Total Synthesis of Cyclosporin O Both in Solution and in the Solid Phase Using Novel Thiazolium-, Immonium-, and Pyridinium-Type Coupling Reagents: BEMT, BDMP, and BEP[1]

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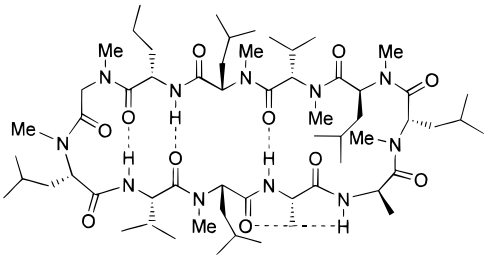
Received October 27, 1999

Cyclosporin O (**1**), an extensively N-methylated immunosuppressive cyclic undecapeptide isolated from *Tolypocladium inflatum* Gams, was synthesized in 20−23% overall yield via 4 + 7 segment condensation and cyclization by the combined utilization of novel thiazolium- and immonium-type peptide coupling reagents 2-bromo-3-ethyl-4-methyl thiazolium tetrafluoroborate (BEMT) and 5-(1*H*-benzotriazol-1-yloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate (BDMP) as well as compound 2-bromo-1-ethyl pyridinium tetrafluoroborate (BEP). BEMT and BEP, which have been proven to be very efficient for the coupling of peptide segments containing N-alkylated amino acid residues with respect to the fast reaction speed, low racemization, and high yields, were used to construct hindered amide bonds in CsO with the addition of HOAt, whereas the most efficient HOBt-derived immonium type reagent, BDMP, was used to perform the coupling of coded amino acids in CsO. Thus, the highly hindered protected 8−11 tetrapeptide **25** was successfully synthesized using BEMT in 65% yield, and the 1−7 heptapeptide **21** was obtained in 52−55% yield by the rationally combined utilization of BDMP, BEMT, and BEP. The synthesis of the linear undecapeptide **27** of CsO in the solid phase using BEMT and BEP was accomplished for the further evaluation of the effectiveness of these reagents.

Introduction

Cyclosporins (Cs), produced as secondary fungal metabolites by *Cylindrocarpon lucidum* Booth and *Tolypocladium inflatum* Gams, are a family of neutral, homodetic, hydrophobic, and immunosuppressive cyclic undecapeptides containing five to seven *N*-methylamino acids.[2] Cyclosporin A (CsA) as the most important member of this family has been well studied and widely used for the prevention of rejection in transplantation surgery.[3] However, the total synthesis of CsA is more cumbersome and expensive than the isolation from fungal metabolites owing to, in particular, the difficult synthesis of MeBmt, an unusual amino acid in position 1 of CsA containing three chiral carbon.[4] Cyclosporin O (CsO), another member of Cs family, is the only analogue not containing MeBmt, but it still exhibits strong immunosuppressive activity.[5] Furthermore, CsO bearing in position 2 a norvaline makes it much less nephrotoxic than CsA.[6] Although it has been enzymatically synthesized by Lawen et al.[5] in *Beauveria nivea* by cyclosporin synthetase, the chemical synthesis has not appeared so far. Therefore, with the seeking for highly active and less nephrotoxic analogues through SAR studies, the chemical synthesis of CsO is demanded.



1 Cyclosporin O (CsO)

It is obvious that the facile synthesis of CsO will rely mainly on the efficiency of coupling reagents for the

(1) Nomenclature and symbols of amino acids and peptides generally follow the recommendations of the IUPAC−IUB Joint Commission of Biochemical Nomenclature in: *Pure Appl. Chem.* **1984**, *56*, 595−624. The following additional abbreviations are used: Aib, α-aminoisobutyric acid; AOMP, 5-(1*H*-7-azabenzotriazol-1-yloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate; BDMP, 5-(1*H*-benzotriazol-1-yloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate; BEMT, 2-bromo-3-ethyl-4-methylthiazolium tetrafluoroborate; BEP, 2-bromo-1-ethyl pyridinium tetrafluoroborate; BOMI, *N*-(1*H*-benzotriazol-1-ylmethylene)-*N*-methylmethanaminium hexachloroantimonate *N*-oxide; BOP, (1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; BPMP, 1-(1*H*-benzotriazol-1-yloxy)phenylmethylenepyrrolidinium hexachloroantimonate; BTFFH, 1,1,3,3-bis(tetramethylene)florouronium hexafluorophosphate; CMMM, chloro(4-morphoino)methylenemorpholinium hexafluorophosphate; DIEA, *N*, *N*′-diisopropylethylamine; HAPyU, 1-(1-pyrrolidinyl-1*H* -1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene)pyrrolidinium hexafluorophosphate *N*-oxide; HBPyU, *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-bis(tetramethylene)-uronium hexafluorophosphate; HBTU, *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HOBt, 1-hydroxybenzotriazole; MeBmt, (4*R*)-4-[(2′*E*)-butenyl]-4,*N*-dimethyl-L-threonine; PyBroP, bromotripyrrolidinophosphonium hexafluorophosphate.

(2) (a) Traber, R.; Loosli, H.-R.; Hofmann, H.; Huhn, M.; Wartburg, A. *Helv. Chim. Acta* **1982**, *65*, 1655−1677. (b) Traber, R.; Kuhn, M.; Loosli, H.-R.; Pache, W.; Wartburg, A. *Helv. Chim. Acta* **1977**, *60*, 1568−1578. (c) Inouye, H.; Jaenicke, L.; Lounasmaa, M.; Marner, F.-J.; SÚquin, U.; Somersalo, P.; Uesato, S.; Wenger, R. M. In *Progress in the Chemistry of Organic Natural Products: Cyclosporin and Analogues-isolation and Synthesis-Mechanism of Action and Structural Requirements for Pharmacological Activity*; Herz, W., Grisebach, H., Kirby, G. W., Tamm, C., Eds.; Springer-Verlag: New York, 1986; Vol. 50, pp 123−168.

(3) (a) Borel, J. F. In *Cyclosporine A*; White, D. J. G., Ed.; Elsevier Biomedical: Amsterdam, 1982; pp 5−17. (b) Loosli, H.-R.; Kessler, H.; Oschkinat, H.; Weber, H.-P.; Petcher, T.; Widmer, A. *Helv. Chim. Acta* **1985**, *68*, 682−704. (c) Kessler, H.; Loosli, H.-R.; Oschkinat, H. *Helv. Chim. Acta* **1985**, *68*, 661−681.

(4) Wenger, R. M. *Helv. Chim. Acta* **1983**, *66*, 2308−2321.

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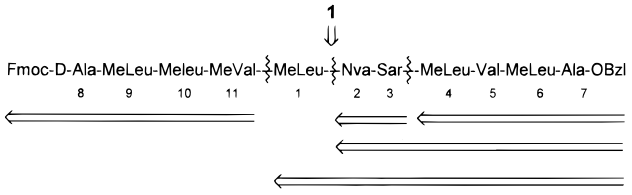
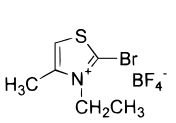
10.1021/jo991687c CCC: $19.00 © 2000 American Chemical Society
Published on Web 04/21/2000

Scheme 1. Synthetic Strategy of Cyclosporin O

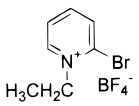


construction of the highly hindered amino acid residues in the molecule.

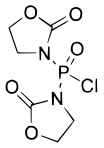
Recently, we have exploited several new types of coupling reagents.[7] Among them, thiazolium-type reagent 2-bromo-3-ethyl-4-methyl thiazolium tetrafluoroborate (BEMT, **2**) and pyridinium salt 2-bromo-1-ethyl pyridinium tetrafluoroborate (BEP, **3**) were shown to be very effective for the coupling of *N*-methyl or C_α,C_α-dialkyl amino acids with high reaction speed, low epimerization, and excellent yields in the presence of HOAt.[8] In our previous studies, we also demonstrated the high efficiency and lower side reactions of the immonium salt 5-(1*H*-benzotriazol-1-yloxy)-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate (BDMP, **9**) during the coupling of normal coded amino acids.[9] Taking advantages of these reagents, we successfully synthesized the cyclic undecapeptide cyclosporin O both in solution and in solid phase.



