Convenient synthesis of Thr and Ser carrying the tumor associated sialyl-(2→3)-T antigen as building blocks for solid-phase glycopeptide synthesis

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Sialyl-T antigens have been synthesized, conveniently and in high yield, by a block condensation approach. Glycosylation of the D-GalN_3 derivative with the suitably protected sialyl-α-(2→3)-D-galactopyranosyl trichloroacetimidate by using trimethylsilyl trifluoromethanesulfonate (TMS-OTf) as a promoter in dichloromethane gave the trisaccharide in high yield. Cleavage of the *tert*-butyldimethylsilyl ether (TBDMS) at the anomeric position of the sialic acid containing trisaccharide derivative, using tetrabutylammonium fluoride (TBAF) in pyridine, occurred in very high yield. After conversion to the trichloroacetimidate coupling of the sialyl-containing trisaccharide derivative with Fmoc-Thr/Ser-OPfp, using silver trifluoromethanesulfonate (AgOTf) as a promoter in dichloromethane, gave the target building blocks in good yields.

Introduction

MUC1 is the major surface molecule in the gastro-intestinal tracts. In normal tissue the MUC1 glycoprotein is glycosylated with β-D-Gal-(1→3)-α-D-GalNAc also named the T antigen on Thr and Ser. In normal tissue this disaccharide is further elongated with lactosamine units. However, in malignant epithelial tumors there is a down regulation of GlcNAc transferase and instead of elongation the T antigen is terminated by sialylation of the 6-position of GalNAc (β-D-Gal-(1→3)-[α-sialyl-(2→6)]-α-D-GalNAc) or the 3-position of Gal (α-sialyl-(2→3)-β-D-Gal-(1→3)-α-D-GalNAc) or it may be fucosylated at the 2-position of Gal (α-L-Fuc-(1→2)-β-D-Gal-(1→3)-α-D-GalNAc).[1–3] These structures, which are absent in normal tissue, can be expected to show antigenic properties[4–6] and they may together with the T and Tn antigens be involved in host rejection of malignant tissue. The negatively charged sialic acids have been considered to shield against host recognition[7,8] and therefore an antigenic response against these abundant antigens elicited upon challenge with a glycopeptide vaccine could be an effective method of cancer treatment.

In order to understand in more detail the physiochemical and biological properties of the MUC1 glycoprotein of malignant tissue, substantial efforts have been dedicated to the chemical synthesis of glycopeptides carrying these antigens. In the recent report on the synthesis of the 2,3-sialyl-T antigen by Nakahara *et al.*[9] the building block was constructed by using N^{α}-(fluoren-9-ylmethoxycarbonyl)-*O*-(2-azido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-galactopyranosyl)-L-serine allyl ester as an acceptor and 5-acetamido-4,7,8,9-tetra-*O*-benzyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid-(2→3)-2,6-di-*O*-benzyl-D-galactopyranosyl trichloroacetimidate-(1c→4b)-lactone as a disaccharide donor. The method published by Nakahara *et al.* showed poor stereoselectivity during the glycosylate reaction. In the present paper a more convenient, more stereoselective and high yielding method for the synthesis of 2,3-sialyl-T antigen is reported. The problem in the synthesis of this antigen is mainly associated with the sialylation reaction and the glycosylation should therefore be performed first while the glycosyl acceptor can be maintained as a simple, small nucleophile. The acceptor should furthermore be ready for easy activation at the anomeric center.

Results and discussion

In the retrosynthetic perspective the first disconnection of the trisaccharide is conveniently placed between the Gal and the GalN_3 residue allowing an easy β-glycosylation to be performed at the highest level of compound complexity. For the synthesis of the sugar part of the two desired sialyl-T antigens **13** and **14**, the *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-acetyl-D-galactopyranosyl trichloroacetimidate **7** was selected as the glycosyl donor, and *tert*-butyldimethylsilyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside **8**[10] was selected as the glycosyl acceptor. For attachment of the trisaccharide to threonine and serine the Fmoc/Pfp ester approach[6,11] was employed. The Pfp ester is stable during the glycosylation and the product can be directly used in peptide coupling reactions in glycopeptide synthesis.

