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Synthesis of Oxytocin Analogues with Replacement of Sulfur by Carbon Gives Potent Antagonists with Increased Stability

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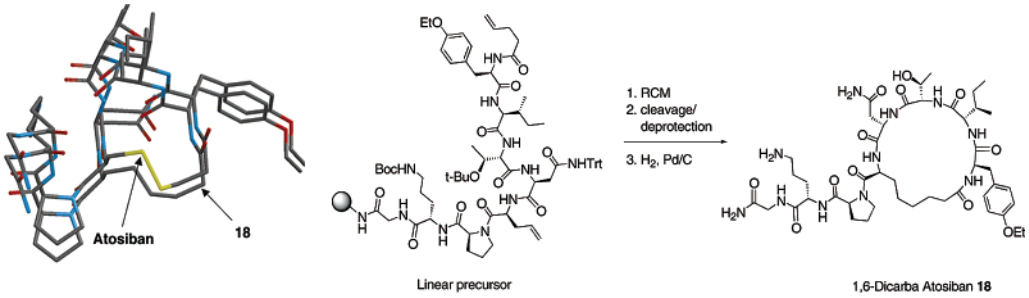

The neuropeptide oxytocin **1** controls mammary and uterine smooth muscle contraction. Atosiban **2**, an oxytocin antagonist, is used for prevention of preterm labor and premature birth. However, the metabolic lifetimes of such peptide drugs are short because of in vivo degradation. Facile production of oxytocin analogues with varying ring sizes wherein sulfur is replaced by carbon (methylene or methine) could be achieved by standard solid-phase peptide synthesis using olefin-bearing amino acids followed by on-resin ring-closing metathesis (RCM). These were tested for agonistic and antagonistic uteronic activity using myometrial strips taken from nonpregnant female rats. Peptide **8** showed agonistic activity in vitro ($EC_{50} = 1.4 \times 10^3 \pm 4.4 \times 10^2$ nM) as compared to **1** ($EC_{50} = 7.0 \pm 2.1$ nM). Atosiban analogues **17** ($pA_2 = 7.8 \pm 0.1$) and **18** ($pA_2 = 8.0 \pm 0.1$) showed substantial activity compared to the parent oxytocin antagonist **2** ($pA_2 = 9.9 \pm 0.3$). Carba analogue **35** ($pA_2 = 6.1 \pm 0.1$) had an agonistic activity over 2 orders of magnitude less than its parent **3** (8.8 ± 0.5). A comparison of biological stabilities of 1,6-carba analogues of both an agonist **8** and antagonist **18** versus parent peptides **1** and **2** was conducted. The half-lives of peptides **8** and **18** in rat placental tissue were shown (Table 2) to be greatly improved versus their parents oxytocin **1** and atosiban **2**, respectively. These results suggest that peptides **8** and **18** and analogues thereof may be important leads into the development of a long-lasting, commercially available therapeutic for initiation of parturition and treatment of preterm labor.

Introduction

Oxytocin **1** (Figure 1, Table 1) is a mammalian nonapeptide hormone synthesized by the magnocellular neurons of the hypothalamus.[1] It has many physiological roles, including mammary and uterine smooth muscle contraction,[1] neurotransmission in the central nervous system, and autocrine and/or paracrine functions in the ovaries and testes.[2,3] Oxytocin **1** is clinically used to

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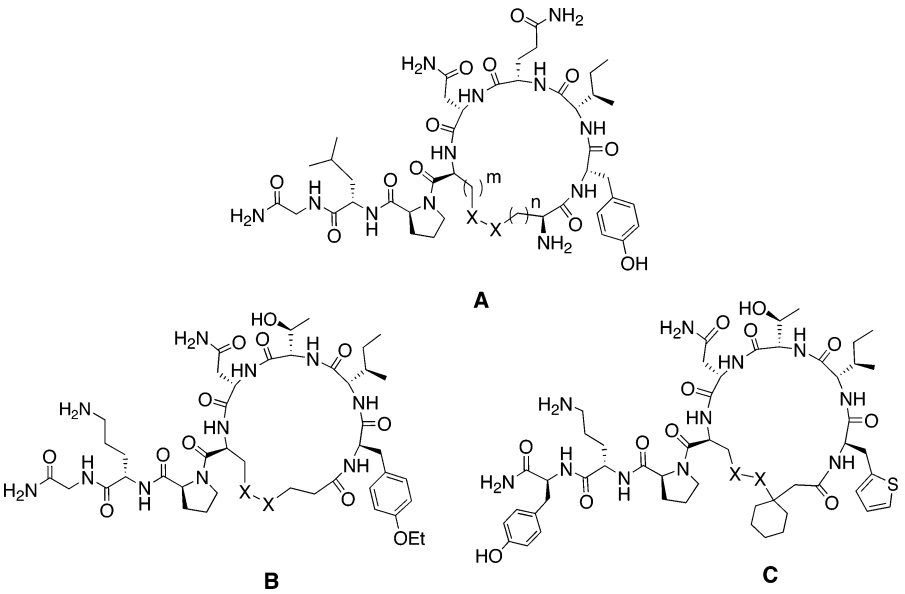

FIGURE 1. General structures of oxytocin (A), atosiban (B), and antagonist **3** (C). See Table 1 below.

TABLE 1. Structures of Carbocyclic Peptides 1−12, 15−18 and 35

structure	compd	*m*	*n*	X−X	isomer	ring size
A	**1**	1	1	S−S		20
	4	1	1	CH=CH	*cis*	20
	5	1	2	$S-SCH_2$		21
	6	2	1	CH=CH	*cis*	21
	7	2	1	CH=CH	*trans*	21
	8	1	2	CH=CH	2:1 *cis/trans*	21
	9	2	2	CH=CH	*cis*	22
	10	2	2	CH=CH	*trans*	22
	11	2	1	$CH_2=CHCH_2O$	*cis*	22
	12	2	1	$CH_2=CHCH_2O$	*trans*	22
	15	2	1	CH_2-CH_2		21
	16	2	2	CH_2-CH_2		22
B	**2**	-	-	S−S		20
	17	-	-	CH=CH	1:4 *cis/trans*	20
	18	-	-	CH_2-CH_2		20
C	**3**	-	-	S−S		20
	20	-	-	CH=CH	*cis/trans*	20
	35	-	-	CH_2-CH_2		20

induce labor and control postpartum hemorrhage,[1,4] but it has a short half-life (2−5 min) in vivo and is administered to patients intravenously.[5,6] Antagonists of oxytocin are of interest as tocolytic agents that inhibit preterm labor and delay premature birth. There are an estimated 13 million premature births worldwide per annum[7] that account for 66% of all neonatal mortality and contribute to serious complications and infant morbidity.[8,9] 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.[10] Atosiban (Tractocile) (Figure 1, Table 1) **2**[11−13] is a competitive antagonist of oxytocin receptors (OTR) that has been approved in Europe for the short-term treatment of preterm labor.[14−18]

However, **2** has a short metabolic half-life (ca. 16−18 min), is usually adminstered intravenously, and can have unwanted side effects due to lack of selectivity for the oxytocin receptor over the vasopressin receptors (VPR).[13,19] Arginine[8]vasopressin (AVP), a hormone synthesized in the posterior pituitary that plays a key role in osmotic regulation and vasoconstriction, is structurally very similar to atosiban **2** and oxytocin **1**.[19] Considerable effort over the past decade has been devoted to the synthesis of nonpeptidic OTR antagonists in an effort to increase oral availability and longevity. However, drug approval and liability issues[20] as well as side effects, lack of potency, poor bioavailability, or insufficient OTR specificity have kept such compounds[21−27] from clinical use.[9,28] To address the selectivity problem with peptide analogues, Manning and co-workers synthesized a series of compounds including **3** (Figure 1, Table 1), which differs

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from atosiban **2** by the presence of a bulky cyclohexyl moiety at position 1 and introduction of a tyrosine residue at position 9 as well as a D-thienylalanine at position 2.[19] Nonapeptide **3** has comparable potency to **2**, but the selectivity for the OTR over the VP receptor set is greatly enhanced,[19] probably due primarily to the increased conformational rigidity as a result of the cyclohexyl group.

