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

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Dedicated to the memory of Professor Raymond U. Lemieux.

Abstract: The lewis b tetrasaccharide binds specifically to the Griffonia simplicifolia lectin GS-IV. In an effort to obtain tighter binding derivatives, we have synthesized a series of analogs of this tetrasaccharide and studied their binding to the lectin. This paper (part I in a series of two) describes the synthesis of and binding studies with analogs where the galactose unit (b) has been altered at the 6-position. Even though this position does not make direct contact with the protein in the crystal structure, the binding was very sensitive to these substitutions.

Key words: lewis^b-tetrasaccharide, lectins, galactose unit

Carbohydrates play a very important role in many biological processes including inflammation, cellular differentiation, hormone-cell recognition and bacterial-host cell attachment, and have been implicated in several life threatening diseases such as cancer, inflammatory disorders, and microbial infection.[1–8] One of the most important present drawbacks of carbohydrate-based therapeutic agents is the low intrinsic affinity of carbohydrates to their protein receptors (normally in the millimolar range). Hence, it is important to investigate the factors which influence carbohydrate-protein binding strength with a view to designing better inhibitors.

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), to the lectin IV of *Griffonia simplicifolia* (GS-IV)[9] is one of the most systematically studied systems in carbohydrate-protein recognition through the pioneering contributons of R. U. Lemieux and his collaborators.[10–20] GS-IV-Le^b recognition therefore represents an ideal system to evaluate approaches to the design of better inhibitors of carbohydrate-protein binding. Numerous tetrasaccharide analogs have been chemically synthesized,[12–18] and the crystal structure of a tetrasaccharide-protein complex is known.[10,13] Briefly, the X-ray crystal structure of GS-IV bound to Le^b-OMe involves both polar and extensive nonpolar interactions (Figure 1). Essential polar interactions occur through hydrogen bonding of OH-3 and OH-4 of galactosyl residue (unit b) with Asp-89 and Asn-135. The 4-OH group of the fucosyl residue c is also critically involved through a hydrogen bond to Ser-49. In addition to these essential interactions, OH-3 (unit c) and OH-2 (unit d) also contribute significantly though the corresponding deoxy-compounds still bind.[11–13]

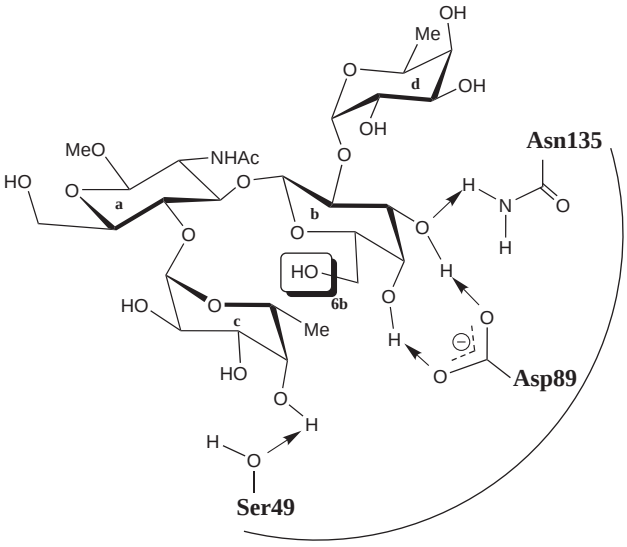

Figure 1 Schematic of the crystalline complex of Leb-OMe bound to lectin GS-IV.[10,13] Essential hydrogen bonding with the protein is shown. OH-6 (unit b), which has been derivatized in the present work, is highlighted.

Since a large portion of the tetrasaccharide resides in the aqueous phase but is close to the protein, several opportunities should exist for strengthening of the binding by adding groups to the this part of the tetrasaccharide. The crystal structure data[10,13] shows that the **6b**-hydroxy group is accepted into a nonpolar region of the combining site through without any direct hydrogen bonding to the protein. In fact, the **6b**-O-methyl analog which was found to bind 0.5 kcal/mol more strongly than the parent compound, Le^b-Ome.[19] The crystal structure of the complex also showed the internuclear distances between the oxygen atom of the hydroxy group at position **6b** and Tyr223, Trp133 and Arg88 (residues not shown in Figure 1) to be in the range of 4–7 Å units.[20] We therefore decided to examine if substitution of OH-**6b** by groups other than methyl might further enhance the binding. To accomplish this, we replaced the **6b** hydroxy group by an amino group, and attached various substituents by N-acylation.

