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Chemoenzymatic synthesis of deca and dodecasaccharide *N*-glycans of the 'bisecting' type†

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Abstract

The synthesis of *N*-glycans carrying the 'bisecting' GlcNAc modification is difficult due to steric hindrance at the central β-mannoside. A facile introduction of a 'bisecting' GlcNAc residue was accomplished by using an excess of a glucosaminyl fluoride. After deprotection of the resulting octasaccharide a 6-amino-hexanoyl spacer was attached. Enzymatic elongation of the carbohydrate chains using three different glycosyltransferases gave full length 'bisecting' *N*-glycans of the complex type terminating with galactose, α-2,6 or α-2,3 linked sialic acid. © 2000 Elsevier Science Ltd. All rights reserved.

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The biological activity of glycoproteins is related to the oligosaccharides present on their surface.[1] Subtle changes in the structures of asparagine-linked oligosaccharides (*N*-glycans) may result in a completely altered functionality of the glycoprotein. In particular, an increase of *N*-glycans containing a 'bisecting' GlcNAc residue has been correlated with malignancy in several types of cancer[2] and also with the ability of the trk receptor to dimerize.[3] Three full length *N*-glycans containing a 'bisecting' GlcNAc residue (**A**, **B** and **C**) were synthesized using a combination of chemical and enzymatic methods (Fig. 1).[4] These model compounds were designed to study the influence of the 'bisecting' GlcNAc substituent on the biological properties of *N*-glycans.[5]

The high diversity of *N*-glycans found on glycoproteins limits the isolation of pure compounds from natural sources. Especially when rare structures are desired, purification procedures tend to be very difficult. Among the rare *N*-glycans are those containing a single ('bisecting') GlcNAc residue at OH-4 of the β-mannosyl unit in the core pentasaccharide.

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† Dedicated to Professor Horst Kessler on the occasion of his 60th birthday.

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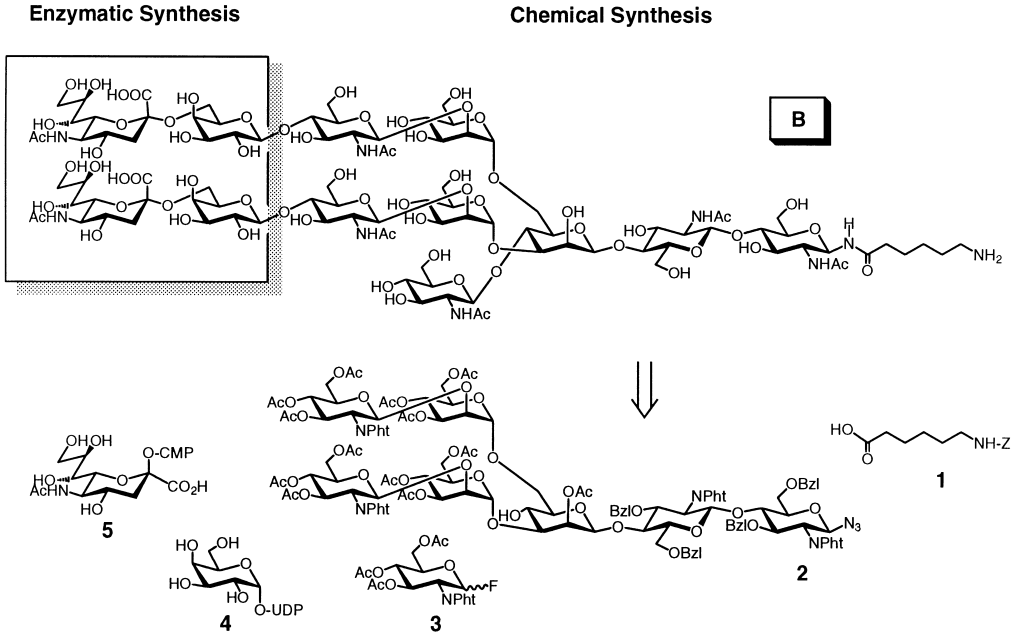

