Preparation of [D-Cysteine]^8-cyclosporin via Intramolecular Sulfur Transfer Reaction†

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Starting from [D-serine]^8-cyclosporin (**2**) the diacetate **3** was prepared. This was treated with Lawesson's reagent. From the reaction mixture, three diacetylated thioamides were isolated in this order of increasing polarity: the 4,7-dithioamide **4a**, the 7-thioamide **4b**, and the 4-thioamide **4c**. The acetate groups were hydrolyzed from all three compounds under basic conditions to give **5a–c**. Treatment of **5b** with tosyl chloride led to the dihydrothiazolobicyclosporin **6**. Hydrolysis under acidic conditions yielded [D-cysteine]^8-cyclosporin (**7**). This was converted to the *O,S*-diacetate **8**. A reductive desulfurization of **8** to the known acetylcyclosporin A (**9**) provided the stereochemical correlation between **7** and cyclosporin A (**1**).

Cyclosporin A[1] (**1**), the active ingredient of Sandimmune, is a powerful immunosuppressant[2] preventing allograft rejections in animals[3] and humans.[4] It was first isolated from natural sources[5] and prepared later through total synthesis.[6] Cyclosporin A inhibits[7] the production of various lymphokines such as interleukin-2 (IL-2). These lymphokines are mainly 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]

To date approximately 40 modified cyclosporins have been isolated from natural sources.[12] Additional examples were the result of considerable synthetic efforts.[13] From these studies has emerged the knowledge that the free hydroxyl group of the amino acid 1 {MeBMT: *N*,4-dimethyl-4(*R*)-[(*E*)-2-butenyl]-L-threonine}[14] is important for good immunosuppressant activity. A "bioactive conformation" of this group as a prerequisite for immunosuppressive activity has been proposed.[15]

While cyclosporin A itself has been the subject of over 15 000 scientific publications so far, we turned our interest to some aspects of the chemistry of [D-serine]^8-cyclosporin[16] (**2**). This natural product analog of cyclosporin A differs from the parent compound at position 8[14] with the D-alanine having been replaced by a D-serine. To the best of our knowledge, the thiol analog [D-cysteine]^8-cyclosporin (**7**) has not been described[17] so far. Therefore, we were interested in preparing this compound synthetically. The knowledge[18] about the regioselective introduction of thioamides into the cyclosporin A (**1**) molecule prompted us to investigate the analogous reaction with [D-serine]^8-cyclosporin[16] (**2**). It was our intention to first insert the sulfur atom regiospecifically into the cyclosporin molecule in the form of a thioamide at the amino acid 7. In a

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(17) Recently some mercapto analogs (R_1 = OH; R_2 = *S*-Alkyl; Chart I) have been described. They were obtained starting from [β-fluoro-D-alanine]^8-cyclosporin: Patchett, A. A.; Taub, D.; Hensens, O. D.; Goegelman, R. T.; Yang, L.; Dumont, F.; Peterson, L.; Sigal, N. *J. Antibiot.* **1992**, *45*, 94.

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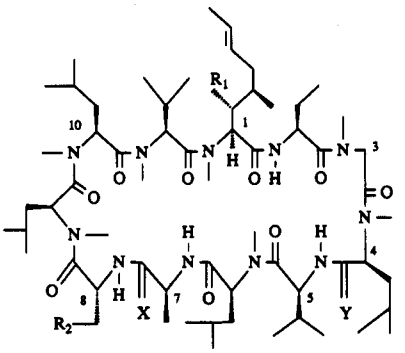
subsequent step the sulfur would then be transferred via an intramolecular sulfur transfer reaction to the methylene group of D-serine of the amino acid 8. In order to put this concept into practice, both alcohol functions of the starting material 2 were protected as their acetates. In analogy[19] to the acetylation of cyclosporin A (1) itself, the protection of 2 had to be carried with acetic anhydride/pyridine in the presence of 4-(dimethylamino)pyridine to assure the formation of the diacetate 3. The protected cyclosporin was treated briefly with Lawesson's reagent[20] in toluene or xylene at elevated temperature. A mixture of thioamides was obtained. By column chromatography over silica gel, two of the three major products were first enriched while the diacetyl 4-thioamide 4c was obtained in pure form. The mixed fractions containing 4a and 4b were further purified by reverse-phase column chromatography on RP-18. Thus we were able to isolate, in the order of increasing polarity and following crystallization of each compound, the diacetylated 4,7-dithioamide 4a in 22.5% yield, the diacetylated 7-thioamide 4b in 8% yield, and diacetylated 4-thioamide 4c in 11% yield. The structural assignments were based on mass spectral and NMR data obtained for these compounds. The least polar of the three compounds showed a $[M + 1]^+$ ion at m/z 1334.6, that is 32 mass units up from the $[M + 1]^+$ ion of the starting material 3 observed at m/z 1302.7, indicating the presence of two thioamide groups in 4a. The other two components, 4b and 4c, showed $[M + 1]^+$ ions at m/z 1318.6 and 1318.5, respectively, both in agreement with the presence of monothioamides. Since the acetate groups remained untouched in all three products (see below) and based upon precedents,[18] it was reasonable to assume that secondary amides had been selectively converted to thioamides.

