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Synthesis of *O*-glycopyranosyl-*N*-hydroxysuccinimides of glucose and lactose and their opening by nucleophiles into prespacer glycosides

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O-Glycopyranosyl-*N*-hydroxysuccinimides of glucose and lactose have been synthesized. Peracetylated sugars, acetobromo sugars and benzoylated thioethyl glycosides have been used as precursors. With boron trifluoride etherate in dichloromethane, peracetylated sugars gave predominantly the β anomer. Trimethylsilyl trifluoromethanesulfonate in nitromethane, however, gave predominantly the α anomer. Treatment of the acetylated *O*-glucopyranosyl-*N*-hydroxysuccinimide with various nucleophiles (methoxide, hydroxide and pentylamine) gave the corresponding deacetylated *N*-(succinyl)glucopyranosylhydroxylamines in almost quantitative yield. These derivatives are suitable for the formation of glycoconjugates or attachment of carbohydrates to solid phases using the opened succinimide as linking arm.
Keywords: *N*-Hydroxysuccinimide, *O*-glycosylhydroxylamide, spacer, glycoconjugates.

Several methods and different linking arms are used for the coupling of carbohydrates to carriers to make glycoconjugates or carbohydrate substituted polymers [1]. No method so far is universal and without drawbacks, and there is still a need for improvements and the developments of new methods. We now report on the synthesis of *O*-glycopyranosyl-*N*-hydroxysuccinimides which, on treatment with various nucleophiles, give a glycoconjugate directly or a derivative easily converted into one.

Results and discussion

The *O*-glycopyranosyl-*N*-hydroxysuccinimides were prepared in a number of ways. The treatment of peracetylated glucose or lactose with *N*-hydroxysuccinimide in the presence of a Lewis acid gave a high yield of the corresponding glycosides (69–81%). The anomeric ratio in the products depended strongly on the solvent and the Lewis acid used. Boron trifluoride etherate in dichloromethane gave almost exclusively the β anomers **1** (glucose, 73%) and **5** (lactose, 78%), while treatment with trimethylsilyl trifluoromethanesulfonate in nitromethane gave predominantly the α anomers **2** (glucose, 64%) and **6** (lactose, 54%). The latter result is probably not due to an *in situ* anomerization, since in experiments where the *O*-glucopyranosyl-*N*-hydroxysuccinimide (**1**) was exposed to the coupling conditions, no anomerization was observed. Good separations of the two anomers were obtained easily on TLC (toluene/ethyl acetate, 1:2 by vol, ΔR_F: glucose, 0.26; lactose, 0.10) and silica gel chromatography.

The *O*-glycopyranosyl-*N*-hydroxysuccinimides can be prepared also from the corresponding glycosyl bromide or thioethyl glycoside. Thus, coupling of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with *N*-hydroxysuccinimide in dichloromethane using silver trifluoromethanesulfonate [2, 3] as promoter gave the *O*-(2,3,4,6-tetra-*O*-acetyl-β,α-D-glucopyranosyl)-*N*-hydroxysuccinimides **1** and **2** in 88% yield (β/α ratio: 5/3.8) and coupling of ethyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside using *N*-iodosuccinimide–silver trifluoromethanesulfonate [4, 5] as promoter gave a β/α mixture of the corresponding *O*-glucopyranosyl-*N*-hydroxysuccinimides **3** and **4** in 77% total yield (β/α ratio: 1/1).

To show the potential of the succinimidyl group as a linking arm precursor, compound **1** was treated with various nucleophiles. Upon treatment with sodium hydroxide in aqueous ethanol or sodium methoxide in methanol, a quantitative yield of the opened derivatives **7** and **8** was obtained, derivatives suitable for formation of glycoconjugates or for attachment to a solid phase via an ester or an amide linkage [1]. Upon treatment with n-pentylamine in tetrahydrofuran, the glycolipid derivative **9** was obtained directly in 95% yield.

The *O*-glycopyranosyl-*N*-hydroxysuccinimides thus seem

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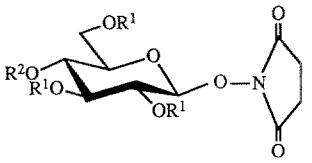
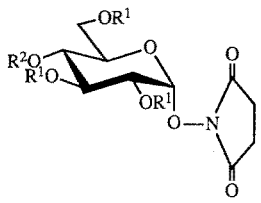


