Head-to-Backbone Cyclization of Peptides on Solid Support by Nucleophilic Aromatic Substitution

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A new versatile synthetic route is presented for the cyclization of tripeptides on solid support using nucleophilic aromatic substitution in the cyclization step. Identification of all conformers within a limit of 3 kcal/mol from the identified global minimum conformations by Monte Carlo conformational searching reveals that five out of six synthesized compounds have well-defined peptide backbone conformational properties. This was determined by clustering the identified conformers against a filter of seven to nine torsion angles in the peptide backbone. Thus, the results meet our goal to find synthetic routes to peptides that are conformationally sufficiently locked to serve as convenient leads for further development of pharmacophoric models. The strategy is based on Fmoc-peptide chemistry on a *N*-aminoethyl-substituted glycine bound to the commercially available Rink amide PS-resin. After deprotection of the *N*-terminus of the tripeptide, it is acylated with a fluoronitrobenzoic acid. Subsequently, a Boc group on the *N*-bound aminoethyl substituent is selectively deprotected allowing cyclization from the head (*N*-terminus) to the backbone substituent, thereby leading to the desired cyclized tripeptides. A number of representative examples of peptides cyclized by this method have been synthesized and characterized by NMR. Protecting groups that allow the incorporation of side chain functionalized amino acids have been found. Thus, the route provides access to generic libraries of conformationally restricted peptide sequences expressing a range of proteinogenic pharmacophores.

Introduction

Peptides and proteins are known to possess extremely important roles in biochemistry, not just as the predominant component in receptors, antibodies, ion channels, enzymes, and carrier proteins but also as chemical compounds recognized by these protein entities (ligands/substrates). Structure-based design has in recent years been extensively used in ligand design for globular proteins. Especially for membrane-bound receptor molecules, the isolation and subsequent identification of the active site is, however, a time-consuming and certainly nontrivial process. As a result, localization and subsequent detailed deduction of the spatial and electronic properties of an active site can be extremely challenging if at all possible. These facts continue to motivate an ongoing search for rational ways for deducing this type of information from leads. The emergence and subsequent wide acceptance of combinatorial chemical methods has inspired many in the scientific community to synthesize generic libraries of peptides.[1] Nevertheless, the fact is that linear peptides can adopt massive numbers of conformations, of which only one or a few are biologically active.[2] Consequently, there are calls for well-considered approaches to the assessment and perhaps alteration of the conformational properties of peptides that are used as drug leads. We are particularly interested in the various turn motifs that large peptides and proteins can adopt. These motifs are poorly mimicked by short, linear peptides since such peptides experience a considerable loss of entropy by undergoing the processes of folding into such conformations. Thus, short, linear peptides predominantly adopt more extended conformations.[3] Since the turn motifs are often present to a large extent on the surface of proteins (up to one third of the backbone in globular proteins can be part of various bent motifs),[4] it is widely believed that these motifs will often be the ones recognized by some receptors.[5] This is one reason that the mimicry of β-turns, in particular, has been attempted by many researchers either by the development of turn-mimicking heterocycles[5,6] or by cyclization of short peptide sequences.[6−8] The first approach is unsuitable for use in combinatorial chemistry since the routes to such turn-promoting heterocycles are multistep nongeneric routes, which are not compatible with combinatorial approaches. In the case of small peptides (less than six amino acids), cyclization is far from trivial[9,10] although an intriguing, systematic approach to reducing this problem has been

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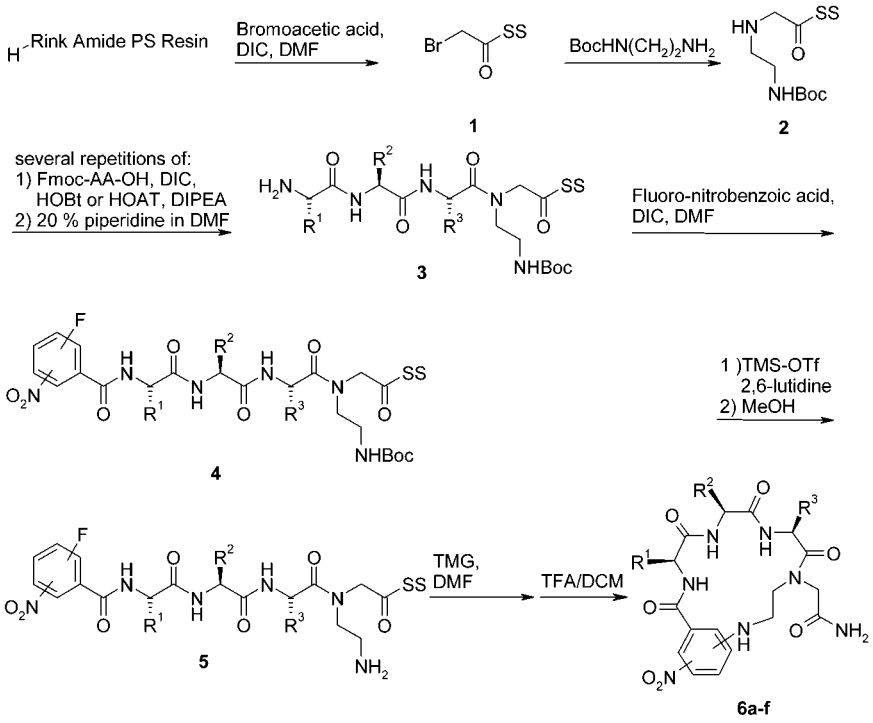
Scheme 1. Synthetic Route to Cyclic Peptides