A comparison of the ^{1}H NMR spectra of 1, 2, 3, and 4a–c revealed that the absorptions due to one or two of the NH protons, following conversion to the respective thioamides, had been shifted to lower field while the remaining amide proton shifts were unaffected. It had been established[21] for 1 that among all the amide protons the one assigned to the amino acid 5 appears as a doublet near δ 7.5 ppm while the one assigned to the amino acid 8 appears as a doublet at highest field near δ 7.2 ppm. Introduction of a hydroxy group at amino acid 8 caused the latter to be shifted to 7.62 ppm. Similar observations were made for the diacetate 3 (see below). In the case of the dithioamide 4a, however, a signal between 7.0 and 7.5 ppm was missing. While the chemical shifts of the remaining amide protons had not changed significantly, two new signals below 8.75 ppm were detected (see Table I). For each of the mono thioamides 4b and 4c, a shift to lower field of only one of the two higher field NH signals was detected. This called for a correlation of the NH signals near δ 7.50 and 7.60 ppm of 3 with the signals near δ 8.75 and 9.35 ppm of either of the compounds 4a–c. For an unequivocal assignment the chemical shifts of the α-protons were taken into consideration. Through a series of systematic decoupling experiments, correlations were established between each NH proton and its neighbor,

Chart I



1 - 5, 7 - 9

1)	R_1 = OH; R_2 = H	X = Y = O
2)	$R_1 = R_2$ = OH	X = Y = O
3)	$R_1 = R_2$ = OAc	X = Y = O
4a)	$R_1 = R_2$ = OAc	X = Y = S
4b)	$R_1 = R_2$ = OAc	X = S; Y = O
4c)	$R_1 = R_2$ = OAc	X = O; Y = S
5a)	$R_1 = R_2$ = OH	X = Y = S
5b)	$R_1 = R_2$ = OH	X = S; Y = O
5c)	$R_1 = R_2$ = OH	X = O; Y = S
7)	R_1 = OH; R_2 = SH	X = Y = O
8)	R_1 = OAc; R_2 = SAc	X = Y = O
9)	R_1 = OAc; R_2 = H	X = Y = O

the α proton of the same amino acid. This allowed an unambiguous assignment of the NH groups for the compounds 3 and 4a–c. The observed shielding effects on the NH signals due to the thioamides were relatively large: 1.77 ± 0.06 ppm for the 7-thioamides and 1.27 ± 0.01 ppm for the 4-thioamides. The accompanying downfield shifts of the signals due to CH groups attached to these NH groups ($CH^8NH^7C{=}S$ or $CH^5NH^4C{=}S$) were somewhat smaller: 0.79 ppm for the 7-thioamides and 0.95 ppm for the 4-thioamides. In the case of compounds 4a and 4b the 7-thioamides had an even smaller deshielding effect of 0.23 ppm on the α-protons of the thioamino acids ($S{=}C^7CH$).

Furthermore, in each of the ^{1}H NMR spectra of 3 and 4c the methylene protons next to the acetoxy group gave rise to an eight-line pattern characteristic for the AB part of an ABX spin system. On the other hand, the corresponding protons of compounds 4a and 4b each gave rise to a doublet. This is consistent with the presence of a 7-thioamide in compounds 4a and 4b, suggesting a degree of rotational freedom not observed for the 7-amides 3 and 4c. Additional support for the presence of the 7-thioamide partial structure was provided by the chemical shifts observed for the L-alanine (amino acid 7) methyl groups listed in the Experimental Section. For the 7-amides 3 and 4c, a doublet appeared near δ 1.33 ppm. When the 7-amide was replaced by a 7-thioamide (compounds 4a and 4b), the signal for the methyl group was consistantly shifted to lower field by approximately 0.25 ppm and was observed near δ 1.6 ppm. Finally, the 4α-proton was independently identified due to the deshielding effect of approximately 0.2 ppm by the 4-thioamide ($S{=}C^4CH$). For each of the compounds 4a and 4c a multiplet near 5.50 ppm was observed while the corresponding signal for 3 and 4b appeared near 5.33 ppm.

