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# Determination of the substituent distribution in *O*-sulfonylbutyl-(1→4)-glucans

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**Abstract**

A method has been developed to determine the distribution of substituents in the glucose units of sulfonylbutylethers of cyclomaltoheptaose (β-cyclodextrin). This method involves hydrolysis of the glucosidic linkages, permethylation, formation of sulfonylchlorides and subsequent transformation to the permethylated sulfonylfluoride derivatives. The latter were thermostable and could be analyzed by GLC and identified by EI and CIMS. For confirmation, the 2-, 3-, and 6-*O*-substituted standard compounds were independently synthesized and characterized by NMR and GLC–MS. 

*Keywords:* Sulfonylbutyl cyclomaltoheptaoses; Substitution pattern; Synthesis of (4′-fluorosulfonyl)butylethers of glucose

## 1. Introduction

In connection with cyclomaltooligosaccharides (cyclodextrins, CDs) the term 'host–guest interaction' becomes more and more important today, especially as among a broad range of guest molecules even hydrophobic drugs can readily be complexed by CDs. As particularly the β-CD is poorly water soluble, CD derivatives are synthesized for use as drug carriers. Although a number of modified CDs have been prepared, only three types of derivatives can be used as pharmaceutical excipients: methyl-, hydroxypropyl- (HP) and sulfonylbutylethers (SBE). Both the HP- and the SBE-β-CDs show excellent safety profiles. An advantage of the negative charge of the sulfonylbutyl substituents is presumably its behaviour against bile salts that are expected to be repelled from the SBE substituent so that the excretion of these salts should be lessened [1]. The derivatization of CDs can occur at all three positions on each glucopyranose unit so that a mixture of multiply substituted CDs is generated. For pharmaceutical applications, these products must be thoroughly characterized. Luna et al. determined the distribution of sulfonylbutyl groups over cyclodextrin molecules by capillary electrophoresis [2]. Isolated monosubstituted CDs were characterized by NMR spectroscopy [3] of mixtures as well by mass spectrometry [2,4]. Other anionic (1→4)-glucan ethers, as sulfonylethyl- or carboxymethyl celluloses, have been analyzed by means of high pH-anion exchange chromatography with pulsed amper-

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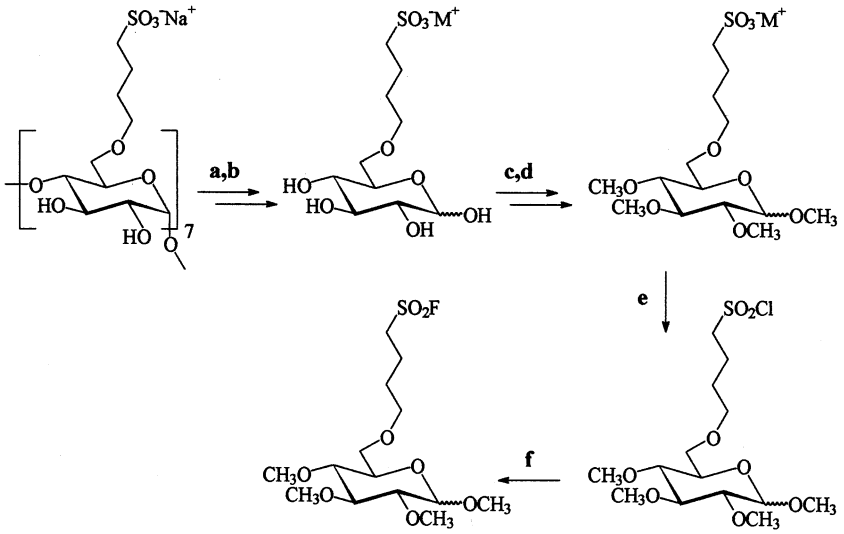
ometric detection (HPAEC-PAD) [5,6]. We now report on the development of a method for the analysis of the regioselectivity of the sulfonylbutylation reaction in the glucose unit.

## 2. Results and discussion

The SBE7-β-CD (DS 0.73/glucose unit) was prepared by addition of 1,4-butane sultone to β-CD in aqueous sodium hydroxide [7] and obtained as the sodium salt. The DS (degree of substitution) was calculated from the sulfur content.

*HPAEC-PAD.*—Due to the anionic character of the substituent, we first applied HPAEC-PAD to the hydrolysate of the SBE-β-CD. Group separation was achieved, but the regioisomers of the mono- and disubstituted glucoses were only partially or not separated. A drawback of pulsed amperometric detection is the strong decrease of the response with increasing number of substituents [5,6,8]. Therefore, it was investigated whether the permethylated SBE-glucoses gave better separation and at the same time would give a more uniform response. However, separation was not significantly improved, and the efficiency of the CarboPac column decreased with each injection. The material of this column also contains a resin with sulfonic acid groups and, via ionic interaction, smaller latex particles with trialkylammonium residues are bound on the surface of the anionic core [9]. We assume that competing interaction of highly sulfonylbutylated analytes with this core structure for the cationic resin particles altered the shape of the stationary phase. The sulfonylethylethers investigated by Kragten et al. [5,6] had a much lower DS and did not contain trisubstituted residues. Besides this problem, peak broadening due to hydrophobic interactions of the butyl chain with the stationary phase reduced separation efficiency.

*Preparation of volatile sulfonic acid derivatives.*—GLC is widely used for the analysis of complex mixtures as they are obtained from cleavage of poly- or oligosaccharide derivatives. Separation efficiency is high and usually superior to liquid chromatographic systems. A further advantage is the well established coupling with mass spectrometry that helps to identify the structure of the expected components of complex mixtures, as well as unexpected side products. However, sufficient volatility of the analytes is required. We therefore started to synthesize appropriate derivatives of the sulfonic acid residues, as we had done already with cationic starch ethers [10,11]. First, ethyl esters were prepared via the reaction sequence methanolysis, sodium–trialkylammonium exchange, permethylation, sodium–proton exchange, and esterification with triethylorthoformate [12]. However, it turned out that these compounds are not thermostable. Therefore we prepared the sulfonylchlorides and subsequently the even more volatile and stable sulfonylfluorides [13]. This procedure, the 'sulfofluoride route' comprises the following steps (see Scheme 1): (a) hydrolysis with perchloric acid, followed by neutralization with KOH; (b) cation-exchange: $Et_3HN^+$ for $K^+$; (c) permethylation with NaH and $CH_3I$ in DMF, followed by purification by size-exclusion chromatography on Sephadex LH20 with methanol; (d) once more cation-exchange: $Et_3HN^+$ for $Na^+$; (e) sulfonylchlorination with sulfuryl chloride and triphenyl phosphine in dichloromethane according to Huang et al. [14]; (f) sulfonylfluorination with KF-18-crown-6–acetonitrile complex in acetonitrile according to Bianchi et al. [15], and subsequent purification by column-chromatorgraphy on silica gel with petrol 1:3 ether–ethyl acetate. Hydrolysis with perchloric acid was preferred to methanolysis or hydrolysis catalyzed by trifluoroacetic acid since the solution could be concentrated under neutral conditions after precipitating the acid with KOH. In contrast, evaporation of the solvent under acidic conditions at room temperature caused significant formation of reversion products, which might even have enhanced the problems with the stability of the CarboPac anion exchange column mentioned above. Transformation of the sulfonylchloride to the sulfonylfluoride derivative was quantitative when phase transfer conditions were applied. The use of an ion exchange resin in the fluoride form according to Borders et al. [16] was less effective.




