dissolved in toluene (0.3 M with respect to **2**) and heated for 72 h at reflux. The solution was then cooled and concentrated. The crude product was then separated by flash chromatography using 6:1 hexanes/ethyl acetate.

3: mp 149–151 °C; 300-MHz NMR ($CDCl_3$) 0.72 (s, 3 H), 1.25 (t, J = 7 Hz, 3 H), 1.59 (s, 3 H), 1.71 (br s, 3 H), 2.06 (m, 2 H), 2.56 (dd, J = 4 and 9 Hz, 1 H), 2.82 (br t, J = 11 Hz, 1 H), 3.61 (dd, J = 3 and 7 Hz, 1 H), 3.78 (br s, 1 H), 4.14 (m, 2 H), 5.70 (br s, 1 H), 5.90 (s, 2 H), 6.71 (s, 3 H); ^{13}C NMR ($CDCl_3$) 14.09, 21.35, 28.69, 29.36, 29.80, 35.20, 44.50, 46.85, 59.43, 61.17, 101.16, 106.21, 108.66, 109.83, 122.74, 123.39, 131.09, 133.03, 147.43, 148.04, 169.53, 171.50; IR ($CDCl_3$) 2990, 1770, 1740 sh, 1730, 1620, 1580, 1450, 1380, 1325, 1250, 1045 cm^{-1}. Anal. Calcd for $C_{23}H_{26}O_8$: C, 64.18; H, 6.09. Found: C, 64.20; H, 6.05.

4: mp 146.5–148 °C; 300-MHz NMR ($CDCl_3$) 1.27 (s, 3 H), 1.27 (t, J = 7 Hz, 3 H), 1.79 (br s, 3 H), 1.83 (br s, 3 H), 2.36 (d of m, J = 16 Hz, 1 H), 2.56 (d, J = 16 Hz, 1 H), 2.61 (m, 1 H), 3.07 (m, 2 H), 3.45 (dd, J = 4 and 6 Hz, 1 H), 4.14 (m, 2 H), 5.72 (br s, 1 H), 5.89 (d, J = 2 Hz, 2 H), 6.69 (s, 1 H), 6.72 (s, 1 H); ^{13}C NMR ($CDCl_3$) 14.18, 22.50, 28.88, 29.11, 31.04, 35.23, 42.29, 42.54, 55.24, 60.85, 100.98, 105.17, 108.15, 109.83, 122.71, 123.62, 130.26, 133.04, 146.91, 147.53, 166.39, 167.65, 172.44; IR (film) 3005, 2995, 1770, 1730, 1620, 1505, 1445, 1380, 1260, 1230, 1180, 930 cm^{-1}. Anal. Calcd for $C_{23}H_{26}O_8$: C, 64.18; H, 6.09. Found: C, 64.19; H, 6.45.

9: 300-MHz NMR ($CDCl_3$) 0.86 (s, 3 H), 1.24 (t, J = 7 Hz, 3 H), 1.28 (t, J = 7 Hz, 3 H), 1.62 (s, 3 H), 1.71 (br s, 3 H), 2.18 (dd, J = 4 and 14 Hz, 1 H), 2.37 (br d, J = 14 Hz, 1 H), 2.59 (dd, J = 7 and 12 Hz, 1 H), 2.74 (br t, J = 12 Hz, 1 H), 3.42 (d, J = 15 Hz, 1 H), 3.85 (br s, 6 H), 3.94 (m, 2 H), 4.08–4.23 (m, 5 H), 5.62 (br s, 1 H), 6.67 (s, 1 H), 6.77 (s, 1 H); ^{13}C NMR ($CDCl_3$) 14.04, 14.17, 21.22, 28.91, 29.97, 31.02, 35.13, 38.26, 42.30, 44.97, 55.78, 55.87, 56.09, 58.41, 60.85, 61.14, 106.21, 111.24, 114.13, 123.84, 126.16, 130.66, 130.83, 148.13, 165.08, 169.75, 171.40, 171.69; IR ($CDCl_3$) 3020, 1770, 1735, 1695, 1610, 1450, 1370, 1200, 1025, 910 cm^{-1}; HRMS for $C_{28}H_{36}O_{10}$ requires 532.23086, measured 532.23245.

10: 300-MHz NMR ($CDCl_3$) 1.22 (t, J = 7 Hz, 3 H), 1.26 (t, J = 7 Hz, 3 H), 1.35 (s, 3 H), 1.77 (s, 3 H), 1.83 (s, 3 H), 2.39 (br d, J = 8 Hz, 1 H), 2.62 (m, 2 H), 3.03 (m, 2 H), 3.35 (br d, J = 6 Hz, 1 H), 3.68–3.87 (m, 2 H), 3.76 (s, 3 H), 3.83 (s, 3 H), 4.11 (m, 4 H), 5.71 (br s, 1 H), 6.64 (s, 1 H), 6.74 (s, 1 H); ^{13}C NMR ($CDCl_3$) 14.08, 14.12, 22.42, 28.83, 29.12, 31.70, 35.06, 38.13, 38.78, 42.99, 53.37, 54.32, 55.59, 55.64, 60.69, 60.78, 105.05, 110.24, 114.09, 124.21, 126.39, 130.02, 130.89, 147.62, 167.00, 167.94, 171.86, 172.31; IR ($CDCl_3$) 3030, 1770, 1740, 1730, 1615, 1445, 1380, 1260, 1030 cm^{-1}.

General Preparation of the Lactones. The Diels–Alder adduct (1.0 equiv) was dissolved in methylene chloride (0.13 M). After the solution was cooled to 0 °C, MCPBA (1.05 equiv) was added as a solid in one portion. The reaction mixture was slowly allowed to warm to room temperature over 2 h. The mixture was diluted with ether (twice the original volume) and washed with saturated sodium bicarbonate solution and then with brine. The organic layer was concentrated, dissolved in THF to make a 0.13 M solution, and treated with 3 N perchloric acid (4 mL/mmol of epoxide). After the solution had stirred for 12 h at room temperature, it was diluted with an equal volume of water and extracted twice with ether. The combined extracts were washed with brine, dried, and concentrated. In the case of the lactone derived from **10**, it was dissolved in methylene chloride and treated with diazomethane in ether until gas evolution ceased. Lactone **5** was produced in 98% yield. Lactone **11** was produced in 76% yield.

