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Adaptable Synthesis of *C*-Glycosidic Multivalent Carbohydrates and Succinamide-Linked Derivatization

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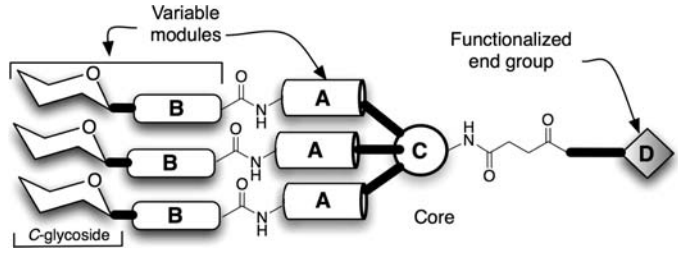
ABSTRACT



A modular approach to the synthesis of trivalent *C*-glycosidic carbohydrates is described. The approach is illustrated employing carboxylate-terminated *C*-glycosidic D-mannose, D-glucose, and D-galactose derivatives with different length C1-linked spacer units and also core units with different length linker units attached. The central core scaffold is additionally functionalized via a succinamide-based, conjugatable linker unit, exemplified in an extended multivalent derivative [31] and a pyrene-bearing fluorsecent-labeled tris-*C*-mannosyl conjugate [33].

Many biological recognition processes involve key binding roles for carbohydrate structures, specifically many cell surface processes, including host cell recognition events (e.g., embryogenesis), various cancer-related processes (e.g., metastasis, mediation of angiogenesis), and many pathogen attachment processes.[1] Understanding carbohydrate-mediated interactions remains at the forefront of chemical biology, with requirements to both probe and model interactions, develop methods for evaluation, and establish new entries to synthetic entities as ligand analogues. Carbohydrate-related mimetics offer significant potential for development of new therapeutic agents.

Many of these interactions are now understood to involve multivalent processes, providing much higher selectivity and affinity through cooperative binding than the relatively low affinities of monovalent carbohydrate ligands. Thus, new methods to generate synthetic multivalent systems in a controlled and variable manner are of importance.

A range of synthetic architectures have been reported to evaluate and explore multivalency in carbohydrate-based recognition events. These have included various oligomeric linear templates,[2] globular/dendritic systems,[3] and cyclic scaffolds.[4] There have been a number of examples of high

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efficacy ligand analogues, for example, with enhanced affinity for bacterial toxins[3b] or cancer-associated cell-surface receptors.[5] In only a few cases is a structural knowledge of the basis for multivalent interaction available, and thus most effective synthetic ligands have been identified through screening numbers of ligand types or serendipity.

It is also evident from several studies that the mode of multivalent binding can vary between ligands/ligand types and that higher-order organization can play a role in the aggregation of ligands at surfaces. Advances in surface immobilization of synthetic multivalent carbohydrates have allowed the cluster glycoside effect to be further investigated.[6] This has recently begun to be exploited in the connection of multivalent ligands to nanoparticles and quantum dots.[7] It is thus important to have syntheses of multivalent architectures amenable to practicable diversity but which also, ideally, are designed to enable surface immobilization and other conjugation.

We report here a synthetic approach to *C*-glycosidic multisugar-bearing ligands designed to enable diversification by variation of several modular components to deliver a virtual matrix of diverse carbohydrate ligand arrays. Figure 1 shows a generic model structure. The A and B modules

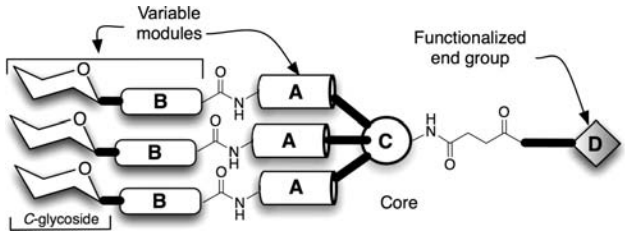


Figure 1. Generic multivalent *C*-glycosidic ligand representation.

