Synthesis and Properties of N-2,2,3,3-Tetrafluoropropionyl-amino Acids

Ichiro TOMIDA and Hiroshi KAYAHARA

Department of Agriculture, Institute of Agricultural Chemistry, Shinshu-University

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Synthesis of N-2,2,3,3-tetrafluoropropionyl-amino acids (abbreviated as tetrafluoropropionyl- or Tfp-amino acids) by the reaction of amino acids with tetrafluoropropionyl anhydride was described. Racemization was observed to occur to a considerable extent during the process of the introduction of this group. Optically active Tfp-amino acids were able to be prepared free from racemization by transesterification of methyl tetrafluoropropionate with amino acid *tert*-butyl esters, followed by treatment of the resulting Tfp-amino acid *tert*-butyl esters with trifluoroacetic acid. Some Tfp-dipeptide esters were prepared from the corresponding dipeptide *tert*-butyl esters in this way. This group was cleavable by mild alkaline hydrolysis or by reduction with sodium borohydride as well as the Tfa-group.

We synthesized a series of N-Tfp-amino acids, because the Tfp-group was expected to be cleaved by very mild alkaline hydrolysis as easily as the Tfa-group.¹⁾ Tfp-amino acids were obtained by treating amino acids with 1~2 equivalent amounts of 2,2,3,3-tetrafluoropropionyl anhydride. When one molar equivalent of the anhydride was applied to each amino acid such as Ala, Val, Leu and Phe at 85~95°C for *ca.* 0.5 hr, about half of the starting amino acid was recovered. The use of tetrafluoropropionic or trifluoroacetic acid as a solvent was not effective, as in the case of trifluoroacetylation.²⁾ The yield of Tfp-DL-Ala-OH was elevated by applying two equivalent amounts of the reagent. With such amino acids as Val, Leu and Ileu, however, the desired compounds were obtained better under a more drastic condition (an equivalent amount of Tfp-anhydride/1 hr under reflux at *ca.* 145°C) and by subsequent fractional distillation of the reaction mixture. Though two molar equivalents of the reagent was applied under the same condition to Val, the yield of the Tfp-amino acid was not improved.

Tfp-Ala-OH which was prepared by acylat-

ing L-Ala-OH with Tfp-anhydride showed a relatively small specific rotation in comparison with that of Tfa-L-Ala-OH ($[\alpha]_D^{18}$: -60.7° ($c=1.98$, H_2O))³⁾ and also showed almost the same melting point as that of Tfp-DL-Ala-OH. We therefore checked the optical purity of this compound. After removal of the Tfp-group by treatment with conc. NH_4OH aq. (s. below), the resulting amino acid was converted to Tfa-Pro-Ala-OMe and then analyzed gaschromatographically, according to the Lit.³⁾ Both isomers (LL and LD) were satisfactorily separated from one other under the experimental condition, and from the ratio of the area of their peaks it was established that racemization occurred to an extent of 54% during the acylation. The racemization of L-Val with Tfp-anhydride was also detected gaschromatographically to an extent of 80%. Possibly in the drastic acylation, the Tfp-amino acids formed were activated under the formation of the mixed anhydride with Tfp-anhydride and racemization occurred easily over the conversion to the pseudooxazolone ring,⁴⁾ the formation being detected by its IR-spectrum.

Since acylation with Tfp-anhydride on amino acids gave partly racemized products, we prepared also the Tfp-amino acids from the corresponding amino acid *tert*-butyl esters

Abbreviation: DCCD, dicyclohexylcarbodiimide; DMF, dimethyl formamide; HOBt, 1-hydroxybenzotriazole; Tfa, trifluoroacetyl; Tfp, 2,2,3,3-tetrafluoropropionyl; Z, carbobenzyloxy.

by means of transesterification⁵⁾ of methyl tetrafluoropropionate with them, followed by cleaving the ester group with trifluoroacetic acid. By gaschromatography the Tfp-amino acids were proved to be prepared free from racemization in this way. Tfp-L-Pro-OH prepared in this way showed perfectly the same melting point and optical rotation as those of the corresponding compound prepared directly from L-Pro by treating with Tfp-anhydride. This agrees with the fact that Pro can not form any oxazolone structure under these conditions and it supports the above statement.

