Glycans

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Highly Efficient Synthesis of Multiantennary Bisected N-glycans Based on Imidates

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Dedicated to Professor Horst Kunz on the occasion of his 75th birthday

Abstract: *The occurrence of N-glycans with a bisecting GlcNAc modification on glycoproteins has many implications in developmental and immune biology. However, these particular N-glycans are difficult to obtain either from nature or through synthesis. We have developed a flexible and general method for synthesizing bisected N-glycans of the complex type by employing modular TFAc-protected donors for all antennae. The TFAc-protected N-glycans are suitable for the late introduction of a bisecting GlcNAc. This integrated strategy permits for the first time the use of a single approach for multiantennary N-glycans as well as their bisected derivatives via imidates, with unprecedented yields even in a one-pot double glycosylation. With this new method, rare N-glycans of the bisected type can be obtained readily, thereby providing defined tools to decipher the biological roles of bisecting GlcNAc modifications.*

The oligosaccharide part plays an important role in the biological activity of recombinant therapeutic N-glycoproteins.[1] In some cases, individual N-glycans on glycoproteins have been linked to a specific function,[2] however, for the majority of N-glycans, the biological roles are difficult to elucidate. Since the isolation of pure N-glycans is feasible only in a few cases,[3] chemical synthesis[4] is needed to provide the particular structures for high-throughput glycomics[5] or chemical glycoprotein synthesis.[6] Based on a chemical and enzymatic approach for complex N-glycans[7] using modular building blocks,[8] even the most demanding examples can be synthesized.[9] However, owing to steric effects, the synthesis of N-glycans bearing a bisecting *N*-acetylglucosamine (GlcNAc) at the central β-mannoside remains particularly challenging.[9,10] The occurrence of bisecting GlcNAc significantly alters the conformational properties of N-glycans,[11] which affects recognition by lectins[12] and thus influences cell proliferation, tumor progression, and the immune response.[13] Herein, we report the highly efficient assembly of bisected N-glycans at a late stage by using trifluoroacetamido (TFAc)-protected building blocks.

Early studies[10a,b] on the synthesis of bisected N-glycans revealed that the N-protecting group on the bisecting GlcNAc strongly influences the subsequent attachment of the antennae (1,3- and 1,6-arm) to the central β-mannoside. In most approaches, the bisecting GlcNAc has thus been installed prior to the antennae, which means that additional protecting-group transformations are required.[9,10] Only a few examples have been reported for the late introduction of a bisecting GlcNAc,[10c,e,i,m] which is analogous to the biosynthesis of bisected N-glycans by GnT-III.[14] The late introduction of a bisecting GlcNAc is hampered by the low reactivity of the 4-OH group buried in the protected N-glycan acceptor and requires a large excess of bisecting GlcNAc donors, which can lead to side reactions and low yields as a result of difficult purifications.[10i]

One of the most demanding targets, a pentaantennary bisected N-glycan with an additional core fucose,[9] was obtained through an early-introduction strategy using a TFAc-protected[15] bisecting GlcNAc donor followed by attachment of the α1,6-branch.[10f,g] A key finding of these syntheses was that the reactivity of the donor for the α1,6-branch increased significantly when using TFAc groups instead of bulky phthalimido groups.[9] This is also reflected in the unsatisfactory outcome of the final glycosylations of the phthalimido-protected bisected N-glycan **1**. Using the early introduction of bisecting GlcNAc, coupling of the 1,6-branch gave poor yields (12%),[10f] but the late-introduction strategy also gave only 46% yield[10i] in the final step (Scheme 1). We hypothesized that replacement of the NPht groups in the antennae of **1** by TFAc should improve the installation of the antennae as well as the late introduction of a bisecting GlcNAc.

In order to investigate the influence of a consistent N-TFAc protection pattern in the antennae of N-glycans, we synthesized the modified modular[8] donors **B**, **C**, and **D** as thioglycosides or trichloroacetimidates[16] (Scheme 2). Disaccharide **3** was conveniently obtained from tetraacetylmannose **2**[17] and donor **E1**,[10f] followed by derivatization as an imidate (**B1**) and a thioglycoside (**B2**).

