Tetrahedron Letters 51 (2010) 2990–2993

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Fully automated synthesis of ^{13}N-labeled nitrosothiols

Vanessa Gómez-Vallejo [a], Koichi Kato [b], Iosu Oliden [a], Javier Calvo [c], Zuriñe Baz [a], José I. Borrell [d], Jordi Llop [a,*]

[a] Radiochemistry Department, CIC biomaGUNE, Parque Tecnológico de San Sebastián, p° Miramón 182, 20009 San Sebastián, Spain
[b] Department of Molecular Probes, Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan
[c] Mass Spectrometry Platform, CIC biomaGUNE, Parque Tecnológico de San Sebastián, p° Miramón 182, 20009 San Sebastián, Spain
[d] Grup d'Enginyeria Molecular, Institut Químic de Sarrià, Universitat Ramon Llull, Via Augusta 390, 08017 Barcelona, Spain

ARTICLE INFO

Article history:
Received 23 February 2010
Revised 25 March 2010
Accepted 30 March 2010
Available online 3 April 2010

Keywords:
Nitrosothiols
Positron emission tomography
Nitrogen-13

ABSTRACT

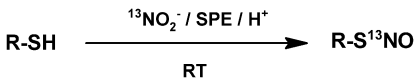
In the present Letter, a fast, automated, and reproducible method for the synthesis of *S*-[^{13}N]nitrosothiols is reported. The labeling strategy is based on trapping [^{13}N]NO_2^- in an anion exchange resin. The reaction with thiols in acidic media led to the formation of the desired nitrosothiols in short reaction times (60 s) with excellent radiochemical conversions (from 48.7% to 74.5%). Final radiotracers were purified by HPLC. Good radiochemical yields (from 33.8% to 60.6%, decay corrected) and radiochemical purities (>99%) were obtained in all cases. Stability of the labeled compounds was checked.

Nitric oxide (NO) is a key signaling molecule involved in the regulation of many biological and physiological processes, such as blood flow and pressure,[1] neurotransmission[2] and inflammation and host defense.[3,4] The importance of NO has promoted a lot of interest in the development of drugs able to generate NO directly and/or indirectly for therapeutic treatments. Such is the case of organic nitrates, which have been used for many years to treat patients with ischemic heart disease; however, they are known to develop tolerance[5] and consequently other NO donor drugs have been developed during the last years. One example is *S*-nitrosothiols, which have several advantages over other NO donors, because they represent endogenous NO reservoirs while they do not induce oxidative stress or vascular tolerance.[6]

In spite of the potential applications in the clinical environment of *S*-nitrosothiols, the exact mechanisms underlying the role developed by such kind of molecules have not been fully elucidated. From this perspective, a technique able to detect and quantify the presence of *S*-nitrosothiols in vivo and at trace levels is highly desirable. Positron emission tomography, a noninvasive imaging technique, fulfills these requirements.

In a recent work,[7] we presented a fast and simple strategy for the generation of the radioactive precursor [^{13}N]NO_2^- and its reaction with glutathione in acidic media to yield *S*-[^{13}N]nitrosoglutathione ([^{13}N]GSNO). In continuation of our work, we used the same radioactive precursor to synthesize *N*-[^{13}N]nitrosamines with high yields by trapping [^{13}N]NO_2^- in an anion exchange resin and performing the reaction with secondary amines in the presence of Ph_3P and Br_2.[8] In the current Letter, we present a general procedure for the radiosynthesis of *S*-[^{13}N]nitrosothiols by trapping [^{13}N]NO_2^- in an anion exchange resin and reacting with the appropriate thiol in acidic conditions (Scheme 1). An automatic remote controlled system to perform the production (synthesis, purification, and formulation) of *S*-[^{13}N]nitrosothiols has been designed and implemented.[9]

In a typical experiment, ^{13}N (30 mCi, 1.11 GBq) was produced in an IBA Cyclone 18/9 cyclotron via the ^{16}O(p,α)^{13}N nuclear reaction. The target, containing 1.75 mL of water (ultrapure, Type I water, ISO 3696) was irradiated with 18 MeV protons at a beam current of 20 μA for 1.5 min (integrated current of 0.5 μAh). The resulting solution was collected in a 5 mL conic vial, loaded in a glass column filled with cadmium[10] and allowed to react for 40 s to obtain a virtually [^{13}N]NO_3^- free solution, which was eluted through an anion exchange cartridge (Sep-Pak® Accell Plus QMA, Waters) to selectively retain [^{13}N]NO_2^-. The column was further eluted with distilled water (2 mL) and the QMA cartridge was dried with inert gas for 15 s. An acidic solution of the adequate thiol (0.5 mL, see precursors, Table 1[11,12]) was loaded into the cartridge, reaction was allowed to occur and then the reaction mixture was pushed directly into a collection vial (no purification) or to the HPLC system (when a purification step was needed).

