

Ethyl 2-acetamido-4,6-di-O-benzyl-2,3-N,O-carbonyl-2-deoxy-1-thio-β-D-glycopyranoside as a versatile GlcNAc donor

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General methods.

All organic solvents were distilled before use, except Et₂O, which was stored over Na. Organic solutions were dried over MgSO₄ before concentration, which was performed under reduced pressure at <40 °C (bath temperature). NMR spectra (JEOL) were recorded at 400 MHz (¹H) or at 100MHz (¹³C), respectively, in CDCl₃. TMS was used as internal standard (δ = 0) for ¹H-spectra in chloroform. ¹³C-spectra were referred to the chloroform signal (δ = 77.17). TLC was performed on silica gel 60 F₂₅₄ (Merck) glass plates with detection by UV-light and/or charring with 8% sulfuric acid. Column chromatography was performed on silica gel (Matrix Silica Si 60A, 35-70m). MALDI-TOF spectra were recorded on a Bruker Biflex III using 2',4',6'-trihydroxy-acetophenone monohydrate (THAP) as matrix.

Ethyl 2-acetamido-4,6-di-O-benzyl-2,3-N,O-carbonyl-2-deoxy-1-thio-β-D-glycopyranoside (1)

Ethyl 2-amino-4,6-di-O-benzyl-2-deoxy-1-thio-β-D-glycopyranoside (500 mg, 1.24 mmol) was dissolved in dichloromethane (20 mL) and saturated NaHCO₃ solution (10 mL) added. The resulting mixture was vigorously stirred and cooled to 0°C. Then triphosgene (147 mg, 0.5 mmol, 0.4 eq) was added and the vigorous stirring continued for approx. 30 min at 0°C.

After completion of the reaction (TLC: toluene/ ethyl acetate 1:1), some more dichloromethane was added, the phases separated and the aqueous one extracted with three portions of dichloromethane. The combined organic phases were washed with saturated sodium NaHCO_3 solution to which a small amount of ethylenediamine had been added (approx. 1 mL) and finally with water. After drying, filtration and concentration ethyl 2-amino-4,6-di-*O*-benzyl-2,3-*N,O*-carbonyl-2-deoxy-1-thio- β -D-glucopyranoside was obtained as a colourless solid (524 mg, 1.22 mmol, 98%). $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ = 7.19-7.40 (10H, m, Ph), 5.30 (1H, s, NH), 4.85 (1H, d, Ph- CHH -), 4.48-4.65 (4H, m, H-1, Ph- CH_2 -), 4.22 (1H, dd \approx t, H-3), 3.92 (1H, dd \approx t, H-4), 3.68-3.82 (2H, m, H-6, H-6'), 3.60 (1H, ddd, H-5), 3.48 (1H, dd \approx t, H-2), 2.68-2.83 (2H, m, H-2, S- CH_2 - CH_3), 1.31 (3H, t, S- CH_2 - CH_3) ppm; $^3J_{1,2} = 9.9$, $^3J_{2,3} = 11.3$, $^3J_{3,4} = 9.5$, $^3J_{4,5} = 8.8$, $^3J_{5,6} = 3.7$, $^3J_{5,6'} = 2.2$ Hz; $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): δ = 158.88 (O-($\text{C}=\text{O}$)-N), 138.11, 137.30, 128.52, 128.46, 128.16, 128.08, 127.81, 127.78 (Ph), 85.62 (C-1), 82.73, 80.28, 73.85, 73.51, 73.13, 68.57 (C-3, C-4, C-5, C-6, Ph- CH_2 -), 59.25 (C-2), 24.95 (S- CH_2 - CH_3), 15.48 (S- CH_2 - CH_3) ppm. Ethyl 2-amino-4,6-di-*O*-benzyl-2,3-*N,O*-carbonyl-2-deoxy-1-thio- β -D-glucopyranoside (520 mg, 1.21 mmol) and diisopropylethylamine (1.1 ml, 6.2 mmol) were dissolved in dry dichloromethane (5 ml). Acetyl chloride (430 μl , 6.2 mmol) were added and the mixture stirred at room temperature. After consumption of the starting material (TLC: toluene/ ethyl acetate 3:1, approx. 2 h), the reaction mixture was poured into saturated NaHCO_3 solution, the phases separated and the aqueous phase extracted with dichloromethane. The combined organic phases were washed with water and brine, dried, filtered and concentrated. The raw product was purified by short column filtration over silica gel (hexanes /ethyl acetate 6:1) to yield compound **1** (520 mg, 1.10 mmol, 91 %) as a colourless solid. $[\alpha]_{\text{D}} -10^\circ$ (c 0.66, CH_2Cl_2); $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ = 7.20-7.39 (10H, m, Ph), 4.85 (1H, d, Ph- CHH -), 4.70 (1H, d, H-1), 4.48-4.62 (3H, m, Ph- CH_2 -), 4.25 (1H, dd \approx t, H-3), 3.88 -3.42 (2H, m, H-2, H-4), 3.68-3.79 (2H, m, H-6, H-6'),

