Unusual Peptide Bond Cleavage Reactions during Acidolytic Deprotection Reactions

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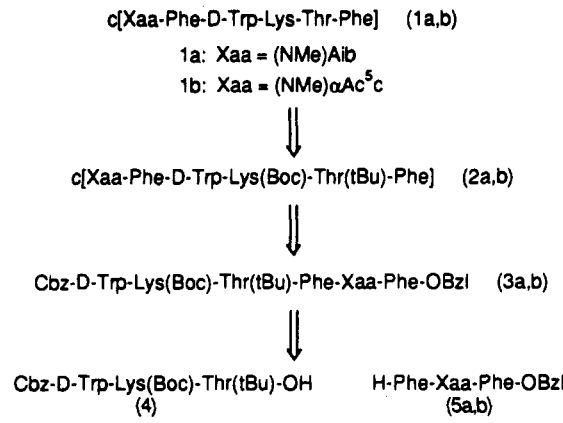
Our studies of structurally constrained peptides have prompted us to design and synthesize compounds which contain the sterically hindered residues *N*-methyl-2-aminoisobutyric acid and *N*-methyl-1-aminocyclopentanecarboxylic acid ((NMe)Aib and (NMe)αAc^{5}c, respectively). The challenges of incorporating such α-amino acid derivatives into peptide compounds were addressed in recent studies.[1] Syntheses of the hexapeptides c[(NMe)Aib-Phe-D-Trp-Lys-Thr-Phe] (**1a**) and c[(NMe)αAc^{5}c-Phe-D-Trp-Lys-Thr-Phe] (**1b**) were planned via a direct application of these findings. During the independent protocols in the synthesis of **1a** and **1b** we observed products of unexpected amide bond cleavage reactions which have not been reported before in similar procedures. Information from the characterization and analysis of the structures of both the desired and unexpected products may provide insight into the effect of highly sterically hindered substituents on peptide backbone stability.

Target hexapeptides **1a,b** were to be obtained (Scheme I) by acidolytic deprotection of the protected derivatives c[(NMe)Aib-Phe-D-Trp-Lys(Boc)-Thr(tBu)-Phe] (**2a**) and c[(NMe)αAc^{5}c-Phe-D-Trp-Lys(Boc)-Thr(tBu)-Phe] (**2b**). Compounds **2a,b** were isolated after two steps including cyclization between the residues Phe and D-Trp. Fully protected linear hexapeptides **3a,b** were obtained from a fragment condensation reaction between Cbz-D-Trp-Lys(Boc)-Thr(tBu)-OH (**4**) and the tripeptide H-Phe-Xaa-Phe-OBzl (**5a,b** where Xaa = (NMe)Aib and (NMe)αAc^{5}c, respectively). Syntheses of the N^{α}-Boc protected derivatives of compounds **5a,b** have been described in detail.[1a]

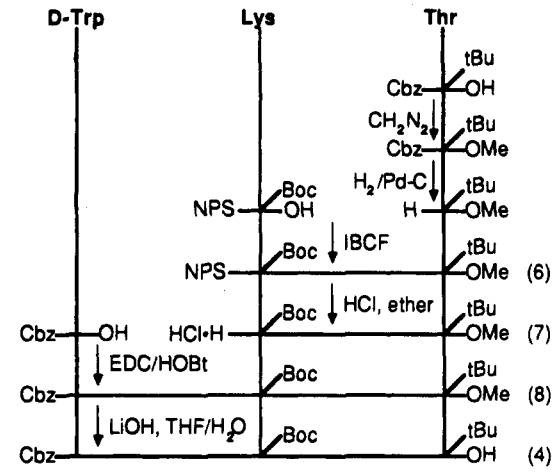
The tripeptide **4** (Scheme II) is a common fragment in both families of peptides and was synthesized in a stepwise manner. Precursor Nps-Lys(Boc)-Thr(tBu)-OMe (**6**) was isolated from a mixed anhydride reaction between Nps-Lys(Boc)-OH and H-Thr(tBu)-OMe using isobutyl chloroformate. The Nps protection was selectively removed in the presence of the *tert*-butyl-based side chain protections with 1 equiv of HCl in Et_2O at 0 °C to give the hydrochloride **7**. By subsequent coupling reaction with Cbz-D-Trp-OH using EDC/HOBt, we obtained the tripeptide **8**. Saponification of the methyl ester with LiOH in THF/H_2O gave the carboxylic acid Cbz-D-Trp-Lys(Boc)-Thr(tBu)-OH (**4**) in good yield (89%). The ^{1}H- and ^{13}C-NMR spectra of carboxylic acid **4** strongly indicate the absence of epimerization under the basic conditions of reaction.