Figure 1. Retrosynthetic disconnection of the 'bisecting' dodecasaccharide **B**

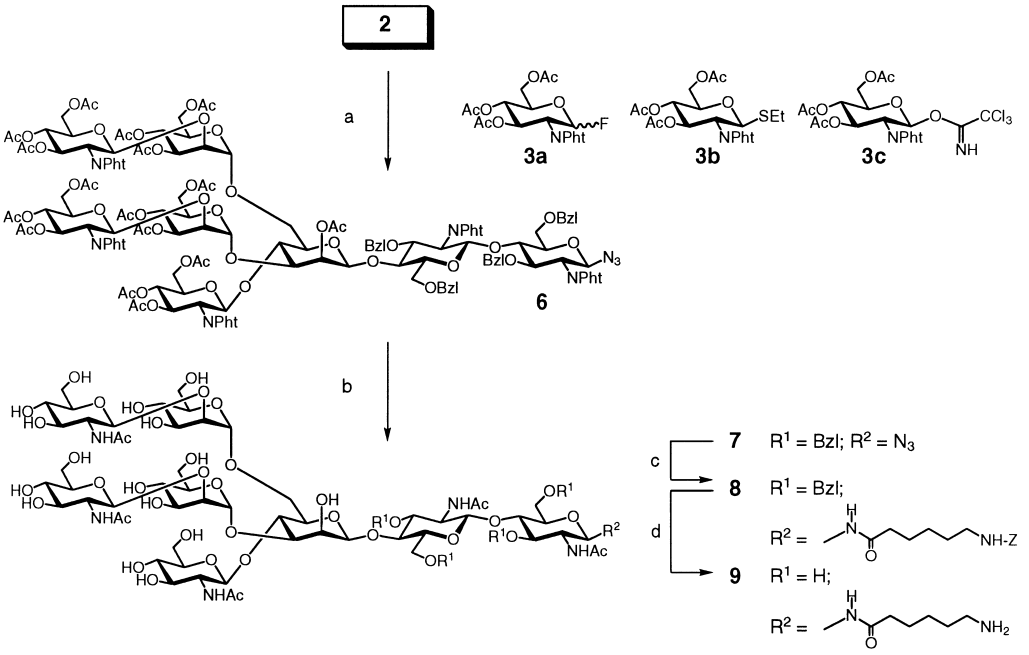

Figure 2. (a) Compound **3a**, CH_2Cl_2, BF_3–OEt_2, (56.0%); (b) (1) ethylenediamine, *n*-BuOH, 80°C; (2) pyridine, Ac_2O; (3) $MeNH_2$ (40% in H_2O), [(1)–(3): 95%]; (c) (1) HS-$(CH_2)_3$-SH, MeOH; DIPEA; (2) **1**, TBTU, HOBt, Et_3N, NMP [(1)–(2): 54%]; (d) PdO×H_2O, MeOH, HOAc (95%). TBTU = (1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate

Previous approaches by the groups of Ogawa[6] and Paulsen[7] to synthesize 'bisecting' *N*-glycans showed that the introduction of three branches to the β-mannoside was sterically hindered. Partial structures of 'bisecting' *N*-glycans could only be obtained when the 1,6-arm was introduced after the attachment of the 1,3-arm and the 'bisecting' residue. To establish a more direct approach we examined the en route conversion of heptasaccharide **2**[8] to a 'bisecting' octasaccharide (Fig. 2). The attempted glycosylation of the heptasaccharide **2** with the trichloroacetimidate[9] **3c** showed only traces of the desired octasaccharide **6**. We assumed that the steric congestion around OH-4 requires a glycosyl donor that remains active in the reaction mixture over a prolonged period of time. In contrast to thioglycosides or imidates that are prone to elimination[10] or rearrangements,[11] glycosylfluorides remain largely unchanged when activated with borontrifluoride–diethyl ether.[12] A suitable donor was found in the fluoride[13] **3a** that was still present in the glycosylation mixture after 16 h. When the heptasaccharide **2** was reacted with ten equivalents of glycosyl fluoride **3a** the 'bisecting' octasaccharide was obtained in 56% yield.[14] Later a similar strategy was used by Ogawa et al.[15] to obtain 'bisecting' core hexasaccharides.

