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Synthesis of 5-methylthioribose specifically bitritiated at the 5-C position

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Summary

An efficient and reliable method for the preparation of bitritiated-methylthioribose from a suitably protected 5-ketoazido-ribose derivative has been developed. This compound is an essential component in the assay of the microbial enzyme methylthioribose kinase, which is involved in the methionine salvage pathway from methylthioadenosine. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: ^{3}H labelling; methylthioribose; methylthioribose kinase; enzymatic assay

Introduction

5-Methylthioribose (MTR) kinase catalyses the ATP-dependent phosphorylation of 5-methylthioribose in the methionine salvage pathway.[1] This enzyme is not present in mammalian cells but it is essential for methionine recycling from 5′-methylthioadenosine in plants[2] and in a number of bacterial and protozoan pathogens of humans.[3–5] In recent years, it has been demonstrated that inhibitors of MTR kinase are potent inhibitors of the growth of MTR kinase-containing microorganisms.[6–9]

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Due to its specific and crucial role in microbial methionine salvage, MTR kinase has emerged as a target enzyme for the molecular design of new selective antibacterial and antiprotozoal agents.[10,11] For characterization of MTR activity from different sources, a radioactive enzymatic assay has been developed.[9] This assay employs ^{3}H or ^{14}C labelled MTR which is phosphorylated during the enzymatic reaction and is thus transformed into the corresponding labelled methylthioribose-1-phosphate (1-P-MTR). MTR kinase activity is assayed by measuring the rate of formation of 1-P-MTR from MTR after their separation on an anion resin exchanger. Until now, the production of 5-[methyl-^{3}H]-methylthioribose or 5-[methyl-^{14}C]-methylthioribose involved preparation of 5′-[methyl-^{3}H]-methylthioadenosine[12,13] or 5′-[methyl-^{14}C]-methylthioadenosine;[14,15] the depurination of which is achieved by acid hydrolysis[16] or by incubation with MTA/SAH nucleosidase (EC.3.2.2.9).[12,13]

For the ongoing determination of the distribution of MTR kinase among protozoa and other microbes and the screening of the inhibitory potency of large series of MTR analogs, substantial amounts of highly labelled MTR are required. For this purpose, the previously described protocols for the synthesis of labelled MTR have serious limitations. The present paper describes a new and reliable method for the preparation of 5-[$^{3}H_2$]-methylthioribose of high specific activity.

Results and discussion

Reduction of an aldehydo or keto group with [^{3}H]-sodium borohydride is widely used for the preparative synthesis of a large variety of tritium labelled compounds. We found that the highly reactive carboxylic azide **7** provided a useful tool for incorporation of two ^{3}H tritium atoms into the target compound **10**. 1,2-*O*-(isopropylidene)-3-*O*-methoxymethyl-α-D-ribofuranoyl azide **7**[†] was prepared in good yield from the diacetonide **1** of D(+)-glucose according to procedures described in the literature[17–21] (Scheme 1).

[†] **7**: ^{1}H-N.M.R ($CDCl_3$, 250 Mz): 1.36 and 1.59 (s, 3H, $(CH_3)_2C$); 3.42 (s, 3H, OCH_3); 4.13 (dd, 1H, H_3, $J_{H3-H4} = 9.2$ Hz, $J_{H3-H2} = 4.2$ Hz); 4.46 (d, 1H, H_4, $J_{H4-H3} = 9.2$ Hz) ; 4.69 (t, 1H, H_2, $J_{H2-H1} = J_{H2-H3} = 3.8$ Hz); 4.75 (s, 2H, O–CH_2–O); 5.86 (d, 1H, H_1, $J_{H1-H2} = 4.0$ Hz). ^{13}C N.M.R ($CDCl_3$, 62.5 Mz) : 26.3 and 26.8 ($(CH_3)_2C$); 55.9 (OCH_3); 78.4 (C4); 78.7 (C3); 79.1 (C2); 96.7 (O–CH_2–O); 104.4 (C1); 113.8 ($(CH_3)_2C$); 176.8 (C=O). IR : 2147, 1719 cm^{-1}. $[\alpha]_D^{20} = +15.2°$ ($c = 0.005$ g/ml; $CHCl_3$).

