Preparation of Supramolecular Devices Using Peptide Synthesis: Design and Synthesis of a Tubular Hexacrown Molecule

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Abstract: The utility of peptide synthesis for the preparation of supramolecular devices is presented. This novel and general methodology uses α-helix peptides as a framework to organize multiple binding sites or reactive centers in a desired spatial orientation. The versatility of the strategy is demonstrated by the synthesis of a tubular hexacrown ether molecule, **1**, designed to mimick the ion-transport properties of channel proteins. The hexacrown peptide **1** is 21 residues long and composed of L-alanine and six 21-crown-7 derivatives of L-phenylalanine. Peptide **1** was synthesized via a segment condensation strategy. The suitably protected segment **4**, was prepared by solid-phase synthesis using the *p*-nitrophenyl oxime resin **3** as support. Circular dichroism studies demonstrated that the hexacrown peptide **1** adopts as predicted an α-helical conformation in trifluoroethanol positioning the ligand side chains on the same side of the helix. The supramolecular arrangement of the crown ligands in **1** is supported by hypsochromic and hypochromic effects observed in its fluorescence emission spectrum.

Supramolecular devices—synthetic molecules bearing multiple binding sites or effectors with a defined three-dimensional geometry—are highly desirable for a wide range of applications such as biosensors, molecular electronics, and energy storage devices as well as for biomimicking natural systems.[2] However, to render these systems functional, a gating mechanism is necessary to control their spatial organization.

In nature, the task of organizing binding sites in a definite geometry is performed particularly by polypeptides. Also, biological systems are often regulated by conformational changes.[3] With those features in mind, we sought to prepare supramolecular devices using peptides as framework. In this article, we demonstrate the utility of peptide synthesis for the preparation of supramolecular devices by describing the synthesis of a tubular shape hexacrown ether peptide[4] **1** designed to mimick ion channel proteins by aligning the crown rings.[5] Solid-phase peptide synthesis[6] has several advantages for the preparation of supramolecular systems. The technique is rapid and versatile, and the recent developments[7] in synthetic methodology and purification techniques make it even more flexible. Also, the possibility of predicting accurately the secondary structure, and thus the orientation of the side chains,[8] from the amino acid sequence[9] adds to the attractiveness of this method. Finally, control over the folding/unfolding equilibrium[10] by thermal or ionic denaturation could eventually be used to regulate the function of peptide-based supramolecular devices.

Results and Discussion

Design. The tubular artificial ion channel **1**, a 21-residue peptide composed of L-alanine and a 21-crown-7 derivative of L-3,4-dihydroxyphenylalanine **2**, was designed by using CPK models and molecular modeling techniques. L-Alanine was chosen for its high intrinsic preference to form helices[9,11] with the expectation that it would induce an α-helix in **1**. Under the latter structure, peptide **1** is calculated to be 3.2 nm long, enough to span a bilayer membrane. Because an α-helix does not have an integral number of amino acids per turn, it is impossible to align perfectly the crown rings. However, spacing the crown ether **2** at positions 2 and 6 of a repeating heptad results in an array of hosts nicely stacked, as illustrated in Figure 1 by the α-helix axial projection and the CPK model of **1**.

Synthesis. The choice of a repeating heptad also minimizes the effort in the synthesis if the desired peptide is to be prepared by segment condensation.[12] Indeed, to avoid the tedious purification steps associated with a stepwise synthesis of a 21-residue peptide, we adopted a strategy involving segment condensation. The basic idea was to prepare first a 7-residue segment suitably protected on both ends. Then, after selective deprotections, trimerize that segment to obtain the desired 21-residue peptide **1**. For the preparation of protected peptide segments, the *p*-nitrophenyl oxime resin[13] **3** is well suited. That resin is stable to trifluoroacetic acid (TFA) and uses amino acids protected with α-*N*-*tert*-butyloxycarbonyl groups (*N*-*t*-BOC). Moreover, the peptides can be cleaved under mild nucleophilic conditions by

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(2) (a) Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 89-112, and references cited therein. (b) Carter, F. L. *Molecular Electronic Devices II*; Marcel Decker: New York, 1987. (c) Carter, F. L. *Molecular Electronic Devices*; Marcel Decker: New York, 1982. (d) Haddon, H. C.; Lamola, A. A. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1874-1878. (e) Murray, R. W. In *Chemical Sensors and Microinstrumentation*; Melchior, D. C., Bassett, R. L., Eds.; ACS Symposium Series 403; American Chemical Society: Washington, DC, 1989; pp 1-19.

(3) Franklin, T. J. In *Design and Synthesis of Organic Molecules Based on Molecular Recognition*; Van Binst, G., Ed.; Springer Verlag: Berlin 1986.

