JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2005; **48**: 117–121.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.904

Note

Synthesis of [$^{13}C_4$]furan

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Summary

Furan is a liver toxicant and carcinogen in laboratory animals. [$^{13}C_4$]Furan was required for *in vivo* metabolism and mechanistic studies. It was prepared in five steps from commercially available [$^{13}C_3$]propargyl alcohol and [^{13}C]paraformaldehyde.

Key Words: furan; ^{13}C labeling; stable isotope

Introduction

Furan is an industrial chemical that has also been detected as an environmental contaminant in smog, tobacco smoke, coffee, and canned foods.[1] It is a liver and kidney toxicant and a hepatocarcinogen in rodents,[2–6] and has been classified as a possible human carcinogen.[7] Metabolic activation plays an important role in the toxicity of furan.[8–11] Furan is converted to a toxic metabolite in a reaction catalyzed by cytochrome P-450 2E1.[8,9] The initial oxidation product of furan is *cis*-2-butene-1,4-dial.[12,13] The chemical reactivity of this α,β-unsaturated dialdehyde indicates that it may be the ultimate reactive metabolite.[10,13,14] However, it is also possible that *cis*-2-butene-1,4-dial could undergo further metabolism to generate the reactive intermediate responsible for triggering the toxic and carcinogenic effects of furan.

Identification of *in vivo* metabolites can reveal activation pathways. Furan (8 mg/kg) is rapidly metabolized in rats, with the majority (40%) being eliminated in the exhaled air as either unchanged furan or carbon dioxide.[15] A significant amount of radioactivity is associated with the urine (22%) and feces (20%). The urine contains multiple hydrophilic furan metabolites but these species have not been chemically characterized.[15] Since subsequent oxidation

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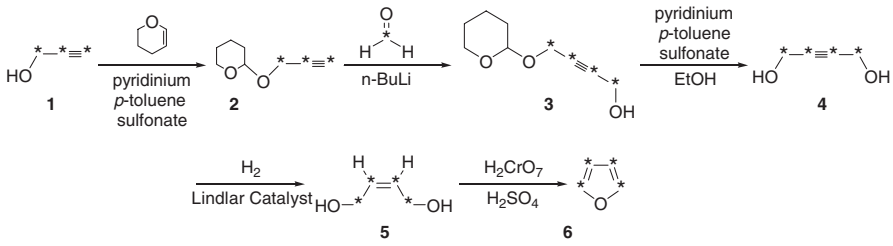
Contract/grant sponsor: NIH; contract/grant number: ES-10577

Received 21 September 2004
Accepted 11 October 2004



Scheme 1.

of *cis*-2-butene-1,4-dial can lead to the formation of maleic acid as well as possibly fumaric acid, we expected that some of the metabolites could also be generated from endogenous process such as the citric acid cycle. Therefore, we decided to take a stable isotope labeling approach to this problem, which required the preparation of [$^{13}C_4$]furan. In this paper, we report the preparation of [$^{13}C_4$]furan in a multi step synthetic approach (Scheme 1) from commercially available [$^{13}C_3$]propargyl alcohol and [^{13}C]paraformaldehyde (Cambridge Isotope Laboratory).

Results and discussion

The goal of this project was to synthesize uniformly [^{13}C]-labeled furan for our metabolic studies. We adapted existing synthetic methods to achieve our target compound. The structures of the intermediates and the product were confirmed and characterized by ^1H and ^{13}C NMR spectroscopy. The hydroxyl group of [$^{13}C_3$]propargyl alcohol (**1**) was protected with 3,4-dihydropyran in presence of pyridinium–paratoluene-sulfonate to give 93% yield of [1,2,3-$^{13}C_3$]3-tetrahydropyran-2-yloxy)-prop-1-yne (**2**).[16] Treatment of **2** with [^{13}C]paraformaldehyde in presence of *n*-butyl lithium led to [$^{13}C_4$]4-(tetrahydropyran-2-yloxy)-but-2-yn-1-ol (**3**) in 92% yield.[17] Deprotection of **3** was accomplished by heating this intermediate with pyridinium–paratoluene-sulfonate at 55 °C for 3–4 h, producing [$^{13}C_4$]2-butyne-1,4-diol (**4**) in 85% yield following column chromatography.[16] Hydrogenation of **4** with Lindlar catalyst followed by flash chromatography gave [$^{13}C_4$]*cis*-2-butene-1,4-diol (**5**) in 77% yield.[18] The progress of the reaction was monitored by TLC analysis to ensure that the reduction proceeded to the alkene, not the alkane. The desired product, [$^{13}C_4$]furan (**6**) was obtained by the oxidation of **5** in presence of sodium dichromate.[19] Distillation of the product provided a 44% yield of pure **6**. The overall yield of **6** from [$^{13}C_3$]-propargyl alcohol was 25%.

