Flow chemistry kinetic studies reveal reaction conditions for ready access to unsymmetrical trehalose analogues†

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Monofunctionalization of trehalose, a widely-found symmetric plant disaccharide, was studied in a microreactor to give valuable kinetic insights that have allowed improvements in desymmetrization yields and the development of a reaction sequence for large scale monofunctionalizations that allow access to probes of trehalose's biological function.

Trehalose (**1**) (Fig. 1), the α,α-1,1 disaccharide of glucose, is an important biological molecule. It is synthesized by plants, bacteria and all invertebrates[1] as a protective agent against stress conditions[2] and can serve as a major carbon source for metabolism. Analogues of trehalose are potential fungicides or antibiotics by virtue of their ability to inhibit trehalase, an enzyme critical to the metabolism of this sugar.[3] Furthermore, the human pathogen *Mycobacterium tuberculosis* incorporates trehalose into the cell wall as an assortment of glycolipids, such as mycolic acid esters, sulfolipids, polyacetylated species and complex lipooligosaccharides.[4] This lipid envelope of *M. tuberculosis* plays important roles in bacterium-host interactions and prevents uptake of potential drugs.[4] Trehalose analogues that disrupt cell wall synthesis may therefore act as novel antitubercular agents.[5]

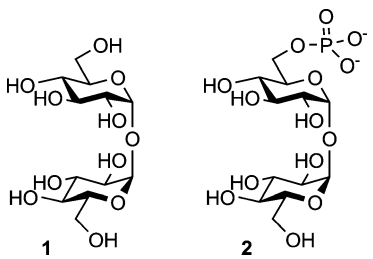

Fig. 1 Trehalose (**1**) and trehalose-6-phosphate (**2**).

In plants, bacteria and yeast, trehalose-6-phosphate (**2**) acts as a signalling molecule controlling carbohydrate metabolism.[1,6] While for bacteria and yeast the mechanism of action is well understood,[6] for plants very little is known.[1] Understanding the role of **2** in plants is crucial since manipulation of this pathway may lead to considerable improvements in crop biomass. To realize this aim, easy access to **2** and other variants is required.

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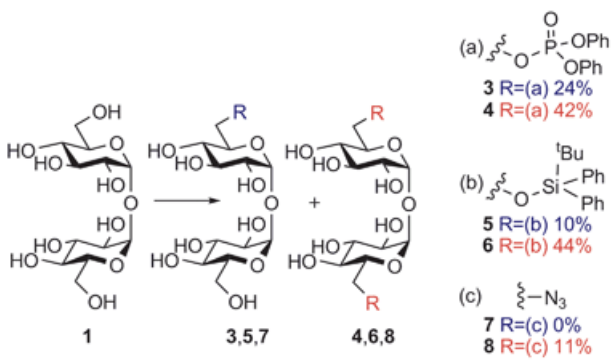
In all the above applications, non-symmetrical trehalose analogues are particularly desirable. The glycosylation of two glucose units would provide a suitable method, but needs the simultaneous formation of two α anomeric linkages. Methodologies to accomplish this have been reported, such as dehydrative glycosylations,[7] and methyl ketoside glycosylations,[8] but are limited to the synthesis of *manno* and C-1 methyl analogues respectively. Intramolecular aglycone delivery[9] is more general, but requires more extensive protecting group manipulations. Alternatively, direct modification of trehalose, a cheap, commercially available starting material avoids difficult glycosylations. A drawback to this strategy is lack of differentiation between the two glucose rings of symmetric trehalose leading to low yields. Nonetheless, even *via* such low yielding routes, several non-symmetrical analogues have been synthesized demonstrating the viability of this strategy.[5,10]

In principle, the mono-functionalization of a symmetric polyol such as **1** can be partitioned into two strategic levels of selectivity: (a) regioselective modification of (primary) hydroxyls within a symmetry unit *and* (b) simultaneous differentiation of the symmetry units (here glucose rings). Mode (a) selectivity is, of course, well established for carbohydrates, where primary hydroxyls can be regioselectively accessed in the presence of secondary alcohols.[11] Efficient monofunctionalizations of symmetric diols (mode (b) selectivity) have been developed and are now widely used.[12,13] Analogous methods for the desymmetrization of trehalose might usefully combine these two modes. The kinetics of mode (b) can be modelled as two consecutive first order reactions (eqn (1)–(4) and Scheme 1). The corresponding rate equations can be solved to calculate the product distributions over time.