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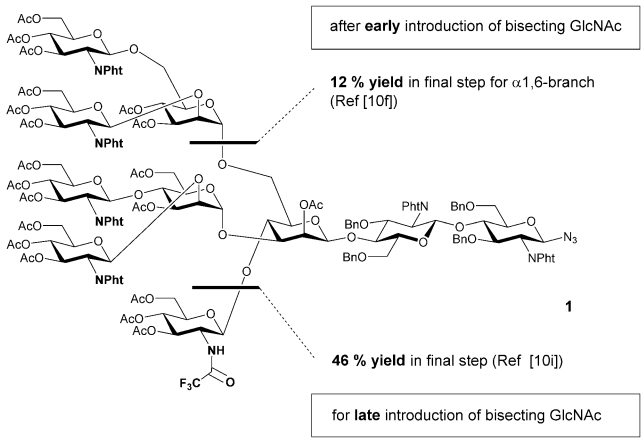
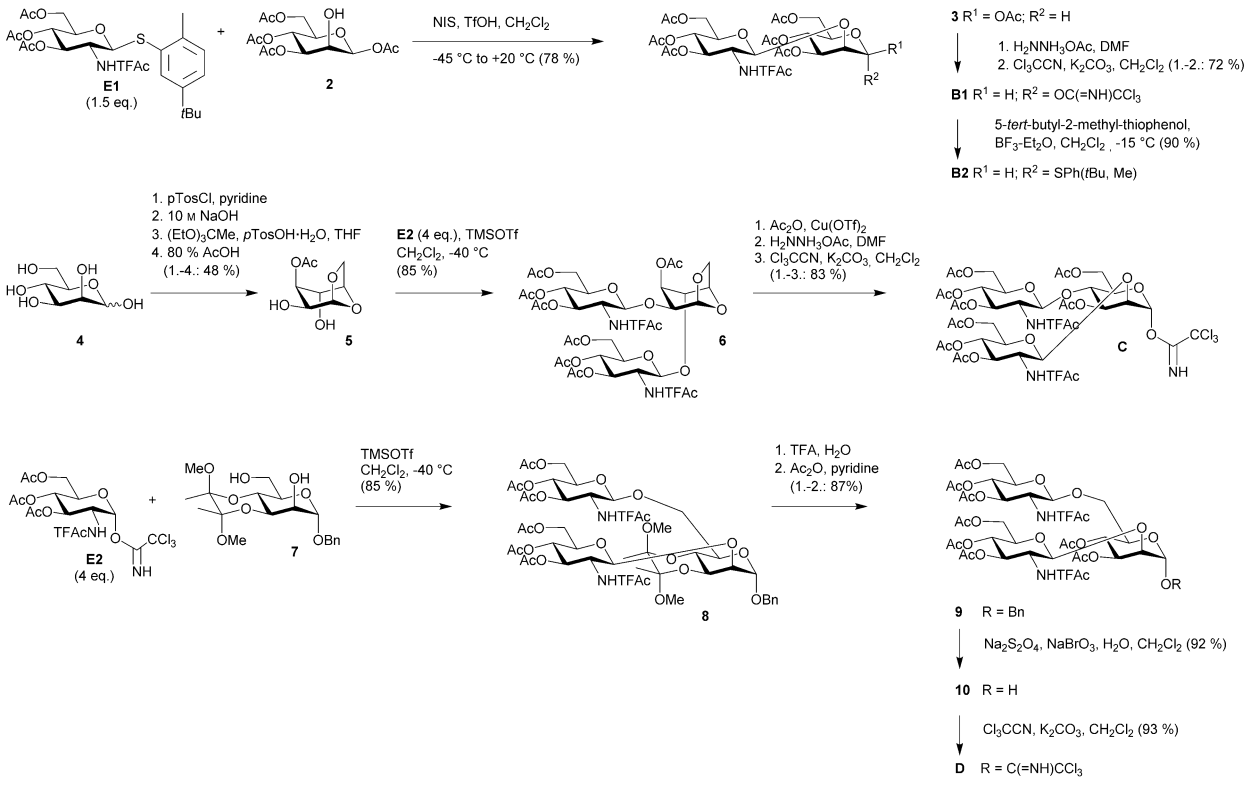


