# Norbornene-Constrained Cyclic Peptides with Hairpin Architecture: Design, Synthesis, Conformation, and Membrane Ion Transport

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A novel family of hairpin cyclic peptides has been designed on the basis of the use of norbornene units as the bridging ligands. The design is flexible with respect to the choice of an amino acid, the ring size, and the nature of the second bridging ligand as illustrated here with the preparation of a large number of norborneno cyclic peptides containing a variety of amino acids in ring sizes varying from 12- to 29-membered, with the choice of the second bridging ligand being a rigid norbornene (**11**, **13a**,**b**), an adamantane unit (**7a**,**b** and **8**), or a flexible cystine residue (**4a**,**b** and **10**). The presence of built-in handles (as protected COOH groups) permits the attachment of a variety of subunits as shown here with the ligation of Leu-Leu, Val-Val, or Aib-Aib pendants in **4b**, **7b**, and **13b**, respectively. This novel class of constrained cyclic peptides are demonstrated to adopt $\beta$-sheet- or hairpin-like conformation as shown by $^1$H NMR and CD spectra. Membrane ion-transport studies have shown that the norborneno cyclic peptides **4b** and **7b** containing Leu-Leu or Val-Val pendants symmetrically placed on the exterior of the ring show high efficiency and selectivity in the transport of specifically monovalent cations. This property can be attributed to the hairpin-like architecture induced by the norbornene unit since the bis-adamantano peptide **15** containing two pairs of Leu-Leu pendants on the exterior is able to transport both monovalent ($Na^+$, $K^+$) and divalent ($Mg^{2+}$/$Ca^{2+}$) cations.

## Introduction

Use of conformationally constrained organic scaffoldings/templates that can have the capability to control the shape of a peptide to a well-defined element of protein secondary structure is fast becoming the most popular approach for the construction of simple mimics of protein secondary structures.[1] Such structural mimics are not only important for a rational design of simple, low molecular weight pharmaceutical agents[2] but also provide important information on complex structure−activity relationships and valuable insights into the forces that control protein folding.[3]

Creation of constrained $\beta$-sheets or $\beta$ hairpin mimics[4] is particularly important because of their demonstrated potential as synthetic templates (TASPs) in the construction of artificial proteins and novel antibiotics. Although a considerable number of nonpeptidic template-based designs are now available[5] for $\beta$-sheet or $\beta$-hairpin mimics, most of them suffer from a common disadvantage of the dependence of $\beta$-sheet content on $\beta$-strand length and display low $\beta$-sheet character. Cyclic peptides with extra imposed constraint can readily overcome this problem. This idea is beautifully illustrated[6] in the design

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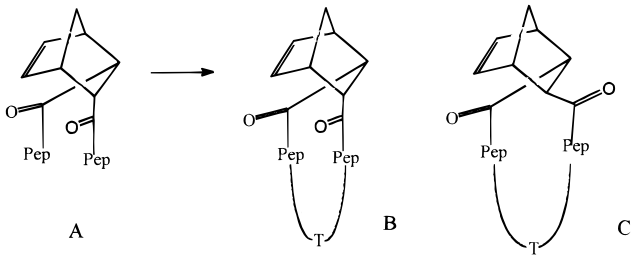
of cyclic peptide analogues of gramicidin S that exhibit a high $\beta$-sheet content.

With the main aim of creating cyclic peptides with high $\beta$-sheet content, we considered the use of a norbornene unit, a low molecular weight monoterpenoid framework, as a hairpin turn template. Our recent[7] demonstration of endo-cis (2*S*,3*R*)-norbornene dicarbonyl unit as an efficient turn-element in the design of simple models of two-stranded parallel $\beta$-sheets suggested an exciting possibility of the creation of constrained cyclic $\beta$-sheets by simply closing the free ends of the two peptide chains in (A) either by another norbornene unit or an equivalent turn template T (B). It was envisaged that while endo-cis (2*S*,3*R*) norbornene dicarbonyl unit would generate a narrow hairpin structure, the 2,3-trans isomer would lead to wider rings (C) that may show modified behavior toward membrane ion transport.




T = cystine/ adamantane/ norbornene

We describe herein the first illustration of this concept and report on the design, synthesis, and conformational preferences of norbornene-constrained macrocycles, a new class of cyclic peptides with hairpin architecture, and demonstrate their efficiency in the selective transport of monovalent alkali metal ions across lipid bilayer membranes. The design draws a parallel with Nature's strategy for the construction of gramicidin S.[8] Thus, while Nature uses two proline residues at almost opposite poles to create a hairpin architecture in gramicidin S, the present design deploys norbornene units for the same purpose. The design strategy is flexible with respect to ring size, nature of amino acid, and nature of the second turn template as shown here with the preparation of a large number of norbornene-constrained cyclic peptides containing a variety of amino acids in assorted ring sizes (12- to 29-membered) with the second template varying from rigid norbornene (2,3-cis or -trans) or adamantane framework to a more flexible cystine unit.

The presence of −COOH handles (as protected esters) in the ring provides an additional advantage for anchoring peptide or metal-complexing ligands that may act as lariats in assisting the metal ion transport through membranes.

## Results and Discussion

The 18-membered endo-cis (2*S*,3*R*) norbornene-constrained cystine-containing cyclic peptides **4a** and **4b** were prepared in a single step by the condensation of the dicarboxylic acid of 2,3-norbornene leucine bispeptide (**3**, prepared[7] from commercially available anhydride **1**, Scheme 1) with, respectively, simple cystine diOMe or its C, C′ bis Leu-Leu dipeptide.[9] The 23- and 29-membered adamantane-constrained cyclodepsipeptides **7a**, **7b** and **8** on the other hand required condensation of corresponding norborneno bis ser peptides **5a**, **5b** and **6**, respectively, with 1,3-adamantane dicarbonyl dichloride (Scheme 1) in the presence of 4, 4′-dimethyl amino pyridine (DMAP).

The synthesis of 2,3-*trans*-norbornene-constrained 12- and 24-membered cystine cyclic peptides **10** and **11** was achieved in a single step by the condensation of 2,3-*trans*-norbornene dicarbonyl dichloride (**9**) with cystine dimethyl ester dihydrochloride in dry dichloromethane in the presence of triethylamine (Scheme 2). The cyclic-(NBE-Cyst) monomer and (NBE-Cyst)$_2$ dimer (**10** and **11**, respectively) were easily separated on a column of silica gel using a gradient elution with chloroform/methanol and identified by FAB MS. A trace amount of trimer cyclo(NBE-Cyst)$_3$ was also isolated as shown by FAB MS. The preparation of Ser-based bisnorborneno cyclic peptides **13a** and **13b** required a two-step procedure. In the first step, Ser-OMe or Ser-Aib-Aib-OMe was condensed with trans norborneno (LeuOH)$_2$ (generated from the diester prepared by direct condensation of LeuOMe with **9**) to yield the corresponding norbornene-supported bis-di- or bis-tetrapeptide. A repeat condensation with norbornene dicarbonyl dichloride in the presence of DMAP gave the desired bisnorborneno peptides **13a** and **13b** in modest yields.

The cyclic peptide **15** containing exclusively the adamantane templates was considered as an appropriate control for comparison with the norborneno cyclic peptides in their structural preferences and ion-transport behavior. For the synthesis of bisadamantano cyclic peptide **15** containing two pairs of Leu-Leu pendants symmetrically placed on the exterior of the ring, C,C′-Leu-Leu extended Cystine peptide **14** was coupled with 1,3-adamantane dicarbonyl dichloride under high dilution conditions (Scheme 3). The structure of **15** was fully supported by spectral and analytical data.

The 2,3-*cis*- and *trans*-norbornene-constrained cyclic peptides thus prepared were fully characterized by spectral and analytical data and were examined for conformational preferences by $^1$H NMR and CD studies.

**Conformational Studies in Solution.** The norbornene-constrained cyclic peptides listed in Schemes 1 and 2 showed high solubility in a wide range of organic solvents, including the apolar solvent chloroform. The 400 MHz $^1$H NMR spectra in $CDCl_3$ and in DMSO-$d_6$ were extremely well resolved (particularly for **4a**,**b**, **7a**, **10**, and **11**) with sharp resonances. The lack of concentration dependence of NH chemical shifts over the range 2−10 mM suggested absence of aggregation effects. The wide dispersion of NH and C$^\alpha$H resonances facilitated complete assignment and indicated a well-defined conformational species in solution. All the backbone proton resonances could easily be assigned using a combination of TOCSY and ROESY experiments. The relevant NMR parameters for NH protons and their significant NOEs in norbornene-constrained cystine cyclic peptides **4a** and **4b** are shown in Table 1. Table 2 presents similar parameters

