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# 3-AZI-1-METHOXYBUTYL β-D-GALACTOPYRANOSIDE, A PHOTOAFFINITY LABEL FOR MONOCLONAL ANTIGALACTAN ANTIBODIES OF THE $V_H$ GAL 39.1/55.1 GENE-FAMILY*

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ABSTRACT

3-Azi-1-methoxybutyl β-D-galactopyranoside, prepared from 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in four steps, had an affinity constant for antigalactans IgA (Fab′) J539 and X24 of 1550 and 1730 $M^{-1}$, respectively. 3-Azi-1-methoxybutyl β-D-(4-$^3$H)galactopyranoside, when photolysed in the presence of IgA X24, specifically labelled the galactan-binding area of the immunoglobulin.

## INTRODUCTION

The evaluation of the role of hydrogen bonding in the binding of saccharides with antibodies has led to a report[1,2] of the affinity of deoxyfluorogalactopyranosides for monoclonal antibodies having galactan specificity. Recently, attention has been directed to the use of affinity labels, such as *p*-nitrophenyl[3] and 2,3-epoxypropyl[4,5] glycosides of (1→6)-linked-β-D-galacto-oligosaccharides in order to gain insight into the antigen–antibody interaction. Of particular interest would be the determination of the distance between galactosyl-binding subsites through a correlation of sequence data on labelled antibody with the known X-ray structure[6] of the IgA J539 (Fab′).

An ideal affinity label must be stable while it is positioned in the combining site of the protein. In photoaffinity labelling[7], the reactivity remains masked until it is activated by photolysis. Indiscriminate reactivity of the ligand with the protein is preferable to selective reactivity with certain amino acid side-chains. Clearly, in the latter case, the (biased) selectivity would give a more ambiguous answer to the question of *sub*-site location. Thus, it would be desirable to use a ligand incorporating a photoactive group which (*a*) is sufficiently small so as not to perturb the

* Dedicated to Professor Hans Paulsen
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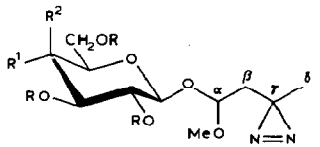
interactions necessary for binding of ligand to antibody, (*b*) is chemically inert until activation by photolysis, (*c*) can be activated at wavelengths >300 nm in order to minimize photo-damage to the protein, and (*d*) gives rise to a highly reactive species capable of indiscriminate reaction with the protein. We were therefore interested in 3-azi-1-methoxybutyl β-D-galactopyranoside (**1**) as a potential photoaffinity label for monoclonal anti-(1,6)-β-D-galactopyranans. Upon irradiation (310–320 nm), the diazirino moiety decomposes to afford a carbene and liberates molecular nitrogen[8–10]. Subsequently, the carbene may be captured by covalent insertion into proximal amino acids lining the combining area, disproportionate to an olefin, or react with water to yield an alcohol.

## RESULTS AND DISCUSSION

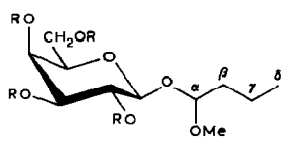
The affinity of antigalactan monoclonal IgA's for such ligands as **1** can be determined by fluorimetric titration[11,12]. Unfortunately, the emission wavelength (~334 nm) of the tryptophan in the protein coincides with a strong absorption band of the diazirine. Thus, it is necessary to correct the observed changes in fluorescence by determining the molar quenching due to the added diazirine. Moreover, for the equilibrium *1*,

$$\text{Fab}' + \text{Ligand} \leftrightarrows \text{Fab}' \cdots \text{Ligand} \qquad (1)$$

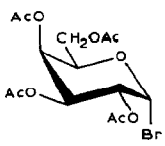
any covalent bonding between the protein and the carbene photogenerated from the diazirine *during* fluorimetric titration would appear as an apparently higher $K_a$ for the ligand in question. The degree of quenching caused by the diazirine can be measured readily by titrating an unrelated monoclonal antibody with a non-binding ligand such as 3-azi-1-methoxybutyl β-D-glucopyranoside (**2**). The $K_a$ of the antigalactan monoclonal antibody can be determined with a ligand mimicking **1** but lacking the diazirino moiety, for example, 1-methoxybutyl β-D-galactopyranoside (**3**). A close similarity in the $K_a$ values for **1** and **3** would indicate little if any covalent



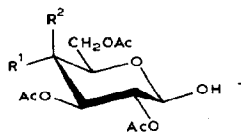
**1** R = R¹ = H, R² = OH
**2** R = R² = H, R¹ = OH
**9** R = Ac, R¹ = H, R² = OAc
**10** R = Ac, R² = H, R¹ = OAc



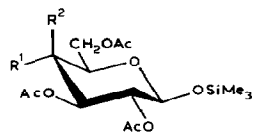
**3** R = H
**11** R = Ac



**4**



**5** R¹ = H, R² = OAc
**6** R¹ = OAc, R² = H



**7** R¹ = H, R² = OAc
**8** R¹ = OAc, R² = H

binding between substrate and protein during the actual fluorimetric titration.

