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Synthesis of a LEC14 nonasaccharide, a core-fucosylated, biantennary *N*-glycan with a novel GlcNAc residue in the core region[†,‡]

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Abstract

The recently found core substitution of *N*-glycans termed LEC14 is characterized by a GlcNAc residue linked β(1,2) to the central β-mannoside. Starting from a pentasaccharide building block functionalized for core-fucosylated *N*-glycans the total synthesis of a protected LEC14 nonasaccharide was accomplished. The key step of the synthesis was the introduction of the additional β(1,2)-linked GlcNAc residue that was highly dependent on the solvent and appears to proceed via an amide acetal intermediate.

Keywords: glycosylation; oligosaccharide; *N*-glycan; LEC14; amide acetal.

Glycoproteins play an important role in higher organisms and subtle changes of the carbohydrate moieties may result in a completely altered functionality of the glycoprotein. Thus, protein oligosaccharides and especially asparagine linked *N*-glycans have been subject to intensive investigations.[1] All *N*-glycans have a $Man_3GlcNAc_2$ core pentasaccharide in common, which can be modified by the addition of single sugar residues. Frequently found motifs[2] are fucose bound to the reducing GlcNAc in α(1,6)- or α(1,3)-linkage, xylose β(1,2)-linked to the β-mannosyl residue, or the addition of a β(1,4)-linked 'bisecting' GlcNAc at the β-mannnoside. Recently, a new glycosylation pattern of the core pentasaccharide termed LEC14 was found in dominant mutants of Chinese hamster ovary cells (CHO-cells) by the group of Pamela Stanley.[3] The unusual residue in LEC14 is an additional GlcNAc β(1,2)-linked to the central β-mannoside, the effective glycosyl transferase was characterized and termed GlcNAc-TVII.[3b] A

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chemical synthesis of LEC14 may help to generate antibodies and facilitate to study the influence of this unusual GlcNAc-residue on the biological properties of *N*-glycans.

The synthesis of complex *N*-glycans by classical chemical methods requires many steps and may be particularly challenging during the final deprotection.[4] Thus we have developed a chemoenzymatic approach, which has been used successfully in the synthesis of sialylated *N*-glycans and their conjugates.[5] We describe herein the synthesis of nonasaccharide **A** as a suitable precursor for the subsequent chemoenzymatic elongation to complex *N*-glycans of the LEC14 type.

Retrosynthetic analysis of **A** (Fig. 1) suggested disconnection to pentasaccharide **1**,[6] a compound originally developed for the synthesis of core-fucosylated *N*-glycans. Regio- and stereoselective glycosylation of a core trisaccharide 2″,3″ diol gave pentasaccharide **1** bearing the desired 2″-OH function. It was envisioned to use fluoride **2** for the glycosylation of the axial hydroxyl group of the pentasaccharide. Fluoride **2** showed favorable stability under glycosylation conditions leading to *N*-glycans of the bisecting type[7] where strong steric hindrance needed to be overcome. Pentasaccharide **1** is functionalized for selective attachment of disaccharide imidate **3** as the 1,6-arm and for fucosylation at the reducing GlcNAc. Furthermore, the azido group at the reducing end allows convenient coupling to aspartic acid[5c] or linkers.[6b]

Pentasaccharide **1** was prepared as described.[6] Initial attempts to glycosylate pentasaccharide **1** using fluoride **2** (Fig. 2) were conducted in analogy to the conditions developed for 'bisecting' *N*-glycans (10 equivalents of donor **2**, dichloromethane, 1 equiv. BF_3–Et_2O).[7] Below a temperature of –25°C) no reaction was observed. The temperature optimum was found between –15 and –20°C. Under these conditions the acceptor **2** was consumed within 15 minutes. However, when the reaction mixture was allowed to attain room temperature, TLC revealed that the newly formed product spot was slowly disappearing upon warming. After workup only the acceptor **2** and hydrolyzed donor were detected.

