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Challenges in the Conversion of Manual Processes to Machine-Assisted Syntheses: Activation of Thioglycoside Donors with Aryl(trifluoroethyl)iodonium Triflimide

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Abstract

The steps needed to adapt a stable iodonium promoter for use in automated fluororous-assisted solution-phase oligosaccharide synthesis are described. Direct adaptation of the originally reported batch procedure resulted in the formation of an orthoester or protecting group transfer to the glycosyl acceptor. Fortunately, the addition of inexpensive β -pinene as an acid scavenger avoided both of these side reactions. The utility of this newly developed protocol was applied to the automated solution-phase synthesis of a β -glucan fragment.

Graphical Abstract

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Supporting Information

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Automated solution-phase synthesis protocols, synthetic procedures, and spectral data (PDF)

With increasing interest in reproducibility and the automation of specialized chemical production comes the need for reactions compatible with the liquid handling platforms used to carry out machine-assisted solution-phase and solid-phase syntheses, including flow chemistry.¹ Unfortunately, the vast majority of reported chemical reactions are not homogeneous from start to finish, but rely on principles such as precipitation of components to drive reactions forward or gradual dissolution of components to limit solvent usage. These issues are becoming especially problematic in the recent push to automate oligosaccharide synthesis. Despite over a decade of work to that end—comprising automated solid-supported synthesis on a commercial synthesizer (Glyconeer 2.1),² HPLC-assisted solid-supported automated synthesis,³ automated glycolipid-assisted solution-phase synthesis,⁴ automated fluororous-assisted solution-phase synthesis,⁵ and automated iterative electrochemical solution-phase synthesis⁶—only a small subset of glycosylation and deprotection procedures have been translated from manual synthetic procedures to machine-assisted processes to create robustly reproducible protocols. Regardless of whether the reactions occur in solution or upon polymeric supports, robotic handling platforms at present are still most reliable when dispensing liquids and solutions rather than solids and precipitates. Therefore, many traditional glycosylation methods that rely on the formation of solids such as silver salts can generally only be performed as manual batch processes that are not readily adapted to iterative machine-assisted protocols. Herein we report the first example of a machine-assisted protocol that uses a hypervalent iodonium species⁷ to carry out a glycosylation reaction, and we show its use in an iterative multistep automated synthesis of a glucan fragment. The development of these processes highlights general strategies for translating traditional manual benchtop processes to machine-assisted procedures.

For a protocol to easily translate to automated synthesis platforms, all reagents and reaction mixtures need to be soluble in a solvent amenable to the desired reaction in order to be transferred via a liquid handling system. Although this constraint is also true of continuous flow systems, all current iterative automation platforms known for biopolymer synthesis are based on batch reactors and therefore precipitates can form during reaction as long as they can be solubilized later in an appropriate solvent during the purification step, whether that be rinsing of a solid-phase or loading of a fluororous solid-phase extraction cartridge. Ideally, any machine-assisted glycosylation would provide the desired product with at least a 2:1 stereoselectivity and a 70% yield to allow ease of product identification and purification using recycling HPLC (RHPLC)⁸ or other methods. In addition, reactions should be conducted in the temperature range most readily accessed in automation platforms (−45 to 250 °C). Finally, all reagents should ideally be stable in solution at room temperature under argon for the length of the automation cycle in order to minimize required interventions. This latter requirement has started turning our focus away from the more reactive trichloroacetimidate donors⁵ toward thioglycosides.⁹ To this end, hypervalent iodonium salts **1A**⁷ and **1B**,^{7b} which are stable to ambient air and moisture, look promising for carrying out glycosylations at ambient temperatures (Figure 1).

Given the general importance of reagent and reactant solubility in machine-assisted protocols, data on the solubility of aryl(trifluoroethyl)iodonium triflimide **1A** in dichloromethane and dichloroethane, the most commonly used solvents in glycosylation, were

needed first. Saturation concentrations were determined by saturating solvent with **1A**, evaporating an aliquot, reconstituting in acetonitrile, and measuring UV absorbance against a calibration curve. UV absorbance was measured using an Agilent 1100 HPLC by injecting the samples directly from the autosampler into the diode array detector.¹⁰ As expected, this promoter was soluble in both solvents but at a low concentration (<50 mM, Table 1, entries 1 and 2). Since this promoter has also been used in nitrile solvents to promote β -stereoselectivity in the glycosylation of donors possessing benzyl ethers at the 2-position,^{7b} the solubility of the promoter in various 1,2-dichloroethane/CH₃CN mixtures was also investigated without the use of added heat. Addition of the nitrile cosolvent proved to increase the solubility of the promoter by 5- to 12-fold (Table 1, entries 3 and 4). From these data, a 1:10 mixture of acetonitrile in 1,2-dichloroethane (DCE) was chosen as the reaction solvent for machine-assisted synthesis trials.

