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Efficient system for the preparation of [^{13}N]labeled nitrosamines

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ABSTRACT

In the present Letter, a fast and reproducible method for the synthesis of *N*-[^{13}N]nitrosamines is reported. The labeling strategy is based on trapping [^{13}N]NO_2^- in an anion exchange resin. The reaction with secondary amines in the presence of Ph_3P and Br_2 led to the formation of the desired nitrosamines in short reaction times (2 min) with excellent radiochemical conversion (>45%). Final radiotracers were obtained after purification in good radiochemical yields (>30%, decay corrected). Radiochemical purity was above 99% in all cases.

Positron emission tomography (PET) is a non-invasive in vivo imaging technique which produces a three-dimensional image of functional processes in a living organism. Commonly used radionuclides for PET imaging include carbon-11 (^{11}C), fluorine-18 (^{18}F), oxygen-15 (^{15}O), nitrogen-13 (^{13}N) or bromine-76 (^{76}Br), which can be easily produced in good yields in cyclotrons for further incorporation into organic molecules to be used as radiotracers.[1] Due to radioactive decay, the preparation of the radiotracers requires the development of fast and efficient synthetic strategies to be carried out in automatic-remote controlled synthesis modules.

Among all PET radionuclides, ^{11}C (half-life of 20.4 min and maximum positron energy of 960.5 keV) and ^{18}F (half-life of 109.8 min and maximum positron energy of 633.5 keV) have been by far the most widely used, due to their versatile chemistry, relatively long half-life and high production yields in commercially available cyclotrons. In spite of the historical importance of ^{11}C and ^{18}F, the use of other radioisotopes (e.g., ^{13}N, with a half-life of 9.97 min and maximum positron energy of 1.19 MeV) might be very advantageous for the development of alternative synthetic strategies to label molecules in different positions, thus giving further understanding of specific biological and/or physiological processes. A clear example is found in nitrosamines. Since Magee and Barnes showed the carcinogenic potential of *N*-nitrosodimethylamine (NDMA) in rats in 1956,[2] a high number of nitrosamines have shown potent carcinogenic effects in experimental studies with tumor induction in different sites like oral cavity, esophagus, stomach, urinary bladder and brain.[3] In spite of the large number of studies in the preclinical area, a better understanding of the in vivo mechanism by which nitrosamines exert their carcinogenic effect is highly desirable. Especially, the carcinogenic potential of nitrosamines has driven the studies about the influence of tobacco and food additives. In this sense, ^{13}N labeling (either combined with ^{11}C labeling or not) would represent a powerful tool to perform in vivo pharmacokinetic studies in animals, including bio-distribution and metabolism.

N-Nitrosation of amines is a well explored reaction in organic synthesis. In the last decades, different nitrosating agents including nitrous acid (HNO_2), nitrosyl chloride (NOCl), dinitrogen trioxide (N_2O_3), dinitrogen tetroxide (N_2O_4), nitrosonium tetrafluoroborate and alkyl nitrites have been applied widely for the synthesis of nitrosamines. More recently, heterogeneous reagent systems have been demonstrated to be an excellent alternative for the synthesis of nitrosamines following simple experimental procedures under mild conditions.[4] However, the [^{13}N]nitrosation of amines is strongly restricted due to the limited availability of cyclotron-generated radioactive precursors ([^{13}N]NH_4^+ and [^{13}N]NO_3^-) and the low amount (sub-micro molar scale) in which they can be produced.

In a recent work, we presented a fast and simple strategy for the generation of the radioactive precursor [^{13}N]NO_2^- and further reaction with glutathione to yield *S*-[^{13}N]nitrosoglutathione.[5] In continuation of our work, we have used the same radioactive precursor to efficiently synthesize different *N*-[^{13}N]nitrosamines following the $Ph_3P/Br_2/NO_2^-$ strategy recently described by

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Nowrouzi and co-workers.[6] Although very high yields were described in this work, in the particular case of using non carrier added-cyclotron produced $[^{13}N]NO_2^-$, the anion is obtained in very low concentration aqueous solution, and thus application of the methodology is not trivial.

