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Synthesis of a O-propargyl glycoside suitable for protein modification by Cu(I)-catalyzed cycloaddition

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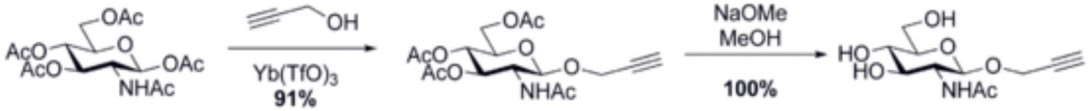
Introduction

Here we describe the efficient and facile synthesis of N-acetyl glucosamine propargyl O-glycoside, a compound which has been prepared previously by conventional methods.[1] In previous syntheses the glycoside was obtained as a mixture of anomers1a) and in moderate to acceptable yield1b). The described improved method exploits a high yielding and strongly β-selective lanthanide triflate catalysed[2] glycosylation reaction. In addition to reaction efficiency the method is characterised by its simplicity and facile compound isolation and purification. The propargyl O-glycoside was prepared for its subsequent use in Cu(I) catalysed protein modification reactions.[3]



Subject terms: **Isolation, Purification and Separation** **Synthetic chemistry**

Keywords: **lanthanide triflate catalyzed reactions** **glycosylation** **alkynyl glycosides**

Reagents

- β-D Glucosamine pentaacetate, 96% (Alfa Aesar cat. no. L09020)
- Dichloromethane, HPLC grade (Fisher cat. no. D/1856/17)
- Propargyl alcohol, 99% (Fisher cat. no. 13145)
- Ytterbium trifluoromethanesulfonate hydrate (Alfa Aesar cat. no. 40314)
- Thin-layer chromatography plates on aluminium backing, silica gel 60 F254 (Merck cat. no. 1.05554.0001)
- Sodium hydrogen carbonate (Fisher cat. no. 12336)
- Magnesium sulphate, >99% (Fisher cat. no. M/1000/62)
- Sodium methoxide, 98% (Alfa Aesar cat. no. L05673)
- Methanol (Rathburn, cat. no. RH1019)

• Ethyl acetate, HPLC grade (Rathburn, cat. no. RH1013)
• Dowex 50X2-100 (Acros cat. no. 20302)
• pH test strips 0-14.0 (Sigma cat. no. P-4786)

Equipment

• Magnetic hotplate stirrer (e.g. IKA® RCT Basic)
• 500 mL round bottom flask
• 250 mL round bottom flask
• Water condenser (to fit neck of flask)
• Rubber septa (to fit neck of flask)
• Disposable syringes
• Disposable needles
• Teflon-coated magnetic stir bar
• 500 mL separatory funnel
• 250 mL Erlenmeyer flasks
• Glass sinter funnel for filtration
• Balloon fitted to disposable 2.5 mL syringe barrel
• Rotary evaporator (Büchi)

Procedure

Propargyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside

1) Place 4.0 g of β-D glucosamine pentaacetate in a 250ml round bottom flask together with a Teflon coated stirrer bar.
2) Dissolve 1.8 mL of propargyl alcohol in 90 mL of dichloromethane and add the freshly prepared solution to the round bottom flask with stirring.
3) Add 0.96 g of Ytterbium(III) trifluoromethanesulfonate hydrate to the stirred mixture and equip the flask with a water cooled condenser.
4) Heat the reaction mixture to refluxing on an oil bath (bath temperature approximately 55 °C) and keep at this temperature for 24 hours.
5) Dilute the solution with a further 50 mL of dichloromethane and transfer to a 500 mL separatory funnel.
6) Wash the organic layer with two 150 mL portions of a saturated aqueous solution of sodium hydrogen carbonate.
7) Wash the combined aqueous phases with one 100 mL portion of dichloromethane and combine all organic layers.
8) Transfer the organic layer to a 500 mL round bottom flask, dry by stirring over approximately 2.5 g of anhydrous magnesium sulfate for 10 minutes and then remove the drying agent by suction filtration.
9) Distil off the solvent on a rotary evaporator at a bath temperature of 40 °C.

Propargyl 2-acetamido-2-deoxy-β-D-glucopyranoside

10) Place a Teflon coated stirrer bar and 1.5g of propargyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (from step 9) in a 250ml round bottom flask.
11) Add 100 mL of methanol and stir the mixture until a clear solution is obtained.
12) Add 21 mg of sodium methoxide to the stirred reaction mixture and monitor the progress of the reaction by an appropriate analytical technique (thin-layer chromatography using ethyl acetate :methanol (8.5 :1.5) as eluent is recommended).
13) If the reaction is not complete within 1 hour, add another portion of 10 mg of sodium methoxide to the reaction mixture.
14) Once completion of the reaction has been confirmed, add strongly acidic cation exchange resin until neutral pH is reached (test with pH paper).
15) Remove the ion exchange resin by suction filtration and distil off the solvent on a rotary evaporator at a bath temperature of 40 °C.

