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Mature homogeneous erythropoietin building blocks by chemical synthesis: the EPO 22–37 glycopeptide domain presenting the full N-linked dodecasaccharide

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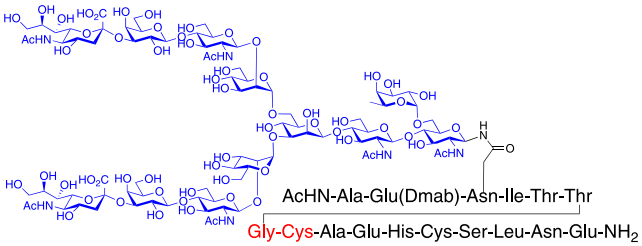
Abstract—A synthesis of EPO 22–37 glycopeptide (**1**), presenting the N-linked dodecasaccharide of erythropoietin, is described.

We have been focusing on preparing homogeneous erythropoietin (EPO) by chemical synthesis.[1] The complexity of the undertaking has served to identify problems in oligosaccharide synthesis and in glycopeptide synthesis, and has prompted new strategies for ligating such synthetic polypeptides en route to larger structures. In addition to enabling access to naturally biosynthesized glycoproteins, chemical synthesis offers the realistic prospect of building homogeneous modified glycopeptides designed through insights from structural biology.[2] The heart of the challenge is that of building glycopolypeptides which are mature in their oligosaccharide domains, and can be ligated with broad structural latitude to peptides and peptidoglycans. With these capabilities, one could target specific, known biologically active glycoproteins while seeking structural advantages through the modalities of medicinal chemistry (Fig. 1).

In an earlier publication directed to the erythropoietin (EPO) problem, we related the synthesis of the protected biantennary dodecamer glycan **4**.[3] The synthesis commenced with the preparation of hexasaccharide (**2**) and trisaccharide (**3**), through recourse to glycal assembly methods developed in our laboratory and through application of the Crich–Kahne direct mannosylation protocol.[4,5] Under Sinay radical cation activation conditions,



(**1**) EPO (22-37)

Figure 1. EPO 22–37 glycopeptide (**1**).

coupling of **2** with excess amounts of **3** successfully provided the fully protected dodecasaccharide **4**[6] (Scheme 1).

Given the degree of functionalization of dodecasaccharide **4**, careful consideration had to be given to the sequence of the deprotection pathway. First, the methyl esters of the erstwhile sialic acid functionalities would be unmasked, along with the ten acetyl protecting groups, in order to achieve compatibility with conditions required for the subsequent phthalamide removal. In practice, hydrolysis of the methyl esters, accompanied by removal of the resident acetyl protecting groups, provided an appropriate substrate for cleavage of the two phthalamide moieties through exposure to ethylenediamine.[7] This intermediate was subjected to peracetylation to afford the bis-lactone intermediate **5**. The next step involved the removal of the anomeric TBS ether through

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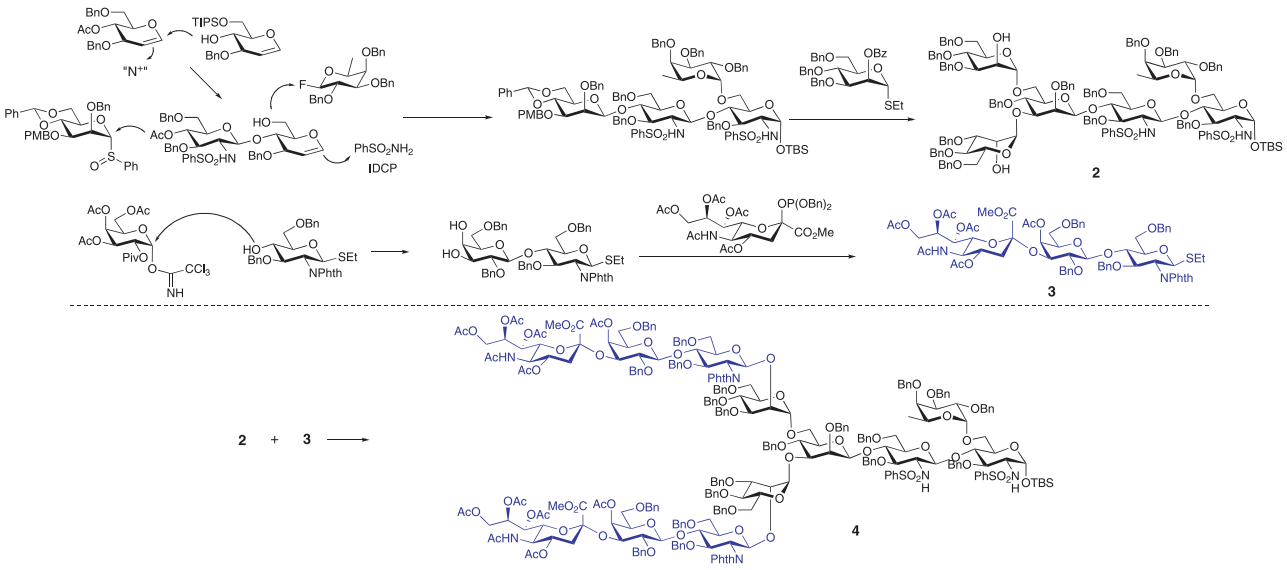