Scheme 1 Conditions: (a) diphenyl chlorophosphate, py, 18 h; (b) *tert*-butyldiphenylchlorosilane, imidazole, DMF, 26 h; (c) HN_3, PPh_3, diisopropyl azodicarboxylate, 1,4-dioxane, 66 h.

$$Tre + R \xrightarrow{k_1} Mono + R \xrightarrow{k_2} Di$$

$$d[Tre]/dt = -k_1[Tre][R] \quad (1)$$

$$d[Mono]/dt = k_1[Tre][R] - k_2[Mono][R] \quad (2)$$

$$d[Di]/dt = k_2[Mono][R] \quad (3)$$

$$d[R]/dt = -k_1[Tre][R] - k_2[Mono][R] \quad (4)$$

For a symmetric diol where modification of one hydroxyl does not perturb the reactivity of the other, $k_1 = 2k_2$ giving a 50% theoretical yield for the monofunctional product for 1 equivalent of reagent *R* at the reaction endpoint. We attempted a range of reactions on **1** in batch, but found substantially lower yields for the desired product (Scheme 1), implying that $k_1 \neq 2k_2$ in all cases. Phosphorylation, silylation and Mitsunobu reaction with hydrazoic acid gave yields of 24%, 10% and 0% respectively for the desired monofunctionalized product. Previous literature accounts of trehalose modifications have reported similar low yields. For example, halogenation of the primary hydroxyl with *N*-halosuccinimides gave 13%, 21% and 37% yields for chloro, iodo and bromo respectively,[14] while tosylation afforded only the doubly functionalized adduct.[15] Together these preliminary results both highlighted and confirmed existing inefficiencies in polyol systems such as **1**; although formally simple, such monofunctionalizations remain an unsolved problem.

We hypothesized that these non statistical yields were due to the lower solubility of trehalose in organic solvents giving a low k_1. In such a scenario, after the first reaction, the monofunctionalized trehalose adduct is rendered more soluble, and as a result $k_2 > k_1$ leading to the observed low yields.‡ With this in mind, we devised strategies to improve yields and to test the validity of this hypothesis.

Flow chemistry is an emerging technology that allows precise control of reaction conditions and is particularly applicable to desymmetrizations.[16] Direct analysis of product distribution by HPLC against residence time can yield valuable kinetic data. High reproducibility allows easy scale-up, a particular problem for this type of regioselective reaction where the optimum end point can be difficult to judge, often requiring a visual estimation of product distribution by TLC. Phosphorylation of **1** in a microreactor to optimize the yield of **3** was performed with excess diphenyl chlorophosphate (4 equiv.) over different residence times. Product distribution was monitored by HPLC (Fig. 2, see ESI†).[17] A maximum yield of 44% was obtained for a 2 min residence time. The reaction was performed on a 2 gram scale with 39% isolation of **3**. Non-linear regression analysis of the data using eqn (1)–(4) (see ESI†) revealed that $k_1 = 0.157$ $mol^{-1}dm^3s^{-1}$ and $k_2 = 0.133$ $mol^{-1}dm^3s^{-1}$, better reflecting the expected theoretical yields and ratio of k_1 to k_2 compared to the batch reaction.

While the improved yield of phosphorylation is beneficial, the use of flow methods here is not a synthetic solution in its own right since the technique is very solvent intensive (the necessity for solubilisation of trehalose required a dilute 25 mM solution); methods minimizing solvent usage are preferable.[18] Furthermore, for instalment of other functionality, conditions would have to be individually re-optimized. Therefore, we chose to use the same principles to develop more general methodology for the desymmetrization of trehalose to give a versatile starting material that could be further modified to other products.

