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Enzymic glycosylation of (±)-(3,5/4,6)-3,6-diazido-4,5-dihydroxycyclohexene. A way to prepare stereochemically pure and enzyme resistant, basic pseudo-disaccharides as competitive enzyme inhibitors ☆

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Abstract

By β-D-galactosylation of (±)-(3,5/4,6)-3,6-diazido-4,5-dihydroxycyclohexene, pure (+)-(3,5/4,6)-3,6-diazido-4-*O*-(β-D-galactopyranosyl)-5-hydroxycyclohexene (**3**) was obtained. The diamine (+)-(1,3/2,6)-3,6-diamino-1-*O*-(β-D-galactopyranosyl)-2-hydroxycyclohexane, derived from compound **3** by catalytic hydrogenation, is stable against enzymic cleavage and competitively inhibits β-D-galactosidase from *Escherichia coli* with a K_i-value of 5.5 mM. Sigmatropic rearrangement of **3** in methanolic solution partially led to an unseparable mixture of the regioisomers (3,5/4,6)-3,4-diazido-5-*O*-(β-D-galactopyranosyl)-6-hydroxycyclohexene and (3,5/4,6)-3,4-diazido-6-*O*-(β-D-galactopyranosyl)-5-hydroxycyclohexene. Catalytic hydrogenation thereof yielded an equally unseparable mixture of the diamines (1,3/2,4)-1,2-diamino-3-*O*-(β-D-galactopyranosyl)-4-hydroxycyclohexane and (1,3/2,4)-1,2-diamino-4-*O*-(β-D-galactopyranosyl)-3-hydroxycyclohexane, inhibiting β-D-galactosidase competitively with K_i 0.9 mM.

Keywords: β-D-Galactosidase; Pseudo-disaccharides; Competitive inhibition; Stereospecific glycosylation

☆ Dedicated to Professor R.R. Schmidt on the occasion of his 60th birthday.

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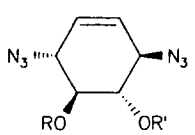
1. Introduction

Substituted cyclohexene derivatives prepared from *p*-benzoquinone [1] were used to synthesise rare hexopyranoses [2] and alkylating pseudo-disaccharides for the site-directed chemical modification of glycoside cleaving enzymes [3]. The glycosylation of such carbocycles, by either chemical or enzymic procedures, could be a source for the preparation of rare disaccharides, or pseudo-disaccharides. The formation of separable diastereomers is a means of obtaining enantiomerically pure compounds either of structurally varying hexoses or cyclitols. As an example, we describe the β-D-galactosylation of (±)-(3,5/4,6)-3,6-diazido-4,5-dihydroxy-cyclohexene [(±)-**1**].

2. Results and discussion

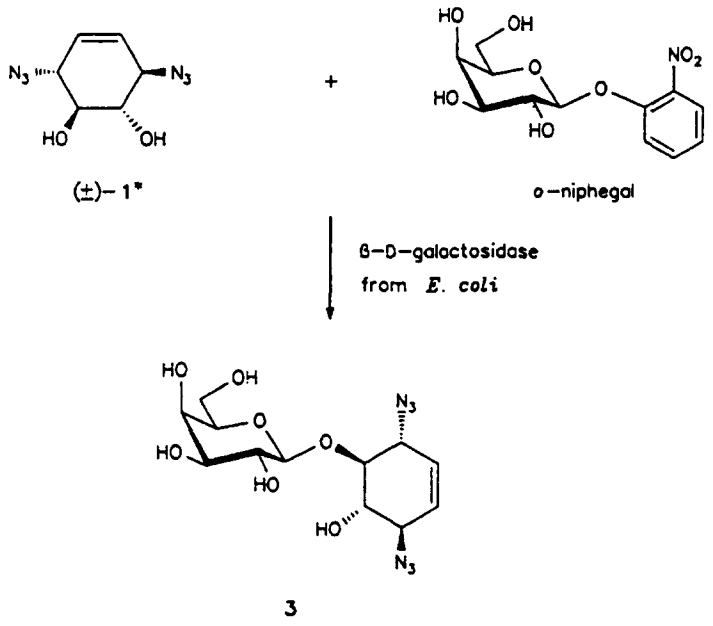
Stereoselective and regioselective enzymic glycosylations of polyhydroxy compounds have been known for a long time [4] and applied, for instance, to the preparation of a diastereomerically pure β-D-galactoside of prochiral glycerol [5] and, more recently, of equally prochiral carbocyclic meso-diols [6] in good yields. The enzyme used was β-D-galactosidase from *Escherichia coli*, and the donor substrates either D-lactose or *o*-nitrophenyl β-D-galactopyranoside (*o*-niphegal).