(4) Some examples of crown ether peptides reported in the literature. (a) Poly(15-crown-5-L-DOPA): Berthet, M.; Sonveaux, E. *Biopolymers* **1986**, *25*, 189-204. (b) Crown ether modified poly(L-glutamate): Anzai, J.; Ueno, A.; Osa, T. *Makromol. Chem. Rapid Commun.* **1982**, *3*, 55-58; and small molecular weight peptides. (c) Detellier, C.; Stöver, H. D. H. *Synthesis* **1983**, 990-992. (d) Berthet, M.; Yordanov, S.; Sonveaux, E. *Makromol. Chem. Rapid Commun.* **1986**, *7*, 205-209.

(5) It has been proposed that an array of stacked crown ether rings could mimick ion-transport processes of channel proteins: (a) Behr, J. P.; Lehn, J.-M.; Dock, A. C.; Moras, D. *Nature* **1982**, *295*, 526-527. (b) Ludovic, J.; Lehn, J.-M. *Tetrahedron Lett.* **1988**, *29*, 3803-3806. (c) Kragten, U. F.; Roks, M. F. M.; Nolte, R. J. M. *J. Chem. Soc., Chem. Commun.* **1985**, 1275-1276, and references cited therein.

(6) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149-2154. (b) Barany, G.; Merrifield, R. B. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 2, pp 1-284.

(7) Barany, G.; Kneib-Cordonier, N.; Mullen, D. G. *Int. J. Pept. Protein Res.* **1987**, *30*, 705-739.

(8) McGregor, M. J.; Islam, S. A.; Sternberg, M. J. E. *J. Mol. Biol.* **1987**, *198*, 295-310.

(9) (a) Chou, P. Y.; Fasman, G. D. *Annu. Rev. Biochem.* **1978**, *47*, 251-276; (b) Argos, P.; MohanaRao, J. K. *Methods Enzymol.* **1986**, *130*, 185-209.

(10) (a) Hirs, C. H. W.; Timasheff, S. N. *Methods Enzymol.* **1986**, *131*, 1-280. (b) Schultz, G. E.; Schirmer, R. H. *Principles of Protein Structure*; Springer-Verlag: New York, 1979; pp 149-165. (c) Anfinsen, C. F.; Scheraga, H. A. *Adv. Protein Chem.* **1975**, *29*, 205-300.

(11) Walton, A. G. *Polypeptides and Protein Structure*; Elsevier: New York, 1981; pp 50-68.

(12) Kaiser, E. T. *Acc. Chem. Res.* **1989**, *22*, 47-54, and references cited therein.

(13) (a) DeGrado, W. F.; Kaiser, E. T. *J. Org. Chem.* **1982**, *47*, 3258-3261. (b) DeGrado, W. F.; Kaiser, E. T. *J. Org. Chem.* **1980**, *45*, 1295-1300.

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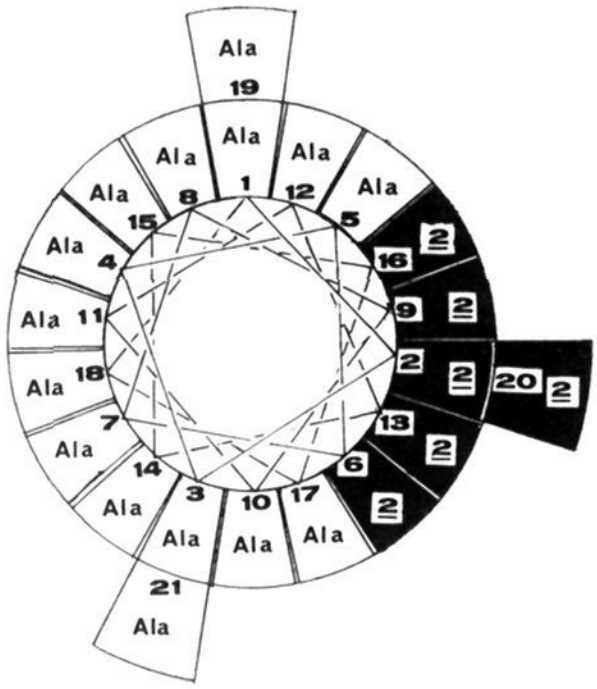

