N.C.A. ^{11}C-LABELLING OF BENZENOID COMPOUNDS IN RING POSITIONS: SYNTHESIS OF NITRO-[1-^{11}C]BENZENE AND [1-^{11}C]ANILINE

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SUMMARY

The paper describes the first method for n.c.a. ^{11}C-ring labelling of benzenoid compounds having a reactive group for further derivatization by use of the known principle of synchronous six-electron cyclization of hexatriene systems into aromatics. Nitro-[^{11}C]methane (1) prepared from cyclotron-produced [^{11}C]carbon dioxide reacts in the presence of t-BuOK with 5-dimethylaminopenta-2,4-dienylidene-dimethylammonium perchlorate (2) to form 6-nitro-1-dimethylamino-[6-^{11}C]hexatriene (3) followed by cyclization/ aromatization into nitro-[1-^{11}C]-benzene (4) at increased temperatures. Starting from 1, nitro-[1-^{11}C]benzene of a radiochemical purity of about 92 % and a mean specific radioactivity of 1 Ci/μmol was obtained within 7 min. Related to [^{11}C]CO_2, the reproducible radiochemical yield of 4 (decay-corrected) was 80$\pm$5 %. Reduction of 4 by heating the above reaction mixture with aqueous Na_2S gave [1-^{11}C]aniline (5) of a radiochemical purity of about 81 %. The reproducible radiochemical yield of 5 (decay-corrected) in relation to [^{11}C]CO_2 was 65$\pm$5 %, the synthesis time from 1 was 18 min.

Keywords: PET, ^{11}C-ring labelling, nitro-[^{11}C]methane, nitro-[1-^{11}C]benzene, [1-^{11}C]aniline

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INTRODUCTION

Since the fifties many syntheses of ^{13}C- and ^{14}C-ring-labelled benzenoid compounds have been published. Among them, however, there was no procedure which would be suited to prepare benzenoid compounds containing the positron-emitting radionuclide ^{11}C ($t_{1/2}$ = 20.4 min) in their ring, even though some attempts have been carried out in the past.

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Early investigations [1] dealing with the reaction of cyclopentadiene and free ^{11}C atoms in "hot" and "thermal" systems gave only mixtures of different products which mainly consisted of ^{11}C labelled polymers, but [^{11}C]benzene was also formed in yields of 8-11 %. When the classic synthesis of benzene, toluene, and xylene was modified by catalytic trimerizing [^{11}C]-acetylene with acetylene and propyne carriers, the result was also a mixture of products of very low specific radioactivities (60 μmol inactive acetylene in the synthesis of [^{11}C]benzene) and low yields (about 25 % [^{11}C]benzene) [2].

Our own experiments to synthesize ^{11}C-ring-labelled benzenoid compounds have been begun with a pyrolytic procedure in accordance to some experimental details on ^{14}C-labelling which were published by Kopinke et al. [3]. We tried to transfer Kopinke's data to our ^{11}C projects. Na-cyclopentadienide synthesized from cyclopentadiene and metallic sodium in liquid ammonia was allowed to react with [^{11}C]CH_3I to form [^{11}C]methylcyclopentadiene. Isomerization and aromatization into [^{11}C]benzene was carried out by pyrolysis at 800 °C. However, the radiochemical yield of [^{11}C]benzene was only about 5 % [4].

Because the named procedures were fully unsuitable we sought for routes which should afford acceptable synthesis times and yields and, in addition to this, include the possibility to prepare ^{11}C-ring-labelled key aromatics with reactive groups for further derivatization at the benzenoid ring. The reasons to prefer ^{11}C-ring labelling of compounds containing an aromatic ring are obvious. The aromatic ring is a metabolically stable component of the molecule. If such a compound is used as a PET tracer, the stability of the ring-labelling may be important with respect to the fate of the label. Moreover, there are compounds which can only be labelled in their aromatic ring.