Scheme 1. (a) $HClO_4$ (70%), rt/10 min; dilution to 7% $HClO_4$, 100 °C/16 h; neutralization with KOH (6 M), $M^+ = K^+$; (b) $M^+ = K^+ \rightarrow Et_3HN^+$ (Amberlite IR-120, $NEt_3H^+$, $H_2O$); (c) $CH_3I$–NaH; DMF/rt/12 h, purification by SEC (Sephadex LH20; methanol), $\rightarrow M^+ = Na^+$; (d) $M^+ = Na^+ \rightarrow Et_3HN^+$ (Amberlite IR-120, $NEt_3H^+$, $H_2O$); (e) $Ph_3P$–$SO_2Cl_2$; $CH_2Cl_2$/rt/12 h; (f) KF/18-crown-6–MeCN complex; MeCN/rt/12 h; Silica Gel 60; 1:3 petrol ether–EtOAc).

*GLC and GLC–MS analysis.*—Sulfonylchloride derivatives were partially degraded when injected in a hot split injector (250 °C). 4′-Chlorobutyl- and 3′-butenylethers of glucose could be identified by GLC–MS. However, the sulfonylfluoride form was completely stable. Fig. 1 shows the gas chromatogram of the components obtained from SBE7-β-CD by the sulfonylfluoride route. The position of substitution could be deduced from the mass spectra of the mono- and disubstituted methyl glucosides. The trisubstituted compound that has been detected by HPAEC was not observed. The order of elution on a 60 m DB5 column was $6\beta > 2\beta > 3\beta > 6\alpha > 2\alpha > 3\alpha$ and $2.3\beta > 2.3\alpha > 3.6\beta > 2.6\beta > 2.6\alpha > 3.6\alpha$. However, 3.6β and 2.6β as well as 2.6α and 3.6α were only partially separated. As a consequence, the integration of these peaks is less reproducible and reliable. Characteristic fragment ions of diagnostic value were $m/z$ 212 and 225 that correspond to $m/z$ 88 and 101 in permethylated methyl glucopyranosides [17]. The mass difference of methyl to (4′-fluorosulfonyl)butyl-(SFB) ethers is 124. The $C_2$ fragment ion with $m/z$ 212 can be formed from C-1 + C-2, C-2 + C-3, and C-3 + C-4 and therefore occurs in the mass spectra of the 2- and 3-mono-*O*-substituted regioisomers, as well as in the 2,6- and the 3,6-di-*O*-substituted methyl glucosides. For

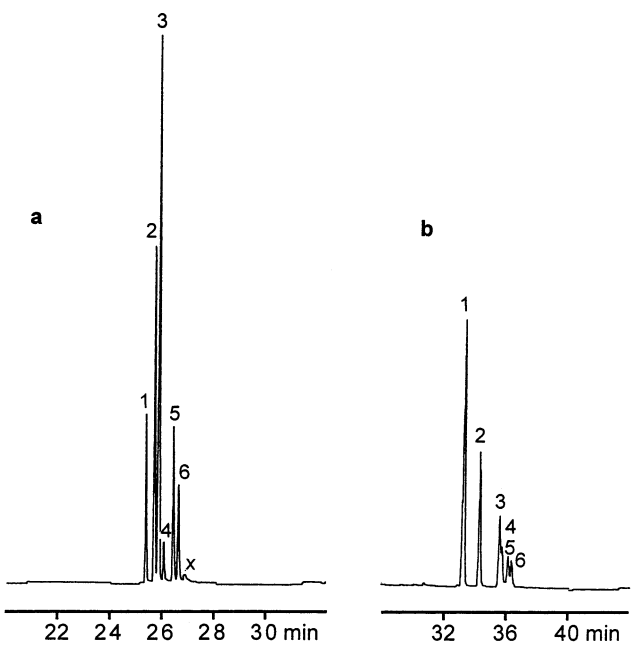



Fig. 1. Gas chromatogram of the permethylated sulfonylfluoride derivatives (SFB-Glc) obtained from β-cyclodextrinsulfonylbutylether SBE7-β-CD. Peaks are assigned according to their mass spectral data and in some cases confirmed by comparison with synthesized standard compounds. (a) Monosubstituted methyl *O*-methyl-D-glucopyranosides; (1) 6-SFB-β (**4b**); (2) 2-SFB-β (**9b**); (3) 3-SFB-β (**7b**); (4) 6-SFB-α (**4a**); (5) 2-SFB-α (**9a**); (6) 3-SFB-α (**7a**); (x) probably methyl 2-*O*-(4′-fluorosulfonyl)butyl-3,5,6-tri-*O*-methy-D-glucofuranoside; (b) disubstituted methyl *O*-methyl-D-glucopyranosides; (1) 2,3-SFB-β; (2) 2,3-SFB-α; (3) 3,6-SFB-β; (4) 2,6-SFB-β; (5): 2,6-SFB-α; (6) 3,6-SFB-α.