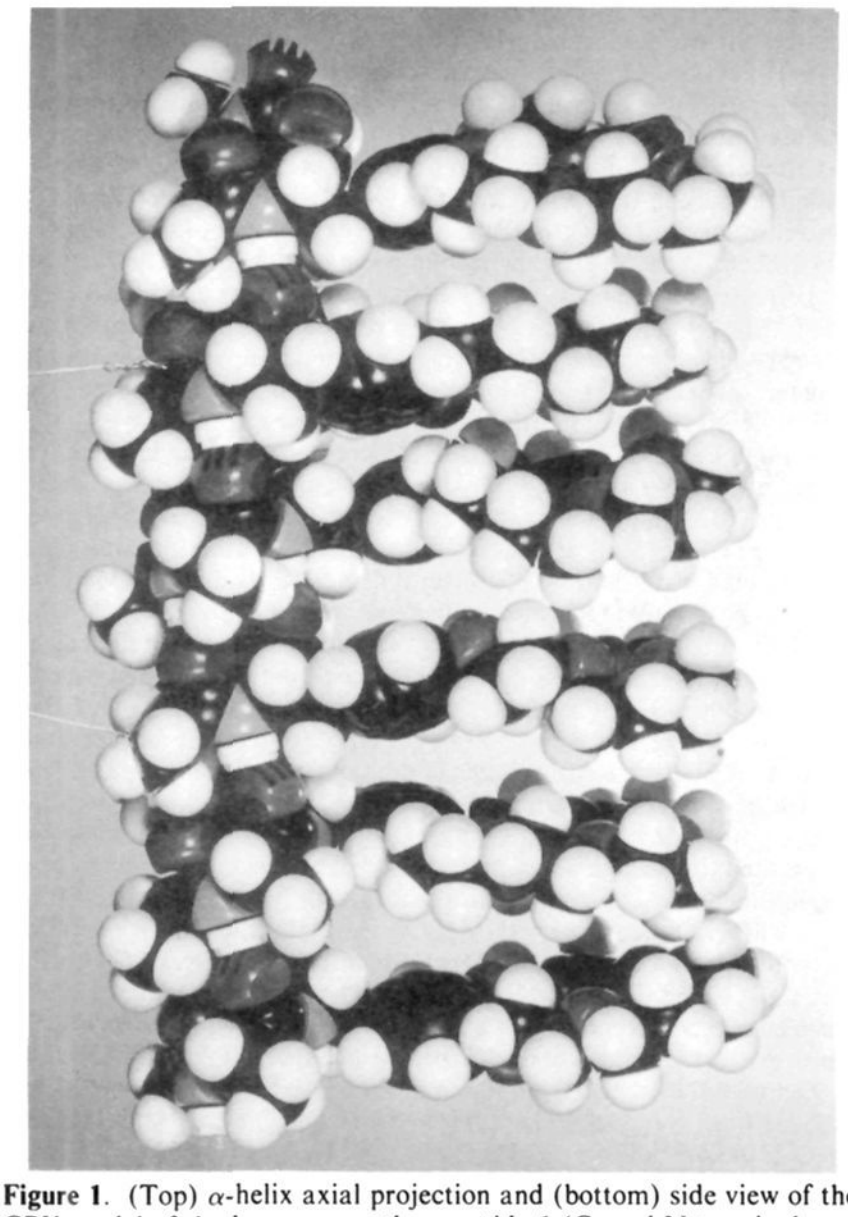

Figure 1. (Top) α-helix axial projection and (bottom) side view of the CPK model of the hexacrown ether peptide **1** (C- and N-terminal protecting groups removed from clarity).

treatment with a free amino acid ester to obtain suitably protected segment.[13a]

Hexacrown peptide **1** was prepared by following the scheme illustrated in Figure 2.[14] The key 7-residue segment **4** was prepared by starting with the *N-t*-BOC amino acids using the oxime resin **3**. The necessary *N-t*-BOC-21-crown-7-L-phenylalanine (**2**) was synthesized from L-DOPA by modifying a described procedure.[4a] After the first 21-crown-7 amino acid was loaded onto the resin by using dicyclohexylcarbodiimide (DCC) as the coupling agent, the *N-t*-BOC group was removed with a 25% solution of TFA in dichloromethane (DCM). The three

