# Bridged Cyclosporins

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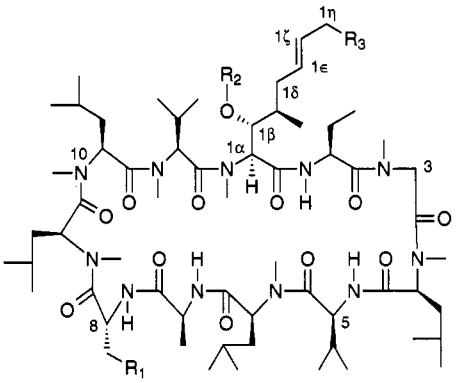
The primary hydroxyl group of [D-serine]$^8$-cyclosporin (**1b**) was alkylated with isopropyl bromoacetate to provide the O-alkylated compound **1c**. Then the secondary hydroxyl group was acetylated to give **1d**. The allylic methyl group of the MeBmt was brominated giving **1e**. Bromide **1e** was converted to the azide **1f**. Transesterification of the isopropyl ester **1f** to the methyl ester **1g** and subsequent hydrolysis provided acid **1h**. Reduction of the azide gave the amino acid **1i**. The bromide of **1e** was also replaced by an acetate to give **1j** which was hydrolyzed to give the hydroxy acid **1k**. The amino acid **1i** and the hydroxy acid **1k** both were cyclized to the bridged cyclosporin lactam **2a** and lactone **2b**, respectively. Degradation of the MeBmt residue of the acid **1l** with ozone followed by reduction gave the hydroxy acid **3** which was cyclized to a mixture of the bridged lactone **4** and the dimeric bislactone **5**.

Cyclosporin A[1] (**1a**) is the active ingredient of the immunosuppressant[2] sandimmune. It prevents allograft rejections in animals[3] and humans.[4] It is available from natural sources[5] and from total synthesis.[6,7] Cyclosporin A binds to cyclophilin[8] which in all likelihood is identical with the enzyme peptidyl-prolyl *cis-trans* isomerase.[9] The cyclophilin–cyclosporin A complex in turn binds to and inhibits the $Ca^{2+}$- and calmodulin-dependent phosphatase calcineurin.[10] Cyclophilin is known to recognize and bind to a region of cyclosporin A (**1a**) consisting of the sequence of amino acids 9–11–4 (Chart 1). This stretch was defined[11] as the binding domain of cyclosporin A. Calmodulin in turns binds to amino acids 5–8 of the cyclosporin A in the cyclophilin–cyclosporin A complex. This region was defined as the effector domain of cyclosporin A.[11]

Cyclosporin A prevails in one particular conformation in both[12] the solid state and in chloroform solution. Mixtures of conformations may be observed by NMR in solutions of different polarity.[13] We were interested in locking the cyclosporin molecule into a more rigid conformation, possibly one mimicking the active conformation of cyclosporin A. We reasoned that the flexibility of the parent compound should be decreased considerably by bridging the monocyclic cyclosporin A. Theoretically, a bridge could be built anywhere between two of 11 amino acids built into cyclosporin A. It was our intention to link two of the 10 possible side chains, thus avoiding the introduction of additional stereogenic centers. Since we had access to greater amounts[14] of [D-serine]$^8$-cyclosporin (**1b**), amino acid 8 was chosen to serve as the frist bridgehead. Previously,[15] we had demonstrated how to activate the allylic methyl group of cyclosporin A for further derivation. That work led to the preparation of OL-17, the main metabolite of cyclosporin A. An analo-

**Chart 1**



**1a-l**

**1a**: $R_1 = R_2 = R_3 = H$
**1b**: $R_1 = OH$; $R_2 = R_3 = H$
**1c**: $R_1 = OCH_2COOCH(CH_3)_2$; $R_2 = R_3 = H$
**1d**: $R_1 = OCH_2COOCH(CH_3)_2$; $R_2 = Ac$; $R_3 = H$
**1e**: $R_1 = OCH_2COOCH(CH_3)_2$; $R_2 = Ac$; $R_3 = Br$
**1f**: $R_1 = OCH_2COOCH(CH_3)_2$; $R_2 = Ac$; $R_3 = N_3$
**1g**: $R_1 = OCH_2COOMe$; $R_2 = H$; $R_3 = N_3$
**1h**: $R_1 = OCH_2COOH$; $R_2 = H$; $R_3 = N_3$
**1i**: $R_1 = OCH_2COOH$; $R_2 = H$; $R_3 = NH_2$
**1j**: $R_1 = OCH_2COOCH(CH_3)_2$; $R_2 = Ac$; $R_3 = OAc$
**1k**: $R_1 = OCH_2COOH$; $R_2 = H$; $R_3 = OH$
**1l**: $R_1 = OCH_2COOH$; $R_2 = R_3 = H$

