Carbohydrates

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Synthesis of Pentaantennary N-Glycans with Bisecting GlcNAc and Core Fucose**

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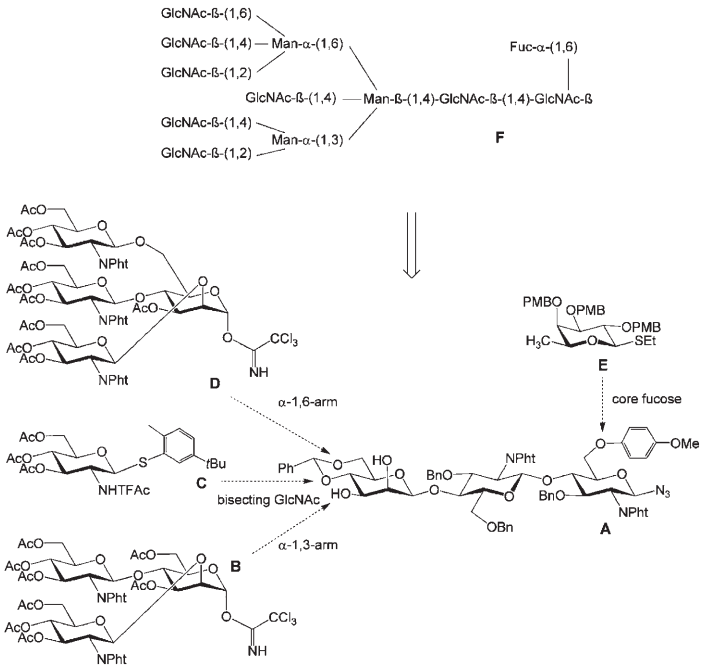
Dedicated to Professor Joachim Thiem on the occasion of his 65th birthday

Recombinant therapeutic glycoproteins contain mainly asparagine-linked oligosaccharides (N-glycans), which are often essential for the proper function of the glycoprotein. The heterogeneity of the glycans in natural glycoproteins is a large obstacle for the entire research in the field of glycobiology,[1] and despite recent advances in chemical synthesis of N-glycans,[2–10] the majority of N-glycans for biological studies are still isolated from natural sources.[11] We have developed modular building blocks for the most abundant complex N-glycans,[12] which after fortuitous results from the test of this modular system allowed the synthesis of complex N-glycans with the maximum number of branches and core substitutions (glycan **F**; Scheme 1).

Previously we have developed modular building blocks for the synthesis of complex N-glycans with up to four antennae,[12] which contained a bisecting GlcNAc moiety[4,13,14] or a core fucose moiety.[15] As a result of the steric hindrance, bisected N-glycans with three or four antennae are especially difficult to obtain.[4] Encouraged by recent improvements[14] we investigated the complex N-glycan **F** (Scheme 1). A particularly high substitution pattern is found at the Manα1,6Manβ unit of **F** where each mannose contains a total of four glycosidic partners. Pentaantennary N-glycans are found in ovomucoid,[16] fish hyosophorin,[17] CHO cells,[18] and HepG2 cells.[19]



Scheme 1. Retrosynthesis of pentaantennary N-glycans with bisecting GlcNAc and core fucose groups. Ac = acetyl, Bn = benzyl, Pht = phthalimido, PMB = *p*-methoxybenzyl, TFAc = trifluoroacetyl.

First, unsubstituted pentaantennary N-glycans were assembled to reduce the synthetic complexity of **F**. The synthesis of tetrasaccharide donor **D** began with the 3-O-allylation of benzylmannoside (**2**) via a stannylene acetal to give **3** (Scheme 2).[20,21] Threefold glycosylation of triol **3** with donor **1** (6 equiv) gave the tetrasaccharide **4** (77%). After deallylation of **4**, the acetylation of alcohol **5** required catalytic amounts of DMAP. After catalytic hydrogenation of **6** to remove the benzyl group, the hemiacetal was converted into imidate **D** and coupled with the hexasaccharide **12** to give the decasaccharide **13** in 65% yield after optimization (Scheme 3).

The high reactivity of donor **D** prompted us to incorporate a bisecting GlcNAc moiety and a core fucose residue. Thus, the branched trisaccharide **B**[12] was coupled to the core trisaccharide **A** (Scheme 4).[15] The resulting hexasaccharide (80%) was acetylated and the benzylidene acetal was cleaved (75% over 2 steps). After the selective chloroacetylation of **14**, the hexasaccharide **15** was coupled with thioglycoside **C**[4] to give the bisected heptasaccharide **16**. Dechloroacetylation yielded the acceptor **17**, which was coupled with the disaccharide **18** (77%). The nonasaccharide **19** was deprotected and fucosylated to give the triantennary decasaccharide **21** in 93% yield.