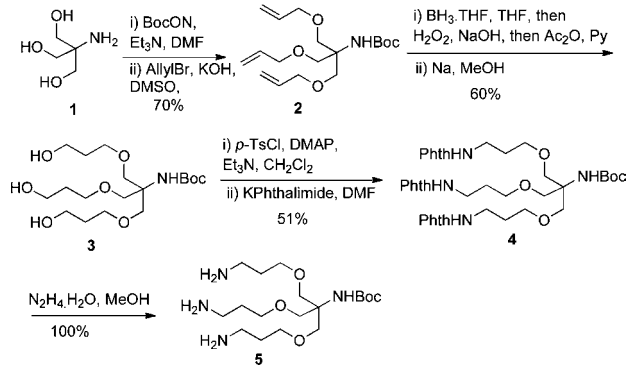
are independently variable to enable changes in spatial array and/or changes in ligand rigidity (and thus an A × B matrix). The C unit is a common trisalkyl core, and an alkyl/polyether/amido "tail" unit is linked from the core nitrogen via a succinimidyl coupling to introduce terminus D to allow direct surface attachment or attachment of other motifs for immobilization studies or conjugations. This end linker module also has highly variable structural options.

C-Glycosidic attachment provides anomerically stable ligands but also provides saccharide-type independent chemistry for attachment to the core, obviating any anomeric stereochemical variability from the multivalent coupling step. Different anomerically pure *C*-glycosides can be prepared separately, and employing conserved attachment chemistry for any saccharides also circumvents any significant differences in reactivity or selectivity between different saccharides (in contrast to *O*-glycosylation attachments).

This letter reports the synthesis of the scaffold/core units, *C*-glycosidic components, and the combination of units to provide variable multivalent systems.

The core scaffolds for this study were prepared from tris(hydroxymethyl)aminomethane **1**. To illustrate modularity, we demonstrate the synthesis of a minimal core type derived from **1** and of an ethylene diamine extended core and their respective coupling to different *C*-glycoside modules with varying length spacers preattached. The first truncated core target was trisamine **5**, prepared through N-protection of **1** and allylation of the hydroxyl units[8] (Scheme 1). Subsequent hydroboration/oxidation of the

Scheme 1. Synthesis of the Tris(aminopropyl) Core



terminal alkenes (with in situ acetylation−deacetylation) provided triol **3**.

Triol **3** was then converted to phthalimide derivative **4** with subsequent hydrazinolysis of this material furnishing target **5**.[9] Unfortunately, purification of this material was problematic (due to a hydrazinolysis by-product), and thus an alternative approach was sought. This objective was achieved by proceeding via trisnitrile intermediate **6**[10] obtained from triol **1** in high yield, through O-selective Michael addition to acrylonitrile.

Since the amino group needed protection and would need further elaboration with linker units for diversification (i.e., linking to terminus **D**, Figure 1), the amine was protected using a motif that addressed both requirements simultaneously. Thus, reaction with succinic anhydride followed by esterification afforded amide **7**. The strategy envisaged amide coupling of the core to *C*-glycosides bearing extended carboxylates, thus the nitriles were converted to amines. Direct conversion to the parent, shorter trisamine **9** was

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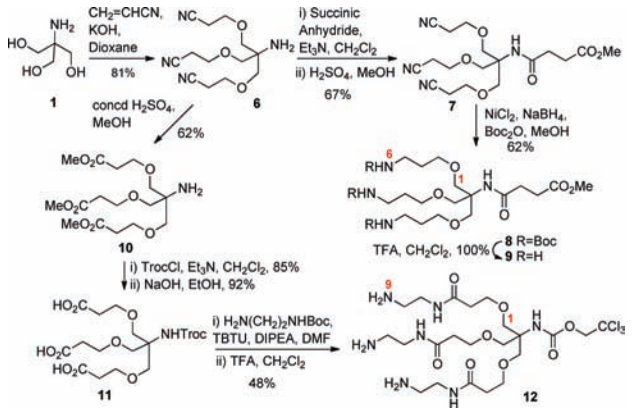
achieved using nickel boride reduction of trisnitrile **7**, and tris-Boc protected derivative **8**[11] was formed in situ (facilitating purification) and then deprotected to provide pure trisamine **9**.

The second type of trisamine core unit (with an extended linker incorporated in the three branches) was prepared starting from the same trisnitrile **6**, but effecting hydrolysis and esterification of the nitrile groups to give **10**, followed by amine protection and saponification to deliver **11** (Scheme 2). The carboxylates were then used to extend the linker arms

