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Stereoselective One-Pot Synthesis of Isomaltooligosaccharides

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A highly stereoselective synthesis of α-linked (1→6)-gluco- di- and trisaccharides has been described. In this procedure two thioglucosides, (5-nitro-2-pyridyl) 1-thio-β-D-glucopyranoside and 6-O-tritylated ethyl 1-thioglucoside were applied as an acceptor and a donor respectively.

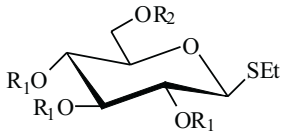
Key words: glycosylation, thioglycosides

Numerous bacterial polysaccharides are capable of inducing a protective antibody response [1]. Therefore, synthetic oligosaccharides mimetics of natural inducers are also needed as models in the study of mechanism of immunological processes, as well as in the design of artificial vaccines [2]. Oligosaccharides containing glucose with α-linked (1→6) glycoside bond are constituents of bacterial outer membranes in *Tremella mesentrica* and *Pullaria pullulans* [3].

Various glycosylation methods for the synthesis of oligosaccharides have been reported [4,5]. Main disadvantages of these metodologies in synthesis of oligoisomaltosides consisting of two and more monomer units are multistep procedures and a low α stereoselectivity [6]. Consequently, isolation and chromatographic purification of higher α-linked (1→6)-oligosaccharides present some specific problems, because of physicochemical similarity of the α and β-linked products. Most synthetic efforts are directed towards preparation of oligosaccharide building blocks, using minimal number of synthetic steps. To facilitate the synthesis of oligosaccharides, some new chemoselective glycosylation strategies have been developed [7,8]. Current methods of choice involve thioglycosides as donors and acceptors, due to their ease of preparation and availability of methods for their activation by a number of thiophilic reagents [5].

RESULTS AND DISCUSSION

Recently, we have found that (5-nitro-2-pyridyl) 1-thio-β-D-glucopyranoside (**2**) is a very stable acceptor in the synthesis of 1,6-D-gluco-disaccharides [9]. We have observed, however, a low α/β selectivity when **1a** was used as a glycosyl donor. Continuing this study, we now describe a highly stereoselective glycosylation procedure that allows the preparation of α-linked (1→6)-trioside **5** in one-pot operation.



1a: R_1=Bn, R_2=Bn
1b: R_1=Ac, R_2=Tr
1c: R_1=Bn, R_2=Tr

Figure 1.

In this procedure thioglucoside **2** with unprotected primary hydroxyl group was used as an acceptor and 6-O-tritylated thioglucoside **1c** as a donor. Model donor **1c** was chosen, taking into account mechanistic considerations. Since carboxonium ion intermediate-state is likely formed, one could expect an influence of a bulky protecting group in position C-6 in shielding an acceptor approach from β-side, thus, enhancing the α stereoselectivity of glycosylation. It has been reported that bulky protecting groups at C-6, for example trityl or *t*-butyldimethylsilyl, of a glucosyl donor improve the α-selectivity in an I^+ promoted glycosylation [10]. Indeed, treatment of an equimolar solution of acceptor **2** and donor **1c** in toluene with N-iodosuccinimide (NIS)/triflic acid (TfOH) at room temperature for 15 minutes resulted rather unexpectedly in formation of trisaccharide **5** as the α anomer (Table 1, entry 1). However, when two-fold excess of acceptor was used in the reaction, only α-linked disaccharide **4** was isolated (Table 1, entry 3). In both procedures no β-linked products were formed (Scheme 1).

The crucial process allowing such an outcome of the glycosylation consists of transetherification of the acceptor molecule **2** by the primary condensation product **4** or by donor **1c** respectively under applied reaction conditions. In each procedure we have observed some amount of **3**, resulting from the intermolecular trityl group transfer. Furthermore, we have found that in the case of acetyl-protected donors, like **1b**, the only isolated product in reaction with selected acceptor **2** was 6-O-tritylated acceptor, but none of desired oligosaccharide was isolated. Interestingly, such 6-O-trityl group transfer was not observed in reaction of **1c** with methyl 2,3,4-tri-O-benzyl-α-D-glucoside as an acceptor, but glycosylation led to the mixture of anomers in this experiment [8].

High stereoselectivity of elaborated one-pot glycosylation is worth noting. In this manner we have synthesized α-linked (1→6)-D-gluco- di- and trisaccharides (**4** and **5**), depending on relative ratio of glycosyl donor and acceptor. These compounds, suitably protected with the trityl at the primary hydroxyl and thiopyridyl groups at the reducing end, can be applied in a block synthesis of oligosaccharides as acceptors and donors alike.

