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Synthesis of the Lewis a Trisaccharide Based on an Anomeric Silyl Fluorous Tag

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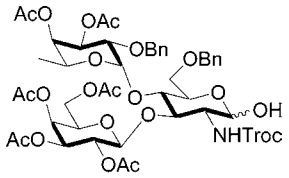
ABSTRACT



Lewis a

The synthesis of the trisaccharide Lewis a was performed using an anomeric fluorous silyl protective group. This methodology allowed us to fully characterize each product (NMR, MS) and monitor each synthetic step (TLC). Although the product purifications could be performed by fluorous-solid-phase extraction (F-SPE) technology, standard chromatography could be used to effect purification if necessary. Trichloroethoxy carbonyl (Troc) protection of the amino group of the glucosamine moiety was found essential to allow protecting group manipulation of the fluorous protected sugar.

Rapid and reliable access to specific oligosaccharides is critical to the development of glycobiology. However, traditional oligosaccharide synthesis is a time-consuming business primarily because of the tedious chromatographic purifications often required of the multiple synthetic intermediates. To overcome these problems the solid-phase synthesis of oligosaccharides has been actively studied,[1] and the synthesis of oligosaccharides using a peptide synthesizer has been reported.[2] However, current solid-phase synthetic technologies suffer from disadvantages, such as difficulty in monitoring the reaction progress and in characterizing the reaction outcome by NMR analysis or mass spectrometry. Recently, fluorous chemistry has become an attractive alternative to solid-phase synthesis.[3] This technology is attractive for strategic separation of reaction mixtures since fluorous-tagged compounds can be quickly separated from nontagged compounds in binary liquid−liquid and solid−liquid extractions. Highly fluorinated acetal, silyl, benzyl, thiol, and novel acyl protecting groups for hydroxy functions used for oligosaccharides synthesis have already been reported.[4] However, such highly fluorinated molecules can have limited or no solubility in organic solvents. Therefore, finding suitable reaction conditions for working with such materials is not trivial. On the other hand, lightly fluorinated compounds are soluble in common organic solvents and can be analyzed by NMR and MS, and the reactions can be monitored by TLC on conventional plates.

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Furthermore, a lightly fluorinated compound is readily purified from nonfluorinated compounds by a simple fluorous-solid-phase extraction (F-SPE)[5] on Fluoro*Flash* silica gel.

Recently, we reported on the fluorous disaccharide synthesis of Galβ(1−3)GlcNAc using an anomeric silyl fluorous tag as a protective group on the glycosylation acceptor.[6] We have shown that all non-fluorinated compounds can be removed from glycosylation crudes by eluting with MeOH/H_2O on Fluoro*Flash* silica, and the fluorinated materials can be recovered using MeOH as the eluant. Thus, with this method the compounds can be purified by a simple F-SPE, and the reaction can be driven to completion by multiple cycles, in analogy to the solid-phase synthesis. Furthermore, all the intermediates can be characterized by NMR and MALDI-TOF analysis, and the reactions can be monitored by TLC.

With the aim of demonstrating the utility of this strategy for speeding up the synthesis of oligosaccharides, we have now performed the synthesis of the Lewis a trisaccharide employing reagents, protecting-group manipulations, and glycosylation conditions that are standard in the field.

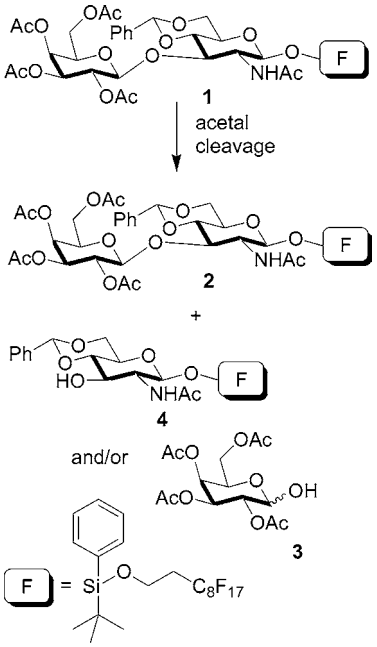
Dramatically important for this approach was the selection of the nitrogen protective group for the glucosamine moiety. Our initial approach began with the known fluorous-tag-protected disaccharide **1**.[6] We began the protecting group manipulation with the aim of obtaining the free 4-OH acceptor **2** ready for the following planned glycosylation (Scheme 1).

