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A sugar aminoacid for the development of multivalent ligands for *Escherichia coli* 0157 verotoxin

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Dedicated to Professor George Fleet on the occasion of his 65th birthday

ABSTRACT

Functionalised carbohydrates for incorporation into multivalent ligands for the *Escherichia coli* 0157 verotoxin are highly desirable. Here, we report the synthesis of a sugar aminoacid based on the known Gal-α-1,4-Gal ligand for verotoxin in eight steps and in ~10% overall yield.

1. Introduction

Escherichia coli O157, which periodically causes outbreaks of potentially lethal diarrhoea, presents a problem for human health worldwide.[1] This organism produces so-called verotoxins (or shiga-like toxins), which are members of the AB_5 class of bacterial toxins, along with cholera and pertussis toxins.[2,3] Verotoxins bind to cell surface globotriaosylceramide epitopes, resulting in toxin uptake by the cell, followed by the catalytically active A-subunit cleaving a single nucleotide base from ribosomal RNA, and hence shutting down protein synthesis.[4] It is now well-established that the most effective means of neutralising the effect of verotoxin is by preventing its interaction with host cells with multivalent carbohydrate-based ligands.[5] Building on our work on the development of carbohydrate-based sensors and imaging agents for protein toxins and bacteria,[6] and extending our interest in verotoxin ligands,[7] we sought novel ligand components suitable for oligomerisation into potential verotoxin inhibitors. Sugar aminoacids present attractive building blocks in this regard, given their potential for conjugation to give multivalent constructs and the ease with which they can be derivatised for ligand optimisation.

α-C-linked galactose, even in multivalent format, does not give rise to potent verotoxin ligands.[8] In contrast, a Gb_3 trisaccharyl C-linked amino acid, and peptides derived therefrom, shows good inhibition of verotoxin, but ligand synthesis is lengthy.[9] Whilst verotoxin binding by the Gal-α-1,4-Gal disaccharide is appreciably weaker than that by Gb_3 trisaccharide,[10] it can still serve as an effective template for inhibitor development.[11] In the Gal-α-1,4-Gal series, amino-[12] and carboxy-derivatives[11,12] have been reported. Importantly, Kitov and Bundle have shown that, with respect to the parent disaccharide, substitution in either the 2- or 6-position of Gal-α-1,4-Gal does not significantly reduce affinity for verotoxin.[11] Herein, we report the synthesis of a Gal-α-1,4-Gal aminoacid with amino substitution at C-2 and a carboxymethyl group at C-6 of the reducing terminal sugar unit (Fig. 1). Our synthetic approach was to first prepare the difunctionalised reducing terminal sugar unit, and then glycosylate to install the inter-sugar α-1,4-glycosidic linkage.

2. Results and discussion

Commercial methyl β-D-galactoside, by way of the 3,4,6-O-acetalated intermediate, was protected as its 3,4-O-acetonide **2** in 93% yield by reaction with 2,2-dimethoxypropane and *p*-toluenesulfonic acid, followed by careful selective cleavage of the mixed sugar-(2-methoxy)propyl acetal at C-6 with 50% aq TFA (Scheme 1). Mono-O-tosylation of the primary alcohol followed by treatment with sodium azide in DMF gave azide **3**.[13] Subsequent alkylation with sodium hydride and methyl bromoacetate then gave azido-ester **4** in 86% yield. Deprotection of the acetal in **4** was initially attempted with 80% aq TFA, but this gave a mixture of products, including those resulting from ester hydrolysis. A further attempt at acetal removal with 50% aq HBF_4 in methanol gave the desired methyl ester **5** in 90% yield.[14]

Numerous attempts were made to directly cyclise ester **5** to the corresponding 2,3-lactone, **6**, but this was largely unsuccessful. Ester hydrolysis followed by diimide-based cyclisation again resulted only in low yields (<20%) or in the desired lactone **6**.[13] In addition, attempts to selectively acetylate or benzoylate the equatorial 3-OH of 3,4-diol **5** typically resulted in a mixture of 3-mono- and 3,4-di-O-acylated material. The change of oxygen to azide functionality at