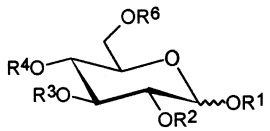
the 2,3-SFB isomer this fragment is partially shifted to *m/z* 336. The ion at *m/z* 225 is present for all 2-*O*-substituted-SFB-glucosides since it contains C-2, C-3, and C-4 with the original pattern at C-2 and C-4. The 6-*O*-monosubstituted methyl glucosides show intensive peaks at *m/z* 88 and 101. 3-SFB derivatives additionally show *m/z* 199 corresponding to *m/z* 75 in permethylated hexopyranosides, formed by rearrangement of the 3-*O*-substituent to C-1 of the methyl glucoside. The molecular masses of 374 (mono-SFB) and 498 (di-SFB) could be deduced from the CI mass spectra with ammonia as reactant gas. One further peak (assigned as component x in Fig. 1), which was detected in this group, showed the same molecular mass as the monosubstituted derivatives and a mass spectrum that was very similar to 2-*O*-SFB-Glc. Since this peak increased with increasing 2-*O*-substitution and showed a fragment ion typical for methyl furanosides, it is assumed that this peak belongs to the methyl 3,5,6-tri-*O*-methyl-2-*O*-SFB-glucofuranoside. A second anomer with the same mass spectrum could be detected when complete separation of all eight isomers was achieved on a special column (Optima-δ3, 60 m). In Fig. 1 it co-elutes with the 2-SFB-α-glucoside. The relative amount of component x was about 1.5%. Usually furanosides play no significant role in the equilibrium mixture of methyl glucosides obtained under acidic conditions. However, 2-*O*-sulfonylbutylation may favour the formation of furanosides. The analysis of an isolated mono-2-*O*-SBE-β-CD [2] supported this interpretation as component x with its characteristic mass spectrum was formed as a by-product of this regioisomer. The anomer of component x co-elutes with 2-SFB-α-Glc and therefore does not interfere with quantification.

*Synthesis of standard compounds.*—All peak assignments of the mono-SFB glucosides and that one of the 2,6-di-SFB glucoside could be confirmed by independent synthesis of the standard compounds. In addition, the order of elution was found to be β before α anomers, as has been observed for other methyl glucoside derivatives before. The strategy of synthesis was aimed at the formation of partially methylated methyl glucosides with one or two OH groups free for sulfonylbutylation. Methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranside (**1a**) was obtained via tritylation, permethylation, and subsequent detritylation of methyl α-D-glucopyranoside. Methyl 2,4,6-tri-*O*-methyl-α,β-D-glucopyranoside (**5**) was accessible from the mainly β-(1→3)-linked glucan laminaran via permethylation and methanolysis. For the 2-*O*-SFB-standard (**9**), 3-*O*-methyl-glucose was transformed to the methyl glucoside, protected as 4,6-*O*-benzylidene acetal and then reacted with the 1,4-butane sultone in aqueous sodium hydroxide to give the corresponding 2-*O*-(4′-sulfonyl)butyl derivative (**8**), which was permethylated after cleavage of the benzylidene group. Since sulfonylbutylation was performed on the methyl glucopyranoside, no furanoside (x) was observed. The α and β anomers of the sulfonylfluoride derivative (**9a** and **9b**) could be separated and characterized by NMR spectroscopy. For the synthesis of the disubstituted standard compound, methyl 3,4-di-*O*-methyl-α,β-D-glucopyranoside was obtained from the (1→2)-linked disaccharide sophorose via *tert*-butyldimethylsilylation of the primary positions, followed by permethylation and methanolysis. The partially methylated compounds were transformed to the sulfonylbutylethers (sodium salt). From these the SFB derivatives **4a**, **7a** and **9a**,**b** were obtained as described above following the sulfofluoride route. The 2,6-SFB-Glc was only obtained as a component of a crude product, but was sufficient for comparison of GLC and MS data (Table 1).

*NMR spectra of sulfonylbutyl derivatives.*—All standard compounds were investigated by $^{1}H$ and $^{13}C$ NMR spectroscopy as well as two-dimensional NMR techniques ($^{1}H-^{1}H$ and $^{1}H-^{13}C$ correlation spectroscopy: COSY and in addition for $^{13}C$: DEPT measurement). NMR data of known intermediates were in agreement with literature data.

*Quantification.*—The relative molar composition of the mono- and disubstituted glucosyl residues could be determined from the peak areas in the gas chromatogram. Since not all steps of the sulfofluoride route are quantitative, discrimination of higher substituted compounds must be taken into consideration. The unsubstituted fraction is also not obtained in a

Table 1




| Compound [a] | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^6$ |
|---|---|---|---|---|---|
| **1** | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | H |
| **2** | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $(CH_2)_4SO_3^-Na^+$ |
| **3** | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $(CH_2)_4SO_2Cl$ |
| **4** | $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ | $(CH_2)_4SO_2F$ |
| **5** | $CH_3$ | $CH_3$ | H | $CH_3$ | $CH_3$ |
| **6** | $CH_3$ | $CH_3$ | $(CH_2)_4SO_3^-Na^+$ | $CH_3$ | $CH_3$ |
| **7** | $CH_3$ | $CH_3$ | $(CH_2)_4SO_2F$ | $CH_3$ | $CH_3$ |
| **8** | $CH_3$ | $(CH_2)_4SO_3^-Na^+$ | $CH_3$ | benzylidene | |
| **9** | $CH_3$ | $(CH_2)_4SO_2F$ | $CH_3$ | $CH_3$ | $CH_3$ |

[a] **a** and **b** were added to the compound number to assign the α and β anomers (see text).

representative ratio due to the non ionic character of these constituents. Therefore, only the relative composition within the mono- and disubstituted groups was determined. Surprisingly, preferred 3-*O*-sulfonylbutylation was found for SBE7-β-CD, followed by 2- and 6-*O*-substitution as is obvious from the gas chromatogram (Fig. 1). The disubstitution is in full agreement with this regioselectivity as 2,3-SBE is the main pattern, followed by 3,6 and 2,6. This unexpected behaviour was further investigated. The results are reported and discussed together with the quantitative analysis of a number of further SBE-β-CDs with different DS values according to the method described in this paper [18].

For the determination of the molar fractions of un-, mono- di- and trisubstituted glucosyl units, capillary electrophoresis (CE) is under investigation. Although group separation was achieved by HPAEC, no quantitative evaluation is possible due to the different response factors of regioisomers in the PAD [5,6,8].

Ratios of α/β anomers were calculated from the gas chromatograms and were found to depend strongly on the position of substitution. Obviously, the β configuration is always the most preferred for the 3-*O*-SFB methyl glucoside (up to 84%). While the absolute β/α values vary, the relative ratio (β/α of 3-SFB-Glc : β/α of 6-SFB-Glc : β/α of 2-SFB-Glc) was quite constant 2.4 : (1.1–1.7) : 1.0. The reason for the wide range for 6-SFB-Glc is not known. With the help of the relative β/α ratios the composition of the monosubstituted fraction could even be calculated when 6β and 3α were not separated as was the case on a 25 m CPSil8CB column. These values were in excellent agreement with the data obtained by complete separation on a 60 m column.

