(1*E*,3*Z*)-1,3-Nonadienyl 8-((2,2-Dimethylpropanoyl)oxy)octanoate (3). A mixture of 0.773 g (2.12 mmol) of **10** and 240 mg of Lindlar's catalyst in 35 mL of benzene was subjected to 20 cycles of evacuation and flushing with hydrogen at 1 atm. The reduction was followed by TLC; after 20 min the reduction was complete. Filtration through Celite and evaporation gave 0.773 g (99%) of **3** as a pale yellow oil. NMR ($CDCl_3$): δ 7.37 (d, J = 12.2 Hz, 1 H, =C'HO), 6.29 (t, 1 H, 2' =CH), 5.91 (t, 1 H, 3' =CH), 5.49 (m, 1 H, 4' =CH), 4.04 (t, 2 H, 8-CH_2O), 2.36 (t, 2 H, 2-CH_2), 2.11 (m, 2 H, 5'-CH_2), 1.72–1.50 (m, 6 H, 3-, 7-, and 6'-CH_2), 1.45–1.30 (m, 10 H, 4-, 5-, 6-, 7'-, and 8'-CH_2), 1.19 (s, 9 H, CMe_3), 0.89 (t, 3 H, 9'-CH_3). IR (neat): 3080 (=CH), 1750 (dienol ester C=O), 1725 (ester C=O), 1650, 1605 (C=C cm^{-1}. UV (EtOH): λ_{max} 240 nM. MS (DCI, NH_3): 384 (M + NH_4^+). HRMS (EI) for $C_{22}H_{38}O_4$: calcd 366.276 99, found 366.275 96.

Enol Phosphate 4. A solution of 0.1270 g (0.35 mmol) of **3** in 1 mL of THF at −108 °C was added dropwise over 4 min via a cold (−108 °C) cannula to a solution of 0.385 mmol of lithium diisopropylamide and 0.7 mL of HMPA in 2 mL of THF at −108 °C. The light yellow solution was stirred for 2 min longer, then 69 μL of diethylphosphorobromidate in 1 mL of THF at −108 °C was added over 1 min. The mixture was kept at this temperature for 1.5 h and then was allowed to warm to 0 °C. The mixture was diluted with 15 mL of ether, washed with water (2 × 4 mL), 1 M potassium carbonate (1 × 4 mL), 1 M lithium chloride (1 × 4 mL), and brine (1 × 4 mL), dried over potassium carbonate, and evaporated. Chromatography on silica (1:1 hexane–ethyl acetate) gave 0.0925 g (53%) of **4**. NMR ($CDCl_3$): δ 6.45 (d, 1 H, 10-CH), 6.14 (t, 1 H, 11-CH), 5.40 (m, 1 H, 12-CH), 4.87 (m, 1 H, 13-CH), 4.26–4.15 (m, 5 H, 7-CH and P-OCH_2), 4.03 (t, 2 H, 1-CH_2), 2.10–1.95 (m, 4 H, 6- and 14-CH_2), 1.63–1.58 (m, 4 H, 5- and 15-CH_2), 1.45–1.20 (m, 27 H, 2-, 3-, 4-, 16-, and 17-CH_2, *t*-Bu and phos-CH_3), 0.89 (t, 3 H, 18-CH_3). IR (neat): 1730 (ester C=O), 1650, 1600 (C=C), 1285 (P=O) cm^{-1}. UV (*c*-C_6H_{12}): λ_{max} 238 nm. MS (DCI NH_3): 503 (M+ NH_4^+).

