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Synthesis of 1D-(1,3,5/2,4)-4-acetamido-5-amino-1,2,3-cyclohexanetriol and its incorporation into a pseudo-disaccharide

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Abstract

The synthesis of the title compound and 1D-(1,3,5/2,4)-4-acetamido-5-amino-3-*O*-(β-D-glucopyranosyluronic acid)-1,2,3-cyclohexanetriol is described. Starting from methyl 2-acetamido-2-deoxy-α-D-glucopyranoside 2L-(2,4,5/3)-4-acetamido-3-benzoyloxy-2-benzyloxy-5-hydroxycyclohexanone was prepared via a series of transformations including the regioselective ring opening of the benzylidene acetal and the mercury(II)-catalyzed carbocyclic ring closure reaction of 5-enopyranoside. Stereoselective reduction of ketone **11** with $NaBH(OAc)_3$ gave 1D-(1,2,4/3,5)-2-acetamido-3-*O*-benzoyl-4-*O*-benzyl-1,3,4,5-cyclohexanetetrol (88%), which was then converted into 1D-(1,3,5/2,4)-4-acetamido-5-azido-3-*O*-benzoyl-2-*O*-benzyl-1-*O*-pivaloyl-1,2,3-cyclohexanetriol through selective 5-OH protection, 1-*O*-mesylation, and subsequent azide displacement. Saponification and hydrogenation of this gave the title compound. Selective *O*-debenzoylation with 1.1 equiv of K_2CO_3 in MeOH gave 1D-(1,3,5/2,4)-4-acetamido-5-azido-2-*O*-benzyl-1-*O*-pivaloyl-1,2,3-cyclohexanetriol (73%). Glycosylation of this compound with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate in CH_2Cl_2, using silver triflate as the promoter, afforded 1D-(1,3,5/2,4)-4-acetamido-5-azido-2-*O*-benzyl-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glu-

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copyranosyluronate)-1-*O*-pivaloyl-1,2,3-cyclohexanetriol (**20**) in 79% yield. Finally, *O*-deacylation and subsequent hydrogenation of this compound gave the basic pseudo-disaccharide.

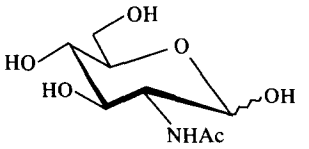
Keywords: Glycosidase inhibitor; Pseudo-aminosugar; Ferrier's carbocylization

1. Introduction

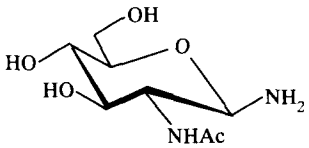
Enzyme inhibitors are invaluable for studying enzyme mechanisms as well as for developing therapeutic agents. In 1973, Lai and Axelrod [1] reported that simple sugar analogs bearing a basic nitrogen attached to the anomeric position (i.e. glycosylamines) are strong inhibitors of glycosidases. This and subsequent studies [2–5] showed that glycosylamines derived from D-glucose, D-galactose, D-mannose, and *N*-acetyl-D-glucosamine inhibit the corresponding glycosidases up to 10^3 times more strongly than their respective non-basic analogs, e.g. free sugars, thioglycosides, *N*-acetyl-or *N*-aryl-glycosylamines. This strong inhibition was assumed to be due to the formation of a tight ion-pair at the catalytic site consisting of the protonated inhibitor and a negatively charged group essential for catalysis [6]. In spite of the good inhibitory activity and ease of preparation of glycosylamines, however, their susceptibility to spontaneous hydrolysis and instability of the α,β-anomeric configurations in aqueous solution prevent them from becoming practical inhibitors. For experiments requiring long incubation time, e.g. with cell cultures or in glycoprotein research, stable and specific inhibitors have to be found. Deoxynojirimycin and other imino sugars with the ring oxygen being replaced by nitrogen represent a class of this kind of inhibitors [7]. In addition, 2-acetamido-1,2-dideoxynojirimycin was synthesized and shown to be an efficient inhibitor of *N*-acetylglucosaminidases [5,8] (Fig. 1). Its simpler analog, with the hydroxymethyl group at C-5 removed, was also described and shown to be a moderate inhibitor but of relatively poor activity compared with 2-acetamido-1,2-dideoxynojirimycin, indicating the contribution of CH_2OH group on C-5 to the inhibitory activity [9] (see accompanying formulas). In another case, β-glucosidase from sweet almonds was shown to have a similar affinity for D-xylose ($K_i = 495$ mM) and D-glucose ($K_i = 160$ mM), showing little effect of the 5-CH_2OH substituent on enzyme–substrate interactions [10]. These findings prompted us to prepare a simple, stable, basic pseudo-sugar analog (**1**) of 2-acetamido-2-deoxy-β-D-glucosylamine and couple it with glucuronic acid to form basic pseudo-disaccharide (**2**). Compounds **1** and **2** were designed in the hope that they might serve as potential inhibitors of certain *N*-acetylhexosaminidases and, in particular, hyaluronidases.

2. Results and discussion

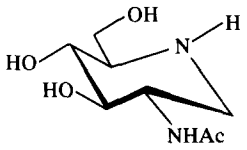
The Ferrier reaction [11] was applied as a key step for the construction of the functionalized cyclitol skeleton. Starting from methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**3**), prepared according to the procedure of Lee [12], methyl 2-acetamido-3-



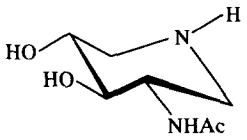
2-acetamido-2-deoxy-D-glucose
(K_i = 4 x 10^{-3} M, with bovine β-*N*-acetylglucosaminidase, ref 5)



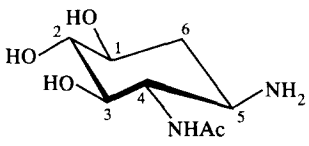
2-acetamido-2-deoxy-β-D-glucosylamine
(K_i = 4.3 x 10^{-5} M, with bovine β-*N*-acetylglucosaminidase, ref 5)



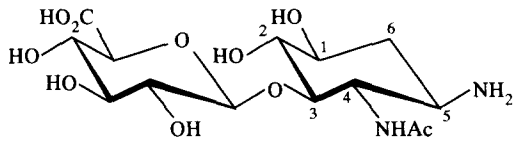
2-acetamido-1,2-dideoxynojirimycin
(K_i = 6 x 10^{-7} M, with bovine β-*N*-acetylglucosaminidase, ref 5)



a simple analog of 2-acetamido-1,2-dideoxynojirimycin
(IC_{50} = 1 x 10^{-4} M, with bovine β-*N*-acetylhexosaminidase, ref 9)



1



2

Fig. 1.