The disaccharide **3** was synthesized[12] as previously described by condensation of 2-(trimethylsilyl)ethyl 4,6-*O*-benzylidene-β-D-galactopyranoside **2** with phenyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate **1** in a yield of 65%. Acetylation of this sialylated galactose derivative afforded compound **4** quantitatively, which was converted *via* reductive cleavage of the benzylidene group (10% Pd/C, 63% yield) to give **5**. Acetylation with acetic anhydride and pyridine gave 98% of 2-(trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside **6**. Selective removal of the 2-(trimethylsilyl)ethyl group of **6** was effected by trifluoroacetic acid[13,14] to give the 1-hydroxy compound (94% yield), which was subsequently transformed into the trichloroacetimidate **7** using trichloroacetonitrile and DBU in 80% yield (Scheme 1).[15,16]

The glycosylation of *tert*-butyldimethylsilyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside[10] **8** with **7** in dichloromethane in the presence of TMS-OTf for 2 days at 0 °C and an additional 1 day at 0 °C to room temperature, afforded stereoselectively the corresponding trisaccharide **9** in 80% yield (Scheme 2). The β-configuration of **9** was confirmed by ^{1}H NMR spectroscopy, which showed the signals at δ 5.11 (1H, dd, $J_{1,2}$ 8.0, $J_{2,3}$ 10.1) for 2b-H of the obtained trisaccharide. The

superior yield and β selectivity of this glycosylation compared with a previous report[9] demonstrate the advantage of this disconnection strategy. Removal of the *tert*-butyldimethylsilyl group in **9** in the presence of TBAF using pyridine as solvent for 20 min at 0 °C afforded the 1-hydroxy compound in 81% yield irrespective of the presence of the benzylidene group. In other solvents premature cleavage of the benzylidene group was observed. Compound **9** was transformed into the trichloroacetimidate **10** using trichloroacetonitrile and DBU in 96% yield (Scheme 3).

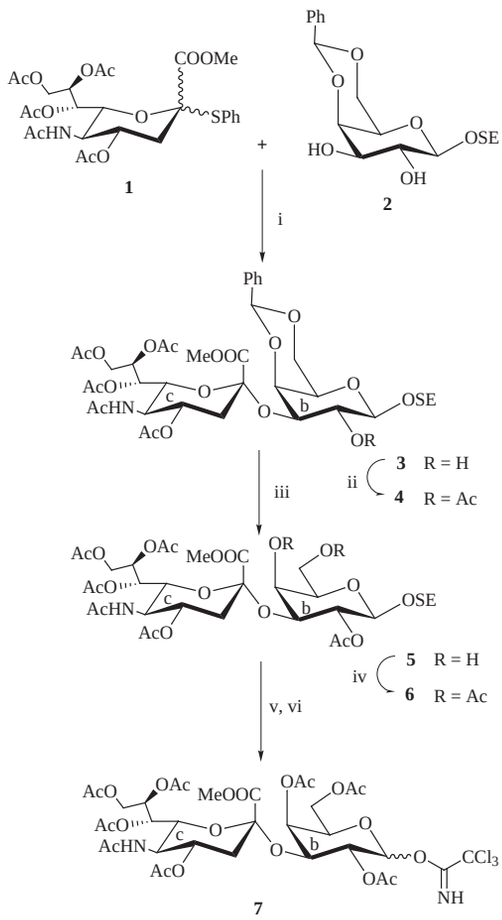
The final glycosylation with compound **10** of N^{α}-(fluoren-9-ylmethoxycarbonyl)-L-threonine pentafluorophenyl ester[11] (**11**) or N^{α}-(fluoren-9-ylmethoxycarbonyl)-L-serine pentafluorophenyl ester[11] (**12**) in dichloromethane in the presence of AgOTf as a promoter for 2 h at 0 °C, gave the α-glycosides **13** (41% yield) and **14** (33% yield), respectively (Scheme 4). During the glycosylation, β-glycosides were also formed in 10% and 24% yield, respectively. The ^{1}H NMR spectra of **13** and **14** showed a one proton doublet at δ 5.23 ($J_{1,2}$ 3.6, 1a-H) and δ 5.10 ($J_{1,2}$ 3.2, 1a-H), respectively, confirming the formed glycosidic linkages to have the α configuration.

For the synthesis of the sialyl-(2→3)-T antigen, two steps were of importance. One is the coupling of the $GalN_3$ acceptor with sialylated Gal donor, the other is the removal of the TBDMS group.