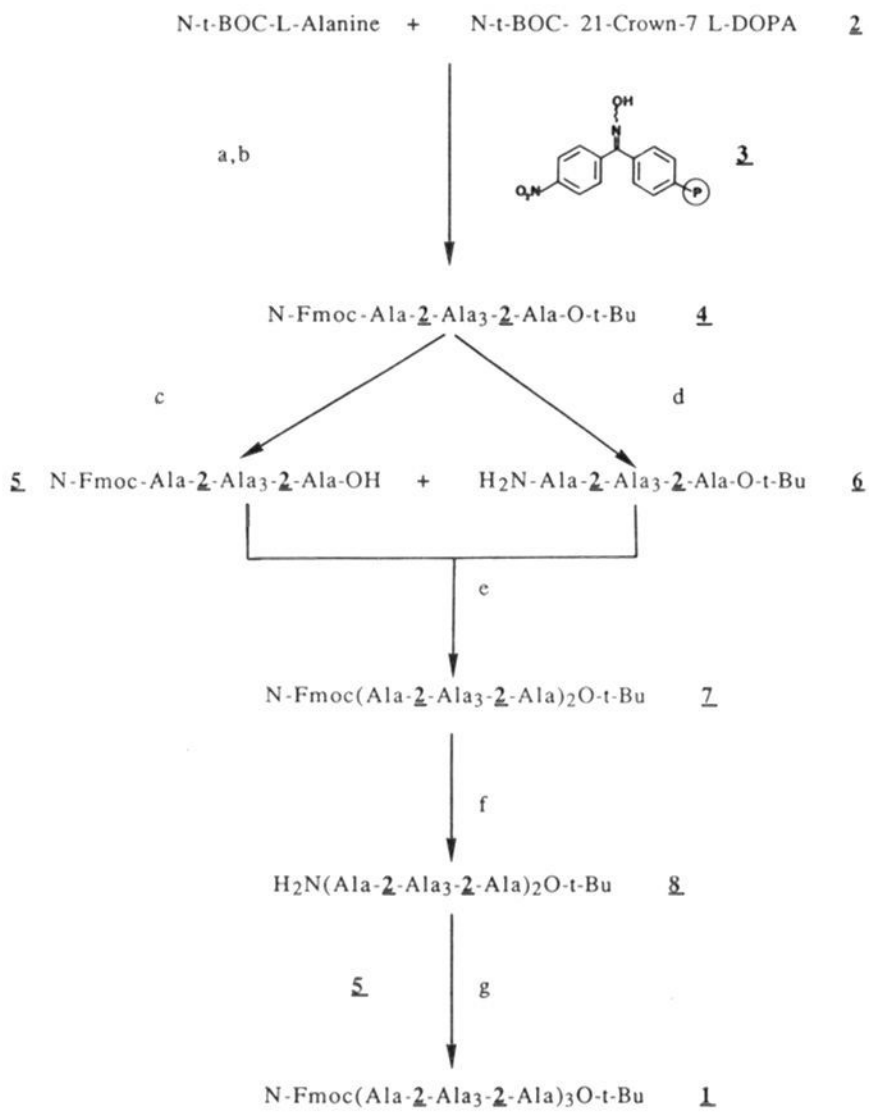

Figure 2. Synthesis of the hexacrown molecule **1**. Reagents and conditions: (a) coupling in DMF, 2 h, via 5 equiv of preformed symmetric anhydride or hydroxybenzotriazole activated esters of *N-t*-BOC amino acids and 1.1 equiv of diisopropylethyl amine, deprotection with 25% TFA/CH_2Cl_2, 1 h (b) Cleavage with AcOH·H_2N-alanine-*tert*-butyl ester, CH_2Cl_2, 24 h (c) TFA/5% *p*-cresol, 30 min (d) 30% diethylamine/CH_2Cl_2–DMF (4/6), 2 h (e) DCC/HOBT in DMF/CH_2Cl_2 (4/1), 85 h (f) diethylamine/DMF, 2 h; (g) DCC/HOBT in DMSO, 21 h.

consecutive alanines were coupled for 1 h by the preformed symmetric anhydride method.[15] The second crown ether **2** was attached via its hydroxybenzotriazole activated ester.[16] Then, the last alanine was added as its α-*N*-fluorenylmethyloxycarbonyl derivative (*N*-FMOC), using the preformed symmetric anhydride method. The 7-residue segment **4** was obtained with an isolated yield of 59% by treating the peptide resin with the acetate salt of L-alanine *tert*-butyl ester in DCM for 24 h.

Selective deprotection of the *tert*-butyl ester of **4** was performed with TFA containing 5% *p*-cresol as a scavenger and gave a quantitative yield of the peptide acid **5**. On the other hand, treatment of **4** with a 30% solution of diethylamine in a mixture of DCM/DMF (4/6) for 2 h at 25 °C afforded the free amino peptide **6** in a 70% yield. Segments **5** and **6** were coupled together by using the HOBT/DCC method in a 1/1 mixture of DMF/DMSO for 48 h. After purification by size-exclusion chromatography on LH-60 lipophilic Sephadex in DMSO, the tetracrown compound **7** was obtained with a 34% isolated yield.

Diethylamine deprotection of **7** gave a 96% yield of the free amino peptide **8**. The latter was coupled with the 7-residue segment **5** in DMSO by using HOBT and DCC for 24 h. Purification, first by gel permeation chromatography and then by reverse-phase HPLC, gave a 14% isolated yield of the desired hexacrown compound **1**, whose structure was confirmed by FAB mass spectroscopy.

Conformational Studies. The ability of peptide **1** to adopt a helical conformation was studied by circular dichroism spectropolarimetry.[17] The circular dichroic spectrum of **1** in tri-

(14) A similar strategy has been used for the preparation of amphiphilic peptides: DeGrado, W. F.; Lear, J. D. *J. Am. Chem. Soc.* **1985**, *107*, 7684–7689.

(15) Hagenmaier, H.; Frank, H. *Z. Physiol. Chem.* **1972**, *353*, 1973.
(16) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.
(17) Adler, A. J.; Greenfield, N. H.; Fasman, G. D. *Methods Enzymol.* **1973**, *27*, 675–735.