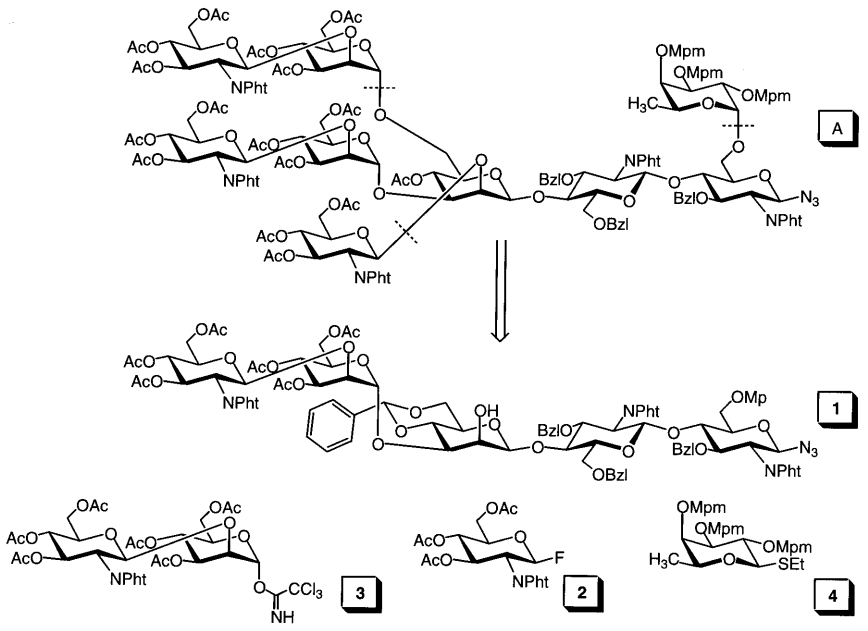

Figure 1. Building blocks employed for the synthesis of LEC14 nonasaccharide **A**. Mp = *p*-methoxyphenyl, MPM = *p*-methoxybenzyl

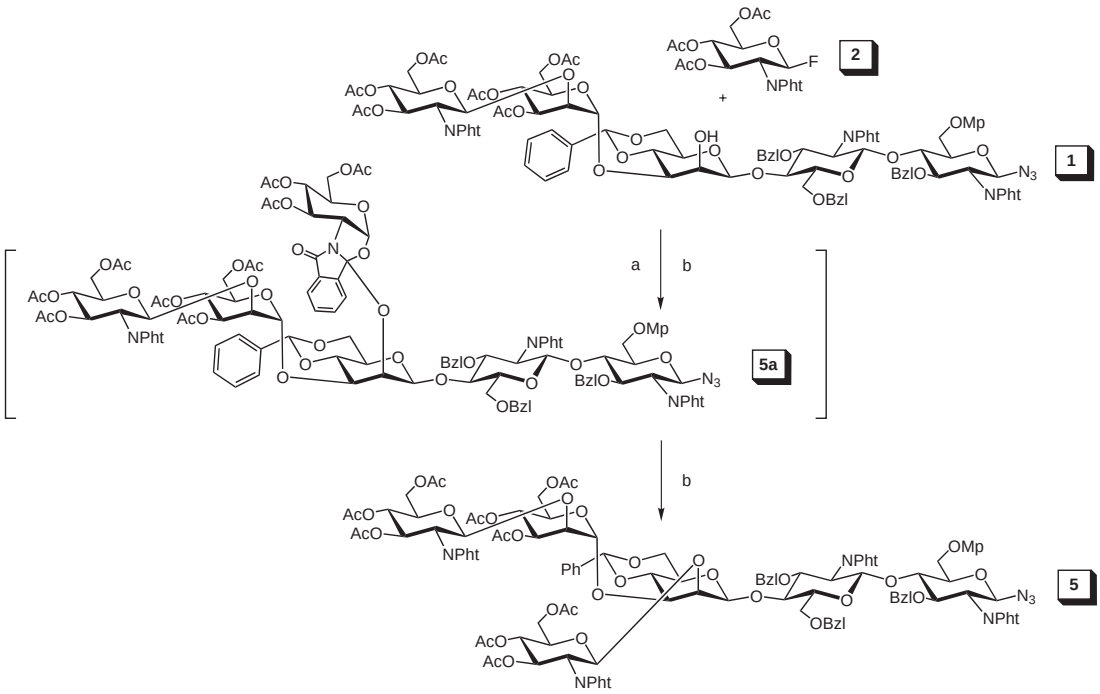

Figure 2. (a) CH_2Cl_2, 1 equiv. BF_3–OEt_2; (b) CH_3CN, 3 equiv. BF_3–OEt_2, −20°C, (78%)

