Glycopeptides (1)

In Pursuit of Carbohydrate-Based HIV Vaccines, Part 1: The Total Synthesis of Hybrid-Type gp120 Fragments**

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Notwithstanding enormous scientific effort, the development of a vaccine against HIV has, thus far, proven to be elusive.[1] To date, commonly utilized vaccine formulations have been unable to elicit potent and broadly neutralizing immune responses.[2] The very high rate of viral variation is often cited as the major impediment to vaccine design. Another serious complication is the very low immunogenicity of the protein domain of the viral surface envelope protein gp120. This phenomenon could be explained by a number of factors.[3] Extensive glycosylation of this envelope protein, gp120, is arguably the most effective viral defense mechanism.[4,5] Indeed, gp120 is typically modified with 24 carbohydrate motifs, which render most of its polypeptide architecture virtually inaccessible to the immune system.[6]

Our thought was that these glycans could themselves serve as targets for an anti-HIV vaccine. In favor of exploring such a course is the fact that some of the glycans are highly conserved, and are located on the outer side of the gp120 trimer—a positioning that could well enhance their accessibility. Thus, if a suitable construct, capable of eliciting a focused immune response to gp120 glycans could be designed and synthesized, a key element of the HIV defense system would have been targeted, and an effective vaccine could be at hand. Indeed, some agents that bind gp120 glycans (e.g. the dendritic cell receptor DC-SIGN and the bacterial protein cyanovirin-N) are actually presently known.[7,8]

Further support for this line of attack arose from investigations on the epitope structure of 2g12, one of the most potent broadly neutralizing antibodies known to date.[9,10] Alanine scanning mutagenesis demonstrated that the presence of a number of glycan-carrying asparagines in gp120 is essential to antibody binding.[11] It was initially suggested that glycans at Asn295 and 332 may form the antibody epitope. In a separate account, the nature of the glycans crucial to 2g12 binding was elucidated. Experiments with a range of mannosidases and endoglycosidases prompted the conclusion that hybrid or high-mannose-type glycans on the gp120 comprise the 2g12 epitope-forming carbohydrates.[12] These findings provide a conceptual launching point for designing an HIV vaccine.

Even in the face of spectacular advances in the field of biotechnology,[13] it is our judgment that, at the present time, chemical synthesis constitutes the *only* prospect for building strictly homogeneous glycopeptides of suitable complexity in the required amounts and with the structural diversity needed to support such a vaccine-targeting discovery program.[14–16] Herein, we describe the first synthesis of a hybrid-type nonasaccharide and its incorporation into HIV gp120$^{316–335}$ N-linked glycopeptides.

We commence by setting forth the essentials of the underlying logic of the strategy. Synthesis of the glycan (Scheme 1) would start with the preparation of trisaccharide **7**, which contains "virtual" and "in-place" acceptor points on the terminal mannose unit. The plan takes recourse to glycal assembly methods that we developed in the past.[17,18] Applicability of the methods to the problem at hand gained enormously from the very powerful β-mannosylation procedure introduced by Crich and co-workers.[19,20] The "lower" (C3′) branching mannose would first be installed, followed by glycosylation with a lactosamine donor (see **7**→**8**). Opening of the benzylidene ring would then provide the opportunity to install the "upper" high-mannose cluster at C6′ (**8**→**9**).

