Synthesis of D-Lysine^8-cyclosporine A. Further Characterization of BOP-Cl in the 2–7 Hexapeptide Fragment Synthesis[1]

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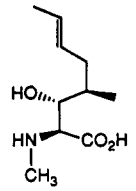
The coupling reagent *N,N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) has been used to synthesize the 2–7 hexapeptide fragment of cyclosporine A. The reagent proved useful in four of the five coupling steps with yields ranging from 78 to 92%. Overall, this BOP-Cl mediated synthesis proved to be more difficult than the previously described 8–11 tetrapeptide fragment synthesis (*J. Org. Chem.* **1986**, *51*, 3350). Prudent choices of both protecting groups and reaction conditions were required. The best approach, which employed Pmz-N-protection of the 5–7 tripeptide and Fmoc-N-protection of the 4–7 tetrapeptide gave the fully protected hexapeptide in 51% overall yield for the coupling steps. The 2–7 fragment, thus obtained, was used to prepare D-lysine^8-cyclosporine A. This analogue, containing D-lysine at the 8-position of cyclosporine A, is useful for isolating and characterizing putative receptors of cyclosporine A.

Introduction

The cyclosporines are a family of extensively N-methylated cyclic undecapeptides, a number of which display a variety of potent, orally active biological activities.[2] These properties, which include T-cell specific immunosuppression[2] and antiparasitic effects,[3,4] have stimulated a great deal of interest in these compounds on the parts of the medicinal chemical and biomedical communities. More recently, cyclosporine A (CsA) has been shown to be a potent inhibitor of the peptidyl-prolyl cis–trans isomerase, cyclophilin, whose function had been previously undetermined.[5–7] Unfortunately, only limited structure–activity data has appeared for these synthetically challenging compounds.[8] That no analogues have been reported to possess greater activity than the parent CsA reflects both the extraordinary sensitivity of this system to modifications and the lengthy times necessary to assemble the portions of this molecule.

The development of chemically simple and temporally efficient methods for the total synthesis of CsA and analogues has been an area of focus in our laboratories during the past several years. We have reported previously the efficient synthesis of two of the three major portions of the cyclosporine ring; the highly functionalized amino acid MeBmt (**1**)[9] and the 8–11 tetrapeptide fragment (D-Ala-MeLeu-MeLeu-MeVal).[1,10] This fragment, previously available by a difficult and racemization-prone N to C terminal extension,[11] was constructed by C to N terminal extension, in high yield with essentially no racemization, by employing activation of carbamate-protected amino acids with the condensing agent BOP-Cl (**2**).[10,12]



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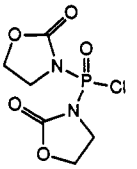

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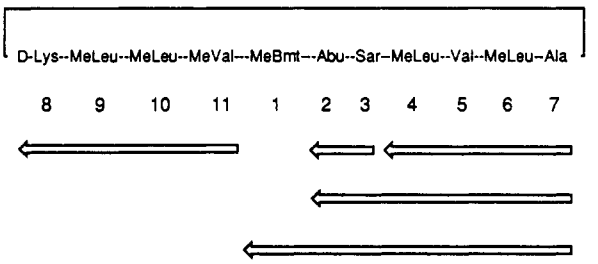
We now report on the range and limitations of BOP-Cl as a coupling reagent for synthesis of the remaining 2–7 hexapeptide fragment and its overall use in the synthesis of D-Lys^8-CsA (**3**), a known analogue containing D-lysine at the 8-position of CsA.[13] We have reported previously on the use of this analogue for protein photoaffinity labeling and affinity chromatography and its importance in isolating and characterizing putative receptors of CsA.[14]

Scheme I. Overall Strategy for Synthesis of D-Lys^8-CsA



Our overall strategy (Scheme I) is similar to that reported by Wenger,[13,15] with the exception that construction of the 8–11 tetrapeptide fragment is by the usual C to N

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† Abstracted in part from the Ph.D. thesis of Roger D. Tung (1987) submitted to the Graduate School of the University of Wisconsin—Madison.

(1) Nomenclature and symbols of amino acids and peptides generally follow the recommendations of the IUPAC-IUB Joint Commission of Biological Nomenclature (*Pure Appl. Chem.* **1984**, *56*, 595–624). Other abbreviations used are: Alloc, allyloxycarbonyl; BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; BOP-Cl, *N,N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride; CsA, cyclosporine A; DIEA, diisopropylethylamine; HOBt, 1-hydroxybenzotriazole; MeBmt, (4*R*)-4-[(*E*)-2-butenyl]-4,*N*-dimethyl-L-threonine or (2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoic acid; NMM, *N*-methylmorpholine; Pmz, *p*-methoxybenzyloxycarbonyl; Teoc, 2-trimethylsilylethyloxycarbonyl.

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(6) Takahashi, N.; Hayano, T.; Suzuki, M. *Nature* **1989**, *337*, 473–475.
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(8) (a) Wenger, R. M. *Prog. Allergy* **1986**, *38*, 46–64. (b) Traber, R.; Hofmann, H.; Loosle, H.-R.; Ponelle, M.; von Wartburg, A. *Helv. Chim. Acta* **1987**, *70*, 13–36. (c) Rich, D. H.; Dhaon, D. H.; Dunlap, B.; Miller, S. P. F. *J. Med. Chem.* **1986**, *29*, 978–984.
(9) (a) Tung, R. D.; Rich, D. H. *Tetrahedron Lett.* **1987**, *28*, 1139–1142. (b) Tung, R. D.; Sun, C.-Q.; Deyo, D.; Rich, D. H. In *Peptides, Chemistry and Biology: Proceedings of the 10th American Peptide Symposium*; Marshal, G. R., Ed.; ESCOM Science: Leiden, 1988; pp 149–151. (c) Aebi, J. D.; Dhaon, M. K.; Rich, D. H. *J. Org. Chem.* **1987**, *52*, 2881–2886.
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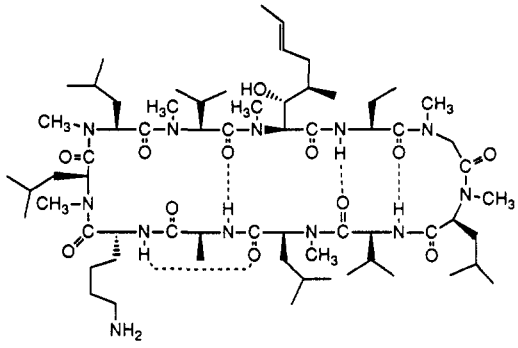

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terminal extension. We chose to use benzyl ester protection of the carboxyl terminal, which in our case would be especially advantageous because it would be removable simultaneously with the N-terminal Fmoc group of the eventual linear undecapeptide.[16]

Results

2–7 Hexapeptide. The yields obtained from coupling of N-protected amino acids with segments of the 2–7 peptide are shown in Table I. Variation in both protecting group and conditions are given for each sequence except for Boc-Abu-Sar-OBzl (entry 1 in Table I), for which optimization of yield was unnecessary. In some cases preactivation conditions, in which the N-protected amino acid was allowed to react with BOP-Cl before adding the amino component, were employed to limit the reaction of the amine component with BOP-Cl and hence increase yields. This proved best for the dipeptides R-MeLeu-Ala-OBzl **6** and **7** (entries 3 and 5 in Table I), and in another instance for the tetrapeptide Boc-MeLeu-Val-MeLeu-Ala-OBzl **15** (entry 16 in Table I). In other cases, when yields were low, we tried alternative N-protecting groups, because previously[17] we had found a dependence of yield on steric bulk of the protecting group. For the tripeptide R-Val-MeLeu-Ala-OBzl (entries 6–14 in Table I) Alloc and Teoc protection led to maximum yields (82 and 88%, respectively), but under conditions of controlled dropwise addition Pmz was found to also give acceptable yields (78%).

As the wide variation in yields presented for entries 15–20 in Table I suggests, the 3 + 1 coupling leading to the tetrapeptide R-MeLeu-Val-MeLeu-Ala-OBzl was the most troublesome step. Yields in the BOP-Cl couplings varied with conditions and also seemed sensitive to the precursor tripeptide deprotection. The best yield (88%) was found for the amino tripeptide derived from Teoc deprotection using HCl/dioxane (entry 18 in Table I). However, the Fmoc-protected tetrapeptide **16** could be prepared in equally good yield (84–86%) by using Fmoc-MeLeu acid chloride as the acylating reagent (entry 20 in Table I). The Fmoc-protected tetrapeptide was useful as it led to better yields in the subsequent BOP-Cl mediated 4 + 2 fragment coupling to give hexapeptide **18** (entries 21 and 22 in Table I).

TFA deprotection of hexapeptide **18** was accomplished in 82–90% yield. Likewise, Boc group deprotection of other peptides that appear in Table I were carried out in TFA. In all cases, these deprotections were accomplished in high yield with minimal contamination from side products. The various other N-protecting groups that appear in Table I were successfully deprotected using

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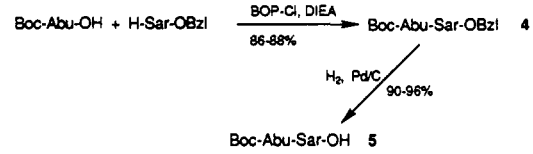
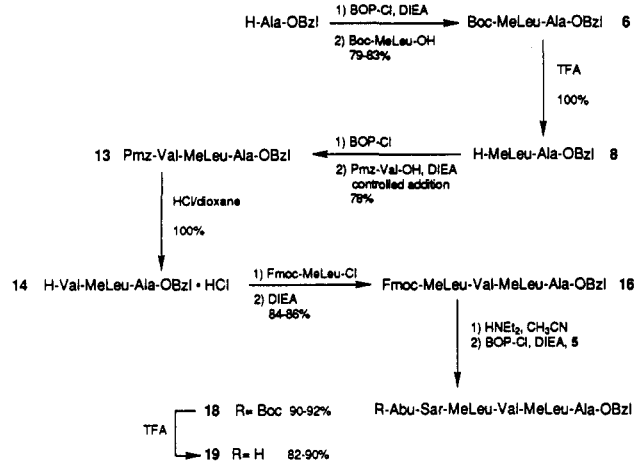
(17) Tung, R. D.; Rich, D. H. *J. Am. Chem. Soc.* **1985**, *107*, 4342–4343.

