Syntheses of model oligosaccharides of biological significance. XI. A short synthesis of fucosylated chitobiosides, also bound to asparagine in a synthon (as in *N*-linked glycoproteins)[1,2]

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We describe a simple and efficient method for the preparation of the trisaccharide GlcNAc(β1-4)-[Fuc(α1-6)-]GlcNAc(β1-) (**1**) and of the protected form of GlcNAc(β1-4)-[Fuc(α1-6)-]GlcNAc(β1-Asn) (**2**). The key intermediate is benzyl 4,6-benzylidene chitobioside **5** giving the desired trisaccharide by *in situ* anomerization–glycosylation reaction with 2,3,4-tribenzylfucosyl bromide. The benzyl glycoside in the trisaccharide **6** has been replaced by acetate and then bromine; this glycosylating agent was used to prepare methyl and 8-methoxycarbonyloctyl glycosides as well as isothiocyanate **12**, in a series of reactions. The latter compound gave, on reaction with 1-benzyl *N*-benzyloxycarbonyl-L-asparate, compound **13** (a protected derivative of **2**), which should serve as a synthon for syntheses of glycopeptides.

Key words: glycopeptide, synthesis; oligosaccharide, synthesis; chitobiosides; fucosylated chitobiosides; *N*-linked oligosaccharides.

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On décrit une méthode simple et efficace de préparer le trisaccharide GlcNAc(β1-4)-[Fuc(α1-6)-]GlcNAc(β1-) (**1**) ainsi que la forme protégée du GlcNAc(β1-4)-[Fuc(α1-6)-]GlcNAc(β1-Asn) (**2**). L'intermédiaire clé est le 4,6-benzylidène chitobioside de benzyle (**5**) qui fournit le trisaccharide désiré grâce à une réaction d'anomérisation et de glycosylation *in situ* à l'aide du bromure de 2,3,4-tribenzylfucosyle. On a remplacé le glucoside de benzyle du trisaccharide **6** par l'acétate et finalement le bromure; dans une série de réactions, on a utilisé cet agent de glycosylation pour préparer les glucosides de méthyle ou de 8-méthoxycarbonyloctyle ainsi que l'isothiocyanate **12**. Ce dernier composé, par réaction avec le *N*-benzyloxycarbonyl-L-aspartate de benzyle, conduit au composé **13** (une forme protégée du composé **2**) qui devrait s'avérer être un synthon utile pour la synthèse de glycopeptides.

Mots clés: glucoside, synthèse; oligosaccharide, synthèse; chitobiosides; chitobiosides fucosylés; oligosaccharides liés à l'azote.

[Traduit par la revue]

As part of our studies of unusual carbohydrate epitopes appearing in neoplasia and cancer (cf. also ref. 1), we are investigating synthetic approaches to complex oligosaccharides and building blocks for syntheses of glycopeptides. The carbohydrate structures in an appropriate form are usually immunogenic and consequently can be used for the preparation of antibodies, which in turn can recognize the original carbohydrate structure. Suitably modified or as such, the antibodies can serve as diagnostic reagents. Glycosylasparagine derivatives also can be used for the construction of glycosylpeptides required as models for immunological and conformational studies. The very common trisaccharide GlcNAc(β1-4)-[Fuc(α1-6)-]GlcNAc(β1-) (**1**) occurs in the core portion of the structure of N-linked oligosaccharides in glycoproteins. The procedures that we previously described for syntheses of trisaccharide glycosides **10** and **11** were lengthy (1, 3). Consequently, we have attempted to synthesize **1** from peracetylated chitobiose (which can be obtained in quantity (2) by acetolysis of chitin). We also wanted to ascertain whether a bromide derived from **1** could be obtained without disturbing the linkage of L-fucose to one of the *N*-acetylglucosamines.[6] We anticipated that such a bromide could be utilized for a synthesis of the synthon GlcNAc(β1-4)-[Fuc(α1-6)-]GlcNAc(β1-Asn) (**2**), which could in turn be used for the preparation of glycopeptides by an extension of the peptide chain.

A major problem connected with the formation of such a synthetic intermediate is the acid lability of α-L-fucosides. To achieve glycosylation with a glycosylating agent based on fucose, fucose has to be protected by benzyl groups. Otherwise the glycosylating agent would not be sufficiently reactive (5). For instance, glycosylation of benzylidene chitobioside **5** with 2,3,4-triacetylfucosyl halogenides does not give any identifiable product. Benzyl protection on the other hand renders fucose sensitive to acid conditions. Therefore, to effect transformation of the 1-*O*-acetyl of trisaccharide **1** into a bromide for eventual use as a glycosylating agent, all hydroxy groups of **1** have to be protected as their acetyls. Thus it can be expected that the peracetylated bromide of **1** should be sufficiently stable to act as a glycosylating agent.

[1]For part 10, see D. M. Whitfield *et al.*, preceding paper.

