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Letter

Synthesis of Mannosidase-Stable Man_3 and Man_4 Glycans Containing S-linked Manα1→2Man Termini

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Cite This: *Org. Lett.* 2021, 23, 3053−3057

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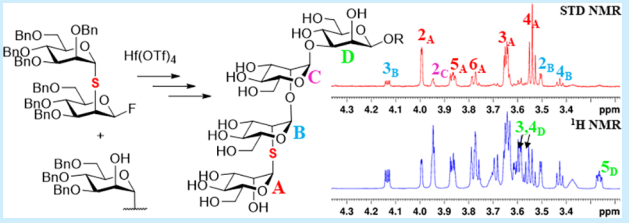
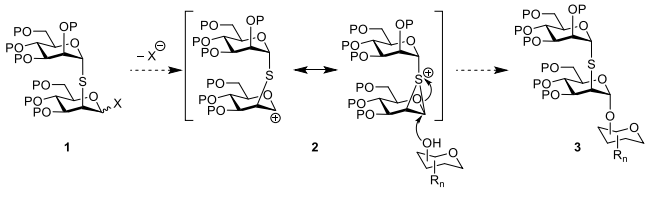
ABSTRACT: Oligomannose glycans are of interest as HIV vaccine components, but they are subject to mannosidase degradation *in vivo*. Herein, we report the synthesis of oligosaccharides containing a thio linkage at the nonreducing end. A thio-linked dimannose donor participates in highly stereoselective glycosylations to afford trimannose and tetramannose fragments. Saturation transfer difference nuclear magnetic resonance (STD NMR) studies show that these glycans are recognized by HIV antibody 2G12, and we confirm that the reducing terminal S-linkage confers complete stability against *x. manihotis* mannosidase.



Carbohydrate or glycoconjugate vaccines[1] are in use or development for the prevention of bacterial infections,[2,3] cancer,[4] and HIV.[5−7] In HIV vaccine development, there is significant interest in elicitation of antibodies that can bind to the Manα1→2Man moieties of high mannose ($Man_9GlcNAc_2$) glycans;[8−11] however, we and others have shown that, for glycoconjugate vaccines, mannosidase trimming degrades this motif, so that the antibody response is directed against the glycan core or other structures in the glycoconjugate.[12,13] A possible solution to this problem is chemical stabilization of the Manα1→2Man linkage against enzymatic hydrolysis, in particular using sulfur[14−20] in the glycosidic linkage. Indeed, antibodies raised against some S-linked glycan analogues exhibit cross-reactivity with the natural oxygen-linked sugars,[21−25] but such analogues have not been tested in the case of oligomannose vaccines.

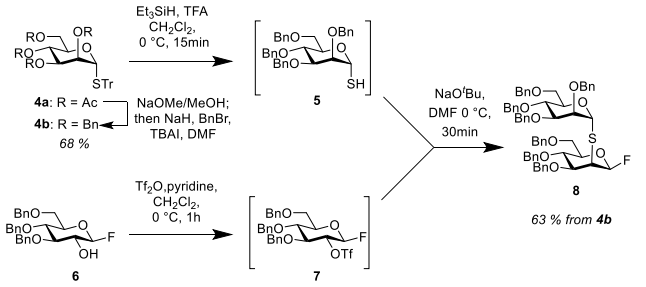
Inspired by a report of anomeric alkylation to produce a sulfur-linked Manα1→2Man disaccharide,[19] we wondered whether a disaccharide donor containing this S-linkage (see **1**, Scheme 1) would participate in stereospecific glycosylation with anchimeric assistance from the thioether linkage. Glycosyl donors containing simple 2-thio substituents are known,[26−33] but only one thio-linked disaccharide donor has been reported, with a gluco- configuration.[34] Glycosylation with dimannose derivative **1** would offer an efficient route to serum-stabilized fragments of $Man_9GlcNAc_2$, or potentially the whole oligosaccharide.