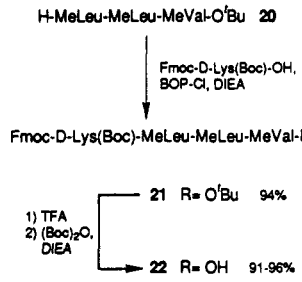
Scheme II. Synthesis of CsA 2–3 Fragment



Scheme III. Synthesis of CsA 2–7 Fragment



Scheme IV. Synthesis of D-Lys^8-CsA 8–11 Fragment



Scheme V. Synthesis of D-Lys^8-CsA

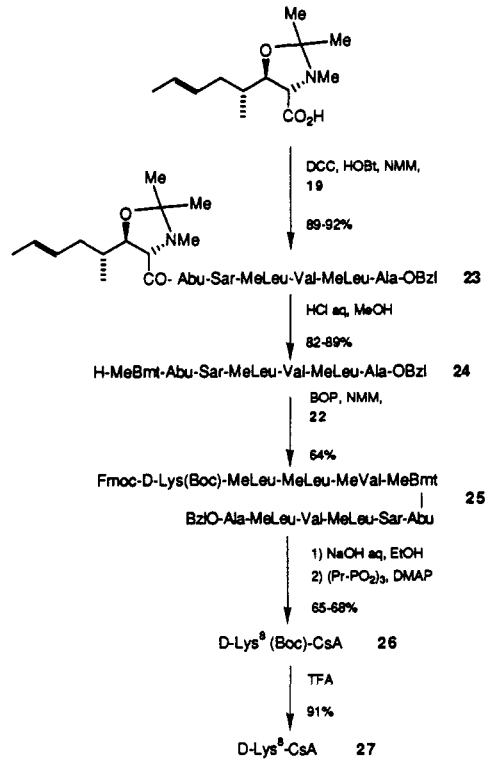


Table I. BOP-Cl and Acid Chloride Coupling of N-Protected Amino Acids with Segments of the 2–7 Peptide

entry	product sequence[a]	compd no.	conditions	yield, %
1	Boc-Abu-Sar-OBzl	4	A[b]	86–88
2	Boc-MeLeu-Ala-OBzl	6	A	69
3	Boc-MeLeu-Ala-OBzl	6	preactivation[c]	79–83
4	Fmoc-MeLeu-Ala-OBzl	7	A	78
5	Fmoc-MeLeu-Ala-OBzl	7	preactivation	86
6	Boc-Val-MeLeu-Ala-OBzl	9	A	67
7	Boc-Val-MeLeu-Ala-OBzl	9	A 1 equiv of HOBt	57
8	Fmoc-Val-MeLeu-Ala-OBzl	10	C,[d] from 7	66
9	Fmoc-Val-MeLeu-Ala-OBzl	10	Fmoc-Val-Cl, DIEA[e]	61
10	Alloc-Val-MeLeu-Ala-OBzl	11	A	78–82
11	Teoc-Val-MeLeu-Ala-OBzl	12	A, 1.1 equiv of Teoc-Val	70–76
12	Teoc-Val-MeLeu-Ala-OBzl	12	A, 1.7 equiv of Teoc-Val	88
13	Pmz-Val-MeLeu-Ala-OBzl	13	A, 1.5 equiv of Pmz-Val	64
14	Pmz-Val-MeLeu-Ala-OBzl	13	controlled addition[f]	78
15	Boc-MeLeu-Val-MeLeu-Ala-OBzl	15	A, from 11[g]	47
16	Boc-MeLeu-Val-MeLeu-Ala-OBzl	15	preactivation, from 11[g]	65
17	Boc-MeLeu-Val-MeLeu-Ala-OBzl	15	A, from 12[h]	71
18	Boc-MeLeu-Val-MeLeu-Ala-OBzl	15	A, from 12[i]	88
19	Fmoc-MeLeu-Val-MeLeu-Ala-OBzl	16	C, from 10	25
20	Fmoc-MeLeu-Val-MeLeu-Ala-OBzl	16	Fmoc-MeLeu-Cl, DIEA, from 13[i]	84–86
21	Boc-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl	18	A, from 15[h]	82
22	Boc-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl	18	C, from 16	90–92

[a] Longer line (–) indicates site of new peptide bond formed from acid–amine starting materials. [b] BOP-Cl, DIEA; in situ activation of the amino acid as described in general procedure A. [c] BOP-Cl, DIEA; preactivation of the amino acid prior to addition of the amine peptide. [d] BOP-Cl, DIEA; sequential removal of Fmoc-protection and BOP-Cl, DIEA coupling as described in general procedure C. [e] Acid chloride method as described in the specific experimental procedure for tetrapeptide 16. [f] 1.5 equiv of Pmz-Val and DIEA were added dropwise to the amino peptide and BOP-Cl. [g] Pd^0–dimedone deprotection of the Alloc protected tripeptide. [h] TFA deprotection of the N-protected amino peptide. [i] HCl/dioxane deprotection of the N-protected amino peptide.

either TFA, HCl/dioxane, Pd^0-dimedone (for Alloc), or diethylamine (for Fmoc) as reported in the footnotes of Table I.

In our judgement, the best overall pathway for synthesis of the 2–7 fragment is as shown in Schemes II and III.

8–11 Tetrapeptide. Synthesis of the D-Lys^8-CsA 8–11 tetrapeptide fragment followed directly from methods previously reported,[10] except that an additional protection step was required. Hence, BOP-Cl-mediated coupling gave 94% of the desired tetrapeptide **21** (Scheme IV), which was deprotected in TFA and then Boc reprotected at the ϵ-nitrogen of lysine to give 91–96% of tetrapeptide acid **22**.

D-Lys^8-CsA Synthesis. The remaining sequence (Scheme V) was carried out employing the strategy first described by Wenger.[15] Heptapeptide **23** was obtained in 92% from MeBmt and deprotected in 89% yield using methanolic/aqueous HCl. Fragment coupling of amino-deprotected heptapeptide **24** to tetrapeptide acid **22** using Castro's BOP reagent[18] yielded 64% of undecapeptide **25** after recovery and recycling of unconsumed heptapeptide. The yield was 84% when based solely upon recovery of this starting material. Cyclization, using propylphosphonic anhydride, was carried out in 68% yield, after base-catalyzed removal of both the benzyl ester and Fmoc protecting groups. Subsequent treatment with TFA gave D-Lys^8-CsA **27** in 91% yield.

Discussion

Synthesis of the CsA 2–7 Hexapeptide. The overall 2–7 hexapeptide synthesis was carried out by using the strategy first described by Wenger[15] and employed a 4 + 2 fragment coupling. BOP-Cl-mediated synthesis of the "left hand" dipeptide fragment Boc-Abu-Sar-OBzl (**4**) proceeded in 86–88% yield (see entry 1 of Table I), when carried out under conditions that rely on in situ activation of the amino acid. These same conditions have proven successful for synthesis of the highly N-methylated 8–11 fragment.[10] The overall synthesis of the 2–3 fragment is depicted in Scheme II. In contrast with this dipeptide, the apparently simple R-MeLeu-Ala-OBzl was obtained in somewhat poorer yields, when synthesized by the same method (entries 2 and 4 of Table I). We suspected that these low yields might have resulted from reaction between the phosphinic acid chloride and the primary amine of Ala-OBzl. Indeed, a byproduct, tentatively identified as the phosphinamidate on the basis of its ^1H NMR spectrum, was isolated from the reaction of BOP-Cl with Ala-OBzl.

In another experiment, when BOP-Cl was mixed with 1 equiv of DIEA and 1 equiv of Leu-O^{t}Bu, a 90% yield of the phosphinamidate was formed in 4 h at 0 °C to ambient temperature. In contrast, reaction with secondary amines was negligible: When MeLeu-O^{t}Bu was subjected to the same conditions, no reaction was evident even after 2 days at ambient temperature. Therefore, the ability of BOP-Cl to phosphorylate amino acyl esters appears to be limited to primary amines.

To circumvent the formation of phosphinamidate, we carried out a preactivation step in which the N-protected amino acid was allowed to react with BOP-Cl for a number of hours before the amine component was added. In this manner, slightly higher yields of the dipeptides **6** and **7** were obtained (entries 3 and 5 of Table I).

As the data in Table I indicate, the success of BOP-Cl in the synthesis of the tripeptide R-Val-MeLeu-Ala-OBzl (entries 6–14) was highly dependent upon a judicious choice of N-protecting groups and coupling conditions. The use of Boc-protection resulted in a relatively low yield of **9** (67%). Preactivation or addition of other reagents (e.g., HOBt and 2-mercaptopyridine), in attempts to form more reactive intermediates, failed to increase the yield. Previously,[17] we had shown a dependence of yield on steric bulk of the N-protecting group. We therefore decided to explore alternative protecting groups for preparation of this tripeptide. BOP-Cl-mediated coupling of Fmoc-Val to the free *N*-methylamino dipeptide derived from **7**, gave a relatively low yield of tripeptide **10**, as did coupling with

(18) Le Nguyen, D.; Seyer, R.; Heitz, A.; Castro, B. *J. Chem. Soc., Perkin Trans. 1* **1985**, 1025–1031.

Table II. Maximum Overall Yields Along Selected Pathways of the 2–7 Hexapeptide Synthesis

pathway	6–7 dipeptide	4 →	5–7 tripeptide	5 →	4–7 tetrapeptide	2–3 →	2–7 hexapeptide	maximum overall yield, %
A	Boc[a] (83)[b]		Pmz (78)		Fmoc (86)		Boc (92)	51
B	Boc (83)		Teoc (88)		Boc (88)		Boc (82)	53
C	Boc (83)		Alloc (82)		Boc (65)		Boc (82)	36
D	Fmoc (86)		Fmoc (66)		Fmoc (25)		Boc (92)	13

[a] N-Protecting group of the peptide O-benzyl ester. [b] Best yield obtained from the coupling methods listed in Table I.

