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Synthesis of novel trivalent α-*C*-mannosides bearing different end-functionalized tethers is described.

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A synthesis of *C*-glycosidic multivalent mannosides suitable for divergent functionalized conjugation

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ABSTRACT

Divergent syntheses of two novel C-glycosidic multivalent mannosides derived from a common trivalent C-mannosyl carboxylate-terminated intermediate are described. This illustrates synthesis of multivalent C-glycosidic architectures bearing variable extended functionalized tethers. One such tether incorporates an embedded fluorescent unit providing a C-mannosyl multivalent epitope offering potential applications though further conjugation or immobilization.

1. Introduction

There are a large number of essential biological processes that rely on carbohydrate-mediated recognition events at the cell surface. Numerous examples of such events include host cell recognition and cancer-mediated processes[1-5] and many of these processes are known to involve the presentation of multivalent carbohydrate species, greatly enhancing overall ligand-receptor affinities through cooperative binding. As a result of this there is a need for robust and efficient syntheses of multivalent carbohydrate architectures as a means to provide useful tools for studying cluster-glycoside-mediated biological recognition processes. This is a rapidly expanding area of glycobiology and there are many examples of different synthetic approaches to[6-13] and applications of multivalent carbohydrates. There is specific importance in synthetic access to new, diverse multivalent carbohydrates with useful functional tethers, providing reagents for conjugation to labelling agents, immobilization and array applications.[14-18] *C*-Glycosidic multivalent saccharides provide the potential to generate libraries of stereochemically-defined multivalent core sections, which can be adapted for a diversity of targets through modular syntheses and which are hydrolytically and enzymatically stable. There are few examples of multivalent *C*-glycosides, with examples of oxazole linked tri- and tetravalent systems[19a] on a pentaerythritol core, systems based on calixarene scaffolds,[19b] and peptide-spacer linked examples.[19c]

We have previously reported the synthesis of a novel tris-*C*-mannoside carboxylate intermediate **1** and its elaboration by attachment of a pyrenyl fluorophore.[20] This letter describes a divergent and modular approach to creating end-functionalized multivalent ligands through attachment of different functionalized groups to **1** (Figure 1).

Specifically, we report elaboration of trivalent-*C*-mannoside **1** with a novel thio-terminated alkyl ether and also a bifunctional fluorophore, suitable for further conjugations and/or immobilizations exploiting an embedded fluorescent reporter group.

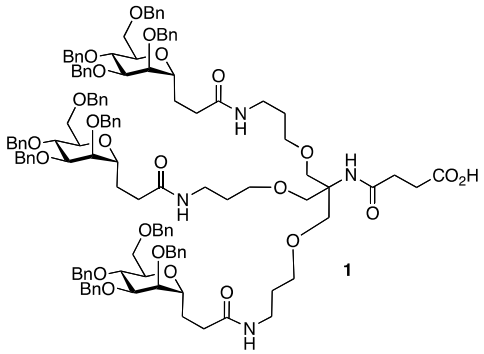

Figure 1. Multivalent *C*-mannosyl carboxylate **1**

2. Results and discussion

2.1. Attachment of an extended thio-tether to core trivalent *C*-mannoside 1

Free thiols and thioethers are well established as suitable functional groups for the immobilisation and study of biomolecules and ligands, including interesting multivalent carbohydrate architectures.[21-23] There have been a growing