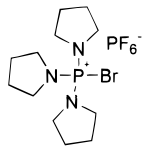
2 BEMT



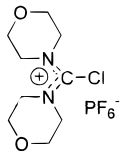
3 BEP



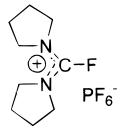
4 BOP-Cl



5 PyBroP



6 CMMM



7 BTFFH

Results and Discussion

The synthesis of cyclosporin O, shown in Scheme 1, was accomplished via a 4 + 7 fragment condensation and cyclization with the choice of ring disconnection between the L-alanine and the D-alanine.[10]

The 8−11 tetrapeptide fragment **25** is constructed by the racemization-minimizing C to N terminal elongation according to the strategy described by Rich et al.[11] rather than the racemization-prone N to C elongation.[12] The synthesis of the 1−7 heptapeptide was carried out using [2 + 4] + 1 segment coupling wherein the *N*-terminal amino acid, greatly affecting the biological activity of Cs, was introduced at the final step in facilitating the variation at that position during the synthesis of the analogues of cyclosporin O or CsA. The Fmoc group was chosen as a unique amino protecting group for the ready availability of racemization-free Fmoc N-methylated amino acids,[13] the easy detection of products by UV monitoring during coupling, and the applicability for solid-phase synthesis.

Table 1. Comparison of Reactivity and Racemization of Halogenerated Coupling Reagents Using HPLC[a]

coupling reagent	reactivity		racemization
	yield[b] (%)	$t^{1/2}$ (min)	D-isomer (%)
PyBroP	6.10	79	22.3
BOP-Cl	5.34	∼90[d]	4.13
CMMM	2.31	>120	31.4
BTFFH	9.35	49	25.9
BEMT	45.9	−[e]	2.72
BEMT[c]	84.3	<2[e]	1.33
BEP	50.8	<2[e]	4.59
BEP[c]	75.9	<2[e]	1.42

[a] Model reaction: Z-Gly-Phe-OH + Val-OCH_3·HCl → Z-Gly-Phe-Val-OCH_3. Reaction conditions: solvent CH_2Cl_2 (0.1 M); T −10 °C; base DIEA. [b] Reaction time t = 2 min. [c] HOAt (1 equiv) was added as an additive. [d] The $t^{1/2}$ value of BOP-Cl cannot be evaluated accurately due to its bad solubility in CH_2Cl_2. [e] The coupling reactions were accomplished within 2 min.

Evaluation and Selection of Coupling Reagents. To improve the effectiveness for the synthesis of CsO, several novel coupling reagents have been examined. The segment condensation of Z-Gly-Phe-OH with Val-OCH_3·HCl was selected as the model reaction with monitoring by HPLC to evaluate the efficiencies of the novel reagents BEMT, BEP, as well as the corresponding halogenerated coupling reagents, such as halouronium and halophosphonium salts. As shown in Table 1, reagents BEMT and BEP were much more reactive than commonly used reagents PyBroP,[14] BTFFH,[15] BOP-Cl,[16] and CMMM. The coupling was accomplished within 2 min under the tested reaction conditions. In addition, the coupling yield could be dramatically improved if predried solvent was used and base was added slowly at lower temperature. It was found that the racemization of product using BEMT and BEP was much lower than other halogenerated coupling reagents except BOP-Cl. Their efficiency could be further improved with the addition of HOAt, especially in the racemization-prone segment condensation.

The effectiveness of these reagents in the coupling of peptide segments containing *N*-methyl and α,α-dialkyl amino acids was also evaluated using the model reactions, Z-MeVal-OH + MeVal-OCH_3·HCl → Z-MeVal-MeVal-OCH_3 and Z-Aib-OH + Aib-OCH_3·HCl → Z-Aib-Aib-OCH_3, and monitoring by HPLC and ^{1}H NMR.[17] It was observed that the hindered amide bonds can be efficiently coupled using BEMT and BEP with fast reaction speeds, excellent yields, and low racemization. Their effectiveness was approved further by the synthesis of a series of oligopeptides containing sterically hindered amino acid residues as shown in Table 2.

(7) (a) Li, P.; Xu, J. C. *Tetrahedron Lett.* **1999**, *40*, 3605−3608. (b) Li, P.; Xu, J. C. *Tetrahedron Lett.* **1999**, *40*, 8301−8304.
(8) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.
(9) Li, P.; Xu, J. C. *Chin. J. Chem.* **2000**, *18*, 85−93.
(10) Wenger, R. M. *Helv. Chim. Acta* **1984**, *67*, 502−525.
(11) Tung, R. D.; Dhaon, M. K.; Rich, D. H. *J. Org. Chem.* **1986**, *51*, 3350−3354.
(12) Wenger, R. M. *Helv. Chim. Acta* **1983**, *66*, 2672−2702.
(13) Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1983**, *48*, 77−81.
(14) Coste, J.; Frerot, E.; Jouin, P. *J. Org. Chem.* **1994**, *59*, 2437−2446.
(15) El-Faham, A. *Chem. Lett.* **1998**, 671−672.
(16) Van der Auwera, C.; Anteunis, M. J. O. *Bull. Soc. Chim. Belg.* **1986**, *95*, 203−205.
(17) The quantity of the D−L isomer can be calculated from the four sharp signals of OMe group. The diastereoisomers: Z-L-MeVal-L-MeVal-OMe, Z-D-MeVal-L-MeVal-OMe, and Z-D,L-Me-Val-L-Me-Val-OMe were synthesized, respectively, to determine the chemical shifts and proportions of the OMe signals of the four conformers of each isomer.

Table 2. Synthesis of Oligopeptides Using BEMT and BEP

peptide[a]	reagent	yield[b] (%)
Z-MeVal*-MeVal-OCH_3	BEMT	88.2
Boc-Val-Val-MeVal*-Pro-Pro-OBzl	BEP	87.5
Z-Aib*-Aib-OCH_3	BEMT	94.6
Z-MeVal*-MeVal-OCH_3	BEP	95.4
Fmoc-Meleu*-MeVal-OBzl	BEMT	89.4
Fmoc-D-Ala*-MeLeu-MeLeu-MeVal-OBzl	BEP	94.3

[a] The amide bond formed in the peptide is indicated with an *. [b] Isolated yield based upon N-protected amino acid except the last example.

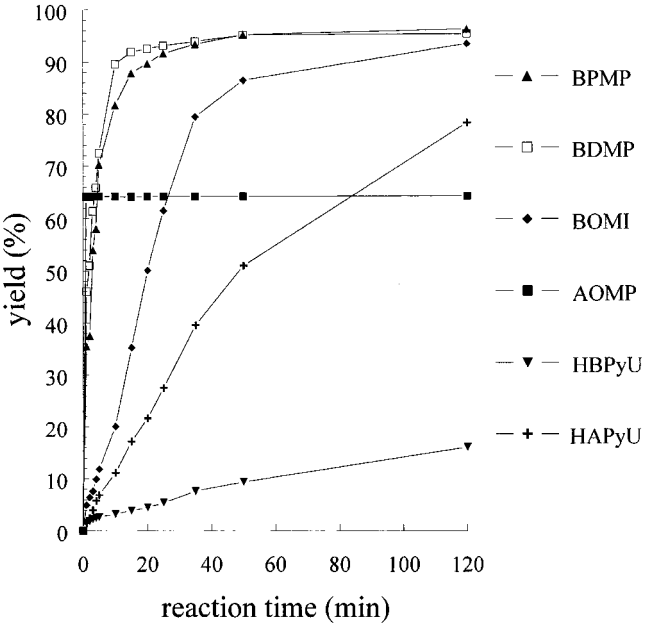


Figure 1. Comparison of reactivity of immonium salts vs HBPyU and HAPyU. Model reaction: Z-Gly-Phe-OH + Val-OCH_3·HCl → Z-Gly-Phe-Val-OCH_3. Reaction conditions: T −10 °C, base 2,6-lutidine, solvent THF, substrate ratio: N-protected amino acid/amino acid ester hydrochloride/coupling reagent/base = 1/1.1/1.1/3 (mol).

On the basis of studies and the above results, BEMT and BEP were chosen as the coupling reagents for the syntheses of hindered amide bonds in cyclosporin O.

The alternate, as the method of choice, HOBt-, HOAt-, and HOPfp-based immonium salts **8**−**12** were selectively used for the coupling of normal coded amino acids with extremely high reactivity and very low racemization.

