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Carbohydrate Research 341 (2006) 2115–2125

Carbohydrate
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1,3-Dideoxynojirimycin-3-yl glycosides of β-(1→3)- and β-(1→6)-linked gluco-oligosaccharides

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Received 23 March 2005; received in revised form 7 April 2006; accepted 7 May 2006

Abstract—Standard chemical methods involving the use of O-acetylated glycosyl trichloroacetimidates as glycosylating agents were used to prepare the five 1,3-dideoxynojirimycin-3-yl β-(1→3)-linked oligo-glucosides (**1**–**5**) and also the β-(1→6)-bonded glucobiose (gentiobiose)-based analogue **6** as potential fungicides. In the course of the work, the β-(1→6), β-(1→6)-linked analogue **8** of **6** and 6-*O*- and 4-*O*-β-glucopyranosyl-deoxynojirimycins **7** and **9**, respectively, were also produced.

Keywords: Fungicides; Glycosylation; Nojirimycin; ^{13}C and ^{1}H NMR; HR-MS/FAB; ES-TOF-MS

1. Introduction

Interest remains high in the development of new antifungal agents, in particular for the treatment of the increasing numbers of immuno-compromised patients suffering from AIDS or coping with cancer chemotherapy or recovery from organ transplant surgery. One important strategy for developing suitable protection against common infections depends on the use of compounds that interfere specifically with the biosynthesis of the key components of cell walls of the relevant pathogenic microorganisms.[1–4] The efficacies of the penicillins and cephalosporins as antibiotics depend on their use in this approach directed against bacterial cell wall biosynthesis; the search continues for fungicides which act by similar mechanisms.

Together with a mannoprotein, a β-(1→3)-linked glucan with DP ~ 1500 and containing up to 10% β-(1→6)-linked branching makes up the major part of fungal cell walls. In addition, a β-(1→6) glucan with about 20% of β-(1→3) branching, and also chitin, are minor components. Whereas the linear part of the β-(1→3) glucan is synthesized on the plasma-side of the cell membrane, branching as well as any putative cross-linking between different polysaccharides take place in the cell wall itself,[1,2,5] and therefore the glycosylases and glycosyl transferases which mediate the branching and cross-linking processes may, in principle at least, be reached by inhibitors which do not have to find means of transport across the cell membranes as is the case with inhibitors of the β-(1→3) glucan synthases.[1,2,4]

On the assumption that, in the course of the branching reactions, glucosyl and oligoglucosyl residues from the non-reducing ends of β-(1→3)-glucans are transferred to 6-positions of non-terminal units of other (1→3)-linked glucan chains,[5,6] and that cross-linking involves glycosidic bonding of the reducing centers of some chains to primary positions of non-terminal units of others,[5–9] we have developed a strategy for the preparation of potential inhibitors of the enzymes implicated. This involves the specific O-glycosylation of the well known glycosidase inhibitor 1-deoxynojirimycin (1,5-dideoxy-1,5-imino-D-glucitol, DNJ)[10] with β-(1→3)- and β-(1→6)-linked glucose oligomers on the principle that in such compounds the oligosaccharide moieties would be recognized and bound by the relevant enzymes, and thereby the inhibiting nitrogen-containing moieties would be directed towards the active sites. The

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doi:10.1016/j.carres.2006.05.004

consequence could be selective interference in the production of the branched glycans during the biosynthesis of the major fungal cell wall polymers.

Previous studies encourage this approach. For example, several bioactive glycosylated DNJ derivatives are known, some having been isolated from natural sources and some having been made by chemical or by enzymic procedures.[10–13] Not surprisingly, several of these compounds inhibit carbohydrate-processing enzymes,[10] as do various nitrogen-containing analogues with somewhat modified structures,[14–16] indicating that the area could be fertile as a source of the enzyme inhibitors required for the present work.

More complex glycosylated DNJ compounds to have been synthesized are analogues of the biologically important sialyl-Lewis X[17] and A[18] tetrasaccharides which have the *N*-heterocyclic compound replacing the structurally related *N*-acetylglucosamine.

Specifically, as far as the strategy to be used in the present work is concerned, homo-glucooligosaccharide 1-deoxynojirimycin glycosides are known to inhibit relevant enzymes. Thus DNJ glycosides of maltooligosaccharides, up to the heptaose, α-(1→4)-linked to the aglycon inhibit some amylases with structure–activity relationships which depend to a major extent on the source of the enzymes.[19] For example, a bacterial saccharifying α-amylase is not inhibited by DNJ itself, but is by the 4-*O*-α-D-glucosyl and 4-*O*-α-maltosyl derivatives to increasing extents; the activities however do not increase further on subsequent elongation of the saccharide chain. On the other hand, a microbial β-amylase is inhibited by 4-*O*-α-D-glucosyl-DNJ, and to some extent by the trisaccharide-linked analogue, but not by other members of the series. Of special relevance to the present work is the observation that the enzyme of *Aerobacter aerogenes* that hydrolyzes the α-(1→3)- and α-(1→6)-linked glucan pullulan is inhibited by 4-O-α-maltosyl-DNJ and, increasingly, by higher members of the series.[19] In a similar fashion, 4-*O*-β-D-glucosyl- and -cellobiosyl-DNJ inhibit cellulases of bacteria and fungi, the latter being much the more potent, and 6-*O*-β-cellobiosyl-DNJ also shows some cellulase inhibiting activity.[20] In the course of that work it was reported that cellooligosaccharide glycosides of DNJ had been made by use of a cellodextrin phosphorylase of *Clostridium thermocellum* expressed by *E. coli*, applied with glucose 1-phosphate as glucose donor and 4-*O*-D-glucosyl- and 6-*O*-cellobiosyl-DNJ as acceptors.[21]

Although known in the first instance as a powerful inhibitor of α-glucosidases, DNJ has also been shown to display considerable activity against fungal β-glucosidases, the β-enzyme of *Aspergillus wentii*, for example, being inhibited with K_i=2.7 μM.[22] DNJ and its derivatives have also been recognized as being of potential value as antidiabetic, antineoplastic and anti-HIV agents.[10]

2. Results and discussion

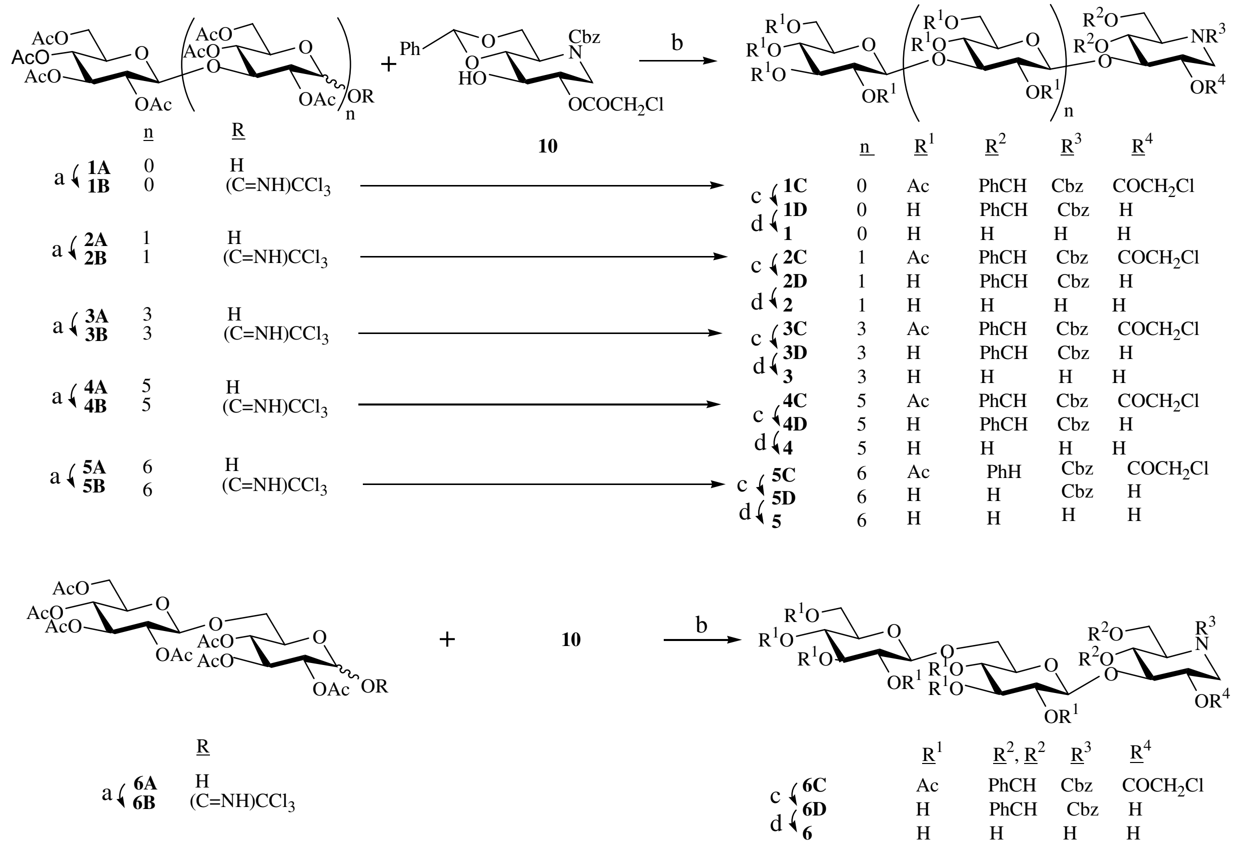
For the purpose outlined above, D-glucose, its β-(1→3)-linked dimer (laminaribiose), the corresponding β-(1→3)-linked tetramer, hexamer and heptamer, and also the β-(1→6)-linked glucobiose, gentiobiose, were separately β-(1→3) bonded to DNJ to give compounds **1**–**6**, respectively (Scheme 1). In addition, corresponding glycosides having glucose and gentiobiose separately linked β-(1→6) to the same amine (compounds **7** and **8**, respectively) and glucose β-(1→4)-bonded to DNJ (compound **9**) were also synthesized (Scheme 2).

