STICS: surface-tethered iterative carbohydrate synthesis†

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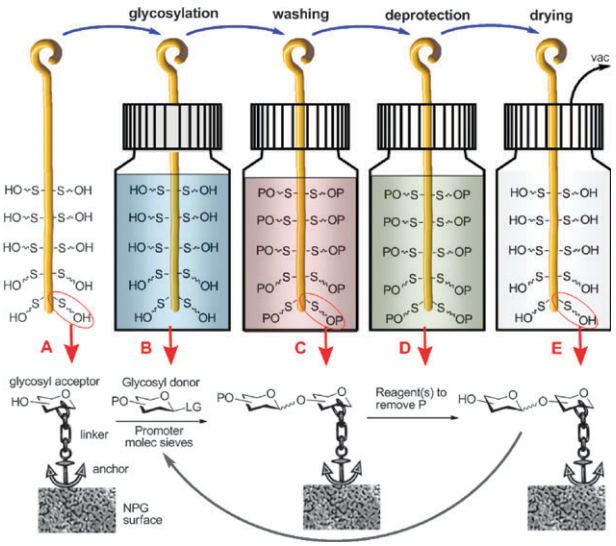
A new surface-tethered iterative carbohydrate synthesis (STICS) technology is presented in which a surface functionalized 'stick' made of chemically stable high surface area porous gold allows one to perform cost efficient and simple synthesis of oligosaccharide chains; at the end of the synthesis, the oligosaccharide can be cleaved off and the stick reused for subsequent syntheses.

Carbohydrates are the most abundant molecules on Earth, yet our ability to chemically synthesize these complex natural compounds to keep pace with rapidly evolving areas of glycomics is still far from being satisfactory.[1–4] In spite of significant progress in the area of synthetic carbohydrate chemistry, there is a constant need for improved methods.[5–14] Recent years have witnessed a new loop of discovery of high throughput technologies for expeditious oligosaccharide assembly; their syntheses in a one-pot fashion,[15–19] by using an automated synthesizer,[20,21] or in a microreactor[22] are only a few of the recent notable breakthroughs.

Herein we describe a new complementary technology that we call STICS (surface-tethered iterative carbohydrate synthesis). This technique is aimed at overcoming some of the limitations associated with traditional solid-phase synthesis of oligosaccharides,[23,24] such as limited use of molecular sieves, large volume of waste solvent, resin poisoning, reagent trapping, and difficulties in the construction of compound libraries. As illustrated in Scheme 1, at the basis of the STICS concept is a surface functionalized 'stick' made of chemically stable high surface area material that would simplify the transformation of the solid support-bound molecules between the reaction vessels. The glycosyl acceptor-anchored stick (A) is placed in the reaction vessel (B), containing sufficient quantities of the glycosyl donor, promoter and molecular sieves. Upon completion of the reaction, the stick, functionalized by a protected terminal sugar moiety, is transferred into another vessel (C) containing an appropriate solvent, wherein excess reagent is being rinsed off. Subsequently, the stick is dipped into vessel D containing a certain reagent to remove a strategically placed temporary substituent P. This transformation results in the formation of the second-generation glycosyl acceptor. To conclude the cycle,



Scheme 1 Outline of the STICS concept.

the stick is then briefly placed in a thick-wall container connected to the vacuum (E). Upon completion of this sequence, again, a hydroxyl-modified stick is available for consequent glycosylation. To repeat the cycle, coupling–washing–deprotection–drying steps are performed once again. At the end of the synthesis, the oligosaccharide can be cleaved off from the stick. Depending on the type of the attachment used, the oligosaccharide can be deprotected directly on the stick to be used for immunoassay or molecular recognition studies.[25]

The "stick" material has been prepared in the form of free-standing plates of very high surface area that is then covered with the organic anchor bound to a carbohydrate acceptor. For the high surface area stable material we chose nanoporous gold (NPG) that excellently suits our purpose as it provides the benefits of high surface area with the chemical stability of gold and access to the well-established chemistry of surface modification using thiol or disulfide derivatives that can form self-assembled monolayers (SAMs).[26] The NPG plates have been prepared by dealloying commercially available 10 carat white gold (Hoover and Strong, 41.8 at% gold, 5 at% Ag, 30–35 at% Cu, 8–9 at% Zn, and 15–20 at% Ni) in nitric acid and characterizing the resulting NPG using field-emission SEM and tapping mode AFM.[27] The thickness of the sheet used was 250 μm for these efforts at supported synthesis and pieces were cut of dimensions of 8.0 × 8.0 mm.

The synthesis of the carbohydrates on NPG makes use of a series of common glycosyl donors (**1–4**,[28–31] Fig. 1). The NPG

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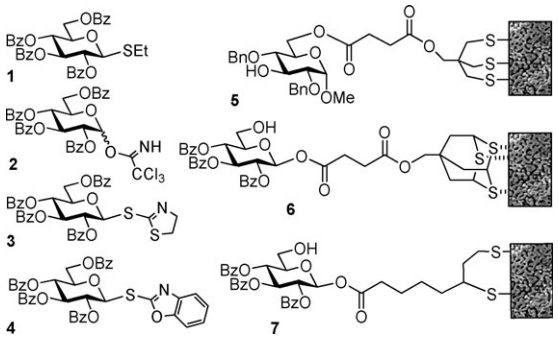


Fig. 1 Glycosyl donors **1–4** and tethered glycosyl acceptors **5–7**.

surface of the plates is then modified with self-assembled monolayers of anchor–linker–monosaccharide conjugates bearing the first partially protected sugar unit (glycosyl acceptor). The anchor compounds are di- or tri-thioderivatives designed to bond strongly to the surface and do not desorb in the organic solvents used in the subsequent preparative steps.