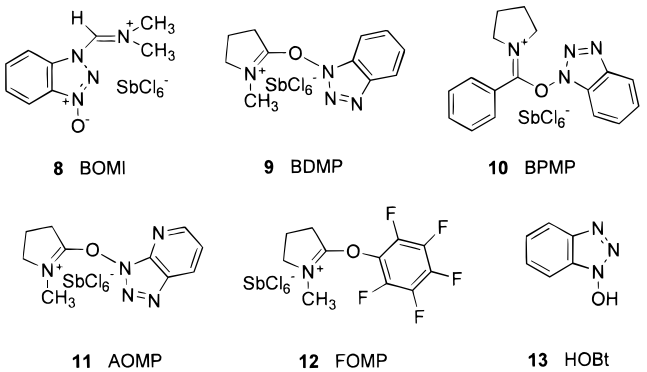


Figure 1 showed that reagents BOMI,[18,19] BDMP, and BPMP were more reactive than uronium/aminium salt HBPyU,[20] even HAPyU, which was broadly regarded as the most efficient coupling reagent up to now.[21,22] Among these HOBt-based immonium-type reagents, the extremely high reactivity of BDMP was presumably due to the tension of intraannular imide bond, which was verified by computer calculations using PCMODEL software.[23] The reactivity of FOMP **12** was rather low, even lower than HBPyU. The reason for the poor coupling yield of AOMP seemed that the extremely high reactivity of AOMP led it to decompose prior to the activation of N-protected amino acid in the presence of base, such as 2,6-lutidine.

Table 3. Comparison of Racemization of Immonium Salts with Other Coupling Reagents[a]

coupling reagent	racemization (DL[b] (%))	
	HPLC method	Young's test
DCCI	19.7	72.1
BOP	9.6	39.6
HBTU	9.8	24.3
HBPyU	7.9	18.0
HBPipU	8.9	20.5
HAPyU	5.7 (3.3[c])	13.9
BOMI	6.4 (3.1[c])	8.8[c]
BPMP	4.8 (2.3[c])	5.4[c]
BDMP	4.6 (2.2[c])	5.3[c]
AOMP	(1.6[c])	3.1[c]

[a] All reactions were carried out under the same conditions that were commonly used for the onium reagents such as BOP, HBTU, and HBPyU.[20a] [b] DL % equal to D-isomer content % multiplied by 2. [c] Reaction conditions were the same as those used in Figure 1 that were suitable for the HOBt-derived immonium-type coupling reagents.

The racemization of these immonium salts was also examined by HPLC and Young's test.[24] It could be seen from Table 3 that BDMP exhibited minimum racemization by comparing with other conventional reagents. Using these reagents, we successfully synthesized a series of oligopeptides and biologically active peptides both in solution and solid phase with low racemization, satisfactory yields and convenient workup.[7a,9] As a consequence, BDMP was selected as the reagent for the coupling of which the amino component was coded amino acid during the synthesis of CsO.

Synthesis of Cyclosporin O. The total synthesis of CsO was carried out following the strategy shown in Scheme 1. The synthesis of the 1−7 heptapeptide **21** was carried out by [2 + 4] + 1 segment condensation (Scheme 2). The Fmoc-Nva-Sar-OBzl **15** was synthesized using BEP in nearly quantitative yield by a one-pot procedure. The benzyl group in **15** was removed by hydrogenolysis to give Fmoc-Nva-Sar-OH **16**. Fmoc-MeLeu-Ala-OBzl **17**

(18) X-ray analysis has shown that BOMI crystallized in N-acylated form. Crystal data for $C_9H_{11}N_4OCl_6Sb$ **BOMI**: M = 525.68, triclinic, a = 9.058(2) Å, b = 15.729(4) Å, c = 6.598(1) Å, α = 94.21(2)°, β = 98.99(2)°, γ = 73.38(2)°, V = 889.4(4) Å^3, T = 293 K, space group $P1$(#2), Z = 2, μ = 24.50 cm^{-1}, D_c = 1.963 g cm^{-3}, 2943 reflections measured, 2727 were unique (R_{int} = 0.076). The final R (R_w) values were 0.045 (0.057) for 2247 reflections [$I > 3.00\sigma(I)$] and 190 variables.

(19) For analogous BDMP, BPMP, and AOMP of which X-ray data have not yet been obtained, the structural representations have arbitrarily been assigned as the O-substituted forms, and their nomenclature was also retained in this paper.

(20) (a) Chen, S. Q.; Xu, J. C. *Tetrahedron Lett.* **1992**, *33*, 647−650. (b) Coste, J.; Frerot, E.; Jouin, P. *Tetrahedron Lett.* **1991**, *32*, 1967−1970.

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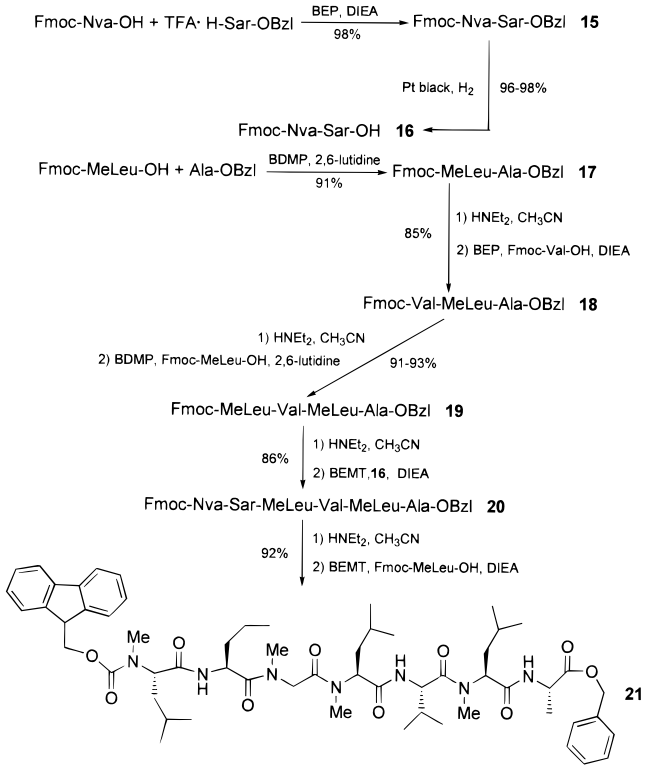
(24) Williams, M. W.; Young, G. T. *J. Chem. Soc.* **1963**, 881−889.

Scheme 2. Synthesis of CsO 1−7 Fragment

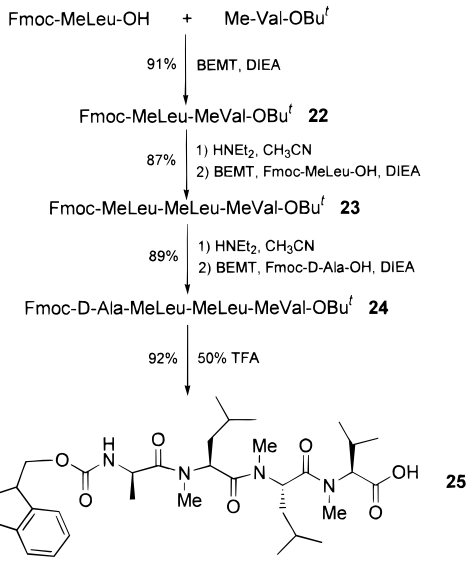


was synthesized using BDMP in which Ala-OBzl·Tos can be used directly, but the yield was diminished from 91% to 80% in comparison with the use of Ala-OBzl. After removal of Fmoc in dipeptide **17** with diethylamine, the N-deprotected dipeptide was reacted with Fmoc-Val-OH using BEP to afford tripeptide Fmoc-Val-MeLeu-Ala-OBzl **18**. Subsequently, the tetrapeptide Fmoc-MeLeu-Val-MeLeu-Ala-OBzl **19** was obtained by the condensation of N-deprotected tripeptide **18** with Fmoc-MeLeu-OH using BDMP in 91−93% yield. Hexapeptide **20** was obtained by 4 + 2 segment coupling using BEMT in 86% yield without the addition of HOAt since Sar is not a chiral amino acid. Finally, the heptapeptide **21** was obtained from the condensation of N-deprotected **20** with Fmoc-MeLeu-OH using BEMT in 92% yield. Thus, the heptapeptide Fmoc-MeLeu-Nva-Sar-MeLeu-Val-MeLeu-Ala-OBzl was constructed in 52−55% overall yield by the rationally selective utilization of the novel immonium-, thiazolium-, and pyridinium-type reagents.

The synthesis of the 8−11 tetrapeptide fragment was accomplished using BEMT. To suppress the spontaneous formation of diketopiperazine,[12] the C-terminal was protected as *tert*-butyl ester. Thus, the tripeptide Fmoc-MeLeu-MeLeu-MeVal-OBu^t **23** was obtained from dipeptide **22** in 87% yield. After sequential deprotection and coupling of **23** with Fmoc-D-Ala-OH, tetrapeptide **24** was obtained. Finally the *tert*-butyl ester was removed by the treatment with 50% TFA at low temperature to afford **25** in 92% yield. Thus, the highly hindered tetrapeptide **25** was obtained in 65% overall yield using BEMT (Scheme 3).

