The Synthesis of C-Trisaccharides Exploiting the Stereochemical Diversity of a Central Sugar

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The interaction between cell surface carbohydrates and their protein receptors[1] is implicated in viral[2] and bacterial[3] adhesion, metastasis,[4] and the recruitment of leukocytes.[5] Carbohydrate mimics represent a class of compounds which can be used to study these cellular interactions and may represent leads for drug discovery.[6] In particular, C-glycosides are useful candidates due to their resistance to glycosidases, greatly enhancing their stability in biological fluids.[7] In reference to the generation of di- and trisaccharide analogs, Kishi has shown that in specific models, C-glycosides are similar in both solution conformation and biological activity to their O-glycosidic counterparts and that hydroxyl deletion can alter the overall conformation and flexibility of the C-saccharide.[8] Presently, we are developing substrates to examine these interactions at the molecular level via *de novo* synthesis of pyranyl derivatives within the context of C-disaccharides[9] and alternatively with the combinatorial synthesis of C-glycopeptide ligands on solid support.[10] In this paper, we develop a general strategy for the synthesis of C-trisaccharides[11] with increasing levels of divergence throughout the later stages of the route. Using this approach, we have accomplished the synthesis of C-trisaccharides based on the H type I blood group determinant **1** (Figure 1), implicated in adhesion involving the pathogenic bacteria *Helicobacter pylori*.[3b]

Our synthetic strategy is based on the C-trisaccharide **2**, a mimic of blood group determinant **1**, and represents a practical method to rapidly sort novel trisaccharides via biological evaluation of diastereomeric mixtures. The absolute structure of a compound of interest can be determined via a recursive stereochemical deconvolution of an active pool of diastereomeric mixtures through further elaboration of archived intermediates and subsequent retesting.[12] Expanding on the concepts of our previous work with 1,6 C-disaccharides,[9] we used two fixed pendant sugars at each terminus of the trisaccharide and created *de novo* the central core hexose in **2**. Strategically, the pendent sugar's identity and connectivity could be easily changed,

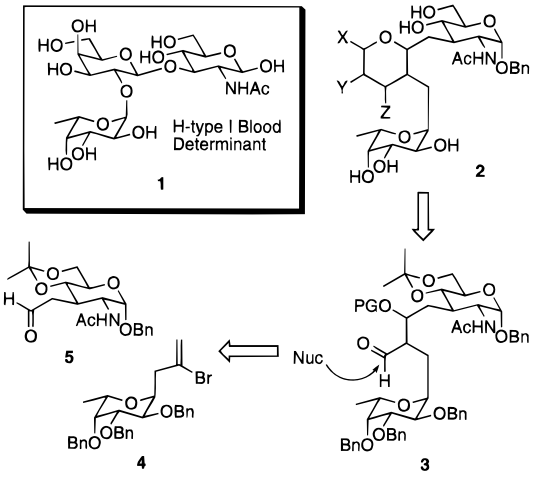
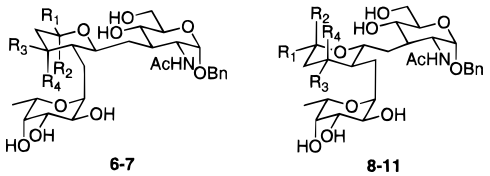


Figure 1. Retrosynthetic analysis to diverse analogs of the H-type I blood group.

Chart 1



Compound	R_1	R_2	R_3	R_4
6	CH_2OH	H	OH	H
7	H	CH_2OH	OH	H
8	CH_2OH	H	OH	H
9	H	CH_2OH	OH	H
10	CH_2OH	H	H	OH
11	H	CH_2OH	H	OH

making this approach general for any trisaccharide of interest with a C-1 and C-2 linkage, a common motif. Permutational alterations of the central hexose should have the most effect on the overall three-dimensional conformation of the trisaccharide. Retrosynthetic analysis of **2** affords the complex "C-disaccharide" **3** that can be modified by the addition of a variety of nucleophiles and then converted synthetically to **2** via an intramolecular cyclization. Synthesis of **3** required an organometallic coupling of C-hexose **4** and aldehyde **5**, both derived from natural sugar precursors. This strategy has thus far yielded the six trisaccharides **6**−**11** through a rapid, convergent approach that can be applied to a variety of cell-surface sugars. Both the central *D*- and *L*-sugars are synthesized with no extra synthetic effort, providing an interesting permutation that would otherwise be financially prohibitive if natural sugar precursors were used as starting materials.