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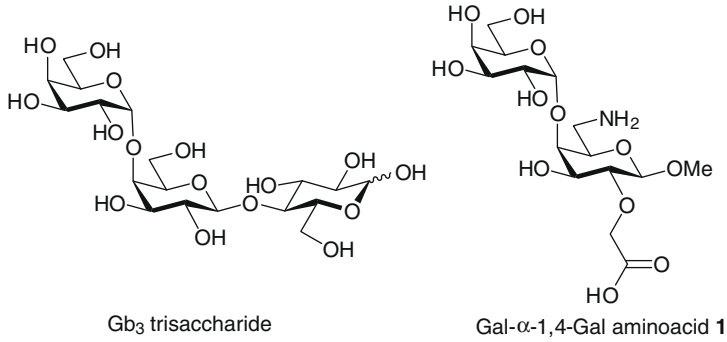
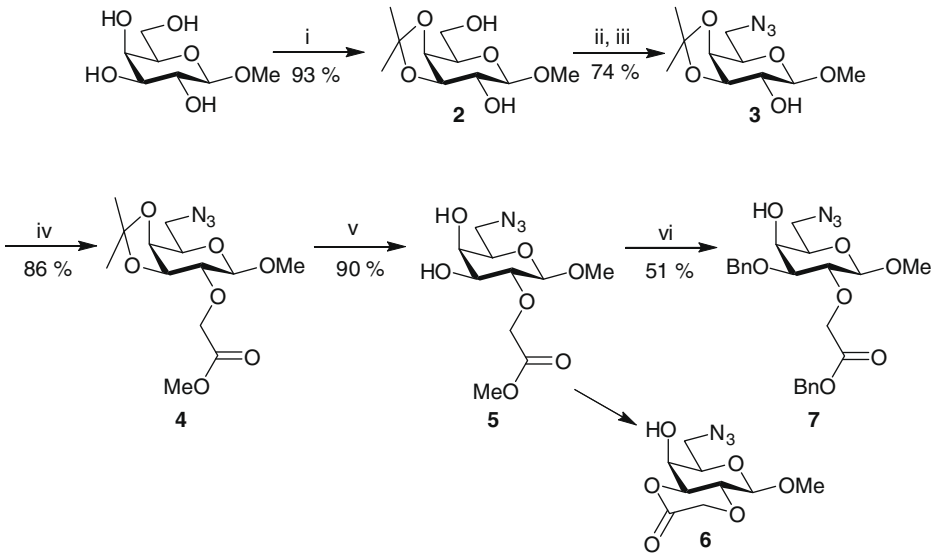
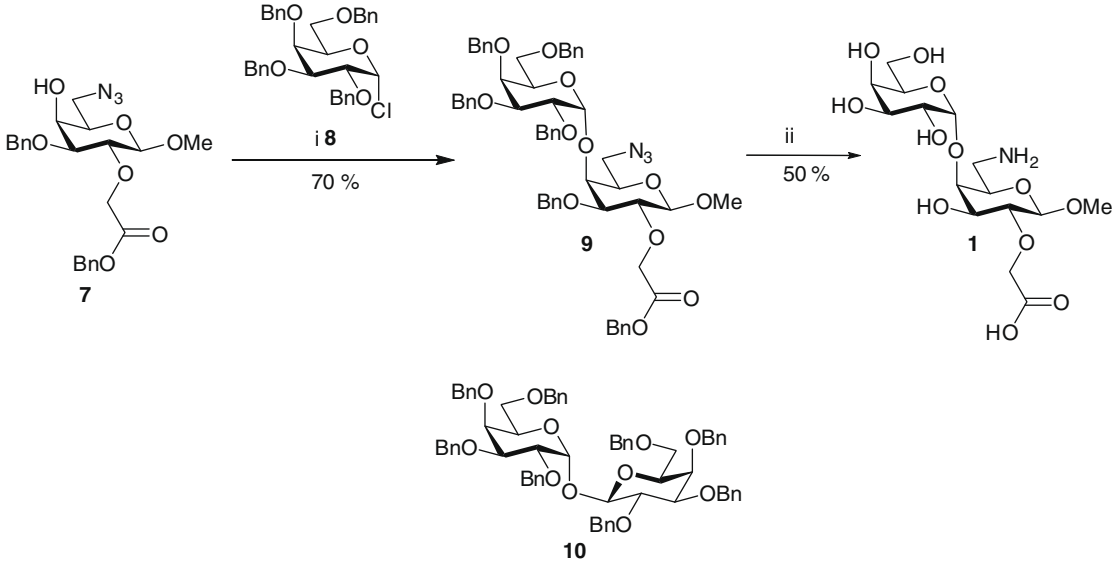


Figure 1. Natural Gb_3 trisaccharide and target sugar aminoacid **1**.



