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Synthesis of Glycopeptides and Neoglycoproteins Containing the Fucosylated Linkage Region of *N*-Glycoproteins

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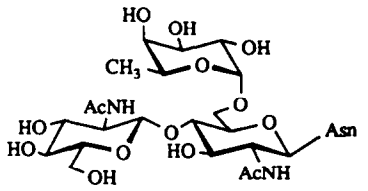
Abstract—*N*-Glycoproteins fucosylated in the core region occur in tumor membranes and virus envelopes. Partial structures of such *N*-glycoproteins containing fucosylated chitobiosyl asparagine conjugates were synthesized using the allyloxycarbonyl (Aloc) and the *tert*-butyl ester protecting groups in the peptide portion. As the α-fucosidic bond of the conjugates revealed to be very sensitive to acids when carrying ether-type protecting groups, a method for exchanging the protecting groups of the fucose portion of saccharides was developed. Conjugates containing *O*-acetyl protected fucose proved to be stable against acids used in glycopeptide syntheses. These methods were applied in the synthesis of a fucosyl chitobiose hexapeptide with the partial sequence of a leukemia virus envelope glycoprotein. The glycopeptide was coupled to bovine serum albumin yielding a neoglycoprotein which contains a glycoconjugate of exactly specified structure.

Introduction

Fucosylation of membrane glycoproteins plays a special role in biological recognition processes.[1] L-Fucose has been found to be one of the important constituents of glycoproteins and glycolipids, e.g. in blood group determinants,[2] tumor-associated antigens,[3] and cell adhesion molecules.[4] For the investigation of such processes, fucosylated glycopeptides with an exactly specified saccharide structure, peptide sequence and stereochemistry of the linkages are of interest.

Among the enzymatic glycosyltransfer reactions, the enzymatic fucosylation belongs to the more problematic conversions. This is due to the relatively high lability of the fucose-1-phosphate. Furthermore, fucosyltransferases are not easily accessible from natural sources.[5] This holds true, in particular, for the enzyme introducing the fucose α(1–6)-glycosidically linked to O-6 of the chitobiose asparagine unit to form structure **1**. *N*-Glycoproteins containing the fucosylated linkage region **1** have been found to be typical membrane components of viruses.[6] Structure **1** has also been reported to occur in tumor-associated glycoprotein antigens.[3a]



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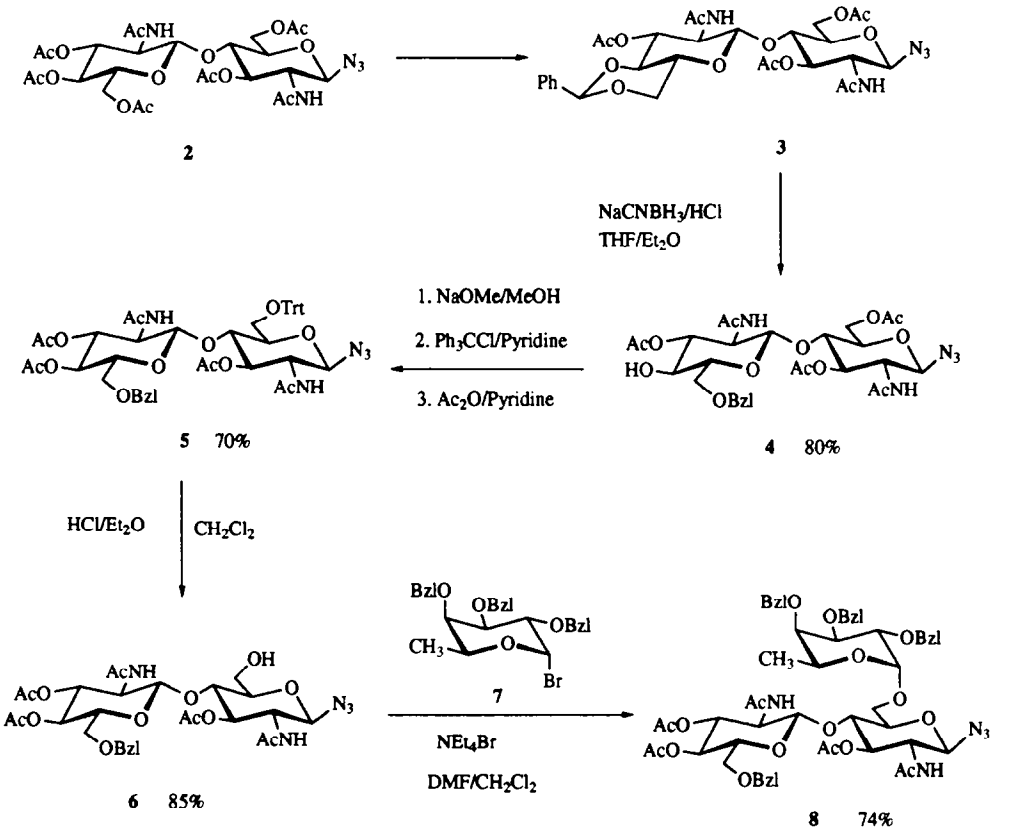
With respect to this situation, glycopeptides of the fucosylated chitobiose asparagine type **1** seemed to be interesting targets for the development and demonstration of efficient methods of chemical syntheses aimed at fucosylated glycopeptides.[7] In these syntheses, the formation of the α-fucosidic bonds requires the use of a fucosyl donor carrying a non-neighboring-group-active protection. As the α-fucosidic linkage is relatively sensitive, the protecting group strategy not only demands the selective orthogonal deprotections within the peptide portion but also has to be compatible with the presence of the fucoside bond.

Synthesis of Fucosyl Chitobiose Derivatives Containing *O*-Benzyl-Protected Fucose

Glycosyl azides are useful precursors of glycosylamines required for the formation of β-*N*-glycosidic conjugates of asparagine.[8–10] Furthermore, anomeric azides constitute advantageously protected anomeric amines which are stable to various reaction conditions applied in protecting group manipulations.[7,11]

Applying a phase transfer-catalyzed reaction,[12] chitobiosyl azide[13] **2** was synthesized from the corresponding glycosyl chloride. After removal of the O-acetyl groups by Zemplén transesterification, benzylidenation and re-acetylation, the 4',6'-*O*-benzylidene derivative[14] **3** was obtained. Reductive opening of the benzylidene acetal selectively gave the 6'-*O*-benzyl compound **4**. Removal of the *O*-acetyl groups, subsequent treatment of the product with triphenylmethyl chloride followed by acetylation using acetic anhydride/pyridine furnished compound **5**. The ^{1}H NMR spectrum of **5** revealed marked shielding and deshielding effects caused by the trityl group: shielding of 4-H resulted in a high-field shift of its signal by $\Delta\delta$= 0.4 ppm relative to the corresponding signal of the detritylated compound **6**. In contrast, H-1' ($\Delta\delta$ = –0.25 ppm), NH' (–2.9 ppm) and N-Ac' (–0.4 ppm) are deshielded. Detritylation of **5** to give **6** was carried out with hydrogen chloride in dichloromethane/diethyl ether. Compound **6** selectively deblocked at 6-OH immediately precipitated from the solution.



Scheme I.

α-Fucosylation of **6** using the *O*-benzyl protected fucosyl bromide[15] **7** according to Lemieux's *in situ*-anomerization method[16] resulted in the stereoselective formation of the trisaccharide azide **8**. Recently, Nifant'ev *et al.*[17] reported on reactions of *O*-benzoylated fucosyl bromide under Helferich conditions in which a preferred formation of α-fucosides was observed. However, usually a mixture of anomers was obtained, and the α preference was only found for acceptors of low reactivity. For fucosylations of primary hydroxy functions the *in situ*-anomerization remains the method of choice.

The selective reduction of the anomeric azide **8** by hydrogenation was successfully achieved with Raney-nickel extensively washed to neutral reaction.[18] Under these conditions, the *O*-benzyl ether groups of the trisaccharide amine **9** remained unaffected. The fucosyl chitobiosylamine was condensed with 1-*tert*-butyl *N*-allyloxycarbonyl aspartate **10** using ethyl 2-ethoxy-1,2-dihydro-quinoline-1-carboxylate (EEDQ)[19] to give the fully protected trisaccharide asparagine conjugate **11**. The corresponding α-anomer was formed as a side product. The Aloc-group was chosen for the syntheses of the target glycopeptides because it can be removed under practically neutral conditions via a palladium(0)-catalyzed allyl transfer to weakly basic nucleophiles irreversibly trapping the allyl moiety.[20] A combination with the C-terminal *tert*-butyl ester appeared promising as the use of the *tert*-butyloxycarbonyl (Boc) group had already been demonstrated successfully in syntheses of chitobiose glycopeptides.[21] However, treatment of the conjugate **11** with trifluoroacetic acid/dichloromethane resulted in a prevailing degradation of the compound.

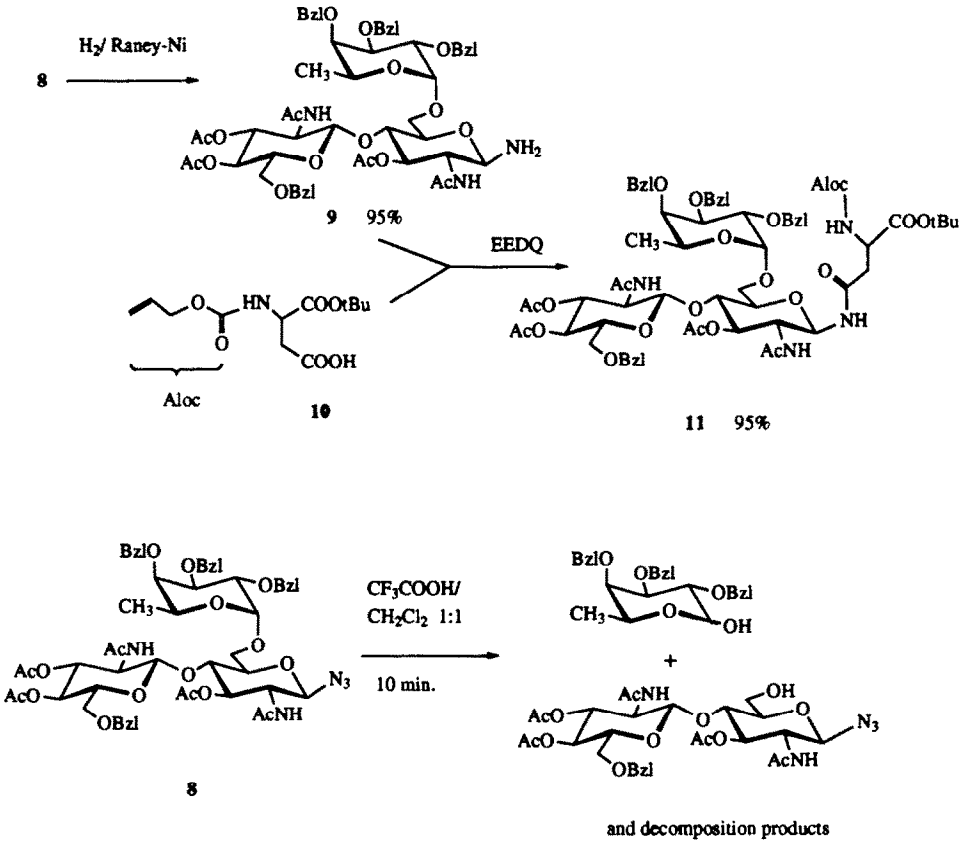
To further examine this decomposition, the trisaccharide azide **8** was treated with trifluoroacetic acid/dichloromethane. Under these conditions the fucosidic bond of **8** was cleaved easily to give **6** and the fucose derivative. Subsequent cleavage of benzyl ether groups and transacylation reactions produced a complex mixture of products. As the fucosidic bond of **8** was slowly attacked even by formic acid, the overall protecting group combination was considered unsuitable for a general strategy to synthesize fucose-containing glycopeptides. As a consequence, we have developed an alternative concept for these glycopeptide syntheses which involves fucosyl chitobiose components exclusively protected by acyl-type groups in the fucose part.