The discovery of antagonistic analogues of **1** that have a longer duration of in vivo activity and greater bioavailability than **2** is highly desirable for development of tocolytic drugs. As the essential disulfide moiety that restricts the conformational mobility of oxytocin and its analogues is inherently sensitive to biochemical reduction, its substitution by a more stable linkage could generate more metabolically robust derivatives. Replacement of disulfide functionality in a variety of biologically active peptides with methylene (CH_2) or amide linkages has afforded analogues that in certain cases retain activity at reduced levels or are antagonists of the natural compound.[29−32] It has been demonstrated that substitution of the disulfide in oxytocin with methylene,[33] methine,[33d] or amide functionalities[33a] yields analogues with potent bioactivity and in some cases increased metabolic stability.[33c] We recently reported that replacement of the disulfide in **1** with a *cis*-alkene to give **4** (Figure 1, Table 1) generates a potent agonist (EC_{50} = 38 nM) that is only 10-fold less active than oxytocin, whereas the corresponding *trans*-alkene and hydrogenated (methylene) analogues are 100-fold less active.[33d] Overlaying energy-minimized structures of **1** and **4** shows that the two peptides are very similar in structure except for a small kink imposed by the preference of the sulfurs for a close to 90° dihedral angle (Figure 2).

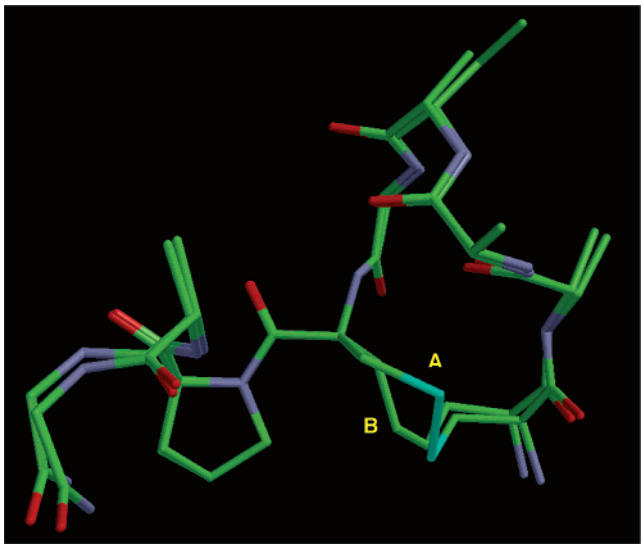

FIGURE 2. Overlaid energy minimized structures of oxytocin **1** (A) and analogue **4** (B). Side chains on Y, I, Q, N, L are omitted for clarity.

In 1976, Smith and Ferger reported that increasing the ring size of **1** by one methylene unit (substitution of a homocysteine for cysteine residue) resulted in an analogue **5** (Figure 1, Table 1) with antagonistic activity ($pA_2 = 6.0$) and greatly reduced agonistic activity.[34] This was attributed to an increase in steric bulk within the cyclic portion of the peptide.

Realizing that a variety of carbocyclic rings are potentially available by ring-closing metathesis (RCM) reactions of resin-bound linear peptides having two alkenyl side chains of varying length,[33d,35−44] we investigated the synthesis, activity, and stability of a series of analogues of oxytocin **1**, atosiban **2**, and the OTR selective antagonist **3**. In each case, the disulfide bridge was replaced with methylene or methine (olefinic) units in an effort to enhance the biological half-life of the analogues without compromising inherent activity.

Results and Discussion

Synthesis of 1,6-Carba Analogues of Oxytocin Antagonists. Ring-closing olefin metathesis (RCM) reactions have been previously done on peptide substrates linked to solid support.[33d,37,42,43] However, the tendency of the organometallic catalysts to bind to resin-held

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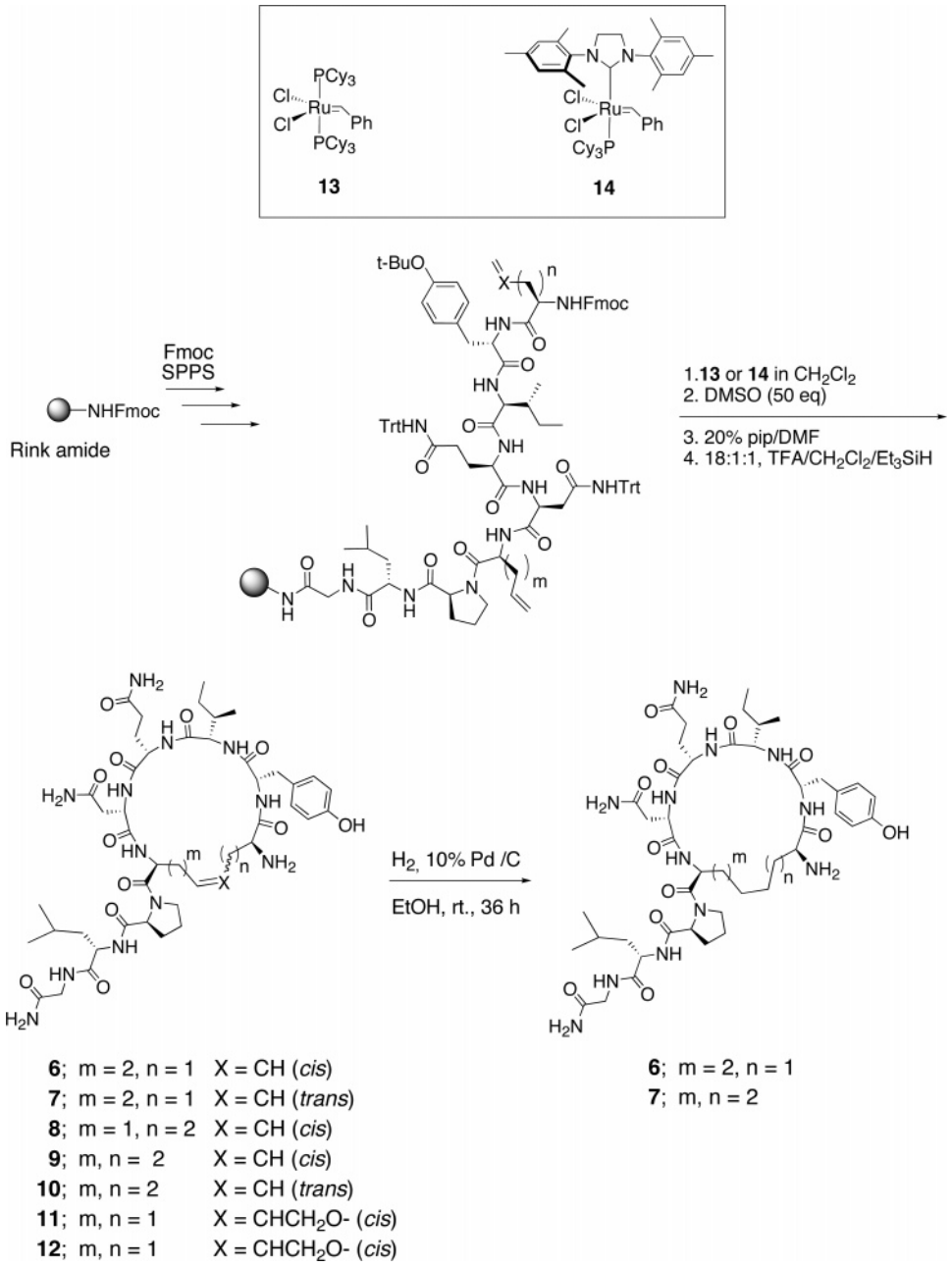
SCHEME 1. Structures of 13 and 14 and General Synthesis of 1,6-Dicarba Analogues Using RCM Reaction



