Synthesis and Pharmacological Evaluation of an Analogue of the Peptide Hormone Oxytocin That Contains a Mimetic of an Inverse γ-Turn

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Oxytocin is a neurohypophyseal peptide hormone that induces labor and lactation in mammals. An inverse γ-turn mimetic corresponding to the tripeptide Ile-Val-Asn has been synthesized and incorporated instead of residues 3−5 of oxytocin to probe the hypothesis that a γ-turn involving these residues is found in the receptor bound conformation of oxytocin. In the turn mimetic, residues *i* and *i* + 1 are connected by a ψ[CH_2O] isostere while a covalent methylene bridge replaces the hydrogen bond that is often found between residues *i* and *i* + 2 in γ-turns. The turn mimetic was assembled from three types of building blocks: an azido epoxide, an α-bromo acid, and a protected β-amino alcohol. The oxytocin analogue did not induce contractions of the uterus nor did it inhibit oxytocin-induced contractions. It is suggested that the loss of bioactivity is mainly due to the presence of a ψ[CH_2O] isostere instead of an amide bond between residues *i* and *i* + 1 in the turn mimetic.

Introduction

Peptides display a multitude of diverse and important biomedicinal activities, but use of peptides as therapeutic agents is often limited by poor pharmacokinetic properties, i.e., low uptake on oral administration, rapid enzymatic degradation, and facile excretion.[1] In addition, conformational flexibility may reduce the biological activity and receptor selectivity of peptides, whereas low solubility can impose restrictions on their use under physiological conditions. Substantial efforts have been devoted to the design and synthesis of mimetics of peptides in order to circumvent the above problems.[2−5] Since peptidomimetics are often conformationally restricted, they may also be used in attempts to establish the bioactive conformation of peptides.

Turns are defined as regions where a peptide chain reverses its overall direction.[6,7] In a γ-turn, this occurs over three residues and a hydrogen bond is often formed between residues *i* and *i* + 2 so that a pseudo-seven-membered ring is formed. Depending on if the side chain of residue *i* + 1 is in an equatorial or axial orientation on the pseudo-seven-membered ring, γ-turns are classified as inverse or classical, respectively. In the related β-turns, chain reversal instead involves four residues and a hydrogen bond may then be formed between residues *i* and *i* + 3. Structural studies have revealed that a large number of naturally occurring small peptides that function as hormones or neurotransmitters, or have other regulatory roles in organisms, may adopt β- or γ-turn conformations. β-Turns are more frequent than γ-turns in biologically active peptides, but vasopressin,[8,9] Leu-enkephalin,[10−12] angiotensin II,[13] and bradykinin[14] have been suggested to populate γ-turn conformations.

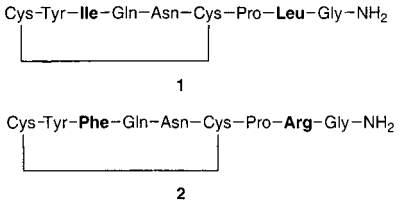

Figure 1. Closely related structures of the hormones oxytocin (**1**) and vasopressin (**2**).

Oxytocin (**1**, Figure 1) is a neurohypophyseal nonamer peptide hormone, the physiological function of which is to regulate milk ejection and uterine contractions in mammals.[15,16] Previous conformational studies have suggested that [Pen^1]oxytocin populates an inverse γ-turn centered at Gln4.[17−19] Gly-[Lys^8]vasopressin, Gly-Gly-Gly-[Lys^8]vasopressin,[20] and the drug desmopressin ([Mpr^1,$DArg^8$]vasopressin),[21] which have closely related structures [cf. vasopressin (**2**), Figure 1], were also found to adopt inverse γ-turns at this position. In addition, the conformation of desmopressin showed large similarities to the solution structure of neurophysin-bound oxytocin[22] for residue Tyr2-Cys6.[21] These observations suggest that it should be interesting to probe the biologically active conformation of oxytocin by replacing the Ile3-Gln4-Asn5 fragment with a mimetic of an inverse γ-turn.

Results and Discussion

We recently reported the design of inverse γ-turn mimetics **3** (Figure 2) and incorporation of such a mimetic into the drug desmopressin.[23] In **3** the amide bond between residue *i* and *i* + 1 of the γ-turn has been replaced by a methylene ether isostere. In addition, a

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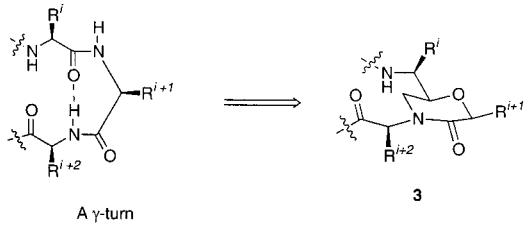
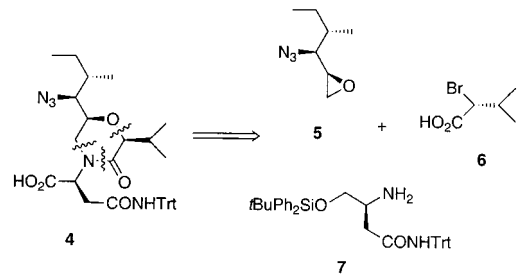


Figure 2. Classical and inverse γ-turns have the C═O of residue i close to the NH of residue $i + 2$ and are often stabilized by a hydrogen bond between these groups. In inverse γ-turn mimetic **3**, the hydrogen bond has been replaced by a methylene bridge that provides conformational restriction.

methylene bridge ensures the close spatial location of residues i and $i + 2$ by forming a six-membered morpholin-3-one ring. Ab initio calculations revealed that this covalent linkage effectively restricts the torsional angles of the $i + 1$ residue to values close to those of an inverse γ-turn.[23,24] We have now focused on preparation of inverse γ-turn mimetic **4** (Scheme 1), which has side chains corresponding to those of the tripeptide Ile-Val-Asn, and on incorporation of **4** in place of residues 3−5 in oxytocin. In oxytocin, Gln4 can be replaced by Val with retention of >25% of the agonistic activity at the uterine receptor.[25,26] Val was therefore used instead of Gln at position $i + 1$ of turn mimetic in order to simplify the synthesis. A retrosynthetic analysis suggested that azido epoxide **5**, (*R*)-2-bromo-3-methylbutanoic acid **6**, and protected asparaginol **7** are suitable building blocks for synthesis of mimetic **4**. The azido group in **5** serves as a precursor of the amino group of residue i in the turn, while the *tert*-butyldiphenylsilyl-protected alcohol in **7** corresponds to the carboxyl group of residue $i + 2$ of the turn.

Scheme 1[a]

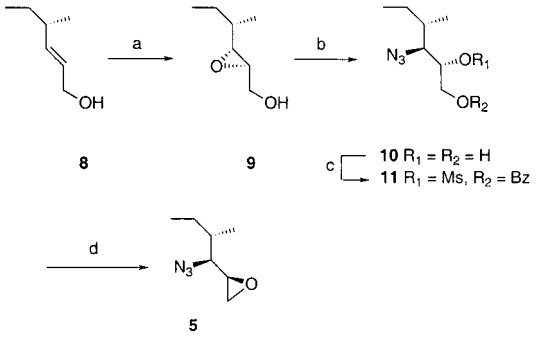


[a] Retrosynthetic analysis showing that the target mimetic **4** may be assembled from three building blocks: azido epoxide **5**, α-bromo acid **6**, and protected β-amino alcohol **7**.