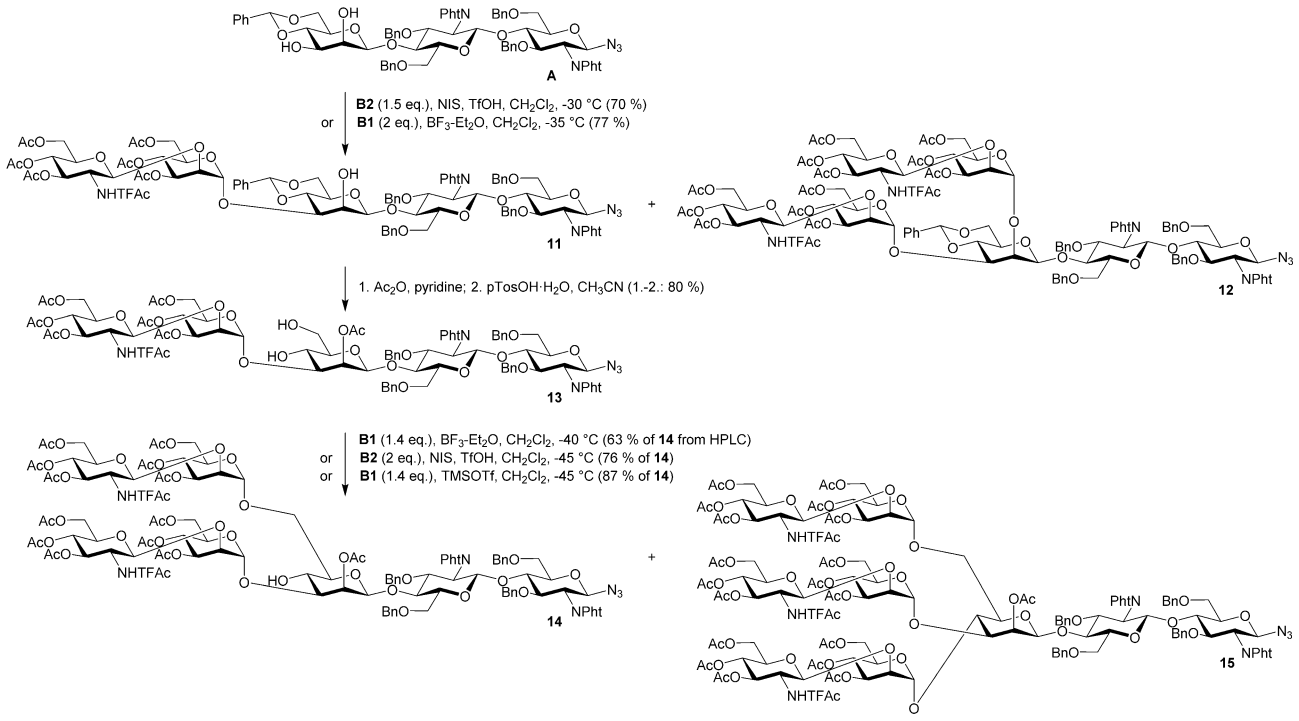
Scheme 1. Comparison of the final glycosylation to give tetraantennary bisected N-glycan **1** containing NPht groups in all antennae. Bn = benzyl, NPht = phthalimido, Ac = acetyl.

For the synthesis of the 2,4-substituted trisaccharide donor **C**, we were able to replace the tin-mediated functionalization of benzyl mannoside[18] with high yielding transformations based on the mild $Cu(OTf)_2$-mediated acetolysis of 1,6-anhydrosugars.[19] The 3-acetylated 1,6-anhydromannose diol **5** was synthesized from mannose **4** in a four-step one-pot procedure. Mannose **4** was converted into 1,6-anhydromannose, followed by selective 2,3-*cis*-orthoacetate formation. Hydrolysis of the 2,3-orthoester gave acetate **5** as the favored axial rearrangement product. Double glycosylation of diol **5** with 4 equiv of donor **E2**[20] furnished the trisaccharide **6** (85% yield) in high purity, followed by one-pot acetolysis and installation of the imidate (83% yield of **C** over three steps). The 2,6-branched trisaccharide donor **D** was obtained from the 3,4-diketal acceptor **7**[21] and imidate **E2** in a double glycosylation, followed by replacement of the diketal with acetates, oxidative debenzylation,[22] and imidate formation.