The procedure adopted (Scheme 1, for example, for compounds **1**–**6**) involved use of the appropriate O-acetylated glycosyl trichloroacetimidates (**1B**–**6B**), made from the corresponding sugar peracetates by selective hydrolysis of the anomeric ester functions by use of hydrazinium acetate in DMF,[23] and treatment of the derived free sugars (**1A**–**6A**) with trichloroacetonitrile in dichloromethane, usually in the presence of potassium carbonate.[24] The glycosyl donors **1B**–**5B** were coupled with the monohydroxy DNJ derivative **10** (Scheme 1), which was made from the corresponding 2,3-diol together with the known 3-ester and the 2,3-diester, and which was also used to make compound **6**. For compounds **7** and **8**, selective glycosylation at the primary position of the diol **11** was employed, and the (1→4)-linked glucoside **9** was made using the 2,3,6-tribenzyl ether **12**.[25] The products were purified by chromatography and deprotected by standard deesterification followed by hydrogenolysis, the alternative deprotection sequence of hydrogenolysis followed by deesterification being less successful.

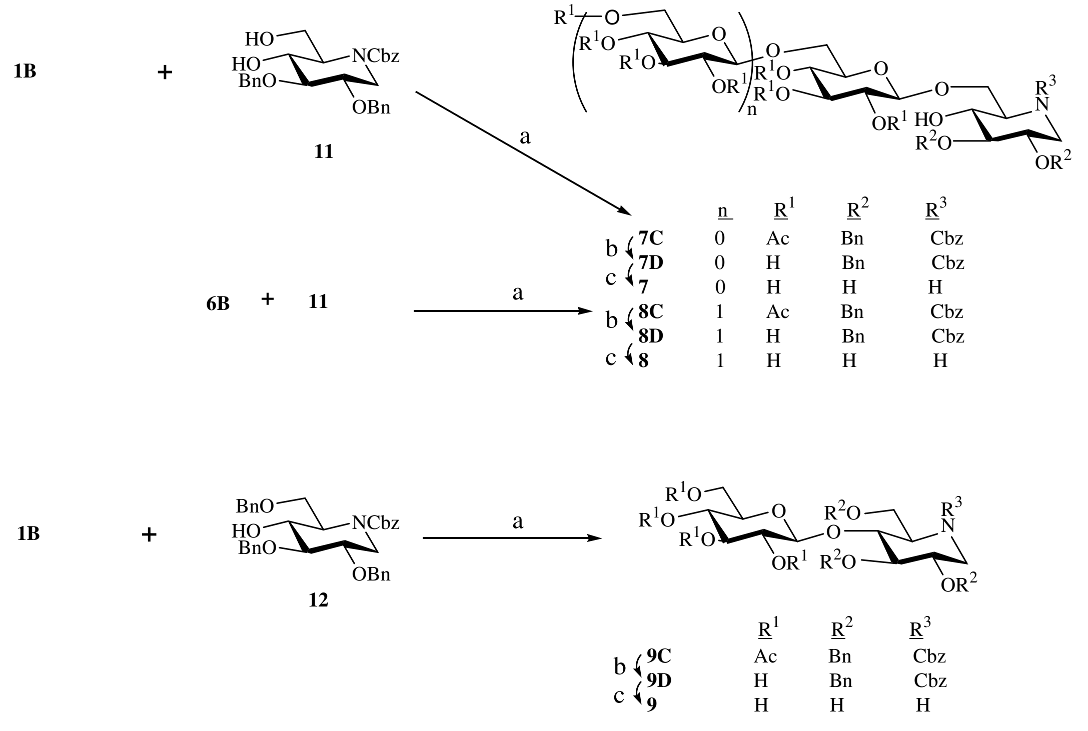
During glycosylation of diol **11** at O-6 with tetra-*O*-acetyl-α,β-D-glucosyl trichloroacetimidate some selective deacetylation of the product occurred to give a 2,3,4-triacetate that afforded the same product (**7D**) on deacetylation as did the tetraacetate (**7C**).

On the basis of the β-configurations assigned by NMR coupling evidence to the glucosides **1**, **7** and **9** ($J_{1,2} = 8.0 \pm 0.1$ Hz), and of expectations founded on many analogous literature precedents,[26] all of the glycosidic bonds formed were assumed also to have this anomeric configuration. The couplings that were observed in the anomeric doublet signals in the ^{1}H NMR spectra concurred, but not all such signals were visible—especially in the spectra of the more complex compounds. Compound **7** has been reported previously,[12] its formation establishing that diol **11** underwent preferential substitution at the primary position in keeping with normal experience.[27] It is assumed that compound **8** was derived similarly.

Although, as already indicated, glycosylated DNJ derivatives can inhibit various enzymes and show a range of bioactivities, it is recognized that their potency against glucosidases from several sources is not high.[10]



Scheme 1. Reagents: (a) CCl_3CN, K_2CO_3; (b) TMSOTf, CH_2Cl_2, molecular sieve; (c) MeONa, MeOH; (d) H_2, Pd/C, H_2O, pressure.



Scheme 2. Reagents: (a) TMSOTf, CH_2Cl_2, molecular sieve; (b) MeONa, MeOH; (c) H_2, Pd/C, H_2O, pressure.

For this reason, and because our primary interests were in the effects of our products on whole organisms, they (except compound **4**) were tested against 20 organisms as follows: **nine fungi** (four plant pathogens, *Botrytis cinerea*, *Colletotrichum acutatum*, *Diplodia pinea*, *Fusarium oxysporum*; three dermatophytes, *Microsporum canis*, *Trichophyton mentagrophytus*, *Trichophyton rubrum*; one yeast *Candida albicans* and one saprophyte, *Rhizopus stolonifer*); **three bacteria** (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*); **three insects** [*Lucilia cuprina* (sheep blowfly), *Heliothis armigera* (tomato fruitworm) and *Spodoptera litura* (tropical armyworm)]; **two algae** (*Chlorella*, *Dunaliella salina*); **one nematode** (*Panagrelius redivivus)*; **one plant cell line** (*BY-2*, tobacco cell line); and **one crustacean** [*Artemia salina* (brine shrimp)].

Compounds were tested at a concentration of 1 mg/mL, and in series of six threefold dilutions. One of the dermatophyte fungi, *Trichophyton rubrum*, was susceptible to compound **1**, but only at the highest concentration, and none of the other test organisms, except the brine shrimp, reacted to any of the compounds at any of the concentrations used. The exceptional shrimp was vulnerable to compounds **1**, **2** and **5**–**9**, the first four at concentrations >1 mg/mL, **5**–**8** at <1 mg > 0.33 mg/mL and the 1,4-linked compound **9** was most potent at < 0.33 mg > 0.11 mg/mL.