The 'bisecting' octasaccharide **6** could also be obtained using silvertriflate/hafnocene-dichloride[16] as a promoter for fluoride **3a**, however, yields remained below 20%. Activation of the donor **3a** with lithiumperchlorate in dichloromethane[17] did not provide the desired glycosylation product.

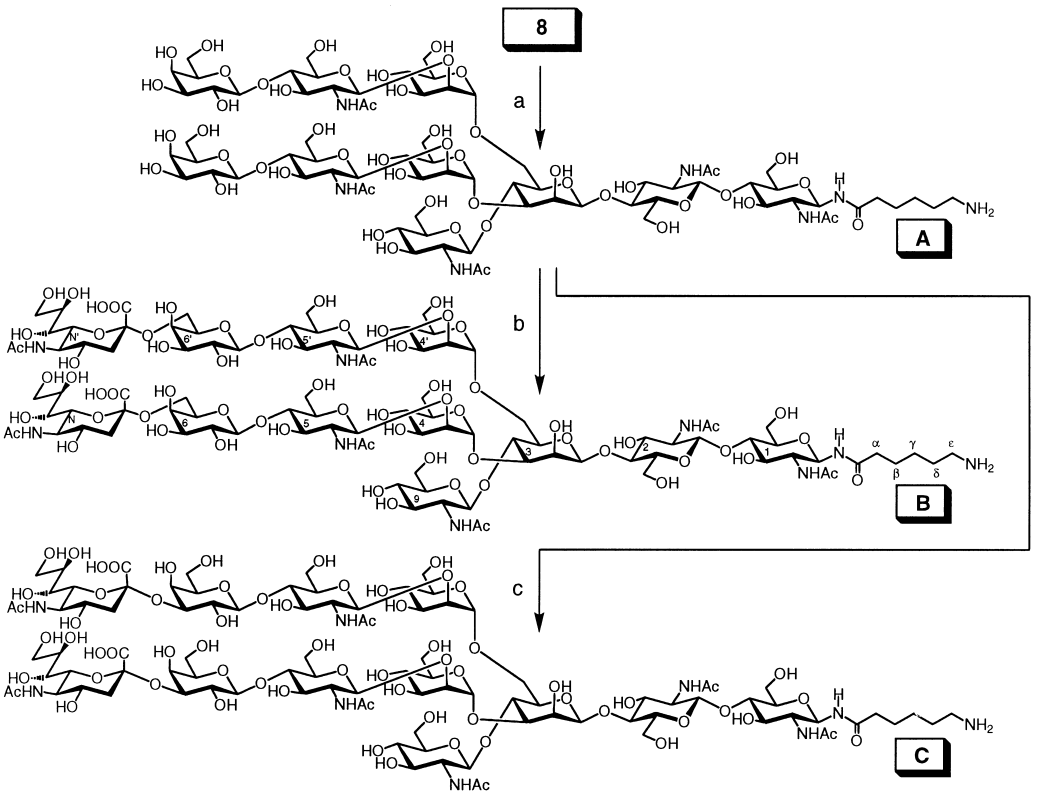

Figure 3. (a) UDP-Gal **3**, galactosyltransferase (E.C. 2.4.1.22), alkaline phosphatase (E.C. 3.1.3.1), pH = 7.4 (89%); (b) CMP-Neu5Ac **4**, β-galactoside-α-2,6-sialyltransferase (E.C. 2.4.99.1), alkaline phosphatase (E.C. 3.1.3.1), pH = 6.5 (a+b: 79%); (c) CMP-Neu5Ac **4**, β-galactoside-α-2,3-sialyltransferase (E.C. 2.4.99.6), alkaline phosphatase (E.C. 3.1.3.1), pH = 6.5 (a+c: 61%)