The *galacto* (1) and *gluco* (2) diazirines were prepared by a modification of the procedure of Kurz *et al.*[13], which led to improvements in the yields. Hydrolysis of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (4) afforded the β-anomer **5** stereoselectively and in excellent yield. Treatment[14] of **5** with *N*-trimethylsilylacetamide yielded a 2:1 mixture of the trimethylsilyl derivative **7** and its α-anomer. However, treatment[15] of **5** with a solution of hexamethyldisilazane, chlorotrimethylsilane, and pyridine in ether furnished 90% of **7** after purification by flash column chromatography[16] and short-path distillation under reduced pressure. Reaction of **7** with 3-azibutyraldehyde dimethyl acetal[17] (**12**, obtained from 3-oxobutyraldehyde dimethyl acetal in 50% yield) by trimethylsilyl trifluoromethanesulfonate (triflate)-catalyzed transacetalation afforded **9** as a 1:1 mixture of diastereoisomers epimeric at C-α in 73% yield. The transacetalation, which was sluggish at −78°, could not be accelerated by warming since higher temperatures promoted the anomerization of **7** (under the conditions here reported, 20% of **7** anomerized during the condensation reaction). Indeed, the reaction stopped typically after 10–11 h and the addition of more (0.1 equiv.) trimethylsilyl triflate was required every 12 h until most of **7** had reacted. The mixture of diastereomers **9** was unresolvable by t.l.c., and was deacetylated (Zemplén) to give 96% of a ~1:1 mixture of diastereomers **1**. The $^1$H- and $^{13}$C-n.m.r. data for **9** and **1** were consistent with the assigned structures.

The known[13] *gluco*-diazirine **2** was prepared in a similar manner, starting with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside[18,19] (**6**). 2D Homo- (COSY) and heteronuclear correlation (HETCOR) n.m.r. experiments were performed on the tetra-acetate **10**. Based on the results and on those of 1D homonuclear decoupling experiments, assignments are presented here for the $^1$H- and $^{13}$C-n.m.r. data for *gluco* derivatives **10** and **2**, and correct an accidental interchange of values in an earlier report[13]. Condensation of **7** with butyraldehyde dimethyl acetal in the presence of trimethylsilyl triflate furnished 58% of the diazirino-defecient *galacto* analog **11**. Zemplén deacetylation of **11** gave the desired compound **3**.

The diastereomeric nature of **1**–**3** and **9**–**11** is evident from the pair of doublets for H-1 in the $^1$H-n.m.r. spectra, and the $J_{1,2}$ values of 7.5–8.0 Hz are indicative of the β configuration.

The tryptophanyl fluorescence[11,12] of monoclonal IgA (Fab′) J539 was perturbed maximally (after appropriate corrections) by 42% ($\Delta F_{max}$) by the *galacto*-diazirine **1** to give a $K_a$ of $1.55 \times 10^3$ M$^{-1}$ (Fig. 1). This affinity constant is essentially identical to that of the diazirino-deficient *galacto* derivative **3** ($K_a$ $1.57 \times 10^3$ M$^{-1}$; $\Delta F_{max}$ 14%; Fig. 1). Thus, no conversion into covalently bound ligand took place during the fluorimetric titration. For IgA X24, the $K_a$ with **1** was $1.73 \times 10^3$ M$^{-1}$ ($\Delta F_{max}$ = 35%).

Radioactively labeled **1** (called **1***), with $^3$H at position 4′, was prepared by NaB$^3$H$_4$ reduction of 2,3:5,6-di-*O*-isopropylidene-D-*xylo*-hexos-4-ulose 1,1-(dimethyl acetal)[20], deprotection, and conversion of the D-(4-$^3$H)galactose into **1*** *via* condensation of the trimethylsilyl glycoside of its 2,3,4,6-tetra-acetate with 3-azibu-

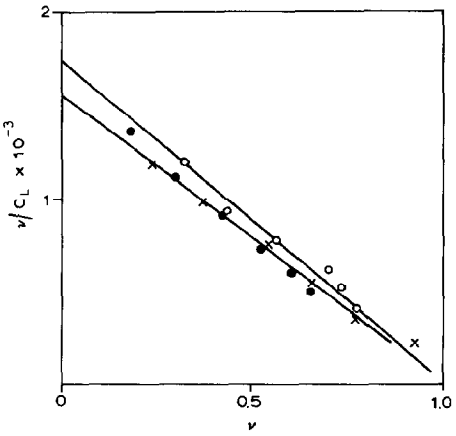



Fig. 1. Scatchard plots for the binding of 3-azi-1-methoxybutyl β-D-galactopyranoside (**1**) to IgA J539 (Fab') (•) and IgA X 24 (o), and 1-methoxybutyl β-D-galactopyranoside (**3**) to IgA J539 (Fab') (×). For details, see text and refs. 11 and 12.

tyraldehyde dimethyl acetal.