Scheme 1. Thioether-Linkage-Assisted Stereospecific Glycosylation



To prepare the requisite thio-disaccharide donor, we began from known trityl thioglycoside **4a** (Scheme 2).[35,18] Exchange to benzyl protecting groups proceeded in 68% overall yield to afford building block **4b**. We wondered whether **5**-derived thiolate could displace a 2-triflate derivative with a relatively inert leaving group such as a fluoride already present at C1.

Scheme 2. Synthesis of S-Linked Disaccharide Donor



Thus, we prepared 1-fluoro glucose derivative **6** by a known protocol including epoxidation of tribenzyl glucal,[36] followed by TBAF treatment.[37] Following triflation of **6** and

Received: March 2, 2021
Published: April 1, 2021

https://doi.org/10.1021/acs.orglett.1c00726
Org. Lett. 2021, 23, 3053−3057

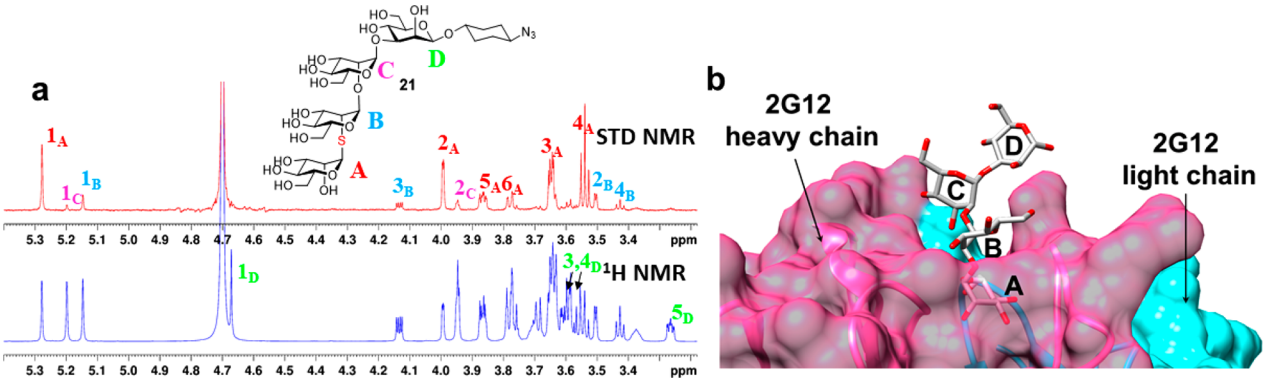
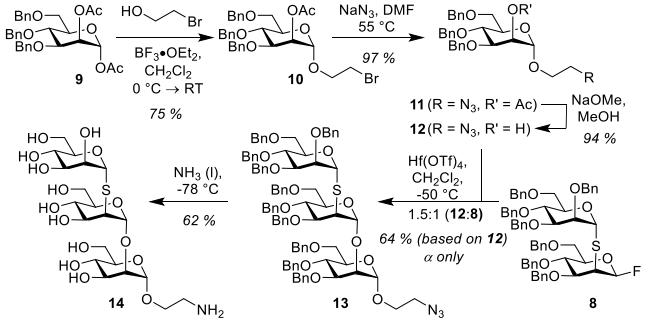


Figure 1. Binding analysis of S-Man$_4$ to HIV broadly neutralizing antibody 2G12: (a) STD-NMR spectrum of S-Man$_4$ (**21**) with 2G12 IgG. Bottom spectrum (blue) shows the reference 800 MHz ^{1}H NMR whereas the top (red) shows corresponding STD spectrum. See the Supporting Information for details. Numbers indicate selected assignments by carbon number and ring letter. (b) Crystal structure for all O-linked Man$_4$ (**22**) bound to 2G12 (PDB ID 6MSY).

triethylsilane/trifluoroacetic acid deprotection of **4b**, **5** and **7** were combined and allowed to react in the presence of sodium *tert*-butoxide to afford the desired disaccharide **8** in 63% yield.