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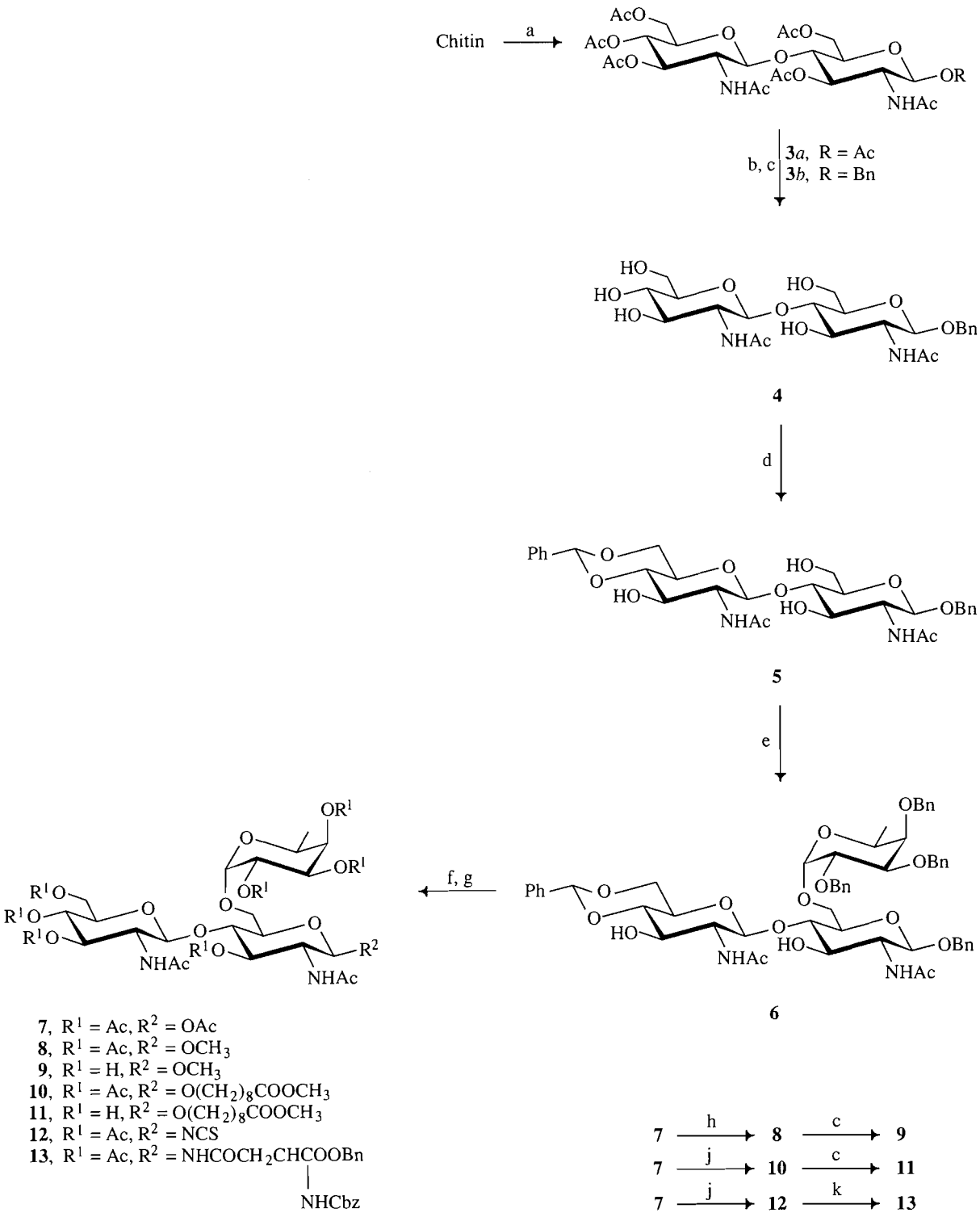
[6]After completion of the experimental work and during the preparation of this manuscript, a publication by Kunz and Unverzagt appeared (4), in which this problem was studied using a different approach.



a, H_2SO_4, Ac_2O; b, $TiBr_4/CH_2Cl_2$; BnOH, *p*-tosyl acid/CH_2Cl_2 or HCl/Ac_2O–AcOH; BnOH, Ag_2CO_3, anh. $CuSO_4$; c, NaOMe/MeOH, RT; d, $PhCH(OMe)_2$, *p*-tosyl acid/CH_3CN–DMF; e, Et_4NBr/DMF+2,3,4–tri-*O*-benzyl-α-L-fucopyranosyl bromide/CH_2Cl_2; f, Pd/C–H_2/AcOH–MeOH; g, Ac_2O/C_5H_5N, RT, h, $TiBr_4/CH_2Cl_2$; *p*-tosyl acid/ anh. MeOH; i, $TiBr_4/CH_2Cl_2$; 8-methoxycarbonyl octanol, *p*-tosyl acid/CH_2Cl_2; j, $TiBr_4/CH_2Cl_2$; KNS, *p*-tosyl acid/CH_3CN; k, 1-Benzyl *N*-(benzyloxy)carbonyl-L-aspartate, Et_3N, toluene

