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Synthesis and Binding to Plant Lectins of Sulfur-containing Analogues of βGal1,3αGalNAc (T-Antigen)

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Abstract—Analogues of the tumor-associated T-antigen (βGal1,3αGalNAc) containing 4,6-epidithio and 4,6-thietan modifications were synthesized from the α-allyl glycoside of βGalα1,3G1cNAc via suitable thiocyanate derivatives. Binding to three leguminous lectins as model systems was investigated in an enzyme-linked lectin assay (ELLA) and IC_{50} values comparable to the corresponding natural disaccharides T-antigen, lactose and *N*-acetyllactosamine were found.

In the design of glycomimetics, an important challenge is the synthesis of biologically active oligosaccharide derivatives that possess new functional properties yet bind to natural receptors. Presently, we report on sulfur-containing analogues of the tumor-associated T-antigen βGal1,3αGalNAc, the carbohydrate determinant of allyl glycoside **1**. Compounds **2** and **3**, potential photooxidants,[1] and immunomodulators,[2] contain fused 4,6-epi-dithiolane and 4,6-epi-thietane rings in the reducing GalNAc moieties.

As a first model experiment, we wished to ascertain whether the relatively bulky sulfur functionalities of compounds **2** and **3** will be accommodated by the binding sites of typical Gal/GalNAc specific legume lectins from peanut *(Arachis hypogaea,* PNA), *Erythrina corallodendron* (EcorL) and soybean *(Glycine max,* SBA).[3–7]

Synthesis

Compound **1** and intermediate **4** (Fig. 2) were prepared[8] by standard glycosylation procedures. For the synthesis of **2** from **4**, sulfur atoms were introduced as thiocyanate groups[9,10] in a stepwise fashion, using mesylate as the leaving group at C-6 and triflate as the leaving group at C-4. For the synthesis of **3** from **4**, the sulfur atom was introduced as a thiocyanate group at C-6, and displacement of mesylate with inversion at C-4 was effected by the action of sodium methoxide on intermediate **6b** (Fig. 2). For reasons of clarity, selected physical data are given of **3** in per-*O*-acetylated form.

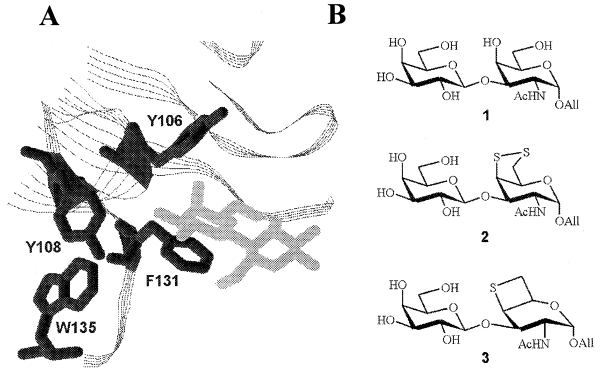

Figure 1. (A) Structure of the carbohydrate binding site *of Erythrina corallodendron* lectin with bound lactose (Protein Data Bank, entry I LTE). Aromatic side chains are displayed in dark grey, the bound lactose in light grey. (B) Structures of the synthesized disaccharides: α-allyl glycoside of T-antigen **1**, 4,6-epidithio modified α-allyl glycoside of T-antigen **2** and 4,6-thietan modified α-allyl glycoside of T-antigen **3**.

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Lectin binding studies

The assay system used is a modified ELLA (Fig. 3) based on binding of the biotinylated lectins, peanut agglutinin (PNA), *Erythrina corallodendron* lectin (EcorL) and soybean agglutinin (SBA) to immobilized asialofetuin (ASF).[11,12] The affinity constants (K_d) for binding of the lectins to immobilized ASF were found to be 140 nM for biotin-PNA, 120 nM for biotin-EcorL, and 195 nM for biotin-SBA (data not shown). IC_{50} values for compounds **1**–**3** and for the standards T-antigen (as the reducing disaccharide), *N*-acetyllactosamine (LacNAc) and lactose (Lac) were calculated from titration curves obtained by inhibition of binding of biotinylated lectin to immobilized ASF and quantification of bound lectin (Fig. 3A). Relative inhibitory potencies were calculated with the inhibitory potency of Lac arbitrarily set at 1 (Fig. 3B). Binding of the biotinylated lectins to ASF was unaffected by up to 25 mM concentrations of *trans* 4,5-dihydroxy- 1,2-dithiane, indicating that the measured values are not artifacts due to a chemical reaction of disulfide with components of the assay system.