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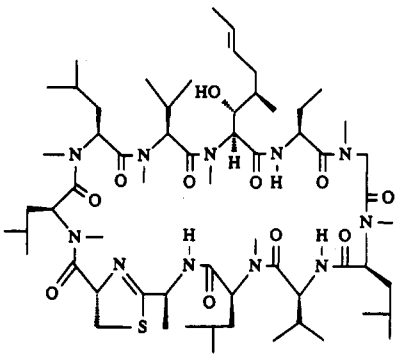
Table I. Chemical Shifts (δ ppm) of NH Groups[a] and α-Protons of Associated Amino Acids[b]

compd	H⁸NCS	H⁵NCS	H²NCO	H⁷NCO	H⁵NCO	H⁸NCO	H²C	H⁷C	H⁵C	H⁸C
1			7.96 (9)	7.68 (8)	7.48 (8)	7.17 (8)	5.03	4.52	4.66	4.83
2			8.10 (9)	7.82 (7)	7.46 (8)	7.62 (8)	5.03	4.52	4.64	4.83
3			8.54 (10)	8.06 (7)	7.49 (9)	7.61 (8)	4.95	4.41	4.76	5.09
4a	9.44 (8)	8.77 (9)	8.50 (10)	8.04 (7)			4.95	4.64	5.71	5.88
4b	9.33 (8)		8.49 (10)	8.03 (6)	7.52 (9)		4.95	4.64	4.79	5.88
4c		8.75 (9)	8.54 (10)	8.06 (7)		7.71 (8)	4.96	4.43	5.72	5.11
5a	9.00 (7)	8.74 (7)	7.63 (10)	7.68 (6)			5.05	4.76	5.17	5.48
5b	9.07 (7)		7.68 (10)	7.74 (7)	7.46 (8)		5.03	4.72	4.63	5.48
5c		8.72 (7)	7.96 (10)	7.80 (7)		7.49 (7)	5.03	4.56	5.24	4.88
7			8.08 (10)	7.73 (7)	7.48 (9)	7.16 (8)	5.02	4.53	4.67	5.05
8			8.56 (10)	8.06 (7)	7.51 (9)	7.48 (8)	4.96	4.44	4.77	4.89
9			8.54 (10)	8.02 (7)	7.48 (9)	7.45 (8)	4.97	4.40	4.77	4.84

[a] Coupling constants (Hz) are shown in parentheses. [b] Center of multiplets.

Chart II



6

The proximity between the primary acetoxy group and the thioamide in **4b** was also demonstrated chemically. Thus compound **4b** was hydrolyzed to the unprotected 7-thioamide **5b** by a transesterification in methanol in the presence of sodium methoxide. The proton NMR spectrum of **5b** was in agreement with the assigned structure. It showed three NH signals between 7.40 and 7.70 ppm. In place of an absorption at δ 7.62 ppm observed for **3**, a new peak appeared at δ 9.07 ppm, consistent with the replacement of the carbonyl group of amino acid 7 by a thioamide. Treatment of the dihydroxy compound **5b** with tosyl chloride in the presence of 4-(dimethylamino)-pyridine led directly to the dihydrothiazolobicyclosporin **6**.[22] This fulfilled the double purpose of forming the crucial bond between the methylene group of the D-serine and sulfur, verifying at the same time the structural assignment of **4b**. The primary hydroxy group of **5b** had undergone a selective reaction with tosyl chloride. In the presence of 4-(dimethylamino)pyridine as base the tosylate of **5b** could not be isolated. Under the reaction conditions it served as an intramolecular alkylating agent for the neighboring thioamide with loss of *p*-toluenesulfonate. We now had formed the crucial bond between the methylene group of the original D-serine (amino acid 8)[14] and a sulfur group, albeit still attached to the amide carbon of amino acid 7 as a thioimino ester. The final step involved opening of the thiazolidine ring in **6** under acidic conditions which led to the desired [D-cysteine]⁸-cyclosporin (**7**). In the NMR spectrum of **7** the amide protons appeared at the positions expected for cyclosporins[21] such as **1** or **2**.

While the stereochemical integrity of [D-cysteine]⁸-cyclosporin (**7**) seemed not to be in doubt as judged from its NMR data, the same cannot be claimed for the cyclic compound **6**. Its NMR spectrum did not show the usually sharp singlets due to the *N*-methyl groups encountered with pure isomers of various cyclosporins including the ones described in this report. Heating a solution of **6** in DMSO as solvent to 180 °C did not sharpen the spectrum significantly. From the data available, the question as to the presence of rotamers or diastereoisomers for the bicyclosporin **6** cannot be answered. If diastereoisomers should be present in **6**, hydrolysis to **7** would be a thermodynamically controlled reaction. For further characterization by ^{1}H NMR spectroscopy, **7** was acetylated to produce the *O,S*-diacetate **8**, thus allowing a direct comparison with the *O,O*-diacetate **3** (see Table I). The chirality of amino acid 8 (D-cysteine) of **7** was secured experimentally via a desulfurization reaction in the presence of Raney nickel.[23] The thioacetate moiety of **8** was reduced leading to the known[19] acetylcyclosporin A (**9**). This was found to be identical in every respect (mp, mixed mp, ^{1}H NMR, *m/z*, HPLC, TLC) with an authentic sample prepared[19] from cyclosporin A (**1**).

The acetyl groups of both thioamides **4a** and **4c** were removed under transesterification conditions in methanol in the presence of catalytic amounts of sodium methoxide for further characterization (see the Experimental Section).

