Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **43**, 557-563 (2000)

SYNTHESIS OF [1-^{11}C]PHENOL

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SUMMARY

The synthesis of no-carrier-added [1-^{11}C]phenol (**3**) which is a further important aromatic ^{11}C unit for potential PET radiotracers is described for the first time. [1-^{11}C]Aniline (**1**) was diazotized and the [1-^{11}C]benzenediazonium bromide (**2**) formed was concentrated *in situ* to give **3**. Before diazotization, **1** had to be purified twice by means of an adsorber resin and a cation exchange resin. Starting from the purified **1**, **3** was obtained in a radiochemical purity of about 79 % within 10 min. Related to crude **1** from the one-pot process, the reproducible radiochemical yield of **3** was about 16 % (decay-corrected). ^{13}C/^{11}C Co-labelling experiments were carried out in order to confirm the identity of **3** and the position of the label.

Keywords: PET, ^{11}C-ring labelling, diazotization, [1-^{11}C]aniline, [1-^{11}C]phenol

INTRODUCTION

Radiotracers labelled with ^{11}C ($t_{1/2}$ = 20.4 min) are used for monitoring specific biological processes by means of positron emission tomography (PET).

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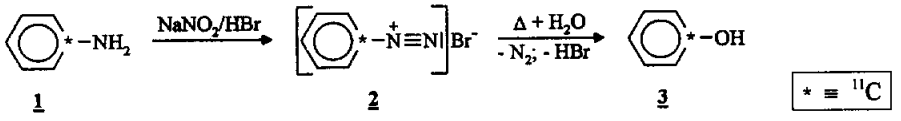
Received 26 March 1999
Revised 22 September 1999
Accepted 24 November 1999

Within our investigations aimed at developing methods for ^{11}C-ring labelling of aromatic metabolically stable compounds [1-3], we dealt with the preparation of [1-^{11}C]phenol (3) as another important aromatic ^{11}C unit for potential PET radiotracers.

The synthesis of nitro-[1-^{11}C]benzene, prepared by reaction of 5-dimethylamino-penta-2,4-dienylidene-dimethylammonium perchlorate with nitro-[^{11}C]methane, and the subsequent reduction of nitro-[1-^{11}C]benzene to [1-^{11}C]aniline (1) has been described in [1]. For preparation of 3 the primary aromatic amine 1 was diazotized. In detail, 1 was converted with aqueous bromohydric nitrous acid to the [1-^{11}C]benzenediazonium bromide (2), which was concentrated *in situ* to 3 by cleavage of nitrogen according to Scheme 1:



Scheme 1

RESULTS AND DISCUSSION

[1-^{11}C]Aniline (1) was synthesized in a one-pot process according to [1]. In the reaction mixture of 1 there were various potentially interfering substances (dimethylamine, potassium perchlorate, by-products from the excess precursor, sodium sulphide, hexamethylphosphoric triamide). For consecutive reactions such as the diazotization of 1 it was necessary to separate these impurities.

For purifying crude 1, we examined several solid-phase extraction methods. First attempts were carried out by using an RP-18-cartridge. For this purpose the crude reaction mixture of 1 was buffered to pH 7 and diluted with water. The adsorption of 1 on the cartridge was on an average of 34 %. Radiochemically pure 1 was extracted with diluted HBr in yields of about 27 %. However, the conversion of purified 1 with nitrous acid according to Scheme 1 did not give the desired 3. Only unidentifiable products were obtained.

The next experiments for purifying 1 were done by means of (i) an adsorber resin (polystyrene which is able to adsorb non-polar and weakly polar substances, such

as aniline) and (ii) a cation exchange resin. In neither of these methods was the desired compound formed.

Finally, we found that significant yields of **3** according to the classical way of Scheme 1 could be obtained only by combining the two purification steps of **1** mentioned above as follows:

(i) Solid phase extraction of **1** from its water-diluted alkaline reaction mixture on a polystyrene cartridge (LiChrolut EN, Merck), acidification of the cartridge, followed by elution of **1** with ethanol (yield of purified **1**: 52 %).

(ii) Adsorption of **1** from its acidified, water-diluted ethanolic solution on a cation exchange resin cartridge (DOWEX 50 WX 8, SERVA) as [1-^{11}C]anilinium cation followed by elution with diluted ammonia (yield of purified **1**: 39 %).

After these two purification steps a very pure **1** (> 99 % radiochemical purity) was obtained in total yields (decay-corrected) of about 20 %, starting from crude **1** from the one-pot process.