Synthesis of azido epoxide **5** began with a Katsuki−Sharpless asymmetric epoxidation[27] of allylic alcohol **8**[28] to furnish epoxy alcohol **9** (Scheme 2) (82%, 88% de according to ^{1}H NMR analysis). Regioselective opening of the epoxide in **9** by $Ti(OiPr)_2(N_3)_2$[29] then yielded azido diol **10**. Benzoylation of the primary hydroxyl group of **10** directly followed by mesylation of the secondary hydroxyl group gave **11**. Finally, treatment of **11** with sodium ethoxide resulted in removal of the benzoyl group and intramolecular ring closure to give building block **5** (41% overall yield from **8**, 90% de based on ^{1}H NMR analysis).

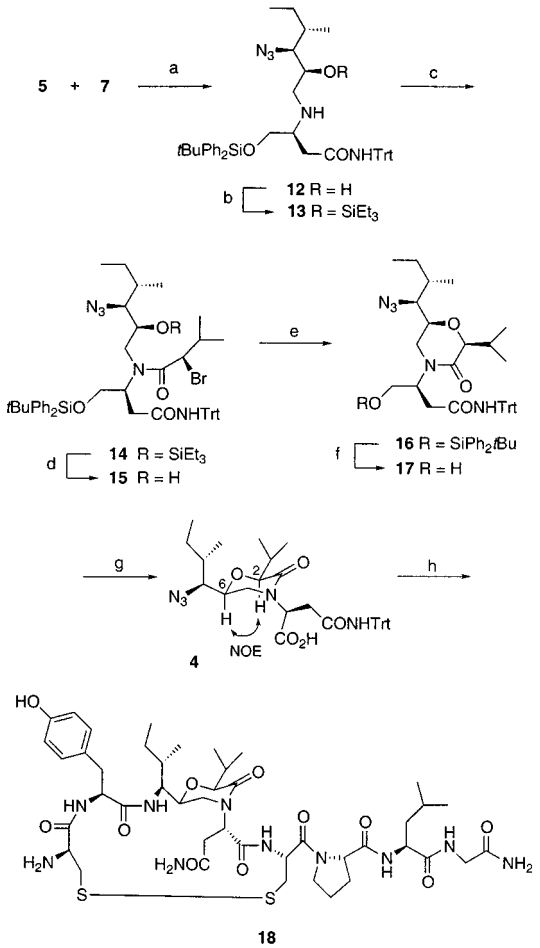
Assembly of inverse γ-turn mimetic **4** began by nucleophilic attack at the primary position of azido epoxide **5** with silylated asparaginol **7**[23] (Scheme 3). At

Scheme 2[a]



[a] (a) D-(−)-Diisopropyl tartrate, $Ti(OiPr)_4$, *t*BuOOH in toluene, CH_2Cl_2, −20 °C, 82%; (b) $Ti(OiPr)_2(N_3)_2$, benzene, reflux, 92%; (c) collidine, BzCl, −10 °C → room temp, then MsCl, CH_2Cl_2, 0 °C → room temp, 66%; (d) NaOEt in EtOH, THF, room temp, 83%.

Scheme 3[a]



[a] (a) EtOH, reflux, 68%; (b) Et_3SiCl, imidazole, CH_2Cl_2, 0 °C → room temp, 86%; (c) (*R*)-(+)-2-bromo-3-methylbutanoic acid (**6**), diisopropylcarbodiimide, CH_2Cl_2, 0 °C → room temp; (d) 2 M HCl(aq)/THF (3:2), room temp, 56% from **13**; (e) KH, THF/DMF (2.4:1), 0 °C, 89%; (f) TBAF·H_2O, THF, room temp, 97%; (g) $NaIO_4$, $RuCl_3$·H_2O, CCl_4/CH_3CN/H_2O (2:2:3), room temp, 61%; (h) solid-phase synthesis, 29%.

this stage, the minor diastereomer originating from the synthesis of **5** could be removed by flash column chromatography so that amino alcohol **12** was obtained in enantiomerically pure form as determined by ^{1}H NMR spectroscopy (68% yield). Attempts to acylate the amino group of **12**, without acylation of the secondary hydroxyl group, were not successful. Therefore, the

Table 1. ^{1}H NMR Data (δ, ppm) for Peptidomimetic **18** in Aqueous Solution[a]

residue	NH	H-α	H-β	H-γ	others
Cys[1]		4.03	3.13, 3.28		
Tyr[2]	9.28	4.75	3.03, 3.07		6.84 and 7.16 (arom)
mimetic					
Ile[3]	7.55	3.70	1.63	0.91, 1.02	0.76 (δ-CH_3), 0.92 (γ-CH_3), 3.86 (CHO)
Val[4]		4.14	2.22		0.87 and 1.01 (γ-CH_3)
Asn[5]		5.32	2.74, 2.91		7.01 and 7.78 ($CONH_2$)
bridge					3.26 and 3.60 (CH_2N)
Cys[6]	8.11	5.05	2.78, 3.26		
Pro[7]		4.40	1.90, 2.27	2.01[b]	3.65 and 3.74 (H-δ)
Leu[8]	8.66	4.28	1.60, 1.65	1.63	0.87 and 0.92 (δ-CH_3)
Gly[9]	8.51	3.84, 3.91			7.16 and 7.47 ($CONH_2$)

[a] Obtained at 400 MHz, 278 K, and pH = 6.2 (phosphate buffer) for a ~14.3 mM solution of **18** in water containing 10% D_2O [HDO (δ = 4.98) as internal standard]. [b] Degeneracy has been assumed.

hydroxyl group of **12** was first protected by treatment with triethylsilyl chloride to furnish **13**. Despite this, coupling of α-bromo acid **6** with secondary amine **13** turned out to be difficult, most likely because of steric hindrance. Conversion of **6** into the corresponding acid chloride allowed rapid and almost complete acylation of **13**, but the product **14** was found to be a mixture of diastereomers, most likely due to epimerization of the stereogenic center of **6**. Initially the yield was low when *N*,*N*′-diisopropylcarbodiimide (DIC) was used as coupling reagent, but **14** was obtained as a single diastereomer according to ^{1}H NMR spectroscopy. To improve the yield, the influence of the ratio of **6** to DIC, the concentrations of the reactants, and the reaction time were investigated carefully. By use of an excess of α-bromo acid **6** (8 equiv), a 2:1 ratio of **6** to DIC, a high concentration of **13** (0.09 M), and a prolonged reaction time (2 days), a satisfactory yield of **14** could be obtained. Despite substantial efforts, **14** could not be obtained completely free from diisopropylurea, the side product of the coupling. However, removal of the triethylsilyl group by treatment with aqueous HCl allowed the remaining contaminants of diisopropylurea to be removed by flash column chromatography and **15** was isolated in 56% yield based on **13**. The key cyclization of **15** was achieved by conversion of **15** into the corresponding alkoxide by treatment with potassium hydride in a mixture of THF and DMF, which induced intramolecular ring closure to give morpholin-3-one **16** in high yield (89%). Formation of side products due to β-elimination or epimerization of the stereocenter adjacent to the carbonyl group in **16** was not detected in the ring-closure step. Finally, deprotection of the primary hydroxyl group in **16** with tetrabutylammonium fluoride and subsequent oxidation of **17** with the biphasic $RuCl_3$−$NaIO_4$[30] system gave the desired inverse γ-turn mimetic **4** (61%) after purification by reversed-phase HPLC. The structure of mimetic **4** was confirmed by ^{1}H NMR spectroscopy, which revealed a strong NOE between hydrogen atoms H-2 and H-6 in the morpholin-3-one ring. This observation is consistent with the expectation that the inverse γ-turn mimetic should adopt a half-chair conformation (cf. **4**, Scheme 3), with H-2 and H-6 in axial orientations.

