Serine-Based Cyclodepsipeptides on an Adamantane Building Block: Design, Synthesis, and Characterization of a Novel Family of Macrocyclic Membrane Ion-Transporting Depsipeptides

Darshan Ranganathan,*,† V. Haridas,† K. P. Madhusudanan,‡ Raja Roy,‡ R. Nagaraj,§ and G. B. John§

Contribution from the Biomolecular Research Unit, Regional Research Laboratory (CSIR), Trivandrum 695019, India, Central Drug Research Institute, Lucknow, India, and Centre for Cellular and Molecular Biology, Hyderabad, India

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Abstract: A simple two-step synthetic strategy provides a straightforward entry to a large variety of adamantane-containing serine-based cyclodepsipeptides. The design is flexible with respect to the choice of an amino acid, the ring size, and the nature of the template as illustrated here with the preparation of a large variety of serine-based macrocycles, for example, 18-membered simple cyclo(Adm-Ser-)$_2$ (**4**), 21-membered, S−S bridged, cyclo(Adm-Ser-Cyst-Ser-) (**10**), 24-membered cyclo(Adm-Ser-Val-)$_2$ (**5a**), cyclo(Adm-Ser-Leu-)$_2$ (**5b**), cyclo(Adm-Ser (Leu)-Val)$_2$ (**6**), pyridine-containing cyclo(Adm-Ser-Val-Py-Val-Ser-) (**13**), 26-membered cyclo(Adm-Ser-Ser-)$_2$ (**8**) and crown ether hybrid cyclo(Adm-Ser-TEG-Ser-) (**12**), and 36-membered cyclo(Adm-Ser-)$_4$ (**7**) and provides built-in handles (in the form of protected NH_2 and COOH groups) for attachment of suitable pendants leading to attractive models that may have multiple uses as membrane ionophores, scaffolds, or templates in the design of artificial proteins and for studying the structure−function relationship in biological receptors. This novel class of macrocyclic peptides are demonstrated to adopt β-turn type conformation and possess high efficiency in transporting Na^+, Ca^{2+}, and Mg^{2+} ions across model membranes. Amongst the cyclodepsipeptides reported here, the 24-membered macrocycle **6**, containing two leucine residues symmetrically placed on the exterior of the ring, was found to be the most efficient ion-transporter in lipid bilayer membranes. Interestingly, no appreciable ion-transport was noticed by 18-membered cyclodepsipeptide (**4**) and by macrocycles **10**, **12**, and **13** possessing only one adamantane unit in their cyclic framework. These results show that a minimum of two adamantane units in a 24-membered ring size appears to be the optimum requirement for efficient membrane ion transport.

Introduction

Cyclodepsipeptides belong to a special class of compounds well-known for their biological functions as antibiotics and regulators of ion-transport in membranes.[1] This special property may be attributed to their capability to complex and release the metal ions at a rate most desirable for good transporters. Amongst the naturally occurring cyclodepsipeptides, valinomycin—a cyclododecadepsipeptide containing three cyclic repeats of a four residue (D-Val-L-Lac-L-Val-D-HyV) segment with alternating amide (CONH) and ester (COO) bonds—occupies a central place because of its special property of selectively transporting potassium ions across biological membranes.[2]

In order to be an efficient ion-transporter, a molecule must not only be able to bind a metal ion selectively and tightly but also must be flexible enough to release the ion fairly rapidly. Additionally, to be compatible with a biomembrane, the ionophore must have a hydrophobic exterior while having a hydrophilic interior capable of binding an ion. Valinomycin meets all these requirements by having a balled hydrophobic exterior provided by a large number of isopropyl groups of valine and hydroxyvaleric acid (HyV) residues and at the same time showing conformational flexibility[3] by striking a balance of more polar amide carbonyl and less polar ester carbonyls to complex with less charge dense potassium ions.

An interesting feature of most naturally occuring cyclopeptides or cyclodepsipeptides is the presence of an *N*-methylamino acid or a D-amino acid or a Proline residue in their cyclic framework.[1] This must be Nature's strategy to create bends in the peptide backbone in order to facilitate cyclization.[4] In valinomycin, Nature has taken particular care to arrange alternating sequence of D and L amino acids introducing repeating bends and resulting in a tennis-ball-seam arrangement without imposing much rigidity on the structure—a feature particularly important to any successful ionophore because not only must a metal ion be bound and transported but also it must be released after transport if the ionophore is to be useful.[5]

In recent years, there has been a virtual explosion in the number of papers that have appeared in literaure on the design of nonpeptidic macrocyclic ionophores, such as crown ethers, cryptands, spherands, calixarenes, and cyclophanes, etc., incor-

* To whom correspondence should be addressed. FAX: +91-0471-490186/491712. E-mail: darshan@csrrltrd.ren.nic.in.

† Biomolecular Research Unit.

‡ Central Drug Rsearch Institute.

§ Centre for Cellular and Molecular Biology.

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(1) Ovchinnikov, Y. A.; Ivanov, V. T.; Shkrob, A. M. *Membrane Active Complexones;* Elsevier: Amsterdam, 1974. Ovchinnikov, Y. A.; Ivanov, V. T. *Tetrahedron Report No.1*; Pergamon Press: New York, 1976.

(2) Izatt, R. M.; Bradshaw, J. S.; Nielson, S. A.; Lamb, J. D.; Christensen, J. J.; *Chem. Rev.* **1985**, *85*, 271−339. Marrone, T. J.; Merz, K. M., Jr., *J. Am. Chem. Soc.* **1995**, *117*, 779.

(3) Smith, G. D.; Daux, W. L.; Langs, D. A.; DeTitta, G. T.; Edmonds, J. W.; Rohrer, D. C.; Weeks, C. M. *J. Am. Chem. Soc.* **1975**, *97*, 7242. Karle, I. L.; Flippen-Anderson, J. L. *J. Am. Chem. Soc.* **1988**, *110*, 3253. Karle, I. L. *J. Am. Chem. Soc.* **1975**, *97*, 4379. Patel, D. J.; Tonelli, A. E. *Biochemistry* **1973**, *12*, 486. Tabeta, R.; Saito, H. *Biochemistry* **1985**, *24*, 7696.