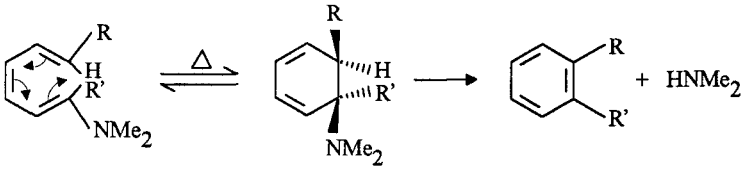
Assessing the different theoretical possibilities for synthesizing ^{11}C-ring-labelled benzenoid compounds [3, 5 - 10], the synchronous six-electron cyclization of hexatriene systems into aromatics [10] seemed to be the most promising one. This method should open a suitable approach to different ^{11}C-ring-labelled benzenoid compounds because it offers the possibility of varying the kind of substituents and their position at the nonradioactive precursor. Therefore, we continued our investigations on the base of this method.

RESULTS AND DISCUSSION

Principle of the labelling

The synchronous six-electron cyclization of hexatriene systems into benzenoid compounds according to [10] takes place in two steps (equ. 1):

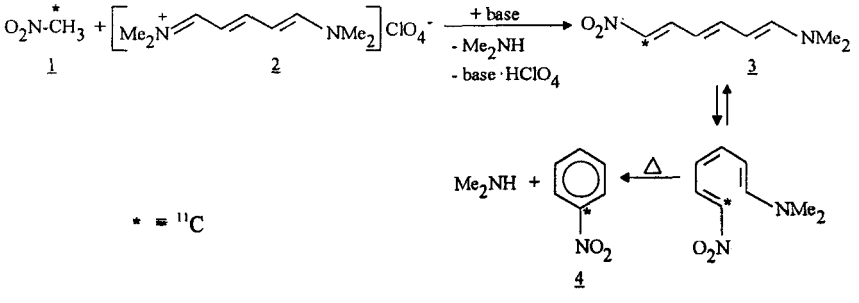
1. thermal cyclization of a hexatriene into a cyclohexadiene as a reversible process
2. irreversible conversion of the cyclohexadiene into a benzenoid compound by an elimination step, provided that the starting hexatriene has a suitable leaving group, e.g. a dimethylamino group, at C-1, as well as a proton at C-6. The dimethylamino group of the cyclohexadiene, however, must be arranged in trans position to the proton.



equ. 1

Prerequisite for the ^{11}C-ring labelling of an benzenoid compound according to equ. 1 is the synthesis of an appropriate [^{11}C]hexatriene, which requires a nonradioactive precursor with 5 methine groups (i.e. 3 conjugated double bonds) and suitable leaving groups at both ends of the molecule. Such a precursor is e.g. a pentamethinium salt, which is able to react with an ^{11}C-synthone containing a C-H-acid methyl group, e.g. nitro-[^{11}C]methane. The principle of the synthesis is as follows:

In the presence of a base, nitro-[^{11}C]methane (1) reacts with 5-dimethylaminopenta-2,4-dienylidene-dimethylammonium perchlorate (2) (as a suitable pentamethinium salt) to form 6-nitro-1-dimethylamino-[6-^{11}C]hexatriene (3) and dimethylamine. The cyclization/ aromatization into nitro-[1-^{11}C]benzene (4) occurs by elimination of the second dimethylamino group at an increased temperature [10] according to equ. 2 :

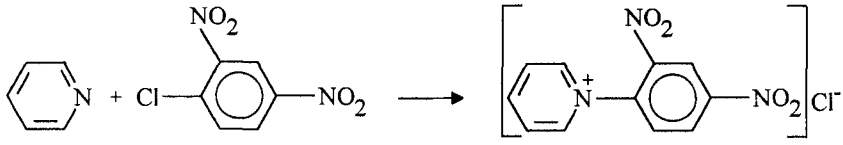


equ.2

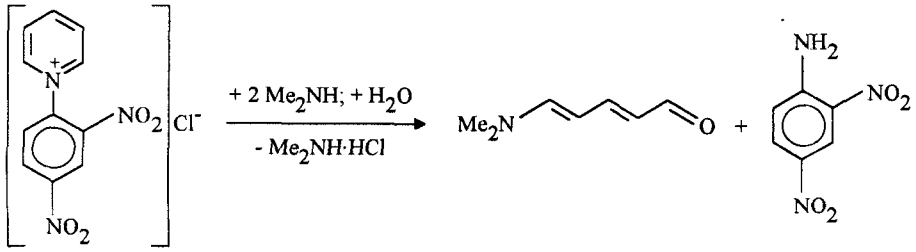
Precursor synthesis

The precursor for the synthesis, 5-dimethylaminopenta-2,4-dienylidene-dimethylammonium perchlorate (2), is prepared as follows:

