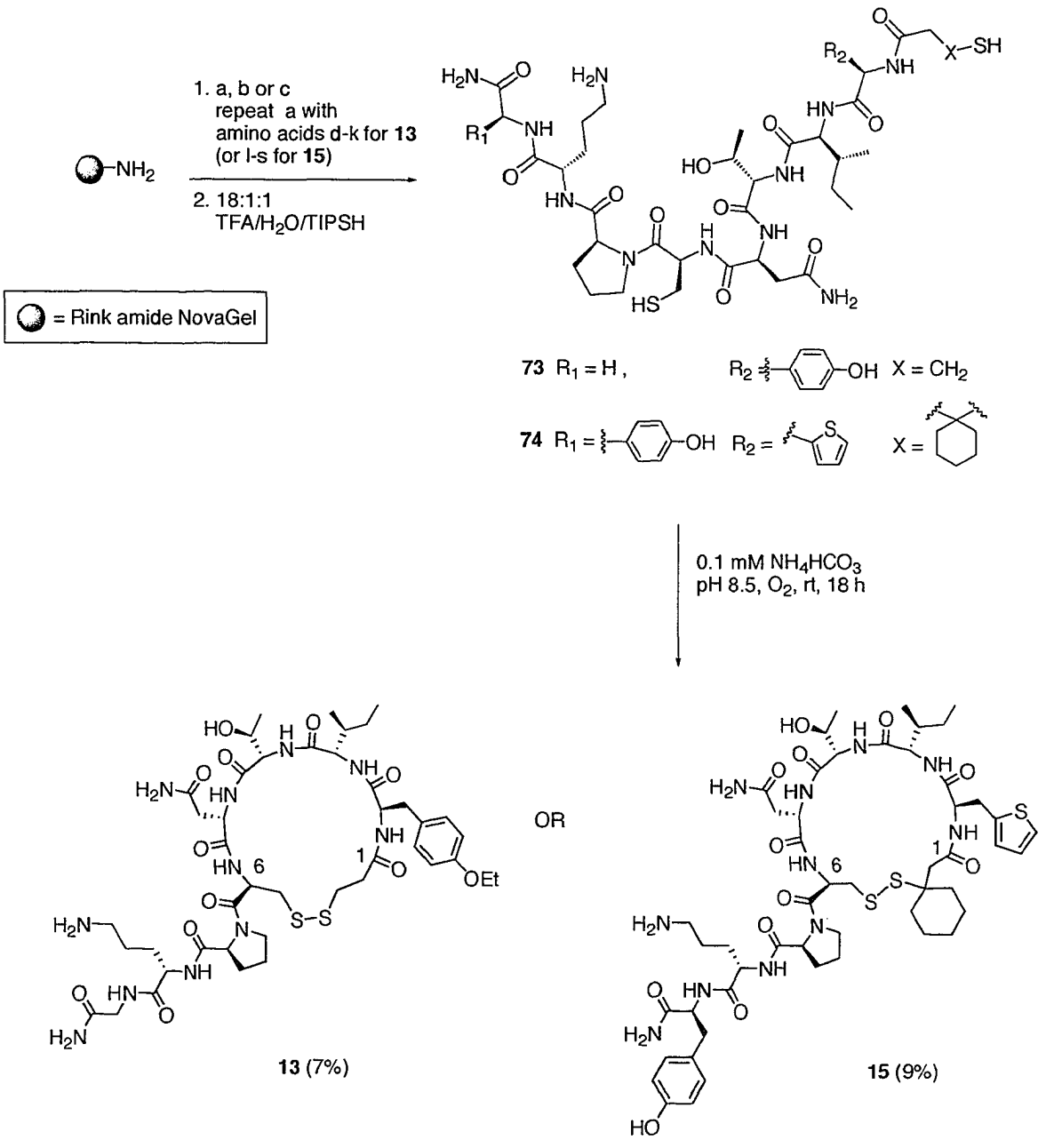
Atosiban (**13**)



Antagonist **15**

The antagonists **13** and **15** are not readily available commercially, and therefore were synthesized using standard Fmoc SPPS (Scheme 17).

Scheme 17 Synthesis of atosiban (**13**) and antagonist **15**.[a]



[a] Conditions: (a) PyBOP, NMM, DMF, (b) Fmoc-Gly-OH, (c) Fmoc-Tyr(*O*-*t*-Bu)-OH, (d) Fmoc-Orn(*N*-Boc)-OH, (e) Fmoc-Pro-OH, (f) Fmoc-Cys(*S*-Trt)-OH, (g) Fmoc-Asn(*N*-Trt)-OH, (h) Fmoc-Thr(*O*-*t*-Bu)-OH, (i) Fmoc-Ile-OH, (j) Fmoc-D-Tyr(*O*-Et)-OH, (k) (*S*-Trt)-3-mercaptopropionic acid (**71**), (l) Fmoc-Orn(*N*-Boc)-OH, (m) Fmoc-Pro-OH, (n) Fmoc-Cys(*S*-Trt)-OH, (o) Fmoc-Asn(*N*-Trt)-OH, (p) Fmoc-Thr(*O*-*t*-Bu)-OH, (q) Fmoc-Ile-OH, (r) Fmoc-D-Thi-OH, (s) (*S*-PMB)-3-mercapto-3,3-cyclopentamethylene-propionic acid (**72**).

The *S*-trityl protected 3-mercaptopropionic acid (**71**)[138] and commercially available *S*-para-methoxybenzyl (PMB) protected 3-mercapto-3,3-cyclopentamethylenepropionic acid (**72**) are used to introduce the residues at position 1 for both peptides **13** and **15** to allow ease of protecting group removal upon acidic cleavage of the peptides from the resin.

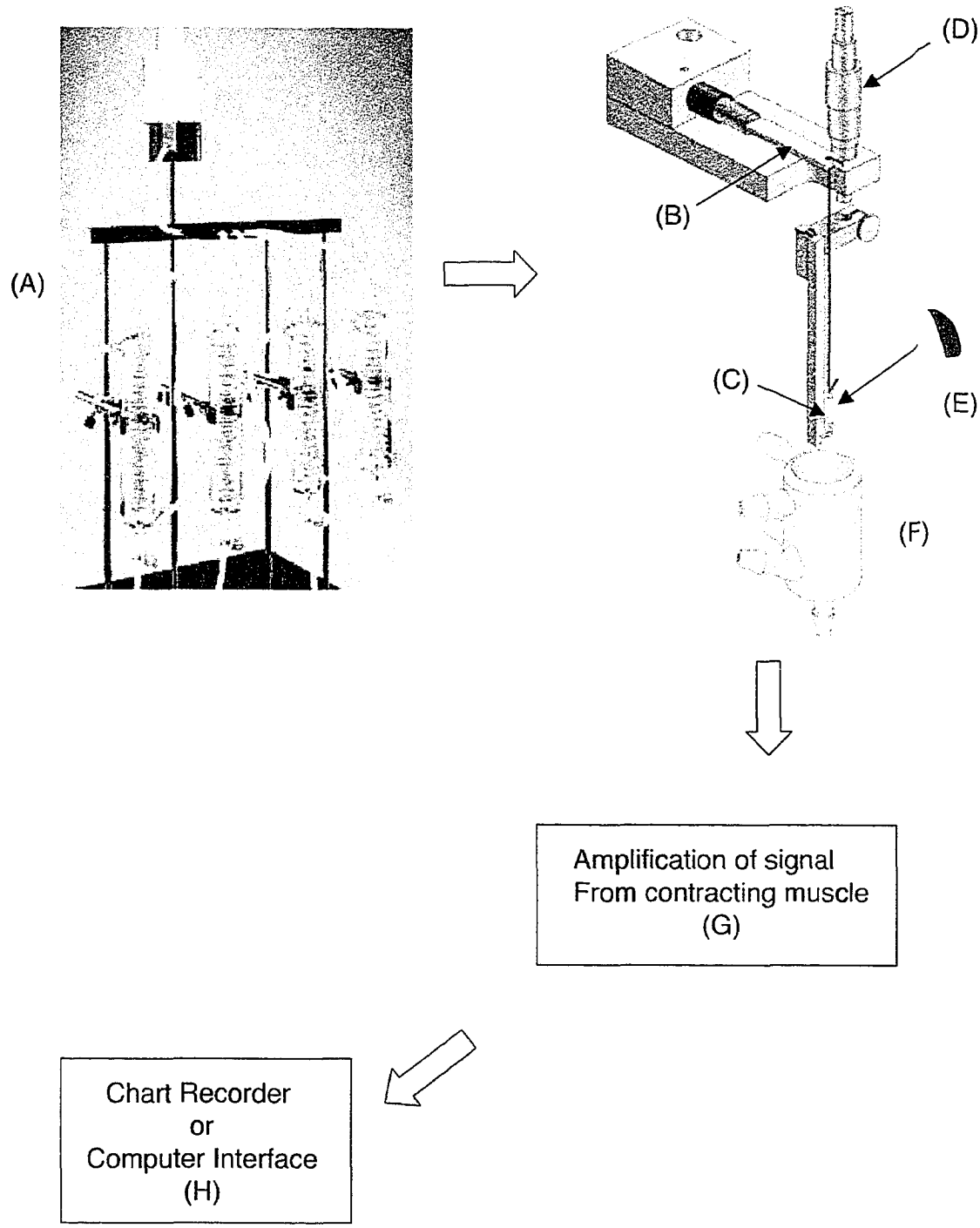
The synthesis of resin-bound linear precursors to **13** and **15** (Scheme 17) using standard Fmoc SPPS on Rink amide resin (0.6 mmol/g) proceeds smoothly. Cleavage of the resin-bound precursors is accomplished with 95% TFA to afford the deprotected linear precursors **73** and **74**. Peptides **73** and **74** are then oxidatively cyclized in ammonium bicarbonate buffer at pH 8.5 with constant oxygen aeration to give antagonists **13** and **15** in 7% and 9% overall yields, respectively.

2.6 Biological Testing of 1,6-Dicarba Analogs as Oxytocin Agonists

Testing for *in vitro* agonistic activity was done on all 1,6-carba analogs **17**-**29** using previously published procedures.[44,102] This methodology requires the use of freshly excised uterine tissue strips (3 x 10 mm) from non-pregnant, female rats. The ends of each strip of tissue are placed on tissue mounts, one of which is anchored and the other attached to a force displacement transducer, which measures the muscle contractions. Immersion of the freshly mounted tissue in buffer approximating physiological conditions preserves the tissue for extended periods of testing. Measurement of the amount of muscle contractility is accomplished by converting the muscle contractions to observable readouts on a chart data recorder (after amplification). Measurement of the area of the resultant data peaks from each muscle contraction can be summed allowing

for the relative determination of the agonist activity of oxytocin (**11**) (or analogs). The greater the area recorded over a given time period, the greater the muscle contractility, and hence the greater the agonistic effect. For the purposes of this study, biological testing of agonists was done on a Biopac Systems Inc. Myobath^{TM} apparatus (Figure 12).

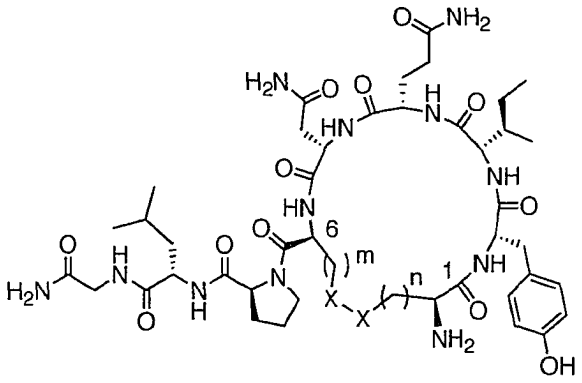
Figure 12.[a] Biopac Systems Inc. Myobath™ Apparatus.[139]



[a] Apparatus: (A) Gravity fed buffer pre-warming system, (B) force transducer, (C) tissue mounts, (D) micrometer (tissue tension adjustment), (E) rat uterine tissue (1.0 x 0.3 cm), (F) warm jacketed muscle bath, (G) Bridge8 Modules low noise transducer amplifier, (H) chart/data recorder interface.

All synthetic, carbocyclic peptides were prescreened as oxytocin agonists using fresh uterine tissue from Sprague-Dawley rats (250 g) up to a concentration of 10 μM, but only those demonstrating significant activity are tabulated (Table 4). The 1,6-dicarba analogs of oxytocin **17**, **18a** and **18b** were tested separately and exhibit oxytocin agonistic activity. Their EC_{50} values are 348, 38 and 250 nM, respectively, in comparison to oxytocin (**11**) (EC_{50} = 3 nM). Both the *trans* and saturated dicarba analogs **18b** and **17** show activity two orders of magnitude less than that of oxytocin. However, the *cis* analog **18a** displays activity ca. 14-fold less than that of oxytocin, in accord with our modeling studies, which suggest that this rigidified analog closely resembles oxytocin (**11**) (Figure 6). As expected none of the 1,6-dicarba analogs of atosiban (**13**) and the antagonist **15** synthesized by Manning show any significant agonistic activity. During biological testing, with the exception of the 2:1 *cis/trans* mixture **19**, all larger ring carbocyclic analogs also display no appreciable agonistic activity. Surprisingly, mixture **19** exhibits agonistic behavior at higher doses (EC_{50} value of $1.4 \pm 0.4 \times 10^3$ nM) and shows no antagonistic behavior when tested (see below). This contrasts with the literature report on the corresponding, larger ring disulfide analogs (e.g., [1-Hcy, 6-Hcy]-oxytocin) **12**, which exhibits no oxytocin agonist properties, but shows moderate antagonistic behavior (pA_2 = 6.0).[82] As mentioned previously, the pA_2 values are defined as the negative logarithm of the concentration of antagonist that diminishes the activity of a double dose of oxytocin to that of a single dose.[100,140] There is uncertainty as to whether the *cis* (**19a**) or *trans* (**19b**) analog is responsible for the agonistic activity as the separation of these isomers is not readily achieved.

Table 4. *In vitro* biological results for oxytocin agonistic activity.[a,b]



Carbocycle	X-X	m	n	EC_{50} (nM)
11	S-S	1	1	3[a], (7 ± 2)[b]
17	CH_2-CH_2	1	1	348[a]
18a	CH=CH	1	1	38[a]
18b	CH=CH	1	1	250[a]
19 2:1 *cis/trans* mixture	CH=CH	1	2	1400 ± 400[b]

[a] Values from uterine tissue from one animal. [b] Values from uterine tissue from 3 separate animals.

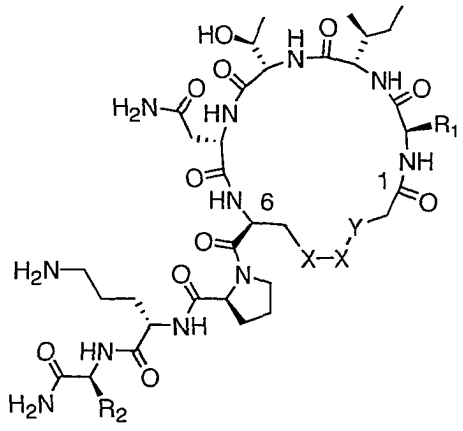
2.7 Biological Testing of 1,6-Dicarba Analogs as Oxytocin Antagonists

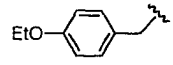
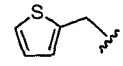
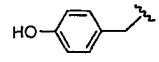
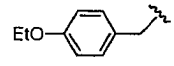
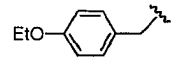
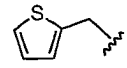
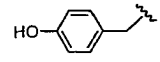
Testing for antagonistic activity is done in a similar manner to the agonistic tests. The same apparatus (Figure 12) is used and rat uterine tissue is prepared in an analogous manner. However, instead of measuring increased muscle contractility to determine the activity of the analogs, the potency of antagonists (nM) is determined by how much they decreased the response of a set dose of oxytocin. To be more precise, pA_2 values are calculated based upon the concentration of antagonist required to diminish the response

of a double dose of oxytocin (**11**) to that of a single dose. These values for the carbocyclic analogs are then compared to the known potent antagonists atosiban (**13**)[86,97] or **15**.[97]

All carbocyclic analogs were pre-screened for antagonistic activity. Only the 1,6-dicarba analogs **26** and **27**, and **29** possess significant antagonistic activity (Table 5). None of the carbocycles with increased ring sizes (21 and 22 members) **19-23** exhibit any appreciable antagonistic activity when tested on fresh rat uterine tissue, even at higher concentration (10 μM). As mentioned above, the 2:1 *cis/trans* mixture **19** shows agonistic behavior at higher concentration (>1 μM) (Table 4). This is interesting as Smith reported that a larger ring (21 membered) disulfide-containing analog **12** has moderate antagonistic behavior ($pA_2 = 6.0$).[82] The pA_2 values for carbocyclic mixture **26a** and **26b** as well as 1,6-dicarba analogs **27** and **29** were calculated using the methodology developed by Schild.[100,140] Carbocyclic atosiban analogs **26** and **27** have potent oxytocin antagonistic activity ($pA_2 = 7.8 \pm 0.1$ and $pA_2 = 8.0 \pm 0.1$, respectively) approaching that of disulfides **13** (atosiban) and **15** (9.9 ± 0.3 and 8.8 ± 0.5, respectively) (Table 5). Interestingly, the olefin functionality in analog **26**, which imposes considerable conformational restraint on the ring system, has only limited influence on activity as the more flexible saturated derivative **27** has nearly the same antagonistic effect on oxytocin receptors (OTRs). This is in contrast with the agonistic effect of oxytocin dicarba analog **18a** (38 nM) and its congeners, where activity is reduced by one order of magnitude for the *trans* and saturated analogs **18b** (250 nM) and **17** (348 nM), respectively. However, separation difficulties led to peptide **26** being tested as a 1:4 mixture of *cis/trans* isomers, and the activity of each isomer is uncertain.

Table 5 *In vitro* biological results for oxytocin antagonistic activity.*



Carbocycle	X-X	Y	R_1	R_2	pA_2	$[pA_2]$ nM
13	S-S	CH_2		H	9.9 ± 0.3	0.2 ± 0.1
15	S-S				8.8 ± 0.5	3 ± 1
26a, 26b 1:4 mixture (*cis/trans*)	CH=CH	CH_2		H	7.8 ± 0.1	17 ± 6
27	CH_2-CH_2	CH_2		H	8.0 ± 0.1	10 ± 1
29	CH_2-CH_2				6.1 ±0.1	750 ± 20

* Values are averaged results from uterus tissue taken from 3 separate trials.

The dicarba analog **29** (pA_2 = 6.1 ± 0.1) is considerably less active than its disulfide parent **15** (pA_2 = 8.8 ± 0.5) (Table 5). A possible explanation for the nearly three orders of magnitude decrease in activity of **29** may be deviation in structure from the preferred 90° dihedral angle between the sulfurs of the disulfide bridge in **29**. This may be

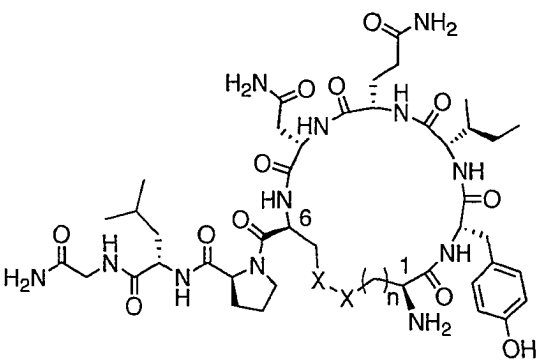
promoted by the steric crowding of the cyclohexyl group. Introduction of the conformationally less constrained cyclohexyl-bearing methylene linker that can readily rotate to alleviate non-bonded interactions may alter the rest of the peptide geometry and thereby hinder the ability of **29** to readily dock with the OTR. The optimal 90° dihedral angle of the disulfide in **15** could potentially be restored through incorporation of a conformational lock, for example an additional ring structure on the carbon bridge.

2.8 Biological Stability of 1,6-Dicarba Analogs 19 and 27

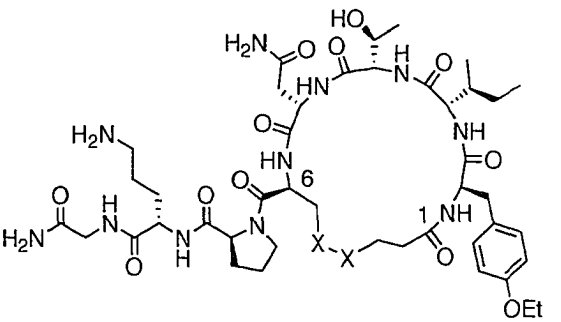
Previous methods for the testing of *in vitro* and *in vivo* biological stability of oxytocin (and analogs) involved the synthesis of radiolabeled peptides (H^3 or I^{125}), which are readily detectable in both *in vitro* and *in vivo* studies.[76,102] The *in vitro* metabolism of oxytocin (**11**) in rat placental tissue has been described.[102] These experiments monitored the degradation of tritium labeled oxytocin derivatives (using HPLC) by enzymes termed "oxytocinases" in fresh rat placental tissue homogenate.[102] Based on this methodology a new technique was developed for testing the biological stability of the 1,6-dicarba analogs of oxytocin and derivatives without the need for radiolabeling. Both agonists **11** (oxytocin) and **19** and antagonists **13** (atosiban) and **27** are incubated (37 °C) in freshly prepared rat placental tissue homogenate[102] for varying lengths of time. The same muscle bath apparatus (Figure 12) used for the agonist and antagonist studies is then employed for determining the remaining activity of the analogs. To determine if this methodology is useful as a general method to examine stability, carbocycles of both an agonist **19** and an antagonist **27** were studied. Tissue from four separate animals was used for the testing, two for each of the agonist and antagonist. The results for analogs **19** and **27** were then

compared to their parent disulfide containing peptides oxytocin (**11**) and atosiban (**13**), respectively. Half-lives are calculated for both the agonists **11** and **19** and antagonists **13** and **27** (Table 6). Values are reported for each experiment as there seem to be variations (based on half-lives recorded) in the levels of enzyme(s) responsible for breaking down oxytocin (**11**) in placental tissue in different animals.[102]

Table 6. Placental tissue half-lives for compounds **11**, **13**, **19** and **27**.*[a-d]



A



B

Carbocycle	Analog	X-X	n	Half-lives (min)
A	**11**	S-S	1	10 ± 6
A	**19a,19b** (2:1, *cis/trans*)	CH=CH	2	20 ± 4
B	**13**	S-S	-	132 ± 24
B	**27**	CH_2-CH_2	-	312 ± 6

* This table illustrates the general trend of increased stability of 1,6-carba analogs of **1** and **2** in placental tissue. Agonists and antagonists were incubated in phosphate buffer (pH 7.5) at 37 °C as control. Incubation in rat placental homogenate was also done at 37 °C. [a-d] Represent placental tissue homogenate from four different rats, two of each for the agonists and antagonists.

The results show that the 1,6-carba analog **19** has a considerably greater half-life (8-11 minutes longer) than oxytocin (**11**) when incubated in placental tissue from two different rats. Similar results are observed with the 1,6-dicarba analog **27** of atosiban (**13**). Analog **27** has a half-life in placental tissue more than twice that of atosiban (**13**). These results demonstrate that replacement of disulfide bridges in peptidic oxytocin agonists or antagonists with carbon linkers (saturated or unsaturated) can significantly stabilize the compounds and hinder degradation in placental tissue while still retaining high levels of inherent activity.

3. INTRODUCTION (BACTERIOCINS)

3.1 Bacteriocins from Lactic Acid Bacteria (LAB)

Bacteriocins from lactic acid bacteria (LAB) are another class of peptides of biological interest due to their potent antimicrobial activity towards a number of strains of Gram-positive bacteria.[141] Many of these peptides such as nisin A,[142-147] lacticin 3147[30,33] and leucocin A UAL-187[148,149] contain cyclic sulfur bridges, either as thio-ethers or disulfides. In principle, these could be replaced with carbon linkers of various lengths. The bis-methylene moiety (saturated and unsaturated) has already been shown to be a suitable replacement for disulfide bridges in oxytocin and related analogs.[44] However, it remains to be determined whether or not an ethylene linker is a plausible surrogate for a thio-ether linkage. Mimicking a thio-ether linkage with this type of linker would result in an analog in which the bridge was extended by one methylene (saturated) or methine (olefin) unit. This may or may not have a detrimental effect on the biological activity due to the inherent differences in bond length between carbon-carbon [1.34 Å (olefin) 1.54 Å saturated)][128] and carbon-sulfur (1.81 Å) bonds.[128]

Bacteriocins produced by lactic acid bacteria (LAB) (~30-60 amino acids) are a heterogeneous group of ribosomally synthesized antimicrobial cationic peptides that display potent antimicrobial activity against certain other Gram-positive organisms.[30,149] A classification of bacteriocins does exist (Table 7).

Table 7. Classification and characteristics of bacteriocins.[31]

Class	*Characteristics*	*Subclasses*
I. Lantibiotics	-Ribosomally synthesized precursor peptides that undergo extensive post-translational modifications of amino acids -Small (20-25 amino acids, < 5 kDa) peptides containing intramolecular thio-ether rings formed by lanthionine[a] and β-methyllanthionine[a]	Ia. Elongated, amphiphilic with pore-forming activity Ib. Globular, consisting of anionic or neutral peptides, no net charge, enzyme-inhibitory
II. Nonlantibiotics[b]	-Ribosomally synthesized inactive prepeptides that undergo minimal post translational modification of cysteine and by cleavage at a double glycine (-2, -1) release a mature cationic peptide	IIa.[c] Conserved amino acid motif, YGNGVXC, near the N-terminus -Referred to as "pediocin-like" or "*Listeria*-active" (i.e. listeriocidal) -Share ~40-60% sequence similarity in the N-terminal half 37-48 residues IIb. Complementary two-peptide bacteriocins; display low or no activity alone but can increase five-fold for combined activity IIc.[d] Other modified bacteriocins
III. Nonlantibiotics	-High-molecular-weight (> 30 kDa), heat labile proteins -Uncommon in LAB	
IV.[e]	-Complex bacteriocins carrying lipid or carbohydrate moieties	

[a] Lanthionine and methyllanthione bridges arise from Michael attack of cysteine thiol onto the modified serine and threonine residues, dehydroalanine (Dha) and dehydrobutyrine (Dhb) respectively.
[b] One or two pairs of cysteine residues that form disulfide bridges are referred to as "cystibiotics"; a single cysteine residue that must be in the reduced thiol form for activity is referred to as a "thiolbiotic"[29]
[c] van Belkum and Stiles divide class II bacteriocins into six groups: IIa. Cystibiotics containing four cysteine residues that form two disulfide bridges, one each in the C- and N- termini; IIb. Cystibiotics with one disulfide bridge spanning the N- and C- sections of the peptide; IIc. Cystibiotics lacking the YGNGVXC motif with the disulfide bridge spanning the N- and C- sections of the peptide; IId. Only one or no cysteine residues, lacking the YGNGVXC motif; IIe. Two separate complementary peptides (enhancing or synergistic); IIf. Atypical bacteriocins: cyclic or leaderless peptides[31]
[d] Previous definitions of IIc: Bacteriocins dependent upon export using the translocase general secretory pathway.
[e] This category has been excluded from Nes' classification because these compounds have not been purified and evidence for them is based upon loss of activity.[150]

However, categorization continues to evolve due to the discovery of new and unusual peptides. To date, these peptides have been divided into four main categories and two distinct families; lantibiotics and nonlantibiotics (Table 7).[31] This classification[31,150-154] is based upon a number of criteria, namely molar mass, thermal stability, enzymatic sensitivity, the presence of post-translationally modified amino acids, the mode of action and structure-function similarities (Table 7).

The way in which bacteriocins exert their antimicrobial activity has been a major topic of research.[141] Although precise mechanistic descriptions are often unknown, investigations have demonstrated that generalized membrane disruption models (barrel stave or carpet) are too simplistic to describe the bactericidal action of bacteriocins.[155,156] It has been proposed that class I and class II bacteriocins exert their antimicrobial response by permeabilizing the cell membrane of target organisms, or in certain cases, targeting intermediates of cell wall biosynthesis, specifically the inhibition of peptidoglycan formation.[143,144,157] Recent investigations indicate that pediocin-like (type IIa) bacteriocins may depend on membrane proteins involved in sugar transport.[155,156,158-162] Some class I bacteriocins, such as nisin A,[142-147] require lipid II as a cell membrane receptor prior to pore formation.[157,163]

The potential application for bacteriocin-producing lactic acid bacteria in food preservation has already been realized. Nisin A is currently approved for use in over 80 countries as a food preservative in meat and dairy products due to its broad activity spectrum and lack of toxicity to humans.[29,31,148,149] Pediocin-like type IIa bacteriocins, and the organisms that produce them are also being investigated as possible therapeutic agents, especially for the treatment of gastrointestinal infections. Due to the lack of

toxicity in humans, both type I and type IIa bacteriocins are currently being tested as antimicrobials against organisms resistant to current antibiotics.[156] However, limited solubility and instability at neutral or basic pH, in addition to inactivation by intestinal proteases limit their possible use as therapeutics.[31] Hence, the replacement of the sulfur bridges in two bacteriocins, leucocin A (type IIa) and lacticin 3147 (type Ia), with carbon bridges was investigated in the hope that active and more stable analogs could be generated. If successful, such compounds may be important leads for the development of therapeutic agents in humans or veterinary medicine.

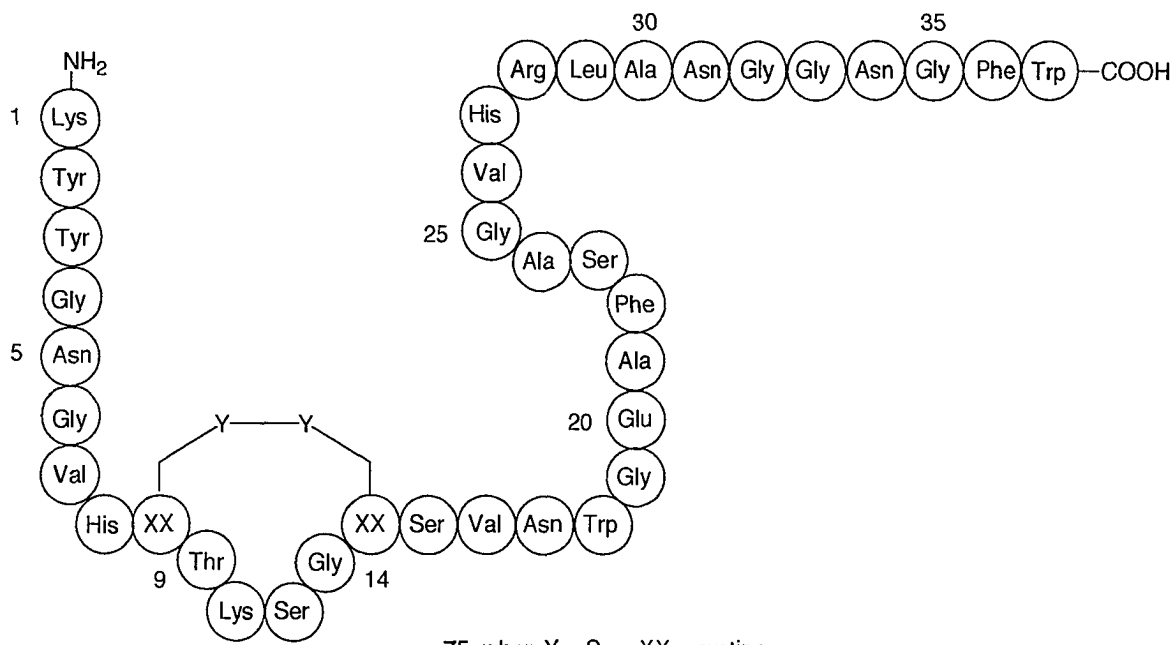
3.2 Leucocin A-UAL 187

Leucocin A-UAL 187 (**75**) (Figure 13), produced by *Leuconostoc gelidum* UAL 187, is a 37 amino acid type IIa bacteriocin (Table 7) that is isolated from vacuum packaged meat.[149] It displays antimicrobial activity against a wide spectrum of LAB, meat spoilage bacteria and food-related human pathogens, including *Listeria monocytogenes*.[149] The biological activity of leucocin A is believed to be mediated through a chiral receptor molecule.[156] A previous study by Vederas and co-workers demonstrated that the D-enantiomer of leucocin A, containing all unnatural D-amino acid residues, is completely inactive.[156] Leucocin A (**75**) is stable at low pH ($\leq$ 7) and heat resistant. However, it is rendered inactive by a wide range of proteolytic enzymes and at higher pH values (7.5-8.5).[149]

3.2.1 Leucocin A Project Goals

The lack of activity of leucocin A (**75**) at slightly basic pH may be due in part to degredation of the disulfide bridge and the subsequent loss of tertiary structure. Stabilization of this compound by replacement of the disulfide bridge with a carbon-carbon linker would produce a dicarba analog **76** (Figure 13), which may display comparable potency, as was the case for the dicarba analogs of oxytocin agonists **18a** and antagonists **26**, **27** and **29**. The goal of this project is to synthesize **76** as a dicarba analog of leucocin and test its activity and stability. If successful, **76** and analogs thereof may prove to be important leads into the development of "pediocin-like" bacteriocins as therapeutics.

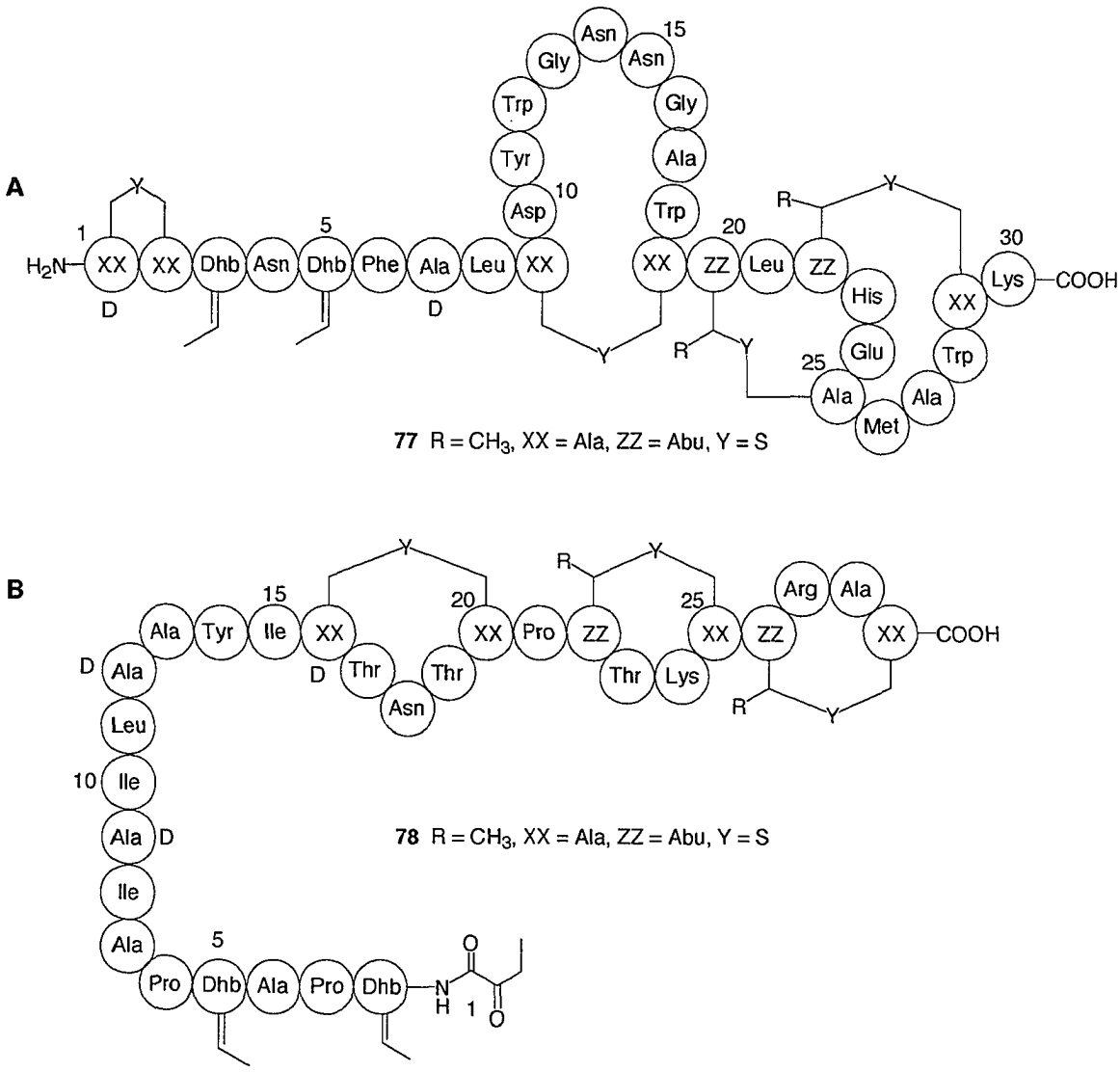
Figure 13 Leucocin A (**75**) and 9,14-dicarba leucocin A (**76**).



3.3 Lacticin 3147

Lacticin 3147, is a two-component bacteriocin consisting of A1 (**77**) and A2 (**78**) peptides (Figure 14).

Figure 14 General structures of lacticin 3147 peptides A1 (A) and A2 (B).

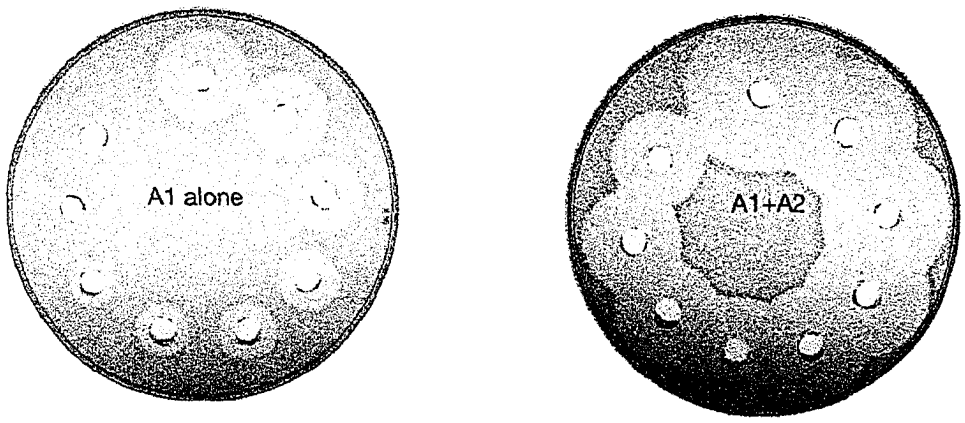


It is produced by *Lactococcus lactis* subspecies *lactis* DPC3147. Both of these peptides were first isolated from the Irish Kefir grain in 1996,[164] which was used to preserve dairy

beverages at room temperature for several weeks without refrigeration. It is believed that the production of peptides **77** and **78** is responsible for the preservative nature of the Kefir grain.[164]

Lacticin 3147 peptide A1 (**77**) (30 amino acids) exhibits antimicrobial activity on its own (Figure 15). However, the addition of lacticin 3147 A2 (**78**) (29 amino acids) greatly enhances the antimicrobial response at nM concentrations. Hence, peptides **77** and **78** are synergistic bacteriocins (Figure 15).[32]

Figure 15 Well diffusion assay of Lacticin 3147 peptides **77** and **78** against *L. Lactis HP* (Nathaniel Martin, Ph. D. Thesis).[32]



The lacticin 3147 peptides are active against *Listeria monocytogenes* (a food born pathogen),[165] a variety of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), penicillin-resistant *Pneumococcus* (PRP), *Propionibacterium*, and *Streptococcus mutans*.[166] Inspection of **77** and **78** reveals extensive post-translational modifications, which include multiple lanthionine and methyl

lanthionine bridges, dehydro amino acid residues (Dha and Dhb) and an N-terminal diketo-amide functionality in lacticin A2 (**78**). There are also "D" residues in each peptide resulting from Michael attack of cysteine sulfhydryl groups onto dehydro amino acid residues. These modifications made it difficult to determine the structures of these peptides by traditional chemical techniques such as Edman degradation. However, NMR solution structures for each peptide have been reported.[33] During this work, it became apparent that the sulfurs in the lanthionine bridges are prone to aerobic oxidation to sulfoxides. This greatly diminishes the antimicrobial activity.

