The synthesis and conformational properties of the diastereoisomeric βDGal(1→4)βDGlcNAc(1→6)6-*C*-CH_3-D-Gal trisaccharides

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The trisaccharide βDGal(1→4)βDGlcNAc(1→6)DGal was known to be bound strongly by the so-called anti-I Ma monoclonal antibody. In order to help assess the conformation about the 1→6 glycosidic linkage that is accepted by the antibody combining site, the conformationally well-defined βDGal(1→4)βDGlcNAc(1→6) derivatives of 7-deoxy-L-*glycero*-D-*galacto*-heptopyranose and 7-deoxy-D-*glycero*-D-*galacto*-heptopyranose were synthesized. The conformational preferences for these trisaccharides were established by ^{1}H nmr spectroscopy and rationalized by computer-assisted molecular modelling.

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On sait que le trisaccharide βDGal(1→4)βDGlcNAc(1→6)DGal est lié fortement à l'anticorps monoclonal anti-I Ma. Dans le but d'aider à déterminer la conformation autour de la liaison glycosidique (1→6) qui est accepté par le site combinant de l'anticorps, on a synthétisé les dérivés conformationnellement bien définis βDGal(1→4)βDGlcNAc(1→6) du déoxy-7-L-*glycéro* D-*galacto*-heptopyrannose et du déoxy-7 D-*glycéro*-D-*galacto*-heptopyrannose. On a établi les préférences conformationnelles de ces trisaccharides par la spectroscopie de rmn du ^{1}H et on les a rationalisées par des modèles moléculaires fournis par ordinateur.

[Traduit par le journal]

Introduction

The myeloma protein which was classified (1) as the monoclonal antibody I Ma (group 1) was found to possess a combining site which binds at the surface of a βDGal(1→4)βDGlcNAc(1→6) structural unit which was common to a number of the oligosacchardies studied.

We have predicted (2) that the conformational preference for the glycosidic linkage at the 4-position of the βDGlcNAc residue should be near $\phi^{C4'/H1''} = 50°$, $\psi^{H4'/C1''} = 10°$. Both for reasons of nonbonded interactions and the *exo*-anomeric effects, it was to be expected that the glycosyl bond of the βDGlcNAc unit to the 6-position of a sugar, which may be either βDGal or αDGalNAc, would possess the $\phi^{C6/H1'}$ torsion angle close to +60°. However, rotation about either the O6—C6 bond (to provide changes in the $\psi^{C5/C1'}$ torsion angle) or the C5—C6 bond (to provide changes in the $\omega^{H5/O6}$ torsion angle) was expected to lead to a number of conformers which differ little in energy (3). Indeed, hard-sphere calculations using the general procedure described by Venkatachalam and Ramachandran (2) while setting $\phi^{C6/H1'} = 60°$ substantiated this expectation.

The procedure was to examine the barriers to rotation about the O6—C6 bond (changes in $\psi^{C5/C1'}$) with the atoms on C5 and C6 in the 3 staggered orientations (namely, $\omega^{H5/O6} = +60°$, 180°, and −60°). In order to simplify the calculation, as in the past (2), the hydroxyl group hydrogen atoms were neglected and the hydroxymethyl groups of the hexose units, when present, were treated as 6-deoxy units. The parameters for the βDGlcNAc unit were derived from the X-ray structures of 4-*N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine (4) and lactose (5). The coordinates for the hydrogen atoms attached to carbon were established by fixing the C—H bond length at 1.1 Å and making equal the three bond angles involving this hydrogen atom. The neutron diffraction data (6) for methyl β-D-galactopyranoside were used for the two βDGal units of βDGal(1→4)βDGlcNAc(1→6)βDGal. Since no interaction can be reasonably expected to exist between the two terminal βDGal units, the conformation of the trisaccharide was estimated by examining the structure as two disaccharide units. The calculations showed that the $\psi^{C5/C1'}$ torsion angle could vary within the following ranges without encountering energy barriers greater than ± 0.5 kcal/mol.