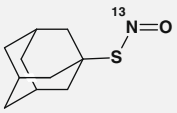
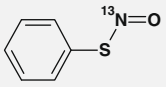
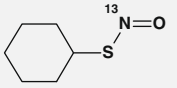
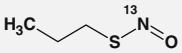
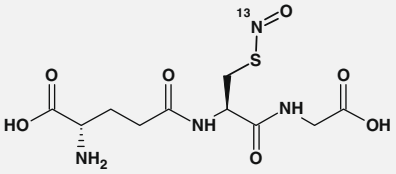
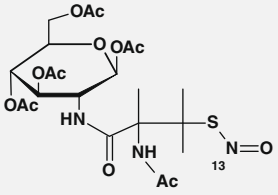


Scheme 1. Synthesis of *S*-[^{13}N]nitrosothiols by trapping [^{13}N]NO_2^- in an anion exchange resin and reacting with thiols in acidic conditions.

* Corresponding author. Tel.: +34 943 005 333; fax: +34 943 005 301.
E-mail address: jllop@cicbiomagune.es (J. Llop).

0040-4039/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2010.03.122

Table 1
Structures of the synthesized *S*-[^{13}N]nitrosothiols and the precursors used

Precursor	Nitrosothiol	
1-Adamantanethiol		**1**
Thiophenol		**2**
Cyclohexanethiol		**3**
1-Propanethiol		**4**
L-Glutathione		**5**
2-(*N*-Acetyl-D-penicillamido)-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose		**6**

During optimization runs, reaction time and acid concentration were modified to optimize radiochemical conversion, calculated as the ratio between the amount of radiotracer before purification and the amount of [^{13}N]NO_2^- obtained after the reduction step. The percentage of radioactivity as *S*-[^{13}N]nitrosothiol in the reaction mixture was determined by HPLC.[13] The presence of the desired radiotracers was confirmed by co-elution with reference compounds, which were prepared on site following the procedure described by Wang et al.[14]

Our first optimization runs were carried out on the synthesis of **1**. In our previous work,[7] the synthesis of [^{13}N]GSNO was achieved by dissolving glutathione in aqueous hydrochloric acid, which was reacted with [^{13}N]NO_2^- in a reaction vial. In the current case, water was not an appropriate solvent due to the hydrophobic character of 1-adamantanethiol and, consequently, acetonitrile was used as a solvent. First attempts were performed by using HCl (final concentration = 1.0 M, prepared by adding the correct amount of 35% aqueous HCl to the precursor solution in acetonitrile), but low radiochemical conversion values were observed, irrespective of the precursor concentration (0.5–2.0 mg/mL) and reaction time (60–120 s). The radioactivity profiles registered during syntheses suggested a strong displacement of the [^{13}N]NO_2^- anion out of the cartridge while the precursor solution was loaded, preventing direct contact between [^{13}N]NO_2^- and 1-adamantanethiol and precluding the reaction to occur.

This strong displacement was attributed to (i) the ionic strength of chloride anion and (ii) the presence of water in the precursor solution, both factors contributing to increase eluotropic power. Trifluoroacetic acid (TFA) was considered a suitable candidate to overcome this problem due to the lower ionic strength of the trifluoroacetate anion and the possibility to prepare the precursor solution in anhydrous conditions. In order to get an experimental proof for the suitability of TFA, [^{13}N]NO_2^- (1 mCi) was loaded in a glass column (5 mm id, column length = 18 cm) prefilled with anion exchange resin; the column was rinsed with TFA (0.5 mL, 1 M solution in acetonitrile) or HCl (0.5 mL, 1 M in acetonitrile,

