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[A031]

Studies towards use of di-*tert*-butyl-dicarbonate both as a protecting and activating group in the synthesis of dipeptides

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Abstract

Amide formation from amino acids was achieved in an easy and convenient one-pot procedure in chloroform or tetrahydrofuran as solvent using di-tert-butyl dicarbonate both as a protecting and an activating agent in the absence of a catalyst or coupling reagent. A number of dipeptide has been thus synthesized in good yields. The structures of the dipeptides were confirmed by IR, NMR and mass spectral data.

Keywords:

Di-tert-butyl-dicarbonate, activation of carboxylic group, amino protecting-group, amide formation

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1. Introduction

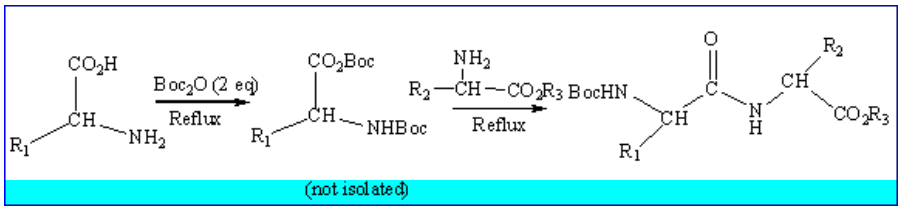
In the realm of amino acid / peptide research, di-*tert*-butyl dicarbonate (Boc_2O) [1] is a widely used reagent for the clean and rapid introduction of acid-labile Boc amino protecting group [2]. In some cases, Boc_2O has been used as an apparent dehydrating agent when it reacts with carboxylic acids [3], certain hydroxyl groups [4] or with primary nitroalkanes [5]. It is also an efficient *tert*-butoxycarbonylating agent for alcohols, thiols, amines and various carbon nucleophiles [6]. Furthermore several reports described the use of Boc_2O in the presence of the super acylation catalyst, DMAP [7-11] in reactions with amines and alcohols. Interestingly, Boc-anhydride has been found to be an effective reagent for the activation of carboxylic acid carbonyl groups towards nucleophilic addition, presumably via a mixed anhydride intermediate [12,13]. It was reported that treatment of an *N*-protected amino acid with Boc_2O might result in the activation of the carboxylic carbonyl, aminolysis

of which with a second amino acid will then culminate in peptide bond formation [14].

The above observations prompted us to investigate the possibility of using Boc_2O simultaneously both as a protecting and activating group in dipeptide synthesis by a one pot-procedure in the absence of a catalyst.

2. Results and Discussion

The use of Boc_2O in the dual role of protecting and activating reagent was investigated by employing 2 mol eq of Boc_2O whereby one mol eq was used for *N*-protection and the other mol eq was used for carboxyl activation (Scheme 1). The reaction mixture was then quenched with one mole eq of another suitably *C*-protected amino acid to yield the protected dipeptide in relatively good yields.



Scheme 1: General Scheme for the synthesis of the dipeptides

The first dipeptide investigagated was dipeptide Boc-glycine-L-phenylalanine ethyl ester (Fig 1). Glycine was treated with 2 mol eq of Boc_2O in $CHCl_3$ and to the reaction mixture, phenylalanine ethyl ester was added, the dipeptide Boc-glycine-L-phenylalanine ethyl ester (1) was obtained only in 34% yield [15]. When the reaction was carried out in the presence of K_2CO_3 as a base, better yields were obtained (e.g. 74%, entry 1, Table 1).

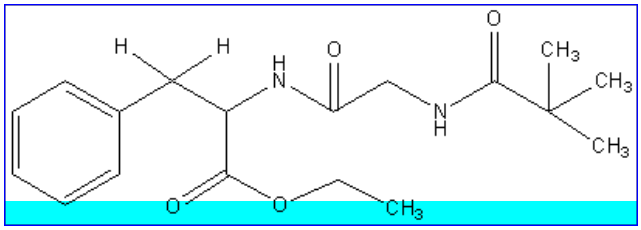


Fig 1: Boc-glycine-L-phenylalanine ethyl ester (1)

The IR spectrum showed three carbonyl peaks at 1740, 1718 and 1688 cm^{-1} corresponding to the ester, urethane and amide carbonyl stretching frequencies respectively.