O-benzoyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**4**) was prepared by acid-catalyzed acetal exchange with α,α-dimethoxytoluene in DMF and subsequent benzoylation. To synthesize compound **5** with a free 6-hydroxyl group, regioselective reductive ring opening of the benzylidene acetal in **4** was attempted with borane–trimethylamine and aluminium chloride in dichloromethane [13]. It was found that, while an excess of both $BH_3 \cdot NEt_3$ and $AlCl_3$ was necessary for the complete reaction of starting material, the yield of the desired product (**5**) depended significantly on the ratio of $BH_3 \cdot NEt_3$ to $AlCl_3$. The major side-reaction was *O*-debenzylidenation. Raising the ratio of reducing agent to Lewis acid led to the increase of the yield of compound **5**. Thus, treatment of **4** with 4:4 mol. equiv of $BH_3 \cdot NEt_3$–$AlCl_3$ in CH_2Cl_2 for 30 min at −15°C gave methyl 2-acetamido-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**5**) in 20% yield,

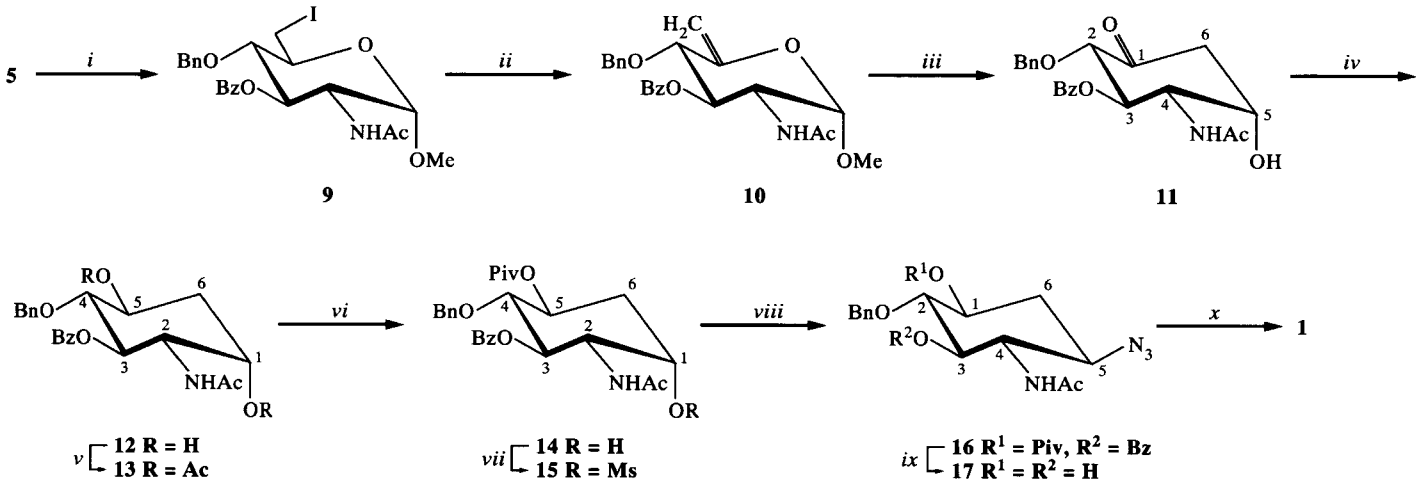
i: $PhCH(OMe)_2$ (1.25 *equiv.*), DMF, cat. TsOH, 60°C, 3 h, then BzCl, pyridine, r.t., 5 h, 86%.
ii: BH_3Me_3N (4 *equiv.*), $AlCl_3$ (2.5 *equiv.*), CH_2Cl_2, MS4A, -15°C, 30 min, 65% for **5**; 16% for **6**.
iii: Ac_2O, pyridine, r.t., 4 h, quantitative.

Scheme 1.

together with methyl 2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (**6**) (51%), after column chromatography. When the ratio of $BH_3 \cdot NEt_3$–$AlCl_3$ was increased to 4:2.5 and the reaction was carried out under the same conditions as above, however, a 65% yield of **5** could be obtained, along with a 16% yield of **6** (Scheme 1). The structure of **5** was elucidated on the basis of its ^{1}H NMR analysis and further confirmed by converting it into the corresponding acetate (**7**). On acetylation, the H-6a,b were shifted from δ 3.86–3.75 (high field) in **5** to δ 4.39–4.30 (relatively low field) in **7** (*O*-acetylated), whereas the H-4 resonated at almost the same place in both **5** and **7** (δ 3.88–3.80). These data clearly indicated that, in compound **5**, the 6-hydroxy group was free and the benzyloxy group was attached at C-4. Compound **5** was then efficiently converted into iodide **9** by reaction with triphenylphosphine and *N*-iodosuccinimide in tetrahydrofuran under reflux, according to the procedure of Hanessian [14]. Dehydroiodination of **9** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DMF gave the 5-enopyranoside **10**.

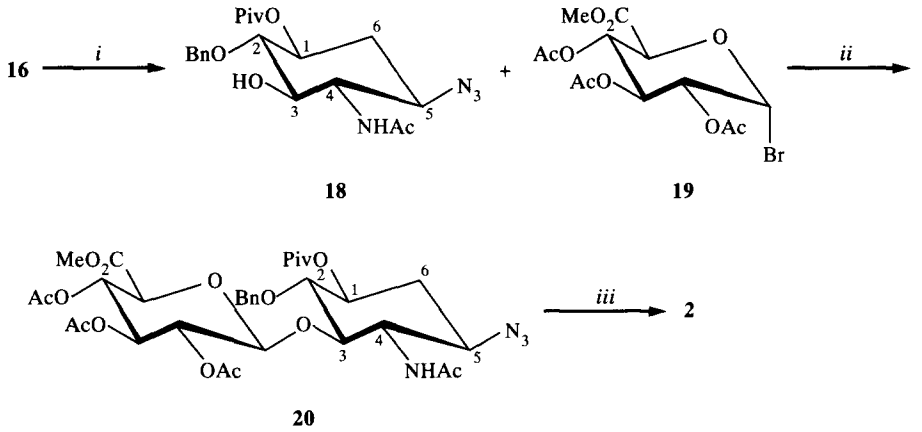
Next, the Ferrier carbocyclic ring-closure reaction [11] was examined. Treatment of enopyranoside **10** with a stoichiometric amount of mercury(II) chloride in aqueous acetone under reflux gave the desired 2L-(2,4,5/3)-4-acetamido-3-benzoyloxy-2-benzyloxy-5-hydroxycyclohexanone (**11**) in a moderate yield. In our hands, however, the separation of **11** from a large amount of mercury salts either by column chromatography or by crystallization seemed cumbersome, especially in the case of large-scale preparation. This problem was efficiently solved by using mercury(II) sulfate as the catalyst [15]. Thus, treatment of **10** with only a catalytic amount of mercury(II) sulfate in dioxane–5 mM sulfuric acid gave the cyclohexanone **11** in excellent yield, and the purification of the product became very simple, either by column chromatography or through direct crystallization from ethanol. In the ^{1}H NMR spectrum of **11**, the large coupling between H-2 and H-3 ($J_{2,3}$ 9.4 Hz), as well as between H-3 and H-4 ($J_{3,4}$ 9.4 Hz), showed that the cyclohexanone took a $^2C_5$ (L) conformation; while the relatively small coupling between H-4 and H-5 ($J_{4,5}$ 2.40 Hz) and that between H-5 and H-6 ($J_{5,6a}$ 3.04, $J_{5,6b}$ 3.62 Hz) gave the evidence that the 5-hydroxyl group in **11** should adopt an axial orientation. Stereoselective reduction of the ketone was achieved by reaction of **11** with sodium triacetoxyborohydride [$NaHB(OAc)_3$] in acetic acid [16], giving diol **12** (88%). Again, the configuration of the newly generated hydroxyl group was determined by NMR spectroscopy. The large coupling constants, $J_{4,5}$ (9.50 Hz) and $J_{5,6b}$ (12.0 Hz), revealed that the new hydroxyl group in **12** was situated in an equatorial position. This was further confirmed by transforming it into the acetate derivative **13**, all the ring protons of which were unambiguously assigned (see Experimental).