3.62 (1H, ddd, H-5), 2.60-2.71 (2H, m, S-CH₂-CH₃), 2.53 (3H, s, NAc), 1.26 (3H, t, S-CH₂-CH₃) ppm; ³J_{2,3} = 11.7, ³J_{3,4} = 9.9 Hz; ¹³C-NMR (100 MHz, CDCl₃): δ = 173.01 (O-(C=O)-CH₃), 154.17 (O-(C=O)-N), 138.04, 137.13, 128.56, 128.47, 128.17, 127.79 (Ph), 84.73 (C-1), 82.99, 80.29, 74.32, 73.53, 68.51 (C-3, C-4, C-5, C-6, Ph-CH₂-), 60.06 (C-2), 25.49 (NAc), 24.95 (S-CH₂-CH₃), 14.33 (S-CH₂-CH₃) ppm.

Cholesteryl 2-acetamido-4,6-di-O-benzyl-2,3-N,O-carbonyl-2-deoxy-β-D-glucopyranoside (2β)

A solution of **1** (25 mg, 53 μmol) and cholesterol (17 mg, 44 μmol) in dry CH₂Cl₂ (2 mL) was stirred with powdered molecular sieves (4Å) under nitrogen for 30 min when NIS (26 mg, 115 μmol) and a catalytic amount AgOTf were added. After 10 min stirring at room temperature, the reaction mixture was quenched with Et₃N (50 μL) and applied onto a silica gel column and eluted (toluene → toluene/ethyl acetate 25:1) to give **2β** (32 mg, 40 μmol, 91 %); [α]_D -32° (c 2.97, CH₂Cl₂); ¹H-NMR (400 MHz, CDCl₃): δ = 7.35-7.20 (10H, m, Ph), 5.35 (1H, d, J = 5.1 Hz, Chol), 5.12 (1H, d, J = 6.6 Hz, H-1), 4.74 (1H, d, Ph-CHH-), 4.61 (1H, d, Ph-CHH-), 4.53 (2H, s, Ph-CH₂), 4.24 (1H, dd, H-3), 4.03 (1H, m, Chol), 3.83-3.76 (2H, m), 3.69 (1H, dd), 3.64-3.55 (2H, m), 2.51 (3H, s, NAc), 2.39-0.67 (Chol) ppm; ¹³C-NMR (100 MHz, CDCl₃): δ = 170.6 (O-(C=O)-CH₃), 153.9 (O-(C=O)-N), 140.4 (Chol), 137.8, 137.4, 128.5-127.9 (Ph), 122.1 (Chol), 98.5 (C-1), 78.8 (2C), 78.6, 76.3, 73.5, 72.1, 70.8 (C-3, C-4, C-5, C-6, Ph-CH₂-, Chol), 61.3 (C-2), 58.9, 56.2, 50.3, 42.4, 39.9, 39.6, 38.2, 37.3, 36.8, 36.3, 35.9, 32.0, 31.9, 29.7, 28.3, 28.1, 24.7, 24.4, 23.9, 22.9, 22.7 (Chol), 21.2 (NAc), 19.5, 18.8, 12.0 (Chol).