Some Tfp-dipeptide esters were also prepared from the corresponding dipeptide *tert*-butyl esters in the same way and used for the test of the cleavability of the Tfp-group.

The Tfp-group could be cleaved under mild alkaline conditions as easily as the Tfa-group¹⁾ (0.5 hr in 0.2 N NaOH or 1.5 hr in 0.1 N NaOH in 0.1% solution of Tfa- or Tfp-Gly-OH or by treatment with 28% or 14% aq. solution of ammonia at room temperature). However, in dilute NaOH (0.001 N) the cleavage of the Tfa-group is easier than that of the Tfp-group. The Tfp-group of Tfp-Val-OH was cleavable only under very drastic conditions (4 N NaOH for 0.5 hr).

The reductive cleavage of this group with sodium borohydride⁶⁾ was successful as well as the Tfa-group. By thin-layer chromatography we found that the Tfp-group was cleaved from Tfp-Pro-O_tBu already by treating at room temperature for 1 hr, and from Tfp-Val-O_tBu under reflux at 80°C for 1 hr in ethanol. This group was also removed from Tfp-Val-Val-O_tBu and Tfp-D-Val-Val-O_tBu to give the corresponding dipeptide-*tert*-butyl esters, respectively, but not perfectly from Tfp-Val-Pro-O_tBu under the above conditions. A more detailed study about the application of this method remains to be carried out.

The Tfp-group is spectroscopically characteristic. The presence of this group is easily detected by the absorption band at 800 and 845 cm⁻¹ in IR. The proton of this group gives a characteristic signals in NMR as a triple-triple type splitting at 6.2 ppm ($J=53$ Hz and apparent $J=6$ Hz)⁷⁾ (Fig. 1).

EXPERIMENTAL

All melting points were uncorrected. The optical rotation was determined with an Automatic Polarimeter, MP 1-T type, of Applied Electric Lab. Gaschromatography was performed with a Hitachi K-53 gaschromatograph equipped with a column (1 m×3 mm *i.d.*) of 5% OV-17 on Chromosorb M

FIG. 1. NMR Spectrum of Tfp-DL-Ala-OH (CDCl₃ + pyr. -d₆).

AW HMDS and a hydrogen flame ionization detector. IR-spectra were recorded on a Hitachi EPI G₂ infrared spectrometer and NMR-spectra at 60 MHz with a Hitachi R-24 spectrometer.

A. Synthesis of Tfp-amino acids

General procedures

A. Treatment of amino acids with Tfp-anhydride: Amino acid (10~50 mm) was added to Tfp-anhydride (1~2 eq) and the reaction mixture was heated under the noted conditions. Unreacted Tfp-anhydride and Tfp-OH were evacuated perfectly. Absolute ether was added to the residue and it was allowed to stand overnight. The precipitated starting amino acid was filtered off. Evaporation of the filtrate *in vacuo* gave an oily substance, Tfp-amino acid, which solidified after a while. It was purified by recrystallization and/or sublimation. A variation in the separation procedure was used in preparing some Tfp-amino acids. The reaction mixture was subjected directly to fractional distillation. After the fraction, bp 52~55°C/21 mmHg, (characterized as Tfp-OH in its IR-spectrum) was distilled off, the main fraction, bp *ca.* 100~130°C/0.5~1.0 mmHg, was taken up. This fraction, the desired compound, solidified after a while. It was purified as above.

B. Transesterification of Tfp-OMe with amino acid *tert*-butyl esters, followed by treatment with CF₃COOH: Amino acid *tert*-butyl ester (or its hydrochloride) (10 mm) was treated with Tfp-OMe (1.5~3 eq) in MeOH (*ca.* 5 ml) (in the presence of Et₃N (1.2 eq)) under the noted conditions. The reaction mixture was evaporated to dryness. The residue was dissolved in EtOAc and washed with 3% aq. citric acid, aq. NaHCO₃, aq. NaCl and H₂O, successively. Evacuation of the solvent gave an oily substance (Tfp-amino acid *tert*-butyl ester). It was treated with CF₃COOH (*ca.* 2 ml) for 1 hr at room temperature. The excess of the reagent was evacuated. The resulting residue was separated between EtOAc and aq. NaHCO₃. The water layer was acidified with conc. HCl and the separated oily substance was extracted with EtOAc. The organic layer was dried over anhydrous Na₂SO₄ and evaporated to dryness. The resulting residue, Tfp-amino acid, was solidified after a while. Purification was performed as above.

