Cyclosporin A: Regioselective Ring Opening and Fragmentation Reactions *via* Thioamides. A Route to Semisynthetic Cyclosporins†

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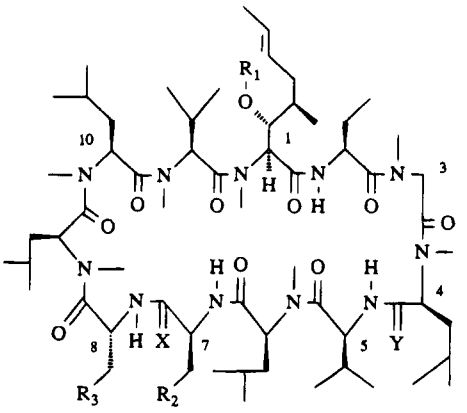
Cyclosporin A (**1a**) served as the starting material for the semisynthetic preparation of a variety of novel cyclosporins. Acetylcyclosporin A (**2**) was treated with Lawesson's reagent. From the reaction mixture, three novel acetylated thioamides were isolated: the 4,7-bis(thioamide) **3a**, the 7-thioamide **3b**, and the 4-thioamide **3c**. The acetylated products **3a–c** were hydrolyzed to the known thioamides **4a–c**, respectively. The 7-thioamide **3b** was alkylated to give the *S*-benzyl thioamidate **5b**. A regioselective ring opening reaction at the activated site was induced by treating **5b** under acidic conditions giving the 7,8-seco-cyclosporin **7a**. The (*R*)-alanine moiety was replaced by (*R*)-phenylalanine via the Edman degradation product **7e** giving **7f**. Removal of the protecting groups led to **7h**. This was cyclized to [(*R*)-phenylalanine]^8-cyclosporin (**1b**). The N-protected 7,8-seco-cyclosporin **7i** was reduced to the aldehyde **7j**, homologated (**7k**), and deprotected to give **7m**. This was cyclized to the vinylogous cyclosporin **8a**. Similarly, the 4,5-seco-cyclosporin **9c** was prepared and converted via several steps (**9d–h**) to the vinylogous cyclosporin **8b**. Finally, under acidic conditions, the dibenzyl bis(thioamidate) **5a** underwent a fragmentation reaction to give the octapeptide **10a** and the tripeptide **11a**. The octapeptide **10a** was coupled with a different tripeptide (**11d**) to **9i** and cyclized via **9j–k** to the [(*S*)-phenylalanine]^7-cyclosporin (**1c**).

Cyclosporin A[1] (**1a**) (see Chart 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] The mechanism of action[7] is based on the inhibition of the production of lymphokines such as interleukine-2 (IL-2). Mainly 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]

Chart 1



1 - 4

1a)	$R_1 = R_2 = R_3 = H$	X = Y = O
1b)	$R_1 = R_2 = H$; $R_3 = Ph$	X = Y = O
1c)	$R_1 = R_3 = H$; $R_2 = Ph$	X = Y = O
2)	$R_1 = Ac$; $R_2 = R_3 = H$	X = Y = O
3a)	$R_1 = Ac$; $R_2 = R_3 = H$	X = Y = S
3b)	$R_1 = Ac$; $R_2 = R_3 = H$	X = S; Y = O
3c)	$R_1 = Ac$; $R_2 = R_3 = H$	X = O; Y = S
4a)	$R_1 = R_2 = R_3 = H$	X = Y = S
4b)	$R_1 = R_2 = R_3 = H$	X = S; Y = O
4c)	$R_1 = R_2 = R_3 = H$	X = O; Y = S

A number of modified cyclosporins have been isolated from natural sources[12] while others were prepared *via* total synthesis[13] for comparison with cyclosporin A (**1a**)

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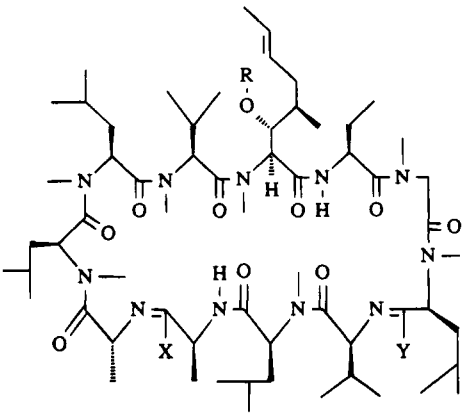
to establish a structure–activity relationship. From these studies has emerged the knowledge that the free hydroxyl group of the amino acid 1 (MeBmt)[14] is important for good immunosuppressant activity. A "bioactive conformation" of this group as a prerequisite for immunosuppressive activity has also been proposed.[15]

Cyclophilin recognizes and binds to a region encompassing the amino acids 9 *via* 1 to 4 (see Chart 1). This stretch was defined as the binding domain of cyclosporin A. Calmodulin, binding to the cyclophilin–cyclosporin A complex, seems to recognize the remaining amino acid sequence of cyclosporin A. This region was defined as the effector domain[16] of cyclosporin A. In conjunction with these findings, it would be highly desirable to have at hand a method allowing manipulation of one of the domains of cyclosporin A as defined above, while preserving the other. Such a method should be applicable to the most abundant of all the natural cyclosporins, namely cyclosporin A itself, thus avoiding laborious total syntheses.

Lawesson's reagent[17] allowed the regioselective introduction of sulfur into cyclosporin A. These transformations preferentially occurred at the non-methylated amino acids 4 and 7, converting these secondary amides to their respective thioamides. Yields, however, were low as published[18] for the unprotected cyclosporin A (**1a**). Yet, the positions of the markers thus introduced into the cyclosporin molecule are strategically located in the vicinity of the borderlines dividing the binding from effector domain. Therefore, the yields of thioamides had to be improved considerably in order to make this approach interesting for further development. Prospects to reach this goal were fair based on our experience[19] for the conversion of [(*R*)-serine]^8-cyclosporin to its thioamides. In that case, acetylation of the hydroxy groups allowed the thioamides to be isolated in combined yields approaching 50%. Activation of the various thioamides as thioamidates would then set the stage for regioselective hydrolytic cleavages[20] of cyclosporin A (**1a**) between amino acids 4 and 5 or between amino acids 7 and 8. A concomitant hydrolytic cleavage at both of these sites should result in a fragmentation reaction of cyclosporin A, yielding an octapeptide and a tripeptide.

Chart 2. Cyclosporin Thioamidates



5 - 6*

5a)	R = Ac	X = Y = SCH_2Ph
5b)	R = Ac	X = SCH_2Ph; Y = OH
5c)	R = Ac	X = OH; Y = SCH_2Ph
6a)	R = H	X = Y = SCH_2Ph
6b)	R = H	X = SCH_2Ph; Y = OH
6c)	R = H	X = OH; Y = SCH_2Ph

*) The notation X and/or Y = OH does not imply the presence of the enol tautomeric form.

The known[21] cyclosporin A acetate (**2**) was treated with Lawesson's reagent[17] at elevated temperature for a short period of time. The mixture was separated by extensive column chromatography on silica gel and, if necessary, reversed phase Rp-18 as described[19] for the analogous conversion of diacetyl-[(*R*)-serine]^8-cyclosporin. Thus, we were able to isolate, in increasing order of polarity, the acetyl 4,7-bis(thioamide) **3a**, the acetyl 7-thioamide **3b**, and the acetyl 4-thioamide **3c** in 34, 10, and 4% yield, respectively (see Chart 2). These yields were calculated for the crystallized products. In addition, appreciable amounts of each compound were obtained from the mother liquors, since the losses due to crystallization are considerable, thus making the workable yields higher than indicated above. Product distribution, especially bis(thioamide) to thioamides, strongly depends upon the ratio of Lawesson's reagent to cyclosporin employed for the particular reaction.

The structural assignments for the acetyl thioamides **3a–c** were based on the chemical shifts[22] observed for the NH groups of the secondary amides as listed in

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Table 1. Chemical Shifts (δ ppm) of NH Groups[a] and α-Protons of Associated Amino Acids[b]

compd	H^8NCS	H^5NCS	H^2NCO	H^7NCO	H^5NCO	H^8NCO	H^2C	H^7C	H^5C	H^8C
1a			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
3a	9.28 (7)	8.76 (9)	8.48 (10)	7.98 (7)			4.96	4.63	5.74	5.56
3b	9.18 (8)		8.47 (9)	7.95 (6)	7.49 (9)		4.95	4.62	4.79	5.56
3c		8.74 (9)	8.52 (10)	8.01 (7)		7.53 (8)	4.97	4.42	5.74	4.85

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

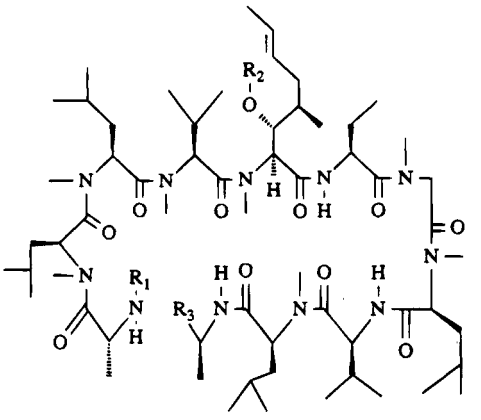
Table 1. These assignments were confirmed by the products obtained following hydrolyses of the acetate groups for all three acetyl thioamides **3a–c** in methanol in the presence of sodium methoxide, leading to the known[18] thioamides **4a–c**.

With the thioamides **3a–c** and **4a–c** at hand, we set out to test their potential for the regioselective ring opening reactions. This was first done *via* S-alkylation of either one of the acetylated mono-thioamides **3b** or **3c**. Suitable conditions for the conversion of these thioamides involved treatment with an alkylating agent, e.g. benzyl bromide, in methylene chloride in the presence of an organic base such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) or in a two-phase system in the presence of aqueous sodium hydroxide. Acetate groups were not hydrolyzed even in the presence of 30% aqueous sodium hydroxide. The products of these S-alkylations (**5b,c**) most likely consisted of mixtures of conformational and/or stereochemical isomers as indicated by their NMR spectra in chloroform solutions at room temperature. NMR experiments in DMSO solutions at temperatures as high as 180 °C did not result in first order spectra. The mono-thioamides **4b** or **4c** with the free hydroxy groups were selectively alkylated on the sulfur groups leading to thioamidates **6b** and **6c**, respectively.

A solution of the *S*-benzyl thioamidate **5b** in acetonitrile was added to a 1:1 mixture of 6 N hydrochloric acid and acetonitrile. This resulted in a clean ring opening reaction with the formation of the *O*-acetyl-7,8-seco-cyclosporin **7a** in 73% yield following chromatography on silica gel (see Scheme 1). Product **7a** featured an unprotected amino terminus (amino acid 8), an acid terminus protected as a benzyl thiol ester (amino acid 7), and a hydroxy group protected as an acetate. This doubly protected undecapeptide proved to be quite stable while being kept in solution. In methylene chloride, **7a** was stored unchanged for further use in subsequent reactions. On the other hand, when solutions of **7a** were evaporated to dryness and the amorphous solids were examined by mass spectroscopy after a few days, products of higher molecular weight, not present prior to evaporation, were detected. For a fresh sample of the open chain product **7a**, mass spectroscopy clearly revealed the amino acid sequence, showing fragments in agreement with a ring opening reaction between amino acids 7 and 8. Mass spectroscopy proved to be a highly informative analytical tool and was routinely used for all new linear peptides described in this paper.

