Synthesis and Cell Growth Inhibitory Activity of Six Non-glycosaminoglycan-Type Heparin-Analogue Trisaccharides

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The design and synthesis of heparin mimetics with high anticancer activity but no anticoagulant activity is an important task in medicinal chemistry. Herein, we present the efficient synthesis of five Glc-GlcA-Glc-sequenced and one Glc-IdoA-Glc-sequenced non-glycosaminoglycan, heparin-related trisaccharides with various sulfation/sulfonylation and methylation patterns. The cell growth inhibitory effects of the compounds were tested against four cancerous human cell lines and two non-cancerous cell lines. Two D-glucuronate-containing tetra-*O*-sulfated, partially methylated trisaccharides displayed remarkable and selective inhibitory effects on the growth of ovary carcinoma (A2780) and melanoma (WM35) cells. Methyl substituents on the glucuronide unit proved to be detrimental, whereas acetyl substituents were beneficial to the cytostatic activity of the sulfated derivatives.

Introduction

Heparin and heparan sulfate (HS) are linear anionic polysaccharides belonging to the family of glycosaminoglycans. Although both are composed of alternating α-D-glucosamine and hexuronic acid (β-D-glucuronic acid, β-D-GlcA, or α-L-iduronic acid, α-L-IdoA) units, there are some structural differences between their saccharide sequences and sulfation degree (Figure 1A). Whereas heparin predominantly consists of the trisulfated L-IdoA-containing disaccharide (highlighted in green in structure **1**), the major repeating unit of HS is a D-GlcA-containing disaccharide with a lower sulfation degree (highlighted in yellow in structure **1**).[1] The most well-known activity of heparin is the anticoagulant effect which is based on the specific interaction of a unique pentasaccharide domain of heparin with the endogenous coagulation inhibitor antithrombin.[2]

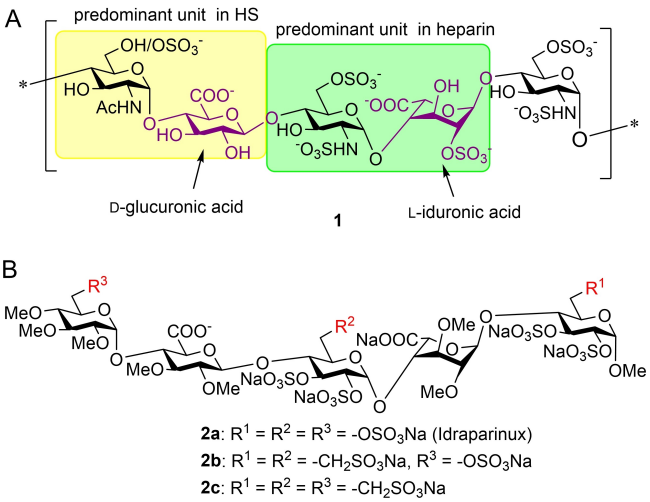

Figure 1. A) Representative pentasaccharide unit of heparin/heparan sulfate (HS; **1**), highlighting the predominant disaccharide repeating units. B) Synthetic non-glycosaminoglycan-type pentasaccharides (**2a-c**) with anticoagulant activity

Beyond antithrombin, a large number of proteins, such as growth factors, cytokines, enzymes, membrane receptors as well as viral proteins can interact with both heparin and HS.[3] Consequently, heparin and HS have many biological effects[4–10] such as anti-inflammatory,[5] cardiovascular and tissue protection,[5] kidney and nerve protection,[6] angiogenic,[7] metastasis and growth factor inhibitory[8] as well as anti-protozoan[9] and antiviral[10] activity which can be translated to therapeutic application.

The HS/heparin-protein interactions are dominated by charge-charge interactions between the anionic carboxylate

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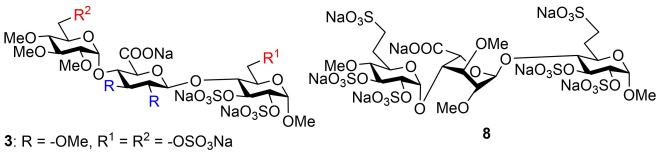
and sulfate group of the polysaccharide and basic amino acids of the proteins, and, importantly, heparin, due to its higher sulfation degree, can outcompete HS for binding to protein ligands. Indeed, heparin and its derivatives are being investigated for the treatment of a number of disorders, including cancer.[11,12]

Polysulfated oligosaccharides (malto- and isomaltooligomers, oligomannuronates) have also been known to be effective in inhibiting the growth, angiogenesis and migration of cancer cells.[13–16]

Based on the above, non-glycosaminoglycan analogues of heparin might be important structures in the development of anticancer agents. Our research group has long been working on the synthesis of heparin-analogue oligosaccharides. Several pathways have been developed to prepare the non-glycosaminoglycan, fully sulfated and fully methylated heparinoid anticoagulant pentasaccharide idraparinux **2a**,[17–20] and its sulfonic acid derivatives **2b** and **2c** (Figure 1) in which several sulfate esters were substituted by sulfonatomethyl moieties to improve the binding affinities.[20,21,22] We envisaged that trisaccharides fragments of these highly sulfated/sulfonylated pentasaccharides (**3**–**8**, Figure 2) might bind to the protein ligands, for example, heparanase, with charge–charge interactions and might display cell growth inhibitory activity. Moreover, advantageously, such smaller oligosaccharides lack anticoagulant activity because pentasaccharide is the minimal unit of heparinoids required for the anticoagulant effect as it is well known from the literature.[23–25]

In this paper, we describe the synthesis of six heparin-analogue trisaccharides (five Glc-GlcA-Glc/one Glc-IdoA-Glc **3**–**8**, Figure 2), including three sulfonic acid derivatives (**6**–**8**), and present their cell growth inhibitory activity on some healthy and cancerous cell lines. We focused primarily on the synthesis of D-glucuronic acid-containing oligosaccharides because such HS-like structures might have heparanase inhibitory activity.[26,27] Moreover, the synthesis of the GlcA moiety is much simpler and faster than the preparation of the L-iduronic acid unit. We assumed that this small set of trisaccharides allows us to compare the effect of iduronic acid versus glucuronic acid and sulfate group *vs* sulfonate group on the biological activity studied.



3: R = -OMe, R^1 = R^2 = -OSO_3Na
4: R = -OAc, R^1 = R^2 = -OSO_3Na
5: R = -OH, R^1 = R^2 = -OSO_3Na
6: R = -OMe, R^1 = -CH_2SO_3Na, R^2 = -OSO_3Na
7: R = -OMe, R^1 = R^2 = -CH_2SO_3Na

Figure 2. Non-glycosaminoglycan heparin-analogue trisaccharides (**3**-**8**) involved in this study.

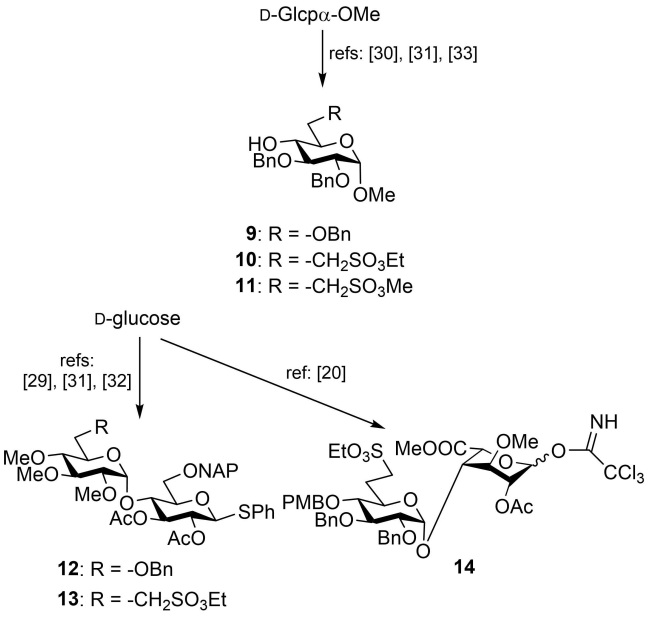
Results and Discussion

Chemistry

Synthesis of compounds **3** and **6** have been described earlier.[28,29] The preparation of the D-glucuronic acid-containing trisaccharides **4**, **5**, **7** and the L-iduronic acid-containing trisaccharide **8** was planned by coupling the precursor, non-uronic disaccharide donors **12**, **13** and **14** to the properly protected glucoside acceptor (**9**, **10** or **11**) and formation of the uronic acid at the trisaccharide level. The mono- (**9**, **10** and **11**)[30,31,33] and disaccharide (**12**, **13** and **14**)[20,29,31,32] building blocks used in the synthesis have already been described in our previous works (Figure 3). The sulfonatomethyl group was always introduced at the monosaccharide level and protected in ester form to facilitate the synthesis. Sulfonic acid methyl ester **11**[30] was formed by free-radical addition of bisulfite to the corresponding 6,7-unsaturated heptoside followed by methyl esterification of the obtained sulfonic acid by diazomethane. Noteworthy, this method, requiring a peroxybenzoate catalysis, was incompatible with thioglycosides bearing an oxidisable thio aglycone.

The sulfonic acid ethyl ester moiety (**10**, **13**, **14**) was introduced by Horner-Wadsworth-Emmons olefination[20,29,31,32] of the corresponding 6-aldehyde derivatives followed by catalytic hydrogenation or by nucleophilic displacement of the corresponding primary carbohydrate triflates with the lithiated ethyl methanesulfonate.[31] These two methods worked equally well on *O*- and *S*-glycosides, and the disaccharide units used (**13**, **14**) were constructed from the sulfonatomethyl-containing thioglycosyl monosaccharide donors.

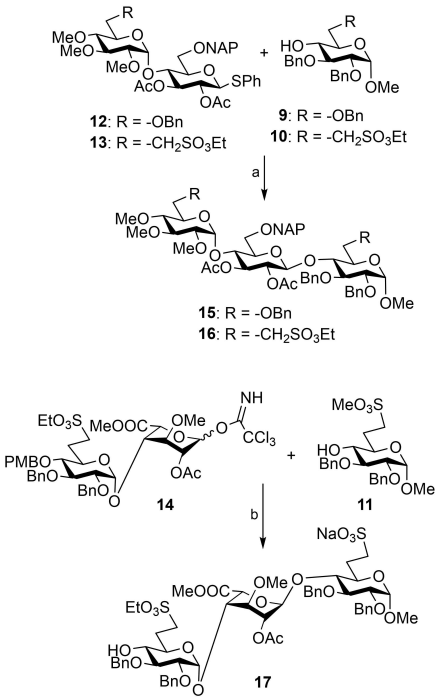
The synthesis was started with the assembly of the protected glucuronic acid containing trisaccharides **15**–**17** (Scheme 1). Condensation of the disaccharide donor **12** and the



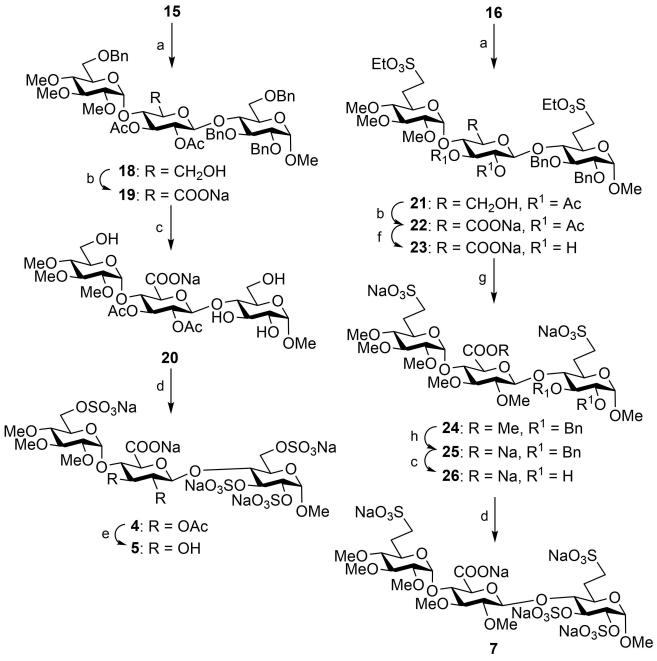
9: R = -OBn
10: R = -CH_2SO_3Et
11: R = -CH_2SO_3Me

12: R = -OBn
13: R = -CH_2SO_3Et

Figure 3. The structure of the used mono- and disaccharide building blocks.