Fragment condensation reactions (Scheme III) were carried out between Cbz-D-Trp-Lys(Boc)-Thr(tBu)-OH (**4**) and H-Phe-Xaa-Phe-OBzl (**5a,b**) using EDC/HOOBt (3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one).[2] Catalytic hydrogenolysis of the linear hexapeptides provided for the removal of benzyloxycarbonyl and benzyl ester protections simultaneously, giving H-D-Trp-Lys(Boc)-Thr(tBu)-Phe-Xaa-Phe-OH (**9a,b**). After cyclization with diphenyl phosphorazidate[3] at 4–6 °C, the cyclic products **2a** and **2b** were obtained.

Scheme I



Scheme II



Simultaneous acidolysis of *tert*-butyl ether and N^{ϵ}-Boc protections from compounds **2a,b** was carried out with TFA in the presence of scavengers. While the deprotected compounds c[Xaa-Phe-D-Trp-Lys-Thr-Phe] (**1a,b**) were expected, linear peptide compounds were isolated from RP HPLC separation of the reaction mixtures. Results of FAB-MS and 2D ROESY ^{1}H-NMR experiments identified these compounds as H-Phe-D-Trp-Lys-Thr-Phe-Xaa-OH (**10a,b**). Sequencing analysis confirmed that both compounds **10a** and **10b** contained a free N-terminal Phe, thus revealing an unanticipated cleavage of the Xaa-Phe amide bond. The extent of these reactions was measured by HPLC integration in each case. Deprotection of the cyclic hexapeptide containing the (NMe)Aib residue (**2a**) resulted in a proportion of linear to cyclic compounds of 42:58 (i.e., peptides **10a**:**1a**). However, after deprotection of cyclic hexapeptide **2b** (containing the (NMe)αAc^{5}c

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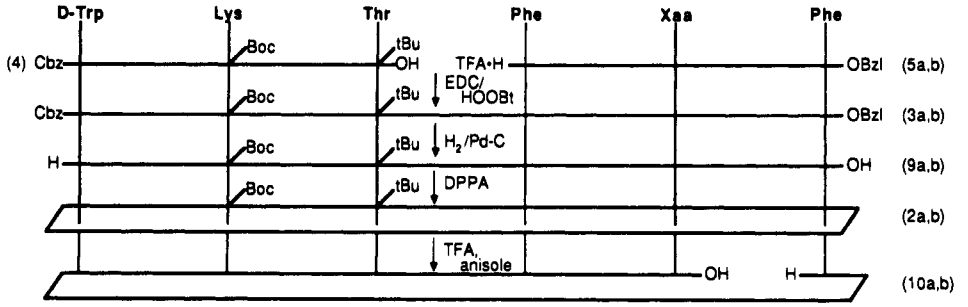
(1) (a) Spencer, J. R.; Antonenko, V. V.; Delaet, N. G. J.; Goodman, M. *Int. J. Pept. Protein Res.* **1992**, *40*, 282–293. (b) Moretto, V.; Valle, G.; Crisma, M.; Bonora, G. M.; Toniolo, C. *Int. J. Biol. Macromol.* **1992**, *14*, 178–184.

(2) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 2034–2040.

(3) Brady, S. F.; Paleveda, W. J.; Arison, B. H.; Freidinger, R.; Nutt, R. F.; Veber, D. F. In Peptides, Structure and Function. *Proceedings of the Eighth American Peptide Symposium*; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co.: Rockford, IL, 1983; pp 127–130.

0022-3263/93/1958-1635$04.00/0

Scheme III



residue) under these conditions, only the linear compound **10b** was isolated.

To our knowledge there are no reports of amide bonds within cyclic homodetic peptides that cleave under deprotection conditions using TFA.[4,5] The presence of water was not rigorously excluded during this procedure because it is critical for such cleavages. Interestingly, in related compounds containing Aib or α-Ac^{5}c at the same position as their *N*-methylated derivatives, amide bond scission during procedures which used TFA was not observed. Thus, the *N*-alkylation, not discernably affecting the Phe-Xaa amide bond, may actually have the effect of destabilizing the neighboring Xaa-Phe amide bond.

