Preparation and in Vitro Activities of Ethers of [D-Serine]^8-cyclosporin

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Received August 12, 1994®

Reactions of the [D-serine]^8-cyclosporin (**2**) with a series of alkylating agents under phase transfer conditions gave the alkylated products **3**–**6**. Alkylations of **2** with hindered esters of bromoacetate gave the crystalline esters **7** and **8**. Hydrolysis under basic conditions gave the acid **10**. Reduction of ester **8** led to a novel cyclosporin **11**. This was transformed in two additional steps to **15**. In a similar two-step sequence **17** was prepared from **15**. From **2** and methyl 2-(bromomethyl)acrylate product **20** was obtained. Alkylation of **2** with **49** followed by deprotection yielded **24**. The linear isomer **27** was prepared. The 3-hydroxypropyl ether **30** was prepared in two steps from **28**. The 4-hydroxybutyl ether **31** was accessible from **2** and 1,4-dibromobutane. The hydroxy group of **11** was converted to the tosylate **32**. Base treatment of **32** led to the bicyclic [3(*R*)-morpholinecarboxylic acid]^8-cyclosporin (**39**). The [2-ethoxy-5-morpholinecarboxylic acid]^8-cyclosporin **40** was prepared via **36**. Base treatment of the bromoacetate **37** gave the morpholinone derivative **41**. [4(*R*)-Oxazolidinecarboxylic acid]^8-cyclosporin (**42**) was obtained from **2** and methylene bromide. From **24** the tosylate **38** was prepared and cyclized to the hexahydrooxazepine derivative **43**. [2(*R*)-Piperidinecarboxylic acid]^8-cyclosporin (**49**) was prepared from **42** and 2(*R*)-piperidinecarboxylic acid **45** *via* **46**–**48**. The bicyclic cyclosporin **39** was found to be about 3–4 times more active than cyclosporin A in our *in vitro* tests.

Cyclosporin A[1] (**1**), the active ingredient of Sandimmune, is a powerful immunosuppressant[2] preventing allograft rejections in animals[3] and humans.[4] It is available from natural sources[5] or through total synthesis.[6] Its mechanism of action[7] is based on the inhibition of the production of lymphokines such as interleukine-2 (IL-2). These lymphokines are secreted by the activated T helper cells, thus stimulating the clonal expansion of activated T cells. These in turn are capable of distinguishing self from nonself in their response against antigens presented to the immune system in association with major histocompatibility complex (MHC) class I or class II gene products. Although it is recognized that cyclosporin A inhibits the transcription of lymphokines, the exact mechanism[8] is not clear. Cyclosporin A binds tightly to cyclophilin,[9] the postulated receptor, which in all likelihood is identical with the enzyme peptidyl–prolyl *cis–trans* isomerase.[10] The cyclophilin–cyclosporin A complex in turn binds to and inhibits the Ca^{2+} and calmodulin-dependent phosphatase calcineurin.[11]

A number of modified cyclosporins have been isolated from natural sources[12] or were obtained through synthetic efforts[13] for comparison with cyclosporin A (**1**) to establish a structure–activity relationship. From these studies has emerged the knowledge that the free hydroxyl group of the amino acid 1 (MeBMT)[14] is important for good immunosuppressant activity. A "bioactive conformation" of this group as a prerequisite for immunosuppressive activity has also been proposed.[15]

Recently, we have described the introduction of additional functional groups into the cyclosporin molecule. This was accomplished via alkylation[16] of the secondary hydroxy group of cyclosporin A (**1**). This led to hitherto unknown modifications of the natural product, while at the same time we gained insight into the kinetics of these alkylations. However, immunosuppressive effects of these O-alkylated cyclosporin A derivatives were decreased 50–100-fold. In our continuous search for more potent cyclosporins, we then wanted to alkylate the primary hydroxy group of [D-serine]^8-cyclosporin (**2**) selectively on the primary hydroxy group.

Chemistry

The known[17] [D-serine]^8-cyclosporin (**2**) was alkylated under phase transfer-catalyzed reaction conditions.[18] Allyl iodide[19] gave [*O*-allyl-D-serine]^8-cyclosporin (**3**) in yields exceeding 80%. These alkylations proceeded much faster on the primary hydroxy than on the secondary hydroxy group. For comparison, when cyclosporin A was alkylated[16] with allyl iodide on the MeBmt (amino acid 1[14]) a yield of 55% was reached after 24 h only. On the other hand, the monoalkylated [*O*-allyl-D-serine]^8-cyclosporin (**3**) was isolated in 84% after 2 h. Contaminations by less polar, possibly bisalkylated products of **2** were readily removed by column chromatography. In the NMR spectrum of **3** the methylene group attached to the oxygen gave rise to a doublet (J = 6 Hz) at δ 3.91 ppm, indicating equivalency of the two diastereotopic protons and suggesting free rotation of the allyloxy group. The same would not be expected[16] if the allyl ether were attached to the secondary hydroxy group of amino acid 1 (MeBmt). This is further supported by the pattern of the β-methylene group of the D-serine itself. In the mass spectrum of **3** the strongest peak observed (100%) was the one associated with the molecular ion at 1258.5 [M + 1] mass units. Of equal importance, however, was the observed fragment (10%) of 1145.4 mass units due to the loss of 112 mass units ($C_7H_{12}O$). This can best be explained by a McLafferty rearrangement of the side chain of the MeBmt with concomitant loss of 2-methyl-4-hexenal. These observations also support a preferential alkyla-

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® Abstract published in *Advance ACS Abstracts,* May 1, 1995.

0022-2623/95/1838-1853$09.00/0 © 1995 American Chemical Society

tion of **2** on the primary hydroxy group. In order to simplify the presentation of our results, these criteria may be assumed to be fulfilled for all the cases of alkylations described below.

The observations describing the formation of the allyl ether **3** also applied for the preparation of the propargyl ether **4**. The benzyl ether **5** was prepared in a similar manner. Although a comparison with the benzyl ether of cyclosporin A was not possible, the NMR spectrum of **5** exhibited a narrow AB pattern, indicating that the benzyl group of **5** was attached to the D-serine as well. The methyl ether **6** was prepared in 52% yield. Alkylations of the primary alcohol of our starting material **2** were also fast with *tert*-butyl bromoacetate[19] and isopropyl bromoacetate[19] and gave the crystalline esters **7** and **8**, respectively, in excellent yields. The *tert*-butyl ester **7** was trans esterified in methanol catalyzed by sodium methoxide to give the corresponding methyl ester **9**. This methyl ester was not available in acceptable yields (10% approximately) from the reaction of methyl bromoacetate and **2** under phase transfer reaction conditions. The *tert*-butyl ester **7** was also hydrolyzed to the acid **10** under basic conditions. While the reduction of the *tert*-butyl ester **7** in the presence of sodium borohydride proved to be slow, the reduction of the isopropyl ester **8** led to the novel cyclosporin **11** (SDZ IMM-125) in good yields. In this product the amino acid at position 8 of the natural product (D-serine) had been replaced by *O*-(2-hydroxyethyl)-D-serine. From this, the monoacetate **12** was prepared for further characterization. The addition of methylmagnesium iodide to the methyl ester **9** led to the tertiary alcohol **13**.

The newly introduced primary hydroxy group of **11** was expected to behave similarly toward alkylating agents as did [D-serine]^8-cyclosporin (**2**) itself. This should allow the extension of the side chain of **11** by additional ethylene glycol units. The phase transfer reaction of **11** with bromoacetic acid isopropyl ester yielded the ester **14** as a crystalline material. However, this was obtained in lower yield than the corresponding product **8** from **2** under similar conditions.

Reduction of **14** in the presence of lithium borohydride yielded the product **15**, with two ethylene glycol moieties attached to the hydroxy group of D-serine in **2**. Extension of the side chain by a third ethylene glycol as exemplified for **17** was carried out in a similar way via **16**. The third alkylation, that is the reaction of **15** with isopropyl bromoacetate, seemed to proceed with yields even lower than the alkylation of **11** producing **14**. From this limited number of experiments it was concluded that the yield for the addition of bromoacetate decreased with the length of the poly(ethylene glycol) chain already present in the substrate.

Cyanomethylation of [D-serine]^8-cyclosporin (**2**) was carried out under similar conditions leading to **18** in 65% yield. The amide **19** was isolated from this reaction as a side product. It was synthesized more conveniently from the methyl ester **9** in ethanol saturated with ammonia. The reaction of methyl 2-(bromomethyl)-acrylate[20] with **2** allowed the preparation of the ether **20** in 85% yield after chromatography. In this case and in contrast to attempts for the direct formation of methyl ester **9** from **2** (see above), but for reasons not completely understood, the methyl ester **20** was not affected by the strong sodium hydroxide used for phase transfer reactions. Treatment of **20** in the presence of lithium borohydride allowed the reduction of the methyl ester group to the corresponding alcohol. Unfortunately, this was accompanied by a nonstereoselective reduction of the double bond, giving **21**. The presence of a mixture of diastereoisomers was discernible from the NMR spectrum of **21**. Among the seven *N*-methyl groups present in the molecule six appeared as singlets and one gave rise to two singlets of equal intensity at δ 3.17/3.18, indicative of a 1:1 mixture of diastereoisomers. For cyclosporin A (**1**) the shift at δ 3.15 ppm was assigned[21] to the *N*-methyl group of amino acid 9. The corresponding *N*-methyl group of **21** is located closest of the stereogenic center introduced by the reduction process. The closest NH to the same stereogenic center is located on amino acid 7. This is the proton giving rise to two doublets near δ 7.15 in the NMR spectrum of **21**.

We then attempted the preparation of the unsaturated allyl ether alcohol **24** via the chloro ether **22**. The latter was prepared in 12% yield from the bisfunctional 1-chloro-2-(chloromethyl)-2-propene[19] and [D-serine]^8-cyclosporin (**2**). In the NMR spectrum the corresponding four allylic methylene protons gave rise to a very narrow multiplet near δ 4.0 ppm. In the corresponding ether formed from cyclosporin A, the chlorine was readily replaced[16] by acetate and hydrolyzed to the alcohol, carrying out both reactions under basic conditions. However, attempts to exchange the chloride of **22** by acetate and a subsequent hydrolysis step were not satisfactory. Under the conditions employed, the reactions were accompanied by the loss of water probably from the MeBMT as indicated by mass spectral data. In order to circumvent this problem, one of the chlorides of 1-chloro-2-(chloromethyl)-2-propene was replaced in two steps by a hydroxy group[22] leading to the known compound **51**. This was protected with *tert*-butyldimethylsilyl chloride producing the ether **52** and converted to the iodide **53** (for details see the Experimental Section). We were now able to obtain ether **23** from **2** and the iodide **53** in 93% yield after chromatography and in 78% yield following crystallization. Hydrolysis of the silyl protecting group then led to the desired ether **24**. Interestingly, after removal of the bulky *tert*-butyldimethylsilyl protecting group both methylene groups were recorded as AB quartets in the NMR spectrum of **24** while in the NMR spectrum of the **23**, presumably a sterically more hindered compound, only one of the new methylene groups gave rise to a quartet.

The linear isomer of **24** was prepared starting from **2** and 1,4-dibromo-2(*E*)-butene.[19] The use of 2.5 equiv of the 1,4-dibromobutene in a phase transfer reaction with [D-serine]^8-cyclosporin (**2**) resulted in the reaction of only one of the bromides with a cyclosporin giving **25** in 60% isolated yield. In a subsequent exchange reaction, the bromide was replaced by an acetoxy group, giving **26**. In a final step, the acetoxy group of **26** was hydrolyzed to the desired linear allyl alcohol **27**. The NMR spectrum of this substance gave rise to two distinct sets of signals associated with the two oxygen-substituted methylene groups of the side chain of D-serine. The lower field doublet at δ 4.17 ppm was assigned to the ether methylene while the higher field multiplet (due

to coupling to the OH) between 3.8 and 4.1 ppm was assigned to the hydroxy-substituted methylene group.