The ^1H NMR spectrum (Fig 2) of (1) showed multiplets at d 7.1-7.3 corresponding to the aromatic protons. The two methylene protons attached to NH appeared at different chemical shift values (d 5.0 and 4.1 ppm). In fact one of the CH has merged with the quartet of the methylene protons of ethyl group at d 4.1 ppm. The methine proton appeared at 4.6 ppm while the benzilic protons appeared at d 3.1 ppm. The Boc protons appeared as a singlet at d 1.4 ppm and the triplet at d 1.2 ppm corresponded to the methyl of the ester moiety. The NH signals have most probably exchange with the solvents and do not appear in the spectrum.

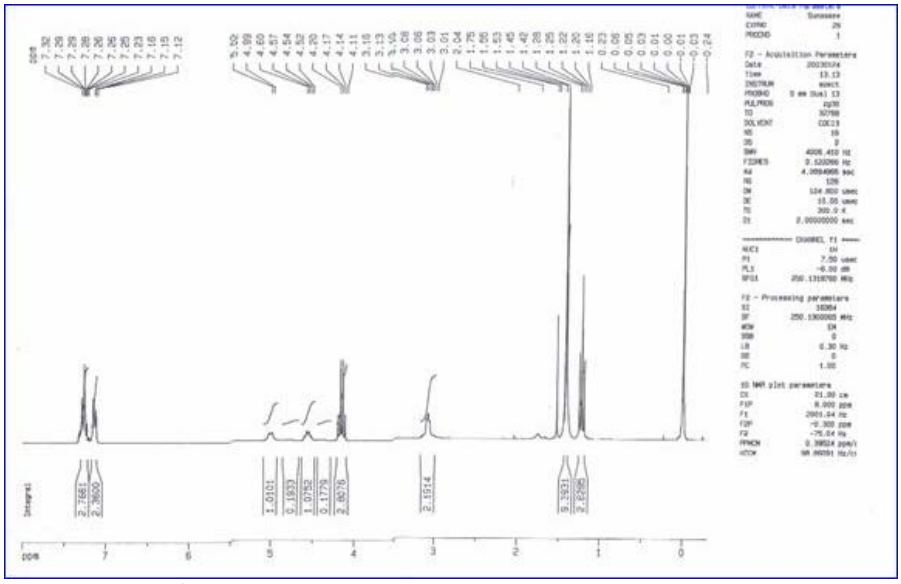


Fig 2: ^{1}H NMR of Boc-glycine-L-phenylalanine ethyl ester

The ^{13}C NMR (Fig 3) of the dipeptide (1) was in concordant with the structure proposed. The spectrum showed three signals at d171.8, 171.0 and 155.1 ppm corresponding to the three different carbonyls. The aromatic carbons appeared at d 146.7, 136.1-126.9 ppm while the other aliphatic protons were at 85.1, 79.7, 61.2, 54.5, 38.3, 29.6-27.3b and 14.1 ppm.