3.3.1 Lacticin 3147 Project Goals

The broad biological spectrum of lacticin 3147, and its lack of human toxicity has attracted much interest for its application as a food preservative. However, the susceptability of lanthionine and β-methyllanthionine bridges present in lacticin 3147 to air oxidation make isolation in an open atmosphere problematic.[32] Replacement of these thio-ether bridges in **77** and **78** with carbon-carbon linkers should yield analogs **79-82** (Table 8) that may prove to be chemically and biologically more stable while maintaining antibiotic activity.

Table 8. Possible carbocyclic analogs of Lacticin 3147 peptide structures A and B (Figure 14).[a]

Structure	Peptide	Y	R	XX	ZZ
A	**77**	S	CH_3	Ala	Abu[b]
B	**78**	S	CH_3	Ala	Abu[b]
A	**79**	CH=CH	H	Ala	Ala
A	**80**	CH_2-CH_2	H	Ala	Ala
B	**81**	CH=CH	H	Ala	Ala
B	**82**	CH_2-CH_2	H	Ala	Ala

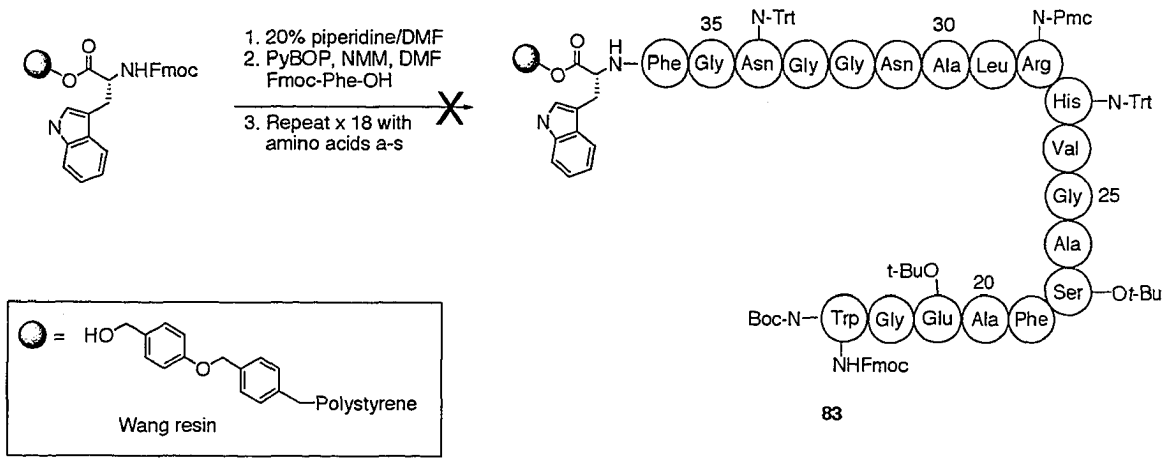
[a] Dhb = dehydrobutyrine [b] Abu = α-aminobutyrine.

3. RESULTS AND DISCUSSION

3.4 Synthesis of 9,14-Dicarba Leucocin A 76

The positive results on stability and activity observed when the disulfide bridge is replaced with bis-methylene (or methine) linkers in oxytocin and derivatives suggested application to larger and more complicated peptide systems. Leucocin A (**75**), a 37 amino acid bacteriocin, contains a sulfur-sulfur bond that bridges amino acid residues 9 and 14 forming a 20 membered ring. Standard Fmoc SPPS with incorporation of L-allylglycine (**10**) in place of cysteine residues 9 and 14 of **75** would provide a bis-olefin linear precursor for RCM reaction to give **76**. Initial attempts at the C- to N-terminal synthesis (Scheme 18) of a linear precursor to **76** using standard Fmoc SPPS on Wang resin pre-loaded with Fmoc-Trp(N-Boc)-OH failed to yield any appreciable amount of peptide after coupling of amino acid 20. The synthesis (Scheme 18) proceeds smoothly from residues 36-24. However, beyond this point the synthesis becomes sluggish, and in many cases couplings do not reach completion based on the Kaiser test.[167] Cleavage of a sample of resin (95% TFA) after coupling of tryptophan 17 yielded no detectable peptide **83** upon analysis by MALDI-TOF and ES mass spectrometry. This result is rather disappointing, as the coupling of 9 more amino acid residues to **83** is required prior to cyclization by on-resin RCM reaction. A previously reported synthesis of the D-enantiomer of leucocin A showed that coupling becomes increasingly more difficult beyond phenylalanine 22, often requiring heating of the resin bound peptide and multiple couplings. This is probably due to the relative hydrophobicity of the protected resin-bound peptide, which may lead to aggregated/coiled conformations that block the N-terminus.[156,168]

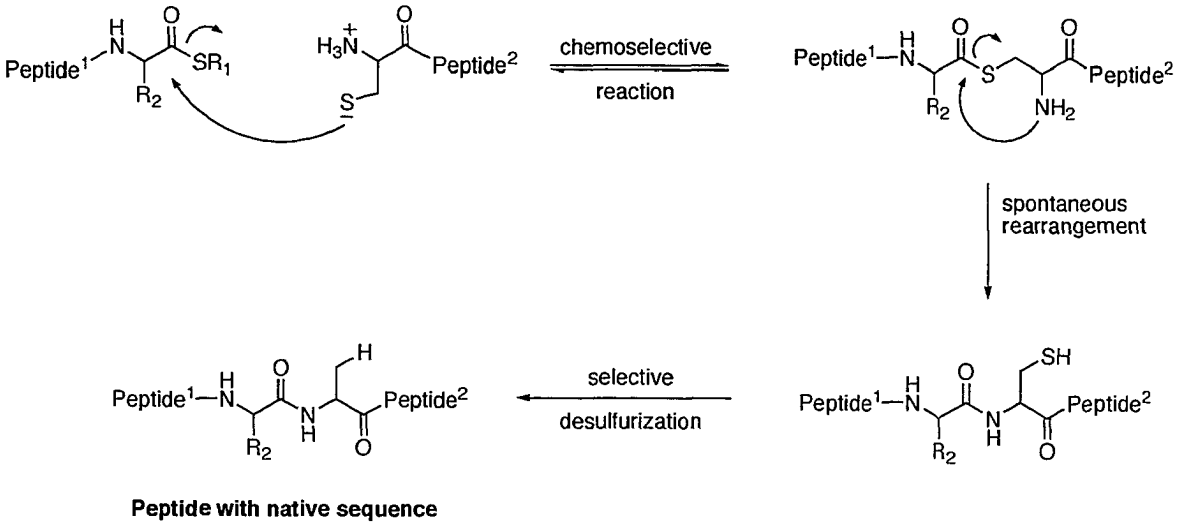
Scheme 18 Attempted linear synthesis of leucocin A fragment **83**.[a,b]



[a] Conditions: (a) Fmoc-Phe-OH, (b) Fmoc-Gly-OH, (c) Fmoc-Asn(*N*-Trt)-OH, (d, e) Fmoc-Gly-OH, (f) Fmoc-Asn(*N*-Trt)-OH, (g) Fmoc-Ala-OH, (h) Fmoc-Leu-OH, (i) Fmoc-Arg(*N*-Pmc)-OH,[b] (j) Fmoc-His(*N*-Trt)-OH, (k) Fmoc-Val-OH, (l) Fmoc-Gly-OH, (m) Fmoc-Ala-OH, (n) Fmoc-Ser(*O*-*t*-Bu)-OH, (o) Fmoc-Phe-OH, (p) Fmoc-Ala-OH, (q) Fmoc-Glu(*O*-*t*-Bu)-OH, (r) Fmoc-Gly-OH, (s) Fmoc-Trp(*N*-Boc)-OH. [b] Pmc = 2,2,5,7,8-pentamethylchroman-6-sulfonyl, a TFA cleavable protecting group for the guanidine functionality.

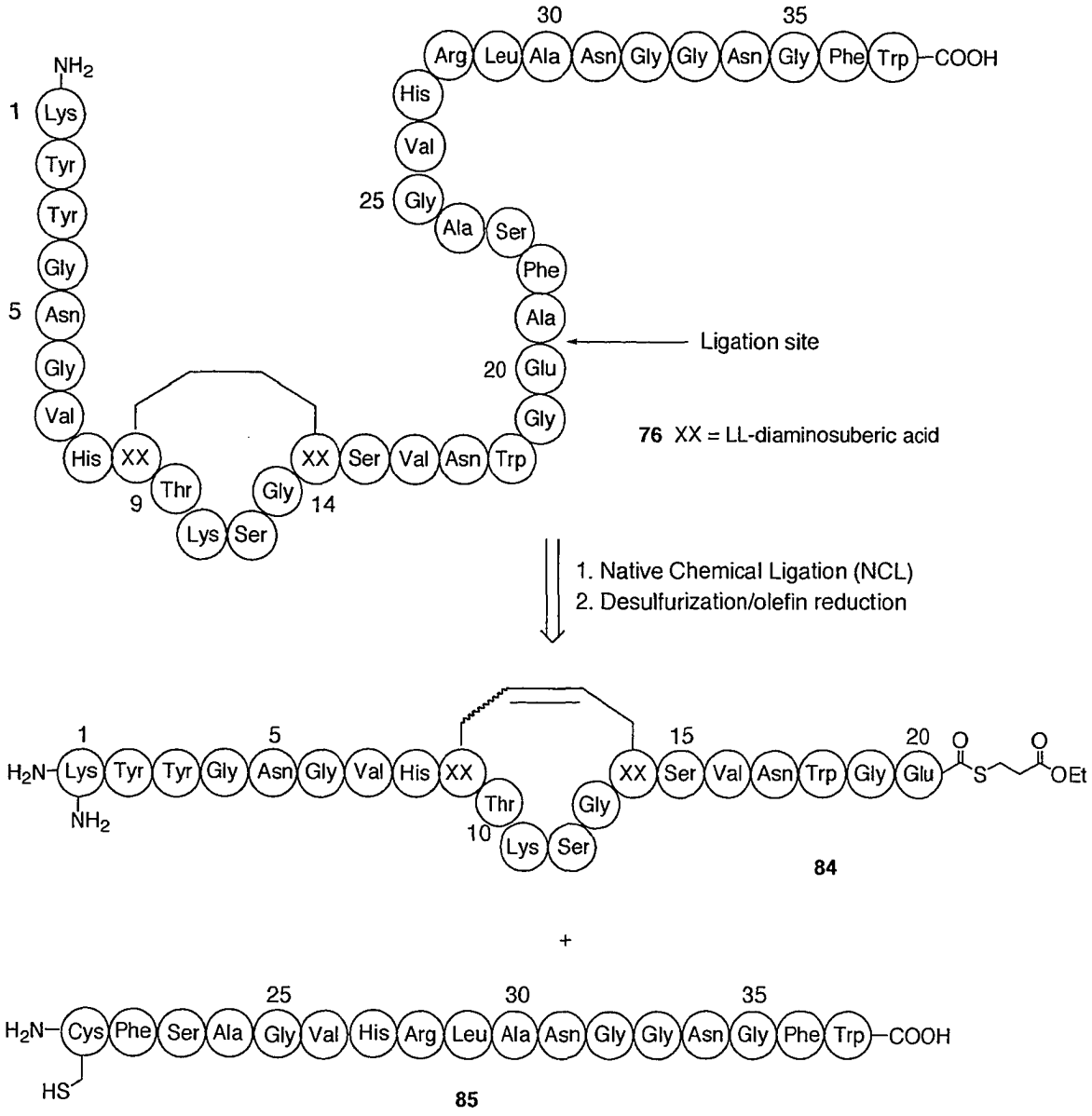
To achieve a 9,14-carba analog of Leu A, an alternative convergent approach was investigated, wherein the peptide would be built as two fragments and coupled using Native Chemical Ligation (NCL) developed by Kent and Dawson.[23] This approach relies on a highly chemoselective reaction between unprotected peptide fragments bearing an N-terminal cysteine residue and a C-terminal thioester. The limitation of this technique has been the requirement of a cysteine residue in the ligation site of the desired peptide.[23] However, recent methodology allows for selective desulfurization of the resultant cysteine residues to alanine following ligation. This broadens the applicability of this method for the synthesis of non-cysteine containing peptides (Scheme 19).[22]

Scheme 19 NCL and selective desulfurization.[22]



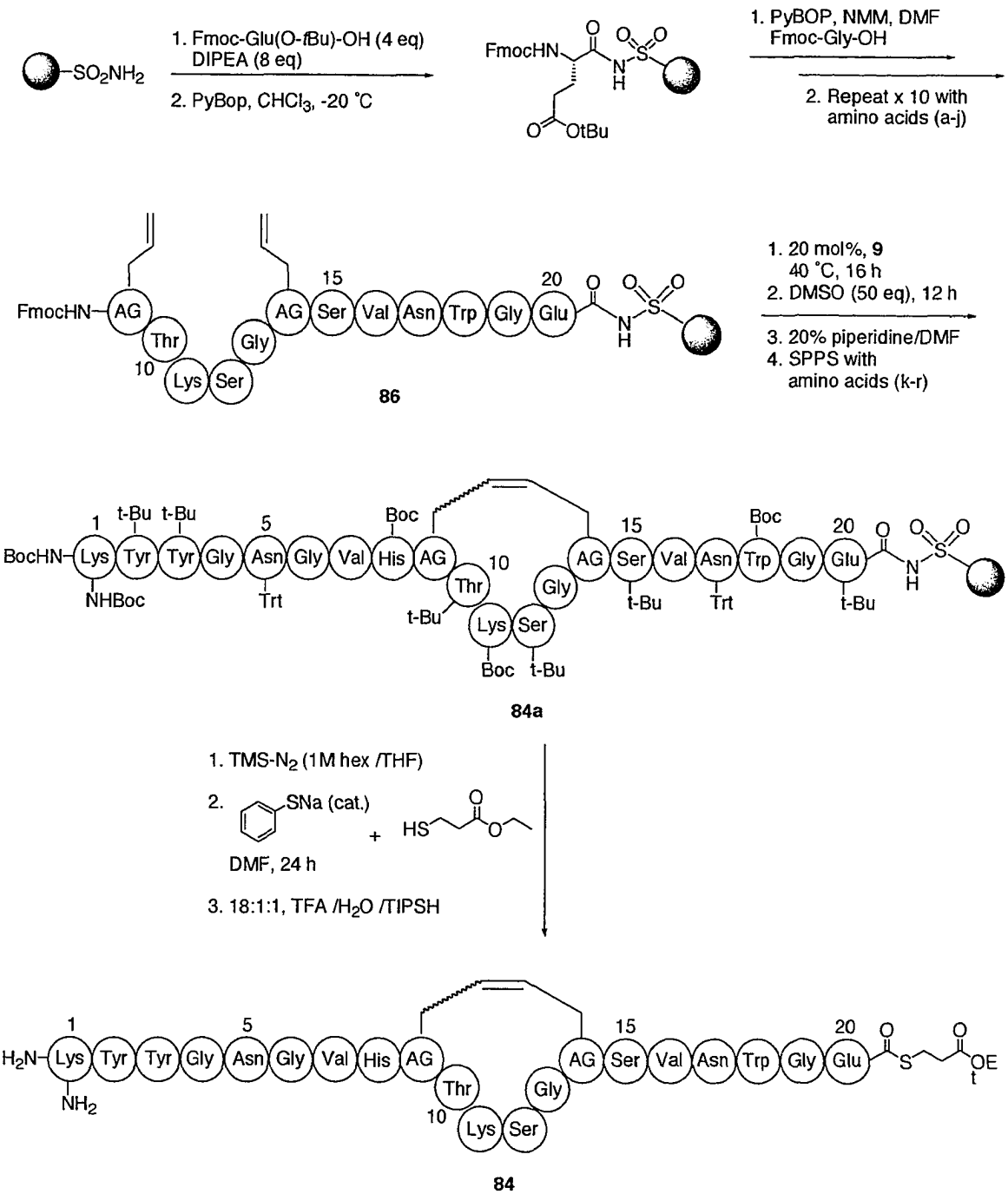
The presence of alanine at position 19 in leucocin A (**75**) may be made by NCL of the peptide thioester **84** and cysteine containing peptide **85** followed by desulfurization (Scheme 20).

Scheme 20 Retrosynthesis of 9,14-dicarba Leu A (**76**).



The synthesis of the N-terminal thioester **84** (Scheme 21) begins with the coupling of N-9*H*-fluorenylmethoxycarbonyl-L-glutamic acid 5-*tert*-butyl ester (Fmoc-Glu(*O*-*t*-Bu)-OH) to 4-sulfamylbutyryl resin, using PyBOP/DIPEA in $CHCl_3$ at –20 °C.[16]

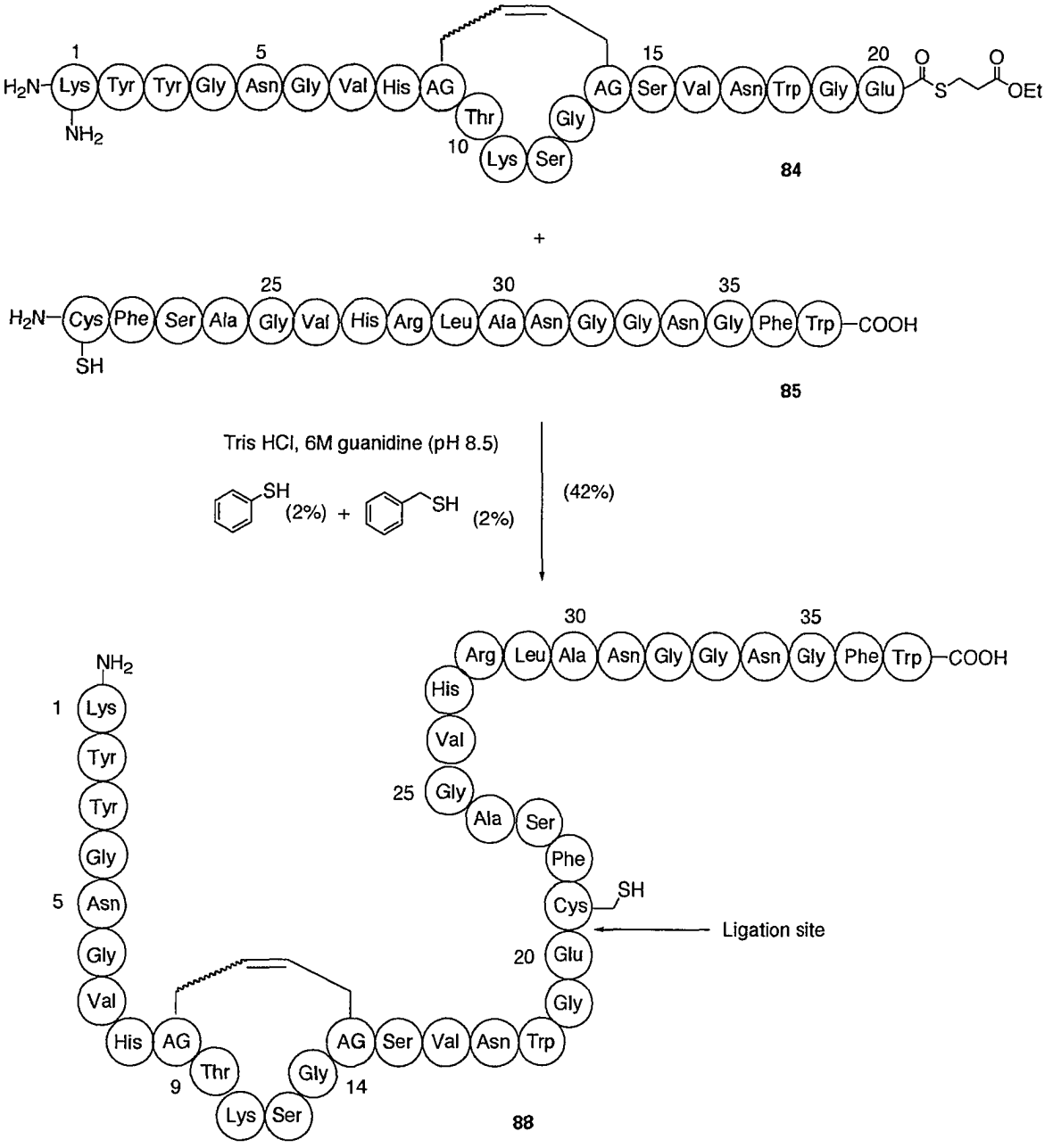
Scheme 21 Synthesis of the N-terminal thioester **84**.[a,b]



[a] Conditions: (a) Fmoc-Trp(*N*-Boc)-OH, (b) Fmoc-Asn(*N*-Trt)-OH, (c) Fmoc-Val-OH, (d) Fmoc-Ser(*O*-*t*-Bu)-OH, (e) Fmoc-L-AllGly-OH, (f) Fmoc-Gly-OH, (g) Fmoc-Ser(*O*-*t*-Bu)-OH, (h) Fmoc-Lys(*N*-Boc)-OH, (i) Fmoc-Thr(*O*-*t*-Bu)-OH, (j) Fmoc-L-AllGly-OH. [b] Conditions: (k) Fmoc-His(*N*-Trt)-OH, (l) Fmoc-Val-OH, (m) Fmoc-Gly-OH, (n) Fmoc-Asn(*N*-Trt)-OH, (o) Fmoc-Gly-OH, (p, q) Fmoc-Tyr(*O*-*t*-Bu)-OH, (r) N-Boc-Lys(*N*-Boc)-OH (**87**).

All other remaining residues (9-19) are coupled using standard Fmoc SPPS chemistry to give the resin-bound bis-allylglycine peptide **86**. Cyclization of **86** under standard RCM conditions using Grubbs second generation catalyst **9** yields resin-bound cyclic peptide **84a** after coupling of amino acids 1-8 using standard SPPS. *N,N'*-Di-*tert*-butoxycarbonyl (Boc) protection at the N-terminal lysine residue **87** prevents side reactions during the cleavage of peptide **84a** from the resin. Fmoc protected amines are known to be problematic in thiol/thiolate cleavage reactions due to premature removal of the Fmoc group.[16,22,23] Cleavage of **84a** from the resin is achieved by methylation of the nitrogen of the sulfonamide with TMS-diazomethane, followed by reaction with ethyl 3-mercaptopropionate in the presence of a catalytic amount of sodium thiophenolate. The addition of thiophenolate initiates the resin cleavage.[16] This latter step is low yielding, however repetitive methylation followed by treatment with thiol yields more of the desired product. Acid labile protecting groups are then removed with 95% TFA to afford the deprotected olefin thioester **84** in 1% overall yield (Scheme 21). This low yield is mostly attributed to the inefficiency of the methylation process, which affords little product even after repeated treatments. Ligation of thioester **84** to the C-terminal fragment **85** (prepared by Darren Derksen on Wang resin) in Tris HCl buffer at pH 8.5[22] yields the ligated product **88** (42%) after RP-HPLC purification (Scheme 22).

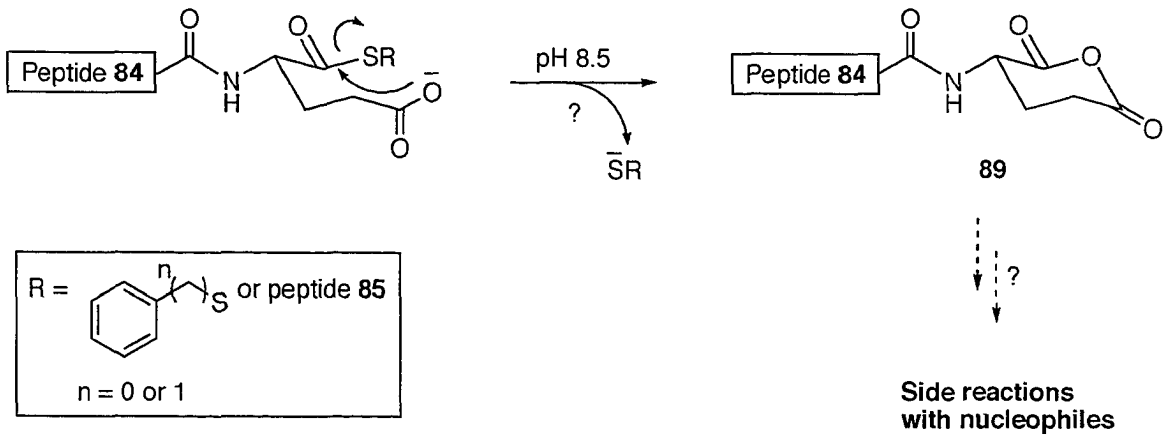
Scheme 22 NCL of peptide thioester **84** and C-terminal peptide **85**.



It has been reported that the coupling of two unprotected peptide fragments using NCL methodology produces high yields of product with little to no side reactions due to its chemoselective nature. However, our low yield, coupled with multiple product peaks

(RP-HPLC), suggest that in our system this is not the case. The glutamic acid residue at position 20 may be interfering with the ligation process by cyclizing onto the thioester functionality of either activated adducts of **84** or the peptide-peptide thioester bond formed during the first equilibrium step of NCL (Scheme 23). This would form anhydride **89**, which then could react with other nucleophiles present in the peptides **84**, **85** or **88** at pH 8.5.

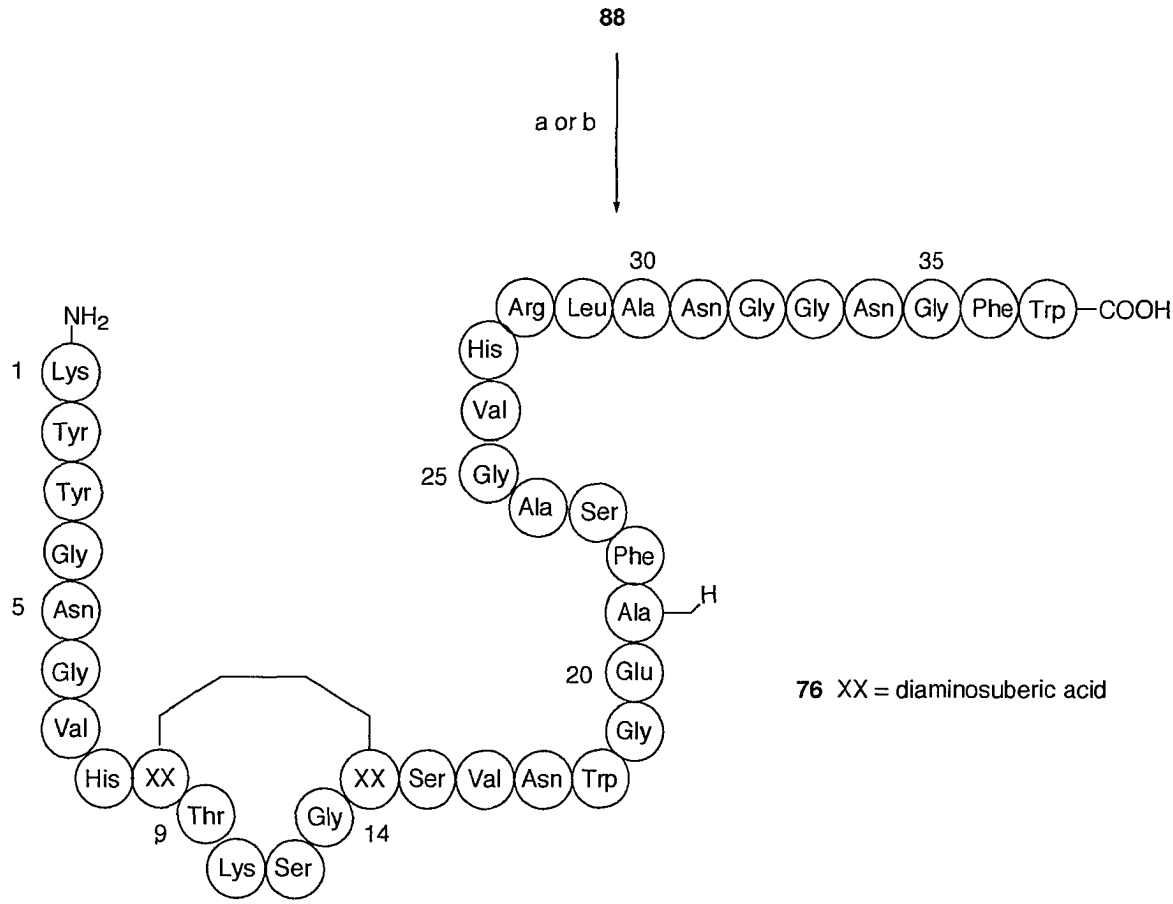
Scheme 23 Possible cyclization of Glu 20 onto activated peptide precursors.



Desulfurization, and concomitant olefin reduction of the ligated peptide **88** was attempted using 10% Pd/C in 20% aqueous AcOH, under H_2 at 760 torr. Isolation of this mixture fails to provide any detectable amounts of the desired peptide by mass spectrometry (MS). It may be that the peptide remains fixed to the heterogeneous catalyst (Scheme 23). Attempts at desulfurization on a small amount of **88** (ca. 1 mg) using $NiBH_4$ ($NiCl_2$ + $NaBH_4$) in methanol, followed by extraction and analysis by MS,

suggested that a small amount of desired peptide **76** formed. However, attempted isolation by HPLC failed to yield any of the desired product (Scheme 24).

Scheme 24 Desulfurization and reduction of peptide **88**.[a]



[a] Conditions: (a) 10% Pd/C, H_2 (760 torr), 20% aqueous AcOH, (b) $NiCl_2$ (10 eq), $NaBH_4$ (30 eq), $MeOH/H_2O$ (1:1).

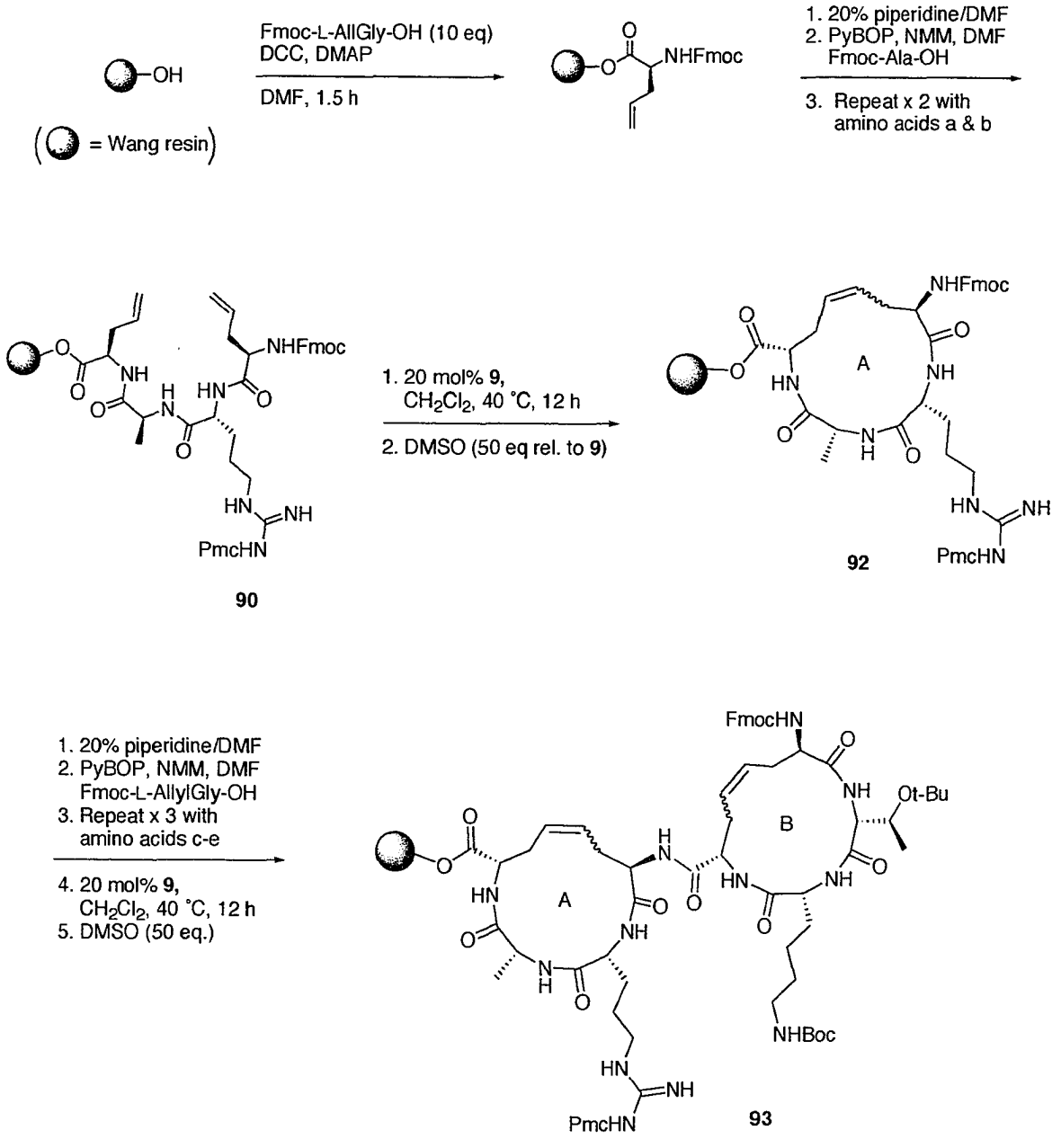
Further work is ongoing to optimize the conditions for both ligation and desulfurization sequences.

3.5 Synthesis of Lacticin 3147 A2 Analog Having 3 Carbocyclic Rings

The methodology for RCM on resin-bound peptides can potentially be applied to lacticin 3147 peptide A2 (**78**). The A1 peptide **77** contains two overlapping thioether bridges, and would require orthogonal olefin protection of linear precursors prior to RCM to ensure the proper ring orientation. To ensure correct ring formation, a sequential set of cyclization reactions was designed to form carbocyclic versions of the ABC ring system of lacticin 3147 A2.

Synthesis of linear precursor **90** (Scheme 25) on Wang resin (0.8 mmol/g) is readily accomplished with incorporation of Fmoc-L-allylglycine (**10a**) at position 29 (Table 8) and commercially available Fmoc-D-allylglycine (**91**) at position 26, using standard Fmoc SPPS. Both the L and D enantiomers of allylglycine are required so that the correct stereocenters are in place at the peptide backbone RCM. Cyclization of **90** is achieved by standard RCM conditions using Grubbs second generation catalyst **9** to give the A ring **92** as the only peptide detectable by MALDI-TOF/MS [(M+H) = 634]. Removal of the N-terminal Fmoc with piperidine, followed by Fmoc SPPS gives a linear precursor to **93**, which is cyclized by RCM using analogous conditions. This affords the AB ring system **93** as the only detectable product by MALDI-TOF/MS [(M+H) = 1030].

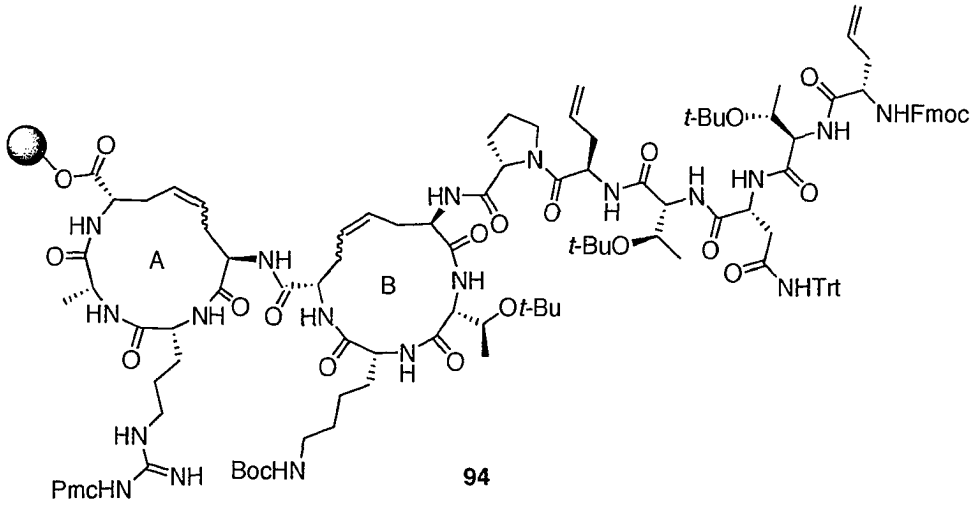
Scheme 25 Synthesis of A **92** and AB **93** carbocyclic fragments.[a]



[a] Amino acids (a) Fmoc-Arg(*N*-Pmc)-OH, (b) Fmoc-L-AllGly-OH (**10a**), (c) Fmoc-Lys(*N*-Boc)-OH, (d) Fmoc-Thr(*O*-*t*-Bu)-OH, (e) Fmoc-D-AllGly-OH (**91**).

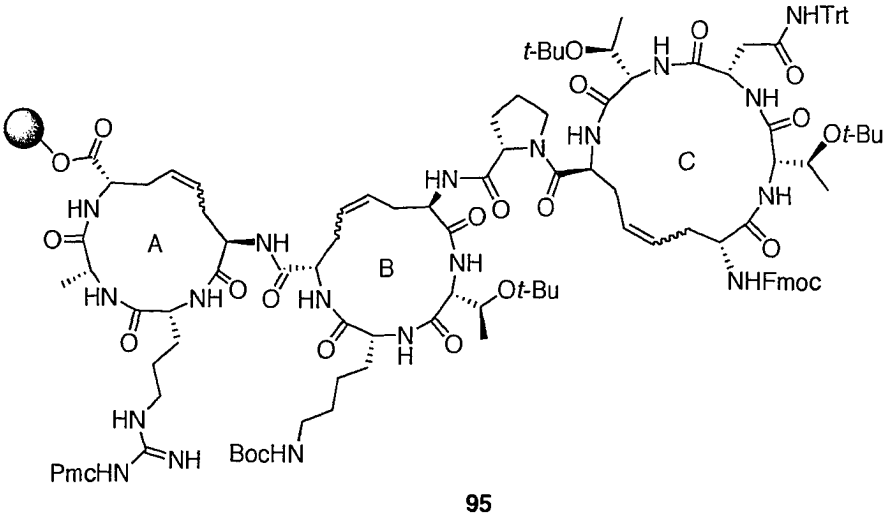
N-Terminal Fmoc deprotection of **93** using piperidine followed by continued SPPS affords the linear precursor **94** (Scheme 26).

Scheme 26 Cyclization of linear precursor **94** to tricycle **95**.[a,b]



1. 50 mol% **9**
CH_2Cl_2, 40 °C, 48 h

2. DMSO (50 eq)
relative to **9**

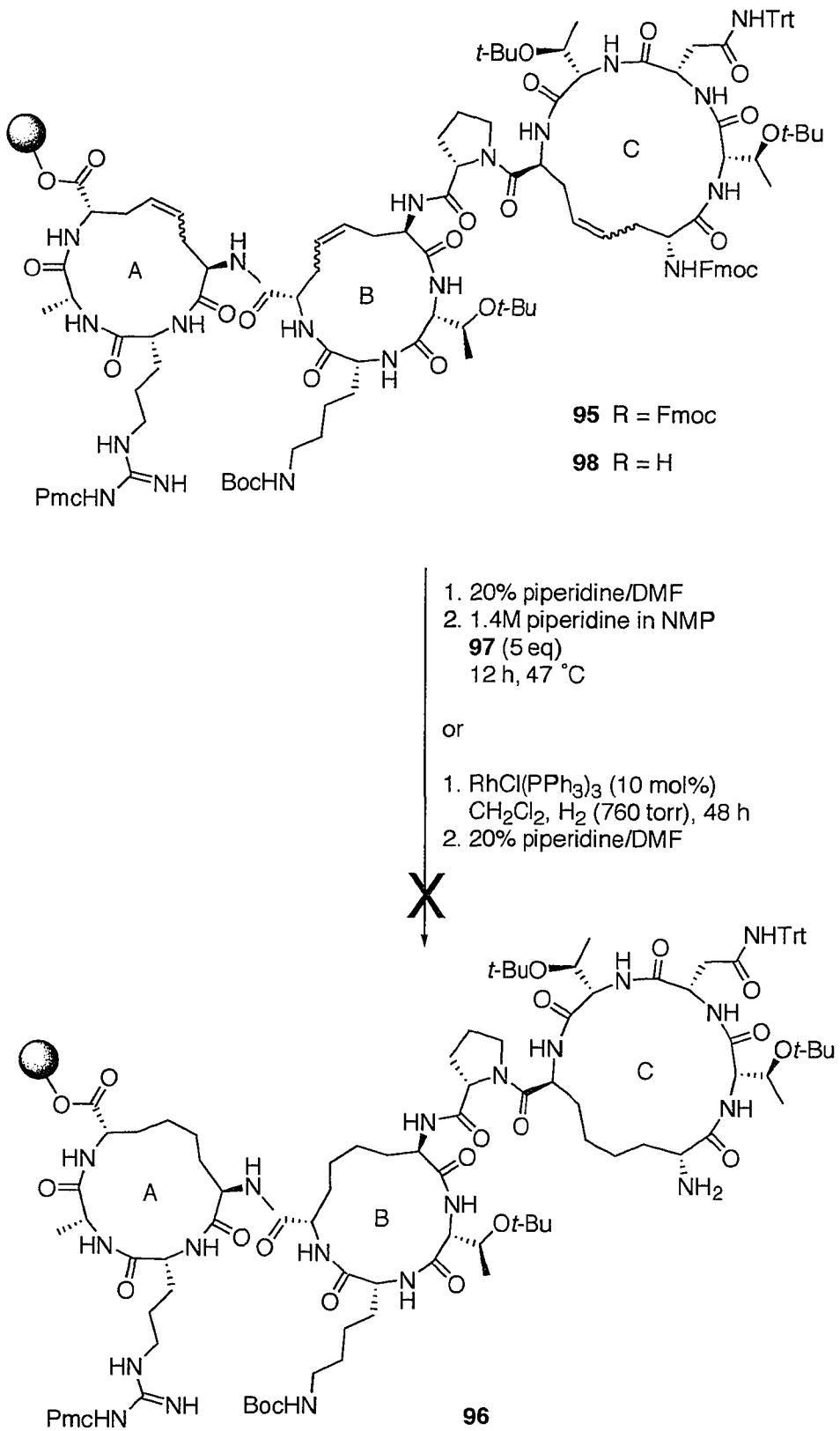


[a] Conditions leading to synthesis of **93** from **94**: (a) 20% piperidine in DMF, (b) PyBOP, NMM, DMF, (c) Fmoc-Pro-OH. [b] Repeat (a) and (b) for amino acids: (d) Fmoc-L-AllGly (**10a**), (e) Fmoc-Thr(*O*-*t*-Bu)-OH, (f) Fmoc-Asn(*N*-Trt)-OH, (g) Fmoc-Thr(*O*-*t*-Bu)-OH, (h) Fmoc-D-AllGly-OH (**91**).

