CHAPTER II

EXPERIMENTAL

2.1 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 or NaCl cell on neat samples. 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 in the solvents at 200 MHz, unless otherwise noted. Chemical shifts are reported in part per million (ppm, δ) down field relative to the internal standard tetramethylsilane. Spectra patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad.

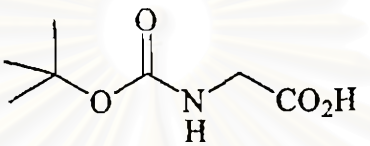
Thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} 0.2 mm pre-coated aluminium plates. Flash column chromatography was carried out on Merck silica gel 60 (0.040-0.063 mm).

Distilled water was used for all chemical experiments. Unless otherwise noted, all chemicals and solvents were obtained from commercial suppliers (Aldrich, Fluka and Merck) and were purified according to the literature[33], if necessary. Fmoc-protected amino acids were obtained from Calbiochem-Novabiochem. Anhydrous DMF was obtained from Fluka and used without further purification. Acetonitrile was hplc grade from Merck and used without further purification. Dichloromethane was analytical grade and stored over calcium chloride. Pyridine was distilled from calcium hydride and stored over 4A molecular sieve. Reactions involving air- and moisture-sensitive reagents were executed under an atmosphere of dry nitrogen.

Removal of solvents was carried out on a Buchi rotary evaporator attached to a water aspirator. Analytical samples were dried under vacuum at room temperature for several hours.

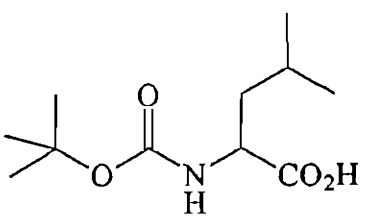
2.2 Synthesis of Starting Materials

N-tert-Butoxycarbonylglycine (Boc-Gly-OH)



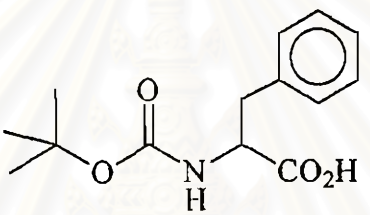
Glycine (0.75 g, 10 mmol) was added with stirring to a mixture of 10% aqueous NaOH (5 mL) and *t*-butanol (5 mL). Di-*t*-Butyl dicarbonate (Boc_2O) (2.18 g, 10 mmol) was then added dropwise. The resulting emulsion was stirred at room temperature overnight. During the course of the reaction, a vigorous evoluation of CO_2 was observed and the reaction mixture became homogeneous. The mixture was evaporated to dryness and the residue was acidified to pH 2 with 10% aqueous HCl. The solution was extracted several times with ethyl acetate. The combined organic phase was dried over $MgSO_4$ and evaporated to give the crude product as an oil. Trituration with hexane gave *N-tert*-butoxycarbonylglycine as a white solid (1.68 g, 96% yield) which was pure enough for practical purposes, m.p. 87-88 °C (lit.[34] m.p. 88-89 °C). ^1H NMR ($CDCl_3$) δ_H 1.43 (9H, s, Boc C<u>H</u>$_3$ (x3)), 3.95 (2H, d, C<u>H</u>$_2$), 5.12 (1H, br m, N<u>H</u>), 8.90 (1H, br s, CO_2<u>H</u>) (Figure 1).

N-tert-Butoxycarbonyl-L-leucine (Boc-L-Leu-OH)



By a method similar to that used in the synthesis of Boc-Gly-OH. Boc-L-Leu-OH was obtained from L-leucine (1.32 g, 10 mmol), di-*t*-butyl dicarbonate (2.18 g, 10 mmol), 10% aqueous NaOH (5 mL) and *t*-butanol (5 mL). Trituration of the crude product with hexane afforded the product as a white solid (2.19 g, 95% yield), m.p. 70-72 °C (lit.[35] m.p.71-72 °C). ^{1}H NMR ($CDCl_3$) δ_H 0.94 (6H, d, isopropyl C<u>H</u>$_3$ (x2)), 1.42 (9H, s, Boc C<u>H</u>$_3$ (x3)), 1.64 (3H, br m, C<u>H</u>, C<u>H</u>$_2$), 4.30 (1H, m, C_α<u>H</u>), 4.92 (1H, br d, N<u>H</u>) (Figure 2).

N-tert-Butoxycarbonyl-L-phenylalanine (Boc-L-Phe-OH)

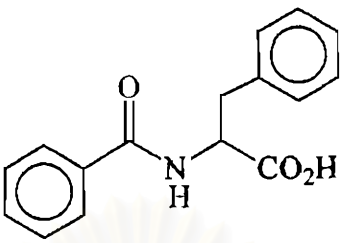


N-tert-Butoxycarbonyl-L-phenylalanine was similarly prepared as a white crystalline solid in quantitative yield (3 mmol scale) starting from L-phenylalanine, m.p. 85-87 °C (lit.[34] m.p. 85-87 °C). ^{1}H NMR ($CDCl_3$) δ_H 1.29, 1.40 (9H, 2xs, Boc C<u>H</u>$_3$ (x3) rotamers), 2.31-3.26 (2H, 2xm, Phe C<u>H</u>$_2$ rotamers), 4.39, 4.59 (1H, 2xm, Phe aliphatic C_α<u>H</u>), 5.05, 6.44 (1H, 2xd, N<u>H</u> rotamers), 7.24 (5H, m, Phe aromatic C<u>H</u>), 7.72 (1H, br s, CO_2<u>H</u>) (Figure 3).

N-tert-Butoxycarbonyl-DL-phenylalanine (Boc-DL-Phe-OH)

N-tert-Butoxycarbonyl-DL-phenylalanine was similarly prepared as a white crystalline solid in quantitative yield (1 mmol scale) starting from DL-phenylalanine, m.p. 113-115 °C. ^{1}H NMR ($CDCl_3$) δ_H 1.30, 1.40 (9H, 2xs, Boc C<u>H</u>$_3$ (x3) rotamers), 2.30-3.26 (2H, 2xm, Phe C<u>H</u>$_2$ rotamers), 4.39, 4.61 (1H, 2xm, Phe aliphatic C_α<u>H</u>), 4.98, 6.22 (1H, 2xd, N<u>H</u> rotamers), 7.24 (5H, m, Phe aromatic C<u>H</u>), 8.45 (1H, br s, CO_2<u>H</u>) (Figure 4).

N-Benzoyl-L-phenylalanine (Bz-L-Phe-OH)[36]

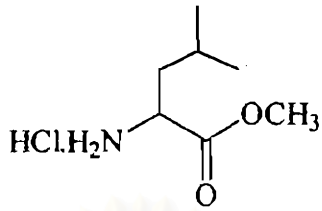


L-Phenylalanine (1.65 g, 10 mmol) was dissolved in diluted NaOH (0.2 g NaOH in 15 mL H_2O). Simultaneously, with stirring, benzoyl chloride (1.16 mL, 10 mmol) and a solution of 0.6 g NaOH in H_2O 3 mL were added dropwise, keeping the temperature below 30 °C and the pH weakly alkaline. Stirring was continued for 1 hour. The solution was acidified with 10% aqueous HCl and the precipitate was collected by filtration. Further recrystallization from ethyl acetate gave Bz-L-Phe-OH as a white crystalline solid (2.42 g, 90% yield), m.p. 139-140 °C, $[\alpha]_D^{20}$ +92.7 (c=1.02, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ_H 3.32 (2H, m, C$\underline{H}_2$), 5.08 (1H, m, Phe aliphatic C$_\alpha\underline{H}$), 6.74 (1H, d, N$\underline{H}$), 7.22 (5H, m, Phe aromatic C$\underline{H}$), 7.38 (2H, t, benzoyl *m*-C$\underline{H}$), 7.51 (1H, t, benzoyl *p*-C$\underline{H}$), 7.68 (2H, d, benzoyl *o*-C$\underline{H}$), 8.39 (1H, br s, $CO_2\underline{H}$) (Figure 5).

N-Benzoyl-DL-phenylalanine (Bz-DL-Phe-OH)

N-Benzoyl-DL-phenylalanine was similarly prepared as a white crystalline solid in quantitive yield (1 mmol scale) starting from DL-phenylalanine, m.p.185-188 °C. ^{1}H NMR (DMSO-d_6) δ_H 3.14 (2H, m, Phe C$\underline{H}_2$), 4.60 (1H, m, Phe aliphatic C$_\alpha\underline{H}$), 7.26 (5H, m, Phe aromatic C$\underline{H}$), 7.49 (3H, m, benzoyl *m*-C$\underline{H}$, benzoyl *p*-C$\underline{H}$), 7.79 (2H, d, benzoyl *o*-C$\underline{H}$), 8.71 (1H, d, N$\underline{H}$) (Figure 6).

L-Leucine methyl ester hydrochloride[36]

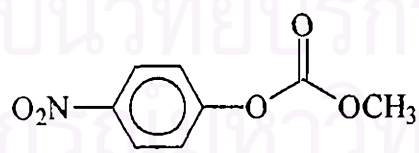


Thionyl chloride ($SOCl_2$) (1.78 g, 15 mmol) was added dropwise with stirring to a cooled (ice bath) methanol suspension (20 mL) of L-leucine (1.31 g, 10 mmol), at such a rate that the reaction mixture refluxes lightly. Refluxing was continued for 2 hours. The solvent was evaporated *in vacuo* and the residue was dissolved in ethanol. Ether was added to precipitate the hydrochloride, which was collected by filtration, washed with ether and dried with P_2O_5 to give colorless crystals (1.78 g, 98% yield), m.p. 150-152 °C (lit.[35] m.p. 151-153 °C). ^{1}H NMR (D_2O) δ_H 0.76 (6H, d, isopropyl C<u>H</u>$_3$ (x2)), 1.56 (3H, br m, C<u>H</u>, C<u>H</u>$_2$), 3.63 (3H, s, OC<u>H</u>$_3$), 3.91 (1H, m, C$_\alpha$<u>H</u>) (Figure 7).

2.3 Synthesis of Reagents

2.3.1 Synthesis of Aryl methyl carbonates

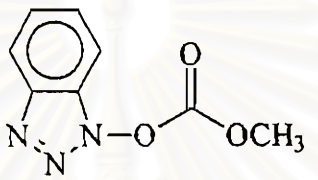
4-Nitrophenyl methyl carbonate (**1a**)



A solution of 4-nitrophenol (1.39 g, 10 mmol) in dry pyridine (10 mL) was cooled in an ice bath. Methyl chloroformate (0.95 g, 10 mmol) was then added dropwise with stirring. The stirring was continued until the reaction mixture became homogeneous. This solution was kept in the refrigerator at 4 °C overnight. The reaction mixture was diluted with dichloromethane, extracted with 5% HCl, 5% $NaHCO_3$, H_2O and brine. It was then dried with $MgSO_4$, filtered, concentrated under

reduce pressure to give the crude product as a white solid. Recrystallization from ethyl acetate-hexane gave a white crystalline solid (1.58 g, 80% yield), m.p. 108-110 °C. ^{1}H NMR ($CDCl_3$) δ_H 3.92 (3H, s, OC<u>H</u>$_3$), 7.34 (2H, d, C<u>H</u>), 8.26 (2H, d, C<u>H</u>) (Figure 8).

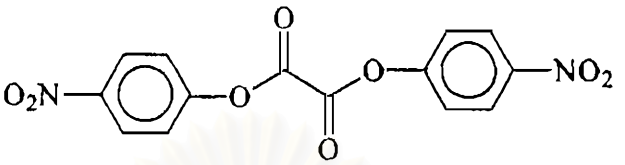
Benzotriazolyl methyl carbonate (**1b**)



1-Hydroxybenzotriazole monohydrate (HOBt.H_2O) (1.53 g, 10 mmol) was dissolved in dichloromethane (10 ml) in a round bottom flask. This solution was cooled in an ice bath at 0 °C with stirring. Triethylamine (1.38 mL, 10 mmol) was added dropwise, followed by methyl chloroformate (0.95 g, 10 mmol). A white precipitate, triethylamine hydrochloride, immediately formed. After stirring the reaction mixture for 1 hour in the ice bath, the mixture was allowed to warm to ambient temperature and stirring continued for 2 hours. The reaction mixture was washed several times with H_2O and brine, dried with $MgSO_4$ and filtered. The solution was evaporated to dryness to give a white solid. Further recrystallization from ethyl acetate-hexane gave the title compound as a white crystalline solid (1.58 g, 82% yield), m.p. 77-79 °C. ^{1}H NMR ($CDCl_3$) δ_H 4.16 (3H, s, OC<u>H</u>$_3$), 7.53 (1H, t, C<u>H</u>), 7.75 (1H, t, C<u>H</u>), 7.98 (1H, d, C<u>H</u>), 8.18 (1H, d, C<u>H</u>) (Figure 9).

