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Automated Solution-Phase Synthesis of *S*-Glycosides for the Production of Oligomannopyranoside Derivatives

Mallory K. Kern and Nicola L. B. Pohl*

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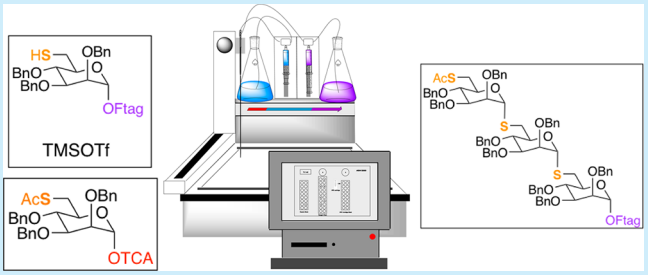
ABSTRACT: Thioglycosides are more resistant to enzymatic hydrolysis than their O-linked counterparts, thereby becoming attractive targets for carbohydrate-based therapeutic development. We report the first development of methods for the site-selective incorporation of S-linkages into automated solution-phase oligosaccharide protocols. The protocols were shown to be compatible with the formation of *S*- or *O*-glycosides for the synthesis of mannopyranoside trimmers that incorporate both S- and O-linkages to allow the selective incorporation of an *S*-glycoside in various stages in an automated program.



Naturally occurring thiosugars are exceedingly rare in living systems despite sulfur being an essential component in other biomolecules. Although naturally rare, carbohydrates that replace the native ring or glycosidic oxygen atoms with sulfur constitute an important class of glycomimetics.[1] Glycosidic linkages that incorporate sulfur are less prone to hydrolysis than their oxygen-containing analogues[2] and can inhibit the activity of some glycosidases.[3] These properties have made thioglycosides attractive therapeutic targets.[4] However, despite great progress in the development of automated methods to string together carbohydrate monomers, these efforts have focused primarily on the creation of the common natural O-linkages.[5−9] The development of protocols to site-selectively incorporate sulfur linkages into these automated oligosaccharide synthesis protocols could greatly expand the possibilities for designing glycomimetics. Herein we report the first automated synthesis protocols that incorporate *S*-glycosides while limiting side reactions and allowing the further incorporation of *O*-glycosidic linkages.

The inclusion of sulfur into the glycosidic linkage poses additional synthetic challenges relative to oxygen. Thioglycoside donors and common glycosylation promoters (*N*-iodosuccinimide, $Ph_3Bi(OTf)_2$, and $AuCl_3$) are incompatible with thioglycoside acceptors for the formation of *S*-glycosidic linkages.[9−11] Also, thiol acceptors can dimerize prior to coupling and are susceptible to oxidation reactions.[11,12] Despite these challenges, a number of manual thioglycoside syntheses have been reported.[5,6,11,13,14]

In choosing targets for automated synthesis, we focused our efforts on the common mannose linkage found in the high-mannose capping structures of *N*-glycans and that is of particular interest in the design of compounds that modulate viral and parasitic infections.[15] D-Mannose has also been useful in glycan-based drug development.[16] Stable enzymes to create mannose linkages are not yet commercially available, thereby making these particularly important targets for chemical synthesis. In addition, the syntheses of 1,6- and 1,2-S-linked disaccharides from thiomannosyl building blocks[14] and a 3,6-*O*,*S*-trimannoside[17] have been manually made as benchmarks.

Despite these prior approaches, no synthetic procedures have yet been demonstrated to work in an automated liquid-handling system.[14,17] The use of machine-assisted liquid-handling systems allows the desired targets to be synthesized without the variability introduced by multiple manual operations, thereby increasing batch-to-batch and lab-to-lab reproducibility.[18] Unfortunately, the transfer of manual to automated processes is not always straightforward.[9] Although solution-phase-based automation platforms can be dosed with solid reagents when other methods fail, ideally all reaction components start and remain in solution.[19]

The automated synthetic method described in this work is applicable for both highly reactive and less reactive trichloroacetimidate donors. The addition of a fluorous tag allows the automated purification of intermediates by fluorous solid-phase extraction (FSPE).[20]

We began our investigation into the automated synthesis of thioglycosides by the design and synthesis of the necessary building blocks. To this end, we decided to make novel thiol acceptor **4** and thiol donor **5** along the same pathway (Scheme 1). First, D-(+)-mannose was converted into the known *O*-allyl,

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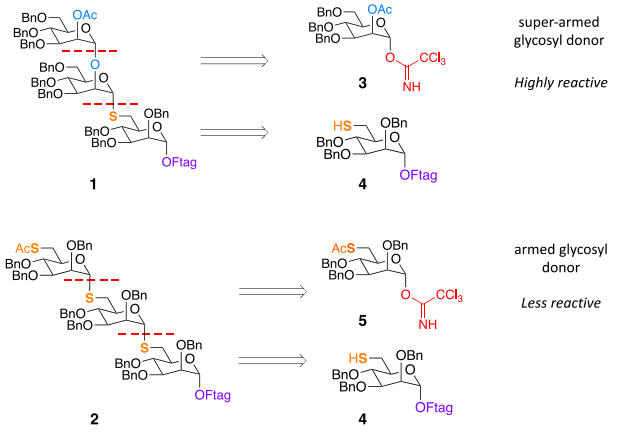
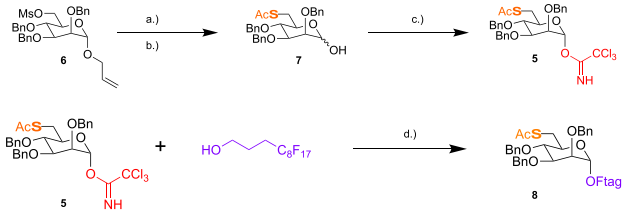
Scheme 1. Trisaccharide Targets Discussed in This Work