Compounds **2** and **3** bind specifically to all three lectins tested (IC_{50}: 1.95 mM and 2.51 mM for PNA, 1.51 mM

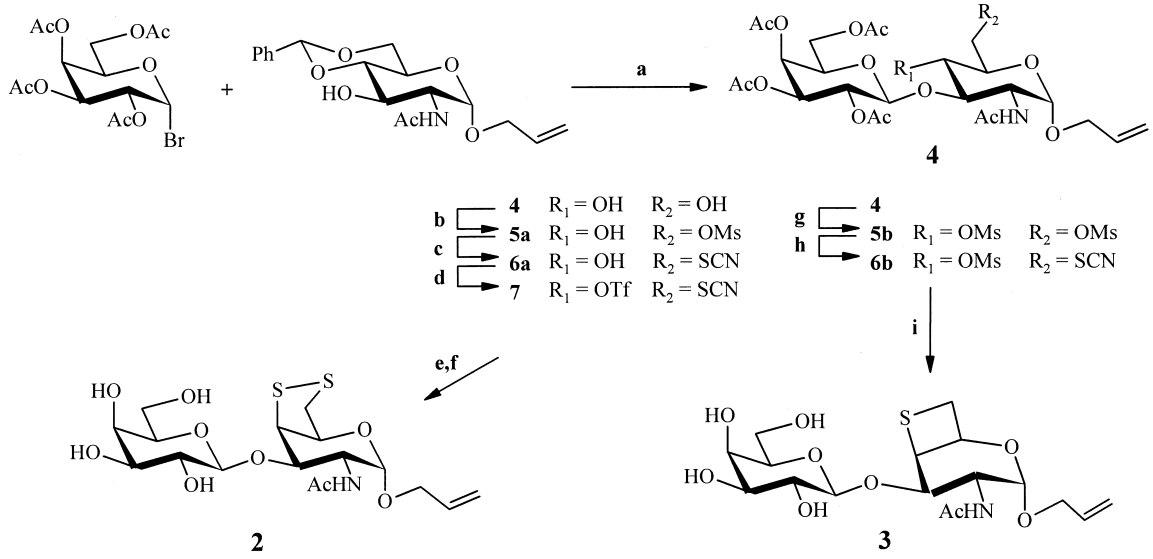

Figure 2. Chemical syntheses of compounds **2** and **3:** (a) $Hg(CN)_2/HgBr_2$; toluene/CH_3NO_2; 60 °C; 23 h; then 80% AcOH; 90 °C; 30 mm; (b) MsCl; pyr; 0 °C; 2 h; (c) KSCN; DME; Δ; 36 h; (d) Tf_20; pyr; −15 to 0 °C; 3 h; (e) KSCN; DME; Δ 12 h; (f) CH_3ONa/CH_3OH; RT; (g) MsCl; pyr; DMAP; 0 °C to rt; 6 h; (h) KSCN; DME; 100 °C; 15 h; (i) CH_3ONa/CH_3OH; reflux; 2 h.