2.3.2 Synthesis of Aryl oxalates

Bis(4-nitrophenyl) oxalate (**2a**)

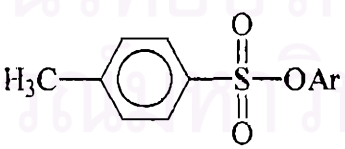


The procedure was carried out in the similar manner to the preparation benzotriazolyl methyl carbonate (2a). Bis(4-nitrophenyl) oxalate was obtained from 4-nitrophenol (1.39 g, 10 mmol, oxalyl chloride (0.43 mL, 5 mmol) and triethylamine (1.38 mL, 10 mmol). Recrystallization from ethyl acetate afforded a pale yellow solid (1.48 g, 89% yield), m.p. 268-270 °C. ^{1}H NMR (DMSO-d_6) δ_H 7.62 (4H, d, C<u>H</u>), 8.43 (4H, d, C<u>H</u>) (Figure 10).

Attempted synthesis of pentachlorophenyl oxalate

The method described in the foregoing preparation of bis(4-nitropenyl) oxalate (**2a**) above was followed , 4-nitrophenyl being replaced by pentachlorophenol. But TLC indicated that no pentachlorophenyl oxalate was obtained by this method.

2.3.3 Synthesis of Aryl *p*-toluenesulfonates



3a Ar = 4-nitrophenyl **3b** Ar = 2,4,5-trichlorophenyl

3c Ar = pentafluorophenyl **3d** Ar = pentachlorophenyl

General Procedure

To a cooled (ice bath) solution of an appropriate phenol (10.1 mmol) in dry pyridine (10 mmol) was slowly added toluenesulfonyl chloride (10 mmol) portionwise

as solid with stirring. The stirring was continued until the reaction mixture became homogeneuos. This solution was left at 4 °C in the refrigerator overnight. The reaction mixture was diluted with dichloromethane, extracted with 5% HCl, 5% $NaHCO_3$, H_2O and brine. It was then dried with $MgSO_4$, filtered and concentrated under reduce pressure to the crude aryl *p*-toluenesulfonate which was purified by recrystallization.

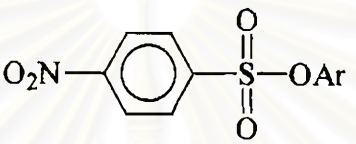
4-Nitrophenyl *p*-toluenesulfonate (**3a**) Purified by recrystallization from ethyl acetate-hexane and obtained as a white crystalline solid (2.55 g, 87% yield), m.p. 93-94 °C. Anal. Calcd. for $C_{13}H_{11}NO_5S$: C, 53.24; H, 3.78; N, 4.78%. Found : C, 53.13; H, 3.81; N, 4.65%. IR ν_{max} (KBr)/cm^{-1} 3118, 1618, 1592, 1532, 1486, 1379, 1353, 1201, 1170 (Figure 11). ^1H NMR ($CDCl_3$) δ_H 2.44 (3H, s, tosyl C$\underline{H}_3$), 7.15 (2H, d, 4-nitrophenyl C$\underline{H}$), 7.32 (2H, d, tosyl C$\underline{H}$), 7.70 (2H, d, 4-nitrophenyl C$\underline{H}$), 8.16 (2H, d, tosyl C$\underline{H}$) (Figure 12).

2,4,5-Trichlorophenyl *p*-toluenesulfonate (**3b**) Purified by recrystallization from ethyl acetate and obtained as a white crystalline solid (3.16 g, 90% yield), m.p. 92-93 °C. Anal. Calcd. for $C_{13}H_9O_3SCl_3$: C, 44.41; H, 2.58%. Found : C, 44.38; H, 2.65%. IR ν_{max} (KBr)/cm^{-1} 3098, 1597, 1460, 1385, 1346, 1260, 1195, 1175 (Figure 13). ^1H NMR ($CDCl_3$) δ_H 2.45 (3H, s, tosyl C$\underline{H}_3$), 7.32 (2H, d, tosyl C$\underline{H}$), 7.42 (1H, s, trichlorophenyl C$\underline{H}$), 7.45 (1H, s, trichlorophenyl C$\underline{H}$), 7.76 (2H, d, tosyl C$\underline{H}$) (Figure 14).

Pentafluorophenyl *p*-toluenesulfonate (**3c**) Purified by recrystallization from ethyl acetate-hexane and obtained as a white crystalline solid (2.87 g, 85% yield), m.p. 63-64 °C. Anal. Calcd. for $C_{13}H_7O_3SF_5$: C, 46.16; H, 2.09%. Found : C, 46.15; H, 2.10%. IR ν_{max} (KBr)/cm^{-1} 3099, 1652, 1599, 1521, 1387, 1198, 1180, 998 (Figure 15). ^1H NMR ($CDCl_3$) δ_H 2.48 (3H, s, tosyl C$\underline{H}_3$), 7.40 (2H, d, tosyl C$\underline{H}$), 7.84 (2H, d, tosyl C$\underline{H}$) (Figure 16).

Pentachlorophenyl *p*-toluenesulfonate (**3d**) Purified by recrystallization from ethyl acetate and obtained as a white crystalline solid (3.70 g, 88% yield), m.p. 154-156 °C. Anal. Calcd. for $C_{13}H_7O_3SCl_5$: C, 37.13; H, 1.68%. Found : C, 37.21; H, 1.55%. IR ν_{max} (KBr)/cm^{-1} 1652, 1381, 1361, 1195, 1180 (Figure 17). ^1H NMR ($CDCl_3$) δ_H 2.49 (3H, s, tosyl C<u>H</u>$_3$), 7.38 (2H, d, tosyl C<u>H</u>), 7.89 (2H, d, tosyl C<u>H</u>) (Figure 18).

2.3.4 Synthesis of Aryl 4-nitrobenzenesulfonates



3a Ar = 4-nitrophenyl **3b** Ar = 2,4,5-trichlorophenyl

3c Ar = pentafluorophenyl **3d** Ar = pentachlorophenyl

General procedure

The general procedure described in the foregoing preparation of aryl *p*-toluenesulfonate was followed, tosyl chloride being replaced by 4-nitrobenzene sulfonyl chloride. Aryl 4-nitrobenzenesulfonate was obtained as crystalline solid starting from substituted phenol (10.1 mmol), 4-nitrobenzenesulfonyl chloride (10 mmol) and dry pyridine (10 mL).

4-Nitrophenyl 4-nitrobenzenesulfonate (**4a**) Purified by recrystallization from ethyl acetate and obtained as a yellow crystalline solid (2.79 g, 86% yield), m.p. 156-157 °C Anal. Calcd. for $C_{12}H_8N_2O_7S$: C, 44.45; H, 2.49; N, 8.64%. Found : C, 44.40; H, 2.46; N, 8.75%. IR ν_{max} (KBr)/ cm^{-1} 3111, 1614, 1588, 1529, 1485, 1395, 1350, 1203 (Figure 19). ^1H NMR ($CDCl_3$) δ_H 7.21 (2H, d, 4-nitrophenyl C<u>H</u>), 8.09 (2H, d, 4-nitrophenyl C<u>H</u>), 8.23 (2H, d, 4-nitrobenzenesulfonyl C<u>H</u>), 8.40 (2H, d, 4-nitrobenzenesulfonyl C<u>H</u>) (Figure 20).

2,4,5-Trichlorophenyl 4-nitrobenzenesulfonate (**4b**) Purified by recrystallization from ethyl acetate and obtained as a yellow crystalline solid (3.10 g, 81% yield), m.p. 143-144 °C. Anal. Calcd. for $C_{12}H_6NO_5SCl_3$: C, 37.67; H, 1.58; N, 3.66%. Found : C, 37.63; H, 1.79; N, 3.59%. IR ν_{max} (KBr)/cm^{-1} 3095, 1532, 1459, 1407, 1393, 1348, 1188 (Figure 21). ^1H NMR ($CDCl_3$) δ_H 7.45 (1H, s, trichlorophenyl C<u>H</u>), 7.54 (1H, s, trichlorophenyl C<u>H</u>), 8.10 (2H, d, 4-nitrobenzenesulfonyl C<u>H</u>), 8.40 (2H, d, 4-nitrobenzenesulfoyl C<u>H</u>) (Figure 22).

Pentafluorophenyl 4-nitrobenzenesulfonate (**4c**) Purified by recrystallization from ethyl acetate-hexane and obtained as a yellow crystalline solid (3.06 g, 83% yield), m.p. 108-109 °C. Anal. Calcd. for $C_{12}H_4NO_5SF_5$: C, 39.04; H, 1.09; N, 3.79%. Found : C, 38.91; H, 1.42; N. 3.69%. IR ν_{max} (KBr)/ cm^{-1} 3117, 1610, 1537, 1400, 1351, 1200, 999 (Figure 23). ^1H NMR ($CDCl_3$) δ_H 8.19 (2H, d, 4-nitrobenzene sulfonyl C<u>H</u>), 8.46 (2H, d, 4-nitrobenzenesulfonyl C<u>H</u>) (Figure 24).

Pentachlorophenyl 4-nitrobenzenesulfonate (**4d**) Purified by recrystallization from ethyl acetate and obtained as a yellow crystalline solid (3.88 g, 86% yield), m.p. 195-196 °C. Anal. Calcd. for $C_{12}H_4NO_5SCl_5$: C, 31.92; H, 0.89; N, 3.10%. Found : C, 31.82; H, 0.93; N, 2.94%. IR ν_{max} (KBr)/cm^{-1} 3103, 1530, 1402, 1361, 1316, 1195 (Figure 25). ^1H NMR ($CDCl_3$) δ_H 8.25 (2H, d, 4-nitrobenzenesulfonyl C<u>H</u>), 8.47 (2H, d, 4-nitrobenzenesulfonyl C<u>H</u>) (Figure 26).

2.4 Synthesis of Aryl Esters

*Attempted synthesis of N-tert-butoxycarbonylglycine 4-nitrophenyl ester using 4-nitrophenyl methyl carbonate (**1a**) as a reagent*

To a mixture of Boc-Gly-OH (87.5 mg, 0.5 mmol) and 4-nitrophenyl methyl carbonate (**1a**) (98.5 mg, 0.5 mmol) in acetonitrile (3 mL) was added triethylamine (0.07 mL, 0.5 mmol) dropwise at room temperature. After stirring overnight, TLC indicated no reaction had occurred.

*Attempted synthesis of N-tert-butoxycarbonylglycine benzotriazolyl ester using benzotriazolyl methyl carbonate (**1b**) as a reagent*

Similar procedures as described above were used to carry out the synthesis of *N-tert*-butoxycarbonylglycine benzotriazolyl ester, 4-nitrophenyl methyl carbonate (**1a**) being replaced by benzotriazolyl methyl carbonate (**1b**). The reaction was completed within 2 hours and the solvent was evaporated to dryness. The residue was dissolved with dichloromethane and washed with 5% HCl, 5%$NaHCO_3$, H_2O and brine. The organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash column chromatography using 40% ethyl acetate-hexane as eluent to give one major product. ^{1}H NMR ($CDCl_3$) δ_H 1.38 (9H, s, Boc C$\underline{H}_3$(x3)), 3.74 (3H, s, OC$\underline{H}_3$), 3.89 (2H, d, C$\underline{H}_2$), 5.02 (1H, m, N$\underline{H}$) (Figure 27). This was indentified as methyl ester of Boc-Gly-OH, not the desired product.

*Attempted synthesis of aryl benzoate using aryl p-toluenesulfonate (**3a-3d**) as reagent*

To a solution of benzoic acid (0.5 mmol) and aryl *p*-toluenesulfonate (0.5 mmol) in dichloromethane (3 mL) was added triethylamine (0.5 mmol) dropwise at room temperature. After stirring overnight, TLC indicated no reaction had taken place. Variation of the reaction conditions including the reagent (**3a-3d**) and the solvent (DMF, acetonitrile, pyridine), all reactions had not occurred. The exception is when pentafluorophenyl *p*-toluenesulfonate (**3c**) was used as a reagent in DMF , reaction occurred to 50% completion to give pentafluorophenyl benzoate according to TLC and ^{1}H NMR analysis.

2.4.1 The Study of Effect of Catalysts

The reaction of benzoic acid with 4-nitrophenyl *p*-toluenesulfonate (**3a**) was chosen to study the effect of catalysts.

4-Dimethylaminopyridine (DMAP) as a catalyst

To a solution of benzoic acid (61 mg, 0.5 mmol), 4-nitrophenyl *p*-toluenesulfonate (**3a**) (146.5 mg, 0.5mmol) and DMAP (61.1 mg, 0.5mmol) in DMF (3 mL) was added triethylamine (0.07 mL, 0.5 mmol) with stirring. After stirring overnight at room temperature, no reaction had occurred (according to TLC).