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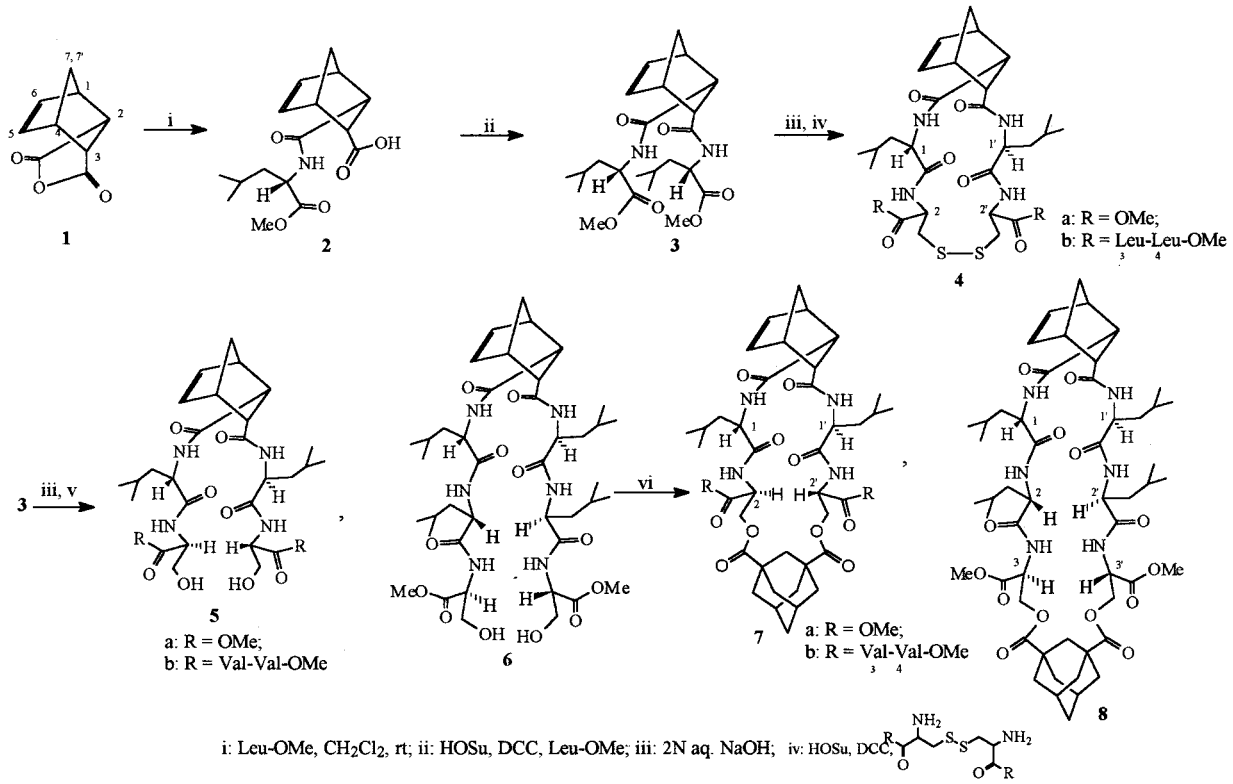
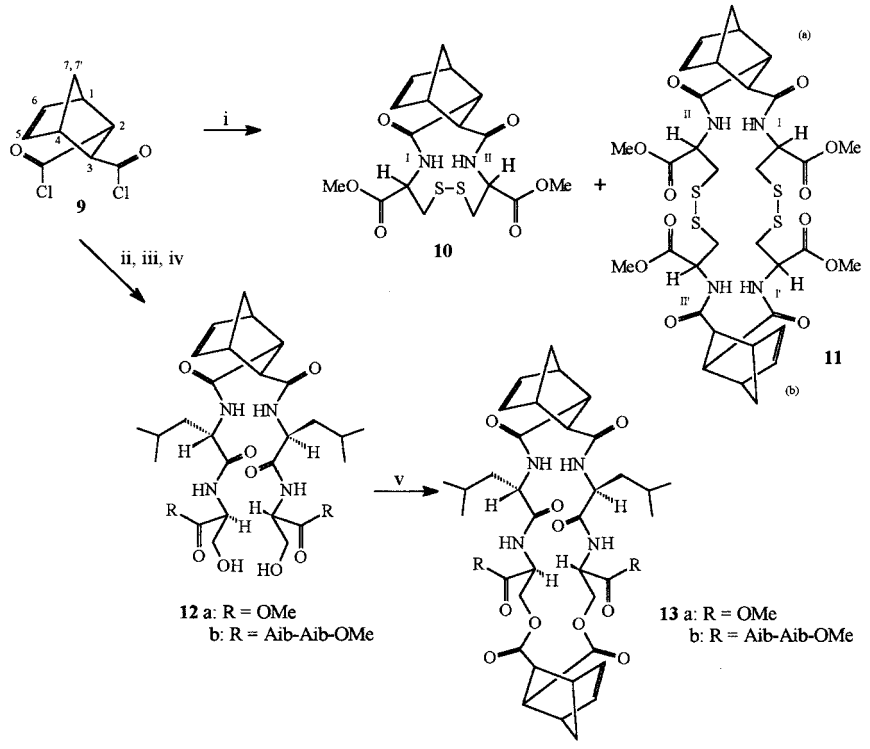
**Scheme 1**




**Scheme 2**




for NH protons in trans norbornene-constrained cystine cyclic peptides **10** and **11**.

The presence of medium to strong intrastrand sequential NOE connectivities $d_{\alpha N(i,i+1)}$ in all the norbornene-supported peptides suggested domination of $\beta$-sheet conformers in solution. The temperature dependence of amide protons in **4a**,**b**, **7a**, **10**, and **11** was studied in DMSO-$d_6$ over the temperature range of 303−343 K

**Scheme 3**

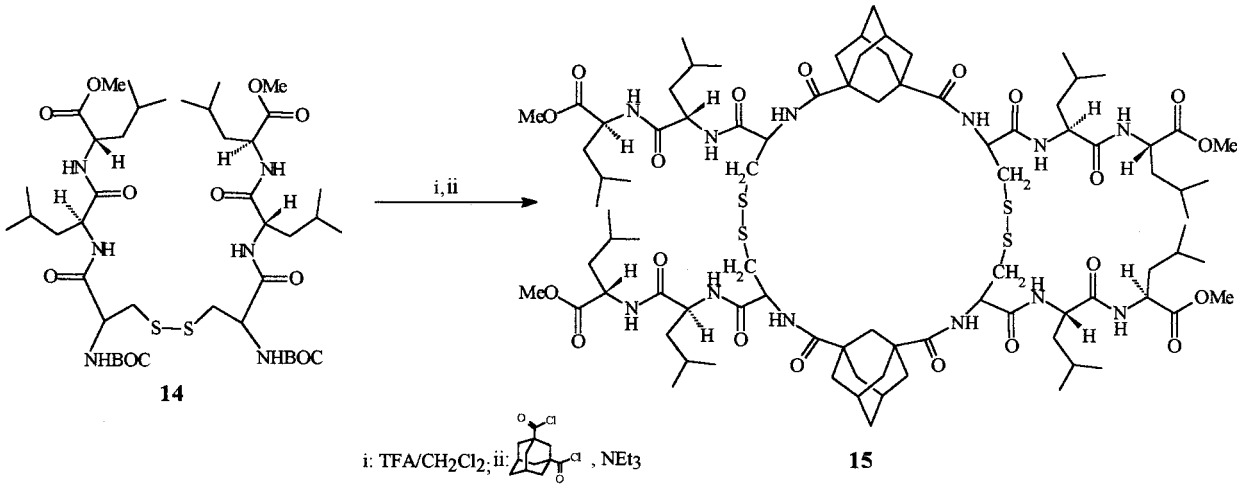



**Table 1. Characteristic $^1$H NMR (400 MHz, DMSO-$d_6$) Parameters for NH Protons and Significant NOEs in 4a and 4b**

| | cyclo(NBE-Leu-Cyst-Leu) (**4a**) | | | | cyclo(NBE-Leu-Cyst(Leu-Leu)$_2$-Leu-) (**4b**) | | | |
|---|---|---|---|---|---|---|---|---|
| NH | $\delta$ (ppm) | $J_{N,C\alpha}$ (Hz) | d$\delta$/d$T$ (ppb/K) | significant NOEs | $\delta$ (ppm) | $J_{N,C\alpha}$ (Hz) | d$\delta$/d$T$ (ppb/K) | significant NOEs |
| 1 | 7.79 | 6.1 | −3.2 | NBE H2 (s) | 8.12 | 5.0 | −3.8 | NBE H2(s); 2 NH (w) |
| 1′ | 7.87 | 7.5 | −2.4 | NBE H3(s); 2′ NH (w) | 8.10 | 5.7 | −3.4 | NBE H3 (s) |
| 2 | 8.29 | 7.6 | −5.0 | 1α (s); 1′$\beta$(w) | 8.65 | 7.1 | −3.2 | 1α (m); 3 NH (w) |
| 2′ | 8.56 | 6.1 | −3.4 | 1′α (s); 1$\beta$(w) | 8.14 | 8.2 | −4.1 | 1′α (m); 3′ NH (w) |
| 3 | | | | | 7.18 | 8.2 | +0.9 | 2 α (w); 4 NH (w) |
| 3′ | | | | | 7.82 | 8.4 | −3.6 | 2′ α (s) |
| 4 | | | | | 7.74 | 7.8 | −2.2 | 3α (m) |
| 4′ | | | | | 8.17 | 7.8 | −4.8 | 3′α (m) |

