Glycopeptides (2)

In Pursuit of Carbohydrate-Based HIV Vaccines, Part 2: The Total Synthesis of High-Mannose-Type gp120 Fragments—Evaluation of Strategies Directed to Maximal Convergence**

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There are strong grounds to suppose that some selected glycosylation patterns of the HIV viral protein gp120 can themselves serve as epitopes for potent, broadly neutralizing antibodies (e.g. 2g12).[1,2] The epitopes in question may comprise several hybrid or high-mannose-type glycans at particular asparagine loci (Asn 295, 332, 339, 386, and 392). The 2g12 antibody has been shown to recognize a cluster of $\alpha1\rightarrow2$ linked mannose residues on the HIV surface. Another argument in favor of the high-mannose-type glycan cluster epitope was reported by Burton, Wilson, and co-workers.[3] These workers described a structure of 2g12 cocrystallized with the high-mannose-type reducing oligosaccharide $Man_9GlcNAc_2$. The crystal structure demonstrated that the antibody may bind up to four individual high-mannose glycans simultaneously, thus favoring a very high affinity recognition. Accordingly, a synthetic construct that is able to elicit a strong immune response to a conserved cluster of gp120 high-mannose glycans could potentially emerge as a valuable candidate for incorporation into an HIV vaccine. In the preceding paper,[4] we related a strategy for the construction of a hybrid type gp120 glycopeptide construct.

Herein we describe the synthesis of gp120 fragments comprising one of key asparagine sites (332) modified with a fully synthetic high-mannose glycan. Although the nonamannose section of the molecule was previously prepared and tested in binding with cyanovirin-N,[5–7] no total chemical synthesis of any $Man_9GlcNAc_2$ containing glycopeptides has been reported.[8]

In our route to the glycan portion of the glycopeptide, we utilized, as proposed earlier, trisaccharide **2**,[9] which already encompasses the synthetically difficult β-mannosidic linkage, as well as differentiated C3 and C6 access points (see asterisks) for the subsequent introduction of the nonsymmetrical mannose branching pattern.

From this point onward, two strategies for progression to the octamannose motif presented themselves. One strategy would start with two consecutive mannosylations of the 3-OH and 6-OH groups of **2**, employing mannoside donors **3** and **4**, respectively, to complete the first "mannose layer". In turn, the second "layer" of three mannose units would be introduced by triple mannosylation of the pentasaccharide triol acceptor with mannoside donor **3**, providing the Man-6 octasaccharide. Saponification of the esters followed by the introduction of another trimannose layer should provide the desired Man-9 undecamer glycan (Scheme 1; "layered approach").

Alternatively, one would construct the "upper" pentamannose **5** and "lower" trimannose **6** building blocks separately, followed by coupling them with the key trisaccharide **2** at the "real" (C3) and the "virtual" (C6) acceptor sites (see asterisks), thus reaching the undecamer **7b** in a highly convergent fashion (Scheme 1; "block approach").

With the glycan matrix assembled, the next phases of the program would involve global deprotection[10] followed by amination at the anomeric site.[11] We initially envisioned that a small (penta)peptide would be introduced by aspartylation.[12,13] Finally, native chemical ligation (NCL) would complete the synthesis of **1** (Scheme 1), paving the way for conjugation to a carrier immunogen en route to fashioning a testable vaccine.[14,15]

The "layered" approach was explored first (Scheme 2). Glycosylation of the 3-OH group of trisaccharide **2** with ethylthiomannoside donor **3** under the Sinaÿ radical activation conditions[16] gave tetrasaccharide **8** bearing the benzylidene group spanning C4 and C6. The acetal linkage was opened in a reductive fashion to afford tetrasaccharide **9**. The primary hydroxy group of **9** was in turn mannosylated with phenylthiomannoside **4**. Saponification of the resulting pentasaccharide **10** exposed the three required acceptor sites (see asterisks). Trimannosylation of **11** delivered octasaccharide **12** in high yield (55 %). This protocol (saponification followed by trimannosylation) was repeated to synthesize the desired protected undecasaccharide **7a**.