Upon exposure to standard RCM conditions the reaction yields a mixture of cyclized product **95** and starting material, as detected by MALDI-TOF/MS. Increasing the loading of catalyst **9** to 50 mol% and extending the reaction time to 48 h affords the tricyclic ABC carbocycles **95** as the only detectable peak by MS [(M+H) = 1608] when a sample is cleaved from the resin with TFA. The use of increased amounts of catalyst **9** presents no problems as excess catalyst can be removed from the reaction mixture using simple filtration, prior to purification.

To avoid future purification difficulties resulting from the eight possible combinations of *cis/trans* isomers of the three olefins present in **95**, an on-resin reduction of the double bonds was attempted to give a fully saturated tri-carbocycle **96**. Attempts to accomplish this reduction using a modified procedure of Verdine and co-workers[36] utilizes an *in situ* diimide formation, resulting from the reaction of 2,4,6-triisopropylbenzenesulfonyl hydrazide (TPSH) (**97**) with piperidine. Multiple attempts at the reduction of resin-bound **95** with *in situ* diimide formation (Scheme 27) yielded only peptide **98** after resin cleavage (95% TFA), as detected by MALDI-TOF/MS. Peptide **98** is simply **95** without the N-terminal Fmoc, which is removed prior to the hydrogenolysis reaction. Hydrogenation using homogeneous Wilkinson's catalyst $[RhCl(P(Ph)_3)_3]$[170-172] in the presence of H_2 of resin-bound peptide **95** shows only starting material, as detected by MALDI-TOF/MS after acidic cleavage (95% TFA) (Scheme 27). Successful reduction to the fully saturated analog **96** in appreciable yields is required to warrant the continued Fmoc SPPS towards a fully carbocyclic analog **82** of the lacticin 3147 A2 peptide.

Scheme 27 Attempted on-resin reduction of **95**.[36,170-172]

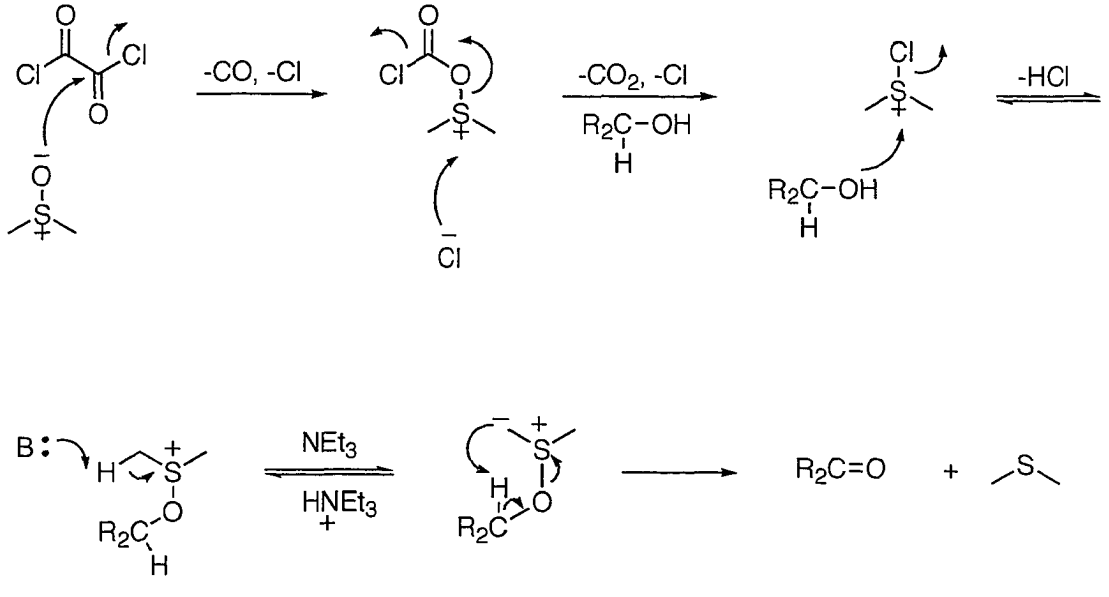


4. INTRODUCTION TO RESIN BOUND SWERN REACTION

4.1 Resin Bound Swern Oxidation

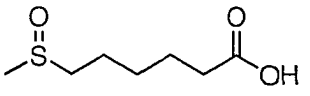
Oxidations of primary and secondary alcohols to aldehydes and ketones using activated DMSO are among the most widely used reactions in organic synthesis.[173-175] Although a number of activating reagents like DCC,[176] acetic anhydride,[177] trifluoroacetic anhydride[178] and sulfur trioxide[175] have been developed, the Swern procedure, which employs DMSO and oxalyl chloride,[179] is especially effective (Scheme 28). The use of oxalyl chloride facilitates purification due to the lack of urea byproduct formation. It also minimizes side reactions (e.g., Pummerer rearrangement) that are commonplace when DMSO is activated with carbodiimides or anhydrides respectively.[179,180]

Scheme 28 The Swern oxidation reaction.

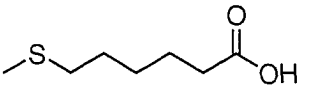


The existing Swern procedure typically uses excess reagent and produces the volatile and noxious byproduct, dimethyl sulfide (bp 37°C).[173-175] Sulfoxides such as 6-(methylsulfinyl)hexanoic acid (**99**) (Figure 16) are reported as being efficient substitutes for DMSO in Swern oxidation reactions.[180] This modification generates the non-volatile sulfide, 6-(methylthio)hexanoic acid (**100**) (Figure 16) which is easily removed by simple extraction or filtration through silica gel, and can be recycled by aqueous periodate oxidation.[180a] It has also recently been reported that preparation of similar fluorous sulfoxides can avoid the production of malodorous byproducts (e.g., dimethylsulfide) to provide oxidized products in high yield, with recovery of the active sulfoxide after workup and oxidation.[180b]

Figure 16 6-(Methylsulfinyl)hexanoic acid (**99**) and 6-(methylthio)hexanoic acid (**100**).



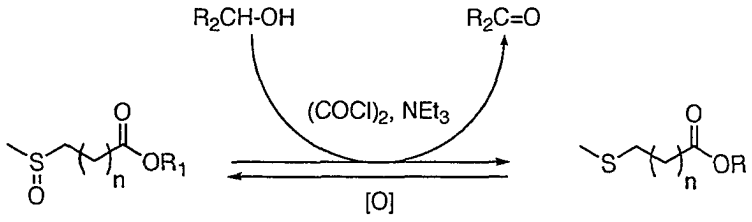
99



100

Immobilization of **99** and similar sulfoxides onto Merrifield,[180] Wang[181] or polyethylene glycol (PEG)[182] resins have also been reported. Like **99**, these resin bound sulfoxides **101-103** (Table 9) are shown to be efficient substitutes for DMSO in the conversion of a variety of primary and secondary alcohols to their respective aldehyde or ketone derivatives (Table 9).[180-182]

Table 9 Oxidation of representative alcohols by various sulfoxides.[180-182]



R_1	n	[O] equivalents	R_2CHOH	Composition (%) R_2CO/R_2CHOH	R_2CO isolated (%)
H	5	1.2	*endo*-Borneol	95/5	90
			3,5-(OMe)-C_6H_3-CH_2-OH	96/1	94
				96/4	92
			n-Dodecanol	97/3	93
			Cinnamyl alcohol	97/3	94
			Benzoin	44/33	31
			Phenethyl alcohol		
Merrifield resin	5	2.0	*endo*-Borneol	>99/1	95
Wang resin	1	2.0	3-Benzyloxybenzyl alcohol	-	71
					80
			Anisoin	-	
			α-Methyl-2-napthylenemethanol	-	82
PEG-2000	5	2.0	*endo*-Borneol	-	91[a]
			3,5-(OMe)-C_6H_3-CH_2-OH	-	94[a]
				-	99[a]
			n-Dodecanol	-	94[a]
			Cinnamyl alcohol	-	99[a]
			Benzoin	-	94[a]
			Cholesterol[a]	-	99[a]
			sec-phenethyl alcohol		

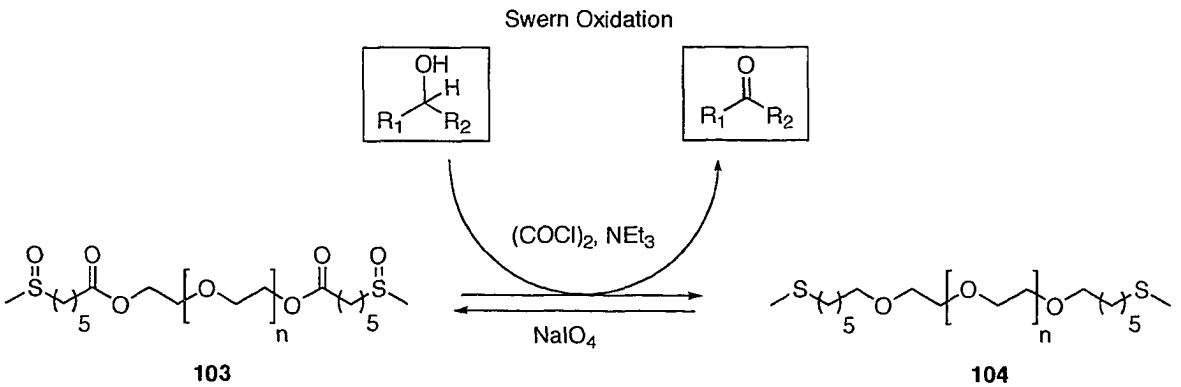
[a] Based on the conversion of 0.75 mmol (NMR).[182]

Purification by simple filtration yields the desired oxidized products in yields comparable to the solution phase Swern oxidation.[179,180,182] Of even greater benefit is that after the reaction, the reduced polymer-bound sulfides can be efficiently recycled by

periodate treatment for PEG bound reagents[182] or peroxide treatment for Wang bound reagents.[181]

This recycling step has been shown to occur in high yield (ca. 98%) for the conversion of PEG-bound sulfide **104** to the PEG-bound sulfoxide **103**, and results in no loss of oxidation potential[181,182] (Scheme 29).

Scheme 29 Recycling of **104** to **103** after Swern oxidation.[182]



The use of soluble scaffolds such as PEG allow for the formation of a homogeneous phase in the reaction medium while maintaining the polymeric properties that simplify product separation and reagent recovery. Thus, the problems encountered with poor swelling resins in polar solvents and lower reactivity often seen when using polymer-bound reagents in solid phase reactions are avoided. Due to the bi-functionality of PEG resins, there is also the added advantage of two oxidative equivalents of sulfoxide for every one equivalent of PEG resin. This gives reagents like **103** greater oxidative potential, as 1 mol equiv of the bi-functional reagent results in 2 oxidative equivalents of

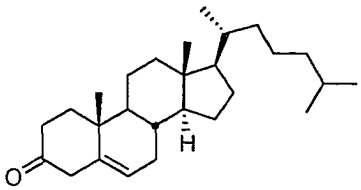
sulfoxide.[182] Recycling of the PEG-bound reagent **104** is also made easier as the extent of oxidation is readily monitored by ^{1}H NMR.[182] The use of polystyrene based sulfoxide requires the cleavage of the supported reagents prior to monitoring by ^{1}H NMR.[180,181]

To date, the use of PEG-bound sulfoxides in Swern oxidation reactions has been limited to relatively small amounts (ca. 0.75 mmol) of a selected group of primary and secondary alcohols.[182] Applying the PEG-bound sulfoxide **103** to the oxidation of more complex alcohols on a multi-gram scale may facilitate this methodology as a possible industrial process. Cholest-5-ene-3-one (**105**) (Figure 17) is available commercially[183] but due to high cost, its efficient synthesis from cholesterol (**106**)[183] (Figure 17) in multi-gram quantities is of economic interest. The oxidation of cholesterol (**106**) to cholest-5-ene-3-one (**105**) has been explored using a variety of methodologies such as the use of platinum catalysts[184] and pyridinium chlorochromate (PCC).[185] However, due to the ease with which **105** undergoes isomerization to the α,β-unsaturated isomer cholest-4-ene-3-one (**107**) (Figure 17) under mildly acidic conditions, lengthy bromination and debromination steps are required for the protection of the double bond during oxidation of cholesterol (**106**) to cholest-5-ene-3-one (**105**).[186,187] The standard Swern oxidation using oxalyl chloride activated DMSO[175,179] has been shown to efficiently convert **106** to **105** in high yield (ca. 95%).[179] The use of the PEG-bound sulfoxide **103** in the modified Swern procedure described has also been used in the conversion of small quantities (ca. 0.75 mmol) of **106** to **105** (Table 9).[182] This methodology has its advantages in that it would avoid the production of unpleasant by-products (dimethyl sulfide) and afford a much simpler, non-aqueous workup, during the synthesis of pure cholest-5-ene-3-one (**105**).

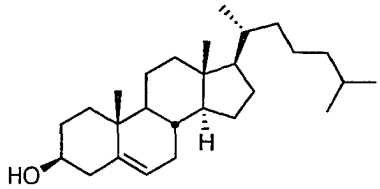
4.2 Modified Swern Oxidation Project Goals

The efficiency of resin-bound sulfoxides like **103** in Swern oxidations offers an efficient alternative for the facile conversion of multi-gram quantities of cholesterol (**106**) to cholest-5-ene-3-one. If successful, reagents like **103** should be readily amendable to reaction scale-up for use on a variety of complex, multi-gram oxidation reactions. The preceding study outlines the details involved in the synthesis of a PEG-bound sulfoxide **103** and its sequential use in the efficient conversion of multi-gram (~10.0 g) quantities of cholesterol (**106**) to cholest-5-en-3-one (**105**).

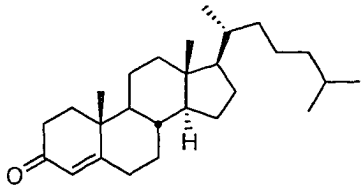
Figure 17 Structures of cholest-5-ene-3-one (**105**), cholesterol (**106**) and cholest-4-ene-3-one (**107**).



Cholest-5-ene-3-one (**105**)



Cholesterol (**106**)

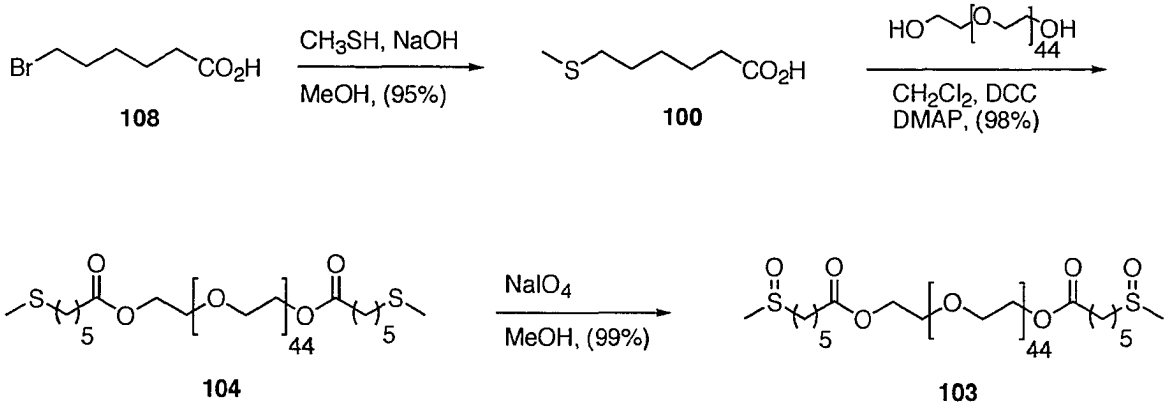


Cholest-4-ene-3-one (**107**)

4.3 RESULTS AND DISCUSSION

Synthesis of multiple gram quantities (>11.0 g) of the PEG-bound sulfoxide **103** (Scheme 30) involves the treatment of commercially available 6-bromohexanoic acid (**108**) with methanethiol in the presence of sodium hydroxide to give 6-(methylthio)hexanoic acid (**100**) in high yield (95%). Two equivalents of the thioether **100** are then coupled to one equivalent of poly(ethylene glycol)-2000 (PEG-2000), using DCC, in the presence of DMAP, to afford the polymer-bound methyl sulfide **104**. Oxidation with sodium metaperiodate (< 4 °C) smoothly leads to the PEG-bound sulfoxide **103**, with no detection of over oxidation products (e.g., sulfone).[182]

Scheme 30 Synthesis of PEG-bound sulfoxide **103**.

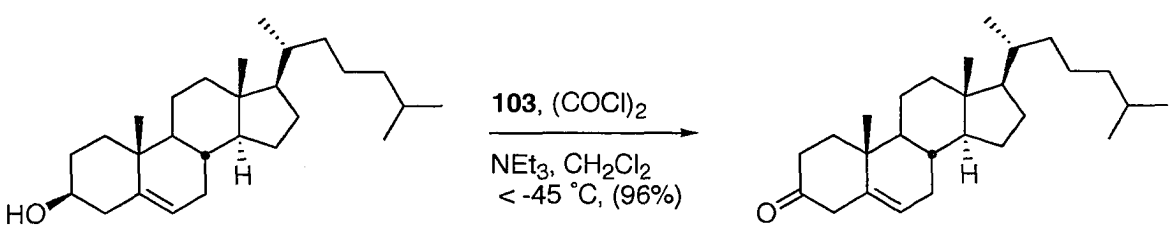


The chemistry employed in the above synthesis is efficient, reproducible and reaction work-ups are generally mild. The only problematic step is the evaporation of methanol during the workup of **103**, which must be done by concentrating the resultant solution at < 10 °C. These conditions ensure that the newly formed PEG-bound sulfoxide **103** is not

over oxidized to the sulfone as has been noted on concentration *in vacuo* at temperatures > 10 °C.[188] It is important to note that sulfide **100** can also be readily oxidized with $NaIO_4$ to give a free sulfoxide **98** and has been used on its own to oxidize alcohols (Table 9).[180] The sulfide produced after oxidation has a less noxious odor than the dimethyl sulfide byproduct produced when DMSO is used.[180]

As mentioned, the previous methodology used to oxidize large quantities of cholesterol (**106**) to cholest-5-ene-3-one (**105**) involves a lengthy, three step procedure whereby cholesterol is first brominated followed by oxidation and subsequent debromination.[186,187] Using the above DMSO substitute **103** it is possible to obtain large amounts of cholest-5-ene-3-one (**105**) (≥10 g) in excellent yield (ca. 96%) in a one-pot, efficient synthesis (Scheme 31).

Scheme 31 Oxidation of cholesterol (**106**) to cholest-5-ene-3-one (**105**).



106 **105**

The modified Swern oxidation reaction (Scheme 31) is done at < 45 °C and involves the activation of the PEG-bound sulfoxide **103** with oxalyl chloride (2.0 eq). Cholesterol (**106**) is then added, followed by triethylamine to yield large quantities of cholest-5-ene-3-one (**105**) (≥10 g) in excellent yield (ca. 96%). The use of a stoichiometric amount of

PEG-supported reagent **103** in Swern oxidation reactions results in a small amount of unreacted alcohol (ca. 4%).[182] Therefore, to drive the reaction to completion, one equivalent (two oxidative equivalents) of the reagent **103** is used.[182] Workup involves concentration of the reaction mixture to one-fifth the volume and addition of excess ether to precipitate the polymer-bound reagent. Cooling this mixture to –20 °C for several hours facilitates the precipitation of reacted polymer-bound reagent, which is readily recovered via filtration in a 1:1 mixture of PEG-bound sulfide to sulfoxide.[182] Concentration of the filtrate affords cholest-5-ene-3-one (**105**), which is slightly contaminated with the polymer-bound reagent mixture (ca. 1%). Subsequent filtration through a pad of silica gives the pure oxidized product, according to ^{1}H NMR, in comparable yields (ca 96%) to the original Swern oxidation.[175] The recovered polymer-supported sulfide/sulfoxide mixture is then re-oxidized with periodate treatment to regenerate the PEG-bound sulfoxide **103** in 91% yield, which, unlike its polystyrene bound counterparts,[180] retains its full oxidation capacity.[181,182] Occasionally, the regenerated polymer-supported sulfoxide has a slight yellow colour due to traces of I_2. This has no deleterious effects on the oxidation capacity of the reagent, but is readily removed by repeated washes of the resin with a dilute solution of sodium thiosulfate during the periodate work-up.[182]

This modified Swern procedure provides considerable improvement over the currently available methods, facilitating scale up through the use of an efficient PEG-bound sulfoxide **103**. Straightforward synthesis of multi-gram quantities of cholest-5-ene-3-one **105** and the potential applicability to other sensitive steroidal systems greatly

facilitates the study of enzymes such as cholesterol oxidase[189] and 3-oxo-Δ^5-steroid isomerase[190] by making substrates readily available in an efficient, short one-pot reaction.

5. SUMMARY AND FUTURE DIRECTIONS

5.1 Oxytocin and Carbocyclic Derivatives

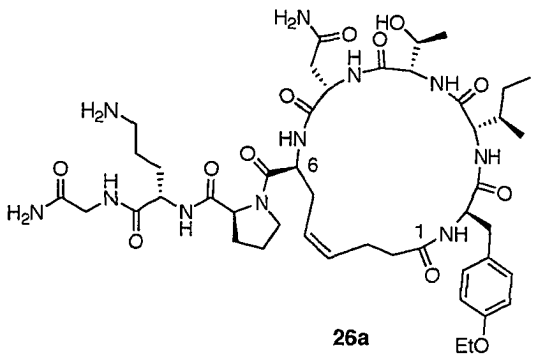
The preceding studies have examined the effects of replacement of sulfur bridges in agonists and antagonists (atosiban (**13**) and cyclohexyl derivative **15**) of oxytocin (**11**) on biological activity and stability. A synthetic strategy involving an RCM reaction on bis-olefin containing, resin-bound peptides has been developed for the replacement of disulfide bridges. Addition of DMSO removes ruthenium by-products after the RCM reaction and facilitates the purification of these analogs by RP-HPLC. For carbocyclic analogs of antagonist **15**, which are inaccessible by the RCM reaction, preformed carbon bridges were made using a methodology developed by the Vederas laboratory[54,55] for the synthesis of DAP and DAS. A derivative of α–aminosuberic acid **60** containing a cyclohexyl moiety was synthesized and incorporated into a linear peptide precursor via Fmoc SPPS. Cyclization of this peptide was accomplished using amide bond forming techniques (PyBOP/HOBt/DIPEA)[46] to afford the carbocyclic peptide derivative **29**, which is the dicarba analog of antagonist **15**.

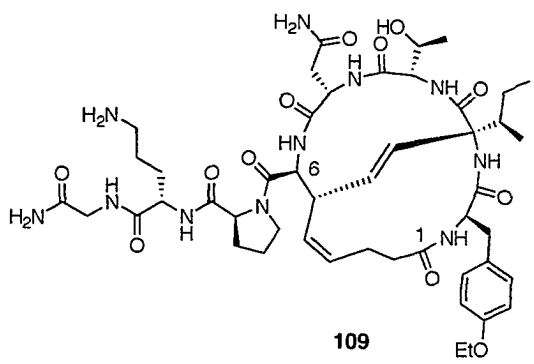
Biological testing of all carbocyclic analogs has shown that replacement of the disulfide bridges in oxytocin (**11**), atosiban (**13**), and antagonist **15** with carbon-carbon linkers (saturated or unsaturated) leads to carbocyclic peptide analogs **18a**, **19**, **26**, **27** and **29** that have only a minimal loss of inherent biological activity. A new methodology for testing the *in vitro* stability of both disulfide-containing and carbocyclic peptide analogs has been developed. This assay involves the use of fresh rat placental tissue which contains enzymes responsible for the metabolism of oxytocin (**11**) *in vivo*.[102] The results

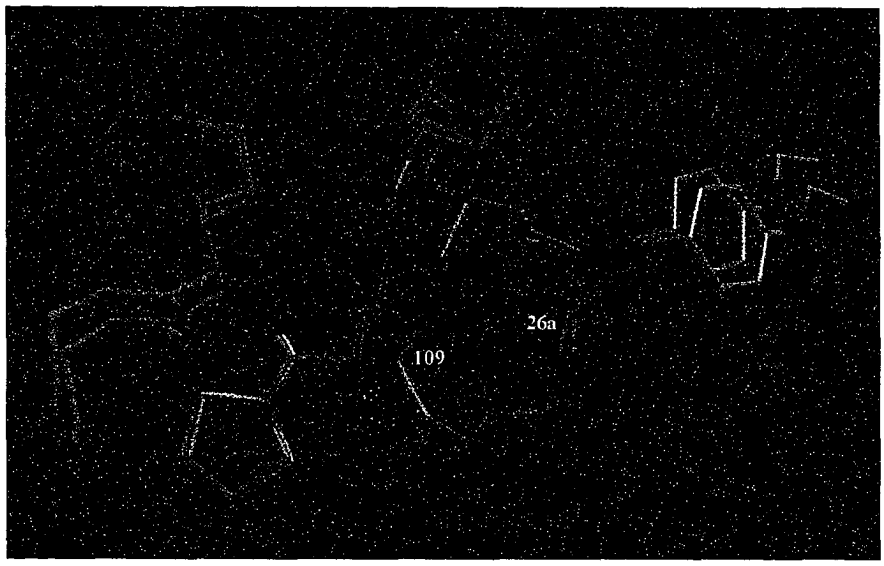
show that carbocyclic agonists as well as antagonists **19** (8-11 min longer) and **27** (> 2 x) have greatly enhanced half-lives in comparison to their parent peptides, oxytocin (**11**) and atosiban (**13**). The observed retention of activity and inherent increased stability of the 1,6-dicarba analogs provides a basis for the design of new oxytocin analogs (especially antagonists) with increased potency and metabolic stability that may prove valuable for the development of an improved therapeutic for treatment of pre-term labor and premature birth.

Future directions may involve the application of this RCM methodology towards a generation of derivatives that are functionalized on the bridge carbons (e.g., via bis-hydroxylation, epoxidation or Diels Alder reactions of the olefin), thereby rigidifying the carbocyle, and mimicking the preference for a nearly 90° dihedral angle in the disulfide moiety. If a bis-hydroxyl moiety is added at the double bond of the olefin carbocyles, possible oxidation to the respective diketone functionality using PEG-bound sulfoxide **103** on the free peptide would provide a convenient method for the synthesis of such derivatives. Over the last decade, a great deal of interest in peptide chemistry has been devoted to the synthesis and study of rigid molecules that lack conformational freedom and often result in a higher receptor binding affinity and/or modulation in biological activity.[191] Bicyclic analogs of oxytocin have been synthesized by Hruby and co-workers,[191b-f] and are shown to have potent antagonistic activity. Through NMR and computational chemistry techniques this was shown to be a result of conformational restriction of the desired peptides bioactive conformation.[115,191d,e] Modeling studies of bicyclic structural analogs of atosiban **13** show that the structure of *cis* dicarba analog **26a** closely resembles a bicyclic derivative **109** (Figure 18).

Figure 18 Structure of bicyclic analog **109** and overlaid energy minimized structures of **26a** and **109** (done by Dr. Kamaljit Kaur)[a, b]





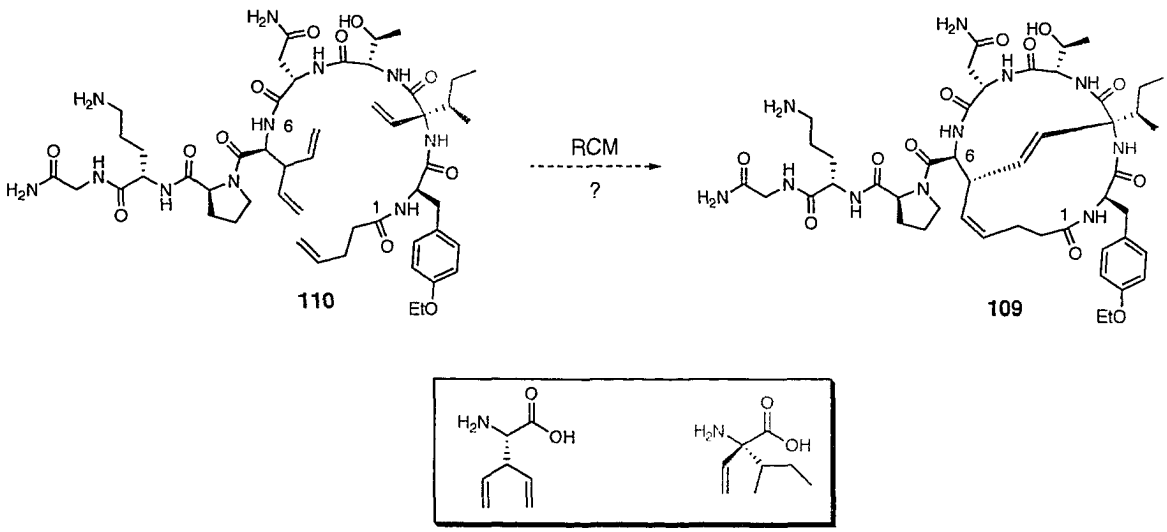


[a] Side-chain on Asn (5) deleted for clarity.
[b] Olefins drawn as they appear in model.

Performing an RCM reaction(s) (Scheme 32) on a tetra-olefin-containing linear precursor **110** may provide the bicyclic tetra-carba analog **109** of atosiban (**13**) with increased conformational constraint. This increased constraint may have a profound effect on activity and stability, and facilitate in the separation of all possible stereo-

isomers.[191] Such changes may offer additional avenues for improvement of drug properties by medicinal chemistry.

Scheme 32 Proposed synthesis of bicyclic peptide analog **109**.[a]

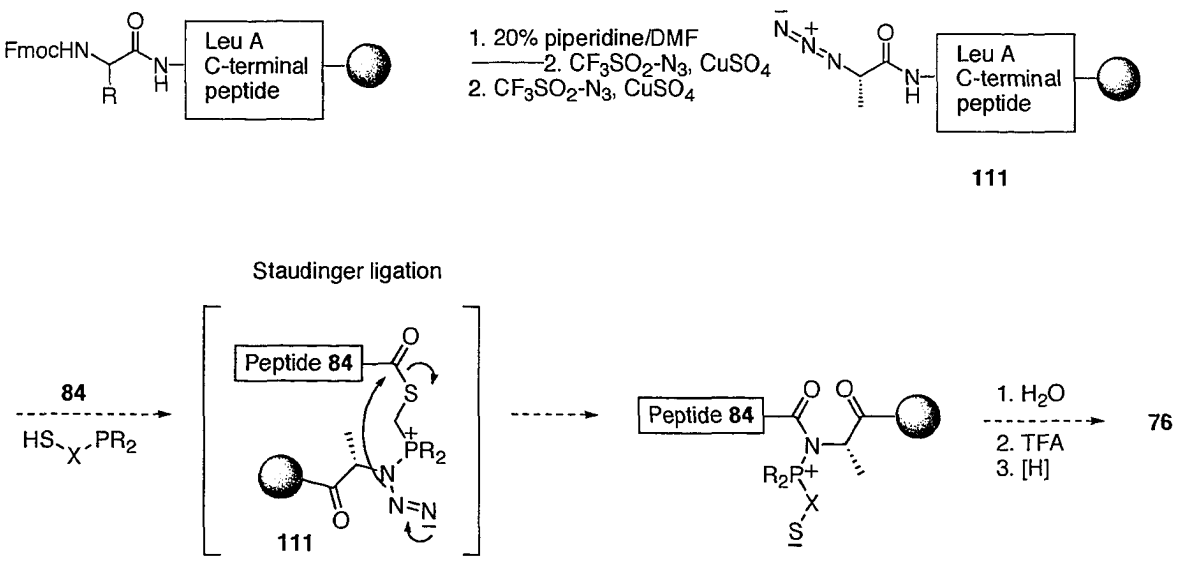


It has also been shown that selective glycosylation of AVP (**14**) on the phenolic functionality of tyrosine at position 2 increases the duration of biological activity.[192] This is presumably due to the delivery of the intact peptide to the receptor site, thus retarding the enzymatic metabolism of AVP **14** *in vivo*.[192] It would be of interest to see if this enhanced duration of activity holds true for oxytocin (**11**) and especially for its active and already more stable carbocyclic derivatives such as **17**, **18a**, **18b**. Glycosylation of atosiban (**13**) and analogs **26** and **27** on asparagine at position 5 or tyrosine at position 1 of antagonist **15** and analog **29** would also be of interest to test for enhanced duration of activity.

5.2 9,14-Dicarba Leucocin A

A small quantity of 9,14-dicarba leucocin A (**76**) has been synthesized and detected by MALDI-TOF/MS. This was accomplished using a convergent approach whereby a carbocyclic analog of the N-terminal portion of leucocin A **84**, synthesized by SPPS and cyclized using the RCM methodology, and the C-terminal peptide **85** were ligated using native chemical ligation (NCL). However, the low yielding ligation step, as well as the problems encountered during the desulfurization of ligated precursor **88** suggests the investigation of a new synthetic strategy. The traceless Staudinger ligation[24-26,193] (Scheme 33) allows for the ligation of peptide fragments without the requirement for a cysteine residue in the peptide backbone, thus requiring no desulfurization step.

Scheme 33 Possible on-resin traceless Staudinger ligation of peptides **84** and **111**.[24-26,193,194]



This methodology would employ the use of the readily available peptide thioester **84** and the synthesis of a C-terminal fragment **111** containing an N-terminal azide functionality. Methodology for the formation of N-terminal azides on resin-bound peptides has recently been developed by Liskamp and co-workers,[194] making this ligation technique very appealing.

5.3 Lacticin 3147 A2 Tricyclic (ABC) Carbocyclic Analog

The synthesis of a tricyclic ABC ring carbocyclic fragment **95** using three sequential RCM reactions has been achieved. To our knowledge, this is the first report of three sequential RCM cyclizations being done on a single resin-bound peptide. On resin reduction of **95** using both *in situ* diimide formation[124,169] or hydrogenation with Wilkinson's catalyst[170-172] fails to produce any of the reduced tricyclic peptide **96**. Further investigation into the on-resin reduction of the olefins in **95** is of interest, and may be accomplished using palladium nanoparticle clusters.[195,196] These clusters are derived from the reaction of H_2PdCl_4 with poly(*N*-vinyl-2-pyrrolidone) (PVP) at reflux to give PVP protected Pd nanoparticles (PVP-Pd). In the presence of an H_2 atmosphere, these PVP-Pd particles may be able to enter the pores of the Wang resin allowing for the reduction of **95** to **96**.

Although not mentioned, the correct connectivity of the olefin rings in **95** will have to be verified using 2-D NMR correlation experiments (COSY, HMBC, HMQC) to ensure ring opening polymerization (ROMP) reactions have not occurred. This polymerization was not detectable by MALDI-TOF/MS but a full NMR study is required to prove this. To ensure this is not the case, synthesis of a saturated ABC tricycle **96** may be done with

reduction of olefins after each cyclization reaction. This might not only forego the problems encountered in the reduction of the three separate carbocycles in **95** at one time, but it would also avoid the possibility of ROMP reaction.

Finally, testing of the olefinic tricycle **95** (and perhaps the bicycle **93**) for synergistic behavior with respect to natural lacticin 3147 A1 **77** would be of interest to determine whether the dehydro amino acid containing tail of lacticin 3147 A2 **78** is necessary for antimicrobial potency.

6. EXPERIMENTAL PROCEDURES

6.1 General Experimental Methods

6.1.1 Reagents, solvents and solutions

All chemicals and solvents were purchased from the Aldrich Chemical Company Inc., (Madison WI), Sigma Chemical Company (St. Louis MO) or Fisher Scientific Ltd. (Ottawa ON). Unless otherwise stated, all protected amino acids, derivatives and SPPS solid supports were purchased from Calbiochem-Novabiochem Corporation (San Diego CA), Sigma-Aldrich Canada Ltd. (Oakville, ON) or Bachem California Inc. (Torrance CA). Solvents for anhydrous reactions were dried according to Perrin *et al.*[197] Tetrahydrofuran (THF) and diethylether were distilled over sodium under an argon atmosphere. Acetonitrile, dichloromethane and triethylamine were distilled over calcium hydride. Methanol was distilled over magnesium turnings and a catalytic amount of iodine. Water was obtained from a Milli-Q reagent water system. All other reagents and solvents were either reagent or HPLC grade and used without further purification. Unless otherwise specified solutions of $NaHCO_3$, HCl, NaOH, KOH and LiOH refer to aqueous solutions. Brine refers to a saturated solution of NaCl. For the Swern reaction modification it is important to note that methanethiol is toxic, volatile (bp 6 °C) and has an obnoxious stench. Therefore, care must be taken to decompose excess reagent with either $Pb(OAc)_2$ or a bleach solution. The mass of condensed gas was calculated from the weight loss of the lecture bottle. PEG-2000 stands for poly(ethylene glycol) of molecular weight 2000.

6.1.2 Purification techniques

Analytical TLC was performed on glass plates (1.5 x 5 cm) precoated (0.25 mm) with silica gel (Merck, Silica Gel 60 F_{254}). Compounds were visualized by exposure to UV light, I_2 or by dipping the plates in solutions of 0.3% ninhydrin/97% EtOH/3% AcOH (w/v/v) or $(NH_4)_2Mo_7O_{24} \cdot 4\ H_2O$ 10% H_2SO_4 followed by heating on a hot plate. Column chromatography was performed using the method of Still,[198] employing silica gel grade 60 (Rose Scientific, 230-400 mesh).

Preparative high performance liquid chromatography (prep-HPLC) was performed on a GILSON high performance liquid chromatograph equipped with a 322 pump, a manual injector and a single wavelength, Gilson 151/152 UV/VIS detector. UniPoint™ System Software was used to record and analyze the chromatograms. Columns used for reversed phase prep-HPLC were Waters radial compression modules with μBondpak™ 10 μm, 125 Å, 25 x 100 mm, PrepPak® C_{18} columns and guard columns with similar packing. Unless otherwise stated, two mobile phases were used for peptide purification. System A: Eluting with 10% MeCN/90% H_2O (0.1% TFA) for 5 min, then a gradient of 10-55% MeCN over 20 min, at a flow rate of 15 mL/min. System B: Eluting with 10% MeCN/90% H_2O (0.1% TFA) for 5 min, then a gradient of 10-90% MeCN over 20 min at a flow rate of 15 mL/min. All peptides were detected by the presence of amide bonds at 220 nm.