Conformers of near equal energy for βDGlcNAc(1→6)βDGal

$\phi^{C6/H1'}$	$\omega^{H5/O6}$	$\psi^{C5/C1'}$
60°	60°	120°–290°
60°	180°	110°–240°
60°	−60°	100°–260°

In view of the very large number of energetically near-equivalent conformers for the βDGlcNAc-(1→6)βDGal disaccharide, no conclusion could be drawn as to which conformer was most likely to be accepted by the I Ma combining site. It was

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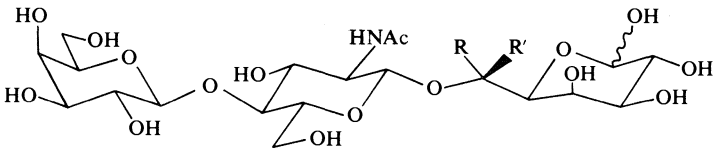
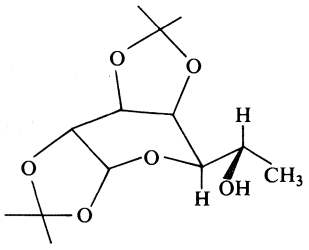


6 R′ = CH_3, R = H
9 R′ = H, R = CH_3

expected, however, that the range of low energy conformers could be narrowed by the substitution of a methyl group for a hydrogen at the C6 atom of the reducing βDGal unit. This would lead to two diastereoisomers depending on which hydrogen atom was substituted; namely, structures **6** and **9**. Since **6** is derived from 7-deoxy-L-*glycero*-D-*galacto*-heptose, it will be referred to as the L-trissaccharide. D-Trisaccharide will be used to refer to the derivative of 7-deoxy-D-*glycero*-D-*galacto*-heptose; namely, **9**. This paper is concerned with the synthesis of the trisaccharides **6** and **9** and a study of their conformational properties. The results obtained in a study of their properties as inhibitors of anti-I Ma is reported in a separate communication (7).

Discussion

In a study of the binding of antibodies specific for the β-D-galactopyranosyl group, Lemieux *et al.* (8) observed that the addition of methylmagnesium bromide to 1,2;3,4-di-*O*-isopropylidene-α-D-*galacto*-1,6-dialdohexopyranose (9) (**1**) provided the L and D isomers **2** and **3**, respectively, in a ratio of



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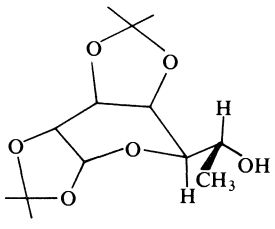

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about 3:1. However, these products were not characterized. Instead, the compounds were converted to their methyl β-glycosides to serve as inhibitors in the immunochemical studies. The configuration of the new asymmetric carbon in the minor component was established to be D by relating its physical properties to the enantiomeric structure which had been previously described by Jackson and Hudson (10). It should be noted at this point that there exists an error in the reporting of these compounds by Lemieux *et al.* (8). The correct names are given in the title of the preparation. The first fraction from the chromatogram should have been reported as the D-isomer and the second fraction as the L-isomer. In the present work, the L-compound **2** was readily isolated by direct crystallization. Compound **3** (the minor D-isomer) was obtained after purification by way of its crystalline benzoate. The spacings for the signals for the hydrogens of the pyranose ring in compounds **2** and **3** were virtually the same as those published by Cone and Hough (11) for 1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranose. The chemical shifts of H1, H2, and H3 were essentially the same. The shift of H4 was 4.26 ppm for both the galactose compound and the L-isomer (**2**), but the signal for H4 of the D-isomer, probably because of electrostatic deshielding by O6, was 0.2 ppm to lower field. In view of the magnitudes of the coupling constants for H5 and H6 (6.8 Hz for **2** and 7.2 Hz for **3** with $CDCl_3$ as solvent), the compounds must exist extensively in the conformer which maintains these two hydrogens in near *anti*-periplanar orientation. It is noted, however, that the pyranose ring in these compounds is strongly distorted away from the $^4C_1$ conformation (11) as is implied in the conformational formulas.

