Synthetic Analogs of the Lewis^b-Tetrasaccharide and their Binding to Griffonia Simplicifolia Lectin-Part II

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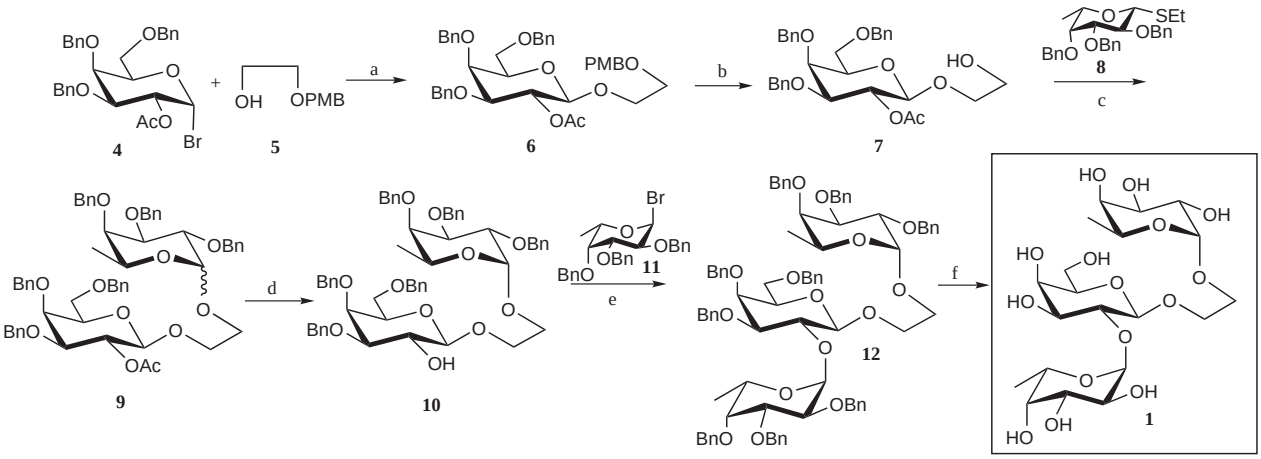
Dedicated to the memory of Professor Raymond U. Lemieux.

Abstract: The lewis b tetrasaccharide is known to bind to lectin IV of Griffonia simplicifolia. In an effort to prepare superior ligands, we have synthesized analogs of this tetrasaccharide and evaluated their binding to the lectin. This paper (part II in a series of two) describes the synthesis of and binding studies with analogs where the GlcNAc unit (a) has been replaced by simpler mimicking residues that are both flexible and rigid. A simple 1,2-*trans* cyclohexanediol residue provided a reasonable simplified inhibitor whereas a flexible 1,2-ethanediol replacement led to a completely inactive ligand.

Key words: lewis b tetrasaccharide, lectins, galactose unit

The recognition and binding of complex carbohydrates by proteins forms a major basis for their biological activities.[1–5] The binding of the Lewis-b (Le^b) tetrasaccharide, α-L-Fuc-(1→2)-β-D-Gal-(1→3)-[α-L-Fuc-(1→4)]-β-D-GlcNAc-OMe (Le^b-OMe), by the lectin IV of *Griffonia simplicifolia* (GS-IV has been extensively studies by Lemieux and co-workers.[6–13] GS-IV-Le^b recognition is therefore an ideal system to test approaches to the design of superior inhibitors of carbohydrate-protein binding. Many tetrasaccharide analogs have been prepared and evaluated, and the crystal structure of a tetrasaccharide bound to the protein has been solved.[7,13] High-resolution crystallographic studies reveal that the main interaction of the tetrasaccharide with the protein occurs via the terminal galactosyl residue, with further participation of both fucosyl residues. The GlcNAc residue, however, is exposed to the bulk solvent and should mainly serve as a scaffold for the correct orientation of the three other sugar rings. This paper deals with synthesis of and binding studies with analogs where the GlcNAc residue has been simplified. Extensive studies by Lemieux et.al have revealed very little about the role of the GlcNAc residue.[9–12] We therefore decided to synthesize analogs (**1**–**3**) of Le^b-OMe by replacing the GlcNAc residue with simpler 'mimicking' structures.

