ORGANIC LETTERS

2003
Vol. 5, No. 25
4839−4842

Sulfo-Protected Hexosamine Monosaccharides: Potentially Versatile Building Blocks for Glycosaminoglycan Synthesis

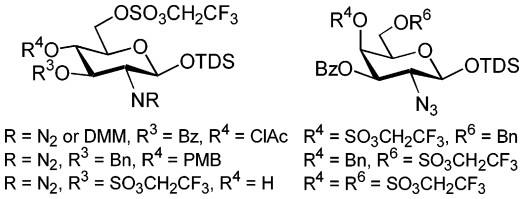
Nathalie A. Karst,‡ Tasneem F. Islam,‡ and Robert J. Linhardt*

Departments of Chemistry, Biology and Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, New York 12180

linhar@rpi.edu

Received September 26, 2003

ABSTRACT



2,2,2-Trifluorodiazoethane was investigated as a reagent for sulfo group protection on hexosamine monosaccharides. The synthesis of glucosamine and galactosamine building blocks fully differentiated for glycosaminoglycan synthesis and the synthesis of glycosyl donors are described. The compatibility of trifluoroethylsulfonate under a variety of reaction conditions has also been investigated.

2,2,2-Trifluorodiazoethane, introduced for sulfonic acid protection,[1] was first used as a reagent for sulfate ester protection in carbohydrates by Flitsch and co-workers in 1997.[2] Simple hexoses, protected as 2,2,2-trifluoroethylsulfonates, were shown to be stable under a variety of reaction conditions and could be removed by using base to recover the corresponding sulfonated hexoses. In our efforts toward the chemoenzymatic synthesis of glycosaminoglycans (GAGs), we decided to investigate the preparation of monomer building blocks carrying sulfo groups protected as trifluoroethylsulfonates. In this approach a sulfo group is either already present in the carbohydrate or introduced at the beginning of the synthesis. Tedious manipulations of sulfo monoester, particularly difficult at the higher oligosaccharide level, are eliminated, thus facilitating protecting group manipulation and purification, giving more straightforward access to oligosaccharides of interest.[3] We planned to begin by investigating the use of trifluoroethylsulfonate in the preparation of hexosamine monomer building blocks, which can be used in the synthesis of chondroitin sulfate, dermatan sulfate, and heparin oligosaccharides. The typical sulfation patterns in hexosamine residues naturally occurring in GAGs directed us toward the selection of our primilary targets (Table 1). Monosaccharide buiding blocks were built with orthogonal protection compatible with elongation at both the reducing and nonreducing ends (the C-4 of glucosamine and

Table 1. Most Common Sulfation Patterns in GAGs[a]

D-glucosamine in HP/HS[b]	D-galactosamine in CS/DS[b]
GlcN*p*6S	**GalN*p*4S**
GlcN*p*3,6S	**GalN*p*6S**
GlcN*p*S6S	**GalN*p*4,6S**
GlcN*p*S3,6S	

[a] Chosen targets are shown in bold. [a] HP: heparin. HS: heparan sulfate. CS: chondroitin sulfate. DS: dermatan sulfate. In HP/HS N can be substituted with acetyl or sulfo and in CS/DS N is always substituted with acetyl.

‡ Present address: Department of Chemistry, University of Iowa, Iowa City, Iowa 52242.

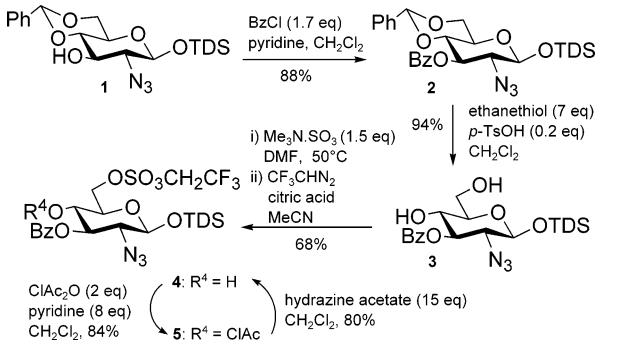
(1) Messe, C. O. *Synthesis* **1984**, 1041−1042.
(2) Proud, A. D.; Prodger, J. C.; Flitsch, S. L. *Tetrahedron Lett.* **1997**, *38*, 7243−7246.
(3) Karst, N.; Linhardt, R. J. *Curr. Med. Chem.* **2003**, *10*, 1993−2031.

