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Design and synthesis of a heparanase inhibitor with pseudodisaccharide structure

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Abstract—Aza-analogue of the basic disaccharide unit of heparane sulfate was designed as a potent inhibitor against heparanase produced by solid tumors cell, and synthesized via a coupling reaction of phenyl 2-azide-1-thio-D-glucopyranoside derivatives with a partially protected 1-deoxynojirimycin derived from D-glucose. The azapseudodisaccharide inhibited tumor cell heparanase with IC_{50} value of 58–63 μM.

1. Introduction

It has been recognized that tumor metastasis occurs via complex multistage process which involves tumor cell adhesion to various basement membrane components and degradation of extracellular matrix and basement membranes.[1] Glycosaminoglycans such as heparan sulfate (HS) are the important constituents in these structures. In 1983, HS degradative activities of murine B 16 melanoma sublines were found to correlate with their metastatic lung colonization and invasive abilities, and this activity was proven to be caused by HS specific *endo*-β-glucronidase (heparanase) cleaving the linkage between glucuronic acid and *N*-acetyl-glucosamine in HS.[2] These results suggest that heparanase plays an important role in cell invasion of some malignant solid tumors through basement membranes. Consequently, molecules that are able to inhibit heparanase might possibily prevent tumor metastasis. Such consideration stimulated researchers to develop heparanase inhibitors. Heparin, modified heparin derivatives,[3] and suramine[4] have been reported to inhibit heparanase and suppress tumor metastatis. However, the diverse functionality and higher-molecular weight nature of these substances have also led to search of low-molecular weight heparanase inhibitors with a potentialy higher specificity.[5–7] In connection with our chemical studies on endoglycosidase inhibitors,[8] we were interested in heparanase inhibitor, and designed a new compound **1**, a mimic of the basic repeating disaccharide unit of heparane sulfate, as a potential heparanase inhibitor. The compound **1** is composed of 2-acetamido-2-deoxy-6-*O*-sulfonyl-α-D-glucopyranosyl moiety and 2,6-dideoxy-2,6-imino-L-gulonic acid (**2**). The latter is known as a naturally occurring powerful enzyme inhibitor against human liver β-D-glucuronidase.[9] Although 1-deoxynojirimycin (**3**)[10] and its stereoisomers have been widely utilized for designing and synthesizing new glycosidase inhibitors,[11] such utilizations of **2** have not been reported.[12] Here we describe the synthesis of **1** and its enzyme inhibitory activities (Fig. 1).[13]

2. Results and discussions

Our synthetic process directed towards **1** involved chemical conversion[14] of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**4**) into 2,3-di-*O*-benzyl-*N*-benzyloxycarbonyl-6-*O*-*t*-butyl-diphenylsilyl (TBDPS)-1,5-dideoxy-1,5-imino-D-glucitol (**14**) and benzyl *N*-benzyloxycarbonyl-4,5-di-*O*-benzyl-2,6-dideoxy-2,6-imino-L-gulonate (**28**) as glycosyl acceptors, their coupling with phenyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-1-thio-D-glucopyranosides (**17**) as a glycosyl donor, and regioselective manipulations such as imino acid formation and *O*-sulfation.

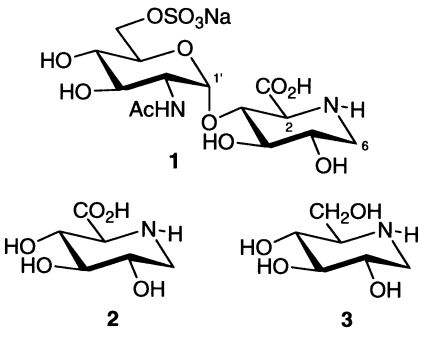

Figure 1.

Keywords: aza compounds; carbohydrate mimetics; enzyme inhibitors; thioglycosides.

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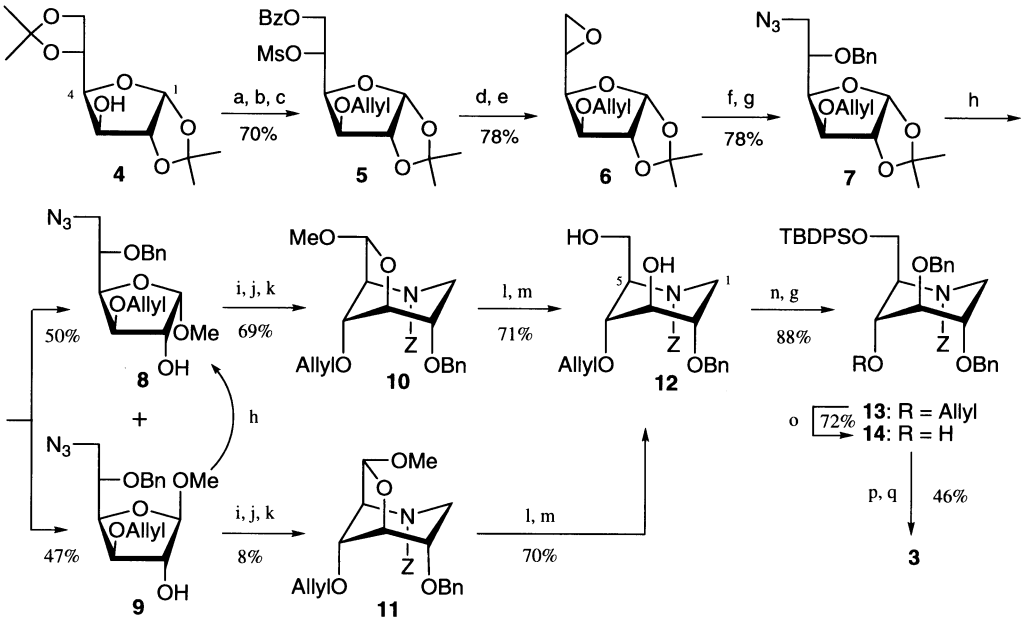
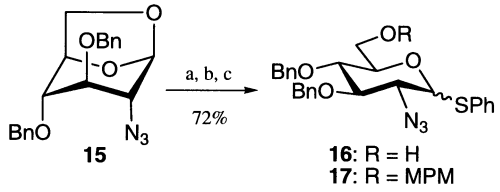


Scheme 1. (a) NaH, allyl bromide, 0°C; (b) 80% AcOH, 60°C; (c) BzCl, and then MsCl, pyridine–CH_2Cl_2, −15–0°C; (d) NaOMe, MeOH, rt; (e) *t*-BuOK, THF–DMF, 0°C–rt; (f) NaN_3, NH_4Cl, aq. DMF, 80°C; (g) BnBr, NaH, *n*-$Bu_4N^+I^-$, DMF, 0°C; (h) 3% HCl in MeOH, rt; (i) Tf_2O, pyridine, CH_2Cl_2, −60°C; (j) Ph_3P, CH_2Cl_2, rt–45°C; (k) K_2CO_3, H_2O, dioxane, MeOH, THF, rt; and then ZCl, 0°C; (l) TFA, H_2O–dioxane, rt; (m) $NaBH_4$, 95% EtOH, 0°C; (n) TBDPSCl, imidazole, DMF, rt; (o) $PdCl_2$, NaOAc, aq. AcOH, 50°C; (p) TBAF, AcOH, THF, rt; (q) 10% Pd–C, H_2, AcOH–EtOH–H_2O, rt.

Initially, 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**4**) was converted into the 6-*O*-benzoyl-5-*O*-mesyl derivative **5** in 70% overall yield by the three-sequence which included (a) 3-*O*-allylation with sodium hydride (NaH) and allyl bromide in *N*,*N*-dimethylformamide (DMF), (b) selective hydrolysis of the 5,6-*O*-isopropylidene group with 90% acetic acid, (c) one-pot esterification with benzoyl chloride followed by methanesulfonyl chloride in pyridine (Scheme 1). The compound **5** was treated with methanolic sodium methoxide to afford an epoxide **6** in 60% yield, while successive treatments of **5** with sodium methoxide and potassium *t*-butoxide in tetrahydrofuran afforded **6** in 76% yield. The oxirane in **6** was opened by the action of sodium azide in aqueous DMF in the presence of NH_4Cl, and subsequent benzylation {benzyl bromide (BnBr), NaH, *n*-tetrabutylammonium iodide (*n*-Bu_4NI)} gave a benzyl ether **7** in 85% yield. This was treated with 3%HCl in methanol to afford an anomeric mixture of methyl glycosides, which was readily separated into each isomer **8** (50% yield) and **9** (47% yield) by chromatography on silica gel. In order to attain the effective construction of the piperidine ring system (vide infra), the β-isomer **9** was again equilibrated under the same conditions to the anomeric mixture, from which **8** was isolated in 52% yield. By repetition of this procedure, 80% yield of **8** was attained. Treatment of **8** with triflic anhydride in pyridine–dichloromethane at −60°C gave an unstable 2-*O*-triflate derivative, from which the piperidine ring system was formed via reduction of the azide group to amine with triphenylphosphine, followed by cyclization through an intramolecular displacement of the trifluoromethanesulfonyl group under basic condition. The bicyclic amine thus formed was isolated, upon treatment with benzyloxycarbonyl chloride, as a carbamate **10** in 69% yield from **8**.[†] On the other hand, the stereoisomer **11** was obtained from **9** in only 8% yield under the same conditions. The formation of the bicyclic amine from **9** as monitored by TLC analysis was sluggish to decompose the intermediary triflate derivative under these conditions. This low reactivity would be explained by the steric hindrance arising from the β-methoxy group. Acidic hydrolysis of **10**, followed by sodium borohydride reduction gave a diol **12** in 71% yield. In the same way, **11** was also converted into **12** in 70% yield. The primary hydroxyl group in **12** was protected as the TBDPS ether, and the resulting secondary hydroxyl group was benzylated to afford a fully protected nojrimycin derivative **13** in 88% overall yield. Cleavage of the allyl group with palladium (II) chloride and sodium acetate in aqueous acetic acid provided the glycosyl acceptor **14**[‡] in 72% yield.



Scheme 2. (a) PhSTMS, ZnI_2, $(CH_2Cl)_2$, rt; (b) K_2CO_3, aq. MeOH, rt; (c) MPMCl, NaH, *n*-$Bu_4N^+I^-$, DMF, 0°C.

[†] All ^1H NMR (400 or 500 MHz, 23°C) spectra of **10**–**14**, **18**–**30** appears as broadened and complicated signals probably due to the presence of some rotamers. This made difficult the ^1H NMR analyses. However, the difficulty was circumvented by measuring such spectra in d_6-dimethyl sulfoxide at the elevated temperature (80–120°C). In addition, the preferred conformation of a series of deoxynojirimycin derivatives having the benzyloxycarbonyl group seems to be near $^1C_4$ rather than $^4C_1$ form (vide supra, see also Ref. 11).

[‡] In order to confirm the piperidine ring system, **14** was also transformed into **3** (see Section 3).

The glycosyl donor **17** was prepared from 1,6-anhydro-2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-glucopyranose **15**[15] as follows (Scheme 2). The anhydro sugar **15** was treated with (phenylthio)trimethylsilane in the presence of zinc iodide,[16] and subsequently with potassium carbonate in methanol to afford thioglycosides **16**. The hydroxyl group in **16** was protected as *p*-methoxybenzyl ether, giving the donor **17** (α/β=2/1) in 72% yield from **15**. Although each anomer was readily separated by chromatography on silica gel, these isomers were employed to the next glycosidation step without separation.

