Synthesis of fully deprotected glyco-MTS reagents

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Method Article

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Abstract

Introduction

Methanethiosulfonates \(MTS) are a group of reagents which allow the site-selective modification of accessible cysteine residues in proteins.[1] Synthesis of compounds carrying the MTS moiety has allowed the covalent attachment of structures to protein surfaces which represent functional mimics of post-translational modifications.[2] Here we describe the synthesis of ethyl-linked O-glycosyl methanethiosulfates which allow the selective incorporation of fully deprotected carbohydrate structures onto protein scaffolds.[3,4] See figure in Figures section.

Reagents

• 40-60º Petroleum spirit, glass distilled grade \(Rathburn, cat. no. RG2031) • Ethyl acetate, HPLC grade \(Rathburn, cat. no. RH1013) • Methanol, HPLC grade \(Rathburn, cat. no. RH1019) • N,N-Dimethylformamide \(DMF) \(Rathburn, cat. no. RG2014) • Boron trifluoride etherate, >98% \(Alfa Aesar cat. no. A15275) • 2-Bromoethanol, 97% \(Alfa Aesar cat. no. A10275) • Acetic anhydride, >97% \(Alfa Aesar cat. no. 36292) • Pyridine, >99% \(Acros cat. no. 13178) • Sodium methoxide, 98% \(Alfa Aesar cat. no. L05673) • Dowex 50X2-100 \(Acros cat. no. 20302) • Sodium methanethiosulfonate \(TRC Biomedical Research cat. no. S645000) • Silica gel for flash column chromatography \(BDH, cat. no. 153325P) • Thin-layer chromatography plates on aluminium backing, silica gel 60 F254 \(Merck) • Deuterated chloroform \(Sigma-Aldrich, cat. no. 151823) • Deuterium oxide, >99.95% \(Alfa Aesar, cat. no. 43700)

Equipment

• Magnetic hotplate stirrer \(eg. IKA® RCT Basic) • Digital temperature probe • Oil bath • 10 and 50 mL two-neck round bottom flask • Water condenser \(to fit neck of flask) • Rubber septa \(to fit neck of flask) • Teflon-coated magnetic stirrer bar • Balloon fitted to disposable 2.5 mL syringe barrel • Rotary evaporator \(Büchi) • Pyrex chromatographic column \(approx. diameter 3 cm) • NMR tubes

Procedure

Synthesis of 2-bromoethyl 2,3,4,6 tetra-O-acetyl-α-D-glucopyranoside 1) Flush a three-neck 50mL round bottom flask containing a Teflon-coated magnetic stirrer bar with dry nitrogen for 5 minutes. Fit one neck with a quickfit adaptor containing a thermometer, the second neck with a rubber septum and equip the main neck with a reflux condenser. 2) Weigh out 1.45g of D-glucose and add to the flask followed by 19 mL of 2-bromoethanol. Place the mixture under a constant pressure of nitrogen with the help of a nitrogen filled balloon. 3) Stir the reaction mixture magnetically and add 145 µL of boron trifluoride etherate dropwise to the reaction mixture. 4) Increase the internal temperature to 105 °C with the help of an oil bath. Keep at this temperature for 8 hours. 5) Allow the mixture to cool to room

temperature and distill off excess solvent on a rotary evaporator at a water bath temperature of 40 °C. 6) Prepare a mixture of 6.4 mL of acetic anhydride and 9.6 mL of pyridine and add the freshly prepared mixture to the remaining residue. This mixture is allowed to stir for 24 h at room temperature. 7) Distill off all volatile components on a rotary evaporator at a water bath temperature of 40 °C. 8) Purify the resin by flash column chromatography on silica eluting with a gradient of ethyl acetate: petrol \(3: 7) to neat ethyl acetate. **Synthesis of 2-\(α-D-glucopyranosyl)ethyl methanethiosulfonate** 9) Place 300 mg of 2-bromoethyl 2,3,4,6 tetra-O-acetyl-α-D-glucopyranoside in a 10 mL round bottom flask together with a Teflon coated stirrer bar. Add 3 mL of methanol and prepare a suspension by magnetic stirring. 10) Separately dissolve 54 mg of sodium methoxide in 10mL of methanol \(0.1 M) and transfer 0.3 mL of the freshly prepared solution to the stirred suspension. 11) Following completion of the reaction after approximately 6 h \(monitor by thin layer chromatography) filter the clear solution through a plug of strongly acidic cation-exchange resin \(2*1 cm). 12) Distill of the solvent on a rotary evaporator at a water bath temperature of 40 °C. 13) Take up the remaining solid in 7 mL of N,N-dimethyl formamide and add 100 mg of sodium methanethiosulfonate to the stirred mixture. Warm the solution to 50 °C for 24 h. 14) Allow the mixture to cool to room temperature and distill of all volatiles on a rotary evaporator at a water bath temperature of 40 °C. 15) Purify the product by flash column chromatography on silica eluting with methanol: ethyl acetate \(1: 9).