O-Acyl-Protected Fucosyl Chitobiose Derivatives

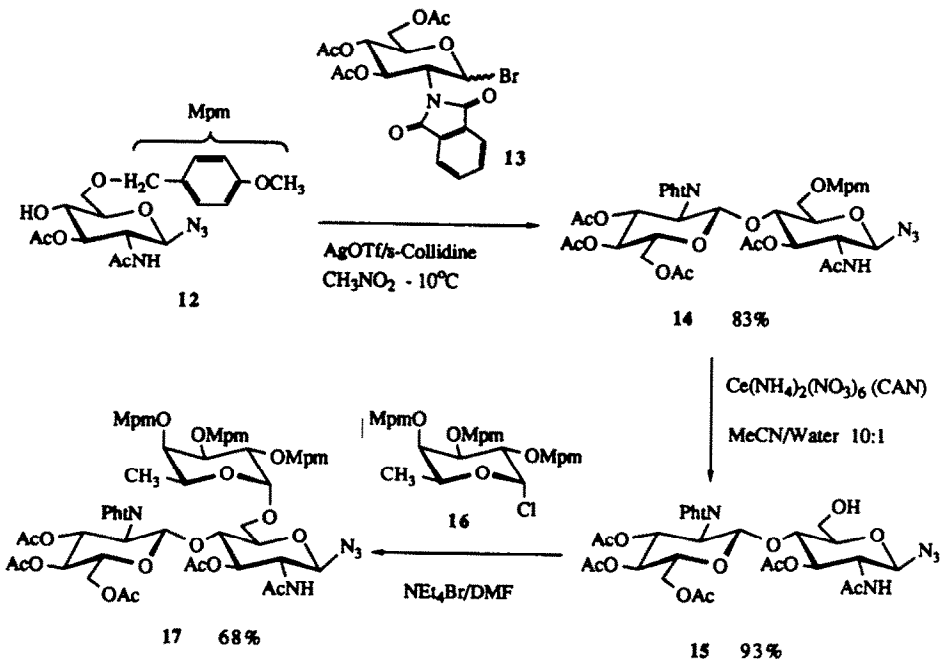
Glycosyl azides constitute an anomeric protection stable under conditions of acylation and deacylation procedures, acid-catalyzed acetalization, Lewis acid-catalyzed regioselective acetal opening and glycosylation.[10] Thus, the selectively deblocked glucosaminyl azide **12** was prepared and glycosylated using the *N*-phthaloyl glucosaminyl bromide[22] **13** to form the chitobiosyl azide **14** in high yield.[7,11,23] Removal of the 6-*O*-(4-methoxy)benzyl (Mpm) group by applying ceric ammonium nitrate (CAN)[24] furnished

the required glycosyl acceptor **15**. In contrast to hydrogenolytic cleavages of benzyl ethers, this oxidative deprotection proceeds without affecting the azide function.

As a fucosyl donor, which ensures the stereoselective formation of an α-fucosidic bond to **15** and allows selective removal of its *O*-protecting groups in the presence of the azide functionality, we have



Scheme II.



Scheme III.

synthesized the *O*-(4-methoxy)benzyl-protected fucosyl chloride[25] **16**. One of the key steps in the synthesis of **16** consists of the isomerization of the Mpm-protected allyl fucopyranoside to the corresponding propenyl fucoside. Although this isomerization catalyzed by tris(triphenylphosphine)rhodium(I) chloride[26] in ethanol/water can be performed without adding a base, the presence of DABCO accelerates the process. In any case, the Wilkinson catalyst should be of good quality. The propenyl fucoside was selectively hydrolyzed using pyridinium tosylate and the resulting product was reacted with tris(dimethylamino)phosphine/carbon tetrachloride[27] to give **16**.[25] Under these conditions, the acid-sensitive Mpm groups remained stable. It should be mentioned that an alternative method consisting of the treatment of a thio fucoside with bromine delivers the corresponding Mpm protected fucosyl bromide.[28,29] Reaction of fucosyl chloride **16** with acceptor **15** according to the *in situ*-anomerization method stereoselectively gave the desired trisaccharide **17**.

The exchange of the ether-type (Mpm) protection for the ester-type of **17** was carried out by initial oxidative cleavage of the Mpm ethers using CAN, again without affecting the azide group of **18**.

Crude **18** was directly subjected to dephthaloylation by treatment with hydrazine in ethanol. Subsequent acetylation furnished the acetyl-protected trisaccharide **19**. Hydrogenation catalyzed by Raney-nickel gave the trisaccharide amine **20**.

Synthesis of Fucosyl Chitobiose Asparagine Glycopeptides Using the Aloc/OtBu Strategy

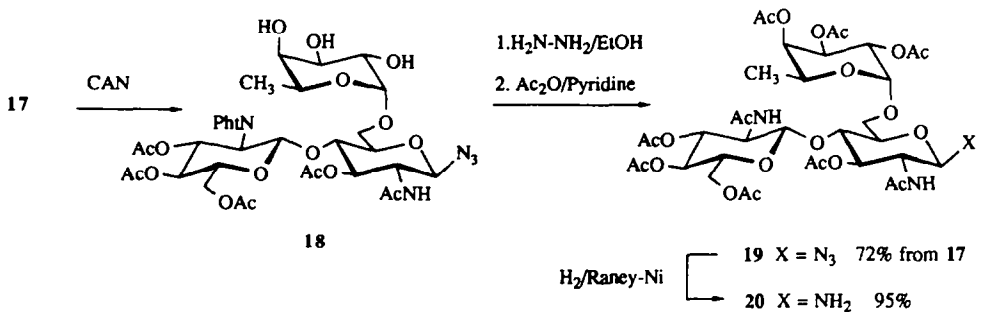
Condensation of the Aloc aspartic acid α-*tert*-butyl ester **10** with the glycosylamine **20** gave the *N*-glycosidic conjugate **21**. In this reaction, a small amount of the corresponding α-anomer of the *N*-glycoside was found which was separated by flash chromatography. The Aloc group was selectively removed from **21** by applying the palladium(0)-catalyzed allyl transfer reaction.[20] *N*,*N*'-Dimethyl barbituric acid[30] was chosen as the nucleophile which, in contrast to dimedone,[20] has practically no carbonyl activity, and can easily be separated from the liberated amino compound **22**. The carbonyl reactivity of dimedone or other C,H acidic carbonyl compounds in some cases may cause side reactions, e.g. the formation of enamines of the amino component just deprotected.

Condensation of **22** with the Aloc-dipeptide **23** by activation with water-soluble 1-ethyl-3-(3-dimethylaminopropyl carbodiimide (EDC) in the presence of 1-hydroxybenzotriazole[31] yielded the trisaccharide tripeptide **24**.

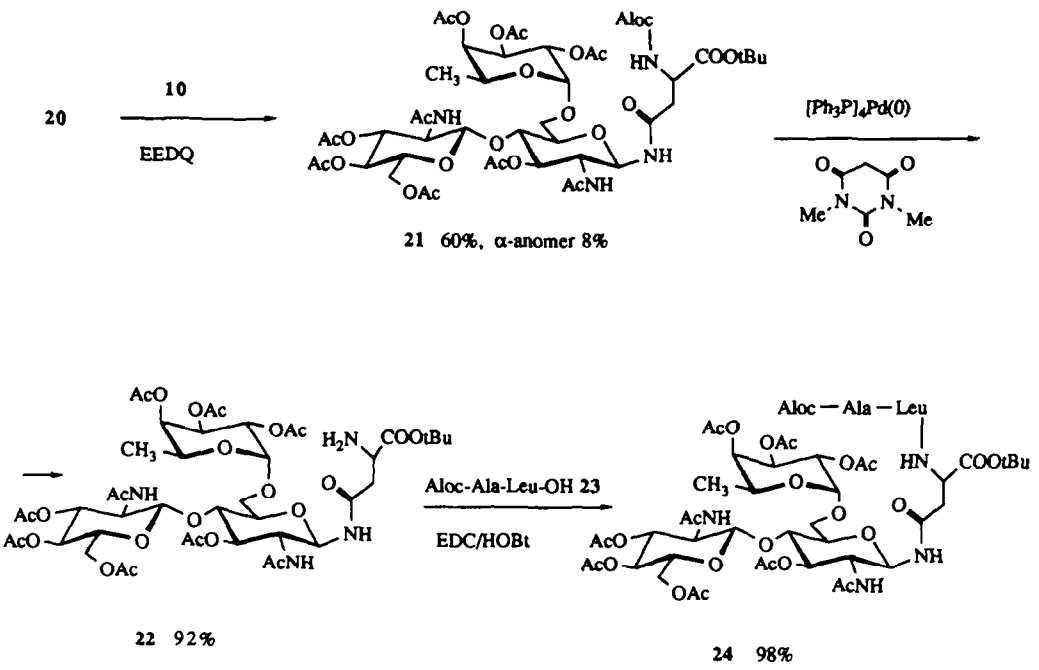
It is very important for the generalization of this concept of glycopeptide syntheses that the C-terminal *tert*-butyl ester of **24** can be cleaved with complete selectivity even by using neat trifluoroacetic acid to give the carboxy-deblocked product **25** without affecting the fucosidic bond. The marked difference in the stability of the fucosidic bond of the glycopeptide **24** carrying *O*-acetyl protection at the fucose moiety in comparison to that of the conjugate **11** or of the trisaccharide azide **8**, both containing *O*-benzyl protected fucose moieties, can be rationalized by an electrostatic effect. Protonation of the *O*-acetyl protected compound should mainly occur at the numerous carbonyl oxygens being centers of high electron density. In this way, a Coulomb repulsion results which protects the internal sensitive intersaccharidic and glycosidic bonds against proton attack.[7] Taking advantage of this indirect but very efficient protection of the glycosidic linkages,[32] the *tert*-butyl-type protection can be removed from the glycopeptides with high selectivity. In this manner, the deblocked compound **25** was isolated in quantitative yield.

