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Peracetylated laminaribiose: preparation by specific degradation of curdlan and its chemical conversion into *N*-acetylhyalobiuronic acid

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ABSTRACT

β-Laminaribiose octaacetate (**2b**) was prepared in greater than 50% yield from the microbial polysaccharide curdlan by specific degradation with a yeast cell-wall lytic enzyme preparation, Kitalase, and subsequent acetylation. Acetolysis of curdlan also gave α-laminaribiose octaacetate (**2a**) in 27% yield. The usefulness of these peracetates **2a** and **2b** as starting materials for organic synthesis was shown by converting **2b** into *N*-acetylhyalobiuronic acid (**23**), the disaccharide repeating unit of hyaluronic acid. The conversion was carried out *via* a series of reactions, which included azidonitration of the glucal derivative and selective alkylidenation or direct tritylation to discriminate two primary hydroxyl groups existing in the disaccharide intermediates.

INTRODUCTION

Di- and trisaccharides with α- or β-(1→4) linkages, such as maltose, cellobiose, chitobiose, and maltotriose, are readily accessible by enzymic or chemical degradation of starch, cellulose, chitin, and pullulan, respectively. They have been employed as substrates for our studies on regioselective protection of oligosaccharides[1–4] and have served as key starting materials for syntheses of several biologically important compounds[5–10].

In order to extend the range of the utilizable oligosaccharides, our attention has now been directed towards a β-(1→3)-linked disaccharide, 3-*O*-(β-D-glucopyranosyl)-D-glucose (laminaribiose, **1**). Since β-(1→3)-linked glycosides have been found in many natural sugar chains as the basic skeleton or a key fragment (*e.g.*, noncytotoxic antitumor polysaccharides[11], the carbohydrate–protein linkage region of glycosaminoglycans[12], T-antigen determinants[13] and so on), an efficient preparation of **1** and its facile transformation to other β-(1→3)-linked disaccharides would be very welcome.

This paper deals with a preparation of **2**, the peracetate of **1**, utilizing regiospecific degradation of the microbial polysaccharide curdlan, and subsequent chemical conversion of **2** into the disaccharide repeating unit of hyaluronic acid, 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid)-D-glucopyranose (*N*-acetylhyalobiuronic acid **23**), as a typical example of the disaccharide transformation.

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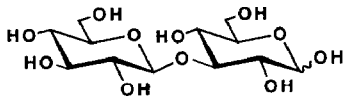

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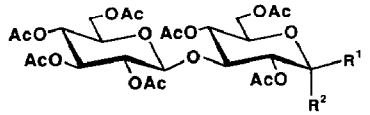
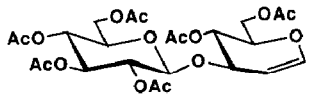


	R^1	R^2
2a	H	OAc
2b	OAc	H
3	H	Br



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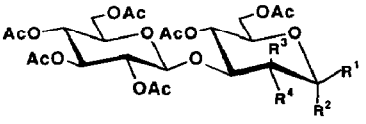
RESULTS AND DISCUSSION

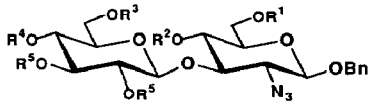
Both chemical and enzymic degradation methods have been tried for preparation of **1** and **2**. Thiem *et al.*[14] obtained **2a** in 30% yield by acetolysis of laminaran and pachyman. We tried to develop a large-scale preparation method by acetolysis of the microbial polysaccharide curdlan. When curdlan was treated with acetic anhydride containing 2.5% conc. sulfuric acid (50–55°, 5 days), α-laminaribiose octaacetate (**2a**) was obtained in 27% yield, together with α-D-glucopyranose pentaacetate (34%) and α-laminaritriose hendecaacetate (11%). These results showed that although the acetolysis of curdlan proceeded in essentially the same way as that reported for laminaran and pachyman, the yield of **2a** was slightly lower. Therefore our attention was next turned to enzymic degradation. Villa *et al.*[15] reported that incubation of laminaran or yeast (1→3)-β-D-glucan with an enzyme isolated from *Kluyveromyces phaseolosporus* gave **1** and D-glucose in 45 and 18% yields, respectively. Instead of using the same purified enzyme, which was laborious to obtain, we tried Kitalase, an enzyme preparation from culture filtrates of *Rhizoctonia solani*, which are known to contain a few separable *exo*- and *endo*-(1→3)-β-D-glucanases[16]. Optimum conditions for the production of **1** were sought using a range of pH values, temperatures, and reaction times with the aid h.p.l.c. for product determination. In each run, the amount of **1** increased with time for a while, then gradually decreased. When the reaction was carried out at 42° and pH 5, h.p.l.c. analysis showed that the yield of **1** reached a maximum of 57% on the basis of curdlan used. For large-scale preparation of **2b**, the mixture produced by the enzymic degrada-

tion was concentrated to dryness, acetylated, and fractionated by flash chromatography.

Among many significant choices of targets of synthesis using **2b** as starting material, *N*-acetylhyalobiuronic acid (**23**) was first chosen. Introduction of a nitrogenous function into the C-2 position and selective oxidation of the 6′-hydroxy group of the starting disaccharide were necessary for the preparation of the target molecule. Similarly to the conversion of **2a** reported by Thiem *et al.*[17], **2b** was changed to the glycal derivative **4** *via* glycosyl bromide **3** in the usual way (HBr–acetic acid, followed by Zn dust). Compound **4** underwent the azidonitration reaction[18] in acetonitrile, and the resulting glycosyl nitrates were converted into acetates by treatment with sodium acetate in acetic acid, giving three compounds that crystallized after purification by



	R^1	R^2	R^3	R^4
5	OAc	H	H	N_3
6	H	OAc	H	N_3
7	OAc	H	N_3	H
8	H	Br	H	N_3



	R^1	R^2	R^3	R^4	R^5
9	Ac	Ac	Ac	Ac	Ac
10	H	H	H	H	H
11	Ac	Ac	PhCH<		Ac
12	PhCH<		Ac	Ac	Ac
13	PhCH<		PhCH<		Ac
14	Ac	Ac	Me_2C<		Ac
15	Me_2C<		Me_2C<		Ac
16	Ac	Ac	Tr	Ac	Ac
17	Ac	Ac	H	H	Ac
18	Tr	Ac	Tr	Ac	Ac
19	Tr	Ac	Ac	Ac	Ac
20	Ac	Ac	H	Ac	Ac

chromatography: 1,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-azido-2-deoxy-β-D-glucopyranose (**5**) (28.5%), 1,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-azido-2-deoxy-α-D-glucopyranose (**6**) (11.5%), and 1,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-azido-2-deoxy-β-D-mannopyranose (**7**) (26.7%). Lemieux and Ratcliffe[18] reported that it took 8–10 h to complete the azidonitration reaction of peracetylated D-glucal and the product was a 1:1 mixture of D-*gluco* and D-*manno* derivatives. It was quite interesting to see that azidonitration of **4** was complete within only 2 h under almost the same conditions, giving a 1.5:1 ratio of D-*gluco* to D-*manno* configurations, despite the presence of bulky D-glucopyranosyl moiety at the C-3 position.