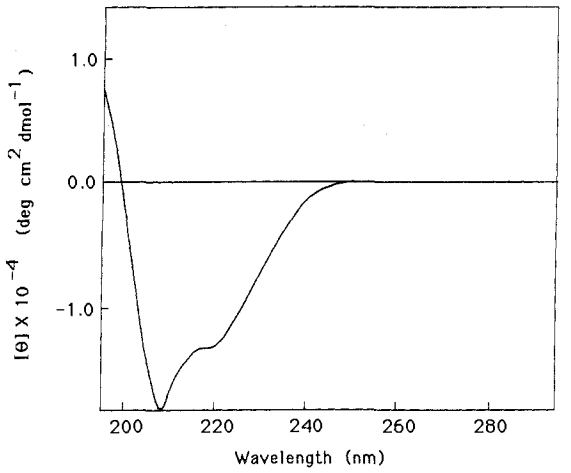

Figure 3. Circular dichroism spectrum of the hexacrown ether peptide **1** at a concentration of 1 mg/mL in trifluoroethanol.

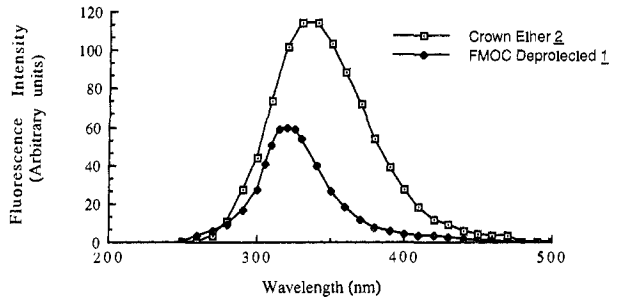

Figure 4. Fluorescence emission spectra of the FMOC-deprotected analogue of **1** and the methyl ester of the single crown ether **2** at 25 °C in degassed methanol. The λ_{max} observed are 320 and 335 nm, respectively.

fluoroethanol (TFE) is illustrated in Figure 3. The curve is typical of an α-helix peptide having characteristic minima at 208 and 222 nm with mean residue molar ellipticities of −18 300 and −13 500 deg cm^2 dmol^{-1}, respectively. This result confirms the initial prediction that a peptide composed of L-alanine and L-DOPA should exist mainly under a helical conformation. Morever, it implies that the receptor moities are located adjacently on the same side of the helix in a tubular arrangement.

Crown Ethers Orientation. A further confirmation of the tubular parallel arrangement of the ligand side chains is obtained by studying the fluorescence behavior of the FMOC-deprotected analogue of peptide **1**.[18] Indeed, the fluorescence spectrum of this derivative in methanol (Figure 4) shows marked differences when compared to the one obtained with the methyl ester of crown monomeric analogue **2**. The intensity of the emission band is characterized by an important hypochromic effect with a quantum yield roughly 2 times smaller relative to the ester of **2**. Also, the maximum wavelength is shifted from 335 nm for the crown ether **2** to 320 nm. Those phenomena may be attributed to the stacking interactions between the adjacent aromatic fluorophores. A similar behavior has been observed in the fluorescence spectrum of face-to-face stacked porphyrin molecules.[19]

Conclusions

In summary, we have demonstrated the versatility of peptide synthesis, especially the oxime resin **3**, for the preparation of supramolecular devices by describing the first synthesis of a tubular hexacrown compound, **1**. Peptide **1** adopts an α-helix structure in TFE, confirming the possibility of predicting the spatial orientation of the receptors. Also, the supramolecular parallel arrangement of the crown ether side chains was ascertained by fluorescence spectroscopy. We are presently working to demonstrate the generality of this methodology by preparing supramolecular devices bearing different receptors or effectors as well as to establish the ion-transport properties and ionic conductivity of the hexacrown ether **1** across planar lipid bilayers.

Experimental Section

All solvents and materials were Reagent, Spectro, or HPLC grade quality purchased commercially and used without any further purification except for DMF, which was distilled from $MgSO_4$ onto 4-Å molecular sieves.

^{1}H and ^{13}C NMR spectra were recorded on a GE QE300 instrument. The chemical shifts are expressed in ppm relative to tetramethylsilane unless otherwise indicated. Positive FAB mass spectra were obtained at a unit resolution with a VG FAB-E spectrometer at 8 kV. Elemental analyses were performed by Microanalysis Inc. (Wilmington, DE).

HPLC was performed on Vydac C_4 and C_{18} reverse-phase HPLC columns. Size-exclusion HPLC was done on a Du Pont semipreparative Zorbax Sil column. All solvents were degassed and gradients of standard buffers A (H_2O/0.1% TFA) and B (90% CH_3CN/9.9% H_2O/0.1% TFA) were used with reverse-phase columns. For size-exclusion chromatography, DMF with 0.1% TFA was utilized. Gel permeation chromatography was performed with a 2.5 × 90 cm column using Sephadex LH-60 in DMSO. The fractions's absorbance was determined at 280 nm. Melting points were measured with a Thomas-Hoover capillary apparatus and are uncorrected.

