PENTAGASTRIN ANALOGS CONTAINING α-AMINOOXY ACIDS

VI. Synthesis of two pentagastrin analogs ^{14}C-labelled at the N-terminal residue

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SUMMARY

Tert.-Butyloxycarbonyl-l-^{14}C-glycyl-L-tryptophyl-L--methionyl-L-aspartyl-L-phenylalaninamide /I/, H-l-^{14}C--glycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalaninamide /II/ and tert.-butyloxycarbonyl-l-^{14}C-aminooxyacetyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalaninamide /V/ of different specific radioactivities have been synthesized for biological studies.

Key Words: Synthesis, ^{14}C-labelling, aminooxyacetic acid, pentagastrin analogs

INTRODUCTION

The mammalian "little" gastrins, consisting of 17 amino acids, have several biological effects in the gastrointestinal tract under physiological conditions [1]. The C-terminal tetrapeptide of these gastrins /H-Trp-Met-Asp-Phe-NH_2/ exhibits the full activity of the original heptadecapeptide [2].

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More than 200 analogs and derivatives of this C-terminal tetrapeptide have been synthesized. Peptide hormones in living organisms are generally inactivated by endo- and exopeptidases. We have earlier synthesized BOC-l-^{14}C-Gly--Trp-Met-Asp-Phe-NH_2 [3] with three different specific activities to study its inactivation in living organism. The biological studies revealed that after hepatic transit this pentagastrin analog is excreted through the bile partly in intact, and partly in deaminated forms [4] . Some new pentagastrin analogs containing α-aminooxy acid at N-terminus have enhanced gastric acid secretory activity in rats [5, 6]. It was shown that the aminooxy acid-containing analogs have a greater secretory activity than the glycyl analog [7]. The increased biological effect may be connected with the slower elimination from the circulation of these analogs. This finding promted us to synthesise one of the most active analogs in ^{14}C-labelled form for a detaited study of metabolism.

RESULTS AND DISCUSSION

BOC-l-^{14}C-Gly-Trp-Met-Asp-Phe-NH_2 /I/ with a specific activity of 4.82 mCi/mmol or 178,3 MBq/mmol was synthesized by the reaction of BOC-l-^{14}C-Gly-OPcp /5.15 mCi/mmol/ and H-Trp-Met-Asp-Phe-NH_2. In addition to the usual chemical controls, /I/ was characterized by radiochromatogram-scanning as a single TLC spot and radioactive peak and further by its biological activity which was identical with that of the unlabelled compound [3].

H-1-^{14}C-Gly-Trp-Met-Asp-Phe-NH_2 /II/ with a specific activity of 4.18 mCi/mmol or 154,7 MBq/mmol was prepared from /I/ using trifluoroacetic acid with subsequent liberation from it trifluoroacetate. It was characterized in the same way as /I/. It showed a single TLC spot and radioactive peak. Its biological activity was identical with that of the unlabelled compound [8].

