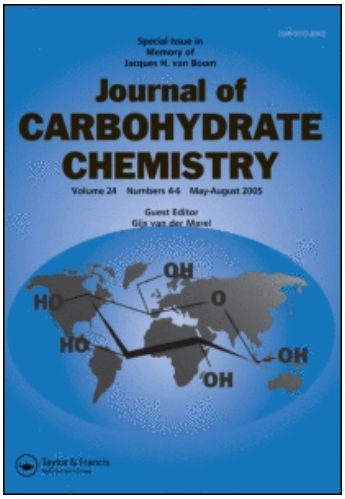

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Synthesis of the Sialyloligosaccharide Gal-β(1→3)-[Neu5Ac-α(2→6)]-GalNAc, The Epitope of the Tumor-Associated Glycoprotein Tag 72

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SYNTHESIS OF THE SIALYLOLIGOSACCHARIDE GAL-β(1→3)-[NEU5AC-α(2→6)]-GALNAC, THE EPITOPE OF THE TUMOR-ASSOCIATED GLYCOPROTEIN TAG 72.

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ABSTRACT

Benzyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside (**3**) was glycosylated with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**2**) to give disaccharide **4**. Reductive ring-opening of the acetal group provided as a major compound (64%) the 6-*O*-(4-methoxybenzyl) derivative (**5**) which was directly converted into benzyl 2-acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-*O*-benzoyl-2-deoxy-β-D-galactopyranoside (**10**) through triflate displacement by tetra-n-butylammonium benzoate, followed by removal of the 4-methoxybenzyl ether group. The minor compound (30%) (**6**) could be also converted into **10** through the 6-*O*-(*tert*-butyldimethylsilyl) ether **8** which was treated similarly to compound **5**. Stannous triflate promoted condensation of methyl (5-acetamido 4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl chloride)onate (**12**) with the glycosyl acceptor **10**, in tetrahydrofuran, afforded a 3:1 mixture of α and β (2→6) linked trisaccharides **13** and **14** in 44% isolated yield (63% based on starting material recovery). Removal of the protecting groups led to trisaccharide Gal-β(1→3)-[Neu5Ac-α(2→6)]-GalNAc (**1**), the epitope of the tumor-associated glycoprotein TAG 72.

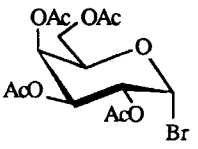
INTRODUCTION

The trisaccharide Gal-β(1→3)-[Neu5Ac-α(2→6)]-GalNAc **1** has been very recently recognized as the epitope of the second generation monoclonal antibody CC49 of the tumor-associated glycoprotein TAG 72 (from human colon cancer).[1] This trisaccharide has also been characterized on acute myelogeneous leukemia cells of human origin,[2] but not on mature glycophorin, the major sialoglycoprotein of human red cells;[3] it has been suggested that this carbohydrate antigen, like antigens Tn and sialyl Tn,[4]

could be a tumor marker.[5] Recently Iijima *et al.* reported the synthesis of the glycopeptide carrying this oligosaccharide-sequence.[6] As part of a program related to synthetic antigens, we now describe, according to a completely different approach, the synthesis of trisaccharide **1**, which was not previously synthesized. By coupling compound **1** to a biological carrier through an α-linked spacer arm,[7] synthetic antigens derived from this trisaccharide should be obtained.

RESULTS AND DISCUSSION

Benzyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside (**3**) was obtained from benzyl 2-acetamido-β-D-glucopyranoside[8] by treatment with 4-methoxybenzaldehyde dimethyl acetal in *N,N*-dimethylformamide, in the presence of 4-toluenesulfonic acid. Condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**2**) with **3** in nitromethane-benzene in the presence of mercuric cyanide gave the crystalline disaccharide **4** in 55% yield. Reductive ring-opening of the 4-methoxybenzylidene acetal by treatment with trifluoroacetic acid and sodium cyanoborohydride in *N,N*-dimethylformamide was not as regioselective as reported for monosaccharide[9] and afforded a mixture of compounds **5** (64%) and **6** (30%) which could be separated by flash chromatography. The desired 6-*O*-(4-methoxybenzyl) derivative (**5**) was converted into galacto-disaccharide **9** in 58% isolated yield by treatment with triflic anhydride in pyridine and then tetra-*n*-butylammonium benzoate in toluene, according to Lubineau *et al*.[8] Removal of the 4-methoxybenzyl ether group with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone[10] afforded glycosyl acceptor **10** in 95% yield after flash chromatography. The regioisomer 4-*O*-(4-methoxybenzyl) (**6**) could also be converted into disaccharide **10**. To this end, compound **6** was treated with *tert*-butylchlorodimethylsilane in *N,N*-dimethylformamide, in the presence of imidazole, to give the crystalline silylated disaccharide **7** in 84% yield. Treatment of **7** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone furnished in 87% yield the alcohol **8**, which was converted into galacto-disaccharide **11** under the conditions described for compound **5**. Compound **11** was not fully characterized but its structure was proved by ^{1}H NMR ($J_{3,4}$ 3.5 Hz) and it afforded the known compound **10** by carefully controlled removal of the silyl group ; treatment with one equivalent of tetra-*n*-butylammonium fluoride in tetrahydrofuran led to benzoyl migration. A good yield (76%) for the deprotection step was obtained by treating compound **11** with a solution of 3% hydrogen chloride in 1:1 (v/v) methanol-ethyl ether at 0 °C for 1h.[11]