3. Experimental

3.1. General methods

All glycosylation reactions were carried out under argon in dry CH_2Cl_2. TLC was performed on Silica Gel HF-254, and column chromatography on Silica Gel 230–400 mesh (Merck). NMR spectra were recorded on solutions in $CDCl_3$, CD_3OD or D_2O (in the last case resonances being referenced to acetone as a secondary standard, δ_H 2.217, δ_C 33.17) with a Bruker Avance NMR spectrometer at 300 MHz (for ^{1}H) or 75 MHz (for ^{13}C). Chemical shifts were recorded in ppm from tetramethylsilane as internal reference. Resonances for ester and aromatic groups were observed as required by the assigned structures but are not recorded. DEPT experiments were conducted to identify the number of hydrogen atoms bonded to carbon atoms; the methylene group resonances are identified in the reported ^{13}C NMR spectral data. High resolution mass spectra (HR-MS) were measured with a VG70-250S double focusing magnetic sector mass spectrometer, a Mariner 8105 electrospray TOF mass spectrometer with ionization effected by use of a caesium ion gun or a Mariner 5158 electrospray TOF instrument. Optical rotations were measured with a Perkin–Elmer 241 automatic polarimeter. Laminaribiose, laminaritetraose, laminarihexaose and laminariheptaose were purchased from Seikagaku Corporation, and gentiobiose was purchased from Sigma Chemical Co.

3.2. 4,6-*O*-Benzylidene-*N*-benzyloxycarbonyl-2-*O*-chloroacetyl-1,5-dideoxy-1,5-imino-D-glucitol (10), 4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-2,3-di-*O*-chloroacetyl-1,5-dideoxy-1,5-imino-D-glucitol and 4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-3-*O*-chloroacetyl-1,5-dideoxy-1,5-imino-D-glucitol

To a suspension of 4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol[25,28] (771 mg, 2.0 mmol) in CH_2Cl_2 (20 mL) at 0 °C were added 2,6-lutidine (2.4 mL, 20 mmol), triethylamine (0.39 mL, 2.8 mmol) and chloroacetic anhydride (430 mg, 2.5 mmol), and the mixture was stirred at 0 °C for 3.5 h, poured into ice-water (30 mL) and extracted with CH_2Cl_2 (3×20 mL). The combined organic extracts were washed with ice-cold HCl (2 M, 40 mL) and water (20 mL), dried (Na_2SO_4) and evaporated to dryness. Column chromatography (CH_2Cl_2/MeOH 300:1→100:1) of the residue gave, in order of elution, the 2,3-di-chloroacetate (165 mg, 15%), the required 2-ester **10** (231 mg, 25%) and the 3-ester (303 mg, 33%).

Compound **10**: mp 50–52 °C, $[\alpha]_D^{20}$ −13.8 (*c* 3.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.54 (s, 1H, CHPh), 5.13, 5.09 (2d, 2H, *J* 12.2 Hz, CH_2Ph), 4.91 (m, 1H, H-2), 4.82 (dd, 1H, $J_{6a,6e}$ 13.3, $J_{5,6e}$ 4.5 Hz, H-6e), 4.21 (dd, 1H, $J_{5,6a}$ 10.7 Hz, H-6a), 4.12 (dd, 1H, $J_{1a,1e}$ 13.9, $J_{1e,2}$ 4.3 Hz, H-1e), 4.03, 3.96 (2d, 2H, *J* 14.9 Hz, CH_2Cl), 3.81 (m, 1H, H-3), 3.70 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.5 Hz, H-4), 3.36 (ddd, 1H, H-5), 3.20 (dd, 1H, $J_{1a,2}$ 8.2 Hz, H-1a); ^{13}C NMR ($CDCl_3$): δ 101.9, 80.0, 73.7, 73.7, 69.4 (CH_2), 67.8 (CH_2), 53.4, 45.2 (CH_2), 40.6 (CH_2). HR-MS/EI *m*/*z* calcd for $C_{23}H_{25}ClNO_7$ $(M+H)^+$: 462.1319, found: 462.1302.

3-Ester: mp 146–148 °C; $[\alpha]_D^{20}$ −7.6 (*c* 1.0, $CHCl_3$), lit.[17] $[\alpha]_D^{20}$ −5.2 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 5.50 (s, 1H, CHPh), 5.12, 5.09 (2d, 2H, *J* 12 Hz, CH_2Ph), 5.03 (dd, 1H, $J_{2,3,}$ 9.2, $J_{3,4}$ 7.7 Hz, H-3), 4.78 (dd, 1H, $J_{6a,6e}$ 10.7, $J_{5,6e}$ 4.4 Hz, H-6e), 4.31 (t, 1H, $J_{5,6a}$ 10.8 Hz, H-6a), 4.16 (dd, 1H, $J_{1a,1e}$ 13.8, $J_{1e,2}$ 4.4 Hz, H-1e), 4.11 (s, 2H, CH_2Cl), 3.8–3.65 (m, 2H, H-2, -4), 3.35 (ddd, 1H, $J_{4,5}$ 10.2 Hz, H-5), 2.98 (dd, 1H, $J_{1a,2}$ 9.2 Hz, H-1a); ^{13}C NMR ($CDCl_3$): δ 101.1, 79.6, 77.5, 69.4 (CH_2), 69.1, 67.8 (CH_2), 54.2, 48.9 (CH_2), 40.8 (CH_2); HR-MS/EI *m*/*z* calcd for $C_{23}H_{25}ClNO_7$ $(M+H)^+$: 462.1319, found: 462.1334.

2,3-Diester: mp 132–135 °C; $[\alpha]_D^{20}$ −22.5 (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.53 (s, 1H, CHPh), 5.25 (dd, 1H, $J_{2,3}$ 7.0, $J_{3,4}$ 9.6 Hz, H-3), 5.17–5.11 (2d, 2H, *J* 12.2 Hz, CH_2Ph), 5.03 (ddd, 1H, $J_{2,1a}$ 8.5, $J_{2,3}$ 7.0, $J_{2,1e}$ 4.3 Hz, H-2), 4.83 (dd, 1H, $J_{6a,6e}$ 11.3, $J_{5,6e}$ 4.5 Hz, H-6e), 4.28 (t, 1H, $J_{5,6a}$ 10 Hz, H-6a), 4.20 (dd, 1H, $J_{1a,1e}$ 14.0 Hz, H-1e), 4.05 (s, 2H, CH_2Cl), 3.99, 3.93 (2d, 2H, *J* 15.0 Hz, CH_2Cl), 3.92 (t, 1H, $J_{4,5}$ 9.6 Hz, H-4), 3.46 (dt, 1H, H-5), 3.30 (dd, 1H, H-1a); ^{13}C NMR ($CDCl_3$): δ 101.45, 77.3, 75.4, 71.4, 69.4 (CH_2), 68.0 (CH_2), 53.8, 45.6 (CH_2), 40.5 (CH_2), 40.4 (CH_2). HR-MS/EI *m*/*z* calcd for $C_{25}H_{26}Cl_2NO_8$ $(M+H)^+$: 538.1035, found: 538.1055.

3.3. 3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-2-*O*-chloroacetyl-1,5-dideoxy-1,5-imino-D-glucitol (1C)

Powdered 4 Å molecular sieves (300 mg) were added to a stirred solution containing compound **10** (88 mg, 0.19 mmol) and 2,3,4,6-tetra-*O*-acetyl-α,β-D-glucopyranosyl trichloroacetimidate **1B**[29,30] [(98 mg, 0.20 mmol), made from tetraacetylglucose **1A**] in dry CH_2Cl_2 (5 mL). The mixture was stirred at room temperature for 30 min, cooled in an ice bath and TMSOTf (30 μL) was added. After 15 min at 0 °C, triethylamine (0.5 mL) was added, and the solvents were evaporated to dryness. Column chromatography (hexane/EtOAc 2:1) of the residue gave the title compound (107 mg, 71%): $[\alpha]_D^{20}$ −34.7 (*c* 0.21, $CHCl_3$), lit.[25] $[\alpha]_D^{20}$ −24 (*c* 0.9, $CHCl_3$); ^1H NMR data in agreement with lit. data;[28] ^{13}C NMR ($CDCl_3$): δ 166.5, 156.0, 101.6, 100.7, 80.25, 79.0, 73.6, 73.2, 72.3, 71.8, 70.1 (CH_2), 68.5, 68.2 (CH_2), 62.1 (CH_2), 52.9, 44.8 (CH_2), 40.8 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{37}H_{41}ClNO_{16}$ $(M-H)^+$: 790.2114, found: 790.2133.

