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N-Trifluoroacetyl-β-alanine in the Synthesis of Carnosine

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Abstract — Conditions have been developed for the synthesis of *N*-trifluoroacetyl-β-alanine, *N*-tifluoroacetyl-β-alanyl chloride, and *N*-trifluoroacetyl-β-alanine 4-nitrophenyl ester. These compounds reacted with histidine methyl ester or sodium salt to give *N*-trifluoroacetyl-β-alanyl-L-histidine methyl ester $CF_3CONHCH_2CH_2 \cdot CONHCH(CH_2C_3H_3N_2)COOCH_3$ and *N*-trifluoroacetyl-β-alanyl-L-histidine $CF_3CONHCH_2CH_2CONHCH \cdot (CH_2C_3H_3N_2)COOH$. Their hydrolysis with a solution of sodium hydroxide in aqueous ethanol, followed by acidification with trifluoroacetic acid, led to the formation of β-alanyl-L-histidine (L-carnosine).
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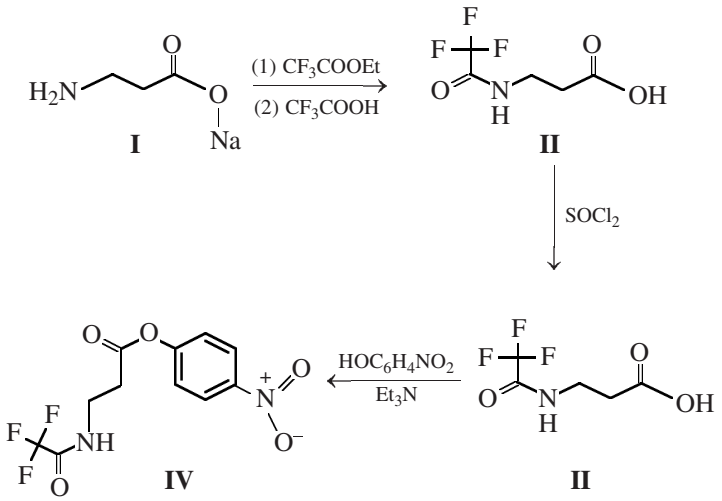
L-Carnosine (β-alanyl-L-histidine) exhibits a broad spectrum of biological activity and is used in the synthesis of medicines [1]. Numerous methods for the preparation of carnosine have been reported [2–11]; the amino group in β-alanine is mainly protected using phthalyl [2, 3], triphenylmethyl [4], or carbamate-like group [5, 6]. Although trifluoroacetyl group has long been used for the protection of amino groups, they have not found wide application in peptide syntheses, in particular due to racemization processes. On the other hand, the ease of introduction and removal of trifluoroacetyl protection makes such procedures quite promising in those cases when no racemization is possible. In the present work we made an attempt to synthesize carnosine on the basis of *N*-trifluoroacetyl-β-alanine and its derivatives.

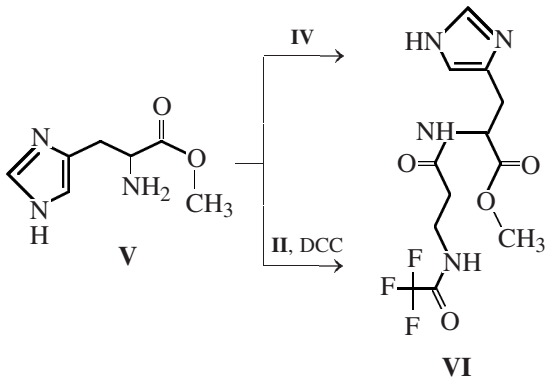
By reaction of β-alanine sodium salt (**I**) with ethyl trifluoroacetate (reactant molar ratio 1 : 1.3) in anhydrous ethanol, followed by acidification of the reaction mixture with trifluoroacetic acid, we isolated *N*-trifluoroacetyl-β-alanine (**II**) which was then converted into *N*-trifluoroacetyl-β-alanyl chloride (**III**) by treatment with boiling thionyl chloride. Compound **III** was brought into reaction with 4-nitrophenol in the presence of triethylamine in tetrahydrofuran to obtain *N*-trifluoroacetyl-β-alanine 4-nitrophenyl ester (**IV**) (Scheme 1).

Scheme 1.