The diazotization of the radiochemically pure **1** in its bromohydric solution and the subsequent concentration of the diazonium salt **2** produced 79.1 % of **3** and 18.2 % of an unidentified ^{11}C-labelled product (percentages are decay-corrected). No [1-^{11}C]aniline (**1**) was found, i.e. it was completely converted. The radiochromatogram of **3** is shown in Fig. 1.

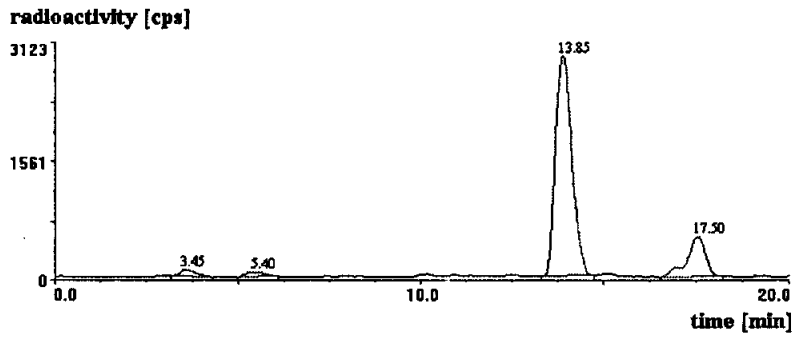


Fig. 1: Radiochromatogram of [1-^{11}C]phenol (**3**) after diazotization of purified [1-^{11}C]aniline (**1**) and concentration of the diazonium salt **2** formed
3.45 min: unidentified product; 1.4 %
5.40 min: unidentified product; 1.3 %
13.85 min: [1-^{11}C]phenol (**3**); 79.1 %
17.50 min: unidentified product; 18.2 %

For simultaneous detection of 1 (13.00 min) and 3 (13.85 min) by means of HPLC a gradient with a small slope (0 min - 100 % buffer/ 0 % acetonitrile; 20 min - 0 % buffer/ 100 % acetonitrile; see Fig. 1) had to be used.

The identity of the radioactive products 1 (6.35 min) and 3 (7.81 min) were confirmed by using a second HPLC system that works isocratically with isopropanol/water (20/80) containing 0.1 M ammonium formate.

$^{13}C/^{11}C$ Co-labelling experiments were carried out in order to unequivocally affirm the identity of 3 and its labelling position. The position of the ^{11}C label was confirmed by analysis of the ^{13}C-NMR spectrum of [1-^{13}C]phenol which was synthesized by the same method used for 3 through addition of nitro-[^{13}C]methane immediately after the trapping of nitro-[^{11}C]methane according to [1]. The strong ^{13}C signal at $\delta = 156.0$ ppm corresponded to the ^{13}C signal of the C-1 of authentic phenol. The [1-^{13}C]phenol was also analysed by MS, indicating a molecular ion peak at m/z 94 (M-1), which corresponded with the molecular ion peak at m/z 93 (M-1) observed with authentic phenol.

EXPERIMENTAL

General

To determine the extent of the reaction conversion and the radiochemical purity of the reaction products and to identify the products, analytical HPLC was used. HPLC was performed with an HPLC system from Merck-Hitachi, including a gradient pump (L-6200A), a Rheodyne injector with a 20 µl loop and a diode array detector (L-4500 DAD) coupled in series with a radioactivity detector FLO-ONE\Beta A500 (Canberra Packard). Two different analytical HPLC systems were used: a gradient system and a isochratic system.

Gradient system:

The column used was a Merck LiChrospher 100 RP-18 endcapped, 5µm, 150 mm x 3.3 mm. The mobile phase consisted of phosphate buffer pH 7 ($c[NaH_2PO_4] = 0.26$ mM; $c[Na_2HPO_4] = 0.51$ mM) and acetonitrile at a flow rate of 0.5 ml/min, with the following linear gradient of the eluents: 0 min - 100 % buffer/ 0 % MeCN; 20 min - 0 % buffer/ 100 % MeCN.

Isochratic system:

These analyses were done with a NUCLEOSIL 120 RP-18 column (5 µm, 125 mm x 4 mm, Macherey-Nagel) eluted isocratically with isopropanol/water (20/80) containing 0.1 M ammonium formate at a flow rate of 0.5 ml/min.

Nitrobenzene p.a. (Fluka, Switzerland), aniline p.a. (Fluka, Switzerland) and phenol p.a. (Berlin Chemie, Germany) were used as reference substances. For co-labelling experiments nitro-[^{13}C]methane (99 atom % ^{13}C) was purchased from Aldrich.

^{13}C-NMR spectra were recorded on a Varian INOVA 400 spectrometer at 100.6 MHz with $CDCl_3$ as internal standard.