The homolog of **11** was prepared allowing [D-serine]8-cyclosporin (**2**) to react with the acetals of 3-bromopropionaldehyde. Alkylation under phase transfer-catalyzed conditions with the ethylene glycol-protected bromide[19] led to **28** in 49% yield while the more stable propylene glycol-protected bromide[19] led to **29** in 75% yield. Acidic hydrolysis of **28** followed by reduction in the presence of sodium borohydride gave the 3-hydroxypropyl ether **30**. The hydroxybutyl ether **31** was prepared from **2** and 1,4-dibromobutane[19] as solvent in a phase transfer-catalyzed reaction. The remaining bromide was exchanged by acetate in a second step. Trans esterification of the acetate in methanol catalyzed by sodium methoxide gave rise to the 4-hydroxybutyl ether **31**.

The D-serine 2-hydroxyethyl ether **11** was converted to the corresponding tosylate **32** with tosyl chloride in either pyridine or, alternatively, in methylene chloride in the presence 30% sodium hydroxide, but in the absence of phase transfer catalyst. The tosylate was replaced by azide giving **33**. This was reduced by sodium borohydride in the presence of Pd on charcoal to the amine **34**. The latter was transformed into the sulfonamide **35**.

In analogy to the preparation of the tosylate **32** or the halides **22** or **25** described above, additional alkylating agents based on [D-serine]8-cyclosporin (**2**) were generated. The bromo acetal **36** was obtained in 44% yield starting from **2** in the presence of a combination[23] of ethyl vinyl ether and *N*-bromosuccinimide. According to the NMR spectrum of **36**, the product consisted of a mixture of diastereoisomers. The bromoacetate **37** was isolated following standard procedures. The tosylate **38** was obtained from **24** under mildly basic conditions.

When the tosylate **32** was exposed to sodium hydroxide in methylene chloride, this time in the presence of a phase transfer catalyst, a smooth cyclization to the bicyclic cyclosporin **39** was accomplished in nearly quantitative yield. Thus, a new cyclosporin was obtained in four steps starting from **2** with 2(*R*)-morpholinecarboxylic acid in place of D-serine as in **2** or in place of D-alanine as in cyclosporin A (**1**). The structural assignment of **39** was supported by its NMR spectrum. The temperature had to be elevated to 180 °C (in deuterated DMSO) in order to obtain a well-resolved spectrum, while in chloroform solution at room temperature the main conformation of **39** was accompanied by detectable amounts of other, as yet undetermined, conformations. Attempts to crystallize this compound have failed so far.

Since we found the morpholine derivative **39** to be of interest pharmacologically (see below), the preparation of a morpholine derivative of a higher oxidation stage than **39** was attempted. Such a compound was accessible through the cyclization of **36** in a phase transfer-catalyzed reaction. The cyclic acetal **40** was obtained as a mixture of diastereoisomers which we could not separate. Surprisingly, we were not able to hydrolyze the acetal **40** under various acidic conditions to the corresponding cyclol. However, we took advantage of the inertness of **40** in the presence of hydrochloric acid, since this allowed unreacted starting material **36** to be hydrolized to **2**, thus rendering the purification of **40** more efficient.

Since access to a lactone via the acetal **40** seemed to be blocked, the bromo ester **37** was subjected to the conditions of anhydrous potassium carbonate in DMF solution. The mixture was heated to 60 °C for 2 h. This resulted in the formation of the bicyclic compound **41** with the 2-morpholinone-5(*R*)-carboxylic acid incorporated in position 8 of the cyclosporin skeleton. We found this ring system to be quite strained, since it underwent ring-opening reactions in the presence of methanol or ethanol, even in the absence of base, to give the corresponding hydroxy methyl or ethyl ester, respectively. In chloroform solution, compound **41** gave rise to a non-first-order NMR spectrum. Uniformity for **41** could be demonstrated by NMR in DMSO solution at 180 °C.

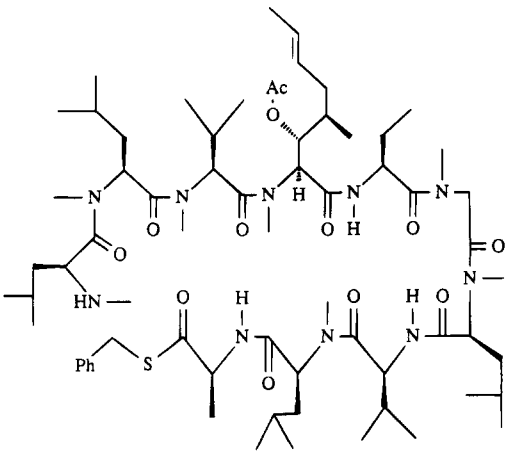
Next, the incorporation of the oxazolidine moiety into the cyclosporin skeleton was attempted. To this end [D-serine]8-cyclosporin (**2**) was subjected to a phase transfer-catalyzed reaction in the presence of methylene bromide. Thus we were able to prepare, albeit in low yield, [4(*R*)-oxazolidinecarboxylic acid]8-cyclosporin (**42**) in a single step. Proton NMR spectroscopy of a chloroform solution of this compound indicated the presence of a considerable amount of a secondary conformation. The same sample in deuterated DMSO at 180 °C gave rise to a first-order spectrum. The presence of an oxazolidine ring in **42** was supported by the ^{13}C spectrum of the compound, featuring 11 amide carbonyls. An alternative cyclization mode to a less likely 1,3,5-dioxazepine derivative should feature 10 carbonyl amides only.

For comparison with the five- and six-membered ring bicyclic cyclosporin derivatives described above, the preparation of a seven-membered ring derivative was attempted. Under phase transfer reaction conditions a cyclization of the tosylate **38** to the hexahydrooxazepine derivative **43** was accomplished in 44% yield.

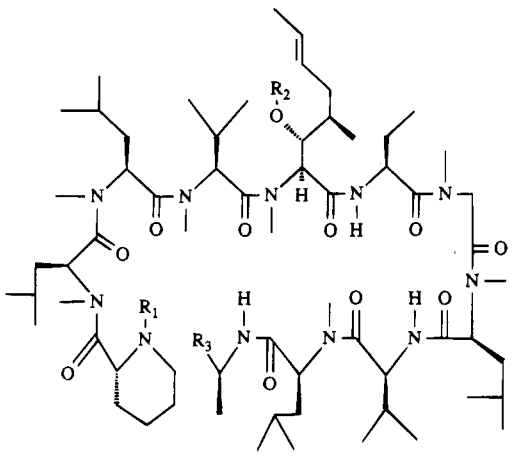
In order to further investigate the role of the ether function of **39**, the oxygen atom of the morpholine ring finally was replaced by a methylene group. [D-Serine]8-cyclosporin (**2**) did not lend itself for an easy transformation. Formally, 3(*R*)-morpholinecarboxylic acid of **39** had to be substituted by 2(*R*)-piperidinecarboxylic acid. This was accomplished making use of the decapeptide **44** recently prepared[24] from cyclosporin A (see Scheme 1). Coupling of **44** with *N*-BOC-2(*R*)-piperidinecarboxylic acid[25] (**45**) in the presence of 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ) gave the fully protected undecapeptide **46**. This was deprotected stepwise to the undecapeptide **48** *via* **47** and then cyclized to the cyclosporin **49**, an isosteric compound of **39**. In order to obtain a well-resolved NMR spectrum for **49** the sample had to be heated (in DMSO-d_6 solution) to 180 °C.

In chloroform solution cyclosporin A (**1**) is present in practically one single conformation.[21] The presence of a second conformation is barely detectable by NMR spectroscopy. The situation is quite different in the case of chloroform solutions of the bicyclic compounds **39**–**43** and **49**. The presence of one or more secondary conformations is quite noticeable. This other conformation or these conformations might well be identical to the conformation observed[26] for cyclosporin A in more

Scheme 1



44



46: R_1 = BOC; R_2 = Ac; R_3 = $COSCH_2Ph$

47: R_1 = H; R_2 = Ac; R_3 = $COSCH_2Ph$

48: R_1 = R_2 = H; R_3 = COOH

49

polar solvents and may possibly resemble the conformation assumed by cyclosporin A when bound to cyclophilin.[9]

Pharmacology

(1) Mixed Lymphocyte Reaction[27] (MLR). Lymphocytes (ca. 5×10^5) from spleens of female Balb/c mice, age 8–10 weeks, were incubated during 5 days with lymphocytes (ca. 5×10^5) from spleens of female CBA mice, also age 8–10 weeks. The cyclosporins to be tested were dissolved (v/v) in ethanol (9 parts) and Tween 80 (1 part) at a final concentration of 20 mg/mL. This solution was diluted with saline to the required concentrations prior to the addition to the incubation medium. Drug efficacy was assessed by the ability to suppress lymphocyte proliferation as measured by the incorporation of a labeled precursor ([^{3}H]thymidine) into DNA. Unstimulated lymphocyte cultures (either CBA or Balb/c) alone yielded 500–2000 dpm. Stimulated cultures (CBA + Balb/c) yielded approximately 200 000–250 000 dpm without drug. Determinations were made in triplicate cultures, and four concentrations of each drug were assessed in the range of 1.8–1000 ng/mL. The effects are expressed as IC_{50} (μg/mL) equaling the calculated concentration of a drug inhibiting the proliferative response by 50%. Results are summarized in Table 1. Control values for cyclosporin A run in parallel are given in parentheses.

(2) Humoral Immune Response[28] (Mishell–Dutton Assay). Lymphocytes (ca. 10×10^6) obtained from the spleens of female OF1 mice were cultured together with sheep erythrocytes (ca. 30×10^6) for 3 days in the presence of the cyclosporins to be tested. The lymphocytes were harvested and plated onto agar with fresh erythrocytes (antigen). Antibodies coating the erythrocytes were secreted by the sensitized lymphocytes. These erythrocytes underwent lysis and formed a plaque in presence of complement. Drug efficacy could be assessed from the number of antibody producing hence plaque forming cells. Effects are expressed as IC_{50} (μg/mL) and summarized in Table 1. Control values for cyclosporin A run in parallel are given in parentheses.

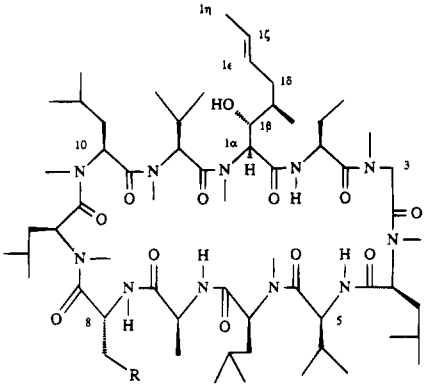
(3) Interleukin-2 Production.[29] Interleukin-2 (Il-2) production was induced by stimulating lymphocytes derived from mouse spleens with the mitogen concanavalin A. Supernatants of these cultures were collected after 48 h and assayed for IL-2 content. Interleukin-2 concentrations were determined by monitoring the growth of an IL-2-dependent cell line (CTLL). The proliferation of these cells was determined after 48 h using tritium-labeled thymidine incorporation as described for the mixed lymphocyte reaction. The amount of IL-2 produced in the primary culture with concanavalin A correlates with the growth of the indicator (CTLL) cells. Cyclosporins to be tested were added together with the mitogen in the primary culture. Inhibitory concentrations (IC_{50}) were determined and are listed in Table 1. Control values for cyclosporin A run in parallel are given in parentheses.

