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A Versatile Strategy for the Synthesis of Complex Type *N*-Glycans: Synthesis of Diantennary and Bisected Diantennary Oligosaccharides[1]

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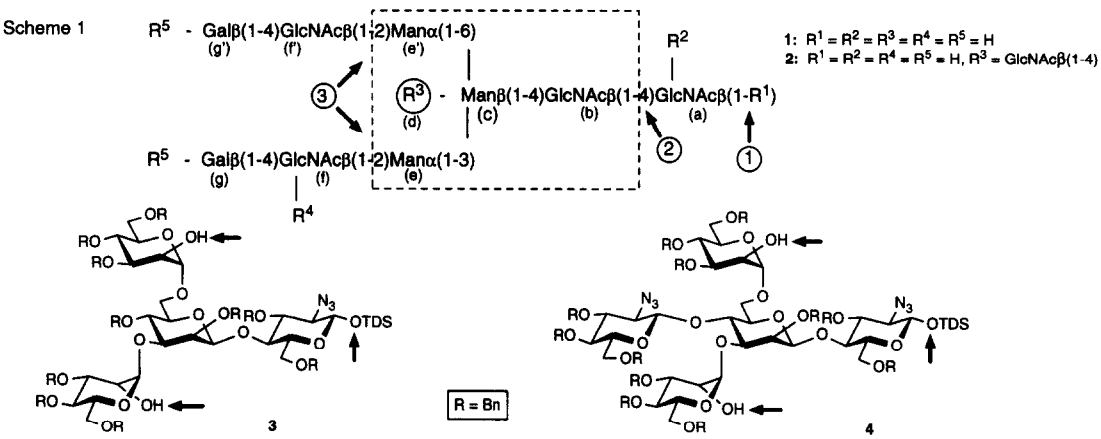
Abstract: Based on readily available glucose, 2-azido-glucose, mannose, and *N*-phthaloyllactosamine building blocks **5, 6, 8,** and **13** a highly versatile strategy for the synthesis of complex type and bisected complex type *N*-glycan residues is established; this is demonstrated for the synthesis of nonasaccharide **1** and decasaccharide **2**, respectively. The glucose residue **5** finally provides regioselective access to the 3-, 4-, and/or 6-hydroxy groups for antenna attachment, introduction of the bisecting *N*-acetylglucosamine residue, and epimerisation at C-2 in order to generate the required β-linked mannosyl residue c.

Most cell surface proteins and proteins present in blood serum of vertebrates are *N*- and/or *O*-glycosylated; the derived glycoproteins often appear in various glycoforms, thus constituting natural product libraries.[2] In order to investigate the biological function of the various oligosaccharide residues, particularly the *N*-glycans gained wide interest.[3,4] The required structurally defined *N*-glycans should be accessible by chemical[5-12] or chemoenzymatic[13-15] synthesis as shown by several groups.

Scheme 1



We have developed over the years efficient syntheses of mono- and disaccharide building blocks which are useful in *N*-glycan synthesis,[16] as also shown in related approaches.[10,14] Here a versatile strategy for the construction of eventually all complex type including bisected type oligosaccharides required for *N*-glycopeptide synthesis is presented. It is based on a flexible protective group pattern and on *O*-glycosyl trichloroacetimidates as powerful glycosyl donors.[16] It is applied to the synthesis of the biantennary nonasaccharide **1** and the corresponding bisected decasaccharide **2** (Scheme 1).[2,3] The strategy (Scheme 1, bond disconnections ①–③) leads to tetra- and pentasaccharides **3** and **4** as basic structures (frame in Scheme 1: R^3 = H or GlcNAcβ(1-4)). They can be employed for the attachment of other (R^4,R^5 = H) and also different antennae at the 2-hydroxy groups of the two α-linked mannose residues e and e' (③); also either an *N*-