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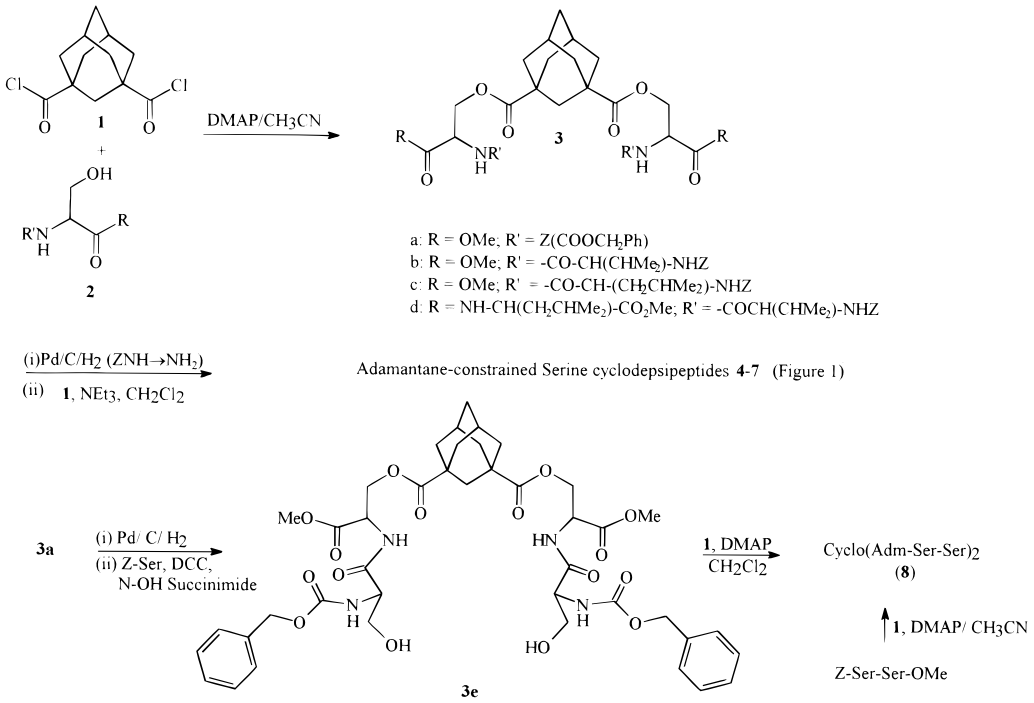
Scheme 1



porating aromatic, non aromatic, carbohydrate, or steroid units in their cyclic frame work, and even pendant attachments have been done in order to increase their potential for forming three-dimensional complexes and as efficient ion-transporters in membranes.[6]

Although a large number of simple structural analogs of valinomycin and some related cyclodepsipeptide antibiotics of natural origin have been prepared,[5] to our knowledge, the reports on the *de novo* design of membrane ion-transporting macrocyclic peptides or depsipeptides are still scarce.[7]

In this paper, we report the design, synthesis, and characterization of a new class of cyclodepsipeptides wherein a serine $-CH_2OH$[8] is used for ester bond formation and rigid, low molecular weight, lipophilic adamantane building blocks provide the desired membrane permeability and conformational constraint for efficient ion transport in membranes. The present strategy[9] is flexible with respect to the ring size, the choice of an amino acid, and the choice of a building block as illustrated here with the preparation of 18-membered (**4**), 21-membered (**10**), 24-membered (**5a**,**b**, **6**, and **13**), 26-membered (**8** and **12**), and 36-membered (**7**) serine-based macrocycles. An additional advantage is provided by the built-in handles in **4**−**13** (in the form of protected NH_2 and COOH groups) that can be ligated via peptide chemistry to a variety of subunits, for example, a long alkyl amine, a polypeptide chain, a polysaccharide unit, or even a peptide dendron, providing attractive models for novel artificial protein design[10] and membrane ion-transport. The design also permits the incorporation of ionophoric pendants such as crown ethers either as part of the cyclic backbone as illustrated here with the preparation of macrocycle **12** or attached to the amino and carboxy side arms.

Results and Discussion

The two-step synthesis envisaged here (Scheme 1) involves first the condensation of an N,C-protected serine amino acid or its peptide (**2**) with 1,3-adamantane dicarbonyl dichloride (**1**) to give the bis-Ser derivative **3a**−**d**, which after N-deprotection and recoupling with **1** affords the desired cyclodepsipeptides

(4) Proline has been established as a frequent cause of chain reversal in globular proteins (Crawford, J. L; Lipscomb, W. N; Schollman, C. G. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 538. Chou, P. Y.; Fasman, G. D. *Biochemistry* **1974**, *13*, 222). This property arises in part from the rigidity of the pyrrolidine ring, which restricts the possible values of the angle of rotation ϕ around the $C^{\alpha}N$ bond to between 105 and 125°, thus diminishing the allowed space on the conformational maps (Venkatachalam, C. M. *Biopolymers* **1969**, *6*, 1425). While proline is a proteinous amino acid, D-amino acids are not known to occur in natural proteins (Stryer, L. *Biochemistry*, 2nd ed.; Freeman: New York, 1981; p 774). Their frequent occurrence in microbial linear or cyclopeptides is truly remarkable and may be necessary to facilitate cyclization. The D-amino acids by virtue of their opposite chirality and hence opposite geometry are likely sites for bends stabilizing the cyclic backbone (Ramakrishnan,C.; Sarathy, K. P. *Int. J. Pep. Protein Res.* **1969**, *1*, 63).

(5) Marrone, T. J.; Merz, K. M., Jr. *J. Am. Chem. Soc.* **1992**, *114*, 7542. Also see: Fyles, T. M. in *Bioorganic Chemistry Frontiers*; Springer Verlag: New York, 1991, Vol. 1, pp 71−113. Gokel, G. W.; Eschegoyen, L. *Ibid.* pp 115−141. Shinkai, S. *Ibid.* pp 161−201.

(6) For recent updates on these topics see: *Comprehensive Supramolecular Chemistry, Vol.1*; *Molecular Recognition: Receptors For Cationic Guests;* Gokel, G. W., Guest Ed.; Pergamon, Elsevier Science Ltd.: Oxford, 1996. *Comprehensive Supramolecular Chemistry, Vol. 2, Molecular Recognition: Receptors for Molecular Guests*; Vogtle, F., Guest Ed.; Pergamon, Elsevier Science Ltd.: Oxford, 1996. Gokel, G. W.; Murillo, O. *Acc. Chem. Res.* **1996**, *29*, 425−432.

(7) See ref 1 and: Roeske, R. W.; Kennedy, S. J. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Marcel Dekker, Inc.: New York, 1983; Vol. 7, p 205−256. Garcia-Echeverria, C.; Albericio, F.; Giralt, E.; Pons, M. *J. Am. Chem. Soc.* **1993**, *115*, 11663. Ghadiri, M. R.; Granja, J. R.; Buchler, L. N. *Nature* **1994**, *369*, 301. Khazanovich, N.; Granja, J. R.; Mc Ree, D. I.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1994**, *116*, 6011. Ranganathan, D.; Haridas, V.; Madhusudanan, K. P.; Roy, R.; Nagaraj, R.; John, G. B.; Sukhaswami, M. B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1105. Karle, I. L; Ranganathan, D.; Haridas, V. *J. Am. Chem. Soc.* **1996**, *118*, 10916.

(8) Interestingly, while hydroxy acids like lactic acid and hydroxyvaleric acids are commonly employed for depsibond formation in cyclodepsipeptides, hydroxy amino acids are rarely used, and there are hardly any examples where proteinous aminoacids like serine and tyrosine are exploited either by nature or in the laboratory for the construction of cyclodepsipeptides (see refs 1 and 14).