(1→6)-β-D-Galactan-binding monoclonal antibodies have H-chains which are encoded by either the $V_H$ GAL 39.1 or the $V_H$ GAL 55.1 gene[21] and have similar affinities and binding sub-site patterns[22]. The latter gene encodes the H-chain of X24 directly, and whole IgA X24 was therefore used in labelling experiments with **1***. A solution [7.2 mg/mL; $A_{280}$ 10; $4.6 \times 10^{-5}$ M] of affinity-purified[23] IgA X24 in phosphate-buffered saline (PBS, pH 7.4) was used to dissolve **1*** to a final concentration of $3.1 \times 10^{-3}$ M. Thus, the excess molar concentration of **1*** over that of antibody sites (equiv. wt. 77,000 Dalton/site) was 33 times, and therefore [free ligand] ≈ [added ligand]. For Absite + L(igand) ⇆ Absite···L, $K_a = c_{\text{Absite···L}}/c_{\text{Absite}} \times c_L$; for X24 and **1**, the $K_a$ was $1.73 \times 10^3$ M$^{-1}$. Thus, the ratio $c_{\text{Absite···L}}/c_{\text{Absite}} = 5.4$, and ~84% of the antibody sites are occupied at all times with **1***, and this was called solution *A*. Solution *A* was diluted 1:1 with PBS (which results in a 73% occupation of its antibody sites by **1***) and was made $0.8 \times 10^{-3}$ M in the methyl β-glycoside of (1→6)-β-D-galactotetraose ($MeGal_4$)[24]. The $K_a$ of the latter compound with X24 is $0.5 \times 10^6$ M$^{-1}$ (see ref. 22). Thus, the ratio $K_a$ ($MeGal_4$)/$K_a$ (**1***) = $0.3 \times 10^3$. Since their concentrations are of the same order of magnitude (0.8 and $1.5 \times 10^{-3}$ M, respectively), the $MeGal_4$ will almost completely displace **1*** from the combining site. This was called solution *B*. Both solutions *A* and *B* were irradiated at 300–350 nm, extensively dialysed, and then subjected to SDS slab gel electrophoresis[25,26] under reducing and denaturing conditions. Two radioactive areas were observed for solution *A* (Fig. 2), one in the general area of the H-chain (~57% of the total incorporated radioactivity) and one in the area of the L-chain (~43% of the incorporated radioactivity). In solution *B*, the incorporation of **1*** was blocked by the addition of the $MeGal_4$, showing that **1*** in solution *A* had bound in the combining site of the immunoglobulin. The carbene formed upon

irradiation of **1*** is extremely reactive and is located on the very flexible acetal-aglycon. Thus, it is not unexpected that this flexibility may result in the insertion of this carbene into both the H and L chain, as the general combining area of antibodies is known to occur near the H/L interface[27]. Alternatively, it is conceivable that the *R* and *S* forms of **1*** may orient themselves differently on the protein, perhaps causing insertion into either the H or the L chain.

## EXPERIMENTAL

*General methods.* — Melting points were determined with a Buchi melting-point apparatus and are uncorrected. Optical rotations were measured at 25° with a Perkin-Elmer automatic polarimeter Model 241 MC. T.l.c. was performed on Silica Gel 60 $F_{254}$ (Merck, 5554), using the solvents indicated, and visualization was effec-

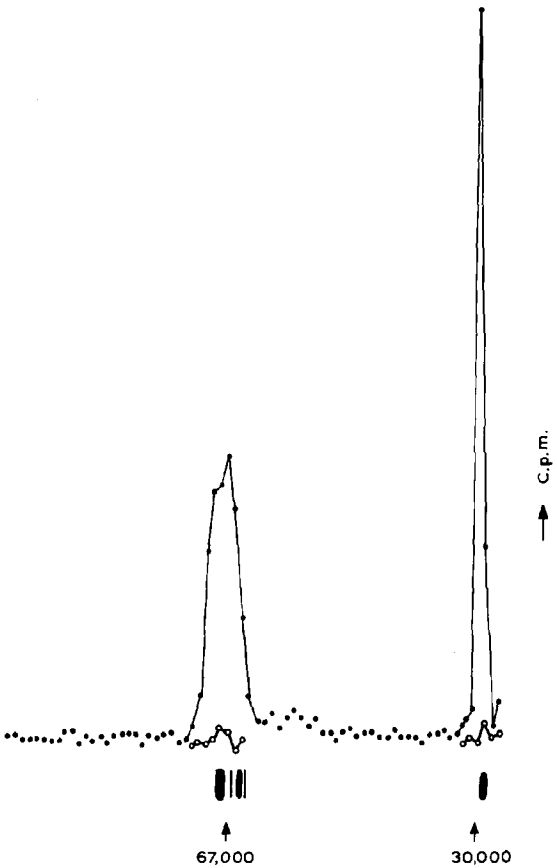



Fig. 2. SDS-PAGE of IgA X-24 after u.v.-catalyzed labelling with **1***. Counts show incorporation of radioactivity in both H- and L-chains, and failure of incorporation when u.v.-catalyzed labelling was attempted in the presence of methyl (1→6)-β-D-galactotetraoside (bold line).

ted by charring with 5% $NH_4Mo_7O_{24}\cdot 4H_2O$ in aqueous 10% sulfuric acid. Silica Gel 60 (230–400 mesh, Merck, 9385) was used for flash column chromatography[17].

$^1H$- and $^{13}C$-n.m.r. spectra (internal $Me_4Si$) were recorded at room temperature with a Varian FX-100, HR-220, or FX-300 spectrometer, as indicated. C.i.(ammonia)-mass spectra were recorded with a Finnegan 1015 mass spectrometer. Elemental analyses were performed either by Atlantic Microlab, Inc. (Atlanta, GA) or Galbraith Laboratories, Inc. (Knoxville, TN).

Radioactive material was detected either radioautographically (Agfa-Geveart Curix X-ray film) or with a Berthold t.l.c.-linear analyzer LB 282. Radioactive samples in solution were assayed in a Berthold BF-815 liquid scintillation counter, using the scintillator indicated. H.p.l.c. was performed using an LKB 2152 h.p.l.c. controller, two LKB 2150 pumps, a Rheodyne 7126 injector, a Knauer/Shandon Hypersil ODS column (250 × 4.0 mm, 5 μm), an LKB 2151 variable wavelength monitor, an LKB 2211 SuperRac fraction-collector, and a Shimadzu C-R2AX integrator.