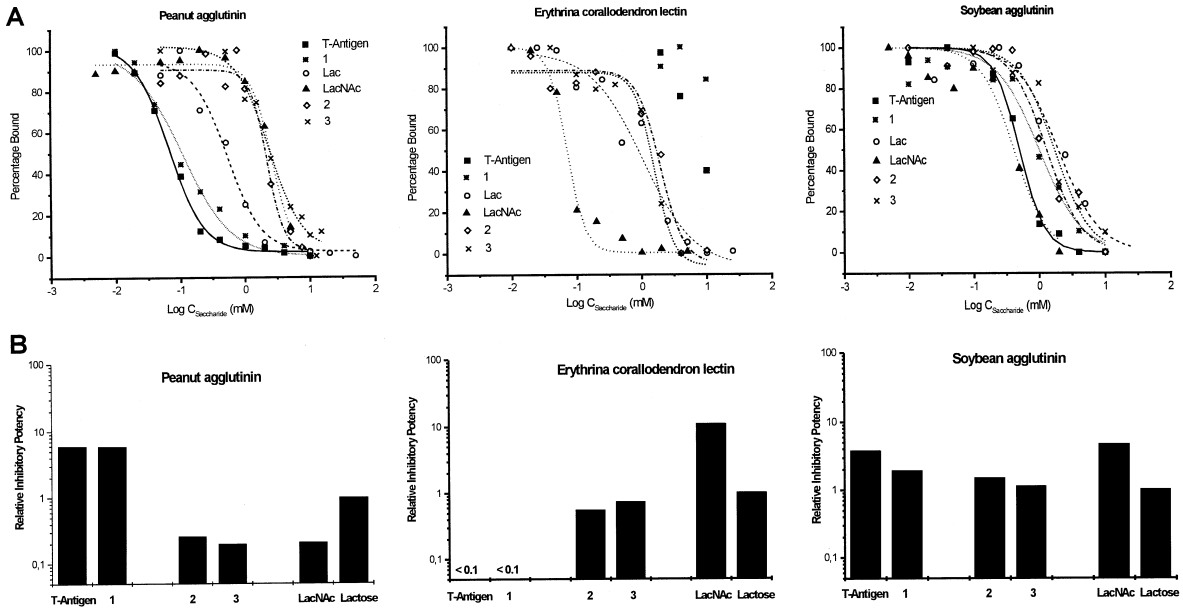

Figure 3. (A) Saccharide competition for binding of peanut agglutinin, *Erythrina corallodendron* lectin and soybean agglutinin to immobilized asialofetuin. The sugars shown are T-antigen (■), **1** (∗), **2** (◇), **3** (x), *N*-acetyllactosamine (▲) and lactose (○). (B) Relative inhibitory potencies of the different disaccharides. The inhibitory potency of lactose was arbitrarily set to 1.

and 1.15 mM for EcorL, 1.29 mM and 1.72 mM for SBA). Binding of the analogues to PNA is ca. 30 times weaker than that of T-antigen, whereas their binding to EcorL and SBA is comparable to that of Lac. These results suggest that binding of both **2** and **3** is mediated primarily through hydrogen bonding and hydrophobic interaction with the non-reducing Gal moieties while the modified GalNAc moieties neither contribute to nor strongly hamper binding. The functional potential of compounds **2**, **3** and related derivatives is significant in view of the clusters of aromatic amino acids typically present at carbohydrate binding sites (cf. Fig. 1). Thus, in the case of tryptophan-135 in EcorL, efficient energy transfer has been demonstrated[13] between the indole and the parallel dansyl group of the bound artificial ligand, *N*-dansylgalactosamine.

Similarly, in the case of cutinase from *Fusarium solani pisi,* the single tryptophan-69 mediates the photoreduction of an adjacent disulfide grouping upon ultraviolet irradiation.[1] Consequently, we are planning to use compounds **2**, **3** and related derivatives in spectroscopic and photochemical explorations of the carbohydrate binding sites in PNA, EcorL, SBA and other lectins.

