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Synthesis of (R) and (S) ^{14}C-labelled Ethyl Nipecotate, for preparation of GABA uptake ligands.

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SUMMARY

^{14}C-labelled (R)- and (S)-ethyl 3-[carboxyl-^{14}C]piperidinecarboxylate were synthesised in 3 steps, including a chiral HPLC separation, starting from 3-[carboxyl-^{14}C]pyridine-carboxylate. The overall radiochemical yield of the labelled (R)- and (S)-enantiomers were 67% and 61%. The radiochemical purities were higher than 98% with specific radioactivities of 48 mCi/mmol.

Key words: ^{14}C, nipecotic acid, GABA, ethyl 3-[carboxyl-^{14}C]piperidinecarboxylate, chiral resolvation, Gabitril®, Tiagabine.

INTRODUCTION

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS)[1,2]. A number of cyclic amino acids such as nipecotic acid inhibit the uptake of GABA and have been shown to enhance the biochemical and behavioral effects produced by GABA. These compounds can be considered as conformationally-restricted GABA analogs[3]. The hydrophilic nature of these cyclic amino acids prevents significant permeation of the blood-brain-barrier[4,5]. Attachment of a lipophilic moiety to the nitrogen atom of the cyclic amino acid leads to a compound that readily cross the blood-brain-barrier. One notable example is (R)-N-(4,4-Bis-(3-methyl-thien-2-yl)-but-3-enyl)-3-piperidinecarboxylic acid[6] (Gabitril®) shown in Figure 1. This compound has recently received regulatory approvals from the European health authorities for treatment of epilepsy.

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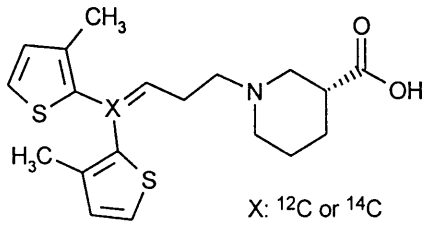


Figure 1 Structure of Gabitril®.

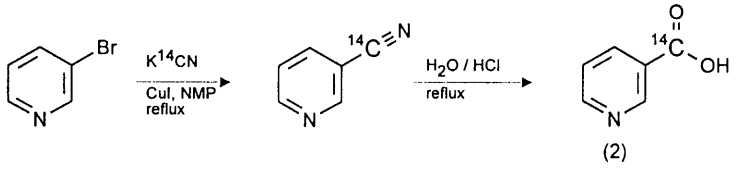
Gabitril® has previously been ^{14}C-labelled as shown in Figure 1 and has been used at Novo Nordisk A/S to examine the degradation pathways and mass balance of the compound[7]. Alternative labelling in the nipecotic acid part of Gabitril® and other GABA uptake inhibitors is of interest in such degradation studies since degradation products resulting from this part of the molecule are nor readily detected e.g. has close to zero UV response.

In the present study we describe the synthesis of ^{14}C-labelled (R)- and (S)-ethyl nipecotate ^{14}C-labelled in the carboxylic-group.

RESULTS AND DISCUSSION

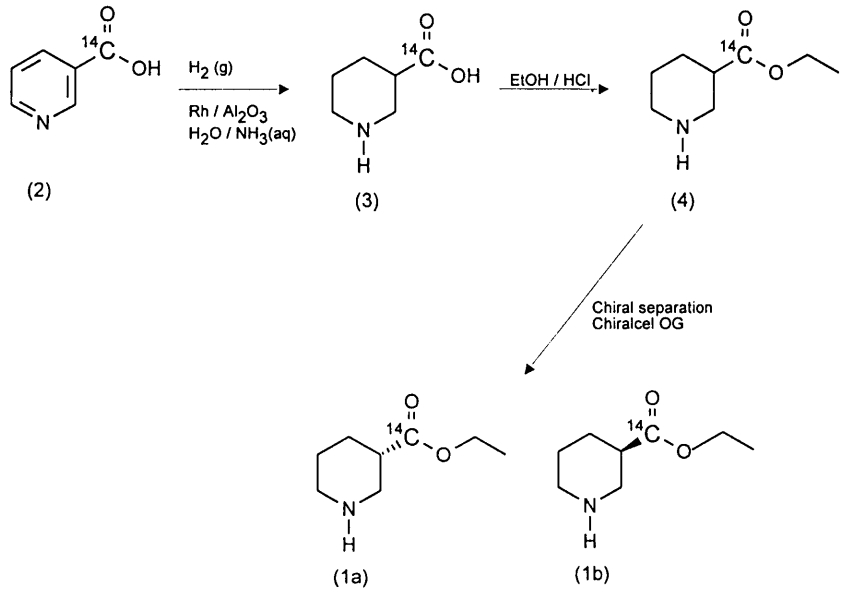
Scheme 2 shows the reaction sequence employed in the synthesis of (R)- and (S)-ethyl 3-[carboxyl-^{14}C]piperidinecarboxylate (1a + 1b).