Vinyl bromide **4**, generated in three steps from commercially available fucose,[13] was coupled to aldehyde **5**[14] with $CrCl_2$/0.5% $NiCl_2$[15] and blocked to obtain separable TBS ethers **12** and **13**,[16] 2:1, respectively (Scheme 1). Diastereoselective hydroboration/oxidation (9-BBN/H_2O_2)[17] and subsequent oxidation with Dess−Martin periodinane (DMP)[18] gave aldehydes **14** and **19**, specific examples of retron **3**.[19]

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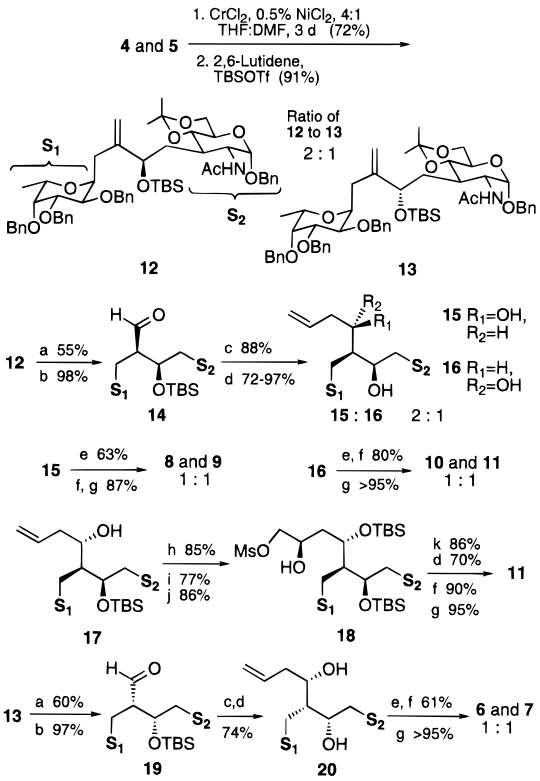
Scheme 1[a]



[a] (a) 9-BBN, THF, 0 °C; (b) Dess−Martin periodinane, CH_2Cl_2; (c) allylmagnesium bromide, THF, −78 °C; (d) TBAF, THF or Et_2O; (e) MCPBA, CH_2Cl_2; (f) cat. CSA, CH_2Cl_2; (g) H_2, $Pd(OH)_2$/C, MeOH; (h) 2,6-lutidene, TBSOTf; (i) OsO_4, $DHQD_2$·PYR, *t*-BuOH/H_2O, 0 °C, 5:1; (j) Et_3N, MsCl, CH_2Cl_2; (k) NaH, Et_2O.

Aldehyde **14** was homologated with the addition of allylmagnesium bromide to yield separable homoallylic alcohols, which were then desilylated with TBAF to give diols **15** and **16** (2:1, respectively). The major diastereomer **15** was expected to result from a Felkin−Ahn addition of the nucleophile to the aldehyde. This stereochemical assignment was confirmed following a chiral crotylboration using the (+) Ipc_2BOMe ligand, giving the homoallylic alcohols in a 6:1 ratio as predicted, in a 48% yield.[20] The minor diastereomer could be favored following an oxidation/reduction (DMP/$LiBH_4$) procedure, giving a 1:3 ratio of diastereomers (not shown, 95% yield). The olefin in diol **15** was oxidized with MCPBA to yield a 1:1 mix of epoxides which was subsequently treated with CSA, resulting in cyclization to the pyran and removal of the isopropylidine protecting group from the *N*-acetylglucosamine. Hydrogenation using H_2 with $Pd(OH)_2$ on carbon removed all but the anomeric benzyl group, limiting the diastereomers formed to the two C-trisaccharides **8** and **9**[21] containing the center sugar 2,4-dideoxy-L-galactose for **8** and 2,4-dideoxy-D-altrose for **9**.[22]