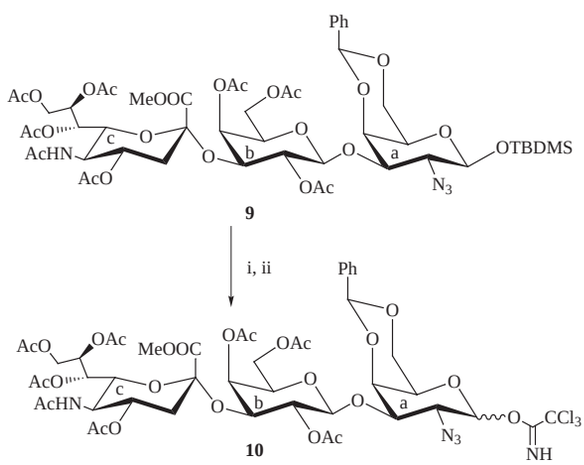
In the case of glycosylation, several kinds of reaction conditions were tried. First the reaction at 0 °C for 1 day was investigated. However, a mixture of target trisaccharide and orthoester compound at a ratio of 1:1 was produced. In order to achieve quantitative conversion from orthoester to target trisaccharide the reaction was performed at 20 °C. Under these conditions the conversion was efficient and quantitative. The optimal reaction temperature and conditions were 0 °C for 2 days followed by 1 day increasing the reaction temperature from 0 °C to room temperature. Under these conditions, no orthoester compound was observed in the product and the reaction yield was in excess of 80%.

For cleavage of the TBDMS group, THF was initially used as a reaction solvent as previously reported.[17] However, under those conditions, the benzylidene group was simultaneously removed. Therefore the solvent was changed from THF to pyridine as a proton scavenger. Using these reaction conditions, no degradation occurred and the reaction yield was in excess of 81%.

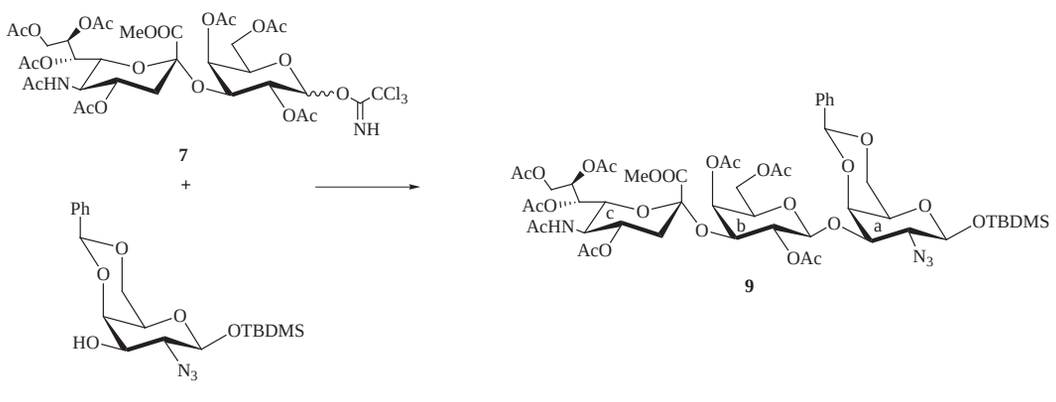
In conclusion a new block condensation approach has been employed for the synthesis of the sialyl-(2→3)-T antigen trisaccharide. The use of a sialylated galactosyl trichloroacetimidate