Trimethylsilyl trifluoromethanesulfonate was distilled at 2 kPa prior to use. Pyridine and triethylamine were distilled from $CaH_2$, ether from Na/benzophenone, dichloromethane from $P_2O_5$, and methanol from Mg/turnings. The light petroleum had b.p. 35–60°. Ligand-induced fluorescence changes of the IgA $(Fab')^2$[28] J539 and of whole IgA X24 were measured[11,12] with a Perkin-Elmer MPF-3 spectrometer.

*2,3,4,6-Tetra-O-acetyl-β-D-galactose* (**5**). — Prepared using the procedure of Bredereck *et al.*[29], **5** (90%) had m.p. 127–128° (from ether–acetone). N.m.r. data ($CDCl_3$): $^1H$ (220 MHz), δ 2.00, 2.05, 2.10, 2.16 (4 s each 3 H, 4 OAc), 3.88 (d, 1 H, *J* 9 Hz, exchanges with $CD_3OD$, OH) 3.98 (dt, 1 H, $J_{4,5}$ 1.5, $J_{5,6a} = J_{5,6b} = 6.5$ Hz, H-5), 4.17 (d, 2 H, $J_{5,6a} = J_{5,6b} = 6.5$ Hz, H-6a,6b), 4.68–4.75 (m, 1 H, H-1), 5.04–5.15 (m, 2 H, H-2,3), 5.39–5.45 (m, 1 H, H-4). On addition of $CD_3OD$, the signal at δ 4.68–4.75 collapsed to a d ($J_{1,2}$ 7 Hz); $^{13}C$ (75 MHz), δ 20.55, 20.63, 20.68, 20.80 (4 $CH_3CO$), 61.49 (C-6), 67.21 (C-4), 70.52, 70.98 (C-2, C-3, and C-5), 95.93 (C-1), 170.09, 170.28, 170.60, 170.95 (4 $CH_3CO$).

*Trimethylsilyl 2,3,4,6-tetra-O-acetyl-β-D-galacto-* (**7**) *and -gluco-pyranoside* (**8**). — To a stirred syspension[15] of 2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranose (1 equiv., 0.17M) in anhydrous ether under argon were added successively hexamethyldisilazane (1 equiv.), chlorotrimethylsilane (1.2 equiv.), and pyridine (1 equiv.). The resulting mixture was stirred at 25°, and the reaction was monitored by t.l.c.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranose (**5**; 3.0 g, 8.62 mmol) was treated as described above. After 30 min, t.l.c. (light petroleum–ethyl acetate, 4:1) indicated the absence of **5**. Concentration of the reaction mixture gave a viscous white suspension, which was subjected to flash column chromatography (same solvent). Concentration of the appropriate fractions, followed by distillation at 130°/133 Pa, furnished **7** as a viscous, colourless syrup (3.26 g, 90%), $[\alpha]_D$ +8.5° (*c* 0.8, chloroform). N.m.r. data ($CDCl_3$): $^1H$ (220 MHz), δ 0.17 (s, 9 H, $Me_3Si$), 1.98, 2.04, 21.4 (3 s, 12 H, 4 OAc), 3.90 (overlapping dd, 1 H, $J_{5,6a}$ 7, $J_{5,6b}$ 6 Hz, H-5), 4.09 (dd, 1 H, $J_{6a,6b}$ 11, $J_{5,6b}$ 6 Hz, H-6b), 4.16 (dd, 1 H, $J_{6a,6b}$ 11, $J_{5,6a}$ 7 Hz, H-6a), 4.70 (d, 1 H,

$J_{1,2}$ 7.5 Hz, H-1), 4.98 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.5 Hz, H-3), 5.15 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 10.5 Hz, H-2), 5.36 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1 Hz, H-4); $^{13}C$ (75 MHz): $\delta$ 0.00 ($CH_3Si$), 20.47, 20.54, 20.68 ($CH_3CO$), 61.51 (C-6), 67.29 (C-4), 70.91, 70.98 (C-2, C-3, C-5), 96.11 (C-1), 169.29, 170.08, 170.16, 170.23 (4 $CH_3CO$). Mass spectrum: $m/z$ 438 $(M + NH_4]^+$.

*Anal.* Calc. for $C_{17}H_{28}O_{10}Si$: C, 48.55; H, 6.72. Found: C, 48.50; H, 6.74.

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranose[18,19] (**6**; 1.0 g, 2.87 mmol) was treated as described above. After 1 h, t.l.c. (light petroleum–ethyl acetate, 2:1) indicated the absence of **6**. Solvent removal and recrystallisation of the residue from light petroleum–ethyl acetate gave **8** (0.77 g, 64%) as white needles, m.p. 104–105°, $[\alpha]_D$ + 0.7° (*c* 1, chloroform); lit.[14] m.p. 98°, $[\alpha]_D$ + 8.0°). N.m.r. data ($CDCl_3$): $^1H$ (220 MHz), $\delta$ 0.16 (s, 9 H, $Me_3Si$), 2.00, 2.02, 2.03, 2.07 (4 s, each 3 H, 4 OAc), 4.12 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6b}$ 2.5 Hz, H-6b), 4.20 (dd, 1 H, $J_{6a,6b}$ 12, $J_{5,6a}$ 5.5 Hz, H-6a), 3.71 (ddd, 1 H, $J_{4,5}$ 10, $J_{5,6a}$ 5.5, $J_{5,6b}$ 2.5 Hz, H-5), 4.74 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.91 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9.5 Hz, H-2), 5.05 (t, 1 H, *J* 9.5 Hz, H-3 or H-4), 5.19 (t, 1 H, *J* 9.5 Hz, H-3 or H-4); $^{13}C$ (75 MHz): $\delta$ 0.00 ($CH_3Si$), 20.62, 20.69 ($CH_3CO$), 62.25 (C-6), 68.77 (C-4), 71.95 (C-2), 72.82 (C-5), 73.28 (C-3), 95.61 (C-1), 169.28, 169.43, 170.31, 170.58 (4 $CH_3CO$). Mass spectrum: $m/z$ 438 $[M + NH_4]^+$.