We suggest that the Xaa-Phe amide bond which is contained within the cyclic, deprotected peptide is subjected to a special strain. Support for this suggestion rests in the observation of scission at low pH of this particular bond. The 1D ^{1}H-NMR spectrum of cyclic compound **1a** reveals the Xaa-Phe NH resonance as a broadened singlet while all other NH resonances are sharp doublets. Such broadening of resonance is witnessed among amide protons which show rapid exchange through acid or base catalysis.[6] In addition, slight perturbations (e.g., diminution) in the amide proton vicinal coupling constant, J_{HN_α}, may be indicative of nonplanar bond distortion.[7] Nonplanarity of the amide bond is both energetically allowable in unstrained amide bond models[8] and well documented by NMR techniques in strained cyclic peptide systems.[9] In short, we believe the observed resonance broadening is related to a diminished amide character of the Xaa-Phe linkage.[10] This property, not observed in amides in less constrained environments, is certainly linked to the unusual susceptibility toward the facile cleavage reactions reported in this manuscript.

Experimental Section

General Information. Analytical data were obtained as described previously.[1a] The N-terminal amino acid analyses were obtained from The Scripps Research Institute, La Jolla, CA. RP HPLC was carried out on a Vydac C_{18} semipreparative column (1.0 × 25 cm) with detection at 215 nm. All materials were reagent grade and were used without further purification, with the following exceptions: HOBt·H_2O was dissolved and reevaporated from ethanol and toluene successively and then dried; EDC·HCl was dried under reduced pressure at 50 °C over P_2O_5; DMF and THF were anhydrous as purchased from Aldrich. For ^{1}H-NMR assignments the phenylalanines are referred to by the following numbering: c[Xaa-*Phe*^{1}-D-Trp-Lys-Thr-*Phe*^{2}].

H-D-Trp-Lys(Boc)-Thr(tBu)-Phe-(NMe)Aib-Phe-OH (9a). To a solution of hexapeptide **3a** (0.38 mmol, 0.46 g) in 6.0 mL of 20% AcOH in MeOH was added 10% Pd/C (71 mg). H_2 was introduced at atmospheric pressure at 20 °C and the mixture allowed to react for 2 h. The catalyst was removed by filtration and the solvent removed under reduced pressure. Purification was carried out by silica gel chromatography ($CHCl_3$/MeOH/AcOH = 88/12/1). Deprotected **9a** was obtained as a white solid (0.33 g, 88%) after crystallization in water: mp 166–170 °C; $[\alpha]^{25}_D$ −1.4° (*c* 0.35, EtOH); R_f 0.48 ($CHCl_3$/MeOH/AcOH = 85/15/3); ^{1}H-NMR δ_H (360 MHz, DMSO-d_6) 10.92 (s, 1 H, indole NH), 8.95 (br s, 1 H, NH), 7.90 (m, 2 H, two NH), 7.60 (d, 1 H, J = 11 Hz, Trp arom), 7.35 (d, 1 H, J = 11 Hz, Trp arom), 7.30–7.05 (m, 13 H, Trp, Phe^1, Phe^2 arom and NH), 7.00 (t, 1 H, J = 11 Hz, Trp arom), 6.82 (br s, 1 H, Lys NH$^\epsilon$), 4.87 (m, 1 H, CH$^\alpha$), 4.27 (m, 1 H, CH$^\alpha$), 4.18 (m, 2 H, two CH$^\alpha$), 3.89 (m, 1 H, CH$^\alpha$), 3.75 (m, 1 H, Thr CH$^\beta$), 3.20–2.75 (m, 11 H, three CH_2^β, NCH_3 and Lys CH_2^ϵ), 1.53 (m, 2 H, Lys CH_2^β), 1.40–1.07 (m, 4 H, two Lys CH_2), 1.36 (s, 9 H, Boc CH_3), 1.21, 1.17 (s, s, 6 H, Aib CH_3^β and $CH_3^{\beta'}$), 1.12 (s, 9 H, tBu CH_3), 1.00 (d, 3 H, J = 9 Hz, Thr CH_3^γ); IR ν_{max} (KBr) 3337, 3030, 2977, 1654, 1522, 1251, 1173, 1081 cm^{-1}; FAB-

(4) Other papers which describe unusual amide bond scissions involve the role of intramolecular catalysis: (a) Anteunis, M. J. O.; Van der Auwera, C. *Int. J. Pept. Protein Res.* **1988**, *31*, 301–310. (b) Geiger, T.; Clarke, S. *J. Biol. Chem.* **1987**, *262*, 785–794. (c) Kluger, R.; Hunt, J. C. *J. Am. Chem. Soc.* **1989**, *111*, 5921–5925.