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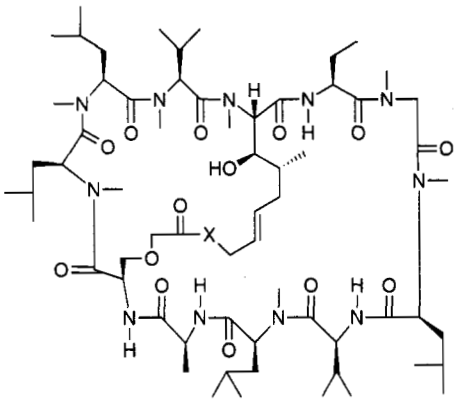
**Scheme 1**



**2a:** X = NH
**2b:** X = O

**1i** ⟶ **2a**
**1k** ⟶ **2b**

gous activation of a derivative of [D-serine]$^8$-cyclosporin (**1b**) might serve as the second bridgehead, thus dividing the original cyclosporin moiety into two approximately equal parts. *A priori*, the formation of two diastereoisomers could be anticipated, since a bridge could be formed above or below the original cyclosporin skeleton.

[D-Serine]$^8$-cyclosporin[14] (**1b**) was treated[16] with isopropyl bromoacetate under phase transfer reaction conditions in methylene chloride and 40% NaOH in the presence of catalytic amounts of benzyltriethylammonium chloride to give the monoalkylated ester **1c** in 88% yield as a crystalline product. In order to prevent the secondary hydroxyl group from participating in undesirable reactions during the following steps, **1c** was acetylated in the presence of 4-(dimethylamino)pyridine (DMAP) giving [*O*-acetyl-MeBmt]-{*O*-[(isopropoxycarbonyl)methyl]-D-serine}$^8$-cyclosporin (**1d**). This was treated with NBS in carbon tetrachloride in the presence of a catalytic amount of the radical initiator azobisisobutyronitrile in carbon tetrachloride in order to oxidize the allylic methyl group to a bromomethyl group, giving the crystalline cyclosporin **1e** in 72% yield. In the $^1$H NMR spectrum, the allylic bromomethyl group gave rise to two triplets centered at $\delta$ 3.92 ppm. The doublet near $\delta$ 1.55 due to the allylic methyl group of the precursor was absent.

In a next step, the bromide of **1e** was replaced by azide in the presence of tetrabutylammonium azide in DMF at 100 °C producing crystalline **1f** in 69% yield. In the NMR spectrum of **1f**, the allylic methylene protons appeared as a multiplet at higher field between $\delta$ 3.6 and 3.8 ppm. The mass spectrum of azide **1f** had a mass unit corresponding to the protonated molecular ion and a peak due to the loss of 41 mass units, indicative of the replacement of the labile azide group by a proton during fast atom bombardment.

The protecting groups on **1f** were then removed stepwise. First, the acetate was hydrolyzed in methanol with catalytic amounts of sodium methoxide which also gave rise to transesterification of the isopropyl ester to provide the methyl ester **1g**. In our experience, the acetyl protecting group on the MeBmt (as in **1f** and cases described below) was best removed by transesterification in methanol. Replacing methanol by ethanol as solvent resulted in lower yields. Methyl ester **1g** was fully characterized (see Experimental Section). In the second step, azido methyl ester **1g** was hydrolyzed to azido acid **1h**. The azido group was reduced to the amino group by sodium borohydride in the presence of palladium on charcoal, giving **1i** in 62% yield. The resolution of the NMR spectrum of this polar compound was found to be marginal at best, and its purity was ascertained by HPLC.

When the monocycle **1i**, an amino acid of high polarity, was treated with benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) in methylene chloride, a much less polar substance was formed in 43% yield and assigned the structure of lactam **2a**. To the best of our knowledge, this represents the first example of a bridged cyclosporin to be described in the literature. The mass spectrum showed the expected molecular ion of 1273.8 $[M + 1]^+$ mass units. The compound was thoroughly examined by NMR spectroscopy (COSY, TOCSY, ROESY). All the signals below 2 ppm, including those due to the *N*-methyl groups, were assigned unequivocally. The shifts for the protons of the bridged lactam **2a** are very similar to those observed for cyclosporin A itself, with subtle differences (see Table 1) being detectable, nevertheless. A noticeable difference was observed for the $^6NCH_3$ group which was shifted upfield by 0.22 ppm. An even bigger change was detected for the signal assigned to the NH group of amino acid 8, which was observed at $\delta$ 6.06 ppm. This represents an upfield shift of 1.1 ppm in comparison to the signal observed for the corresponding NH proton of cyclosporin A in the same solvent. A similar 1 ppm upfield shift was observed for the NH group of amino acid 7 at $\delta$ 6.74 ppm. These upfield shifts might be due to the loss of internal hydrogen bridges in the case of the bridged cyclosporin **2a**. A slight downfield shift of 0.3 ppm was observed for the NH group of amino acid 2, which now appeared at $\delta$ 8.28 ppm. The signal due to the NH group of amino acid 5 was not affected. The signal assigned to the NH group of the newly formed amide bond was observed at $\delta$ 7.20 ppm. The signal due to the OH group, exchangeable in $D_2O$, was found at 5.80 ppm and showed a coupling to the 1$\beta$-proton found at 3.79 ppm. The bridged cyclosporin **2a** features a total of five methylene groups which are built into the periphery of the ring skeleton. For each of these five pairs of diastereotopic protons the NMR spectrum should reveal relatively large differences in their chemical shifts. The largest differences of 1.45, 1.32, and 1.38 ppm were observed for the signals due to the diastereotopic protons of the two allylic methylene groups and the glycine methylene group, respectively. These large differences may render decoupling experiments rather difficult. For this reason, the coupling constant between the two vinylic protons could not be measured directly. However, from the fully coupled spectrum, the size of the coupling constant was estimated to be between 14.5 and 16.5 Hz in agreement with the presence of a trans-substituted double bond. ROESY correlations were detected from the 1$\epsilon$ proton at 5.61 ppm to only one of the two 1$\eta$ protons at 3.10 ppm and to the 1$\eta$-NH, also from the 1$\zeta$ proton at 5.50 ppm to both of the 1$\delta$ protons at 1.52 and 2.85 ppm. These observations lend further support to the presence of a trans-substituted double bond in **2a**. The newly formed bridge was also manifested by a ROESY correlation between the 1$\eta$-NH and the lower field proton (3.70 ppm) of the two 8$\beta$ hydrogens. Weaker correlations between the 1$\eta$-NH and the two $OCH_2COO$ protons were discernible as well. It was between these two amino acids that the lactam had been formed.