To introduce a nitrogenous function at the pseudo-anomeric position in **12**, regioselective protection of the equatorial hydroxy group was required. This was done by selective pivaloylation (trimethylacetylation). Thus, reaction of diol **12** with 1.20 equiv of pivaolyl chloride in pyridine at 0°C gave 1D-(1,2,4/3,5)-2-acetamido-3-*O*-benzoyl-4-*O*-benzyl-5-*O*-pivaloyl-1,3,4,5-cyclohexanetetrol (**14**) (87%). Compound **14** was then reacted with mesyl chloride in pyridine to give mesylate **15** which, on treatment with lithium azide in DMF, gave the key intermediate 1D-(1,3,5/2,4)-4-acetamido-5-azido-3-*O*-benzoyl-2-*O*-benzyl-1-*O*-pivaloyl-1,2,3-cyclohexanetriol (**16**) in excellent yield. *O*-Deacylation of **16** with methanolic potassium hydroxide afforded compound **17** (92%).



i: N-iodosuccinimide, PPh_3, THF, reflux, 4 h, 92%.
ii: DBU, DMF, 70°C, 1 h, 81%.
iii: cat. $HgSO_4$, 5 mM H_2SO_4-dioxane (1:2, v/v), 60°C, 1.5 h, 91%; or $HgCl_2$ (1.1 *equiv.*), H_2O-acetone (1:2, v/v), reflux, 3.5 h, 63%.
iv: $NaBH(OAc)_3$, AcOH, 20°C, 1 h, 88%.
v: Ac_20, NEt_3, r.t., overnight quantitative.
vi: Pivaloyl chloride, pyridine, 0-20°C, 16 h, 87%.
vii: Mesyl chloride, pyridine, r.t., overnight, 96%.
viii: LiN_3, DMF, 70-80°C, 12 h, 88.7%.
ix: KOH, THF-MeOH (1:1, v/v), r.t., 16 h, 92%.
x: H_2/Pd-C, EtOH, r.t., 24 h, 85%.

Scheme 2.



i: K_2CO_3 (1.1 *equiv.*), THF-MeOH (1:1, v/v), 20°C, 4.5 h, 71%.
ii: AgOTf, MS4A, CH_2Cl_2, -15°C, 3h, 79%.
iii: (a) 0.6 M NaOH, MeOH-H_2O (9:1, v/v), r.t., 16 h; (b) Dowex 50w-x8 (H^+); (c) H_2, Pd-C, H_2O, r.t., 24 h, 88%.

Scheme 3.

Finally, catalytic hydrogenolysis of **17** in the presence of 10% palladium on charcoal in ethanol furnished the desired 1*D*-(1,3,5/2,4)-4-acetamido-5-amino-1,2,3-cyclohexanetriol (**1**) in 85% yield (Scheme 2).

To conjugate **1** with glucuronic acid through a β-(1 → 3)-glycosidic linkage, the benzoyl protecting group in **16** was selectively removed by treatment with 1.1 equiv of potassium carbonate in mixed THF–MeOH, giving compound **18** (71%). Condensation of **18** with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (**19**) [17] in CH_2Cl_2 in the presence of silver triflate and MS4A at −15°C proceeded smoothly to give the pseudo-disaccharide derivative **20** in 79% yield. The β-glycosidic linkage in **20** was apparent as judged by the large coupling between H-1′ and H-2′ ($J_{1',2'}$ 7.6 Hz). Saponification of **20** with sodium hydroxide in MeOH–H_2O, followed by catalytic hydrogenation in H_2O afforded the target pseudo-disaccharide **2** (88%) as a white powder (Scheme 3).

Preliminary bioassays showed that compound **1** behaved as a weak inhibitor against *N*-acetylglucosaminidase from *Charonia lampas* (IC_{50} = 2 mM), but neither compound **1** nor compound **2** showed inhibitory activities to the hyaluronidase from beef testes up to 5 mM.

3. Experimental

General methods.—Melting points were determined with a Yamato micro melting point apparatus and are uncorrected. Optical rotations were determined with a Perkin–

Elmer Model 241 MC polarimeter at 23°C. Infrared (IR) spectra were recorded with a Shimadzu IR-27 spectrophotometer, using KBr disks for solid samples and KRS (thallium bromide–iodide) for liquid samples. ^{1}H NMR spectra were recorded with either Jeol JNM-GX 400 or Jeol JNM-GX 500 spectrometers, using Me_4Si as the internal standard for solutions in $CDCl_3$, unless otherwise specified. High-resolution fast-atom bombardment mass spectra (HR-FABMS) were obtained on a Jeol SX-102 spectrometer. Reactions were monitored by TLC on precoated plates of silica gel $60F_{254}$ (layer thickness, 0.25 mm, E. Merck, Darmstadt, Germany). Glycoside derivatives were detected by spraying a solution of MeOH–concd H_2SO_4–*p*-anisaldehyde (85:10:5), then heating at 200°C on a hot plate; cyclitol derivatives were detected by spraying a 5% solution of phosphomolybdic acid in EtOH, then 5% solution of H_2SO_4 in ethanol, and heating at 200°C on a hot plate. All the cyclitol derivatives appeared as sky-blue spots on TLC. Flash column chromatography was performed on silica gel 60 (230–400 mesh, E. Merck, Darmstadt, Germany). Ratios of eluting solvents in chromatography are specified by volume. Solutions were evaporated in vacuo below 45°C under diminished pressure.

*Methyl 2-acetamido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-α-*D*-glucopyranoside* (**4**).—To a solution of compound **3** (8.22 g, 35.0 mmol), prepared by refluxing *N*-acetylglucosamine in MeOH in the presence of Dowex 50w-x8 (H^+ form), in DMF (120 mL) were added α,α-dimethoxytoluene (6.25 mL, 41.0 mmol) and *p*-toluenesulfonic acid (200 mg). The resulting mixture was evacuated with stirring by an aspirator for 3 h at 60°C, and then cooled to room temperature. Pyridine (30 mL) and BzCl (8.5 mL, 73.2 mmol) were added. The mixture was stirred for 5 h, poured into ice–water, and extracted with $CHCl_3$ (3 × 250 mL). The extracts were washed with aq $NaHCO_3$, brine, and water, dried (Na_2SO_4), and concentrated. The residue was purified by flash column chromatography (25:1 $CHCl_3$–EtOH) to give compound **4** (12.9 g, 86%) as white crystals, which were recrystallized from EtOH, mp 205–206°C; lit. mp 212–216°C [18]; $[\alpha]_D$ −3.2°(*c* 0.7, $CHCl_3$); lit. $[\alpha]_D$ + 8 ± 2° [18] (*c* 1.04, $CHCl_3$); IR (ν_{max}): 3350 (NH), 1715 (OBz), 1660 and 1530 cm^{-1} (NHAc); ^{1}H NMR: δ 8.03–7.24 (m, 10 H, 2 C_6H_5), 5.90 (d, 1 H, $J_{2,NH}$ 9.05 Hz, NH), 5.58 (t, 1 H, $J_{2,3}$ 9.46, $J_{3,4}$ 9.80 Hz, H-3), 5.56 (s, 1 H, benzylidene), 4.79 (d, 1 H, $J_{1,2}$ 3.36 Hz, H-1), 4.52 (dt, 1 H, H-2), 4.34 (dd, 1 H, $J_{5,6a}$ 4.88 Hz, $J_{6a,6b}$ 10.5 Hz, H-6a), 3.95 (dt, 1 H, $J_{4,5}$ 10.5, $J_{5,6b}$ 10.4 Hz, H-5), 3.88 (t, 1 H, H-4), 3.83 (t, 1 H, H-6b), 3.45 (s, 3 H, OCH_3), 1.87 (s, 3 H, $NCOCH_3$). Anal. Calcd for $C_{23}H_{25}NO_7$: C, 64.62; H, 5.90; N, 3.27. Found: C, 64.89; H, 5.99; N, 3.32.