Discussion

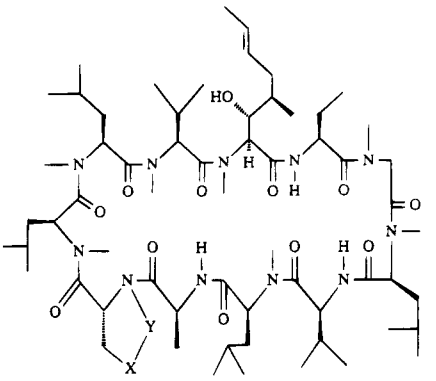
In order to define scope and limitations of the structure–activity relationship for cyclosporins, the novel derivatives of [D-serine]^8-cyclosporin described in this report were evaluated pharmacologically. *In vitro* tests like the mixed lymphocyte reactions (MLR), the primary humoral immune response to sheep red blood cells (Mishell–Dutton, MD), and the formation of Il-2 were used to evaluate the biological potency of the synthetic derivatives. These were run in parallel and compared directly with the effects of cyclosporin A (**1**) in these same tests. The results are listed in Table 1.

The [D-serine]-8-substituted ethers **3**–**10** were found to be equipotent in one of the *in vitro* tests and less active by a factor of 10 at most in one of the other tests. The reduction product **11**, obtained from the esters **8** or **9**, was judged to be as potent as **1** in these *in vitro* tests. The acetate derivative **12** was found to be of comparable potency in these assays. Extension by a second ethylene glycol of **11** to **15** led to a decrease in activity. A further reduction in activity was observed when a third ethylene glycol moiety was attached to **15** to give compound **17**. A potency judged to be equivalent to that of **1** was observed for compound **24**. On the other

Table 1. In Vitro Test Results and Substituents



1 - 38



39 - 43, 49

	MLR[a]		MD[b]		Il-2[c]		R
1	<0.008		0.020		<0.008		H
2	<0.008		0.026		<0.008		OH
3	0.088	(0.008)	0.032	(0.028)	0.115	(0.008)	$OCH_2CH{=}CH_2$
4	0.072	(0.008)	0.036	(0.018)	0.09	(0.008)	$OCH_2C{\equiv}CH$
5	0.25	(0.008)	0.46	(0.029)	0.095	(0.020)	$OCH_2C_6H_5$
6	<0.008	(0.008)	<0.01	(0.003)	0.024	(0.008)	OCH_3
7	0.24	(0.080)	0.41	(0.030)	>1.0	(0.020)	$OCH_2COOC(CH_3)_3$
8	0.1	(0.027)	0.725	(0.032)			$OCH_2COOCH(CH_3)_2$
9	0.044	(0.008)	0.065	(0.025)			OCH_2COOCH_3
10	0.44	(0.018)	0.48	(0.024)	0.53	(0.008)	OCH_2COOH
11	<0.008	(0.008)	0.018	(0.015)	0.006	(0.012)	OCH_2CH_2OH
12	0.018	(0.012)	0.028	(0.028)	0.012	(0.008)	OCH_2CH_2OAc
13	0.05	(0.008)	0.078	(0.032)			$OCH_2C(CH_3)_2OH$
14	0.05	(0.008)	0.078	(0.032)			$OCH_2CH_2OCH_2COOCH(CH_3)_2$
15	0.17	(0.008)	0.035	(0.023)	0.075	(0.008)	$OCH_2CH_2OCH_2CH_2OH$
16	0.35	(0.027)	0.53	(0.031)			$OCH_2CH_2OCH_2CH_2OCH_2COOCH(CH_3)_2$
17	0.47	(0.002)	0.1	(0.015)			$OCH_2CH_2OCH_2CH_2OCH_2CH_2OH$
18	0.01	(0.008)	0.023	(0.027)			$OCH_2C{\equiv}N$
19	0.064	(0.012)	0.063	(0.014)	0.07	(0.008)	OCH_2CONH_2
20	0.04	(0.008)	0.016	(0.006)	0.1	(0.008)	$OCH_2C(=CH_2)COOCH_3$
21	0.013	(0.008)	0.024	(0.022)	0.017	(0.008)	$OCH_2CH(CH_3)CH_2OH$
22	0.27	(0.012)	0.23	(0.024)	>0.1	(0.008)	$OCH_2C(=CH_2)CH_2Cl$
23	0.45	(0.027)	>1.0	(0.032)	>1	(0.029)	$OCH_2C(=CH_2)CH_2OSi(CH_3)_2C(CH_3)_3$
24	<0.008	(0.008)	0.032	(0.029)	0.048	(0.008)	$OCH_2C(=CH_2)CH_2OH$
25	0.09	(0.008)	0.24	(0.028)			$OCH_2CH{=}CHCH_2Br$
26	0.04	(0.006)	0.04	(0.026)			$OCH_2CH{=}CHCH_2OAc$
27	0.07	(0.006)	0.036	(0.026)			$OCH_2CH{=}CHCH_2OH$
28	0.117	(0.008)	0.105	(0.026)	0.086	(0.020)	$OCH_2CH_2CH(OCH_2CH_2O)$
29	0.12	(0.014)	0.21	(0.018)			$OCH_2CH_2CH(OCH_2CH_2CH_2O)$
30	0.02	(0.002)	0.009	(0.015)			$OCH_2CH_2CH_2OH$
31	0.094	(0.008)	0.09	(0.020)			$OCH_2CH_2CH_2CH_2OH$
32	0.050	(0.028)			0.014	(0.020)	OCH_2CH_2OTos
33	0.11	(0.012)	0.087	(0.015)			$OCH_2CH_2N_3$
34	0.22	(0.012)	0.095	(0.028)	0.38	(0.008)	$OCH_2CH_2NH_2$
35	0.11	(0.012)	0.048	(0.024)	0.057	(0.014)	$OCH_2CH_2NHSO_2C_{10}H_6$-5-$N(CH_3)_2$
36							$OCH(OEt)CH_2Br$
37							$OC(=O)CH_2Br$
38							$OCH_2C(=CH_2)CH_2OSO_2C_6H_4CH_3$
39	0.006	(0.028)			0.003	(0.010)	X = O; Y = CH_2CH_2
40	0.040	(0.038)			0.010	(0.010)	X–Y = $OCH(OEt)CH_2$
41	0.167	(0.038)			0.024	(0.010)	X–Y = $OC(=O)CH_2$
42	0.118	(0.038)					X = O; Y = CH_2
43	0.036	(0.038)			0.012	(0.010)	X = O; Y = $CH_2C(=CH_2)CH_2$
49	0.029	(0.038)					X = CH_2; Y = CH_2CH_2

[a] MLR = mixed lymphocyte reaction[27] (IC_{50}, μg/mL). [b] MD = Mishel–Dutton assay[28] (IC_{50}, μg/mL). [c] Il-2 = interleukin-2 production [29](IC_{50}, μg/mL).

hand, its linear isomer **27** was found to be weaker in the MLR test by a factor of 10.

When the bicyclic cyclosporin **39** was tested in our *in vitro* tests, it was consistently found to be approximately 3–4 times more active than cyclosporin A. It inhibited the release of Il-2 at concentrations approximately 3 times lower than did cyclosporin A. In the MLR test, it was about 4 times as potent as cyclosporin A. To the best of our knowledge only one other cyclosporin derivative[30] has been reported to reach this level of potency. The activities of the remaining bicyclic cyclosporins were found to be equipotent to cyclosporin A at best.

Conclusions

[D-Serine]⁸-cyclosporin (**2**) was found to tolerate a fairly wide variety of substituents on the primary

hydroxy group without undue loss of activity as manifested by the *in vitro* tests described above. In fact, the bicyclic cyclosporin **39**, prepared in four steps from the natural product **2**, was found to be approximately 3–4 times more active than cyclosporin A. The oxygen atom of the morpholine derivative **39** seems to play an important role for the *in vitro* activity. When this oxygen was replaced by a methylene group, the resulting compound **49** which is isosteric to **39** was found to be less active than **39** but still equipotent to cyclosporin A. When the oxygen atom of **39** was conjugated with a carbonyl group as exemplified by the lactone **41**, the result was again a weaker immunosuppressive activity than measured for **39**. Incorporating the endocyclic oxygen atom into an acetal function as exemplified by **40**, also resulted in an immunosuppressant less active than **39**. Ring contraction to a five-membered amino acid derivative in position 8 showed similar effects: the oxazolidine derivate **42** also was found to be inferior to the morpholine derivative **39**. Ring enlargement to the seven-membered oxazepine derivative **43** again resulted in a drop of activity as tested relative to **39**. From this limited number of bicyclic cyclosporins including the compound described by Schreiber *et al.*,[30] it may be concluded that a heteroatom with its lone pairs of electrons like the oxygen atom (or a sulfur atom for the Schreiber compound) of the morpholine derivate **39** seems to enhance the immunosuppressive activity as measured in our *in vitro* tests. But the ring size accommodating the heteroatom plays an important role as well.

Cyclosporin A essentially exists in both the solid state and in chloroform solution as a single conformation.[21] This conformation differs from the cyclosporin A conformation when complexed with cyclophilin.[9] On the other hand, chloroform solutions of the bicyclic cyclosporins described above are populated to a considerable amount by at least one other low energy conformation. So far, the nature of these conformations has not been determined experimentally. It may be speculated that such conformations, e.g., of the morpholine derivative **39**, might mimick active conformations of cyclosporin A. However, the presence of such low-energy conformations alone cannot be the only reason for an improvement in activity as indicated by the results obtained for the bicyclic derivatives **40–43** or **49** of cyclosporin, which are all weaker than cyclosporin A. From the limited number of cyclic amino acids incorporated into position 8 of the cyclosporin skeleton one might conclude that further attempts toward shifting the conformational equilibrium[31] away from the "ground" state conformation of cyclosporin A, as manifested by NMR spectroscopy in chloroform solution, could lead to cyclosporins with higher immunosuppressive activity.

Experimental Section

General. Thin layer chromatography (TLC) was carried out on glass plates coated with silica gel F-60 (E. Merck) and, usually, were developed in ethyl acetate saturated with water. For flash column chromatography, silica gel 60 (230–400 mesh ASTM), E. Merck, Darmstadt, was used. High-pressure liquid chromatography (HPLC) analyses were carried out on a Merck-Hitachi HPLC system using a RP-18 reverse phase column at 75 °C and monitored with a UV detector at 204 nm. Mixtures of acetonitrile and water were used as mobile phase. The amount of water might vary between 15 and 40%. The aqueous phase (3.7 L) contained 85% phosphoric acid (1 mL). Nuclear magnetic resonance spectra (NMR) for the new compounds were obtained in deuterated chloroform solutions on a Bruker-360 spectrometer and were recorded in δ values relative to TMS (tetramethylsilane) as internal standard. Except for *N*-methyl groups, chemical shifts are listed only if they differ significantly from those corresponding to the starting material. The assignments of the signals are tentative and based on the chemical shifts observed for the corresponding signals of cyclosporin A, some of which are added for convenience (in parentheses). For the complete spectrum of cyclosporin A (**1**) see ref 21. Mass spectra were measured on a VG 70-SE high-resolution mass spectrometer. Melting points are not corrected.

[*O*-Propenyl-D-serine]^8-cyclosporin (3). A solution of [D-serine]^8-cyclosporin (**2**) (2.4 g, 2 mmol), tetrabutylammonium chloride[19] (1.13 g, 4 mmol), and allyl iodide[19] (0.46 mL, 5 mmol) in methylene chloride (100 mL) was stirred at room temperature (rt) for 2 h in the presence of 30% sodium hydroxide (10 mL). Ether was added and washed with water. The organic phase was separated, dried over Na_2SO_4, and evaporated to dryness to give the crude product (2.5 g). This was purified on silica gel with ether/ethyl acetate (95:5) to give the monoalkylated product (2.1 g): yield 84%; *m/z* calcd for $C_{65}H_{115}N_{11}O_{13}$ 1257.9, found 1258.5 $[MH]^+$, 1145.4 $[M - 112]^+$; $[\alpha_D] = -179.9°$ ($c = 0.732$ in MeOH); NMR δ 2.68 (2.70), 2.72 (2.70), 3.12 (3.11), 3.15 (3.11), 3.27 (3.27), 3.35–3.60, 3.40 (3.39), 3.50 (3.51), 3.73–3.80, 3.91, 5.15–5.25, 5.75–5.85, 7.45, 7.58, 8.09, 8.52.

