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Synthesis of prospective disaccharide ligands for *Escherichia coli* O157 verotoxin

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ABSTRACT

The synthesis of new potential ligands for *Escherichia coli* 0157 verotoxin is reported, based on disaccharide fragments of the tetrasaccharide glycan portion of Gb_4 glycolipid. Intramolecular aglycone delivery was employed for the high-yielding and stereoselective production of the azidopropyl-tethered α-galactoside building block.

Infections caused by strains of *Escherichia coli* O157, giving rise to symptoms such as diarrhoea, are frequently reported worldwide. In severe cases (approximately 10%), the infection can lead to a haemolytic uremic syndrome (HUS), which for children has a significant mortality rate (approx. 3–6%).[1] The toxic effects of the infection process are due to the production of Shiga-like toxins (SLTs), also known as verotoxins (VTs), which are members of the AB_5 class of bacterial toxins, along with cholera and pertussis toxins.[2,3] The B-subunits (five of them in each toxin molecule) recognise a cell surface carbohydrate epitope, globotriaosylceramide, Gb_3 (for Gb_3 trisaccharide structure, see Fig. 1). After binding to this entity and entering the cell, the catalytically active A-subunit of verotoxin shuts down protein synthesis by specifically removing a single nucleotide base from ribosomal RNA.[4]

The design of synthetic antagonists capable of neutralising verotoxins relies on understanding the molecular basis of ligand–toxin interactions. Three Gb_3 trisaccharide binding sites per verotoxin B-subunit monomer were identified by X-ray crystallography,[5] with consideration of atomic distances and hydrogen bonding abilities implicating site 2 as the dominant site for Gb_3 binding. In contrast, computational approaches implicate site 1,[5] whereas transfer NOE[6a] and residual dipolar coupling[6b] experiments between a ^{13}C-labelled ligand[6c] and verotoxin also suggest site 2.[6] Further studies indicate that the interaction of VT-1 with Gb_3 is 'highly context dependent'.[7] Nonetheless, in 2000, Bundle and colleagues demonstrated that the millimolar binding potency of the Gb_3 trisaccharide can be increased over a million-fold by its incorporation into a multivalent 'starfish' format.[8] This strategy has spawned numerous other efforts to prepare multivalent verotoxin ligands[9] although, where investigated, in vivo efficacy did not truly reflect in vitro potency.[10] Recently, Bundle and colleagues showed that in vivo protein-mediated supramolecular templating can be used to enhance the activity of polymer-based multivalent ligands such that they can protect transgenic mice from verotoxin.[11]

In addition to therapeutic applications per se, there is significant interest in the detection of verotoxins. A number of ligand-based approaches have been reported, all based around the Gb_3 trisaccharide, derivatives or fragments thereof.[12] We noted literature reports that whereas the Gb_4 glycolipid is the preferred receptor for VT**2e** (for Gb_4 tetrasaccharide structure, see Fig. 1), it is not recognised by VT-**1**, VT-**2** or VT-**2c**. In contrast, N-deacetylated Gb_4 is an efficient receptor for all VTs.[4] In connection with our work on glyconanoparticle-based sensors,[13] we have developed chemical syntheses of the non-reducing terminal disaccharide fragments of Gb_4/de-*N*-acetyl-Gb_4 illustrated in Figure 1.

The two target compounds initially selected, **1** and **2**, (Fig. 1) were to be equipped with an azidopropyl spacer that can be transformed straightforwardly into an aminopropyl group for conjugation to surfaces of various types.[14] An obvious challenge in the synthesis of **1** and **2**, therefore, is the installation of the α-linkage to the azidopropyl tether. This could of course be achieved by glycosylation of 3-azidopropanol using a galactosyl donor bearing a non-participating group at O-2, but glycosylations involving reactive primary alcohols do not always exhibit a high degree of