The tetrapeptide **25** was also synthesized in an alternative way using BEP, in which the C-terminal was protected as a benzyl ester. A 48% yield was still obtained during the synthesis of Fmoc-MeLeu-MeLeu-MeVal-OBzl by sequential deprotection and coupling of Fmoc-MeLeu-MeVal-OBzl with Fmoc-MeLeu-OH. However, the same

Scheme 3. Synthesis of CsO 8−11 Fragment



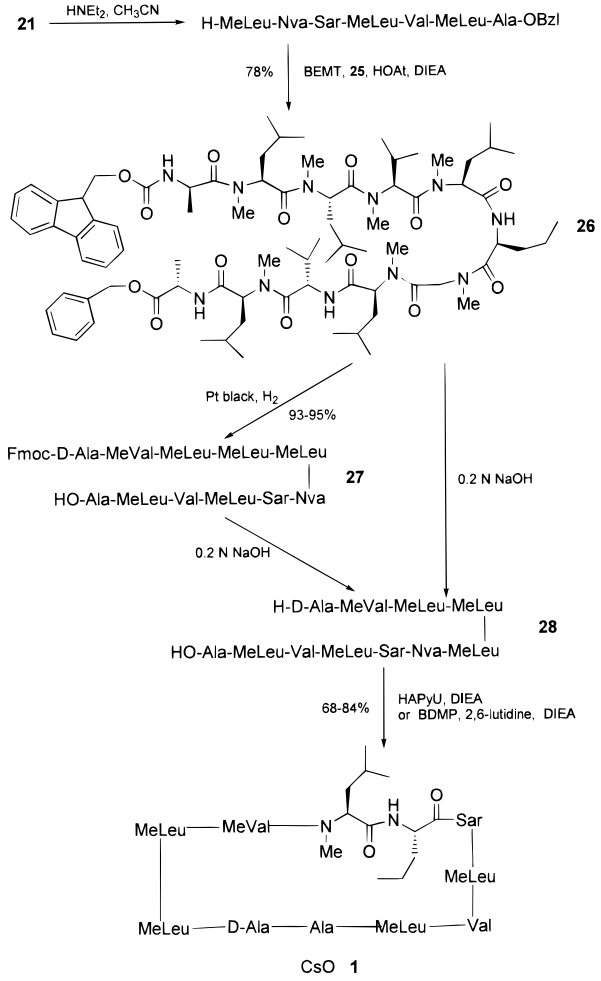
tripeptide sequence was not obtained at all during the synthesis of cyclosporin A using a modified mixed pivalic anhydride method according to the literature[12] due to the spontaneous formation of diketopiperazine.

Finally, the linear undecapeptide **26** was synthesized via 4 + 7 segment coupling in 78% yield using BEMT. HOAt was added to suppress the racemization of MeVal at position 11. Then, the benzyl ester of peptide **26** was removed by hydrogenolysis with Pt black to give peptide **27** in 93−95% yield. After removal of Fmoc by the treatment with 0.2 M NaOH, the N-free undecapeptide **28** was cyclized in a 0.2 mM dilute solution using HAPyU or BDMP to give cyclosporin O **1** in 68−84% yield (Scheme 4). The deprotected undecapeptide **28** can also be obtained from peptide **26** according to the known procedure.[25] Thus, CsO was obtained in 20−23% overall yield starting from corresponding amino acid derivatives. The structure of the final product was verified by ^{1}H NMR, ^{13}C NMR, ESI-MS, and IR.

Synthesis of Cyclosporin O in the Solid Phase. In establishing the structure/immunosuppressive activity relationship, it is essential to have a large number of Cs analogues with structural diversity to address the issues generated from the emerging knowledge of interactions of Cs with its receptors cyclophilin and calcineurin. These encouraged us to synthesize cyclosporin analogues in the solid phase. Also, for the further judgment of the usefulness of reagents BEMT and BEP, we synthesized the linear undecapeptide of CsO in the solid phase. The commercially available Wang resin was chosen as the solid support. The first residue Fmoc-Ala-OH was anchored using DCC/HOAt/DMAP in CH_2Cl_2. Considering the inapplicability of the ninhydrin test for monitoring the coupling toward *N*-methylamino acids, the double coupling strategy (2 × 3 h) was arbitrarily adopted. The cleavage of peptide from resin was carried out using 50% TFA in CH_2Cl_2 at low temperature (−15 to −18 °C). Although the results of UV analysis[26] during Fmoc deprotection indicated that the coupling yield of each step was above 90%, the purity of the final linear undecapeptide was far below the theoretical value, only approxi-

(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.

Scheme 4. Synthesis of Cyclosporin O



mately 5% shown by the ESI-MS spectrum. It is most likely that the amide bonds between hindered amino acids are prone to undergo hydrolysis under strong acidic medium.[27]

The extremely hindered 8−11 tetrapeptide was also synthesized in the solid phase using BEP and BEMT from Fmoc-MeVal-Wang resin. The purity of the obtained crude peptide was 95% and 74%, respectively, although peptides such as this sequence were in favor of the formation of diketopiperrazine during N-deprotection of the dipeptide resin.[28]

Conclusions

The synthesis of the immunosuppressive cyclic undecapeptide cyclosporin O has been achieved in 20−23% overall yield using novel coupling reagents BEMT, BDMP, and BEP. This demonstrated that thiazolium-type reagent BEMT and pyridinium type reagent BEP were very compatible on the syntheses of peptides containing hindered N-alkylated amino acid residues with fast reaction speeds and low racemization, whereas the most efficient coupling reagent among the HOBt-derived immonium salts, BDMP, was used for the coupling of coded amino acids. The effectiveness of these reagents were demonstrated by the easy synthesis of the highly hindered protected 8−11 tetrapeptide fragment in 65% overall yield using BEMT, and the 1−7 heptapeptide in 52−55% overall yield by the rationally combined utilization of BDMP, BEMT, and BEP. The efficiency of these reagents in SPPS was also judged by the synthesis of linear undecapeptide of CsO and the hindered 8−11 fragment of CsO using BEMT and BEP.

Experimental Section

Melting points were determined in capillary tubes and uncorrected. Because in most cases ^{1}H NMR spectra demonstrated multiple conformations, generally with different coupling constants for the different rotamers, *J* values are for the most part omitted. The amino acids of CsO and its intermediates are uniformly numbered (see Scheme 1) and marked in superscript style. TLC elution solvents are abbreviated in the text by letters followed by the ratio of components (v/v), with components as follows: A, ethyl acetate/petroleum; B, methanol/ethyl acetate. Solvents and reagents were purified by standard methods as necessary.

HOAt, HOBt, $SbCl_5$, DCC, and 2-bromopyridine were purchased from Aldrich Chemical Co., Milwaukee, WI, and used without purification. Fmoc-Nva-OH and Fmoc-Sar-OH were purchased from Peptide International Inc. HBTU, BOP, and PyBOP were purchased from Calbiochem-Novabiochem Corp. HBPyU, HAPyU, and HBPipU were prepared according to literatures. Cbz-*N*-methylamino acids were synthesized by the procedure of McDermott and Benoiton.[29] Fmoc-*N*-methylamino acids were synthesized by the procedure of Freidinger et al.[13]

Synthesis of Coupling Reagents. BEMT (2). 2-Bromo-4-methylthiazole[30] (3.17 mL, 22.8 mmol) was added dropwise into a solution of $Et_3O^+BF_4^-$ (4.33 g, 22.8 mmol) in $ClCH_2$-CH_2Cl (15 mL) at room temperature. After being stirred at 50 °C for 30 min, the reaction mixture was cooled, diluted with anhydrous ether, filtered, and washed with ether. The crude product was crystallized from acetone/ether to yield 6.42 g (95.7%) of **2** as colorless crystals: mp 189−189.5 °C; IR (KBr) 3134, 1581, 1065, 522; ^{1}H NMR (300 MHz, acetone-d_6) δ 1.55 (t, J = 7.5 Hz, 3H), 2.77 (s, 3H), 4.72 (q, J = 7.5 Hz, 2H), 8.15 (s, 1H); ^{13}C NMR (300 MHz, acetone-d_6) δ 14.0, 15.0, 50.0, 123.5, 145.9, 148.7; FAB-MS *m*/*z* 206, 208. Anal. Calcd for $C_6H_9BBrF_4NS$: C, 24.52; H, 3.09; N, 4.77. Found: C, 24.35; H, 2.92; N, 4.71.