Scheme 1. Synthesis of the protected trisaccharides (**15**-**17**). a) dry CH_2Cl_2, NIS, **15**: TfOH, **16**: AgOTf, −50 to −15 °C, 4 h (**15**: 78%; **16**: 86%); b) dry CH_2Cl_2, TMSOTf, −20 to 0 °C, 2 h (63%).



Scheme 2. Transformations of the D-glucuronic acid containing protected trisaccharides **15** and **16**. a) CH_2Cl_2, H_2O, DDQ, RT, 30 min (**18**: 84%, **21**: 82%); b) CH_2Cl_2, H_2O, TEMPO, BAIB, RT, 24 h (**19**: 61%, **22**: 79%); c) 96% EtOH, AcOH, Pd(C), H_2, RT, 24 h (**20**: 98%, **26**: 92%); d) dry DMF, SO_3·Et_3N, 50 °C, **4**: 24 h; **7**: 48 h (**4**: 74%, **7**: 68%); e) MeOH, 3 M NaOH, 0 °C to RT, 24 h (92%); f) i. MeOH, NaOMe, RT, 24 h, ii. acetone, NaI, RT, 24 h (90% over two steps); g) dry DMF, NaH, MeI, 0 °C to RT, 24 h (72%); h) THF, MeOH, 0.5 M NaOH, RT, 24 h (82%).

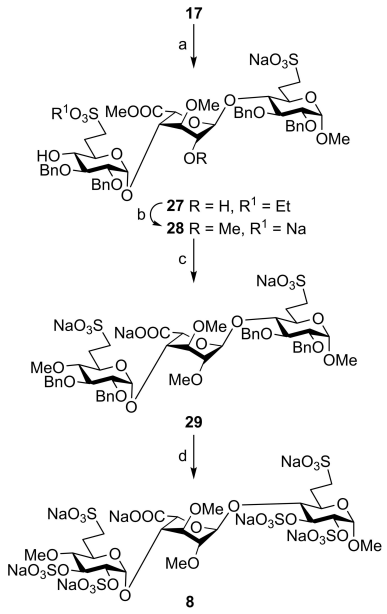
monosaccharide acceptor **9**[33] using the NIS-TfOH promoter system in dry CH_2Cl_2 provided the needed protected trisaccharide **15** with exclusive β-selectivity and good yield. The glycosylation reaction was also performed with the sulfonic acid containing building blocks **13** and **10**. In this reaction, the expected sulfonic acid containing protected trisaccharide **16** was formed with excellent yield and complete stereoselectivity. For the synthesis of the L-iduronic acid containing trisaccharide, the monosaccharide acceptor **11** bearing a C-6-sulfonatomethyl moiety was glycosylated with the disaccharide imidate **14** using TMSOTf activation. Under the acidic conditions of the glycosylation reaction, the *O*-PMB group was cleaved from position 4 of the non-reducing end, and the sulfonic acid methyl ester was converted to the corresponding sodium sulfonate by the alkaline work-up procedure. This one-pot three-step transformation afforded the L-iduronate-containing partially protected trisaccharide **17** with 63% yield.

Deprotection and formation of the uronic acid, methyl ether and sulfate ester functional groups on trisaccharides **15**–**17** were then performed. Starting from compound **15**, two trisaccharides (**4** and **5**) were prepared (Scheme 2). First, the 6-*O*-NAP group was selectively removed from the glucuronic acid precursor unit under oxidative conditions using DDQ, then the liberated hydroxyl group of **18** was oxidized into carboxylic acid using TEMPO/BAIB reagent combination to result in the D-glucuronic acid containing trisaccharide **19**.

Removal of the benzyl groups by catalytic hydrogenation afforded **20**, the liberated hydroxyl groups of which were then sulfated at 50 °C using SO_3·Et_3N complex in DMF to obtain the first trisaccharide product **4** which contains two acetyl groups on the glucuronic acid moiety. Careful alkaline hydrolysis of the two acetyl groups in the presence of sulfate esters provided the partially methylated tetra-*O*-sulfated trisaccharide **5** in 92% yield.[34]

For the preparation of the permethylated trisaccharide disulfonic acid **7**, the 6-*O*-NAP ether of the middle unit was selectively removed from compound **16**. The liberated hydroxyl group of **21** was oxidized into carboxylic acid to produce **22**. Next, the acetyl groups were cleaved under Zemplén conditions, and the ethyl ester protecting groups were removed from the methylene sulfonates by nucleophilic displacement reaction using NaI reagent (**24**). Subsequently, the free 2- and 3-OH groups of the glucuronic acid unit were methylated using NaH and MeI in dry DMF. Beside the hydroxyls, the carboxylic acid moiety was also methylated in the reaction. The methyl ester was hydrolyzed under alkaline conditions (**25**) and the benzyl groups were removed by catalytic hydrogenation (**26**). Finally, the liberated hydroxyl groups were sulfated using SO_3·Et_3N complex to produce trisaccharide **7** bearing two methylene sulfonic acid moieties.

Towards synthesis of the L-iduronic acid containing trisaccharide **8**, compound **17** was deacetylated and the obtained **27** was treated with methyl iodide in the presence of NaH (Scheme 3). Parallel to the *O*-methylation conversion of the ethyl sulfonate ester into sodium sulfonate was also observed giving rise to the disodium salt **28**. The uronate ester was then



Scheme 3. Transformation of the L-iduronic acid containing trisaccharide **17**. a) MeOH, NaOMe, RT, 4 h (99%); b) dry DMF, NaH, MeI, 0 °C, 2 h (52%); c) MeOH, 0.2 M NaOH, RT, 24 h (76%); d) i. 96% EtOH, Pd(C), AcOH, H_2, RT, 24 h, ii. dry DMF, SO_3·Et_3N, 50 °C, 24 h (59% over two steps).

converted to the uronate salt **29** using aqueous NaOH solution in methanol.

Finally, the benzyl groups were removed by catalytic hydrogenation and *O*-sulfation of the liberated hydroxy groups was carried out under the previously described conditions. As a result, the L-iduronate-containing trisaccharide disulfonic acid **8** has successfully been prepared. After completion of the synthesis, the cell growth inhibitory activity and cytotoxicity of these six trisaccharide derivatives were investigated.

Biological evaluation

The biological effect of the above six trisaccharide derivatives **3**–**8** to the cellular viability of A2780 human ovarian carcinoma, WM35 human melanoma and HaCaT spontaneously immortalized human keratinocyte cell lines were investigated by MTT assay. Doxorubicin, a generally used chemotherapeutic agent[35] was used as positive control. The dose-response relationship of doxorubicin was investigated on each cell line prior to testing the trisaccharides and 1 μM, as a maximal effective concentration (Figure S1 in the Supporting information), was used as positive control in the subsequent experiments.

Among the investigated trisaccharides, compound **4** and **5** reduced the viability of the WM35 melanoma cell line and compound **4** also inhibited the growth of the A2780 ovarian carcinoma cells in a concentration dependent manner (Figure 4A, B). Experimentally determined IC_{50} values of compound **4** were 0.55 ± 0.46 and 1.63 ± 0.61 μM on WM35 and A2780 cells, respectively. Compound **5** was less effective when tested on A2780 cells and its potency is reduced compared to

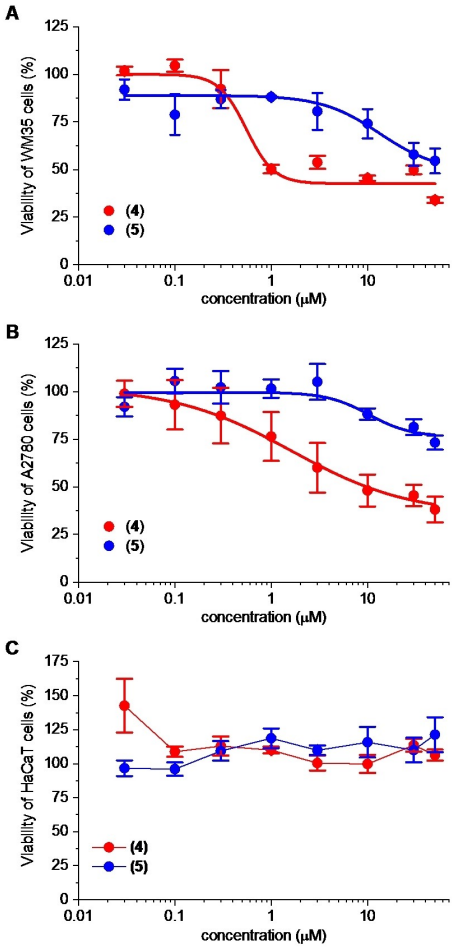


Figure 4. Effect of compounds **4** and **5** on the viability of cancerous and non-cancerous cell lines. Concentration-dependent effect of compound **4** and **5** on the viability of A) WM35 melanoma, B) A2780 ovarian carcinoma and C) HaCaT keratinocyte cell lines. Viability was determined by MTT assay after 72 h of treatment with the compounds applied in the indicated concentrations and, when it was possible logistic dose-response curves were fitted and IC_{50} values were determined as described in the Experimental Section. Data are presented as mean ± SEM, $n = 6$ at each data point.

compound **4**: IC_{50} values of compound **5** were 13.28 ± 8.57 and 10.27 ± 5.62 μM on WM35 and A2780 cells, respectively. Efficacy of these compounds was lower than that of doxorubicin: they induced only a partial decrease in viability even at the highest concentration (50 μM) applied (Figure 4A, B). Importantly, compound **4** and **5** were discriminative between the tumor-driven and nontumorigenic cell lines: In contrast to doxorubicin (Figure S1C), the viability of the non-tumorigenic HaCaT cells did not decline applying these compounds up to 50 μM (Figure 4C).

The other four trisaccharides (**3**, **6**, **7** and **8**) only moderately affected the growth of the cancerous cell lines and did not exhibit dose dependent effect on viability. Considering that compounds **3**, **4** and **5** differ only in the substitution pattern of the glucuronic acid unit, the inactivity of compound **3** demonstrates that the methyl substitution on the uronic acid is detrimental to the inhibitory activity against the cancerous cell

lines studied. Moreover, the higher activity of compound **4** relative to **5** shows that acetyl substituents on the uronic acid are advantageous to the biological effect. The growth of HaCaT cells was not influenced by compounds **3**, **6**, **7** and **8** (Figures S2–S5). The compounds selectivity was also studied on human cancerous cell lines (Ebc-1 and MonoMac6) from different origin and on non-cancerous non-human primate Vero E6 cells (Figure S6A–F). In this study, compound **4** and **5** showed a modest, concentration-dependent cytostatic effect on the cancerous MonoMac6 cell line. Except for that, the compounds were ineffective on all three cell lines.

Conclusions

Using our previous synthetic experience, the targeted four new heparin-analogue trisaccharides were successfully synthesized with excellent yields. The cell growth inhibitory study showed clearly that the six tested trisaccharides have no effect on the growth of healthy keratinocyte derived cells (HaCaT). They do not adversely affect the growth of these cells as opposed to the chemotherapeutic agent Doxorubicin which is used in medicine. Moreover, our results have also shown that two of our compounds of glucuronic acid content (**4**, **5**) displayed remarkable cell growth inhibitory effects on ovary carcinoma (A2780) and melanoma (WM35) cells.