6.1.3 Instrumentation for compound characterization

NMR spectra were recorded on a Varian Inova 600, Inova 500, Inova 400, Inova 300 or Unity 500 spectrometer. For ^1H (300, 400, 500 or 600 MHz) spectra, δ values were

referenced to H_2O (4.79 ppm), CH_2Cl_2 (5.32 ppm), $CHCl_3$ (7.24 ppm), CH_3OH (3.34 ppm) or DMSO (3.53 ppm), and for ^{13}C (75, 100, 125 or 150 MHz) spectra, δ values were referenced to CD_2Cl_2 (53.1 ppm), $CDCl_3$ (77.0 ppm), CD_3OD (49.0 ppm) or DMSO-d_6 (39.7 ppm). First order behavior was assumed in all analysis, and ^1H NMR multiplets (in peptides) were reported as a single value for narrow peaks and a over a range for larger multiplets. Additional assignments were done using pulsed field gradient versions of shift correlation spectroscopy (gCOSY), total correlation spectroscopy (gTOCSY), heteronuclear multiple quantum coherence (gHMQC) and heteronuclear multiple bond correlation (gHMBC).

Selective multiple site homonuclear decoupling experiments on olefinic peptides (in D_2O) were accomplished using shifted laminar pulses on a Varian Inova 600 MHz spectrometer running at 599.933 MHz. The instrument was equipped with a Sun microsystems® Ultra 5, running VNMR 6.1C software, and a wave form generator to produce shaped pulses. The program PBOX, part of the spectrometer software package VNMR 6.1C, was used to calculate the WURST-2 pulse shape required for the decoupling experiments. To calculate the WURST-2 shape, peaks belonging to protons that were to be decoupled from the olefin protons were interactively input into the program PBOX, along with a reference coupling constant between these protons. Additionally, a transmitter power of 53 and a pulse width of 5.5 for a 90° rectangular ^1H pulse, was required as input for PBOX. The parameters calculated by PBOX were then input as standard decoupling parameters, using a decoupling power of 21, in a normal ^1H pulse sequence. Finally, the transmitter and decoupler offsets were set to be equal (tof = dof). The spectrum was acquired with a total of 16 scans, a spectral width of 8000 Hz and

a total of 48000 points. Coupling constants between the adjacent olefin protons were measured directly and accurately from the acquired spectrum, and used to assign *cis/trans* geometry about the double bond. Unless otherwise mentioned, the chemical shifts for the adjacent olefin protons are reported for cyclic peptides as "hidden AB quartets" as the fully coupled spectrum was used for ^{1}H NMR assignments.

Microanalyses were obtained on Perkin Elmer 240 or Carlo Erba 1180 elemental analyzers. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a micro cell (100 mm path length 1 mL). IR spectra were recorded on a Nicolet Magna-IR 750 with Nic-Plan microscope FT-IR spectrometer. Unless otherwise specified, spectra were recorded on a Kratos AEI MS-50 (HREIMS), ZabSpec Isomass VG (HRESMS) in positive mode or a Perspective Biosystems Voyager™ Elite MALDI-TOF using either 4-hydroxy-α-cyanocinnamic acid (HCCA) or 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid) as matrices.

6.1.4 Computer Modelling Experiments

Computer modelling experiments for oxytocin (**11**), dicarba analog **18a** dicarba atosiban analog **26a** and bicyclic analog **114** were done using Spartan™ for Mactintosh 2002. Lowest energy structures were constructed using Hartree-Fock, 3-21G calculations to provide for energy minimized structures which were then overlaid with each other to determine structural similarity.

6.1.5 General method for solid phase peptide syntheses (SPPS)

All peptides were synthesized either manually using a 50 mL SPPS vessel equipped with a 3-way stop-cock and 'C' fritted ground glass joint or on a Rainin Protein Technologies PS3 automated peptide synthesizer. All peptides were prepared using "L" configured amino acids with N-terminal 9*H*-fluorenylmethoxycarbonyl (Fmoc) protection on Rink amide,[20] Wang[19] or Sieber amide[21] resins (Calbiochem-Novabiochem Corporation (San Diego CA)). All amino acids were coupled using either benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) or dicyclohexyldiimide (DCC) as the activating agent, 1.15 equivalents of amino acid (compared to PyBOP) or 2.10 equivalents of amino acid (compared to DCC) and 2-3 h coupling times. The coupling of successive amino acids to the resin using PyBOP or DCC was done as follows. For the coupling of amino acids with PyBOP a 25% solution of *N*-methylmorpholine (NMM) was added to the N-Fmoc protected amino acid (1.15 eq relative to the resin) followed by PyBOP (1.00 eq relative to the resin). This mixture was stirred for 10 min and added to the resin which was previously swollen in *N,N*-dimethylformamide (DMF) and agitated with bubbling argon for 2-3 h. For the coupling of amino acids to the resin with DCC, the *N*-Fmoc acid (4.00 eq relative to the resin) in CH_2Cl_2 was added to a solution of DCC (2.00 eq) in CH_2Cl_2 at 0 °C. This mixture was stirred for 20 min at 0 °C and concentrated *in vacuo*. The residue was taken up in DMF and added to the resin, previously swollen in DMF and agitated with bubbling argon for 2-3 h.

The Kaiser test[167] for free amines was used to determine the completion of the reaction as follows. To a small sample of resin bound peptide in a test tube was added 2

drops of ninhydrin (5 g in 100 mL EtOH), 90% aqueous phenol (80 g in 20 mL EtOH) and KCN (2 mL 1 mM KCN in 98 mL pyridine). This mixture was then heated at 110 °C for 6 min. The presence of blue coloured beads (positive test) is an indication of the presence of free amine and thus incomplete coupling. The coupling procedure was repeated until no more blue colour is observed in the resin beads. Once no colour is seen in the beads (negative test), the resin bound peptide is treated with 20% Ac_2O/DMF to end-cap any remaining free amine. N-Terminal Fmoc removal from the resin bound amino acid was accomplished with a 20% solution of piperidine in DMF (5 min x 2). The Kaiser test was used to determine the presence of free amine as described above, only this time blue coloured beads (positive test) were desired.

6.1.6 Ring closing metathesis (RCM) of peptides using catalysts 8 or 9

All peptides cyclized using RCM reactions were done in degassed CH_2Cl_2 on fully protected, linear resin-bound precursors prior to N-terminal Fmoc removal. Degassing was accomplished by vigorous bubbling of argon through anhydrous CH_2Cl_2 for 45-60 min. The general procedure for RCM on these linear bound precursors is as follows. To a suspension of resin-bound peptide in degassed CH_2Cl_2 was added a solution of either **8** or **9** (20 mol%) in CH_2Cl_2. This entire mixture was then heated to gentle reflux for 16 h. The reaction mixture was then cooled to rt, DMSO (50 eq relative to **8** or **9**) was added and the mixture was stirred for an additional 12 h. The resin-bound peptide was then filtered and washed successively with CH_2Cl_2 and MeOH. Final N-terminal Fmoc removal was accomplished on the resin-bound peptide using a solution of 25% piperidine in DMF (5 min x 2). The cyclic peptides were then cleaved from the resin using cleavage cocktails

with the following composition: (A) 18:1:1 TFA/CH_2Cl_2/Et_3SiH, (B) 18:1:1 TFA/CH_2Cl_2/iPr_3SiH or (C) TFA/H_2O/iPr_3SiH with gentle swirling for 3-4 h. This mixture was then filtered and the filtrate concentrated *in vacuo* to 0.5 mL. Precipitation with ice cold Et_2O yields the cyclic peptides as off-white solids for purification by prep-HPLC as described.

6.1.7 Reduction of olefinic peptides using 10% Pd/C

Reductions of olefinic peptides **18a**, **19 (a,b)**, **22a** and **26a** were performed using 10% Pd/C under an H_2 atmosphere as follows: 10% Pd/C (10% w/w) was added to a solution of cyclic olefinic peptide in anhydrous EtOH. This solution was then stirred vigorously at rt under H_2 at 760 torr for 36 h. This suspension was then filtered through Celite, concentrated *in vacuo* and subjected to prep-HPLC as mentioned to give the reduced peptides **17**, **20**, **23** and **27** in quantitative yield according to HPLC and ^1H NMR.

6.1.8 Assay for agonistic activity of oxytocin analogs

Oxytocin analogs **17**, **18a** and **18b** and antagonist analogs **19-29** were tested for agonistic as well as antagonistic activity. Testing of peptides for agonistic activity was done using freshly excised uteri from mature non-pregnant female Sprague Dawley rats (~250 g), and when possible the experiments were done in triplicate using tissue samples from three separate animals. Muscle bath preparations were done based on published methodology.[44,102] Rat uteri were cut into strips (3 x 10 mm) and mounted vertically on a Biopac Systems Inc. Myobath™ apparatus, using wire hooks in separately jacketed organ baths at 30 °C. These baths contained 10 mL of Krebs buffer (118 mM NaCl; 4.7 mM

KCl; 2.5 mM $CaCl_2$; 1.2 mM KH_2PO_4; 0.59 mM $MgSO_4$; 25 mM $NaHCO_3$; 11.7 mM D-glucose) at pH 7.4 with constant CO_2 purging. One end of each strip was anchored in the bath and the other end was attached to a FT-03C force-displacement transducer connected to a World Precision Instruments Inc. transducer/computer interface. Resting tension was set to 1g to provide maximum active tension. Oxytocin (**11**) injections were made directly into the muscle baths every 5 min with the following increasing oxytocin concentrations; 0.78, 3.12, 12.50, 50.00 and 200 nM, respectively. The percent activity values were measured for oxytocin (**11**) at these concentrations, and dose-response curves were constructed for each separate trial. All analogs were injected into the muscle baths in the same manner as oxytocin. Measurements were recorded in 5-min blocks with analog injections of increasing concentration being added at the end of each 5-min time interval. Oxytocin analogs were measured for uteronic activity up to 10 μM. Dose-response curves for the analogs were constructed at the conclusion of each 60 min experiment, and compared to oxytocin (**11**). Approximate EC_{50} values were calculated from regression calculations using Microsoft Excel™.

6.1.9 Assay for antagonistic activity of oxytocin analogs

Biological testing for oxytocin antagonistic activity was done on atosiban (**13**), antagonist (**15**) and carbocyclic peptides **19-29** using fresh uterine tissue as prepared above. Oxytocin (**11**), and analog injections were made directly into the baths. Muscle strips were incubated for 4 min at 30 °C directly in the baths with a known concentration (ng/mL) of antagonist. Oxytocin (**11**) (0.78 nM) is then added directly into the baths containing incubated antagonists and the data was recorded over a 3 min interval

immediately after incubation. At the end of the 3 min interval, oxytocin (**11**) of a higher concentration (3.0 nM) is added and data was again collected over 3 min. This process is repeated every 3 min with increasing concentrations of oxytocin at 13.0, 50.0 and 200 nM, respectively. Oxytocin-inhibitory curves (Schild plots[100]) were constructed from the data collected from each experiment and the results were compared to the known oxytocin antagonists atosiban (**13**) and the antagonist **15** using Microsoft Excel™. The pA_2 values (defined as the negative logarithm of the concentration of antagonist that diminished the OT activity of a double dose to that of a single dose) for each OT antagonist analog were calculated using the Schild plot analysis method.[100] These results are reported in Table 5.

6.1.10 Preparation of fresh placental tissue homogenate.

Placental tissue from freshly killed pregnant Sprague Dawley rats at day 19 of gestation, is excised and placed in 40 mM sodium phosphate buffer at pH 7.0 to give a final concentration of 0.1-0.2 g of tissue in 0.5 mL of buffer. This mixture is then homogenized using a Model PRO 200 homogenizer from PRO Scientific Co. The resulting suspension is centrifuged for 15 min at 4 °C and 2000 g. The supernatant is then decanted and used as the placental homogenate for *in tissue* stability tests.

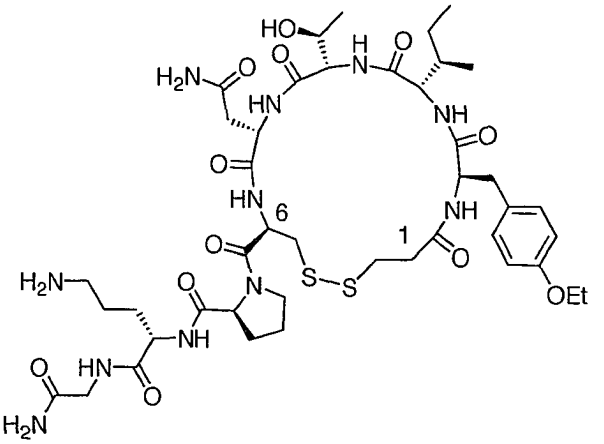
6.1.11 Assaying for *in tissue* biological stability of oxytocin analogs.

A new method was developed for testing the duration of activity of oxytocin analogs in fresh placental homogenate. Biological stability tests were done on compounds **11**, **13**, mixture **21** and **29** using freshly excised uteri from non-pregnant, female Sprague Dawley

rats (~250 g). Muscle bath preparations were carried out as mentioned above for agonistic and antagonistic assays. Placental tissue homogenate from freshly killed rats provided for maximum quantities of oxytocinase. Separate solutions containing 1 μM of oxytocin (**11**) and agonist mixture **21** were incubated with the homogenate serum for 0, 5, 10, 15, 20, 25 and 30 minutes at 37 °C. Samples of oxytocin (10 nM) and **21** (1.0 μM) were then taken directly from these homogenate solutions and added directly into the muscle baths. Maximum activity was measured at time 0 min for each compound in phosphate buffer alone at pH 7.0. The decrease in percent activity of the analogs in placental homogenate was measured at each 5 min incubation interval. Percent activity versus incubation time curves were constructed for compounds **11** and **21(a, b)** and half-lives (in minutes) were calculated for each trial using regression calculations from Microsoft Excel™. The values reported are from two separate trials using placental homogenate from two separate animals. Atosiban (**13**) and antagonist **27** were tested in a similar manner to the above agonists. Both atosiban (**13**) and analog **27** were incubated as 100 nM solutions in the placental homogenate. Incubation times were set at 0, 1, 2, 3, 4 and 6 h respectively. Muscle baths were incubated for 4 min with samples of atosiban (10 nM) and **27** (100 nM) from the respective antagonist-homogenate solutions (100 nM) followed by addition of oxytocin (**11**) every 3 min using the following concentrations in ascending order; 0.98, 3.9, 15.6, 62.5 and 250 nM respectively. Percent activity of oxytocin response was measured at a concentration of 3.9 nM of **11**, and these trials were repeated on homogenate samples from two separate animals. The maximum inhibition for compounds **13** and **27** were measured in phosphate buffer alone at pH 7.0 and were recorded as time 0 h. Any decrease in percent inhibition was measured at each of the 3

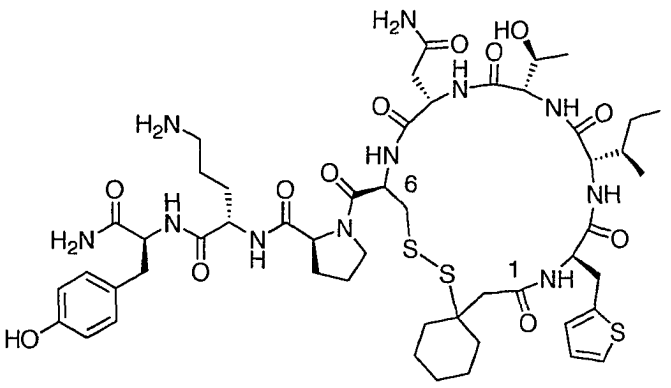
min interval with the concentrations of **11** indicated. From these results, percent activity graphs were constructed for compounds **13** and **27** using regression calculations from Microsoft Excel™, for each separate trial. The half-lives (in min) for atosiban (**13**) and **27** were measured separately and compared for each trial (Table 6).

6.2 Synthesis and Characterization of Compounds



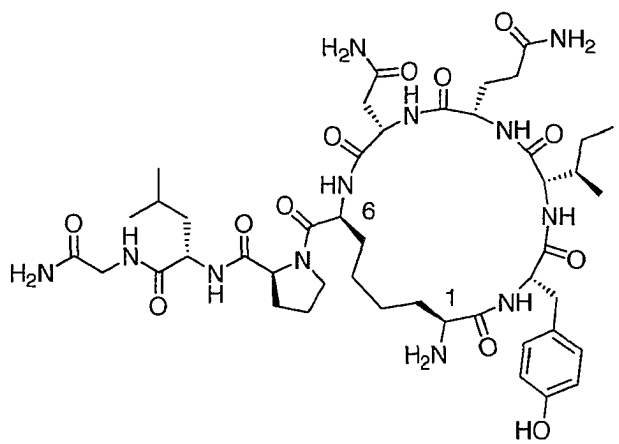
Glycinamide, *O*-ethyl-N-(3-mercapto-1-oxopropyl)-D-tyrosyl-L-isoleucyl-L-threonyl-L-asparaginyl-L-cysteinyl-L-propyl-L-ornithyl-, cyclic (1→5)-disulfide (Atosiban) (13).[86] The linear precursor to **13** was synthesized on Rink amide NovaGel™ (0.66 g, 0.40 mmol) using standard Fmoc SPPS. The following *N*-Fmoc protected amino acids were coupled using PyBOP in this order: Fmoc-Gly-OH, Fmoc-Orn(*N*-Boc)-OH, Fmoc-Pro-OH, Fmoc-Cys(*S*-Trt)-OH, Fmoc-Asn(*N*-Trt)-OH, Fmoc-Thr(*O*-*t*-Bu)-OH, Fmoc-Ile-OH, Fmoc-D-Tyr(*O*-Et)-OH, (*S*-Trt)-3-mercaptopropionic acid (**67**). The linear precursor **69** was cleaved from the resin by treatment of the polymer-bound peptide with cleavage cocktail (C) (15 mL) for 3 h at rt. Concentration of this cleaved mixture *in vacuo* followed by precipitation with cold Et_2O yielded the crude linear peptide **69** as an off-white solid; MALDI-TOF (MS) 996.8 (M+H). The solid was dissolved in 0.1 mM

NH_4HCO_3 (100 mL, 1 mg/mL) buffer at pH 8.0, and treated with O_2 and vigorous stirring for 18 h. This mixture was then concentrated *in vacuo* to one third of the original volume, and the remainder of the buffer was removed by lyophilization. Peptide **13** was isolated as a single peak by prep-HPLC (t_R = 14.94 min) using solvent system B to afford atosiban (**13**) as a white fluffy solid after lyophilization (11 mg, 7.3% based on 1/4 (~0.10 mmol) of cleaved resin-bound linear precursor **69**). ^{1}H NMR assignments can be found in Table 15; ^{1}H NMR (D_2O, 600 MHz) δ 7.22 (ap. d, 2H, *J* = 8.6 Hz), 6.96 (ap. d, 2H, *J* = 8.6 Hz), 4.85 (dd, 1H, *J* = 3.2, 10.0 Hz), 4.65 (dd, 1H, *J* = 6.3, 9.9 Hz), 4.61 (m, 1H), 4.44 (dd, 1H, *J* = 6.0, 8.0 Hz), 4.35 (m, 2H), 4.17 (m, 1H), 4.14 (d, 1H, *J* = 4.9 Hz), 4.10 (q, 2H, *J* = 7.1 Hz), 3.92 (m, 2H), 3.84 (m, 1H), 3.73 (m, 1H), 3.13 (m, 1H), 3.20 (m, 3H), 2.96 (m, 2H), 2.92-2.82 (m, 3H), 2.81-2.68 (m, 3H), 2.58 (m, 1H), 2.32 (m, 1H), 2.03 (m, 2H), 1.92 (m, 2H), 1.80 (m, 4H), 1.37 (t, 3H, *J* = 7.1 Hz), 1.20 (d, 3H, *J* = 6.4 Hz), 1.05 (m, 1H), 0.83 (m, 1H), 0.74 (t, 3H, *J* = 7.2 Hz), 0.53 (d, 3H, *J* = 7.0 Hz); ^{13}C NMR (D_2O, 150 MHz) δ 175.3, 175.1, 174.9, 174.8, 174.4, 173.4, 171.9, 171.2, 158.0, 131.4, 129.5, 116.0, 68.4, 65.2, 59.9, 59.5, 56.7, 54.3, 51.9, 48.9, 43.2, 39.6, 36.5, 31.0, 30.3, 28.5, 25.6, 25.0, 24.0, 19.5, 17.5, 15.1, 14.4, 11.9; MALDI-TOF (MS) calcd. for $C_{43}H_{67}N_{11}O_{12}S_2$ 993.4. Found 994.4 (M+H, 100%), 1016.4 (M+Na, 73%), 1032.3 (M+K, 48%).



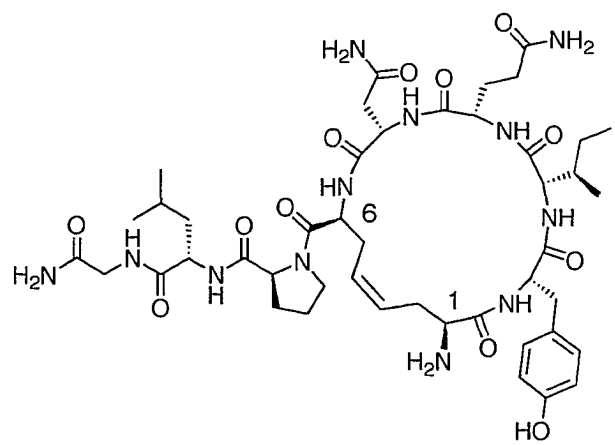
L-Tyrosinamide, *N*-(3-vinyl-3,3-cyclohexyl-1-oxopropyl)-D-thienyl-L-isoleucyl-L-threonyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-ornithyl-, cyclic (1→5)-disulfide (15).[97] The linear precursor to **15** was synthesized and cleaved in the same manner as peptide **13** using Rink amide NovaGel (0.66 g, 0.40 mmol) and standard Fmoc SPPS. The following N-Fmoc protected amino acids were coupled with PyBOP in the order: Fmoc-Tyr(*O*-*t*-Bu)-OH, Fmoc-Orn(*N*-Boc)-OH, Fmoc-Pro-OH, Fmoc-Cys(*S*-Trt)-OH, Fmoc-Asn(*N*-Trt)-OH, Fmoc-Thr(*O*-*t*-Bu)-OH, Fmoc-Ile-OH, Fmoc-D-Thi-OH, (*S*-PMB)-1-(3-mercapto-3,3-cyclopentamethylene)propionic acid (**68**). The resin bound linear precursor **70** was cleaved from the resin and cyclized in the same manner as **69**. Lyophilization and purification by prep-HPLC (t_R = 15.76 min) using the same solvent system (B) as for **13** gave **15** (15 mg, 9.0% based on 1/4 (~ 0.10 mmol) of cleaved resin) as a white fluffy solid. ^{1}H NMR assignments can be found in Table 16; ^{1}H NMR (D_2O, 600 MHz) δ 7.31 (dt, 1H, J = 1.2, 5.1 Hz), 7.13 (ap. d, 2H, J = 7.9 Hz), 7.01 (m, 1H), 6.95 (m, 1H), 6.83 (ap. d, 2H, J = 7.5 Hz), 4.56 (m, 1H), 4.51 (m, 1H), 4.36 (dd, 1H, J = 7.0, 7.7 Hz), 4.24 (m, 2H), 4.15 (m, 2H), 3.81 (m, 2H), 3.47 (dd, 1H, J = 5.0, 14.9 Hz), 3.20 (dd, 1H, J = 8.6, 15.1 Hz), 3.12 (dd, 1H, J = 5.7, 14.0 Hz), 3.07 (dd, 1H, J = 2.7, 14.4 Hz), 2.99-2.89 (m, 4H), 2.86 (dd, 1H, J = 6.4, 15.8 Hz), 2.72 (m, 2H), 2.68 (d, 1H, J = 14.1 Hz), 2.51 (d, 1H, J = 14.1 Hz), 2.22 (m, 1H), 2.01 (m, 4H), 1.90-1.43 (m, 16H), 1.31 (m, 2H), 1.22 (m,

2H), 1.19 (m, 1H), 1.12 (m, 1H), 0.89 (d, 3H, J = 6.8 Hz), 0.83 (t, 3H, J = 7.4 Hz); ^{13}C NMR (D_2O, 150 MHz) δ 178.1, 176.4, 175.7, 174.8, 174.4, 174.2, 173.9, 173.8, 173.3, 172.4, 172.0, 155.3, 139.4, 131.4, 127.9, 127.6, 126.1, 116.6, 70.4, 67.4, 61.6, 58.9, 55.6, 55.4, 54.9, 53.8, 53.2, 51.1, 49.1, 44.5, 39.5, 37.7, 37.1, 35.9, 35.2, 32.0, 31.0, 30.3, 28.5, 25.7, 24.8, 24.0, 17.5, 16.1, 11.2; MALDI-TOF (MS) calcd for $C_{51}H_{75}N_{11}O_{12}S_3$ 1129.5. Found 1130.7 (M+H, 74%), 1152.7 (M+Na, 100%), 1168.7 (M+K, 79%).



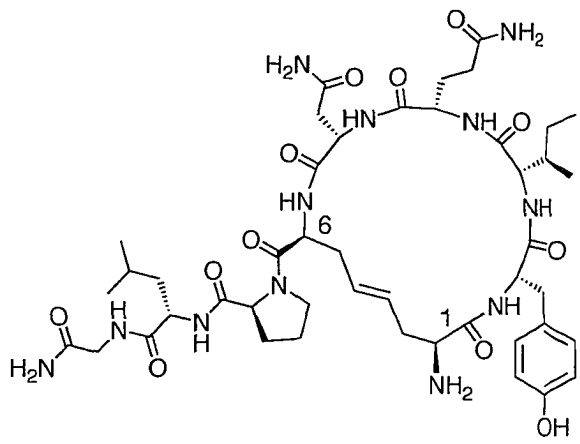
[1,6-α,α'-L,L-Diaminosuberic acid]oxytocin (17).[44] A sample of peptide **18a** (8 mg, 8 μmol) was reduced as described in the general methods (section 6.1.6) to give peptide **17** as a single peak (t_R = 14.81 min) by prep-HPLC using solvent system A to afford **17**, upon lyophilization, as a white fluffy solid (8 mg, quant.). ^{1}H NMR assignments can be found in Table 10; ^{1}H NMR (D_2O, 600 MHz) δ 7.21 (ap. d, 2H, J = 8.6 Hz), 6.88 (ap. d, 2H, J = 8.6 Hz), 4.68 (dd, 1H, J = 6.0, 6.0 Hz), 4.55 (dd, 1H, J = 5.0, 5.5 Hz), 4.42 (m, 1H), 4.39 (dd, 1H, J = 6.0, 6.5 Hz), 4.26 (m, 1H), 4.21 (d, 1H, J = 5.5 Hz), 4.03 (dd, 1H, J = 6.1, 8.0 Hz), 3.90 (m, 1H), 3.86 (d, 1H, J = 17.1 Hz), 3.82 (d, 1H, J = 17.1 Hz), 3.73 (m, 1H), 3.59 (m, 1H), 3.15 (dd, 1H, J = 5.9, 14.3 Hz), 2.87 (dd, 1H, J = 9.0, 14.7 Hz), 2.84-2.78 (m, 2H), 2.35 (m, 2H), 2.28 (m, 1H), 2.01-1.90 (m, 4H), 1.88-1.82 (m, 4H),

1.80-1.62 (m, 4H), 1.55 (m, 1H), 1.48-1.22 (m, 5H), 1.04 (m, 1H), 0.90 (d, 3H, J = 6.3 Hz), 0.93-0.82 (m, 9H). ^{13}C NMR (D_2O, 125 MHz) δ 178.4, 176.1, 175.3, 174.8, 174.4, 174.2, 173.3, 172.5, 172.2, 169.5, 155.4, 131.3, 129.2, 118.4, 116.4, 61.2, 61.1, 59.0, 56.0, 55.6, 53.4, 52.8, 51.5, 48.5, 42.9, 42.5, 40.3, 38.1, 37.1, 35.7, 31.8, 31.0, 30.5, 30.0, 26.7, 26.1, 25.6, 25.1, 25.0, 24.6, 23.0, 21.5, 15.6, 11.4, 11.2; ES (MS) calcd. for $C_{45}H_{70}N_{12}O_{12}$ 970.52. Found 971 (M+H).

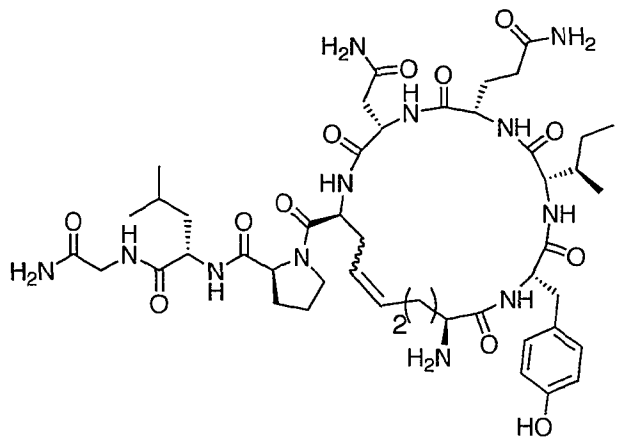


(*cis*)-[1,6-α,α'-L,L-Diamino-γ,γ'-dehydrosuberic acid]oxytocin (**18a**).[44] Peptide synthesis of **17-18b** was done on 0.5 mmol scale using standard Fmoc SPPS conditions on Rink amide NovaGel™ resin (0.80 g, 0.50 mmol). The amino acids for peptides **17-18b** were coupled in the order: Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Pro-OH, Fmoc-L-AllGly-OH (**10a**), Fmoc-Asn(*N*-Trt)-OH, Fmoc-Gln(*N*-Trt)-OH, Fmoc-Ile-OH, Fmoc-Tyr(*O*-*t*-Bu)-OH, Fmoc-L-AllGly-OH (**10a**). A small sample of the linear precursor to peptides **17-18b** was cleaved by treatment of the resin bound peptide with cleavage cocktail (A) for 3 h at rt. Concentration of this mixture followed by precipitation and filtration gave the crude linear peptide **33** as a white solid [ES (MS) 1241 (M+Na)]. Two fifths of the resin bound precursor **33** (~ 0.2 mmol) was treated under standard RCM

conditions using catalyst **8** (34 mg, 20 mol%). After RCM, the mixture was cooled to rt and DMSO (71 μL, 50 eq relative to **8**) was added and the suspension was gently stirred for 12 h. A small portion of the resin was subjected to the TFA cleavage conditions using cocktail (A) and the *N*-Fmoc cyclic peptide was subjected to MS analysis [MALDI-TOF (MS) 1213.6 (M+Na)]. The N-terminal Fmoc group was removed from the remainder of the resin-bound cyclic peptide with a solution of 20% piperidine in DMF and subjected to the same cleavage conditions as above. The crude mixture of isomers **18a** and **18b** was isolated as a white solid after precipitation with cold Et_2O and purification. Peptide **18a** was separately isolated as a single peak (t_R = 12.83 min) by prep-HPLC using eluent system A, to give **18a**, upon lyophilization, as a white fluffy solid (28 mg, 14 % overall from ~ 0.20 mmol resin bound peptide cleaved). ^{1}H NMR assignments can be found in Table 10; ^{1}H NMR (D_2O, 500 MHz) δ 7.20 (ap. d, 2H, *J* = 8.3 Hz), 6.83 (ap. d, 2H, *J* = 8.3 Hz), 5.68-5.60 (hidden AB quartet, 2H, *J* = 10.9 Hz), 4.70 (m, 1H), 4.64 (m, 1H), 4.44-4.42 (m, 2H), 4.30-4.27 (m, 2H), 4.04-4.03 (m, 2H), 3.94 (d, 1H, *J* = 17.2 Hz), 3.87 (d, 1H, *J* = 17.2 Hz), 3.72 (m, 1H), 3.61 (m, 1H), 3.09 (m, 1H), 3.00 (m, 1H), 2.82-2.78 (m, 4H), 2.59-2.50 (m, 2H), 2.40 (m, 2H), 2.30 (m, 1H), 2.02-1.92 (m, 6H), 1.68 (m, 2H), 1.61 (m, 1H), 1.22 (m, 1H), 1.02 (m, 1H), 0.95 (d, 3H, *J* = 5.8 Hz), 0.89-0.82 (m, 9H); ^{13}C NMR (D_2O, 150 MHz) δ 186.9, 184.1, 182.1, 178.1, 176.0, 175.2, 174.7, 173.9, 173.1, 172.0, 171.2, 170.5, 155.6, 131.1, 130.0, 128.3, 125.2, 116.2, 61.1, 59.1, 56.0, 55.0, 53.1, 52.8, 52.1, 51.0, 48.5, 42.5, 40.0, 39.1, 36.2, 35.6, 35.5, 31.8, 30.2, 29.8, 26.5, 25.0, 24.9, 22.8, 21.2, 16.0, 12.0; ES/MS calcd. for $C_{45}H_{68}N_{12}O_{12}$ 968.5. Found 969 (M+H, 36%), 991 (M+Na, 100%), 1008 (M+K, 12%).

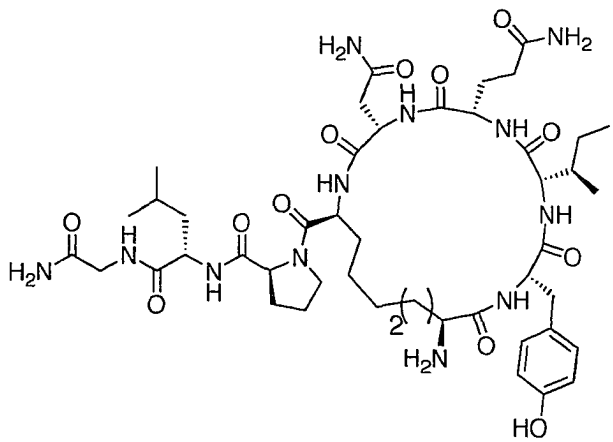


(*trans*)-[1,6-α,α'-L,L-Diamino-γ,γ'-dehydrosuberic acid]oxytocin (18b).[44] Peptide **18b** was isolated as a single peak (t_R = 13.32 min) using prep-HPLC eluting with solvent system A to give **18b**, upon lyophilization, as a white fluffy solid (7 mg, 2% based on ~ 0.20 mmol resin cleaved). ^{1}H NMR assignments can be found in Table 10; ^{1}H NMR (D_2O, 600 MHz) δ 7.17 (ap. d, 2H, *J* = 8.5 Hz), 6.84 (ap. d, 2H, *J* = 8.5 Hz), 5.60-5.49 (hidden AB quartet, 2H, *J* = 15.4 Hz), 4.69 (dd, 1H, *J* = 4.7, 9.8 Hz), 4.65 (dd, 1H, *J* = 7.0, 8.6 Hz), 4.58 (dd, 1H, *J* = 3.5, 5.3 Hz), 4.38 (dd, 1H *J* = 5.7, 8.4 Hz), 4.27 (dd, 1H, *J* = 6.1, 7.0), 4.09-4.07 (m, 2H), 3.95 (d, 1H, *J* = 6.1 Hz) 3.89 (d, 1H, *J* = 17.2 Hz), 3.83 (d, 1H, *J* = 17.2 Hz), 3.69 (m, 1H), 3.58 (m, 1H), 3.11 (dd, 1H, *J* = 7.0, 14.4 Hz), 2.99 (dd, 1H, *J* = 8.6, 14.4 Hz), 2.93 (dd, 1H, *J* = 4.8, 15.8 Hz), 2.71 (dd, 1H, *J* = 9.8, 15.8 Hz), 2.69 (m, 1H), 2.56 (m, 1H), 2.48 (m, 1H), 2.42-2.22 (m, 4H), 2.05 (m, 1H), 1.98 (m, 3H), 1.86-1.81 (m, 2H), 1.67 (m, 2H), 1.56 (m, 1H), 1.34 (m, 1H), 1.07 (m, 1H), 0.90 (d, 3H, *J* = 6.3 Hz), 0.86 (d, 3H, *J* = 6.3 Hz), 0.85-0.80 (m, 6H); ^{13}C NMR (D_2O, 125 MHz) δ 193.3, 193.1, 189.4, 184.8, 176.1, 175.4, 174.8, 174.3, 172.5, 171.6, 170.2, 155.5, 142.7, 131.6, 131.2, 128.6, 126.2, 122.9, 119.2, 116.6, 66.3, 61.2, 60.9, 56.4, 55.6, 53.5, 53.0, 52.2, 51.3, 48.6, 46.6, 42.9, 40.2, 36.9, 34.6, 34.2, 31.8, 30.1, 26.4, 25.7, 25.1, 22.9, 21.6, 15.3, 11.4. ES/MS calcd. $C_{45}H_{68}N_{12}O_{12}$ 968.5. Found 969 (M+H).



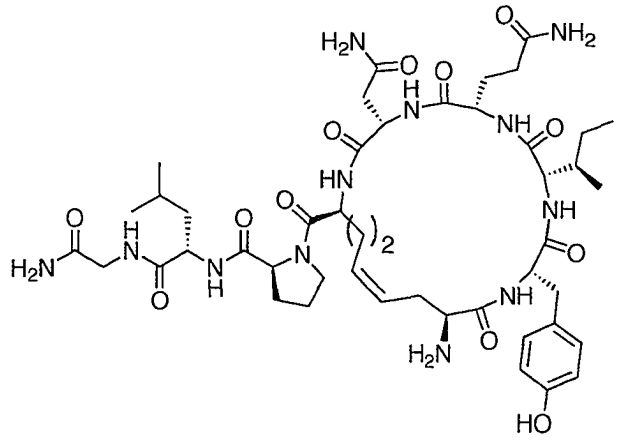
(2:1, *cis/trans*)-[1,6-α,α'-L,L-Diaminonon-γ-enedioic acid]oxytocin (19a and **19b)**. Peptide mixture **19** was synthesized in the same manner as peptides **18a** and **18b** using Rink amide NovaGel (0.66 g, 0.40 mmol) and standard Fmoc SPPS. The following *N*-Fmoc protected amino acids were used in the synthesis, and coupled with PyBOP in this order: Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Pro-OH, Fmoc-L-AllGly-OH (**10a**), Fmoc-Asn(*N*-Trt)-OH, Fmoc-Gln(*N*-Trt)-OH, Fmoc-Ile-OH, Fmoc-Tyr(*O*-*t*-Bu)-OH, Fmoc-L-Hag-OH (**35**). A mixture of 2:1 *cis/trans* isomers, **19** was isolated as a single peak by prep-HPLC (t_R = 13.18 min) using solvent system A. Mixture **19**, after lyophilization, was recovered as a fluffy white solid (17 mg, 8% from 3/5 (~ 0.30 mmol) of cleaved resin bound peptide). ^{1}H NMR assignments can be found in Table 12; ^{1}H NMR (D_2O, 600 MHz) major isomer δ 7.27 (ap. d, 2H, *J* = 8.2 Hz), 6.91 (ap. d, 2H, *J* = 8.5 Hz), 5.57 (m, 2H), 5.45 (m, 1H), 4.84 (dd, 1H, *J* = 6.4, 10.3 Hz), 4.52 (dd, 1H, *J* = 5.1, 8.8 Hz), 4.44 (m, 1H), 4.35 (dd, 1H, *J* = 4.8, 9.8 Hz), 4.14 (AB quartet, 1H, *J* = 4.9 Hz), 4.06 (m, 2H), 3.95 (m, 1H), 3.95 (d, 1H, *J* = 17.2 Hz), 3.88 (d, 1H, *J* = 17.2 Hz), 3.64 (m, 2H), 3.50 (m, 1H), 3.35 (dd, 1H, *J* = 5.8, 14.7 Hz), 3.18 (dd, 1H, *J* = 6.7, 14.4 Hz), 3.00 (dd, 2H, *J* = 9.5, 14.3 Hz), 2.78 (m, 2H), 2.66 (dd, 1H, *J* = 10.8, 14.7 Hz), 2.50-2.25 (m, 5H), 2.10-1.86 (m, 9H), 1.80 (m, 1H), 1.66 (m, 4H), 1.40 (m, 1H), 1.22 (m, 2H), 0.96-0.83 (m,

12H); minor isomer δ 7.19 (ap d, 2H, J = 8.2 Hz), 6.87 (ap d, 2H, J = 8.4 Hz), 5.57 (m, 2H), 4.70 (m, 1H), 4.30 (dd, 1H, J = 5.2, 9.6 Hz), 4.00 (t, 1H, J = 6.6 Hz), 3.94 (d, 1H, J = 17.2 Hz), 3.87 (d, 1H, J = 17.2 Hz), 3.76 (m, 2H), 2.19 (m, 2H), 2.90 (dd, 0.67H, J = 4.5, 15.2 Hz), 1.03 (m, 0.33H); Diagnostic peaks only. ^{13}C NMR (D_2O, 150 MHz) major isomer δ 134.2, 117.8, 130.0, 128.5; minor isomer δ 133.5, 117.8, 130.0; MALDI-TOF (MS) calcd. for $C_{46}H_{70}N_{12}O_{12}$ 982.5. Found 983.5 (M+H, 17%), 1005 (M+Na, 100%), 1021 (M+K, 22%).