BEP (3). 2-Bromopyridine was treated with $Et_3O^+BF_4^-$ according to the above procedure and the literature[31] to give **3** in 95.2% yield: mp 103−104 °C; IR (KBr) 3106, 1617, 1050, 521; ^{1}H NMR (300 MHz, acetone-d_6) δ 1.69 (t, J = 7.3 Hz, 3H), 5.01 (q, J = 7.3 Hz, 2H), 8.24 (m, 1H), 8.53−8.57 (m, 2H), 9.32 (d, J = 6.9 Hz, 1H); FAB-MS *m*/*z* 186, 188.

General Procedure I. Synthesis of Immonium-Type Coupling Reagents. A solution of *N*,*N*-dialkylamide (10 mmol) in CH_2Cl_2 was added dropwise to a solution of bis-(trichloromethyl)carbonate (0.989 g, 3.33 mmol) in CH_2Cl_2 (5 mL) at 0 °C under nitrogen atmosphere. After being stirred for approximately 1 h, when the evolution of carbon dioxide had ceased, a 0.89 M solution of $SbCl_5$ in $CHCl_3$ (10.7 mL) was added dropwise at −30 °C under vigorous stirring. The reaction mixture was stirred at 0 °C for 2 h, and the resultant suspension was filtered under nitrogen atmosphere, washed with cold CH_2Cl_2, and dried in vacuo. Recrystallization from

(26) (a) For quantitation of Fmoc-protected peptide segment on resin, UV absorbance of the fulvene−piperidine adduct at 301 nm was measured after cleavage of the Fmoc group with piperidine. (b) Meienhofer, J.; Waki, M.; Heimer, E.; Lambros, T. J.; Makofske, R.; Chang, C.-D. *Int. J. Pept. Protein Res.* **1979**, *13*, 35.

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$CH_3CN/CHCl_3$ gave the corresponding intermediate α-chloro immonium hexachloroantimonate as a crystalline solid.

KOBt (0.173 g, 1 mmol) or KOPfp (0.222 g, 1 mmol) was added to a solution of α-chloro immonium hexachloroantimonate (1 mmol) in dry CH_3CN (3 mL) at − 30 °C with stirring under argon atmosphere. After the reaction mixture was stirred at room temperature for 2 h, it was filtered, the filtrate was concentrated under reduced pressure, and the residue was recrystallized from CH_3CN/Et_2O to give HOBt or HOPfp-derived immonium-type coupling reagent as crystalline solid.

BOMI (8). Synthesized from *N*,*N*-dimethylformamide and KOBt in 76% overall yield: mp 152−153 °C dec; IR (KBr) 3050, 1702, 1496, 1446; ^{1}H NMR (300 MHz, acetone-d_6) δ 2.81 (s, 3H), 2.97 (s, 3H), 7.95−7.45 (m, 4H), 7.99 (s, 1H); ^{13}C NMR (300 MHz, acetone-d_6) δ 32.7, 38.2, 111.8, 117.5, 129.0, 129.2, 129.5, 139.8, 164.7. Anal. Calcd for $C_9H_{11}Cl_6N_4OSb$: C, 20.54; H, 2.09; N, 10.65. Found: C, 20.78; H, 2.12; N, 10.63.

BDMP (9). Synthesized from *N*-methylpyrrolidone and KOBt in 80% yield: mp 165−166 °C dec; IR (KBr) 3127, 1655, 1496, 1445; ^{1}H NMR (300 MHz, acetone-d_6) δ 1.98−2.09 (m, 2H), 2.39 (t, J = 8 Hz, 2H), 2.83 (s, 3H), 3.48 (t, J = 7 Hz, 2H), 7.34−7.95 (m, 4H); FAB-MS *m*/*z* 217. Anal. Calcd for $C_{11}H_{13}Cl_6N_4OSb$: C, 23.94; H, 2.36; N, 10.15. Found: C, 23.83; H, 2.13; N, 10.24.

BPMP (10). Synthesized from *N*-benzoylpyrrolidine and KOBt in 75% yield: mp 93−94 °C dec; IR (KBr) 3100, 1616, 1494, 1467, 1331; ^{1}H NMR (300 MHz, acetone-d_6) δ 1.95 (m, 4H), 3.57 (m, 4H), 7.34−7.97 (m, 9H); ^{13}C NMR (300 MHz, acetone-d_6) δ 25.0, 26.6, 48.4, 51.2, 111.3, 118.4, 127.6, 128.2 (2C), 129.0, 129.4, 129.5 (2C), 132.0, 134.4, 141.3, 170.8. Anal. Calcd for $C_{17}H_{17}Cl_6N_4OSb$: C, 32.52; H, 2.71; N, 8.92. Found: C, 32.23; H, 2.61; N, 8.84.

AOMP (11). The solution of HOAt (0.136 g, 1 mmol) and NEt_3 (0.14 mL, 1 mmol) in CH_2Cl_2 (5 mL) was added to a suspension of 5-chloro-3,4-dihydro-1-methyl 2*H*-pyrrolium hexachloroantimonate (0.453 g, 1 mmol) in CH_2Cl_2 (5 mL) at 0 °C. After being violently stirred at room temperature for 2 h, it was filtered, and the filter cake was washed with cooled CH_2Cl_2, dried, and recrystallized from acetone/Et_2O to yield 0.459 g (83%) of **11** as crystalline solid: mp 108−110 °C dec; IR (KBr) 1667, 1496, 1459; ^{1}H NMR (300 MHz, acetone-d_6) δ 2.13 (m, 2H), 2.59 (t, J = 8 Hz, 2H), 2.93 (s, 3H), 3.62 (t, J = 7 Hz, 2H), 7.53 (dd, J = 4 Hz, J = 8 Hz, 1H), 8.45 (dd, J = 8 Hz, J = 1 Hz, 1H) 8.76 (dd, J = 4 Hz, J' = 1 Hz, 1H). Anal. Calcd for $C_{10}H_{12}Cl_6N_5OSb$: C, 21.73; H, 2.17; N, 12.66. Found: C, 21.60; H, 2.11; N, 12.74.

FOMP (12). Synthesized from *N*-methylpyrrolidone and KOPfp in 77.7% overall yield: mp 182−183 °C dec; IR (KBr) 1705, 1530, 1520; ^{1}H NMR (300 MHz, acetone-d_6) δ 2.02 (m, 2H), 2.35 (t, J = 8 Hz, 2H), 2.81 (s, 3H), 3.46 (t, J = 7 Hz, 2H); ^{19}F NMR (300 MHz, acetone-d_6, CF_3COOH) δ −76.8 to −77.1 (m, 2F), −80.2 to −80.3 (m, 2F), −91.1 to −91.4 (m, 1F); FAB-MS *m*/*z* 266. Anal. Calcd for $C_{11}H_9Cl_6F_5NOSb$: C, 21.99; H, 1.50; N, 2.33. Found: C, 21.50; H, 1.34; N, 2.16.

Evaluation of Reactivity and Racemization of Different Coupling Reagents by HPLC Using the Model Reaction: Z-Gly-Phe-OH+Val-OMe·HCl → Z-Gly-D/L-Phe-Val-OMe. Z-Gly-Phe-OH (50 mg, 0.14 mmol) and Val-OMe·HCl (26 mg, 0.154 mmol) were coupled with the tested coupling reagent (0.154 mmol). Boc-Phe-Val-OMe (66 mg, 0.18 mmol) was added as the internal reference. Reaction tests were performed in a 1.5 mL scale. Aliquots (10 μL) from the reaction mixture were quenched and dissolved in 100 μL of buffer solution (CH_3OH/H_2O/TFA 50/50/1). The resultant samples were analyzed by HPLC to give the following results: Z-Gly-Phe-OH (t_R= 4.04 min); Z-Gly-L-Phe-Val-OMe (t_R = 9.24 min); Z-Gly-D-Phe-Val-OMe (t_R = 10.28 min); Boc-Phe-Val-OMe (t_R = 15.82 min) by comparing to the prepared reference compounds. Peak areas were compared in order to obtain the chemical yields (yield (%) = $[(LL/X_1 + DL/X_2)/a{\cdot}S] \times 100\%$). Percentage of epimers was calculated according to the equation: D (%) = $[DL/X_2/(LL/X_1 + DL/X_2)] \times 100\%$; where LL refers to the peak area of Z-Gly-L-Phe-Val-OMe, DL refers to that of Z-Gly-D-Phe-Val-OMe, S refers to that of Boc-Phe-Val-OMe, a = 0.778, which is the molar ratio between Z-Gly-OH and Boc-Phe-Val-OMe; X_1 = 1.269, and X_2 = 1.254, which are the determined correction factors for absorption difference (220 nm) between the references. HPLC conditions: Column: Kromasil KR 100-10 C18 (4.6 × 25 cm). Eluent: 48% CH_3CN (0.1% TFA). Flow rate: 1.5 mL/min. Detection: 220 nm (0.5 AUFS).