Next we investigated the coupling of the heptasaccharide **17** with the donor tetrasaccharide **D** (5 equiv; Scheme 5). After purification by flash chromatography and HPLC, only 9% of the bisected pentaantennary compound **22** was obtained. To evaluate the activation using thioglycosides, imidate **D** was converted into the thioglycoside **8**. Reaction of the donor **8** (3.3 equiv) with the acceptor **17** gave the product

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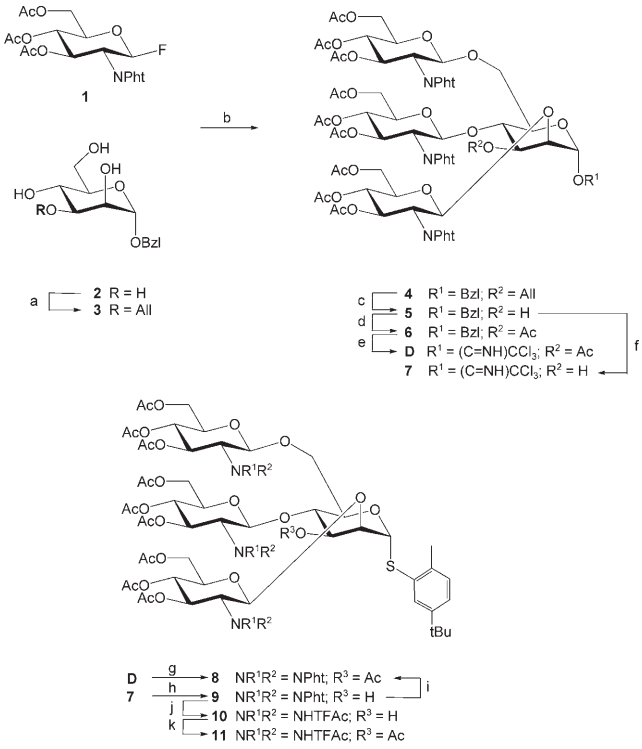
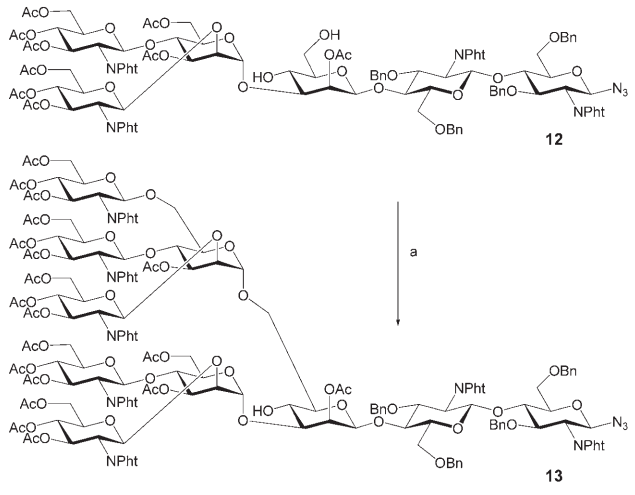
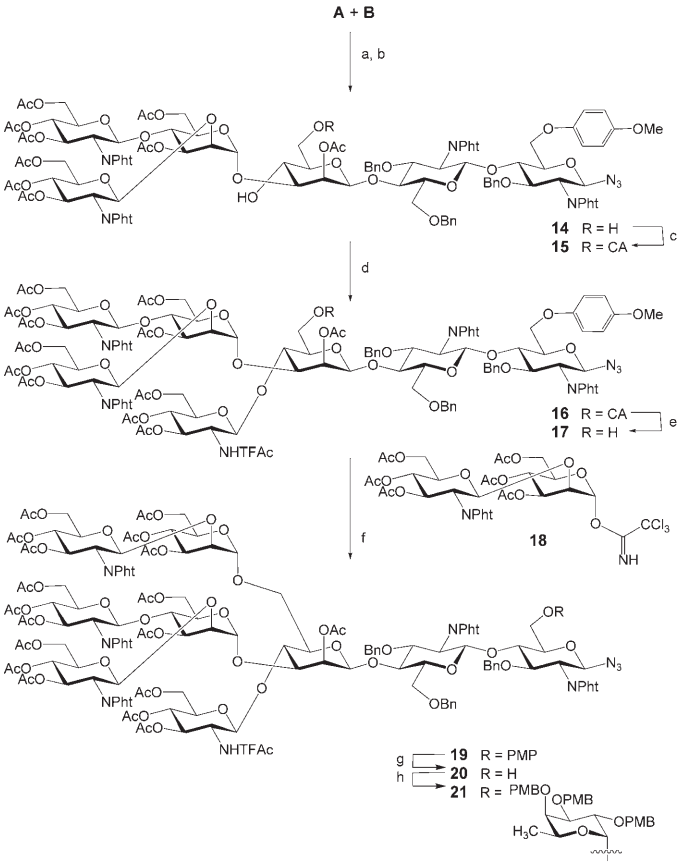