1. Tfp-Gly-OH (I)

a) According to procedure A: H-Gly-OH (3.38 g, 45 mm) was added to Tfp-anhydride (15.5 ml, 2 eq) and the reaction mixture was heated gradually to 110°C in an oil bath for 15 min with occasional shaking, until H-Gly-OH was dissolved. It was heated for further 15 min. The color of the reaction mixture changed to brown to some extent. Crude I, mp 65~82°C (recrystallized from benzene): yield, 6.46 g, 70%.

Recovered H-Gly-OH: yield, 0.64 g, 19%. A portion (3.38 g) was sublimated (100°C/3 mmHg). I, white crystalline, (2.70 g). mp 94.5~95.0°C. Found: C, 29.29; H, 2.48; F, 37.16. Calcd. for C₆H₅NO₃F₄: C, 29.57; H, 2.48; F, 37.42%.

b) From H-Gly-O_tBu (bp 43~44°C/7 mmHg)⁸⁾ according to procedure B. Conditions for transesterification: at 50~60°C for 0.5 hr. Crude I, mp 89°C (yield, 32%). After sublimation, mp 92~94°C.

2. Tfp-DL-Ala-OH (II)

a) From H-DL-Ala-OH according to procedure A. i) Reaction conditions: Tfp-anhydride (2 eq) at 95°C for 0.5 hr. Recovered H-DL-Ala-OH (9%). The ethereal residue, crude II (yield, 39%), which solidified after a while. After sublimation (95~100°C/2 mmHg) and subsequent recrystallization from *n*-hexane/benzene/EtOAc, II, mp 124~127°C. Recrystallization was very difficult, because this compound clings tightly to the wall of a flask. NMR, *s.* Fig. 1. Found: C, 33.19; H, 3.17; F, 35.16. Calcd. for C₆H₇NO₃F₄: C, 33.19; H, 3.25; F, 35.00%. ii) Reaction conditions: Tfp-anhydride (1 eq) otherwise under the same conditions as above. Recovered H-DL-Ala-OH (79%). II, mp 117.0~119.0°C (yield, 17%). iii) Reaction conditions: Tfp-anhydride (1 eq) at 145°C for 1 hr, followed by fractional distillation. The main fraction, II, bp 100~127°C/1.1 mmHg, (bath temp. <130°C), mp 122~124°C (after solidification), (yield, 46%).

b) From H-DL-Ala-O_tBu (bp 46~47°C/15 mmHg)⁹⁾ according to procedure B. Conditions for transesterification: at 50~60°C for 0.5 hr. II, mp 122~123°C (yield, 36%). After sublimation, mp 123~124.5°C.

3. Tfp-L-Ala-OH (III)

a) From H-L-Ala-OH according to procedure A. i) Reaction conditions: Tfp-anhydride (2 eq) at 95~145°C for 20 min. Recovered H-L-Ala-OH (15%). The ethereal residue, crude III (yield, 87%), which was then dissolved in sat. aq. NaCl and extracted with ether extensively. Evaporation of the ether gave a crystalline solide, III, mp 120~121.5°C (at 115°C, *sint.*). $[\alpha]_{20}^D$: $-18.3 \pm 0.2^\circ$ (*c*=2.9, H₂O). Found: C, 33.00; H, 3.34; N, 6.27. Calcd. for C₆H₇O₃NF₄: C, 33.19; H, 3.25; N, 6.45%.