Acetonitrile is known to favor β -selectivity in glycosylation via a nitrilium intermediate, potentially competing with the attachment of the acceptor to the activated donor, thus decreasing the rate of the reaction.¹¹ To investigate the effect of the acetonitrile solvent, a glycosylation reaction between acceptor **2** and donor **3A** was conducted on a solution-phase automated synthesis platform (Figure 2). The synthesizer, consisting of a robotic arm coupled to two syringe pumps, transfers reagents in solution to an array of double-jacketed reactors under argon that can be vortexed and either heated or cooled (see Supporting Information). Small aliquots were picked at different time points from the reactor, transferred to a sample rack, and quenched in order to be further analyzed by HPLC. This experiment proved that the small amount of acetonitrile did not impact the apparent reaction rate significantly. Total disappearance of the limiting acceptor was observed by HPLC after only 30 min. However, to our surprise two products of the same molecular weight but different retention times were formed (see Supporting Information). Further isolation identified the second more apolar product as the orthoester **5** (Figure 2).

Because orthoester **5** was the major side product of the reaction with the addition of the more polar solvent, other reaction parameters were investigated (Figure 2). Switching the *tert*-*tert*-butylpyrimidine **9** (TTBP) to a base with a higher pK_a such as 2,6-di-*tert*-butylpyridine **7** (DTBP) or 2,6-di-*tert*-butyl-4-methyl-pyridine **8** (DTBMP)¹² did slightly improve the formation of the desired product. Diluting the reaction also slightly improved the conversion to the desired product **4** (Figure 2, columns 1–7). However, elimination of the base entirely only resulted in the transfer of an acetate protecting group to the free hydroxyl of the acceptor (Figure 2, columns 11 and 12, compound **6**). Given the evidence for these hindered bases being potentially nucleophilic,¹³ strained olefins as nonbasic proton scavengers¹⁴ were explored as an alternative to amine bases. To this end, β -pinene **10**, of comparable cost to the solvent DCE, was tested for its ability to hinder formation of the orthoester as well as the transfer of the protecting group in the glycosylation reaction. Interestingly, 4 equiv of this nonbasic proton scavenger did inhibit the formation of the orthoester completely, but the acetate transfer only partially (Figure 2, column 8). However, increasing the amount of β -pinene proved to inhibit both side reactions (Figure 2, columns 9 and 10). Therefore, all subsequent reactions were run at 15 mM in the presence of 20 equiv of the β -pinene.

In theory at least, the reaction conditions should now be amenable to robotic liquid handling systems required by solid-phase- and solution-phase-based automated synthesis platforms. To probe this glycosylation reaction in practice and to test the compatibility of the new conditions with common deprotection steps without the need for purification between these two reaction types, several donors with different protecting groups at their 6-positions that are commonly used in carbohydrate chemistry were procured. Each iterative cycle of oligosaccharide synthesis is commonly composed of a glycosylation step, followed by the selective deprotection of the next position to be glycosylated, and finally purification of the newly formed acceptor intermediate by some means such as, in our case, fluorous solid-phase extraction (FSPE, Table 2). Purification between the glycosylation and the deprotection steps is usually not required for successful removal of most masking groups and therefore is avoided to save time. However, these new glycosylation conditions produce a new set of reagents and byproducts that could potentially inhibit the usual deprotection programs.^{5b} A levulinate ester (Lev), chloroacetate ester (ClAc), and a *tert*-butyldimethylsilyl ether (TBDMS) were chosen for testing due to their ability to potentially be removed in the presence of benzoyl esters and benzyl ethers (Table 2). In each case, compatibility between the glycosylation and the deprotection steps^{5b,15} was observed in the automation cycles, giving **11** as a major product after FSPE purification as seen by HPLC analysis. Purification by HPLC allowed us to obtain the disaccharide **11** in 26% to 72% unoptimized yield depending on the protecting group and the glycosylation conditions used (Table 2). For most automated syntheses, optimization of yields is less important than the ability of a general set of conditions to provide sufficient material for further study in the first attempt.