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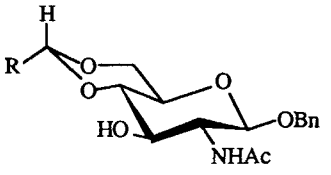
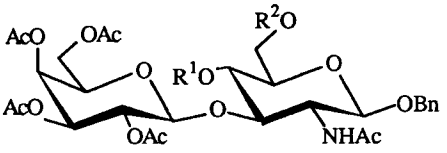
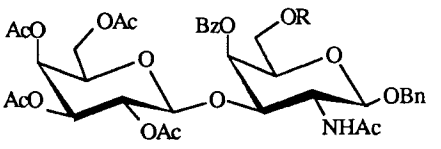


R=C_6H_4OMe-(4) **3**



R^1, R^2 = CHC_6H_4OMe-(4) **4**
R^1 = H , R^2 = $CH_2C_6H_4OMe$-(4) **5**
R^1 = $CH_2C_6H_4OMe$-(4) , R^2 = H **6**
R^1 = $CH_2C_6H_4OMe$-(4) , R^2 = Bu^tMe_2Si **7**
R^1 = H , R^2 = Bu^tMe_2Si **8**



R = $CH_2C_6H_4OMe$-(4) **9**
R = H **10**
R = Bu^tMe_2Si **11**

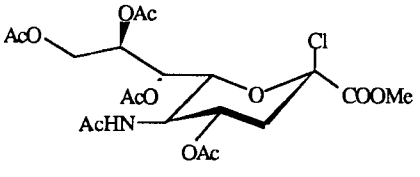
Glycosylation of acceptor **10** with sialic acid donor **12**,[12] obtained from enzymatically prepared *N*-acetylneuraminic acid,[13] was studied with various promotors (mercuric salts, silver triflate and tin(II) triflate[14]), in two solvents (dichloromethane or tetrahydrofuran). The results are gathered in Table.[15]

The proportion of α and β (2→6) isomeric trisaccharides was evaluated from ^1H NMR spectra (H-3e signal appeared at δ 2.73 for α and at 2.37 for β isomer). A spectacular solvent effect was observed : by changing dichloromethane to

TABLE. Sialylation of disaccharide **10.**

Entry	Catalyst	Solvent	Isolated yield α + β (%)	Ratio (α:β)
1	$Sn(OTf)_2$	CH_2Cl_2	32	1:3
2	AgOTf	CH_2Cl_2	44	2:3
3	$Sn(OTf)_2$	THF	44	3:1
4	AgOTf	THF	36	2:1
5	$Hg(CN)_2$ (3) $HgBr_2$ (1)	CH_2Cl_2	53	1:1

tetrahydrofuran, the ratio of α anomer **13** versus β anomer **14** was completely reversed (compare entries 1 and 3, 2 and 4). A better stereoselectivity was obtained with stannous triflate than with silver triflate (compare entries 1 and 2, 3 and 4). High stereoselectivity in favor of the α glycoside by the use of silver triflate in tetrahydrofuran has been reported.[16] By using, in this solvent , silver triflate and stannous chloride together, higher α stereoselectivity has been obtained,[17] which is in close connection with the result we obtained with tin(II) triflate. In the glycosylation reaction, tetrahydrofuran like acetonitrile[18] might be involved in complexation at the axial face of the intermediate oxocarbenium, allowing the nucleophile attack preferably from the equatorial face and giving rise to a preferential α glycoside formation. Optimum conditions ($Sn(OTf)_2$, THF), were selected for synthesis of the desired trisaccharide **13**.



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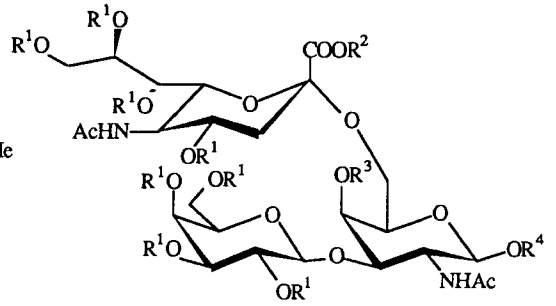
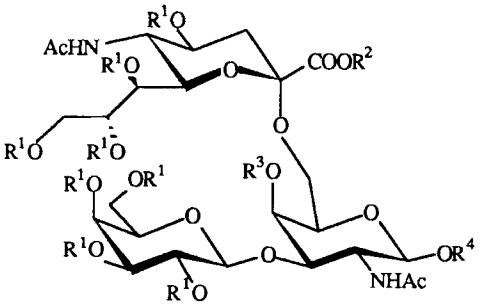


R^1 = Ac, R^2 = Me, R^3= Bz, R^4= Bn **13**

R^1 = R^2 = R^3= H, R^4= Bn **15**

R^1 = R^2 = R^3= R^4= H **1**



R^1 = Ac, R^2 = Me, R^3= Bz, R^4= Bn **14**

R^1 = R^2 = R^3= H, R^4= Bn **16**

A 3:1 mixture of trisaccharides **13** and **14** that could not be separated at this stage, was obtained in 44% isolated yield (63% based on starting material recovery). Hydrolysis of ester groups was carried out by treatment with M NaOH in methanol to afford trisaccharides **15** and **16** which were obtained after flash chromatography respectively in 69% and 21% yield. The α anomeric configuration of compound **15** was confirmed by

incubating an aliquot with neuraminidase. Finally, compound **15** was hydrogenolysed with 10% palladium-carbon in 90% ethanol to give the target compound **1** in quantitative yield.