*Methyl 2-acetamido-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (**5**) *and methyl 2-acetamido-3-*O*-benzoyl-2-deoxy-α-*D*-glucopyranoside* (**6**).—(*a*) A mixture of compound **4** (427 mg, 1.0 mmol), borane–trimethylamine (292 mg, 4.0 mmol), and MS4A (1.0 g) in CH_2Cl_2 (25 mL) was stirred at room temperature for 30 min, cooled to −15°C, and then powdered $AlCl_3$ (533 mg, 4.0 mmol) was added. TLC showed the disappearance of compound **5** within 30 min. The reaction mixture was then filtered through a Celite pad. The filtrate was stirred with 1 M HCl (10 mL) for 10 min. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 30 mL). The organic layer and extracts were combined, dried, and concentrated. The residue was put on a column of silica gel. Elution of the column with 20:1 $CHCl_3$–EtOH

gave compound **5** (87.6 mg, 20.4%); further elution with 10:1 $CHCl_3$–EtOH gave compound **6** (173 mg, 51%).

(*b*) A mixture of compound **4** (5.34 g, 12.5 mmol), molecular sieves 4A (15 g), and borane–trimethylamine (3.65 g, 50 mmol) in CH_2Cl_2 (150 mL) was stirred at room temperature for 30 min. Then the mixture was cooled to −15°C, powdered anhydrous $AlCl_3$ (4.17 g, 31.3 mmol) added, and the suspension stirred at that temperature for 30 min. Processing as described in procedure *a* gave compounds **5** (3.50 g, 65%) and **6** (0.85 g, 15.8%).

Compound **5**: mp 134–135°C, $[\alpha]_D$ +47.0° (*c* 0.8, $CHCl_3$); IR (ν_{max}): 3350 (OH and NH), 1720 (OBz), 1665 and 1535 cm^{-1} (NHAc); ^1H NMR: δ 8.02–7.15 (m, 10 H, 2 C_6H_5), 5.85 (d, 1 H, $J_{2,NH}$ 9.46 Hz, NH), 5.57 (dd, 1 H, $J_{2,3}$ 9.46 Hz, $J_{3,4}$ 10.5 Hz, H-3), 4.76 (d, 1 H, $J_{1,2}$ 3.66 Hz, H-1), 4.64 (d, 1 H, J_{gem} 11.0 Hz, 1/2 CH_2Ph), 4.60 (d, 1 H, 1/2 CH_2Ph), 4.39 (dt, 1 H, H-2), 3.88 (t, 1 H, $J_{4,5}$ 9.60 Hz, H-4), 3.86–3.75 (m, 3 H, H-5, 6a, 6b), 3.40 (s, 3 H, OCH_3), 1.85 (s, 3 H, $NCOCH_3$). Anal. Calcd for $C_{23}H_{27}NO_7$: C, 64.32; H, 6.34; N, 3.26. Found: C, 64.08; H, 6.36; N, 3.17.

Compound **6**: mp 95–100°C; $[\alpha]_D$ +96.5° (*c* 0.3, $CHCl_3$); lit. [18] $[\alpha]_D$ +99° (*c* 2.93, $CHCl_3$); lit. [19] $[\alpha]_D$ +91.2° (*c* 1.70, $CHCl_3$); IR (ν_{max}): 3340 (OH and NH), 1715 (OBz), 1660 and 1540 cm^{-1} (NHAc); ^1H NMR δ 8.05–7.45 (m, 5 H, C_6H_5), 5.82 (d, 1 H, $J_{2,NH}$ 9.46 Hz, NH), 5.30 (t, 1 H, $J_{2,3} = J_{3,4} = 9.70$ Hz, H-3), 4.76 (d, 1 H, $J_{1,2}$ 3.66 Hz, H-1), 4.45 (dt, 1 H, H-2), 3.93–3.86 (m, 3 H, H-4,5,6a), 3.78–3.74 (m, 1 H, H-6b), 3.43 (s, 3 H, OCH_3), 1.88 (s, 3 H, $NCOCH_3$). Anal. Calcd for $C_{16}H_{21}NO_7$: C, 56.63; H, 6.24; N, 4.13. Found: C, 56.50; H, 6.38; N, 4.05.

*Methyl 2-acetamido-6-*O*-acetyl-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (**7**).—A solution of compound **5** (200 mg, 0.465 mmol) in pyridine–Ac_2O (2:1, 3 mL) was stirred at room temperature for 4 h. Conventional work-up followed by column chromatography (25:1 $CHCl_3$–EtOH) gave compound **7** (200 mg, 91%) as white crystals, mp 118–119°C, $[\alpha]_D$ +62.7° (*c* 0.8, $CHCl_3$); IR (ν_{max}): 3380 (NH), 1735 (OAc), 1720 (OBz), 1660 and 1535 cm^{-1} (NHAc); ^1H NMR: δ 8.06–7.13 (m, 10 H, 2 C_6H_5), 5.83 (d, 1 H, $J_{2,NH}$ 9.50 Hz, NH), 5.59 (t, 1 H, $J_{2,3} = J_{3,4} = 9.60$ Hz, H-3), 4.75 (d, 1 H, $J_{1,2}$ 3.66 Hz, H-1), 4.63 and 4.53 (each d, each 1 H, J_{gem} 11.2 Hz, CH_2Ph), 4.46 (dt, 1 H, H-2), 4.39 (dd, 1 H, $J_{5,6a}$ 2.00, $J_{6a,6b}$ 11.5 Hz, H-6a), 4.30 (dd, 1 H, $J_{5,6b}$ 4.05 Hz, H-6b), 3.94 (m, 1 H, H-5), 3.82 (t, 1 H, H-4), 3.42 (s, 3 H, OCH_3), 2.11 (s, 3 H, $OCOCH_3$), 1.84 (s, 3 H, $NCOCH_3$). Anal. Calcd for $C_{25}H_{29}NO_8$: C, 63.68; H, 6.20; N, 2.97. Found: C, 63.78; H, 6.20; N, 2.88.

*Methyl 2-acetamido-4,6-di-*O*-acetyl-3-*O*-benzoyl-2-deoxy-α-*D*-glucopyranoside* (**8**). —A solution of compound **6** (180 mg, 0.530 mmol) in pyridine–Ac_2O (2:1, 3 mL) was stirred at room temperature for 5 h. Conventional treatment followed by column chromatography (20:1 $CHCl_3$–EtOH) gave compound **8** (224 mg, quantitative) as white crystals: mp 138–139°C; lit. [18] mp 140–141°C; lit. [19] mp 133–134°C; $[\alpha]_D$ +54.5° (*c* 0.7, $CHCl_3$); lit. [18] $[\alpha]_D$ +52° (*c* 0.8, $CHCl_3$); lit. [19] $[\alpha]_D$ +55.5° (*c* 0.8, $CHCl_3$); IR (ν_{max}): 3350 (NH), 1730 (OAc), 1715 (OBz), 1665 and 1530 cm^{-1} (NHAc); ^1H NMR: δ 7.99–7.42 (m, 5 H, C_6H_5), 5.83 (d, 1 H, $J_{2,NH}$ 9.46 Hz, NH), 5.47 (t, 1 H, $J_{2,3} = J_{3,4} = 9.46$ Hz, H-3), 5.32 (t, 1 H, $J_{4,5}$ 9.80 Hz, H-4), 4.81 (d, 1 H, $J_{1,2}$ 3.36 Hz, H-1), 4.50 (dt, 1 H, H-2), 4.29 (dd, 1 H, $J_{5,6a}$ 4.57, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.15 (dd, 1 H, $J_{5,6b}$ 2.20 Hz, H-6b), 4.02 (ddd, 1 H, H-5), 3.44 (s, 3 H, OCH_3), 2.13,

1.92, and 1.86 (each s, each 3 H, 2 $OCOCH_3$ and $NCOCH_3$). Anal. Calcd for $C_{20}H_{25}NO_9$: C, 56.73; H, 5.95; N, 3.31. Found: C, 56.62; H, 5.95; N, 3.12.