products complicates purification and drastically lowers or abolishes yields. Fortunately, we recently found that treatment of the resin after RCM reaction with dimethyl sulfoxide (DMSO) removes organometallic byproducts and allows facile recovery of the desired cyclized compounds.[33d] It has also been uncertain whether a variety of larger rings would be available via the RCM process because conformational preferences of the precursors could hinder the cyclization. However, the syntheses of analogues **6**−**12** (Figure 1, Table 1), containing rings larger than oxytocin **1** (i.e., >20 members) proceed readily as outlined in Scheme 1. For 21-membered rings **6**−**8**, the syntheses employed SPPS of linear precursors using Fmoc methodology with incorporation of L-homoallylglycine[48,49] at either positions 1 or 6, with L-allylglycine added to the opposing position. Use of L-homoallylglycine at both residues 1 and 6 generates the linear precursor to analogues **9** and **10** having 22-membered rings. To examine the influence of a ring oxygen on biological activity, L-*O*-allylserine[50] was placed at position 1 with L-allylglycine at position 6 (compounds **11** and **12**). RCM reactions on suspensions of resin-bound peptides using 10 mol % solutions of either Grubbs first-generation

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catalyst **13**[51,52] (Scheme 1) or second-generation catalyst **14**[53] (Scheme 1) in dry degassed CH_2Cl_2 required reflux at 40 °C for 18 h. Treatment with DMSO (50 equiv relative to catalyst) for another 12 h at 20 °C assists removal of byproducts and facilitates purification.[33d] Following removal of the N-terminal Fmoc with piperidine, acidic cleavage from the resin with concomitant removal of acid-labile side-chain protecting groups gives cyclic peptides in overall yields of 8−28% (Scheme 1) after purification by RP-HPLC. The lower yields can be attributed to cyclizations with the less active Grubbs first-generation catalyst **13**. The *cis*/*trans* isomers of all but one set of olefin-containing peptides, compound **8**, are readily separated by HPLC. In each case, the cyclic olefinic peptides elute prior to their linear precursors during RP-HPLC analysis.

The assignment of *cis* and *trans* configuration could be accomplished by a novel simultaneous ^{1}H NMR double-decoupling experiment at 500 MHz. In these experiments, two sets of allylic protons at different chemical shifts are simultaneously irradiated, thereby allowing for the observation of the decoupled AB quartet due to the adjacent olefinic protons. The coupling constants of the two olefinic protons are then readily measurable, and are 8.5−10.7 Hz for the *cis* isomers and 14.9−15.9 Hz for the *trans* isomers. Saturated derivatives of both 21- and 22-membered ring analogues are available by quantitative reduction of olefinic peptides **8** and **9** using standard hydrogenation to yield peptides **15** and **16** (Figure 1, Table 1), respectively. The peptides were then examined for both agonistic and antagonistic activity in comparison to peptides **1** and **2** (see below).

Synthesis of 1,6-dicarba analogues of atosiban **2** relies on analogous methodologies. The salient features are that position 1 of **2** contains 3-mercaptopropionic acid, which was replaced with commercially available 4-pentenoic acid. L-Allylglycine was placed at position 6 for the purposes of RCM reaction. Fmoc SPPS yields a linear resin-bound precursor that could be cyclized and subsequently cleaved from the resin to give analogue **17** (Figure 1, Table 1) as a 1:4 mixture of *cis*/*trans* isomers in 21% overall yield. Various attempts to separate these isomers using RP-HPLC failed, and the two component mixture **17** was tested directly. Reduction of **17** quantitatively gives a fully saturated pure dicarba analogue **18** (Figure 1, Table 1), which was also tested for antagonistic activity and compared to atosiban **2**.

Based on the successful syntheses of these carbocyclic peptide analogues by RCM reactions, it appeared that a similar approach should provide access to an analogue of **3**, which has superior selectivity for OT vs VP receptors, with methine or methylene groups in place of sulfur. Synthesis of a linear precursor with 1-(vinylcyclohexyl)-1-acetic acid at position 1 and L-allylglycine at postion 6 using standard SPPS on Rink amide resin (0.6 mmol/g) proceeds readily (Scheme 2). However, RCM reaction of this resin-bound precursor **19** under a variety of conditions generated no cyclized derivative **20** (Figure 1, Table 1), but instead gave a dimerized product. It appears that this dimerization occurs beween allylglycine residues between neighboring peptides due to the steric bulk of the 1-(vinylcyclohexyl)-1-acetic acid residue. To counteract this, a linear precursor **21** having L-crotylglycine at position 6 was made so as to provide additional steric bulk to the olefin terminus and thereby prevent dimerization. However, attempted RCM on **21** yielded no metathesis products. Linear precursor **19** was also made on Rink amide resin with a much lower loading (0.12 mmol/g) to achieve greater dilution and thereby retard dimerization. Unfortunately, this also yielded no metathesis products under RCM conditions. Finally, linear precursor was made on Sieber amide resin which permitted cleavage of the peptide from the resin under mild conditions (1% TFA) without side-chain deprotection to give **22**. Numerous RCM reaction attempts on dilute solutions of this free peptide did not generate any monomeric cyclized product. Clearly, the steric crowding caused by the cyclohexyl group completely inhibits intramolecular cyclization by the bulky organometallic reagent.

To overcome the difficulty of making a carbocyclic analogue of **3** via RCM reaction, the strategy was changed to a more classical approach. This involved incorporation an orthogonally protected cyclohexyl derivative of α-aminosuberic acid **23** with intramolecular amide bond formation as the ring-closing step.[54] Compound **23** could be readily made as shown in Scheme 3. The key step, photolytic decomposition of a diacyl peroxide at low temperature in the absence of solvent, is based on a novel methodology recently reported by us that allows facile synthesis of amino acid derivatives from two carboxylic acids without crossover products.[55] The known[56] anhydride **24** is readily opened to acid **25** using sodium methoxide in refluxing methanol. Acid **25** reacts with 50% hydrogen peroxide in the presence of sulfuric acid to give the peracid **26** in 78% yield. Coupling of peracid **26** with *N*-carboxybenzylglutamic acid 1-benzyl ester (*N*-Cbz-Glu-OBn) **27** using dicyclohexyldicarboximide (DCC) produces the corresponding diacyl peroxide **28** in 80% yield. Photolysis of neat **28** at −78 °C for 48 h affords **29** in 34% yield with recovery of 36% of **28**, which could be recycled. Quantitative deprotection of **29** by hydrogenolysis gives amine **30**, and subsequent Fmoc protection generates **23**.