Experimental

[$^{13}C_3$]Propargyl alcohol (99%) and [^{13}C]paraformaldehyde (99%) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA).

n-Butyl lithium in *n*-hexane (2.8 M) was purchased from Alfa Aesar (Ward Hill, MA). All other compounds were purchased from Aldrich (Milwaukee, WI). ^{1}H and ^{13}C NMR spectra were obtained either at 300 MHz or at 500 MHz on a Varian Inova-300 or 500 NMR spectrometer in $CDCl_3$, CD_3OD, or acetone-d_6 and are reported in ppm relative to an external standard. Thin layer chromatography was performed on silica gel aluminum sheets (EM Science, Silica Gel 60 F_{254}). The chromatograms were visualized by staining with phosphomolybdic acid reagent/$KMnO_4$ solution, followed by heating. Column chromatography was performed using silica gel (70–230 mesh, EM Science).

[1,2,3-$^{13}C_3$]3-(Tetrahydropyran-2-yloxy)-prop-1-yne, **2**

A solution of pyridinium paratoluene sulfonate (1.6 g, 6.2 mmol) in anhydrous dichloromethane was added to an ice-cooled mixture of 3,4-dihydropyran (22.5 ml, 248 mmol), and [$^{13}C_3$]propargyl alcohol (**1**, 3.7 g, 62 mmol) in anhydrous dichloromethane (250 ml) under N_2 atmosphere. The mixture was stirred under N_2 at 0–5°C for 30 min, then at 20°C for 2 h. The mixture was diluted with ether (400 ml), washed with saturated $NaHCO_3$ solution (150 ml), brine (150 ml), and water (300 ml). The aqueous solutions were combined and extracted with ether (2 × 200 ml). The combined organic solutions were dried over $MgSO_4$, and evaporated to give **2** (8.25 g, 57.7 mmol, 93% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.81 (dd, 1 H, H-2′, $^3J_{H,H}$ = 6.7 Hz, $^3J_{H,H}$ = 3.0 Hz), 4.25 (dm, 2 H, H-3, $^1J_{H,C}$ = 149.4 Hz), 3.84 (m, 1 H, H-6′$_a$), 3.54 (m, 1 H, H-6′$_b$), 2.40 (ddd, 1 H, H-1, $^1J_{H,C}$ = 248 Hz, $^2J_{H,C}$ = 52 Hz, $^3J_{H,C}$ = 2 Hz), 1.85 (m, 2 H, H-4′), 1.75 (m, 1 H, H-3′$_a$), 1.65 (m, 1 H, H-3′$_b$), 1.60 (m, 1 H, H-5′$_a$), 1.55 (m, 1 H, H-5′$_b$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 96.8 (C-2′), 79.8 (dd, C-2, $^1J_{C,C}$ = 173.1 Hz, $^1J_{C,C}$ = 73.4 Hz), 73.8 (dd, C-1, $^1J_{C,C}$ = 172.1 Hz, $^2J_{C,C}$ = 13.0 Hz), 62.0 (C-6′), 53.9 (dd, C-3, J_1 = 73.4 Hz, J_2 = 13.0 Hz), 30.2 (C-3′), 25.3 (C-5′), 19.0 (C-4′).

[$^{13}C_4$]4-(Tetrahydropyran-2-yloxy)-but-2-yn-1-ol, **3**

A 2.79 M solution of *n*-butyl lithium in *n*-hexane (18 ml, 50 mmol) was added to a stirred solution of **2** (6.0 g, 42 mmol) in anhydrous ether (400 ml) at −78°C and under a N_2 atmosphere. The mixture was stirred under N_2 for 1 h at −78°C and then allowed to warm to room temperature with continuous stirring. [^{13}C]Paraformaldehyde (2.6 g, 27 mmol) was heated and the resulting formaldehyde bubbled through the stirred reaction mixture in a stream of nitrogen. After overnight at room temperature, reaction was quenched with saturated NH_4Cl (400 ml). The aqueous solution was extracted with ether (2 × 200 ml), and the ether phase was dried over $MgSO_4$. Removal of ether under reduced pressure gave **3** (6.7 g, 38.5 mmol, 92% yield). ^{1}H NMR