5: 300-MHz NMR ($CDCl_3$) 1.28 (t, J = 7 Hz, 3 H), 1.57 (s, 3 H), 1.98–2.15 (m, 2 H), 2.36 (d, J = 3 Hz, 1 H), 3.04 (d, J = 4 Hz, 2 H), 3.22 (t, J = 4 Hz, 1 H), 3.99 (br s, 1 H), 4.14 (q, J = 7 Hz, 2 H), 5.90 (s, 2 H), 6.68 (d, J = 6 Hz, 1 H), 6.83 (d, J = 6 Hz, 1 H), 6.89 (s, 1 H); IR ($CDCl_3$) 3650–2400, 1775, 1740 sh, 1725, 1620, 1440, 1380, 1250, 905 cm^{-1}. Anal. Calcd for $C_{20}H_{22}O_9$: C, 59.11; H, 5.46. Found: C, 58.88; H, 5.43.

11: 300-MHz NMR ($CDCl_3$) 1.28 (t, J = 7 Hz, 6 H), 1.58 (s, 3 H), 2.10 (m, 2 H), 2.79 (br s, 1 H), 3.08 (m, 1 H), 3.24 (br t, J = 8 Hz, 1 H), 3.52 (s, 3 H), 3.70 (m, 1 H), 3.83 (s, 3 H), 3.84 (s, 3 H), 3.82–4.08 (m, 4 H), 4.14 (m, 4 H), 6.67 (s, 1 H), 7.13 (s, 1 H); ^{13}C NMR ($CDCl_3$) 14.13, 20.65, 29.31, 31.02, 37.72, 39.33, 50.76, 52.38, 55.71, 55.84, 59.62, 60.91, 61.02, 67.90, 71.13, 84.12, 110.22, 113.88, 125.11, 128.15, 128.95, 132.12, 147.17, 168.49, 172.31, 172.63; IR ($CDCl_3$) 3030, 1780, 1740 sh, 1730, 1615, 1450, 1385, 1280, 1120, 1010 cm^{-1}; HRMS for $C_{26}H_{34}O_{11}$ requires 522.21012, measured 522.21083.

Synthesis of Aldehyde 7. The bromo ester (1.67 g, 5.30 mmol) was dissolved in 15 mL of chloroform at room temperature. Tetrabutylammonium dichromate (7.58 g, 10.60 mmol) was added and the mixture was heated at reflux for 2 h. The cooled mixture was filtered through 30 g of silica gel using 400 mL of ether to afford 1.24 g (93% yield) of pure **7**: NMR ($CDCl_3$) 1.21 (t, J = 7 Hz, 3 H), 3.93 (s, 6 H), 3.96 (s, 2 H), 4.14 (q, J = 7 Hz, 2 H), 6.75 (s, 1 H), 7.35 (s, 1 H), 10.08 (s, 1 H); IR (CCl_4) 3030, 1740, 1730, 1695, 1680, 1470, 1280, 1170, 1115, 1030 cm^{-1}.

Acknowledgment. We thank the National Institutes of Health (Grant CA30623) for financial assistance.

Registry No. **1**, 87212-59-7; **2**, 72827-05-5; **3**, 103225-52-1; **4**, 103225-53-2; **5**, 103225-54-3; **6**, 93-40-3; **7**, 103225-55-4; **7** (lactone), 16135-41-4; **8**, 103225-56-5; **9**, 103225-57-6; **10**, 103225-58-7; **11**, 103225-59-8; **11** (acid), 103225-60-1; ethyl 2-(bromoethyl)-4,5-dimethoxyphenylacetate, 73053-80-2; quassimarin, 59938-97-5.

BOP-Cl Mediated Synthesis of the Cyclosporine A 8–11 Tetrapeptide Fragment

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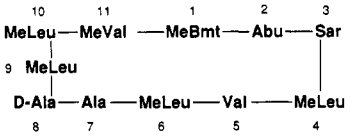
With the bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) coupling reagent, synthesis of the cyclosporine A 8–11 tetrapeptide has been carried out utilizing both Fmoc- and Cbz-N-protection of the intermediates. The former protecting group allows a very time efficient sequence where isolation of the free imino fragment is obviated and demonstrates the potential for applications to solid phase. Cbz protection is more economical for larger scale synthesis, however, and provides more stable intermediates. In both cases remarkable chemical efficiency was achieved, the fully protected tetrapeptide being obtained in 66% and 73% yields, respectively, from the constituent protected amino acids. By means of the Fmoc procedure, diastereomers at each stage of coupling have been separately prepared. HPLC analysis, using these compounds as internal standards, has shown that racemization in all cases was less than 1%.

The cyclosporines are a family of extensively N-methylated cyclic undecapeptides, first isolated as fungal metabolites, a number of which possess strong, orally active immunosuppressive properties.[1] Due to the recent FDA

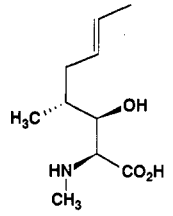
0022-3263/86/1951-3350$01.50/0

approval of one member, cyclosporine A (CsA, **1**, marketed under the trade name Sandimmune) for use in the prevention of transplant rejections, as well as preliminary indications that CsA may be effective in the treatment of such diverse diseases as malaria and rheumatoid arthritis, a facile entry to new analogues that might possess more closely defined activities and fewer side effects would be highly desirable.