Acid labile glycosylation products are known from the formation of orthoesters when neighboring group participation is employed.[8] Orthoesters may rearrange to glycosides under acidic reaction conditions. We thus attempted to rearrange the labile glycosylation intermediate using two additional equivalents of borontrifluoride etherate for activation. This gave the desired hexasaccharide **5**, albeit in low yield. A significant improvement of the yields was finally achieved when acetonitrile was applied as an alternate solvent. By stepwise optimization of the reaction conditions, mainly to avoid side reactions (3 equiv. BF_3–Et_2O, acetonitrile, −20°C), the isolated yield of the LEC14 hexasaccharide **5** could be raised to 78%. Careful TLC analysis showed that even in acetonitrile the labile intermediate was formed first and then disappeared giving rise to the hexasaccharide **5**. These observations would be consistent with the formation and subsequent rearrangement of an intermediate amide acetal[9] as a labile intermediate. We thus propose the occurrence of an intermediate imide acetal **5a** as an aza-analog of a glycosyl orthoester in the course of the glycosylation reaction of **1** using the phthalimido substituted donor **2**.

With hexasaccharide **5** in hands the following course of the synthesis was straight forward (Fig. 3). Debenzylidenation of **5** gave hexasaccharide diol **6**, which was regioselectively glycosylated using donor **3** under dilute conditions to afford the α(1,6)-linked octasaccharide **7**. To ensure regioselective core-fucosylation, the remaining 4″-hydroxyl substituent was acetylated and the *p*-methoxyphenyl group was oxidatively cleaved affording octasaccharide **9** with a single hydroxyl group in high yield. The final coupling of octasaccharide **9** and thiofucoside **4**[10] activated with $Bu_4NBr/CuBr_2$[11] provided the target nonasaccharide **A** in 82% yield. The structure of **A**[12] was confirmed by 2D-NMR spectroscopy[13] (TOCSY, NOESY, HMQC-DEPT, HMCQ-COSY) and ESI-MS.