Racemization test of the above obtained III: III (50 mg) was treated with conc. NH₄OH (0.5 ml) for 24 hr at room temperature. (No unreacted starting substance could be detected by tlc). The reaction mixture was evaporated to dryness and it was repeated after addition of EtOH to remove H₂O perfectly. The residue was then converted to Tfa-Pro-Ala-OMe according to the Lit.⁸⁾ and separated by gaschromatography (column temperature, 180°C). *t_R* of Tfa-L-Pro-L-Ala-OMe=11.2 min and that of Tfa-L-Pro-D-Ala-OMe=13.8 min. Separation degree, 100%.⁹⁾

The overlapping grade of both peaks, 0%. The ratio of the area, LD/LL=32/68 (blank test, LD/LL=5/95 for the starting H-L-Ala-OH, $[\alpha]_D^{21}$: +12.1° ($c=10.0$, 6 N HCl) (Lit.¹⁰) $[\alpha]_D^{20}$: +14.7° ($c=10.0$, 6 N HCl); LD/LL=53/47 for the used H-DL-Ala-OH and LD/LL=52/48 for the Tfp-DL-Ala-OH, II, which was prepared in 2 a). The racemization degree during the synthesis of this compound was estimated to be $(32-5) \times 2 = 54\%$.

ii) Reaction conditions: Tfp-anhydride (1 eq) at 145°C for 1 hr, followed by fractional distillation. The fraction, III, bp -126°C/0.5 mmHg, mp 121~123°C (at 111°C, sint.) (after solidification), (yield, 38%).

b) From H-L-Ala-O₂Bu·HCl according to procedure B. Conditions for transesterification: Tfp-OME (2 eq) in the presence of Et₃N (2 eq) at 60~70°C for ca. 3 hr. The obtained oil, crude III, was subjected to fractional distillation. An oil, III, bp 103~104°C/0.5 mmHg, mp 59~64°C (after solidification), (yield, 54%). $[\alpha]_D^{21}$: +44.4° ($c=1.0$, MeOH). IR $\frac{KBr}{max}$ cm⁻¹: 805, 840 (Tfp); 1550, 1760 (CONH); 1700, 2500~3500 (COOH). NMR (CDCl₃) δ : 1.50 (3H, d, $J=8$ Hz, CH₃); 4.63 (1H, quin. $J=8$ Hz, CH); 6.08 (1H, tt, $J=53$ and app. $J=6$ Hz, Tfp); 7.28 (1H, d, $J=8$ Hz, NH); 8.80 (1H, s, COOH). Found: C, 33.39; H, 3.27; F, 35.30. Calcd. for: C₈H₉O₃NF₄: C, 33.19; H, 3.25; F, 35.00%. No racemization during the reactions was detected by the test described above. (D/L=7/93; blank test, D/L=7/93).

4. Tfp-DL-Val-OH (IV)

a) From H-DL-Val-OH according to the procedure A. Reaction conditions: Tfp-anhydride (1 eq) at 145°C for 1 hr, followed by fractional distillation. The fraction, bp 127~136°C/0.8 mmHg, mp 90.0~91.0°C (after solidification), IV (yield, 32%). After sublimation and recrystallization from EtOAc/*n*-hexane, mp 91~93°C (at 89°C, sint.). IR $\frac{NaCl}{max}$ cm⁻¹: 795, 840 (Tfp); 1550 (CONH); 1690 (COOH); 1735 (CONH); 2400~3170 (COOH); 3350 (NH). NMR (CDCl₃ + pyr. -d₆) δ : 1.0 (6H, d, $J=6$ Hz, 2CH₃); 1.9~2.7 (1H, m, β -CH); 4.62 (1H, dd, $J=8$ and 5 Hz, α -CH); 6.23 (1H, tt, $J=53$ and app. $J=6$ Hz, Tfp); 8.10 (1H, d, $J=8$ Hz, NH); 15.0 (1.2 H, s, COOH). Found: C, 39.48; H, 4.54; F, 30.92. Calcd. for C₈H₁₁O₃NF₄: C, 39.19; H, 4.52; F, 31.00%.

Using Tfp-anhydride (2 eq) otherwise under the same conditions as above: The fraction, bp 60~75°C/20 mmHg, which was characterized as 2-tetrafluoroethyl-4-isopropyl-pseudooxazolone-(5) by its IR-spectrum,^{4a)} (yield, 46%) and the residue, from which an oil, crude IV (yield, 19%) was separated by fractionation.

5. Tfp-L-Val-OH (V)

a) From H-L-Val-OH according to procedure A.