Scheme 1

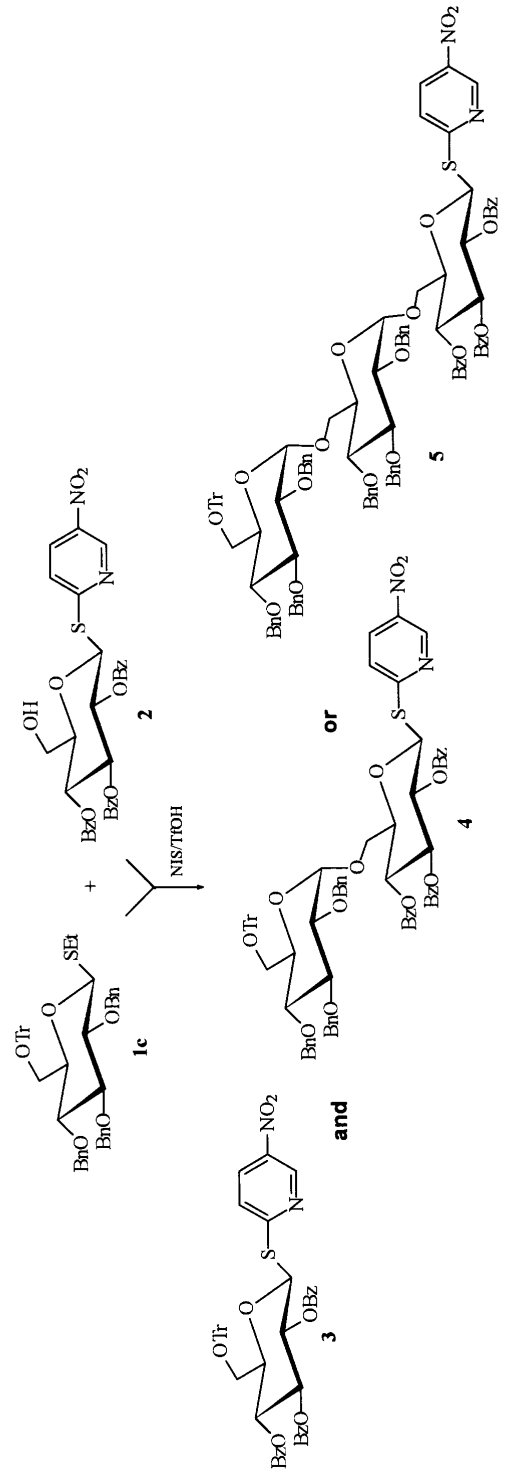


Table 1. Glycosylation reactions of **1c** with **2**.

Entry	Donor **1c** : Acceptor **2**	Yield of isolated products [%]		
		Tritylated acceptor **3**	Disaccharide **4**	Trisaccharide **5**
1	1 : 1	44	–	42
2	2 : 1	52	–	16
3	1 : 2	23	21	–

A scope and the applicability of the newly observed glycosylations, promoted by concomitant intermolecular trityl group transfer for synthesis of bacterial oligosaccharides, is subject of further study.

EXPERIMENTAL

General methods. Optical rotations were measured with Perkin-Elmer 141 polarimeter using sodium lamp (589 nm) at room temperature. Elemental analyses were performed on a Perkin-Elmer 2400 analyzer. ^{13}C NMR and ^{1}H NMR spectra were recorded for solutions in $CDCl_3$ (internal TMS) on Varian 300 MHz spectrometer. Reactions were monitored by TLC on precoated plates of silica gel 60 (70–230 mesh, Merck), components were detected by spraying the plates with 3% palladium(II) chloride in water (sulphur compounds) or 10% sulphuric acid in ethanol followed by heating. Chromatographic purification was done on silica gel 60 columns (Merck) 0.063–0.2 mm. All organic solutions were concentrated under reduced pressure at 40°C.

Starting materials. Ethyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-glucopyranoside (**1a**), ethyl 2,3,4-tri-O-acetyl-6-O-trityl-1-thio-β-D-glucopyranoside (**1b**), ethyl 2,3,4-tri-O-benzyl-6-O-trityl-1-thio-β-D-glucopyranoside (**1c**) [11], (5-nitro-2-pyridyl) 2,3,4-tri-O-benzoyl-1-thio-β-D-glucopyranoside (**2**) [9] were prepared according to the published procedures. NIS, TfOH and molecular sieves 4 Å are commercially available (Aldrich, Merck, POCh) and were used without purification.

Typical procedure for glycosidation. Donor **1a**, **1b** or **1c** (0.05–0.1 mmol) and acceptor **2** (0.05–0.1 mmol) were dissolved in a dry toluene (3 ml) containing micronised molecular sieves 4 Å and vigorously stirred at room temperature for 30 minutes. Promoter NIS/TfOH (1 equiv./0.1 equiv.) was added and mixture was stirred at room temperature until completion (TLC, hexane/ethyl acetate 2:1 v/v). The mixture was filtered, dissolved in toluene (10 ml), washed with 10% aqueous $Na_2S_2O_3$ and water, dried, filtered and concentrated. The crude product was purified by column chromatography (hexane/ethyl acetate 8:1 and 6:1 v/v).