Cholesteryl 2-acetamido-4,6-di-O-benzyl-2,3-N,O-carbonyl-2-deoxy-α-D-glucopyranoside (2α)

A solution of **1** (25 mg, 53 μmol) and cholesterol (17 mg, 44 μmol) in dry CH₂Cl₂ (2 mL) was stirred with powdered molecular sieves (4Å) under nitrogen for 30 min when NIS (26 mg,

115 μmol) and AgOTf (5 mg, 20 μmol) were added. After 30 min stirring at room temperature, the reaction mixture was applied onto a silica gel column and eluted (toluene \rightarrow toluene/ethyl acetate 25:1) to give **2 α** (31 mg, 39 μmol , 89 %); $[\alpha]_{\text{D}} +79^{\circ}$ (c 1.08, CH_2Cl_2); $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ = 7.35-7.20 (10H, m, Ph), 5.83 (1H, d, J = 2.7 Hz, H-1), 5.28 (1H, d, J = 4.6 Hz, Chol), 4.82 (1H, d, Ph-CHH-), 4.71 (1H, dd, H-3), 4.61 (1H, d, Ph-CHH-), 4.48 (2H, t, Ph-CH₂), 3.98 (1H, m, Chol), 3.83-3.76 (3H, m), 3.66 (1H, d), 3.50 (1H, m), 2.51 (3H, s, NAc), 2.35-0.66 (Chol) ppm; $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): δ = 171.1 (O-(C=O)-CH₃), 153.7 (O-(C=O)-N), 140.4 (Chol), 138.0, 137.3, 128.5-127.9 (Ph), 122.2 (Chol), 94.3 (C-1), 78.6, 78.1, 74.9, 73.6, 73.4, 72.3, 67.9 (C-3, C-4, C-5, C-6, Ph-CH₂-, Chol), 60.2 (C-2), 56.8, 56.2, 50.1, 42.4, 40.0, 39.8, 39.6, 37.0, 36.7, 36.3, 35.9, 32.0 (2C), 28.3, 28.1, 28.0, 24.4, 23.9, 22.9, 22.7 (Chol), 21.1 (NAc), 19.4, 18.8, 11.9 (Chol).

Cholesteryl 2-acetamido-4,6-di-*O*-benzyl-2-deoxy-D-glucopyranoside (3)

A small sample of compound **2** was dissolved in MeOH and a catalytic amount NaOMe (1M) was added. The mixture was kept at ambient temperature. A sample for MALDI-TOF MS was taken after 4h. MALDI-TOF MS: 792.5 $[\text{M}+\text{Na}]^+$, 808.5 $[\text{M}+\text{K}]^+$; found: 792.5 $[\text{M}+\text{Na}]^+$, 808.5 $[\text{M}+\text{K}]^+$.

Cholesteryl 4,6-di-*O*-benzyl-2,3-*N,O*-carbonyl-2-deoxy-D-glucopyranoside (4)

A small sample of compound **2** was dissolved in THF and NaOH (0.5 M) was added. The mixture was kept at ambient temperature. Samples for MALDI-TOF MS were taken after 4 h and 8 h. MALDI-TOF MS: 776.5 $[\text{M}+\text{Na}]^+$, 792.5 $[\text{M}+\text{K}]^+$; found: 776.5 $[\text{M}+\text{Na}]^+$, 792.5 $[\text{M}+\text{K}]^+$.