10.1021/ol035882w CCC: $25.00
Published on Web 11/12/2003

the C-3 of galactosamine). Hydroxyl groups were protected by using various combinations of ester or ether protecting groups and both azido and *N*,*N*-dimethylmaleimido series were studied for their compatibility with the trifluoroethylsulfonate group.

Starting from D-glucosamine hydrochloride, compound **1** was prepared as described in the literature[4] and subsequently treated with benzoyl chloride to afford the 3-benzoylated derivative **2** in good yield (Scheme 1). Cleavage of the

Scheme 1

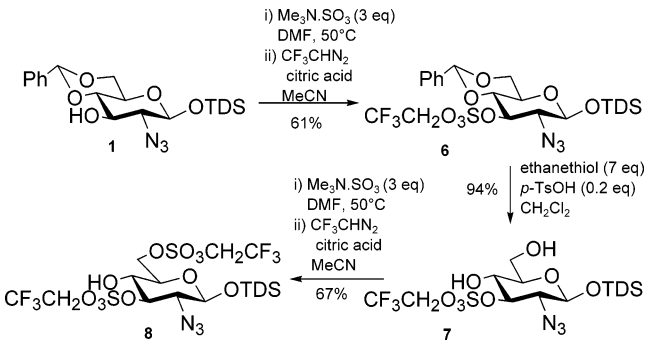


benzylidene acetal gave the 4,6-diol **3**, which was selectively 6-***O***-sulfonated with sulfur trioxide−trimethylamine complex in *N*,*N*-dimethylformamide.

Treatment of the intermediate sulfo monoester with an excess of a fresh solution of trifluorodiazoethane[5] in acetonitrile in the presence of citric acid afforded the desired 6-trifluoroethylsulfonate (sulfo diester) derivative **4** in 68% overall yield. The reaction proceeded smoothly after 1−2 days at room temperature requiring excess citric acid for the reaction to go to completion. No side products were detected, demonstrating the azido group to be compatible with the conditions of the sulfo-protection reaction. The selectivity of the trifluorodiazoethane for the sulfo ester was also verified as the 4-hydroxyl group remained untouched in this reaction. Chloroacetylation of the free 4-hydroxyl group provided the first GAG building block, **5**. Acceptor **4** could be later recovered from building block **5** by selective cleavage of the 4-chloroacetyl group with hydrazine acetate.

Preparation of disulfo derivative **8** was next undertaken from the common intermediate **1** (Scheme 2). 3-*O*-Sulfonation followed by treatment with trifluorodiazoethane afforded derivative **6** in 61% yield. Conditions for the cleavage of the benzylidene acetal were compatible with the trifluoro-

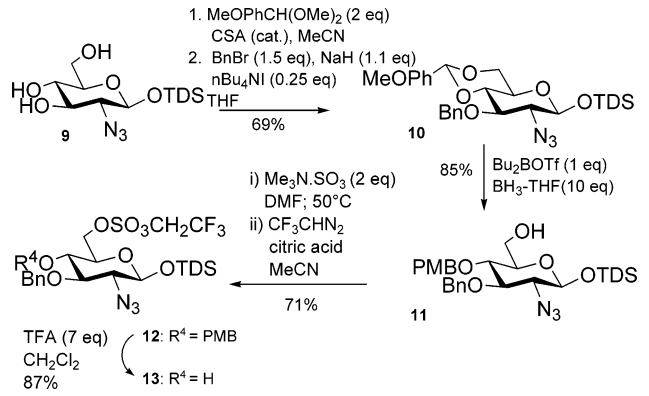
Scheme 2



ethylsulfonate moiety and the 4,6-diol **7** was obtained as the only product in good yield. When diol **7** was then submitted to a 2-step sulfonation/sulfo-protection sequence compound **8** was obtained in 67% yield.