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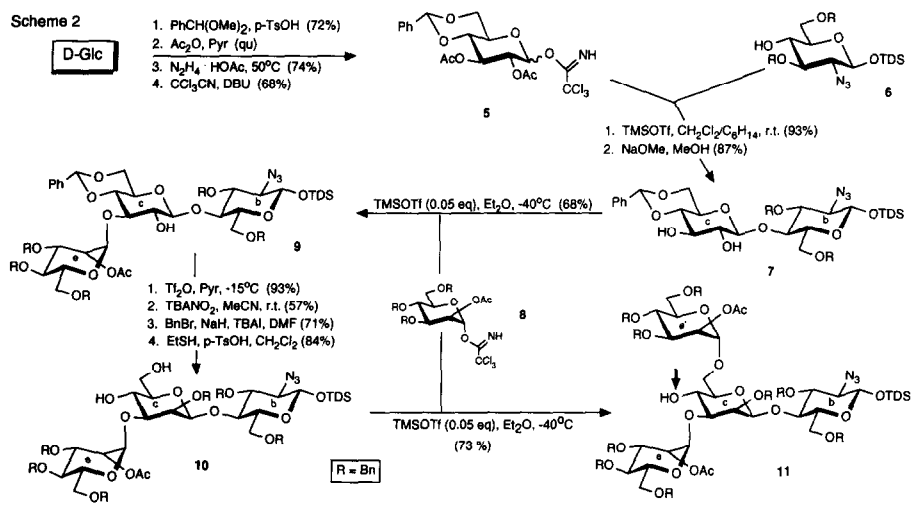
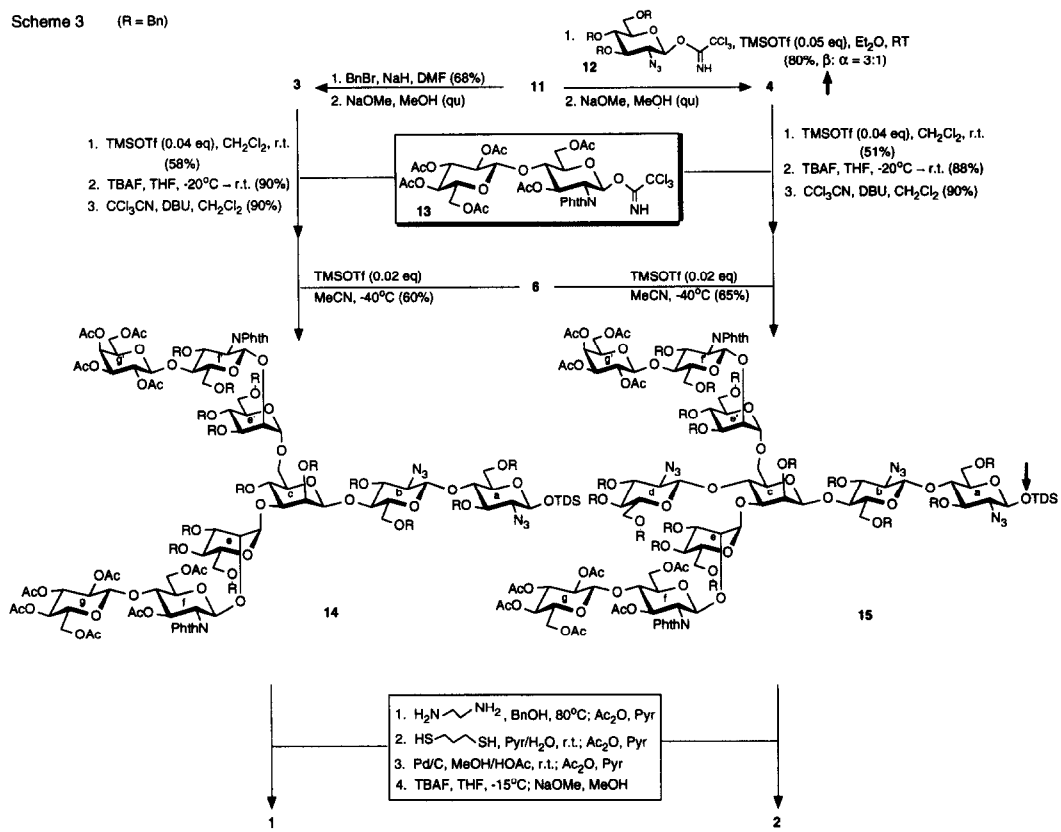
acetylglucosamine (R^2 = H) or a fucosylα(1-6)-*N*-acetylglucosamine [R^2 = Fucα(1-6)] residue can be attached in β(1-4)-linkage at the reducing end (②); with *N*-linked asparagine (R^1 = Asn), these compounds can be the directly employed for *N*-glycopeptide synthesis (①).[17]