Scheme 2. Synthesis of the Amino-Extended Core

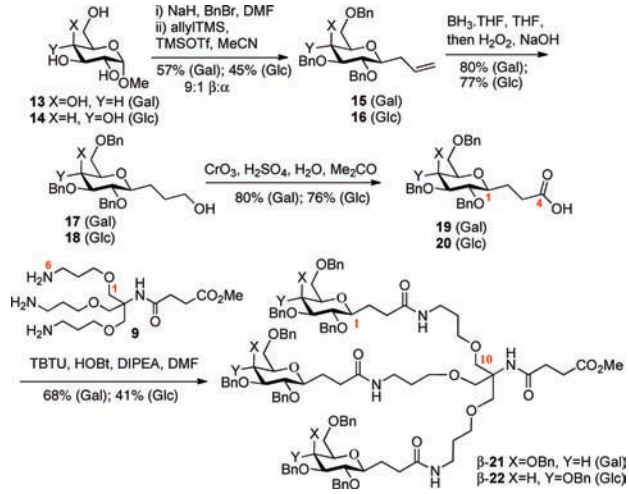


by amidation. This enables extension using an array of differentially protected diamino spacers. In this work, trisacid **11** was coupled with mono-Boc protected ethylenediamine, and the Boc groups were removed, providing an extended trisamine system **12**. This extended the core component of the A unit (Figure 1) by three atoms but retained the same attachment chemistry as employed with the shorter parent example **9**, to allow for addition of any type of *C*-glycosidic sugar carboxylate. These syntheses thus illustrate entry into two scaffold sections containing 6- and 9-atom branches, respectively. Each is terminated with an amine group, facilitating a modular access to multivalent systems though one common coupling process.

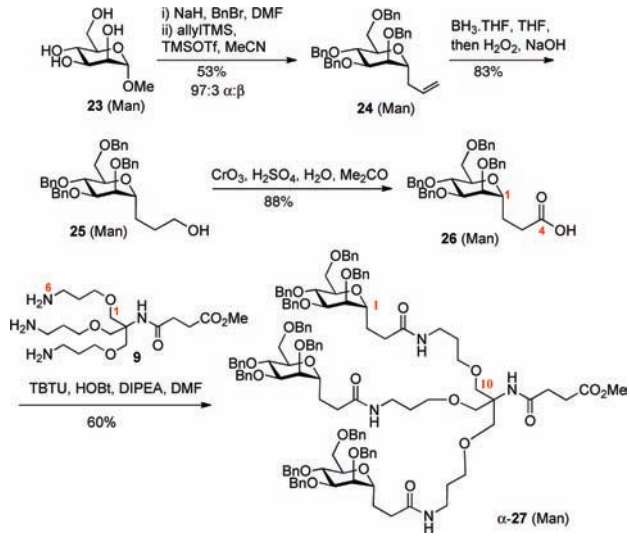
To illustrate the matrix of potential coupling partners, *C*-glycosidic units suitable for coupling with core intermediates **9** and **12** were prepared with a 4- or 9-atom linker. Both forms of *C*-glycosidic units were prepared via a common sequence, and examples were prepared for three different monosaccharides (Gal, Man, and Glc). The first, shorter spacer-bearing *C*-glycoside modules were prepared through allylation (after benzylation) at C-1 of methyl glycosides **13**, **14**, and **23** and subsequent oxidative manipulations to furnish *C*-glycosidic acids **19**, **20** (Scheme 3) and **26** (Scheme 4).

The C-allylation reactions proceeded with the anticipated anomeric selectivities, affording *C*-galactoside (**15**) and *C*-glucoside (**16**) with 9:1 β:α selectivity (Scheme 3) and the α-*C*-mannoside (**24**) [α:β 97:3] (Scheme 4). These

Scheme 3. *C*-Glycoside Propionate Synthesis and Application to 9-Atom Linked Trivalent Glycosides



Scheme 4. Trivalent *C*-Mannoside Synthesis



anomerically defined *C*-glycosidic carboxylate modules thus provided reagents to target a matrix of amide-linked multivalent systems through coupling to trisamines **9** and **12**.

Thus, these shortest chain *C*-glycoside types **19**, **20**, and **26** were coupled with the shortest chain trisamine core **9** (Schemes 3 and 4). This afforded examples of multivalent saccharides β-**21**, β-**22**, and α-**27** of the minimal structure, with a 10-atom length of branch to sugar C1.