EXPERIMENTAL

General procedures. Melting points are uncorrected. Optical rotations were measured with a Roussel-Jouan electronic, digital micropolarimeter. ^{13}C NMR and ^{1}H NMR spectra were recorded with a Bruker AM-250 spectrometer and TMS as the internal standard (for solutions in $CDCl_3$), or as the external standard (for solutions in D_2O). Reactions were followed by TLC on silica gel plates with fluorescence indicator (Merck). Detection was done by UV absorption and by spraying the plates with 5% ethanolic sulfuric acid. Silica gel Merck (6-35μ) was used for preparative HPLC and flash chromatography. Elemental analyses were performed by the Laboratoire Central de Micro-analyse du CNRS.

Benzyl 2-Acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside (3). A mixture of benzyl 2-acetamido-2-deoxy-β-D-glucopyranoside[8] (12.5 g, 40 mmol), 4-methoxybenzaldehyde dimethyl acetal (18.5 g, 85 mmol), and 4-toluenesulfonic acid (550 mg) in dry *N,N*-dimethylformamide (160 mL) was stirred at room temperature for 5 h. The solution was neutralized with few drops of triethylamine and concentrated to give a solid, which was triturated with water. The precipitate was filtered off and recrystallized from ethanol (14.05 g, 82%) ; mp 271-273 °C, $[\alpha]_D^{20}$ - 94° (*c* 1.06, pyridine) ; ^{1}H NMR (DMSO) δ 1.84 (s, 3H, NAc), 3.77 (s, 3H, OMe), 4.22 (dd, 1H, $J_{6,6'}$ = 10 Hz, $J_{6,5}$ = 4.5 Hz, H-6), 4,54 (d, 1H, J_{gem} = 12.5 Hz, CH_2Ph), 4.58 (d, 1H, $J_{1,2}$ = 8 Hz, H-1), 4.78 (d, 1H, CH_2Ph), 5.56 (s, 1H, *CH*Ph), 6.94 (d, 2H, Ph) and 7,35 (m, 7H, Ph).

Anal. Calcd for $C_{23}H_{27}NO_7$; 0.25 H_2O : C, 63.65 ; H, 6.38 ; N, 3.22. Found : C, 62.93 ; H,6.07 ; N, 3.27.

Benzyl 2-Acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside (4). A solution of **3** (2 g, 4.7 mmol) in 1:1 (v/v) toluene - nitromethane (200 mL) was boiled in a Dean-Stark until 50 mL of the solvent had been distilled. To this solution adjusted to 60 °C, were added mercuric cyanide (1.8 g, 7 mmol) and a solution of 2,3,4,6 tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**2**, 2.9 g, 7 mmol) in 1:1 (v/v) toluene - nitromethane (20 mL).The mixture was stirred at 60 °C under nitrogen for 15 h. Mercuric cyanide (1.3 g , 4.7 mmol) and bromide **2** (2 g, 4.7 mmol) were added again and the solution was stirred for a additional 24 h. The reaction mixture was washed successively

with ice-cold saturated aqueous sodium hydrogencarbonate, 20% potassium iodide and water, dried (sodium sulfate) and concentrated to dryness. The residue was purified by flash chromatography on silica gel with 2:1 (v/v) toluene - acetone to give pure **4** which crystallized from acetone - ether (1.95 g, 55%), mp 171-172 °C, $[\alpha]_D^{20}$ - 48° (*c* 1, dichloromethane) ; ^{1}H NMR ($CDCl_3$) δ 1.93, 1.94, 1.96, 1.97, 2.14 (5s, 15H, NAc, 4 OAc), 3.10 (dt, 1H, $J_{1,2} = J_{NH,2} = 8$ Hz, $J_{2,3} = 10$ Hz, H-2), 3.56 (m, 2H, H-5, H-5'), 3.67 (t, 1H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 3.80 (m, 4H, MeO, H-6a), 3.91 (dd, 1H, $J_{5,6'a} = 6$ Hz, $J_{6'a,6'b} = 11$ Hz, H-6'a), 4.03 (dd, 1H, $J_{5,6'b} = 8$ Hz, H-6'b), 4.33 (dd, 1H, $J_{5,6e} = 4.5$ Hz, $J_{6e,6a} = 10.5$ Hz, H-6e), 4.56 (d, 1H, $J_{gem} = 12$ Hz, C*H*$_2$Ph), 4.69 (dd, 1H, H-3), 4.72 (d, 1H, $J_{1',2'} = 8$ Hz, H-1'), 4.87 (d, 1H, C*H*$_2$Ph), 4.91 (dd, 1H, $J_{3',4'} = 3.5$ Hz, $J_{2',3'} = 10$ Hz, H-3'), 5.15 (dd, 1H, H-2'), 5.26 (d, 1H, H-1), 5.29 (d, 1H, H-4'),5.49 (s, 1H, *CH*Ph) 5.75 (d, 1H, NH), 6.90 (d, 2H, Ph) and 7.34 (m, 7H, Ph) .