The result of the ring opening reaction for the non-acetylated cyclosporin thioamidate **6b** was essentially the same as described above for **5b**, producing the undecapeptide **7b** in 49% yield with the amino and the hydroxy groups both unprotected and with an acid terminus protected as the benzyl thiol ester. A second hydrolytic step in aqueous methanol of either compound **7a** or **7b**, this time in the presence of sodium hydroxide, led to the known 7,8-seco-cyclosporin **7c**. This was described[6] as

Scheme 1. 7,8-Secocyclosporins



7a - d, 7i - m

5b ⟶ 7a: R_1 = H; R_2 = Ac; R_3 = $COSCH_2Ph$

6b ⟶ 7b: R_1 = R_2 = H; R_3 = $COSCH_2Ph$

7a ⟶ 7c: R_1 = R_2 = H; R_3 = COOH

7a ⟶ 7d: R_1 = CSNHPh; R_2 = Ac; R_3 = $COSCH_2Ph$

7b ⟶ 7i: R_1 = BOC; R_2 = H; R_3 = $COSCH_2Ph$

7i ⟶ 7j: R_1 = BOC; R_2 = H; R_3 = CHO

7j ⟶ 7k: R_1 = BOC; R_2 = H; R_3 = CH=CHCOOCH$_3$

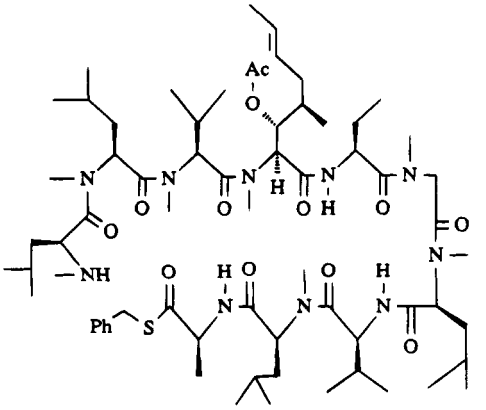
7k ⟶ 7l: R_1 = R_2 = H; R_3 = CH=CHCOOCH$_3$

7l ⟶ 7m: R_1 = R_2 = H; R_3 = CH=CHCOOH

the last intermediate prior to its cyclization to cyclosporin A during the course of the total synthesis.

The 7,8-seco-cyclosporin **7a** was treated with phenyl isothiocyanate forming the phenylthiourea **7d**. In the presence of 6 N hydrochloric acid, this thiourea readily underwent an Edman degradation to the decapeptide **7e** lacking the D-alanine (see Scheme 2). This product was

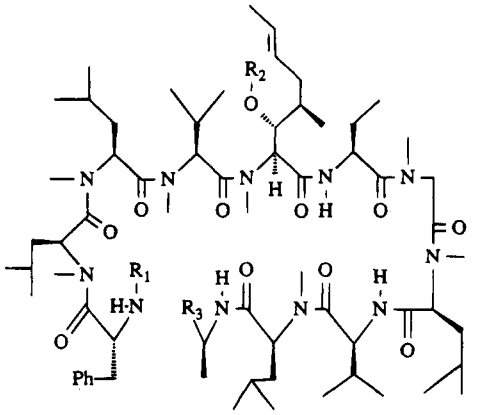
Scheme 2. 7,8-Seco-8-norcyclosporin



7e

7d ⟶ 7e

Scheme 3. 7,8-Secocyclosporins



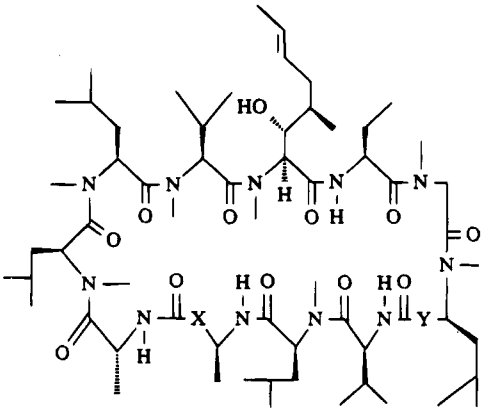
7f - h

7e ⟶ 7f: R_1 = BOC; R_2 = Ac; R_3 = $COSCH_2Ph$

7f ⟶ 7g: R_1 = H; R_2 = Ac; R_3 = $COSCH_2Ph$

7g ⟶ 7h: R_1 = R_2 = H; R_3 = COOH

Chart 3. Vinylogous Cyclosporins



8

8a: X = $_{trans}$CH=CH; Y = bond

8b: X = bond; Y = $_{trans}$CH=CH

used as starting material for the preparation of semisynthetic cyclosporins with a variety of amino acids at position 8 of the natural product not currently available from fermentation reactions. In order to demonstrate this point, we set out to replace (*R*)-alanine of cyclosporin A by (*R*)-phenylalanine. Employing standard peptide forming conditions, the decapeptide **7e** was treated with BOC-protected (*R*)-phenylalanine in the presence of EDC as the condensing agent (see Scheme 3). This resulted in the formation of the fully protected, novel undecapeptide **7f** in 75% yield. The amino protecting group of **7f** was removed in the presence of trifluoroacetic acid giving **7g**. The fully deprotected linear undecapeptide **7h** was obtained following treatment of **7g** with sodium hydroxide. This was cyclized in the presence of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) to the hitherto unknown, crystalline [(*R*)-phenylalanine]^8-cyclosporin (**1b**) in 29% yield. The NMR spectrum of this compound indicated the presence of the usual cyclosporin conformation in chloroform solution. No major changes of chemical shifts due to the presence of the phenyl group were detected.

The stability of ring opening product **7b** was improved by protecting the free amino terminus in the form of the *tert*-butoxycarbonyl group (BOC), as exemplified by compound **7i**. This opened the possibility for a selective manipulation of the carboxy terminus in **7i**. As an example we chose the reduction of the benzyl thiol ester group of **7i** to the carboxaldehyde **7j** in the presence of triethylsilane and Pd/C, a technique described[23] only recently. We have modified the procedure slightly employing homogeneous catalysis. Palladium(II) acetate was added to the substrate followed by the addition of the reducing agent (triethylsilane). The endpoint of the reduction of the thiol ester was indicated by a precipitation of the black Pd(0) catalyst, thus allowing visualization of the progress of the reduction. When the reaction mixture was worked up immediately, undesirable double bond reduction could be avoided. The aldehyde **7j** was obtained in 61% yield following column chromatography. As a next step, the aldehyde **7j** was homologated via Wittig reaction in the presence of commercial methyl (triphenylphosphoranylidene)acetate leading to the α,β-unsaturated ester **7k** in practically quantitative yield. The amino protecting group was removed in the presence of trifluoroacetic acid to give **7l** in 65% yield. The fully deprotected peptide **7m** was obtained following hydrolysis under basic conditions. This set the stage for a cyclization in the presence of 2-ethoxy-1-ethoxy-1,2-dihydroquinoline (EEDQ) to a new class of cyclosporins with a double bond built into the peptide backbone. The newly formed cyclosporin **8a** (see Chart 3), with the amino acid 8 [(*R*)-alanine] having been replaced by a vinylogous alanine, was obtained as a crystalline material in 10% yield. The preparation of vinylogous polypeptides[24] has been described by Schreiber *et al.*

In analogy to the cyclosporin A ring opening reaction between amino acids 7 and 8 described above, we next examined the ring opening between the amino acids 4 and 5 (see Scheme 4). For this purpose the 4-thioamide **4c** was converted to the benzyl thioamidate **6c**. Addition of **6c** to an excess of 6 N hydrochloric acid in acetonitrile again resulted in a clean ring opening reaction. The linear 4,5-seco-cyclosporin **9a**, protected as the benzyl thiol ester, was obtained in 52% yield. In contrast to any of the NMR spectra of the 7,8-seco-cyclosporins described above, the spectrum of **9a** in chloroform solution at room temperature was strikingly similar to the spectrum of cyclosporin A itself in chloroform solution. However, small differences were detectable. In the case of **9a**, five of the seven NCH_3 signals gave rise to sharp singlets. The two NCH_3 signals which gave rise to two signals each, were assigned to the $^4NCH_3$ and the $^6NCH_3$ groups, respectively, since these are located closest to the site of cleavage. This may be interpreted as evidence that this particular compound is folded like cyclosporin A, the transannular hydrogen bonds locking the molecule into a cyclosporin A like conformation.

Hydrolysis of **9a** under basic conditions resulted in the formation of the deprotected, linear 4,5-seco-cyclosporin **9b**. In chloroform solution at room temperature this molecule was present in more than one conformation as

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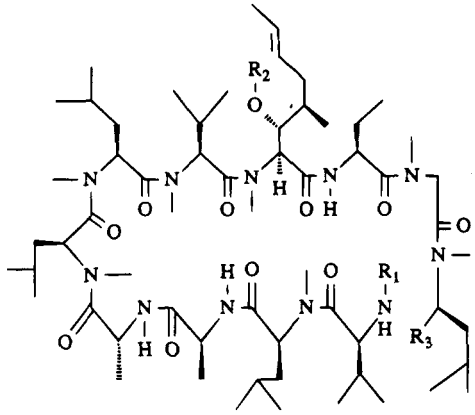
Scheme 4. 4,5-Secocyclosporins



9a - h

6c ⟶ 9a: $R_1 = R_2 = H$; $R_3 = COSCH_2Ph$

9a ⟶ 9b: $R_1 = R_2 = H$; $R_3 = COOH$

5c ⟶ 9c: $R_1 = H$; $R_2 = Ac$; $R_3 = COSCH_2Ph$

9c ⟶ 9d: $R_1 = BOC$; $R_2 = Ac$; $R_3 = COSCH_2Ph$

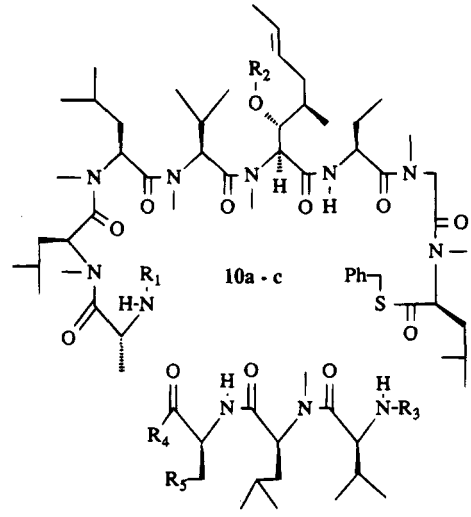
9d ⟶ 9e: $R_1 = BOC$; $R_2 = Ac$; $R_3 = CHO$

9e ⟶ 9f: $R_1 = BOC$; $R_2 = Ac$; $R_3 = CH{=}CHCOOCH_3$

9f ⟶ 9g: $R_1 = H$; $R_2 = Ac$; $R_3 = CH{=}CHCOOCH_3$

9g ⟶ 9h: $R_1 = R_2 = H$; $R_3 = CH{=}CHCOOH$

Scheme 5. Fragmentation



11a - d

5a ⟶ 10a: $R_1 = H$; $R_2 = Ac$

10a ⟶ 10b: $R_1 = BOC$; $R_2 = Ac$

6a ⟶ 10c: $R_1 = R_2 = H$

5a ⟶ 11a: $R_3 = H$; $R_4 = SCH_2Ph$; $R_5 = H$

11a: ⟶ 11b: $R_3 = BOC$; $R_4 = SCH_2Ph$; $R_5 = H$

12b ⟶ 11c: $R_3 = BOC$; $R_4 = OCH_2Ph$; $R_5 = Ph$

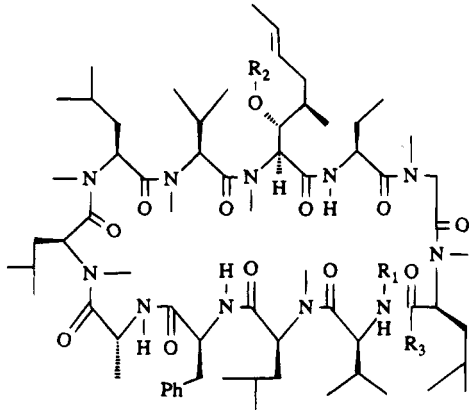
11c ⟶ 11d: $R_3 = BOC$; $R_4 = OH$; $R_5 = Ph$

evidenced from its NMR spectrum. When the 4,5-seco-cyclosporin **9b** was treated with (benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) in methylene chloride, the cyclization to **1a** was accomplished in 33% yield of crystalline product. It was found to be identical in every respect with cyclosporin A. This represents the first example of a linear undecapeptide being cyclized to a cyclosporin between amino acids 4 and 5.