SCHEME 1

The formation of *N*-glycosidic linkages between glucose (6–8), 2-acetamido-2-deoxy glucose (or chitobiose) (9–13) or derivatives (16), and asparagine has been previously reported. Most often glycosylamides (4, 6–16) were used in reaction with 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate. However, in alternate approaches glycosylamines were reacted with *N*-(benzyloxycarbonyl)-L-aspartic anhydride (14), with 1-benzyl *N*-[2-(-triphenylphosphonio)ethoxycarbonyl]aspartate (15), or 1-*tert*-butyl *N*-allyloxycarbonylaspartate (4). The glycosylamines were prepared either by reduction of azides (4, 6–16, 20) or by reaction of reducing aldoses with ammonium hydrogen carbonate (19). These procedures are all relatively time-consum-

ing, and thus our objective has been to reduce the complexity and shorten time requirements for the synthesis. Glycosylisothiocyanate (17) was successfully reacted with 1-benzyl *N*-benzyloxycarbonyl-L-aspartate (18), which served as precedent for our final coupling to give **13**.

Octaacetylchitobiose (**3***a*) obtained by acetolysis of chitin (2) can be transformed efficiently into either heptaacetylchitobiosyl chloride by treatment with anhydrous HCl or heptaacetylchitobiosyl bromide by treatment with titanium tetrabromide. Both of these halogenides yield benzyl glycosides on reaction with benzyl alcohol. The 4- and 6-hydroxy groups on the "nonreducing" terminal monosaccharide unit of this benzyl glycoside are protected as their benzylidene derivative, rendering the other 6-hydroxy group (residing on the "reducing" monosaccharide unit) as the only available primary hydroxyl for the reaction with 2,3,4-tri-*O*-benzyl-L-fucosyl bromide (1, 3). The secondary OH-3 groups do not react with the fucosyl residue to any appreciable extent under these conditions.

The protected trisaccharide **6**, made in just four steps, is then debenzylated and acetylated. The acetylated intermediates are suitable for chromatography, assuring that the starting material for the preparation of the bromide, by a reaction with titanium tetrabromide (21), is indeed pure. The bromide was then used without purification as the glycosylating synthon to prepare methyl glycoside **8** and 8-methoxycarbonyloctyl glycoside **10** by reaction with appropriate alcohols, and isothiocyanate **12** by reaction with potassium thiocyanate. The two former compounds were identical to samples synthesized previously by a substantially more tedious procedure (the acetyl protecting groups were smoothly removed using Zemplen's conditions) (1, 3). The isothiocyanate served as one of the starting materials for the simple and efficient synthesis of the protected derivative **13** of GlcNAc(β1-4)-[Fuc(α1-6)-]GlcNAc(β1-Asn). The anomericity of 2-acetamido-2-deoxy D-glucosides in both **12** and **13** is β, as can be seen from the diaxial coupling constants of H-1 and H-1′: J = 8.3 Hz (**12**), and 9.0 and 9.7 Hz (**13**), cf. Experimental. The synthetic design is portrayed in Scheme 1.

The choice of the isothiocyanate reaction for the introduction of the amino function into the anomeric position at the trisaccharide reducing end deserves a comment. Most importantly, it was chosen because of the mild reaction conditions required. An alternate pathway via glycosyl azides (22) (in this case the azide derived from **7** (R^1 = Ac, R^2 = N_3)) involves the reduction of the N_3 group to an amino group. The resulting amine (R^1 = Ac, R^2 = NH_2) is unstable because of the complex pattern of acetyl migrations to the amino group (cf. ref. 22). Considering the overall requirements of the azide route (cf. ref. 22 for an excellent discussion of the azide procedure), we believed that, although it gives moderate yields, the isothiocyanate approach is preferable since it is experimentally simple and easily reproducible.

After appropriate deprotection, compound **13** can be used in oligopeptide synthesis or, alternatively, the glycosylated asparagine can be obtained. The use of **13** and its variations in glycopeptide synthesis will be described in a future communication.

Experimental

General methods

Melting points were determined with a Reichert Thermovar melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer polarimeter (model 243B) at 26 ±1°C. Microanalyses were performed by the Microanalytical Laboratory Ltd., Markham, Ontario. ^{1}H NMR spectra were recorded at 360.06 MHz with a Nicolet spectrometer or at 500 MHz with a Bruker AM spectrometer at the NMR spectroscopy laboratory of the Carbohydrate Research Centre, University of Toronto. Spectra were obtained at 23 ±2°C; chemical shifts are cited in δ (ppm) scale relative to TMS (0 ppm) and coupling constants are reported in hertz (Hz). The IR spectra were recorded on a Perkin–Elmer (model 1430) spectrometer using thin film or NaCl plates. Fast Atom Bombardment mass spectra (FAB-MS) were performed with a VG Analytical ZAB-SE high-mass high-resolution mass spectrometer at the mass spectrometry laboratory of the Carbohydrate Research Centre, University of Toronto.

