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A concise route to the core pentasaccharide of N-linked glycoproteins

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Abstract—A concise preparation of the common pentasaccharide core of the N-linked glycoproteins is described. The reducing end glycal is functionalized at the level of chitobiose, which is then β-mannosylated using Crich's direct coupling protocol. Deprotection of the branching mannose residue, and di-α-mannosylation complete the synthesis.

Our laboratory is currently developing a major program in the total synthesis of glycopolypeptides. Such targets can potentially be used in the development of new diagnostic assays and synthetic vaccines.[1,2] Thus, the preparation of glycopolypeptides is no longer viewed from the primary perspective of a purely academic challenge. While the challenges are indeed daunting, possibilities for building constructs which carry medically valuable information abound with the increasing maturity of the dynamic field of glycobiology.[3] At the present time, chemical synthesis is arguably the only viable way to prepare significant quantities of homogeneous samples of complex glycopeptide targets where the structure of the oligosaccharide can be varied at will.

The abundance of N-linked glycoproteins in nature and the important role they play in cellular interactions prompt major interest in their synthesis by chemical means.[4] It has also been one of the focal research goals of this laboratory.[5] The glycal assembly method[6] has proven itself to be applicable in the preparation of the 'symmetrical' N-linked glycans.[7] Indeed two years ago we reported a route to the signature core region of N-linked glycans.[8,9] In that synthesis a β-linked glucose was introduced at C4 of the chitobiose. Subsequently, epimerization at C2 of the glucose was accomplished by oxidation to a 2-ketoderivative followed by reduction to produce the interior mannose. While this method did provide the core pentasaccharide, the synthesis lacked the directness required to service our growing program. Fortunately, recent developments in carbohydrate synthesis now allow for major simplifications in the elaboration of the core region saccharides. In particular, triflate-mediated direct β-mannosylation methodology, developed by Crich and associates,[10–12] was envisioned for the formation of the key β-mannosyl–chitobiose linkage.[13,14] The Kochetkov amination reaction[15] followed by the Lansbury peptide conjugation procedure[16] enables access to glycopeptides without reliance on intermediate anomeric azides. Moreover, we have recently discovered that such glycans with all of the hydroxyl groups liberated are readily available from the global deprotection of reducing polybenzylated precursors using sodium in liquid ammonia.[17] Figure 1 expresses globally our new synthetic approach taking the advantage of the above findings.

We started with the known disaccharide **1**,[7] which is available from 3,6-di-*O*-benzylglycal.[18] In order to simplify the final steps of the synthesis, it was preferable to

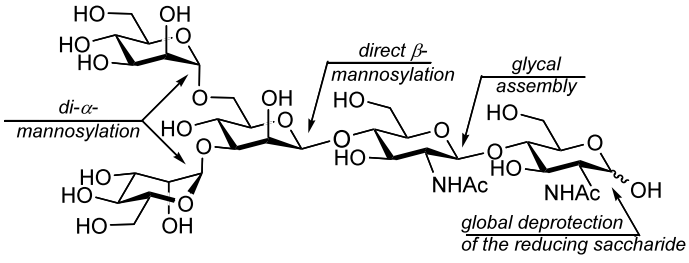

Figure 1.

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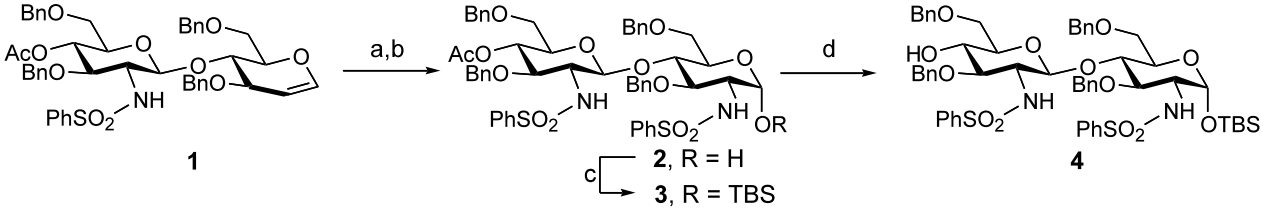
functionalize the reducing end double bond at an early stage. Towards this end, glycal **1** was subjected to iodosulfonamidation (Scheme 1).[19] Following exposure of the addition products to hydrolysis in THF, both isomeric iodosulfonamides were cleanly converted into the reducing disaccharide **2**. Interestingly, the anomeric hydroxyl group in **2** adopts exclusively the α configuration, as evidenced by ^{1}H NMR measurements. This finding can be explained by the intramolecular hydrogen bonding stabilization of the α anomer. Furthermore, this anomeric configuration can be preserved during silylation with excess TBSOTf which gives **3** as a clean α anomer. Deacetylation of **3** following the Zemplén protocol afforded the disaccharide acceptor **4** in 53–60% for the four steps starting from **1**.

Happily, triflate-mediated mannosylation of **4** (Scheme 2) was highly efficient.[20] The coupling reaction, performed at −78°C with 1.5 equiv. of **4** in dichloromethane following the activation of the sulfoxide **5**[21] with triflic anhydride, afforded the mixture of anomers in 85–91% yield and 8/1 β/α ratio.[22] Although an excess of glycosyl acceptor is normally employed in this type of coupling in order to achieve better diastereomeric ratios, the unreacted acceptor is recovered and can be recycled. The isomeric trisaccharides were separated following the removal of the *p*-methoxybenzyl group from mannose 3-OH using ceric ammonium nitrate in wet acetonitrile, providing alcohol **7** as a single anomer in 74% yield.