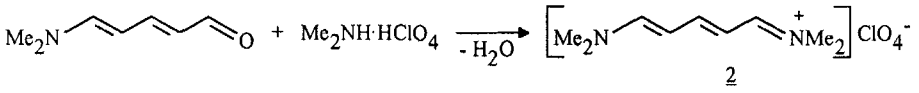
1. Pyridine is reacted with 1-chloro-2,4-dinitrobenzene to form N-(2,4-dinitrophenyl)pyridinium chloride [11]:



2. The pyridinium salt formed is very unstable in the presence of bases, which open the pyridine ring by removing the pyridine nitrogen. Reaction of this pyridinium salt with dimethylamine produces the 5-dimethylaminopenta-2,4-dienal, a so-called merocyanine, and 2,4-dinitroaniline [12]:



3. The merocyanine reacts with dimethylamine perchlorate to form the desired pentamethinium salt (2), a so-called azacyanine, by elimination of water [12]:



2

The structures of the merocyanine and the azacyanine were confirmed by ^{13}C-NMR data.

Synthesis of nitro-[1-^{11}C]benzene

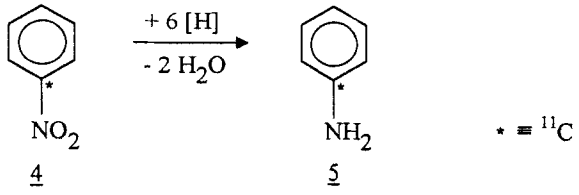
Initial attempts to synthesize nitro-[1-^{11}C]benzene (4) according to equ. 2 in the presence of triethylamine in DMF at 100 °C showed, that the conditions worked out on a nonradioactive scale are unsuitable for fast synthesis with ^{11}C. Triethylamine, the base used, proved to be too weak. For this reason many nonradioactive experiments were carried out to test potassium tert.butylate in various aprotic solvents (e.g. MeCN, DMF, DMSO, Dioxan, THF, HMPT)

at various temperatures (80 °C, 110 °C, 150 °C, 170 °C) with various reaction times. The extent of conversion was investigated by HPLC. The optimized conditions were successfully transferred to the radioactive scale.

The best results for synthesis of nitro-[1-^{11}C]benzene as regards conversion extent and reaction time were obtained by using hexamethylphosphoric triamide (HMPT) as solvent in the presence of t-BuOK. Starting from nitro-[^{11}C]methane (1), nitro-[1-^{11}C]benzene of a radiochemical purity of about 92 % and a mean specific radioactivity of 1 Ci/μmol was obtained within 7 min. The reproducible radiochemical yield of 4 (decay-corrected) in relation to [^{11}C]CO_2 was in the range of 80±5 %. An HPLC radiogram of unpurified 4 is shown in Fig. 1.

Synthesis of [1-^{11}C]aniline

[1-^{11}C]Aniline (5) was synthesized by reduction of nitro-[1-^{11}C]benzene (4) according to equ. 3. For this purpose the above reaction mixture was treated with an appropriate reducing agent while heating in a one-pot process:



4 5 equ. 3

At the beginning the reducing agents $Na_2S_2O_4$, $SnBr_2$/HBr, $LiAlH_4$/THF, $FeSO_4$/NaOH, $PhNHNH_2$, H_2NNH_2 and Na_2S [14] have been tested in nonradioactive experiments using suitable solvents (e.g. DMF, MeCN, THF, Dioxan, DMSO, HMPT) at various temperatures (110 °C, 150 °C, 170 °C) with various reaction times. The extent of conversion was investigated by HPLC. The conditions of successful nonradioactive reduction were transferred to the radioactive scale:

- In experiments using $Na_2S_2O_4$ in DMF/H_2O at 110 °C only a small amount of [1-^{11}C]aniline was obtained. Mainly radioactive ionic compounds were produced, probably a mixture of N-[^{11}C]phenylsulphamic and amino-[^{11}C]benzenesulphonic acid.
- Experiments with $SnBr_2$/HBr in MeCN, DMF or HMPT at 110 °C or 170 °C gave an indefinable ^{11}C-labelled reaction product (probably a colloid) which was irreversibly absorbed at the top of the HPLC column.