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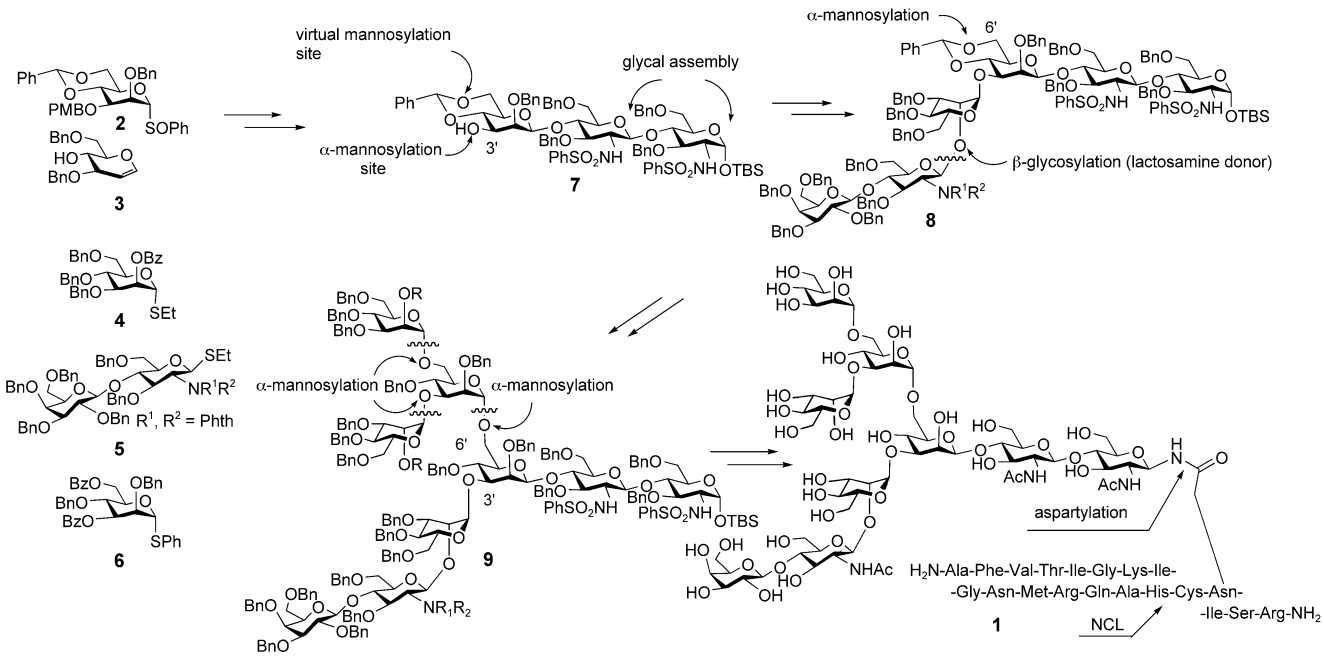
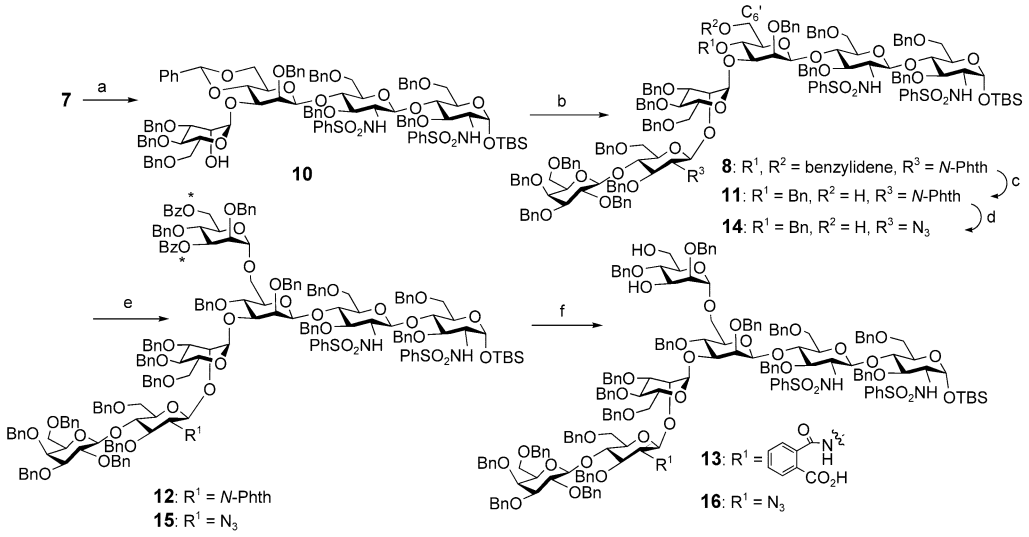


Scheme 1. Synthetic strategy for the preparation of glycopeptide **1**. PMB = *p*-methoxybenzyl; TBS = *tert*-butyldimethylsilyl; Phth = phthaloyl. NCL = native chemical ligation.

We had earlier documented the use of an amination–aspartylation native chemical ligation (NCL) sequence (drawing from earlier findings by the groups of Kochetkov,[21] Lansbury,[22] and Kent and Muir[23]) for the synthesis of large N-linked glycopeptides of high complexity.[24–26] The 331 Cys adjacent to the aspartylation site (332) in gp120 could provide a convenient disconnection point for NCL. Accordingly, we sought initially to prepare a glycan pentapeptide construct expecting to use it in conjugation with the pentadecapeptide thioester (Scheme 1).

