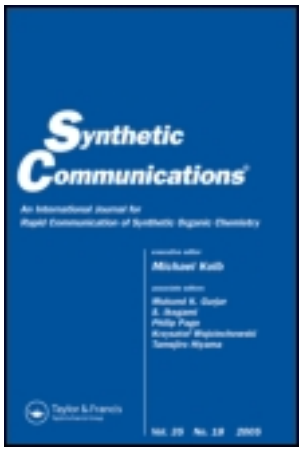


Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

PENTAFLUOROPHENYL 4-NITROBENZENESULFONATE AS A PEPTIDE COUPLING REAGENT

Khanitha Pudhom [a] & Tirayut Vilaivan [b]

[a] Organic Synthesis Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Phayathai Road, Patumwan, Bangkok, 10330, Thailand

[b] Organic Synthesis Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Phayathai Road, Patumwan, Bangkok, 10330, Thailand

Version of record first published: 09 Nov 2006.

To cite this article: Khanitha Pudhom & Tirayut Vilaivan (2001): PENTAFLUOROPHENYL 4-NITROBENZENESULFONATE AS A PEPTIDE COUPLING REAGENT, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 31:1, 61-70

To link to this article: http://dx.doi.org/10.1081/SCC-100000180

SYNTHETIC COMMUNICATIONS, 31(1), 61–70 (2001)

PENTAFLUOROPHENYL 4-NITROBENZENESULFONATE AS A PEPTIDE COUPLING REAGENT

Khanitha Pudhom and Tirayut Vilaivan*

Organic Synthesis Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Phayathai Road, Patumwan, Bangkok 10330, Thailand

ABSTRACT

Protected dipeptides were obtained in good yield by coupling Boc- or Fmoc-protected amino acids with amino acid esters in the presence of pentafluorophenyl 4-nitrobenzenesulfonate (PFNB) as peptide coupling agent and 1-hydroxybenzotriazole as catalyst.

Peptide bond formation between an *N*-protected amino acid and an amino acid ester is perhaps the most crucial step in peptide synthesis. Among many peptide coupling reagents known to date (1–3), sulfonate esters of 1-hydroxybenzotriazoles have been reported to be efficient reagents for peptide and oligonucleotide synthesis (4–9), and for sulfonylation of amines (10). The more stable and crystalline sulfonate esters of strongly acidic phenols were reported to be unreactive in peptide coupling reactions (4–6), although they have been successfully used as sulfonyl transfer agents to phenols and amines (11). We have recently shown that aryl esters of *N*-protected amino acids can be prepared in good yield by reactions of the amino acids with sulfonate esters of phenols bearing electron-withdrawing groups

*To whom correspondence should be addressed.

such as *p*-nitrophenol and pentafluorophenol in the presence of an organic base and a catalytic amount of HOBt (12). Since these aryl esters of amino acids are good acylating agents, it is therefore of interest to investigate the possibility of using aryl sulfonates to assist peptide bond formation without prior isolation of the aryl esters.

Boc-glycine reacted slowly and incompletely with pentafluorophenyl 4-nitrobenzenesulfonate (PFNB, **1**) (12) in the presence of triethylamine in DMF, but the addition of 0.1 eq of HOBt at room temperature caused rapid disappearance of the starting materials. After stirring for 15 min at room temperature, an equivalent of glycine ethyl ester hydrochloride was added, followed by another equivalent of triethylamine, and the reaction mixture was stirred for another 60 min. Simple aqueous work-up afforded Boc-glycylglycine ethyl ester in 90% yield. Under similar conditions, many other Boc- and Fmoc-dipeptides were obtained in good yield and purity after simple aqueous work-up followed by chromatography, if necessary (Table 1). All products gave the expected ^{1}H NMR spectra and satisfactory elemental analysis (C, H, N). It was found also that pre-activation of the amino acid was not necessary and all components may be added simultaneously to give similar yields of the same products. Interestingly, no *N*-nitrobenzenesulfonyl amino acid ester was observed as a side product, although the amino group is expected to be a better nucleophile than the carboxylate group.

