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Syntheses of Two [^{14}C]-Labeled Disodium 4-[(*N*-*tert*-butylimino)methyl]benzene-1,3-disulfonate *N*-oxides

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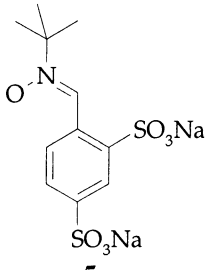
SUMMARY

NXY-059 (**5**) is a new chemical entity developed as a putative agent for the treatment of acute and chronic neurological degenerative disorders. It is currently undergoing clinical evaluation for the treatment of stroke. In order to study the metabolic transformation and organ distribution of the compound, radiolabeled NXY-059 was needed. In this paper, we describe the synthesis of two differently carbon-14 labeled disodium 4-[(*N*-*tert*-butylimino)methyl]benzene-1,3-disulfonate *N*-oxides.

Keywords: NXY-059, nitrone, acetone *O*-benzyloxime, degenerative disorders.



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INTRODUCTION

NXY-059 belongs to the chemical class of nitrones. Nitrones have been used as radical trapping agents, giving spin-adducts that are long-lived enough to be studied by spectroscopic methods. It has also been shown that nitrones react with radicals formed *in vivo*[1], and this property has attracted attention as a possible way of treating diseases manifested by oxidative stress. In addition to the ability to trap radical species, other properties of nitrones have been reported such as calcium-channel blocking[2], enzyme inhibition[3] and nootropic[4] effects. The potential of this class of compound has been demonstrated in a stroke model in the rat, where NXY-059 was shown to have a profound effect on infarct volume[5].

RESULTS AND DISCUSSION

In order to study the pharmacokinetic and metabolic properties of NXY-059, radiolabeled material was needed. Cleavage of the nitrone function, either chemically or enzymatically could be anticipated. Therefore, in order to study the distribution of degradation products both the aromatic and the aliphatic parts of the molecule needed to be labeled.

For labeling of the aromatic moiety, the carbon-14 label was introduced as described by Perry[6]. In brief, 1-bromo-2,4-dichlorobenzene was reacted with butyllithium generating the desired organometallic intermediate to which [^{14}C]carbon dioxide was added. The formed 2,4-dichlorobenzoic acid **1** was converted to the desired aldehyde by reduction of the carboxylic acid with lithium aluminum hydride to the corresponding alcohol **2**, followed by oxidation of the alcohol with lead tetraaceteate[7]. The aromatic halogens of **3** were then substituted by reaction with sodium sulfite[8] at moderately elevated pressure affording [1-alpha-^{14}C]benzaldehyde-2,4-disulfonic acid disodium salt **4**. Finally, the aldehyde was reacted with *N-tert*-butylhydroxylamine to afford NXY-059 labeled at the nitronyl carbon.

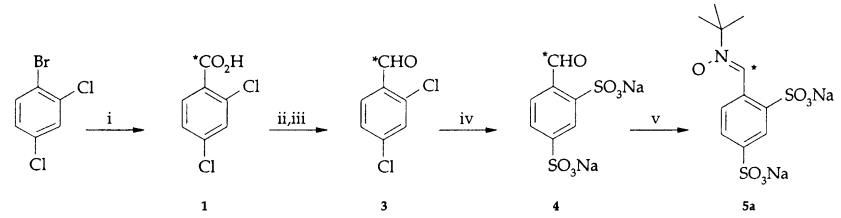

Figure 1 i) n-BuLi followed by $^{14}CO_2$ ii) $LiAlH_4$ iii) $Pb(OAc)_4$ iv) Na_2SO_3 v)*N-tert*-butylhydroxylamine

In the retrosynthetic analysis of the synthesis of labeled *N-tert*-butylhydroxylamine, acetone oxime was first considered as a starting material since it has been shown in the literature that oximes treated with an organolithium compound give in low or moderate yields the alkyl or aryl hydroxylamine[9]. However, when acetone oxime was reacted with methyllithium the desired *N-tert*-butylhydroxylamine could not be isolated. It is likely that the basicity of the organolithium reagent converts the oxime to the anion, which then would be less prone to undergo a nucleophilic attack. To circumvent this, [2-^{14}C]acetone was converted to [2-^{14}C]acetone *O*-benzyloxime by reaction with *O*-benzylhydroxylamine. The resulting imine **6**, was reacted with methyllithium using boron trifluoride ethyl etherate as catalyst,[10] affording *O*-benzyl-*N-tert*-butylhydroxylamine in 55% yield. The *O*-benzyl-protecting group was removed by catalytic hydrogenation over Pd in methanol, affording [^{14}C]-labeled *N-tert*-butylhydroxylamine. The *N-tert*-butylhydroxylamine obtained was then condensed with benzaldehyde-2,4-disulfonic acid disodium salt in methanol to afford the desired [^{14}C]NXY-059, **5b**.