With the modular set of N-TFAc-protected donors in hand, the synthesis of biantennary N-glycans through double regio- and stereoselective glycosylation of core trisaccharide **A**[23] was investigated (Scheme 3). The reaction of thioglycoside **B2** with **A** and NIS/TfOH activation selectively gave the desired α1,3-linked pentasaccharide **11** (70%), along with debenzylidenation of **11** and formation of the double-glycosylation product **12** (12%). The yield of **11** was raised to 77% by using imidate **B1** and $BF_3{\cdot}Et_2O$ activation (+15% of **12**), whereas TMSOTf gave lower yields due to an increase in double glycosylation. Pentasaccharide **11** was acetylated and debenzylidenated to diol **13**, which was subsequently reacted with the disaccharide imidate **B1** (1.4 equiv) in the presence of $BF_3{\cdot}Et_2O$. LC-MS analysis of the crude reaction mixture showed the desired heptasaccharide **14** (63%), remaining acceptor **13** (15%), and considerable double glycosylation to nonasaccharide **15** (22%).[24] With the thioglycoside **B2**, the yield of heptasaccharide **14** increased to 76%, but 16% of the nonasaccharide **15** was also obtained. The formation of the undesired side product **15** could be virtually eliminated by reacting the diol **13** and imidate **B1** in the presence of dilute TMSOTf (87% of **14**).



Scheme 2. Synthesis of the N-TFAc-protected donors **B**, **C**, and **D**.



Scheme 3. Regioselectivity of the TFAc-protected donors **B1** and **B2**.

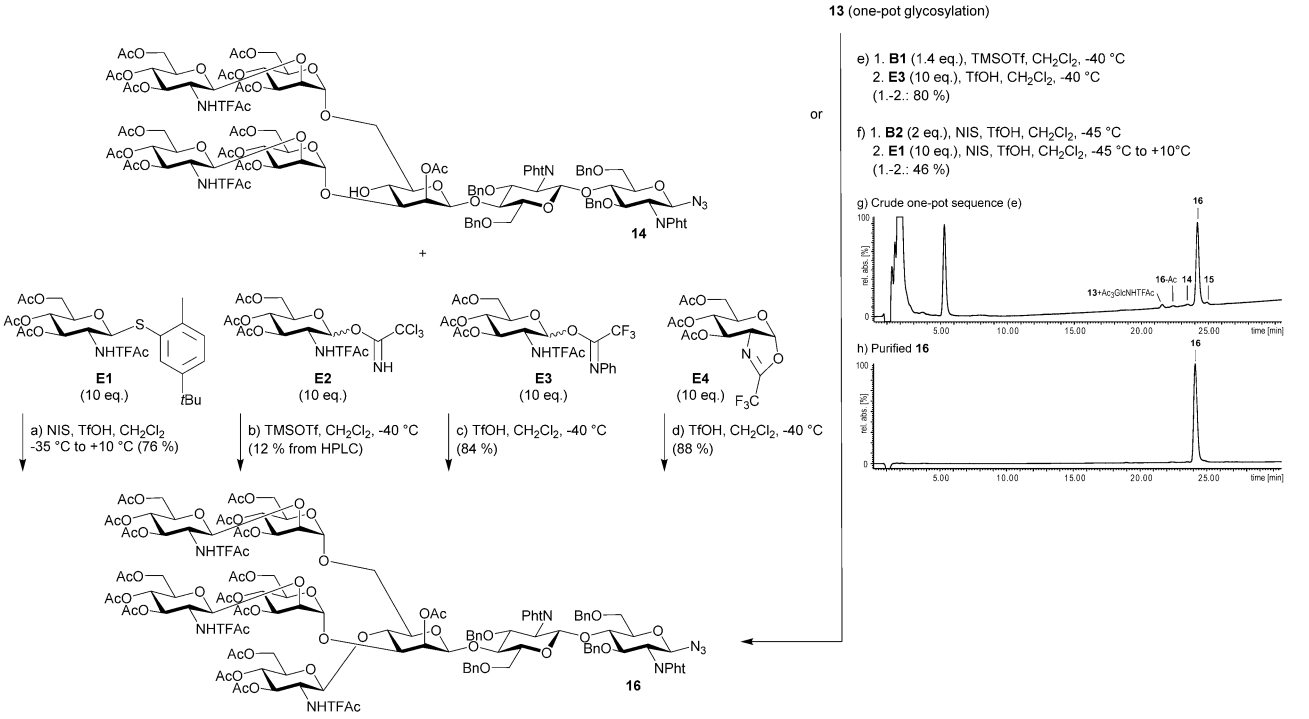
In all glycosylations of the TFAc-protected donors **B1** and **B2**, complete α-selectivity was obtained. Other stereoisomers were not detectable by HPLC–MS or NMR, which is in accordance with the analogous donors with NAc or NPht protection.[24] In contrast to the NPht-protected donor,[24] regioselective glycosylations with the unbranched TFAc donors **B1** or **B2** are prone to double glycosylation, thus suggesting a significant steric contribution from the remote NPht group. On the other hand, the N-glycans **11** and **14** with remote TFAc groups appear to provide good accessibility of the remaining OH-2[3] and OH-4[3] functions to excess donors and should thus facilitate the late introduction of a bisecting GlcNAc.

