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Research Article

Radiosynthesis of a 2-substituted 4,5-dihydro-1H-[2-^{11}C] imidazole: the I_2 imidazoline receptor ligand [^{11}C] benazoline

D. Roeda*, F. Hinnen and F. Dollé
Département de Recherche Médicale, Service Hospitalier Frédéric Joliot, CEA, 4 Place du Général Leclerc, Orsay F-91401, France

Summary

Benazoline (2-naphthalen-2-yl-4,5-dihydro-1H-imidazole) is a selective high-affinity ligand for the imidazoline I_2 receptor. This compound was labelled with carbon-11 ($T_{1/2} = 20.4$ min) at the number two carbon atom of its 2-imidazoline ring. Cyclotron-produced [^{11}C]carbon dioxide reacted with 2-naphthylmagnesium bromide to give 2-[carboxyl-^{11}C]naphthoic acid in 60% radiochemical yield. The latter was heated with a mixture of ethylenediamine and its dihydrochloride at 300°C to give [^{11}C]benazoline in 16% overall yield, relative to [^{11}C]carbon dioxide and with a specific radioactivity of 54 GBq/μmol, decay corrected for end of irradiation. The procedure requires about 45 min from end of cyclotron irradiation. This method should be extendable to other imidazolines.

Key Words: [2-^{11}C]imidazolines; [^{11}C]benazoline; imidazoline receptor

Introduction

2-Imidazoline (4,5-dihydro-1H-imidazole), bearing an alkyl or an aryl group at the 2-position, frequently constitutes a compound of interest in medicinal chemistry. About 25% of nearly 4000 published compounds of this structure, are reported on for their pharmacological activity.[1]

*Correspondence to: D. Roeda, Département de Recherche Médicale, Service Hospitalier Frédéric Joliot, CEA, 4 Place du Général Leclerc, F-91401 Orsay, France. E-mail: roeda@dsvidf.cea.fr

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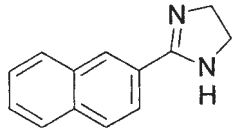
One of these compounds is 2-naphthalen-2-yl-4,5-dihydro-1H-imidazole (benazoline, **1**). It has a high affinity for the imidazoline I_2 receptor ($pK_i = 9.07$) with a high selectivity for I_2 relative to the α_1- and the α_2 receptors (2691- and 18621-fold, respectively).[2]



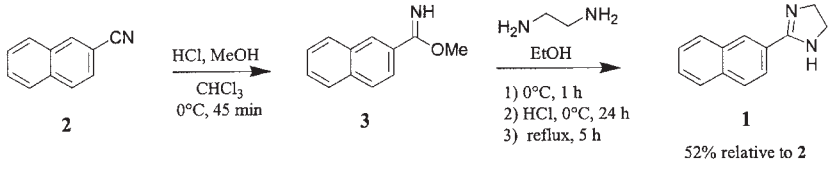
Benazoline (**1**)

Our interest in the study of receptor systems in the living human brain by positron emission tomography (PET) prompted us to envisage labelling of compound **1** with the short-lived positron-emitting radioisotope carbon-11 (half-life = 20.4 min). To date, no suitable PET ligand has been available for the imidazoline receptor system.

Carbon atom number two of the 2-imidazoline ring, part of the molecule's amidine substructure, is the only candidate for labelling with carbon-11. The other two are practically inaccessible with current carbon-11 labelling technology, let alone those of the naphthalene system. To our knowledge, introduction of a carbon-11 atom in this position has not been published before. Thus, the exploration of the synthesis of [^{11}C]benazoline may not only provide us with an interesting new research tool in PET, but also give us a novel labelling pathway that possibly can be extended to other 2-substituted 2-imidazolines.