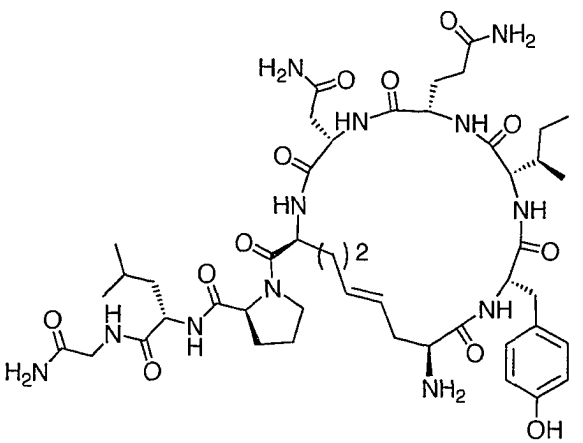
[1,6-α,α'-L,L-Diaminononanedioic acid]oxytocin (20). Pd/C 10% (3 mg) was added to mixture **19** (3 mg, 3 μmol) in EtOH (3 mL). Peptide mixture **19** was reduced in the same manner as peptide **18a** using standard hydrogenolysis techniques. Peptide **20** was isolated by prep-HPLC (t_R = 13.25 min) using solvent system A. This yielded peptide **20** (~ 3 mg, quant.) as a fluffy white solid after lyophilizaton. ^{1}H NMR assignments can be found in Table 11; ^{1}H NMR (D_2O, 600 MHz) δ 7.16 (ap. d, 2H, J = 8.4 Hz), 6.83 (ap. d, 2H, J = 8.5 Hz), 4.70 (m, 2H), 4.40 (m, 2H), 4.28 (m, 1H), 4.14 (d, 1H, J = 5.1 Hz), 4.08 (dd, 1H, J = 5.6, 8.8 Hz), 3.98 (dd, 1H, J = 5.1, 6.8 Hz), 3.90 (d, 1H, J = 17.1 Hz), 3.83 (d, 1H, J = 17.1 Hz), 3.74 (m, 1H), 3.60 (m, 1H), 3.15 (dd, 1H, J = 6.1, 14.4 Hz), 2.95 (dd, 1H, J =

8.6, 14.3 Hz), 2.85 (dd, 1H, J = 5.4, 15.7 Hz), 2.75 (dd, 1H, J = 8.8, 15.7 Hz), 2.37 (m, 2H), 2.25 (m, 1H), 2.00 (m, 3H), 1.88 (m, 5H), 1.66 (m, 3H), 1.58 (m, 2H), 1.42-1.21 (m, 7H), 1.14 (m, 1H), 1.05 (m, 1H), 0.91 (d, 3H, J = 6.2 Hz), 0.89 (d, 3H, J = 6.9 Hz), 0.86 (d, 3H, J = 6.5 Hz), 0.84 (t, 3H, J = 7.4 Hz); ^{13}C NMR (D_2O, 150 MHz) δ 178.4, 176.1, 175.7, 175.2, 174.9, 174.3, 173.6, 173.2, 172.5, 155.2, 131.3, 128.5, 116.5, 72.3, 61.2, 59.5, 56.0, 55.4, 53.9, 53.5, 53.0, 50.9, 48.2, 43.1, 40.3, 39.2, 37.5, 36.5, 32.0, 31.2, 30.7, 30.0, 27.1, 26.0, 25.5, 24.1, 23.2, 21.5, 15.7, 11.7; ES (MS) calcd. for $C_{46}H_{72}N_{12}O_{12}$ 984.5. Found: 986 (M+H, 100%), 1008 (M+Na, 69%).



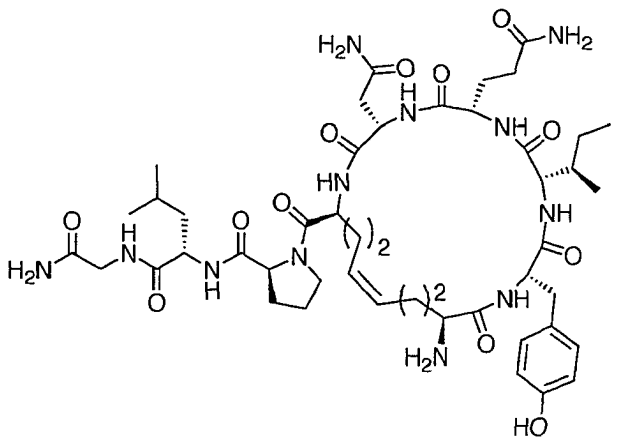
(*cis*)-[1,6-α,α'-L,L-Diaminonon-γ-enedioic acid]oxytocin (21a). Peptides **21a** and **21b** were synthesized on Rink amide NovaGel (0.66 g, 0.40 mmol) using standard Fmoc SPPS conditions and PyBOP as coupling agent as described. The following amino acids were coupled in the order: Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Pro-OH, Fmoc-L-Hag-OH (**35**), Fmoc-Asn(*N*-Trt)-OH, Fmoc-Gln(*N*-Trt)-OH, Fmoc-Ile-OH, Fmoc-Tyr(*O*-*t*-Bu)-OH, Fmoc-L-AllGly-OH (**10a**). Cleavage of the peptide from the resin was accomplished using standard cleavage conditions with cocktail (A). Peptide **21a** was isolated as a single peak using prep-HPLC eluting with solvent system A. Peptide **21a**

was isolated as a fluffy white solid after lyophilization (24 mg, 10% from 3/5 (~ 0.30 mmol) of cleaved resin bound peptide). ^{1}H NMR assignments can be found in Table 11; ^{1}H NMR (D_2O, 600 MHz) δ 7.19 (ap. d, 2H, J = 8.2 Hz), 6.87 (ap. d, 2H, J = 8.2 Hz), 5.68 (hidden AB quartet, 1H, J = 8.5 Hz), 5.51 (hidden AB quartet, 1H, J = 8.5 Hz), 4.64 (m, 1H), 4.42 (m, 1H), 4.30 (m, 1H), 4.16 (m, 1H), 4.12 (m, 1H), 3.93 (m, 1H), 3.92 (d, 1H, J = 17.3 Hz), 3.86 (d, 1H, J = 17.3 Hz), 3.74 (m, 1H), 3.61 (m, 1H), 3.10 (dd, 1H, J = 6.8, 14.3 Hz), 3.04 (dd, 1H, J = 7.2, 14.3 Hz), 2.90 (m, 1H), 2.75 (m, 3H), 2.36 (m, 3H), 2.26 (m, 1H), 2.16 (m, 1H), 2.02 (m, 6H), 1.92 (m, 3H), 1.76-1.56 (m, 5H), 1.17 (m, 1H), 0.94 (d, 3H, J = 5.9 Hz), 0.89 (d, 3H, J = 6.0 Hz), 0.86 (d, 3H, J = 6.8 Hz), 0.81 (m, 3H); ^{13}C NMR (D_2O, 150 MHz) δ 176.1, 175.6, 175.2, 174.9, 174.1, 173.9, 172.7, 155.1, 135.1, 131.4, 128.5, 123.0, 116.5, 61.2, 60.8, 56.7, 55.6, 53.4, 52.8, 50.8, 49.0, 48.8, 43.3, 40.2, 36.0, 32.1, 29.6, 27.0, 26.3, 25.4, 23.2, 21.7, 16.0, 11.6; MALDI-TOF (MS) calcd. for $C_{46}H_{70}N_{12}O_{12}$ 982.5. Found 983.5 (M+H, 15%), 1005.5 (M+Na, 100%), 1021.5 (M+K, 44%).



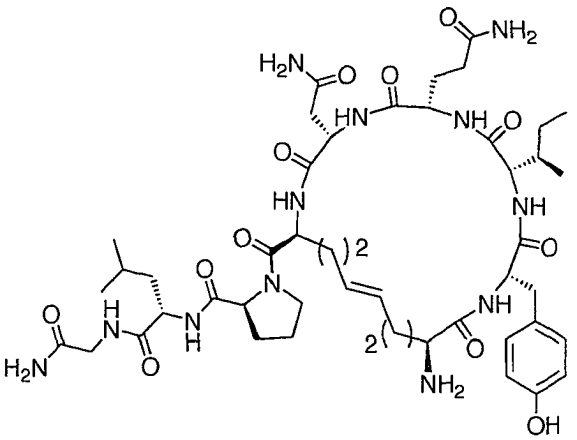
(*trans*)-[1,6-α,α'-L,L-Diamino-δ-non-γ-enedioic acid]oxytocin (21b). Peptide **21b** was isolated as a single peak by prep-HPLC (t_R = 13.32 min) using solvent system A. Peptide

21b was recovered as a fluffy white solid after lyophilization (16 mg, 6% from 3/5 (~ 0.30 mmol) cleaved resin bound peptide). ^{1}H NMR assignments can be found in Table 11; ^{1}H NMR (D_2O, 600 MHz) δ 7.19 (ap. d, 2H, J = 7.1 Hz), 6.87 (ap. d, 2H, J = 8.3 Hz), 5.70 (hidden AB quartet, 1H, J = 15.6 Hz), 5.45 (hidden AB quartet, 1H, J = 15.6 Hz), 4.65 (m, 1H), 4.44 (m, 2H), 4.28 (m, 1H), 4.12 (m, 1H), 4.07 (m, 1H), 4.03 (d, 1H, J = 6.2 Hz), 3.94 (m, 1H), 3.92 (d, 1H, J = 17.2 Hz), 3.87 (d, 1H, J = 17.2 Hz), 3.76 (m, 1H), 3.62 (m, 1H), 3.16 (dd, 1H, J = 7.0, 14.4 Hz), 2.98 (dd, 1H, J = 8.42, 14.4 Hz), 2.78 (m, 3H), 2.58 (m, 1H), 2.38 (m, 2H), 2.29 (m, 1H), 2.12 (m, 1H), 2.04 (m, 5H), 1.86 (m, 2H), 1.70 (m, 2H), 1.66 (m, 2H), 1.60 (m, 1H), 1.30 (m, 1H), 1.04 (m, 1H), 0.94 (d, 3H, J = 6.1 Hz), 0.91 (d, 3H, J = 7.1 Hz), 0.89 (d, 3H, J = 6.1 Hz), 0.84 (t, 3H, J = 7.3 Hz); ^{13}C-NMR (D_2O, 150 MHz) 178.5, 176.0, 175.1, 174.9, 174.1, 173.9, 172.2, 170.0, 155.3, 137.0, 131.5, 128.8, 122.5, 116.5, 61.4, 60.4, 56.3, 55.5, 53.6, 53.2, 52.3, 51.2, 48.7, 42.9, 40.8, 37.0, 36.5, 36.1, 34.6, 32.0, 30.2, 29.9, 27.3, 25.8, 25.3, 23.2, 22.3, 16.2, 11.3; MALDI-TOF (MS) calcd. for $C_{46}H_{70}N_{12}O_{12}$ 982.5. Found 1005.5 (M+Na, 100%), 1021.5 (M+K, 32%).



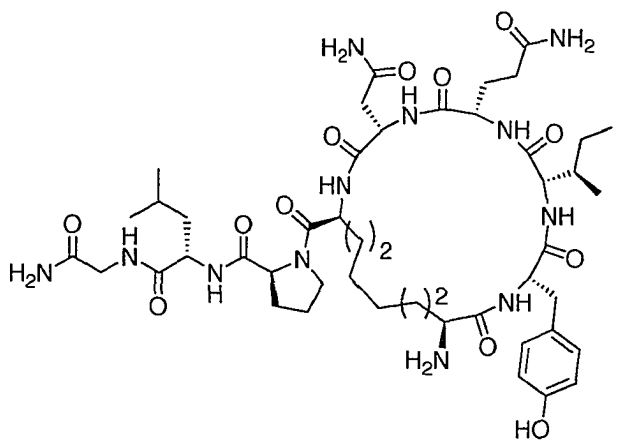
(*cis*)-[1,6-α,α'-L,L-Diaminodec-δ-enedioic acid]oxytocin (22a). Peptides **22a** and **22b** were synthesized in the same manner as peptides **18**, **19** and **21** using Rink amide NovaGel (0.66 g, 0.40 mmol) and standard Fmoc SPPS. The following *N*-Fmoc protected amino acids were used in the synthesis, and coupled with PyBOP in this order: Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Pro-OH, Fmoc-L-Hag-OH (**35**), Fmoc-Asn(*N*-Trt)-OH, Fmoc-Gln(*N*-Trt)-OH, Fmoc-Ile-OH, Fmoc-Tyr(*O*-*t*-Bu)-OH, Fmoc-L-Hag-OH (**35**). Peptide **22a** was cleaved from the resin using cleavage cocktail (A) as previously mentioned. Peptide **22a** was isolated as a single peak by prep-HPLC (t_R = 15.28 min) using solvent system A. Peptide **22a** was isolated as a fluffy white solid after lyophilization (17 mg, 8% from 3/5 (~ 0.30 mmol) of the cleaved resin bound peptide). ^{1}H NMR assignments can be found in Table 13; ^{1}H NMR (D_2O, 600 MHz) δ 7.26 (ap. d, 2H, J = 8.6 Hz), 6.93 (ap. d, 2H, J = 8.6 Hz), 5.52 (hidden AB quartet, 2H, J = 10.4 Hz), 4.90 (m, 1H), 4.82 (dd, 1H, J = 8.9, 10.8 Hz), 4.48 (m, 2H), 4.35 (m, 1H), 4.24 (m, 1H), 4.12 (m, 1H), 4.01 (m, 1H), 3.97 (d, 1H, J = 17.3 Hz), 3.89 (d, 1H, J = 17.3 Hz), 3.78 (m, 1H), 3.66 (m, 1H), 3.22 (dd, 1H, J = 7.0, 14.5 Hz), 3.07-2.96 (m, 2H), 2.70 (m, 1H), 2.40 (m, 2H), 2.32 (m, 1H), 2.29-1.80 (m, 14H), 1.70 (m, 3H), 1.39 (m, 1H), 1.17 (m, 1H),

0.99 (d, 3H, J = 6.1 Hz), 0.97-0.92 (m, 9H); ^{13}C NMR (D_2O, 150 MHz) (HMQC data only) δ 131.3, 130.8, 130.2, 116.6, 62.3, 61.3, 60.6, 56.0, 55.2, 53.6, 51.3, 50.3, 48.5, 43.3, 40.7, 32.0, 30.0, 25.6, 23.2, 21.4, 15.8, 11.1; ES (MS) calcd. for $C_{47}H_{72}N_{12}O_{12}$ 996.5. Found 998 (M+H, 62%), 1020 (M+Na, 100%).



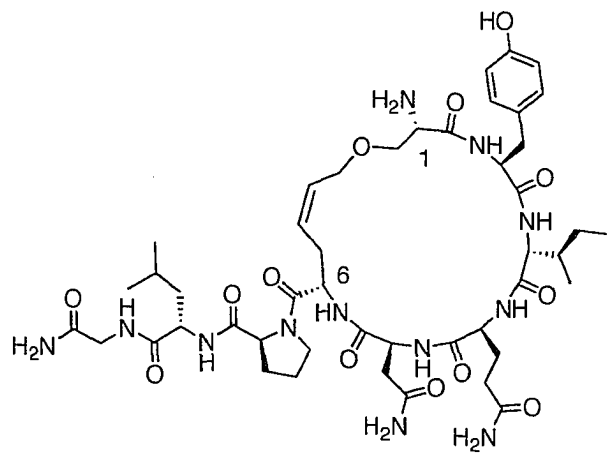
(*trans*)-[1,6-α,α'-LL-Diaminodec-δ-enedioic acid]oxytocin (**22b**). Peptide **22b** was isolated as a single peak by prep-HPLC (t_R = 15.67 min) using solvent system A. Peptide **22b** was isolated as a fluffy white solid after lyophilization (56 mg, 23% from 3/5 (~ 0.30 mmol) of cleaved resin bound peptide). ^{1}H NMR assignments can be found in Table 13; ^{1}H NMR (D_2O, 600 MHz) δ 7.26 (ap. d, 2H, J = 8.6 Hz), 6.95 (ap. d, 2H, J = 8.5 Hz), 5.56-5.46 (m, 2H), 5.02 (m, 1H), 4.91 (dd, 1H, J = 5.7, 10.3 Hz), 4.52 (m, 2H), 4.33 (dd, 1H, J = 5.0, 9.8 Hz), 4.27 (m, 1H), 4.00 (m, 1H), 3.91 (d, 1H, J = 17.2 Hz), 3.85 (d, 1H, J = 17.2 Hz), 3.82 (m, 1H), 3.68 (m, 1H), 3.30 (dd, 1H, J = 5.4, 14.8 Hz), 3.17 (dd, 1H, J = 3.6, 15.9 Hz), 2.99 (dd, 1H, J = 10.2, 14.5 Hz), 2.78 (dd, 1H, J = 11.9, 15.6 Hz), 2.51 (m, 2H), 2.34 (m, 4H), 2.10 (m, 5H), 1.92 (m, 5H), 1.72 (m, 5H), 1.50 (m, 1H), 1.36 (m, 1H), 1.02-0.93 (m, 12H); ^{13}C NMR (D_2O, 150 MHz) δ 178.6, 178.4, 176.2, 175.6, 175.0, 174.3, 172.4, 170.8, 155.8, 131.3, 128.5, 116.6, 61.3, 60.6, 56.0, 55.6, 54.0, 53.6, 52.1,

51.5, 50.9, 48.8, 47.9, 43.1, 42.7, 40.2, 36.5, 32.2, 30.0, 28.7, 25.7, 23.0, 20.9, 15.8, 11.8; ES (MS) calcd. for $C_{47}H_{72}N_{12}O_{12}$ 996.5. Found 998 (M+H, 50%), 1020 (M+Na, 100%).



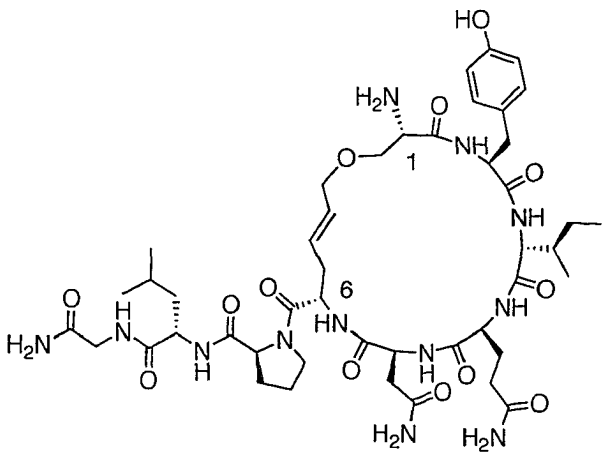
[1,6-α,α'-L,L-Diaminodecanedioic acid]oxytocin (23). Pd/C 10% (20 mg) was added to a solution of peptide **22a** (20 mg, 28 μmol) in EtOH (10 mL). Peptide **23** was reduced in the same manner as peptides **18a** and **19(a, b)** using standard hydrogenolysis techniques. The mixture was stirred for 36 h at rt under H_2 (760 torr). Peptide **23** was isolated by prep-HPLC (t_R = 15.99 min) using solvent system A, which yielded peptide **23** (20 mg, quant.) as a white fluffy solid after lyophilization. ^{1}H NMR assignments can be found in Table 13; ^{1}H NMR (D_2O, 600 MHz) δ 7.21 (ap d, 2H, J = 8.5 Hz), 6.87 (ap d, 2H, J = 8.6 Hz), 4.93 (m, 2H), 4.60 (m, 1H), 4.43 (dd, 1H, J = 6.0, 8.2 Hz), 4.28 (dd, 1H, J = 4.9, 9.8 Hz), 4.16 (dd, 1H, J = 4.8, 8.0 Hz), 3.94 (m, 2H), 3.93 (d, 1H, J = 17.2 Hz), 3.86 (d, 1H, J = 17.2 Hz), 3.80 (m, 1H), 3.18 (dd, 1H, J = 6.0, 14.4 Hz), 3.13 (dd, 1H, J = 3.9, 16.2 Hz), 2.92 (dd, 1H, J = 9.8, 14.2 Hz), 2.84 (dd, 1H, J = 11.0, 16.2 Hz), 2.43 (m, 1H), 2.32 (m, 2H), 2.05 (m, 3H), 1.91 (m, 5H), 1.77 (m, 1H), 1.73-1.58 (m, 4H), 1.50-1.24 (m, 7H), 1.18 (m, 1H), 1.08 (m, 1H), 0.97-0.88 (m, 12H); ^{13}C NMR (D_2O, 150 MHz) δ 178.7, 176.6, 175.4, 174.9, 174.4, 173.2, 171.0, 155.5, 131.1, 128.8, 116.2, 62.0, 61.4, 56.4,

54.9, 50.8, 48.7, 43.2, 40.2, 37.4, 31.8, 26.0, 25.5, 24.9, 21.5, 15.7, 11.6; ES (MS) calcd. for $C_{47}H_{72}N_{12}O_{12}$ 998.5. Found 1000 (M+H, 58%), 1022 (M+Na, 100%).



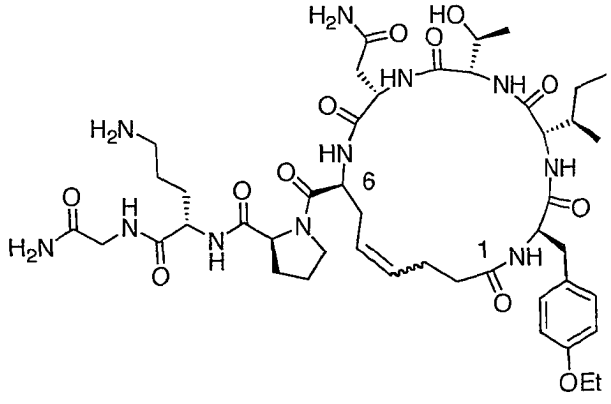
(*cis*)-[1,6-α,α'-LL-Diamino-δ'-carboxy-ethoxy)-γ-hexendioic acid]oxytocin (24a). Peptides **24a** and **24b** were synthesized in the same manner as peptides **18**, **19**, **21** and **23** on Rink amide NovaGel™ (0.66 g, 0.40 mmol) using standard Fmoc SPPS. The *N*-Fmoc protected amino acids were coupled using PyBOP in the following order: Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Pro-OH, Fmoc-L-AllGly-OH (**10a**), Fmoc-Asn(*N*-Trt)-OH, Fmoc-Gln(*N*-Trt)-OH, Fmoc-Ile-OH, Fmoc-Tyr(*O*-*t*-Bu)-OH, Fmoc-Ser(*O*-allyl)-OH (**40**). Peptide **24a** was purified and isolated as its *N*-Fmoc protected derivative after resin cleavage using cleavage cocktail (A). Purification by prep-HPLC gives the *N*-Fmoc precursor to **24a** as a single peak (t_R = 18.01) using solvent system A. The *N*-Fmoc protected precursor was then treated with a 20% solution of piperidine in DMF (2.5 mL) for 5 min and concentrated *in vacuo* at rt. The residue was resuspended in H_2O (2.5 mL) and subjected to prep-HPLC (t_R = 12.44 min) under the same conditions as above. Peptide **24a** was isolated as a fluffy white solid after lyophilization (2 mg, 1% from 1/2 (~ 0.20 mmol) of resin bound peptide cleaved). ^{1}H NMR assignments can be found in

Table 14; ^{1}H NMR (D_2O, 600 MHz) δ 7.21 (ap. d, 2H, *J* = 8.4 Hz), 6.88 (ap. d, 2H, *J* = 8.5 Hz), 5.68 (m, 2H), 4.70 (m, 1H), 4.64 (m, 1H), 4.44 (m, 1H), 4.30 (m, 1H), 4.19 (m, 2H), 4.12 (m, 3H), 3.96 (m, 1H), 3.92 (d, 1H, *J* = 17.2 Hz), 3.88 (d, 1H, *J* = 17.2 Hz), 3.89 (m, 1H), 3.73 (m, 1H), 3.62 (m, 1H), 3.21 (dd, 1H, *J* = 6.0, 14.4 Hz), 3.01 (dd, 1H, *J* = 8.6, 14.3 Hz), 2.88 (dd, 1H, *J* = 5.3, 15.7 Hz), 2.78 (dd, 1H, *J* = 8.5, 15.8 Hz), 2.55 (m, 2H), 2.38 (m, 2H), 2.29 (m, 1H), 2.02 (m, 4H), 1.90 (m, 3H), 1.69 (m, 2H), 1.62 (m, 1H), 1.34 (m, 1H), 1.08 (m, 1H), 0.95 (d, 3H, *J* = 6.2 Hz), 0.90 (m, 9H); ^{13}C NMR (D_2O, 150 MHz) (HMQC data only) δ 131.4, 129.4, 116.5, 68.2, 67.6, 61.5, 59.5, 56.2, 55.7, 53.6, 52.1, 51.2, 48.6, 43.0, 39.5, 36.4, 32.0, 30.0, 25.8, 23.3, 22.3, 16.2, 11.9. MALDI-TOF (MS) calcd. for $C_{46}H_{70}N_{12}O_{13}$ 998.52. Found 999.3 (M+H, 100%), 1021.3 (M+Na, 100%), 1037.3 (M+K, 72%).



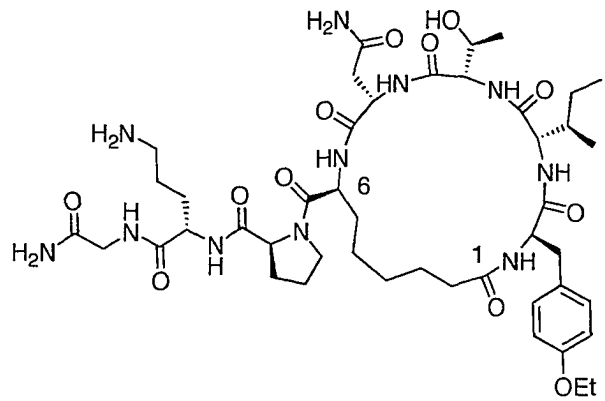
(*trans*)-[1,6-α,α'-LL-Diamino-δ'-carboxy-ethoxy)-γ-hexendioic acid]oxytocin (24b). Peptide **24b** was purified and isolated as its *N*-Fmoc protected derivative. After resin cleavage using cocktail (A), peptide **24b** was purified by prep-HPLC (t_R = 17.76) using solvent system A. The *N*-Fmoc protected precursor was then treated with a solution of 20% piperidine in DMF (2.5 mL) for 5 min and concentrated *in vacuo* at rt. The residue was resuspended in H_2O (2.5 mL) and subjected to HPLC (t_R = 12.44 min) under the

same conditions as above. Peptide **24b** was isolated as a fluffy white solid after lyophilization (3 mg, 2% from 1/2 (~ 0.20 mmol) of cleaved resin bound peptide). ^{1}H NMR assignments can be found in Table 14. ^{1}H NMR (D_2O, 600 MHz) δ 7.21 (ap. d, 2H, J = 8.5 Hz), 6.88 (ap. d, 2H, J = 8.6 Hz), 5.69 (hidden AB quartet, 2H, J = 15.9 Hz), 4.82 (m, 1H), 4.63 (dd, 1H, J = 3.8, 8.6 Hz), 4.44 (dd, 1H, J = 5.5, 8.3 Hz), 4.31 (m, 1H), 4.16 (m, 3H), 4.03 (m, 2H), 3.97 (m, 1H), 3.92 (d, 1H, J = 17.2 Hz), 3.86 (d, 1H, J = 17.2 Hz), 3.83 (dd, 1H, J = 4.9, 11.4 Hz), 3.74 (m, 1H), 3.20 (dd, 1H, J = 6.0, 14.4 Hz), 2.98 (m, 2H), 2.82 (dd, 1H, J = 9.8, 15.7 Hz), 2.52 (m, 2H), 2.42 (m, 1H), 2.36 (m, 1H), 2.29 (m, 1H), 2.08-1.85 (m, 7H), 1.68 (m, 3H), 1.61 (m, 1H), 1.40 (m, 1H), 1.18 (m, 1H), 0.95 (d, 3H, J = 6.2 Hz), 0.93-0.87 (m, 9H); ^{13}C NMR (D_2O, 150 MHz) (HMQC data only) δ 131.3, 130.5, 128.9, 116.6, 72.6, 68.2, 61.5, 61.0, 58.6, 55.8, 55.6, 53.7, 52.8, 51.0, 48.5, 43.5, 40.3, 39.7, 36.9, 36.2, 33.8, 32.0, 30.2, 25.7, 25.2, 23.1, 21.8, 15.8, 11.6; MALDI-TOF (MS) calcd. for $C_{46}H_{70}N_{12}O_{13}$ 998.52. Found 1021.4 (M+Na, 100%), 1037.4 (M+K, 57%).

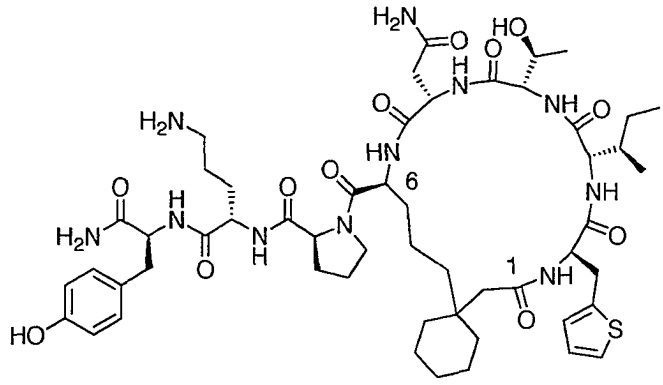


(1:4, *cis/trans*)-[1,6-α-L-Amino-α'-deamino-γ,γ'-dehydrosuberic acid]atosiban (26 a and **b**). The peptide mixture **26** was synthesized on Rink amide NovaGel resin (0.66 g, 0.40 mmol) using standard Fmoc SPPS conditions. The following *N*-Fmoc amino acids

were coupled with PyBOP in the order: Fmoc-Gly-OH, Fmoc-Orn(*N*-Boc)-OH, Fmoc-Pro-OH, Fmoc-L-AllGly-OH (**10a**), Fmoc-Asn(*N*-Trt)-OH, Fmoc-Thr(*O*-*t*-Bu)-OH, Fmoc-Ile-OH, Fmoc-D-Tyr(*O*-Et)-OH, 4-pentenoic acid. Cyclization was done on the resin-bound linear precursor to **26** using catalyst **9** (68 mg, 20 mol%) under standard RCM conditions. Cleavage of the cyclic peptide **26** was accomplished using cocktail (A) to yield the crude peptide mixture **26** as an off-white solid. This solid was dissolved in 0.1% aqueous TFA and subjected to prep-HPLC purification (t_R = 14.35 min) using solvent system A. All attempts to separate the peptide isomers were unsuccessful and the peptides **26a** and **26b** were isolated as a 4:1 mixture of *cis/trans* isomers (0.12 g, 21% overall, from 0.40 mmol of cleaved resin) after lyophilization. ^{1}H NMR assignments can be found in Table 15; ^{1}H NMR (D_2O, 600 MHz) major isomer δ 7.20 (ap. d, 2H, J = 8.4 Hz), 6.94 (ap. d, 2H, J = 8.5 Hz), 5.56 (hidden AB quartet, 1H, J = 14.9 Hz), 5.34 (hidden AB quartet, 1H, J = 14.9 Hz), 4.68-4.57 (m, 3H), 4.42 (dd, 1H, J = 6.4, 7.1 Hz), 4.34 (dd, 1H, J = 5.5, 8.1 Hz), 4.30 (d, 1H, J = 5.2 Hz), 4.13 (m, 1H), 4.09 (q, 2H, J = 7.0 Hz), 4.13 (m, 1H), 3.91 (m, 2H), 3.74 (m, 1H), 3.63 (m, 1H), 3.02 (m, 3H), 2.89 (m, 1H), 2.40 (m, 3H), 2.36-2.21 (m, 5H), 2.02 (m, 2H), 1.90 (m, 2H), 1.84-1.68 (m, 5H), 1.35 (t, 3H, J = 7.0 Hz), 1.18 (d, 3H, J = 6.4 Hz), 1.20 (m, 1H), 0.79 (m, 1H), 0.71 (t, 3H, J = 7.5 Hz), 0.61 (d, 3H, J = 6.7 Hz); minor isomer δ 5.59 (hidden AB quartet, 1H, J = 10.7 Hz), 5.51 (hidden AB quartet, 1H, J = 10.7 Hz), 0.89 (m, 1H), 0.52 (m, 3H); ^{13}C NMR (D_2O, 150 MHz) (HMQC diagnostic peaks for major isomer only) δ 134.5, 131.4, 125.8, 116.3; ES(MS) calcd. for $C_{45}H_{69}N_{11}O_{12}$ 955.5. Found 956.5 (M+H).

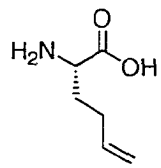


[1,6-α-L-Amino-α'-deaminosuberic acid]atosiban (27). To a solution of mixture **26** (~ 9 mg, 10 μmol) in EtOH was added 10% Pd/C (9 mg). The entire mixture was reduced in the same manner as peptides **18a**, **19** and **22a** to give the crude peptide **27** upon filtration. The crude peptide was dissolved in 1% aqueous TFA (6 mL) and subjected to prep-HPLC (t_R = 14.61 min) using the same eluent conditions employed for purification of mixture **26**, which yielded **27** (5 mg, 55%) after HPLC purification. ^{1}H NMR assignments can be found in Table 15; ^{1}H NMR (D_2O, 600 MHz) δ 7.22 (ap. d, 2H, J = 8.6 Hz), 6.96 (ap. d 2H, J = 8.5 Hz), 4.60 (m, 3H), 4.42 (dd, 1H, J = 6.4, 8.1 Hz), 4.33 (dd, 1H, J = 5.3, 9.9 Hz), 4.28 (m, 1H), 4.20 (m, 2H), 4.15 (q, 2H, J = 7.0 Hz), 3.92 (m, 2H), 3.78 (m, 1H), 3.63 (m, 1H), 3.42 (m, 3H), 2.98 (dd, 1H, J = 9.6, 13.5 Hz), 2.84 (dd, 1H, J = 6.8, 15.6 Hz), 2.74 (dd, 1H, J = 7.9, 15.7 Hz), 2.32 (m, 2H), 2.20 (m, 1H), 2.02 (m, 2H), 1.91 (m, 2H), 1.80 (m, 5H), 1.64 (m, 1H), 1.56 (m, 2H), 1.37 (t, 3H, J = 7.0 Hz), 1.34-1.28 (m, 3H), 1.26-1.16 (m, 4H), 1.09 (m, 1H), 0.86 (m, 1H), 0.76 (t, 3H, J = 7.2 Hz), 0.60 (d, 3H, J = 6.9 Hz); ^{13}C NMR (D_2O, 150 MHz) δ 178.3, 175.5, 174.7, 174.5, 172.8, 170.5, 168.2, 157.9, 131.2, 129.5, 116.0, 68.1, 65.2, 63.3, 60.1, 59.2, 56.8, 51.6, 48.8, 43.0, 39.8, 36.2, 28.2, 25.5, 24.0, 20.0, 15.9, 15.1, 11.8; MALDI-TOF (MS) calcd. for $C_{45}H_{71}N_{11}O_{12}$ 957.5. Found 958.5 (M+H).

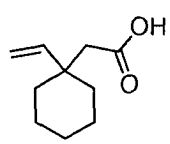


[(1,6-α-L-Amino-α'-deamino-β',β'-cyclohexylsuberic acid)-2-D-thienyl-9-L-tyrosyl]-atosiban (29). The linear precursor to **29** was synthesized on Sieber amide resin (0.24 g, 0.15 mmol) with 0.62 mmol/g loading. The first amino acid, Fmoc-Tyr-(*O*-*t*Bu)-OH (0.35 g, 0.75 mmol) was dissolved in DMF (10 mL) followed by addition of HOBt (0.10 g, 0.75 mmol) and DIPCDI (95 mg, 0.75 mmol). The mixture was added to the Sieber amide resin which was pre-swollen in DMF, and the entire reaction mixture was agitated with bubbling argon for 4 h. The Kaiser test was used to monitor the extent of coupling, and once complete, the resin-bound amino acid was end-capped with Ac_2O (10 mL, 20 min). All other amino acids were coupled with PyBOP in this order; Fmoc-D-Thi-OH, Fmoc-Ile-OH, Fmoc-Thr(*O*-*t*-Bu)-OH, Fmoc-Asn(*N*-Trt)-OH, (2S)-*N*-(9*H*-fluorenylmethoxycarbonyl-amino)-5-(1-methoxycarbonylmethyl-cyclohexyl)pentanoic acid (**60**), Fmoc-Pro-OH and Fmoc-Orn(*N*-Boc)-OH. The *N*-Fmoc protecting group on Fmoc-D-Thi-OH was removed using a solution of 20% piperidine in DMF (15 mL, 2 x 5 min) to afford the resin-bound, linear peptide **67**. The side chain protected linear precursor **67** was cleaved from the resin using the same conditions as for peptide **56**. This gave the crude peptide methyl ester **68** as an off-white solid. Purification was accomplished using prep-HPLC (t_R = 24.52 min), using solvent system B to yield pure peptide methyl ester **68** as a white solid (42 mg, 18%); MALDI-TOF (MS) calcd. for

$C_{87}H_{125}N_{11}O_{15}SNa$ 1602.9. Found 1603.0 (M+Na). The methyl ester **68** (42 mg, 0.03 mmol) was treated with 2M LiOH (2 mL) for 3 d at rt and purified with prep-HPLC as above (t_R = 23.88 min) to give the peptide acid **69** (25 mg, 59%) as a white solid; MALDI-TOF (MS) calcd. for $C_{85}H_{119}N_{11}O_{15}SNa$ 1589.9. Found 1588.9 (M+Na). The peptide acid **69** (25 mg, 18 μmol) was dissolved in dry DMF (15 mL) and PyBOP (56 mg, 0.11 mmol), HOBt (15 mg, 0.11 mmol) and NMM (24 μL, 0.22 mmol) were added. The reaction mixture was then stirred in the dark for 2.5 d, concentrated and purified by prep-HPLC (t_R = 26.92 min) as above to give the protected cyclized product **70** (8 mg, 29%); MALDI-TOF (MS) calcd. for $C_{85}H_{117}N_{11}O_{14}SNa$ 1570.8. Found 1571.8 (M+Na). The protected, cyclized peptide **70** (8 mg, 50 μmol) was treated with cleavage cocktail (A) for 4 h at rt. Purification with prep-HPLC as above, (t_R = 16.39 min) yielded the fully deprotected peptide, **29** (4 mg, 76%) as a white solid. ^{1}H NMR assignments can be found in Table 16. ^{1}H NMR (D_2O, 600 MHz) δ 7.31 (d, 1H, *J* = 4.4 Hz), 6.98 (m, 4H), 6.55 (ap. d, 2H, *J* = 8.1 Hz), 4.75 (m, 1H), 4.62 (m, 1H), 4.55 (m, 1H), 4.51 (m, 1H), 4.46 (t, 1H, *J* = 7.8 Hz), 4.41-4.31 (m, 2H), 4.16 (m, 2H), 3.77 (m, 1H), 3.62 (m, 1H), 3.32 (m, 2H), 2.94 (m, 2H), 2.76 (m, 2H), 2.56 (m, 1H), 2.45 (m, 1H), 2.25 (m, 2H), 2.12 (m, 1H), 2.19 (m, 4H), 1.84-1.61 (m, 5H), 1.52 (m, 1H), 1.40 (m, 5H), 1.26 (m, 8H), 1.15 (m, 6H), 0.83 (m, 6H); ^{13}C NMR (D_2O, 150 MHz) (HMQC diagnostic peaks only) δ 131.2 (D-Thi), 128.2 (Tyr), 125.9 (D-Thi), 119.4 (Tyr); MALDI-TOF (MS) calcd. for $C_{53}H_{79}N_{11}O_{12}S$ 1093.6, found 1094.2 (M+H, 100%), 1116.2 (M+Na, 35%), 1132.1 (M+K, 30%).