Coupling reaction of **14** and **17** was examined under several conditions[17–19] as shown in Table 1 (Scheme 3). Best result was obtained by the action of *N*-iodosuccinimide (NIS) in the presence of triflic acid in ether–dichloromethane (5:1)[18] at −50°C to afford an α-glycoside **19** and the corresponding β-isomer **18**, in 77 and 15% yield, respectively (entry 5).

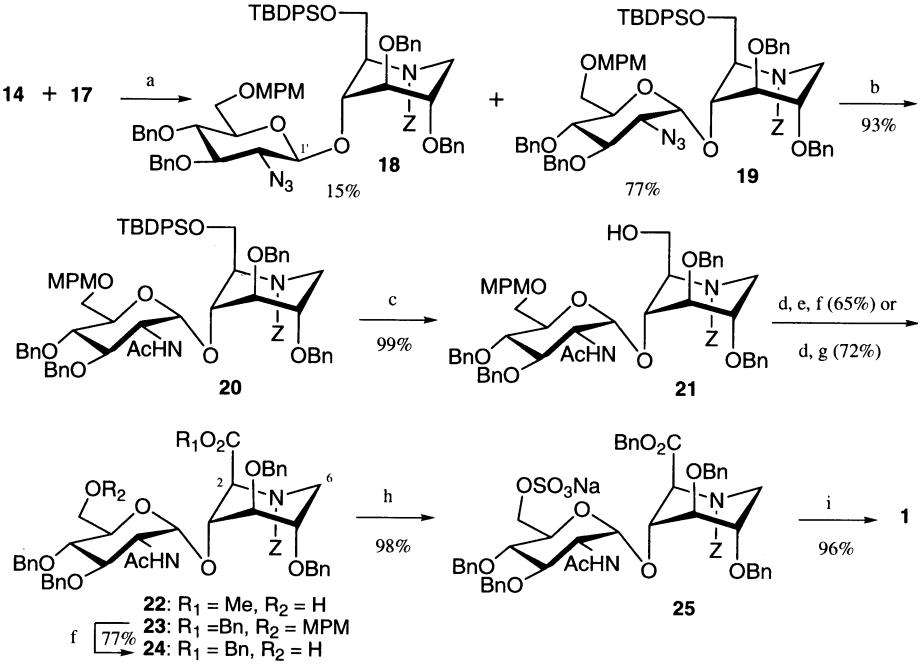
Table 1. Glycosidation reactions

Entry	Donor **17** (equiv.)	Conditions	Yield (%)[a]	
			18	**19**
1	1.2	NBS, CH_2Cl_2, MS4A, rt, 16 h	6	28
2	1.2	NBS, CH_3CN, MS4A, rt, 16 h	18	12
3	1.2	NBS, $LiClO_4$, Et_2O, MS4A, −15–0°C, 5 h	3	27
4	1.3	MeOTf, Et_2O, MS4A, 0°C, rt, 30 h	5	54
5	1.1	NIS, TfOH, Et_2O–CH_2Cl_2 (5:1), MS4A, −50°C, 50 min	15	77
6	1.1	NIS, TESOTf, Et_2O–$(CH_2Cl)_2$ (5:1), MS4A, −10°C, 30 min	8	67
7	1.3	DMTST, CH_2Cl_2, MS3A, −10°C, 2 h	21	62

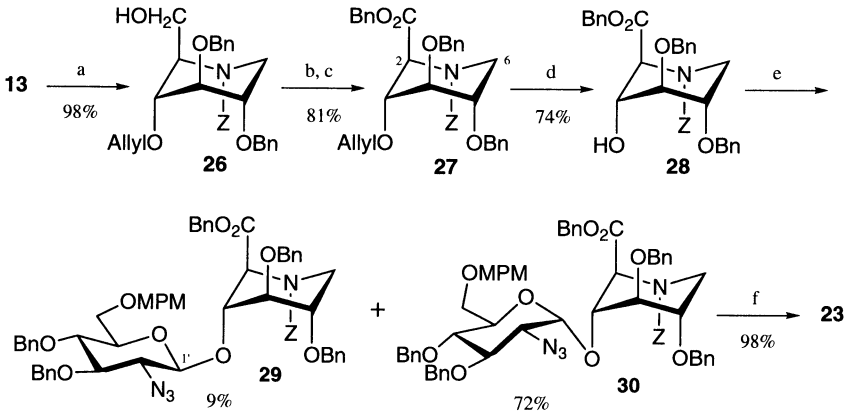
[a] The calculation was based on the acceptor **14**.

High α-selectivity (**19**/**18**=92/8, 59% yield) was observed when methyl triflate[19] was used as a promoter in ether. The stereochemistry of newly created glycosidic linkage in **18** and **19** was confirmed by the ^1H NMR analyses in d_6-dimethylsulfoxide at the elevated temperature (110–120°C). Thus, the spectrum of **19** showed the H-1′ resonance at δ 5.17 as a doublet with $J_{1',2'}$=3.7 Hz, whereas one of **18** exhibited a signal, due to H-1′, at δ 4.52 as a doublet with 7.8 Hz.

Constructing a desired glycosidic linkage between **14** and **17**, we turned our attention next to functionalization of this pseudodisaccharide **19**. Initially, the azide **19** was reduced with triphenylphosphine, after *N*-acetylation, giving a 2′-acetamido derivative **20** in 93% yield. Treatment of **19** with thioacetic acid in pyridine afforded **20** in a single step (85% yield). This compound **20** underwent de-silylation with tetrabutylammonium fluoride (TBAF) in the presence of acetic acid to give **21** in 99% yield. Deprotection in the absence of acetic acid resulted in the product contaminated with an *N*,*O*-cyclic carbonate produced by an attack of 6-*O*-alkoxide anion to benzyloxycarbonyl group. Conversion of the hydroxyl group in **21** into the carboxylic function was achieved by Jones oxidation at room temperature in a short time. The resulting carboxylic acid was, after methylation, subject to de-*O*-*p*-methoxybenzylation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to afford an alcohol **22** in 65% overall yield. Attempts to synthesize **1** from **22**, however, gave unsatisfactory results because of contamination of side-products encountered in alkaline hydrolysis at the later stage of total synthesis. This problem was finally overcome by the replacement of protection for the carboxyl group. Thus, the carboxylic acid obtained from **21** reacted with BnBr in the presence of cesium carbonate, giving the corresponding benzyl ester **23** in 72% yield. Upon treatment with DDQ, **23** afforded



Scheme 3. (a) NIS, TfOH, MS4A, ether–CH_2Cl_2, −50°C; (b) Ph_3P, THF, 60°C, and then H_2O and Ac_2O, rt; (c) TBAF, AcOH, THF, rt; (d) Jones reagent, rt; (e) diazomethane in Et_2O, rt; (f) DDQ, aq. CH_2Cl_2, 0°C; (g) BnBr, $CsCO_3$, DMF, rt; (h) SO_3·Me_3N, DMF, 0°C–rt; (i) 10% Pd–C, H_2, AcOH–MeOH–H_2O, rt.



Scheme 4. (a) TBAF, AcOH, THF, rt; (b) Jones reagent, rt; (c) BnBr, $CsCO_3$, DMF, rt; (d) $PdCl_2$, NaOAc, aq. AcOH, 50°C; (e) **17**, NIS, TESOTf, MS4A, ether– $(CH_2Cl)_2$, −78°C; (f) AcSH, pyridine, 0°C–rt.

an alcohol **24** in 77% yield.[§] Finally, the alcohol **24** was suffered to *O*-sulfation with sulfur trioxide trimethylamine complex ($Me_3N{\cdot}SO_3$) to provide a 6′-*O*-sulfated derivative **25**, in which all *O*-benzyl groups were removed by hydrogenolysis with 10% palladium carbon under hydrogen atmosphere in aqueous methanol–acetic acid to give **1** in high yield.

The structure of **1** was unambiguously elucidated on the basis of NMR analyses. The vicinal coupling constants, $J_{2,3}=J_{3,4}=J_{4,5}=J_{5,6a}$=ca. 7–9 Hz, in the ^{1}H NMR spectra strongly supported the[4] C_1 conformation of the imino acid moiety in **1**, which was the same as for glucronic acid unit in heparin oligosaccharides.[20]

Furthermore we have also developed an improved route to **1** (Scheme 4). The first step was de-silylation of **13**, giving an alcohol **26** in 98% yield. Jones oxidation of **26** and subsequent benzylation afforded a benzyl ester **27** in 81% yield. This was subject to de-allylation to give an imino acid derivative **28** in 74% yield. The imino acid **28** was allowed to react with **17** in the presence of NIS-triethylsilyl triflate in ether-1,2-dichloroethane (5:1) at −78°C to give a desired pseudodisaccharide **30** in 72% yield along with the corresponding β-anomer **29** (9%). Nonetheless the two anomers were easily distinguished by comparisons of the anomeric protons in the ^{1}H NMR spectra; δ 5.22 ($J_{1',2'}$=3.9 Hz) for **30** vs 4.69 ($J_{1',2'}$= 8.3 Hz) for **29**. The azide residue in **30** was cleanly reduced with thioacetic acid to produce the known acetamide **23** almost quantitatively; the overall yield from **13** attained to 41% (cf. 36% in the previous route). Transformation of **23** into **1** has already established. This quite convergent strategy would make it feasible to attach a variety of sugar residues to the C-3 position of **2**, and therefore could be of interest as a concise and flexible method for preparation of new pseudooligosaccharides.

Heparanse inhibition of **1** was examined according to the method previously reported by Nakajima et al.[4] Thus, [^{3}H] HS was incubated at 42°C with partially purified heparanase in the presence or absence of **1**, and the degradation course of [^{3}H] HS was monitored by HPLC. In the case of B16-F10 melanoma heparanase, this degradation was completely inhibited by 2.1 mM of **1**, but it was almost unaffected by 2.1 μM concentration. The concentration of **1** required for 50% inhibition of the heparanase was 140 μM. In addition, compound **1** also showed more potent activity (IC_{50}= 58–63 μM) against the heparanase derived from colon 26N-17 cells. The activity was comparable to those of siastatin B analogs previously reported as a heparanase inhibitor.[5,6]

In conclusion, we have developed a new type of heparanase inhibitor by taking advantage of glycosidation reaction between a partially protected nojrimycin derivatives **14** or **28** and phenyl 2-azido-2-deoxy-1-thio-D-glucopyranosides **17**.

3. Experimental

3.1. General procedures

Melting points were determined in a capillary with an Ishii melting-point apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-370 polarimeter. IR spectra were recorded with a Shimazu-FT-IR-8100M spectrophotometer. ^{1}H NMR spectra were recorded at 400 or 500 MHz with JEOL JNM-α400 or GX 500 spectrometers. Column chromatography was performed on silica gel 60 (230–400 mesh; E. Merck, Darmstadt, Germany). Merk precoated silica gel 60 F_{254} plates, 0.25 or 0.5 mm thickness, were used for analytical or preparative thin layer chromatography, respectively. Organic solutions obtained after extractive work-up were dried over $MgSO_4$, filtered through a pad of Celite, and evaporated under reduced pressure.