*Anal.* Found: C, 48.62; H, 6.72.

*Preparation of glycopyranosides* **9–11**. — With some modifications of the reported procedures[13,30], the β-glycopyranosides **9–11** were prepared as follows. To a stirred solution of a dimethyl acetal (0.5M, 2 equiv.) in anhydrous dichloromethane at −78° under argon was added successively trimethylsilyl triflate (0.1 equiv.) and a 0.16M solution of the trimethylsilyl β-D-glycopyranoside (1.0 equiv.) in the same solvent. An aliquot (0.1 equiv.) of trimethylsilyl triflate was added every 12 h until only a trace of the starting glycoside remained (t.l.c.). Triethylamine (slight excess over the total amount of trimethylsilyl triflate) was added to the mixture, which was then allowed to warm to room temperature, and poured into a 1:1 mixture of saturated aqueous sodium hydrogencarbonate and ether. The aqueous layer was extracted thoroughly with ether, and the combined extracts were washed with saturated aqueous sodium chloride, dried ($MgSO_4$), and concentrated at 2 kPa to give a viscous oil, which was subjected to flash column chromatography.

(*a*) *3-Azi-1-methoxybutyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside* (**9**). — Compound **7** (0.476 mmol) was treated for 73.5 h with 3-azibutylraldehyde dimethyl acetal (**12**) as described above. Flash column chromatography, using light petroleum–ethyl acetate (4:1), gave, initially, trimethylsilyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (40 mg), $R_F$ 0.5, m.p. 115–116° (from light petroleum), $[\alpha]_D$ +124° (*c* 0.8, chloroform). N.m.r. data: $^1H$ (220 MHz, $C_6H_6$), $\delta$ 0.068 (s, 9 H, $Me_3Si$), 1.66, 1.70, 1.77 (3 s, 12 H total, 4 OAc), 4.11–4.18 (m, 2 H, H-6a,6b), 4.27 (t, 1 H, $J_{5,6a} = J_{5,6b}$ = 6.5 Hz, H-5), 5.43 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 11 Hz, H-2), 5.61 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.64 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.5 Hz, H-4), 5.70 (dd, 1 H, $J_{2,3}$ 11, $J_{3,4}$ 3.5 Hz, H-3); $^{13}C$ (25 MHz, $CDCl_3$): $\delta$ 0.29 ($CH_3Si$), 20.97 ($CH_3CO$), 62.22 (C-6), 66.37 (C-4), 67.92, 68.57 (C-2 or C-3), 69.38 (C-5), 91.12 (C-1), 170.45,

170.56 ($CH_3CO$).

*Anal.* Calc. for $C_{17}H_{28}O_{10}Si$: C, 48.55; H, 6.72. Found: C, 48.64; H, 6.73.

Further elution afforded **9** (0.16 g, 73%), which $^1$H-n.m.r. spectroscopy revealed to be a ~1:1 mixture of diastereomers, $R_F$ 0.2 (light petroleum–ethyl acetate). N.m.r. data: $^1$H (220 MHz, $CDCl_3$), δ 1.04, 1.05 (2 s, 3 H, Me-C-N), 1.55–1.77 (m, 2 H, $CH_2$-C-N), 1.98, 2.04, 2.05, 2.15 (4 s, each 3 H, 4 OAc), 3.34 (s, 0.5 H, OMe), 3.40 (s, 0.5 H, OMe), 3.94 (t, 1 H, $J_{5,6a} = J_{5,6b}$ = 6 Hz, H-5), 4.06–4.22 (m, 2 H, H-6a,6b), 4.68, 4.73 (2 d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.56 (dd, 0.5 H, *J* 5 Hz, *J* 6.5 Hz, MeO-C*H*-O), 4.78 (t, 0.5 H, *J* 6 Hz, MeO-C*H*-O), 5.02, 5.03 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.5 Hz, H-3), 5.22, 5.25 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10.5 Hz, H-2), 5.39 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4); $^{13}$C (25 MHz, $CDCl_3$), δ 20.30 ($CH_3$C-N) 20.65 ($CH_3CO$), 23.11, 29.69 (N-C-N), 39.43, 40.10 ($CH_2$C-N), 53.21, 54.90 ($OCH_3$), 61.37, 61.57 (C-6), 67.07 (C-4), 68.77 (C-2), 70.96, 71.11 (C-3, C-5), 96.71, 97.38 (C-1), 99.75, 101.79 (MeO-*C*H), 169.22, 170.10, 170.24, 170.33 (4 $CH_3CO$).

*Anal.* Calc. for $C_{19}H_{28}N_2O_{11}$: C, 49.55; H, 6.13; N, 6.09. Found: C, 49.44; H, 6.17; N, 6.00.