Thin-layer chromatography (TLC) was performed on silica gel $60F_{254}$ (Merck) plastic plates, which were developed using 30% sulfuric acid followed by heating at 150°C. Silica gel (230–400 mesh; Toronto Research Chemicals) was used for flash chromatography. All starting materials were dried under high vacuum over P_2O_5 or KOH overnight prior to use. Solvents were distilled under argon from appropriate drying agents onto anhydrous 3 Å molecular sieves and were transferred via syringe. Solvents were removed at reduced pressures using a rotary evaporator at temperatures not exceeding 40°C.

Benzyl 2-acetamido-4-O-(2′-acetamido-2′-deoxy-3′, 4′, 6′-tri-O-acetyl-β-D-glucopyranosyl)-2-deoxy-3,6-di-O-acetyl-β-D-glucopyranoside (3b)

Method A

To solid titanium(IV) bromide (12.2 g) stirring at 0°C was added a solution of chitobiosyl octaacetate (2) (3.00 g, 4.23 mmol) in dry dichloromethane (120 mL), followed by ethyl acetate (30 mL) (21). The resulting homogeneous reddish brown mixture was further stirred at room temperature for 6 h. It was diluted with dry dichloromethane (50 mL) and anhydrous sodium acetate (12 g) was added. After stirring for 15 min the reaction mixture was poured onto ice-water (100 mL) and the product was extracted into dichloromethane (300 mL), dried over $MgSO_4$, and evaporated and coevaporated with toluene (2 × 30 mL) to give a colourless solid residue (3.08 g). This was dissolved in dichloromethane (300 mL) and treated with benzyl alcohol (14.4 mL, 141 mmol) and *p*-toluenesulfonic acid monohydrate (160 mg, 0.85 mmol) at room temperature for 69 h. The reaction mixture was washed with ice-water (150 mL), dried over $MgSO_4$, and evaporated to dryness giving a light orange liquid residue. It was subjected to chromatography on a silica gel column ($CHCl_3$–acetone, 2:1), giving pure **3***b* (1.11 g, 36%) as a colourless solid, mp 277–280°C (dichloromethane–acetone); $[\alpha]_D$ −63.0° (*c* 0.60, $CHCl_3$); R_f 0.58 (threefold development in chloroform–acetone, 2:1); IR (neat); 3295 (NH), 3075 (aromatic CH), 1750 (COOR), and 1665 (CONH) cm^{-1}; ^{1}H NMR (δ): 7.32 (m, 5, $C_6H_5CH_2$), 6.02 (d, 1H, J = 9.0 Hz, N*H*′), 5.62 (d, 1H, J = 9.3 Hz, N*H*′), 5.18 (t, 1H, J= 9.3 Hz, H-3′), 5.04 (t, 2H, J = ca. 9.02 Hz, overlapping H-3 and H-4′), 4.86 and 4.57 (2d, 2H, J = 12.2 Hz, $C_6H_5CH_2$), 4.56 (d, 1H, J = 8.6 Hz, H-1′), 4.44 (d, 1H, J = 7.9 Hz, H-1), 4.09 (td, 1H, J = 9.3 and 7.9 Hz, H-2), 3.87 (br q, 1H, J = 9.0 Hz, H-2′), 3.74 (t, 1H, J = 8.6 Hz, H-4), 3.59–3.67 (m, 2H, H-5, H-5′), 2.16, 2.07, 2.03, 2.01, and 1.96 (5s, 21H, CH_3CO). Anal. calcd. for $C_{33}H_{44}N_2O_{16}$: C 54.69, H 6.12, N 3.87; found: C 54.60, H 6.23, N 3.72.

Method B

To a solution (14 mL) of acetic acid and acetic anhydride (1:1, v/v) saturated with anhydrous HCl at 0°C was added chitobiosyloctaacetate (1.1 g) and the reaction mixture was slowly stirred for 60 h at room temperature. Then the reaction mixture was diluted with ice-cold $CHCl_3$ (50 mL), ice was added, and the solution was washed successively with ice-cold water, saturated aqueous $NaHCO_3$, and water, dried over Na_2SO_4, and evaporated to dryness at temperatures <35°C. The residue was triturated with dry diethyl ether and recrystallized from chloroform–acetone mixture, giving a white crystalline chloride, mp 212–214°C, 420 mg (11, 23). The chloride (0.2 g) was dissolved in benzylalcohol (12 mL; dried over 4 Å molecular sieve), anhydrous Ag_2CO_3 (65 mg) and anhydrous $CaSO_4$ (680 mg) were added, and the mixture was slowly stirred for 48 h at room temperature. After filtering the reaction mixture through a bed of Celite that was subsequently washed with $CHCl_3$, the combined washings were evaporated to dryness *in vacuo*, and the residue was

subjected to chromatography on a silica gel column as described in Method A (cf. above).

