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Synthesis of three Biantennary *N*-Glycans containing the α-1,6 Core-Fucosyl Motif

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Abstract: Combined chemical and enzymatic synthesis gave access to the biantennary core-fucosylated *N*-glycans **A**, **B** and **C** terminating with galactose, α-2,6 or α-2,3 linked sialic acid. The oligosaccharides were functionalized at the anomeric center with a 6-aminohexanoyl spacer to facilitate their incorporation into neoglycoproteins. As a versatile precursor served the chemically synthesized core-fucosylated octasaccacharide **2**. © 1997 Elsevier Science Ltd.

Many important biological processes in mammals are mediated by glycoproteins.[1] Changes in their glycosylation pattern may interfere with cellular functions and may thus lead to health disorders. An increase of asparagine-linked oligosaccharides (*N*-glycans) with an α-1,6 linked core-fucosyl unit has been related to malignancy in several types of cancer.[2] To systematically elucidate the biological roles of core-fucosylated *N*-glycans, a series of three model compounds (**A**, **B** and **C**) containing the entire oligosaccharide portion was assembled using a combined chemical and enzymatic approach.[3]

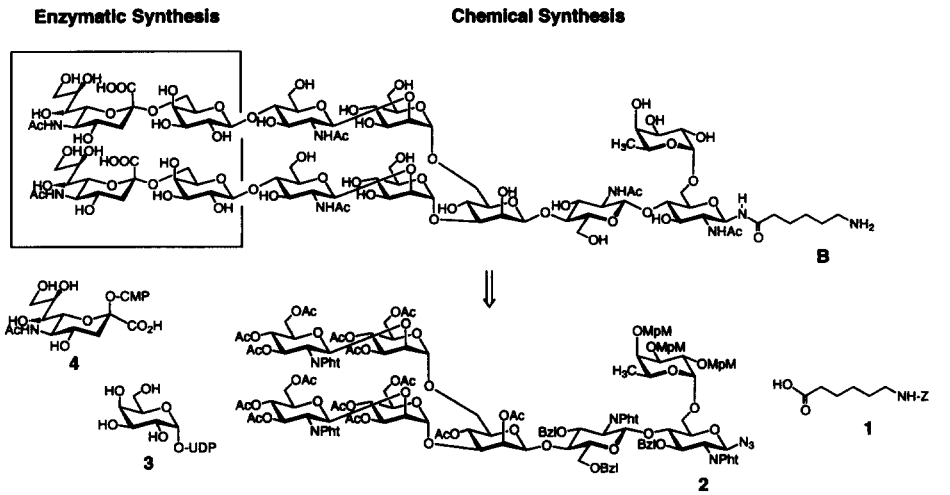

Figure 1: Retrosynthetic disconnection of the biantennary core-fucosylated dodecasaccharide **B**.

The availability of natural *N*-glycans with defined structures is limited by tedious isolation procedures[4] and difficulties still encountered in plain chemical synthesis.[5] We have therefore developed a combined chemical and enzymatic approach that gave rise to sialylated biantennary *N*-glycans conjugated to asparagine[6a] and to glycopeptides.[6b] This chemoenzymatic methodology reduced the overall number of steps, facilitated deprotection and provided a flexible basis for rapid enzymatic derivatization of the

deprotected compounds. In this paper the first chemoenzymatic total synthesis of core-fucosylated biantennary *N*-glycans (**A-C**) using the aforementioned methodology is shown (Fig. 1). Starting from the recently described[7] core-fucosylated octasaccharide **2** the assembly of *N*-glycans with biorelevant termini was examined. The chemically synthesized octasaccharide **2** was designed to facilitate several synthetic goals: a) rapid deprotection of the oligosaccharide without affecting the acid labile core-fucosyl unit; b) attachment of a desired linker at the anomeric center via an azido group[8] and c) the enzymatic elongation of the oligosaccharide chains using glycosyltransferases.