(*b*) *3-Azi-1-methoxybutyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranoside*[13] (**10**). — Compound **8** (0.295 g, 0.702 mmol) was allowed to react with 3-azibutyraldehyde dimethyl acetal (**12**) for 6 days as described above. Flash column chromatography (light petroleum–ethyl acetate, 1:1) and recrystallization from the same solvents furnished **10** (0.180 g, 56%), m.p. 76–77°, $[\alpha]_D$ −37° (*c* 0.4, chloroform). N.m.r. data: $^1$H (300 MHz, $CDCl_3$), δ 1.01, 1.02 (2 s, 3 H, $CH_3$C-N), 1.59–1.73 (m, 2 H, MeOC-C$H_2$), 1.98, 2.00, 2.01, 2.02, 2.05, 2.06 (6 s, 12 H, Ac), 3.32 (s, 0.5 H, OMe), 3.40 (s, 0.5 H, OMe), 3.64–3.74 (m, 1 H, H-5), 4.07–4.25 (m, 2 H, H-6a,6b), 4.53 (dd, 0.5 H, *J* 5 Hz and 6.5 Hz, MeO-C*H*-O), 4.69, 4.74 (d, d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.71–4.78 (m, 0.5 H, MeO-C*H*-O), 4.95–5.10 (m, 2 H, H-2,4), 5.18, 5.19 (2 t, 1 H, $J_{2,3} = J_{3,4}$ = 9 Hz, H-3); $^{13}$C (75 MHz, $CDCl_3$), δ 20.28, 20.41, 20.49, 20.59, 20.66 ($CH_3CO$, $CH_3$-C-N), 23.03 (N-C-N), 39.38, 40.09 (MeOC-$CH_2$), 53.03, 54.96 ($OCH_3$), 62.02, 62.20 (C-6), 68.53 (C-4), 71.34 (C-2), 72.01 (C-5), 72.97, 73.03 (C-3), 96.02, 96.90 (H-1), 99.70, 101.66 (MeO*C*-O), 169.10, 169.39, 170.24, 170.53 (4 $CH_3CO$). Mass spectrum: *m/z* 478 $[M + NH_4]^+$.

*Anal.* Calc. for $C_{19}H_{28}N_2O_{11}$: C, 49.55; H, 6.13; N, 6.09. Found: C, 49.67; H, 6.15; N, 6.07.

(*c*) *1-Methoxybutyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranoside* (**11**). — Compound **7** (0.406 g, 0.952 mmol) was allowed to react with butyraldehyde dimethyl acetal for 6 days as described above. Flash column chromatography (toluene–acetone, 7:1) afforded **7** (0.12 g) and **11** (0.172 g, 58% based on unrecovered **7**). N.m.r. data for **11**: $^1$H (300 MHz, $CDCl_3$), δ 0.89–0.94 (m, 3 H, $CH_3CH_2$), 1.34–1.41 (m, 2 H, $CH_3CH_2$), 1.57–1.67 (m, 2 H, MeO-C-$CH_2$), 1.99, 2.04, 2.05, 2.16 (4 s, each 3 H, 4 OAc), 3.31 (s, 0.6 H, OMe), 3.38 (s, 0.4 H, OMe), 3.89–3.95 (m, 1 H, H-5), 4.09–4.20 (m, 2 H, H-6a,6b), 4.69, 4.75 (2d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.57, 4.78 (t, t, 1 H, *J* 6 Hz, MeO-C*H*-O), 5.03, 5.04 (2 dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 3.5 Hz, H-3) 5.24, 5.26 (2 dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10 Hz, H-2), 5.39 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0

Hz, H-4); $^{13}C$ (75 MHz): δ 13.80, 13.89 ($CH_3CH_2$), 17.58, 17.83 ($CH_3CH_2$), 20.54, 20.64, 20.73 ($CH_3CO$), 35.36, 36.69 (MeO-C-$CH_2$), 52.21, 54.66 ($OCH_3$), 61.43, 61.64 (C-6), 67.25 (C-4), 69.02, 69.14 (C-2), 70.85, 70.94 (C-3), 71.23 (C-5), 96.95, 97.20 (C-1), 102.40, 105.80 (MeOCH), 169.24. 170.09, 170.25, 170.33 (4 $CH_3CO$). Mass spectrum: *m/z* 452 $[M + NH_4]^+$.

*Anal.* Calc. for $C_{19}H_{30}O_{11}$: C, 52.51; H, 6.96. Found: C, 52.57; H, 6.99.

*3-Azi-1-methoxybutyl β-D-galactopyranoside* (**1**). — A solution of **9** (0.20 g, 0.435 mmol) in anhydrous methanol (5 mL) was stirred with methanolic M sodium methoxide (0.5 mL) for 0.5 h, when t.l.c. (light petroleum–ethyl acetate, 2:1) indicated the absence of **9**. The mixture was neutralized with Amberlite IR-120 ($H^+$) resin, filtered, and concentrated to give **1** (0.122 g, 96%). Recrystallization from light petroleum–2-propanol afforded a white solid, m.p. 125–128°, $[\alpha]_D$ −26° (*c* 0.9, methanol). N.m.r. data: $^1H$ (300 MHz, $CD_3OD$), δ 1.04 (s, 3 H, Me-C-N), 1.56 (dd, 0.6 H, *J* 7.0, *J* 14.5 Hz, MeOC-C*H*), 1.66–1.73 (m, 1.4 H, MeOCH-C*H*), 3.40–3.56 (m, 3 H, H-2,3,5), 3.43, 3.47 (2 s, 3 H, OMe), 3.72 (d, 2 H, $J_{5,6}$ 7.2 Hz, H-6), 3.82 (unresolved d, 1 H, *J* 2.7 Hz, H-4), 4.32, 4.46 (2 d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.63 (dd, 0.4 H, *J* 5 and 6.7 Hz, MeO-C*H*), 4.89–4.92 (m, MeOC*H*, obscured by HOD peak); $^{13}C$ (25 MHz), δ 20.39, 20.51 ($CH_3C$-N), 25.44 (N-C-N), 41.04, 41.28 (MeOC-$CH_2$), 54.09, 55.90 ($OCH_3$), 62.40 (C-6), 70.18 (C-4), 72.46 (C-2), 74.98 (C-3), 76.73 (C-5), 101.19, 101.42 (C-1), 102.01, 103.41 (MeOCH). Mass spectrum: *m/z* 310 $[M + NH_4]^+$.

