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Synthesis of a *N*-glycan nonasaccharide of the bisecting type with additional core-fucose

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Abstract—A chemical synthesis was developed for *N*-glycans substituted with core-fucose and an additional bisecting GlcNAc (LEC10 motif). The synthesis was conducted using a suitably functionalized *N*-glycan pentasaccharide assembled from modular building blocks. Selective introduction of the bisecting GlcNAc residue was followed by attachment of the α1,6-arm and core-fucose. After introduction of an aminohexanoyl spacer the nonasaccharide was completely deprotected providing a substrate for enzymatic elongation and conjugation to proteins for further biological studies.

Cell surface and secretory proteins typically bear asparagine-linked sugar chains (*N*-glycans). These carbohydrates are involved in numerous biological events, such as cell–cell recognition, differentiation, immune response and inflammation.[1] The modification of *N*-glycans with a bisecting GlcNAc residue is frequently found in aberrant cells, but the function of this substitution still remains unclear.[2] A similar scenario is observed for the occurrence of core-fucose,[3] which is known to influence antibody-dependent cellular cytotoxicity (ADCC). Unfortunately, the isolation of homogeneous *N*-glycans from natural sources is difficult due to the high natural heterogeneity[4] impairing studies of the structure–function relationships not only of this class of carbohydrates. We herein describe the synthesis of biantennary *N*-glycans (**A**) carrying both a bisecting-GlcNAc and a core-fucose (LEC10 motif)[5] and the preparation of derivatives suitable for enzymatic elongation and coupling to proteins.

It was planned to synthesize the double substituted *N*-glycan[6] by combining the strategies developed for core-fucosylated[7] and multiantennary bisected *N*-glycans.[8] Based on our modular *N*-glycan building block system,[9] retrosynthetic analysis suggested the disconnection to the complex pentasaccharide **B**,[7] thioglycoside **C** serving as a donor for the bisecting GlcNAc,[8] disaccharide donor **D**[9b] and fucosyl donor **E**[10] (Fig. 1).

To assure the compatibility of the building blocks **B** and **C** the introduction of the bisecting-GlcNAc was investigated first. The advanced pentasaccharide building block **B** was selectively chloroacetylated at the primary OH-group with chloroacetic acid anhydride under dilute conditions furnishing **1** in a yield of 93% (Fig. 2). The attachment of the bisecting donor **C** was conducted using *N*-iodosuccinimide and trifluoromethanesulfonic acid,[11] maintaining a strict temperature range from −40 to −35 °C to obtain optimum results. Addition of 3 equiv of donor **C** gave the desired hexasaccharide **2** in 77% yield. Subsequently, the chloroacetyl group was removed in the presence of the base sensitive trifluoroacetamide under mild conditions in a two phase reaction with potassium carbonate in dichloromethane/methanol (94%).[12] The resulting alcohol was coupled with the disaccharide trichloroacetimidate **D** promoted by borontrifluoride and afforded the bisected octasaccharide **4** in 84% yield. Up to this point the presence of the *p*-methoxyphenyl group required for the introduction of the core-fucosyl moiety did not appear to influence the outcome of the different glycosylation procedures and the protective group manipulations. Oxidative cleavage of the *p*-methoxyphenyl group was conducted with cerium(IV)–ammoniumnitrate (CAN)[13] to afford the acceptor **5** almost quantitatively.

For the transfer of the core-fucose onto the acceptor **5** the robust MPM-protected fucosyldonor **E**[10] was activated

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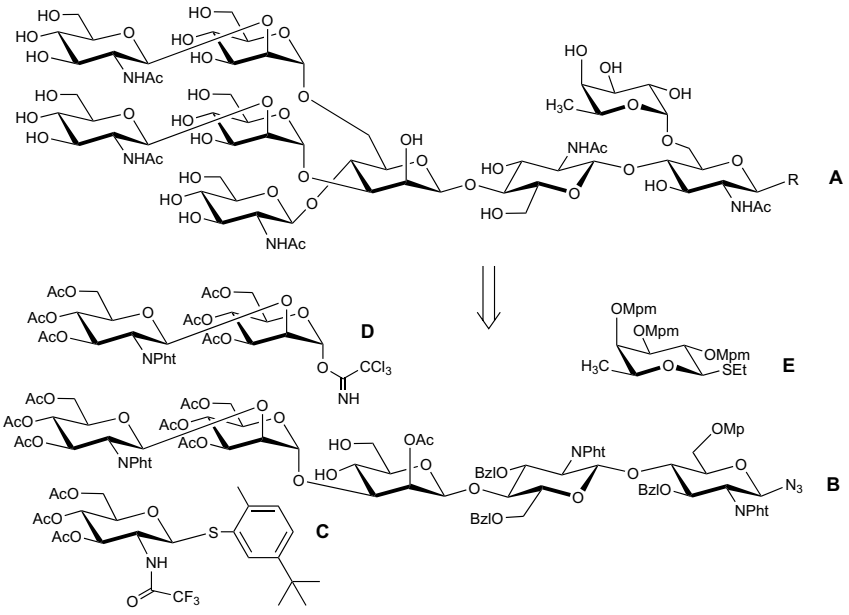