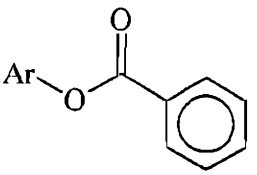
Reaction of 4-nitrophenyl p-toluenesulfonate (3a) with DMAP

4-Nitrophenyl *p*-toluenesulfonate (**3a**) (130.5 mg, 0.5 mmol) and DMAP (61.1 mg, 0.5 mmol) were dissolved in DMF (3mL) with stirring at room temperature. Triethylamine (0.07 mL, 0.5 mmol) was then added dropwise and the mixture was stirring overnight. TLC indicated no reaction had taken place.

1-Hydroxybenzotriazole (HOBt) as a catalyst

The method described in the foregoing procedure for the reaction between benzoic acid with 4-nitrophenyl *p*-toluenesulfonate (**3a**) was followed, the DMAP being replaced by $HOBt.H_2O$. The reaction was completed within 30 min at room temperature and was worked up by diluting with dichloromethane, washing with 5% HCl, 5% $NaHCO_3$, H_2O and brine. The combined organic phase was dried over $MgSO_4$ and evaporated to dryness and the residue was purified by flash column chromatography using 10% ethyl acetate-hexane as eluent. The desired product, 4-nitrophenyl benzoate was obtained as a colorless solid (109.4 mg, 90% yield).

2.4.2 Synthesis of Aryl benzoates



Ar = 4-nitrophenyl, 2,4,5-trichlorophenyl, pentafluorophenyl, pentachlorophenyl

General procedure

A mixture of benzoic acid (0.5 mmol) , aryl 4-nitrobenzenesulfonate (0.5 mmol) and HOBt.H_2O (0.05 mmol) in dichloromethane (5 mL) was stirred at room temperature. Triethylamine (0.5 mmol) was then added and the stirring was continued for 20-30 min. After the reaction was completed (as judged by TLC), the reaction mixture was extracted with 5% HCl, 5% $NaHCO_3$. The organic phase was then washed with H_2O and brine, dried with $MgSO_4$, filtered and concentrated under reduced pressure to give, after purification by short column chromatography, pure aryl benzoate.

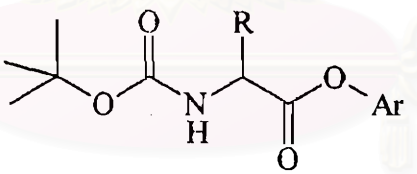
4-Nitrophenyl benzoate Purified by short column chromatography with 30% chloroform-hexane as eluent and obtained as a colorless solid (111.8 mg, 92% yield), m.p. 88-89 °C. IR ν_{max} (KBr)/cm^{-1} 3115, 3078, 1742, 1616, 1593, 1522, 1347, 1173 (Figure 28). ^{1}H NMR ($CDCl_3$) δ_H 7.42 (2H, d, 4-nitrophenyl C<u>H</u>), 7.55 (2H, t, benzoyl *m*-C<u>H</u>), 7.59 (1H, t, benzoyl *p*-C<u>H</u>), 8.20 (2H, d, benzoyl *o*-C<u>H</u>), 8.32 (2H, d, 4-nitrophenyl C<u>H</u>) (Figure 29).

2,4,5-Trichlorophenyl benzoate Purified by short column chromatography with 30% chloroform-hexane as eluent and obtained as a colorless solid (135.7 mg, 90% yield), m.p. 145-146 °C. IR ν_{max} (KBr)/cm^{-1} 3096, 1749, 1651, 1602, 1559, 1465, 1270, 1247, 1181 (Figure 30). ^{1}H NMR ($CDCl_3$) δ_H 7.43 (1H, s, trichlorophenyl C<u>H</u>), 7.52 2H, t, benzoyl *m*-CH), 7.60 (1H, s, trichlorophenyl C<u>H</u>), 7.68 (1H, t, benzoyl *p*-C<u>H</u>), 8.19 (2H, d, benzoyl *o*-C<u>H</u>) (Figure 31).

Pentafluorophenyl benzoate Purified by short column chromatography with 30% chloroform-hexane as eluent and obtained as a colorless solid (139.7 mg, 97% yield), m.p. 74-75 °C. IR ν_{max} (KBr)/cm^{-1} 3070, 1757, 1600, 1521, 1453, 1243, 1049, 1019 (Figure 32). ^{1}H NMR ($CDCl_3$) δ_H 7.56 (2H, t, benzoyl *m*-CH), 7.71 (1H, t, benzoyl *p*-CH), 8.20 (2H, d, benzoyl *o*-CH). IR ν_{max} (KBr)/cm^{-1} 3070, 1757, 1600, 1521, 1453, 1243, 1049, 1019 (Figure 33).

Pentachlorophenyl benzoate Purified by short column chromatography with 30% chloroform-hexane as eluent and obtained as a colorless solid (161.2 mg, 87% yield), m.p. 163-164 °C. IR ν_{max} (KBr)/cm^{-1} 3068, 1749, 1599, 1541, 1454, 1387, 1238, 1089, 1073 (Figure 34). ^{1}H NMR ($CDCl_3$) δ_H 7.55 (2H, t, benzoyl *m*-CH), 7.69 (1H, t, benzoyl *p*-CH), 8.20 (2H, d, benzoyl *o*-CH) (Figure 35).

2.4.3 Synthesis of Aryl esters of *N-tert*-butoxycarbonyl amino acids



Glycine R = H L-Leucine R = 

General procedure

Triethylamine (0.5 mmol) was added dropwise with stirring to a solution of *N-tert*-butoxycarbonyl amino acid (0.5 mmol), aryl 4-nitrobenzenesulfonate (0.5 mmol) and HOBt.H_2O (0.05 mmol) as a catalyst in DMF (3 mL) at room temperature. The reaction mixture was allowed to stir at room temperature for 30 min. The solution was diluted with dichloromethane, washed with 5% HCl, 5% $NaHCO_3$, H_2O and brine and dried over $MgSO_4$. It was then evaporated to dryness and the residue chromatographed on silica gel column to give pure aryl ester of *N-tert*-butoxycarbonyl amino acid.

N-tert-Butoxycarbonylglycine 4-nitrophenyl ester (Boc-Gly-OPnp) Purified by short column chromatography with 50% chloroform-hexane as eluent and obtained as a colorless solid (120 mg, 81% yield), m.p. 55-57 °C. Anal. Calcd. for $C_{13}H_{16}O_6N_2$: C, 52.70; H, 5.44; N, 9.46%. Found : C, 52.77; H, 5.55; N, 9.25%. IR ν_{max} (KBr)/cm^{-1} 3436, 3119, 3084, 2980, 2936, 1777, 1702, 1617, 1594, 1527, 1368, 1291, 1254, 1212, 1159 (Figure 36). ^{1}H NMR ($CDCl_3$) δ_H 1.44 (9H, s, Boc C<u>H</u>$_3$ (x3)), 4.17 (2H, d, Gly C<u>H</u>$_2$), 5.18 (1H, br m, N<u>H</u>), 7.28 (2H, d, 4-nitrophenyl C<u>H</u>), 8.24 (2H, d, 4-nitrophenyl C<u>H</u>) (Figure 37).

N-tert-Butoxycarbonylglycine 2,4,5-trichlorophenyl ester (Boc-Gly-OTcp) Purified by short column chromatography with 50% chloroform-hexane with as eluent and obtained as a colorless solid (161.0 mg, 91% yield), m.p. 104-105 °C (lit.[20] m.p. 106-107 °C). Anal. Calcd. for $C_{13}H_{14}O_4NCl_3$: C, 44.03; H, 3.98; N, 3.95%. Found : C, 44.05; H, 3.89; N, 3.99%. IR ν_{max} (KBr)/cm^{-1} 3375, 3098, 3011, 2980, 2939, 1775, 1712, 1511, 1459, 1417, 1370, 1352, 1281, 1255, 1167, 1142, 1083 (Figure 38). ^{1}H NMR ($CDCl_3$) δ_H 1.45 (9H, s, Boc C<u>H</u>$_3$ (x3)), 4.20 (2H, d, Gly C<u>H</u>$_2$), 5.07 (1H, br t, N<u>H</u>), 7.30 (1H, s, trichlorophenyl C<u>H</u>), 7.53 (1H, s, trichlorophenyl C<u>H</u>) (Figure 39).

N-tert-Butoxycarbonylglycine pentafluorophenyl ester (Boc-Gly-OPfp) Puried by short column chromatography with 50% chloroform-hexane as eluent and obtained as a colorless solid (159.1 mg, 93% yield), m.p. 74-75 °C (lit.[20] m.p. 79-80 °C). Anal. Calcd. for $C13H_{12}O_4NF_5$: C, 45.76; H, 3.54; N, 4.11%. Found : C, 45.80; H, 3.51; N, 4.10%. IR ν_{max} (KBr)/cm^{-1} 3397, 2987, 2944, 1783, 1723, 1657, 1524, 1473, 1414, 1371, 1285, 1258, 1144, 993 (Figure 40). ^{1}H NMR ($CDCl_3$) δ_H 1.46 (9H, s, Boc C<u>H</u>$_3$ (x3)), 4.27 (2H, d, Gly C<u>H</u>$_2$), 5.09 (1H, br m, N<u>H</u>) (Figure 41).

N-tert-Butoxycarbonylglycine pentachlorophenyl ester (Boc-Gly-OPcp) Purified by short column chromatography with 60% chloroform-hexane as eluent and obtained as a colorless solid (169.3 mg, 80% yield), m.p. 132-133 °C. Anal. Calcd. for $C_{13}H_{12}O_4NCl_5$: C, 38.87; H, 2.86; N, 3.31%. Found : C, 38.76; H, 2.99; N, 3.24%. ^{1}H NMR ($CDCl_3$) δ_H 1.46 (9H, s, Boc C$\underline{H}_3$ (x3)), 4.24 (2H, d, Gly C$\underline{H}_2$), 5.04 (1H, br m, N$\underline{H}$) (Figure 42).

N-tert-Butoxycarbonyl-L-leucine 4-nitrophenyl ester (Boc-L-Leu-OPnp) Purified by short column chromatography with 40% chloroform-hexane as eluent and obtained as a colorless solid (114.2 mg, 65% yield), m.p. 64-66 °C. Anal. Calcd. for $C_{17}H_{24}O_6N_2$: C, 57.94; H, 6.87; N, 7.95%. Found : C, 57.95; H, 6.94; N, 7.85%. IR ν_{max} (KBr)/ cm^{-1} 3430, 3119, 3085, 2963, 2936, 1771, 1713, 1618, 1549, 1528, 1349, 1277, 1255, 1210, 1164, 1120 (Figure 43). ^{1}H NMR ($CDCl_3$) δ_H 0.98 (6H, d, isopropyl C$\underline{H}_3$ (x2)), 1.44 (9H, s, Boc C$\underline{H}_3$ (x3)), 1.75 (3H, m, Leu C$\underline{H}$, C$\underline{H}_2$), 4.49 (1H, m, Leu C$_\alpha\underline{H}$), 4.95 (1H, br d, N$\underline{H}$), 7.27 (2H, d, 4-nitrophenyl C$\underline{H}$), 8.23 (2H, d, 4-nitrophenyl C$\underline{H}$) (Figure 44).

N-tert-Butoxycarbonyl-L-leucine 2,4,5-trichlorophenyl ester (Boc-L-Leu-OTcp) Purified by short column chromatography with 30% chloroform-hexane as eluent and obtained as a colorless solid (185.1 mg, 90% yield), m.p. 62-63 °C, $[\alpha]_D^{26}$ -23.9 (c=1.42, $CHCl_3$). Anal. Calcd. for $C_{17}H_{22}O_4NCl_3$: C, 49.71; H, 5.40; N, 3.41%. Found : C, 49.72; H, 5.32; N, 3.32%. ^{1}H NMR ($CDCl_3$) δ_H 1.00 (6H, d, isopropyl C$\underline{H}_3$ (x2)), 1.45 (9H, s, Boc C$\underline{H}_3$ (x3)), 1.80 (3H, m, Leu C$\underline{H}$, C$\underline{H}_2$), 4.52 (1H, m, Leu C$_\alpha\underline{H}$), 4.94 (1H, br t, N$\underline{H}$), 7.29 (1H, s, trichlorophenyl C$\underline{H}$), 7.54 (1H, s, trichlorophenyl C$\underline{H}$) (Figure 45).