(9) The first illustration of the template-based strategy for the synthesis of a large number of adamantane-containing cystine cyclic peptides was recently reported by us (Ranganathan, D.; Haridas, V.; Madhusudanan, K. P.; Roy, R.; Nagaraj, R.; John, G. B.; Sukhaswami, M. B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1105. Karle, I. L; Ranganathan, D.; Haridas, V. *J. Am. Chem. Soc.* **1996**, *118*, 10916).

(10) Carey, R. T.; Mutter, M. In *Molecular conformation and Biological Interactions*; Balaram, P., Ramaseshan, S., Eds.; Indian Academy of Sciences: Bangalore, 1991; pp 457−468. Grove, A.; Mutter, M.; Rivier, J. E.; Montal, M. *J. Am. Chem. Soc.* **1993**, *115*, 5919. Zhang, L.; Tam, J. P. *J. Am. Chem. Soc.* **1997**, *119*, 2363.

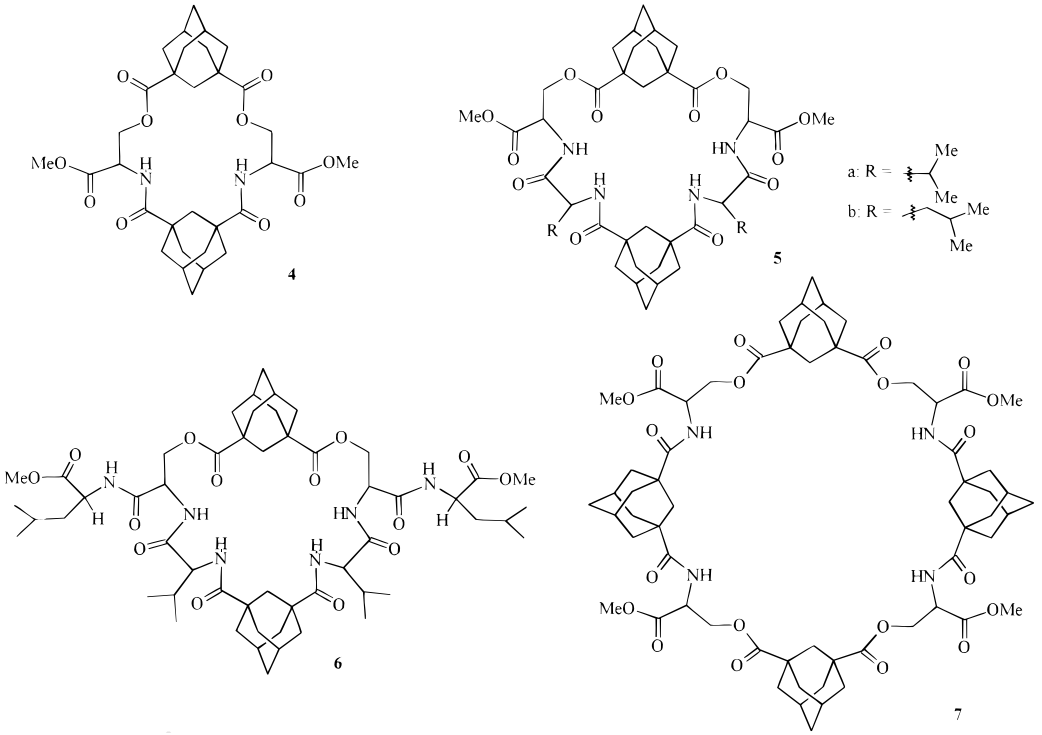


Figure 1.

4−**7** in good yields (Scheme1). Thus, the 18-membered serine cyclodepsipeptide (**4**), the simplest member of the family, was obtained in an overall yield of 59% from Z-Ser-OMe (**2**, R = OMe, R′ = Z) and **1** via the bis-ser intermediate **3a**. A noteworthy feature of this reaction was the formation of a small amount (∼2%) of 2 + 2 cyclization product **7** (Figure 1) containing four cyclic repeats of adamantane and serine units connected with alternating pairs of ester and amide bonds in a 36-membered cyclic framework. In terms of ring size, the macrocycle **7** can be considered as equivalent to valinomycin—a 36-membered cyclododecadepsipeptide. Interestingly, the FAB-MS spectrum (Supporting Information) showed the presence of $(M + H)^+$, $(M/2 + H)^+$, and $(M/4 + H)^+$ peaks, which supported the 4-fold symmetry. The presence of only a single set of proton resonances for adamantane and serine units in the ^{1}H NMR of **7** also indicated the highly symmetrical nature of the macrocycle.

For the preparation of 24-membered macrocycles **5a** and **5b**, the intermediate bis-depsipeptides **3b** and **3c**—obtained by the direct condensation of serine peptides Z-Val-Ser-OMe and Z-Leu-Ser-OMe, respectively, with the adamantane template **1**—were N-deprotected (Pd/C, 5%, H_2) and recoupled with **1**. The cyclodepsipeptide **6** containing two leucine residues on the exterior of the macrocycle **5a** was crafted with the aim of increasing the lipophilicity, most desirable for efficient membrane penetration. The macrocycle **6** was prepared from Z-Val-Ser-Leu-OMe using essentially the same procedure as for **5a** and **5b** (Scheme 1). The 26-membered all-Ser cyclodepsipeptide **8** could be obtained either by a single-step condensation of Z-Ser-Ser-OMe with **1** or by an alternate route with a two-step sequence (Scheme 1) involving first the conversion of **3a** into **3e** (by N-deprotection followed by coupling with Z-SerOH) and the subsequent depsicoupling of **3e** with adamantane template **1** (Scheme 1). The cyclodepsipeptides from both the routes were found to be identical in all respects; the yield,[11] however, was much better (53%) in the two-step procedure. The bis-Z macrocycle **8** could easily be N-deprotected (Pd/C, 5%, H_2) to give a novel bis-amino derivative of **8**, potentially useful for ion-complexation studies.

The versatility of the present strategy was further demonstrated by the preparation of novel macrocyclic cyclodepsipeptides, for example, with an S−S bridge (**10**), a crown ether hybrid (**12**), and containing a pyridyl unit in the cyclic framework (**13**). This group of macrocycles (Figure 2) provided another set of attractive molecules for ion-transport studies.

The N,N′ bis ser (N^{α}-Boc) cystine diOMe (**9**)—prepared from cystine diOMe and Boc-SerOH using DCC/NOH succinimide coupling—was treated with adamantane dicarbonyl dichloride (**1**) in dry CH_3CN at 0 °C in the presence of DMAP (Scheme 2). The 21-membered cystine containing cyclodepsipeptide **10**, obtained in moderate yields (30%), was fully characterized. The Boc-protection on the amino groups can easily be removed (TFA/CH_2Cl_2) without touching the S−S linkage, which is not the case with carbobenzoxy deprotection.