3.1.1. 3-*O*-Allyl-6-*O*-benzoyl-1,2-*O*-isopropylidene-5-*O*-methanesulfonyl-α-D-glucofuranose (5). To a stirred solution of **4** (150 g, 0.58 mol) in *N*,*N*-dimethylformamide (1000 mL) was added sodium hydride (60% mineral oil dispersion, 30 g, 0.75 mol) by portions at 0°C, and then the mixture was stirred for 0.5 h. Allyl bromide (75 ml, 0.87 mol) was added dropwise, and stirring was continued for 3.5 h at 0°C. After quenching with sat. NH_4Cl solution,

[§] Transesterification of **22** with titanium(iv) isopropoxide-benzyl alcohol into **24** failed.

the resulting mixture was extracted with ether. The extracts were washed with water and brine, dried, and evaporated. The residual syrup (188 g) was dissolved in acetic acid–water (4:1, 1000 ml). The mixture was heated at 60°C for 16 h with stirring, cooled, evaporated, and then co-evaporated with toluene to dryness. To a stirred solution of the residual syrup (175 g) in dichloromethane–pyridine (3:5; 800 ml) was added dropwise a solution of benzoyl chloride (67 mL, 0.58 mol) in dichloromethane (70 ml) at −15°C. After 1 h, methanesulfonyl chloride (90 ml, 1.17 mol) was added and the mixture was stirred at −15°C–rt for 12 h. More methanesulfonyl chloride (90 ml, 1.17 mol) was added and the mixture was stirred for 8 h, poured into ice–water and allowed to stand overnight. The resulting mixture was extracted with ether. The extracts were washed with dil. HCl solution, sat. $NaHCO_3$ solution, water and brine, dried, and evaporated. The residue was treated with ether–hexane to give 210 g of crystalline mass, which was recrystallized from ether–hexane to give 179 g of **5** (70% yield from **4**): mp 132–133°C (ether–hexane); $[\alpha]_D^{26}$= −10.9° (*c* 1.25, $CHCl_3$); IR ν_{max}(KBr): 1730, 1350, 1270, 1170, 1070 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.34, 1.50 (6H, each s, acetonide), 3.03 (3H, s, Ms), 4.03 (1H, d, $J_{3,4}$= 3.0 Hz, H-3), 4.09–4.18 (2H, m, allyl), 4.45 (1H, dd, $J_{4,5}$=7.8 Hz, H-4), 4.49 (1H, dd, $J_{5,6a}$=6.4 Hz, $J_{6a,6b}$= 13 Hz, H-6a), 4.62 (1H, d, $J_{1,2}$=3.9 Hz, H-2), 4.93 (1H, dd, $J_{5,6b}$=2.5 Hz, H-6b), 5.22 (1H, br.qd, *J*=10 Hz, olefin), 5.32 (1H, qd, *J*=17, 1.5 Hz, olefin), 5.38 (1H, ddd, H-5), 5.93 (1H, d, H-1), 5.97 (1H, m, olefin), 7.44 (2H, br.t, *J*=7.8 Hz, Ph), 7.57 (1H, td, *J*=7.4, 1.5 Hz, Ph), 8.07 (2H, dd, *J*=8.8 Hz, Ph).

Anal. found: C, 54.17; H, 5.94; S, 7.42. Calcd for $C_{20}H_{26}O_9S$ requires C, 54.29; H, 5.92; S, 7.25%.

3.1.2. 3-*O*-Allyl-5,6-anhydro-1,2-*O*-isopropylidene-β-L-idofuranose (6). To a stirred solution of **5** (7.5 g, 16.9 mmol) in methanol–tetrahydrofuran–dichloromethane (5:2:2, 90 ml) was added sodium methoxide (150 mg, 2.8 mmol). The mixture was stirred at rt for 3 h, treated with Dowex-50W X-8 (H^+) resin, and then filtered. The filtrate was evaporated in high vacuum, and then co-evaporated with toluene to give a syrup (6.8 g). To a stirred solution of the syrup (6.8 g) in *N*,*N*-dimethylformamide–tetrahydrofuran (2:1; 60 ml) was added potassium *t*-butoxide (2.9 g, 25.4 mmol) at 0°C, and the mixture was stirred at 0°C–rt for 6 h, poured into ice–water, and extracted with ether. The extracts were washed with water and brine, dried, and evaporated. The residual syrup was chromatographed on silica gel with hexane–ethyl acetate (8:1→4:1) to give **6** (3.2 g, 78%): $[\alpha]_D^{26}$=−52.4° (*c* 0.28, $CHCl_3$); IR ν_{max}(film): 1647 cm^{-1}; ^1H NMR ($CDCl_3$) δ= 1.32, 1.46, (6H, each s, acetonide), 2.65 (1H, dd, $J_{5,6a}$= 2.8 Hz, $J_{6a,6b}$=5.8 Hz, H-6a), 2.84 (1H, dd, $J_{5,6b}$=4.3 Hz, H-6b), 3.25 (1H, ddd, $J_{4,5}$=6.1 Hz, H-5), 3.82 (1H, dd, $J_{3,4}$=3.4 Hz, H-4), 3.95 (1H, br.d, H-3), 4.01 (1H, dd, *J*=13, 5.9 Hz, allyl), 4.18 (1H, dd, *J*=4.9 Hz, allyl), 4.58 (1H, br.d, $J_{1,2}$=3.7 Hz, H-2), 5.22 (1H, br.d, *J*= 10 Hz, olefin), 5.30 (1H, br.d, *J*=17 Hz, olefin), 5.86 (1H, m, olefin), 5.98 (1H, d, H-1).

Anal. Found: C, 59.39; H, 7.45. Calcd for $C_{12}H_{18}O_5$ requires C, 59.49; H, 7.49%.

3.1.3. 3-*O*-Allyl-6-azido-5-*O*-benzyl-6-deoxy-1,2-*O*-isopropylidene-β-L-idofuranose (7). To a stirred mixture of **6** (3.2 g, 13.2 mmol) and NH_4Cl (5.66 g, 0.11 mol) in *N*,*N*-dimethylformamide–water (10:1, 55 ml) was added sodium azide (2.58 g, 39.7 mmol). The mixture was stirred at 80°C for 2 h, cooled, poured into ice–water and extracted with ether. The extracts were washed with water and brine, dried, evaporated and then co-evaporated with toluene to dryness. To a stirred mixture of the residual syrup (3.70 g), benzyl bromide (2.36 ml, 19.8 mmol) and *n*-tetrabutylammonium iodide (4.88 g, 13.2 mmol) in *N*,*N*-dimethylformamide (30 ml) was added sodium hydride (60% mineral oil dispersion, 793 mg, 19.8 mmol) at 0°C under Ar and the mixture was stirred for 2.5 h. Sat. NH_4Cl solution was added and the resulting mixture was extracted with ether. The extracts were washed with water and brine, dried, and evaporated. The residual syrup was chromatographed on silica gel with hexane–ethyl acetate (10:1) to give **7** (3.84 g, 78%): $[\alpha]_D^{27}$=−57.4° (*c* 1.10, $CHCl_3$); IR ν_{max}($CHCl_3$): 2103, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.32, 1.49, (6H, each s, acetonide), 3.30 (1H, dd, $J_{5,6a}$=6.2 Hz, $J_{6a,6b}$=13 Hz, H-6a), 3.39 (1H, dd, $J_{5,6b}$= 3.3 Hz, H-6b), 3.84 (1H, br.d, $J_{3,4}$=3.4 Hz, H-3), 3.90 (1H, br.dd, *J*=13, 3.4 Hz, allyl), 3.92 (1H, ddd, $J_{4,5}$= 7.5 Hz, H-5), 4.14 (1H, br.dd, *J*=5.4 Hz, allyl), 4.31 (1H, dd, H-4), 4.56 (1H, br.d, $J_{1,2}$=4.0 Hz, H-2), 4.68, 4.87 (2H, each d, *J*=12 Hz, $PhCH_2$), 5.22 (1H, br.dd, *J*=11, 1.5 Hz, olefin), 5.28 (1H, br.dd, *J*=17 Hz, olefin), 5.85 (1H, m, olefin), 5.97 (1H, d, H-1). 7.25–7.41 (5H, m, Ph).

Anal. Found: C, 60.79; H, 6.70; N, 11.15. Calcd for $C_{19}H_{25}O_5N_3$ requires C, 60.79; H, 6.71; N, 11.19%.

3.1.4. Methyl 3-*O*-allyl-6-azido-5-*O*-benzyl-6-deoxy-α-L-idofuranoside (8) and methyl 3-*O*-allyl-6-azido-5-*O*-benzyl-6-deoxy-β-L-idofuranoside (9). A mixture of **7** (163 mg, 0.43 mmol) in 3% HCl in methanol (5 ml) was stirred at rt for 18 h, made neutral anhydrous Na_2CO_3, the mixture filtered, and the filtrate evaporated. The residual syrup was chromatographed on silica gel with hexane–ethyl acetate (4:1) to give **8** (75 mg, 50%) and **9** (71 mg, 47%). The β-anomer **9** (42 mg, 0.12 mmol) was treated with 3% HCl in methanol (5 ml) at rt for 8 h and treated as described above to give **8** (22 mg, 52%) and **9** (19 mg, 45%). By repetition of this procedure once again, total 121 mg (80%) of **8** was obtained. **8**: $[\alpha]_D^{27}$=+88.8° (*c* 1.21, $CHCl_3$); IR ν_{max}($CHCl_3$): 3500, 2103 cm^{-1}; ^1H NMR ($CDCl_3$) δ=2.75 (1H, d, *J*=7.8 Hz, OH), 3.37 (1H, dd, $J_{5,6a}$=5.9 Hz, $J_{6a,6b}$=13 Hz, H-6a), 3.39 (1H, dd, $J_{5,6b}$= 5.4 Hz, H-6b), 3.49 (3H, s, OMe), 3.81 (1H, ddd, $J_{4,5}$= 7.5 Hz, H-5), 3.91 (1H, dd, $J_{2,3}$=3.9 Hz, $J_{3,4}$=5.9 Hz, H-3), 4.01 (1H, br.dd, *J*=12, 5.7 Hz, allyl), 4.25 (1H, dd, H-4), 4.26 (1H, m, allyl), 4.28 (1H, ddd, $J_{1,2}$=4.9 Hz, H-2), 4.67, 4.82 (2H, each d, *J*=11 Hz, $PhCH_2$), 4.99 (1H, d, H-1), 5.21 (1H, br.d, *J*=10, 1.5 Hz, olefin), 5.30 (1H, br.dd, *J*=17 Hz, olefin), 5.90 (1H, m, olefin), 7.28–7.40 (5H, m, Ph).

Anal. Found: C, 58.14; H, 6.70; N, 11.98. Calcd for $C_{17}H_{23}O_5N_3$ requires C, 58.44; H, 6.64; N, 12.03%.