The stereochemistry of **5**, **6**, and **7** was elucidated mainly from their ^{1}H-n.m.r. spectra. In the spectrum of **5**, the H-1 proton resonates at δ 5.51 as a doublet ($J_{1,2}$ 8.3 Hz), showing that the anomeric OAc group was located at β-position. The coupling constant, $J_{2,3}$ (10 Hz) of H-2 at δ 3.54 also reveals the $H_{2,3}$-*trans* relationship, indicating that the azido group at C-2 should be in an equatorial orientation (β-D-*gluco* configuration). The spectrum of **6** reveals the H-1 proton at δ 6.32 with $J_{1,2}$ 3.7 Hz and the H-2 proton at δ 3.60 with $J_{2,3}$ 10 Hz, showing that **6** should have the α-D-*gluco* configuration. In the spectrum of **7**, H-1 and H-2 resonate at δ 5.84 with $J_{1,2}$ 1.7 Hz and δ 3.98 with $J_{2,3}$ 3.7 Hz, respectively. These data clearly show that **7** has a β-D-*manno* configuration.

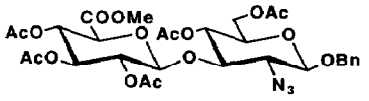
An anomeric mixture of D-*gluco* compounds, **5** and **6**, was treated with hydrogen bromide in acetic acid, and the resulting glycosyl bromide **8** was treated with benzyl alcohol in nitromethane in the presence of silver carbonate to give benzyl 4,6-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-azido-2-deoxy-β-D-glucopyranoside (**9**) in 54% overall yield. *O*-Deacetylation of **9** by the Zemplén procedure afforded compound **10** in quantitative yield. Prior to the oxidation of the C-6′ hydroxyl group of **10**, protection of other hydroxyl groups was necessary, which included the difficult problem of discriminating between the two primary hydroxyl groups at the C-6 and C-6′ positions. In order to overcome this situation, we first attempted selective alkylidenation of either the 4,6- or 4′,6′-hydroxyl groups of **10**. Evans′ benzylidenation[19] through the acetal exchange reaction is known to give kinetically controlled products. Thus, **10** was treated with α,α-dimethoxytoluene (1.25 mol. equiv.) in *N*,*N*-dimethylformamide (DMF) in the presence of *p*-toluenesulfonic acid at 40–45° under diminished pressure. The reaction process was monitored by t.l.c. ($CHCl_3$–methanol, 5:1), which showed the formation of three products. After 6.5 h of such treatment, the mixture was acetylated in the usual way and was subjected to column chromatography, giving respectively a mixture of the 4′,6′-*O*-benzylidene **11** and 4,6-*O*-benzylidene derivatives **12** (40.6% total yield, **11**:**12** = 4.5:1), the 4,6:4′,6′-di-*O*-benzylidene derivative **13** (9.6%), and **9** (peracetate of **10**, 26.5%). Compounds **11** and **12** could be easily separated by fractional crystallization of the mixture from ethanol. When **10** was treated with an excess of α,α-dimethoxytoluene (2.5 mol. equiv.), **13** was the sole product (in 83% yield). The white amorphous compound **13** had very low solubility in various organic solvents (*e.g.*, CH_2Cl_2, $CHCl_3$, dioxane, THF, toluene, ethyl acetate, acetone, ethanol, *etc.*). Structure elucidation of these products was

achieved mainly on the basis of ^{1}H-n.m.r. analyses. As shown in Tables I and II, all ring protons of compounds **11–13** were unambiguously assigned using the decoupling technique.

Isopropylidenation of **10** gave regioselectivity and chemical yields similar to the benzylidenation. Thus the reaction of **10** with 2,2-dimethoxypropane (1.35 mol. equiv.) in DMF in the presence of *p*-toluenesulfonic acid at 50° for 8 h, gave a mixture of the 4′,6′-*O*-isopropylidene derivative **14** contaminated with a small amount of an unidentified product (probably the 4,6-*O*-isopropylidene derivative) (~ 53%), the 4,6:4′,6′-di-*O*-isopropylidene derivative **15** (14.8%), and **9** (peracetate of **10**, 15%). Compound **14** could be isolated crystalline from the crude mixture by agitation in ethanol, but the other product was not isolated from the mother liquor. Structures of **14** and **15** were elucidated on the basis of ^{1}H-n.m.r. analyses (see Tables I and II). Attempts to selectively remove one of the acetal groups in **13** or **15** by using $CuCl_2 \cdot H_2O$ in ethanol or 2-propanol[20], Dowex-50W × 8 (H^+) in DMF–methanol, or 80% aq. acetic acid, all proved unsuccessful.

Since the selective alkylidenations attempted did not give fully satisfactory results, our attention was next directed towards selective tritylation of the primary hydroxy groups. Thus **10** was treated with trityl chloride (1.20 mol. equiv.) in pyridine containing a catalytic amount of 4-dimethylaminopyridine (DMAP) at room temperature for one week, and then acetylated in the usual way, giving four products in 45.6, 16.7, 10.7, and 9.2% yields, respectively. The product obtained in the best yield (45.6%) was shown to be the 6′-*O*-trityl derivative **16** that was identical with the compound derived from **11** by the sequence of debenzylidenation (to give the diol **17**), tritylation, and acetylation. The product obtained in 10.7% yield was **9**, which is simply acetylated **10**. The remaining two compounds obtained in 16.7 and 9.2% yields were elucidated as the 6,6′-di-*O*-trityl derivative **18** and 6-*O*-trityl derivative **19**, respectively, by comparing their ^{1}H-n.m.r. spectra with that of **9** (see Tables I and II).

O-Detritylation of **16** with 80% aq. acetic acid gave **20** in 80% yield. Oxidation of **20** with Jones' reagent, and subsequent esterification with diazomethane afforded



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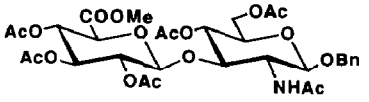

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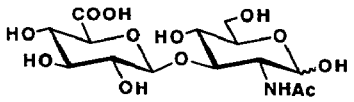

23
(*N*-acetylhyalobiuronic acid)

TABLE I

^{1}H-N.m.r. data for laminaribiose derivatives[a]