Molecular modeling studies using the structure-based drug design technique LUDI[21–24] were carried out focused on modifications at the **6b** position of galactose. A data-

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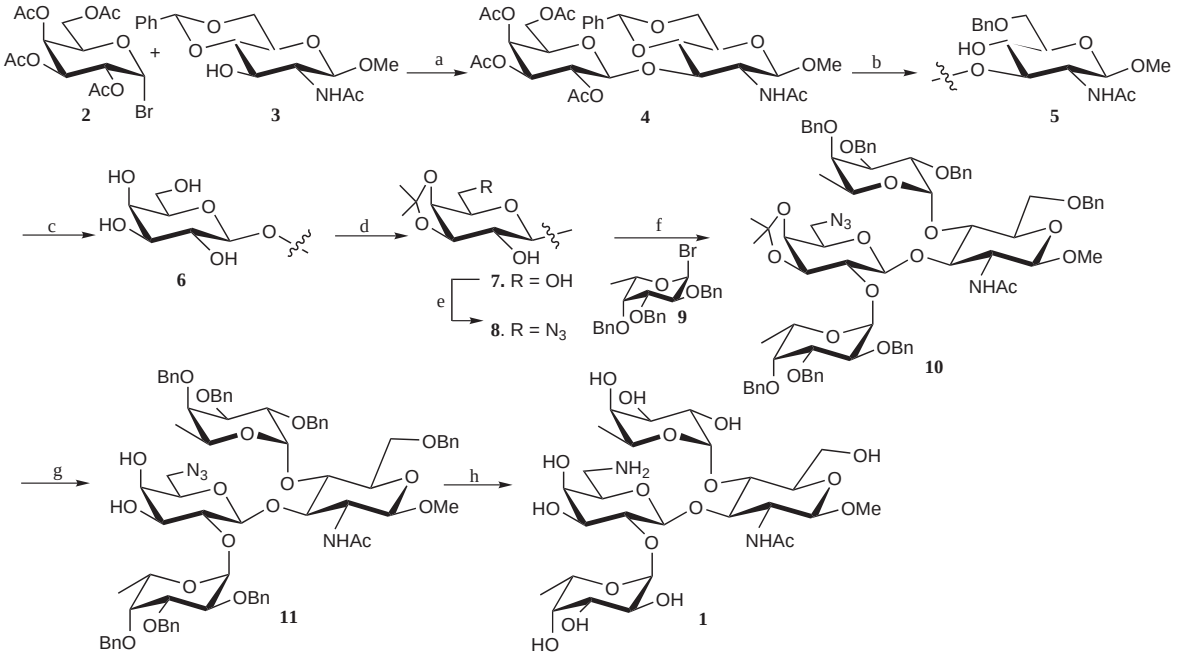
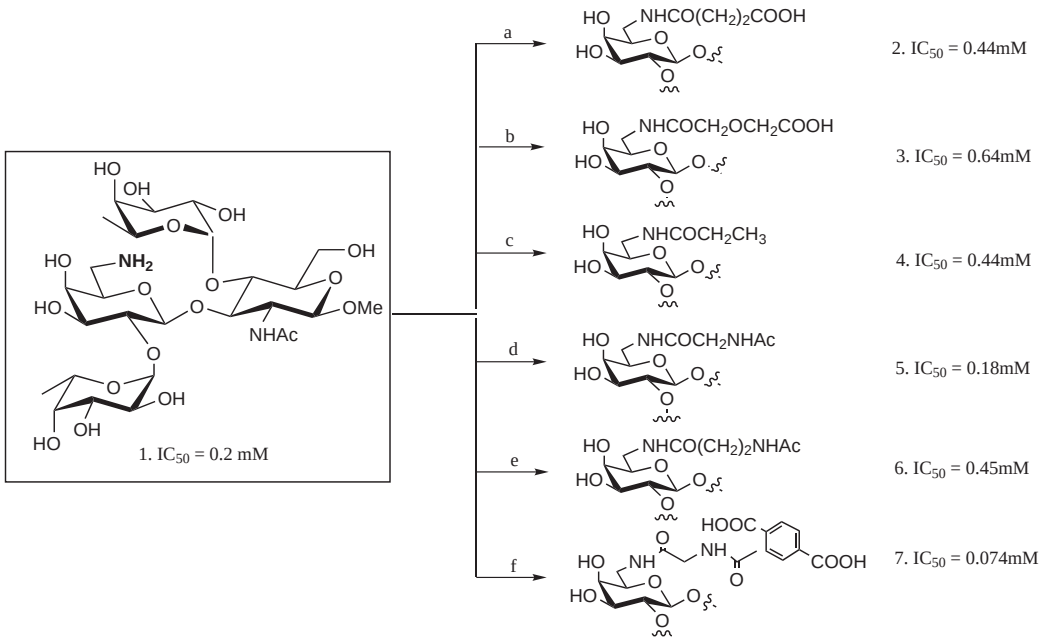