In the case described here, the acceptor diol is a racemic mixture of a substituted cyclohexene (±)-**1**, which can be prepared in one step from (±)-(3,4/5,6)-diepoxy-cyclohexene [1] by treatment with sodium azide [7]. Compound (±)-**1** can be converted either into a diaminocyclitol after exhaustive catalytic reduction or, after ozonolysis and subsequent reduction, into a nojirimycin-type compound [8]. By enzymic β-D-galactosylation, we can primarily test the stereoselectivity of the transfer reaction, but also obtain after catalytic hydrogenation pseudo-oligosaccharides with an aminocyclitol as the aglycone. These basic compounds are potential competitive inhibitors.



	R	R'
(±)-**1***	H	H
(±)-**2***	Ac	Ac
3	H	β-D-galactopyranosyl
4	Ac	tetra-*O*-acetyl-β-D-galactopyranosyl

* only one enantiomer (+) is depicted



Scheme 1.

The enzymic β-D-galactosylation with *o*-niphegal as donor and (±)-**1** as acceptor yielded stereochemically pure (+)-(3,5/4,6)-3,6-diazido-4-*O*-(β-D-galactopyranosyl)-5-hydroxycyclohexene (**3**) (Scheme 1). The optical purity of compound **3** is deduced from its ^{1}H NMR spectra, which show no signals for the other possible diastereomer, the absolute configuration of which can be derived from the specific optical rotation of $[\alpha]_D$ +320° for the carbocyclic alcohol (+)-(3,5/4,6)-3,6-diazido-4,5-dihydroxycyclohexene [(+)-**1**] after it has been isolated from the mixture of enzymic hydrolysis of **3**. In agreement with investigations on cyclitols [9], a +*synclinal* 1,2-diol grouping results in a positive increment in optical rotatory power.

Based on the outcome of the described transgalactosylation to **3**, we postulate a diasteromeric binding mode of the cyclohexene ring, in which the diol grouping of the aglycone is +*synclinal* disposed (Fig. 1). This is in agreement with recent investigations by Lopez et al. [10], who demonstrate a selective 3-galactosylation of a β-D-xyloside where the 2,3-diol grouping also has a +*synclinal* orientation.

Catalytic hydrogenation turns galactoside **3** into the corresponding galactosylated aminocyclitol, (+)-(1,3/2,6)-3,6-diamino-1-*O*-(β-D-galactopyranosyl)-2-hydroxycyclohexane (**11**), in which one amino group is placed adjacent to the glycosidic oxygen atom and is therefore in a position to compete for an activating proton (Fig. 2). In a series of earlier investigations we were able to show that amino groups, suitably positioned in the aglyconic moiety of glycosides [11] or oligosaccharides [12], can protect the glycosidic bond, which is otherwise susceptible to enzyme-catalysed hydroly-

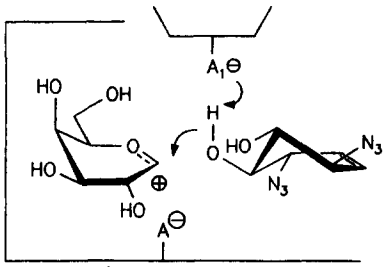
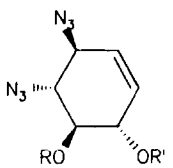


Fig. 1. Mechanism of the transgalactosylation to **3**. The 4,5-diol grouping in compound **1** has a + *synclinal* orientation.

sis. This protection is also found in compound **11**. Being resistant against enzymic hydrolysis, the β-D-galactoside **11** is a competitive inhibitor, with an inhibition constant of 5.5 mM.