Having established that common deprotection conditions could be directly carried out after glycosylation with the hypervalent iodonium salt **1A** and β -pinene **10** without the use of any intermediate purification, we applied this new automated strategy for the formation of a larger oligosaccharide. Starting with acceptor **2**, three iterative cycles were implemented on the automation platforms using only 2 equiv of donor **3B** in each glycosylation step and the conditions for the levulinoyl cleavage (Figure 3). After the last FSPE and further reversed-phase purification, the desired β -1,6-glucan tetrasaccharide **12** was obtained in 16% over 6 steps (74% average per reaction step).

In conclusion, we have demonstrated that the air- and water-stable iodinated promoter **1A** can be translated for use in an automation protocol using robotic liquid handling to activate bench stable thioglycoside donors. In addition, the new conditions used here for glycosylation have been shown to be compatible with common deprotection conditions without the need for intermediate purification. The use of iterative glycosylation/6-*O*-Lev-deprotection/FSPE cycles was applied to achieve the formation of a β -1,6-glucan tetramer. This strategy extends the possible activation chemistries that can be implemented in the known automated oligosaccharide protocols to a variety of thioglycoside donors and glycoside acceptors to potentially access a wider range of structures. Key to the successful adaption of the manual protocol to automation was careful reaction monitoring of undesired glycosylation side products and the use of a strained olefin acid scavenger, a process that

holds lessons for future attempts to develop new chemistries for automated synthesis platforms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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1A **1B**

Figure 1.
Hypervalent iodonium promoters.

Figure 2.

Optimization of the reaction conditions. (1) Base = 7 (4 equiv), [**2**] = 113 mM; (2) base = 7 (4 equiv), [**2**] = 50 mM; (3) base = 7 (4 equiv), [**2**] = 15 mM; (4) base = 8 (4 equiv), [**2**] = 50 mM; (5) base = 8 (4 equiv), [**2**] = 15 mM; (6) base = 8 (4 equiv), [**2**] = 5 mM; (7) base = 9 (4 equiv), [**2**] = 50 mM; (8) base = 10 (4 equiv), [**2**] = 15 mM; (9) base = 10 (10 equiv), [**2**] = 15 mM; (10) base = 10 (20 equiv), [**2**] = 15 mM; (11) no base, [**2**] = 50 mM; (12) no base, [**2**] = 15 mM.

Figure 3.
Automated solution-phase synthesis of β -1,6-glucan tetramer.

Table 1

Solubility of Aryl(trifluoroethyl)iodonium Triflimide 1A in Different Solvents at 23 °C

entry	solvent	saturation (mM) ^a	saturation (mg·mL ⁻¹) ^a
1	CH ₂ Cl ₂	44 ± 2	25 ± 1
2	ClCH ₂ CH ₂ Cl (DCE)	46 ± 2	26 ± 1
3	1:20, CH ₃ CN/DCE	246 ± 6	140 ± 3
4	1:10, CH ₃ CN/DCE	582 ± 1	330 ± 1

^aErrors bars represent duplicate measurements.

Table 2

Results for the Glycosylation/Deprotection/FSPE Cycle with Donors 3A–D

Legend:

- Glycosylation:** 3B-D (2 equiv), $\text{CF}_3\text{CH}_2\text{I}/\text{Ph}\cdot\text{NTf}_2$ (2.4 equiv), **8** (4 equiv) or **10** (20 equiv), DCE, 25 °C, 2 h
- Deprotection:**
 - conditions 1:** R = Lev, $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ (5 equiv), AcOH (7.5 equiv), 25 °C, 1.5 h
 - conditions 2:** R = ClAc, DABCO (20 equiv), MeCN:EtOH (1:5), 70 °C, 3 h
 - conditions 3:** R = TBDMS, TBAF: $\text{NEt}_3 \cdot 3\text{HF}$ (4:1), DMSO:THF, 45 °C, 12 h
- FSPE:** loading = DMSO, Fluorophobic = MeOH: H_2O (4:1), Fluorophilic = Acetone

entry	donor	proton scavenger	deprotection condition	yield (%)
1	3B	8	1	26
2	3B	10	1	72
3	3C	10	2	58
4	3D	10	3	47