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Synthesis of the fucosylated biantennary *N*-glycan of erythropoietin

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Abstract—A synthesis of the protected biantennary *N*-glycan of the naturally occurring glycoprotein, erythropoietin, is described.

Our laboratory is currently committed to a major initiative seeking to accomplish the de novo synthesis of the naturally occurring glycoprotein, erythropoietin (EPO). Aside from the obvious synthetic allure of this highly complex and challenging target, we were particularly drawn to EPO as a function of its remarkable therapeutic profile. EPO, a 166-residue, multiply glycosylated protein, is widely used in the treatment of anemia.[1] EPO possesses four carbohydrate domains, three of which are N-linked to asparagines and one of which is O-linked to a serine. Various studies have established the importance of the carbohydrate motifs in mediating the stability and in vivo activity of EPO. However, EPO is currently available only as a mixture of glycoforms. Without access to homogeneous EPO, it is difficult to perform rigorous comparative studies on the properties of the different glycoforms. Through recourse to total synthesis, we will be in the unique position of having potential access to a number of different EPO glycoforms for biological studies.

The total synthesis of a glycoprotein such as EPO would be a complicated matter. Success would be predicted on the ability to devise solutions to a range of synthetic challenges that will arise. In order to address some anticipated issues, we first applied a cysteine-based glycopeptide ligation strategy.[2] This was recently extended to accommodate reiterative couplings, where glycopeptides are sequentially coupled to form a tri-glycosylated peptide.[3] In light of the paucity of cysteine residues on the backbone of EPO, we have developed a novel cysteine-free glycopeptide coupling protocol.[4] With these enabling methodologies in hand, we next approached the syntheses of the carbohydrate domains of EPO. We report herein the synthesis of the biantennary N-linked type glycan (cf. **1**), incorporating the complicating fucose and sialic acid motifs, which are believed to be important for the in vivo activity of EPO (Fig. 1).

Our laboratory has had a longstanding interest in developing improved methods for the synthesis of complex carbohydrates.[5] In 2003, we disclosed a synthesis of a pentasaccharide *N*-glycan featuring application of Crich's direct coupling method for the formation of the key mannose–chitobiose linkage.[6] More recently, we completed the synthesis of multibranched *N*-acetyllactosamine type PSA glycans.[7] In the course of this endeavor, we developed a useful and direct protocol for the appendage of complex glycans to peptides,

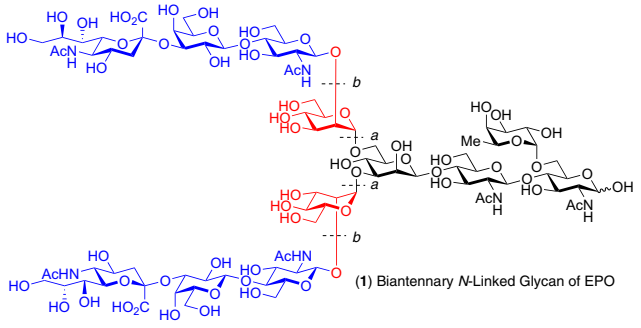

Figure 1. Biantennary N-linked glycan (**1**).