Hydrogen atom	*Chemical Shift (δ) and multiplicity*					
	5	**6**	**7**	**9**	**11**	**12**
H-1	5.51d	6.32d	5.84d	4.35d	4.35d	4.46d
H-2	3.54dd	3.60dd	3.98dd	3.43dd	3.42[b]	3.45[b]
H-3	3.62t	3.95t	4.04dd	4.47t	3.45[b]	3.45[b]
H-4	4.98dd	5.06t	5.22t	4.9[b]	4.95[b]	3.62t
H-5	3.7[b]	4.0m[b]	3.75ddd	3.56ddd	3.55m[b]	3.38m[b]
H-6a	4.04dd	4.15dd	4.18dd	4.16dd	4.14dd	3.84m
H-6b	4.22dd	4.22dd	4.28dd	4.21dd	4.22dd	4.32dd
H-1′	4.80d	4.78d	4.74d	4.78d	4.81d	4.75d
H-2′	4.96dd	4.97dd	4.96dd	4.9[b]	4.95m[b]	5.02dd
H-3′	5.21t	5.22t	5.22t	5.19t	5.30t	5.15t
H-4′	5.08t	5.12t	5.12t	5.06t	3.66t	5.06t
H-5′	3.7[b]	3.75m[b]	3.73ddd	3.64ddd	3.47m[b]	3.5m[b]
H-6′a	4.09dd	4.08dd	4.12dd	4.01dd	3.70t	4.01t
H-6′b	4.37dd	4.43dd	4.33dd	4.32dd	4.31dd	4.18dd
	13	**14**	**15**	**16**	**17**	
H-1	4.48d	4.34d	4.39d	4.39d	4.36d	
H-2	3.47dd	3.42dd	3.36dd	4.48dd	3.42dd	
H-3	3.65[b]	3.45t	3.44t	3.67t	3.60t	
H-4	3.65m[b]	4.92t	3.66t	5.02t	4.85t	
H-5	3.4m[b]	3.51ddd	3.18dt	3.58ddd	3.5m[b]	
H-6a	3.7m[b]	4.13dd	3.82t	4.18dd	4.15dd	
H-6b	4.12dd	4.20dd	3.94dd	4.27dd	4.22dd	
H-1′	4.85d	4.72d	4.78d	4.89[b]	4.76d	
H-2′	5.05dd	4.87dd	4.94dd	4.90m[b]	4.82dd	
H-3′	5.27t	5.10t	5.11t	5.13dt[c]	4.99t	
H-4′	3.71t	3.65t	3.75t	5.04t	3.67dt	
H-5′	3.4m[b]	3.27dt	3.29ddd	3.54ddd	3.4m[b]	
H-6′a	3.81t	3.70t	3.79dd	3.08dd	3.75dt	
H-6′b	4.36dd	3.89dd	3.90dd	3.34dd	3.89ddd	
	18	**19**	**20**	**21**	**22**	
H-1	4.39d	4.36[b]	4.36d	4.34d	5.01d	
H-2	3.49dd	3.4[b]	3.42dd	4.43dd	3.22dt	
H-3	3.60t	3.4[b]	3.6[b]	3.48t	4.52t	
H-4	4.89t	4.85m	4.85t	4.9[b]	4.97t	
H-5	3.5m[b]	3.4[b]	3.65m[b]	3.53ddd	3.67ddd	
H-6a	3.14dd	3.16bd	4.17dd	4.14dd	4.12dd	
H-6b	3.22dd	3.16bd	4.22dd	4.21dd	4.24dd	
H-1′	4.85[b]	4.75d	4.79d	4.81d	4.65d	
H-2′	4.85[b]	4.91dd	4.96[b]	4.94[b]	4.82dd	
H-3′	5.09dt[c]	5.17t	5.26t	5.25t	5.20t	
H-4′	4.99t	5.03t	4.89[b]	5.13t	5.12t	
H-5′	3.45m[b]	3.61ddd	3.46m[b]	3.95d	3.95d	
H-6′a	3.03dd	3.97dd	3.59ddd	—	—	
H-6′b	3.29dd	4.27dd	3.67[b]	—	—	

[a] ^{1}H-N.m.r. spectra were measured for solutions in $CDCl_3$, using tetramethylsilane as the internal standard; the n.m.r. data of protecting groups were described in the Experimental part; the data for compounds **10** and **23** in D_2O were also described in the Experimental section. [b] The multiplicity is uncertain due to low resolution and/or overlapping of signals. [c] The H-3′ proton appears as a doublet of triplets. The reason for this was not clear.

TABLE II

$^1H - ^1H$ Coupling constants of laminaribiose derivatives

Coupled protons	*J-values (Hz)*					
	5	**6**	**7**	**9**	**11**	**12**
1,2	8.3	3.7	1.7	7.9	7.8	8.0
2,3	10.0	10.0	3.7	10.0	[a]	[a]
3,4	9.8	10.0	8.8	10.0	[a]	9.0
4,5	9.8	10.0	8.8	10.0	[a]	9.0
5,6a	2.2	2.2	2.7	2.4	2.4	[a]
5,6b	4.6	4.2	5.6	4.9	4.6	4.5
6a,6b	12.5	12.0	12.2	12.4	12.2	12.0
1',2'	8.0	7.8	7.8	7.9	8.0	8.0
2',3'	9.6	9.3	9.3	9.5	9.5	9.6
3',4'	9.6	9.5	9.3	9.5	9.5	9.6
4',5'	9.6	9.5	9.3	9.5	9.6	9.6
5',6'a	2.2	2.0	2.4	2.2	9.8	11.3
5',6'b	4.4	4.0	4.6	4.2	4.9	4.85
6'a,6'b	12.5	12.3	12.5	12.2	10.0	12.2
	13	**14**	**15**	**16**	**17**	
1,2	7.9	7.6	7.8	7.9	8.0	
2,3	9.5	9.3	9.5	9.3	9.5	
3,4	[a]	9.3	9.5	9.3	9.8	
4,5	[a]	10.0	9.5	9.3	9.8	
5,6a	[a]	2.4	9.6	2.4	2.4	
5,6b	4.9	4.6	5.2	4.9	4.8	
6a,6b	11.0	12.3	10.0	12.2	12.2	
1',2'	7.6	7.9	7.8	[a]	7.8	
2',3'	8.5	9.3	9.2	9.3	9.4	
3',4'	8.5	10.2	9.3	9.3	9.4	
4',5'	8.5	10.2	9.3	9.3	9.4	
5',6'a	9.3	9.7	9.7	4.9	4.8	
5',6'b	4.9	5.1	5.4	2.4	2.8	
6'a,6'b	10.0	11.2	10.2	10.5	11.0	
	18	**19**	**20**	**21**	**22**	
1,2	8.1	[a]	8.0	7.9	8.0	
2,3	9.5	[a]	9.5	9.6	9.6	
3,4	9.5	[a]	9.8	9.6	9.6	
4,5	9.5	[a]	9.8	9.5	9.6	
5,6a	2.2	3.9	2.1	2.4	2.4	
5,6b	5.9	3.9	4.6	4.7	4.9	
6a,6b	10.5	—	12.1	12.5	12.3	
1',2'	[a]	7.8	8.0	7.8	7.8	
2',3'	9.5	9.5	9.5	9.5	9.0	
3',4'	9.5	9.5	9.5	9.5	9.4	
4',5'	9.5	9.5	[a]	9.0	9.7	
5',6'a	5.0	2.2	2.8	—	—	
5',6'b	2.5	4.5	[a]	—	—	
6'a,6'b	10.5	12.4	11.2	—	—	

[a] Not analyzed due to low resolution or the overlapping of signals.

benzyl 4,6-di-*O*-acetyl-2-azido-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-β-D-glucopyranoside (**21**) in 87% yield. Compound **21** was also obtainable in 41% yield when the trityl derivative **16** was directly oxidized according to the procedure of Sinaÿ[21]. Compound **21** was converted into the 2-acetamido derivative **22** in 76% yield by treatment with nickel boride [nickel(II) chloride–sodium borohydride] in ethanol[22] and subsequent treatment with acetic anhydride. Finally, **22** was successively subjected to saponification with sodium hydroxide, catalytic hydrogenolysis (palladium-on-charcoal), neutralization with Dowex-50W × 8 (H^+), and lyophylization, giving the known *N*-acetylhyalobiuronic acid (**23**) (refs. 23, 24) in 74% yield as a hygroscopic, amorphous solid.

