Carbohydrate Research, 205 (1990) c12–c17
Elsevier Science Publishers B.V., Amsterdam

Preliminary communication

The effect of substituting key hydroxyl groups by amino groups on the binding of the Lewis b tetrasaccharide by a lectin and a monoclonal antibody*

Raymond U. Lemieux, Roman Szweda†, Eugenia Paszkiewicz-Hnatiw†, and Ulrike Spohr‡

Department of Chemistry, University of Alberta, Edmonton, Alberta, T6G 2G2 (Canada)

(Received April 13th, 1990; accepted for publication June 20, 1990)

In an early binding study[1], using an affinity-purified polyclonal anti-β-D-Gal antibody reagent, both methyl 4-deoxy- and 4-chloro-4-deoxy-β-D-galactopyranoside were found to be much inferior inhibitors of the binding of a β-D-galactopyranoside artificial antigen [0.2–0.3% of the potency exhibited by β-D-Gal-OMe (**1**)] than was methyl 4-amino-4-deoxy-β-D-galactopyranoside (**2** 14% of the potency of **1**). In view of our more recent studies of the binding of oligosaccharides by monoclonal antibodies and lectins[2], it can be expected that OH-4 together with OH-3 provided the key polar grouping for the binding reaction of **1**. Furthermore, it seems likely that the interaction was with the carboxylate group of an aspartate unit, as was found to be the case for the key OH-3 and OH-4 groups of the β-D-Gal unit of the Lewis b human blood group determinant when bound by the lectin IV of *Griffonia simplicifolia* (GSIV)[3–5]. The key polar interaction for this binding reaction is displayed in Fig. 1 for the methyl glycoside (Le^b-OMe) of the Lewis b blood group related tetrasaccharide.

In view of the appreciable activity exhibited by the aminogalactoside **2**, it was apparent that substantial activity may be maintained upon substitution of a key hydroxyl group by an amino group. This possibility found support in the observation by Rahuel *et al.*[6] that *N*-deacetylation of the α-D-GalNAc unit of the A human blood group determinant led to an acquired B-activity. The B human blood group determinant has a hydroxyl group in the place of the acetamido group of the A epitope. Later, it was found that this OH-2 of the α-D-Gal unit of the B trisaccharide was strongly involved in the key polar grouping for binding by two different monoclonal anti-B antibodies[7]. Therefore, it became of interest to establish the effect of replacing OH-3b and OH-4b of the Le^b-OMe by an amino group on the binding by GSIV, especially since the crystal

* Dedicated to Professor Leslie Hough in the year of his 65th birthday.
† University of Alberta postdoctoral fellow, 1987–89.
‡ Research Associate, 1984–88.

0008-6215/90/$ 03.50

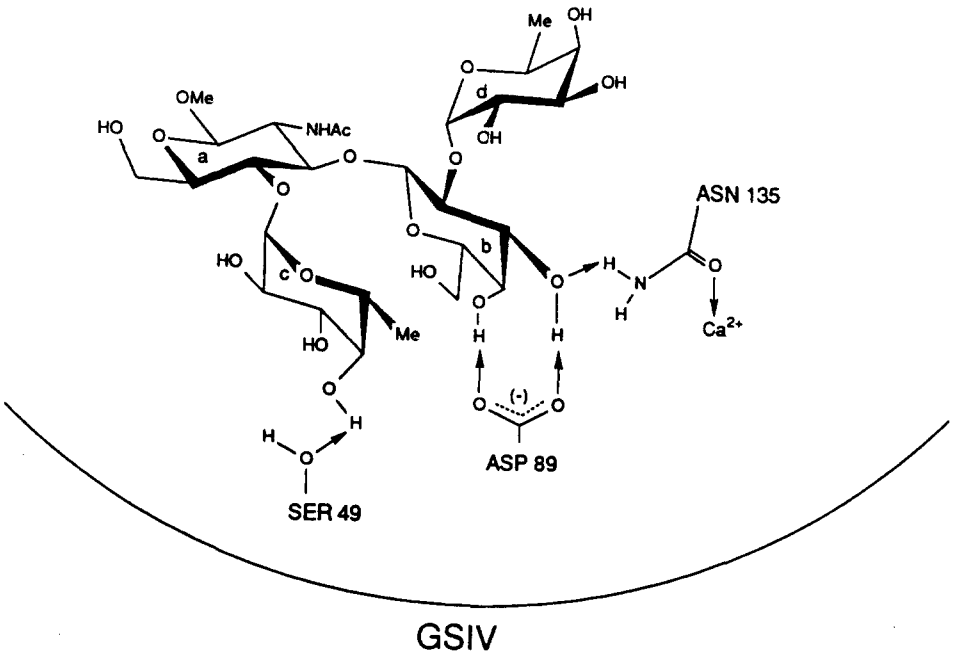


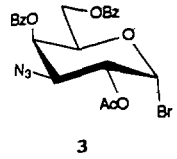
Fig. 1. The key polar interaction of Le^b-OMe(α-L-Fuc(1→2)-β-D-Gal(1→3)-[α-L-Fuc(1→4)]-β-D-GlcNAc-OMe) with the combining site of the lectin GSIV[5].

structure for the GSIV (Le^b-OMe)$_2$ complex is known[5] and the key polar interaction is definitely that displayed in the partial structure presented in Fig. 1.