## 3. Conclusions

A method is presented that allows the determination of the regioisomer composition in the mono- and disubstituted fractions of sulfonylbutylethers of (1→4)-glucans. The permethylated (4′-fluorosulfonyl)butylethers of methyl glucosides were prepared as volatile and thermostable compounds that could be analyzed by GLC and GLC–MS. HPAEC-PAD was not applicable since hydrophobic interactions of the resin with the butyl chains prevented regioisomer separation, and the cooperative effect of multiply SBE-substituted analyte residues presumably impaired the stationary phase. Peak identity deduced from the mass spectra could be confirmed by independent synthesis of standard compounds.

## 4. Experimental

*General.*—The SBE7-β-CD (sodium form) was obtained from Cydex, Kansas, USA. For preparation, see Refs. [7,18]. All reagents were of highest purity available and purchased from Fluka, Aldrich or E. Merck.

*GLC.*—GLC separations were carried out on a Carlo Erba GC 6000 Vega Series 2 instrument equipped with an on-column injector, a flame ionization detector (FID), a 25 m capillary column CPSil 8CB (Chrompack) connected with a retention gap (2 m), and a E. Merck Hitachi D-2500 integrator. Hydrogen was used as carrier gas (80 kPa). Complete separation of all monosubstituted SBE-Glc derivatives was obtained with a 60 m DB5, i.d. 0.25 mm, helium as carrier gas at 2.0 kPa, splitless injection (40 s) and the following temperature program: 100 °C (1 min), with 7 °C/min to 240 (10 min isotherm), with 10 °C/min to 290 °C (hold). For quantification of the disubstituted fraction the following program was used: 120 °C (1 min), with 15 °C/min to 260 °C, 20 min hold, then with 10 °C/min to 290 °C (hold). Data from the FID were collected with a Shimadzu Techlab C-R6A Chromatopac integrator.

*GLC–MS.*—EI (70 eV) mass spectra were recorded on a VG Analytical VG/70-250S instrument. For CI, ammonia was used as a reactant gas. Separation on an Optima column-δ3 (Machery and Nagel, 60 m) was followed with an Ion Trap detector (ITD, Finnigan MAT).

*HR-FABMS.*—High resolution FAB-mass spectra (positive ion mode) were recorded on a VG Analytical VG/70-250S instrument with a xenon-gun and *m*-nitrobenzylalcohol as matrix. Calibration was performed with polyethyleneglycol. Precise masses were obtained by linear interpolation between two reference masses after electrical scanning of the mass range [19]. Resolution: 6000 (for compound **7** and **9**), 4000 (for compound **4**). 10–20 spectra were accumulated.

*ESIMS.*—Electrospray mass spectra (positive mode) were recorded on a LC Esquire instrument (Bruker/HP). The sample was dissolved in 1:1 MeOH–$CH_2Cl_2$ and introduced directly via a syringe.

*NMR spectroscopy.*—Proton NMR spectra were measured with a Bruker WM 400 instrument ($^1$H: 400 MHz, $^{13}$C: 100.62 MHz); the solvents $D_2O$ (internal standard: acetone, $\delta$ 2.18) and $CDCl_3$ (internal standard: TMS) were purchased from E. Merck/Aldrich.

*Column chromatography.*—For purification Silica Gel 60 (63–200 μm, E. Merck) was used.

*Size-exclusion chromatography.*—For size-exclusion chromatography Sephadex LH20 (Pharmacia) in MeOH was used.

*HPAEC.*—For the ion-exchange chromatography a Dionex 300 system equipped with a semipreparative Carbo-Pac PA1 column (250 × 9 mm) and a PE-detector was used. The pulse potentials were 0.05 V (E1), 0.6 V (E2) and 0.6 V (E3). The response time was 1 s, the pulse times were 480, 120 and 60 ms. The following elution programme was used (eluent A: 0.1 M NaOH; eluent B: 0.1 M NaOAc): from 0 to 5 min: 95% A and 5% B; at 30 min: 75% A and 25% B and from 45 to 60 min: 0% A and 100% B. The flow was 4 mL/min.

*General procedures — ion-exchange.*—The ion-exchange resin (Amberlite IR-120, $H^+$, E. Merck) was treated with an aq satd triethylamine solution until the pH was alkaline. The excess of triethylamine was washed out with distilled water. For regeneration the resin was washed with 1 M HCl (volume: 20 fold volume of that of the resin). To transform the sodium form of the sulfonic acids to the triethylammonium salts the samples were treated with the $Et_3HN^+$ loaded resin and freeze-dried afterwards.

*$HClO_4$ hydrolysis [5].*—The SBE-β-CD sample (2–5 mg) was stirred in a 1 mL V-vial with $HClO_4$ (70%, 100 μL) at room temperature (rt) for 10 min. The solution was diluted with distilled water (900 μL) and stirred for 16 h at 100 °C. For neutralisation, a solution of 6 M KOH (about 300 μL) was added. The precipitated $KClO_4$ was removed by decantation and the supernatant, which contained the products, was dried in a stream of nitrogen.

*TFA hydrolysis.*—The SBE-β-CD sample (2–5 mg) was stirred in a 1 mL V-vial with 2 M trifluoroacetic acid (1 mL) at 120 °C for 3 h. Afterwards the acid was removed in a

stream of nitrogen. Residues of the acid were removed by co-distillation with toluene (three times).

*Methanolysis.*—The SBE-β-CD sample (2–5 mg) was stirred in a 1 mL V-vial with 1.5 M methanolic HCl (1 mL) at 100 °C for 2 h. The acid and alcohol then were removed in a stream of nitrogen.

*Permethylation.*—For permethylation, the sugar derivatives were dissolved in DMF (30 mg/mL) and NaH (5 molar equivalents/OH group) was added at 0 °C. The mixture was stirred for 0.5 h and then $CH_3I$ was added (5 molar equiv/OH group). The reaction mixture was stirred overnight at rt. After quenching with MeOH–water, the reaction was neutralized with dilute aq HCl, and afterwards the solvents were evaporated. The crude product was purified by size-exclusion chromatography with MeOH as eluent.

*Sulfonylchlorination [14].*—For the sulfonylchlorination of the sulfonic acid (triethylammonium form, 1 molar equiv), triphenyl phosphine (1.8 molar equiv) was dissolved in $CH_2Cl_2$ (0.22 mmol/mL). At 0 °C freshly distilled $SO_2Cl_2$ (2 molar equiv) was added. The educt — dissolved in $CH_2Cl_2$ (0.12 mmol/mL) — was added dropwise at rt. The mixture was stirred overnight at rt and the reaction was stopped by adding an excess of 1:1 petrol ether–diethyl ether. Triphenylphosphine oxide precipitated and the supernatant was decanted. The solvents were removed by distillation and the crude sulfonyl chloride mixture was used for the sulfonylfluorination reaction.