Fmoc-Val acid chloride. On the other hand, the linear and exceptionally unhindered allyloxycarbonyl group[19] (Alloc, entry 10) gave quite acceptable yields of tripeptide **11** (78–82%). The successful couplings obtained with the acid- and fluoride-labile 2-(trimethylsilyl)ethyl carbamate protected amino acids[20] prompted us to utilize Teoc-Val to synthesize the corresponding tripeptide **12**. Yields in this case were moderate (70–76%) when 1.2 equiv of Teoc-Val were used, but could be considerably improved (88%) using 1.7 equiv (compare entries 11 and 12 in Table I).

For larger scale reactions we utilized the commercially available and acid labile protecting group *p*-methoxy-Cbz-valine (Pmz-Val). BOP-Cl-mediated condensation employing in situ activation of the amino acid (condition A in Table I) resulted in a low yield of tripeptide **13** because of formation of large amounts of the symmetrical valine anhydride. This byproduct was stable through workup and chromatographed closely with the product, making purification difficult. Fortunately, this contaminant could be greatly reduced by employing a dropwise addition of a mixture containing Pmz-Val and tertiary amine base into a solution of BOP-Cl and the amino dipeptide. In this manner, 78% of the corresponding tripeptide **13** was obtained.

As indicated by the wide variation in yields shown in Table I for entries 15–20, synthesis of the tetrapeptide R-MeLeu-Val-MeLeu-Ala-OBzl was an even more troublesome step. BOP-Cl-mediated coupling was sensitive not only to reaction conditions but was also dependent upon the source and deprotection of the amino tripeptide. For instance, the Alloc derived amino tripeptide gave a lower yield in coupling to Boc-MeLeu than did the Teoc derived amino tripeptide under similar conditions (compare entries 15 and 16 with 17 and 18). The Fmoc-tetrapeptide **16**, prepared from tripeptide **10** and Fmoc-MeLeu by the one-pot Fmoc-deprotection/BOP-Cl-coupling method, was obtained in exceedingly poor yield (25%). The best yield (88%) was obtained from BOP-Cl coupling of Boc-MeLeu to the amino tripeptide derived from HCl/dioxane deprotection of **12**.

Because of the considerable variation in yields encountered when employing BOP-Cl as coupling reagent for formation of tetrapeptides **15** and **16**, as well as the desire to use the one-pot Fmoc-deprotection/BOP-Cl-coupling procedure in the 4 + 2 fragment condensation, we chose to examine the use of Fmoc-MeLeu acid chloride as the acylating reagent. Fmoc amino acyl chlorides are stable at ambient temperature under an inert atmosphere and are highly reactive acylating reagents.[10,21] Carpino's group has described the use of Fmoc acyl chlorides and reported them to be highly crystalline substances, stable in storage for months without decomposition.[21] When Fmoc-MeLeu acid chloride was added to the amino tripeptide derived from HCl/dioxane deprotection of **13**, and the mixture neutralized by slow addition of DIEA, the desired tetrapeptide **16** was isolated in acceptable and reproducible yields (84–86% over the deprotection and coupling). We have successfully applied this methodology to the synthesis of multigram quantities (>8 g in one instance) of the tetrapeptide **16** and believe that, of the routes that we have explored, this is the most useful for routine application.

Finally, BOP-Cl-mediated 4 + 2 fragment coupling was found to proceed with a slight variation in yields (entries 21 and 22 in Table I) when carried out by either deprotection of Boc-tetrapeptide **15** to its free amine and a separate coupling step (82%) or by one-pot deprotection/coupling of Fmoc-protected **16** (90–92%).

As indicated by the results shown in Table II, when the optimized yields for all coupling steps in synthesis of the 2–7 hexapeptide are considered, two pathways (A and B) emerge as equally reasonable approaches. Of these two, for the reasons stated above, we chose Pmz protection of the 5–7 tripeptide and Fmoc protection of the 4–7 tetrapeptide, during preparation of the title compound. The best overall combination of conditions, for synthesis of the 2–7 fragment, is shown in Scheme III.

Synthesis of the D-Lys[8]-CsA 8–11 Tetrapeptide. Synthesis of the 8–11 tetrapeptide Fmoc-D-Lys(Boc)-MeLeu-MeLeu-MeVal (**21**, Scheme IV) followed directly from methods previously reported for synthesis of the CsA 8–11 fragment.[10] These methods have as their basis BOP-Cl activation and benzyloxycarbonyl (Cbz) amino protection in a series of highly efficient couplings and catalytic deprotections. Hence, *N*-methylamino tripeptide **20** was synthesized in 56% overall yield from Cbz-MeVal, and condensed, via BOP-Cl, with commercially available N^{α}-Fmoc-D-lysine(N^{ϵ}-Boc) amino acid to give the tetrapeptide **21** in 94% yield. Removal of the *tert*-butyl ester proceeded smoothly in TFA and was accompanied by loss of the Boc group, which was readily replaced in a second step using di-*tert*-butyl dicarbonate, to give the N^{α}-Fmoc-N^{ϵ}-Boc-protected acid **22** in 96% yield from **21**.

Synthesis of the CsA 1–7 Heptapeptide. Synthesis of the N-deprotected 1–7 fragment **24** was carried out according to procedures described by Wenger.[15] These procedures employ DCC coupling of MeBmt (protected as the acetonide) to amino hexapeptide **19**, with subsequent deprotection in dilute methanolic/aqueous HCl (Scheme V). MeBmt was synthesized in six steps using the method of Evans and Weber.[22] Their asymmetric glycine enolate aldol reaction required multigram quantities of the chiral aldehyde (2*R*,4*E*)-2-methyl-4-hexenal, which were obtained through the methods of Deyo et al.[23] Accordingly, heptapeptide **23** was obtained in 92% yield from MeBmt, while acetonide deprotection gave 89% of N-deprotected heptapeptide **24**.

Fragment Coupling and Cyclization. The remaining 4 + 7 fragment coupling (Scheme V) continues to be the

(19) (a) Kunz, H.; Unverzagt, C. *Angew. Chem., Intl. Ed. Engl.* **1984**, *23*, 436–437. (b) Guibe, F.; Dangles, O.; Balavoine, G. *Tetrahedron Lett.* **1986**, *27*, 2365–2368. (c) Hayakawa, Y.; Kato, H.; Uchiyama, M.; Kajino, H.; Noyori, R. *J. Org. Chem.* **1986**, *51*, 2402–2404.

(20) Shute, R. E.; Rich, D. H. *Synthesis* **1987**, 346–348.

(21) Carpino, L. A.; Cohen, B. J.; Stephens, K. E., Jr.; Sadat-AAlaee, S. Y.; Tien, J.-H.; Landridge, D. C. *J. Org. Chem.* **1986**, *51*, 3734–3736.

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most problematic step in the overall synthesis of cyclosporine analogues. The best available method, that of Wenger,[15] which utilizes Castro's BOP reagent,[18] seldom gives better than 50% yield of undecapeptide. For this reason, we chose to reexamine this critical, yet somewhat unavailing, reaction. A careful accounting for material balance, after the 3 days of reaction, revealed 55% of desired undecapeptide, 11% of a major impurity (believed to be the diastereomer resulting from racemization of MeVal at the 11-position[15]), and the remaining mass in recovered starting materials. In other runs, we attempted to drive the reaction to completion after the third day, by additions of BOP, *N*-methylmorpholine, and tetrapeptide **22**, without success.[24] Fortunately, starting material (heptapeptide) could be recycled; and in this way the yield of desired undecapeptide **25** was increased to 64% (or 84% when based upon recovery of heptapeptide).

The final cyclization was also carried out according to methods described by Wenger.[25] Saponification of the benzyl ester of **25**, using ethanolic/aqueous NaOH, conveniently transpired with concomitant removal of the Fmoc protecting group. After workup, the crude deprotected undecapeptide was cyclized, using propylphosphonic anhydride and 4-(dimethylamino)pyridine, in a dilute solution, to give D-Lys[8](Boc)-CsA **26** in 68% yield. Subsequent Boc-deprotection in TFA went without incident to give the title compound **27** in 91% yield after purification by preparative TLC.

Caveats Regarding Use of BOP-Cl. From the preceding results, it is evident that BOP-Cl is, in general, an excellent reagent for the activation of carbamate protected amino acids and their subsequent coupling to N-alkylated amino acids. Nevertheless, it does suffer from several drawbacks, some of which were not apparent in our earlier work. First, is the limitation when using BOP-Cl with β-branched amino acids having bulky N-protecting groups; for example, BOP-Cl is not compatible with BOC-Val couplings. This incompatibility is a notable deficiency due to the great popularity of the Boc group and limits the use of the reagent to cases where one is willing to design the synthesis around the properties of the condensing agent. Second, BOP-Cl will react with primary amines, albeit somewhat more slowly than with carboxylates. In contrast, reaction with secondary amines is negligible. In the case of primary amines, preactivation of the carboxylate component is possible and moderately increases yields with no noticeable racemization of the activated component.

It should be noted that, due to the exceedingly poor solubility of BOP-Cl in nearly all organic solvents (e.g., methylene chloride, THF, acetonitrile, and DMF), this preactivation step may be a somewhat capricious procedure.[26] Other groups[27] have also noted the reaction of BOP-Cl with primary amines and have suggested the use of notably shorter preactivation intervals for optimal yields. Whereas, greater solubility would be useful for reproducible reaction times, as well as for solid-phase work, this insolubility generally does not pose a significant limitation to the use of BOP-Cl, as it goes into solution as it reacts with the carboxylate component to form the mixed anhydride.

In general, it seems fair to state that, given a judicious choice of protecting groups and reaction conditions, BOP-Cl provides an efficient method for the condensation of both N-alkylated and nonalkylated carbamate-protected amino acids. Yet, in cases where the nucleophile is a primary amine, due to the formation of phosphinamidates as a side reaction, other coupling reagents (e.g., diethyl cyanophosphate,[28] propylphosphonic anhydrides,[29] Castro's BOP reagent,[18] or Fmoc-amino acyl chlorides[21]) might well be considered. However, where N-alkylated nucleophiles are involved, BOP-Cl is a first-choice reagent, giving very high yields with negligible levels of racemization.