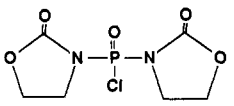
1

A total synthesis of the cyclosporines has been reported by Wenger[2-4] and has been utilized by our laboratory in the synthesis of some novel analogues.[5] However, the originally formulated coupling methodology is quite cumbersome, requiring in our hands some months of work for each analogue. The main constraints to the synthesis of these compounds have been (1) the lack of ready availability of the so-called "MeBmt" (**2**, position 1 in the cyclosporines), an unusual amino acid possessing three contiguous asymmetric centers and a trans olefinic group, and (2) the difficulty of activating the α-amino carboxylates of the amino acid acids sufficiently to allow facile ammonolysis in these highly hindered, N-methylated systems without significant loss of stereochemical fidelity. The former problem is currently being addressed in this laboratory and will be reported in due course; the latter comprises the subject matter for this paper.



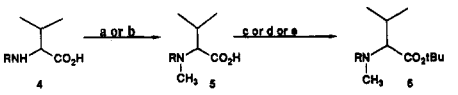
2

We have previously noted that bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl, **3**)[6] provides an efficient method for the nearly racemization-free condensation of urethane-protected α-amino and N-alkylated α-amino acids.[7] We have also reported, in a preliminary communication, the use of **3** in a highly efficient synthesis of the key CsA 8–11 tetrapeptide fragment, utilizing a novel one-pot deprotection–coupling of the intermediate Fmoc-protected species.[8,9] In this report we more fully



3

Scheme I[a]



4a, (L) R = Fmoc	**5a**, (L) R = Fmoc	**6a**, (L) R = Fmoc
b, (D) R = Fmoc	**b**, (D) R = Fmoc	**b**, (D) R = Fmoc
c, (L) R = Cbz	**c**, (L) R = Cbz	**c**, (L) R = Cbz
d, (L) R = Boc	**d**, (L) R = Boc	**d**, (L) R = H
e, (D) R = Boc	**e**, (D) R = Boc	**e**, (D) R = H

[a] (a, for **4a,b**) 3 equiv of paraformaldehyde, catalytic *p*-TsOH, $PhCH_3$, reflux ($-H_2O$), then 3 equiv of Et_3SiH, $CF_3CO_2H/CHCl_3$; (b, for **4c–e**) 8 equiv of MeI, 3 equiv of NaH, THF, 0 °C to ambient temperature; (c, for **5a,b**) 1.05 equiv of $(COCl)_2$, catalytic DMF, CH_2Cl_2 or THF, 0 °C, and then 3 equiv of *t*-BuOH, 2 equiv of AgCN, PhH, reflux; (d, for **5c**) excess isobutylene, catalytic H_2SO_4, CH_2Cl_2, −78 °C to ambient temperature; (e, for **5d,e**), 1–2 equiv of *p*-TsOH or H_2SO_4, dioxane, reflux, followed by excess isobutylene, −78 °C to ambient temperature.

delineate the reactions involved and demonstrate, by separate syntheses of the appropriate diastereomers and HPLC analysis, that stereochemical purity is essentially fully retained throughout the process. We also report the use of *N*-Cbz protection in a more economical synthesis of the same fragment which is fully amenable to scaling up to the order of tens of grams.

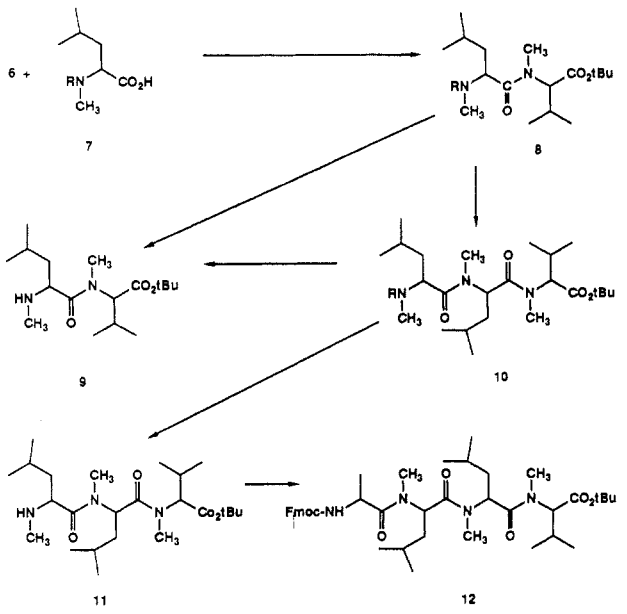
Results and Discussion

The success of our procedure relied largely on the choice of protecting groups. As Wenger noted,[3] carboxylate protection as the benzyl ester is precluded in a synthesis proceeding toward the N terminus due to formation of diketopiperazine upon N-deprotection of the MeLeu-MeVal (N-Boc, O-Bzl) fragment. In contrast, also as reported, the same sequence is fully stable as the *tert*-butyl ester. Our early attempts at a synthesis of MeVal-O-*t*-Bu (**6c**) by standard acid catalyzed reaction with isobutylene resulted in poor yields, but we were able to circumvent the problem by employing a silver cyanide assisted esterification[10] of the stable Fmoc-MeVal acid chloride (Scheme I).[11] Remarkably, less than 1% racemization occurred in the preparation of **6a** and **6b**, again confirming the stabilizing influence of *N*-carbamate protection. We later found (not surprisingly in retrospect) that our initial attempts failed due to the sluggishness of reaction; it provides a useful measure of the degree to which steric hindrance is introduced by N-methylation that the reaction of valine with isobutylene is finished in 4 h,[12] whereas in the cases of either MeVal or its Cbz-protected derivative it is incomplete at 4 days.