*Sulfonylfluorination [15].*—The sulfonylchlorides of the permethylated glucosides were dissolved in acetonitrile (0.12 mmol/mL) and KF in a molar excess of 1.2 was added. After adding a catalytic amount of the 18-crown-6–acetonitrile complex that was freshly prepared according to Gokel and Cram [20], the mixture was stirred overnight at rt. The products were purified by column chromatograhpy (3:4 petrol ether–1:3 EtOAc; $R_f$: disubstituted methyl glucosides: 0.25-monosubstituted methyl glucosides: 0.49).

*Sulfonylbutylation [21].*—The partially methylated methyl glucoside was dissolved in a system of 10:1 isopropanol–water, powdered NaOH (excess of 1.8 molar equiv/OH) was added and the solution was stirred for 0.5 h at rt. Then 1,4-butane sultone (2 molar equiv/OH) was added and the reaction mixture was stirred overnight at rt. A further amount of NaOH and butane sultone (2 molar equiv/OH) was added and the system was stirred for 2 days at rt. To stop the reaction and to destroy the unreacted sultone, more NaOH (7 molar equiv per mol of glucoside) and water were added and the mixture was heated to 100 °C for 2.5 h. The solution was neutralised with dilute aq HCl, the solvents were removed by distillation, and the products were purified by column chromatography.

*Esterification of the sulfonylbutylethers [12].*—The methyl *O*-methyl-*O*-sulfonylbutyl-α,β-D-glucosides (free acid form) obtained by methanolysis, ion exchange, permethylation and once again ion exchange of SBE-β-CD were treated with triethyl orthoformate at 50 °C for 2 days. Thin-layer chromatography (TLC) control (1:1 petrol ether–EtOAc) showed complete reaction. The excess of orthoformate was removed by distillation.

*Synthesis of standard compounds.*—Besides the permethylated 6-*O*-substituted standard compound (6-SFB-Glc, **4**) the intermediates formed on the way to it on the analytical pathway were isolated and characterized by NMR and MS (the sulfonic acid derivative **2**, the sulfonylchloride derivative **3**). As the sulfonylchloride derivative is a reactive intermediate, the work-up procedure (column chromatography) could cause losses. For that reason the other standard compounds were only isolated and characterized in their sodium sulfonate or the sulfonylfluoride form.

*Methyl 6-O-(4′-fluorosulfonyl)butyl-2,3,4-tri-O-methyl-α-D-glucopyranoside (6-SFB-Glc,* **4a***).*—Methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranoside (**1a**) was obtained via 6-*O*-tritylation, permethylation and detritylation of commercial methyl α-D-glucopyranoside. NMR data were in accordance with the literature [22]. Intermediate **1a** (154.4 mg, 0.65 mmol) was sulfonylbutylated according to the general method (see above). The crude product (**2a**) was purified by column chromatography (eluent: 5:1 $CHCl_3$–MeOH; $R_f$ 0.21). Yield: 148.5 mg (0.38 mmol, 58%). $^1H$ NMR ($D_2O$, acetone as internal standard): $\delta$ 4.90 (d, 1 H, H-1,

$J_{1,2}$ 3.56 Hz); 3.62 (m, 2 H, H-6); 3.60 (m, 1 H, H-5); 3.52 (m, 2 H, H-1′); 3.50 (s, 3 H, –$CH_3$); 3.47 (s, 3 H, –$CH_3$); 3.41 (dd, 1 H, H-3, $J_{3,4}$ 3.56 Hz); 3.38 (s, 3 H, –$CH_3$); 3.31 (s, 3 H, –$CH_3$); 3.27 (dd, 1 H, H-2); 3.22 (dd, 1 H, H-4, $J_{4,3}$ 3.56 Hz); 2.88 (m, 2 H, H-4′); 1.71 (m, 2 H, H-3′); 1.66 (m, 2 H, H-2′). $^{13}$C NMR ($D_2O$): $\delta$ 96.90 (C-1); 82.60 (C-3); 80.20 (C-2); 79.30 (C-4); 71.10 (C-1′); 69.70 (C-5); 68.90 (C-6); 60.40 (–$CH_3$); 60.30 (–$CH_3$); 58.20 (–$CH_3$); 55.30 (–$CH_3$); 51.10 (C-4′); 27.60 (C-2′); 21.40 (C-3′). From **2a**, first the methyl (4′-chlorosulfonyl)butyl 2,3,4-tri-*O*-methyl-α-D-glucoside (**3a**) was synthesized according to the general method (see above) (94.66 mg, 0.24 mmol). The sulfonylchloride derivative was purified by column chromatography (1:3 petrol ether–EtOAc; $R_f$ 0.35). Yield: 81.4 mg (0.21 mmol; 88.0%). $^1$H NMR ($CDCl_3$, TMS as internal standard): $\delta$ 4.82 (d, 1 H, H-1, $J_{1,2}$ 3.56 Hz); 3.81 (dd, 2 H, H-4′); 3.63 (m, 2 H, H-6); 3.62 (m, 4 H, H-5/–$CH_3$); 3.60 (m, 2 H, H-1′); 3.54 (s, 3 H, –$CH_3$); 3.51 (s, 3 H, –$CH_3$); 3.50 (vt, 1 H, H-3); 3.41 (s, 3 H, –$CH_3$); 3.20 (dd, 1 H, H-2); 3.14 (dd, 1 H, H-4); 2.17 (m, 2 H, H-3′); 1.82 (m, 2 H, H-2′). $^{13}$C NMR ($CDCl_3$): $\delta$ 97.50 (C-1); 83.50 (C-3); 81.80 (C-2); 79.50 (C-4); 70.70 (C-1′); 69.90 (C-5); 69.80 (C-6); 65.40 (C-4′); 60.90 (–$CH_3$); 60.50 (–$CH_3$); 59.00 (–$CH_3$); 55.20 (–$CH_3$); 27.50 (C-2′); 22.20 (C-3′). The methyl (4′-chlorosulfonyl)butyl 2,3,4-tri-*O*-methyl-α-D-glucoside (81.4 mg, 0.21 mmol) was dissolved in MeCN and transformed to the sulfonylfluoride derivative by means of an anion exchange resin ($F^-$ form, MeCN) prepared according to Ref. [16]. Sulfonylchloride **3a** was filtered twice through the resin in a column to achieve complete transformation. The solvent was evaporated and the crude product purified by column chromatography (2:1 EtOAc–EtOH; $R_f$ 0.69). Yield of **4a**: 34.0 mg (0.09 mmol, 43%). $^1$H NMR ($CDCl_3$): $\delta$ 4.81 (d, 1 H, H-1, $J_{1,2}$ 3.56 Hz); 3.62 (m, 4 H, H-6/–$CH_3$); 3.61 (m, 1 H, H-5); 3.58 (m, 2 H, H-1′); 3.56 (m, 1 H, H-6); 3.54 (s, 3 H, –$CH_3$); 3.51 (s, 3 H, –$CH_3$); 3.53 (m, 1 H, H-3); 3.40 (s, 3 H, –$CH_3$); 3.39 (m, 2 H, H-4′); 3.19 (m, 1 H, H-4); 3.13 (m, 1 H, H-2); 2.10 (m, 2 H, H-3′); 1.82 (m, 2 H, H-2′). $^{13}$C NMR ($CDCl_3$): $\delta$ 97.50 (C-1); 83.50 (C-3); 81.80 (C-2); 79.60 (C-4); 70.60 (C-1′); 69.90 (C-5); 69.80 (C-6); 60.90 (–$CH_3$); 60.50 (–$CH_3$); 59.00 (–$CH_3$); 55.20 (–$CH_3$); 27.90 (C-2′); 21.20 (C-3′). GLC–MS: $m/z < 102$ (5%); $m/z$ 102–173 ($>$ 1%); $m/z$ 173–343 ($> 0.1$%); $m/z$ 41 (10); 45 (19); 55 (23); 71 (11); 73 (12); 75 (27); 88 (100); 89 (8); 101 (50); 111 (2); 117 (1.1); 127 (1.5); 131 (2); 139 (6); 145 (1.9); 187 (0.1); 195 (0.2); 225 (0.3); 251 (0.2); 279 (0.1); 283 (0.3); 311 (1.3); 312 (0.2). CIMS (ammonia): $m/z$ 375 [M + 1], 392 [M + 18]. ESIMS (positive mode): $m/z$ 397 [M + Na], 413 [M + K]. HR-FABMS (positive mode): $m/z$ 343.1260 [M − MeOH + H]$^+$, ($C_{14}H_{27}FO_8S$ − MeOH + H) requires 343.1226); $m/z$ 311.0906 [M − 2MeOH + H]$^+$, ($C_{14}H_{27}FO_8S$ − 2MeOH + H requires 311.0965).

