MATERIALS AND METHODS

Following general procedures were employed in all reactions unless otherwise stated. All solvents were freshly distilled before use. Amino acids were used as received from Sigma-Aldrich Company, USA. All the amino acids used, except glycine, are of L-configuration unless otherwise specified. IR spectra were recorded on Schimadzu model FT-IR spectrophotometer (KBr pellets, 3 cm⁻¹ resolution). High resolution mass spectra were recorded on a Micromass Q-TOF micro mass spectrometer and PE-SCIEX 150 EX LCMS using electron spray ionization mode, ¹H NMR and ¹³C NMR spectra were recorded on a Bruker AMX 300 MHz and 100 MHz spectrometer using TMS as an internal standard. The ultrasound bath (Elma, T 310/H) was German made and operated at 35 kHz. The RP-HPLC experiments were carried out in Agilent 1100 series instrument having G1311A VWD at λ = 254 nm, flow 0.5 mL/min, column: Agilent Eclipse XDB-C18, pore size-5µm, diameter x length = 4.6 x 150 mm; method: gradient 0.1% TFA, water-acetonitrile; 30-100% in 30 min. Melting points were determined in an open capillary and are uncorrected. TLC experiments were done using MERCK TLC aluminium sheets (silica gel 60 F₂₅₄) and chromatograms were visualized by exposing in iodine chamber, UV-lamp or spraying with KMnO₄ and heating. Column chromatography was performed on silica gel (100-200 mesh) using ethyl acetate and hexane mixtures as eluent.

The solvents used for the present work were purified as follows:

1) Acetic acid (glacial): Acetic anhydride (1 mL/lit.) was added to react with water present. Heated for 1 h at 110 °C in the presence of CrO₃ (20.0 g/lit.) and distilled at constant boiling point.

2) Acetonitrile: Treated with anhydrous CaCl₂ for 24 h and distilled over P₃O₅.

3) Chloroform: Distilled over CaSO₄ or from P₃O₅ and passed through a column of basic Al₂O₃ and stored in a brown bottle over molecular sieves (4 Å).
4) **Dichloromethane**: As described for chloroform.
5) **Diethylamine**: Distilled and stored in a brown bottle.
6) **Diethyl ether**: Kept over anhydrous CaCl₂ overnight, decanted, refluxed over sodium wire for 1 hr, distilled and stored over sodium wire.
7) **1,4-Dioxane**: Distilled and stored in a brown bottle.
8) **N,N-Dimethylformamide**: Shaken with KOH pellets for 1 h, decanted, distilled *in vacuo* (1-2 mm) and stored in a brown bottle (flushed with nitrogen) over molecular sieves.
9) **Ethyl acetate**: Treated with K₂CO₃ overnight, filtered and distilled over P₂O₅.
10) **Ethyl chloroformate**: Distilled and stored in a brown bottle.
11) **n-Hexane**: Distilled and stored over sodium wire.
12) **Methanol**: Refluxed over magnesium turnings (5.0 g/lit.) in presence of traces of iodine and distilled.
13) **N-Methylmorpholine**: Refluxed over sodium wire for 2 h, distilled and stored over sodium wire.
14) **Petroleum ether (60-80 °C)**: Treated with 10% by volume of conc. H₂SO₄ (2 or 3 times) followed by conc. KMnO₄ in 10% H₂SO₄ till permanganate color is retained, washed with water, dried and distilled.
15) **Pyridine**: Shaken with NaOH or KOH pellets for 2 h, decanted, and distilled over ninhydrin (1.0 g/lit.).
16) **Tetrahydrofuran**: Shaken with KOH pellets for 2 h, decanted, refluxed over sodium wire in presence of benzophenone till the solution became blue, distilled and stored over sodium wire.
17) **Toluene**: Washed with 10% by volume of conc. H₂SO₄ till the H₂SO₄ layer became pale yellow, washed, distilled and stored over sodium wire.
18) **Triethylamine**: As described for N-methylmorpholine.

**9-Fluorenylmethoxycarbonylamino acids**

**Method A: Employing Fmoc-Cl**

To an ice-cold solution (or suspension) of the amino acid (10 mmol) in 10% Na₂CO₃ solution (20 mL), Fmoc-Cl (2.63 g, 10 mmol) in 1,4-dioxane (25 mL) was added drop wise over
a period of 30 min. The mixture was stirred at 0 °C for 2 h and at room temperature for 5-6 h. It was diluted with water (100 mL) and extracted with ether (3 x 50 mL). The aqueous phase was acidified using cold 6N HCl to pH 2. The liberated Fmoc-amino acid was extracted with EtOAc (4 x 25 mL). The organic phase was washed with 1N HCl, water and saturated brine solution, dried over anhydrous Na₂SO₄ and evaporated under reduced pressure. The resulting gum was crystallized from a suitable solvent.