**Table 2. Characteristic $^1$H NMR (400 MHz, $CDCl_3$) Parameters for NH Protons and Significant NOEs in 10 and 11**

| | cyclo(NBE-Cyst-) (**10**) | | | | cyclo[(NBE- Cyst-)$_2$] (**11**) | | | |
|---|---|---|---|---|---|---|---|---|
| NH | $\delta$ (ppm) | $J_{N,C\alpha}$ (Hz) | d$\delta$/d$T$ (ppb/K) | significant NOEs | $\delta$ (ppm) | $J_{N,C\alpha}$ (Hz) | d$\delta$/d$T$ (ppb/K) | significant NOEs |
| I | 6.77 | 9.3 | −5.2 | NBE H2(s) | 7.41 | 7.8 | −2.7 | NBE H3a(s); I$\beta$ (s) |
| II | 6.81 | 8.6 | −4.7 | NBE H3(s) | 7.30 | 7.7 | −3.6 | NBE H2a(s); NBE H6a(s); II$\beta$ (s) |
| I′ | | | | | 7.23 | 7.6 | −3.8 | NBE H2b(s); NBE H6b(s); I′$\beta$ (s) |
| II′ | | | | | 7.42 | 8.0 | −2.6 | NBE H3b(s); II′$\beta$ (s) |

(Supporting Information). The spread over of d$\delta$/d$T$ values from as low as 0.9 to >5 ppb/K (Tables 1−3) suggested involvement of considerable number of NH protons in intramolecular hydrogen bonding.[10] This observation was supported by relatively low-field chemical shift (7−9 ppm) values for most NH protons. Although weak, the presence of long-range NOEs (interstrand) between the NH($i$ + 1) of strand I and the $\beta$($i$) proton of strand II in the ROESY spectra of **4a** (Supporting Information) and **4b** (Figure 1b) provided further evidence for parallel $\beta$-sheet conformation.

Interestingly, cyclic peptide **4b** showed weak NH−NH NOEs (Figure 1a), indicating some population of conformers in the helical region presumably involving Leu-Leu pendants. In the NMR spectra of 2,3-*trans*-norbornene-supported cyclic peptides **10** and **11**, strong NOEs were observed between the NH protons and norbornene ring protons in $d_{\alpha N(i,i+1)}$ relationship. Additional NOEs seen in the ROESY spectrum of **11** (Figure 3) particularly between NH and the cystine $\beta$ protons suggested $\beta$-turn features. Strong NOEs observed between NH II−NBE H6a and NH I′-NBE H6b (NBE = norbornene ring) supported the trans orientation of 2,3 substituents in **11**. Although cyclic peptide **10** exhibited high-temperature coefficients (∼5ppb/K) ruling out any internal NH- - -O═C bonding, the relatively low values for temperature coefficients (2−4 ppb/K) exhibited by **11** suggested involvement of NH protons in interstrand intramolcular hydrogen bonding. Both **10** and **11** showed consistently high values (7.6−9.3 Hz) for the $^3J_{HN\alpha}$ coupling constants (Table 2), supporting $\beta$-sheet conformation.

The adamantane-containing norborneno cyclic peptides **7a** and **7b** also exhibited strong $d_{\alpha N(i,i+1)}$ NOEs, suggesting $\beta$-sheet features. The presence of weak NH−NH intrastrand cross-peaks in **7b** (as was observed in **4b**) indicated some population of helical conformers (due to participation of Val-Val pendants). Most NH protons in **7a** and **7b** showed high (>8 Hz) $^3J_{HN\alpha}$ coupling constants and low-temperature coefficients (1−4 ppb/K), which further supported $\beta$-sheet structure (Table 3). The presence of long-range interstrand NOEs between Leuα and

(10) In polar aprotic solvents such as DMSO-$d_6$, the −d$\delta$/d$T$ values > 5 ppb/K are interpreted for a solvent-exposed NH while values < 3ppb/K suggest an amide NH that is shielded from solvent through either intramolecular hydrogen bonding or steric shielding ((a) Zerkout, S.; DuPont, V.; Aubrey, A.; Vidal, J.; Collet, A.; Vicherat, A.; Marraud, M. *Int. J. Pept. Protein Res.* **1994**, *44*, 378. (b) Prasad, S.; Rao, R. B.; Balaram, P. *Biopolymers* **1995**, *35*, 11. (c) Toniolo, C.; Bonora, G. M.; Stavropoulous, G.; Cordopatis, P.; Theodoropoulos, D. *Biopolymers* **1986**, *25*, 281. (d) Aubrey, A.; Chung, M. T.; Marraud, M. *J. Am. Chem. Soc.* **1985**, *107*, 1825. (e) Sakakibara, S. *Biopolymers* **1995**, *37*, 17).

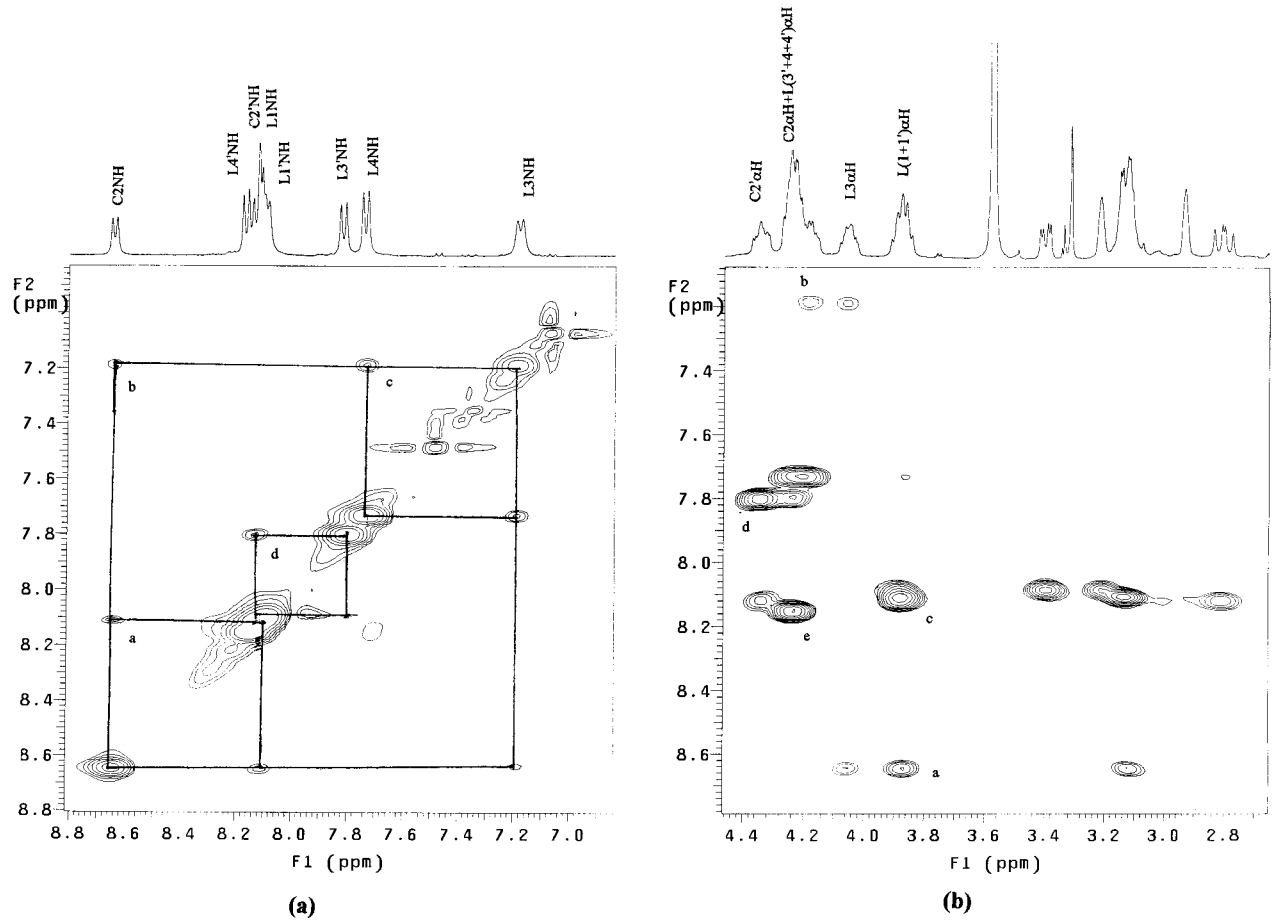



**Figure 1.** Partial 400 MHz ROESY spectra of **4b** in DMSO-$d_6$ showing (a) NH−NH (b) NH−C$^{\alpha}$H ROEs. Significant ROEs in (a) L1NH−C2NH, C2NH−L3NH, L3NH−L4NH, and C2′NH−L3′NH are denoted by a, b, c, and d, respectively, and in (b) C2NH−L1α, L3NH−C2α, C2′NH−L1α, L3′NH−C2′α, and L4′NH−L3′α are denoted by a, b, c, d, and e, respectively.

Ser$\beta$ protons in the ROESY spectrum of **7a** (Figure 2) provided conclusive evidence for the $\beta$-sheet structure. Figure 2 presents the ROESY spectrum of **7a**.