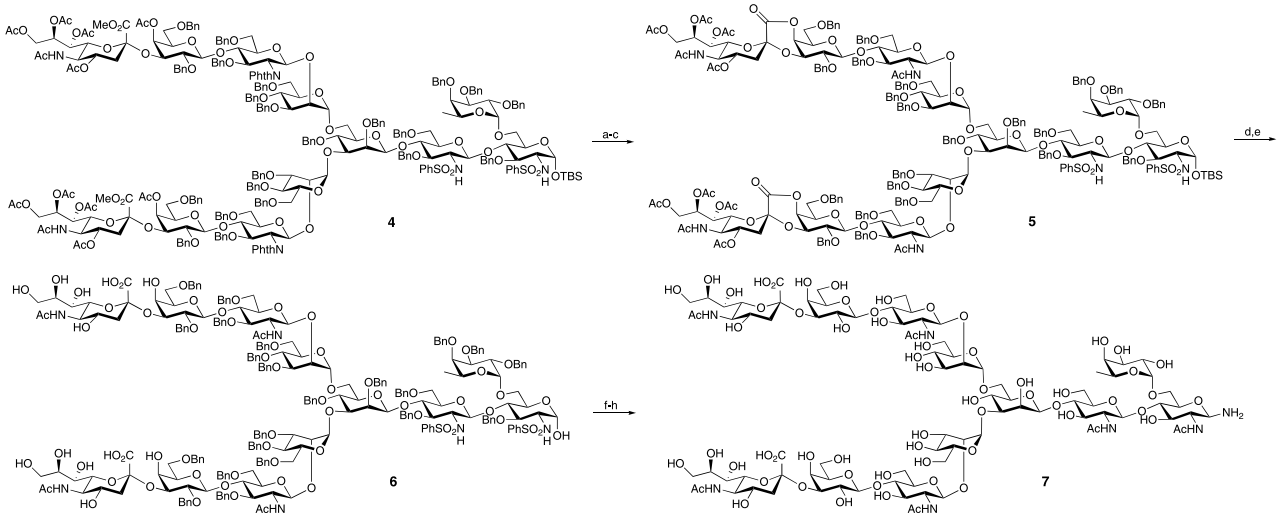
Scheme 1. Synthesis of the protected glycan of the EPO dodecasaccharide.

exposure to TBAF. Subsequent solvolysis gave rise to intermediate **6** (Scheme 2).