Vinyl Ether 11. A solution of **4** (92.5 mg, 184 μmol) in 2.6 mL of hexane was cooled to 0 °C, and 0.37 mL of 1 M triethylaluminum in hexane was added by syringe, followed at once by 11 mg of tetrakis(triphenylphosphine)palladium(0) suspended in 0.3 mL of 1,2-dichloroethane. The reaction mixture was brought to 20 °C, and 1,2-dichloroethane was added dropwise until a homogeneous solution was obtained (0.6 mL required). After 10.5 h at 20 °C, the reaction was terminated by the addition of 15 mL of ether, and the solution was washed with 3 M sodium hydroxide (2 × 4 mL), water (2 × 4 mL), and brine (1 × 4 mL), dried, and filtered through Florisil. Chromatography on silica gave 24.3 mg of recovered **4** and 33.3 mg (70% corrected for recovered **4**) of **11**. NMR ($CDCl_3$): δ 6.50 (m, 1 H, 10-CH), 6.26 (m, 1 H, 8-CH), 6.00 (m, 1 H, 11-CH), 5.85 (m, 1 H, 12-CH), 5.31 (m, 1 H, 13-CH), 5.10 (m, 1 H, 7-CH), 4.04 (t, 2 H, 1-CH_2), 2.12 (m, 2 H, 14-CH_2), 1.95 (m, 2 H, 6-CH_2), 1.64 (m, 4 H, 5- and 15-CH_2), 1.45–1.20 (m, 10 H, 2-, 3-, 4-, 16-, and 17-CH_2), 1.19 (s, 9 H, *t*-Bu), 0.89 (t, 3 H, CH_3). IR (neat): 1730 (ester C=O), 1650, 1605 (C=C) cm^{-1}. UV (*c*-C_6H_{12}): λ_{max} 247 nM.

Colneleonitrile (12). Potassium hydroxide (60 mg, 1.07 mmol) was dissolved in 300 μL of water, and the ester **11** (9.3 mg, 26.5 μmol) was added in 2.0 mL of methanol. The mixture was heated at reflux for 4 h, after which it was cooled and the methanol was evaporated. Ether (8 mL) was added, and the mixture was washed with water (1 × 1 mL), 1 M potassium carbonate (1 × 1.5 mL), and brine (1 × 1.5 mL), dried, and evaporated to yield 6.8 mg (96%) of the corresponding alcohol. NMR ($CDCl_3$): δ 6.50 (m, 1 H, 10-CH), 6.21 (m, 1 H, 8-CH), 6.00 (m, 1 H, 11-CH), 5.85 (m, 1 H, 12-CH), 5.31 (m, 1 H, 13-CH), 5.10 (m, 1 H, 7-CH), 3.63 (t, 2 H, 1-CH_2), 2.12 (m, 2 H, 14-CH_2), 1.95 (m, 2 H, 6-CH_2), 1.64 (m, 4 H, 5- and 15-CH_2), 1.45–1.20 (m, 10 H, 2-, 3-, 4-, 16-, and 17-CH_2), 0.89 (t, 3 H, CH_3). IR (neat): 3600–3100 (OH), 1650, 1610 (C=O) cm^{-1}. UV (C_6H_{12}): λ_{max} 246 nM. MS (DCI, NH_3): 267 (M + H^+).

A solution of the above alcohol (6.5 mg, 24.3 μmol) in 800 μL of dichloromethane was cooled to −23 °C, and 5.1 μL (36.4 μmol) of triethylamine was added, followed by 2.1 μL (26.7 μmol) of methanesulfonyl chloride. After 0.5 h at −23 °C, TLC analysis indicated complete conversion to the mesylate had occurred. The mixture was warmed to 20 °C, diluted with 3.5 mL of pentane, washed with 1 M potassium carbonate (2 × 1 mL) and brine (1 × 1 mL), dried, and evaporated. The residue was dissolved in 1.5 mL of DMSO, and 60 mg (1.22 mmol) of finely powdered sodium cyanide was added. After 5 h at 20 °C, the mixture was diluted with 5 mL of ether and washed with water (2 × 1 mL). The water was back-extracted with ether (2 mL). The combined ether extracts were washed with water (2 × 2 mL) and brine (1 × 2 mL), dried, and evaporated to yield 7.0 mg (100%) of **12**. NMR ($CDCl_3$): δ 6.52 (m, 1 H, 10-CH), 6.23 (m, 1 H, 9-CH), 6.00 (m, 1 H, 11-CH), 5.85 (m, 1 H, 12-CH), 5.31 (m, 1 H, 13-CH), 5.10 (m, 1 H, 8-CH), 2.34 (t, 2 H, 2-CH_2), 2.12 (m, 2 H, 14-CH_2), 1.95 (m, 2 H, 7-CH_2), 1.64 (m, 6 H, 3-, 6-, and 15-CH_2), 1.45–1.20 (m, 8 H, 4-, 5-, 16-, and 17-CH_2), 0.89 (t, 3 H, CH_3). IR (neat): 2240 (C≡N), 1650, 1605 (C=C) cm^{-1}. UV (C_6H_{12}): λ_{max} 248 nm. MS (EI): 275 (M^+).