3.4. 4,6-*O*-Benzylidene-*N*-benzyloxycarbonyl-1,5-dideoxy-3-*O*-β-D-glucopyranosyl-1,5-imino-D-glucitol (1D)

Sodium methoxide in MeOH (0.1 mL containing 1.5 mg of the alkoxide) was added to a solution of compound **1C** (105 mg, 0.13 mmol) in MeOH (5 mL) and the mixture was stirred at 20 °C overnight. The solvent was removed to afford the title pentaol as a white solid (70 mg, 100%): mp 155–157 °C; $[\alpha]_D^{20}$ +1.8 (*c* 0.36, $CHCl_3$/MeOH 1:1), lit.[25] $[\alpha]_D^{20}$ +0.2 (*c* 1.3, $CHCl_3$/MeOH 1:1); ^1H NMR data in agreement with lit. data;[25] ^{13}C NMR ($CDCl_3$/CD_3OD 1:1): δ 154.8, 103.1, 100.6, 84.4, 78.3, 75.9, 75.8, 73.6, 69.4, 68.8, 68.8 (CH_2), 67.1 (CH_2), 61.0 (CH_2), 54.3, 48.3 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{27}H_{34}NO_{11}$ $(M+H)^+$: 548.2132, found: 548.2125.

3.5. 1,5-Dideoxy-3-*O*-β-D-glucopyranosyl-1,5-imino-D-glucitol (1)

Pd/C (100 mg, 10%) was added to a solution of compound **1D** (59 mg, 0.11 mmol) in water (3 mL) and hydrogenolysis was carried out under 55 psi for 3 days. The catalyst was filtered off, and the solution was freeze dried to afford compound **1** (32 mg, 91%): $[\alpha]_D^{20}$ +12.0 (*c* 0.2, H_2O); lit.[12] $[\alpha]_D^{20}$ +18.1 (*c* 0.74, H_2O); The ^1H NMR (D_2O) spectral data were identical to lit. data;[25] ^{13}C NMR (D_2O) all 12 resonances had δ values within the range -2.7 ± 0.3 ppm with respect to lit. values.[12] HR-MS/FAB *m*/*z* calcd for $C_{12}H_{24}NO_9$ $(M+H)^+$: 326.1451, found: 326.1442.

3.6. 2,4,6-Tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α,β-D-glucopyranose (2A)

Laminaribiose (200 mg, 0.585 mmol) was peracetylated under standard conditions (pyridine, acetic anhydride, DMAP as catalyst) and after 24 h the solvents were evaporated to dryness. The crude peracetates were dissolved in DMF (5 mL), hydrazinium acetate (1.3 mol equiv)[23] was added and the mixture was stirred at 20 °C for 3 h. Ethyl acetate (50 mL) was added, the organic layer was washed with $NaHCO_3$ (10 mL, satd aq) and water (10 mL), dried (Na_2SO_4) and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc 1:2) to give the title heptaacetate (310 mg, 84%, mainly α-anomer): ^{13}C NMR ($CDCl_3$): δ 101.1, 90.6, 76.2, 73.4, 73.1, 72.1, 71.75, 68.5, 68.4, 67.9, 62.5 (CH_2), 62.2 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{26}H_{35}O_{17}$ $(M-OH)^+$: 619.1874, found: 619.1857; calcd for $C_{26}H_{36}O_{18}Na$ $(M+Na)^+$: 659.1800, found: 659.1753.

3.7. 2,4,6-Tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α,β-D-glucopyranosyl trichloroacetimidate (2B)

Anhydrous potassium carbonate (140 mg, 1 mmol) and trichloroacetonitrile (0.5 mL) were added to a solution of laminaribiose heptaacetate (**2A**, 141 mg, 0.22 mmol) in CH_2Cl_2 (5 mL), the mixture was stirred at room temperature overnight and solvents were evaporated to dryness. Column chromatography (hexane/EtOAc 1:2) of the residue gave the title trichloroacetimidates (175 mg, 100%) as a mixture of anomers (α,β 1:1.15): ^1H NMR ($CDCl_3$): δ 8.70, 8.68 (2s, 2 × 0.5H, NH), 6.46 (d, 0.5H, *J* 3.6 Hz, H-1α), 5.74 (d, 0.5H, *J* 8.3 Hz, H-1β); ^{13}C NMR ($CDCl_3$): δ 161.3 (β), 160.65 (α), 101.0 (α,β), 95.6 (β), 93.2 (α), 78.7 (β), 72.9 (α), 72.8 (β), 71.8 (α), 71.6 (β), 71.5 (α), 71.15 (β), 70.3 (α), 68.2 (α), 68.1 (β), 67.9 (β), 67.3 (α), 61.8 (CH_2), 61.6 (CH_2), 60.4 (β). α,β-Assignments were made by use of the spectrum of the pure α-compound.[31] HR-MS/FAB *m*/*z* calcd for $C_{28}H_{36}Cl_3NO_{18}Cs$ $(M+Cs)^+$: 912.0052, found: 912.0016.

3.8. 3-*O*-[2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-2-*O*-chloroacetyl-1,5-dideoxy-1,5-imino-D-glucitol (2C)

Compound **10** (120 mg, 0.260 mmol) was glycosylated with the trichloroacetimidate **2B** (192 mg) as in the preparation of compound **1C**. Purification by flash chromatography (hexane/EtOAc 1:1) gave the title compound (133 mg, 50%): $[\alpha]_D^{20}$ −38.2 (*c* 0.28, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.66 (d, 1H, *J* 8.1 Hz, H-1′ or H-1″), 4.55 (d, 1H, *J* 8.1 Hz, H-1′ or H-1″); ^{13}C NMR ($CDCl_3$): δ 166.5, 156.3; 101.7, 101.3, 100.6, 80.0, 79.2, 79.0, 73.9, 73.3, 72.2, 72.1, 71.4, 70.2 (CH_2), 68.45, 68.3, 68.2 (CH_2), 62.3 (CH_2), 62.1(CH_2), 52.0, 44.2 (CH_2), 40.8 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{49}H_{58}ClNO_{24}Na$ $(M+Na)^+$: 1102.2935, found: 1102.2824.

3.9. 4,6-*O*-Benzylidene-*N*-benzyloxycarbonyl-1,5-dideoxy-3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-1,5-imino-D-glucitol (2D)

The fully protected laminaribiosyl compound **2C** (128 mg, 0.116 mmol) was deacylated in the usual way to give the title compound (86 mg, 100%): $[\alpha]_D^{20}$ −13.0 (*c* 0.2, MeOH); ^1H NMR (CD_3OD): δ 4.63 (d, 1H, *J* 7.7 Hz, H-1′ or H-1″), 4.55 (d, 1H, *J* 7.7 Hz, H-1′ or H-1″); ^{13}C NMR (CD_3OD): δ 156.0, 105.8, 104.8, 102.8, 88.6, 86.1, 80.6, 78.6, 78.4, 78.1, 76.0, 75.6, 72.0, 71.4, 71.0, 70.2, 68.9, 63.1, 62.9, 55.6; HR-MS/FAB *m*/*z* calcd for $C_{33}H_{44}NO_{16}$ $(M+H)^+$: 710.2660, found: 710.2677.

3.10. 1,5-Dideoxy-3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-1,5-imino-D-glucitol (2)

Compound **2D** (86 mg, 0.121 mmol) was hydrogenolyzed as for the preparation of compound **1** and gave the title compound (44 mg, 75%): $[\alpha]_D^{20}$ +1.0 (*c* 0.29, H_2O); ^{13}C NMR (D_2O): δ 103.2, 103.1, 87.3, 84.8, 76.4, 76.05, 76.0, 73.9, 73.7, 70.2, 70.0, 69.6, 68.5, 61.1 (CH_2), 61.1 (CH_2), 60.9 (CH_2), 60.65, 48.4 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{18}H_{34}NO_{14}$ $(M+H)^+$: 488.1979, found: 488.1997.