(5-Nitro-2-pyridyl) 2,3,4-tri-O-benzoyl-6-O-trityl-1-thio-β-D-glucopyranoside (3), white crystals, m.p. 103–107°C, $[\alpha]_D$ +65.1° (c 0.8, $CHCl_3$). Anal. calc. for $C_{51}H_{40}N_2O_{10}S$ (872.94): C, 70.17; H, 4.62; N, 3.21; S, 3.67. Found: C, 70.48, H, 4.72, N, 3.15, S, 3.57; ^{13}C NMR δ: 165.8, 165.3, 164.9, 164.5 (C-2_{pyr}, 3 $C_6H_5\underline{C}O$), 145.0 (C-6_{pyr}), 143.5 (3 C-1_{ar} in $(\underline{C}_6H_5)_3C$), 141.9 (C-5_{pyr}), 133.7, 133.5, 133.3 (3 C-1_{ar} in $\underline{C}_6H_5CO$), 131.2 (C-4_{pyr}), 130.0–127.0 (3 $\underline{C}_6H_5CO$, 3 $(\underline{C}_6H_5)_3C$), 122.3 (C-3_{pyr}), 86.9 ($\underline{C}(C_6H_5)_3$), 81.6 (C-1), 78.6, 74.3, 70.3, 69.4, 62.9 (C-2, 3, 4, 5, 6); ^{1}H NMR δ: 3.29 (dd, 1H, J = 10.9 Hz, J = 2.7 Hz, H-6a), 3.36 (dd, 1H, J = 10.9 Hz, J = 5.4 Hz, H-6), 4.09 (ddd, 1H, J = 10.1 Hz, J = 5.4 Hz, J = 2.5 Hz, H-5), 5.59 (dd~t, 1H, J = 9.8 Hz, H-4), 5.76 (dd, 1H, J = 10.3 Hz, J = 9.5 Hz, H-2), 5.97 (dd, 1H, J = 9.3 Hz, J = 9.5 Hz, H-3), 6.11 (d, 1H, J = 10.3 Hz, H-1), 7.25–7.45 (m, 25H, Ph, H-3_{pyr}), 7.70–7.86 (m, 6H, Ph), 8.22 (dd, 1H, J = 2.7 Hz, J = 8.8 Hz, H-4_{pyr}), 9.31 (d, 1H, J = 2.7 Hz, H-6_{pyr}).

(5-Nitro-2-pyridyl) 2,3,4-tri-O-benzyl-6-O-trityl-α-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-1-thio-β-D-glucopyranoside (4), syrup, $[\alpha]_D$ +54.1° (c 1.0, $CHCl_3$). Anal. calc. for $C_{78}H_{68}N_2O_{15}S$ (1305.45): C, 71.76; H, 5.25; N, 2.15; S, 2.46. Found: C, 71.52, H, 5.28, N, 2.37, S, 2.39; ^{13}C NMR δ: 165.8, 165.3, 164.9, 164.5 (C-2_{pyr}, 3 $C_6H_5\underline{C}O$), 145.0 (C-6_{pyr}), 143.5(3 C-1_{ar} in $\underline{C}_6H_5)_3C$), 141.9 (C-5_{pyr}), 138.0, 137.9 (3 C-1_{ar} in $\underline{C}_6H_5$-CH_2), 133.5, 133.3, 133.2 (3 C-1_{ar} in $\underline{C}_6H_5CO$), 131.2 (C-4_{pyr}), 129.9–127.0 (3 $\underline{C}_6H_5$-CH_2, 3 $\underline{C}_6H_5CO$, 3 $(\underline{C}_6H_5)_3C$), 122.3 (C-3_{pyr}), 100.6 (C-1′), 86.9 ($\underline{C}(C_6H_5)_3$), 81.6 (C-1), 78.7, 76.8, 76.2, 76.1, 74.4, 74.3, 72.0, 71.8, 71.2, 70.3, 69.4, 65.4, 62.9 (3 C_6H_5-$\underline{C}H_2$, C-2, 3, 4, 5, 6, 2′, 3′, 4′,