N-tert-Butoxycarbonyl-L-leucine pentafluorophenyl ester (Boc-L-Leu-OPfp) Purified by short column chromatography with 30% chloroform-hexane as eluent and obtained as a colorless oil (158.9 mg, 80% yield) (lit.[38] m.p. 48-50 °C), $[\alpha]_D^{26}$ -16.4 (c=2.34, $CHCl_3$). Anal. Calcd. for $C_{17}H_{20}O_4NF_5$: C, 51.39; H, 5.07; N, 3.53%. Found : C, 51.32; H, 5.35; N,3.52%. ^{1}H NMR ($CDCl_3$) δ_H 1.00 (6H, d, isopropyl C$\underline{H}_3$ (x2)), 1.46 (9H, s, Boc C$\underline{H}_3$ (x3)), 1.73 (3H, m, Leu C$\underline{H}$, C$\underline{H}_2$), 4.60 (1H, m, Leu C$_\alpha\underline{H}$), 4.91 (1H, br d, N$\underline{H}$) (Figure 46).

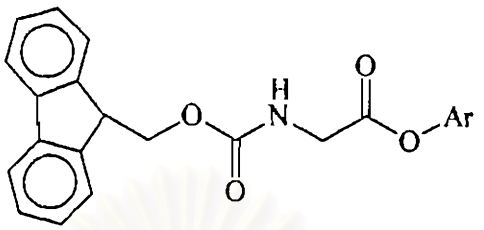
N-tert-Butoxycarbonyl-L-leucine pentachlorophenyl ester (Boc-L-Leu-OPcp) Purified by short column chromatography with 40% chloroform-hexane as eluent and obtained as a colorless oil (65.0 mg, 27% yield). Anal. Calcd. for $C_{17}H_{20}O_4NCl_5$: C, 42.57; H, 4.20; N, 2.92%. Found : C, 42.53; H, 4.31; N, 3.08%. ^{1}H NMR ($CDCl_3$) δ_H 1.00 (6H, d, isopropyl C$\underline{H}_3$ (x2)), 1.47 (9H, s, Boc C$\underline{H}_3$ (x3)), 1.72 (3H, m, Leu C$\underline{H}$, C$\underline{H}_2$), 4.63 (1H, m, Leu C$_\alpha\underline{H}$), 4.89 (1H, br d, N$\underline{H}$) (Figure 47).

2.4.4 Synthesis of Aryl esters of *N*-9-Fluorenylmethoxycarbonyl amino acids

General procedure

The general procedure was carried out in the similar manner to the preparation of aryl ester of *N-tert*-butoxycarbonyl amino acid, except that triethylamine being replaced by diisopropylethylamine (DIEA). Aryl ester was obtained from *N*-9-fluorenyl-methoxycarbonyl amino acid (0.3 mmol), pentafluorophenyl 4-nitrobenzenesulfonate (**4b**) or 2,4,5-trichlorophenyl 4-nitrobenzenesulfonate (**4c**) (0.3 mmol), HOBt.H_2O (0.03 mmol) and DIEA (0.3 mmol) in DMF (3mL). The residue was purified by flash column chromatography with suitable solvent system to give analytically pure aryl ester.

Aryl esters of N-9-fluorenylmethoxycarbonylglycine

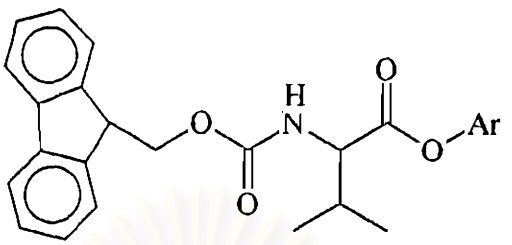


Ar = 2,4,5-trichlorophenyl, pentafluorophenyl

N-9-Fluorenylmethoxycarbonylglycine 2,4,5-trichlorophenyl ester (Fmoc-Gly-OTcp)
Purified by short column chromatography with 50 % chloroform-hexane as eluent and obtained as a colorless solid (140.0 mg, 98% yield), m.p. 143-144 °C (lit.[17] m.p. 146 °C). Anal. Calcd. for $C_{23}H_{16}O_4NCl_3$: C, 57.95; H, 3.38; N, 2.94%. Found : C, 57.95; H, 3.48; N, 2.91%. IR ν_{max} (KBr)/cm^{-1} 3322, 3091, 3064, 2980, 2947, 1768, 1687, 1539, 1462, 1384, 1351, 1308, 1268, 1229, 1193, 1164, 1124 (Figure 48). ^{1}H NMR ($CDCl_3$) δ_H 4.24 (1H, t, Fmoc aliphatic C<u>H</u>), 4.30 (2H, d, Gly C<u>H</u>$_2$), 4.47 (2H, d, Fmoc C<u>H</u>$_2$), 5.35 (1H, br t, N<u>H</u>), 7.34 (5H, m, trichloro-phenyl C<u>H</u>, Fmoc aromatic C<u>H</u>), 7.57 (1H, s, trichlorophenyl C<u>H</u>), 7.59 (2H, d, Fmoc aromatic C<u>H</u>), 7.75 (2H, d, Fmoc aromatic C<u>H</u>) (Figure 49).

N-9-Fluorenylmethoxycarbonylglycine pentafluorophenyl ester (Fmoc-Gly-OPfp)
Purified by short column chromatography with 50% chloroform-hexane as eluent and obtained as a colorless solid (120.9 mg, 87% yield), m.p. 154-156 °C (lit.[37] m.p. 157-158 °C). Anal. Calcd. for $C_{23}H_{14}O_4NF_5$: C, 59.62; H, 3.05; N, 3.02%. Found : C, 59.64; H, 3.05; N, 3.04%. IR ν_{max} (KBr)/cm^{-1} 3344, 3069, 3026, 2986, 2952, 1791, 1704, 1525, 1451, 1292, 1257, 1170, 999 (Figure 50). ^{1}H NMR ($CDCl_3$) δ_H 4.25 (1H, t, Fmoc aliphatic C<u>H</u>), 4.37 (2H, d, Gly C<u>H</u>$_2$), 4.48 (2H, d, Fmoc C<u>H</u>$_2$), 5.34 (1H, br t, N<u>H</u>), 7.37 (4H, m, Fmoc aromatic C<u>H</u>), 7.60 (2H, d, Fmoc aromatic C<u>H</u>), 7.78 (2H, d, Fmoc aromatic C<u>H</u>) (Figure 51).

Aryl esters of N-9-fluorenylmethoxycarbonyl-L-valine



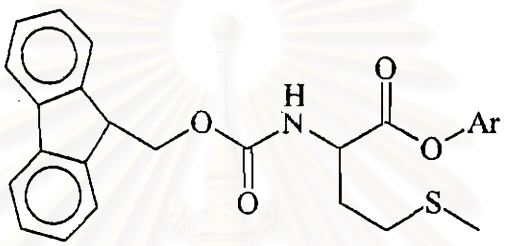
Ar = 2,4,5-trichlorophenyl, pentafluorophenyl

N-9-Fluorenylmethoxycarbonyl-L-valine 2,4,5-trichlorophenyl ester (Fmoc-L-Val-OTcp) Purified by short column chromatography with 50% dichloromethane-hexane as eluent and obtained as a colorless solid (150.0 mg, 96% yield), m.p. 145-146 °C (lit.[16] m.p. 143-144 °C), $[\alpha]_D^{26}$ -30.2 (*c*=1.32, $CHCl_3$) (lit.[37] $[\alpha]_D^{25}$ -30.2 (*c*=1.00, $CHCl_3$)). Anal. Calcd. for $C_{26}H_{22}O_4NCl_3$: C, 60.19; H, 4.27, N, 2.70%. Found : C, 60.21; H, 4.33; N, 2.64%. IR ν_{max} (KBr)/cm^{-1} 3309, 3065, 2971, 2938, 1771, 1697, 1543, 1455, 1349, 1289, 1259, 1234, 1170, 1121, 1083 (Figure 52). ^1H NMR ($CDCl_3$) δ_H 1.09 (6H, dd, isopropyl C<u>H</u>$_3$ (x2)), 2.43 (1H, m, Val C<u>H</u>), 4.26 (1H, t, Fmoc aliphatic C<u>H</u>), 4.48 (2H, d, Fmoc C<u>H</u>$_2$), 4.60 (1H, dd, Val C$_\alpha$<u>H</u>), 5.29 (1H, d, N<u>H</u>), 7.34 (5H, m, trichlorophenyl C<u>H</u>, Fmoc aromatic C<u>H</u>), 7.55 (1H, s, trichlorophenyl C<u>H</u>), 7.60 (2H, d, Fmoc aromatic C<u>H</u>), 7.79 (2H, d, Fmoc aromatic C<u>H</u>) (Figure 53).

N-9-Fluorenylmethoxycarbonyl-L-valine pentafluorophenyl ester (Fmoc-L-Val-OPfp) Purified by short column chromatography with 60% dichloromethane-hexane as eluent and obtained as a colorless solid (120.7 mg, 80% yield), m.p. 119-121 °C (lit.[16]m.p. 121-123 °C), $[\alpha]_D^{26}$ -22.4 (*c*=1.32, $CHCl_3$) (lit.[15] $[\alpha]_D^{25}$ -21.9 (*c*=1.00, $CHCl_3$)). Anal. Calcd. for $C_{26}H_{20}O_4NF_5$: C, 61.79; H, 3.99; N, 2.77%. Found : C, 61.78; H, 4.03; N, 2.79%. IR ν_{max} (KBr)/cm^{-1} 3393, 3041, 2980, 1797, 1713, 1522, 1450, 1397, 1348, 1312, 1229, 1089, 995 (Figure 54). ^1H NMR ($CDCl_3$) δ_H 1.06 (6H, dd, isopropyl C<u>H</u>$_3$ (x2)), 2.40 (1H, m, Val C<u>H</u>), 4.24 (1H, t, Fmoc aliphatic C<u>H</u>), 4.48

(2H, d, Fmoc C$\underline{H}_2$), 4.70 (1H, dd, Val C$_\alpha\underline{H}$), 5.29 (1H, d, N$\underline{H}$), 7.36 (4H, m, Fmoc aromatic C$\underline{H}$), 7.60 (2H, d, Fmoc aromatic C$\underline{H}$), 7.78 (2H, d, Fmoc aromatic C$\underline{H}$) (Figure 55).

Aryl esters of N-9-fluorenylmethoxycarbonyl-L-methionine



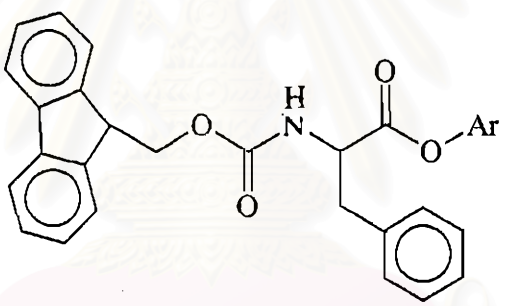
Ar = 2,4,5-trichlorophenyl, pentafluorophenyl

N-9-Fluorenylmethoxycarbonyl-L-methionine 2,4,5-trichlorophenyl ester (Fmoc-L-Met-OTcp) Purified by short column chromatography with 50% dichloromethane-hexane as eluent and obtained as a colorless solid (145.0 mg, 88% yield), m.p. 152-153 °C, $[\alpha]_D^{26}$ -15.7 (*c*=1.36, $CHCl_3$). Anal. Calcd. for $C_{26}H_{22}O_4NSCl_3$: C, 56.69; H, 4.03; N, 2.54%. Found : C, 56.53; H, 3.90; N, 2.51%. IR ν_{max} (KBr)/cm^{-1} 3316, 3087, 2967, 2936, 1782, 1687, 1539, 1452, 1349, 1306, 1284, 1261, 1215, 1124, 1080, 1058 (Figure 56). ^{1}H NMR ($CDCl_3$) δ_H 2.12 (3H, s, C$\underline{H}_3$), 2.36 (2H, m, Met C$\underline{H}_2$), 2.66 (2H, t, Met C$\underline{H}_2$), 4.24 (1H, t, Fmoc aliphatic C$\underline{H}$), 4.49 (2H, d, Fmoc C$\underline{H}_2$), 4.80 (1H, m, Met C$_\alpha\underline{H}$), 5.46 (1H, d, N$\underline{H}$), 7.35 (5H, m, trichlorophenyl C$\underline{H}$, Fmoc aromatic C$\underline{H}$), 7.55 (1H, s, trichlorophenyl C$\underline{H}$), 7.60 (2H, d, Fmoc aromatic C$\underline{H}$), 7.76 (2H, d, Fmoc aromatic C$\underline{H}$) (Figure 57).