Reaction conditions: Tfp-anhydride (1 eq) at 145°C for 1 hr, followed by fractional distillation. The fraction, bp 117~135°C/0.5 mmHg, mp 78~88°C (after solidification), V (yield, 34%). After sublimation, mp 81 (sint.) -89°C. IR, perfectly identical with that of IV in 4.a).

Racemization test for the above obtained IV: IV (0.12 g, 0.5 mm) was treated with aq. 4 N NaOH (0.5 ml) for 20 hr room temperature. The reaction mixture was neutralized by adding aq. 4 N AcOH (0.5 ml) and evaporated to dryness *in vacuo* and this was repeated after addition of H₂O and then EtOH to remove the AcOH and water. The residue was converted to Tfa-Pro-Val-OME according to the Lit.⁸⁾ and separated by gaschromatography (column temp., 140°C).

t_R of Tfa-L-Pro-L-Val-OME=13.2 min and that of Tfa-L-Pro-D-Val-OME=10.7 min. Both peaks were perfectly separated. The ratio of the area, LD/LL=51/49. (blank test: LD/LL=8.6/91.4 for the used H-L-Val-OH ($[\alpha]_D^{20}$: +25.7°, Lit.¹⁰) $[\alpha]_D^{20}$: +28.8° ($c=3.0$, 6 N HCl) and LD/LL=10.5/89.5 for the H-L-Val-OH, which was treated with 4 N NaOH for 20 hr at room temperature). The racemization degree during the synthesis of this compound was estimated to be 80%.

b) From H-L-Val-O₂Bu·HCl according to procedure B. Conditions for the transesterification: Tfp-OME (1.1 eq) in the presence of Et₃N (1.1 eq) under reflux (bath temp. 100~115°C) for 6 hr. An oily substance, bp 107~115°C/0.5 mmHg, V (yield, 45%). A portion of it solidified after a long time, mp 87~88°C (ca. at 62°C, sint.). $[\alpha]_D^{23}$: +9.1° ($c=3.3$, MeOH). IR $\frac{Film}{max}$ cm⁻¹: 800, 840 (Tfp); 1540 (CONH); 1680~1740 (broad) (COOH and CONH); 2980 (CH₃); 2500~3500 (broad) (COOH). NMR (CDCl₃ + pyr. -d₆) δ : 1.05 (6H, d, $J=6$ Hz, 2CH₃); 1.0~1.7 (1H, m, β -CH); 4.70 (1H, dd, $J=6$ and 9 Hz, α -CH); 6.44 (1H, tt, $J=53$ Hz and app. $J=6$ Hz, Tfp); 8.96 (1H, d, $J=9$ Hz, NH); 9.47 (s, COOH). Found: C, 38.92; H, 4.79; F, 31.15. Calcd. for C₈H₁₁O₃NF₄: C, 39.19; H, 4.52; F, 31.00%. No racemization during the synthesis could be detected by the test described above. (D/L=13/87; blank test, D/L=13/87).

6. Some other Tfp-amino acids (VI-XI)

The Tfp-amino acids listed in Table I were prepared according to procedure A. Reaction conditions: Tfp-anhydride (1 eq) at 145°C for 1 hr.

7. Tfp-L-Pro-OH (XII)

a) From H-L-Pro-OH according to procedure A. Reaction conditions: Tfp-anhydride (2 eq) at 90~100°C for 15 min. The etheral residue solidified after a while. A white crystalline solid, mp 111~117°C (after sublimation and the following recrystallization from benzene/*n*-hexane), XII (yield, 48%). mp 125~129.5°C (after further recrystallization from MeOH/H₂O)

TABLE I. SYNTHESIS OF TFP-AMINO ACIDS (VI~XI)

No.	Tfp-amino acid	Main fraction			Mp, after purification °C	Elemental analysis		
		Bp °C/mmHg	Mp °C	Yield %		Calcd./Found C	H	% F
1.	Tfp-DL-Leu-OH (VI)	135~139/0.7	80~83	43	90~91	41.70	5.05	29.32
2.	Tfp-L-Leu-OH (VII) ^{a)}	80~132/0.5	semisolid	33		41.97	5.34	29.59
3.	Tfp-DL-Ile-OH (VIII)	117~135/0.7	100~102	38	98 (sint.) 102~109	41.70	5.06	29.32
4.	Tfp-L-Ile-OH (IX) ^{a)}	80~137/1.0	81~93	29		41.67	5.20	29.47
5.	Tfp-DL-Norval-OH (X) ^{b)}			36	74~75	39.18	4.49	31.02
						39.33	4.39	31.10
6.	Tfp-DL-Norleu-OH (XI) ^{b)}			51	91~92	41.70	5.02	29.34
						41.81	4.96	29.57

^{a)} Since these compounds existed as a mixture of the L- and the D-form by racemization; they were not purified and analysed.