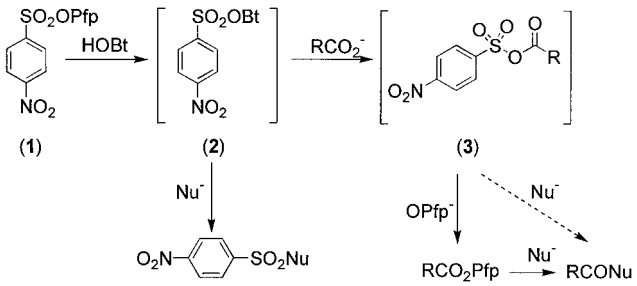
Although HOBt is a well-known acylation catalyst in peptide synthesis (13), catalysis of sulfonation reactions is much less common (14). We propose that HOBt catalyzes this reaction by first reacting with pentafluorophenyl

Table 1. Protected Dipeptides Obtained from Coupling of Protected Amino Acids Employing Pentafluorophenyl 4-Nitrobenzenesulfonate (PFNB) as Coupling Agent

N-Protected Amino Acid	Amino Acid Ester	Dipeptide Product[a]	Yield (%)[b]
Boc-Gly-OH	H-Gly-OEt	Boc-Gly-Gly-OEt	90
Boc-Gly-OH	H-L-Ala-OMe	Boc-Gly-L-Ala-OMe	82
Boc-Gly-OH	H-Sar-OEt	Boc-Gly-Sar-OEt	92
Boc-L-Leu-OH	H-Gly-OEt	Boc-L-Leu-Gly-OEt	95
Boc-L-Leu-OH	H-L-Ala-OMe	Boc-L-Leu-L-Ala-OMe	84
Fmoc-Gly-OH	H-Gly-OEt	Fmoc-Gly-Gly-OEt	96
Fmoc-L-Val-OH	H-Gly-OEt	Fmoc-L-Val-Gly-OEt	97
Fmoc-L-Val-OH	H-L-Ala-OMe	Fmoc-L-Val-L-Ala-OMe	91
Fmoc-L-Val-OH	H-Sar-OEt	Fmoc-L-Val-Sar-OEt	95
Fmoc-L-Phe-OH	H-Gly-OEt	Fmoc-L-Phe-Gly-OEt	94
Fmoc-L-Lys(Boc)-OH	H-Sar-OEt	Fmoc-L-Lys(Boc)-Sar-OEt	92
Fmoc-L-Ser(^{t}Bu)-OH	H-L-Leu-OMe	Fmoc-L-Ser(^{t}Bu)-L-Leu-OMe	96
Fmoc-L-Trp(Boc)-OH	H-Gly-OEt	Fmoc-L-Trp(Boc)-Gly-OEt	94

[a]All products gave clean ^{1}H NMR spectra.
[b]Yield after purification by passing through a short silica gel column.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016



Scheme 1.

4-nitrobenzenesulfonate to give benzotriazol-1-yl 4-nitrobenzensulfonate (**2**) as the reactive intermediate. Benzotriazolyl sulfonates are known to react with carboxylate ions to give mixed sulfonic-carboxylic anhydrides (4–6), which could undergo a nucleophilic attack by the amino component either directly or after a reaction with the pentafluorophenoxide ion generated in the first step to form pentafluorophenyl esters (Scheme 1). Formation of the aryl ester by an alternative S_NAr pathway also might be considered possible, due to the presence of electron-withdrawing groups on the aromatic ring of the phenol. Indeed, it was previously noted that 4-nitrophenyl 4-nitrobenzenesulfonate reacted with morpholine under harsh conditions and in the absence of catalysts to give both 4-nitrophenyl morpholine and 4-nitrobenzenesulfonylmorpholine (15). However, we have evidence against this mechanism, since 4-nitrophenyl 4-nitrobenzensulfonate and similar aryl 4-nitrobenzenesulfonates reacted with morpholine in the presence of HOBt to give only 4-nitrobenzenesulfonylmorpholine, resulting from nucleophilic attack at the sulfonyl group and not arylmorpholines, which would be the S_NAr product. Furthermore, even though the product from sulfonylation of morpholine has no ability to react further with nucleophiles, the rate of reaction increased substantially in the presence of HOBt (a reaction between 4-nitrophenyl 4-nitrobenzenesulfonate and morpholine was completed in 30 min in the presence of 0.1 eq HOBt, and less than 10% completed after 120 min without HOBt). This suggests that, in the PFNB-mediated peptide bond formation, HOBt catalyzes the nucleophilic attack of the sulfonate ester rather than subsequent acylation steps. The putative mixed sulfonic-carboxylic anhydrides or benzotriazol-l-yl 4-nitrobenzenesulfonate intermediates have not yet been successfully isolated, probably due to their high reactivities.

Racemization of the *N*-terminal amino acid during coupling reactions also was studied using two model couplings between an *N*-acyl-protected amino acid (*N*-Bz-L-Phe-OH) and a urethane protected amino acid (Boc-L-Phe-OH) with H-L-Leu-OMe. Control reactions between *N*-Bz-DL-Phe-OH or *N*-Boc-DL-Phe-OH and H-L-Leu-OMe gave crude products that showed clearly distinguishable ^{1}H-NMR signals, due to each diastereomeric dipeptide product in equal amounts.