Experimental Section

Instrumentation. Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded using Bruker WP-200, WP-270, AM-300, or AM-500 instruments in $CDCl_3$ unless otherwise indicated. Chemical shifts are reported in ppm (δ units) downfield from tetramethylsilane. Because in most cases the spectra showed multiple conformations, generally with different coupling constants for the different rotamers, *J* values are for the most part omitted. Likewise, because of complexity, spectra for some of the larger peptides are not reported, but are available as supplemental material. Optical rotations were recorded with a Perkin-Elmer 241 automatic polarimeter; and IR spectra with a Perkin-Elmer Model 599B instrument.

Chromatography. Column chromatography was carried out using a modified flash system packed with 230–400-mesh Merck grade 60 silica gel. Analytical TLC was performed on glass-backed Merck grade 60, UV-indicating silica plates of 0.25-mm thickness, while preparative runs were made on 0.50-mm plates. For compound visualization, 4% phosphomolybdic acid in ethanol was employed with heating. TLC elution solvents are abbreviated in the text by letters followed by percentages of minor component, with components as follows (minor/major): A, ethyl acetate/toluene; B, methanol/CH_2Cl_2; C, acetone/hexanes; D, 85:10:5 $CHCl_3$/methanol/acetic acid; E, acetone/CH_2Cl_2; F, 4:1:1 1-butanol/acetic acid/water.

Solvents. Diethyl ether and tetrahydrofuran (THF) were distilled from sodium/benzophenone. Acetone (HPLC grade, Aldrich Chemical Co., Milwaukee, WI) was stored over 4-Å molecular sieves for 24 h and distilled prior to use. Methylene chloride was distilled from P_2O_5. Ethyl acetate was reagent grade (Baker, Phillipsburg, NJ).

Reagents. Diisopropylethylamine (DIEA), trifluoroacetic acid (TFA), *N*-methylmorpholine (NMM), 4-(dimethylamino)pyridine (DMAP), oxalyl chloride, anisole, anhydrous dioxane, and 10% palladium on carbon (10% Pd–C) were purchased from Aldrich Chemical Co. of Milwaukee, WI, and used without subsequent purification. Prior to use, 1-hydroxybenzotriazole (Aldrich) was dehydrated by three successive azeotropic distillations employing 1:1 toluene/THF on a rotary evaporator. *N,N'*-Dicyclohexylcarbodiimide (DCC, Aldrich) was distilled under an inert atmosphere. HCl–dioxane (5.8 M) was prepared by bubbling gaseous HCl into anhydrous dioxane cooled on ice. Propylphosphonic anhydride (50% w/w in CH_2Cl_2) was purchased from Fluka Chemical Corp. of Ronkonkoma, NY, and used without subsequent purification. *N,N'*-Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) were purchased from Chemical Dynamics Corp. of South Plainfield, NJ, and used without subsequent purification.

Amino Acids. *N*-(4-Methoxybenzyloxycarbonyl)valine (Pmz-Val) was purchased from Omni Biochemicals of National City, CA, under the name "Moz-Val". All other N-protected amino

(24) We are presently unable to explain these results and are continuing to investigate this enigmatic reaction.

(25) Rosenthaler, J.; Wenger, R.; Ball, P. E.; Schreier, M. H.; Quesniaux, V. International Patent WO 86/02080, filled 9/27/85, published 4/10/86; assigned to Sandoz AG.

(26) Recent experiences by our and other groups have indicated that the reaction can be dependent on the source of the reagent. Notably, one batch of BOP-Cl from Aldrich (lot no. 04422DT KT) has been found to produce inferior yields and incomplete reactions. We have used BOP-Cl from Chemical Dynamics and Advanced Chemtech with good results.

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acids, including N^{α}-[(9-fluorenylmethyl)oxy]carbonyl-N^{ϵ}-(*tert*-butyloxycarbonyl)-D-lysine [Fmoc-D-Lys(Boc)] were purchased from Bachem Inc. of Torrance, CA. Boc-*N*-methyl amino acids were synthesized by the procedure of McDermott and Benoiton;[30] while Fmoc-*N*-methyl amino acids were synthesized using the procedure of Freidinger et al.[31]

General Experimental Procedures. General Procedure A. Peptide Couplings Using BOP-Cl as the Condensing Reagent. A solution of an N-protected amino acid (11 mmol) and the amino acid or peptide benzyl ester (10 mmol) in methylene chloride (50–100 mL) was cooled in an ice bath with stirring under an inert atmosphere. The cold mixture was treated with diisopropylethylamine (22 mmol) followed, in one portion, by BOP-Cl (11 mmol). The mixture was stirred in the cold until TLC analysis indicated that consumption of the amino component was complete or no longer proceeding, and then it was poured into ether (3× the reaction volume) and water (2× the reaction volume). The organic layer was separated, washed with 10% aqueous $KHSO_4$, H_2O, 1 N $NaHCO_3$, 50% brine, and brine. After drying over $MgSO_4$ it was concentrated in vacuo to an oil or foam, which was purified by flash chromatography.

General Procedure B. Removal of *N*-*tert*-Butyloxycarbonyl Protection with TFA. The *N*-*tert*-butyloxycarbonyl-protected peptide, either neat or as a solution in methylene chloride, was chilled under an inert atmosphere in an ethylene glycol/CO_2 bath held at −15 to −18 °C by periodic additions of CO_2. To this flask was added, via cannula and with stirring, TFA which was precooled in the same bath. The mixture was transferred to a cold room maintained at −13 to −18 °C and allowed to stand under a balloon of argon until TLC analysis indicated that consumption of the starting material was complete (4–18 h). The chilled reaction mixture was then cautiously added dropwise to a rapidly stirring slurry of methylene chloride, ice, and $NaHCO_3$ (1–1.1 g/mL of TFA), and, if necessary, the pH of the aqueous layer was adjusted to above 6 using 1 M $NaHCO_3$. The layers were separated, and the aqueous portion washed with additional methylene chloride (2×). The combined organics were dried over $MgSO_4$ and concentrated in vacuo to an oil, which was generally used without subsequent purification.

General Procedure C. Sequential Removal of *N*-[(9-Fluorenylmethyl)oxy]carbonyl Protection and BOP-Cl-Mediated Coupling. A solution of the *N*-Fmoc-protected compound in CH_3CN (ca. 200 mM) was treated with an equal volume of diethylamine under an inert atmosphere until TLC analysis indicated that consumption of the starting material was complete (20 min to 3 h), and the solution was concentrated in vacuo. The residue was treated with additional CH_3CN (ca. 1/2 the reaction volume) and again concentrated to yield a yellow oil. This oil was treated with CH_2Cl_2 (to ca. 50 mM) and 2.4 equiv of diisopropylethylamine and then chilled with stirring in an ice/H_2O bath under N_2. The cold solution was treated sequentially, with single portions of the appropriate N-protected amino acid (1.1 equiv) and BOP-Cl (1.2 equiv).

In larger scale preparations (>ca. 3 mmol) it may be advantageous to employ an ice/salt bath due to the exothermic nature of the reaction. Also, in these cases, the equivalents of N-protected amino acid, BOP-Cl, and diisopropylethylamine may be reduced to 1.05, 1.1, and 2.2, respectively. Ammonolysis of the (soluble) mixed phosphinic–carboxylic anhydride is fast relative to its formation from the (insoluble) BOP-Cl; therefore, upon dissolution of all solids, the reaction may generally be presumed to be complete, subject to TLC analysis. Upon completion, the reaction mixture was worked up as described in general procedure A. It is generally advantageous to complete the workup and chromatography within 24 h of the reaction in order to avoid formation of insoluble polymers from the dibenzofulvene byproduct. If polymer formation is a problem, generally filtering the reaction prior to workup or chromatography is helpful.

Specific Experimental Procedures. *N*-(*tert*-Butyloxycarbonyl)-α-aminobutyrylsarcosine Benzyl Ester (4). Via general procedure A, sarcosine benzyl ester (3.166 g, 17.76 mmol) and Boc-Abu (2.972 g, 15.87 mmol) were condensed to yield, after workup and chromatography on 400 g of silica gel (20% acetone/hexanes eluant), 4.836 g (88%) as a pale yellow, viscous oil: TLC R_f (A, 25%) 0.43; $[\alpha]_D$ −6.7° (*c* 1.0, $CHCl_3$) (lit.[15] $[\alpha]_D$ −4.2° (*c* 1.0, $CHCl_3$)); ^1H NMR (270 MHz, $CDCl_3$) δ 0.86, 0.95 (2 t, *J* = 7.8 Hz, 3 H total), 1.22–1.65 (m, 2 H), 1.42, 1.43 (2 s, 9 H), 2.98 (2 s, 3 H total), 3.87 (d, *J* = 18 Hz, 1 H), 4.48 (d, *J* = 18 Hz, 1 H), 4.33–4.42, 4.56–5.67 (2 m, 1 H total), 5.18, 5.20 (2 s, 2 H total), 5.34 (d, *J* = 8.8 Hz, 1 H), 7.35 (s, 5 H).

***N*-(*tert*-Butyloxycarbonyl)-α-aminobutyrylsarcosine (5).** A solution of 4 (2.444 g, 7.01 mmol) in 60 mL of ethanol was hydrogenated using 247 mg of 10% Pd–C catalyst. After filtering through Celite and concentrating in vacuo, the white, foamy residue, 1.854 g (96%), was used without subsequent purification: TLC R_f (D) 0.46; $[\alpha]_D$ −3.5° (*c* 3.0, $CHCl_3$) (lit.[15] $[\alpha]_D$ −5.3° (*c* 1.0, $CHCl_3$)); ^1H NMR (270 MHz $CDCl_3$) δ 0.96 (t, *J* = 6.8 Hz, 3 H), 1.43 (s, 9 H), 1.34–1.93 (m, 2 H), 3.00, 3.18 (2 s, 3 H total), 3.90 (d, 1 H, *J* = 17 Hz, 1 H), 4.44 (d, *J* = 17 Hz, 1 H), 4.57–4.75, 4.15–4.29 (2 m, 1 H total), 5.48–5.72 (m, 1 H), ca. 5.7–7.5 (broad, 1 H, COO*H*).