The target oxytocin analogue **18** was prepared on a polystyrene resin grafted with polyethylene glycol chains that were functionalized with the Rink linker.[31,32] The synthesis was performed by coupling of N^{α}-Fmoc amino acids, which carried standard side chain protecting groups and had been preactivated as benzotriazolyl esters.[33] In the first attempt to prepare oxytocin analogue **18**, mimetic **4** was activated as an azabenzotriazolyl ester by treatment with HOAt and DIC in DMF.[34] Only a slight excess (~30%) of activated **4** relative to the capacity of the resin was used in the coupling. After attachment of **4** to the solid phase, the azido group was reduced by treatment with tin(II) chloride in the presence of thiophenol and triethylamine.[35,36] The reduction could be monitored conveniently by the disappearance of the N_3 stretch in the IR spectrum obtained from a few resin beads.[37] After reduction of the azido group had reached completion, it was found that it is essential to wash the resin with 20% piperidine in DMF before proceeding with the synthesis. If washing with piperidine was omitted, incorporation of the following amino acid did not reach completion. This was assumed to be due to complexation of tin salts to the liberated amino group in the attached mimetic. The azido group thus served as a masked amino group throughout the synthesis of **4** and during attachment to the peptide resin. After solid-phase synthesis had been completed, the resin was treated with trifluoroacetic acid containing water, thioanisole, and ethanedithiol as scavengers to liberate the peptide analogue from the solid support and to deprotect the amino acid side chains.[38] Disulfide bond formation was effected by oxidation with iodine in methanol,[39] and purification by reversed-phase HPLC furnished peptide analogue **18** (33% yield based on the capacity of the resin). The product was homogeneous when analyzed by reversed-phase HPLC and had the correct molecular weight according to FAB mass spectrometry. However, ^{1}H NMR spectroscopy revealed two sets of resonances, both of which were compatible with the structure of **18.** N-Alkylated amino acids, such as turn mimetic **4**, are more susceptible to epimerization during activation and coupling than ordinary amino acids.[40,41] We therefore assumed that the heterogeneity of the product could be due to epimerization of the stereocenter adjacent to the carboxyl group of **4**. Indirect support for this assumption was provided by the observation that the chirality of the Tyr, Cys, Pro, and Leu residues in the product was intact, as determined by chiral amino acid analysis.[42] Use of HATU[43,44] as coupling reagent, in combination with collidine as base and dichloromethane as solvent, has been shown to suppress epimerization in coupling of peptide segments.[45] When mimetic **4** was incorporated using these conditions, **18** was obtained in 29% yield based on the capacity of the resin. In this case, ^{1}H NMR spectroscopy revealed only one set of signals, which was in complete

agreement with the structure of **18** (Table 1). The structure of **18** was further confirmed by means of fast-atom bombardment mass spectrometry and amino acid analysis.

Oxytocin (**1**) is a potent inducer of uterine contractions, and the ability of analogue **18** to induce contractions of rat uterine tissue segments, or inhibit oxytocin-induced contractions, was investigated in an organ bath.[46] It was found that **18** did not act as an oxytocin agonist even at the highest concentration (2.0 μM) evaluated in this assay. Furthermore, **18** did not display any antagonistic effect at concentrations ranging from 0.49 to 7.8 μM when oxytocin was used as agonist. These findings could be viewed in the context of early observations, which showed that modifications in the macrocyclic ring of oxytocin, for instance by replacement of Gln4 by isoglutamine, resulted in loss of activity at the uterine receptor.[47−49]

The lack of activity of **18** may be due to incorporation of turn mimetic **4** having a drastic effect on the conformation of **18** compared to oxytocin. It is also possible that the structural modifications introduced by mimetic **4** prevent **18** from binding to receptors in the uterus. As outlined in the preceding paper,[50] conformational studies of an analogue of the drug desmopressin ([Mpr^1,D-Arg^8]vasopressin) in which a γ-turn mimetic similar to **4** has been incorporated revealed that the desmopressin analogue was able to populate similar conformations as desmopressin itself. In view of the close structural and conformational[21] similarities between oxytocin and desmopressin, it therefore appears unlikely that the lack of activity of **18** is due only to conformational influences from the γ-turn mimetic. It should, however, be kept in mind that **18** is expected to be more rigid than oxytocin and that this could contribute to the low activity of **18**. It may therefore be proposed that structural features of the γ-turn mimetic also are important when accounting for the loss of activity of **18**. For instance, the methylene bridge might provide steric hindrance for binding of **18** to the uterine receptor. However, replacement of the amide bond between residues 3 and 4 in desmopressin with a methylene ether isostere was found to result in a substantial loss of activity at the vasopressin V_2 receptor.[50] Accordingly, it is possible that the lack of an amide bond between residues 3 and 4 of **18** significantly contributes to the loss of bioactivity. This, in turn, would suggest that the corresponding amide bond in oxytocin is involved in hydrogen bonding with the uterine G-protein-coupled receptor, a situation that is regarded to be uncommon for peptide hormones that interact with transmembrane receptors.[51] Finally, these results may also suggest that the receptor bound conformation of oxytocin does not contain an inverse γ-turn at residues 3−5.

In conclusion, an inverse γ-turn mimetic, **4**, which has side chains functionalities corresponding to an Ile-Val-Asn tripeptide, has been synthesized from three building blocks: an azido epoxide, an O-silylated amino alcohol, and an α-bromo acid. Mimetic **4** was then incorporated in place of residues 3−5 in the peptide hormone oxytocin by Fmoc solid-phase synthesis to give oxytocin analogue **18**. Analogue **18** did not induce contractions of uterine tissue segments nor did it act as an inhibitor of oxytocin-induced contractions. This may be due to the structural features of the γ-turn mimetic, in particular the presence of a methylene ether isostere instead of an amide bond between residues 3 and 4 in **18**.

Experimental Section

General Methods and Materials. All reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions unless otherwise stated. CH_2Cl_2 and THF were distilled from calcium hydride and potassium benzophenone, respectively. DMF was distilled and then dried over 3 Å molecular sieves. TLC was performed on silica gel 60 F_{254} (Merck) with detection by UV light and charring with phosphomolybdic acid in EtOH. Flash column chromatography (eluents given in brackets) was performed on silica gel (Matrex, 60 Å, 35−70 μm, Grace Amicon).