Methyl Colneleate. Six milligrams (21.7 μmol) of **12** was dissolved in 1 mL of ethanol, and 50 mg (0.89 mmol) of potassium hydroxide was added. The mixture was heated at reflux for 5 h. Ether (5 mL) was added to the cooled flask, and the solution was brought to pH 4.0 with 2 M citric acid. The layers were separated, and the aqeous phase was extracted with ether (1 mL) again. The combined ether extracts were washed with water (3 × 1 mL) and brine (1 × 1 mL), dried over sodium sulfate, and transferred to a clean flask. Diazomethane in nitrogen was bubbled through the solution at 0 °C for 10 min. The solution was then evaporated to dryness. The residue was dissolved in 2:1 hexane–ether and filtered through a short plug of silica. Evaporation gave 4.1 mg (61%) of methyl colneleate. The natural 8*E* isomer was separated by preparative HPLC (DuPont Zorbax silica, 4.6 mm × 250 mm, 0.2% THF in hexane, 2 mL min^{-1}, R_t = 21.6 min (8*Z* isomer, R_t = 19.0 min). NMR ($CDCl_3$): δ 6.50 (d, J = 11.8 Hz, 1 H, 11-CH), 6.26 (d, J = 12.2 Hz, 1 H, 9-CH), 5.99 (t, J = 11.8 Hz, 1 H, 12-CH), 5.85 (t, J = 11.1 Hz, 1 H, 13-CH), 5.29 (d of t, J_d = 10.3 Hz, 1 H, 13-CH), 5.14 (d of t, J_d = 12.2 Hz, 1 H, 8-CH), 2.30 (t, 2 H, 2-CH_2), 2.09 (m, 2 H, 15-CH_2), 1.94 (m, 2 H, 7-CH_2), 1.62 (m, 2 H, 3-CH_2), 1.38–1.22 (m, 12 H, 4-, 5-, 6-, 16-, 17-, and 18-CH_2), 0.88 (t, 3 H, CH_3). IR (neat): 3020 (=CH), 1740 (ester C=O), 1645, 1605 (C=C) cm^{-1}. UV (C_6H_{12}): λ_{max} 248 nm. MS (EI): 308 (M^+). HRMS (DCI, NH_3) for $C_{19}H_{32}O_3$: calcd 308.23497, found 308.23498. Synthetic **1** and its methyl ester were identical with reference samples prepared from linoleic acid by incubation with the 15 000 g supernatant from potato homogenate as previously described.[2,3]

Registry No. **1**, 52761-34-9; **3**, 125076-99-5; (8*E*)-**4**, 125077-00-1; (8*Z*)-**4**, 125108-31-8; **5**, 3884-92-2; **6**, 125076-95-1; **7**, 125076-97-3; **8**, 125076-94-0; **9**, 87745-64-0; **10**, 125076-98-4; (8*E*)-**11**, 125077-01-2; (8*Z*)-**11**, 125108-32-9; (8*E*)-**12**, 125077-04-5; (8*Z*)-**12**, 125077-08-9; H_2C=$CHCH_2C$≡$C(CH_2)_4CH_3$, 24948-66-1; $HO(CH_2)_7CO_2Me$, 20257-95-8; $(CH_3)_3CCO_2(CH_2)_7CO_2H$, 125076-96-2; (*E*,*E*,*Z*)-$H_3C(CH_2)_4CH$=$CHCH$=$CHOCH$=$CH(CH_2)_6OH$, 125077-02-3; (*Z*,*E*,*Z*)-$H_3C(CH_2)_4CH$=$CHCH$=$CHOCH$=$CH(CH_2)_6OH$, 125077-06-7; (*E*,*E*,*Z*)-$H_3C(CH_2)_4CH$=$CHCH$=$CHOCH$=$CH(CH_2)_6OSO_2Me$, 125077-03-4; (*Z*,*E*,*Z*)-$H_3C(CH_2)_4CH$=$CHCH$=$CHOCH_2(CH_2)_6OSO_2Me$, 125077-07-8; (*E*,*E*,*Z*)-$H_3C(CH_2)_4CH$=$CHCH$=$CHOCH$=$CH(CH_2)_6CO_2Me$, 52077-21-1; (*E*,*Z*,*Z*)-$H_3C(CH_2)_4CH$=$CHCH$=$CHOCH$=$CH(CH_2)_6CO_2Me$, 125077-05-6.

