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Synthesis of [$^2H_4$]oxymetazoline and [^{14}C]oxymetazoline

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An efficient synthesis of [$^2H_4$] and [^{14}C]oxymetazoline has been developed. Both compounds follow the same synthetic route with the introduction of the label occurring at different synthetic steps. The synthesis of [$^2H_4$]oxymetazoline from [$^2H_4$]ethylene diamine was achieved in one step with a 40% yield. The synthesis of [^{14}C]oxymetazoline from potassium [^{14}C]cyanide was achieved in two steps with an overall radiochemical yield of 67%.

Keywords: [$^2H_4$]oxymetazoline; [^{14}C]oxymetazoline; Afrin; SCH 9384; carbon-14; synthesis

Introduction

Oxymetazoline is an over-the-counter nasal spray used to ease nasal congestion caused by rhinitis, hay fever, allergies, colds, or other sinus problems. It provides symptomatic relief of nasal congestion by stimulating the α-adrenergic receptors in the arterioles of the nasal mucosa and producing vasoconstriction. The generic name is oxymetazoline hydrochloride. It is commonly known as Afrin® and marketed by Merck in the United States.

Experimental

General

All reactions were carried out under an atmosphere of nitrogen unless otherwise stated. Purifications were carried out by using flash column chromatography on a Teledyne Isco CombiFlash R_f. [$^2H_4$]Ethylene diamine (98 atom% ^{2}H) was purchased from MSD Isotopes. Potassium [^{14}C]cyanide was purchased from GE Healthcare. 2,4-Dimethyl-6-*tert*-butyl phenol was purchased from Maybridge and was purified before use. All other commercially available reagents and solvents were purchased from Aldrich and were used without further purification. Radioactivity measurements were performed on a Packard 2200CA liquid scintillation analyzer using Scintiverse BD as liquid scintillation cocktail. TLC was performed with Whatman LK6DF (silica gel 60 Å) 5 × 20 cm, 0.25-mm plates. The plates were scanned on a Bioscan 1000 linear analyzer. Mass spectra were acquired on the JEOL MStation magnetic sector mass spectrometer operating in the fast atom bombardment (FAB) ionization mode.

[$^2H_4$]Oxymetazoline and [^{14}C]oxymetazoline were analyzed by HPLC conducted on a Waters 600 multisolvent delivery system with Waters 2487 dual channel absorbance detector. Radiochemical purity of [^{14}C]oxymetazoline was determined by using a Radiomatic 525TR radioflow detector with Flo-Scint III liquid scintillation cocktail (3:1). The following systems were used:

System 1

Phenomenex Gemini C18, 4.6 × 100 mm, 3 μm, 280 nm; MeOH:MeCN:H_2O:diethylamine (45:20:35:0.3) for 10 min followed by a step gradient to MeCN, 1.0 ml/min.

System 2

Phenomenex Luna CN, 4.6 × 100 mm, 3 μm, 280 nm; 20 mM ammonium acetate (pH 5.0):MeCN (50:50) for 10 min followed by a step gradient to MeCN, 1.0 ml/min.

Specific activity of [^{14}C]oxymetazoline was determined by LC/MS on a Waters 2695 XE Separations module multisolvent delivery system with Waters PDA 996 absorbance detector. Mass spectra were acquired on the ZQ 2000 mass spectrometer operating in electrospray positive mode (ES^+). The following system was used: Phenomenex NX C18, 4.6 × 50 mm, 3 μm, 280 nm; 10 mM ammonium bicarbonate (pH 9.7):MeCN (55:45) for 4 min, 1.0 ml/min.

Synthesis of 3-(bromomethyl)-6-*tert*-butyl-2,4-dimethylphenol (2)

To a round bottom flask containing 2,4-dimethyl-6-*tert*-butylphenol **1** (4.56 g, 25.6 mmol) was added paraformaldehyde (768 mg, 25.6 mmol) followed by the addition of AcOH (10 ml, 200 mmol). To this suspension was added 33% wt. HBr solution in AcOH (4.63 ml, 25.6 mmol). This was stirred at 45°C until no starting material remained by TLC (10% ether in hexanes). The reaction was cooled to room temperature and was poured into H_2O (30 ml) with vigorous stirring. The aqueous layer was extracted with CH_2Cl_2 (2 × 30 ml). The organics were pooled, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give an orange oil. This was purified by flash column chromatography (silica gel, gradient from 100% hexanes to 100% ether) to give 2.86 g (41%) of compound **2** as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$):δ6.97 (s, 1H), 4.74 (s, 1H), 4.56 (s, 2H), 2.36 (s, 3H), 2.29 (s, 3H), 1.42 (s, 9H).