2,3,4-tribenzyl, and 6-mesyl mannoside in 29% over five steps (**S.6** in the Supporting Information (SI)).[21] The allyl group of the mannopyranoside was then removed using $PdCl_2$, followed by the introduction of a thioacetate group at the six-position to give mannopyranoside **7** in 42% yield over two steps (Scheme 2). **7** was then converted into the trichloroacetimidate (TCA)

Scheme 2. Synthesis of Mannopyranoside Analog 8[a]



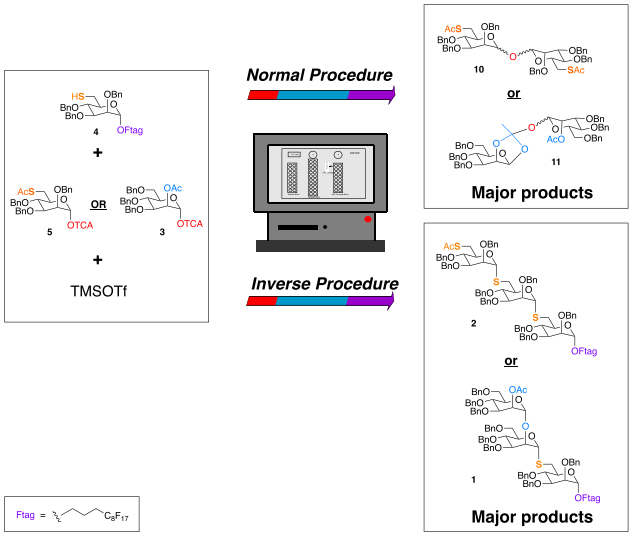
[a](a) $PdCl_2$, MeOH, 18 h; (b) KSAc, DMF, 80 °C, 3 h; (c) TCA, DBU, CH_2Cl_2, 12 h; and (d) BF_3OEt_2, CH_2Cl_2/TFT, 2 h.

mannopyranoside donor **5**. Fluorinated mannopyranoside analog **8** was then realized by coupling thiol donor **5** with the partially fluorinated undecanol in 78% yield. The fluorous tag addition allows for the facile isolation of target compounds by FSPE.[20] (See the SI.)

Initially the deprotection of the thioacetate moiety of mannoside analog **8** with $LiAlH_4$, was attempted; however, yields of thiol acceptor **4** were poor (<20%, see the SI), which prompted us to explore other deprotection pathways. The thiomannopyranoside was instead deprotected with hydrazine monohydrate and acetic acid, yielding the free thiol mannoside acceptor **4** in 72% yield. (See the SI.) Finally, mannosyl donor **3** was synthesized over six steps with an overall yield of 8% following previously published procedures[20,22] to incorporate a standard O-linked sugar into the automated synthesis protocols.

Prior to developing our strategy for the automated synthesis of S-linked glycosides, potential glycosylation conditions were screened. The Lewis acid promoter trimethylsilyl triflate (TMSOTf) was employed because it has been shown to work well in the formation of *O*-glycosidic linkages[7,8] as well as S-linkages[17] and works well as a solution for delivery by automated liquid-handling platforms.[6−9,23] Mannosyl acceptor **4** was coupled to TCA donor **3** in the presence of TMSOTf to form the expected disaccharide (**S.9** in the SI). However, upon workup, the disaccharide was obtained in unexpectedly low yields (<20%), with an appreciable amount of the 1,1-linked homodimer (observed: m/z 1021.2 [M + Na^+]) and orthoester (observed: m/z 989.1 [M + Na^+], ^{13}C peak at 120 ppm) side products having been formed, as seen by LRMS and NMR analyses (**10** and **11**, Scheme 3). These types of side products

Scheme 3. Initial Glycosylation Strategy Using the "Traditional" Glycosylation Method



are known in the literature with *O*-glycosides and fairly common in glycosylation reactions with reactive TCA donors.[24] The three benzyl protecting groups and an acetate moiety appended to mannosyl donor **3** make this building block especially reactive, making it a superarmed donor.[25,26] Likewise, thiol donor **5** was shown to be reactive as well, suggesting armed reactivity. (See the SI for further discussion.)