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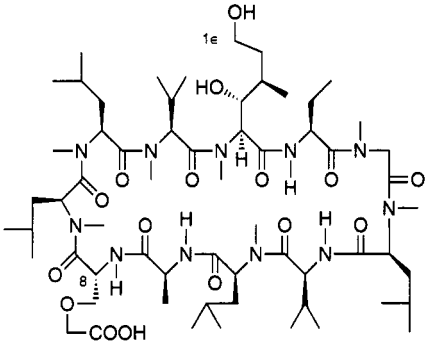
**Table 1. Chemical Shifts ($\delta$ ppm) in $CDCl_3$ of NH and *N*-Methyl Groups [Coupling Constants (Hz) Are Shown in Parentheses]**

| compd | $H^2NCO$ | $H^7NCO$ | $H^5NCO$ | $H^8NCO$ | $^1NCH_3$ | $^3NCH_3$ | $^6NCH_3$ | $^4NCH_3$ | $^9NCH_3$ | $^{10}NCH_3$ | $^{11}NCH_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1a** | 7.96 (9) | 7.68 (8) | 7.48 (8) | 7.17 (8) | 3.51 | 3.39 | 3.27 | 3.11 | 3.11 | 2.70 | 2.70 |
| **2a** | 8.28 (8) | 6.74 (7) | 7.52 (8) | 6.06 (8) | 3.37 | 3.38 | 3.05 | 3.03 | 3.27 | 2.76 | 2.56 |
| **2b** | 8.29 (9) | 6.79 (7) | 7.49 (10) | 6.15 (8) | 3.28 | 3.38 | 3.04 | 3.07 | 3.33 | 2.75 | 2.55 |
| **4** | 8.08 (6) | 6.95 (8) | 7.67 (9) | 7.22 (6) | 2.90 | 3.33 | 3.05 | 3.02 | 3.44 | 2.73 | 2.40 |
| **5** | 8.66 (10) | 7.88 (7) | 7.50 (9) | 8.16 (8) | 3.48 | 3.42 | 3.45 | 3.09 | 3.21 | 2.63 | 2.62 |

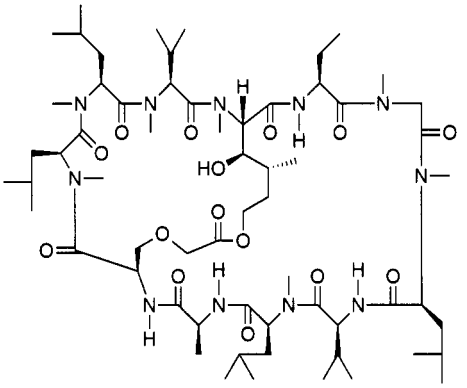
**Scheme 2**




**3**

**11 → 3**

**Scheme 3**



**4**

**3 → 4**

We had shown[15] earlier that the allylic bromide of [$\eta$-bromo-*O*-acetyl-MeBmt]$^1$-cyclosporin A was readily replaced by an acetoxy group. Therefore, bromide **1e** was transformed in a similar way. Upon being heated to 60 °C in 2-butanone in the presence of tetrabutylammonium acetate and catalytic sodium iodide the diacetate **1j** was isolated in 76% yield after purification. In the $^1H$ NMR two singlets were observed for the two acetate groups. In addition, the two protons adjacent to the primary acetate group gave rise to a multiplet near $\delta$ 4.42 ppm. The integrity of the trans double bond in **1j** was verified by irradiation at $\delta$ 4.42 ppm which caused the multiplet between 5.4 and 5.5 ppm to collapse to a doublet revealing a coupling constant of 16 Hz characteristic for a *trans* disubstituted double bond. The protecting groups of **1j** were removed by acetate hydrolysis in methanol at 50 °C and subsequent ester hydrolysis in water to give acid **1k**.