N-9-Fluorenylmethoxycarbonyl-L-methionine pentafluorophenyl ester (Fmoc-L-Met-OPfp) Purified by short column chromatography with 60% dichloromethane-hexane as eluent and obtained as a colorless solid (149.2 mg, 93% yield), m.p. 110-111 °C (lit.[15] m.p. 102-104 °C), $[\alpha]_D^{26}$ -11.9 (*c*=1.32, $CHCl_3$) (lit.[37] $[\alpha]_D^{25}$ -12.6 (*c*=1.00,

$CHCL_3$)). Anal. Calcd. for $C_{26}H_{20}O_4NSF_5$: C, 58.10; H, 3.75; N, 2.61%. Found : C, 58.15; H, 3.74; N, 2.65%. IR ν_{max} (KBr)/cm^{-1} 3326, 3066, 2921, 1789, 1695, 1524, 1450, 1274, 1169, 1124, 1085, 998 (Figure 58). ^1H NMR ($CDCl_3$) δ_H 2.12 (3H, s, C<u>H</u>$_3$), 2.30 (2H, m, Met C<u>H</u>$_2$), 2.61 (2H, t, Met C<u>H</u>$_2$), 4.24 (1H, t, Fmoc aliphatic C<u>H</u>), 4.49 (2H, d, Fmoc C<u>H</u>$_2$), 4.88 (1H, m, Met C$_\alpha$<u>H</u>), 5.48 (1H, d, N<u>H</u>), 7.35 (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>) (Figure 59).

Aryl esters of N-9-fluorenylmethoxycarbonyl-L-phenylalanine

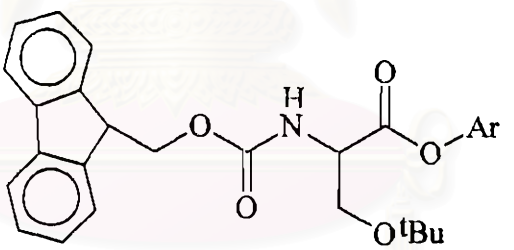


Ar = 2,4,5-trichlorophenyl, pentafluorophenyl

<u>*N*-9-Fluorenylmethoxyarbonyl-L-phenylalanine 2,4,5-trichlorophenyl ester (Fmoc-L-Phe-OTcp)</u> Purified by short column chromatography with 50% dichloromethane-hexane as eluent and obtained as a colorless solid (155.3 mg, 93% yield), m.p. 184-185 °C (lit.[16] m.p. 181-182 °C), $[\alpha]_D^{26}$ -29.4 (*c*=1.15, $CHCl_3$) (lit.[37] $[\alpha]_D^{25}$ -29.3 (*c*=1.60, $CHCl_3$)). Anal. Calcd. for $C_{30}H_{22}O_4NCl_3$: C, 63.57; H, 3.91; N, 2.47%. Found : C, 63.61; H, 3.94; N, 2.44%. IR ν_{max} (KBr)/cm^{-1} 3309, 3086, 3066, 2981, 2957, 1779, 1684, 1546, 1458, 1349, 1266, 1212, 1136, 1107, 1084 (Figure 60). ^1H NMR ($CDCl_3$) δ_H 3.29 (2H, m, Phe C<u>H</u>$_2$), 4.20 (1H, t, Fmoc aliphatic C<u>H</u>), 4.40 (2H, d, Fmoc C<u>H</u>$_2$), 4.91 (1H, m, Phe aliphatic C$_\alpha$<u>H</u>), 5.22 (1H, d, N<u>H</u>), 7.29 (11H, m, Phe aromatic C<u>H</u>, Fmoc aromatic C<u>H</u>, trichlorophenyl C<u>H</u>), 7.52 (2H, d, Fmoc aromatic C<u>H</u>), 7.76 (2H, d, Fmoc aromatic C<u>H</u>) (Figure 61).

N-9-Fluorenylmethoxycarbonyl-L-phenylalanine pentafluorophenyl ester (Fmoc-L-Phe-OPfp) Purified by short column chromatography with 55% dichloromethane-hexane as eluent and obtained as a colorless solid (147.8 mg, 91% yield), m.p. 148-149 °C (lit.[15] 154-157 °C), $[\alpha]_D^{26}$ -20.7 (*c*=1.42, $CHCl_3$) (lit.[15] $[\alpha]_D^{25}$ -20.3 (*c*=1.00, $CHCl_3$)). Anal. Calcd. for $C_{30}H_{20}O_4NF_5$: C, 65.10; H, 3.64; N, 2.53%. Found : C, 65.19; H, 3.71; N, 2.56%. IR ν_{max} (KBr)/cm^{-1} 3335, 3067, 3034, 2959, 1786, 1701, 1524, 1451, 1318, 1270, 1179, 1129, 1000 (Figure 62). ^1H NMR ($CDCl_3$) δ_H 3.30 (2H, m, Phe C$\underline{H}_2$), 4.20 (1H, t, Fmoc aliphatic C$\underline{H}$), 4.42 (2H, d, Fmoc C$\underline{H}_2$), 5.02 (1H, m, Phe aliphatic C$_\alpha\underline{H}$), 5.20 (1H, d, N$\underline{H}$), 7.33 (9H, m, Phe aromatic C$\underline{H}$, Fmoc aromatic C$\underline{H}$), 7.56 (2H, d, Fmoc aromatic C$\underline{H}$), 7.78 (2H, d, Fmoc aromatic C$\underline{H}$) (Figure 63).

Aryl esters of N-9-fluorenylmethoxycarbonyl-(O-t-butyl)-D-serine



Ar = 2,4,5-trichlorophenyl, pentafluorophenyl

N-9-Fluorenylmethoxycarbonyl-(*O-t*-butyl)-D-serine 2,4,5-trichlorophenyl ester (Fmoc-D-Ser(O^{t}Bu)-OTcp) Purified by short column chromatography with 50% dichloromethane-hexane as eluent and obtained as a colorless oil (140.1 mg, 83% yield), $[\alpha]_D^{26}$ -16.2 (*c*=3.28, $CHCl_3$). Anal. Calcd. for $C_{28}H_{26}O_5NCl_3$: C, 59.75; H, 4.66; N, 2.49%. Found : C, 59.76; H, 4.94; N, 2.43%. IR ν_{max} (neat)/cm^{-1} 3439, 3327, 3068, 2974, 1785, 1727, 1510, 1454, 1393, 1340, 1289, 1233, 1193, 1124, 1083 (Figure 64). ^1H NMR ($CDCl_3$) δ_H 1.21 (9H, s, Boc C$\underline{H}_3$ (x3)), 3.70 (1H, dd, Ser C$\underline{H}_2$), 4.04 (1H, dd, Ser C$\underline{H}_2$), 4.25 (1H, t, Fmoc aliphatic C$\underline{H}$), 4.42 (2H, m, Fmoc C$\underline{H}_2$), 4.78 (1H, d, Ser C$_\alpha\underline{H}$), 5.72 (1H, d, N$\underline{H}$), 7.34 (5H, m, trichlorophenyl C$\underline{H}$, Fmoc

aromatic CH), 7.56 (1H, s, trichlorophenyl CH), 7.60 (2H, d, Fmoc aromatic CH), 7.76 (2H, d, Fmoc aromatic CH) (Figure 65).

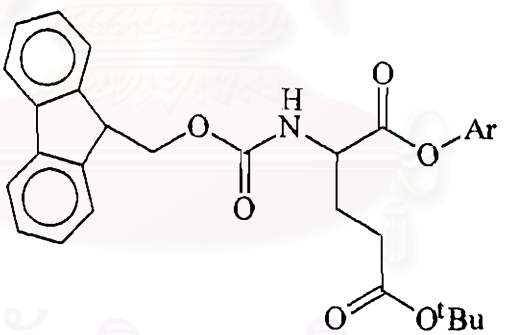
N-9-Fluorenylmethoxycarbonyl-(*O*-*t*-butyl)-D-serine pentafluorophenyl ester (Fmoc-D-Ser(O^{t}Bu)-OPfp) Purified by short column chromatography with 50% dichloromethane-hexane as eluent and obtained as a colorless oil (107.1 mg, 65% yield), $[\alpha]_D^{26}$ -11.1 (*c*=2.66, $CHCl_3$). Anal. Calcd. for $C_{28}H_{24}O_5NF_5$: C, 61.20; H, 4.40; N, 2.55%. Found : C, 61.25; H, 4.56; N, 2.53%. IR ν_{max} (neat)/cm^{-1} 3451, 3325, 3069, 2978, 1800, 1731, 1523, 1476, 1451, 1366, 1339, 1288, 1239, 1195, 1147, 1103, 1058, 996 (Figure 66). ^{1}H NMR ($CDCl_3$) δ_H 1.21 (9H, s, Boc CH$_3$ (x3)), 3.70 (1H, dd, Ser CH$_2$), 4.04 (1H, dd, Ser CH$_2$), 4.28 (1H, t, Fmoc aliphatic CH), 4.45 (2H, m, Fmoc CH$_2$), 4.88 (1H, d, Ser C$_\alpha$H), 5.72 (1H, d, NH), 7.36 (4H, m, Fmoc aromatic CH), 7.61 (2H, d, Fmoc aromatic CH), 7.78 (2H, d, Fmoc aromatic CH) (Figure 67).

Aryl esters of N-9-fluorenylmethoxycarbonyl-(O-t-butyl)-L-serine

N-9-Fluorenylmethoxycarbonyl-(*O*-*t*-butyl)-L-serine 2,4,5-trichlorophenyl ester (Fmoc-L-Ser(O^{t}Bu)-OTcp) Purified by short column chromatography with 50% dichloromethane-hexane as eluent and obtained as a colorless oil (146.8 mg, 87% yield), $[\alpha]_D^{26}$ -16.2 (*c*=3.28, $CHCl_3$). Anal. Calcd. for $C_{28}H_{26}O_5NCl_3$: C, 59.75; H, 4.66; N, 2.49%. Found : C, 57.73; H, 4.72; N, 2.35%. IR ν_{max} (neat)/cm^{-1} 3438, 3325, 3068, 2974, 1785, 1727, 1510, 1454, 1393, 1340, 1289, 1233, 1193, 1124, 1083 (Figure 68). ^{1}H NMR ($CDCl_3$) δ_H 1.20 (9H, s, Boc CH$_3$ (x3)), 3.70 (1H, dd, Ser CH$_2$), 4.04 (1H, dd, Ser CH$_2$), 4.25 (1H, t, Fmoc aliphatic CH), 4.42 (2H, m, Fmoc CH$_2$), 4.78 (1H, d, Ser C$_\alpha$H), 5.72 (1H, d, NH), 7.33 (5H, m, trichlorophenyl CH, Fmoc aromatic CH), 7.55 (1H, s, trichlorophenyl CH), 7.60 (2H, d, Fmoc aromatic CH), 7.75 (2H, d, Fmoc aromatic CH) (Figure 69).

N-9-Fluorenylmethoxycarbonyl-(*O*-*t*-butyl)-L-serine pentafluorophenyl ester (Fmoc-L-Ser(O^{t}Bu)-OPfp) Purified by short column chromatography with 50% dichloromethane-hexane as eluent and obtained as a colorless oil (130.0 mg, 80% yield), $[\alpha]_D^{26}$ -11.1 (c=2.66, $CHCl_3$) (lit.[37] $[\alpha]_D^{25}$ -10.5 (*c*=5.00, $CHCl_3$)). Anal. Calcd. for $C_{28}H_{24}O_5NF_5$: C, 61.20; H, 4.40; N, 2.55%. Found : C, 59.59; H, 4.42; N, 2.46%. IR ν_{max} (neat)/cm^{-1} 3451, 3323, 3069, 2978, 1800, 1732, 1523, 1476, 1452, 1394, 1366, 1340, 1288, 1239, 1195, 1147, 1103, 1082, 1058, 996 (Figure 70). ^{1}H NMR ($CDCl_3$) δ_H 1.20 (9H, s, Boc C$\underline{H}_3$ (x3)), 3.70 (1H, dd, Ser C$\underline{H}_2$), 4.03 (1H, dd, Ser C$\underline{H}_2$), 4.26 (1H, t, Fmoc aliphatic C$\underline{H}$), 4.45 (2H, m, Fmoc C$\underline{H}_2$), 4.88 (1H, d, Ser C$_\alpha\underline{H}$), 5.70 (1H, d, N$\underline{H}$), 7.36 (4H, m, Fmoc aromatic C$\underline{H}$), 7.60 (2H, d, Fmoc aromatic C$\underline{H}$), 7.76 (2H, d, Fmoc aromatic C$\underline{H}$) (Figure 71).