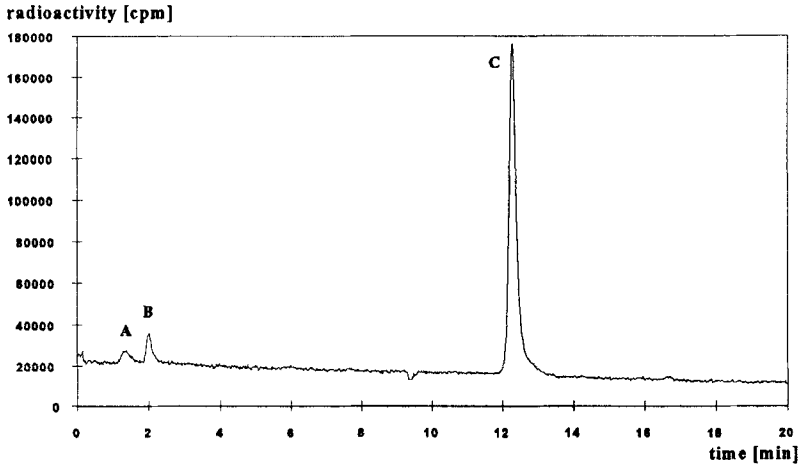


Fig. 1: HPLC radiogram obtained from the reaction mixture of the nitro-[1-^{11}C]benzene synthesis

A (1.31 min): unidentified product, probably [^{11}C]CH_3ONO [13]; 2.81 %
B (2.01 min): [^{11}C]CH_3OH; 5.33 %
C (12.27 min): nitro-[1-^{11}C]benzene; 91.86 %
(radioactivity [%] is decay-corrected)

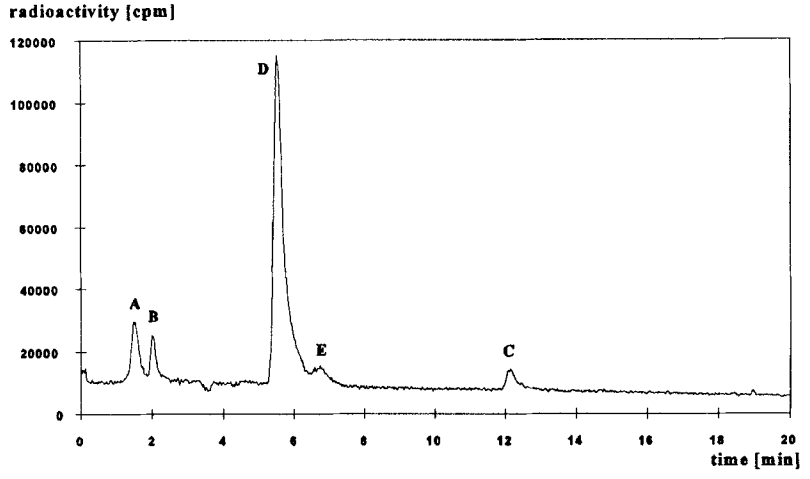


Fig. 2: HPLC radiogram obtained from the reaction mixture of the [1-^{11}C]aniline synthesis

A (1.47 min): unidentified product, probably [^{11}C]CH_3ONO [13]; 7.82 %
B (2.01 min): [^{11}C]CH_3OH; 4.29 %
C (12.12 min): nitro-[1-^{11}C]benzene; 5.00 %
D (5.51 min): [1-^{11}C]aniline; 80.61 %
E (6.73 min): unidentified product, probably [1,1'-^{11}C]hydrazobenzene; 2.28 %
(radioactivity [%] is decay-corrected)

- An incomplete conversion of nitro-[1-^{11}C]benzene into [1-^{11}C]aniline with many by-products was achieved in another experiment using phenylhydrazine in HMPT at 170 °C.
- The use of THF-$LiAlH_4$ solution in HMPT at 110 °C yielded about 60 % [1-^{11}C]aniline and two by-products, probably azoxy-[1,1'-^{11}C]benzene and azo-[1,1'-^{11}C]benzene.