To our disappointment we found that the standard, widely used procedures for regioselective cleavage of the benzylidene group (HCl, $NaCNBH_3$ or Et_3SiH, TFA)[7,8] afforded the desired product in a very poor yield (5−10%), the main isolated product being the tagged monosaccharide **3**. To test the reactivity/stability of this disaccharide, we tried to completely remove the benzylidene group via hydrogenolysis (H_2/Pd). To our surprise **4** was again the main isolated product.[9,10] A similar pattern of behavior was observed when the disaccharide **1** was submitted to a transacetalyzation reaction (1,3-propanditiol, CSA or MeOH, CSA). These findings suggested that the fluorous tag produced an inversion of the normal reactivity[11] between the benzylidene acetal and the glycosyl linkage.[12]

Recognizing that the glycosyl linkage might be stabilized by stereoelectronic effects, we used a different amino protecting group for the glucosylamine. We chose for our purpose the *N*-trichloroethoxycarbonyl derivative (Troc), as this is a useful protecting group in oligosaccharide synthesis.[13] The Troc group can be removed in high yield by reduction.[14] In addition, this group is stable under a range of standard conditions used for carbohydrate synthesis, and *N*-Troc-protected glycosyl donors have been used for a convergent synthesis of oligosaccharides.

Starting from the known *N*-Troc-protected glucosamine **5**[11] the fluorous tag was attached to the anomeric hydroxy group by using triethylamine and DMAP; the fluorous compound **6** was obtained in quantitative yield (Scheme 2). Removal of the acetyl groups from **6** under Zemplén conditions[15] followed by treatment of the crude product with benzaldehyde dimethylacetal in the presence of CSA afforded the fluorous glycosyl acceptor **7**. As a result of the limited number of fluorine atoms **6** and **7** show normal chromato-

Scheme 1. Cleavage of the Benzylidene Group[a]



[a] Regioselective cleavage: $NaCNBH_3$, HCl or Et_3SiH, TFA. Hydrogenolytic cleavage: H_2, Pd/C. Transacetalyzation: 1,3-propanditiol, CSA or MeOH, CSA.

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(8) DeNinno, M. P.; Etienne, J. B.; Duplantier, K. C. *Tetrahedron Lett*. **1995**, *36*, 669.

(9) When a reductive cleavage was used the galactose moiety was detected as a pyran derivative, whereas in the case of hydrolytic cleavage the hydrolyzed galactose has been detected.

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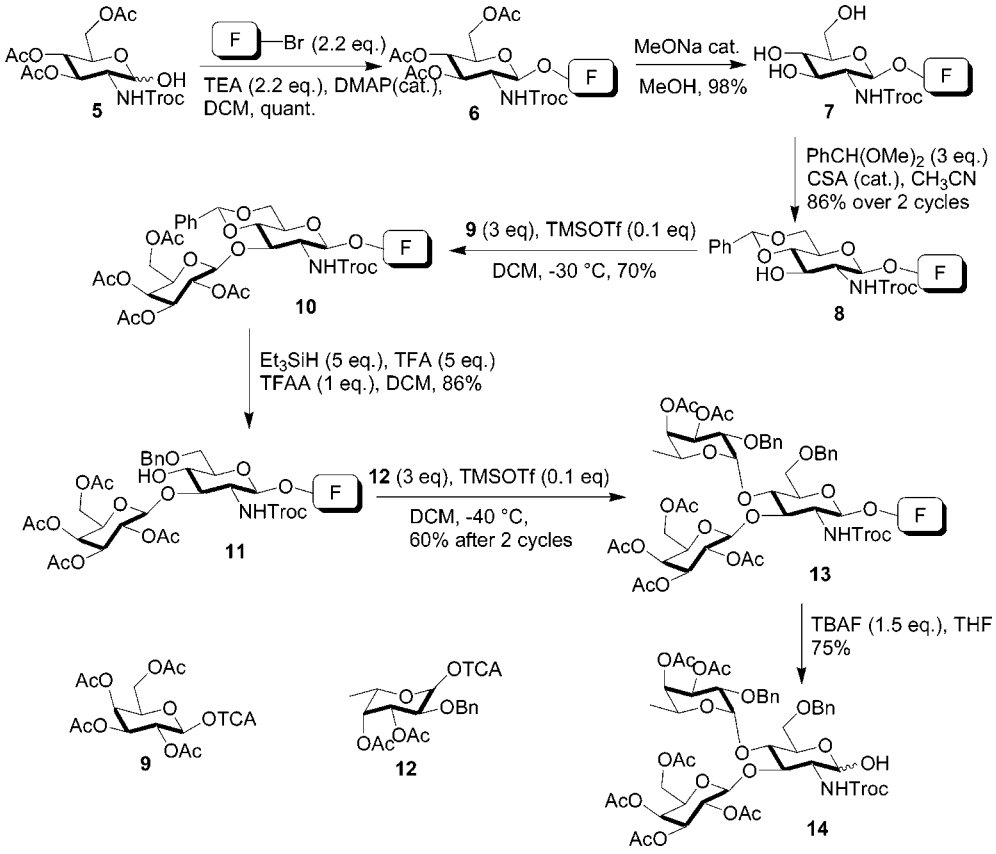
Scheme 2. Synthesis of the Trisaccharide Lewis a