Methyl substituents on the glucuronide unit proved to be detrimental to the cytostatic activity of the sulfated derivatives. As it is well demonstrated that the methyl substitution of the uronic acids does not adversely affect the anticoagulant activity of heparinoids,[20–22,36,37] this effect was unexpected and worthy of further study. The sulfonic acid derivatives, including the iduronate-containing disulfonic acid **8** showed very low or no activity, that, however, can probably be attributed to the methyl ether content of their uronate residue.

In summary, in this short study we identified two glucoronate-containing heparinoid trisaccharides of simplified structure that display promising and selective cell growth inhibitory activity.

Experimental Section

General information: Optical rotations were measured at room temperature on a Perkin-Elmer 241 automatic polarimeter. TLC analysis was performed on Kieselgel 60 F_{254} (Merck) silica-gel plates with visualization by immersing in a sulfuric-acid solution (5% in EtOH) followed by heating. Column chromatography was performed on silica gel 60 (Merck 0.063–0.200 mm) and Sephadex LH-20 (Sigma-Aldrich, bead size: 25–100 mm). Organic solutions were dried over $MgSO_4$ and concentrated under vacuum. ^{1}H and ^{13}C NMR spectroscopy (^{1}H: 360, 400 and 500 MHz; ^{13}C: 90.54, 100.28 and 125.76 MHz) were performed on Bruker DRX-360, Bruker DRX-400 and Bruker Avance II 500 spectrometers at 25 °C. Chemical shifts are referenced to $SiMe_4$ or sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS, $\delta = 0.00$ ppm for ^{1}H nuclei) and to residual solvent signals ($CDCl_3$: $\delta = 77.00$ ppm, CD_3OD: $\delta = 49.15$ ppm for ^{13}C nuclei). MALDI-TOF MS analyses of the compounds were carried out in the positive reflektron mode using a BIFLEX III mass spectrometer (Bruker, Germany) equipped with delayed-ion extraction. 2,5-Dihydroxybenzoic acid (DHB) was used as matrix and $F_3CCOONa$ as cationising agent in DMF. ESI-TOF MS spectra were recorded by a microTOF–Q type QqTOFMS mass spectrometer (Bruker) in the positive ion mode using MeOH as the solvent.

Penta-sodium [methyl (2,3,4-tri-*O*-methyl-6-*O*-sulfonato-α-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-acetyl-β-D-glucopyranosyl-uronate)-(1→4)-2,3,6-tri-*O*-sulfonato-α-D-glucopyranoside] (4): Compound **20** (140 mg, 0.206 mmol) was treated with $SO_3{\cdot}Et_3N$ complex (747 mg, 4.12 mmol) in dry DMF (5.0 mL). After 24 h of stirring at 50 °C, the reaction mixture was neutralized with saturated aqueous solution of $NaHCO_3$ (1.73 g, 20.59 mmol), and the resulting mixture was concentrated in vacuo. The crude product was purified by Sephadex gel G-25 in H_2O and then treated with Dowex ion exchange resin (Na^+ form) to give **4** (165 mg, 74%) as a white solid. $[\alpha]_D = +50.0$ ($c = 0.12$, H_2O); R_f 0.21 (6:7:1 EtOAc/MeOH/H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta = 5.30$ (t, $J = 8.8$ Hz, 1H, H-3-E), 5.20 (d, $J = 3.4$ Hz, 1H, H-1-D), 5.14 (d, $J = 3.2$ Hz, 1H, H-1-F), 4.96-4.92 (m, 2H, H-1-E, H-2-E), 4.58 (t, $J = 8.7$ Hz, 1H, H-3-F), 4.38-4.34 (m, 3H, H-2-F, H-6a,b-F), 4.27 (d, $J = 10.5$ Hz, 1H, H-6a-D), 4.16-4.12 (m, 2H, H-4-E, H-6b-D), 4.03-3.98 (m, 2H, H-4-F, H-5-F), 3.90 (d, $J = 9.7$ Hz, 2H, H-5-E, H-5-D), 3.62, 3.58, 3.48, 3.45 (4 x s, 12H, 4 x OCH_3), 3.55-3.50 (m, 1H, H-3-D), 3.33-3.28 (m, 2H, H-2-D, H-4-D), 2.15, 2.13 (2 x s, 6H, 2 x Ac-CH_3) ppm; ^{13}C NMR (100 MHz, D_2O): $\delta = 174.0$, 173.0 (3 C, 2 x Ac-*C*O, *C*OONa), 98.8 (1 C, C-1-E), 97.4 (1 C, C-1-D), 97.2 (1 C, C-1-F), 82.5 (1 C, C-3-D), 80.0 (1 C, C-2-D), 78.1 (1 C, C-4-D), 76.9 (1 C, C-5-E), 75.7 (2 C, C-3-E, C-3-F), 75.1 (2 C, C-2-F, C-4-E), 73.8 (1 C, C-4-F), 72.6 (1 C, C-2-E), 68.9, 68.8 (2 C, C-5-D, C-5-F), 65.9, 65.8 (2 C, C-6-D, C-6-F), 60.2, 60.0, 59.6 (3 C, 3 x OCH_3), 55.4 (1 C, C-1-OCH_3), 20.5, 20.3 (2 C, 2 x Ac-*C*H_3) ppm; ESI-TOF-MS: *m/z* calcd for $C_{26}H_{37}Na_3O_{31}S_4$: [*M*-2Na]$^{2-}$ 520.995; found: 520.996.

Penta-sodium [methyl (2,3,4-tri-*O*-methyl-6-*O*-sulfonato-α-D-glucopyranosyl)-(1→4)-(β-D-glucopyranosyl-uronate)-(1→4)-2,3,6-tri-*O*-sulfonato-α-D-glucopyranoside] (5): Compound **4** (40 mg, 0.036 mmol) dissolved in MeOH (1.5 mL) and 3 M solution of NaOH (0.8 mL) was added at 0 °C. After 24 h stirring at room temperature, the reaction mixture was neutralized with AcOH and the resulting mixture was concentrated in vacuo. The crude product was purified by Sephadex gel G-25 in H_2O and then treated with Dowex ion exchange resin (Na^+ form) to give **5** (34 mg, 92%) as a white solid. $[\alpha]_D = +68.7$ ($c = 0.08$, H_2O); R_f 0.01 (8:2 MeCN/H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta = 5.64$ (d, $J = 3.7$ Hz, 1H, H-1-D), 5.14 (d, $J = 3.6$ Hz, 1H, H-1-F), 4.64 (d, $J = 7.9$ Hz, 1H, H-1-E), 4.62 (t, $J = 9.4$ Hz, 1H, H-3-F), 4.36 (d, $J = 3.1$ Hz, 2H, H-6a,b-F), 4.34 (dd, $J = 3.4$ Hz, $J = 9.8$ Hz, 1H, H-2-F), 4.27 (d, $J = 10.2$ Hz, 1H, H-6a-D), 4.13 (d, $J = 10.1$ Hz, 1H, H-6b-D), 4.07-3.99 (m, 2H, H-4-F, H-5-F), 3.90-3.82 (m, 3H, H-4-E, H-5-E, H-5-D), 3.74-3.70 (m, 1H, H-3-E), 3.60, 3.57, 3.52, 3.45 (4 x s, 12H, 4 x OCH_3), 3.55-3.50 (m, 1H, H-3-D), 3.40 (dd, $J = 8.1$ Hz, $J = 9.1$ Hz, 1H, H-2-E), 3.36-3.30 (m, 2H, H-2-D, H-4-D) ppm; ^{13}C NMR (100 MHz, D_2O): $\delta = 101.7$ (1 C, C-1-E), 98.1 (1 C, C-1-F), 96.8 (1 C, C-1-D), 82.4 (1 C, C-3-D), 81.2 (1 C, C-2-D), 78.9 (1 C, C-4-D), 77.4, 77.2, 77.1 (4 C, C-3-F, C-3-E, C-4-E, C-5-E), 76.1 (1 C, C-2-F), 74.4 (1 C, C-2-E), 73.9 (1 C, C-4-F), 69.8, 69.7 (2 C, C-5-D, C-5-F), 67.2 (1 C, C-6-F), 66.9 (1 C, C-6-D), 61.0, 60.8, 59.0 (3 C, 3 x OCH_3), 56.2 (1 C, C-1-OCH_3) ppm; ESI-TOF-MS: *m/z* calcd for $C_{22}H_{33}Na_5O_{29}S_4$: [*M*-2Na]$^{2-}$ 478.985; found: 478.985.

Penta-sodium [methyl (2,3,4-tri-*O*-methyl-6-deoxy-6-*C*-sulfonatomethyl-α-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-methyl-β-D-glucopyranosyl-uronate)-(1→4)-6-deoxy-6-*C*-sulfonatomethyl-2,3-di-*O*-sulfonato-α-D-glucopyranoside] (7): Compound **26** (98 mg, 0.119 mmol) was treated with $SO_3{\cdot}Et_3N$ complex (215 mg, 1.19 mmol) in dry DMF (2.9 mL). After 48 h stirring at 50 °C, the reaction mixture was neutralized with saturated aqueous solution of $NaHCO_3$ (499 mg, 5.940 mmol) and the resulting mixture was concentrated in vacuo. The crude product was purified by Sephadex gel G-25 in H_2O and then treated with Dowex ion

exchange resin (Na^+ form) to give **7** (83 mg, 68%) as a white solid. $[\alpha]_D = +66.9$ ($c = 0.13$, H_2O); R_f 0.19 (6:7:1 EtOAc/MeOH/H_2O); ^1H NMR (500 MHz, D_2O): $\delta = 5.49$ (d, $J = 3.7$ Hz, 1H, H-1″), 5.11 (d, $J = 3.7$ Hz, 1H, H-1), 4.69 (d, $J = 7.8$ Hz, 1H, H-1′), 4.59 (t, $J = 9.1$ Hz, 1H, H-3), 4.36 (dd, $J = 3.7$ Hz, $J = 9.6$ Hz, 1H, H-2), 3.96-3.89 (m, 3H, H-4′, H-5, H-5′), 3.76 (t, $J = 9.1$ Hz, 1H, H-4), 3.62-3.52 (m, 17H, H-3′, H-5″, 5 x OCH_3), 3.50 (t, $J = 9.7$ Hz, 1H, H-3″), 3.44 (s, 3H, C-1O-CH_3), 3.33 (dd, $J = 3.9$ Hz, $J = 10.2$ Hz, 1H, H-2″), 3.30 (t, $J = 9.0$ Hz, 1H, H-2′), 3.18-2.96 (m, 5H, H-4″, H-7a,b, H-7″a,b), 2.46-2.44 (m, 1H, H-6a), 2.21-2.16 (m, 1H, H-6″a), 1.98-1.88 (m, 2H, H-6b, H-6″b) ppm, ^{13}C NMR (500 MHz, D_2O): $\delta = 173.3$ (1 C, *C*O), 102.0 (1 C, C-1′), 96.9 (1 C, C-1), 95.3 (1 C, C-1″), 85.5 (1 C, C-3′), 82.6 (1 C, C-2′), 82.2 (1 C, C-4″), 81.4 (1 C, C-3″), 80.4 (1 C, C-2″), 78.4 (1 C, C-4), 76.3 (1 C, C-3), 75.2 (1 C, C-2), 75.1 (1 C, C-5′), 72.8 (1 C, C-4′), 69.3 (1 C, C-5), 68.4 (1 C, C-5″), 60.3, 60.2, 59.6, 59.1, 58.9 (5 C, 5 x OCH_3), 55.3 (1 C, C-1-OCH_3), 47.2, 47.0 (2 C, 2 x C-7), 26.3 (1 C, C-6′), 26.0 (1 C, C-6) ppm; ESI-TOF-MS: *m/z* calcd for $C_{26}H_{41}Na_6O_{27}S_4$: $[M+H]^+$ 1051.0099; found: 1051.0091.