Anal. Calcd for $C_{37}H_{45}O_{16}N$: C, 58.49 ; H, 5.97 ; N, 1.85 ; O, 33,69. Found : C, 58.32 ; H, 5.81 ; N, 1.91 ; O, 33.88.

Benzyl 2-Acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (5) and benzyl 2-acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-4-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (6). To a mixture of **4** (1.8 g, 2.2 mmol), sodium cyanoborohydride (0.7 g, 11 mmol) and powdered molecular sieves 4 A (5 g) in DMF (20 mL) was added a solution of trifluoroacetic acid (1.7 mL, 22 mmol) in DMF (15 mL). The mixture was stirred at room temperature under nitrogen. The mixture was diluted with dichloromethane, and washed with saturated aqueous sodium hydrogencarbonate, water, dried (sodium sulfate) and concentrated to dryness. Flash chromatography of the residue with 4:1 (v/v) dichloromethane-acetone afforded in the first fractions pure amorphous compound **5** (1.1 g, 64%); $[\alpha]_D^{20}$ +25° (*c* 0.49, dichloromethane); ^{1}H NMR ($CDCl_3$) δ 1.90, 1.95, 2.00, 2.05, 2.15 (5s, 15H, NAc, 4 OAc), 3.11 (dt, 1H, $J_{1,2} = J_{NH,2} = 8$ Hz, $J_{2,3} = 10$ Hz, H-2), 3.48 (m, 2H, H-4, H-5), 3.66 (m, 1H, $J_{6a,6b} = 12$ Hz, H-6a), 3.80 (s, 3H, MeO), 3.85 (m, 1H, H-6b), 4.01 (m, 1H, H-5'), 4.11 (m, 2H, H-6'a, H-6'b), 4.39 (dd, 1H, $J_{3,4} = 9.5$ Hz, H-3), 4,53 (d, 1H, $J_{gem} = 12$ Hz, C*H*$_2$Ph), 4.55 (d, 1H, $J_{1',2'} = 8$ Hz, H-1'), 4.55 (d, 1H, J_{gem} = 12 Hz, C*H*$_2$Ph), 4.59 (d, 1H, C*H*$_2$*Ph)*, 4.89 (d, 1H, C*H*$_2$Ph), 4.96 (d, 1H, $J_{1,2} = 8$ Hz, H-1), 4.98 (dd, 1H, $J_{2',3'} = 10.5$ Hz, $J_{3',4'} = 3.5$ Hz, H-3'), 5.22 (dd, 1H, H-2'), 5.37 (d, 1H, H-4'), 5.63 (d, 1H, NH), 6.87 (d, 2H, Ph) and 7.30 (m, 7H, Ph).

Anal. Calcd for $C_{39}H_{43}O_{17}N$: C, 58.34 ; H, 6.22 ; N, 1.84 ; O, 33.60. Found : C, 58.08 ; H, 6.33 ; N, 1.91 ; O, 33.38.

Pure amorphous compound **6** (0.5 mg, 30%) was obtained from the following fractions; $[\alpha]_D^{20}$ -38° (*c* 0.66, dichloromethane); ^{1}H NMR ($CDCl_3$) d 1.98, 2.00, 2.04, 2.05, 2.12 (5s, 15H, NAc, 4 OAc), 3.80 (s, 3H, OMe), 4.51 (d, 1H, J_{gem} = 11 Hz, C*H*$_2$Ph), 4.56 (d, 1H, J_{gem} = 12 Hz, C*H*$_2$Ph), 4.65 (d, 1H, $J_{1',2'}$ = 8 Hz, H-1), 4.78 (d, 1H, $J_{1,2}$ = 8 Hz, H-1'), 4.84 (d, 1H, C*H*$_2$Ph), 4.85 (d, 1H, C*H*$_2$Ph), 5,00 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 5.19 (dd, 1H, H-2'), 5.37 (d, 1H, H-4'), 5.85 (d, 1H, $J_{NH,1}$ = 9 Hz, NH), 6.88 (d, 2H, Ph) and 7.32 (m, 7H, Ph).

Anal. Calcd for $C_{39}H_{43}O_{17}N$: C, 58.34 ; H, 6.22 ; N, 1.84 ; O, 33.60. Found : C, 58.30 ; H, 6.34 ; N, 1.95 ; O, 33.53 .