Synthesis of Cyclosporin O in Solution. General Procedure II. Synthesis of Peptide Using Thiazolium Salt BEMT or Pyridinium Salt BEP as a Coupling Reagent. DIEA (3.2 equiv) was added to a cooled mixture (−10 °C) of N-protected amino acid (1 equiv), amino acid, or peptide ester hydrochloride (1.1 equiv) and BEMT or BEP (1.1 equiv) in CH_2Cl_2 (3−5 mL/mmol). The mixture was stirred at −10 °C for 5 min and then reacted at room temperature until the consumption of the carboxylic component was complete trailing by TLC. Then the reaction mixture was concentrated and purified by flash chromatography on silica gel to afford the desired product.

General Procedure III. Synthesis of Peptides Using Immonium Salt BDMP as a Coupling Reagent. 2,6-Lutidine (3.2 equiv) was added to a cooled mixture (−10 °C) of N-protected amino acid (1 equiv), amino acid ester hydrochloride (1.1 equiv), and BDMP (1.1 equiv) in THF (2−4 mL/mmol), stirred at −10 °C for 1 min and at room temperature for 2 h. The reaction mixture was diluted with THF, the resultant suspension was filtered, and the filtered cake was washed with THF. The combined filtrates were concentrated under reduced pressure to give the crude product, which was purified by flash chromatography on silica gel to afford the desired product.

General Procedure IV. Removal of *N*-(9*H*-Fluoren-9-ylmethoxy)carbonyl Protective Group. *N*-Fmoc-protected peptide was dissolved in CH_3CN (ca. 100 mmol/L) and treated with an equal volume of diethylamine under nitrogen atmosphere until TLC analysis indicated that the starting material disappeared (ca. 40 min) trailing by TLC. The solution was concentrated in vacuo, and the residue was dissolved in CH_3CN and concentrated again to give *N*-Fmoc-deprotected peptide, which was further dried in vacuo for 2 h and utilized for the following coupling reaction without further purification.

General Procedure V. Removal of the Protective Group of the *C*-Benzyl Ester of Protected Peptide. *N*-Fmoc protected peptide benzyl ester was hydrogenated over Platinum black (15% (w/w)) in CH_3OH (5 mL/mmol) for 48 h. The catalyst was filtered off, and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography on silica gel to afford the desired product.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]norvalylsarcosine (16).** Via general procedure **V**, **15** (0.680 g, 1.385 mmol) was deprotected by hydrogenlysis over Platinum black to yield, after workup and purified by flash chromatography on 75 g of silica gel (AcOEt/petroleum 1/2 eluant), 0.588 g (98.2%) of **16** as foamy solid, which did not exhibit a distinct melting point: TLC R_f (B, 1/4) 0.65; $[\alpha]^{25}_D$ −5.9° (*c* 1, $CHCl_3$); IR (KBr) 3305, 2961, 1720, 1644; ^{1}H NMR (300 MHz, DMSO-d_6) two conformers δ 0.81−0.96 (m, 3H), 1.20−1.41 (m, 2H), 1.42−1.61 (m, 2H), 3.06, 3.17 (2s, 3H), 3.77, 4.16 (2d, J = 17 Hz, 2H), 4.18−4.36 (m, 3H), 4.42−4.53 (m, 1H), ca. 3.3−4.6 (br, 1H), 7.32 (t, J = 7 Hz, 2H), 7.42 (t, J = 7 Hz, 2H), 7.50−7.65 (m, 1H), 7.73 (m, 2H), 7.89 (d, J = 7 Hz, 2H); EI-MS *m*/*z* 411 $[M + H]^+$, 392, 178, 179. Anal. Calcd for $C_{23}H_{26}N_2O_5{\cdot}0.5H_2O$: C, 65.85; H, 6.49; N, 6.68. Found: C, 65.56; H, 6.43; N, 6.49.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*-methylleucylvalyl-*N*-methylleucylalanine Benzyl Ester (19).** Via general procedures **IV** and **III**, **18** (0.411 g, 0.655 mmol) was sequentially deprotected and coupled with Fmoc-MeLeu-OH (0.289 g, 0.786 mmol) using reagent BDMP (0.434 g, 0.786 mmol) in the presence of 2,6-lutidine (182 μL, 1.57 mmol) to yield, after workup and chromatography on silica gel (AcOEt/petroleum 1/2 eluant), 0.459 g (92.9%) of **19** as a white foamy solid: mp 44−46 °C; TLC R_f (A, 1/1) 0.61; $[\alpha]^{20}_D$ −91.8° (*c* 0.5, CH_3OH); IR (KBr) 3327, 2959, 1746, 1678, 1627; ^{1}H NMR (300 MHz, $CDCl_3$) two conformers: δ 0.82−1.02 (m, 18H), 1.36 (d, J = 7 Hz, 3H), 1.38−1.80 (2m, 6H) 1.90−2.30 (m, 1H), 2.78, 2.82, 2.84, 3.01 (4s, 6H) 4.27 (t, J = 6.5 Hz, 1H) 4.40−4.65 (m,