	R	R'
(±)-**5***	H	H
(±)-**6***	Ac	Ac
7	β-D-galactopyranosyl	H
8	tetra-*O*-acetyl-β-D-galactopyranosyl	Ac
9	H	β-D-galactopyranosyl
10	Ac	tetra-*O*-acetyl-β-D-galactopyranosyl

* only one enantiomer (+) is depicted

In solution, and at room temperature, acceptor (±)-**1** and the aglycon in galactoside **3** undergo a very slow sigmatropic rearrangement to yield (±)-(3,5/4,6)-3,4-diazido-5,6-dihydroxycyclohexene [(±)-**5**] and a mixture of galactosides: (3,5/4,6)-3,4-diazido-5-*O*-(β-D-galactopyranosyl)-6-hydroxycyclohexene (**7**) and (3,5/4,6)-3,4-diazido-6-*O*-(β-D-galactopyranosyl)-5-hydroxycyclohexene (**9**) in a ratio of 3:1 (Scheme 2). The

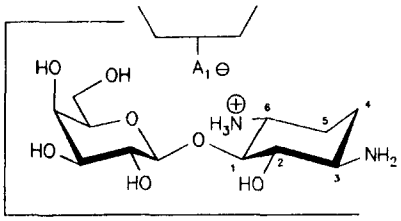
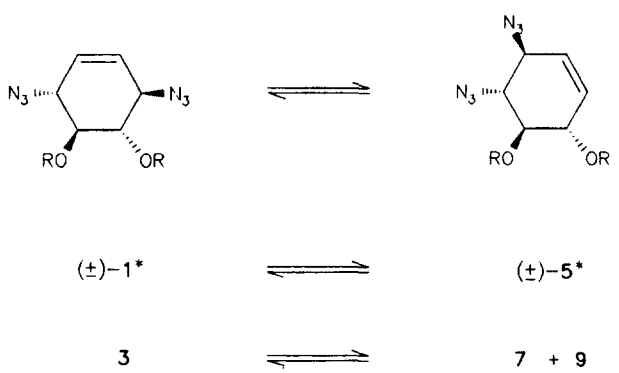


Fig. 2. Protection against enzymic hydrolysis in compound **11** is by the aglyconic 6-NH_2 group competing for the activating proton of A_1-H.

ratio between (±)-**1** and (±)-**5**, and between **3** and the mixture of **7** and **9** on equilibration, was not determined. Catalytic hydrogenation of the mixture of **7** and **9** yielded the diamines (1,3/2,4)-1,2-diamino-3-*O*-(β-D-galactopyranosyl)-4-hydroxycyclohexane (**13**) and (1,3/2,4)-1,2-diamino-4-*O*-(β-D-galactopyranosyl)-3-hydroxycyclohexane (**15**), which could also not be separated. The mixed diamines **13** and **15** as their isomer **11** were stable against enzyme-catalysed hydrolysis. The inhibition constant for the mixture is 0.9 mM. We assume that the major component **13** is the actual competitive inhibitor, and that the minor component **15** is mainly protected because of the enzyme inhibition by compound **13**. Under this assumption, the actual K_i for compound **13** would be even lower than 0.9 mM. According to Lopez et al. [10], a complex leading to the 3-galactosylation of a β-D-xyloside is stabilised by polar interaction between the xyloside acetal oxygen atoms and a polar group A_2–H (Fig. 3). This may explain the stronger binding of **13** as compared to **11**.



* only one enantiomer (+) is depicted

Scheme 2.

