Pergamon

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Tetrahedron Letters 44 (2003) 1787–1789

TETRAHEDRON LETTERS

A short synthesis of the trisaccharide building block of the N-linked glycans

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Received 12 December 2002; revised 9 January 2003; accepted 10 January 2003

Abstract—An efficient preparation of the core trisaccharide of N-linked glycoproteins containing β-azido functionality at the reducing terminus is described. In the synthesis, triflate-mediated direct β-mannosylation was employed for the formation of the β-D-Man-(1→4)-GlcNAc linkage; the anomeric azide installation was achieved through oxazoline ring opening.

In natural N-linked glycoproteins, the side chain of Asn in a segment of the peptide containing the Asn-Xxx-Ser(Thr) unit is glycosylated with a highly conserved saccharide core. All types of N-linked glycans include a trisaccharide comprising β-mannosylated chitobiose.[1] The mannose can be further di- or tri-glycosylated with various carbohydrate chains. The efficient assembly of this core structure opens the way for the development of practical synthesis of N-linked glycoproteins.[2]

Since the first synthesis of an N-linked glycan fragment was disclosed by Jeanloz in 1976,[3] there have been many attempts targeting efficient preparations of these structures.[4–8] The ever increasing need for practically useful quantities of glycopeptide samples comprising well-defined single glycoforms demands simplified procedures for their chemical synthesis.[2] Such methodology would ultimately include a number of common building blocks together with unambiguous coupling methods, which have potential for automation. The appropriately protected trisaccharide core containing a convenient handle for peptide conjugation would undoubtedly be useful as one of these building blocks. The design of such compounds (Fig. 1) would include orthogonally exposable 3-, 4-, and 6-OH groups of the mannose residue, whereas the anomeric azido functionality would serve as a point of the peptide attachment either through the Staudinger-type ligation[9,10] or through reduction to the corresponding amine.[8] Azides have been demonstrated to be convenient precursors in the preparation of both natural and unnatural glycan/peptide linkages, and, recently, in direct cell-surface modification.[11,12]

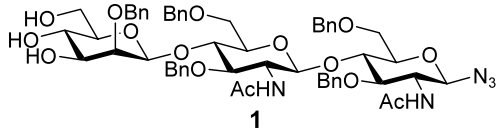

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Figure 1.

Despite the significant amount of research devoted to the synthesis of core structures, the problem of the formation of the more difficult β-D-Man(1→4)GlcNAc linkage which is central to any N-linked glycans, still lacks a direct and universally accepted approach.[1] Novel glycosylations based on substitution of anomeric triflates, developed in this laboratory, proved to give excellent results in the formation of some of the most challenging β-mannosides.[13–15] In a recent study in this group, a number of GlcNAc surrogates were tested as acceptors in the synthesis of the β-D-Man(1→4)GlcNAc bond: 2-azido-2-deoxyglucose derivatives, providing both good yields and complete anomeric selectivity, were found to be optimal.[16] A readily available alcohol **5**[17] was therefore the acceptor of choice in the present synthesis. 4,6-Benzylidene protection of the donor molecule provides torsional rigidity to the pyranose ring necessary for high glycosylation stereoselectivity and is readily removed under acidic conditions. A silyl group would be the optimal orthogonal protection for the mannose 3-OH; however, these adversely affect the stereochemical outcome of the coupling reaction.[18] Consequently, a *p*-methoxybenzyl ether serves as a less sterically demanding temporary substitute for the OTBS group (Scheme 1). Various methods for the

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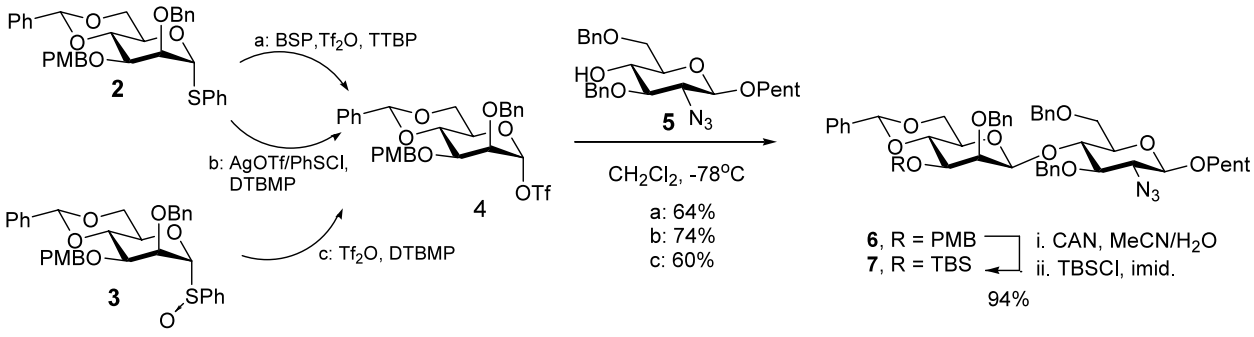