[*O*-Propinyl-D-serine]^8-cyclosporin (4). A solution of [D-serine]^8-cyclosporin (**2**) (1.2 g, 1 mmol), propargyl bromide[19] (0.1 mL, 1 mmol), and tetrabutylammonium chloride (50 mg, 0.2 mmol) in methylene chloride (50 mL) was stirred overnight at rt in the presence of 30% sodium hydroxide (7 mL). Then *tert*-butyl methyl ether was added, washed with water, separated, and dried over $MgSO_4$. The solvent was evaporated to leave the crude product (1.4 g). This was purified on silica gel with ether/ethyl acetate (5:1) to give the pure product (1.0 g): yield 81%; *m/z* calcd for $C_{65}H_{113}N_{11}O_{13}$ 1255.9, found 1257.0 $[MH]^+$, 1143.8 $[M - 112]^+$; $[\alpha_D] = -168.5°$ ($c = 0.55$ in MeOH); NMR δ 2.42, 2.68 (2.70), 2.72 (2.70), 3.12 (3.11), 3.15 (3.11), 3.27 (3.27), 3.45–3.50, 3.65–3.70, 3.42 (3.39), 3.52 (3.51), 3.70–3.80, 4.08, 7.04, 7.48, 7.75, 8.10.

[*O*-Benzyl-D-serine]^8-cyclosporin (5). A solution of [D-serine]^8-cyclosporin (**2**) (1.2 g, 1 mmol), benzyltriethylammonium chloride (0.2 g, 0.5 mmol), and benzyl bromide (5 mL, 37 mmol) in methylene chloride (100 mL) was stirred for 3 h in the presence of 40% aqueous NaOH (20 mL). The mixture was diluted with ether (300 mL) and washed with water. The organic phase was dried over $MgSO_4$ and evaporated. The crude product was chromatographed on silica gel to give the pure product (1.0 g): yield 76.5%; *m/z* calcd for $C_{69}H_{117}N_{11}O_{13}$ 1307.9, found 1308.5 $[MH]^+$, 1195.3 $[M - 112]^+$; $[\alpha_D] = -173.1°$ ($c = 0.741$ in MeOH); NMR δ 2.70 (2.70), 2.73 (2.70), 3.13 (3.11), 3.15 (3.11), 3.27 (3.27), 3.43 (3.39), 3.52 (3.51), 3.4–3.6, 3.72–3.80, 4.4–4.5, 7.35–7.45, 7.02, 7.48, 7.76, 8.13.

[*O*-Methyl-D-serine]^8-cyclosporin (6). A solution of [D-serine]^8-cyclosporin (**2**) (1.2 g, 1 mmol), tetrabutylammonium chloride (0.454 g, 2 mmol), and methyl iodide (282 mg, 2 mmol) in methylene chloride (100 mL) was stirred overnight at rt in the presence of 30% sodium hydroxide (50 mL). More solvent was added, and the mixture was washed with water, separated, dried over $MgSO_4$, and evaporated. The crude product was purified on silica gel to give the pure product (650 mg) as a white foam: yield 52%; *m/z* calcd for $C_{63}H_{113}N_{11}O_{13}$ 1231.9, found 1232.9 $[MH]^+$, 1119.5 $[M - 112]^+$; $[\alpha_D] = -175.1°$ ($c = 0.562$ in MeOH); NMR δ 2.68 (2.70), 2.72 (2.70), 3.11 (3.11), 3.13 (3.11), 3.27 (3.27), 3.28, 3.40 (3.39), 3.50 (3.51), 3.3–3.6, 3.70–3.75, 3.75–3.80, 7.02, 7.49, 7.75, 8.11.

[*O*-[(*tert*-Butoxycarbonyl)methyl]-D-serine]^8-cyclosporin (7). A solution of [D-serine]^8-cyclosporin (**2**) (60 g, 50 mmol), *tert*-butyl bromoacetate[19] (40 g, 200 mmol), and benzyltriethylammonium chloride (2 g, 5 mmol) in methylene chloride (750 mL) was stirred vigorously for 2 h at rt with 30% sodium hydroxide (150 mL). The reaction mixture was diluted with

water (200 mL) and extracted with two portions of *tert*-butyl methyl ether (1 L each). The organic phase was dried over $MgSO_4$, filtered, and evaporated to yield the crude product (84 g). This was treated with ethyl ether to give the pure product as a crystalline material (58.2 g). The mother liquors were chromatographed on silica gel to give a second batch (7.5 g) of pure product: yield 95%; mp 183–184 °C; *m/z* calcd for $C_{68}H_{121}N_{11}O_{15}$ 1331.9, found 1333.1 $[MH]^+$, 1219.9 $[M - 112]^+$; $[\alpha_D] = -168.4°$ ($c = 0.41$ in MeOH); NMR δ 1.47, 2.68 (2.70), 2.71 (2.70), 3.10 (3.11), 3.19 (3.11), 3.25 (3.27), 3.40 (3.39), 3.50 (3.51), 3.65–3.80, 3.81–3.93, 7.03, 7.48, 7.76, 8.11.

[*O*-[(Isopropoxycarbonyl)methyl]-D-serine]⁸-cyclosporin (8). A 6 L flask was charged with a mixture of [D-serine]⁸-cyclosporin (**2**) (244 g, 0.2 mol), isopropyl bromoacetate[19] (181 g, 1 mol), tetraethylammonium chloride (45 g, 0.2 mol), methylene chloride (2 L), and 30% NaOH solution (500 mL). This was stirred vigorously at rt for 45 min. Then, the aqueous phase was diluted with water (1 L), separated from the organic phase, and extracted with three portions of ether (1 L each). The combined organic phase was acidified with acetic acid, washed with brine until neutral, and dried over $MgSO_4$. The aqueous phase was extracted twice with ether and dried as above. The organic solvents were evaporated. The residue was dissolved in a minimal amount of methylene chloride and poured on a silica gel column 19 cm in diameter and 11 cm in height. The column was eluted in this order with methylene chloride, ethyl ether, ether/ethyl acetate (2:1), and ethyl acetate (2 L each). The fractions containing the product were evaporated. The residue was treated with ether/pentane to give the pure crystalline product (231 g): yield 88%; mp 169–171 °C; *m/z* calcd for $C_{67}H_{119}N_{11}O_{15}$ 1317.9, found 1319.0 $[MH]^+$, 1205.7 $[M - 112]^+$; $[\alpha_D] = -162.4°$ ($c = 0.37$ in MeOH); NMR δ 1.26, 2.68 (2.70), 2.72 (2.70), 3.11 (3.11), 3.20 (3.11), 3.25 (3.27), 3.40 (3.39), 3.50 (3.51), 3.45–3.75, 3.75–3.85, 3.9–4.02, 4.9–5.2, 7.05, 7.49, 7.77, 8.10.

[*O*-(Carbomethoxymethyl)-D-serine]⁸-cyclosporin (9). A solution of *tert*-butyl ester **7** (68 g, 66 mmol) in absolute methanol (250 mL) was added at rt to a freshly prepared solution of sodium (1.5 g, 65 mmol) in methanol (50 mL). After 3 h the mixture was acidified with acetic acid and the solvent was evaporated to dryness. The residue was dissolved in methylene chloride and washed with water. The organic phase was dried over $MgSO_4$ and evaporated. The residue was crystallized from ether to give the pure methyl ester (65.8 g): yield 97%; mp 139 °C dec; *m/z* calcd for $C_{65}H_{115}N_{11}O_{15}$ 1289.9, found 1290.8 $[MH]^+$, 1177.7 $[M - 112]^+$; $[\alpha_D] = -187.3°$ ($c = 0.54$ in MeOH); NMR δ 2.68 (2.70), 2.71 (2.70), 3.10 (3.11), 3.18 (3.11), 3.25 (3.27), 3.40 (3.39), 3.50 (3.51), 3.45–3.75, 3.73, 3.75–3.80, 3.97–4.08, 7.04, 7.48, 7.74, 8.10. This product was also obtained, albeit in 10–15% yield only, starting from [D-serine]⁸-cyclosporin (**2**) via alkylation with methyl bromoacetate[19] in a phase transfer-catalyzed reaction.

[*O*-(Carboxymethyl)-D-serine]⁸-cyclosporin (10). A solution of the *tert*-butyl ester **7** (580 mg, 0.44 mmol) was added to a solution of KOH (2.5 g, 4.5 mmol) in methanol (75 mL) and 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 the mixture was washed with water. The organic phase was dried over $MgSO_4$ and evaporated 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.

[*O*-(2-Hydroxyethyl)-D-serine]⁸-cyclosporin (11) (SDZ IMM-125). Under an atmosphere of argon, absolute ethanol (1 L) was externally cooled with the help of a dry ice bath to 5 °C internal temperature. Lithium borohydride (25 g, 1.15 mol) was added slowly. When the addition was completed, the solution was allowed to warm to rt. A solution of the isopropyl ester **8** (98.8 g, 75 mmol) in absolute ethanol (750 mL) was added within 5 min with rapid stirring. The formation of a suspension was observed. After being kept at rt for 3 h, the mixture was diluted with ethanol (1 L) and cooled to 5 °C. A solution of acetic acid (70 mL) in water (250 mL) was added while taking care with the aid of a dry ice bath that the internal temperature did not exceed 25 °C. The clear solution was evaporated under reduced pressure. The residue was dissolved in a 4:1 mixture of *tert*-butyl methyl ether and ethyl acetate and then washed with water. The organic phase was dried over $MgSO_4$ and evaporated. The crude product was crystallized from ether/water to give the pure product (77 g): yield 81%; mp 126–128 °C dec; *m/z* calcd for $C_{64}H_{115}N_{11}O_{14}$ 1261.9, found 1262.8 $[MH]^+$, 1149.6 $[M - 112]^+$; $[\alpha_D] = -185.9°$ ($c = 0.632$ in MeOH); NMR δ 2.68 (2.70), 2.70 (2.70), 3.10 (3.11), 3.17 (3.11), 3.23 (3.27), 3.40 (3.39), 3.50 (3.51), 3.54, 3.58–3.65, 3.65–3.70, 3.8–3.85, 7.31, 7.48, 7.82, 8.02. The same product was also obtained when the methyl ester **9** was reduced under similar conditions.

[*O*-(2-Acetoxyethyl)-D-serine]⁸-cyclosporin (12). A mixture of the alcohol **11** (600 mg, 0.47 mmol) in pyridine (30 mL) and acetic anhydride (30 mL) was kept at rt for 2.5 h. The solvent was evaporated under reduced pressure. The crude product was chromatographed on silica gel with ethyl ether/ethyl acetate (4:1) as eluent to yield the pure product (430 mg); yield 70%; mp 153–155 °C; *m/z* calcd for $C_{66}H_{117}N_{11}O_{15}$ 1304.7, found 1305.1 $[MH]^+$, 1192.0 $[M - 112]^+$; $[\alpha_D] = -175.8°$ ($c = 0.25$ in MeOH); NMR δ 2.08, 2.69 (2.70), 2.72 (2.70), 3.11 (3.11), 3.15 (3.11), 3.26 (3.27), 3.40 (3.39), 3.50 (3.51), 3.55–3.65, 3.70–3.80, 4.1–4.2, 7.01, 7.49, 7.75, 8.10.