Methyl 2-acetamido-4,6-di-*O*-benzyl-2,3-*N,O*-carbonyl-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 6)-2,3,4-tri-*O*-benzyl- α -D-mannopyranoside (5)

A solution of **1** (210 mg, 45 μmol) and methyl 2,3,4-tri-*O*-benzyl- β -D-mannopyranoside (145 mg, 31 μmol) in dry CH_2Cl_2 (20 mL) was stirred with powdered molecular sieves (4Å) under nitrogen for 30 min when NIS (215 mg, 0.96 mmol) and a catalytic amount AgOTf were added. After 10 min stirring at room temperature, the reaction mixture was quenched with Et_3N (50 μL) and applied onto a silica gel column and eluted (toluene \rightarrow toluene/ethyl acetate 25:1) to give **5** (245 mg, 28 μmol , 90 %); $[\alpha]_{\text{D}} +15^\circ$ (*c* 2.64, CH_2Cl_2); $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ = 7.35-7.15 (25H, m, Ph), 5.00 (1H, d, J = 6.4 Hz, $\beta\text{H-1}'$), 4.89 (1H, d, Ph- CHH-), 4.75-4.65 (8H, m), 4.60 (2H, s, Ph- CH_2), 4.20 (1H, dd, H-3'), 4.05 (1H, dd), 3.95 (1H, m), 3.89-3.74 (8H, m), 3.65 (1H, dd), 3.27 (3H, s, OMe), 2.45 (3H, s, NAc) ppm; $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): δ = 170.3 (O-($\text{C}=\text{O}$)- CH_3), 154.1 (O-($\text{C}=\text{O}$)-N), 138.9, 138.6, 138.5, 138.1, 137.4, 128.8-127.9 (Ph), 99.7, 99.1 (C-1, C-1'), 80.4, 78.9, 78.8, 75.9, 74.9 (2C), 74.7, 73.3, 72.8, 72.2, 71.6, 70.3, 68.2 (C-2 – C-6, C-3' – C-6'Ph- CH_2 -), 60.6 (C-2'), 54.8 (OMe), 23.6 (NAc). ESI: 896.3622 $[\text{M}+\text{Na}]^+$; found: 896.3622 $[\text{M}+\text{Na}]^+$.

Methyl 2-acetamido-4,6-di-*O*-benzyl-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 6)-2,3,4-tri-*O*-benzyl- α -D-mannopyranoside (6)

A small sample of compound **5** was dissolved in MeOH and a catalytic amount NaOMe (1M) was added. The mixture was kept at ambient temperature. A sample for MALDI-TOF MS was taken after 4h. MALDI-TOF MS: 870.4 $[\text{M}+\text{Na}]^+$; Found: 870.55 $[\text{M}+\text{Na}]^+$.

Methyl 2-acetamido-4,6-di-*O*-benzyl-2,3-*N,O*-carbonyl-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 3)-2-*O*-benzyl-4,6-*O*-benzylidene- α -D-glucopyranoside (7 β)

A solution of **1** (25 mg, 53 μmol) and methyl 2-*O*-benzyl-4,6-*O*-benzylidene- α -D-glucopyranoside (13 mg, 35 μmol) in dry CH_2Cl_2 (2 mL) was stirred with powdered molecular sieves (4Å) under nitrogen for 30 min when NIS (26 mg, 115 μmol) and a catalytic amount AgOTf were added. After 10 min stirring at room temperature, the reaction mixture was quenched with Et_3N (50 μL) and applied onto a silica gel column and eluted (toluene \rightarrow

