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Synthesis of a Core-Fucosylated, Biantennary Octasaccharide as a Precursor for Glycopeptides of Complex *N*-Glycans

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Abstract: A convergent synthesis of the biantennary and core-fucosylated octasaccharide **20** (a protected form of **A**) is described. Octasaccharide **20** is designed to serve as a precursor for dodecasaccharide **1**, a complex *N*-glycan frequently found in glycoproteins of the serum and the cell surface. Copyright © 1996 Elsevier Science Ltd

The oligosaccharides present on glycoproteins and glycolipids are participating in the flow of biological information in the organism[1,2]. Many of those effects remain to be understood, especially the relevance of core-fucosylation on *N*-linked oligosaccharides (*N*-glycans). To examine the biological consequences of core-fucosylation, we are interested in comparing the properties of the parent *N*-glycans with their fucosylated analogues. We describe herein the synthesis of octasaccharide **20** as a precursor for core-fucosylated model compounds such as **1**.

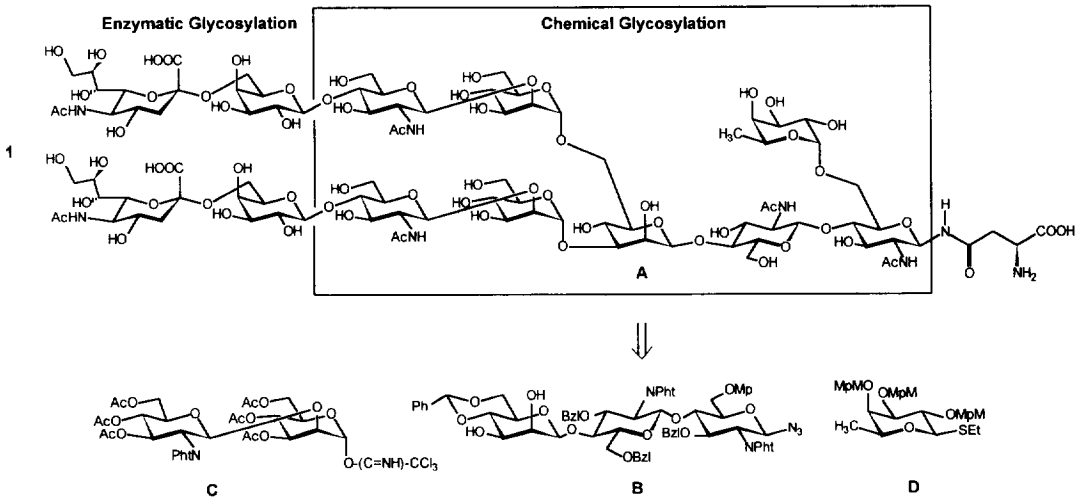

Figure 1: Retrosynthetic analysis of dodecasaccharide-asparagine **1** as a model compound for core-fucosylated *N*-glycans of the complex type. A protected form of compound **A** was synthesized from the building blocks **B**, **C** and **D**.

The synthesis of complex *N*-glycans by classical chemical methods[3,4,5] requires many steps and may lead to severe problems during final deprotection, especially for sialylated compounds. A combination of

chemical and enzymatic glycosylation may reduce the number of synthetic steps and the difficulties related to protective groups. The synthesis of octasaccharide **20** was designed to facilitate subsequent chemoenzymatic elongation[6,7] to dodecasaccharide-asparagine **1**. Retrosynthetic analysis of **A** (Fig. 1) suggested disconnection to core trisaccharide **B**, disaccharide donor **C** for attachment of the antennae and fucosyldonor **D**. Core trisaccharide **B** combines several features: the azido group[8] at the reducing end is maintained throughout the synthesis to allow coupling with an aspartic acid moiety at a desired stage[7]. The benzylidene protected ß-mannoside **B** is suited for facile connection of the side chains in position 3'' and 6'' via double regioselective glycosylation[6]. Furthermore the hydroxyl group at position 4'' becomes accessible for the introduction of a bisecting GlcNAc-moiety[9]. The *O*-6 p-methoxyphenyl group was chosen for the construction of the α-(1→6)-fucosidic linkage at a late stage of the synthesis[4b]. Core trisaccharide **B** was obtained from monosaccharides **3**, **8** and **11**[10a] using the procedure for ß-mannosylation developed by Kunz[11]. Compounds **3**[12] and **8** were both synthesized via thioglycoside **2**[11b].