^{b)} Separated by sublimation.

IR Nujol cm^{-1} : VI; 800, 840 (w) (Tfp); 1560, 1735 (CONH); 1695, 2500~3200 (COOH); 3300 (NH). VII; identical with that of VI. VIII; 800, 830 (w) (Tfp); 1560~1570, 1735 (CONH); 1695, 2400~3200 (COOH); 3350 (NH). IX; identical with that of VIII.

NMR (CDCl_3 + pyr. - d_5) δ : VI; 0.6~1.05 (6H, 2CH_3); 1.5~2.0 (3H, CH_2 and CH); 4.5~5.1 (1H, m, α -CH); 6.35 (1H, tt, $J=53$ Hz and app. $J=6$ Hz, Tfp); 9.60 (1H, d, $J=8$ Hz, NH); 11.80 (ca. 1.5H, s, COOH); VIII; 0.7~1.2 (6H, m, 2CH_3); 1.2~1.8 (2H, m, CH_2); 1.8~2.5 (1H, m, β -CH); 4.6~5.1 (1H, m, α -CH); 6.52 (1H, tt, $J=53$ Hz and app. $J=6$ Hz, Tfp); 9.0~9.5 (1H, NH); 12.45 (s, COOH). X; 0.7~2.2 (ca. 7H, CH_3 - CH_2 - CH_2 -); 4.4~4.85 (1H, m, α -CH); 6.22 (1H, tt, $J=53$ Hz and app. $J=6$ Hz, Tfp); 8.26 (1H, d, $J=8$ Hz, NH); 14.0 (ca. 1.2H, s, COOH). XI; 0.4~2.2 (ca. 9H, CH_3 -(CH_2) $_3$ -); 4.45~4.95 (1H, m, α -CH); 6.30 (1H, tt, $J=53$ Hz and app. $J=6$ Hz, Tfp); 9.14 (1H, d, $J=8$ Hz, NH); 13.94 (ca. 1.5H, s, COOH).

(yield, 30%). Mix. mp 125~128.5°C with XII in b), mp 125~126.5°C. $[\alpha]_D^{17}$: +59.3° ($c=4.5$, MeOH). IR Nujol cm^{-1} : 820, 860 (w) (Tfp); 1665 (CONH); 1725 (COOH); 2400~3400 (COOH). NMR (CDCl_3 + pyr. - d_5) δ : 1.8~2.4 (4H, m, ring- CH_2 -); 3.5~4.0 (2H, m, ring- CH_2 -); 4.5~4.8 (1H, m, α -CH); 6.40 (1H, tt, $J=53$ and app. $J=6$ Hz, Tfp); 12.0 (s, COOH). Found: C, 39.57; H, 3.89; N, 5.95. Calcd. for $\text{C}_9\text{H}_9\text{NO}_3\text{F}_4$: C, 39.52; H, 3.73; N, 5.76%.

b) From H-L-Pro-O $_t$ Bu·HCl according to procedure B. Conditions for the transesterification: Tfp-OMe (3 eq) in the presence of Et $_3$ N (1 eq) at room temperature overnight. A neutral fraction, crude Tfp-L-Pro-O $_t$ Bu, XIII (yield, 55%). mp 58~60°C (after sublimation). mp 63.0~64.5°C (from MeOH/H $_2$ O). $[\alpha]_D^{24}$: +70.0° ($c=1.8$, MeOH). IR Nujol cm^{-1} : 795, 830 (Tfp); 1660 (CONH); 1740 (COO $_t$ Bu). Found: C, 48.45; H, 6.00; N, 4.88. Calcd. for $\text{C}_{12}\text{H}_{17}\text{O}_3\text{NF}_4$: C, 48.16; H, 5.73; N, 4.68%. De-*tert*-butoxylation of XIII (mp 58~60°C) gave an oily substance, crude XII (yield, 88% from XIII), mp 117.5~124°C (after solidification). mp 125.5~126.5°C (after sublimation and the following recrystallization from EtOH/*n*-hexane). $[\alpha]_D^{24}$: +61.6° ($c=3.25$, MeOH). IR: identical with that of XII in a).