Aryl esters of N-9-fluorenylmethoxycarbonyl-(tert-butyl ester)-L-glutamic acid



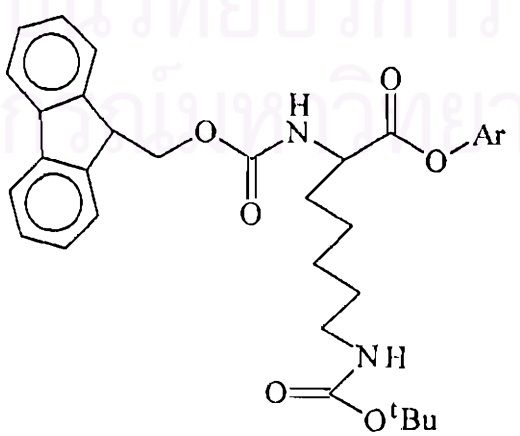
Ar = 2,4,5-trichlorophenyl, pentafluorophenyl

N-9-Fluorenylmethoxycarbonyl-(*tert*-butyl ester)-L-glutamic acid 2,4,5-trichlorophenyl ester (Fmoc-L-Glu(O^{t}Bu)-OTcp) Purified by short column chromatography with 50% chloroform-hexane as eluent and obtained as a colorless solid (143.8 mg, 79% yield), m.p. 140-140 °C, $[\alpha]_D^{26}$ -18.5 (*c*=1.05, $CHCl_3$). Anal. Calcd. for $C_{30}H_{28}O_6NCl_3$: C, 59.57; H, 4.67; N, 2.32%. Found : C, 59.59; H, 4.56; N, 2.30%. IR ν_{max} (KBr)/cm^{-1} 3400, 3067, 2976, 2954, 1786, 1709, 1522, 1453, 1387, 1368, 1347, 1317, 1263, 1225, 1197, 1153, 1125, 1083, 1047 (Figure 72). IR ν_{max} (KBr)/cm^{-1} 3400, 3067, 2976, 2954, 1786, 1709, 1522, 1453, 1387, 1368, 1347, 1317, 1263,

1225, 1197, 1153, 1125, 1083, 1047 (Figure 72). ^{1}H NMR ($CDCl_3$) δ_H 1.45 (9H, s, Boc C<u>H</u>$_3$ (x3)), 2.06-2.51 (4H, m, Glu C<u>H</u>$_2$), 4.22 (1H, t, Fmoc aliphatic C<u>H</u>), 4.43 (2H, m, Fmoc C<u>H</u>$_2$), 4.65 (1H, m, Glu C$_\alpha$<u>H</u>), 5.64 (1H, d, N<u>H</u>), 7.34 (5H, m, trichlorophenyl C<u>H</u>, Fmoc aromatic C<u>H</u>), 7.53 (1H, s, trichlorophenyl C<u>H</u>), 7.60 (2H, d, Fmoc aromatic C<u>H</u>), 7.76 (2H, d, Fmoc aromatic C<u>H</u>) (Figure 73).

<u>*N*-9-Fluorenylmethoxycarbonyl-(*tert*-butyl ester)-L-glutamic acid pentafluorophenyl ester (Fmoc-L-Glu(O^{t}Bu)-OPfp)</u> Purified by short column chromatography with 50% chloroform-hexane as eluent and obtained as a colorless solid (108.8 mg, 61% yield), m.p. 116-117 °C (lit.[16] m.p. 119-120 °C), $[\alpha]_D^{26}$ -17.7 (*c*=1.06, $CHCl_3$) (lit.[37] $[\alpha]_D^{25}$ -18.4 (*c*=5.00, $CHCl_3$)). Anal. Calcd. for $C_{30}H_{26}O_6NF_5$: C, 60.92; H, 4.43; N, 2.37%. Found : C, 60.93; H, 4.58; N, 2.37%. IR ν_{max} (KBr)/cm^{-1} 3368, 3068, 2980, 1792, 1724, 1702, 1524, 1452, 1390, 1370, 1271, 1159, 1117, 1084, 1044, 996 (Figure 74). ^{1}H NMR ($CDCl_3$) δ_H 1.46 (9H, s, Boc C<u>H</u>$_3$ (x3)), 2.01-2.50 (4H, m, Glu C<u>H</u>$_2$), 4.23 (1H, t, Fmoc aliphatic C<u>H</u>), 4.43 (2H, m, Fmoc C<u>H</u>$_2$), 4.70 (1H, m, Glu C$_\alpha$<u>H</u>), 5.68 (1H, d, N<u>H</u>), 7.35 (4H, m, Fmoc aromatic C<u>H</u>), 7.58 (2H, d, Fmoc aromatic C<u>H</u>), 7.75 (2H, d, Fmoc aromatic C<u>H</u>) (Figure 75).

Aryl esters of N-9-fluorenylmethoxycarbonyl-N-ε-tert-butoxycarbonyl-L-lysine



Ar = 2,4,5-trichlorophenyl, pentafluorophenyl

N-9-Fluorenylmethoxycarbonyl-*N*-ε-*tert*-butoxycarbonyl-L-lysine 2,4,5-trichlorophenyl ester (Fmoc-L-Lys (Boc)-OTcp) Purified by short column chromatography with 20% ethyl acetate-hexane as eluent and obtained as a colorless solid (185.2 mg, 93% yield), m.p. 109-110 °C, $[\alpha]_D^{26}$ -17.7 (*c*=1.37, $CHCl_3$). Anal. Calcd. for $C_{33}H_{35}O_6N_2Cl_3$: C, 59.87; H, 5.33; N, 4.23%. Found : C, 59.56; H, 5.22; N, 4.29%. IR ν_{max} (KBr)/cm^{-1} 3350, 3066, 2973, 2937, 1775, 1692, 1527, 1455, 1391, 1367, 1348, 1274, 1252, 1170, 1121, 1082 (Figure 76). ^1H NMR ($CDCl_3$) δ_H 1.94 (9H, s, Boc C$\underline{H}_3$ (x3)), 1.76-2.19 (8H, m, Lys C$\underline{H}_2$), 3.14 (2H, br d, Lys C$\underline{H}_2$), 4.20 (1H, t, Fmoc aliphatic C$\underline{H}$), 4.42 (2H, m, Fmoc C$\underline{H}_2$), 4.59 (1H, m, Lys C$_\alpha\underline{H}$), 5.52 (1H, d, N$\underline{H}$), 7.33 (5H, m, trichlorophenyl C$\underline{H}$, Fmoc aromatic C$\underline{H}$), 7.52 (1H, s, trichlorophenyl C$\underline{H}$), 7.50 (2H, d, Fmoc aromatic C$\underline{H}$), 7.75 (2H, d, Fmoc aromatic C$\underline{H}$) (Figure 77).

N-9-Fluorenylmethoxycarbonyl-*N*-ε-*tert*-butoxycarbonyl-L-lysine pentafluorophenyl ester (Fmoc-L-Lys (Boc)-OPfp) Purified by short column chromatography with 5% ethyl acetate-dichloro-methane as eluent and obtained as a colorless solid (171.2 mg, 88% yield), m.p. 103-104 °C (lit.[16] m.p. 102-105 °C), $[\alpha]_D^{26}$ -15.0 (*c*=1.41, $CHCl_3$) (lit.[15] $[\alpha]_D^{26}$ -14.2 (*c*=1.00, $CHCl_3$)). Anal. Calcd. for $C_{33}H_{33}O_6N_2F_5$: C, 61.11; H, 5.13; N, 4.32%. Found : C, 61.08; H, 5.02; N, 4.37%. IR ν_{max} (KBr)/cm^{-1} 3355, 3095, 2978, 2940, 1792, 1695, 1523, 1451, 1369, 1274, 1253, 1173, 1106, 1000 (Figure 78). ^1H NMR ($CDCl_3$) δ_H 1.43 (9H, s, Boc C$\underline{H}_3$ (x3)), 1.75-2.14 (8H, br m, Lys C$\underline{H}_2$), 3.15 (2H, br d, Lys C$\underline{H}_2$) 4.23 (1H, t, Fmoc aliphatic C$\underline{H}$), 4.43 (2H, m, Fmoc C$\underline{H}_2$), 4.60 (1H, m, Lys C$_\alpha\underline{H}$), 5.56 (1H, d, N$\underline{H}$), 7.33 (4H, m, Fmoc aromatic C$\underline{H}$), 7.60 (2H, d, Fmoc aromatic C$\underline{H}$), 7.75 (2H, d, Fmoc aromatic C$\underline{H}$) (Figure 79).

2.5 Synthesis of Dipeptides by using pentafluorophenyl 4-nitrobenzenesulfonate (4c) and HOBt

General procedure

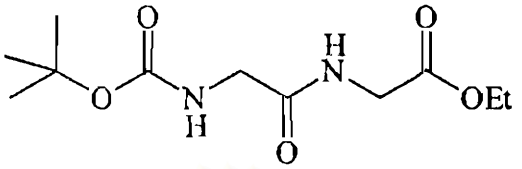
Procedure A

A solution of *N*-protected amino acid (0.3 mmol), pentafluorophenyl 4-nitro benzenesulfonate (**4c**) (0.3 mmol), HOBt.H_2O (0.03 mmol) in DMF (3 mL) was added 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 (0.3mmol) was added to the reaction mixture. The reaction mixture was allowed to react for 1 hour 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 to give analytically pure dipeptide.

Procedure B

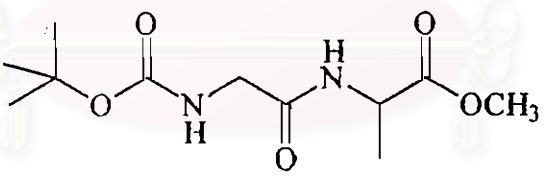
To a solution of *N*-protected amino acid (0.3 mmol), pentafluorophenyl 4-nitro benzenesulfonate (**4c**) (0.3 mmol), an amino acid methyl or ethyl ester hydrochloride (0.3 mmol) and HOBt.H_2O (0.03 mmol) in DMF (3 mL) was added triethylamine (0.6 mmol) when using *N*-Boc protected amino acid or DIEA (0.6 mmol) when using *N*-Fmoc protected amino acid with stirring at room temperature. After being stirred for 2 hour, the mixture was worked up by diluting with dichloromethane, washing with 5%HCl, 5% $NaHCO_3$, H_2O and brine and drying with $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography to give analytically pure dipeptide.

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



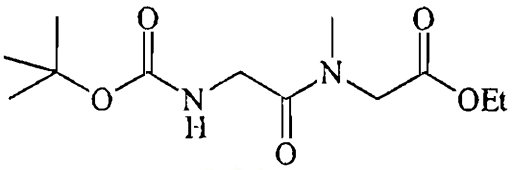
Purified by short column chromatography with 50% ethyl acetate-hexane as eluent and obtained as a colorless oil (70.0 mg, 90% yield). Anal. Calcd. for $C_{11}H_{20}O_5N_2$: C, 50.76; H, 7.74; N, 10.76%. Found : C, 50.83; H, 7.72; N, 10.69%. IR ν_{max} (neat)/cm^{-1} 3330, 2981, 2936, 1682, 1531, 1455, 1371, 1251, 1205, 1171, 1029, 947, 864 (Figure 80). ^1H NMR ($CDCl_3$) δ_H 1.26 (3H, t, ethyl C<u>H</u>$_3$), 1.42 (9H, s, Boc C<u>H</u>$_3$ (x3)), 3.81 (2H, d, Gly1 C<u>H</u>$_2$), 4.01 (2H, d, Gly2 C<u>H</u>$_2$), 5.30 (1H, br t, N<u>H</u>), 6.78 (1H, br t, N<u>H</u>) (Figure 81).

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



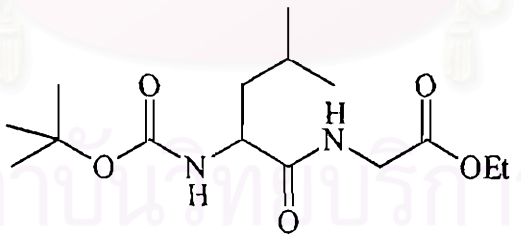
Purified by short column chromatography with 60% chloroform-hexane as eluent and obtained as a colorless oil (67.4 mg, 82% yield), $[\alpha]_D^{20}$ +10.7 (c=1.00, $CHCl_3$). Anal. Calcd. for $C_{11}H_{20}O_5N_2$: C, 50.76; H, 7.74; N, 10.76%. Found : C, 50.73; H, 7.76; N, 10.46%. IR ν_{max} (neat)/cm^{-1} 3320, 2980, 2938, 1744, 1674, 1528, 1456, 1369, 1280, 1249, 1217, 1168, 1055 (Figure 82). ^1H NMR ($CDCl_3$) δ_H 1.40 (3H, d, Ala C<u>H</u>$_3$), 1.44 (9H, s, Boc C<u>H</u>$_3$ (x3)), 3.72 (3H, s, OC<u>H</u>$_3$), 3.80 (2H, d, Gly C<u>H</u>$_2$), 4.57 (1H, m, Ala C$_\alpha$<u>H</u>), 5.24 (1H, br m, Ala N<u>H</u>), 6.75 (1H, br d, Gly N<u>H</u>) (Figure 83).