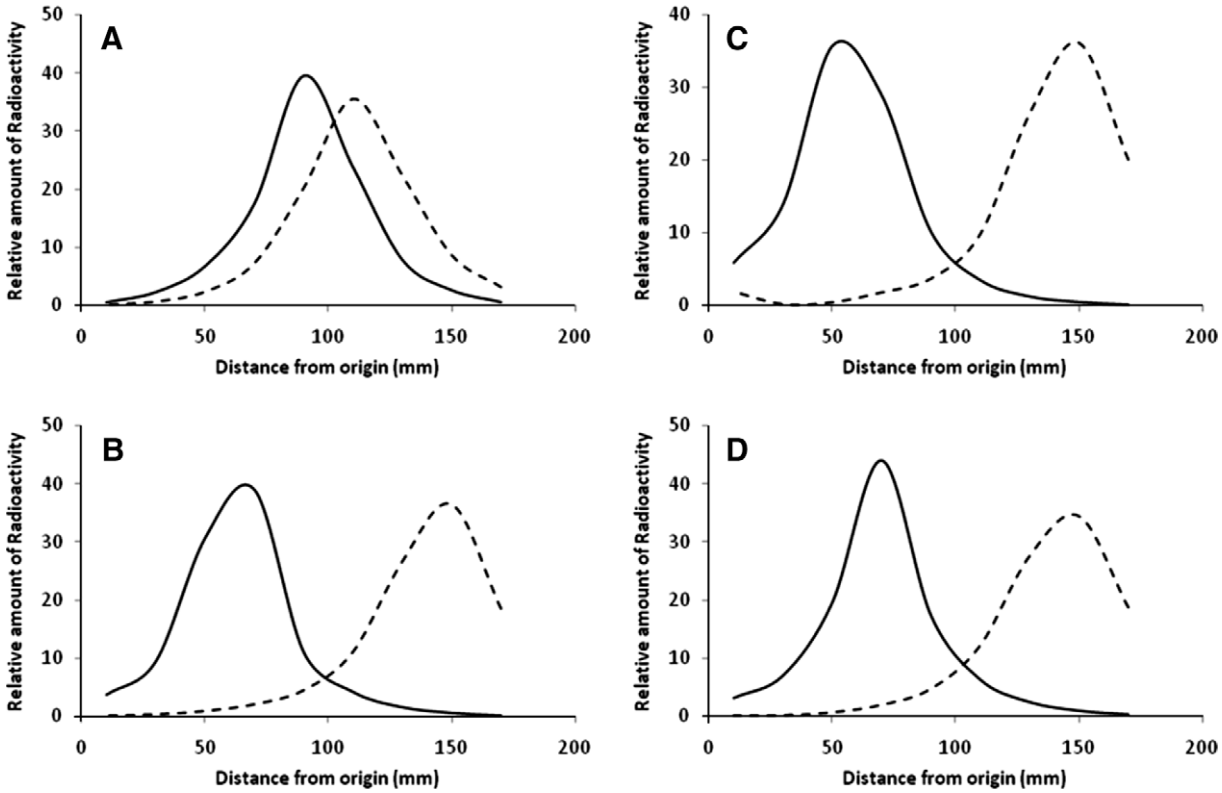


Figure 1. Distribution of radioactivity ([^{13}N]NO_2^-) in the column (5 mm id, column length = 18 cm) prefilled with anion exchange resin; Solid lines show radioactivity distribution before elution. Dotted lines show distribution after elution with: (A) 0.5 mL of 1 M TFA solution in acetonitrile, (B) 0.5 mL of 1 M HCl solution in acetonitrile prepared from 35% aqueous HCl solution, (C) 0.5 mL of 1 M TFA solution in water, and (D) 0.5 mL of 1 M HCl solution in water.