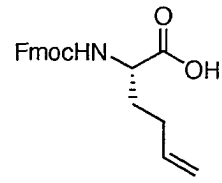


(2S)-2-Amino-5-hexenoic acid (L-homoallylglycine) (30).[125,126] A solution of (R,S)-N-Acetamido-5-hexenoic acid (**39**) (7.30 g, 56.5 mmol) in distilled H_2O (250 mL) was basified to pH 7.5 with conc. NH_4OH to give a final amino acid concentration of 0.25 M. Porcine kidney acylase I (2040 units /mg, 167 mg) was added and the reaction mixture was stirred for 24 h at 37 °C. The mixture was then acidified to pH 5 with 1 M HCl and charcoal was added. The mixture was heated to 60 °C and filtered through a pad of Celite. The aqueous filtrate was further acidified to pH 1.5 with conc. HCl, and washed with EtOAc (3 x 100 mL) and concentrated *in vacuo*. The residue was dissolved in a minimal amount of MeOH, filtered, and treated with excess propylene oxide to give **30** (3.45 g, 47%) as a white solid upon filtration; IR (microscope) 3350–2550, 1582, 1450, 1352 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 5.87 (tdd, 1H, J = 6.5, 10.4, 17.1 Hz, CH_2=C<u>H</u>), 5.25 (m, 2H, C<u>H</u>$_2$=CH), 3.70 (dd, 1H, J = 5.5, 10.7 Hz, AcHNC<u>H</u>), 2.15 (m, 2H, CH_2=CHC<u>H</u>$_2$$CH_2$), 1.95 (m, 2H, CH_2=CHCH_2C<u>H</u>$_2$); ^{13}C NMR (D_2O, 125 MHz) δ 175.4, 137.8, 116.7, 55.2, 30.6, 29.5; MS (EI) calcd. for $C_6H_{11}NO_2$ 129.0790, Found 127.0790 (M^+); $[\alpha]_D$ (*c* 1.0, H_2O) = + 14.8. [lit.[125] $[\alpha]_D$ (*c* 0.9, H_2O) = + 13.5]



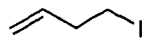
1-(Vinylcyclohexyl)acetic acid (32).[130] A solution of crude ester **46** (15.5 g, 92.0 mmol) in dry Et_2O (120 mL) was added dropwise to a suspension of $LiAlH_4$ (5.24 g, 140 mmol) in dry Et_2O (60 mL) at 0 °C. The mixture was warmed to rt and stirred for an additional 8

h. The reaction was quenched with ice and the aqueous layer was extracted with Et_2O (2 x 180 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to give the crude allylic alcohol **47** (11.21 g, 89%) as a colourless liquid. Triethyl orthoacetate (79.6 mL, 409 mmol) and propionic acid (0.39 mL, 5 mmol) were added to the crude alcohol **47** (10.9 g, 86.6 mmol) and the reaction mixture was heated to reflux using a Dean-Stark apparatus to collect the ethanol (8.5 mL) formed during the reaction. The reaction mixture was cooled to rt, and MeOH (150 mL) and solid KOH (9.7 g, 0.16 mol) were added. The entire mixture was then heated at reflux for 6 h. The resulting solution was concentrated *in vacuo* and the residual oil was partitioned between $NaHCO_3$ (175 mL) and Et_2O (175 mL). The aqueous layer was then acidified to pH 1 with conc. HCl and extracted with CH_2Cl_2 (3 x 150 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* and the residue was subjected to flash chromatography (SiO_2, 13:7 EtOAc /hexanes) to give **32** as a semi-solid (7.57 g, 52%) from the allylic alcohol **47**; IR ($CHCl_3$, cast) 3400-2500, 1706, 1447, 1411 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 11.88 (br s, 1H, $CO_2\underline{H}$), 5.75 (dd, 1H, J = 11.0, 17.7 Hz, CH_2=C$\underline{H}$), 5.10 (dd, 1H, J = 1.2, 11.0 Hz, C$\underline{H}_2$=CH), 5.00 (dd, 1H, 1.1, 17.7 Hz, C$\underline{H}_2$=CH), 2.30 (s, 2H, C$\underline{H}_2CO_2H$), 1.63 (m, 2H, C$\underline{H}_2(CH_2)_4C\underline{H}_2$), 1.60-1.30 (m, 8H, C$\underline{H}_2(C\underline{H}_2)_3C\underline{H}_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 178.5, 144.5, 113.5, 45.5, 39.1, 35.6, 26.1, 22.0; HRMS (EI) calcd for $C_{10}H_{16}O_2$ 168.1150. Found 168.1149 (M^+)

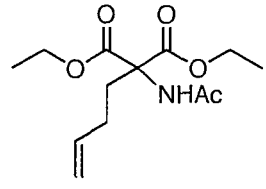


(2S)-*N*-(9*H*-Fluorenylmethoxycarbonyl)-2-amino-5-hexenoic acid (Fmoc-L-homoallylglycine) (35). $NaHCO_3$ (1.61 g, 19.2 mmol) was added to a solution of L-homoallylglycine (**30**) (0.62 g, 4.8 mmol) in H_2O (50 mL) at 0 °C. The reaction mixture was allowed to stir at 0 °C for 10 min. A solution of 9*H*-fluorenylmethyl chloroformate (1.41 g, 5.27 mmol) in acetone (25 mL) was added dropwise over 15 min. The reaction mixture was stirred at 0 °C for 1 h and then allowed to warm to rt and was maintained at rt for a further 4 h. The mixture was then concentrated *in vacuo* and the resulting residue was taken up in H_2O (50 mL). The aqueous layer was extracted with EtOAc (2 x 75 mL). The aqueous layer was then acidified to pH 2 with 1 M HCl and extracted with EtOAc (4 x 75 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to yield **35** as a white solid. Recrystallization from CH_2Cl_2 and hexanes gave pure **35** (1.21 g, 72%); mp 127-130 °C [lit.[199] mp 128-129.5 °C (racemic mixture)]; IR ($CHCl_3$, cast) 3400-2400, 1717, 1521, 1417 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 10.63 (br s, 1H, $CO_2\underline{H}$), 7.78 (ap. d, 2H, J = 7.5 Hz, ArH), 7.58 (m, 2H, ArH), 7.38 (ap. t, 2H, J = 7.4 Hz, ArH), 7.28 (ap. t, 2H, J = 7.4 Hz, ArH), 5.75 (m, 1H, CH_2=C$\underline{H}$), 5.28 (d, 1H, J = 8.3 Hz, N$\underline{H}$CH), 5.04 (m, 2H, C$\underline{H_2}$=CH), 4.32 (m, 3H, NHC$\underline{H}CO_2H$ and $Ar_2CHC\underline{H_2}$), 4.22 (t, 1H, J = 6.9 Hz, ArC$\underline{H}$Ar), 2.18 (m, 2H, CH_2=CHC$\underline{H_2}CH_2$), 2.02 (m, 1H, CH_2=$CHCH_2C\underline{H_2}$), 1.80 (m, 1H, CH_2=$CHCH_2C\underline{H_2}$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.9, 156.0, 143.6, 141.2, 136.5, 127.6, 127.0, 124.9, 119.9, 115.9, 67.1, 53.3, 47.2, 31.6, 29.4; MS (ES)

calcd. for $C_{21}H_{21}NO_4Na$ 374.1362, Found 374.1363 (M+Na); $[\alpha]_D$ (*c* 1.0, $CHCl_3$) = + 13.3.

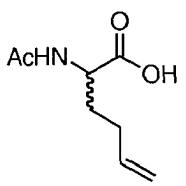


4-Iodo-1-butene (37).[127] To a solution of NaI (29.2 g, 0.200 mol) in anhydrous acetone (200 mL) was added 4-bromo-1-butene (17.5 g, 133 mmol) dropwise via an addition funnel and the reaction mixture was stirred while heating at reflux for 20 h. The mixture was cooled to room temperature and diluted with Et_2O (380 mL). The organic layer was washed with brine (3 x 150 mL), and the combined organic layers were dried (Na_2SO_4). The volatiles were distilled up to 45 °C at 760 torr. The remaining liquid was transferred to a 100 mL round bottom flask and distilled up to 80 °C at 760 torr. The remaining oil was then distilled at 59 °C at 69 torr to yield a pink liquid (18.1 g, 74%); IR ($CHCl_3$, cast): 3078, 3001, 2977-2926, 2372-2320, 1638, 1424, 1247, 1178, 991, 919 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.73 (m, 1H, CH_2=C<u>H</u>), 5.08 (m, 2H, C<u>H$_2$</u>=CH), 3.17 (m, 2H, C<u>H$_2$</u>I), 2.59 (m, 2H, C<u>H$_2$</u>CH_2I); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 136.8, 117.0, 37.7, 4.8; HRMS (EI) calcd. for C_4H_7I 181.9593. Found 181.9590 (M^+).



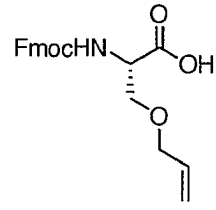
Diethyl (*R,S*)-2-(*N*-Acetamido)-5-hexenoate (38).[200] Sodium (1.70 g, 74.0 mmol) was added in portions to a 250 mL round bottom flask containing anhydrous EtOH (150 mL). Diethyl *N*-acetamidomalonate (**36**) (16.1 g, 74.0 mmol) was then added and the solution

turned to a pale orange colour. 4-Iodo-1-butene (**37**) (14.7 g, 81.0 mmol) was quickly added and the reaction mixture was heated to reflux and stirred for 23 h. The mixture was then concentrated *in vacuo* and the residue was dissolved in H_2O (200 mL). The aqueous layer was acidified to pH 4 with AcOH, and extracted with $CHCl_3$ (3 x 200 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to yield an orange liquid. Purification was performed using flash chromatography (SiO_2, 40% EtOAc/hexanes) to give a colourless oil **38** (13.1 g, 65%); IR ($CHCl_3$, cast) 3384, 2981, 1751, 1504, 1447, 1278, 1201 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.78 (br s, 1H, CONHCH$_3$), 5.75 (tdd, 1H, J = 20.0, 10.4, 6.5 Hz, CH_2=CHCH$_2$), 4.98 (m, 2H, CH$_2$=CH), 4.23 (q, 4H, J = 7.1 Hz, CH$_2$CH$_3$), 2.41 (m, 2H, CH_2=CHCH$_2$CH$_2$), 2.01 (s, 3H, CH$_3$CONH), 1.89 (m, 2H, CH_2=CHCH$_2$CH$_2$), 1.25 (t, 6H, J = 7.1 Hz, CH$_2$CH$_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.9, 168.0, 137.0, 115.4, 66.4, 62.6, 31.5, 28.2, 23.2, 14.1; HRMS (EI) calcd. for $C_{13}H_{21}NO_5$ 271.1420. Found 271.1420 (M^+).



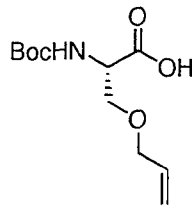
(*R*,*S*)-2-Acetamido-5-hexenoic acid (39).[125] To a solution of compound **38** (13.1 g, 48.1 mmol) in 1:1 EtOH/H_2O (175 mL) was added NaOH (2.12 g, 52.9 mmol) and the reaction mixture was heated to reflux and stirred for 16 h. The reaction mixture was concentrated *in vacuo* and the residue was diluted with H_2O (150 mL). The aqueous layer was extracted with EtOAc (150 mL), and then acidified to pH 1 with 1M HCl. This layer was then further extracted with EtOAc (4 x 150 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to yield **39** as a white solid (7.30 g, quant.); IR

(microscope) 3346, 3080-2200, 1927, 1716, 1594, 1547 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 5.86 (tdd, 1H, J = 6.7, 10.4, 17.0 Hz, CH_2=C<u>H</u>), 5.05 (m, 2H, C<u>H</u>$_2$=CH), 4.31 (dd, 1H, J = 4.6, 9.3 Hz, AcHNC<u>H</u>), 2.22-2.06 (m, 2H, CH_2=CHC<u>H</u>$_2$$CH_2$), 2.02 (s, 3H, C<u>H</u>$_3$C(O)), 2.00-1.72 (m, 2H, CH_2=CHCH_2C<u>H</u>$_2$); ^{13}C NMR (D_2O, 75 MHz) 176.8, 175.0, 138.1, 116.6, 53.0, 30.5, 30.1, 22.4; MS (EI) calcd. for $C_8H_{13}NO_3$ 171.0895, Found 171.0896 (M^+).



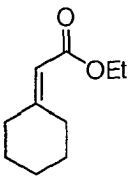
***N*-Fmoc-L-*O*-allylserine (40).**[129] TFA (30 mL) was added to a solution of *N*-Boc-Ser(*O*-allyl)-OH (**42**) (3.50 g, 14.2 mmol) in CH_2Cl_2 (150 mL) at rt. The reaction mixture was stirred for 2 h after which the solvent was removed *in vacuo* to give a colourless oil. The oil was repeatedly taken up in CH_2Cl_2 and concentrated *in vacuo* to yield the TFA salt **43** (3.32 g, 90%) as a white crystalline solid. This salt was dissolved in H_2O (60 mL) and $NaHCO_3$ (4.31 g, 51.1 mmol) was added at 0 °C. After 5 min, a solution of Fmoc-Cl (3.65 g, 14.1 mmol) in acetone (60 mL) was added and the reaction mixture was stirred for 4 h. The reaction mixture was washed with EtOAc (2 x 50 mL), and the aqueous layer was acidified with 1M HCl. The aqueous layer was extracted with EtOAc (4 x 50 mL) and the combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to yield a yellow oil. The oil was subjected to flash chromatography (SiO_2, 1% AcOH in 1:2 EtOAc/hexanes) and the residue was recrystallized from CH_2Cl_2/hexanes to give **40** as brilliant white crystals get (2.94 g, 63%); mp 107-109 °C (lit.[201] mp 108-109 °C); IR

($CHCl_3$ cast) 3065, 2949, 1721, 1519, 1477, 1450, 1420, 1336, 1216, 1105, 1085 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.79 (ap d, 2H, J = 7.5 Hz, ArH), 7.61 (m, 2H, ArH), 7.34 (ap. t, 2H, J = 7.5 Hz, ArH), 7.26 (ap t, 2H, J = 7.3 Hz, ArH), 5.24 (dd, 1H, J = 1.0, 17.1 Hz, CH_2=CHCH$_2$), 5.12 (dd, 1H, J = 1.0, 10.5 Hz, CH_2=CHCH$_2$), 5.01-5.22 (m, 1H, CH_2=CHCH$_2$), 4.32-4.40 (m, 3H, Ar_2CHCH_2O, NHCHCO$_2$H), 4.21 (t, 1H, J = 6.7 Hz, Ar_2CH-CH$_2$-O-), 3.92-4.00 (m, 1H, CH_2=CHCH_2O), 3.82 (d, 1H, J = 5.2, 9.6 Hz, OCH_2CH), 3.70 (dd, 1H, J = 4.0, 9.8 Hz, OCH_2CH); ^{13}C NMR (75 MHz, CD_3OD) δ 173.6, 158.6, 145.3, 142.6, 135.8, 128.9, 128.2, 126.3, 120.9, 117.5, 73.2, 70.7, 68.2, 55.8, 48.2; HRMS (EI) calcd. for $C_{21}H_{21}O_5N$ 367.1419, found 367.1427 (M^+); $[\alpha]_D$ +23.0° (*c* 1.0, $CHCl_3$)

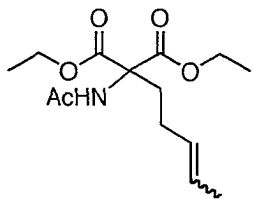


***N*-Boc-L-*O*-allylserine (42).**[129] NaH (0.50 g, 19.6 mmol) was added to a solution of N-Boc-L-serine (**41**) (2.00 g, 9.80 mmol) in anhydrous DMF (25 mL) at 0 °C. After stirring for 30 min, allyl bromide (0.900 mL, 10.7 mmol) was added and the reaction mixture was then allowed to warm to rt over 4 h. The solvent was removed *in vacuo* and the residue was dissolved in 1M NaOH (20 mL) and washed with EtOAc (2 x 20 mL). After acidifying with 1M HCl, the aqueous layer was extracted with EtOAc (3 x 30 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2, 1% AcOH in 1:1 EtOAc/hexanes) to afford **42** as a colourless oil (4.06 g, 85%); IR (microscope) 2979,

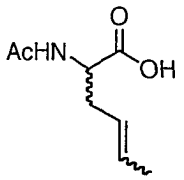
2932, 1717, 1511, 1455, 1394, 1368, 1163, 1109 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 9.48 (br s, 1H, $CO_2\underline{H}$), 5.82 (m, 1H, $CH_2=C\underline{H}$), 5.42 (d, 1H, J = 8.7 Hz, CON$\underline{H}$), 5.22 (dd, 1H, J = 1.6, 15.7 Hz, $C\underline{H}_2$=CH), 5.18 (dd, 1H, J = 1.6, 10.0 Hz, $C\underline{H}_2$=CH), 4.42 (m, 1H, NHC$\underline{H}CO_2H$), 3.98 (d, 2H, J = 5.6 Hz, CH=CHC$\underline{H}_2$), 3.82 (m, 1H, $C\underline{H}_2O$), 3.63 (dd, 1H, J = 3.6, 9.5 Hz, $C\underline{H}_2O$), 1.42 (s, 9H, $C(C\underline{H}_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.3, 155.6, 133.8, 117.6, 80.3, 72.3, 69.7, 53.8, 28.4; HRMS (ES) calcd. for $C_{11}H_{19}O_5NNa$ 268.1161. Found 268.1154 (M+Na); $[\alpha]_D$ = -17.3° (*c* 2.6, MeOH).



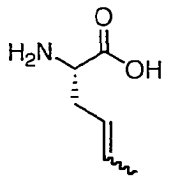
Ethyl cyclohexylidine-2-acetate (46).[130] A solution of triethyl phosponoacetate (**45**) (22.4 g, 100.0 mmol) in 1,2-dimethoxyethane (120 mL) was added dropwise over 30 min to a suspension of NaH (4.00 g, 60% in mineral oil) in 1,2-dimethoxyethane (80 mL) at 0 °C. The mixture was stirred for 15 min at 0 °C and warmed to rt. Cyclohexanone (9.82 g, 0.100 mol) was then added and the mixture was stirred for 30 min. The reaction mixture was washed with H_2O (2 x 200 mL) and the organic layer was dried (Na_2SO_4). Concentration of the organic layer *in vacuo* yields **46** as a liquid (15.6 g, 92%), which was used without any further purification; IR ($CHCl_3$, cast) 2980, 2932, 2857, 1737, 1716, 1648, 1447, 1157 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.55 (s, 1H, C$\underline{H}CO_2Et$), 4.94 (q, 2H, J = 7.1 Hz, $OC\underline{H}_2CH_3$), 2.78 (t, 2H, J = 6.3 Hz, $(C\underline{H}_2)_2C$=CH), 2.15 (t, 2H, J = 5.4 Hz, $(C\underline{H}_2)_2C$=CH), 1.64-1.52 (m, 6H, $CH_2(C\underline{H}_2)_3CH_2$), 1.23 (t, 3H, J = 7.1 Hz, $OCH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.6, 163.3, 113.0, 59.4, 38.0, 29.9, 28.7, 27.9, 26.3, 14.4; HRMS (EI) calcd. for $C_{10}H_{16}O_2$ 168.1150. Found 168.1150 (M^+).



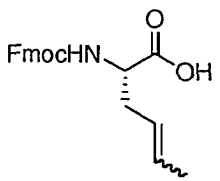
Diethyl-(4:1, *E*/*Z*)-(*R*,*S*)-2-Acetamido-4-hexenoate (53a).[133] Sodium (1.38 g, 60.0 mmol) was added in portions to anhydrous EtOH (125 mL) and allowed to completely dissolve. Diethyl *N*-acetamidomalonate (**36**) (13.0 g, 60.0 mmol) was then added and the solution went from colourless to pale orange. Crotyl bromide (85%, 10.0 g, 72.0 mmol) was added and the reaction mixture was heated to reflux and stirred for 12 h. The reaction was filtered, and the filtrate was concentrated *in vacuo* to give a dark orange oil. The oil was suspended in H_2O (100 mL), and acidified to pH 4 with glacial AcOH. The aqueous layer was extracted with CH_2Cl_2 (4 x 100 mL), and the combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to give crude **53a** as a yellow oil. The oil was subjected to flash chromatography (SiO_2, 2:1, EtOAc/hexanes) to afford **53a** as a colourless oil. Recrystallization from ether/petroleum ether afforded pure **53a** as a 1:4 mixture of *cis*/*trans* isomers in the form of a white solid (15.30 g, 94%), which was used without further purification; IR ($CHCl_3$, cast) 3304, 2983, 1743, 1667, 1445, 1301, 1202 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) for *trans* (major) isomer only δ 6.68 (s, 1H, AcNH), 5.47 (m, 1H, CH_3CH=CH), 5.17 (m, 1H, CH_3CH=CH), 4.20 (m, 4H, CH_2CH_3), 2.95 (d, 2H, J = 7.4 Hz, CH=CHCH_2), 1.99 (s, 3H, CH_3CONH), 1.59 (m, 3H, CH_3CH=CH), 1.21 (t, 6H, J = 7.2 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.9, 167.8, 130.5, 123.5, 66.4, 62.4, 35.7, 22.9, 17.9, 14.0; HRMS (EI) calcd. for $C_{13}H_{21}NO_5$ 271.1420. Found 271.1428 (M^+).



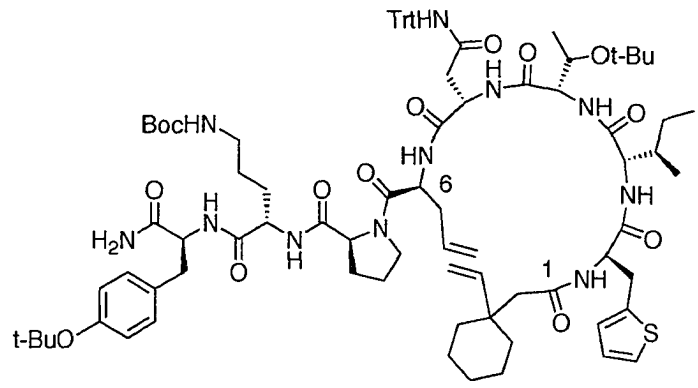
(1:4, *cis/trans*)-(*R,S*)-2-Acetamido-4-hexenoic acid (53b).[133] Compound **53a** (10.4 g, 38.0 mmol) was dissolved in a 1:1 mixture of EtOH/H_2O (125 mL) and solid NaOH (1.69 g, 46.0 mmol) was added. This mixture was heated to reflux and stirred for 12 h. The EtOH was removed *in vacuo* and the remaining aqueous layer was diluted with H_2O (125 mL) and washed with EtOAc (200 mL). The aqueous layer was then acidified to pH 1 with 1M HCl, and extracted with EtOAc (125 mL x 4). The combined organic layers were dried (Na_2SO_4) and concentrated to give **53b** as a white solid (5.28 g, 81%) as a 1:4 mixture of *cis/trans* isomers; IR (microscope) 3342, 3005-2200, 1921, 1720, 1545, 1433, 1378, 1341, 1301, 1221 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) for *trans* (major) isomer only δ 11.35 (br s, 1H, $CO_2\underline{H}$), 6.31 (d, 1H, J = 8.0 Hz, AcN$\underline{H}$), 5.54 (m, 1H, $CH_3C\underline{H}$=$CHCH_2$); 5.31 (m, 1H, CH_3CH=$C\underline{H}CH_2$), 4.58 (dt, 1H, J = 6.0, 7.5 Hz, NHC$\underline{H}CO_2H$), 2.50 (m, 2H, CH=$CHC\underline{H}_2$), 2.03 (s, 3H, $C\underline{H}_3C(O)NH$), 1.64 (dd, 3H, J = 1.0, 6.5 Hz, $C\underline{H}_3CH$=CH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.6, 171.1, 130.2, 124.2, 52.3, 34.9, 22.9, 18.1; HRMS (ES) in negative mode calcd. for $C_8H_{12}NO_3$ 170.0812. Found 170.0810 (M-H).



(4:1, *E*/*Z*)-(2*S*)-2-Amino-4-hexenoic acid (L-crotylglycine) (53).[133] (1:4, *cis*/*trans*)-(*R*,*S*)-2-Acetamido-4-hexenoic acid (**53b**) (5.28 g, 30.8 mmol) was dissolved in distilled H_2O (250 mL) and basified to pH 7.5 with conc. NH_4OH to give a final amino acid concentration of 0.12 M. Porcine kidney acylase I (2040 units /mg, 160 mg) was added and the reaction mixture was stirred for 24 h at 37 °C. The mixture was then acidified to pH 5 with 1 M HCl and charcoal was added. The reaction mixture was then heated to 60 °C and filtered through a pad of Celite. The aqueous filtrate was acidified to pH 1.5 with conc. HCl, and extracted with EtOAc (3 x 100 mL). The aqueous layer was then concentrated *in vacuo*, and the residue was taken up in a minimal amount of MeOH, filtered, and treated with excess propylene oxide to give **53** (1.42 g, 36%) as a white solid upon filtration as a 1:4 mixture of *cis*/*trans* isomers; IR (microscope) 3250-2400, 2130, 1580, 1503, 1433, 1407, 1360, 1333, 1315 cm^{-1}; ^1H NMR (D_2O, 500 MHz) for major isomer only δ 5.72 (m, 1H, $CH_3C\underline{H}$=CH), 5.37 (m, 1H, CH_3CH=C$\underline{H}$), 3.73 (dd, 1H, J = 4.9, 7.0 Hz, $H_2NC\underline{H}CO_2H$), 2.52 (m, 2H, CH=CHC$\underline{H}_2$), 1.69 (d, 3H, J = 6.6 Hz, C$\underline{H}_3$CH=CH); ^{13}C NMR (D_2O, 125 MHz) δ 174.9, 132.9, 124.0, 55.2, 34.5, 18.2; HRMS (ES) in negative mode calcd. for $C_6H_{10}NO_2$ 128.0706. Found 128.0708 (M-H).

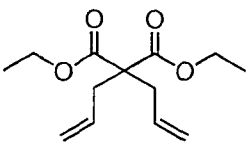


(1:4, *cis/trans*)-(2*S*)-*N*-(9*H*-Fluorenylmethoxycarbonyl)-2-amino-4-hexenoic acid (Fmoc-L-crotylglycine) (54). A solution of 9*H*-fluorenylmethyl chloroformate (6.12 g, 23.0 mmol) in dioxane (30 mL) was added dropwise over 15 min to a solution of L-crotylglycine (**53**) (2.70 g, 21.0 mmol) in 10% aqueous $NaHCO_3$ (60 mL) and dioxane (20 mL) at 0 °C. The mixture was then stirred at 0 °C for 2.5 h and then warmed to rt and stirred for a further 8 h. H_2O (600 mL) was added and the reaction mixture was washed with EtOAc (2 x 150 mL). The aqueous layer was acidified to pH 1 with conc. HCl and further extracted with EtOAc (3 x 250 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to yield **54** as a coloured, residue which was recrystallized from CH_2Cl_2 to give pure **54** as a white solid (3.00 g, 41%); IR ($CHCl_3$, cast) 3500-2650, 1719, 1518, 758 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) for major isomer only δ 10.82 (br s, 1H, $CO_2\underline{H}$), 7.75 (ap d, 2H, J = 7.4 Hz, ArH), 7.57 (m, 2H, ArH), 7.38 (ap t, 2H, J = 7.4 Hz, ArH), 7.30 (ap t, 2H, J = 7.4 Hz, ArH), 5.70-5.50 (m, 1H, $CH_3C\underline{H}$=CH), 5.34 (m, 2H, CON$\underline{H}$CH, CH_3CH=C$\underline{H}$), 4.45 (m, 3H, NHC$\underline{H}CO_2H$, $Ar_2CHC\underline{H}_2$), 4.22 (t, 1H, J = 7.0 Hz, ArC$\underline{H}$Ar), 2.78-2.32 (m, 2H, CH=CHC$\underline{H}_2$), 1.66 (d, 3H, J = 6.1 Hz, C$\underline{H}_3$CH=CH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.8, 156.0, 143.8, 143.7, 141.3, 130.6, 127.8, 127.1, 125.1, 124.1, 120.1, 67.2, 53.5, 47.2, 35.2, 18.0; HRMS (ES) calcd. for $C_{21}H_{21}NO_4Na$ 374.1363. Found 374.1367 (M+Na).

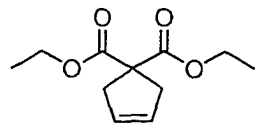


(*O-tert*-Butoxycarbonyl)-L-tyrosinamide, [*N*-(3-vinyl-3,3-cyclohexylacetyl)-D-thienyl-L-isoleucyl-(*O-tert*-butyl)-L-threonyl-L-asparaginyl-L-allylglycyl-L-prolyl-(5-*N-tert*-butoxycarbonyl)-L-ornithinyl-] (56). Peptide **56** was synthesized on Sieber amide resin (0.50 g, 0.30 mmol) with 0.62 mmol/g loading using standard Fmoc SPPS conditions. The first amino acid was loaded onto the resin as follows. To a solution of Fmoc-Tyr-(*O-t*-Bu)-OH (0.69 g, 1.5 mmol) in DMF (10 mL) was added HOBt (0.20 g, 1.5 mmol) and DIPCDI (0.19 g, 1.5 mmol). This mixture was added to the Sieber amide resin, pre-swollen in DMF and the entire reaction mixture was agitated with bubbling argon for 4 h. The mixture was filtered and the resin washed with DMF (4 x 10 mL). The Kaiser test was used on a small sample of resin to indicate the presence of no free amine and thus completion coupling. The resin was end-capped with Ac_2O (10 mL, 20 min) and washed with DMF. The remaining amino acids were coupled using PyBOP in the order: Fmoc-Orn(*N*-Boc)-OH, Fmoc-Pro-OH, Fmoc-L-AllGly-OH (**10a**), Fmoc-Asn(*N*-Trt)-OH, Fmoc-Thr(*O-t*-Bu)-OH, Fmoc-Ile-OH, Fmoc-D-Tyr(*O*-Et)-OH, 1-vinylcyclohexyl-1-acetic acid (**49**). The side chain protected linear precursor was cleaved from the resin by adding 1% TFA/CH_2Cl_2 (10 mL) into the SPPS vessel, sealing and gently shaking it for 5 min. The filtrate was then drained into a solution of 10% pyridine in MeOH (2 mL) and the resin was thoroughly washed. This process was repeated (x 10) and the filtrate

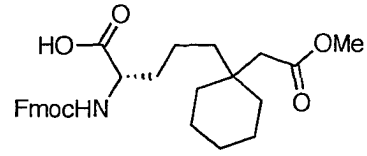
was concentrated *in vacuo*. The residue was then triturated with cold Et_2O to give the crude product **56** as an off-white solid. Purification was accomplished using prep-HPLC (t_R = 32.81 min), using solvent system B. This yielded **56** as a white solid (0.11 g, 21%) after concentration and drying *in vacuo*. MS data only; ES (MS) calcd. for $C_{87}H_{119}N_{11}O_{14}SNa$ 1597. Found 1597 (M+Na).



Diethyl diallylmalonate (57).[135] Sodium (0.58 g, 24 mmol) was added to a solution of diethylmalonate (2.0 g, 12 mmol) in anhydrous EtOH (25 mL). This mixture was stirred until all the Na had dissolved. 1-Bromo-2-propene (3.2 g, 25 mmol) was added and the mixture was heated to reflux and stirred for 8 h. The EtOH was removed *in vacuo* and the residue was taken up in H_2O (60 mL) and acidified with to pH 1 with 1 M HCl. The aqueous layer was extracted with EtOAc (3 x 60 mL). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to yield a slightly yellow liquid, which was further purified by flash chromatography (SiO_2, 5% EtOAc/hexanes) to give the product **57** as colourless liquid (1.7 g, 57%); IR (neat film) 2982, 1735, 1286, 1217, 1196 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.60 (m, 2H, CH_2=C<u>H</u>), 5.02 (m, 4H, C<u>H</u>$_2$=CH), 4.10 (q, 4H, *J* = 7.1 Hz, OC<u>H</u>$_2$CH$_3$), 2.57 (d, 4H, *J* = 7.4 Hz, =CHC<u>H</u>$_2$), 1.18 (t, 6H, *J* = 7.1 Hz, OCH$_2$C<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.9, 132.5, 119.2, 61.3, 57.4, 36.9, 14.2; HRMS (EI) calcd. for $C_{13}H_{20}O_4$ 240.1362, found 240.1364 (M^+); Anal. calcd. for $C_{13}H_{20}O_4$ C, 64.98; H, 8.39, found C, 64.78; H 8.50.

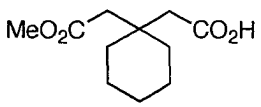


Diethyl cyclopent-3-ene-1,1-dicarboxylate (58).[119,135,201] Catalyst **9** (0.10 g, 20 mol%) was added to a solution of diethyl diallylmalonate (**57**) (0.30 g, 1.3 mmol) in CH_2Cl_2 (100 mL). The reaction mixture was heated to reflux and stirred for 14 h and then cooled to rt. DMSO (0.98 mL, 6.25 mmol) was added and the mixture was stirred for an additional 12 h at rt. The solvent was removed *in vacuo* and the residue was purified by flash chromatography (SiO_2, 1:8 EtOAc/hexanes) to give the product **58** as a clear liquid (0.24 g, 92%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.55 (m, 2H, C<u>H</u>=C<u>H</u>), 4.13 (q, 4H, J = 7.1 Hz, C<u>H</u>$_2$CH$_3$), 2.94 (s, 4H, =CHC<u>H</u>$_2$), 1.20 (t, 6H, J = 7.1 Hz, CH$_2$C<u>H</u>$_3$); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 172.2, 127.8, 61.5, 58.8, 40.8, 14.0. The reaction was shown to be quantitative by TLC and ^{1}H NMR of crude reaction mixture of **58**.



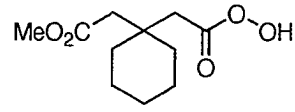
(2*S*)-2-(9*H*-Fluorenylmethoxycarbonyl-amino)-5-(1-methoxycarbonylmethyl-cyclohexyl)pentanoic acid (60). A solution of 9*H*-fluorenylmethyl chloroformate (0.37 g mg, 1.4 mmol) in dioxane (15 mL) was added dropwise over 20 min to a suspension of (2*S*)-2-amino-5-(methoxycarbonylmethyl-cyclohexyl)pentanoic acid (**66**) (0.330 g, 1.21 mmol) in 10% aqueous $NaCO_3$ (20 mL) and dioxane (10 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2.5 h, warmed to rt and stirred for an additional 1.5 h. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in water (50 mL) and washed with Et_2O (2 x 50 mL). The aqueous layer was acidified to pH 3 with

1M HCl, and extracted with EtOAc (4 x 50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to give the product **60** as a white foam (0.220 g, 38%); IR ($CHCl_3$ cast) 3325-2860, 1722, 1525, 1450 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.40 (br s, 1H, $CO_2\underline{H}$), 7.75 (ap. d 2H, J = 7.4 Hz, Ar$\underline{H}$), 7.58 (m, 2H, Ar$\underline{H}$), 7.39 (ap. td, 2H, J = 1.1, 7.4 Hz, Ar$\underline{H}$), 7.23 (ap. t, 2H, J = 7.4 Hz, Ar$\underline{H}$), 5.43 (d, 1H, J = 8.3 Hz, C(O)N$\underline{H}$), 4.40 (m, 3H, $Ar_2CHC\underline{H}_2$, NHC$\underline{H}CO_2H$), 4.21 (t, 1H, J = 7.0 Hz, $Ar_2C\underline{H}CH_2$), 3.59 (s, 3H, $CO_2C\underline{H}_3$), 2.25 (s, 2H, $C\underline{H}_2CO_2CH_3$), 1.88 (m, 1H, $C\underline{H}_2CH_\alpha$), 1.69 (m, 1H, $C\underline{H}_2CH_\alpha$), 1.54-1.21 (m, 14H, $(C\underline{H}_2)_2C(C\underline{H}_2)_5$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.0, 173.1, 156.2, 143.8, 141.3, 127.8, 127.7, 127.1, 125.1, 120.0, 67.2, 53.8, 51.3, 47.2, 36.0, 35.8, 32.9, 26.1, 21.6, 21.6, 18.9; HRMS (ES) calcd. for $C_{29}H_{35}NO_6Na$ 516.2362. Found 516.2362 (M+Na); $[\alpha]_D$ (*c* 1.0, $CHCl_3$) = + 8.1.



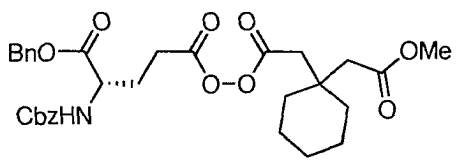
(1-Methoxycarbonylmethyl-cyclohexyl)acetic acid (62). Methyl ester **62** was synthesized using a modification of a U.S. patent.[202] To neat 1,1-cyclohexanediacetic acid **61** (5.00 g, 25.0 mmol) was added AcCl (7.00 mL, 98.0 mmol). The reaction mixture was heated at 60 °C for 3 h, resulting in a homogeneous solution. The volatiles were removed up to 100 °C at 40 torr to afford the cyclohexylglutaric anhydride **62a** as a semi translucent solid (4.42 g, 97%). The anhydride **62a** was used immediately in the next reaction without further purification. NaOMe (1.56 g, 24.0 mmol) was added to a solution of the anhydride **62a** (4.42 g, 24.0 mmol) in anhydrous MeOH (100 mL) and the reaction mixture was heated to reflux and stirred for 16 h. The reaction mixture was

acidified to pH 4 with glacial AcOH and the MeOH was removed *in vacuo*. The residue was dissolved in H_2O (100 mL) and extracted with EtOAc (2 x 125 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated to yield **62** as a colourless, viscous oil (6.27 g, quant. from **62a**); IR (cast, $CHCl_3$) 3450-2350, 1740, 1705, 1440, 1409, 1336, 1286, 1254, 1166 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 11.61 (br s, 1H, $CO_2\underline{H}$), 3.60 (s, 3H, $CO_2C\underline{H}_3$), 2.53 (s, 2H, $C\underline{H}_2CO_2$), 2.49 (s, 2H, $C\underline{H}_2CO_2$), 1.20 (m, 10H, $C\underline{H}_2(C\underline{H}_2)_3C\underline{H}_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.9, 173.1, 51.3, 35.9, 35.3, 25.7, 21.6, 21.5; HRMS (EI) calcd. for $C_{11}H_{18}O_4Na$ 237.1097. Found 237.1097 (M+Na).



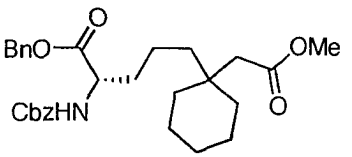
(1-Hydroperoxycarbonylmethylcyclohexyl)acetic acid methyl ester (63). Aqueous H_2O_2 (50%, 2.50 mL) was slowly added to a solution of compound **62** (5.04 g, 24.0 mmol) in conc. H_2SO_4 (3 mL) at 0 °C, and the mixture was stirred at 0 °C for 2 h. The mixture was then warmed to rt and stirred for an additional 1 h. It was then quenched with ice and diluted with Et_2O (50 mL). The organic layer was separated, washed with H_2O (50 mL) and saturated $NaHCO_3$ (50 mL), dried (Na_2SO_4) and concentrated *in vacuo* to yield the peracid **63** as a colourless viscous oil (4.22 g, 78%). The peracid was used without further purification in the next step due to volatility; IR (cast, $CHCl_3$) 3274, 2930, 2857, 1733, 1456, 1440; Where possible NMR signals for each chair conformer are shown: ^1H NMR ($CDCl_3$, 300 MHz) δ 11.12 (br s, 1H, $CO_3\underline{H}$), 3.60 (s, 3H, $CO_2C\underline{H}_3$), 2.59 (s, ~1H, $C\underline{H}_2CO_3H$ (one chair conformer)), 2.51 (s, ~1H, $C\underline{H}_2CO_3H$ (one chair conformer)), 2.50 (s, ~1H, $C\underline{H}_2CO_2CH_3$ (one chair conformer)), 2.46 (s, ~1H,

$C\underline{H}_2CO_2CH_3$ (one chair conformer)), 1.40 (m, 10H, $C\underline{H}_2(C\underline{H}_2)_3C\underline{H}_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.4 (one chair conformer), 172.2 (one chair conformer), 171.9, 51.3, 40.7, 37.4, 35.7 (one chair conformer), 35.6 (one chair conformer), 35.4 (one chair conformer), 35.2 (one chair conformer), 25.4 (one chair conformer), 25.2 (one chair conformer), 21.2 (one chair conformer), 21.1 (one chair conformer); HRMS (ES) calcd. for $C_{11}H_{19}O_5$ 231.1227. Found 231.1228 (M+H).