Next, a fully protected 4,5-seco-cyclosporin was prepared starting from **3c**. The sulfur of this thioamide was benzylated in a two-phase system in the presence of 30% aqueous sodium hydroxide to give *S*-benzyl amidate **5c** in 78% yield. The NMR spectra of this compound indicated the presence of double bond isomers associated with the thioamidate. The ring opening occurred readily in the presence of 6 N hydrochloric acid yielding the novel linear undecapeptide **9c**. We did not encounter undue losses due to hydrolysis of either of the two ester groups. No migratory tendency of the acetate protecting group toward the free amino group was detected. The amino group was treated with BOC anhydride to give the fully protected 4,5-seco-cyclosporin **9d**. This was reduced with triethylsilane in the presence of $Pd(OAc)_2$ to give the aldehyde **9e**. Treatment with methyl (triphenylphosphoranylidene)acetate led to the fully protected unsaturated peptide **9f**. The amino group was liberated with trifluoroacetic acid to give **9g**. The completely deprotected undecapeptide **9h** was isolated following hydrolysis under basic conditions. Cyclization of **9h** led to the cyclosporin **8b**. In this case, the unsaturation is located on amino acid 4, the *N*-methyl-(*S*)-leucine having been substituted by a vinylogous *N*-methyl-(*S*)-leucine.

From our reactions of acetylcyclosporin A (**2**) with Lawesson's reagent, the acetyl 4,7-dithioamide **3a** was the product most readily available. It could be prepared in yields approaching 50%, depending upon the ratio between Lawesson's reagent and cyclosporin. The *O*-acetyl 4,7-dithioamide **3a** was benzylated to give the dibenzyl 4,7-bis(thioamidate) **5a**. When this compound was added to an excess of hydrochloric acid, this resulted in a two-tier hydrolytic cleavage with concomitant fragmentation of cyclosporin A into the octapeptide **10a** and the tripeptide **11a** (see Scheme 5). Both these fragments featured unprotected amino termini and thioacid termini protected as *S*-benzyl esters, respectively. The tripeptide **11a** proved to be quite unstable and could not be characterized satisfactorily. Therefore, the products of the fragmentation reaction of **5a** were treated *in situ* with BOC anhydride, following neutralization of the acidic reaction medium with sodium hydroxide. In addition, the BOC-protected octapeptide **10b** and the now stable BOC-protected tripeptide **11b** exhibited greater differences in their polarities facilitating their separation. This was easily accomplished by a filtration through a short silica gel column. However, since we were primarily interested in the octapeptide **10a**, the fragmentation products were usually separated as the free amines. The octapeptide **10a** was then coupled with a tripeptide, e.g. **11d**. This *N*-BOC protected tripeptide was prepared employing conventional techniques (see Experimental Section). The (*S*)-phenylalanine containing linear undecapeptide **9i** (see Scheme 6) was isolated in 47% yield and deprotected stepwise to **9j** in 92% then to **9k** in 70% yield. The latter was cyclized in the presence of commercial (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) to the novel [(*S*)-phenylalanine]^7-cyclosporin (**1c**) in 22% yield.

Scheme 6. 4,5-Secocyclosporins



9i - k

10a + 11d ⟶ 9i: R_1 = BOC; R_2 = Ac; R_3 = SCH_2Ph

9i ⟶ 9j: R_1 = H; R_2 = Ac; R_3 = SCH_2Ph

9j ⟶ 9k: R_1 = R_2 = H; R_3 = OH

Experimental Section

General. Thin layer chromatography (TLC) plates were developed in ethyl acetate saturated with water. High-pressure liquid chromatography (HPLC) analyses were carried out using a RP-18 reversed-phase column at 75 °C. The spectra were monitored at 204 nm. The mobile phase consisted of aqueous acetonitrile with the amount of water varying between 15 and 40%. In addition, the aqueous phase contained 1 mL of 85% phosphoric acid per 3.7 L. Unless listed otherwise nuclear magnetic resonance (NMR) spectra were measured in deuterated chloroform solution on a 360-MHz spectrometer with TMS as reference. The assignments of most of the signals are tentative and based on the chemical shifts observed for the corresponding signals of cyclosporin A. For the complete spectra of cyclosporin A (**1a**) see ref 22. Column chromatography was carried out on silica gel columns with water-saturated diethyl ether or as specified, on RP-18 reversed phase columns with methanol/water 88:12.

Cyclosporin A (1a) from 4,5-Secocyclosporin (9b). A mixture of **9b** (110 mg, 0.09 mmol), 4-(dimethylamino)pyridine (500 mg, 4 mmol), (benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluorophosphate (BOP) (220 mg, 0.5 mmol) in methylene chloride (100 mL) was kept at room temperature overnight. Ether was added and washed sequentially with water, 2 N HCl solution, saturated Na_2CO_3 solution, and water. The organic phase was dried over magnesium sulfate and evaporated. The crude product (80 mg) was chromatographed on silica gel to give the pure product (36 mg), which was identical in every respect (NMR, IR, mass spectrum, mp, mixed mp, TLC, HPLC) with authentic material: yield 33%.

[(*R*)-Phenylalanine]^8-cyclosporin (1b). A mixture of 7,8-seco-[(*R*)-phenylalanine]^8-cyclosporin (**7h**) (1.4 g, 1.08 mmol), 4-(dimethylamino)pyridine (5 g, 41 mmol) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, 2.5 g, 5.6 mmol) in methylene chloride (800 mL) was kept at room temperature overnight. Ether was added and washed sequentially with water, 1 N HCl solution, water, and $NaHCO_3$ solution. The organic phase was dried over magnesium sulfate and evaporated. The crude product (1.0 g) was chromatographed on silica gel to give pure product (360 mg): yield 29%. A sample was crystallized from ethyl acetate/hexane: mp 136–139 °C; *m/z* calcd for $C_{68}H_{115}N_{11}O_{12}$ 1277.8, found 1278.7 $[MH]^+$; $[\alpha_D]$ = −170.2° (*c* = 0.455 in MeOH); NMR δ 1.33, 1.62, 2.66, 2.67, 2.96, 3.09, 3.25, 3.39, 2.9–3.0, 3.46, 7.07, 7.15–7.30, 7.48, 7.72, 8.17.

[(S)-Phenylalanine]^7-cyclosporin (1c). A mixture of 4,5-seco-[(*S*)-phenylalanine]^7-cyclosporin (**9k**) (360 mg, 0.28 mmol), 4-(dimethylamino)pyridine (1.5 g, 12.6 mmol), and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP, 620 mg, 1.4 mmol) in methylene chloride (400 mL) was kept at room temperature overnight. Ether was added and washed sequentially with water, 2 N HCl solution, water, and $NaHCO_3$ solution. The organic phase was dried over magnesium sulfate and evaporated. The crude product (450 mg) was chromatographed on silica gel to give pure product (80 mg): yield 22%; *m/z* calcd for $C_{68}H_{115}N_{11}O_{12}$ 1277.7, found 1278.9 $[MH]^+$; $[\alpha_D]$ = −149.2° (*c* = 0.250 in MeOH); NMR δ 0.62, 0.73, 1.23, 1.62, 2.72, 2.74, 3.09, 3.13, 3.16, 3.38, 2.7–3.0, 3.4–3.5, 3.53, 7.07, 7.10–7.30, 7.57, 7.65, 7.87.

Acetylcyclosporin Thioamides 3a–c. A solution of acetylcyclosporin[21] (**2**) (180.0 g, 0.145 mol) in xylene (1.8 L) was heated to 130 °C internal temperature. To the clear solution, Lawesson's reagent[17] (35.0 g, 0.087 mol) was added in small portions. After 30 min at 130 °C, the reaction mixture was cooled to room temperature and evaporated under reduced pressure. The residue was dissolved in methylene chloride and chromatographed as described for the preparation of the diacetyl-[D-serine]^8-cyclosporin thioamides.[19]

Acetylcyclosporin 4,7-Bis(thioamide) 3a. Crystalline product (63 g): yield 34%; mp 226–228 °C; *m/z* calcd for $C_{64}H_{113}N_{11}O_{11}S_2$ 1275.7, found 1276.6 $[MH]^+$; $[\alpha_D]$ = −242.7° (*c* = 0.261 in MeOH); NMR δ 1.39, 1.56, 1.59, 2.0, 2.65, 2.68, 3.01, 3.24, 3.28, 3.38, 3.45, 5.40–5.60, 7.98, 8.48, 8.76, 9.28.

Acetylcyclosporin 7-Thioamide 3b. Crystalline product (18.8 g): yield 10.3%; mp 224–225 °C; *m/z* calcd for $C_{64}H_{113}N_{11}O_{12}S$ 1259.9, found 1260.6 $[MH]^+$; $[\alpha_D]$ = −211.7° (*c* = 0.286 in MeOH); NMR δ 1.38, 1.56, 1.59, 2.00, 2.65, 2.68, 3.08, 3.23, 3.24, 3.28, 3.45, 5.40–5.60, 7.49, 7.95, 8.47, 9.17.

Acetylcyclosporin 4-Thioamide 3c. Crystalline product (6.0 g): yield 3.6%; mp 219–220 °C; *m/z* calcd for $C_{64}H_{113}N_{11}O_{12}S$ 1259.9, found 1260.6 $[MH]^+$; $[\alpha_D]$ = −293.7° (*c* = 0.317 in MeOH); NMR δ 1.27, 1.33, 1.61, 2.00, 2.66, 2.67, 3.02, 3.21, 3.26, 3.38, 3.45, 5.45–5.60, 7.51, 8.01, 8.52, 8.72.

Cyclosporin 4,7-Bis(thioamide) 4a. To a solution prepared by dissolving sodium (1.0 g, 45 mmol) in methanol (35 mL) was added a solution of **3a** (6.37 g, 5 mmol) in methanol (75 mL). The mixture was kept at room temperature for 2.5 h. Acetic acid was added to adjust to pH 7. Then, *tert*-butyl methyl ether was added (150 mL) and washed with water and brine. The organic phase was dried over $MgSO_4$ and evaporated. The crude product (6.6 g) was chromatographed on silica gel with water saturated *tert*-butyl methyl ether to give the pure product (5.5 g): yield 92%. A sample was crystallized from ether/hexane to give a crystalline product: mp 139 °C dec; *m/z* calcd for $C_{62}H_{111}N_{11}O_{10}S_2$ 1233.8, found 1234.4 $[MH]^+$; $[\alpha_D]$ = −201.8° (*c* = 0.280 in MeOH); $[\alpha_D]$ = −233.3° (*c* = 0.392 in $CHCl_3$); lit.[18] $[\alpha_D]$ = −235.7° (*c* = 1.06 in $CHCl_3$); NMR identical with NMR of authentic material.