EXPERIMENTAL

General methods and materials. — Melting points were determined with a Yamato micro melting point apparatus, and are uncorrected. Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter. I.r. spectra were recorded with a Shimasu IR-27 spectrophotometer, using potassium bromide disks for solid samples and KRS (thallium bromide–iodide) cells for liquid samples. ^{1}H-N.m.r. spectra were recorded at 400 MHz or 500 MHz with JEOL JNM-GX 400 or JEOL JNM-GX 500 spectrometers, with solutions in $CDCl_3$, unless otherwise specified. δ Values are expressed in p.p.m. downfield of the internal standard, tetramethylsilane (Me_4Si). δ (D_2O) Values were expressed in p.p.m. by reference to an internal standard of HDO (δ 4.70). Reactions were monitored by t.l.c. on precoated plates of Silica Gel $60F_{254}$ (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany). Flash chromatography was performed on columns of Silica Gel 60 (70–230 mesh; E. Merck, Darmstadt, Germany). Solvent extracts were dried with anhydrous magnesium sulfate ($MgSO_4$) or sodium sulfate (Na_2SO_4), and solutions were concentrated under diminished pressure below 45°. Curdlan was purchased from Wako Pure Chemical Industries, Ltd. and used without purification. Kitalase (300 units/g) was purchased from K.I. Chemical Industries, Co. Ltd., Japan. Buffer solutions were prepared to cover the pH range 4–8: 10mM acetate buffer (pH 4.0–6.4) and 10mM phosphate buffer (pH 6.0–8.0). High-performance liquid chromatography (h.p.l.c.) was performed with a Kaseisorb LC NH_2-300-5 (4.6 × 150 mm) column in a Hitachi liquid chromatograph equipped with a Shodex RI SE-11 differential refractometer. The solvent was 8:2 (v/v) acetonitrile–water pumped at 0.4 mL/min. High-resolution fast-atom bombardment mass spectra (h.r.f.a.b.-m.s.) were obtained on a JEOL SX-102 spectrometer.

*1,2,4,6-Tetra-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-*D*-glucopyranose (laminaribiose octaacetate)* (**2**). — *Method A.* To a stirred suspension of curdlan (90%, 200 g) in acetic anhydride (1300 mL) was added dropwise a solution of conc. sulfuric acid (32.5 mL) in acetic acid (100 mL) at room temperature. The suspension was then stirred at 50–55°, and it gradually became a viscous, clear solution after 3 h. During reaction, t.l.c. [1:1 (v/v) benzene–ethyl acetate] showed the formation of three major components (R_F 0.8, 0.5, and 0.1, corresponding to mono-, di-, and

trisaccharide derivatives, respectively), together with immobile components (R_F 0). After the component at R_F 0.5 seemed to reach the maximum (5 days), the reaction was stopped by adding anhydrous sodium acetate (100 g) to the mixture. The dark brown solution was then poured into ice-water, stirred overnight, and extracted with chloroform (3 × 700 mL). The extracts were combined, washed successively with brine, aq. sodium hydrogencarbonate ($NaHCO_3$), and water, dried ($MgSO_4$), and concentrated. The resulting dark-brown syrup was applied to a silica gel column (9.0 × 100 cm). First, elution of the column with 2:1 (v/v) toluene–ethyl acetate gave 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (160 g, 34%), m.p. 112–113° (ethanol), $[\alpha]_D^{23}$ + 101.7° (*c* 1, $CHCl_3$) [lit.[25] m.p. 112–113°, $[\alpha]_D$ +102° ($CHCl_3$)].

Continued elution of the column with 3:2 (v/v) toluene–ethyl acetate afforded a brown foam (130 g), which was dissolved in hot ethanol, decolorized with charcoal, and allowed to crystallize from ethanol, giving α-laminaribiose octaacetate (**2a**) (105 g, 27%) as white needles. The ^{1}H-n.m.r. spectrum showed that it was a 1:1 molecular complex of **2a** with ethanol: m.p. 78–80°, $[\alpha]_D^{20}$ +22.1 (*c* 0.3, $CHCl_3$) [lit.[26] m.p. 77–78°, $[\alpha]_D^{17}$ +20° (*c* 3.6, $CHCl_3$); lit.[14] m.p. 82.5° $[\alpha]_D^{20}$ +21.9° (*c* 1.0, $CHCl_3$)].

Further elution of the column with 2:3 (v/v) toluene–ethyl acetate gave a light-brown amorphous solid (45 g). Zemplén deacetylation of the product, followed by acetylation with sodium acetate–acetic anhydride and decolorization with charcoal, gave a white powder, which was identified as β-laminaritriose hendecaacetate (42 g, 11%), m.p. 115–120°, $[\alpha]_D^{20}$ −36.5° (*c* 0.7, $CHCl_3$) [lit.[27] m.p. 120–121°, $[\alpha]_D$ −40° ($CHCl_3$)].

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.63. Found: C, 49.52; H, 5.62.

Method B. A suspension of curdlan (90%, 10.0 g) in 10mM acetate buffer (pH 5, 500 mL) containing Kitalase (1 g, 300 units/g) was incubated for 12 h at 42°. Then the Kitalase was deactivated by heating the mixture for 30 min at 100°. The reaction mixture was then concentrated *in vacuo* to dryness, and to the residue was added sodium acetate (5 g) and acetic anhydride (35 mL). The resulting mixture was heated for 3 h at 100°, cooled, poured into ice-water, and extracted with chloroform. The extracts were washed with aq. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The residue was subjected to flash chromatography. Elution of the column with 2:1 (v/v) toluene–ethyl acetate gave 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (8.03 g, 37%), m.p. 132–133° (ethanol) [lit.[27] m.p. 132–133°]. Further elution of the column with 3:2 (v/v) toluene–ethyl acetate gave β-laminaribiose octaacetate (**2b**) (9.6 g, 51%), m.p. 158–160° (ethanol), $[\alpha]_D^{23}$ −26.5° (*c* 0.5, $CHCl_3$) [lit.[27] m.p. 160–161°, $[\alpha]_D^{17-21}$ −28.6° ($CHCl_3$); lit.[26] m.p. 156–158°, $[\alpha]_D^{16}$ −25.3° (*c* 2.2, $CHCl_3$)].

*4,6-Di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,5-anhydro-2-deoxy-*D*-arabino-hex-1-enitol* (**4**). — To a solution of **2** (20.5 g, 30 mmol) in acetic acid (100 mL) and dichloroethane (50 mL) was added dropwise a solution of hydrogen bromide in acetic acid (25%, 30 mL). The mixture was stirred for 3 h at room temperature, poured into ice–water, and extracted with chloroform (3 × 250 mL). The extracts were combined, successively washed with brine, aq. $NaHCO_3$, and water, dried (Na_2SO_4), and filtered. The filtrate was concentrated to dryness. Flash chromatography

of the residue with 3:1 (v/v) toluene–ethyl acetate gave the glycosyl bromide **3** (18.4 g, 87%) as a white foam, which was directly used for next step without further purification. To a suspension of zinc dust (20 g), sodium acetate (8.0 g), and copper(II) sulfate pentahydrate (0.8 g) in 60% aqueous acetic acid (120 mL) was added dropwise a solution of **3** (14.0 g, 20 mmol) in ethyl acetate (30 mL). The mixture was stirred for 30 min at room temperature, filtered through a Celite pad, and the filtrate was concentrated. The residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with aq. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. Flash chromatography of the residue with 4:1 (v/v) toluene–ethyl acetate gave **4** (7.85 g, 70%) as a colorless syrup, $[\alpha]_D^{23}$ −48.5° (*c* 0.7, $CHCl_3$) [lit.[17] $[\alpha]_D^{20}$ −47.2° (*c* 1, $CHCl_3$)]. The ^{1}H-n.m.r. spectrum was in agreement with its structure.

*1,4,6-Tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-2-azido-2-deoxy-β-*D*-glucopyranose* (**5**), *-α-*D*-glucopyranose* (**6**), and *1,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-2-azido-2-deoxy-β-*D*-mannopyranose* (**7**). — To a suspension of ammonium cerium(IV) nitrate (16.4 g, 30 mmol) and sodium azide (1.04 g, 16 mmol) in anhydrous acetonitrile (100 mL) at −15° was added a solution of **4** (4.80 g, 8.5 mmol) in anhydrous acetonitrile (30 mL) under nitrogen atmosphere. The mixture was vigorously stirred for 2 h at that temperature, diluted with cold water (100 mL), and extracted with cold ether (3 × 350 mL). The extracts were combined, washed with cold water (3 × 100 mL), dried (Na_2SO_4), and concentrated to dryness, yielding a white powder. A solution of the product in glacial acetic acid (100 mL) containing anhydrous sodium acetate (6.0 g) was heated for 2 h at 100°, cooled, and diluted with chloroform. The organic layer was successively washed with brine, aq. $NaHCO_3$, and water, dried (Na_2SO_4), and filtered. The filtrate was concentrated. The residue was chromatographed with 5:1 (v/v) toluene–ethyl acetate, to give **5** (1.61 g, 28.5%), **6** (0.65 g, 11.5%), and **7** (1.50 g, 26.7%).