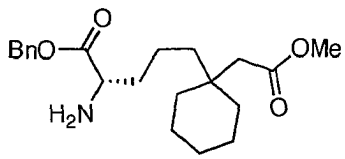
(2*S*)-2-Benzyloxycarbonylamiono-5-((1-methoxycarbonylmethylcyclohexyl)-acetylperoxy)-5-oxo-pentanoic acid benzyl ester (64). Cbz-Glu-OBn (**7**) (1.34 g, 3.60 mmol) and DCC (0.93 g, 4.5 mmol) were added to a solution of **63** (1.00 g, 4.50 mmol) in CH_2Cl_2 (25 mL) at 0 °C. The mixture was stirred at 0 °C for 2 h, slowly warmed to rt and filtered through celite. The filtrate was concentrated and the residue was dissolved in EtOAc. The solution was cooled to –20 °C for 6 h, filtered, and the filtrate was concentrated *in vacuo* to yield a yellow gum. This was subjected to flash chromatography (SiO_2, 1:1 EtOAc/hexanes) to give **64** as a colourless gum (1.69 g, 80%); IR ($CHCl_3$, cast) 3346, 3033, 2931, 1806, 1776, 1731, 1524, 1454 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.31 (m, 10H, ArH), 5.49 (d, 1H, J = 7.6 Hz, C(O)N$\underline{H}$CH), 5.18 (s, 2H, Ar$C\underline{H}_2O$), 5.08 (s, 2H, Ar$C\underline{H}_2O$), 4.42 (m, 1H, HN$C\underline{H}$C(O)), 3.60 (s, 3H, $CO_2C\underline{H}_3$), 2.63 (s, 2H, $C\underline{H}_2CO_2$), 2.54 (s, 2H, $C\underline{H}_2CO_2$), 2.43 (m, 2H, $CH_2C\underline{H}_2CO_3$), 2.30 (m, 1H, $C\underline{H}_2CH_2CO_3$), 2.06 (m, 1H, $C\underline{H}_2CH_2CO_3$), 1.60-1.38 (m, 10H, $C\underline{H}_2(C\underline{H}_2)_3C\underline{H}_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.1, 171.2, 168.3, 167.4, 167.0, 156.0, 136.1, 135.0, 128.7, 128.6,

128.5, 128.4, 128.2, 128.1, 67.6, 67.2, 53.2, 51.3, 40.8, 37.0, 36.1, 35.6, 35.5, 35.4, 27.6, 26.2, 25.6, 25.4, 21.4, 21.3; HRMS (ES) calcd. for $C_{31}H_{37}NO_{10}$ 583.2490 (M+H), Found 584.2488; $[\alpha]_D$ (*c* 1.0, $CHCl_3$) = + 2.5.

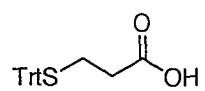


(2*S*)-2-Benzyloxycarbonylamino-5-(1-methoxycarbonylmethylcyclohexyl)pentanoic acid benzyl ester (65). A solution of **64** (0.50 g, 0.86 mmol) in CH_2Cl_2 (5 mL) was spread out over the bottom of a 500 mL Pyrex recrystallization dish. The solvent was removed with a steady stream of argon to afford a thin layer of neat diacyl peroxide **64**. The vessel was then covered with a quartz glass plate and sealed under an argon atmosphere. The reaction vessel was then immersed in a cold bath at – 78 °C. A 0.9 Amp UV lamp was placed directly onto the quartz plate and the neat sample was irradiated at 254 nm for 36 h. The reaction mixture was warmed to rt, and the reaction vessel was extracted extensively with CH_2Cl_2. The solvent was removed *in vacuo* to yield a yellow oil, which was subjected to flash chromatography (SiO_2, 1:6 to 1:1 EtOAc/hexanes). This afforded the product **65** as colourless gum (0.15 g, 34%, R_f = 0.61 in 1:1 EtOAc/hexanes) as well as some recovered starting material **64** (0.18 mg, 36%, R_f = 0.51 in 1:1 EtOAc/hexanes); For **65**: IR ($CHCl_3$, cast) 3349, 3064, 3032, 2929, 2855, 1728, 1523, 1455 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.35 (m, 10H, ArH), 5.39 (d, 1H, *J* = 8.2 Hz, CONHCH), 5.24-5.08 (m, 4H, $ArCH_2O$), 4.42 (m, 1H, CONHCH), 3.60 (s, 3H, CO_2CH_3), 2.21 (s, 2H, $CH_2CO_2CH_3$), 1.86 (m, 1H, CH_2CH_α), 1.68 (m, 1H, CH_2CH_α), 1.45-1.10 (m, 14H, $(CH_2)_2C(CH_2)_5$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.7, 172.4, 155.9,

136.3, 135.4, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 67.1, 54.0, 51.0, 41.4, 35.9, 35.6, 35.2, 33.4, 26.0, 21.6, 18.7; HRMS (ES) calcd for $C_{29}H_{38}NO_6$ 496.2694 (M+H), Found 496.2690; $[\alpha]_D$ (*c* 1.0, $CHCl_3$) = - 8.0.

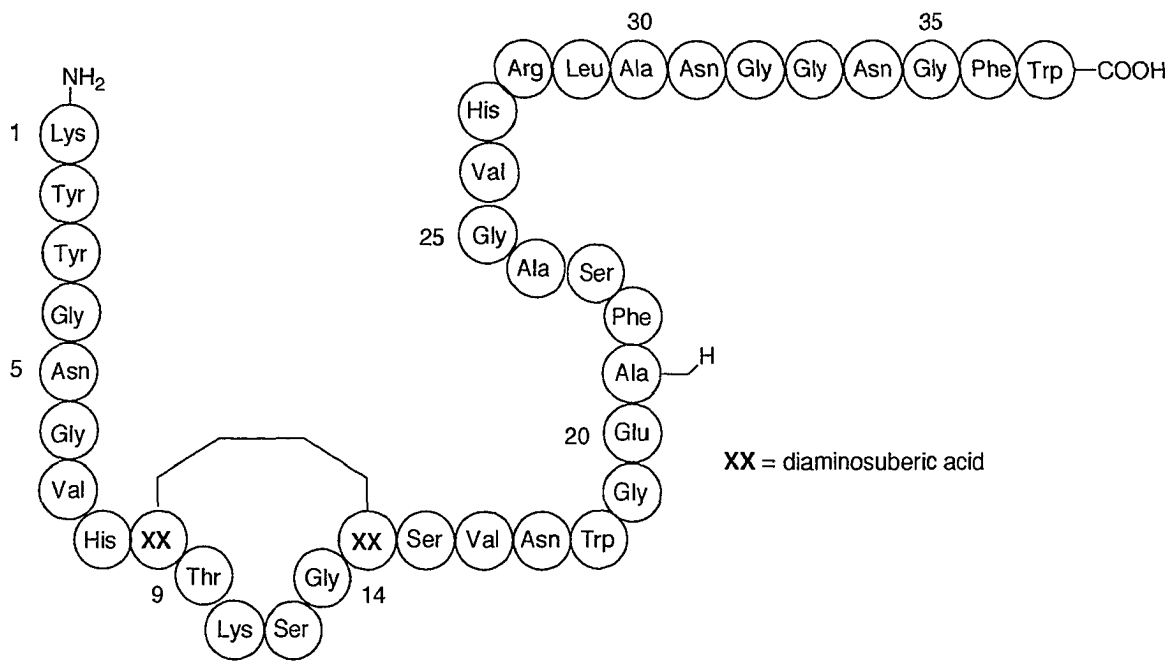


(2*S*)-2-Amino-5-(methoxycarbonylmethylcyclohexyl)pentanoic acid [(α-L-amino-6,6-suberic acid) 8-monomethyl ester] (66). Pd/C (10%, 58 mg) was added to a solution of compound **65** (0.60 g, 1.2 mmol) in anhydrous EtOH (10 mL). The reaction mixture was then stirred under H_2 (760 torr) for 12 h. The mixture was filtered through a pad of Celite, and the filtrate concentrated *in vacuo* to give the amine **66** as a white solid (0.33 g, quant.) which was used in the next step without any further purification; IR (microscope) 3450, 3400-2450, 1733, 1586, 1455 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 3.62 (s, 3H, $CO_2C\underline{H}_3$), 3.56 (dd, 1H, *J* = 5.3, 6.9 Hz, $NH_2C\underline{H}$), 2.32 (s, 2H, $C\underline{H}_2CO_2CH_3$), 1.81 (m, 2H, $CH_2C\underline{H}_2$), 1.61-1.30 (m, 14H, $(C\underline{H}_2)_2C(C\underline{H}_2)_5$); ^{13}C NMR (CD_3OD, 100 MHz) δ 174.6, 173.4, 63.6, 51.7, 43.2, 42.3, 33.1,27.2, 22.7, 19.9; HRMS (ES) calcd. for $C_{14}H_{26}NO_4$ 272.1856. Found 272.1852 (M+H). $[\alpha]_D$ (*c* 1.0, CH_3OH) = + 7.4.



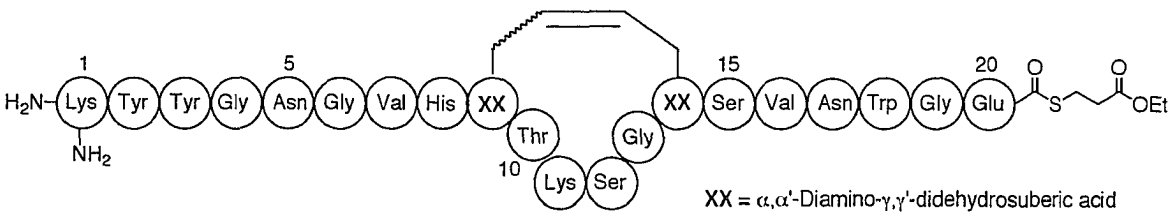
(*S*-Trt)-3-Mercaptopropionic acid (71). This compound was synthesized using a modified procedure from Yuen and coworkers.[138] Triphenylmethanol (9.39 g, 35 mmol) followed by BF_3OEt_2 (6.5 mL) were added to a solution of 3-mercaptopropionic acid

(3.18 g, 30 mmol) in glacial AcOH (25 mL). The mixture was stirred at rt for 12 h. The mixture was cooled to 0 °C, and saturated NaOAc (80 mL) and H_2O (160 mL) were added. The mixture was stirred for 15 min and then allowed to stand for 1 h. The precipitate was collected by filtration. The precipitate was dissolved in 1:1 Et_2O/H_2O (100 mL), stirred for 5 min and separated by filtration. Successive washings of the precipitate with Et_2O/H_2O yielded the product as a white solid (9.29 g, 89%); mp 179-182 °C (lit.[138] mp 177-183 °C); IR (microscope) 3061, 3032, 2933, 2913, 1704, 1592, 1488, 1255 cm^{-1}; ^1H NMR ($DMSO_{d6}$, 400 MHz) δ 7.33-7.29 (m, 12H, ArH), 7.23 (m, 3H, ArH), 2.27 (t, 2H, J = 6.7 Hz, $SC\underline{H}_2CH_2CO_2H$), 2.15 (t, 2H, J = 6.7 Hz, $SCH_2C\underline{H}_2CO_2H$); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 172.5, 144.2, 129.0, 127.9, 126.6, 66.1, 32.8, 26.6; HRMS (ES) calcd. for $C_{22}H_{20}O_2SNa$ 371.1076, found 371.1077 (M+Na).



[9,14-α,α'-L,L-Diaminosuberic acid]leucocin A (76). $NiCl_2$ (1 mg, ~ 5 μmol) was added to a solution of cysteinyl peptide **88** (2 mg, 0.5 μmol) in 1:1 $MeOH/H_2O$ (2 mL). The

reaction mixture was stirred at rt for 15 min. Sodium borohydride (1 mg, ~ 15 μmol) was then quickly added to the reaction mixture and H_2 gas was evolved immediately. The reaction vessel was sealed and heated at 50 °C for 1 h. The reaction mixture was centrifuged, and the supernatant was removed. The solid precipitate was then taken up in 1:1 MeOH/H_2O (1 mL), sonicated and centrifuged. The supernatant was removed and the process was repeated. The combined supernatants were concentrated to 2 mL *in vacuo* and subjected to MALDI-TOF (MS) and RP-HPLC analysis. On one attempt **76** was detected by MS; MALDI-TOF (MS) calcd. for $C_{176}H_{249}N_{52}O_{50}$ 3890.9. Found 3893.1 (M+H). However no product was isolated during attempted purification by prep-HPLC using solvent system B.



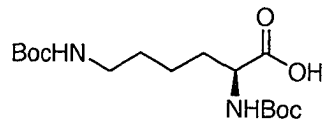
20-(1-ethyl mercapopropionate), [L-lysyl-L-tyrosyl-L-tyrosyl-L-glycyl-L-aspariginyl-L-glycyl-L-valyl-L-histidyl-L-α-aminobutyrl-L-threonly-L-lysyl-L-serinyl-L-glycyl-L-(α-aminobutyrl)-L-serinyl-L-valyl-L-asparaginyl-L-tryptophanyl-L-glycyl-L-glutamyl-], (9→14) (*E/Z*)-α,α'-L,L-diamino-γ,γ'dehydrosuberic acid (84).

I) Resin bound peptide thioester (84a). A solution of Fmoc-Glu(*O-t*-Bu)-OH (0.87 g, 2.0 mmol) and DIPEA (0.70 mL, 4.0 mmol) in $CHCl_3$ (20 mL) was added to 4-sulfamylbutyryl AM NovaGel resin (0.83 g, 0.50 mmol) in $CHCl_3$ (20 mL) at –20 °C. PyBOP (1.0 g, 2.0 mmol) was added and the mixture was allowed to stand at –20 °C,

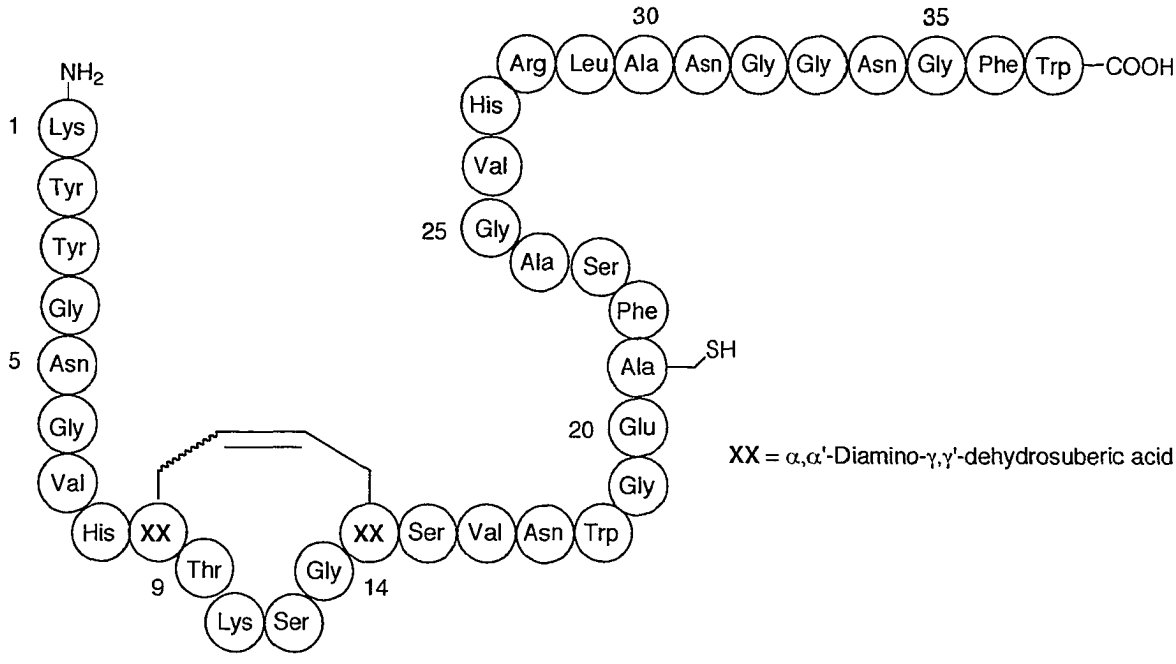
with occasional swirling for 2.5 h. The reaction mixture was warmed to rt, washed with $CHCl_3$ (3 x 20 mL), EtOH (3 x 20 mL) and then resuspended in DMF (20 mL) for 30 min to allow for resin swelling. Amino acids (9-19) were coupled as their *N*-Fmoc protected derivatives using PyBOP and standard SPPS in this order: Fmoc-Gly-OH, Fmoc-Trp(*N*-Boc)-OH, Fmoc-Asn(*N*-Trt)-OH, Fmoc-Val-OH, Fmoc-Ser(*O*-*t*-Bu)-OH, Fmoc-L-AllGly-OH (**10a**), Fmoc-Gly-OH, Fmoc-Ser(*O*-*t*-Bu)-OH, Fmoc-Lys(*N*-Boc)-OH, Fmoc-Thr(*O*-*t*-Bu)-OH, Fmoc-L-AllGly-OH (**10a**). The resin bound peptide (1/2 of the resin, ~ 0.25 mmol) was subjected to standard RCM conditions using catalyst **9** (50 mg, 20 mol%). Deprotection of the N-Fmoc protecting group was accomplished with 20% piperidine/DMF (2 x, 10 mL, 5 min) and the free amine was detected using the Kaiser test. After cyclization, standard Fmoc SPPS was continued for *N*-Fmoc amino acids (2-8) using PyBOP in this order: Fmoc-His(*N*-Trt)-OH, Fmoc-Val-OH, Fmoc-Gly-OH, Fmoc-Asn(*N*-Trt)-OH, Fmoc-Gly-OH, Fmoc-Tyr(*O*-*t*-Bu)-OH, Fmoc-Tyr(*O*-*t*-Bu)-OH. The last amino acid, lysine at position 1, required the *N*-terminal Boc protection to avoid side reactions during the cleavage of **84a** from the resin, and thus was coupled as its symmetrical anhydride as follows. Boc-Lys(*N*-Boc)-OH (**87**) (1.1 g, 3.1 mmol) was dissolved in dry CH_2Cl_2 (20 mL) and cooled to 0 °C. DIPCDI (0.24 mL, 1.6 mmol) was added and the reaction mixture was stirred for 20 min at 0 °C. The reaction mixture was concentrated *in vacuo* and the residue was taken up in DMF (20 mL). The *N*-terminal Fmoc protecting group on the resin bound peptide was removed and the above mixture was added to the SPPS vessel. After 2 h of agitation with bubbling argon at rt, the resin was filtered, washed with DMF (4 x 20 mL) and tested for free amine (Kaiser test). The

resin-bound peptide was end-capped with 20% Ac_2O/DMF (10 mL, 20 min) to give resin-bound peptide **84a**.

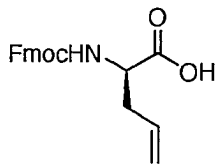
II) Cleavage and deprotection of 84a (84). The resin-bound peptide **84a** (0.25 mmol) was treated with dry THF (15 mL) for 1 h to ensure maximum resin swelling. Activation of the 4-sulfamylbutyryl resin was achieved by adding a 1M solution of TMS-diazomethane in 1:1 THF/hexane (10 mL) to the resin bound peptide with gentle swirling for 2.5 h. The resin was then washed with THF (5 x 10 mL) followed by DMF (5 x 10 mL). The resin bound peptide was resuspended in DMF (12 mL) and ethyl 3-mercaptopropionate (1.7 mL, 12 mmol) was added to make a solution with a final concentration of 1M. Thiophenolate sodium salt (1.0 mg, 0.2 mmol) was added and the reaction mixture was gently swirled for 24 h at rt.[16] The resin was filtered and washed with DMF (7 x 5 mL), and the filtrate was concentrated *in vacuo*. Deprotection of the peptide was done by treating the residue with cleavage cocktail (A) (5 mL) for 4 h at rt. Concentration of this mixture *in vacuo*, followed by precipitation with cold Et_2O yielded crude **84** as a light brown solid. Purification was accomplished with prep-HPLC (t_R = 14.31 min) as a single peak using solvent system B. This yielded **84** as a fluffy white solid upon lyopholization (6 mg, 1% based on 1/2 (~0.25 mmol) of resin cleaved). MS data for **84** only; MALDI-TOF (MS) calcd. for $C_{101}H_{146}N_{27}O_{31}S$ 2264.1, found 2266.5 (M+H).



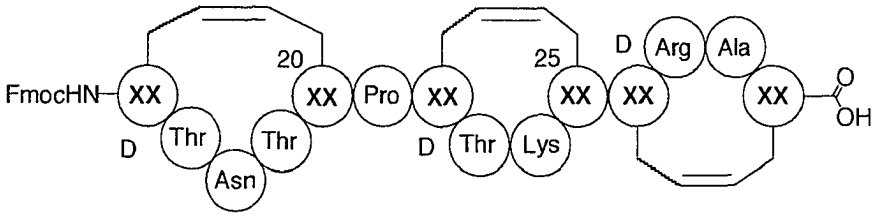
Boc-Lys(*N*-Boc)-OH (87).[203] A solution of Boc_2O (6.74 g, 30.0 mmol) in dioxane (40 mL) was added dropwise over 30 min to a solution of L-lysine (1.50 g, 10.0 mmol) in 1M NaOH (40 mL). The reaction mixture was stirred for 5 h at rt and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (100 mL), and washed with H_2O (100 mL), 10% aqueous citric acid (100 mL) and brine (100 mL). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was triturated with petroleum ether/Et_2O, and the precipitate filtered and dried to yield di-Boc protected lysine **87** as a white foam (2.70 g, 76%). This compound was used without further purification; IR (microscope) 3338, 2978, 1693, 1524, 1455, 1393, 1367, 1250, 1167; ^{1}H NMR ($CHCl_3$, 300 MHz) δ 9.85 (br s, 1H, $CO_2\underline{H}$), 6.20 (m, 1H, $CH_2N\underline{H}CO$), 5.30 (d, 1H, J = 7.7 Hz, $N\underline{H}CHCO_2H$), 4.24 (m, 1H, $NHC\underline{H}CO_2H$), 3.05 (m, 2H, $C\underline{H}_2NHCO$), 1.80 (m, 1H, $CH_\alpha C\underline{H}_2$), 1.65 (m, 1H, $CH_\alpha C\underline{H}_2$), 1.45 (m, 2H, $CH_2C\underline{H}_2CH_2$), 1.39 (m, 18H, $C(C\underline{H}_3)_3$); ^{13}C NMR ($CHCl_3$, 100 MHz) δ 176.2, 156.3, 155.8, 80.0, 79.3, 53.2, 40.1, 32.1, 29.5, 28.4, 28.3, 22.4; HRMS (ES) calcd. for $C_{16}H_{30}N_2O_6Na$ 369.1996, found 369.1989 (M+Na); $[\alpha]_D$ = + 12.1 (*c* 1.1, $CHCl_3$).



(*E/Z*)-[9,14-(α,α'-L,L-Diamino-γ,γ'-dehydrosuberic acid)-21-L-cysteinyl]leucocin (88). The ligation of peptide thioester **84** and N-terminal cysteine containing peptide **85** was done using a modification of a literature procedure.[22] Peptides **84** (4 mg, 2 μmol) and **85** (3 mg, 2 μmol) were dissolved in Tris-HCl, 6 M guanidine buffer (4 mL) at pH 8.5. MeCN (500 μL) was added to help in the solubilization of the peptides. Thiophenol (90 μL, 2% v/v) and benzylmercaptan (90 μL, 2% v/v) were added and the entire mixture was stirred at rt for 18 h. The mixture was directly subjected to purification by prep-HPLC (t_R = 14.12 min) analysis using solvent system B, which afforded the ligated peptide **88** as a white fluffy solid upon lyophilization (3 mg, 42%). MS data only; MALDI-TOF (MS) calcd. for $C_{176}H_{249}N_{52}O_{50}S$ 3922.8. Found 3924.3 (M+H).



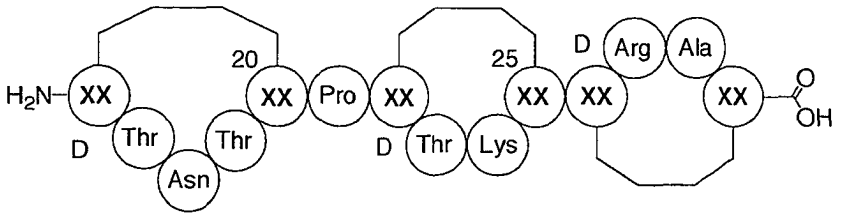
***N*-Fmoc-D-allylglycine (91).** Fmoc-Cl (5.18 g, 21.0 mmol) in dioxane (20 mL) was added dropwise over 15 min to a solution of D-allylglycine (2.00 g, 17.0 mmol) in 10% $NaHCO_3$ (30 mL) at 0 °C. This mixture was stirred at 0 °C for 3 h, allowed to warm to rt and stirred for an additional 1 h. The reaction mixture was washed with Et_2O (4 x 100 mL) and the aqueous layer acidified with 1M HCl. The aqueous layer was extracted with EtOAc (4 x 50 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The residue was then crystallized from CH_2Cl_2/hexanes to give **91** as a fluffy white solid (4.09 g, 70%); IR (microscope) 3404, 3085, 2966, 1722, 1645, 1525, 1450, 1396, 1234, 1190, 1048 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.74 (ap. d, 2H, J = 7.6 Hz, ArH), 7.56 (m, 2H, ArH), 7.38 (ap. t, 2H, J = 7.5 Hz, ArH), 7.30 (ap. t, 2H, J = 7.5 Hz, ArH), 5.71 (m, 1H, CH_2=CH), 5.30, (d, 1H, J = 7.9 Hz, NH), 5.15 (m, 2H, CH_2=CH), 4.49 (m, 1H, NHCH), 4.40 (d, 2H, J = 7.0 Hz, Ar_2CHCH_2), 4.22 (t, 1H, J = 7.0 Hz, Ar_2CHCH_2), 2.64 (m, 1H, =CHCH$_2$), 2.55 (m, 1H, =CHCH$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.2, 156.0, 143.8, 141.4, 131.8, 127.8, 127.1, 125.1, 120.1, 119.8, 67.3, 53.1, 47.2, 36.4; HRMS (ES) calcd. for $C_{20}H_{19}NO_4Na$ 360.1206. Found 360.1203; $[\alpha]_D$ = -13.6 (*c* 1.0, $CHCl_3$).



XX = α,α'-Diamino-γ,γ'-dehydrosuberic acid

***N*-(9*H*-Fluorenylmethoxycarbonyl)-D-(α-aminobutyrl)-L-threonyl-L-asparaginyl-L-threonyl-L-(α-aminobutyrl)-L-prolyl-D-(α-aminobutyrl)-L-threonyl-L-lysyl-D-(α-aminobutyrl)-L-arginyl-L-alanyl-L-(α-aminobutyrine), [(16→ 20), (22→25), (26→29)] (*E*/*Z*)-α,α'-D,L-diamino-γ,γ'-dehydrosuberic acid (95)**. The linear precursor to **95** was synthesized on Wang resin (0.75 g, 0.60 mmol) with a loading of 0.81 mmol/g. The first amino acid, Fmoc-L-allylglycine (2.0 g, 7.5 mmol) was dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C. DIPCDI (0.38 g, 3.8 mmol) was then added and the reaction mixture was stirred at 0 °C for 20 min. The mixture was then concentrated *in vacuo* and the residue was taken up in DMF and added to the Wang resin previously swollen in DMF. The reaction mixture was agitated with bubbling argon for 3 h, filtered and washed with DMF (4 x 20 mL). Amino acids 26-28 were coupled using PyBOP in this order: Fmoc-Ala-OH, Fmoc-Arg(*N*-Pmc)-OH, Fmoc-D-AllGly-OH (**91**) to yield the resin-bound linear fragment **90**. The resin-bound precursor **90** was subjected to standard RCM conditions using catalyst **9** (0.10 g, 20 mol%). A small sample of resin bound peptide was cleaved with cocktail (B) at rt for 2.5 h. Concentration of the reaction mixture *in vacuo*, followed by precipitation with cold Et_2O yields carbocycle **92** as an off-white solid; MALDI-TOF (MS) calcd. for $C_{32}H_{39}N_7O_7$ 633.3. Found 634.3 (M+H). None of the linear precursor **92** was detected by MS. The resin-bound cyclic peptide **92** (0.60 mmol) was resuspended in DMF (20 mL) and Fmoc SPPS was continued using PyBOP to couple

amino acids 22-25 in this order: Fmoc-L-AllGly-OH (**10a**), Fmoc-Lys(*N*-Boc)-OH, Fmoc-Thr(*O*-*t*-Bu)-OH, Fmoc-D-AllGly-OH (**91**). Upon completion of coupling, an RCM reaction was done in the same manner as mentioned above for carbocycle **92** to give the resin-bound bicycle **93**. A small sample of **93** was cleaved using cocktail (B) and subjected to MS analysis; MALDI-TOF/(MS) calcd. for $C_{50}H_{68}N_{12}O_{12}$ 1028.5. Found 1229.5 (M+H). Only the desired bicycle **93** was detected by MS analysis. Resin-bound bicycle **93** (0.30 mmol) was resuspended in DMF and SPPS was continued using the following protected amino acids in this order: Fmoc-Pro-OH, Fmoc-L-AllGly-OH (**10a**), Fmoc-Thr(*O*-*t*-Bu)-OH, Fmoc-Asn(*N*-Trt)-OH, Fmoc-Thr(*O*-*t*-Bu)-OH, Fmoc-D-AllGly-OH (**91**). This yields the resin-bound linear precursor **94**. An RCM reaction on precursor **94** was then conducted using modified conditions of the RCM reaction. Cyclization times were increased to 48 h, to allow for the RCM reaction to go to completion and cleavage [cocktail (B)] of a small sample resin yields the tricyclic peptide **95** as a white solid and the only product detectable by MS. MS data for **95** only; MALDI-TOF (MS) calcd. for $C_{75}H_{105}N_{19}O_{21}$ 1607.8. Found 1608.9 (M+H).



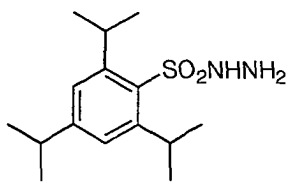
XX = Diamiosuberic acid

D-(α-aminobutyrl)-L-threonyl-L-asparaginyl-L-threonyl-L-(α-aminobutyrl)-L-prolyl-D-(α-aminobutyrl)-L-threonyl-L-lysyl-D-(α-aminobutyrl)-L-arginyl-L-alanyl-L-(α-aminobutyrine), [(16→20), (22→25), (26→29)] α,α'-D,L-diaminosuberic acid (96).

I) Synthesis of 95 using diimide reduction.[36] 2,4,6-Triisopropylbenzenesulfonyl hydrazide (**97**) (60.0 mg, 0.200 mmol) and 1.4 M piperidine/NMP (3 mL) were added to resin-bound **95** (0.120 g, 40.0 μmol) in a 50 mL SPPS vessel. The vessel was sealed and placed in a water bath at 47 °C for 2 h. The solution was filtered, and the resin washed with NMP (4 x 5 mL). The process was repeated (x 4) for a total of 10 h of reduction time. Cleavage of a small sample was done by treating the resin bound peptide with cocktail (B) (2 mL) for 3 h. Concentration of this mixture *in vacuo*, followed by precipitation with cold Et_2O yielded a white solid as a product, which was subjected to MALDI-TOF (MS). Analysis by MS showed only the presence of *N*-Fmoc deprotected derivative **98**. MALDI-TOF (MS) calcd. for $C_{60}H_{95}N_{19}O_{19}$ 1385.7. Found 1386.8 (M+H).

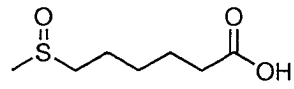
II) Attempted reduction of 94 using Wilkinson's catalyst.[170-172] Wilkinson's catalyst (4 mg, 4 μmol) was added to a sample of resin-bound **95** (0.120 g, 40.0 μmol) suspended in dry, degassed CH_2Cl_2 (3 mL). The reaction mixture was swirled gently under H_2 at 760 torr for 48 h. The resin was filtered, washed with CH_2Cl_2 (4 x 3 mL) and dried under a stream of argon. A small sample of resin-bound peptide (~20 mg) was cleaved by

treatment of the resin with cocktail (B) at rt for 4 h. Concentration of the mixture *in vacuo* followed by precipitation with cold Et_2O yielded an off-white solid, which was subjected to MALDI-TOF (MS). Analysis by MS showed that the precipitate was cleaved starting material **95**; MALDI-TOF MS calcd. for $C_{75}H_{105}N_{19}O_{21}$ 1607.8. Found 1609.7 (M+H, 100%) and 1630.7 (M+Na, 43%).

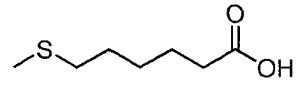


2,4,6-Triisopropylbenzenesulfonyl hydrazide (97).[124] Hydrazine hydrate (0.68 g, 14.7 mmol) was added dropwise over 15 min to a solution of 2,4,6-triisopropylbenzenesulfonyl chloride (2.00 g, 6.70 mmol) in THF (10 mL) at -10 °C. The reaction mixture was warmed to 0 °C and stirred for 3 h where upon a white precipitate formed. Water was added dropwise until all the precipitate was dissolved. Et_2O (50 mL) was added to the mixture and the aqueous layer was removed. The organic layer was washed with brine (3 x 10 mL), dried (Na_2SO_4) and filtered through Celite. The filtrate was concentrated *in vacuo* at < 20 °C. Petroleum ether (30 mL) was added and the precipitate formed was collected by filtration and washed thoroughly with petroleum ether. Trituration with ice-cold H_2O followed by filtration and drying *in vacuo* afforded **97** as a white solid (1.60 g, 80%); mp 117-119 °C (lit.[124] mp 118-120 °C) IR (cast, $CHCl_3$) 3356, 3281, 3175, 2957, 2870, 1602, 1463, 1427, 1364, 1322, 1152 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.19 (s, 2H, Ar$\underline{H}$), 5.44 (br s, 1H, N$\underline{H}NH_2$), 4.12 (sept, 2H, J = 6.9 Hz, ortho C$\underline{H}(CH_3)_2$), 3.26 (br s, 2H, NHN$\underline{H}_2$), 2.88 (sept, 1H, J = 6.9 Hz, para C$\underline{H}(CH_3)_2$), 1.25 (d, 12H, J = 6.9 Hz, ortho CH(C$\underline{H}_3)_2$), 1.24 (d, 6H, J = 6.9 Hz, para

$CH(C\underline{H}_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.9, 151.9, 128.7, 124.1, 122.8, 34.4, 29.9, 25.0, 23.8; HRMS (ES) calcd. for $C_{15}H_{26}N_2O_2SNa$ 321.1607. Found 321.1605 (M+Na).

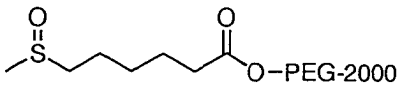


6-(Methylsulfoxide)hexanoic acid (99). A 0.5 M aqueous solution of sodium metaperiodate (500 mL, 0.25 mol) was added dropwise to a solution of 6-(methylthio)-hexanoic acid (**100**) (40.0 g, 0.250 mol) in MeOH (500 mL) at 0 °C. The reaction mixture was warmed to 4 °C and stirred for 12 h. The mixture was then concentrated *in vacuo* (< 5 °C) to prevent over oxidation, and the residue was extracted with CH_2Cl_2 (1 L) in the presence of $MgSO_4$. The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to yield **99** as a light yellow oil (46.0 g, quant.) after drying *in vacuo* for 20 h; IR (microscope) 2932, 2870, 2517, 1707, 1469, 1418, 1296, 1270, 1225, 1121 cm^{-1}; ^1H NMR (CD_2Cl_2, 400 MHz) δ 11.18 (br. s, 1H, $CO_2\underline{H}$), 2.82 (ddd, 1H, J = 4.7, 14.1, 12.8 Hz, $S(O)C\underline{H}_2$), 2.79 (ddd, 1H, J = 6.0, 8.8, 11.3 Hz, $S(O)C\underline{H}_2$), 2.58 (s, 3H, $C\underline{H}_3S(O)$), 2.28 (t, 2H, J = 7.4 Hz, $C\underline{H}_2CO_2H$), 1.74 (m, 2H, $S(O)CH_2C\underline{H}_2$), 1.64 (p, 2H, J = 7.5 Hz, $(CH_2)_2C\underline{H}_2(CH_2)_2$), 1.46 (m, 2H, $C\underline{H}_2CH_2CO_2H$); ^{13}C NMR (CD_2Cl_2, 75 MHz) δ 176.8, 54.0, 38.1, 34.0, 28.4, 24.7, 22.6; HRMS (EI) calcd for $C_7H_{14}O_3S$ 178.0664, found 178.0664 (M^+).

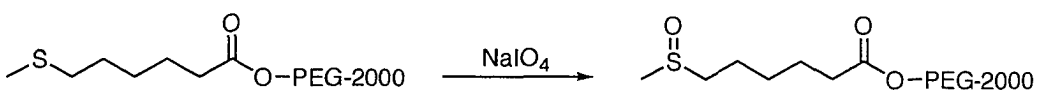


6-(Methylthio)-hexanoic acid (100).[180] See note in general experimental (section 6.1.1) regarding the toxicity of methanethiol. A 1L, three-necked, round-bottomed flask was

equipped with a magnetic stir bar, two 100 mL pressure-equalizing addition funnels and an adaptor with a stopcock, connected to an empty gas-washing bottle, which in turn was connected to a lecture bottle containing methanethiol. One addition funnel was charged with 6M NaOH aqueous solution while the other was fitted with a dry-ice condenser with an outlet connected to a trap containing either a $Pb(OAc)_2$ or bleach solution. The flask was charged with 6-bromohexanoic acid (51.0 g, 0.260 mol) followed by methanol (300 mL) and cooled to 0 °C. Methanethiol (40.0 g, 0.860 mol) was condensed into the second addition funnel and was added carefully, with stirring, to the reaction mixture followed by the addition of 6M NaOH aqueous solution (98 mL). The mixture was allowed to warm to 20 °C and stirred for 16 h. Excess methanethiol was removed by bubbling argon into the mixture with a vent to a bleach solution, followed by removal of methanol *in vacuo*. The residue was acidified to pH 1 with conc. HCl and extracted with hexane (5 x 150 mL). The combined organic extracts were dried over sodium sulfate and concentrated *in vacuo* to give 6-(methylthio)hexanoic acid (**100**) (40.2 g, 95%) as a colourless oil; IR ($CHCl_3$, cast) 3037, 1708 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 11.85 (br, 1H, $CO_2\underline{H}$), 2.48 (t, 2H, J = 7.3 Hz, $SC\underline{H}_2$), 2.36 (t, 2H, J = 7.4 Hz, $C\underline{H}_2CO_2H$), 2.06 (s, 3H, $C\underline{H}_3S$), 1.62 (m, 4H, $SCH_2C\underline{H}_2$, $C\underline{H}_2CH_2CO_2H$), 1.44 (m, 2H, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 180.5, 34.3, 34.3, 29.2, 28.5, 24.7, 15.5; HRMS (EI) calcd. for $C_7H_{14}O_2S$ 162.0715, found 162.0714 (M^+). Anal. calcd. for $C_7H_{14}O_2S$: C, 51.82; H, 8.70; S, 19.76. Found: C, 51.66; H, 9.07; S, 19.51.