Cyclosporin 7-Thioamide 4b. The acetoxy compound **3b** was hydrolyzed as described above to give the known compound **4b**: yield 80%; mp 140 °C dec; *m/z* calcd for $C_{62}H_{111}N_{11}O_{11}S$ 1217.8, found 1218.8 $[MH]^+$; $[\alpha_D]$ = −165.6° (*c* = 0.471 in MeOH); $[\alpha_D]$ = −195.6° (*c* = 0.439 in $CHCl_3$); lit.[18] $[\alpha_D]$ = −192.3° (*c* = 0.30 in $CHCl_3$); NMR identical with NMR of authentic material.

Cyclosporin 4-Thioamide 4c. The acetoxy compound **3c** was hydrolyzed as described above to give the known compound **4b**: yield 83%; mp 142 °C dec; *m/z* calcd for $C_{62}H_{111}N_{11}O_{11}S$ 1217.8, found 1218.8 $[MH]^+$; $[\alpha_D]$ = −220.0° (*c* = 0.23 in MeOH); $[\alpha_D]$ = −280.4° (*c* = 0.314 in $CHCl_3$); lit.[18] $[\alpha_D]$ = −284.8° (*c* = 1.22 in $CHCl_3$); NMR identical with NMR of authentic material.

Acetylcyclosporin 4,7-Bis(benzyl thioamidate) 5a. A mixture of acetyl 4,7-dithioamide **3a** (50.0 g, 39 mmol) and benzyl bromide (50 mL, 422 mmol) in methylene chloride (500 mL) was stirred vigorously during 30 min, together with 30% NaOH (125 mL). Ether was added and washed thoroughly with water, dried over magnesium sulfate, and evaporated to give the crude product (125.1 g). This was purified on silica gel to give the pure product (30.6 g): yield 54%; *m/z* calcd for $C_{78}H_{125}N_{11}O_{11}S_2$ 1455.7, found 1456.9 $[MH]^+$; $[\alpha_D]$ = −159.9° (*c* = 0.294 in MeOH); NMR δ 1.98, 2.65, 2.83, 2.85, 3.03, 3.10, 3.11, 6.80, 7.15–7.40, 7.48; NMR (DMSO-d_6 at 180 °C) δ 1.19, 1.23, 1.58, 1.94, 2.77, 2.85, 2.95, 2.97, 3.06, 4.23, 4.25, 5.00, 6.85, 7.05, 7.20–7.40.

Acetylcyclosporin 7-(Benzyl thioamidate) 5b. A solution of **3b** (5.0 g, 4 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 6 mL, 40 mmol) and benzyl bromide (3.8 mL, 32 mmol) in methylene chloride (100 mL) was kept at room temperature for 1 h. Then, *tert*-butyl methyl ether was added, washed three times with water, dried over magnesium sulfate, and evaporated. The crude product (6.1 g) was chromatographed on silica gel with water-saturated ether to give the product (4.4 g): yield 81.5%; *m/z* calcd for $C_{71}H_{119}N_{11}O_{12}S$ 1349.7, found 1350.5 $[MH]^+$; $[\alpha_D] = -125.8°$ (c = 0.240 in MeOH); NMR (DMSO-d_6 at 180 °C) δ 1.15, 1.20, 1.59, 1.94, 2.72, 2.88, 2.90, 2.95, 2.97, 3.03, 4.32, 7.25–7.45.

Acetylcyclosporin 4-(Benzyl thioamidate) 5c. A mixture of **3c** (2.55 g, 2 mmol) and benzyl bromide (2 mL, 17 mmol) in methylene chloride (50 mL) was stirred vigorously for 30 min in the presence of a 30% NaOH solution (5 mL). The reaction was worked up the usual way with ether to give the crude product (5.6 g), which was chromatographed on silica gel to give the pure product (2.01 g): yield 74%; *m/z* calcd for $C_{71}H_{119}N_{11}O_{12}S$ 1349.7, found 1350.7 $[MH]^+$; $[\alpha_D] = -212.7°$ (c = 0.455 in MeOH); NMR δ 1.98, 2.80, 2.83, 2.98, 3.06, 3.10, 3.12, 3.17; NMR (DMSO-d_6 at 180 °C) δ 1.95, 2.79, 2.88, 2.96, 2.98, 3.00, 3.03, 4.18, 4.33, 5.00, 6.71, 7.04, 7.20, 7.2–7.4.

Cyclosporin 4,7-Bis(benzyl thioamidate) 6a. A mixture of **4a** (1.5 g, 1.2 mmol) and benzyl bromide (1.5 mL, 13 mmol) in methylene chloride (50 mL) was stirred vigorously in the presence of 30% NaOH (15 mL) for 30 min. The usual workup in ether gave the crude product (3.0 g) which was purified on silica gel to give the pure product (1.0 g): yield 59%; *m/z* calcd for $C_{76}H_{123}N_{11}O_{10}S_2$ 1413.7, found 1414.9 $[MH]^+$; $[\alpha_D] = -146.2°$ (c = 0.281 in MeOH); NMR (DMSO-d_6 at 180 °C) δ 0.74, 1.18, 1.22, 1.57, 2.8–3.1, 4.25, 5.02, 6.7–7.4.

Cyclosporin 7-(Benzyl thioamidate) 6b. A mixture of **4b** (6.0 g, 5.0 mmol) and benzyl bromide (5 mL, 43 mmol) in methylene chloride (100 mL) was stirred vigorously in the presence of 30% NaOH (25 mL) for 30 min. The usual workup in ether (250 mL) gave the crude product which was purified on silica gel with ethyl acetate/hexane 3:1 to give the pure product (5.1 g): yield 78%; *m/z* calcd for $C_{69}H_{117}N_{11}O_{11}S$ 1307.7, found 1308.8 $[MH]^+$; $[\alpha_D] = -114.3°$ (c = 0.463 in MeOH); NMR (DMSO-d_6 at 180 °C) δ 0.75, 1.16, 1.20, 1.60, 2.72–3.10, 4.30, 6.7–7.1, 7.2–7.4.

Cyclosporin 4-(Benzyl thioamidate) 6c. A mixture of **4c** (800 mg, 0.66 mmol) and benzyl bromide (1 mL, 8 mmol) in methylene chloride (50 mL) was stirred vigorously in the presence of 30% NaOH (10 mL) for 30 min. The usual workup in ether (100 mL) gave the crude product which was purified on silica gel with ethyl acetate/hexane 4:1 to give the pure product (430 mg): yield 50%; *m/z* calcd for $C_{69}H_{117}N_{11}O_{11}S$ 1307.7, found 1308.7 $[MH]^+$; $[\alpha_D] = -191.7°$ (c = 0.372 in MeOH); NMR (DMSO-d_6 at 180 °C) δ 0.74, 1.20, 1.22, 1.58, 2.72–3.10 [7 NCH_3], 4.21, 4.30–4.40, 5.07, 6.7, 7.0–7.1, 7.2–7.4.

***O*-Acetyl-7,8-secocyclosporin-7-thiocarboxylic Acid *S*-Benzyl Ester (7a).** The solution of *S*-benzylimino ester **5b** (3.95 g, 2.9 mmol) in acetonitrile (80 mL) was added during 2 min to a mixture of 6 N HCl (20 mL) in acetonitrile (80 mL) and stirred at room temperature for 20 min. The solution was adjusted to pH 8 and extracted with *tert*-butyl methyl ether (70 mL). The organic phase was dried over magnesium sulfate and evaporated to dryness to give the crude seco compound (4.6 g). This was chromatographed on silica gel with *tert*-butyl methyl ether/methanol 9:1 to give the pure product (2.9 g): yield 73%; *m/z* calcd for $C_{71}H_{121}N_{11}O_{13}S$ 1367.7, found 1368.5 $[MH]^+$, 664 [M − Abu-Sar-MeLeu-Val-MeLeu-Ala-SCH_2Ph]$^+$ = [439.1 + Ac-MeBmt]$^+$, 604.2 [664 − AcOH]$^+$, 439.1 [326.0 + MeVal]$^+$, 326.0 [Ala-MeLeu-MeLeu]$^+$; $[\alpha_D] = -144.2°$ (c = 0.260 in MeOH); NMR δ 0.77, 1.26, 1.34, 2.00, 2.94, 2.97, 2.98, 3.02, 3.05, 3.09, 3.17, 4.10, 7.20–7.35.

7,8-Secocyclosporin-7-thiocarboxylic Acid *S*-Benzyl Ester (7b). The solution of *S*-benzylimino ester **6b** (900 mg, 0.7 mmol) in acetonitrile (25 mL) was added to a mixture of 2 N HCl (15 mL) in acetonitrile (25 mL) and stirred at room temperature for 20 min. The solution was extracted with *tert*-butyl methyl ether (70 mL). The organic phase was dried over magnesium sulfate and evaporated to dryness to give the crude seco compound (540 mg). This was chromatographed on silica gel with *tert*-butyl methyl ether/methanol 85:15 to give the pure product (450 mg): yield 49%; *m/z* calcd for $C_{69}H_{119}N_{11}O_{12}S$ 1325.7, found 1326.9 $[MH]^+$, 1202.8 [M − $C_6H_5CH_2S$]$^+$, 1131.8 [778.5 + MeLeu-Val-MeLeu]$^+$, 778.5 [439.3 + MeBmt-Abu-Sar]$^+$, 604.5 [439.3 + MeBmt − HOH]$^+$, 439.3 [326.2 + MeVal]$^+$, 326.2 [Ala-MeLeu-MeLeu]$^+$; $[\alpha_D] = -174.2°$ (c = 0.884 in MeOH); NMR δ 0.54, 0.77, 1.26, 1.31, 1.61, 3.00, 3.03, 3.08, 3.17, 3.32, 3.45, 4.08, 7.15, 7.2–7.4, 7.30.

7,8-Secocyclosporin-7-carboxylic Acid[6] (7c). A solution of **7a** (2.9 g, 2.1 mmol) in methanol (10 mL) was added to a solution prepared by dissolving sodium (330 mg) in methanol (100 mL). After 3 h at room temperature, water (20 mL) was added followed by acetic acid (10 mL). Methanol was evaporated and replaced by methylene chloride and washed with water. The organic phase was dried over magnesium sulfate and evaporated to give the crude product (3.7 g). This was chromatographed on silica gel with *tert*-butyl methyl ether/MeOH/NH_4OH (80:20:5) to give the pure product (2.0 g): yield 78%; *m/z* calcd for $C_{62}H_{113}N_{11}O_{13}$ 1219.8, found 1220.7 $[MH]^+$, 622 [M − 597]$^+$ (loss of Abu-Sar-MeLeu-Val-MeLeu-Ala-OH) = [439 + MeBmt]$^+$, 604.4 [622 − HOH]$^+$, 439.3 [326 + MeVal]$^+$, 326.2 [Ala-MeLeu-MeLeu]$^+$; $[\alpha_D] = -161.6°$ (c = 0.580 in MeOH); NMR (DMSO-d_6 at 180 °C) δ 0.77, 1.13, 1.28, 2.90, 2.93, 2.94, 2.96, 2.98, 3.78–3.82, 3.93–3.98, 4.61–4.68, 5.18, 6.9–7.1. The same product was also obtained when **7b** was hydrolyzed under similar conditions.