**Circular Dichroism Studies.** CD studies were carried out in trifluroethanol (TFE) at 298K. Table 4 summarizes the maxima and minima in the CD spectra of the norbornene-constrained cyclic peptides listed in Schemes 1−3. Norbornene-constrained cystine cyclic peptides **4a** and **4b** containing only one norbornene unit in the ring showed a positive CD band at 218 nm attributed to a type-II $\beta$-turn conformation. The shape of the CD spectra in **4a** and **4b** is quite similar, indicating that the Leu-Leu pendant chromophores hardly make any contribution except for increase in the ellipticity value (Table 4). Introduction of an adamantane ring into the backbone causes a marked change in the CD spectra. The adamantane-containing norborneno cyclic peptide **7a** and **7b** showed a negative CD band at ∼220 nm. The intense minimum at 222 nm in **7a** with high ellipticity value is typical of a $\beta$-sheet structure. The sheet structure was maintained in aqueous methanol as shown by almost identical CD values. The CD spectrum of **7b** (with Val-Val pendants) showed some resemblance to the α-helix CD, consistent with NMR results. Introduction of another leucine residue into the ring of **7a** shifted the minima toward red. Thus, the CD of cyclic peptide **8** showed a negative band at ∼235 nm with approximately the same ellipticity value. The simple norborneno cystine cyclic peptides **10** and **11** were shown to adopt typical type II $\beta$-turn structures with red shifts of $\lambda$ in **11** of 14 nm. However, more interesting results were obtained with bisnorborneno cyclic peptides **13a** and **13b**. Both open (**12a**) and cyclized (**13a**) compounds showed remarkably similar CD with minima at 222 nm and ellipticity values of 38 000 and 51 400, respectively, thus indicating that the Leu-Ser sequence even with one norbornene unit is able to adopt a sheet structure. Cyclization with a second norbornene unit, however, increases the ellipticity value. While open norborneno bispeptide **12b** showed unordered structure, the cyclic peptide **13b** (with Aib-Aib pendants on the ring) showed development of features of a helical conformation. A large wavelength minimum at ∼224 nm having an ellipticity of −32 000, accompanied by a minimum at 205 nm having an ellipticity value −70 000 in **13b** is completely consistent with NMR results. The CD spectrum of **13b** is typical of type III $\beta$-turn normally found in Aib-containing peptides. As a control, the CD spectrum of bis-adamantano cyclic peptide **15** (with Leu-Leu pendants anchored on the exterior of the ring) showed a typical type II $\beta$-turn CD maxima at ∼220 nm. Interesingly, the cyclic peptide **15** with or without Leu-Leu pendants showed an almost similar CD spectrum showing negligible influence of side-chain chromophores on the overall secondary structure. The above results have clearly shown that CD spectra of norborneno constrained cyclic peptides indicate largely $\beta$-sheet conformation as also shown by NMR studies.

**FT-IR Studies.** The FT-IR studies conducted in $CHCl_3$ at 298 K at 10 mM concentration showed the presence

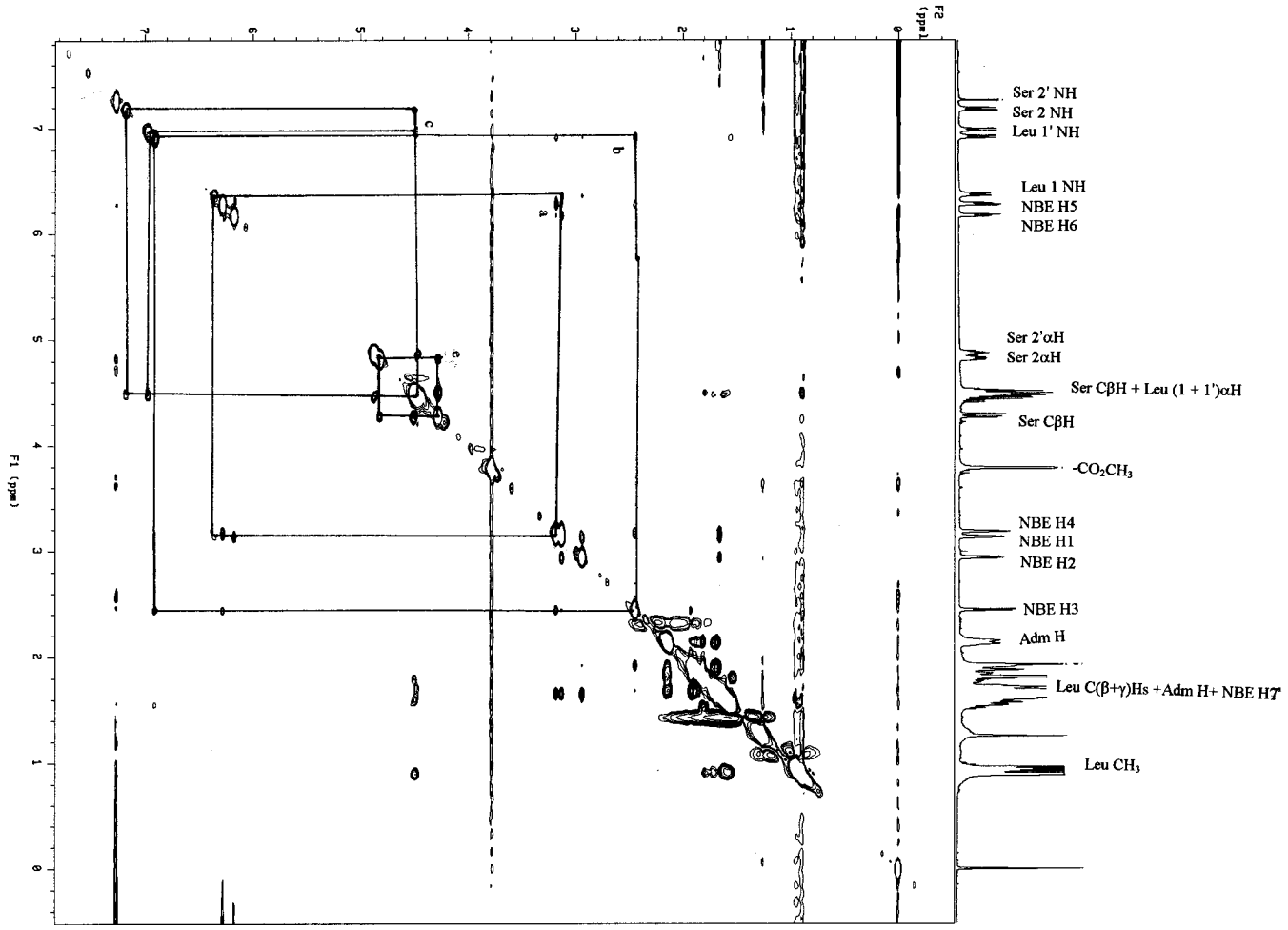



**Figure 2.** 400 MHz ROESY spectrum of **7a** in $CDCl_3$. The prominent ROE cross-peaks L1NH−NBEH1, L1′NH−NBEH3, S2NH−L1αH, S2′NH−L1′αH, and L1′αH−S2$\beta$H are denoted by the letters a, b, c, d, and e, respectively.

of intramolecular hydrogen bonding, thus corroborating NMR results. All norborneno cyclic peptides (with the sole exception of cyclo (NBE-Cyst), **10**, which showed only one band at ~3420 $cm^{-1}$) exhibited two bands in the NH stretch region. While the more intense band at ~3320 $cm^{-1}$ was attributed to intramolecularly hydrogen-bonded NH because of its concentration independent nature, the band at ~3430 $cm^{-1}$ was assigned to the free NH group.

Interestingly, while bisnorborneno cyclic peptides show strong intramolecular hydrogen bonding with the ~3320 $cm^{-1}$ band almost double the intensity of the 3420 $cm^{-1}$ band, the adamantane-containing norborneno cyclic peptides show just the reverse trend with 3420 $cm^{-1}$ band becoming more intense. The FT-IR results are in agreement with CD data.

**Membrane Ion-Transport Studies.** With a variety of norbornene-constrained cyclic peptides containing a single norbornene unit (**4a**,**b**, **10**) or a norbornene unit in conjunction with an adamantane ring (**7a**,**b**, **8**) or two norbornene units (**11**, **13a**,**b**), we set out to measure the capability of these molecules to transport ions in model membranes. Using fluorescent dye method[11] with valinomycin as a standard reference, it was found that while most cyclic peptides failed to show appreciable ion transport, the cyclic peptides **4b** and **7b** with a pair of, respectively, Leu-Leu and Val-Val pendants anchored on the exterior of the ring showed high promise. As shown in Figure 4A, the cyclic peptide **7b** was able to dissipate the diffusion potential created by valinomycin (Vm), indicating its capability to conduct $Na^+$ ions across the lipid bilayer. Figure 4B shows that **7b** was also able to transport $Li^+$ ions across the lipid bilayer with almost the same efficiency. However, **7b** was totally unable to transport the divalent cations $Ca^{2+}$ and $Mg^{2+}$ as indicated in Figure 4C. That **7b** is specific for monovalent cations was also shown by the fact that $K^+$ ions are transported out in the presence of $CaCl_2$ or $MgCl_2$ in the external medium. Figure 4D indicates that even Tris is transported by **7b**. In this experiment, the diffusion potential is set up by Vm in the presence of sucrose in the external medium. Addition of **7b** results in change of fluorescence only when Tris is present in the internal medium. Furthermore, cyclic peptide **7b** was able to set up a diffusion potential in POPC vesicles when sucrose was present in the external medium.

