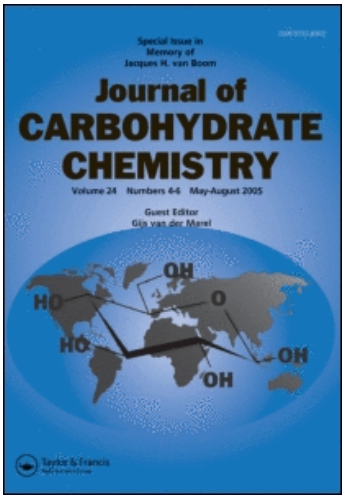

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SYNTHESIS OF THE *VIBRIO CHOLERAE* O1 OGAWA AND INABA TERMINAL DISACCHARIDES WITH DIOXOLANE-TYPE SPACERS AND THEIR COUPLING TO PROTEINS[1]

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ABSTRACT

The disaccharide, which corresponds to the terminal fragment of the *Vibrio cholerae* O1 LPS, was prepared starting from the corresponding trichloroacetimidate derivative of the monosaccharide in the presence of trimethylsilyl triflate. After selective reduction of the azido group, the reaction with 2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronic acid in the presence of EEDQ afforded the corresponding amides. The cleavage of dioxolane protecting group followed by careful deacetylation and coupling with Bovine Serum Albumin or Meningococcal Outer Membrane Protein in the presence of sodium cyanoborohydride gave the corresponding neoglycoconjugates.

INTRODUCTION

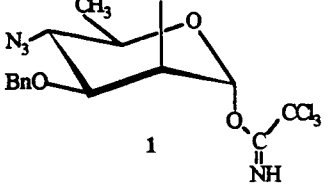
Only three serotypes of *Vibrio cholerae*, O1 Ogawa, O1 Inaba and O139 Bengal,[2] are able to cause cholera in humans. Current vaccines against cholera afford only short-term and low efficacy protection. Among the antigens that should be considered for

vaccine development are the lipopolysaccharides. These compounds are important targets for vibriocidal antibodies and are also responsible for the serotype specificity.[3] The polysaccharides belonging to the two first serotypes are linear homopolymers composed of α-(1→2)-linked *N*-(3-deoxy-L-*glycero*-tetronyl)-D-perosamine.[4,5] It was demonstrated that 2-*O*-methyl-D-perosamine occurred at the nonreducing terminus,[6,7] suggesting that its antigenic determinant is associated with the methyl group.

The lack of a protective vaccine against cholera prompted the synthesis of LPS oligosaccharidic fragments[8,9] as part of several approaches to develop a conjugated vaccine. In a previous paper,[8] we described the synthesis of the terminal disaccharide using coupling of 2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronic acid as the key step. Here, we describe the synthesis of terminal disaccharides with dioxolane-type spacer arms and demonstrate their usefulness for the preparation of neoglycoproteins.

RESULTS AND DISCUSSION

A new perosamine donor **1** was obtained in 84% yield from the corresponding 1,2-di-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranose[10] by chemoselective deacetylation using ethanolamine in ethyl acetate[11] followed by imidation with trichloroacetonitrile and potassium carbonate in dichloromethane. The reaction of **1** with 4-(1,3-dioxolan-2-yl)-3-oxabutanol[12] (**2**) proceeded smoothly in the presence of trimethylsilyl triflate affording the spacer-arm perosaminide **3** in 68% yield. The presence of the spacer in compound **3** and throughout the synthetic sequence was confirmed by the observation of triplets at 5.00 - 5.15 ppm in the ^{1}H NMR spectra and



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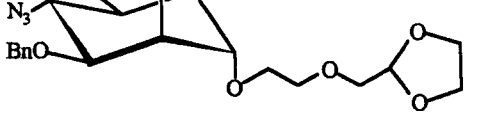
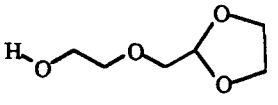


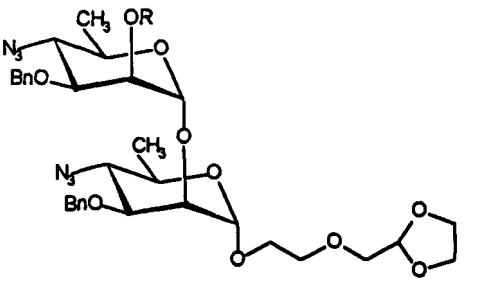
3 R = Ac
4 R = H
5 R = CH_3



2

also by signals at 102 and 65 ppm in the ^{13}C NMR spectra, corresponding to the dioxolane carbons.