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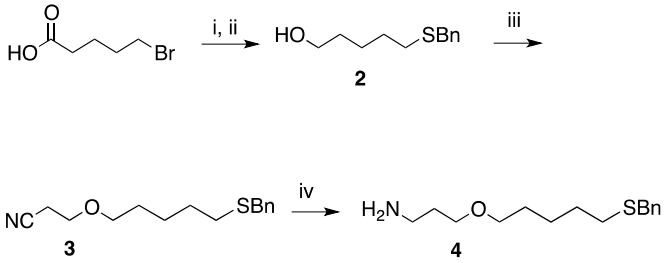
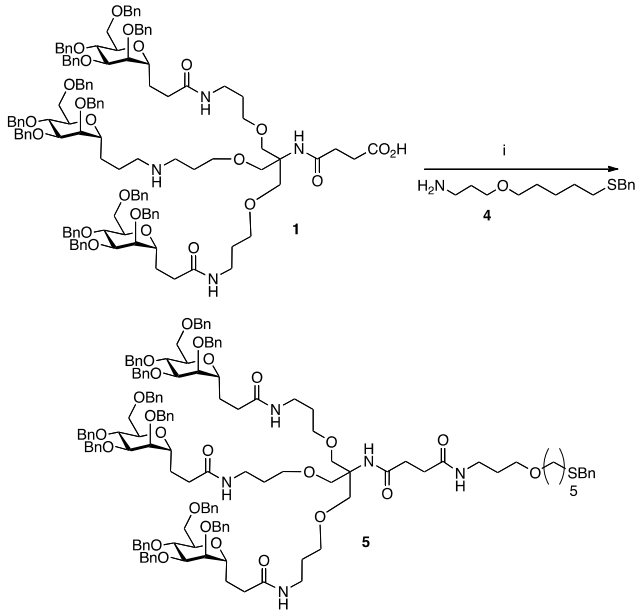
number of examples of multivalent carbohydrate ligand immobilization using SPR arrays, and several valuable reports of attachment to Quantum dots[24] or gold nanoparticles.[25] Thus we sought to illustrate that trivalent *C*-mannoside **1** could be elaborated with an extended tether bearing a terminal sulfur functionality. We sought to demonstrate this using a novel extended thiol-terminated unit with lipid-like characteristics (Scheme 1).



Scheme 1. Reagents and conditions: (i) BnSH, NaOH, EtOH, 40 °C, 2 d, 69% (ii) BH_3.THF, THF, RT, 5 h, 96% (iii) CH_2=CHCN, NaH, dioxane, RT, 18 h, 89% (iv) $BH_3.Me_2S$, THF, reflux, 2 h, 98%.

Thus, *S*-alkylation of 5-bromovaleric acid with benzyl mercaptan gave the desired thioether[26] and subsequent reduction of the carboxylic acid using BH_3.THF successfully delivered alcohol **2**.[27] This material was then extended through *O*-alkylation with acrylonitrile to furnish novel nitrile **3**. Finally, reduction of **3** with $BH_3.SMe_2$ gave novel amine **4** in excellent yield, suitable for coupling with multivalent carboxylate **1**. Coupling of amine **4** to acid **1** efficiently afforded the target trivalent *C*-mannoside **5**,[28] bearing the novel thio-tether end group (Scheme 2).

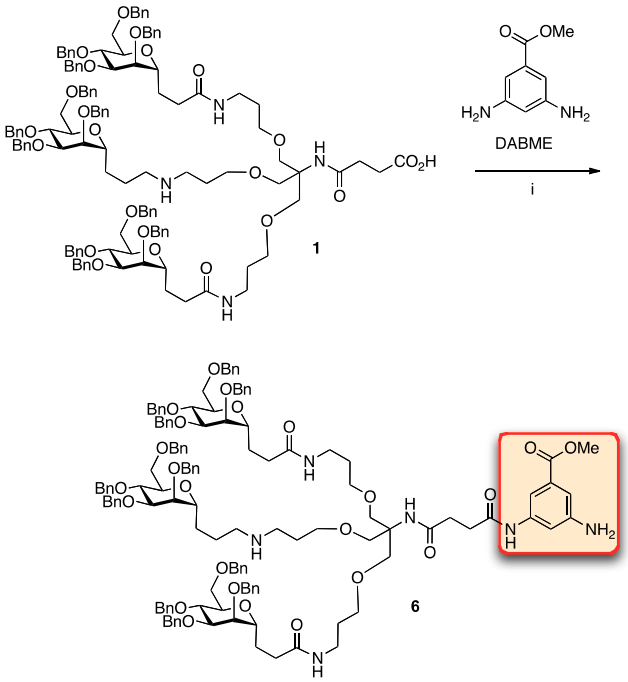


Scheme 2. Reagents and conditions: (i) TBTU, HOBt, DIPEA, DMF, RT, 24 h, 68%.

2.2 Attachment of a bifunctional fluorescent labelling tether

The second type of multivalent architecture reported incorporates a tether which contains an embedded fluorescent tag which has two orthogonal functional groups. The interest here lay in delivering both the capability for a further divergence to heterogeneous multivalent targets, but also for embedding a fluorescent label in the linker, whilst retaining reactivity for further conjugations without any further functional group manipulations. Recently, the utility of simpler monovalent *N*-glycans bearing an AEAB-derived unit within a linker has been illustrated in applications to generating glycan arrays with embedded fluorophores.[18]

Thus, coupling of 3,5-diaminobenzoic acid methyl ester (DABME) with trivalent **1** demonstrated that mono-amidation could be intercepted (anticipated from the predicted slower second amidation with a second large dendron) thus affording the target mono-amide derivative **6** (Scheme 3).[29]



Scheme 3. Reagents and conditions: (i) TBTU, HOBt, DIPEA, DMF, RT, 24 h, 44%.