Its condensation with the pre-formed tripeptide *tert*-butyl ester **26** synthesized by usual Z/-OtBu-strategy gave the trisaccharide hexapeptide **27** representing a fully protected partial structure of an envelope glycoprotein of a murine leukemia virus.[6]

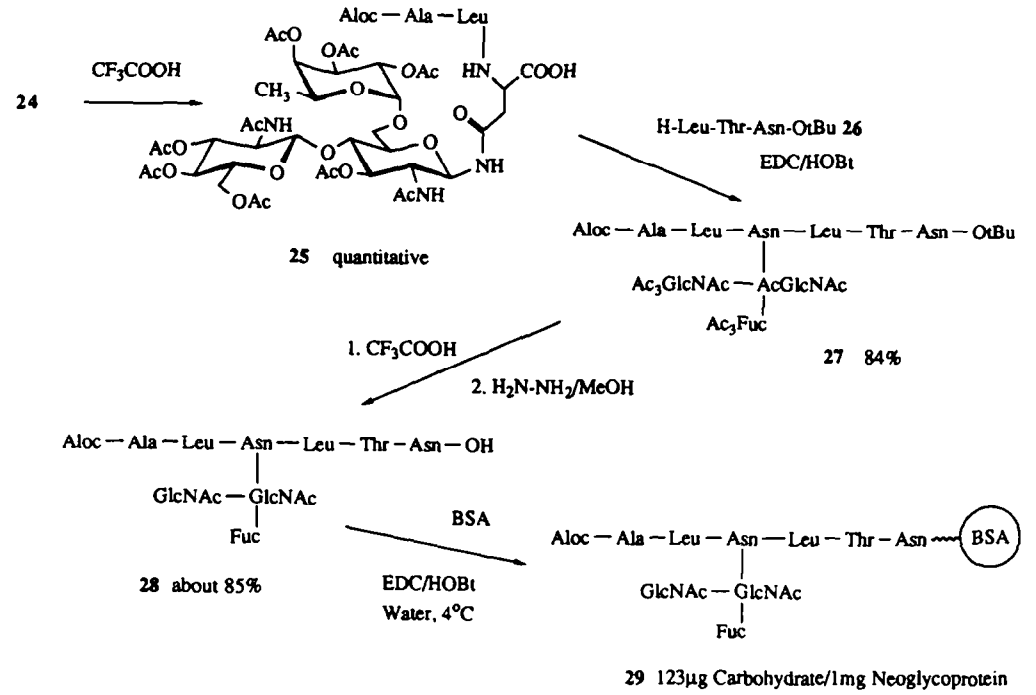
Treatment of **27** with trifluoroacetic acid and, subsequently, with hydrazine in methanol[33] resulted in the removal of the *tert*-butyl ester protection and methanolysis of the numerous ester groups of the saccharide portion to give the practically deblocked glycopeptide **28**. The small Aloc group is considered to have only little influence on immunological properties. Applying water-soluble carbodiimide/1-hydroxybenzotriazole in water solution[34] the glycopeptide **28** containing the fucosylated linkage region of *N*-glycoproteins was coupled to bovine serum albumin (BSA) as the carrier protein in water/dimethylformamide.



Scheme IV.



Scheme V.



Scheme VI.

The synthetic neoglycoprotein **29** was separated from low molecular weight components by ultrafiltration. According to the carbohydrate analysis (phenol/sulfuric acid method[35]) the neoglycoprotein **29** contained 12.3 % of carbohydrate. A mixture of *N*-acetylglucosamine and fucose (2:1) was used as a standard.

The methods developed in this synthesis offer a general access to fucose containing glycopeptide partial structures of biologically interesting glycoproteins. Analogous strategies were applied successfully in the chemical synthesis of Lewis^a antigen[28] and Lewis^x antigen[29] glycopeptides which also involve a protecting group exchange at the fucose portion.

Experimental Section

The general methods used have been described previously.[11,12,25,34]

Melting points were determined using a Büchi apparatus (according to Dr Tottoli) and are uncorrected.

Optical rotation values were measured with a Perkin Elmer polarimeter 241. 400 MHz ^1H and 100.6 MHz ^{13}C NMR spectra were recorded using a Bruker AM 400 (tetramethylsilane as the internal standard). Flash chromatography was carried out on silica gel Kieselgel 60 (0.04–0.063 mm) purchased from E. Merck, Darmstadt, Germany. TLC were recorded using silica gel Kieselgel 60 - F_{254} (E. Merck, Darmstadt, Germany). Indication was performed by UV light or using a mixture (1:1) of 2 N H_2SO_4 and a solution of 3-methoxy-phenol (0.2 %) in ethanol.

2-Acetamido-4-O-(2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl azide (4)

A suspension of sodium cyanoborohydride[36] (3 g, 47 mmol) and 2-acetamido-4-*O*-(2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide[14] **3** (2.1 g, 3.16 mmol), synthesized from *O*-acetylated chitobiosyl azide[12] **2** was stirred in freshly distilled tetrahydrofuran (200 mL). HCl/diethyl ether was added dropwise until the evolution of hydrogen ceased. After completion of the reaction (TLC monitoring: $CHCl_3$:MeOH, 5:1), the solvent was evaporated *in vacuo*. The remaining residue was dissolved in dichloromethane (300 mL) and washed with 1 M $KHCO_3$ solution. After drying with $MgSO_4$ the solvent was evaporated *in vacuo* and the crude **4** was purified by chromatography in dichloromethane: methanol (10:1). Recrystallization from methanol/-chloroform/petroleum ether yielded colorless crystals: 1.85 g (80 %); mp 197 °C; $[\alpha]_D^{22} = -34.6°$ (c = 0.5, methanol); R_f = 0.53 ($CHCl_3$:MeOH, 5:1). 400 MHz ^1H NMR (DMSO-d_6): δ = 8.0 (d, $J_{2',NH'}$ = 9Hz, 1H, NH'); 7.9 (d, $J_{2,NH}$ = 9 Hz, 1H, NH), 7.4–7.25 (m, 5H, Ar), 5.44 (d, $J_{4,OH}$ = 5.4 Hz, 1H, OH), 4.95 (dd, $J_{2,3} = J_{3,4}$ = 9 Hz, 1H, H-3), 4.85 (m, 1H, H-3'); 4.73 (d, $J_{1,2}$ = 9 Hz, 1H, H-1), 4.5 (m, 3H, CH_2O, H-1'), 4.39 (d, J_{gem} = 12 Hz, 1H, H-6a), 4.08 (dd, J_{vic} = 5.6 Hz, 1H, H-6b), 3.81–3.68 (m, 4H, H-2, H-4, H-5, H-6a'), 3.6 (m, 1H, H-6b'), 3.44 (m, 1H, H-2'), 3.4–3.28 (m, 2H, H-4', H-5'), 2.03, 1.94, 1.92 (3s, 9H, OAc), 1.77, 1.71 (2s, 6H, NAc); 100.6 MHz ^{13}C NMR (DMSO-d_6): δ = 169.9–169.0 C=O; 138.5 C-i Ar; 128.2; 127.1 Ar; 100.1 C-1'; 87.3 C-1; 72.3 CH_2O; 68.9 C-6'; 62.1 C-6; 53.8 C-2'; 52.7 C-2; 22.5; 22.45 NAc; 20.6; 20.5; 20.35 OAc. Anal. calcd for $C_{29}H_{39}N_5O_{13}$ (665.7): C 52.33, H 5.91, N 10.52; found: C 51.99, H 6.01, N 10.21.

2-Acetamido-4-O-(2-acetamido-3,4-di-O-acetyl-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-6-O-tripheny-methyl-2-deoxy-β-D-glucopyranosyl azide (5)

To a solution of **4** (3 g, 4.5 mmol) in methanol (100 mL) were added 20 drops of 1 M sodium methanolate in methanol. After 3 h, the solution was neutralized by addition of Amberlite IR-120 (H+), filtered and the solvent was evaporated *in vacuo* to give quantitatively 2-acetamido-4-*O*-(2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosyl azide.

This product (2 g, 3.7 mmol) was stirred with triphenylmethyl chloride (4 g) in pyridine (40 mL) at 80 °C until the educt was completely consumed (3 days, TLC: $CHCl_3$:MeOH, 5:1). After addition of 20 mL of acetic anhydride, the mixture was allowed to react at room temperature for 16 h. The solution was diluted with dichloromethane (500 mL), extracted three times with 1 M HCl (500 mL) and with 1 M $KHCO_3$ solution (250 mL), dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by chromatography on 100 g of silica gel in diethyl ether → ethyl acetate. Stirring with diethyl ether gave **5** as colorless crystals, yield: 2.35 g (70 %); mp 145–152 °C; $[\alpha]_D^{22}$ = –50.1° (c = 0.5, CH_2Cl_2); R_f = 0.33 (ethyl acetate). 400 MHz ^1H NMR (DMSO-d_6): δ = 8.0 (d, $J_{2,NH}$ = 9.3 Hz, 1H, NH), 7.4–7.25 (m, 20H, Ar), 5.31 (d, $J_{2',NH'}$ = 9.4 Hz, 1H, NH'), 4.93 (dd, $J_{2,3} = J_{3,4}$ = 9.8 Hz, 1H, H-3), 4.83–4.78 (m, 2H, H-3', H-4'), 4.73 (d, $J_{1,2}$ = 9 Hz, 1H, H-1), 4.5, 4.43 (2d, J_{gem} = 11.7 Hz, 2H, CH_2O) 4.34 (d, $J_{1',2'}$ = 8.4 Hz, 1H, H-1'), 4,04 (dd, $J_{3,4} = J_{4,5}$ = 9.6 Hz, 1H, H-4), 3.88 (ddd, 1H, H-2), 3.7 (m, 1H, H-5), 3.62 (dd, J_{vic} = 3 Hz, J_{gem} = 12 Hz, 1H, H-6a'), 3.5–3.4 (m, 3H, H-6a, H-6b', H-5', H-2'), 3.0 (dd, J_{vic} = 3 Hz, J_{gem} = 10.5 Hz, 1H, H-6b), 1.90, 1.89, (2s, 9H, OAc), 1.82 (s, 3H, NAc), 1.43 (s, 3H, NAc'); 100.6 MHz ^{13}C NMR (DMSO-d_6): δ = 169.6–168.5 C=O; 143.3 C-i Tr; 137.8 C-i Bzl; 128.2–127.1 Ar; 98.9 C-1'; 87.1 C-1; 85.7 C-q Tr; 72.3 CH_2O; 68.04 C-6'; 61.4 C-6; 53.4 C-2'; 52.8 C-2; 22.4; 22.3 NAc; 20.27; 20.2; 20.1 OAc. Anal. calcd for $C_{48}H_{53}N_5O_{13}$ H_2O (925.9): C 62.23, H 5.77, N 7.56; found: C 61.96, H 5.76, N 7.72.