For the preparation of cyclodepsipeptide−crown hybrid (**12**), tetraethylene glycol (TEG) was treated with N^{α}-Boc-Ser-(CH_2-OBzl)OH in the presence of DMAP under DCC/NOH succinimide coupling conditions. The N,O-protected bis-Ser derivative (**11**) was selectively O-deprotected ($Pd/C/H_2$) and directly treated with **1** and DMAP under high dilution conditions to give the crown depsipeptide **12** in 25% yield (Scheme 2). The macrocycle **13** with a pyridyl unit in the backbone was constructed from bis-Ser intermediate **3b** by N^{α}-deprotection ($Pd/C/H_2$) followed by treatment with 2,6-pyridinedicarbonyl dichloride (Scheme 2).

Interestingly, while the 18-membered macrocycle **4** did not show any significant features in its ^{1}H NMR and CD spectra, the ROESY NMR of 24-membered macrocycles **5a**,**b** and **6** indicated development of secondary structural features. A particularly diagnostic feature that signifies the formation of a β-turn-type structure in peptides was the presence of strong Ser NH-Val/Leu $C^{\alpha}H$ cross-peaks in the ROESY spectra (supporting

(11) The one-step condensation of Z-Ser-Ser-OMe with **1** gave a mixture of products, of which macrocycle **8** formed only a small portion (∼10%). The major compound (∼30%) of the reaction was tentatively identified (FAB-MS) as the 3 + 3 macrocycle—a 39-membered cyclodepsipeptide containing three cyclic repeats of alternating 1,3-adamantanedicarbonyl and Z-Ser-Ser-OMe units.

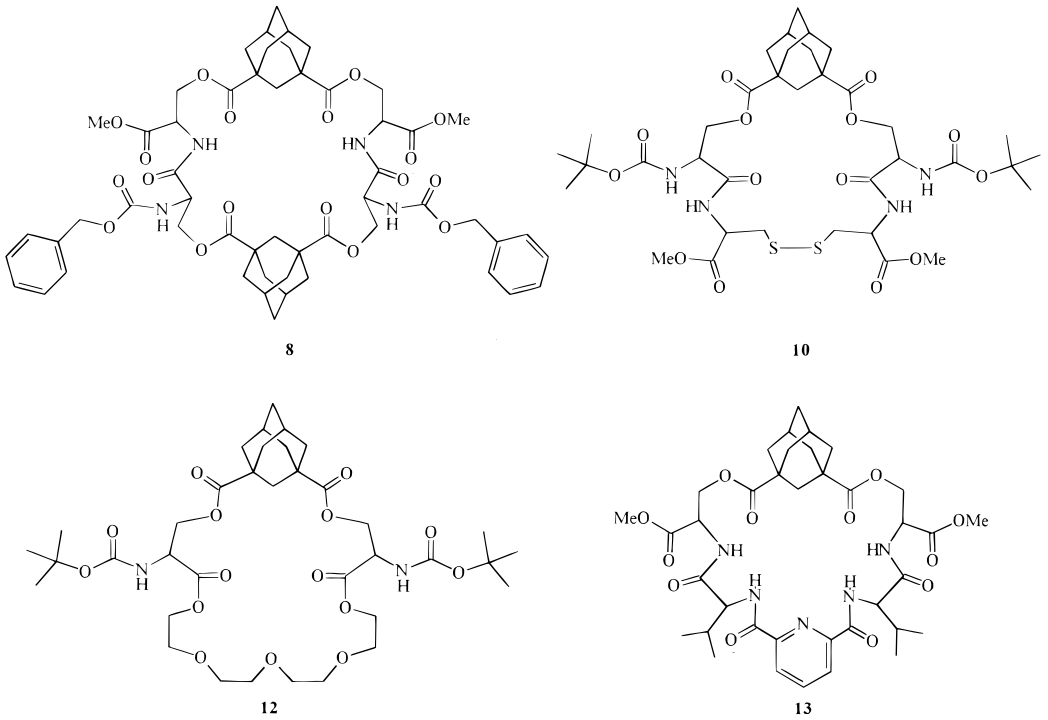
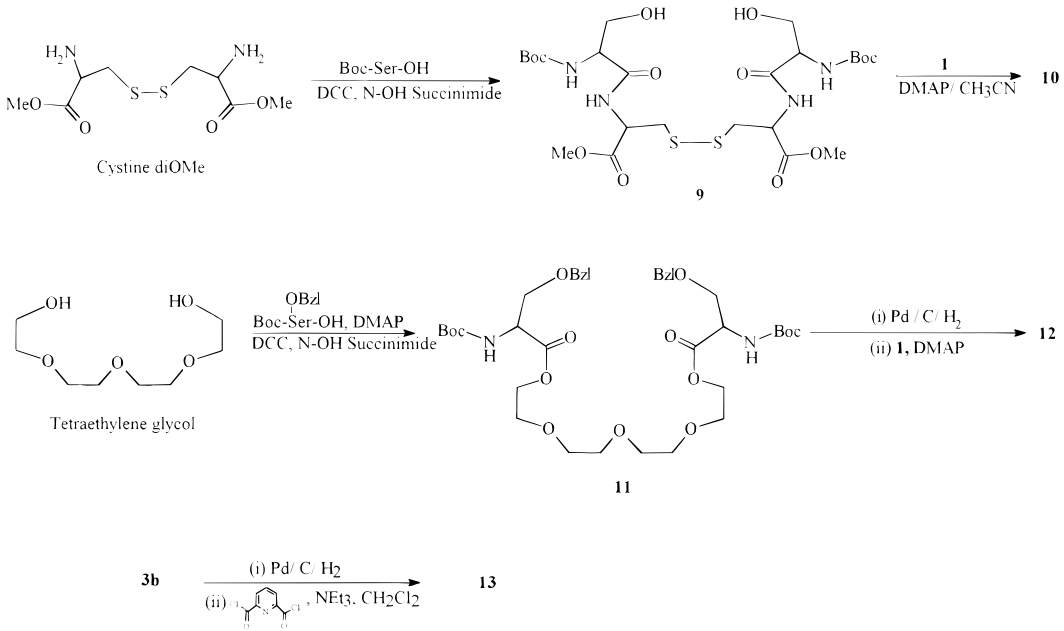


Figure 2.

Scheme 2



information) of **5a**,**b** and **6**. A very enhanced ROE between Val/Leu $C^{\alpha}H$ and Ser $C^{\beta}H_2$ protons further supported the β-turn-type conformation. The syn orientation of amide carbonyls at the Val/Leu end was indicated by the presence of only a weak ROE effect between the Val/Leu NH and the adamantane methylene protons. Variable-temperature (VT) studies conducted in $CDCl_3$ with **5a**,**b** and **6** over the temperature range 298−328 K showed relatively low temperature coefficients for ring Val/Leu NH ($d\delta/dT$ in ppb/K = −2.8 (**5a**), −1.5 (**5b**) and −3 (**6**)), indicating possible involvement of these protons in stabilizing turn conformation via 10-membered hydrogen-bonded rings.