3.11. 2,4,6-Tri-*O*-acetyl-3-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-α,β-D-glucopyranose (3A)

Laminaritetraose was peracetylated and the ester (460 mg, 0.380) specifically deacetylated at C-1 as described for laminaribiose peracetate. Chromatographic purification (hexane/EtOAc 1:5) gave the title compound (395 mg, 91%, mainly α-anomer): ^1H NMR ($CDCl_3$): δ 4.54 (d, 1H, *J* 8.1 Hz, H-1′ or H-1″ or H-1‴β), 4.50 (d, *J* 8.0 Hz, H-1′ or H-1″ or H-1‴), 4.38 (d, 1H, *J* 8.1 Hz, H-1′ or H-1″ or H-1‴); ^{13}C NMR ($CDCl_3$): δ 101.1, 100.8, 100.5, 90.1, 78.9, 78.5, 75.4, 73.2, 72.9, 72.6, 71.65, 70.9, 68.3, 68.1, 67.45, 62.2 (CH_2), 62.2 (CH_2), 62.0 (CH_2), 61.7 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{50}H_{68}O_{34}Cs$ $(M+Cs)^+$: 1345.2646, found: 1345.2556.

3.12. 2,4,6-Tri-*O*-acetyl-3-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-α-D-glucopyranosyl trichloroacetimidate and 2,4,6-tri-*O*-acetyl-3-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-β-D-glucopyranosyl trichloroacetimidate (3B)

The 1-hydroxylaminaritetraose derivative **3A** (285 mg) was converted to the mixed trichloroacetimidates as for the laminaribiose heptaacetate. Purification by column chromatography (hexane/EtOAc 1:4) gave the title compounds (279 mg, 87%) as a 1:1 mixture of anomers which were separated by further chromatography:

α-Anomer: ^1H NMR ($CDCl_3$): δ 8.73 (s, 1H, NH), 6.45 (d, 1H, *J* 3.7 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 160.9, 101.4, 101.1, 101.0, 93.45, 79.2, 78.6, 76.1, 73.5, 73.2, 72.9, 72.2, 72.1, 72.0, 71.2, 70.65, 68.8, 68.6, 68.4, 67.6, 62.5 (CH_2), 62.3 (CH_2), 62.0 (CH_2), 62.0 (CH_2), 60.7.

β-Anomer: ^1H NMR ($CDCl_3$): δ 8.72 (s, 1H, NH), 5.75 (d, 1H, *J* 8.2 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 161.4, 101.4, 101.1, 100.9, 95.85, 78.6, 78.35, 73.2, 73.15, 72.8, 72.3, 72.2, 72.0, 71.2, 68.7, 68.6, 68.4, 68.2, 62.45 (CH_2), 62.3 (CH_2), 62.15 (CH_2), 62.05 (CH_2), 60.7; HR-MS/FAB *m*/*z* calcd for $C_{52}H_{68}Cl_3NO_{34}Cs$ $(M+Cs)^+$: 1488.1743, found: 1488.1748.

3.13. 3-*O*-[2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-2-*O*-chloroacetyl-1,5-dideoxy-1,5-imino-D-glucitol (3C)

Compound **10** (62 mg, 0.134 mmol) was glycosylated with the trichloroacetimidates **3B** (192 mg) derived from laminaritetraose as in the preparation of compound **1C**. Chromatographic purification (hexane/EtOAc 1:2) gave the title compound (104 mg, 47%): $[\alpha]_D^{20}$ −36.1 (*c* 0.59, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 166.4, 156.2, 101.6, 101.3, 101.0, 100.8, 100.5, 80.0, 79.2, 78.9, 78.6, 78.4, 74.3, 73.9, 73.5, 73.15, 72.8, 72.2, 71.9, 71.1, 70.1 (CH_2), 68.6, 68.5, 68.3, 68.1 (CH_2), 62.2 (CH_2), 62.2 (CH_2), 62.0 (CH_2), 62.0 (CH_2), 52.0, 44.1 (CH_2), 40.7 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{73}H_{91}ClNO_{40}$ $(M+H)^+$: 1656.4806, found: 1656.4718.

3.14. 4,6-*O*-Benzylidene-*N*-benzyloxycarbonyl-1,5-dideoxy-3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-1,5-imino-D-glucitol (3D)

The laminaritetraose compound **3C** (115 mg, 0.175 mmol) was deacylated in the usual way to give the title compound (73 mg, 100%): $[\alpha]_D^{20}$ −12.0 (*c* 0.05, DMSO);

^{13}C NMR (DMSO-d_6): δ 156.0, 104.2, 103.5, 103.4, 102.4, 100.5, 87.4, 87.1, 86.5, 83.3, 78.55, 77.3, 76.9, 76.7, 76.4, 74.2, 73.2, 73.1, 70.4, 69.4, 69.2 (CH$_2$), 68.8, 68.3, 66.9 (CH$_2$), 61.4 (CH$_2$), 61.2 (CH$_2$), 61.2 (CH$_2$), 60.9 (CH$_2$), 52.3, 47.3 (CH$_2$); HR-MS/FAB *m/z* calcd for $C_{45}H_{64}NO_{26}$ (M+H)$^+$: 1034.3717, found: 1034.3694.

3.15. 1,5-Dideoxy-3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-1,5-imino-D-glucitol (3)

Compound **3D** (46 mg, 0.044 mmol) was hydrogenolyzed as for the preparation of compound **1** to give the title compound (25 mg, 69%): $[\alpha]_D^{20}$ −7.4 (*c* 0.27, H$_2$O); ^{13}C NMR (D$_2$O): δ 103.2, 103.1, 103.0, 103.0, 88.0, 84.75, 76.4, 76.1, 73.9, 73.7, 71.0, 70.4, 70.0, 68.5, 61.7 (CH$_2$), 61.1 (CH$_2$), 61.1 (CH$_2$), 61.1 (CH$_2$), 61.1 (CH$_2$), 60.8, 49.0 (CH$_2$); HR-MS/ESI *m/z* calcd for $C_{30}H_{54}NO_{24}$ (M+H)$^+$: 812.3036, found: 812.3015.

3.16. 2,4,6-Tri-*O*-acetyl-3-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-*O*-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-*O*-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-*O*-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-*O*-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-α,β-D-glucopyranose (4A)

Laminarihexaose was acetylated and the perester (366 mg, 0.220 mmol) specifically deacetylated at C-1 as described for laminaribiose peracetate. Chromatographic purification of the product (hexane/EtOAc/MeOH 1:3:0.5) gave the title compound (360 mg, 99%, mainly α-anomer): ^{13}C NMR (CDCl$_3$): δ 101.4, 101.0, 90.4, 79.3, 78.6, 73.6, 73.3, 72.1, 71.25, 68.6, 68.45, 67.55, 62.4, 62.0 (CH$_2$). HR-MS/ESI *m/z* calcd for $C_{74}H_{100}O_{50}Cs$ (M+Cs)$^+$: 1921.4337, found: 1921.4499.

3.17. 2,4,6-Tri-*O*-acetyl-3-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-*O*-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-*O*-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-*O*-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-*O*-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-α,β-D-glucopyranosyl trichloroacetimidate (4B)

The 1-hydroxylaminarihexaose peracetate **4A** (308 mg, 0.185 mmol) was converted to the mixed trichloroacetimidates as for laminaribiose heptaacetate. Chromatographic purification (hexane/EtOAc/MeOH 1:3:0.2) gave the title compounds (290 mg, 87%): ^{1}H NMR (CDCl$_3$): δ 8.7 (s, 1H, NH), 6.45 (d, 0.5H, $J_{1,2}$ 3.7 Hz, H-1α), 5.75 (d, 0.5H, $J_{1,2}$ 8.2 Hz, H-1β), 5.30 (s, 2 H); ^{13}C NMR (CDCl$_3$): δ 101.1, 100.7, 95.5, 93.1, 79.0, 78.2, 73.3, 72.9, 72.7, 72.5, 71.7, 70.9, 70.3, 68.45; 68.1, 67.2, 62.1, 61.7 (CH$_2$), 60.4; HR-MS/FAB *m/z* calcd for $C_{76}H_{100}Cl_3NO_{50}Cs$ (M+Cs)$^+$: 2064.3433, found: 2064.3605.