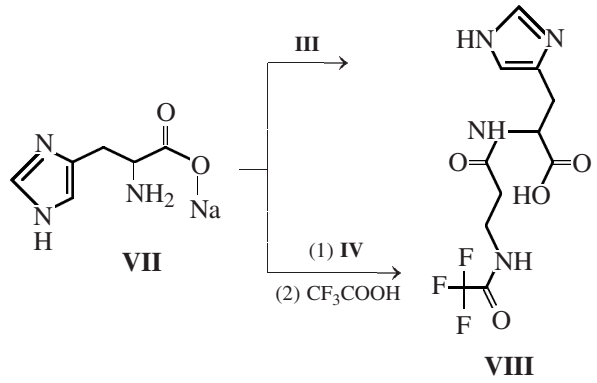
Compounds **II**–**IV** were used as starting materials in the synthesis of L-carnosine. Addition of acid chloride **III** to a solution of L-histidine methyl ester (**V**) and triethylamine in THF led to the formation of *N*-trifluoroacetyl-β-alanyl-L-histidine methyl ester (**VI**) in a yield of about 20%. In the reaction of compound **V** with nitrophenyl ester **IV**, the yield of product **VI** attained 65%. Finally, ester **V** reacted with *N*-trifluoroacetyl-βalanine (**II**) in the presence of dicyclohexylcarbodiimide (DCC) as condensing agent to give about 80% of **VI** (Scheme 2).

Scheme 2.

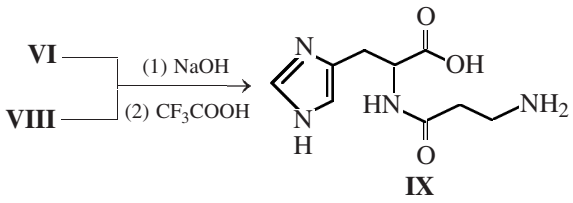


We also tried to use L-histidine sodium salt (**VII**) which was prepared by treatment of L-histidine with sodium ethoxide. Sodium salt **VII** was subjected to subsequent transformations without isolation as individual substance. Addition of acid chloride **III** to a solution of sodium salt **VII** in ethanol at 0°C afforded 55% of *N*-trifluoroacetyl-β-alanyl-L-histidine (VIII). When a solution of **VII** in ethanol was treated with *p*-nitrophenyl ester **IV**, followed by acidification of the reaction mixture with trifluoroacetic acid, we isolated about 65% of amino acid derivative **VIII** (Scheme 3).

Scheme 3.



Compounds **VI** and **VIII** were subjected to hydrolysis with sodium hydroxide in aqueous ethanol. The subsequent acidification of the reaction mixtures with trifluoroacetic acid gave β-alanyl-L-histidine (**IX**); the optical rotation of samples of **IX** thus obtained coincided with that reported for the best samples of L-carnosine.



The structure of the isolated products was confirmed by the IR and NMR spectra and elemental analyses.

EXPERIMENTAL

The IR spectra were recorded in KBr on a Protege-460 spectrometer with Fourier transform. The NMR spectra were measured on a Bruker Avance-400 instrument at 20°C. L-Histidine methyl ester was synthesized by the procedure reported in [3]. Ethanol was purified by distillation over calcium hydride. Tetrahydrofuran, diethyl ether, and hexane were distilled over metallic sodium. The other solvents and reagents were used without additional purification.

***N*-Trifluoroacetyl-β-alanine (II).** β-Alanine, 8.91 g, was added to a solution of sodium ethoxide prepared from 2.3 g of sodium and 150 ml of ethanol. The mixture was stirred for 1 h, 18.46 g of ethyl trifluoroacetate was added dropwise, and the mixture was stirred for 10 h, treated with 14.82 g of trifluoroacetic acid, stirred for 1 h, and evaporated under reduced pressure. The residue was extracted with diethyl ether (3×150 ml), the extracts were combined, washed with 150 ml of water, dried over sodium sulfate, and filtered, 50 ml of hexane was added, and the solvent was distilled off under reduced pressure to a volume of 70 ml. The precipitate was filtered off, washed with hexane, and dried under reduced pressure. Yield 14.81 g (80%), mp 114–116°C. IR spectrum, ν, cm^{-1}: 1716 (C=O, acid), 1692 (C=O, amide), 1561 (C–N, amide). ^1H NMR spectrum (D_2O), δ, ppm (*J*, Hz): 2.73 t (2H, *J* = 6.5), 3.57 t (2H, *J* = 6.3). Found, %: C 32.56; H 3.48; N 7.41. $C_5H_6F_3NO_3$. Calculated, %: C 32.44; H 3.27; N 7.57.

***N*-Trifluoroacetyl-β-alanyl chloride (III).** A mixture of 18.51 g of compound **II** and 29.75 g of thionyl chloride was heated for 7 h under reflux. Excess thionyl chloride was distilled off, and the residue was distilled under reduced pressure. Yield 17.71 g (87%), bp 101–102°C (6 mm), n_D^{17} = 1.4220. IR spectrum,

ν, cm^{-1}: 1794 (C=O), 1709 (C=O, amide), 1562 (C–N, amide). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 3.22 t (2H, *J* = 6.0), 3.63 q (2H, *J* = 6.1), 7.55 br.s (1H, NH). Found, %: C 29.39; H 2.61; N 6.73. $C_5H_5ClF_3NO_2$. Calculated, %: C 29.50; H 2.48; N 6.88.

