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Note

Glycosylation of allyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside with bulky substituted glycosyl donors

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Abstract—Glycosylation of allyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside with bulky substituted glycosyl donors leads to the formation of derivatives of the disaccharide α-D-Glc-(1→3)-D-GlcNAc with different yields.

Keywords: Bulky protecting groups; 2-Acetamido-2-deoxy-D-glucopyranose; Triflates

The disaccharide fragment, α-D-Glc*p*A-(1→3)-D-Glc*p*NAc, of lipopolysaccharides (LPSs) was found in the bacterial cell walls of *Shigella boydii* 5[1] and *Hafnia alvei* PCM 1185[2] and *Proteus mirabilis* 010.[3] Recently, we described its synthesis.[4] Here, we present the results of the study on the influence of bulky groups at C-6 in the glycosyl donor molecule on the glycosylation reaction of the title compound.

The aim of this work was to improve the yield of the reaction for synthesis of derivatives of the disaccharide α-D-Glc-(1→3)-D-GlcNAc. These disaccharides, after selective deprotection at C-6 and oxidation, are suitable for preparation of α-D-GlcA-(1→3)-D-GlcNAc.

Results presented in Table 1 clearly indicate that the best glycosyl donor in this reaction is trichloroacetimidate **1**. This means that the more reactive the glycosyl donor, the higher the yield of the reaction. On the other hand, the type of glycosyl donor is not the only factor influencing the reaction. Replacing an *O*-benzyl group (ether type, **1**) with *O*-acetyl (ester type, **3**) causes a distinct lowering of the reaction yield. The result is in agreement with the theory of armed and disarmed glycosyl donors.[5] The other factor is the steric bulk of the terminal protecting group. A very bulky protecting group like *tert*-butyldimethylsilyl (**6**) or trityl (**8**) can sterically hinder the anomeric centre, which results in a lowering of the yield or even stops the reaction.

Very important for the reaction is the configuration of the anomeric carbon atom as confirmed by the results of the glycosidation of **9** with the thioglycosides (**6** and **7**[6]) with the 6-*O*-TBDMS group (α and β anomers). These results strongly support the proposed explanation in the literature[7] and in our publication[4] the role of a β-triflate byproduct in the mechanism of the reaction.

1. Experimental

1.1. General methods

All reactions were carried out in commercially available dry solvents (Fluka, water <0.005%). Thin-layer chromatography was performed with E. Merck pre-coated Silica Gel 60 F-254 plates and detection of compounds was achieved by charring after spraying with 5% H_2SO_4 in EtOH. Column chromatography was carried out with Kieselgel 60 Silica Gel (E. Merck, <200 mesh). ^1H and ^{13}C NMR spectra were recorded at 25 °C with a Varian Mercury spectrometer at 400 and 100 MHz, respectively, with Me_4Si as internal standard. Assignments were based on homonuclear decoupling experiments

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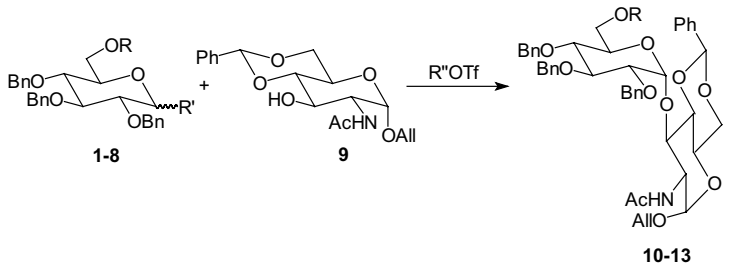
Table 1. The results of a glycosidation of **9** with different protected at C-6 glycosyl donors **1**–**8** in the presence of silver or trimethylsilyltriflate (R″ = Ag or TMS)



Compound	Protecting group (R)	Leaving group (R′)	Yield (**10**–**13**) (%)
1	Bn	α-O(C=NH)CCl_3	70
2	Bn	α-SPh	24
3	Ac	α-O(C=NH)CCl_3	45
4	Ac	α-Br	28
5	Ac	α-SPh	14
6	TBDMS	α-SPh	9
7	TBDMS	β-SPh	0
8	Tr	α-SPh	0

and homo- and heteronuclear correlations. Mass spectra were measured using MALDITOF with α-cyano-4-hydroxycinnamic acid (CCA) as a matrix. Optical rotations were measured with a JASCO J-20 polarimeter. Elemental analyses were carried out with a Carlo Erba apparatus.