After deacetylation of compound **3**, the resulting free hydroxyl group was either methylated to give the terminal Ogawa monosaccharide **5**[6,7] in 65% yield or glycosidated with the same perosamine donor **1** in the presence of triethylsilyl triflate as catalyst to afford the corresponding disaccharide **6** in 54% yield. The structure of **6** was ascertained by the presence of two doublets due to H-1' and H-1 at 4.80 and 4.70 ppm in the ^1H NMR spectrum that correlated in the H-C COSY spectrum with ^{13}C NMR signals at 98.3 and 98.7 ppm, due to C-1' and C-1, respectively.



6 R = Ac
7 R = H
8 R = CH_3

The migration of an acetyl group to the glycosyl acceptor previously detected when coupling was performed using a glycosyl halide and silver triflate[8] was not observed under these conditions. Deacetylation of **6** under Zemplen conditions followed by methylation gave the terminal Ogawa disaccharide **8** in 65% yield.

The previously described[8] selective reduction of the azido function in the presence of benzyl groups was then used for monosaccharides **3** and **5** and for disaccharides **6** and **8**, followed by the reaction with 2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronic acid (prepared from L-malic acid as previously described[8]) in the presence of EEDQ in dichloromethane. The amides **9**, **10**, **11** and **12** were formed in yields of 55-85% and were characterized by the presence in the ^1H NMR spectra of one or two doublets in the 6-7 ppm region.

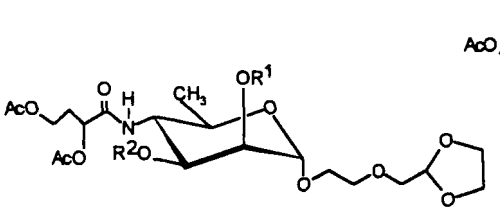
Hydrogenolysis of benzyl groups proceeded successfully in the presence of 5% Pd/C for the amides. Next, re-*O*-acetylation gave the per-*O*-acetylated mono- (**13**, **14**) and disaccharides (**15**, **16**) in 82 - 88% yields.

Deprotection of the aldehyde function was performed with formic acid as previously established.[12,13] Careful deacetylation with 0.1 M sodium methoxide in methanol at 0 °C afforded the free monosaccharides (**17**, **18**) and disaccharides (**19**, **20**)

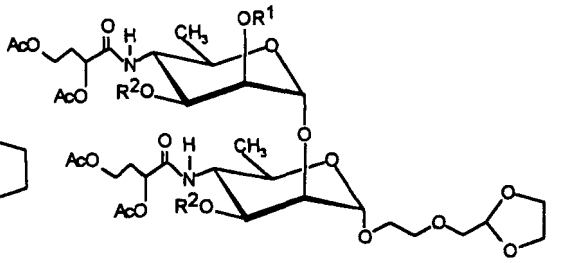
with the spacer aldehyde group. The coupling with BSA using reductive amination[14] gave conjugates containing 23-34 mol of the oligosaccharide per mol of BSA. These neoglycoproteins were remarkably useful[15] for the study of the molecular specificity of anti-Ogawa[16] and anti-Inaba antibodies.[16]

In order to obtain neoglycoproteins with vaccine potential, the same reaction was performed with the highly immunogenic meningococcal Outer Membrane Protein complex (OMP). The carbohydrate to protein ratio 1/5-10 (mg/mg) obtained confirm the efficiency of the conjugation procedure through dioxolane-type spacer arms.

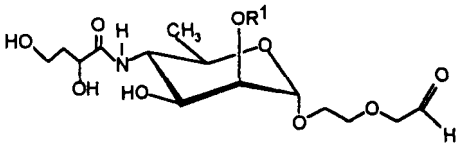
The immunogenicity of disaccharide-OMP conjugates and their use as potential vaccines is now under investigation in laboratory animals and will be published in a separate communication.



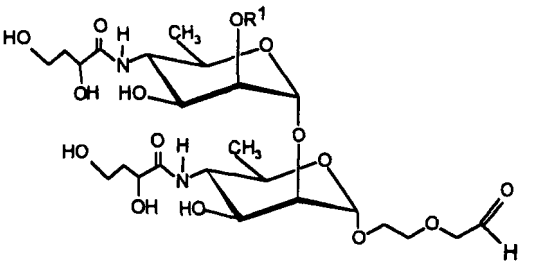
9 R^1 = Ac; R^2 = Bn
10 R^1 = CH_3; R^2 = Bn
13 R^1 = Ac; R^2 = Ac
14 R^1 = CH_3; R^2 = Ac



