Tetrahedron Letters 51 (2010) 2648–2651

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Convenient introduction of a bisecting GlcNAc residue into multiantennary N-glycans as the ultimate residue

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ARTICLE INFO

Article history:
Received 20 October 2009
Revised 3 March 2010
Accepted 9 March 2010
Available online 15 March 2010

Keywords:
Glycosylation
N-Glycan
Bisecting
Glycoprotein
Oligosaccharide

ABSTRACT

A straightforward chemical synthesis was developed for multiantennary N-glycans of the complex-type containing a bisecting GlcNAc moiety. It was found that a bisecting GlcNAc can be introduced as the final residue despite considerable steric hindrance of the buried 4-hydroxyl group of the N-glycan acceptor. This approach circumvents the need for a temporary protecting group on the primary hydroxyl group of the central β-mannoside resulting in a shorter and more flexible synthesis. Thus the generation of N-glycans with an optional bisecting GlcNAc can be accomplished by a unified synthetic path using the same precursors and intermediates.

The attachment of N-glycans to asparagine residues of cell surface and secretory proteins is one of the most frequently found posttranslational modifications of higher organisms. The carbohydrates not only influence the physicochemical properties of the resulting glycoproteins but are also involved in biological recognition events.[1] The occurrence of complex-type N-glycans containing a bisecting GlcNAc residue is common on antibodies,[2] however, remains rare on other glycoproteins[3] and has been associated with malignancy in several cases.[4,5] Typically the isolation of N-glycans from natural sources is quite difficult[6] and renders small amounts only.[7] When particular structures are desired this approach may not be feasible and thus chemical synthesis[8] provides a good alternative in many cases. We herein describe the synthesis of multiantennary N-glycans with up to four branches carrying a bisecting GlcNAc. In contrast to previous approaches the introduction of the bisecting GlcNAc moiety was accomplished as the final step and required only the fully elaborated branched N-glycan acceptors, which were accessible conveniently from modular building blocks (Fig. 1).[9,10]

In the course of our studies aimed at the modular synthesis of complex-type N-glycans with a bisecting GlcNAc modification we established two related approaches. The direct route takes advantage of the glycosylation of the remaining OH-4 at the β-mannoside[11–13] but was found to be restricted to biantennary N-glycans.[14] Thus a more general approach was developed for N-glycans with up to five branches where the bisecting GlcNAc moiety was introduced prior to the attachment of the α1,6-arm requiring two additional steps for the selective protection and deprotection of OH-6 of the β-mannoside.[8,14–16] Owing to the findings that the introduction of the bisecting GlcNAc moiety is highly dependent on the N-protecting groups of the bisecting donor[17,13] and the antennae,[8] we reinvestigated the introduction of a bisecting GlcNAc into the biantennary N-glycan **1** using the *N*-trifluoroacetamido-protected donor **B**[8] and compared it to the phthalimido-protected glycosyl fluoride **E** (Fig. 2).

Initially, the heptasaccharide **1** was assembled by double regio- and stereoselective glycosylation from the building blocks **A** and **C**.[18] When heptasaccharide **1** was reacted with the bisecting GlcNAc donor **B** in moderate excess (3 equiv) and *N*-iodosuccinimide/trifluoromethane sulfonic acid (NIS/TfOH)[19] only little conversion to the desired product was found. Surprisingly, under more stringent conditions using 10 equiv of donor **B** and an acceptor concentration of 11 mM complete conversion of the acceptor **1** occurred (according to TLC) when keeping the reaction temperature at −40 °C for 1 h followed by warming up to −10 °C over a period of 2 h. Flash chromatography afforded the desired bisected octasaccharide **D** in a yield of 75%, which significantly surpassed the yield obtained previously with the phthalimido-protected donor **E** (56%).[11]

After this encouraging finding we investigated the scope of this procedure for multiantennary N-glycans with more branches (Fig. 3). We were pleased to see that under similar conditions the bulky triantennary acceptors **3** and **5** also reacted with **B** affording the bisected nonasaccharides **4** and **6** in yields of 86% and 50%, respectively. These are the first examples for the direct

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doi:10.1016/j.tetlet.2010.03.031