2-Acetamido-4-O-(2-acetamido-3,4-di-O-acetyl-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-2-deoxy-β-D-glu-copyranosyl azide (6)

To a solution of **5** (2.3 g, 2.5 mmol) in dichloromethane (10 mL) and diethyl ether (30 mL) was added a saturated solution of HCl in diethyl ether (4 mL). After 5 min the solvent was evaporated *in vacuo*. Dichloromethane (20 mL) was distilled off from the residue, which was purified by chromatography on silica gel (100 g) in dichloromethane:methanol 20:1 to give **6**; yield: 1.46 g (85 %); mp 192 °C; $[\alpha]_D^{22}$ = –24.5 °C (c = 1, methanol); R_f = 0.2 (CH_2Cl_2:MeOH 20:1). 400 MHz ^1H NMR (DMSO-d_6): δ = 8.19 (d, $J_{2',NH'}$ = 10 Hz, 1H, NH'), 8.06 (d, $J_{2,NH}$ = 10 Hz, 1H, NH), 7.38–7.24 (m, 5H, Ar), 5.05 (dd, $J_{2',3'} = J_{3',4'}$ =10 Hz, 1H, H-3'), 4.93 (dd, $J_{2,3} = J_{3,4}$ = 10 Hz, 1H, H-3), 4.87 (dd, $J_{3',4'} = J_{4',5'}$ = 10 Hz, 1H, H-4'), 4.82 (t, J = 5.5 Hz, 1H, OH), 4.67 (d, $J_{1,2}$ = 9.5 Hz, 1H, H-1), 4.60 (d, $J_{1',2'}$ = 8.4 Hz, 1H, H-1'), 4.46, 4.36 (2d, J_{gem} = 11.6 Hz, 2H, CH_2O), 3.76–3.86 (m, 2H, H-2, H-5), 3.76–3.61 (m, 2H, H-6a, H-5'), 3.60–3.51 (m, 3H, H-2', H-4, H-6a'), 3.49–3.40 (m, 2H, H-6b', H-6b), 1.90, 1.87, 1.85 (3s, 9H, OAc), 1.78, 1.75 (2s, 6H, NAc); 100.6 MHz ^{13}C NMR (DMSO-d_6) δ =169.4–169.0 C=O; 137.8 C-i Ar; 128.1–127.3 Ar; 100.3 C-1'; 87.6 C-1; 72.3 CH_2O; 68.1 C-6'; 58.8 C-6; 53.5 C-2'; 52.8 C-2; 22.5 NAc; 20.3; 20.28; 20.21 OAc. Anal.

calcd for $C_{29}H_{39}N_5O_{13}$ (665.7): C 52.33, H 5.91, N 10.52; found: C 52.22, H 6.17, N 10.38.

2-Acetamido-4-O-(2-acetamido-3,4-di-O-acetyl-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-6-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranosyl azide (8)

Tetraethylammonium bromide (4 g, 19 mmol), compound **6** (1.25 g, 1.88 mol) and molecular sieves 4 Å (6 g) were stirred in 15 mL of dimethylformamide:dichloromethane (2:1) for 30 min. Using a syringe, 2,3,4-tri-*O*-benzyl-α-D-fucopyranosyl bromide[15] **7** (3 g, 6 mmol) dissolved in dichloromethane (5 mL) was added dropwise. After 4 days, the mixture was filtered through Celite, which subsequently was washed with dichloromethane (300 mL). The combined organic solutions were extracted with 1 M $KHCO_3$ solution (3 × 100 mL), dried with $MgSO_4$, concentrated *in vacuo* and the remainder was dried in high *vacuo*. Purification by chromatography (100 g silica gel) in petroleum ether:ethyl acetate 2:1 → dichloromethane:methanol 20:1 and by recrystallization from dichloromethane: diisopropyl ether gave **8**: 1.5 g (74 %); mp 175 °C; $[\alpha]_D^{22}$ = −38.65° (*c* = 0.5, chloroform); R_f = 0.4 ($CHCl_3$:MeOH, 10:1). 400 MHz ^{1}H NMR (DMSO-d_6): δ = 8.03 (d, $J_{2',NH'}$ = 9.5 Hz, 1H, NH'), 7.98 (d, $J_{2,NH}$ = 9.5 Hz, 1H, NH) 7.4–7.2 (m, 20H, Ar), 5.08 (dd, $J_{2',3'}$ = $J_{3',4'}$ = 9.5 Hz, 1H, H-3'), 5.03 (d, $J_{1'',2''}$ = 2.9 Hz, 1H, H-1''), 4.93 (dd, $J_{2,3}$ = $J_{3,4}$ = 9 Hz, 1H, H-3), 4.88–4.77 (m, 3H, CH_2O'', H-4'), 4.7–4.62 (m, 3H, CH_2O''), 4.68 (d, $J_{1',2'}$ = 8 Hz, 1H, H-1') 4.61 (d, $J_{1,2}$ = 10.3 Hz, 1H, H-1), 4.52 (d, J_{gem} = 12 Hz, 1H, CH_2O'') 4.38, 4.28 (2d, J_{gem} = 1.7 Hz, 2H, CH_2O'), 3.97 (m, 1H, H-5''), 3.9–3.7 (m, 7H, H-2, H-4, H-6a/b, H-2'', H-3'', H-4''), 3.67 (m, 1H, H-5), 3.58 (ddd, 1H, H-2), 3.4 (m, 2H, H-5', H-6a'), 3.24 (dd, J_{vic} = 5 Hz, J_{gem} = 11.8 Hz, 1H, H-6b'), 1.9, 1.88, 1.81 (3s, 9H, OAc), 1.79 (s, 3H, NAc), 1.75 (s, 3H, NAc'), 1.09 (d, $J_{5'',6''}$ = 6.2 Hz, 3H, CH_3 Fuc), 100.6 MHz ^{13}C NMR (DMSO-d_6), δ = 169.4–168.8 C=O; 138.8, 138.6, 137.7 C-i Ar; 127.97–127.0 Ar; 99.4 C-1'; 96.3 C-1''; 87.2 C-1; 74.2; 72.2; 71.6; 71.4 CH_2O; 67.9 C-6'; 64.2 C-6; 53.8 C-2'; 52.6 C-2; 22.5; 22.4 NAc; 20.19; 20.15 OAc; 16.1 CH_3 Fuc. Anal. calcd for $C_{56}H_{67}N_5O_{17}$ (1082.2): C 62.15, H 6.24, N 6.47; found: C 62.06, H 6.57, N 6.26.

2-Acetamido-4-O-(2-acetamido-3,4-di-O-acetyl-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-6-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranosyl-amine (9)

The trisaccharide azide **8** (1 g, 0.92 mmol) was dissolved in methanol (20 mL). After addition of Raney-nickel (200 mg, E. Merck, Darmstadt, Germany), which was washed 10 times with water, hydrogenation was performed for 3 h. After filtration and concentration *in vacuo* the glycosylamine **9** was obtained: yield: 927 mg (95 %); $[\alpha]_D^{22}$ = −24.8° (*c* =0.5, CH_2Cl^2); R_f = 0.2 ($CHCl_3$:MeOH, 10:1). 400 MHz ^{1}H NMR (DMSO-d_6): δ = 8.03 (d, $J_{2',NH'}$ = 9.5 Hz, 1H, NHAc'), 7.7 (d, $J_{2,NH}$ = 9.5 Hz, 1H, NHAc), 7.4–7.2 (m, 20H, Ar), 5.08 (dd, $J_{2',3'}$ = $J_{3',4'}$ = 9.5 Hz, 1H, H-3'), 5.03 (d, $J_{1'',2''}$ = 3 Hz, 1H, H-1'') 4.9–4.6 (m, 8H, CH_2O'', H-3, H-4', H-1'), 4.53 (d, J_{gem} = 12 Hz, 1H, CH_2O''), 4.39; 4.29 (2d, J_{gem} = 12 Hz, 2H, CH_2O'), 1.88, 1.85, 1.82 (3s, 9H, OAc), 1.75, 1.74 (2s, 6H, NAc), 1.07 (d, $J_{5'',6''}$ = 6Hz, 3H, CH_3 Fuc). Anal. calcd for $C_{56}H_{69}N_3O_{17}$ (1056.2): C 63.68, H 6.59, N 3.98; found: C 62.05, H 6.52, N 3.96.

N-*Allyloxycarbonyl-aspartic acid* α-tert-*butyl ester (10)*

To a solution of 1-*O*-*tert*-butyl aspartate[37] (3 g, 15.9 mmol) and $KHCO_3$ (3 g, 32 mmol) in water (50 mL) was added at 0 °C allyl chloroformate (1.7 mL, 16 mmol, E. Merck, Darmstadt, Germany). After stirring for 1 h, the mixture was extracted with diethyl ether(100 mL). The aqueous layer was acidified to pH 2 using 1 M HCl and extracted with diethyl ether (4 × 50 mL). These ether solutions were combined and dried with $MgSO_4$. The solvent was evaporated *in vacuo* to give **10** as an oil; 3.87 g (89 %); $[\alpha]_D^{22}$ = +21 (*c* = 1, CH_2Cl_2), R_f = 0.25 (petroleum ether:ethyl acetate, 2:1). 400 MHz ^{1}H NMR (DMSO-d_6): δ = 12.4 (s, 1H, COOH), 7.55 (d, *J* = 8.3 Hz, 1H, NH urethane), 5.9 (m, 1H, =CH-), 5.27 (dd, J_{trans} = 17.2 Hz, J_{gem} = 1.5 Hz, 1H, CH_2=), 5.16 (dd, J_{cis} = 10.4 Hz, J_{gem} = 1.5 Hz, 1H, CH_2=), 4.46 (m, 2H, CH_2O), 4.25 (m, 1H, -α-CH Asp), 2.65 (dd, J_{vic} = 5.7 Hz, J_{gem} = 16.3 Hz, 1H, β-CHa-Asp), 2.51 (dd, J_{vic} = 8 Hz, 1H, β-CHb Asp): 1.37 (s, 9H, tBu). Anal. calcd for $C_{12}H_{19}NO_6$ (273.3): C 52.74, H 7.01, N 5.13; found: C 52.87, H 7.18, N 5.00.

