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Synthesis of an *N*-glycan decasaccharide of the hybrid type

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Abstract—A hybrid-type *N*-glycan decasaccharide $GlcNAcMan_7GlcNAc_2$ was synthesized from the pentasaccharide $GlcNAcMan_2GlcNAc_2$ as an advanced intermediate and an acyl-protected pentamannosyl donor. Benzyl mannoside was regioselectively benzoylated and glycosylated at OH-3 and OH-6 with a dimannoside to give the 3,6-branched pentamannoside. Coupling of the two pentasaccharides furnished the target decasaccharide in 60% yield. Deprotection of the base labile functions furnished a hybrid-type *N*-glycan decasaccharide functionalized for the conjugation with peptides or proteins.

The sugar moieties of glycoproteins are responsible for many of their biological and physicochemical properties.[1] *O*-Glycans are oligosaccharides from glycoproteins linked to the hydroxyl groups of serine or threonine whereas *N*-glycans are bound to the amide nitrogen of the side chain of asparagine. *N*-glycans share a common pentasaccharide core structure ($Man_3GlcNAc_2$) and can be distinguished according to their carbohydrate extensions into high-mannose, complex and hybrid type.[2] Hybrid-type *N*-glycans were found to be essential in neuronal development[3] and also to be associated with tumor glycoproteins.[4] By use of an engineered strain of *P. pastoris* [5] glycoproteins can be obtained carrying predominantly mammalian-like hybrid-type *N*-glycans. Recently, a first chemical synthesis of a hybrid-type *N*-glycan linked to a peptide was published.[6] We have previously developed efficient chemical and enzymatic syntheses for a series of *N*-glycans of the complex-type including the incorporation of core-fucose or bisecting residues.[7] The resulting modular system of building blocks was successfully extended to yield hybrid-type *N*-glycans (**D**). Retrosynthetic analysis of the decasaccharide **D** led to pentasaccharide **A**,[8] dimannosyl donor **B** and selectively acylated benzylmannoside **C** (Fig. 1).

It was planned to incorporate the oligomannosyl part of the hybrid-type *N*-glycan as a single pentamannoside building block (**5**, Fig. 2). For the synthesis of the required branched pentamannoside highly diverse approaches were reported.[9] However, nearly all of these strategies utilize permanent benzyl-type protective groups, which may lead to difficulties in the final deprotection steps. In order to establish a synthetic access to a pentasaccharide donor bearing exclusively acyl protection we focussed on the disaccharide **B** and the appropriately protected acceptor **C** as key building blocks. The peracetylated dimannoside **1** was obtained according to the published procedure[10] and subsequently converted to the corresponding ethylthioglycoside **B** by activation with tin(IV) chloride (Fig. 2).

For the synthesis of the desired pentamannoside a building block for the central mannoside with unprotected 3,6-OH-groups was prepared. Kong and co-workers[9f] used a 1,2-ethylidene mannose building block, which requires the conversion into a donor and additional protection of the free hydroxy functions after the glycosylation step. In an earlier publication[9e] a four-step reaction sequence was applied to obtain the anomeric allyl derivative of the desired acceptor **C**. To shorten this route we envisioned the orthoester-isomerization approach[11] as suggested by Oscarsson and Svahnberg.[12] The selective protection was carried out by reacting α-benzylmannoside[13] with trimethylorthobenzoate in the presence of an acid catalyst. In a one-pot procedure the intermediate bis-orthobenzoate was hydrolyzed in aqueous TFA to give the 2,4-dibenzoyl-protected acceptor **C** (48%), which could easily be separated from the 2,6-isomer (37%) by column chromatography. The analogous reaction was also performed with triethylorthoacetate, however, the separation of the resulting isomeric acetates by flash chromatography was not possible. When reacting the acceptor **C** with three