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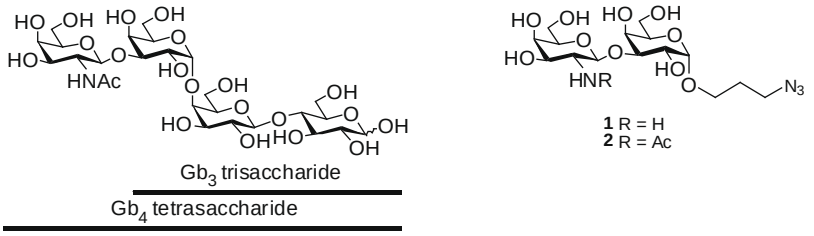
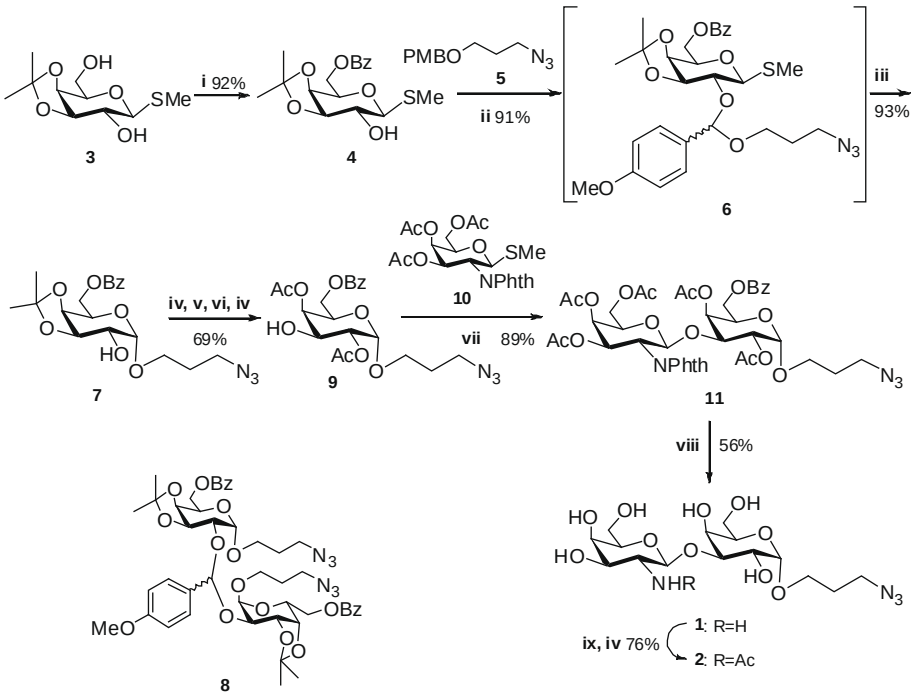


Figure 1. Glycolipid glycans and the target disaccharides **1** and **2**.



Scheme 1. Reagents and conditions: (i) BzCN, Et_3N; (ii) DDQ; (iii) IDCP; (iv) Ac_2O, pyridine; (v) CH_3COCH_3, CSA; (vi) $(MeO)_3CMe$, CSA; (vii) NIS, TfOH; (viii) $H_2NCH_2CH_2NH_2$, EtOH, Δ; (ix) NaOMe, MeOH.

α/β-selectivity in such reactions. Furthermore, classical Fischer glycosylation (heating a free monosaccharide in the presence of acid together with the acceptor alcohol, the latter generally used as the solvent) which usually favours *cis*-glycosides is not practical in this case since galactose has a tendency to also form furanosides. In addition, the alcohol (3-azidopropanol) is not commercially available which also restricts its use in excess. In order to overcome this potential selectivity problem, we adopted an intramolecular aglycone delivery approach (IAD),[15] based on the use of a *p*-methoxybenzylidene acetal (Scheme 1).[16] Another feature of our approach is the use of an inverse approach to IAD, that is, the *p*-methoxybenzyl group was first attached to the acceptor instead of the donor. Although subsequent acetal formation yields the same intermediate as achieved by initial attachment to the donor, an inverse approach offers the advantage of fewer reaction steps in this particular case.

The primary hydroxy group of the known thioglycoside **3**[17] was selectively protected in high yield using benzoyl cyanide. The resulting benzoate ester, **4**, was subsequently coupled with 3-azidopropyl *p*-methoxybenzyl ether, **5**,[18] under oxidative conditions to give mixed acetal **6**, which was used directly in the next step. Intramolecular glycosylation using iodonium dicollidine perchlorate (IDCP), which proved superior to both dimethyl(thiomethyl)sulfonium triflate (DMTST) and *N*-iodosuccinimide (NIS), gave the desired α-glycoside, **7**, along with the symmetric acetal **8** (as judged by mass spectrometry). Work-up of the crude reaction mixture with acidic acetone gave smooth hydrolysis of **8** into **7**, which was obtained with excellent stereocontrol and in excellent overall yield. Conventional orthoester formation and rearrangement then gave 3-OH acceptor **9**, which was glycosylated with known thioglycoside donor **10**[19] in the presence of NIS/TfOH giving protected disaccharide **11**. Removal of the phthalimido group in **11** proved troublesome, with initial attempts with hydrazine hydrate giving glycoside hydrolysis. However, swapping to ethylenediamine[20] gave amino-disaccharide **1** in acceptable yield. Subsequent N-acetylation then gave acetamido-disaccharide **2**.

In summary, we have developed a short synthesis of tethered disaccharide fragments of Gb_4 and its de-N-acetylated counterpart based on an efficient use of intramolecular aglycone delivery. Biological and structural studies to evaluate these compounds are ongoing.