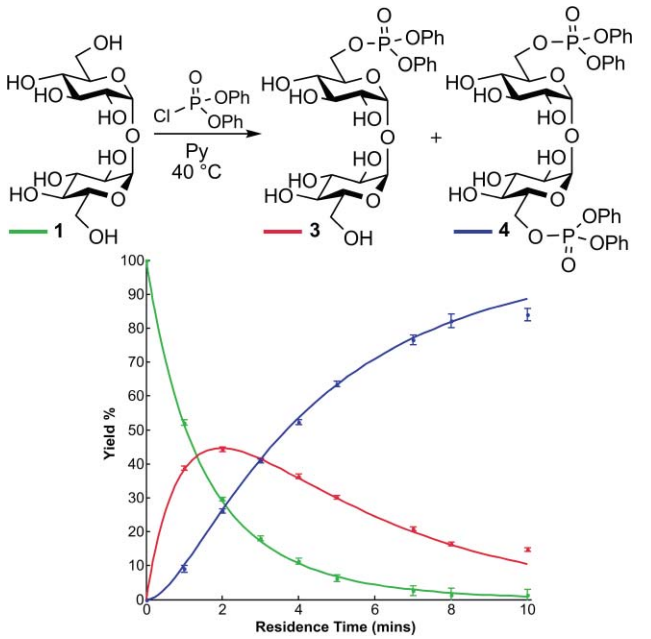
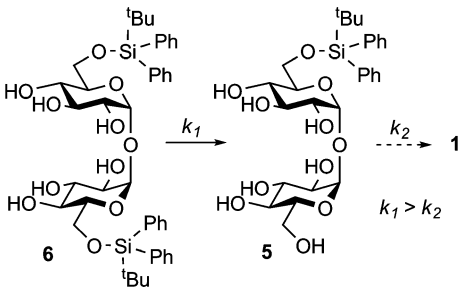


Fig. 2 Product distribution at various residence times for the phosphorylation of **1** in a microreactor chip.

In this, we exploited the poor solubility of deprotected trehalose analogues to achieve more efficient desymmetrizations. Rather than attempting a single modification on **1** based on simultaneous mode (a) and mode (b) selectivity, a reverse approach was used applying *first* mode (a) *then* mode (b) selectivity (Scheme 2). Removal of a silyl group from **6** gives **5**, which is far less soluble. Thus the subsequent deprotection to **1** occurs at a much slower rate leading to higher yields for **5**.



Scheme 2 Reverse modification strategy for desymmetrizations. Desilylation reactions performed with TBAF on a microreactor.

The kinetics of this reverse approach were probed using a microreactor chip. Two solvent conditions were explored: (i) high solubility conditions (methanol:pyridine 1 : 1) where **6** and **5** show similar solubility and (ii) low solubility conditions (THF:petrol 1 : 1) where the solubility of **5** is significantly reduced (Fig. 3). Product distributions were again analyzed using non-linear regression based on eqn (1)–(4) (see ESI†) to calculate rate constants. For the high solubility case $k_1 = 21.9 \times 10^{-3}$ $mol^{-1}dm^3s^{-1}$ and $k_2 = 17.9 \times 10^{-3}$ $mol^{-1}dm^3s^{-1}$. For the low solubility case $k_1 = 21.6 \times 10^{-3}$ $mol^{-1}dm^3s^{-1}$, effectively unaltered by the solvent. On the other hand $k_2 = 7.67 \times 10^{-3}$ $mol^{-1}dm^3s^{-1}$, substantially reduced from the high solubility case. Thus, by altering the