It was planned to confirm the configurations of the products (**2** and **3**), formed in the reaction of the aldehyde (**1**) with methylmagnesium iodide, by nuclear Overhauser enhancement studies (2, 12) since the C6-methyl group could be expected to reside closer to H4 in the L-isomer than in the D-isomer. However, although saturation of the signals assigned to these methyl groups did, in fact, cause a greater enhancement of H4 in the case of the L-isomer, the significance of this result proved to be obscured by the fact that the experiment could not avoid simultaneous saturation of the isopropylidene groups. This interference by the isopropylidene groups was not present in the case of the trisaccharides **6** and **9** and, as will be seen below, the ^1H nmr spectra for these compounds further confirmed these configurational assignments.

The chemical syntheses of compounds **6** and **9** were accomplished by condensation of hexa-*O*-acetyl-2-deoxy-2-phthalimidolactosyl chloride (13) with the alcohols **2** and **3** under the conditions

reported (14) for the preparation of 6-*O*-[hexa-*O*-acetyl-2-deoxy-2-phthalimido-β-lactopyranosyl]-1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranose. The products (**4** and **7**) were treated first with hydrazine to remove the acyl groups and the resulting amines were then *N*-acetylated to provide **5** and **8**. Removal of the isopropylidene groups by hydrolysis in aqueous trifluoroacetic acid afforded the desired trisaccharides **6** and **9**. The overall yields are poor and not to be taken as representative of the methods used, primarily since the preparations were made only once and no effort was made to optimize the reaction and isolation procedures.

The hard-sphere calculations (5° steps) for the D- and L-trisaccharides (**6** and **9**) employed the same atomic parameters mentioned above in connection with the molecular modelling for the βDGal(1→4)-βDGlcNAc(1→6)βDGal trisaccharide, except that the *C*-methyl group was substituted for the particular H6 atom with a C—C bond length of 1.536 Å. In the case of the D-trisaccharide (**9**), the calculation showed the most favorable conformer to be near that with $\phi^{C6/H1'} = 60°$, $\omega^{H5/O6} = 60°$, and $\psi^{C5/C1'} = 130°$. The minimum energy conformers with $\omega^{H5/O6} = 180°$ and 300° were indicated to be about 1 kcal and 3 kcal/mol, respectively, less favorable. The $\omega^{H5/O6} = 60°$ torsion angle requires that the torsion angle be defined by H5 and that H6 be near 180°. Thus, should this inference as to conformational preference be correct, there should exist a coupling constant between the vicinal *anti*-periplanar H5 and H6 atoms of near 10 Hz. Indeed, a coupling of 8.5 Hz was observed. Examination of a molecular model in this conformation shows the C6-methyl group to be close to H5 and H6 only. In fact, saturation of the methyl group led to nuclear Overhauser enhancement of only these two hydrogens. The signal for H4 at 4.17 and 4.24 ppm (α and β anomers) was not affected. When $\omega^{H5/O6}$ was set at 60° and the $\psi^{C5/C1'}$ torsion angles in the range 120–160° examined by HSEA calculations, the best value for the φ torsion angle was found to be +50° with ψ = 130°. In the context of these calculations, the difference of near 0.1 kcal/mol between this conformation and that with $\phi^{C6/H1'} = 60°$ and $\psi^{C5/C1'} = 130°$ is not significant. A projection formula for the D-trisaccharide (**9**) is presented in Fig. 1.

The hard-sphere calculations for the L-trisaccharide (**6**) indicated the staggered conformation with $\omega^{H5/O6} = 190°$ to be most favorable by nearly 1 kcal and that this conformer involved $\psi^{C5/C1'} = 230°$ (−130°) (for this calculation, $\phi^{C6/H1'}$ had been arbitrarily set at 60°). When this value for ω was