Experimental Section

General. Thin-layer chromatography (TLC) plates were developed in ethyl acetate saturated with water. Column chromatography was carried out on silica gel columns with water-saturated diethyl ether or, as specified, on RP-18 with methanol/water, 88:12. 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 proton nuclear magnetic resonance (NMR) spectra were measured in deuterated chloroform solution on a 360-MHz spectrometer with TMS as reference. The assignment of the chemical shift, e.g., 2.67 (2.70) [s, 3 H, $^{11}NCH_3$] refers to the observed signal for the methyl group attached to the nitrogen of amino acid 11. Except for *N*-methyl groups chemical shifts of proton NMR spectra are listed only if they differ significantly from those of the starting material or if they are crucial for later assignments. The assignments for most 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 comparison (2.70). Correlations between all NH groups and their respective α-protons were secured individually for com-

(22) The oxygen analog has previously been prepared: Wenger, R. M. Sandoz Ltd. Unpublished results.

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pounds **3**, **4a–c**, **5a–c**, **7**, **8**, and **9** through a series of systematic decoupling experiments (see Table I and below). Assignments marked with a double dagger (‡) may be interchangeable if they are listed consecutively within a group of similar chemical shifts. For the complete spectra of cyclosporin A (**1**) see ref 21. Melting points are not corrected. Molecular weights were determined by high-resolution FAB mass spectroscopy.

Diacetyl-[D-serine]^8-cyclosporin (3). A solution of [D-serine]^8-cyclosporin[16] (**2**) (24.3 g, 20 mmol) and 4-(dimethylamino)pyridine (2.4 g, 20 mmol) in 250 mL each of pyridine and acetic anhydride was kept at room temperature overnight. The solvents were evaporated under reduced pressure, at the end with the aid of two portions of 150 mL of toluene. The residue was chromatographed on silica gel with ethyl acetate/ethyl ether, 4:1. The pure fractions were evaporated and crystallized by the addition of ether: yield 25.0 g (95%); R_f = 0.45; mp 186–187 °C; *m/z* calcd for $C_{66}H_{115}N_{11}O_{15}$ 1301.9, found 1302.7 (M + 1)$^+$; $[\alpha]_D$ = −217.3° (*c* = 0.689 in MeOH); NMR δ 1.33 (1.36) [d, *J* = 7 Hz, 3 H, $^7CH_3$], 2.00 [s, 3 H, CH_3CO], 2.06 [s, 3 H, CH_3CO], 2.65‡ (2.70) [s, 3 H, $^{10}NCH_3$], 2.67‡ (2.70) [s, 3 H, $^{11}NCH_3$], 3.09 (3.11) [s, 3 H, $^4NCH_3$], 3.23‡ (3.11) [s, 3 H, $^9NCH_3$], 3.26‡ (3.27) [s, 3 H, $^6NCH_3$], 3.27‡ (3.39) [s, 3 H, $^3NCH_3$], 3.45 (3.51) [s, 3 H, $^1NCH_3$], 4.10–4.22 [m, 2 H, CH_2OAc], 4.36–4.46 (4.52) [m, 1 H, 7α-H], 4.64 (4.74) [d, *J* = 14 Hz, 1 H, 3α′-H], 4.72–4.80 (4.66) [m, 1 H, 5α-H], 4.90–5.00 (5.03) [m, 1 H, 2α-H], 4.97 (5.14) [d, *J* = 11 Hz, 1 H, 11α-H], 5.02–5.16 (4.83) [m, 1 H, 8α-H], 5.29–5.33 (5.34) [m, 1 H, 4α-H], 5.45–5.60 [m, 2 H, ^{1}NCHCHOAc], 7.49 [d, *J* = 9 Hz, 1 H, ^{5}NH], 7.61 [d, *J* = 8 Hz, 1 H, ^{8}NH], 8.06 [d, *J* = 7 Hz, 1 H, ^{7}NH], 8.54 [d, *J* = 10 Hz, 1 H, ^{2}NH].

Diacetyl-[D-serine]^8-cyclosporinthioamides (4a–c). A solution of the diacetate **3** (19.5 g, 15 mmol) in 150 mL of xylene was heated to 133 °C (internal temperature). To the clear solution Lawesson reagent[20] (3.61 g, 9 mmol) was added in small portions. After 30 min the reaction mixture was cooled to room temperature and evaporated under reduced pressure. The residue of 24.57 g was chromatographed on silica gel with fractions of approximately 200 mL being collected. Fractions 44–65 were combined and evaporated to give 2.5 g of a foam which crystallized upon treatment with ether/hexane to give pure **4c**, fractions 14–35 were combined and evaporated to give 11.5 g of a slightly yellow foam which was chromatographed on a RP-18 reversed-phase column with methanol/water, 85:15, as eluent leading to a separation of **4b** and **4a**. Fractions 36–43 contained another 860 mg of a mixture of **4b** and **4c**.