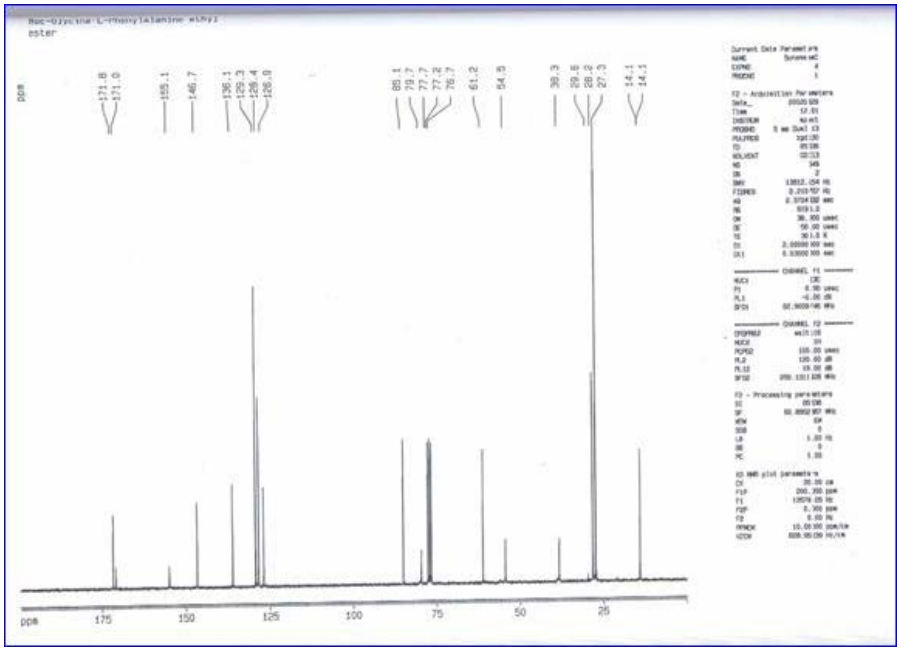


Fig 3: ^{13}C NMR of Boc-glycine-L-phenylalanine ethyl ester (1)

The structure was further confirmed by mass spectrum analysis. The mass spectum analysis by chemical ionization method did not show the molecular ion. However the signal at m/z =294 corresponded to the fragment due to the loss of butene molecule from the molecular ion confirming the formation of the dipeptide. Further the spectral data was in concordance with what has been reported earlier [15] with slight shifts.

Having achieved the initial objective of peptide bond formation using Boc_2O both as a protecting and activating group simultaneously, we proceeded to study the generality and efficiency of this method. Thus, using the same strategy, we have been able to synthesize successfully the dipeptides Boc-glycine-glycine ethyl ester (entry 2), Boc-L-alanine-L-phenylalanine

ethyl ester (entry 3) and Boc-L-alanine-L-alanine ethyl ester (entry 4) (Table 1**)** [16-18].

Our method was also extended to a secondary α-amino acid L-proline, but coupling of proline with tyrosine methyl ester under the described conditions afforded the protected dipeptide only in low yields. Therefore, the reaction conditions were modified by carrying the reaction out in THF and in the absence of K_2CO_3 since proline was completely soluble. The desired protected dipeptide Boc-L proline-L tyrosine methyl ester (entry 5) [19] was obtained as a yellow solid in 85% yield. The dipeptide Boc-L-pro-L-isoleucine (entry 6) has also been prepared successfully in 71% yield and this dipeptide has not been reported earlier. The IR spectrum showed the NH vibrational frequency at 3380 cm^{-1} whereas the carbonyl peaks appeared at 1736, 1719 and 1685 cm^{-1}. The ^1H NMR spectrum of (6) (Fig 4) showed a broad singlet corresponding to the NH proton. The methine protons attached to the amide groups appeared at d 5.1 ppm and d 4.3 ppm. The peak at d 4.2 ppm was related to the methelene protons of the ester group. The prolinyl protons and one of the methine protons of the isoleucine appeared as multiplets at d 3.5 and 2.0 ppm.