Starting from the fully protected tetrapeptide, the sequence of reactions leading to the cyclosporines involves C-deprotection of that fragment and coupling to the 1–7 fragment to form the linear undecapeptide, followed by N and C termini deprotection and cyclization. Given this reaction scheme, the use of either N-Boc or Cbz protection is rendered inadvisable; the former due to *tert*-butyl protection of the tetrapeptide and the latter due to the olefinic side chain of **2**. Fmoc protection, however, represents a completely orthogonal group[13] which has the potential to

(1) Borel, J. F. In *Cyclosporine A*; White, D. J. G., Ed.; Elsevier Biomedical: Amsterdam, 1982; pp 5–17.
(2) Wenger, R. M. *Helv. Chim. Acta* **1983**, *66*, 2308–2321.
(3) Wenger, R. M. *Helv. Chim. Acta* **1983**, *66*, 2672–2702.
(4) Wenger, R. M. *Helv. Chim. Acta* **1984**, *67*, 502–525.
(5) Rich, D. H.; Dhaon, M. K.; Dunlap, B.; Miller, S. P. F. *J. Med. Chem.* **1986**, *29*, 978–984.
(6) Diago-Meseguer, J.; Palomo-Coll, A. L.; Fernandez-Lizarbe, J. R.; Zugaza-Bilbao, A. *Synthesis* **1980**, 547–551.
(7) Tung, R. D.; Rich, D. H. *J. Am. Chem. Soc.* **1985**, *107*, 4342–4343.
(8) Tung, R. D.; Rich, D. H. In *Peptides: Structure and Function*; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Co.: Rockford, IL, 1985; pp 217–220.
(9) Abbreviations used follow IUPAC-IUB tentative rules as described in: *J. Biol. Chem.* **1972**, *247*, 977–983. Additionally, Fmoc is used as the abbreviation for [(9-fluorenylmethyl)oxy]carbonyl.
(10) Takimoto, S.; Inanaga, J.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 2335–2336.
(11) We have also found this procedure to be highly effective for acylation of other poorly nucleophilic species such as carbamates and highly branched amines; Tung, R. D.; Rich, D. H., unpublished observations.
(12) Roeske, R. *J. Org. Chem.* **1963**, *28*, 1251–1253.

Scheme II



	protecting group	configuration Ala	MeLeu	MeLeu	MeVal
7a	Fmoc			L	
7b	Fmoc			D	
7c	Cbz			L	
8a	Fmoc			L	L
8b	Fmoc			L	D
8c	Fmoc			D	L
8d	Cbz			L	L
9				L	L
10a	Fmoc		L	L	L
10b	Fmoc		D	L	L
10c	Cbz		L	L	L
11			L	L	L
12a	Fmoc	L	L	L	L
12b	Fmoc	D	L	L	L

be applied to solid-phase methodology.[14] With this possibility in mind and with the ready availability of N-methylated Fmoc amino acids,[15] we carried out a complete synthesis of the tetrapeptide using this protecting group (Scheme II). Making use of our previously reported[8] one-pot deprotection-coupling, excellent yields (85–90% over both reactions) were obtained. A full assessment of the stereochemical fidelity of this process was carried out by synthesis of the compounds with the N-terminal (and in the case of the dipeptides, the C-terminal) enantiomeric amino acids and HPLC analysis under conditions that gave full resolution of the diastereomeric impurities. Since silica gel chromatography was carried out with solvent systems that did not provide separation of the diastereomeric pairs, presumably no inadvertent removal of diastereomeric impurities occurred, and the HPLC analysis should therefore reflect the actual ratio of products formed. It should be noted though, that in the case of the dipeptides, kinetic enrichment of the LL/DD enantiomeric pair was possible since coupling of Fmoc-D-MeLeu with MeVal-O-*t*-Bu resulted in a 58% yield in 2 h and only 70% over 18 h, whereas the LL dipeptide was formed in over 90% yield and was generally complete in 2–3 h. However, it seems reasonable to assume that the main source of optical impurity was the esterification reaction and in any event, no more that 0.7% diastereomeric impurity was found in any of the cases examined.

Although the Fmoc procedure has the virtues of speed (one residue per day may be added to the peptide chain if desired) and potential adaptability to solid phase, there are also a number of disadvantages associated with Fmoc protection. If multigram quantities or many analogues are desired, its expense can be considerable. Also, Freidinger's[15] N-alkylation methodology is a two-step procedure requiring chromatography or crystallization at each step, whereas McDermott and Benoiton's[16] N-methylation of Cbz amino acids provides compounds of good purity simply upon aqueous workup. Furthermore, we have noted that the Fmoc–protected peptides, especially those which exist as oils, undergo a slow and evidently self-catalytic decomposition at room temperature, which appears to be accelerated by exposure to sunlight. When stored in the cold under a N_2 atmosphere, the Fmoc peptides are stable for many months without apparent decomposition, but for the above reasons we chose to carry out larger scale work with the Cbz group.

Coupling of the Cbz-protected intermediates, as well as hydrogenolytic removal of the N-protection, proceeded in uniformly excellent yields (93–99%) (scheme II).[17] In the final step of the synthesis, a first run of coupling with Fmoc-D-Ala to give the finished tetrapeptide proceeded in relatively poor yield (76%). During the course of this condensation, stirring failed, and the reaction did not proceed to completion. To assure that the problem had indeed been of mechanical origin a second run was carried out; in this case, deprotection of **10c** and coupling to Fmoc-D-Ala yielded 85% of the desired compound over the two steps. This result points out the necessity for continuous stirring of these heterogeneous reactions.

The BOP-Cl coupling reagent has provided a remarkably simple and efficient (66–73% overall yield) synthesis of this challenging peptide. Further experiments indicate its applicability to the entire cyclosporine molecule; these will be reported shortly.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker WP-200 or WP-270 instrument in $CDCl_3$ unless otherwise noted. Chemical shifts are reported in ppm (δ units) downfield of tetramethylsilane. Since in all cases except that of compounds **6d** and **6e** the spectra showed multiple conformations, generally with different coupling constants for the different rotamers, J values are for the most part omitted. Silica gel chromatography was carried out under low pressure (5–15 psi) using Merck grade 60 silica, 230–400 mesh. TLCs were run on Merck Kieselgel 60 plates with fluorescent indicator. Cbz-N-methyl amino acids were synthesized by the procedure of McDermott and Benoiton.[16] Fmoc-N-methyl amino acids were synthesized by the procedure of Freidinger et al.[15] HPLC analyses were carried out on a Waters instrument using either a Waters μBondapak C18 or a Vydec C18 analytical column.