Compound **5**: m.p. 175–176°, $[\alpha]_D^{20}$ −11.3° (*c* 0.3, $CHCl_3$); ν_{max}^{KBr} 2080 (N_3) and 1730 cm^{-1} ($OCOCH_3$); δ_H: 2.00, 2.02, 2.04, 2.08 (2), 2.085, and 2.20 (each s, each 3 H, 7 $OCOCH_3$).

Anal. Calc. for $C_{26}H_{35}N_3O_{17}$: C, 47.20; H, 5.33; N, 6.35. Found: C, 47.18; H, 5.34; N, 6.31.

Compound **6**: m.p. 172–173°, $[\alpha]_D^{20}$ +37.0° (*c* 0.3, $CHCl_3$); ν_{max}^{KBr} 2100 (N_3) and 1740 cm^{-1} ($OCOCH_3$); δ_H: 2.01, 2.03, 2.07, 2.075, 2.08, 2.09, and 2.21 (each s, each 3 H, 7 $OCOCH_3$).

Anal. Calc. for $C_{26}H_{35}N_3O_{17}$: C, 47.20; H, 5.33; N, 6.35. Found: C, 47.48; H, 5.47; N, 6.01.

Compound **7**: m.p. 185–187°, $[\alpha]_D^{20}$ −49.4° (*c* 0.4, $CHCl_3$); ν_{max}^{KBr} 2090 (N_3) and 1735 cm^{-1} ($OCOCH_3$); δ_H: 2.02, 2.04, 2.06, 2.066, 2.09 (2), and 2.20 (each s, each 3 H, 7 $OCOCH_3$).

Anal. Calc. for $C_{26}H_{35}N_3O_{17}$: C, 47.20; H, 5.33; N, 6.35. Found: C, 47.12; H, 5.31; N, 6.18.

*Benzyl 4,6-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-2-azido-2-deoxy-β-*D*-glucopyranoside* (**9**). — To a solution of the mixture of **5** and **6** (2.70 g, 4.08

mmol) in acetic acid (40 mL) was added a solution of hydrogen bromide in acetic acid (25%, 3.2 mL). The resulting mixture was stirred for 4 h at room temperature, then poured into ice-water and extracted with chloroform (3 × 120 mL). The extracts were combined, washed with aq. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. Flash chromatography of the residue with 5:1 (v/v) toluene–ethyl acetate gave the glycosyl bromide **8** (2.84 g, 85%) as a white foam, which was used for next glycosylation without further purification. To the suspension of **8** (2.80 g, 4.10 mmol), benzyl acohol (2.5 mL), and Drierite (5 g) in nitromethane (15 mL) in a dark-brown (amber) flask was added silver carbonate (4.5 g, 16.4 mmol) under argon. The mixture was stirred at −15° for 20 h, then diluted with chloroform (200 mL), and filtered through a Celite pad. The filtrate was washed with brine and water, dried (Na_2SO_4), and concentrated. The residue was chromatographed with 5:1 (v/v) toluene–ethyl acetate as eluent, giving **9** (1.58 g, 54%), m.p. 169–171° (ethanol), $[\alpha]_D^{23}$ −13.7° (*c* 0.2, $CHCl_3$); ν_{max}^{KBr} 2100 (N_3) and 1735 cm^{-1} ($OCOCH_3$); δ_H: 1.99, 2.007, 2.01, 2.06, 2.07, and 2.10 (each s, each 3 H, 6 $OCOCH_3$), 4.68 (d, 1 H, J_{gem} 12 Hz, 1/2 PhCH_2), 4.90 (m, 1 H, 1/2 PhCH_2, overlapping with H-2′,4), 7.35 (bs, 5 H, C_6H_5).

Anal. Calc. for $C_{31}H_{39}N_3O_{16}$: C, 52.46; H, 5.54; N, 5.92. Found: C, 52.37; H, 5.54; N, 5.84.

O-Deacetylation of **9**. — To a solution of **9** (1.75 g, 2.46 mmol) in 1:1 (v/v) tetrahydrofuran–methanol (40 mL) was added 0.2M methanolic sodium methoxide (0.2 mL). The mixture was stirred at room temperature for 5 h, neutralized with Dowex-50W × 8 (H^+) and filtered. The filtrate was concentrated to dryness, giving **10** (1.20 g, quantitative) as a white solid, which was directly used for the next reactions; m.p. 147–149°, $[\alpha]_D^{23}$ −14.2° (*c* 0.3, H_2O); ν_{max}^{KBr} 3350 (OH) and 2080 cm^{-1} (N_3). ^{1}H-N.m.r. data (D_2O): δ 3.26 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.3 Hz, H-2), 3.31–3.51 (m, 7 H, H-2′,3,3′,4,4′,5,5′), 3.65 (dd, 1 H, $J_{5,6a}$ 5.6, $J_{6a,6b}$ 12.4 Hz, H-6a), 3.70 (dd, 1 H, $J_{5',6'a}$ 5.4, $J_{6'a,6'b}$ 12.4 Hz, H-6′a), 3.83 (dd, 1 H, $J_{5,6b}$ 2.3 Hz, H-6b), 3.87 (dd, 1 H, $J_{5',6'b}$ 2.4 Hz, H-6′b) (the assignment of H-6 and H-6′ might be reversible), 4.58 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1′), 4.61 (d, 1 H, H-1), 4.76 (d, 1 H, J_{gem} 11.5 Hz, 1/2 PhCH_2), 4.92 (d, 1 H, 1/2 PhCH_2), 7.45 (m, 5 H, C_6H_5).

*Benzyl 4,6-di-*O*-acetyl-3-*O*-(2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-*D*-glucopyranosyl)-2-azido-2-deoxy-β-*D*-glucopyranoside* (**11**), *benzyl 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranoside* (**12**), and *benzyl 3-*O*-(2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-*D*-glucopyranosyl)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranoside* (**13**). — A solution of **10** (1.15 g, 2.51 mmol), α,α-dimethoxytoluene (0.46 mL, 3.0 mmol), and *p*-toluenesulfonic acid (20 mg) in *N*,*N*-dimethylformamide (DMF) (50 mL) was evacuated by a water pump at 40–50°. The reaction was monitored by t.l.c. [5:1 (v/v) chloroform–methanol]. After the reaction was continued for 6.5 h, the mixture was cooled, pyridine (5 mL) and acetic anhydride (2 mL) were added, stirred at room temperature overnight, then poured into ice-water, and extracted with chloroform. The extracts were successively washed with M hydrochloric acid, aq. $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated. The residue was subjected to flash chromatography. Elution of the column with 7:1 (v/v) toluene–ethyl acetate gave **13** (176 mg, 9.6%) as a white amorphous solid, m.p. 212–213°, $[\alpha]_D^{23}$ −70.2°

(*c* 0.3, $CHCl_3$); δ_H: 2.04 and 2.08 (each s, each 3 H, 2 $OCOCH_3$), 4.70 (d, 1 H, J_{gem} 11 Hz, 1/2 PhCH_2), 4.94 (d, 1 H, 1/2 PhCH_2), 5.38 and 5.55 (each s, each 1 H, 2 PhCH), 7.32–7.48 (m, 15 H, 3 C_6H_5).