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



Purified by short column chromatography with 70% chloroform-hexane as eluent and obtained as a colorless oil (75.6 mg, 92% yield). Anal. Calcd. for $C_{12}H_{22}O_5N_2$: C, 52.54; H, 8.08; N, 10.21%. Found : C, 52.69; H, 8.14; N, 10.17%. IR ν_{max} (neat)/ cm^{-1} 3422, 2980, 2937, 1715, 1664, 1488, 1412, 1370, 1204, 1172, 1052 (Figure 84). ^1H NMR ($CDCl_3$) δ_H 1.22 (3H, 2xt, C<u>H</u>$_3$ rotamers), 1.40 (9H, s, Boc C<u>H</u>$_3$ (x3)), 2.95, 2.99 (3H, 2xs, Sar C<u>H</u>$_3$ rotamers), 3.84, 3.99 (2H, 2xd, Gly C<u>H</u>$_2$ rotamers), 3.95, 4.08 (2H, 2xs, Sar C<u>H</u>$_2$ rotamers), 4.14 (2H, q, ethyl C<u>H</u>$_2$), 5.42 (1H, br m, N<u>H</u>) (Figure 85).

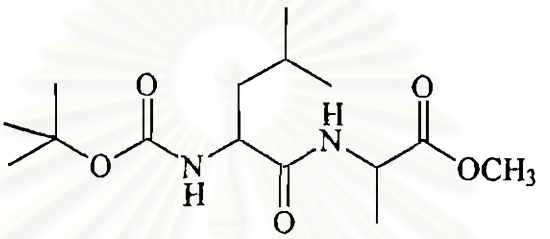
N-tert-Butoxycarbonyl-L-leucylglycine ethyl ester (Boc-L-Leu-Gly-OEt)



Purified by short column chromatography with 50% chloroform-hexane as eluent and obtained as a colorless 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, 56.94; H, 8.92; N, 8.85%. Found : C, 56.95; H, 8.82; N, 8.91%. IR ν_{max} (KBr)/cm^{-1} 3325, 2959, 2871, 1757, 1664, 1541, 1468, 1392, 1370, 1304, 1250, 1198, 1174, 1100, 1044, 1024 (Figure 86). ^1H NMR ($CDCl_3$) δ_H 0.92 (6H, dd, isopropyl C<u>H</u>$_3$ (x2)), 1.28 (3H, t, ethyl C<u>H</u>$_3$), 1.41 (9H, s, Boc C<u>H</u>$_3$ (x3)), 1.55-1.90 (3H, br m, Leu C<u>H</u>,C<u>H</u>$_2$), 4.00 (2H, d, Gly C<u>H</u>$_2$),

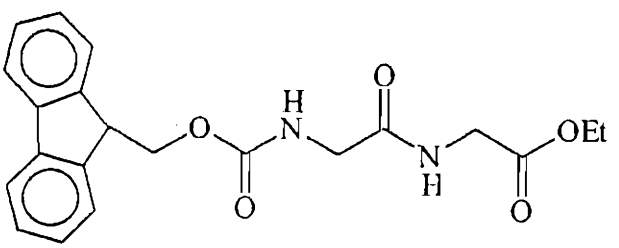
4.15 (1H, m, Leu $C_\alpha\underline{H}$), 4.19 (2H, q, ethyl $C\underline{H}_2$), 4.96 (1H, br d, Gly N<u>H</u>), 6.70 (1H, br m, Leu N<u>H</u>) (Figure 87).

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



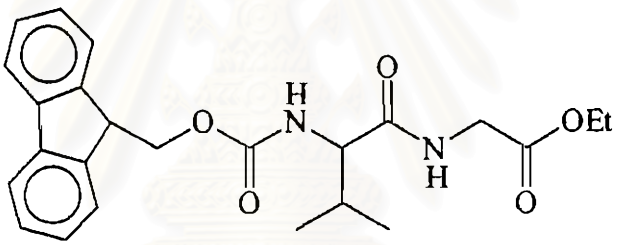
Purified by short column chromatography with 60% chloroform-hexane as eluent and obtained as a colorless solid (79.6 mg, 84% yield), m.p. 97-99 °C, $[\alpha]_D^{20}$ -31.7 (c=1.00, $CHCl_3$). Anal. Calcd. for $C_{15}H_{28}O_5N_2$: C, 56.94; H, 8.92; N, 8.85%. Found : C, 56.78; H, 8.71; N, 8.70%. IR ν_{max} (KBr)/cm^{-1} 3313, 2961, 1756, 1687, 1655, 1531, 1457, 1392, 1368, 1293, 1250, 1206, 1168, 1050, 1025 (Figure 88). ^{1}H NMR ($CDCl_3$) δ_H 0.95 (6H, dd, isopropyl $C\underline{H}_3$ (x2)), 1.39 (3H, d, Ala $C\underline{H}_3$), 1.44 (9H, s, Boc $C\underline{H}_3$ (x3)), 1.65 (3H, br m, Leu C<u>H</u>, $C\underline{H}_2$), 3.73 (3H, s, $OC\underline{H}_3$), 4.08 (1H, m, Leu $C_\alpha\underline{H}$), 4.55 (1H, m, Ala $C_\alpha\underline{H}$), 4.87 (1H, br d, Ala N<u>H</u>), 6.60 (1H, br d, Leu N<u>H</u>) (Figure 89).

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



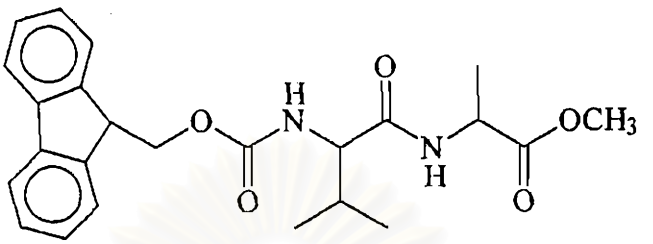
Purified by short column chromatogrphy with 70% chloroform-hexane as eluent and obtained as a colorless solid (110.0 mg, 96% yield), m.p. 115-116 °C. Anal. Calcd. for $C_{21}H_{22}O_5N_2$: C, 65.96; H, 5.80; N, 7.33%. Found : C, 65.95; H, 5.78; N, 7.28%. IR ν_{max} (KBr)/cm^{-1} 3426, 3068, 2982, 1718, 1674, 1536, 1451, 1403, 1252, 1215, 1050 (Figure 90). ^1H 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>) (Figure 91).

<u>*N*-9-Fluorenylmethoxycarbonyl-L-valylglycine ethyl ester (Fmoc-L-Val-Gly-OEt)</u>



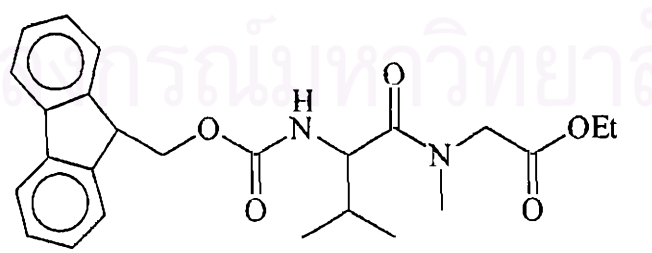
Purified by short column chromatography with 70% chloroform-hexane as eluent and obtained as a colorless solid (122.8 mg, 97% yield), m.p. 197-198 °C, $[\alpha]_D^{20}$ -18.0 (*c*=1.00, $CHCl_3$). Anal. Calcd. for $C_{24}H_{28}O_5N_2$: C, 67.91; H, 6.65; N, 6.60%. Found : C, 67.87; H, 6.53; N, 6.67%. IR ν_{max} (KBr)/cm^{-1} 3440, 3069, 2966, 1705, 1661, 1535, 1451, 1296, 1214, 1111, 1030 (Figure 92). ^1H 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, Gly N<u>H</u>), 6.47 (1H, br t, Val 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>) (Figure 93).

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



Purified by short column chromatography with 60% chloroform-hexane as eluent and obtained as a colorless 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, 67.91; H, 6.65; N, 6.60%. Found : C, 67.91; H, 6.60; N, 6.53%. IR ν_{max} (KBr)/cm^{-1} 3432, 3067, 2962, 1704, 1661, 1532, 1452, 1336, 1295, 1226, 1151 (Figure 94). ^1H 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, Ala N<u>H</u>), 6.47 (1H, d, Val 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>) (Figure 95).

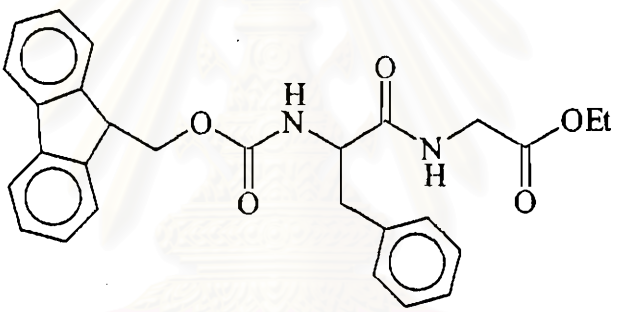
N-9-Fluorenylmethoxycarbonyl-L-valylsarcosine ethyl ester (Fmoc-L-Val-Sar-OEt)



Purified by short column chromatography with 30% ethyl acetate-hexane as eluent and obtained as a 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, 68.47; H, 6.90; N, 6.39%. Found : C, 68.45; H,

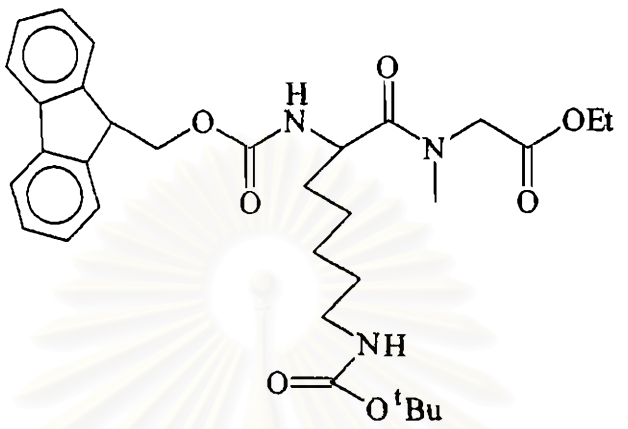
6.84; N, 6.31%. IR ν_{max} (neat)/cm^{-1} 3431, 3314, 3065, 2967. 1718, 1640, 1529, 1479, 1452, 1374, 1206, 1111, 1093 (Figure 96). ^{1}H NMR ($CDCl_3$) δ_H 1.00 (6H, dd, isopropyl C<u>H</u>$_3$ (x2)), 1.26 (3H, t, ethyl C<u>H</u>$_3$), 2.07 (1H, m, Val C<u>H</u>), 3.18 (3H, s, Sar C<u>H</u>$_3$), 3.71, 3.80 (1H, 2xs, Sar C<u>H</u>$_2$ rotamers), 4.05-4.64 (8H, m, ethyl C<u>H</u>$_2$, Sar C<u>H</u>$_2$ rotamers, Val C$_\alpha$<u>H</u>, Fmoc C<u>H</u>$_2$, Fmoc aliphatic C<u>H</u>), 5.57 (1H, d, N<u>H</u>), 7.35 (4H, m, Fmoc aromatic C<u>H</u>), 7.59 (2H, d, Fmoc aromatic C<u>H</u>), 7.74 (2H, d, Fmoc aromatic C<u>H</u>) (Figure 97).

<u>*N*-9-Fluorenylmethoxycarbonyl-L-phenylalanylglycine ethyl ester (Fmoc-L-Phe-Gly-OEt)</u>



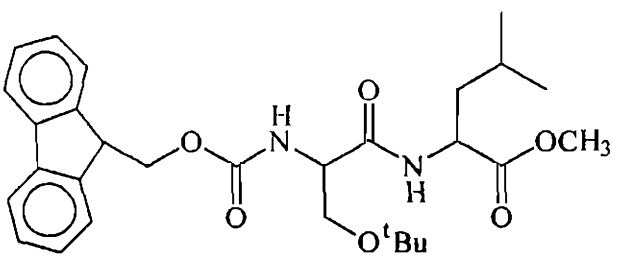
Purified by short column chromatography with chloroform as eluent and obtained as a colorless solid (129.2 mg, 94% yield), m.p. 185-187 °C, $[\alpha]_D^{20}$ -14.2 (*c*=1.02, $CHCl_3$). Anal. Calcd. for $C_{28}H_{28}O_5N_2$: C, 71.17; H, 5.97; N, 5.93%. Found : C, 71.12; H, 6.00; N, 5.80%. IR ν_{max} (KBr)/cm^{-1} 3468, 3304, 1738, 1695, 1654, 1541, 1262, 1210, 1038 (Figure 98). ^{1}H NMR ($CDCl_3$) δ_H1.26 (3H, t, ethyl C<u>H</u>$_3$), 3.10 (2H, br d, Phe C<u>H</u>$_2$), 4.20 (3H, m, ethyl C<u>H</u>$_2$, Fmoc aliphatic C<u>H</u>), 4.42 (3H, br m, Phe aliphatic C$_\alpha$<u>H</u>, Fmoc C<u>H</u>$_2$), 5.32 (1H, br m, Gly N<u>H</u>), 6.30 (1H, br m, Phe N<u>H</u>), 7.10-7.47 (9H, m, Phe aromatic C<u>H</u>, Fmoc aromatic C<u>H</u>), 7.52 (2H, m, Fmoc aromatic C<u>H</u>), 7.76 (2H, d, Fmoc aromatic C<u>H</u>) (Figure 99).

