THE USE OF N-TRICHLOROACETOXYPHTHALIMIDE IN THE SYNTHESIS OF AN ENKEPHALIN ANALOG WITH THE Tyr-D-Ala-Gly-Ph-NH_2 SEQUENCE

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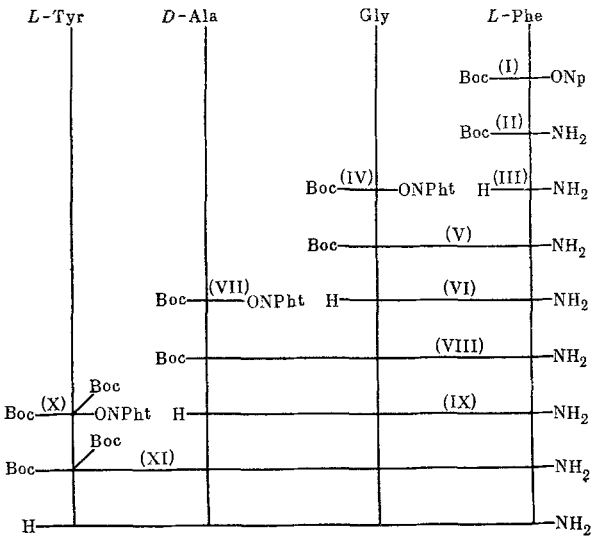
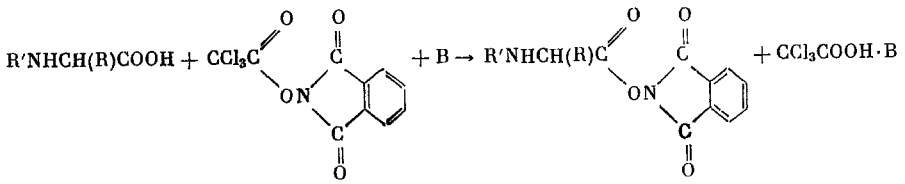
In our previous work [1], we described a synthesis for N-trichloroacetoxyphthalimide, which is a reagent for the preparation of activated N-oxyphthalimide esters of N-protected amino acids and was used for the synthesis of a tetrapeptide enkephalin analog. Coy et al. [2] have reported that the peptide bond between the Tyr and Gly residues in the sequence of enkephalin-like peptides is not stable to the action of peptidases. Susumu et al. [3] have shown that Gly^2 may be replaced by a D-amino acid residue such as D-Ala, thereby considerably enhancing resistance of the bond to the action of peptidases. The protection of the C-terminal part of the molecule relative to the action of enzymes is achieved by amidation. A number of enkephalin analogs have now been obtained [4] with powerful analgetic action and enhanced resistance to the action of peptidases. The tetrapeptide (TP) synthesized in the present work is such a peptide.

The synthesis of TP was carried out by the activated ester method by the following scheme:



N-Oxyphthalimide esters of N-protected amino acids were used as the activated esters to obtain the peptide bond. These esters were obtained by the following scheme:



where R' = $CO_2C(Me)_3$, R is the amino acid sidechain, and B is a base such as pyridine, N-methylmoprholine, and Et_3N.

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N-Oxyphthalimide esters are highly reactive in aminolysis reactions, which permitted the synthesis of TP under mild conditions with good yield. In addition, these derivatives readily crystallize and hydroxyphthalimide which forms in the aminolysis is easily removed from the peptide product by washing with aqueous $NaHCO_3$.

The activated esters of (IV), (VII), and (X) were obtained in 99%, 82%, and 90% yields, respectively. The aminolysis was carried out with a slight excess of the amino component (up to 15%). Trifluoroacetic acid was used to remove the Boc protective group. Purification of TP was carried out by liquid chromatography on a Zorbax C-8 column in 8% MeCN + 92% 0.2 M $AcONH_4$. The product was homogeneous on thin-layer chromatography and its physicochemical properties were in accord with literature data [5].