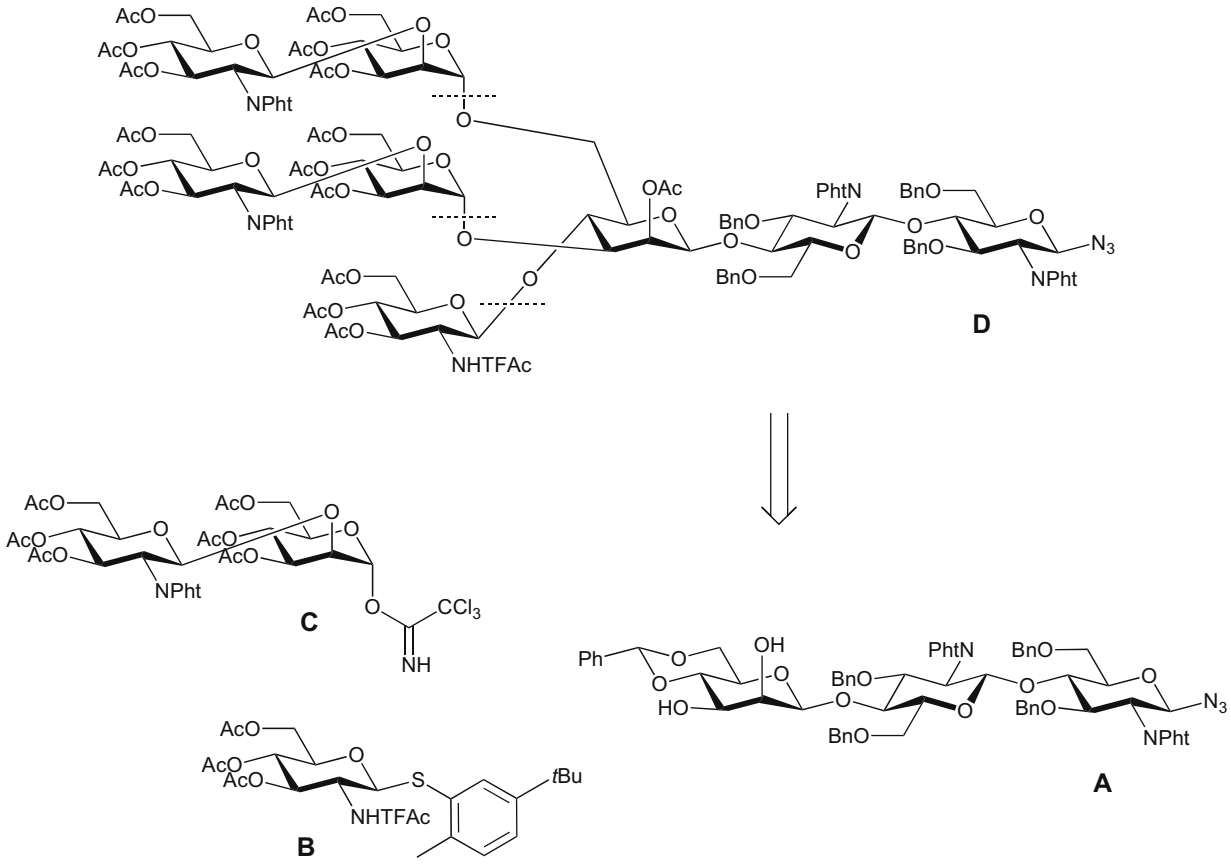

Figure 1. Retrosynthesis of the bisected octasaccharide **D**.