***N*-(*tert*-Butyloxycarbonyl)-*N*-methylleucylalanine Benzyl Ester (6)** (route A, without preactivation of the caroxylic acid). Via general procedure A, Boc-MeLeu (1.68 g, 7.26 mmol) and Ala-OBzl (1.30 g, 7.25 mmol) were condensed to yield, after chromatography on a 240-g silica gel column (13% acetone/hexanes eluant), 2.25 g (78%) of colorless oil: TLC R_f (A, 25%) 0.59; $[\alpha]_D$ −75.6° (*c* 1.0, $CHCl_3$) (lit.[15] $[\alpha]_D$ −67.0° (*c* 1.0, $CHCl_3$)); ^1H NMR (270 MHz, $CDCl_3$) δ 0.84–0.99 (t, *J* = 3.3 Hz, 6 H), 1.39 (d, *J* = 7.5 Hz, 3 H), 1.47 (s, 9 H), 1.56–1.74 (m, 3 H), 2.73 (s, 3 H), 4.47–4.72 (m, 2 H), 5.09–5.23 (m, 2 H), 6.33–6.69 (m, 1 H), 7.27–7.39 (m, 5 H).

***N*-(*tert*-Butyloxycarbonyl)-*N*-methylleucylalanine Benzyl Ester (6)** (route B, preactivating the carboxylic acid). A solution of Boc-MeLeu (10.31 g, 42.02 mmol) and diisopropylethylamine (7.67 mL, 44.0 mmol) in 250 mL of CH_2Cl_2 was cooled in an ice/water bath under N_2 and treated with 11.21 g (44.02 mmol) of BOP-Cl, and the suspension was stirred vigorously for 2.5 h. To this mixture was added, in one portion, a solution of alanine benzyl ester (7.298 g, 40.72 mmol) and diisopropylethylamine (7.67 mL, 44.0 mmol) in 6 mL of CH_2Cl_2. The mixture was placed under a $CaSO_4$ drying tube and stirred overnight in a 5 °C cold room. The solution was then worked up as described in general procedure A and purified by chromatography on 400 g of silica gel, eluting with 10% acetone/hexanes to yield, as the pure compound, 13.65 g (83%) of a very pale yellow oil; physical characteristics identical with that produced by route A.

***N*-[(9-Fluorenylmethyl)oxy]carbonyl-*N*-methylleucylalanine Benzyl Ester (7).** Via general procedure A, Fmoc-MeLeu (1.29 g, 3.50 mmol) and Ala-OBzl (690 mg, 3.85 mmol) were condensed to yield, after workup and chromatography on 120 g of silica (15% acetone/hexanes eluant), 1.29 g (69%) of a colorless glass: TLC R_f (A, 25%) 0.41, $[\alpha]_D$ −63.3° (*c* 1.0, $CHCl_3$); ^1H NMR (90 MHz, $CDCl_3$ δ 0.62–1.08 (m, 6 H), 1.18–1.76 (m, 3 H), 1.33 (d, *J* = 6.3 Hz, 3 H), 2.79 (s, 3 H), 3.95–4.82 (m, 5 H), 5.17 (s, 2 H), 6.30–6.55 (m, 1 H), 7.22–7.93 (m, 8 H). Anal. Calcd for $C_{32}H_{36}N_2O_5$: C, 72.70; H, 6.86; N, 5.30. Found: C, 72.41; H, 6.87; N, 5.32.

***N*-Methylleucylalanine Benzyl Ester (8).** Via general procedure B, 6 (8.946 g, 22.00 mmol) was deprotected with 58 mL of 50% TFA in methylene chloride to yield, after neutralization, extraction into methylene chloride, and evaporation, a quantitative yield of a viscous, yellow oil which was used without purification for the next step. In other runs, chromatographic purification on silica gel was carried out, using 7% MeOH/$CHCl_3$ eluant, to give in essentially quantitative yield, a light yellow oil: TLC R_f (B, 6%) 0.27; $[\alpha]_D$ −42.3° (*c* 1.0, $CHCl_3$) (lit.[15] $[\alpha]_D$ −44.5° (*c* 1.0, $CHCl_3$)); ^1H NMR (200 MHz, $CDCl_3$) δ 0.90 (t, *J* = 6.1 Hz, 6 H), 1.51 (d, *J* = 7.3 Hz, 3 H), 1.54–1.74 (m, 3 H), 2.68 (3 s, 3 H total), 3.00 (dd, 1 H), 4.53 (m, 1 H), 5.05–5.20 (dd, *J* = 12 Hz, 2 H), 7.25–7.39 (s, 5 H), 8.21 (d, *J* = 7.4 Hz, 1 H).

***N*-(*tert*-Butyloxycarbonyl)valyl-*N*-methylleucylalanine Benzyl Ester (9).** Via general procedure A, Boc-valine (407 mg, 1.87 mmol) and 8 (518 mg, 1.69 mmol) were condensed with BOP-Cl to yield, after workup and purification on a silica gel column (16% acetone/hexanes eluant), 576 mg (67%) of a nearly colorless viscous oil: TLC R_f (C, 20%), 0.31, $[\alpha]_D$ −102.9° (*c* 1.0,

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(31) Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1982**, *48*, 77–81.

$CHCl_3$) (lit.[15] $[\alpha]_D$ -97.2° (c 1.0, $CHCl_3$)); ^1H NMR (200 MHz, $CDCl_3$) δ 0.84–1.06 (m, 12 H), 1.28–1.48 (m, 12 H), 1.66 (t, J = 7.5 Hz, 2 H), 1.77–1.89 (m, 1 H), 1.89–2.06 (m, 1 H), 2.80, 3.80 (2 s, 3 H total), 4.21–4.33 (m, 1 H), 4.37, 4.43 (2 d, J = 7.0, 6.8 Hz, 1 H total), 4.54 (t, J = 6.5 Hz, 1 H) 5.07–5.25 (m, 3 H), 6.52 (d, J = 7.5 Hz, 1 H), 7.33 (s, 5 H).

***N*-[(9-Fluorenylmethyl)oxy]carbonylvalyl-*N*-methylleucylalanine Benzyl Ester (10)** (route A, from MeLeu-Ala-OBzl A solution of 8 (613 mg, 2 mmol) in methylene chloride (10 mL) was cooled in an ice/H_2O bath under Ar and treated sequentially with diisopropylethylamine (383 mL, 2.20 mmol) and Fmoc-valine acid chloride[21] (788 mg, 2.20 mmol). The mixture was transferred into a 6 °C cold room and stirred overnight at that temperature. The next day, the reaction was worked up as described in general procedure A and the product was purified chromatographically (80 g of silica gel, 20% acetone/hexanes eluant) to yield 773 mg (61%) of a nearly colorless, viscous oil: TLC R_f (A, 25%) 0.38, $[\alpha]_D$ -84.8° (c 1.0, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.80–1.11 (m, 12 H), 1.24, 1.36 (2 d, both J = 6.5 Hz, 3 H total), 1.59–1.73 (m, 3 H), 1.90–2.09 (m, 1 H), 2.84, 3.00 (2 s, 3 H total), 4.14–4.30, 4.30–4.44, 4.44–4.63 (3 m, 5 H total), 5.05–5.19 (m, 3 H), 5.54 (d, J = 10 Hz, 1 H), 6.53 (d, J = 6.5 Hz, 1 H), 7.21–7.46 (m, 9 H), 7.58 (d, J = 6.3 Hz, 2 H), 7.76 (d, J = 7 Hz, 2 H). Anal. Calcd for $C_{37}H_{45}N_3O_6 \cdot 1.25H_2O$: C, 68.34; H, 7.36; N, 6.30. Found: C, 68.29; H, 6.92; N, 6.16.

***N*-[(9-Fluorenylmethyl)oxy]carbonylvalyl-*N*-methylleucylalanine Benzyl Ester (10)** (route B, from Fmoc-MeLeu-Ala-OBzl). Via general procedure C, 7 (1.22 g, 2.31 mmol) was sequentially deprotected and condensed with Fmoc-Val. Workup and chromatography (75 g of silica, 20% acetone/hexanes eluant) were carried out to yield 950 mg, 66%, of a white foamy solid, identical with that produced by route A.

***N*-(Allyloxycarbonyl)valyl-*N*-methylleucylalanine Benzyl Ester (11).** Via general procedure A, Alloc-valine (2.187 g, 10.87 mmol) and 8 (3.027 g, 9.88 mmol) were condensed with BOP-Cl to yield, after workup and purification on a 310-g silica gel column (30% acetone/hexanes eluant), 3.982 g (82%) of a nearly colorless, viscous oil: TLC R_f (A, 25%) 0.40, $[\alpha]_D$ -104.4° (c 1.0, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.83–1.06 (m, 12 H), 1.36 (d, J = 7.5 Hz, 3 H), 1.58–1.73 (m, 3 H), 1.88–2.06 (m, 1 H), 2.83, 3.00 (2 s, 3 H total), 4.38–4.65 (m, 4 H), 5.07–5.39 (m, 4 H), 5.46 (d, J = 9.3 Hz, 1 H), 5.79–6.03 (m, 1 H), 6.59 (d, J = 7.3 Hz, 1 H), 7.36 (s, 5 H). Anal. Calcd for $C_{26}H_{39}N_3O_6$: C, 63.78; H, 8.03; N, 8.58. Found: C, 63.55; H, 8.06; N, 8.45.