*Anal.* Calc. for $C_{11}H_{20}N_2O_7$: C, 45.19; H, 6.90; N, 9.59. Found: C, 45.06; H, 6.94; N, 9.52.

*3-Azi-1-methoxybutyl β-D-glucopyranoside*[13] (**2**). — A solution of **10** (0.20 g, 0.435 mmol) was treated with methanolic sodium methoxide as described for the preparation of **1**. The product was recrystallized from light petroleum–2-propanol to give **2** (0.120 g, 94%), m.p. 143–145°, $[\alpha]_D$ −43° (*c* 0.4, water). N.m.r. data: $^1H$ (300 MHz, $CD_3OD$) δ 1.04 (s, 3 H, MeC-N), 1.56 (dd, 0.5 H, *J* 7.5 Hz and 15 Hz, MeO-C*H*), 1.66–1.74 (m, 1.5 H, MeOC-C*H*), 3.14–3.40 (m, 4 H, H-2,3,4,5), 3.42, 3.48 (2 s, 3 H, $OCH_3$), 3.65 (dd, 1 H, $J_{5,6a}$ 4.2, $J_{6a,6b}$ 12 Hz, H-6a), 3.85 (d, 1 H, $J_{6a,6b}$ 12 Hz, H-6b), 4.38, 4.51 (2 d, 3 H, $J_{1,2}$ 7.8 Hz, H-1), 4.64 (t, 0.5 H, *J* 6 Hz, MeOC*H*), 4.88–4.93 (m, MeOC*H*, obscured by HOD peak); $^{13}C$ (75 MHz) δ 20.57 ($CH_3C$-N), 24.25 (N-C-N), 41.04, 41.34 (MeOC-$CH_2$), 54.21, 56.02 ($OCH_3$), 62.81 (C-6), 71.64 (C-4), 75.03 (C-2), 78.08 (C-3,5), 100.89, 101.24 (C-1), 101.42, 103.53 (MeOCH).

*Anal.* Calc. for $C_{11}H_{20}N_2O_7$: C, 45.19; H, 6.90; N, 9.59. Found: C, 45.15; H, 6.93; N, 9.50.

*1-Methoxybutyl β-D-galactopyranoside* (**3**). — A solution of **11** (0.535 g, 1.23 mmol) was *O*-deacetylated and processed as described for the preparation of **1**. Recrystallization of the product from ethanol gave **3** (0.292 g, 89%), m.p. 157–162°, $[\alpha]_D$ −12° (*c* 0.7, water). N.m.r. data: $^1H$ (300 MHz, $CD_3OD$), δ 0.92 (t, 3 H, *J* 7.3 Hz, $CH_3CH_2$), 1.35–1.50 (m, 2 H, $CH_3CH_2$), 1.58–1.75 (m, 2 H, MeOC-$CH_2$), 3.39, 3.43 (2 s, 1.2 H, 1.8 H, OMe), 3.40–3.59 (m, 3 H, H-2,3,5), 3.73 (d, 2 H, $J_{5,6}$ 6 Hz,

H-6,6), 3.85 (d, 1 H, $J_{3,4}$ 3 Hz, H-4), 4.35, 4.48 (2 d, 1 H $J_{1,2}$ 7.0 Hz, H-1), 4.62 (dd, 0.5 H, $J$ 4.9 and 6.5 Hz, MeOC*H*), 4.82–4.85 (m, MeOC*H*, obscured by HOD peak); $^{13}C$ (75 MHz), $\delta$ 14.28 ($CH_3CH_2$), 18.63, 18.77 ($CH_3CH_2$), 37.22, 38.06 (MeOC-$CH_2$), 53.23, 55.71 ($OCH_3$), 62.39 (C-6), 70.21 (C-4), 72.59 (C-2), 75.05 (C-3), 76.63 (C-5), 101.72, 101.90 (C-1), 103.68, 107.02 (MeO*C*H). Mass spectrum: $m/z$ 284 $[M + NH_4]^+$.

*Anal.* Calc. for $C_{11}H_{22}O_7$: C, 49.60; H, 8.33. Found: C, 49.37; H, 8.30.