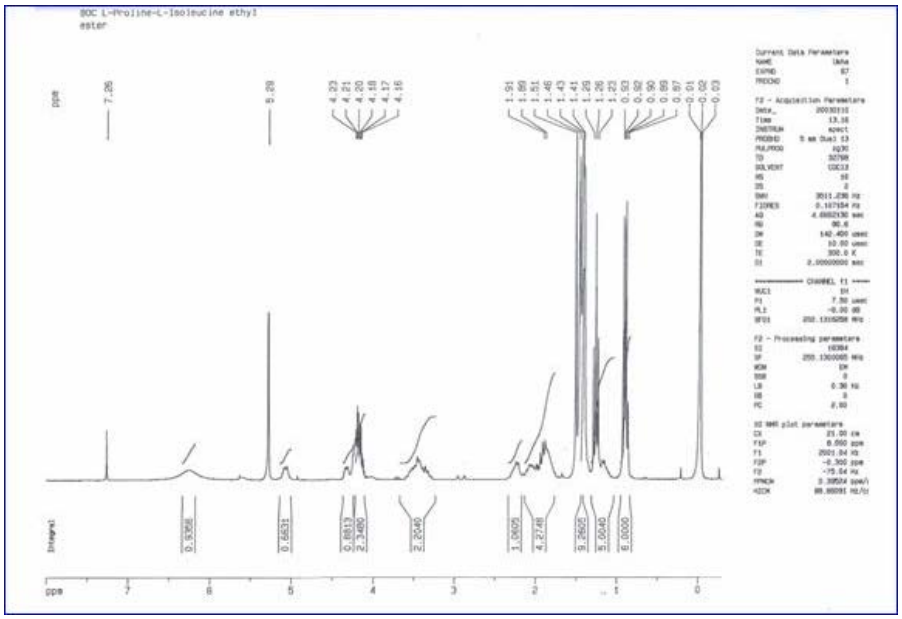


Fig 4: ^1H NMR of Boc-L-pro-L-isoleucine (6)

The ^{13}C NMR (Fig 6) spectrum was in concordant with the structure proposed and the chemical shift values (in ppm) have been assigned as illustrated in (Fig 5).

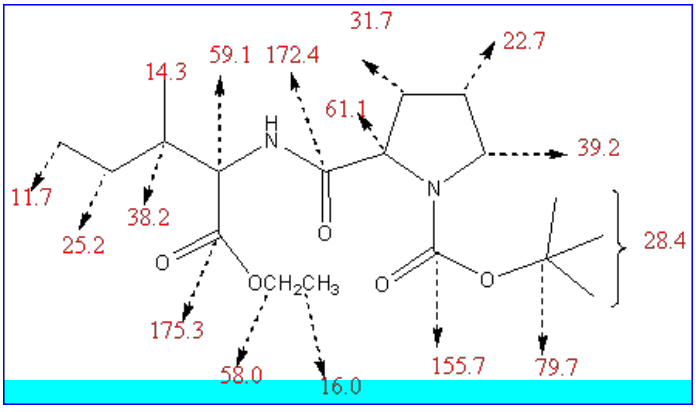


Fig 5: Dipeptide Boc-L-pro-L-isoleucine with ^{13}C NMR chemical shift values

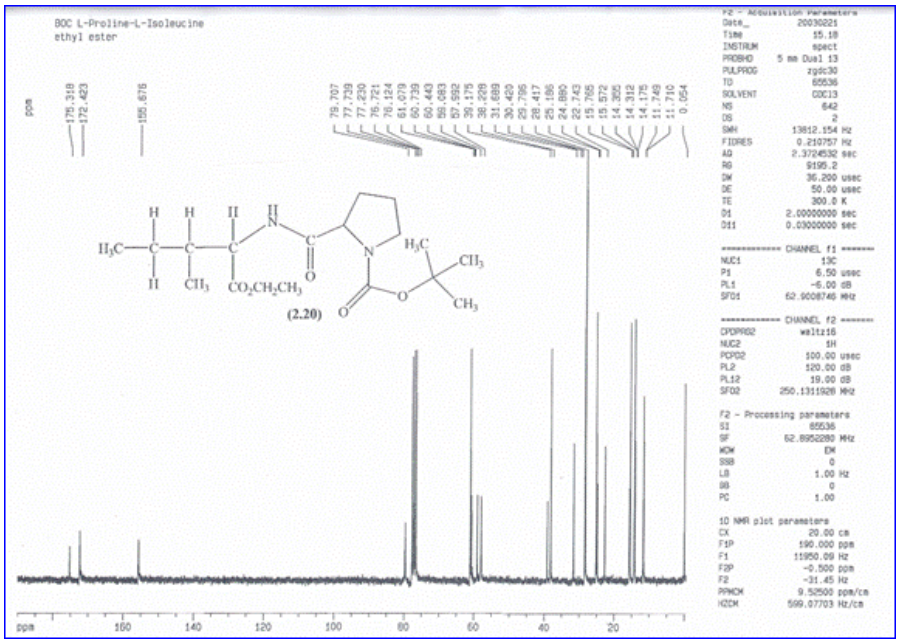


Fig 6: ^{13}C NMR of Boc-L-pro-L-isoleucine (6)