Table 2. Racemization Studies

Test System	Reagent[a]	Ratio of L/L:D/L Isomer
N-Boc-L-Phe-OH	DCC + HOBt · H_2O 0.1 eq	>98:2
+ H-L-Leu-OMe	HBTU	>98:2
	(**1**) + HOBt · H_2O 0.1 eq	>98:2
N-Bz-L-Phe-OH	DCC + HOBt · H_2O 0.1 eq	65:35
+ H-L-Leu-OMe	HBTU	68:32
	(**1**) + HOBt · H_2O 0.1 eq	50:50
	(**1**) + HOBt · H_2O 2 eq	50:50

[a]All reactions were carried out in DMF unless otherwise indicated.

Therefore the ratio of products containing L-Phe and D-Phe could be measured directly from integration of ^{1}H-NMR spectra. The results revealed that no significant racemization took place when Boc-L-Phe-OH, but not *N*-Bz-L-Phe-OH, was employed as the carboxy component (Table 2), which is consistent with other known sulfonyl-based peptide coupling agents (6,7), except in one case where intromolecular reaction is possible (16). Changing the solvent (DMF, MeCN) and the base (Et_3N, DIEA), and increasing the amount of HOBt up to 2 eq did not yield any improvements, indicating that racemization of the mixed carboxylic-sulfonic anhydride of the *N*-acylamino acid takes place much faster than its trapping by the auxiliary nucleophile. HBTU and DCC in the presence of HOBt gave a somewhat lower degree of racemization under similar conditions, while those of DCC alone gave very impure product in poor yield. The use of (**1**) as peptide coupling reagent should therefore be limited to coupling of urethane-protected amino acid in a stepwise fashion and should not be used for fragment coupling.

In conclusion, pentafluorophenyl 4-nitrobenzensulfonate (**1**), which is a stable and crystalline reagent, may be used as an efficient peptide coupling agent in the presence of a tertiary organic base and a catalytic amount of 1-hydroxybenzotriazole. The speed of the reaction and simplicity of product purification make this reagent an alternative to established peptide coupling agents, especially when urethane-protected amino acids are employed as the carboxyl component where racemization would not be a problem. The possibility of using (**1**) in solid phase peptide synthesis and detailed mechanistic aspects of the coupling reaction are currently under investigation.

EXPERIMENTAL

General

Melting points were recorded on a Fisher-John melting point apparatus and are quoted uncorrected. Specific rotations were measured on a Perkin-Elmer 341

polarimeter and $[\alpha]_D$-values are given in units of 10^{-1} deg · cm^2 · g^{-1}. IR spectra were recorded on a Nicolet Model Impact 410 Fourier Transform Infrared Spectrometer using KBr disk. Elemental Analyses were performed on a Perkin Elmer Elemental Analyzer 2400 CHNS/O at the Research Equipment Centre, Chulalongkorn University. Routine ^{1}H NMR spectra were obtained on a Bruker ACF 200 (Chulalongkorn University, Bangkok) operating at 200 MHz (^{1}H) and 50.28 MHz (^{13}C). High field NMR experiments were performed on a JEOL JNM500 at at the Research Equipment Centre, Chulalongkorn University. Chemical shifts are reported in parts per million (ppm, δ) downfield relative to the internal standard tetramethylsilane. Unless otherwise noted, all chemicals and solvents were obtained from commercial suppliers (Aldrich, Fluka, and Merck) and were used as received. Reactions were performed under an atmosphere of dry nitrogen.

Pentafluorophenyl 4-nitrobenzenesulfonate (**1**) was prepared from pentafluorophenol and 4-nitrobenzenesulfonyl chloride in pyridine (12) (83% yield) as a yellow crystalline solid after recrystallization from ethyl acetate-hexane. It can be stored at room temperature for years without deterioration. m.p. 108°–109°C; Anal. calcd. C: 39.0, H: 1.1, N: 3.8 found : C: 38.9, H: 1.4, N: 3.7%; IR ν_{max} (KBr)/cm^{-1} 3117, 1610, 1537, 1400, 1351, 1200, 999. δ_H ($CDCl_3$, 200 MHz) 8.20 (2H, d, $J = 7.5$ Hz), 8.45 (2H, d, $J = 7.5$ Hz).