Benzyl 2-acetamido-4-O-(2'-acetamido-4,6-O-benzylidene-2'-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranoside (5)

Compound **3***b* (300 mg) in CH_3OH (25 mL) was stirred with 0.1 N sodium methoxide at room temperature for 4 h. Methanol was evaporated at <30°C, water (60 mL) was added to the residue, and the resulting solution was washed with diethyl ether (3×). The combined ether washings were washed with water, and all aqueous fractions were combined, neutralized with 1 M aqueous HCl, treated with mixed bed resin AG 501 X-8 for 45 min, filtered, and evaporated to dryness to give **4**. To a clear solution of compound **4** (537 mg, 1.04 mmol) and *p*-toluenesulfonic acid monohydrate (145 mg) in acetonitrile–DMF (1:4, 25 mL) was added benzaldehyde dimethyl acetal (0.50 mL) (24) and the reaction mixture was stirred in the dark at room temperature. After 22 h additional acetal (0.30 mL) was added and the reaction mixture was stirred for another 74 h. Triethylamine (1 mL) was added and the solution was stirred for 5 min, evaporated to dryness, and the light brown residue was taken up into DMF (1.5 mL) and applied to a silica gel column (chloroform–methanol, 10:1) to give compound **5** (457 mg, 73%) as a colourless solid; mp 287–290°C (chloroform–methanol; dec.); $[\alpha]_D$ −81.7° (*c* 0.59, MeOH–H_2O, 2:1); R_f 0.30 (chloroform–methanol, 8:1); ^1H NMR (δ): 7.20–8.00 (m, 10, H, C_6H_5), 5.60 (s, 1H, PhC*H*(-O)$_2$), 4.77 (d, 1H, *J* = 12.5 Hz, PhC*H*H), 4.59 (d, 1H, *J* = 7.6 Hz, H-1'), 4.51 (d, 1H, *J* = 12.5 Hz, PhCH*H*), 4.38 (d, 1H, *J* = 8.4 Hz, H-1), 1.84 and 1.81 (2s, 6H, C*H*$_3$CO). Anal. calcd. for $C_{30}H_{38}N_2O_{11}$: C 59.79, H 6.36, N 4.65; found: C 59.82, H 6.38, N 4.71.

Benzyl 2-acetamido-4-O-(2'-acetamido-4,6-O-benzylidene-2'-deoxy-β-D-glucopyranosyl)-2-deoxy-6-O-(2'',3'',4''-tri-O-benzyl-α-L-fucopyranosyl)-β-D-glucopyranoside (6)

Dry dichloromethane (9 mL) and powdered 4 Å molecular sieve (1.12 g) were added to a mixture of compound **5** (570 mg, 0.943 mmol) and tetraethylammonium bromide (1.12 g) in DMF (9 mL) and the resulting suspension was stirred for 0.5 h. Then a solution of 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide (0.562 g, 1.13 mmol) in dichloromethane (2 mL) was added. The residue of bromide in the flask was rinsed into the reaction vessel with additional dichloromethane (2 × 1 mL). To this reaction mixture, after being slowly stirred for 19 h, triethylamine (2 mL) was added and the mixture was stirred for an additional few minutes. The solids were filtered off and washed with dichloromethane (3 × 50 mL). The combined filtrate and washings were washed with water (2 × 50 mL) and the aqueous portions back-extracted with chloroform (2 × 30 mL). The combined organic extracts were dried over $MgSO_4$, then evaporated to dryness to give a light brown syrup (1.40 g), which was purified by chromatography on a silica gel column (ethyl acetate eluted nonpolar impurities, and chloroform–methanol, 20:1, eluted the required product) to yield compound **6** (474 mg, 49%) as a colorless solid, mp 256–260°C (dec.) (methanol – ethyl acetate); $[\alpha]_D$ −88.3° (*c* 0.53, $CHCl_3$); R_f 0.36 (chloroform–methanol, 10:1); ^1H NMR (δ): 7.20–7.60 (m, 25H, C_6H_5), 6.53 (d, 1H, *J* = 8.7 Hz, exchangeable with D_2O, N*H*), 5.47 (s, 1H, PhC*H*(-O$_2$), 5.46 (d, 1H, partly overlapped with the signal at 5.47, *J* approx. 6 Hz, exchangeable with D_2O, N*H*), 4.87 (d, 1H, *J* = 3.5 Hz, H-1''), 4.47 (d, 1H, *J* = 8.4 Hz, H-1'), 4.29 (d, 1H, *J* = 8.2 Hz, H-1), 4.15 (q, 1H, *J* = 6.5 Hz, H-5''), 2.02 and 1.80 (2s, 6H, C*H*$_3$CO), 1.17 (d, 3H, *J* = 6.5 Hz, H-6''). Anal. calcd. for $C_{57}H_{66}N_2O_{15}$: C 67.18, H 6.53, N 2.75; found: C 67.21, H 6.60, N 2.68.