^{1}H NMR spectra for compounds **4**−**17** were recorded at 400 or 500 MHz, whereas ^{13}C NMR spectra were obtained at 100 MHz, for solutions in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.26 ppm) or $CHCl_3$ (δ_C 77.0 ppm) as internal standard] at 298 K. The ^{1}H NMR spectrum of oxytocin analogue **18** was recorded at 400 MHz and 278 K in a mixture of phosphate buffer (500 μL, 40 mM, pH = 6.2, ∼15 mM in NaN_3) and D_2O (50 μL) using the resonance of H_2O (δ_H 4.98 ppm) as internal standard. Proton resonances were assigned from appropriate combinations of COSY,[52] TOCSY,[53] HSQC,[54] and NOESY[55−57] experiments. Diastereomeric excesses and ratios of the rotameric mixtures were derived from the ^{1}H NMR spectra. Ions for positive fast-atom bombardment mass spectra were produced by a beam of xenon atoms (6 keV) from a matrix of glycerol and thioglycerol. Melting points are uncorrected.

The purity of oxytocin analogue **18** and building block **4** was analyzed by reversed-phase HPLC using a Kromasil C-8 column (100 Å, 5 μm, 4.6 × 250 mm). Linear gradients of 0% to 100% of solvent B in solvent A over 60 min and 40% to 100% of solvent B in solvent A over 30 min were used for **17** and **4**, respectively, with a flow rate of 1.5 mL/min and detection at 214 nm (solvent systems: A, 0.1% aqueous trifluoroacetic acid; B, 0.1% trifluroacetic acid in CH_3CN). Purification of crude **18** and **4** by HPLC was performed on a Kromasil C-8 column (100 Å, 5 μm, 20 mm × 250 mm) using the same eluent and a flow rate of 11 mL/min.

(2*R*,3*R*,4*S*)-2,3-Epoxy-4-methylhexan-1-ol (9). D-(−)-Diisopropyl tartrate (30.3 μL, 0.145 mmol) and Ti(O*i*Pr)$_4$ (28.4 μL, 96.4 μmol) were added sequentially to a mixture of CH_2Cl_2 (7 mL) and crushed 4 Å molecular sieves (55 mg) at −20 °C. After the mixture was stirred for 15 min at −20 °C, *t*BuOOH in toluene (3.55 M, 1.19 mL, 4.24 mmol) was added slowly. After a further 30 min, allylic alcohol **8**[28] (0.220 g, 1.93 mmol) was added dropwise. The reaction was allowed to run overnight at −20 °C. A cooled (0 °C) solution of $FeSO_4 \cdot 7H_2O$ (0.64 g) and tartaric acid (0.193 g) in water (5 mL) was subsequently added, and the mixture was stirred for 10 min. The phases were separated, and the aqueous phase was extracted twice with CH_2Cl_2. The combined organic phases were cooled to 0 °C and treated with aqueous 30% NaOH saturated with NaCl (1.2 mL) and water (8.8 mL), and the mixture was stirred vigorously for 1 h until two clear phases were formed. The aqueous phase was extracted with CH_2Cl_2 (2 × 8 mL), and the combined organic phases were dried with Na_2SO_4, filtered, and concentrated. Flash chromatography (pentane/diethyl ether, 10:1 → 1:1) of the residue gave epoxy alcohol **9** as a colorless oil (0.205 g, 82%, 88% de as determined by integration of the ^{1}H NMR resonances for the CH_3CHCHO protons): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89 (1H, brd, J = 12.6 Hz, C*H*$_2$OH), 3.58 (1H, brd, J = 12.4 Hz, C*H*$_2$OH), 2.96 (1H, ddd, J = 4.8, 2.4, and 2.4 Hz, OC*H*CH$_2$OH), 2.69 (1H, dd, J = 7.3, 2.4 Hz, CH$_3$CHC*H*O), 2.38 (1H, s, OH), 1.47−1.37 (1H, m, C*H*$_2$CH$_3$), 1.36−1.23 (2H, m, C*H*CH$_3$ and C*H*$_2$CH$_3$), 0.99 (3H, d, J = 6.5 Hz, CHC*H*$_3$), 0.90 (3H, t, J = 7.3 Hz, CH$_2$C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 61.9, 60.5, 58.3, 37.1, 26.4, 16.6, 11.6; HRMS (FAB) calcd for $C_7H_{15}O_2$ 131.1072 ($M + H^+$), found 131.1071.

(2*S*,3*S*,4*S*)-3-Azido-4-methylhexan-1,2-diol (10). Epoxy alcohol **9** (3.19 g, 24.5 mmol) in benzene (32 mL) was added to a suspension of $Ti(OiPr)_2(N_3)_2$ (7.56 g, 30.2 mmol) in benzene (200 mL) at 85 °C. After 30 min, the mixture was concentrated, then diethyl ether (400 mL) and 5% aqueous H_2SO_4 (200 mL) were added, and the mixture was stirred for 10 min until two clear phases were formed. The aqueous phase was extracted with CH_2Cl_2 (4 × 20 mL), and the combined organic phases were dried with Na_2SO_4, filtered, and concentrated. The residue was washed with pentane and then purified by flash chromatography (pentane/Et_2O, 5:1 → 1:3) to give azido diol **10** as a colorless oil (3.93 g, 92%, 88% ∼de): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85−3.76 (2H, m, C*H*OH and C*H*$_2$OH), 3.71 (1H, dd, J = 11.3, 6.6 Hz, C*H*$_2$OH), 3.39 (1H, t, J = 6.3 Hz, CHN_3), 2.34 (2H, brs, OH), 1.75−1.64 (1H, m, C*H*CH_3), 1.63−1.56 (1H, m, C*H*$_2CH_3$), 1.33−1.17 (1H, m, C*H*$_2CH_3$), 1.00 (3H, d, J = 6.9 Hz, CHC*H*$_3$), 0.92 (3H, t, J = 7.4 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 71.4, 70.1, 63.2, 36.0, 24.7, 16.2, 11.2; IR (neat) 3365, 2969, 2100, 1462, 1269 cm^{-1}; HRMS (FAB) calcd for $C_7H_{16}N_3O_2$ 174.1243 (M + H$^+$), found 174.1245.