9: $[\alpha]_D^{27}$=−47.3° (*c* 0.55, $CHCl_3$); IR ν_{max}($CHCl_3$): 3490, 2103 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.87 (1H, d, *J*=4.4 Hz,

OH), 3.33 (1H, dd, $J_{5,6a}$=5.9 Hz, $J_{6a,6b}$=13 Hz, H-6a), 3.45 (3H, s, OMe), 3.47 (1H, dd, $J_{5,6b}$=3.4 Hz, H-6b), 3.83 (1H, dd, $J_{2,3}$=2.4 Hz, $J_{3,4}$=5.4 Hz, H-3), 3.91 (1H, ddd, $J_{4,5}$= 7.3 Hz, H-5), 3.95 (1H, br.dd, J=13, 6.5 Hz, allyl), 4.15 (1H, br.dd, J=5.4 Hz, allyl), 4.25 (1H, dd, H-2), 4.37 (1H, dd, H-4), 4.72, 4.86 (2H, each d, J=11 Hz, $PhCH_2$), 4.85 (1H, br.s, H-1), 5.22 (1H, br.dd, J=10, 1.5 Hz, olefin), 5.28 (1H, br.dd, J=17 Hz, olefin), 5.88 (1H, m, olefin), 7.27–7.43 (5H, m, Ph).

Anal. Found: C, 58.03; H, 6.78; N, 11.91. Calcd for $C_{17}H_{23}O_5N_3$ requires C, 58.44; H, 6.64; N, 12.03%.

3.1.5. Methyl 3-*O*-allyl-5-*O*-benzyl-*N*-benzyloxycarbonyl-2,6-dideoxy-2,6-imino-α-L-gulofuranoside (10). To a stirred mixture of **8** (31.5 g, 90 mmol) and pyridine (19.8 ml, 0.25 mol) in dichloromethane (300 ml) was added dropwise triflic anhydride (33.3 g, 0.12 mol) at −60°C under Ar and then the mixture was stirred at −60°C for 1.6 h. After quenching with methanol (5.0 ml), the resulting mixture was warmed to rt, diluted with ether, washed with oxalic acid solution, satureted $NaHCO_3$ solution, water, and brine, dried, and evaporated to give a syrup (44.1 g), which was employed to the next step without further purification. A mixture of the triflate (44.1 g) and triphenylphospine (26.2 g, 0.10 mol) in dichloromethane (263 ml) was stirred at rt for 1.5 h and at 45°C for 2.5 h, cooled and stirred vigorously with 50% potassium carbonate solution (263 ml) in tetrahydrofuran–methanol–water (3:1:1; 550 ml) for 4 d. To the resulting suspension was added benzyloxycarbonyl chloride (30.8 g, 0.18 mol) at 0°C, and then the mixture was stirred for 7 h, diluted with dichloromethane. The organic layer was separated and the aqueous phase was extracted with dichloromethane. The combined organic phase was washed with water and brine, dried, and evaporated. The residue was treated with hexane–ether to remove phosphine oxide as semicrystalline solids. The mother liquid was purified by chromatography on silica gel with hexane–ethyl acetate (10:1→5:1) to give **10** (27.3 g, 69%): $[\alpha]_D^{27}$=+28.5° (*c* 1.20, $CHCl_3$); IR ν_{max}($CHCl_3$): 1700, 1649 cm^{-1}; ^1H NMR (d_6-DMSO, 110°C) δ=3.26 (3H, s, OMe), 3.63 (1H, dd, $J_{5,6a}$=4.9 Hz, $J_{6a,6b}$=13 Hz, H-6a), 3.74 (1H, ddd, $J_{4,5}$=2.0 Hz, $J_{5,6b}$= 6.8 Hz, H-5), 3.82 (1H, dd, H-6b), 4.00-4.12 (2H, m, allyl), 4.26–4.30 (2H, br.s, H-2,3), 4.36 (1H, ddd, $J_{3,4}$= 3.9 Hz, $J_{2,4}$=2.0 Hz, H-4), 4.53, 4.58 (2H, each d, J= 12 Hz, $PhCH_2$), 4.76 (1H, br.s, H-1), 5.10, 5.13 (2H, each d, J=12 Hz, $PhCH_2$), 5.07–5.25 (2H, m, olefin), 5.84 (1H, m, olefin), 7.24–7.35 (10H, m, Ph).

Anal. Found: C, 68.22; H, 6.63; N, 3.17. Calcd for $C_{25}H_{29}O_6N$ requires C, 68.32; H, 6.65; N, 3.19%.

3.1.6. Methyl 3-*O*-allyl-5-*O*-benzyl-*N*-benzyloxycarbonyl-2,6-dideoxy-2,6-imino-β-L-gulofuranoside (11). To a stirred mixture of **9** (630 mg, 1.81 mmol) and pyridine (0.44 ml, 5.44 mmol) in dichloromethane (5 ml) was added dropwise triflic anhydride (0.76 g, 2.69 mol) at −60°C under Ar and the mixture was stirred at −60°C for 45 min. After quenching with methanol (0.8 ml), the resulting mixture was warmed to rt, diluted with ether, washed with oxalic acid solution, sat. $NaHCO_3$ solution, water, and brine, dried, and evaporated to give a triflate as a syrup (880 mg). A mixture of the crude triflate (880 mg) and triphenylphospine (473 mg, 1.81 mmol) in dichloromethane (6 ml) was stirred at rt for 0.5 h and then at 45°C for 2 h. The reaction mixture was cooled and stirred vigorously with 50% potassium carbonate solution (5 ml) in tetrahydrofuran–methanol–water (3:1:1; 11 ml) for 2 d. To the resulting suspension was added benzyloxycarbonyl chloride (0.31 g, 1.82 mmol) at 0°C, and then the mixture was stirred for 1 hr, diluted with dichloromethane. The organic layer was separated and the aqueous phase was extracted with dichloromethane. The combined organic phase was washed with water and brine, dried and evaporated. The residue was chromatographed on silica gel with benzene–ethyl acetate (15:1) and then purified by preparative TLC with carbontetrachloride–ethyl acetate (2:1) to give **11** (64 mg, 8%); $[\alpha]_D^{25}$=−40.0° (*c* 0.43, $CHCl_3$); IR ν_{max}($CHCl_3$): 1700, 1650 cm^{-1}; ^1H NMR (d_6-DMSO, 120°C) δ=3.34 (3H, s, OMe), 3.64 (1H, m, H-6a), 3.76 (1H, m, H-5), 3.84 (1H, dd, $J_{5,6b}$=7.1 Hz, $J_{6a,6b}$=13 Hz, H-6b), 3.99–4.09 (3H, m, H-3, allyl), 4.29 (1H, m, H-4), 4.56 (2H, br.s, $PhCH_2$), 4.65 (1H, m, H-2), 5.02 (1H, d, $J_{1,2}$=2.3 Hz, H-1), 5.07–5.15 (3H, m, $PhCH_2$, olefin), 5.22 (1H, dd, J=17, 1.5 Hz, olefin), 5.85 (1H, m, olefin), 7.25–7.36 (10H, m, Ph).

Anal. Found: C, 67.96; H, 6.66; N, 3.09. Calcd for $C_{25}H_{29}O_6N$ requires C, 68.32; H, 6.65; N, 3.19%.

3.1.7. 4-*O*-Allyl-2-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-gulcitol (12). (i) From **10**. A mixture of **10** (3.5 g, 7.97 mmol) in dioxane–trifluoroacetic acid–water (2:1:1; 20 ml) was stirred at rt for 11 h, and poured into ice–sodium acetate solution. The resulting mixture was extracted with chloroform. The extracts were washed with sat. $NaHCO_3$ solution, water and brine, dried, and evaporated. The residual syrup was dissolved in 95% ethanol (40 ml) and treated with sodium borohydride (1 g, 26.4 mmol) at 0°C. After 2 h, the reaction mixture was quenched with excess acetic acid, and then evaporated. The residue was diluted with dichloromethane–water, and extracted with dichloromethane. The extracts were washed with water and brine, dried, and evaporated. The residual syrup was chromatographed on silica gel with benzene–ethyl acetate (1:1) to give **12** (2.42 g, 71%).

(ii) From **11**. A mixture of **11** (60 mg, 0.14 mmol) in dioxane–trifluoroacetic acid–water (2:1:1; 1.2 ml) was stirred at rt for 1 h, and treated with the procedure as described above to give a crude hemiacetal, which was reduced with sodium borohydride (10 mg, 0.26 mmol) in 95% ethnaol (5 ml) and purified by prepartive TLC with carbon tetrachloride–ethyl acetate (2:1) to give **12** (41 mg, 70%): $[\alpha]_D^{24}$=+7.7° (*c* 0.49, $CHCl_3$); IR ν_{max}($CHCl_3$): 3400, 1690 cm^{-1}; ^1H NMR (d_6-DMSO, 80°C) δ=3.32 (1H, dd, $J_{1a,1b}$=14 Hz, $J_{1a,2}$=3.4 Hz, H-1a), 3.49 (1H, q, $J_{2,3}$= 4.2 Hz, H-2), 3.53 (1H, dd, $J_{3,4}$=5.2 Hz, $J_{4,5}$=4.9 Hz, H-4), 3.61 (1H, dd, $J_{5,6a}$=5.5 Hz, $J_{6a,OH}$=5.5 Hz, $J_{6a,6b}$= 11 Hz, H-6a), 3.63 (1H, dd, $J_{5,6b}$=5.5 Hz, $J_{6b,OH}$=5.5 Hz, H-6b), 3.74 (1H, q, $J_{2,3}$=4.2 Hz, $J_{3,OH}$=5.2 Hz, $J_{3,4}$= 5.2 Hz, H-3), 3.92 (1H, dd, $J_{1b,2}$=3.7 Hz, H-1b), 4.00 (1H, q, H-5), 4.06 (1H, ddt, J=13, 5.5, 1.5 Hz, allyl), 4.14 (1H, ddt, J=5.2, 1.5 Hz, allyl), 4.53, 4.59 (2H, each d, J=12 Hz, $PhCH_2$), 4.56 (1H, t, 6-OH), 5.05–5.14 (4H, m, 3-OH,

$PhCH_2$, olefin), 5.90 (1H, m, olefin), 7.25–7.34 (10H, m, Ph).

Anal. Found: C, 67.61; H, 6.60; N, 3.16. Calcd for $C_{24}H_{29}O_6N$ requires C, 67.43 H, 6.84; N, 3.28%.

3.1.8. 4-*O*-Allyl-*N*-benzyloxycarbonyl-2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenyl-silyl-1,5-dideoxy-1,5-imino-D-gulcitol (13). To a stirred solution of **12** (2.42 g, 5.67 mmol) and imidazole (1.24 g, 18.2 mmol) in *N*,*N*-dimethylformamide (20 ml) was added *tert*-butylchlorodiphenylsilane (2.39 g, 8.70 mmol) at rt and the mixture was stirred at rt for 1 h, poured into ice–water. The resulting mixture was extracted with ether. The extracts were washed with water and brine, dried and evaporated. To a stirred mixture of the residual syrup, benzyl bromide (4.26 g, 24.9 mmol) and tetrabutylammonium iodide (3.07 g, 8.31 mmol) in *N*,*N*-dimethylformamide (50 ml) was added sodium hydride (a 60% mineral oil dispersion, 1 g, 25 mmol) at 0°C. After 3 h, the reaction mixture was quenched with methanol, and poured into ice–water. The resulting mixture was extracted with ether. The extracts were washed with water and brine, dried and evaporated. The residual syrup was chromatographed on silica gel with hexane–ethyl acetate (10:1) to give **13** (3.76 g, 88%); $[\alpha]_D^{21}=+14.9°$ (*c* 0.95, $CHCl_3$); IR ν_{max}(film): 1701, 1425, 1111 cm^{-1}; ^1H NMR (d_6-DMSO, 80°C) δ=0.99 (9H, s, *t*-butyl), 3.24 (1H, dd, $J_{1a,1b}$=14 Hz, $J_{1a,2}$=2.8 Hz, H-1a), 3.66–3.68 (2H, m, H-2,3), 3.81–3.88 (3H, m, H-4,6), 3.98 (1H, dd, $J_{1b,2}$=3.7 Hz, H-1b), 4.00 (1H, m, allyl), 4.12 (1H, ddt, *J*=13, 4.9, 1.5 Hz, allyl), 4.15 (1H, q, H-5), 4.47, 4.57 (2H, each d, *J*=12 Hz, $PhCH_2$), 4.61 (2H, br.s, $PhCH_2$), 4.98 (1H, d, *J*=13 Hz, $PhCH_2$), 5.06–5.09 (2H, m, $PhCH_2$, olefin), 5.17 (1H, qd, *J*=17, 5.8, 1.8 Hz, olefin), 5.84 (1H, m, olefin), 7.24–7.60 (25H, m, Ph).