Scheme 1. Reagents and conditions: (i) (a) *p*-TsOH, $(CH_3)_2C(OCH_3)_2$; (b) (i) 50% TFA (aq), DCM; (ii) *p*-TsCl, $(CH_3)_2CO$, pyridine; (iii) NaN_3, DMF; (iv) methyl bromoacetate, NaH, DMF; (v) 50% HBF_4 (aq), MeOH; (vi) (a) MeOH, Bu_2SnO; (b) toluene, BnBr, Bu_4NBr.

C-6 appears to reduce 3/4-O-acylation selectivity. Tin acetal chemistry was therefore considered.[15] Reaction of diol **5** with dibutyltin oxide in refluxing methanol and subsequent addition of 5 mol equiv of benzyl bromide and 0.5 mol equiv of tetrabutylammonium bromide gave selective 3-O-benzylation resulting in **7** in moderate yield. A small sample of the benzyl ether was acetylated



Scheme 2. Reagents and conditions: (i) toluene/diethyl ether (2:1), AgOTf, collidine, 4 Å MS, −78 C; (ii) EtOH, AcOH, H_2, Pd-C.

to confirm the regiocontrol of the benzylation [^{1}H NMR data showed a downfield shift for H-4 upon acetylation (3.79–5.36 ppm)]. However, on closer inspection it became apparent that the 3-O-benzylated product **6** was in fact the benzyl ester, the methyl ester functionality having been transformed during the benzylation process. Presumably, residual moisture in conjunction with the tin reagent contributed to methyl ester hydrolysis in situ, followed by carboxyl group benzylation, giving **6**.

Glycosylation of the sugar aminoacid **7** with 1.5 mol equiv of galactosyl chloride **8**[16] was effected with silver triflate and collidine in anhydrous toluene/diethyl ether[17] to promote glycosylation α-selectivity (Scheme 2). The reaction gave the desired α-linked glycoside, **9**, in 70% isolated yield, accompanied by the formation of 1,1′-linked disaccharide **10** (20%),[18] presumably arising from donor quenching and coupling with further donor. Altering the donor:acceptor stoichiometry did not impact productively on the yield of the desired glycoside **9**. Final reductive deprotection of azido-ester **9** proceeded in moderate yield, reflecting issues associated with partial removal and/or reduction of benzyl ether-protecting groups.

3. Conclusion

In conclusion, the synthesis of di-functionalised, Gal-α-1,4-Gal aminoacid **1**[20] from methyl β-D-galactoside was successfully achieved in eight steps and in ~10% overall yield. Studies exploring the use of this reagent in verotoxin inhibitor development will be reported in due course.