The ion transport experiments with **4b** showed (Figure 5) that like **7b**, only the monovalent cations are transported by **4b**. Figure 5A shows the dissipation of diffusion

(11) (a) Loew, L. M.; Rosenberg, I.; Bridge, M.; Gitter, C. *Biochemistry*, **1983**, *22*, 837. (b) Loew, L. M.; Benson, L.; Lazorovici, P.; Rosenberg, I. *Biochemistry* **1985**, *24*, 2101. (c) Shai, Y.; Bach, D.; Yanovsky, A. *J. Biol. Chem.* **1990**, *265*, 20202. (d) Glaser, S. M.; Cumsky, M. G. *J. Biol. Chem.* **1990**, *265*, 8808, (e) Roise, D. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 608. (f) Epand, R. M.; Shai, Y.; Segrest, J. P.; Anantharamaiah, G. M. *Biopolymers* **1995**, *37*, 319. (g) 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.

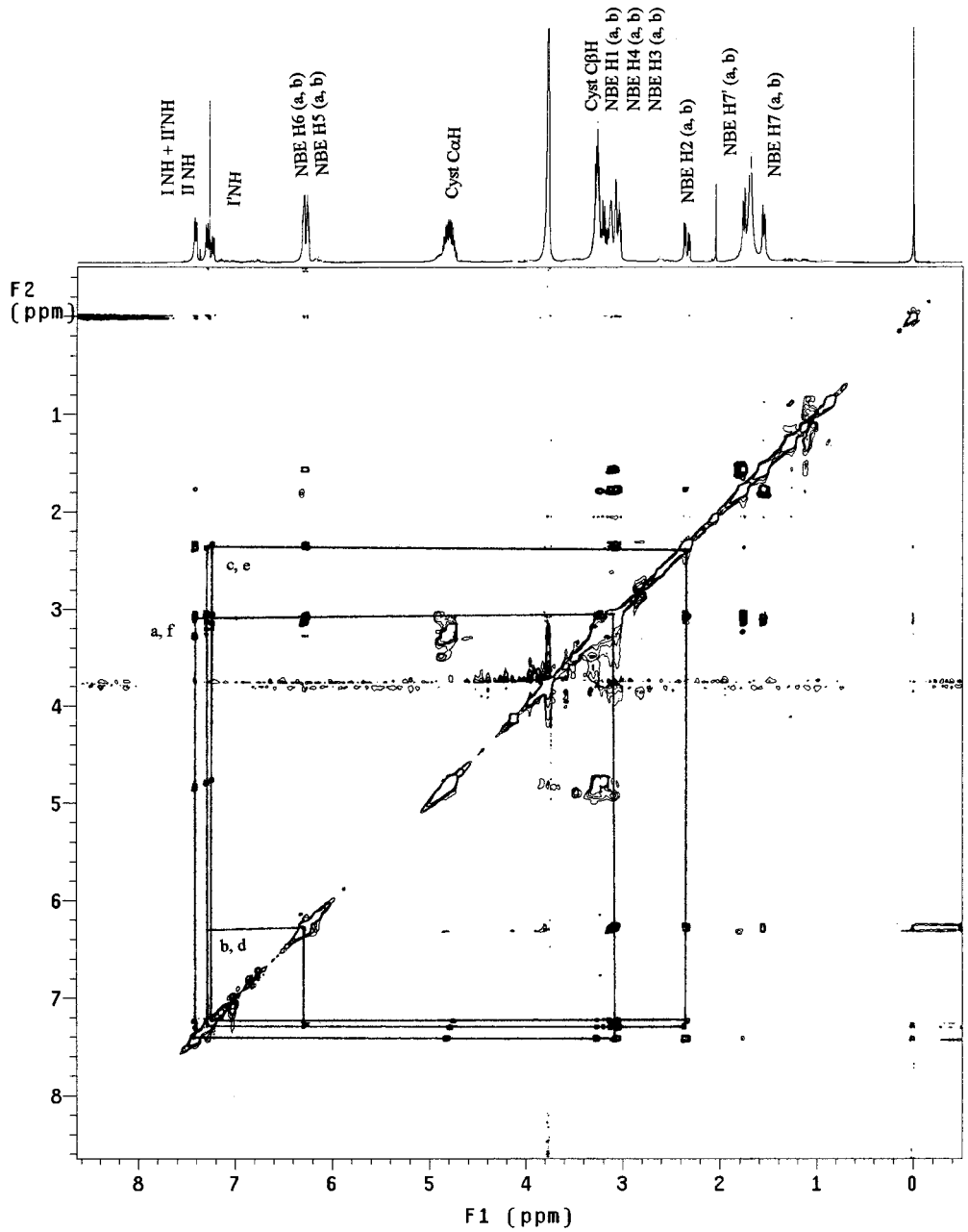



**Figure 3.** 400 MHz ROESY spectrum of **11** in $CDCl_3$. The prominent ROE cross-peaks INH−H3a, I′NH−H6b, I′NH−H2b, IINH−H6a, IINH−H2a, and II′NH−H3b are denoted by the letters a, b, c, d, e, and f, respectively. The NH−C$^{\beta}$Hs cross-peaks are not marked.

**Table 3. Characteristic $^1$H NMR (400 MHz, $CDCl_3$) Parameters for NH Protons and Significant NOEs in 7a[a]**

| | cyclo(NBE-Leu-Ser-Adm-Ser-Leu-) (**7a**) | | | |
|---|---|---|---|---|
| NH | $\delta$ (ppm) | $J_{N,C\alpha}$ (Hz) | d$\delta$/d$T$ (ppb/K) (DMSO-$d_6$) | significant NOEs |
| **1** | 6.37 | 8.8 | −1.3 | NBE (H1, H2) (s) |
| **1′** | 6.92 | 8.2 | −1.4 | NBE (H3, H4) (s) |
| **2** | 6.98 | 8.3 | −1.8 | 1α (s) |
| **2′** | 7.18 | 8.3 | −2.0 | 1′α (s) |

[a] The VT studies were carried out in DMSO-$d_6$.

**Table 4. CD Spectral Parameters (TFE, 298 K) for Norbornene-Supported Cyclic Peptides Listed in Schemes 1−3**

| cyclic peptide | $\lambda$ (nm) | $[\theta]_M \times 10^{-3}$ |
|---|---|---|
| **4a** | 218 | 9.4 |
| **4b** | 218 | 19.8 |
| **7a** | 222 | −19.5 |
| **7b** | 219 | −13.2 |
| **8** | 235 | −13.0 |
| **10** | 208 | 120 |
| **11** | 222 | 150 |
| **13a** | 222 | −51.4 |
| **13b** | 205, 224 | −70, −32 |
| **15** | 220 | 24.2 |

potential set up by Vm in lipid vesicles by **4b**. Even at a concentration of lipid/peptide ratio of 3000:1, **4b** was found to be active in transport of $Na^+$ ions. The cyclic peptide **4b** could also set up a diffusion potential (unlike **7b**) as shown in Figure 5B, indicating that it can transport $K^+$ ions from the lumen of the vesicles to the exterior. The drop, however, is less than the Vm experiment presumably due to influx of $Na^+$ ions into the vesicles.