8. Tfp-DL-Phe-OH (XIV)

From H-DL-Phe-OH according to procedure A. Reaction conditions: Tfp-anhydride (2 eq) at 90°C for 15 min. The recovered H-DL-Phe-OH (12%). Sublimation of the ethereal residue gave a crystalline substance, XIV, mp 106~111°C (yield, 45%). mp 109.0~111.0°C (after further sublimation). IR Nujol cm^{-1} : 710, 760 (C_6H_5); 800, 860 (Tfp); 1555 (CONH); 1695 (COOH), 1725 (CONH); 2400~3000 (COOH); 3300 (NH). NMR (CDCl_3 + pyr. - d_5) δ : 3.0~3.7 (2H, m, - CH_2 -); 4.7~5.2 (1H, m, α -CH); 6.3 (1H, tt, $J=53$ and app. $J=6$ Hz, Tfp); 7.15 (5H, s, C_6H_5); 9.25 (1H, d, $J=8$ Hz, NH); 11.6 (1H, s, COOH). Found: C, 48.68; H, 3.64; F, 25.95. Calcd. for $\text{C}_8\text{H}_7\text{O}_3\text{NF}_4$: C, 49.15; H, 3.78; F, 25.92%.

9. Tfp-L-Phe-OH (XV)

From H-L-Phe-O $_t$ Bu (bp 107°C/0.25 mmHg)¹¹⁾ according to procedure B. Conditions for the transesterification: Tfp-OMe (2 eq) under reflux for 3 hr. After the transesterification, an oil (crude Tfp-L-Phe-O $_t$ Bu) (yield, 96%). De-*tert*-butoxylation of this oil gave XV, mp 90°C (from EtOAc/*n*-hexane) (yield, 50%). mp 95~96°C (after sublimation). $[\alpha]_D^{21}$: -7.0° ($c=1.0$, MeOH). IR and NMR: identical with those

of XIV. Found: C, 48.72; H, 4.10; N, 4.88; F, 25.69. Calcd. for $C_{12}H_{11}O_3NF_4$: C, 49.15; H, 3.78; N, 4.78; F, 25.91%.

B. Synthesis of some Tfp-dipeptide-esters

1. Tfp-L-Val-L-Pro-O_tBu (XVI)

Z-L-Val-L-Pro-O_tBu (XVII), an oil (3.55 g, 8.8 mm), which was prepared from Z-L-Val-OH and H-L-Val-O_tBu with the DCCD/HOBt-method,¹²⁾ was hydrogenated in MeOH in the presence of Pd-black under successive addition of 6.7 N methanolic HCl (1.31 ml, 1 eq). After removal of the catalyst, the resulting solution was concentrated *in vacuo*. It gave an oily substance, crude H-L-Val-L-Pro-O_tBu·HCl (XVIII·HCl), (2.90 g, yield, 83%). tlc; *n*-BuOH/AcOH/H₂O (4:1:1) with ninhydrine, one spot, *R*_f=0.50. This oil was dissolved in MeOH (3 ml) and treated with Tfp-OMe (3 ml) in the presence of Et₃N (1.5 ml) overnight. After the same working-up as described in procedure B in section A, the neutral fraction was obtained as an oil, crude XVI (0.95 g, yield from XVII, 27%), which solidified after a while. glc (column temperature, 200°C), *t*_R=13.00 min, a single peak. mp 99~107°C (from *n*-hexane). mp 107.5~108.5°C (after sublimation). [α]_D²⁵: +97.5° (*c*=1.00, MeOH). Found: C, 51.51; H, 6.55; N, 7.19. Calcd. for $C_{17}H_{28}O_4N_2F_4$: C, 51.25; H, 6.57; N, 7.03%.