***O*-Acetyl-*N*-(phenylthiocarbamoyl)-7,8-secocyclosporin-7-thiocarboxylic Acid *S*-Benzyl Ester (7d).** The solution of **5b** (13.8 g, 10 mmol) in acetonitrile (150 mL) was added slowly to a mixture of 6 N HCl (80 mL) and acetonitrile (200 mL). After 30 min it was adjusted to pH 7 with carbonate solution, and then commercial phenyl isothiocyanate (14 mL, 117 mmol) was added. After 1 h, ether (300 mL) was added, separated, dried over magnesium sulfate, and evaporated. The crude product was chromatographed on silica gel to give pure product (13 g): yield 87%; *m/z* calcd for $C_{78}H_{126}N_{12}O_{13}S_2$ 1502.8, found 1504.1 $[MH]^+$, 1309.0 [M − Ala-SCH_2Ph]$^+$ = [1082.9 + Val-MeLeu]$^+$, 1297 [M − PhNHC(=S)-Ala]$^+$, 1170.9 [M − 334]$^+$, 1082.9 [955.8 + MeLeu]$^+$, 1043 [M − 461]$^+$, 955.8 [799.7 + Abu-Sar]$^+$, 799.7 [574.5 + Ac-MeBmt]$^+$, 739.6 [799.7 − AcOH]$^+$, 574.5 [461.4 + MeVal]$^+$, 461.4 [334.3 + MeLeu]$^+$, 334.3 [PhNHC(=S)-Ala-MeLeu]$^+$; $[\alpha_D] = -170.7°$ (c = 0.420 in MeOH); NMR (DMSO-d_6 at 180 °C) δ 0.77, 1.23, 1.31, 1.60, 1.94, 2.85–3.05, 4.08, 5.10, 6.9–7.5.

Decapeptide 7e. A solution of **7d** (10.0 g, 6.7 mmol) in acetonitrile (50 mL) was added dropwise during 5 min to a mixture of acetonitrile (100 mL) and 6 N HCl solution (50 mL). After 1 h at room temperature the pH was adjusted to neutral with 2 N Na_2CO_3. Then, *tert*-butyl methyl ether was added and washed with water then with brine, dried over magnesium sulfate, and evaporated to give the crude product (10 g). This was chromatographed on silica gel with water saturated ethyl acetate to give 6.4 g of the product: yield 75%; *m/z* calcd for $C_{68}H_{116}N_{10}O_{12}S$ 1296.7, found 1297.5 $[MH]^+$, 1102.5 [M − Ala-SCH_2Ph]$^+$ = [975.5 + MeLeu]$^+$, 975.5 [876.5 + Val], 876.5 [749.5 + MeLeu]$^+$, 749.5 [368.4 + AcMeBmt-Abu-Sar]$^+$, 533.5 [368.4 + AcMeBmt − HOAc]$^+$, 368.4 [H-MeLeu-MeLeu-MeVal]$^+$; $[\alpha_D] = -138.6°$ (c = 0.220 in MeOH); NMR (DMSO-d_6 at 180 °C) δ 0.77, 1.32, 1.61, 1.96, 2.27, 2.90, 2.97, 3.00, 3.48, 4.09, 4.45–4.55, 4.55–4.65, 4.65–4.70, 4.75–4.85, 4.90–5.00, 5.12, 5.56, 6.92, 7.01, 7.15–7.35, 7.46.

***O*-Acetyl-*N*-BOC-7,8-seco-[(*R*)-phenylalanine][8]-cyclosporin-7-thiocarboxylic Acid *S*-Benzyl Ester (7f).** A mixture of the decapeptide **7e** (2.0 g, 1.5 mmol), commercial *N*-BOC-(*R*)-phenylalanine (1 g, 3.75 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC, 750 mg, 3.9 mmol) in methylene chloride (50 mL) was kept at room temperature overnight. The solvent was diluted with *tert*-butyl methyl ether, washed with water and brine, dried over magnesium sulfate, and evaporated. The crude (3.1 g) was chromatographed on silica gel to give pure product (1.5 g): yield 75%; *m/z* calcd for $C_{82}H_{133}N_{11}O_{15}S$ 1543.7, found 1566.9 [M + Na]$^+$, 1544.8 $[MH]^+$, 1445.7 [MH − BOC]$^+$, 1349.7 [M − Ala-SCH_2Ph]$^+$ = [996.6 + MeLeu-Val-MeLeu]$^+$, 996.6 [840.4 + Abu-Sar]$^+$, 840.4 [615.4 + Ac-MeBmt]$^+$, 780.5 [840.4 −

HOAc]$^+$, 615.4 [502.3 + MeVal]$^+$, 502.3 [375.2 + MeLeu]$^+$, 375.2 [BOC-Phe-MeLeu]$^+$; $[\alpha_D] = -163.8°$ ($c = 0.210$ in MeOH); NMR (DMSO-d_6 at 180 °C) δ 0.76, 1.31, 1.34, 1.93, 2.83, 2.88, 2.91, 2.92, 2.93, 2.97, 4.08, 5.10, 5.85, 6.85, 6.95, 7.15–7.30, 7.40.

O-Acetyl-7,8-seco-[(*R*)-phenylalanine]^8-cyclosporin-7-thiocarboxylic Acid *S*-Benzyl Ester (7g). A cold solution of **7f** (800 mg, 0.52 mmol) and trifluoroacetic acid (4 mL) in methylene chloride (20 mL) was kept at rt for 1.5 h after which time 2 N Na_2CO_3 was added. Then, *tert*-butyl methyl ether was added, washed with water and brine, dried over $MgSO_4$, and evaporated. The crude (850 mg) was chromatographed with ethyl acetate to give 660 mg of pure product: yield 88%; *m/z* calcd for $C_{77}H_{125}N_{11}O_{13}S$ 1443.7, found 1444.2 [MH]$^+$, 1023 [M – Val-MeLeu-Ala-SBz]$^+$ = [896 + MeLeu]$^+$, 896 [515.5 + AcMeBmt-Abu-Sar]$^+$, 680.5 [515.5 + AcMeBmt – HOAc]$^+$, 515.5 [402.4 + MeVal]$^+$, 402.4 [275.3 + MeLeu]$^+$, 275.3 [H-Phe-MeLeu]$^+$; $[\alpha_D] = -163.0°$ ($c = 0.215$ in MeOH); NMR δ 0.76, 1.34, 1.62, 2.00, 2.82, 2.94, 2.95, 3.02, 3.03, 3.09, 3.17, 4.10, 6.75, 6.95, 7.1–7.4.

7,8-Seco-[(*R*)-phenylalanine]^8-cyclosporin (7h). A solution of **7g** (1.4 g, 1 mmol) and 2 N NaOH (20 mL) in methanol (50 mL) was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure, methylene chloride was added, washed with water, 1 N HCl solution, water, and brine, and dried over magnesium sulfate and evaporated to give the product (1.2 g): yield 78%; *m/z* calcd for $C_{68}H_{117}N_{11}O_{13}$ 1295.8, found 1296.9 [MH]$^+$, 698.5 [515.4 + MeBmt]$^+$, 680.5 [698.5 – HOH]$^+$, 515.4 [402.3 + MeVal]$^+$, 402.3 [275.2 + MeLeu]$^+$, 275.2 [H-Phe-MeLeu]$^+$; $[\alpha_D] = -140.7°$ ($c = 0.348$ in MeOH); NMR (DMSO-d_6 at 180 °C) δ 0.76, 1.28, 1.60, 2.81, 2.88, 2.90, 2.93, 2.98, 3.05, 2.8–3.1, 3.91, 4.60, 4.65–4.75, 5.13, 6.85–7.05, 7.15–7.30.

***N*-BOC-7,8-secocyclosporin-7-thiocarboxylic Acid *S*-Benzyl Ester (7i).** The ring opening reaction was carried out as described for the preparation of **7b** starting from **6b** (6.8 g, 5.2 mmol). The solution of crude **7b** in aqueous acetonitrile, however, was neutralized with 2 N Na_2CO_3 and treated with commercial di-*tert*-butyl dicarbonate (BOC anhydride, 7 g, 32 mmol). After 2 h, the reaction mixture was extracted with ether. The organic phase was separated, washed first with saturated carbonate solution and then with brine and dried over $MgSO_4$ to give the crude product (12 g). This was chromatographed on silica gel with ether/hexane 3:1 to give the pure product (4.1 g): yield 55%; *m/z* calcd for $C_{74}H_{127}N_{11}O_{14}S$ 1425.8 found 1449.0 [M + Na]$^+$, 1425.7 [M]$^+$, 1231.3 [M – Ala-SCH_2Ph]$^+$ = [878.1 + MeLeu-Val-MeLeu]$^+$, 878.1 [722.1 + Abu-Sar]$^+$, 722.1 [539.0 + MeBmt]$^+$, 539.0 [426.0 + MeVal]$^+$, 426.0 [298.9 + MeLeu]$^+$, 298.9 [BOC-Ala-MeLeu]$^+$; $[\alpha_D] = -155.0°$ ($c = 0.340$ in MeOH); NMR δ 2.98, 3.00, 3.07, 3.15, 3.30, 3.43; NMR (DMSO-d_6 at 180 °C) δ 0.76, 1.20, 1.30, 1.38, 2.88, 2.92, 2.93, 2.94, 2.97, 3.05, 4.09, 5.12, 5.82, 6.9–7.1, 7.2–7.3, 7.4–7.5.

***N*-BOC-7,8-secocyclosporin-7-carboxaldehyde (7j).** Under an atmosphere of argon, a solution of **7i** (2.0 g, 1.4 mmol) and $Pd(OAc)_2$ (100 mg, 0.45 mmol) in acetone (50 mL) was treated with triethylsilane (3 mL, 19 mmol). After 10 min, the solution turned dark and the reaction was quenched with methanol. The solvent was evaporated and the residue was chromatographed on silica gel to give the aldehyde (1.1 g): yield 61%; *m/z* calcd for $C_{67}H_{121}N_{11}O_{14}$ 1303.7, found 1326.7 [M + Na]$^+$, 1304.8 [MH]$^+$, 1231.7 [M – Ala-CH=O]$^+$ = [878 + MeLeu-Val-MeLeu]$^+$, 878 [722.2 + Abu-Sar]$^+$, 722.2 [539.2 + MeBmt]$^+$, 539.2 [426.1 + MeVal]$^+$, 426.1 [299.0 + MeLeu]$^+$, 299.0 [BOC-Ala-MeLeu]$^+$; $[\alpha_D] = -160.0°$ ($c = 0.095$ in MeOH); NMR (DMSO-d_6 at 180 °C) δ 0.76, 1.19, 1.20, 1.38, 2.88, 2.92, 2.93, 2.94, 2.97, 3.05, 5.12, 5.82, 6.91, 7.04, 7.37, 9.44.