N²-(Allyloxycarbonyl)-N⁴-(2-acetamido-4-O-(2-acetamido-3,4-di-O-acetyl-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-6-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranosyl)-L-asparagine tert-butyl ester (11)

The trisaccharide amine **9** (0.5 g, 0.47 mmol), Aloc-Asp-OtBu **10** (0.2 g, 0.73 mmol) and EEDQ[19] (0.6 g, 2.4 mmol) were stirred in dimethylformamide (5 mL). After 3 days the solvent was distilled off in high *vacuo*. The remainder was purified by flash chromatography on silica gel (60 g) in petroleum ether:ethyl acetate 2:1→ dichloromethane:methanol 50:1 to give **11**; 466 mg (75 %); $[\alpha]_D^{22}$ = −28.4° (*c* = 0.75, CH_2Cl_2); R_f = 0.38 ($CHCl_3$:MeOH, 10:1); 400 MHz ^{1}H NMR (DMSO-d_6): δ = 8.45 (d, $J_{1,NH}$ = 9 Hz, 1H, γ-NH Asn), 8.03 (d, $J_{2',NH'}$ = 9.2 Hz, 1H, NHAc'), 7.78 (d, $J_{2,NH}$ = 9.3 Hz, 1H, NHAc), 7.4–7.2 (m, 21H, Ar, NH urethane), 5.85 (m, 1H, =CH-), 5.25 (dd, J_{trans} = 17 Hz, J_{gem} = 1.5 Hz, 1H, CH_2=), 5.14 (dd, J_{cis} = 10.6 Hz, 1H, CH_2=), 5.07 (dd, $J_{2',3'}$ = $J_{3',4'}$ = 10 Hz, 1H, H-3'), 5.02 (d, $J_{1'',2''}$ = 3 Hz, 1H, H-1''), 4.98 (dd, $J_{1,NH}$ = $J_{1,2}$ = 9 Hz, 1H, H-1), 4.88 (dd, $J_{2,3}$ = $J_{3,4}$ = 10 Hz, 1H, H-3), 4.85–4.6 (m, 7H, CH_2O'', H-4', H-1'), 4.52 (d, J_{gem} = 11 Hz, 1H, CH_2O''), 4.43 (m, 2H, CH_2O allyl), 4.41, 4.3 (2d, J_{gem} = 12 Hz, 2H, CH_2O'), 4.26 (m, 1H, α-CH Asn), 4.0 (m, 1H, H-5''), 3.86–3.62 (m, 7H, H-2, H-4, H-6a/b, H-2'', H-3'', H-4''), 3.52–3.28 (m, 5H, H-2', H-5', H-5, H-6a'/b'), 2.5 (m,

2H, β-CH_2 Asn), 1.9, 1.86, 1.85 (3s, 9H, OAc), 1.75, 1.72 (2s, 6H, NAc), 1.35 (s, 9H, tBu), 1.08 (d, $J_{5'',6''}$ = 6.8 Hz, 3H, CH_3 Fuc); 100.6 MHz ^{13}C NMR (DMSO-d_6): δ = 170.3–168.9 C=O; 155.4 C=O urethane; 138.8–137.7 C-i Ar; 133.3 =CH-; 128.0–127.0 Ar; 116.8 CH_2=; 99.4 C-1'; 96.8 C-1''; 80.5 C-q tBu; 78.3 C-1; 74.2; 72.2; 71.6; 71.0 CH_2O; 67.9 C-6'; 65.4 C-6; 64.3 CH_2O allyl; 53.8 C-2'; 52.0 C-2; 50.75 α-C Asn; 36.8 β-C Asn; 27.4 tBu; 22.5 NAc; 20.35; 20.23; 20.21 OAc; 16.3 CH_3 Fuc. Anal. calcd for $C_{68}H_{86}N_4O_{22}$ (1311.5): C 62.27, H 6.61, N 4.27; found: C 62.04, H 6.69, N 4.17.

The corresponding α anomer was isolated by this chromatography as a side product:

*N^2-(Allyloxycarbonyl)-N^4-(2-acetamido-4-O-(2-acetamido-3,4-di-O-acetyl-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-6-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-2-deoxy-α-D-glucopyranosyl)-L-asparagine tert-butyl ester (**11a**)*

Yield: 62 mg (10 %); R_f = 0.42 ($CHCl_3$:MeOH, 10:1). 400 MHz ^1H NMR (DMSO-d_6): δ = 8.63 (d, $J_{1,NH}$ = 9.5 Hz, 1H, γ-NH Asn), 8.03 (d, $J_{2',NH'}$ = 9.3 Hz, 1H, NHAc'), 7.51 (d, $J_{2,NH}$ = 9.2 Hz, 1H, NHAc), 7.43 (d, J = 8.2 Hz, 1H, NH urethane), 7.4–7.2 (m, 20H, Ar), 5.9 (m, 1H, =CH-), 5.36 (dd, $J_{1,2}$ = 5.1 Hz, 1H, H-1α), 5.3–5.14 (m, 3H, CH_2= H-3), 5.07 (d, $J_{1'',2''}$ = 3.2 Hz, 1H, H-1''), 5.04 (m, 1H, H-3'), 4.88 (d, J_{gem} = 12 Hz, 1H, CH_2O''), 4.82 (dd, $J_{3',4'} = J_{4',5'}$ = 9.6 Hz, 1H, H-4'), 4.77 (d, J_{gem} = 11 Hz, 1H, CH_2O''), 4.72–4.58 (m, 4H, CH_2O'', H-1'), 4.5–4.43 (m, 3H, CH_2O allyl, CH_2O''), 4.36, 4.26 (2d, J_{gem} = 12 Hz, 2H, CH_2O'), 4.25 (m, 1H, α-CH Asn), 4.14 (m, 1H, H-2), 2.6 (m, 2H, β-CH_2 Asn), 1.90, 1.88, 1.80 (3s, 9H, OAc), 1.78, 1.76 (2s, 6H, NAc), 1.37 (s, 9H, tBu), 1.06 (d, $J_{5'',6''}$ = 6.4 Hz, 3H, CH_3 Fuc).

*2-Acetamido-4-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-3-O-acetyl-6-O-(2,3,4-tri-O-(p-methoxybenzyl)-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranosyl azide (**17**)*

To a stirred mixture of tetraethylammonium bromide (3 g, 14.2 mmol), powdered molecular sieves 4 Å (3 g) and dry dimethylformamide (11 mL) was added 2-acetamido-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-3-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide[11] **15** (1.1 g, 1.56 mmol). After 30 min, 2,3,4-tri-*O*-(*p*-methoxybenzyl)-α-L-fucopyranosyl chloride[25] **16** (2.5 g, 3.66 mmol) dissolved in dichloromethane (11 mL) was added dropwise. After completion of the reaction (TLC monitoring CH_2Cl_2:MeOH, 20:1), dichloromethane (200 mL) was added and the solution was filtered through Celite. The filtrate was extracted with 1 M $KHCO_3$ (100 mL), dried with $MgSO_4$ and concentrated *in vacuo*. Traces of dimethylformamide were removed in high *vacuo* and the residue was subjected to flash chromatography on silica gel (100 g) in petroleum ether:ethyl acetate 1:1 → ethyl acetate. About 1.5 g of 2,3,4-tri-*O*-Mpm fucopyranose[25] could be recovered. Crude **17** was purified by an additional flash chromatography in CH_2Cl_2:MeOH 80:1. Yield: 1.29 g (68 %), amorphous solid; $[\alpha]_D^{22}$ = −80.6° (*c* = 1, CH_2Cl_2); R_f = 0.4 (CH_2Cl_2:MeOH, 20:1). 400 MHz ^1H NMR (DMSO-d_6): δ = 8.0–7.84 (m, 5H, Ar Pht, NH), 7.4–7.3 (m, 6H, Ar Mpm), 6.96–6.85 (m, 6H, Ar Mpm), 5.54 (dd, $J_{2',3'}$ = 10.6 Hz, $J_{3',4'}$ = 9.3 Hz, 1H, H-3'), 5.47 (d, $J_{1',2'}$ = 9.3 Hz, 1H, H-1'), 4.92 (m, 2H, H-3, H-4'), 4.83 (d, $J_{1'',2''}$ = 2.6 Hz, 1H, H-1'') 4.83 (d, J_{gem} = 11.7 Hz, 1H, CH_2O), 4.75–4.42 (m, 6H, CH_2O, H-1), 4.17 (dd, J_{vic} = 3.4 Hz, J_{gem} = 12.3 Hz, 1H, H-6a'), 3.96 (dd, $J_{3,4} = J_{4,5}$ = 9.4 Hz, 1H, H-4), 3.92–3.53 (m, 19H, CH_3O, H-2, H-2', H-2'', H-3'', H-4'', H-5, H-5', H-5'', H-6a, H-6b'), 3.32 (dd, J_{vic} = 3.2 Hz, J_{gem} = 11.6 Hz, 1H, H-6b), 2.00, 1.96, 1.98 (3s, 9H, OAc), 1.78, 1.75 (2s, 6H, NAc, OAc), 1.01 (d, $J_{5'',6''}$ = 6.4 Hz, 3H, CH_3 Fuc). 100.6 MHz ^{13}C NMR (DMSO-d_6): δ = 169.7–167.0 C=O; 158.6–158.5 C-p Mpm; 135.1 C-4/5 Pht; 130.9–130.4 C-i Mpm, C-1/2 Pht; 129.3–128.7 C-o Mpm; 123.6 C-3/6 Pht; 113.6-113.4 C-m Mpm; 96.0; 95.8 C-1',C-1''; 87.0 C-1; 73.8; 71.4; 71.3 CH_2O; 63.5 C-6; 60.9 C-6'; 54.9–54.5 CH_3O, C-2'; 52.6 C-2; 22.5 NAc; 20.2; 20; 19.9; OAc; 16.2 CH_3 Fuc. Anal. calcd for $C_{60}H_{69}N_5O_{22}$ (1212.3): C 59.45, H 5.74, N 5.77; found: C 59.05, H 5.69, N 5.72.

*2-Acetamido-4-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-3-O-acetyl-6-O-(α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranosyl azide (**18**)*

A solution of **17** (1.1 g, 0.907 mmol) in 30 mL of acetonitrile:water 9:1 was stirred with ceric ammonium nitrate (3 g, 5.47 mmol). After completion of the reaction (TLC monitoring $CHCl_3$:MeOH, 10:1), acetonitrile (30 mL) was added, concentrated *in vacuo* and the remainder was subjected to chromatography on silica gel (50 g) in CH_2Cl_2:MeOH 30:1 → 6:1. Yield: 3 g; contaminated with inorganic material, R_f = 0.15 ($CHCl_3$:MeOH, 10:1). The crude product was converted to **19**.