General Procedure for Peptide Coupling Using (1) as Coupling Reagent

A solution of *N*-protected amino acid (0.3 mmol), pentafluorophenyl 4-nitrobenzenesulfonate (0.3 mmol), and HOBt · H_2O (0.03 mmol) in DMF (3 mL) was added to triethylamine (0.3 mmol) when using *N*-Boc protected amino acid or DIEA (0.3 mmol) when using *N*-Fmoc protected amino acid as the carboxyl component with stirring. After stirring for 15 min at room temperature, a solution of an amino acid methyl or ethyl ester hydrochloride (0.3 mmol) and triethylamine or DIEA (0.3 mmol) was added to the reaction mixture. The reaction mixture was allowed to react for 1 h at room temperature and diluted with dichloromethane. This solution was washed with 5% HCl, 5% $NaHCO_3$, H_2O, and brine and then dried over $MgSO_4$. The dried solution was evaporated under reduced pressure and the residue was purified by flash column chromatography (SiO_2, CH_2Cl_2:Hexane) to give analytically pure dipeptide.

In an alternative modification, the reaction was performed and worked up identically except that all reactants were added simultaneously at the beginning.

N-tert-Butoxycarbonylglycylglycine ethyl ester (Boc-Gly-Gly-OEt)

Colorless oil (70.0 mg, 90% yield). Anal. Calcd. for $C_{11}H_{20}O_5N_2$: C, 50.8; H, 7.7; N, 10.8%. Found: C, 50.8; H, 7.7; N, 10.7%. IR ν_{max} (neat)/cm^{-1} 3330,

2981, 2936, 1682, 1531, 1455, 1371, 1251, 1205, 1171, 1029, 947, 864. ^{1}H NMR ($CDCl_3$) δ_H 1.26 (3H, t, ethyl C$\underline{H}_3$), 1.42 (9H, s, Boc C$\underline{H}_3$ (x3)), 3.81 (2H, d, Glyl C$\underline{H}_2$), 4.01 (2H, d, Gly2 C$\underline{H}_2$), 4.18 (2H, q, ethyl C$\underline{H}_2$), 5.30 (1H, br t, N$\underline{H}$), 6.78 (1H, br t, N$\underline{H}$).

N-tert-Butoxycarbonylglycyl-L-alanine methyl ester (Boc-Gly-L-Ala-OMe)

Colorless oil (67.4 mg, 82% yield), $[\alpha]_D^{20}$ + 10.7 (c = 1.00, $CHCl_3$). Anal. Calcd. for $C_{11}H_{20}O_5N_2$: C, 50.8; H, 7.7; N, 10.8%. Found: C, 50.7; H, 7.8; N, 10.5%. IR ν_{max} (neat)/cm^{-1} 3320, 2980, 2938, 1744, 1674, 1528, 1456, 1369, 1280, 1249, 1217, 1168, 1055. ^{1}H NMR ($CDCl_3$) δ_H 1.40 (3H, d, Ala C$\underline{H}_3$), 1.44 (9H, s, Boc C$\underline{H}_3$ (x3)), 3.72 (3H, s, OC$\underline{H}_3$), 3.80 (2H, d, Gly C$\underline{H}_2$), 4.57 (1H, m, Ala C$_\alpha\underline{H}$), 5.24 (1H, br m, Gly N$\underline{H}$), 6.75 (1H, br d, Ala N$\underline{H}$).

N-tert-Butoxycarbonylglycylsarcosine ethyl ester (Boc-Gly-Sar-OEt)

Colorless oil (75.6 mg, 92% yield). Anal. Calcd. for $C_{12}H_{22}O_5N_2$: C, 52.5; H, 8.1; N, 10.2% Found: C, 52.7; H, 8.1; N, 10.2%. IR ν_{max} (neat)/cm^{-1} 3422, 2980, 2937, 1715, 1664, 1488, 1412, 1370, 1204, 1172, 1052. ^{1}H NMR ($CDCl_3$) δ_H 1.22 (3H, 2xt, C$\underline{H}_3$ rotamers), 1.40 (9H, s, Boc C$\underline{H}_3$ (x3)), 2.95, 2.99 (3H, 2xs, Sar C$\underline{H}_3$ rotamers), 3.84, 3.99 (2H, 2xd, Gly C$\underline{H}_2$ rotamers), 3.95, 4.08 (2H, 2xs, Sar C$\underline{H}_2$ rotamers), 4.14 (2H, q, ethyl C$\underline{H}_2$), 5.42 (1H, br m, N$\underline{H}$)

N-tert-Butoxycarbonyl-L-leucylglycine ethyl ester (Boc-L-Leu-Gly-OEt)

