Note

Simple Procedure for Preparing Tri-fluoroacetylprolyl Amino Acids, Acyl Components in the Racemization Test for Peptide Synthesis by GLC

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Among the methods for racemization testing in peptide synthesis, the GLC method is a simple one and the most practicable, especially when a packed column is used. In this method Tfa-Pro-X-OH (X = amino acids) is coupled with H-Val-Pro-OMe, the resulting Tfa-tetrapeptide-OMe is separated by GLC, and the degree of racemization is estimated from the ratio of the peaks separated to the diastereoisomers LDLL and LLLL.^{1,2)} The Tfa-Pro-X-OH used in this method has been prepared in several ways: for example, Tfa-Pro-Val-OH was prepared *via* Tfa-Pro-Val-O^tBu, which was obtained by direct condensation^{1,2)} of both the amino acid derivatives or by replacement of Z- with the Tfa group from Z-Pro-Val-O^tBu.²⁾ It has also been prepared from Z-Pro-Val-OMe by saponification and subsequent replacement of Z- with the Tfa group.³⁾ These methods were, however, fairly laborious in starting from amino acids. In this paper, we report a simpler procedure for preparing such compounds, Tfa-Pro-OH being coupled with H-X-OBzl to give Tfa-Pro-X-OBzl, which was then converted to the desired compound.

In this procedure, Tfa-Pro-OH was prepared according to the literature.⁴⁾ It was, however, not isolated and condensed directly in the same pot with amino acid benzyl ester *p*-tosylate by the DCC/HOBt method in DMF to give Tfa-Pro-X-OBzl, which was obtained from the neutral fraction as a crystalline substance in a reasonable yield. The product showed a single peak by GLC, in which the other diastereoisomer, DL or LD, could be detected.⁵⁾ It was therefore estimated to have been formed without racemi-

zation in this coupling reaction and to be optically pure. It is an advantage in this reaction that the base not need to be added again to liberate the benzyl ester from its *p*-tosylate, owing to the presence of the TMG already added for the synthesis of Tfa-Pro-OH. It was then hydrogenated catalytically to the desired Tfa-Pro-X-OH in a reasonable yield. The methyl ester of this product also showed a single peak by GLC, *i.e.*, this compound was also optically pure

Table I. YIELDS AND PROPERTIES OF THE SYNTHESIZED COMPOUNDS^a

Compound Tfa-Pro-X-OR X-OR =	Yield	mp (°C)	$[\alpha]_D^{20}$ $c = 1, \text{ MeOH}$	q_i^k
-Gly-OBzl	45.5 ^b	139–142	–76.5	—
-Ala-OBzl	65 ^b	115	–122	0.856
-Val-OBzl	72.5 ^b	54–56	–86	1.000
-Leu-OBzl	51 ^b	95.0	–82	1.12
-Ile-OBzl	81 ^{bc}	oil	—	1.22
-Phe-OBzl	48 ^b	123–124	–35.5	— ^l
-Gly-OH	80 ^d	oil ^e	—	—
-Ala-OH	52 ^d	177.0 ^f	–88.5	—
-Val-OH	78 ^d	120–121 ^g	–58	—
-Leu-OH	70 ^d	130–132 ^h	–65	—
-Ile-OH	—	oil ⁱ	—	—
-Phe-OH	55 ^d	146–147 ^j	+4.2	—

^a All the compounds except Tfa-Pro-Gly-OH, Tfa-Pro-Ile-OBzl and Tfa-Pro-Ile-OH gave a satisfactory elemental analysis result for C and H. NMR data of all these compounds agreed with their respective structures.

^b Yield from amino acids X.

^c Yield for the neutral fraction, which showed a single peak by GLC.

^d Yield from Tfa-Pro-X-OBzl.

^e Tfa-Pro-Gly-OH · DCHA, mp 189–191°C.

^f Yield of the crude product, mp 168–175°C 74%. Lit.,³⁾ mp 175–178°C $[\alpha]_D^{20} - 83$ ($c = 1, \text{ MeOH}$).

^g Lit.,¹⁾ mp 118–120°C, $[\alpha]_{546}^{20} 69.8$ ($c = 1, \text{ MeOH}$).

^h Lit.,³⁾ mp 131–133.5°C, $[\alpha]_D^{20} - 68$ ($c = 1, \text{ MeOH}$).

ⁱ Tfa-Pro-Ile-OH · DCHA^a, mp 169–170°C, 72% yield of the acidic fraction.

^j Lit.,³⁾ mp 144.0–146.0°C, $[\alpha]_D^{20} + 3$.