Diacetyl-[D-serine]^8-cyclosporin-4,7-dithioamide (4a): yield 4.5 g (22.5%) of the crystalline product; R_f = 0.65; mp 156–157 °C; *m/z* calcd for $C_{66}H_{115}N_{11}O_{13}S_2$ 1333.9, found 1334.6 (M + 1)$^+$; $[\alpha]_D$ = −209.0° (*c* = 0.531 in MeOH); NMR δ 1.57 (1.36) [d, *J* = 7 Hz, 3 H, $^7CH_3$], 2.00 [s, 3 H, CH_3CO], 2.08 [s, 3 H, CH_3CO], 2.65‡ (2.70) [s, 3 H, $^{10}NCH_3$], 2.68‡ (2.70) [s, 3 H, $^{11}NCH_3$], 3.02 (3.11) [s, 3 H, $^4NCH_3$], 3.25‡ (3.11) [s, 3 H, $^9NCH_3$], 3.37‡ (3.27) [s, 3 H, $^6NCH_3$], 3.38 (3.39) [s, 3 H, $^3NCH_3$], 3.46 (3.51) [s, 3 H, $^1NCH_3$], 4.28 [d, *J* = 6 Hz, 2 H, CH_2OAc], 4.63 [d, *J* = 14 Hz, 1 H, 3α′-H], 4.59–4.69 [m, 1 H, 7α-H], 4.90–5.00 [m, 1 H, 2α-H], 4.97 [d, *J* = 11 Hz, 1 H, 11α-H], 5.48–5.51 [m, 2 H, ^{1}NCHCHOAc], 5.46–5.54 [m, 1 H, 4α-H], 5.64–5.78 [m, 2 H, 5α-H, 9α-H], 5.84–5.92 [m, 1 H, 8α-H], 8.04 [d, *J* = 7 Hz, 1 H, ^{7}NH], 8.50 [d, *J* = 10 Hz, 1 H, ^{2}NH], 8.77 [d, *J* = 9 Hz, 1 H, ^{5}NH], 9.44 [d, *J* = 8 Hz, 1 H, ^{8}NH].

Diacetyl-[D-serine]^8-cyclosporin-7-thioamide (4b): yield 1.5 g (7.6%) of the crystalline product; R_f = 0.58; mp 146–147 °C; *m/z* calcd for $C_{66}H_{115}N_{11}O_{14}S$ 1317.9, found 1318.6 (M + 1)$^+$; $[\alpha]_D$ = −176.3° (*c* = 0.452 in MeOH); NMR δ 1.57 (1.36) [d, *J* = 7 Hz, 3 H, $^7CH_3$], 2.00 [s, 3 H, CH_3CO], 2.07 [s, 3 H, CH_3CO], 2.65‡ (2.70) [s, 3 H, $^{10}NCH_3$], 2.67‡ (2.70) [s, 3 H, $^{11}NCH_3$], 3.08 (3.11) [s, 3 H, $^4NCH_3$], 3.23‡ (3.11) [s, 3 H, $^9NCH_3$], 3.24‡ (3.27) [s, 3 H, $^6NCH_3$], 3.35 (3.39) [s, 3 H, $^3NCH_3$], 3.45 (3.51) [s, 3 H, $^1NCH_3$], 4.27 [d, *J* = 6 Hz, 2 H, CH_2OAc], 4.58–4.70 [m, 1 H, 7α-H], 4.65 [d, *J* = 14 Hz, 1 H, 3α′-H], 4.75–4.83 [m, 1 H, 5α-H], 4.90–5.00 [m, 1 H, 2α-H], 4.97 [d, *J* = 11 Hz, 1 H, 11α-H], 5.31–5.36 [m, 1 H, 4α-H], 5.44–5.60 [m, 2 H, ^{1}NCHCHOAc], 5.85–5.91 [m, 1 H, 8α-H], 7.52 [d, *J* = 9 Hz, 1 H, ^{5}NH], 8.03 [d, *J* = 6 Hz, 1 H, ^{7}NH], 8.49 [d, *J* = 10 Hz, 1 H, ^{2}NH], 9.33 [d, *J* = 8 Hz, 1 H, ^{8}NH].