Having demonstrated that the protected undecasaccharide could be assembled by the "layered approach" in an efficient manner (7 steps, 11 % overall yield), we explored a still more convergent "block approach" (Scheme 3). Pentasaccharide block **5** was assembled efficiently through two consecutive dimannosylation reactions starting from phenylthiol mannoside **14** and chloromannose donor **15**. The "lower" two trisaccharide "blocks" were joined by a MeOTf-mediated glycosylation to afford hexasaccharide **18** efficiently. Reduction of **18** released the primary hydroxy group to give **19**, which was then subjected to a 6+5 glycosylation with donor **5**. Following the examination of

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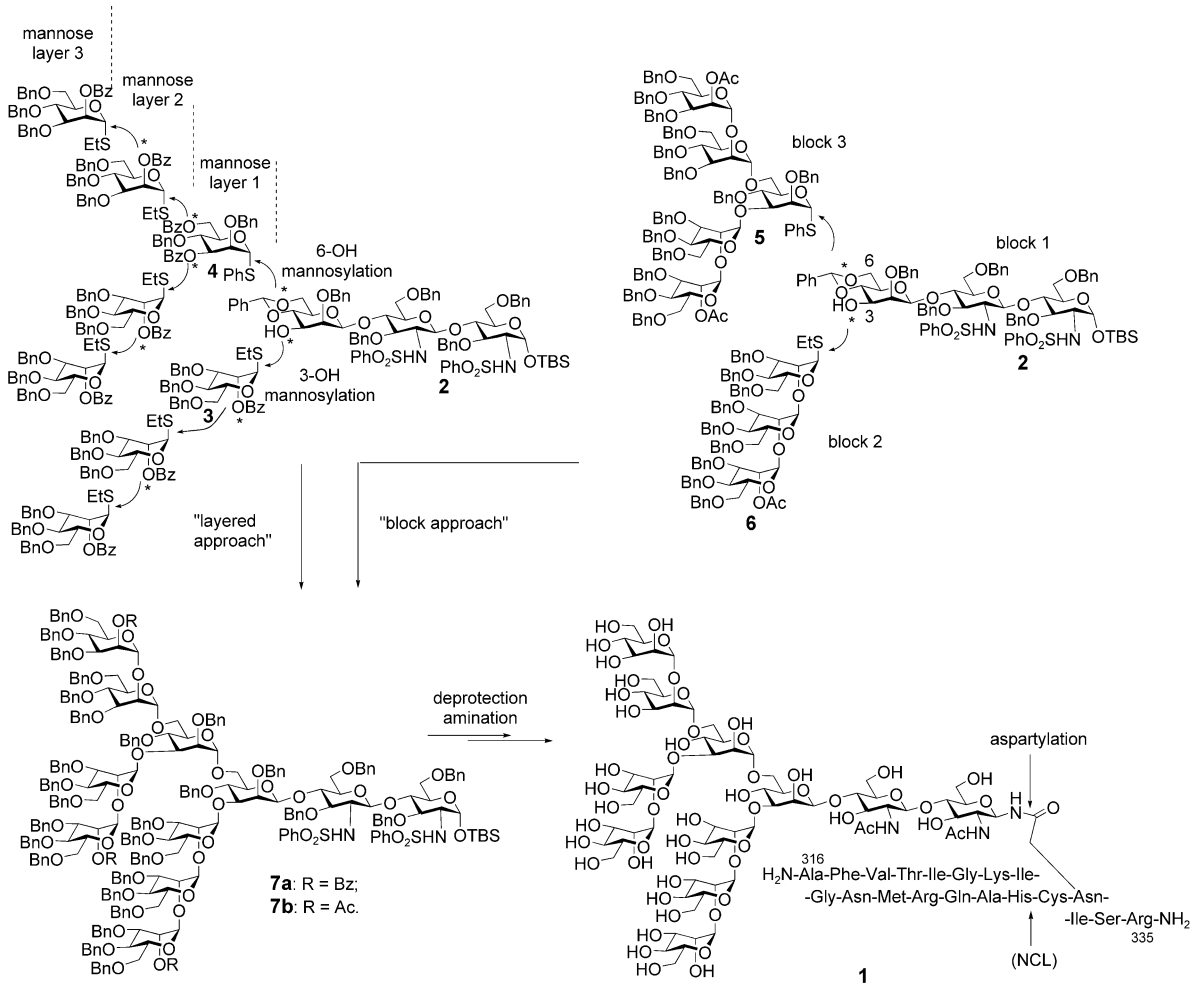
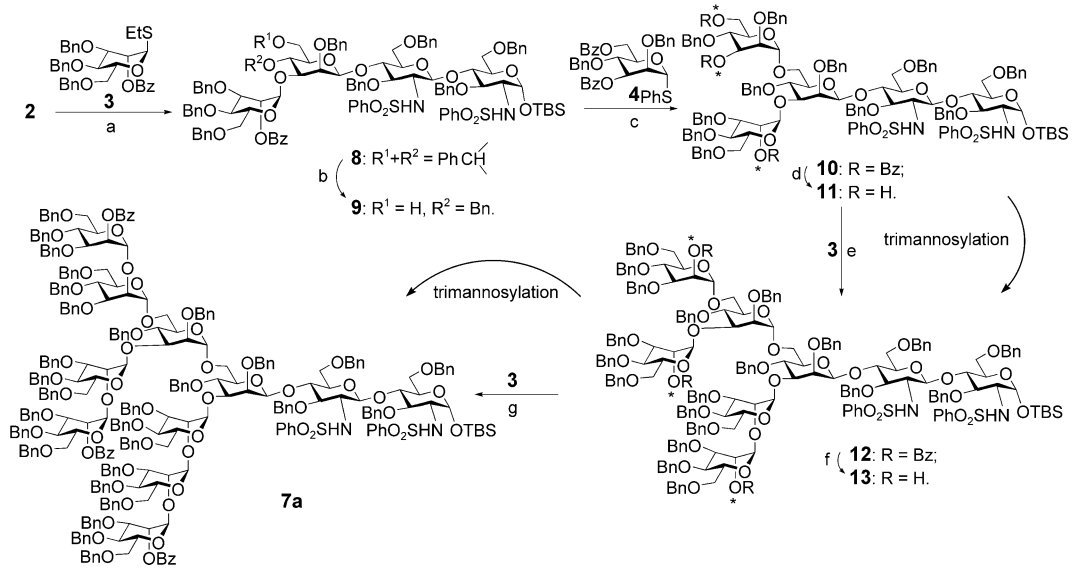
[**] This work was supported by the National Institutes of Health (CA-28824). US Army Breast Cancer Foundation postdoctoral fellowship support is gratefully acknowledged by X.G. (BC022120) and V.D. (BC020513). We thank Ms. Anna Dudkina and Ms. Sylvi Rusli (NMR Core Facility, CA-02848) for mass spectrometric analyses, Dr. George Sukenic for NMR spectroscopic analyses, and Dr. J. David Warren for help in the preparation of starting materials. We also thank Dr. Justin S. Miller, Dr. Tom Muir, Mr. Michael Hahn, and Mr. Matthew Sekedat for their generous help in the preparation of the peptides.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