Monosaccharide building block **12**, with ether protecting groups, was next synthesized to broaden the scope of our protection chemistry (Scheme 2). Introduction of the *p*-methoxybenzylidene at the 4,6-position of **9**, followed by benzylation at the 3-position gave **10**. Treatment of **10** with dibutylborane triflate and 1 M borane solution in THF[6] regioselectively opened the benzylidene ring and provided compound **11** in good yield and stereoselectivity. The remaining free 6-position was sulfonated and sulfo-protected to afford building block **12** in 71% overall yield. The *p*-methoxybenzyl could later be selectively removed, under acidic conditions, to give access to glycosylation acceptor **13**.

Scheme 3



D-Glucosamine hydrochloride was used to prepare 1,3,4,6-*O*-acetyl-2-deoxy-2-dimethylmaleimido *β*-D-glucopyranoside **14**,[7] which was treated with *p*-methoxyphenol in the presence of catalytic trifluoromethanesulfonic acid and transesterified to afford the *β*-MP derivative **15** (Scheme 4). Benzylidena-

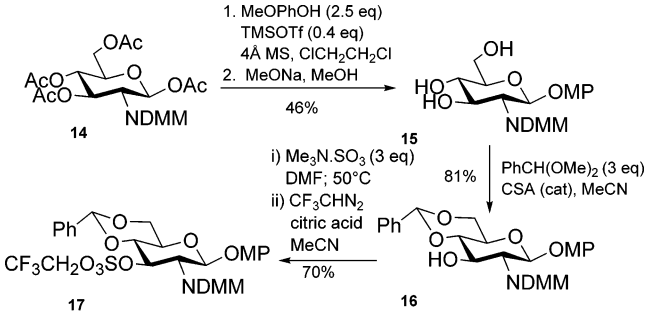
(4) La Ferla, B.; Prosperi, D.; Lay, L.; Russo, G.; Panza, L. *Carbohydr. Res.* **2002**, *337*, 1333−1342.

(5) **Typical Procedure.** Monosaccharide sulfo ester (500 mg) in solution in acetonitrile (5 mL) was treated with a fresh solution of 2,2,2-trifluorodiaozethane (40 mL) prepared as described in ref 2. (CAUTION: This reagent should be considered as potentially explosive and highly toxic.) Citric acid (2 g) was added and the reaction mixture was stirred at room temperature until TLC analysis showed complete consumption of the starting material (1−2 days). The solution was filtered over Celite and concentrated. The residue, dissolved in dichloromethane, was washed successively with water, a saturated solution of sodium bicarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. The product was purified by silica gel chromatography.

(6) Jiang, L.; Chan, T.-H. *Tetrahedron Lett.* **1998**, *39*, 355−358.

(7) Aly, M. R. E.; Castro-Palomino, J. C.; Ibrahim, E.-S. I.; El-Ashry, E.-S. H.; Schmidt, R. R. *Eur. J. Org. Chem.* **1998**, *11*, 2305−2316.

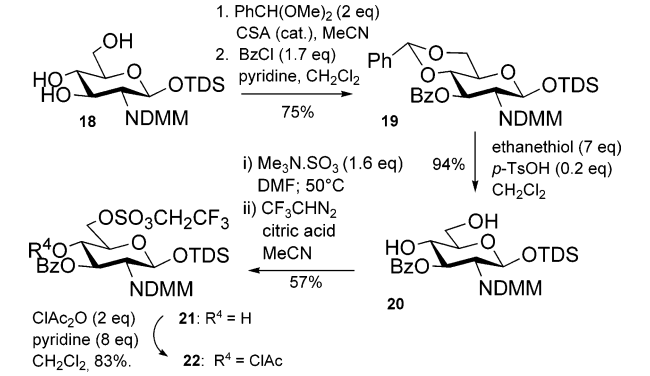
Scheme 4



tion, leaving the 3-position differentiated, was followed by sulfonation and sulfo-protection to afford derivative **17** in 70% overall yield. No side products were detected during these reactions and both the MP and DMM protecting groups were stable under the reaction conditions.