Hepta-sodium [methyl (6-deoxy-4-*O*-methyl-2,3-di-*O*-sulfonato-6-*C*-sulfonatomethyl-α-D-glucopyranosyl)]-(1→4)-(2,3-di-*O*-methyl-α-L-idopyranosyl-uronate)-(1→4)-(6-deoxy-2,3-di-*O*-sulfonato-6-*C*-sulfonatomethyl-α-D-glucopyranoside)] (8): A mixture of **29** (34 mg, 0.030 mmol) and Pd/C (10%, 30 mg) was dissolved in 96% EtOH-AcOH (30:1, 3.1 mL) and stirred in an autoclave under H_2 atmosphere (at 10 bar) for 24 h. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated. The crude product (23 mg, 99%, R_f 0.17 (7:6:1 CH_2Cl_2/MeOH/H_2O)) was used for further reaction without purification. A solution of the crude product (23 mg, 0.029 mmol) in dry DMF (1.5 mL) was treated with $SO_3{\cdot}Et_3N$ complex (105 mg, 0.580 mmol, 5.0 equiv./OH). After 24 h stirring at 50 °C, the reaction mixture was neutralized with aqueous solution of $NaHCO_3$ (244 mg, 25.0 equiv./OH). The resulting mixture was concentrated. The crude product was purified by Sephadex gel G-25 in H_2O and then treated with Dowex ion exchange resin (Na^+ form), to give **8** (20 mg, 59% for two steps) as a white solid. $[\alpha]_D = +41.3$ ($c = 0.08$, H_2O); R_f 0.12 (7:6:1 CH_2Cl_2/MeOH/H_2O); ^1H NMR (D_2O, 360 MHz): $\delta = 5.39$ (d, $J_{1,2} = 3.1$ Hz, 1H, H-1-F), 5.24 (s, 1H, H-1-G), 5.13 (d, $J_{1,2} = 3.4$ Hz, 1H, H-1-H), 4.86 (s, 1H, H-5-G), 4.63-4.57 (m, 2H, H-3-H, H-3F), 4.37 (dd, $J_{1,2} = 3.3$ Hz, $J_{2,3} = 9.6$ Hz, 1H, H-2-H), 4.28 (dd, $J_{1,2} = 3.0$ Hz, $J_{2,3} = 9.4$ Hz, 1H, H-2-F), 4.13 (s, 1H, H-4-G), 3.92-3.77 (m, 4H), 3.59, 3.57, 3.53, 3.45 (4 x s, 12H, 4 x OCH_3), 3.30 (t, $J = 8.9$ Hz, 1H), 3.20-3.00 (m, 5H), 2.39-1.92 (m, 4H, H-6a,b-F, H-6a,b-H) ppm; ^{13}C NMR (D_2O, 90 MHz): $\delta = 97.9$ (2 C, C x C-1), 94.9 (1 C, C-1), 82.4, 80.0, 78.2, 77.6, 76.6, 76.5, 75.9, 72.7, 71.3, 70.2, 70.1 (12 C, skeleton carbons), 61.3, 60.1, 58.9, 56.3 (4 C, 4 x OCH_3), 48.2, 47.9 (2 C, 2 x C-7), 27.3, 27.2 (2 C, 2 x C-6) ppm; ESI-TOF-MS: *m/z* calcd for $C_{24}H_{35}Na_8O_{33}S_6$: $[M+Na]^+$ 1226.8561; found: 1226.8538.

Methyl (6-*O*-benzyl-2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)-(1→4)-[2,3-di-*O*-acetyl-6-*O*-(2′-naphthyl)methyl-β-D-glucopyranosyl]-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (15): To a solution of compound **12**:[29] (440 mg, 0.948 mmol, 1.5 equiv.) and compound **9**[33] (500 mg, 0.632 mmol) in dry CH_2Cl_2 (15 mL) was added 4 Å molecular sieves (0.5 g). After stirring for 30 min at room temperature, the mixture was cooled to −50 °C and the solutions of NIS (213 mg, 0.948 mmol, 1.5 equiv. for donor) in dry THF (400 μL) and TfOH (25 μL, 0.294 mmol, 0.3 equiv. for donor) were added. Allowed to warm up the solution to −15 °C and the mixture were stirred for 4 h at that temperature. When the TLC analysis (1:1 *n*-hexane/EtOAc) showed complete consumption of the donor, the reaction mixture was neutralized with Et_3N (500 μL), diluted with CH_2Cl_2 (150 mL), and filtered. The filtrate was washed with an aqueous solution of $Na_2S_2O_3$ (10%, 2×50 mL), a saturated aqueous solution of $NaHCO_3$ (2×50 mL), and water (2×50 mL), dried, and concentrated. The crude product was purified by column chromatography on silica gel (1:1 *n*-hexane/EtOAc) to give compound **15** (562 mg, 78%) as a colourless syrup. $[\alpha]_D = +62.2$ ($c = 0.09$, $CHCl_3$); R_f 0.47 (1:1 *n*-hexane/EtOAc); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.81$-7.13 (m, 27H, arom), 5.14 (t, $J = 9.2$ Hz, 1H, H-3-E), 5.08 (d, $J = 3.5$ Hz, 1H, H-1-D), 5.02 (d, $J = 11.6$ Hz, 1H, Bn-CH_{2a}), 4.86 (dd, $J = 9.3$ Hz, $J = 8.2$ Hz, 1H, H-2-E), 4.76 (d, $J = 11.7$ Hz, 1H, Bn-CH_{2b}), 4.71 (dd, $J = 12.1$ Hz, $J = 5.5$ Hz, 2H, Bn-CH_2), 4.59-4.53 (m, 4H, H-1-E, H-1-F, NAP-CH_2), 4.48-4.41 (m, 3H, Bn-CH_2), 4.28 (d, $J = 12.2$ Hz, 1H, Bn-CH_{2b}), 3.95-3.84 (m, 3H, H-3-F, H-4-F, H-4-E), 3.76 (dd, $J = 10.5$ Hz, $J = 3.0$ Hz, 1H, H-6a-F), 3.68-3.60 (m, 5H, H-5-D, H-5-F, H-6a,b-E, H-6b-F), 3.58, 3.42, 3.40, 3.34 (4 x s, 12H, 4 x OCH_3), 3.46-3.35 (m, 4H, H-2-F, H-3-D, H-6a,b-D), 3.25-3.21 (m, 1H, H-5-E), 3.19-3.15 (m, 1H, H-4-D), 3.05 (dd, $J = 9.8$ Hz, $J = 3.5$ Hz, 1H, H-2-D), 2.01, 1.94 (2 x s, 6H, 2 x Ac-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.0$, 169.7 (2 C, 2 x Ac-CO), 139.5, 138.3, 138.1, 137.7, 136.1, 133.3, 132.9 (7 C, 7 x C_q arom), 128.7-125.7 (27 C, arom), 99.9 (1 C, C-1-F), 98.4 (1 C, C-1-E), 97.8 (1 C, C-1-D), 83.2 (1 C, C-3-D), 81.8 (1 C, C-2-D), 80.2 (1 C, C-3-F), 79.3, 79.2 (2 C, C-2-F, C-4-D), 77.0 (1 C, C-4-F), 75.1, 74.9 (3 C, C-3-E, C-4-E, C-5-E), 72.9 (1 C, C-2-E), 71.2 (1 C, C-5-D), 69.9 (1 C, C-5-F), 73.7, 73.6, 73.3 (5 C, 4 x Bn-CH_2, NAP-CH_2), 68.8, 68.4 (2 C, C-6-D, C-6-E), 67.9 (1 C, C-6-F), 60.7, 60.4, 59.3 (3 C, 3 x OCH_3), 55.3 (1 C, C-1-OCH_3), 21.1, 20.8 (2 C, 2 x Ac-CH_3) ppm; MALDI-TOF-MS: *m/z* calcd for $C_{65}H_{76}NaO_{18}$: $[M+Na]^+$ 1167.492; found: 1167.657.

Methyl [ethyl (2,3,4-tri-*O*-methyl-6-deoxy-6-*C*-sulfonatomethyl-α-D-glucopyranosyl)]-(1→4)-[2,3-di-*O*-acetyl-6-*O*-(2′-naphthyl)methyl-β-D-glucopyranosyl]-(1→4)-[ethyl (2,3-di-*O*-benzyl-6-deoxy-6-*C*-sulfonatomethyl-α-D-glucopyranoside)] (16): To a solution of compound **13**[31] (1.343 g, 1.667 mmol, 1.6 equiv.) and compound **10**[31] (500 mg, 1.041 mmol) in dry CH_2Cl_2 (30 mL) was added 4 Å molecular sieves (1.0 g). After stirring for 30 min at room temperature, the mixture was cooled to −50 °C and the mixture of the solutions of NIS (412 mg, 1.833 mmol, 1.5 equiv. for donor) in dry THF (1.0 mL) and AgOTf (103 mg, 0.400 mmol, 0.38 equiv. for donor) in dry toluene (1.0 mL) were added. Allowed to warm up the solution to −15 °C and the mixture were stirred for 4 h at that temperature. When the TLC analysis (1:1 *n*-hexane/EtOAc) showed complete consumption of the donor, the reaction mixture was diluted with CH_2Cl_2 (200 mL), and filtered through a pad of Celite®. The filtrate was washed with an aqueous solution of $Na_2S_2O_3$ (10%, 2×75 mL), a saturated aqueous solution of $NaHCO_3$ (2×75 mL), and water (2×75 mL), dried, and concentrated. The crude product was purified by column chromatography on silica gel (1:1 *n*-hexane/EtOAc) to give compound **16** (1.058 g, 86%) as a colourless syrup. $[\alpha]_D = +36.5$ ($c = 0.20$, $CHCl_3$); R_f 0.28 (1:1 *n*-hexane/EtOAc); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.87$-7.76 (m, 3H, arom), 7.69 (s, 1H, arom), 7.48-7.33 (m, 3H, arom), 7.31-7.08 (m, 17H, arom), 5.17 (t, $J = 9.2$ Hz, 1H, H-3-E), 5.07-4.73 (m, 5H, H-1-D, H-1-E, H-2-E, BnCH_2), 4.70-4.43 (m, 5H, H-1-F, NAPCH_2, BnCH_2), 4.27 (q, $J = 7.1$ Hz, 2H, $SO_3CH_2CH_3$), 4.11 (q, 2H, $SO_3CH_2CH_3$), 3.97-3.80 (m, 2H, H-3-F, H-4-E), 3.69-3.59 (m, 1H, H-5-F), 3.53-3.22 (m, 7H, H-3-D, H-5-D, H-2-F, H-4-F, H-7_a-F, H-6_a-E, H-6_b-E), 3.53, 3.50, 3.38, 3.31 (4 s, 12H, 4 x CH_3), 3.21-2.98 (m, 3H, H-5-E, H-7_b-F, H-7_a-D), 2.95-2.78 (m, 2H, H-2-D, H-7_b-D), 2.67 (t, $J = 9.3$ Hz, 1H, H-4-D), 2.39-2.27 (m, 1H, H-6_a-F), 2.26-2.11 (m, 1H, H-6_a-D), 2.07, 2.00 (2 s, 6H, 2 x Ac-CH_3), 1.88-1.72 (m, 2H, H-6_b-F, H-6_b-D), 1.39 (t, $J = 6.9$ Hz, 3H, $SO_3CH_2CH_3$), 1.28 (t, 3H, $SO_3CH_2CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 169.8$, 169.7 (2 C, 2 x Ac-CO), 139.1, 137.7, 135.2, 133.1, 132.9 (5 C, C_q arom), 128.3, 128.1, 128.2, 129.0, 127.9, 127.8, 127.5, 126.8, 126.5, 126.0, 125.9, 125.8, 125.6 (17 C, arom), 100.9 (1 C, C-1-D), 97.6 (1 C, C-1-F), 96.8 (1 C, C-1-E), 83.2 (1 C, C-4-D), 82.3 (1 C, C-3-D), 81.9 (1 C, C-2-D), 81.8 (1 C, C-4-F), 79.7 (1 C, C-2-F), 79.7 (1 C, C-3-F), 75.0 (1 C, C-5-E), 74.5 (1 C, C-3-E), 74.1 (1 C, C-4-E), 74.2, 73.3, 73.1 (3 C, 2 x BnCH_2, NAPCH_2), 72.7 (1 C, C-2-E), 69.2 (1 C, C-5-D), 67.5 (1 C, C-5-F), 67.1 (1 C, C-6-E), 66.1, 65.8 (2 C, $SO_3CH_2CH_3$), 60.5, 60.2, 58.9, 55.3 (4 C, OCH_3), 46.7, 46.6 (2 C, C-7-D, C-7-F), 25.9 (C-6-D), 25.9 (C-6-F), 20.8, 20.6 (2 C, $COCH_3$), 15.0, 14.1 (2 C, $SO_3CH_2CH_3$) ppm; MALDI-TOF-MS: *m/z* calcd for $C_{57}H_{76}NaO_{22}$: $[M+Na]^+$ 1199.42; found: 1200.73.