(2*S*,3*S*,4*S*)-3-Azido-2-methanesulfonyloxy-4-methylhex-1-yl benzoate (11). 2,4,6-Collidine (2.03 mL, 15.4 mmol) and benzoyl chloride (1.51 mL, 13.0 mmol) were added sequentially to a solution of azido diol **10** (2.05 g, 11.8 mmol) in CH_2Cl_2 (55 mL) at −10 °C. The solution was allowed to attain room temperature overnight, then it was cooled again to 0 °C, and methanesulfonyl chloride (1.00 mL, 12.8 mmol) was added. After being allowed to attain room temperature overnight, the mixture was poured into saturated aqueous NaCl (50 mL). The aqueous phase was extracted with EtOAc (4 × 20 mL), and the combined organic phases were dried with Na_2SO_4, filtered, and concentrated. Flash chromatography (heptane/ethyl acetate, 15:1 → 5:1) of the residue gave benzoate **11** as a colorless oil (2.53 g, 66%, 88% de as determined by integration of the ^{1}H NMR resonances for the CH_2O protons): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09−8.05 (2H, m, Ph), 7.62−7.57 (1H, m, Ph), 7.50−7.43 (2H, m, Ph), 5.22−5.17 (1H, m, CHOMs), 4.71 (1H, ABX-type dd, J = 12.7, 2.3 Hz, CH_2O), 4.49 (1H, ABX-type dd, J = 12.7, 7.8 Hz, CH_2O), 3.69 (1H, dd, J = 8.3, 4.4 Hz, CHN_3), 3.06 (3H, s, SCH_3), 1.78−1.67 (1H, m, C*H*$_2CH_3$), 1.66−1.55 (1H, m, C*H*CH_3), 1.33−1.21 (1H, m, C*H*$_2CH_3$), 1.08 (3H, d, J = 6.8 Hz, CHC*H*$_3$), 0.94 (3H, t, J = 7.4 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 133.5, 129.7, 129.2, 128.6, 79.2, 69.4, 62.4, 38.8, 36.2, 25.4, 15.5, 10.7; IR (neat) 2966, 2104, 1724, 1344, 1269, 1176, 916 cm^{-1}; HRMS (FAB) calcd for $C_{15}H_{22}N_3O_5S$ 356.1580 (M + H$^+$), found 356.1293.

(2*R*,3*S*,4*S*)-3-Azido-1,2-epoxy-4-methylhexane (5). NaOEt in EtOH (2 M, 2.36 mL, 4.72 mmol) was added dropwise to benzoate **11** (1.39 g, 4.29 mmol) in THF (28 mL). After being stirred for 30 min at room temperature, the solution was poured into saturated aqueous NH_4Cl (15 mL). The mixture was extracted with CH_2Cl_2 (2 × 10 mL), and the combined organic phases were dried with Na_2SO_4, filtered, and concentrated. Flash chromatography (pentane/Et_2O, 60:1 → 40:1) of the residue gave epoxide **5** as a colorless oil (0.56 g, 83%, 90% de as determined by integration of the ^{1}H NMR resonances for the CH_2O protons): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.08 (1H, ddd, J = 6.6, 4.0, and 2.6 Hz, CHO), 2.92−2.84 (2H, m, CH_2O and CHN_3), 2.66 (1H, dd, J = 4.8, 2.6 Hz, CH_2O), 1.72−1.64 (2H, m, C*H*CH_3 and C*H*$_2CH_3$), 1.33−1.20 (1H, m, C*H*$_2CH_3$), 0.99 (3H, d, J = 6.9 Hz, CHC*H*$_3$), 0.92 (3H, t, J = 7.3 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 69.3, 53.4, 45.6, 37.3, 25.8, 15.3, 11.1; IR (c = 0.7 in CH_2Cl_2) 2968, 2099 1461, 1252 cm^{-1}; HRMS (FAB) calcd for $C_7H_{17}N_4O$ 173.1483 (M + NH_4+), found 173.1413.

***N*-Triphenylmethyl-(3*S*)-3-[(2*S*,3*S*,4*S*)-3-azido-2-hydroxy-4-methylhexylamino]-4-(*tert*-butyldiphenylsilyloxy)butyramide (12).** Azido epoxide **5** (0.225 g, 1.45 mmol) and amine **7**[23] (0.87 g, 1.45 mmol) were dissolved in EtOH (35 mL), and the solution was refluxed for 96 h. The solvent was evaporated, and flash chromatography (heptane/ethyl acetate, 10:1 → 1:1) of the residue yielded azido alcohol **12** as a white solid (0.74 g, 68%): mp 145−148 °C; $[\alpha]_D^{20}$ −6.8° (c 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (1H, s, NHTrt), 7.70−7.60 (4H, m, Ph), 7.49−7.33 (6H, m, Ph), 7.31−7.19 (15H, m, Ph), 3.77 (1H, ABX-type dd, J = 10.4, 4.2 Hz, CH_2O), 3.63 (1H, ABX-type dd, J = 10.4, 4.9 Hz, CH_2O), 3.59−3.52 (1H, m, CHO), 3.02−2.93 (1H, m, C*H*NH), 2.80 (1H, dd, J = 7.1, 3.9 Hz, CHN_3), 2.65 (1H, ABX-type dd, J = 11.6, 9.6 Hz, CH_2CO), 2.56−2.40 (3H, m, CH_2CO and C*H*$_2$NH), 2.30 (2H, bs, OH and NH), 1.79−1.67 (1H, m, C*H*CH_3), 1.50−1.39 (1H, m, C*H*$_2CH_3$), 1.26−1.13 (1H, m, C*H*$_2CH_3$), 1.09 (9H, s, $C(CH_3)_3$), 0.94 (3H, d, J = 6.8 Hz, CHC*H*$_3$), 0.90 (3H, t, J = 7.4 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 144.9, 135.5, 133.0, 129.9, 128.6, 127.8, 127.8, 126.8, 70.4, 70.3, 67.0, 64.0, 56.4, 50.4, 38.9, 35.6, 26.9, 24.9, 19.2, 16.2, 11.1; IR (neat) 3319, 2958, 2929, 2100, 1657, 1537, 1109, 698 cm^{-1}; HRMS (FAB) calcd for $C_{46}H_{56}N_5O_3Si$ 754.4152 (M + H$^+$), found 754.4163.

***N*-Triphenylmethyl-(3*S*)-3-[(2*S*,3*S*,4*S*)-3-azido-4-methyl-2-triethylsilyloxyhexylamino]-4-(*tert*-butyldiphenylsilyloxy)butyramide (13).** Imidazole (0.155 g, 2.28 mmol) was added to a solution of azido alcohol **12** (0.735 g, 0.990 mmol) in CH_2Cl_2 (50 mL) at room temperature. The solution was cooled to 0 °C, and chlorotriethylsilane (0.366 mL, 2.18 mmol) was added. After 20 h, the mixture was poured into saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were dried with Na_2SO_4, and the solvents were evaporated. Flash chromatography (heptane/ethyl acetate, 15:1 → 5:1) of the residue furnished silylated alcohol **13** as a white solid (0.712 g, 86%): mp 49−52 °C; $[\alpha]_D^{20}$ +1.4° (c 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.02 (1H, s, NHTrt), 7.66−7.60 (4H, m, Ph), 7.47−7.31 (6H, m, Ph), 7.29−7.18 (15H, m, Ph), 3.82−3.74 (2H, m, CHOSi and CH_2OSi), 3.55 (1H, ABX-type dd, J = 10.4, 5.2 Hz, CH_2OSi), 3.04−2.97 (1H, m, CHN), 2.75 (1H, dd, J = 7.3, 4.4 Hz, CHN_3), 2.71 (1H, ABX-type dd, J = 11.4, 6.8 Hz, CH_2N), 2.64 (1H, ABX-type dd, J = 11.6, 5.7 Hz, CH_2N), 2.46−2.42 (2H, m, CH_2CO), 1.94 (1H, s, NH), 1.74−1.65 (1H, m, C*H*CH_3), 1.49−1.39 (1H, m, C*H*$_2CH_3$), 1.17−1.08 (1H, m, C*H*$_2CH_3$), 1.06 (9H, s, $C(CH_3)_3$), 0.96−0.84 (15H, m, CHC*H*$_3$, CH_2CH_3, and $OSi(CH_2CH_3)_3$), 0.56 (6H, q, J = 7.9 Hz, $OSi(CH_2CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 144.9, 135.5, 133.0, 132.8, 129.9, 128.6, 127.8, 126.8, 73.0, 70.1, 69.8, 64.1, 56.7, 49.5, 38.8, 34.5, 26.9, 24.9, 19.2, 16.5, 11.1, 6.9, 5.2; IR (neat) 3319, 2958, 2102, 1668, 1489, 1105 cm^{-1}; HRMS (FAB) calcd for $C_{52}H_{70}N_5O_3Si_2$ 868.5017 (M + H$^+$), found 868.5022.