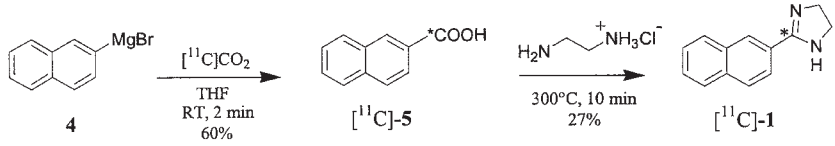
Results and discussion

Benazoline (**1**) was synthesized according to a literature procedure[2] in 52% yield from 2-cyanonaphthalene (**2**) via naphthalene-2-carboximidic acid methyl ester (**3**) (Scheme 1). This is an example of the usual method



Scheme 1.

of synthesis of 2-substituted 2-imidazolines, namely the cyclization reaction of ethylenediamine with a carboxylic acid or rather one of its derivatives.[3] The reaction sequence in Scheme 1 is rather unsuitable for our labelling purpose (too many steps) but the approach in general is convenient to the extent that [^{11}C]carboxylic acids are normally easy to make in good yields from [^{11}C]carbon dioxide and the appropriate Grignard reagent. Its disadvantage lies in the fact that the radioactivity introduction is to be followed by at least one chemical step, but this drawback looks inevitable for this type of molecule in any case. So we have developed a radiosynthesis for [^{11}C]benazoline ([^{11}C]-**1**) according to the more straightforward route outlined in Scheme 2.



4 [^{11}C]-**5** [^{11}C]-**1**

Scheme 2.

2-Naphthylmagnesium bromide (**4**) was freshly prepared from 2-bromonaphthalene before each [^{11}C]benazoline run, following a literature procedure that employs a substantial amount of 1,2-dibromoethane as an initiator.[4] The final reaction mixture of Grignard in tetrahydrofuran (THF) was used as such in the radiosynthesis. Before this, samples of the Grignard preparation were analysed by hydrolysis to naphthalene and by carbonation to 2-naphthoic acid (**5**), giving average yields of 76 and 69%, respectively (HPLC), relative to the theoretical 0.62 M Grignard concentration.

2-[^{11}C]naphthoic acid ([^{11}C]-**5**) could be made in about 60% radiochemical yield from cyclotron-produced no-carrier-added [^{11}C]carbon dioxide by passing the latter with an inert vector gas into 400 µl of the Grignard solution at room temperature. The radioactivity trapping efficiency was 95%. When the reaction mixture was acidified, 37% of the radioactivity, most likely non-reacted [^{11}C]carbon dioxide, could be chased by bubbling through an inert gas. Radio-HPLC analysis showed that the remainder was only 2-[^{11}C]naphthoic acid. This is in contrast with an early report on the similar synthesis of 2-[^{14}C]naphthoic acid (yield 73%), reporting the side product [^{14}C]di-2-naphthylketone.[5] 2-Naphthylmagnesium bromide (**4**) is only moderately reactive, seen the important amount of non-reacted [^{11}C]carbon dioxide. Indeed, at −20°C we found no reaction at all and increasing the reaction

temperature to 60°C did not improve the yield. When less Grignard solution was used (100 µl) the [^{11}C]carbon dioxide trapping efficiency dropped considerably, also at −20°C.

Aryllithium compounds are generally more reactive than arylmagnesium compounds and usually give ketones with carbon dioxide.[6] Nevertheless, we tried to obtain 2-[^{11}C]naphthoic acid also through carbonation of 2-naphthyllithium, made from 2-bromonaphthalene and *n*-butyllithium, in order to avoid the rather laborious Grignard preparation before each radiosynthesis. However, this preparation did not react at all with [^{11}C]carbon dioxide. Possibly the concentration we used was too low or the 2-naphthyllithium, which was reported to be particularly sensitive to the Wurtz-coupling, was lost, as was reported for the reaction of 2-bromonaphthalene and lithium metal that gave exclusively di-2-naphthyl.[7] Although formation of 2-naphthyllithium from *n*-butyllithium has been reported in the literature (e.g. ref. 8), we did not further explore this option, the more so because there would be a risk of attack of non-reacted *n*-butyllithium on the naphthalene system of the formed 2-[^{11}C]naphthoic acid.[9]