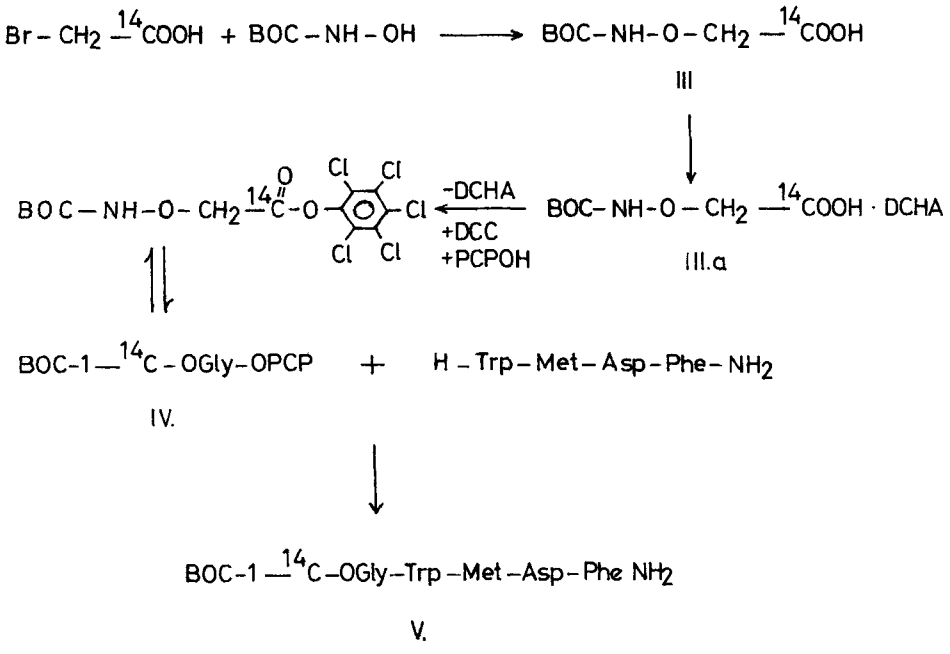
BOC-1-^{14}C-OGly-Trp-Met-Asp-Phe-NH_2 /V/ with a specific activity of 500 μCi/mmol or 18,43 MBq/mmol was prepared by the reaction of BOC-1-^{14}C-OGly-OPcp /IV/ and H-Trp-Met-Asp--Phe-NH_2 [5]. Its chemical and biological identity to the unlabelled compound [5] and its purity as a single TLC spot and radioactive peak were established.

For the synthesis of /IV/, 1-^{14}C-bromoacetic acid was reacted with BOC-NH-OH according to the method described previously for benzyloxycarbonyl derivative [9]. The BOC-1--^{14}C-OGly-OH /III/ obtained was isolated and characterized as its dicyclohexylammonium salt /III./ /580 μCi/mmol/ or 21,46 MBq/mmol/. After liberation of BOC-1-^{14}C-OGly-OH from its salt, BOC-1-^{14}C-OGly-OPcp /IV/ was prepared and chemically characterized in the same way as for the unlabelled compound [10] [SCHEME 1].

Studies on the metabolisms of these compounds will be published later.

Abbverivations: BOC- = tert.-butyloxycarbonyl-; OGly = aminooxyacetic acid; DCHA = dicyclohexylamine, DCCI = dicyclohexylcarbodiimide; HOPcp = pentachlorophenol,

SCHEME 1.



EXPERIMENTAL

All common L-configuration amino acids were commercial products. Melting points are uncorrected, measured using a Tottoli /Büchi/ apparatus. Optical rotations were measured with a Perkin-Elmer 141 automatic digital readout polarimeter, tube length 1 dm. One dimensional thin-layer chromatography was carried out on 5x20 cm plates using 0.2 mm thick Kieselgel PF_{254} /Merck/ and the following solvent systems were used.

S 1, n-BuOH/AcOH/H_2O 4:1:1

S 2, $CHCl_3$/MeOH 8:2

S 3, $CHCl_3$/n-hexane/AcOH 8:1:1

Solvents S 4 to S 7 were made by mixing EtOAc with a stock solution of pyridine/AcOH/H_2O=20:6:11 in the following ratios:

S 4, EtOAc/stock	9.5:0.5
S 5, EtOAc/stock	9:1
S 6, EtOAc/stock	4:1
S 7, EtOAc/stock	7:3

Thin-layer chromatograms were controlled with a Packard Chromatogram-scanner Modell 7201. The activities were measured with a Packard TRI-CARB liquid scintillation system.

Tert.-Butyloxycarbonyl-1-^{14}C-glycyl-L-tryptophyl-L-methionyl--L-aspartyl-L-phenylalaninamide /I/