(5) Efficient acidolytic deprotections using TFA and the ethanedithiol/anisole system (Sakakibara, S. In Peptides. *Proceedings of the Fifth American Peptide Symposium*; Goodman, M., Meienhofer, J., Eds.; John Wiley and Sons: New York, 1977; pp 436–447. Pallai, P.; Struthers, S.; Goodman, M. *Biopolymers* **1983**, *22*, 2523–2538) have been carried out in our laboratories on related cyclic hexapeptides. A typical procedure is analogous to the one presented in this manuscript for the deprotection of compound **2a**. On the basis of our analyses of cyclic peptide **1a** (unpublished), we feel that other reagents and/or scavengers would provide similar results. Indole is also preferred as a scavenger during acidolytic reactions of peptides containing tryptophan. (Stewart, J. M.; Young, J. D. In *Solid-Phase Peptide Synthesis*, 2nd ed.; Pierce Chemical Co.: Rockford, IL, 1984; p 77). For the deprotection of compound **2b**, we obtained only the linear peptide **10b**. This result appears to be related to the additional steric bulk of the (NMe)αAc^{5}c residue.

(6) Berger, A.; Loewenstein, A.; Meiboom, S. *J. Am. Chem. Soc.* **1959**, *81*, 62–67. Perrin, C. L. *Acc. Chem. Res.* **1989**, *22*, 268–275 and references cited therein.

(7) Wüthrich, K. *NMR in Biological Research: Peptides and Proteins*; North-Holland: Amsterdam, 1976; pp 74–80.

(8) Winkler, F. K.; Dunitz, J. D. *J. Mol. Biol.* **1971**, *59*, 169–182. Ramachandran, G. N.; Lakshminarayanan, A. V.; Kolaskar, A. S. *Biochim. Biophys. Acta* **1973**, *303*, 8–13.

(9) Grathwohl, C.; Tun-kyi, A.; Bundi, A.; Schwyzer, R.; Wüthrich, K. *Helv. Chim. Acta* **1975**, *58*, 415–423. Wüthrich, K.; Grathwohl, C.; Schwyzer, R. In *Peptides, Polypeptides and Proteins*; Blout, E. R., Bovey, F. A., Goodman, M., Lotan, N., Eds.; John Wiley and Sons: New York, 1974; pp 300–307.

(10) It appears that the amide bond in this environment, aside from the other five in the same molecule, responds uniquely to a combination of effects from macrocyclization and adjacent residue properties of steric bulk and/or electronics. We are currently carrying out a physicochemical and computational based investigation of these and other peptides to assess the relative impact of each of these factors on the observed hydrolytic cleavage reactions. We are comparing information from our ongoing study to that presented in recent reports on the characteristics of several resonance impaired amides in nonpeptide compounds: Bennet, A. J.; Somayaji, V.; Brown, R. S.; Santarsiero, B. D. *J. Am. Chem. Soc.* **1991**, *113*, 7563–7571. Greenberg, A. In *Structure and Reactivity (Molecular Structure and Energetics*, Vol. 7); Liebman, J. F., Greenberg, A., Eds.; VCH: New York, 1988; pp 139–178. For additional information on nonenzymatic hydrolyses of both planar and distorted amide containing compounds, see: (a) Brown, R. S.; Bennet, A. J.; Ślebocka-Tilk, H. *Acc. Chem. Res.* **1992**, *25*, 481–488 and references cited therein. (b) Bennet, A. J.; Wang, Q.-P.; Ślebocka-Tilk, H.; Somayaji, V.; Brown, R. S. *J. Am. Chem. Soc.* **1990**, *112*, 6383–6385. (c) Keillor, J. W.; Brown, R. S. *J. Am. Chem. Soc.* **1992**, *114*, 7983–7989.