Anal. Found: C, 74.57; H, 6.91; N, 1.94. Calcd for $C_{47}H_{53}O_6NSi$ requires C, 74.67; H, 7.07; N, 1.85%.

3.1.9. *N*-Benzyloxycarbonyl-2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-1,5-dideoxy-1,5-imino-D-gulcitol (14). A mixture of **13** (3.10 g, 4.10 mmol), sodium acetate (1.72 g, 21.0 mmol) and palladium chloride (931 mg, 5.25 mmol) in 90% acetic acid was heated at 50°C with stirring for 3.5 h, cooled and concentrated. The residue was diluted with chloroform and washed with water, sat. $NaHCO_3$ solution, water, and brine, dried and evaporated. The residual syrup was chromatographed on silica gel with hexane–ethyl acetate (7:1) to give **14** (2.10 g, 72%); $[\alpha]_D^{26}=+27.4°$ (*c* 0.59, $CHCl_3$); IR ν_{max}($CHCl_3$): 3500, 1700 cm^{-1}; ^1H NMR (d_6-DMSO, 80°C) δ=0.98 (9H, s, *t*-butyl), 3.21 (1H, dd, $J_{1a,1b}$=14 Hz, $J_{1a,2}$=2.9 Hz, H-1a), 3.54 (1H, dd, $J_{2,3}$=3.9 Hz, $J_{3,4}$=5.8 Hz, H-3), 3.70 (1H, q, H-2), 3.86 (1H, dd, $J_{5,6a}$=4.9 Hz, $J_{6a,6b}$=10 Hz, H-6a), 3.92 (1H, dd, $J_{5,6b}$=5.4 Hz, H-6b), 3.98 (1H, m, H-4), 4.05 (1H, q, $J_{4,5}$=6.3 Hz, H-5), 4.12 (1H, dd, $J_{1b,2}$=3.0 Hz, H-1b), 4.46, 4.59 (2H, each d, *J*=12 Hz, $PhCH_2$), 4.60, 4.64 (2H, each d, *J*=12 Hz, $PhCH_2$), 4.67 (1H, br.s, OH), 4.99, 5.09 (1H, d, *J*=13 Hz, $PhCH_2$), 7.23–7.61 (25H, m, Ph).

Anal. Found: C, 73.67; H, 6.92; N, 2.01. Calcd for $C_{44}H_{49}O_6NSi$ requires C, 73.81; H, 6.90; N, 1.96%.

3.1.10. 1,5-Dideoxy-1,5-imino-D-glucitol (3). To a stirred mixture of **14** (440 mg, 0.61 mmol) and acetic acid (48 mg, 0.80 mmol) in tetrahydrofuran (3 ml) was added a 1.0 M solution of tetrabutylammonium fluoride (0.68 mL, 0.68 mmol) at rt under Ar. The mixture was stirred at rt for 8 h, diluted with ethyl acetate, successively washed with water and brine, dried and evaporated. The residual syrup was chromatographed on silica gel with hexane–ethyl acetate (2:1→1:1) to give 2,3-di-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol[21] (255 mg, 87%), $[\alpha]_D^{23}=+23.2°$ (*c* 0.57, $CHCl_3$); ^1H NMR (d_6-DMSO, 100°C) δ=3.30 (1H, dd, $J_{1a,1b}$=14 Hz, $J_{1a,2}$=3.1 Hz, H-1a), 3.55 (1H, dd, $J_{2,3}$=4.3 Hz, $J_{3,4}$=5.5 Hz, H-3), 3.66-3.69 (2H, m, H-6), 3.70 (1H, q, H-2), 3.88–3.92 (2H, m, H-4, 5), 4.07 (1H, dd, $J_{1b,2}$=3.1 Hz, H-1b), 4.37 (1H, br.t, *J*=5.1 Hz, 6-OH), 4.44 (1H, d, *J*=6.4 Hz, 4-OH), 4.48 (1H, d, *J*=12 Hz, $PhCH_2$), 4.59–4.73 (3H, m, $PhCH_2$), 5.10 (2H, s, $PhCH_2$), 7.26–7.37 (15H, m, Ph). A mixture of the above glucitol (174 mg, 0.36 mmol) and 10% Pd–C (30 mg) in acetic acid–ethanol–water (1:1:1; v/v/v, 3 ml) was stirred at rt under a hydrogen atmosphere for 45 hr. The catalyst was then filtered off and washed with aq. methanol. The filtrate and washings were combined, concentrated in vacuo and then treated with dil. hydrochloric acid solution, concentrated in vacuo. The residue was crystallized from ethanol–methanol to give **3** (31 mg, 53%) as the hydrochloride. mp 207–209°C (dec., aq. EtOH) [lit.[22] mp 196–198°C, lit.[14] mp 204–205°C]; $[\alpha]_D^{23}=+35.0°$ (*c* 0.45, H_2O), [lit.[22] $[\alpha]_D^{22}=+38.0°$]; ^1H NMR (D_2O) δ=2.99 (1H, t, $J_{1a,1b}=J_{1a,2}$=12 Hz, H-1a), 3.22 (1H, ddd, H-5), 3.52 (1H, dd, $J_{1b,2}$=4.9 Hz, H-1b), 3.53 (1H, dd, $J_{2,3}$=9.3 Hz, $J_{3,4}$=9.7 Hz, H-3), 3.62 (1H, dd, $J_{4,5}$=9.3 Hz, H-4), 3.80 (1H, ddd, H-2), 3.89 (1H, dd, $J_{5,6a}$=5.4 Hz, $J_{6a,6b}$=13 Hz, H-6a), 3.96 (1H, dd, $J_{5,6b}$=2.9 Hz, H-6b); ^{13}C NMR (D_2O) δ=46.3, 58.1, 60.4, 67.4, 68.2, 76.7.

3.1.11. Phenyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-*p*-methyoxybenzyl-1-thio-D-glucopyranosides (17). To a stirred solution of **15** (1.28 g, 3.48 mmol) and (phenylthio)-trimethylsilane (1.90 g, 10.4 mmol) in 1,2-dichloroethane (30 ml) was added zinc iodide (4.0 g, 12.5 mmol) at rt. The mixture was stirred at rt for 12 h, diluted with chloroform and washed with dil. HCl solution, sat. $NaHCO_3$ solution, water, and brine, dried and evaporated. The residual syrup (2.6 g) was dissolved in methanol–water (10:1; 20 ml) and treated with potassium carbonate (964 mg, 6.97 mmol) at rt for 0.5 hr. The resulting mixture was concentrated, and diluted with chloroform, washed with water, and brine, dried, and evaporated to give **16** (2.0 g). To a stirred mixture of **16** (2.0 g), *p*-methoxybenzyl chloride (1.50 g, 9.58 mmol) and tetrabutylammonium iodide (1.29 g, 3.48 mmol) in *N*,*N*-dimethylformamide (20 ml) was added sodium hydride (a 60% oil suspension, 420 mg, 10.5 mmol) at 0°C. After 4 h, the reaction mixture was quenched with methanol, and poured into sat. NH_4Cl solution. The resulting mixture was extracted with ether. The extracts were washed with water and brine, and evaporated. The residual syrup was chromatographed on silica gel with hexane–ethyl acetate (20:3) to give **17** (1.49 g, 72% from **15**) as an anomeric mixture (α/β=2:1, ^1H NMR analysis). A small quantity of the anomeric mixture was separated by chromatography on silica gel with hexane–ethyl acetate (10:1).

α-anomer: mp 63–64°C (diisopropylether); $[\alpha]_D^{22}$=+128° (*c* 1.01, $CHCl_3$); ^1H NMR ($CDCl_3$) δ=3.59 (1H, dd, $J_{5,6a}$=2.0 Hz, $J_{6a,6b}$=11 Hz, H-6a), 3.72–3.79 (3H, m, H-4, 5, 6b), 3.76 (3H, s, OMe), 3.81 (1H, dd, $J_{2,3}$=10 Hz, $J_{3,4}$=8.9 Hz, H-3), 3.94 (1H, dd, $J_{1,2}$=5.5 Hz, H-2), 4.34 (1H, m, H-5), 4.36, 4.56 (2H, each d, *J*=11 Hz, $PhCH_2$), 4.48, 4.78 (2H, each d, *J*=11 Hz, $PhCH_2$), 4.88, 4.91 (2H, each d, *J*=11 Hz, $PhCH_2$), 5.61 (1H, d, H-1), 6.82 (2H, d, *J*=8.5 Hz, Ph), 7.13–7.40 (15H, m, Ph), 7.50 (2H, dd, *J*=1.8 and 7.5 Hz, Ph).

Anal. Found: C, 68.16; H, 5.87; N, 6.86; S, 5.37. Calcd for $C_{34}H_{35}O_5N_3S$ requires C, 68.32; H, 5.90; N, 7.03; S, 5.36%.

β-isomer: mp 86–87°C (diisopropylether); $[\alpha]_D^{22}$=−43.9° (*c* 1.03, $CHCl_3$); ^1H NMR ($CDCl_3$) δ=3.34 (1H, dd, $J_{1,2}$=10 Hz, $J_{2,3}$=9.4 Hz, H-2), 3.45 (1H, ddd, H-5), 3.49 (1H, dd, $J_{3,4}$=9.2 Hz, H-3), 3.58 (1H, dd, $J_{4,5}$=9.5 Hz, H-4), 3.70 (1H, dd, $J_{5,6a}$=4.1 Hz, $J_{6a,6b}$=11 Hz, H-6a), 3.73 (1H, dd, $J_{5,6b}$=2.1 Hz, H-6b), 3.79 (3H, s, OMe), 4.40 (1H, d, H-1), 4.46, 4.54 (2H, each d, *J*=11 Hz, $PhCH_2$), 4.54, 4.76 (2H, each d, *J*=11 Hz, $PhCH_2$), 4.81, 4.84 (2H, each d, *J*=11 Hz, $PhCH_2$), 6.85 (2H, d, *J*=8.6 Hz, Ph), 7.17–7.32 (15H, m, Ph), 7.59 (2H, dd, *J*=1.2 and 8.2 Hz, Ph).

Anal. Found: C, 68.16; H, 5.87; N, 6.96; S, 5.36. Calcd for $C_{34}H_{35}O_5N_3S$ requires C, 68.32; H, 5.90; N, 7.03; S, 5.36%.

