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POSSIBLE DRUGS LABELLED WITH ^{14}C. IV.[1] SYNTHESIS OF D-PHENYLALANINYL-PROLYL-ARGININE ALDEHYDE LABELLED IN THE GUANIDINO GROUP

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SUMMARY

α-tert-Butyloxycarbonyl-ω-benzyloxycarbonyl-[guanido-^{14}C]arginine was prepared by reacting α-tert-butyloxycarbonyl-ornithine with N-benzyloxycarbonyl-S-methyl-[^{14}C]isothiourea, and it was transformed to D-phenylalaninyl-prolyl-[guanido-^{14}C]arginine aldehyde, via ω-benzyloxycarbonyl-[guanido-^{14}C]-arginine lactam.

Key Word: α-BOC-ω-Cbz-[guanido-^{14}C]arginine

INTRODUCTION

In a preceeding paper (1) we reported on the synthesis of a new anticoagulant, D-phenylalaninyl-prolyl-arginine aldehyde (GYKI-14166)[x] labelled in the phenylalanine group. In order to complete the pharmacokinetic and metabolism studies, labelling of another site of the molecule was required. Hereby we report on the synthesis of GYKI-14166 labelled in the guanidino group of arginine. This synthesis proved to be more advantageous as compared to the earlier one, avoiding the losses of resolution.

Although there have been several methods for labelling

[x] Unless otherwise stated amino acids should be considered to be L enantiomers.

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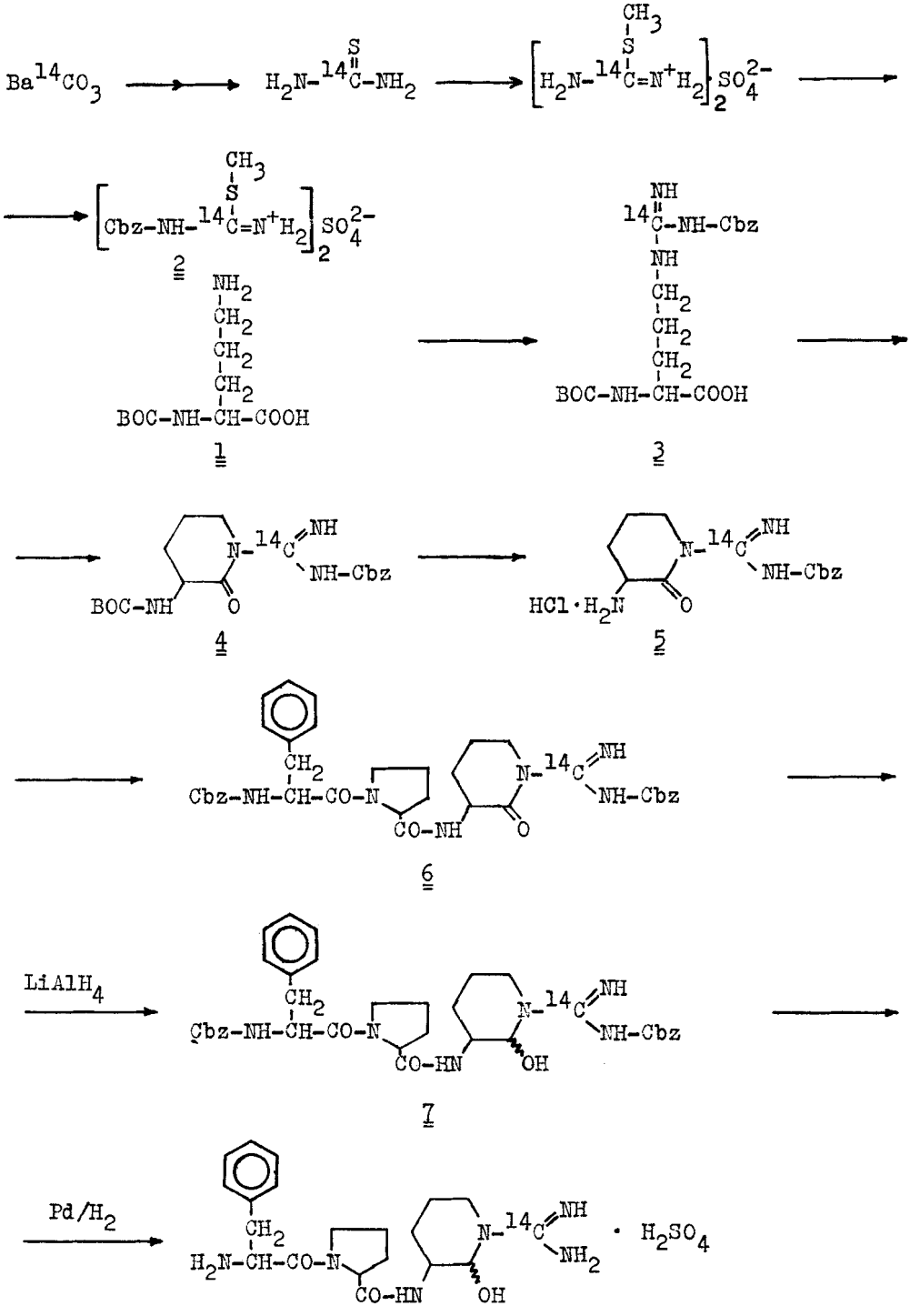