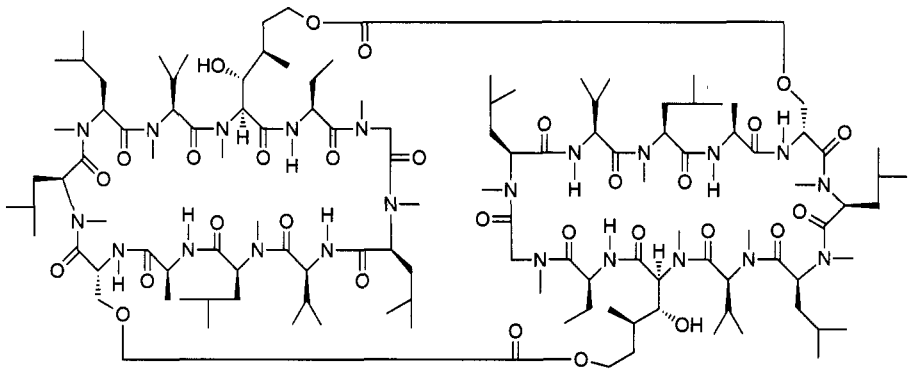
When the polar dihydroxy acid **1k** was treated with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and DMAP in methylene chloride, a less polar product was formed. The bridged lactone **2b** was isolated in 24% yield after column chromatography and showed the expected molecular ion of 1274.9 $[M + 1]^+$ mass units. Examination by NMR spectroscopy (COSY, TOCSY, ROESY) revealed the similarity between this compound and the lactam **2a**. All the signals below 2 ppm, including those due to the *N*-methyl groups, were again assigned unequivocally. Among the *N*-methyl and the remaining NH groups, no significant shift differences were descernible between the lactam **2a** and the lactone **2b**. Replacement of nitrogen by oxygen caused the diastereotopic protons at the 1$\eta$ position to shift downfield by 0.42 and 0.95 ppm, respectively. The presence of a trans-disubstituted double bond was confirmed by the observed ROESY interaction between the 1$\epsilon$ proton, detected at 5.85 ppm, and only one of the diastereotopic 1$\eta$ protons, detected at 4.05 ppm. Similarly, a ROESY interaction was observed between the 1$\zeta$ proton at 5.60 ppm and one and only one of the diastereotopic 1$\delta$ protons, detected near 1.62 ppm. The fact that the lactone formation had taken place with the primary hydroxyl group could be seen from the coupling patterns of the two diastereotopic protons at position 1$\eta$ ($\delta$ 4.00–4.10 and 4.95–5.00 ppm) and from the chemical shift observed for the 1$\beta$ proton ($\delta$ 3.75–3.80 ppm). When the secondary hydroxyl group was acetylated (see **1d**) the NMR signal corresponding to the 1$\beta$ proton was shifted downfield and was observed near 5.5 ppm. A similar downfield shift might be expected if the lactone had formed from **1k** between the acid function and the secondary hydroxyl group.

A lactone bridge shortened by two carbon atoms was prepared next. Ester **1c** was hydrolyzed to acid **1l** under basic conditions. Treating this acid (**1l**) with ozone followed by reductive cleavage of the ozonide in the presence of sodium borohydride gave the dihydroxy acid **3** in 20% yield as a crystalline compound that showed the expected molecular ion of 1266.8 $[M + 1]^+$ mass units.

When the polar dihydroxy acid **3** was treated with EDC in the presence of DMAP in methylene chloride for 5 h, bridged lactone **4** was isolated in 19% yield and showed the expected molecular ion of 1249.3 $[M + 1]^+$ mass units. The bridged lactone **4** was fully characterized by NMR spectroscopy (COSY, TOCSY, ROESY). When lactone **4** was compared with lactone **2b**, the largest chemical shift difference for the *N*-Me groups was seen for the signal due to the $^1NCH_3$ group which was shifted upfield by 0.38 ppm. Interestingly, the signal due to NH of amino acid 8 in **2b** was found to be unchanged from the chemical shift of the same NH in cyclosporin A. This represents a downfield shift of 1.07 ppm in comparison to the larger bridged lactone **2b**, indicating the renewed presence of a hydrogen bridge between the $H^8NCO$ and another amide group in compound **4** (but absent in **2b**). From the observed coupling between the OH group and the 1$\beta$-proton, detected at 3.70 ppm, it was concluded that the secondary alcohol, exchangeable with $D_2O$ and detectable in the vicinity of 5.90 ppm, had not participated in the cyclization reaction. Therefore, the lactone in **4** had formed, as anticipated, between the acid group and the primary alcohol group. However, it was also noticeable that this compound did not exist as a single conformational isomer in chloform solution.