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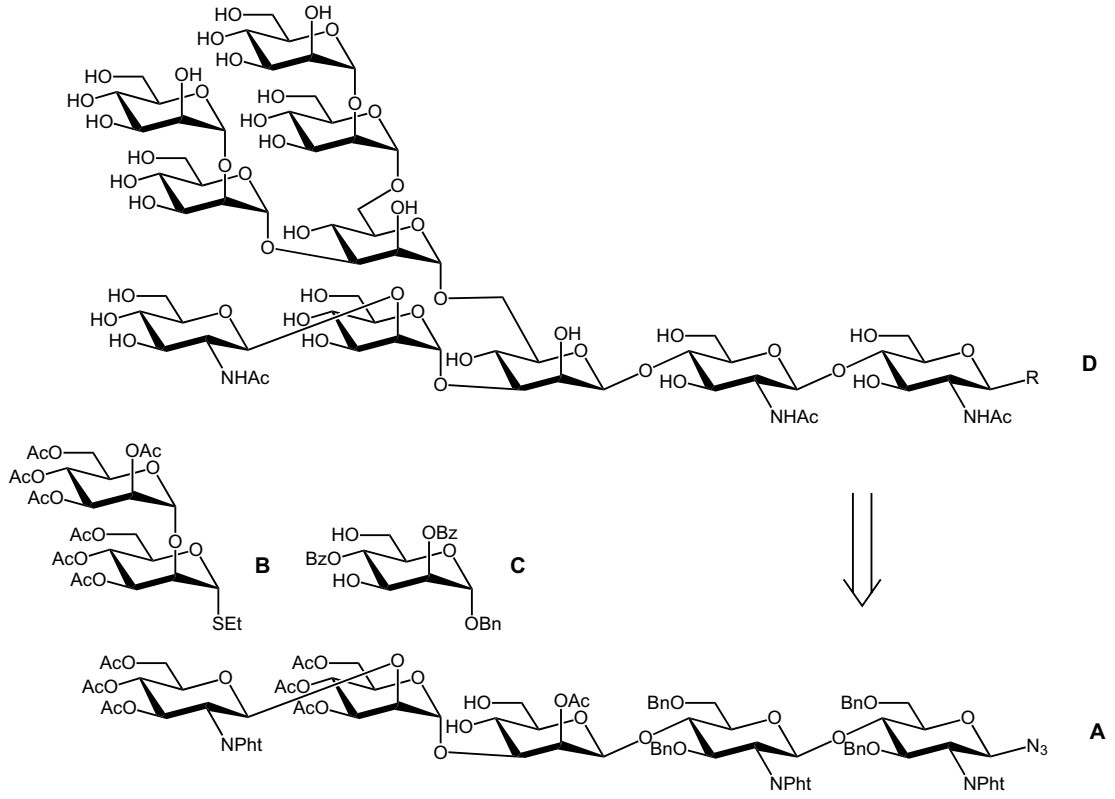

Figure 1. Building blocks employed for the synthesis of the hybrid decasaccharide **D**.