Tris(2-aminoethyl)amine as a Substitute for 4-(Aminomethyl)piperidine in the FMOC/Polyamine Approach to Rapid Peptide Synthesis

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Received September 28, 1989

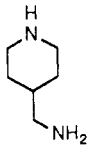
Recently[1,2] we described the use of FMOC amino acid chlorides as coupling agents in combination with de-

0022-3263/90/1955-1673$02.50/0

Table I. Synthesis of Peptide Segments via the FMOC/TAEA Method

compound	yield, %	mp, °C	$[\alpha]_D$; temp, °C
FMOC-Gly-Gly-Phe-OBn, 4	69.6	162–3	−8.4° (c 1, DMF); 23
FMOC-Tyr(Bn)-Gly-Gly-Phe-Leu-OBn, 5	45.8	191–2	−17.6° (c 0.5, DMF); 21
H-Lys-Pro-Lys-Pro-NH-$C_{12}H_{25}$, 6	60.9	100–4	−47.6° (c 0.5, DMF); 23
H-Phe-Phe-Val-Gly-Leu-Met-OBn, 7	40	210–16	−43.8° (c 0.5, $CHCl_3$); 23
H-Phe-Phe-Val-Gly-Leu-Met-NH_2, 8	32.4	228–32	−33.6° (c 0.3, DMF); 23

blocking by 4-(aminomethyl)piperidine (4-AMP) **1** as a convenient new technique for the rapid assembly of short peptide segments. Decisive to the success of the method



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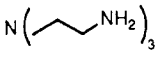

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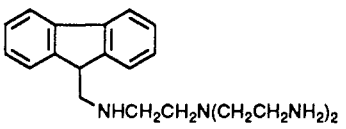
was extraction with a phosphate buffer of pH 5.5 to remove the byproduct arising from dibenzofulvene (DBF) and 4-AMP. In the case of certain sequences, especially if methylene dichloride is used as solvent, separation of the growing peptide from the 4-AMP/DBF adduct was complicated by formation of emulsions or separation of a voluminous precipitate during buffer extraction.

It has now been found that the formation of such precipitates can be avoided by substitution of tris(2-aminoethyl)amine (TAEA) **2** for 4-AMP as deblocking/scavenging agent. In this case adduct **3** is presumably formed and extracted by the phosphate buffer. Examples of peptides synthesized by the new protocol include **4**–**8** (Table I).



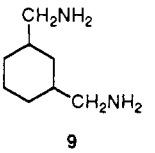
In the previous study trifunctional amino acids were incorporated into the sequence being assembled via benzyl- rather than *tert*-butyl-based side chain protection since it has not been generally possible to synthesize stable FMOC amino acid chlorides bearing protecting groups derived from *tert*-butyl alcohol. Since *tert*-butyl-based side chain protecting groups are more convenient to handle in the final deblocking step (use of TFA vs catalytic hydrogenolysis), we have now substituted the corresponding commercially available stable pentafluorophenyl esters[3] for the acid chlorides where necessary. This required only that the appropriate coupling step be extended from 10–20 min to about 45 min.