Methyl 2-acetamido-3-O-benzoyl-4-O-benzyl-2,6-dideoxy-6-iodo-α-D-glucopyranoside (**9**).—A mixture of compound **5** (9.0 g, 21.0 mmol), Ph_3P (11.0 g, 42 mmol), and *N*-iodosuccinimide (9.45 g, 42 mmol) in tetrahydrofuran (150 mL) was stirred under reflux for 4 h. The mixture was then cooled to 0°C, MeOH (6 mL) was added, and stirring was continued for 1 h. Solvents were removed to give a white residue, which was dissolved in a minimum amount of $CHCl_3$ and the solution put on a silica gel column. The column was eluted with 5:1 to 2:1 toluene–EtOAc to give the iodide **9** (10.4 g, 92%) as a white solid, which was utilized for the next step directly. ^{1}H NMR: δ 8.04–7.13 (m, 10 H, 2 C_6H_5), 5.80 (d, 1 H, $J_{2,NH}$ 9.76 Hz, NH), 5.58 (dd, 1 H, $J_{2,3}$ 10.7, $J_{3,4}$ 9.03 Hz, H-3), 4.76 (d, 1 H, $J_{1,2}$ 3.41 Hz, H-1), 4.69 (d, 1 H, J_{gem} 10.8 Hz, 1/2 CH_2Ph), 4.64 (d, 1 H, 1/2 CH_2Ph), 4.45 (m, 1 H, H-2), 3.68 (t, 1 H, $J_{4,5}$ 9.23 Hz, H-4), 3.56–3.50 (m, 2 H, H-5,6a), 3.45 (s, 3 H, OCH_3), 3.40 (dd, 1 H, $J_{5,6b}$ 6.05, $J_{6a,6b}$ 11.2 Hz, H-6b), 1.84 (s 3 H, $NCOCH_3$).

Methyl 2-acetamido-3-O-benzoyl-4-O-benzyl-2,6-dideoxy-α-D-xylo-hex-5-enopyranoside (**10**).—The iodide **9** (10.0 g, 18.5 mmol) was dissolved in DMF (100 mL) and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) (12.8 mL, 84.0 mmol) was added. The mixture was stirred at 60–70°C for 1.5 h. TLC (1:1 benzene–EtOAc) showed the complete reaction of compound **9**. The mixture was cooled to room temperature, diluted with $CHCl_3$ (600 mL), washed with aq $Na_2S_2O_3$ and brine, dried, and concentrated. The residue was purified byflash column chromatography (3:1 toluene–EtOAc) to give compound **10** (6.18 g, 81%), which was crystallized from diethyl ether; mp 155–156°C, $[\alpha]_D$ −2.9° (*c* 0.3, $CHCl_3$); IR (ν_{max}): 3300 (NH and OH), 1715 (OBz), 1660 and 1530 cm^{-1} (NHAc); ^{1}H NMR: δ 7.96–7.15 (m, 10 H, 2 C_6H_5), 5.85 (d, 1 H, $J_{2,NH}$ 9.46 Hz, NH), 5.45 (dd, 1 H, $J_{2,3}$ 9.46, $J_{3,4}$ 9.81 Hz, H-3), 4.97 (bs, 1 H, H-6a), 4.84 (bs, 1 H, H-6b), 4.79 (d, 1 H, $J_{1,2}$ 3.36 Hz, H-1), 4.74 (d, 1 H, J_{gem} 10.6 Hz, 1/2 CH_2Ph), 4.58 (d, 1 H, 1/2 CH_2Ph), 4.51 (dt, 1 H, H-2), 4.13 (dt, 1 H, $J_{4,6a} = J_{4,6b} = 1.80$ Hz, H-4), 3.45 (s, 3 H, OCH_3), 1.83 (s, 3 H, $NCOCH_3$). Anal. Calcd for $C_{23}H_{25}NO_6$: C, 67.14; H, 6.12; N, 3.40. Found: C, 67.40; H, 6.08; N, 3.13.

2L-(2,4,5 / 3)-4-Acetamido-3-benzoyloxy-2-benzyloxy-5-hydroxycyclohexanone (**11**). —(*a*) A solution of compound **10** (5.0 g, 12.2 mmol) and mercuric(II) sulfate (300 mg) in 1,4-dioxane-5 mM aqueous H_2SO_4 (210 mL, 2:1) was heated for 1.5 h at 60°C, cooled, and concentrated in vacuo. The residue was dissolved in $CHCl_3$ (500 mL), washed with water (80 mL), dried, and concentrated to dryness. Flash column chromatography (10:1 $CHCl_3$–EtOH) of the residue gave the product (4.40 g, 91%). Crystallization of the crude product from EtOH–$CHCl_3$ gave crystalline compound **11** (3.52 g, 73%): mp 189–192°C (dec.), $[\alpha]_D$ −72.0° (*c* 0.2, $CHCl_3$); IR (ν_{max}): 3320 (OH and NH), 1725 (ketone), 1710 (OBz), 1640 and 1535 cm^{-1} (NHAc); ^{1}H NMR: δ 7.98–7.17 (m, 10 H, 2 C_6H_5), 6.40 (d, 1 H, $J_{4,NH}$ 8.60 Hz, NH), 5.70 (t, 1 H, $J_{2,3} = J_{3,4} = 9.40$ Hz, H-3), 5.11 (d, 1 H, J_{gem} 12.2 Hz, 1/2 CH_2Ph), 4.60 (ddd, 1 H, $J_{4,5}$ 2.40 Hz, H-4), 4.54 (d, 1 H, 1/2 CH_2Ph), 4.37 (m, 1 H, H-5), 4.20 (d, 1 H, $J_{2,3}$ 9.40 Hz, H-2), 2.79 (dd, 1 H, $J_{5,6a}$ 3.04, $J_{6a,6b}$ 15.0 Hz, H-6a), 2.73 (dd, 1 H, $J_{5,6b}$ 3.62 Hz, H-6b), 2.47 (m, 1 H, OH), 1.90 (s, 3 H, $NCOCH_3$). Anal. Calcd for $C_{22}H_{23}NO_6$: C, 66.48; H, 5.83; N, 3.52. Found: C, 66.50; H, 5.87; N, 3.56.

(*b*) A mixture of compound **10** (2.06 g, 5 mmol) and mercury(II) chloride (1.49 g, 5.5 mmol) in water–acetone (1:2, 45 mL) was refluxed for 3.5 h. The mixture was then cooled, filtered through a Celite pad and the filtrate concentrated in vacuo to dryness. The residue containing mercury salts was subjected to repeated column chromatography (15:1 $CHCl_3$–EtOH) to give compound **11** (1.25 g, 63%), which was identical to the product obtained in procedure *a*.