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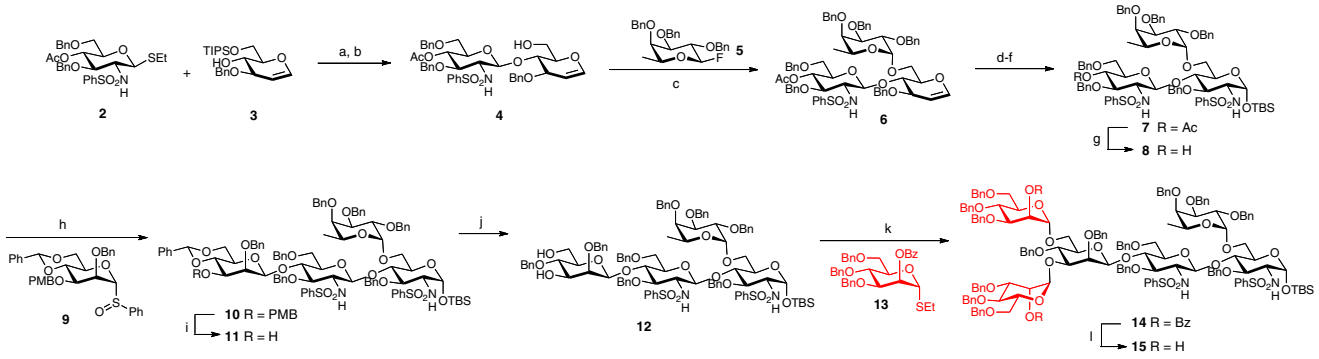
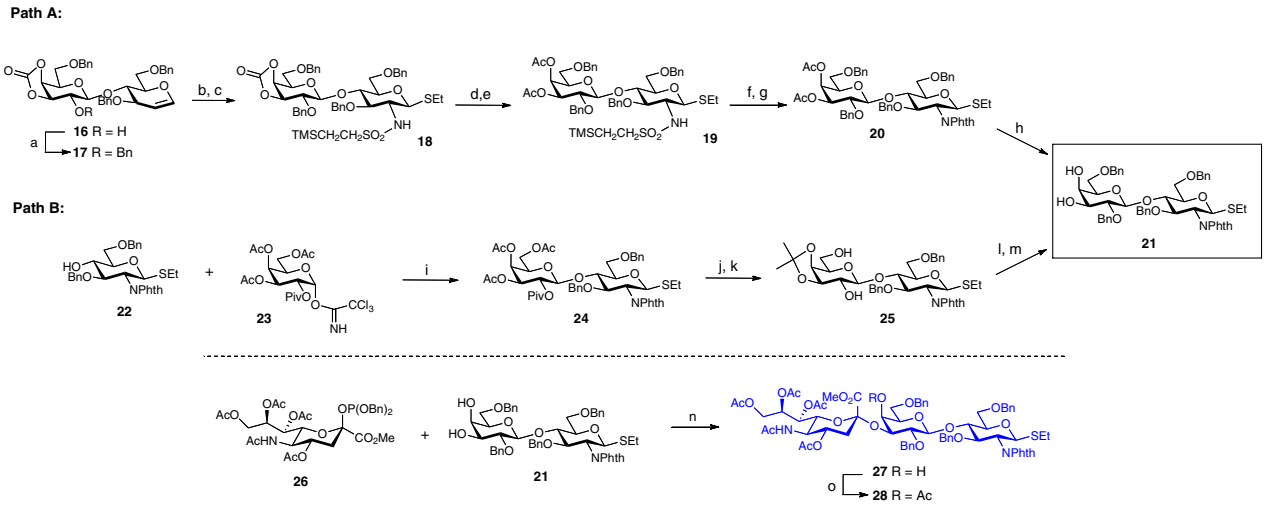
commencing with global deprotection of the glycan domain (Na/NH_3) followed by a Kochetkov amination–Lansbury aspartylation sequence.[8] We expect to apply these advances to the synthesis of **1** and its appendage to the peptide domain. We envisioned a synthetic pathway to **1** that would build from the tetrasaccharide core and would involve sequential diglycosylation steps (a and b) for the installation of the antennary portions of the molecule.

Our synthesis commenced with known monosaccharides **2** and **3**,[9] which are available from D-glucal (Scheme 1). Methyl triflate-mediated glycosylation between **2** and **3**, followed by removal of the C_6 –TIPS group, provided disaccharide **4**. The fucose motif was introduced through glycosyl fluoride **5**. To simplify the final steps of the synthesis, we elected to functionalize the reducing end double bond at this early trisaccharide stage. To that end, **6** was subjected to iodosulfonamidation, hydrolysis, and TBS protection to provide protected trisaccharide **7**. Saponification of **7** furnished the requisite acceptor, **8**. Subsequent triflate-mediated mannosylation of **8** with a slight excess of donor **9** proceeded smoothly at −78 °C to give an anomeric mixture of **10** with an α/β ratio of 1:5 (75–84% yield). The undesired α–isomer could be removed through deprotection of the C_4 PMB group with cerium ammonium nitrate (CAN), to yield tetrasaccharide **11** as a single isomer. Regioselective cleavage of the benzylidene acetal with borane in the presence of dibutyl boron triflate afforded the 3,6-diol, **12**. Twofold glycosylation of the latter was accomplished through Sinaÿ radical activation, to afford hexasaccharide **14**.[10] Subsequent cleavage of the two benzoates provided **15**.