With dimannose donor **8** in hand, we prepared a suitable monomannose acceptor to produce Man$_3$. Starting from mannose building block **9**,[19] installation of an azidoethyl linker and deprotection at the 2-position efficiently afforded acceptor **12**. Glycosylation of **12** with 1.5 equiv of **8**, in the presence of hafnium trifluoromethanesulfonate[38] afforded the desired trisaccharide **13** in 64% yield as a single stereoisomer. After global deprotection with sodium in liquid ammonia, the desired S-Man$_3$ **14** was isolated in 62% yield (Scheme 3).

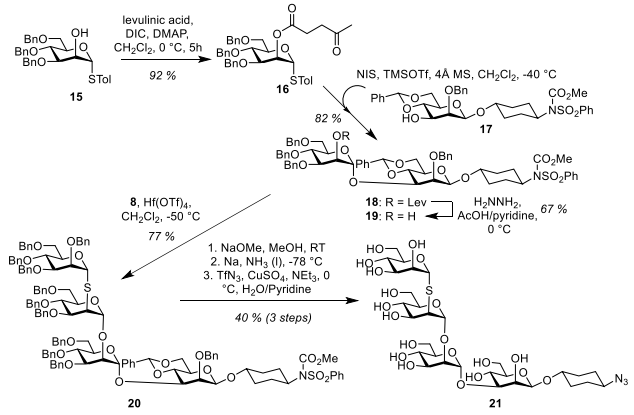
Scheme 3. Synthesis of S-Linked Man$_3$



The stability of the thio linkage under dissolving metal conditions has been observed previously,[19,20] but is nevertheless noteworthy. The α configuration of all mannose units was confirmed by carbon-coupled HSQC, which showed all $^1J_{CH}$ to be in the range of 171−178 Hz (see the Supporting Information).[39,40]

Similarly, we set about preparation of an S-Man$_4$ containing a reducing-terminal β-mannose analogous to the core mannose in the natural Man$_9$GlcNAc$_2$. We prepared dimannose acceptor **19** by coupling our previously described β-mannose core **17**[41] to known building block **16**,[42] followed by Lev deprotection. **19** coupled smoothly to Man$_2$ fluoride donor **8** (see Scheme 4) in 77% yield, again as single stereoisomer. This tetrasaccharide was globally deprotected and converted to azide **21** in three steps with an overall yield of 40%. **21** exhibited three anomeric $^1J_{CH}$ values from 169 to

Scheme 4. Synthesis of S-Linked Man$_4$



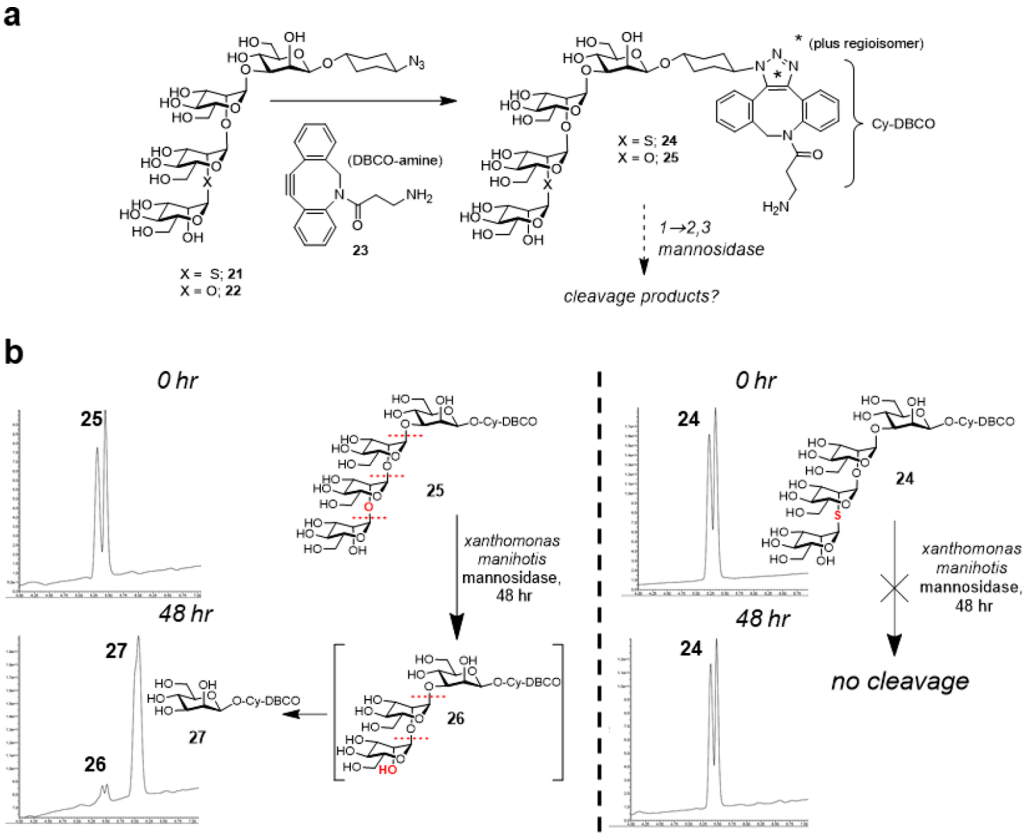
174 Hz for the α linkages, and, as expected, a value of 158 Hz for β linkage (see the Supporting Information).

