Drug Development

Reactivity-Based One-Pot Synthesis of Oligomannoses: Defining Antigens Recognized by 2G12, a Broadly Neutralizing Anti-HIV-1 Antibody**

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The design of immunogens capable of eliciting broadly neutralizing antibodies is a major, but elusive, goal of HIV vaccine research.[1] However, a small panel of broadly neutralizing human monoclonal antibodies (mAbs)[2] isolated from seropositive donors may be very valuable in guiding the design of such immunogens.[3] One antibody from this panel is mAb 2G12, which recognizes a conserved and unusually dense cluster of oligomannose residues on the "silent face" of gp120, the envelope protein of HIV-1.[4]

The crystal structure of the Fab fragment of 2G12 shows that the antibody adopts a highly unusual domain-exchanged dimeric structure.[5] The structure produces an array of antibody combining sites in proximity to one another (Figure 1). Two conventional combining sites composed of heavy (V_H) and light (V_L) variable domains sandwich an unconventional site composed of residues from the two neighboring V_H domains.

The structure of Fab 2G12 complexed with $Man_9GlcNAc_2$ (**1**) shows that the conventional binding sites are occupied by the D1 arms of the $Man_9GlcNAc_2$ moieties, and that the terminal Manα1-2Man residues make 85% of the protein

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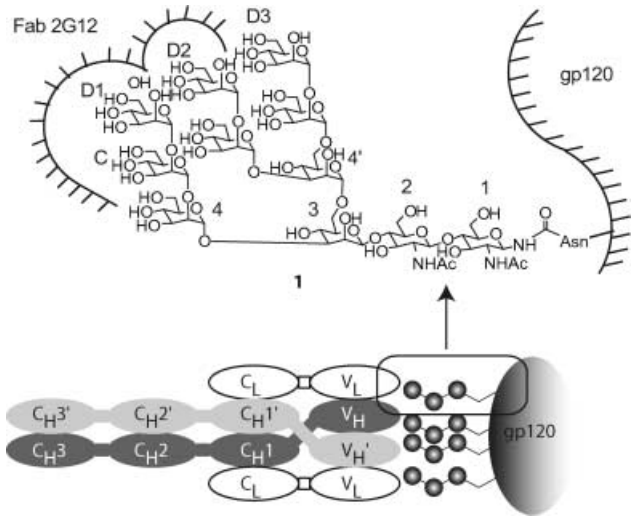


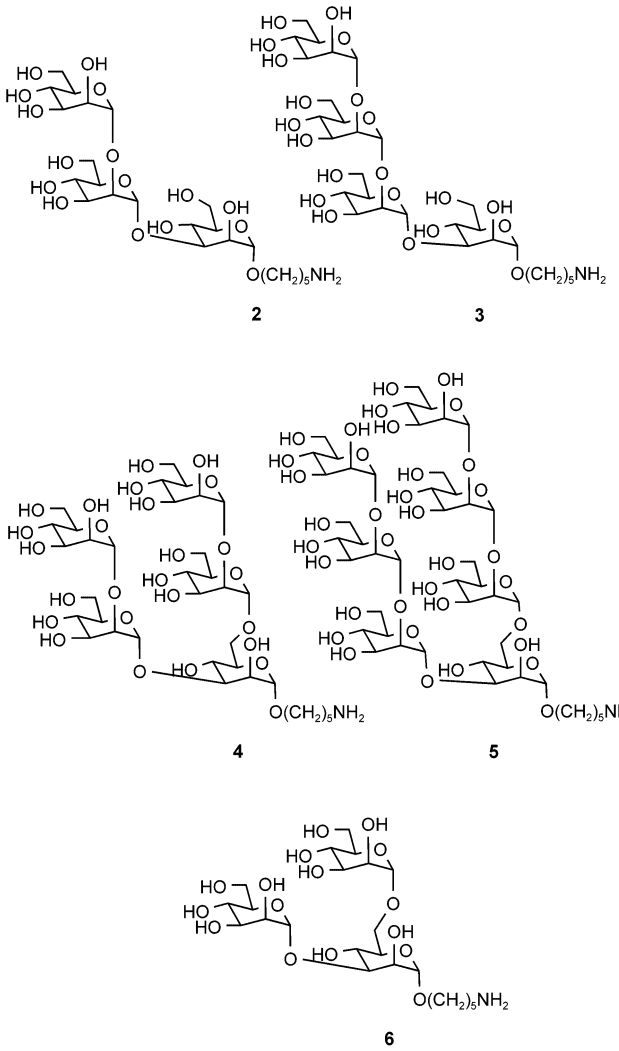
Figure 1. The structure of $Man_9GlcNAc_2$ (**1**; Man = mannose; Glc = glucose; Ac, acetyl) on gp120 interacting with the Fab fragment of the broadly neutralizing 2G12 antibody.

contacts. In the crystal, the D2 arms from two neighboring $Man_9GlcNAc_2$ moieties occupy the nonconventional site.