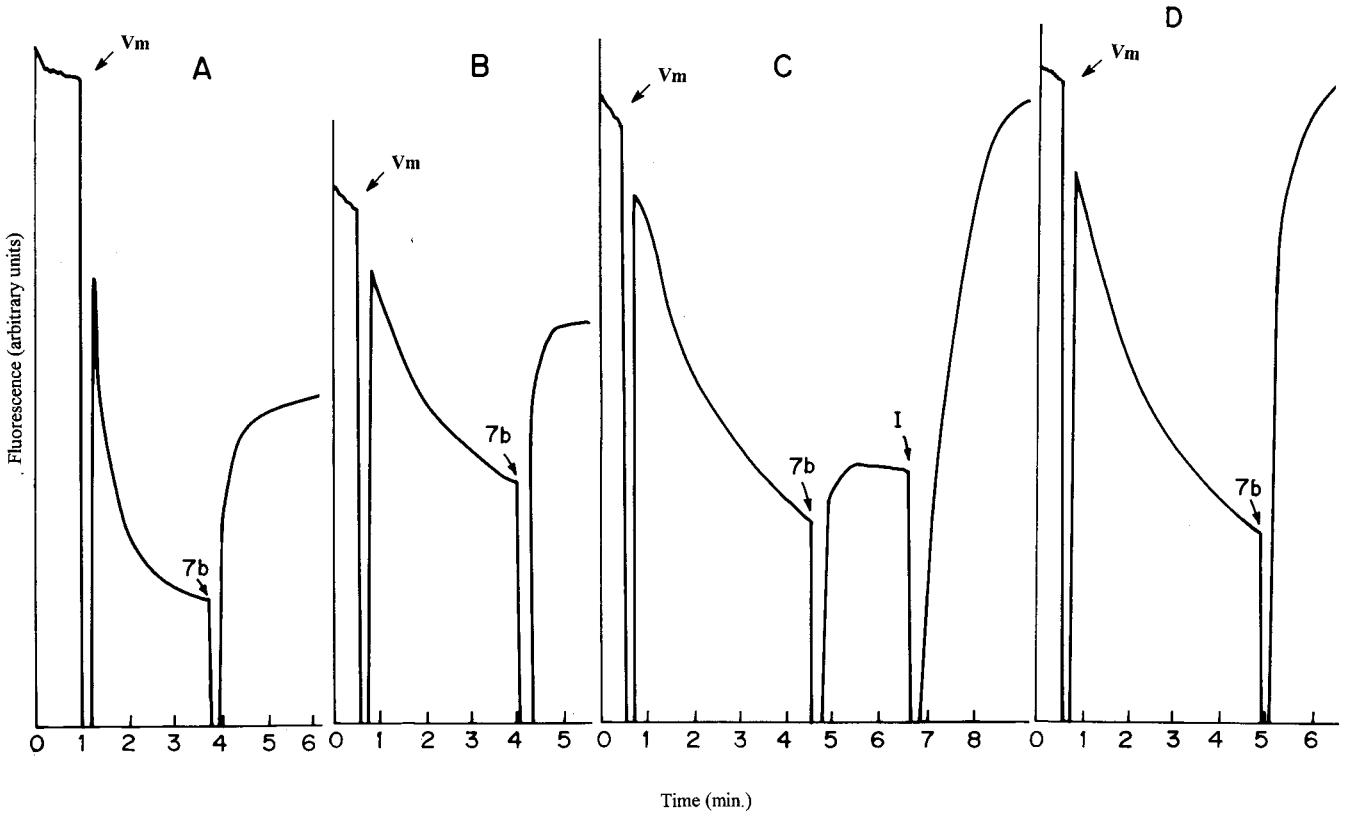



**Figure 4.** Effect of the addition of norbornene-cyclic peptide **7b** on the diffusion potential set up by valinomycin in large unilamellar vesicles (LUVs) of palmitoyl oleoyl phosphatidyl choline (POPC). LUVs were diluted into media of different composition: (A) 150 mM NaCl, (B) 150 mM LiCl, (C) 150 mM $CaCl_2/MgCl_2$ and (D) 300 mM sucrose in 10 mM Tris. Parts A, B, and D show that **7b** (1.2 $\mu$M) is effective in transporting $Na^+$, $Li^+$, and $Tris^+$ at a lipid/Vm ratio of 50:1. $Ca^{2+}/Mg^{2+}$ ions are not transported as shown in part C. The arrows Vm, **7b** and I denote the points of addition of valinomycin, norborneno cyclic peptide **7b**, and laslocid (a calcium ionophore), respectively.

As a control, cyclic peptide containing two adamantane units (**15**) was studied for ion transport capability (Supporting Information). The results showed that despite the presence of four Leu-Leu pendants on the exterior, the efficiency of **15** was found to be almost same as its parent analogue (without Leu-Leu pendants). The adamantano cyclic peptide **15**, unlike norbornenopeptides **4b** and **7b**, was able to transport both monovalent and divalent cations showing only slight preference for $Na^+$ ions (Supporting Information). Thus, in conclusion, membrane ion-transport studies have shown that the norbornene unit alone is not able to assist the cyclic peptides in metal ion transport as was shown by the inability of mono norborneno peptides **4a** and **10** and by bisnorborneno peptides **13a** and **13b**. The presence of adamantane unit in norborneno peptides improves the required membrane permeability but that in itself is not sufficient as was shown by the failure of adamantane-containing norborneno cyclic peptides **7a** and **8**. Attachment of hydrophobic peptide ligands, for example, Leu-Leu (as in **4b**) and Val-Val (as in **7b**), creates the optimum structure for membrane transport of metal ions. Although adamantane-containing cyclic peptides have previously been shown[12,13] by us to be efficient metal ion transporters in membranes, the present design incorporating the norbornene nucleus clearly shows the additional advantage of selectivity only for monovalent cations. Further experiments to ascertain the nature and mechanism of ion transport are under study.

In summary, the norbornene-constrained cystine or adamantane-bridged cyclic peptides described here represent a new class of membrane ionophores. The propensity of the norborneno-cyclic peptides to adopt a $\beta$-sheet like conformation has been demonstrated by $^1H$ NMR and CD studies. The novel hairpin architecture and the demonstrated high efficiency of these molecules for selective monovalent cation transport in model membranes combined with their extremely simple and direct synthesis from commercially available starting materials is expected to provide additional incentives for future designs of norbornene-based ionophores.

## Experimental Section

All amino acids used were of L-configuration. Melting points are uncorrected. $^1H$ NMR ROESY experiments were performed using 0.2 and 0.3 s mixing time with pulsed spin locking with 30° pulses and 2 kHz spin locking field. FAB MS were obtained using *m*-nitrobenzyl alcohol as the matrix. The circular dichroism (CD) spectra were recorded on JASCO J 20 spectropolarimeter in quartz cells of 1 mm path length at 25 °C ($\theta_M$ = molar ellipticity). 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.

For ion transport study, large unilamellar vesicles (LUVs) of palmitoyl oleoyl phosphatidyl choline (POPC) were prepared

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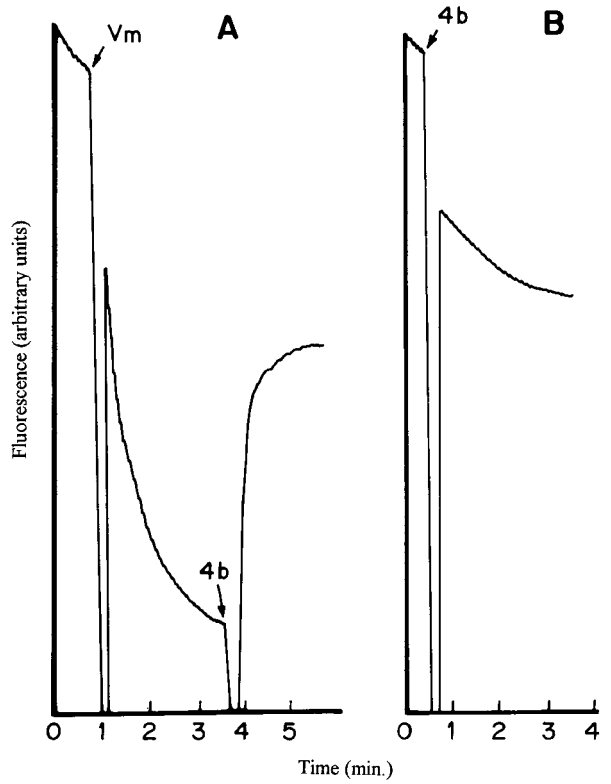


**Figure 5.** (A) Dissipation of the diffusion potential set up by valinomycin (at a lipid/Vm ratio 50:1) by norborneno cyclic peptide **4b** (1.2 $\mu$M) indicating transport of $Na^+$. (B) **4b** is able to set up a diffusion potential on its own, indicating its capability to transport $K^+$ from the lumen of the vesicles to the exterior.

by the extrusion method[14] in Hepes (5 mM, pH 7.4, 150 mM KCl). Vesicles were diluted 100 fold into 5mM Hepes (pH 7.4) containing either 150 mM NaCl, 150 mM LiCl, 150 mM $CaCl_2$/$MgCl_2$, or 300 mM sucrose. Fluorescent dye 3,3′-bis (ethylthio)-dicarbocyanine iodide [dis-$C_2$-(5)] was then added followed by valinomycin (1.2 $\mu$M; lipid:Vm ratio 50:1). Fluorescent spectra were recorded on a Hitachi 4010 spectrofluorimeter at 25 °C with $\lambda_{ex} = 620$ and $\lambda_{em} = 670$ nm.. The ion-transport capability of norborneno cyclic peptides was evaluated by monitoring the dissipation of a valinomycin-mediated $K^+$ diffusion potential and creating a diffusion potential like valinomycin.[11]

**Preparation of Cyclo(NBE-Leu-Cyst-Leu-) (4a) and Cyclo(NBE-Leu-Cyst(Leu-Leu)$_2$-Leu) (4b).** To a well-stirred and ice-cooled solution of biscarboxylic acid of **3** (1 mmol) in dry $CH_2Cl_2$ (~10 mL) or a mixture of dry DMF (~0.5 mL) and $CH_2Cl_2$ (~10 mL) was added, sequentially, N-OH succinimide and DCC (2 mmol each). After 15 min of stirring, the reaction mixture was treated with freshly prepared free base of cystine diOMe[9] (1 mmol) or C,C′ extended cystine bispeptide [$NH_2$-Cyst(Leu-Leu-OMe)$_2$-$NH_2$, (1 mmol)- prepared by N,N′ deprotection of bis-Boc-Cyst(Leu-Leu-OMe)$_2$: mp 130−132 °C; IR (KBr) 3319, 1752, 1703, 1669, 1655, 1548, 1523 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 0.90 (24H, d, $J$ = 5.5 Hz), 1.47 (18H, s), 1.64 (12H, m), 2.87 (2H, m), 3.01 (2H, m), 3.72 (6H, s), 4.54 (4H, m), 4.74 (2H, m), 5.58 (2H, d, $J$ = 8.6 Hz), 6.81 (2H, d, $J$ = 7.0 Hz), 7.84 (2H, d, $J$ = 7.5 Hz)] and left stirred at room temperature for 24 h. The precipitated DC-urea was filtered, the residue was washed with $CH_2Cl_2$ (3 × 20 mL), and the combined filtrates were washed, sequentially, with ice-cold 2 N $H_2SO_4$, $H_2O$, and saturated aqueous $NaHCO_3$ (20 mL each) and dried (anhyd $MgSO_4$), and solvent was removed in vacuo. The residue was purified by column chromatography on silica gel and eluted with $CHCl_3$/MeOH (gradient) to afford the title norbornene-supported cyclic peptides.