*2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-6-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranosyl azide (**19**)*

The crude **18** (3 g) in ethanol (40 mL) and hydrazine hydrate (10 mL) was stirred at 80 °C for 1 h. Subsequently, acetone (30 mL) was added and the mixture was concentrated *in vacuo*. This procedure was repeated twice with 30 mL of acetone. The remainder was dried in high *vacuo*. Subsequently, pyridine:acetic anhydride 2:1 (50 mL) was added and the mixture was stirred for 18 h. The volatile components were evaporated by codistillation with toluene (30 mL) *in vacuo*, which was repeated twice. Purification was achieved by flash chromatography on silica gel (70 g) eluting first with acetone and, subsequently, with CH_2Cl_2:MeOH 30:1. Yield: 581 mg (72 % based on **17**); mp 137 °C; $[\alpha]_D^{22}$ = −72.5° (*c* = 0.25, CH_2Cl_2); R_f

= 0.4 ($CHCl_3$:MeOH, 10:1). 400 MHz ^1H NMR (DMSO-d_6): δ = 8.04 (d, $J_{2',NH'}$ = 9.2 Hz, 1H, NHAc'), 7.96 (d, $J_{2,NH}$ = 9.4 Hz, 1H, NHAc), 5.28 (dd, $J_{2'',3''}$ = 10.5 Hz, $J_{3'',4''}$ = 3.5 Hz, 1H, H-3''), 5.2 (dd, $J_{4'',5''}$ = 0.8 Hz, 1H, H-4''), 5.09 (dd, $J_{2',3'}$ = 10.2 Hz, $J_{3',4'}$ = 9.7 Hz, 1H, H-3'), 5.03–4.93 (m, 3H, H-1'', H-2'', H-3), 4.83 (dd, $J_{3',4'} = J_{4',5'}$ = 9.7 Hz, 1H, H-4'), 4.67 (d, $J_{1',2'}$ = 8.5Hz, 1H, H-1'), 4.64 (d, $J_{1,2}$ = 9.4 Hz, 1H, H-1), 4.31 (dq, $J_{5'',6''}$ = 6.5 Hz, 1H, H-5''), 4.27 (dd, J_{vic} = 3.7 Hz, J_{gem} = 12.5 Hz, 1H, H-6a'), 3.96 (dd, J_{vic} = 1.8 Hz, 1H, H-6b'), 3.83–3.66 (m, 6H, H-2, H-4, H-5, H-5', H-6a/b), 3.4 (ddd, 1H, H-2'), 2.12, 2.09, 1.99, 1.95, 1.94, 1.92, 1.90 (7s, 21H, OAc), 1.78, 1.76 (2s, 6H, NAc), 1.04 (d, $J_{5'',6''}$ = 6.5 Hz, 3H, CH_3 Fuc). 100.6 MHz ^{13}C NMR (DMSO-d_6): δ [= 170.0–169.0 C=O; 99.6 C-1'; 95.6 C-1''; 87.2 C-1; 65.5 C-6; 61.5 C-6'; 53.6 C-2'; 52.5 C-2; 22.5 NAc; 20.5–20.2 OAc; 15.4 CH_3 Fuc. Anal. calcd for $C_{36}H_{51}N_5O_{21}$ (889.8): C 48.59, H 5.78, N 7.87; found: C 48.50, H 5.72, N 7.85.

2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-6-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranosylamine (20)

The trisaccharide azide **19** (400 mg, 0.45 mmol) dissolved in 30 mL of dioxane:ethanol 5:1 was hydrogenated over Raney-nickel (200 mg, E. Merck, Darmstadt, Germany, 10 times washed with H_2O). After 3 h, the catalyst was filtered off and the solvents was evaporated *in vacuo*. Compound **20** was directly transformed to **21**. Yield: 369 mg (95 %), amorphous; $[\alpha]_D^{22}$ = –50.6° (*c* = 0.25, CH_2Cl_2); R_f = 0.15 ($CHCl_3$:MeOH, 10:1). 400 MHz ^1H NMR (DMSO-d^6): δ = 8.04 (d, $J_{2',NH'}$ = 9.2 Hz, 1H, NHAc'), 7.73 (d, $J_{2,NH}$ = 9.4 Hz, 1H, NHAc), 5.28 (dd, $J_{2'',3''}$ = 10.5 Hz, $J_{3'',4''}$ = 3.5 Hz, 1H, H-3''), 5.2 (dd, $J_{4'',5''}$ = 0.8 Hz, 1H, H-4''), 5.08 (dd, $J_{2',3'}$ = 10.1 Hz, $J_{3',4'}$ = 9.8 Hz, 1H, H-3') 5.01 (d, $J_{1'',2''}$ = 3.4 Hz, 1H, H-1''), 4.95 (m, 1H, H-2''), 4.85 (dd, $J_{2,3} = J_{3,4}$ = 9.6 Hz, 1H, H-3), 4.81 (dd, $J_{3',4'} = J_{4',5'}$ = 9.8 Hz, 1H, H-4'), 4.65 (d, $J_{1',2'}$ = 8.4 Hz, 1H, H-1'), 4.4 (dq, $J_{5'',6''}$ = 6.4 Hz, 1H, H-5''), 4.26 (dd, J_{vic} = 3.5 Hz, J_{gem} = 12 Hz, 1H, H-6a'), 3.99 (d, $J_{1,2}$ = 9.1 Hz, 1H, H-1), 3.93 (dd, J_{vic} = 1.5 Hz, 1H, H-6b'), 2.12, 2.09, 1.99, 1.94, 1.92, 1.90 (6s, 21H, OAc), 1.75, 1.73 (2s, 6H, NAc), 1.02 (d, $J_{5'',6''}$ = 6.4 Hz, 3H, CH_3 Fuc). 100.6 MHz ^{13}C NMR (DMSO-d_6): δ = 170.1–169.0 C=O; 99.7 C-1'; 95.9 C-1''; 84.7 C-1; 65.7 C-6; 61.5 C-6'; 54.5 C-2'; 53.7 C-2; 22.7; 22.5 NAc; 20.5–20.2 OAc; 15.3 CH_3 Fuc.

N^2-(Allyloxycarbonyl)-N^4-(2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-6-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranosyl)-L-asparagine tert-*butyl ester (21)*

Trisaccharide amine **20** (369 mg, 0.47 mmol), Aloc-Asp-OtBu **10** (200 mg, 0.73 mmol) and 600 mg (2.4 mmol) of EEDQ[19] were stirred in 2 mL of dimethylformamide. After 5 days the solvent was evaporated in high *vacuo* and the residue was purified by flash chromatography on silica gel (60 g) in petroleum ether:ethyl acetate 2:1 → CH_2Cl_2:MeOH 35:1. Yield: 321 mg (60 %) **21**, amorphous; $[\alpha]_D^{22}$ = –51.8° (c = 0.5, CH_2Cl_2); R_f = 0.45 ($CHCl_3$:MeOH, 10:1). 400 MHz ^1H NMR (DMSO-d_6): δ = 8.51 (d, $J_{1,NH}$ = 9.3 Hz, 1H, γ-NH Asn), 8.04 (d, $J_{2',NH'}$ = 9.2 Hz, 1H, NHAc'), 7.86 (d, $J_{2,NH}$ = 9.4 Hz, 1H, NHAc), 7.27 (d, *J* = 8.3 Hz, 1H, NH urethane), 5.87 (m, 1H, =CH-), 5.3–5.2 (m, 3H, H-3'', H-4'', CH_2= *trans*), 5.15 (dd, J_{cis} = 10.5 Hz, J_{gem} = 1.4 Hz, 1H, CH_2=), 5.09 (dd, $J_{2',3'}$ = 10.2 Hz, $J_{3',4'}$ = 9.7 Hz, 1H, H-3'), 5.02–4.97 (m, 2H, H-1, H-2''), 4.93 (d, $J_{1'',2''}$ = 3.5 Hz, 1H, H-1''), 4.89 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, 1H, H-3), 4.81 (dd, $J_{3',4'} = J_{4',5'}$ = 9.7 Hz, 1H, H-4'), 4.68 (d, $J_{1',2'}$ = 8.5 Hz, 1H, H-1'), 4.5–4.4 (m, 3H, CH_2O, H-5''), 4.3–4.2 (m, 2H, α-CH Asn, H-6a'), 3.93 (dd, J_{vic} = 1.5 Hz, J_{gem} = 12.2 Hz, 1H, H-6b'), 3.83 (ddd, 1H, H-2), 3.75–3.63 (m, 4H, H-4, H-5, H-5', H-6a), 3.52 (ddd, 1H, H-2'), 3.4 (m, 1H, H-6b), 2.6–2.35 (m, 2H, β-CH_2 Asn), 2.12, 2.10, 2.00, 1.94, 1.93, 1.92, 1.90 (7s, 21H, OAc), 1.75, 1.72 (2s, 6H, NAc), 1.35 (s, 9H, tBu), 0.98 (d, $J_{5'',6''}$ = 6.4 Hz, 3H, CH_3 Fuc). 125.75 MHz ^{13}C NMR (DMSO-d_6): δ = 170.4–169.1 C=O; 155.5 C=O urethane; 133.5 =CH-; 116.9 CH_2=; 99.4 C-1'; 95.3 C-1''; 80.6 C-q tBu; 77.6 C-1; 65.2 C-6; 64.4 CH_2O; 61.6 C-6'; 53.6 C-2'; 52.1 C-2; 50.7 α-C Asn; 36.8 β-C Asn; 27.5 tBu; 22.64; 22.59 NAc; 20.6-20.3 OAc; 15.4 CH_3 Fuc. Anal. calcd for $C_{48}H_{70}N_4O_{26}{\cdot}H_2O$ (1137.1): C 50.70, H 6.38, N 4.92; found: C 50.70, H 6.60, N 5.51.

As a side product the corresponding α anomer was isolated:

N^2-(Allyloxycarbonyl)-N^4-(2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-6-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-2-deoxy-α-D-glucopyranosyl)-L-asparagine tert-*butyl ester (21a)*

Yield: 46.5 mg (8 %), amorphous, $[\alpha]_D^{22}$ = –44.5° (c = 1.5, CH_2Cl_2); R_f = 0.47 ($CHCl_3$:MeOH, 10:1). 400 MHz ^1H NMR (DMSO-d_6): δ= 8.62 (d, $J_{1,NH}$ = 9.6 Hz, 1H, γ-NH Asn), 8.04 (d, $J_{2',NH'}$ = 9.6 Hz, 1H, NHAc'), 7.40 (d, *J* = 8.3 Hz, 1H, NH urethane), 7.36 (d, $J_{2,NH}$ = 9.4 Hz, 1H, NHAc), 5.88 (m, 1H, =CH-), 5.37 (dd, $J_{1,2}$ = 5.2 Hz, 1H, H-1), 5.3–5.15 (m, 5H, H-3, H-3'', H-4'', CH_2=), 5.09–4.99 (m, 3H, H-1'', H-2'', H-3'), 4.85 (dd, $J_{3',4'}$= $J_{4',5'}$ = 9.7 Hz, 1H, H-4'), 4.64 (d, $J_{1',2'}$ = 8.4 Hz, 1H, H-1'), 4.47 (m, 2H, CH_2O), 4.32–4.24 (m, 3H, α-CH Asn, H-5'', H-6a'), 4.14 (ddd, $J_{2,3}$ = 10 Hz, 1H, H-2), 3.97 (m, 1H, H-6b'); 3.8–3.7 (m, 4H, H-4, H-5, H-5', H-6a), 3.68–3.61 (m, 2H, H-2', H-6b), 2.96 (dd, J_{vic} = 5.1 Hz, J_{gem} = 15 Hz, 1H, β-CHa-Asn), 2.52 (dd, J_{vic} = 8.8 Hz, 1H, β-CHb Asn), 2.09, 2.05, 2.02, 1.99, 1.96, 1.95, 1.90 (7s, 21H, OAc), 1.77 (s, 6H, NAc), 1.38 (s, 9H, tBu), 1.04 (d, $J_{5'',6''}$ = 6.4 Hz, 3H, CH_3 Fuc). 125.75 MHz ^{13}C NMR (DMSO-d_6): δ = 170.3–169.1 C=O; 155.4 C=O urethane; 133.4 =CH-; 116.8 CH_2=; 99.8 C-1'; 95.4 C-1''; 80.7 C-q tBu; 65.5 C-6; 64.4 CH_2O; 61.5 C-6'; 53.4 C-2';

51.3 C-2; 50.0 α-C Asn; 37.2 β-C Asn; 27.5 tBu; 22.6; 22.4 NAc; 20.6–20.2 OAc; 15.4 CH_3 Fuc. Anal. calcd for $C_{48}H_{70}N_4O_{26}\cdot H_2O$ (1137.1): C 50.70, H 6.38, N 4.92; found: C 50.84, H 6.74, N 5.27.