Diacetyl-[D-serine]^8-cyclosporin-4-thioamide (4c): yield 2.1 g (11%) of the crystalline product; R_f = 0.50; mp 162–163 °C; *m/z* calcd for $C_{66}H_{115}N_{11}O_{14}S$ 1317.9, found 1318.5 (M + 1)$^+$; $[\alpha]_D$ = −260.0° (*c* = 0.566 in MeOH); NMR δ 1.34 (1.36) [d, *J* = 7 Hz, 3 H, $^7CH_3$], 2.00 [s, 3 H, CH_3CO], 2.06 [s, 3 H, CH_3CO], 2.65‡ (2.70) [s, 3 H, $^{10}NCH_3$], 2.66‡ (2.70) [s, 3 H, $^{11}NCH_3$], 3.02 (3.11) [s, 3 H, $^4NCH_3$], 3.26‡ (3.11) [s, 3 H, $^9NCH_3$], 3.27‡ (3.27) [s, 3 H, $^6NCH_3$], 3.38 (3.39) [s, 3 H, $^3NCH_3$], 3.45 (3.51) [s, 3 H, $^1NCH_3$], 4.10–4.25 [m, 2 H, CH_2OAc], 4.38–4.48 [m, 1 H, 7α-H], 4.62 [d, *J* = 14 Hz, 1 H, 3α′-H], 4.92–5.00 [m, 1 H, 2α-H], 4.97 [d, *J* = 11 Hz, 1 H, 11α-H], 5.07–5.15 [m, 1 H, 8α-H], 5.48–5.51 [m, 2 H, ^{1}NCHCHOAc], 5.46–5.54 [m, 1 H, 4α-H], 5.64–5.76 [m, 2 H, 5α-H, 9α-H], 7.71 [d, *J* = 8 Hz, 1 H, ^{8}NH], 8.06 [d, *J* = 7 Hz, 1 H, ^{7}NH], 8.54 [d, *J* = 10 Hz, 1 H, ^{2}NH], 8.75 [d, *J* = 9 Hz, 1 H, ^{5}NH].

[D-Serine]^8-cyclosporin-4,7-dithioamide (5a). To a solution prepared by dissolving sodium metal (910 mg, 40 mmol) in 25 mL of methanol there was added a solution of **4a** (4.0 g, 3.0 mmol) in methanol (50 mL). This mixture was kept at room temperature for 4 h. Acetic acid was added to neutralize the base. The solvent was evaporated under reduced pressure. The residue was dissolved in *tert*-butyl methyl ether and washed with water. The organic phase was dried over magnesium sulfate and evaporated. The crude product was chromatographed on silica gel with water-saturated *tert*-butyl methyl ether to give the pure product (3.1 g, 83%): *m/z* calcd for $C_{62}H_{111}N_{11}O_{11}S_2$ 1249.7, found 1250.9 (M + 1)$^+$; $[\alpha]_D$ = −173.8° (*c* = 0.511 in MeOH); NMR δ 1.60 (1.36) [d, *J* = 7 Hz, 3 H, $^7CH_3$], 2.69‡ (2.70) [s, 3 H, $^{10}NCH_3$], 2.70‡ (2.70) [s, 3 H, $^{11}NCH_3$], 3.06 (3.11) [s, 3 H, $^4NCH_3$], 3.22‡ (3.11) [s, 3 H, $^9NCH_3$], 3.30‡ (3.27) [s, 3 H, $^6NCH_3$], 3.38 (3.39) [s, 3 H, $^3NCH_3$], 3.52 (3.51) [s, 3 H, $^1NCH_3$], 4.74 [d, *J* = 14 Hz, 1 H, 3α′-H], 4.73–4.79 [m, 1 H, 7α-H], 4.95–5.11 [m, 3 H, 2α-H, 6α-H, 10α-H], 5.12–5.22 [m, 1 H, 5α-H], 5.18 [d, *J* = 11 Hz, 1 H, 11α-H], 5.42–5.52 [m, 2 H, 1α-H, 8α-H], 7.63 [d, *J* = 10 Hz, 1 H, ^{2}NH], 7.68 [d, *J* = 6 Hz, 1 H, ^{7}NH], 8.74 [d, *J* = 7 Hz, 1 H, ^{5}NH], 9.00 [d, *J* = 7 Hz, 1 H, ^{8}NH].

[D-Serine]^8-cyclosporin-7-thioamide (5b). Similarly, **4b** (1.00 g, 0.93 mmol) was hydrolyzed to give the product (890 mg, 95%) as a foam: *m/z* calcd for $C_{62}H_{111}N_{11}O_{12}S$ 1233.9, found 1234.7 (M + 1)$^+$; $[\alpha]_D$ = −166.1° (*c* = 0.502 in MeOH); NMR δ 1.59 (1.36) [d, *J* = 7 Hz, 3 H, $^7CH_3$], 2.69‡ (2.70) [s, 3 H, $^{10}NCH_3$], 2.70‡ (2.70) [s, 3 H, $^{11}NCH_3$], 3.10 (3.11) [s, 3 H, $^4NCH_3$], 3.21‡ (3.11) [s, 3 H, $^9NCH_3$], 3.25‡ (3.27) [s, 3 H, $^6NCH_3$], 3.38 (3.39) [s, 3 H, $^3NCH_3$], 3.50 (3.51) [s, 3 H, $^1NCH_3$], 4.60–4.66 [m, 1 H, 5α-H], 4.68–4.76 [m, 1 H, 7α-H], 4.74 [d, *J* = 14 Hz, 1 H, 3α′-H], 4.98–5.12 [m, 3 H, 2α-H, 6α-H, 10α-H], 5.18 [d, *J* = 11 Hz, 1 H, 11α-H], 5.44–5.52 [m, 1 H, 8α-H], 7.46 [d, *J* = 8 Hz, 1 H, ^{5}NH], 7.68 [d, *J* = 10 Hz, 1 H, ^{2}NH], 7.74 [d, *J* = 7 Hz, 1 H, ^{7}NH], 9.07 [d, *J* = 7 Hz, 1 H, ^{8}NH].