For the second step, the conversion of 2-[^{11}C]naphthoic acid ([^{11}C]-**5**) into [^{11}C]benazoline ([^{11}C]-**1**), we based ourselves on a German patent from 1938.[10] Our method consisted in hydrolysis of the reaction mixture of [^{11}C]-**5**, not with HCl as above, but with an aqueous solution of ethylenediamine and its dihydrochloride. The mixture was then heated at 300°C during 10 min, with the reaction vessel open to atmosphere, giving [^{11}C]-**1** in 27% radiochemical yield with respect to [^{11}C]-**5** and in an overall radiochemical yield of 16% with respect to [^{11}C]carbon dioxide. There is no reaction at lower temperatures. The reaction mixture was taken up in *N*,*N*-dimethylformamide (DMF) for HPLC injection. Water, which perturbs less the HPLC system, can also be used, but leaves more undissolved solids which can give problems with the injection. [^{11}C]Benazoline ([^{11}C]-**1**) was isolated using reversed-phase semi-preparative HPLC. Apart from very little 2-[^{11}C]naphthoic acid ([^{11}C]-**5**), two radioactive side products were seen on the chromatogram in-between [^{11}C]-**5** and [^{11}C]-**1**. The first apparently comes from reaction of [^{11}C]carbon dioxide with ethylenediamine and is most likely [^{11}C]ethyleneurea.[11] The second one may very well be *N*-(2-aminoethyl)-2-[^{11}C]naphthamide.[10] [^{11}C]Benazoline ([^{11}C]-**1**) was formulated by a C18-Seppak®-cartridge procedure. It was eluted from the cartridge with a small amount of ethanol followed by physiological serum or citric acid buffer (pH = 4.7), the latter giving a slightly better

result. The specific radioactivity was about 54 GBq/μmol (decay corrected for end of irradiation) for a 30 min/30 μA irradiation. The whole procedure can be carried out in 45 min.

A reagent that can serve as an alternative to ethylenediamine is ethyleneurea (2-imidazolidone),[12] which we confirmed by a non-radioactive experiment. The yield is higher, but the solidified melt, after cooling, is difficult to dissolve for HPLC injection and we discarded this potentially interesting approach for this technical reason. Two other methods employing ethylenediamine and carboxylic acids did not work for 2-naphthoic acid in our hands, the first using toluene as a solvent[13] and the second using triphenylphosphine, tetrachloromethane and triethylamine at room temperature.[14]

The condensation of a carboxylic acid with ethylenediamine, which we have adopted for the [^{11}C]benazoline synthesis, has a wide scope.[10] We assume that the presented procedure can be extended to a general method to obtain labelled 2-substituted 2-imidazolines.

Experimental

All chemicals were purchased from Aldrich, France except for 2-cyanonaphthalene (Lancaster, UK) and magnesium turnings (Farmitalia Carlo Erba, Italy) and were used without further purification. An isotonic citrate buffer (pH 4.7) was prepared from citric acid (0.12 g) and sodium citrate (0.24 g) in sterile water (10 ml), and then submitted to sterile filtration. Air- or moisture sensitive reactions were conducted in oven-dried glassware and under an inert atmosphere. ^{1}H-NMR and ^{13}C-NMR were recorded on a Bruker AMX (300 MHz) apparatus at room temperature using TMS as an internal standard. The chemical shifts are reported in ppm, downfield from TMS. Abbreviations used: s, m, br for singlet, multiplet and broad, respectively. HPLC was performed on a reversed-phase column (Xterra MS C18, 7 μm, 7.8 × 300 mm) using a mixture of water, acetonitrile and triethylamine (70/30/0.05 v/v/v = system A or 60/40/0.1 v/v/v = system B) as the mobile phase (5 ml/min) and employing a UV (254 nm)- and a Geiger–Müller detector.