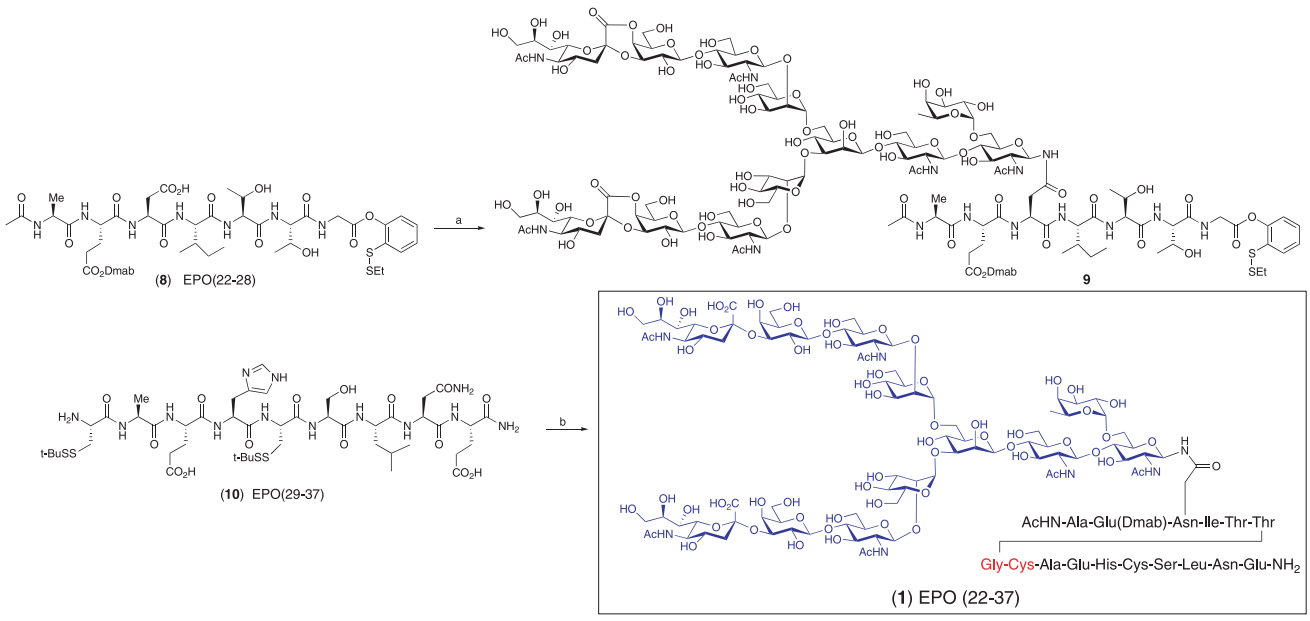
At this stage, we were obliged to face a key challenge in this sequence—the concomitant removal of the 22 benzyl ethers and the two phenyl sulfonamides of the ensemble *with maintenance of the anomeric hydroxyl group*. Fortunately, exposure of **6** to Birch reduction conditions (Na/NH_3), following previously developed procedures, indeed accomplished the global deprotection.[8] Finally, Kochetkov anomeric amination conditions readily provided the glycosylamine **7** in good overall yield from the fully protected **4**.[9] The realization of compatibility of the sialic acid residues with the Kochetkov amination conditions was particularly welcome.

With the deprotected dodecasaccharide in hand, we turned our attentions to the ultimate goal of merging the glycan with the EPO peptide fragment. Toward that end, peptide **8**, which corresponds to EPO (22–28), was prepared under previously described conditions, and equipped with a C-terminal phenolic ester (Scheme 3).[10] Aspartylation between **7** and **8**, with concurrent lactone formation, provided **9**.[11] At this stage, we investigated the feasibility of native chemical ligation between **9** and the EPO (29–37) peptide (**10**), itself available through solid-phase peptide synthesis. Happily, under improved conditions (PBS, PhSH, TECP), ligation using our O → S migration based method proceeded smoothly to afford **1**, corresponding to the full EPO 22–37 glycopeptide domain, in good overall yield.[10,12]

In summary, the synthesis of a key fragment contained in the naturally occurring glycoprotein, erythropoietin, has been accomplished by strictly chemical means. Toward this end, the complex dodecasaccharide domain of EPO has been successfully assembled, deprotected, and coupled to the requisite peptide fragment through



Scheme 2. Reagents and conditions: (a) NaOMe, MeOH; (b) $H_2NCH_2CH_2NH_2$; (c) Ac_2O, py.; (d) TBAF, AcOH; (e) NaOMe, $MeOH/H_2O$; (f) Na, NH_3; (g) Ac_2O, $NaHCO_3$; (h) NH_4HCO_3, H_2O (63% overall yield).



Scheme 3. Reagents and conditions: (a) **7**, HATU, iPr$_2$NEt, DMSO, 40%, (b) PBS, 1% PhSH, TCEP, 56%.

aspartylation. Furthermore, the peptide domain has been elongated following application of our recently developed native chemical ligation methodology. The assembly of **1** clearly lays the groundwork for the realization of the ultimate goals, as described above. A corresponding advancement in the construction of mature O-linked glycopeptides is described in the letter which will follow.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.09.050.

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