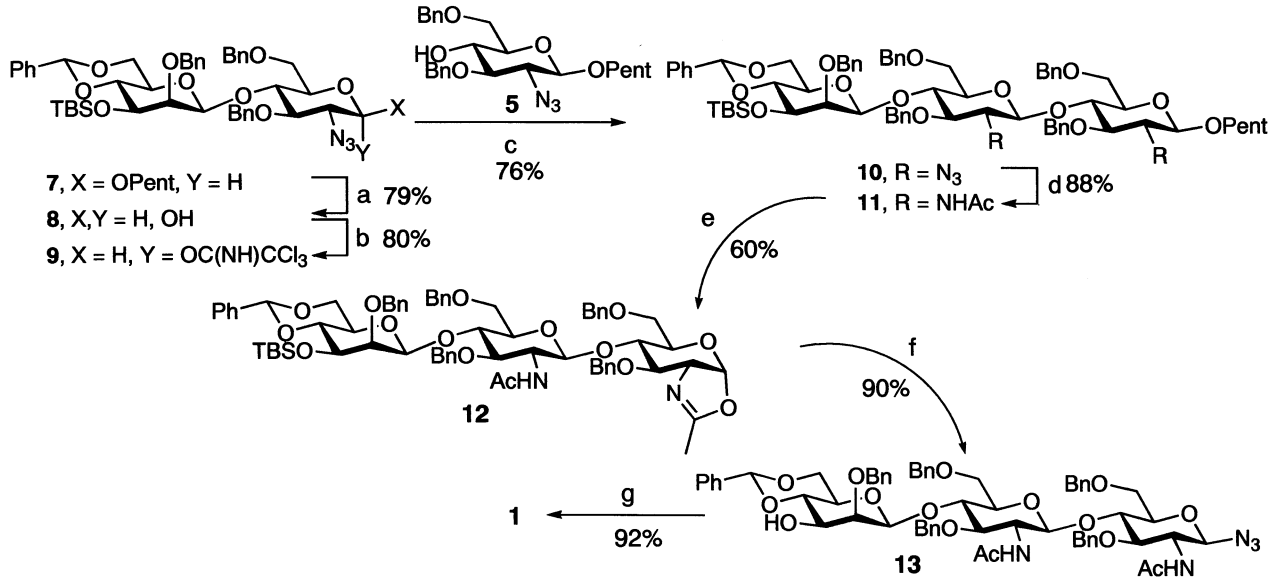
Scheme 1.

generation of the triflate **4** were investigated. Among these, the benzenesulfinylpiperidine (BSP)/triflic anhydride combination[19] was found to be the most practical method to activate the thiomannoside donor **2**,[20] despite providing the desired disaccharide **6** in slightly lower yield than the AgOTf/PhSCl couple[21] (64% versus 74%). The latter procedure suffers from low stability of starting materials as well as difficulty in product isolation, which detract from its applicability in large-scale syntheses.[22]

Sulfoxide **3**[20] was also used in the preparation of triflate **4** but was somewhat inferior to the above methods in terms of both yield and product purification.[23] Complete stereocontrol was achieved with all three activation protocols. After glycosylation, the PMB protection was easily exchanged for TBS in 94% overall yield.

To complete the chitobiose moiety at the reducing terminus, the disaccharide **7** was converted into the anomeric α-trichloroacetimidate **9** and then coupled with a second aliquot of acceptor **5** (Scheme 2). Since the azido substituent in the donor is incapable of aiding *trans*-glycosylation, conditions favoring S_N2 substitution of the α-trichloroacetimidate were needed for the coupling. In the event, treatment of the reagent mixture with $BF_3{\cdot}OEt_2$ in toluene afforded the desired β-glucoside in 76% yield. Attempts at utilizing the 'nitrile effect' to achieve stereochemical control failed in this case, as the reaction was poorly reproducible and typically afforded only 1/5 mixture of α/β anomers. Following the glycosylation, the two azides in the trisaccharide **10** were converted into acetamides through a convenient (if not particularly pleasant) one-step reaction with potassium thioacetate/thioacetic acid.[24]

Introduction of the anomeric azido group into the chitobiose unit is usually achieved by multi-step sequences such as developed in the Danishefsky laboratory.[25] We envisioned a much shorter route capitalizing on the recent discovery of an efficient oxazoline ring opening with a combination of trimethylsilyl azide and tetrabutylammonium fluoride.[26] Toward this end, the terminal *n*-pentenyl group in **11** was activated with NIS/TESOTf in anhydrous dichloromethane to afford oxazoline **12** which was converted into azide **13** immediately following purification. The mannose 3-OH was simultaneously liberated in this step. Compound **13**



Scheme 2. *Reagents and conditions:* a: NIS, $MeCN/H_2O$; b: CCl_3CN, DBU, CH_2Cl_2; c: $BF_3{\cdot}OEt_2$, toluene; d: AcSK/AcSH, DMF; e: NIS, TESOTf, CH_2Cl_2; f: $TMSN_3$/TBAF, THF; g: 80% aq. AcOH.

itself can be viewed as a valuable building block in the synthesis of N-linked glycans as it provides an isolated α-mannose attachment point. Additional glycosylation can be performed after reductive cleavage of the benzylidene acetal, or its complete removal. The latter is easily achieved by treatment with 80% aqueous AcOH which, in the case of **13**, provided triol **1** in 92% yield.

In conclusion, a short synthesis of the N-glycan trisaccharide building block with a terminal azido group is reported. The procedure can readily be adopted to access a wide range of glycans with any substitution pattern at the central mannose residue of the core region.

Acknowledgements

We thank the NIH (GM 62160) for support of this work.

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