2. Tfp-L-Val-L-Val-O_tBu (XIX)

Hydrogenation of Z-L-Val-L-Val-O_tBu (XX) was performed in the same way as described above and gave an oily substance, crude H-L-Val-L-Val-O_tBu·HCl (XXI·HCl), (yield, 60%). Its transesterification with Tfp-OMe in the same way as above gave an oily substance, crude XIX. After standing for a long time, a crystalline substance was formed in the oily product. It was separated from the mother liquor and crystallized from MeOH/H₂O mp 122.0~123.0°C. [α]_D²⁵: +69.0° (*c*=0.94, MeOH). Found: C, 51.24; H, 7.34; N, 7.19. Calcd. for $C_{17}H_{28}O_4N_2F_4$: C, 50.99; H, 7.05; N, 7.00%.

3. Tfp-D-Val-L-Val-O_tBu (XXII)

Z-D-Val-L-Val-O_tBu (XXIII) was prepared from Z-D-Val-OH and H-L-Val-O_tBu by the DCCD/HOBt-method.¹²⁾ mp 90~93°C (yield, 90%). mp 98~99°C (from *n*-hexane). Found: C, 64.72; H, 8.44; N, 7.16. Calcd. for $C_{22}H_{34}O_5N_2$: C, 65.00; H, 8.43; N, 6.89%. Hydrogenation of XXIII in the usual manner gave an oily substance, crude H-D-Val-L-Val-O_tBu (XXIV), (yield, ca. 100%). Transesterification of crude XXIV with Tfp-OMe in the same manner as above gave XXII, mp 95~105°C (from MeOH/H₂O) (yield, 33% from XXIII). mp 110~111.5°C (several times from MeOH/H₂O). [α]_D²⁵: -12.8° (*c*=1.51, MeOH). Found: C, 51.10; H, 7.03; N, 7.01. Calcd. for $C_{17}H_{28}N_2O_4F_4$:

C, 50.99; H, 7.05; N, 7.00%.

C. Cleavability of the Tfp-group

1. From II with 1 N NaOH or with NH₄OH

Several mg of II were treated with one drop of 1 N NaOH and allowed to stand at room temperature. At a certain time interval, the reaction mixture was chromatographed on tlc (CMA=CHCl₃/MeOH/AcOH (760:40:24)¹³⁾). The plate was sprayed with aq 1 N NaOH, heated at 120°C, in order to cleave the Tfp-group from unreacted II perfectly on the plate, and then again with 0.6% ninhydrine solution (AcOH). (*R*_f: H-L-Ala-OH, 0.0 and unreacted II, 0.3). After 30 min the spot at *R*_f=0.3 was no longer detectable.

II (10 mg) was treated with a few drops of aq NH₄OH at room temperature. The reaction mixture was checked in the same way as described above. Spot II was no longer detectable after 60 min of the treatment with conc. NH₄OH (28%) and 240 min of the treatment with half-diluted aq NH₄OH (14%).

2. From Tfp-Val-O_tBu, XIII, XVI, XIX and XXII with NaBH₄⁶⁾

Tfp-Val-O_tBu or XIII (ca. 20 mg, respectively) was treated with a saturated solution (ca. 0.5 ml) of NaBH₄ (4 mm) in abs. EtOH (5 ml) at room temperature for 1 hr and then under reflux for 1 hr. At a certain time interval, the reaction mixture was chromatographed on tlc (silica gel) (CMA-system). The starting Tfp-ester derivatives were visualized with the morin reagent¹⁴⁾ or the acridine reagent (0.5% in MeOH) under the UV-lamp and the resulting amino acid *tert*-butyl esters with the ninhydrine reagent. (*R*_f: Tfp-Val-O_tBu, 0.89; and H-Val-O_tBu, 0.17~(tailing); XIII, 0.80 and H-Pro-O_tBu, 0.14). The spot (XIII) was no longer detectable after standing for 1 hr (more rapidly with the diluted aq NaOH) and that of Tfp-Val-O_tBu after reflux for 1 hr.

The same test was carried out for the compounds, XVI, XIX, and XXII. It was observed that the two latter substances disappeared perfectly, but the former one did not perfectly under the above conditions (*R*_f: XIX and XXII 0.86; XVI, 0.87; XXI, 0.1~0.42).

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