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2-(4-*tert*-butyl-3-hydroxy-2,6-dimethylphenyl)acetonitrile (3)

To a round bottom flask containing 3-(bromomethyl)-6-*tert*-butyl-2,4-dimethylphenol **2** (2.86 g, 10.5 mmol) was added DMSO (19.4 ml). To this solution was added KCN (682 mg, 10.5 mmol) dissolved in H_2O (9.7 ml). The solution was stirred at 50°C for 6 h or until no starting material remained by TLC (10% ether in hexanes). The reaction was cooled to room temperature and diluted with H_2O. The aqueous phase was extracted with ether (3 × 30 ml). The organics were pooled, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give an oily yellow solid. Crystallization from hexanes gave 1.48 g (65%) of compound **3** as an off-white powder. ^{1}H-NMR (400 MHz, $CDCl_3$):δ6.99 (s, 1H), 4.78 (s, 1H), 3.63 (s, 2H), 2.33 (s, 3H), 2.28 (s, 3H), 1.41 (s, 9H).

[$^2H_4$]Oxymetazoline (5)

To a pressure tube containing 2-(4-*tert*-butyl-3-hydroxy-2,6-dimethylphenyl)acetonitrile **3** (350 mg, 1.60 mmol) was added thioacetamide (12 mg, 0.16 mmol) and [$^2H_4$]ethylene diamine **4** (500 µl, 8.0 mmol). The tube was sealed and heated at 118°C for 3.5 h or until no starting material remained by TLC (10% 7 N NH_3 in MeOH in CH_2Cl_2). The reaction was poured into ice and was extracted with CH_2Cl_2 (3 × 5 ml). The organics were pooled, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a dark yellow oil. This was purified by flash column chromatography (silica gel, gradient from 100% CH_2Cl_2 to 70% 7 N NH_3 in MeOH in CH_2Cl_2) to give 170.5 mg (40%) of compound **5** as a light yellow solid.

Crystallization of [$^2H_4$]Oxymetazoline (5)

Several batches of yellow solid [$^2H_4$]Oxymetazoline **5** (329.4 mg) were combined and suspended in acetone. Hexane was added to further crystallize the compound. The suspension was placed at −20°C for 1 h. The white solid collected was dried to constant weight to give 240 mg (73%) of 99.4% pure compound **5** as determined by HPLC system 1. FAB-MS: *m/z* 265 $(M+H)^+$. ^{1}H-NMR (600 MHz, $CDCl_3$):δ6.96 (s, 1H), 3.66 (s, 2H), 2.27 (s, 3H), 2.21 (s, 3H), and 1.40 (s, 9H).

2-(4-*tert*-butyl-3-hydroxy-2,6-dimethylphenyl)aceto[^{14}C]nitrile (6)

To a round bottom flask containing 3-(bromomethyl)-6-*tert*-butyl-2,4-dimethylphenol **2** (248 mg, 0.915 mmol) was added DMSO (1.6 ml). To this solution was added potassium [^{14}C]cyanide (51.8 mCi, 0.909 mmol) dissolved in H_2O (200 µl). The vial that contained the potassium [^{14}C]cyanide was rinsed with H_2O (2 × 205 µl) and the rinses were added to the reaction. The yellow solution was stirred at 50°C for 5.5 h or until no starting material remained by TLC (10% ether in hexanes). The reaction was cooled to room temperature and H_2O was added. The aqueous phase was extracted with ether (3 × 5 ml). The organics were pooled, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give 41.1 mCi (79%) of compound **6** as a yellow oil with 98.1% RCP. Radio-TLC: R_f (ether:hexanes = 20:80) 0.146. The crude product was used directly in the next step without further purification.