Further support for the turn structure in 24-membered macrocycles **5a**,**b** and **6** was provided by their CD spectra (supporting Information). Thus, in trifluroethanol (TFE) solution, while **5b** showed a minimum at 220 nm and **6** showed minima at ∼200 and 210 nm—attributed to a distorted β-turn structure—the compound **5a** exhibited a broad minimum at ∼215 nm with a shoulder at 202 nm, a feature typical of a type I β-turn in cyclic peptides.[12] The macrocycles maintain their β-turn conformation even in aqueous solvents as was shown by their CD spectra in 15% aqueous methanol (Supporting Information).

With a variety of cyclodepsipeptides in hand, we set out to measure the capability of these molecules to transport ions in model membranes.

Using the fluorescent dye method[13] with valinomycin as a standard reference,[9] it was found that while the 18-membered cyclodepsipeptide (**4**) showed near absence of any ion transport

(12) Woody, R. W. In *Peptides: Analysis, Synthesis, Biology;* Udenfriend, S., Meienhofer, J., Eds.; Academic Press: NY, 1985; Vol. 7, pp 16−115.

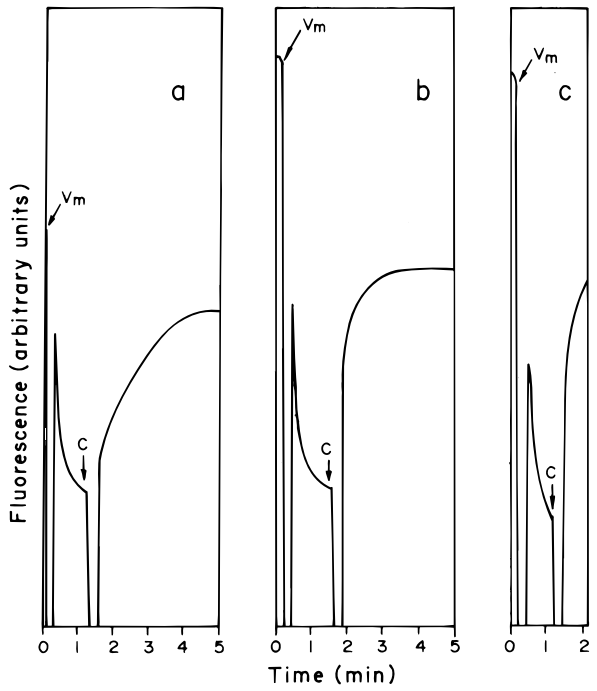


Figure 3. Effect of the addition of cyclodepsipeptide **6** (5.6 μM) on the diffusion potential set up by valinomycin (at a lipid: υ_m ratio of 300:1) for Na^+ (a), Ca^{2+} (b), and Mg^{2+} (c) as monitored by the fluorescence of cyanine dye dis-C_2 (5). Maximum efficiency was seen at a lipid:peptide ratio of 300:1. The points υ_m and *c* denote the points of addition of valinomycin and cyclodepsipeptide **6**, respectively.

capability,[14] the larger macrocycles **5a**,**b** and **6**−**8** were all found to be very effective in translocating Na^+, Mg^{2+}, and Ca^{2+} ions across model membranes (small unilamellar vesicles). Amongst these macrocycles, the cyclodepsipeptide **6**, with two leucine residues on the exterior of the ring, showed maximum efficiency and was demonstrated (Figure 3) to be almost as efficient as valinomycin (with respect to the lipid:ionophore ratio) in transporting Na^+, Mg^{2+}, and Ca^{2+} ions. None of these macrocycles caused the release of entrapped carboxyfluorescein, indicating that the movement of ions across the lipid bilayer was not due to the formation of large pores or detergent-like action. Although the transport mechanism is yet to be established, the nonselective nature of ion-influx suggests encapsulation of the ions by macrocyclic peptides. Interestingly, the cyclodepsipeptides **10**, **12**, and **13**, all containing only one adanantane unit, and despite having strong metal-complexing ligands in their cyclic framework, were found to to be totally devoid of any membrane ion-transport capability, thus supporting our earlier notion[9] that a minimum number of two adamantane units in the appropriate ring size are necessary for membrane penetration.

In summary, the adamantane-constrained, serine-based cyclodepsipeptides described here represent a new class of peptide ionophores. The intrinsic propensity of these macrocycles (as exemplified with **5a**,**b**, **6**, and **8**) to adopt a β-turn-type conformation and the presence of built-in handles for ligating lipophilic chains or secondary structural elements on their exterior would make them attractive models for studying ion-transport in membranes, structure−function relationship in receptors, and protein-folding mechanisms and as conformationally well-defined scaffolds or templates for use in the synthesis of artificial proteins. The novel architecture and the demonstrated high efficiency of these molecules for ion-transport in model membranes combined with their extremely simple and straightforward synthesis from commercially available starting materials is expected to provide additional incentives for the design of future serine-based cyclodepsipeptides on an adamantane building block.

Experimental Section

All amino acids used were of L-configuration. Melting points were recorded on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured with an automatic JASCO polarimeter; concentrations are given in grams/100 mL. Infrared spectra were recorded on a Perkin-Elmer/1600-FT spectrometer either in chloroform solutions, as neat liquids, or as KBr pellets, and prominent peaks are expressed in cm^{-1}. ^{1}H NMR spectra were recorded on Varian UNITY-400, Bruker WM-300, Hitachi R-600, and JEOL 90 MHz instruments. The chemical shifts are reported in δ (ppm) with TMS at 0.00 as an internal reference. ROESY experiments were performed using 0.2 s mixing time with pulsed spin locking with 30° pulses and 2 kHz spin-locking field. FAB-MS were obtained on a JEOL SX-120/DA-6000 instrument using *m*-nitrobenzyl alcohol as the matrix. Reactions were monitored wherever possible by TLC. Silica gel G (Merck) was used for TLC and column chromatography was done on silica gel (100−200 mesh) columns, which were generally made from a slurry in hexane or a mixture of hexane and ethyl acetate. Products were eluted with either a mixture of ethyl acetate / hexane or chloroform / methanol. The circular dichroism (CD) spectra were recorded on JASCO J- 715 instrument in quartz cells of 1 mm path length at 25 °C. For ion-transport study, small unilamellar vesicles of palmitoyloleoylphosphatidylcholine were prepared by sonication of a suspension of multilamellar vesicles with a Branson sonifier having a microtip. The vesicles were loaded with K^+ (300 mM). The diffusion potential was set up by diluting the concentrated stock of the lipid vesicles 100-fold into buffer (Hepes, 10 mM, pH 7.4) containing either 300 mM NaCl, 250 mM $CaCl_2$, or 200 mM $MgCl_2$. Fluorescent dye 3,3′-bis(ethylthio)-dicarbocyanine iodide [dis-C_2-(5)] was then added followed by valinomycin (0.3 μg/mL; lipid: υ_m ratio 300:1). Fluorescence spectra were recorded in a Hitachi 4010 spectrofluorimeter at 25 °C. The ion-transport capability of cyclodepsipeptides (**5a**−**8**) was evaluated by monitoring the dissipation of a valinomycin-mediated K^+ diffusion potential, creating a diffusion potential-like valinomycin (ref 13) and by the chlorotetracycline−Ca^{2+} assay (ref 15).