To overcome the shortage of complex *N*-glycans for investigations in the field of glycobiology we have developed a generally applicable chemoenzymatic approach. This methodology allows the synthesis of full length biantennary *N*-glycans and their conjugation to asparagine,[18a] glycopeptides[18b] or proteins.[5] Thus, the 'bisecting' octasaccharide **6** was deprotected in a three step sequence[19] to give the intermediate octasaccharide **7**. The azido function of compound **7** was reduced and coupled to *Z*-aminohexanoic acid.[20] Final debenzylation of **8** furnished the 'bisecting' octasaccharide **9** (95% yield) equipped with a spacer containing a terminal amino group. Elongation of the two antennae of the watersoluble octasaccharide **9** with galactose and sialic acid was accomplished by enzymatic transfer reactions. Galactosyltransferase in combination with alkaline phosphatase[21] utilizes uridine-diphosphogalactose **4** (UDP-Gal) to efficiently transfer two galactose residues to the acceptor **9** in 89% yield. The decasaccharide **A** served as an acceptor for two different sialyltransferases[22] giving the desired α-2,6 and the α-2,3 sialylated dodecasaccharides **B** and **C** (Fig. 3). Structural confirmation of the products was obtained by electrospray ionization mass spectroscopy (ESI-MS) and NMR spectroscopy.[23] The full length biantennary *N*-glycans of the 'bisecting' type have been synthesized for the first time and provide valuable tools to study the glycobiology of core-modifications on a molecular level.