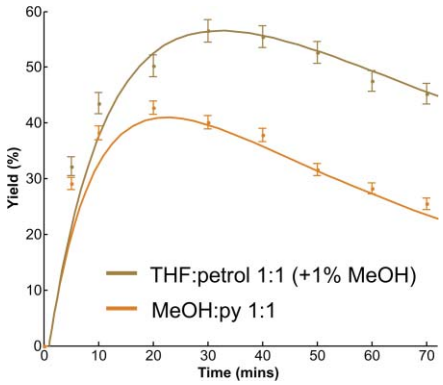
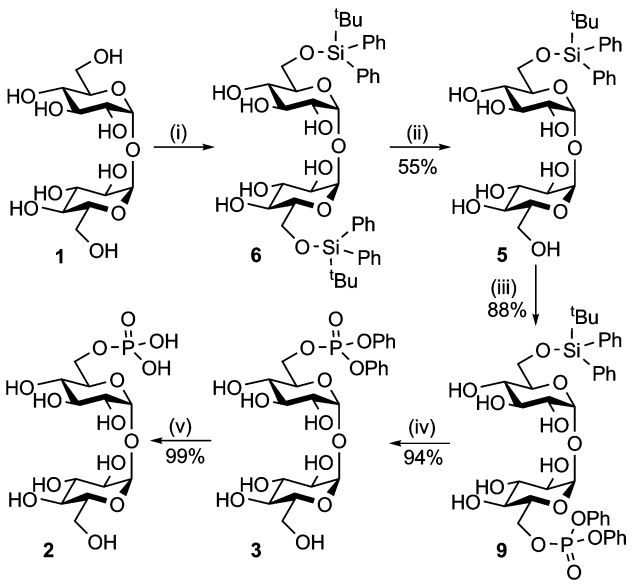


Fig. 3 Yield of **5** *versus* time for different solvents.

solvent to exploit solubility differences of trehalose adducts, yields of monofunctionalization were improved through manipulation of k_2.‡

Such "phase control" of reactions has been utilized in enzymatic synthesis to shift reaction equilibria towards the product by judicious choice of solvent.[19] Understanding the physics of attrition grinding deracemizations (another "phase controlled" process)[20] has allowed for the enantiopure synthesis of the non-steroidal anti-inflammatory drug Naproxen.[21] Analogously, by understanding the kinetics of trehalose desymmetrizations, we have optimized conditions for the synthesis of **2**, an important natural signalling molecule.

To avoid the potential for precipitation under such reduced solubility conditions (with subsequent blockage of the microreactor) and to create a more generally applicable route for increased scale, we used the kinetic data and conditions discovered using the flow methods to develop a batch method. In this way, flow discovery allowed batch optimization. Thus under these newly discovered conditions, silylation of **1** smoothly gave **6** (Scheme 3). The crude product was used directly after removal of pyridine. **6** was suspended in diethyl ether and methanol to give a saturated solution, which was deprotected with HCl generated *in situ* from acetyl chloride.[22] Regular monitoring of the reaction allowed isolation of **5** in 55% yield over 2 steps. Compound **5** was phosphorylated regioselectively affording **9** in 88%. Subsequent deprotection of the silyl group under acidic conditions proceeded smoothly in 94% yield. Hydrogenation of **3** allowed near quantitative phosphate deprotection to furnish **2** in a 45% yield over five steps. Thus, we obtained phosphorylation yields similar to those discovered through flow chemistry using a convenient and scalable batch process that offers a significant improvement in yield over the conventional reaction (Scheme 1a).



Scheme 3 Conditions: (i) *tert*-butyldiphenylchlorosilane, py, 14 h; (ii) AcCl, MeOH–Et_2O 1 : 3 18 h; (iii) diphenyl chlorophosphate, py, 15 h; (iv) AcCl, MeOH, 18 h; (v) H_2, PtO_2, AcOH, EtOH, 14 h.

In conclusion, we have used flow chemistry to study the desymmetrization of trehalose. This has produced kinetic data that has given vital insights into the mechanism of these seemingly simple reactions. This data can explain previous literature reports of low yielding monofunctionalizations on deprotected trehalose. Strategies to improve dissolution of trehalose, such as dilute reaction mixtures, or protection of secondary hydroxyls are possible workarounds. Alternatively, a reverse modification route can exploit phase effects to give greater than statistical monofunctionalization yields. The facile, scalable synthesis of **5** can be readily incorporated into synthetic routes for other non-symmetrical trehalose analogues and, together with the reverse modification approach in general, could lead to significant improvements in yield. The potential also exists for extension of the principle, for example, to pseudosymmetric disaccharide polyols allowing access to modifications at one primary hydroxyl in the presence of another.

Acknowledgements

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Notes and references

‡ The rate constants k_1 and k_2 refer to the *apparent* rate constants. Due to the poor solubility of the trehalose analogues, the actual concentration of these compounds in solution cannot be determined. Thus, while the actual rate constants cannot be measured, it is mathematically convenient in the context of these discussions to express the reductions in rate as apparent rate constants.

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