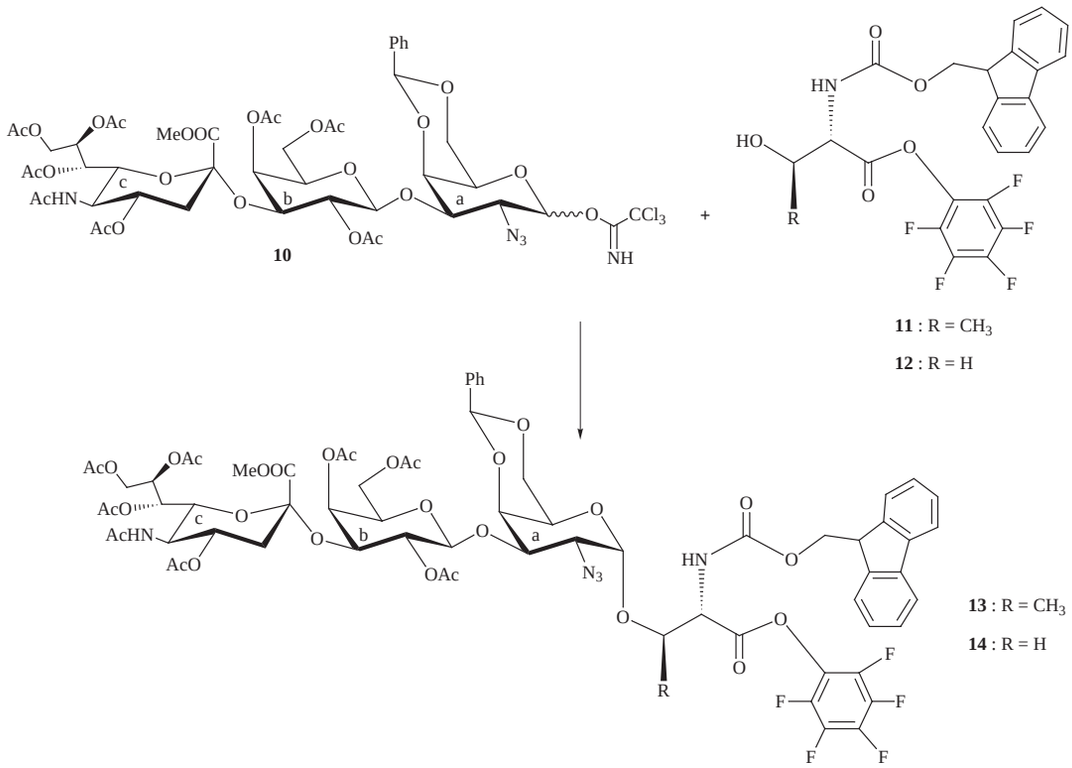
Scheme 1 *Reagents, conditions and yields*: i, 3.5 equiv. NIS, 0.4 equiv. TfOH, MS 3 Å, CH_2Cl_2, −30 °C, 65%; ii, Ac_2O, py, rt, quant.; iii, Pd/C, AcOH, rt, 63%; iv, Ac_2O, py, rt, 98%; v, CF_3CO_2H, CH_2Cl_2, rt, 94%; vi, CCl_3CN, 1 equiv. DBU, CH_2Cl_2, 0 °C, 80%.



Scheme 3 *Reagents*, *conditions and yields*: i, TBAF (1.0 M solution in THF), py, 0 °C, 81%; ii, CCl_3CN, 1 equiv. DBU, CH_2Cl_2, 0 °C, 96%.



Scheme 2 *Reagents*, *conditions and yield*: 0.1 equiv. TMS-OTf, CH_2Cl_2, MS 4 Å (AW300), 0 °C to rt, 80%.



Scheme 4 *Reagents*, *conditions and yield*: 2 equiv. AgOTf, CH_2Cl_2, MS 4 Å (AW300), 0 °C, 41% (R = CH_3), 33% (R = H). At the same time β glycoside linkages were also formed, 10% (R = CH_3), 24% (R = H).

donor ensured high yield in the glycosylation of the TBDMS protected GalNAc precursor. The use of TBDMS allowed high yield conversion to the trichloroacetimidate and glycosylation of Fmoc amino acid OPfp esters. The building blocks **13** and **14** can directly be used in automated solid phase glycopeptide synthesis.

Experimental

General procedures

Optical rotations were determined with a Perkin-Elmer 241 polarimeter at 25 °C (and are given in units of 10^{-1} deg cm^2 g^{-1}), and IR spectra were recorded with a Perkin-Elmer 1600 series FTIR. ^{1}H NMR spectra were recorded at 250 MHz with a Bruker DRX 250 spectrometer. Preparative chromatography was performed on silica gel [Merck silica gel 60 (0.040–0.063 mm)] with the solvent systems specified. Concentrations were conducted *in vacuo*.

2-(Trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranoside 4

A solution of 2-(trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-4,6-*O*-benzylidene-β-D-galactopyranoside[12] (**3**, 1.0 g, 1.19 mmol) in pyridine (5 cm^3) was added to acetic anhydride (5 cm^3) and the mixture was stirred for 2 h at room temperature. The product was dissolved in CH_2Cl_2 (50 cm^3) and the solution was washed with 2 M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography ($CHCl_3$–MeOH 50:1) of the residue on silica gel gave **4** (1.04 g, quant.) as an amorphous mass; $[\alpha]_D$ +2.5 (*c* 0.9, $CHCl_3$); δ_H(250 MHz, $CDCl_3$) 0.96 (2H, m, $Me_3SiCH_2CH_2$), 1.86 (3H, s, AcN), 2.00, 2.04, 2.11, 2.17, 2.19 (15H, 5s, 5AcO), 2.67 (1H, dd, J_{gem} 12.9, $J_{3eq,4}$ 4.5, 3c-H_{eq}), 3.63 (3H, s, MeO), 4.56 (1H, d, $J_{1,2}$ 8.0, 1b-H), 4.78 (1H, m, 4c-H), 5.04 (1H, d, $J_{NH,5}$ 10.2, NH), 5.19 (1H, dd, $J_{1,2}$ 8.0, $J_{2,3}$ 10.1, 2b-H), 5.35 (1H, s, PhC*H*), 5.52 (1H, m, 8c-H), 7.32–7.50 (5H, m, Ph) (Found: C, 54.33; H, 6.22; N, 1.38. Calc. for $C_{40}H_{57}NO_{19}Si$: C, 54.35; H, 6.50; N, 1.58%).