Methyl [ethyl (2,3-di-*O*-benzyl-6-deoxy-6-*C*-sulfonatomethyl-α-D-glucopyranosyl)]-(1→4)-[methyl (2-*O*-acetyl-3-*O*-methyl-α-L-idopyranosyl)-uronate]-(1→4)-[sodium (2,3-di-*O*-benzyl-6-deoxy-6-*C*-sulfonatomethyl-α-D-glucopyranoside)] (17): To a solution of compound **14**[20] (147 mg, 0.315 mmol, 1.5 equiv.) and compound **11**[30] (200 mg, 0.210 mmol) in dry CH_2Cl_2 (6 mL) was added 4 Å molecular sieves (1.0 g). After 30 min, the mixture was cooled to −20 °C and a solution of TMSOTf (5 μL, 0.021 mmol) in dry CH_2Cl_2 (190 μL) was added. After stirring for 2 h, TLC analysis showed the complete consumption of the donor. The reaction mixture was neutralized with Et_3N (150 μL), diluted with CH_2Cl_2 (100 mL) and filtered. The filtrate was washed with a saturated aqueous solution of $NaHCO_3$ (2×25 mL) and water (2×25 mL), dried and concentrated. The crude product was purified by column chromatography on silica gel (1:1 *n*-hexane/EtOAc) to give compound **17** (151 mg, 63%) as a colourless syrup. R_f 0.12 (1:1 *n*-hexane/EtOAc); ^{1}H NMR (360 MHz, CD_3OD): δ = 7.37-7.26 (m, 20H; arom), 5.20 (d, 1H, J = 2.6 Hz), 4.91-4.57 (m, 12H; 4 x Bn-CH_2, 2 x H-1, H-2′, H-5′), 4.26 (q, J = 7.1 Hz, 2H, $SO_3CH_2CH_3$), 3.92-3.18 (m, 10H), 3.49, 3.34 (3 x s, 9H, 3 x OCH_3), 3.07-2.84 (m, 4H; 2 x H-7a,b), 2.34-2.24 (m, 3H, 2 x H-6a), 2.06 (s, 3H, Ac-CH_3), 1.94-1.90 (m, 2H, 2 x H-6b), 1.35 (t, J = 7.1 Hz, 3H, $SO_3CH_2CH_3$) ppm; ^{13}C NMR (90 MHz, CD_3OD): δ = 172.0, 171.3 (2 C, 2 x CO), 140.3, 140.2, 139.7, 139.5 (4 C, 4 x C_q arom), 129.5-128.3 (20 C, arom), 100.8, 99.4, 98.7 (3 C, 3 x C-1), 82.4, 81.7, 81.1, 80.6, 79.5, 78.1, 76.6, 74.8, 71.8, 70.5, 70.3, 69.8 (12 C, skeleton carbons), 76.2, 75.9, 74.5, 74.0 (4 C, 4 x Bn-CH_2), 68.0 (1 C, $SO_3CH_2CH_3$), 58.8, 55.6, 52.6 (3 C, 4 x OCH_3), 47.0 (2 C, 2 x C-7), 28.3, 26.7 (2 C, 2 x C-6), 21.4 (1 C, Ac-CH_3), 15.5 (1 C, $SO_3CH_2CH_3$) ppm.

Methyl (6-*O*-benzyl-2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (18): To a vigorously stirred solution of **15** (550 mg, 0.480 mmol) in CH_2Cl_2 (8.0 mL) and H_2O (0.8 mL) DDQ (163 mg, 0.720 mmol) was added. After 30 min the mixture was diluted with CH_2Cl_2 (200 mL) and extracted with saturated aqueous solution of $NaHCO_3$ (2×30 mL), and H_2O (2×30 mL), dried and concentrated. The crude product was purified by silica gel chromatography (9:1 CH_2Cl_2/acetone) to give compound **18** (405 mg, 84%) as a colourless syrup. $[\alpha]_D$ = +25.0 (c = 0.10, $CHCl_3$); R_f 0.40 (9:1 CH_2Cl_2/acetone); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40-7.24 (m, 20H, arom), 5.10 (t, J = 9.2 Hz, 1H), 5.02 (d, J = 3.6 Hz, 1H), 4.90-4.43 (m, 11H), 3.83-3.81 (m, 2H), 3.75-3.71 (m, 2H), 3.63-3.61 (m, 7H), 3.59, 3.45, 3.44, 3.36 (4 x s, 12H, 4 x OCH_3), 3.50-3.38 (m, 3H), 3.17 (d, J = 9.1 Hz, 1H), 3.09 (dd, J = 9.7 Hz, J = 3.6 Hz, 2H), 2.01, 1.93 (2 x s, 6H, 2 x Ac-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.8, 169.5 (2 C, 2 x Ac-*C*O), 139.2, 138.2, 137.9, 137.5 (4 C, 4 x C_q arom), 128.7-127.2 (20 C, arom), 99.8, 98.4, 98.1 (3 C, 3 x C-1), 83.3, 81.5, 79.8, 79.4, 79.0, 77.0, 75.2, 74.9, 74.7, 72.7, 71.3, 69.8 (12 C, skeleton carbons), 75.3, 73.7, 73.5, 73.4 (4 C, 4 x Bn-CH_2), 68.5, 67.5, 61.3 (3 C, 3 x C-6), 60.6, 60.4, 59.4 (3 C, 3 x OCH_3), 55.3 (1 C, C-1-OCH_3), 20.9, 20.7 (2 C, 2 x Ac-CH_3) ppm; MALDI-TOF-MS: m/z calcd for $C_{54}H_{68}NaO_{18}$: $[M+Na]^+$ 1027.430; found: 1027.886.

Methyl (6-*O*-benzyl-2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)-(1→4)-[sodium (2,3-di-*O*-acetyl-β-D-glucopyranosyl)-uronate]-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (19): To a vigorously stirred solution of **18** (390 mg, 0.388 mmol) in CH_2Cl_2 (8.0 mL) and H_2O (4.0 mL) TEMPO (12 mg, 0.077 mmol) and BAIB (375 mg, 1.164 mmol) were added. After 24 h stirring at room temperature, the reaction mixture was quenched by addition of 10% aqueous solution of $Na_2S_2O_3$ (35 mL). The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were dried, and concentrated. The crude product was purified by silica gel chromatography (95:5 CH_2Cl_2/MeOH) to give **19** (245 mg, 61%) as a colourless syrup. $[\alpha]_D$ = +46.1 (c = 0.18, $CHCl_3$); R_f 0.33 (95:5 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ = 7.33-7.08 (m, 20H, arom), 5.01 (t, J = 9.2 Hz, 1H), 4.98 (d, J = 2.1 Hz, 1H), 4.89-4.34 (m, 11H), 3.97 (t, J = 9.2 Hz, 1H), 3.74-3.47 (m, 9H), 3.45, 3.34, 3.32, 3.21 (4 x s, 12H, 4 x OCH_3), 3.30-3.25 (m, 2H), 3.15 (d, J = 9.4 Hz, 1H), 2.93 (dd, J = 9.7 Hz, J = 3.4 Hz, 1H), 1.91, 1.82 (2 x s, 6H, 2 x Ac-CH_3) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 172.7, 171.5, 171.0 (3 C, 2 x Ac-*C*O, *C*OONa), 140.4, 139.5, 139.1 (4 C, 4 x C_q arom), 129.8-128.3 (20 C, arom), 101.3, 99.1, 98.8 (3 C, 3 x C-1), 84.4, 82.9, 81.0, 80.6, 80.2, 78.3, 77.8, 77.7, 75.7, 73.5, 72.3, 71.2 (12 C, skeleton carbons), 76.4, 74.6, 74.5, 74.2 (4 C, 4 x Bn-CH_2), 69.3, 69.0 (2 C, 2 x C-6), 61.0, 60.8, 59.6 (3 C, 3 x OCH_3), 55.6 (1 C, C-1-OCH_3), 21.1, 20.7 (2 C, 2 x Ac-CH_3) ppm; MALDI-TOF-MS: m/z calcd for $C_{54}H_{65}Na_2O_{19}$: $[M+Na]^+$ 1063.391, found 1063.853.

Methyl (2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)-(1→4)-[sodium (2,3-di-*O*-acetyl-β-D-glucopyranosyl)-uronate]-(1→4)-α-D-glucopyranoside (20): A mixture of 19 (235 mg, 0.226 mmol) in 96% EtOH/AcOH (19:1, 15 mL), and Pd(C) (10%, 180 mg) was stirred in an autoclave under H_2 atmosphere (at 10 bar) for 24 h. The catalyst was filtered off through a pad of Celite®, washed with MeOH, and the filtrate was concentrated under reduced pressure. The crude product was purified by Sephadex LH-20 gel chromatography (MeOH) to give **20** (150 mg, 98%) as a white powder. $[\alpha]_D$ = +106.7 (c = 0.03, H_2O); R_f 0.38 (1:1 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ = 5.31 (t, J = 9.9 Hz, 1H, H-3-E), 5.18 (d, J = 3.3 Hz, 1H), 4.90-4.83 (m, 2H), 4.75 (d, J = 3.4 Hz, 1H), 4.09 (t, J = 9.2 Hz, 1H), 3.94 (d, J = 9.4 Hz, 1H), 3.82-3.67 (m, 6H), 3.63-3.47 (m, 4H), 3.59, 3.52, 3.45, 3.38 (4 x s, 12H, 4 x OCH_3), 3.26-3.21 (m, 2H), 2.12, 2.10 (2 x s, 6H, 2 x Ac-CH_3) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 177.6, 173.5, 173.3 (3 C, 2 x Ac-*C*O, *C*OONa), 100.7, 100.0, 98.2 (3 C, 3 x C-1), 83.4, 81.3, 80.0, 79.4, 76.2, 75.7, 73.4, 72.5, 72.0, 71.9, 71.2 (12 C, skeleton carbons), 60.8, 60.6 (2 C, 2 x C-6), 61.0, 60.5, 60.2 (3 C, 3 x OCH_3), 55.9 (1 C, C-1-OCH_3), 21.3, 20.9 (2 C, 2 x Ac-CH_3) ppm; MALDI-TOF-MS: m/z calcd for $C_{26}H_{41}Na_2O_{19}$: $[M+Na]^+$ 703.203, found 703.520.