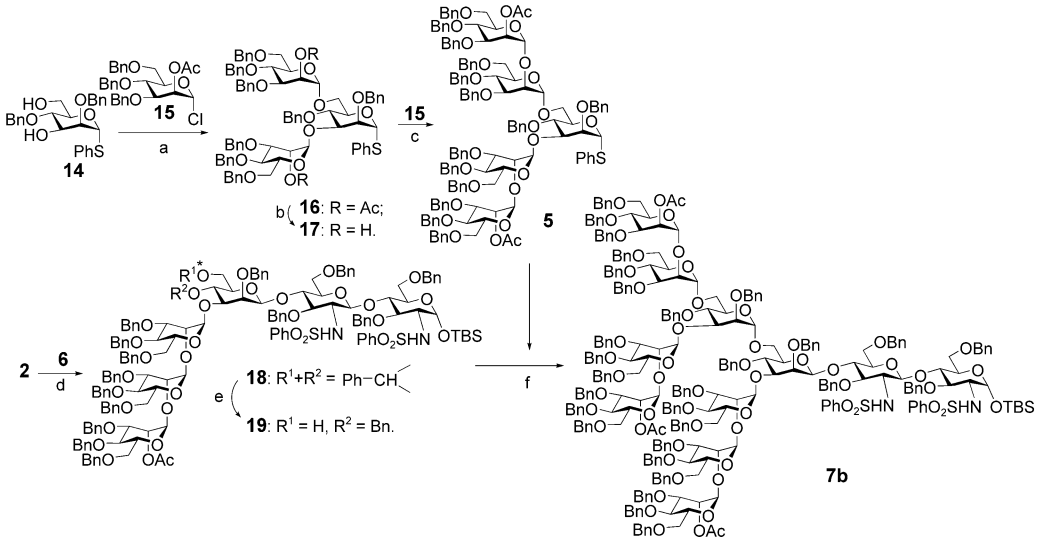
  DOI: 10.1002/ange.200353626



Scheme 1. Synthetic strategy for the assembly of the gp120 glycopeptide fragments. TBS = *tert*-butyldimethylsilyl.