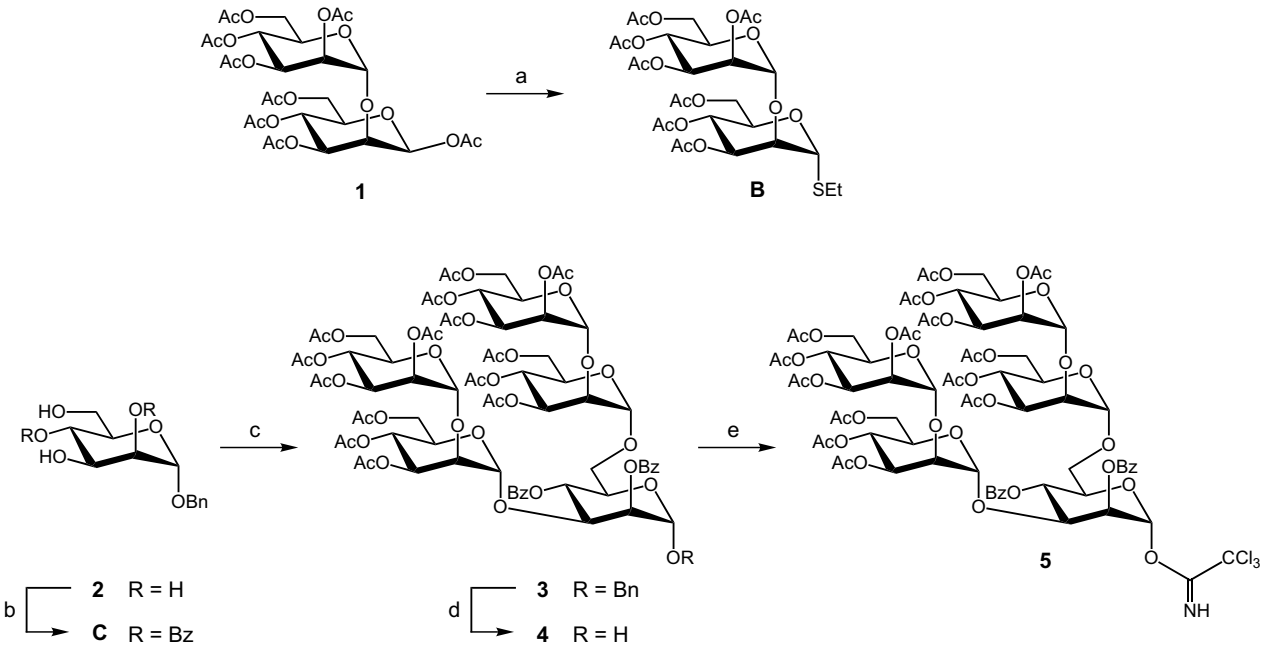

Figure 2. Reagents and conditions: (a) EtSH, $SnCl_4$, CH_2Cl_2, (68%); (b) (1) $PhC(OMe)_3$, TFA, MeCN; (2) 80% TFA in H_2O, [(1)–(2) 48%]; (c) **B**, NIS, TfOH, molecular sieves 4 Å, CH_2Cl_2, −10 °C (65%); (d) H_2, $PdOxH_2O$, MeOH, AcOH (63%); (e) DBU, Cl_3CCN, CH_2Cl_2, 0 °C (88%).

equivalents of donor **B** both hydroxyl functions were elongated with a disaccharide leading to the desired pentamannoside **3** in 65% yield. The benzyl protecting group at the anomeric position of **3** was removed by hydrogenolysis and the resulting hemiacetal was converted to the trichloroacetimidate **5** by addition of trichloroacetonitrile and DBU (Fig. 3).

With donor **5** in hands we investigated the final coupling step of the two pentasaccharides **A** and **5**. Using 2