***N*-(2,2,2-Trimethylsilylethyloxycarbonyl)valyl-*N*-methylleucylalanine Benzyl Ester (12).** Via general procedure A, Teoc-valine (491 mg, 1.88 mmol) and 8 (used without purification from the deprotection of 455 mg, 1.12 mmol of 6) were condensed with BOP-Cl to yield, after workup and purification on a 60-g silica gel column (27% EtOAc/hexanes eluant), 541 mg (88% from 6) of a colorless, viscous oil: TLC R_f (A, 15%) 0.22, $[\alpha]_D$ -100.9° (c 1.0, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.0 (s, 9 H), 0.83–1.05 (m, 14 H), 1.34 (d, J = 7.2 Hz, 3 H), 1.90–2.03 (m, 3 H), 2.81, 2.99 (2 s, 3 H total), 4.04–4.18 (m, 2 H), 4.41–4.57 (m, 2 H), 5.09–5.21 (m, 3 H), 5.30 (d, J = 9.8 Hz, 1 H), 6.49 (d, J = 6.8 Hz, 1 H), 7.34 (s, 5 H). Anal. Calcd for $C_{28}H_{47}N_3O_6Si$: C, 61.17; H, 8.62; N, 7.64. Found: C, 60.98; H, 8.88; N, 7.74. In other runs on approximately the same scale, using 1.2 equiv of Teoc-valine, a 70–76% yield of 12 was obtained.

***N*-(4-Methoxybenzyloxycarbonyl)valyl-*N*-methylleucylalanine Benzyl Ester (13).** Employing a dropwise addition of Pmz-Val; 1.90 g (6.20 mmol) of 8 and BOP-Cl (2.21 g, 8.68 mmol) were added to 30 mL of methylene chloride. The suspension was cooled to 0 °C under N_2, and a mixture of Pmz-Val (2.44 g, 8.68 mmol) and diisopropylethylamine (3.0 mL, 17 mmol) in 30 mL of methylene chloride was added dropwise, over 6 h, to the rapidly stirred suspension. After complete addition most BOP-Cl had dissolved. The reaction was stirred for an additional 14 h at 5 °C and then concentrated to a thick oily residue, which was applied directly to 180 g of silica gel, and eluted with 20–25–30% EtOAc/hexanes (1–1–2 L each in a step gradient), to give 2.75 g (78%) of a colorless, viscous oil: TLC R_f (A, 25%) 0.33; $[\alpha]_D$ -83.6° (c 1.0, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.84–1.06 (m, 12 H), 1.36 (d, J = 6.8 Hz, 3 H), 1.60–1.74 (m, 3 H), 1.89–2.07 (m, 1 H), 2.85, 3.00 (2 s, 3 H total), 3.83 (s, 3 H), 4.46–4.65 (m, 3 H), 5.00–5.25 (m, 4 H), 5.53 (d, J = 9 Hz, 1 H), 6.59 (d, J = 7.5 Hz, 1 H), 6.84–6.94 (m, 2 H), 7.27–7.42 (m, 7 H). Anal. Calcd for $C_{31}H_{43}N_3O_7$: C, 65.36; H, 7.61; N, 7.38. Found: C, 65.18; H, 7.85; N, 7.21.

Valyl-*N*-methylleucylalanine Benzyl Ester (14) (route A, from Pmz-Val-MeLeu-Ala-OBzl). A mixture of 13 (5.70 g, 10 mmol) and anisole (1.52 mL, 14 mmol) in 7.8 mL of dioxane was cooled to 0 °C under N_2 and treated with a precooled, 0 °C, solution of 5.8 M HCl–dioxane (17.2 mL, 100 mmol of HCl). After being stirred for 2 h at 0 °C and an additional 12 h at 5 °C, the mixture was rotary evaporated under reduced pressure (≤0.3 mmHg), employing a -78 °C trap, and allowing for evaporative self-cooling of the flask contents. After additional vacuum drying (4 h at room temperature, ≤0.3 mmHg) the mobile, light yellow oil that resulted was triturated with 4 × 50 mL of petroleum ether to give 4.42 g (100%) of the peptide amine hydrochloride as a white solid. This material was used without subsequent purification, as residual anisole and Pmz-derived impurities are really removed chromatographically in the next step.

Valyl-*N*-methylleucylalanine Benzyl Ester (14) (route B, from Alloc-Val-MeLeu-Ala-OBzl) [*Note*: Tetrakis(triphenylphosphine)palladium is oxygen-sensitive. It should be weighed rapidly and stored refrigerated under an inert atmosphere (preferably argon)]. Via essentially the literature procedure,[19a] a solution of 11 (2.982 g, 6.09 mmol) in THF (50 mL) was treated, under nitrogen, with dimedone (5.978 g, 42.65 mmol) followed by $(PPh_3)_4Pd$ (352 mg, 0.305 mmol). At 20 min, TLC analysis (25% acetone/hexanes) demonstrated the absence of starting material. At 35 min, the mixture was rotary evaporated to yield an orange residue, which was taken up in ether (100 mL), filtered, and washed with 0.5 N HCl (4 × 100 mL). The combined aqueous extracts were made basic with $NaHCO_3$ and extracted with ether (2 × 100 mL). These latter organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to yield 2.179 g (88%) of a viscous yellow oil, which was used without purification for the next condensation: TLC R_f (D) 0.58 with minor impurities as 0.74, 0.98; $[\alpha]_D$ -106.2° (c 1.0, $CHCl_3$) (lit.[15] $[\alpha]_D$ -102° (c 1.0, $CHCl_3$)); ^1H NMR (200 MHz, $CDCl_3$) δ 0.70–0.93 (m, 12 H), 0.98, 1.27 (2 d, J = 0.74, 3 H total), 1.16–1.74 (m, 4 H), 2.70, 2.95 (2 s, 3 H total), 3.65, 3.94 (2 d, J = 5.0, 7.4, 1 H total), 4.28–4.90 (m, 5 H), 4.90–5.16 (m, 2 H), 6.99 (d, J = 8.5 Hz, 1 H), 7.30 (s, 5 H).

Valyl-*N*-methylleucylalanine Benzyl Ester (14) (route C, from Teoc-Val-MeLeu-Ala-OBzl). Via general procedure A, a solution of 12 (528 mg, 0.96 mmol) in methylene chloride (0.6 mL) was treated in the cold with TFA (2 mL) and deprotected overnight. Workup by basification and organic extraction yielded, after drying and concentrating in vacuo, a green oil which was used without further purification.

***N*-(*tert*-Butyloxycarbonyl)-*N*-methylleucylvalyl-*N*-methylleucylalanine Benzyl Ester (15).** Boc-MeLeu (1.569 g, 6.40 mmol) was preactivated with BOP-Cl as described in the synthesis of 6 (route B), allowing the preactivation to run for 2 h. A solution of 14 (from deprotection of the Alloc tripeptide 11; 2.160 g, 5.36 mmol, assuming a quantitative yield from the deprotection) in CH_2Cl_2 (15 mL) was added in one portion, and the mixture was stirred overnight in a 6 °C cold room. Workup, as described in general procedure A, and chromatography on 250 g of silica gel (32% EtOAc/hexanes eluant) yielded, as a pale yellow oil, 2.192 g (65%): TLC R_f (A, 25%) 0.43, $[\alpha]_D$ -136.5° (c 1.0, $CHCl_3$) (lit.[15] $[\alpha]_D$ -126.7° (c 1.0, $CHCl_3$)); ^1H NMR (270 MHz, $CDCl_3$) δ 0.80–1.03 (m, 14 H), 1.35 (d, J = 7.8 Hz, 3 H), 1.48 (s, 9 H), 1.56–1.76 (m, 6 H), 1.94–2.10 (m, 1 H), 2.75, 2.78, 2.82, 3.00 (4 s, 3 H total), 4.41–4.68, 4.68–4.84 (2 m, 3 H total), 5.08–5.25 (m, 3 H), 6.47 (d, J = 6.8 Hz, 1 H), 6.63–6.78 (m, 1 H), 7.35 (s, 5 H).

This procedure, applied to 14 obtained by other routes, gave yields in the range of 62–71%. In one run where the Boc-MeLeu was not preactivated, a 47% yield of tetrapeptide 15 was obtained.

***N*-[(9-Fluorenylmethyl)oxy]carbonyl-*N*-methylleucylvalyl-*N*-methylleucylalanine Benzyl Ester (16)** (route A, from HCl-Val-MeLeu-Ala-OBzl). **Fmoc-MeLeu-Cl.** A solution of Fmoc-MeLeu (4.41 g, 12 mmol) in 120 mL of methylene chloride was chilled to 0 °C, under N_2. To this solution was added oxalyl chloride (2.30 mL, 26.4 mmol) in one portion followed, after several minutes, by a catalytic amount of DMF (ca. 120 μL, dropwise until a steady bubbling was observed). After 2 h the mixture was concentrated on a rotary evaporator as described for 14. After

vacuum drying for 2 h the oil that resulted was triturated to a yellow solid with 3 × 75 mL of petroleum ether at 0 °C. (Filtration through a Schlenk fritted-glass filter tube is recommended to avoid atmospheric moisture.) This material was dried under reduced pressure and used without further purification. (For characterization, a sample of the acid chloride was recrystallized from CH_2Cl_2/hexanes: mp 88.5–90.0 °C; $[\alpha]_D$ –6.8° (*c* 1.0, CH_2Cl_2); IR (CH_2Cl_2) 1780 (s, C(O)Cl), 1690 (s, O–C(O)–N).

Tetrapeptide 16. A solution of **14** (10.0 mmol assuming a quantitative yield from Pmz-tripeptide deprotection) in 30 mL of methylene chloride was cooled to 0 °C under N_2 and treated with the entire yield of Fmoc-MeLeu-Cl (12.0 mmol assuming a quantitative yield) as a solution in 30 mL of methylene chloride. The stirred solution was treated dropwise over 1 h with diisopropylethylamine (4.2 mL, 24 mmol) in 30 mL of methylene chloride. After 3 h at 0 °C the reaction was diluted with 90 mL of methylene chloride and washed with 1 M $KHSO_4$ and 50% brine. The organic layer was concentrated, diluted with diethyl ether/ethyl acetate mixture, and washed with saturated $NaHCO_3$, 50% brine, and brine. After drying ($MgSO_4$) and concentrating in vacuo, the resulting foamy white solid was immediately chromatographed on 700 g of silica gel (33% acetone/hexanes eluant) to yield 6.40 g (85%) of a foamy white solid: TLC R_f (A, 25%) 0.27; $[\alpha]_D$ –123.8° (*c* 1.0, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 0.84–1.04 (m, 18 H), 1.35 (d, *J* = 6.8 Hz, 3 H), 1.53–1.85 (m, 6 H), 1.92–2.89 (m, 6 H total), 3.00 (s, 3 H), 4.27 (t, *J* = 7.4 Hz, 1 H), 4.40–4.60 (m, 3 H), 4.73 (dd, *J* = 6.7, 7.1 Hz, 2 H total), 5.07–5.26 (m, 3 H), 6.49 (d, *J* = 6.4 Hz, 1 H), 6.63 (d, *J* = 8.8 Hz, 1 H), 7.24–7.46 (m, 9 H), 7.58 (d, *J* = 8.1 Hz, 2 H), 7.77 (d, *J* = 8.1 Hz, 2 H). Anal. Calcd for $C_{44}H_{58}N_4O_7$: C, 70.00; H, 7.74; N, 7.42. Found: C, 70.07; H, 8.03; N, 7.17.