reported.[3] These facts motivated us to develop versatile combinatorial synthetic routes to rigid, cyclized peptides containing a nonpeptidic tether. We hereby present a novel route to cyclized tripeptides linked with an ethylamino benzoyl moiety using nucleophilic substitution in the cyclization step leading to peptides, which are linked from the head (*N*-terminal) to a backbone nitrogen. Recent reports indicate that nucleophilic aromatic substitution is a convenient method for cyclization of peptide sequences of various lengths.[10−13] However, so far, mainly cyclization from the head to a side chain nucleophile has been reported. We chose to introduce the linkage between the head and a backbone nitrogen, since none of the side chains are occupied by tether formation in this way, maximizing the number of pharmacophoric interactions between the side chains and the targeted biomolecule. By introducing the aromatic moiety in the tether, we obtain yet another diversity element in the scaffold. Focusing on the substituents linking the peptide to the aromatic tether, the three substitution patterns ortho, meta, and para have all been found to be accessible via this strategy.

Synthetic Methods

The synthetic strategy is shown in Scheme 1.

The commercially available Rink amide PS-resin[14] was acylated with bromoacetic acid via the method of symmetrical anhydrides leading to substrate **1**. Nucleophilic substitution with mono-Boc-protected ethylenediamine afforded an *N*-substituted glycine **2**, onto which an amino acid was coupled using a preactivated mixture of amino acid, *N*,*N*′-diisopropylcarbodiimide (DIC), *N*,*N*-diisopropylethylamine (DIPEA), and 1-hydroxy-7-azabenzotriazole (HOAt)[15] in *N*,*N*-dimethylformamide (DMF)/dichloromethane (DCM). The remainder of the peptide sequence was synthesized via standard Fmoc chemistry by preactivation with 1-hydroxybenzotriazole (HOBt),[16,17,18] when the exposed amino group was primary, or HOAt when it was secondary (Pro). Deprotection of the Fmoc group was accomplished by shaking the resin in piperidine/DMF (1:4) for 30 min. After deprotection of the *N*-terminal amino acid giving rise to substrate **3**, the amino group was acylated with a premixed solution of the appropriate fluoronitrobenzoic acid and diisopropylcarbodiimide in DCM, affording resin-bound peptides of type **4**. The Boc group was subsequently selectively removed without cleavage from the resin by treatment with a solution of trimethylsilyl trifuoromethanesulfonate/2,6-lutidine in DCM for 30 min twice according to a procedure described by Zhang et al.[19] leading to immobilized peptides **5**. Cyclization of the molecule was accomplished by heating the resin to 70 °C in DMF with *N*,*N*,*N*′,*N*′-tetramethylguanidine (TMG, 5 equiv) for 17 h, resulting in cyclic peptides **6a**−**f** (Chart 1). Peptides, which were not carrying functional groups in the side chains, were cyclized without further complications (Table 1, entry 1), whereas peptides that were carrying side-chain functionalities had to be protected. In the case of arginine, Pbf (2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl) was found to afford suitable protection for the guanidyl group. Removal of the protecting group was accomplished by storing the cleaved peptide in TFA for 2 h (Table 1, entry 2). The sequence containing methionine was synthesized