Timing

30 hours (including purification)

Troubleshooting

Low yield/impurities:
Confirm purity of the product from step 9) by 1H- and 13C-NMR spectroscopy.
Only proceed to step 14) once completion of the deacetyltion reaction has been confirmed. Use freshly activated strongly acidic cation-exchange resin [H+ form].

Anticipated Results

A typical yield for the glycosylation reaction is 90%. The sodium methoxide catalysed deacetylation reaction typically proceeds in quantitative yield.

Propargyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside

-44.1 (c 1.0, $CHCl_3$); m.p. = 180-182 ºC; m/z (ESI^+): 386.2 (100%, $M+H^+$) ^1H NMR (400 MHz, $CDCl_3$): δ 2.01 (12H, 4xs, 3x$OCOCH_3$, 1x$NHCOCH_3$), 2.48 (1H, t, *J* 1.9 Hz, OCH_2CCH), 3.73 (1H, ddd, $J_{5,4}$ 9.9, $J_{5,6}$ 4.4, $J_{5,6'}$ 2.2 Hz, H-5), 3.91-3.97 (1H, m, H-2), 4.12 (1H, dd, $J_{6,6'}$ 12.3, $J_{5,6'}$ 2.0 Hz, H-6'), 4.26 (1H, dd, $J_{6,6'}$ 12.3, $J_{5,6}$ 4.6 Hz, H-6), 4.36 (2H, d, *J* 2.0 Hz, OCH_2CCH), 4.85 (1H, d, $J_{1,2}$ 8.4 Hz, H-1), 5.06 (1H, app t, J 9.6 Hz, H-4), 5.21-5.32 (1H, m, H-3), 5.90 (1H, d, $J_{H2,NH}$ 9.0 Hz, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.6, 20.7, 20.8, 23.4 (3x$OCOCH_3$, 1x$NHCOCH_3$), 54.7 (C-2), 55.9 (OCH_2CCH), 61.9 (C-6), 68.4 (C-4), 71.9 (C-5), 72.4 (C-3), 75.39 (OCH_2CCH), 78.5 (OCH_2CCH), 98.3 (C-1), 169.4, 170.4, 170.7, 171.0 (3x$OCOCH_3$, 1x$NHCOCH_3$)

Propargyl 2-acetamido-2-deoxy-β-D-glucopyranoside

-29.1 (c 1.0, H_2O); m.p. = 146 ºC; ^1H NMR (400 MHz, MeOH-d4): δ 2.00 (4H, s, 1x$NHCOCH_3$, OCH_2CCH), 3.30-3.42 (2H, m, H-4, H-5), 3.43-3.50 (3H, m, H-3, OCH_2CCH), 3.66-3.73 (2H, m, H-2, H-6'), 3.81 (1H, dd, $J_{6,6'}$ 12.3, $J_{5,6}$ 1.8 Hz, H-6), 4.55 (1H, d, $J_{1,2}$ 9.2 Hz, H-1), 5.21-5.32 (1H, m, H-3), 5.90 (1H, d, $J_{H2,NH}$ 9.0 Hz, NH).

References

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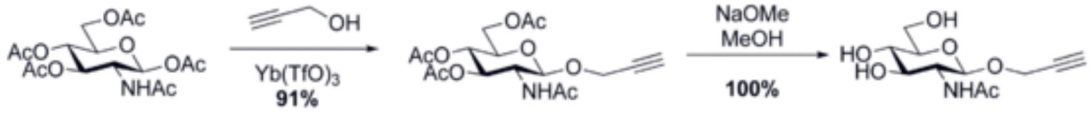
b) D.-L. Ma, T. Y.-T. Shum, F. Zhang, C.-M. Che, M. Yang, *Chem. Commun*. 4675 (2005)
2. J. Inanaga, Y. Yokoyama, T. Hanamoto, *Tetrahedron Lett*. **34**, 2791 (1993)
3. S. I. van Kasteren, H. B. Kramer, H. H. Jensen, S. J. Campbell, J. Kirkpatrick, N. J. Oldham, D. C. Anthony, B. G. Davis, *Nature* **446**, 1105 (2007)

Figures

Figure 1



Associated Publications

This protocol is related to the following articles:

- Expanding the diversity of chemical protein modification allows post-translational mimicry
  Sander I. van Kasteren, Holger B. Kramer, Henrik H. Jensen, Sandra J. Campbell, Joanna Kirkpatrick, Neil J. Oldham, Daniel C. Anthony, and Benjamin G. Davis

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Competing financial interests

The authors declare no competing financial interests.

Readers' Comments

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