Figure 1. Building blocks employed for the synthesis of the bisected and core-fucosylated nonasaccharide **A**.

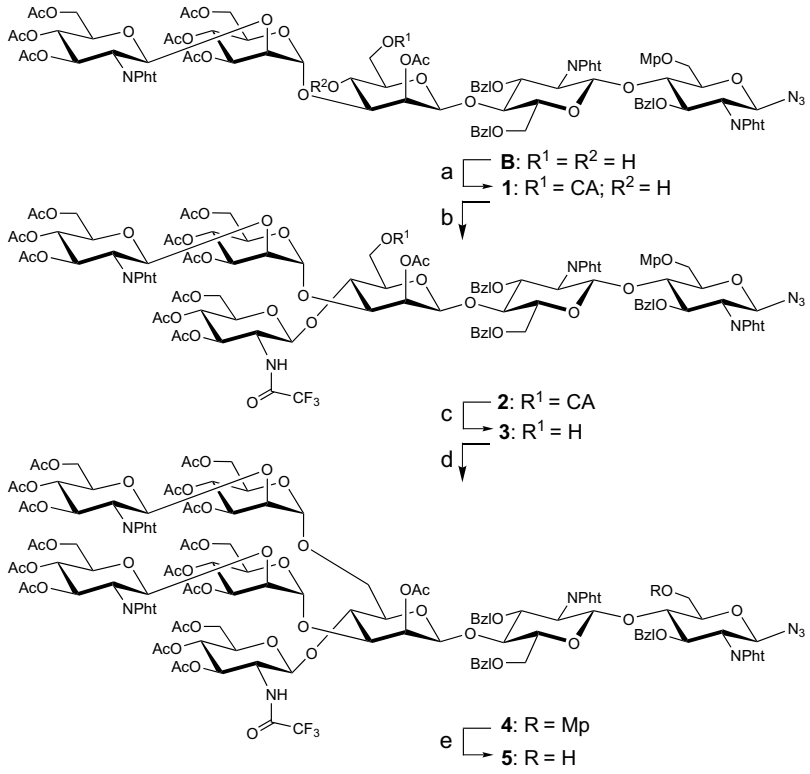

Figure 2. Reagents and conditions: (a) pyridine, $(ClAc)_2O$, CH_2Cl_2, 0 °C (93 %); (b) **C**, NIS, TfOH, molecular sieves 4 Å, CH_2Cl_2, −40 °C (77%); (c) K_2CO_3, CH_2Cl_2/MeOH (10:1), −10 °C (94%); (d) **D**, BF_3–OEt_2, molecular sieves 4 Å, CH_2Cl_2, −40 °C (84%); (e) $(NH_4)_2Ce(NO_3)_6$, CH_3CN, toluene, H_2O (98%).

with $CuBr_2/Bu_4NBr$[14] providing the bisected α-fucosylated nonasaccharide **6**[15] in 83% yield (Fig. 3). To ascertain the deprotection of the complex *N*-glycan **6** the *p*-methoxybenzyl groups were first cleaved with CAN in 65% yield (**7**).[16] For the global deprotection of the base labile acetates, phthalimides and the trifluoroacetamide we used a one-pot three step procedure[8] involving treatment with ethylene diamine[17] followed by peracetylation and *O*-deacetylation. After purification by size exclusion chromatography the nonasaccharide **8** was obtained in 78% yield. At this stage the modification of the anomeric azido group with a spacer was envisioned to allow the conjugation with proteins. In line with the series of previously synthesized neoglycoproteins[2,3a,18] we