2-(Trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2-*O*-acetyl-β-D-galactopyranoside 5

A solution of **4** (1.05 g, 1.19 mmol) in AcOH (30 cm^3) was hydrogenolysed in the presence of 10% Pd/C (1.2 g) for 8 h at room temperature then filtrate and washings were combined and concentrated. Column chromatography ($CHCl_3$–MeOH 50:1) of the residue on silica gel gave **5** (0.60 g, 63%) as an amorphous mass; $[\alpha]_D$ −13.1 (*c* 0.7, $CHCl_3$); δ_H(250 MHz, $CDCl_3$) 0.99 (2H, m, $Me_3SiCH_2CH_2$), 1.86 (3H, s, AcN), 2.02, 2.04, 2.09, 2.14, 2.17 (15H, 5s, 5AcO), 2.63 (1H, dd, J_{gem} 12.9, $J_{3eq,4}$ 4.5, 3b-H_{eq}), 3.80 (3H, s, MeO), 4.27 (1H, dd, $J_{2,3}$ 9.8, $J_{3,4}$ 2.6, 3b-H), 4.36 (1H, dd, $J_{8,9'}$ 2.3, J_{gem} 12.3, 9′c-H), 4.52 (1H, d, $J_{1,2}$ 7.9, 1b-H), 4.82 (1H, m, 4c-H), 5.06 (1H, dd, $J_{1,2}$ 8.2, $J_{2,3}$ 9.6, 2b-H), 5.30 (1H, d, $J_{NH,5}$ 9.9, NH), 5.34 (1H, dd, $J_{6,7}$ 2.3, $J_{7,8}$ 9.1, 7c-H), 5.52 (1H, m, 8c-H) (Found: C, 49.64; H, 6.42; N, 1.70. Calc. for $C_{33}H_{53}NO_{19}Si$: C, 49.80; H, 6.71; N, 1.76%).

2-(Trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside 6

A solution of **5** (0.60 g, 0.75 mmol) in pyridine (3 cm^3) was added to acetic anhydride (3 cm^3) and the mixture was stirred for 2 h at room temperature. The product was dissolved in CH_2Cl_2 (50 cm^3) and the solution was washed with 2 M HCl and water, dried (Na_2SO_4) and concentrated. Column chromatography (ethyl acetate–hexane 4:1) of the residue on silica gel gave **6** (0.65 g, 98%) as an amorphous mass; $[\alpha]_D$ –12.8 (*c* 1.1, $CHCl_3$); δ_H(250 MHz, $CDCl_3$) 0.93 (2H, m, $Me_3SiCH_2CH_2$), 1.70 (1H, t, $J_{3ax,4} = J_{gem}$ 12.5, 3c-H_{ax}), 1.84 (3H, s, AcN), 1.99, 2.02, 2.04, 2.07, 2.07, 2.17, 2.20 (21H, 7s, 7AcO), 2.57 (1H, dd, J_{gem} 12.7, $J_{3eq,4}$ 4.6, 3c-H_{eq}), 3.84 (3H, s, MeO), 4.35 (1H, dd, $J_{8,9'}$ 2.6, J_{gem} 12.3, 9′c-H), 4.52 (1H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.3, 3b-H), 4.57 (1H, d, $J_{1,2}$ 8.1, 1b-H), 4.90 (1H, d, $J_{3,4}$ 2.7, 4b-H), 5.01

(1H, dd, $J_{1,2}$ 8.1, $J_{2,3}$ 10.1, 2b-H), 5.36 (1H, dd, $J_{6,7}$ 2.6, $J_{7,8}$ 9.2, 7c-H), 5.53 (1H, m, 8c-H) (Found: C, 50.24; H, 6.40; N, 1.53. Calc. for $C_{37}H_{57}NO_{21}Si$: C, 50.50; H, 6.53; N, 1.59%).