**Selected Data.** Cyclo(NBE-Leu-Cyst-Leu-) (**4a**): yield 40%; mp 109−110 °C; $[\alpha]^{29}_D$ +16.783 (*c* 1.18, $CHCl_3$); IR (KBr) 3321-(br), 1748, 1696(sh), 1672(sh), 1658, 1555, 1532 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.83 (12H, m), 1.21 (1H, d, $J$ = 8.1 Hz), 1.26 (1H, d, $J$ = 8.1 Hz), 1.36−1.69 (6H, m), 2.86 (1H, m), 2.96 (4H, m), 3.12 (1H, m), 3.15 (1H, m), 3.26 (1H, m), 3.59 (3H, s), 3.61 (3H, s), 3.94 (1H, m), 4.09 (1H, m), 4.25 (1H, m), 4.43 (1H, m), 5.92 (1H, m), 6.22 (1H, m), 7.79 (1H, d, $J$ = 6.1 Hz), 7.87 (1H, d, $J$ = 7.5 Hz), 8.29 (1H, d, $J$ = 7.6 Hz), 8.56 (1H, d, $J$ = 6.1 Hz); FAB MS *m/z* 641 (100) $(MH)^+$. Anal. Calcd for $C_{29}H_{44}N_4O_8S_2$: C, 54.35; H, 6.92; N, 8.74. Found: C, 54.59; H, 7.09; N, 8.65.

**Cyclo(NBE-Leu-Cyst(Leu-Leu)$_2$-Leu) (4b):** yield 29%; syrup; $[\alpha]^{29}_D$ −52.038 (*c* 0.9, $CHCl_3$); $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.74−0.92 (36H, m), 1.22−1.24 (2H, m), 1.37−1.66 (18H, m), 2.80 (1H, m), 2.93 (1H, brs), 3.13 (4H, m), 3.22 (1H, brs), 3.40 (1H, m), 3.58 (6H, s), 3.89 (2H, m), 4.19 (1H, m), 4.26 (4H, m), 4.33 (1H, m), 5.88 (1H, m), 6.25 (1H, m), 7.18 (1H, d, $J$ = 8.2 Hz), 7.74 (1H, d, $J$ = 7.8 Hz), 7.82 (1H, d, $J$ = 8.4 Hz), 8.10−8.17 (4H, m), 8.65 (1H, d, $J$ = 7.1 Hz); FAB MS *m/z* 1093 (100) $(MH)^+$, 1115 (47) $(M + Na^+)$. Anal. Calcd. for $C_{53}H_{88}N_8O_{12}S_2$: C, 58.21; H, 8.11; N, 10.24. Found: C, 57.98; H, 8.07; N, 10.30.

**Preparation of Cyclo(NBE-Leu-Ser-Adm-Ser-Leu-) (7a) and Cyclo(NBE-Leu-Ser(Val-Val)-Adm-Ser-(Val-Val)-Leu-) (7b).** A freshly prepared solution of 1,3-adamantane dicarbonyl dichloride[12] (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-serpeptide[7] **5a**,**b** or **6** (1 mmol in 150 mL of dry $CH_2Cl_2$) containing DMAP (2 mmol) and the reaction mixture stirred for 12 h at room temperature. Workup as for **4a**,**b** and purification of the residue on a short column of silica gel using $CHCl_3$/MeOH as eluent afforded the title cyclic peptide in modest yields.

**Selected Data. Cyclo(NBE-Leu-Ser-Adm-Ser-Leu-) (7a):** yield 17%; mp 100−101 °C; IR (KBr) 3336 (br), 1744, 1680, 1647, 1557 (sh), 1534 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 0.94 (6H, brd), 0.89 (6H, brd), 1.50−2.13 (16H, m), 2.44 (1H, d, $J$ = 5.8 Hz), 2.94 (1H, m), 3.13 (1H, brs), 3.18 (1H, brs), 3.78 (6H, s), 4.28 (1H, dd, $J$ = 4.1, 11.4 Hz), 4.40−4.56 (5H, m), 4.82 (1H, m), 4.87 (1H, m), 6.17 (1H, m), 6.28 (1H, m), 6.37 (1H, d, $J$ = 8.8 Hz), 6.92 (1H, d, $J$ = 8.2 Hz), 6.98 (1H, d, $J$ = 8.3 Hz), 7.18 (1H, d, $J$ = 8.3 Hz); FAB MS *m/z* 799 (100) $(MH)^+$. Anal. Calcd for $C_{41}H_{58}N_4O_{12}$: C, 61.63; H, 7.31; N, 7.01. Found: C, 61.78; H, 7.45; N, 7.21.

**Cyclo(NBE-Leu-Ser(Val-Val)-Adm-Ser-(Val-Val)-Leu-) (7b):** yield 15%; mp 172−173 °C; $[\alpha]^{29}_D$ −37.368 (*c* 2.25, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 0.96 (36H, m), 1.21 (2H, m), 1.40−2.29 (24H, m), 3.22 (2H, brs), 3.31 (2H, brs), 3.76 (6H, brs), 4.15−4.76 (12H, m), 6.20 (1H, m), 6.38 (1H, m), 6.73 (4H, m), 7.53 (1H, brd). 7.65 (3H, m); FAB MS *m/z* 1195 (32) $(MH)^+$. Anal. Calcd for $C_{61}H_{94}N_8O_{16}$: C, 61.28; H, 7.92; N, 9.37. Found: C, 61.09; H, 7.79; N, 9.29.

**Preparation of Cyclo(NBE-Leu-Leu-Ser-Adm-Ser-Leu-Leu-) (8).** Prepared by condensation of 1,3-adamantane dicarbonyl dichloride with norborneno (Leu-Leu-Ser)$_2$ (**6**) using same procedure as for **7a** and **7b**.

**Selected data for 8:** yield 25%; syrup; IR (neat) 3306 (br), 1742, 1647, 1546 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 0.93 (24H, m), 1.08−2.33 (28H, m), 3.09 (4H, m), 3.74 (6H, brs), 4.10−4.87 (10H, m), 6.16 (1H, brs), 6.34 (1H, brs), 6.33 (2H, m), 7.33 (1H, d, $J$ = 7.72 Hz), 7.54 (1H, brd), 7.80 (1H, d, $J$ = 6.5 Hz), 8.06 (1H, d, $J$ = 9.0 Hz), 8.43 (1H, brd); FAB MS 1025 (82) $(MH)^+$.

**Preparation of Cyclo(NBE-Cyst-) (10) and Cyclo[(NBE-Cyst)$_2$] (11).** A solution of *trans*-(2,3)-norbornene dicarbonyl dichloride (**9**, 2 mmol) in dry $CH_2Cl_2$ (100 mL) was added dropwise over 0.5 h to a well-stirred solution of L-cystine dimethyl ester dihydrochloride (2 mmol) and triethylamine (9 mmol) in dry $CH_2Cl_2$ (~150 mL) at 0 °C. The reaction mixture was stirred at room temperature for 12 h and washed, sequentially, with ice-cold 2 N $H_2SO_4$, $H_2O$, and 5% $NaHCO_3$ (~20 mL each). The organic layer was dried over anhyd $MgSO_4$

(14) MacDonald, R. C.; MacDonald, R. I.; Menco, B. Ph. M.; Takeshita, K.; Subbarao, N. K.; Hu, L.-r. *Biochim. Biophys. Acta* **1991**, *1061*, 297.

and concentrated in vacuo. The residue was chromatographed on silica gel with ethyl acetate/benzene (80:20) as eluent to afford the norbornene-containing cystine peptides **10** and **11** as white microcrystalline solid.

**Selected Data. Cyclo(NBE-Cyst-) (10):** yield 25%; mp 238−240 °C; IR (KBr) 3435, 3294, 1748, 1688, 1658, 1609 (sh), 1552 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 1.54 (1H, d, $J$ = 8.4 Hz), 1.92 (1H, d, $J$ = 8.4 Hz), 2.17 (1H, d, $J$ = 6.0 Hz), 2.89 (3H, m), 3.00 (1H, brs), 3.14 (1H, brs), 3.46 (2H, brs), 3.78 (6H, s), 4.93 (2H, m), 6.25 (1H, m), 6.31 (1H, m), 6.77 (1H, d, $J$ = 9.3 Hz), 6.81 (1H, d, $J$ = 8.6 Hz); ES MS $m/z$ 415 (100) $(MH)^+$; HRMS $MH^+$ found 415.100724, $C_{17}H_{22}N_2O_6S_2$ requires 415.099755.