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arginine in the guanidino group (2-4), strangely enough, there is no method for labelling protected arginine derivatives useable in peptide syntheses. As α-tert-butyloxycarbonyl-ω-benzyloxy-carbonyl-arginine (3) was required for the synthesis of GYKI-14166 on our patented route (5), this key intermediate was prepared by reacting N-benzyloxycarbonyl-S-methyl-[^{14}C]isothiourea (2) with tert-butyloxycarbonyl-ornithine (1). This one-pot method for preparing 3 was superior to the other one (when S-methyl-iso-thiourea was reacted with 1 and the tert-butyloxycarbonyl-arginine obtained was benzyloxycarbonylated) and the yield was somewhat higher, too.

[^{14}C]Thiourea was prepared from barium[^{14}C]carbonate via barium[^{14}C]cyanamide in a yield of 89.7% as described earlier (6,7). It was then methylated with dimethyl sulfate, and the resulted S-methyl-[^{14}C]isothiuronium sulfate - without purification - was reacted with N-benzyloxycarbonyloxy-succinimide[x] to give 2. Formation of the product was monitored by TLC and it was reacted in situ with 1 to give 3, which was transformed to α-tert-butyloxycarbonyl-ω-benzyloxycarbonyl arginine lactam (4), followed by conversion into GYKI-14166 as described in the previous paper (1). The radiochemical yield was 12.7% based on $Ba^{14}CO_3$.

EXPERIMENTAL

Thin layer chromatography was carried out on silica gel 60 F_{254} (MERCK). The spots were located by chlorine-tolidine spray reagent and quantified by a Berthold TLC scanner. Radioactivity was measured in a Packard Tri-Carb 2660 liquid scintillation spectrometer.

[^{14}C]Thiourea

[^{14}C]Thiourea (460 mg; 6.04 mmoles; 12.61 GBq) was prepared

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[x] It was prepared according to Frankel et al. (8).

from $Ba^{14}CO_3$ (1.3737 g; 6.656 mmoles; 14.06 GBq) by a known method (6,7) in a radiochemical yield of 89.7%. Purity was checked by TLC (ethyl acetate - pyridine - acetic acid - water 30:20:6:11; R_f 0.5).

α-tert-Butyloxycarbonyl-ω-benzyloxycarbonyl-[guanido-^{14}C] arginine (3)

S-Methyl-[^{14}C]isothiourea was prepared according to Oliverio et al. (9) and used without purification. 460 mg (6.04 mmoles; 12.61 GBq) of [^{14}C]thiourea, 0.28 ml of water and 0.416 mg (3.3 mmoles) of dimethyl sulfate were stirred at 130°C for 30 minutes, then - after cooling - the product was dissolved in a mixture of water (15 ml) and ethanol (15 ml). To the solution, 1.4 ml (10 mmoles) of triethylamine and 1.7 g (6.8 mmoles) of N-benzyloxycarbonyloxysuccinimide (8) were added and stirring was continued at room temperature for 20 minutes. The reaction was monitored by TLC (ethyl acetate - benzene 1:1; R_f 0.6) and, if it was necessary, an additional portion of N-benzyloxycarbonyloxysuccinimide was added. When the reaction was completed 2.32 g (10 mmoles) of 1 and another 1.4 ml (10 mmoles) of triethylamine were added to the mixture, it was refluxed for 1.5 hours and the ethanol was removed by evaporation. The residue was acidified with acetic acid (30 ml) and after the addition of 20 ml of ethyl acetate the mixture was stirred for 15 minutes. Then the layers were separated, the aqueous phase was extracted with ethyl acetate (2x20 ml) and the combined extracts were washed with water (4x40 ml) and 25% NaCl (2x40 ml), successively. The organic phase was dried over Na_2SO_4 and evaporated. The residue crystallized on treatment with ether. Yield: 1.66 g (4.1 mmoles; 8.49 GBq) of 3 (68%). The material showed only one spot by TLC (ethyl acetate - pyridine - acetic acid - water 60:20:6:11; R_f 0.5).