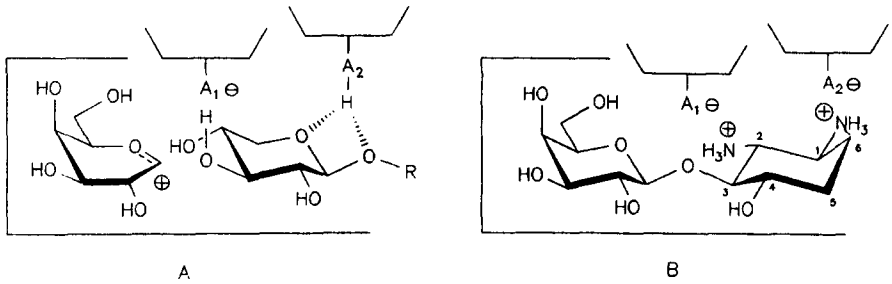
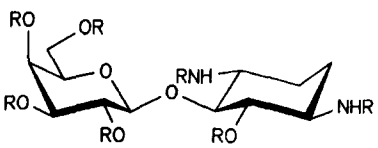
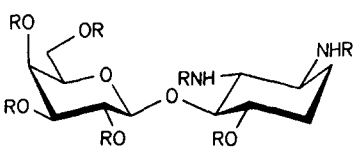


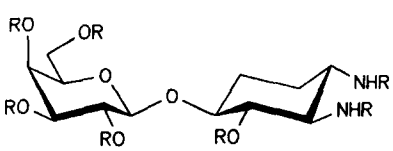
Fig. 3. Polar interactions by A_2–H lead to the preferential formation of β-D-galactopyranosyl-(1 → 3)-β-D-xylopyranoside (A) [10]. Similar interaction with the 1-NH_2 group in **13** (B) may be responsible for enhanced affinity in comparison with **11**.



	R
11	H
12	Ac



	R
13	H
14	Ac



	R
15	H
16	Ac

3. Experimental

General methods.—All reactions were monitored by TLC on Silica Gel 60 F_{254} (E. Merck) with detection by UV absorption and by charring with 2% H_2SO_4 in MeOH. Column chromatography was performed on Silica Gel 32-63, 60 A (ICN). Optical rotations were obtained with a Schmidt and Haensch Polartronic I polarimeter and measured at 25°C. ^{1}H NMR spectra were recorded with a Bruker AM 400 spectrometer at 400 MHz for solutions in $CDCl_3$ (internal Me_4Si). Kinetic data were obtained with an Eppendorf photometer at 405 nm connected to a transformation unit and a SE 120 recorder (BBC).

Enzyme.—β-D-Galactosidase [EC 3.2.1.23, 640 U/mg protein] from *E. coli* was purchased from Boehringer Mannheim.

Determination of the inhibition constants (K_i).—*o*-Nitrophenyl β-D-galactopyranoside was used as substrate (1–10 mM, K_M 120 μM) in 100 mM Na–K–phosphate buffer (pH 6.80, 1 mM $MgCl_2$) at 30°C. Inhibitors were used in the following concentrations (mM): **11** 2.5, 6.0, 10.0; **13/15** 0.6, 1.2, 1.8, 2.4. K_i-Values were determined by a Dixon plot [13].

(±)-(3,5/4,6)-3,6-Diazido-4,5-dihydroxycyclohexene [(±)-**1**].—To a solution of (±)-(3,4/5,6)-diepoxycyclohexene [1] (2.7 g, 24.5 mmol) in dry MeOH (165 mL), NaN_3 (6.4 g, 98 mmol) and $ZnSO_4 \cdot H_2O$ (17.6 g, 98 mmol) were added. The mixture was refluxed for 90 min and then filtered through Celite. The filtrate was evaporated and the residue chromatographed (1:1 EtOAc–cyclohexane) to give (±)-**1** (2.3 g, 47%); R_f 0.44 (1:1 EtOAc–cyclohexane); mp 57°C (from ether–petroleum ether); IR (KBr): 2100 cm^{-1}. Anal. Calcd for $C_6H_8O_2N_6$: C, 36.74; H, 4.11; N, 42.84. Found: C, 36.85; H, 4.14; N, 44.00. For ^{1}H NMR spectra compound (±)-**1** (50 mg, 0.255 mmol) was acetylated (2:1 pyridine–Ac_2O, 3 mL). The solution was evaporated to dryness and the residue chromatographed (1:10 EtOAc–cyclohexane) yielding (±)-(3,5/4,6)-4,5-di-*O*-acetyl-3,6-diazidocyclohexene [(±)-**2**] (53 mg; 75%); R_f 0.37 (1:5 EtOAc–cyclohexane). ^{1}H NMR data: δ 5.79 (s, 2 H, H-1, H-2), 5.23 (dd, 2 H, $J_{6,5}$ 8.25, $J_{6,4}$ 2.7, $J_{3,4}$ 8.25, $J_{3,5}$ 2.7 Hz, H-6, H-3), 4.22 (dd, 2 H, H-4, H-5), 2.12 (s, 6 H, 2 Ac).