This assumption was tested by reacting heptasaccharide **14** with 10 equiv of bisecting donor **E1** and NIS/TfOH activation (Scheme 4). To our delight, the reaction proceeded smoothly and furnished the desired bisected octasaccharide **16** in good yield and purity (76%). In combination with TMSOTf, donor **E2** was too reactive and showed only 12% conversion into **16**. The less reactive *N*-phenylimidate **E3**[25] (10 equiv + 4 equiv TfOH) provided nearly complete conversion of the acceptor **14** and raised the yield of isolated **16** to 84%. Even higher yields (88% of **16**) could be obtained with the sensitive but difficult-to-handle oxazoline **E4**.[26] The high yields of bisected N-glycan **16** result from a nearly complete conversion of the TFAc-protected acceptor **14** and favorable separation properties of the bisected product **16** during flash chromatography.

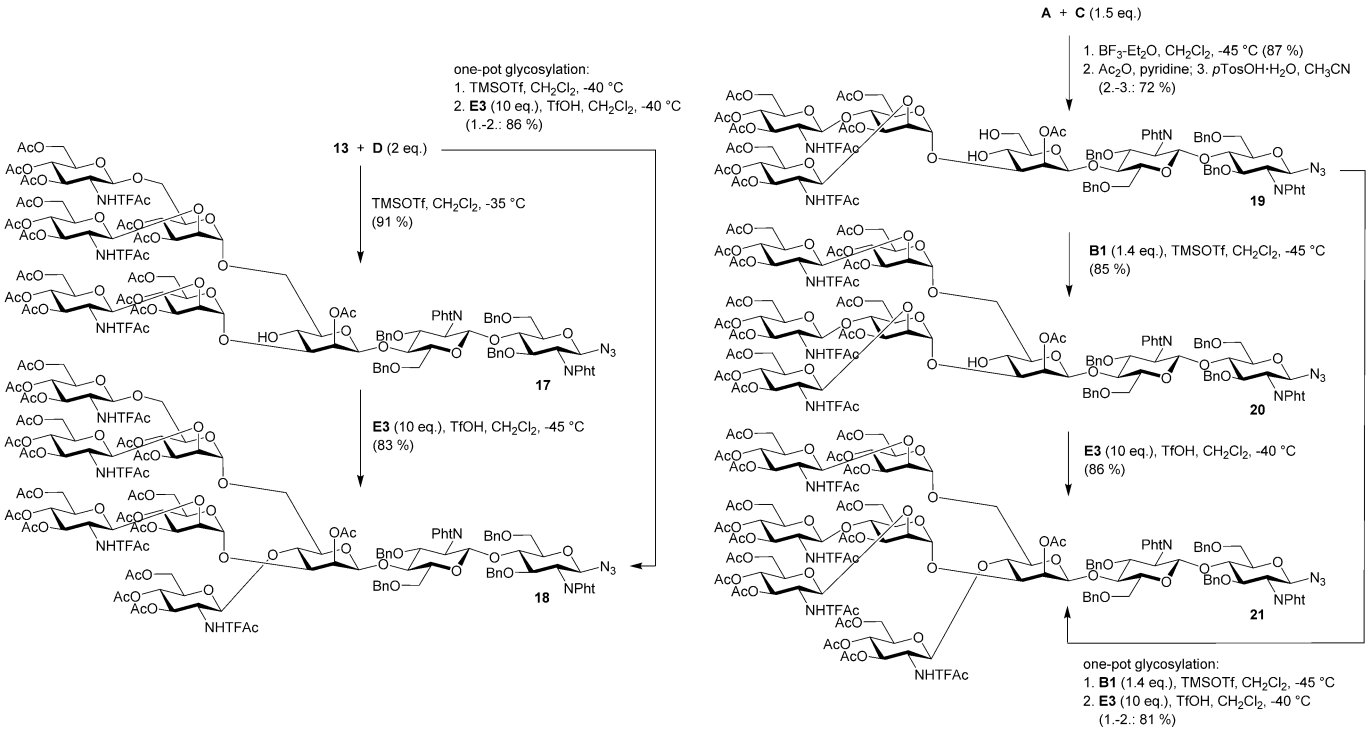
Since the TFAc-protected acceptor **14** was amenable to the efficient late introduction of a bisecting GlcNAc, we assumed that the introduction of the 1,6-arm and bisecting GlcNAc might be combined in a one-pot procedure. Pentasaccharide **13** was first reacted with disaccharide imidate **B1** (0.05 equiv of TMSOTf) until completion (TLC), followed by the addition of 10 equiv of bisecting donor **E3** and 4 equiv of TfOH (Scheme 4e). Remarkably, after a regio- and stereoselective glycosylation, followed by a demanding glycosylation with large excess of donor, HPLC–MS analysis of the crude reaction product (Scheme 4g) showed very few side products. After flash chromatography, pure bisected octasaccharide **16** was isolated in a yield of 80% (vs. 73% for the sequential procedure). In contrast, the one-pot glycosylation of **13** with the thioglycoside donors **B2** (2 equiv) and **E1** (10 equiv), which requires stoichiometric NIS activation, yielded only 46% of **16** because of side reactions.