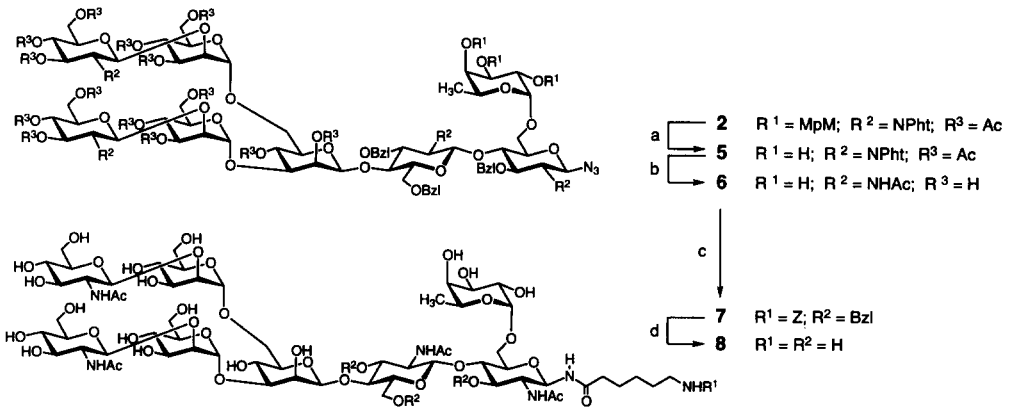

Figure 2: a) CAN, CH_3CN, H_2O (85.0 %); b) 1. H_2N-$(CH_2)_2$-NH_2, *n*-BuOH, 80°C; 2. pyridine, Ac_2O; 3. $MeNH_2$ (40 % in H_2O), (1.-3.: 95 %); b) 1. HS-$(CH_2)_3$-SH, MeOH; DIPEA; 2. **1**, TBTU, HOBt, Et_3N, NMP (1.-2.: 52 %); d) $PdOxH_2O$, MeOH, HOAc (92 %); CAN = $(NH_4)_2Ce^{IV}(NO_3)_6$; MpM = p-methoxybenzyl.

The conversion of the octasaccharide azide **2** to the glycoconjugate **8** required several synthetic steps (Fig. 2). The majority of protective functions except the three benzyl groups and the anomeric azide were removed prior to coupling to the aminohexanoyl spacer. First the three *p*-methoxybenzyl groups of the fucose residue[8] in compound **2** were oxidatively cleaved by CAN[8] in acetontrile-water (9:1) at 0°C. To avoid side reactions during deprotection the original conditions were modified giving compound **5** in 85 % yield. Octasaccharide **5** was dephthaloylated in a one-pot reaction composed out of three steps developed for *N*-glycans containing anomeric azido groups.[5a] The reaction of octasaccharide **5** with ethylene diamine in *n*-butanol at 80° according to Hindsgaul et al.[9] gave an intermediate tetraamino compound without affecting the anomeric azido group. Subsequent peracetylation and O-deacetylation furnished the watersoluble octasaccharide **6** in 95 % overall yield after solid phase extraction. The octasaccharide azide **6** was then converted to the glycosylamine and coupled with Z-6-aminohexanoic acid **1**. Reduction of the azido function was accomplished by excess propanedithiol in methanol-triethylamine[10,5a] followed by removal of the volatile reagents. To the crude glycosylamine was added a solution of Z-aminohexanoic acid **1** activated with TBTU[11] ((1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate)/HOBt. After RP-HPLC purification the desired β-*N*-glycosidically linked glycoconjugate **7** was obtained in 52 % yield. Catalytic hydrogenation over palladiumhydroxide removed the four remaining benzylic protective groups furnishing the deprotected aminohexanoylated octasaccharide **8** in 95 % yield after size exclusion chromatography.

The chemically prepared core-fucosylated octasaccharide **8** served as an acceptor in the enzymatic elongation of the oligosaccharide chains (Fig. 3). An efficient method for the enzymatic transfer of sugars using glycosyltransferases in combination with alkaline phosphatase[12] allowed the rapid completion of biantennary *N*-glycans.[5] This methodology was successfully adapted to the preparative galactosylation of the core-fucosylated acceptor **8**. Incubation of **8** with UDP-galactose **3**, galactosyltransferase (E.C. 2.4.1.22) and alkaline phosphatase (E.C. 3.1.3.1) gave a 75 % yield of decasaccharide **A**[13] containing two galactose residues. In natural *N*-glycans the terminal sialic acid is attached to galactose via an α-2,3 or α-2,6 linkage. The availability of the two sialyltransferases permitted convenient sialylation of the intermediate **A**. In a one-pot reaction the acceptor **8** was first galactosylated as shown above followed by incubation of the reaction mixture with CMP-*N*-acetylneuraminic acid **4**, α-2,6-sialyltransferase (E.C. 2.4.99.1) and alkaline phosphatase. After size exclusion chromatography a 62 % yield of the sialylated dodecasaccharide **B**[13] was

obtained. Analogous use of recombinant α-2,3 sialytransferase[14] (E.C. 2.4.99.6) gave the desired *N*-glycan conjugate **C**[13] with two α-2,3 linked sialic acid residues.