Unfortunately, the yield of the last step is low, possibly because of solubility problems, but recovered starting material could be reacted again to afford additional product. This could then be incorporated in the synthesis of the linear precursor **31** on Sieber amide resin using standard SPPS (Scheme 4). Peptide **31** is cleaved from the resin using 1% TFA to yield the fully protected peptide methyl ester **32**, which could be hydrolyzed using 2 N lithium hydroxide to yield peptide acid **33**. This reaction proceeds to only 59% completion after 3 days at room temperature, but attempts to increase the yield by heating at 50 °C for 3 h lead to decomposition of the

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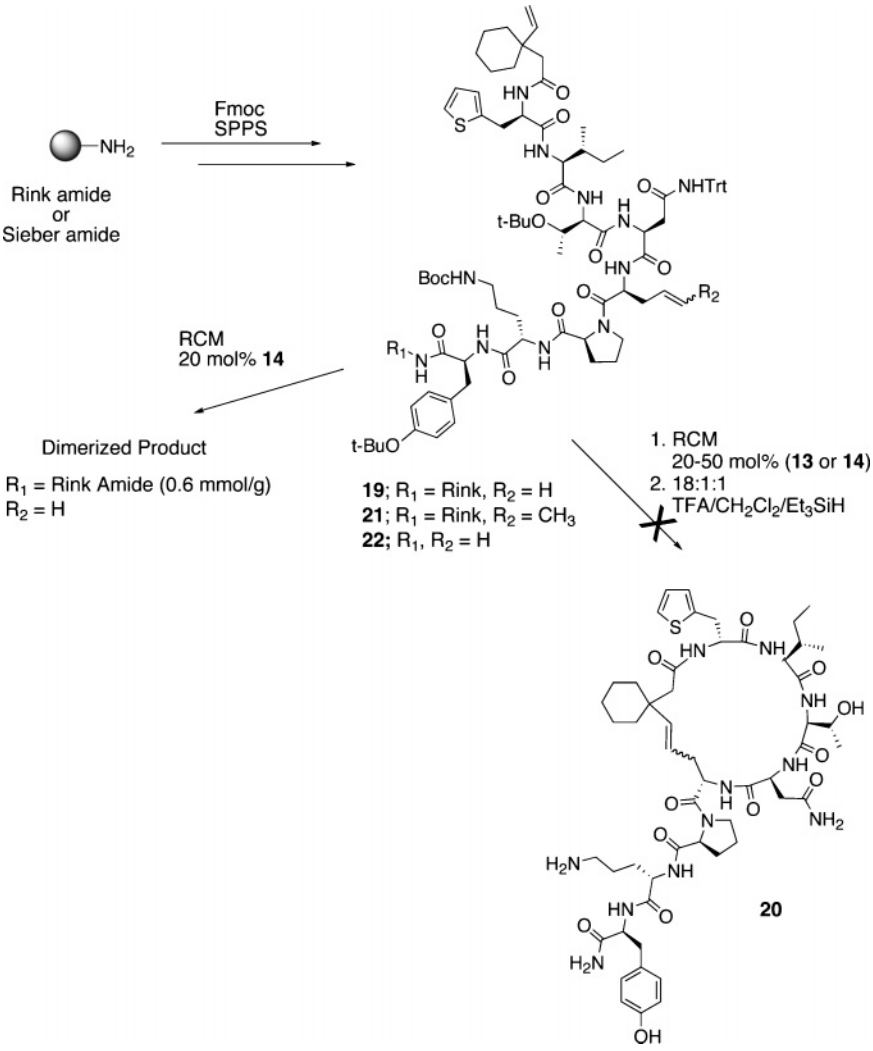
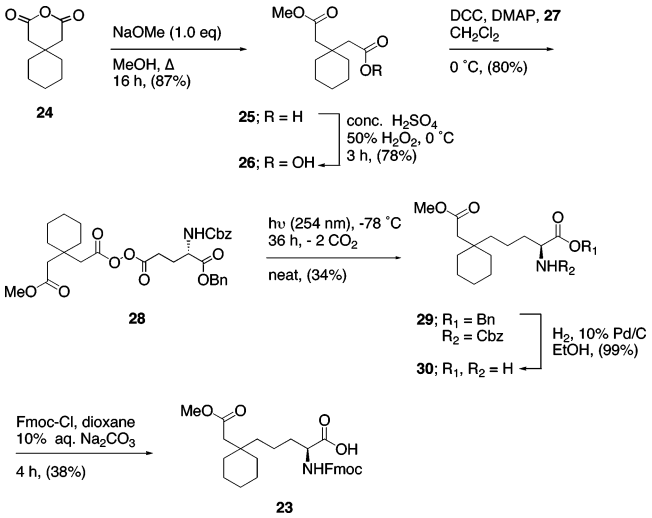
SCHEME 2. Attempted Syntheses of 1,6-Dicarba Analogue 20 via RCM



SCHEME 3. Synthesis of α-Aminosuberic Acid Derivative 23



peptide. The acid **33** undergoes intramolecular cyclization upon reaction with 1:1 PyBOP/HOBt in DMF at room temperature for 2.5 days.[54] Treatment of the resulting cyclic analogue **34** (29% yield) with 95% TFA removes all side chain protecting groups. The dicarba analogue **35** (Figure 1, Table 1) could be isolated in 76% yield after HPLC purification and was tested for biological activity and stability.