The late installation of a bisecting GlcNAc was subsequently tested with TFAc-protected triantennary N-glycans (Scheme 5). Glycosylation of pentasaccharide **13** with trisaccharide donor **D** gave the triantennary octasaccharide **17** in 91% yield. Subsequent reaction with the bisecting donor **E3** furnished 83% of the bisected nonasaccharide **18**. When **13** was glycosylated in a one-pot sequence, the yield of isolated **18** was 86% (vs. 75% for the sequential procedure).

As an access to 2,4-branched N-glycans, core trisaccharide **A** was reacted with imidate **C** to give the α1,3-linked hexasaccharide intermediate (87% when using $BF_3{\cdot}OEt_2$ with nearly no double glycosylation, 72% when using TMSOTf because of double glycosylation). The hexasaccharide was converted into diol **19**, which was glycosylated with donor **B1** to the triantennary octasaccharide **20** (85%). Late introduction of the bisecting donor **E3** proceeded smoothly and furnished the bisected N-glycan **21** in 86% yield. Following the one-pot method, 81% of **21** was obtained directly from **19** (vs. 73% for the sequential procedure).

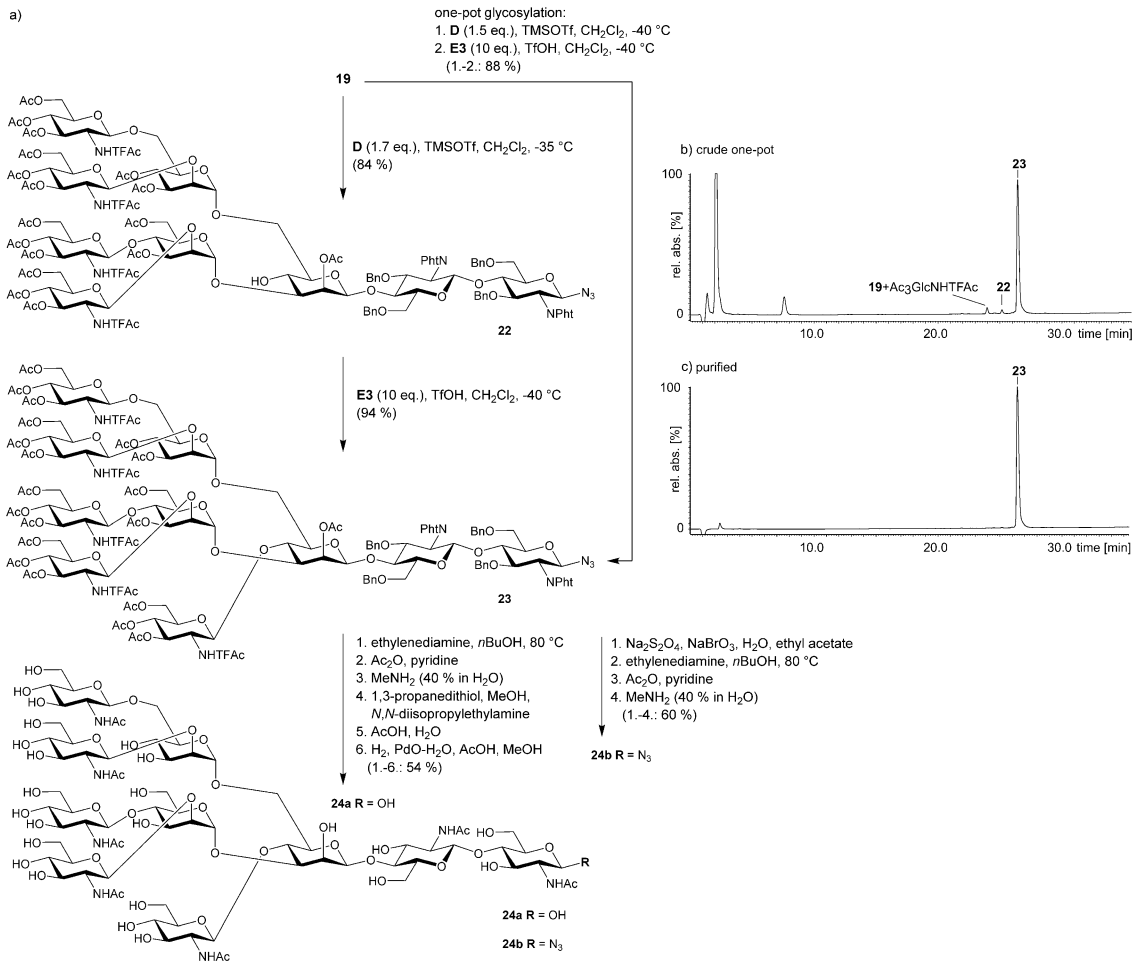


Scheme 4. a–f) Sequential and one-pot synthesis of **16** through late introduction of bisecting GlcNAc. g) HPLC–MS of crude one-pot reaction (e) and h) purified **16** (UV traces).



Scheme 5. Sequential and one-pot synthesis of triantennary bisected N-glycans **18** and **21**.

Acceptor **19** was also extended to the tetraantennary nonasaccharide **22** (84%) and efficiently converted into bisected decasaccharide **23** (94%, Scheme 6). Very high overall yields (88% of **23**) and excellent purities (Scheme 6b,c) were also obtained with the one-pot procedure (vs. 78% for the sequential procedure).



Scheme 6. a) Synthesis and deprotection of tetraantennary bisected N-glycan **23**. b) HPLC–MS of crude one-pot reaction and c) purified **23** (UV traces).