The best results for synthesis of [1-^{11}C]aniline as regards conversion extent and reaction time were obtained by using Na_2S. In a one-pot process nitro-[1-^{11}C]benzene was reduced by adding an aqueous Na_2S solution to the reaction mixture mentioned above and heating. Thus, starting from nitro-[^{11}C]methane (1), [1-^{11}C]aniline of a radiochemical purity of about 81 % was prepared within 18 min. Based on [^{11}C]CO_2, the reproducible radiochemical yield of 5 (decay-corrected) was in the range of 65±5 %. An HPLC radiogram of unpurified 5 is shown in Fig. 2.

EXPERIMENTAL

The ^{13}C NMR spectra were recorded on a FT-NMR-spectrometer MSL 300 (Bruker spectrospin) at 75 MHz.

1-Chloro-2,4-dinitrobenzene, 40 % aqueous dimethylamine, t-BuOK and HMPT were purchased from Merck, Germany and had for synthesis quality. Pyridine p.a. was obtained from PCK Schwedt, Germany, $AgNO_2$ and $Na_2S{\cdot}5H_2O$ p.a. from Riedel-de Haën, Germany. Dimethylamine perchlorate was self-made from 40 % aqueous dimethylamine and 70 % perchloric acid (p.a., Laborchemie Apolda, Germany) in ethanol, followed by evaporation of the solvents, washing the crystals with ethanol and diethyl ether and drying under vacuum.

For HPLC investigations the following reference substances were used: methyl iodide for synthesis (Merck, Germany), nitromethane 99 % (Aldrich, Germany), nitrobenzene p.a. (Fluka, Switzerland) and aniline p.a. (Laborchemie Apolda, Germany).

To determine the extent of the reaction conversion, the radiochemical purity of the reaction products and the specific radioactivity of nitro-[1-^{11}C]benzene, an HPLC system (Merck-Hitachi) was used, including a gradient pump (L-6200A), a Rheodyne injector with a 20 µl loop, a Separon SGX C18 column (RP-18, 5µm, 150 x 3.3 mm, LP Prague) and a diode array detector (L-4500 DAD) coupled in series with a radioactivity detector. The mobile phase consisted of phosphate buffer (2.6 mmol/l NaH_2PO_4 + 5.1 mmol/l Na_2HPO_4; pH 7.0) and acetonitrile at a flow rate of 0.5 ml/min, with the following linear gradient of the eluents:

0 min - 70 % buffer/ 30 % MeCN; 10 min - 0 % buffer/ 100% MeCN; 20 min - 0 % buffer/ 100 % MeCN.

Synthesis of the precursor

N-(2,4-Dinitrophenyl)pyridinium chloride

In ethanol (50 ml) a mixture of 1-chloro-2,4-dinitrobenzene (24.1 g; 0.12 mol) and pyridine (12 g; 0.15 mol) were heated under reflux for 5 h. Then the solvent was evaporated. The solid residue was washed with diethyl ether and filtered through a frit glass filter. The dried solid was recrystallized from ethanol, washed with diethyl ether and dried. The weakly yellow crystals had m.p. 228-230 °C (lit. m.p. < 200 °C dec. [11]).

Yield: 26.7 g ≙ 80 %.

5-Dimethylaminopenta-2,4-dienal

N-(2,4-Dinitrophenyl)pyridinium chloride (25 g; 0.089 mol) in ethanol (250 ml) was treated with 40 % aqueous dimethylamine (18.75 ml; 0.167 mol). The mixture was heated at 70 °C for 30 min, evaporated under reduced pressure, and treated with cold water (150 ml). The precipitated 2,4-dinitroaniline was separated by filtration and the filtrate made alkaline with sodium hydroxide (5 g; 0.125 mol) in water (25 ml). As a result the solution turned dark violet and violet crystals separated. This mixture was extracted with methylene chloride (4 x 50 ml), the combined extracts were dried with Na_2SO_4.