***N*-BOC-7,8-secocyclosporin-7-acrylic Acid Methyl Ester (7k).** A mixture of **7j** (900 mg, 0.69 mmol) and commercial methyl (triphenylphosphoranylidene)acetate (340 mg, 1.0 mmol) in toluene (50 mL) was heated to 100 °C for 1 h. The mixture was diluted with ether and washed with water. The solvent was evaporated and the residue was chromatographed on silica gel to give the pure product (790 mg): yield 84%; *m/z* calcd for $C_{70}H_{125}N_{11}O_{15}$ 1359.7, found 1382.2 [M + Na]$^+$, 1360.2 [MH]$^+$, 1231.3 [M – NHC_4H_6COOMe]$^+$ = [1005.1 + Val-MeLeu]$^+$, 1005.1 [878.0 + MeLeu]$^+$, 878.0 [722.0 + Abu-Sar]$^+$, 722.0 [539.0 + MeBmt]$^+$, 539.0 [426.0 + MeVal]$^+$, 426.0 [298.9 + MeLeu]$^+$, 298.9 [BOC-Ala-MeLeu]$^+$; $[\alpha_D] = -157.3°$ ($c = 0.295$ in MeOH); NMR (DMSO-d_6 at 180 °C) δ 0.75, 1.19, 1.20, 1.38, 2.88, 2.91, 2.92, 2.93, 2.97, 3.05, 3.65, 4.10–4.20, 4.30–4.38, 4.43–4.55, 4.61, 4.66–4.74, 4.80, 4.90, 5.00, 5.13, 5.78–5.85, 5.85, 6.80, 6.91, 7.03, 7.11.

7,8-Secocyclosporin-7-acrylic Acid Methyl Ester (7l). A mixture of **7k** (670 mg, 0.49 mmol), trifluoroacetic acid (5 mL), CH_2Cl_2 (25 mL), and water (1 mL) was stirred at room temperature for 1.5 h. It was slowly neutralized with saturated Na_2CO_3 solution (100 mL). Then, *tert*-butyl methyl ether was added. The organic phase was dried, evaporated, and chromatographed on silica gel to give the product (400 mg): yield 65%; *m/z* calcd for $C_{65}H_{117}N_{11}O_{13}$ 1259.8, found 1260.9 [MH]$^+$, 778.5 [M – MeLeu-Val-MeLeu-NHCH(Me)CH=CHCOOMe]$^+$ = [622 + Abu-Sar]$^+$, 622 [439.3 + MeBmt]$^+$, 604.4 [622 – HOH]$^+$, 439.3 [326.2 + MeVal]$^+$, 326.2 [H-Ala-MeLeu-MeLeu]$^+$; $[\alpha_D] = -163.6°$ ($c = 0.518$ in MeOH); NMR (DMSO-d_6 at 180 °C) δ 0.77, 1.13, 1.20, 1.60, 2.88, 2.92, 2.93, 2.94, 2.98, 3.07, 3.65, 3.73–3.81, 4.10–4.18, 4.28–4.37, 4.43–4.53, 4.60, 4.65–4.72, 4.79, 4.89, 4.99, 5.13, 5.70–5.80, 6.75–6.85, 6.88, 7.00, 7.09.

7,8-Secocyclosporin-7-acrylic Acid (7m). To a mixture of **7l** (400 mg, 0.32 mmol) and 2 N Na_2CO_3 (10 mL) in methanol (25 mL) was added water to obtain a clear solution. This was kept at room temperature overnight. The solution was adjusted to pH 4 with 2 N HCl solution. The methanol was evaporated under reduced pressure, methylene chloride was added, and the solution was dried over Na_2SO_4. The solution was filtered and evaporated to give the crude product (400 mg). This was chromatographed on silica gel to give pure product (320 mg): yield 81%; *m/z* calcd for $C_{64}H_{115}N_{11}O_{13}$ 1245.8, found 1246.9 [MH]$^+$, 622 [439.2 + MeBmt]$^+$, 604 [622 – HOH]$^+$, 439.2 [326.1 + MeVal]$^+$, 326.1 [H-Ala-MeLeu-MeLeu]$^+$.

[4(*S*)-Amino-2(*E*)-pentenoic Acid]^7-cyclosporin (8a). A solution of **7m** (320 mg, 0.26 mmol) in CH_2Cl_2 (100 mL) was treated with commercially available 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ, 1.09 g, 4.4 mmol) and kept at room temperature for 2 h. The solution was diluted with *tert*-butyl methyl ether, washed sequentially with water, 2 N HCl solution, water, and saturated $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4 and evaporated to yield 360 mg of crude product. This was chromatographed on silica gel to give 34 mg of pure product: yield 10%; mp 168–169 °C; *m/z* calcd for $C_{64}H_{113}N_{11}O_{12}$ 1227.7; found 1228.8 [MH]$^+$, 1115.7 [M – $C_7H_{12}O$]$^+$ (loss of MeBmt side chain); $[\alpha]_D = -169.8°$ ($c = 0.258$ in MeOH); NMR δ 0.68, 1.20, 1.30, 1.62, 2.66, 2.75, 3.08, 3.15, 3.21, 3.36, 3.39, 5.78–5.86, 6.41, 6.71, 7.23, 7.50, 7.87.

[4(*S*)-(Methylamino)-6-methyl-2(*E*)-heptenoic acid]^4-cyclosporin (8b). A solution of **9h** (130 mg, 0.1 mmol) in CH_2Cl_2 (15 mL) was treated with commercially available 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC, 130 mg, 0.68 mmol) and kept at room temperature for 2 h. The solution was diluted with *tert*-butyl methyl ether, washed sequentially with water, 2 N HCl solution, water, and saturated $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4 and evaporated to yield crude product (360 mg). This was chromatographed on silica gel to give pure product (12 mg): yield 10%; *m/z* calcd for $C_{64}H_{113}N_{11}O_{12}$ 1227.7; found 1228.3 [MH]$^+$; $[\alpha]_D = -150.7°$ ($c = 0.275$ in MeOH); NMR δ 0.53, 1.27, 1.32, 2.68, 2.75, 2.87, 3.10, 3.21, 3.40, 3.50, 6.12–6.20, 6.39, 7.05, 7.20, 7.74, 7.94.

4,5-Secocyclosporin-4-thiocarboxylic Acid *S*-Benzyl Ester (9a). A solution of **6c** (3.1 g, 2.2 mmol) in acetonitrile (25 mL) was added dropwise to a mixture of 6 N HCl (30 mL) and acetonitrile (50 mL) and stirred at room temperature for 30 min. The solution was adjusted to pH 8 by the addition of saturated Na_2CO_3 solution and extracted with ether. The organic phase was dried over magnesium sulfate and evaporated to dryness. The crude product (2.4 g) was chromatographed on silica gel with ether/methanol 7:1 to give pure product (1.5 g): yield 52%; *m/z* calcd for $C_{69}H_{119}N_{11}O_{12}S$ 1325.7, found 1326.9 [MH]$^+$, 1202.8 [M – *S*-benzyl]$^+$ = [1075.7 + MeLeu]$^+$, 1075.7 [1004 + Sar]$^+$, 1004 [919.6 + Abu]$^+$, 919.6

[736.5 + MeBmt]$^+$, 736.5 [623.3 + MeVal]$^+$, 623.3 [496.3 + MeLeu]$^+$, 496.3 [298 + Ala-MeLeu]$^+$, 298 [H-Val-MeLeu-Ala]$^+$, $[\alpha_D] = -163.2°$ ($c = 0.205$ in MeOH); NMR (DMSO-d_6 at 180 °C) δ 0.75, 0.83, 1.21, 1.60, 2.87, 2.88, 2.90, 2.91, 2.93, 2.95, 3.41, 4.11, 5.11, 6.9–6.95, 7.15–7.30, 7.30–7.4.

4,5-Secocyclosporin (9b). A mixture of benzyl ester **9a** (750 mg, 0.57 mmol) and 2 N NaOH solution (5 mL) in methanol (30 mL) was kept at room temperature for 2.5 h. It was acidified to pH 3 with 2 N HCl and extracted with CH_2Cl_2, washed with brine, dried over magnesium sulfate, and evaporated to dryness to give the crude product (810 mg). This was chromatographed on silica gel to give pure product (510 mg): yield 74%; *m/z* calcd for $C_{62}H_{113}N_{11}O_{13}$ 1219.8, found 1220.7 [MH]$^+$, 919.6 [M − Abu-Sar-MeLeu-OH] = [736.5 + MeBmt]$^+$, 901 [919.6 − HOH]$^+$, 736.5 [623.4 + MeVal]$^+$, 623.4 [496 + MeLeu]$^+$, 496.4 [H-Val-MeLeu-Ala-Ala-MeLeu]$^+$; $[\alpha_D] = -134.8°$ ($c = 0.508$ in MeOH); NMR (DMSO-d_6 at 150 °C) δ 0.74, 1.20, 1.22, 1.60, 2.83, 2.87, 2.89, 2.90, 2.92, 2.93, 2.94,3.90–3.95, 5.10, 6.95–7.05, 7.45–7.55.

***O*-Acetyl-4,5-secocyclosporin-4-thiocarboxylic Acid *S*-Benzyl Ester (9c).** A solution of **5c** (8.6 g, 6.3 mmol) in acetonitrile (100 mL) was added dropwise to a mixture of 6 N HCl (45 mL) and acetonitrile (80 mL) and stirred at room temperature for 30 min. The solution was adjusted to pH 8 by the addition of saturated Na_2CO_3 solution (150 mL) and extracted with *tert*-butyl methyl ether. The organic phase was dried over magnesium sulfate and evaporated to dryness. The crude product (10 g) was chromatographed on silica gel with *tert*-butyl methyl ether/methanol 10:1 to give pure product (4.3 g): yield 51%; *m/z* calcd for $C_{71}H_{121}N_{11}O_{13}S$ 1367.7, found 1368.6 [MH]$^+$, 1244.6 [M − SCH_2Ph]$^+$ = [1117.5 + MeLeu]$^+$, 1117.5 [1046.5 + Sar]$^+$, 1046.5 [961.4 + Abu]$^+$, 961.4 [736.3 + Ac-MeBmt]$^+$, 901.4 [961.4 − AcOH]$^+$, 736.3 [623.2 + MeVal]$^+$, 623.2 [496.2 + MeLeu]$^+$, 496.2 [369.1 + MeLeu]$^+$, 369.1 [298 + Ala]$^+$, 298 [H-Val-MeLeu-Ala]$^+$; $[\alpha_D] = -112.2°$ ($c = 0.270$ in MeOH); NMR (DMSO-d_6 at 180 °C) δ 1.22, 1.60, 1.95, 2.88, 2.90, 2.91, 2.93, 2.94, 2.96, 3.42, 4.11, 4.6–4.7, 4.7–4.8, 5.11, 6.92, 7.1–7.3, 7.32.