Scheme 2. a) 1. Bu_2SnO, MeOH, reflux; 2. AllBr, CsF, DMF, (1.–2., 70%); b) $BF_3{\cdot}OEt_2$, CH_2Cl_2, −10°C, MS (4 Å), 77%; c) $PdCl_2$, MeOH, 77%; d) Ac_2O, pyridine, DMAP, 81%; e) 1. H_2, $PdO{\cdot}H_2O$, AcOH, MeOH, 82%; 2. Cl_3CCN, DBU, CH_2Cl_2, 0°C, 85%; f) 1. H_2, $PdO{\cdot}H_2O$, AcOH, MeOH, 77%; 2. Cl_3CCN, K_2CO_3, CH_2Cl_2, 82%; g) 5-*tert*-butyl-2-methyl-thiophenol, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0°C, MS (4 Å), 69%; h) 5-*tert*-butyl-2-methyl-thiophenol, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −10°C, MS (4 Å), 86%; i) Ac_2O, pyridine, DMAP, 58%; j) 1. ethylenediamine, *n*BuOH, 80°C, 68%; 2. PfpOTFAc, NEt_3, THF; 3. Ac_2O, pyridine, (2.–3., 63%); k) Ac_2O, pyridine, 84%. All = allyl, DBU = 1,8-diazabicyclo[5.4.0]-undec(7)ene, DMAP = 4-dimethylamino-pyridine, MS = molecular sieves, Pfp = pentafluorophenyl, THF = tetrahydrofuran.



Scheme 3. a) **D**, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −20°C, MS (4 Å), 65%.

in 16% yield after preparative HPLC. However, analytical HPLC-MS showed the product to be a 1:1 mixture of the undecasaccharide **22** and a second undecasaccharide, which

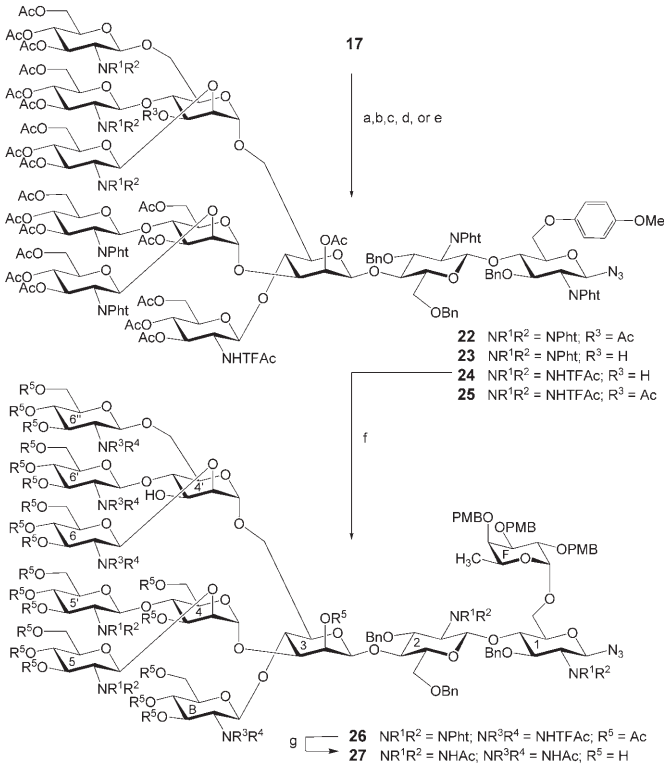


Scheme 4. a) **A**, **B**, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −25°C, MS (4 Å), 80%; b) 1. Ac_2O, pyridine; 2. *p*TosOH·H_2O, CH_3CN, (1.–2., 75%); c) chloroacetic anhydride, pyridine, CH_2Cl_2, 0°C, 77%; d) **C**, NIS, TfOH, CH_2Cl_2, −30°C, MS (4 Å), 61%; e) K_2CO_3, CH_2Cl_2/MeOH (10:1), −10°C, 84%; f) $BF_3{\cdot}OEt_2$, CH_2Cl_2, −25°C, MS (4 Å), 77%; g) CAN, CH_3CN, toluene, H_2O, 89%; h) **E**, $CuBr_2$, Bu_4NBr, CH_2Cl_2, DMF, 93%. CA = chloroacetyl, CAN = ceric ammonium nitrate, DMF = *N*,*N*-dimethylformamide, NIS = *N*-iodosuccinimide, PMP = *p*-methoxyphenyl, TfOH = trifluormethanesulfonic acid, Tos = toluene-4-sulfonyl.