3.18. 3-*O*-[2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-2-*O*-chloroacetyl-1,5-dideoxy-1,5-imino-D-glucitol (4C)

Compound **10** (36 mg, 0.078 mmol) was glycosylated with the trichloroacetimidate **4B** as for the preparation of compound **1C**. Chromatographic purification (hexane/EtOAc/MeOH 1:3:0.2) gave the title compound **4C** (92 mg, 52%): $[\alpha]_D^{20}$ −33.5 (*c* 0.23, CHCl$_3$); ^{13}C NMR (CDCl$_3$): δ 101.6, 101.3, 101.1, 100.9, 100.8, 100.4; HR-MS/FAB *m/z* calcd for $C_{97}H_{122}ClNO_{56}Cs$ (M+Cs)$^+$: 2364.5472, found: 2364.5339.

3.19. 4,6-*O*-Benzylidene-*N*-benzyloxycarbonyl-1,5-dideoxy-3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-1,5-imino-D-glucitol (4D)

The above laminarihexaose derivative **4C** (61 mg, 0.026 mmol) was deacylated in the usual manner to give the title compound **4D** (37 mg, 100%): $[\alpha]_D^{20}$ −18.0 (*c* 0.1, H$_2$O); HR-MS/FAB *m/z* calcd for $C_{57}H_{84}NO_{36}$ (M+H)$^+$: 1358.4773, found: 1358.4729.

3.20. 1,5-Dideoxy-3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-1,5-imino-D-glucitol (4)

Compound **4D** (27 mg, 0.020 mmol) was hydrogenolyzed as for the preparation of glycoside **1** to give the deprotected compound **4** (18 mg, 78%): $[\alpha]_D^{20}$ +15.2 (*c* 0.33, H$_2$O); ^{13}C NMR (D$_2$O): δ 103.2, 102.95, 84.6, 76.4, 76.05, 73.9, 73.7, 70.0, 68.5, 68.1, 67.2, 66.7, 61.1 (CH$_2$), 60.2, 58.0, 46.2 (CH$_2$); HR-MS/FAB *m/z* calcd for $C_{42}H_{74}NO_{34}$ (M+H)$^+$: 1136.4092, found: 1136.4144.

3.21. 2,4,6-Tri-*O*-acetyl-3-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-α,β-D-glucopyranose (5A)

Laminariheptaose was acetylated and the perester (184 mg, 0.082 mmol) specifically deacetylated at C-1 as described for laminaribiose peracetate. Chromatographic purification of the product (hexane/EtOAc/MeOH 2:6:1) gave the title compound (148 mg, 82%, mainly α-anomer): ^{13}C NMR (CDCl$_3$): δ 101.1, 101.0, 100.8, 90.2, 79.3, 78.9, 78.7, 76.0, 73.7, 73.2, 72.9, 72.6, 71.9, 71.2, 68.45, 67.2, 62.63 (CH_2), 62.28, (CH_2), 61.93 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{86}H_{116}O_{58}Cs$ $(M+Cs)^+$: 2209.5182, found: 2209.5078.

3.22. 2,4,6-Tri-*O*-acetyl-3-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-α,β-D-glucopyranosyl trichloroacetimidate (5B)

The 1-hydroxylaminariheptaose peracetate **5A** (120 mg, 0.054 mmol) was converted to the mixed trichloroacetimidates as for laminaribiose heptaacetate. Chromatographic purification (hexane/EtOAc/MeOH 5:15:1) gave the title compounds (106 mg, 83%): ^{1}H NMR ($CDCl_3$): δ 8.68 (s, 1H, NH), 6.45 (d, 0.5 H, *J* 3.6 Hz, H-1α), 5.75 (d, 0.5H, *J* 8.4 Hz, H-1β); ^{13}C NMR ($CDCl_3$): δ 161.35, 160.9, 101.4, 101.2, 101.0, 95.8, 93.4, 79.3, 78.7, 77.9, 76.2, 73.5, 73.25, 73.1, 72.75, 72.1, 71.2, 70.6, 68.6, 68.4, 62.4 (CH_2), 62.0 (CH_2), 60.7 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{88}H_{116}Cl_3NO_{58}Cs$ $(M+Cs)^+$: 2352.4278, found: 2352.4318.

3.23. 3-*O*-[2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-2-*O*-chloroacetyl-1,5-dideoxy-1,5-imino-D-glucitol (5C)

Compound **10** (28 mg, 0.061 mmol) was glycosylated with the trichloroacetimidates **5B** (112 mg) as in the preparation of compound **1C**. Chromatographic purification (hexane/EtOAc/MeOH 5:15:1) gave the title compound (58 mg, 45%): $[\alpha]_D^{20}$ −42.2 (*c* 0.23, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 166.1, 155.9, 101.3, 101.0, 100.6, 100.2, 79.65, 78.9, 78.6, 78.2, 73.6, 72.9, 72.7, 72.4, 71.7, 70.8, 69.8 (CH_2), 68.2, 68.0, 67.8 (CH_2), 62.0 ($3\times CH_2$), 61.6 (CH_2), 60.3 (CH_2), 43.8 (CH_2), 40.4 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{109}H_{138}ClNO_{64}Cs$ $(M+Cs)^+$: 2652.6317, found: 2652.6380.

3.24. 4,6-*O*-Benzylidene-*N*-benzyloxycarbonyl-1,5-dideoxy-3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-1,5-imino-D-glucitol (5D)

The above peracylated laminariheptaose derivative **5C** (32 mg, 0.012 mmol) was deacylated in the usual way to give the title compound (20 mg, 100%): $[\alpha]_D^{20}$ −18.3 (*c* 0.23, H_2O); ^{13}C NMR (D_2O): δ 157.0, 103.3, 103.1, 101.85, 84.8, 78.4, 76.45, 76.1, 73.9, 73.8, 70.0, 69.3 (CH_2), 68.55, 61.1 (CH_2), 53.4, 49.3 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{63}H_{94}NO_{41}$ $(M+H)^+$: 1520.5301, found: 1520.5190.

3.25. 1,5-Dideoxy-3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-1,5-imino-D-glucitol (5)

Compound **5D** (35 mg, 0.023 mmol) was hydrogenolyzed as for the preparation of compound **1** to give the title compound (17 mg, 57%): $[\alpha]_D^{20}$ +2.5 (*c* 0.28, H_2O); ^{13}C NMR (D_2O): δ 103.2, 103.0, 84.6, 76.5, 76.1, 73.9, 73.7, 70.0, 68.55, 68.1, 67.3, 66.7, 61.1 (CH_2), 60.3, 58.0 (CH_2), 46.2 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{48}H_{84}NO_{39}$ $(M+H)^+$: 1298.4620, found: 1298.4684.

3.26. 2,3,4-Tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranosyl trichloroacetimidate (6Bα) and 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl trichloroacetimidate (6Bβ)

To a stirred solution of gentiobiose 2,2′,3,3′,4,4′,6′-hepta-*O*-acetate (**6A**)[23] (1.50 g, 2.37 mmol) in dry CH_2Cl_2 (15 mL) at 0 °C under Ar, trichloroacetonitrile (1.5 mL, 15 mmol) was added, followed after 5 min by sodium hydride (60%, 250 mg, 3.6 mmol). After 15 min at 0 °C, the reaction mixture was filtered through Celite and applied to a column of silica gel (hexane/EtOAc 1:1) to give the α-trichloroacetimidate as a foam (1.41 g, 76%) which crystallized from hexane: mp 156–158 °C; $[\alpha]_D^{20}$ +51.6 (*c* 1.82, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 8.64 (s, 1H, NH), 6.54 (d, 1H, *J* 3.6 Hz, H-1), 5.55 (t, 1H, *J* 9.8 Hz), 5.18 (t, 1H, *J* 9.4 Hz), 5.0–5.1 (m, 3H, H-2, -4, -4′), 4.90 (dd, 1H, *J* 9.2, 8.0, Hz, H-2′), 4.56 (d, 1H, *J* 7.9 Hz, H-1′), 4.27 (dd, 1H, *J* 12.4, 4.5 Hz, H-6), 4.17 (ddd, 1H, *J* 10.5, 5.5, 2.0 Hz, H-5′), 4.12 (dd, 1H, *J* 12.4, 2.4 Hz, H-6), 3.96 (dd, 1H, *J* 11.3, 2.1 Hz, H-6′), 3.65 (ddd, 1H, *J* 9.9, 4.5, 2.4 Hz, H-5), 3.58 (dd, 1H, *J* 11.3, 5.4 Hz, H-6′); ^{13}C NMR ($CDCl_3$): δ 160.8, 100.5, 92.8, 72.8, 71.9, 71.2, 71.0, 69.9, 69.8, 68.3, 68.3, 67.3 (CH_2), 61.8 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{28}H_{36}Cl_3NO_{18}$ $(M-H)^+$: 778.0919, found: 778.0878.