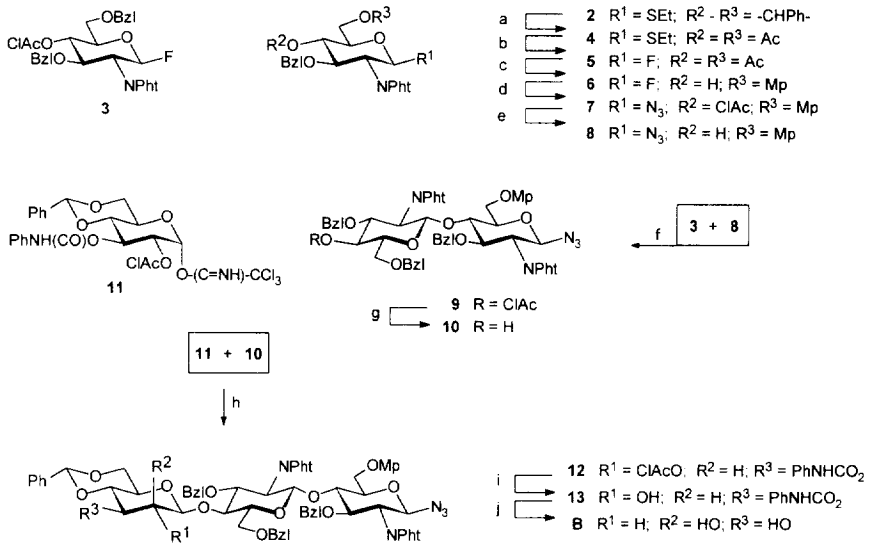

Figure 2: a) 1. p-TosOHxH$_2$O, CH$_3$CN (92 %); 2. pyridine, Ac$_2$O (quantitative); b) HF-pyridine, NBS, CH$_2$Cl$_2$, 0°C (89 %); c) 1. K$_2$CO$_3$, CH$_2$Cl$_2$, MeOH (95 %); 2. p-methoxyphenol, DEAD, Ph$_3$P, CH$_2$Cl$_2$, 0°C (85 %); d) 1. pyridine, (ClAc)$_2$O, CH$_2$Cl$_2$, 0°C (97 %); 2. TMS-N$_3$, BF$_3$-OEt$_2$, molecular sieves 4 Å, CH$_2$Cl$_2$ (91 %); e) K$_2$CO$_3$, CH$_2$Cl$_2$, MeOH (96 %); f) BF$_3$-OEt$_2$, molecular sieves 4 Å, CH$_2$Cl$_2$; g) K$_2$CO$_3$, CH$_2$Cl$_2$, MeOH (68 % **8** →**10**); h) TMSOTf, molecular sieves 4 Å, CH$_2$Cl$_2$; i) K$_2$CO$_3$, CH$_2$Cl$_2$, MeOH (65 % **10** →**13**); j) 1. Tf$_2$O, pyridine, CH$_2$Cl$_2$, -20°C; 2. pyridine, DMF, 60°C; 3. AcOH, dioxane, H$_2$O, 0°C; 4. NaOMe, MeOH, CH$_2$Cl$_2$ (62 % **13** → **B**).

The synthesis of building block **8** (Fig. 2) for the reducing end required special efforts. First, the thioglycoside **2** was converted into fluoride **5** by debenzylidenation and acetylation, followed by fluorination at the anomeric center using Nicolaou's procedure[13]. The crystalline ß-fluoride **5** was deacetylated and the p-methoxyphenyl residue (Mp) was regioselectively introduced at *O*-6 under Mitsunobu[14] conditions yielding **6**. When alternate reaction sequences were used, unexpected side reactions occurred. The most notable are the halogenation of the p-methoxyphenyl group during anomeric fluorination and the hydrolysis of the ß-fluoride when removing the benzylidene acetal by mild acid treatment. Chloroacetylation of **6** and subsequent treatment of the intermediate with trimethylsilyl azide and catalytic amounts of borontrifluoride ether[8] gave ß-azide **7**. Removal of the chloroacetyl group furnished the desired building block **8** in high yield.

