



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

Christian Bernlind^a, Steven W. Homans^a, Robert A. Field^{b,*}

^a Institute of Molecular and Cell Biology, University of Leeds, Leeds, LS2 9JT, UK

^b Department of Biological Chemistry, John Innes Centre, Colney Lane, Norwich, NR4 7UH, UK

ARTICLE INFO

Article history:

Received 19 January 2009

Revised 10 February 2009

Accepted 18 February 2009

Available online 21 February 2009

Keywords:

Gb₃

Gb₄

Verotoxin

Glycosylation

Disaccharide

Intramolecular aglycone delivery (IAD)

ABSTRACT

The synthesis of new potential ligands for *Escherichia coli* O157 verotoxin is reported, based on disaccharide fragments of the tetrasaccharide glycan portion of Gb₄ glycolipid. Intramolecular aglycone delivery was employed for the high-yielding and stereoselective production of the azidopropyl-tethered α -galactoside building block.

© 2009 Elsevier Ltd. All rights reserved.

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 AB₅ 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, Gb₃ (for Gb₃ 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 Gb₃ 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 Gb₃ binding. In contrast, computational approaches implicate site 1,⁵ whereas transfer NOE^{6a} and residual dipolar coupling^{6b} experiments between a ¹³C-labelled ligand^{6c} and verotoxin also suggest site 2.⁶ Further studies indicate that the interaction of VT-1 with Gb₃ is ‘highly context dependent’.⁷ Nonetheless, in 2000, Bundle and colleagues demonstrated that the millimolar binding potency

of the Gb₃ 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 ligands⁹ 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 Gb₃ trisaccharide, derivatives or fragments thereof.¹² We noted literature reports that whereas the Gb₄ glycolipid is the preferred receptor for VT2e (for Gb₄ tetrasaccharide structure, see Fig. 1), it is not recognised by VT-1, VT-2 or VT-2c. In contrast, N-deacetylated Gb₄ 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 Gb₄/de-N-acetyl-Gb₄ 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

* Corresponding author. Tel.: +44 1603 450720; fax: +44 1603 450018.

E-mail address: rob.field@bbsrc.ac.uk (R.A. Field).

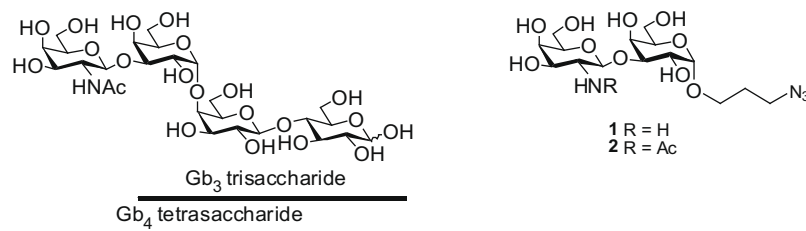
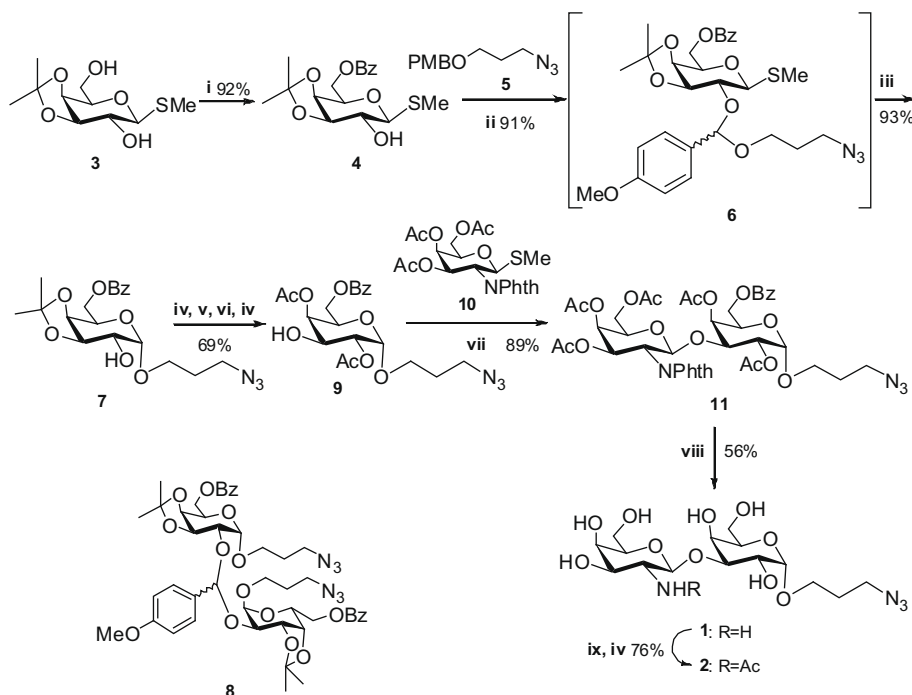


Figure 1. Glycolipid glycans and the target disaccharides **1** and **2**.