Prior to our selection of TAEA as deblocking/scavenging agent two other potentially useful polyamines including 1,3-cyclohexanebis(methylamine), CBMA **9**, and 1,4-bis-(3-aminopropyl)piperazine, BAPP **10**, were examined as substitutes for 4-AMP. Results of the deblocking/scavenging studies on a model urethane, 9-fluorenylmethyl *N*-*p*-chlorophenyl carbamate (FMOC–PCA), are recorded in Table II. Of the three bases, only TAEA led to the successful avoidance of emulsions and/or precipitates upon application to the synthesis of protected tetrapeptide **4**. It was therefore adopted for use with other difficult cases (Table I), and in no case were permanent precipitates or emulsions observed.

Table II. Deblocking of FMOC–PCA via Polyamines[a]

quantity of FMOC–PCA, mmol	quantity of polyamine, mL (equiv)	$CDCl_3$, vol, mL	time for complete deblocking, min	time for complete scavenging, min[a]
		TAEA		
0.14	1 (50)	2[b]	2	15
0.14	2 (100)	2	2	2
		CBMA		
0.10	1.45 (100)	1	2	15
0.14	2 (100)	2	2	50
		BAPP		
0.14	1.44 (50)	2[b]	2	300
0.14	1.44 (50)	1	2	60
0.14	2.88 (100)	2	2	50

[a] Reactions were followed by ^1H NMR analysis; in all cases, TLC analysis showed a light spot due to DBF at the time of complete scavenging according to NMR analysis. For the method used see Experimental Section. [b] These runs are at nearly the same concentration as used in the FMOC/4-AMP process.



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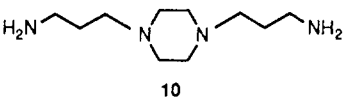

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Experimental Section

General. Melting points and boiling points were uncorrected. Infrared spectra were determined on Perkin-Elmer Model 237B, 1310, 1420, or 1600 FT spectrometers and ^1H NMR spectra on Varian XL-200 (200-MHz) or XL-300 (300-MHz) instruments with Me_4Si as internal standard. Rough kinetic studies of deblocking reactions were carried out on Varian A-60A or Perkin-Elmer R-12 NMR instruments. Column flash chromatography was performed with use of silica gel 60 (Merck, mesh size 230–400). TLC was effected with silica gel 60 F254 (Merck) on precoated glass or aluminum plates. Analytical HPLC was carried out on a Waters system incorporating a 720 controller, 730 data module, U6K injector, 6000A pumps, 441 detector, and Z-module radial compression unit. Preparative HPLC was carried out with a Waters Delta Prep unit using a C18-300 Å, 15 μm column (7.8 × 30 cm) using as eluant H_2O (0.1% TFA)/CH_3CN, 62/38. Optical rotations were determined on a Rudolph Autopol-III digital polarimeter using quartz cells.

Treatment of FMOC–PCA with 1,3-Cyclohexanebis(methylamine). ^1H NMR Examination. A solution of 50 mg of FMOC–PCA in 0.2 mL of CH_2Cl_2 was treated with 0.2 g of CBMA in an NMR tube. The first examination of the NMR spectrum 5 min later showed a strong 2-proton singlet for DBF at δ 6.1 as well as the upfield portion of the AA′BB′ system of the free *p*-chloroaniline at δ 6.5. At the concentration used deblocking via simple aliphatic amines is usually complete just after mixing so that the amount of DBF present can be determined by integrating against the 2-proton upfield PCA "doublet". Over the next 10–15 min the DBF peak dropped gradually as a doublet began to build up near δ 3.0 due to the DBF/CBMA adduct. After 60 min the DBF peak intensity had dropped to about one-fourth that of the PCA "doublet". With 40 mg of urethane, 0.4 mL of $CDCl_3$, and 0.2 g of CBMA, examination after 1 min showed only

(1) Carpino, L. A.; Cohen, B. J.; Stephens, K. E., Jr.; Sadat-Aalaee, S. Y.; Tien, J.-H.; Langridge, D. C. *J. Org. Chem.* **1986**, *51*, 3732.
(2) Beyermann, M.; Bienert, M.; Niedrich, H.; Carpino, L. A.; Sadat-Aalaee, D. *J. Org. Chem.* **1990**, *55*, 721.
(3) (a) Kisfaludy, L. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press, New York, 1980; Part A, Vol. 2, p 417. (b) Atherton, E.; Cameron, L. R.; Sheppard, R. C. *Tetrahedron* **1988**, *44*, 843.