***O*-Acetyl-*N*-BOC-4,5-secocyclosporin-4-thiocarboxylic Acid *S*-Benzyl Ester (9d).** A solution of **9c** (4.3 g, 3.1 mmol) and triethylamine (4 mL, 28 mmol) in CH_2Cl_2 (120 mL) was treated with commercial di-*tert*-butyl dicarbonate (BOC anhydride, 4.3 g, 20 mmol). After 3 h, the reaction mixture was extracted with *tert*-butyl methyl ether. The organic phase was separated, washed sequentially with water, 2 N HCl solution, water, and brine, and dried over Mg_2SO_4 to give the crude product (8 g). This was chromatographed on silica gel with water saturated *tert*-butyl methyl ether to give the pure product (4.3 g): yield 94.5%; *m/z* calcd for $C_{76}H_{129}N_{11}O_{15}S$ 1467.7, found 1490.3 [M + Na]$^+$, 1468.8 [MH]$^+$, 1217.2 [M − MeLeu-SCH_2Ph]$^+$ = [1061.0 + Abu-Sar]$^+$, 1061.0 [836.1 + Ac-MeBmt]$^+$, 1001 [1061.0 − AcOH]$^+$, 836.1 [723.1 + MeVal]$^+$, 723.1 [596.0 + MeLeu]$^+$, 596.0 [469 + MeLeu]$^+$, 469 [327.0 + Ala-Ala]$^+$, 327.0 [BOC-Val-MeLeu]$^+$; $[\alpha_D] = -154.3°$ ($c = 0.290$ in MeOH); NMR (DMSO-d_6 at 180 °C) δ 1.21, 1.22, 1.40, 1.60, 1.93, 2.88, 2.90, 2.93, 2.94, 2.95, 4.11, 5.12, 5.60, 6.88, 6.95, 7.2–7.25, 7.28.

***O*-Acetyl-*N*-BOC-4,5-secocyclosporin-4-carboxaldehyde (9e).** Under an atmosphere of argon, a solution of **9d** (500 mg, 0.35 mmol) and $Pd(OAc)_2$ (50 mg) in acetone (30 mL) was treated with triethylsilane (0.7 mL, 4.4 mmol). After 1 min, the solution turned dark. After 10 min, the reaction was quenched with methanol. The solvent was evaporated and the residue was chromatographed on silica gel with water saturated *tert*-butyl methyl ether to give the pure product (400 mg): yield 87%; *m/z* calcd for $C_{69}H_{123}N_{11}O_{15}$ 1345.7, found 1368.7 [M + Na]$^+$, 1346.8 [MH]$^+$, 1217.6 [1061.4 + Abu-Sar]$^+$ = [M − 128]$^+$ (loss of $CH_3NC_5H_{10}CHO$), 1061.4 [836.4 + Ac-MeBmt]$^+$, 1001.5 [1061.4 − AcOH]$^+$, 836.4 [723.3 + MeVal]$^+$, 723.3 [596.2 + MeLeu]$^+$, 596.2 [469.2 + MeLeu]$^+$, 469.2 [327.0 + Ala-Ala]$^+$, 327.0 [BOC-Val-MeLeu]$^+$; $[\alpha_D] = -141.8°$ ($c = 0.190$ in MeOH); NMR (DMSO-d_6 at 180 °C) δ 0.77, 1.22, 1.23, 1.40, 1.61, 1.95, 2.90, 2.93, 2.95, 2.98, 5.10, 5.6–5.7, 6.90–7.05, 7.3–7.4, 9.50.

***O*-Acetyl-*N*-BOC-4,5-secocyclosporin-4-acrylic Acid Methyl Ester (9f).** A mixture of **9e** (700 mg, 0.52 mmol) and methyl (triphenylphosphoranylidene)acetate (300 mg, 0.9 mmol) in toluene (70 mL) was kept at room temperature for 1 h. The solvent was evaporated and the residue was chromatographed on silica gel to give the product (550 mg): yield 75%; *m/z* calcd for $C_{72}H_{127}N_{11}O_{16}$ 1401.7, found 1424.0 [M + Na]$^+$, 1403.0 [MH], 1217.7 [1061.0 + Abu-Sar]$^+$ = [M − 184]$^+$ (loss of $CH_3NC_7H_{12}COOMe$), 1061.0 [836.1 + Ac-MeBmt]$^+$, 1001.2 [1061.0 − AcOH]$^+$, 836.1 [723.1 + MeVal]$^+$, 723.1 [596.0 + MeLeu]$^+$, 596.0 [397.0 + Ala-MeLeu]$^+$, 397.0 [327.0 + Ala]$^+$, 327.0 [BOC-Val-MeLeu]$^+$; $[\alpha_D] = -143.8°$ ($c = 0.320$ in MeOH); NMR (DMSO-d_6 at 180 °C) δ 0.77, 1.21, 1.23, 1.40, 1.61, 1.95, 2.82, 2.90, 2.93, 2.94, 2.95, 2.98, 3.68, 4.68–4.75, 5.10, 5.60–5.70, 5.85, 6.74, 6.9–7.1, 7.3–7.4.

***O*-Acetyl-4,5-secocyclosporin-4-acrylic Acid Methyl Ester (9g).** A mixture of **9f** (250 mg, 0.18 mmol), trifluoroacetic acid (3 mL), CH_2Cl_2 (10 mL) and water (1 drop) was stirred at room temperature for 1.5 h. It was slowly neutralized with saturated Na_2CO_3 solution and extracted with *tert*-butyl methyl ether. The organic phase was dried, evaporated (250 mg), and chromatographed on silica gel to give the product (220 mg): yield 96%; *m/z* calcd for $C_{67}H_{119}N_{11}O_{14}$ 1301.7, found 1302.6 [MH]$^+$, 961 [736.3 + Ac-MeBmt]$^+$, 901 [961 − AcOH]$^+$, 736.3 [623.2 + MeVal]$^+$, 623.2 [496.0 + MeLeu]$^+$, 496.0 [H-Val-MeLeu-Ala-Ala-MeLeu]$^+$; $[\alpha_D] = -143.2°$ ($c = 0.235$ in MeOH); NMR (DMSO-d_6 at 180 °C) δ 0.79, 1.21, 1.22, 1.60, 1.93, 2.80, 2.88, 2.90, 2.93, 2.95, 2.97, 3.67, 4.68–4.78, 5.10, 5.85, 6.73, 6.85–6.95, 7.38–7.43.

4,5-Secocyclosporin-4-acrylic Acid (9h). A mixture of **9g** (220 mg, 0.17 mmol) and 2 N NaOH (7 mL) in methanol (10 mL) was kept at room temperature for 1 h. The solution was adjusted to pH 4 with 2 N HCl solution. Most of the methanol was evaporated under reduced pressure. The aqueous phase was extracted with methylene chloride, separated, and dried over Na_2SO_4. The solvent was evaporated to give the crude product (140 mg): yield 67%; *m/z* calcd for $C_{64}H_{115}N_{11}O_{13}$ 1245.7, found 1246.5 [MH]$^+$, 1076.4 [919.3 + Abu-Sar-H]$^+$, 919.3 [736.3 + MeBmt]$^+$, 901.3 [919.3 − HOH]$^+$, 736.3 [623.2 + MeVal]$^+$, 623.2 [496.2 + MeLeu]$^+$, 496.2 [H-Val-MeLeu-Ala-Ala-MeLeu]$^+$; $[\alpha_D] = -131.6°$ ($c = 0.220$ in MeOH).

***O*-Acetyl-*N*-BOC-4,5-seco-[(*S*)-phenylalanine]^7-cyclosporin-4-thiocarboxylic Acid *S*-Benzyl Ester (9i).** A mixture of octapeptide **10a** (5.0 g, 4.7 mmol), tripeptide **11d** (2.3 g, 4.7 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC, 1 g, 5.25 mmol) in CH_2Cl_2 (75 mL) was kept at room temperature for 2 h. The mixture was directedly chromatographed on silica gel to give pure undecapeptide (3.4 g): yield 47%; *m/z* calcd for $C_{82}H_{133}N_{11}O_{15}S$ 1543.7, found 1568.2 [M + Na]$^+$, 1544.8 [MH]$^+$, 1445.6 [MH − BOC + H]$^+$, 1293.9 [M − MeLeu-SCH_2Ph]$^+$ = [1137.7 + Abu-Sar]$^+$, 1137.7 [912.6 + Ac-MeBmt]$^+$, 1077.8 [1137.7 − AcOH]$^+$, 912.6 [799.6 + MeVal]$^+$, 799.6 [672.5 + MeLeu]$^+$, 672.5 [474 + Ala-MeLeu]$^+$, 474 [327 + Phe]$^+$, 327 [BOC-Val-MeLeu]$^+$, 271.2 [327 − C_4H_8]$^+$; $[\alpha_D] = -163.0°$ ($c = 0.260$ in MeOH); NMR δ 0.77, 1.11, 1.42, 1.63, 1.93, 2.87, 2.88, 2.96, 2.97, 3.08, 3.13, 4.10; NMR (DMSO-d_6 at 150 °C) δ 0.74, 1.14, 1.37, 1.60, 1.93, 2.75–3.05, 4.10, 7.05–7.55.

***O*-Acetyl-4,5-seco-[(*S*)-phenylalanine]^7-cyclosporin-4-thiocarboxylic Acid *S*-Benzyl Ester (9j).** The solution of **9i** (2.3 g, 1.5 mmol) in CH_2Cl_2 (100 mL) was treated with trifluoroacetic acid (10 mL) and kept at room temperature for 1.5 h. The aqueous phase was adjusted to pH 8 with 2 N Na_2CO_3 and extracted with *tert*-butyl methyl ether. The organic phase was washed with brine, dried over magnesium sulfate, and evaporated to leave the crude product (2.9 g) which was chromatographed with ether/MeOH 93:7 to give the pure product (2.0 g): yield 92%; *m/z* calcd for $C_{77}H_{125}N_{11}O_{13}S$ 1443.7, found 1444.7 [MH]$^+$, 1193.9 [M − MeLeu-SCH_2Ph]$^+$ = [1037.8 + Abu-Sar]$^+$, 1037.8 [812.6 + Ac-MeBmt]$^+$, 977.8 [1037.8 − AcOH]$^+$, 812.6 [699.5 + MeVal]$^+$, 699.5 [572.4 + MeLeu]$^+$, 572.4 [H-Val-MeLeu-Phe-Ala-MeLeu]$^+$; $[\alpha_D] = -123.9°$ ($c = 0.155$ in MeOH).

4,5-Seco-[(*S*)-phenylalanine]^7-cyclosporin (9k). A mixture of **9j** (2.0 g, 1.4 mmol) in methanol (80 mL) and 2 N NaOH (20 mL) was kept at room temperature overnight. Methylene chloride was added and washed first with 2 N HCl solution and then with water. The organic phase was dried over

magnesium sulfate and evaporated and chromatographed on a Rp-18 column with methanol/water 85:15 to give the product (1.27 g): yield 70%; *m/z* calcd for $C_{68}H_{117}N_{11}O_{13}$ 1295.8, found 1296.7 $[MH]^+$, 1278 $[MH - HOH]^+$, 995.5 [M − Abu-Sar-MeLeu-OH]$^+$ = [812.4 + MeBmt]$^+$, 812.4 [699.3 + MeVal]$^+$, 699.3 [572 + MeLeu]$^+$; $[\alpha_D] = -93.8°$ (*c* = 0.320 in MeOH).

Fragmentation of 5a to *O*-Acetyl Octapeptide 4-Thiocarboxylic Acid *S*-Benzyl Ester 10a and Tripeptide 7-Thiocarboxylic Acid *S*-Benzyl Ester 11a. A solution of **5a** (29.1 g, 20 mmol) in acetonitrile (200 mL) was added dropwise to a mixture of acetonitrile (250 mL) and 6 N HCl solution (100 mL). After stirring for 15 min the mixture was treated with 2 N Na_2CO_3 (400 mL). The mixture was extracted with *tert*-butyl methyl ether (500 mL), washed with brine, dried over magnesium sulfate, and evaporated. The crude mixture was chromatographed immediately on silica gel to give the less polar tripeptide **11a** (8 g) (not stable enough to be fully characterized) followed by the octapeptide **10a** (16 g).