N^4-(2-Acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-6-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranosyl)-L-asparagine tert-*butyl ester (22)*

Trisaccharide asparagine conjugate **21** (300 mg, 0.305 mmol) and *N,N'*-dimethylbarbituric acid[30] (1 g, 7.1 mmol) were dissolved under argon atmosphere in oxygen-free tetrahydrofuran (15 mL). After addition of tetrakis(triphenylphosphine)palladium(0) (50 mg, 0.043 mmol), the solution was stirred for 2 h under exclusion of light. The solvent was evaporated *in vacuo*, the residue dissolved in dichloromethane (200 mL) and extracted twice with 1 M $KHCO_3$ (100 mL). After drying with $MgSO_4$ and concentration *in vacuo*, the remaining residue was purified by chromatography on silica gel (30 g) in CH_2Cl_2:MeOH 20:1 → 15:1 → 10:1. Yield: 250 mg (92 %), amorphous; $[\alpha]_D^{22} = -61.9°$ (c = 0.25, CH_2Cl_2); R_f = 0.25 ($CHCl_3$:MeOH, 10:1). 400 MHz ^{1}H NMR (DMSO-d_6): δ = 8.55 (d, $J_{1,NH}$ = 9.2 Hz, 1H, γ-NH Asn), 8.04 (d, $J_{2',NH'}$ = 9.1 Hz, 1H, NHAc') 7.88 (d, $J_{2,NH}$ = 9.4 Hz, 1H, NHAc), 7.27 (d, J = 8.3 Hz, 1H, NH urethane), 5.25–5.18 (m, 2H, H-3", H-4"), 5.09 (dd, $J_{2',3'}$ = 10 Hz, $J_{3',4'}$ = 9.7 Hz, 1H, H-3'), 5–4.92 (m, 3H, H-1, H-1",H-2"), 4.89 (dd, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 9.5 Hz, 1H, H-3), 4.81 (dd, $J_{3',4'} = J_{4',5'}$ = 9.7 Hz,1H, H-4'), 4.66 (d, $J_{1',2'}$ = 8.6 Hz, 1H, H-1'), 4.46 (m, 1H, H-5"), 4.26 (dd, J_{vic} = 4.7 Hz, J_{gem} = 12.5 Hz, 1H, H-6a), 3.93 (dd, J_{vic} = 1.7 Hz, J_{gem} = 12.2 Hz, 1H, H-6b'), 3.82 (ddd, $J_{2,3}$ = 10.1 Hz, 1H, H-2), 3.75–3.66 (m, 3H, H-5, H-5', H-6a), 3.65 (dd, $J_{3,4} = J_{4,5}$ = 9.5 Hz, 1H, H-4), 3.52 (ddd, 1H, H-2'), 3.45–3.37 (m, 2H, H-6b, α-CH Asn), 2.37 (dd, J_{vic} = 4.6 Hz, J_{gem} = 15.2 Hz, 1H, β-CHa-Asn), 2.25 (dd, J_{vic} = 7.8 Hz, 1H, β-CHb Asn), 2.12, 2.09, 2.00, 1.94, 1.93, 1.92, 1.0 (7s, 21H, OAc), 1.75, 1.72 (2s, 6H, NAc), 1.37 (s, 9H, tBu), 0.99 (d, $J_{5",6"}$ = 6.4 Hz, 3H, CH_3 Fuc). Anal. calcd for $C_{44}H_{66}N_4O_{24}\cdot H_2O$ (1053.1): C 50.18, H 6.51, N 5.32; found: C 50.22, H 6.52, N 4.94.

N-*Allyloxycarbonyl-alanyl-leucine* tert-*butyl ester*

This was synthesized according to lit.[20]: Yield: 95 %, oil; $[\alpha]_D^{22} = +25.2°$ (c = 1, $CHCl_3$); Anal. calcd for $C_{17}H_{30}N_2O_5$ (342.4): C 59.62, H 8.83, N 8.18; found: C 59.72, H 8.71, N 8.03.

N-*Allyloxycarbonyl-alanyl-leucine (23)*

This was synthesized from its *tert*-butyl ester by treatment of 1 mmol with trifluoroacetic acid (5 mL) for 30 min, removal of the trifluoroacetic acid *in vacuo* and distillation of dioxane (4 times 40 mL) from the residue. After drying in high *vacuo*, **23** was isolated (quantitative); mp 106°C; $[\alpha]^{22}_D = -40.0°$ (c = 1, CH_2Cl_2). Anal. calcd for $C_{13}H_{22}N_2O_5$ (286.3): C 54.53, H 7.74, N 9.78; found: C 54.82, H 7.94, N 9.99.

N^2-(Allyloxycarbonyl-L-alanyl-L-leucyl)-N^4-(2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-acetyl-6-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranosyl)-L-asparagine tert-*butyl ester (24)*

Glycosyl asparagine ester **22** (104 mg, 98.8 μmol), Aloc-dipeptide **23** (90 mg, 0.31 mmol), HOBt (60 mg, 0.44 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 150 mg, 0.78 mmol) were dissolved and stirred at 0 °C in dimethylformamide (2.5 mL). After 10 min, diisopropylethylamine (45 μL, 258 μmol) was added and the mixture was allowed to warm up to room temperature. After 18 h, the solvent was evaporated in high *vacuo* and the residue was purified by chromatography on silica gel (20 g) in CH_2Cl_2:MeOH 30:1 → 20:1. Yield: 126 mg (98 %), amorphous; $[\alpha]_D^{22} = -43.3°$ (c = 0.75, methanol); R_f = 0.45 ($CHCl_3$:MeOH, 10:1). 400 MHz ^{1}H NMR (DMSO-d_6): δ = 8.55 (d, $J_{1,NH}$ = 9.3 Hz, 1H, γ-NH Asn), 8.18 (d, J = 7.9 Hz, 1H, α-NH Asn), 8.04 (d, $J_{2',NH'}$ = 9.2 Hz, 1H, NHAc'), 7.88 (d, $J_{2,NH}$ = 9.4 Hz, 1H, NHAc), 7.82 (d, J = 8.4 Hz, 1H, NH Leu), 7.34 (d, J = 7.7 Hz, 1H, NH urethane), 5.9 (m, 1H, =CH-), 5.3–5.2 (m, 3H, H-3", H-4", CH_2= *trans*), 5.15 (dd, J_{cis} = 10.5 Hz, J_{gem} = 1.5 Hz, 1H, CH_2=), 5.1 (dd, $J_{2',3'}$ = 10.1 Hz, $J_{3',4'}$ = 9.7 Hz, 1H, H-3'), 5.03–4.97 (m, 2H, H-1, H-2"), 4.93 (d, $J_{1",2"}$ = 3.5 Hz, 1H, H-1"), 4.9 (dd, $J_{2,3} = J_{3,4}$ = 9.8 Hz, 1H, H-3), 4.81 (dd, $J_{3',4'} = J_{4',5'}$ = 9.7 Hz, 1H, H-4'), 4.69 (d, $J_{1',2'}$ = 8.4 Hz, 1H, H-1'), 4.5–4.38 (m, 4H, CH_2O, α-CH Asn, H-5"), 4.33–4.23 (m, 2H, α-CH Leu, H-6a'), 4.02 (dq, 1H, α-CH Ala), 3.93 (m, 1H, H-6b'), 3.83 (m, 1H, H-2), 3.74–3.64 (m, 4H, H-4, H-5, H-5', H-6a), 3.52 (ddd, 1H, H-2'), 3.4 (m, 1H, H-6b), 2.54 (dd, J_{vic} = 6.1 Hz, J_{gem} = 16.5 Hz, 1H, β-CHa-Asn), 2.4 (dd, J_{vic} = 5.5 Hz, 1H, β-CHb Asn), 2.12, 2.11, 2.00, 1.94, 1.93, 1.92, 1.90 (7s, 21H, OAc), 1.75, 1.73 (2s, 6H, NAc), 1.6 (m, 1H, γ-CH Leu), 1.4 (m, 2H, β-CH_2 Leu), 1.32 (s, 9H, tBu), 1.17 (d, J = 7 Hz, 3H, CH_3 Ala), 0.96 (d, $J_{5",6"}$ = 6.4 Hz, 3H, CH_3 Fuc), 0.85, 0.81 (2d, J = 6.5 Hz, 6H, CH_3 Leu). 100.6 MHz ^{13}C NMR (DMSO-d_6): δ = 172.0–169.0 C=O; 155.3 C=O urethane; 133.5 =CH-; 116.9 CH_2=; 99.3 C-1'; 95.2 C-1"; 80.3 C-q tBu; 77.6 C-1; 64.9 C-6; 64.3 CH_2O; 61.6 C-6'; 53.6 C-2'; 52.2 C-2; 50.5; 49.9; 48.7 α-C, Asn, Leu, Ala; 41.2 β-C Leu; 36.7 β-C Asn; 27.5 tBu; 24.0 γ-C Leu; 23.0 δ-C-Leu; 22.6; 22.5 NAc; 21.5 δ-C-Leu; 20.6–20.3 OAc; 18.1 CH_3 Ala; 15.3 CH_3 Fuc. Anal. calcd for $C_{57}H_{86}N_6O_{28}$ (1303.4): C 52.53, H 6.65, N 6.45; found: C 52.95, H 7.04, N 6.69.