In the event, the progression began with the trisaccharide acceptor **7**,[27] which was prepared from 3,6-dibenzylglucal **3** and the sulfoxide donor **2**.[20] Glycosylation of **7** with donor **4** by using the Sinaÿ radical activation conditions[28] proceeded uneventfully to afford the tetrasaccharide in 80% yield. Debenzoylation provided alcohol **10** and set the stage for coupling with the lactosamine thio donor **5** (Scheme 2). Hexasaccharide **8** was obtained in 60% yield, thereby completing the synthesis of the "lower" part of the glycan (projecting from C3′ of the initial trisaccharide) in an efficient



Scheme 2. Synthesis of heptasaccharides **13** and **16**; a) 1. **4**, $(BrC_6H_4)_3NSbCl_6$, MeCN, 80%; 2. NaOMe/MeOH, 89%; b) **5**, $(BrC_6H_4)_3NSbCl_6$, MeCN, 60%; c) BH_3·THF, Bu_2BOTf, CH_2Cl_2, 75%; d) 1. $NH_2CH_2CH_2NH_2$, toluene, 90°C; 2. TfN_3, MeOH/CH_2Cl_2, 50%; e) **6**, $(BrC_6H_4)_3NSbCl_6$, MeCN, 58% for **12**, and 48% for **15**; f) NaOMe/MeOH, 62% for **16**. Tf = trifluoromethanesulfonyl.

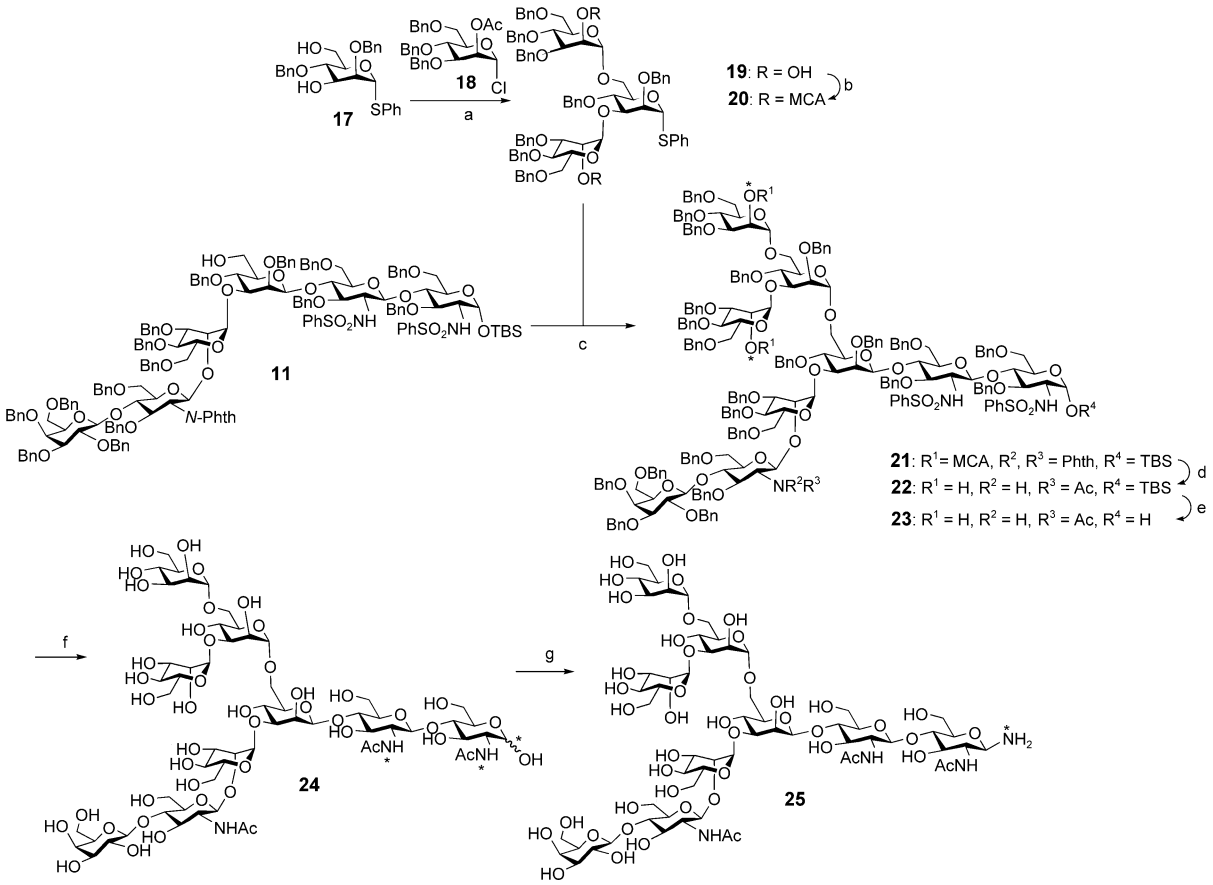
manner. To incorporate the high mannose segment, the primary hydroxy group (C6′) in **8** was liberated by regioselective opening of the 4,6-benzylidene acetal with Bu_2BOTf/BH_3,[29] and the resulting acceptor, **11**, was glycosylated with diester thio donor **6** to give **12** in 58% yield.