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18. All synthetic intermediates gave combustion analysis or high resolution mass spectrometry data consistent with their proposed structures. Selected analytical data for key compounds are as follows:
3-Azidopropyl p-methoxybenzyl ether (**5**): Colourless liquid, bp 180–185 °C; R_f 0.43 (hexane/EtOAc 9:1); δ_H (250 MHz, $CDCl_3$): δ 1.90 (m, 2H, CH_2), 3.44 (t, 2H, *J* 6.7 Hz, spacer CH_2), 3.57 (t, 2H, *J* 6.0 Hz, spacer CH_2), 3.34 (s, 3H, CH_3O), 4.48 (s, 2H, $ArCH_2O$), 6.93 (d, 2H, *J* 8.7 Hz, aromatic H), 7.30 (d, 2H, *J* 8.7 Hz, aromatic H); δ_C (62.9 MHz, $CDCl_3$): δ 29.2 ($CH_2CH_2CH_2$), 48.5 (CH_2N_3), 55.2 (CH_3O), 66.5 (CH_2CH_2O), 72.7 ($ArCH_2O$), 113.7, 129.2, 130.2, 159.2 (aromatic C). Anal. Calcd for $C_{15}H_{11}N_3O_2$: C, 59.71; H, 6.83; N, 18.99. Found: C, 59.70; H, 6.80; N, 19.30.
3-Azidopropyl (2-amino-2-deoxy-β-D-galactopyranosyl)-(1→3)-α-D-galactopyranoside (**1**): $[\alpha]_D$ +137 (*c* 0.3, H_2O); R_f 0.53 (EtOAc/HOAc/MeOH/H_2O 5:3:3:2); δ_H (500 MHz, D_2O) 2.02 (m, 2H, spacer-CH_2), 3.02 (dd, 1H, $J_{1',2'}$ 8 Hz, $J_{2',3'}$ 10 Hz, H2′), 3.56 (m, 2H, spacer-CH_2N_3), 3.66 (dd, 1H, $J_{2',3'}$ 10 Hz, $J_{3',4'}$ 3 Hz, H3′), 3.69 (m, 1H, spacer-CH_2O), 3.76 (dd, 1H, $J_{5',6'a}$ 4 Hz, $J_{5',6'b}$ 7 Hz, H5′), 3.82–3.87 (m, 4H, H6a, H6b, H6′a, H6′b), 3.91 (m, 1H, spacer-CH_2O), 3.95 (m, 1H, H4), 4.07 (m, 3H, H2, H3, H5), 4.34 (m, 1H, H4′), 4.61 (d, 1H, $J_{1',2'}$ 8 Hz, H1′), 5.05 (d, 1H, $J_{1,2}$ 4 Hz, H1); δ_C (D_2O) 28.8 ($CH_2CH_2CH_2$), 49.0 (CH_2N_3), 54.0 (C2′), 61.9 (2 × C; C6, C6′), 65.8 (CH_2CH_2O), 68.0 (C2), 68.6 (C4′), 70.0 (C4), 71.4 (C5), 73.5 (C3′), 76.0 (C5′), 80.5 (C3), 99.1 (C1), 105.9 (C1′); ESI-MS calcd for $C_{15}H_{28}N_4O_{10}$ 424.18, *m/z* 447.17 $[M+Na]^+$.
3-Azidopropyl (2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1,3)-α-D-galactopyranoside (**2**): $[\alpha]_D$ +110 (*c* 0.18, H_2O); R_f 0.68 (EtOAc/HOAc/MeOH/H_2O 5:3:3:2); δ_H (500 MHz, D_2O) 2.01 (m, 2H, spacer-CH_2), 2.12 (s, 3H, NHAc), 3.55 (m, 2H, spacer-CH_2N_3), 3.67 (m, 1 H, spacer-CH_2O), 3.75 (dd, 1H, $J_{5',6'a}$ 4 Hz, $J_{5',6'b}$ 8 Hz, H5′), 3.80–3.86 (m, 5H, H3′, H6a, H6b, H6′a, H6′b), 3.89 (m, 1H, spacer-CH_2O), 3.96 (dd, 1H, $J_{1,2}$ 4 Hz, $J_{2,3}$ 10 Hz, H2), 4.00 (dd, 1H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 2 Hz, H3), 4.01 (m, 3H, H5, H2′, H4′), 4.28 (m, 1H, H4), 4.72 (d, 1H, $J_{1',2'}$ 8 Hz, H1′), 5.00 (d, 1H, $J_{1,2}$ 4 Hz, H1); δ_C (D_2O) 23.1 (CH_3CON), 28.8 ($CH_2CH_2CH_2$), 49.1 (CH_2N_3), 53.5 (C2′), 61.9 and 62.0 (C6, C6′), 65.9 (CH_2CH_2O), 68.2 (C2), 68.6 (C4′), 70.1 (C4), 71.4 (C5), 71.7 (C3′), 75.8 (C5′), 79.9 (C3), 99.4 (C1), 104.1 (C1′), 176.1 (CH_3CON); ESI-MS calcd for $C_{17}H_{30}N_4O_{11}$ 466.19, *m/z* 489.2 $[M+Na]^+$.
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