With the three monosaccharides **3**, **8** and **11** the assembly of trisaccharide **13** (Fig. 2) was examined. Coupling of glycosyl fluoride **3**[12] with acceptor **8**, using borontrifluoride ether as promotor, followed by a

dechloroacetylation step gave the chitobiosylazide **10** in 68 % yield. The acceptor disaccharide **10** was then treated with the glucosyl imidate **11**[10a] promoted by trimethylsilyl triflate[10b]. To facilitate workup the reaction mixture was dechloroacetylated affording trisaccharide **13** in 65 % yield over both steps. Glucosyldonor **11** introduces two valuable features: a) the benzylidene acetal required for the inversion; b) a 2-chloroacetyl moiety that can be removed from the trisaccharide in the presence of the base-sensitive phthalimido groups in high yield. In analogy to the inversion sequence described earlier[6,11], the ß-gluco-configurated trisaccharide **13** was converted in four steps to the ß-manno-configurated trisaccharide diol **B** in 62 % yield (Fig. 2). First, compound **13** was activated as a triflate and then inverted at C-2'' to a cyclic iminocarbonate by heating in DMF/pyridine (60 °C). Acid hydrolysis gave the cyclic 2'', 3''-carbonate which was removed by mild base treatment furnishing ß-mannosyl trisaccharide **B**.

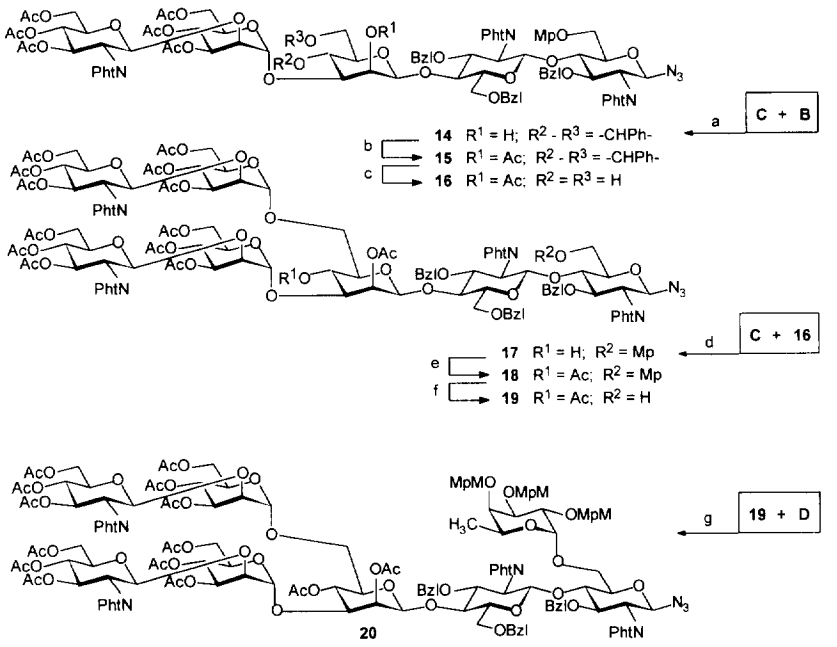

Figure 3: a) BF_3-OEt_2, molecular sieves 4 Å, CH_2Cl_2, -20 °C, (80 %); b) pyridine, Ac_2O (quantitative); c) p-TosOHxH_2O, CH_3CN, (80 %); d) BF_3-OEt_2, molecular sieves 4 Å, CH_2Cl_2, -40 °C, (73 %); e) pyridine, Ac_2O (quantitative); f) CAN, CH_3CN, toluene, H_2O, (90 %); g) $CuBr_2$, Bu_4NBr, molecular sieves 4 Å, DMF, CH_2Cl_2, (85 %); CAN = $(NH_4)_2Ce^{IV}(NO_3)_6$.