For aromatic amino acid the coupling reaction was found to be very sluggish in $CHCl_3$. However, when the reaction was carried out in THF (to higher solubility of the amino acid) and the time allowed for the reaction was increased the coupling reaction was achieved successfully. L-phenylalanine ester condensed with protected Boc-L-phenylamine and the target dipeptide (7), which has not been reported earlier, was formed in reasonable yield (entry 7). The IR spectrum of (7) showed peaks at 3385 corresponding to the NH stretching, 1738 (ester carbonyl), 1718 (amide) and 1686 cm^{-1} (amide carbonyl). The ^1H NMR spectrum of (7) (Fig 7) showed signals at d 7.4-7.2 corresponding to the aromatic protons. The methine protons appeared at d 5.5 and 4.6 ppm. The methylene protons appeared as a quartet at d 4.2 ppm and the benzilic correlated to a multiplet at d 3.2 ppm. The Boc protons (9 H) appeared as a singlet at d 1.5 ppm while the methyl protons of the ester group appeared at d 1.3 ppm.

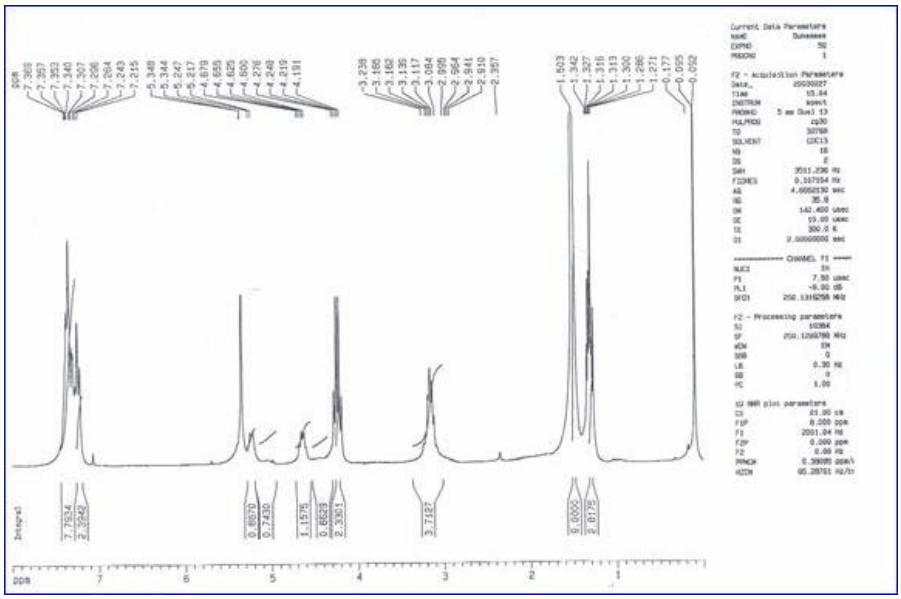


Fig 7: ^1H NMR Boc-Phe-Phe-OEt (7)

The C NMR (Fig 8) spectrum showed three carbonyl peaks at d 175 (C=O ester), 171.9 (C=O amide) and, 155.1 ppm (C=O urethane), 137.4- 126.8 (aromatic), 79.7 (*t*-butyl carbon), 61.3 and 55.9 (methine carbons), 54.6 ($OOCCH_2$), 41.6 and 38.4 (benzilic carbons), 28.3 (CH_3 of BOC) and 14.1 ppm (CH_3CH_2) which were correlated with the structure proposed.