Table 2
Experimental conditions, radiochemical conversion, radiochemical yield, radiochemical purity and specific radioactivity for the preparation of ^{13}N-labeled nitrosothiols

Compound	Acid	Radiochemical conversion[a,c,e] (%)	Radiochemical yield[b,c,e] (%)	Radiochemical purity[d,e] (%)	Specific radioactivity[e,f] (MBq/μmol)
1	TFA	72.5 ± 3.9	53.3 ± 2.9	99.8 ± 0.1	5900 ± 230
2	TFA	21.9 ± 9.9	—	—	—
3	TFA	60.3 ± 5.5	52.3 ± 1.6	99.8 ± 0.1	6450 ± 225
4	TFA	74.5 ± 2.1	60.6 ± 2.1	99.6 ± 0.2	7210 ± 340
5	HCl	48.7 ± 6.3	33.8 ± 3.1	99.2 ± 0.2	185 ± 25
6	TFA	66.2 ± 5.8	59.5 ± 4.8	99.1 ± 0.7	8200 ± 410

[a] Calculated as the ratio between the amount of radiotracer before purification and the amount of $[^{13}N]NO_2^-$ obtained after the reduction step.
[b] Calculated as the ratio between the amount of radiotracer after purification and the amount of ^{13}N generated in the cyclotron.
[c] Decay corrected values.
[d] Calculated from chromatographic profiles after purification.
[e] AVG ± STDV (range).
[f] End of synthesis.

Table 3
Stability of ^{13}N-labeled nitrosothiols

Compound	RCP[a] (%) t = 12 min	RCP[a] (%) t = 24 min	RCP[a] (%) t = 36 min	RCP[a] (%) t = 48 min
1[b]	98.4 ± 0.2	97.7 ± 0.6	97.6 ± 0.6	97.4 ± 0.4
3[b]	99.3 ± 0.5	99.0 ± 0.8	98.7 ± 1.2	98.5 ± 1.0
4[b]	98.8 ± 0.4	98.8 ± 0.6	98.5 ± 0.2	98.4 ± 0.4
5[b]	98.7 ± 0.1	98.2 ± 0.3	98.0 ± 0.6	98.0 ± 0.3
6[b]	98.4 ± 0.5	97.9 ± 0.8	97.2 ± 0.3	97.1 ± 0.5

[a] Radiochemical purity, calculated from chromatographic profiles.
[b] See Table 2 for radiochemical purity at end of synthesis.

prepared from 35% aqueous HCl solution), and the radioactivity distribution within the column was measured by using a TLC plate reader before and after rinsing (Fig. 1A and B). As expected, almost no displacement of the $[^{13}N]NO_2^-$ anion was observed when TFA was used (Fig. 1A), while displacement became significant when the column was rinsed with HCl (Fig. 1B).

Optimization runs were then repeated for the synthesis of **1** using TFA instead of HCl. Radiochemical conversion values were considerably higher but again no significant changes were observed when precursor concentration (0.5–2.0 mg/mL) and reaction time (60–120 s) were modified. A precursor concentration of 1 mg/mL and a reaction time of 60 s were considered to be adequate for the preparation of *S*-[^{13}N]nitrosothiols, and these experimental conditions were adopted for the synthesis of **2**, **3**, **4**, and **6**. In the particular case of **5**, and with the aim of obtaining results comparable to those reported previously,[7] the precursor was dissolved in water and hydrochloric acid was used.

As it can be seen in Table 2, radiochemical conversion values were above 48% in all cases except for compound **2**, where several radioactive unidentified by-products were detected in the chromatographic profile. For compounds **1**, **3**, **4**, and **6** values over 60% were reached. These results point out to the conclusion that either the formation of aliphatic *S*-[^{13}N]nitrosothiols is favored with respect to the formation of aromatic ones or the latter are less stable, undergoing fast decomposition. The relatively low radiochemical conversion obtained in the case of **5** (48.7% ± 6.3) was first attributed to the small molar concentration of precursor. However, the use of substantially higher precursor molar amounts did not significantly increase radiochemical conversion while might lower the efficiency of the later purification step. Careful analysis of radioactivity profiles again suggested a strong displacement of the $[^{13}N]NO_2^-$ anion while the precursor solution was loaded; experiments performed to obtain information regarding eluotropic power of aqueous HCl and TFA solutions showed that, in this case, radioactivity displacement was very similar in both scenarios (Fig. 1C and D) and the use of trifluoroacetic acid was not considered an option to enhance radiochemical conversion.

After establishing the synthetic procedure, and due to the presence of the precursor and radioactive impurities in the reaction mixture, the implementation of a purification step was essential. It is well known that solid phase extraction purification methods are highly desirable when dealing with short-lived isotopes. However, with the aim of establishing a general procedure which could be potentially extended to the preparation of other *S*-[^{13}N]nitrosothiols, semi-preparative HPLC[15] was chosen as a purification method. The fact that the reaction is carried out in a cartridge facilitates direct loading of the mixture in the HPLC loop with minimal radioactivity loss, while accurate adjustment of chromatographic conditions allowed completion of purification in less than 6 min in all cases.