The synthesis of **1** (Scheme 1) began with condensation of the glycosyl bromide **4** (obtained from 3,4,6-tri-*O*-benzyl-1,2-*O*-(1-methoxyethylidine)-α-D-galactopyranose by treatment with acetyl bromide in presence of tetraethylammonium bromide[10]) with the alcohol **5**[14] under Helferich conditions. The glycoside **6** was obtained in 89% yield. Deprotection of the primary alcohol using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), followed by DMTST-promoted reaction with the fucosyl donor **8** gave compound **9** as an α/β mixture where the α isomer was purified by chromatography after de-O-acetylation. Tetraethylammonium bromide-promoted[10] reaction with the fucosyl donor **11** gave compound **12** in



Scheme 1 *Reagents*: a) $Hg(CN)_2$–nitromethane–toluene (89%); b) DDQ (88%); c) **8**, DTBMP–DMTST, CH_2Cl_2 (81%); d) NaOMe–MeOH (82%); e) **11**, Bu_4NBr (68%); f. H_2–$Pd(OH)_2$ (92%).

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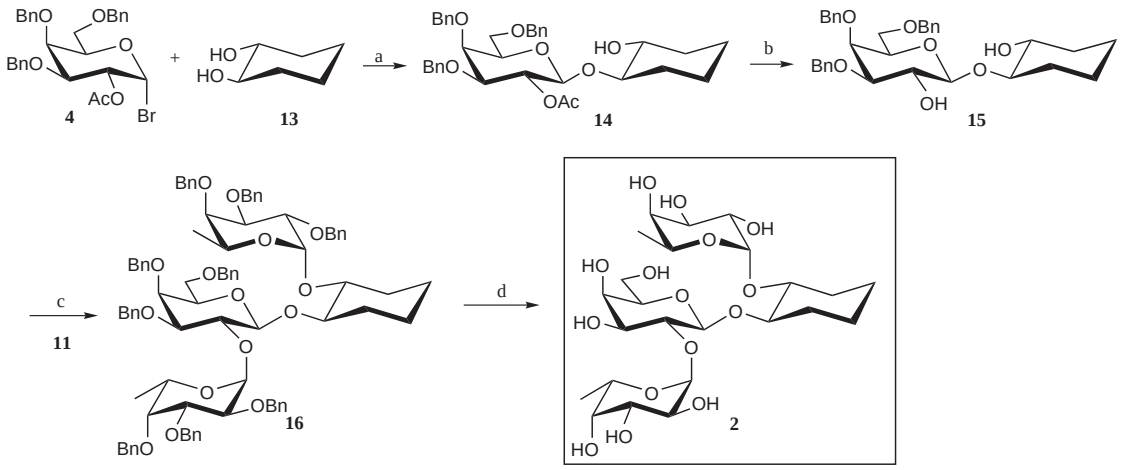