Benzyl 2-Acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-4-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (7). To a solution of **6** (1.44 g, 1.9 mmol) in DMF (4 mL) were added *tert*-butylchlorodimethylsilane (314 mg, 2.1 mmol) and imidazole (400 mg, 5.8 mmol) and the mixture was stirred at room temperature for 1 day. TLC showed that starting material was still present; *tert*-butylchlorodimethylsilane (150 mg, 1 mmol) and imidazole (70 mg, 1.03 mmol) were added again and the stirring mixture was kept at room temperature for 1 more day. The solution was concentrated to dryness and the residue was purifed by preparative HPLC to give pure **7** as a crystalline compound (1.4 g, 84%; mp 76-78 °C; $[\alpha]_D^{20}$ - 40° (*c* 1.17, dichloromethane); ^{1}H NMR ($CDCl_3$) δ 0.09 (s, 6H, $Si(CH_3)_2$, O.90 (s, 9H, Si $C(CH_3)_3$), 1.95, 1.96, 1.99, 2.01, 2.13 (5s, 15H, NAc, 4 OAc, 3.49 (m, 1H, H-2), 3.69 (m, 2H, H-4, H-6a), 3.81 (s, 3H, OAc), 3.86 (m, 3H, H-5, H-5', H-6b), 4.02 (dd, 1H, $J_{6'a,6'b}$ = 11 Hz, $J_{6'a,5'}$ = 5 Hz, H-6'a), 4.10 (dd, 1H, $J_{6'b,5}$ = 3 Hz, H-6'b), 4.17 (t, 1H, $J_{3,2} = J_{4,3}$ = 7.5 Hz, H-3), 4.52 (d, 1H,J_{gem} = 10.5 Hz, C*H*$_2$Ph), 4.55 (d, 1H, J_{gem} = 12 Hz, C*H*$_2$Ph), 4.68 (d, 1H, $J_{1,2}$ = 7 Hz, H-1), 4.79 (d, 1H, C*H*$_2$Ph), 4.80 (d, 1H, $J_{1',2}$ '= 8 Hz, H-1'), 4.84 (d, 1H, C*H*$_2$Ph), 4.99 (dd, 1H, $J_{2',3'}$ = 9.5 Hz, $J_{3',4'}$ = 3 Hz, H-3'), 5.21 (dd, 1H, H-2'), 5.37 (d, 1H, H-4'), 5.59 (d, 1H, $J_{NH,1}$ = 8 Hz, NH), 6.88 (d, 2H, Ph) and 7.30 (m, 7H, Ph).

Anal. Calcd for $C_{43}H_{61}O_{16}NSi$: C, 58.95 ; H, 7.02 ; N, 1.60 ; Si, 3.20. Found : C, 58.89 ; H, 6.91 ; N, 1.89 ; Si, 3.06.

Benzyl 2-Acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-β-D-glucopyranoside (8). To a solution of **7** (1 g, 1.15 mmol) in 10 : 1 (v/v) dichloromethane-water (100 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (1.2 g, 5.25 mmol) and the mixture was vigorously stirred at room temperature for 3 h. The organic phase was successively washed with saturated aqueous sodium hydrogencarbonate, water, dried and concentrated to dryness. Flash chromatography of the residue with 2:1 (v/v) toluene-acetone as the

eluant afforded pure **8** as a crystalline compound (750 mg, 87%) ; mp 208-211°C; $[\alpha]_D^{20}$ -19° (*c* 0.4, dichloromethane) ; ^1H NMR ($CDCl_3$) δ 0.09 (s, 6H, $Si(CH_3)_2$), 0.90 (s, 9H, Si $C(CH_3)_3$), 1.94, 1.99, 2.04, 2.15, 2.17 (5s, 15H, NAc, 4 OAc), 3.45 (t, 1H, $J_{3,4} = J_{4,5}$ = 10 Hz, H-4), 4.38 (dd, 1H, $J_{2,3}$ = 8 Hz, H-3), 4.55 (d, 1H, J_{gem} = 12 Hz, CH_2Ph), 4.56 (d, 1H, $J_{1,2}$ = 8 Hz, H-1), 4.88 (d, 1H, CH_2Ph), 4.98 (d, 1H, $J_{1',2'}$ = 8 Hz, H-1'), 4.98 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 5.22 (dd, 1H, H-2'), 5.37 (d, 1H, H-4'), 5.60 (d, 1H, $J_{NH,1}$ = 7 Hz, NH) and 7.29 (m, 5H, Ph).

Anal. Calcd for $C_{35}H_{53}O_{15}NSi$: C, 55.61 ; H, 7.07 ; N, 1.85. Found : C, 55,65 ; H, 7,09 ; N, 2.13.

Benzyl 2-Acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-*O*-benzoyl-2-deoxy-6-*O*-(4-methoxybenzyl)-β-D-galactopyranoside (9). To a cooled (-40 °C) solution of **5** (300 mg, 0.4 mmol) in pyridine (10 mL) was added dropwise triflic anhydride (0.11 mL, 0.71 mmol). The temperature was allowed to rise to - 15 °C and the mixture was kept for 3 days at this temperature under nitrogen. The solution was poured in an ice cooled mixture of 0.1 M phosphate buffer at pH 7 and dichloromethane. The organic phase was washed with water, concentrated to dryness and the residue was coevaporated with toluene (three times). The residue was then dissolved in toluene (10 mL) and tetra-*n*-butylammonium benzoate (730 mg, 2 mmol) was added. The mixture was stirred for 3 days at room temperature under nitrogen. The mixture was diluted with dichloromethane, washed with O.1 M phosphate buffer pH 7 and water, dried and concentrated to dryness. The residue was purified by flash chromatography on silica gel with 4:1 (v/v) toluene - acetone to afford pure **9** as a crystalline compound (200 mg, 58%), mp 90-95 °C, $[\alpha]_D^{20}$ + 9° (*c*, dichloromethane) ; ^1H NMR ($CDCl_3$) δ 1.94, 1.95, 1.98, 2.01 (4s, 15H, NAc, 4 OAc), 3.78 (s, 3H, OMe), 4.42 (d, 1H, J_{gem} = 12 Hz, CH_2Ph), 4.48 (d, 1H, CH_2Ph), 4.61 (d, 1H, J_{gem} = 12 Hz, CH_2Ph), 4.66 (d, 1H, $J_{1',2'}$ = 8 Hz, H-1'), 4.78 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 4.92 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 4.95 (d, 1H, CH_2Ph), 5.07 (dd, 1H, H-2'), 5.19 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 5.29 (d, 1H, H-4'), 5.70 (d, 1H, $J_{NH,1}$ = 9 Hz, NH), 5.72 (d, 1H, H-4), 6.82 (d, 2H, Ph), 7.27 (m, 7H, Ph), 7.45 (m, 2H, Bz), 7.55 (m, 1H, Bz) and 8.07 (d, 2H, Bz).