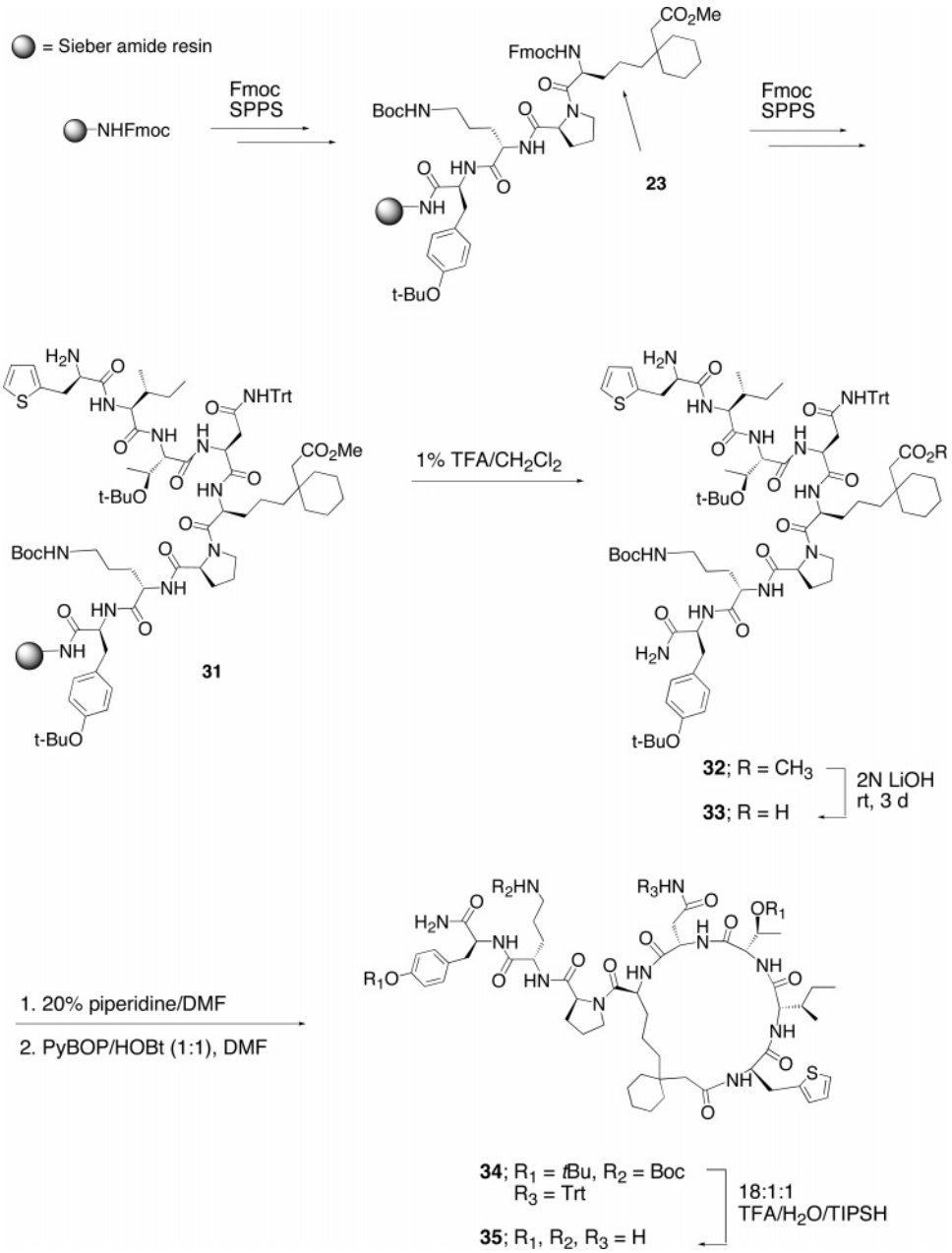
TABLE 2. Oxytocin Agonism and Antagonism[a] of Compounds 1, 2, 3, 8, 17, 18, and 35 and Placental Tissue Half-Lives[b] for 1, 2, 8, and 18

compd	EC_{50} (nM)	[pA_2] (nM)	pA_2	half-life (min)
1	7 ± 2			4,[a] 16[b]
8	$(1.4 \pm 0.4) \times 10^3$			24,[a] 15[b]
2		0.2 ± 0.1	9.9 ± 0.3	156,[c] 108[d]
3		3 ± 2	8.8 ± 0.5	
17		17 ± 6	7.8 ± 0.1	
18		10 ± 1	8.0 ± 0.1	318,[c] 306[d]
35		750 ± 20	6.1 ± 0.1	

[a] Experiments done in triplicate. [b] Four different animals used, two each for agonists **1** and **8** (a and b) and atagonists **2** and **18** (c and d).

Testing of Analogues as Agonists and Antagonists of Oxytocin.[33d,46] Both atosiban **2** and the selective antagonist **3** were tested to compare their activities to the 1,6-carba analogues of oxytocin (Table 2). As expected, none of the carba analogues of **2** and **3** show any significant agonistic activity in vitro.[12,19] During biological testing, with the exception of **8**, all of the larger ring carbocyclic compounds also display no agonistic behavior.

SCHEME 4. Incorporation of 23 into Peptide Chain via SPPS and Cyclization to 35



Surprisingly, **8** exhibits agonistic activity at higher doses ($EC_{50} = (1.4 \pm 0.4) \times 10^3$ nM) and shows no antagonistic behavior when tested. This contrasts with the literature report[34] on the corresponding disulfide analogue **5**, which indicates that it has no oxytocin agonist properties but is a potent antagonist ($pA_2 = 6.0$). The pA_2 values are defined as the negative logarithm of the concentration of antagonist that diminishes the OT activity of a double dose to that of a single dose.[12,47] The exact interpretation of this result is uncertain as the 2:1 mixture of *cis/trans* isomers of **8** could not be easily separated, and it is unclear which isomer is exhibiting the agonistic activity.

All carbocyclic analogues were screened for antagonistic activity. Only the 1,6-dicarba analogues **17**, **18**, and **35** possess significant antagonistic activity, and these values were compared to atosiban **2** and the selective antagonist **3**, respectively (Table 2). The pA_2 values were calculated for active analogues **17**, **18**, and **35** using the methodology developed by Schild[47] (see the Supporting Information). Peptides **17** and **18** have potent OT antagonistic activity ($pA_2 = 7.8 \pm 0.1$ and 8.0 ± 0.1, respectively) approaching that of **2** ($pA_2 = 9.9 \pm 0.3$) (Table 2). Interestingly, the presence of the olefin functionality in **17**, which imposes considerable conformational restraint on the ring system, appears to have only a slight influence on the activity as the more flexible saturated bis-methylene derivative **18** has nearly the same antagonistic effect on the OT receptors. This contrasts the agonistic effect of oxytocin analogue **4** and its congeners, where activity is reduced by an order of magnitude for the trans isomer and the saturated counterpart. The dicarba analogue **35** ($pA_2 = 6.1 \pm 0.1$) is considerably less active than its disulfide parent **3** ($pA_2 = 8.8 \pm 0.5$). A possible explanation for the nearly 3 orders of magnitude decrease in activity of **35** may be deviation in structure from the preferred 90° dihedral

angle between the sulfurs of the disulfide bridge in **3**. 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 nonbonded interactions may alter the rest of the peptide geometry and thereby hinder the ability of **35** to readily dock with the OTR. Potentially the optimal 90° dihedral angle of the disulfide in **3** could be restored through incorporation of a conformational lock, for example, an additional ring structure on the carbon bridge.

Stability of Analogues in Rat Placental Tissue. A new methodology was developed for testing the stability of 1,6-dicarba analogues of oxytocin using fresh rat placental tissue. To determine if this methodology is useful as a general method to examine stablility, we tested both an agonist **8** and antagonist **18**. Four separate animals were used for the testing, two each for the agonist and antagonist. The results for analogues **8** and **18** were then compared to their parent disulfide containing peptides, oxytocin **1** and atosiban **2**, respectively. Half-lives were calculated for both the agonists **1** and **8** and the antagonists **2** and **18** (Table 2). Values are reported for each experiment as there is variation in the levels of enzyme(s) responsible for breaking down oxytocin in placental tissue in different animals.[46] The results show that the 1,6-carba analogue **8** has a considerably greater half-life (8−11 min longer) than **1** when incubated in placental tissue from two different rats. Similar results are observed with the 1,6-dicarba analogue **18** of atosiban. Analogue **18** has a half-life in placental tissue more than twice that of atosiban **2**. 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.