Scheme 1 *Reagents*: a) $Hg(CN)_2$–nitromethane–toluene (89%); b) $NaCNBH_3$–HCl (75%); c) NaOMe–MeOH (95%); d) Acetone/TsOH (78%); e) Ph_3P–DEAD–DPPA (72%); f) **9**–Bu_4NBr (68%); g) $HOAc/H_2O$; h) Na–NH_3 (64%, two steps).

base of chemically relevant fragments was searched for suitable candidates for incorporation into the Lewis b tetrasaccharide structure so as to enhance the binding. Changes resulting from substitutions at the **6b** position of the galactosyl residue was the focus of the design, and the lectin active site was defined as including all amino acids of the surrounding protein within a radius of 15 Å from this atom. This value for the radius was considered to be sufficient as the essential residues important for the activity of the tetrasaccharide were within this distance. The result from the search was pruned down to a subset of seven candidates (Scheme 2) which were then synthesized and evaluated as inhibitors.

The synthesis (Scheme 1) began with the condensation of the peracetylated galactosyl bromide **2** with the blocked GlcNAc derivative **3** using Helferich conditions to furnish the blocked disaccharide **4** (89%).[25] The benzylidene ring



Scheme 2 *Reagents*: a) Succinic anhydride, DMF/MeOH; b) Diglycolic acid anhydride; c) Propionic acid anhydride; d) 1. PfpO-Gly-NH-Fmoc, 2. morpholine, 3. 10% Ac_2O/MeOH; e) 1. PfpO-β-Ala-NH-Fmoc, 2. morpholine, 3. 10% Ac_2O/MeOH; f) 1. PfpO-Gly-NH-Fmoc, 2. morpholine, 3. 1,2,4-benzenetricarboxylic acid anhydride.

in **4** was reductively opened with sodium cyanoborohydride-hydrogen chloride[26] to provide compound **5** (75%). Deacetylation of **5** followed by isopropylidination using standard conditions furnished **7** (78%). Treatment of **7** with triphenyl phosphine (Ph_3P), DEAD, and diphenylphosphoryl azide (DPPA)[27] gave the azido compound **8** (72%). Finally, introduction of the two fucosyl residues by reacting **8** with **9** and tetrabutylammonium bromide gave **10** (68%). The isopropylidene group was selectively removed using 80% acetic acid followed by debenzylation and reduction of the azide using Birch conditions[28] to furnish the desired amino analog **1** in 64% yield over the 2 steps (Scheme 1).

Scheme 2 describes the synthesis of six N-acyl derivatives of **1**. They were generated from **1** and readily available N-acylating reagents under standard conditions. All analogs (compounds **1**–**7**) were purified by column chromatography, lyophilized and structurally characterized by ^{1}H NMR and mass spectroscopy (see Table 1). The reaction yields were in the 80–90% range.