Anal. Calcd. for $C_{44}H_{51}O_{17}N$: C, 61.03 ; H, 5.94 ; N, 1.62 ; O, 31.41. Found : C, 60.05 ; H, 6.00 ; N, 1.75 ; O, 31.82.

Benzyl 2-Acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-*O*-benzoyl-2-deoxy-β-D-galactopyranoside (10). (a) To a solution of **9** (730 mg, 0.844 mmol) in 10:1 (v/v) dichloromethane - water (100 mL) was added 2,3-

dichloro-5,6-dicyano-1,4-benzoquinone (766 mg, 3.37 mmol) and the mixture was vigorously stirred at room temperature for 5 h. Workup as described for compound **8** and flash chromatography of the residue with 3:1 (v/v) toluene-acetone as the eluant afforded pure compound 10 (600 mg, 95%) as an amorphous solid ; $[\alpha]_D^{20}$ + 30° (*c* 1, dichloromethane) ; ^{1}H NMR ($CDCl_3$) δ 1.80, 1.91, 2.05 (3s, 15H, NAc, 4 OAc), 4.58 (d, 1H, J_{gem} = 12 Hz, C*H*$_2$Ph), 4.65 (d, 1H, $J_{1',2'}$ = 8 Hz, H-1'), 4.87 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 4.87 (d, 1H, C*H*$_2$Ph), 4.91 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 5.06 (dd, 1H, H-2'), 5.19 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1),5.24 (d, 1H, H-4'), 5.57 (d,1H, H-4), 5.77 (d, 1H, $J_{NH,1}$ = 7.5 Hz, NH), 7.27 (m, 5H, Ph), 7.45 (m, 2H, Bz), 7.56 (m, 1H, Bz) and 8.07 (d, 2H, Bz).

Anal. Calcd for $C_{36}H_{43}O_{16}N$: C, 57.98 ; H, 5.81 ; N, 1.88 ; O, 34.33.
Found : C, 57.53 ; H, 5.82 ; N, 1.96 ; O, 34.68.

(b). To a cooled (- 40 °C) solution of **8** (200 mg, 0.265 mmol) in pyridine (2 mL) was added dropwise triflic anhydride (0.07 mL, 0.4 mmol) and the reaction was allowed to proceed as described for the preparation of compound **9**. The residue was then dissolved in toluene (2 mL) and treated with tetra-*n*-butylammonium benzoate (480 mg, 1.325 mmol) as described for **9** . The mixture was worked up in the same way and purified by flash chromatography on silica gel with 4:1 (v/v) toluene-acetone to afford compound **11** (130 mg, 57%) ; ^{1}H NMR ($CDCl_3$) δ 0.03 (s, 6H, $Si(CH_3)_2$, 0.88 (s, 9H, $SiC(CH_3)_3$), 1.93, 1.98, 2.01, 2.04 (4s, 15H, NAc, 4 OAc), 3.51 (dt, 1H, $J_{1,2}$ = $J_{NH,1}$ = 8 Hz, $J_{2,3}$ = 11 Hz, H-2), 4.61 (d, 1H, J_{gem} = 12 Hz, C*H*$_2$Ph), 4.70 (d, 1H, $J_{1',2'}$ = 8 Hz, H-1'), 4.79 (dd, 1H, $J_{3,4}$ = 3.5 Hz, H-3), 4.92 (dd, 1H, $J_{3',4'}$ = 3.5 Hz, $J_{2',3'}$ = 10 Hz, H-3'), 4.94 (d, 1H, C*H*$_2$Ph), 5.08 (dd, 1H, H-2'), 5.16 (d, 1H, H-1), 5.28 (d, 1H, H-4'), 5.68 (d, 1H, H-4), 5.77 (d, 1H, NH), 7.31 (m, 8H, Ph, Bz) and 8.05 (d, 2H, Bz). This compound (50 mg, 0.058 mmol) was dissolved in methanol (1 mL) ; a solution of 3% HCl in methanol (0.07 mL) was added and the mixture was stirred at 0 °C for 1 h. The reaction was followed by TLC in 1:1 (v /v) toluene - acetone and it was stopped when nearly complete conversion of the starting material into one product having R_f of compound **10** had occurred. After concentration of the solution, the residue was purified by flash chromatography to afford an amorphous compound (38 mg, 76%), identified to **10**.