1 $R^1 = R^2 = Ac$



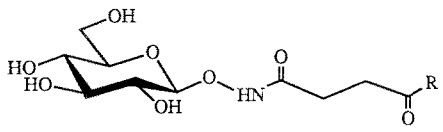
2 $R^1 = R^2 = Ac$

3 $R^1 = R^2 = Bz$

4 $R^1 = R^2 = Bz$

5 R^1 = Ac, R^2 = 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl

6 R^1 = Ac, R^2 = 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl



7 R = OH

8 R = OCH_3

9 R = $NH(CH_2)_4CH_3$

to be promising precursors for the formation of glycoconjugates. They are stable under non-nucleophilic conditions and can be stored without precautions. The versatility in coupling reactions that can be used for their formation should make it possible to synthesize them both from synthetic oligosaccharides and from oligosaccharides isolated from nature (after peracetylation). Furthermore, their conversion into glycoconjugates or precursors thereof is simple and clean. These compounds may probably also be transformed by removal of the succinyl group into unsubstituted *O*-glycosylhydroxylamines, which then could be involved in, e.g., thiourea or amide linkages [1]. *O*-Glycosylhydroxylamines have been obtained previously from *N*-hydroxyphthalimido glycosides [6], but so far we have not been able to find suitable conditions for their synthesis from the succinimide derivatives.

Materials and methods

General methods

Melting points are corrected. Concentrations were performed at <40 °C bath temperature. For optical rotations a Perkin-Elmer 241 polarimeter was used, and measurements were performed at room temperature. NMR spectra were recorded at 30 °C with a JEOL GSX-270 instrument. The following reference signals were used: Me_4Si δ 0.00 ^{13}C in C^2HCl_3 and dioxane δ 67.40 in ^2H_2O. Gel filtrations were performed on a column (2.6 cm × 90 cm) of Bio-Gel P-2 irrigated with water/(1% n-butanol) unless otherwise stated, and a differential refractometer was used for monitoring the column eluants. Silica gel 60 F-254 (Merck, Darmstadt, Germany) was used for TLC and the spots were detected by charring with sulfuric acid. Column chromatography was performed on Matrex™ Silica gel 60 (0.035–0.070 mm, Amicon Corp.). Predried powdered molecular sieves (4 Å, Union Carbide) were used.

O-(*2,3,4,6-Tetra*-O-*acetyl-β-D-glucopyranosyl*)-N-*hydroxysuccinimide* (**1**) *from boron trifluoride etherate coupling*

Boron trifluoride etherate (630 µl, 5.12 mmol) in dichloromethane (2 ml), was added dropwise to a stirred, cooled (ice-bath) solution of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranoside [7] (100 mg, 0.256 mmol) and *N*-hydroxysuccinimide (117 mg, 1.02 mmol) in dichloromethane (10 ml) containing dried molecular sieves, whereafter the reaction mixture was put in a refrigerator. When TLC (ethyl acetate–toluene, 2:1 by vol) showed that almost all starting material was consumed (24 h), chloroform (20 ml) was added and the mixture filtered through Celite. The filtrate was washed with saturated sodium hydrogen carbonate (2 × 5 ml) and water (5 ml). After extracting the water phases with chloroform (5 ml), the organic phases were combined and dried ($MgSO_4$), filtered, and concentrated. The residue was dissolved in chloroform (1 ml) and put on a silica gel column. Elution (ethyl acetate–toluene, 2:1 by vol) gave **1** (83 mg, 73%) together with the α anomer **2** (9.2 mg, 8%). **1** was crystallized from ethanol–water to give material with m.p. 201–202 °C, $[\alpha]_D$ −39° (c 1.0, chloroform). ^{13}C-NMR data (C^2HCl_3): δ 20.6–20.7 (OAc), 25.4 (CH_2), 61.7 (C-6), 68.2, 69.7, 72.3, 72.4 (C-2–C-5), 103.9 (C-1, J_{CH} 170 Hz), 169.3–170.6 (carbonyl C).

Analytical data. Calculated for $C_{18}H_{23}O_{12}N$: C, 48.54; H, 5.20; O, 43.11; N, 3.14. Found: C, 48.47; H, 5.13; N, 3.10.

O-(*2,3,4,6-Tetra*-O-*acetyl-α-D-glucopyranosyl*)-N-*hydroxysuccinimide* (**2**) *from trimethylsilyl trifluoromethanesulfonate coupling*

A solution of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranoside (169 mg, 0.43 mmol) and *N*-hydroxysuccinimide (199 mg, 1.73 mmol) in nitromethane (5 ml) containing dried molecular sieves was stirred at room temperature for 30 min before it was cooled in an ice-bath. Trimethylsilyl trifluoromethanesulfonate (768 µl, 4.3 mmol) was added dropwise over a period of 10 min, after which the reaction mixture was put in a refrigerator. When TLC (ethyl acetate/toluene, 2:1 by vol) showed that almost no starting material remained (24 h), the reaction was worked up as described above to give **2** (124 mg, 64%) together with the β anomer **1** (19 mg, 10%). A sample of **2** was crystallized from ethanol–water to give a compound with m.p. 145–147 °C, $[\alpha]_D$ +172° (c 1.0, chloroform). ^{13}C-NMR data (C^2HCl_3): δ 20.6–20.7 (OAc), 25.4 (CH_2), 61.2 (C-6), 67.6,

69.1, 69.5, 69.5 (C-2–C-5), 100.6 (C-1, J_{CH} 182 Hz), 169.6–170.5 (carbonyl C).