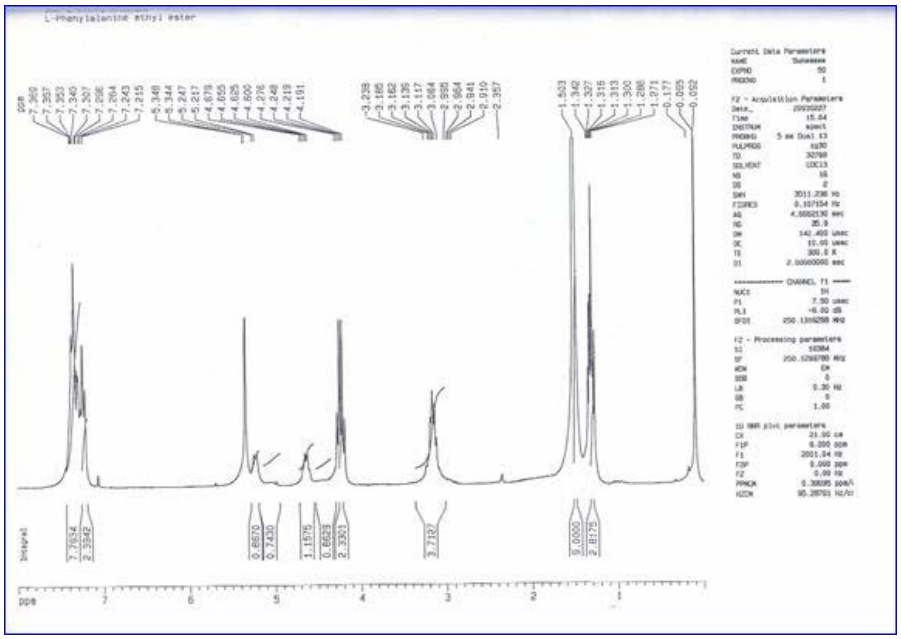


Fig 8: ^{13}C NMR Boc-Phe-Phe-OEt (7)

Table 1. Dipeptides prepared using Boc_2O

Entry	Amino acid	Amino acid ester	Dipeptide	Solvent used	Yield (%)
1	Gly	Phe-OEt	Boc-Gly-Phe-OEt	$CHCl_3$	74
2	Gly	Gly-OEt	Boc-Gly-Gly-OEt	$CHCl_3$	57
3	Ala	Phe-OEt	Boc-Ala-Phe-OEt	$CHCl_3$	78
4	Ala	Ala-OEt	Boc-Ala-Ala-OEt	$CHCl_3$	69
5	Pro	Tyr-OMe	Boc-Pro-Tyr-OMe	THF	85
6	Pro	Ile-OEt	Boc-Pro-Ile-OEt	THF	71
	Phe	Phe-OEt	Boc-Phe-Phe-OEt	THF	51

3. Conclusions

In conclusion, we have developed an easy and convenient method for the synthesis of dipeptides in good yields using the commercially available Boc_2O. Being a one-pot coupling procedure, the above method involves only one purification step. Boc_2O is used extensively for *N*-protection in amino acid chemistry and our results will add to its utility. Improvement of the reaction conditions, yields and application of this methodology to the synthesis of more complex peptides are currently in progress.

4. Materials and Methods

All chemicals were used without further purifications and they were from Aldrich chemical companies. Freshly distilled sample of thionyl chloride was used. Anhydrous sodium sulfate and magnesium sulfate were used as drying agents. Infrared spectra were recorded on a Mattson 1000 FTIR spectrometer. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker Spectrospin at 250 MHz. Chemical shift values were reported in ppm relative to TMS as internal standard.Mass spectra were recorded on MS-50 Kratos for Electron Impact spectra, VG7070E for chemical ionization. Carbon, hydrogen and nitrogen contents were obtained using a LECO 932 CHNS Mattson 1000 spectrophotometer. Melting points were determined using an Electrothermal melting point apparatus and were uncorrected. Chromatographic purification by 'flash column method' was carried out over neutral alumina. Thin Layer chromatography (TLC) was done on 0.25 mm precoated glass plate silica gel. The optical rotation was recorded on a model D polarimeter.