1.2. General procedures for a glycosidation of allyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (9)

1.2.1. Procedure A for glycosyl trichloroacetimidates. Allyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**9**, prepared per the Warren and Jeanloz procedure[9]) in dry DMF was stirred at −60 °C under dry nitrogen and trimethylsilyl triflate was added. After 10 min a glycosyl trichloroacetimidate in dry CH_2Cl_2 was added dropwise and the mixture was stirred for 1 h at −60 °C and then 16 h at rt. Next Pr_2EtN (0.1 mL) was added. The solution was concentrated and co-concentrated with toluene to dryness. The residue was dissolved in CH_2Cl_2, washed with aq $NaHCO_3$ and water, dried with $MgSO_4$ and concentrated. For details of specific compounds, see Sections 1.5.1 and 1.6.1, which follow.

1.2.2. Procedure B for glycosyl bromides. Allyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**9**) in dry DMF was stirred at 0 °C under dry nitrogen and silver triflate was added. After 10 min a glycosyl bromide in dry CH_2Cl_2 was added, and the mixture was stirred for 1 h at 0 °C and then for 16 h at rt. Next Pr_2EtN (0.1 mL) was added. The solution was concentrated and co-concentrated with toluene to dryness. The residue was dissolved in CH_2Cl_2, washed with aq $NaHCO_3$ and water, dried with $MgSO_4$ and concentrated. For details of specific compounds, see Section 1.6.2, which follows.

1.2.3. Procedure C for thioglycosides. Allyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**9**) in dry DMF was stirred at 0 °C under dry nitrogen. After 10 min a thioglycoside in dry CH_2Cl_2 and NIS were added. After 0.5 h trimethylsilyl triflate was added and the mixture was stirred for 1 h at 0 °C and then 16 h at rt. Next Pr_2EtN (0.1 mL) was added. The solution was concentrated and co-concentrated with toluene to dryness. The residue was dissolved in CH_2Cl_2, washed with aq $NaHCO_3$ and water, dried with $MgSO_4$ and concentrated. For details of specific compounds, see Sections 1.5.2, 1.6.1 and 1.7.1, which follow.

1.3. Phenyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-1-thio-α-D-glucopyranoside (6)

To a mixture of phenyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside[8] (60 mg, 0.11 mM) and imidazole in dry DMF stirred at 0 °C *tert*-butylchlorodimethylsilane was added (45 mg, 0.27 mM). After 1 h the mixture was diluted with EtOAc (25 mL) and washed with aq $NaHCO_3$ (20 mL), water (20 mL), dried with $MgSO_4$ and concentrated. The residue was eluted from a column of silica gel with 3:7 acetone–hexanes to give **6** (30 mg, 42%) as an oil: ^{1}H NMR ($CDCl_3$): δ 7.51–7.23 (m, 20H, Ph), 5.62 (d, 1H, $J_{1,2}$ 4.8, H-1), 4.99 (d, 1H, OCH_2Ph), 4.86 (d, 1H, OCH_2Ph), 4.81 (d, 1H, OCH_2Ph), 4.76 (d, 1H, OCH_2Ph), 4.67 (d, 2H, OCH_2Ph), 4.12 (m, 1H, H-5), 3.92–3.82 (m, 3H, H-2, H-3, H-6′), 3.75 (dd, 1H, $J_{5,6}$

11.2, $J_{6,6'}$ 2.0, H-6), 3.59 (t, 1H, $J_{3,4}$ 8.8, $J_{4,5}$ 10.0, H-4), 0.87 (s, 9H, Si–C–(CH_3)$_3$) 0.01 (s, 3H, Si(CH_3)$_2$), 0.01 (s, 3H, Si(CH_3)$_2$). ^{13}C NMR δ 138.89–127.14 (Ph), 87.05 (C-1), 82.76 (C-3), 80.30 (C-2), 77.64 (C-4), 76.07 (OCH_2Ph), 75.30 (OCH_2Ph), 72.87 (C-5), 72.67 (OCH_2Ph), 62.48 (C-6), 26.16 (Si–C–(CH_3)$_3$), 18.57 (Si–C–(CH_3)$_3$), −4.96 (Si(CH_3)$_2$), −5.20 (Si(CH_3)$_2$). This compound was used directly in synthesis as described in Sections 1.5.1, 1.5.2, 1.6.1, 1.6.2, 1.6.3 and 1.7.1, which follow.