**Method B: Employing Fmoc-OSu**

A solution (or suspension) of the amino acid (10 mmol) in 10% Na₂CO₃ solution (20 mL) was allowed to cool at 0 °C and a solution of Fmoc-OSu (3.37 g, 10 mmol) in 1,4-dioxane (25 mL) was added slowly over a period of 30 min and stirring was continued for 2 h at the same temperature and further continued for another 2 h at room temperature. Then, it was worked up as described in the method A.

**Benzyloxy carbonyl chloride (Cbz-Cl)**

Phosgene was bubbled through well-stirred benzyl alcohol (200 g, 125 mL) at −10 °C till an increment of 200 g was achieved. The mixture was slowly allowed to attain room temperature and stirred at that temperature overnight. Excess phosgene and solvent was removed with the aid of water aspirator and the residual colorless liquid was stored over anhydrous Na₂SO₄ in a glass stoppered bottle.

**Benzyloxy carbonyl amino acids**

To a vigorously stirred solution of amino acid (10 mmol) in 4N NaOH (2.5 mL) and acetone (2.5 mL) at 0 °C, benzyloxy carbonyl chloride (1.8 mL, 10 mmol) was added in ten small portions during 2 h period maintaining the pH at 9-10 using 4N NaOH. Stirring was continued at 0 °C and at room temperature overnight. The reaction mixture was diluted with water (10 mL)
and extracted with ether (3 x 50 mL). The aqueous phase was acidified with 6N HCl and the compound separated was extracted with EtOAc (3 x 20 mL). The organic extract was washed with water and saturated brine solution, dried over anhydrous Na₂SO₄ and the solvent removed in vacuo.

**tert-Butyloxycarbonyl amino acids**

A mixture of the amino acid (10 mmol), Boc-ON (11 mmol), Et₃N (13 mmol), dioxane (7 mL) and water was stirred at room temperature till it becomes homogeneous. Water (7 mL) was added to the reaction mixture which was then extracted with ether (3 x 50 mL). The aqueous phase was acidified with 10% KHSO₄ or citric acid solution and the Boc-amino acid was extracted into EtOAc (4 x 20 mL). The combined organic layer was washed with water (3 x 20 mL), dried over anhydrous Na₂SO₄ and evaporated in vacuo to get the title compound.

**Methyl esters of amino acids**

Thionyl chloride (1.5 mmol) was added slowly with stirring into methanol (10 mL) cooled in an ice-bath. Amino acid (10 mmol) was then added in portions during half an hour and stirring was continued for 2 h below 0 °C. After an additional stirring for 2 h at room temperature and the reaction mixture was refluxed for an hour and cooled. The solvent was evaporated and the residue was triturated with dry ether. The separated solid was filtered, washed with ice-cold alcohol and dried to yield amino acid ester hydrochloride salt.

**Benzyl esters of amino acids**

A homogeneous mixture of amino acid (10 mmol), benzyl alcohol (3 mL) and p-TsOH (11 mmol) was placed in a 100 mL glass beaker and exposed for about 30 sec to microwave irradiation operating at its 40% power. After the completion of the reaction, the mixture was
cooled at rt and precipitated with dry ether (25 mL). The crystallized \( p \)-toluenesulfonate salt of amino acid benzyl ester was collected by filtration, and washed with dry ether.

**General procedure for the synthesis of \( N^\alpha \)-protected peptide esters**

A solution of \( N^\alpha \)-protected amino acid (17.8 mmol), EDC (17.8 mmol) and HOBr (18.7 mmol) in \( \text{CH}_2\text{Cl}_2 \) was cooled to 0 °C. To this solution was added DIPEA (35.2 mmol), then activated amino acid ester (17.8 mmol). The solution was allowed to warm to room temperature and stirred overnight. The solution was then diluted with 20 mL of \( \text{CH}_2\text{Cl}_2 \) and was washed with 5% \( \text{Na}_2\text{CO}_3 \) (2 × 10 mL), 10% citric acid (2 × 10 mL), water (2 × 10 mL), and brine (1 × 10 mL), and dried over anhydrous sodium sulfate. Solvent was removed under reduced pressure, and the products were purified by column chromatography. Melting points and optical rotations are compared with the literature data.

**References**