Scheme 1. Reagents and conditions: (i) BzCN, Et₃N; (ii) DDQ; (iii) IDCP; (iv) Ac₂O, pyridine; (v) CH₃COCH₃, CSA; (vi) (MeO)₃CMe, CSA; (vii) NIS, TfOH; (viii) H₂NCH₂CH₂NH₂, 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(thio-

methyl)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 ethylenediamine²⁰ 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 Gb₄ 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.

Acknowledgements

We acknowledge support from the BBSRC and the Knut and Alice Wallenberg Foundation (fellowship to C.B.).

References and notes

- (a) Paton, J. C.; Paton, A. W. *Clin. Microbiol. Rev.* **1998**, *11*, 450–479; (b) Karch, H.; Tarr, P. I.; Blelaszewski, M. *Int. J. Med. Microbiol.* **2005**, *295*, 405–418.
- Merritt, E. A.; Hol, W. G. *J. Curr. Opin. Struct. Biol.* **1995**, *5*, 165–171.
- Lacy, D. B.; Stevens, R. C. *Curr. Opin. Struct. Biol.* **1998**, *8*, 778–784.
- Endo, Y.; Tsurugi, K.; Yutsudo, T.; Takeda, Y.; Ogasawara, K.; Igarashi, K. *Eur. J. Biochem.* **1988**, *171*, 45–50.
- Nyholm, P.-G.; Magnusson, G.; Zheng, Z.; Norel, R.; Binnington-Boyd, B.; Lingwood, C. A. *Chem. Biol.* **1996**, *3*, 263–275.
- (a) Shimizu, H.; Field, R. A.; Homans, S. W.; Donohue-Rolfe, A. *Biochemistry* **1998**, *37*, 11078–11082; (b) Thompson, G. S.; Shimizu, H.; Homans, S. W.; Donohue-Rolfe, A. *Biochemistry* **2000**, *39*, 13153–13156; (c) Shimizu, H.; Brown, J. M.; Homans, S. W.; Field, R. A. *Tetrahedron* **1998**, *54*, 9489–9506.
- Soltyk, A. M.; MacKenzie, C. R.; Wolski, V. M.; Hiram, T.; Kitov, P. I.; Bundle, D. R.; Brunton, J. L. *J. Biol. Chem.* **2002**, *277*, 5351–5359.
- Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000**, *403*, 669–672.
- For instance: (a) Lundquist, J. J.; Debenham, S. D.; Toone, E. J. *J. Org. Chem.* **2000**, *65*, 8245–8250; (b) Nishikawa, K.; Matsuoka, K.; Kita, E.; Okabe, N.; Mizuguchi, M.; Hino, K.; Miyazawa, S.; Yamasaki, C.; Aoki, J.; Takashima, S.; Yamakawa, Y.; Nishijima, M.; Terunuma, D.; Kuzuhara, H.; Natori, Y. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 7669–7674; (c) Watanabe, M.; Igai, K.; Matsuoka, K.; Miyagawa, A.; Watanabe, T.; Yanoshita, R.; Samejima, Y.; Terunuma, D.; Natori, Y.; Nishikawa, K. *Infect. Immun.* **2006**, *74*, 1984–1988; (d) Isobe, H.; Cho, K.; Solin, N.; Werz, D. B.; Seeburger, P. H.; Nakamura, E. *Org. Lett.* **2007**, *9*, 4611–4614; (e) Neri, P.; Tokoro, S.; Yokoyama, S.; Miura, T.; Murata, T.; Nishida, Y.; Kajimoto, T.; Tsujino, S.; Inazu, T.; Usui, T.; Mori, H. *Biol. Pharm. Bull.* **2007**, *30*, 1697–1701.