We are attempting to use the structural information obtained from the complexes of 2G12 with oligomannose chains to design novel immunogens that will elicit a 2G12-like antibody response. As part of these efforts toward development of an HIV vaccine, we have designed and synthesized the Manα1-2Man-containing oligomannoses **2**–**6** (Scheme 1) by using a reactivity-based, modular one-pot synthesis method that requires a minimal number of building blocks.

The programmable reactivity-based one-pot method for oligosaccharide synthesis[6] has since been successfully applied to the synthesis of several biologically significant oligosaccharides, which include Globo H,[7] Lewis Y,[8] *N*-acetyllactosamine oligomers,[9] and fucosyl-GM_1.[10] Before the one-pot synthesis could be applied to oligomannosides the relative reactivity value (RRV) of each monomer had first to be determined by a competitive HPLC assay. The RRV of each mannoside is then used as a guide for the selection of thioglycoside building blocks (Figure 2a).

An analysis of the RRVs of mannose building blocks for the one-pot synthesis of trimannose Manα1-2Manα1-2Man, the D1 arm of $Man_9GlcNAc_2$, suggests that D-mannose thioglycosides are less reactive than other thioglycosides, such as fucose and galactose.[6] In addition, 2-hydroxymannose thioglycosides are in general much more reactive than the corresponding 2-protected derivatives (Scheme 2), which makes a one-pot synthesis with a universal leaving group (used to simplify the protocol and conditions) difficult to carry out. This problem was overcome by Ley and co-workers, who synthesized trimannose with a one-pot strategy by



Scheme 1. The structures of oligomannoses **2**–**6**.

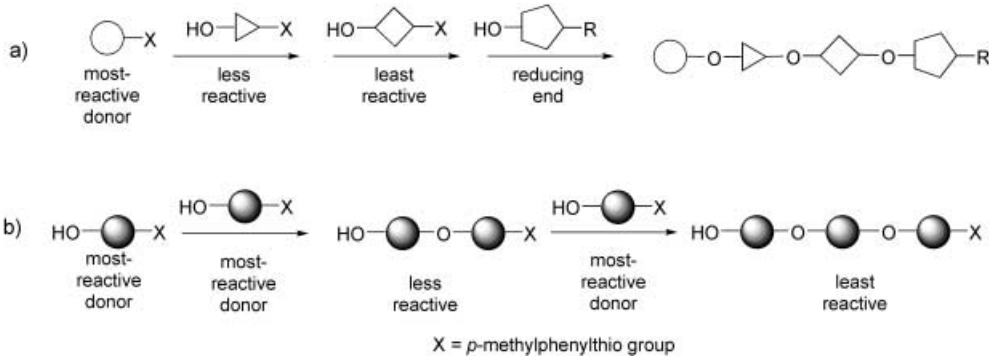
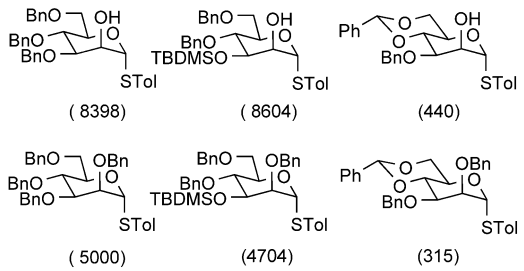


Figure 2. Strategy for a) sequential one-pot synthesis and b) one-pot self-condensation synthesis.



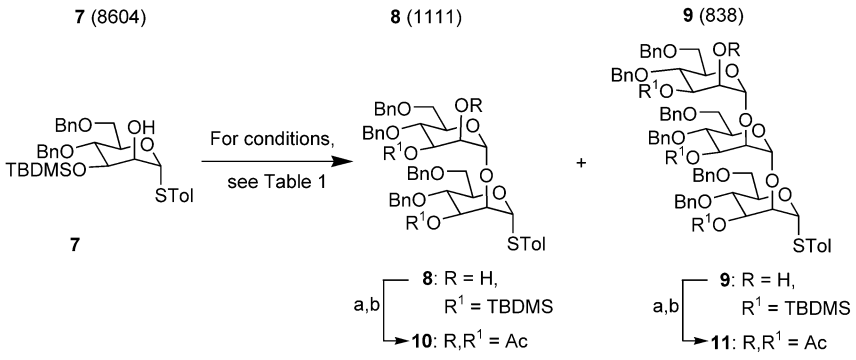
Scheme 2. RRV values of 2-hydroxymannose thioglycosides. Bn = benzyl, Tol = tolyl, TBDMS = *tert*-butyldimethylsilyl.