Conclusion

The ring-closing metathesis (RCM) reaction is currently one of the most popular methods for carbon ring formation. Its utility for cyclization of protected peptides bound to solid phase to produce large (20−22 members) carbocyclic olefins is demonstrated by synthesis of a series of oxytocin analogues. The resulting compounds have an olefin, or after reduction, methylene groups, in place of the essential disulfide moiety. A key feature is the use of DMSO after RCM cyclization to remove organometallic impurities that otherwise lead to product degradation during cleavage from the resin. A limitation of the RCM reaction is that the steric bulk of the catalysts can prevent cyclization in cases where the substrate is already sterically hindered (e.g., **22** → **20**). Fortunately, the use of solvent-free photolysis of diacyl peroxides permits linking of sterically crowded moieties and affords rapid access to functionalized and selectively protected amino acids (e.g., **23**). These can then be incorporated via linear solid-phase synthesis to make carbocyclic peptides (e.g., **35**) via standard amide bond formation. Analogues of atosiban **2** and antagonist **3**, wherein sulfur is replaced by carbon (e.g., **17**, **18**, and **35**) display reduced, but still very high, levels of activity as antagonists of the oxytocin receptor (OTR). Interestingly, such replacement of sulfur by carbon markedly increases the half-life of the peptide analogues in placental tissue. This observation provides a basis for design of new oxytocin 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. It will also be interesting to determine whether similar carbon for sulfur substitution in other peptides will give analogues with improved properties. Finally, the methodology would also be applicable for generation of derivatives that are functionalized on the bridge carbons (e.g., via bis-hydroxylation of the olefin), thereby offering additonal avenues for improvement of drug properties by medicinal chemistry.

Experimental Section

Simultaneous Double-Decoupling for NMR Assignment of *Cis/Trans* Stereochemistry. 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 waveform 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 48 000 points. Coupling constants between the adjacent olefin protons were measured directly and accurately from the aquired spectrum and used to assign *cis/trans* geometry about the double bond.

Assays for OTR Agonistic Activity. Testing of peptides employed freshly excised uteri from mature nonpregnant, female Sprague−Dawley rats (~250 g), and the experiments were done in triplicate using tissue samples from three separate animals. Muscle bath preparations were done on the basis of published methodology.[33d,46] Rat uteri were cut into strips (1 cm × 0.3 cm) and mounted vertically on a Biopac Systems, Inc., myobath apparatus, using wire hooks in separately jacketed organ baths at 30 °C containing 10 mL of Krebs buffer with the following composition: 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 aeration. 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., detection system. Resting tension was set to 1 g to provide maximum active tension. Oxytocin **1** and analogue injections were made directly into the baths. Measurements were recorded in 5 min blocks with injections of increasing concentration being added at the end of each 5 min interval. Dose−response curves for **1** and analogues were constructed at the conclusion of each 60 min experiment. Microsoft Excel was used to calculate regression outputs for these curves in order to determine analogue EC_{50} values.

Assays for OTR Antagonistic Activity. The same basic procedure as for agonist tests was used with the following modifications. Muscle strips were incubated with antagonists

at varying concentrations for 4 min. Oxytocin **1** was then added directly into the baths containing antagonists every 3 min with increasing concentration (0.78, 3.12, 12.50, 50.00, and 200 nM, respectively). All antagonist results were compared to either **2** or **3**, and oxytocin-inhibitory curves were constructed from the data 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 analogue were calculated using the Schild plot analysis method.[47]

Biological Stability. A new method was developed to test the duration of activity of oxytocin analogues in placental tissue. Stability tests were conducted on compounds **1**, **2**, **8**, and **18**. Homogenate was made from placental tissue taken from pregnant female Sprague−Dawley rats at day 19 of gestation to provide for maximum oxytocinase activity. Solutions containing 1 μM of agonists **1** and **8** were incubated with the homogenate for 0, 5, 10, 15, 20, 25, and 30 min at 37 °C. Freshly excised uteri from mature, nonpregnant, female Sprague−Dawley rats (∼250 g) were cut into strips as in the agonistic assays. Muscle bath preparations and the general procedure were as described for agonistic and antagonistic assays. Samples of **1** (10 nM) and **8** (1 μM) were then taken from placental homogenate solutions and added directly into the muscle baths. Maximum activity was measured at time 0 min for (each compound), and the decrease in percent activity of the analogues was measured at each 5 min incubation interval. Percent activity versus incubation time curves were constructed for compounds **1** and **8**, and half-lives (in minutes) were calculated for each trial and averaged. Antagonists **2** and **18** were tested in a manner similar to that of the above agonists with both being 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 each antagonist−homogenate solution (**2**; 10 nM, **18**; 100 nM) followed by addition of **1** every 3 min at increasing concentrations of 0.98, 3.9, 15.6, 62.5, and 250 nM, respectively. Percent activity of oxytocin response was measured at 4 nM of **1**, and these trials were conducted on four separate animals, two of each for the agonist and antagonist. The maximum inhibitions for compounds **2** and **18** were measured at time 0 h, and any decrease in percent inhibition was measured at each of the incubation intervals indicated above.

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) (2). This is the general procedure used for the synthesis of disulfide peptides **2** and **3**. The linear precursor to peptide **2** was synthesized in a manner analogous to that of the other OT analogues using standard Fmoc SPPS. (*S*-Trt)-3-mercaptopropionic acid was prepared as according to literature precident.[57] Disulfide formation of the linear precursor to **2** was done in 0.1 mM NH_4HCO_3 buffer at pH 8.0 with constant O_2 aeration and vigorous stirring for a period of 18 h. This mixture was then concentrated to one-third of the original volume, and the remainder of the buffer was removed by lyopholization. Peptide **2** was isolated as a single peak using C_{18}, RP-HPLC, 10% MeCN, 90% H_2O (0.1% TFA), 5 min, 10−90% MeCN over 20 min, t_R = 14.94 min (11 mg, 7.3% overall based on 0.10 mmol of resin cleaved): ^{1}H NMR (D_2O, 600 MHz) δ 7.22 (apparent doublet, 2H, J = 8.6 Hz), 6.96 (apparent doublet, 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).

(2*S*)-*N*-(9*H*-Fluorenylmethoxycarbonyl)-2-amino-5-hexenoic Acid (Fmoc-L-homoallylglycine). (2*S*)-2-Amino-5-hexenoic acid (L-homoallylglycine) was prepared according to literature precident.[48] To a solution of L-homoallylglycine (0.62 g, 4.8 mmol) in H_2O at 0 °C was added $NaHCO_3$ (1.61 g, 19.1 mmol). This reaction mixture was allowed to stir at 0 °C for 10 min. A solution of 9*H*-fluorenylmethyl chloroformate (Fmoc-Cl) (1.41 g, 5.3 mmol) in acetone was then added dropwise over 15 min. The reaction mixture was stirred at 0 °C for 1 h and then allowed to warm to room temperature where stirring continued for another 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 × 75 mL) and acidified to pH 2 with 1 M HCl and extracted with EtOAc (4 × 75 mL). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo to yield a white solid, which was recrystallized from CH_2Cl_2 and hexanes (1.21 g, 72%): IR ($CHCl_3$, cast) 3400−2400, 1717, 1521, 1417 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.63 (br. s, 1H), 7.78 (apparent doublet, 2H, J = 7.5 Hz), 7.58 (m, 2H), 7.38 (apparent triplet, 2H, J = 7.4 Hz), 7.28 (apparent triplet, 2H, J = 7.4 Hz), 5.75 (m, 1H), 5.28 (d, 1H, J = 8.3 Hz), 5.04 (m, 2H), 4.32 (m, 3H), 4.22 (t, 1H, J = 6.9 Hz), 2.18 (m, 2H), 2.02 (m, 1H), 1.8 (m, 1H); ^{13}C ($CDCl_3$, 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 $C_{21}H_{21}NO_4$ 351.3957, found (M^+); $[\alpha]_D$ (*c* 1.0, $CHCl_3$) = +13.32.