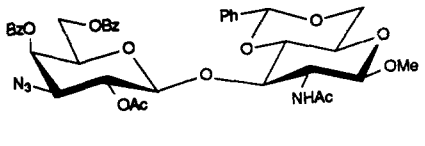
3b-Amino-3b-deoxy-Le^b-OMe (**8**) was synthesized starting from the known 3-azido-3-deoxy-1,2-*O*-isopropylidene-6-*O*-benzoyl-α-D-glucofuranose[8]. The isopropylidene group was removed by hydrolysis in 90% trifluoroacetic acid and the product*, 3-azido-6-*O*-benzoyl-3-deoxy-D-glucopyranose, was isopropylidenated in the usual manner and, as expected from the work of Meyer zu Reckendorf and Spohr[9], provided 3-azido-6-*O*-benzoyl-3-deoxy-1,2-*O*-isopropylidene-α-D-glucopyranose, m.p. 129–130°, $[\alpha]_D$ +132° ($CHCl_3$). The presence of the azido group was displayed by i.r. absorption at 2050 cm^{-1}. The free hydroxyl group was mesylated for replacement by benzoate with inversion of C-4. Removal of the isopropylidene group and acetylation followed by treatment with HBr in AcOH then provided the reagent (**3**) desired for glycosylation of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside using $Hg(CN)_2$ as promoter to form the substituted disaccharide **4**, m.p. >245° (dec.), $[\alpha]_D$ −19° ($CHCl_3$).

The benzylidene group of **4** was removed by hydrolysis in 70% aq. AcOH at 70° and the resulting diol was preferentially benzyloxymethylated[10] at the free primary

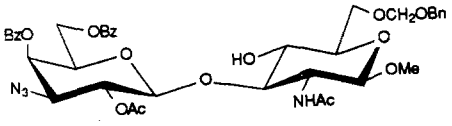
* All products described were chromatographically homogeneous, gave satisfactory elemental analysis, and their ^{1}H-n.m.r. spectra (300 or 400 MHz) substantiated their homogeneity and could be assigned in detail.



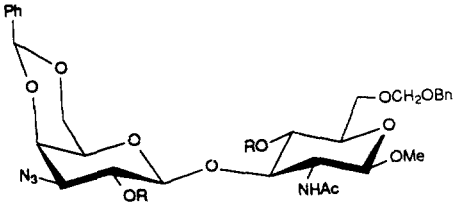
3



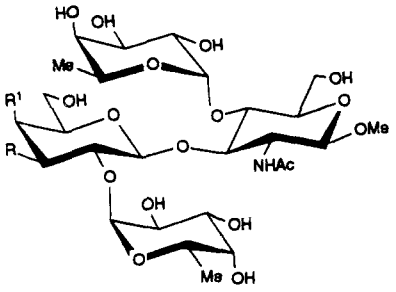
4



5



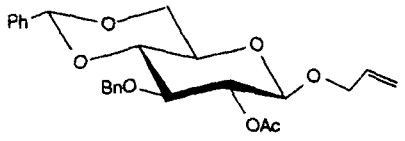
6 R = H
7 R = Bn_3-α-L-Fuc



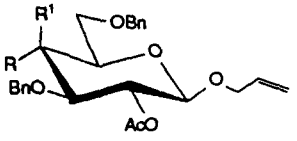
8a R = NH_3^+, R^1 = OH
8b R = NH_2, R^1 = OH
17a R = OH, R^1 = NH_3^+
17b R = OH, R^1 = NH_2

hydroxyl group of the β-D-GlcNAc residue to afford crystalline **5**, m.p. 206–207°, $[\alpha]_D$ +42° ($CHCl_3$) in 93% yield. Zemplén deacylation followed by benzylidenation using α,α-dimethoxytoluene in the presence of *p*-toluenesulfonic acid then provided compound **6**, which was subjected to bromide-ion catalyzed tri-*O*-benzyl-α-L-fucopyranosylation under the usual conditions[4] to form **7** in 90% yield. Reduction of **7** with hydrogen over palladium-on-carbon in the presence of one equivalent of HCl produced the desired product (**8a**) in 63% yield after purification by gel filtration.

