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Regioselective Sulfation of Disaccharides Using Dibutylstannylene Acetals

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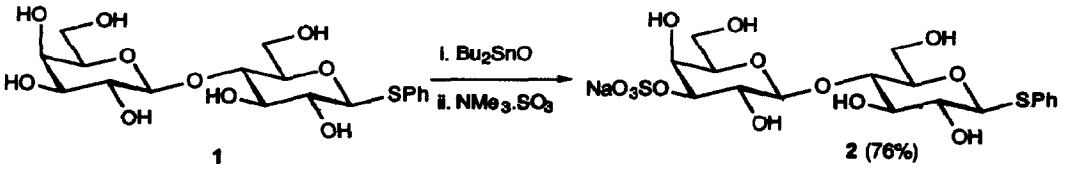
Abstract: Regioselective sulfation of partially protected disaccharides was achieved from their dibutylstannylene acetals by treatment with sulfur trioxide/trimethylamine. Using this methodology 3'-sulfo-N-acetyllactosaminide **5**, which is a substrate for fucosyltransferases and is the partial structure of 3'-sulfo-Lewis^x, can be synthesised in two steps from the N-acetylglucosaminide **3** without the need for hydroxyl group protection. Regioselective sulfation was also observed for partially protected maltosides **6** and **7**.

Sulfated oligosaccharides such as heparin, heparan sulfate, the carrageenans, chondroitin sulfate or sulfoglycolipids play important roles in biological recognition processes. For example, interactions between sulfated Lewis antigens and selectins at the surface of the endothelium have recently been shown to be involved in the recruitment of leucocytes to the site of injury during the inflammatory response.[1] Binding studies suggest that the affinity of E-selectin for the 3'-sulfated Lewis^a or Lewis^x tetrasaccharides appeared to be greater than for the Lewis^a or Lewis^x antigens themselves and 3'-sulfated Lewis^a tetra- and penta-saccharides were found to be better ligands for E-selectins than the 3'-sialyl Lewis^x and Lewis^a analogues. A lot of attention has therefore been focused on the chemical synthesis of these sulfated oligosaccharides[2] in order to study their mechanism of action and to develop inhibitors of this process. Typically, chemical syntheses have involved extensive protection group strategies using orthogonal protecting groups which can be cleaved at a late stage of the synthesis, exposing only those hydroxyl groups that are to be sulfated to the reagent, typically sulfur trioxide/trimethylamine. This strategy has resulted in lengthy syntheses and we report here a selective sulfation method which avoids the use of such protection groups.

Organotin complexes have been shown to be useful reagents for the regioselective acylation and alkylation of saccharides, *via* formation of stannyl complexes, which are preferentially formed with *cis* diols if they are present in the saccharide.[3] This methodology was first used for the selective activation of the 2' and 3'-hydroxyl groups of ribonucleotides towards electrophiles.[4] A striking example of the use of stannylene acetals was demonstrated by the exclusive formation of 3'-O-allyl derivatives directly from unprotected methyl-β-lactoside[5], which has indeed been a standard method of selectively protecting the 3' position of lactosides.

These results led us to investigate the reaction of stannylene acetals with sulfur trioxide/trimethylamine complex, since this would allow us to regioselectively sulfate oligosaccharides. The first reaction was attempted on thiophenyllactoside **1**, which was easily obtained from bromolactose heptaacetate[6] and thiophenol, followed by standard deacetylation. Compound **1** was converted to the stannylene acetal by stirring with dibutyltin oxide[7], the solvent removed and the dry stannylene was then treated with two equivalents of $Me_3N.SO_3$ in dioxane. Complete conversion could be observed by tlc after 30 hrs and purification resulted in 76% of the 3'-sulfated lactoside **2**[8] and 10% of the 3',6' disulfated lactoside as a side product (Scheme 1). The structure of the