2-Acetamido-4-O-(2'-acetamido-2'-deoxy-3',4'-6'-tri-O-acetyl-β-D-glucopyranosyl)-2-deoxy-1,3-di-O-acetyl-6-O-(2'',3'',4''-tri-O-acetyl-α-L-fucopyranosyl)-α-D-glucopyranose (7)

Compound **6** (97 mg, 0.095 mmol) in acetic acid – methanol (10 mL, 3:2) was hydrogenolysed on Pd–C (10%). After the reaction was completed (monitored by TLC), the catalyst was filtered off using a Celite bed, the bed was washed with methanol–water (1:1, 5 × 20 mL), and the combined filtrates were evaporated to dryness. The residue was treated with acetic anhydride (3 mL) and pyridine (3 mL) at room temperature. The white precipitate formed was brought into solution by warming up the reaction mixture to 40°C. The homogeneous mixture was then stirred at room temperature for 16 h. It was diluted with ethyl acetate (100 mL) and washed with cold (0°C) water (3 × 30 mL). The organic layer was dried over $MgSO_4$ and evaporated to give compound **7** as a colourless solid (67 mg, 78%), homogeneous by TLC (ethyl acetate – methanol, 9:1, R_f 0.47); mp 262–265°C (from ethyl acetate – hexane); $[\alpha]_D$ −25.1° (*c* 0.55, $CHCl_3$). ^1H NMR (δ): 6.11 (d, 1H, *J* = 3.9 Hz, H-1), 5.64 (d, 1H, *J* = 8.4 Hz, N*H*), 5.52 (d, 1H, *J* = 9.0 Hz, N*H*), 5.11 (d, 1H, *J* = 3.6 Hz, H-1''), 4.89 (d, 1H, *J* = 8.2 Hz, H-1'), 2.18, 2.17, 2.16, 2.09, 2.08, 2.02, 2.01, 2.00, 1.94, and 1.93 (10s, 30H, C*H*$_3$CO), 1.15, (d, 3H, *J* = 6.5 Hz, H-6''). Anal. calcd. for $C_{38}H_{54}N_2O_{23}$: C 50.33, H 6.00, N 3.09; found: C 50.52, H 6.12, N 3.15.

Methyl 2-acetamido-4-O-(2'-acetamido-2'-deoxy-β-D-glucopyranosyl)-2-deoxy-6-O-(α-L-fucopyranosyl)-α-D-glucopyranoside (9)

To solid titanium(IV) bromide (1.29 g) was added a solution of α-1-*O*-acetyl trisaccharide **7** (204 mg, 0.225 mmol) in dry dichloromethane (9 mL) stirring at 0°C, followed by addition of ethyl acetate (2.5 mL) (21). The resulting homogeneous reddish brown mixture was stirred at room temperature for 4.5 h. Then it was diluted with dry dichloromethane (10 mL), anhydrous sodium acetate (4 g) was added, and the mixture was stirred for 5 min, poured onto ice-water (50 mL), and the product extracted into dichloromethane (200 mL). It was then dried over $MgSO_4$, and evaporated and coevaporated with toluene (2 × 50 mL) to give a pure yellow solid (159 mg), which was treated with dry methanol (0.564 mL of 1 mmol per mL CH_2Cl_2 solution, 0.564 mmol) and *p*-toluenesulfonic acid monohydrate (4 mg, 0.019 mmol) in dichloromethane (2 mL) at room temperature for 18 h. It was diluted with dichloromethane (30 mL), poured into ice-water (20 mL), separated, dried over $MgSO_4$, and evaporated to give a pale yellow solid (129 mg), which was purified on a silica gel column ($CHCl_3$–acetone, 1:1), yielding peracetylated compound **8** (67 mg, 35%); R_f 0.47 (twofold development in $CHCl_3$–acetone, 1:1; identical with an authentic sample prepared by acetylation of compound **8** prepared by another method (3); ^1H NMR (δ): 5.74 (d, 1H, *J* = 8.3 Hz, exchangeable with D_2O, N*H*), 5.67 (d, 1H, *J* = 9.4 Hz, exchangeable with D_2O, N*H*), 5.09 (d, 1H, *J* = 3.6 Hz, H-1''), 4.93 (d, 1H, *J* = 8.3 Hz, H-1'), 4.41 (d, 1H, *J* = 7.6 Hz, H-1), 4.30 (br, 1, 1H, *J* approx. 6.8 Hz, H-5''), 3.45 (s, 3H, OCH_3), 2.18, 2.15, 2.07, 2.01, 2.00, 1.98, and 1.92 (7s, 27H, C*H*$_3$CO), 1.15 (d, 3H, *J* = 6.5 Hz, H-6'').

