SYNTHESIS OF [^{3}H] AMINOCYCLOPROPANE CARBOXYLIC ACID (ACC) AS A LIGAND FOR THE GLYCINE B RECEPTOR

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SUMMARY

The synthesis of [^{3}H] Aminocyclopropane carboxylic acid (ACC, 1) at high specific activity and high purity is described. The compound has been developed as a specific ligand for the Glycine-B binding site.

Key words: Aminocyclopropane carboxylic acid, Tritium, Glycine-B binding, Aminocyclopropene carboxylic acid

INTRODUCTION

Since the discovery of the modulation by glycine of the electrophysiological response induced by N-methyl-D-aspartic acid (NMDA)[1], research effort has been aimed at characterizing the interaction of glycine with the NMDA-phencyclidine (PCP) receptor complex. From the analysis of the structure-activity relationships at this site[2], it was quickly shown that the interaction was independent of the strychnine-sensitive inhibitory glycine receptor (glycine A)[3]. The new Glycine B site was characterized as a modulatory site of the NMDA receptor complex[4] which can influence the binding of PCP ligands[5]. Various types of antagonists[6, 7, 8, 9], partial agonists[10] and agonists have been identified. Amongst them aminocyclopropane carboxylic acid (ACC)[11, 12] appears to be a perfect candidate for development as a ligand to label the glycine B site because it manifests a high affinity for this site, it is devoid of affinity for the glycine A site as measured by [^{3}H] strychnine binding, and it is therefore likely to be a more specific ligand for the glycine B receptor than glycine itself. As a natural product found in plants, ACC is an intermediate in the enzymatic conversion of methionine to ethylene[13], but this interaction should not interfere with the binding of [^{3}H] ACC (1) to brain receptors because mammalian tissues are devoid of this specific enzymatic machinery.

We have designed a new route to [^{3}H] ACC which allows access to the compound at the high specific activity required for binding experiments in high yield and high purity. We report herein the chemical synthesis and purification of [^{3}H] ACC of high specific activity. The biological evaluation of [^{3}H] ACC as a ligand for the glycine B receptor will be reported elsewhere.

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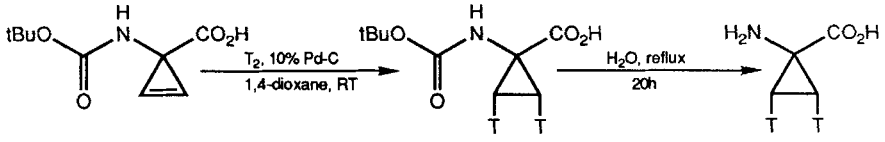
CHEMISTRY

For the preparation of [^{3}H] ACC we envisioned tritiation of a cyclopropene precursor. Scheme 1 describes the synthetic procedure used. The previously described[14] N-BOC aminocyclopropene carboxylic acid **2** was chosen as the starting material for our synthesis. The N-BOC protecting group provides solubility in aprotic solvents necessary for the tritiation and its removal is facile and described for ACC.[15] Thus **2** was treated with tritium gas in the presence of 10% palladium on carbon to rapidly (1 h) reduce the cyclopropene

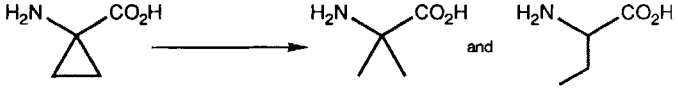
Scheme 1



2 **3** **1**

to provide [^{3}H] N-BOC ACC **3**. The choice of catalyst was based on reports of reduction of ACC to provide a mixture of 2-methyl alanine **4** and 2-amino butanoic acid **5** (Scheme 2).[16] Use of palladium on carbon is reported to reduce cyclopropenes to cyclopropanes without overreduction.[17]

Scheme 2



4 **5**

These overreduction products were not observed in this synthesis.

Compound **3** was purified by preparative reverse-phase HPLC prior to deprotection. A solution of **3** in water/acetonitrile [70:30, 0.05% trifluoroacetic acid (TFA)] was heated to 60-90 °C for 20 h to provide **1**, which was also purified by reverse-phase HPLC. The analysis of **1** follows.

ANALYSIS

Amino acids: 1-aminocyclopropane carboxylic acid, 1-aminocyclopropene carboxylic acid, 2-aminobutanoic acid, and 2-methyl alanine were analyzed via reverse-phase HPLC (Method A) using a Vydac C-18 column (pore diameter = 80Å, particle size = 10 micron, 4.6 mm (I. D.) x 25 cm) using 100% water (0.05% TFA) as eluent (flow rate = 1.00 mL/min). UV detection was used, monitoring at 205 nm. Retention times are listed in Table 1.

Table 1. Retention times of amino acids using isocratic elution (Method A).

Compound	R_t (min)
ACC	3.56
2-aminocyclopropene carboxylic acid	3.03
2-amino butanoic acid	3.76
2-methyl alanine	3.91

For monitoring the hydrogenation reaction the same reverse phase column was used, but a gradient-elution program was used (Method B, monitor at 205 nm). A 40:60 mixture of acetonitrile/water (0.05% TFA) was adjusted to 70:30 acetonitrile/water over 6 min, then held at this ratio for 3 min (flow rate = 1.20 mL/min). Retention times are listed in Table 2. N-BOC Aminocyclopropene carboxylic acid appears as a shoulder on the 1,4-dioxane signal, complicating the analysis until the dioxane is removed.

Table 2. Retention times of N-BOC amino acids using gradient elution (Method B).