In the present study we used the commercially available 3-[carboxyl-^{14}C]pyridine-carboxylate as starting material since we only needed a small amount of the desired ethyl 3-[carboxyl-^{14}C]piperidinecarboxylate. For larger scale preparations ^{14}C-labelled pyridinecarboxylate could easily be synthesised by cyanation of 3-brompyridine with a mixture of potassium [^{14}C]cyanide and copper (I) iodide in N-methylpyrrolidine (NMP)[8]. Subsequent hydrolysis of the nitrile results in the desired ^{14}C-labelled pyridine-carboxylate. This procedure was performed in our laboratory in approximately 80% overall labelling yield (Scheme 1).



Scheme 1 Synthetic route to 3-[carboxyl-^{14}C]pyridinecarboxylate.

The reduction of the ^{14}C-labelled pyridinecarboxylate was done in a mixture of water and ammonia with rhodium on alumina as catalyst. The reaction was performed at atmospheric pressure. The reduction time of 3-[carboxyl-^{14}C]pyridinecarboxylate was approximately 48 hours resulting in a 88% radiochemical yield. Carrying out the hydrogenation in aqueous solution containing a slight excess of ammonia prevented decarboxylation as seen under acidic conditions[9,10]. Concentration of the solution after reduction yielded the desired free acid.



Scheme 2 Synthetic route to (R)- and (S)-ethyl 3-[carboxyl-^{14}C]piperidinecarboxylate.

Esterification of 3 was carried out in ethanol and a mixture of HCl in ethanol was added to the reaction as catalyst. This method provided ethyl 3-[carboxyl-^{14}C]piperidinecarboxylate (4) in a 82% radiochemical yield. The crude product had a radiochemical purity >95%, as determined by radio-TLC. Sulphuric acid can also be used as catalyst, but pilot studies showed that poorly soluble salts of 3 precipitated.

Chiral purification of 4 using a chiralcel OG column (Figure 2) resulted in the two enantiomers 1a and 1b, each with a chiral purity of >98% as determined by chiral radio-HPLC analysis. TLC showed a radiochemical purity of >98%. The identity of the

compounds were determined by TLC and MS: Ethyl 3-[carboxyl-^{14}C]piperidine-carboxylate eluted with the same retention time as a standard reference sample. The specific radioactivity was 48 mCi/mmol as determined by MS using a standard reference sample.

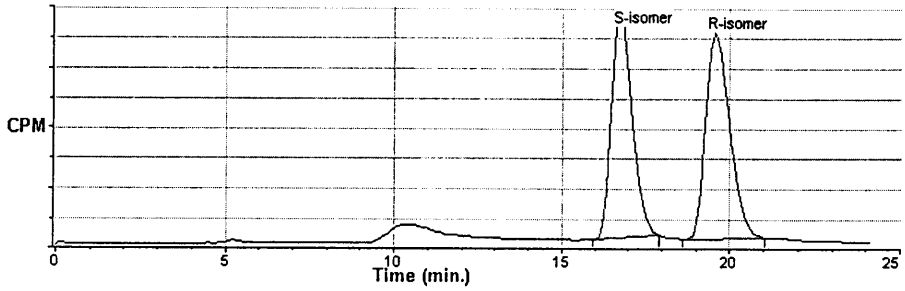


Figure 2 Semi-preparative chiral separation of (R,S)-ethyl 3-[carboxyl-^{14}C]piperidinecarboxylate using a Chiralcel OG column.

In conclusion, the ^{14}C-labelled enantiomers of ethyl 3-[carboxyl-^{14}C]piperidine-carboxylate were synthesised and resolved in a 3 step procedure, starting from 3-[carboxyl-^{14}C]pyridinecarboxylate. The overall radiochemical yield of the (R)- and (S)-enantiomers were 67% and 61%, the radiochemical purities were >98% and the specific radioactivities were 48 mCi/mmol. The chiral radiochemical purities were >98%.

EXPERIMENTAL

3-[Carboxyl-^{14}C]piperidinecarboxylic acid (3).