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References and Notes

1. Prompers, J. J.; Hilbers, C. W.; Pepermans, H. A. M. *FEBS Lett*. **1999**, *456*, 409.
2. Unger, F. M.; Liehl, E. German Patent Appl. DE 3438585 A1, May 2, 1985.
3. Lis, H.; Sharon, N. *Chem. Rev*. **1998**, *98*, 637.
4. Banerjee, R.; Das, K.; Ravishankar, R.; Suguna, K.; Surolia, A.; Vijayan, M. *J. Mol. Biol*. **1996**, *259*, 281.
5. Shaanan, B.; Lis, H.; Sharon, N. *Science* **1991**, *254*, 862.
6. Dessen, A.; Gupta, D.; Sabesan, S.; Brewer, C. F.; Sacchettini, J. C. *Biochemistry* **1995**, *34*, 4933.
7. Elgavish, S.; Shaanan, B. *J. Mol. Biol*. **1998**, *277*, 917.
8. Details of the chemical syntheses will be reported elsewhere. Physical data of compound **1** correspond to those reported in the recent literature: e.g., Lee, R. T.; Lee, Y. C. *Carbohydr. Res*. **1994**, *251*, 69. Gambert, U.; Lio, R. G.; Farkas, E.; Thiem, J.; Bencomo, V. V.; Liptak, A. *Bioorg. Med. Chem*. **1997**, *5*(7), 1285.
Selected physical data for **2**: TLC (CH_2CI_2/MeOH, 91:9): R_f = 0.15; IR: ν = 3319.5, 2948.3, 2162.7, 1629.0, 1538.1 cm^{-1} — ^1H NMR (400 MHz, D_2O): δ 6.03 (m, IH, H2), 5.37 (m, 2H, H3″), 5.09 (s, 1H, H5), 5.01 (d, 1H, $J_{1,2}$ = 3.01, H1), 4.59 (m, 3H, $J_{2,3}$ = 10.54, H2, H3, H4), 4.48 (d, 1H, $J_{1'2'}$ = 8.0, H1′), 4.31 (m, 1H, H1″), 4.13 (m, 1H, H1″), 3.95 (d, 1H, $J_{3',4'}$ = 3.51, H4′), 3.81 (m, 2H, H6′), 3.71 (dd, 1H, $J_{5',6a'}$ = 4.51, $J_{5',6b'}$ = 7.53, H5′), 3.65 (dd, 1 H, $J_{2',3'}$ = 10.03, H3′), 3.52 (m, 2H, H2′, H6a), 3.42 (dd, 1H, $J_{5,6b}$ = 1.01, $J_{6a,6b}$ = 12.55, H6b), 2.06 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, D_2O): δ 175.13 (CO), 133.91 (C2″), 118.58 (C3″), 105.43 (Cl′), 97.80 (C1), 75.54 (C5′), 75.29 (C3′), 75.11 (C5), 72.95, 70.93 (C2′), 69.24 (C1″), 69.01 (C4′), 63.06 (C4), 61.37 (C6′), 48.81 (C2), 43.70 (C6), 22.38 (CH_3). Selected physical data for peracetylated **3**: TLC (ethyl acetate): R_f = 0.29; LR: ν = 3308.0, 2938.0, 1750.1, 1654.2, 1544.2 cm^{-1}. ^1H NMR (400 MHz) δ 5.84(m, 1H, H2″), 5.52 (d, 1H, $J_{NH,2}$ = 8.95, NHAc), 5.34 (dd, 1H, $J_{3',4'}$ = 3.53, $J_{4',5'}$ = 0.95, H4′), 5.23 (m, 2H, H3″), 5.06 (dd, 1H, $J_{1',2'}$ = 8.01, $J_{2'3'}$ = 10.36, H2′), 4.99(d, 1H, $J_{1,2}$ = 2.83, H1), 4.94 (dd, 1H, H3′), 4.72 (ddd, 1H, $J_{2,3}$ = 10.13, H2), 4.59 (d, 1H, H1′), 4.56 (t, 1H, $J_{5,6a}$ = 4.71, H5), 4.40 (t, 1H, H4*), 4.14 (m, 3H, H6′*, H1″), 3.97 (m, 1H, H1″), 3.91 (dd, 1H, *J* = 6.12, H3*), 3.85 (dt, 1H, H5′), 3.42 (dd, 1H, H6a), 2.59 (d, 1H, $J_{6a,6b}$ = 10.12, H6b), 2.19, 2.10, 2.08, 2.03, 1.99, (5s, 15H, $5COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.37, 170.27, 170.18, 169.54, 133.54 (C2″), 117.94 (C3″), 100.95 (C1), 97.21 (C1), 72.96, 72.04, 70.86, 70.84, 68.63 (C1″), 68.55, 66.92, 61.27 (C6′), 49.54, 43.45, 28.48 (C6), 23.45, 20.68, 20.60, 20.55; MS (FL, 7 kV, 3 mA, 120–170 °C): *m*/*z* (rel. intensity) 590.2 (100), 488.3 (10), 142.1 (20); $C_{25}H_{35}NO_{13}S$ (589.62). Coupling constants are given in Hz, the assignment of signals containing (*) may be interchanged. Assignments containing (′) refer to the galactose moiety, (″) to the allyl aglycon and the others to the GalNAc residue.
9. Hill, J.; Hough, L.; Richardson, A. C. *Carbohydr. Res*. **1968**, *8*, 19.
10. Wenzl, I.; Kählig, U.; Unger, F. M.; Schmid, W. *Monatsh. Chem*. **1999**, *130*, 1137.
11. Bayer, E. A.; Wilchek, M. *Meth. Enzymol*. **1990**, *184*, 138.
12. Duk, M.; Lisowska, E.; Wu, J. H.; Wu, A. M. *Anal. Biochem*. **1994**, *221,* 266. For quantification of bound biotinylated lectin, streptavidin-horseradish peroxidase/ABTS/H_2O_2 was used in the assay system. IC_{50} values given are mean values of at least three independent experiments.
13. Adar, R.; Moreno, E.; Streicher, H.; Karlsson, K.-A.; Angstrom, J.; Sharon, N. *Prot. Sci*. **1998**, *7*, 52.