***N*-Trifluoroacetyl β-alanine 4-nitrophenyl ester (IV).** Compound **III**, 16.28 g, was added dropwise to a mixture of 11.13 g of 4-nitrophenol and 8.58 g of triethylamine in 150 ml of THF. The mixture was stirred for 5 h, the solvent was removed under reduced pressure, and the residue was extracted with diethyl ether. The extract was filtered, diluted with 100 ml of heptane, and concentrated under reduced pressure to a volume of 70 ml. The precipitate was filtered off, washed with hexane, and dried under reduced pressure. Recrystallization from diethyl ether gave 17.15 g (70%) of compound **IV** with mp 84–86°C. IR spectrum, ν, cm^{-1}: 1757 (C=O, ester), 1704 (C=O, amide), 1562 (C–N, amide). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 2.96 t (2H, *J* = 6.1), 3.77 q (2H, *J* = 6.0), 7.31 d (2H, *J* = 9.1), 8.28 d (2H, *J* = 9.2). Found, %: C 43.27; H 3.11; N 9.02. $C_{11}H_9F_3N_2O_5$. Calculated, %: C 43.15; H 2.96; N 9.15.

***N*-Trifluoroacetyl-β-alanyl-L-histidine methyl ester (VI).** *a*. A solution of 8.46 g of L-histidine methyl ester and 5.25 g of triethylamine in 100 ml of THF was cooled to 0°C, 10.58 g of compound **III** was added, and the mixture was stirred for 1 h at 0°C and for 5 h at room temperature. The solvent was distilled off under reduced pressure, the residue was dissolved in water, and the solution was extracted with methylene chloride (3 × 150 ml). The extract was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The precipitate was filtered off, washed in succession with diethyl ether and hexane, and dried under reduced pressure. Reprecipitation from methylene chloride with hexane gave 3.36 g (20%) of compound **VI** with mp 130–132°C, $[\alpha]_D^{20}$ = 21.05° (*c* = 3.0, THF). IR spectrum, ν, cm^{-1}: 1757 (C=O, ester), 1704 (C=O, amide), 1642 (C=O, amide), 1565 (C–N, amide), 1536 (C–N, amide). ^1H NMR spectrum (CD_3COCD_3), δ, ppm (*J*, Hz): 2.54 m (2H), 3.04 m (2H), 3.57 t (2H, *J* = 6.0), 3.65 s (3H), 4.70 m (1H), 6.93 s (1H), 7.62 s (1H), 7.85 br.s (1H), 9.17 br.s (1H). ^{13}C NMR spectrum, δ_C, ppm: 29.03, 34.37, 36.00, 51.32, 52.69, 113.30 q (CF_3, $^1J_{CF}$ = 287.74 Hz), 115.30, 134.76, 135.00, 156.71, 170.09, 171.64. Found, %: C 43.02; H 4.41; N 16.52. $C_{12}H_{15}F_3N_4O_4$. Calculated, %: C 42.86; H 4.50; N 16.66.

b. Compound **IV**, 15.31 g, was added to a solution of 8.46 g of L-histidine methyl ester in 150 ml of THF, and the mixture was stirred for 12 h at room temperature, filtered, concentrated under reduced pressure to a volume of 50 ml, and diluted with 200 ml of diethyl ether. The precipitate was filtered off, washed with diethyl ether and hexane, and dried under reduced pressure. Reprecipitation from methylene chloride with hexane gave 8.4 g (50%) of compound **VI** with mp 129–132°C, $[\alpha]_D^{20}$ = 21.0° (*c* = 3.0, THF). Found, %: C 43.08; H 4.64; N 16.83.

c. A solution of 18.51 g of compound **II** in 100 ml of THF was cooled to 0°C, a solution of 20.6 g of *N,N'*-dicyclohexylcarbodiimide in 100 ml of THF and a solution of 16.9 g of compound **V** in 100 ml of THF were added, and the mixture was stirred for 2 h at 0°C and for 15 h at room temperature. The precipitate was filtered off, and the solvent was removed by distillation under reduced pressure. The residue was extracted with methylene chloride, the extract was filtered, concentrated to a volume of 60 ml, and diluted with 200 ml of diethyl ether, and the precipitate was filtered off, washed with diethyl ether and hexane, and dried under reduced pressure. Reprecipitation from methylene chloride gave 26.9 g (80%) of compound **VI** with mp 131–132°C, $[\alpha]_D^{20}$ = 21.15° (*c* = 3.0, THF). Found, %: C 42.98; H 4.54; N 16.57.