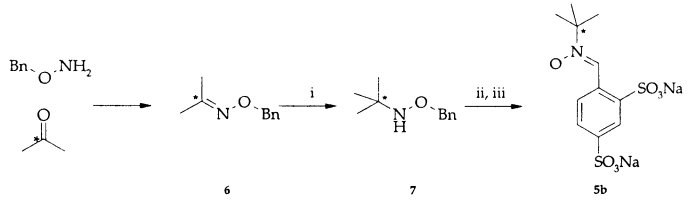

Figure 2 i) MeLi, BF_3 etherate ii) H_2/Pd iii) benzaldehyde-2,4-disulfonic acid disodium salt

EXPERIMENTAL

Radiochemical purity was determined by TLC (silica gel 60 F_{254}, Merck, glass plates) using a Bioscan System 200 Imaging Scanner and with a Packard 500 TR Flow Scintillation Analyzer connected to a HPLC instrument. Radioactivity was measured on a Packard 1000 TR liquid scintillation spectrometer using Packard Ultima Gold as a counting medium. ^{1}H and ^{13}C NMR spectra were obtained either on a Varian 300 MHz or 400 MHz spectrometer with CD_3OD as solvent. Samples containing radionuclei were run in Teflon®-tubes (Wilmad Glass Co., Inc., USA). Mass spectra were recorded on a Finnigan Mat SSQ 710 instrument operating at an electron energy (EI) of 70 eV or a Fisons VG Quattro II instrument, equipped with an electro-spray ionization trap source using the direct inlet technique. Barium [^{14}C]carbonate and [2-^{14}C]acetone were purchased from Amersham International plc, Amersham, England. All products were compared with corresponding unlabeled materials. HPLC analyses were carried out on a C_{18} reversed phase analytical column (YMC, 4.6x100 mm, ODS-A) using UV detection (220 nm). The eluent used was phosphate buffer (0.1 M, pH 6.2): CH_3CN (5:1) containing tetrabutylammonium hydrogensulfate (4 mmol).

2,4-Dichloro-[1-alpha-^{14}C]benzoic acid (1)[6].

1-Bromo-2,4-dichlorobenzene (619 mg, 2.74 mmol) in diethyl ether was cooled to -78 °C and n-butyllithium in hexane (1.6 M, 1.4 mL, 2.24 mmol) was added. The mixture was stirred at -78 °C for 20 min, and then evacuated in a vacuum manifold. $^{14}CO_2$, from $Ba^{14}CO_3$ (100 mCi, 56 mCi/mmol) and concentrated H_2SO_4 (3.5 mL), was introduced and the reaction mixture was stirred at -78 °C for 45 min. NaOH (1 M, 3 mL) was added to the cold solution, and stirring was further continued at room temperature for 15 min. The ether phase was separated, washed twice with NaOH (1 M, 2 mL) and the basic water phases were

combined. The aqueous solution was acidified to pH 2 by the addition of HCl (5 M) and was then extracted with diethyl ether (4x1.5 mL). The combined ether phases were washed with water (1.5 mL), extracted with $NaHCO_3$ (sat, 3x1.5 mL) and the water phase was washed with diethyl ether (1.5 mL) and acidified to pH 2 by the addition of HCl (5 M) and extracted with ether (4x1.5 mL). The combined ether phases were washed with water (1.5 mL), brine (1.5 mL), dried over anhydrous Na_2SO_4 and filtered. Evaporation of the solvent in a stream of nitrogen gave the title compound as a solid material (205 mg, 60%), which was used without further purification. Radiochemical purity of **1** was 96% (TLC, SiO_2; CH_2Cl_2-EtOAc-HOAC 8:1:1, r_f: 0.83) and chemical purity was 98 % (GC, Chrompack CP sil 5 column, 10 m, FID-detection).

2,4-Dichloro-[1-alpha-^{14}C]benzyl alcohol (2).