4.1 Synthesis of dipeptides

To a stirred solution of amino acid (2 mmol) in chloroform or THF (20 ml), a saturated solution of potassium carbonate (5 ml) was added. Boc_2O (2 mmol) was then added to the resulting solution. The reaction mixture was refluxed for 5 hours and monitored by TLC (hexane: ethyl acetate, 3:1). Amino acid ester (2 mmol) was added to the reaction flask and the resulting mixture was refluxed for a further 5 hours. Removal of solvent yielded a pale yellow paste to which a minimum amount of water (10 ml) was added and it was extracted with DCM (5 x 15 mL). The organic extracts were combined and dried over anhydrous magnesium sulphate. Solvent was removed to afford the crude product, which was purified by flash column chromatography. Elution with hexanes to remove unreacted Boc_2O followed by (hexanes - ethyl acetate, 3:1) gave the pure product.

4.1.1 Boc-Gly-Phe-OEt (1)

yellow paste; R_f = 0.74 (hexane - ethyl acetate, 3:1); IR (neat) ν: cm^{-1} 3366 (NH), 1740 (C=O of ester), 1718 (C=O of urethane), 1688 (C=O of amide); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.3-7.1 (5 H, m), 5.0 (1 H, d, J = 8.0 Hz), 4.5 (1 H, m, J = 7.0 Hz), 4.1 (3 H, q, J = 7.0 Hz), 3.1 (2 H, m), 1.4 (9 H, s), 1.2 (3 H, t, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$): δ (ppm) 171.8, 171.0, 155.1, 146.7, 136.1, 129.3, 128.4, 126.9, 85.1, 79.7, 61.2, 54.5, 38.3, 29.6, 28.2, 27.3, 14.1; $[\alpha]^{20}_D$ = -8.9 (c = 1, MeOH), MS (CI) m/z 294 $(M\text{-butene})^+$ (5 %)

4.1.2 Boc-Gly-Gly-OEt (2)

Colourless paste; R_f = 0.61(hexane - ethyl acetate, 3:1); IR (neat) ν: cm^{-1} 3373 (NH), 1751(C=O of ester), 1720(C=O of urethane), 1685 (C=O of amide); ^{1}H NMR ($CDCl_3$): δ (ppm) 6.6(1 H, br, s), 5.2 (1 H, br, s), 4.2 (2 H, q, J = 7 .0 Hz), 4.0 (2 H, d, J = 5.0 Hz), 3.8 (2 H, d, J = 5.0 Hz), 1.4 (9 H, s), 1.2 (3 H, t, J = 7 Hz); ^{13}C NMR ($CDCl_3$): δ (ppm) 169.9, 162.9, 162.6, 61.5, 41.5, 36.5, 31.4, 30.9, 29.6, 28.3 and 14.1

4.1.3 Boc-Ala-Phe-OEt (3)

Viscous yellow oil; R_f = 0.61 (hexane - ethyl acetate, 3:1); $[\alpha]^{20}_D$ = +30 (c = 0.9, $CHCl_3$), IR (neat) ν: cm^{-1} 3369 (NH), 1751 (C=O of ester), 1713(C=O of urethane), 1685 (C=O of amide); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.3-7.1 (m, 5H) 5.2 (1 H, d,

J = 8.0 Hz), 4.9 (1 H, brs), 4.5 (1 H, q, J = 7.0 Hz), 4.3 (1 H, brs), 4.1 (2 H, q, J = 7.0 Hz), 3.1 (2 H, m), 1.4 (12 H, br s), 1.2 (3 H, t, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$): δ (ppm) 175.0, 171.9, 155.1, 137.3, 136.2, 129.4, 128.5, 126.9, 126.8, 79.8, 61.3, 54.5, 53.4, 41.1, 38.4, 28.3, 14.1; MS (CI) m/z 262 $(MH)^+$ (5 %)