MeVal-O-*t*-Bu (6d). A solution of **5d** (3.01 g, 13.0 mmol) in 20 mL of dioxane was treated with 4.95 g (26.0 mmol) of *p*-toluenesulfonic acid monohydrate and heated under reflux for 40 min ($CaCO_3$ drying tube), at which time complete disappearance of starting material was observed by TLC (10% MeOH/$CHCl_3$). The mixture was cooled in an ice/H_2O bath, then

(13) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404–3409, 4218.

(14) For leading references, see: Atherton, E.; Dryland, A.; Sheppard, R. C.; Wade, J. D. In *Peptides, Structure and Function*; Hruby, V. J.; Rich, D. H., Eds.; Pierce Chemical Co.: Rockford, IL, 1983; pp 45–54.

(15) Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1983**, *48*, 77–81.

(16) McDermott, J. R.; Benoiton, N. L. *Can. J. Chem.* **1973**, *51*, 1915–1919.

(17) In some cases, filtration of the reaction mixture and evaporation of the filtrate resulted in a white solid, but after acid–base workup colorless oils were always obtained. The nature of this solid is currently under investigation.

transferred to a thick-walled pressure flask, and treated with 25 mL of isobutylene which had been condensed at −78 °C. The flask was capped and vigorously stirred for 19 h at ambient temperature and then chilled and uncapped, and the contents were poured into cold dilute aqueous NaOH. The mixture was adjusted to pH ~10 (narrow range pH paper) and then extracted with ether (2 × 25 mL). The organic layers were combined, dried over K_2CO_3, and evaporated, and the residue was distilled (short-path condenser) to yield 449 mg (18%) of a colorless oil: bp 72–75 °C (14 mmHg), $[\alpha]_D$ +4.4° (*c* 1.0, $CHCl_3$) [lit.[3] bp 78 °C (16 mmHg), $[\alpha]_D$ +4.5° (*c* 1.0, $CHCl_3$)]; ^1H NMR δ 0.96 (dd, 6 H, *J* = 2.1, 7.4 Hz), 1.43 (br s, NH), 1.48 (s, 9 H), 1.86 (br s, 1 H), 2.37 (s, 3 H), 2.76 (d, 1 H, *J* = 6.4 Hz).

The procedure was later modified in a synthesis of the D-enantiomer **6e**. Thus, removal of the Boc group from **5e** using 1.2 equiv of H_2SO_4 in dioxane followed by treatment with additional H_2SO_4 as a cosolvent and reaction with isobutylene for 4 days at room temperature yielded, after workup and distillation, a 74% yield of the analytically pure amine *tert*-butyl ester, $[\alpha]_D$ −3.5° (*c* 1.0, $CHCl_3$). Considerable ninhydrin-positive material was still present in the extracted aqueous phase, so presumably longer reaction times (6 or more days) would result in yet higher yields.

Fmoc-MeVal-O-*t*-Bu (6a). A suspension of **5a** (4.95 g, 14.0 mmol) in 60 mL of 20% THF/Et_2O was cooled in an ice/H_2O bath under N_2 and treated with oxalyl chloride (1.30 mL, 15.0 mmol), followed by a catalytic amount (10 drops) of DMF. The mixture was stirred for 2 h in the cold and then 45 min at ambient temperature, and the volatiles were removed in vacuo. The yellow, oily residue was taken up in 25 mL of benzene, flushed with N_2, and treated with 2.0 mL (19.6 mmol) of *tert*-butyl alcohol, followed immediately by addition of 3.75 g (25.0 mmol) of AgCN. The suspension was heated under reflux (100 °C oil bath) for 1.5 h, then cooled, diluted with ~30 mL of hexanes, and filtered through a pad of Celite. The filtrate was concentrated to a yellow oil, which was purified by silica gel chromatography (170 g of silica, 5.5% acetone/hexanes eluent), to yield 4.97 g (87%) of a viscous, colorless oil: $[\alpha]_D$ −63.3° (*c* 1.0, $CHCl_3$); ^1H NMR (two major conformations, ~10:1 ratio) δ 0.73–1.04 (m, 6 H), 1.27–1.59 (m, 9 H), 2.12 (br m, 1 H), 2.90 (s, 3 H), 4.06–4.61 (m, 4 H), 7.24–7.46 (m, 4 H), 7.61 (d, 2 H, *J* = 6.2 Hz), 7.78 (d, 2 H, *J* = 6 Hz). Anal. Calcd for $C_{25}H_{31}NO_4$: C, 73.32; H, 7.63; N, 3.42. Found: C, 73.16; H, 7.73; N, 3.14.

Fmoc-D-MeVal-O-*t*-Bu (6b). This compound was prepared from **5b** by the procedure outlined above for compound **6a**, except that CH_2Cl_2 was used as the reaction solvent for the preparation of the acid chloride. Also, the filtrate obtained after Celite filtration was washed with 1 N $NaHCO_3$, H_2O, and brine and then dried over Na_2SO_4 before chromatography. It was later determined that some loss of product had occurred at the washing stage due to decomposition of the stock $NaHCO_3$ solution to Na_2CO_3, with resultant removal of some of the Fmoc group. Yield of the pure material was 60%; $[\alpha]_D$ +61.4° (*c* 1.0, $CHCl_3$). The ^1H NMR was identical with that of the L enantiomer.