Acknowledgements

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13. All synthetic intermediates gave NMR data and combustion analysis or high-resolution mass spectrometry data consistent with their proposed structures. *Selected data for azide* **3**: $[\alpha]_D = -16.0$ (*c* 0.95, $CHCl_3$); ν_{max}/cm^{-1} 2097 (N_3); δ_H ($CDCl_3$, 400 MHz): 1.36 (3H, s, CH_3), 1.48 (3H,s, CH_3),2.85 (1H, br s, OH), 3.39 (1H, dd, $J_{5,6/6'}$ 5, $J_{6,6'}$ 13 Hz, H-6/6′) 3.49 (1H, dd, $J_{1,2}$ 8.1, $J_{2,3}$ 8.4 Hz, H-2), 3.51 (3H, s, OMe), 3.69 (1H, dd, $J_{5,6/6'}$, $J_{6,6'}$, H-6/6′), 3.90 (1H, m H-5), 4.01–4.08 (2H, m, H-3, 4), 4.08 (1H, d, $J_{1,2,}$ H-1); δ_C ($CDCl_3$, 100 MHz): 26.2, 27.9, 51.1, 57.0, 72.9, 73.5, 73.8, 78.9, 103.3, 110.5.
Selected data for lactone **6**: $[\alpha]_D = -15.9$ (*c* 0.36, $CHCl_3$); ν_{max}/cm^{-1} 2100 (N_3); δ_H ($CDCl_3$, 400 MHz): 3.35 (1H, dd, $J_{5,6/6'}$ 2, $J_{6,6'}$ 10 Hz, H-6/6′), 3.60 (3H, s, OMe), 3.74 (1H, m, $J_{5,6/6'}$, H-5), 3.75 (2H, m, H-2, 6/6′), 4.07 (1H, dd, $J_{3,4}$ 3 Hz, H-4), 4.38 (1H, dd, $J_{2,3}$ 7 Hz, $J_{3,4,}$ H-3), 4.42 (1H, d, $J_{1,2}$ 8 Hz, H-1), 4.43 (1H, d, *J* 18.0 Hz, OCH_2COOMe) 4.62 (1H, d, *J* 18.0 Hz, OCH_2COOMe); δ_C ($CDCl_3$, 100 MHz): 50.7, 57.1, 66.2, 67.1, 71.5, 74.0, 79.7, 101.3, 165.8.
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18. Deprotection of disaccharide **10** using catalytic hydrogenolysis gave the known unprotected 1,1′-linked disaccharide, the α/β-stereochemistry of which was confirmed by ^{1}H NMR spectroscopy. Selected data: $[\alpha]_D$ +58 (*c* 0.22, H_2O), (lit.,[19] +56); ^{1}H NMR: δ_H 4.20 ($J_{1a,2a}$ 7.8, H-1a), 5.15 ($J_{1b,2b}$ 3.0, H-1b).
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20. *Selected data for protected penultimate and final compounds*: Methyl 2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl-(1,4)-3-O-benzyl-6-azido-2-benzylacetate-6-deoxy-β-D-galactopyranoside **9**: $[\alpha]_D = +22.7$ (*c* 0.99, $CHCl_3$); ν_{max}/cm^{-1} 1757 (*C*OOBn), 2100 (N_3); δ_H($CDCl_3$): 3.18 (1H, dd, $J_{5a,6a/6a'}$ 4.5, $J_{6a,6a'}$ 8.1, H-6a/6a′), 3.32 (1H, t, $J_{5b,6b/6b'}$5.1, $J_{6b,6b'}$5.1, H-6b/6b′), 3.48 (3H, s, OMe), 3.42–3.47 (2H, m, H-3a + H-5a), 3.59 (2H, m, H-2a + H-6b/6b′), 3.68 (1H, dd, $J_{5a,6a/6a'}$, $J_{6a,6a'}$, H-6a/6a′), 3.75 (1H, d, $J_{3a,4a}$, 3.5, H-4a), 3.99–4.01 (3H, m, H-2b, 3b + 4b), 4.20–4.25 (2H, dd, O*C*H_2Ar), 4.25 (1H, d, $J_{1a,2a}$, 8.0, H-1a), 4.35 (3H, m, H-5b + O*C*H_2), 4.54–4.80 (4H, m, 2 × O*C*H_2Ar), 4.86–4.94 (2H, m, O*C*H_2Ar), 4.94 (1H, d, $J_{1b,2b}$ 3.3, H-1b), 5.15 (2H, dd, *J* 9.3 + 12.6, O*C*H_2), 7.04–7.36 (30H, m, 6 × OCH$_2$*Ar*); δ_C($CDCl_3$): 50.9, 56.9 (OMe), 66.4, 68.2, 69.8, 70.25, 72.2, 73.0, 73.3, 74.4, 74.7, 74.8, 74.9, 76.4, 77.8, 78.9, 79.6, 80.7, 100.8 (C-1b), 104.3 (C-1a), 127.6, 127.7, 127.8, 127.9, 128.2, 128.2, 128.3, 128.5, 128.7, 135.8, 138.3, 138.5, 138.7, 138.8, 139.1, 170.3.
Gal-α-1,4-Gal aminoacid **1**: characteristic NMR data—δ_H (CD_3OD, 400 MHz): 3.51 (3H, s, OMe), 4.31 (2H, m, O*C*H_2), 4.35 (1H, d, $J_{1,2}$ 7.6 Hz, H-1), 5.01 (1H, d, $J_{1',2'}$ 3.4 Hz, H-1′); δ_C (CD_3OD, 100 MHz): 101.7 (C-1′), 104.7 (C-1).