Mass spectrometric analyses were carried out on a Micromass tandem quadrupole mass spectrometer (Quattro LC) operated in the MS mode. Mass spectral data were recorded in the negative ESI mode using a cone voltage of 35 V. A solution of phenol or [1-^{13}C]phenol in MeOH was infused at a flow rate of 5 µl/min.

Radiosyntheses

Nitro-[^{11}C]methane was prepared as previously described [1], starting from [^{11}C]CO_2 *via* [^{11}C]CH_3I. [1-^{11}C]Aniline (1) was synthesized according to the one-pot procedure which is also described in [1].

Purification of [1-^{11}C]aniline (1)

The reaction mixture containing 1 was diluted with water (1 ml) and passed through an activated polystyrene cartridge (LiChrolut EN, Merck). After washing the cartridge with water (10 ml) and with aqueous HBr (2 ml, 10 %), 1 was eluted with ethanol (2 ml).

The acidic ethanolic [1-^{11}C]aniline solution was diluted with water (8 ml) and passed through a cartridge filled with cation exchange resin DOWEX 50 WX 8 (SERVA, 100-200 mesh, H^+ form, neutral). The resin was washed with water to make it neutral. Radiochemically pure 1 was eluted with ammonia (2 M).

[1-^{11}C]Phenol (3)

The ammoniacal solution (2 ml) was acidified with HBr (0.5 ml, 40 %) and cooled to 0 °C. 1 was diazotized by adding $NaNO_2$ (5 mg, 72 µmol) in water (50 µl) to the cooled solution. After 5 min at 0 °C the reaction mixture was heated at 100 °C for 5 min. The diazonium salt 2 formed was then concentrated and 3 was obtained.

^{11}C/^{13}C Co-labelling experiments

Nitro-[1-^{11}C/^{13}C]benzene

Nitro-[^{11}C]methane was trapped in a cooled 2 ml vessel (10 °C) containing HMPT (250 µl), 5-dimethylaminopenta-2,4-dienylidene-dimethylammonium perchlorate (8 mg, 32 µmol) and t-BuOK (3.5 mg, 31 µmol). Nitro-[^{13}C]methane (1 µl, 18.5 µmol) was added. Cyclization/ aromatization into nitro-[1-^{11}C/^{13}C]benzene was achieved by heating the well sealed vessel at 170 °C for 7 min.

[1-^{11}C/^{13}C]Aniline

The reduction to obtain [1-^{11}C/^{13}C]aniline was performed by adding an excess of $Na_2S{\cdot}9H_2O$ (28 mg, 117 µmol) in water (100 µl) to the above reaction mixture and heating at 170 °C for 30 min.

Then the reaction mixture was diluted with water (1 ml) and passed through an activated polystyrene cartridge (LiChrolut EN, Merck). After washing the cartridge with water (20 ml) and with aqueous HBr (2 ml, 10 %), [1-^{11}C/^{13}C]aniline was eluted with ethanol (2 ml).

The acidic ethanolic [1-^{11}C/^{13}C]aniline solution was diluted with water (8 ml) and passed through a cartridge filled with cation exchange resin DOWEX 50 WX 8 (SERVA, 100-200 mesh, H^+ form, neutral). The resin was washed with water to make it neutral. Pure [1-^{11}C/^{13}C]aniline was eluted with ammonia (2 M).

[1-^{11}C/^{13}C]Phenol

The ammoniacal solution (3 ml) was acidified with HBr (1 ml, 40 %) and cooled to 0 °C. [1-^{11}C/^{13}C]Aniline was diazotized by adding $NaNO_2$ (10 mg, 145 µmol) in water (100 µl) to the cooled solution. After 5 min at 0 °C the reaction mixture was

heated at 100 °C for 5 min. The diazonium salt formed was then concentrated and [1-^{11}C/^{13}C]phenol was obtained. It was analysed by two different HPLC systems mentioned above.

After decay of the ^{11}C radioactivity, the acid solution was passed through an activated polystyrene cartridge (LiChrolut EN, Merck). The cartridge was washed with water to make the resin neutral. Then the polystyrene cartridge was dried by means of a nitrogen stream (3 l/h). [1-^{13}C]Phenol was eluted from the cartridge with $CDCl_3$ (1 ml) and analysed by ^{13}C-NMR. A part of the $CDCl_3$ solution of [1-^{13}C]phenol was evaporated for MS investigations in MeOH.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. J. Römer of our institute for his helpful discussion of this work. The authors also thank Dr. M. Scheunemann for recording the NMR spectra and Mrs. K. Fischer for recording the mass spectra.

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