1.4. Phenyl 2,3,4-tri-*O*-benzyl-1-thio-6-*O*-trityl-α-D-glucopyranoside (8)

Sodium hydride (65 mg, 2.71 mM) was added to a cooled solution of phenyl 1-thio-6-*O*-trityl-α-D-glucopyranoside[4] (0.12 g, 0.23 mM) in dry DMF (4 mL) and the mixture was stirred for 1 h at 0 °C. Next BnBr (0.14 mL; 1.4 mM) was added dropwise and the mixture was stirred. After 2 h, excess of sodium hydride was decomposed with MeOH and the mixture was concentrated. The residue was eluted from a column of silica gel with 3:7 acetone–hexanes to give **8** (0.14 g, 78%) as an oil: ^{1}H NMR ($CDCl_3$): δ 7.61–6.95 (m, 35H, Ph), 4.74 (s, 1H, $J_{1,2}$ 4.8, H-1), 4.97–4.77 (m, 4H, OCH_2Ph), 4.71–4.65 (m, 2H, OCH_2Ph), 3.80–3.43 (m, 6H, H-5, H-2, H-3, H-6′, H-6, H-4). ^{13}C NMR δ 144.72–126.52 (Ph), 87.62 (C-1), 86.94 ($C(C_6H_5)_3$), 81.01 (C-3), 79.27 (C-2), 77.99 (C-4), 76.03 (OCH_2Ph), 75.62 (OCH_2Ph), 75.25 (OCH_2Ph), 73.60 (C-5), 69.20 (C-6).

1.5. Allyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (10)

1.5.1. Procedure A. The mixture after the reaction of **9** (0.19 g, 0.54 mM) with **1**[10] (0.47 g, 0.68 mM) in DMF (17 mL) and CH_2Cl_2 (17 mL) in the presence of TMSOTf (0.12 mL, 0.69 mM) was eluted from a column of silica gel with 1:2 EtOAc–toluene to give **10** (0.33 g, 70%) as an oil: $[\alpha]_D^{20}$ +50 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.41–7.24 (m, 25H, Ph), 6.25 (d, 1H, NH), 5.77 (m, 1H, =CH), 5.44 (d, 1H, $J_{1,2}$ 4.0, H-1^{II}), 5.42 (s, 1H, CHPh), 5.21 (m, 2H, =CH_2), 4.94 (d, 1H, OCH_2Ph), 4.87 (d, 1H, $J_{1,2}$ 3.2, H-1^{I}), 4.81 (d, 1H, OCH_2Ph), 4.70 (d, 1H, OCH_2Ph), 4.5 (m, 3H, OCH_2Ph), 4.39 (m, 1H, H-2^{I}), 4.24 (m, 3H, H-3^{I}, H-6$^{II'}$, OCH_2Ph), 4.20 (m, 2H, H-6$^{I'}$, OCH_2), 3.85 (m, 4H, H-3^{II}, H-4^{I}, H-5^{II}, OCH_2), 3.77 (m, 2H, H-6^{I}, H-6^{II}), 3.5 (m, 2H, H-2^{II}, H-5^{I}), 3.19 (dd, 1H, $J_{3,4}$ 10.0, $J_{4,5}$ 10.4, H-4^{II}), 1.86 (s, 3H, NHCOCH_3). ^{13}C NMR δ 170.69 (NCOCH$_3$), 138.89 (=CH), 138.4–126.64 (Ph), 118.07 (=CH_2), 102.44 (CHPh), 97.24 (C-1^{I}), 96.36 (C-1^{II}), 83.10 (C-3^{II}), 81.88 (C-4^{I}), 78.73 (C-2^{II}), 77.99 (C-4^{II}), 75.97 (OCH_2Ph), 7.75 (OCH_2Ph), 74.17 (OCH_2Ph), 72.45 (C-3^{I}), 71.03 (OCH_2Ph), 70.49 (C-6^{II}), 70.31 (C-5^{I}), 69.26 (C-6^{I}), 68.7 (OCH_2), 62.92 (C-5^{II}), 52.27 (C-2^{I}), 23.26 (NHCOCH_3). Anal. Calcd for $C_{52}H_{57}NO_{11}$: C, 71.64; H, 6.54; N, 1.6. Found: C, 70.95; H, 6.87; N, 1.01.