[*O*-(2-Hydroxy-2-methylpropyl)-D-serine]⁸-cyclosporin (13). A solution of methyl ester **9** (500 mg, 0.387 mmol) in ether (20 mL) was added to the Grignard reagent prepared from Mg (2.4 g, 0.1 mol) and methyl iodide (14.2 g, 0.1 mol) in ethyl ether (200 mL). After 2 h at rt there was added saturated aqueous NH_4Cl until a clear solution was obtained. The organic phase was washed with water, dried over $MgSO_4$, and evaporated. The crude product (700 mg) was chromatographed on silica gel with ethyl acetate as eluent to give the pure product (200 mg): yield 40%; *m/z* calcd for $C_{66}H_{119}N_{11}O_{14}$ 1289.9, found 1291.0 $[MH]^+$, 1177.8 $[M - 112]^+$; $[\alpha_D] = -171.2°$ ($c = 0.509$ in MeOH); NMR δ 1.17, 1.18, 2.68 (2.70), 2.70 (2.70), 3.10 (3.11), 3.16 (3.11), 3.23 (3.27), 3.40 (3.39), 3.50 (3.51), 3.25–3.35, 3.60, 3.75–3.85, 7.41, 7.48, 7.73, 8.09.

{*O*-[(3-Oxapentanoic acid)-5-yl]-D-serine}⁸-cyclosporin Isopropyl Ester (14). A mixture of [O-(2-hydroxyethyl)-D-serine]⁸-cyclosporin (**11**) (6.25 g, 5 mmol), isopropyl bromoacetate[19] (6.8 g, 38 mmol), and benzyltriethylammonium chloride (1 g, 2.4 mmol) in a mixture of methylene chloride (150 mL) and 30% sodium hydroxide (50 mL) was stirred at rt for 18 h. The mixture was diluted with *tert*-butyl methyl ether (300 mL) and water (200 mL). The organic phase was washed with saturated NaCl solution. The aqueous phase was extracted again with ether (150 mL). The organic phase was dried over $MgSO_4$, filtered, and evaporated to give crude product (7.7 g). This was chromatographed on silica gel to give the pure product (3.7 g, 54%), which solidified following the addition of a small amount of ethyl ether to give the crystalline product (2.6 g): yield 38%; mp 119 °C dec; *m/z* calcd for $C_{69}H_{123}N_{11}O_{16}$ 1361.7, found 1362.5 $[MH]^+$, 1249.2 $[M - 112]^+$; $[\alpha_D] = -173.2°$ ($c = 0.731$ in MeOH); NMR δ 1.26, 2.68 (2.70), 2.71 (2.70), 3.10 (3.11), 3.16 (3.11), 3.25 (3.27), 3.40 (3.39), 3.50 (3.51), 3.35–3.75, 3.75–3.85, 4.05, 4.95–5.15, 7.02, 7.47, 7.76, 8.10.

[*O*-(5-Hydroxy-3-oxapentyl)-D-serine]⁸-cyclosporin (15). Lithium borohydride (1.86 g, 55 mmol) was added in portions to a solution of **14** (2.1 g, 1.5 mmol) in absolute ethanol (175 mL) under an atmosphere of argon (slightly exothermic reaction). After 3 h, the solution was neutralized with acetic acid. The solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (150 mL) and washed with 2 portions of water (75 mL each). The organic phase was dried over $MgSO_4$, filtered, and evaporated under reduced pressure to leave the crude product (2.0 g). This was chromatographed on silica gel to give the pure product (1.6 g): yield 82%; *m/z* calcd for $C_{66}H_{119}N_{11}O_{15}$ 1305.9, found 1306.7 $[MH]^+$, 1193.6 $[M - 112]^+$; $[\alpha_D] = -183.2°$ ($c = 0.528$ in MeOH); NMR δ 2.6–2.65, 2.68 (2.70), 2.71 (2.70), 3.10 (3.11), 3.16 (3.11), 3.25 (3.27), 3.40 (3.39), 3.50 (3.51), 3.5–3.7, 3.65–3.75, 7.06, 7.48, 7.76, 8.09.

{*O*-[(3,6-Dioxaoctanoic acid)-8-yl]-D-serine}⁸-cyclospor-

in Isopropyl Ester (16). A mixture of the cyclosporin **15** (1.6 g, 1.3 mmol), isopropyl bromoacetate[19] (3.6 g, 20 mmol), benzyltriethylammonium chloride (260 mg, 1.15 mmol), methylene chloride (50 mL), and 30% sodium hydroxide (30 mL) was stirred at rt for 18 h. After diluting with *tert*-butyl methyl ether (150 mL) the organic phase was washed with water (50 mL), dried over $MgSO_4$, filtered, and evaporated to give the crude product (1.56 g, 85%). This was chromatographed on silica gel to give the pure product (520 mg): yield 29%; *m/z* calcd for $C_{71}H_{127}N_{11}O_{17}$ 1405.7, found 1406 $[MH]^+$, 1293 $[M - 112]^+$; $[\alpha_D] = -162.2°$ ($c = 0.384$ in MeOH); NMR δ 1.27, 2.68 (2.70), 2.71 (2.70), 3.09 (3.11), 3.15 (3.11), 3.24 (3.27), 3.39 (3.39), 3.50 (3.51), 3.45–3.75, 3.75–3.85, 4.08, 4.9–5.15, 7.00, 7.47, 7.74, 8.08.

{*O*-(8-Hydroxy-3,6-dioxaoctyl)-D-serine}^{8}-cyclosporin (17). Under an atmosphere of argon was added lithium borohydride (0.49 g, 15 mmol) (exothermic reaction) to a solution of **16** (0.46 g, 0.33 mmol) in absolute ethanol (25 mL). After 2 h, excess of reducing agent was decomposed by the addition of acetic acid. The solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (150 mL), washed with two portions of water (75 mL each), and dried over $MgSO_4$. The filtered solution was evaporated under reduced pressure to leave the crude product (2.0 g) which was chromatographed on silica gel to give the pure product (0.280 g): yield 64%; *m/z* calcd for $C_{68}H_{123}N_{11}O_{16}$ 1349.9, found 1350 $[MH]^+$, 1237 $[M - 112]^+$; $[\alpha_D] = -170.4°$ ($c = 0.40$ in MeOH); NMR δ 2.68 (2.70), 2.71 (2.70), 3.10 (3.11), 3.15 (3.11), 3.25 (3.27), 3.39 (3.39), 3.50 (3.51), 3.55–3.80, 7.00, 7.47, 7.76, 8.08.

[*O*-(Cyanomethyl)-D-serine]^8-cyclosporin (18). A solution of [D-serine]^8-cyclosporin (**2**) (2.4 g, 2 mmol), benzyltriethylammonium chloride (0.9 g, 3.3 mmol), and iodoacetonitrile[19] (0.668 g, 4 mmol) in methylene chloride (100 mL) was stirred vigorously overnight in the presence of a 30% sodium hydroxide solution (50 mL). Then, more phase transfer catalyst (227 mg, 1 mmol) and chloroacetonitrile (0.5 mL) were added, and the mixture was stirred for an additional 6 h. The organic phase was washed with water until neutral, then dried over Na_2SO_4, and evaporated. The residue (2.8 g) was chromatographed on silica gel with ether/acetone (92:8) to give the pure product (1.62 g): yield 67%; *m/z* calcd for $C_{64}H_{112}N_{12}O_{13}$ 1256.9, found 1258.0 $[MH]^+$, 1144.8 $[M - 112]^+$; $[\alpha_D] = -167.1°$ ($c = 0.976$ in MeOH); NMR δ 2.68 (2.70), 2.71 (2.70), 3.07 (3.11), 3.15 (3.11), 3.25 (3.27), 3.40 (3.39), 3.50 (3.51), 3.50 and 3.70–3.75, 3.75–3.80, 4.16–4.26, 7.10, 7.49, 7.77, 8.05.

{*O*-[(Aminocarbonyl)methyl]-D-serine}^{8}-cyclosporin (19). From the above reaction, the title compound (**19**) was isolated as a side product (more polar than **18**): *m/z* calcd for $C_{64}H_{114}N_{12}O_{14}$ 1274.9, found 1275.9 $[MH]^+$; $[\alpha_D] = -156.1°$ ($c = 0.532$ in MeOH); NMR δ 1.39, 1.63, 2.71 (2.70), 2.72 (2.70), 3.11 (3.11), 3.20 (3.11), 3.23 (3.27), 3.40 (3.39), 3.51 (3.51), 3.56–3.65, 3.67–3.75, 3.78–3.90, 4.46, 4.64, 5.52, 6.83, 7.33, 7.48, 7.80, 7.94. The same amide was obtained more readily from the methyl ester **9** in ethanol saturated with anhydrous ammonia in 70% yield.

[*O*-(2-Carbomethoxy-2-propen-1-yl)-D-serine]^8-cyclosporin (20). A mixture of [D-serine]^8-cyclosporin (**2**) (17.0 g, 14 mmol), methyl 2-(bromomethyl)acrylate[20] (12.6 g, 14 mmol), tetrabutylammonium chloride (0.53 g, 2 mmol), methylene chloride (500 mL), and 40% sodium hydroxide (100 mL) was stirred mechanically at rt for 45 min. Then the organic phase was washed twice with water, dried over $MgSO_4$, and evaporated under reduced pressure. The crude material (19 g) was chromatographed on silica gel with ethyl ether/*tert*-butyl methyl ether to give the product (15.6 g): yield 85%; *m/z* calcd for $C_{67}H_{117}N_{11}O_{15}$ 1315.9, found 1316.7 $[MH]^+$, 1203.6 $[M - 112]^+$; $[\alpha_D] = -161.4°$ ($c = 0.53$ in MeOH); NMR δ 2.68 (2.70), 2.72 (2.70), 3.10 (3.11), 3.15 (3.11), 3.25 (3.27), 3.40 (3.39), 3.50 (3.51), 3.45–3.65, 3.77, 3.7–3.8, 4.15, 5.80, 6.30, 7.04, 7.49, 7.75, 8.11.

{*O*-[2-(Hydroxymethyl)-1-propyl]-D-serine}^{8}-cyclosporin (21). A solution of methyl ester **20** (1 g, 0.76 mmol) in ethanol (100 mL) was treated with lithium borohydride (0.860 g, 39 mmol). After 3 h, excess reducing agent was decomposed by the addition of water and ethyl acetate (100 mL) plus a small amount of 2 N HCl in order to dissolve all the solids. The aqueous phase was extracted with more ethyl acetate. The combined organic phase was dried over $MgSO_4$ and evaporated to dryness to leave the crude product (2.5 g). This was chromatographed on silica gel with ethyl acetate as eluant to give the pure product (0.470 g): yield 48%; *m/z* calcd for $C_{66}H_{119}N_{11}O_{14}$ 1289.9, found 1291.0 $[MH]^+$, 1177.8 $[M - 112]^+$; $[\alpha_D] = -174.4°$ ($c = 0.28$ in MeOH); NMR δ 2.70 (2.70), 2.73 (2.70), 3.08 (3.11), 3.17, 3.18 (3.11), 3.23 (3.27), 3.37 (3.39), 3.50 (3.51), 3.4–3.6, 3.75–3.80, 7.12–7.17, 7.49, 7.77, 8.06.