White solid (89.6 mg, 95% yield), m.p. 76°–78°C, $[\alpha]_D^{20}$ −20.0 (c = 1.02, $CHCl_3$). Anal. Calcd. for $C_{15}H_{28}O_5N_2$: C, 56.9; H, 8.9; N, 8.8%. Found: C, 56.9; H, 8.8; N, 8.9%. IR ν_{max} (KBr)/cm^{-1} 3325, 2959, 2871, 1757, 1664, 1541, 1468, 1392, 1370, 1304, 1250, 1198, 1174, 1100, 1044, 1024. ^{1}H NMR ($CDCl_3$) δ_H 0.92 (6H, dd, isopropyl C$\underline{H}_3$ (x2)), 1.28 (3H, t, ethyl C$\underline{H}_3$), 1.41 (9H, s, Boc C$\underline{H}_3$ (x3)), 1.55–1.90 (3H, br m, Leu C$\underline{H}$, C$\underline{H}_2$), 4.00 (2H, d, Gly C$\underline{H}_2$), 4.15 (1H, m, Leu C$_\alpha\underline{H}$), 4.19 (2H, q, ethyl C$\underline{H}_2$), 4.96 (1H, br d, Leu N$\underline{H}$), 6.70 (1H, br m, Gly N$\underline{H}$).

N-tert-Butoxycarbonyl-L-leucyl-L-alanine methyl ester (Boc-L-Leu-L-Ala-OMe)

White solid (79.6 mg, 84% yield), m.p. 97°–99°C, $[\alpha]_D^{20}$ −31.7 (c = 1.00, $CHCl_3$). Anal. Calcd. for $C_{15}H_{28}O_5N_2$: C, 56.9; H, 8.9; N, 8.8%. Found: C, 56.8; H, 8.7; N, 8.7%. IR ν_{max} (KBr)/cm^{-1} 3313, 2961, 1756, 1687, 1655, 1531, 1457,

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

1392, 1368, 1293, 1250, 1206, 1168, 1050, 1025. ^{1}H NMR ($CDCl_3$) δ_H 0.95 (6H, dd, isopropyl C<u>H</u>$_3$ (x2)), 1.39 (3H, d, Ala C<u>H</u>$_3$), 1.44 (9H, s, Boc C<u>H</u>$_3$ (x3)), 1.65 (3H, br m, Leu C<u>H</u>, C<u>H</u>$_2$), 3.73 (3H, s, OC<u>H</u>$_3$), 4.08 (1H, m, Leu C$_\alpha$<u>H</u>), 4.55 (1H, m, Ala C$_\alpha$<u>H</u>), 4.87 (1H, br d, Leu N<u>H</u>), 6.60 (1H, br d, Ala N<u>H</u>).

N-9-Fluorenylmethoxycarbonylglycylglycine ethyl ester (Fmoc-Gly-Gly-OEt)

White solid (110.0 mg, 96% yield), m.p. 115°–116°C. Anal. Calcd. for $C_{21}H_{22}O_5N_2$: C, 66.0; H, 5.8; N, 7.3%. Found: C, 65.9; H, 5.8; N, 7.3%. IR ν_{max} (KBr)/cm^{-1} 3426, 3068, 2982, 1718, 1674, 1536, 1451, 1403, 1252, 1215, 1050. ^{1}H NMR ($CDCl_3$) δ_H 1.27 (3H, t, ethyl C<u>H</u>$_3$), 3.92 (2H, d, Gly1 C<u>H</u>$_2$), 4.02 (2H, d, Gly2 C<u>H</u>$_2$), 4.18 (3H, m, ethyl C<u>H</u>$_2$, Fmoc aliphatic C<u>H</u>), 4.41 (2H, d, Fmoc C<u>H</u>$_2$), 5.62 (1H, br t, N<u>H</u>), 6.62 (1H, br m, N<u>H</u>), 7.35 (4H, m, Fmoc aromatic C<u>H</u>), 7.58 (2H, d, Fmoc aromatic C<u>H</u>), 7.74 (2H, d, Fmoc aromatic C<u>H</u>).