EXPERIMENTAL

Reanal amino acid derivatives were used. The melting points were determined on a Koefler block and the specific optical activity was measured on a Perkin–Elmer 141 polarimeter in a cell with 1 dm pathlength. The IR spectra were taken on an IRS Hitachi 260-10 spectrometer. The amino acid composition after hydrolysis in 6 M HCl for 12 h at 110°C was determined on a Liquimat-III amino acid analyzer with detection by o-phthaldialdehyde. The purity of the compounds obtained was monitored by thin-layer chromatography on Merck F-254 silica gel 60 plates with the following eluents: 1) 100:50:2 benzene–acetone–acetic acid; 2) 95:5:3 chloroform–methanol–acetic acid; 3) 7:3 1-propanol–25% ammonia; 4) 4:1:1 1-butanol–acetic acid–water; 5) 60:20:6:11 ethyl acetate–pyridine–acetic acid–water; 6) 4:1:5 1-butanol–acetic acid–water (upper phase). The amino acid derivatives and peptides were developed by ninhydrin and fluorescamine.

Typical Synthesis of N-Oxyphthalimide Esters of N-Substituted Amino Acids. A sample of 10 mmoles N-trichloroacetoxyphthalimide was added with stirring to a solution of 10 mmoles N-substituted amino acid and 10 mmoles pyridine in 10 ml polar solvent (acetonitrile, DMF, THF, CH_2Cl_2, or ethyl acetate) and maintained at 22-23°C for 2-12 h and then concentrated in vacuum. The residue was diluted with 50 ml ethyl acetate and washed with three 50-ml portions of water and three 10-ml portions of aq. $NaHCO_3$. The ethyl acetate solution was dried over anh. Na_2SO_4 and distilled in vacuum. The residue was crystallized from a suitable solvent. This method gave (IV) in 99% yield, mp 195-196°C, E_f 0.8 (1), 0.72 (2); (VII) in 82% yield, mp 125-127°C, $[\alpha]_{589}^{23}$ +30.4° (C 1, ethyl acetate), R_f 0.8 (1), 0.75 (3); and (X) in 90% yield, mp 76-77°C, $[\alpha]_{589}^{24}$ −29.7° (C 1, ethyl acetate), R_f 0.75 (1), 0.85 (3).

N-tert-Butyloxycarbonyl-L-phenylalanine Amide (II). A sample of 5 g (12.93 mmoles) (I) was suspended in 70 ml methanol and ammonia was bubbled in until completely dissolved. The mixture was maintained for 3 h at 22°C. The solvent was removed in vacuum and the residue was dissolved in 100 ml of a 4:1 mixture of ethyl acetate and water. The extract was washed with five 20-ml portions of 5% aq. $NaHCO_3$ and dried over anhydrous Na_2SO_4. The solvent was removed in vacuum to give 99% (II), mp 108-110°C (from ethyl acetate), $[\alpha]_{589}^{23}$ +8.6° (C 1, methanol), R_f 0.8 (2), 0.5 (1). IR spectrum: 3360, 1650, 1590 cm^{-1}.

N-tert-Butyloxycarbonylglycyl-L-phenylalanine Amide (V). A sample of 3 g (10.72 mmoles) trifluoroacetate (III) and 1.08 g (10.72 mmoles) N-methylmorpholine was added to a solution of 3 g (9.38 mmoles) (IV) in 15 ml DMF and the mixture was left for 18 h at 22°C, and then the solvent was removed in vacuum. The oily residue was dissolved in 100 ml ethyl acetate and washed with three 15-ml portions of water and three 15-ml portions of 5% aq. $NaHCO_3$. The solvent was removed in vacuum to give 73% (V), mp 172-173°C (from ethyl acetate). $[\alpha]_{589}^{23}$ +4.5° (C 1, methanol), R_f 0.2 (1), 0.8 (3). Amino acid analysis: Gly 1.00; Phe 1.08.