*Methyl 2,4,6-tri-*O*-methyl-α-*D*-glucoside* (**5a**).—Laminaran from *Laminaria digitata* (Sigma, L9634), a mainly (95%) β-(1→3)-linked D-glucan (206.1 mg, 1.27 mmol AGU), was dissolved in $Me_2SO$ (2 mL) and powdered NaOH (440.0 mg, 11.0 mmol) was added. The suspension was stirred for 0.5 h at rt and then methyl iodide (510 μL, 8.2 mmol) was added. The suspension was stirred overnight and, to prevent undermethylation, permethylation was performed twice. The permethylated laminaran was dialysed for 2 days against tap water and for another 2 days against distilled water (batchwise) in a cellulose membrane from Spectra Por with a MWCO of 3500, and freeze dried. Yield: 234.4 mg (1.15 mmol, 90%). $^1$H NMR ($CDCl_3$): $\delta$ 4.72 (d, 1 H, H-1, $J_{1,2}$ 7.63 Hz); 3.77 (vt, 1 H, H-3, $J_{2,3}$ 8.6 Hz); 3.62 (m, 1 H, H-6); 3.6 (s, 3 H, –$CH_3$); 3.53 (s, 3 H, –$CH_3$); 3.41 (m, 4 H, H-6, –$CH_3$); 3.31 (m, 1 H, H-5); 3.21 (m, 1 H, H-4); 3.05 (vt, 1 H, H-2). For analytical control the permethylated laminaran (2 mg, 9.8 μmol) was submitted to methanolysis, and the products were trimethylsilylated. GLC analysis (60 °C (1 min), with 25 °C/min to 130 °C, with 4 °C/min to 290 °C) yielded 81.7% of the permethylated 3-*O*-TMS-glucoside, 13.3% di-*O*-TMS derivatives and 4.5% of permethylated methyl glucoside. The permethylated laminaran (234.4 mg, 1.15 mmol AGU) was degraded to monomers by methanolysis (see Section 4.10). Methyl 2,4,6-tri-*O*-methyl-α-D-glucoside (**5a**) was isolated by column chromatography (1:9

petrol ether–EtOAc; $R_f$ 0.22). Yield: 105.7 mg (0.45 mmol, 39%).$^{13}$C NMR ($CDCl_3$): $\delta$ C-1 (97.22); 81.74 (C-2); 79.47 (C-4); 73.57 (C-3); 71.52 (C-6); 70.06 (C-5); 60.89 (–$CH_3$); 59.65 (–$CH_3$); 58.82 (–$CH_3$); 55.66 (–$CH_3$). Analytical data were in accordance with the literature [23].