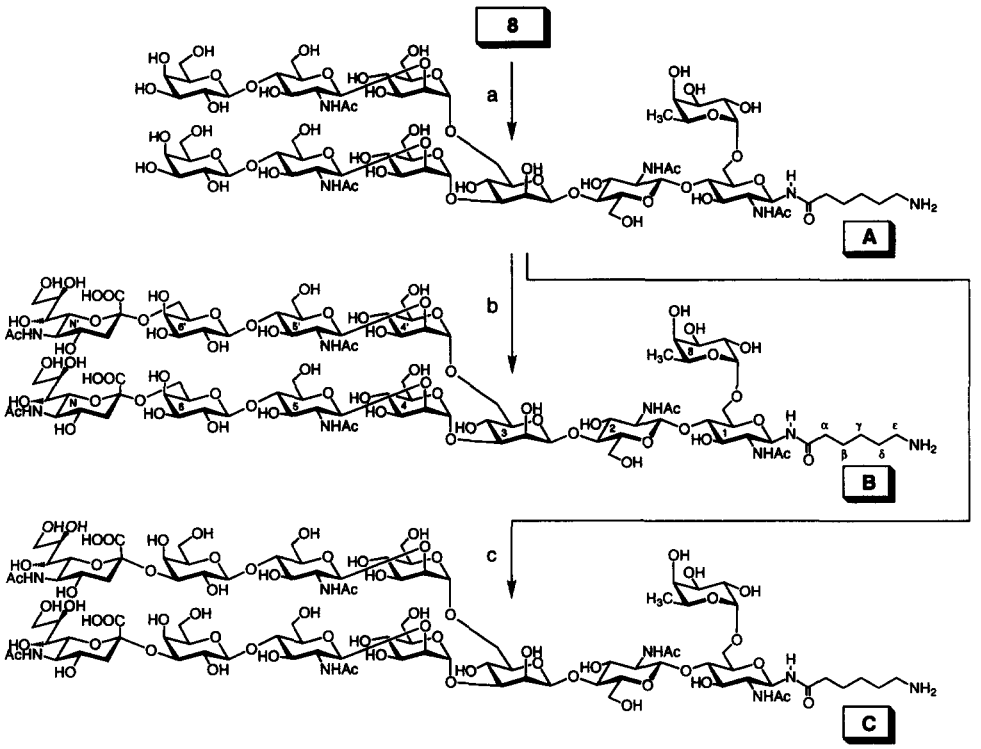

Figure 3: a) UDP-Gal **3**, galactosyltransferase (E.C. 2.4.1.22), alkaline phosphatase (E.C. 3.1.3.1), pH = 7.4 (75 %); b) CMP-Neu5Ac **4**, ß-galactoside-α-2,6-sialyltransferase (E.C. 2.4.99.1), alkaline phosphatase (E.C. 3.1.3.1), pH = 6.5 (a+b: 62 %); c) CMP-Neu5Ac **4**, ß-galactoside-α-2,3-sialyltransferase (E.C. 2.4.99.6), alkaline phosphatase (E.C. 3.1.3.1), pH = 6.5 (a+c: 63 %).

The structures of the core-fucosylated *N*-glycans **A**, **B** and **C** were confirmed by NMR spectroscopy,[13] electrospray ionization mass spectroscopy (ESI-MS) and by comparison with NMR data from isolated reference compounds.[15] A combination of chemical and enzymatical methods allowed for the first time the synthesis of sialylated complex type *N*-glycans containing the α-1,6 core-fucosyl motif. Currently, the incorporation of these core-fucosylated compounds into neoglycoproteins is being examined.