Besides the monomeric bridged lactone **4**, we were able to isolate a second nonpolar compound which was as-

**Scheme 4**



**5**

3 → 5

signed structure **5** based on the following observations. The mass spectrum of **5** showed a molecular ion of 2497 $[M + 1]^+$ mass units expected for a dimer of **4**. The NMR spectra of **5** indicated the presence of a fully symmetrical molecule ($C_2$ symmetry). With the aid of COSY, ROESY, and TOCSY spectra, it was possible to unambiguously assign, among others, all the signals due to the *N*-methyl groups. The chemical shifts of this compound were compared with the chemical shifts seen for the larger bridged lactone **2b**. One of the signals due to a $NCH_3$ group was shifted downfield by 0.41 ppm. This was assigned to the *N*-methyl group of amino acid 6. Upfield shifts of 0.6 and 0.3 ppm were seen for the signals of amino acids 10 and 11, respectively. The lowest field signal among the α protons was now due to 1α proton, although its downfield shift, in relation to that of cyclosporin A, amounted to less than 0.3 ppm. The signal due to NH of amino acid 7 was observed at δ 6.95, an upfield shift of 0.73 ppm. The other NH signals were marginally shifted downfield (0.12 and 0.19 ppm for amino acids 2 and 5, respectively) or remained unchanged, all in comparison to the corresponding shifts observed for the cyclosporin A signals.

In each of the three cases described above, we were able to isolate only one monomeric bridged cyclosporin so far. The conformational aspects of these products have not been determined.

The bridged cyclosporins **2a–b**, **4**, and **5** were tested in vitro for their biological activity in a direct comparison with cyclosporin A (**1a**). Test models used were interleukin-2 production (Il-2), mixed lymphocyte reaction (MLR), and cyclophilin A binding (Cyph). All bridged cyclosporins were found to be considerably less active than the parent compound. In the Il-2 release, they were found to be more than 100 times weaker, in the MLR test they were 50 times weaker, and in the Cyph binding they were about 30 times weaker, all relative to cyclosporin A.

## Experimental Section

**General.** Thin layer chromatography (TLC) plates were developed in ethyl acetate saturated with water. High-pressure liquid chromatography (HPLC) analyses were carried out using a RP-18 reversed-phase column at 75 °C. The spectra were monitored at 204 nm. The mobile phase consisted of aqueous acetonitrile with the amount of water varying between 15 and 40%. In addition, the aqueous phase contained 1 mL of 85% phosphoric acid per 3.7 L. Unless listed otherwise, nuclear magnetic resonance (NMR) spectra were measured in deuterated chloroform solution on a 360-MHz spectrometer with TMS as reference. The assignments of most of the signals are tentative and based on the chemical shifts observed for the corresponding signals of cyclosporin A. The corresponding values for cyclosporin A, e.g., 2.70, were added for convenience. For the complete spectra of cyclosporin A (**1a**) see ref 12. Column chromatography was carried out using silica gel columns with ethyl acetate saturated with water as eluent or as specified. For preparative RP-18 reversed-phase columns the eluent usually was methanol/water 88:12.

***O*-Acetyl-{*O*-[(isopropoxycarbonyl)methyl]-D-serine}$^8$-cyclosporin (1d).** A mixture of **1c**[16] (33.2 g, 25.2 mmol), 4-(dimethylamino)pyridine (1.44 g, 12 mmol), acetic anhydride, and pyridine (150 mL each) was kept at rt for 3 h. The mixture was evaporated to dryness and then dissolved in mehtanol/water 7:1 (240 mL) and allowed to crystallize overnight to give 26.1 g of the product: yield 76%; mp 252–254 °C; *m/z* calcd for $C_{69}H_{121}N_{11}O_{16}$ 1359.9, found 1361.3 $[MH]^+$; $[\alpha_D] = -223.0°$ ($c = 0.490$ in MeOH); NMR δ 1.28, 1.33, 1.59, 2.01, 2.65 (2.70), 2.68 (2.70), 3.08 (3.11), 3.23 (3.11), 3.27 (3.27, 3.39), 3.45 (3.51), 3.58–3.63, 3.72–3.79, 3.92–4.06, 5.45–5.60, 7.37, 7.49, 8.07, 8.53.

**[*O*-Acetyl-η-bromo-MeBmt]$^1$-{*O*-[(isopropoxycarbonyl)methyl]-D-serine}$^8$-cyclosporin (1e).** Compound **1d** (3.9 g, 2.9 mmol) was brominated following the procedures described[15] for acetyl-cyclosporin A to give the product (3.0 g): yield 72%; mp 248–253 °C; *m/z* calcd for $C_{69}H_{120}N_{11}O_{16}Br$ 1440.9, found 1442.1 (100%) $[MH]^+$, 1362.1 (67%) $[MH - Br]^+$; $[\alpha_D] = -271.3°$ ($c = 0.460$ in MeOH); NMR δ 1.27, 1.33, 2.02, 2.65 (2.70), 2.68 (2.70), 3.10 (3.11), 3.25 (3.11), 3.27 (3.27, 3.39), 3.45 (3.51), 3.56–3.63, 3.70–3.80, 3.92, 3.92–4.05, 5.04–5.15, 5.28–5.33, 5.69, 7.35, 7.58, 8.04, 8.53.