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



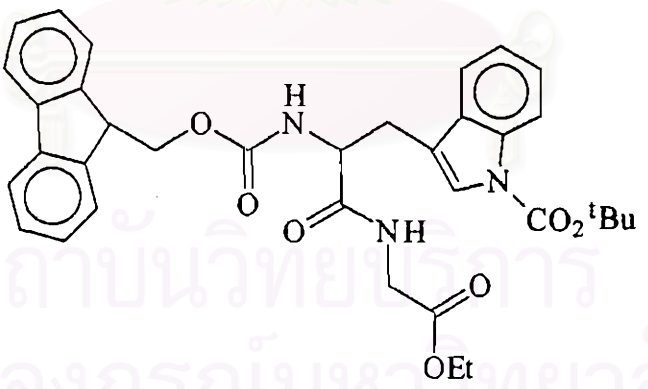
Purified by short column chromatography with 50% ethyl acetate-hexane as eluent and obtained as a clear oil (160.5 mg, 92% yield), $[\alpha]_D^{20}$ +0.5 (c=1.02, $CHCl_3$). Anal. Calcd. for $C_{31}H_{43}O_7N_3$: C, 65.36; H, 7.61; N, 7.38%. Found : C, 65.56; H, 7.50; N, 7.43%. IR ν_{max} (neat)/cm^{-1} 3431, 3066, 2977, 2938, 1711, 1649, 1517, 1453, 1409, 1252, 1173 (Figure 100). ^1H NMR ($CDCl_3$) δ_H 1.26 (3H, 2xt, ethyl C<u>H</u>$_3$ rotamers), 1.41 (9H, s, Boc C<u>H</u>$_3$ (x3)), 1.48-1.95 (8H, br m, Lys C<u>H</u>$_2$), 2.96, 3.14 (3H, 2xs, Sar C<u>H</u>$_3$ rotamers), 3.78, 3.86 (1H, 2xs, Sar C<u>H</u>$_2$ rotamers), 4.20 (4H, m, ethyl C<u>H</u>$_2$, Sar C<u>H</u>$_2$ rotamers, Fmoc aliphatic C<u>H</u>), 4.35 (2H, d, Fmoc C<u>H</u>$_2$), 4.73 (1H, m, Lys C$_\alpha$<u>H</u>), 5.74 (1H, d, Lys N<u>H</u>), 7.35 (4H, m, Fmoc aromatic C<u>H</u>), 7.59 (2H, d, Fmoc aromatic C<u>H</u>), 7.75 (2H, d, Fmoc aromatic C<u>H</u>) (Figure 101).

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



Purified by short column chromatography with 95% chloroform-hexane as eluent and obtained as a colorless 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, 68.21; H, 7.50; N, 5.49%. Found : C, 68.42; H, 7.40; N, 5.37%. IR ν_{max} (KBr)/cm^{-1} 3427, 3249, 3064, 3036, 2962, 2873, 1756, 1727, 1661, 1508, 1451, 1361, 1281, 1224, 1200, 1153, 1096, 1066 (Figure 102). ^1H NMR ($CDCl_3$) δ_H 0.93 (6H, d, isopropyl C<u>H</u>$_3$ (x2)), 1.22 (9H, s, Boc C<u>H</u>$_3$ (x3)), 1.44-1.80 (3H, br m, Leu C<u>H</u>, C<u>H</u>$_2$), 3.38 (1H, t, Ser C<u>H</u>$_2$), 3.71 (3H, s, OC<u>H</u>$_3$), 3.81 (1H, dd, Ser C<u>H</u>$_2$), 4.24 (2H, m, Leu C$_\alpha$<u>H</u>, Fmoc aliphatic C<u>H</u>), 4.40 (2H, d, Fmoc C<u>H</u>$_2$), 4.60 (1H, br m, Ser C$_\alpha$<u>H</u>), 5.76 (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>) (Figure 103).

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



Purified by short column chromatography with chloroform as eluent and obtained as a colorless 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, 68.73; H, 6.09; N, 6.87%. Found : C, 68.92; H, 6.33; N, 7.04. IR ν_{max} (KBr)/cm^{-1} 3329, 3066, 2980, 2936, 1733, 1672, 1531, 1478, 1454, 1374, 1336, 1309, 1257, 1160, 1088, 1027, 939 (Figure 104). ^1H NMR ($CDCl_3$) δ_H 1.23 (3H, t, ethyl C<u>H</u>$_3$), 1.64 (9H, s, Boc C<u>H</u>$_3$ (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<u>H</u>), 4.38 (2H, d, Fmoc $C\underline{H}_2$), 4.54 (1H, m, Trp $C_\alpha\underline{H}$), 5.04 (1H, br m, Gly N<u>H</u>), 6.26 (1H, br m, Trp N<u>H</u>), 7.17-7.65 (10H, m, Fmoc aromatic C<u>H</u>, Trp indole C<u>H</u>), 7.75 (2H, d, Fmoc aromatic C<u>H</u>), 8.11 (1H, d, Trp indole C<u>H</u>) (Figure 105).

2.6 The Study of Mechanism

2.6.1 Synthesis of the mixed carboxylic-sulfonic acid anhydride

Attempted synthesis of mixed carboxylic-sulfonic acid anhydride from the reaction of tosyl chloride with benzoic acid[31]

To a solution of benzoic acid (0.12 g, 1 mmol) and triethylamine (0.14 mL) in dichloromethane (5 mL) at 0 °C was added *p*-toluenesulfonyl chloride (0.19 g, 1 mmol) and the resulting solution was stirred for 10 min at room temperature. The reaction mixture was washed with 5% HCl, 5% $NaHCO_3$, H_2O and brine and dried over $MgSO_4$. The solvent was evaporated and the residue was chromatographed using 60% chloroform-hexane as eluent. The product was shown to be benzoic anhydride by IR which was confirmed by comparison with authentic benzoic anhydride obtained from a DCC-mediated coupling of benzoic acid (Figure 106).

2.6.2 Study of nucleophilic substitution of aryl 4-nitrobenzenesulfonates with various nucleophiles

In this section, nucleophiles chosen were piperidine, *p*-toluidine, and morpholine. The each reaction was divided into two parts. $HOBt.H_2O$ was employed as a catalyst in Part I, but in Part II no catalyst was added.

General procedure

To a solution of 2,4,5-trichlorophenyl 4-nitrobenzenesulfonate (**4b**) (0.3 mmol), nucleophile (0.3 mmol) and $HOBt.H_2O$ (0.03 mmol) (Part I only) in DMF (3 mL) was added triethylamine (0.3 mmol) with stirring at room temperature. After

the reaction was completed (indicated by TLC), the mixture was diluted with dichloromethane ,washed with 5% HCl, 5% $NaHCO_3$, H_2O and brine and then dried with $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography using suitable solvent system as eluent. The product was determined by ^1H NMR.

4-Nitrobenzenesulfonyl piperidine ^1H NMR ($CDCl_3$) δ_H 1.46 (2H, m, CH_2), 1.65 (4H, m, CH_2), 3.02 (4H, t, CH_2), 7.94 (2H, d, 4-nitrobenzenesulfonyl CH), 8.38 (2H, d, 4-nitrobenzenesulfonyl CH) (Figure 107).

4-Nitrobenzenesulfonyl morpholine ^1H NMR ($CDCl_3$) δ_H 3.05 (4H, t, CH_2), 3.78 (4H, t, CH_2), 7.95 (2H, d, 4-nitrobenzenesulfonyl CH), 8.40 (2H, d, 4-nitrobenzenesulfonyl CH) (Figure 108).

2.7 Study of Racemization

The reagent, pentafluorophenyl 4-nitrobenzenesulfonate (**4c**)+HOBt, have been evaluated in two different racemization tests and compared to other conventional peptide coupling reagents such as DCC, DCC+HOBt, HBTU using ^1H NMR to investigate the ratio of D-/L-isomer formed during the coupling reaction. In order to evaluate the effectiveness of our reagent, the model peptide couplings, Bz-L-Phe-L-Leu-OMe and Boc-L-Phe-L-Leu-OMe, were selected.

2.7.1 Synthesis of Bz-L-Phe-L-Leu-OMe and Boc-L-Phe-L-Leu-OMe using different peptide coupling reagents

Dicyclohexylurea (DCC) as activating reagent

To a stirred, ice-cooled solution of *N*-protected phenylalanine (0.25 mmol) and LeuOMe.HCl (0.25 mmol) in DMF (3 mL), triethylamine (0.25 mmol) was added,

followed by DCC (0.25 mmol). The stirring was continued for 1 hour at 0 °C and for overnight at room temperature. Dicyclohexylurea (DCU) was filtered off and the solvent was evaporated to give the crude product.

DCC+HOBt as activating reagent

The foregoing procedure was followed using *N*-protected phenylalanine (0.25 mmol), LeuOMe.HCl (0.25 mmol), triethylamine (0.25 mmol), $HOBt.H_2O$ (0.25 mmol) and DMF (3 mL).

HBTU as activating reagent

To a solution of *N*-protected phenylalanine (0.25 mmol), LeuOMe.HCl (0.25 mmol) and triethylamine (0.5 mmol) in DMF (3 mL) was added HBTU (0.25 mmol). The mixture was stirred at room temperature for 1 hour. The organic phase was worked up by diluting with dichloromethane, washing with 5% HCl, 5% $NaHCO_3$, H_2O and brine and drying with $MgSO_4$. The solvent was removed under reduced pressure to give the crude dipeptide.

*Pentafluorophenyl 4-nitrobenzenesulfonate (**4c**)+HOBt as activating reagent*

The procedure for preparation Bz-L-Phe-L-Leu-OMe and Boc-L-Phe-L-Leu-OMe was similar to the method that was used prepare other dipeptides in section 2.5. The effect of varying of the reaction conditions including changing the solvent (DMF,acetonitrile), the amount of $HOBt.H_2O$ (0.1, 0.5, 1.0, 2.0 eq) and base (DIEA, Et_3N) was examined.

<u>*N*-Benzoyl-DL-phenylalanyl-L-leucine methyl ester (Bz-DL-Phe-L-Leu-OMe)</u> ^1H NMR ($CDCl_3$) δ_H 0.84 (6H, d, isopropyl C<u>H</u>$_3$ (x2)), 1.29-1.67 (3H, m, Leu C<u>H</u>, C<u>H</u>$_2$), 3.17 (2H, d, Phe C<u>H</u>$_2$), 3.58 (3H, s, D-isomer OC<u>H</u>$_3$), 3.69 (3H, s, L-isomer OC<u>H</u>$_3$), 4.52 (1H, m, Leu C$_\alpha$<u>H</u>), 5.06 (1H, m, Phe C$_\alpha$<u>H</u>), 7.0-7.51 (8H, m, Phe aromatic C<u>H</u>, benzoyl C<u>H</u>), 7.72 (2H, d, benzoyl C<u>H</u>).

N-tert-Butoxycarbonyl-L-phenylalanyl-L-leucine methyl ester (Boc-L-Phe-L-Leu-OMe) ^{1}H NMR ($CDCl_3$) δ_H 0.85 (6H, dd, isopropyl C<u>H</u>$_3$ (x2)), 1.36 (9H, s, Boc C<u>H</u>$_3$(x3)), 1.52 (3H, m, Leu C<u>H</u>, C<u>H</u>$_2$), 3.67 (3H, s, OC<u>H</u>$_3$), 4.35 (1H, m, Leu C$_\alpha$<u>H</u>), 4.54 (1H, m, Phe C$_\alpha$<u>H</u>), 5.10 (1H, d, Leu N<u>H</u>), 6.45 (1H, d, Phe N<u>H</u>), 7.20 (10H, m, Phe aromatic C<u>H</u>, benzoyl C<u>H</u>).

2.7.2 Investigation of Racemization during coupling

The normal work-up procedure after each coupling reaction was to wash the solution product in the organic solvent (CH_2Cl_2) with 5% HCl, 5% $NaHCO_3$, H_2O and the solution was dried ($MgSO_4$) and evaporated. The residue was directly examined by ^{1}H NMR without further purification and the percentage of D-/L-isomer was calculated from the ratio of the two diastereomeric products, Bz-D/L-Phe-L-Leu-OMe and Boc-D/L-Phe-L-Leu-OMe, present. ^{1}H NMR analysis of diastereomeric mixtures was investigated by comparison with the coupling reactions of Bz-DL-Phe-OH and Boc-DL-Phe-OH and L-Leu-OMe which are positive control. In both cases, splitting of the signals due to the presence of diastereomeric dipeptides were clearly observed.