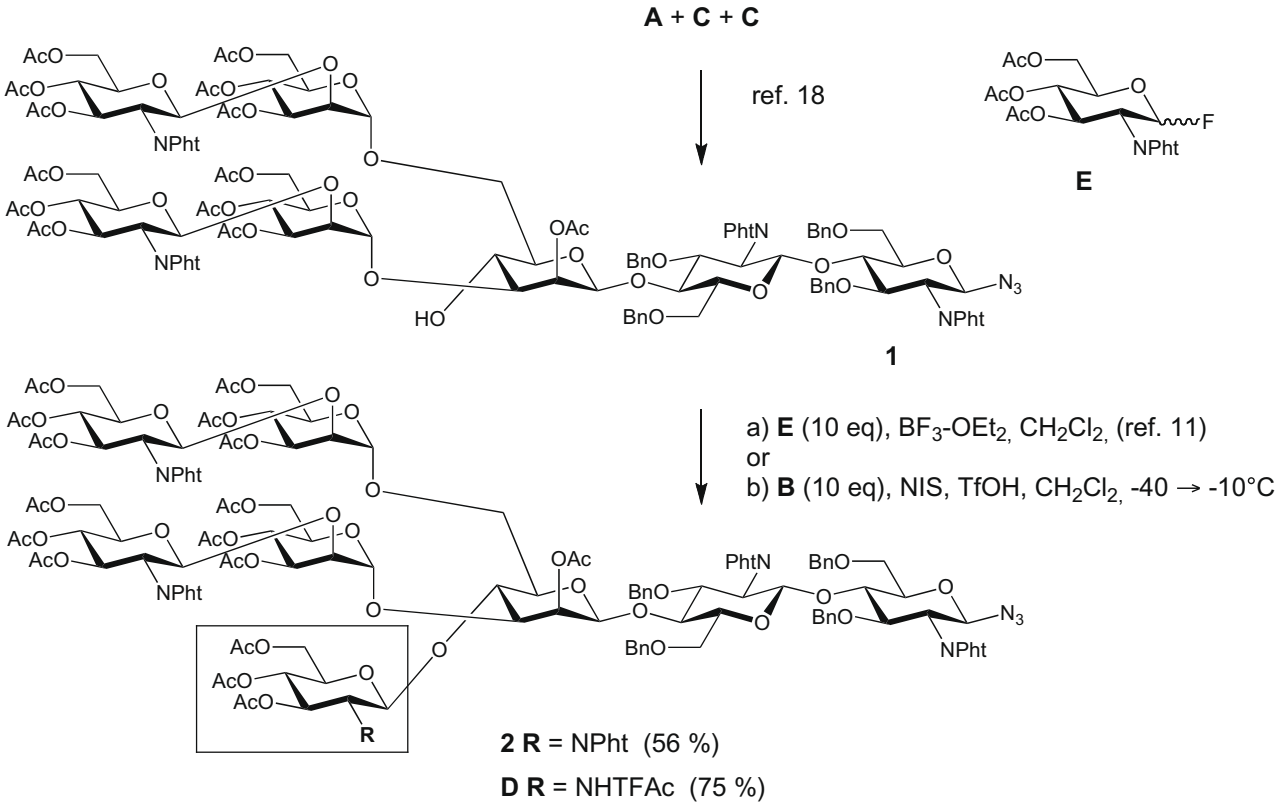

Figure 2. Comparison of the donors **B** and **E** in the late introduction of a bisecting GlcNAc residue.