Methyl (2,3,4-tri-*O*-methyl-6-deoxy-6-*C*-ethylsulfonatomethyl-α-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-deoxy-6-*C*-ethylsulfonatomethyl-α-D-glucopyranoside (21): To a vigorously stirred solution of **16** (1.00 g, 0.850 mmol) in CH_2Cl_2 (12.5 mL) and H_2O (1.25 mL) DDQ (289 mg, 1.28 mmol) was added. After 30 min the mixture was diluted with CH_2Cl_2 (250 mL) and extracted with saturated aqueous solution of $NaHCO_3$ (2×50 mL), and H_2O (2×50 mL), dried and concentrated. The crude product was purified by silica gel chromatography (6:4 *n*-hexane/acetone) to give compound **21** (725 mg, 82%) as a colourless syrup. $[\alpha]_D$ = +48.7 (c = 0.15, $CHCl_3$); R_f 0.28 (6:4 *n*-hexane/acetone); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43-7.22 (m, 10H, arom), 5.24 (t, J = 9.3 Hz, 1H, H-3-E), 5.03 (d, J = 3.7 Hz, 1H, H-1-D), 4.98 (d, J = 11.6 Hz, 1H, BnCH_2), 4.90-4.80 (m, 2H, H-2-E, BnCH_2), 4.74 (d, J = 12.1 Hz, 1H, BnCH_2), 4.68 (d, J = 8.0 Hz, 1H, H-1-E), 4.62 (d, J = 12.1 Hz, 1H, BnCH_2), 4.51 (d, J = 3.6 Hz, 1H, H-1-F), 4.29 (q, J = 7.1 Hz, 4H, $SO_3CH_2CH_3$), 3.92-3.82 (m, 2H, H-3-F, H-4-E), 3.73 (dt, J = 10.3 Hz, J = 2.6 Hz, 1H, H-5-F), 3.62-3.15 (m, 10H, H-5-E, H-6$_a$-E, H-6$_b$-E, H-2-F, H-4-F, H-7$_a$-F, H-3-D, H-5-D, H-7$_a$-D, H-7$_b$-D), 3.56, 3.53, 3.43, 3.35 (4 s, 12H, 4 x CH_3), 3.14-3.05 (m, 1H, H-7$_b$-F), 3.02 (dd, J = 9.8 Hz, J = 3.6 Hz, 1H, H-2-D), 2.74 (t, J = 9.2 Hz, 1H, H-4-D), 2.44-2.34 (m, 1H, H-6$_a$-F), 2.34-2.24 (m, 1H, H-6$_a$-D), 2.05, 2.03 (2 s, 6H, 2 x AcCH_3), 1.93-1.74 (m, 3H, H-6$_b$-F, H-6$_b$-D), 1.42 (t, J = 7.1 Hz, 6H, $SO_3CH_2CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.0, 169.7 (2 x *C*O), 138.9, 137.8 (2 C, C_q arom), 128.5, 128.5, 128.1, 128.0, 127.6, 126.4 (10 C, arom), 100.2 (1 C, C-1-D), 97.9 (1 C, C-1-F), 96.8 (1 C, C-1-E), 83.8 (1 C, C-4-D), 82.7 (1 C, C-3-D), 81.8 (1 C, C-4-F), 81.6 (1 C, C-2-D), 79.5 (1 C, C-2-F), 78.8 (1 C, C-3-F), 75.3 (1 C, C-4-E), 74.9 (1 C, C-5-E), 74.6, 73.4 (1 C, 2 C, BnCH_2), 72.6 (1 C, C-2-E), 72.1 (1 C, C-4-E), 69.5 (1 C, C-5-D), 67.7 (1 C, C-5-F), 66.2, 66.2 (2 C, 2 x $SO_3CH_2CH_3$), 60.3 (1 C, C-6-E), 60.8, 60.6, 59.5, 55.5 (4 C, 4 x OCH_3), 46.7, 46.7 (2 C, C-7-D, C-7-F), 26.3 (1 C, C-6-D), 25.7 (1 C, C-6-F), 20.9, 20.6 (2 C, 2 x AcCH_3), 15.1,

15.1 (2 C, 2 x $SO_3CH_2CH_3$) ppm; MALDI-TOF-MS: *m/z* calcd for $C_{46}H_{68}NaO_{22}S_2$: $[M+Na]^+$ 1059.35; found: 1060.06.

Methyl (2,3,4-tri-*O*-methyl-6-deoxy-6-*C*-ethylsulfonatomethyl-α-D-glucopyranosyl)-(1→4)-[sodium (2,3-*O*-acetyl-β-D-glucopyranosyl)-uronate]-(1→4)-2,3-di-*O*-benzyl-6-deoxy-6-*C*-ethylsulfonatomethyl-α-D-glucopyranoside (22): To a vigorously stirred solution of compound **21** (700 mg, 0.680 mmol) in CH_2Cl_2 (15 mL) and H_2O (7.5 mL) TEMPO (21 mg, 0.2 equiv., 0.14 mmol) and BAIB (870 mg, 4.0 equiv., 2.70 mmol) were added and stirred for 24 h at room temperature. The reaction mixture was quenched by the addition of 10% aqueous solution of $Na_2S_2O_3$ (10.0 mL). The mixture was then extracted twice with CH_2Cl_2 (20 mL), and the combined organic layers were dried and concentrated. The crude product was purified by column chromatography to give **22** (625 mg; 79%) as a white foam. $[\alpha]_D = +47.3$ ($c = 0.11$, $CHCl_3$); R_f 0.40 (98:2 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.40$-7.21 (m, 10H, arom), 5.20 (m, 1H, H-3-E), 5.01 (d, $J = 3.6$ Hz, 1H, H-1-D), 4.92-4.84 (m, 4H, H-2-E, H-1-E, 2 x BnCH_2), 4.68, (d, $J = 12.0$ Hz, 1H, BnCH_2), 4.53 (d, $J = 12.0$ Hz, 1H, BnCH_2), 4.47 (d, $J = 3.6$ Hz, 1H, H-1-F), 4.31-4.29 (m, 4H, $SO_3CH_2CH_3$), 4.03 (t, $J = 8.6$ Hz, 1H, H-5-E), 3.96-3.83 (m, 2H, H-3-F, H-4-E, 3.63 (dt, $J = 9.9$, 2.5 Hz, 1H, H-5-F), 3.59-3.58 (m, 7H, H–3-D, H -5-D, H-2-F, H-4-F, H-7_a-D, H-7_b-D, H-7_a-F), 3.56, 3.53, 3.42, 3.31 (4 s, 12H, 4 x CH_3), 3.08-3.00 (m, 2H, H-7_b-F, H-2-D), 2.73 (t, $J = 9.3$ Hz, 1H, H-4-D), 2.34-2.21 (m, 2H, H-6_a-D, H-6_a-F), 2.06, 2.02 (2 x s, 6H, 2 x AcCH_3), 1.90-1.77 (m, 2H, H-6_b-D, H-6_b-F), 1.41, 1.40 (2 x t, $J = 6.9$ Hz, 6H, 2 x $SO_3CH_2CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.0$, 169.6 (2 C, 2 x *C*O), 138.8, 137.9 (2 C, C_q arom), 128.5, 128.4, 128.2, 128.1, 127.6 (10 C, arom), 100.5 (1 C, C-1-D), 97.9 (1 C, C-1-F), 97.6, 83.8, 82.7, 81.8, 81.4, 79.9, 79.9, 79.5, 75.3, 74.0, 72.5, 69.5, 67.8 (12 C, skeleton carbons), 75.1, 73.5 (2 C, 2 x BnCH_2), 66.7, 66.3 (2 C, 2 x $SO_3CH_2CH_3$), 60.7, 60.7, 59.6, 55.6 (4 C, 4 x OCH_3), 46.9, 46.6 (2 C, C-7-D, C-7-F), 26.0, 25.9 (2 C, C-6-D, C-6-F), 20.9, 20.7 (2 C, 2 x AcCH_3), 15.2, 15.2 (2 C, 2 x $SO_3CH_2CH_3$) ppm, MALDI-TOF-MS: *m/z* calcd for $C_{46}H_{65}Na_2O_{23}S_2$: $[M+Na]^+$ 1095.3; found: 1095.0.

Methyl [sodium (2,3,4-tri-*O*-methyl-6-deoxy-6-*C*-sulfonatomethyl)-α-D-glucopyranosyl]-(1→4)-[sodium (β-D-glucopyranosyl)-uronate]-(1→4)-[sodium (2,3-di-*O*-benzyl-6-deoxy-6-*C*-sulfonatomethyl)-α-D-glucopyranoside] (23): To the solution of compound **22** (530 mg, 0.490 mmol) in MeOH (20 mL) NaOMe (5 mg, 0.075 mmol, 0.15 equiv.) was added and the mixture was stirred at room temperature for 24 h. The reaction mixture was neutralized with acetic acid and all volatiles were evaporated. The crude product was dissolved in acetone (20 ml) and NaI (222 mg, 1.48 mmol, 3.0 equiv.) was added to the solution and stirred at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure and the crude product was purified by column chromatography (7:6:1 CH_2Cl_2/MeOH/H_2O) and gel chromatography (Sephadex LH-20, MeOH) to give **23** (434 mg, 90% for two steps) as a colourless syrup. $[\alpha]_D = +71.0$ ($c = 0.10$, MeOH); R_f 0.41 (95:5 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.43$-7.36 (m, 2H, arom), 7.35-7.19 (m, 8H, arom), 5.52 (d, $J = 3.7$ Hz, 1H, H-1-D), 4.97 (d, $J = 11.2$ Hz, 1H, BnCH_2), 4.76 (d, $J = 11.2$ Hz, 1H, BnCH_2), 4.69-4.62 (m, 2H, H-1-E, H-1-F), 4.61-4.53 (m, 2H, BnCH_2), 3.91-3.69 (m, 5H), 3.68-3.44 (m, 5H), 3.57, 3.53, 3.53, 3.34 (4 s, 12H, 4 x OCH_3), 3.32-3.29 (m, 1H), 3.15 (dd, $J = 3.7$ Hz, 1H, H-2-D), 3.10-2.99 (m, 3H, 3 x H-7), 2.89-2.75 (m, 2H, H-7, H-4-D), 2.60-2.47 (m, 1H, H-6), 2.32-2.18 (m, 1H, H-6), 1.98-1.75 (m, 3H, 2 x H-6, O*H*) ppm; ^{13}C NMR (100 MHz, CD_3OD): $\delta = 140.2$, 139.5 (2 C, C_q arom), 129.4, 129.3, 129.2, 129.1, 128.8, 128.4 (10 C, arom), 103.6, 98.6, 98.6 (3 C, 3 x C-1), 85.2, 83.9, 83.7, 81.6, 81.4, 80.8, 79.2, 78.6, 78.2, 75.5, 70.8, 70.0 (12 C, skeleton carbons),76.0, 73.9 (2 C, 2 x BnCH_2), 60.8, 59.3, 55.5 (4 C, CH_3), 28.1, 28.1 (2 C, C-6-D, C-6-F) ppm; MALDI-TOF-MS: *m/z* calcd for $C_{38}H_{51}Na_4O_{21}S_2$: $[M+Na]^+$ 999.19; found: 999.44.