{*O*-[2-(Chloromethyl)-2-propenyl]-D-serine}^{8}-cyclosporin (22). A solution of [D-serine]^8-cyclosporin (**2**) (2.4 g, 2 mmol), tetrabutylammonium chloride (260 mg, 1 mmol), and 1-chloro-2-(chloromethyl)-2-propene[19] (1.25 g, 10 mmol) in methylene chloride (100 mL) was stirred at rt over the weekend in the presence of 40% sodium hydroxide (10 mL). Then, *tert*-butyl methyl ether was added, and the mixture was washed with water, dried over $MgSO_4$, and evaporated to give the crude product (2.8 g). Pure product (300 mg) was obtained following column chromatography on silica gel: yield 12%; *m/z* calcd for $C_{66}H_{116}N_{11}O_{13}Cl$ 1306.4, found 1306.9 $[MH]^+$, 1193.8 $[M - 112]^+$; $[\alpha_D] = -164.6°$ ($c = 0.771$ in MeOH); NMR δ 2.70 (2.70), 2.72 (2.70), 3.11 (3.11), 3.17 (3.11), 3.26 (3.27), 3.40 (3.39), 3.35–3.60, 3.50 (3.51), 3.7–3.8, 4.0–4.1, 5.18, 5.29, 7.02, 7.48, 7.76, 8.10.

{*O*-[2-[[(*tert*-Butyldimethylsilyl)oxy]methyl]-2-propenyl]-D-serine}^{8}-cyclosporin (23). The solution of [D-serine]^8-cyclosporin (**2**) (24.0 g, 20 mmol), tetrabutylammonium chloride[19] (23.0 g, 0.100 mol), and 2-(iodomethyl)-2-propen-1-ol *tert*-butyldimethylsilyl ether (**49**) (31.2 g, 0.100 mol) in methylene chloride (850 mL) was stirred at rt for 3 h in the presence of 30% sodium hydroxide (250 mL). The mixture was diluted with diethyl ether (1 L) and washed three times with water. The organic phase was dried over $MgSO_4$ and evaporated to dryness to leave a yellow viscous liquid (46 g). This was chromatographed on silica gel with diethyl ether/ethyl acetate (4:1) to give the pure product (25.8 g, 93%). Addition of diethyl ether/pentane led to crystalline material (21.4 g): yield 78%: mp 167–169 °C; *m/z* calcd for $C_{72}H_{131}N_{11}O_{14}Si$ 1402.0, found 1402.7 $[MH]^+$, 1289.7 $[M - 112]^+$; $[\alpha_D] = -168.2°$ ($c = 0.29$ in MeOH); NMR δ 0.05, 0.85, 2.68 (2.70), 2.70 (2.70), 3.10 (3.11), 3.13 (3.11), 3.25 (3.27), 3.40 (3.39), 3.50 (3.51), 3.30–3.55, 3.65–3.75, 3.8–3.9, 4.08, 5.02, 5.17, 6.94, 7.43, 7.71, 8.08.

{*O*-[2-(Hydroxymethyl)-2-propenyl]-D-serine}^{8}-cyclosporin (24). A mixture of silyl ether **23** (21.4 g, 15 mmol), tetrabutylammonium fluoride tetrahydrate (7.25 g, 23 mmol), and absolute tetrahydrofuran (250 mL) was kept at rt for 2 h. Then, *tert*-butyl methyl ether was added and the organic phase was washed first with water then with a 2 N $NaHCO_3$ solution, dried over $MgSO_4$, and evaporated to dryness. The crude product (27 g) was chromatographed on silica gel to give the pure product (17.5 g, 75%) as a foam which was crystallized from ether/hexane (15.6 g): yield 67%; mp 137 °C dec; *m/z* calcd for $C_{66}H_{117}N_{11}O_{14}$ 1287.9, found 1289.0 $[MH]^+$, 1175.5 $[M - 112]^+$; $[\alpha_D] = -181.8°$ ($c = 0.54$ in MeOH); NMR δ 2.68 (2.70), 2.70 (2.70), 3.12 (3.11), 3.17 (3.11), 3.27 (3.27), 3.40 (3.39), 3.50 (3.51), 3.35–3.60, 3.7–3.75, 3.75–3.85, 3.95–4.03, 4.08–4.19, 5.07, 5.22, 7.09, 7.49, 7.82, 8.06.

[*O*-(4-Bromo-2(*E*)-butenyl)-D-serine]^8-cyclosporin (25). A solution of [D-serine]^8-cyclosporin (**2**) (5.0 g, 4 mmol), tetrabutylammonium chloride (2.33 g, 10 mmol), and *trans*-1,4-dibromo-2-butene[19] (2.2 g, 10 mmol) in methylene chloride (250 mL) was stirred at rt for 2 h in the presence of 30% sodium hydroxide (25 mL). Then, *tert*-butyl methyl ether was added, and the mixture was washed with water, dried over $MgSO_4$, and evaporated to give the crude product (6.2 g). This was chromatographed on silica gel with ethyl ether/ethyl acetate to give the pure product (3.3 g): yield 60%; *m/z* calcd for $C_{66}H_{116}N_{11}O_{13}Br$ 1350.8, found 1352.8 $[MH]^+$, 1239.8 $[M - 112]^+$; $[\alpha_D] = -159.4°$ ($c = 0.840$ in MeOH); NMR δ 2.68 (2.70), 2.72 (2.70), 3.10 (3.11), 3.15 (3.11), 3.27 (3.27), 3.40 (3.39), 3.50 (3.51), 3.35–3.6, 3.9–4.0, 5.75–5.95, 7.01, 7.49, 7.70, 8.11.

[*O*-(4-Acetoxy-2(*E*)-butenyl)-D-serine]^8-cyclosporin (26). A solution of bromide **25** (2.0 g, 1.5 mmol), sodium iodide (2.25 g, 15 mmol), and tetraethylammonium acetate (18.9 g, 72

mmol) in ethyl methyl ketone (100 mL) was heated to 100 °C overnight. The mixture was diluted with ether, washed with water, and dried over $MgSO_4$ to give the crude acetate (2.2 g). This was purified on silica gel to give pure product (1.55 g): yield 79%; *m/z* calcd for $C_{68}H_{119}N_{11}O_{15}$ 1329.9, found 1330.8 $[MH]^+$, 1270.8 $[M - HOAc]^+$, 1217.7 $[M - 112]^+$; $[\alpha_D] = -172.1°$ (*c* = 0.54 in MeOH); NMR δ 2.08, 2.68 (2.70), 2.72 (2.70), 3.10 (3.11), 3.15 (3.11), 3.25 (3.27), 3.40 (3.39), 3.50 (3.51), 3.35–3.45, 3.55–3.60, 3.93, 4.55, 5.7–5.8, 7.00, 7.49, 7.77, 8.11.

[*O*-(4-Hydroxy-2(*E*)-butenyl)-D-serine]⁸-cyclosporin (27). A solution of acetate **26** (1.0 g, 1 mmol) in methanol (50 mL) was stirred at rt for 1 h in the presence of 2 N sodium hydroxide (1 mL). The solvent was evaporated under reduced pressure. Ethyl acetate was added, and the mixture was washed with water, dried over $MgSO_4$, and evaporated to give the crude product (1.4 g). This was purified on silica gel with ethyl ether/ethyl acetate (3:1) to give the pure compound (750 mg): yield 78%; *m/z* calcd for $C_{66}H_{117}N_{11}O_{14}$ 1287.9, found 1288.8 $[MH]^+$, 1175.7 $[M - 112]^+$; $[\alpha_D] = -170.7°$ (*c* = 0.53 in MeOH); NMR δ 2.68 (2.70), 2.71 (2.70), 3.10 (3.11), 3.17 (3.11), 3.25 (3.27), 3.40 (3.39), 3.50 (3.51), 3.35–3.6, 3.65–3.85, 3.85–4.05, 4.11–4.21, 5.70–5.90, 7.03, 7.50, 7.82, 8.08.

{*O*-[2-(1,3-Dioxolanyl)-2-ethyl]-D-serine}⁸-cyclosporin (28). A mixture of [D-serine]⁸-cyclosporin (**2**) (2.4 g, 2 mmol), benzyltriethylammonium bromide (450 mg, 1.7 mmol), 2-(2-bromoethyl)-1,3-dioxolane[19] (4.7 g, 40 mmol), and methylene chloride (100 mL) was stirred at rt for 4 days in the presence of 30% NaOH (25 mL). Then, water was added. The organic phase was separated, washed with water, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel to give pure product (1.29 g): yield 49%; *m/z* calcd for $C_{67}H_{119}N_{11}O_{15}$ 1317.9, found 1319.0 $[MH]^+$, 1205.9 $[M - 112]^+$; $[\alpha_D] = -142.2°$ (*c* = 0.245 in MeOH); NMR δ 2.68 (2.70), 2.72 (2.70), 3.10 (3.11), 3.13 (3.11), 3.25 (3.27), 3.40 (3.39), 3.50 (3.51), 3.35–3.60, 3.7–4.0, 4.91, 6.99, 7.48, 7.75, 8.12.

{*O*-[2-(1,3-Dioxanyl)-2-ethyl]-D-serine}⁸-cyclosporin (29). A mixture of [D-serine]⁸-cyclosporin (**2**) (1.2 g, 1 mmol), benzyltriethylammonium chloride (0.4 g), 2-(2-bromoethyl)-1,3-dioxane[19] (5 mL, 37 mmol), and methylene chloride (25 mL) was stirred overnight in the presence of 40% aqueous KOH (10 mL). The mixture was diluted with ether and washed with water. The organic phase was dried over $MgSO_4$ and evaporated. The crude product was chromatographed on silica gel to give pure product (1.0 g): yield 75%; *m/z* calcd for $C_{68}H_{121}N_{11}O_{15}$ 1331.9, found 1332.5 $[MH]^+$, 1219.4 $[M - 112]^+$; $[\alpha_D] = -169.4°$ (*c* = 0.39 in MeOH); NMR δ 2.68 (2.70), 2.71 (2.70), 3.10 (3.11), 3.13 (3.11), 3.25 (3.27), 3.40 (3.39), 3.50 (3.51), 3.4–3.6, 3.65–3.8, 4.0–4.1, 4.58, 7.00, 7.48, 7.74, 8.09.

[*O*-(3-Hydroxypropyl)-D-serine]⁸-cyclosporin (30). A solution of acetal **28** (470 mg, 0.36 mmol) in acetone (25 mL) was kept at rt overnight in the presence of 2 N HCl solution (5 mL). This was neutralized by the addition of 2 N sodium bicarbonate solution (5 mL) and extracted with ethyl acetate. The organic phase was dried over $MgSO_4$, filtered, and evaporated. The crude intermediate was dissolved in ethanol (25 mL) and then treated with sodium borohydride (220 mg, 6 mmol) during 1 h. The solution was cooled externally. Excess reducing agent was destroyed by the careful addition of acetic acid. The solvent was evaporated under reduced pressure. The residue was extracted with ethyl acetate, dried over $MgSO_4$, and filtered. The solvent was evaporated and the residue thus obtained was chromatographed on silica gel to give the product (130 mg): yield 29%; *m/z* calcd for $C_{65}H_{117}N_{11}O_{14}$ 1275.7, found 1276 $[MH]^+$, 1163 $[M - 112]^+$; $[\alpha_D] = -173.1°$ (*c* = 0.238 in MeOH); NMR δ 2.68 (2.70), 2.70 (2.70), 3.10 (3.11), 3.15 (3.11), 3.23 (3.27), 3.39 (3.39), 3.50 (3.51), 3.45–3.65, 3.65–3.85, 7.10, 7.48, 7.74, 8.04.