1D-(1,2,4/3,5)-2-Acetamido-3-O-benzoyl-4-O-benzyl-1,3,4,5-cyclohexanetetrol (**12**). —Sodium borohydride (1.98 g, 52.3 mmol) was added to a solution of AcOH–tetrahydrofuran (3:2, 80 mL) at 5°C (ice–water bath). After the evolution of hydrogen gas had ceased (about 5 min), a suspension of ketone **11** (2.60 g, 6.54 mmol) in acetic acid–tetrahydrofuran (20 mL) was added and stirred at that temperature. The mixture gradually became a clear solution. TLC (10:1 $CHCl_3$–MeOH) showed that the reaction of compound **11** was finished within 30 min. Water (30 mL) was then added. The mixture was concentrated to 40 mL, and extracted with $CHCl_3$ (3×150 mL). The extracts were combined, dried, and concentrated to dryness to give a white solid. Crystallization from EtOH afforded compound **12** (2.30 g, 88%) as white crystals: mp 225–226°C, $[\alpha]_D$ −30.0° (*c* 0.3, $CHCl_3$); IR (ν_{max}): 3300 (OH and NH), 1715 (OBz), 1650 and 1540 cm^{-1} (NHAc); ^1H NMR: δ 8.10–7.38 (m, 10 H, 2 C_6H_5), 6.20 (d, 1 H, $J_{2,NH}$ 8.85 Hz, NH), 5.54 (t, 1 H, $J_{2,3} = J_{3,4} = 9.46$ Hz, H-3), 4.78 and 4.64 (each d, each 1 H, J_{gem} 11.3 Hz, $PhCH_2$), 4.26 (dt, 1 H, $J_{1,2}$ 3.01 Hz, H-2), 4.20 (m, 1 H, H-1), 4.10 (m, 1 H, H-5), 3.60 (t, 1 H, $J_{4,5}$ 9.50 Hz, H-4), 2.41 (d, 1 H, *J* 4.40 Hz, OH), 2.23 (dt, 1 H, $J_{1,6a} = J_{5,6a}$ 2.88, $J_{6a,6b}$ 12.8 Hz, H-6a), 2.06 (d, 1 H, *J* 5.45 Hz, OH), 1.87 (s, 3 H, $NCOCH_3$), 1.72 (dt, 1 H, $J_{1,6b}$ 3.10, $J_{5,6b}$ 12.3 Hz). Anal. Calcd for $C_{22}H_{25}NO_6$: C, 66.15; H, 6.31; N, 3.51. Found: C, 66.06; H, 6.32; N, 3.36.

1D-(1,2,4 / 3,5)-2-Acetamido-1,5-di-O-acetyl-3-O-benzoyl-4-O-benzyl-1,3,4,5-cyclohexanetetrol (**13**).—A mixture of compound **12** (120 mg, 0.30 mmol), Et_3N (2 mL), Ac_2O (1 mL), and catalytic amount of 4-dimethylaminopyridine in CH_2Cl_2 (6 mL) was stirred overnight at room temperature, poured into ice–water, and extracted with CH_2Cl_2 (3×20 mL). The extracts were washed with dilute HCl and water, dried (Na_2SO_4), and concentrated in vacuo. Column chromatography (35:1 $CHCl_3$–EtOH) of the residue gave compound **13** (145 mg, quantitative) as crystals: mp 179–180°C, $[\alpha]_D$ −18.6° (*c* 0. 3, $CHCl_3$); IR (ν_{max}): 3350 (NH), 1735 (OAc), 1715 (OBz), 1665 and 1540 cm^{-1} (NHAc); ^1H NMR: δ 8.02–7.12 (m, 10 H, 2 C_6H_5), 5.87 (d, 1 H, $J_{2,NH}$ 8.54 Hz, NH), 5.50 (dd, 1 H, $J_{2,3}$ 9.16, $J_{3,4}$ 10.3 Hz, H-3), 5.27 (m, 1 H, $J_{1,2}$ 3.0, $J_{1,6a}$ 2.8, $J_{1,6b}$ 2.1 Hz, H-1), 5.21 (ddd, 1 H, $J_{5,6a}$ 11.6, $J_{5,6b}$ 4.88, $J_{4,5}$ 9.46 Hz, H-5), 4.67 (s 2 H, CH_2Ph), 4.42 (dt, 1 H, H-2), 3.83 (t, 1 H, H-4), 2.38 (dt, 1 H, H-6a), 2.17 and 1.99 (each s, each 3 H, 2 $OCOCH_3$), 1.81 (s, 3 H, $NCOCH_3$), 1.71 (m, 1 H, H-6b). Anal. Calcd for $C_{26}H_{29}NO_8$: C, 64.58; H, 6.05; N, 2.90. Found: C, 64.82; H, 6.16; N, 2.75.

1D-(1,2,4 / 3,5)-2-Acetamido-3-O-benzoyl-4-O-benzyl-5-O-pivaloyl-1,3,4,5-cyclohexanetetrol (**14**).—A solution of compound **12** (600 mg, 1.50 mmol) in pyridine (6 mL) was cooled to 0°C under argon, pivaloyl chloride (0.22 mL, 1.80 mmol) was added, and the mixture was stirred for 16 h at 0–20°C. TLC showed the formation of a major component. The reaction mixture was then poured into ice–water and extracted with CH_2Cl_2. The extracts were washed with dilute HCl and water, dried, and concentrated. The residue was purified by column chromatography (25:1 $CHCl_3$–EtOH) to give

compound **14** (632 mg, 87%) which was crystallized from $CHCl_3$–EtOH, mp 145–146°C, $[\alpha]_D$ −55.0° (*c* 0.2, $CHCl_3$); IR (ν_{max}): 3340 (OH and NH), 1725 (pivalate), 1710 (OBz), 1660 and 1535 cm^{-1} (NHAc); ^1H NMR: δ 7.99–7.07 (m, 10 H, 2 C_6H_5), 6.41 (d, 1 H, $J_{2,NH}$ 8.85 Hz, NH), 5.61 (dd, 1 H, H-3), 5.35 (ddd, 1 H, $J_{5,6a}$ 4.58, $J_{5,6b}$ 14.0, $J_{4,5}$ 9.16 Hz, H-5), 4.71 (d, 1 H, J_{gem} 11.0 Hz, 1/2 CH_2Ph), 4.62 (d, 1 H, 1/2 CH_2Ph), 4.32 (ddd, 1 H, $J_{1,2}$ 2.74, $J_{2,3}$ 10.4 Hz, H-2), 4.19 (bs, 1 H, H-1), 3.85 (t, 1 H, $J_{3,4}$ 9.16 Hz, H-4), 2.32 (dt, 1 H, $J_{1,6a}$ 4.58, $J_{6a,6b}$ 13.7 Hz, H-6a), 1.85 (s, 3 H, $NCOCH_3$), 1.72–1.64 (m, 2 H, H-6b and OH), 1.19 [s, 9 H, $C(CH_3)_3$]. Anal. Calcd for $C_{27}H_{33}NO_7$: C, 67.06; H, 6.88; N, 2.89. Found: C, 67.02; H, 6.92; N, 2.89.