Saponification of the two benzoates in **12** (see asterisks) followed by dimannosylation of the corresponding diol would have completed the nonasaccharide assembly. However, this seemingly routine deprotection step, when conducted in the multifaceted setting of **12**, turned out to be problematic. In particular we found that the cleavage of the second benzoate group in **12** was very slow, and further complicated by concurrent hydrolysis of the phthalimide group (giving **13**). To circumvent this problem, the phthalimide group of **11** was replaced by an azide function to give **14**,[30] which was coupled with **6** to provide heptasaccharide **15** in 48% yield.

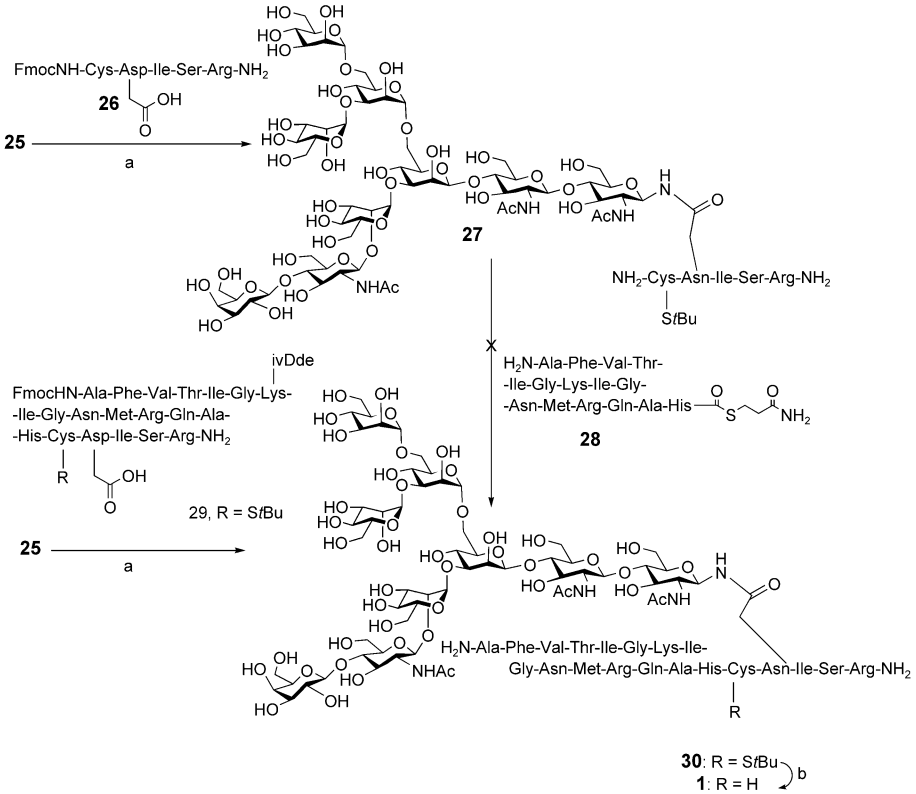
Benzoate deprotection in the presence of excess NaOMe now proceeded smoothly in 62% yield. Although the phthalimide instability problem had been overcome by recourse to an azide function at the level of **11**, we decided to explore a still more convergent approach, featuring condensation of a preassembled trimannose block with the primary hydroxy group in **11**, retaining the intact phthalimide group (Scheme 3).

The required trisaccharide was prepared from thiomannoside diol **17** and chloride **18** in 65% yield following the removal of the two acetates. The resulting alcohols were reprotected as their monochloroacetates in 79% yield, anticipating that the MCA protecting groups could be selectively removed without affecting phthalimide group. In this way, clean access to the required branching point acceptor sites (see asterisks in **21**) would have been provided.