Cbz-MeVal-O-*t*-Bu (6c). A thick-walled pressure flask was charged with a solution of **5c** (6.53 g, 25 mmol), 30 mL of CH_2Cl_2, and 75 mL of isobutylene (condensed at −78 °C). This mixture was cautiously treated with 15 drops of concentrated H_2SO_4, stoppered, and stirred vigorously for 8 days at ambient temperature. The flask was then chilled and opened, and the contents were partitioned between 1 N $NaHCO_3$ (90 mL) and CH_2Cl_2 (3 × 50 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated in vacuo to a yellow-brown oil, which was taken up in 60 mL of isopropyl alcohol, flushed with N_2, and treated with 600 mg of 10% Pd on carbon. Hydrogenation for 2 days at ambient pressure and 1 atm of H_2 resulted in only partial reaction, so additional Pd on carbon (600 mg) was introduced to the mixture and stirring was resumed for 18 h. Filtration of the reaction mixture through Celite and concentration yielded a brown oil, which was short-path distilled [70–74 °C (13mm)] to yield 3.32 g (71% overall) of **6d** which was identical in all respects with that obtained via the Boc-MeVal (**5d**) route (vide supra). In a separate run, the Cbz-MeVal *tert*-butyl ester (**6c**) was isolated by silica gel chromatography using 5% acetone/hexanes as eluant to yield 71% of the theoretical amount of a yellow oil: $[\alpha]_D$ −78.6° (*c* 1.0, $CHCl_3$); ^1H NMR (two conformations) δ 0.88, 0.99 (2 dd, 6 H, *J* = 7, 14 and 7, 11.9 Hz), 1.43, 1.45 (2 s, 9 H total), 2.17 (br m, 1 H), 2.92 (br s, 3 H), 4.20, 4.40 (2 d, 1 H total, *J* = 11, 10 Hz), 5.10–5.28 (m, 2 H), 7.26–7.32 (br s, 5 H). Anal. Calcd for $C_{18}H_{27}NO_4$: C, 67.26; H, 8.47; N, 4.35. Found: C, 67.17; H, 8.31; N, 4.35.

General Procedure for Sequential N-Deprotection and Coupling of Fmoc-Protected Amino Acids and Peptides. A solution of the N-Fmoc-protected compound in CH_3CN (ca. 50 mM) was flushed with N_2, treated with an equal volume of diethylamine, and then stirred for 40 min–1 h (the reaction may be followed by TLC using acetone/hexanes solvent systems), 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 simultaneously, in one portion, with 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. We have found that the 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 of the reaction, the volatiles were removed in vacuo, and the residue was partitioned between EtOAc and water. The organic layer was separated and then washed sequentially with 1 N $NaHCO_3$ (solutions should be made up every 2 weeks to avoid the presence of Na_2CO_3), H_2O, and brine, then dried over Na_2SO_4, and concentrated in vacuo. The residue was then purified by silica gel chromatography using acetone/hexanes as the eluant. It was found to be advantageous to complete the workup and chromatography within 24 h of the reaction in order to avoid formation of insoluble polymers from the dibenzolfulvene byproduct and resultant clogging of the column.

Fmoc-MeLeu-MeVal-O-*t*-Bu (8a). Compound **6a** (1.65 g, 4.00 mmol) was N-deprotected and coupled with **7a** as described above in the general procedure. Chromatographic purification using 7.5% acetone/hexanes as eluant yielded 1.94 g (90%) of a colorless glass which began to form a white solid on standing in the refrigerator for several months: $[\alpha]_D$ −117.3° (*c*, 1.0, $CHCl_3$); ^1H NMR δ 0.53–1.07 (m, 12 H), 1.36–1.47 (m, 10 H), 1.59–1.68 (m, 2 H), 2.05 (br m, 1 H), 2.70–2.96 (m, 6 H), 4.18–5.17 (m, 5 H), 7.30–7.79 (m, 8 H). Anal. Calcd for $C_{32}H_{44}N_2O_5$: C, 71.61; H, 8.26; N, 5.22. Found: C, 71.40; H, 8.41; N, 5.35.

Fmoc-MeLeu-D-MeVal-O-*t*-Bu (8b). Compound **6b** (1.26 g, 3.08 mmol) was N-deprotected and coupled with **7a** as described above in the general procedure. Chromatographic purification using 7.5% acetone/hexanes as eluant yielded 1.31 g (79%) of a colorless glass which began to solidify on standing for several weeks in the refrigerator; $[\alpha]_D$ +3.8° (*c* 1.0, $CHCl_3$); ^1H NMR δ 0.56–1.04 (m, 12 H), 1.28–1.78 (m, 12 H), 2.14 (br m, 1 H), 2.69–2.95 (m, 6 H), 4.21–5.38 (series of m, 5 H), 7.27–7.79 (m, 8 H). Anal. Calcd for $C_{32}H_{44}N_2O_5$: C, 71.61; H, 8.26; N, 5.22. Found: C, 71.67; H, 8.50; N, 5.00.

Fmoc-D-MeLeu-MeVal-O-*t*-Bu (8c). Compound **6a** (206 mg, 0.500 mmol) was N-deprotected and coupled with **7b** as described above in the general procedure (2 h total reaction time). Chromatographic purification using 7.5% acetone/hexanes as eluant yielded 189 mg (70%) of a white solid: mp 87.5–89 °C; $[\alpha]_D$ −6.8° (*c* 1.0, $CHCl_3$); ^1H NMR δ 0.53–1.05 (m, 12 H), 1.27–1.46 (m, 10 H), 1.48–1.80 (m, 2 H), 2.16 (br m, 1 H), 2.70–2.96 (m, 6 H), 4.19–5.37 (m, 5 H), 7.26–7.78 (m, 8 H). Anal. Calcd for $C_{32}H_{44}N_2O_5 \cdot {}^1/_4H_2O$: C, 71.02; H, 8.28; N, 5.18. Found: C, 71.20; H, 8.30; N, 5.02.

Fmoc-MeLeu-MeLeu-MeVal-O-*t*-Bu (10a). Compound **8a** (1.89 g, 3.52 mmol) was N-deprotected and coupled with **7a** as described above in the general procedure (18-h reaction time). Chromatographic purification using 8.5% acetone/hexanes as eluant yielded 2.04 g (87%) of a colorless glass: $[\alpha]_D$ −153.4° (*c* 1.0, $CHCl_3$); ^1H NMR δ 0.52–1.06 (m, 18 H), 1.25–1.81 (m, 6 H), 1.42–1.48 (m, 9 H), 2.19 (br m, 1 H), 2.56–2.98 (m, 9 H), 3.98–5.55 (series of m, 6 H), 7.26–7.79 (m, 8 H). Anal. Calcd for $C_{39}H_{57}N_3O_6$:

C, 70.56; H, 8.65; N, 6.33. Found: C, 70.92; H, 8.47; N, 6.26.