2-*Naphthalen-2-yl-4,5-dihydro-1H-imidazole (benazoline,* **1***)*

A solution of 2-cyanonaphthalene (1 g, 6.54 mmol) and methanol (0.53 ml, 13.08 mmol) in chloroform (10 ml) was cooled in an ice/water

bath and gaseous hydrochloric acid was passed through over 45 min. The yellow reaction mixture was kept for 12 h in a refrigerator at 0–4°C. Diethyl ether (50 ml) was added and the formed precipitate was filtered off (pale-yellow solid; 2.2 g). This solid, which is highly impure naphthalene-2-carboximidic acid methyl ester hydrochloride, was added to a solution of ethylenediamine (6.7 ml, 100 mmol) in absolute ethanol (300 ml) cooled in an ice/water bath. After the mixture had been stirred over 1 h at 0°C, a mixture of concentrated hydrochloric acid (7 ml) and ethanol (20 ml) was added carefully, causing a white precipitate. The mixture was stored in the refrigerator for 24 h and then, after dilution with ethanol (100 ml), refluxed over 5 h. The precipitate was filtered off and discarded. The filtrate was evaporated till dryness and the residue was taken up in chloroform. This organic solution was washed with 3 N sodium hydroxide and dried on sodium sulphate. Evaporation of the solvent gave a yellow solid (**1**,667 mg, 52%). ^{1}H-NMR (d_6-DMSO): δ: 4.14 (4H, s), 6.80–7.20 (1H, br), 7.56–7.58 (2H, m), 7.93–8.02 (4H, m), 8.35 (1H, s). ^{13}C-NMR (d_6-DMSO): δ: 49.4 (CH_2), 124.3 (CH), 125.9 (CH), 126.2 (CH), 126.4 (CH), 127.0 (CH), 127.1 (CH), 127.9 (CH), 132.0 (C_q), 133.4 (C_q), 163.2 (C_q).

2-*Naphthylmagnesium bromide* (**4**)

Magnesium turnings (214 mg, 8.79 mmol) were placed in a reaction vessel, equipped with magnetic stirring and an addition funnel and provided with a nitrogen atmosphere, and were covered with dry THF (3 ml). The addition funnel was charged with 2-bromonaphthalene (1.668 g, 8.04 mmol) in dry THF (12 ml). 1,2-Dibromoethane (98 µl, 1.14 mmol) was added at once into the reaction vessel, which was then immersed in a heating bath at 80°C. As soon as a refluxing condition was reached the contents of the addition funnel were added dropwise over 15 min. Refluxing was continued for 1 h after which all magnesium had been consumed. The red–brown reaction mixture was used as such in the radiochemistry procedure (see below). Its quality was checked in two ways: (1) An aliquot (50 µl) was hydrolyzed with HPLC mobile phase and analysed for naphthalene (system B, retention time: 6.8 min). (2) Ten millilitres of carbon dioxide gas were passed through an aliquot of the Grignard solution (400 µl) in 1 min, followed by hydrolysis with HPLC mobile phase. The mixture was analysed for 2-naphthoic acid on HPLC (system A, retention time: 3.8 min).

2-*Naphthyllithium*

2-Bromonaphthalene (1 mg, 5 μmol) was dissolved in dry THF (200 μl) in a septum-capped vial. The solution was cooled in an ice/water bath and *n*-butyllithium (7 μl, 2.5 M in hexanes, 15 μmol) was added by syringe through the septum. The mixture was left at 0°C for 5 min and was then used in its totality for trapping [^{11}C]carbon dioxide at −10°C.

[^{11}C]Carbon dioxide

No-carrier-added [^{11}C]carbon dioxide was produced using the nuclear reaction $^{14}N(p,\alpha)^{11}C$ by irradiation of a pressurized nitrogen gas target (6 bar) with a cyclotron-generated 20 MeV proton beam.[15] A current of 30 μA during 30 min gave rise to ∼48 GBq (1.3 Ci) of radioactive carbon dioxide. After irradiation, the radioactive gas was released from the target holder through a drying column (70 mm × 4 mm) filled with phosphorus pentoxide. The [^{11}C]carbon dioxide was frozen out in a liquid-argon cooled stainless steel coil that was positioned in a shielded hot cell in which all further radioactivity manipulations were performed remotely controlled.