Circular dichroism spectra were recorded at 25 °C on an ISA Jobin-Yvon Dichograph III with Aldrich Co. Gold Label trifluoroethanol. The concentration used was 1 mg/mL and the path length of the quartz cell was 0.02 cm. Spectra are expressed in mean residue molar ellipticity and are corrected for the background. Mean residue molecular mass utilized was 172.1 g. Fluorescence studies were carried out at room temperature in degassed methanol solution using an Aminco-Bowman spectrophotometer. The intensity of fluorescence is normalized for a constant optical density at 280 nm, the excitation wavelength used.

Methyl Ester of *N*-*t*-BOC-3,4-(21-crown-7)-L-phenylalanine (2). Under argon, *N*-*t*-BOC-3,4-dihydroxy-L-phenylalanine methyl ester[20] (22.3 g, 0.072 mol) and cesium carbonate (23.5 g, 0.072 mol) were dissolved in 400 mL of dry degassed methanol. The mixture was gently warmed on a water bath to 45 °C until complete dissolution. Methanol was then evaporated to dryness under reduced pressure at 45 °C. DMF was added and evaporated under high vacuum at the same temperature to remove the residual methanol. The green product obtained was dissolved in 300 mL of DMF and transferred in a 3-L three-neck flask containing 1 L of DMF under argon at 60 °C to generate a deep green solution. A 300-mL DMF solution of dibromohexaethylene glycol[21] (29.0 g, 0.072 mol) was then added dropwise over a 1-h period. The resulting solution was stirred at 60 °C overnight. The brown suspension was evaporated under high vacuum at 45 °C. The residue was taken up in dichloromethane and washed three times with 5% $NaHCO_3$ and three times with H_2O then dried with $MgSO_4$. After filtration and evaporation, the crude brown oil was purified by flash chromatography on SiO_2 using first CH_2Cl_2 and then CH_2Cl_2 with 2 and 4% methanol. Trituration with petroleum ether/ether (10/1) gave the desired crown ether as a white powder: yield 39%; mp 66–67 °C (petroleum ether/ether); $[\alpha]^{23}_D$ = +4.2 ± 0.8° (c = 0.91, MeOH), 546 nm = +4.6 ± 0.8°, 436 nm = +10.9 ± 0.8°, 365 nm = +23.4 ± 0.8°; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9 H), 3.01 (m, 2 H), 3.63–3.82 (m, 19 H), 3.89 (t, 4 H), 4.12 (t, 4 H), 4.55 (m, 1 H), 4.97 (d, 1 H, J = 7.7 Hz), 6.66 (br s, 2 H), 6.79 (d, 1 H); ^{13}C NMR ($CDCl_3$) δ 27.79, 37.11, 51.59, 54.01, 68.73, 69.17, 69.97, 70.49, 79.12, 113.75, 114.82, 121.46, 128.63, 147.41, 148.22, 154.50, 171.74; FAB MS (acetone, *m*-nitrobenzyl alcohol) m/z 580 (M + Na$^+$), 558 (M + H$^+$). Anal. Calcd for $C_{27}H_{43}O_{11}N$: C, 58.17; H, 7.72; H, 2.52. Found; C, 57.91; H, 8.05; N, 2.62.

***N*-*t*-BOC-3,4-(21-crown-7)-L-phenylalanine (2).** The above ester (14.8 g, 0.027 mol) was suspended in 30 mL of 1 M NaOH solution and stirred at room temperature for 1.5 h. The clear alkaline solution was washed with ether and then acidified with ice-cold 1 M HCl to pH 4, producing a milky solution. The aqueous layer was extracted with three times with 300 mL of CH_2Cl_2. The organic phase was washed with water, dried with $MgSO_4$, filtered, and evaporated to give a yellowish oil.

(18) Prepared by a 2-h treatment of **1** with a 30% diethylamine/DMF solution. Synthetic details will be published elsewhere.

(19) Dubowchik, G. M.; Hamilton, A. D. *J. Chem. Soc., Chem. Commun.* **1986**, 665–666.

(20) Banerjee, S. M.; Ressler, C. *J. Org. Chem.* **1976**, *41*, 3056–3058.

(21) Prepared from hexaethylene glycol with dibromotriphenylphosphorane in acetonitrile; adapting a preparation of: Schaefer, J. P.; Higgins, J. G.; Shenoy, P. K. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V, pp 249–251. The dibromo compound is extracted from the crude product with petroleum ether. Spectral data were in accordance with the proposed structure.