Timing

72 h

Troubleshooting

Low yield: Ensure that a solution of sodium methoxide in methanol is prepared freshly each time. Proceed with the protocol after step 10) only once completion of the reaction has been confirmed. Use freshly activated strongly acidic cation-exchange resin \[H+ form].

Anticipated Results

A typical isolated yield of methanethiosulfonate product is 144 mg \(73 %). Analytical data: $[\alpha]_D^{27}$ -15.8 \(c 0.9, H2O); IR \(KBr) 3400\(OH), 1310, 1131\(S-SO_2) 1H NMR \(500 MHz, D2O) δ 3.07 \(dd, J1,2 8.1, J2,3 9.4 Hz, 1H, H-2), 3.16 \(dd, J3,4 9.0, J4,5 9.8 Hz, 1H, H-4), 3.24 \(ddd, J4,5 9.8, J5,6 6.0, J5,6’ 2.3 Hz, 1H, H-5), 3.27 \(t, J 9.0 Hz, 1H, H-3), 3.30-3.33 \(m, 2H, CH_2S), 3.34 \(s, 3H, CH_3SO_2), 3.50 \(dd, J5,6 6.0, J6,6’ 12.4 Hz, 1H, H-6), 3.69 \(dd, J5,6 2.3, J6,6’ 12.4 Hz, 1H, H-1, H-6’), 3.81 \(dt, Jt 5.8, Jd11.5 Hz, 1H, OCHH’-), 4.00 \(dt, Jt 5.7, Jd11.4 Hz, 1H, OCHH’-), 4.30 \(d, J1,2 8.1 Hz, 1H, H-1); ^{13}C NMR \(50 MHz, D_2O) δ 36.9 \(CH_2S), 51.0 \(CH_3SO_2), 62.0 \(OCH_2), 69.5, 70.9, 74.3, 76.7, 77.3 \(C-2, C-3, C-4, C-5, C-6), 103.7 \(C-1); HRMS m/z \(FAB+): found 341.0351 \(M+Na+); $C_9H_{18}O_8S_2Na$ requires 341.0341

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Figures

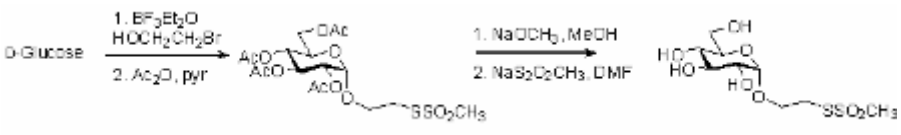

Figure 1

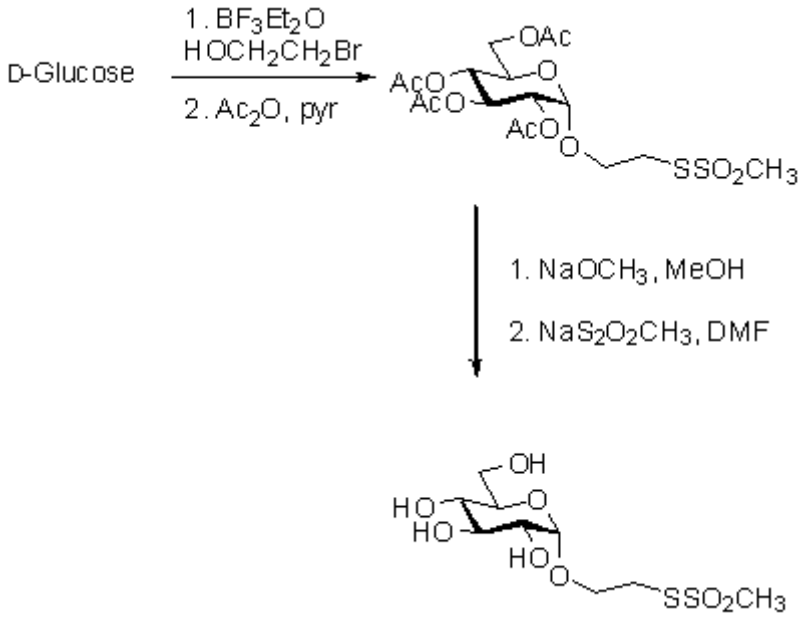

Figure 2