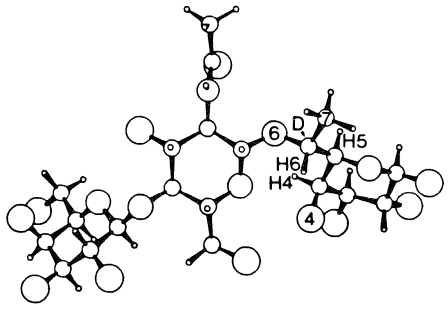

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FIG. 1. Computer drawing of the D-trisaccharide (**9**) in the conformation anticipated by HSEA calculation in order to display the *anti*-periplanar orientation of H5 and H6 and the orientation of the C7-methyl group relative to H4.

used in a second iterative HSEA calculation[3] to estimate the most favorable values for φ and ψ, the values $\phi^{C6/H1'} = 50°$ and $\psi^{C5/C1'} = 230°$ were obtained and the total conformational energy was −0.6 kcal/mol for the compound in the conformation (**6***a*) represented in Fig. 2. On applying the HSEA calculation to the conformer with $\omega^{H5/O6} = 290°$ (−70°), the conformer (**6***b*) with $\phi^{C6/H1'} = 45°$ and $\psi^{C5/C1'} = 250°$ (−110°) was indicated as energetically most favorable with a total conformational energy of 0 kcal/mol.

Thus, it appeared that conformer **6***a* should have a substantially higher abundance than **6***b*. However, the ^{1}H nmr properties of the L-isomer clearly demand that the conformational equilibrium favor conformer **6***b*. This followed from the observations that (*a*) the coupling constant for the vicinal H5 and H6 atoms was 8.0 Hz, in keeping with a high population of the conformer **6***b*, which has these atoms in *anti*-periplanar orientation, but not **6***a*, where these atoms are in *syn*-clinal orientations, and (*b*) saturation of the signal for the C-methyl group strongly enhanced the signal for H4 as well as those for H5 and H6 in keeping with conformation **6***b* but not **6***a*.

That the HSEA calculations predicted a greater conformational stability for **6***a* whereas **6***b* is undoubtedly more favorable is not necessarily surprising. The calculations may well have underestimated the *syn*-axial like nonbonded interaction between O4 and O6 in **6***a*. The HSEA calculations assume that the van der Waals radius for an oxygen atom is 1.5 Å. However, evidence has been presented that the effective radius for oxygen can vary

[3]The procedure for hard-sphere, *exo*-anomeric (HSEA) calculations (2) has been improved and ref. 15 should be consulted in this regard.

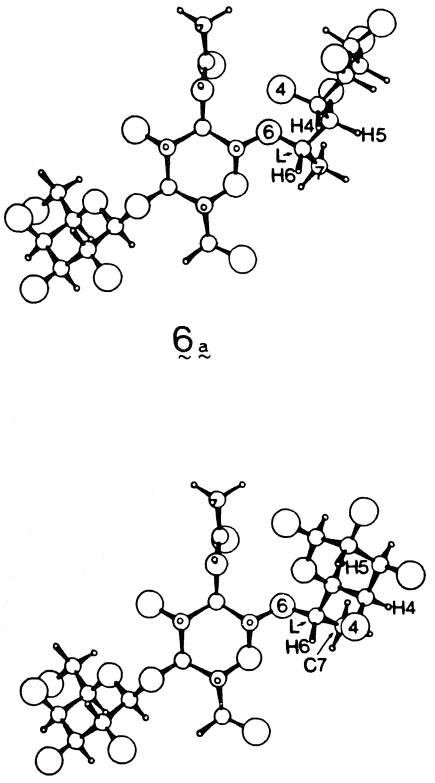

FIG. 2. Computer drawing of the L-trisaccharide (**6**) in the conformation (**6***a*) anticipated to be most favorable by HSEA calculations and in the conformation (**6***b*) best indicated by the ^{1}H nmr spectrum. In the case of **6***a*, note the *syn*-axial like arrangement of O4 and O6, the *syn*-clinal orientation of H5 and H6, and that the C7-methyl group is distant from H4. In the case of **6***b*, note that H5 and H6 are in *anti*-periplanar orientation and that the C7-methyl group is in *syn*-axial like orientation with H4.

depending on its substituents (16). Moreover, the calculations do not include either electrostatic terms or a consideration of the effect on the apparent volume of hydroxyl groups when these are hydrogen bonded to the solvent water. We were fortunate in this regard in that **6** proved to be a very poor inhibitor (7) and, therefore, the possible availability of the compound to the antibody combining site in conformation **6***a* did not become an important question.