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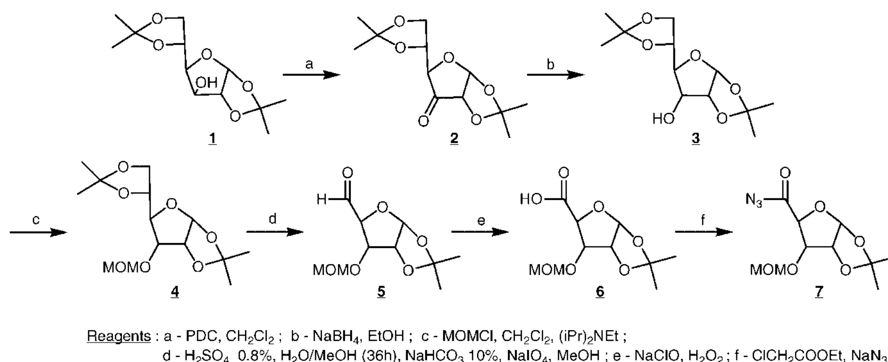
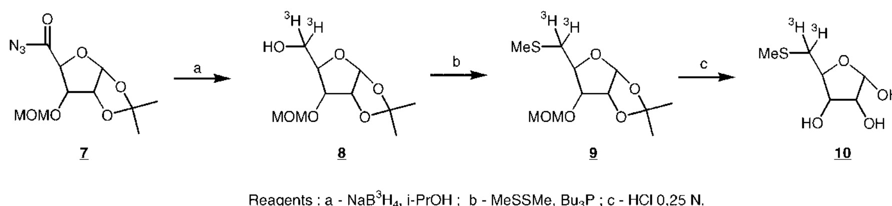


Scheme 1.

As outlined in Scheme 2, this acylazidosugar derivative 7, suitably protected with acid removable protecting groups was an attractive precursor for a short synthesis of bitritiated MTR **10**.



Scheme 2.

The procedures for each step in this synthetic route and the methods of purification of each intermediate were adapted for secure manipulation of radioactive material.

Reduction of an excess of 7 (0.36 mmol) with ^{3}H-$NaBH_4$ (925 MBq, 19.3 GBq/mmol) was readily achieved in isopropanol at room temperature. The progress of the reaction was monitored quantitatively by TLC radiochromatography using aliquots (1 μl) of the reaction mixture. The resulting bitritiated ribose derivative was easily separated from excess starting material by column chromatography on silica gel. This provided pure compound 8 in 91% yield (23 mg, 840 MBq, specific activity 6.7 MBq/mmol).

The methylthio group was introduced into the 5-C position of the ribose 8 derivative *via* a single step using a combination of dimethyldisulfide and tri-*n*-butylphosphine.[22] However, when performed at μmol scale, this reaction was more efficient without additional

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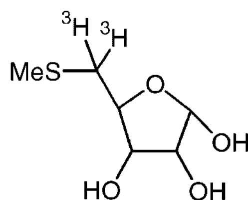

solvent. When an aliquot of compound **8** (107.3 MBq, 16 μmol) was treated under argon at 50°C with five equivalents of dimethyldisulfide and 10 equivalents of tri-*n*-butylphosphine, the reaction proceeded smoothly and was complete after 12 h.

Further purification of the resulting crude product by chromatography provided pure labelled MTR derivative **9** (85 MBq) in 80% yield. The deprotection of the 1,2- and 3-hydroxyl groups of compound **9** was achieved by treatment of **9** with aqueous 0.25 M HCl. After neutralization of the solution with NH_4OH, desalting and purification of the deprotected MTR were performed on HP 20 SS hydrophobic resin. Similarly, chemically and radiochemically pure 5-[$^3H_2$]-MTR **10** (69 MBq, 81% yield) with a specific activity of 6.7 MBq/mmol was obtained. Unlabelled MTR was also synthesized using the same reaction sequence. The structures of the unlabelled intermediates as well as their corresponding tritiated derivatives were established using spectroscopic techniques (^{1}H, ^{13}C NMR and mass spectrometry).

Experimental

All chemicals were of the highest purity available. [^{3}H]-$NaBH_4$ (925 MBq, 19.3 GBq/mmol) was purchased from NEN Life Science Products. ^{1}H, ^{13}C NMR were recorded on a Bruker AC 250 spectrometer. Chemical shifts (δ) are reported in ppm using TMS as internal standard and J values in Hz. Mass spectra were obtained using an AUTOSPEC (VG instruments) spectrometer. HP 20 SS Dianion resin was from Mitsubishi Fine Chemicals. Counting was carried out with a Packard Liquid Scintillation Counter using a Packard Scintillation cocktail Ultima-Flo AP.

The chemical homogeneity of all of the radioactive compounds was checked by TLC (silica gel precoated plates no. 5545 Merck) and radiochemical purity was moniotored by radiochromatography using a LB 285 BERTHOLD Scanner equipped for TLC-CHROMA 2D.

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1,2-O-isopropylidene-3-O-methoxymethyl-5-[$^3H_2$]-α-D-ribopentofuranose: **8**

[^{3}H]-$NaBH_4$ (925 MBq, 19.3 GBq/mmol), in powder form, was combined in a 5 ml flask with 2.5 ml of 1,2-*O*-(isopropylidene)-3-*O*-methoxymethyl-α-D-ribofuranoyl azide **7** (100 mg, 0.36 mmol). The solution was magnetically stirred at room temperature. To monitor the progress of the reduction reaction, 1 μl aliquots were removed from the reaction mixture at regular intervals and analyzed using TLC radiochromatography. Once all of the [^{3}H]-$NaBH_4$ had been consumed (30 min), the reaction mixture was neutralized with a few drops of acetic acid and evaporated under vacuum. The resulting crude labelled compound **8** was extracted by trituration of the residue with 4×5 ml of chloroform.

