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Rapid synthesis of oligosaccharides using an anomeric fluorous silyl protecting group†

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A fluorous silyl ether tag as protecting group at the anomeric position of sugar acceptors allows rapid synthesis of oligosaccharides by reducing the purification procedures to a simple and fast fluorous solid-phase extraction.

Carbohydrates on cell surfaces play important roles in biological processes such as cell–cell interaction, cell adhesion, and immunogenic recognition.[1] It would be of great importance for the development of glycobiology to have easy access to pure synthetic oligosaccharides. However, the synthesis of oligosaccharides is a lengthy process that typically involves iterative sequences of protecting group removal and glycosylation steps starting from suitably protected and activated glycosyl acceptors and donors. In classical conditions, each of these steps requires a chromatographic purification of mixtures that can be rather complex. Solid-phase synthesis of oligosaccharides has been extensively studied[2] as a means to speed up this process. However, solid-phase synthesis suffers from some disadvantages, such as difficulties in monitoring the reaction process and in characterizing the reaction products by NMR analysis, or mass spectrometry. The use of a soluble PEG-based support[3] can overcome some of these disadvantages, however the reactions still could not be monitored by TLC and the intermediates would not be easily purified from other PEG-linked byproducts. Furthermore, in the NMR spectra the signals due to the PEG protons extensively overlap with the region typical of carbohydrate protons, thus complicating the characterization of the compounds.

Recently, fluorous chemistry has been developed for use in several fields such as combinatorial chemistry, parallel synthesis, and the discovery of catalysts.[4] This technology is very attractive for strategic separation of reaction mixtures since fluorous-tagged compounds can be quickly separated from non-tagged compounds in binary liquid–liquid and solid–liquid extractions.[4d] A large number of fluorine atoms can be required to induce tagged molecules to partition into a fluorous solvent. Such highly fluorinated molecules can have little or no solubility in organic solvents, and therefore finding suitable reaction conditions for this material is not trivial. On the other hand, the recently introduced technique of fluorous solid-phase extraction (SPE) is proving far superior to liquid–liquid extractions for compounds with fewer fluorine atoms.[5] Highly fluorinated acetal, silyl, benzyl, and novel acyl protecting groups for hydroxy functions have already been reported[6–8] or are commercially available.

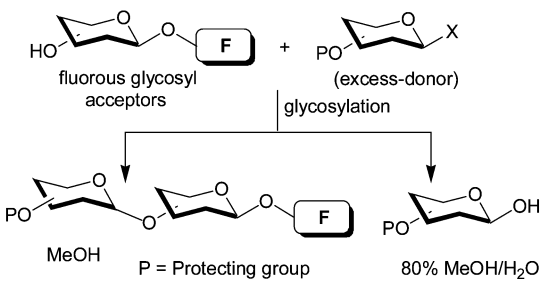
We would like to report here the application of a known acid- and base-stable silyl protecting group developed by Wipf and co-workers[6c] to fluorous oligosaccharide synthesis.

Our aim is to exploit a fluorous silyl protecting group appended at the anomeric position of glycosyl acceptors in a growing oligosaccharide chain to avoid the tedious, expensive, and time consuming flash chromatography purification of the reaction product after every step. Our concept of oligosaccharide synthesis using a fluorous tag is shown in Scheme 1. Introduction of the tag at the anomeric position of the glycosyl acceptor should enable the efficient synthesis of branched and long-chain oligosaccharides. The glycosyl acceptor bound to the fluorous tag can be coupled with the glycosyl donor to afford the fluorous disaccharide which in principle should be separable from non-fluorous material, such as excess donor, by solid-phase extraction without the need for column chromatography. Thus, the reaction mixture could be passed through fluorous silica gel in a two-stage extraction which would first remove the non-tagged products and then elute the fluorous-tagged one. The fluorous tag is removed at the end of the synthetic sequence to give the desired oligosaccharide. In this scheme, unreacted glycosyl aceptor, which is fluorous tagged, cannot be easily separated by fluorous extraction from the tagged reaction product. However, the reaction could be monitored, *e.g.* by MALDI MS, and driven to completion by multiple cycles, similar to the practice of solid-phase synthesis.

To put this scheme into practice, the fluorous protecting group must be easy to prepare and to introduce on the sugar, capable of withstanding the typical reaction conditions required for protection/deprotection of the acceptors and for the glycosylation reactions, and finally it must be easily removed from the final oligosaccharide. Given the peculiar reactivity of the anomeric oxygen, esters were deemed unsuitable, and we focused our attention on the use of silyl ethers. Among the known lightly fluorinated silyl halides,[9] *tert*-butyl-phenyl-1*H*,1*H*,2*H*,2*H*-heptadecafluorodecyloxysilyl bromide[6c] was chosen because it is easily synthesized and relatively stable to acidic and basic conditions.[10]

The *tert*-butyl-phenyl-1*H*,1*H*,2*H*,2*H*-heptadecafluorodecyloxysilyl bromide was synthesized as described in the literature[6c] and the fluorous tag was attached to the anomeric hydroxy group of the glucosamine derivative **1** (Scheme 2) by using imidazole and DMAP to give the fluorous compound **4** in 85% yield. This silyl ether proved to be sufficiently robust to withstand the classical reaction conditions of sugar chemistry, as shown by the synthesis of the Galβ1,3-GlcNAc disaccharide **8** reported in Scheme 2.