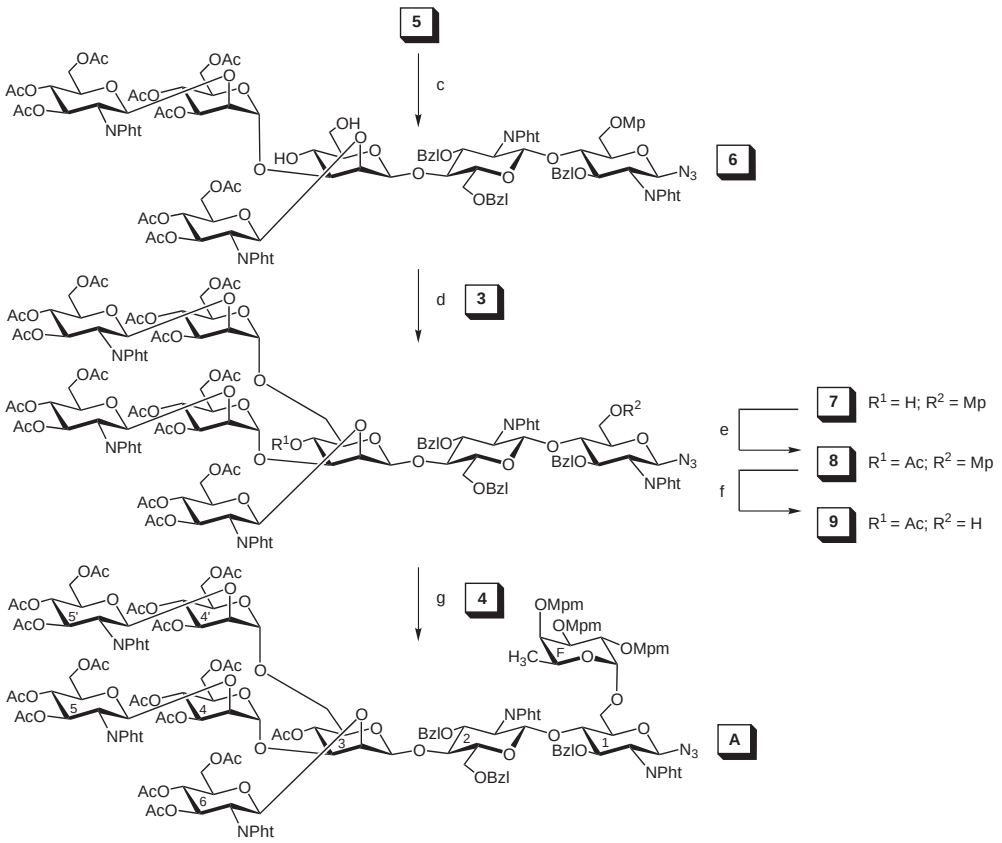

Figure 3. (c) CH_3CN, *p*-TosOH*x*H_2O, (89%); (d) **3**, CH_2Cl_2, BF_3–OEt_2, −40°C, (76%); (e) pyridine, Ac_2O, (quant.); (f) CH_3CN, toluene, H_2O, $(NH_4)_2Ce(NO_3)_6$, (94%); (g) **4**, DMF, CH_2Cl_2, $CuBr_2$, Bu_4NBr, 3 days, (82%)

In conclusion, we have developed an efficient strategy based on a system of modular building blocks to access the rare LEC14 variations of core-fucosylated *N*-glycans in substantial amounts. Work to deprotect the final compound **A** and convert it into novel probes for glycobiology[14] is in progress.

Acknowledgements

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13. ESI-MS (methanol): $C_{171}H_{180}N_8O_{68}$ M_r (calcd) 3433.09 M_r (found) 3456.51 $(M+Na)^+$; $[\alpha]_D^{22} = -4.05$ ($c = 0.5$, CH_2Cl_2); ^1H NMR (500 MHz, [D_6] DMSO): $\delta = 8.02–6.48$ (m, 47H, Ar), 5.77–5.66 (m, 2H, H-3^6, H-$3^{5'}$), 5.62 (dd, 1H, H-3^5), 5.53 (d, $J_{1,2} = 7.80$ Hz, 1H, H-1^6), 5.33 (d, $J_{1,2} = 7.80$ Hz, 1H, H-1^2), 5.29 (d, $J_{1,2} = 7.80$ Hz, 1H, H-1^5), 5.24 (d, $J_{1,2} = 8.80$ Hz, 1H, H-$1^{5'}$), 5.21 (d, $J_{1,2} = 8.80$ Hz, 1H, H-1^1), 5.10–4.94 (m, 3H, H-4^5, H-4^4, H-$4^{5'}$), 4.91 (m, 1H, H-3^4), 4.88–4.62 (m, 9H, H-4^6, H-$4^{4'}$, CH_2O, H-4^3, H-1^F, H-1^3), 4.62 (m, 6H, H-$3^{4'}$, CH_2O, H-1^4), 4.42–4.23 (m, 3H, CH_2O, H-$6a^5$), 2.92 (m, 1H, H-$5^{4'}$), 2.79–2.66 (m, 2H, H-$6a^3$, H-$6b^3$), 2.25–1.71 (16s, 48H, OAc), 1.01 (d, $J_{5,6} = 5.90$ Hz, 3H, H-6^F). ^{13}C NMR (125 MHz, [D_6] DMSO): $\delta = 170.18–168.37$ (C=O), 101.07 (C-1^3), 98.59 (C-1^4), 97.38 (C-1^F), 97.24 (C-$1^{4'}$), 96.93 (C-1^7), 96.29 (C-1^2), 96.18 (C-1^5), 96.06 (C-$1^{5'}$), 84.52 (C-1^1), 20.45–19.91 (16 OAc), 16.28 (C-6^F). Compound **5**: ^{13}C NMR (125 MHz, [D_6] DMSO): $\delta = 97.50$ ($J_{C\text{-}1,H\text{-}1} = 168.84$ Hz, C-1^7 β).
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