Evaporation of the filtered extract left a violet solid. Recrystallization from cyclohexane gave brownish-violet hygroscopic crystals. The aldehyde had m.p. 49-52 °C (lit. m.p. 59 °C [12]).
Yield: 7 g ≙ 63 %.
^{13}C-NMR: 75.475 MHz, $CDCl_3$, TMS ≙ 0, δ in ppm
C(1): 191.43 (s, CHO); C(2): 118.61 (s, CH); C(3): 152.68 (s, CH); C(4): 96.63 (s, CH);
C(5): 156.70 (s, CH); (-N(CH_3)$_2$): 40.65 (broad s)

5-Dimethylaminopenta-2,4-dienylidene-dimethylammonium perchlorate (2)
5-Dimethylaminopenta-2,4-dienal (2.5 g; 0.02 mol), dimethylamine perchlorate (3.5 g; 0.02 mol), and ethanol (8 ml) were heated under reflux for 2 h. The salt separated after cooling was recrystallized from ethanol. The violet crystals had m.p. 163-165 °C (lit. m.p. 167 °C [12]). The salt is soluble in methanol, chloroform, methylene chloride, acetone, acetonitrile and insoluble in diethyl ether and carbon tetrachloride.
Yield: 3.8 g ≙ 76 %.
^{13}C-NMR: 75.475 MHz, $CDCl_3$, TMS ≙ 0, δ in ppm
C(1), C(5): 161.98 (s, CH); C(2), C(4): 103.54 (s, CH); C(3): 163.94 (s, CH);
(-N(CH_3)$_2$), (=N$^+$(CH_3)$_2$): 38.18 (s), 46.23 (s)

Radiosyntheses

[^{11}C]Carbon dioxide
^{11}C was produced on the modified U-120 cyclotron at the Research Center Rossendorf Inc. by the ^{14}N(p,α)^{11}C nuclear reaction giving [^{11}C]CO_2. The nitrogen target was irradiated with 13 MeV protons at various beam currents and bombardment times. The used amounts of radioactivity of the starting [^{11}C]CO_2 were in the range of 1 - 2 GBq for the normal attempts. For the determination of the specific activity amounts of 15 - 20 GBq [^{11}C]CO_2 were used.

[^{11}C]Methyl iodide
[^{11}C]Methyl iodide was prepared by the classic one-pot method [15] involving the reduction of [^{11}C]CO_2 with $LiAlH_4$ to lithium aluminium-[^{11}C]methylate, hydrolysis of this intermediate organometallic complex and subsequent iodization of the formed [^{11}C]methanol with HI.

Nitro-[^{11}C]methane (1)
The gas-solid reaction of [^{11}C]methyl iodide with silver nitrite according to [13] resulted in nitro-[^{11}C]-methane (1). In this procedure the [^{11}C]CH_3I was driven by an N_2 stream (flow rate = 40 ml/ min) through a heated glass column (i.d. 3 mm, length 4 cm, oven temp. 80 °C) containing 0.4 g $AgNO_2$.

Nitro-[1-^{11}C]benzene (4)
The [^{11}C]CH_3NO_2 (1) thus produced was trapped in a cooled 2 ml vessel (10 °C) containing 250 μl HMPT, 8 mg 5-dimethylaminopenta-2,4-dienylidene-dimethylammonium perchlorate (2) and 3.5 mg t-BuOK. Cyclization/ aromatization into nitro-[1-^{11}C]benzene (4) was achieved by heating the well sealed vessel at 170 °C for 7 min.

[1-^{11}C]Aniline (5)
The reduction to obtain [1-^{11}C]aniline (5) as performed by adding an excess of 8 mg $Na_2S{\cdot}5H_2O$ in 100 μl water to the above reaction mixture and heating at 170 °C for 10 min.

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