***N*-Triphenylmethyl-(3*S*)-3-{*N*′-[(2*S*,3*S*,4*S*)-3-azido-2-hydroxy-4-methylhexyl]-*N*′-[(1*R*)-1-bromo-2-methylpropylcarbonyl]amino}-4-(*tert*-butyldiphenylsilyloxy)butyramide (15).** 1,3-Diisopropylcarbodiimide (DIC, 86.0 μL, 0.432 mmol) was added to a solution of (*R*)-(+)-2-bromo-3-methylbutanoic acid (**6**, 157 mg, 0.865 mmol, Aldrich) in CH_2Cl_2 (0.6 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min, after which amine **13** (90.8 mg, 0.108 mmol) in CH_2Cl_2 (0.6 mL) was added. After a further 28 h at 0 °C, the diisopropylurea was filtered off and the filtrate was extracted with saturated aqueous $NaHCO_3$ (2 × 5 mL), 10% aqueous citric acid (2 × 5 mL), and brine (5 mL). The organic solution was dried over Na_2SO_4 and concentrated. Flash chromatography (heptane/ethyl acetate, 25:1 → 15:1) of the residue gave somewhat impure triethylsilylated amide **14** (84.4 mg). Compound **14** (84.4 mg, ∼81.8 μmol) was then dissolved in a mixture of 2 M aqueous HCl (3 mL) and THF (2 mL), and the resulting mixture was stirred at room temperature for 5 days. The mixture was poured into saturated aqueous $NaHCO_3$ (5 mL) and was extracted with CH_2Cl_2 (5 × 10 mL). The combined organic solutions were dried over Na_2SO_4 and concentrated. Flash chromatography (heptane/ethyl acetate, 15:1 → 5:1) of the residue yielded **15** as a white solid (54.6 mg, 56% over two steps): mp 65−68 °C; $[\alpha]_D^{20}$ −45° (c 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 4:1 mixture of rotamers) δ (major) 7.66−7.60 (3.2H, m, Ph), 7.41−7.34 (4.8H, m, Ph), 7.30−7.20 (7.2H, m, Ph), 7.20−7.16 (4.8H, m, Ph), 6.69 (0.8H, s, NHTrt), 4.30−4.20 (1.6H, m, CHBr and CH_2OSi), 4.04−3.95 (1.6H, m, CHN and C*H*OH), 3.66 (0.8H, ABX-type dd, J = 10.2, 5.5 Hz, CH_2OSi), 3.55 (0.8H, ABX-type dd, J = 15.3, 10.0 Hz, CH_2N), 3.44−3.24 (1.6H, m, CH_2N and CH_2CO), 2.67 (0.8H, dd, J = 7.4, 3.7 Hz, CHN_3), 2.37 (0.8H, ABX-type dd, J = 15.7, 3.2 Hz, CH_2-

CO), 2.33−2.23 (0.8H, m, C*H*$(CH_3)_2$), 1.84−1.73 (0.8H, m, C*H*CH_3), 1.61−1.50 (0.8H, m, CH_2CH_3), 1.31−1.17 (0.8H, m, CH_2CH_3), 1.15 (2.4H, d, J = 6.6 Hz, CH(CH_3)$_2$), 1.05 (7.2H, s, C$(CH_3)_3$), 1.00 (2.4H, d, J = 6.6 Hz, CH(CH_3)$_2$), 0.94 (2.4H, t, J = 7.4 Hz, CH_2CH_3), 0.91−0.81 (2.4H, m, CHCH_3); ^{1}H NMR (400 MHz, $CDCl_3$, 4:1 mixture of rotamers) δ (minor) 7.60−7.54 (0.8H, m, Ph), 7.46−7.42 (1.2H, m, Ph), 7.30−7.20 (1.8H, m, Ph), 7.16−7.12 (1.2H, m, Ph), 6.83 (0.2H, s, NHTrt), 4.64 (0.2H, d, J = 6.9 Hz, CHBr), 4.62−4.54 (0.2H, m, C*H*OH), 4.16−4.10 (0.4H, m, CHN and CH_2OSi), 3.72 (0.2H, ABX-type dd, J = 10.7, 4.3 Hz, CH_2N), 3.60−3.50 (0.2H, m, CH_2N), 3.44−3.24 (0.2H, m, CH_2OH), 2.88−2.78 (0.4H, m, CHN_3 and CH_2CO), 2.50 (0.2H, ABX-type dd, J = 15.5, 6.7 Hz, CH_2CO), 2.33−2.23 (0.2H, m, C*H*$(CH_3)_2$), 1.84−1.73 (0.2H, m, C*H*CH_3), 1.67−1.62 (0.2H, m, CH_2CH_3), 1.31−1.17 (0.2H, m, CH_2CH_3), 1.05 (1.8H, s, C$(CH_3)_3$), 0.91−0.81 (1.8H, m, CH_2CH_3, CH(CH_3)$_2$), 0.83 (0.6H, d, J = 6.8 Hz, CHCH_3); IR (neat) 3359, 2962, 2102, 1685, 1649, 1489, 1111 cm^{-1}; HRMS (FAB) calcd for $C_{51}H_{63}BrN_5O_4Si$ 916.3833 (M + H$^+$), found 916.3845.

