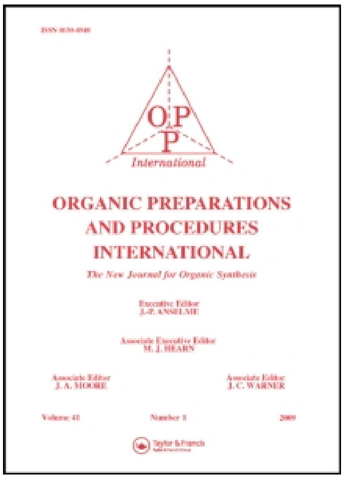

Organic Preparations and Procedures International
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t902189982

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To cite this Article Pardanani, N. H. and Muller, N.(1978) 'SYNTHESIS OF THE TRIPEPTIDE AMIDE CARBOBENZOXY-L-PROLYL-TRIFLUORO-DL-LEUCYL GLYCINAMIDE', Organic Preparations and Procedures International, 10: 6, 279 — 283

To link to this Article: DOI: 10.1080/00304947809355050

URL: http://dx.doi.org/10.1080/00304947809355050

ORGANIC PREPARATIONS AND PROCEDURES, INT. 10(6), 279-283 (1978)

SYNTHESIS OF THE TRIPEPTIDE AMIDE CARBOBENZOXY-L-PROLYL-TRIFLUORO-DL-LEUCYL GLYCINAMIDE

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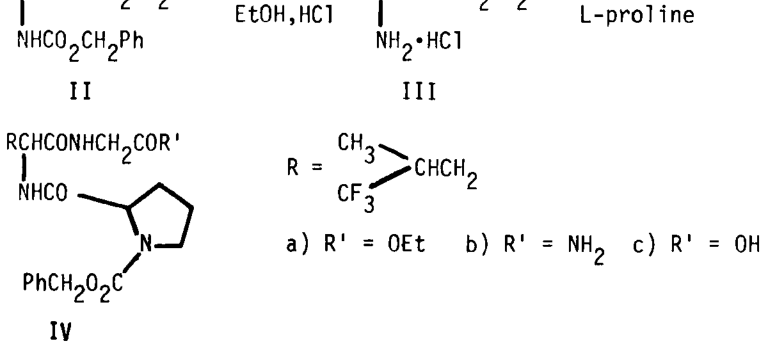
The finding[1] that leucine requiring mutants of E. Coli can be grown in media containing 5,5,5-trifluoroleucine in place of leucine indicates that substitution of trifluoroleucine for leucine in proteins and peptides has relatively little effect on their biological activities. We are undertaking to prepare several such materials in the expectation that their ^{19}F nmr spectra will be useful in investigating conformational changes or intermolecular interaction in which they take part. Although the main objective is to study macromolecular substances, it seems worthwhile also to examine



$$\underset{\displaystyle NH_2}{RCHCO_2H} + ClCO_2CH_2Ph \xrightarrow{NaOH} \underset{\displaystyle NHCO_2CH_2Ph}{RCHCO_2H}\ (\text{I}) \xrightarrow{EtO_2CCH_2NH_2\cdot HCl}$$

$$\underset{\displaystyle NHCO_2CH_2Ph}{RCHCONHCH_2CO_2Et}\ (\text{II}) \xrightarrow[EtOH,HCl]{H_2,\ Pd/C} \underset{\displaystyle NH_2\cdot HCl}{RCHCONHCH_2CO_2Et}\ (\text{III}) \xrightarrow[\text{L-proline}]{\text{Carbobenzoxy-}}$$

IV

$R = (CH_3)(CF_3)CHCH_2$

a) $R' = OEt$ b) $R' = NH_2$ c) $R' = OH$

one or more small peptides containing trifluoroleucine. The tripeptide amide, L-prolyl-trifluoro-DL-leucyl glycinamide was chosen because the unfluorinated analog, melanostatin, has known biological activity[2] and has been studied by X-ray and nmr methods.[3]

5,5,5-Trifluoro-DL-leucine on carbobenzoxylation with carbobenzoxy chloride and sodium hydroxide[4,5] afforded carbobenzoxy-5,5,5-trifluoro-DL-leucine(I) which was condensed with ethyl glycinate by the mixed anhydride technique[6] using isovaleryl chloride to give ethyl carbobenzoxy-trifluoro-DL-leucylglycinate(II). Removal of the carbobenzoxy group by catalytic hydrogenation furnished III, which on condensation with carbobenzoxy-L-proline also by the mixed anhydride method gave ethyl carbobenzoxy-L-prolyl-trifluoro-DL-leucylglycinate(IVa). Amination of IVa was accomplished by allowing its solution in ethanolic ammonia to stand at room temperature for four days to afford carbobenzoxy-L-prolyl-trifluoro-DL-leucyl glycinamide(IVb). Hydrolysis of IVa with 1N sodium hydroxide at room temperature gave carbobenzoxy-L-prolyl-trifluoro-DL-leucyl glycine(IVc).

EXPERIMENTAL

5,5,5-trifluoro-DL-leucine was purchased from Aldrich Chemical Company, Inc. Melting points (uncorrected) were taken using open capillaries. The electron impact mass spectra were obtained with a Dupont 21-110 instrument. Specific rotations were kindly determined by Dr. Y. A. Shaikh with a Perkin-Elmer 241 polarimeter using materials which had been stored as solids at room temperature for about 9 months. Since the stereoisomers of trifluoro-DL-leucine may react at different rates, and the purification procedures may effect some fractionation of the product mixture, the optical purity of the final products is unknown; the specific rotations are included at the insistence of a referee.