Methyl [sodium (2,3,4-tri-*O*-methyl-6-deoxy-6-*C*-sulfonatomethyl)-α-D-glucopyranosyl]-(1→4)-[methyl (2,3-*O*-methyl-β-D-glucopyranosyl)-uronate]-(1→4)-[sodium (2,3-di-*O*-benzyl-6-deoxy-6-*C*-sulfonatomethyl)-α-D-glucopyranoside] (24): To the solution of compound **23** (482 mg, 0.490 mmol) in dry DMF (15 mL) NaH (36 mg, 1.48 mmol, 60 m/m%, 3.0 equiv.) was added at 0 °C. After 30 min stirring at that temperature 315 µl MeI (2.22 mmol, 4.5 equiv.) was added to the mixture and stirred for 24 h at room temperature. The reaction mixture was quenched by the addition of MeOH (1.5 mL) and acetic acid (1-2 drops). The solution was concentrated and the crude product was purified by gel chromatography (Sephadex LH-20, MeOH) to give **24** (354 mg, 72%) as a white foam. $[\alpha]_D = +33.3$ ($c = 0.09$, MeOH); R_f 0.36 (7:3 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.27$-7.13 (m, 10H, arom), 5.34 (d, $J = 3.5$ Hz, 1H, H-1-D), 4.88 (d, $J = 11.1$ Hz, 1H, BnCH_2), 4.58-4.46 (m, 5H, H-1-E, H-1-F, BnCH_2), 3.81 (d, $J = 9.6$ Hz, 1H), 3.74 (t, $J = 9.1$ Hz, 1H), 3.68 (t, $J = 9.2$ Hz, 1H), 3.63-3.55 (m, 1H), 3.49, 3.49, 3.49, 3.47, 3.44, 3.41, 3.26 (7 s, 21H, 7 x OCH_3), 3.54-3.31 (m, 4H), 3.30-3.06 (m, 4H), 3.03-2.66 (m, 4H), 2.54-2.41 (m, 1H, 1 x H-6a), 2.20-2.08 (m, 1H, 1 x H-6_a), 1.89-1.85 (m, 2H, 2 x H-6b) ppm; ^{13}C NMR (100 MHz, CD_3OD): $\delta = 170.5$ (1 C, *C*O), 140.3, 139.4 (2 C, C_q arom), 129.2, 129.1, 128.9, 128.7, 128.2 (10 C, arom), 104.7, 98.6, 96.6 (3 C, 3 x C-1), 86.9, 85.3, 84.5, 84.0, 83.5, 82.8, 81.0, 80.9, 75.3, 74.9, 70.6, 70.2 (12 C, skeleton carbons), 76.2, 74.1 (2 C, 2 x BnCH_2), 61.1, 61.1, 60.9, 60.5, 59.4, 55.6, 53.1 (7 C, 7 x OCH_3), 48.9, 48.5 (2 C, C-7-D, C-7-F), 37.0, 28.2, 27.7 (2 C, C-6-D, C-6-F) ppm; MALDI-TOF-MS: *m/z* calcd for $C_{41}H_{58}Na_3O_{21}S_2$: $[M+Na]^+$ 1019.26; found: 1019.47.

Methyl [sodium (2,3,4-tri-*O*-methyl-6-deoxy-6-*C*-sulfonatomethyl)-α-D-glucopyranosyl]-(1→4)-[sodium (2,3-*O*-methyl-β-D-glucopyranosyl)-uronate]-(1→4)-[sodium (2,3-di-*O*-benzyl-6-deoxy-6-*C*-sulfonatomethyl)-α-D-glucopyranoside] (25): To the solution of compound **24** (480 mg, 0.480 mmol) in the mixture of THF (3.0 mL) and MeOH (3.0 mL) 0.5 M NaOH solution (2.9 mL) was added and stirred at room temperature for 24 h. The reaction was neutralized by the addition of 60 v/v% acetic acid solution and the mixture was concentrated. The crude product was purified by gel chromatography (Sephadex, LH-20, MeOH) and converted to sodium salt by ion exchange resin (Dowex, Na^+ form, MeOH) to give **25** (398 mg, 82%) as colorless syrup. $[\alpha]_D = +42.8$ ($c = 0.10$, $CHCl_3$); R_f 0.94 (6:4 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.31$ (d, $J = 7.2$ Hz, 2H, arom), 7.26-7.08 (m, 8H, arom), 5.36 (d, $J = 3.7$ Hz, 1H, H-1-D), 4.88 (d, $J = 11.4$ Hz, 1H, BnCH_2), 4.66-4.52 (m, 3H, H-1-E, H-1-F, BnCH_2), 4.47 (s, 2H, BnCH_2), 3.81-3.69 (m, 2H), 3.68-3.65 (m, 3H), 3.48, 3.47, 3.45, 3.42, 3.40, 3.08 (6 x s, 18H, 6 x OCH_3), 3.52-3.16 (m, 4H), 3.04-2.87 (m, 5H), 2.81-2.63 (m, 2H), 2.54-2.40 (m, 1H, H-6a), 2.21-2.08 (m, 1H, H-6a), 1.88-1.68 (m, 2H, 2 x H-6b) ppm; ^{13}C NMR (100 MHz, CD_3OD): $\delta = 139.2$, 138.2 (2 C, C_q arom), 127.9, 127.5, 126.9 (10 C, arom), 97.3, 97.2, 95.5 (3 C, C-1-D, C-1-E, C-1-F), 86.2, 84.4, 83.9, 82.6, 81.9, 81.2, 79.8, 79.4, 77.0, 74.2, 69.2, 68.8 (12 C, skeleton carbons), 72.5, 72.5 (2 C, 2 x BnCH_2), 59.6, 59.5, 59.0, 58.3, 54.6, 54.3 (6 C, 6 x OCH_3), 29.4, 27.0 (2 C, C-6-D, C-6-F) ppm; MALDI-TOF-MS: *m/z* calcd for $C_{40}H_{55}Na_4O_{21}S_2$: $[M+Na]^+$ 1027.23; found: 1027.90.

Methyl [sodium (2,3,4-tri-*O*-methyl-6-deoxy-6-*C*-sulfonatomethyl)-α-D-glucopyranosyl]-(1→4)-[sodium (2,3-*O*-methyl-β-D-glucopyranosyl)-uronate]-(1→4)-[sodium (6-deoxy-6-*C*-sulfonatomethyl)-α-D-glucopyranoside] (26): To the solution of compound **25** (398 mg, 0.400 mmol) in EtOH (15 mL, 96%) 10%-os Pd/C (300 mg) and acetic acid (350 µL) were added. The mixture was stirred at room temperature for 24 h under 10 bar H_2 atmosphere. The mixture was diluted with MeOH and the catalyst was filtrated through a pad of Celite® and then the solution was concentrated. The crude product was purified by gel chromatography (Sephadex, G-25, H_2O) to give **26** (121 mg, 92%) as white powder. $[\alpha]_D = +77.7$ ($c = 0.13$, MeOH); R_f 0.53 (7:6:1 CH_2Cl_2/MeOH/H_2O); ^{1}H NMR

(400 MHz, D_2O): $\delta = 5.35$ (d, $J = 3.7$ Hz, 1H, H-1″), 4.46 (d, $J = 7.7$ Hz, 1H, H-1′), 3.77-3.51 (m, 5H), 3.51-3.31 (m, 19H), 3.31-3.07 (m, 6H), 3.07-2.77 (m, 5H), 2.32-2.19 (m, 1H, H-6a), 2.09-1.97 (m, 1H, H-6a), 1.82-1.63 (m, 2H, 2 x H-6b) ppm, ^{13}C NMR (100 MHz, D_2O): $\delta = 174.4$ (1 C, *C*O), 102.3, 98.7, 95.1 (3 C, 3 x C-1), 85.8, 83.4, 82.9, 82.3, 81.2, 80.6, 75.8, 72.9, 71.5, 70.9, 68.9, 68.4 (12 C, skeleton carbons), 60.5, 60.1, 59.5, 59.4, 58.9, 55.1 (6 C, 6 x O*C*H$_3$), 47.3, 47.1 (2 C, 2 x C-7), 26.2, 25.8 (2 C, 2 x C-6) ppm; MALDI-TOF-MS: *m/z* calcd for $C_{26}H_{43}Na_4O_{21}S_2$: $[M+H]^+$ 847.13; found: 847.64.

Methyl [ethyl (2,3-di-*O*-benzyl-6-deoxy-6-*C*-sulfonatomethyl-α-D-glucopyranosyl)]-(1→4)-[methyl (3-*O*-methyl-α-L-idopyranosyl)-uronate]-(1→4)-[sodium (2,3-di-*O*-benzyl-6-deoxy-6-*C*-sulfonatomethyl-α-D-glucopyranoside)] (27): To a solution of compound **17** (103 mg, 0.088 mmol) in MeOH (2.5 mL) was added $NaOCH_3$ (25 mg, 0.462 mmol). The reaction mixture was stirred for 4 h and monitored by TLC. After the complete disappearance of the starting material, the mixture was neutralized with AcOH and concentrated. The crude product was purified by column chromatography on Sephadex LH-20 (MeOH) to give compound **27** (99 mg, 99%) as a colourless syrup. $[\alpha]_D = +167.5$ ($c = 0.08$, $CHCl_3$); R_f 0.38 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (CD_3OD, 360 MHz): $\delta = 7.36$-7.24 (m, 20H, arom), 5.16 (s, 1H), 5.10 (d, $J = 3.4$ Hz, 1H), 5.04 (d, $J = 2.1$ Hz, 1H), 4.90-4.61 (m, 8H), 4.29 (q, $J = 7.1$ Hz, 2H, $SO_3CH_2CH_3$), 3.95 (s, 1H), 3.83 (s, 1H), 3.76-3.73 (m, 3H), 3.60-3.21 (m, 9H), 3.49, 3.41, 3.35 (3 x s, 9H, 3 x O*C*H$_3$), 3.19-2.83 (m, 4H, 2 x H-7a,b), 2.34-1.92 (m, 4H, 2 x H-6a,b), 1.37 (t, $J = 7.1$ Hz, 3H, $SO_3CH_2CH_3$) ppm; ^{13}C NMR (CD_3OD, 90 MHz): $\delta = 171.7$ (1 C, CO), 140.2, 140.0, 139.4, 138.9 (4 C, 4 x C_q arom), 129.6-128.6 (20 C, arom), 102.4, 98.6, 96.1 (3 C, 3 x C-1), 82.3, 81.9, 80.6, 80.0, 79.2, 75.6, 74.5, 72.4, 71.4, 70.5, 69.1, 67.0 (12 C, skeleton carbons), 76.3, 76.0, 74.8, 73.8 (4 C, 4 x Bn*C*H$_2$), 67.9 (1 C, $SO_3CH_2CH_3$), 58.5 (1 C, C-3′-O*C*H$_3$), 55.6 (C-1-O*C*H$_3$), 52.9 (1 C, COO*C*H$_3$), 48.6, 47.1 (2 C, 2 x C-7), 28.4, 26.8 (2 C, 2 x C-6), 15.5 (1 C, $SO_3CH_2CH_3$); ESI-TOF-MS: *m/z* calcd for $C_{53}H_{68}KO_{21}S_2$: $[M+H]^+$ 1143.333; found: 1143.394.

Methyl [sodium (2,3-di-*O*-benzyl-6-deoxy-4-*O*-methyl-6-*C*-sulfonatomethyl-α-D-glucopyranosyl)]-(1→4)-[methyl (2,3-di-*O*-methyl-α-L-idopyranosyl)-uronate]-(1→4)-[sodium (2,3-di-*O*-benzyl-6-deoxy-6-*C*-sulfonatomethyl-α-D-glucopyranoside)] (28): To a solution of **27** (95 mg, 0.084 mmol) in dry DMF (3 mL) was slowly added NaH (8 mg, 0.201 mmol) at 0 °C. After stirring for 30 min at 0 °C, MeI (11 μL, 0.201 mmol) was added. When complete conversion of the starting material into a main spot had been observed by TLC analysis (2 h at 0 °C), CH_3OH (1.0 mL) was added. The reaction mixture was stirred for 5 min and the solvents were evaporated. The crude product was purified by column chromatography on Sephadex LH-20 (MeOH) to give compound **28** (50 mg, 52%) as a colourless syrup. R_f 0.44 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (CD_3OD, 360 MHz): $\delta = 7.39$-7.25 (m, 20H, arom), 5.19 (d, $J = 2.1$ Hz, 1H), 5.16 (d, $J = 3.3$ Hz, 1H), 4.86 (d, $J = 2.7$ Hz, 1H), 4.86-4.61 (m, 8H), 3.85-3.84 (m, 1H), 3.76-3.72 (m, 4H), 3.61-3.30 (m, 4H), 3.51, 3.47, 3.46, 3.39, 3.37 (5 x s, 15H, 5 x O*C*H$_3$), 3.08-2.98 (m, 3H) 2.91-2.77 (m, 4H, 2 x H-7a,b), 2.36-1.89 (m, 4H, 2 x H-6a,b) ppm; ^{13}C NMR (90 MHz, CD_3OD): $\delta = 171.6$ (1 C, CO), 140.1, 140.0, 139.5, 139.4 (4 C, 4 x C_q arom), 129.5-128.6 (20 C, arom), 99.8, 98.7, 95.5 (3 C, 3 x C-1), 84.9, 82.2, 81.9, 80.6, 80.5, 79.4, 78.2, 75.7, 71.9, 71.1, 70.7, 69.8 (12 C, skeleton carbons), 76.2, 76.1, 73.9 (4 C, 4 x Bn*C*H$_2$), 61.4, 59.9, 58.9, 55.6 (4 x O*C*H$_3$), 52.7 (1 C, COO*C*H$_3$), 28.5, 28.4 (2 C, 2 x C-6).