The tetraantennary azide **23** was fully deprotected in six steps to furnish the reducing decasaccharide **24a** (54%). The versatile bisected N-glycan azide **24b** was accessible through selective debenzylation[22] in four steps (60%). Comparison of the key proton resonances of **24a** and **24b** with isolated **24a**[28] showed almost perfect identity (max. ±0.02 ppm, Table S1).

NMR characterization of the four bisected N-glycans was carried out through a combination of 2D-NMR experiments[27] (see the Supporting Information), which allowed full assignment of the resonances and confirmed the regio- and stereoselectivity of the glycosidic linkages. For bisected N-glycans, the typical ^{13}C downfield shift of 7 ppm for C-4[3] was observed.

Remarkably, the glycosylation yield for the late introduction of a bisecting GlcNAc was independent of the number of antennae for the TFAc-protected N-glycan acceptors (**14**, **17**, **20** and **22**), thus indicating a similar accessibility of OH-4[3]. Owing to the nearly quantitative conversions and the favorable flash chromatography properties of the TFAc-protected N-glycans, the one-pot reactions reproducibly achieved higher overall yields than two separate glycosylations.

In summary, a flexible and general access to complex N-glycans was developed by employing modular N-TFAc-protected donors for all antennae. The resulting N-glycans are suitable acceptors for the highly efficient late introduction of a bisecting GlcNAc. The strategy provides a single route to multiantennary N-glycans as well as their bisected derivatives via imidates, with unprecedented yields even in a one-pot double glycosylation. With the new method, rare N-glycans of the bisected type can be readily obtained, providing defined tools to decipher the biological roles of bisecting GlcNAc modifications.

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