Preparation of target compound **22** was next undertaken (Scheme 5). Triol **18**, obtained from **14** by literature

Scheme 5

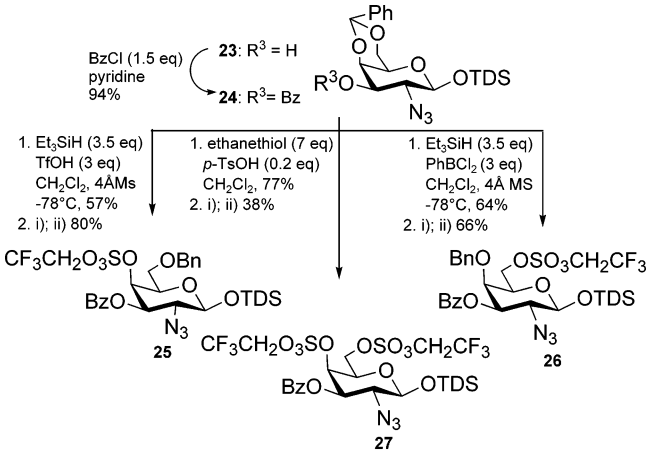


methods,[8] was benzylidenated and benzoylated affording **19** in 75% overall yield. Cleavage of the benzylidene acetal, selective 6-*O*-sulfonation, and sulfo-protection afforded the expected compound **21**. Chloroacetylation at the 4-position gave access to the desired building block **22** in good yield.

The azido GalN derivative **23** was prepared from D-galactosamine hydrochloride according to known procedures[9] and benzoylated at the 3-position to afford common intermediate **24** (Scheme 6). Regioselective opening of benzylidene acetal under different conditions gave access to either 6- or 4-benzylated compounds in good yield and with good selectivity. The corresponding 4,6-diol could be obtained in 77% yield from cleavage of the benzylidene ring.

The intermediate free hydroxyl derivatives were sulfonated and sulfo-protected to afford the corresponding 4-, 6-, and 4,6-trifluoroethylsulfonates **25**, **26**, and **27** in good to moderate yields.

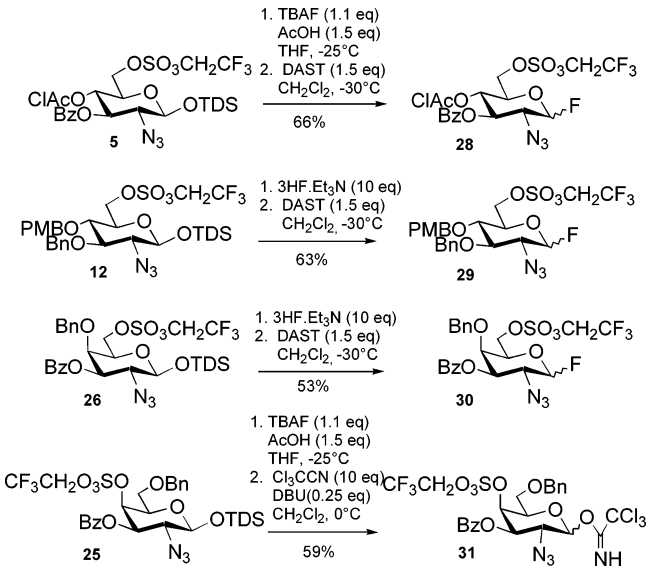
Scheme 6[a]



[a] Reagents and conditions: (i) $Me_3N{\cdot}SO_3$ (3−4.5 equiv), DMF; 50 °C; (ii) CF_3CHN_2, citric acid, MeCN.