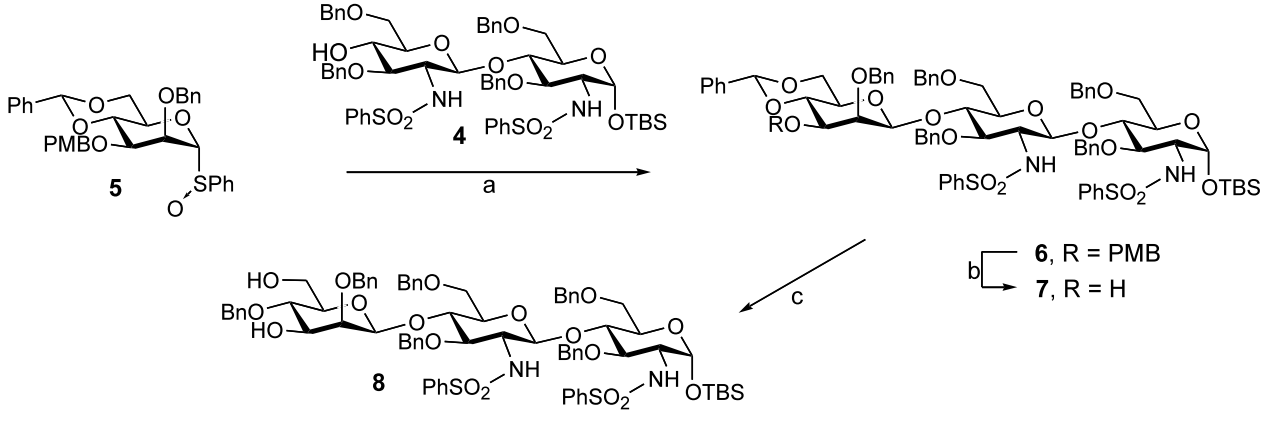
Regioselective cleavage of the 4,6-benzylidene ring was achieved by borane reduction in the presence of dibutylboron triflate.[23] Addition of 3 equiv. of Lewis acid was required to complete the conversion of **7** into the desired 3,6-diol **8**. Simultaneous glycosylation of the primary and secondary hydroxyl groups in **8** was then necessary to form the desired pentasaccharide. Several donors containing ester protection at mannose O2 were screened using various coupling conditions. Glycosylation of **8** with an excess of thiomannoside **9** in acetonitrile using Sinaÿ radical cation activation[24,25] was the most convenient, and provided di-α-mannoside **10** in good yield and complete anomeric selectivity (Scheme 3).

No indication of orthoester formation was found as pentasaccharide **10** was readily saponified producing 'symmetrical' diol **11**. The C2 hydroxyls of the terminal mannose residues in **11** serve as attachments points for lactosamine spacer units, enabling it to be used in the preparation of complex-type glycopeptides. On the other hand, anomeric silyl protection can easily be removed using tetrabutylammonium fluoride to give reducing pentasaccharide **12**.[26]

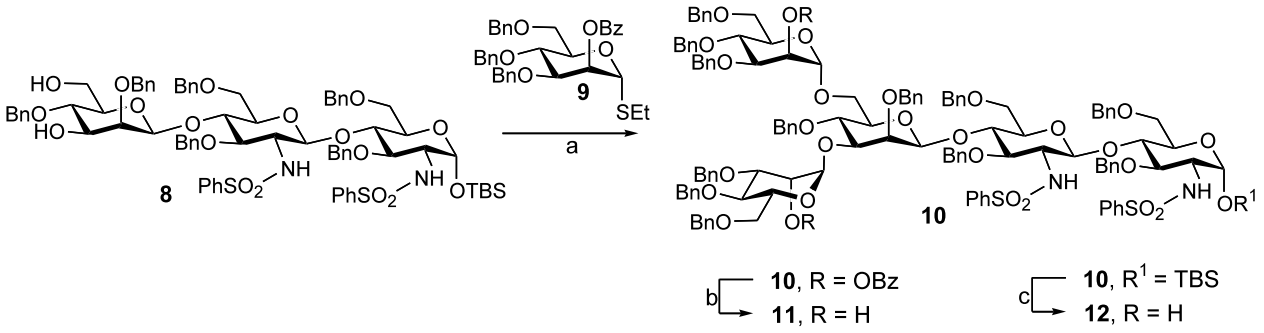
Compound **12**, now reachable in a robust way, serves as a flexible building block in a variety of constructions of highly complex and potentially valuable N-linked glycans. Such extensions and applications of the chemistry described above will be reported in due course.



Scheme 1. *Reagents and conditions*: (a) $I(coll)_2ClO_4$, $PhSO_2NH_2$; (b) Et_3N, H_2O/THF; (c) TBSOTf, 2,6-lutidine, CH_2Cl_2; (d) NaOMe/MeOH; 53–60% for four steps.



Scheme 2. *Reagents and conditions*: (a) i. Tf_2O, DTBMP, CH_2Cl_2, −78°C, ii. **4**, 85–91% (β/α = 8/1); (b) CAN, MeCN/H_2O, 74%; (c) Bu_2BOTf, BH_3·THF, THF, 72%.



Scheme 3. *Reagents and conditions*: (a) $(BrC_6H_4)_3NSbCl_6$, MeCN, 74%; (b) NaOMe/MeOH, 89%; (c) TBAF/AcOH, THF, 81%.

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