3-[Carboxyl-^{14}C]pyridinecarboxylate (0.98 mCi, 50 mCi/mmol; specific radioactivity given by American Radiolabeled Chemicals Inc.) was dissolved in water (2 ml) and 25% NH_3(aq) (0.50 ml). 5% Rhodium on alumina (29.5 mg) was added, and the mixture was hydrogenated at room temperature and atmospheric pressure. The reaction was followed using TLC (system I and II). After 2 days of hydrogenation TLC showed a radiochemical conversion of >95%. The solution was filtered using a Millex® filter (0.45 μm) and evaporated *in vacuo*.

Radiochemical yield: 0.86 mCi (88%). Radiochemical purity >95%, determined by radio-TLC analysis (system II).

Ethyl 3-[carboxyl-^{14}C]piperidinecarboxylate (4).

To crude 3-[carboxyl-^{14}C]piperidinecarboxylate (0.6 mCi) (3) was added ethanol (2 ml) and HCl in ethanol (0.3 ml, approximately 1.5 M). The mixture was stirred at room temperature for 21 hours. TLC (system I) showed complete conversion of the starting material. The solvent was removed by evaporation and the residue was dissolved in 2-propanol (0.2 ml) and n-hexane (1 ml).

Radiochemical yield: 0.49 mCi (82%). Radiochemical purity >95%, determined by radio-TLC analysis (system I).

Ethyl (R)-3-[carboxyl-^{14}C]piperidinecarboxylate (1a).

The 2-propanol/n-hexane mixture containing 4 (0.49 mCi) was purified by 5 injections on a Chiralcel OG column (250 x 4.6 mm, 10 µm). The collected fractions of relevant isomer were combined and evaporated *in vacuo*. The residue was dissolved and stored in ethanol (9.0 ml).

Radiochemical yield: 0.23 mCi (94%). Chiral radiochemical purity >98%, determined by radio-HPLC analysis. Radiochemical purity >98%, determined by radio-TLC (System I).

Ethyl (S)-3-[carboxyl-^{14}C]piperidinecarboxylate (1b).

Using the same procedure as described above for the R-isomer, 0.21 mCi (86%) of ethyl (S)-3-[carboxyl-^{14}C]piperidinecarboxylate (1b) was isolated with a chiral radiochemical purity >98%, determined by radio-HPLC. Radiochemical purity >98%, determined by radio-TLC (System I).

MATERIALS

3-[Carboxyl-^{14}C]pyridinecarboxylate was purchased from American Radiolabeled Chemicals Inc. The material had a radiochemical purity >99%, as determined byTLC. The specific radioactivity of the material was 50 mCi/mmol as determined by mass spectrometry. All solvents used were of analytical grade.

RADIOACTIVITY COUNTING

Radioactivity in the column effluent from the HPLC was monitored with a Radiomatic/Canberra Flo-One beta detector (A-515), using a 500 µl liquid flow cell. The ratio of column effluent to liquid scintillator (Opti-fluor™, Packard) was 1:2. Data collection was done by Flo-One data software on a PC-80386 computer.

Determination of total radioactivity was carried out on a Packard 1000 CA tri-carb liquid scintillation analyzer, using 20 ml counting vials and Opti-fluor™ Packard liquid scintillator.

MASS SPECTROSCOPY

The mass spectrometer was a Triple Quadrupole LC/MS/MS mass spectrometer from Perkin-Elmer Sciex Instruments.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC analyses were performed using a Merck HPLC pump L-6200 with a rheodyne injector (20 μl loop) and a Merck UV-detector L-4000 (operating at 232 nm). Preparative HPLC separations were performed using a Merck HPLC pump L6200 with a rheodyne injector (200 μl loop) and a Merck UV-detector L-4000 (operating at 232 nm).

Chiral HPLC was accomplished with a Chiralcel OG column (250 x 4.6, 10 μm) from Chiralcel Industries. The mobile phase was a mixture of n-hexan, 2-propanol, 2-butanol and diethyl amine (94/5/2/1). The flow rate was 0.8 ml/min.

TLC

TLC was performed on glass plates coated with 0.25 mm silica gel 60 F_{254} (Merck). The mobile phases were a mixture of CH_2Cl_2, MeOH, HCOOH (70:25:5, system I) or a mixture of n-butanol, ethanol, water (20:20:10, system II). Radio-TLC analysis were performed using a Bioscan Imaging Scanner System 200-IBM with an Autochanger 1000. The collimator grid contained 10 strings/mm and the P10 gas (10% methane in argon) flow was 1.5 l/min.

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