To a suspension of lithium aluminum hydride (51 mg, 1.34 mmol) in diethyl ether (4 mL) **1** (205 mg, 1.06 mmol) in diethyl ether (4 mL) was added dropwise at room temperature and under nitrogen. The reaction mixture was stirred at ambient temperature for 1 h, cooled to 4 °C, and HCl (1 M, 3 mL) was added. Stirring was continued for 20 min and then the ether phase was separated. The aqueous phase was extracted with diethyl ether (2x3 mL) and the organic extracts were combined, washed with water (2 mL), $NaHCO_3$ (2 mL), dried over anhydrous Na_2SO_4 and filtered. Evaporation of the solvent in a stream of nitrogen, afforded **2** (173 mg, 91%) as crystals. The radiochemical purity was 95.5% (TLC, toluene-EtOAc 3:1, r_f: 0.58) and chemical purity 94% (GC, as above). The material obtained was used in the next step without further purification.

2,4 -Dichloro-[1-alpha-^{14}C]benzaldehyde (3).

To lead tetraacetate (524 mg, 1.18 mmol) in pyridine (3.5 mL) under nitrogen atmosphere and at room temperature was added a solution of **2**

(173 mg, 0.97 mmol) in diethyl ether. The mixture was then stirred at reflux for 95 min, cooled and treated with HCl (1 M, 7.5 mL) and diethyl ether (3 mL). The mixture was filtered, washed with diethyl ether (3 mL) and the ether phase was separated. The water phase was extracted with diethyl ether (3x3 mL) and the combined ether phases were washed with HCl (2 M, 7 mL), water (3 mL), brine (3 mL), filtered through a silica-plug, dried over anhydrous Na_2SO_4 and filtered. Evaporation of the solvent by carefully applying a stream of nitrogen afforded crude **3** (144 mg, 84%) with a radiochemical purity of 80% (TLC, SiO_2; CH_2Cl_2-hexane 1:1, r_f: 0.47). The crude material was then purified by preparative TLC chromatography (SiO_2, E. Merck, 2 mm thickness, CH_2Cl_2-hexane 1:1). The product-containing band was scraped off and extracted with diethyl ether (30 mL). Evaporation of the solvent in a stream of nitrogen, gave the title compound (123 mg, 72%) as colorless crystals, with a radiochemical purity >99% (TLC). EIMS, m/z: (relative intensity) 178 (42), 177 (70, M^+).

[1-alpha-^{14}C]Benzaldehyde-2,4-disulfonic acid disodium salt (4). The above aldehyde (**3**; 123 mg, 0.70 mmol), unlabeled 2,4-dichloro-benzaldehyde (155 mg, 0.88 mmol), water (16 mL) and sodium sulfite (503 mg, 3.97 mmol) were stirred at 155-160 °C for 3 h in a pressure vessel. After cooling in an ice-bath for 1 h the reaction mixture was transferred to a round bottom flask. The solution was concentrated to ca. 1 mL volume overnight in a stream of nitrogen. The solution was cooled to 4 °C, when a sodium hypochlorite solution (0.4 mL, 8-12%) was added. The mixture was stirred for 15 min, centrifuged (15 min, 4000 r/m), and the supernatant was discarded. The crystalline material obtained was taken to dryness *in vacuo* at 50 °C, affording 403 mg (ca 82%) of a weakly yellow product with a chemical purity of 98.6% and a radiochemical purity of 96.5% according to analytical HPLC. The product was used directly in the next step of the synthesis.

Disodium 4-[(*N-tert*-butylimino)[^{14}C]methyl]benzene-1,3-disulfonate *N*-oxide, 5a, [^{14}C]NXY-059.

A solution of **4** (401 mg, ca. 1.3 mmol) in methanol (4.5 mL) was mixed with *N-tert*-butylhydroxylamine (168 mg, 1.89 mmol) and acetic acid (24.5 mg, 0.40 mmol). The solution was kept at 50 °C under a nitrogen atmosphere overnight and then centrifuged. The supernatant was removed and the residue was washed with methanol (1 mL). The combined solution was taken to dryness *in vacuo* giving 537 mg of a crude product. Crude **5a** dissolved in water (0.7 mL) was treated with 2-propanol (5 mL) to give a milky solution. The solution was kept at room temperature for 2 h and at 4 °C overnight. The crystals obtained were collected by centrifugation, washed with ice-cold 2-propanol (1 mL), and dried, affording the title compound (314 mg, 64%) with a specific radioactivity of 36 mCi/mmol, a chemical purity of 99.6% and a radiochemical purity of 98.2% (analytical HPLC). MS (DI, ESP) m/z 358. ^{1}H NMR (300 MHz, CD_3OD) δ 9.28 (d, *J*=8.2 Hz, 1 H), 8.99 (s, 1 H), 8.55 (d, *J*=1.9 Hz, 1 H), 7.94 (dd, *J*=8.3 Hz, *J*=1.9 Hz, 1 H), 1.61 (s, 9 H). ^{13}C NMR (75 MHz, CD_3OD) δ 147.4, 145.8, 131.6, 130.2, 129.9, 128.4, 126.4, 73.2, 28.3.