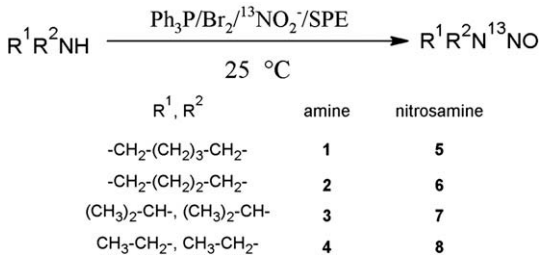
Our first attempts to synthesize *N*-[^{13}N]nitrosamines followed the general procedure in which nitrous acid, (generated from nitrite solution and mineral acid in water) is reacted with secondary amines. In this case, using piperidine (**1**) as secondary amine, under non radioactive conditions and for a wide range of temperatures ($0\ ^\circ C \leqslant T \leqslant 100\ ^\circ C$) and acid concentrations ($1 \leqslant pH \leqslant 4.5$, NO_2^-/amine ratio = 1.2/1), the reaction of formation of *N*-nitrosopiperidine (**5**) was very slow and chemical conversion (NO_2^- into **5**) was very poor (under 5% in all cases) after 10 min of reaction. The reaction was also carried out at lower NO_2^-/amine ratios (to simulate radioactive conditions) and chemical conversion was strongly decreased. These results were confirmed, also using **1** as secondary amine, under radioactive conditions. No radioactive peak corresponding to [^{13}N]**5** was detected in any case.

With the aim of improving our results, the resin-supported methodology for the synthesis of *N*-[^{13}N]nitrosamines previously reported by Vavrek and Mulholland[7] was used;[8] under these conditions, the formation of [^{13}N]**5** could not be detected after 10 min of reaction. Due to this fact, the combined approach (resin-supported NO_2^- + Ph_3P/Br_2/amine strategy) was assayed (Scheme 1).

In a typical experiment, ^{13}N (25 mCi, 925 GBq) was produced in a cyclotron via the $^{16}O(p,\alpha)^{13}N$ nuclear reaction. The target (containing 3.2 mL of purified water) was irradiated with 18 MeV protons at a beam current of 10 μA for 2 min. The resulting solution, containing $[^{13}N]NO_2^-$ (16.5 ± 3.8%), $[^{13}N]NO_3^-$ (74.1 ± 3.5%) and $[^{13}N]NH_4^+$ (9.4 ± 0.4%) was passed through a glass column filled with cadmium/sand 3:1 mixture (w/w, column length = 16.5 cm) to obtain a virtually $[^{13}N]NO_3^-$ free solution (95.5 ± 1.1% $[^{13}N]NO_2^-$, 0.7 ± 0.45% $[^{13}N]NO_3^-$ and 3.8 ± 1.4% $[^{13}N]NH_4^+$).[9]

The solution was directed to an anion exchange solid phase extraction (SPE) cartridge (Sep-Pak® Accell Plus QMA, Waters) to trap $[^{13}N]NO_2^-$ quantitatively. The cartridge was dried with nitrogen, washed with tetrahydrofuran (2 mL) and dried again. A freshly prepared solution (0.8 mL) containing Ph_3P/Br_2/amine (25:25:20 μmol) in dry dichloromethane was circulated through the cartridge at a flow rate of 0.4 mL/min. The eluted solution was recovered in a vial, dried under continuous nitrogen flow and the residue was reconstituted with water (2 mL).

The resulting solution was analyzed by HPLC using a YMC J'sphere ODS-H80 column (4 μm particle size, 15 × 0.46 mm) as stationary phase and water/acetonitrile/methanol (28:18.5:3.5) as mobile phase.[10] Simultaneous UV (λ = 254 nm) and isotopic detection were used. The presence of *N*-[^{13}N]nitrosamine was confirmed by co-elution with reference compound solution (retention times = 3.43, 2.56, 5.62, and 3.23 min for **5**, **6**, **7**, and **8**, respectively).



Scheme 1. Synthesis of *N*-[^{13}N]nitrosamines by following the combined approach (resin-supported NO_2^- + Ph_3P/Br_2/amine).

Table 1

Entry	R^1R^2NH	R^1R^2NNO	RCC[a,c,d]	RCY[b,c,d]
1	1	5	53.4 ± 1.3 (51.9–54.4)	37.8 ± 3.1 (34.7–40.9)
2	2	6	50.2 ± 9.8 (38.8–56.2)	40.7 ± 8.0 (31.6–46.1)
3	3	7	45.6 ± 7.4 (38.1–52.8)[e]	34.0 ± 7.3 (27.3–41.9)[e]
4	4	8	45.9 ± 5.4 (42.0–49.7)	36.4 ± 5.0 (32.9–40.0)

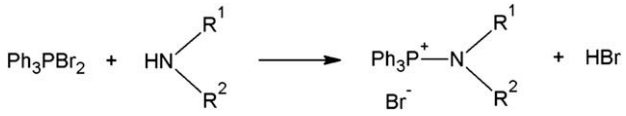
[a] RCC: Radiochemical conversion (%), calculated as the ratio between the amount of radioactivity present as *N*-[^{13}N]nitrosamine and the amount of radioactivity trapped in the SPE cartridge.
[b] RCY: Radiochemical yield (%), calculated as the ratio between the amount of radioactivity present as *N*-[^{13}N]nitrosamine (after purification) and the amount of radioactivity generated in the cyclotron.
[c] Decay corrected values.
[d] AVG ± STDV (range).
[e] Amount of Ph_3P/Br_2/amine was 50/50/40 μmol.