a trace of both DBF and free PCA. The DBF peak grew gradually until after 60 min it reached a maximum and then dropped in intensity only slowly thereafter. After 12 h the DBF peak was still strong although the adduct doublet near δ 3.0 was now visible. In the case of TAEA similar results were obtained. In this case a peak at δ 3.1, not well separated from a major peak due to TAEA itself, may have arisen from the DBF adduct. NMR experiments of this type were completed for a variety of simple amines and polyamines in addition to CBMA, BAPP, and TAEA, e.g., 4-AMP, *N*-(2-aminoethyl)morpholine, *N*-(3-aminopropyl)morpholine, *n*-propylamine, ethanolamine, 2-methoxyethylamine, and 4-picolylamine. With most simple primary amines large excesses and high concentrations of amine were needed to effect significant adduct formation. With 4-picolylamine both deblocking and adduct formation were relatively slow (no PCA or DBF visible after 1 h). Data for compounds tested in connection with the present study are collected in Table II.

FMOC-Gly-Gly-Phe-Leu-OBn, 4. Leucine benzyl ester *p*-toluenesulfonic acid salt (0.4 g, 1.00 mmol) was suspended in 5% aqueous $NaHCO_3$ (20 mL), and 10 mL of methylene chloride was added. The mixture was stirred vigorously and FMOC-Phe-Cl (0.5 g, 1.1 mmol) was added in small portions. After stirring for 10 min the methylene dichloride layer was separated. Tris(2-aminoethyl)amine (7.5 mL, 50 equiv)[4] was added, and the solution was stirred for 30 min. During this time a white precipitate separated. The precipitate dissolved readily in saturated NaCl solution. The reaction mixture was extracted with saturated NaCl solution (3 × 10 mL) and phosphate buffer, pH 5.5 (3 × 15 mL). There was no interference by either an emulsion or a precipitate. Additional CH_2Cl_2 was used for back extraction. The clear organic layer was collected in a clean round-bottomed flask, the volume was reduced to 10 mL (rotary evaporator), and two new cycles were begun using 1.1 mmol of FMOC-Gly-Cl in each cycle.

After the last coupling was completed the organic layer was collected. *N*-Methylpiperazine (0.1 mL) was added, and the solution was stirred for 2 min. The reaction mixture was washed with 5% HCl (2 × 10 mL), 5% $NaHCO_3$ (2 × 10 mL), saturated NaCl (2 × 10 mL), and H_2O (2 × 10 mL), dried over $MgSO_4$, and filtered, and the solvent was removed on a rotary evaporator. A pale yellow solid (630 mg, 89.4%) was obtained which by TLC analysis (90% EtOAc/10% hexane/1% HOAc) was found to contain dibenzofulvene (R_f 0.74), two unidentified components (very light spots, at R_f 0.0 and 0.35), and tetrapeptide **4** (R_f 0.20). The yellow solid was dissolved in 5 mL of CH_2Cl_2, and the solution was evaporated onto 1 g of silica gel and loaded onto the top of a 4 × 60-cm column packed with 150 g of silica gel. The column was eluted with EtOAc/hexane/HOAc, 90/10/01 v/v/v. Fractions containing the tetrapeptide were collected, evaporated, and recrystallized from EtOAc/hexane, 80/20 v/v, to give 490 mg (69.6%) of the tetrapeptide as a white solid: mp 162–3 °C (lit.[1] mp 163–4 °C); $[\alpha]^{23}_D$ −8.4° (*c* 1, DMF) [lit.[1] $[\alpha]^{25}_D$ −8.2° (*c* 1, DMF)]; ^{1}H NMR ($CDCl_3$) δ 0.85 (d, 6, $CH(CH_3)_2$), 1.6 (m, 3, $CH_2CH(Me)_2$), 3.00 (q, 1, $CHHC_6H_5$), 3.2 (q, 1, $CHHC_6H_5$), 3.9 (d, 4, NCH_2CO), 4.2 (t, 1, $CHCH_2O$), 4.4 (d, 2, $CHCH_2O$), 4.6 (q, 1, NCHCO), 4.8 (q, 1, NCHCO), 5.1 (s, 2, $OCH_2C_6H_5$), 6.5 (m, 1, NH), 7.2–7.8 (m, 21, NH and aryl).