[D-Serine]^8-cyclosporin-4-thioamide (5c). Similarly, **4c** (1.22 g, 0.93 mmol) was hydrolyzed to give the product (610 mg, 54%) as a foam: *m/z* calcd for $C_{62}H_{111}N_{11}O_{12}S$ 1233.9, found 1234.9 (M + 1)$^+$; $[\alpha]_D$ = −205.1° (*c* = 0.448 in MeOH); NMR δ 1.37 (1.36) [d, *J* = 7 Hz, 3 H, $^7CH_3$], 2.69‡ (2.70) [s, 3 H, $^{10}NCH_3$], 2.70‡ (2.70) [s, 3 H, $^{11}NCH_3$], 3.05 (3.11) [s, 3 H, $^4NCH_3$], 3.20‡ (3.11) [s, 3 H, $^9NCH_3$], 3.35‡ (3.27) [s, 3 H, $^6NCH_3$], 3.38 (3.39) [s, 3 H, $^3NCH_3$], 3.50 (3.51) [s, 3 H, $^1NCH_3$], 4.50–4.62 [m, 1 H, 7α-H], 4.73 [d, *J* = 14 Hz, 1 H, 3α′-H], 4.86–4.90 [m, 1 H, 8α-H], 4.97–5.08 [m, 3 H, 2α-H, 6α-H, 10α-H], 5.16 [d, *J* = 11 Hz, 1 H, 11α-H], 5.20–5.28 [m, 1 H, 5α-H], 7.49 [d, *J* = 7 Hz, 1 H, ^{8}NH], 7.80 [d, *J* = 7 Hz, 1 H, ^{7}NH], 7.96 [d, *J* = 10 Hz, 1 H, ^{2}NH], 8.72 [d, *J* = 7 Hz, 1 H, ^{5}NH].

Dihydrothiazolo[2.1.30]bicyclosporin (6). A solution of the 7-thioamide **5b** (210 mg, 0.17 mmol) and 4-(dimethylamino)-pyridine (1.0 g, 8.2 mmol) in methylene chloride (15 mL) was allowed to react with tosyl chloride (1.0 g, 5.3 mmol) at room temperature for 1.5 h. Ether was added and washed with water and brine. The organic phase was dried over magnesium sulfate and evaporated to give the crude product (400 mg). This was chromatographed on silica gel to give the pure product (170 mg, 82%): *m/z* calcd for $C_{62}H_{109}N_{11}O_{11}S$ 1215, found 1216 (M + 1)$^+$; $[\alpha]_D$ = −231.8° (*c* = 0.217 in MeOH).

[D-Cysteine]^8-cyclosporin (7). A solution of the thiazolidine **6** (170 mg, 0.14 mmol) in acetonitrile (15 mL) was kept at room temperature for 5 h in the presence of 1 N HCl solution (1 mL). The solution was neutralized with 1 N sodium bicarbonate solution and extracted with *tert*-butyl methyl ether. The organic phase was dried over magnesium sulfate, filtered, and evaporated

to leave 170 mg of crude product. This was purified on a silica gel column to give 150 mg (87%) of pure product: m/z calcd for $C_{62}H_{111}N_{11}O_{12}S$ 1233.9, found 1234.7 $(M + 1)^+$; $[\alpha]_D = -153.4°$ (c = 0.155 in MeOH); NMR δ 1.37 (1.36) [d, J = 7 Hz, 3 H, $^7CH_3$], 2.68‡ (2.70) [s, 3 H, $^{10}NCH_3$], 2.69‡ (2.70) [s, 3 H, $^{11}NCH_3$], 2.7–2.8 [m, 2 H, CH_2S], 3.10 (3.11) [s, 3 H, $^4NCH_3$], 3.22‡ (3.11) [s, 3 H, $^9NCH_3$], 3.23‡ (3.27) [s, 3 H, $^6NCH_3$], 3.40 (3.39) [s, 3 H, $^3NCH_3$], 3.48 (3.51) [s, 3 H, $^1NCH_3$], 4.48–4.58 [m, 1 H, 7α-H], 4.63–4.71 [m, 1 H, 5α-H], 4.72 [d, J = 14 Hz, 1 H, 3α'-H], 4.96–5.12 [m, 4 H, 2α-H, 6α-H, 8α-H, 10α-H], 5.12 [d, J = 11 Hz, 1 H, 11α-H], 7.16 [d, J = 8 Hz, 1 H, ^{8}NH], 7.48 [d, J = 9 Hz, 1 H, ^{5}NH], 7.73 [d, J = 7 Hz, 1 H, ^{7}NH], 8.08 [d, J = 10 Hz, 1 H, ^{2}NH].