11 R^1 = Ac; R^2 = Bn
12 R^1 = CH_3; R^2 = Bn
15 R^1 = Ac; R^2 = Ac
16 R^1 = CH_3; R^2 = Ac



17 R^1 = H
18 R^1 = CH_3



19 R^1 = H
20 R^1 = CH_3

Table 1. ^{13}C NMR spectral data[a] for compounds **1-16**

compd	Perosamine						Perosamine'					
	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C1'**	**C2'**	**C3'**	**C4'**	**C5'**	**C6'**
1	94.1	69.9	75.2	63.3	65.8	18.5						
3	97.8	67.3	76.1	64.0	66.9	18.5						
5	97.3	78.0	76.2	64.1	66.9	18.3						
6	98.7	73.7	75.3	63.8	67.1	18.5	98.3	66.7	77.6	64.1	67.2	18.5
8	98.8	73.4	77.0	64.1	67.0	18.5	98.9	76.4	78.1	64.4	67.7	18.5
9	97.4	66.8	73.3	52.0	67.2	17.6						
10	97.7	75.9	75.6	52.6	67.4	17.9						
11	98.6	74.1	74.9	51.5	67.5	17.6	99.5	66.7	72.3	52.1	68.5	17.8
12	98.9	73.8	75.8	52.2	68.6	18.0	99.6	74.7	75.9	52.9	67.7	18.0
13	97.1	69.2	68.3	51.3	67.8	17.7						
14	97.5	77.8	71.0	51.4	68.2	17.8						
15	98.2	76.1	71.9	51.5	68.0	17.7	99.1	69.7	70.8	51.5	67.0	17.5
16	98.1	74.8	71.1	51.8	69.1	17.8	99.3	77.9	70.5	51.7	68.0	17.9

a. Chemical shifts for other carbons are as follows: **Spacer-arm, dioxolane ring** 102.3 102.5, 65.1- 64.8 ppm, **OCH_2CH_2O** 72.3-70.7 ppm; **2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronyl, C-2** 71.1-70.8 ppm, **C-3** 30.6-30.8 ppm, **C-4** 50.7-50.9 ppm; **Bn, C_6H_5** 137.6-137.4 and 127.4-129.3 ppm, **PhCH_2** 71.6-71.4 ppm; **CH_3CO, C=O** 169.9-169.3 ppm, **CH_3** 20.5-20.0 ppm; **OCH_3** 59.3-58.8 ppm.

EXPERIMENTAL

General procedures. Optical rotations were measured at 25 °C with a POLAMAT A automatic polarimeter, using a 5 cm 5 mL cell. NMR spectra were recorded at 25 °C with a BRUKER AC-250F spectrometer. Chemical shifts (δ) are given in ppm relative to the signal for internal tetramethylsilane for ^{1}H NMR spectra and indirectly to $CDCl_3$, δ 77.03 for ^{13}C NMR spectra. Assignments were performed on the basis of homonuclear and heteronuclear correlation experiments. The following notation is used to define the NMR signals: **p** for the perosamine linked to the spacer, **p'** or just **'** for the second perosamine unit, **t** for the L-*glycero*-tetronyl moiety.

All compounds were purified by column chromatography on Kieselgel 60 (Fluka, < 230 mesh ASTM) and fractions were monitored by TLC on Kieselgel 60 F_{254} (Merck). Detection was effected by charring with a 5% solution of concentrated sulfuric acid in ethanol after examination under UV light. Concentrations were conducted under reduced pressure at 40 °C (bath).

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 2-*O*-Acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (3). 2-Aminoethanol (0.06 mL) was added to a solution of 1,2-di-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranose[17] (188 mg, 0.5 mmol) in ethyl acetate (3 mL). After the solution had been stirred for 4 h, TLC (dichloromethane/acetone 8:1 v/v) showed complete transformation of the starting diacetate into a more polar compound (R_f 0.7). The solution was washed with water until the washings were neutral and the organic phase was dried (anhydrous Na_2SO_4), filtered, concentrated and dried *in vacuo*.

The resulting syrup was stirred in the presence of K_2CO_3 (0.142 g) and trichloroacetonitrile (0.2 mL) in dry dichloromethane (3 mL) for 24 h. Then the suspension was filtered through Celite and the filtrate concentrated *in vacuo* to give *2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl trichloroacetimidate* (**1**), isolated as a syrup (0.202 g, 84%): R_f 0.65 (dichloromethane/acetone 4:1 v/v); $[\alpha]_D$ -47.0° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 8.29 (s, 1H, $NHCCl_3$), 7.40-7.20 (m, 5H, Ph), 6.28 (d, 1H, H-2), 5.50 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1), 4.60 (s, 2H, PhCH_2), 3.89 (dd, 1H, H-3), 3.75 (m, 1H, H-5), 3.5 (dd, 1H, H-4), 2.25 (s, 3H, CH_3CO), 1.49 (d, 3H, H-6s).