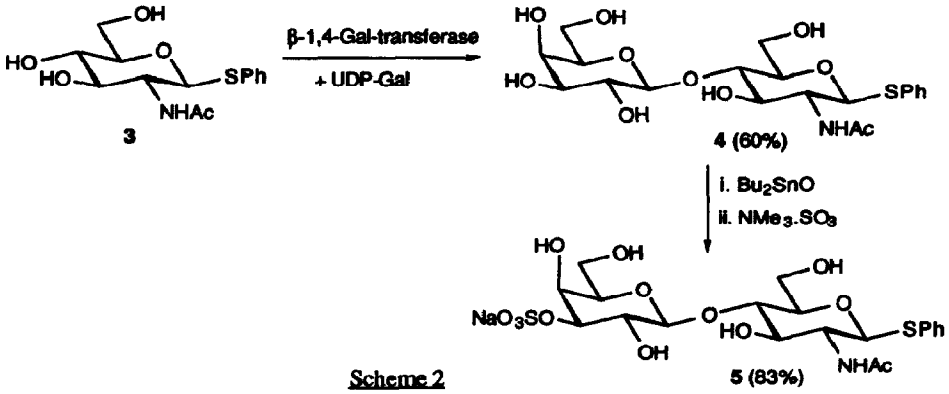


Scheme 1

product (2) was confirmed by ^{1}H NMR which showed the expected downfield shift for the 3'- and 4' protons at 4.01 ppm and 3.87-3.89 ppm respectively.[9] High resolution mass spectrometry confirmed the presence of only one sulfate in the molecule. The structure of **2** was also confirmed by an alternative synthesis of **2**, *via* a more conventional 5 step route from **1**, involving full use of protection groups which gave identical material as judged by ^{1}H NMR spectroscopy.

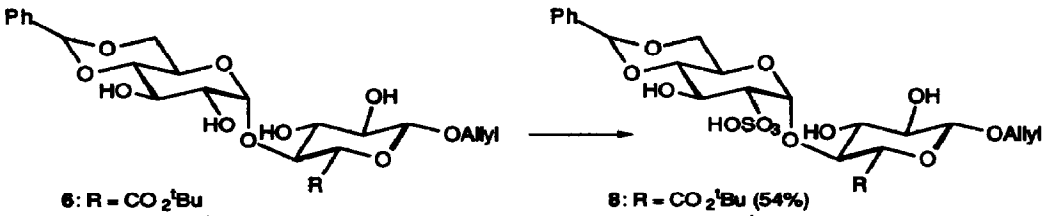
In order to confirm that the regioselectivity observed was due to the presence of the tin complex, lactoside **1** was stirred with $Me_3N.SO_3$ in dioxane, which gave no product, presumably due to the poor solubility of **1**. When the reaction was repeated in DMF it yielded a mixture of sulfated regioisomers, of which the main components, as expected, appeared to be the 6 and 6' primary mono- and di-sulfate esters.

This selective sulfation method was particularly useful in a chemoenzymatic synthesis of 3'-sulfo N-acetyl-lactosaminide **5** (Scheme 2) which could be achieved in only two steps. The target oligosaccharide **5** has recently been shown to be useful in detecting high levels of serum α-1,3-L-fucosyltransferase in ovarian cancer patients[10], since it is a selective substrate for this enzyme. The disaccharide **4** was synthesised in one step (60% isolated yield) by stereo- and regioselective galactosylation of **3** using β-1,4-galactosyltransferase from bovine milk (obtained from Sigma) using previously described procedures.[11] The structure of **4** was confirmed by NMR spectroscopy.[12] Sulfation of **4** to **5** was achieved in 83% isolated yield. It appeared that the selectivity for sulfation of the 3' hydroxyl group of **4** was higher than for the lactoside **1**, since no side products could be isolated. The structure of **5** was confirmed by spectroscopic methods.[13]



Scheme 2

Regioselective sulfation was further explored on maltosides, as part of an ongoing programme concerned with the synthesis of partial heparan sulfate structures.[14] Maltosides do not contain a cis diol, and hence the major sulfate esters from the reaction of unprotected maltoside with the tin reagent would be expected to be mixtures of mono- and di- 6 and 6' sulfate esters. When both primary hydroxyl groups were protected such as in compounds **6** and **7**, selective sulfation of the 2' hydroxyl group was observed in reasonable isolated yield (54% for **8**[15] and 56% for **9**[16]) (Scheme 3). This selectivity might either be due to the increased reactivity of the 2' hydroxyl group, which has been documented,[17] or due to the C1'-C2' cis dioxy configuration next to the α–glycosidic linkage. Structure **8** is of interest since it is part of a recently characterised minimal binding sequence of heparin sulfate to bFGF.[18]



Scheme 3

In summary, we have shown that hydroxyl groups in carbohydrates can be activated for sulfation by formation of dibutyltin stannylene acetals. This procedure provides a highly regioselective sulfation method of unprotected or partially protected saccharides and helps to avoid extensive use of protection groups in the synthesis of sulfated oligosaccharides. This is particularly useful in combination with enzymatic strategies as was shown for the synthesis of 3'-sulfo-N-acetyl-lactosaminide **5** which is now accessible by a two step chemoenzymatic route.