***N*-[(9-Fluorenylmethyl)oxy]carbonyl-*N*-methylleucyl-valyl-*N*-methylleucylalanine Benzyl Ester (16)** (route B, from deprotection/coupling of the Fmoc-Val-MeLeu-Ala-OBzl). Via general procedure C, **10** (902 mg, 1.44 mmol) was deprotected and condensed with Fmoc-MeLeu (556 mg, 1.51 mmol). After workup and chromatogaphy (95 g of silica gel, 22% acetone/hexanes eluant), 274 mg (25%) of the tetrapeptide was obtained as a white foam, identical with that obtained by route A.

***N*-Methylleucylvalyl-*N*-methylleucylalanine Benzyl Ester (17).** Via general procedure B, a solution of **15** (1.956 g, 2.52 mmol) was treated with TFA (3.3 mL) and deprotected for 29 h in the cold. A viscous yellow oil was obtained, which was recrystallized from ether/hexanes (using seed crystals obtained from a sample crystallization in $^iPr_2O$/hexanes) to yield 1.190 g (86%) of white crystals: mp 73.5–75.5 °C; TLC R_f (B, 6%) 0.29; $[\alpha]_D$ –124.0° (*c* 1.0, $CHCl_3$) (lit.[15] mp 76–78 °C; $[\alpha]_D$ –130.9° (*c* 1.0, $CHCl_3$)); ^1H NMR (270 MHz, $CDCl_3$) δ 0.88–1.07 (m, 18 H), 1.36 (d, *J* = 6.8 Hz, 3 H), 1.50–1.79 (m, 6 H), 1.96–2.13 (m, 1 H), 2.48, 2.80, 3.32–3.47 (2 s, m, 6 H total), 3.32–3.47 (m, 1 H), 4.54 (q, *J* = 7.4 Hz, 1 H), 4.76 (t, *J* = 7.1 Hz, 1 H), 5.06–5.21 (m, 3 H), 6.03–6.10, 6.46–6.57 (2 m, 1 H total), 6.65 (d, *J* = 7.4 Hz, 1 H), 7.33 (s, 5 H), 7.80, 8.09 (2 d, *J* = 9.1, 8.8 Hz, 1 H total). This material is difficult to recrystallize, and in other runs was used without purification.

***N*-(*tert*-Butyloxycarbonyl)-α-aminobutyrylsarcosyl-*N*-methylleucylvalyl-*N*-methylleucylalanine Benzyl Ester (18)** (route A, from one-pot deprotection/coupling of **16**). Via general procedure C, a solution of *N*-Fmoc-protected peptide **16** (3.20 g, 6.00 mmol) in 30 mL of CH_3CN was treated with an equal volume of diethylamine while cooling on ice, under N_2. After being stirred for 3 h at 0 °C the solution was concentrated in vacuo, and the residue was treated with 20 mL of CH_3CN and again concentrated to yield a yellow oil. This oil was treated with 60 mL of methylene chloride and 2.31 mL (13.2 mmol) of diisopropylethylamine and then chilled to 0 °C under N_2. Dipeptide acid **5** (1.81 g, 6.60 mmol) and BOP-Cl (1.83 g, 7.20 mmol) were then added, sequentially, to the ice-cold stirred solution. After 5 h at 0 °C the reaction mixture was concentrated in vacuo and the residue was dissolved in diethyl ether/ethyl acetate mixture, which was extracted as described in general procedure A. After flash chromatography on 400 g of silica gel (30% acetone/hexanes eluant), 4.33 g (92%) of a light yellow, foamy solid was obtained: TLC R_f (A, 25%) 0.33, $[\alpha]_D$ –137.8° (*c* 1.0, $CHCl_3$) (lit.[15] $[\alpha]_D$ –137.9° (*c* 1.0, $CHCl_3$)); ^1H NMR (200 MHz, $CDCl_3$) δ 0.67–1.07 (m, 21 H), 1.33 (d, *J* = 7.3 Hz, 3 H), 1.42 (s, 9 H), 1.53–1.89 (m, 8 H), 2.02–2.30 (m, 1 H), 2.67–3.35 (m, 9 H), 3.74–5.26 (m, 9 H), 5.55 (d, *J* = 8.3 Hz, 1 H), [5.93 (d, *J* = 8.8 Hz), 6.51 (d, *J* = 7.0 Hz), 6.76 (d, *J* = 9 Hz), 7.91 (d, *J* = 6.0 Hz), 2 H total], 7.32 (s, 5 H). Anal. Calcd for $C_{41}H_{68}N_6O_9 \cdot 0.5H_2O$: C, 61.71; H, 8.72; N, 10.53. Found: C, 62.85; H, 8.69; N, 10.40.

***N*-(*tert*-Butyloxycarbonyl)-α-aminobutyrylsarcosyl-*N*-methylleucylvalyl-*N*-methylleucylalanine Benzyl Ester (18)** (route B, from MeLeu-Val-MeLeu-Ala-OBzl). Via general procedure A, a solution of dipeptide acid **5** (89 mg, 0.32 mmol) and amino tetrapeptide **17** (167 mg, 0.313 mmol) were coupled with BOP-Cl to yield, after workup and chromatography on 25 g of silica gel (29% acetone/hexanes eluant), 203 mg (82%) of a colorless, glassy foam: physical data was identical to that produced by route A.

L-2-Aminobutyrylsarcosyl-*N*-methylleucylvalyl-*N*-methylleucylalanine Benzyl Ester (19). Via general procedure B, **18** (868 mg, 1.10 mmol) was N-deprotected with TFA (5.5 mL) in 1.5 mL of methylene chloride for 14 h at –15 °C to yield, after workup, 680 mg (90%) of a white foamy solid, which required no further purification: TLC R_f (D) 0.27; $[\alpha]_D$ –134.5° (*c* 1.0, $CHCl_3$) (lit.[25] $[\alpha]_D$ –134.6° (*c* 1.0, $CHCl_3$)); ^1H NMR (270 MHz, $CDCl_3$) spectrum available as supplemental material.

N^{α}-[(9-Fluorenylmethyl)oxy]carbonyl-N^{ϵ}-(*tert*-butyloxycarbonyl)-D-lysyl-*N*-methylleucyl-*N*-methylleucyl-*N*-methylvaline *tert*-Butyl Ester (21). Via general procedure A, tripeptide amine **20** (1.77 g, 4.00 mmol) and N^{α}-[(9-fluorenylmethyl)oxy]carbonyl-N^{ϵ}-(*tert*-butyloxycarbonyl)-D-lysine (2.06 g, 4.40 mmol) were condensed with BOP-Cl to yield, after workup, 3.34 g (94%) of a white, foamy solid requiring no further purification: TLC R_f (A, 30%) 0.39; $[\alpha]_D$ –104.9° (*c* 0.45, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) spectrum available as supplemental material. Anal. Calcd for $C_{50}H_{77}N_5O_9$: C, 67.31; H, 8.70; N, 7.85. Found: C, 67.37; H, 8.91; N, 7.68.

N^{α}-[(9-Fluorenylmethyl)oxy]carbonyl-N^{ϵ}-(*tert*-butyloxycarbonyl)-D-lysyl-*N*-methylleucyl-*N*-methylleucyl-*N*-methylvaline (22). Via general procedure B, 280 mg (0.314 mmol) of **21** was deprotected with 5 mL of TFA for 24 h. The TFA was removed at –17 °C by distillation into a CO_2/acetone trap under high vacuum, and then the residue was treated with CCl_4 (2 mL) and again concentrated in vacuo. This residue was taken up in CH_2Cl_2 (ca. 5 mL) and treated dropwise with diisopropylethylamine until a pH of 6 was reached (moist pH paper). The solution was then treated with additional diisopropylethylamine (66 μL, 0.38 mmol), followed by 83 mg (0.38 mmol) of di-*tert*-butyl dicarbonate, and the mixture was stirred for 20 h under N_2. The solution was then poured into ether (15 mL), and the organics were washed with 5% $KHSO_4$ (2×), water, and brine, dried over $MgSO_4$, and concentrated in vacuo to a brown foam. After chromatography on 25 g of silica gel (packed in neat CH_2Cl_2, 5.5% MeOH/CH_2Cl_2 eluant), 240 mg (91%) of a light tan foam was obtained: TLC R_f (B, 7.5%) 0.45; $[\alpha]_D$ –109.5° (*c* 1.0, $CHCl_3$) (lit.[25] $[\alpha]_D$ –129.1° (*c* 1.0, $CHCl_3$)); ^1H NMR (270 MHz, $CDCl_3$) spectrum available as supplemental material. Anal. Calcd for $C_{46}H_{69}N_5O_9$: C, 66.08, H, 8.32; N, 8.38. Found: C, 65.89; H, 8.57; N, 8.02.