Compound **8** was dried and treated with 1% sodium methoxide (1 mL) in dry methanol (92 mL) at room temperature for 20 min. Then the reaction mixture was neutralized with acidic resin (0.5 mL), filtered, washed with MeOH–H_2O (1:1, 3 × 10 mL), and evaporated to give compound **9** (45 mg, 34%) as a colourless solid; mp 300–302°C (from ethanol–water); $[\alpha]_D$ −67.9° (*c* 0.67, H_2O) (lit. (3) mp 298–300°C (dec.); $[\alpha]_D$ −65.8° (*c* 0.53, H_2O). The ^1H NMR spectrum of **9** was identical with that of the authentic sample (3).

8-Methoxycarbonyloctyl 2-acetamido-4-O-(2'-acetamido-2'-deoxy-3',4'-6'-tri-O-acetyl-β-D-glucopyranosyl)-3-O-acetyl-2-deoxy-6-O-(2'',3'',4''-tri-O-acetyl-α-L-fucopyranosyl)-β-D-glucopyranoside (10)

To solid titanium(IV) bromide (0.79 g) was added a solution of α-1-*O*-acetyl trisaccharide **7** (124 mg, 0.137 mmol) in dry dichloromethane (4 mL) stirring at 0°C, followed by an addition of ethyl acetate (1 mL) (21). The reaction mixture was stirred at room temperature for 17 h. Then it was diluted with dry dichloromethane (15 mL), anhydrous sodium acetate (1.5 g) was added, and after stirring for 15 min it was shaken with ice-water (25 mL). The product was then extracted into dichloromethane (70 mL), dried over $MgSO_4$, and the solvents were evaporated and coevaporated with toluene (100 mL) to give pale yellow solid bromide (96 mg). It was treated with 8-methoxycarbonyl-octanol (25) (400 mg, 2.12 mmol) and *p*-toluenesulfonic acid (3 mg) in dichloromethane (2 mL) at room temperature for 26 h. The reaction mixture was then diluted with dichloromethane (70 mL) and poured into ice-water (20 mL). The usual work-up gave a crude product, which after chromatography on a silica gel column (ethyl acetate) yielded pure

10 (38 mg, 27%); $[\alpha]_D$ −68.5° (*c* 0.99, $CHCl_3$) (lit. (1) $[\alpha]_D$ −69.2° (*c* 1.0, $CHCl_3$)); R_f and ^1H NMR were identical with those of an authentic sample (1).

*8-Methoxycarbonyloctyl 2-acetamido-4-*O*-(2′-acetamido-2′-deoxy-β-*D*-glucopyranosyl)-2-deoxy-6-*O*-α-fucopyranosyl-p-*D*-glycopyranoside (**11**)*

Zemplen deacetylation of **10** (52 mg, 0.050 mmol) in dry methanol (2 mL) was done as described previously (1), using 1% sodium methoxide (1 mL) and neutralizing acidic resin (0.5 mL). The trisaccharide **11** (37 mg, 100%) was obtained as a colourless solid, mp 282–286°C (aqueous ethanol); $[\alpha]_D$ −60.6° (*c* 0.31, 50% aqueous ethanol); (lit. (1) mp 283–286°C; $[\alpha]_D$ −63.5° (*c* 0.42, 50% aqueous ethanol). The ^1H NMR was identical with that of the authentic sample (1).

*2-Acetamido-4-*O*-(2′-acetamido-2′-deoxy-3′,4′-6′-tri-*O*-acetyl-β-*D*-glucopyranosyl)-3-*O*-acetyl-2-deoxy-6-*O*-(2″,3″,4″-tri-*O*-acetyl-α-*L*-fucopyranosyl)-β-*D*-glucopyranosyl isothiocyanate (**12**)*