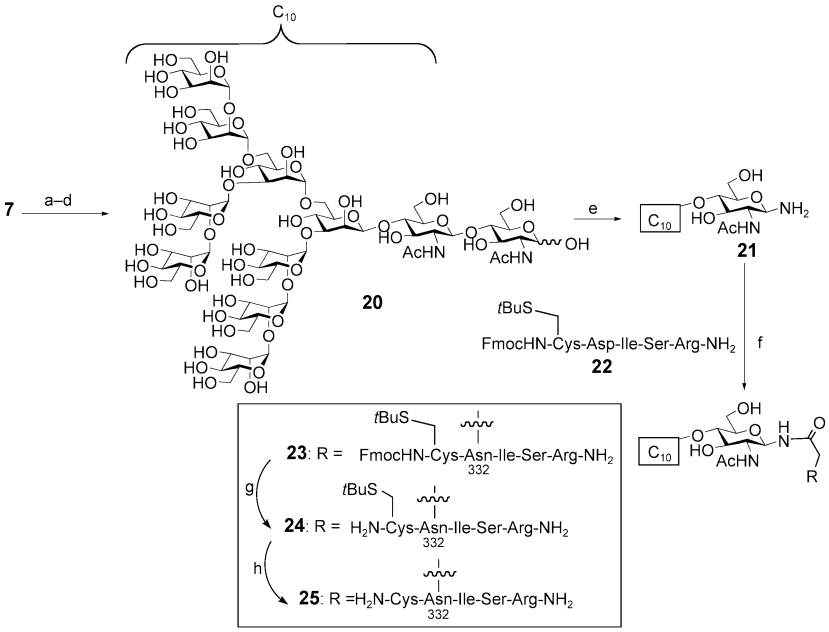
Scheme 2. Synthesis of Undecasaccharide **7a** through the "layered" approach. a) **3**, $(BrC_6H_4)_3NSbCl_6$, CH_3CN, 4 h, 78%; b) BH_3, Bu_2BOTf, THF, 0°C, 7 h, 90%; c) **4**, $(BrC_6H_4)_3NSbCl_6$, CH_3CN, 4 h, 74%; d) NaOMe, MeOH, 12 h, 91%; e) **3**, $(BrC_6H_4)_3NSbCl_6$, CH_3CN, 12 h, 55%; f) NaOMe, MeOH, 12 h, 84%; g) **3**, $(BrC_6H_4)_3NSbCl_6$, CH_3CN, 12 h, 51%. Tf = trifluoromethanesulfonyl.



Scheme 3. Synthesis of undecasaccharide **7b** through "block" approach. a) **15**, AgOTf, DTBP, CH_2Cl_2, −10°C→RT, 18 h; b) NaOMe, MeOH, 10 h, 50% for two steps; c) **15**, AgOTf, DTBP, CH_2Cl_2, −10°C to RT, 18 h; 87%; d) MeOTf, DTBP, CH_2Cl_2, −40°C to RT, 12 h, 70%; e) BH_3, Bu_2BOTf, THF, 0°C, 7 h, 86%; f) **5**, $(BrC_6H_4)_3NSbCl_6$, CH_3CN, 10 h, 63% (85% based on recovered **19**). DTBP = 2,6-di-*tert*-butylpyridine.

several protocols for coupling, it was found that the Sinaÿ radical activating conditions worked best,[16] delivering the desired undecasaccharide **7b** in 63% yield (85% based on recovered acceptor **19**). At least in this endeavor, the ultimately convergent "block approach" was indeed shown to be more concise, bringing forward the protected high-mannose **7b** in 51% yield over three steps (starting from **2**).