The study of the interactions of **6** and **9** with the anti-I Ma monoclonal antibody are reported in a separate communication (7) which also reports the results obtained with a number of related compounds. It proved that compound **9** reacts with the antibody even more strongly (about 0.5 kcal/mol) than does the βDGal(1→4)βDGlcNAc(1→6)DGal trisaccharide. This appears to be the first instance in which a complex synthetic structure that is closely related to a natural antigenic determinant binds the antibody more strongly. It is considered that this exceptional event likely occurs, at least in part, because compound **9** is held in the specific conformation that is required for the binding. This matter has a bearing on the notion at times expressed that ground state conformations are not necessarily implicated in antibody–antigen interactions. As expected, the preferred conformation for **9** is amongst the most favorable of the conformations available to the βDGal(1→4)βDGlcNAc-(1→6)DGal trisaccharide.

Experimental

The purification of the solvents, chromatographic and analytical procedures were the same as previously reported (13).

1,2;3,4-Di-O-*isopropylidene*-α-D-galacto-*1,6-dialdo-hexopyranose (1)*

1,2:3,4-Di-*O*-isopropylidene-α-D-galactose was oxidized using the chromium trioxide – pyridine complex in dichloromethane as was described by Arrick *et al.* (9). Examination of the dark green syrupy crude product (95% yield) showed it to be of as good a quality as that obtained by distillation. The ^{1}H nmr spectrum was in accord with that published by Horton *et al.* (17).

1,2;3,4-Di-O-*isopropylidene-7-deoxy*-β-L-glycero-D-galacto-*heptopyranose(2)*

Methylmagnesium iodide was prepared under standard conditions using magnesium (1.42 g, 58 mmol) and methyl iodide (8.3 g, 58 mmol) in ether (60 mL). A solution of the aldehyde (**1**) (8.0 g, 31 mmol) in diethyl ether (45 mL) was added dropwise with stirring and the mixture was kept at room temperature for 15 h. The reaction mixture was then cooled in ice-water and a 10% solution of ammonium chloride in water (50 mL) was added. Work-up in the usual manner using diethyl ether as extractant, drying over magnesium sulfate, and solvent removal, left a syrup which was decolorized by passing a solution in ethyl acetate through a short column of silica gel. Removal of the solvent left a syrup (7.3 g) which was dissolved in hexane (15 mL). On storage, the solution deposited 3.17 g of crystals which, after three recystallizations from hexane, provided 2.2 g (26%) of **2**, mp 90–91°C; $[\alpha]_D^{23}$ −58.5° (*c* 1.8, chloroform); ^{1}H nmr ($CDCl_3$) δ: 5.58 (d, 1H, H-1, $J_{1,2}$ = 4.6 Hz), 4.59 (dd, 1H, H-3, $J_{2,3}$ = 2.1 Hz, $J_{3,4}$ = 8.0 Hz), 4.32 (dd, 1H, H-2), 4.26 (dd, 1H, H-4, $J_{4,5}$ = 2.0 Hz), 3.99 (m, 1H, H-6), 3.48 (dd, 1H, H-5, $J_{5,6}$ = 6.8 Hz), 1.50, 1.44, 1.32 (3s, 12H, O—C(=O)—$(CH_3)_2$), 1.26 (d, 3H, (C-7)H_3, $J_{6,CH_3} \simeq$ 6 Hz). *Anal.* calcd. for $C_{13}H_{22}O_6$: C 56.92, H 8.08; found: C 56.87, H 8.05.