After washing of the chloroform solution with brine, the organic layer was dried over anhydrous $MgSO_4$. Evaporation of chloroform followed by column chromatography with ethyl acetate/hexane (7/3) gave pure ribose derivative **8** (840 MBq, 23 mg, 91%) as a colorless oil. The specific activity of the product was 6.7 GBq/mmol and the combined ^{1}H NMR and TLC radiochromatography indicated the material to be >98% chemically and >99% radiochemically pure.

^{1}H-N.M.R (δ, $CDCl_3$): 1.37 and 1.59 (s, 3H, $(CH_3)_2C$); 3.45 (s, 3H, OCH_3); 3.68 (dd, 1H, H_5, $J_{H5-H5'} = 12.0$ Hz, $J_{H5-H4} = 2.7$ Hz); 3.97 (dd, 1H, $H_{5'}$, $J_{H5'-H5} = 12.0$ Hz, $J_{H5'-H4} = 2.7$ Hz); 4.06 (dd, 1H, H_3, $J_{H3-H4} = 9.0$ Hz, $J_{H3-H2} = 4.0$ Hz); 4.13 (dt, 1H, H_4, $J_{H4-H3} = 9.0$ Hz, $J_{H4-H5} = J_{H4-H5'} = 2.7$ Hz); 4.66 (t, 1H, H_2, $J_{H2-H1} = J_{H2-H3} = 4.0$ Hz); 4.73 and 4.78 (d, 1H, O–CH_2–O, $J_{H-H} = 6.7$ Hz); 5.76 (d, 1H, H_1, $J_{H1-H2} = 4.0$ Hz).

1,2-O-isopropylidene-3-O-methoxymethyl-5-deoxy-5-S-methylthio-5-[$^3H_2$]-α-D-ribofuranose: **9**

An aliquot of 107.3 MBq (16 μmol) of **8**, prepared as described, was mixed in a stoppered micro vial (flushed with argon), with dimethyldisulfide (5 eq.) and tri-*n*-butylphosphine (10 eq.). The reaction mixture was then stirred at 50°C until total transformation of **8** was achieved (12 h, TLC radio-chromatography control, eluant: ethyl acetate/hexane : 50/50). The resulting crude material was taken with 1 ml of chloroform and applied to a silica gel column (0.5–10 cm). In this way, pure labelled protected MTR **9** (85 MBq, 80%) was obtained.

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^{1}H-N.M.R (δ, $CDCl_3$): 1.37 and 1.59 (s, 3H, $(CH_3)_2C$); 2.11 (s, 3H, SCH_3); 2.70 (dd, 1H, H_5, $J_{H5-H5'} = 13.1$ Hz, $J_{H5-H4} = 4.6$ Hz); 2.90 (dd, 1H, $H_{5'}$, $J_{H5'-H5} = 13.1$ Hz, $J_{H5'-H4} = 2.7$ Hz); 3.45 (s, 3H, OCH_3); 3.92 (dd, 1H, H_3, $J_{H3-H4} = 9.0$ Hz, $J_{H3-H2} = 4.0$ Hz); 4.29 (m, 1H, H_4); 4.66 (t, 1H, H_2, $J_{H2-H1} = J_{H2-H3} = 4.0$ Hz); 4.73 and 4.78 (d, 1H, O–CH_2–O, $J_{H-H} = 6.7$ Hz); 5.76 (d, 1H, H_1, $J_{H1-H2} = 4.0$ Hz).

*5-[$^3H_2$]-5-deoxy-5-S-methylthio-*D*-ribofuranose:* **10**

85 MBq of **9** was dissolved in 1 ml of aqueous 0.25 M HCl and the solution was heated at 50°C. The removal of 1,2-*O*-isopropylidene and 1-methoxymethyl groups was completed in 5 h (checked by TLC radio-chromatography, eluant: ethyl acetate/methanol : 90/10). After neutralization of the aqueous solution with a few drops of 7N NH_4OH, desalting and purification of compound **10** was carried out using a column (0.5–10 cm) containing HP 20 SS hydrophobic resin. Elution was performed successively with 50 ml of water and 50 ml of methanol/water : 10/90. Fractions containing the radioactive product were combined, the methanol was removed and the resulting solution lyophilized to afford 69 MBq of pure **10**, specific activity: 67 MBq/mmol (81% yield). The chemical homogeneity and radiochemical purity was checked by ^{1}H NMR and TLC radio-chromatography using ethyl acetate/methanol : 90/10 as solvent.

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