*Methyl 3-*O*-(4′-fluorosulfonyl)butyl-2,4,6-tri-*O*-methyl-α-*D*-glucopyranoside (3-SFB-Glc*, **7a**).*—The methyl 2,4,6-tri-*O*-methyl-α-D-glucoside (**5a**) was sulfonylbutylated following the general procedure. The crude product was purified by column chromatography (5:1 $CHCl_3$–MeOH; $R_f$ 0.21). Yield of **6a**: 51.6 mg (0.13 mmol, 29%). $^1$H NMR ($CDCl_3$): $\delta$ 4.11 (d, 1 H, H-1, $J_{1,2}$ 8.14 Hz); 3.77 (m, 2 H, H-1′); 3.62–3.07 sugar protons; 3.48–3.53 (2 s, 6 H, 2 × –$CH_3$); 3.39 (s, 3 H, –$CH_3$); 3.40 (s, 3 H, –$CH_3$); 2.98 (m, 2 H, H-4′); 1.86 (m, 2 H, H-3′); 1.67 (m, 2 H, H-2′). The 3-SBE-Glc (**6a**) was transformed to the sulfonylfluoride derivative following the analytical pathway (see Section 4.10). The sulfonylfluoride derivative was purified by column chromatography (1:3 petrol ether–EtOAc; $R_f$: 0.38). Yield of **7a**: 32.3 mg (0.09 mmol, 66.2%). $^1$H NMR ($CDCl_3$): $\delta$ 4.86 (d, 1 H, H-1, $J_{1,2}$ 3.56); 3.81 (m, 2 H, H-4′); 3.58 (vd, 2 H, H-6); 3.55 (m, 2 H, H-3/H-5); 3.41 (s, 3 H, –$CH_3$); 3.48 (m, 2 H, H-1′); 3.47 (s, 3 H, –$CH_3$); 3.42 (s, 3 H, –$CH_3$); 3.41 (s, 3 H, –$CH_3$); 3.25 (m, 1 H, H-4); 3.20 (dd, 1 H, H-2, $J_{2,1}$ 3.56); 2.09 (m, 2 H, H-3′); 1.77 (m, 2 H, H-2′). $^{13}$C NMR ($CDCl_3$): $\delta$ 97.56 (C-1); 82.12 (C-2); 81.82 (C-3); 79.82 (C-4); 72.07 (C-4′); 71.38 (C-6); 70.37 (C-5); 60.93 (–$CH_3$); 59.61 (–$CH_3$); 58.86 (–$CH_3$); 55.61 (–$CH_3$); 51.10 (C-1′); 28.69 (C-3′); 21.19 (C-2′). GLC–EIMSMS: $m/z < 102$ ($> 5\%$); $m/z$ 102–173 ($> 1\%$); $m/z$ 173–343 ($> 0.5\%$); $m/z$ 39 (6); 41 (17); 43 (7); 45 (66); 55 (100); 59 (6); 71 (39); 72 (5); 73 (11); 74 (64); 75 (35); 87 (16); 88 (24); 89 (8); 101 (73); 102 (12); 103 (1.7); 111 (3.5); 113 (1.8); 114 (1.8); 115 (1.2); 117 (2.6); 123 (1.1); 127 (5); 139 (69); 140 (4); 141 (4); 143 (1.7); 145 (4); 159 (1.2); 187 (4); 199 (26); 200 (2); 201 (1.4); 212 (84); 231 (8); 214 (5); 225 (6); 226 (0.7); 255 (0.9); 311 (0.8). CIMS (ammonia): $m/z$ 375 [M + 1]; $m/z$ 392 $[M + 18]^+$. ESIMS (positive mode): $m/z$ 397 $[M + Na]^+$, 413 $[M + K]^+$. HRFABMS (positive mode): $m/z$ 375.1439 $[M + H]^+$ ($C_{14}H_{27}FO_8S + H$ requires 375.1489), 373.1346 ($C_{14}H_{27}FO_8S + 2\ H + H^+$ requires 373.1332), 343.1149 ($[M - MeOH + H]^+$ $C_{14}H_{27}FO_8S + 2$ MeOH + H requires 343.1226); 311.1013 [2 MeOH + H]$^+$ $C_{14}H_{27}FO_8S + 2$ MeOH + H requires 311.0965).

*Methyl 2-*O*-(4′-fluorosulfonyl)butyl-3,4,6-tri-*O*-methyl-α,β-*D*-glucopyranoside (2-SFB-Glc*, **9**).*—Commercially available 3-*O*-methyl-α,β-D-glucopyranose (251.2 mg, 1.3 mmol) was transformed into the methyl glucoside by a methanolysis (see Section 4.10). The crude product was dissolved in DMF and benzaldehyde dimethylacetal (1.1 equiv/equiv glucose) and a catalytic amount of *p*-toluene sulfonic acid was added. The reaction mixture was stirred for 3 h at 60 °C under reduced pressure (30 mbar). The temperature was increased to 85 °C to remove the DMF, and the crude methyl 4,6-*O*-benzylidene-3-*O*-methyl-α,β-D-glucoside was purified by column chromatography (1:1 petrol ether–EtOAc; $R_f$ 0.18). Yield: 209.7 mg (0.7 mmol, 54.5%). $^{13}$C NMR ($CDCl_3$): $\delta$ 128.99/128.25/126.06/126.03/101.29 (bn); 99.83 (C-1); 82.04/80.66 (C-3/C-4); 72.32 (C-2); 69.03 (C-6); 62.56 (C-5). This intermediate was sulfonylbutylated following the general procedure. The methyl 4,6-*O*-benzylidene-3-*O*-methyl-2-*O*-(4′-sulfonyl)butyl-α,β-D-glucoside was purified by column chromatography (3:1 $CHCl_3$–MeOH; $R_f$ 0.28). Yield of **8**: 94.4 mg (0.22 mmol, 32%). $^1$H NMR ($CDCl_3$): $\delta$ 7.46 (m, 2 H, Ph); 7.33 (m, 3 H, Ph); 5.47 (s, 1 H, C*H*Ph); 4.78 (d, 1 H, H-1, $J_{1,2}$ 3.56 Hz); 4.25 (m, 1 H, sugar); 3.30–3.76 (m, 5 H, sugar); 3.70 (m, 2 H, H-1′); 3.58 (s, 3 H, *O*Me); 3.39 (s, 3 H, *O*Me); 2.97 (m, 2 H, H-4′); 1.86 (m, 2 H, H-2′); 1.70 (m, 2 H, H-3′). The benzylidene group of **8** was cleaved according to Hann et al. [24]. Compound **8** (94.4 mg, 0.22 mmol) was dissolved in 0.01 M $H_2SO_4$ and heated for 3 h to 100 °C. The reaction mixture was neutralised with diluted NaOH and the solvent evaporated. After exchange of $Na^+$ for $Et_3HN^+$ (see Section 4.10) 69.9 mg (0.16 mmol, 77%) of methyl 3-*O*-methyl-2-*O*-(4′-sulfonyl)butyl-α,β-D-glucoside ($NEt_3H^+$ form) was obtained. After permethylation (see Section 4.10) it was transformed into the sulfonyl-