[^{14}C]Propane-2-one *O*-benzyloxime (6).

To [2-^{14}C]acetone (50 mCi, 0.86 mmol) in ethanol (1.5 mL) were added acetone (37 mg, 0.64 mmol), pyridine (243 mg, 3.07 mmol) and *O*-benzylhydroxylamine (251 mg, 1.57 mmol). The mixture was stirred at reflux under a N_2 atmosphere for 3 h and then left overnight at room temperature. The solvents were removed in a stream of nitrogen. Diethyl ether (6 mL) was added, and the organic phase was washed with water (3x1 mL), HCl (2M, 2x1 mL), water (1 mL), $NaHCO_3$ (sat, 1 mL), brine (1 mL), dried over anhydrous Na_2SO_4 and finally evaporated in a stream of nitrogen to give the crude title compound (205 mg, ca. 82%). MS m/z: (M^+) 165.

***O*-Benzyl-*N*-*tert*-butylhydroxylamine Hydrochloride (7).**

To **6** (205 mg, 1.2 mmol) in dry toluene (10 mL) and under an argon atmosphere was added boron trifluoride diethyl etherate (190 μL, 1.5 mmol) at -78 °C. The solution was stirred for 10 min when methyllithium (1.6 M; diethyl ether, 0.9 mL, 1.5 mmol) was added dropwise. Stirring was continued for 45 min and then the solution was cooled to -78 °C and more BF_3 etherate (190 μL) and methyllithium (1.6 M; 1.8 mL, 3.0 mmol) were added. Stirring was continued for 1.5 h while the temperature reached 0 °C. $NaHCO_3$ (sat., 1 mL) was added and the solvent was evaporated. Water (3 mL) was added to the remainder and the aqueous phase was extracted with diethyl ether (6x1 mL). The organic phase was washed with water (1 mL), brine (1 mL), dried over anhydrous Na_2SO_4, filtered and evaporated at reduced pressure until ca. 4 mL remained. Ethereal hydrogen chloride was added and the solid material was collected, affording the title compound (147 mg, 55%).

Disodium 4-[(*N*-tert-1-[^{14}C]butylimino)methyl]benzene-1,3-disulfonate *N*-oxide (5b).

7 (138, 0.56 mmol) in methanol (0.75 mL) was hydrogenated at room temperature over Pd/C (10%) for 65 min. The mixture was filtered and evaporated. The remainder was dissolved in $NaHCO_3$ (sat, 1 mL) and the aqueous phase was extracted with diethyl ether (6x1 mL). The combined organic phase was washed with brine (sat., 1 mL), dried over anhydrous Na_2SO_4, filtered and evaporated in a stream of nitrogen to give *N*-*tert*-butylhydroxylamine (98 mg) as white crystals.

To the crystals were added methanol (1.5 mL), water (2 drops), HOAc (10 mg) and 4-formyl-1,3-benzenedisulfonic acid disodium salt (119 mg, 0.36 mmol). The mixture was stirred under a nitrogen atmosphere at 50 °C overnight, and then cooled and centrifuged. The supernatant was collected and evaporated. The remainder was crystallized from 2-propanol and water to give the title compound (86 mg, 39%), with a

specific activity of 29.7 mCi/mmol, a chemical purity of 97% and a radiochemical purity of 98%.

^{1}H NMR (300 MHz, CDO_3D) δ 9.28 (d, *J*=8.4 Hz, 1 H), 8.98 (s, 1 H), 8.54 (d, *J*=1.5 Hz, 1 H), 7.93 (dd, *J*=8.2 Hz, *J*=1.6 Hz, 1 H), 1.61 (s, 9 H).

^{13}C NMR (75 MHz, CD_3OD) δ 147.2, 145.6, 131.4, 130.0, 129.8, 128.3, 126.2, 28.3.

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