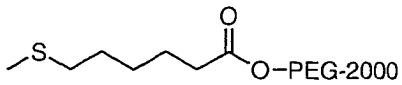


Poly(ethylene glycol) bis-[6-(methylsulfinyl)hexanoate] (103).[180] A 0.5 M aqueous solution of sodium metaperiodate (19.0 mL, 9.70 mmol) was added dropwise to a solution of **104** (10.5 g, 4.60 mmol) in MeOH (80 mL) and H_2O (20 mL) at 0 °C. The mixture was then stirred at 0 °C for 2 h, during which time a white precipitate formed, and then stored in the fridge at 4 °C for 16 h. The MeOH was then removed *in vacuo* (< 10°C) and the residue diluted with water to dissolve the precipitate. This aqueous solution was then extracted with CH_2Cl_2 (5 x 50 mL) and the combined organic layers were dried (Na_2SO_4). Concentration *in vacuo* yielded poly(ethylene glycol) bis-[6-(methylsulfinyl)hexanoate] (**103**) (10.6 g, 99%) as a white solid; IR (CH_2Cl_2, cast) 1732, 1114 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.20 (t, 2H, J = 4.7 Hz, $OC\underline{H}_2PEG$), 3.61 (s, 88H, PEG-2000), 2.66 (m, 2H, $S(O)CH_2$), 2.53 (s, 3H, $C\underline{H}_3S(O)$), 2.33 (t, 2H, J= 7.3 Hz, $C\underline{H}_2CO_2PEG$), 1.76 (m, 2H, $S(O)CH_2C\underline{H}_2$), 1.65 (m, 2H, $C\underline{H}_2CH_2CO_2$), 1.48 (m, 2H, $CH_2C\underline{H}_2CH_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1, 70.4, 68.9, 63.3, 54.2, 38.4, 33.6, 28.0, 24.2, 22.1. Anal. calcd. for $C_{104}H_{206}O_{50}S_2$: C, 53.84; H, 8.89; S, 2.76. Found: C, 54.00; H, 9.01; S, 3.01.

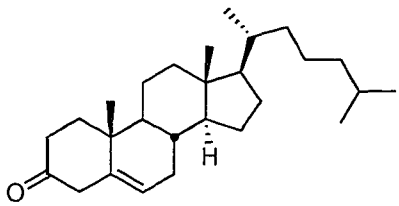


Recovery and recycling of 103. The reacted PEG-bound sulfide/sulfoxide mixture from oxidation of cholesterol (**106**) was dissolved in CH_2Cl_2 (500 mL) and washed with water (3 x 500 mL) and brine (500 mL), to remove NEt_3 salts formed during the oxidation. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo* to give, according to ^1H

NMR, a 7:3 mixture of bound sulfide/sulfoxide (57.6 g, 97%). This mixture was then treated with a 0.5 M aqueous solution of sodium metaperiodate (19.4 mL, 9.7 mmol) in analogous conditions to those mentioned above, to give the regenerated PEG-sulfoxide **103** (52.6 g, 91%) which by ^{1}H NMR showed solely the presence of sulfoxide.[180,182]



Poly(ethylene glycol) bis-[6-(methylthio)hexanoate] (104).[180] 1,3-Dicyclohexylcarbodiimide (DCC) (3.60 g, 17.5 mmol) and 4-*N*,*N*-dimethylaminopyridine (DMAP) (0.21 g, 1.7 mmol) and **100** (2.80 g, 17.5 mmol) were added to a solution of poly(ethylene glycol)-2000 (PEG-2000) (10.0 g, 5.00 mmol) in anhydrous CH_2Cl_2 (50 mL). The mixture was then stirred at room temperature for 16 h. The white urea precipitate that formed during the course of the reaction was removed by filtration through Celite and washed with CH_2Cl_2 (150 mL). The filtrate was concentrated to ~15 mL and Et_2O (150 mL) was added with vigorous stirring. The solution was stirred at 0 °C for 2 h and the resulting precipitate was removed by filtration, washed (Et_2O) and dried *in vacuo* to give poly(ethylene glycol) bis-[6-(methylthio)hexanoate] (**103**) (11.17 g, 98%) as a white solid; IR (CH_2Cl_2 cast) 1733, 1113 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.21 (t, 2H, J = 4.8 Hz, OC$\underline{H}_2$PEG-2000), 3.64 (s, 88H, PEG-2000), 2.48 (t, 2H, J = 7.3 Hz, SC$\underline{H}_2$), 2.33 (t, 2H, J = 7.4 Hz, C$\underline{H}_2$CO$_2$-PEG), 2.08 (s, 3H, C$\underline{H}_3$S), 1.63 (m, 4H, -C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_2$), 1.42 (m, 2H, C$\underline{H}_2$C$\underline{H}_2$C$\underline{H}_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4,70.4, 69.1, 63.3, 33.9, 28.6, 28.1, 24.4, 15.4.



Cholest-5-en-3-one (105). Oxalyl chloride (3.4 mL, 39.0 mmol) in CH_2Cl_2 (25 mL) was added dropwise to a solution of **103** (60.0 g, 25.9 mmol) in anhydrous CH_2Cl_2 (600 mL) at –60 °C. The mixture was then stirred at –60 °C for 5 min. A solution of cholesterol (**106**) (10.0 g, 25.9 mmol) in anhydrous CH_2Cl_2 (275 mL) was added to the mixture which was stirred at –60 °C for 45 min. Triethylamine (21.6 mL, 155 mmol) was added dropwise at –60 °C and the mixture was stirred between –55 °C and –45 °C for 2.5 h. The reaction mixture was warmed to rt and concentrated *in vacuo* to a volume of 200 mL. Et_2O (800 mL) was added to precipitate the polymer and the reaction mixture was cooled to –20 °C. The precipitated polymer was removed by filtration and washed with Et_2O (800 mL). The filtrate was concentrated to give 11.0 g of crude cholest-5-en-3-one (**105**) as a yellow solid containing a trace of polymer contamination (ca. 1%). The product was further purified by addition of Et_2O (300 mL) and passing the resulting suspension through a short pad of silica (75 g). The silica pad was then washed with diethyl ether (200 mL) and concentration of the filtrate *in vacuo* gave cholest-5-en-3-one (**105**) (9.54 g, 96%) as a white solid. ^{1}H NMR spectroscopy showed the product to be >98% pure; IR ($CHCl_3$, cast) 2946, 2867, 1719, 1467 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.32 (m, 1H, C=C$\underline{H}$), 3.25 (m, 1H, C$\underline{H}_2$C(O)), 2.79 (dd, 1H, J = 16.5, 2.1 Hz, C$\underline{H}_2$C(O)), 2.43 (ddd, 1H, J = 15.0, 13.7, 5.8 Hz, $CH_2C\underline{H}_2C(O)$), 2.28 (m, 1H, C$\underline{H}_2CH_2C(O)$), 2.00 (m, 3H, C=CHC$\underline{H}_2$, C$\underline{H}(CH_3)_2$), 1.80 (m, 1H, C$\underline{H}(CH_3)_2$), 1.60-1.40 (m, 7H), 1.39-1.22 (m, 3H), 1.18-0.92 (m, 10H), 1.15 (s, 3H, CC$\underline{H}_3$), 0.89 (d, 3H, J = 6.5 Hz, CHC$\underline{H}_3$), 0.83 (d, 6H, J

= 6.6 Hz, $CH(C\underline{H}_3)_2$), 0.68 (s, 3H, $CC\underline{H}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.3, 138.6, 122.9, 56.6, 56.2, 49.2, 48.3, 42.4, 39.7, 39.5, 37.6, 36.9, 36.8, 36.2, 35.8, 31.9, 31.8, 28.2. 28.0, 24.3, 23.8, 22.8, 22.5, 21.4, 19.2, 11.9; MS (ES) 385.4 (MH^+), consistent with the literature.[180,182]

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8. APPENDIX

8.1 ^{1}H-NMR Assignments for Oxytocin Analogs

Table 10 ^{1}H NMR assignments for oxytocin agonists **17**, **18a** and **18b**

Residue	**18a**	**18b**	**17**
Allylglycine[1]			Reduced (AG)
$C_\alpha H$	4.03	4.07	3.90 (J = 5.5 Hz)
$C_\beta H_2$	2.78	2.56, 2.69	1.82
CH=CH	5.60 (J = 10.9 Hz)	5.49 (J = 15.4 Hz)	1.41 (-CH_2-CH_2-)
Tyr[2]			
$C_\alpha H$	4.70	4.65 (J = 7.0, 8.6 Hz)	4.68 (J = 5.9, 7.4 Hz)
$C_\beta H_2$	3.00, 3.09	2.99 (J = 8.6, 14.4 Hz)	2.94 (J = 5.9, 14.7 Hz)
		3.11 (J = 7.0, 14.4 Hz)	3.21 (J = 7.4, 14.7 Hz)
ArH	7.20 (J = 8.3 Hz)	7.17 (J = 8.5 Hz)	7.21 (J = 8.6 Hz)
ArH	6.83 (J = 8.3 Hz)	6.84 (J = 8.5 Hz)	6.88 (J = 8.6 Hz)
Ile[3]			
$C_\alpha H$	4.27	3.95 (J = 6.1 Hz)	4.21 (J = 5.5 Hz)
$C_\beta H$	1.98	1.81	1.83
$C_\gamma CH_2$	1.02, 1.22	1.07, 1.34	1.04, 1.30
$C_\gamma CH_3$	0.82-0.89	0.80-0.85	0.82-0.89
$C_\delta CH_3$	0.82-0.89	0.80-0.85	0.82-0.89
Gln[4]			
$C_\alpha H$	4.04	4.09	4.03 (J = 6.1, 8.1 Hz)
$C_\beta H_2$	2.02	1.98, 2.05	1.96
$C_\gamma H_2$	2.40	2.35	2.35
Asn[5]			
$C_\alpha H$	4.64	4.69 (J = 4.7, 9.8 Hz)	4.55 (J = 5.0, 5.5 Hz)
$C_\beta H_2$	2.79	2.71 (J = 4.7, 15.8 Hz)	2.80, 2.84
		2.93 (J = 9.8, 15.8 Hz)	

Allylglycine[6]			Reduced (AG)
$C_\alpha H$	4.42	4.58 (J = 3.5, 5.3 Hz)	4.42
$C_\beta H_2$	2.30, 2.50	2.30, 2.48	1.71
CH=CH	5.68 (J = 10.9 Hz)	5.60 (J = 15.4 Hz)	1.22
Pro[7]			
$C_\alpha H$	4.43	4.38 (J = 5.7, 8.4 Hz)	4.39 (J = 6.0, 6.5 Hz)
$C_\beta H_2$	1.92	1.86, 2.23	1.96, 2.01
$C_\gamma H_2$	2.59	1.98	2.25, 2.35
$C_\delta H_2$	3.61, 3.72	3.58, 3.69	3.59, 3.73
Leu[8]			
$C_\alpha H$	4.30	4.27 (J = 6.1, 7.0 Hz)	4.26
$C_\beta H_2$	1.61, 1.68	1.56, 1.67	1.55, 1.63
$C_\gamma H$	1.68 (J = 5.8 Hz)	1.67 (J = 6.3 Hz)	1.63 (J = 6.3 Hz)
$C_\delta CH_3$	0.82-0.89	0.86 (J = 6.3 Hz)	0.82-89
$C_\delta CH_3$	0.95 (J = 5.8 Hz)	0.90 (J = 6.3 Hz)	0.90 (J = 6.3 Hz)
Gly[9]			
$C_\alpha H_2$	3.87-3.93 (J = 17.2 Hz)	3.82-3.88 (J = 17.2 Hz)	3.82-3.88 (J = 17.1 Hz)

Table 11 ^{1}H NMR assignments for oxytocin analogs **21a**, **21b** and **20**

Residue	**21a**	**22b**	**20**
Allylgycine[1]			
			Reduced
$C_\alpha H$	4.16	4.12	3.90 (J = 5.5 Hz)
$C_\beta H_2$	2.75	2.58, 2.78	1.88
CH=CH	5.42 (J = 8.5 Hz)	5.45 (J = 15.6 Hz)	1.42-1.21
Tyr[2]			
$C_\alpha H$	4.64	4.65	4.70
$C_\beta H_2$	3.10 (J = 6.8, 14.3 Hz)	2.98 (J = 8.4, 14.4 Hz)	2.95 (J = 8.6, 14.3 Hz)
	3.04 (J = 7.2, 14.3 Hz)	3.16 (J = 7.0, 14.4 Hz)	3.15 (J = 6.1, 14.4 Hz)
ArH	7.19 (J = 8.2 Hz)	7.19 (J = 7.1 Hz)	7.16 (J = 8.4 Hz)
ArH	6.87 (J = 8.2 Hz)	6.87 (J = 8.3 Hz)	6.83 (J = 8.5 Hz)
Ile[3]			
$C_\alpha H$	4.30	4.03 (J = 6.3 Hz)	4.14 (J = 5.1 Hz)
$C_\beta H$	1.76	1.86	1.88
$C_\gamma CH_2$	1.56, 1.82	1.04, 1.30	1.05, 1.30
$C_\gamma CH_3$	0.86 (J = 6.8 Hz)	0.91 (J = 7.1 Hz)	0.89 (J = 6.9 Hz)
$C_\delta CH_3$	0.81	0.84 (J = 7.3 Hz)	0.84 (J = 7.4 Hz)
Gln[4]			
$C_\alpha H$	4.64	4.65	4.70
$C_\beta H_2$	2.75, 2.90	2.78	2.75
		2.92 (J = 5.2, 15.9 Hz)	(J = 8.8, 15.7 Hz)
			2.85 (J = 5.4, 15.7 Hz)
$C_\gamma H$	3.42, 3.49	2.78	3.60, 3.74
Asn[5]			
$C_\alpha H$	4.72	4.72	4.70
$C_\beta H_2$	3.04 (J = 6.8, 14.3)	2.98 (J = 8.4, 14.4 Hz)	2.95 (J = 8.6, 14.3 Hz)

	3.10 (J = 6.8, 14.3 Hz)	3.16 (J = 7.0, 14.4 Hz)	3.15 (J = 6.1, 14.3 Hz)
Homoallylglycine[6]			Reduced
$C_\alpha H$	4.42	4.44	4.42
$C_\beta H_2$	1.91, 2.02	2.04, 2.29	1.88
$C_\gamma CH_2$	3.61, 3.74	3.62, 3.76	1.42-1.21
CH=CH	5.71 (J = 8.5 Hz)	5.63 (J = 15.4 Hz)	1.42-1.21 (-CH_2-CH_2-)
Pro[7]			
$C_\alpha H$	4.33	4.12 (J = 5.7, 8.4 Hz)	4.28 (J = 6.0, 6.5 Hz)
$C_\beta H_2$	2.02	2.04	2.00
$C_\gamma H_2$	2.36	2.38	2.25, 2.35
$C_\delta H_2$	3.41, 3.52	3.62, 3.76	3.59, 3.73
Leu[8]			
$C_\alpha H$	4.12	4.03 (J = 6.2 Hz)	4.08
$C_\beta H_2$	1.17, 1.56	1.86	2.25, 2.37
$C_\gamma H$	1.72	1.66	1.30
$C_\delta CH_3$	0.94 (J = 6.0 Hz)	0.84 (J = 7.3 Hz)	0.86 (J = 6.2 Hz)
$C_\delta CH_3$	0.89 (J = 6.0 Hz)	0.91 (J = 7.1 Hz)	0.91 (J = 6.5 Hz)
Gly[9]			

Table 12 ^{1}H NMR assignments for 2:1 mixture of **19a** and **19b**

Residue	**19a** (*cis*)	**19b** (*trans*)
Homoallylgycine[1]		
$C_\alpha H$	4.52 (J = 5.1, 8.8 Hz)	4.61
$C_\beta H_2$	1.86-2.10	1.86-2.10
$C_\gamma H_2$	2.19-2.25	
CH=CH	5.45 (J = 10.8 Hz) 5.55	5.55, 5.60 (J = 15.4 Hz)
Tyr[2]		
$C_\alpha H$	4.84 (J = 6.0, 9.5 Hz)	
$C_\beta H_2$	3.00 (J = 9.5, 14.3 Hz) 3.35 (J = 6.0, 14.4 Hz)	
ArH	7.27 (J = 8.2 Hz)	7.19 (J = 8.2 Hz)
ArH	6.91 (J = 8.5 Hz)	6.87 (J = 8.4 Hz)
Ile[3]		
$C_\alpha H$	4.12	4.12
$C_\beta H$	1.80, 2.29	1.80, 2.29
$C_\gamma H_2$	1.17, 1.39	-
$C_\gamma CH_3$	0.83-0.96	0.83-0.96
$C_\delta CH_3$	0.83-0.96	0.83-0.96
Gln[4]		
$C_\alpha H$	4.78	-
$C_\beta H_2$	2.66 (J = 10.8, 14.7 Hz), 2.78	2.90 (J = 4.5, 15.2 Hz)
$C_\gamma H$	2.78	2.78
Asn[5]		
$C_\alpha H$	4.62	4.70
$C_\beta H_2$	3.00 (J = 8.4, 14.4 Hz) 3.18 (J = 6.7, 14.4 Hz)	-

Allylglycine[6]		
$C_\alpha H$	4.61	4.40
$C_\beta H_2$	2.19, 2.25	-
CH=CH	5.45, 5.55 (J = 10.8 Hz)	5.55, 5.60 (J = 15.4 Hz)
Pro[7]		
$C_\alpha H$	4.24	4.14 (J = 4.9 Hz)
$C_\beta H_2$	1.86-2.10	1.86-2.10
$C_\gamma H_2$	1.86-2.10	1.86-2.10
$C_\delta H_2$	3.66, 3.78	-
Leu[8]		
$C_\alpha H$	4.35 (J = 4.8, 9.8 Hz)	4.30 (J = 5.2, 9.6 Hz)
$C_\beta H_2$	1.86-2.10	1.86-2.10
$C_\gamma H$	1.86-2.10	1.86-2.10
$C_\delta CH_3$	0.83-0.96	0.83-0.96
$C_\delta CH_3$	0.83-0.96, 0.91 (J = 6.1 Hz)	0.83-0.96
Gly[9]		
$C_\alpha H_2$	3.91 (J = 17.2 Hz)	3.89 (J = 17.2 Hz)

Table 13 ^{1}H NMR assignments for oxytocin analogs **22a**, **22b** and **23**

Residue	**22a**	**22b**	**23**
Homoallylgycine[1]			
$C_\alpha H$	4.52	4.01	3.94
$C_\beta H_2$	2.10	1.80-2.29	1.58-1.73
$C_\gamma CH_2$	2.10	1.80-2.29	1.24-1.50
CH=CH	5.46 (J = 9.3 Hz)	5.52	1.24-1.50 (reduced)
Tyr[2]			
$C_\alpha H$	4.91 (J = 5.7, 10.3 Hz)	4.82 (6.8, 9.2 Hz)	4.93
$C_\beta H_2$	2.99 (J = 10.2, 14.5 Hz) 3.30 (J = 5.7, 14.5 Hz)	2.96-3.07 3.22 (J = 6.8, 14.5 Hz)	2.92 (J = 9.8, 14.4 Hz) 3.18 (J = 6.0, 14.4 Hz)
ArH	7.26 (J = 8.6 Hz)	7.21 (J = 8.6 Hz)	7.16 (J = 8.5 Hz)
ArH	6.95 (J = 8.5 Hz)	6.93 (J = 8.6 Hz)	6.87 (J = 8.6 Hz)
Ile[3]			
$C_\alpha H$	4.27	4.12	3.94
$C_\beta H$	1.92	1.80-2.29	1.24-1.50
$C_\gamma CH_2$	1.36, 1.50	1.17-1.39	1.24-1.50
$C_\gamma CH_3$	0.94 (J = 6.3 Hz)	0.92-0.97	0.91 (J = 7.6 Hz) 0.92-0.98
$C_\delta H_3$	0.97 (J = 7.3 Hz)	0.92-0.97	0.92-0.98
Gln[4]			
$C_\alpha H$	4.33 (J = 5.0, 9.8 Hz)	4.24	4.16 (J = 4.8, 8.0 Hz)
$C_\beta H_2$	2.90	1.80-2.29	1.91, 2.05
$C_\gamma H_2$	3.49	2.32, 2.40	2.32, 2.43
Asn[5]			
$C_\alpha H$	5.02	4.90	4.93
$C_\beta H_2$	2.78 (J = 11.9, 15.6 Hz) 3.17	2.96-3.07 2.70	3.13 (J = 3.9, 16.2 Hz) 2.84

	(J = 3.6, 15.6 Hz)		(J = 11.0, 16.2 Hz)
Homoallylglycine[6]			Reduced
$C_\alpha H$	4.27	4.48	
$C_\beta H_2$	2.10	1.80-2.29	1.88
$C_\gamma CH_2$	2.34	1.80-2.29	1.24-1.50
CH=CH	5.56 (J = 9.3 Hz)	5.52	1.24-1.50
Pro[7]			
$C_\alpha H$	4.00	4.42	4.43 (J = 6.0, 8.2 Hz)
$C_\beta H_2$	1.92, 2.10	1.80-2.29	2.05
$C_\gamma H_2$	1.92, 2.10	1.80-2.29	1.91
$C_\delta H_2$	3.68, 3.82	3.66-3.78	3.62 (6.8, 10.0 Hz) 3.80 (7.1, 10.0 Hz)
Leu[8]			
$C_\alpha H$	4.52	4.35	4.60
$C_\beta H_2$	1.50	1.70	1.58-1.73
$C_\gamma H$	1.72	1.70	1.58-1.73, 1.77
$C_\delta CH_3$	1.02-0.98	0.92-97 0.99 (J = 6.1 Hz)	0.89 (J = 6.1 Hz) 0.92-0.98
Gly[9]			
$C\alpha H_2$	3.93 (J = 17.2 Hz)	3.96 (J = 17.3 Hz)	3.90 (J = 17.2 Hz)
Pro[7]			

Table 14 ^{1}H NMR assignments for oxytocin analogs **24a** and **24b**

Residue	**24a**	**24b**
Ser(O-All)[1]		
$C_\alpha H$	4.19	4.20
$OC_\beta H_2$	3.89, 3.96	3.90, 3.97
$OC_\gamma H_2$	4.12, 4.19	4.16
CH=CH	5.68 (J = 10.8 Hz)	5.69 (J = 15.9 Hz)
Tyr[2]		
$C_\alpha H$	4.73	4.74
$C_\beta H_2$	3.01 (J = 8.6, 14.3 Hz) 3.21 (J = 6.0, 14.4 Hz)	2.98, 3.20 (J = 6.0, 14.4 Hz)
ArH	7.21 (J = 8.4 Hz)	7.21 (J = 8.5 Hz)
ArH	6.95 (J = 8.5 Hz)	6.88 (J = 8.6 Hz)
Ile[3]		
$C_\alpha H$	4.12	4.11
$C_\beta H$	1.90	1.85-1.90
$C_\gamma CH_2$	1.08, 1.34	1.18, 1.40
$C_\gamma CH_3$	0.90	0.87-0.93
$C_\delta H_3$	0.90	0.87-0.93
Gln[4]		
$C_\alpha H$	4.12	4.12
$C_\beta H_2$	2.02	1.85-2.01
$C_\gamma H_2$	2.38	2.36, 2.42
Asn[5]		
$C_\alpha H$	4.70	4.71
$C_\beta H_2$	2.78 (J = 8.5, 15.8 Hz) 2.88 (J = 5.3, 15.7 Hz)	2.82 (J = 9.8, 15.7 Hz), 2.98

Allylglycine[6]		
$C_\alpha H$	4.64	4.63 (J = 3.8, 8.6 Hz)
$C_\beta H_2$	2.55	2.52
CH=CH	5.68 (J = 10.8 Hz)	5.69 (J = 15.9 Hz)
Pro[7]		
$C_\alpha H$	4.44	4.44 (J = 5.5, 8.3 Hz)
$C_\beta H_2$	1.90, 2.29	2.08, 2.36
$C_\gamma H_2$	1.90, 2.02	1.85-2.08
$C_\delta H_2$	3.62, 3.73	3.65, 3.74
Leu[8]		
$C_\alpha H$	4.30	4.31
$C_\beta H_2$	1.62, 1.69	1.61, 1.68
$C_\gamma H$	1.72	1.68
$C_\delta CH_3$	0.90 0.95 (J = 6.2 Hz)	0.91, 0.95 (J = 6.2 Hz)
Gly[9]		
$C_\alpha H_2$	3.91 (J = 17.2 Hz)	3.91 (J = 17.2 Hz)

Table 15 ^{1}H NMR assignments for atosiban (**13**) and analogs **26b** and **27***

Residue	Atosiban (**13**)	**26b** (*trans only*)	**27**
Mpa[1] or 4-PA[1]			
$C_\alpha H$	2.58, 2.61-2.68	2.21-2.36	2.32
$C_\beta H_2$	2.82-2.92	2.40, 3.02	$C\beta H_2$, $C\gamma H_2$
CH=CH	-	5.35 (J = 14.9 Hz)	1.64, 2.20
D-Tyr[2](OEt)			
$C_\alpha H$	4.61 (J = 6.3, 9.9 Hz)	4.68	4.63
$C_\beta H_2$	2.96, 3.13	2.72, 3.02	2.98 (J = 9.6, 13.5 Hz) 3.02
ArH	7.22 (J = 8.6 Hz)	7.20 (J = 8.4 Hz)	7.22 (J = 8.6 Hz)
ArH	6.96 (J = 8.6 Hz)	6.94 (J = 8.5 Hz)	6.96 (J = 8.5 Hz)
$OC\underline{H_2}CH_3$	4.10 (J = 7.1 Hz)	4.09 (J = 7.0 Hz)	4.15 (J = 7.0 Hz)
$OCH_2C\underline{H_3}$	1.37 (J = 7.1 Hz)	1.35 (J = 7.0 Hz)	1.37 (J = 7.0 Hz)
Ile[3]			
$C_\alpha H$	4.14 (J = 4.9 Hz)	4.13	4.20
$C_\beta H$	1.80	1.70	1.80
$C_\gamma H_2$	1.80	0.80, 1.00	0.86, 1.09
$C_\gamma H_3$	0.53 (J = 7.0 Hz)	0.61 (J = 6.7 Hz)	0.60 (J = 6.9 Hz)
$C_\delta H_3$	0.74 (J = 7.2 Hz)	0.71 (J = 7.5 Hz)	0.76 (J = 7.2 Hz)
Thr[4]			
$C_\alpha H$	4.30	4.30 (J = 5.2 Hz)	4.28
$C_\beta H$	4.17	4.13	4.20
$C_\gamma H_3$	1.20 (J = 6.4 Hz)	1.18 (J = 6.4 Hz)	1.23 (J = 6.7 Hz)
Asn[5]			
$C_\alpha H$	4.85	4.68	4.61

	(3.2, 10.0 Hz)		
$C_\beta H_2$	2.82-2.92, 3.10	2.89, 3.02	2.74 (J = 7.9, 15.7 Hz) 2.84 (J = 6.8, 15.6 Hz)
Cys[6]/Allylglycine[6]			
$C_\alpha H$	4.61	4.57	4.58
$C_\beta H_2$	2.68-2.81, 2.82-2.92	2.21-2.36	CβH$_2$, Cγ H$_2$
CH=CH	-	5.58 (*trans*, major) (J = 14.9 Hz) 5.51 (*cis*) (J = 10.9 Hz)	1.56, 1.80
Pro[7]			
$C_\alpha H$	4.44	4.42	4.42 (J = 6.4, 8.1 Hz)
$C_\beta H_2$	2.03-2.32	2.21-2.36	2.32
$C_\gamma H_2$	1.92	1.90, 2.02	1.91, 2.02
$C_\delta H_2$	3.73, 3.84	3.63, 3.74	3.63, 3.78
Orn[8]			
$C_\alpha H$	4.35	4.34	4.32
$C_\beta H_2$	1.80	1.68-1.84, 1.90	1.91, 2.02
$C_\gamma H_2$	1.80	2.21-2.36, 2.40	1.91, 2.02
$C_\delta H_2$	3.20	3.02	3.02 (J = 6.1 Hz) 0.92-0.98
Gly[9]			
$C_\alpha H_2$	3.92	3.91	3.92

* Mpa = 3-mercaptopropionic acid, 4-PA = 4-pentenoic acid

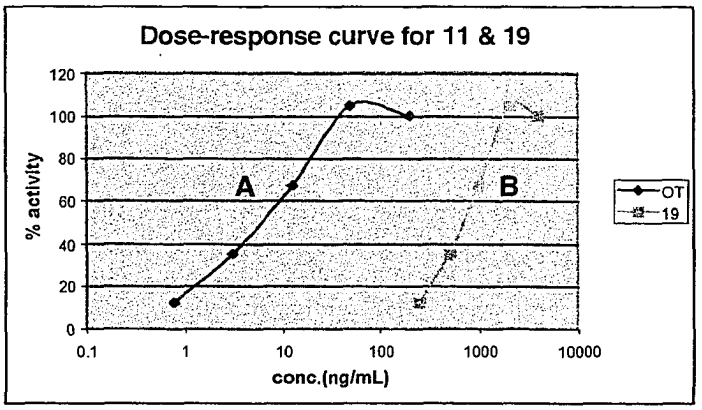
Table 16 ^{1}H NMR assignments for antagonist **15** and analog **29***

Residue	**15**	**29**
Mca[1] or Vca[1]		
$C_\alpha H$	2.51 (J = 14.1 Hz) 2.68 (J = 14.1 Hz)	2.01
$(CH_2)_5$	1.12, 1.19, 1.31, 1.43-1.93	1.40, 1.64-1.84
CH_2CH_2	-	1.12,1.15
D-Thi[2]		
$C_\alpha H$	4.76	4.73
$C_\beta H_2$	3.20 (J = 8.6, 15.0 Hz) 3.47 (J = 5.0, 15.0 Hz)	3.13
ArH	7.01 7.31 (J = 1.2, 5.12 Hz)	7.31 (J = 4.4 Hz)
ArH	6.95	6.98 7.00 (J = 5.6 Hz)
Ile[3]		
$C_\alpha H$	4.15	4.16
$C_\beta H$	1.12	1.26
$C_\gamma CH_2$	1.75	1.61-1.84
$C_\gamma CH_3$	0.89 (J = 8.9 Hz)	0.83
$C_\delta CH_3$	0.83 (J = 7.4 Hz)	0.83
Thr[4]		
$C_\alpha H$	4.24	4.31-4.41
$C_\beta H$	4.15	4.16
$C_\gamma H_3$	1.21	1.26
Asn[5]		

$C_\alpha H$	4.85	4.46
$C_\beta H_2$	2.72 2.86 (J = 6.4, 15.0 Hz)	2.94, 3.32
Cys[6]/XY[6]		
$C_\alpha H$	4.56	4.62
$C\beta H_2$	2.89-2.99	1.61-1.84
$C_\gamma H_2 C_\gamma H_2$	-	1.26
Pro[7]		
$C_\alpha H$	4.36 (J = 7.0, 7.7 Hz)	4.46
$C_\beta H_2$	2.01, 2.22	2.19
$C_\gamma H_2$	1.70	2.12, 2.25
$C_\delta H_2$	3.81	3.62, 3.77
Orn[8]		
$C_\alpha H$	4.24	4.31-4.41
$C_\beta H_2$	1.43-1.93	1.61-1.84
$C_\gamma H_2$	2.01, 2.22	1.61-1.84, 2.19
$C_\delta H_2$	2.89-2.99	2.45, 2.76
Tyr[9]		
$C_\alpha H$	3.56	4.55
$C_\beta H_2$	2.89-2.99 3.07 (J = 2.7, 14.4 Hz)	2.94, 3.32
ArH	7.13 (J = 7.9 Hz)	6.98
ArH	6.83 (J = 7.5 Hz)	6.55 (J = 8.1 Hz)

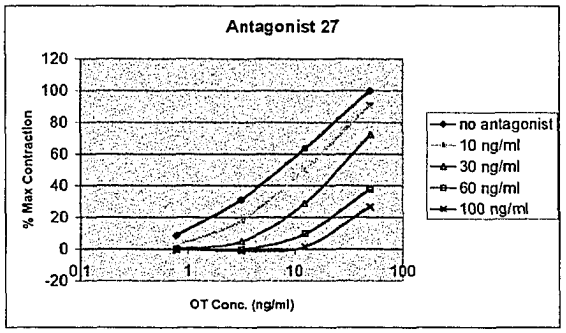
* Mca = 3-mercapto-3,3-cyclohexylpropionic acid, Vca = 1-vinyl-1,1-cyclohexylacetic acid

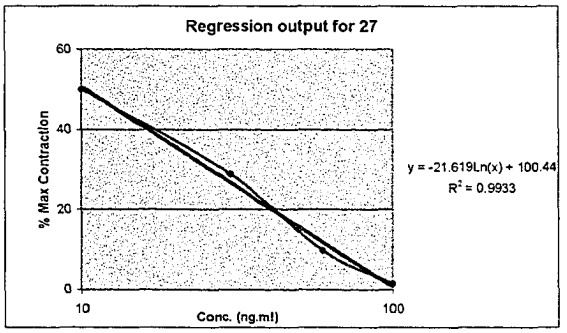
8.2 Sample dose-response curveS for oxytocin (11) and compound 19



(*EC_{50} values were measured directly from these response curves)

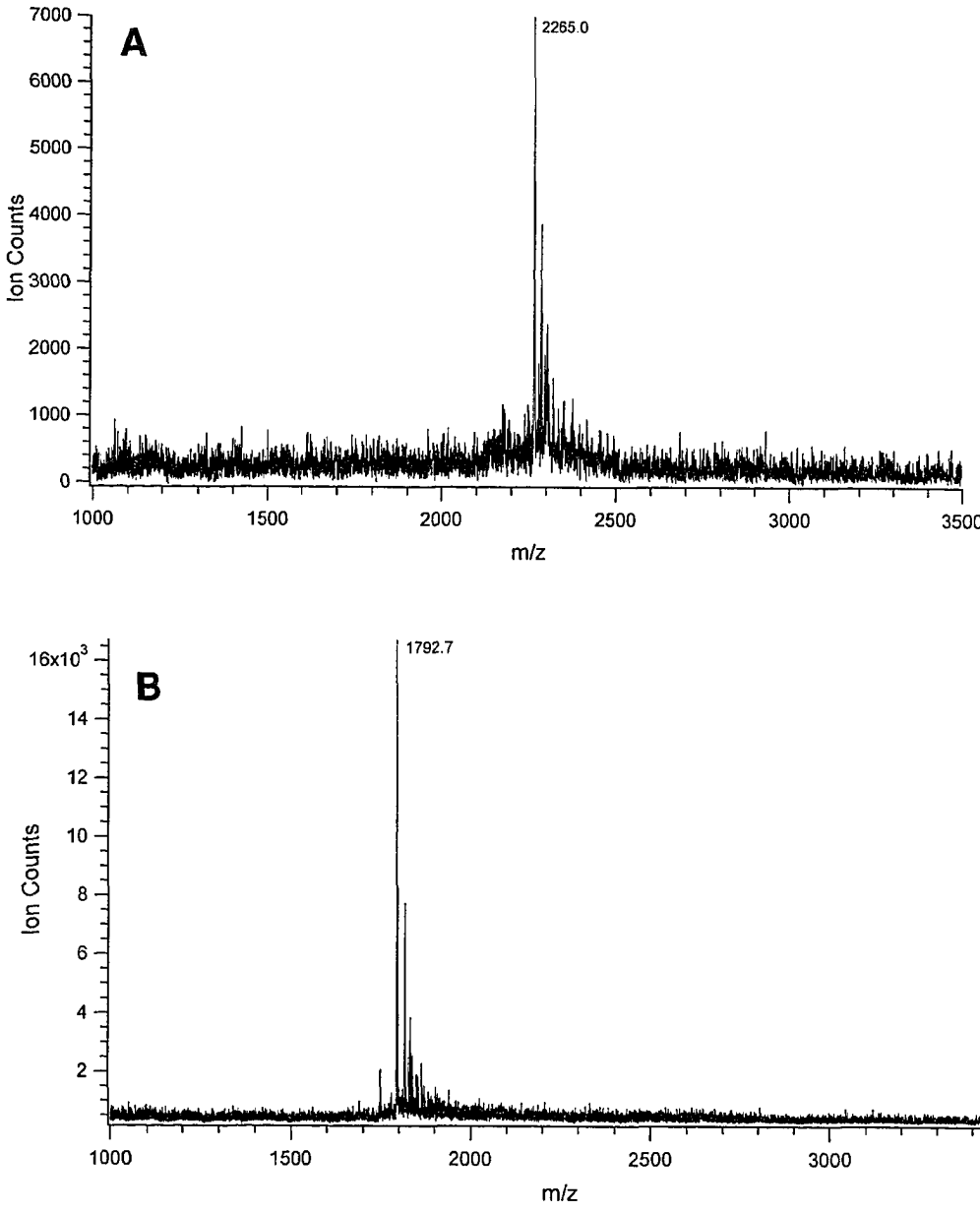
8.3 Sample inhibitory curve for dicarba antagonist 27, regression output and calculation of pA_2 value



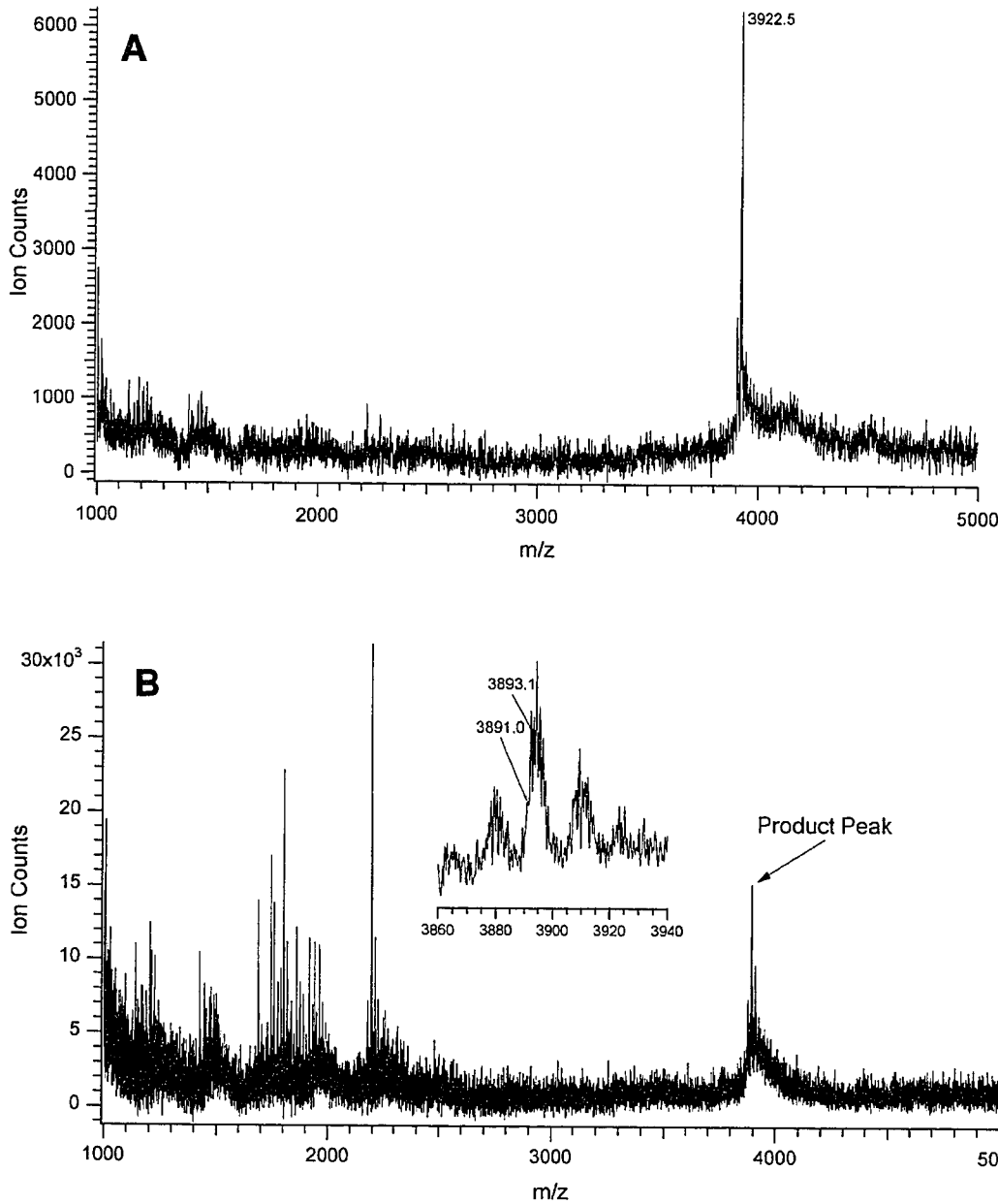


Y(%)	Ln(x)	x (ng/mL)	x (nM) ([pA_2])	pA_2
47.4	2.44	11.47	11.97	7.92

8.4 MALDI-TOF (MS) spectra of Leu A fragments 84 (A) and 85 (B)



8.5 MALDI-TOF (MS) spectra of ligated product 88 [(M+H); A] and crude dicarba Leu A 76 [(M+H); B]



8.6 MALDI-TOF (MS) spectrum of ABC carbotricycle 95 [(M+H); C]