For the β-mannopyranoside linkage between sugar residues b and c in **1** and **2**, respectively, transformation of a β-linked glucopyranosyl to a β-mannopyranosyl residue was envisaged; yet, different from related approaches,[8,11,17-19] this epimerisation was planned after the attachment of α(1-3)-linked mannosyl residue e to a 4,6-*O*-benzylidene protected glucose. Then benzylidene group manipulation will provide entire flexibility as to further regioselective linkages to the 2-, 4-, and/or 6-position of the mannosyl residue c.[20] To this end, 4,6-*O*-benzylidene glucose was prepared;[21] per-*O*-acetylation, ensuing regioselective removal of the anomeric *O*-acetyl group by treatment with hydrazinium acetate,[16] and then reaction with CCl_3CN in the presence of DBU as base furnished donor **5** (α:β = 10/1) (Scheme 2). Glycosylation of known azidoglucose derivative **6**[22] with **5** in the presence of TMSOTf as the catalyst afforded the β-linked disaccharide in very high yield. Removal of the *O*-acetyl groups gave 2c,3c-*O*-unprotected disaccharide **7** which proved to be an ideal acceptor for the next glycosylation reactions. Known mannosyl donor **8**[23] was directly employed in the presence of TMSOTf as the catalyst yielding in ether as solvent at -40 °C trisaccharide **9**. The mannosyl residue at 3c-*O* now also served as protective group, thus the remaining 2c-hydroxy group could be conveniently epimerised at this stage: treatment with Tf_2O in pyridine at -15 °C, ensuing addition of tetrabutylammonium nitrite ($TBANO_2$), and then hydrolysis[18] furnished 2-*O*-unprotected mannosyl residue c. Reaction with BnBr/NaH in the presence of TBAI led to 2c-*O*-benzylation. Then the 4c,6c-*O*-benzylidene group was removed under acid catalysis in the presence of EtSH as nucleophile, affording 4c,6c-*O*-unprotected trisaccharide **10**. Glycosylation with mannosyl donor **8** led to regioselective 6c-*O* reaction, furnishing tetrasaccharide **11**, which turned out to be an ideal intermediate: it offers via direct glycosylation of the 4c-hydroxy group generation of bisected structures; removal of the *O*-acetyl groups at mannosyl residues e and e' permits antenna attachment and also the anomeric position at the b residue is selectively accessible.

For the synthesis of diantennary structures, reaction of **11** with BnBr/NaH was performed leading to 4c-*O*-benzylation; ensuing treatment with NaOMe/MeOH afforded building block **3** (Scheme 3). For the synthesis of bisected structures, **11** was glycosylated with known azidoglucose donor **12**[24] resulting in an unexpectedly high glycosylation yield; only some α-product had to be separated. Removal of the *O*-acetyl groups afforded

building block **4**. Both compounds, **3** and **4**, were treated in the same way: glycosylation with known lactosamine donor **13**[25] in the presence of TMSOTf as the catalyst afforded the octa- and nonasaccharides.[26]



Then the anomeric TDS groups were removed with TBAF in THF as solvent; addition of CCl_3CN in the presence of DBU as the base furnished the trichloroacetimidates. Their reaction with **6**[22] as the acceptor in the presence of TMSOTf as the catalyst in MeCN at -40 °C led exclusively to β-linkage,[27] thus furnishing nona- and decasaccharide **14** and **15**. Their structures could be assigned with the help of NMR data.[28]

For the transformation of **14** and **15** into target molecules **1** and **2**, firstly treatment with ethylenediamine in butanol at 80 °C was performed, in order to remove the *N*-phthaloyl groups;[29] this led also to loss of the *O*-acetyl groups; ensuing treatment with Ac_2O/pyridine led to *N,O*-acetylation. The azido groups were reduced with propane-1,3-dithiol in pyridine/water. Hydrogenolytic debenzylation with Pd/C as the catalyst in MeOH/HOAc (1/1) was followed by complete acetylation. Thus, only the TDS group at the anomeric carbon was still retained, thus permitting selective access to this position. Treatment with TBAF in THF at -15 °C led to removal of the TDS group. Then de-*O*-acetylation furnished target molecules **1** and **2**. Their structures were assigned by MALDI-TOFand comparison with the NMR data of structurally related compounds.[30]

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28. ^{13}C NMR (150.91 MHz, $CDCl_3$): **14**: δ = (d, $^1J_{C,H}$ = 166 Hz, C-1f), 96.8 (d, $^1J_{C,H}$ = 168 Hz, C-1f), 97.2 (d, $^1J_{C,H}$ = 161 Hz, C-1a), 97.9 (d, $^1J_{C,H}$ = 172 Hz, C-1e'), 98.5 (d, $^1J_{C,H}$ = 173 Hz, C-1e), 101.5 (d, ^1J = 161 Hz, C-1g'), 101.5 (d, $^1J_{C,H}$ = 161 Hz, C-1b), 101.6 (d, $^1J_{C,H}$ = 163 Hz, C-1g), 101.7 (d, $J_{1C\text{-}1H}$ = 158 Hz, C-1c); C-1b(C-1g interchangeable. **15**: δ = 96.1 (d, $^1J_{C,H}$ = 165 Hz, C-1f), 96.8 (d, $^1J_{C,H}$ = 161 Hz, C-1a), 97.0 (d, $^1J_{C,H}$ = 167 Hz, C-1f), 98.1 (d, $^1J_{C,H}$ = 171 Hz, C-1e'), 98.6 (d, $^1J_{C,H}$ = 178 Hz, C-1e), 100.9 (d, $^1J_{C,H}$ = 166 Hz, C-1b), 101.2 (D, $^1J_{C,H}$ = 163 Hz, C-1d), 101.6 (d, $^1J_{C,H}$ = 162 Hz, C-1g'), 101.8 (d, $^1J_{C,H}$ = 159 Hz, C-1c), 102.2 (d, $^1J_{C,H}$ = 163 Hz, C-1d); C-1b/C-1d interchangeable.
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