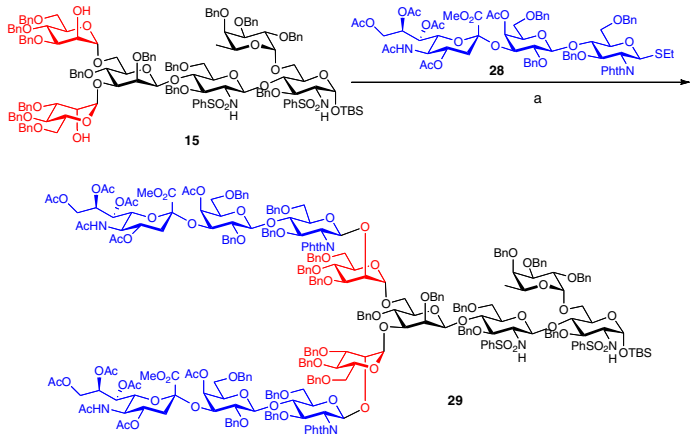
With the hexasaccharide in hand, we turned our attention to the preparation of trisaccharide thiol donor (Scheme 2). The key disaccharide intermediate in this sequence (**21**) could be prepared through two alternate pathways, as shown. According to Path A, the route to **21** commenced with the known disaccharide **16**, available through DMDO mediated glycal epoxidation fol-



Scheme 1. Reagents and conditions: (a) MeOTf, DTBP, DCM, 64%; (b) TBAF, AcOH, THF, 96%; (c) $Sn(OTf)_2$, DTBP, THF, 65–72%; (d) IDCP, $PhSO_2NH_2$, THF; (e) H_2O, Et_3N, THF; (f) TBSOTf, 2,6-lutidine, DCM; (g) NaOMe, MeOH, 44–55% (four steps); (h) Tf_2O, TBP, DCM, 75–84%; (i) CAN, MeCN, H_2O, 65–70%; (j) BH_3·THF, Bu_2BOTf, 70–74%; (k) $(BrC_6H_4)_3NSbCl_6$, MeCN, 90%; (l) NaOMe, MeOH, 83–85%.



Scheme 2. Reagents and conditions: (a) BnBr, NaH, DMF, 70–80%; (b) IDCP, $TMSCH_2CH_2NH_2$, THF, (c) LHMDS, EtSH, DMF, 92% (two steps) (d) NaOMe, MeOH, 90%; (e) Ac_2O, pyr, 92%; (f) CsF, DMF, 64–77%; (g) phthalic anhydride, pyr, Piv_2O, 74–85%; (h) NaOMe, MeOH, 86–90%; (i) TMSOTf, DCM, −78 °C, 79%; (j) NaOMe, MeOH, 97%; (k) DMP, CSA, 68%; (l) NaH, BnBr, TBAI, THF, 85%; (m) aq HOAc, 60 °C, 92%; (n) TMSOTf, MeCN, 55–62%; (o) Ac_2O, pyr, 84–87%.



Scheme 3. Reagents: (a) $(BrC_6H_4)_3NSbCl_6$, MeCN, 50%.

lowed by direct glycosylation.[11] Following protection at C_2, the reducing end olefin was functionalized through iodosulfonamidation followed by roll-over, as shown. Intermediate **18** was advanced to disaccharide **20** through a series of protecting group manipulations. Following saponification of **20**, the requisite diol **21** was in hand. An alternate route to **21** (Path B) commenced with monosaccharide **22**.[12] Coupling with trichloroimidate donor **23**, equipped with a pivaloate at C_2, provided disaccharide **24**. Hydrolysis followed by selective acetonide formation yielded **25**, which was then converted to disaccharide **21** in two steps, as shown.[13] Under previously reported conditions, the regioselective coupling between **21** and glycosyl phosphite **26** proceeded smoothly to afford trisaccharide **27** in 55–62% yield.[14] Acetylation at C_4 furnished the requisite trisaccharide donor **28**.

In the event, we were pleased to find that glycosylation of **15** with excess amounts of thioglycoside **28** under Sinaÿ radical cation activation provided the dodecasaccharide **29** in 50% yield, along with a small amount of the monocoupled product (Scheme 3).

In summary, we have developed a convergent strategy toward the synthesis of complex biantennary N-linked glycan of EPO. Studies which interface the total synthesis of the fucose and sialic acid dodecameric oligosaccharides described above with various ligation strategies are well underway and the results will be reported in due course.

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