graphic behavior on standard silica gel, which allows monitoring of the course of the reactions by TLC. Starting from **7**, compound **10** was obtained with the same procedure described for the synthesis of compound **1**.[6]

Using Troc for glucosamine *N*-protection the normal reactivity was restored, and the regioselective cleavage of the benzylidene acetal proceeded smoothly with Et_3SiH in the presence of TFA and TFAA. The main product observed (TLC, MALDI, NMR) was the disaccharide **11**. Regioselective opening was confirmed by acetylating the free 4-OH and observing the downfield shift of the 4-H proton in the glucosamine moiety. A very small quantity of the 4,6-diol byproduct derived from hydrolysis of the benzylidene group was easily removed by standard chromatography.

The suitably protected disaccharide **11** was then submitted to a glycosylation reaction with the fucosyl donor **12**, in the presence of TMSOTf, affording the Lewis a trisaccharide **13** in 60% yield. The crude product was purified by F-SPE with the procedure described above, and the reaction was driven to completion by a second cycle of glycosylation with 3 equiv of the fucosyl donor **12**, affording the trisaccharide **13** in 60% unoptimized yield. The relatively low yield in this step is due to the low stability of **13** for which we observed 30% decomposition in 3 days at −18 °C.

Finally, the fluorous silyl protecting group of **13** was removed by reaction with TBAF to afford crude **14**, which was purified by fluorous silica gel chromatography, eluting with 80% MeOH−H_2O.

In conclusion, we have expanded the possibilities for using a fluorous tag as a protecting group at the anomeric position of a glycosyl acceptor. The methodology has allowed rapid synthesis of a trisaccharide by a F-SPE purification. The trisaccharide **14** was obtained in only 3 days, in 23% overall yield, from **5** with a reduced number of silica gel chromatographic purification (average of 81% every step). All fluorinated compounds synthesized retain normal chromatographic behavior on standard silica gel, allowing easy monitoring of the course of reactions by TLC. Purification of the desired products obtained from multistep synthesis was significantly simplified by the fluorous moiety. The reaction mixtures were subjected to F-SPE on fluorous silica gel. The fluorous compounds could also be purified by normal silica gel chromatography if necessary, which was advantageous compared to solid-phase synthesis.

In addition, each synthetic intermediate could be easily characterized by NMR and mass spectroscopy (MALDI-TOF). The NMR signals originating from the fluorous tag did not interfere with the carbohydrate region, as is often observed in the case of PEG-supported synthesis. The optimization of the reaction conditions using the fluorous technology is easier than with the solid-phase synthesis because the reactions are run in liquid phase. Only standard apparatus is required for such oligosaccharides syntheses, and the reactions are easily scalable.

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The reactivity of the fluorous tagged compounds appears to depend critically on the protecting group on the glucosamine moiety. Using the appropriate group, standard manipulation of the carbohydrate functionality and glycosylation conditions could be adopted.

The study of new silyl fluorous tags and further applications to the synthesis of several complex carbohydrates and glycoconjugates are now in progress.

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Supporting Information Available: Experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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