[^{14}C]Oxymetazoline (8)

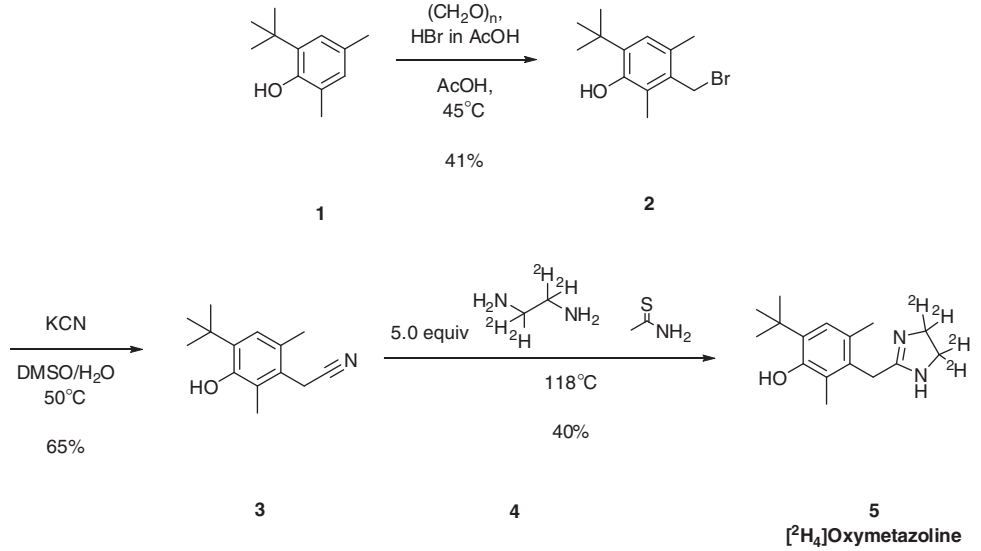
To a pressure tube containing 2-(4-*tert*-butyl-3-hydroxy-2,6-dimethylphenyl)aceto[^{14}C]nitrile **6** (41.1 mCi, 0.721 mmol) was added thioacetamide (5.41 mg, 0.072 mmol) and ethylene diamine **7** (483 µl, 7.21 mmol). The tube was sealed and heated at 118°C until the reaction was complete by TLC (10% 7 N NH_3 in MeOH in methylene chloride). The reaction was checked by TLC every 2–3 h. Additional thioacetamide (5.41 mg, 0.072 mmol) was added until the reaction was complete. The total time for completion was 16 h and a total of 37.9 mg of thioacetamide was added. The reaction was poured into ice and extracted with CH_2Cl_2 (3 × 5 ml). The organics were pooled, washed with H_2O (25 ml), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give 38.4 mCi (93.4%) of compound **8** as a yellow solid with 97.8% RCP by TLC and 92.4% RCP by HPLC system 1. Radio-TLC: R_f (7 N NH_3 in MeOH: CH_2Cl_2 = 10:90) 0.203. The crude was purified by flash column chromatography (silica gel, gradient from 100% CH_2Cl_2 to 70% 7 N NH_3 in MeOH in CH_2Cl_2). For our purposes, the most pure fractions containing product were combined and concentrated down under reduced pressure to give 3.6 mCi (8.8%) of compound **8** as a white solid with RCP of 98.5 and 99.0% by HPLC system 1 and system 2, respectively. The specific activity of [^{14}C]oxymetazoline is 60.4 mCi/mmol as determined by LC/MS system. The less pure fractions gave 31.2 mCi (76%) of compound **8** as a white solid with 89.9% RCP by HPLC system 1. The total RCY for this step was 85%. The overall RCY of compound **8** over two steps was 67%.

Results and discussion

[$^2H_4$]Oxymetazoline was prepared for use as an internal standard in a bioanalytical liquid chromatography/tandem mass spectrometry method. [^{14}C]Oxymetazoline was synthesized to support drug disposition studies. [$^2H_4$] and [^{14}C]oxymetazoline were synthesized, using a modified literature procedure,[1] in 40 and 67% yield, respectively.