To extend this coupling matrix and illustrate the modular potential of this approach, an example of an 9-atom chain-extended *C*-glycosidic carboxylate was prepared by divergence from the synthesis described in Scheme 3. *C*-Propyl alcohol **18** was homologated by azidation to **28**, reduction to *C*-glucosidic amine **29**, and coupling with methyl hydrogenphthalate, to afford, after saponification, extended carboxylate **30** (Scheme 5). Coupling the longer-chain trisamine

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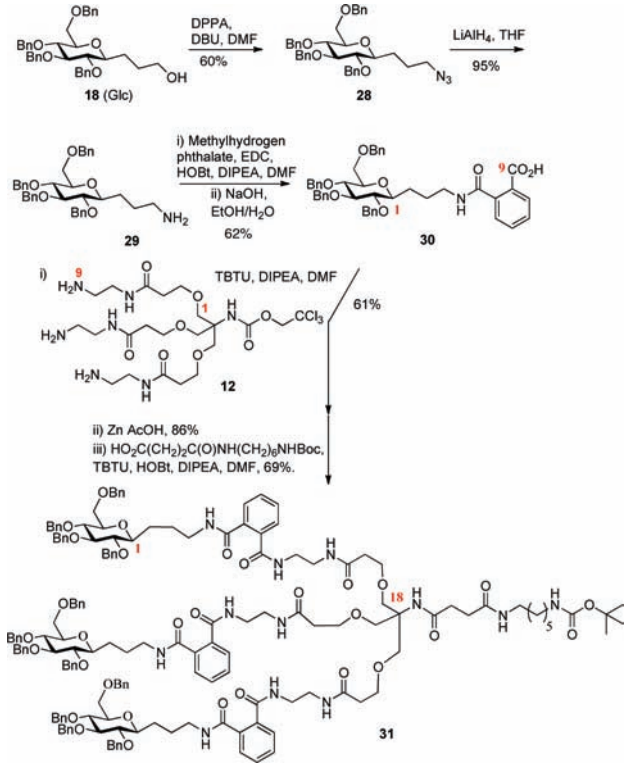
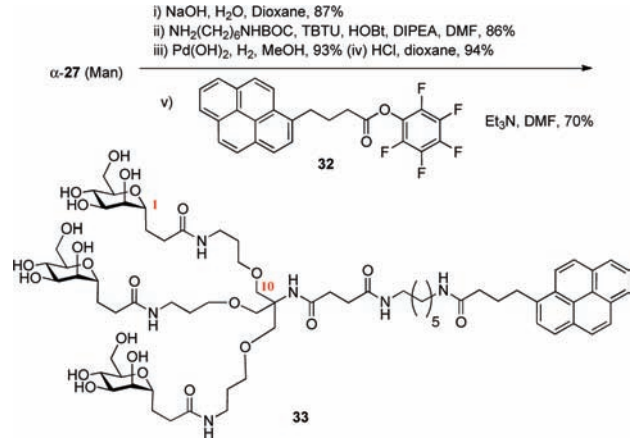
Scheme 5. Synthesis of 18-Atom Branch Multivalent *C*-Glycoside **31**, Bearing a Succinimide-Linked Funtionalized Tether



core **12** (Scheme 2) with the phthalate-extended longer *C*-glycoside system **30** (Scheme 5) then afforded the maximum extended structure (from the modules reported here), with an 18-atom length of branch to C1. After multivalent coupling, the core amino terminus of the larger construct was deprotected and an *N*-Boc-terminated polyalkyl unit appended to give **31** (Scheme 5), a precursor for immobilization/conjugation of this multivalent epitope.

To illustrate application to conjugates bearing free sugar ligands, the tris-*C*-mannosyl system α-**27** was elaborated to a fluorescent-label bearing conjugate **33**, by extension onto the carboxylate of α-**27** (analogous to the extension for **31**) with hydrogenloysis of the sugar benzyls and removal of the terminal NHBoc allowing amidation using the pentafluorophenyl ester-activated pyrene reagent **32** to yield conjugate **33** in 70% yield (Scheme 6).

Scheme 6. Synthesis of the Tris-*C*-mannsosyl Pyrene Conjugate



In conclusion, synthetic entry is described to facilitate the modular assembly of a matrix of trivalent *C*-glycosides (cf. Figure 1). Examples are demonstrated for systems with differing sugar-core distances of 10−18 atoms (and linker functionality), showing that modification of the succinimide unit after multivalent assembly facilitates divergent tether derivatization. The methodology is designed to utilize preprepared *C*-glycosides, thus avoiding anomeric coupling issues and using the same amide coupling steps for any further examples. This significantly enhances applicability to a diversity of further modules and to parallel or automated syntheses using arrays of different *C*-glycosides (including disaccharides) without the need to develop any modified coupling chemistry. An example illustrating application to free sugar bearing multivalent conjugates is described, and such systems offer scope for generating on-chip sugar arrays and further conjugates.

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Supporting Information Available: ^{1}H/COSY, ^{13}C/DEPT NMR spectra, and mass spectra are available for compounds in **2**−**33** and some other intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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