MS m/z 983 $(M + H)^+$; HR FAB-MS calcd for $C_{53}H_{74}N_8O_{10}K$ $(M + K)^+$ 1021.5165, found 1021.5206.

c[(NMe)Aib-Phe-D-Trp-Lys(Boc)-Thr(tBu)-Phe] (2a). A solution of compound **9a** (0.25 mmol, 250 mg) in 95 mL of dry DMF was chilled to −10 °C. To this solution were added diphenyl phosphorazidate (0.36 mmol, 0.10 g) dissolved in DMF (5 mL) and K_2HPO_4 (1.3 mmol, 0.23 g). After 20 min at −10 °C, the reaction was allowed to stir at 4–6 °C for 2 d. The mixture was treated with an ion-exchange resin (2.5 g of Biorad AG 501-X8 mixed bed, 20–50 mesh) for 3 h at 4–6 °C. Solid materials were removed by filtration, and the solvent was removed under reduced pressure. Purification was carried out by silica gel chromatography ($CHCl_3$/MeOH/AcOEt/AcOH = 90/5/5/1), and cyclic peptide **2a** was obtained as a pale yellow powder (150 mg, 61%) after crystallization in water: mp 144–147 °C; $[\alpha]^{25}_D$ +46.9° (c 0.35, $CHCl_3$); R_f 0.24 ($CHCl_3$/MeOH/AcOH = 95/5/3); ^{1}H-NMR δ_H (360 MHz, DMSO-d_6) 10.80 (s, 1 H, indole NH), 8.37 (d, 1 H, J = 11 Hz, NH), 8.15 (d, 1 H, J = 11 Hz, NH), 7.58–6.93 (m, 18 H, Trp, Phe^1, and Phe^2 arom and three NH), 6.75 (t, 1 H, J = 11 Hz, Lys NH$^\epsilon$), 4.67 (m, 1 H, CH$^\alpha$), 4.49 (m, 1 H, CH$^\alpha$), 4.27 (m, 2 H, two CH$^\alpha$), 4.00 (m, 1 H, CH$^\alpha$), 3.85 (m, 1 H, Thr CH$^\beta$), 3.15–2.72 (m, 11 H, three CH$_2^\beta$, NCH$_3$ and Lys CH$_2^\epsilon$), 1.60 (m, 2 H, Lys CH$_2^\beta$), 1.37 (s, 9 H, Boc CH$_3$), 1.25 (m, 2 H, Lys CH$_2$), 1.20, 1.13 (s, s, 6 H, Aib CH$_3^\beta$ and CH$_3^{\beta'}$), 1.03 (s, 12 H, tBu CH$_3$ and Thr CH$_3^\gamma$), 0.92 (m, 2 H, Lys CH$_2$); IR ν_{max} (KBr) 3324, 3030, 2977, 2933, 1655, 1511, 1366, 1249, 1173, 1081 cm^{-1}; FAB-MS m/z 965 $(M + H)^+$; HR FAB-MS calcd for $C_{53}H_{73}N_8O_9$ $(M + H)^+$ 965.5501, found 965.5557.

Deprotection of c[(NMe)Aib-Phe-D-Trp-Lys(Boc)-Thr(tBu)-Phe] (2a). To a solution of cyclic peptide **2a** (0.027 mmol, 26 mg) in CH_2Cl_2 (4.5 mL) were added anisole (10% v/v, 0.9 mL) and ethanedithiol (5% v/v, 0.45 mL). The mixture was chilled (0 °C) and treated with TFA (4.5 mL) for 1 h at 0 °C and 2 h at 20 °C. The solvent was removed under reduced pressure (using a bleach trap to oxidize the ethanedithiol), and toluene (3 × 10 mL) was added and removed under reduced pressure. The residue was crystallized in cold (0 °C) ether, isolated by filtration, and purified by RP HPLC. Two compounds were obtained after successive chromatographies employing gradient elutions of aqueous acetonitrile containing 0.1% TFA. Initial separation was carried out with a 25 min elution of 32 → 42% MeCN/H_2O (0.1% TFA). Linear compound **10a** was repurified by a 20-min elution of 28 → 38% MeCN/H_2O (0.1% TFA) and cyclic compound **1a** by a 20-min elution of 30 → 60% MeCN/H_2O (0.1% TFA).