(1:4, *cis/trans*)-(2*S*)-*N*-(9*H*-Fluorenylmethoxycarbonyl)-2-amino-4-hexenoic (Fmoc-L-crotylglycine). (2*S*)-2-Amino-4-hexenoic acid (L-crotylglycine) was prepared according to literature precident.[58] A solution of L-crotylglycine (2.70 g, 20.9 mmol) in 10% aqueous $NaHCO_3$ (60 mL) and dioxane (20 mL) was cooled to 0 °C and stirred for 15 min. Fmoc-Cl (6.12 g, 23.0 mmol) in dioxane (30 mL) was then added dropwise over 15 min, and the mixture was stirred at 0 °C for 2.5 h. The reaction was warmed to rt and stirred for a further 8 h. H_2O (600 mL) was added, and the aqueous layer was extracted with EtOAc (2 × 150 mL). The aqueous layer was acidified to pH 1 with concd HCl and extracted with EtOAc (3 × 250 mL). The combined organic layers were dried (Na_2SO_4) and concentrated yielding a colored residue which was recrystallized from CH_2Cl_2 and hexanes to give Fmoc-L-crotylglycine as a white solid in a 1:4 *cis/trans* mixture (3.00 g, 41%): IR ($CHCl_3$, cast) 3500−2650, 1719, 1518, 758 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) for major isomer only δ 10.82 (br s, 1H, CO_2H), 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_3CH=CH), 5.34 (m, 2H, CONHCH, CH_3CH=C*H*), 4.45 (m, 3H, NHC*H*CO_2H, Ar_2CHCH_2), 4.22 (t, 1H, J = 7.0 Hz, ArC*H*Ar), 2.78−2.32 (m, 2H, CH=CHCH_2), 1.66 (d, 3H, J = 6.1 Hz, CH_3CH=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).

Synthesis of Peptides 6 and 7. This is a general method used for cyclization of oxytocin analogues **6**−**12** and **17** using ring-closing metathesis.[33d] All cyclizations involving resin-bound oxytocin analogues were done on Rink amide NovaGel. To a suspended solution of resin-bound peptide precursor to **6** and **7** (645 mg, 0.25 mmol) in degassed CH_2Cl_2 (5 mL) was added 10 mol % of either bis(tricyclohexylphosphine)benzylideneruthenium(IV) dichloride (Grubbs first-generation

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catalyst) **13**[51,52] (21 mg, 0.03 mmol) or 1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(phenylmethylene)-(tricyclohexylphosphine)ruthenium (Grubbs second-generation catalyst) **14**[53] (21 mg, 0.03 mmol) dissolved in degassed CH_2Cl_2 (2 mL). This reaction mixture was then allowed to gently reflux for ~18 h. The mixture was then cooled to room temperature, DMSO (50 equiv relative to the catalyst) was then injected into the sample, and the mixture was allowed to stir at room temperature for an additional 12 h. The resin-bound peptide was then filtered through a sintered glass frit and washed successively with CH_2Cl_2 and MeOH. Fmoc removal was carried out while the peptide was on resin using 20% (v/v) piperidine/DMF followed by filtration and washing as mentioned above. Cleavage of the peptide from the resin was done using a cocktail of 18:1:1 TFA/CH_2Cl_2/Et_3SiH. Upon purification, the cyclized olefinic peptides **6** and **7** elute from the column just prior to their linear precursor. In all cases, except for **8** and **17**, the peptides were isolated as their *cis*/*trans* isomers. It is important to note that Fmoc deprotection prior to cleavage is essential not only for the separation of *cis*/*trans* isomers but also for reduction via hydrogenation.

***cis*-[1,6-α,α′-L,L-Diaminonon-γ-enedioic acid]oxytocin (6).** Peptide **6** was isolated as a single peak using C_{18}, RP-HPLC, 10% MeCN, 90% H_2O (0.1% TFA), 5 min, 10−55% MeCN over 20 min, t_R = 12.99 min (24 mg, 10% from 0.25 mmol of resin bound peptide cleaved): ^{1}H NMR (D_2O, 600 MHz) δ 7.19 (apparent doublet, 2H, J = 8.2 Hz), 6.87 (apparent doublet, 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), 3.89 (AB quartet, 2H, 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 (7).** Peptide **7** was isolated as a single peak using C_{18}, RP-HPLC, 10% MeCN, 90% H_2O (0.1% TFA), 5 min, 10−55% MeCN over 20 min, t_R = 13.32 min (16 mg, 6% from 0.25 mmol resin bound peptide cleaved): ^{1}H NMR (D_2O, 600 MHz) δ 7.19 (apparent doublet, 2H, J = 7.1 Hz), 6.87 (apparent doublet, 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), 3.90 (AB quartet, 2H, 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).

[1,6-α,α′-L,L-Diaminononanedioic acid]oxytocin (15). Hydrogenation of cyclic olefinic peptides **8**, **9,** and **17** was done using standard hydrogenation techniques. The general procedure for the reduction is as follows. The olefinic peptides were dissolved in anhydrous EtOH followed by the addition of 10% Pd/C. The reaction mixture was then stirred under a hydrogen atmosphere at atmospheric pressure for ~36 h. The reaction mixtures were then filtered through a pad of Celite and concentrated to give a white precipitate. The precipitate was dissolved in H_2O and subjected to the same RP-HPLC purification. The saturated products eluted from the column after their olefin precursors under the same conditions. In all cases, yields were excellent (approximately quantitative) according to HPLC and ^{1}H NMR analyses. A sample of mixture **8** (3 mg, 31 μmol) was reduced as mentioned above with 10% Pd/C (3 mg) to give peptide **15** (~3 mg, quant). Peptide **15** was isolated as a single peak using C_{18}, RP-HPLC, 10% MeCN, 90% H_2O (0.1% TFA), 5 min, 10−55% MeCN over 20 min, t_R = 13.25 min: ^{1}H NMR (D_2O, 600 MHz) δ 7.16 (apparent doublet, 2H, J = 8.4 Hz), 6.83 (apparent doublet, 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), 3.86 (AB quartet, 2H, 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).