With these S-Man$_3$ and S-Man$_4$ derivatives in hand, we proceeded to study their recognition by HIV broadly neutralizing antibody 2G12, which binds primarily to the linear trimannose (D1) arm of Man$_9$GlcNAc$_2$. STD-NMR (Saturation Transfer Difference nuclear magnetic resonance (NMR)) spectroscopy with 25 μM 2G12 IgG and a 200:1 ratio of sugar:antibody showed that, as expected, the greatest saturation transfer is seen for the nonreducing mannose unit in either Man$_3$ or Man$_4$ (Figure S1 in the Supporting Information and Figure 1a). In the case of the Man$_4$ derivative, negligible STD is observed for the reducing-terminal mannose unit. These data are closely analogous to STD NMR data previously acquired for oxygen-linked oligomannose fragments,[43,44] and are consistent with crystal structure data for Man$_4$ bound to 2G12, in which little if any interaction is evident between the antibody and residue D (Figure 1b).

Lastly, we tested natural and sulfur-substituted Man$_4$ derivatives against the action of *Xanthomonas manihotis* mannosidase, which cleaves oligomannose Manα1→2Man and Manα1→3Man linkages. S-Man$_4$ derivative **21** and its oxygen analogue **22** were labeled by strain-promoted azide/alkyne cycloaddition[45] with DBCO[46] amine linker **23**, in order to facilitate separation and detection of degradation products by LC/MS. After incubation with mannosidase, LC/MS analysis showed no degradation of sulfur-substituted

https://doi.org/10.1021/acs.orglett.1c00726
Org. Lett. 2021, 23, 3053−3057

Scheme 5. Stability of S-Linked Man_4 Against Mannosidase Cleavage



derivative **24** after 48 h, but nearly complete digestion of natural Man_4 derivative **25** to Man_1 **27** (Scheme 5).

In conclusion, we have demonstrated a facile synthetic route to Man_3 and Man_4 derivatives with a nonreducing-terminal sulfur linkage that is highly resistant to enzymatic degradation. These derivatives are recognized by an HIV antibody, 2G12, through contacts that are similar to those it makes with the natural oligomannose structure. This synthetic strategy should be readily amenable to preparation of higher branched stabilized oligomannose analogues, suitable for immunogenicity studies in the near future.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.1c00726.

Procedures, spectra for compounds **4b**, **5**−**8**, **10**−**21**, and additional experiments (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This project was supported by NIH Award Nos. R01AI090745 and R01AI113737 and Brandeis' SPROUT program. The 800 MHz NMR in the Landsman Research Facility was purchased with a grant from the NCRR High-End Instrumentation program (No. S10RR017269) and updated with funds from HHMI and Brandeis University. Dr. Susan Pochapsky of the Brandeis NMR Facility is gratefully acknowledged for assistance with STD NMR.

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