FMOC-Tyr(Bn)-Gly-Gly-Phe-Leu-OBn, 5. The intermediate tetrapeptide was prepared as described above from 1 mmol of H-Leu-OBn·TsOH. Following the final coupling with FMOC-Tyr(Bn)-Cl, the organic layer was collected and worked up as given for 1. Removal of solvent gave a pale yellow solid (1.18 g), which was chromatographed on silica gel (elution by EtOAc/hexane, 100/1, v/v). Fractions containing the pentapeptide (R_f 0.3) were collected and evaporated, and the residue was recrystallized from EtOAc/hexane to give 439 mg (45.8%) of the protected pentapeptide as a white solid: mp 191–2 °C (lit.[1] mp 178 °C; $[\alpha]^{21}_D$ −17.6° (*c* 0.5, DMF) [lit.[1] $[\alpha]^{25}_D$ −16.9° (*c* 0.9, DMF)]; ^{1}H NMR ($CDCl_3$–DMSO-d_6) δ 0.8 (s, 6, $HC(CH_3)_2$), 1.5 (m, 3, $CH_2CH(Me)_2$), 2.8–3.1 (m, 4, $CH_2C_6H_5$), 3.8 (d, 4, NCH_2CO), 4.0–4.5 (m, 5, $CHCH_2O$, 2 NCHCO), 4.75 (m, 1, NCHCO), 4.85 (s, 2 $COOCH_2C_6H_5$), 5.00 (q, 2, $C_6H_4OCH_2C_6H_5$), 6.35 (br s, 1, NH), 6.7–7.7 (m, 31, NH, aryl).

(4) TAEA was obtained as a clear light green liquid from Aldrich Chemical Co. We also thank the Grace Chemical Co. for a sample of this material (98%) obtained as a yellow-colored liquid.

H-Lys-Pro-Lys-Pro-NH-$C_{12}H_{25}$, 6. To a solution of dodecylamine (190 mg, 1 mmol) in 10 mL of $CHCl_3$ and 3 mL of 10% aqueous Na_2CO_3 was added dropwise a solution of FMOC-Pro-Cl (430 mg, 1.2 mmol) in 10 mL of $CHCl_3$. The reaction mixture was stirred for 20 min, and the deblocking process via TAEA was initiated. The protocol followed that described above except that the lysine residue was added as FMOC-Lys(BOC)-OPfp and due to the lesser reactivity of pentafluorophenyl esters relative to acid chlorides the acylation reaction was allowed to proceed for 45 min (TLC). Crude FMOC-Lys(BOC)-Pro-Lys-(BOC)-Pro-NH-$C_{12}H_{25}$ was obtained as a pale yellow oil that was analyzed by TLC ($CHCl_3/CH_3OH$/HOAc, 90/10/1, v/v/v): R_f's 0.9 (UV dark, Cl_2/tolidine dark), 0.70 (UV dark, Cl_2/tolidine dark), 0.55 (UV light, Cl_2/tolidine light), 0.12 (ninhydrin light, Cl_2/tolidine dark).

The oil was dissolved in 5 mL of $CHCl_3$, the solution was evaporated with 1 g of silica gel, and the mixture was loaded onto the top of a 4 × 60-cm column packed with 150 g of silica gel. The column was eluted with $CHCl_3/CH_3OH$/HOAc, 90/10/01, v/v/v. Fractions containing FMOC–tetrapeptide amide contaminated with some FMOC-Lys(BOC)-O-Pfp (R_f 0.7 and 0.9) were collected, washed with 5% $NaHCO_3$, and dried over $MgSO_4$, and the solvent was removed in vacuo. A white foam (1.18 g) was obtained that was redissolved in $CHCl_3$ (10 mL). TAEA (7.5 mL) was added, and the solution was stirred for 45 min and worked up as usual with phosphate buffer. The crude bis-*t*-BOC tetrapeptide amide, obtained as a yellow solid (1.05 g), was dissolved in CH_2Cl_2/TFA, 50/50, v/v (10 mL), and the solution was stirred for 1 h. Excess TFA and solvent were removed in vacuo. Trituration of the viscous oil with Et_2O gave the tris(trifluoroacetate) as an off-white solid (680 mg, 69.6%). The pure salt was obtained after preparative HPLC separation in 60.9% yield. ^{1}H NMR and other spectral data and physical properties agreed with those of the sample prepared earlier.[2]