1.5.2. Procedure C. The mixture after the reaction of **9** (0.12 g, 0.34 mM) with **2**[11] (0.26 g, 0.41 mM) in DMF (10 mL) and CH_2Cl_2 (10 mL) in the presence of NIS (0.1 g) and TMSOTf (30 μL) was eluted from a column of silica gel with 1:2 EtOAc–toluene to give **10** (0.07 g, 24%) as an oil. The compound was identical to that from Procedure A, above.

1.6. Allyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (11)

1.6.1. Procedure A. The mixture after the reaction of **9** (0.17 g, 0.49 mM) with **3**[12] (0.39 g, 0.61 mM) in DMF (15 mL) and CH_2Cl_2 (15 mL) in the presence of TMSOTf (0.16 mL; 0.89 mM) was eluted from a column of silica gel with 2:3 EtOAc–toluene to give **11** (0.18 g, 45%) as an oil: $[\alpha]_D^{20}$ +66 (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.40–6.90 (m, 20H, Ph), 6.0 (d, 1H, NH), 5.89 (m, 1H, =CH), 5.49 (d, 1H, $J_{1,2}$ 3.6 H-1^{II}), 5.44 (s, 1H, CHPh), 5.32 (m, 2H, =CH_2), 4.97 (d, 1H, OCH_2Ph), 4.89 (d, 1H, $J_{1,2}$ 4.0, H-1^{I}), 4.87 (d, 1H, OCH_2Ph), 4.77 (d, 1H, OCH_2Ph), 4.53 (m, 2H, OCH_2Ph), 4.48 (m, 1H, H-2^{I}), 4.3 (d, 1H, OCH_2Ph), 4.27–4.16 (m, 4H, H-3^{I}, H-5^{II}, H-6^{II}, OCH_2), 4.12 (m, 2H, H-4^{I}, H-6$^{II'}$), 4.04 (m, 1H, OCH_2), 3.95 (m, 3H, H-3^{II}, H-6^{I}, H-6$^{I'}$), 3.77 (m, 1H, $J_{4,5}$ 9.6, $J_{5,6}$ 10.4, H-5^{I}), 3.38 (dd, 1H, $J_{2,3}$ 9.2, H-2^{II}), 3.25 (t, 1H, $J_{3,4}$ 9.2, $J_{4,5}$ 9.6, H-4^{II}), 2.08 (s, 3H, COCH_3), 2.02 (s, 3H, NCOCH_3). ^{13}C NMR δ 171.18 (COCH$_3$), 170.35 (NCOCH$_3$), 138.86 (=CH), 138.27–126.61 (Ph), 118.72 (=CH_2), 102.41 (CHPh), 97.66 (C-1^{I}), 96.09 (C-1^{II}), 83.22 (C-3^{II}), 81.48 (C-4^{I}), 79.32 (C-2^{II}), 78.64 (C-4^{II}), 75.9 (OCH_2Ph), 74.77 (OCH_2Ph), 72.06 (C-5^{II}), 71.15 (OCH_2Ph), 69.20 (C-5^{I}), 68.91 (OCH_2), 68.31 (C-3^{I}), 64.58 (C-6^{II}), 62.99 (C-6^{I}), 51.86 (C-2^{I}), 23.31 (COCH$_3$), 21.15 (NHCOCH$_3$). MALDITOF-MS: Calcd for $C_{47}H_{53}NO_{12}$: 823.9 [M]. Found: 846.3 $[M+Na]^+$.