In Table 1, the radiochemical conversion of $[^{13}N]NO_2^-$ into *N*-[^{13}N]nitrosamine is shown for amines **1**–**4**. In all cases, radiochemical conversion (decay corrected) was above 45%. Importantly, irrespectively of the structure of the secondary amine, only the presence of one major chemical impurity (retention time = 10.5 min) was detected in all cases in the final solution before purification. As previously reported in the literature,[11] Ph_3P quantitatively reacts with Br_2 to immediately yield bromotriphenylphosphonium bromide. The slow reaction of this reagent with the secondary amine yields the precursor for the nitrosation reaction (triphenyl(amin-1-yl)phosphonium bromide, see Scheme 2 for structure). The excess of bromotriphenylphosphonium bromide constituted the undesired impurity observed in the HPLC at the end of the synthesis, and could be removed from the bulk solution by eluting the reaction mixture through a C-18 SPE cartridge, rinsing with purified water and eluting the desired radiotracer with 3 mL of ethanol/water solution (25/75 in the case of [^{13}N]**7**, 20/80 in the case of [^{13}N]**5** and 15/85 in the other cases). This purification step was also successful in removing unreacted $[^{13}N]NO_2^-$ and $[^{13}N]NO_3^-$. Final reconstitution with 5 mL of injectable physiologic solution and filtering through a 0.22 μm filter left the radiotracer ready for putative in vivo studies in animals.

Average radiochemical yields (calculated as the ratio between the amount of radiotracer and the amount of radioactivity generated in the cyclotron, decay corrected) for the preparation of *N*-[^{13}N]nitrosamines were in the range 34.0–40.7% (Table 1). Total synthesis time (including purification) was less than 10 min and radiochemical purity was above 99% in all cases.

As can be seen in Table 1, the lowest radiochemical conversion and yield were obtained for [^{13}N]**7**, despite a higher concentration of Ph_3P/Br_2/amine was used. We believe that this is due to steric hindrance exerted by diisopropylamine after formation of triphenyl(diisopropylamin-1-yl)phosphonium bromide.

In summary, we present here a simple, fast and easy to automate procedure for the preparation of [^{13}N]labeled nitrosamines. By using a combined approach (resin-supported $^{13}NO_2^-$ + Ph_3P/Br_2/amine), $^{13}NO_2^-$ (aqueous solution) can be reacted with secondary amines to synthesize nitrosamines with excellent conversion and good radiochemical yields. Despite $[^{13}N]NO_2^-$ is obtained as aqueous solution from the cyclotron, the amine is solved in dichloromethane; thus, the method is applicable to the preparation of



Scheme 2. Reaction of formation of the precursor triphenyl(amin-1-yl)phosphonium bromide.

other nitrosamines with more complex structure, even if they are not water-soluble.

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8. In the previous work by Vavrek and Mullholand many details about the procedure are omitted and reaction conversions are not specified for the particular cases. In the present work, for the synthesis of *N*-[^{13}N]nitrosopiperidine the solution coming from the cyclotron, after reduction step, was directed to an anion exchange solid phase extraction cartridge (Accell plus QMA, Waters). A freshly prepared solution (2.0 mL) containing piperidine (20–200 μmol) and hydrochloric acid ($1.0 \leqslant$ final pH $\leqslant 4$) in water was circulated through the cartridge at a flow rate of 0.4 mL/min. The eluted solution was analyzed by HPLC under the same conditions as described in the main text.
9. *Stationary phase*: HP Asahipak ODP-50 column (5 μm particle size, 125 × 4 mm, Teknokroma, Spain); *mobile phase*: solution containing additive for ionic chromatography (P/N 5062-2480, Agilent Technologies, 15 mL) in a mixture water/acetonitrile (86/14, 1L), adjusted to pH = 8.6 with 1 M NaOH solution; flow rate 1 mL/min; simultaneous UV ($\lambda = 254$ nm) and isotopic detection.
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