3.1.12. 4-*O*-(2-Azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl)-*N*-benzyloxycarbonyl-2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-1,5-dideoxy-1,5-imino-D-gulcitol (18) and 4-*O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-α-D-glucopyranosyl)-*N*-benzyloxycarbonyl-2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-1,5-dideoxy-1,5-imino-D-gulcitol (19). To a stirred suspension of **14** (1.01 g, 1.41 mmol), **17** (942 mg, 1.58 mmol) and pulverized activated 4 Å molecular sieves (2.40 g) in ether–dichloromethane (32:5, 37 ml) was added *N*-iodosuccinimide (887 mg, 3.94 mmol) at −50°C under Ar. A sat. solution of trifluoromethanesulfonic acid in dichloromethane (1.2 ml) was then added dropwise. After 50 min, the reaction mixture was poured into sat. $NaHCO_3$ and extracted with ether. The extract was successively washed with sat. $Na_2S_2O_3$, sat. $NaHCO_3$, water and brine, dried, and evaporated. The residual syrup was chromatographed on silica gel with benzene–ethyl acetate (200:1) to give **19** (1.30 g, 77%) and **18** (257 mg, 15%).

18: $[\alpha]_D^{26}$=+14.7° (*c* 0.28, $CHCl_3$); IR ν_{max}($CHCl_3$): 2103, 1700 cm^{-1}; ^1H NMR (d_6-DMSO, 110°C) δ=0.99 (9H, s, t-butyl), 3.29 (1H, dd, $J_{1',2'}$=7.8 Hz, $J_{2',3'}$=9.3 Hz, H-2′), 3.30 (1H, dd, $J_{1a,1b}$=14 Hz, $J_{1a,2}$=3.4 Hz, H-1a), 3.47 (1H, m, H-5′), 3.48 (1H, dd, $J_{3',4'}$=7.8 Hz, H-3′), 3.53 (1H, dd, $J_{4',5'}$=9.5 Hz, H-4′), 3.60 (2H, br.d, H-6′), 3.64 (1H, br.d, H-2), 3.68 (3H, s, OMe), 3.77 (1H, dd, $J_{1b,2}$=5.9 Hz, H-1b), 3.86 (2H, br.d, H-6), 3.92 (1H, dd, $J_{2,3}$=4.4 Hz, $J_{3,4}$=2.4 Hz, H-3), 4.31 (1H, $J_{4,5}$=2.0 Hz, H-4), 4.35, 4.40 (2H, each d, *J*=12 Hz, $PhCH_2$), 4.51 (1H, m, H-5), 4.52 (1H, d, H-1′), 4.50–4.80 (8H, m, $PhCH_2$), 5.00, 5.04 (2H, each d, *J*=13 Hz, $PhCH_2$), 6.76 (2H, dd, *J*=6.4, 2.3 Hz, Ph), 7.13–7.61 (37H, m, Ph).

Anal. Found: C, 71.59; H, 6.62; N, 4.69. Calcd for $C_{72}H_{78}O_{11}N_4Si$ requires C, 71.86; H, 6.53; N, 4.66%.

19: $[\alpha]_D^{26}$=+41.9° (*c* 0.88, $CHCl_3$); IR ν_{max}($CHCl_3$): 2103, 1700 cm^{-1}; ^1H NMR (d_6-DMSO, 120°C) δ=0.99 (9H, s, *t*-butyl), 3.28 (1H, dd, $J_{1a,1b}$=14 Hz, $J_{1a,2}$=3.1 Hz, H-1a), 3.45 (1H, dd, $J_{1',2'}$=3.7 Hz, $J_{2',3'}$=10 Hz, H-2′), 3.52 (1H, br.d, $J_{6'a,6'b}$=14 Hz, H-6′a), 3.58 (1H, dd, $J_{5',6'b}$=3.4 Hz, H-6′b), 3.66 (1H, dd, $J_{3',4'}$=8.8 Hz, $J_{4',5'}$=9.8 Hz, H-4′), 3.68 (1H, m, H-2), 3.72 (3H, s, OMe), 3.79 (1H, dd, H-3′), 3.80 (1H, m, H-5′), 3.86 (1H, t, $J_{2,3}$=2.4 Hz, $J_{3,4}$=2.4 Hz, H-3), 3.89 (2H, br.d, H-6), 3.90 (1H, dd, $J_{1b,2}$=5.2 Hz, H-1b), 4.13 (1H, t, $J_{4,5}$=2.4 Hz, H-4), 4.30, 4.39 (2H, each d, *J*=12 Hz, $PhCH_2$), 4.54–4.69 (10H, m, $PhCH_2$), 5.03 (2H, br.s, $PhCH_2$), 5.17 (1H, d, H-1′), 6.83 (2H, dd, *J*=6.7, 2.1 Hz, Ph), 7.6–7.41 (33H, m, Ph), 7.60 (4H, br.t, *J*=6.2 Hz).

Anal. Found: C, 71.52; H, 6.59; N, 4.72. Calcd for $C_{72}H_{78}O_{11}N_4Si$ requires C, 71.86; H, 6.53; N, 4.66%.

3.1.13. 4-*O*-(2-Acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-α-D-glucopyranosyl)-*N*-benzyloxycarbonyl-2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-1,5-dideoxy-1,5-imino-D-gulcitol (20). (a) A mixture of **19** (1.15 g, 0.96 mmol) and triphenylphosphine (276 mg, 1.05 mmol) in tetrahydrofuran (38 ml) was heated at 60°C for 16 h with stirring, cooled to rt. Water (4.0 ml) was added and stirring was continued for further 20 h. Acetic anhydride (0.3 ml) was added to the resulting mixture and the solution was stirred at rt for 3 h, and evaporated. The residual syrup was chromatographed on silica gel with hexane–ethyl acetate (2:1) to give **20** (1.09 g,93%).

(b) To a stirred mixture of **19** (7.4 g, 6.15 mmol) in pyridine (10 ml) was added dropwise thioacetic acid (20 ml) at 5°C and then the mixture was stirred at rt for 18 h, evaporated. The residual syrup was co-evaporated with toluene in 3 times, and then chromatographed on silica gel with hexane–ethyl acetate (4:1→1:1) to give **20** (6.40 g, 85%); $[\alpha]_D^{23}$=+30.2° (*c* 0.42, $CHCl_3$); IR ν_{max}($CHCl_3$): 1699, 1684, 1110 cm^{-1}; ^1H NMR (d_6-DMSO, 110°C) δ=0.97 (9H, s, *t*-butyl), 1.58 (3H, s, NAc), 3.21 (1H, br.d, $J_{1a,1b}$=14 Hz, H-1a), 3.50 (1H, br.d, $J_{6'a,6'b}$=11 Hz, H-6′a), 3.55–4.10 (6H, m, H-2, 3, 6, 3′, 4′), 3.60 (1H, dd, $J_{5',6b}$=3.4 Hz, H-6′b), 3.71 (3H, s, OMe), 3.85 (1H, br.d, H-5′), 3.98 (1H, m, H-2′), 4.03 (1H, br.s, H-4), 4.05 (1H, br.d, H-1b), 4.30, 4.40 (2H, each d, *J*=12 Hz, $PhCH_2$), 4.55 (1H, br.s, H-5), 4.45–4.71 (8H, m, $PhCH_2$), 4.87 (1H, d, $J_{1',2'}$=3.0 Hz, H-1′), 5.04, 5.07 (2H, each d, *J*=13 Hz, $PhCH_2$), 6.63 (1H, d, $J_{2'NH'}$=7.3 Hz, NH′), 6.82 (2H, br.d, *J*=8.5 Hz, Ph), 7.16–7.59 (37H, m, Ph).

Anal. Found: C, 72.63 H, 6.67 N, 2.31. Calcd for $C_{74}H_{82}O_{12}N_2Si$ requires C, 72.88; H, 6.78; N, 2.30%.

3.1.14. 4-*O*-(2-Acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-α-D-glucopyranosyl)-*N*-benzyloxycarbonyl-2,3-di-*O*-benzyl-1,5-dideoxy-1,5-imino-D-gulcitol (21). To a stirred mixture of **20** (6.0 g, 4.92 mmol) and acetic acid (0.50 g, 8.33 mmol) in tetrahydrofuran (100 ml) was added dropwise a 1.0 M solution of tetrabutylammonium fluoride (7.87 ml, 7.87 mmol) at rt. The mixture was

stirred at rt for 22 h, diluted with chloroform and washed with water and brine, dried, and evaporated. The residual syrup was chromatographed on silica gel with hexane–ethyl acetate (1:1) to give **21** (4.80 g, 99%). $[\alpha]_D^{25}=+20.1°$ (*c* 0.67, $CHCl_3$); IR $\nu_{max}(CHCl_3)$: 3400, 1698, 1680, 1612 cm^{-1}; ^1H NMR (d_6-DMSO, 120°C) δ=1.58 (3H, s, NAc), 3.54 (1H, dd, $J_{3',4'}$=9.3 Hz, $J_{4',5'}$=9.2 Hz, H-4′), 3.35 (1H, dd, $J_{1a,1b}$=14 Hz, $J_{1a,2}$=2.9 Hz, H-1a), 3.59–3.67 (5H, m, H-6, 3′, 6′), 3.69–3.75 (2H, m, H-2,3), 3.74 (3H, s, OMe), 3.87 (1H, ddd, $J_{5',6'a}$=3.5 Hz, $J_{5',6'b}$=2.5 Hz, H-5′), 3.98 (1H, m, H-2′), 4.00 (1H, br.t, $J_{3,4}$=3.0 Hz, $J_{4,5}$=3.0 Hz, H-4), 4.06 (1H, dd, $J_{1b,2}$=3.9 Hz, H-1b), 4.34 (1H, dt, $J_{5,6a}$=6.3 Hz, $J_{5,6b}$=6.3 Hz, H-5), 4.36–4.71 (10H, m, $PhCH_2$), 4.88 (1H, d, $J_{1',2'}$=3.4 Hz, H-1′), 5.07, 5.12 (2H, each d, *J*=13 Hz, $PhCH_2$), 6.62 (1H, d, $J_{2'NH'}$=7.3 Hz, NH′), 6.85 (2H, br.d, *J*=8.2 Hz, Ph), 7.17–7.32 (12H, m, Ph).

Anal. Found: C, 70.89 H, 6.60 N, 2.86. Calcd for $C_{58}H_{64}O_{12}N_2$ requires C, 71.00; H, 6.58; N, 2.86%.

