SYNTHESIS OF [^{14}C]-N-[(TRIMETHYLAMINEBORYL)CARBONYL]-PHENYLALANINE-METHYL ESTER

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SUMMARY

Reported is the synthesis of [^{14}C]-L-N-[(trimethylamine-boryl)carbonyl]-phenylalanine methyl ester, a boron containing α-amino acid dipeptide analog with anti-neoplastic, anti-inflammatory, and hypolipidemic activities. The ^{14}C label is universally distributed among all carbon positions of the phenylalanine portion of the molecule. Briefly, the dipeptide is prepared by reacting the phenylalanine methyl ester hydrochloride salt with trimethylamine-carboxyborane, triphenylphospine (TPP), CCl_4, and triethylamine (TEA) in acetonitrile, for 24 hours. The final product afforded a specific activity of 5.71 mCi/mmol.

Key words: boron, dipeptide, [^{14}C]-L-N-[(trimethylamine-boryl)-carbonyl]-phenylalanine methyl ester

INTRODUCTION

Boron analogs of α-amino acids, such as trimethylamine-carboxyborane, $(CH_3)_3N{\cdot}BH_2COOH$ (the protonated boron analog of betaine, $(CH_3)_3N^+CH_2COO^-$), have demonstrated antineoplastic, anti-inflammatory, and hypolipidemic activities (1-4). An investigation into the biological activity of dipeptide analogs demonstrated similar biological activities (5). The presence of a

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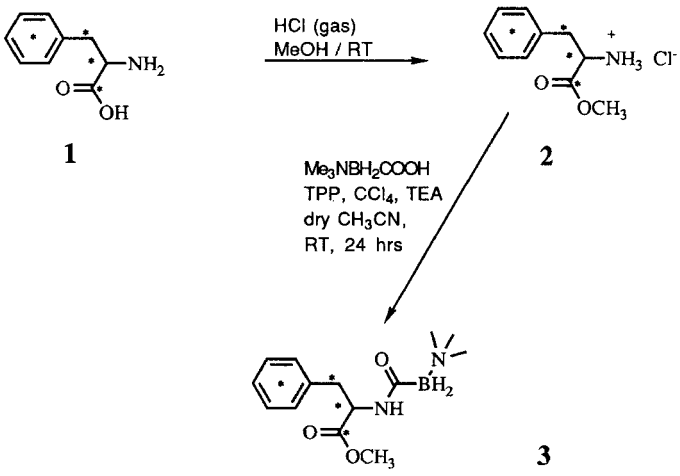
boron atom in these compounds may also make them useful in boron neutron capture therapy (BNCT) as an additional cytotoxic mechanism of action in human tumor treatment(6). The synthesis of one of the more active dipeptides, L-N-[(trimethylamineboryl)-carbonyl]-phenylalanine methyl ester, with a ^{14}C-label randomly occurring at all positions of the phenylalanine molecule is described in this paper. This compound is to be used, in the future, in rodent tissue distribution and elimination studies.

DISCUSSION

The synthesis of [^{14}C]-L-N-[(trimethylamine-boryl)-carbonyl]-phenylalanine methyl ester begins with the preparation of phenylalanine methyl ester hydrochloride, by treatment of a methanolic suspension of [^{14}C]-L-phenylalanine with hydrogen chloride gas. Following ester formation, the phenylalanine methyl ester hydrochloride salt was converted to its free base with triethylamine. Condensation to form the amide linkage was accomplished by adding trimethylamine-carboxyborane in the presence of triphenylphosphine and CCl_4. A second equivalent of triethylamine is present to trap the HCl generated by the interaction of triphenylphosphine and CCl_4.

Scheme 1:



EXPERIMENTAL PROCEDURE

All chemicals were used as received from manufacturers, with the exception of acetonitrile and methanol which were dried by distillation prior to use. [^{14}C]-L-phenylalanine, NEC-284E, (specific activity 485.7 mCi/mmol) was obtained from New England Nuclear, Boston, Mass. Triphenylphosphine and triethylamine were obtained from Sigma Chemical Co. Trimethylamine-carboxyborane was obtained from Boron Biologicals Inc., Raleigh, NC. HCl gas was obtained from Aldrich Chemical Co. Radiopurity was determined using a Bioscan BID-100 Image Analyser and Whatman Diamond Series TLC plates (60Å silica gel with fluorescent indicator). ^{14}C was counted, using a Packard Tricarb 4000 liquid scintillation spectrometer with Scintiverse II® counting solution, and corrected for quenching.

[^{14}C]-L-phenylalanine methyl ester hydrochloride **2**. [^{14}C]-L-phenylalanine **1**, 485.7 mCi/mmol was diluted with unlabelled L-phenylalanine (33.9mg, 0.205 mmol) to a calculated specific activity of 4.83 mCi/mmol. The diluted amino acid (34.2 mg, 0.207 mmol) was suspended in 15 ml dry methanol. HCl gas was allowed to bubble through this suspension for 1 hour, after which methanol and any remaining HCl gas was removed under reduced pressure (7). This process was repeated, resulting in a clear solution, immediately following solvent removal. The resultant methyl ester was obtained in nearly quantitative yield, R_f=0.25 in 7:3 ethyl acetate/hexanes [for unlabelled **2**, R_f=0.25 in 7:3 ethyl acetate/hexanes: H^1 NMR (DMSO-d_6) δ 8.58 (s,2H,NH_2), δ 7.3 (m,5H,C_6H_5), δ 4.27 (t,1H,CH), δ 3.66 (s,3H,OCH_3), δ 3.1 (m,2H,CH_2)].

[^{14}C]-L-N-[(trimethylamine-boryl)-carbonyl]-phenylalanine methyl ester **3**. Compound **2** (44.44 mg, 0.207 mmol), trimethylamine-carboxyborane (24.2 mg, 0.207 mmol), and

triphenylphosphine (78.7 mg, 0.300 mmol) were dissolved in 10ml of dry acetonitrile with triethylamine (0.08 ml, 60.7 mg, 0.600 mmol) and carbon tetrachloride (0.04 ml, 70.8 mg, 0.460 mmol). The reaction was stirred at room temperature for 24 hours under nitrogen and the solvent was removed under reduced pressure. The product was purified on silica gel using two void volumns of ethyl acetate/hexanes (1:1), two void volumns ethyl acetate/hexanes 6:4, and then ethyl acetate/hexanes (7:3). Unreacted starting material was recycled and the above procedure was repeated twice. The combined weight of purified product yielded 21.4 mg (37%) of a white solid, R_f=0.20 in 7:3 ethyl acetate/hexanes [for unlabelled **3**, R_f=0.20 in 7:3 ethyl acetate/hexanes: H^1 NMR ($CDCl_3$) δ 7.2 (m,5H,C_6H_5), δ 5.97 (d,1H,NH), δ 5.0 (q,1H,CH), δ 3.67 (s,3H,OCH_3), δ 3.05 (o,2H,CH_2), δ 2.72 (s,9H,NMe_3), δ 1.7 (m,2H,BH_2)]. The specific activity was 5.71 mCi/mmol, with a radiopurity of greater than 99% as determined by radioscan, using a Bioscan BID-100 Image Analyser.

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