H-Phe-D-Trp-Lys-Thr-Phe-(NMe)Aib-OH (**10a**). Obtained as a white powder (10.2 mg, 36%) after lyophilization: mp 158–163 °C; R_f 0.24 ($CHCl_3$/MeOH/NH_4OH = 75/25/5); HPLC t_R 10.7 min (20 → 80% MeCN/H_2O, 0.1% TFA over 20 min); ^{1}H-NMR δ_H (500 MHz, DMSO-d_6) 11.93 (br s, 1 H, COOH), 10.82 (s, 1 H, indole NH), 8.83 (d, 1 H, J = 8.5 Hz, Trp NH), 8.50 (d, 1 H, J = 8.0 Hz, Lys NH), 8.04 (d, 1 H, J = 8.0 Hz, Phe^2 NH), 7.93 (br s, 3 H, Phe^1 NH), 7.87 (d, 1 H, J = 8.5 Hz, Thr NH), 7.73 (d, 1 H, J = 8.0 Hz, Trp arom), 7.69 (br s, 3 H, Lys NH$_3^\epsilon$), 7.30–6.97 (m, 12 H, Trp, Phe^1 and Phe^2 arom), 6.87 (d, 2 H, J = 7.5 Hz, Phe^1 arom), 4.85 (m, 3 H, Phe^2 CH$^\alpha$, Trp CH$^\alpha$, and Thr OH), 4.32 (m, 1 H, Lys CH$^\alpha$), 4.15 (m, 1 H, Thr CH$^\alpha$), 4.01 (br s, 1 H, Phe^1 CH$^\alpha$), 3.85 (m, 1 H, Thr CH$^\beta$), 3.03, 2.85 (m, m, 2 H, Trp CH$^\beta$ and CH$^{\beta'}$), 2.92, 2.75 (m, m, 2 H, Phe^2 CH$^\beta$ and CH$^{\beta'}$), 2.82 (s, 3 H, NCH$_3$), 2.74, 2.55 (m, m, 2 H, Phe^1 CH$^\beta$ and CH$^{\beta'}$), 2.67 (br s, 2 H, Lys CH$_2^\epsilon$), 1.56, 1.42 (m, m, 2 H, Lys CH$^\beta$ and CH$^{\beta'}$), 1.45 (m, 2 H, Lys CH$_2^\delta$), 1.25 (s, 6 H, Aib CH$_3^\beta$ and CH$_3^{\beta'}$), 1.13 (m, 2 H, Lys CH$_2^\gamma$), 0.96 (d, 3 H, J = 6.0 Hz, Thr CH$_3^\gamma$); FAB-MS m/z 827 $(M + H)^+$; HR FAB-MS calcd for $C_{44}H_{59}N_8O_8$ $(M + H)^+$ 827.4456, found 827.4441. N-terminal amino acid analysis: Phe.

c[(NMe)Aib-Phe-D-Trp-Lys-Thr-Phe] (**1a**). Obtained as a white powder (9.2 mg, 37%) after lyophilization: mp 135–139 °C; R_f 0.52 ($CHCl_3$/MeOH/NH_4OH = 75/25/5); HPLC t_R 13.3 min (20 → 80% MeCN/H_2O, 0.1% TFA over 20 min); ^{1}H-NMR δ_H (500 MHz, DMSO-d_6) 10.79 (s, 1 H, indole NH), 8.80 (d, 1 H, J = 8.4 Hz, Trp NH), 8.48 (d, 1 H, J = 8.0 Hz, Lys NH), 8.11 (d, 1 H, J = 8.2 Hz, Phe^2 NH), 7.91 (br s, 1 H, Phe^1 NH), 7.81 (d, 1 H, J = 8.2 Hz, Thr NH), 7.70 (d, 1 H, J = 8.0 Hz, Trp arom), 7.66 (br s, 3 H, Lys NH$_3^\epsilon$), 7.27–6.95 (m, 12 H, Trp, Phe^1 and Phe^2 arom), 6.83 (d, 2 H, J = 7.5 Hz, Phe^1 arom), 4.90 (m, 1 H, Phe^2 CH$^\alpha$), 4.79 (m, 1 H, Trp CH$^\alpha$), 4.74 (d, 1 H, J = 4.7 Hz, Thr OH), 4.29 (m, 1 H, Lys CH$^\alpha$), 4.13 (m, 1 H, Thr CH$^\alpha$), 3.98 (br s, 1 H, Phe^1 CH$^\alpha$), 3.80 (m, 1 H, Thr CH$^\beta$), 3.00, 2.82 (m, m, 2 H, Trp CH$^\beta$ and CH$^{\beta'}$), 2.92 (s, 3 H, NCH$_3$), 2.90, 2.74 (m, m, 2 H, Phe^2 CH$^\beta$ and CH$^{\beta'}$), 2.72, 2.51 (m, m, 2 H, Phe^1 CH$^\beta$ and CH$^{\beta'}$), 2.64 (br s, 2 H, Lys CH$_2^\epsilon$), 1.52, 1.38 (m, m, 2 H, Lys CH$^\beta$ and CH$^{\beta'}$), 1.41 (m, 2 H, Lys CH$_2^\delta$), 1.27, 1.25 (s, s, 6 H, Aib CH$_3^\beta$ and CH$_3^{\beta'}$), 1.10 (m, 2 H, Lys CH$_2^\gamma$), 0.94 (d, 3 H, J = 6.2 Hz, Thr CH$_3^\gamma$); FAB-MS m/z 809 $(M + H)^+$; HR FAB-MS calcd for $C_{44}H_{57}N_8O_7$ $(M + H)^+$ 809.4350, found 809.4346.