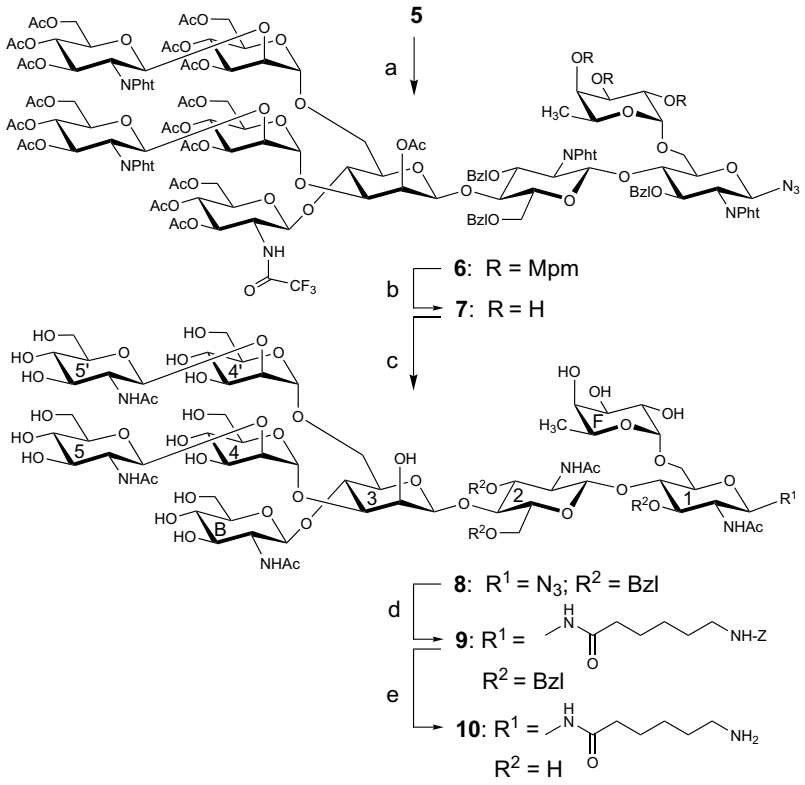

Figure 3. Reagents and conditions: (a) **E**, $CuBr_2$, Bu_4NBr, molecular sieves 4 Å, DMF, CH_2Cl_2 (83%); (b) $(NH_4)_2Ce(NO_3)_6$, CH_3CN, H_2O (65%); (c) (1) ethylenediamine, *n*-BuOH, 80 °C; (2) pyridine, Ac_2O; (3) $MeNH_2$ (41% in H_2O), [(1)–(3): 78%]; (d) (1) HS–$(CH_2)_3$–SH, MeOH, DIPEA; (2) *Z*-aminohexanoic acid, PyBOP, DIPEA, dioxane/DMF (1:1) [(1)–(2): 79%]; (e) H_2, $PdOxH_2O$, MeOH, HOAc (64%). PyBOP═(benzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)phosphonium-hexafluorophosphate).

chose 6-amino hexanoic acid as a spacer. First, the anomeric azido group was reduced with propanedithiol[19] and the intermediate β-glycosylamine was directly coupled with *Z*-aminohexanoic acid using PyBOP[20] in dioxane/DMF (1:1) providing **9** in 79% yield. Final hydrogenolysis of the benzyl protective groups afforded the target molecule **10** in 64% yield after size exclusion chromatography. The structure of **10** was confirmed by 2D NMR spectroscopy (COSY, TOCSY, NOESY, HMQC–COSY and HMQC–TOCSY)[21] and ESI-MS.[22]

In summary we have developed an efficient synthesis for a biantennary *N*-glycan nonasaccharide with core-fucosyl and bisecting substitution using a modular building block system. The deprotected *N*-glycan-spacer-conjugate **10** is suitable for enzymatic elongation and conjugation to proteins.