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Chart 1

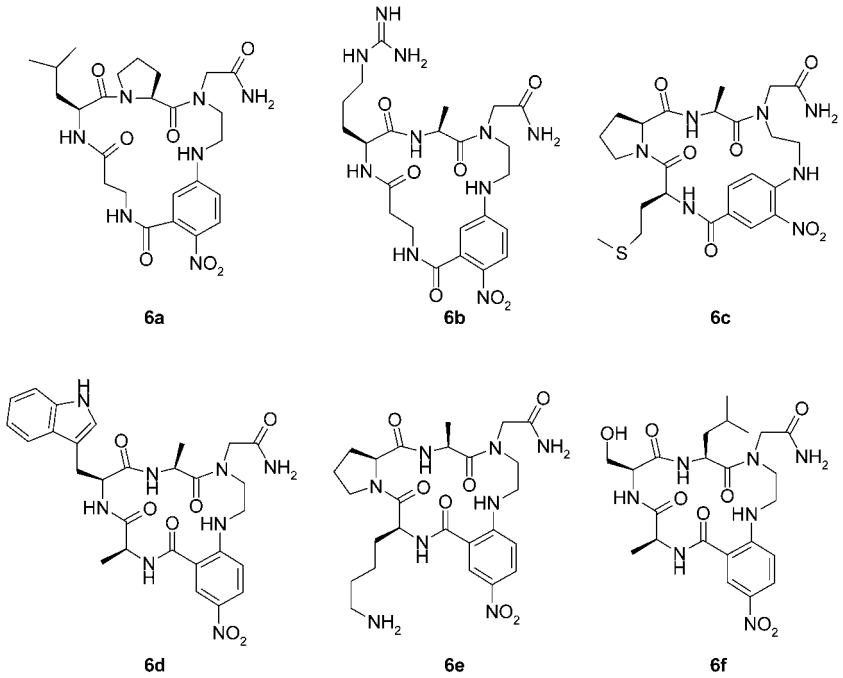

Table 1. Crude Purity and Yield

compd	crude purity (%) (ELS)	yield (%)
6a	86	44
6b	30	10
6c	24	6.3
6d	75	16
6e	*a*	9.4
6f	35	5.5

[a] An unambiguous determination of the purity could not be performed.

by using *S*-unprotected methionine (Table 1, entry 3)—a small amount (<10%) of the corresponding sulfoxide was removed in the HPLC purification. Attempts to incorporate tryptophan in the peptide sequence were successful when the indole moiety was protected with a Boc group. The observed selectivity of the deprotection of the alkylamine may be due to the formation of a *N*-carboxyindole intermediate[20] or an *O*-silylated carboxyindole reducing the susceptibility of reactions at the hetero-aromat. Serine was introduced unprotected, and right after the coupling the hydroxyl group was silylated with *tert*-butyldimethylsilyl chloride. Since both the Boc- and Fmoc-protecting groups were already employed in the synthetic strategy, it was necessary to find a protecting group orthogonal to both these groups for the amino group in the lysine side chain. We also wanted this protection to be removable either on-resin or by TFA, which we used to cleave the peptides from resin. The Teoc (2-(trimethylsilyl)ethyloxycarbonyl) group[21] turned out to be appropriate for this purpose. The structures of the purified compounds were unambiguously verified using 2D NMR (COSY, TOCSY, NOESY, and ROESY), high-resolution mass spectrometry (fast atom bombardment), and elemental analysis. The purities of the crude compounds were estimated by LC—MS in connection with evaporative light scattering, ELS, since it is a very sensitive method that should be independent of the compound type.[22,23]

Table 1 shows the crude purities of the synthesized peptides and the yields after purification by reversed-phase HPLC.

Molecular Modeling

Macromodel v. 7.0[24] was used to determine the low energy conformations of the synthesized compounds. The global and local minima for each compound were estimated using a Truncated Newton Conjugate Gradient (TNCG) algorithm applying the Merck Molecular Force Field (MMFF)[25−28]—the GB/SA continuum model was used to simulate water solvation.[29] All conformations within 3 kcal/mol of the identified lowest energy conformer were collected over 10000−25000 iterations in Monte Carlo multiple minimum searches—3 kcal/mol has been suggested as the maximally acceptable energy penalty upon binding to a receptor site.[30] No torsional constraints were applied. To determine the statistical similarity of the found conformers with respect to backbone-shape, the found low-energy conformations were clustered against a filter of seven to nine torsion angles (±20°) along the peptide backbone. The torsion angles of the predominant clusters are shown in Table 3 and

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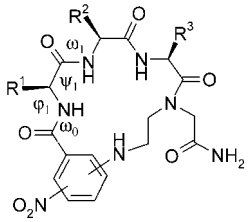
Table 2. Numbers of Found Low-Energy Conformers versus Total Number of Conformers

compd	6a	6b	6c	6d	6e	6f
identified conformers[a]	102	610	311	49	189	61
conformers in main cluster	76 (75%)	573 (94%)	221 (71%)	20 (41%)	179 (95%)	54 (86%)

[a] Number of conformers identified by Monte Carlo searches (10000−25000 iterations).

Table 3. Found Torsion Angles along the Peptide Backbone in Main Clusters[a]



	ω_0	φ_1	ψ_1	ω_1	φ_2	ψ_2	ω_2	φ_3	ψ_3
6a	−169			174	−87	150	−174	−72	154
6b	179			178	−79	−33	180	−161	150
6c	170	−153	70	3	−90	−18	−167	−157	164
6d	179	−89	−22	−169	−90	−45	−176	−167	74
6e	177	−108	133	−2	−78	−28	178	−170	150
6f	176	72	28	175	68	24	174	−162	83

[a] Torsion angles are measured in degrees along the peptide backbone according to standard conventions.

the results in Table 2. The torsion angles χ_1−χ_n are, however interesting, not taken into account when clustering the conformers.