A solution of **1** (378 mg, 0.81 mmol) and 4-(1,3-dioxolan-2-yl)-3-oxabutanol (**2**) (132 mg, 0.89 mmol) in anhydrous dichloromethane (5 mL) was stirred in the presence of molecular sieves (4Å, 315 mg) at rt for 5 min. Trimethylsilyl triflate (0.059 mL, 0.32 mmol) was added dropwise. After 15 min, the reaction was quenched with triethylamine. The mixture was filtered and the filtrate concentrated under reduced pressure. The residue was purified by column chromatography (hexane/ethyl acetate 5:1 v/v) to give **3**, isolated as a syrup (251 mg, 68%): $[\alpha]_D$ +83° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.40-7.20 (m, 5H, Ph), 5.45 (dd, 1H, $J_{2,3}$ = 3.3 Hz, H-2), 5.10 (t, 1H, dioxolane), 4.87 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.64 (AB q, 2H, PhCH_2), 4.06-3.90 (m, 4H, 2CH_2 dioxolane), 3.90-3.85 (m, 5H, 2CH_2 spacer and H-3), 3.70-3.65 (m, 1H, H-5), 3.54 (d, 2H, CH_2 spacer), 3.48 (dd, 1H, H-4), 2.19 (s, 3H, CH_3CO), 1.47 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6s).

Anal. Calcd for $C_{21}H_{29}O_8N_3$ (451.46): C, 55.87; H, 6.47; N, 9.31. Found: C, 56.01; H, 6.52; N, 9.10.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 4-Azido-3-*O*-benzyl-2-*O*-methyl-4,6-dideoxy-α-D-mannopyranoside (5). To a solution of **3** (0.33 mg, 0.73 mmol) in methanol (2 mL) was added methanolic sodium methoxide (3 mL) and the reaction was stirred for 1 h at rt. The reaction mixture was neutralized with Dowex-50 (H^+) resin, then filtered and the filtrate concentrated to give *4-(1,3-dioxolan-2-yl)-3-oxabutyl 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranoside* (**4**) as a syrup.

Compound **4** (0.3 g, 0.7 mmol) was dissolved in *N,N*-dimethylformamide (5 mL) and sodium hydride (35 mg, 1.4 mmol) was added at 0 °C. After 30 min, methyl iodide (0.05 mL, 0.8 mmol) was added at 0 °C and the mixture was stirred for an additional 24 h at 25 °C. Methanol was then added to destroy the excess sodium hydride. The resulting solution was concentrated and the residue purified by column chromatography (hexane/ethyl acetate 2:1 v/v). Compound **5** was isolated as a syrup (0.29 g, 65%): $[\alpha]_D$ +92° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.40-7.29 (m, 5H, Ph), 5.05 (t, 1H, dioxolane), 4.86 (s, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 4.68 (s, 2H, PhCH_2), 3.97-3.95 (m, 2H, CH_2 spacer), 3.93 (dd, 1H, H-3), 3.89-3.78 (m, 2H, CH_2 spacer), 3.75-3.62 (m, 6H, 2CH_2 spacer, H-2 and H-5), 3.55 (dd, 1H, H-4), 3.53-3.50 (d, 2H, CH_2 spacer), 3.50 (s, 3H, OCH_3), 1.26 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6s).

Anal. Calcd for $C_{20}H_{29}O_7N_3$ (423.43): C, 56.72; H, 6.90; N, 9.92. Found: C, 56.40; H, 6.87; N, 10.01.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 2-*O*-(2-*O*-Acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (6). A solution of compound **4** (100 mg, 0.24 mmol) and trichloroacetimidate **1** (137 mg, 0.29 mmol) in dry dichloromethane (10 mL) was stirred in the presence of molecular sieves (4Å, 0.5 g) for 15 min. The mixture was cooled to -60 °C under nitrogen, then trimethylsilyl triflate (0.008 mL) was added and the mixture was stirred while the temperature was allowed to rise slowly (-60 °C → 25 °C). TLC (hexane/ethyl acetate 2:1 v/v) showed the presence of one major product (R_f 0.59). The mixture was diluted with dichloromethane (10 mL), neutralized with triethylamine, filtered and the filtrate was concentrated. The residue was purified by column chromatography (hexane/ethyl acetate 6:1 v/v) to afford **6**, isolated as a syrup (90 mg, 54%): $[\alpha]_D$ +42° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.40–7.25 (m, 10H, 2Ph), 5.47 (dd, 1H, $J_{2',3'}$ = 3.5 Hz, H-2'), 5.15 (t, 1H, dioxolane), 4.85 (d, 1H, $J_{1',2'}$ = 1.3 Hz, H-1'), 4.76 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.75-4.60 (2AB q, 4H, 2PhCH_2), 4.00-3.92 (m, 3H, H-2 and CH_2 spacer), 3.89-3.82 (m, 2H, CH_2 spacer), 3.80-3.75 (m, 2H, H-3, 3'), 3.75-3.65 (m, 4H, 2CH_2 spacer), 3.60-3.50 (m, 2H, H-5, 5'), 3.55 (d, 2H, CH_2 spacer), 3.45-3.32 (m, 2H, H-4, 4'), 2.28 (s, 3H, CH_3CO), 1.45-1.32 (2d, 6H, H-6, 6').