**[*O*-Acetyl-η-azido-MeBmt]$^1$-{*O*-[(isopropoxycarbonyl)methyl]-D-serine}$^8$-cyclosporin (1f).** A mixture of the bromide **1e** (7.27 g, 5.06 mmol), sodium azide (3.4 g, 52 mmol), and sodium iodide (270 mg, 1.8 mmol) in DMF (85 mL) was heated to 100 °C for 2 h. The solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and washed with water and brine. The crude product was chromatographed on silica gel to give the pure product (4.9 g): yield 69%; mp 240–242 °C; *m/z* calcd for $C_{69}H_{120}N_{14}O_{16}$ 1400.9, found 1402.4 (100%) $[MH]^+$, 1361.3 (38%) $[MH - N_3 + H]^+$; $[\alpha_D] = -279.6°$ ($c = 0.280$ in $CHCl_3$); NMR δ 1.25–1.31, 1.33, 2.02, 2.65 (2.70), 2.68 (2.70), 3.10 (3.11), 3.23 (3.11), 3.26 (3.27, 3.39), 3.45 (3.51), 3.93–4.07, 7.38, 7.59, 8.05, 8.57.

**[η-Azido-MeBmt]$^1$-[*O*-(carbomethoxymethyl)-D-serine]$^8$-cyclosporin (1g).** A solution of **1f** (2.5 g, 1.79 mmol) was added to a solution of sodium (200 mg, 8 mmol) in methanol (10 mL) and kept at rt overnight. The mixture was neutralized with acetic acid. The solvent was evaporated. The residue was dissolved in ethyl acetate and washed with water until neutral. The solution was dried over $Na_2SO_4$ and evaporated to give the crude product (2.2 g). This was chromatographed on silica gel to give the pure product (2.04 g): yield 86%. A sample was crystallized from ether: mp 147–149 °C; *m/z* calcd for $C_{65}H_{114}O_{15}$ 1330.9, found 1354.2 (27%) $[M + Na]^+$, 1332.2 (100%) $[MH]^+$, 1291.1 (23%) $[MH - N_3 + H]^+$; $[\alpha_D] = -173.7°$ ($c = 0.245$ in MeOH); NMR δ 1.35, 2.68 (2.70), 2.73 (2.70), 3.11 (3.11), 3.19 (3.11), 3.25 (3.27), 3.40 (3.39), 3.52 (3.51), 3.75, 3.96–4.08, 7.07, 7.50, 7.80, 8.14.

**[η-Azido-MeBmt]$^1$-[*O*-(carboxymethyl)-D-serine]$^8$-cyclosporin (1h).** A mixture of the methyl ester **1g** (4.59 g, 3.48

mmol) and 2 N sodium hydroxide (10 mL, 20 mmol) in methanol (75 mL) was kept at rt for 1 h. The solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and washed sequentially with 1 N HCl, water, and brine (100 mL each). The solvent was dried over $Na_2SO_4$ and then evaporated to give the crude acid (4.8 g) which was chromatographed on silica gel to give the pure product (3.98 g): yield 87%; *m/z* calcd for $C_{64}H_{112}N_{14}O_{15}$ 1316.8, found 1317.6 $[MH]^+$, 1276.6 (41%) $[MH - N_3 + H]^+$; $[\alpha_D] = -168.2°$ (c = 0.307 in MeOH); NMR δ 1.35, 2.68 (2.70), 2.71 (2.70), 3.10 (3.11), 3.19 (3.11), 3.24 (3.27), 3.40 (3.39), 3.51 (3.51), 4.05, 7.22, 7.50, 7.84, 8.12.

**[η-Amino-MeBmt]$^1$-[*O*-(carboxymethyl)-D-serine]$^8$-cyclosporin (1i).** The solution of **1h** (1.32 g, 1 mmol) in methanol (25 mL) was treated in small portions with $NaBH_4$ (1.9 g, 50 mmol) in the presence of 10% Pd/C (190 mg). After 90 min at rt, the catalyst was filtered off and the solution was evaporated. Excess hydrochloric acid was added, and the mixture was extracted with methylene chloride. The solution was dried over $Na_2SO_4$ and evaporated to give the crude product (1.10 g). This was chromatographed on an RP-18 reversed-phase column to give the pure product (800 mg): yield 62%; *m/z* calcd for $C_{64}H_{114}N_{12}O_{15}$ 1290.8, found 1291.9 (100%) $[MH]^+$, 1276.9 (36%) $[M - NH_2]^+$; $[\alpha_D] = -125.3.°$ (c = 0.139 in MeOH); NMR δ 2.69 (2.70), 2.78 (2.70), 2.98 (3.11), 3.03 (3.11), 3.20 (3.27), 3.23 (3.39), 3.33 (3.51).