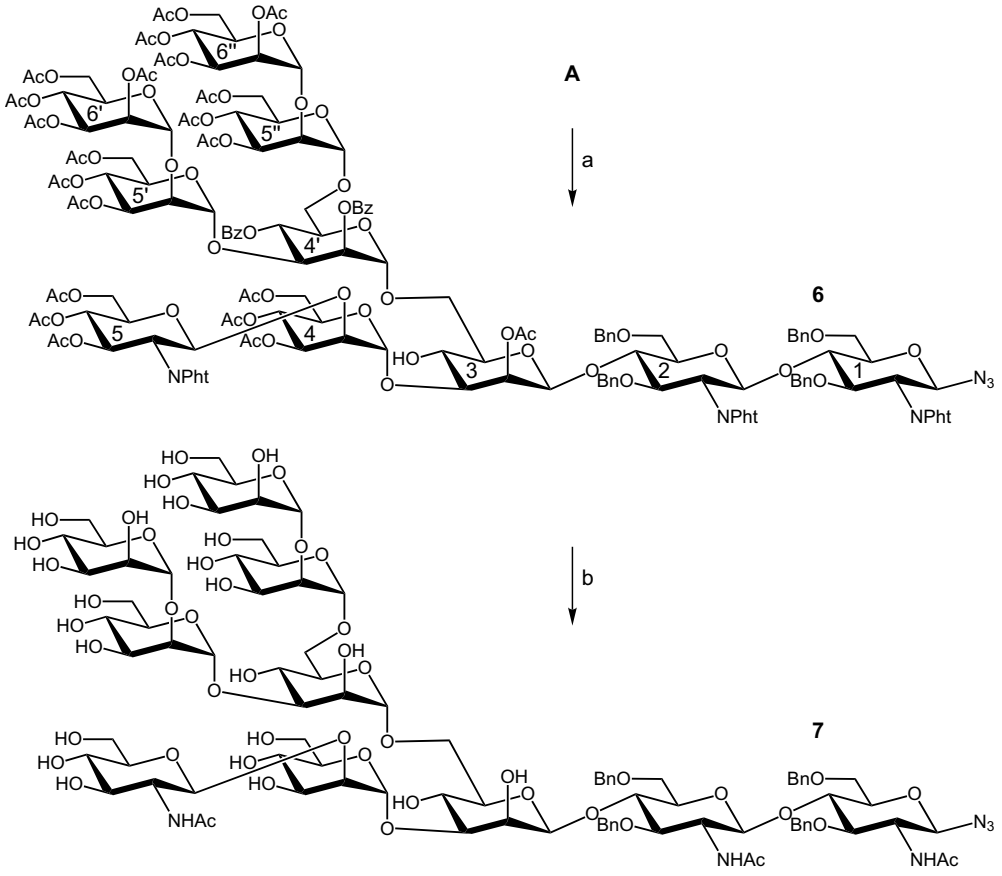

Figure 3. Reagents and conditions: (a) **5**, BF_3–OEt_2, molecular sieves 4 Å, CH_2Cl_2; −45 °C (60%); (b) (1) ethylenediamine, *n*-BuOH, 90 °C, 48 h; (2) Ac_2O, pyridine; (3) $MeNH_2$ (41% in H_2O) [(1)–(3) 78%].

equivalents of donor **5** at −45 °C in dichloromethane and BF_3–OEt_2 as an activator, the decasaccharide **6** was obtained in a yield of 60% without optimization. The structure of the hybrid-type *N*-glycan **6** was confirmed by 2D NMR spectroscopy (TOCSY, NOESY, HMBC, HMQC-COSY, HMQC-TOCSY)[14] and ESI-MS.[15] Cleavage of the esters and phthalimido groups was performed by heating the oligosaccharide **6** with ethylene diamine in *n*-butanol[16] and was monitored by HPLC/ESI-TOF-MS. It was found that after 24 h of deprotection about one third of the oligosaccharide still carried one benzoyl group, presumably linked to the sterically less accessible O-4 of the 4'-mannose residue. After heating for an additional 24 h the deprotection went to completion. The decasaccharide was then acetylated with acetic anhydride/pyridine and the O-acetates were subsequently cleaved by addition of methylamine (41% in water). The deprotected decasaccharide was purified by adsorption on a SepPak-C-18-cartridge and elution with a step gradient of acetonitrile in water furnishing the target molecule **7**, in 78% yield over the three steps. The decasaccharide **7** displays a hybrid-type *N*-glycan (**D**) functionalized with an anomeric azido group, which allows incorporation of the *N*-glycan into glycopeptides and other glycoconjugates.[17]

In summary a rapid access was developed to *N*-glycans of the hybrid-type following a modular approach. The key building block for the oligomannosyl part displaying only ester protection was obtained by a short synthesis and was efficiently coupled to give the target decasaccharide suitable for studies in glycobiology.