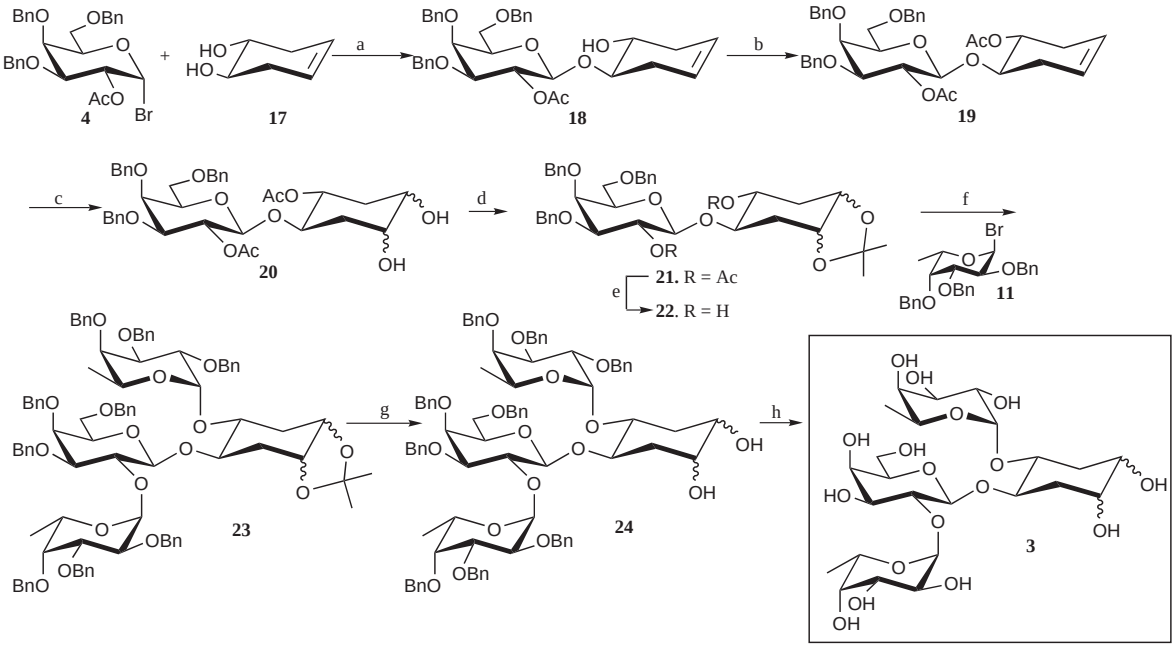
Scheme 2 *Reagents*: a) $Hg(CN)_2$–nitromethane–toluene (74%); b) NaOMe–MeOH (94%); c) **11**–Bu_4NBr (70%); d. H_2–$Pd(OH)_2$ (85%).

68% yield (attempts to introduce the two fucosyl residues in one step gave a complex mixture of compounds which could not be separated by chromatography). Finally, debenzylation by catalytic hydrogenation gave the desired compound **1**[15] in 92% yield.

Le^b-OMe analogs **2** were synthesized in a similar fashion (Scheme 2). (1*R*,2*R*)-*trans*-1,2-Cyclohexane diol **13** was prepared as described in the literature.[16] The diol **4** was glycosylated with **13** using mercuric cyanide to furnish **14** in 74% yield. De-O-acetylation, followed by introduction of the two fucosyl residues in one step using tetrabutylammonium bromide gave **16** in 70% yield. Finally, debenzylation gave the desired tetrasaccharide **2**[15] in 82% yield.

Based on the crystal complex of the native Le^b-OMe tetrasaccharide,[8] we anticipated that if the cyclohexanediol analog **2** were to bind to GS-IV, it would leave a hydrophobic cyclohexane ring projecting into solvent. This might well lead to very unfavorable hydration properties so we also decided to prepare the control derivative **3** where hydrophilic OH groups were added to the more hydrophobic parent structure **2**.

For the synthesis of analog **3**, (1*R*, 2*R*)-*trans*-cyclohex-4-enediol (**17**) was used (Scheme 3). Donor **4** was reacted with **17** using mercuric cyanide to furnish **18** in 70% yield. Initial attempts to introduce the two fucosyl residues in one step (on de-O-acetylated **18**) followed by selective hydroxylation in the last step were unsuccessful. Therefore, compound **18** was O-acetylated and subsequently hydroxylated (using osmium tetroxide under standard conditions) to give a mixture of two compounds (**20**). Reaction of **20** with 2,2-dimethoxypropane gave **21**,



Scheme 3 *Reagents*: a) $Hg(CN)_2$–nitromethane–toluene (70%); b) acetic anhydride–pyridine (93%); c) OsO_4–NMO (81%); d) 2,2-dimethoxypropane–TsOH (87%); e) NaOMe–MeOH (94%); f) **11**, Bu_4NBr (72%); g) $HOAc/H_2O$; h) H_2–$Pd(OH)_2$ (64%, two steps).

which was de-O-acetylated to give the diol **22**. Fucosylation of **22** using **11** and tetraethylammonium bromide gave **23** in 72% yield. Hydrolysis of the ketal followed by debenzylation generated the desired compound **3**[15] in 64% yield (mixture of two isomers **3a** and **3b**).