**Cyclo[(NBE-Cyst)$_2$] (11):** yield 23%; mp 111−113 °C; IR (KBr) 3347, 1745, 1669, 1646, 1535 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 1.56 (2H, m), 1.76 (2H, m), 2.31 (1H, d, $J$ = 5.8 Hz), 2.36 (1H, d, $J$ = 5.8 Hz), 3.04 (2H, m), 3.08 (2H, m), 3.12 (2H, m), 3.25 (8H, m), 3.80 (12H, s), 4.80 (4H, m), 6.25 (2H, m), 6.30 (2H, m), 7.23 (1H, d, $J$ = 7.6 Hz), 7.30 (1H, d, $J$ = 7.7 Hz), 7.41 (2H, m); ES MS $m/z$ 829 (100) $(MH)^+$; HRMS $MH^+$ found 829.193700, $C_{34}H_{44}N_4O_{12}S_4$ requires 829.191686.

Continued elution of the column afforded small amounts (2% yield) of cyclo(NBE-Cyst)$_3$: mp 148−150 °C; IR (KBr) 3350, 1750, 1654, 1575, 1533 $cm^{-1}$; ES-MS $m/z$ 1243 (100) $(MH)^+$.

**Preparation of Cyclo[(NBE-Leu-Ser)$_2$] (13a) and Cyclo[(NBE-Leu-Ser(Aib-Aib))$_2$] (13b). a. Preparation of Norbornene-Supported Bispeptides 12a and 12b.** Norbornene dicarbonyl dichloride (**9**, 1 mmol) was first converted into bis Leu peptide by reaction with LeuOMe (freshly generated from LeuOMe·HCl and triethylamine (2 mmol each) at 0 °C in $CH_2Cl_2$) in the presence of triethylamine (2 mmol), in $CH_2Cl_2$, using a procedure similar to that for compound **10**. The crude product was purified by column chromatography.

**Selected data for norbornene bis LeuOMe (NBE-(LeuOMe)$_2$):** yield 99%; mp 180−185 °C; IR (KBr) 3306,1752, 1646, 1553 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 0.94 (12H, m), 1.45−1.95 (8H, m), 2.09 (1H, d, $J$ = 6.0 Hz), 2.48 (1H, d, $J$ = 4.0 Hz), 2.79 (1H, m), 3.05 (1H, m), 3.71 (6H, s), 4.58 (2H, m), 6.28 (1H, m), 6.36 (1H, m), 6.49 (1H, d, $J$ = 7.8 Hz), 7.31 (1H, d, $J$ = 7.8 Hz).

The title ser peptides **12a** and **12b** were prepared by condensation of the dicarboxylic acid (foamy solid; IR (KBr) 3269, 1732, 1647, 1557, 1550 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) $\delta$ 0.90 (12H, brs), 1.36−1.86 (8H, m), 2.18 (1H, d, $J$ = 5.3 Hz), 2.86 (1H, brs), 2.93 (1H, brs), 3.06 (1H, brs), 4.45 (2H, m), 6.15 (1H, brs), 6.23 (1H, brs), 7.54 (2H, m)) of NBE-Leu(OMe)$_2$ with SerOMe or Ser-Aib-Aib-OMe (prepared by deprotection of Z-Ser-Aib-Aib-OMe; mp 159−160 °C; IR (KBr) 3437, 3359, 3283, 1745, 1723, 1669, 1557 (sh), 1535 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.47 (12H, brs), 3.68 (3H, s), 3.95 (2H, m), 4.14 (1H, m), 5.08 (2H, s), 5.96 (1H, s), 6.90 (1H, s), 7.13 (1H, s), 7.25 (5H, brs)) using DCC/HOSu coupling procedure and workup as for compounds **4a** and **4b**.

**Selected Data. NBE(Leu-Ser)$_2$ (12a):** yield 35%; mp 174−175 °C; IR (KBr) 3431, 3293, 1760, 1670, 1647, 1556, 1534 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 0.88 (12H, brs), 1.30−1.79 (8H, m), 2.61 (1H, brs), 2.89 (1H, brs), 3.06 (1H, brs), 3.37 (1H, brs), 3.78 (6H, s), 3.85 (4H, m), 4.50 (2H, m), 4.70 (2H, m), 6.01 (1H, brs), 6.15 (1H, brs), 7.64 (4H, m); ES MS $m/z$ 611 (100) $(MH)^+$; HRMS $MH^+$ found 611.331563, $C_{29}H_{46}N_4O_{10}$ requires 611.329219.

**NBE(Leu-Ser-Aib-Aib)$_2$ (12b):** yield 36%; mp 170−172 °C; IR (KBr) 3313, 1752, 1672, 1628 (sh), 1539 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$ + DMSO-$d_6$) $\delta$ 0.93 (12H, m), 1.36−1.83 (32H, m), 2.86−3.33 (4H, m), 3.66 (8H, brs), 3.93 (2H, m), 4.26 (4H, m), 6.23 (2H, m), 7.26−7.73 (8H, m); FAB MS $m/z$ 973 (100) $(M + Na^+)$.

b. **Condensation of Bispeptide 12a and 12b with Norbornene Dicarbonyldichloride (9) To Give 13a and 13b.** Using a procedure as described for compounds **7a** and **7b**, the title cyclopeptides were prepared, purified, and characterized.

**Selected Data. Cyclo[(NBE-Leu-Ser)$_2$] (13a):** yield 35%; mp 96−100 °C; IR (KBr) 3650 (sh), 3336, 1746, 1684, 1663, 1643 (sh), 1543 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) $\delta$ 0.95 (12H, m), 1.40−1.86 (10H, m), 2.23−2.61 (2H, m), 2.86−3.36 (6H, m), 3.77 (6H, brs), 4.20−4.90 (8H, m), 5.90−6.31 (4H, m), 6.68−7.50 (4H, m); HRMS $MH^+$ found 757.367168, $C_{38}H_{52}N_4O_{12}$ requires 757.365999.

**Cyclo[(NBE-Leu-Ser-Aib-Aib)$_2$] (13b):** yield 20%; mp 130−132 °C; IR (KBr) 3512 (sh), 3355, 1741, 1700 (sh), 1672, 1570, 1534 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$ + DMSO-$d_6$) $\delta$ 0.86 (12H, m), 1.30−1.86 (34H, m), 2.60−3.26 (8H, m), 3.63 (8H, brs), 3.86 (2H, m), 4.20 (4H, m), 6.00−6.26 (4H, m), 7.06−7.58 (8H, m); FAB MS $m/z$ 1097 (95) $(MH)^+$, 1119 (75) $(M + Na^+)$. Anal. Calcd for $C_{54}H_{80}N_8O_{16}$: C, 59.10; H, 7.34; N, 10.21. Found: C, 58.94; H, 7.29; N, 10.01.

**Preparation of Adamantane-Constrained Leu-Leu Anchored Cystine Cyclopeptide 15.** Prepared by condensing freshly made 1,3-adamantane dicarbonyl dichloride (2 mmol in 50 mL dry $CH_2Cl_2$) with deprotected (TFA/ $CH_2Cl_2$/0 °C) bis-Boc-Cyst-Leu-Leu-OMe (**14**, prepared by coupling bis-Boc-Cystine with Leu-Leu-OMe in 78% yield; mp 130−132 °C; IR (KBr) 3319, 1752, 1703, 1669, 1655, 1548 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 0.90 (24H, d, $J$ = 5.5 Hz), 1.47 (18H, s), 1.64 (12H, m), 2.87 (2H, m), 3.01 (2H, m), 3.72 (6H, s), 4.54 (4H, m), 4.71 (2H, m), 5.58 (2H, d, $J$ = 8.6 Hz), 6.81 (2H, d, $J$ = 7.0 Hz), 7.84 (2H, d, $J$ = 7.5 Hz)) in dry $CH_2Cl_2$ (~300 mL) containing triethylamine (4 mmol). Workup as for **4a**,**b** afforded the title compound in 46% yield: mp 173−174 °C; $[\alpha]^{26}_D$ −35.73 (c, 1.06, $CHCl_3$); IR (KBr) 3336, 1743, 1672, 1649, 1558, 1535 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.84 (48H, m), 1.29−1.96 (48H, m), 2.05 (4H, brs), 2.88 (4H, m), 3.07 (4H, m), 3.60 (12H, s), 4.29 (8H, m), 4.51 (4H, m), 7.66 (8H, m), 8.22 (4H, brd); FAB MS $m/z$ 1840 (100) $(M + Na^+)$. Anal. Calcd for $C_{88}H_{144}N_{12}O_{20}S_4$: C, 58.12; H, 7.98; N, 9.24. Found: C, 58.23; H, 7.91; N, 9.13.

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**Supporting Information Available:** $^1H$ NMR spectra of **4a**,**b**, **7a**, **10**, **11**, **15**; ROESY spectra of **4a**, **10**; VT-NMR spectra of **4a**,**b**, **7a**, **10**, **11**; FAB MS of **4b**, **7a**, **8**, **10**, **11**, **13b**; CD spectra of **4a**,**b**, **7a**, **13a**, **10**, **11**, **12b**, **13b**; ion transport diagram of **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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