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15. Compound **6**: ESI-MS (acetonitrile, 0.1% formic acid): $C_{168}H_{184}N_6O_{76}$ M_r (calcd) 3501.1, M_r (found) 3524.3 $(M+Na)^+$; $[\alpha]_D^{24}$ +0.25 (*c* 2, CH_2Cl_2); ^1H NMR (500 MHz, DMSO-d_6): δ = 8.00–7.48 (m, 22H, Pht, Bz), 7.25–6.57 (m, 20H, Bn), 5.73–5.63 (m, 2H, H-3^5, H-$4^{4'}$), 5.57 (d, $J_{OH,H-4}$ = 5.9 Hz, 1H, OH), 5.47 (dd, $J_{1,2}$ < 1 Hz, $J_{2,3}$ < 1 Hz, 1H, H-$2^{4'}$), 5.36 (d, $J_{1,2}$ = 8.2 Hz, 1H, H-1^5), 5.29–4.84 (m, 19H, H-1^1, H$1^{5'}$, H-$3^{5''}$, H-$3^{6''}$, H-$3^{5'''}$, H$1^{4'}$, H-1^2, H-$2^{6''}$, H-$4^{6''}$, H-4^5, H-$3^{6'}$, H-2^3, H-$4^{5'}$, H-4^4, H$1^{6''}$, H-$4^{6'}$, H$1^{5''}$, H-$2^{6'}$, H-1^4), 4.81–4.75 (m, 3H, H-$3^{5'}$, CH_2Oa, H-3^4), 4.70 (d, $J_{1,2}$ < 1 Hz, 1H, H-1^3), 4.58 (d, J_{gem} = 13 Hz, 1H, CH_2Oa′), 4.50–4.36 (m, 7H, H-$3^{4'}$, 4 × CH_2O, H$1^{6'}$, CH_2Ob′), 4.34–4.12 (m, 4H, CH_2Ob, H-2^4, H-6a^5, H-2^5), 4.10–3.43 (m, 33H, H-3^1, H-$2^{5''}$, H-$5^{6''}$, H-6b^5, H-3^2, H-6a/b^4, H-6a/b$^{5'}$, H-6a/b$^{5''}$, H-6a/b$^{6'}$, H-6a/b$^{6''}$ H-4^1, H-4^2, H-5^5, H-2^2, H-$5^{5''}$, H-$5^{6'}$, H-$5^{5'}$, H-5^4, H-6a$^{4'}$, H-6b$^{4'}$, H-3^3, H-2^1, H-$5^{4'}$, H-4^3, H-6a^2, H-$2^{5'}$, H-5^1, H-6b^2), 3.40–3.15 (m, 6H, H-6a^1, H-6a^3, H-5^3, H-6b^1, H-6b^3, H-5^2), 2.1–1.7 (21s, 63H, Me), ^{13}C NMR (125 MHz, DMSO-d_6): δ = 170.2–169.2 (21 C═OOAc), 167.6, 167.2 (2C, C═ONPht), 165.0, 164.8 (C═OBz), 138.1, 138.0 (2C), 137.7 (C_qBn), 135.0–133.6 (C4/5Pht, C-4Bz), 130.8–130.5 (C_qPht), 129.5–128.7 (Ar), 128.6, 128.5 (C_qBz), 128.3–127.3 (Ar), 123.6 (C3/6Pht), 99.2 (C-$1^{5'}$), 98.0 (C-$1^{6''}$), 97.9 (C-1^4), 97.8 (C-$1^{6'}$), 97.6 (C-$1^{3''}$), 97.5 (C-$1^{5''}$), 97.1 (C-$1^{4'}$), 96.5 (C-1^2), 96.1 (C-1^5), 84.8 (C-1^1), 77.6 (C-3^3), 76.4 (C-3^1), 76.1 (C-$2^{5''}$), 75.7–75.5 (5C, C-3^2, C-$2^{5'}$, C-5^1, C-4^1, C-4^2), 75.3 (C-$3^{4'}$), 75.2 (C-5^3), 74.1 (C-5^2), 73.8, 73.6 (CH_2O), 73.5 (C-2^4), 72.2, 71.7 (CH_2O), 71.3 (C-$2^{4'}$), 71.1 (C-5^5), 69.7 (2C, C-3^5, C-$3^{5''}$), 68.8–68.4 (11C, C-3^4, C-$2^{6''}$, C-$3^{5'}$, C-$5^{5''}$, C-$5^{4'}$, C-4^5, C-2^3, C-$5^{6'}$, C-5^4, C-$2^{6'}$, C-$5^{6''}$), 68.2 (C-$5^{5'}$), 68.1 (C-$3^{6'}$), 67.9 (C-$3^{6''}$), 67.8 (C-$4^{4'}$), 67.7 (C-6^2), 67.5 (2C, C-6^1, C-6^3), 66.3 (C-4^3), 65.8 (C-$6^{4'}$), 65.5 (C-$4^{6''}$), 65.3 (C-$4^{5'}$), 65.0 (C-$4^{6'}$), 64.8 (C-$4^{5''}$), 64.7 (C-4^4), 55.9 (C-2^2), 54.6 (C-2^1), 53.8 (C-2^5), 61.8–61.1 (6C, C-6^4, C-6^5, C-$6^{5'}$, C-$6^{5''}$, C-$6^{6'}$, C-$6^{6''}$), 20.7–20.0 (21C, Me).
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