General Procedure for the Preparation of 1, 3-Adamantane Bis-Ser Depsipeptides 3a−d Listed in Scheme 1. A solution of 1,3-adamantanedicarbonyl dichloride (**1**, 1 mmol, freshly prepared from dicarboxylic acid by refluxing with 3 M excess of $SOCl_2$ for 3 h, followed by drying in vacuo) in dry CH_2Cl_2 (10 mL) was added dropwise over a period of 0.5 h to a well stirred and ice-cooled solution of N^{α}-Z-Ser-OMe or its di- or tripeptide (2 mmol) in dry CH_3CN (30 mL) containing *N*,*N*′-dimethyl-4-aminopyridine (DMAP, 2 mmol) and the mixture stirred at room temperature for 12 h. Solvents were evaporated *in vacuo*, the residue was triturated with ethyl acetate (∼100 mL), the extract was washed sequentially with 20 mL each of ice cold 2 N H_2SO_4, H_2O, and 5% aqueous $NaHCO_3$, and the organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified on a short column of silica gel using a mixture of ethyl acetate and hexane as eluent to afford the adamantane-supported bis-Ser peptides **3a**−**d** in nearly quntitative yields.

3a: yield 99%; syrup; $[\alpha]^{25}_D = +32.82$ (*c* 6.35, $CHCl_3$); IR (KBr) 3380, 2958, 2920, 2871, 1744, 1712, 1645, 1522, 1454, 1391, 1349, 1212 cm^{-1}; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.53−2.20 (m, 14H), 3.70

(13) Loew, L. M.; Rosenberg, I.; Bridge, M.; Gitter, C. *Biochemistry* **1983**, *22*, 837. Loew, L. M.; Benson, L.; Lazarovici, P.; Rosenberg, I. *Biochemistry* **1985**, *24*, 2101. Shai, Y.; Bach, D.; Yanovsky, A. *J. Biol. Chem.* **1990**, *265*, 20202. Glaser, S. M.; Cumsky, M. G. *J. Biol. Chem.* **1990**, *265*, 8808. Roise, D. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 608. Epand, R. M.; Shai,Y.; Segrest, J. P.; Anantharamaiah, G. M. *Biopolymers* **1995**, *37*, 319. Minn, A. J.; Velez, P.; Schendel, S. L.; Liang, H.; Muchmore, S. W.; Fesik. S. W.; Fill, M.; Thompson, C. B. *Nature* **1997**, *385*, 353.

(14) Cyclodepsipeptides of ring size smaller than eight residues do not normally form complexes with metal ions; their carbonyls are oriented outward. Roeske, R. W.; Kennedy, S. J. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Marcel Dekker, Inc.: New York, 1983, Vol. 7, pp 205−256.

(15) Nagaraj, R.; Mathew, M. K.; Balaram, P. *FEBS Lett.* **1980**, *121*, 365.

(s, 6H), 4.39 (m, 4H), 4.65 (m, 2H), 5.10 (s, 4H), 5.97 (d, J = 7.5 Hz, 2H), 7.31 (s, 10H).

3b: yield 90%; mp 128−130 °C; $[\alpha]^{25}_D$ = +53.24 (*c* 2.05, $CHCl_3$); IR (KBr) 3315, 3075, 2965, 2870, 1746, 1695, 1661, 1558, 1536, 1513, 1458, 1395 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 0.93 (m, 12H), 1.53−2.24 (m, 16H), 3.81 (s, 6H), 4.42 (m, 6H), 4.92 (m, 2H), 5.09 (brs, 4H), 5.65 (d, J = 7.5 Hz, 2H), 7.55 (s + m, 12H).

3c: yield 93%; mp 118−119 °C; IR (KBr) 3391, 3298, 2964, 2871, 1736, 1718, 1666, 1538, 1457 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89 (brs, 12H), 1.40−2.16 (m, 20H), 3.80 (s, 6H), 4.27 (brd, 2H), 4.51 (m, 4H), 4.92 (brs, 2H), 5.04 (brs, 2H), 5.11 (brs, 2H), 5.54 (brs, 2H), 7.32 (brs, 10H), 7.47 (brs, 2H).

3d: yield 82%; mp 148−150 °C; IR (KBr) 3312, 3078, 2965, 1747, 1697, 1660, 1544, 1532, 1461, 1378, 1249, 1211 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (brs, 24H), 1.62−2.18 (m, 22H), 3.71 (s, 6H), 4.15 (m, 2H), 4.26 (m, 2H), 4.46 (m, 2H), 4.96 (m, 4H), 5.15 (m, 2H), 6.03 (brs, 2H), 7.30 (s + m, 12H), 7.61 (brs, 2H).

Preparation of Cyclodepsipeptides 4−7. General Procedure: (a) N-Deprotection of Adamantane-Supported Bis-*N*$^\alpha$-Z-Ser-Depsipeptides 3a−d. A solution of bis N$^\alpha$-Z-peptide **3a−d** (1 mmol) in dry ethyl acetate (∼10 mL) was admixed with Pd/C, 5% (peptide/catalyst 1:0.5 w/w) and hydrogenolyzed using Parr hydrogenation apparatus. The reaction mixture after complete N$^\alpha$-deprotection (TLC) was filtered through a sintered funnel, the residue was washed with dry ethyl acetate (20 mL), and the filtrate was directly used for the coupling reaction in the next step.

(b) Condensation of N$^\alpha$-Deprotected 1,3-Adamantane Bis-Ser Depsipeptides 3a−d with 1,3-Adamantanedicarbonyl Dichloride (1). To a well-stirred and ice-cooled solution of N$^\alpha$-deprotected bis-Serdepsipeptide (1 mmol in ∼100 mL of dry EtOAc) containing 2 mmol of triethylamine was added dropwise a solution of freshly prepared 1,3-adamantanedicarbonyl dichloride (1 mmol in 50 mL of dry CH_2Cl_2) over a period of 0.5 h and the mixture stirred at room temperature for 12 h. The solvents were removed *in vacuo, and* the residue was taken up in CH_2Cl_2 (∼100 mL) and washed, sequentially, with 20 mL each of the ice-cold 2 N H_2SO_4, H_2O, and 5% aqueous $NaHCO_3$. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo* and the residue purified on a column of silica gel using either a mixture of EtOAc/hexane or $CHCl_3$/MeOH as eluent to afford adamantane-constrained Ser cyclodepsipeptides **4−7** in good yields.