The binding of GS IV lectin to compounds **1**–**7** and to the parent compound Le[b]-OMe was then studied by an ELISA inhibition technique. Briefly, GS IV lectin, purified using a Synsorb-Lewis[b] affinity column,[9,14,29,30] was coated on 96-well ELISA plates. Lewis-b-BSA glycoconjugate premixed with inhibitor solution was then added to the wells. The effective inhibitor concentrations were in the range 1 nM–1 mM. After incubation at r.t. for 18 h and washing, the Lewis-b-BSA-glycoconjugate that remained bound to the wells was detected by adding first mouse monoclonal anti Lewis-b antibody, then goat anti-mouse IgG-horseradish peroxidase conjugate, and finally substrate (3,3′5,5′-tetramethylbenzidine). Absorbance was read at 450 nm and percent inhibition was calculated using wells containing no inhibitor as the reference points.

The IC_{50} values for **1**–**7** were as shown in Scheme 2. The best new inhibitor was compound **7** (0.074 mM). However, this value is not as good as that of the parent compound, Le[b]-OMe (0.036 mM). Thus, none of the analogs synthesized were any better inhibitors than the parent compound. Of the substituted amino compounds **2**–**7**, only **7** (0.074 mM) and **5** (0.18 mM) were better inhibitors than the amino compound itself **1** (0.2 mM). These results further confirm the importance of the **6b** position for binding, but clearly groups other than those investigated here will have to be used to obtain a tighter binding to the GS-IV lectin.

Table 1 Selected Low-Resolution MS and 360 MHz NMR Data ([a]295 K, D_2O, $\delta_{acetone}$ = 2.225 ppm) for the Le[b]-OMe Analogs

Analogs	^{1}H NMR data (360 MHz)[a]	$[M + H]^+$
1	1.25 and 1.26 (d × 2, 6 H, H-6c, H-6d); 2.07 (s, 3 H, NHAc); 3.47 (s, 3 H, OMe); 4.33 (q, 1 H, H-5d); 4.32 (d, 1 H, *J* = 8 Hz, H-1a); 4.66 (d, 1 H, *J* = 8 Hz, H-1b); 4.80 (q, 1 H, *J* = 6.5 Hz, H-5c); 5.01 (d, 1 H, *J* = 3.5 Hz, H-1c); 5.15 (d, 1 H, *J* = 4 Hz, H-1d)	689.2
2	1.25 and 1.26 (d × 2, 6 H, H-6c, H-6d); 2.44–2.56 [m, 4 H, -$(CH_2)_2$-]; 3.47 (s, 3 H, OMe); 4.35 (q, 1 H, H-5d); 4.42 (d, 1 H, *J* = 8 Hz, H-1a); 4.61 (d, 1 H, *J* = 8 Hz, H-1b); 4.84 (q, 1 H, *J* = 6.5 Hz, H-5c); 5.02 (d, 1 H, *J* = 3.5 Hz, H-1c); 5.14 (d, 1 H, *J* = 4 Hz, H-1d)	789.1
3	1.24 and 1.27 (d × 2, 6 H, H-6c, H-6d); 3.48 (s, 3 H, OMe); 4.04 (s, 4 H, -CH_2-O-CH_2-); 4.33 (q, 1 H, H-5d); 4.34 (d, 1 H, *J* = 8 Hz, H-1a); 4.64 (d, 1 H, *J* = 8 Hz, H-1b); 4.84 (q, 1 H, *J* = 6.5 Hz, H-5c); 5.00 (d, 1 H, *J* = 3.5 Hz, H-1c); 5.14 (d, 1 H, *J* = 4 Hz, H-1d)	805.2
4	1.13 (t, 3 H, CH_3); 1.24 and 1.26 (d × 2, 6 H, H-6c, H-6d); 2.30 (q, 2 H, -CH_2-); 3.48 (s, 3 H, OMe); 4.33 (q, 1 H, H-5d); 4.34 (d, 1 H, *J* = 8 Hz, H-1a); 4.61 (d, 1 H, *J* = 8 Hz, H-1b); 4.82 (q, 1 H, *J* = 6.5 Hz, H-5c); 5.01 (d, 1 H, *J* = 3.5 Hz, H-1c); 5.15 (d, 1 H, *J* = 4 Hz, H-1d)	745.1
5	1.25 and 1.27 (d × 2, 6 H, H-6c, H-6d); 2.05 and 2.07 (2 × s, 6 H, NHAc); 3.48 (s, 3 H, OMe); 4.33 (q, 1 H, H-5d); 4.33 (d, 1 H, *J* = 8 Hz, H-1a); 4.61 (d, 1 H, *J* = 8 Hz, H-1b); 4.81 (q, 1 H, *J* = 6.5 Hz, H-5c); 5.01 (d, 1 H, *J* = 3.5 Hz, H-1c); 5.14 (d, 1 H, *J* = 4 Hz, H-1d).	788.2
6	1.25 and 1.27 (d × 2, 6 H, H-6c, H-6d); 2.04 and 2.06 (2 × s, 6 H, NHAc); 3.49 (s, 3 H, OMe); 4.31 (d, 1 H, *J* = 8 Hz, H-1a); 4.34 (q, 1 H, H-5d); 4.61 (d, 1 H, *J* = 8 Hz, H-1b); 4.82 (q, 1 H, *J* = 6.5 Hz, H-5c); 5.01 (d, 1 H, *J* = 3.5 Hz, H-1c); 5.14 (d, 1 H, *J* = 4 Hz, H-1d)	802.2
7	1.25 and 1.27 (d × 2, 6 H, H-6c, H-6d); 2.04 and 2.06 (2 × s, 6 H, NHAc); 4.20 (d, 1 H, *J* = 8 Hz, H-1a); 4.35 (q, 1 H, H-5d); 4.61 (d, 1 H, *J* = 8 Hz, H-1b); 4.82 (q, 1 H, *J* = 6.5 Hz, H-5c); 4.95 (d, 1 H, *J* = 3.5 Hz, H-1c); 5.15 (d, 1 H, *J* = 4 Hz, H-1d); 7.50–8.40 (m, aromatic)	938.2