The synthesis of the deuterium-labeled oxymetazoline is shown in Scheme 1. 2,4-Dimethyl-6-*tert*-butylphenol was bromomethylated by heating with paraformaldehyde and HBr in acetic acid in 41% yield using modified literature procedures.[2,3] Cyanide was introduced via nucleophilic displacement of bromine in 65% yield after crystallization from hexanes.[4–6] Deuterium was incorporated by the cyclocondensation of 2-(4-*tert*-butyl-3-hydroxy-2,6-dimethylphenyl)acetonitrile with [$^2H_4$]ethylene diamine in the presence of a catalytic amount of thioacetamide in a pressure tube.[7]

The cyclocondensation reaction with the deuterium label proved to be the most challenging step. Literature conditions[7] require a ten-fold excess of the ethylene diamine, as it also acts as solvent in the reaction. In an effort to reduce cost and conserve the labeled reagent, the [$^2H_4$]ethylene diamine was dropped to a five-fold excess. However, under these conditions, we were unable to drive the reaction to completion. At best, there was a 20% yield of product and 57% recovery of unreacted starting material. Dash *et al.* reported smooth transformations of various nitriles to their corresponding 2-imidazolines via thioacetamide catalysed cyclocondensations.[7] Their proposed mechanistic pathway involves the initial reaction of thioacetamide with ethylene diamine producing 2-methyl-2-imidazoline and H_2S; with the H_2S acting as the actual catalyst for the cyclocondensation reaction. Based on this theory, we ran this reaction in a pressure tube in order to contain the H_2S and drive the reaction to completion. As shown in Table 1, this change doubled the yield of [$^2H_4$]oxymetazoline to 40% after column purification, all starting material was consumed, and it allowed

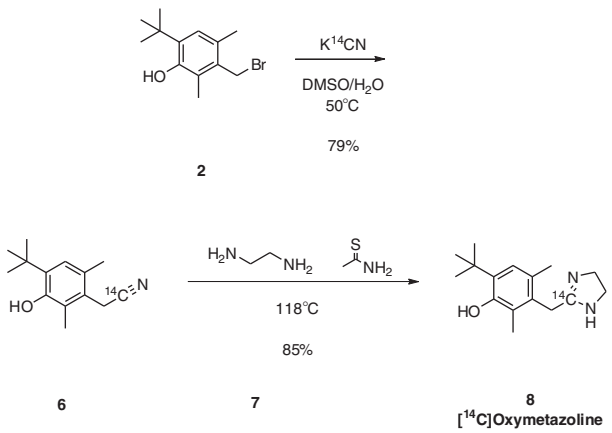


Scheme 1. Synthesis of [$^2H_4$]oxymetazoline.

Table 1. Optimization of reaction to produce [$^2H_4$]oxymetazoline

Reaction	Eq. of 4	Comments	Yield (%)[a]
1	5.0	Refluxed (RB flask, condensor), 118°C, 3 h. TLC analysis of reaction mixture showed incomplete reaction and six major components	20
2	5.0	Refluxed (RB flask, condensor), 118°C, 16 h. TLC analysis of reaction mixture showed incomplete reaction and six major components. Additional thioacetamide did not change the reaction profile	18
3	5.0	Heated (Sealed tube), 118°C, 3.5 h. TLC analysis of the crude reaction showed complete consumption of **3** and formation of product **5** and two additional components	40

[a]After silica gel chromatography.



Scheme 2. Synthesis of [^{14}C]oxymetazoline.

for the conservation of the label by using a five-fold rather than a ten-fold excess. Additionally, TLC showed a much cleaner reaction.

Further attempts to optimize the reaction were set aside due to an accelerated delivery timeline requested by the customer group for the deuterated standard. A 40% yield was acceptable to deliver sufficient material.

The [^{14}C] label was incorporated one step earlier, as in Scheme 2, by nucleophilic displacement of bromine from 3-(bromomethyl)-6-*tert*-butyl-2,4-dimethylphenol with [^{14}C]cyanide from potassium [^{14}C]cyanide.[4–6] The remaining steps were identical to the SIL synthesis.

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