Benzyl 2-Acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-*O*-benzoyl-2-deoxy-6-*O*-[methyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-β-D-galactopyranoside (13) and benzyl 2-acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-*O*-benzoyl-2-deoxy-6-*O*-[methyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonu-

lopyranosyl)onate]-β-D-galactopyranoside (14). Powdered molecular sieves 4A (1g) and stannous triflate (216 mg, 0.52 mmol) were added to a solution of **10** (260 mg, 0.35 mmol) in anhydrous THF (1 mL) ; the mixture was cooled to -10 °C and a solution of methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl chloride)onate (**12**) (260 mg, 0.52 mmol) in anhydrous THF (1.5 mL) was added dropwise. The reaction mixture was stirred under nitrogen at -10 °C for 1h and then at room temperature. The reaction was monitored by TLC (7:7:1, v/v/v toluene-ether-methanol). After 24 h some more chloride **12** (260 mg, 0.52 mmol) and stannous triflate (216 mg, 0.52 mmol) were added and the reaction mixture was kept under stirring for 24 h.The mixture was diluted with dichloromethane, filtered through Celite and successively washed with saturated aqueous sodium hydrogencarbonate, water, dried (Na_2SO_4) and concentrated to dryness. The residue (500 mg) was purified by preparative HPLC with 7:7:1 (v/v/v) toluene-ether-methanol as eluent to give a mixture of α (**13**) and β (**14**) trisaccharide (180 mg, 44%, **13** : **14** = 3:1 cf. entry 3 in Table). Reactions in entries 1, 2, 4 and 5 were performed in the same way. ^1H NMR ($CDCl_3$) δ 1.78-2.19 (m, 30H,2 NAc, 8 OAc), 2.39 (dd, 0.25H, $J_{3"e,4"}$ = 5Hz,$J_{3"e,3"a}$ = 13 Hz, H-3"e β), 2.56 (dd, 0.75 H, $J_{3"e,4"}$ = 4.5 Hz, $J_{3"e,3"a}$ = 13 Hz, H-3"e α), 3.56 (s, CH_3 α), 3.83 (s, CH_3 β), 5.69 (d, 1H, $J_{2,NH}$ = 7.5 Hz, NH), 5.82 (d, 0.75 H, $J_{3,4}$ = 3 Hz, H-4 α), 5.85 (d, 0.25 H, $J_{3,4}$ = 3 Hz, H-4 β), 6.00 (d, 0.75H,$J_{5",NH}$ = 8 Hz, NH α), 6.15 (d, 0.25 H, $J_{5",NH}$ = 10 Hz, NH β), 7.15–7.60 (m, 8H, Ph, Bz) and 8.05 (m, 2H, Bz).

Anal. Calcd for $C_{56}H_{70}O_{28}N_2$: C, 55.17 ; H, 5.79 ; O, 36.75 ; N, 2.30. Found : C, 55.35 ; H, 6.05 ; O, 36.88 ; N, 2.14.

Some starting material **10** was recovered from the first fractions of HPLC (78 mg, 31%).

Benzyl 2-Acetamido-6-*O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-2-deoxy-3-*O*-(β-D-galactopyranosyl)-β-D-galactopyranoside (15) and benzyl-2-acetamido-6-*O*-(5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosylonic acid)-2-deoxy-3-*O*-(β-D-galactopyranosyl)-β-D-galactopyranoside (16). To a solution of the 3:1 mixture of **13** and **14** (150 mg, O.123 mmol) in methanol (20 mL) was added dropwise M aq. NaOH (2.4 mL). The mixture was stirred for 6 h at room temperature, then neutralized with Dowex 50-X8 (H^+) resin, filtered through Celite and the filtrate concentrated to dryness. The residue was chromatographed on silica gel (flash chromatography) in 2:2:1 v/v/v propanol-2-ethyl acetate-water, to afford pure **15** (65 mg, 69%), a mixture of **15** and **16** (8 mg, 9%) and pure **16** (20 mg, 21%).

Compound **15** : $[\alpha]_D^{20}$ - 13° (*c* 0.76, water) ; ^1H NMR (D_2O) δ 1.69 (t, 1H, $J_{3"a,4"} = J_{3"e,3"a}$ = 12.5 Hz, H-3"a), 1.82 (s, 3H, NAc), 2.01 (s, 3H, NAc), 2.73 (dd,

1H, $J_{3"e,4}$ = 4.5 Hz, H-3"e), 4.17 (d, 1H, $J_{3,4}$ = 3 Hz, H-4), 4.36 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1'), 4.49 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 4.65 (d, 1H, J_{gem} = 12 Hz, CH_2Ph), 4.86 (d, 1H, CH_2Ph) and 7.40 (m, 5H, Ph).

Compound **16** :$[\alpha]_D^{20}$ - 21° (*c* 0.81, water) ; ^{1}H NMR (D_2O) δ 1.64 (dd, 1H, $J_{3"a,4"}$ = 12Hz, $J_{3"a,3e"}$ = 13 Hz, H-3"a), 1.88 (s, 3H, NAc), 2.05 (s, 3H, NAc), 2.37 (dd, 1H, $J_{3"e,4}$ = 5 Hz, H-3"e), 4.22 (d, 1H, $J_{3,4}$ = 3 Hz, H-4), 4.38 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1'), 4.54 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 4.66 (d, 1H, J_{gem} = 12 Hz, CH_2Ph), 4.86 (d, 1H, CH_2Ph) and 7.40 (m, 5H, Ph).