After optimization of the coupling conditions, it was found that the glycosylation of hexamer acceptor **11** with trisaccharide donor **20** proceeded in 78% yield to afford the nonasaccharide **21**. The MCA protecting groups were then quantitatively removed by thiourea. This step was followed by conversion (see structure **22**) of the phthalimide group into the required acetamide function and liberation of anomeric hydroxy site with TBAF/AcOH. Compound **23**, bearing the free reducing end, was then subjected to global deprotection with sodium in liquid ammonia. At this stage we exploited the remarkable stability of the anomeric reducing end (see asterisk) to a massive excess of sodium—ammonia, which was reported earlier by our group.[31–33] The free amines in the product were then acetylated with acetic anhydride in saturated $NaHCO_3$ (see asterisks) to afford the glycan **24** bearing the anomeric OH (see asterisk) in 70% yield.



Scheme 3. Synthesis of free glycosyl amine **25**; a) 1. AgOTf, DTBP, CH_2Cl_2, 2. NaOMe/MeOH, 65%; b) $(ClCH_2CO)_2O$, py, CH_2Cl_2, 79%; c) $(BrC_6H_4)_3NSbCl_6$, MeCN, 78%; d) 1. $NH_2CH_2CH_2NH_2$, *n*-BuOH/toluene, 90°C; 2. Ac_2O, py; 3. NaOMe/MeOH, 69%; e) TBAF/AcOH, 89%; f) 1. Na/NH_3, −78°C, 2. $NaHCO_3$, Ac_2O, 70%; g) NH_4HCO_3/H_2O. DTBP = 2,6-di-*tert*-butylpyridine; MCA = monochloroacetate; TBAF = tetra-*n*-butylammonium fluoride.



Scheme 4. Synthesis of glycopeptide; a) 1. HATU, DIPEA, DMSO; 2. NH_2NH_2, piperidine, DMF, 30% for **27** and 20% for **30**; b) MES-Na, DMF, DIPEA, H_2O, 95%. HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; DIPEA = *N*,*N*-diisopropylethylamine; DMSO = dimethyl sulfoxide; DMF = *N*,*N*-dimethylformamide; Fmoc = 9-fluorenylmethyloxycarbonyl; ivDde = 4,4-dimethyl-2,6-dioxocyclohex-1-ylidine-3-methylbutyl.

Compound **25** bearing the free anomeric amine, (see asterisk) was obtained by following the Kochetkov amination procedure[21] and was used for peptide coupling without further purification.

Coupling of **25**, with excess pentapeptide **26**, under previously developed conditions[24] was followed by cleavage of the Fmoc group to afford glycopeptide **27** (30% yield for two steps). Unfortunately, despite numerous attempts, we were unable to produce **1** by native chemical ligation of **27** and **28** (Scheme 4).[34] This unexpected failure obliged us to investigate the possibility of direct coupling of the non-asaccharide amine **25** and eicosapeptide **29**. The latter was prepared by using automated Fmoc synthesis.[35] We were pleased to find that conjugation of such large substrates is in fact feasible, though not yet efficient. In the event, glycopeptide **30** was obtained in 20% yield following the removal of Fmoc and ivDde groups from the resulting glycopeptide.

Deprotection of the S*t*Bu blocking the sulfhydryl moiety of Cys in **30** was the only step remaining for the preparation of **1**. Cleavage of this disulfide bond proved to be unexpectedly challenging. All attempts to cleave the disulfide of **30** by using MES—Na (2-sulfanylethanesulfonic acid, sodium salt),[36,37] DTT (dithiothreitol),[38–40] or TCEP (tris(2-carboxyethyl)-phosphane hydrochloride)[41–43] as reducing agents in phosphate or guanidine buffer either led to no reaction or provided complex mixtures.

Finally, we attempted to carry out the reduction in organic solvent. When the glycopeptide **30** was stirred with MES-Na in DMF with diisopropylethylamine as base at room temperature, the reduction proceeded in quantitative conversion and the corresponding product **1** was isolated in homogeneous form (NMR and LC–MS analyses[44]) in 95% yield after purification by HPLC. Full characterizations are provided in the Supporting Information.

In summary, we have, for the first time, synthesized mature hybrid type HIV gp120 glycopeptide fragments.[45] This project is now at the stage of optimal conjugation to various immunogens and en route to detailed exploration of the immunological issues discussed above.

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[45] In the following paper in this issue, we describe our efforts for the synthesis of high-mannose-type HIV gp120 glycopeptides fragments: X. Geng, V. Y. Dudkin, M. Mandal, S. J. Danishefsky, *Angew. Chem.* **2004**, *116*, 2616 – 2619; *Angew. Chem. Int. Ed.* **2004**, *43*, 2562 – 2565.