***N*-Triphenylmethyl-(3*S*)-3-{(2*S*,6*S*)-6-[(1*S*,2*S*)-1-azido-2-methylbutyl]-2-isopropylmorpholin-3-one-4-yl}-4-(*tert*-butyldiphenylsilyloxy)butyramide (16).** KH (4.7 mg, 0.12 mmol) was suspended at 0 °C in a mixture of THF (2.4 mL) and DMF (1.0 mL). Bromo alcohol **15** (70 mg, 76 μmol) in THF (3.8 mL) was added dropwise to this suspension. After 90 min at 0 °C, the mixture was poured into saturated aqueous $NaHCO_3$ (5 mL) and extracted with CH_2Cl_2 (6 × 10 mL). The combined extracts were dried over Na_2SO_4, and the solvents were evaporated under reduced pressure. Flash chromatography (heptane/ethyl acetate, 10:1 → 3:1) of the residue gave the cyclic morpholinone derivative **16** as a white solid (57 mg, 89%): mp 84−88 °C; $[\alpha]_D^{20}$ −43° (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68−7.60 (4H, m, Ph), 7.48−7.34 (6H, m, Ph), 7.32−7.21 (9H, m, Ph), 7.21−7.15 (6H, m, Ph), 6.78 (1H, s, NHTrt), 4.14 (1H, ABX-type dd, J = 9.7, 8.0 Hz, CH_2O), 3.84 (1H, d, J = 2.3 Hz, COCHO), 3.64−3.75 (4H, m, CHN_3C*H*O, CH_2N, CHN, and CH_2O), 3.33 (1H, ABX-type dd, J = 15.3, 10.3 Hz, $COCH_2$), 3.22 (1H, ABX-type dd, J = 17.5, 9.4 Hz, CH_2N), 2.75 (1H, ABX-type dd, J = 7.1, 3.5 Hz, CHN_3), 2.54 (1H, ABX-type dd, J = 15.3, 4.5 Hz, $COCH_2$), 2.48−2.35 (1H, m, C*H*$(CH_3)_2$), 1.83−1.71 (1H, m, C*H*CH_3), 1.55−1.42 (1H, m, CH_2CH_3), 1.25−1.13 (1H, m, CH_2CH_3), 1.08 (3H, d, J = 7.2 Hz, CH(CH_3)$_2$), 1.05 (9H, s, C$(CH_3)_3$), 0.95 (3H, d, J = 6.7 Hz, CHCH_3), 0.9 (3H, d, J = 6.8 Hz, CH(CH_3)$_2$), 0.87 (3H, t, J = 7.4 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 169.4, 144.6, 135.5, 133.1, 129.8, 128.6, 127.9, 127.8, 127.0, 81.8, 73.3, 70.4, 67.1, 63.3, 61.2, 52.2, 37.1, 34.2, 30.6, 26.8, 24.8, 19.2, 19.1, 16.2, 15.9, 10.9; IR (neat) 3313, 2962, 2102, 1684, 1633, 1489, 1107 cm^{-1}; HRMS (FAB) calcd for $C_{51}H_{62}N_5O_4Si$ 836.4571 (M + H$^+$), found 836.4561.

***N*-Triphenylmethyl-(3*S*)-3-{(2*S*,6*S*)-6-[(1*S*,2*S*)-1-azido-2-methylbutyl]-2-isopropylmorpholin-3-one-4-yl}-4-hydroxybutyramide (17).** Tetrabutylammonium fluoride (19 mg, 74 μmol) was added to a solution of protected alcohol **16** (56 mg, 67 μmol) in THF (2.0 mL) at room temperature. After the mixture was stirred for 2.5 h, the solvent was evaporated. Flash chromatography (heptane/ethyl acetate, 1:1 → 1:5) of the residue furnished alcohol **17** as a white solid (39 mg, 97%): mp 85−87 °C; $[\alpha]_D^{20}$ −72° (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31−7.21 (9H, m, Ph), 7.21−7.17 (6H, m, Ph), 7.07 (1H, s, NHTrt), 4.08 (1H, bs, OH), 3.90 (1H, d, J = 2.3 Hz, COCHO), 3.81−3.67 (4H, m, CH_2OH, CHN_3C*H*O, and CHN), 3.52 (1H, t, J = 11.1 Hz, CH_2N), 3.30 (1H, ABX-type dd, J = 11.6, 2.6 Hz, CH_2N), 3.15 (1H, ABX-type dd, J = 15.2, 8.1 Hz, CH_2CO), 2.83 (1H, ABX-type dd, J = 15.0, 6.0 Hz, CH_2CO), 2.80 (1H, dd, J = 6.7, 4.5 Hz, CHN_3), 2.47−2.34 (1H, m, C*H*$(CH_3)_2$), 1.80−1.69 (1H, m, C*H*CH_3), 1.52−1.40 (1H, m, CH_2CH_3), 1.24−1.10 (1H, m, CH_2CH_3), 1.06 (3H, d, J = 7.0 Hz, CH(CH_3)$_2$), 0.95 (3H, d, J = 6.7 Hz, CHCH_3), 0.88 (3H, d, J = 6.6 Hz, CH(CH_3)$_2$), 0.85 (3H, t, J = 7.4 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 169.5, 144.6, 128.7, 128.1, 127.2, 81.7, 73.4, 70.7, 67.2, 64.1, 60.7, 51.3, 36.3, 34.3, 31.0, 24.8, 19.2, 16.4, 15.7, 10.9; IR (neat) 3319, 2962, 2106, 1684, 1668, 1633, 1491, 1449, 1036 cm^{-1}; HRMS (FAB) calcd for $C_{35}H_{44}N_5O_4$ 598.3393 (M + H$^+$), found 598.3409.

(2*S*)-2-{(2*S*,6*S*)-6-[(1*S*,2*S*)-1-Azido-2-methylbutyl]-2-isopropylmorpholin-3-one-4-yl}-3-(*N*-triphenylmethylcarbamoyl)propionic Acid (4). A catalytic amount (2.2 mol %) of ruthenium trichloride hydrate was added to a mixture of alcohol **17** (72 mg, 0.12 mmol), CCl_4 (0.3 mL), acetonitrile (0.3 mL), H_2O (0.45 mL), and sodium metaperiodate (80 mg, 0.37 mmol). The black mixture was stirred vigorously for 3.5 h at room temperature, then CH_2Cl_2 (5 mL) and H_2O (3 mL) were added, and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 5 mL), and the combined organic extracts were dried with $MgSO_4$ and concentrated. The residue was diluted with diethyl ether (5 mL), filtered through a Celite pad, and concentrated. Flash chromatography (toluene/ethanol, 10:1 → 1:1) followed by purification by reversed-phase HPLC (linear gradient 40% → 100% solvent B in solvent A during 30 min) furnished acid **4** as a white solid (45 mg, 61%): mp 105−107 °C; $[\alpha]_D^{20}$ −90° (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27−7.14 (15H, m, Ph), 4.21−4.13 (1H, m, CHN), 4.03 (1H, d, J = 1.5 Hz, COCHO), 3.76−3.68 (1H, m, CHN_3C*H*O), 3.68 (1H, t, J = 10.8 Hz, CH_2N), 3.39 (1H, bd, J = 9.5 Hz, CH_2N), 3.05 (1H, ABX-type dd, J = 16.5, 5.4 Hz, CH_2CO), 2.96 (1H, ABX-type dd, J = 16.8, 8.3 Hz, CH_2CO), 2.87 (1H, dd, J = 6.5, 4.7 Hz, CHN_3), 2.47−2.34 (1H, m, C*H*$(CH_3)_2$), 1.78−1.66 (1H, m, C*H*CH_3), 1.46−1.36 (1H, m, CH_2CH_3), 1.22−1.10 (1H, m, CH_2CH_3), 1.13 (3H, d, J = 7.0 Hz, CH(CH_3)$_2$), 1.02 (3H, d, J = 6.7 Hz, CHCH_3), 0.96 (3H, d, J = 6.8 Hz, CH(CH_3)$_2$), 0.89 (3H, t, J = 7.4 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 170.1, 169.4, 144.6, 128.8, 127.8, 127.0, 81.6, 73.6, 70.6, 67.3, 58.7, 51.5, 36.4, 34.2, 30.6, 24.5, 19.3, 16.3, 15.7, 10.9; IR (neat) 3323, 2962, 2104, 1732, 1687, 1643, 1491, 1184, 1024 cm^{-1}; HRMS (FAB) calcd for $C_{35}H_{41}N_5O_5Na$ 634.3006 (M + Na$^+$), found 634.3074.