Fmoc-D-MeLeu-MeLeu-MeVal-O-*t*-Bu (10b). Compound **8a** (268 mg, 0.500 mmol) was N-deprotected and coupled with **7b** as described above in the general procedure (18 h reaction time). Chromatographic purification, eluting with 7.5% acetone/hexanes yielded 282 mg (85%) of a colorless glass, which began to solidify on standing in the refrigerator for several weeks: $[\alpha]_D$ -44.0° (*c* 1.0, $CHCl_3$); ^1H NMR δ 0.55–1.02 (m, 18 H), 1.26–1.87 (m, 15 H), 2.10 (br m, 1 H), 2.56–3.08 (m, 9 H), 4.18–5.54 (series of m, 6 H), 7.25–7.78 (m, 8 H). Anal. Calcd for $C_{39}H_{57}N_3O_6$: C, 70.56; H, 8.65; N, 6.33. Found: C, 70.66; N, 8.86; N, 6.04.

Fmoc-Ala-MeLeu-MeLeu-MeVal-O-*t*-Bu (12a). Compound **10a** (332 mg, 0.500 mmol) was N-deprotected and coupled with Fmoc-Ala as described above in the general procedure. Chromatographic purification using 15% acetone/hexanes as eluant yielded 286 mg (78%) of a white, foamy solid, which did not exhibit a distinct melting point: $[\alpha]_D$ -155.5° (*c* 1.0, $CHCl_3$); ^1H NMR δ 0.80 (d, 3 H, J = 6.8 Hz), 0.83–1.05 (m, 18 H), 1.23–1.78 (m, 15 H), 2.17 (br m, 1 H), 2.82–3.05 (m, 9 H), 3.96–4.76 (m, 5 H), 5.44–5.78 (m, 3 H), 7.27–7.74 (m, 4 H), 7.50 (d, 2 H, J = 7 Hz), 7.76 (d, 2 H, J = 7 Hz). Anal. Calcd for $C_{42}H_{62}N_4O_7$: C, 68.64; H, 8.50; N, 7.26. Found: C, 68.73; H, 8.54; N, 7.28.

Fmoc-D-Ala-MeLeu-MeLeu-MeVal-O-*t*-Bu (12b, from 10a). Compound **10a** (350 mg, 0.527 mmol) was N-deprotected and coupled with Fmoc-D-Ala as described above in the general procedure. Chromatographic purification using 15% acetone/hexanes as eluant yielded 331 mg (85%) of a white, foamy solid, which did not exhibit a distinct melting point: $[\alpha]_D$ -152.6° (*c* 1.0, $CHCl_3$); ^1H NMR δ 0.79 (d, 3 H, J = 6.8 Hz), 0.84–1.08 (m, 18 H), 1.27–1.87 (m, 15 H), 2.17 (br m, 1 H), 2.81–3.13 (m, 9 H), 3.96–4.77 (m, 5 H), 5.48–5.76 (m, 3 H), 7.26–7.42 (m, 6 H), 7.60 (d, 1 H, J = 7 Hz), 7.78 (d, 1 H, J = 7 Hz). Anal. Calcd for $C_{42}H_{62}N_4O_7$: C, 68.64; H, 8.50; N, 7.62. Found: C, 68.60; H, 8.47; N, 7.63.

Cbz-MeLeu-MeVal-O-*t*-Bu (8d). A solution of **6d** (2.28 g, 15.0 mmol) and **7c** (4.19 g, 15.1 mmol) in 200 mL of CH_2Cl_2 was cooled with stirring under an atmosphere of N_2 in an ice/H_2O bath. The cold mixture was treated with diisopropylethylamine (5.75 mL, 32.3 mmol) followed, in one portion, by BOP-Cl (4.19 g, 16.5 mmol). The mixture was stirred for 2 h in the cold and then concentrated in vacuo. The residue was partitioned between H_2O and EtOAc, and the organic layer was separated and washed with $KHSO_4$, H_2O, 1 N $NaHCO_3$, 50% brine, and brine. After drying over Na_2SO_4 it was concentrated in vacuo to a yellow oil, which was purified by flash chromatography on 300 g of silica gel, eluting with 7.5% acetone/hexanes: yield, 6.26 g (93%) of colorless oil; $[\alpha]_D$ -138.9° (*c* 1.0, $CHCl_3$) [lit.[3] $[\alpha]_D$ -136.5° (*c* 1.0, $CHCl_3$)]. ^1H NMR δ 0.67–1.10 (m, 12 H), 1.34–1.50 (m, 9 H), 1.56–1.82 (m, 3 H), 2.17 (br m, 1 H), 2.72–3.00 (series of s, 6 H), 4.13 (br m, 1 H), 4.75 (m, 1 H), 5.07–5.23 (m, 2 H), 7.34 (br s, 5 H).

MeLeu-MeVal-O-*t*-Bu (9). A solution of **8d** (4.65 g, 10.4 mmol) in 40 mL of 2-propanol was flushed with N_2 and treated with 500 mg of 10% Pd on carbon. The mixture was placed under an atmosphere of H_2, stirred for 14 h, then flushed with N_2, filtered through a pad of Celite, and concentrated in vacuo. The residue was treated with 150 mL of 0.5 N HCl, which was then washed with ether (2x), made basic with 5% NH_4OH (to pH ~9 by narrow range paper), and again washed with ether (3×). These latter organic extracts were combined, washed with 50% brine and brine, and dried over $MgSO_4$. Concentration in vacuo yielded 3.04 g (93%) of a colorless oil: $[\alpha]_D$ -115.4° (*c* 1.0, $CHCl_3$) [lit.[3] $[\alpha]_D$ -118° (*c* 1.0, $CHCl_3$)]; ^1H NMR (Me_2SO-d_6) δ 0.74–0.95 (m, 12 H), 1.12–1.24 (m, 3 H), 1.37, 1.40 (2 s, 9 H total), 1.61 (br s, NH), 1.74 (septet, 1 H, J = 7.7 Hz), 2.04–2.17 (m, 4 H), 2.78, 2.89 (2 s, 3 H total), 3.42 (br s), 4.63 (d, 1 H, J = 10.5 Hz).