Analytical data. Calculated for $C_{18}H_{23}O_{12}N$: C, 48.54; H, 5.20; O, 43.11; N, 3.14. Found: C, 48.65; H, 5.17; N, 3.10.

O-(*2,3,4,6-Tetra*-O-*acetyl-β/α-*D-*glucopyranosyl*)-N-*hydroxysuccinimide* (**1** *and* **2**) *from silver trifluoromethanesulfonate coupling*

Silver trifluoromethanesulfonate (1.53 g, 5.96 mmol) in toluene (12 ml) was added dropwise to a stirred, cooled (ice-bath) solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide [8] (2.13 g, 5.18 mmol) and *N*-hydroxysuccinimide (1.19 g, 10.35 mmol) in dichloromethane (40 ml) containing dried molecular sieves. When the addition was completed (30 min), the reaction mixture was concentrated to about half of its original volume and put directly on top of a silica gel column. Elution (ethyl acetate–toluene 2:1 by vol) gave **1** (1.16 g, 50%) and **2** (781 mg, 38%). ^{13}C-NMR spectra were indistinguishable from the ones above.

O-(*2,3,4,6-Tetra*-O-*benzoyl-β/α-*D-*glucopyranosyl*)-N-*hydroxysuccinimide* (**3** *and* **4**) *from* N-*iodosuccinimide coupling*

Silver trifluoromethanesulfonate (80 mg, 0.31 mmol) and *N*-iodosuccinimide (77 mg, 0.34 mmol) were added to a stirred solution of thioethyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside [9] (205 mg, 0.31 mmol) and *N*-hydroxysuccinimide (72 mg, 0.63 mmol) in dichloromethane (10 ml) containing molecular sieves. After 5 min the reaction mixture was diluted with dichloromethane (10 ml) and filtered through Celite. Washing with 0.1 M sodium thiosulfate (5 ml), saturated sodium hydrogen carbonate (5 ml), water (5 ml) and drying (magnesium sulphate) gave after concentration a syrup (240 mg) which was put on a silica gel column. Elution (ethyl acetate–toluene, 1:3 by vol) gave **3** (85 mg, 40%) and **4** (80 mg, 37%).

Analytical data for **3**: m.p. 216–18 °C (from ethanol–ethyl acetate), $[\alpha]_D$ −48° (c 0.94, chloroform). ^{13}C-NMR data (C^2HCl_3): δ 25.3 (CH_2), 63.2 (C-6), 69.7, 70.4, 72.6, 72.8 (C-2–C-5), 103.3 (C-1, J_{CH} 172 Hz), 128.3–133.5 (aromatic C), 165.1–165.3, 170.0 (carbonyl C). Calculated for $C_{38}H_{31}O_{12}N$: C, 65.80; H, 4.50; O, 27.68; N, 2.02. Found: C, 65.7; H, 4.55; N, 1.95.

Analytical data for **4**: $[\alpha]_D$ +152° (c 0.76, chloroform). ^{13}C-NMR data (C^2HCl_3): δ 25.4 (CH_2), 62.1 (C-6), 68.3, 69.7, 70.1, 70.6 (C-2–C-5), 100.7 (C-1, J_{CH} 183 Hz); 128.3–133.5 (aromatic C), 165.1–166.1, 170.4 (carbonyl C). Calculated for $C_{38}H_{31}O_{12}N$: C, 65.80; H, 4.50; O, 27.68; N, 2.02. Found: C, 65.95; H, 4.60; N, 2.0.

O-(*2,2',3,3',4',6,6'-Hepta*-O-*acetyl-β-*D-*lactopyranosyl*)-N-*hydroxysuccinimide* (**5**) *from boron trifluoride etherate coupling*

Starting from 1,2,2',3,3',4',6,6'-octa-*O*-acetyl-β-D-lactose [10] and using the same experimental conditions and workup procedure as those in the boron trifluoride etherate promoted formation of **1** above, **5** (134 mg, 78%) was obtained after column chromatography (ethyl acetate–toluene, 4:1 by vol). $[\alpha]_D$ −17° (c 0.63, chloroform). ^{13}C-NMR data (C^2HCl_3): δ 20.5–20.9 (OAc), 25.4 (CH_2), 60.9, 61.9 (C-6,6'), 66.7–76.6 (C-2,3,4,5,2',3',4',5'), 101.2 (C-1', J_{CH} 161 Hz), 102.2 (C-1, J_{CH} 174 Hz), 169.2–170.4 (carbonyl C).