Acknowledgements

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13. AH = 6-aminohexanoyl; **A**: ESI-MS [MeOH/H_2O]: $C_{74}H_{126}N_6O_{50}$ M_r(calcd) 1898.76; M_r(found) 950.7 $(M+2H)^{2+}$; $[\alpha]_D^{22}$ = -2.2° (0.63; H_2O).
^{1}H-NMR (500MHz, D_2O/CD_3CN as internal standard): δ = 4.90 (d, $J_{1,2}$ < 1.0Hz, 1H, H-1^4 α), 4.85 (d, $J_{1,2}$ = 9.7Hz, 1H, H-1^1 ß), 4.70 (d, $J_{1,2}$ < 1.0Hz, 1H, H-1$^{4'}$α), 4.66 (d, $J_{1,2}$ = 3.2Hz, 1H, H-1^{8}α), 4.56 (d, $J_{1,2}$ < 1.0Hz, 1H, H-1^3 ß), 4.46 (d, $J_{1,2}$ = 8.1Hz, 1H, H-1^2 ß), 4.36 (d, $J_{1,2}$ = 7.5Hz, 2H, H-1^5 ß, H-1^5 ß), 4.25 (d, $J_{1,2}$ = 7.6Hz, 1H, H-1^6 ß), 4.24 (d, $J_{1,2}$ = 7.6Hz, 1H, H-1^6 ß), 2.73 (t, $J_{\delta,\varepsilon}$ = 7.7Hz, 2H, ε-CH_2), 2.06 (t, $J_{\alpha,\beta}$ = 7.2Hz, 2H, α-CH_2), 1.87, 1.83, 1.82, 1.78 (4s, 12H, NAc), 1.43-0.98 (d, $J_{5,6}$ = 6.6Hz, 3H, H-6^8).
^{13}C-NMR (125MHz, [D_6]-DMSO):δ =103.82 C-1^6, C-1$^{6'}$, 101.90 C-1^2, 101.32 C-1^3 ($J_{C\text{-}1,H\text{-}1}$ =162.4Hz), 100.36 C-1^{5}ß, C-1$^{5'}$ß, 100.29 C1^{8}α, 100.27 C-1$^{4'}$α ($J_{C\text{-}1,H\text{-}1}$ = 172.3Hz), 97.89 C-1^{4}α ($J_{C\text{-}1,H\text{-}1}$ = 171.4Hz), 79.20 C-1^1 ß, 40.16 C-6 AH, 36.33 C-2 AH, 27.63 C-5 AH, 25.90 C-4 AH, 25.39 C-3 AH, 23.23, 22.90 NAc, 16.30 C-6^8.
B: ESI-MS [MeOH/H_2O]: $C_{96}H_{160}N_8O_{66}$ M_r(calcd) 2480.94; M_r(found) 1241.6 $(M+2H)^{2+}$; $[\alpha]_D^{22}$ = -5.6° (0.78; H_2O).
^{1}H-NMR (500MHz, D_2O/CD_3CN as internal standard): δ 4.93 (d, $J_{1,2}$ < 1.0Hz, 1H, H-1^{4}α), 4.84 (d, $J_{1,2}$ = 9.7Hz, 1H, H-1^1 ß), 4.75 (d, $J_{1,2}$ < 1.0Hz, 1H, H-1$^{4'}$α), 4.68 (d, $J_{1,2}$ = 3.7Hz, 1H, H-1^{8}α), 4.58 (d, $J_{1,2}$ < 1.0Hz, 1H, H-1^{3}ß), 4.48 (d, $J_{1,2}$ = 7.8Hz, 1H, H-1^{2}ß), 4.41 (d, $J_{1,2}$ = 7.4Hz, 2H, H-1^{5}ß, H-1$^{5'}$ß), 4.24 (d, $J_{1,2}$ = 7.8Hz, 1H, H-1^{6}ß), 4.23 (d, $J_{1,2}$ = 7.8Hz, 1H, H-1^{6}ß), 2.78 (t, $J_{\delta,\varepsilon}$ = 7.6Hz, 2H, ε-CH_2), 2.48 (dd, J_{vic} = 4.4Hz, J_{gem} = 12.4Hz, 2H, H-3eq^N, H-3eq$^{N'}$), 2.08 (t, $J_{\alpha,\beta}$ = 7.2Hz, 2H, α-CH_2), 1.90, 1.87, 1.86 1.84, 1.83, 1.80 (6s, 18H, NAc), 1.52 (t, J_{vic} = 12.2Hz, 2H, H-3ax^N, H-3ax$^{N'}$), 1.01 (d, $J_{5,6}$ = 6.6Hz, 3H, H-6^8).