Anal. Calcd for $C_{34}H_{44}O_{11}N_6$ (712.74): C, 57.29; H, 6.22; N, 11.79. Found: C, 57.42; H, 6.14; N, 11.86.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 2-*O*-(4-Azido-3-*O*-benzyl-2-*O*-methyl-4,6-dideoxy-α-D-mannopyranosyl)-4-azido-3-*O*-benzyl-2-*O*-methyl-4,6-dideoxy-α-D-mannopyranoside (8). Transformation of **6** (0.172 g, 0.24 mmol), as described above for **5**, afforded **8**, also isolated as a syrup (95 mg, 65%): $[\alpha]_D$ +51° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.45-7.30 (m, 5H, Ph), 5.05 (t, 1H, CH dioxolane), 4.93 (d, 1H, $J_{1',2'}$ = 1.6 Hz, H-1'), 4.87 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.83-4.59 (2AB, 4H, PHCH_2), 4.05-3.85 (m, 5H, 2CH_2 dioxolane and H-2), 3.80-3.65 (m, 6H, H-3, 3', 2CH_2 spacer), 3.55 (d, 2H, CH_2 spacer), 3.55-3.48 (m, 3H, H-4, 5, 5'), 3.40 (dd, 1H, H-2'), 3.25 (dd, 1H, H-4'), 3.05 (s, 3H, OCH_3), 1.38, 1.25 (2d, 6H, H-6, 6').

Anal. Calcd for $C_{33}H_{44}O_{10}N_6$ (684.73): C, 57.88; H, 6.48; N, 12.27. Found: C, 57.92; H, 6.40; N, 12.21.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 2-*O*-Acetyl-3-*O*-benzyl-4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronyl)-α-D-mannopyranoside (9). A solution of azide **3** (0.130 g, 0.2 mmol) in absolute ethanol (2 mL) was stirred in the presence of Pd/C (5%, 63 mg) under a hydrogen atmosphere. After 2 h, TLC (hexane/ethyl acetate 1:1 v/v) revealed complete conversion of the starting material into a new compound (R_f 0.0, ninhydrin test). The mixture was filtered, and the filtrate was concentrated and dried *in vacuo*.

To a solution of the resulting amine (0.10 g, 0.23 mmol) and 2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronic acid (0.095 g, 0.47 mmol) in dichloromethane (0.5 mL) was added EEDQ (0.116 g, 0.47 mmol) and the resulting solution was stirred for 15 min, when TLC (hexane/ethyl acetate 3:1 v/v) showed complete conversion of the starting material. The reaction mixture was concentrated and the residue purified by column chromatography on silica gel (hexane/ ethyl acetate 4:1 v/v) to give the title compound **9**, isolated as a syrup: yield 0.120 g (85%); $[\alpha]_D$ +63° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.4–7.2 (m, 5H, Ph), 6.13 (d, 1H, $J_{4,NH}$ = 9.6 Hz, NHCO), 5.39 (d, 1H, $J_{2,3}$ = 3.3 Hz, H-2p), 5.18 (dd, 1H, H-2t), 5.03 (t, 1H, dioxolane), 4.82 (s, 1H, $J_{1,2}$ = 1.9 Hz, H-1p), 4.65, 4.37 (AB q, 2H, PhCH_2), 4.14-4.08 (m, 2H, H-4t), 4.03 (m, 1H, H-4p), 3.98-3.95 (m, 4H, 2CH_2 dioxolane), 3.87-3.86 (m, 2H, H-3p, 5p), 3.80 (m, 2H, CH_2 spacer), 3.69-3.60 (m, 2H, CH_2 spacer), 3.55 (dd, 2H, CH_2 spacer), 2.15, 2.10, 2.05 (3s, 9H, 3CH_3CO), 2.15-2.10 (m, 2H, H-3t), 1.28 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6p).