α-tert-Butyloxycarbonyl-ω-benzyloxycarbonyl-[guanido-^{14}C]arginine lactam (4)

To a solution of 3 (1.66 g; 4.1 mmoles; 8.49 GBq) in anhydrous tetrahydrofuran (15 ml) triethylamine (0.6 ml; 4.1 mmoles) and, after cooling to -10°C, isobutyl chloroformate (0.53 ml; 4.1 mmoles) were added. The mixture was stirred for 15 minutes and triethylamine (0.6 ml; 4.1 mmoles) was added again, then stirring was continued at -10°C for 10 minutes, at 0°C for one hour and for an additional hour at room temperature. Thereafter 75 ml of ice water was poured to the mixture and this was stirred for 20 minutes. The precipitated crystals were filtered off and washed with ice water (2x15 ml), dissolved in $CHCl_3$ (50 ml) and after shaking with ice water (30 ml), the organic solution was separated and dried over $CaCl_2$ with stirring for 45 minutes. The mixture was cleared with silica gel and filtered. After evaporation the residue was triturated with petroleum ether to produce crystals of 4. Yield: 1.157 g, (74%). Total activity: 5.00 GBq. The material showed only one spot by TLC (ethyl acetate - pyridine - acetic acid - water 60:20:6:11; R_f 0.8).

Benzyloxycarbonyl-D-phenylalaninyl-prolyl-[guanido-^{14}C]arginine-(ω-benzyloxycarbonyl) lactam (6)

To a suspension of 4 (1.157 g; 2.96 mmoles; 5.00 GBq) in $CHCl_3$ (2 ml), 6 ml of ethyl acetate containing 0.35 g HCl per ml were added; the mixture was stirred at room temperature for 3 hours, then diluted with anhydrous ether (30 ml). The precipitated crystals of [guanido-^{14}C]arginine lactam HCl (5) were collected and dried in a desiccator over P_2O_5[x].

[x] 5 was not purified; its purity was estimated to be about 90% by TLC (ethyl acetate - pyridine - acetic acid - water 60:20:6:11; R_f 0.2)

To a solution of Cbz-D-Phe-Pro-OH (1.96 g; 3.3 mmoles) in dimethylformamide (5 ml) N-methylmorpholine (0.37 ml; 3.3 mmoles) and isobutyl chloroformate (0.44 ml; 3.3 mmoles) were added at -15°C and stirred for 10 minutes. Thereafter a solution of 5 in dimethylformamide (5 ml) and triethylamine (1.4 ml; 10 mmoles) were added, stirring was continued at -15°C for one hour and at 0°C for an additional hour, then the mixture was processed as described in a previous paper (1). The crude 6 (1.79 g; 4.83 GBq; 96%) was purified by chromatography (silica gel 60; Merck; 70-230 mesh; benzene - tetrahydrofuran 8:2) yielding 1.596 g (2.38 mmoles; 4.52 GBq) of 6 as a white powder, which proved to be homogenous by TLC (ethyl acetate - pyridine - acetic acid - water 960:20:6:11; R_f 0.8).

Cbz-D-Phe-Pro-[guanido-^{14}C]Arg(Cbz) aldehyde (7)

The reduction with $LiAlH_4$ was carried out as described in the preceeding paper (1); 1.296 g (1.93 mmoles) of 7 was obtained as a white powder. Yield was 81%. The material analysed by TLC showed one spot with a tail (ethyl acetate - pyridine - acetic acid - water 120:20:6:11; R_f 0.4).

H-D-Phe-Pro-[guanido-^{14}C]Arg-H sulfate (GYKI-14166)

Deprotection was performed by catalytic hydrogenation as described earlier (1); 553 mg (1.1 mmoles) of GYKI-14166 was obtained as a voluminous white solid (57%). The product was identical with the inactive samples by TLC (ethyl acetate - pyridine - acetic acid - water 30:20:6:11; R_f 0.5) and by biological activity (130%). The overall radiochemical yield was 12.7% calculated relative to $Ba^{14}CO_3$. Total activity: 1.78 GBq.

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