c[(NMe)αAc^{5}c-Phe-D-Trp-Lys(Boc)-Thr(tBu)-Phe] (2b). To a solution of hexapeptide **3b** (0.16 mmol, 201 mg) in 20 mL of 5% AcOH in MeOH was added 10% Pd/C (20 mg). H_2 was introduced at atmospheric pressure at 20 °C and the mixture allowed to react for 2 h. The catalyst was removed by filtration and the solvent removed under reduced pressure. Purification was carried out by silica gel chromatography ($CHCl_3$/MeOH/AcOH = 90/10/1, TLC R_f 0.50) and deprotected **9b** was obtained as a solid (67 mg, 39%) and was used directly.

A solution of compound **9b** (0.063 mmol, 67 mg) in dry DMF (42 mL) was chilled to −10 °C. To this solution were added diphenyl phosphorazidate (0.097 mmol, 21 μL) and K_2HPO_4 (0.32 mmol, 55 mg). After 15 min at −10 °C, the reaction was allowed to stir at 4–6 °C for 3 d. The solvent was removed under reduced pressure, and the resulting residue was suspended in AcOEt (250 mL) which was washed with saturated NaCl (3 × 30 mL) and dried ($MgSO_4$). Purification was carried out by silica gel chromatographies (5% MeOH/$CHCl_3$ and 2.5% MeOH/$CHCl_3$), and cyclic peptide **2b** was obtained as a pale yellow solid (42 mg, 68%) after crystallization in water: mp 128–132 °C; $[\alpha]^{25}_D$ +28.3° (c 0.35, $CHCl_3$); R_f 0.44 ($CHCl_3$/MeOH/AcOH = 95/5/3); ^{1}H-NMR δ_H (360 MHz, DMSO-d_6) 10.87 (s, 1 H, indole NH), 8.60 (br s, 1 H, NH), 8.39 (br s, 1 H, NH), 7.70 (d, 1 H, J = 11 Hz, Trp arom), 7.40–6.97 (m, 12 H, Trp, Phe^1, Phe^2 arom and three NH), 6.87 (m, 2 H, arom), 6.75 (br s, 1 H, Lys NH$^\epsilon$), 6.59 (m, 2 H, arom), 6.35 (m, 1 H, arom), 4.86 (m, 1 H, CH$^\alpha$), 4.57 (m, 1 H, CH$^\alpha$), 4.28 (m, 1 H, CH$^\alpha$), 4.10 (m, 2 H, two CH$^\alpha$), 3.89 (m, 1 H, Thr CH$^\beta$), 3.07–2.57 (m, 11 H, three CH$_2^\beta$, NCH$_3$, and Lys CH$_2^\epsilon$), 1.93, 1.62 (m, 2 H, Lys CH$_2^\beta$), 1.55–0.80 (m, 12 H, two Lys CH$_2$ and four Ac^{5}c CH$_2$), 1.36 (s, 9 H, Boc CH$_3$), 1.05 (s, 9 H, tBu CH$_3$), 0.90 (d, 3 H, J = 11 Hz, Thr CH$_3^\gamma$); IR ν_{max} (KBr) 3328, 3030, 2975, 1654, 1508, 1250, 1171, 1080 cm^{-1}; FAB-MS m/z 992 $(M + H)^+$; HR FAB-MS calcd for $C_{55}H_{75}N_8O_9$ $(M + H)^+$ 991.5657, found 991.5688.