1,2;3,4-Di-O-*isopropylidene-7-deoxy*-α-D-glycero-D-galacto-*heptopyranose (3)*

The mother liquor from the first crystallization of the L-isomer (**2**) was taken to a syrup (3.86 g) which was dissolved in dichloromethane (10 mL), pyridine (4 mL), and benzoyl chloride (2 mL). The solution was stirred at room temperature for 3 h and the product was isolated in the usual manner. Solvent removal left a syrup which was taken up in hexane (3 mL) and the solution was kept at −4°C for 2 days. Crystals (0.290 g) were deposited which were debenzoylated in methanol – sodium methoxide without further purification. The product was ap-

plied to a column of silica gel and eluted with ethyl acetate – hexane (6:4). The main fractions were combined and dissolved in a small amount of hexane and the solution deposited a white solid (0.188 g, 2%) when stored at −4°C overnight. This product, mp 57–58°C, $[\alpha]_D^{23}$ −34.8° (*c* 0.3, chloroform), was not further purified since the ^{1}H nmr spectrum revealed it to be a nearly pure substance; ^{1}H nmr ($CDCl_3$) δ: 5.55 (d, 1H, H-1, $J_{1,2}$ = 5 Hz), 4.62 (dd, 1H, H-3, $J_{2,3}$ = 2.2 Hz, $J_{3,4}$ = 8.0 Hz), 4.46 (dd, 1H, H-4, $J_{4,5}$ = 2.0 Hz), 4.30 (dd, 1H, H-2), 3.96 (m, 1H, H-6), 3.50 (dd, 1H, H-5, $J_{5,6}$ = 7.2 Hz), 1.51, 1.45, 1.36, 1.32, 1.29 (5 O ‖ peaks, 15H, O—C(CH$_3$)$_2$ and (C-7)H$_3$ overlapping). *Anal.* calcd. for $C_{13}H_{22}O_6$: C 56.92, H 8.08; found: C 56.42, H 7.77.

*1,2;3,4-Di-*O*-isopropylidene-6-*O*-[3,6-di-*O*-acetyl-2-deoxy-4-*O*-(tetra-*O*-acetyl-*β*-*D*-galactopyranosyl)-2-phthalimido-*β*-*D*-glucopyranosyl]-7-deoxy-*β*-*L*-glycero-*D*-galacto-heptopyranose (4)*

3,6-Di-*O*-acetyl-4-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-α,β-D-glucopyranosyl chloride (13) (1.3 g, 1.8 mmol) in nitromethane (5 mL) was added to a solution of the alcohol **2** (0.5 g, 1.8 mmol), *sym*-collidine (0.22 g, 1.8 mmol), and silver triflate (0.49 g, 1.9 mmol) in dry nitromethane (15 mL) cooled to −25°C (18). The mixture was stirred at −25°C for 2 h. The reaction mixture was then stirred at room temperature for 8 h. The solids that were deposited after the addition of chloroform (100 mL) were removed by filtration and the filtrate was washed with sodium thiosulfate solution, cold water, dilute aqueous HCl, and saturated sodium bicarbonate solution. Solvent removal, after drying over sodium sulfate, left a foam which was applied to a silica gel column and eluted with ethyl acetate – hexane (1:1). Solvent removal of the second fraction left a white solid (0.63 g, 36%), mp 119–123°C; $[\alpha]_D^{23}$ −21.6° (*c* 1.1, chloroform); ^{1}H nmr ($CDCl_3$) δ: 7.80–7.52 (broad m, 4H, phthalimido), 5.70 (dd, 1H, H-3′, $J_{3',4'}$ = 8.0 Hz, $J_{2',3'}$ = 10.5 Hz), 5.39 (d, 1H, H-1′, $J_{1',2'}$ = 8.5 Hz), 5.23 (broad d, 1H, H-4″, $J_{3'',4''}$ = 3.1 Hz), 5.04 (dd, 1H, H-2″, $J_{1'',2''}$ = 7.5 Hz, $J_{2'',3''}$ = 10.5 Hz), 4.87 (dd, 1H, H-3″), 4.73 (d, 1H, H-1, $J_{1,2}$ = 5.0 Hz), 4.44 (d, 1H, H-1″), 3.18 (broad dd, 1H, H-5, $J_{5,6}$ = 8.2 Hz), 2.06, 2.05, 1.99, 1.97, 1.88, 1.80 (6s, 18H, acetyl CH$_3$), 1.32, 1.18, 1.12, O ‖ 0.80, 0.75 (5 peaks, 15H, O—C—(CH$_3$)$_2$ and (C-7)H$_3$). *Anal.* calcd. for $C_{45}H_{57}O_{23}N$: C 55.16, H 5.86, N 1.43; found: C 55.02, H 5.83, N 1.31.