1.6.2. Procedure B. The mixture after the reaction of **9** (0.20 g, 0.57 mM) with **4**[12] (0.25 g, 0.88 mM) in DMF (15 mL) and CH_2Cl_2 (15 mL) in the presence of AgOTf (0.40, 1.56 mM) was eluted from a column of silica gel with 2:3 EtOAc–toluene to give **11** (0.13 g, 28%) as an oil. The compound was identical to that from Procedure A, above.

1.6.3. Procedure C. The mixture after the reaction of **9** (0.15 g, 0.42 mM) with **5**[4] (0.30 g, 0.51 mM) in DMF (7 mL) and CH_2Cl_2 (7 mL) in the presence of NIS

(0.12 g) and TMSOTf (30 μL) was eluted from a column of silica gel with 1:2 EtOAc–toluene to give **11** (0.049 g, 14%) as an oil. The compound was identical with that from Procedure A, above.

1.7. Allyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-α-D-glucopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (12)

1.7.1. Procedure C. The mixture after the reaction of **9** (57 mg, 0.16 mM) with **6** (0.15 g, 0.19 mM) in DMF (7 mL) and CH_2Cl_2 (7 mL) in the presence of NIS (45 mg) and TMSOTf (15 μL) was eluted from a column of silica gel with 1:2 EtOAc–toluene to give **12** (19 mg, 9%) as an oil: $[\alpha]_D^{20}$ +75 (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.38–6.88 (m, 20H, Ph), 6.03 (d, 1H, NH), 5.85 (m, 1H, =CH), 5.43 (s, 1H, CHPh), 5.65 (d, 1H, $J_{1,2}$ 4.0 H-1^{II}), 5.24 (m, 2H, $=CH_2$), 4.94 (d, 1H, OCH_2Ph), 4.93 (d, 1H, $J_{1,2}$ 4.0, H-1^{I}), 4.83 (d, 1H, OCH_2Ph), 4.73 (d, 1H, OCH_2Ph), 4.50 (m, 2H, OCH_2Ph), 4.43 (m, 1H, H-2^{I}), 4.25–4.05 (m, 5H, H-3^{I}, H-3^{II}, H-6^{II}, OCH_2, OCH_2Ph), 4.01 (m, 1H, OCH_2), 3.95–3.84 (m, 4H, H-4^{I}, H-5^{I}, H-6^{I}, H-6$^{I'}$), 3.76 (t, 1H, $J_{6,6'}$ 10.4, $J_{5,6}$ 9.6, H6^{II}), 3.40 (m, 2H, H-5^{I}, H-2^{II}), 3.25 (t, 1H, $J_{3,4}$ 9.2, $J_{4,5}$ 9.2, H-4^{II}), 2.04 (s, 3H, $NCOCH_3$), 0.90 (s, 9H, Si–C–(CH_3)$_3$) 0.11 (s, 3H, $Si(CH_3)_2$), 0.08 (s, 3H, $Si(CH_3)_2$). ^{13}C NMR: δ 170.64 ($NCOCH_3$), 138.96 (=CH), 138.51–126.65 (Ph), 118.83 ($=CH_2$), 102.51 (CHPh), 97.97 (C-1^{II}), 96.41 (C-1^{I}), 83.26 (C-3^{II}), 82.04 (C-4^{I}), 79.03 (C-2^{II}), 78.18 (C-4^{II}), 76.04 (OCH_2Ph), 74.74 (OCH_2Ph), 72.52 (C-3^{I}), 71.33 (C-5^{II}), 71.05 (OCH_2Ph), 69.33 (C-6^{II}), 68.75 (OCH_2), 64.06 (C-5^{I}), 62.80 (C-6^{I}), 52.55 (C-2^{I}), 26.35 (Si–C–(CH_3)$_3$), 23.18 ($NHCOCH_3$), 18.70 (Si–C–(CH_3)$_3$), −4.75 ($Si(CH_3)_2$), −5.17 ($Si(CH_3)_2$). MALDITOF-MS: Calcd for $C_{51}H_{65}NO_{11}$: 896.1 [M]. Found: 918.2 $[M+Na]^+$.

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