changing the anomeric leaving group on mannose from F to SePh to SEt, and thus changing the reactivity of this group.[11]

While we continue to find new protecting groups to tune the reactivity of mannose thioglycosides for the one-pot synthesis of oligomannoses, we report herein a new one-pot strategy for the synthesis of both mannose dimer **8** and trimer **9**. In our strategy, the most reactive monomer undergoes self-condensation to give a less-reactive dimer. The dimer then serves as an acceptor for another monomer molecule, which leads to formation of the trimer (Figure 2b). Several 2-hydroxymannose thioglycosides were designed and tested for self-condensation but their reactions were not clean and separation of the monomer, dimer, and trimer by column chromatography was required. This problem was overcome by introducing the nonpolar protecting group *tert*-butyldimethylsilyl to form **7**, which undergoes self-condensation to give dimer **8** and trimer **9** (Scheme 3).

Entry 5 in Table 1 lists the optimal conditions for the one-pot self-condensation of **7** to produce **8** and **9**. The reaction temperature is key to the degree of self-condensation. No self-condensation occurred at −60 °C (Entry 1) and mainly dimer **8** and monomer **7** were found at −50 °C (Entry 2). Uncontrollable self-condensation was observed at temperatures over −20 °C (Entry 8). The overall yield was reduced when more than 0.6 molar equivalents NIS were added (Entry 4), probably as a result of decomposition of the thioglycosides, dimer **8**, and trimer **9**.

The RRVs of **7**–**9** were determined and, as expected, monomer **7** (RRV = 8604) is the most reactive and undergoes self-condensation to give the less-reactive dimer **8** (RRV = 1111), which is then glycosylated by monomer **7** again to give the least reactive molecule in the series, trimer **9** (RRV = 838). Subsequent removal of the TBDMS group and acetylation of **8** and **9** gave **10** and **11**, respectively (Scheme 3).



Scheme 3. One-pot self-condensation synthesis of building blocks **10** and **11**. a) Tetrabutylammonium fluoride, tetrahydrofuran, RT, 24 h; b) Ac_2O, Et_3N, 4-dimethylaminopyridine, CH_2Cl_2, RT, 2 h.

Table 1: The reaction conditions used for reactivity-based one-pot self-condensation of **7**.

Entry	*T* [°C]	NIS [molar equiv][a]	*t* [h]	Yield of **8** [%]	Yield of **9** [%]
1	−60	0.7	24	0	0
2[b]	−50	0.7	24	70	5
3	−45	0.7	2	40	20
4	−40	0.7	1	35	25
5	−40	0.6	1	38	30
6	−40	0.5	1	50	15
7	−30	0.7	0.5	25	15
8[c]	−20	0.7	0.5	0	10

[a] NIS = *N*-iodosuccinimide. [b] The yield was calculated based on the amount of **7** recovered. [c] Polymerization was observed.

Donors **10**–**12**[12] were coupled to acceptor **13** or **14** (Bz, benzoyl)[13] to give trimannose **15**, tetramannose **16**, pentamannose **17**, heptamannose **18**, or trimannose **19**. Details of these syntheses are shown in Table 2. Donors **10**–**12** exhibited excellent Manα1-6Man or Manα1-3Man selectivity as a result of steric bulk at the 2-position of **10** and **11** and participation of the acetyl group of **12**. As shown in Table 2, deprotection of oligomannoses **15**–**19** gave the corresponding deprotected oligomannoses **2**–**6**, respectively, in good yields.[9]

$Man_9GlcNAc_2$ (**1**)[5] and deprotected oligosaccharides **2**–**6** were evaluated for their ability to inhibit the interaction between 2G12 and gp120 in an enzyme-linked immunosorbent assay (see Figure 3).[4a] We will ultimately take the best synthetic inhibitors found in this assay forward into experiments to develop multivalent constructs as potential HIV vaccine candidates for eliciting 2G12-like antibodies. The results for trimannose **2** (15% inhibition at 2 mM) and tetramannose **3** (79%) indicate that an extra α1-2 linked mannose unit significantly enhances the inhibitory effect to a level comparable with that achieved by $Man_9GlcNAc_2$ (71%). This result is consistent with crystallographic studies that identified the D1 arm of $Man_9GlcNAc_2$ as the primary carbohydrate recognition motif for 2G12; compound **3** contains the same arrangement of mannose units as the D1 arm. The Manβ1-4GlcNAcβ1-4GlcNAc core of $Man_9GlcNAc_2$ appears not to be critical for binding as it is absent from compound **3**. Pentamannose **4** (79% inhibition at 2 mM), though lacking two sequential Manα1-2Man units, is divalent which may explain its more efficient inhibition of 2G12–gp120 binding compared to that of compound **2** (15%). Surprisingly, heptamannose **5** (65% inhibition at 2 mM), which contains both residues mimicking the D1 arm of $Man_9GlcNAc_2$ and an additional Manα1-2Manα1-2Manα1-6Man-linked branch, does not offer a further increased affinity over that of compound **3**. The second branch, not found in mammalian glycans, may force compound **5** to adopt an unusual conformation that is not optimal for 2G12 recognition.