Anal. Calc. for $C_{37}H_{39}N_3O_{12}$: C, 61.92; H, 5.48; N, 5.85. Found: C, 61.81; H, 5.48; N, 5.79.

Elution of the column with 5:1 (v/v) toluene–ethyl acetate afforded a white solid, ^{1}H-n.m.r. spectrum of which showed that it was a mixture of compound **11** and **12** (727 mg, 40.6%, **11**:**12** = 4.5:1). Fractional crystallization of the mixture from ethanol gave crystalline **11** and **12**.

Compound **11**: m.p. 210–211°, $[\alpha]_D^{23}$ −32.7° (*c* 0.2, $CHCl_3$); δ_H: 2.01, 2.03, 2.09, and 2.10 (each s, each 3 H, 4 $OCOCH_3$), 4.68 (d, 1 H, J_{gem} 12 Hz, 1/2 PhCH_2), 4.95 (m, 1 H, 1/2 PhCH_2, overlapping with H-2′,4), 5.48 (s, 1 H, PhCH), 7.35–7.42 (m, 10 H, 2 C_6H_5).

Anal. Calc.for $C_{34}H_{39}N_3O_{14}$: C, 57.22; H, 5.51; N, 5.89. Found: C, 57.00; H, 5.50; N, 5.87.

Compound **12**: m.p. 172–174°, $[\alpha]_D^{23}$ −50.6° (*c* 0.15, $CHCl_3$); δ_H: 1.96, 1.98, 1.99, and 2.05 (each s, each 3 H, 4 $OCOCH_3$), 4.68 (d, 1 H, J_{gem} 12 Hz, 1/2 PhCH_2), 4.92 (d, 1 H, 1/2 PhCH_2), 5.55 (s, 1 H, PhCH), 7.15–7.38 (m, 10 H, 2 C_6H_5).

Anal. Calc. for $C_{34}H_{39}N_3O_{14}$: C, 57.22; H, 5.51; N, 5.89. Found: C, 57.12; H, 5.60; N, 5.81.

Further elution of the column with 3:1 (v/v) toluene–ethyl acetate recovered **10** as **9** (472 mg, 26.5%).

In another experiment, **10** (320 mg, 0.7 mmol) and α,α-dimethoxytoluene (0.275 mL, 1.82 mmol) in DMF (5 mL) were reacted for 3 h at 60°, as described above, to give **13** (430 mg, 85%), after flash chromatography.

*Benzyl 4,6-di-*O*-acetyl-3-*O*-(2,3-di-*O*-acetyl-4,6-*O*-isopropylidene-β-*D*-glucopyranosyl)-2-azido-2-deoxy-β-*D*-glucopyranoside* (**14**) and *benzyl 3-*O*-(2,3-di-*O*-acetyl-4,6-*O*-isopropylidene-β-*D*-glucopyranosyl)-2-azido-2-deoxy-4,6-*O*-isopropylidene-β-*D*-glucopyranoside* (**15**). — A solution of **10** (210 mg, 0.46 mmol) and 2,2-dimethoxypropane (76.2 μL, 0.62 mmol) in DMF (3 mL) containing *p*-toluenesulfonic acid (10 mg) was stirred for 8 h at 50°, and then pyridine (2 mL) and acetic anhydride (0.8 mL) were added. The mixture was stirred overnight, poured into water, and extracted with chloroform. The extracts were washed with water, dried (Na_2SO_4), and concentrated. Flash chromatography of the residue with 6:1 (v/v) toluene–ethyl acetate gave **15** (43.5 mg, 14.8%), m.p. 85–86°, $[\alpha]_D^{23}$ −44.3° (*c* 0.2, $CHCl_3$); δ_H: 1.39 and 1.47 (each bs, each 6 H, 2 CMe_2), 2.05 and 2.08 (each s, each 3 H, 2 $OCOCH_3$), 4.66 (d, 1 H, J_{gem} 12 Hz, 1/2 PhCH_2), 4.90 (d, 1 H, 1/2 PhCH_2), 7.38 (bs, 5 H, C_6H_5).

Anal. Calc. for $C_{29}H_{39}N_3O_{13}$: C, 54.62; H, 6.17; N, 6.59. Found: C, 54.41; H, 6.32; N, 6.49.

Elution of the column with 5:1 (v/v) toluene–ethyl acetate afforded crude **14** (167 mg, ~53%), the ^{1}H-n.m.r. spectrum of which showed that it contained a small amount of another, unidentified component. Crystallization of the crude product from ethanol gave pure **14** as needles, m.p. 172–173°, $[\alpha]_D^{20}$ −22.5° (*c* 0.3, $CHCl_3$); δ_H: 1.36 and 1.44

(each s, each 3 H, CMe_2), 1.98, 2.02, 2.06, and 2.09 (each s, each 3 H, 4 $OCOCH_3$), 4.67 (d, 1 H, J_{gem} 12 Hz, 1/2 PhCH_2), 4.93 (d, 1 H, 1/2 PhCH_2), 7.38 (bs, 5 H, C_6H_5).

Anal. Calc. for $C_{30}H_{39}N_3O_{15}$: C, 52.86; H, 5.77; N, 6.16. Found: C, 52.98; H, 5.89; N, 5.96.

Further elution of the column with 3:1 (v/v) toluene–ethyl acetate recovered **10** as **9** (48.9 mg, 15%).

*Benzyl 4,6-di-*O*-acetyl-3-*O*-(2,3-di-*O*-acetyl-β-*D*-glucopyranosyl)-2-azido-2-deoxy-β-*D*-glucopyranoside* (**17**). — *From* **11**. A mixture of **11** (160 mg, 0.224 mmol) in 80% aq. acetic acid (5 mL) was heated for 1.5 h at 70–80°. The solution was concentrated *in vacuo* to dryness. Chromatography of the residue with 20:1 (v/v) chloroform–ethanol gave **17** (120 mg, 85%) as a white foam, m.p. 70–73°, $[\alpha]_D^{20}$ −13.5° (*c* 0.2, $CHCl_3$); ν_{max}^{KBr} 3500 (OH), 2100 (N_3), and 1740 cm^{-1} ($OCOCH_3$); δ_H: 2.06, 2.09, 2.10, and 2.11 (each s, each 3 H, 4 $OCOCH_3$), 2.76 (d, 1 H, $J_{4',OH}$ 4.6 Hz, OH at C-4'), 4.69 (d, 1 H, J_{gem} 12 Hz, 1/2 PhCH_2), 4.95 (d, 1 H, 1/2 PhCH_2), 7.41 (bs, 5 H, C_6H_5).

Anal. Calc. for $C_{27}H_{35}N_3O_{14}$: C, 51.84; H, 5.64; N, 6.72. Found: C, 51.58; H, 5.78; N, 6.51.

From **14**. A solution of **14** (200 mg, 0.293 mmol) in 80% aq. acetic acid (8 mL) was stirred for 1 h at 50°. Then the mixture was concentrated *in vacuo* to dryness. Chromatography as described above gave **17** (162 mg, 88%).