(+)-(3,5/4,6)-3,6-Diazido-4-O-(β-D-galactopyranosyl)-5-hydroxycyclohexene (**3**).—To a mixture of (±)-**1** (2 g, 10.2 mmol) and *o*-niphegal (1.6 g, 5.3 mmol) in Na–K–phosphate buffer (20 mL, 100 mM, 1 mM $MgCl_2$, pH 6.80), β-D-galactosidase from *E. coli* (2.1 mg protein/5 mL buffer) was added. After 5 h at room temperature, more enzyme (1 mg protein/2.5 mL buffer) was added and the mixture stirred for an additional hour. The ratio between *o*-niphegal and transfer product **3** was approximately 2:1 when the reaction was stopped by heating for 5 min at 95°C. The solution was extracted with ether (3 × 10 mL), freeze-dried and the residue chromatographed (7:2:1 EtOAc–MeOH–H_2O). The mixed fraction of unchanged (±)-**1** and product **3**, R_f 0.25 (27:2:1 EtOAc–MeOH–H_2O), was acetylated (2:1 pyridine–Ac_2O, 15 mL) at room temperature. The solution was poured into ice-water and extracted with CH_2Cl_2 (4 × 5 mL). The organic layer was washed sequentially with satd aq $NaHCO_3$ and water, dried and concentrated. Chromatography of the residue (1:3 EtOAc–cyclohexane) gave (+)-(3,5/4,6)-5-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-3,6-diazidocyclohexene (**4**) (0.55 g) as a colourless oil. By recovering unchanged (±)-**1** (1.5 g,

7.65 mmol), the total yield of **4** was 38%; $[\alpha]_D$ + 116° (*c* 5.43, $CHCl_3$); R_f 0.37 (1:1 EtOAc–cyclohexane). Anal. Calcd for $C_{22}H_{28}O_{12}N_6$: C, 46.49; H, 4.96; N, 14.78. Found: C, 46.12; H, 5.02; N, 14.57. ^{1}H NMR data: δ 5.77 (m, 2 H, H-1, H-2), 5.39 (dd, 1 H, $J_{4',3'}$ 3.3, $J_{4'5'}$ 1.2 Hz, H-4'), 5.17 (dd, 1 H, $J_{5,4}$ 10.2, $J_{5,6}$ 8.25 Hz, H-5), 5.13 (dd, 1 H, $J_{2',3'}$ 10.5, $J_{2',1'}$ 7.5 Hz, H-2'), 5.03 (dd, 1 H, H-3'), 4.75 (d, 1 H, H-1'), 4.20 (dd, 1 H, $J_{6'a,6'b}$ 11.25, $J_{6'a,5'}$ 6.3 Hz, H-6'a), 4.14–4.08 (m, 3 H, H-3, H-6, H-6'b), 3.92 (ddd, 1 H, H-5'), 3.78 (dd, 1 H, $J_{4,3}$ 7.8 Hz, H-4), 2.16, 2.14, 2.07, 2.06, 1.99 (5 s, each 3 H, 4 Ac).