3H), 4.72−4.85 (m, 2H), 5.06−5.28 (m, 3H), 6.48 (d, J = 5.3 Hz, 1H), 6.63 (d, J = 9.1 Hz, 1H), 7.25−7.43 (m, 9H), 7.59 (d, J = 7.2 Hz, 2H), 7.76 (d, J = 7.5 Hz, 2H); EI-MS m/z 754 $M^{\cdot+}$, 576, 179, 91. Anal. Calcd for $C_{44}H_{58}N_4O_7 \cdot 0.5H_2O$: C, 69.18; H, 7.78; N, 7.33. Found: C, 68.97; H, 7.77; N, 7.07.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]norvalylsarcosyl-*N*-methylleucylvalyl-*N*-methylleucylalanine Benzyl Ester (20).** Via general procedures **IV** and **II**, **19** (0.120 g, 0.159 mmol) was sequentially deprotected and coupled with **16** (0.078 g, 0.191 mmol) using BEMT (0.056 g, 0.19 mmol) in the presence of DIEA (67 μL, 0.38 mmol) to yield, after workup and chromatography on silica gel (AcOEt/petroleum 1/1 eluant), 0.126 g (85.7%) of **20** as a white foamy solid: mp 80−81 °C; TLC R_f (A, 1/1) 0.37; $[\alpha]^{22}_D$ −114.6° (c 1, $CHCl_3$); IR (KBr) 3315, 2960, 1710, 1636; ^{1}H NMR (300 MHz, $CDCl_3$) more than two conformers δ 0.68−1.10 (m, 21H), 1.20−1.88 (m, 13H), 1.99−2.37 (m, 1H), 2.68−3.36 (m, 9H), 3.85−5.24 (m, 12H), [6.03 (d, J = 8.4 Hz), 6.51 (d, J = 7.4 Hz) 6.80 (d, J = 8.1 Hz), 7.65 (overlapped), 7.92 (d, J = 6 Hz), total 3 H], 7.20−7.42 (m, 9H), 7.45−7.65 (m, 2H), 7.75 (d, J = 7.4 Hz, 2H); ESI-MS m/z 1872 $[M + M + Na]^+$, 964 $[M + K]^+$, 948 $[M + Na]^+$, 925 $[M + H]^+$, 482 $[(M + H + K)/2]^+$, 474 $[(M + H + Na)/2]^+$. Anal. Calcd for $C_{52}H_{72}N_6O_9 \cdot 1.5H_2O$: C, 65.59; H, 7.94; N, 8.83. Found: C, 65.58; H, 7.72; N, 8.73.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*-methylleucylnorvalylsarcosyl-*N*-methylleucylvalyl-*N*-methylleucylalanine Benzyl Ester (21).** Via general procedures **IV** and **II**, **20** (0.156 g, 0.169 mmol) was sequentially deprotected and coupled with Fmoc-MeLeu-OH (81 mg, 0.22 mmol) using BEMT (64 mg, 0.22 mmol) in the presence of DIEA (76 μL, 0.44 mmol) to yield, after workup and chromatography on silica gel (AcOEt/petroleum 1/1 eluant), 0.163 g (92.1%) of **21** as white foamy solid: mp 48−49 °C; TLC R_f (A, 1/1) 0.33; $[\alpha]^{22}_D$ −114.8° (c 0.5, $CHCl_3$); IR (KBr) 3319, 2959, 1746, 1675, 1630; ^{1}H NMR (400 MHz, $CDCl_3$) HMQC spectrum available as Supporting Information, more than two conformers, δ 0.70−0.98 (m, 27H), 1.20−1.40 (m, 3H), 1.40−1.85 (m, 13H), 2.18 (m, 1H), 2.60−3.44 (m, 12H), 3.88−4.27 (m, 3H), 4.35−4.62 (m, 3H), 4.65−4.77 (m, 1H), 4.78−5.24 (m, 6H), 6.99 (d, J = 8.9 Hz, 1H), 7.22−7.42 (m, 9H), 7.45 (d, J = 8.9 Hz, 1H), 7.58 (m, 2H), 7.75 (d, J = 7.5 Hz, 2H), 8.04 (d, J = 6.9 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) more than two conformers: δ 17.20, 17.37, 17.75, 17.90, 18.16, 18.38, 18.63, 19.18, 19.38, 19.89, 21.57, 21.75, 22.02, 22.13, 22.19, 22.43, 22.80, 23.07, 23.14, 23.22, 23.31, 23.57, 24.79, 24.84, 24.98, 25.13, 25.19, 25.55, 29.90, 30.54, 30.82, 31.03, 34.33, 34.53, 36.13, 36.35, 36.99, 37.54, 37.67, 37.83, 38.55, 47.31, 47.36, 47.96, 48.25, 48.54, 48.61, 49.83, 54.38, 54.61, 55.01, 55.17, 56.91, 58.31, 66.86, 67.01, 67.79, 120.00, 125.06, 127.12, 127.73, 128.11, 128.31, 128.56, 135.52, 135.74, 141.35, 141.38, 143.99, 144.14, 157.06, 168.39, 169.66, 170.46, 170.57, 171.62, 171.66, 172.30, 172.44, 172.73, 172.83; ESI-MS m/z 1091 $[M + K]^+$, 1075 $[M + Na]^+$, 546 $[(M + H + K)/2]^+$, 538 $[(M + H + Na)/2]^+$. Anal. Calcd for $C_{59}H_{85}N_7O_{10} \cdot 1.25H_2O$: C, 65.92; H, 8.17; N, 9.12. Found: C, 65.61; H, 8.12; N, 9.41.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-D-Alanyl-*N*-methylleucyl-*N*-methylleucyl-*N*-methylvalyl *tert*-Butyl Ester (24).** Via general procedures **IV** and **II**, **23** (0.094 g, 0.14 mmol) was sequentially deprotected and coupled with Fmoc-D-Ala-OH (0.053 g, 0.17 mmol) using BEMT (0.050 g, 0.17 mmol) in the presence of DIEA (59 μL, 0.34 mmol) to yield, after workup and chromatography on 20 g of silica gel (AcOEt/petroleum 1/4 eluant), 0.093 g (89%) of **24** as a white foamy solid: mp 51−54 °C; TLC R_f (A, 1/2) 0.56; $[\alpha]^{22}_D$ −168° (c 0.1, $CHCl_3$); IR (KBr) 3317, 2961, 1730, 1642; ^{1}H NMR (400 MHz, $CDCl_3$) HMQC spectrum available as Supporting Information, two conformers, δ 0.78−1.02 (m, 21H), 1.25−1.80 (m, 15H), 2.17 (m, 1H), 2.81−3.13(m, 9H), 4.20 (t, J = 7.1 Hz, 1H), 4.29−4.41 (m, 2H), 4.67 (t, J = 7.0 Hz, 1H), 4.74 (d, J = 10.4 Hz, 1H), 5.46−5.56 (m, 2H), 5.71, 5.77 (2d, J = 7.8 Hz, 1H), 7.26 (t, J = 7.5 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.59 (d, J = 6.0 Hz, 2H), 7.75 (d, J = 7.5 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) two conformers δ 18.69, 18.76, 18.80, 18.85, 19.70, 20.03, 22.17, 22.31, 22.36, 22.76, 22.86, 22.92, 23.75, 24.47, 24.69, 24.96, 25.02, 27.25, 27.56, 28.05, 28.09, 29.76, 30.03, 30.24, 30.43, 30.57, 31.23, 37.76, 37.98, 38.07, 38.77, 47.19, 47.37, 51.14, 51.64, 51.89, 62.59, 67.01, 81.44, 81.99, 120.03, 125.16, 127.11, 127.76, 141.35, 143.85, 143.97, 155.57, 168.77, 169.66, 170.05, 170.91, 171.71, 172.62, 172.86; EI-MS m/z 548, 422, 178, 179, 57. Anal. Calcd for $C_{42}H_{62}N_4O_7$: C, 68.64; H, 8.50; N, 7.62. Found: C, 68.54; H, 8.71; N, 7.46.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-D-alanyl-*N*-methylleucyl-*N*-methylleucyl-*N*-methylvaline(25).** The flask containing **24** (0.564 g, 0.767 mmol) was chilled under argon atmosphere in an ethylene glycol/CO_2 bath held at −15 to −20 °C. TFA (5 mL), which was precooled in the same bath, was added to the flask, and the reaction mixture was stirred at the low temperature until TLC trailing indicated the completed deprotection of peptide **24** (16 h). The TFA was removed at −15 °C by distillation into a CO_2/acetone trap under vacuum, and then the residue was treated with AcOEt (5 mL) and concentrated repeatedly in vacuo. The residue was purified by flash chromatography on silica gel (AcOEt/Petroleum 1/1 eluant) to give 0.478 g (91.7%) of **25**: mp 80−82 °C; TLC R_f (B, 1/10) 0.62; $[\alpha]^{20}_D$ −102.0° (c 0.25, $CHCl_3$); IR (KBr) 3316, 2960, 1726, 1643; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.76−1.20 (m, 21H), 1.24−1.91 (m, 6H), 2.32 (m, 1H), 2.77−3.9(m, 9H), 4.06−4.49 (m, 4H), 4.72 (m, 1H), 5.44−5.56 (m, 2H), 5.92, 6.60 (2d, J = 8.0 Hz, 1H), 7.31 (m, 2H), 7.40 (t, J = 7.3 Hz, 2H), 7.60 (m, 2H), 7.76 (d, J = 7.6 Hz, 2H); EI-MS m/z 661, 421, 178. Anal. Calcd for $C_{38}H_{54}N_4O_7 \cdot H_2O$: C, 65.49; H, 8.10; N, 8.04. Found: C, 65.66; H, 8.01; N, 7.96.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-D-Alanyl-*N*-methylleucyl-*N*-methylleucyl-*N*-methylvalyl-*N*-methylleucylnorvalylsarcosyl-*N*-methylleucyl-valyl-*N*-methylleucylalanine Benzyl Ester (26).** Via general procedures **IV** and **II**, **21** (0.205 g, 0.195 mmol) was sequentially deprotected and coupled with **25** (0.159 g, 0.230 mmol) using BEMT (86 mg, 0.293 mmol) and HOAt (40 mg, 0.293 mmol) in the presence of DIEA (92 μL, 0.53 mmol) to yield, after workup and chromatography on silica gel (AcOEt/petroleum 6/1 eluant), 0.227 g (78.3%) of **26** as white foamy solid: mp 92−94 °C; TLC R_f (A, 2/1) 0.32; $[\alpha]^{25}_D$ −217.7° (c 0.3, $CHCl_3$); IR (KBr) 3322, 2960, 1715, 1643; ^{1}H NMR (400 MHz, acetone-d_6) HMQC spectrum available as Supporting Information, more than two conformers, δ 0.72−1.08 (m, 45H), 1.20−1.40 (m, 6H), 1.40−1.83 (m, 19H), 2.05−2.41 (m, 2H), 2.75−3.29 (m, 21H), 4.18−4.38 (m, 5H), 4.45 (m, 1H), 4.59−4.75 (m, 2H), 4.93 (m, 1H), 5.10−5.27 (m, 6H), 5.46−5.57 (m, 2H) 7.32−7.43 (m, 9H), 7.71 (m, 2H), 7.87 (d, J = 7.5 Hz, 2H); ^{13}C NMR (400 MHz, acetone-d_6) more than two conformers, δ 14.14, 14.32, 17.51, 17.55, 17.75, 18.43, 18.86, 19.15, 19.58, 19.95, 20.20, 21.72, 22.05, 22.35, 22.47, 22.80, 23.12, 23.23, 23.50, 23.59, 23.73, 24.48, 25.39, 26.08, 27.77, 30.87, 31.13, 31.56, 35.18, 35.35, 37.17, 37.24, 37.72, 38.12, 39.63, 47.81, 47.94, 48.50, 48.84, 49.00, 49.26, 50.35, 50.45, 51.96, 52.31, 54.99, 55.10, 55.22, 55.50, 55.64, 55.74, 55.82, 59.06, 59.29, 67.14, 67.27, 68.10, 120.79, 126.11, 126.18, 127.93, 127.97, 128.53, 128.83, 128.93, 129.30, 137.07, 137.14, 142.05, 144.94, 145.01, 154.50, 168.09, 169.51, 169.76, 170.30, 170.55, 170.94, 171.21, 171.26, 171.48, 172.01, 172.24, 173.06, 173.61, 173.80; ESI-MS m/z 1530 $[M + K]^+$, 1514 $[M + Na]^+$, 1492 $[M + H]^+$, 785 $[(M + K + K)/2]^+$, 776 $[(M + Na + K)/2]^+$, 768 $[(M + Na + Na)/2]^+$, 765 $[(M + H + K)/2]^+$, 758 $[(M + H + Na)/2]^+$. Anal. Calcd for $C_{82}H_{127}N_{11}O_{14} \cdot H_2O$: C, 65.27; H, 8.62; N, 10.21. Found: C, 65.21; H, 8.67; N, 10.08.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-D-Alanyl-*N*-methylleucyl-*N*-methylleucyl-*N*-methylvalyl-*N*-methylleucylnorvalylsarcosyl-*N*-methylleucylvalyl-*N*-methylleucylalanine (27).** Via general procedure V, **26** (96 mg, 0.064 mmol) was deprotected by hydrogenlysis over Platinum black to yield, after workup and purification by flash chromatography on silica gel (CH_3OH/AcOEt 1/20 eluant), 84 mg (93.1%) of product as white solid: mp 111−113 °C; TLC R_f (B, 1/10) 0.50; $[\alpha]^{26}_D$ −165.4° (c 0.5, CH_3OH); IR (KBr) 3323, 2960, 1724, 1644; ^{1}H NMR (400 MHz, acetone-d_6) more than two conformers δ 0.75−1.11 (m, 45H), 1.21−1.42 (m, 6H), 1.43−1.85 (m, 19H), 2.02−2.42 (m, 2H), 2.75−3.29 (m, 21H), 4.17−4.40 (m, 6H), 4.60−4.77 (m, 2H), 4.87−5.28 (m, 5H), 5.46−5.59 (m, 2H), 7.34 (t, J = 7.8 Hz, 2H), 7.42 (t, J = 7.4 Hz, 2H),