4: yield 59%; mp 205−206 °C; $[\alpha]^{25}_D$ = +72.82 (*c* 3.85, $CHCl_3$); IR (KBr) 3426, 2922, 2859, 1749, 1715, 1670, 1556 (sh), 1512, 1457, 1385, 1356 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.65−2.23 (m, 28H), 3.78 (s, 6H), 4.44 (m, 4H), 4.84 (m, 2H), 6.34 (d, J = 7.5 Hz, 2H); FAB MS *m/z* 615 (100) $(M + H)^+$.

5a: yield 60%; mp 149−151 °C; $[\alpha]^{25}_D$ = −2.47 (*c* 2.2, $CHCl_3$); IR (KBr) 3324, 2917, 2864, 2807, 1743, 1653 (br), 1558, 1523, 1505, 1459, 1377 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.93, 0.95 (d, d, J = 6.9 Hz, 6.7 Hz, 6H, 6H), 1.67−2.24 (m, 30H), 3.78 (s, 6H), 4.24 (dd, J = 3.4, 7.7 Hz, 2H), 4.29 (m, 2H), 4.49 (dd, J = 3.0, 8.5 Hz, 2H), 4.89 (m, 2H), 6.15 (d, J = 8.3 Hz, 2H), 6.94 (d, J = 8.1 Hz, 2H); FAB MS *m/z* 813 (46) $(M + H)^+$.

5b: yield 55%; mp 104−105 °C; IR (KBr) 3374, 2938, 2865, 1744, 1678, 1647,1529, 1456 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.89, 0.95 (d, d, J = 6.4 Hz, 6.4 Hz, 6H, 6H), 1.55−2.24 (m, 34H), 3.78 (s, 6H), 4.32 (dd, J = 4.5, 6.8 Hz, 2H), 4.40 (dd, J = 2.2, 8.6 Hz, 2H), 4.49 (m, 2H), 4.93 (m, 2H), 6.07 (d, J = 7.7 Hz, 2H), 7.30 (d, J = 8.6 Hz, 2H); FAB MS *m/z* 841 (100) $(M + H)^+$.

6: yield 50%; mp 151−153 °C; $[\alpha]^{25}_D$ = −32.81 (*c* 1.3, $CHCl_3$); IR (KBr) 3318, 3072, 2942 (br), 2867, 1747, 1700, 1678, 1650 (br), 1544, 1514, 1474 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (m, 24H), 1.50−2.23 (m, 36H), 3.68 (s, 6H), 4.16 (t, J = 7.1 Hz, 2H), 4.31 (d, J = 5.3 Hz, 4H), 4.52 (m, 2H), 4.73 (m, 2H), 6.08 (d, J = 7.5 Hz, 2H), 6.69 (d, J = 8.3 Hz, 2H), 6.97 (d, J = 8.1 Hz, 2H); FAB MS *m/z* 1039 (100) $(M + H)^+$.

7: yield 2%; syrup; $[\alpha]^{25}_D$ = +29.49 (*c* 1.1, $CHCl_3$); IR (KBr) 3416, 2927, 2865, 1742, 1671, 1648, 1557, 1524, 1459, 1353 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.60−2.32 (m, 56H), 3.79 (s, 12H), 4.43 (m, 8H), 4.82 (m, 4H), 6.53 (d, J = 7.6 Hz, 4H); FAB MS *m/z* 1229 (65) $(M + H)^+$, 615 (23) $(M/2 + H)^+$, 308 (26) $(M/4 + H)^+$.

Preparation of Cyclo(Adm-Ser-Ser-)$_2$ (8). (a) 1,3-Adamantane Bis-Ser-Ser Depsipeptide (3e). To a well-stirred and ice-cooled mixture of Z-Ser (2 mmol), DCC, and *N*-hydroxysuccinimide (2 mmol each) in dry dichloromethane (30 mL) was added, in one lot, the *N*$^\alpha$-Z-deprotected (Pd/C, 5%, H_2) solution of **3a** (1 mmol) in dry ethyl acetate (30 mL) and the reaction mixture stirred at room temperature for 24 h. Usual peptide workup and purification of the crude product (silica gel column with $CHCl_3$/MeOH eluents) afforded the title compound in 79% yield: thick syrup; IR (KBr) 3385, 2944, 1738, 1687, 1675, 1569, 1535, 1456, 1246, 1231 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.56−2.22 (m, 14H), 3.78 (s + m, 8H), 3.99 (m, 2H), 4.38 (m, 6H), 4.83 (m, 2H), 5.11 (s, 4H), 6.03 (d, J = 6.31 Hz, 2H), 7.33 (s, 10H), 7.57 (d, J = 6.31 Hz, 2H).

(b) Coupling of Adm-Supported Bis-Ser-Ser Peptide 3e with 1,3-adamantanedicarbonyl dichloride. A freshly prepared solution of 1,3-adamantanedicarbonyl dichloride (**1**, 1 mmol) in dry dichloromethane (50 mL) was added dropwise over a period of 0.5 h to a well-stirred and ice-cooled solution of **3e** (1 mmol) in dry CH_3CN (100 mL) containing DMAP (2 mmol). After 12 h of stirring at room temperature, the reaction mixture was worked up by evaporating the solvents *in vacuo*, triturating the residue with ethyl acetate (100 mL), and washing the extract, sequentially, with 2 N H_2SO_4, H_2O, and 5% $NaHCO_3$ solution (20 mL each), drying the organic layer with anhydrous $MgSO_4$, and evaporating *in vacuo*. The residue was chromatographed on a small column of silica gel and eluted with a mixture of $CHCl_3$/MeOH to afford the title compound **8** in 53% yield: mp 200−203 °C; $[\alpha]^{25}_D$ = +63.69 (*c* 1.6, $CHCl_3$); IR (KBr) 3392, 3278, 3042, 2943, 2865, 1742, 1718 (sh), 1679, 1533, 1502 (sh), 1452 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.50−2.25 (m, 28H), 3.88 (m, 2H), 4.00 (s, 6H), 4.39 (d, J = 11.2 Hz, 2H), 4.53 (m, 2H), 4.73 (d, J = 11.2 Hz, 2H), 4.85 (brs, 2H), 4.95 (brs, 2H), 5.10 (s, 4H), 5.45 (d, J = 8.6 Hz, 2H), 7.35 (brs, 10H), 7.88 (d, J = 6.25 Hz, 2H); FAB MS *m/z* 1057 (50) $(M + H)^+$.