- For instance: (a) Armstrong, G. D.; Rowe, P. C.; Orrbine, E.; Klassen, T. P.; Wells, G.; Mackenzie, A.; Lior, H.; Blanchard, C.; Auclair, F.; Thompson, B.; Rafter, D. J.; McLaine, P. N. *J. Infect. Dis.* **1995**, *171*, 1042–1045; (b) Paton, A. W.; Morona, R.; Paton, J. C. *Nat. Med.* **2000**, *6*, 265–270; (c) Mulvey, G. L.; Marcato, P.; Kitov, P. I.; Sadowska, J.; Bundle, D. R.; Armstrong, G. D. *J. Infect. Dis.* **2003**, *187*, 640–649.
- Kitov, P. I.; Mulvey, G. L.; Griener, T. P.; Lipinski, T.; Solomon, D.; Paszkiewicz, E.; Jacobson, J. M.; Sadowska, J. M.; Suzuki, M.; Yamamura, K. I.; Armstrong, G. D.; Bundle, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 16837–16842.
- For instance: (a) Goldman, E. R.; Clapp, A. R.; Anderson, G. P.; Uyeda, H. T.; Mauro, J. M.; Medintz, I. L.; Mattoussi, H. *Anal. Chem.* **2004**, *76*, 684–688; (b) Uzawa, H.; Ito, H.; Neri, P.; Mori, H.; Nishida, Y. *ChemBioChem* **2007**, *8*, 2117–2124; (c) Chien, Y.-Y.; Jan, M. D.; Adak, A. K.; Tzeng, H. C.; Lin, Y. P.; Chen, Y. J.; Wang, K. T.; Chen, C. T.; Chen, C. C.; Lin, C. C. *ChemBioChem* **2008**, *9*, 1100–1109; (d) Kale, R. R.; McGannon, C. M.; Fuller-Schaefer, C.; Hatch, D. M.; Flagler, M. J.; Gamage, S. D.; Weiss, A. A.; Iyer, S. S. *Angew. Chem., Int. Ed.* **2008**, *47*, 1265–1268.
- Thioctic acid amide tethers for achieving low non-specific protein binding to gold glyconanoparticles. (a) Karamanska, R.; Mukhopadhyay, B.; Russell, D. A.; Field, R. A. *Chem. Commun.* **2005**, 3334–3336; Silver and gold glyconanoparticles for colorimetric bioassays. (b) Schofield, C. L.; Haines, A. H.; Field, R. A.; Russell, D. A. *Langmuir* **2006**, *22*, 6707–6711; Glyconanoparticles for the colorimetric detection of cholera toxin: (c) Schofield, C. L.; Field, R. A.; Russell, D. A. *Anal. Chem.* **2007**, *79*, 1356–1361; Detection of *Ricinus communis* agglutinin 120 using carbohydrate-stabilised gold nanoparticles. (d) Schofield, C. L.; Mukhopadhyay, B.; Hardy, S. M.; McDonnell, M. B.; Field, R. A.; Russell, D. A. *Analyst* **2008**, *133*, 626–634; *E. coli* detection using carbohydrate-functionalised CdS quantum dots. (e) Mukhopadhyay, B.; Martins, M. B.; Karamanska, R.; Russell, D. A.; Field, R. A. *Tetrahedron Lett.* **2009**, *50*, 886–889.
- For example, in the generation of carbohydrate microarrays via biotinylation (Karamanska, R.; Clarke, J.; Blixt, O.; MacRae, J. I.; Zhang, J. Q.; Crocker, P. R.; Laurent, N.; Wright, A.; Flitsch, S. L.; Russell, D. A.; Field, R. A. *Glycoconjugate J.* **2008**, *25*, 69–74) or via amide coupling to self-assembled monolayers (Zhi, Z.-L.; Laurent, N.; Powell, A. K.; Karamanska, R.; Fais, M.; Voglmeir, J.; Wright, A.; Blackburn, J. M.; Crocker, P. R.; Russell, D. A.; Flitsch, S. L.; Field, R. A.; Turnbull, J. E. *ChemBioChem* **2008**, *9*, 1568–1575).