[*O*-(4-Hydroxybutyl)-D-serine]⁸-cyclosporin (31). A mixture of [D-serine]⁸-cyclosporin (**2**) (3.6 g, 3 mmol), tetrabutylammonium bisulfate (340 mg), 1,4-dibromobutane[19] (100 mL), and 30% NaOH (50 mL) was stirred at rt for 2 h. Then, water and methylene chloride were added. The organic phase was separated, dried over $MgSO_4$ and evaporated. The residue (6.0 g) was chromatographed on silica gel to give the pure monobromide (3.0 g): yield 64%; *m/e* calcd for $C_{66}H_{118}N_{11}O_{13}Br$ 1551.7, found 1352.8 $[MH]^+$. This was heated to reflux for 1 h in methyl ethyl ketone (50 mL) in the presence of tetraethylammonium acetate tetrahydrate (3.0 g) and a trace of sodium iodide. The solvent was evaporated under reduced pressure. The residue was dissolved in ether and washed with water. The ether layer was dried over $MgSO_4$, filtered, and evaporated. The crude product was treated with absolute methanol (100 mL) to which a trace of freshly prepared sodium methoxide had been added. After 1.5 h at rt the solution was acidified with acetic acid and evaporated to dryness. The residue was dissolved in ether and washed with water. The organic phase was dried over $MgSO_4$ to give the crude product (3.0 g) which was chromatographed on silica gel to yield the pure product (1.6 g): yield 41%; *m/z* calcd for $C_{66}H_{119}N_{11}O_{14}$ 1289.7, found 1290 $[MH]^+$, 1177 $[M - 112]^+$; $[\alpha_D] = -178.4°$ (*c* = 0.505 in MeOH); NMR δ 2.70 (2.70), 2.73 (2.70), 3.12 (3.11), 3.16 (3.11), 3.27 (3.27), 3.42 (3.39), 3.52 (3.51), 3.35–3.70, 3.7–3.8, 7.06, 7.48, 7.77, 8.08.

{*O*-[2-(Tosyloxy)ethyl]-D-serine}⁸-cyclosporin (32). A mixture of the alcohol **11** (5.04 g, 4.0 mmol), 4-(dimethylamino)pyridine (2.44 g, 20 mmol), and tosyl chloride (1.91 g, 10 mmol) in methylene chloride (100 mL) was kept at rt for 6 h. Then the reaction mixture was chromatographed on silica gel (250 g) with ethyl acetate to give the pure product (5.4 g): yield 95%; *m/z* calcd for $C_{71}H_{121}N_{11}O_{16}S$ 1415, found 1416 $[MH]^+$, 1303 $[M - 112]^+$; $[\alpha_D] = -163.3°$ (*c* = 0.523 in MeOH); NMR δ 0.71, 1.07, 1.36, 1.63, 2.45, 2.67 (2.70), 2.68 (2.70), 3.05 (3.11), 3.09 (3.11), 3.23 (3.27), 3.39 (3.39), 3.50 (3.51), 3.35 3.55, 3.58, 4.00–4.15, 3.67, 3.79, 4.47, 4.64, 4.71, 5.10, 5.51, 5.69, 6.98, 7.36, 7.48, 7.72, 7.79, 8.08.

[*O*-(2-Azidoethyl)-D-serine]⁸-cyclosporin (33). A mixture of the tosylate **32** (2.24 g, 1.58 mmol), sodium iodide (380 mg, 2.54 mmol), and sodium azide (340 mg, 5.23 mmol) in DMF (80 mL) was heated to 60 °C for 4 h and then to 110 °C for 2 h. *tert*-Butyl methyl ether (250 mL) was added. The organic phase was washed with water and brine, then dried over sodium sulfate, and evaporated to give the crude product (2.4 g). This was purified on silica gel to give the pure product (2.0 g): yield 98%; *m/z* calcd for $C_{64}H_{114}N_{14}O_{13}$ 1286.8, found 1287.1; NMR δ 0.70, 1.07, 1.37, 1.63, 2.68 (2.70), 2.72 (2.70), 3.10 (3.11), 3.16 (3.11), 3.25 (3.27), 3.40 (3.39), 3.50 (3.51), 3.35–3.55, 3.70–3.80, 4.49, 4.66, 4.72, 5.52, 5.72, 7.03, 7.50, 7.77, 8.11.

[*O*-(2-Aminoethyl)-D-serine]⁸-cyclosporin (34). Sodium borohydride (640 mg, 17 mmol) was added in portions to a stirred mixture of the azide **33** (2.0 g, 1.56 mmol) and 10% palladium on charcoal (100 mg) in methanol (100 mL) and water (5 mL).[32] After 15 min the catalyst was filtered off, and the solution was concentrated. The residue was dissolved in methylene chloride and washed with water. The organic phase was dried over $MgSO_4$ and evaporated to give the product (1.6 g) as a white foam: yield (82%); *m/z* calcd for $C_{64}H_{116}N_{12}O_{13}$ 1260.8, found 1261.7; $[\alpha_D] = -163.6°$ (*c* = 0.516 in MeOH); NMR δ 0.71, 1.37, 1.63, 2.72 (2.70), 2.74 (2.70), 3.13 (3.11), 3.18 (3.11), 3.28 (3.27), 3.43 (3.39), 3.53 (3.51), 3.40–3.50, 3.70–3.80, 4.51, 4.65, 4.73, 4.95–5.10, 5.52, 5.73, 7.08, 7.49, 7.76, 8.09.

[*O*-(2-Aminoethyl)-D-serine]⁸-cyclosporin *N*-[5-(Dimethylamino)-1-naphthalenesulfonamide] (35). A mixture of the amine **34** (140 mg, 0.11 mmol), 4-(dimethylamino)-pyridine (122 mg, 1 mmol), and dansyl chloride[19] (135 mg, 0.5 mmol) in methylene chloride was kept at rt for 4 h. More solvent was added, and the mixture was washed with water and brine. The organic phase was dried over $MgSO_4$ and evaporated. The pure product (120 mg) was obtained following chromatography on silica gel: yield 72%; *m/z* calcd for $C_{76}H_{127}N_{13}O_{15}S$ 1493.9, found 1495.3; $[\alpha_D] = -163.3°$ (*c* = 0.750 in MeOH); NMR δ 1.38, 1.62, 2.67 (2.70), 2.68 (2.70), 2.90, 3.02 (3.11), 3.10 (3.11), 3.26 (3.27), 3.40 (3.39), 3.50 (3.51), 3.10–3.40, 3.50–3.55, 4.49, 4.64, 4.70, 5.12, 5.48, 5.64, 5.95–6.04, 7.20, 7.29, 7.45–7.56, 7.79, 8.03, 8.25, 8.38, 8.56.

{*O*-[2-(Bromoethyl)-1-ethoxy]-D-serine}⁸-cyclosporin (36). A solution of [D-serine]⁸-cyclosporin (**2**) (6.08 g, 5 mmol) and ethyl vinyl ketone (20 mL) in carbon tetrachloride (20 mL) was stirred overnight with *N*-bromosuccinimide (3.65 g, 20 mmol). This was diluted with *tert*-butyl methyl ether and

washed with Na_2CO_3 solution. The organic phase was concentrated and then chromatographed on silica gel to give the product (3.0 g): yield 44%; *m/z* calcd for $C_{66}H_{118}N_{11}O_{14}Br$ 1367/1369, found 1368/1370 $[MH]^+$; NMR δ 2.73, 2.77, 2.80, 3.15, 3.21, 3.29, 3.44, 3.55, 7.03, 7.50, 7.79, 8.11.

[*O*-(Bromoacetyl)-D-serine]^8-cyclosporin (37). A solution of bromoacetyl bromide (10 mL, 115 mmol) in methylene chloride (25 mL) was added slowly to a solution of [D-serine]^8-cyclosporin (**2**) (9.0 g, 7.4 mmol) and triethylamine (25 mL, 180 mmol) in methylene chloride (150 mL). After 2 h *tert*-butyl methyl ether was added. The organic phase was washed with water and then with brine, dried over Na_2SO_4, and evaporated. The crude product was chromatographed on silica gel to give the pure product (7.43 g): yield 75%; *m/z* calcd for $C_{64}H_{112}N_{11}O_{14}Br$ 1337/1339, found 1338/1340 $[MH]^+$; NMR δ 2.72, 3.12, 3.23, 3.28, 3.41, 3.52, 3.85, 7.21, 7.49, 7.72, 8.07.

{*O*-[2-[(Tosyloxy)methyl]-2-propen-1-yl]-D-serine}^{8}-cyclosporin (38). A mixture of the alcohol **24** (1.3 g, 1 mmol), 4-(dimethylamino)pyridine (610 mg, 5 mmol), and tosyl chloride (570 mg, 3 mmol) in methylene chloride was kept at rt for 1 h. The mixture was chromatographed directly on silica gel with *tert*-butyl methyl ether as eluent to give the pure product (1.0 g): yield 69%; *m/z* calcd for $C_{73}H_{123}N_{11}O_{16}S$ 1441.8, found 1442.6 $[MH]^+$; NMR δ 0.70, 1.35, 1.63, 2.20, 2.69, 2.70, 3.07, 3.11, 3.25, 3.41, 3.51, 3.86, 4.48, 7.00, 7.36, 7.49, 7.72–7.82, 8.12.

[3(*R*)-Morpholinecarboxylic acid]^8-cyclosporin (39). A solution of the tosylate **32** (13.3 g, 9.4 mmol), and tetrabutylammonium chloride (2.1 g, 9.25 mmol) in methylene chloride (130 mL) was stirred vigorously at rt for 1 h in the presence of 50% aqueous NaOH solution (30 mL). Then, *tert*-butyl methyl ether (250 mL) and water (100 mL) were added. The organic phase was separated and dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The crude product was chromatographed on silica gel (450 g) with water-saturated ethyl acetate as eluent to give the pure product (10.5 g): yield 90%; *m/z* calcd for $C_{64}H_{113}N_{11}O_{13}$ 1243.8, found 1244.6 $[MH]^+$, 1131.5 $[M - 112]^+$; $[\alpha_D] = -162.2°$ (*c* = 0.558 in MeOH); NMR δ 0.56, 0.79, 1.24, 1.63, 2.70 (2.70), 2.80 (2.70), 3.10 (3.11), 3.12 (3.11), 3.30 (3.27), 3.40 (3.39), 3.50 (3.51), 4.33, 5.15, 5.57, 5.77, 7.40, 7.63, 8.30; NMR δ (DMSO-d_6 at 180 °C) 0.75, 1.20, 1.59, 2.85, 2.90, 2.91, 2.92, 2.96, 3.04; ^{13}C NMR (amino acid 8) 42.575, 49.924, 66.348, 66.576, and 169.4–173.8 (11 C=O).

[2(*RS*)-Ethoxy-5(*R*)-morpholinecarboxylic acid]^8-cyclosporin (40). A mixture of the bromo acetal **36** (5.1 g, 3.7 mmol), tetrabutylammonium chloride (2.9 g), and 40% sodium hydroxide (20 mL) in methylene chloride (50 mL) was stirred at rt for 1 h. The mixture was cooled and diluted with water, and then *tert*-butyl methyl ether was added. The organic phase was dried. The solvent was evaporated, and the residue was treated with 2 N HCl (2 mL) in methanol (75 mL). After 1 h the solution was adjusted to pH 8 with 2 N Na_2CO_3 solution and extracted with ethyl acetate to give the crude product (4.2 g). This was chromatographed on silica gel to give the pure product (1.95 g): yield 41%, *m/z* calcd for $C_{66}H_{117}N_{11}O_{14}$ 1287, found 1288 $[MH]^+$; $[\alpha_D] = -156.3°$ (*c* = 0.585 in MeOH); NMR δ 2.69, 2.72, 2.78, 2.81, 3.07, 3.09, 3.10, 3.17, 3.28, 3.31, 3.38, 3.39, 3.48, 3.50 (NCH_3 only).