(+)-(1,3 / 2,6)-3,6-Diamino-1-O-(β-D-galactopyranosyl)-2-hydroxycyclohexane, dihydrochloride (**11**).—Compound **4** (225 mg, 0.396 mmol) was deacetylated [14] and the solution demineralised by elution (MeOH) from a column of silica gel. The eluate was concentrated and chromatographed (27:2:1 EtOAc–MeOH–H_2O) to give **3** (128 mg, 91%) as a colourless foam. Compound **3** was hydrogenated in MeOH (10 mL) and concd HCl (0.2 mL) over Pt (10 mg PtO_2) for 1 h. The mixture was filtered and concentrated to give **11** (134 mg, 98%) as a colourless foam; R_f 0.12 (2:2:1 EtOAc–MeOH–25% aq. NH_3). Anal. Calcd for $C_{12}H_{30}O_9N_2Cl_2 \cdot 2\,H_2O$: C, 34.55; H, 7.24; N, 6.71. Found: C, 35.10; H, 7.48; N, 6.33. For an analytical sample, compound **11** (41 mg, 0.11 mmol) was acetylated (2:1 pyridine–Ac_2O, 3 mL) at room temperature. The solution was evaporated to dryness and chromatographed (27:2:1 EtOAc–MeOH–H_2O) yielding (+)-(1,3/2,6)-3,6-diacetamido-2-*O*-acetyl-1-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)cyclohexane (**12**) (39 mg, 58%) as a colourless oil; $[\alpha]_D$ −31° (*c* 0.98, $CHCl_3$); R_f 0.26 (17:2:1 EtOAc–MeOH–H_2O). ^{1}H NMR data: δ 6.38 (d, 1 H, NH), 6.16 (d, 1 H, NH), 5.39 (dd, 1 H, $J_{4',3'}$ 3.3, $J_{4',5'}$ 1.35 Hz, H-4'), 5.06 (dd, 1 H, $J_{2',3'}$ 10.35, $J_{2',1'}$ 7.5 Hz, H-2'), 5.01 (d, 1 H, H-3'), 4.84 (t, 1 H, $J_{2,3} = J_{2,1}$ 7.2 Hz, H-2), 4.65 (d, 1 H, H-1'), 4.19 (dd, 1 H, $J_{6'a,6'b}$ 11.25, $J_{6'a,5'}$ 6.3 Hz, H-6'a), 4.15 (dd, 1 H, $J_{6'b,5'}$ 7.35 Hz, H-6'b), 4.1–3.9 (m, 3 H, H-3, H-6a, H-6b), 3.91 (ddd, 1 H, H-5'), 3.78 (t, 1 H, $J_{1,6}$ 7.2 Hz, H-1), 2.16, 2.11 (2 s, each 3 H, 2 NHAc), 2.10, 2.08, 2.03, 2.01, 1.99 (5 s, each 3 H, 5 Ac), 1.95–1.8 (m, 2 H, H-4a, H-4b), 1.4–1.52 (m, 2 H, H-5a, H-5b).

Determination of the optical activity of the aglycon in galactoside **3**.—To compound **3** (56 mg, 1.58 mmol) in Na–K–phosphate buffer (5 mL, 100 mM, 1 mM $MgCl_2$, pH 6.80), β-D-galactosidase from *E. coli* (0.15 mg protein) was added. After 1 h at room temperature, the solution was freeze-dried and the residue acetylated (2:1 pyridine–Ac_2O, 3 mL) at room temperature. The solution was evaporated to dryness and the aglycon isolated by chromatography (1:3 EtOAc–cyclohexane), (37 mg, 84%). ^{1}H NMR data were identical with those of compound (±)-**2** but with optical activity of $[\alpha]_D$ +320° (*c* 1.86, $CHCl_3$).

(±)-(3,5 / 4,6)-3,4-Diazido-5,6-dihydroxycyclohexene [(±)-**5**].—Compound (±)-**1** (100 mg, 0.51 mmol) was dissolved in MeOH (10 mL) and stirred at 30°C for 2 days. The solution was evaporated and the residue purified by chromatography (1:1 EtOAc–cyclohexane) to give (±)-**5** as a colourless oil (62 mg; 62%); R_f 0.65 (1:1 EtOAc–cyclohexane); IR (film): 2100 cm^{-1}. Anal. Calcd for $C_6H_8O_2N_6$: C, 36.74; H, 4.11; N, 42.84. Found: C, 36.87; H, 4.20; N, 43.51. For ^{1}H NMR compound (±)-**5** (20 mg, 0.1 mmol) was acetylated (2:1 pyridine–Ac_2O, 2 mL) at room temperature and then concentrated. Chromatography of the residue (1:10 EtOAc–cyclohexane) gave (±)-