Procedure for Solid-Phase Synthesis of 18. Peptide **18** was synthesized in a mechanically agitated reactor on a polystyrene resin grafted with aminated poly(ethylene glycol) chains (TentaGel-S−NH_2, Rapp Polymere, Germany; 0.276 g, 0.25 mmol/g, 69 μmol). The resin was functionalized with the linker *p*-[α-[(9-fluorenylmethoxy)formamido]-2,4-dimethoxybenzyl]phenoxyacetic acid (Novabiochem, Switzerland). Reagent solutions and DMF for washing were added manually to the reactor. N^α-Fmoc amino acids (Bachem, Switzerland) with the following side chain protective groups were used: triphenylmethyl (Trt) for Cys and *tert*-butyl (*t*Bu) for Tyr.

The linker and the N^α-Fmoc amino acids were activated as 1-benzotriazolyl esters and then added to the resin. Activation was performed by reaction of the appropriate N^α-Fmoc amino acid (0.275 mmol), 1-hydroxybenzotriazole (HOBt, 55.8 mg, 0.414 mmol), and 1,3-diisopropylcarbodiimide (41.6 μL, 0.269 mmol) in DMF (1.0 mL) for 30 min. Acylation was performed for 1−2 h and monitored by addition of bromophenol blue (0.05% of the resin capacity).[58] Fmoc deprotection was performed by treatment with 20% piperidine in DMF (3 + 7 min). The inverse γ-turn mimetic **4** (55.0 mg, 90 μmol) was activated using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate[43,44] (HATU; 40 mg, 0.105 mmol) and collidine (24.0 μL, 0.173 mmol) in CH_2Cl_2 (1.0 mL). Activated **4** was then coupled for 60 h, and the coupling was monitored by bromophenol blue. Before proceeding further, the resin was capped with acetic anhydride (0.5 mL) in CH_2Cl_2 for 45 min. After incorporation of **4** into the peptide, reduction of the azido group[35,36] was achieved by sequential addition of triethylamine (240 μL, 1.73 mmol), thiophenol (145 μL, 1.38 mmol), and $SnCl_2$ (66 mg, 0.345 mmol) to the suspended resin in THF (1 mL). After 19 h at room temperature, the resin was washed with THF (5 × 1 mL), 20% piperidine (2 × 1 mL), DMF (6 × 1 mL), and CH_2Cl_2 (5 × 1 mL) and was dried under vacuum. The disappearance of the N_3 stretch at 2100 cm^{-1} (IR spectrum of a few resin beads[37]) indicated successful reduction. The resin was then swelled in DMF (1 mL), and synthesis of **18** was continued as described above.

After completion of the synthesis, the resin carrying the protected peptide was washed with CH_2Cl_2 (5 × 1 mL) and

dried under vacuum. The peptide−resin was then cleaved, and the amino acid side chains were deprotected by treatment with trifluoroacetic acid/water/thioanisole/ethanedithiol [87.5:5:5:2.5, 30 mL] for 2.5 h, followed by filtration. Acetic acid was added to the filtrate, the solution was concentrated, and acetic acid was added twice more followed by concentration. The residue was triturated with diethyl ether, which gave a solid crude peptide that was dissolved in a mixture of acetic acid and water and was freeze-dried. Cyclization[39] was performed immediately by alternating additions of portions of the crude peptide in acetic acid and a 0.1 M solution of I_2 in methanol to a 10% solution of acetic acid in methanol (2 mL/mg cleaved resin). After the final addition of I_2, a light-brown solution was obtained that was neutralized and decolorized by stirring with Dowex 2 × 8-100 anion-exchange resin (Fluka, Cl^- form, 50−100 mesh; converted into acetate form by washing with 1 M aqueous NaOH, water, acetic acid, water, and methanol), filtered, and concentrated. The residue was then dissolved in a mixture of acetic acid and water and freeze-dried. Purification by preparative reversed-phase HPLC using a linear gradient of 0 → 100% of solvent B in solvent A over 60 min gave **18** as a white solid after free-drying (20 mg, 29%): ^{1}H NMR data are given in Table 1; MS (FAB) calcd for $C_{44}H_{69}N_{10}O_{11}S_2$ 978 ($M + H^+$), found 977. Amino acid analysis gave the following: Cys 2.00 (2), Gly 1.01 (1), Leu 1.01 (1), Pro 0.99 (1), Tyr 0.99 (1).

Pharmacological Evaluation of 18.[46] Sprague−Dawley rats (body weight ∼250 g, M & B A/S, Denmark) were selected at natural estrus by investigation of vaginal smears. The selected animals were sacrificed, and the whole uterus was removed and dissected free from fat and blood vessels. Four equal segments from each uterus were cut longitudinally, and two or three of these were then used in the evaluation. The uterine segments were mounted in organ baths containing 10 mL of de Jalon's nutrient solution aerated with carbogen gas. All segments were allowed to stabilize for 60 min at a resting tension of 1.0 g (10 mN). The isometric contractions were measured using a Grass force displacement transducer (model FT03) connected to an amplifier and recorded on a Grass polygraph (model 7D). The net maximal increase in uterine contraction was chosen as the index of response. The Grass system was calibrated so that 1.0 g equaled 20 mm (10 mN). An oxytocin house standard was used as agonist and control substance. The oxytocin house standard (616.5 IU/mg) and analogue **18** were both dissolved in aqueous 0.9% NaCl solution.

Evalution of **18** as an oxytocin agonist was performed against the oxytocin house standard using a 2 + 2 test. At least one group of doses consisting of $standard_{low}$, $standard_{high}$, $test_{low}$, and $test_{high}$ was given in randomized order to each uterine segment. Analogue **18** was evaluated on two different occasions in a total of nine experiments but did not show any agonistic effect even at the highest concentration evaluated in the organ bath (2.0 μM).

Each evaluation of **18** as an antagonist of oxytocin was started by administration of an exercise series of oxytocin consisting of six different concentrations. This series was not used for calculations. Six new oxytocin concentrations ranging from 3.2 nM to 1.6 μM were then administered at constant time intervals (45 s), and a cumulative dose−response curve was constructed. The uterine segments were rinsed only after the whole series had been completed. Then analogue **18** was administered, and after 60 s the oxytocin series was repeated and the uterine segment was rinsed again. The procedure was repeated with concentrations of **18** ranging from 0.49 to 7.84 μM in the organ bath. Analogue **18** was evaluated at three different occasions in a total of 16 experiments but did not show any antagonistic activity at any of the evaluated concentrations.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **4**, **5**, **9**−**13**, and **15**−**17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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