Results and Discussion

The peptides were, as seen in Table 1, isolated in yields ranging from 6% to 44% after purification with reversed-phase HPLC. The reliable results for the synthesis of head-to-backbone cyclized peptides, also with the functionalized amino acids Lys, Arg, Trp, Met, and Ser, suggest that even more diverse peptides can be synthesized by our methods.

The outcome of the conformational search reveals that despite existing in a large number of low-energy conformations the majority of these have considerable conformational similarity. It has been found that most of the identified conformations differ mainly in the side chains. The peptide backbones in compounds **6a**−**f** have strong statistical representation in their main clusters except for compound **6d**, where only 41% of the identified conformers can be found in the main cluster (Figure 1). Thus, our aim to develop a method that reduces the number of possible conformers in the backbone has widely been achieved. The most abundant torsion angles do, however, not match the idealized torsion angles for β-turns as determined by Venkatachalam.[31] On the other hand, the backbones are locked sufficiently so that a biologically active cyclized peptide of this type can be used for further design of pharmacophore models. The possibility of altering the backbone conformations by the use of fluoro nitrobenzoic acids with different substitution patterns might also allow the vital study of the influence of the backbone on the activity, which can be included in such a pharmacophoric hypothesis. A graphical representation of the distinct biases for certain conformational families can be seen in Figure 2, which shows superimpositions of the found low energy conformers.

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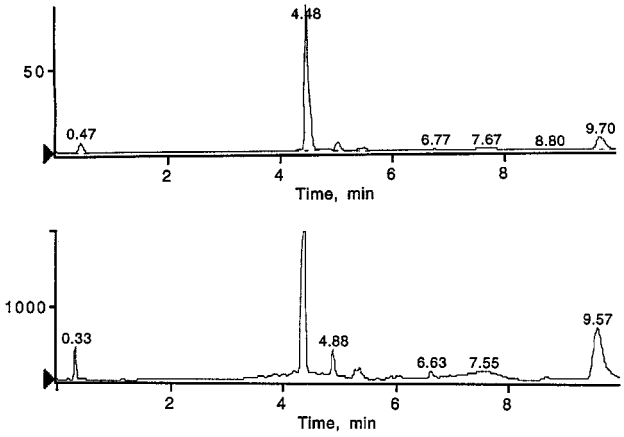

Figure 1. RP-HPLC analysis of crude compound **6d**. Top: evaporative light-scattering chromatogram. Bottom: UV-absorbance chromatogram (= 214 nm).

Conclusion

In conclusion, we have demonstrated a novel generic strategy leading to constrained cyclic peptides. The strategy has been proven versatile allowing incorporation of a range of functionalized amino acids. Furthermore, modeling shows that the cyclic peptides have well-defined conformational properties. Very high ratios of the low energy conformers are found in the main clusters when filtered against seven to nine torsion angles in the peptide backbone. Thus, the high numbers of low energy conformers are due to flexibility in other parts of the molecules (side chains and nonpeptidic tether). This opens up for a more straightforward deduction of the bioactive conformation of the peptide sequence if a compound is found to give a positive response in an assay.

Experimental Section

Rink amide resin and all amino acids were purchased from Bachem AG, HOBt was purchased from Acros, and HOAt was purchased from Perseptive Biosystems. DIC, DIPEA, piperidine, *N*-Boc-ethylenediamine, and 2-(trimethylsilyl)ethyl *p*-nitrophenyl carbonate were purchased from Fluka. 2-Fluoro-5-nitrobenzoic acid and trimethylsilyl trifluoromethanesulfonate were purchased from Aldrich. 5-Fluoro-2-nitrobenzoic acid was purchased from Interchim (France) and 4-fluoro-3-nitrobenzoic acid from Lancaster (England). High-resolution mass spectra were recorded at the University of Copenhagen. Elemental analyses were performed at the Structural Chemistry laboratory at Novo Nordisk A/S, Måløv.

General Procedure. Standard Wash. A standard wash series consisting of DMF (3 × 20 mL), DCM (2 × 20 mL), MeOH (1 × 20 mL), 2-propanol (1 × 20 mL), DCM (1 × 20 mL), and *tert*-butyl methyl ether (2 × 20 mL) followed each step. The suspension was shaken in each solvent portion for at least 2 min.