O-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-acetyl-D-galactopyranosyl trichloroacetimidate 7

To a solution of **6** (674.3 mg, 0.77 mmol) in CH_2Cl_2 (6 cm^3), cooled to 0 °C, was added CF_3CO_2H (5 cm^3) and the mixture was stirred for 1 h at room temperature and concentrated. The product was purified by chromatography on a column of silica gel with ethyl acetate–hexane 4:1 to give the 1-hydroxy compound (561.6 mg, 94%). To a solution of this in CH_2Cl_2 (10 cm^3), cooled to 0 °C, were added trichloroacetonitrile (2.14 cm^3) and DBU (108 mm^3, 0.72 mmol), and the mixture was stirred for 2 h at 0 °C then concentrated. Column chromatography (ethyl acetate–hexane 4:1) of the residue on silica gel gave **7** (532.2 mg, 80%) as an amorphous mass which was used directly in the glycosylation reaction; ν_{max}(film)/cm^{-1} 3450–3200 (NH), 1750 (ester), 1670 and 1550 (amide). The anomeric ratio (α:β) was estimated as ~1:5 from the ratio of the intensities of the anomeric proton signals of galactose residue in the ^{1}H NMR spectrum (Found: C, 44.12; H, 4.74; N, 2.94. Calc. for $C_{34}H_{45}Cl_3N_2O_{21}$: C, 44.19; H, 4.91; N, 3.03%).

tert-Butyldimethylsilyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside 9

To a solution of *tert*-butyldimethylsilyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside[10] (**8**, 225 mg, 0.55 mmol) and **7** (514 mg, 0.56 mmol) in dry CH_2Cl_2 (1.8 cm^3) were added MS 4 Å (AW-300, 700 mg), and the mixture was stirred for 5 h at room temperature, then cooled to 0 °C. Trimethylsilyl trifluoromethanesulfonate (TMS-OTf, 10 mm^3, 55 μmol) was added to the mixture and this was stirred for 2 days at 0 °C and for an additional 1 day at 0 °C to room temperature. After completion of the reaction, the mixture was neutralised with Et_3N, and filtered, and the residue was washed with CH_2Cl_2. The combined filtrate and washings were concentrated. Column chromatography (ethyl acetate–hexane 4:1) of the residue on silica gel gave **9** (519.1 mg, 80%) as an amorphous mass; $[\alpha]_D$ +5.8 (*c* 0.6, $CHCl_3$); δ_H(250 MHz, $CDCl_3$) 0.16, 0.17 (6H, 2s, Me_3CMe_2Si), 0.94 (9H, s, Me_3CMe_2Si), 1.72 (1H, t, $J_{3ax,4} = J_{gem}$ 12.4, 3c-H_{ax}), 1.86 (3H, s, AcN), 2.01, 2.03, 2.04, 2.07, 2.10, 2.18, 2.23 (21H, 7s, 7AcO), 2.59 (1H, dd, J_{gem} 12.6, $J_{3eq,4}$ 4.6, 3c-H_{eq}), 3.51 (1H, dd, $J_{2,3}$ 10.5, $J_{3,4}$ 3.4, 3a-H), 3.67 (1H, dd, $J_{1,2}$ 7.5, $J_{2,3}$ 10.5, 2a-H), 3.86 (3H, s, MeO), 4.36 (1H, dd, $J_{8,9'}$ 2.8, J_{gem} 12.5, 9′c-H), 4.53 (1H, d, $J_{1,2}$ 7.6, 1a-H), 4.60 (1H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.4, 3b-H), 4.93 (1H, d, $J_{1,2}$ 8.0, 1b-H), 4.94 (1H, d, $J_{3,4}$ 3.9, 4b-H), 5.03 (1H, d, $J_{NH,5}$ 10.2, NH), 5.11 (1H, dd, $J_{1,2}$ 8.0, $J_{2,3}$ 10.1, 2b-H), 5.40 (1H, dd, $J_{6,7}$ 2.6, $J_{7,8}$ 9.0, 7c-H), 5.52 (1H, s, PhC*H*), 5.56 (1H, m, 8c-H), 7.31–7.55 (5H, m, Ph) (Found: C, 52.37; H, 6.18; N, 4.79. Calc. for $C_{51}H_{72}N_4O_{25}Si$: C, 52.39; H, 6.21; N, 4.79%).