Analytical data. Calculated for $C_{30}H_{40}O_{20}N$: C, 49.05; H, 5.49; O, 43.56; N, 1.91. Found: C, 49.12; H, 5.43; N, 1.85.

O-(*2,2',3,3',4',6,6'-Hepta*-O-*acetyl-α-*D-*lactopyranosyl*)-N-*hydroxysuccinimide* (**6**) *from trimethylsilyl trifluoromethanesulfonate coupling*

Starting from 1,2,2',3,3',4',6,6'-octa-*O*-acetyl-β-D-lactose [10] and using the same experimental conditions and workup procedure as those in the trimethylsilyl trifluoromethanesulfonate promoted formation of **2** above, **6** (74 mg, 54%) and **5** (26 mg, 15%) were obtained after column chromatography (ethylacetate–toluene, 4:1 by vol). Data for **6**: $[\alpha]_D$ +76° (c 1.15, chloroform). ^{13}C-NMR data (C^2HCl_3): δ 20.5–20.8 (OAc), 25.4 (CH_2), 60.9, 61.2 (C-6,6'), 66.8–75.2 (C-2,3,4,5,2',3',4',5'), 100.6 (C-1, J_{CH} 184 Hz), 100.8 (C-1', J_{CH} 164 Hz), 169.1–170.3 (carbonyl C).

Analytical data. Calculated for $C_{30}H_{40}O_{20}N$: C, 49.05; H, 5.49; O, 43.56; N, 1.91. Found: C, 48.70; H, 5.21; N, 1.99.

*N-(Succinyl)-O-β-*D-*glucopyranosyl-hydroxylamine* (**7**)

A solution of **1** (74 mg, 0.137 mmol) in 0.25 M aqueous sodium hydroxide–ethanol, 1:1 by vol (8 ml) was stirred at room temperature for 1 h, then neutralized with Dowex-H^+ resin, filtered and concentrated to give **7** (43 mg, quantitative yield). Before elementary analysis, the sample was further purified on a Bio-Gel P-2 column and converted into its sodium salt by passing it through a short cation exchange column in the sodium form. For optical rotation the compound in its sodium form was used and dissolved in water without any pH adjustments. $[\alpha]_D$ −43° (c 1.0, water). ^{13}C-NMR data (^2H_2O, p^{2}H2): δ 28.6, 30.6 (CH_2), 61.4 (C-6), 70.0, 72.1, 76.3, 77.0 (C-2–C-5), 106.2 (C-1), 172.6, 178.0 (carbonyl C).

Analytical data. Calculated for $C_{10}H_{16}O_9NNa$: C, 37.86; H, 5.08; O, 45.39; N, 4.42; Na, 7.25. Found: C, 37.85; H, 5.04; N, 4.33.

N-(*Succinylmethylester*)-O-*β*-D-*glucopyranosylhydroxylamine* (**8**)

0.1 M sodium methoxide (1 ml) was added to a solution of **1** (61.4 mg, 0.138 mmol) in methanol (4 ml), and the solution was stirred at room temperature for 1 h, then neutralized with Dowex resin (H^+), filtered and concentrated to give **8** (43 mg, quantitative yield). Before elementary analysis, the sample was further purified on a Bio-Gel P-2 column. The overall yield was 95%. $[\alpha]_D$ −39° (c 0.8, water). ^{13}C-NMR data (^2H_2O): δ 28.0, 29.6 (CH_2), 53.2 (OCH_3), 61.4 (C-6), 70.0, 72.1, 76.3, 77.0 (C-2–C-5), 106.2 (C-1), 172.6, 176.0 (carbonyl C).

Analytical data. Calculated for $C_{11}H_{19}O_9N$: C, 42.72; H, 6.19; O, 46.56; N, 4.53. Found: C, 42.58; H, 6.29; N, 4.45.

N-(*Succinylpentylamide*)-O-*β*-D-*glucopyranosylhydroxylamine* (**9**)

n-Pentylamine (1.2 ml, 10.3 mmol) was added to a stirred solution of **1** (121 mg, 0.27 mmol) in tetrahydrofuran (5 ml) containing molecular sieves. After 36 h the reaction mixture was concentrated and the residue was purified on a silica gel column (chloroform–methanol–ammonia$_{(aq)}$, 65:40:10 by vol) and was further purified on a Bio-Gel P-2 column eluted with aqueous 0.1 M pyridinium acetate, pH 5.5, to give **9** (86 mg, 95%). $[\alpha]_D$ −42° (c 1.0, water). ^{13}C-NMR data (^2H_2O): δ 14.2 (CH_3), 22.5, 28.8, 29.0, 29.2, 31.5, 40.2 (CH_2), 61.4 (C-6), 70.1, 72.2, 76.3, 77.0 (C-2–C-5), 106.2 (C-1), 172.4, 174.7 (carbonyl C).

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