(3,5/4,6)-5,6-di-*O*-acetyl-3,4-diazidocyclohexene [(±)-**6**] (24 mg, 85%) as a colourless oil; R_f 0.25 (1:5 EtOAc–cyclohexane). ^{1}H NMR data: δ 5.73 (m, 1 H, $J_{1,2}$ 10.13, $J_{1,6}$ 2.44, $J_{1,4}$ 2.45, $J_{1,3}$ −0.064 Hz, H-1), 5.70 (m, 1 H, $J_{2,6}$ 2.21, $J_{2,4}$ −2.22, $J_{2,3}$ 0.065 Hz, H-2), 5.54 (ddt, 1 H, $J_{6,5}$ 8.21, $J_{6,4}$ 3.12, $J_{6,3}$ −0.014 Hz, H-6), 5.19 (dd, 1 H, $J_{5,3}$ 11.13, $J_{5,4}$ −0.03 Hz, H-5), 4.01 (ddt, 1 H, $J_{4,3}$ 8.98 Hz, H-4), 3.69 (dd, 1 H, H-3), 2.14, 2.05 (2 s, each 3 H, 2 Ac).

*(3,5 / 4,6)-3,4-Diazido-5-*O*-(β-*D*-galactopyranosyl)-6-hydroxycyclohexene* (**7**) *and (3,5 / 4,6)-3,4-diazido-6-*O*-(β-*D*-galactopyranosyl)-5-hydroxycyclohexene* (**9**).—Compound **3** (241 mg, 0.673 mmol) was dissolved in 1:1 MeOH–H_2O (8 mL) and stirred at 50°C for 4 days. The ratio between **3** and the mixture of **7/9**, R_f 0.32 (17:2:1 EtOAc–MeOH–H_2O), was about 1:2, as indicated by TLC analysis. The solution was evaporated to dryness and acetylated (2:1 pyridine–Ac_2O, 4 mL) at room temperature and then concentrated. Chromatography (1:3 EtOAc–cyclohexane) of the residue gave a mixture of (3,5/4,6)-6-*O*-acetyl-5-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-3,4-diazidocyclohexene (**8**) and (3,5/4,6)-5-*O*-acetyl-6-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-3,4-diazidocyclohexene (**10**) as a colourless oil, (229 mg, 60%); R_f 0.35 (1:1 EtOAc–cyclohexane). ^{1}H NMR data (**8**): δ 5.77–5.65 (m, 2 H, H-1, H-2), 5.36 (dd, 1 H, $J_{4',3'}$ 3.3, $J_{4',5'}$ 1.2 Hz, H-4′), 5.12 (dd, 1 H, $J_{4,5}$ 11.25, $J_{4,3}$ 7.8 Hz, H-4), 5.11 (dd, 1 H, $J_{2',3'}$ 10.5, $J_{2',1'}$ 7.8 Hz, H-2′), 5.00 (dd, 1 H, H-3′), 4.56 (d, 1 H, H-1′), 4.45–4.40 (m, 1 H, H-3), 4.20–4.05 (m, 2 H, H-6′a, H-6′b), 4.05–3.97 (m, 1 H, H-6), 3.90 (ddd, 1 H, $J_{5',6'a} = J_{5',6'b}$ 6.75 Hz, H-5′), 3.62 (dd, 1 H, $J_{5,6}$ 9 Hz, H-5), 2.14, 2.13, 2.06, 2.03, 1.98 (5 s, each 3 H, 5 Ac). ^{1}H NMR data (**10**): δ 5.77–5.67 (m, 2 H, H-1, H-2), 5.46 (m, 1 H, H-6), 5.37 (dd, 1 H, H-4′), 5.19 (dd, 1 H, $J_{2',3'}$ 10.5, $J_{2',1'}$ 7.8 Hz, H-2′), 5.03 (dd, 1 H, $J_{3',4'}$ 3.75 Hz, H-3′), 4.81 (d, 1 H, H-1′), 4.20–4.05 (m, 2 H, H-6′a, H-6′b), 4.05–3.97 (m, 1 H, H-3), 3.91 (m, 1 H, H-5′), 3.81 (dd, 1 H, $J_{4,5}$ 11.25, $J_{4,3}$ 7.9 Hz, H-4), 3.55 (dd, 1 H, $J_{5,6}$ 9 Hz, H-5), 2.10, 2.09, 2.05, 2.04, 1.99 (5 s, each 3 H, 5 Ac).