N-9-Fluorenylmethoxycarbonyl-L-valylglycine ethyl ester (Fmoc-L-Val-Gly-OEt)

White solid (122.8 mg, 97% yield), m.p. 197°–198°C, $[\alpha]_D^{20}$ −18.0 (c = 1.00, $CHCl_3$). Anal. Calcd. for $C_{24}H_{28}O_5N_2$: C, 67.9; H, 6.6; N, 6.6%. Found: C, 67.9; H, 6.5; N, 6.7%. IR ν_{max} (KBr)/cm^{-1} 3440, 3069, 2966, 1705, 1661, 1535, 1451, 1296, 1214, 1111, 1030. ^{1}H NMR ($CDCl_3$) δ_H 0.95 (6H, dd, isopropyl C<u>H</u>$_3$ (x2)), 1.27 (3H, t, ethyl C<u>H</u>$_3$), 2.16 (1H, m, Val C<u>H</u>), 4.04 (3H, dd, Gly C<u>H</u>$_2$, Val C$_\alpha$<u>H</u>), 4.20 (3H, m, ethyl C<u>H</u>$_2$, Fmoc aliphatic C<u>H</u>), 4.42 (2H, m, Fmoc C<u>H</u>$_2$), 5.38 (1H, d, Val N<u>H</u>), 6.47 (1H, br t, Gly N<u>H</u>), 7.34 (4H, m, Fmoc aromatic C<u>H</u>), 7.59 (2H, d, Fmoc aromatic C<u>H</u>), 7.76 (2H, d, Fmoc aromatic C<u>H</u>).

N-9-Fluorenylmethoxycarbonyl-L-valyl-L-alanine methyl ester (Fmoc-L-Val-L-Ala-OMe)

White solid (116.3 mg, 91% yield), m.p. 208°–209°C, $[\alpha]_D^{20}$ −18.3 (c = 1.02, $CHCl_3$). Anal. Calcd. for $C_{24}H_{28}O_5N_2$: C, 67.9; H, 6.6; N, 6.6%. Found: C, 67.9; H, 6.6; N, 6.5%. IR ν_{max} (KBr)/cm^{-1} 3432, 3067, 2962, 1704, 1661, 1532, 1452, 1336, 1295, 1226, 1151. ^{1}H NMR ($CDCl_3$) δ_H 0.98 (6H, dd, isopropyl C<u>H</u>$_3$ (x2)), 1.40 (3H, d, Ala C<u>H</u>$_3$), 2.11 (1H, m, Val C<u>H</u>), 3.72 (3H, s, OC<u>H</u>$_3$), 4.04 (1H, m, Val C$_\alpha$<u>H</u>), 4.23 (1H, m, Fmoc aliphatic C<u>H</u>), 4.40 (2H, m, Fmoc C<u>H</u>$_2$), 4.59 (1H, m, Ala C$_\alpha$<u>H</u>), 5.48 (1H, d, Val N<u>H</u>), 6.47 (1H, d, Ala N<u>H</u>), 7.32 (4H, m, Fmoc aromatic C<u>H</u>), 7.57 (2H, d, Fmoc aromatic C<u>H</u>), 7.76 (2H, d, Fmoc aromatic C<u>H</u>).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

N-9-Fluorenylmethoxycarbonyl-L-valylsarcosine ethyl ester (Fmoc-L-Val-Sar-OEt)

Colorless oil (117.8 mg, 95% yield), $[\alpha]_D^{20}$ −14.5 (c = 1.03, $CHCl_3$). Anal. Calcd. for $C_{25}H_{30}O_5N_2$: C, 68.5; H, 6.9; N, 6.4%. Found: C, 68.4; H, 6.8; N, 6.3%. IR ν_{max} (neat)/cm^{-1} 3431, 3314, 3065, 2967, 1718, 1640, 1529, 1479, 1452, 1374, 1206, 1111, 1093. ^{1}H NMR ($CDCl_3$) δ_H 1.00 (6H, dd, isopropyl C$\underline{H}_3$ (x2), 1.26 (3H, t, ethyl C$\underline{H}_3$), 2.07 (1H, m, Val C$\underline{H}$), 3.18 (3H, s, Sar C$\underline{H}_3$), 3.71, 3.80 (1H, 2xs, Sar C$\underline{H}_2$ rotamers), 4.05–4.64 (8H, m, ethyl C$\underline{H}_2$, Sar C$\underline{H}_2$ rotamers, Val C$_\alpha\underline{H}$, Fmoc C$\underline{H}_2$, Fmoc aliphatic C$\underline{H}$), 5.57 (1H, d, N$\underline{H}$), 7.35 (4H, m, Fmoc aromatic C$\underline{H}$), 7.59 (2H, d, Fmoc aromatic C$\underline{H}$), 7.74 (2H, d, Fmoc aromatic C$\underline{H}$).