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7 *General sulfation procedure:* Disaccharide (4.6 x 10^{-4} mol) and Bu_2SnO (4.6 x 10^{-4} mol) were stirred in refluxing dry methanol (4 ml) for 2 hours. The reaction mixture was concentrated *in vacuo* and the dry dibutylstannylene intermediate treated with $Me_3N.SO_3$ (9.2 x 10^{-4} mol) in dioxane (THF for compound **4**) (4ml) at room temperature for 30 -93 hours. The reaction mixture was diluted with methanol (3 ml), filtered and concentrated *in vacuo*. The residue was then redissolved in 3 ml methanol and either directly chromatographed on silica (compounds **8** and **9**) or (compounds **2** and **5**) firstly loaded onto a cation exchange resin column (AG50W-X8, sodium form, 1 x 4 cm). After elution of the product with methanol the eluant was concentrated *in vacuo* and chromatographed on a silica column using $MeOH/CHCl_3/H_2O$ (5/8/1).

8 spectroscopic data for **2**: ν_{max} (KBr)/cm^{-1} 1250 (SO_3^-); δ_H(500 MHz; CD_3OD) 4.21-4.25 (2H, m, 3'-H, 4'-H), 4.48 (1H, d, *J* 7.8, 1'-H), 4.62 (1H, d, *J* 9.8, 1-H); δ_C(125.78 MHz, CD_3OD) 89.12 (1-C), 104.82 (1'-C); *m/z* (FAB^-) 513.0738 (M^-), $C_{18}H_{25}O_{13}S_2^-$ requires 513.0737.

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12 Spectroscopic data for **4**: δ_H(500 MHz; CD_3OD) 2.01 (3H, s, Ac), 3.50 (1H, dd, *J* 3.2, 9.7, 3'-H), 3.83 (1H, d, *J* 3.2, 4'-H), 4.41 (1H, d, *J* 7.5, 1'-H), 4.81 (1H, d, *J* 10.5, 1-H); δ_C(125.78 MHz; CD_3OD) 88.49 (1-C), 105.03 (1'-C), 173.37 (CO); *m/z* (DCI) 366 [$(M\text{-}SPh)^+$, 36%], 204 (100). The β1-4 linkage was confirmed by NOE experiments after acylation of compound **4**.

13 Spectroscopic data for **5**: ν_{max} (KBr)/cm^{-1} 1250 (SO_3^-); δ_H(500 MHz; CD_3OD) 2.00 (3H, s, Ac), 4.22 (1H, d, *J* 3.2, 4'-H), 4.25 (1H, dd, *J* 3.2, 9.7, 3'-H), 4.51 (1H, d, *J* 7.8, 1'-H), 4.79 (1H, d, *J* 10.5, 1-H); δ_C(50 MHz, CD_3OD) 87.86 (1-C), 104.25 (1'-C), 173.33 (CO); *m/z* (FAB^-) 554.0999 (M^-), $C_{20}H_{28}NO_{13}S_2^-$ requires 554.1002.

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15 Spectroscopic data for **8**: δ_H (500 MHz; CD_3OD) 1.52 (9 H, s, $-C(CH_3)_3$), 4.24-4.32 (2H, m, 2'-H and $-OCH_aH_bCH{=}CH_2$), 4.40 (1H, d, *J* 7.9, 1-H), 5.56 (1H, s, PhCH-), 5.89 (1H, d, *J* 4.0, 1'-H); δ_C(125.78 MHz; CD_3OD) 28.54 ($-C(CH_3)_3$), 83.73 (-*C*Me3), 98.09, 103.01 and 103.76 (1-C, 1'-C, *C*HPh), 169.16 (CO); *m/z* (FAB^-) 619.1708 (M^-), $C_{26}H_{35}O_{15}S^-$ requires 619.1697.

16 Spectroscopic data for **9**: δ_H (500 MHz; CD_3OD) 0.11 and 0.12 (6H, 2xs, $SiMe_2$), 0.92 (9H, s, *t*Bu), 4.29 (1H, dd, *J* 4.0, 9.6, 2'-H), 4.33 (1H, d, *J* 7.9, 1-H), 5.59 (1H, s, PhCH), 5.83 (1H, d, *J* 4.0, 1'-H); δ_C(125.78 MHz; CD_3OD) -4.89 and -4.81 ($SiMe_2$), 19.38 (*C*Me3), 26.58 (C*Me*3), 98.78, 102.83 and 103.02 (1-C, 1-C', *C*HPh); *m/z* (FAB^-) 663.2149 (M^-), $C_{28}H_{43}O_{14}SiS^-$ requires 663.2143.

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