Compound **6** exhibited fluorescence at 380 nm (with excitation at 241 nm, Figure 2), thus confirming this novel multivalent *C*-glycoside ligand has an effective embedded fluorescent motif. A terminal anilino function is widely employed for array synthesis/immobilization or conjugations and thus **6** is well suited as a new tool for such applications. Additionally, this material could be used further to create more elaborate multivalent compounds through manipulation of the amino and ester functionalities with suitable pre-formed multivalent constructs. For example, the amino group of **6** would be suitable for coupling to different carboxylate terminated multivalent saccharide modules to provide heterogeneous higher-valent structures. The aryl ester would also remain available for attaching a handle for immobilization once the amino group were employed for conjugation, providing potential for double reported group attachment. Thus, compound **6** offers potential access to novel diversity for applications to arrays of higher valent, fluorescently labelled *C*-glycosidic ligands.

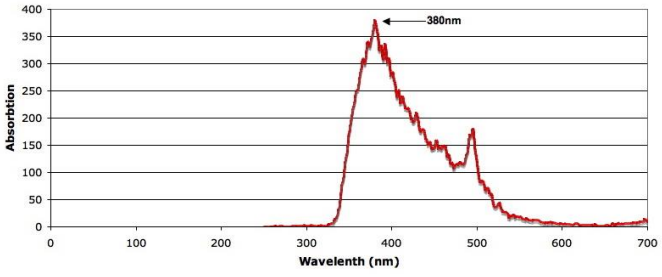
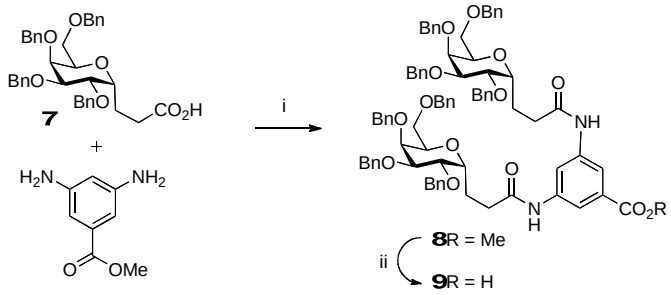


Figure 2: Fluorescence spectrum for trivalent C-mannoside **6**.

2.4 Addressing individual saccharide valency

Having demonstrated that DABME could be singly conjugated with a larger trivalent mannoside and in order to investigate the assembly of more complex/higher valency multivalent architectures, we undertook the synthesis of bivalent *C*-glycosidic derivative **9** through amidation of DABME with a smaller, monovalent *C*-glycoside unit (Scheme 4). In this case we employed the known *C*-galactoside monomer **7**,[30] which was efficiently di-coupled with DABME to furnish **8** and following



saponification, yielded the desired acid **9**.

Scheme 4. Reagents and conditions: (i) TBTU, HOBt, DIPEA, DMF, RT, 16 h, 57% (ii) NaOH, THF/H_2O, RT, 3 h, 72%.

3. Conclusion

We report the synthesis of two novel multivalent *C*-glycosidic mannosyl ligands, **5** and **6**, whose differing functional tethers facilitate future utilisation as probes to investigate multivalent carbohydrate interactions. Inclusion of an embedded fluorescent label combined with bidirectional further functionalization options provides a valuable and versatile tool to explore the potential of novel *C*-glycosidic ligands.