fluoride derivative following the analytical pathway (see Section 4.10). The α and β standard compounds could be separated by column chromatography (1:3 petrol ether–EtOAc; $R_f$ α: 0.5; $R_f$ β 0.39). The yield of the 2-SFB-α-Glc (**9a**) was 6.7 mg (0.018 mmol) and of the 2-SFB-β-Glc (**9b**) 15.5 mg (0.041 mmol). In addition a fraction of the α and β anomer mixture (**9a**,**b**) could be isolated (13.9 mg, 0.037 mmol). The total yield of **9** was 36.2 mg (0.096 mmol, 60%). $^1$H NMR ($CDCl_3$); **9a**, δ 4.80 (d, 1 H, H-1, $J_{1,2}$ 3.56); 3.67 (m, 2 H, H-1′); 3.61 (s, 3 H, –$CH_3$); 3.575 (m, 3 H, 2 H-6/H-5); 3.53 (s, 3 H, –$CH_3$); 3.49 (m, 2 H, H-4′); 3.46 (vt, 1 H, H-3); 3.42 (3s, H, –$CH_3$); 3.40 (s, 3 H, –$CH_3$); 3.268 (dd, 1 H, H-2, $J_{2,1}$ 3.56); 2.09 (m, 2 H, H-3′); 1.79 (m, 2 H, H-2′). $^{13}$C NMR ($CDCl_3$); **9a**, δ 98.01 (C-1); 83.50 (C-3); 80.74 (C-2); 80.06 (C-4); 71.36 (C-6); 70.37 (C-5); 70.32 (C-1′); 61.35 (–$CH_3$); 60.83 (–$CH_3$); 59.63 (–$CH_3$); 55.53 (–$CH_3$); 51.11/50.94 (C-4′); 28.27 (C-2′); 21.42 (C-3′). $^1$H NMR (**9b**): δ 4.12 (d, 1 H, H-1, $J_{1,2}$ 7.63); 3.82/3.70 (m, 2 H, H-1′); 3.64 (dd, 1 H, H-6, $J_{6,6}$ 10.68, $J_{6,5}$ 2.03); 3.61 (s, 3 H, –$CH_3$); 3.56 (dd, 1 H, H-6, $J_{6,6}$ 10.68, $J_{6,5}$ 4.58); 3.53 (s, 3 H, –$CH_3$); 3.51 (s, 3 H, –$CH_3$); 3.47 (m, 2 H, H-4′); 3.26 (m, 1 H, H-5, $J_{5,6}$ 2.03, $J_{5,6}$ 4.58); 3.15 (m, 2 H, H-3/H-4, $J_{3,2}$ 3.56, $J_{3,4}$ 2.03); 3.03 (m, 1 H, H-2); 2.09 (m, 2 H, H-3′); 1.74 (m, 2 H, H-2′). $^{13}$C NMR (**9b**): δ 104.56 (C-1); 86.77 (C-3); 82.42 (C-2); 80.13 (C-5); 75.05 (C-4); 71.60 (C-6)/(C-1′); 61.37 ($CH_3$); 60.76 ($CH_3$); 59.77 ($CH_3$); 57.34 ($CH_3$); 51.04/50.88 (C-4′); 28.42 (C-2′); 21.10 (C-3′). GLC–MS: ($m/z < 102$ (> 5%); $m/z$ 102–173 (> 1%); $m/z$ 173–343 (> 0.5%); $m/z$ 39 (5); 41 (15); 43 (6); 45 (59); 55 (100); 59 (6); 59 (7); 71 (32); 73 (9); 74 (65); 75 (97); 87 (18); 88 (41); 89 (13); 101 (27); 102 (8); 111 (3); 113 (2); 114 (1.8); 115 (2); 117 (4); 123 (1.2); 127 (2.5); 139 (62); 140 (3); 141 (3); 143 (1.7); 149 (1.6); 187 (0.9); 199 (0.7); 212 (52); 213 (4.5); 214 (3); 225 (65); 226 (6); 227 (3.5); 235 (0.9); 251 (0.7); 255 (0.9); 269 (0.7); 300 (2.7); 311 (4). CIMS (ammonia): $m/z$ 375 $[M+1]^+$, 392 $[M+18]^+$. ESIMS (positive mode): $m/z$ 397 $[M+Na]^+$, 413 $[M+K]^+$. HRFABMS (positive mode): $m/z$ 375.1478 $[M+H^+$ requires $(C_{14}H_{27}FO_8S)+H]^+$, 375.1489), 373.1346 $[M-2\ H+H]^+$, $C_{14}H_{27}FO_8S-2$ MeOH + H requires 373.1332), 343.1136 $[M-MeOH+H]^+$, ($C_{14}H_{27}FO_8S$ + MeOH + H requires 343.1226); 311.1000 $[M-2$ MeOH + H$]^+$, $C_{14}H_{27}FO_8S-2$ MeOH + H requires 311.0965).

*Disubstituted methyl glucosides.*—Methyl 2,6-di-*O*-(4′-fluorosulfonyl)butyl-3,4-di-*O*-methyl-α,β-D-glucopyranoside (2,6-SFB-Glc) was prepared as follows. From sophorose methyl 3,4-di-*O*-methyl-α,β-D-glucopyranoside was obtained via 6,6′-di-*O*-*tert*-butyldimethylsilylation, permethylation, and methanolysis as one component of the disaccharide. Sulfonylbutylation and transformation to the sulfonylfluoride yielded two components, which co-eluted with the corresponding components obtained from SBE7-β-CD by the analytical procedure and which had been identified as the 2,6-SFB-Glc derivatives from their EI-mass spectra: EIMS: ($m/z < 102$ (> 5%), $m/z < 300$ (> 1%) $m/z$ 41 (16), 42 (7), 43 (6), 45 (20), 55 (100), 56 (5), 59 (5), 64 (8), 71 (17), 72 (7), 73 (6), 74 (34), 75 (45), 87 (13), 88 (21), 89 (5), 101 (8), 117 (2), 139 (61), 140 (3), 141 (3), 212 (25) 225 (39), 226 (3). CIMS (ammonia): $m/z$ 499 $[M+1]^+$, 516 $[M+18]^+$. ESIMS (positive mode): $m/z$ 521 $[M+Na]^+$, 537 $[M+K]^+$. The substitution pattern of the other disubstituted regioisomers were deduced from their mass spectra (for interpretation of the mass spectra, see text): GLC–EIMS (ion trap) of methyl 2,3-di-*O*-(4′-fluorosulfonyl)butyl-4,6-di-*O*-methyl-α,β-D-glucopyranoside (2,3-SFB-Glc): $m/z$ 55 (100), 71 (14), 101 (5), 139 (77), 199 (3), 212 (3), 225 (17), 336 (19). CIMS (ammonia): $m/z$ 499 [M + 1], 516 [M + 18]. Methyl 3,6-di-*O*-(4′-fluorosulfonyl)butyl-2,4-di-*O*-methyl-α,β-D-glucopyranoside (3,6-SFB-Glc). EIMS (ion trap): $m/z$ 55 (100), 71 (16), 74 (24), 75 (16), 101 (42), 111 (3), 139 (76), 199 (3), 212 (36), 225 (5), 435 (2). CIMS (ammonia): $m/z$ 499 $[M+1]^+$, 516 $[M+18]^+$.

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