Activation of the anomeric position and preparation of the glycosyl donors was next studied. Selective removal of the TDS[10] group was found to be more troublesome than expected. Indeed, the trifluoroethylsulfonate group, when present at the 6-position, acted as a good leaving group under basic conditions in both GlcN and GalN series and the corresponding 1,6-anhydro sugars were recovered as side products. When excess acetic acid was added to tetrabutylammonium fluoride, in the case of compound **5**, or a milder reagent such as trihydrofluoride triethylamine was used, the corresponding hemiacetals could be obtained in good yields (Scheme 7).

Scheme 7

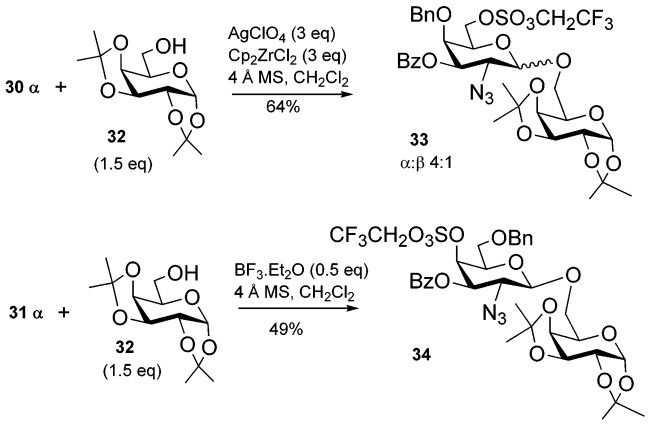


Activation of the 6-trifluoroethylsulfonate hemiacetals under basic conditions to prepare trichloroacetimidates also

(8) Chiesa, M. V.; Schmidt, R. R. *Eur. J. Org. Chem.* **2000**, *21*, 3541−3554.

(9) Herzner, H.; Eberling, J.; Schultz, M.; Zimmer, J.; Kunz, H. *J. Carbohydr. Chem.* **1998**, *17*, 759−776.

Scheme 8



led to partial loss of the sulfo-protecting group. Milder bases such as cesium carbonate did not afford the trichloroacetimidates. In contrast, halogens, including fluoride, were easily introduced at the anomeric position of 6-sulfo-protected derivatives. Thus, treatment of the hemiacetals with dimethylammonium sulfur trifluoride afforded the corresponding fluorides **28**, **29**, and **30** in good yield.

Removal of the TDS group in the 4-sulfo-protected series was achieved with tetrabutylammonium fluoride in the presence of an excess or equimolar amount of acetic acid. Since the configuration of the GalN sugar did not allow easy intramolecular substitution and ring closure, no side product was observed under those conditions. The hemiacetal intermediate was treated with trichloroacetonitrile and catalytic DBU to afford a separable mixture of α- and *β*-trichloroacetimidate **31** in 59% overall yield.

Preliminary glycosylation attempts, achieved by the coupling of α-fluoride **30** and α-imidate **31** with the 6-hydroxyl acceptor **32**, showed promising results (Scheme 8). Despite the electron-withdrawing character of the trifluoroethylsulfonate, which disarmed the glycoside donor, encouraging yields were obtained in both series.

In conclusion, mono- and disulfo-protected hexosamine building blocks were prepared in good yields. Trifluoroethylsulfonate moieties were shown to be compatible with both azido and *N*,*N*-dimethylmaleoyl groups and to a wide array of protection and deprotection chemistry. These 6-*O*-trifluoroethylsulfonate derivatives can rearrange under basic conditions so that care must be taken to avoid basic conditions in the preparation of glycosyl donors. Glycoside fluorides are easily prepared in the presence of the sulfo protecting group, and fluoride donors have shown good behavior in both glycopeptide and oligosaccharide synthesis and can be activated by using a large range of reaction conditions.[11] Additional glycosylation studies are currently underway on the applications of these donors in GAG synthesis and the results of these studies will be reported in due course.

Supporting Information Available: Spectral data for compounds **4**, **8**, **12**, **17**, **21** and **25**−**27**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL035882W

(10) The abbreviation used are the following: DMM, dimethylmaleimido; TDS, thexyldimethylsilyl.

(11) For a review on glycosyl fluoride see: Toshima, K. *Carbohydr. Res*. **2000**, *327*, 15−26.