Carbobenzoxy-5,5,5-trifluoro-DL-leucine(I).- 5,5,5-Trifluoro-DL-leucine (18.5 g., 0.1 mole) was carbobenzoxylated with carbobenzoxy chloride (18.7 g., 0.11 mole) and sodium hydroxide (8.8 g., 0.22 mole) at 0° as described in the literature.[5] The syrup which separated was crystallized from ethyl

Ethyl carbobenzoxy-L-prolyl-trifluoro-DL-leucylglycinate(IVa).- Carbobenzoxy-L-proline (5 g., 0.02 mole) was condensed with III (6.1 g., 0.02 mole) by the mixed anhydride procedure described above. Compound IVa was isolated as a syrup. Attempts to crystallize it from several solvents were unsuccessful, but a portion stored at 25° for several months eventually became a waxy semisolid mass, $[\alpha]_D^{25}$ -33.20 (c, 1.00, EtOH). High resolution mass: Calcd for $C_{23}H_{30}F_3N_3O_6$: 501.217; found 501.215.

Carbobenzoxy-L-prolyl-trifluoro-DL-leucylglycinamide(IVb).- To 200 ml of abs. ethanol, saturated with anhydrous ammonia at 0°, was added 5 g. (0.01 mole) of IVa, and the solution was kept at room temperature for 4 days. The solvent was removed under reduced pressure; the syrupy residue crystallized slowly after standing for a few days. Recrystallization from water yielded 3.8 g. (80%) of IVb, mp. 132-136°, $[\alpha]_D^{25}$ -10.10 (c, 1.00, EtOH). High resolution mass: Calcd for $C_{21}H_{27}F_3N_4O_5$: 472.192; found: 472.192. The air dried sample analyzed for hydrate containing 1.5 mole of water.

Anal. Calcd for $C_{21}H_{27}F_3N_4O_5 \cdot 1.5H_2O$: C, 50.50; H, 6.01; N, 11.22; F, 11.42. Found: C, 50.50; H, 5.91; N, 11.38; F, 11.31.

Carbobenzoxy-L-prolyl-trifluoro-DL-leucylglycine(IVc).- 1 g. (0.002 mole) of IVa was suspended in a mixture of 5 ml acetone and 2 ml water, and one equivalent of 1N sodium hydroxide was added with stirring. After 30 minutes at room temperature the solution was acidified. The compound was isolated by filtration and dissolved in aq. sodium bicarbonate, and the solution was filtered and acidified. The precipitated product was recrystallized from aq. ethanol, giving 0.8 g. (85%) of IVc, mp. 178-182, $[\alpha]_D^{25}$ -34.80 (c, 1.00, EtOH).

acetate - pet. ether to give 27 g. (84%) of I, mp. 65-68°.

Anal. Calcd for $C_{14}H_{16}F_3NO_4$: C, 52.66; H, 5.01; N, 4.38; F, 17.86

Found: C, 52.54; H, 4.96; N, 4.27; F, 18.10.

Ethyl Carbobenzoxy-trifluoro-DL-leucylglycinate(II).- A solution of I (23.9 g., 0.075 mole) and triethylamine (7.65 g., 0.075 mole) in a mixture of 80 ml dry toluene and 80 ml chloroform was cooled to -5°. Isovaleryl chloride (9 g., 0.075 mole) was added with stirring and the solution left at -5° for 1.5 hr. A precooled solution of ethyl glycinate hydrochloride (10.46 g., 0.075 mole) and triethylamine (7.65 g., 0.075 mole) in 160 ml dry chloroform was added and the mixture stored overnight in the refrigerator. The solution was washed with water and 3% aq. sodium bicarbonate, concentrated under reduced pressure to half of its original volume, and diluted with pet. ether until cloudy. On cooling, the product separated as a thick syrup which on two recrystallizations from aq. ethanol gave 19.7 g. (65%) of II, mp. 85-86°.

Anal. Calcd for $C_{18}H_{23}F_3N_2O_5$: C, 53.41; H, 5.69; N, 6.92; F, 14.09.

Found: C, 53.20; H, 5.80; N, 6.81; F, 14.30

Ethyl trifluoro-DL-leucylglycinate hydrochloride(III).- 10 g. (0.025 mole) of II dissolved in 300 ml of abs. ethanol containing 2.7 ml of conc. hydrochloric acid were reduced with hydrogen over 2 g. of 5% palladium/charcoal catalyst at atmospheric pressure. When evolution of carbon dioxide had ceased, the catalyst was filtered off and the solvents removed under reduced pressure. The residue was dried by addition of benzene and evaporation under reduced pressure. On recrystallization from ethanol-ether it gave 7 g. (91%) of III, mp. 180-182°.

Anal. Calcd for $C_{10}H_{17}F_3N_2O_3 \cdot HCl$: C, 39.47; H, 5.54; N, 9.13; F, 18.59; Cl, 11.58.

Found: C, 39.24; H, 5.81; N, 8.93; F, 18.60; Cl, 11.80.

Anal. Calcd for $C_{21}H_{26}F_3N_3O_6$: C, 53.27; H, 5.49; N, 8.87; F, 12.05. Found: C, 53.29; H, 5.70; N, 8.70; F, 12.01.

Acknowledgement.- This work was supported by NSF grant BMS74-15490.

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(Received October 27, 1977; in revised form June 23, 1978)