**[*O*-Acetyl-η-acetoxy-MeBmt]$^1$-{*O*-[(isopropoxycarbonyl)methyl]-D-serine}$^8$-cyclosporin (1j).** The title compound (2.1 g) was prepared from **1e** (2.8 g, 1.9 mmol) following procedures described[15] for the preparation of OL-17: yield 76%; *m/z* calcd for $C_{71}H_{123}N_{11}O_{18}$ 1417.9, found 1419.4 $[MH]^+$, 1359.4 $[MH - AcOH]^+$; $[\alpha_D] = -213.0°$ (c = 0.553 in MeOH); NMR δ 1.26, 1.27, 1.32, 2.00, 2.03, 2.65 (2.70), 2.67 (2.70), 3.08 (3.11), 3.18, 3.23 (3.11), 3.25 (3.27, 3.39), 3.44 (3.51), 3.60, 3.76, 3.92–4.06, 4.34–4.50, 4.64, 4.72, 4.96, 5.39–5.48, 5.68, 7.36, 7.54, 8.05, 8.54.

**[η-Hydroxy-MeBmt]$^1$-[*O*-(carboxymethyl)-D-serine]$^8$-cyclosporin (1k).** A solution of **1j** (900 mg, 0.64 mmol) in methanol (50 mL) was added to a solution of sodium (320 mg, 14 mmol) in methanol (25 mL) and kept at rt for 2 h and then at 50 °C for 1.5 h. Water was added. After 2 h, the cold solution was evaporated under reduced pressure. The residue was dissolved in methylene chloride and then acidified with 2 N HCl solution and washed with brine. The organic phase was dried over magnesium sulfate, filtered, and evaporated to give the product (740 mg) of 92% purity (HPLC): yield 89%; *m/z* calcd for $C_{71}H_{123}N_{11}O_{18}$ 1291.9, found 1293.2 $[MH]^+$; $[\alpha_D] = -144.2°$ (c = 0.541 in MeOH); NMR δ 1.35, 2.67 (2.70), 2.68 (2.70), 3.10 (3.11), 3.22 (3.11), 3.24 (3.27), 3.42 (3.39), 3.43 (3.51), 3.60–3.75, 4.00–4.17, 4.40–4.50, 4.63, 7.56, 7.65, 7.75, 8.18.

**[*O*-(Carboxymethyl)-D-serine]$^8$-cyclosporin (1l).** A solution of the ester **1c** (580 mg, 0.44 mmol) and KOH (2.5 g, 45 mmol) in methanol (75 mL) was kept at rt for 2 h. Acetic acid was added. Most of the methanol was evaporated under reduced pressure. Ethyl acetate was added and washed with water. The organic phase was dried over $MgSO_4$, evaporated, and dried under high vacuum to give the product (480 mg): yield 86%; *m/z* calcd for $C_{64}H_{113}N_{11}O_{15}$ 1275.7, found 1276.8 $[MH]^+$; $[\alpha_D] = -172.8°$ (c = 0.926 in MeOH); NMR δ 0.72, 1.38, 1.63, 2.70 (2.70), 2.72 (2.70), 3.12 (3.11), 3.20 (3.11), 3.24 (3.27), 3.41 (3.39), 3.51 (3.51), 3.6–3.7, 3.75–3.85, 4.00–4.14, 4.48, 4.65, 4.73, 7.25–7.35, 7.49, 7.82, 8.06.

**[η-Amino-MeBmt]$^1$-[*O*-(carboxymethyl)-D-serine]$^8$-cyclosporin Lactam (2a).** A mixture of **1i** (200 mg, 0.155 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, 340 mg, 0.77 mmol) in methylene chloride (25 mL) was kept at rt overnight and then chromatographed on silica gel to give the pure product (86 mg): yield 43%; *m/z* calcd for $C_{64}H_{112}N_{12}O_{14}$ 1272.8, found 1273.8 (40%) $[MH]^+$; $[\alpha_D] = -228.6°$ (c = 0.137 in MeOH); NMR δ 1.08 (1.01), 1.38 (1.36), 1.50–1.55, 2.56 (2.70), 2.76 (2.70), 2.80–2.90, 3.03 (3.11), 3.05 (3.27), 3.07–3.13, 3.13–3.30, 3.27 (3.11), 3.37 (3.51), 3.38 (3.39), 3.49–3.54, 3.65–3.72, 3.75–3.82, 3.97, 4.25–4.35, 4.48–4.65, 5.02–5.09, 5.10, 5.23, 5.43, 5.45–5.55, 5.55–5.66, 5.66, 5.80, 5.76, 6.06 (7.17), 6.74 (7.68), 7.20, 7.52 (7.48), 8.28 (7.96).