*Benzyl 4,6-di-*O*-acetyl-3-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-trityl-β-*D*-glucopyranosyl)-2-azido-2-deoxy-β-*D*-glucopyranoside* (**16**). — A mixture of **17** (110 mg, 0.176 mmol) and trityl chloride (78.5 mg, 0.282 mmol) in pyridine (2 mL) containing 4-dimethylamino pyridine (DMAP) (10 mg) was heated for 20 h at 50°, then cooled. To the mixture was added acetic anhydride (0.5 mL). The mixture was stirred for 2 h at room temperature, poured into ice-water, and extracted with ethyl acetate. The extracts were washed with aq. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The residue was chromatographed with 7:1 (v/v) toluene–ethyl acetate, giving **16** (130 mg, 81%), m.p. 186–187° (ethanol), $[\alpha]_D^{20}$ +18.2° (*c* 0.2, $CHCl_3$); δ_H: 1.72, 1.80, 1.97, 2.07, and 2.10 (each s, each 3 H, 5 $OCOCH_3$), 4.69 (d, 1 H, J_{gem} 12 Hz, 1/2 PhCH_2), 4.96 (d, 1 H, 1/2 PhCH_2), 7.21–7.42 (m, 20 H, 4 C_6H_5).

Anal. Calc. for $C_{48}H_{51}N_3O_{15}$: C, 63.35; H, 5.65; N, 4.62. Found: C, 63.22; H, 5.64; N, 4.40.

Tritylation of **10**. — To a solution of **10** (320 mg, 0.7 mmol) in pyridine (3 mL) containing DMAP (10 mg) was added trityl chloride (234 mg, 0.84 mmol) at 0°. Then the mixture was stirred at room temperature for 7 days. Acetic anhydride (0.8 mL) was added, and the resulting mixture was stirred at room temperature overnight, poured into ice-water, and extracted with ethyl acetate. The extracts were washed with brine and water, dried (Na_2SO_4), and concentrated. The residue was applied to a column of silica gel (230–400 mesh). Elution of the column with 9:1 (v/v) toluene–ethyl acetate gave **18** (130 mg, 16.7%), m.p. 196–198° (ethanol), $[\alpha]_D^{20}$ +21.2° (*c* 0.3, $CHCl_3$); δ_H: 1.54, 1.70, 1.95, and 2.05 (each s, each 3 H, 4 $OCOCH_3$), 4.81 (d, 1 H, J_{gem} 11.5 Hz, 1/2 PhCH_2), 5.01 (d, 1 H, 1/2 PhCH_2), 7.19–7.38 (m, 35 H, 7 C_6H_5).

Anal. Calc. for $C_{65}H_{63}N_3O_{14}$: C, 70.32; H, 5.72; N, 3.78. Found: C, 69.99; H, 5.71; N, 3.48.

Elution of the column with 7:1 (v/v) toluene–ethyl acetate afforded a mixture of **16** and **19** (365 mg), which was separated by repeated chromatography with 9:1 (v/v) benzene–ethyl acetate, giving **16** (290 mg, 45.6%) and **19** (58.6 mg, 9.2%).

Compound **19**: m.p. 111–112° (ethanol), $[\alpha]_D^{23}$ 0° (*c* 0.2, $CHCl_3$); δ_H: 1.74, 1.98, 1.99, 2.03, and 2.07 (each s, each 3 H, 5 $OCOCH_3$), 4.79 (d, 1 H, J_{gem} 12 Hz, 1/2 PhCH_2), 5.00 (d, 1 H, 1/2 PhCH_2), 7.21–7.41 (m, 20 H, 4 C_6H_5).

Anal. Calc. for $C_{48}H_{51}N_3O_{15}$: C, 63.35; H, 5.65; N, 4.62. Found: C, 63.15; H, 5.68; N, 4.43.

Further elution of the column with 4:1 (v/v) toluene–ethyl acetate recovered **10** as **9** (76 mg, 10.7%).

*Benzyl 4,6-di-*O*-acetyl-3-*O*-(2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyl)-2-azido-2-deoxy-β-*D*-glucopyranoside* (**20**). — A solution of **16** (132 mg, 0.145 mmol) in 80% aq. acetic acid (3 mL) was heated for 40 min at 70°, then cooled, and concentrated to dryness. Chromatography of the residue with 1:1 (v/v) toluene–ethyl acetate gave **20** (77.5 mg, 80%), m.p. 179–181° (dec.), $[\alpha]_D^{20}$ −12.8° (*c* 0.2, $CHCl_3$); ν_{max}^{KBr} 3450 (OH), 2100 (N_3), and 1735 cm^{-1} ($OCOCH_3$); δ_H: 2.01, 2.06, 2.08, 2.10, and 2.11 (each s, each 3 H, 5 $OCOCH_3$), 2.47 (dd, 1 H, $J_{6'a,OH}$ 4.2 Hz, $J_{6'b,OH}$ 9.8 Hz, OH at C-6'), 4.69 (d, 1 H, J_{gem} 12 Hz, 1/2 PhCH_2), 4.93 (d, 1 H, 1/2 PhCH_2), 7.38 (bs, 5 H, C_6H_5).

Anal. Calc. for $C_{29}H_{37}N_3O_{15}$: C, 52.17; H, 5.59; N, 6.29. Found: C, 51.92; H, 5.68; N, 6.10.

*Benzyl 4,6-di-*O*-acetyl-2-azido-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-β-*D*-glucopyranoside* (**21**). — *From* **16**. To a cooled solution of **16** (130 mg, 0.143 mmol) in acetone (5 mL) was added dropwise a solution of chromium trioxide (145 mg, 1.43 mmol) in 3.5M sulfuric acid (0.4 mL) at 0°. Then the mixture was stirred at room temperature for 4.5 h, poured into ice-water, and extracted with chloroform. The extracts were washed with water, dried (Na_2SO_4), and concentrated to dryness. The resulting residue was dissolved in ethyl acetate and directly esterified with diazomethane in ether. Solvents were evaporated, and chromatography of the residue with 5:1 (v/v) toluene–ethyl acetate gave **21** (40.8 mg, 41%): m.p. 194–196° (ethanol), $[\alpha]_D^{23}$ −17.3° (*c* 0.2, $CHCl_3$); δ_H: 2.00, 2.01, 2.07, 2.08, and 2.10 (each s, each 3 H, 5 $OCOCH_3$), 3.71 (s, 3 H, OCH_3), 4.68 (d, 1 H, J_{gem} 12 Hz, 1/2 PhCH_2), 4.93 (m, 1 H, 1/2 PhCH_2, overlapping with H-2', 4), 7.39 (bs, 5 H, C_6H_5).

Anal. Calc. for $C_{30}H_{37}N_3O_{16}$: C, 51.79; H, 5.36; N, 6.04. Found: C, 51.70; H, 5.39; N, 6.01.

From **20**. To a cooled solution of **20** (60 mg, 0.090 mmol) in acetone (3 mL) was added a solution of chromium trioxide (150 mg, 1.50 mmol) in 3.5M sulfuric acid (0.5 mL). The mixture was stirred at 0° for 1.5 h, then it was treated as described above to give **21** (55 mg, 87%).

*Benzyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-β-*D*-glucopyranoside* (**22**). — To a green solution of **21** (30 mg, 43.1 μmol), nickel(II) chloride hexahydrate (200 mg), and boric acid (100 mg) in 4:1 (v/v) ethanol–ethyl acetate (5 mL) was added a filtered solution of sodium borohydride in ethanol until the black color remained for 1 h. Then acetic anhydride (0.2 mL) was

added. The mixture was stirred for 2 h at room temperature, diluted with chloroform, and filtered through a Celite pad. The filtrate was washed with aq. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. Chromatography of the residue with 1:1 (v/v) toluene–ethyl acetate gave **22** (23.5 mg, 76%): m.p. 180–181° (ethanol), $[\alpha]_D^{20}$ −31.3° (*c* 0.1, $CHCl_3$); δ_H: 1.96, 1.98, 1.99, 2.00, 2.07, and 2.08 (each s, each 3 H, 5 $OCOCH_3$ and $NCOCH_3$), 3.70 (s, 3 H, OCH_3), 4.54 (d, 1 H, J_{gem} 11.5 Hz, 1/2 $PhCH_2$), 4.87(d, 1 H, 1/2 $PhCH_2$), 5.63 (d, 1 H, $J_{2,NH}$ 7.3 Hz, NH), 7.20–7.39 (bs, 5 H, C_6H_5).