4.1.4 Boc-Ala-Ala-OEt (4)

Colourless viscous liquid; R_f = 0.54 (hexane - ethyl acetate, 5:1); Yield 69 %; $\alpha]^{20}_D$ = -51.4 (c = 1, EtOH), IR (neat) ν: cm^{-1} 3369 (NH), 1752 (C=O of ester), 1716 (C=O of urethane), 1686 (C=O of amide); ^{1}H NMR ($CDCl_3$): δ (ppm) 5.2 (2 H, br s), 4.4-4.1(4H, m), 1.6 (3 H, d, J = 7.2 Hz), 1.5 (9 H, s), 1.4 (3 H, d, J = 7.2 Hz), 1.3 (3 H, t, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$): δ (ppm) 173.4, 155.2, 146.8, 85.1, 79.7, 61.3, 49.3, 28.3(3 C), 27.4, 18.6, 14.2

4.1.5 Boc-Pro-Tyr-OMe (5)

Yellow solid; R_f = 0.35 (hexane - ethyl acetate, 3:1); $[\alpha]^{20}_D$ = -13.1 (c = 1, MeOH); IR (neat) ν: cm^{-1} 3391 (NH), 1736 (C=O of ester), 1716 (C=O of urethane), 1689 (C=O of amide); ^{1}H NMR ($CDCl_3$): δ (ppm) 8.0 (1 H, s), 7.0 (2 H, d, J = 9.0 Hz), 6.8 (2 H, d, J = 8.0 Hz), 5.4 (1 H, br s), 5.0 (1 H, d, J = 8.0 Hz), 4.5 (1 H, q, J = 8.0 Hz), 4.1 (2 H, m), 3.7 (3 H, s), 3.1 (2 H, m), 1.6 (4 H, m), 1.4 (9 H, s); ^{13}C NMR ($CDCl_3$): δ (ppm) 172.6, 171.4, 162.9, 155.4, 130.3, 127.3, 115.5, 80.2, 60.5, 54.7, 52.2, 37.6, 29.7, 28.3 (3 C), 21.0, 14.2; Anal. Calc. (Found) $C_{20}H_{28}N_2O_6$: C: 61.21 (61.25); H: 7.19 (7.23); N: 7.14 (7.28); MS (CI) m/z 393 $(MH)^+$ (5 %)

4.1.6 Boc-Pro-Ile-OEt (6)

Colourless paste; R_f = 0.43 (hexane - ethyl acetate, 3:1); $[\alpha]^{20}_D$ = + 9.0 (c = 0.5, $CHCl_3$); IR (neat) ν: cm^{-1} 3381 (NH), 1736 (C=O of ester), 1719 (C=O of urethane), 1685 (C=O of amide); ^{1}H NMR ($CDCl_3$): δ (ppm) 6.2 (1 H, br s), 5.1 (1 H, d, J = 7.0 Hz), 4.3 (1 H, d, J = 5.0 Hz), 4.2 (2 H, m), 3.5 (2 H, m), 2.3 (1 H, m), 2.2-1.8 (4 H, m), 1.4 (9 H, s), 1.3-1.2 (5 H, m), 0.9 (6 H, m); ^{13}C NMR ($CDCl_3$): δ (ppm) 175.3, 172.4, 155.7, 79.7, 61.1, 59.1, 58.0, 39.2, 38.2, 31.7, 28.4, 25.2, 22.7, 15.6, 14.3, 11.7

4.1.7 Boc-Phe-Phe-OEt (7)

Thick colourless paste; R_f = 0.51 (hexane - ethyl acetate, 2:1); $[\alpha]^{20}_D$ = -13.1 (c = 1, MeOH), IR (neat) ν: cm^{-1} 3385 (NH), 1738 (C=O of ester), 1718 (C=O of urethane), 1686 (C=O of amide); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.4-7.3 (8 H, m), 7.2 (2 H, m), 5.3 (1 H, d, J = 7.0 Hz), 5.1 (1 H, br s), 4.6 (1 H, q, J = 7.0 Hz), 4.3 (1 H, br s), 4.2 (2 H, q, J = 7.0 Hz), 3.2 (4 H, m), 1.5 (9 H, s), 1.3 (3 H, t, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$): δ (ppm) 175.0, 171.9, 155.1, 137.4, 136.2, 129.4, 129.3, 128.5, 128.4, 126.9, 126.8, 79.7, 55.9, 54.6, 53.4, 41.2, 38.4, 28.3, 14.1

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