***O,S*-Diacetyl-[D-cysteine]^8-cyclosporin (8).** [D-Cysteine]^8-cyclosporin (7) (90 mg, 0.07 mmol) was acetylated as described above for the preparation of **3**: yield 22.0 mg (23%); m/z calcd for $C_{66}H_{115}N_{11}O_{14}S$ 1317.9, found 1319.1 $(M + 1)^+$; $[\alpha]_D = -180.6°$ (c = 0.366 in MeOH); NMR δ 1.32 (1.36) [d, J = 7 Hz, 3 H, $^7CH_3$], 2.00 [s, 3 H, CH_3COO], 2.35 [s, 3 H, CH_3COS], 2.65‡ (2.70) [s, 3 H, $^{10}NCH_3$], 2.67‡ (2.70) [s, 3 H, $^{11}NCH_3$], 3.08 (3.11) [s, 3 H, $^4NCH_3$], 2.80–2.90 and 3.10–3.20 [m, 2 H, CH_2SAc], 3.23‡ (3.11) [s, 3 H, $^9NCH_3$], 3.25‡ (3.27) [s, 3 H, $^6NCH_3$], 3.28‡ (3.39) [s, 3 H, $^3NCH_3$], 3.44 (3.51) [s, 3 H, $^1NCH_3$], 4.40–4.48 [m, 1 H, 7α-H], 4.66 [d, J = 14 Hz, 1 H, 3α'-H], 4.74–4.80 [m, 1 H, 5α-H], 4.86–4.92 [m, 1 H, 8α-H], 4.92–5.00 [m, 1 H, 2α-H], 4.98 [d, J = 11 Hz, 1 H, 11α-H], 5.50–5.54 [m, 2 H, ^{1}NCHCHOAc], 7.48 [d, J = 8 Hz, 1 H, ^{8}NH], 7.51 [d, J = 9 Hz, 1 H, ^{5}NH], 8.06 [d, J = 7 Hz, 1 H, ^{7}NH], 8.56 [d, J = 10 Hz, 1 H, ^{2}NH].

Acetylcyclosporin A (9). A solution of 8 (100 mg, 0.076 mmol) in absolute ethanol (20 mL) was heated to reflux for 30 min in the presence of Raney nickel which had been washed three times with the same solvent. The catalyst was filtered off, and the solution was evaporated. The residue was dissolved on *tert*-butyl methyl ether and washed with 2 N HCl solution, water, and brine. The organic phase was dried over sodium sulfate and evaporated to give the crude product (100 mg) which was chromatographed over silica gel to give pure product: yield 53 mg (56%); mp 226–228 °C; authentic sample[19] mp 226–228 °C; mixed mp 227–228 °C; m/z calcd for $C_{64}H_{113}N_{11}O_{13}$ 1243.9, found 1244.9 $(M + 1)^+$; $[\alpha]_D = -215°$ (c = 0.116 in MeOH); lit.[19] $[\alpha]_D = -246°$ (c = 0.81 in MeOH); NMR δ 1.27 (1.26) [d, J = 7 Hz, 3 H, $^8CH_3$], 1.32 (1.36) [d, J = 7 Hz, 3 H, $^7CH_3$], 2.00 [s, 3 H, CH_3CO], 2.64‡ (2.70) [s, 3 H, $^{10}NCH_3$], 2.67‡ (2.70) [s, 3 H, $^{11}NCH_3$], 3.08 (3.11) [s, 3 H, $^4NCH_3$], 3.20‡ (3.11) [s, 3 H, $^9NCH_3$], 3.23‡ (3.27) [s, 3 H, $^6NCH_3$], 3.25‡ (3.39) [s, 3 H, $^3NCH_3$], 3.43 (3.51) [s, 3 H, $^1NCH_3$], 4.35–4.45 [m, 1 H, 7α-H], 4.65 [d, J = 14 Hz, 1 H, 3α'-H], 4.74–4.80 [m, 1 H, 5α-H], 4.82–4.86 [m, 1 H, 8α-H], 4.93–5.01 [m, 1 H, 2α-H], 4.98 [d, J = 11 Hz, 1 H, 11α-H], 5.50–5.54 [m, 2 H, ^{1}NCHCHOAc], 7.45 [d, J = 8 Hz, 1 H, ^{8}NH], 7.48 [d, J = 9 Hz, 1 H, ^{5}NH], 8.02 [d, J = 7 Hz, 1 H, ^{7}NH], 8.54 [d, J = 10 Hz, 1 H, ^{2}NH]; also identical by TLC, HPLC.

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Supplementary Material Available: 360-MHz ^{1}H NMR spectra of **3**, **4a–c**, **5a–c**, **7**, **8**, and **9** (10 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.