O-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-D-galactopyranosyl trichloroacetimidate 10

To a solution of **9** (1.59 g, 1.36 mmol) in pyridine (10 cm^3), cooled to 0 °C, was added tetrabutylammonium fluoride [1.0 M solution in tetrahydrofuran (1 cm^3)], and the mixture was stirred for 20 min at 0 °C. The product was dissolved in CH_2Cl_2 (200 cm^3) and the solution was washed with 2 M HCl, dried (Na_2SO_4) and concentrated. The product was purified by chromatography on a column of silica gel with ethyl acetate–hexane 4:1 to give the 1-hydroxy compound (1.16 g, 81%). To a solution of this in CH_2Cl_2 (7 cm^3), cooled to 0 °C, were added trichloroacetonitrile (3.3 cm^3) and DBU (165 mm^3, 1.10 mmol), and the mixture was stirred for 2 h at 0 °C then concentrated. Column chromatography (ethyl acetate–hexane 4:1) of the residue on silica gel gave **10** (1.27 g, 96%) as an amorphous mass which was immediately used for glycosylation reactions; ν_{max}(film)/cm^{-1} 3450–3200 (NH), 2120 (N_3), 1740 (ester), 1680 and 1540 (amide), 710 (Ph). The anomeric ratio (α:β) was estimated as ~7:5 from the ratio of the intensities of the anomeric proton signals of N_3-galactosamine residue in the ^{1}H NMR spectrum (Found: C, 47.02; H, 4.83; N, 5.59. Calc. for $C_{47}H_{58}Cl_3N_5O_{25}$: C, 47.07; H, 4.87; N, 5.84%).

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-*O*-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl]-L-threonine pentafluorophenyl ester 13

To a solution of **10** (300 mg, 0.25 mmol) and N^{α}-(fluoren-9-ylmethoxycarbonyl)-L-threonine pentafluorophenyl ester[11] (**11**, 151 mg, 0.30 mmol) in dry CH_2Cl_2 (6 cm^3) were added MS 4 Å (AW-300, 900 mg), and the mixture was stirred for 5 h at 0 °C. Silver trifluoromethanesulfonate (AgOTf) (128 mg, 0.50 mmol) was added, and the mixture was stirred for a further 2 h at 0 °C. The solids were filtrated off and washed with CH_2Cl_2. The combined filtrate and washings were concentrated. Column chromatography (ethyl acetate–hexane 4:1) of the residue on silica gel gave **13** (159.9 mg, 41%) as an amorphous mass. At the same time the β glycoside linkage was also formed (38.0 mg, 10%); $[\alpha]_D$ +19.1 (*c* 1.3, $CHCl_3$); δ_H(250 MHz, $CDCl_3$) 1.44 (3H, d, $J_{\gamma CH_3,\beta H}$ 6.4, γCH_3), 1.70 (1H, t, $J_{3ax,4} = J_{gem}$ 12.4, 3c-H_{ax}), 1.84 (3H, s, AcN), 1.99, 2.00, 2.01, 2.04, 2.05, 2.05, 2.11 (21H, 7s, 7AcO), 2.59 (1H, dd, J_{gem} 12.7, $J_{3eq,4}$ 4.6, 3c-H_{eq}), 3.63 (1H, dd, $J_{2,3}$ 10.6, $J_{3,4}$ 2.4, 3a-H), 3.85 (3H, s, MeO), 4.60 (1H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 3.4, 3b-H), 4.78 (1H, dd, $J_{\alpha H,\beta H}$ 2.1, $J_{\alpha H,NH}$ 9.3, αH), 4.91 (1H, d, $J_{1,2}$ 9.2, 1b-H), 4.94 (1H, d, $J_{3,4}$ 3.5, 4b-H), 5.13 (1H, d, $J_{NH,5}$ 10.0, N*H*Ac), 5.15 (1H, t, $J_{1,2} = J_{2,3}$ 10.0, 2b-H), 5.23 (1H, d, $J_{1,2}$ 3.6, 1a-H), 5.34 (1H, dd, $J_{6,7}$ 2.6, $J_{7,8}$ 8.8, 7c-H), 5.52 (1H, m, 8c-H), 5.55 (1H, s, PhC*H*), 6.22 (1H, d, $J_{\alpha H,NH}$ 9.3, N*H*Fmoc), 7.30–7.77 (13H, m, Ph and fluorene) (Found: C, 54.15; H, 4.63; N, 4.51. Calc. for $C_{70}H_{74}F_5N_5O_{29}$: C, 54.44; H, 4.83; N, 4.53%).