7.72 (m, 2H), 7.87 (d, $J = 7.5$ Hz, 2H); ESI-MS *m/z* 1440 $[M + K]^+$, 1424 $[M + Na]^+$, 1402 $[M + H]^+$, 732 $[(M + Na + K)/2]^+$, 720 $[(M + H + K)/2]^+$, 712 $[(M + H + Na)/2]^+$. Anal. Calcd for $C_{75}H_{121}N_{11}O_{14}\cdot 2H_2O$: C, 62.69; H, 8.77; N, 10.72. Found: C, 62.69; H, 8.65; N, 10.21.

Cyclosporin O (1) (Cyclization Using HAPyU). The protected peptide **26** (90 mg, 0.060 mmol) was dissolved in 2.5 mL of ethanol, cooled to 0 °C, and flushed with argon. A 0.59 mL portion of 0.2 M NaOH was added to the solution and the mixture stirred for 1.5 h, then a second portion of NaOH (0.29 mL) was added and the mixture stirred for an additional 3.5 h. The reaction mixture was neutralized to pH 6 with 0.2 M HCl, treated with brine (9 mL), and extracted with methylene chloride (5 × 20 mL). The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo to give a pale yellow glass. This deprotected peptide was dissolved in 300 mL of CH_2Cl_2 and then HAPyU (131 mg, 0.302 mmol) and DIEA (105 μL, 0.604 mmol) were added. The reaction mixture was stirred for 2 h at room temperature, concentrated in vacuo, and purified by flash chromatography on silica gel (AcOEt/petroleum 4/1 eluant) to yield 59 mg (84%) of CsO as white solid: mp 124−126 °C; TLC R_f ($AcOEt/CH_3OH/H_2O$, 20/1/1) 0.63; IR (KBr) 3422, 3321, 2962, 1631, 852; ^{1}H NMR (400 MHz, $CDCl_3$) HMQC spectrum available as Supporting Information δ 0.65−1.09 (m, 45H), 1.18−2.21 (m, 26H), 2.47 (m, 1H), 2.69 (br, 6H), 3.10 (s, 3H), 3.19 (s, 3H), 3.28 (s, 3H), 3.36 (s, 3H), 3.40 (s, 3H), 3.16, 4.71 (2d, $J = 14.1$ Hz, 2H), 4.44 (m, 1H), 4.69 (t, $J = 9$ Hz, 1H), 4.86 (m, 1H), 5.03−5.21(m, 5H), 5.34 (dd, $J_1 = 11.5$ Hz, $J_2 = 3.5$ Hz, 1H), 5.69 (dd, $J_1 = 11.2$ Hz, $J_2 = 4.0$ Hz, 1H), 7.45−7.56 (m, 2H), 8.06 (d, $J = 6.9$ Hz, 1H), 8.45 (d, $J = 9.7$ Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 14.02, 15.09, 17.73, 18.25, 18.45, 18.47, 19.70, 21.00, 21.23, 21.89, 22.18, 23.48, 23.66, 23.79 (2C), 23.84 (2C), 24.36, 24.47, 24.54, 24.74, 24.91, 29.67, 29.72, 29.87, 30.00, 31.20 (2C), 31.32, 31.73, 33.84, 36.16, 37.48, 38.88, 39.29 (2C), 40.66, 44.67, 46.83, 47.91, 48.31, 49.91, 54.09, 55.01, 55.13, 55.22, 57.18, 58.38, 170.17, 170.69, 170.78, 170.94, 171.28, 171.56, 171.67, 172.81, 173.14, 173.55, 173.81; ESI-MS *m/z* 1183.8 $[M + Na]^+$, 1161.6 $[M + H]^+$, 603.3 $[(M + 2\ Na)/2]^+$, 592.4 $[(M + H + Na)/2]^+$.

Cyclosporin O (1) (Cyclization using BDMP). The undecapeptide **26** (40 mg, 0.027 mmol) was deprotected according to above procedure. This deprotected peptide was dissolved in 150 mL CH_2Cl_2, then treated with BDMP (74 mg, 0.13 mmol) and 2,6-lutidine (31 μL, 0.27 mmol) was added at −10 °C. The reaction mixture was stirred for 4 h at room temperature, then DIEA (23 μL, 0.13 mmol) was added. After the reaction mixture was further stirred for 2 h, it was concentrated in vacuo and purified by flash chromatograph on silica gel (AcOEt/petroleum 4/1 eluant), then rechromatographed (AcOEt/petroleum 2/1→ 4/1 eluant) to yield 21 mg (68%) of CsO as white solid. Physical data and spectra data are shown to be identical with the sample synthesized by HAPyU.

Synthesis of 8−11 Fragment of CsO and the Total Synthesis of Linear Undecapeptide of CsO in the Solid Phase. The commercially available Wang resin was chosen as the solid support. The first amino acid residue was anchored using DCC/HOAt/DMAP in CH_2Cl_2. Fmoc was used for the N_α-protection, and the elongation of peptide segment was realized using coupling reagents BEMT and BEP. The cleavage of peptide from resin was carried out using 50% TFA in CH_2Cl_2 at low temperature. For experimental details see the Supporting Information.

Acknowledgment. This work was supported by the National Natural Science Foundation of China (9772045).

Supporting Information Available: Experimental details for the synthesis of compounds **15**, **17**, **18**, **22**, and **23**. HMQC spectra for sompounds **1**, **21**, **24**, and **26**. ^{1}H NMR assignment for compounds **1** and **15**−**27**. Experimental procedure for solid-phase synthesis of the 8−11 fragment of CsO and the linear undecapeptide of CsO. This material is available free of charge via the Internet at http://pubs.acs.org.

JO991687C