As shown previously[6], the equatorial 3''-OH group in benzylidene protected β-mannoside **B** is preferred in glycosylation reactions over the axial 2''-OH function. Accordingly disaccharide donor **C**[6,7] reacted with trisaccharide **B** to the α-(1→3)-linked pentasaccharide **14** in 80 % yield. Prior to further elongation, pentasaccharide **14** was acetylated and then debenzylidenated to give acceptor **16**. Regioselective glycosylation of pentasaccharide diol **16** at the primary hydroxyl group using donor **C** under dilute conditions afforded the α-(1→6)-linked heptasaccharide **17** in 73 % yield. To ensure regioselective core-fucosylation, the remaining hydroxyl function was acetylated and the p-methoxyphenyl group was cleaved with CAN[15] affording heptasaccharide **19** with a free hydroxyl group at position 6^1 in high yield. The p-methoxybenzyl (MPM) substituted thiofucoside **D** was chosen for α-fucosylation[16] because the MPM residues can be selectively removed by oxidation, thus circumventing the difficulties frequently encountered during deprotection of benzylated fucosides. Donor **D** was prepared from L-fucose in four steps[17]. The final

coupling (Fig. 3) of heptasaccharide **19** and thiofucoside **D** activated with $Bu_4NBr/CuBr_2$[18] provided the target octasaccharide **20** in 85 % yield. The structure of **20**[19] was confirmed by 2D-NMR spectroscopy (TOCSY, NOESY, HMQC-DEPT, HMQC-COSY) and FAB-MS.

In conclusion, the presented strategy gives a synthetic access to a core fucosylated *N*-glycan bearing the option for enzymatic elongation and attachment of amino acids at the reducing end[7]. We are at present investigating these reactions to provide probes for biological studies.

Acknowledgements

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10. a) **11** is available in four steps from 3-*O*-(*N*-phenylcarbamoyl)-D-glucopyranose[11b]: 1. $PhCH(OMe)_2$, p-TosOH, CH_3CN; 2. pyridine, $(ClAc)_2O$, CH_2Cl_2, 0°C; 3. N_2H_4xHOAc, DMF, 0°C; 4. Cl_3CCN, DBU, CH_2Cl_2, 0°C; b) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem* **1994**, *50*, 21-123.
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12. Fluoride **3** was prepared from compound **2** in three steps: 1. $Na(CN)BH_3$, molecular sieves 4Å, THF, 0°C (86 %); 2. pyridine, $(ClAc)_2O$, CH_2Cl_2, 0°C (95%); 3. HF-pyridine, NBS, CH_2Cl_2 0°C, (91 %).
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19. **20:** FAB-MS [3-nitrobenzylalcohol]: $C_{153}H_{163}N_7O_{60}$ M_r (calcd) 3058.0; M_r (found) 3081 (M+Na).
^{1}H-NMR (600 MHz, d_6-DMSO): δ 5.42 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1^2ß), 5.27 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1^5ß), 5.18 (d, 1H, $J_{1,2}$ = 9.0 Hz, H-1ß), 5.16 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-$1^{5'}$ß), 4.78 (d, $J_{1,2}$ < 1.0 Hz, 1H, H-1^3), 4.58 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1^{Fuc}α), 4.56 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1^4), 4.28 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-$1^{4'}$α), 3.74, 3.73, 3.68 (3s, 9H, OMe), 2.25, 2.03, 1.98, 1.97, 1.94, 1.92, 1.91, 1.83, 1.79, 1.75 (10s, 42 H, OAc).
^{13}C-NMR (125 MHz, d_6-DMSO): δ 97.82 C-1^4α ($J_{C,H}$ = 174.3 Hz), 97.40 C-1^3β ($J_{C,H}$ = 165.1 Hz), 97.08 C-$1^{4'}$α ($J_{C,H}$ = 173.6Hz), 96.66 C-1^{Fuc}, 96.40 C-1^2, 96.16 C-$1^{5'}$, 96.12 C-1^5, 84.42 C-1^1.

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