4b-Amino-4b-deoxy-Le^b-OMe (**17**) was synthesized starting from 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-α-D-glucopyranosyl bromide[11] to prepare allyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranoside, m.p. 81.5–82°, $[\alpha]_D$ −34° ($CHCl_3$) under Koenigs–Knorr type conditions. Deacetylation to selectively remove the 4- and 6-*O*-acetyl groups was achieved by treatment with 3.2% Et_3N in 84% aq. MeOH for 26 h at 4°. The



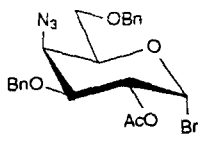
9



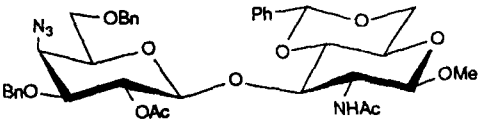
10 R = OH, R^1 = H
11 R = H, R^1 = N_3

^{1}H-n.m.r. singlet for the remaining 2-*O*-acetyl group was at 2.02 p.p.m. The oily product was reacted with α,α-dimethoxytoluene in acetonitrile containing *p*-toluenesulfonic acid to provide the 4,6-*O*-benzylidene derivative **9** in 77% yield, m.p. 120–121°, $[\alpha]_D$ −25° ($CHCl_3$).

Reduction of **9** with sodium cyanoborohydride in the presence of HCl[4,12] provided allyl 2-*O*-acetyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (**10**), m.p. 60.5–61.5°, $[\alpha]_D$ −28° ($CHCl_3$). The hydroxyl group of **10** was replaced by azide with inversion of C-4 in near 50% yield by way of either the 4-*O*-mesylate or 4-*O*-triflate derivative. The allyl aglycon of the 4-azido-D-*galacto* compound (**11**), m.p. 47.5–48.5°, $[\alpha]_D$ −16.5 ($CHCl_3$), was replaced by hydrogen by rearrangement to the 1-*O*-propenyl glycoside using tris-(triphenylphosphine)rhodium (I) chloride in the presence of 1,4-diazabicyclooctane followed by hydrolysis using $HgCl_2$–HgO[13,14]. The product was treated with oxalyl bromide in CH_2Cl_2 and *N*,*N*-dimethylformamide to produce the glycosyl bromide **12**, which was used for the preparation of **13** in 71% yield, m.p. 258–259° (dec.), $[\alpha]_D$ +0.2° ($CHCl_3$), under the conditions described for the preparation of **4**.



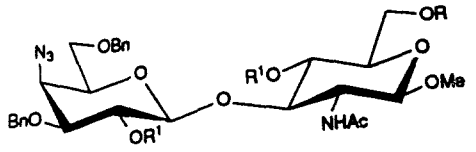
12



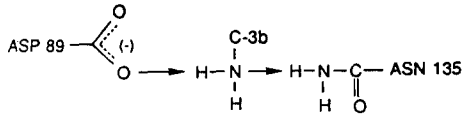
13

As already described for the preparation of **5**, compound **13** was deacetylated and hydrolyzed to provide the triol **14**, which was preferentially benzyloxymethylated to form **15**. Tri-*O*-benzyl-α-L-fucopyranosylation under bromide-ion catalyzed conditions then gave **16**, which was reduced in the presence of HCl to provide the desired amine **17a**. The hydroxyl group at the 2′-position proved highly resistant to fucosylation and the yield of **16** was only 34%.

A comparison of the ^{1}H-n.m.r. parameters for the free bases **8b** and **17b** with those previously reported[4] for Le^b-OMe confirmed the structural assignments. The chemical shifts for the hydrogens of the β-D-GlcNAc-OMe unit were the same within 0.02 p.p.m., except for the acetyl group of **8b**, which was shielded by 0.05 p.p.m. The situation was



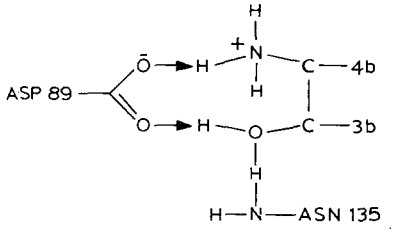
14 R = R^1 = H
15 R = $BnOCH_2$, R^1 = H
16 R = $BnOCH_2$, R^1 = Bn_3-α-L-Fuc



18

similar for the ring hydrogens of the two fucose units, except that the chemical shift for H-1c of **17b** was deshielded by 0.08 p.p.m. from the shift observed for H-1c of Le^b-OMe. In the case of the H-1d hydrogens, that of **8b** was shielded by 0.13 p.p.m. As expected, the major changes were in the chemical shifts for the ring hydrogens of the β-D-Gal b-unit. In the case of **8b**, H-3b was shielded by 0.64 p.p.m. whereas for **17b** H-4b was shielded by 0.74 p.p.m. For the 3-amino compound **8b**, H-2b was shielded by 0.07 p.p.m. and H-4b shielded by 0.09 p.p.m. In the case of the 4-amino compound **17b**, H-2b was shielded by 0.20 p.p.m. and H-3b deshielded by 0.10 p.p.m. The signals for the other hydrogens were not assigned.