Anal. Calcd for $C_{29}H_{41}N_1O_{13}$ (611.62): C, 56.94; H, 6.70; N, 2.29. Found: C, 56.77; H, 6.61; N, 2.31.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 3-*O*-Benzyl-2-*O*-methyl-4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronyl)-α-D-mannopyranoside (10). Treatment of azide **5** (0.140 g, 0.32 mmol) as for the preparation of **9** gave, after chromatography using (hexane/ethyl acetate 1:1 v/v) as eluent, compound **10**, isolated as a syrup: yield 0.158 g (82%); $[\alpha]_D$ + 98° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.43-7.26 (m, 5H, Ph), 6.28 (d, 1H, $J_{4,NH}$ = 9.6 Hz, NHCO), 5.23 (dd, 1H, H-2t), 5.15 (t, 1H, CH dioxolane), 4.92 (s, 1H, $J_{1,2}$ = 1.3 Hz, H-1p), 4.46 (AB q, 2H, PhCH_2), 4.18 (m, 2H, H-4t), 4.09 (dd, 1H, H-4p),

4.05-3.91 (m, 4H, $2CH_2$ dioxolane), 3.88-3.80 (m, 2H, H-3p, 5p), 3.75-3.66 (m, 4H, $2CH_2$ spacer), 3.64 (dd, 1H, H-2p), 3.55 (d, 2H, CH_2 spacer), 3.56 (s, 3H, OCH_3), 2.26-2.17 (m, 2H, H-3t), 2.15-2.05 (2s, 6H, $2CH_3CO$), 1.29 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6s).

Anal. Calcd for $C_{28}H_{41}N_1O_{12}$ (583.62): C, 57.62; H, 7.08; N, 2.40. Found: C, 57.64; H, 7.12; N, 2.36.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 2-*O*-[2-*O*-Acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronyl)-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl]- 4- (2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronyl)-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (11). Treatment of azide **6** (0.189 g, 0.26 mmol) as for the preparation of **9** after chromatography using (hexane/ethyl acetate 1:1 v/v) as eluant gave compound **11**, isolated as a syrup: yield 0.160 g (59%); $[\alpha]_D$ +37° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.46-7.29 (m, 10H, Ph), 6.16 (d, 1H, $J_{4,NH}$ = 9.8 Hz, NHCO), 5.99 (d, 1H, $J_{4,NH}$ = 9.5 Hz, NHCO), 5.59 (dd, 1H, $J_{2',3'}$ = 3.6 Hz, H-2p'), 5.28 (dd, 1H, H-2t), 5.15 (t, 1H, CH dioxolane), 4.98 (s, 1H, $J_{1',2'}$ = 1.9 Hz, H-1p'), 4.85 (s, 1H, $J_{1,2}$ = 1.8 Hz, H-1p), 4.60, 4.50 (AB q, 4H, $2PhCH_2$), 4.28-4.05 (m, 5H, H-4t, 4t', 2p, 4p, 4p'), 4.08-3.95 (m, 4H, $2CH_2$ dioxolane), 3.95-3.78 (m, 6H, H-3p, 3p', 5p, 5p', CH_2 spacer), 3.69-3.50 (m, 2H, CH_2 spacer), 3.58 (d, 2H, CH_2 spacer), 2.15-2.00 (m, 6H, H-3t), 2.2-2.0 (5s, 15H, $5CH_3CO$), 1.27 (2d, 6H, H-6p, 6p').

Anal. Calcd for $C_{50}H_{63}O_{21}N_2$ (730.66): C, 82.28; H, 9.38; N, 3.83. Found: C, 82.29; H, 9.20; N, 3.90.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 2-*O*-[4-(2,4-Di-*O*-acetyl-3-deoxy-L-*glycero*-tetronyl)-3-*O*-benzyl-2-*O*-methyl-4,6-dideoxy-α-D-mannopyranosyl]-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronyl)-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (12). Treatment of azide **8** (0.046 g, 0.067 mmol) as for the preparation of **9** gave, after chromatography using (hexane/ethyl acetate 1:1 v/v) as eluent, compound **12**, isolated as a syrup: yield 0.037 g (55%); $[\alpha]_D$ +42° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.48-7.20 (m, 10H, Ph), 6.26 (d, 1H, $J_{4,NH}$ = 9.6 Hz, NHCO), 5.98 (d, 1H, $J_{4,NH}$ = 9.4 Hz, NHCO), 5.24 (dd, 1H, H-2t), 5.19 (t, 1H, CH dioxolane), 5.07 (s, 1H, $J_{1',2'}$ = 1.5 Hz, H-1p'), 4.88 (s, 1H, $J_{1,2}$ = 1.7 Hz, H-1p), 4.60, 4.50 (2AB q, 4H, $2PhCH_2$), 4.25-4.08 (m, 5H, H-4t, 4t', 2p, 4p, 4p'), 4.08-3.90 (m, 4H, $2CH_2$ dioxolane), 3.96-3.75 (m, 6H, H-3p, 3p', 5p, 5p', CH_2 spacer), 3.75-3.66 (m, 3H, H-2p', CH_2 spacer), 3.58 (dd, 2H, CH

spacer), 3.35 (s, 3H, OMe), 2.15-2.20 (m, 6H, H-3t), 2.25-2.09 (4s, 12H, $4CH_3CO$), 1.26 (2d, 6H, H-6p, 6p').