Synthesis of Monoadamantyl Ser-Based Cyclodepsipeptides 10, 12, and 13. Preparation of Cyclo(Adm-Ser-Cyst-Ser-) (10). (a) *N*$^\alpha$-Boc-Ser-Cyst-Ser *N*$^\alpha$-Boc-bis-depsipeptide (9). To a well-stirred and ice-cooled solution of *N*$^\alpha$-Boc-Ser-OH (10 mmol) in dry CH_2Cl_2 (∼50 mL) admixed with *N*-hydroxysuccinimide (10 mmol) and dicyclohexylcarbodiimide (DCC, 10 mmol) was added a freshly prepared solution of cystine diOMe (generated *in situ* at 0 °C from 5 mmol of cystine dimethyl ester dihydrochloride and 10 mmol of dry triethylamine in dry CH_2Cl_2). After 2 days of stirring at room temperature, the reaction mixture was filtered, the residue washed with CH_2Cl_2, the filtrate washed, sequentially, with 1 N H_2SO_4, H_2O, and 5% aqueous $NaHCO_3$ (20 mL each), dried (anhydrous Mg SO_4), and evaporated *in vacuo*, and the residue was cleaned up on a small column of silica gel using $CHCl_3$/MeOH as eluents to give the title bis-Ser peptide in 75% yield: syrup; IR (KBr) 3402, 2988, 1750, 1730, 1679, 1548, 1533, 1500 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 1.46 (s, 18H), 3.17 (d, J = 6.3 Hz, 4H), 3.78 (s, 6H), 4.0−4.49 (m, 6H), 4.87 (m, 2H), 5.88 (d, J = 7.6 Hz, 2H), 7.63 (d, J = 7.6 Hz, 2H); FAB MS *m/z* 643 (14) $(MH)^+$, 543 (42) $(M - Boc + H)^+$, 443 (100) $(M - 2 \times Boc + H)^+$.

(b) Condensation of the Bis-Ser peptide 9 with 1,3-Adamantanedicarbonyl dichloride. A dilute solution of freshly prepared 1,3 adamantanedicarbonyl dichloride (**1**, 1 mmol in 50 mL of dry CH_2Cl_2) was added dropwise over a period of 0.5 h to a well-stirred and ice-cooled solution of the bis-Ser peptide **9** (1 mmol in 100 mL of dry CH_3CN) containing DMAP (2 mmol) and the reaction mixture stirred for 12 h at room temperature. Workup as for **8** and purification of the residue on a short column of silica gel using $CHCl_3$/MeOH as eluent afforded the title cyclodepsipeptide **10** in 30% yield: thick syrup; IR (KBr) 3387, 2940, 2866, 1740, 1688, 1536, 1514, 1458, 1371, 1251 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.49 (s, 18H), 1.67−2.17 (m, 14H), 3.14 (brd, 2H), 3.35 (brd, 2H), 3.78 (s, 6H), 4.17 (m, 2H), 4.45 (brd, 2H), 4.62 (brd, 2H), 4.86 (m, 2H), 5.43 (d, J = 6.51 Hz, 2H), 7.19 (brd, 2H); FAB MS *m/z* 831 (10) $(MH)^+$, 731 (42) $(M - Boc + H)^+$, 631 (100) $(M - 2 \times Boc + H)^+$.

Preparation of Crown Ether-Containing, Adamantane-Constrained Ser Cyclodepsipeptide (12). (a) Preparation of the Precursor Bis-*N*$^\alpha$-Boc-Ser-(CH$_2$-OBzl)-Tetraethylene Glycol Ester (11). To a well-stirred and ice-cooled mixture of Boc-Ser(CH_2-OBzl)-OH (2 mmol), DCC (2 mmol), and *N*-hydroxysuccinimide (2 mmol) in dry CH_2Cl_2 (20 mL) was added a solution of tetraethylene glycol (1 mmol) in CH_2Cl_2 (∼20 mL) containing DMAP (2 mmol). After 24 h of stirring

at room temperature, the reaction mixture was workedup as for **9** and the residue chromatographed over a short column of silica gel with a mixture of ethyl acetate/hexane as eluent to afford the title compound in 60% yield: syrup; IR (KBr) 3440 (br), 2985, 2940, 2879, 1757, 1722, 1633, 1579, 1503, 1458, 1370, 1350, 1291, 1252, 1169, 1115 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 18H), 3.57−3.87 (m, 16H), 4.28 (brs, 4H), 4.48 (m, 6H), 5.43 (brd, 2H), 7.28 (brs, 10H); FAB MS *m/z* 755 (20) $(M + Li^+)$, 649 (31) $(M - Boc + H)^+$, 549 (14) $(M - 2 \times Boc + H)^+$.

(b) Condensation of O-Deprotected Bis-Ser-Tetraethylene Glycol Ester 11 with 1,3-Adamantanedicarbonyl Dichloride (1). A solution of 1,3-adamantanedicarbonyl dichloride (1 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise to a well-stirred and ice-cooled solution of 1 mmol of O-deprotected (Pd/C, 5%, H_2) **11** in dry ethyl acetate (∼100 mL) containing DMAP (2 mmol). The reaction mixture was stirred at room temperature for 12 h and worked up as described in the general procedure for **4**−**7**. The residue was chromatographed on a small column of silica gel and eluted with a mixture of ethyl acetate/hexane to afford the crown hybrid **12** in 25% yield: thick syrup; IR (KBr) 3356 (br), 2932, 2863, 1733, 1718, 1641, 1576, 1555, 1523, 1501, 1466, 1372 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 18H), 1.67−2.06 (m, 14H), 3.64 (m, 12H), 4.59 (m, 8H), 4.61 (m, 2H), 5.45 (br, 2H); FAB MS *m/z* 779 (100) $(M + Na^+)$.

Preparation of Cyclo(Adm-Ser-Val-Pyr-Val-Ser-) (13). A solution of 2,6-pyridinedicarbonyl dichloride (1 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise to a well-stirred and ice-cooled solution of N-deprotected (Pd/C, 5%, H_2) 1,3-adamantane bis Ser-Val depsipeptide **3b** (1 mmol) in dry ethyl acetate (100 mL) containing 2 mmol of triethylamine and the reaction mixture stirred for 12 h. The solvents were removed *in vacuo*, and the residue was dissolved in CH_2Cl_2 (100 mL), washed with 5% $NaHCO_3$ (20 mL), dried (anhydrous $MgSO_4$), and evaporated *in vacuo*. The residue on purification on a small column of silica gel and elution with $CHCl_3$/MeOH gave the title compound **13** in 42% yield: mp 136−137 °C; IR (KBr) 3315, 2965, 2941, 1742, 1670, 1544, 1447 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.02 (m, 12H), 1.27−1.83 (m, 14H), 2.53 (m, 2H), 3.77 (s, 6H), 4.26 (m, 2H), 4.46 (m, 4H), 4.80 (m, 2H), 7.35 (d, $J = 7.1$ Hz, 2H), 8.03 (1H, t, $J = 7.1$ Hz), 8.32 (d, $J = 7.1$ Hz, 2H), 8.67 (d, $J = 8.6$ Hz, 2H); FAB MS *m/z* 756 (71) $(M + H)^+$.

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Supporting Information Available: ^1H NMR of **5a**,**b** and **6**, ROESY NMR of **5a**,**b** and **6**, FAB-MS of **4**, **5a**,**b**, **6**−**8**, **10**, **12**, and **13**, CD spectra of **5a** and **6** in TFE, CD spectra of **5a** and **6** in aqueous MeOH, and ion-transport diagrams of **7** and **8** (22 pages). See any current masthead page for ordering and Internet access instructions.

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