lacked an acetyl group. Since the acceptor **17** was pure, we examined the donor **8** by HPLC-MS, which revealed the presence of a second compound, which lacked an acetyl group (10%). The impurity was confirmed as compound **9**, which had resulted from an incompletely 3-O-acetylated batch of donor **D**. The outcome of the glycosylation with the 9:1 mixture of donor **8** and **9** indicated that the side product **9** must serve as a very potent donor because the undecasaccharide **23** accounted for 50% of the product mixture. In contrast, HPLC-MS analysis of the reaction mixture obtained with the impure imidate **D** (which contained 10% of **7**) revealed only traces of the undecasaccharide **23** along with the undecasaccharide **22**.

Thus the pure donors **8** and **9** were synthesized from the intermediate **5**. Unexpectedly, formation of imidate **7** from **5** was only successful using K_2CO_3 as a base to prevent additional formation of the imidate at the 3-OH position.[22,23] Imidate **7** was converted into the thioglycoside **9**, which was acetylated to give **8**.

Glycosylation experiments using the donors **8** and **9** showed marked differences. We were pleased to see that,



Scheme 5. a) **D**, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −10 °C, MS (4 Å), (**22**, 9%); b) **8**, NIS, TfOH, CH_2Cl_2, −30 °C, MS (4 Å) (**22,** 8%); c) **9**, NIS, TfOH, CH_2Cl_2, −30 °C, MS (4 Å) (**23**, 31%); d) **10**, NIS, TfOH, CH_2Cl_2, −30 °C, MS (4 Å) (**24**, 47%); e) **11**, NIS, TfOH, CH_2Cl_2, −30 °C, MS (4 Å) (**25**, 38%); f) 1. CAN, CH_3CN, toluene, H_2O, 82%; 2. **E**, $CuBr_2$, Bu_4NBr, CH_2Cl_2, DMF, 75%; g) 1. ethylenediamine, *n*BuOH, 80 °C; 2. Ac_2O, pyridine; 3. $MeNH_2$ (40% in H_2O) (1.–3., 59%).

when pure donor **9** was used, the yield of undecasaccharide **23** increased to 31%. In contrast, pure donor **8** gave only 8% of the undecasaccharide **22** after an identical workup. We assumed that the low yields obtained with imidate **D** and thioglycoside **8** were linked to the steric bulk of the 3-O-acetylated tetrasaccharide moiety. To further reduce the overall bulk of donor **9**, the peripheral phthalimido groups were replaced by the smaller trifluoroacetamide groups. The N-trifluoroacetylated donors **10** and **11** were synthesized from donor **9** (Scheme 2). The undecasaccharide **25** was obtained in 38% yield from the 3-O-acetylated donor **11**. Donor **10**, which lacked the acetate at the O3 position, gave 47% of undecasaccharide **24**. The acetyl moiety at the O3 position can lower the reactivity of donors **D**, **8**, and **11** through electronic and steric effects. Thus the increased yields of the bisected undecasaccharides using the modified donors **9** and **10** may benefit from the inverted effects. The peripheral phthalimido groups and the acetate at the O3 position of the activated mannoside are key positions that simultaneously affect the reactivity of the donors.

Removal of the PMP group (82%) of **24** and subsequent core fucosylation (75%) finally gave the dodecasaccharide **26**. Global deprotection of the base labile groups and N-acetylation was achieved in three steps and in a one-pot reaction to furnish the dodecasaccharide **27**. Compound **27** is a derivative of **F** and contains an azide group for the generation of neoglycoproteins or glycopeptides.

Herein we have reported the first chemical synthesis of highly branched pentaantennary N-glycans and derivatives with bisecting and core fucosyl modifications. The hindered glycosylation reactions were systematically optimized after the identification of key protecting groups which reduce the proximal and peripheral crowding. The use of a universally applicable modular set of building blocks is expected to facilitate the general chemical synthesis of branched N-glycans.

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