Anal. Calc. for $C_{32}H_{41}NO_{17}$: C, 54.00; H, 5.81; N, 1.97. Found: C, 53.70; H, 5.71; N, 2.01.

*2-Acetamido-2-deoxy-3-*O*-(β-*D*-glucopyranosyluronic acid)-*D*-glucopyranose (N-acetylhyalobiuronic acid)* (**23**). — A solution of **22** (12 mg, 16.8 μmol) in 9:1 (v/v) methanol–water (1.5 mL) was treated with 6M sodium hydroxide (0.15 mL) for 3 h at room temperature, then applied to a short column (6 × 50 mm) of Dowex-50W × 8 (H^+) and eluted with 9:1 (v/v) methanol–water to give a product, which was neutralized with aq. $NaHCO_3$ then concentrated *in vacuo*. A solution of the residue in 4:1 (v/v) ethanol–water (3 mL) was stirred with 10% Pd-on-charcoal (20 mg) under hydrogen atmosphere for 10 h, then filtered and washed with 4:1 (v/v) ethanol–water. The filtrate and washings were concentrated *in vacuo* to give a residue, which was dissolved in 4:1 (v/v) methanol–water, applied to a column of Dowex-50W × 8 (H^+) and eluted with 4:1 (v/v) methanol–water. The fractions containing the product were collected and concentrated to dryness. The residue was redissolved in water and lyophilized, to give **23** (5.0 mg, 74%) as a hygroscopic, white amorphous solid, $[\alpha]_D^{23}$ −33.5° (*c* 0.2, H_2O) [lit.[23] $[\alpha]_D^{25}$ −32° (H_2O)]. H.r.f.a.b.-m.s.: Calc. for $C_{14}H_{24}NO_{12}$ $(M + H)^+$: 398.1298. Found: 398.1315. ^{1}H-N.m.r. data (D_2O): δ 1.96 (s, 3 H, CH_3CO), 3.31 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.15 Hz, H-2′), 3.42–3.52 (m, 3 H, H-3′,4′,5), 3.65–3.87 (m, 4.35 H, H-2 of β anomer and H-3,4,6a,b), 3.93 (d, 0.35 H, $J_{4',5'}$ 9.8 Hz, H-5′ of β anomer), 3.94 (d, 0.65 H, $J_{4',5'}$ 9.5 Hz, H-5′ of α anomer), 4.00 (dd, 0.65 H, $J_{1,2}$ 3.6, $J_{2,3}$ 10.7 Hz, H-2 of α anomer), 4.47 (dd, 0.35 H, $J_{1',2'}$ 7.9 Hz, H-1′ of β anomer), 4.52 (d, 0.65 H, $J_{1',2'}$ 7.9 Hz, H-1′ of α anomer), 4.68 (d, 0.35 H, $J_{1,2}$ 8.5 Hz, H-1 of β anomer), 5.11 (d, 0.65 H, $J_{1,2}$ 3.6 Hz, H-1 of α anomer).

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REFERENCES

1 I. Fujimaki, Y. Ichikawa, and H. Kuzuhara, *Carbohydr. Res.*, 101 (1982) 148–151.
2 Y. Ichikawa, A. Manaka, and H. Kuzuhara, *Carbohydr. Res.*, 138 (1985) 55–64.
3 N. Sakairi, M. Hayashida, and H. Kuzuhara, *Carbohydr. Res.*, 185 (1989) 91–104.
4 S. I. Nishimura, H. Kuzuhara, Y. Takiguchi, and K. Shimahara, *Carbohydr. Res.*, 194 (1989) 223–231.
5 N. Sakairi and H. Kuzuhara, *Tetrahedron Lett.*, 23 (1982) 5327–5330.

6 Y. Ichikawa, R. Monden, and H. Kuzuhara, *Tetrahedron Lett.*, 27 (1986) 611–614.
7 M. Hayashida, N. Sakairi, and H. Kuzuhara, *Carbohydr. Res.*, 158 (1986) c5–c8.
8 Y. Ichikawa, R. Monden, and H. Kuzuhara, *Carbohydr. Res.*, 172 (1988) 37–64.
9 N. Sakairi, M. Hayashida, A. Amano, and H. Kuzuhara, *J. Chem. Soc., Perkin Trans. 1*, (1990) 1301–1313.
10 S. I. Nishimura and H. Kuzuhara, *Carbohydr. Res.*, 206 (1990) 207–217.
11 R. L. Whistler, A. A. Bushway, P. P. Singh, W. Nakahara, and R. Tokuzen, *Adv. Carbohydr. Chem. Biochem.*, 32 (1976) 235–275.
12 (a) K. Sugahara, I. Yamashina, P. D. Waard, H. V. Halbeek, and J. F. G. Vliegenthart, *J. Biol. Chem.*, 263 (1988) 10168–10174. (b) G. Ekborg, T. Curenton, N. R. Krishna, and L. Rodén, J. Carbohydr. Chem., 9 (1990) 15–37.
13 S. Hakomori, *Ann. Rev. Immunol.*, 2 (1984) 103–126.
14 J. Thiem, A. Sievers, and H. Karl, *J. Chromatogr.*, 130 (1977) 305–313.
15 T. G. Villa, H. J. Phaff, and V. Notario, *Carbohydr. Res.*, 74 (1979) 369–370.
16 (a) A. Totsuka and T. Usui, *Agric. Biol. Chem.*, 50 (1986) 543–550; (b) T. Usui, K. Totani, A. Totsuka, and M. Oguchi, *Biochim. Biophys. Acta*, 840 (1985) 255–263. (c) N. Ohno, I. Nono, and T. Yadomae, *Carbohydr. Res.*, 194 (1989) 261–271.
17 J. Thiem and H. Karl, *Chem. Ber.*, 112 (1979) 1046–1056.
18 R. U. Lemieux and R. M. Ratcliffe, *Can. J. Chem.*, 57 (1979) 1244–1251.
19 M. E. Evans, *Carbohydr. Res.*, 21 (1972) 473–475.
20 M. Iwata and H. Ohrui, *Bull. Chem. Soc. Jpn.*, 54 (1981) 2837–2838.
21 J. C. Jacquinet, M. Petitou, P. Duchaussoy, I. Lederman, J. Choay, G. Torri, and P. Sinaÿ, *Carbohydr. Res.*, 130 (1984) 221–241.
22 H. Paulsen and V. Sinnwell, *Chem. Ber.*, 111 (1978) 869–889.
23 B. Weissmann and K. Meyer, *J. Am. Chem. Soc.*, 76 (1954) 1753–1757.
24 S. Takanashi, T. Kawaguchi, and M. Kawada, *Chem. Pharm. Bull.*, 14 (1966) 1433–1434.
25 M. L. Wolfrom and A. Thompson, *Methods Carbohydr. Chem.*, 2 (1963) 211–215.
26 P. Bachli and E. G. V. Percival, *J. Chem. Soc.* (1952) 1243–1246.
27 S. Peat, W. J. Whelman, and H. G. Lawley, *J. Chem. Soc.*, (1958) 724–728.