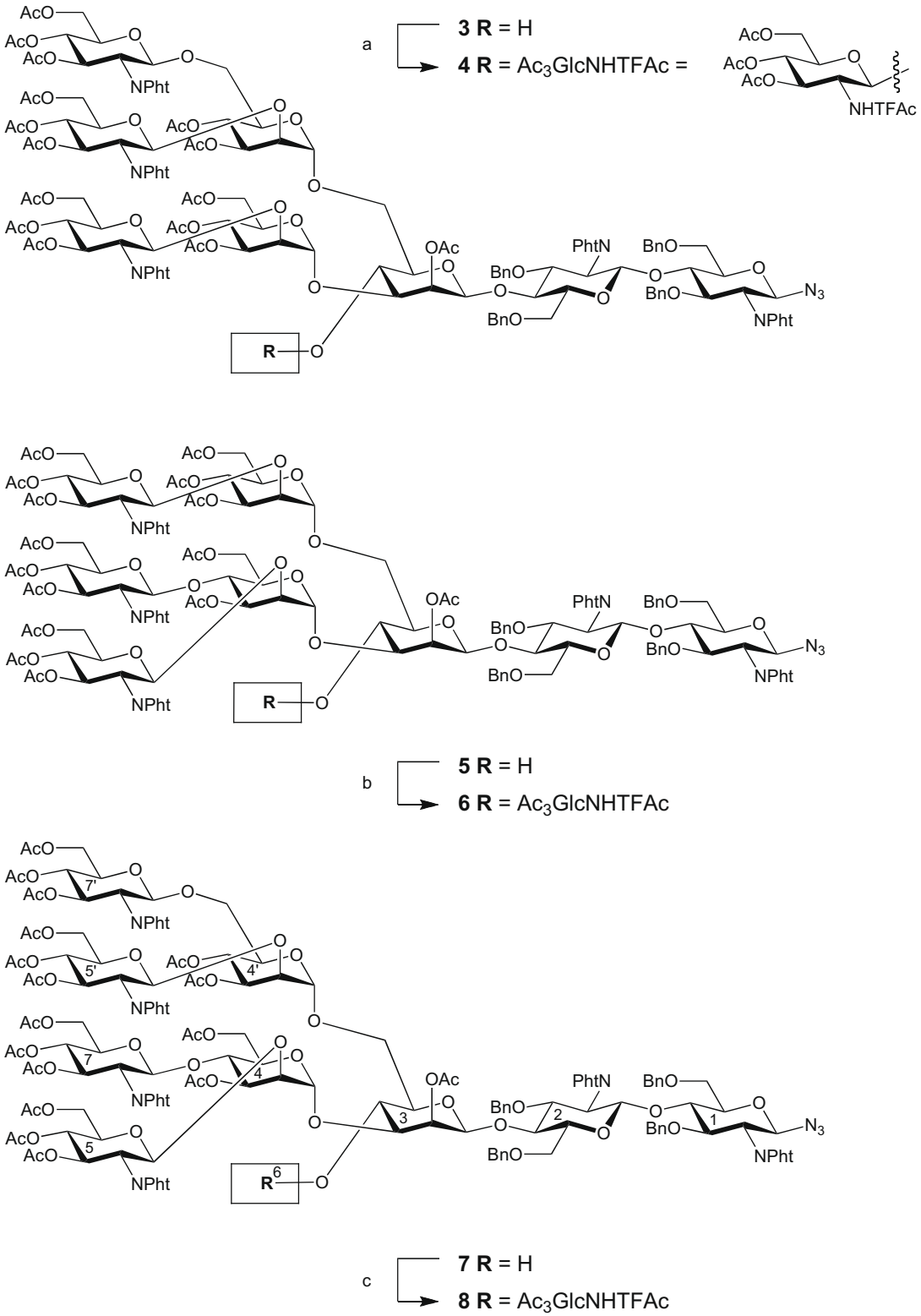

Figure 3. (a) **B** (10 equiv), NIS, TfOH, CH_2Cl_2, −40→−10 °C, molecular sieves 4 Å, (86%); (b) **B** (10 equiv), NIS, TfOH, CH_2Cl_2, −40→−5 °C, molecular sieves 4 Å, (50%); (c) **B** (10 equiv), NIS, TfOH, CH_2Cl_2, −40 °C→room temperature, molecular sieves 4 Å, (24% after HPLC) or **B** (10 equiv), NIS, TfOH, CH_2Cl_2, −30→−25 °C, molecular sieves 4 Å, (46% after HPLC).

conversion of triantennary N-glycans to their bisected derivatives. When the same reaction was tested with the even more sterically congested tetraantennary acceptor **7** only low conversion to the bisected decasaccharide **8** (24% yield after HPLC) was found due to side reactions. From the HPLC–MS data the major side product could not be identified but about 5% of the acceptor **7** was converted to a trifluoroacetylated derivative. Subsequent inspection of the HPLC–MS data of the reactions with acceptors **1**, **3**, and **5** also showed the masses of the trifluoroacetylated transacylation products. To the best of our knowledge the transacylation[20] from amides in glycosylation reactions has not been reported before.

In related studies on the synthesis of multiantennary bisected N-glycans we found that donor **B** was best employed in a narrow temperature range otherwise deactivation may occur. We thus applied modified glycosylation conditions to the tetraantennary compound **7** as the most demanding example. When acceptor **7** was present in higher concentration (30 mM) and the reaction temperature was kept between −30 and −25 °C we were pleased to find that about 70% of **7** was converted to the desired product **8** according to HPLC–MS. Despite the good conversion the bisected decasaccharide **8** could not be isolated as a pure compound by flash chromatography and required preparative RP-HPLC in order to remove the remaining acceptor and side products (46% final yield).