N-tert-Butyloxycarbonyl-D-alanylglycyl-L-phenylalanine Amide (VIII). A sample of 2.1 g (6.22 mmoles) trifluoroacetate (VI) and 0.63 g (6.22 mmoles) N-methylmorpholine was added to a solution of 1.87 g (5.6 mmoles) (VII) in 10 ml DMF and maintained for 16 h at 22°C. Ethyl acetate was added until the volume was 100 ml and the mixture was washed with three 10-ml portions of water, three 10-ml portions of 5% aq. $NaHCO_3$, and three 10-ml portions of 3% aq. $KHSO_4$. The solution was dried over anhydrous Na_2SO_4 and the solvent was removed in vacuum to give 73% (VIII), mp 118-120°C (from ethyl acetate), $[\alpha]_{589}^{23}$ +10.7° (C 1, methanol), R_f 0.1 (1), 0.8 (3). Amino acid analysis: Gly 1.00; Ala 1.00; Phe 1.38.

N,O-tert-Butyloxycarbonyl-L-tyrosyl-D-alanylglycyl-L-phenylalanine Amide (XI). A sample of 1.3 g (3.19 mmoles) trifluoroacetate (IX) and 0.32 g (3.19 mmoles) N-methylmorpholine was added to a solution of 1.5 g (2.87 mmoles) (X) in 12 ml DMF and left for 16 h at 22°C. The solution was diluted with ethyl acetate to 100 ml and then washed with three 10-ml portions of water, three 10-ml portions of 5% $NaHCO_3$ and three 10-ml portions of aq. $KHSO_4$ and dried over anh. Na_2SO_4. The solvent was removed in vacuum to give 90% (XI), mp 180-181°C (from ethyl acetate), $[\alpha]_{589}^{23}$ +12° (C 0.5, methanol), R_f 0.1 (1), 0.82 (3).

L-Tyrosyl-D-alanylglycyl-L-phenylalanine Amide (TP). A sample of 0.1 g TP trifluoroacetate in a mixture of 8% acetonitrile and 92% 0.2 M aq. $AcONH_4$ was purified by high-resolution liquid chromatography on a 4.6 × 150 mm column packed with Zorbax C-8 at an elution rate of 2 ml/min. The yield of TP was 65%, $[\alpha]_{589}^{23}$ +43.2° (C 0.5, methanol), R_f 0.63 (3), 0.5 (4), 0.47 (5), 0.5 (6). Amino acid analysis: Gly 1.00; Ala 1.00; Phe 1.13; Tyr 1.16.

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CONCLUSIONS

An enkephalin analog was synthesized with the sequence Tyr-D-Ala-Gly-Phe-NH_2 by using activated N-oxyphthalimide esters and N-trichloroacetoxyphthalimide.

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EFFECT OF THE EXTENT OF REDUCTION OF NAPHTHALENE – AND PHENANTHRENE – CYCLOPENTADIENYL IRON COMPLEXES ON THEIR ELECTRONIC ABSORPTION SPECTRA

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There have been a number of studies on the reduction and oxidation of arene–cyclopentadienyl iron complexes, in which the reduction potentials [1, 2], ESR spectra [3, 4], and electronic absorption spectra (EAS) were investigated [5, 6].

The reduction of the cations of naphthalene– and phenanthrene–cyclopentadienyl iron complexes in Na/1,2-dimethoxyethane showed that these cations may be converted quantitatively at low temperature to electroneutral complexes and then to anions

$$[AreneFeC_5H_5]^+BF_4^- \xrightarrow{e} [AreneFeC_5H_5]^{\cdot} \xrightarrow{e} [AreneFeC_5H_5]^-.$$

These conversions are indicated by the ESR spectra: the ESR spectrum of the electroneutral complex is detected upon the reduction of the cation to give this complex [3]. The intensity of this signal then decreases to zero, indicating transformation of the paramagnetic electroneutral complex to the diamagnetic anion. This process is monitored by oxidation of the anion by atmospheric oxygen. After oxidation, the ESR spectrum of the electroneutral complex reappears.

Analysis of the ESR spectra [3, 4] and of the d–d transitions [5, 6] in the EAS of these complexes showed that the Fe atom has the following arrangement of d levels:

$$e_{1g}^*(d_{xz}, d_{yz}) > e_{2g}(d_{xy}, d_{x^2-y^2}) \gtrsim a_{1g}(d_{z^2}).$$

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