Tert.-Butyloxycarbonyl-1-^{14}C-glycine /126.5 mg, 72 %, m.p. 79-81°C, 4.52 mCi/mmol or 167.42 MBq/mmol/ was obtained by tert.-butyloxycarbonylation of 33.5 mg 1-^{14}C-glycine and 42 mg glycine. To obtain the pentachlorophenyl ester of tert.--butyloxycarbonyl-1-^{14}C-glycine /110 mg, 52 %, m.p. 114-116°C/ we used the method earlier described [3]. 119.2 mg /0,2 mmol/ L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalaninamide and 105.8 mg /0.25 mmol/ BOC-1-^{14}C-glycine pentachlorophenyl ester /5.15 mCi/mmol/ were dissolved in 15 ml DMF saturated with argon. After a 48 h reaction time under stirring at room temperature, the reaction mixture was concentrated to 3 ml under reduced pressure and poured into 30 ml ether saturated with argon. After 30 min stirring, the protected pentapeptidamide was collected by centrifugation, washed with

ether, and dried in a desiccator over P_2O_5. The crude product /143 mg, 95 %, m.p. 199-203°C/, containing minimal contamination, was dissolved in 2 ml methanol, precipitated by adding water, centrifuged off, washed with ether, and dried in a desiccator over P_2O_5. Yield: 129 mg; a chromatographycally /S4, S5, S6/ and radiochromatographically homogeneous product, m.p. 207-209°C $[\alpha]_D^{22}$=-29° c=1 DMF. Amino acid analysis: Phe 1.00; Asp 0.97; Met 0.93; Gly 1.02; Trp 0.87.

H-1-^{14}C-glycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalaninamide /II/

22.5 mg /0.03 mmol, 4.82 mCi/mmol or 178.3 MBq/mmol/ /I/was dissolved in 0.5 ml 2.2 N acetic acid/HCl, shaken for 30 min., poured into 7 ml cooled ether saturated with argon, centrifuged, washed with ether and dried in a desiccator over KOH. The pentapeptidamide salt was dissolved in water containing 1.1 equivalents of 1 N NaOH, and the solution was acidified to pH 7 with 1 N HCl. The precipitate was filtered off and washed thorougly with water in an argon atmosphere. Yield: 18.5 mg /94.8 %/; a chromatographically /S4, S7/ and radiochromatographically homogeneous product, m.p. 204-206°C.

Tert.-Butyloxycarbonyl-1-^{14}C-aminooxyacethyl-L-tryptophyl-L--methionyl-L-aspartyl-L-phenylalaninamide /V/

215 mg /0.36 mmol/ L-tryptophyl-L-methionyl-L-aspartyl--L-phenylalaninamide [5] and 113 mg /0.44 mmol, 0.58 mCi/mmol or 21.46 MBq/mmol/ /IV/ were dissolved in 7.5 ml DMF saturated with argon. After 72 h reaction time under stirring at room

temperature, the reaction mixture was concentrated to 2 ml under reduced pressure and poured into 20 ml ether saturated with argon. After centrifugation the protected pentapeptid-amide was washed with ether, and dried in a desiccator over P_2O_5. The crude product /275 mg, 100 %, m.p. 187-188°C/ contained minimal contamination; 155 mg crude peptide dissolved in 3 ml methanol, precipitated by adding water, centrifuged, washed with ether, and dried. Yield: 124 mg, 80 %; a chromatographically /S1 , S3 , S5 , S6 / and radiochromatography-cally homogeneous product, m.p. 193-196°C.

Tert.-Butyloxycarbonyl-l-^{14}C-aminooxyacetic acid pentachlorophenyl ester /IV/

373 mg /1 mmol/ tert.-butyloxycarbonyl-l-^{14}C-aminooxy-acetic acid /prepared by E. Koltai et al.,/ was isolated as the DCHA /III a/ salt chromatographically /S1 , S4 / and radiochromatographically homogeneous, m.p. 190-193°C, 580 μCi/mmol or 21.46 MBq/mmol/. It was liberated from salt form in the usual way /III/. The homogeneous oil and 266 mg /1 mmol/ pentachlorophenol were dissolved in 5 ml dioxane, cooled to 0°C and 206 mg /1 mmol/ DCCI was added. After 4 h stirring at room temperature the precipitated dicyclohexylurea was removed by filtration and washed with dioxane, the dioxane was evaporated under reduced pressure, and the residue was recrystallized from methanol, filtered off and dried.

Yield: 204.2 mg, 46.5 %; a chromatographically /S1 , S3 , S4 / and radiochromatographically homogeneous product, m.p. 143-144°C, $[\alpha]_D^{25}$=-16.3°, c=1 DMF.

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