***N*-Boc-aminoethylglycine Handle.** Fmoc-protected Rink amide resin (2.00 g, 0.94 mmol total loading) was suspended in DMF and shaken for 3 min. After drainage of the reactor, the resin was shaken in DMF (20 mL) and piperidine (5 mL) for 30 min. After removal of the liquid, a solution of bromoacetic acid (3.27 g, 23.5 mmol) in DCM (15 mL) and DIC (1.47

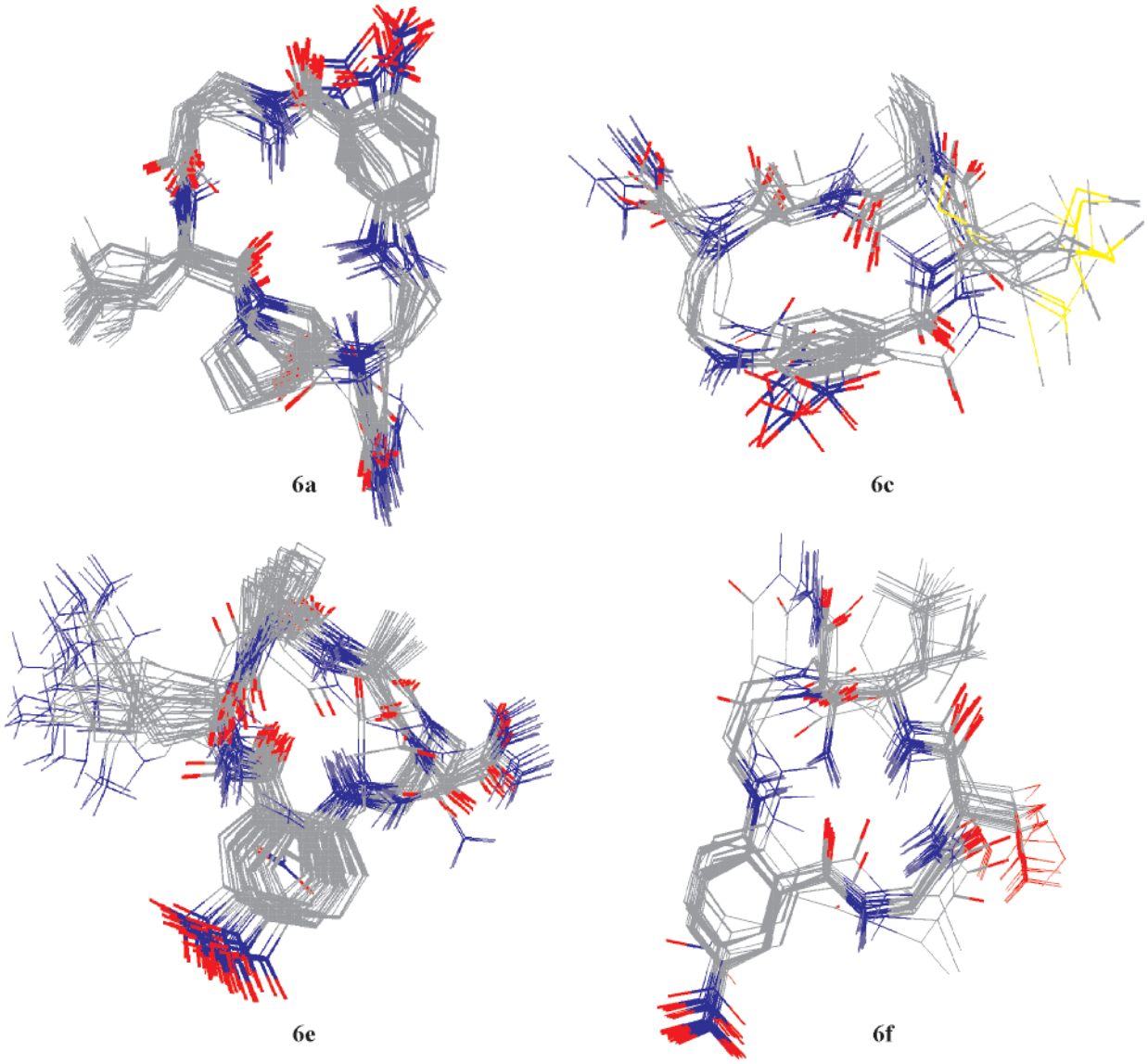

Figure 2. Superimpositions of identified low-energy conformers.

mL, 9.40 mmol) in DCM (15 mL) was added to the resin, and the suspension was shaken overnight. The resin was washed with DCM (5 × 20 mL); the resin was shaken in each solvent-portion for at least 2 min. A solution of *N*-(*tert*-butyloxycarbonyl)-1,2-diaminoethane (903.6 mg, 5.64 mmol) and DIPEA, (321.8 μL, 1.88 mmol) in DCM (20 mL) was added, and the suspension was shaken overnight. A standard washing procedure as described above was applied.