***N*-Trifluoroacetyl-β-alanyl-L-histidine (VIII).** *a*. L-Histidine, 7.76 g, was added to a solution of sodium ethoxide prepared from 1.15 g of metallic sodium and 150 ml of ethanol. The mixture was stirred for 1 h and cooled to 0°C, 10.18 g of compound **III** was added dropwise, and the mixture was stirred for 5 h, filtered, and evaporated under reduced pressure. The residue was washed with acetone and diethyl ether, and dried under reduced pressure. Reprecipitation from ethanol with diethyl ether gave 8.86 g (55%) of compound **VIII** with mp 190–192°C, $[\alpha]_D^{20}$ = 18.9° (*c* = 3.0, water). IR spectrum, ν, cm^{-1}: 1717 (C=O, acid), 1671 (C=O, amide), 1630 (C=O, amide), 1558 (C–N, amide), 1538 (C–N, amide). ^1H NMR spectrum (D_2O), δ, ppm (*J*, Hz): 2.42 t (2H, *J* = 4), 2.94 m (1H), 3.11 m (1H), 3.40 t (2H, *J* = 8.0), 4.36 q (1H, *J* = 4), 7.12 s (1H), 8.44 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 27.13, 34.19, 35.93, 53.91, 115.69 q (CF3, $^1J_{CF}$ = 286.03 Hz), 116.50, 129.67, 133.11, 158.48, 172.54, 176.31. Found, %: C 41.24; H 4.19; N 17.21. $C_{11}H_{13}F_3N_4O_4$. Calculated, %: C 41.00; H 4.07; N 17.39.

b. L-Histidine, 7.76 g, was added to a solution of sodium ethoxide prepared from 1.15 g of metallic sodium and 150 ml of ethanol. The mixture was stirred for 1 h, 15.31 g of compound **IV** was added, and the mixture was stirred for 2 days, filtered, and treated with 5.7 g of trifluoroacetic acid. The resulting solu-

tion was stirred for 2 h, filtered, concentrated under reduced pressure to a volume of 30 ml, and diluted with 150 ml of acetone. The precipitate was filtered off, washed with acetone and diethyl ether, and dried under reduced pressure. Reprecipitation from ethanol with diethyl ether gave 10.47 g (65%) of compound **VIII** with mp 188–191°C, $[\alpha]_D^{20}$ = 18.1° (*c* = 3.0, water). Found, %: C 41.21; H 4.25; N 17.48.

β-Alanyl-L-histidine (IX). A solution of 4.2 g of sodium hydroxide in 25 ml of water was added to a solution of 16.81 g of compound **VI** in 75 ml of ethanol. The mixture was stirred for 24 h, filtered, acidified by adding 6.27 g of trifluoroacetic acid, stirred for 2 h, and evaporated under reduced pressure. The residue was washed with acetone and ethanol, dried under reduced pressure, and dissolved in a minimal amount of water, the solution was filtered and diluted with 150 ml of acetone, and the precipitate was filtered off and washed with acetone. Reprecipitation from water with ethanol gave 8.48 g (75%) of compound **IX** with mp 259–262°C (decomp.), $[\alpha]_D^{20}$ = 21.9° (*c* = 3.0, water); published data: mp 259–263°C, $[\alpha]_D^{20}$ = 22° [6]; 250°C (decomp.), $[\alpha]_D^{20}$ = 20.9° [3]. IR spectrum, ν, cm^{-1}: 1660 (C=O, acid), 1648 (C=O, amide), 1584 (C–N, amide). ^1H NMR spectrum (D_2O), δ, ppm (*J*, Hz): 2.62 t (2H, *J* = 6), 3.02 m (2H), 3.18 t (2H, *J* = 6.0), 4.43 m (1H), 6.92 s (1H), 7.68 s (1H). ^{13}C NMR spectrum, δ_C, ppm: 28.86, 32.16, 35.66, 55.09, 117.31, 133.16, 135.63, 171.48, 177.84. Found, %: C 47.84; H 6.33; N 24.68. $C_9H_{14}N_4O_3$. Calculated, %: C 47.78; H 6.24; N 24.76.

Following an analogous procedure, from 16.11 g of *N*-trifluoroacetyl-β-alanyl-L-histidine, 2.2 g of sodium hydroxide, and 0.57 g of trifluoroacetic acid we obtained 9.27 g (82%) of compound **IX** with mp 256–260°C (decomp.), $[\alpha]_D^{20}$ = 21.2° (*c* = 3.0, water).

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