^{13}C-NMR (125MHz, D_2O/CD_3CN as internal standard): δ 104.42 C-1^6, C-1$^{6'}$, 101.89 C-1^2, 101.33 C-1^3 ($J_{C\text{-}1,H\text{-}1}$ = 161.1Hz, determined from a coupled HMQC-experiment), 101.06 C-1^{4}α ($J_{C\text{-}1,H\text{-}1}$ = 171.4Hz), 100.44 C1^{8}α, 100.29 C-1^{5}ß, 100.14 C-1$^{5'}$ß, 97.80 C-1$^{4'}$α ($J_{C\text{-}1,H\text{-}1}$ = 172.5Hz), 79.21 C-1^{1}ß, 40.97 C-3^N, C-3$^{N'}$, 40.07 C-6 AH, 36.30 C-2 AH, 27.21 C-5 AH, 25.85 C-4 AH, 25.35 C-3 AH, 23.31, 22.93 NAc, 16.31 C-6^8.
C: ESI-MS [MeOH/H_2O]: $C_{96}H_{160}N_8O_{66}$ M_r(calcd) 2480.94; M_r(found) 1241.6 $(M+2H)^{2+}$; $[\alpha]_D^{22}$ = -5.2° (0.78; H_2O).
^{1}H-NMR (500MHz, D_2O/CD_3CN as internal standard): δ 4.91 (d, $J_{1,2}$ < 1.0Hz, 1H, H-1^4 α), 4.85 (d, $J_{1,2}$ = 9.7Hz, 1H, H-1^1 ß), 4.73 (d, $J_{1,2}$ < 1.0Hz, 1H, H-1$^{4'}$α), 4.68 (d, $J_{1,2}$ = 3.6Hz, 1H, H-1^{8}α), 4.56 (d, $J_{1,2}$ < 1.0Hz, 1H, H-1^{3}ß), 4.46 (d, $J_{1,2}$ = 8.0Hz, 1H, H-1^{2}ß), 4.37 (d, $J_{1,2}$ = 7.5Hz, 2H, H-1^{5}ß, H-1$^{5'}$ß), 4.35 (d, $J_{1,2}$ = 8.0Hz, 1H, H-1^{6}ß), 4.34 (d, $J_{1,2}$ = 8.0Hz, 1H, H-1^{6}ß), 2.79 (t, $J_{\delta,\varepsilon}$ = 7.7Hz, 2H, ε-CH_2), 2.56 (dd, J_{vic} = 4.4Hz, J_{gem} = 12.4Hz, 2H, H-3eq^N, H-3eq$^{N'}$), 2.08 (t, $J_{\alpha,\beta}$ = 7.3Hz, 2H, α-CH_2), 1.90, 1.85, 1.84, 1.83, 1.81 (6s, 18H, NAc), 1.60 (t, J_{vic} = 12.1Hz, 2H, H-3ax^N, H-3ax$^{N'}$), 1.01 (d, $J_{5,6}$ = 6.6Hz, 3H, H-6^8).
^{13}C-NMR (125MHz, D_2O/CD_3CN as internal standard): δ 103.55 C-1^6, 103.50 C-1$^{6'}$, 101.90 C-1^2, 101.28 C-1^{3}ß ($J_{C\text{-}1,H\text{-}1}$ = 161.7Hz), 100.72 C-1^{4}α ($J_{C\text{-}1,H\text{-}1}$ = 171.9Hz), 100.43 C-1^5 ß, C-1$^{5'}$ ß, 100.30 C1^{8}α, 97.96 C-1$^{4'}$α ($J_{C\text{-}1,H\text{-}1}$ = 172.5Hz), 79.23 C-1^1, 40.52 C-3^N, C-3$^{N'}$, 40.08 C-6 AH, 36.30 C-2 AH, 27.23 C-5 AH, 25.86 C-4 AH, 25.36 C-3 AH, 23.24, 22.92 NAc, 16.31 C-6^8.
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