N-9-Fluorenylmethoxycarbonyl-L-phenylalanylglycine ethyl ester (Fmoc-L-Phe-Gly-OEt)

White solid (129.2 mg, 94% yield), m.p. 185°–187°C, $[\alpha]_D^{20}$ −14.2 (c = 1.02, $CHCl_3$). Anal. Calcd. for $C_{28}H_{28}O_5N_2$: C, 71.2; H, 6.0; N, 5.9%. Found: C, 71.1; H, 6.0; N, 5.8%. IR ν_{max} (KBr)/cm^{-1} 3468, 3304, 1738, 1695, 1654, 1541, 1262, 1210, 1038. ^{1}H NMR ($CDCl_3$) δ_H 1.26 (3H, t, ethyl C$\underline{H}_3$), 3.10 (2H, br d, Phe C$\underline{H}_2$), 4.20 (3H, m, ethyl C$\underline{H}_2$, Fmoc aliphatic C$\underline{H}$), 4.42 (3H, br, m, Phe aliphatic C$_\alpha\underline{H}$, Fmoc C$\underline{H}_2$), 5.32 (1H, br m, Gly N$\underline{H}$), 6.30 (1H, br m, Phe N$\underline{H}$), 7.10–7.47 (9H, m, Phe aromatic C$\underline{H}$, Fmoc aromatic C$\underline{H}$), 7.52 (2H, m, Fmoc aromatic C$\underline{H}$), 7.76 (2H, d, Fmoc aromatic C$\underline{H}$).

N-9-Fluorenylmethoxycarbonyl-*N*-ε-*tert*-butoxycarbonyl-L-lysylsarcosine ethyl ester (Fmoc-L-Lys(Boc)-Sar-OEt)

Colorless oil (160.5 mg, 92% yield), $[\alpha]_D^{20}$ +0.5 (c = 1.02, $CHCl_3$). Anal. Calcd. for $C_{31}H_{43}O_7N_3$: C, 65.4; H, 7.6; N, 7.4%. Found: C, 65.6; H, 7.5; N, 7.4%. IR ν_{max} (neat)/cm^{-1} 3431, 3066, 2977, 2938, 1711, 1649, 1517, 1453, 1409, 1252, 1173. ^{1}H NMR ($CDCl_3$) δ_H 1.26 (3H, 2xt, ethyl C$\underline{H}_3$ rotamers), 1.41 (9H, s, Boc C$\underline{H}_3$ (x3)), 1.48–1.95 (8H, br m, Lys C$\underline{H}_2$), 2.96, 3.14 (3H, 2xs, Sar C$\underline{H}_3$ rotamers), 3.78, 3.86 (1H, 2xs, Sar C$\underline{H}_2$ rotamers), 4.20 (4H, m, ethyl C$\underline{H}_2$, Sar C$\underline{H}_2$ rotamers, Fmoc aliphatic C$\underline{H}$), 4.35 (2H, d, Fmoc C$\underline{H}_2$), 4.73 (1H, m, Lys, C$_\alpha\underline{H}$), 5.74 (1H, d, Lys N$\underline{H}$), 7.35 (4H, m, Fmoc aromatic C$\underline{H}$), 7.59 (2H, d, Fmoc aromatic C$\underline{H}$), 7.75 (2H, d, Fmoc aromatic C$\underline{H}$).

N-9-Fluorenylmethoxycarbonyl-(*O*-*tert*-butyl)-L-seryl-L-leucine methyl ester (Fmoc-L-Ser(^{t}Bu)-L-Leu-OMe)

White solid (146.7 mg, 96% yield), m.p. 105°–106°C, $[\alpha]_D^{20}$ +22.5 (c = 1.01, $CHCl_3$). Anal. Calcd. for $C_{29}H_{28}O_6N_2$: C, 68.2; H, 7.5; N, 5.5%. Found: C,

68.4; H, 7.4; N, 5.4%. IR ν_{max} (KBr)/cm^{-1} 3427, 3249, 3064, 3036, 2962, 2873, 1756, 1727, 1661, 1508, 1451, 1361, 1281, 1224, 1200, 1153, 1096, 1066. ^{1}H NMR ($CDCl_3$) δ_H 0.93 (6H, d, isopropyl C$\underline{H}_3$ (x2)), 1.22 (9H, s, Boc C$\underline{H}_3$ (x3)), 1.44–1.80 (3H, br m, Leu C$\underline{H}$, C$\underline{H}_2$), 3.38 (1H, t, Ser C$\underline{H}_2$), 3.71 (3H, s, OC$\underline{H}_3$), 3.81 (1H, dd, Ser C$\underline{H}_2$), 4.24 (2H, m, Leu C$_\alpha\underline{H}$, Fmoc aliphatic C$\underline{H}$), 4.40 (2H, d, Fmoc C$\underline{H}_2$), 4.60 (1H, br m, Ser C$_\alpha\underline{H}$), 5.76 (1H, br m, N$\underline{H}$), 7.35 (4H, m, Fmoc aromatic C$\underline{H}$), 7.58 (2H, d, Fmoc aromatic C$\underline{H}$), 7.74 (2H, d, Fmoc aromatic C$\underline{H}$).