The structures of the bisected N-glycans **D**, **4**, **6**, and **8** were confirmed by ESI-MS and NMR spectroscopy[21] and proved identical to the reference compounds obtained previously by the alternative strategy following the early introduction of the bisecting GlcNAc moiety.[14]

In summary we have developed an efficient and straightforward procedure for the direct conversion of multiantennary N-glycans to their bisected derivatives. The convenient introduction of the bisecting moiety at a late stage was generally applicable to complex-type N-glycans with up to four phthalimido-protected antennae.[10] Thus a unified synthetic path using modular building blocks provides rapid access to N-glycans with an optional bisecting GlcNAc moiety.

Acknowledgments

We are grateful to the Deutsche Forschungsgemeinschaft (DFG) and the Fonds der Chemischen Industrie for financial support.

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21. *Compound* **D**: ESI-MS (acetonitrile, 0.1% formic acid): $C_{142}H_{149}F_3N_8O_{60}$ M_r (calcd) 2982.88, M_r (found) 3006.18 $(M+Na)^+$; $[\alpha]_D^{23}$ −4.3 (*c* 0.5, CH_2Cl_2); ^{13}C NMR (90.6 MHz, DMSO-d_6): δ 156.4 (q, C=O TFAc), 100.6 (C-$1^6\beta$, $J_{C\text{-}1,H\text{-}1}$ = 169.0 Hz from a coupled HMQC spectrum), 97.4 (C-$1^2\beta$, $J_{C\text{-}1,H\text{-}1}$ = 168.0 Hz from a coupled HMQC spectrum), 97.3 (C-$1^{4'}$), 96.0 (C-1^5), 95.4 (C-1^3), 95.2 (C-$1^{5'}$), 94.4 (C-1^4), 84.6 (C-1^1). *Compound* **6**: ESI-MS (acetonitrile, 0.1% formic acid): $C_{160}H_{166}F_3N_9O_{68}$ M_r (calcd) 3357.98, M_r (found) 3359.1 $(M+H)^+$, 3380.9 $(M+Na)^+$; $[\alpha]_D^{23}$ −5.3 (*c* 0.5, CH_2Cl_2); ^{13}C NMR (90.6 MHz, DMSO-d_6): δ 156.3 (q, C=O TFAc), 97.4 (C-$1^2\beta$, $J_{C\text{-}1,H\text{-}1}$ = 164.4 Hz from a coupled HSQC spectrum), 97.4 (C-1^4), 96.3 (C-$1^6\beta$, $J_{C\text{-}1,H\text{-}1}$ = 162.3 Hz from a coupled HSQC spectrum), 96.0 (C-1^5), 95.3 (C-$1^{5'}$), 95.1 (C-1^3), 95.0 (C-$1^{4'}$), 94.8 (C-1^7), 84.6 (C-1^1). *Compound* **8**: ESI-MS (acetonitrile, 0.1% formic acid): $C_{178}H_{183}F_3N_{10}O_{76}$ M_r (calcd) 3733.07, M_r (found) 3755.89 $(M+Na)^+$; $[\alpha]_D^{23}$ +16.6 (*c* 0.5, CH_2Cl_2); ^{13}C NMR (90.6 MHz, DMSO-d_6): δ 156.3 (q, C=O TFAc), 100.9 (C-$1^6\beta$, $J_{C\text{-}1,H\text{-}1}$ = 164.7 Hz from a coupled HSQC spectrum), 97.4 (C-$1^2\beta$, $J_{C\text{-}1,H\text{-}1}$ = 165.9 Hz from a coupled HSQC spectrum), 97.2 (C-1^4), 96.7 (C-$1^{7'}$), 96.0 (C-1^5), 95.1 (C-$1^{5'}$), 95.0 (C-1^3), 94.8 (C-1^7), 94.7 (C-$1^{4'}$), 84.6 (C-1^1).