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22. Compound **10**: ESI-MS (H_2O, 0.1% formic acid): $C_{70}H_{119}N_7O_{45}$ M_r (calcd) 1777.7, M_r (found) 1778.8 $(M+H)^+$, 1800.9 $(M+Na)^+$; $R_f = 0.28$ (*iso*-propanol/1 M NH_4OAc, 2:1); $[\alpha]_D^{22}$ −9.0 (*c* 0.9, H_2O); ^1H NMR (500 MHz, D_2O with DMSO-d_6 as internal standard): $\delta = 4.86$ (d, $J_{1,2} < 1$ Hz, 1H, H-1^4), 4.84 (d, $J_{1,2} = 9.2$ Hz, 1H, H-$1^1\beta$), 4.81 (d, $J_{1,2} < 1$ Hz, 1H, H-$1^{4'}$), 4.68 (d, $J_{1,2} = 3.3$ Hz, 1H, H-$1^F\alpha$), 4.51–4.46 (m, 2H, H-1^3, H-1^2), 4.35 (2d, $J_{1,2} = 8.3$ Hz, 2H, H-$1^5\beta$, H-$1^{5'}\beta$), 4.27 (d, $J_{1,2} = 7.9$ Hz, 1H, H-$1^B\beta$), 4.05 (dd, $J_{1,2} < 1$ Hz, $J_{2,3} < 3$ Hz, 1H, H-2^4), 3.98 (dd, $J_{1,2} < 2$ Hz, $J_{2,3} < 3$ Hz, 1H, H-2^3), 3.95–3.92 (m, 2H, H-$2^{4'}$, H-5^F), 3.87 (dd, $J_{3,4} = J_{4,5} = 9.7$ Hz, 1H, H-4^3), 3.81–3.74 (m, 3H, H-$6a^B$, H-$6a^4$, H-$6a^{4'}$), 3.72–3.63 (m, 11H, H-$6a^5$, H-$6a^{5'}$, H-3^4, H-$6a^3$, H-$6b^3$, H-$6a^1$, H-$6a^2$, H-3^3, H-2^1, H-$3^{4'}$, H-2^2), 3.59–3.45 (m, 18H, H-$6b^5$, H-3^2, H-3^F, H-2^F, H-3^1, H-4^1, H-$6b^{5'}$, H-$6b^2$, H-4^2, H-4^F, H-5^4, H-$6b^1$, H-2^5, H-$2^{5'}$, H-$5^{4'}$, H-$6b^B$, H-5^1, H-2^B), 3.42–3.33 (m, 6H, H-$6b^4$, H-$6b^{4'}$, H-3^5, H-5^2, H-3^B, H-5^3), 3.32–3.19 (m, 8H, H-$3^{5'}$, H-4^4, H-4^5, H-$4^{4'}$, H-$4^{5'}$, H-5^5, H-$5^{5'}$, H-5^B), 3.06 (dd, $J_{3,4} = J_{4,5} = 9.2$ Hz,1H, H-4^B), 2.76 (t, $J_{vic} = 7.4$ Hz, 2H, εCH_2, AH), 2.08 (t, $J_{vic} = 6.8$ Hz, 2H, αCH_2, AH), 1.90, 1.87, 1.86, 1.85, 1.80 (5s, 15H, CH_3, NAc), 1.45 (m, 2H, δCH_2, AH), 1.40 (m, 2H, βCH_2, AH), 1.15 (m, 2H, γCH_2, AH), 1.01 (d, $J_{5,6} = 6.3$ Hz, 3H, H-6^F); ^{13}C NMR (90.6 MHz, D_2O with DMSO-d_6 as internal standard): $\delta = 178.87$ (C═O, Amide), 176.25, 176.19, 176.08, 176.06, 175.99 (C═O, NAc), 102.64 (C-1^2), 102.21 (C-1^B), 101.73 (C-1^3), 101.54 (C-1^4), 101.47 (C-1^5), 101.06 (C-$1^{5'}$), 101.00 (C-1^F), 99.20 (C-$1^{4'}$), 80.22 (C-4^1), 80.19 (C-4^2), 80.16 (C-3^3), 79.88 (C-1^1), 78.31 (C-5^B), 78.08 (C-2^4), 77.75 (C-$2^{4'}$), 77.42 (C-5^5), 77.32 (C-$5^{5'}$), 76.80 (C-5^1), 76.17 (C-5^2), 75.81 (C-5^3), 75.13 (C-5^4), 75.04 (C-$3^{5'}$), 74.90 (C-$5^{4'}$), 74.57 (C-3^B), 74.21 (C-3^5), 73.59 (C-3^1), 73.43 (C-4^F), 73.41 (C-3^2), 73.24 (C-4^3), 72.69 (C-4^B), 71.89 (C-2^3), 71.47 (C-$4^{5'}$), 71.34 (C-4^5), 71.13 (C-$3^{4'}$), 71.09 (C-3^F), 70.91 (C-3^4), 69.71 (C-2^F), 69.14 (C-$4^{4'}$), 68.95 (C-4^4), 68.41 (C-6^1), 68.37 (C-5^F), 66.99 (C-6^3), 63.50 (C-6^4), 63.41 (C-$6^{4'}$), 63.24 (C-6^B), 62.16 (C-6^5), 62.13 (C-$6^{5'}$), 61.44 (C-6^2), 57.72 (C-2^B), 56.89 (C-2^5), 56.86 (C-$2^{5'}$), 56.72 (C-2^2), 55.39 (C-2^1), 40.80 (C-6, AH), 37.01 (C-2, AH), 28.16 (C-5, AH), 26.59 (C-4, AH), 26.11 (C-3, AH), 24.04, 23.99, 23.96, 23.76, 23.62 (CH_3, NAc), 17.05 (C-6^F).