After collection of the desired fraction into a vial containing 2 mL of 0.5 mM EDTA aqueous solution, reformulation was carried out (**1**–**4** and **6**) by trapping the radiotracer in a C-18 cartridge, eluting with ethanol and reconstituting with physiologic saline solution. In the case of **5**, direct dilution of the collected fraction and pH adjustment with phosphate buffer left the final solution with ethanol concentration below 10%.

Final solutions were analyzed by means of HPLC.[13] All undesired radiochemical impurities (mainly unreacted $[^{13}N]NO_2^-$ and $[^{13}N]NO_3^-$) were successfully removed from the solution in all cases (Table 2, radiochemical purity). No undesired peaks were detected in the UV profile. Hence, concentration of precursor in the final solutions was, according to the limit of detection of the analytical method, below 150, 60, 20, 1500, and 450 ng/mL for **1** and **3–6**, respectively. The presence of labeled species (**5** and **6**) was confirmed by UPLC-MS (ESI-TOF).[16] This analytical technique was not suitable for the identification of **1**, **3**, and **4**. In this case, co-elution with reference compound in two different chromatographic scenarios confirmed the presence of the desired labeled species.[17]

Average radiochemical yields (calculated as the ratio between the amount of radiotracer after purification and the amount of ^{13}N generated in the cyclotron, decay corrected) for the preparation of *S*-[^{13}N]nitrosothiols were above 50% for **1**, **3**, **4**, and **6** while they were slightly lower in the case of **5** (Table 2) as expected according to radiochemical conversions. Total synthesis time (including purification) was less than 13 min in all cases and radiochemical purities (determined by HPLC[13]) were above 99% for **1** and **3**–**6**. In the case of **2**, radiochemical purity was under 85% immediately after purification, pointing out to a fast decomposition of *S*-[^{13}N]nitrosothiophenol. Specific activities (Table 2) were in the range 5900–8200 MBq/μmol for **1**, **2**, **4**, and **6**, and were significantly lower

(185 MBq/μmol) in the case of **5**, probably due to the presence of NO_3^- and/or NO_2^- as impurities in the hydrochloric acid solution.

Stability tests performed on **1** and **3**–**6** showed that, in all cases, radiochemical purities after 48 min (from end of synthesis) were above 97% (Table 3). For compound **2**, radiochemical purity quickly decreased with time (results not shown) confirming the instability of *S*-[^{13}N]nitrosothiophenol; different additives were assayed to improve stability with poor results.

To the best of our knowledge, the synthesis of *S*-[^{13}N]nitrosothiols has been reported previously only by us[7] and by Vavrek and Mulholland,[18] who investigated the formation of water-soluble *S*-[^{13}N]nitrosothiols following a similar procedure. In that case, purification step was not performed and the chemical purity of final radiotracers was not precisely reported.

In the work we are presenting here, decay corrected radiochemical yield for the synthesis of **5** (33.8 ± 3.1%) is slightly higher than the one reported in our previous work[7] (24.2%). Radiochemical purity is somewhat higher than in the previous work (99.2 ± 0.2% vs 96.4 ± 0.6%) and the method has been extended to the preparation of non water-soluble *S*-[^{13}N]nitrosothiols. Therefore, the general procedure here reported should allow the preparation of a wide range of *S*-[^{13}N]nitrosothiols with high chemical and radiochemical purity.

Acknowledgments

The authors would like to thank Dr. Peñuelas (Nuclear Medicine Department, Clínica Universitaria de Navarra) for developing and transferring to our research group the original idea of synthesizing *S*-[^{13}N]nitrosothiols. The authors also thank the Departamento de Industria, Comercio y Turismo of the Vasc Government for financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.03.122.