Anal. Calcd for $C_{49}H_{68}O_{20}N_2$ (1005.05): C, 58.55; H, 6.82; N, 2.79. Found: C, 58.39; H, 6.93; N, 2.85.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 2,3-Di-*O*-acetyl-4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronyl)-α-D-mannopyranoside (13). The protected amide **9** (50 mg, 0.08 mmol) in absolute ethanol was hydrogenated over Pd/C (5%, 45 mg). After 24 h, TLC (hexane/ethyl acetate 1:2 v/v) showed complete conversion into a new compound (R_f 0.2). The reaction mixture was filtered and the filtrate concentrated.

A solution of the residue in pyridine (1 mL) was cooled to 0-5 °C and acetic anhydride (0.4 mL) was added. The resulting solution was stirred for 2 h, then concentrated and co-concentrated with toluene (3 x 10 mL). Column chromatography (hexane/ethyl acetate 1:1 v/v) of the residue afforded **13** as a syrup (34 mg, 90%): $[\alpha]_D$ +33° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 6.25 (d, 1H, $J_{4,NH}$ = 9.5 Hz, NHCO), 5.32 (dd, 1H, $J_{3,4}$ = 10.3 Hz, H-3p), 5.15 (dd, 1H, $J_{2,3}$ = 3.3 Hz, H-2p), 5.11-5.05 (m, 2H, CH dioxolane and H-2t), 4.85 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1p), 4.28-4.19 (m, 1H, H-4p), 4.15-4.10 (m, 2H, H-4t), 4.08-3.96 (m, 4H, $2CH_2$ dioxolane), 3.85-3.75 (m, 4H, $2CH_2$ spacer), 3.8 (m, 1H, H-5p), 3,55 (d, 2H, CH_2 spacer), 2.15 (m, 2H, C-3t), 2.15-2.05 (4s, 12H, $4CH_3CO$), 1.26 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6s).

Anal. Calcd for $C_{24}H_{37}O_{14}N_1$ (563.55): C, 51.15; H, 6.62; N, 2.48. Found: C, 51.31; H, 6.79; N, 2.55.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 3-*O*-Acetyl-4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronyl)-2-*O*-methyl-α-D-mannopyranoside (14). Compound **14** was prepared from compound **10** (0.025 g, 0.04 mmol) by the same sequence as described for **13**. The yield of **14** isolated as a syrup was 0.020 g (88%): $[\alpha]_D$ +37° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 6.36 (d, 1H, $J_{4,NH}$ = 9.5 Hz, NHCO), 5.27 (dd, 1H, $J_{3,4}$ = 10.5 Hz, H-3p), 5.15-5.10 (m, 2H, CH dioxolane and H-2t), 4.88 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-1p), 4.28-4.15 (m, 3H, H-4p, H-4t), 4.08-3.95 (m, 4H, $2CH_2$ dioxolane), 3.85 (m, 2H, CH_2 spacer), 3.85-3.70 (m, 3H, H-5p, CH_2 spacer), 3.60 (d, 2H, CH_2 spacer), 3.55 (dd, 1H, H-2p), 3.50 (s, 3H, OCH_3), 2.15 (m, 2H, H-3t), 2.15-2.05 (3s, 9H, $3CH_3CO$), 1.26 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6s).

Anal. Calcd for $C_{27}H_{37}O_{13}N_1$ (583.58): C, 55.56; H, 6.39; N, 2.40. Found: C, 55.48; H, 6.11; N, 2.32.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 2-*O*-[2,3-di-*O*-Acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronyl)-4,6-dideoxy-α-D-mannopyranosyl]- 4 -(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronyl)-3-*O*-acetyl-4,6-dideoxy-α-D-mannopyranoside (15). The title compound (**15**) was prepared from compound **11** (0.035 g, 0.034 mmol) by the same sequence as described for **13**. The yield of **15**, a syrup, was 0.026 g (82%): $[\alpha]_D$ +48° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 6.35 (d, 1H, $J_{4,NH}$ = 9.6 Hz, NHCO), 6.08 (d, 1H, $J_{4,NH}$ = 9.7 Hz, NHCO), 5.46 (d, 1H, $J_{2,3}$ = 3.5 Hz, H-2p'), 5.24 (m, 2H, H-3p, 3p'), 5.18-5.00 (m, 3H, H-2t, 2t', CH dioxolane), 4.95 (s, 1H, $J_{1,2}$ = 1.3 Hz, H-1p), 4.88 (s, 1H, $J_{1',2'}$ = 1.5 Hz, H-1p'), 4.35-4.18 (m, 6H, H-4t, 4t', 4p, 4p'), 4.03-3.95 (m, 5H, H-2p and $2CH_2$ dioxolane), 3.95-3.78 (m, 6H, H-5p, 5p' and $2CH_2$ spacer), 3.59 (dd, 2H, CH_2 spacer), 2.38-2.32 (m, 4H, H-3t, 3t'), 2.35-2.00 (7s, 21H, $7CH_3CO$), 1.28 (2d, 6H, H-6p, 6p').