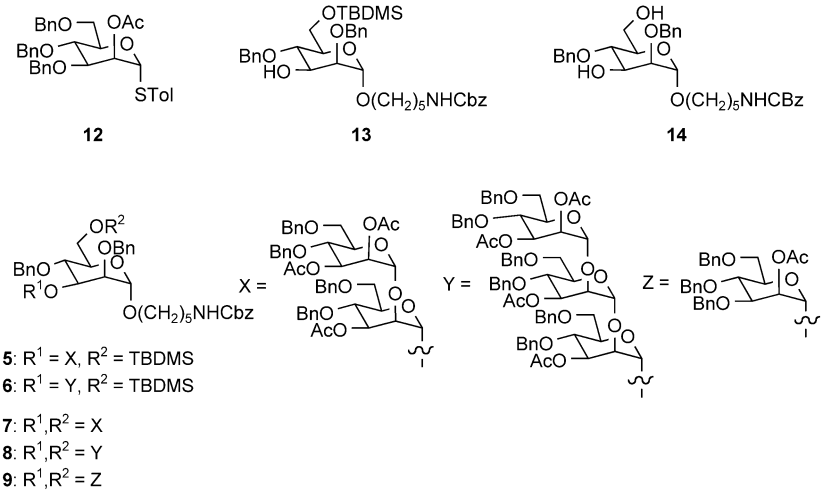


Table 2: The reaction conditions for the synthesis of oligosaccharides **15**–**18** and **2**–**6**.

Donor	Acceptor	NIS [equiv]	TfOH[a] [equiv]	T [°C]	t [h]	Protected oligosaccharide (yield [%])	Deprotected oligosaccharide (yield [%])
10	**13**	1.3	0.13	−20	2	**15** (85)	**2** (75)[b]
11	**13**	1.3	0.13	−10	4	**16** (83)	**3** (72)[b]
10	**14**	2.6	0.26	−20	2	**17** (65)	**4** (68)[c]
11	**14**	2.6	0.26	−10	4	**18** (63)	**5** (65)[c]
12	**14**	2.6	0.26	0	24	**19** (50)	**6** (60)[c]

[a] TfOH, trifluoroacetic acid. [b] a) 80% acetic acid, RT, 4 h; b) NaOMe, RT, 2 h; c) Pd black, 5% formic acid/MeOH, H_2, RT, 24 h. [c] a) NaOMe, RT, 2 h; b) Pd black, 5% formic acid/MeOH, H_2, RT, 24 h.

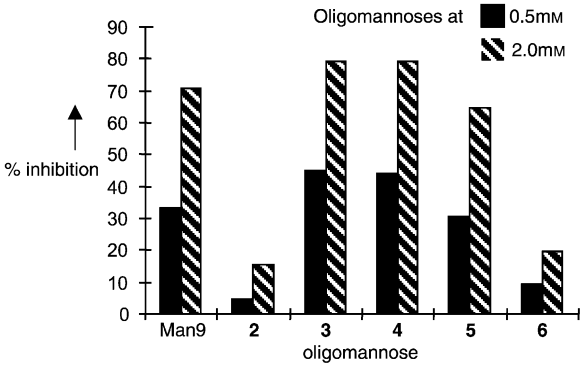


Figure 3. Inhibition (%) of 2G12 binding to gp120. Man9 = $Man_9GlcNAc_2$ (**1**).

In conclusion, we have developed a novel and efficient route to high-mannose oligosaccharides through a reactivity-based one-pot self-condensation reaction. By using this method we have prepared several Manα1-2Man-containing oligosaccharides and found they effectively inhibit the binding of 2G12 to gp120. We have identified new synthetic epitope mimics that are as effective as, or better than, $Man_9GlcNAc_2$ at inhibiting this interaction. Work is in progress to develop multivalent constructs as candidates for the development of an HIV vaccine.

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Keywords: antibodies · drug design · oligosaccharides · self-condensation · synthetic methods

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