3.1.15. Methyl 3-*O*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-*N*-benzyloxycarbonyl-4,5-di-*O*-benzyl-2,6-dideoxy-2,6-imino-L-gulonate (22). To a stirred solution of **21** (427 mg, 0.44 mmol) in acetone (15 ml) was added dropwise Jones reagent (ca. 0.5 ml) at rt. After 10 min, 2-propanol was added, and the resulting mixture was poured into water, extracted with chloroform. The extracts were washed successively with water and brine, dried, and evaporated, co-evaporated with toluene. The residue was treated with diazomethane in ether and the solution evaporated. The residue was passed through a short colomn of silica gel {chloroform–ethyl acetate (6:1)} to give a syrup (420 mg), which was dissolved in dichloromethane–water (19:1, 20 ml). To this solution was added 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (283 mg, 1.25 mmol) at 0°C. The mixture was stirred at 0°C for 2 h, diluted with dichloromethane, and washed successively with sat. $Na_2S_2O_3$, sat. $NaHCO_3$, water and brine, dried, and evaporated. The residual syrup was chromatographed on silica gel with hexane–ethyl acetate (1:2) to give **22** (252 mg, 65%), $[\alpha]_D^{24}=+50.2°$ (*c* 0.67, $CHCl_3$); IR $\nu_{max}(CHCl_3)$: 3360, 1755, 1710, 1690 cm^{-1}; ^1H NMR (d_6-DMSO, 80°C) δ=1.64 (3H, s, NAc), 3.48–3.69 (6H, m, H-6a, 3′, 4′, 5′, 6′), 3.54 (3H, s, Me), 3.78–3.84 (2H, m, H-4, 5), 3.98 (1H, br.t, H-2′), 4.07 (1H, dd, $J_{5,6b}$=3.9 Hz, $J_{6a,6b}$=14 Hz, H-6b), 4.34 (1H, br.s, H-3), 4.38–4.73 (9H, m, OH, $PhCH_2$), 4.95 (1H, br.s, H-2), 4.96 (1H, d, $J_{1',2'}$=3.4 Hz, H-1′), 5.07, 5.16 (2H, each d, *J*=13 Hz, $PhCH_2$), 6.87 (1H, d, $J_{2'NH'}$=9.3 Hz, NH′), 7.16–7.33 (25H, m, Ph).

Anal. Found: C, 68.57; H, 6.27; N, 3.03. Calcd for $C_{51}H_{56}O_{12}N_2$ requires C, 68.90; H, 6.35; N, 3.15%.

3.1.16. Benzyl 3-*O*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-α-D-glucopyranosyl)-*N*-benzyloxycarbonyl-4,5-di-*O*-benzyl-2,6-dideoxy-2,6-imino-L-gulonate (23). To a stirred solution of **21** (54 mg, 0.06 mmol) in acetone (2 ml) was added dropwise Jones reagent (ca. 50 μl) at rt. After 20 min, 2-propanol was added, and the resulting mixture was poured into water, extracted with chloroform. The extracts were washed successively with water and brine, dried, and evaporated, co-evaporated with toluene. To a stirred solution of the residual syrup and benzyl bromide (13 μl, 0.11 mmol) in *N*,*N*-dimethylformamide (1.0 ml) was added cesium carbonate (27 mg, 0.06 mmol) at rt. The mixture was stirred for 6 h, and poured into water, extracted with chloroform. The extracts were successively washed with water and brine, dried, and evaporated. The residual syrup was chromatographed on silica gel with hexane–ethyl acetate (2:1) to give **23** (43 mg, 72%); $[\alpha]_D^{25}=+53.1°$ (*c* 0.56, $CHCl_3$); IR $\nu_{max}(CHCl_3)$: 1748, 1703, 1684 cm^{-1}; ^1H NMR (d_6-DMSO, 120°C) δ=1.62 (3H, s, NAc), 3.50–3.60 (2H, m, H-6a, 4′), 3.49 (1H, dd, $J_{5',6'a}$=8.8 Hz, $J_{6'a,6'b}$=11 Hz, H-6′a), 3.54 (1H, t, $J_{2',3'}$=9.3 Hz, $J_{3',4'}$=9.3 Hz, H-3′), 3.59 (1H, dd, $J_{5',6'b}$=3.9 Hz, H-6′b), 3.72 (3H, s, OMe), 3.74–3.84 (3H, m, H-4, 5, 5′), 3.98 (1H, m, H-2′), 4.08 (1H, dd, $J_{6a,6b}$=13 Hz, H-6b), 4.32 (1H, d, *J*=12 Hz, $PhCH_2$), 4.39 (1H, br.s, H-3), 4.39–4.67 (9H, m, $PhCH_2$), 4.93 (1H, d, *J*=13 Hz, $PhCH_2$), 4.94 (1H, d, $J_{1',2'}$=3.9 Hz, H-1′), 4.99 (1H, br.s, H-2), 5.05–5.14 (3H, m, $PhCH_2$), 6.68 (1H, d, $J_{2'NH'}$=8.3 Hz, NH′), 6.82 (2H, br.d, *J*=8.8 Hz, Ph), 7.17–7.32 (32H, m, Ph).

Anal. Found: C, 71.90 H, 6.38 N, 2.52. Calcd for $C_{65}H_{68}O_{13}N_2$ requires C, 71.94; H, 6.32; N, 2.58%.

3.1.17. Benzyl 3-*O*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-*N*-benzyloxycarbonyl-4,5-di-*O*-benzyl-2,6-dideoxy-2,6-imino-L-gulonate (24). To a stirred solution of **23** (1.30 g, 1.20 mmol) in dichloromethane–water (19:1, 20 ml) was added 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (544 mg, 2.40 mmol) at 0°C. The mixture was stirred at 0°C for 2 h, diluted with dichloromethane, and washed successively with sat. $Na_2S_2O_3$, sat. $NaHCO_3$, water and brine, dried, and evaporated. The residual syrup was chromatographed on silica gel with hexane–ethyl acetate (1:1) to give **24** (0.89 g, 77%), $[\alpha]_D^{25}=+44.4°$ (*c* 0.12, $CHCl_3$); IR $\nu_{max}(CHCl_3)$: 3350, 1745, 1710, 1680 cm^{-1}; ^1H NMR (d_6-DMSO, 110°C) δ=1.65 (3H, s, NAc), 3.55–3.69 (6H, m, H-6a, 3′, 4′, 5′, 6′), 3.78–3.84 (2H, m, H-4, 5), 4.00 (1H, br.t, H-2′), 4.05 (1H, dd, $J_{5,6b}$=4.0 Hz, $J_{6a,6b}$=14 Hz, H-6b), 4.21 (1H, br.s, OH), 4.38 (1H, br.t, H-3), 4.43–4.71 (8H, m, $PhCH_2$), 4.96 (1H, d, *J*=12 Hz, $PhCH_2$), 4.97 (1H, d, $J_{1',2'}$=3.4 Hz, H-1′), 4.99 (1H, br.s, H-2), 5.09-5.15 (3H, m, $PhCH_2$), 6.74 (1H, d, $J_{2'NH'}$=8.5 Hz, NH′), 7.16–7.33 (30H, m, Ph).

Anal. Found: C, 70.68 H, 6.19; N, 2.86. Calcd for $C_{57}H_{60}O_{12}N_2$ requires C, 70.94; H, 6.27; N, 2.90%.

3.1.18. Benzyl 3-*O*-(2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-*N*-benzyloxycarbonyl-4,5-di-O-benzyl-2,6-dideoxy-2,6-imino-L-gulonate sodium salt (25). Sulfur trioxide–trimethylamine complex (177 mg, 1.27 mmol) was added to a solution of **24** (612 mg, 0.64 mmol) in *N*,*N*-dimethylformamide (3 ml) at 0°C, and the mixture was stirred for 12 h at rt, diluted with methanol (0.5 ml), placed on a column of Sephadex LH-20 pre-equilibrated with 1:1 (v/v) chloroform–methanol, and eluted with the same solvent. The product was chromatographed on a column of Dowex 50 W X-8 (Na^+) resin, preequilibrated with 80% methanol by using the same solvent to give a crude product, which was then chromatographed on silica gel with chloroform–methanol (10:1) to give **25** (665 mg, 98%): $[\alpha]_D^{26}=+18.7°$ (*c* 0.52, $CHCl_3$); IR

ν_{max}(film): 1740, 1703, 1653, 1431, 1215 cm^{-1}; ^1H NMR (d_6-DMSO, 80°C) δ=1.62 (3H, s, NAc), 3.51–3.58 (3H, m, H-6a, 3′, 4′), 3.75 (1H, m, H-5′), 3.77 (1H, br.t, H-5), 3.79 (1H, br.t, H-4), 3.94 (1H, m, H-2′), 3.95 (1H, dd, $J_{5',6'a}$= 1.7 Hz, $J_{6'a,6'b}$=11 Hz, H-6′a), 4.04 (1H, dd, $J_{5,6b}$=4.7 Hz, $J_{6a,6b}$=14 Hz, H-6b), 4.13 (1H, dd, $J_{5',6'b}$=2.9 Hz, H-6′b), 4.38 (1H, br.s, H-3), 4.36–4.65 (7H, m, $PhCH_2$), 4.83 (1H, d, J=11 Hz, $PhCH_2$), 4.96 (1H, d, $J_{1',2'}$=3.4 Hz, H-1′), 4.94–4.99 (2H, m, H-2, $PhCH_2$), 5.07–5.15 (3H, m, $PhCH_2$), 6.83 (1H, d, $J_{2'NH'}$=8.6 Hz, NH′), 7.12–7.36 (30H, m, Ph).

Anal. Found: C, 63.43 H, 5.60; N, 2.56; S, 2.55. Calcd for $C_{57}H_{59}O_{15}N_2SNa\cdot H_2O$ requires C, 63.09; H, 5.66; N,2.59; S, 2.95%.

3.1.19. 4-*O*-Allyl-*N*-benzyloxycarbonyl-2,3-di-*O*-benzyl-1,5-dideoxy-1,5-imino-D-gulcitol (26). To a stirred mixture of **13** (890 mg, 1.18 mmol) and acetic acid (0.11 ml, 2.00 mmol) in tetrahydrofuran (15 ml) was added dropwise a 1.0 M solution of tetrabutylammonium fluoride (1.88 ml, 1.88 mmol) at rt. The mixture was stirred at rt for 22 h, diluted with ethyl acetate and washed with water and brine, dried, and evaporated. The residual syrup was chromatographed on silica gel with hexane–ethyl acetate (4:1) to give **26** (599 mg, 98%); $[\alpha]_D^{26}$=+15.1° (*c* 0.85, $CHCl_3$); IR ν_{max}(film): 3447, 1699, 1428, 1072 cm^{-1}; ^1H NMR (d_6-DMSO, 100°C) δ=3.34 (1H, dd, $J_{1a,1b}$=14 Hz, $J_{1a,2}$=4.2 Hz, H-1a), 3.58–3.68 (4H, m, H-2,3, 6a, 6b), 3.75 (1H, dd, $J_{3,4}$=5.4 Hz, $J_{4,5}$=4.9 Hz, H-4), 3.92 (1H, dd, $J_{1b,2}$=4.4 Hz, H-1b), 4.01–4.15 (3H, m, H-5, allyl), 4.47 (1H, t, J=5.5 Hz, OH), 4.49, 4.58 (2H, each d, J= 12 Hz, $PhCH_2$), 4.63, 4.67 (2H, each d, J=12 Hz, $PhCH_2$), 5.06–5.24 (4H, m, $PhCH_2$, olefin), 5.84 (1H, m, olefin), 7.24–7.60 (15H, m, Ph).

Anal. Found: C, 71.53; H, 6.84; N, 2.51. Calcd for $C_{31}H_{35}O_6N$ requires C, 71.93; H, 6.82; N, 2.71%.