*(1,3 / 2,4)-1,2-Diamino-3-*O*-(β-*D*-galactopyranosyl)-4-hydroxycyclohexane, dihydrochloride* (**13**) *and (1,3 / 2,4)-1,2-diamino-4-*O*-(β-*D*-galactopyranosyl)-3-hydroxycyclohexane, dihydrochloride* (**15**).—The mixture of **8/10** (119 mg, 0.209 mmol) was deacetylated as described for the preparation of **3** to give, after usual workup and chromatography (27:2:1 EtOAc–MeOH–H_2O), a mixture of **7** and **9** (68 mg, 91%). Hydrogenation in MeOH (5 mL) and concd HCl (0.1 mL) over Pt (8 mg PtO_2) for 1 h afforded after filtration and evaporation to dryness the diamines **13/15** (71 mg, 98%); R_f 0.13 (2:2:1 EtOAc–MeOH–25% aq NH_3). For ^{1}H NMR the mixture of **13/15** (35 mg, 0.094 mmol) was acetylated (2:1 pyridine–Ac_2O, 3 mL) at room temperature and then concentrated. Chromatography (27:2:1 EtOAc–MeOH–H_2O) of the residue yielded the mixture of (1,3/2,4)-1,2-diacetamido-4-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)cyclohexane (**14**) and (1,3/2,4)-1,2-diacetamido-3-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)cyclohexane (**16**) (36 mg, 65%) as colourless foams; R_f 0.31 (17:2:1 EtOAc–MeOH–H_2O). ^{1}H NMR data (**14**): δ 6.55 (d, 1 H, NH), 6.18 (d, 1 H, NH), 5.37 (dd, 1 H, $J_{4',3'}$ 3.3, $J_{4',5'}$ 1.35 Hz, H-4′), 5.06 (dd, 1 H, $J_{2',3'}$ 10.5, $J_{2',1'}$ 7.65 Hz, H-2′), 4.99 (dd, 1 H, H-3′), 4.89 (m, 1 H, H-4), 4.65 (d, 1 H, H-1′), 4.18–4.05 (m, 2 H, H-6′a, H-6′b), 3.95–3.85 (m, 2 H, H-5′, H-3), 3.80–3.67 (m, 2 H, H-1, H-2), 2.1–1.9, 1.5–1.2 (m, 4 H, H-5a, H-5b, H-6a, H-6b), 2.15, 2.08 (2 s,

each 3 H, 2 NHAc), 2.07, 2.01, 1.98, 1.94, 1.93 (5 s, each 3 H, 5 Ac). ^{1}H NMR data (**16**): δ 6.38 (d, 1 H, NH), 6.12 (d, 1 H, NH), 5.38 (dd, 1 H, $J_{4',3'}$ 3.45, $J_{4',5'}$ 1.35 Hz, H-4'), 5.08 (dd, 1 H, $J_{2',3'}$ 10.5, $J_{2',1'}$ 7.8 Hz, H-2'), 5.00 (dd, 1 H, H-3'), 4.77 (dd, 1 H, $J_{3,4}$ 9, $J_{3,2}$ 10.5 Hz, H-3), 4.51 (d, 1 H, H-1'), 4.15 (dd, 1 H, $J_{6'a,6'b}$ 11.25, $J_{6'a,5'}$ 6.3 Hz, H-6'a), 4.08 (dd, 1 H, $J_{6'b,5'}$ 7.2 Hz, H-6'b), 3.95–3.85 (m, 2 H, H-5', H-4), 3.8–3.67 (m, 2 H, H-1, H-2), 2.1–1.9, 1.5–1.2 (m, 4 H, H-5a, H-5b, H-6a, H-6b), 2.16, 2.09 (2 s, each 3 H, 2 NHAc), 2.06, 2.02, 1.99, 1.95, 1.92 (5 s, each 3 H, 5 Ac).

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