Peptide Assembly. A solution of Fmoc-amino acid (3.76 mmol), DIC (588.7 μL, 3.76 mmol), and HOAt (511.7 mg, 3.76 mmol) for secondary amines or HOBt (508.1 mg, 3.76 mmol,) for primary amines in DMF (10 mL) and DCM (5 mL) was added. After the solution was shaken for 45 min, DIPEA (482.8 μL, 2.82 mmol) was added and the mixture shaken overnight, except for Fmoc-Ser-OH, which was only allowed to react for 4 h. The standard washing procedure described above was performed.

The Fmoc protection was removed by shaking the resin in a solution of DMF (20 mL) and piperidine (5 mL) for 30 min followed by the standard washing procedure.

Serine Protection. After coupling with Fmoc-Ser-OH, the resin was washed with DMF (5 × 10 mL), and a solution of *tert*-butyldimethylsilyl chloride (708.4 mg, 4.70 mmol), 4-(*N*,*N*-dimethylamino)pyridine (DMAP, 11.5 mg, 0.094 mmol), and DIPEA (805 μL, 4.70 mmol) in DCM (25 mL) was added, after which time the suspension was shaken overnight followed by the usual washing procedure. No control of reaction completion was performed.

Cyclization. After deprotection of the terminal amino group, a solution of fluoronitrobenzoic acid (1392.0 mg, 7.52 mmol) and DIC (588.7 μL, 3.76 mmol) in DMF (10 mL)/DCM (5 mL) was added. The suspension was shaken overnight. The standard washing procedure was performed. A mixture of trimethylsilyl trifluoromethanesulfonate (3.6 mL, 20 mmol) and 2,6-lutidine (3.5 mL, 30 mmol) in DCM (20 mL) was added to the resin.[20] The liquid was removed after 30 min, and an identical mixture of trimethylsilyl trifluoromethanesulfonate (3.6 mL, 20 mmol) and 2,6-lutidine (3.5 mL, 30 mmol) in DCM (20 mL) was subsequently added to the resin. The liquid was removed after 30 min followed by addition of methanol (20 mL). The reactor was drained after 30 min, and the resin was washed. A solution of *N*,*N*,*N*′,*N*′-tetramethylguanidine (589.7 μL, 4.70 mmol) in DMF (30 mL) was added to the resin, and the resulting suspension was shaken at 70 °C for 17 h. The standard washing procedure was applied. The resin was shaken in a solution of glacial acetic acid (10 mL) in THF (20 mL) for 15 min and subsequently subjected to the usual standard washing procedure. Peptides containing serine were treated with a solution of tetrabutylammonium fluoride (TBAF, 4.70 mmol, 4.7 mL of a 1 M solution in THF) in THF (15 mL)

for 2.5 h and subsequently by the usual standard washing procedure. Peptides were cleaved from the resin by shaking the resin in TFA (10 mL)/DCM (10 mL) for 30 min except for compound **6b**, which was exposed to the TFA mixture for 2 h in order to remove the Pbf group efficiently. After evaporation of the solvent, the compound was dissolved in water (25 mL), and hydrochloric acid (83 μL, 12 M in water, 2.82 mmol) was added. The resulting solution was lyophilized. The residue referred to as Gly in the NMR data is the carbamoylmethyl substituent.

(6′*S*,9′*S*)-1,3-(4-Carbamoylmethyl-6,7-propano-9-(2-methylpropyl)-5,8,11,15-tetraoxo-1,4,7,10,14-pentaazapentadecano)-4-nitrobenzene (6a): yield 248 mg (0.411 mmol, 44%); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.27 (m, 2H, Leu, β-Ala), 7.92 (d, J = 9.1 Hz, 1H, Ar), 7.37 (b, 1H), 7.22 (m, 1H, Gly), 7.00 (s, 1H, Gly), 6.89 (s, 1H, Ar), 6.60 (dd, J = 9.1 Hz, 2.0 Hz, 1H, Ar), 4.71 (dd, J = 14.8 Hz, 7.4 Hz, 1H, Pro), 4.61 (m, 1H, Leu), 4.25−3.95 (m, Gly, Et, water), 3.76 (m, 1H, Pro), 3.70 (d, J = 16.3 Hz, 1H, Gly), 3.55−3.40 (m, 4H, Pro, β-Ala, Et), 3.20 (m, 1H, β-Ala), 2.69 (m, 1H, β-Ala), 2.23 (m, 1H, Pro), 2.17 (d, J = 14.8 Hz, 1H, β-Ala), 2.04 (m, 1H, Pro), 1.89 (m, 1H, Pro), 1.65 (m, 2H, Pro, Leu), 1.47 (m, 1H, Leu), 1.37 (m, 1H, Leu), 0.89 (m, 6H, Leu); HRMS (FAB+, m/z) exact mass of $[M + H]^+$ calcd for $C_{25}H_{35}N_7O_7$ 546.2676, found 546.2647. Anal. Calcd for $C_{25}H_{35}N_7O_7$·1.0 HCl·2.0H_2O: C, 48.58; H, 6.52; N, 15.86. Found: C, 48.33; H, 6.64; N, 15.93.