[2-Morpholinone-5(*R*)-carboxylic acid]^8-cyclosporin (41). The mixture of [*O*-(bromoacetyl)-D-serine]^8-cyclosporin (**37**) (2.7 g, 2 mmol) and solid K_2CO_3 (1.38 g, 10 mmol) in DMF (50 mL) was heated to 60 °C for 2 h. The cold solution was poured on 2 N HCl and extracted with *tert*-butyl methyl ether. The organic phase was first washed with water and then with saturated sodium bicarbonate solution, dried over Na_2SO_4, and evaporated. The crude product was chromatographed on silica gel with ethyl acetate as eluent to give the pure product (800 mg): yield 32%; *m/z* calcd for $C_{64}H_{111}N_{11}O_{14}$ 1257.8, found 1258.8 $[MH]^+$; $[\alpha_D] = -208.8°$ (*c* = 0.245 in $CHCl_3$); NMR δ 0.57, 0.76, 1.25, 1.63, 2.70, 2.81, 3.10, 3.25, 3.32, 3.39, 3.50, 4.36, 4.38, 4.43, 4.47, 4.48, 4.50, 4.53, 4.57, 7.40, 7.68, 8.30; NMR δ (DMSO-d_6 at 180 °C) 0.75, 1.20, 1.60, 2.88, 2.91, 2.93, 2.97, 3.02, 3.05, 4.43.

[4(*R*)-Oxazolidinecarboxylic acid]^8-cyclosporin (42). A mixture of [D-serine]^8-cyclosporin (**2**) (2.4 g, 2 mmol), tetrabutylammonium bisulfate (1.0 g), methylene bromide[19] (25 mL), methylene chloride (10 mL), and 40% NaOH (20 mL) was stirred at rt overnight. Then, water and *tert*-butyl methyl ether (100 mL) were added. The organic phase was separated, and the mixture was washed with water and then with brine. The organic phase was dried over Na_2SO_4 and evaporated. The residue (2.4 g) was chromatographed on silica gel to give the pure product (50 mg): yield 2%; *m/z* calcd for $C_{63}H_{111}N_{11}O_{13}$ 1229, found 1230 $[MH]^+$; $[\alpha_D] = -164.7°$ (*c* = 1.900 in MeOH); NMR δ 0.63, 0.80, 1.27, 1.63, 2.72, 2.81, 3.08, 3.20, 3.27, 3.38, 3.46, 7.42, 7.60, 8.19; NMR δ (DMSO-d_6 at 180 °C) 0.77, 1.19, 1.60, 2.05–2.15, 2.88, 2.93, 2.94, 2.95, 2.99, 3.05, 3.70–3.77, 3.92–3.98, 4.10–4.30, 4.30–4.35, 4.35–4.45, 4.60–4.66, 4.90–4.95, 5.07, 5.10–5.15, 6.6–6.7, 6.75–6.85, 7.05–7.10; ^{13}C NMR (amino acid 8) 54.321, 69.602, 80.171 and 168.69–173.96 (11 C=O).

[Hexahydro-6-methylene-1,4-oxazepine-3(*R*)-carboxylic acid]^8-cyclosporin (43). A mixture of the tosylate **38** (650 mg, 0.45 mmol), tetraethylammonium chloride (580 mg), and 40% sodium hydroxide solution (15 mL) in methylene chloride (50 mL) was stirred at rt overnight. This was diluted with *tert*-butyl methyl ether, washed sequentially with water, 2 N HCl solution, and water. The organic phase was dried over sodium sulfate and evaporated to give the crude product (530 mg). This was chromatographed on silica gel to give the pure product (250 mg): yield 44%; *m/z* calcd for $C_{66}H_{115}N_{11}O_{13}$ 1269.8, found 1270.2 $[MH]^+$; $[\alpha_D] = -160.7°$ (*c* = 0.329 in MeOH); NMR δ 0.57, 0.73, 1.64, 2.71, 2.81, 3.12, 3.18, 3.33, 3.42, 3.51, 7.40, 7.59, 8.38; NMR (DMSO-d_6 at 150 °C) δ 0.75, 1.18, 1.60, 2.87, 2.90, 2.92, 2.96, 3.03, 3.85, 3.87, 4.02, 4.03, 4.22, 4.43, 6.53, 6.80, 7.07.

***O*-Acetyl-*N*-BOC-7,8-seco-[2(*R*)-piperidinecarboxylic acid]^8-cyclosporin-7-thiocarboxylic Acid *S*-Benzyl Ester (46).** A mixture of the decapeptide **44**[24] (1.3 g, 1.0 mmol), N-BOC-2(*R*)-piperidinecarboxylic acid **45**[25] (500 mg, 2.2 mmol), and 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ, 500 mg, 2.0 mmol) in methylene chloride (30 mL) was kept at rt overnight. The solvent was diluted with *tert*-butyl methyl ether and washed with 2 N HCl, water, and then brine. The organic phase was dried over magnesium sulfate and evaporated. The crude (2.0 g) was chromatographed on silica gel to give pure product (1.1 g): yield 73%; *m/z* calcd for $C_{79}H_{133}N_{11}O_{15}S$ 1507, found 1508 $[MH]^+$, 1408 $[MH - BOC]^+$, 1313 $[M - Ala\text{-}SCH_2Ph]^+$ = [1186 + MeLeu]$^+$, 1186 [1087 + Val]$^+$, 1087 [960 + MeLeu]$^+$, 960 [804 + Abu-Sar]$^+$, 804 [579 + AcMeBmt]$^+$, 579 [466 + MeVal]$^+$, 466 [339 + MeLeu]$^+$, 339 [BOC-Pipec-MeLeu]$^+$; NMR (DMSO-d_6 at 150 °C) δ 0.76, 0.83, 1.30, 1.39, 1.95, 2.88, 2.92, 2.93, 2.95, 4.08, 4.47, 4.58, 5.10, 7.00–7.08, 7.10–7.20, 7.2–7.3, 7.6–7.7.

***O*-Acetyl-7,8-seco-[2(*R*)-piperidinecarboxylic acid]^8-cyclosporin-7-thiocarboxylic Acid *S*-Benzyl Ester (47).** A cold solution of **46** (1.0 g, 0.77 mmol) and trifluoroacetic acid (7 mL) in methylene chloride (20 mL) was kept at rt for 3 h. The solution was neutralized with 2 N Na_2CO_3 solution. The organic phase was separated, dried over $MgSO_4$, and evaporated. The crude (2 g) was chromatographed on silica gel to give the pure product (560 mg): yield 60%; *m/z* calcd for $C_{74}H_{125}N_{11}O_{13}S$ 1407, found 1408 $[MH]^+$, 1086 [987 + Val]$^+$, 987 [860 + MeLeu]$^+$, 860 [789 + Sar]$^+$, 789 [704 + Abu]$^+$, 704 [479 + AcMeBmt]$^+$, 644 [704 − AcOH]$^+$, 479 [366 + MeVal]$^+$, 366 [H-Pipec-MeLeu-MeLeu]$^+$; NMR (DMSO-d_6 at 150 °C) δ 0.77, 1.31, 1.62, 2.89, 2.95, 2.96, 2.97, 3.60, 4.08, 4.58, 5.10, 7.00–7.08, 7.10–7.12, 7.20–7.30, 7.58–7.65.

7,8-Seco-[2(*R*)-piperidinecarboxylic acid]^8-cyclosporin (48). A solution of **47** (540 mg, 0.44 mmol) and 2 N NaOH (10 mL) in methanol (15 mL) was stirred at rt for 2 h. The solution was neutralized with 2 N HCl solution. The solvent was evaporated under reduced pressure. Methylene chloride was added, and the mixture was washed with water, dried over sodium sulfate, and evaporated to give the product (460 mg); yield 97%; *m/z* calcd for $C_{65}H_{117}N_{11}O_{13}$ 1259, found 1260 $[MH]^+$, 1242 $[MH - HOH]^+$, 1044 [945 + Val]$^+$, 945 [818 + MeLeu]$^+$, 818 [662 + Abu-Sar]$^+$, 662 [479 + MeBmt]$^+$, 644 [662 − HOH]$^+$, 479 [366 + MeVal]$^+$, 366 [H-Pipec-MeLeu-MeLeu]$^+$; NMR (DMSO-d_6 at 150 °C) δ 0.76, 1.28, 1.61, 2.89, 2.93, 2.94, 2.97, 2.98, 3.07, 3.72, 4.58, 5.12, 7.00, 7.12, 7.18.

[2(*R*)-Piperidinecarboxylic acid]⁸-cyclosporin (49). A mixture of 7,8-seco-[2(*R*)-piperidinecarboxylic acid]⁸-cyclosporin (**48**) (230 mg, 0.18 mmol), 4-(dimethylamino)pyridine (300 mg, 2.4 mmol), and (benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluorophosphate (BOP reagent, 600 mg, 1.4 mmol) in methylene chloride (20 mL) was kept at rt overnight. *tert*-Butyl methyl ether was added, and the mixture was washed sequentially with 2 N HCl solution, water, saturated $NaHCO_3$ solution, and brine. The organic phase was dried over magnesium sulfate and evaporated. The crude product (220 mg) was chromatographed on silica gel to give the pure product (110 mg): yield 49%; *m/z* calcd for $C_{65}H_{115}N_{11}O_{12}$ 1241.8, found 1242.6 $[MH]^+$; $[\alpha_D] = -164.5°$ ($c = 0.22$ in MeOH); NMR δ 1.62 (1.62), 2.71 (2.70), 2.81 (2.70), 3.08 (3.11), 3.10 (3.11), 3.29 (3.27), 3.39 (3.39), 3.49 (3.51), 7.40 (7.47), 7.58 (7.75), 8.33 (7.93); NMR δ (DMSO-d_6 at 180 °C) 0.75, 1.18, 1.59, 2.86, 2.90, 2.92, 2.97, 3.03, 3.67–3.72.

1-Acetoxy-2-(chloromethyl)-2-propene[22] (50). A mixture of 1-chloro-2-(chloromethyl)-2-propene[19] (125 g, 1 mol), sodium acetate (82 g, 1 mol), and ethanol (500 mL) was heated to reflux for 24 h. The solids were filtered off. The solution was concentrated under reduced pressure. The residue was dissolved in pentane and washed with water. The organic phase was dried over $MgSO_4$ and evaporated to give the crude product as a yellow oil (75 g).

2-(Chloromethyl)-2-propen-1-ol[22] (51). The chloro acetate **50** was dissolved in a mixture of methanol (250 mL) and 2 N NaOH (10 mL). After 2 h at rt the solution was cooled in an ice bath and acidified with a 2 N HCl solution. Most of the methanol was evaporated under reduced pressure. Then, ether was added, and the mixture was washed with water, dried over magnesium sulfate, and evaporated to leave the crude chloro alcohol (31 g). This was chromatographed to yield the pure product (22 g); yield 21% over two steps; NMR δ 1.4–1.8, 4.15, 4.28, 5.27.

2-(Chloromethyl)-2-propen-1-ol *tert*-Butyldimethylsilyl Ether (52). A solution of chloro alcohol **51** (21.3 g, 0.2 mol), triethylamine (22.2 g, 0.22 mol), *tert*-butyldimethylchlorosilane (33.1 g, 0.22 mol), and 4-(dimethylamino)pyridine (1 g, 8 mmol) in methylene chloride (250 mL) was stirred at rt during 4 h. Then, water was added. The organic phase was dried over magnesium sulfate and evaporated to give the crude product (56 g) which was chromatographed on silica gel to give a colorless oil (42 g): NMR δ 0.1, 0.9, 4.1 and 4.3, 5.2.

2-(Iodomethyl)-2-propen-1-ol *tert*-Butyldimethylsilyl Ether (53). A solution of chloride **52** (42 g, 0.2 mol) and sodium iodide (45 g, 0.3 mol) in acetone (350 mL) was heated to reflux for 24 h. The solids were filtered off, and the solution was concentrated under reduced pressure. The residue was dissolved in ether, washed with water, and dried over magnesium sulfate to yield crude iodide (58 g) which was used without purification for the alkylation reaction.

Acknowledgment. We would like to thank Mr. Charles Quiquerez for providing the mass spectral data and Mrs. M. Ponelle for recording and interpreting the NMR spectra.

Supplementary Material Available: 360 MHz proton NMR spectra of compounds **3–43** and **46–49** (50 pages). Ordering information is given on any current masthead page.

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JM9405351