(1-Methoxycarbonylmethylcyclohexyl)acetic Acid (25). To 1,1-cyclohexanediacetic acid (5.00 g, 25.0 mmol) was added acetyl chloride (7 mL, 98.0 mmol). This mixture was then heated at 60 °C for 3 h and was observed to go from a suspension to a homogeneous solution. The volatiles were then removed at 100 °C under reduced pressure leaving behind a clear liquid which solidified upon cooling to room temperature to give cyclohexylglutaric anhydride **24** as a semitransparent solid (4.42 g, 97%). This was used immediately in the next reaction without further purification. The anhydride **24** was dissolved in dry MeOH (100 mL), NaOMe (1.56 g, 25.0 mmol) was added, and the reaction mixture was stirred at reflux for 12 h. The mixture was then acidified to pH 4 with AcOH, and the methanol was removed under reduced pressure. The residue was dissolved in water (100 mL) and extracted with EtOAc (2 × 125 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated in vacuo to yield a colorless, viscous oil, **25** (6.27 g, quant): IR (cast, $CHCl_3$) 3450−2350, 1740, 1705, 1440, 1409, 1336, 1286, 1254, 1166 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.61 (br s, 1H), 3.60 (s, 3H), 2.52 (s, 2H), 2.49 (s, 2H), 1.20 (m, 10H); ^{13}C ($CDCl_3$, 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 (26). Compound **25** (5.04 g, 23.5 mmol) was dissolved in concd H_2SO_4 (3 mL) at 0 °C. Aqueous H_2O_2 (50%) was then slowly added, and the mixture was stirred at 0 °C for 2 h. The mixture was 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 **26** as a colorless, 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 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.12 (br s., 1H), 3.60 (s, 3H), 2.59 (s, 1H), 2.55 (d, 2H, 36.3 Hz), 2.48 (d, 2H, 17.61 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.4, 171.9, 51.3, 40.7, 37.4, 35.7, 35.4, 25.3, 21.2; 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-oxopentanoic Acid Benzyl Ester (28). To a solution of **26** (1.03 g, 4.51 mmol) in CH_2Cl_2 (25 mL) at 0 °C were added (*N*-Cbz-Glu-OBn)

27 (1.34 g, 3.61 mmol) and DCC (0.93 g, 4.51). The reaction mixture was stirred at 0 °C for 2 h, slowly warmed to rt, and filtered through Celite. The filtrate was concentrated in vacuo and the residue dissolved in EtOAc and cooled to −20 °C for several hours and filtered. The filtrate was concentrated in vacuo to yield a gum which was then subjected to column chromatography (SiO_2, 1:1 hexanes/EtOAc) to give **28** as a colorless gum (1.69 g, 80%): IR (cast, $CHCl_3$) 3346, 3033, 2931, 1806, 1776, 1731, 1524, 1454 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.31 (m, 10H), 5.49 (d, 1H, J = 7.6 Hz), 5.18 (s, 2H), 5.08 (s, 2H), 4.42 (m, 1H), 3.60 (s, 3H), 2.63 (m, 2H), 2.54 (s, 2H), 2.43 (m, 1H), 2.30 (m, 1H), 2.06 (m, 1H), 1.60−1.38 (m, 10H); ^{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-methoxycarbonylmethyl)cyclohexyl)pentanoic Acid Benzyl Ester (29). A solution of **28** (500 mg, 0.86 mmol) in CH_2Cl_2 (5 mL) was spread out over the bottom of a 500 mL Pyrex reaction vessel. The solvent was removed with a steady stream of argon to afford a thin layer of neat diacyl peroxide. 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 residue was extracted extensively with CH_2Cl_2. The solvent was removed in vacuo to yield a yellow oil which was subjected to column chromatography (SiO_2, 1:6 to 1:1 EtOAc/hexanes) to afford **29** as a colorless gum (146 mg, 34%) as well as some recovered starting material (~30%): IR (cast, $CHCl_3$) 3349, 3064, 3032, 2929, 2855, 1728, 1523, 1455 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.35 (m, 10H), 5.39 (d, 1H, J = 8.2 Hz), 5.24−5.08 (m, 4H), 4.42 (m, 1H), 3.60 (s, 3H), 2.21 (s, 2H), 1.86 (m, 1H), 1.68 (m, 1H), 1.45−1.10 (m, 14H); ^{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-((methoxycarbonylmethyl)cyclohexyl)-pentanoic Acid (30). Compound **29** (602 mg, 1.21 mmol) was dissolved in anhydrous EtOH (10 mL), and 10% Pd/C (58 mg) was added. The reaction mixture was then stirred under H_2 at 1 atm for 12 h. The mixture was then filtered through a pad of Celite, and the filtrate was concentrated in vacuo to give the amine **30** as a white solid (328 mg, quant), which was used in the next step without any further purification: IR (μscope) 3450−2450, 1733, 1586, 1455 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 3.62 (s, 3H), 3.56 (dd, 1H, J = Hz), 2.32 (s, 2H), 1.81 (m, 2H), 1.61−1.30 (m, 14H); ^{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.

(2*S*)-2-(9*H*-Fluorenylmethoxycarbonylamino)-5-(1-methoxycarbonylmethylcyclohexyl)pentanoic Acid (23). Compound **30** (328 mg, 1.21 mmol) was dissolved in 10% aqueous $NaCO_3$ (20 mL) and dioxane (10 mL) and cooled to 0 °C. A solution of 9*H*-fluorenylmethoxyl chloroformate (374 mg, 1.39 mmol) in dioxane (15 mL) was then added dropwise over 20 min, and the mixture was stirred at 0 °C for 2.5 h upon completion. The reaction mixture was then warmed to rt and stirred for 1.5 h. Concentration of the mixture in vacuo yielded a residue that was dissolved in water (50 mL) and extracted with Et_2O (2 × 50 mL). The aqueous layer was acidified to pH 3 with 1 M HCl and extracted with EtOAc (4 × 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give the product **23** as a white foam (224 mg, 38%): IR ($CHCl_3$ cast) 3325−2860, 1722, 1525, 1450 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.40 (br s, 1H), 7.75 (apparent doublet, 2H, J = Hz), 7.58 (m, 2H), 7.39 (apparent triplet, 2H, J = Hz), 7.23 (apparent triplet, 2H, J = Hz), 5.43 (d, 1H, J = Hz), 4.40 (m, 3H), 4.21 (dd, 1H, J = Hz), 3.59 (s, 3H), 2.25 (s, 2H), 1.88 (m, 1H), 1.69 (m, 1H), 1.54−1.21 (m, 14H); ^{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.

[(1,6-α-L-Amino-α′-deamino-β′,β′-cyclohexylsuberic acid)-2-D-thienyl-9-L-tyrosyl]atosiban (35). Peptide sythesis was done on a 0.15 mmol scale using Sieber amide resin (0.62 mmol/g) as solid support. The general method for Fmoc SPPS was similar to that for the other peptides. The fully protected linear precursor **31** was cleaved from the resin using 1% TFA in CH_2Cl_2 Purification by C_{18}, RP-HPLC, 10% MeCN, 90% H_2O (0.1% TFA), 5 min, 10−90% MeCN over 20 min (t_R = 24.52 min) gave pure peptide methyl ester **32** 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 **32** (42 mg, 0.03 mmol) was treated with 2 M 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 **33** (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 **33** (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 RP-HPLC (t_R = 26.92 min) as above to give the protected cyclized product **34** (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 **34** (8 mg, 50 μmol) was treated with 18:1:1, TFA/CH_2Cl_2/Et_3SiH for 4 h at rt. Purification with RP-HPLC as above (t_R =16.39 min) yielded the fully deprotected peptide **35** (4 mg, 76%) as a white solid: ^1H NMR (D_2O, 600 MHz) δ 7.31 (d, 1H, J = 4.4 Hz), 6.98 (m, 4H), 6.55 (apparent doublet, 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).

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Supporting Information Available: All general procedures, full experimental details (peptides **3**, **8**−**12**, and **16**−**18**), ^1H NMR assignments for peptides **2**, **3**, **6**−**12**, **16**−**18**, and **35**, Schild plots for **2**, **3**, **17**, **18**, and **35**, and a sample double decoupled spectrum of peptide **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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