*[Carboxyl-^{11}C]2-naphthoic acid ([^{11}C]-**5**)*

A solution of freshly prepared 2-naphthylmagnesium bromide (**4**) in THF (400 μl, see above) was placed in a small conical reaction vial equipped with a septum to accommodate the needles connected to the appropriate in- and outlet Teflon® tubing. [^{11}C]carbon dioxide was released from its cooled trap by warming to room temperature and carried by a nitrogen gas stream (3 ml/min) into the Grignard solution at room temperature. A trap, containing soda lime, had been placed in series to collect any [^{11}C]carbon dioxide not trapped in the reaction vial. When the radioactivity accumulation in the vial had levelled out, the gas flow was discontinued and the reaction mixture was left to stand for 2 min. At this point the solution was either further used in the synthesis of [^{11}C]benazoline (see below) or hydrolyzed for HPLC analysis. In the latter case aqueous HC1 (10 N, 50 μl) in acetonitrile (450 μl) was added. A nitrogen stream was bubbled through the resulting acidic mixture until all volatile radioactivity had been chased. A sample of the mixture was then analysed with HPLC (system A, retention time

2-[^{11}C]naphthoic acid = 3.8 min; note that [^{11}C]carbon dioxide elutes almost indistinguishably close to 2-[^{11}C]naphthoic acid).

*2-Naphthalen-2-yl-4,5-dihydro-1H-[2-^{11}C]-imidazole ([^{11}C]benazoline, [^{11}C]-***1***)*

A solution of ethylenediamine dihydrochloride (200 μmol, 26.6 mg) and ethylenediamine (200 μmol, 13.4 μl) in water (100 μl) was added to the 2-[^{11}C]naphthoic acid ([^{11}C]-**5**) reaction mixture (see above) followed by another 100 μl of water to rinse all ethylenediamine from the addition line into the reaction vial. After nitrogen had been set bubbling through the mixture (20 ml/min), the reaction vial was placed in a heating block at 320°C (effective maximum temperature inside the vial: 300°C). All liquid evaporated quickly and the vapours were guided and condensed via a wide-bore (2 mm id) outlet Teflon® tube into a receiver. It was important that this outlet line had a less than 90° bend close to the reaction vial in order to prevent refluxing of liquid back into the vial. After 2 min all liquids had evaporated. The nitrogen flow was stopped and heating was continued for another 8 min. The vial was carefully cooled down after the nitrogen flow had been restored (to avoid blocking of the inlet needle). DMF (1 ml) was added and nitrogen bubbling was continued for 1 min to promote solubilization of the solids. The DMF solution was then transferred into an HPLC injector loop and injected into the HPLC (system A). The [^{11}C]benazoline peak (retention time ~11 min) was collected in a reservoir containing water (40 ml). The [^{11}C]benazoline was absorbed on a reversed-phase Seppak® cartridge by passing the above aqueous solution through it, followed by water (10 ml) to wash out any organic solvents. The [^{11}C]benazoline was eluted from the cartridge with ethanol (0.5 ml) followed by physiological saline (4.5 ml) or an isotonic citrate buffer (pH = 4.7, 4.5 ml). HPLC analysis (system A; retention time of benazoline = 10.8 min) of this solution showed a single radioactive product co-eluting with co-injected authentic synthesized benazoline. The UV trace did not show any products but benazoline. The specific radioactivity of [^{11}C]benazoline was determined with the same HPLC system by comparing the absorbance of [^{11}C]benazoline to that of a known benazoline standard and correlating the calculated mass with the measured radioactivity. The radiochemical yield was deduced from the total recovered radioactivity (waste, soda lime, reaction vial, etc.) that corresponded well with the expected amount of [^{11}C]carbon dioxide produced.

Conclusion

We have developed a radiosynthesis for the imidazoline I_2 receptor ligand [^{11}C]benazoline for further exploration with PET. The synthesis consists in the condensation of a carbon-11-labelled carboxylic acid with ethylenediamine, introducing the label at the 2-carbon atom position of a 2-substituted 2-imidazoline ring. We expect that this method can be extended to many other imidazolines of this type.

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