*1D-(1,2,4 / 3,5)-2-Acetamido-3-*O*-benzoyl-4-*O*-benzyl-1-*O*-mesyl-5-*O*-pivaloyl-1,3,4,5-cyclohexanetetrol* (**15**).—To a solution of compound **14** (1.20 g, 2.48 mmol) in pyridine (20 mL) was added mesyl chloride (0.96 mL, 12.4 mmol). The mixture was stirred overnight at room temperature, then poured into ice–water, extracted with CH_2Cl_2. The extracts were washed with M HCl, brine, and water, dried (Na_2SO_4), and concentrated. Column chromatography (30:1 $CHCl_3$–EtOH) of the residue gave compound **15** (1.34 g, 96%) which was crystallized from EtOAc–diisopropyl ether, mp 184–185°C, $[\alpha]_D$ −38.7° (*c* 0.3, $CHCl_3$); IR (ν_{max}): 3350 (NH), 1730 (pivalate), 1720 (OBz), 1655 and 1530 (NHAc), 1340 and 1160 cm^{-1} (OMs); ^1H NMR: δ 7.99–7.05 (m, 10 H, 2 C_6H_5), 6.18 (d, 1 H, $J_{2,NH}$ 8.85 Hz, NH) 5.47 (t, 1 H, $J_{2,3} = J_{3,4} = 9.25$ Hz, H-3), 5.17 (ddd, 1 H, $J_{4,5}$ 9.30, $J_{5,6a}$ 4.40, $J_{5,6b}$ 14.0 Hz), 5.12 (bs, 1 H, H-1), 4.73 (d, 1 H, J_{gem} 11.0 Hz, 1/2 CH_2Ph), 4.64 (d, 1 H, 1/2 CH_2Ph), 4.47 (dt, 1 H, $J_{1,2}$ 2.80 Hz, H-2), 3.90 (t, 1 H, H-4), 3.21 (s, 3 H, OSO_2CH_3), 2.60 (dt, 1 H, $J_{1,6a} = J_{5,6a} = 4.40$, $J_{6a,6b}$ 14.0 Hz, H-6a), 1.86 (s, 3 H, $NCOCH_3$), 1.75 (dt, 1 H, $J_{1,6b}$ 2.10 Hz, H-6b), 1.21 [s, 9 H, $C(CH_3)_3$]. Anal. Calcd for $C_{28}H_{35}NO_9S$: C, 59.87; H, 6.28; N, 2.49; S, 5.71. Found: C, 59.68; H, 6.26; N, 2.48; S, 5.61.

*1D-(1,3,5 / 2,4)-4-Acetamido-5-azido-3-*O*-benzoyl-2-*O*-benzyl-1-*O*-pivaloyl-1,2,3-cyclohexanetriol* (**16**).—To a solution of compound **15** (1.39 g, 2.48 mmol) in DMF (25 mL) was added LiN_3 (0.85 g, 17.4 mmol). The mixture was stirred at 70–80°C under argon for 12 h. The mixture was then cooled, diluted with CH_2Cl_2 (200 mL), washed with water (50 mL), brine (50 mL), and water (30 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was subjected to chromatography (4:1 toluene–EtOAc), giving compound **16** (1.12 g, 88.7%) which was crystallized from EtOAc–diisopropyl ether: mp 165–166°C, $[\alpha]_D$ −41.9° (*c* 0.8, $CHCl_3$); IR (ν_{max}): 3350 (NH), 2080 (N_3), 1730 (pivalate), 1715 (OBz), 1655 and 1530 cm^{-1} (NHAc); ^1H NMR: δ 8.00–7.05 (m, 10 H, 2 C_6H_5), 5.99 (d, 1 H, $J_{4,NH}$ 10.0 Hz, NH), 5.26 (t, 1 H, $J_{2,3} = J_{3,4} = 9.46$ Hz, H-3), 4.97 (ddd, 1 H, $J_{1,2}$ 9.50, H-1, $J_{1,6a}$ 4.88, $J_{1,6b}$ 14.0 Hz), 4.74 and 4.54 (each d, each 1 H, J_{gem} 10.7 Hz, Ch_2Ph), 4.33 (q, 1 H, $J_{4,5}$ 10.3 Hz, H-4), 3.83 (t, 1 H, H-2), 3.28 (dt, 1 H, $J_{5,6a}$ 4.20, $J_{5,6b}$ 11.0 Hz, H-5), 2.33 (dt, 1 H, $J_{6a,6b}$ 10.5 Hz, H-6a), 1.84 (s, 3H, $NCOCH_3$), 1.56 (dt, 1 H, H-6b), 1.22 [s, 9 H, $C(CH_3)_3$]. Anal. Calcd for $C_{27}H_{32}N_4O_6$: C, 63.76; H, 6.34; N, 11.02. Found: C, 63.72; H, 6.41; N, 10.87.

*1D-(1,3,5/2,4)-4-Acetamido-5-azido-2-*O*-benzyl-1,2,3-cyclohexanetriol* (**17**).—To a solution of compound **16** (350 mg, 0.688 mmol) in 1:1 tetrahydrofuran–MeOH (4 mL) was added a solution of KOH (84.8 mg, 1.51 mmol) in MeOH (1 mL). The mixture was stirred at room temperature for 16 h and then AcOH (0.5 mL) was added, and the

resulting mixture was concentrated in vacuo to dryness. Column chromatography (10:1 $CHCl_3$–MeOH) of the residue gave compound **17** (203 mg, 92%) which was crystallized from EtOAc–2-propanol: mp 201–202°C, $[\alpha]_D$ +5.5° (*c* 0.1, $CHCl_3$); IR (ν_{max}): 3340 (OH and NH), 2080 (N_3), 1650 and 1540 cm^{-1} (NHAc); ^1H NMR: δ 7.40–7.25 (m, 5H, C_6H_5), 5.63 (d, 1 H, $J_{4,NH}$ 8.50 Hz, NH), 5.20 and 4.78 (each d, each 1 H, J_{gem} 11.2 Hz, $PhCH_2$), 3.71–3.57 (m, 3 H, H-1,3,4), 3.55–3.50 (m, 1 H, H-5), 3.36 (br.s, 1 H, OH), 3.26 (t, 1 H, $J_{1,2} = J_{2,3} = 9.46$ Hz, H-2), 2.38 (m, 1 H, OH), 2.35 (dt, 1 H, $J_{1,6a} = J_{5,6a} = 4.60$, $J_{6a,6b}$ 13.5 Hz, H-6a), 2.08 (s, 3 H, $NCOCH_3$), 1.55 (q, 1 H, $J_{1,6b} = J_{5,6b} = 13.2$ Hz, H-6b). Anal. Calcd for $C_{15}H_{20}N_4O_4$: C, 56.24; H, 6.29; N, 17.49. Found: C, 56.10; H, 6.34; N, 17.28.

1D-(1,3,5/2,4)-4-Acetamido-5-amino-1,2,3-cyclohexanetriol (**1**).—To a solution of compound **17** (128 mg, 0.4 mmol) in ethanol (6 mL) and AcOH (2 mL) was added a suspension of 10% Pd–charcoal (200 mg) in EtOH (2 mL). The mixture was then vigorously stirred under hydrogen for 24 h. TLC showed the absence of starting **17** and the formation of compound **1** (red color with ninhydrin spray). The catalyst was removed by filtration through a Celite pad. The filtrate and washings were combined and concentrated to dryness. The residue was dissolved in water, passed over a column of Dowex 50w-x8 (H^+ form, 5 mL). Elution of the column with 0.5 M NH_4OH gave, after evaporation of the solvent, compound **1** (69.5 mg, 85%) as a white solid: mp 195–198°C (dec.), $[\alpha]_D$ −35° (*c* 0.2, H_2O); IR (ν_{max}): 3350 (OH and NH), 1635 and 1550 cm^{-1} (NHAc); ^1H NMR (D_2O): δ 3.45 (t, 1 H, 4 $J_{3,4} = J_{4,5} = 9.70$ Hz, H-4), 3.48–3.40 (m, 1 H, H-1), 3.24 (t, 1 H, $J_{1,2} = J_{2,3} = 9.10$ Hz, H-2), 3.17 (t, 1 H, H-3), 2.58 (dt, 1 H, $J_{5,6a}$ 4.10, $J_{5,6b}$ 9.85 Hz, H-5), 2.05 (dt, 1 H, $J_{1,6a}$ 4.20, $J_{6a,6b}$ 10.3 Hz, H-6a), 1.95 (s, 3 H, $NCOCH_3$), 1.31 (q, 1 H, $J_{1,6b}$ 9.70 Hz, H-6b). HR-FABMS Calcd for $C_8H_{17}N_2O_4$ (M + 1): 205.1188. Found: 205.1176.