^k $q_i (= t_{Ri}/t_{Rst})$, $t_{Rst} = t_R$ of Tfa-Pro-Val-OBzl (6.31 min). Column, OV-17 (5%) 1 m; oven temp, 240°C, N₂ carrier gas, inlet 1.0 kg/cm²; FID. The fact that these compounds gave a respective single peak shows that no racemization occurred during the preparation, because a pair of diastereomers, for example, Tfa-Pro-Val-OR, LL and LD, would be separable under the GLC conditions.⁵⁾

^l $t_R = 5.94$ min; at 280°C oven temp.

Abbreviations: Bzl, benzyl; DCC, dicyclohexylcarbodiimide; DCHA, dicyclohexylamine; DMF, dimethyl formamide; HOBt, *N*-hydroxybenzotriazole; Tfa, trifluoroacetyl; TMG, tetramethylguanidine; Z, carbobenzyoxy. The L-optical isomer of the amino acid can be assumed, except for Gly.

(LL). We prepared several Tfa-Pro-X-OH easily and rapidly by this procedure, as shown in Table I.

Experimental

All melting points (mp) are uncorrected. Optical rotation was measured with an MP-1T automatic polarimeter (Applied Electric Lab.). NMR spectra were recorded with a Bruker 250 A instrument (250 MHz) using TMS as an internal standard.

Tfa-Pro-Val-OBzl

L-Proline (10 mm) was suspended in Tfa-OMe (4.7 ml, 5 eq) in a round-bottomed flask, and TMG (1.34 ml, 1 eq) was added dropwise at room temperature. The reaction mixture were added HOBt (1 eq), H-Val-OBzl. *p*-Tosylate mixture were added HOBt (1 eq), H-Val-OBzl, *p*-tosylate (1 eq) and DMF (13–15 ml), this solution being stirred in an ice bath. To this solution was then added a solution of DCC (1.05 eq) in DMF (15 ml), which had also been previously chilled in an ice bath. Stirring was continued for 1 hr with ice cooling and then overnight at room temperature. The precipitate formed was removed by filtration, ethyl acetate (40 ml) and water (100 ml) were added to the filtrate, and the organic layer was separated and successively washed with 2 *N* HCl aq, sat. NaHCO₃ aq and water. The neutral fraction was then dried over anhydrous MgSO₄ and evaporated to dryness to give an oil, which solidified after a while. Yield, 2.90 g (72.5%). NMR δ_H (CDCl₃): 0.86 (3H, d, $J=6.9$ Hz, CH₃), 0.91 (3H, d, $J=6.9$ Hz, CH₃), 1.8–2.4 (5H, m, 2CH₂, -P and C _{β} H, -V), 3.1–3.4 (2H, m, C _{δ} H₂, -P), 4.5–4.7 (2H, m, 2C _{α} H, -P and -V), 5.14 and 5.20 (ABd, 2H, $J=13.2$ Hz, CH₂Ph), 6.37 (d, 1H, $J=8$ Hz, CONH), 7.36 (5H, s, CH₂Ph). NMR δ_C (CDCl₃): 17.41 and 18.96 (2CH₃), 25.18 (C _{γ} , -P),

27.42 (C _{β} , -P), 31.20 (C _{β} , -V), 47.36 and 47.46 (C _{δ} , -P), 57.44 (C _{α} , -V), 61.31 (C _{α} , -P), 67.10 (CH₂, CH₂Ph), 128.40, 128.62, 128.68 and 135.31 (C_{arom}, CH₂Ph), 169.57 and 171.50 (2C=O, -P and -V).

Tfa-Pro-Val-OH

Tfa-Pro-Val-OBzl (5 mm) was dissolved in MeOH (10 ml), and hydrogen gas was introduced in the presence of Pd-C (5%, 0.7 g) for 14 hr. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate, and the acidic fraction was obtained in the usual way. After solidifying in hexane, it was recrystallized from AcOEt/hexane. Yield, 1.21 g (78%). NMR δ_H (CDCl₃): 0.94 (3H, d, $J=6.6$ Hz, CH₃), 0.97 (3H, d, $J=6.6$ Hz, CH₃), 1.85–2.35 (5H, m, 2CH₂, -P + C _{β} H, -V), 3.65–3.90 (2H, m, C _{δ} H₂, -P), 4.53 (1H, dd, $J=4.7$ and 8.7 Hz, C _{α} H, -V), 4.58–4.67 (1H, m, C _{α} H, -P), 6.98 (d, $J=8.7$ Hz, CONH), 8.32 (1H, bs, COOH, vanished after adding D₂O). NMR δ_C (CDCl₃): 17.41 and 18.97 (2CH₃), 25.15 (C _{γ} , -P), 27.67 (C _{β} , -P), 30.97 (C _{β} , -V), 47.57 and 47.63 (C _{δ} , -P), 57.42 (C _{α} , -V), 61.40 (C _{γ} , -P), 170.29 and 175.31 (2C=O, -P and -V).

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