(6′*S*,9′*S*)-1,3-(4-Carbamoylmethyl-9-(3-guanidinopropyl)-5,8,11,15-tetraoxo-6-methyl-1,4,7,10,14-pentaazapentadecano)-4-nitrobenzene (6b): yield 55.0 mg (0.092 mmol, 10%, calculated as HCl salt); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.83 (s, 1H, Ala), 8.28 (m, 1H, β-Ala), 8.21 (d, J = 8.0 Hz, 1H, Arg), 7.93−7.88 (m, 2H, Ar, Arg), 7.50−7.02 (several broad signals, 6H, Arg, Gly), 6.88 (s, 1H, Ar), 6.63−6.60 (dd, J = 9.2 Hz, 2.2 Hz, 1H, Ar), 4.46−4.42 (m, 2H, Ala, Arg), 4.05 (d, J = 16.2, 1H, Gly), 3.87 (m, 1H, Et), 3.70 (d, J = 16.3, 1H, Gly), 3.45−3.34 (m, 3H, Et, β-Ala), 3.26 (m, 1H, β-Ala), 3.12 (m, 2H, Arg), 2.72 (m, 1H, β-Ala), 2.16 (d, J = 14.9, 1H, β-Ala), 1.71 (m, 1H, Arg), 1.56 (m, 3H, Arg), 1.24 (d, J = 6.9 Hz, 1H, Ala); HRMS (FAB+, m/z) exact mass of $[M + H]^+$ calcd for $C_{23}H_{35}N_{10}O_7$ 563.2690, found 563.2697. A correct elemental analysis for this compound could not be obtained.

(6′*S*,9′*S*,12′*R*)-1,4-(4-Carbamoylmethyl-5,8,11,14-tetraoxo-9,10-propano-6-methyl-12-(2-(methylsulfanyl)ethyl)-1,4,7,-10,13-pentaazatetradecano)-2-nitrobenzene (6c): yield 39.0 mg (0.060 mmol, 6.3%); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.69 (d, J = 9.3 Hz, 1H, Met), 8.60 (b, 1H, Et), 8.45 (s, 1H, Ar), 7.81 (d, J = 8.7 Hz, 1H, Ar), 7.67 (d, J = 9.1 Hz, 1H, Ar), 7.51−7.06 (several broad signals, Gly), 6.70 (d, 1H, Ala), 4.78 (m, 1H, Met), 4.24 (m, 1H, Et), 4.08−3.92 (m, Pro,Et, Gly), 3.81 (m, 1H), 3.67 (m, 1H, Pro), 3.51 (m, 2H, Et), 3.38 (m, 1H, Pro), 3.29 (m, 1H, Ala), 3.19 (t, J = 10.3 Hz, 1H, Pro), 2.47 (m, 1H, Met), 2.09 (m, Met), 1.93−1.68 (m, Pro, Met), 1.50 (m, 1H, Pro), 1.05 (s, 3H, Met), 0.95 (d, J = 5.84 Hz, 3H, Ala); HRMS (FAB+, m/z) exact mass of $[M + H]^+$ calcd for $C_{24}H_{33}N_7O_7S$ 564.2240, found 564.2220. Anal. Calcd for $C_{24}H_{33}N_7O_7S$·1.0HCl·3.0H_2O: C, 44.07; H, 6.16; N, 14.99. Found: C, 44.46; H, 6.14; N, 14.75.

(6′*S*,9′*S*,12′*S*)-1,2-(4-Carbamoylmethyl-9-(indol-3-ylmethyl)-5,8,11,14-tetraoxo-6,12-dimethyl-1,4,7,10,13-pentaazatetradecano)-4-nitrobenzene (6d): yield 97.0 mg (0.149 mmol, 16%); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.86 (s, 1H, Trp), 8.98 (s, 1H, Et), 8.88 (d, J = 5.2 Hz, 1H, Ala2), 8.74 (d, J = 2.5 Hz, 1H, Ar), 8.14 (dd, J = 9.4 Hz, 2.3 Hz, 1H, Ar), 7.92 (m, 2H, Ala1, Trp), 7.66 (s, 1H, Gly), 7.51 (d, J = 7.8 Hz, 1H, Trp), 7.32 (d, J = 8.0 Hz, 1H, Trp), 7.25 (s, 1H, Gly), 7.05 (m, 2H, Trp), 6.98 (m, 1H, Trp), 6.90 (d, J = 9.5 Hz, 1H, Ar), 4.51−4.35 (m, Ala1, Trp, Et), 4.05 (d, J = 17.7 Hz, 1H, Gly), 3.93−3.83 (m, 2H, Ala2, Gly), 3.59 (m, 1H, Et), 3.42 (m, 1H, Et), 3.03 (d, J = 7.5 Hz, 2H, Trp), 2.72 (d, J = 11.9 Hz, 1H, Et), 1.15 (d, J = 7.33 Hz, 3H, Ala2), 1.09 (d, J = 6.37 Hz, 3H, Ala1); HRMS (FAB+, m/z) exact mass of $[M + H]^+$. Anal. Calcd for $C_{28}H_{32}N_8O_7$·1.0HCl·1.0H_2O: C, 51.97; H, 5.45; N, 17.32. Found: C, 51.92; H, 5.64; N, 17.09.