Trituration overnight with petroleum ether/5% ether gave a white fluffy solid: yield 95%; mp 69–71 °C (petroleum ether). DCHA derivative: mp 72–73 °C (needles from MeOH/H_2O); $[\alpha]^{23}_D$ = +10.2 ± 1.1° (*c* = 0.74, MeOH), 546 nm = +13.0 ± 1.1°, 436 nm = +23.1 ± 1.1°, 365 nm = +45.9 ± 1.1°; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9 H), 3.04 (d, 2 H), 3.58–3.85 (m, 16 H), 3.93 (t, 4 H), 4.18 (m, 4 H), 4.53 (br m, 1 H), 5.00 (d, 1 H, *J* = 8.3 Hz), 6.72 (d, 1 H, J_0 = 8.3 Hz), 6.74 (s, 1 H), 6.80 (d, 1 H, J_0 = 8.0 Hz), 9.70–9.90 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 28.31, 37.39, 54.25, 69.19, 69.77, 70.93, 79.93, 114.40, 115.69, 122.21, 147.92, 148.75, 115.28, 174.39; FAB MS (acetone, *m*-nitrobenzyl alcohol) *m/z* 566 (M + Na^+), 544 (M + H^+). Anal. Calcd for $C_{26}H_{41}O_{11}N$: C, 57.46; H, 7.55; N, 2.58. Found: C, 57.57; H, 7.62; N, 2.44.

***N*-*t*-BOC-3,4-(21-crown-7)-L-phenylalanine–Resin.** The acid **2** (3.3 g, 6.1 mmol) was dissolved in dichloromethane (25 mL) and mixed in a polypropylene container with 6.9 g (6.2 mmol) of *p*-nitrophenyl oxime resin[13] (substitution level = 0.96 mmol/g) swollen in 35 mL of dichloromethane. After cooling to 0 °C, dicyclohexylcarbodiimide (1.3 g, 6.2 mmol) was added and the resulting suspension was stirred on a rotary shaker overnight at 25 °C. The next day, the resin was filtered and carefully washed as follows: 3× 100 mL of DMF, 3× 100 mL of MeOH, 3× 100 mL of DMF, 3× 100 mL of MeOH, and suction dried. The resin was then acetylated by using acetic anhydride (5 mL) and diisopropylethylamine (0.5 mL) in DMF for 1 h. Washing and drying as described above gave the resin used in the next step. Yield, 7.5 g; substitution level, 0.152 mmol/g (quantitative ninhydrin test).

***N*-FMOC-Ala–2–Ala-Ala-Ala–2–Resin.** The title compound was prepared by using 5 g of the above acylated resin **3** (0.76 mmol) in a reaction vessel bearing an opening at the top and a glass fritted disk at the bottom. The vessel was shaken on a rotating shaker at 20 rpm and drained on a vacuum-filtering flask. Deprotection of the *N*-*tert*-butyloxycarbonyl group was performed by treating the resin with a 25% TFA solution in dichloromethane for 1 h. The washing steps in all cases were as follows: 3× 25 mL of DMF, 3× 25 mL of MeOH, 3× 25 mL of DMF, 3× 25 mL of MeOH. Protected alanine derivatives were doubly coupled in DMF for 1 h by using 5 equiv of their preformed symmetric anhydride.[15] The crown ether **2** was also doubly coupled as its HOBT-activated ester[16] (1.5 equiv). The acids were activated in DMF at 0 °C for 15 min prior to their addition to the resin. Then, diisopropylethylamine (1.2 equiv, 0.92 mmol, 160 μL) was added to neutralize the TFA salts. Completion of the coupling reactions was monitored by the ninhydrin test. Yield 6.04 g of peptide resin.

***N*-FMOC-Ala–2–Ala-Ala-Ala–2–Ala-*O*-*tert*-butyl (4).** The peptide resin (6.04 g) was swollen in 35 mL of dichloromethane and treated overnight with the acetate salt of L-alanine *tert*-butyl ester (1.05 g, 5.2 mmol, 7 equiv) prepared in situ by neutralization of the HCl salt. The suspension was filtered and the filtrate was evaporated to give 2.2 g of a yellowish clear oil. Trituration with a 1/1 mixture of petroleum ether/ether gave a solid, which was recrystallized in chloroform/methanol (9/1). The gel formed was filtered and dried to yield 0.67 g of desired peptide **4**: yield 59%; TLC (CH_2Cl_2/10% MeOH) R_f = 0.5, ($CHCl_3$/MeOH/CH_3COOH, 85/10/5) R_f = 0.15; FAB MS (CH_2Cl_2, *m*-nitrobenzyl alcohol) *m/z* 1541 (M + K^+), 1525 (M + Na^+).

***N*-FMOC-Ala–2–Ala-Ala-Ala–2–Ala-OH (5).** The protected peptide **4** (0.3 g, 0.2 mmol) was dissolved in a solution of trifluoroacetic acid (7 mL) containing 1 mL of *p*-cresol. The resulting mixture was stirred at 25 °C for 30 min and then evaporated under reduced pressure. The remaining traces of trifluoroacetic acid were coevaporated twice with DMF under high vacuum. Trituration using ether (4 × 15 mL) and vacuum drying gave 0.28 g of the free carboxylic acid peptide **5**, which was crystallized from methanol: yield 99%; TLC (CH_2Cl_2/20%MeOH) R_f = 0.27; RP HPLC (anal., C_{18}) R_t = 35.5 min (0–100% B in 50 min); size-exclusion HPLC R_t = 5.5 min; FAB MS (DMF, *m*-nitrobenzyl alcohol) *m/z* 1468 (M + Na^+), 1446 (M + H^+). Anal. Calcd for $C_{72}H_{98}O_{24}N_7 \cdot 2H_2O$: C, 58.34; H, 6.95; N, 6.62. Found; C, 58.65; H, 7.07; N, 6.58.