(500 MHz, $CDCl_3$) δ 4.80 (dd, 1 H, H-2′, $^1J_{H,H}$ = 7 Hz, $^1J_{H,H}$ = 3.5 Hz), 4.31 (dm, 4 H, H-4, H-1, $^1J_{H,C}$ = 145.4 Hz), 3.84 (m, 1 H, H-6′$_a$), 3.54 (m, 1 H, H-6′$_b$), 1.85 (m, 2 H, H-4′), 1.75 (m, 1 H, H-3′$_a$), 1.65 (m, 1 H, H-3′$_b$), 1.60 (m, 1 H, H-5′$_a$), 1.55 (m, 111, H-5′$_b$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 88.2 (C-2′), 84.4 (ddd, C-3, $^1J_{C,C}$ = 174.1 Hz, $^1J_{C,C}$ = 67.4 Hz, $^2J_{C,C}$ = 15.1 Hz), 81.6 (ddd, C-2, $^1J_{C,C}$ = 174.0 Hz, $^1J_{C,C}$ = 67.4 Hz, $^2J_{C,C}$ = 15.1 Hz), 62.0 (C-6′), 54.3 (dd, C-4, $^1J_{C,C}$ = 70.4 Hz , $^2J_{C,C}$ = 17.1 Hz), 51.2 (dd, C-1, $^1J_{C,C}$ = 69.5 Hz, $^2J_{C,C}$ = 17.1 Hz), 30.2 (C-3′), 25.3 (C-5′), 19.0 (C-4′).

[$^{13}C_4$]2-Butyne-1,4-diol, **4**

Pyridinium *p*-toluene sulfonate (0.5 g, 2 mmol) was added to a solution of **3** (3.4 g, 19.4 mmol) in absolute ethanol (150 ml), and stirred at 55°C for 3–4 h. Silical gel (10.0 g) was added to the mixture and evaporated to give a complex of silica and crude product. The silica complex was loaded on the top of a silica gel (85 g) chromatography column in hexanes. The desired product **4** was eluted with ethyl acetate/hexanes (1:1). Removal of the solvent yielded **4** as a white solid (1.49 g, 16.5 mmol, 85% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 4.87 (bs, 2 H, -OH) , 4.19 (d, 4 H, H-I, $^1J_{H,C}$ = 145.5 Hz). ^{13}C NMR (75.47 MHz, CD_3OD) δ 82.8 (dd, C-2, $^1J_{C,C}$ = 48.2 Hz, $^1J_{C,C}$ = 37.5 Hz), 49.3 (dd, C-1, $^1J_{C,C}$ = 48.2 Hz, $^1J_{C,C}$ = 37.5 Hz).

[$^{13}C_4$]cis-2-Butene-1,4-diol, **5**

Lindlar catalyst (160 mg, Aldrich, Milwaukee, WI) was added to a solution of **4** (1.7 g, 18 mmol) in ethyl acetate (50 ml). The mixture was hydrogenated until 1 equivalent of hydrogen (410 ml) had been taken up. The mixture was filtered through celite, washing the catalyst with ethyl acetate (15 ml). The eluant was concentrated under reduced pressure. Flash chromatography (100 g of silica gel packed) with ethyl acetate gave **5** (1.29 g, 14.0 mmol, 77% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 5.63 (dm, 2 H, H-2, $^1J_{H,C}$ = 157.9 Hz), 4.87 (s, 2 H, -OH), 4.13 (dm, 4 H, H-1, $^1J_{H,C}$ = 143.4 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 130.2 (dd, C-2, $^1J_{C,C}$ = 30 Hz, $^2J_{C,C}$ = 17 Hz), 57.1 (cc, C-1, $^1J_{C,C}$ = 30 Hz, $^1J_{C,C}$ = 16 Hz).

[$^{13}C_4$]Furan, **6**

A solution of **5** (4.3 g, 46.8 mmol) in water (8 ml) was heated rapidly to 90 °C. The heat was removed and a solution of sodium dichromate (6.0 g of the dihydrate) in aqueous sulfuric acid (2.4 ml of concentrated sulfuric acid in 14 ml of water) was added dropwise with efficient stirring at such a rate that the temperature was kept at 90–92 °C. The mixture was then heated to 100°C for 5 min. The desired product was collected by distillation to yield 95% pure **6** (1.48 g, 20.5 mmol, 44% yield). ^{1}H NMR (300 MHz, acetone-d_6) δ 7.61 (dm,

2 H, H-1, $^{1}J_{H,C}$ = 203.7 Hz), 6.47 (dm, 2 H, H-2, $^{1}J_{H,C}$ = 178.8 Hz). ^{13}C NMR (75 MHz, acetone-d_6) δ 142.6 (m, C-1), 109.3 (m, C-2).

Acknowledgements

This work was supported by NIH research grant ES-10577.

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