Cbz-MeLeu-MeLeu-MeVal-O-*t*-Bu (10c). A solution of **9** (2.75 g, 8.75 mmol) and diisopropylethylamine (3.20 mL, 18.4 mmol) in 120 mL of CH_2Cl_2 was cooled with stirring under an atmosphere of N_2 in an ice/H_2O bath. The cold mixture was treated simultaneously, in one portion, with **7c** (2.57 g, 9.20 mmole and BOP-Cl (2.34 g, 9.20 mmol). The mixture was stirred for 24 h, slowly warming to 10 °C. After being washed with H_2O, 10% $KHSO_4$, 1 N $NaHCO_3$, 50% brine, and brine, it was dried over $MgSO_4$ and concentrated in vacuo to a yellow oil. Purification on a column of 250 g of silica gel, eluting with 7.5% acetone/hexanes, yielded 5.00 g (99%) of a colorless syrup, $[\alpha]_D$ -173.5° (*c* 1.0, $CHCl_3$) [lit.[3] $[\alpha]_D$ -165.5° (*c* 1.0, $CHCl_3$)]; ^1H NMR δ 0.76–1.01 (m, 18 H), 1.28–1.75 (m, 15 H), 2.08 (br m, 1 H), 2.71–3.00 (m, 9 H), 4.68–5.57 (m, 5 H), 7.33–7.37 (m, 5 H).

MeLeu-MeLeu-MeVal-O-*t*-Bu (11). Hydrogenation of **10c** (4.20 g, 6.95 mmol) was carried out as described above for compound **9**, using 30 mL of 2-propanol and 300 mg of 10% Pd on carbon. However, in this case, treatment of the residue from the hydrogenation mixture with aqueous HCl resulted in formation of the hydrochloride salt as a white, water-insoluble solid. This solid was treated with 5% NH_4OH, and the mixture was extracted with ether (3x). The organic layers were combined, washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo to yield 2.94 g (95%) of a colorless syrup: $[\alpha]_D$ -183.0° (*c* 1.0, $CHCl_3$); ^1H NMR (Me_2SO-d_6) δ 0.70 (d, J = 7 Hz) and 0.82–0.96 (m) (18 H total), 1.13–1.81 (m, 16 H), 2.13 (br s, 4 H), 2.64 (s) and 2.83–2.90 (m) (6 H total), 3.41 (br m, 1 H), 4.12, 4.46 (2 d, 1 H total, J = 13, 10 Hz), 5.52 (m, 1 H). Anal. Calcd for $C_{24}H_{47}N_3O_4$: C, 65.27; H, 10.73; N, 9.51. Found: C, 65.05; H, 10.76; N, 9.55.

Fmoc-D-Ala-MeLeu-MeLeu-MeVal-O-*t*-Bu (12b, from 11). A solution of **11** (2.33 g, 5.44 mmol) and 1.99 mL (11.1 mmol) of diisopropylethylamine in 75 mL of CH_2Cl_2 was cooled with stirring under an atmosphere of N_2 in an ice/H_2O bath. The cold mixture was treated simultaneously, in one portion, with Fmoc-D-Ala (1.78 g, 5.72 mmol) and BOP-Cl (1.46 g, 5.74 mmol). The mixture was transferred to a 4–6 °C cold room and allowed to react for 17 h. Sometime during that period the stirring ceased, and the reaction did not go to completion. Workup as described above for **10c** and chromatography on 200 g of silica gel, eluting with 15% acetone/hexanes, yielded 2.96 g (76%) of an analytically pure white foam, identical with that obtained from **10a** (vide supra). In a second run, hydrogenation of **10c** (283 mg, 0.500 mmol) was carried out as described earlier, except that acid–base workup was not attempted. Instead, the residue was directly coupled to Fmoc-D-Ala with BOP-Cl to yield, after silica gel chromatography, 311 mg (85% from **10c**) of a white foam, identical in all respects with that described above.

HPLC Studies. Dipeptides. These compounds were run as the fully protected species and were detected at 254 nm. Using the Waters column with an isocratic system of 57% CH_3CN/43% H_2O, analysis was carried out on a 20-μL sample of a 1 mg/mL solution of the appropriate compound in CH_3CN. With these conditions, base line resolution was obtained between the LL and DL/LD enantiomeric pair of dipeptides. Estimates of diastereomeric impurities were as follows: LL, 0.7%; DL, 0.5%; LD, <0.1%.

Tripeptides. These compounds were run as the fully protected species and were detected at 254 nm. Using the Vydec column with an isocratic system of 67% CH_3CN/37% H_2O, analysis was carried out on 15-μL samples of a 1 mg/mL solution of the appropriate compound in CH_3CN. Using these conditions, resolution to >95% of peak height (equal height peaks) was obtained. For both the LLL and DLL diastereomers, <0.1% optical impurities were observed.

Tetrapeptides. These compounds were not amenable to separation when fully protected, so a sample of each diastereomer was N-deprotected in the following manner: 25 mg of the appropriate tetrapeptide was taken up in 0.5 mL of CH_3CN, flushed with N_2, and treated with 0.5 mL of diethylamine. The mixture was stirred for 40 min at ambient temperature under N_2, and then the volatiles were removed in vacuo. The free amino tetrapeptide was then purified from the Fmoc-related byproducts on a column of 14 g of silica gel, by eluting with 7% MeOH/$CHCl_3$. In the case of these compounds detection at 214 nm was employed. Using the Waters column with an isocratic system of 35% CH_3CN/67% 50 mM triethylammonium phosphate pH 2.25 buffer, analysis was carried out on 15-μL samples of a 1 mg/mL solution of the appropriate compound in CH_3CN. With these conditions, resolution to >95% of peak height (equal-sized peaks) was obtained. Estimates of the diastereomeric impurities were as follows: LLLL, ≪0.1%; DLLL, 0.5%. It was not determined to what extent impurities in the commercial Fmoc-D-Ala contributed to this figure.

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