This scheme employs many of the reagents, protecting-group manipulations, and glycosylation conditions that are standard in the field. Removal of the acetyl groups from **4** under Zemplén conditions[11] followed by treatment of the crude product with benzaldehyde dimethylacetal in presence of CSA afforded the fluorous glycosyl acceptor **6**. Due to the limited number of fluorine atoms on the tag, **5** and **6** maintain a normal



Scheme 1 Concept of oligosaccharide synthesis using a fluorous tag at the anomeric position of the glycosyl acceptor.

† Electronic supplementary information (ESI) available: experimental data. See http://www.rsc.org/suppdata/cc/b3/b311448a/

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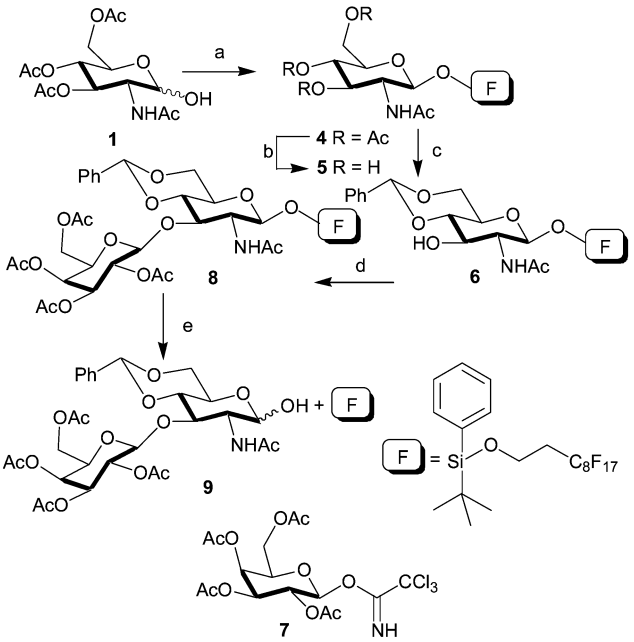

Scheme 2 a) *tert*-Butyl-phenyl-1*H*,1*H*,2*H*,2*H*-heptadecafluorodecyloxysilyl bromide, imidazole, DMAP, DCM, 85%; b) MeONa, MeOH, quant.; c) benzaldehyde dimethylacetal, CSA, CH_3CN, 75% after 2 cycles; d) **7**, TMSOTf, DCM, 78% after 2 cycles; e) TBAF, THF, 62%.

chromatographic behavior on standard silica gel, which allows monitoring of the course of the reactions by TLC. After a first cycle of acetal formation, TLC and MALDI-TOF analysis of the reaction crude revealed a small quantity of non reacted starting material **5**. This could have been removed by standard flash chromatography on regular silica gel. However, as a proof of principle, the reaction mixture was subjected to SPE on fluorous silica gel. Elution with 80% MeOH–H_2O removed all non-fluorous material, and the fluorous tagged compounds were eluted from the column using pure MeOH. The mixture was resubmitted to the acetal formation. This second cycle consumed all the starting material and **6** was isolated in 75% yield.

The disaccharide **8** was obtained by reaction of the pure acceptor **6** with the glycosyl donor **7**[12] (3 equiv.) in the presence of TMSOTf (0.1 equiv.) in CH_2Cl_2. Again, the course of the reaction could be monitored by TLC on standard plates. The fluorous-tagged material could be isolated from by-products by eluting first with 80% MeOH–H_2O (to remove the nontagged products) and then with MeOH (to remove the tagged products), and the reaction was driven to completion by a second cycle of glycosylation with 2 equivalents of the glycosyl donor **7**.

Finally, the fluorous silyl protecting group of **8** was removed by TBAF to afford crude **9**, which was passed through fluorous silica gel, eluting with MeOH–H_2O.

In conclusion, the use of a fluorous tag as protecting group at the anomeric position of a glycosyl acceptor allowed rapid synthesis of a disaccharide by a fluorous solid-phase extraction purification. The disaccharide **9** was obtained in 36% overall yield from **4** (four steps) without silica-gel chromatographic purification. Each synthetic intermediate could be easily purified and characterized by NMR, mass spectroscopy (MALDI-TOF), and TLC on standard silica gel plates. This is a major advantage over classical solid-phase synthesis conditions, and allows rapid optimization of the reaction conditions for each synthetic step. Compared to reaction of PEG-supported carbohydrates, the fluorous tag strategy has the advantage of allowing the use of silica gel TLC to monitor the reaction process. Furthermore, the NMR signals originating from the fluorous tag do not interfere with the carbohydrate region, thus allowing an easy characterization of the tagged compounds. The optimization of the glycosidation conditions, such as the study of new silyl fluorous tags, and further applications to the synthesis of several carbohydrates and glyconjugates are now in progress.

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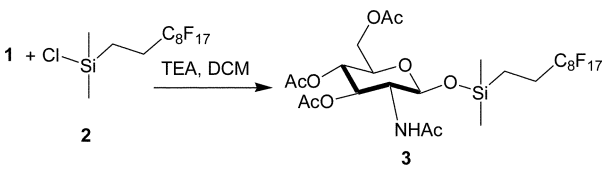
10 The commercially available fluorous silyl chloride **2** yields a relatively unstable silyl ether, which cannot survive, for instance, the methoxide catalyzed deacetylation conditions. Compounds tagged at the anomeric position with **2** could nonetheless be separated from untagged material as described in this paper. For instance, the fully protected fluorous silyl glucosamine **3** could easily be separated on fluorous silica gel from tetra-*O*-acetylglucose by first eluting the non-tagged compound with 80% MeOH–H_2O, followed by pure MeOH that cleanly removed the tagged product **3** from the column.



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