Compounds **10** and **11** were generated from diol **16** in a similar manner combining the epoxidation, cyclization, and deprotection in one step by adding CSA directly to the flask containing MCPBA after 16 h had elapsed. Deprotection of the benzyl groups on the fucose gave two trisaccharides **10** and **11** that could be separated by normal flash silica gel chromatography.[23] These two compounds have the center sugars 2,4-dideoxy-L-allose in **10** and 2,4-dideoxy-D-mannose in **11**.[24]

Deconvolution of the C-5 stereocenter was addressed by generating a stereochemically pure epoxide. Compound **17**, precursor to **16**, was further protected with TBSOTf and then submitted to Sharpless asymmetric dihydroxylation conditions,[25] generating the desired chiral diol which was monomesylated to yield compound **18**. The dihydroxylation with the DHQD ligand proceeded in good yield providing a 5:1 ratio of separable diastereomers. The alternative DHQ ligand gives the diols in an opposite 1:2 ratio. Base-mediated ring closure of **18** formed the epoxide,[26] and removal of both TBS protecting groups gave a stereochemically pure epoxide-diol that converged on the nonstereoselective route. As expected, when this diol was treated with the previous conditions of acid catalyzed cyclization and hydrogenation, C-trisaccharide **11** was generated as a single compound.

Aldehyde **19** was allylated, and the crude reaction mixture was treated with TBAF to remove the TBS ether, providing the exclusive compound **20** as expected from Felkin−Ahn addition. Compound **20** was treated with the one pot epoxidation and cyclization procedure to give two separable trisaccharides. These two compounds were individually analyzed via 2D-COSY, 2D-ROESY, 2D-HMQC, and selective homonuclear ^1H decoupling NMR experiments to determine the stereochemical makeup of the products. At this point the stereochemical outcome of the precedented stereoselective hydroboration and Felkin−Ahn addition for this series was confirmed.[27] Both compounds were selectively hydrogenated and separated to give pure trisaccharides **6** (2,4-dideoxy-D-galactose) and **7** (2,4-dideoxy-L-altrose).[28]

In conclusion, we have presented a general approach to C-trisaccharides with 1,2 sugar branching. The synthesis is both convergent with respect to the pendant sugars and divergent so that modifications of steps in the later portions of the route can be used to generate a variety of structures. Ideally, these procedures could be repeated using a variety of sugars in the S_1 and S_2 locations, archiving portions of the material at each prochiral stage (i.e., **13**, **19**, and **20**) for stereochemical deconvolution as needed. These and other extensions of the present methodology should result in the synthesis of more trisaccharides within this series. Biological assays in parallel with detailed solution conformations of these compounds should provide insight into the binding requirements of selected carbohydrate receptors.[29]

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Supporting Information Available: Experimental procedures and spectroscopic data for reported compounds (36 pages). See any current masthead page for ordering and Internet access instructions.

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(19) Hydroboration of **12** and **13** with BH_3 would provide the opposite regiochemistry and should permit entry into these diastereomers. See ref 16.

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(21) Compounds **8** and **9** could be separated via chromatography following an acetylation step.

(22) These sugars may alternatively be referred to as 2,4-dideoxy-L-glucose for **8** and 2,4-dideoxy-D-idose for **9** due to the lack of the hydroxyl on C-4 found in the natural hexoses.

(23) Two fractions were obtained, one with 10% contamination by the other diastereomer and the other with a 15% contamination of the previous diastereomer.

(24) Or 2,4-dideoxy-L-gulose for **10** and 2,4-dideoxy-D-talose for **11**.

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(27) H-1′ (for the center sugar) in the precursor to **6** is coupled to H-2′ with a large *J* value of 9.8 Hz, indicative of a trans-trans diaxial relationship. H-3′ is coupled to H-2′ (10.4 Hz), H-4′ axial (10.4 Hz), and to H-4′ equatorial (4.2 Hz). H-4′ equatorial, in addition to H-3′ coupling and a geminal coupling to H-4′ axial (12.0 Hz), is weakly coupled to H-5′, a pattern similar to the H-4 proton of galactose found in a previously synthesized C-1, C- 2 branched C-trisaccharide (see ref 8a).

(28) Or 2,4-dideoxy-D-glucose for **6** and 2,4-dideoxy-L-idose for **7**.

(29) Sufficient quantities for biological assays were obtained for all compounds.