**[η-Hydroxy-MeBmt]$^1$-[*O*-(carboxymethyl)-D-serine]$^8$-cyclosporin 1η-Lactone (2b).** A mixture of **1k** (130 mg, 0.1 mmol), 4-(dimethylamino)pyridine (DMAP, 310 mg, 2.5 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC, 200 mg, 1.0 mmol) in methylene chloride (25 mL) was kept at rt overnight and then chromatographed on silica gel to give the pure product (30 mg): yield 24%; *m/z* calcd for $C_{64}H_{111}N_{11}O_{15}$ 1273.8, found 1274.9 $[MH]^+$; $[\alpha_D] = -178.1°$ (c = 0.189 in MeOH); NMR δ 1.10 (1.01), 1.36 (1.36), 1.55–1.70, 2.55 (2.70), 2.75 (2.70), 2.85–2.95, 3.04 (3.27), 3.07 (3.11), 3.20–3.30, 3.28 (3.51), 3.33 (3.11), 3.38 (3.39), 3.30–3.40, 3.75–3.80, 4.00–4.10, 4.30–4.35, 4.55–4.65, 4.95–5.02, 5.05–5.15, 5.15–5.25, 5.22, 5.37–5.45, 5.55–5.65, 5.67, 5.65–5.75, 5.80–5.90, 5.90–5.95, 6.15 (7.17), 6.79 (7.68), 7.49 (7.48), 8.29 (7.93).

**[3(*R*),6-Dihydroxy-4(*R*)-methyl-2(*S*)-(methylamino)hexanoic acid]$^1$-[*O*-(carboxymethyl)-D-serine]$^8$-cyclosporin (3).** A cold solution (−50 °C) of **1l** (1.4 g, 1.1 mmol) in methylene chloride (100 mL) was treated with ozone. The solvent was evaporated and replaced by ethanol. Sodium borohydride (1.4 g, 37 mmol) was added in small portions. The solvent was evaporated under reduced pressure. The reaction mixture was acidified by the addition of 2 N HCl solution and then extracted with ethyl acetate and washed with water and brine. The organic phase was dried over $Na_2SO_4$ to give the crude product (1.3 g), which was crystallized from acetone/ether to give the crystalline product (280 mg): yield 20%; mp 200–202 °C; *m/z* calcd for $C_{62}H_{111}N_{11}O_{16}$ 1265.8, found 1266.8 $[MH]^+$; $[\alpha_D] = -155.2°$ (c = 0.058 in MeOH); NMR δ 2.70 (2.70), 2.72 (2.70), 3.09 (3.11), 3.20 (3.11), 3.31 (3.27), 3.36 (3.39), 3.41 (3.51), 4.00–4.15, 7.39, 7.70, 7.77, 7.80.

**[3(*R*),6-Dihydroxy-4(*R*)-methyl-2(*S*)-(methylamino)hexanoic acid]$^1$-8-[*O*-(carboxymethyl)-D-serine]$^8$-cyclosporin 1ε-Lactone (4).** A mixture of the hydroxy acid **3** (900 mg, 0.71 mmol), 4-(dimethylamino)pyridine (DMAP, 1.1 g, 9 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC, 950 mg, 5 mmol) in methylene chloride (50 mL) was stirred at rt for 5 h. The mixture was diluted with *tert*-butyl methyl ether, washed with 2 N HCl and bicarbonate, and dried. The solvent was evaporated, and the crude product (900 mg) was chromatographed over silica gel to give the product (170 mg): yield 19%; *m/z* calcd for $C_{62}H_{109}N_{11}O_{15}$ 1247.9, found 1249.3 $[MH]^+$; $[\alpha_D] = -157.8°$ (c = 0.250 in MeOH); NMR δ 1.40, 2.40 (2.70), 2.73 (2.70), 2.90 (3.51), 3.02 (3.11), 3.05 (3.27), 3.22, 3.33 (3.39), 3.44 (3.11), 3.42–3.52, 3.70, 3.80–3.95, 4.22, 4.60–4.80, 4.92, 4.95–5.05, 5.20–5.30, 5.32–5.40, 5.42–5.55, 5.75 (5.48), 6.95 (7.68), 7.22 (7.17), 7.67 (7.48), 8.08 (7.96).

**Dimer 5.** From the above chromatogram was obtained a fraction containing the dimeric product (56 mg): yield 6%; *m/z* calcd for $C_{124}H_{218}N_{22}O_{30}$ 2495.8, found 2497 $[MH]^+$; $[\alpha_D] = -251.4°$ (c = 0.265 in MeOH); NMR δ 0.63, 0.77, 1.06, 1.14, 1.31, 2.45–2.60, 2.62 (2.70), 2.63 (2.70), 3.09 (3.11), 3.17, 3.21 (3.11), 3.20–3.30, 3.42 (3.39), 3.45 (3.27), 3.48 (3.51), 4.00–4.10, 4.16, 4.38–4.46, 4.55–4.68, 4.67, 4.73–4.79, 4.79, 4.97–5.08, 5.16–5.23, 5.28, 5.41–5.48, 5.58–5.66, 7.50 (7.48), 7.88 (7.68), 8.16 (7.17), 8.66 (7.96).

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**Supporting Information Available:** 360 MHz $^1$H NMR spectra of compounds **1d–1l**, **2a,b**, and **3–5** and COSY, ROESY, and TOCSY spectra of compounds **2a,b**, **4**, and **5** (27 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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