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23. AH = 6-Aminohexanoyl; compound **A**: ESI-MS [MeOH/H_2O]: $C_{76}H_{129}N_7O_{51}$ M_r (calcd) 1955.79; M_r (found) 978.9 $(M+2H)^{2+}$; $[\alpha]_D^{22} = 2.6$ (0.46; H_2O). ^{1}H NMR (600 MHz, D_2O/CD_3CN as internal standard): $\delta = 4.87$ (d, $J_{1,2} < 1.0$ Hz, 1H, H-1^4), 4.86 (d, $J_{1,2} = 8.7$ Hz, 1H, H-1^1), 4.82 (d, $J_{1,2} < 1.0$ Hz, 1H, H-1$^{4'}$), 4.49 (d, $J_{1,2} < 1.0$ Hz, 1H, H-1^3), 4.42 (d, $J_{1,2} = 8.1$ Hz, 1H, H-1^2), 4.41 (d, $J_{1,2} = 8.6$ Hz, 1H, H-1^5), 4.38 (d, $J_{1,2} = 8.6$ Hz, 1H, H-1$^{5'}$), 4.29 (m, 1H, H-1^6), 4.28 (m, 1H, H-1^9), 4.27 (m, 1H, H-1$^{6'}$), 2.08 (t, $J_{\alpha,\beta} = 7.2$ Hz, 2H, α-CH_2), 1.89, 1.87, 1.86, 1.85, 1.81 (5s, 15H, NAc). ^{13}C NMR (150 MHz, D_2O/CD_3CN, 9:1): $\delta = 103.88$ C-1^6, 103.78 C-1$^{6'}$, 102.20 C-1^2, 101.46 C-1^9, 100.99 C-1^{3}β, 100.79 C-1^4, 100.61 C-1^5, 100.25 C-1$^{5'}$, 98.56 C-1$^{4'}$α, 79.14 C-4^2, C-1^1, 36.42 C-2 AH, 23.32, 23.18, 23.02, 22.90 NAc. Compound **B**: ESI-MS [MeOH/H_2O]: $C_{98}H_{163}N_9O_{67}$ M_r (calcd) 2537.96; M_r (found) 1270.3 $(M+2H)^{2+}$; $[\alpha]_D^{22} = 8.1$ (0.28; H_2O). ^{1}H NMR (500 MHz, D_2O/CD_3CN as internal standard): $\delta = 4.86$ (d, $J_{1,2} < 1.0$ Hz, 1H, H-1^4), 4.85 (d, $J_{1,2} = 9.5$ Hz, 1H, H-1^1), 4.83 (d, $J_{1,2} < 1.0$ Hz, 1H, H-1$^{4'}$), 4.48 (d, $J_{1,2} < 1.0$ Hz, 1H, H-1^3), 4.48 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1^2), 4.41–4.38 (m, 3H, H-1^2, H-1^5, H-1$^{5'}$), 4.26–4.22 (m, 3H, H-1^9, H-1^6 β, H-1^6), 2.47 (m, 2H, H-3eq^N, H-3eq$^{N'}$), 2.07 (t, $J_{\alpha,\beta} = 6.6$ Hz, 2H, α-CH_2), 1.88, 1.87, 1.854, 1.850, 1.826, 1.825, 1.79 (7s, 18H, NAc), 1.50 (2 t, $J_{vic} = 12.2$ Hz, 2H, H-3ax^N, H-3ax$^{N'}$). ^{13}C NMR (125 MHz, D_2O/CD_3CN as internal standard): $\delta = 104.45$ C-1^6, C-1$^{6'}$, 102.23 C-1^2, 101.55 C-1^9, 101.12 C-1^3, 100.83 C-1^4, 100.42 C-1^5, 99.98 C-1$^{5'}$, 98.33 C-1$^{4'}$, 79.17 C-1^1, 40.97 C-3^N, C-3$^{N'}$, 40.07 C-6 AH, 36.30 C-2 AH, 23.31, 22.93 NAc. Compound **C**: ESI-MS [MeOH/H_2O]: $C_{98}H_{163}N_9O_{67}$ M_r (calcd) 2537.96; M_r (found) 1270.3 $(M+2H)^{2+}$; $[\alpha]_D^{22} = 11.2$ (0.1; H_2O). ^{1}H NMR (500 MHz, D_2O/CD_3CN as internal standard): $\delta = 4.91$ (d, $J_{1,2} < 1.0$ Hz, 1H, H-1^4), 4.85 (d, $J_{1,2} = 9.7$ Hz, 1H, H-1^1), 4.73 (d, $J_{1,2} < 1.0$ Hz, 1H, H-1$^{4'}$), 4.68 (d, $J_{1,2} = 3.6$ Hz, 1H, H-1^8), 4.56 (d, $J_{1,2} < 1.0$ Hz, 1H, H-1^3), 4.46 (d, $J_{1,2} = 8.0$ Hz, 1H, H-1^2), 4.37 (d, $J_{1,2} = 7.5$ Hz, 2H, H-1^5, H-1$^{5'}$), 4.35 (d, $J_{1,2} = 8.0$ Hz, 1H, H-1^6), 4.34 (d, $J_{1,2} = 8.0$ Hz, 1H, H-1^6), 2.56 (dd, $J_{vic} = 4.4$ Hz, $J_{gem} = 12.4$ Hz, 2H, H-3eq^N, H-3eq$^{N'}$), 2.08 (t, $J_{\alpha,\beta} = 7.3$ Hz, 2H, α-CH_2), 1.90, 1.85, 1.84, 1.83, 1.81 (6s, 18H, NAc), 1.60 (t, $J_{vic} = 12.1$ Hz, 2H, H-3ax^N, H-3ax$^{N'}$), 1.38 (m, 2H, β-CH_2). ^{13}C NMR (125 MHz, D_2O/CD_3CN as internal standard): $\delta = 103.50$ C-1^6, C-1$^{6'}$, 102.22 C-1^2, 101.54 C-1^9, 100.91 C-1^3, 100.74 C-1^4, 100.72 C-1^5, 100.25 C-1$^{5'}$, 98.72 C-1$^{4'}$, 79.16 C-1^1, 40.51 C-3^N, C-3$^{N'}$, 36.32 C-2 AH, 23.29, 23.20, 23.03, 22.92 NAc.