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(29) 100 g of peeled and finely ground seeds were stirred with 200 mL of MeOH (5×15min) and CH_2Cl_2 (3×15 min). The filtrates were discarded and the powder was dried overnight at r.t. All subsequent procedures were carried out at 4 °C. The dry meal (54 g) was extracted with 400 mL P_1 buffer for 2 h with gentle stirring. The extract was centrifuged for 1 h at 12k rpm and the sediment was re-extracted with P_1 buffer (400 mL) overnight. The aqueous extracts were combined and solid ammonium sulfate was added to 30% saturation. The mixture was gently stirred for 2 h. The precipitated proteins were spun (12k rpm, 20 min) and then discarded. More ammonium sulfate was added to 75% saturation and left stirring overnight. The precipitate was collected by centrifugation, suspended in 25 mL P_1 buffer and dialyzed against P_1 buffer. The extract was loaded on the Synsorb-Le^b affinity column (30 g) and the column was washed with P_1 buffer until the OD_{280} reading was less than 0.02. The lectin was eluted with 0.05 M carbonate/bicarbonate buffer pH 10 with 0.015M NaCl (yield 26mg). The lectin was dialyzed against P_2 buffer, and then concentrated using a Centriprep10 (Amicon). The concentrated lectin was loaded on Synsorb A affinity column (30 g) and recovered flow through was then run through Synsorb B affinity column (30 g). In both cases P_2 buffer was used as eluent. The purity of lectin obtained was confirmed on SDS-PAGE with 2% 2ME where two protein bands were observed of apparent molecular weights of approximately 28 and 26 KD. (P_1 buffer: 0.08 M Na_2HPO_4, 0.02 M KH_2PO_4, 0.15 M NaCl, 0.015 M NaN_3, 0.5 mM $Na_2S_2O_4$, 0.14 mM $CaCl_2$. P_2 buffer: 0.072 M Na_2HPO_4, 0.028 M NaH_2PO_4, 0.15 M NaCl, 3 mM NaN_3, 0.1 mM $CaCl_2$, and 0.1 mM $MnCl_2$). pH of both buffers was adjusted to 7.2.
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