N-9-Fluorenylmethoxycarbonyl-*N*^{in}-*tert*-butoxycarbonyltryptophanylglycine ethyl ester (Fmoc-Trp(Boc)-Gly-OEt)

White solid (172.1 mg, 94% yield), m.p. 80°–81°C, $[\alpha]_D^{20}$ −10.3 (c = 1.11, $CHCl_3$). Anal. Calcd. for $C_{35}H_{37}O_7N_3$: C, 68.7; H, 6.1; N, 6.9%. Found: C, 68.9; H, 6.3; N, 7.0%. IR ν_{max} (KBr)/cm^{-1} 3329, 3066, 2980, 2936, 1733, 1672, 1531, 1478, 1454, 1374, 1336, 1309, 1257, 1160, 1088, 1027, 939. ^{1}H NMR ($CDCl_3$) δ_H 1.23 (3H, t, ethyl C$\underline{H}_3$), 1.64 (9H, s, Boc, C$\underline{H}_3$ (x3)), 3.20 (2H, br d, Trp C$\underline{H}_2$), 3.90 (2H, d, Gly C$\underline{H}_2$), 4.16 (3H, m, ethyl C$\underline{H}_2$, Fmoc aliphatic C$\underline{H}$), 4.38 (2H, d, Fmoc C$\underline{H}_2$), 4.54 (1H, m, Trp C$_\alpha\underline{H}$), 5.50 (1H, br m, Trp N$\underline{H}$), 6.26 (1H, br m, Gly N$\underline{H}$), 7.17–7.65 (10H, m, Fmoc aromatic C$\underline{H}$, Trp indole C$\underline{H}$), 7.75 (2H, d, Fmoc aromatic C$\underline{H}$), 8.11 (1H, d, Trp indole C$\underline{H}$).

ACKNOWLEDGMENTS

We acknowledge financial support from the Department of Chemistry and Rachadaphisek Somphot Endowment (grants for development of new faculty staff) from Chulalongkorn University. We also thank the Research Equipment Centre, Chulalongkorn University, for microanalysis service.

REFERENCES AND NOTES

1. Bodanszky, M. *Peptide Chemistry, A Practical Textbook*; Springer-Verlag: Berlin, 1993.
2. Atherton, E.; Sheppard, R.C. *Solid Phase Peptide Synthesis, A Practical Approach*; IRL Press: Oxford, 1989.
3. Fields, G.B.; Noble R.L. Int. J. Peptide Protein Res. **1990**, *35*, 161.
4. Itoh, M.; Nojima, H.; Notani, J.; Hagiwara, D.; Takai, K. Tetrahedron Lett. **1974**, *35*, 3089.
5. Itoh, M.; Hagiwara, D.; Notani, J. Synthysis **1975**, 456.
6. Itoh, M.; Nojima, H.; Notani, J.; Hagiwara, D.; Takai, K. Bull. Chem. Soc. Jpn. **1978**, *51*, 3320.

7. Devadas, B.; Kundu, B.; Srivastava, A.; Mathur, K.B. Tetrahedron Lett. **1993**, *34*, 6455.
8. Reese, C.B.; Pei-Zhou, Z. J. Chem. Soc., Perkin Trans. I **1993**, 2291.
9. Furukawa, M.; Hokama, N.; Okawara, T. Synthesis **1983**, 42.
10. Kim, S.Y.; Sung, N.-D.; Choi J.-K.; Kim, S.S. Tetrahedron Lett. **1999**, 117.
11. Neuvile, L.; Bigot, A.; Dau, M.E.T.H.; Zhu, J. J. Org. Chem. **1999**, *64*, 7638.
12. Pudhom, K.; Vilaivan, T. Tetrahedron Lett. **1999**, *40*, 5939.
13. Konig, W.; Geiger, R. Ber. **1987**, 788.
14. Fujii, T.; Sakakibara, S. Bull. Chem. Soc. Jpn. **1974**, *47*, 3146.
15. Kotsuki, H.; Kobayashi, S.; Suenaga, H.; Nishizawa, H. Synthesis **1990**, 1145.
16. Cabaret, D.; Wakselman, M. Tetrahedron Lett. **1994**, *35*, 9613.

Received in the U.K. January 31, 2000