toluene/ethyl acetate 10:1) to give **7** (26 mg, 33 μ mol, 94 %); $[\alpha]_D +18^\circ$ (c 0.23, CH_2Cl_2); $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ = 7.45-7.05 (20H, m, Ph), 5.68 (1H, d, J = 7.1 Hz, $\beta\text{H-1}'$), 5.48 (1H, s, Ph-CH), 4.66 (1H, d, J = 3.6 Hz, H-1), 4.58 (4H, m, Ph-CHH-), 4.36 (1H, t), 4.24 (1H, dd, H-3'), 4.13 (1H, m), 3.94 (1H, m), 3.87-3.59 (9H, m), 3.46 (1H, dd), 3.32 (3H, s, OMe), 2.35 (3H, s, NAc) ppm; $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): δ = 171.0 (O-($\text{C}=\text{O}$)- CH_3), 154.2 (O-($\text{C}=\text{O}$)-N), 138.3, 138.2, 137.6, 137.5, 129.5-126.8 (Ph), 102.5, 99.9, 98.0 (C-1, C-1', Ph-CH), 81.7, 79.8, 78.9, 78.3, 76.4, 73.7, 72.7, 72.2, 71.9, 70.6, 69.3, 62.3, 60.2 (C-2-C-6, C-2'-C-6', Ph- CH_2 -), 55.3 (OMe) 24.7 (NAc).

Methyl 2-acetamido-4,6-di-*O*-benzyl-2,3-*N,O*-carbonyl-2-deoxy- α -D-glucopyranosyl-(1 \rightarrow 3)-2-*O*-benzyl-4,6-*O*-benzylidene- α -D-glucopyranoside (7 α**)**

A solution of **1** (25 mg, 53 μ mol) and methyl 2-*O*-benzyl-4,6-*O*-benzylidene- α -D-glucopyranoside (13 mg, 35 μ mol) in dry CH_2Cl_2 (2 mL) was stirred with powdered molecular sieves (4 \AA) under nitrogen for 30 min when NIS (26 mg, 115 μ mol) and AgOTf (5 mg, 20 μ mol) were added. After 30 min stirring at room temperature, the reaction mixture was applied onto a silica gel column and eluted (toluene \rightarrow toluene/ethyl acetate 10:1) to give **7 α** (25 mg, 32 μ mol, 91 %); $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ = 7.58 (2H, m, Ph), 7.34-7.20 (18H, m, Ph), 6.31 (1H, d, J = 2.7 Hz, $\alpha\text{H-1}'$), 5.54 (1H, s, Ph-CH), 4.83 (1H, d, Ph-CHH-), 4.64 (3H, m, Ph-CHH-, H-1), 4.53 (3H, m), 4.33 (1H, d, Ph-CHH-), 4.26 (1H, t), 4.22 (1H, d, Ph-CHH-), 4.01 (2H, m), 3.82-3.64 (4H, m), 3.57-3.46 (3H, m), 3.35 (3H, s, OMe), 2.35 (3H, s, NAc) ppm; $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): δ = 170.3 (O-($\text{C}=\text{O}$)- CH_3), 153.8 (O-($\text{C}=\text{O}$)-N), 138.0, 137.7, 137.4, 137.3, 129.5-126.8 (Ph), 101.4, 98.2, 94.5 (C-1, C-1', Ph-CH), 82.6, 78.3, 77.9, 74.7, 73.5, 73.3, 72.9, 72.2, 69.0, 67.6, 61.7, 60.3 (C-2-C-6, C-2'-C-6', Ph- CH_2 -), 55.3 (OMe) 23.9 (NAc).

Methyl 2-acetamido-4,6-di-*O*-benzyl-2-deoxy-D-glucopyranosyl-(1 \rightarrow 3)-2-*O*-benzyl-4,6-*O*-benzylidene- α -D-glucopyranoside (8**)**

Supplementary Material (ESI) for Chemical Communications
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A small sample of compound **7** was dissolved in MeOH and a catalytic amount NaOMe (1M) was added. The mixture was kept at ambient temperature. A sample for MALDI-TOF MS was taken after 4h. MALDI-TOF MS: 778.3 [M+Na]⁺, 794.3 [M+K]⁺; found: 778.2 [M+Na]⁺, 794.3 [M+K]⁺.