***O*-Acetyl Octapeptide 4-Thiocarboxylic Acid *S*-Benzyl Ester 10a**: yield 75%; *m/z* calcd for $C_{56}H_{94}N_8O_{10}S$ 1070.6, found 1071.8 $[MH]^+$, 820.7 [439.6 + Ac-MeBmt-Abu-Sar]$^+$, 604.7 [439.6 + Ac-MeBmt − AcOH]$^+$, 439.6 [326.5 + MeVal]$^+$, 326.5 [H-Ala-MeLeu-MeLeu]$^+$; $[\alpha_D] = -170.8°$ (*c* = 0.255 in MeOH); NMR δ 0.78, 1.33, 1.62, 2.01, 2.87, 2.97, 2.99, 3.03, 3.09, 3.12, 3.55, 4.08, 4.75, 5.17, 6.7, 7.2−7.35.

Fragmentation of 5a to *O*-Acetyl-*N*-BOC Octapeptide 4-Thiocarboxylic Acid *S*-Benzyl Ester 10b and *N*-BOC-Val-MeLeu-Ala-thiocarboxylic Acid *S*-Benzyl Ester 11b. A solution of **5a** (2.2 g, 1.5 mmol) in acetonitrile (25 mL) was added dropwise to a mixture of acetonitrile (50 mL) and 6 N HCl solution (10 mL). After stirring for 15 min the mixture was treated with 2 N NaOH (40 mL). After 5 min, a solution of di-*tert*-butyl dicarbonate (BOC anhydride, 1.1 g, 5 mmol) in acetonitrile (25 mL) was added. After stirring for 2 h ether was added. The organic phase was separated, washed with brine, dried over magnesium sulfate, and evaporated. The crude mixture (2.2 g) was chromatographed on silica gel to give the less polar tripeptide **11b** (310 mg) followed by the octapeptide **10b** (1.0 g) both as pure products.

***O*-Acetyl-*N*-BOC Octapeptide 4-Thiocarboxylic Acid *S*-Benzyl Ester (10b)**: yield 57%; *m/z* calcd for $C_{61}H_{102}N_8O_{12}S$ 1170.7, found 1171.6 $[MH]^+$, 1071.7 [M − BOC]$^+$, 920.6 [M − MeLeu-SCHPh]$^+$ = [539.6 + Abu-Sar]$^+$, 764.6 [539.6 + Ac-MeBmt]$^+$, 704.6 [764.6 − AcOH]$^+$, 539.6 [426.5 + MeVal]$^+$, 426.5 [299.4 + MeLeu]$^+$, 299.4 [BOC-Ala-MeLeu]$^+$; $[\alpha_D] = -152.5°$ (*c* = 0.318 in MeOH); NMR δ 2.87, 2.97, 2.98, 3.09, 3.13; NMR (DMSO-d_6 at 155 °C) δ 0.77, 1.21, 1.41, 1.61, 1.95, 2.85−3.15, 4.13, 4.4−4.5, 4.6−4.7, 5.10, 5.8−5.9, 6.9−7.0, 7.15−7.30.

***N*-BOC-Val-MeLeu-Ala-thiocarboxylic Acid *S*-Benzyl Ester (11b)**: yield 40%; *m/z* calcd for $C_{27}H_{43}N_3O_5S$ 521.3, found 522.4 $[MH]^+$, 422.3 [MH − BOC]$^+$, 398.3 [M − SCHPh]$^+$ = [327.3 + Ala]$^+$, 327.3 [BOC-Val-MeLeu]$^+$, 271.2 [327.3 − C_4H_9]$^+$, 227.2 [327.3 − BOC]$^+$; $[\alpha_D] = -77.1°$ (*c* = 1.33 in MeOH); NMR (DMSO-d_6 at 180 °C) δ 1.31, 1.40, 2.94, 4.08, 4.25−4.32, 4.45−4.53, 4.92−5.04, 5.65−5.75, 7.15−7.30, 7.4−7.5.

***N*-Boc-Val-MeLeu-Phe-OH Benzyl Ester (11c).** A solution of BOC-Val-OH (2.25 g, 10 mmol) in methylene chloride (50 mL) was treated with 1,3-dicyclohexylcarbodiimide (1.05 g, 5 mmol). After 5 h, the solids were filtered off and the solution was treated with unprotected dipeptide **12b** (2.0 g, 5 mmol) in the same solvent. The mixture was kept overnight. Ether was added and washed sequentially with Na_2CO_3 solution, water, and brine. The organic phase was dried over magnesium sulfate and evaporated. The crude was chromatographed on silica gel to give the tripeptide (2.8 g): yield 96%; *m/z* calcd for $C_{33}H_{47}N_3O_6$ 581.4, found 582.3 $[MH]^+$, 482 [MH − BOC]$^+$, 327.3 [BOC-Val-MeLeu]$^+$, 271.2 [327.3 − C_4H_8]$^+$, 227.2 [Val-Leu]$^+$; $[\alpha_D] = -85.4°$ (*c* = 0.240 in MeOH); NMR (DMSO-d_6 at 150 °C) δ 1.38, 1.4−1.7, 1.9−2.0, 2.78, 2.95−3.15, 4.15−4.25, 4.6−4.7, 4.9−5.0, 5.60, 5.75−5.85, 7.1−7.4.

Fragmentation of 6a to Octapeptide 4-Thiocarboxylic Acid *S*-Benzyl Ester 10c and Tripeptide 7-Thiocarboxylic Acid *S*-Benzyl Ester 11a. A solution of **6a** (2.2 g, 1.6 mmol) in acetonitrile (25 mL) was added dropwise to a mixture of acetonitrile (50 mL) and 6 N HCl solution (15 mL). After stirring for 15 min the mixture was neutralized with 2 N sodium carbonate solution (50 mL). Then, *tert*-butyl methyl ether was added. The organic phase was separated and evaporated. The crude mixture was chromatographed immediately on silica gel with *tert*-butyl methyl ether/MeOH 95:5 to give the less polar tripeptide **11a** (480 mg) (not stable enough to be fully characterized) and with *tert*-butyl methyl ether/MeOH 90:10 to give the octapeptide **10c** (1.34 g).

Octapeptide 4-Thiocarboxylic Acid *S*-Benzyl Ester 10c: yield 83.8%; *m/z* calcd for $C_{54}H_{92}N_8O_9S$ 1028.5, found 1029.4 $[MH]^+$, 778.3 [439.2 + MeBmt-Abu-Sar]$^+$, 622 [439.2 + MeBmt]$^+$, 604.3 [439.2 + MeBmt − HOH]$^+$, 439.2 [326.1 + MeVal]$^+$, 326.1 [H-Ala-MeLeu-MeLeu]$^+$, 199.0 [H-Ala-MeLeu]$^+$; $[\alpha_D] = -180.9°$ (*c* = 0.280 in MeOH); NMR δ 0.73, 0.78, 1.30, 1.64, 2.87, 2.98, 3.00, 3.05, 3.15, 3.25, 3.68, 4.08, 4.70, 5.22, 6.84, 7.2−7.35.

***N*-BOC Octapeptide 4-Thiocarboxylic Acid *S*-Benzyl Ester 10d.** Protection of **10c** in the presence of $(BOC)_2O$ and triethylamine in methylene chloride; yield 56%; *m/z* calcd for $C_{59}H_{100}N_8O_{11}S$ 1128.7, found 1129.8 $[MH]^+$, 878.7 [722.7 + Abu-Sar]$^+$, 722.7 [539.6 + MeBmt]$^+$, 539.6 [426.5 + MeVal]$^+$, 426.5 [299.4 + MeLeu]$^+$, 299.4 [BOC-Ala-MeLeu]$^+$; $[\alpha_D] = -130.0°$ (*c* = 0.255 in MeOH); NMR δ 0.73, 0.78, 1.30, 1.43, 1.64, 2.87, 2.98, 3.00, 3.15, 3.25, 3.68, 4.08, 4.71, 5.22, 6.86, 7.2−7.3.

***N*-BOC-Val-MeLeu-Phe-OH (11d).** The benzyl ester **11c** was hydrogenated in 30 mL of methanol in the presence of 280 mg of 10% Pd/C. The catalyst was filtered off and the solvent was evaporated to give the acid (2.3 g): yield 96%; *m/z* calcd for $C_{26}H_{41}N_3O_6$ 491.2, found 514.3 [M + Na]$^+$, 492.4 $[MH]^+$, 414.3 [514.3 − BOC]$^+$, 327.3 [BOC-Val-MeLeu]$^+$, 271.2 [327.3 − C_4H_8]$^+$, 227.2 [Val-Leu]$^+$; $[\alpha_D] = -80.0°$ (*c* = 0.230 in MeOH); NMR (DMSO-d_6 at 150 °C) δ 1.38, 1.4−1.6, 1.90−2.05, 2.95−3.05, 3.10−3.20, 4.2−4.3, 4.5−4.6, 4.9−5.0, 5.80, 7.0−7.1, 7.15−7.30.

***N*-BOC-MeLeu-Phe-OH Benzyl Ester (12a).** A mixture of the commercial products phenylalanine benzyl ester *p*-toluenesulfonate (5.0 g, 20 mmol), BOC-MeLeu (7.3 g, 30 mmol), 4-(dimethylamino)pyridine (7.3 g, 60 mmol), and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC, 5.75 g, 30 mmol) in CH_2Cl_2 (250 mL) was stirred at room temperature overnight. Ether (500 mL) was added and washed sequentially with water, 2 N HCl solution, water, and 2 N Na_2CO_3 solution. The aqueous phases were extracted with fresh ether. The combined organic phase was dried over magnesium sulfate, filtered, and evaporated to give the crude product (7.8 g). This was chromatographed on silica gel to give the pure dipeptide (5.0 g): yield 53%; *m/z* calcd for $C_{28}H_{38}N_2O_5$ 482.0, found 483.0 $[MH]^+$, 382.9 [MH − BOC]$^+$, 255.9 [M − BOC-MeLeu]$^+$ = [H-Phe-O-CH_2Ph]$^+$; $[\alpha_D] = -62.3°$ (*c* = 0.620 in MeOH); NMR (DMSO-d_6 at 120 °C) δ 0.8−0.9, 1.38, 1.35−1.60, 2.55, 2.95−3.15, 4.4−4.5, 4.6−4.7, 5.1, 7.1−7.4, 7.44.

MeLeu-Phe-OH Benzyl Ester (12b). A solution of **12a** (2.5 g, 5.2 mmol) in methylene chloride (100 mL) was cooled with ice and treated with CF_3COOH (15 mL). After 1.5 h at room temperature, the mixture was neutralized with $NaHCO_3$ solution, extracted with ether, washed first with water, and then with brine, dried over magnesium sulfate, and evaporated to dryness to give a low melting solid (2.0 g), which was used immediately for the coupling reaction: yield 98%; *m/z* calcd for $C_{23}H_{30}N_2O_3$ 382.5, found 383.3 $[MH]^+$; $[\alpha_D] = -21.0°$ (*c* = 0.240 in MeOH); NMR δ 0.87, 0.91, 1.15−1.25, 1.35−1.45, 2.28, 2.9−2.95, 3.0−3.2, 4.9−5.0, 5.1−5.2, 7.0−7.4, 7.58.

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Supplementary Material Available: 360 MHz ^1H NMR spectra of **1b,c**, **3a−c**, **5a−c**, **6a,b**, **7a−l**, **8a,b**, **9a−g**, **9i**, **10a−d**, **11b−d**, and **12a,b** (42 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.