The trithiol derivative **5** and the lipoic acid derivative **7** are expected to be covalently bound to the gold surface, while the derivative **6** with its three sulfide groups should be adsorbed through multiple attractive Au–S interactions, as found for dialkylsulfides.[32,33] The formation of self-assembled monolayers of trithiols,[34–36] lipoic acid[37] including derivatives linked to carbohydrates,[38] resorcin[4]arene tetrasulfides[39] and a trithiaadamantyl derivative[35] have been reported. Depending on the structure, the anchor is covalently bonded to the monosaccharide acceptor either *via* a succinoyl linker segment (**5** and **6**) or directly (**7**) designed so that after the desired series of glycosylation reactions is completed, it can be easily cleaved.

The surface coverage of these anchor-linker species was assessed gravimetrically and was determined to be ~0.9–1.1 μmol per 8 × 8 mm plate. Typical results of single-step glycosylation experiments on a single plate are summarized in Table 1. The main motivation for these preliminary experiments was to identify the best donor-tethered acceptor–promoter combination. This was achieved by direct comparison of donors **1–4** and acceptors **5–7**. The glycosyl donor was used in excess (5 equiv.) and the reaction was only performed once. Expectedly, repetitive glycosylations could positively affect the yield,[40] yet this was not our primary intention at this stage of the method development. As evident from the results presented in Table 1, *S*-benzoxazolyl glycoside **4** provided marginally higher yields than other glycosyl donors tested (**1–3**), and was particularly effective with the primary glycosyl acceptors (**6** and **7**, entries 8 and 12, respectively). Since the disaccharides **8** and **9** in the single-plate experiments were obtained in amounts less than 1 mg, the most reliable technique to determine the yields was HPLC using a standardized calibration plot. Detailed information on this approach can be found in the ESI†.

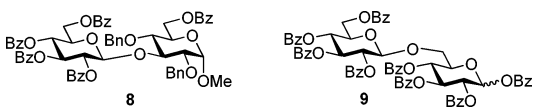


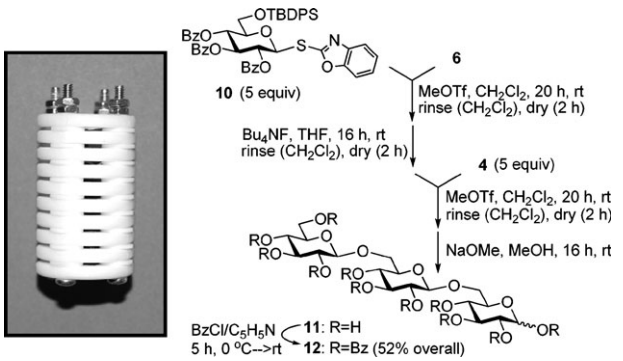
Table 1 STICS of disaccharides **8** and **9**

Entry	Donor	Acceptor	Promoter	Product	Yield (%)
1	**1**	**5**	MeOTf	**8**	31
2	**2**	**5**	TMSOTf	**8**	39
3	**3**	**5**	MeOTf	**8**	32
4	**4**	**5**	MeOTf	**8**	42
5	**1**	**6**	MeOTf	**9**	35
6	**2**	**6**	TMSOTf	**9**	33
7	**3**	**6**	MeOTf	**9**	44
8	**4**	**6**	MeOTf	**9**	65[a]
9	**1**	**7**	MeOTf	**9**	48
10	**2**	**7**	TMSOTf	**9**	50
11	**3**	**7**	MeOTf	**9**	62
12	**4**	**7**	TMSOTf	**9**	65

[a] When this reaction was reproduced on the 10-plate assembly, an improved yield of 72% was achieved.

The free-standing plates can be also slotted into Teflon assemblies (sticks) of ten plates (or greater). For example, the reaction between **4** and **6** performed on the 10-plate stick assembly yielded disaccharide **9** in 72% (7.8 mg), which was a marginal improvement in comparison to the single plate experiment (65%, entry 8, Table 1). The estimated cost of material required to prepare the 10-plate assembly is $18.

To demonstrate further versatility of the developed technique, we performed the synthesis of trisaccharide **11**. For this purpose 6-*O*-TBDPS-protected glycosyl donor **10** was reacted with the anchored glycosyl acceptor **6** in the presence of MeOTf (Scheme 2). The tethered disaccharide intermediate was then treated with Bu_4NF in THF and the resulting 6′-OH acceptor was glycosylated with glycosyl donor **4**. After that, trisaccharide **11** was cleaved off with NaOMe in MeOH, and characterized as per-benzoate **12** obtained in 52% overall yield.



Scheme 2 STICS of trisaccharide **11**.

It is important to note that the use of a stack of NPG plates also allows a split-and-mix combinatorial approach, single plate analysis, conduct of biological assays, *etc*. The stick can be disassembled and shuffled as needed for the creation of combinatorial libraries of carbohydrates. To illustrate this, we performed the synthesis of an alternative trisaccharide derivative (see ESI†). It has been also demonstrated that the remaining stick can be reused with high efficiency (see ESI†).

In conclusion, we have developed a new technology for expeditious oligosaccharide synthesis that elaborates on the advantageous features of traditional solid-phase synthesis. This new approach offers a useful alternative for high throughput

directed and combinatorial synthesis of carbohydrates or other bioorganic molecules as well as biological screening thereof. Considering the manipulative character of STICS strategy, further automation can be envisaged, and is under pursuit in our laboratories.

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