Trisaccharide **7** (308 mg, 0.340 mmol) was treated with titanium(IV) bromide (1.68 g) in dichloromethane (15 mL) and ethyl acetate (4 mL) as described for compound **10**. The reaction mixture was diluted after 4.5 h with dry dichloromethane (50 mL) and after the usual work-up (using anhydrous sodium acetate (5 g), ice-water (100 mL), dichloromethane (220 mL), and toluene (2 × 50 mL)) a pale yellow solid bromide (229 mg) was obtained. It was treated (26) with potassium thiocyanate (0.5 g) and *p*-toluenesulfonic acid monohydrate (50 mg) in acetonitrile (10 mL) at 70°C (bath temperature) for 15 min. Then the reaction mixture was cooled, diluted with dichloromethane (100 mL), washed with water (50 mL), and the organic layer was dried over $MgSO_4$ and evaporated to give a light yellow solid (220 mg). By chromatography on a silica gel column using chloroform–acetone, 37:13 (followed by solvent ratio 2:1), pure **12** (161 mg, 53%) was obtained as a colourless solid; mp 155–157°C (ethyl acetane – hexane); $[\alpha]_D$ −74.2° (*c* 0.67, $CHCl_3$); R_f 0.34 (twofold development in chloroform–acetone, 2:1); IR (neat). 3320 (NH), 2022(-NCS), 1750 (COOR), 1670 (CONH) cm^{-1}; ^1H NMR (δ): 6.03 (br, d, 1H *J* = 8.6 Hz, exchangeable with D_2O, N*H*), 5.81 (d, 1H, *J* = 7.9 Hz exchangeable with D_2O, N*H*′), 5.39 (dd, 1H, *J* = 10.8 and 3.2 Hz, H-3″), 5.32 (br d, 1H, *J* = 3.2 Hz, H-4″), 5.18 (dd, 1H, *J* = 10.8 and 3.6 Hz, H-2″), 5.09 (d, 1H, *J* = 3.6 Hz, H-1″), 5.06 (d, 1H, *J* = 8.3 Hz, H-1), 4.92 (d, 1H, *J* = 8.3 Hz, H-1′), 4.27 (br q, 1H, *J* = 6.5 Hz, H-5″), 3.57 (m, 1H, became dd after exchange with D_2O, *J* = 10.4 and 8.3 Hz, H-2′), 2.18, 2.16, 2.10, 2.08, 2.02, 2.01, and 1.92 (7s, 27H, C*H*$_3$CO), 1.17 (d, 3H, *J* = 6.5 Hz, H-6″). Anal. calcd. for $C_{37}H_{51}N_3SO_{21}$: C 49.06, H 5.68, N 4.64, S 3.54; found: C 49.26, H 5.56, N 4.81, S 3.62.

*2-Acetamido-4-*O*-(2′-acetamido-2′-deoxy-3′,4′-6′-tri-*O*-acetyl-β-*D*-glucopyranosyl)-3-*O*-acetyl-2-deoxy-6-*O*-(2″,3″,4″-tri-*O*-acetyl-α-*L*-fucopyranosyl)-1-*N*-[1-benzyl *N*-(benzyloxy)carbonyl-*L*-aspart-4-oyl]-β-*D*-glucopyranosylamine (**13**)*

A mixture of isothiocyanate **12** (114 mg, 0.126 mmol), 1-benzyl *N*-(benzyloxy)carbonyl-L-aspartate (134 mg, 0.378 mmol), and triethylamine (0.03, 0.192 mmol) in dry toluene (2.5 mL) was stirred (18) at room temperature for 71 h. Then it was diluted with chloroform (70 mL), and the chloroform solution was washed successively with cold (0°C) aqueous sodium bicarbonate (20 mL) and water (20 mL). The chloroform solution was dried over $MgSO_4$ and evaporated to give a pale yellow syrup (185 mg), which after chromatography on a silica gel column (chloroform–acetone, 37:13) yielded pure **13** (49 mg, 32%) as a colourless solid, mp 185°C (with sintering at 128°C; recrystallized from hexane – methanol); $[\alpha]_D$ −43.7° (*c* 0.51, $CHCl_3$); R_f 0.35 (fivefold development in $CHCl_3$–acetone, 37:13); IR (neat):3360 (NH), 3062 and 3036 (aromatic C—H), 1750 (COOR), 1678 (CONH) cm^{-1}; ^1H NMR (δ): 7.30 (m, 10H, $C_6H_5CH_2$), 5.50 (t, 1H, *J* = 9.7 Hz, H-3′), 5.36 (dd, 1H, *J* = 10.8 and 3.2 Hz, H-3″), 5.29 (br d, 1H, *J* = 3.0 Hz, H-4″), 5.21 (d, 1H, *J* approx. 9.0 Hz, H-1′), 5.06 (d, 1H, *J* = 3.6 Hz, H-1″), 5.01 (t, 1H, *J* = 9.7 Hz, H-4′), 4.73 (br t, 1H, *J* approx. 8.6 Hz; became d after exchange with D_2O, *J* = 9.7 Hz, H-1) 4.64 (m, 1H, became t after exchange with D_2O, *J* = 3.6 Hz, H-2 (asparagine)), 4.29 (br q, 1H, *J* = 6.5 Hz, H-5″), 4.02 (br q, 1H, became t after exchange with D_2O, *J* = 10.0 Hz, H-2), 3.90 (t, 1H, *J* = 9.4 Hz, H-4), 3.54 (br d, 1H, *J* = 9.4 Hz, H-5), 3.20 (m, 1H, became br t after exchange with D_2O, *J* approx. 9.3 Hz, H-2′), 2.85 (dd, 1H, *J* = 16.2 and 4.0 Hz, H-3 (asparagine)), 2.68 (dd, 1H, *J* = 16.2 and 4.0 Hz, H-3) (asparagine)), 2.15, 2.13, 2.05, 2.00, 1.99, 1.91, and 1.86 (7s, 27H, C*H*$_3$CO), 1.03 (d, 3H, *J* = 6.5 Hz, H-6″). Anal. calcd. for $C_{55}H_{70}N_4O_{26}$: C 54.91, H 5.87, N 4.66; found: C 54.72, H 5.71, N 4.83.

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