Acknowledgments

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28. Selected data for **5**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.33-7.18 (m, 65H, Ar CH), 7.04 (s, 1H, NH), 6.60 (s, 1H, NH), 6.35 (t, *J* = 5.4, 3H, NH), 4.71 (d, *J* = 11.4, 3H, OCH_2Ph), 4.63-4.47 (m, 21H, OCH_2Ph), 3.98 (td, *J* = 9.6, 4.7, 3H, H1 sugar), 3.79-3.60 (m, 26H, sugar ring + CH_2O + $PhCH_2S$), 3.39-3.20 [m, 16H, OCH_2 core, $CH_2NHC(O)$, 2 x CH_2O tether], 2.97-3.04 [m, 2H, $CH_2NHC(O)$ tether], 2.57-2.38 [m, 6H, 2 x $CH_2C(O)NH$ + $BnSCH_2$], 2.32-2.20 [m, 6H, $CH_2C(O)NH$], 1.82-1.87 (m, 6H, CH_2 alkyl), 1.76-1.46 (m, 14H, CH_2 alkyl); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.1 (C=O amide), 172.8 (2 x C=O amide), 138.6-138.5 (4° Ar C), 130.4-127.3 (Ar CH), 77.6 (CH sugar), 76.4 (CH sugar), 75.3 (CH sugar), 74.3 (CH_2), 73.6 (CH sugar), 73.5 (CH_2), 72.5 (CH_2), 72.4 (CH sugar), 72.1 (CH_2), 71.2 (CH_2 tether), 70.0 (CH_2), 69.5 (CH_2 core), 69.3 (CH_2, tether), 69.1 (CH_2 core), 60.1 (4° C core), 40.5 [$CH_2NHC(O)$], 37.0 [$CH_2NHC(O)$], 36.7 ($PhCH_2S$), 32.9 [$CH_2C(O)$], 32.6 (CH_2 alkyl), 31.6 ($BnSCH_2$), 30.1 [$CH_2C(O)$], 30.1 [$CH_2C(O)$], 29.7 [$CH_2CH_2C(O)NH$], 29.7 (CH_2 alkyl), 29.4 (CH_2 alkyl), 25.9 (CH_2 alkyl), 25.9 (CH_2 alkyl); MS *m/z* 2400 (MNa^+, 100%); IR (neat) υ_{max} 3433, 1654, 1456, 1269, 1102 cm^{-1}; Specific Rotation $[\alpha]^{26}$ = + 28.3 (c 0.5, $CHCl_3$).
29. Selected data for **6**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.53-7.18 (m, 63H, Ar CH), 6.97 (s, 1H, NH), 6.52 (s, 1H, NH), 6.31 (t, *J* = 5.7, 3H, NH), 4.73-4.45 (m, 24H, OCH_2Ph), 3.95-4.01 (m, 3H, H1 sugar), 3.79-3.61 (m, 24H, sugar ring, CH_2O, OCH_3), 3.37-3.34 (t, *J* = 5.6, 6H, OCH_2), 3.23-3.21 [m, 6H, $CH_2NHC(O)$], 2.65-2.48 [m, 4H, 2 x $CH_2C(O)NH$], 2.32-2.24 [m, 6H, $CH_2C(O)NH$], 1.91-1.85 (m, 6H, CH_2 alkyl), 1.61-1.57 (m, 6H, CH_2 alkyl); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.2 (C=O amide), 172.9 (2 x C=O amide), 171.4 (C=O ester), 140.2 (Ar CH), 138.6-138.5 (4° Ar C), 128.7-128.1 (Ar CH), 77.6 (CH sugar), 76.6 (CH sugar), 75.4 (CH sugar), 74.2 (CH_2), 73.6 (CH sugar), 73.6 (CH_2), 72.6 (CH_2), 72.5 (CH sugar), 72.2 (CH_2), 70.2 (CH_2), 69.5 (CH_2 core), 69.2 (CH_2 core), 60.0 (4° C core), 52.3 (OCH_3), 37.2 [$CH_2NHC(O)$], 33.0 [$CH_2C(O)$], 30.1 [$CH_2C(O)$], 30.1 [$CH_2C(O)$], 29.8 [$CH_2CH_2C(O)NH$], 25.9 (CH_2 alkyl core); MS *m/z* 2299 (MNa^+, 100%), 2178 (40%); IR (neat) υ_{max} 3338, 3089, 3062, 3027, 2929, 2863, 1723, 1645, 1548, 1450, 1365, 1217, 1096 cm^{-1}; Specific Rotation $[\alpha]^{26}$ = + 37.7 (c 0.3, $CHCl_3$).
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Supplementary Information

Experimental details for the synthesis of compounds **3-6, 8** and **9**, details of UV and fluorescence experiments for compound **6** and copies of ^{1}H and ^{13}C NMR spectra for compounds **3-6, 8** and **9** and MS spectra for compounds **5, 6, 8** and **9**, and COSY, NOESY, TOCSY spectra of the *C*-allyl precursor of **7**, are available as supporting information.