*1,2;3,4-Di-*O*-isopropylidene-6-*O*-[2-acetamido-2-deoxy-4-*O*-(β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-7-deoxy-β-*L*-glycero-*D*-galacto-heptopyranose (5)*

A solution of compound **4** (0.56 g, 0.57 mmol) and 85% hydrazine hydrate (0.22 g, 4.39 mmol) in ethanol (5 mL) was refluxed for 3 h. Solvent removal left a syrup which was redissolved in water (5 mL). The solution was neutralized with glacial acetic acid and the precipitate formed was removed by filtration. Dialysis of the filtrate through a UMO5 Diaflo membrane (Amicon, Lexington, MA 02173) afforded, after freeze-drying of the dialyzed solution, a solid (0.25 g). This substance was taken up in methanol–water (1:1, 20 mL) containing acetic anhydride (4 mL) and left at room temperature for 2 h. Solvent removal and crystallization from methanol–ether gave a white solid (0.152 g, 42%), mp 165–169°C; ^{1}H nmr (D_2O, HOD δ = 4.80) δ: 5.62 (d, 1H, H-1, $J_{1,2}$ = 5.0 Hz), 4.44 (2d, 2H, H′ and H″ overlapping, *J* = 7.5 Hz and 8.0 Hz, 2.07 (s, 3H, NHAc O ‖ methyl), 1.52, 1.48, 1.38 (3s, 12H, O—C(CH$_3$)$_2$), 1.30 (d, 3H, (C-7)H$_3$), 4.02–3.48 (m, 17H, remaining protons).

*6-*O*-[2-Acetamido-2-deoxy-4-*O*-(β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-7-deoxy-*L*-glycero-*D*-galacto-heptopyranose (6)*

A solution of compound **5** (50 mg, 0.078 mmol) in 95% trifluoroacetic acid (2 mL) was stirred for 3 min at room temperature and rapidly evaporated *in vacuo* to a syrup which was applied to a Sephadex LH-20 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) column and eluted with ethanol–water (1:1). Solvent removal and freeze-drying of the main fraction gave a white solid (13.5 mg, 0.024 mmol, 31%); ^{1}H nmr (D_2O) δ: 5.28 (d, H-1α, $J_{1,2}$ = 2.5 Hz, H-1β not observed, probably obscured by the HOD peak at 4.80), 4.54 (2d, 2H, H-1′ and H-1″ overlapping, *J* = 7.5 Hz and 7.5 Hz), 2.14, 2.13 (2s, NHAc-α and NHAc-β methyl), 1.33 (2d, 3H, (C-7)H$_3$ α and β), 4.08–3.44 (m, 17H, remaining protons).

*1,2;3,4-Di-*O*-isopropylidene-6-*O*-[3,6-di-*O*-acetyl-2-deoxy-4-*O*-(tetra-*O*-acetyl-β-*D*-galactopyranosyl)-2-phthalimido-β-*D*-glucopyranosyl]-7-deoxy-α-*D*-glycero-*D*-galacto-heptopyranose (7)*