Anal. Calcd for $C_{40}H_{59}O_{23}N_2$ (935.89): C, 51.33; H, 6.35; N, 2.87. Found: C, 51.51; H, 6.49; N, 2.94.

4-(1,3-Dioxolan-2-yl)-3-oxabutyl 2-*O*-[3-*O*-Acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronyl)-2-*O*-methyl-4,6-dideoxy-α-D-mannopyranosyl]-3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronyl)-4,6-dideoxy-α-D-mannopyranoside (16). The title compound (**16**) was prepared from compound **12** (0.028 g, 0.040 mmol) by the same sequence as described for **13**. Compound **16**, a syrup, was isolated in a yield of 0.030 g (85%): $[\alpha]_D$ +25° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 6.45 (d, 1H, $J_{4,NH}$ = 9.7 Hz, NHCO), 6.33 (d, 1H, $J_{4,NH}$ = 9.5 Hz, NHCO), 5.38 (d, 1H, H-3p'), 5.22 (dd, 1H, H-3p), 5.15-5.10 (m, 3H, H-2t, 2t', CH dioxolane), 5.10 (s, 1H, $J_{1,2}$ = 1.7 Hz, H-1p), 4.86 (s, 1H, $J_{1,2}$ 1.5 Hz, H-1p'), 4.33-4.08 (m, 8H, H-4t, 4t', 4p, 4p', $2CH_2$ dioxolane), 4.00-3.85 (m, 5H, H-2p and $2CH_2$ spacer), 3.85-3.60 (m, 6H, H-5p, 5p', 2p'), 3.55 (d, 2H, CH_2 spacer), 3.50 (s, 3H, OCH_3), 2.28-2.00 (m, 4H, H-3t, 3t'), 2.20-2.00 (6s, 18H, $6CH_3CO$), 1.24-1.16 (2d, 6H, H-6p, 6p').

Anal. Calcd for $C_{39}H_{60}O_{22}N_2$ (908.89): C, 51.53; H, 6.65; N, 3.08. Found: C, 51.21; H, 6.69; N, 3.13.

General Procedure for Cleavage of Dioxolane Rings, for Deacetylation, and for Conjugation with Proteins. The oligosaccharide derivatives (**13-16**) were dissolved

in formic acid at rt and the solution was stirred for 2 h. TLC (hexane/ethyl acetate 1:6 v/v) indicated the disappearance of the starting material and the appearance of a new spot (aniline phthalate positive). The acid was removed with nitrogen gas at rt and toluene was coevaporated several times from the residue; ^{1}H NMR ($CDCl_3$) δ 9.75-9.72 (s, 1H, HC=O).

The residues (free of formic acid) were dissolved in a solution of sodium methoxide in anhydrous methanol (0.1 M, pH 9) at 0 °C. After 45 min, TLC (ethyl acetate/methanol 20:1 v/v) revealed the complete de-*O*-acetylation of the starting material. The mixture was neutralized with acetic acid, concentrated at room temperature and used directly for conjugation.

The oligosaccharide content was estimated before and after conjugation using the phenol-sulfuric acid method, with 4-(1,3-dioxolan-2-yl)-3-oxabutyl 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronyl)-α-D-mannopyranoside as standard. A solution of the oligosaccharide (0.023 mmol) in a borate buffer (150 μL, 0.2M pH 9) was added to a solution of BSA (9.1 mg, 1.34 x 10^{-4} mmol) in the same buffer (150 μL) or OMP (9.1 mg, 1mL buffer). Sodium cyanoborohydride (1.79 mg, 0.029 mmol) was added and the resulting suspension was stirred at 50 °C for 48 h. After several dyafiltrations, the final products were analyzed by their carbohydrate and protein content.

Compound	hapten/BSA (mol/mol)	OMP/hapten (mg/mg)
17	34	12
19	27	7
18	28	5
20	23	8

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