3.1.20. Benzyl 3-*O*-allyl-*N*-benzyloxycarbonyl-4,5-di-*O*-benzyl-2,6-dideoxy-2,6-imino-L-gulonate (27). To a stirred solution of **26** (165 mg, 0.33 mmol) in acetone (7 ml) was added dropwise Jones reagent (ca. 0.4 ml) at rt. After 5 min, 2-propanol was added, and the resulting mixture was poured into water, extracted with dichloromethane. The extracts were washed successively with water and brine, dried, and evaporated, co-evaporated with toluene. To a stirred solution of the residual syrup (179 mg) and benzyl bromide (0.11 ml, 0.93 mmol) in *N*,*N*-dimethylformamide (2.0 ml) was added cesium carbonate (195 mg, 0.40 mmol) at rt. The mixture was stirred for 3 h, and poured into water, extracted with ether. The extracts were washed successively with water and brine, dried, and evaporated. The residual syrup was chromatographed on silica gel with hexane–ethyl acetate (10:1→ 5:1) to give **27** (160 mg, 81%); $[\alpha]_D^{25}$=+25.1° (*c* 0.56, $CHCl_3$); IR ν_{max}(film): 1747, 1705, 1455, 1132 cm^{-1}; ^1H NMR (d_6-DMSO, 100°C) δ=3.57 (1H, dd, $J_{6a,6b}$=14 Hz, $J_{6a,5}$=3.2 Hz, H-6a), 3.70 (1H, m, H-5), 3.77 (1H, dd, $J_{3,4}$=3.4 Hz, $J_{4,5}$=3.9 Hz, H-4), 3.90 (1H, dd, $J_{5,6b}$= 4.4 Hz, H-6b), 4.04–4.10 (2H, m, allyl), 4.11 (1H, $J_{2,3}$= 3.0 Hz, H-3), 4.51, 4.59 (2H, each d, J=14 Hz, $PhCH_2$), 4.53 (2H, s, $PhCH_2$), 4.82 (1H, d, H-2), 4.97, 5.07 (2H, each d, J=12 Hz, $PhCH_2$), 5.05–5.25 (4H, m, olefin and $PhCH_2$), 5.82 (1H, m, olefin), 7.20–7.33 (20H, m, Ph). HRFABMS: Found; 644.2612, Calcd for $C_{38}H_{39}O_7NNa$: 644.2624 $(M+Na)^+$.

3.1.21. Benzyl *N*-benzyloxycarbonyl-4,5-di-*O*-benzyl-2,6-dideoxy-2,6-imino-L-gulonate (28). A mixture of **27** (85 mg, 0.14 mmol), sodium acetate (47 mg, 0.57 mmol) and palladium chloride (25 mg, 0.14 mmol) in 90% acetic acid (1 ml) was heated 50°C with stirring for 1.5 h, cooled, diluted with ethylacetate, and then filtered through a pad of Celite. The filtrate was evaporated, and then co-evaportaed with toluene. The residue was chromatographed on silica gel with hexane–ethyl acetate (10:1→4:1→2:1) to give **28** (59 mg, 74%); $[\alpha]_D^{26}$=+36.6° (*c* 0.39, $CHCl_3$); IR ν_{max} (film): 3487, 1744, 1705 cm^{-1}; ^1H NMR (d_6-DMSO, 100°C) δ=3.52 (1H, dd, $J_{6a,6b}$=14 Hz, $J_{6a,5}$=3.0 Hz, H-6a), 3.67 (1H, t, J=4.0 Hz, H-4), 3.76 (1H, m, H-5), 4.03 (1H, dd, $J_{5,6b}$=3.5 Hz, H-6b), 4.28 (1H, m, H-3), 4.51, 4.59 (2H, each d, J=12 Hz, $PhCH_2$), 4.52 (2H, s, $PhCH_2$), 4.70 (1H, d, $J_{2,3}$=3.9 Hz, H-2), 4.93, 5.03 (2H, each d, J=12 Hz, $PhCH_2$), 5.10 (2H, s, $PhCH_2$), 7.20–7.32 (20H, m, Ph). HRFABMS: Found; 604.2308, Calcd for $C_{35}H_{35}O_7NNa$: 604.2311 $(M+Na)^+$.

3.1.22. Benzyl 3-*O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl)-*N*-benzyloxycarbonyl-4,5-di-*O*-benzyl-2,6-dideoxy-2,6-imino-L-gulonate (29) and benzyl 3-*O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-α-D-glucopyranosyl)-*N*-benzyloxycarbonyl-4,5-di-*O*-benzyl-2,6-dideoxy-2,6-imino-L-gulonate (30). To a stirred suspension of **28** (44 mg, 77 μmol), **17** (60 mg, 100 μmol), *N*-iodosuccinimide (43 mg, 190 μmol) and pulverized activated 4 Å molecular sieves (100 mg) in ether–1,2-dichloroethane (5:1, 0.6 ml) was added dropwise triethylsilytriflate (23 μl, 100 μmol) at −78°C under Ar. After 2.5 h, the reaction mixture was poured into sat. $NaHCO_3$ and extracted with ether. The extracts were washed successively with sat. $Na_2S_2O_3$, sat. $NaHCO_3$, water and brine, dried, and evaporated. The residual syrup was chromatographed on silica gel with hexane–ethyl acetate (10:1→4:1) and then purified by preparative TLC {chloroform–ethyl acetate (200:1)} to give **29** (7 mg, 9%) and **30** (58 mg, 72%).

29: $[\alpha]_D^{26}$=+18.6° (*c* 0.30, $CHCl_3$); IR ν_{max}(film): 2113, 1748, 1705, 1455, 1249 cm^{-1}; ^1H NMR (d_6-DMSO, 110°C) δ=3.26 (1H, dd, $J_{1',2'}$=8.3 Hz, $J_{2',3'}$=9.3 Hz, H-2′), 3.45–3.72 (7H, m, H-5, 6a, 3′, 4′, 5′, 6′), 3.68 (3H, s, OMe), 3.82 (1H, $J_{5,6}$=5.4 Hz, $J_{6a,6b}$=14 Hz, H-6b), 3.96 (1H, dd, $J_{3,4}$=3.4 Hz, $J_{4,5}$=4.4 Hz,, H-4), 4.36, 4.40 (2H, each d, J=12 Hz, $PhCH_2$), 4.44–4.56 (6H, m, $PhCH_2$), 4.56 (1H, dd, $J_{2,3}$=2.0 Hz, H-3), 4.67–4.75 (3H, m, $PhCH_2$), 4.69 (1H, d, H-1′), 4.94–5.10 (3H, m, $PhCH_2$), 5.01 (1H, d, H-2), 6.78 (2H, d, J=8.4 Hz, Ph), 7.25–7.31 (32H, m, Ph); HRFABMS: Found; 1091.4386, Calcd for $C_{63}H_{64}O_{12}N_4Na$: 1091.4418 $(M+Na)^+$.

30: $[\alpha]_D^{26}$=+64.4° (*c* 0.10, $CHCl_3$); IR ν_{max}(film): 2103, 1748, 1705, 1455, 1124 cm^{-1}; ^1H NMR (d_6-DMSO, 110°C) δ=3.44 (1H, dd, $J_{1',2'}$=3.9 Hz, $J_{2,3}$=9.8 Hz, H-2′), 3.48–3.78 (10H, m, H-5, 6a, 3′, 4′, 5′, 6′), 3.72 (3H, s, MeO), 3.43 (1H, br.t, J=2.5 Hz, H-4), 3.98 (1H, $J_{5,6}$=3.9 Hz,

$J_{6a,6b}$=14 Hz, H-6b), 4.29, 4.38 (2H, each d, J=12 Hz, $PhCH_2$), 4.46 (1H, t, J=2.1 Hz, H-3), 4.47–4.67 (8H, m, $PhCH_2$), 4.93–5.14 (4H, m, $PhCH_2$), 5.06 (1H, d, H-2), 5.22 (1H, d, H-1′), 6.82 (2H, d, J=8.4 Hz), 7.25–7.31 (32H, m, Ph); HRFABMS: Found; 1091.4436, Calcd for $C_{63}H_{64}O_{12}N_4Na$: 1091.4418 $(M+Na)^+$.

3.1.23. Chemical transformation of 30 into 23. To a stirred mixture of **30** (14 mg, 0.01 mmol) in pyridine (0.2 ml) was added dropwise thioacetic acid (0.4 ml) at 0°C and then the mixture was stirred at 0°C→rt for 18 h, evaporated. The residual syrup was co-evaporated with toluene in 3 times, and then chromatographed on silica gel with hexane–ethyl acetate (4:1→1:1) to give **23** (14 mg, 98%).

3.1.24. 3-*O*-(2-Acetamido-2-deoxy-6-*O*-sulfo-α-D-gluco-pyranosyl)-2,6-dideoxy-2,6-imino-L-gulonic acid sodium salt (1). A mixture of **25** (524 mg, 0.49 mmol) and 10% Pd–C (400 mg) in methanol–acetic acid–water (4:1:1; 18 ml) was stirred under a hydrogen atmosphere at rt for 2 d. The mixture was filtered and the filtrate was evaporated. The residue was chromatographed on a gel-permeation column (Sephadex G-10), with water as the eluent, to give **1** (229 mg, 96%) as a hygroscopic white powder: $[\alpha]_D^{25}$= +84.4° (*c* 0.44, H_2O); IR ν_{max}(KBr): 3480, 1650, 1560, 1250, 1230, 1060 cm^{-1}; ^1H NMR (D_2O) δ=2.07 (3H, s, NAc), 2.98 (1H, dd, $J_{5,6a}$=8.9 Hz, $J_{6a,6b}$=13 Hz, H-6a), 3.56 (1H, dd, $J_{5,6b}$=4.0 Hz, H-6b), 3.61 (1H, dd, $J_{3',4'}$= 9.4 Hz, $J_{4',5'}$=10 Hz, H-4′), 3.65 (1H, d, $J_{2,3}$=7.6 Hz, H-2), 3.76 (1H, dd, $J_{2',3'}$=10 Hz, H-3′), 3.81 (1H, dd, $J_{3,4}$=7.3 Hz, $J_{4,5}$=7.6 Hz, H-4), 3.89 (1H, ddd, H-5), 3.96 (1H, br.d, H-5′), 3.98 (1H, dd, $J_{1',2'}$=3.7 Hz, H-2′), 4.08 (1H, dd, H-3), 4.26 (1H, br.d, $J_{6'a,6'b}$=11 Hz, H-6′a), 4.34 (1H, dd, $J_{5',6'b}$=2.9 Hz, H-6′b), 5.42 (1H, d, H-1′). ^{13}C NMR (D_2O) δ=22.8 (Me), 44.9 (C-6), 54.1 (C-2′), 61.3 (C-2), 67.4 (C-6′), 67.5 (C-5), 69.9 (C-4′), 71.3 (C-5′), 71.6 (C-3′), 72.9 (C-4), 74.3 (C-3), 97.7 (C-1′), 171.4 (COOH), 175.2 (NHCO). HRFABMS: Found; 459.0941, Calcd for $C_{14}H_{23}O_{13}N_2SNa$: 459.0921 $(M-Na)^-$.

3.1.25. Heparanase assay. The assay was performed according to the method previously reported by Nakajima et al.[4] In the presence or absence of **1**, *N*-^3H-acetylated heparan sulfate (bovine lung) was incubated at 42°C for 4 h with heparanase partially purifed from colon 26 N-17 or B16-F10 melanoma cells in 0.1% Triton X-100 and 0.1 M sodium acetate (pH 5.0). Incubation products were analyzed by size exclusion chromatography using a Bio-Gel TSK-30 XL column. Percent inhibition was determined by measuring the decrease in the area of the first one-half of the peak of intact [^3H] heparan sulfate on the chromatogram.

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