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-*O*-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl]-L-serine pentafluorophenyl ester 14

Glycosylation of **10** (300 mg, 0.25 mmol) with N^{α}-(fluoren-9-ylmethoxycarbonyl)-L-serine pentafluorophenyl ester[11] (**12**, 148 mg, 0.30 mmol) in dry CH_2Cl_2 (6 cm^3) in the presence of AgOTf (128 mg, 0.50 mmol) and MS 4 Å (AW-300, 900 mg) as described for **13** gave **14** (125.7 mg, 33%) as an amorphous mass. At the same time the β glycoside linkage was also formed (91.1 mg, 24%); $[\alpha]_D$ +26.9 (*c* 2.0, $CHCl_3$); δ_H(250 MHz, $CDCl_3$) 1.85 (3H, s, AcN), 1.98, 2.00, 2.00, 2.04, 2.04, 2.10, 2.20 (21H, 7s, 7AcO), 2.60 (1H, dd, J_{gem} 12.6, $J_{3eq,4}$ 4.5, 3c-H_{eq}), 3.66 (1H, dd, $J_{2,3}$ 10.7, $J_{3,4}$ 2.5, 3a-H), 3.86 (3H, s, MeO), 4.61 (1H, dd, $J_{2,3}$ 10.1, $J_{3,4}$ 3.3, 3b-H), 4.90 (1H, d, $J_{1,2}$ 8.0, 1b-H), 4.95 (1H, d, $J_{3,4}$ 3.2, 4b-H), 5.09 (1H, d, $J_{NH,5}$ 8.7, N*H*Ac), 5.10 (1H, d, $J_{1,2}$ 3.2, 1a-H), 5.15 (1H, t, $J_{1,2}$ 8.0, $J_{2,3}$ 9.9, 2b-H), 5.38 (1H, dd, $J_{6,7}$ 2.6, $J_{7,8}$ 9.1, 7c-H), 5.53 (1H, m, 8c-H), 5.55 (1H, s, PhC*H*), 6.29 (1H, d, $J_{\alpha H,NH}$ 9.3, N*H*Fmoc), 7.28–7.78 (13H, m, Ph and fluorene) (Found: C, 54.14; H, 4.52; N, 4.46. Calc. for $C_{69}H_{72}F_5N_5O_{29}$: C, 54.16; H, 4.74; N, 4.58%).

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References

1 S. Hakomori, *Adv. Cancer Res.*, 1989, **52**, 257.
2 G. F. Springer, *Science*, 1984, **224**, 1198.
3 E. Dabelsteen, *J. Pathol.*, 1996, **179**, 358.
4 K. Frische, T. Jensen, L. Galli-Stampino, S. Mouritsen, O. Werdelin and M. Meldal, in *Peptides in Immunology*, ed. C. H. Schneider, pp. 219–221, John Wiley and Sons, Ltd., Chichester, UK, 1995.
5 T. Jensen, L. Galli-Stampino, S. Mouritsen, K. Frische, S. Peters, M. Meldal and O. Werdelin, *Eur. J. Immunol.*, 1996, **26**, 1342.
6 E. Meinjohanns, M. Meldal, T. Jensen, O. Werdelin, L. Galli-Stampino, S. Mouritsen and K. Bock, *J. Chem. Soc.*, *Perkin Trans. 1*, 1997, 871.
7 P. A. Murray, M. J. Levine, L. A. Tabak and M. S. Reddy, *Biochem. Biophys. Res. Commun.*, 1982, **106**, 390.
8 M. J. Levine, M. C. Herzberg, M. S. Levine, S. A. Ellison, M. W. Stinson, H. C. Li and T. van Dyke, *Infect. Immun.*, 1978, **19**, 107.
9 Y. Nakahara, Y. Ito and T. Ogawa, *Carbohydr. Res.*, 1998, **309**, 287.
10 G. Grundler and R. R. Schmidt, *Liebigs Ann. Chem.*, 1984, 1826.
11 A. Vargas-Berenguel, M. Meldal, H. Paulsen and K. Bock, *J. Chem. Soc.*, *Perkin Trans. 1*, 1994, 2615.
12 S. Komba, H. Ishida, M. Kiso and A. Hasegawa, *Bioorg. Med. Chem.*, 1996, **4**, 1833.
13 A. Kameyama, H. Ishida, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, 1991, **209**, c1.
14 K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, J. Dahmen, G. Noori and K. Stenvall, *J. Org. Chem.*, 1988, **53**, 5629.
15 R. R. Schmidt and J. Michel, *Angew. Chem.*, *Int. Ed. Engl.*, 1980, **19**, 732.
16 T. Murase, H. Ishida, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, 1989, **209**, 71.
17 E. J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc.*, 1972, **94**, 6190.

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