*3-Azi-1-methoxybutyl β-D-(4-$^3$H)galactopyranoside* (**1***). — A solution of 2,3:5,6-di-*O*-isopropylidene-D-*xylo*-hexos-4-ulose 1,1-(dimethyl acetal) (12 mg) in 1,4-dioxane (200 μL) was added to a fresh solution of $NaB^3H_4$ (100 mCi; spec. act., 9.9 Ci/mmol; Amersham, U.K.) in M NaOH (20 μL). After 24 h, the mixture was neutralized (acetic acid) and concentrated to dryness, and a solution of the residue in aqueous 40% trifluoroacetic acid (500 mL) was kept for 5 h at 60°. The D-(4-$^3$H) galactose formed was separated from the corresponding *gluco* compound (which had formed in a slightly larger amount) by p.c. on Whatman No. 3 paper (1-butanol–pyridine–water, 6:4:3). The two monosaccharides accounted almost quantitatively for the radioactivity employed. The sugars were eluted from the paper with water, the solutions were lyophilized, and the residues were acetylated for 24 h with 1:1 acetic anhydride–pyridine (500 μL). After removal of the reagents *in vacuo*, ice-cold 30% HBr in glacial acetic acid (4 mL) was added to the cooled flask containing D-(4-$^3$H)galactose pentaacetate. After 10 h at 0°, the mixture was taken up in dichloromethane (100 mL), washed with ice–water and aqueous sodium hydrogencarbonate, dried ($CaSO_4$), and concentrated *in vacuo*. The residue was stirred with a suspension of silver carbonate (400 mg) in aqueous acetone (400 μL of water in 10 mL of acetone) until no starting material was detectable (~30 min). The mixture was then filtered and concentrated to give nearly homogeneous 2,3,4,6-tetra-*O*-acetyl-β-D-(4-$^3$H)galactose by comparison with an authentic unlabelled sample (t.l.c.; ethyl acetate–light petroleum, 1:1). The residue was thoroughly dried at room temperature and a solution in *N,O*-bis(trimethylsilyl)trifluoroacetamide (3 mL) was kept for 20 h at room temperature. All volatile material was removed at 20°→50°/ $< 5 \times 10^{-3}$ mmHg. The residue was allowed to react under dry nitrogen with a solution of 3-azibutyraldehyde dimethyl acetal (200μL) in dry dichloromethane (2 mL) at −70°. A solution of trimethylsilyl triflate in dry dichloromethane (200 μL, 0.5M) was then added under dry nitrogen, and the mixture was kept for 30 h at −70°. Triethylamine (100 μL) was added, and the mixture was allowed to warm up to room temperature, diluted with dichloromethane (4 mL), washed with cold aqueous 5% sodium hydrogencarbonate and saturated aqueous sodium chloride, and co-concentrated with toluene (3 mL) under reduced pressure. The residue was subjected to Zemplén *O*-deacetylation which also cause desilylation. Preparative t.l.c. (7:2:1 ethyl acetate–methanol–water) gave two major radioactive bands, $R_F$ 0.17 and 0.51, in the ratio ~1:4. The former band co-chromatographed with D-galactose, the latter with unlabelled 3-azi-1-methoxybutyl β-D-galactopyranoside. Preparative t.l.c. on silica gel, with elution of the faster moving component with

aqueous methanol (1:1), followed by h.p.l.c., using water as the solvent with monitoring at 342 nm, gave **1***, which contained 90% of the applied radioactivity [$2.2 \times 10^{10}$ d.p.m., corresponding to 10 mCi (2.4 Ci/mmol)]. The radioactivity co-chromatographed two-dimensionally (t.l.c.: ethyl acetate–methanol–water, 7:2:1) with 3-azi-1-methoxybutyl β-D-galactopyranoside, and **1*** was homogeneous in h.p.l.c. and high-performance t.l.c., but the *R* and *S* diastereomers could not be resolved. Compound **1*** was completely cleaved at pH 6.8 with β-D-galactosidase from *E. coli* (Boehringer Mannheim) to give radioactive galactose, whereas it remained unchanged over 24 h when incubated at pH 6.8 with α-D-galactosidase from green coffee beans (Boehringer Mannheim).

*Measurement of affinity constants* ($K_a$). — IgA J539 (Fab') or IgA X24 ($0.714 \times 10^{-6}$M) in PBS (pH 7.4) was irradiated in a Perkin–Elmer fluorimeter at 295 nm. The changes in fluorescence of the protein were measured at 334 nm as a function of the additions of aliquots of 0.1M **1**. The fraction of antibody sites occupied by ligand ($\nu$) divided by the concentration of free ligand ($c_L$) was plotted *versus* $\nu$, and the slope yielded[11,12] the affinity constant (Fig. 1). The $K_a$ reported herein has been computed taking into account the quenching of protein fluorescence by the ligand. The $K_a$ for **3** and IgA (J539 (Fab') was measured in the same manner as for **1** except that fluorescence was monitored at 340 nm.

*Reaction of 3-azi-1-methoxybutyl β-D-(4-$^3$H)galactopyranoside with IgA X24.* Compound **1*** (0.927 μmol) was dissolved in a solution of affinity-purified anti-galactan IgA 24 (300 μL, 10 $A_{280}$/mL) (solution *A*). Part (50 μL) of solution *A* was diluted 1:1 with PBS and made $0.81 \times 10^{-3}$M in methyl (1→6)-β-D-galactotetraoside (solution *B*). Solutions *A* and *B* were deoxygenated with bubbling nitrogen gas for 2 min, irradiated at 300–350 nm for 10 min, then dialysed against PBS, and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) after denaturation (2% SDS, 5% 2-mercaptoethanol). Parts of the gels were stained with Coomassie Blue; the remainder was cut into 2-mm slices, each of which was submerged in Biolute-S (Zinsser, 0.5 mL) and left overnight. Quickscint-501 (4 mL, Zinsser) was then added, the mixture was kept for 1 h in the cold, and the radioactivity was then counted.

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