N^2-(Allyloxycarbonyl-L-alanyl-L-leucyl)-N^4-(2-acetamido-4-O-(2-acetamido-3,4,6- tri- O-acetyl-2-deoxy-β-D-gluco-pyranosyl)-3-O-acetyl-6-O-(2,3,4-tri-O-acetyl-α-L-fuco-pyranosyl)-2-deoxy-β-D-glucopyranosyl)-L-asparagine (25)

Glycopeptide ester **24** (110 mg, 84.3 μmol) was

dissolved in trifluoroacetic acid (5 mL). After 2 h, TFA was evaporated *in vacuo* and the residue dried in high vacuum. Compound **25** was used for further conversions. Yield: quantitative; 400 MHz ^{1}H NMR (DMSO-d_6) δ = 8.53 (d, $J_{1,NH}$ = 9.3 Hz, 1H, γ-NH Asn), 8.08 (m, 2H, α-NH Asn, NHAc'), 7.88–7.8 (m, 2H, NH Leu, NHAc), 7.35 (d, J = 9.4 Hz, 1H, NH urethane), 5.9 (m, 1H, =CH-), 5.3–5.14 (m, 4H, H-3", H-4", CH_2= *trans*), 5.09 (dd, $J_{2',3'} = J_{3',4'}$ = 10 Hz, 1H, H-3'), 5.05–4.96 (m, 2H, H-1, H-2"), 4.92 (d, $J_{1",2"}$ = 3.5 Hz, 1H, H-1"), 4.89 (dd, $J_{2,3} = J_{3,4}$ = 10 Hz, 1H, H-3), 4.82 (dd, $J_{3',4'} = J_{4',5'}$ = 10 Hz, 1H, H-4'), 4.69 (d, $J_{1',2'}$ = 8 Hz, 1H, H-1'), 4.5–4.4 (m, 4H, CH_2O, α-CH Asn, H-5"), 4.3–4.23 (m, 2H, α-CH Leu, H-6a'), 4.03 (dq, 1H, α-CH Ala), 3.95 (m, 1H, H-6b'), 3.84 (ddd, $J_{1,2}$ = 10 Hz, 1H, H-2), 3.75–3.65 (m, 4H, H-4, H-5, H-5', H-6a), 3.53 (ddd, 1H, H-2'), 3.4 (m, 1H, H-6b); 2.56–2.42 (m, 2H, β-CH_2-Asn), 2.12, 2.09, 2.02, 1.96, 1.94, 1.92, 1.0 (7s, 21H, OAc), 1.75, 1.72 (2s, 6H, NAc), 1.58 (m, 1H, γ-CH Leu), 1.4 (m, 2H, β-CH_2 Leu), 1.18 (d, J = 7 Hz, 3H, CH_3 Ala), 0.95 (d, $J_{5",6"}$ = 6.4 Hz, 3H, CH_3 Fuc), 0.86, 0.81 (2d, J = 6.5 Hz, 6H, CH_3 Leu).

N-*Benzyloxycarbonyl-leucyl-threonyl-asparagine* tert-*butyl ester*

This was synthesized according to the Z/OtBu-technique:[38] Yield: 89 %; mp 142 °C (recrystallized from methanol/ diethyl ether/diisopropyl ether); $[\alpha]_D^{22}$ = –27.4° (c = 1, methanol).

Leucyl-threonyl-asparagine tert-*butyl ester (26)*

Z-Leu-Thr-Asn-OtBu (400 mg, 0.75 mmol) dissolved in methanol (5 mL) was hydrogenated over Pd/C (5 %, 100 mg) for 1 h. After filtration, the solvent was evaporated *in vacuo* and the residue was recrystallized from methanol/diisopropyl ether. Yield: 98 %; mp 152–157 °C, $[\alpha]^{22}_D$ = –46.7° (c = 0.5, methanol).

N^2-(Allyloxycarbonyl-L-alanyl-L-leucyl)-N^4-(2-acetamido-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranosyl)-L-asparaginyl-L-leucyl-L-threonyl-L-asparagine tert-butyl ester (27)

A solution of the Aloc glycopeptide **25** (105 mg, 89.2 μmol), leucyl-threonyl-asparagine *tert*-butyl ester **26** (100 mg, 250 μmol), HOBt (60 mg, 0.44 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 200 mg, 1.04 mmol) in dimethylformamide (2 mL) was stirred at 0 °C. After 10 min, diisopropylethylamine (60 μL, 344 μmol) was added, and the mixture was allowed to react for 18 h. After evaporation of the solvent in high vacuum, the residue was purified by chromatography on silica gel (10 g) in dichloromethane:methanol 30:1 → 5:1 first, and then in petroleum ether:acetone 1:2 → dichloromethane:methanol 5:1 to give **27**; yield 110 mg (84 %), amorphous solid; $[\alpha]^{22}_D$ = –42.3° (c = 0.1, methanol); R_f = 0.6 ($CHCl_3$:MeOH, 5:1). FAB-MS: m/z_{calcd} = 1631; m/z_{found} = 1632 (M + 1); 1654 (M + Na). 400 MHz ^{1}H NMR (DMSO-d_6), δ = 8.54 (d, $J_{1,NH}$ = 8.6 Hz, 1H, γ-NH Asn^3), 8.04 (d $J_{2',NH'}$ = 9.2 Hz, 1H, NHAc'); 7.97 (d, J = 7.9 Hz, 1H, α-NH Asn^6), 7.84–7.8 (m, 3H, NHAc, NH: Thr, Leu^2), 7.57 (d, J = 8.4 Hz, 1H, NH Leu^4), 7.37 (m, 2H, NH urethane, γ-NH Asn^6), 6.89 (s, 1H, γ-NH Asn^6), 5.9 (m, 1H, =CH), 5.3–5.2 (m, 3H, H-3", H4", CH_2= *trans*), 5.16 (m, 1H, CH_2= *cis*), 5.09 (dd, $J_{2',3'}$ = 10.1 Hz, $J_{3',4'}$ = 9.6 Hz, 1H, H-3'), 5.03–4.97 (m, 2H, H-1, H-2"), 4.94–4.86 (m, 3H, H-1", H-3, OH Thr), 4.81 (dd, $J_{3',4'} = J_{4',5'}$ = 9.6 Hz, 1H, H-4'=); 4.68 (d, $J_{1',2'}$ = 8.4 Hz, 1H, H-1'), 4.52–4.44 (m, 5H, CH_2O, α-CH Asn^3, α-CH Asn^6, H-5"), 4.36 (m, 1H, α-CH Leu^4), 4.3–4.22 (m, 2H, α-CH Leu^2, H-6a'), 4.16 (dd, $J_{\alpha,\beta}$ = 4.3 Hz, 1H, α-CH Thr), 4.02 (m, 1H, α-CH Ala), 3.97–3.89 (m, 2H, H-6b', β-CH Thr), 3.84 (m, 1H, H-2), 3.75–3.65 (m, 4H, H-4, H-5, H-5', H-6a), 3.53 (ddd, 1H, H-6b), 2.62 (dd, J_{vic} = 6 Hz, J_{gem} = 16.4 Hz, 1H, β-CHa-Asn^3), 2.51–2.4 (m, 2H, β-CH_2 Asn^6), 2.34 (dd, J_{vic} = 5.4 Hz, 1H, β-CHb Asn^3), 2.12, 2.10, 2.00, 1.95, 1.93, 1.92, 1.90 (7s, 21H, OAc), 1.744, 1.740 (2s, 6H, NAc), 1.6–1.35 (m, 6H, γ-CH Leu, β-CH_2 Leu), 1.35 (s, 9H, tBu), 1.16 (d, J = 7 Hz, 3H, CH_3 Ala), 1.03 (d, J = 6.2 Hz, 3H, Thr), 0.98 (d, $J_{5",6"}$ = 6.3 Hz, 3H, CH_3 Fuc), 0.84–0.8 (m, 12H, CH_3 Leu). 100.6 MHz ^{13}C NMR (DSMO-d_6), δ = 172.0–169.0 C=O; 155.5 C=O urethane; 133.4 =CH-; 116.9 CH_2=; 99.3 C-1'; 95.1 C-1", 80.4 C-q tBu; 77.5 C-1; 66.5 β-C Thr; 64.9 C-6; 64.3 CH_2O; C-6'; 58.0 α-C Thr; 53.6 C-2'; 52.2 C-2; 50.8; 50.5; 49.9; 49.3; 49.0 α-C, Asn, Leu, Ala; 41.2 β-C Leu; 36.7 β-C Asn; 27.5 tBu; 23.9; 23.8 γ-C Leu; 22.6; 22.5 NAc; 21.5 δ-C Leu; 20.6–20.3 OAc; 19.3 CH_3 Thr; 18.0 CH_3 Ala; 15.3 CH_3 Fuc. Anal. calcd for $C_{71}H_{110}N_{10}O_{33}$ (1631.7): C 51.13, H 6.89, N 8.40; Found: C 51.07, H 7.56, N 8.41. The compound was hygroscopic.

N^2-(Allyloxycarbonyl-L-alanyl-L-leucyl)-N^4-(2-acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-O-(-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranosyl)-L-asparaginyl-L-leucyl-L-threonyl-L-asparagine (28)

Glycopeptide **27** (20 mg, 12.2 μmol) was dissolved in trifluoroacetic acid (1 mL) and stirred for 2 h. After evaporation *in vacuo*, the residue was dried in high vacuum, dissolved in methanol (2 mL) and hydrazine hydrate (85 %, 0.5 mL) and stirred for 1 h. Acetone (10 mL) was added and the mixture was concentrated *in vacuo*. Acetone (3 × 10 mL) was distilled off from the residue. The remainder was dissolved in water (5 mL) and the aqueous solution was taken up by using a plastic syringe and passed through a Millipore SEP-PAK C18 cartridge, which had been washed with acetonitrile → water. The loaded cartridge was washed twice with 5 mL of warm water (40 °C). Subsequently, the glycopeptide **28** was eluted from the cartridge using 10 mL of acetonitrile:water 10:1. Evaporation *in vacuo* and lyophilization gave **28**, which contained water and small amounts of impurities of lower molecular weight.

Yield: 14.7 mg (94 %). 400 MHz ^{1}H NMR (D_2O, broad signals): δ = 5.8 (m, 1H, =CH), 5.2 (m, 1H, CH_2= *trans*), 5.05 (m, 1H, CH_2= *cis*), 5.0 (d, $J_{1'',2''}$ = 2.5 Hz, 1H, H-1''), 4.8–4.65 (m, 4H, H-1, H-5'', α-CH Asn), 4.55 (d, $J_{1',2'}$ = 8 Hz, 1H, H-1'), 2.8–2.62 (m, 2H, β-CH_2-Asn[3]), 2.51–2.4 (m, 2H, β-CH_2 Asn[6]), 1.95–1.85 (2s, 6H, NAc), 1.2 (d, J = 7 Hz, 3H, CH_3 Ala), 1.05 (d, J = 6 Hz, 3H, CH_3 Thr), 1.1 (d, $J_{5'',6''}$ = 6.5 Hz, 3H, CH_3 Fuc), 0.9–0.80 (m, 12H, CH_3 Leu).

Coupling of glycopeptide **28** *to bovine serum albumin to give the neoglycoprotein* (**29**)

The glycopeptide **28** (14.5 mg) was dissolved in water (4 mL). To this solution were added BSA (20 mg) and HOBt[17] (10 mg, 73 μmol, dissolved in one drop of dimethylformamide) and, subsequently, at 4 °C EDC (50 mg, 0.26 mmol). The mixture was allowed to react at 0 °C for 14 days. The low-molecular weight components were separated by ultrafiltration. The conjugate was isolated by lyophilization. Yield: 25.8 mg of conjugate **29**. Determination of the carbohydrate content was carried out from 2 mg dissolved in water (10 mL), from which 2 mL were used for photometrical analysis with aqueous phenol (80 %, 50 μL) and conc. sulfuric acid.[35] Measurement was carried out at λ = 490 nm. A 2:1 mixture of *N*-acetylglucosamine and L-fucose was used as the standard. Carbohydrate content of **29**: 123 μg/1 mg neoglycoprotein.

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