5′, 6′); ^{1}H NMR δ: 3.26–3.38 (m, 4H, H-6, H-6a, H-6′, H-6a′), 3.59 (m, 1H, H-5′), 3.68 (dd, 1H, J = 6.1 Hz, J = 7.1 Hz, H-3′), 3.91 (dd, 1H, J = 7.1 Hz, J = 0.5 Hz, H-2′), 4.09 (ddd, 1H, J = 10.2 Hz, J = 5.6 Hz, J = 2.7 Hz, H-5), 4.40; 4.45 (AB, 2H, J = 12.0 Hz, $\underline{CH_2}$Ph), 4.53; 4.57 (AB, 2H, J = 12.4 Hz, $\underline{CH_2}$Ph), 4.56; 4.63 (AB, 4H, J = 12.7 Hz, $\underline{CH_2}$Ph), 4.51–4.64 (m, 1H, H-4′), 5.46 (s, 1H, H-1′), 5.59 (dd, 1H, J = 10.1 Hz, J = 9.5 Hz, H-4), 5.75 (dd, 1H, J = 10.2 Hz, J = 9.5 Hz, H-2), 5.96 (dd, 1H, J = 9.5 Hz, J = 9.3 Hz, H-3), 6.10 (d, 1H, J = 10.5 Hz, H-1), 7.08–7.53 (m, 40H, Ph, H-3$_{pyr}$), 7.70–7.94 (m, 6H, Ph), 8.21 (dd, 1H, J = 2.7 Hz, J = 8.8 Hz, H-4$_{pyr}$), 9.30 (d, 1H, J = 2.7 Hz, H-6$_{pyr}$).

(5-Nitro-2-pyridyl) 2,3,4-tri-O-benzyl-6-O-trityl-α-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzyl-α-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-1-thio-β-D-glucopyranoside (5), syrup, $[\alpha]_D$ +43.0° (c 1.0, $CHCl_3$), Anal. calc. for $C_{105}H_{96}N_2O_{20}S$ (1737.95): C, 72.56; H, 5.57; N, 1.61; S, 1.85. Found: C, 72.74, H, 5.72, N, 1.92, S, 1.74; ^{13}C NMR δ: 165.8, 165.3, 164.9, 164.5 (C-2$_{pyr}$, 3 $C_6H_5\underline{C}O$), 145.0 (C-6$_{pyr}$), 143.5 (3 C-1$_{ar}$ in $\underline{C}_6H_5)_3C$), 141.9 (C-5$_{pyr}$), 138.0, 137.9, 137.8 (6 C-1$_{ar}$ in $\underline{C}_6H_5$-CH_2), 133.5, 133.3, 133.2 (3 C-1$_{ar}$ in $\underline{C}_6H_5CO$), 131.2 (C-4$_{pyr}$), 129.9–127.0 (6 $\underline{C}_6H_5$-CH_2, 3 $\underline{C}_6H_5CO$, 3 $(\underline{C}_6H_5)_3C$), 122.3 (C-3$_{pyr}$), 100.6 (C-1′, 1′′), 86.9 ($\underline{C}(C_6H_5)_3$), 81.6 (C-1), 78.7, 77.2, 76.8, 76.2, 76.1, 74.4, 74.3, 72.0, 71.8, 71.2, 70.3, 69.4, 65.4, 62.9 (6 C_6H_5-$\underline{C}H_2$, C-2, 3, 4, 5, 6, 2′, 3′, 4′, 5′, 6′, 2′′, 3′′, 4′′, 5′′, 6′′); ^{1}H NMR δ: 3.26–3.37 (m, 6H, H-6, H-6a, H-6′, H-6a′, H-6′′, H-6a′′), 3.57–3.61 (m, 2H, H-5, H-5′′), 3.68–3.70 (m, 2H, H-3′, H-3′′), 3.90–3.92 (m, 2H, H-2′, H-2′′), 4.09 (ddd, 1H, J = 9.6 Hz, J = 5.3 Hz, J = 2.9 Hz, H-5), 4.40; 4.43 (2×AB, 4H, J = 12.1 Hz, 2×$\underline{CH_2}$Ph), 4.53; 4.63 (2×AB, 4H, J = 12.6 Hz, 2×$\underline{CH_2}$Ph), 4.55; 4.59 (2× AB, 4H, J = 7.0 Hz, 2×$\underline{CH_2}$Ph), 4.51–4.63 (m, 2H, H-4′, H-4′′), 5.46 (s, 2H, H-1′, H-1′′), 5.59 (dd, 1H, J = 9.8 Hz, J = 9.7 Hz, H-4), 5.75 (dd, 1H, J = 10.2 Hz, J = 9.4 Hz, H-2), 5.96 (dd, 1H, J = 9.4 Hz, J = 9.9 Hz, H-3), 6.10 (d, 1H, J = 10.2 Hz, H-1), 7.08–7.55 (m, 55H, Ph, H-3$_{pyr}$), 7.70–7.96 (m, 6H, Ph), 8.21 (dd, 1H, J = 2.7 Hz, J = 9.0 Hz, H-4$_{pyr}$), 9.30 (d, 1H, J = 2.7 Hz, H-6$_{pyr}$).

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