This was followed by the β-isomer (0.33 g, 18%): mp 180–181 °C (from hexane/EtOAc); $[\alpha]_D^{20}$ −7.3 (*c* 1.50, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 8.81 (s, 1H, NH), 5.87 (d, *J* 7.3 Hz, H-1), 5.28, 5.24 and 5.02 (3t, 3H, *J* ~ 9.5 Hz), 5.15, 5.06 and 4.97 (3t, 3H, *J* ~ 9.5 Hz), 4.63 (d, 1H, *J* 8 Hz, 1H′), 4.26 (dd, 1H, *J* 12.4, 7.4 Hz, H-6), 4.11 (dd, 1H, *J* 12.4, 2.4 Hz, H-6), 3.87 (dd, 1H, *J* 12.2, 2.0 Hz, H-6′), 3.85 (m, 1H, H-5), 3.70 (dd, 1H, *J* 12.2, 7.1 Hz, H-6′), 3.63 (m, 1H, H-5′); ^{13}C NMR ($CDCl_3$): δ 160.8, 100.4, 95.4, 74.5, 72.8, 72.6 72.0, 71.0, 70.2, 68.6, 68.3, 67.6 (CH_2), 61.8 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{28}H_{36}Cl_3NO_{18}$ $(M-H)^+$: 778.0919, found: 778.0897.

3.27. 3-*O*-[2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl]-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-2-*O*-chloroacetyl-1,5-dideoxy-1,5-imino-D-glucitol (6C)

Compound **10** (138 mg, 0.300 mmol) was glycosylated with the above gentiobiose-derived α-trichloroacetimidate (**6Bα**) (240 mg, 0.310 mmol) as in the preparation of compound **1C** to give the title compound (147 mg, 44%): $[\alpha]_D$ −11.4 (*c* 0.7, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 166.1, 155.1, 100.9, 100.3, 100.3, 78.7, 78.6, 75.7, 73.2, 73.1, 72.2, 72.1, 71.0, 69.8, 69.1 (CH_2), 68.7, 68.0 (CH_2), 67.9 (CH_2), 67.5, 61.15 (CH_2), 54.55; 46.3 (CH_2), 40.7 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{49}H_{58}ClNO_{24}Na$ $(M+Na)^+$: 1102.2935, found: 1102.2934.

3.28. 4,6-*O*-Benzylidene-*N*-benzyloxycarbonyl-1,5-dideoxy-3-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-1,5-imino-D-glucitol (6D)

The above fully protected gentiobiose DNJ derivative (**6C**) (137 mg, 0.124 mmol) was deacylated in the usual way to give the title compound (87 mg, 97%): $[\alpha]_D^{20}$ −26.7 (*c* 0.15, MeOH/H_2O 3:1); ^1H NMR (CD_3OD/D_2O 6:1): δ 5.66 (s, 1H, CHPh), 5.14 (s, 2H, CH_2Ph), 4.63 (d, 1H, *J* 7.5 Hz, H-1 or H-1′), 4.48 (d, 1H, *J* 7.8 Hz, H-1 or H-1′); ^{13}C NMR (CD_3OD/D_2O 6:1): δ 157.7, 105.0, 104.2, 102.8, 84.5, 80.5, 78.1, 78.0, 77.9, 77.45, 75.8, 75.4, 71.7, 71.6, 71.2, 71.0 (CH_2), 69.95 (CH_2), 69.2 (CH_2), 62.8 (CH_2), 55.4, 49.7 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{33}H_{43}NO_{16}$ $(M+H)^+$: 710.2660, found: 710.2677.

3.29. 1,5-Dideoxy-3-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-1,5-imino-D-glucitol (6)

The above gentiobiose-DNJ octaol **6D** (72 mg, 0.101 mmol) was hydrogenolyzed as for the preparation of compound **1** to give the title compound (37 mg, 73%): $[\alpha]_D^{20}$ −2.2 (*c* 0.56, H_2O); ^1H NMR (D_2O): δ 4.49 (d, 1H, *J* 7.8 Hz, H-1′ or H-1″), 4.19 (dd, 1H, *J* 11.4, 1.7 Hz);^{13}C NMR (D_2O): δ 103.5, 103.1, 87.75, 76.3, 76.0, 75.9, 75.25, 73.8, 73.6, 70.0, 69.9, 69.5, 69.35, 69.2 (CH_2), 61.1 (CH_2), 60.5, (CH_2), 60.5, 48.0 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{18}H_{34}NO_{14}$ $(M+H)^+$: 488.1979, found: 488.1995.

3.30. 2,3-Di-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (11)

A suspension of 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol[25] (7.35 g, 13.0 mmol) in aqueous acetic acid (80%, 200 mL) was stirred at 25–30 °C for 48 h. The solvents were evaporated and column chromatography (hexane/EtOAc 1:1) of the residue gave the title compound **11** (5.58 g, 90%) as an oil: $[\alpha]_D^{20}$ +21.9 (*c* 1.1, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 157.0, 75.35, 73.8, 72.8 (CH_2), 71.1 (CH_2), 67.55 (CH_2), 67.4, 61.3 (CH_2), 60.3, 38.5 (CH_2). Anal. Calcd for $C_{28}H_{31}NO_6$: C, 70.42; H, 6.54; N, 2.93. Found: C, 70.19; H, 6.32; N, 3.03.

3.31. 6-*O*-[2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl]-2,3-di-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (7C) and 6-*O*-[2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl]-2,3-di-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol

The dibenzyl derivative **11** (0.87 g, 1.82 mmol) was glycosylated with tetra-*O*-acetyl-α,β-D-glucopyranosyl trichloroacetimidates (**1B**) (0.50 g, 1.02 mmol) as described for the preparation of compound **1C**. The crude product was fractionated by flash chromatography (hexane/EtOAc 2:1→1:2) to give, in order of elution, the unreacted acceptor **11** (420 mg, 48%) and the tetraacetylated title glycoside **7C** (250 mg, 35%) as a foam: $[\alpha]_D^{20}$ −3.5 (*c* 0.68, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 4.8–5.1 (m, 5H), 4.3–4.7 (m, 6H), 4.15 (dd, 1H, *J* 12.3, 4.3 Hz), 4.0–4.15 (m, 3H), 3.4–3.8 (m, 5H), 3.08 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 155.6, 99.9, 73.8, 72.9, 71.8, 71.3 (CH_2), 70.8, 70.1, 70.0 (CH_2), 67.3, 66.5 (CH_2), 65.8 (CH_2), 64.5, 60.8 (CH_2), 56.4, 36.1 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{42}H_{50}NO_{15}$ $(M+H)^+$: 808.3180, found: 808.3187.

This was followed by the title triacetate (108 mg, 14%): $[\alpha]_D^{20}$ +54 (*c* 0.62, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 4.8–5.2 (m, 5H), 4.3–4.7 (m, 6H), 4.21 (dd, 1H, *J* 12.2, 3.9 Hz), 3.9–4.2 (m, 3H), 3.4–3.9 (m, 5H), 3.15 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 156.3, 102.6, 73.7, 73.1, 72.8, 72.3, 71.4 (CH_2), 70.9, 70.0 (CH_2), 69.5 (CH_2), 67.4, 67.4 (CH_2), 65.6, 61.0 (CH_2), 57.1, 36.5 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{40}H_{48}NO_{14}$ $(M+H)^+$: 766.3075, found: 766.3148.