Compound **15** (2 mg) was dissolved in 0.1 M citrate buffer pH 5.O and incubated at 37 °C with neuraminidase (Sigma, 10 µL, 0.01 U). After 30 min, TLC in 2:2:1 v/v/v propanol-2--ethyl-acetate-water showed in addition to compound **15** (Rf = O.32) two new spots : Rf = 0.67 (*N*-acetylneuraminic acid) and Rf = 0.22 (disaccharide derivative). Compound **16** was totally stable under these conditions.

2-Acetamido-6-*O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-2-deoxy-3-*O*-β-D-galactopyranosyl)-D-galactopyranose (1). A mixture of **15** (23 mg) and 10% Pd-C (25mg) in 90% ethanol (1 mL) was stirred under hydrogen for 24 h at room temperature, filtered through Celite and the filtrate concentrated to give **1** (20 mg, quantitative yield) ; $[\alpha]_D^{20}$ +19° (c 1.13, water); ^{1}H NMR (D_2O) δ 1.66 (t, 1H, $J_{3"a,4"} = J_{3"e,3"a}$ = 12 Hz, H-3"a), 2,02 (s, 3H, 2 NAc), 2.69 (dd, 1H, $J_{3"e,4}$ = 4 Hz, H-3"e), 4.41 (d, 0.35H, $J_{1',2'}$ = 7.5 Hz, H-1' β), 4.47 (d, 0.65H, $J_{1',2'}$ = 7.5 Hz, H-1' α), 4.65 (d, 0.35H, $J_{1,2}$ = 8 Hz, H-1 β) and 5.18 (d, 0.65H, $J_{1,2}$ = 3.5 Hz, H-1 α) ; ^{13}C NMR (D_2O) δ 22.84, 23.06 (NHCOCH_3), 41.OO (C-3"), 49.74 (C-2 α), 52.64 (C-5"), 53.14 (C-2 β), 61.76 (C-6'), 63.43 (C-9"), 64.45 (C-6 β), 64.61 (C-6 α), 69.16 (C-4 β), 69.02 (C-4",7"), 69.42 (C-4',5 α), 69.57 (C-4 α), 71.41 (C-2'), 72.48 (C-8'), 73.41 (C-3',6"), 74.07 (C-5 β), 75.76 (C-5'), 77.85 (C-3 α), 80.89 (C-3 β), 92.01 (C-1 α), 95.97 (C-1 β), 101.18 (C-2"), 105.54 (C-1' α), 105.71 (C-1' β), 174.27 (C-1") and 174.43, 175.81 (NH*C*OCH$_3$).

REFERENCES AND NOTES

1. F.G.Hanisch, G.Uhlenbruck, H.Egge and J.P.Katalinic, *Biol. Chem. Hoppe-Seyler*, **370**, 21 (1989).

2. M.Fukuda, S.R.Carlsson, J.C.Klock and A.Dell, *J. Biol. Chem.*, **261**, 12796 (1986).

3. M.Fukuda, M.Lauffenburger, H.Sasaki, M.E.Rogers and A.Dell, *J. Biol. Chem.*, **262**, 11952 (1987).

4. T.Kjeldsen, H.Clausen, S.Hirohashi, T.Ogawa, H.Iijima and S.Hakomori, *Cancer Res.*, **48**, 2214 (1988).

5. A.Molino, J.F.Simpson, A.Thor and J.Schlom, *Cancer Res.*, **50**, 1291 (1990).

6. H.Iijima and T.Ogawa, *Carbohydr. Res.*, **186**, 95 (1986).

7. A.Lubineau, H.Bienaymé and J.Le Gallic, *J. Chem. Soc., Chem. Commun.*, 1918 (1989).

8. A.Lubineau and H.Bienaymé, *Carbohydr.Res.*, **212**, 267 (1991).

9. R.Johansson and B.Samuelsson, *J. Chem. Soc. , Chem. Commun.*, 201 (1984).

10. V.Pozsgay, H.J.Jennings and H.Kasper, *J. Carbohydr. Chem.*, **6**, 41 (1987).

11. E.M.Nashed and C.P.J.Glaudemans, *J. Org. Chem.*, **52**, 5255 (1987).

12. Compound **12** was prepared free of unsaturated derivative by treatment of *N*-acetylneuraminic methyl ester with 1:1 (v/v) CH_3COCl : CH_3COOH saturated at 0 ° C with anhydrous HCl in 96 % isolated yield after silica gel chromatography. A. Lubineau, J.Le Gallic, *J. Carbohydr. Chem.*, **10**, 263 (1991).

13. C.Augé, S.David, C.Gautheron, A.Malleron and B.Cavayé, *Nouv. J. Chim.*, **12**, 733 (1988).

14. T.Mukaiyama, N.Iwasawa, R.W.Stevens and T.Haga, *Tetrahedron*, **40**, 1381 (1984).

15. This work was reported on XIII ème Journées de la Chimie et Biochimie des glucides, may 1990, Avignon, France.

16. H.Iijima and T.Ogawa, *Carbohydr. Res.*, **172**, 183 (1988).

17. M.Numata, M.Sugimoto, K.Koike and T.Ogawa, *Carbohydr. Res.*, **163**, 209 (1987).

18. E.Kirchner, F.Thiem, R.Dernick, J.Heukeshoven and J.Thiem, *J. Carbohydr. Chem.*, **7**, 453 (1988).