The binding of GS IV to compounds **1**–**3** and to the parent compound Le^b-OMe was studied by ELISA inhibition as described in the preceding communication.[8] The results are summarized in Scheme 4. The IC_{50} of the reference compound Le^b-OMe was 36 μM. All of the three synthesized analogs **1**–**3** poorly inhibited lectin binding compared to the parent compound, Le^b-OMe. The poorest inhibitor was analog **1**, for which no inhibition could be observed at the concentrations used. Since **1** has the most flexible linkage between the galactosyl and fucosyl residues (b and c), this shows the importance of a more conformationally rigid linkage between these residues. However, even though analogs **2** and **3** both have this more rigid linkage, they were still poor inhibitors compared to Le^b-OMe. However, even the analog **2** where Fuc and Gal residues can adopt similar if not identical relative orientations on a more rigid template results in a 6-fold decrease in potency. Hydration of the complex is not likely the reason, since the more hydrophilic **3** was 3-fold worse again.

Clearly, replacement of the 3,4-disubsituted GlcNAc residue in the Leb tetrasaccahride by a flexible ethane diol as in **1** can be expected to result in a dramatic decrease in activity. However, when this identical strategy was applied to the development of inhibitors of E-selectin binding to the tetrasaccharide sialyl-LeX (SLeX), replacement of a 3,4-diglycosylated GlcNAc residue by ethane diol yielded a greatly simplified structure that was as potent as the parent compound.[14] Replacement of the same GlcNAc residue in SLeX by cyclohexane diol (as was done for **2**) yielded a structure that was almost an order of magnitude more active.[17] In conclusion, and as expounded by Lemieux,[12] the binding of oligosaccharides by proteins remains poorly understood and rules for the design of potent inhibitors are in the early stages of development. One of the most important and difficult factors to incorporate into inhibitor design is the difference in hydration of the free protein and sugar vs the complex they form on binding.

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(15) ^{1}H NMR (D_2O): **1**. δ: 4.09 (q, 1 H, H-5b), 4.31 (q, 1 H, H-5c), 4.57 (d, 1 H, H-1a), 4.93 (d, 1 H, H-1b), 5.23 (d, 1 H, H-1c); **2**. δ: 4.48 (q, 1 H, H-5d), 4.56 (d, 1 H, H-1b), 4.70 (q, 1 H, H-5c), 4.99 (d, 1 H, H-1c), 5.28 (d, 1 H, H-1d); **3a**. δ: 4.35 (d, 1 H, H-5d), 4.52 (d, 1 H, H-1b), 4.60 (q, 1 H, H-5c), 5.00 (d, 1 H, H-1c), 5.28 (d, 1 H, H-1d); **3b**. δ: 4.57 (d, 1 H, H-1b), 4.73 (q, 1 H, H-5c), 4.91 (d, 1 H, H-1c), 5.27 (d, 1 H, H-1d).
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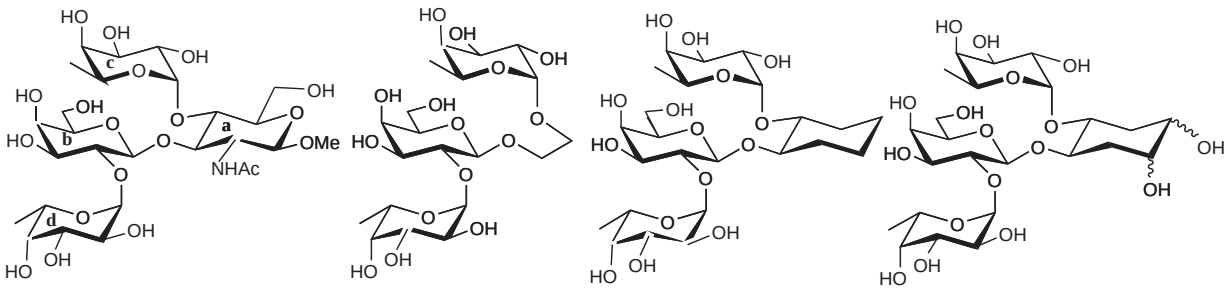

Scheme 4