- Reviewed in: (a) Fairbanks, A. J. *Synlett* **2003**, 1945–1958; (b) Cumpstey, I. *Carbohydr. Res.* **2008**, *343*, 1553–1573.
- Dan, A.; Ito, Y.; Ogawa, T. *J. Org. Chem.* **1995**, *60*, 4680–4681.
- Pozsgay, V.; Jennings, H. J. *Carbohydr. Res.* **1988**, *179*, 61–76.
- 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, CDCl₃): δ 1.90 (m, 2H, CH₂), 3.44 (t, 2H, J 6.7 Hz, spacer CH₂), 3.57 (t, 2H, J 6.0 Hz, spacer CH₂), 3.34 (s, 3H, CH₃O), 4.48 (s, 2H, ArCH₂O), 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, CDCl₃): δ 29.2 (CH₂CH₂CH₂), 48.5 (CH₂N₃), 55.2 (CH₃O), 66.5 (CH₂CH₂O), 72.7 (ArCH₂O), 113.7, 129.2, 130.2, 159.2 (aromatic C). Anal. Calcd for C₁₅H₁₁N₃O₂: 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 $\rightarrow$ 3)- α -D-galactopyranoside (**1**): [α]_D +137 (c 0.3, H₂O); R_f 0.53 (EtOAc/HOAc/MeOH/H₂O 5:3:3:2); δ_H (500 MHz, D₂O) 2.02 (m, 2H, spacer-CH₂), 3.02 (dd, 1H, $J_{1,2}$ 8 Hz, $J_{2,3}$ 10 Hz, H₂'), 3.56 (m, 2H, spacer-CH₂N₃), 3.66 (dd, 1H, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3 Hz, H₃'), 3.69 (m, 1H, spacer-CH₂O), 3.76 (dd, 1H, $J_{5,6a}$ 4 Hz, $J_{5,6b}$ 7 Hz, H₅'), 3.82–3.87 (m, 4H, H_{6a}, H_{6b}, H_{6'a}, H_{6'b}), 3.91 (m, 1H, spacer-CH₂O), 3.95 (m, 1H, H₄), 4.07 (m, 3H, H₂, H₃, H₅), 4.34 (m, 1H, H_{4'}), 4.61 (d, 1H, $J_{1,2}$ 8 Hz, H₁'), 5.05 (d, 1H, $J_{1,2}$ 4 Hz, H₁); δ_C (D₂O) 28.8 (CH₂CH₂CH₂), 49.0 (CH₂N₃), 54.0 (C_{2'}), 61.9 (2 $\times$ C₆, C_{6'}), 65.8 (CH₂CH₂O), 68.0 (C₂), 68.6 (C_{4'}), 70.0 (C₄), 71.4 (C₅), 73.5 (C_{3'}), 76.0 (C_{5'}), 80.5 (C₃), 99.1 (C₁), 105.9 (C_{1'}); ESI-MS calcd for C₁₅H₂₈N₄O₁₀ 424.18, m/z 447.17 [M+Na]⁺.
3-Azidopropyl (2-acetamido-2-deoxy- β -D-galactopyranosyl)-(1,3)- α -D-galactopyranoside (**2**): [α]_D +110 (c 0.18, H₂O); R_f 0.68 (EtOAc/HOAc/MeOH/H₂O 5:3:3:2); δ_H (500 MHz, D₂O) 2.01 (m, 2H, spacer-CH₂), 2.12 (s, 3H, NHAc), 3.55 (m, 2H, spacer-CH₂N₃), 3.67 (m, 1H, spacer-CH₂O), 3.75 (dd, 1H, $J_{5,6a}$ 4 Hz, $J_{5,6b}$ 8 Hz, H₅'), 3.80–3.86 (m, 5H, H_{3'}, H_{6a}, H_{6b}, H_{6'a}, H_{6'b}), 3.89 (m, 1H, spacer-CH₂O), 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, H_{2'}, H_{4'}), 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 (D₂O) 23.1 (CH₃CON), 28.8 (CH₂CH₂CH₂), 49.1 (CH₂N₃), 53.5 (C_{2'}), 61.9 and 62.0 (C₆, C_{6'}), 65.9 (CH₂CH₂O), 68.2 (C₂), 68.6 (C_{4'}), 70.1 (C₄), 71.4 (C₅), 71.7 (C_{3'}), 75.8 (C_{5'}), 79.9 (C₃), 99.4 (C₁), 104.1 (C_{1'}), 176.1 (CH₃CON); ESI-MS calcd for C₁₇H₃₀N₄O₁₁ 466.19, m/z 489.2 [M+Na]⁺.
19. Hasegawa, A.; Nagahama, T.; Ohki, H.; Kiso, M. *J. Carbohydr. Chem.* **1992**, *11*, 699–714.
20. Kanie, O.; Crawley, S. C.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *243*, 139–164.