Automated oligosaccharide synthesis protocols rely on normal glycosylation reactions in which the donor and activator solutions are added to a tagged or solid-phase-linked acceptor. However, in light of the reactivities of TCA donors **3** and **5**, an inverse glycosylation procedure was investigated for the formation of the thioglycoside targets. The inverse glycosylation procedure was developed by Schmidt and coworkers and involves the incubation of the acceptor moiety with the promoter prior to donor addition.[27] A batch-mode solution-phase automated synthesis platform should be amenable to programming of the inverse glycosylation more readily than solid-phase-based machines. Indeed, the use of the inverse procedure significantly improved our glycosylation yields (73 and 78% of trisaccharides **1** and **2**) with minimal orthoester and homodimer formation.

With a viable path toward S-linked di- and trisaccharides using only soluble reactants and reagents, we began to develop automation protocols for their synthesis using an automated liquid-handling platform. The platform consists of a robotic arm coupled to two syringe pumps, which transfer reagents in solution to an array of double-jacketed reactors vessels under argon. These vessels can be vortexed and either heated or cooled.[7,9] (See the Supporting Information.) To enable a successful reaction completion on a solution-phase liquid-handling platform, all reagents must be stable in solution at room temperature for the length of the automation cycle.[7,9] A promoter solution of TMSOTf was employed for the

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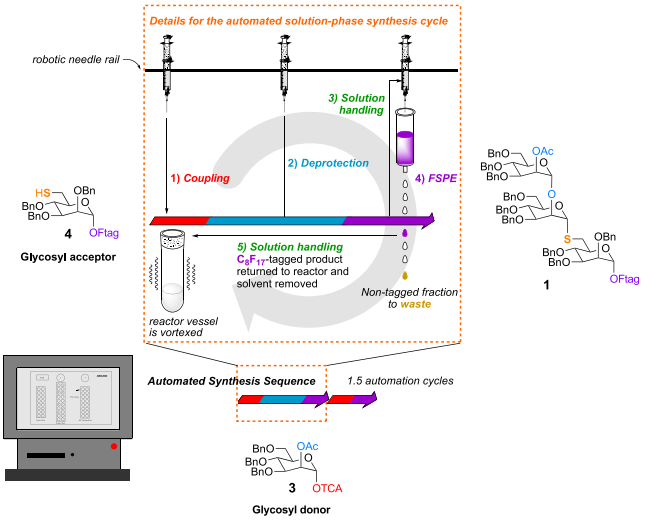
glycosylation steps, followed by hydrazine monohydrate and acetic acid to deprotect the thioacetate moiety. Hydrazine monohydrate is a relatively mild liquid reagent, and acetic acid is not corrosive to the robotic needle, even in the concentrated form.

The automated synthesis of **1** was carried out by successive cycles of coupling, deprotection, and purification (Scheme 4).

Scheme 4. Automated Solution-Phase Synthesis of Trisaccharide 1



First, the disaccharide unit (**S.9**; see the SI) was synthesized by the addition of solutions of TMSOTf and **3** to a cooled (−40 °C) solution of acceptor **4**. The solution of **4** was vortexed for 10 min at −40 °C before the introduction of TMSOTf and the dropwise addition of **3**. Following the deprotection and FSPE purification steps to generate and isolate **12** on the platform, the acceptor was once more cooled to −40 °C, and TMSOTf was added, followed by donor **3** after 10 min. Upon purification of the crude mixture by FSPE, trisaccharide **1** was achieved in 73% isolated yield with evidence of only the α-linked product. These conditions for the formation of the O-linked glycoside did not lead to any noticeable epimerization or cleavage of the S-linked glycosides.

The synthesis of trisaccharide **2**, which contains two thioglycoside linkages, was carried out in the same fashion as trisaccharide **1** from mannosyl acceptor **4** and mannosyl donor **5**. Upon the completion of two automation cycles and FSPE purification, the desired target **2** was synthesized in 78% yield. Again, only the α-linked product was seen. The use of TCA donor **3** allowed for the selective formation of the α-anomer due to anchimeric assistance from the acetate group at the two-position. TCA donor **5** additionally allowed for the selective formation of the α-anomer, possibly due to the influence of the thioacetate moiety at the six-position.[25,28] (See the Supporting Information.)

In conclusion, the first methods for the synthesis of S-linked glycosides on an automated liquid handling are reported with specific application to the synthesis of mannose-containing compounds. The key to the success of these syntheses was the development of an automated protocol for using inverse rather than normal glycosylation protocols for the formation of S-linked oligomannopyranoside derivatives using very reactive glycosyl donors. The protocols can be used in the presence of O-linked glycosides and avoid the formation of side products such as orthoesters and homodimers. With the demonstration of such an automated protocol, glycomimetic production with the site-selective incorporation of *S*-glycosidic linkages can become routine.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c01236.

Experimental procedures, characterization data, and ^{1}H and ^{13}C spectra for all new compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Nicola L. B. Pohl − *Department of Chemistry, Indiana University, Bloomington, Indiana 47405, United States;* orcid.org/0000-0001-7747-8983; Email: npohl@indiana.edu

Author

Mallory K. Kern − *Department of Chemistry, Indiana University, Bloomington, Indiana 47405, United States;* orcid.org/0000-0003-4878-4636

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.0c01236

Author Contributions

Both authors contributed to the design and reporting of this project. M.K.K. carried out all experimental work.

Notes

The authors declare no competing financial interest.

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