3.32. 2,3-Di-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-6-*O*-β-D-glucopyranosyl-1,5-imino-D-glucitol (7D)

Normal deacetylation of the 6-linked tetraacetate (**7C**) and the 2,3,4-triacetate, followed by purification of the crude products by flash chromatography (CH_2Cl_2/EtOAc/MeOH 6:6:1) gave the title compound as a foam (91%, combined yield): $[\alpha]_D^{20}$ +5.5 (*c* 1.31, CH_2Cl_2); ^1H NMR (CD_3OD): δ 5.15, 5.10 (2d, 2H, *J* 12.2 Hz, CH_2Ph), 4.68, 4.59 (2d, 2H, *J* 11.7 Hz, CH_2Ph), 4.59, 4.40 (2d, 2H, *J* 11.6 Hz, CH_2Ph), 4.2–4.3 (m, 3H), 4.13 (dd, 1H, *J* 10.6, 5.0 Hz), 4.04 (t, 1H, *J* 5.5 Hz), 3.91 (dd, 1H, *J* 11.9, 5.5 Hz), 3.82 (dd, 1H, *J* 11.9, 2.1 Hz), 3.55–3.7 (m, 3H), 3.1–3.4 (m, 5H); ^{13}C NMR (CD_3OD):

δ 158.6, 104.8, 80.3, 78.1, 78.0, 78.0, 75.1, 73.7 (CH_2), 71.9 (CH_2), 71.5, 68.6 (CH_2), 68.3 (CH_2), 67.8, 62.8 (CH_2), 59.6, 41.0 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{34}H_{42}NO_{11}$ $(M+H)^+$: 640.2758, found: 640.2772.

3.33. 1,5-Dideoxy-6-*O*-β-D-glucopyranosyl-1,5-imino-D-glucitol (7)

The above pentaol (**7D**) (72.5 mg, 0.113 mmol) was hydrogenolyzed in MeOH (6 mL) and AcOH (1.5 mL) in the presence of Pd/C catalyst (50 mg, 10%) at 50 psi for 16 h, and the pale yellow syrup obtained after removal of the solids and solvents was purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH 2:4:1) to give compound **7** (30 mg, 83%): $[\alpha]_D^{20}$ +8.1 (*c* 0.34, H_2O); the ^1H NMR spectrum (D_2O) was identical to one previously published[12] except that an additional proton resonance (as required by the assigned structure) was observed at δ 3.26–3.40; ^{13}C NMR (D_2O), all δ values were within 0.1 of literature values;[25] HR-MS/FAB *m*/*z* calcd for $C_{12}H_{24}NO_9$ $(M+H)^+$: 326.1451, found: 326.1459.

3.34. 1,5-Dideoxy-6-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-1,5-imino-D-glucitol (8)

Diol **11** (300 mg, 0.63 mmol) was glycosylated with the gentiobiosyl α-trichloroacetimidate (**6B**) (96 mg, 0.12 mmol) as described for the preparation of compound **1C**. The crude product was fractionated by flash chromatography (hexane/EtOAc 1:1→0:1). The main fraction (325 mg), consisting of an inseparable mixture of glycosylation product (**8C**) and unreacted acceptor **11**, was dissolved in MeOH (15 mL) and treated with methanolic MeONa at pH 11 for 18 h. Removal of the solvent and purification of the oily residue by flash chromatography (CH_2Cl_2/EtOAc/MeOH 3.5:3.5:1) gave 2,3-di-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-6-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-1,5-imino-D-glucitol (**8D**) as a foam (58 mg, 59%): $[\alpha]_D^{20}$ −11.1 (*c* 1.8, CH_2Cl_2); ^{13}C NMR (CD_3OD): δ 159.2, 105.9, 80.9, 79.0, 78.0, 76.1, 74.6 (CH_2), 72.9, 72.6 (CH_2), 72.5 (CH_2), 70.8 (CH_2), 70.8 (CH_2), 69.6, 69.6, 68.6, 63.8 (CH_2), 60.6, 41.8 (CH_2). This octaol (50 mg) was hydrogenolyzed as described for compound **7D** in MeOH (6 mL) and AcOH (1.5 mL) in the presence of Pd/C catalyst (40 mg, 10% for 16 h), and the pale yellow syrup obtained after removal of the solids and solvent was purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH 2:4:1) to give the title compound **8** (29 mg, 95%) as a foam: $[\alpha]_D^{20}$ −7.3 (*c* 0.85, H_2O); ^1H NMR (D_2O): δ 4.49 (d, 1H, *J* 7.8 Hz), 4.41 (d, 1H, *J* 7.7 Hz, 1H), 4.21 (dd, 1H, *J* 2.8, 11.9 Hz), 4.19 (dd, 1H, *J* 1.6, 11.7 Hz), 4.02 (dd, 1H, *J* 5.2, 11.9 Hz), 3.89 (dd, 1H, *J* 2.1, 12.4 Hz), 3.82 (dd, 1H, *J* 5.1, 11.7 Hz), 3.70 (dd, 1H, *J* 5.3, 12.4 Hz), 2.97 (t, 1H, *J* 11.9 Hz); ^{13}C NMR (D_2O): δ 105.5, 105.1, 78.5, 78.3, 78.0, 77.3, 75.7, 75.5, 72.2, 71.7, 71.2 (CH_2), 70.2, 69.5, 68.3 (CH_2), 63.3 (CH_2), 61.2, 48.5 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{18}H_{34}NO_{14}$ $(M+H)^+$: 488.1979, found: 488.2002.

3.35. 4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol (9C)

Glycosylation of compound **12**[25] (539 mg) was carried out with tetra-*O*-acetyl-α,β-D-glucopyranosyl trichloroacetimidate (**1B**) (492 mg) as for compound **1C**. Column chromatographic purification (hexane–EtOAc 2:1→1:1) afforded the title compound (**9C**) (370 mg, 43%): $[\alpha]_D^{20}$ +4.4 (*c* 0.41, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 156.2, 100.0, 79.1, 75.6, 74.35, 73.5 (CH_2), 73.2, 72.7 (CH_2), 72.2, 71.4, 71.2 (CH_2), 68.8, 68.5 (CH_2), 67.7 (CH_2), 62.3 (CH_2), 54.6, 41.05 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{49}H_{56}NO_{15}$ $(M+H)^+$: 898.3650, found: 898.3638.

3.36. 2,3,6-Tri-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-dideoxy-4-*O*-β-D-glucopyranosyl-1,5-imino-D-glucitol (9D)

Usual deacetylation of the above 4-linked tetraacetate (**9C**) (185 mg, 0.206 mmol) gave the title compound (**9D**) as an oil (148 mg, 98%); $[\alpha]_D^{20}$ +3.3 (*c* 0.3, MeOH); ^{13}C NMR (CD_3OD): δ 158.8, 104.9, 78.4, 78.1, 77.2, 76.05, 75.3, 74.3 (CH_2), 74.1 (CH_2), 72.4 (CH_2), 72.1, 68.9 (CH_2), 68.7 (CH_2), 63.25 (CH_2), 56.6, 41.3 (CH_2); HR-MS/FAB *m*/*z* calcd for $C_{41}H_{48}NO_{11}$ $(M+H)^+$: 730.3227, found: 730.3231.

3.37. 1,5-Dideoxy-4-*O*-β-D-glucopyranosyl-1,5-imino-D-glucitol (9)

The above tetraol (**9D**) (159 mg, 0.218 mmol) was hydrogenolyzed as described for compound **7D** [MeOH/AcOH 10:1 (3 mL), Pd/C (250 mg, 10%, 40 h)]. The catalyst was removed, the solvents were evaporated and the product was stirred in water (3 mL) with Amberlyst A26 (OH^-) resin for 4 h. Filtration and freeze drying afforded **9** (67 mg, 96%) as a hygroscopic amorphous solid: $[\alpha]_D^{20}$ +20.7 (*c* 0.41, H_2O), lit.[11] $[\alpha]_D$ +25.0 (*c* 0.42, H_2O); the ^1H and ^{13}C NMR spectra were identical to previously reported spectra.[11] Anal. Calcd for $C_{12}H_{23}NO_9{\cdot}H_2O$: C, 41.98; H, 7.34; N, 4.08. Found: C, 42.36; H, 7.40; N, 3.57; HR-MS/FAB *m*/*z* calcd for $C_{12}H_{24}NO_9$ $(M+H)^+$: 326.1451, found: 326.1444.

3.38. Biological testing

Testing was conducted by Michael Surrey and Peter Wigley, BioDiscovery NZ Ltd.

Samples of compounds **1**–**3** and **5**–**9** were dissolved in filter-sterilized deionized water at 2 mg/mL and the solutions were serially diluted six times by a factor of three. Double dilution then resulted on the addition of the organisms in their nutrient media.

Acknowledgements

We thank Dr. H. Wong, Industrial Research Ltd, for providing NMR services and Professor R. J. Ferrier who assisted with the preparation of the manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.05.004.

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