(6′*S*,9′*S*,12′*S*)-1,2-(4-Carbamoylmethyl-12-(4-aminobutyl)-6-methyl-9,10-propano-5,8,11,14-tetraoxotetradecano)-4-nitrobenzene (6e): yield 57.8 mg (0.088 mmol, 9.4%); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.14 (b, 1 H, Et), 8.95 (d, J = 7.0 Hz, 1H, Lys), 8.78 (d, J = 2.5 Hz, 1H, Ar), 8.14−8.10 (m, 1H, Ar), 8.00 (b, 3 H, Lys), 7.68 (s, 1H, Gly), 7.28 (s, 1H, Gly), 7.23 (d, J = 6.1 Hz, 1H, Ala), 6.84 (d, J = 9.5 Hz, 1H, Ar), 4.67 (d, J = 6.4 Hz, 1H, Ala), 4.58 (m, 1H, Lys), 4.47 (d, J = 7.8 Hz, 1H, Pro), 4.43−4.35 (m, 2H, Et), 4.25−4.20 (b, Gly, Et), 3.99 (d, J = 17.7 Hz, 1H, Gly), 3.54 (m, 2H, Pro, Et), 3.49−3.30 (m, 2H, Pro, Et), 2.87 (d, J = 9.2 Hz, 1H, Et), 2.77 (b, 3H, Lys), 2.21 (m, 1H, Pro), 2.09 (m, 1H, Pro), 1.89 (m, 1H, Pro), 1.84−1.43 (3 m, 8H, Lys, Pro), 1.28 (m, 2H, Lys), 1.18 (d, J = 6.6 Hz, 3H, Ala); HRMS (FAB+, m/z) exact mass of $[M + H]^+$ calcd for $C_{25}H_{36}N_8O_7$ 561.2785, found 561.2805. Anal. Calcd for $C_{25}H_{36}N_8O_7$·2.0HCl·3.0H_2O: C, 43.67; H, 6.45; N, 16.30 Found: C, 43.55; H, 6.47; N, 15.95.

(6′*S*,9′*S*,12′*S*)-1,2-(4-Carbamoylmethyl-9-hydroxymethyl-12-methyl-6-(2-methylpropyl)-5,8,11,14-tetraoxo-1,4,7,10,-13-pentaazatetradecano)-4-nitrobenzene (6f). After cyclization, the resin was treated with a solution of tetrabutylammonium fluoride (TBAF, 5 equiv, 4.70 mmol, 4.7 mL of a 1 M solution) in tetrahydrofuran (15 mL) for 2.5 h and subsequently washed: yield 32.6 mg (0.052 mmol, 5.5%); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.90 (d, J = 5.2 Hz, 1H), 8.86 (b, 1H, Et), 8.72 (d, J = 2.5 Hz, 1H, Ar), 8.13 (dd, J = 9.4 Hz, 2.5 Hz, 1H, Ar), 7.76 (m, 2H, Leu, Ser), 7.62 (s, 1H, Gly), 7.21 (s, 1H, Gly), 6.88 (d, J = 9.5 Hz, 1H, Ar), 4.55−4.18 (b, 3H, Leu, Et, Ser, water), 4.10 (d, J = 17.8 Hz, Gly), 3.98 (m, 1H, Ala), 3.85 (d, J = 17.8 Hz, Gly), 3.57−3.43 (m, 4H, Ser, Et), 2.67 (dd, J = 10.4 Hz, 3.7 Hz, 1H, Et), 1.41 (2 m, 5H, Ala, Leu), 1.27 (m, 1H, Leu), 0.79 (d, J = 5.8 Hz, 6H, Leu); HRMS (FAB+, m/z) exact mass of $[M + H]^+$ calcd for $C_{25}H_{37}N_8O_7$ 536.2469, found 536.2475. Anal. Calcd for $C_{23}H_{34}N_7O_8$·1.0HCl·3.5H_2O: C, 43.50; H, 6.51; N, 15.44. Found: C, 43.50; H, 6.33; N, 15.21.

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Supporting Information Available: ^{1}H NMR spectra (1D, COSY, TOCSY, and ROESY/NOESY) of compounds **6a**−**f**. This material is available free of charge via the Internet at http://pubs.acs.org

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