1D-(1,3,5/2,4)-4-Acetamido-5-azido-2-O-benzyl-1-O-pivaloyl-1,2,3-cyclohexanetriol (**18**).—To a solution of compound **16** (330 mg, 0.648 mmol) in 1:1 tetrahydrofuran–MeOH (10 mL) was added a suspension of K_2CO_3 (107.6 mg, 0.778 mmol) in MeOH (2 mL). The mixture was stirred for 4.5 h at 20°C, then poured into water, and extracted with CH_2Cl_2 (3 × 20 mL). The extracts were dried (Na_2SO_4), filtered, and concentrated to dryness. Column chromatography (15:1 $CHCl_3$–EtOH) of the residue gave **18** (186 mg, 71%) as a syrup which became a solid on being kept; $[\alpha]_D$ −10.8° (*c* 0.2, $CHCl_3$); IR (ν_{max}): 3340 (NH and OH), 2080 (N_3), 1730 (pivalate), 1655 and 1535 cm^{-1} (NHAc); ^1H NMR: δ 7.35–7.25 (m, 5 H, C_6H_5), 5.75 (d, 1 H, $J_{4,NH}$ 8.60 Hz, NH), 4.87 (ddd, 1 H, $J_{1,2}$ 9.50, $J_{1,6a}$ 4.88, $J_{1,6b}$ 13.2 Hz, H-1), 4.81 and 4.75 (each d, each 1 H, J_{gem} 11.3 Hz, PhCH), 3.74 (q, 1 H, $J_{3,4} = J_{4,5} = 9.50$ Hz, H-4), 3.68–3.55 (m, 2 H, H-3,5), 3.50 (t, 1 H, $J_{1,2} = J_{2,3} = 9.05$ Hz, H-2), 3.10 (br.s, 1 H, OH), 2.36 (dt, 1 H, $J_{5,6a}$ 4.20, $J_{6a,6b}$ 12.8 Hz, H-6a), 2.05 (s, 3 H, $NCOCH_3$), 1.55 (q, 1 H, $J_{5,6b}$ 12.6 Hz, H-6b), 1.21 [s, 9 H, $C(CH_3)_3$]. Anal. Calcd for $C_{20}H_{28}N_4O_5$: C, 59.39; H, 6.98; N, 13.85. Found: C, 59.38; H, 6.88; N, 13.63.

1D-(1,3,5/2,4)-4-Acetamido-5-azido-2-O-benzyl-3-O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-1-O-pivaloyl-1,2,3-cyclohexanetriol (**20**).—A mixture of compound **18** (145 mg, 0.358 mmol), freshly prepared methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (**19**) (213.6 mg, 0.537 mmol), and activated MS4A (600 mg) in anhydrous CH_2Cl_2 (4 mL) was stirred for 15 min at room temperature, then cooled to

−15°C. Silver triflate (165.6 mg, 0.644 mmol) was added. The mixture was stirred for 3 h at that temperature, Et_3N (0.2 mL) was added, and the suspension was filtered through a Celite pad and washed with CH_2Cl_2 (2 × 10 mL). The filtrate and washings were combined and concentrated to dryness in vacuo. The residue was subjected to column chromatography (2:1 benzene–EtOAc to give **20** (205 mg, 79.3%) as a white solid; $[\alpha]_D$ −20.2° (*c* 0.3, $CHCl_3$); IR (ν_{max}): 3300 (NH), 2080 (N_3), 1730 (ester), 1660 and 1530 cm^{-1} (NHAc); ^1H NMR: δ 7.35–7.25 (m, 5 H, C_6H_5), 5.73 (d, 1 H, $J_{4,NH}$ 8.14 Hz, NH), 5.22–5.18 (m, 2 H, H-3′,4′), 4.98 (dd, 1 H, $J_{1',2'}$ 7.63, $J_{2',3'}$ 9.60 Hz, H-2′), 4.95 (m,1 H, H-1), 4.91 (d, 1 H, J_{gem} 11.0 Hz, 1/2 $PhCH_2$), 4.87 (d, 1 H, H-1′), 4.64 (d, 1 H, 1/2 $PhCH_2$), 4.06 (t, 1 H, $J_{2,3} = J_{3,4} = 9.36$ Hz, H-3), 3.95 (d, 1 H, $J_{4',5'}$ 9.42 Hz, H-5′), 3.80–3.65 (m, 2 H, H-4,5), 3.62 (t, 1 H, $J_{1,2}$ 8.24 Hz, H-2), 3.56 (s, 3 H, OCH_3), 2.32 (dt, 1 H, $J_{1,6a} = J_{5,6a} = 4.60$, $J_{6a,6b}$ 10.5 Hz, H-6a), 2.08, 2.06, and 2.02 (2) (each s, each 3 H, 3 $OCOCH_3$ and $NCOCH_3$), 1.51 (q, 1 H, $J_{1,6b}$ 11.0 Hz, H-6b), 1.14 [s, 9 H, $C(CH_3)_3$]. Anal. Calcd for $C_{33}H_{44}N_4O_{14}$: C, 54.99; H, 6.15; N, 7.77. Found: C, 54.87; H, 6.12; N, 7.68.

1D-(1,3,5 / 2,4)-4-Acetamido-5-amino-3-O-(β-D-glucopyranosyluronic acid)-1,2,3-cyclohexanetriol (**2**).—A solution of compound **20** (72 mg, 0.10 mmol) in 9:1 MeOH–water (10 mL) was treated with 6 M NaOH (1 mL) for 16 h at room temperature, then applied to a column (80 × 20 mm) of Dowex50w-x8 (H^+ form), and eluted with 9:1 MeOH–water. The eluates containing the product were concentrated. A solution of the residue in water (6 mL) was vigorously stirred with 10% Pd/C (100 mg) under hydrogen for 24 h and then filtered through a Celite pad. The filtrate was concentrated in vacuo to dryness. The residue was dissolved in water (15 mL), washed with $CHCl_3$ (3 × 4 mL) to remove pivalic acid. The water layer was concentrated in vacuo and the residue was dried over P_2O_5 under diminished pressure to give compound **2** (33.5 mg, 88%) as a white powder; $[\alpha]_D$ −101.2° (*c* 0.1, H_2O); IR (ν_{max}): 3400–2850 (NH, NH_3^+, and OH), 1640 and 1530 (NHAc), 1600 and 1400 cm^{-1} (carboxylate ion); ^1H NMR (D_2O): δ 4.45 (d, 1 H, $J_{1',2'}$ 7.93 Hz, H-1′), 4.04 (t, 1 H, $J_{3,4} = J_{4,5} = 10.2$ Hz, H-4), 3.66 (d, 1 H, $J_{4',5'}$ 9.46 Hz, H-5′), 3.62–3.56 (m, 2 H, H-3,3′), 3.45–3.41 (m, 3 H, H-1,2,4′), 3.30–3.25 (m, 2 H, H-2′,5), 2.26 (dt, 1 H, $J_{1,6a} = J_{5,6a} = 4.15$, $J_{6a,6b}$ 10.2 Hz, H-6a), 1.97 (s, 3 H, $NCOCH_3$), 1.65 (q, 1 H, $J_{1,6b} = J_{5,6b}$ 9.78 Hz, H-6b). Anal. Calcd for $C_{14}H_{24}N_2O_{10} \cdot 1.8\ H_2O$: C, 40.74; H, 6.74; N, 6.79. Found C, 40.96; H, 6.81; N, 6.52.

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