H-Phe-Phe-Val-Gly-Leu-Met-OBn, 7. H-Met-OBn·TsOH[2] (206 mg, 0.5 mmol) was dissolved in 5% aqueous Na_2CO_3 solution (5 mL) followed by addition of $CHCl_3$ (5 mL). The mixture was stirred vigorously while FMOC-Leu-Cl (223 mg, 0.6 mmol) in $CHCl_3$ (5 mL) was added dropwise. After 15 min of stirring the organic layer was collected and treated with TAEA (3.5 mL), and the solution was stirred for 15 min. After extractions with saturated NaCl solution and phosphate buffer in the normal manner and a volume reduction to 5 mL a new cycle was begun. In this and all subsequent cycles the deblocking time was increased to 30 min. After the last cycle the organic layer was evaporated, the residual oil was triturated with benzene (2 × 15 mL) and CCl_4 (2 × 15 mL), and the solvent was removed to give a pale yellow solid. Precipitation from $CHCl_3$ by Et_2O followed by recrystallization from methanol gave the peptide ester (40%) as a white solid: mp 210–216 °C; $[\alpha]^{23}_D$ − 43.8° (*c* 0.5, $CHCl_3$); MS/FAB 803.7 (MH^+), calcd 802.4 (M); ^{1}H NMR (DMSO-d_6) δ 0.9 (m, 12, CH_3), 1.4 (m, 2, CH_2CH_2S), 1.6 (m, 1, CH_2CHMe_2), 1.8–2.05 (m, 6, $CHMe_2$, $CHCH_2CH$, SMe), 2.5 (m, CH_2S), 2.8 (m, 2, $CH_2C_6H_5$), 3.0 (m, 2 $CH_2C_6H_5$), 3.4 (m, $NHCH_2CO$), 3.7–4.5 (m, 5, $NHCHCO$), 4.7 (br s, 1, NH), 5.1 (s, 2, $OCH_2C_6H_5$), 7.1–7.4 (m, 15, aryl), 7.9–8.5 (m, 6, NH). Subsequent ammonolysis gave the peptide amide (Table I), identified by comparison with a sample prepared by the 4-AMP technique.[2]

Acknowledgment. We thank the National Science Foundation (Grant CHE-8609176) for support of this work. The National Science Foundation also is thanked for supporting the purchase of the high-field NMR spectrometers used in this work. We thank Dr. Robert Cotter of the Middle Atlantic Mass Spectrometry Laboratory for MS/FAB data.

Registry No. **2**, 4097-89-6; **4**, 124482-87-7; **5**, 88099-29-0; **6**·3 TFA, 124482-88-8; **7**, 124482-89-9; **8**, 97559-40-5; **9**, 2579-20-6; **10**, 7209-38-3; FMDC-PCA, 68089-94-1; $CH_3(CH_2)_{11}NH_2$, 124-22-1; H-Leu-OBn·TsOH, 1738-77-8; FMOC-Phe-Cl, 103321-57-9; FMOC-Gly-Cl, 103321-49-9; FMOC-Tyr(Bn)-Cl, 103321-60-4; FMOC-Pro-Cl, 103321-52-4; FMOC-Lys(BOC)-OPfp, 86060-98-2; H-Met-OBn·TsOH, 68739-90-2; FMOC-Leu-Cl, 103321-59-1; FMOC-ValHCl, 103321-53-5; FMOC-Lys(BOC)-Pro-Lys-(BOC)-Pro-NH($C_{12}H_{25}$), 124482-90-2.