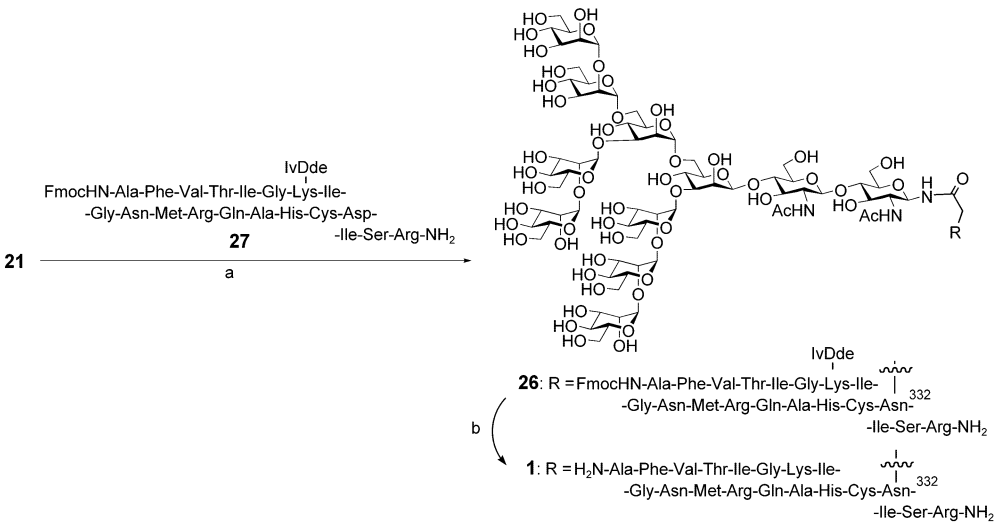
With the protected oligosaccharide in hand, we proceeded to the next phase, global deprotection (Scheme 4). Deacetylation, desilylation, and reduction (dissolving metal) of **7** afforded free glycan **20**.[10] The latter was advanced to glycosylamine **21** through Kochetkov amination.[11] This compound was first coupled with $gp120^{331–335}$ pentapeptide segment **22** (bearing Asp with the protected cysteine thiol and the N-terminal amino groups). Following removal of the Fmoc group and reduction of the disulfide, $gp120^{331–335}$ pentapeptide–high-mannose glycan conjugate **25** was in hand.



Scheme 4. Synthesis of gp120 glycosylated fragments **25**. a) NaOMe/MeOH, 12 h, 96%; b) TBAF, HAc, THF, 0°C, 1 h, 98%; c) Na/NH_3(L), −78°C, 2 h; d) Ac_2O, $NaHCO_3$(sat. aqueous solution), 87% for two steps; e) NH_4HCO_3(sat. aqueous solution), 2 d, 40°C; f) **22**, HATU, DIPEA, DMSO, 7 h; g) piperidine, DMF, 15 min, 24% from **20**; h) $HSCH_2CH_2SO_3Na$, TCEP, 3 days, 60%. Fmoc = 9-fluorenylmethyloxycarbonyl. TBAF = tetra-*n*-butylammonium fluoride; HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; DIPEA = diisopropylethylamine; DMSO = dimethyl sulfoxide; DMF = *N*,*N*-dimethylformamide; TCEP = tris(2-carboxyethyl)phosphane hydrochloride.

Our initial expectation was to utilize native chemical ligation to complete the assembly of the $gp120^{316–335}$ peptide–high-mannose glycan conjugate. However, NCL with **24** (thiol on Cys protected) or **25** (free thiol on Cys) failed to deliver the desired $gp120^{316–335}$ peptide–high-mannose glycan conjugate after several attempts. We recall that in the preceding manuscript,[4] a similar breakdown was noted with the same polypeptide elements and a related complex glycan. Together, these cases underscore unexpected limitations in the applicability of NCL in such highly ornate settings.

Fortunately, as reported earlier,[4] direct coupling with a $gp120^{316–335}$ eicosapeptide fragment **27** (bearing Asp at 332 with protected Lys and the N-terminus amino groups) was feasible (Scheme 5). The desired conjugate $gp120^{316–335}$ peptide–high-mannose glycan, **1**, was isolated following asparty-



Scheme 5. Synthesis of gp120 glycosylated fragments **1**. a) **27**, HATU, DIPEA, DMSO, 7 h; b) N_2H_4, piperidine, DMF, 15 min, 16% from **20**. ivDde = 4,4-dimethyl-2,6-dioxocyclohex-1-ylidine-3-methylbutyl.

lation and deprotection in 16% yield over 3 steps. Compound **1** was purified by reverse-phase HPLC and ^{1}H NMR spectroscopic analysis and MS data[17] are consistent with the desired structure as a homogeneous entity. Full characterizations are provided in the Supporting Information.

In summary, we have reported the first chemical synthesis of gp120 glycopeptide fragments (high-mannose-type conjugate $gp120^{316–335}$ **1** and $gp120^{331–335}$ **25**). The glycan was assembled through two efficient methods, that is, a "layered approach" and a "block approach", and then conjugated with gp120 peptide segments through direct aspartylation. In combination with the preceding manuscript,[4] our total synthesis program provides direct access to mimics of the epitope of broadly neutralizing antibody 2g12, that is, high-mannose and hybrid-type gp120 glycopeptide fragments. With the organic synthesis phase complete, the project has entered the immunogen conjugation phase, en route to a thorough evaluation of the immunological issues discussed earlier.[4]

Received: December 29, 2003 [Z53626]

Keywords: antigens · glycoconjugates · glycopeptides · mannosylation · total synthesis

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