(4*S*,5*R*,1′*R*,3′*E*)-2,2,3-Trimethyl-5-(1′-methyl-3′-pentenyl)-4-(oxazolidinylcarbonyl)-L-2-aminobutyrylsarcosyl-*N*-methylleucylvalyl-*N*-methylleucylalanine Benzyl Ester (23). Via a literature procedure,[15] 161 mg (0.80 mmol) of MeBmt was suspended in 250 mL of freshly distilled acetone (Aldrich HPLC grade, previously stored for 24 h over 4-Å molecular sieves) and heated to reflux, under N_2 for 20 h. The resulting clear solution was concentrated in vacuo down to a 6-mL volume and added to 12 mL of THF. This mixture was treated successively with *N*-methylmorpholine (100 μL, 0.88 mmol), 1-hydroxybenzotriazole (238 mg, 1.76 mmol), and hexapeptide amine **19** (606 mg, 0.88 mmol). The solution was cooled to 0 °C under a N_2 blanket, treated with DCC (182 mg, 0.88 mmol) in one portion, and stirred vigorously at 0 °C until homogeneous. It was then warmed to room temperature and stirred an additional 14 h, after which time DCU, which had precipitated, was removed by filtration and washed with small portions of methylene chloride. The combined filtrate was washed with saturated $NaHCO_3$ and dried over $MgSO_4$. Concentration in vacuo and resuspension of the residue in ethyl acetate yielded more DCU. The residue remaining after a second filtration and concentration in vacuo was purified by

chromatography on 50 g of silica gel (10–20–30% acetone/hexanes). Trituration of the foamy yellow product with pentane yielded 670 mg (92% based on MeBmt) of a white, foamy solid: TLC R_f (C, 20%) 0.36; $[\alpha]_D$ −128.4° (*c* 1.0, $CHCl_3$) (lit.[25] $[\alpha]_D$ −126° (*c* 1.0, $CHCl_3$)); ^1H NMR (200 MHz, $CDCl_3$) spectrum available as supplemental material.

(2*S*,3*R*,4*R*,6*E*)-3-Hydroxy-4-methyl-2-(methylamino)-6-octenoyl-L-2-aminobutyrylsarcosyl-*N*-methylleucylvalyl-*N*-methylleucylalanine Benzyl Ester (24). Via a literature procedure,[15] a solution of **23** (570 mg, 0.625 mmol) in 10 mL of MeOH was treated with 2.5 mL of 1.0 M HCl and stirred for 12 h at room temperature. The mixture was treated with $NaHCO_3$ (710 mg) and concentrated in vacuo to a white solid. This residue was slurried in several milliliters of 4% MeOH/CH_2Cl_2, and the soluble portion was applied directly to 54 g of silica gel and then eluted with 4% MeOH/CH_2Cl_2 to yield 480 mg (89%) of a white, foamy solid: TLC R_f (B, 10%) 0.48; ^1H NMR (270 MHz, $CDCl_3$) spectrum available as supplemental material.

N^{α}-[(9-Fluorenylmethyl)oxy]carbonyl-N^{ϵ}-(*tert*-butyloxycarbonyl)-D-lysyl-*N*-methylleucyl-*N*-methylleucyl-*N*-methylvalyl-(2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl-L-2-aminobutyrylsarcosyl-*N*-methylleucylvalyl-*N*-methylleucylalanyl Benzyl Ester (25). Via essentially a literature procedure,[15] a solution of tetrapeptide acid **22** (253 mg, 0.303 mmol) and heptapeptide amine **24** (252 mg, 0.289 mmol) in 6 mL of methylene chloride was treated under N_2 with BOP (Castro's reagent, 192 mg, 0.433 mmol) followed by NMM (33.4 μL, 0.303 mmol). The flask was flushed with N_2, sealed tightly, and stirred at room temperature. Reaction progress was monitored by TLC (10% MeOH/CH_2Cl_2, 4% phosphomolybdic acid/EtOH visualization) and had ceased after 3 days. At that time, the mixture was concentrated in vacuo to a thick oil, which was applied directly to 29 g of silica gel and eluted with 0–10% MeOH/CH_2Cl_2 (100 mL each of a 2% step gradient). Fractions containing product (a mixture of diastereomers inseparable in this solvent system) were pooled, concentrated, and rechromatographed on 29 g of silica gel, eluted with 10–25% acetone (freshly distilled) in hexanes (800–500–1500–800 mL at a 5% step gradient), to give 270 mg (55%) of desired product and 53 mg of a side product having a higher R_f. Fractions containing heptapeptide were also pooled and concentrated, triturated with 4 × 10 mL of pentane, and dried in vacuo. This residue (representing about 35% recovery) was recycled through the coupling procedure, and the purification process was repeated. Residual starting material from the second cycle was also recovered and recycled. After purification on silica gel (acetone/hexanes) the combined total yield was 27 mg of byproduct and 310 mg (64%) of undecapeptide recovered as a white, foamy solid: TLC R_f (E, 20%) 0.37 (enlongated spot); $[\alpha]_D$ −154.8° (*c* 1.0, $CHCl_3$) (lit.[25] $[\alpha]_D$ −143.8° (*c* 1.0, $CHCl_3$)); ^1H NMR (300 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (DTT/DTE matrix) *m/z* $[M + H]^+$ 1690; HR-FABMS exact mass calcd for $C_{92}H_{145}N_{12}O_{17}$ $[M + H]^+$ 1690.0850, found 1690.0844. Anal. Calcd for $C_{92}H_{144}N_{12}O_{17}$: C, 65.38; H, 8.59; N, 9.94. Found: C, 65.13; H, 8.65; N, 9.66. HR-FABMS for higher R_f side product (DTT/DTE matrix) exact mass calcd for presumed diastereomer of **25**: $[M + H]^+$ 1690.0850, found 1690.0886.

[N^{ϵ}-(*tert*-Butyloxycarbonyl)-D-lysyl[8]]cyclosporine (26). In a modification of the patented procedure,[25] a solution of the protected undecapeptide **25** (110 mg, 0.065 mmol) in 3 mL of ethanol was flushed with N_2 and cooled to 0 °C. The mixture was treated with 0.72 mL of 0.2 M NaOH and stirred for 1.5 h, treated with a second portion of NaOH (0.36 mL), and stirred for an additional 3.5 h. The mixture was then brought to pH 6 using 0.2 M HCl (ca. 1.1 mL), treated with brine (11 mL), and extracted with methylene chloride (30 mL then 4 × 15 mL) (*Note*: If the aqueous pH is greater than 6 an intractable emulsion may result). The combined organics were dried over $MgSO_4$ and concentrated in vacuo to a yellow oil. This residue was taken up in 308 mL of DCM and treated sequentially with 4-(dimethylamino)pyridine (44 mg, 0.36 mmol) and propylphosphonic anhydride (48 μL of a 50% solution in CH_2Cl_2, 0.099 mmol of trimer). The solution was stirred for 2 days at room temperature, concentrated in vacuo to a yellow oil, which was applied directly to 12 g of silica gel and eluted with 10–15–20% acetone (freshly distilled) in hexanes, and then rechromatographed using 1–2–3% MeOH/CH_2Cl_2 as eluant to give 60 mg (68%) of a white amorphous solid: TLC R_f (B, 4%) 0.30; TLC R_f (C, 43%) 0.39; $[\alpha]_D$ −240.8° (*c*, 0.12, $CHCl_3$) (lit.[25] $[\alpha]_D$ −198.3° (*c* 1.0, $CHCl_3$)); ^1H NMR (500 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (HFBA matrix) *m/z* $[M + H]^+$ 1361; HR-FABMS exact mass calcd for $C_{70}H_{127}N_{12}O_{14}$ $[M + H]^+$ 1359.9594, found 1359.9606. Anal. Calcd for $C_{70}H_{126}N_{12}O_{14}\cdot 0.5H_2O$: C, 61.42; H, 9.35; N, 12.28. Found: C, 61.39; H, 9.28; N, 11.93.

[D-Lysyl[8]]cyclosporine (27). Via general procedure B, compound **26** (40 mg, 29 μmol) was deprotected in 0.1 mL of methylene chloride and 0.9 mL of TFA to yield, after workup and purification by preparative thick-layer chromatography (0.5 mm × 20 cm × 20 cm plate), using TLC eluant F, 34 mg (91%) of a white, foamy solid: TLC R_f (F) 0.67; $[\alpha]_D$ −197.0° (*c* 0.2, $CHCl_3$) (lit.[25] $[\alpha]_D$ −204.3° (*c* 0.2, $CHCl_3$)); ^1H NMR (500 MHz, $CDCl_3$) spectrum available as supplemental material. FABMS (3-NBA/Gly matrix) *m/z* $[M + H]^+$ 1260; HR-FABMS exact mass calcd for $C_{65}H_{119}N_{12}O_{12}$ $[M + H]^+$ 1259.9070, found 1259.9109.

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Registry No. **2**, 68641-49-6; **4**, 81135-22-0; **5**, 81135-23-1; **6**, 81135-24-2; **7**, 125714-53-6; **8**, 81135-25-3; **9**, 81135-26-4; **10**, 125714-54-7; **11**, 125734-65-8; **12**, 125714-55-8; **13**, 125714-56-9; **14** (free base), 81135-27-5; **14**·HCl, 125714-59-2; **15**, 81143-02-4; **16**, 125714-57-0; **17**, 81135-28-6; **18**, 81135-29-7; **19**, 81135-30-0; **20**, 103478-69-9; **21**, 125714-58-1; **22**, 104186-80-3; **23**, 81135-32-2; **24**, 81135-33-3; **25**, 104186-79-0; **26**, 104186-76-7; **27**, 104200-97-7; MeBmt, 59865-23-5; BOC-Abu-OH, 34306-42-8; H-Sar-OBzl, 54384-05-3; BOC-MeLeu-OH, 53363-89-6; H-Ala-OBzl, 17831-01-5; Fmoc-MeLeu-OH, 103478-62-2; BOC-Val-OH, 13734-41-3; Fmoc-Val-Cl, 103321-53-5; Fmoc-Val-OH, 68858-20-8; Alloc-Val-OH, 115491-96-8; Teoc-Val-OH, 113306-60-8; PMZ-Val-OH, 4125-82-0; Fmoc-MeLeu-Cl, 125714-60-5; Fmoc-D-Lys(Boc)-OH, 92122-45-7; Me_2CO, 67-64-1.

Supplementary Material Available: NMR spectra of key intermediates and D-Lys[8]-CsA (10 pages). Ordering information is given on any current masthead page.