H_2N-Ala–2–Ala-Ala-Ala–2–Ala-O-*tert*-butyl (6). The *N*-FMOC peptide **4** (0.23 g, 0.15 mmol) was treated with 4 mL of diethylamine in 4 mL of dichloromethane and 6 mL of DMF for 2 h at room temperature. The solvents were evaporated under vacuum and the mixture was coevaporated twice with DMF. The crude solid obtained was triturated with a 1/1 mix of ether/petroleum ether (3 × 25 mL) and then overnight with ether. The product, 0.138 g, was used in the next step without any further purification: yield 70%; TLC (CH_2Cl_2/20% MeOH) R_f = 0.22 (positive with ninhydrin); RP HPLC (anal., C_{18}): R_t = 29.5 min (0–100% B in 50 min); size-exclusion HPLC R_t = 5.5 min; FAB MS (DMF, *m*-nitrobenzyl alcohol) *m/z* 1318 (M + K^+), 1302 (M + Na^+), 1280 (M + H^+).

***N*-FMOC-(Ala–2–Ala-Ala-Ala–2–Ala)$_2$-*O*-*tert*-butyl (7).** The *N*-FMOC-protected peptide **5** (0.159 g, 0.11 mmol) was dissolved in 2 mL of a dichloromethane/DMF mixture (1/1), and HOBT·H_2O (17 mg, 0.11 mmol) was added. The solution was cooled to 5 °C, then DCC (23 mg, 0.11 mmol) was introduced, and the cooling bath was removed. After 30 min of activation, the free amino peptide **6** (0.138 g, 0.11 mmol) was added in 2 mL of DMF. The thick gel was stirred overnight at 25 °C and the reaction progress was monitored by size-exclusion HPLC. Almost no coupling occurred after 17 h, and 1 equiv each of HOBT·H_2O and DCC was added with 2 mL of DMF. After 24 h, only a small amount of the desired 14-residue peptide **7** could be detected. The pH was brought from 4 to 7 with 110 μL of *N*-methylmorpholine, and 3 mL of DMSO was added. The reaction reached a maximum and was stopped 12 h later. The crude mixture was loaded onto a Sephadex LH-60 column and eluted with DMSO to obtain 89 mg of the desired compound **7**. The latter was used in the next step without any further purification: yield 34%; RP HPLC (anal., C_{18}) R_t = 44.6 min (0–100% B in 50 min); size-exclusion HPLC R_t = 5.2 min; FAB MS (DMF, thioglycerol) *m/z* 2754 (M + 2Na^+), 2747 (M + K^+), 2731 (M + Na^+).

H_2N-(Ala–2–Ala-Ala-Ala–2–Ala)$_2$-*O*-*tert*-butyl (8). The *N*-FMOC-protected peptide **7** (89 mg, 33.3 μmol) was dissolved in 5 mL of DMF and the resultant mixture treated with 1 mL of diethylamine for 2 h at 25 °C. The solvents were evaporated under high vacuum and coevaporated twice with 5 mL of DMF. The gel obtained was triturated with ether (3 × 25 mL) and dried under vacuum to give 78 mg of the title peptide **8**, which was used as is in the next reaction: yield 96%; RP HPLC (anal., C_{18}) R_t = 34.1 min (0–100% B in 50 min); size-exclusion HPLC R_t = 5.2 min; FAB MS (DMF, thioglycerol) *m/z* 2532 (M + 2Na^+), 2508 (M + Na^+), 2487 (M + H^+).

***N*-FMOC-(Ala–2–Ala-Ala-Ala–2–Ala)$_3$-*O*-*tert*-butyl (1).** Peptide **5** (50 mg, 34.6 μmol) was dissolved in 1 mL of DMSO and activated with HOBT·H_2O (5.4 mg, 34.6 μmol) and DCC (12 mg, 58.1 μmol) for 45 min at 25 °C. The resulting solution was added to the free amino peptide **8** (69 mg, 27.8 μmol) in 2 mL of DMSO and then rinsed with 0.5 mL of DMSO. The reaction was followed by size-exclusion HPLC. After 7 h, the pH was raised from 4 to 7 with 10 μL of *N*-methylmorpholine. The reaction reached a maximum after 17 h and was stopped. The crude mix was poured on a Sephadex LH-60 column and eluted with DMSO. Lyophylization gave a gummy solid, which was purified by reverse-phase HPLC to obtain 16.3 mg of a white fluffy solid: yield 14%; RP HPLC (anal., C_4) R_t = 44.2 min (0–100% B in 50 min); size-exclusion HPLC R_t = 5.0 min; FAB MS (DMF, thioglycerol) *m/z* 3938 (M + Na^+).

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