Compound	R_t (min)
N-BOC ACC	3.92
2	3.24[a]
1,4-dioxane	2.88

a) Analysis of the starting N-BOC aminocyclopropene carboxylic acid indicated the presence of other small signals at R_t=3.10, 3.63, and 4.35 min. The material is clean by proton NMR analysis, and thus these signals are deemed minor impurities in that they do not interfere with the hydrogenation reaction.

Tritium labelled compounds were analyzed either by Method A or isocratically (Method C) using a Vydac C-18 column (4.5 mm x 25 cm). A 30:70 mixture of acetonitrile/water(0.05% TFA) was the eluant. For standards, UV detection at 205 nm was used. Radiochromatograms were obtained from timed fractions assayed by liquid scintillation counting.

Table 3. Retention times using isocratic elution (Method C)

Compound	Rt(min)
t-BOC-ACC	9.93
2	8.26

EXPERIMENTAL

Cold Synthesis

Hydrogenation of N-BOC aminocyclopropene carboxylic acid. N-BOC aminocyclopropene carboxylic acid **2** (6.0 mg, 0.030 mmol) was dissolved in 1,4-dioxane (6.0 mL) and 10% palladium on carbon (3.0 mg) was added. After briefly evacuating the reaction vessel (to partially de-gas the mixture), the vessel was filled with hydrogen gas (estimate 20 psi H_2 overpressure) and the mixture was vigorously stirred for 60 min. The mixture was filtered to remove the catalyst and the filter bed was further washed with dioxane (3 mL). The dioxane was evaporated in vacuo to leave crude N-BOC ACC (5.2 mg). ^{1}HNMR (300 MHz, $CDCl_3$) δ 1.58 (broad q, 2H), 1.44 (s, 9H), 1.22 (broad q, 2H).

Deprotection to Form aminocyclopropane carboxylic Acid.[15] Crude N-BOC ACC (5.2 mg) was suspended in water (3.0 mL) and heated to reflux for 20 h. The solvent was removed by rotary evaporation to give material identical to authentic ACC (HPLC analysis, Method A).

Hot Synthesis

Tritium reduction of N-BOC aminocyclopropene carboxylic acid. N-BOC aminocyclopropene carboxylic acid **2** (10.0 mg, 0.050 mmol) was combined with 1,4-dioxane (6.0 mL) and 10% palladium on carbon (5.0 mg). After exposure to carrier free tritium gas (120 Ci) for 1 h at room temperature an uptake of 1.0 cc was observed. The mixture was filtered to remove the catalyst and dried in a stream of nitrogen gas. The residue was dissolved in MeOH:dioxane (1/1) and again dried in a stream of nitrogen to remove labile tritium. The residue was redissolved in 10 mL of dioxane, assayed at 2.0 Ci of tritium at DuPont/NEN, and shipped to our laboratory at -70 °C.

Purification of [^{3}H] N-BOC aminocyclopropane carboxylic acid. Preparative HPLC was done on a Vydac C-18 column (10 mm x 25 cm) with the Method C eluant at 2.0 mL/min. An aliquot of crude [^{3}H] N-BOC ACC **3** (100 mCi) was evaporated, redissolved in 0.250 mL of eluant and injected. The column effluent corresponding to standard N-BOC ACC from 12.5 to 13.7 min contained 61.8 mCi of tritium and the response at 205 nm was equivalent to 0.271 mg of the standard. Radiochemical purity using HPLC Method C was 97.7% [^{3}H] N-BOC ACC, **3**.

Deprotection to form [^{3}H] aminocyclopropane carboxylic acid. The HPLC effluent containing 61.8 mCi of [^{3}H] N-BOC ACC (above) was diluted to five mL with Method C eluant and heated at temperatures between 60-90 °C for 7 h. Progress of the reaction followed by HPLRC (Method C) indicated complete conversion to [^{3}H] ACC with no by-products formed. The mixture was cooled to room temperature and concentrated in a stream of nitrogen gas to remove acetonitrile. An aliquot of 0.250 mL (11.3 mCi) was injected on a Vydac C-18 column (10 mm x 25 cm) and eluted with 100% water (0.00% TFA) at 4.0 mL/min.

Fractions were collected at 20 sec intervals and aliquots of 1.00 uL were removed from each to provide a radiochromatogram which corresponded to 99.8% [^3H] ACC at 4.00 min. Detection at 205 nm indicated 86% corresponding to **1**.

Purification of [^3H] Aminocyclopropane carboxylic acid. Two aliquots crude [^3H] ACC totalling 22.7 mCi in 0.500 mL of water (0.05% TFA) were injected on the Vydac C-18 column (10 mm x 25 cm) and eluted with 100% water (0.00% TFA) at 4.0 mL/min. The peaks corresponding to standard ACC at 3.7 to 3.9 min were collected and reduced in volume on a rotary evaporator at 50 ^oC. The residue (0.700 mL) was re-injected in 0.1 mL aliquots. The combined effluents corresponding to standard ACC were reduced in volume to 1.8 mL and diluted with 2.2 mL of water and 1.0 mL of EtOH to afford 5.0 mL of solution containing 4.75 mCi of [^3H] ACC. Radiochemical purity by HPLRC (Method A) was 99.0 $\pm$ 0.01% corresponding to co-injected standard ACC. Analytical recovery of injected tritium was 98.8 $\pm$ 0.97% for the three chromatograms. Radiochemical purity in 100% water (0.00% TFA) was 98.8 $\pm$ 0.31% with an analytical recovery of 99.8 $\pm$ 0.42%. Specific activity, determined from the preparative HPLC of compound **2**, was 46.8 Ci/mmol.

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