3,6-Di-*O*-acetyl-4-*O*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-α,β-D-glucopyranosyl chloride (0.213 g, 0.29 mmol) in dry nitromethane (2 mL) was added to a solution of the alcohol (**3**) (60 mg, 0.22 mmol), *sym*-collidine (33 mg, 0.27 mmol), and silver triflate (60 mg, 0.24 mmol) in dry nitromethane (5 mL) cooled to −25°C. The mixture was stirred at −25°C for 2 h and then warmed to room temperature for 16 h. The reaction mixture was diluted with chloroform (20 mL) and the solids were removed by filtration. The filtrate was washed with cold water, aqueous sodium bicarbonate solution, and dilute aqueous HCl. Solvent removal after drying over sodium sulfate left a foam which was applied to a silica gel column and eluted with ethyl acetate – hexane (1:1). Solvent removal of the second fraction left a white solid (0.14 g, 0.14 mmol, 65%), mp 114–119°C, $[\alpha]_D^{23}$ −20° (*c* 0.5, chloroform); ^{1}H nmr ($CDCl_3$) δ: 7.80–7.62 (broad m, 4H, phthalimido), 5.66 (dd, 1H, H-3′, $J_{3',4'}$ = 8.0 Hz, $J_{2',3'}$ = 10.5 Hz), 5.41 (d, 1H, H-1′, $J_{1',2'}$ = 8.5 Hz), 5.34 (d, 1H, H-1, $J_{1,2}$ = 4.5 Hz), 5.25 (broad d, 1H, H-4″, $J_{3'',4''}$ = 3.1 Hz), 5.04 (dd, 1H, H-2″, $J_{1'',2''}$ = 7.5 Hz, $J_{2'',3''}$ = 10.5 Hz), 4.86 (dd, 1H, H-3″), 4.46 (d, 1H, H-1″), 2.07, 1.99, 1.98, 1.89, 1.84 (5s, 15H, acetyl CH$_3$), O ‖ 1.32, 1.22, 1.20 (3s, 12H, O—C(CH$_3$)$_2$), 0.88 (d, 3H, (C-7)H$_3$). *Anal.* calcd. for $C_{45}H_{57}O_{23}N$: C 55.16, H 5.86, N 1.43; found: C 54.51, H 5.76, N 1.34.

*1,2;3,4-Di-*O*-isopropylidene-6-*O*-[2-acetamido-2-deoxy-4-*O*-(β-*D*-galactopyranosyl)-α-*D*-glucopyranosyl]-7-deoxy-β-*D*-glycero-*D*-galacto-heptopyranose (8)*

Treatment of compound **7** (0.119 g, 0.12 mmol) with 85% hydrazine hydrate, work-up and *N*-acetylation as described above for the preparation of **5**, resulted in the isolation of a white solid (72 mg) which was applied to a Sephadex LH-20 column and eluted with ethanol–water (1:1). Solvent removal from the main fraction gave a white solid (34 mg, 0.053 mmol, 44%), mp 155–158°C; ^{1}H nmr (D_2O) δ: 5.66 (d, 1H, H-1, $J_{1,2}$ = 5.0 Hz), 4.50 (2d, 2H, H-1′ and H-1″ overlapping, *J* = 7.5 Hz), 2.10 (s, O ‖ 3H, NHAc methyl), 1.65, 1.48, 1.45, 1.43 (4s, 12H, O—C(CH$_3$)$_2$), 1.25 (d, 3H, (C-7)H$_3$), 4.20–3.50 (m, 17H, remaining protons).

*6-*O*-[2-Acetamido-2-deoxy-4-*O*-(β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-7-deoxy-*D*-glycero-*D*-galacto-heptopyranose (9)*

A solution of compound **8** (21 mg, 0.033 mmol) in 70% trifluoroacetic acid (2 mL) was stirred at room temperature for 4 min and rapidly evaporated *in vacuo* to about 0.5 mL, and 3

drops of triethylamine was added. Filtration through a column of Sephadex LH-20, as described above for the preparation of **6**, provided a main fraction which was freeze-dried to a white solid (14.5 mg, 79%); ^{1}H nmr (D_2O) δ: 5.24, 4.66 (2d, 1H, H-1α and H-1β, J = 3.0 Hz and 7.5 Hz), 4.58, 4.49 (2d, 2H, H-1′ and H-1″ which shows up at this field position, J = 7.5 Hz and 7.5 Hz), 2.08 (s, 3H, NHAc methyl), 1.26, 1.24 (2d, 3H, (C-7)H_3α and (C-7)H_3β), 4.26–3.42 (m, 17H, remaining protons).

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