It could be imagined that substitution of OH-3b of Le^b-OMe by an amino group to form **8** would lead to the hydrogen-bond network (**18**) on binding by the GSIV lectin, especially at pH 8 where the amino group should exist extensively as the free base. However, the compound was found to be completely inactive in the pH range 5–8 using a solid-phase competitive radioimmunoassay[3]. Indeed, a 1500μM solution provided slightly negative potencies, indicating a slight increase in nonspecific binding of the radioactive artificial antigen to the immobilized lectin. Therefore, the confines of the combining site cannot entertain an amino group to form the type of complex illustrated by **18**. Substitution of OH-4b of Le^b-OMe by an amino group to form **17** also provided a



19

highly inactive compound. Consequently, probably because of the need for hydration, the combining site also cannot adopt the type of complex represented by **19**.

The key hydroxyl groups for the binding of Le^b-OMe by a monoclonal antibody were found to be OH-3b of the β-D-Gal unit and OH-2d of the α-L-Fuc (1→2b) unit[15]. In view of the above-mentioned results with the lectin GSIV, it could be expected that **8** would be inactive as an inhibitor. This was the case at pH 5, since a 3000μM concentration produced no detectable inhibition. The inhibition rose with increasing pH and, by extrapolation, it was estimated that the 6000μM concentration would provide near 50% inhibition at pH 8. On this basis, **8** is almost 300 times less potent than Le^b-OMe at pH 8 and even less potent with protonation of the amino group at lower pH values.

The replacement of OH-4b by hydrogen led to a weak inhibitor of the binding of the Le^b artificial antigen by the monoclonal anti-Le^b antibody[15,16]. It was concluded that the involvement of the hydroxyl group in the binding reaction was marginal in character and to become involved as proton acceptor for intramolecular hydrogen bonding with OH-6b when the Le^b-OMe tetrasaccharide was bound by the antibody. On the basis of these observations, it was to be expected that replacement of OH-4b by an amino group to provide **17** would result in a weaker inhibitor, but especially so in acid media where the hydrogen bonding of the ammonium group with water would be strong and participation of OH-4b as a proton acceptor for hydrogen bonding with OH-6b would be effectively eliminated. In fact, in agreement with expectation, the potency of **17** relative to that for Le^b-OMe was only 8% at pH 5 but rose to 48% at pH 8. It is to be noted in this regard that interactions with combining sites which appear to require intramolecular hydrogen bonding[2] [judging from the crystal structure at 2.8 Å resolution for the $(Le^b\text{-OMe})_2$ GSIV complex[5]] may actually be the result of changes in the hydration of the complex about the periphery of the combining site, since these hydroxyl groups appear to be in only marginal contact with nonpolar groups of the protein.

ACKNOWLEDGMENTS

This research was supported by the Natural Sciences and Engineering Research Council of Canada (Grant A-172 to R.U.L.) and a special grant to R.U.L. from the Alberta Heritage Foundation for Medical Research.

REFERENCES

1 R. U. Lemieux, P. H. Boullanger, D. R. Bundle, D. A. Baker, A. Nagpurkar, and A. Venot, *Nouv. J. Chim.*, 2 (1978) 321–329.
2 R. U. Lemieux, *Chem. Soc. Rev.*, 18 (1989) 347–374.
3 U. Spohr, O. Hindsgaul, and R. U. Lemieux, *Can. J. Chem.*, 63 (1985) 2644–2652.
4 U. Spohr and R. U. Lemieux, *Carbohydr. Res.*, 174 (1988) 211–237.
5 L. T. J. Delbaere, M. Vandonselaar, L. Prasad, J. W. Quail, P. V. Nikrad, J. R. Pearlstone, M. R. Carpenter, L. B. Smillie, U. Spohr, and R. U. Lemieux, *Can. J. Chem.*, 68 (1990) 1116–1121.
6 C. Rahuel, A. Lubineau, S. David, C. Salmon, and J. P. Cartron, *Rev. Fr. Transfus. Immuno-hematologie*, 26 (1983) 347–358.
7 R. U. Lemieux, A. P. Venot, U. Spohr, P. Bird, G. Mandal, N. Morishima, and O. Hindsgaul, *Can. J. Chem.*, 63 (1985) 2664–2668.