Deprotection of c[(NMe)αAc^{5}c-Phe-D-Trp-Lys(Boc)-Thr(tBu)-Phe] (2b). Cyclic peptide **2b** (0.013 mmol, 12.8 mg) was added to a chilled (0 °C) mixture of TFA (5 mL), anisole (10% v/v, 0.5 mL), and indole (0.085 mmol, 10 mg). Stirring was continued for 1 h at 0 °C and 3 h at 20 °C. The solvent was removed under reduced pressure, and toluene (2 × 10 mL) was added and removed under reduced pressure. The residue was crystallized in cold ether, isolated, and purified by RP HPLC (a 20-min gradient elution of 36 → 40% MeCN/H_2O, 0.1% TFA).

H-Phe-D-Trp-Lys-Thr-Phe-(NMe)αAc^{5}c-OH (**10b**). Obtained as a white powder (4.3 mg, 31%) after lyophilization: mp 135–137 °C dec; R_f 0.38 ($CHCl_3$/MeOH/NH_4OH = 75/25/5); HPLC t_R 13.9 min (36 → 40% MeCN/H_2O, 0.1% TFA over 20 min); ^{1}H-NMR δ_H (500 MHz, DMSO-d_6) 11.80 (br s, 1 H, COOH), 10.81 (s, 1 H, indole NH), 8.82 (d, 1 H, J = 8.2 Hz, Trp NH), 8.50 (d, 1 H, J = 8.0 Hz, Lys NH), 8.02 (d, 1 H, J = 8.0 Hz, Phe^2 NH), 7.94 (br s, 3 H, Phe^1 NH), 7.87 (d, 1 H, J = 8.6 Hz, Thr NH), 7.71 (m, 4 H, Lys NH$_3^\epsilon$ and Trp arom), 7.33–6.97 (m, 12 H, Trp, Phe^1 and Phe^2 arom), 6.89 (d, 2 H, J = 7.0 Hz, Phe^1 arom), 4.93 (m, 1 H, Phe^2 CH$^\alpha$), 4.81 (m, 2 H, Trp CH$^\alpha$ and Thr OH), 4.32 (m, 1 H, Lys CH$^\alpha$), 4.16 (m, 1 H, Thr CH$^\alpha$), 4.02 (br s, 1 H, Phe^1 CH$^\alpha$), 3.85 (br s, 1 H, Thr CH$^\beta$), 3.04, 2.86 (m, m, 2 H, Trp CH$^\beta$ and CH$^{\beta'}$), 2.94, 2.78 (m, m, 2 H, Phe^2 CH$^\beta$ and CH$^{\beta'}$), 2.94 (s, 3 H, NCH$_3$), 2.78, 2.57 (m, m, 2 H, Phe^1 CH$^\beta$ and CH$^{\beta'}$), 2.66 (m, 2 H, Lys CH$_2^\epsilon$), 2.17, 2.08 (m, m, 2 H, Ac^{5}c CH$_2^\beta$), 1.77 (m, 2 H, Ac^{5}c CH$_2^{\beta'}$), 1.58 (m, 6 H, Ac^{5}c CH$_2^\gamma$, CH$_2^{\gamma'}$ and Lys CH$_2^\beta$), 1.45 (m, 2 H, Lys CH$_2^\delta$), 1.14 (m, 2 H, Lys CH$_2^\gamma$), 0.90 (d, 3 H, J = 6.2 Hz, Thr CH$_3^\gamma$); FAB-MS m/z 892 $(M + K)^+$. N-terminal amino acid analysis: Phe.

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Supplementary Material Available: Methods of synthesis and spectroscopy for compounds **3a**, **3b**, **4**, **6–8**, and Cbz-Thr-(tBu)-OMe, 500-MHz ^{1}H-NMR COSY spectra for compounds **1a** and **10a** and HOHAHA spectrum for compound **10b**, and 360-MHz ^{1}H-NMR 1D spectra for compounds **2a**, **2b**, **4**, **6**, **7**, and **9a** (16 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.