References and notes

1. Lundberg, J. O.; Weitzberg, E.; Gladwin, M. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 156–167.
2. Grisham, M. B.; Jourd'Heuil, D.; Wink, D. A. *Am. J. Physiol.* **1999**, *276*, G-315–G-321.
3. Al-Sa'doni, H. H.; Ferro, A. *Mini-Rev. Med. Chem.* **2005**, *5*, 247–254.
4. Khan, M.; Jatana, M.; Elango, C.; Paintlia, A. S.; Singh, A. K.; Singh, I. *Nitric Oxide* **2006**, *15*, 114–124.
5. Al-Sa'doni, H.; Ferro, A. *Clin. Sci.* **2000**, *98*, 507–520.
6. Jaworski, K.; Kinard, F.; Goldstein, D.; Holvoet, P.; Trouet, A.; Schneider, Y.-J.; Remacle, C. *Eur. J. Pharmacol.* **2001**, *425*, 11–19.
7. Llop, J.; Gómez-Vallejo, V.; Bosque, M.; Quincoces, G.; Peñuelas, I. *Appl. Radiat. Isot.* **2009**, *67*, 95–99.
8. Gómez-Vallejo, V.; Kato, K.; Hanyu, M.; Minegishi, K.; Borrell, J. I.; Llop, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1913–1915.
9. The scheme of the automated system, the characteristics of the individual components and details about synthetic steps are included as Supplementary data.
10. Cadmium (20 g., granular, 5–20 mesh) was introduced in a glass column (10 mm id, 8 cm in length) and sequentially washed with 1 M HCl (2 × 20 mL), distilled water (3 × 20 mL), 0.5 M aqueous $CuSO_4$ solution (2 × 20 mL), 0.1 M aqueous NH_4Cl solution (2 × 20 mL), and distilled water (3 × 20 mL). The column showed excellent reductive properties for up to 10 consecutive runs within one day. Reconditioning on a daily basis ensured optimal performance for up to 100 runs.
11. Precursors for the radiosynthesis of *S*-[^{13}N]nitrosothiols **1**–**5** were purchased from Sigma–Aldrich and used without further purification. For the synthesis of 2-(*N*-acetyl-D-penicillamido)-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (precursor for **6**), the methodology described in Ref. 12 was followed.
12. Butler, A. R.; Greig, I. R.; Megson, I. L. WO/1998/020015, 1998.
13. For analytical HPLC, an Agilent 1200 Series HPLC system with a multiple wavelength detector (λ = 220 nm) and a radiometric detector was used. A Mediterranean SeaRP-18 column (5 μm, 150 mm, 4.6 mm) was used as stationary phase. For compounds **1**–**4** and **6**, water/methanol/acetonitrile (10:15:75) was used as mobile phase at a flow rate of 1 mL/min. For compound **5**, aqueous TFA solution/acetonitrile (95:5) was used at a flow rate of 1 mL/min.
14. Wang, K.; Hou, Y.; Zhang, W.; Ksebati, M. B.; Xian, M.; Cheng, J. P.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2897–2902.
15. For semi-preparative HPLC, an isocratic pump (Knauer) with a radiometric detector was used. A Mediterranean Sea18 column (5 μm, 250 mm, 10 mm) was used as stationary phase. For compounds **1**–**4** and **6**, water/ethanol/acetonitrile (10:15:75) was used as mobile phase at a flow rate of 6 mL/min. For compound **5**, aqueous TFA solution/ethanol (85:15) was used at a flow rate of 4 mL/min.
16. UPLC/MS analyses were performed using an AQUITY UPLC separation module coupled to a LCT TOF Premier XE mass spectrometer (Waters, Manchester, UK). An Acquity BEH C18 column (1.7 μm, 5 mm, 2.1 mm) was used as stationary phase. The elution buffers were A (methanol and 0.1% formic acid) and B (water and 0.1% formic acid). The column was eluted with a linear gradient consisted of 95% A to 1% over 2.5 min, 1% over 2.5–3.5 min, returned to 95 for 0.5 min and kept for a further 1 min. Total run was 5 min, injection volume was 5 μL and the flow rate 600 μL/min. Detection was performed in positive ion mode in the range of 50–1000, with a scan time of 1 s and a delay time of 0.1 s in centered mode. GSNO was detected as protonated molecule (m/z = 377.08, retention time = 0.81 min) and RIG was detected as sodium adduct (m/z = 572.15, retention time = 1.78 min).
17. Scenario 1: as reported in Ref. 13; scenario 2: as reported in Ref. 16.
18. Vavrek, M. T.; Mulholland, G. K. *J. Labelled. Compd. Radiopharm.* **1995**, *37*, 118.