Methyl [sodium (2,3-di-*O*-benzyl-6-deoxy-4-*O*-methyl-6-*C*-sulfonatomethyl-α-D-glucopyranosyl)]-(1→4)-[sodium (2,3-di-*O*-methyl-α-L-idopyranosyl)-uronate]-(1→4)-[sodium (2,3-di-*O*-benzyl-6-deoxy-6-*C*-sulfonatomethyl-α-D-glucopyranoside)] (29): A solution of the trisaccharide **28** (45 mg, 0.040 mmol) in MeOH (2 mL) was treated with 0.2 M aqueous solution of NaOH (1.0 mL). After 24 h stirring at room temperature the TLC showed complete conversion of the carboxylic esters into sodium salts. The mixture was neutralized with acetic acid, and concentrated. The crude product was purified by Sephadex gel LH-20 in MeOH to give **29** (34 mg, 76%). R_f 0.12 (8:2 CH_2Cl_2/MeOH); ^{1}H NMR (360 MHz, D_2O): $\delta = 7.40$-7.31 (m, 20H, arom), 5.13 (d, $J = 2.6$ Hz, 1H), 5.06 (d, $J = 3.4$ Hz, 1H), 4.86-4.55 (m, 10H), 4.05 (s, 1H), 3.90-3.74 (m, 6H), 3.61-3.37 (m, 4H), 3.57, 3.45, 3.38, 3.37 (4 x s, 12H, 4 x O*C*H$_3$), 3.17-2.99 (m, 4H, 2 x H-7a,b), 2.40-1.92 (m, 4H, 2 x H-6a,b) ppm; ^{13}C NMR (D_2O, 90 MHz): $\delta = 181.5$ (1 C, CO), 138.7, 138.5, 138.3 (4 C, 4 x C_q arom), 129.8-129.2 (20 C, arom), 98.0, 94.6 (3 C, 3 x C-1), 83.6, 81.1, 80.4, 80.2, 79.9, 78.8, 73.3, 71.6, 70.1, 70.0 (12 C, skeleton carbons), 76.6, 76.1, 74.2, 73.8 (4 C, 4 x Bn*C*H$_2$), 61.0, 60.1, 58.8, 56.0 (4 x O*C*H$_3$), 48.3, 48.1 (2 C, 2 x C-7), 27.2 (2 C, 2 x C-6) ppm.

Biological evaluation: Biological activity of compounds **3-8** was tested by investigating their effect on the cellular viability of A2780 human ovarian carcinoma, WM35 human melanoma and HaCaT spontaneously immortalized human keratinocyte cell lines. WM35 and A2780 cells were cultured in RPMI1640 Medium (ThermoFisher) supplemented with 10% fetal bovine serum (FBS. ThermoFisher) and antibiotics penicillin and streptomycin (Pen-Strep, ThermoFisher). HaCaT cell were cultured in DMEM (ThermoFisher) supplemented with FBS and Pen-Strep. During culturing, cells were incubated in a humified thermostat at constant 37 °C and in the presence of 5% CO_2. Media were refreshed every 2-3 days and cells were subcultured when reached ca. 80% confluence. Cellular viability was determined by MTT assay as described earlier.[38,39,40] Briefly, the number of viable cells was indirectly determined by measuring the conversion of the tetrazolium salt MTT (3-{4,5-dimethilthiasol-2-il}-2,5-diphenyltetrasolium bromide, Sigma-Aldrich) to formazan by mitochondrial dehydrogenases. Cells were plated in 96-well microplates (10.000 cells per well density) and were cultured for 3 days and treated by the compounds daily. Negative control group was treated with equal amount of vehicle solvent (DMSO, Sigma-Aldrich) and positive control group was treated with 1 μg/mL doxorubicin. Cells were then incubated with 1 mg/ml MTT for 3 h, precipitated formazan crystals were dissolved in acidic isopropanol (10% 1 M HCl dissolved in isopropanol and supplemented with 10% Triton X 100, all from VWR) and concentration of formazan was assessed colorimetrical way measuring absorbance at 565 nm. Viability was calculated based on the measured absorbance and given as percentage where 100% viability is determined as the mean absorbance of the negative control (i.e., vehicle treated) samples and 0% viability is determined as the mean absorbance of the positive control samples measured in parallel on the same microplate. Dose-response relationship of the above compounds was assessed in a concentration range from 0.03-50 μM by fitting logistic dose-response curves and calculating the IC_{50} values using the equation $y = A2 + (A1 - A2)/(1 + (x/x0)^p)$ where the parameters are: A1: initial value (y_{min}), A2: final value (y_{max}), x0: center (EC/IC_{50}) and p is the calculated power. Fittings were carried out and parameters were calculated using Origin 8.6 software (OriginLab Corporation, Northampton, MA, USA).

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Conflict of Interest

The authors declare no conflict of interest.

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[1] a) *Heparin: A Century of Progress* (Eds.: R. Lever, B. Mulloy, C. P. Page), Springer, Heidelberg, **2012**; b) B. Casu, U. Lindahl, *Adv. Carbohydr. Chem. Biochem.* **2001**, *57*, 159-206.
[2] a) L. Jin, J. P. Abrahams, R. Skinner, M. Petitou, R. N. Pike, R. W. Carrell, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 14683-14688.; b) U. R. Desai, M. Petitou, I. Bjork, S. T. Olson, *J. Biol. Chem.* **1998**, *273*, 7478-7487.
[3] a) I. Capila, R. J. Linhardt, *Angew. Chem. Int. Ed.* **2002**, *41*, 390-412; *Angew. Chem.* **2002**, *114*, 426-450; b) L. Kjelle, U. Lindahl, *Curr. Opin. Struct. Biol.* **2018**, *50*, 101-108; c) R. J. Weiss, J. D. Esko, Y. Tor, *Org. Biomol. Chem.* **2017**, *15*, 5656-5668.
[4] B. Mulloy, J. Hogwood, E. Gray, R. Lever, C. P. Page, *Pharmacol. Rev.* **2016**, *68*, 76-141.
[5] G. Cassinelli, A. Naggi, *Int. J. Cardiol.* **2016**, *212S1*, S14-S21.
[6] M. J. Glantz, P. C. Burger, A. H. Friedman, R. A. Radtke, E. W. Massey, S. C. Schold, *Neurology* **1994**, *44*, 2020-2020.
[7] a) B. Casu, M. Guerrini, S. Guglieri, A. Naggi, M. Perez, G. Torri, G. Cassinelli, D. Ribatti, P. Carminati, G. Giannini, S. Penco, C. Pisano, M. Belleri, M. Rusnati, M. Presta, *J. Med. Chem.* **2004**, *47*, 838-848; b) C. Pisano, C. Aulicino, L. Vesci, B. Casu, A. Naggi, G. Torri, D. Ribatti, M. Belleri, M. Rusnati, M. Presta, *Glycobiology* **2005**, *15*, 1 C-6 C; c) P. Chiodelli, A. Bugatti, C. Urbinati, M. Rusnati, *Molecules* **2015**, *20*, 6342-6388.
[8] a) R. J. Ludwig, *Curr. Drug Discovery Technol.* **2009**, *6*, 281-289; b) H. Zhou, S. Roy, E. Cochran, R. Zouaoui, C. L. Chu, J. Duffner, G. Zhao, S. Smith, Z. Galcheva-Gargova, J. Karlgren, N. Dussault, R. Y. Q. Kwan, E. Moy, M. Barnes, A. Long, C. Honan, Y. W. Qi, Z. Shriver, T. Ganguly, B. Schultes, G. Venkataraman, T. K. Kishimoto, *PLoS One* **2011**, *6*, e21106.
[9] A. M. Vogt, F. Pettersson, K. Moll, C. Jonsson, J. Normark, U. Ribacke, T. G. Egwang, H.-P. Ekre, D. Spillmann, Q. Chen, M. Wahlgren, *PLoS Pathog.* **2006**, 2, e100.
[10] a) P. S. Kwon, H. Oh, S.-J. Kwon, W. Jin, F. Zhang, K. Fraser, J. J. Hong, R. J. Linhardt, J. S. Dordick, *Cell Discovery* **2020**, *6*, 50; b) S. Y. Kim, W. Jin, A. Sood, D. W. Montgomery, O. C. Grant, M. M. Fuster, L. Fu, J. S. Dordick, R. J. Woods, F. Zhang, R. J. Linhardt, *Antiviral Res.* **2020**, *181*, 104873.
[11] C. Lanzi, G. Cassinelli, *Molecules* **2018**, *23*, 2915.
[12] S. Morla, *Int. J. Mol. Sci.* **2019**, *20*, 1963.
[13] R. Sasisekharan, Z. Shriver, G. Venkataraman, U. Narayanasami, *Nat. Rev. Cancer* **2002**, *2*, 521–528.
[14] V. Ferro, L. Liu, K. D. Johnstone, N. Wimmer, T. Karoli, P. Handley, J. Rowley, K. Dredge, C. P. Li, E. Hammond, K. Davis, L. Sarimaa, J. Harenberg, I. Bytheway, *J. Med. Chem.* **2012**, *55*, 3804-3813.
[15] C.-Li Xiao, Z.-H. Tao, L. Guo, W.-W. Li, J.-L. Wan, H.-C. Sun, L. Wang, Z.-Y. Tang, J. Fan, W.-Z. Wu, *BMC Cancer* **2011**, *11*, 1-11.
[16] B. Miao, Y. Chen, J. Li, X. Xin, L. Gan, X. Fu, M. Geng, *Cancer Biol. Ther.* **2010**, *10*, 89-98.
[17] M. Herczeg, E. Mező, L. Lázár, A. Fekete, K. E. Kövér, S. Antus, A. Borbás, *Tetrahedron* **2013**, *69*, 3149-3158.
[18] M. Herczeg, E. Mező, D. Eszenyi, S. Antus, A. Borbás, *Tetrahedron* **2014**, *70*, 2919-2927.
[19] F. Demeter, F. Veres, M. Herczeg, A. Borbás, *Eur. J. Org. Chem.* **2018**, *48*, 6901-6912.
[20] M. Herczeg, L. Lázár, Zs. Bereczky, K. E. Kövér, I. Timári, J. Kappelmayer, A. Lipták, S. Antus, A. Borbás, *Chem. Eur. J.* **2012**, *18*, 10643-10652.
[21] G. Balogh, T. Gyöngyösi, I. Timári, M. Herczeg, A. Borbás, K. Fehér, K. E. Kövér, *J. Chem. Inf. Model.* **2019**, *59*, 4855-4867.
[22] G. Balogh, T. Gyöngyösi, I. Timári, K. Sadiq, M. Herczeg, A. Borbás, K. Fehér, K. Kövér, *J. Chem. Inf. Model.* **2021**, (under submission). .
[23] C. A. A. van Boeckel, T. Beetz, J. N. Vos, A. J. M. Dejong, S. F. van Aelst, R. H. van den Bosch, J. M. R. Mertens, F. A. van der Vlugt, *J. Carbohydr. Chem.* **1985**, *4*, 293-321.
[24] C. A. A. van Boeckel, M. Petitou, *Angew. Chem. Int. Ed.* **1993**, *32*, 1671-1690; *Angew. Chem.* **1993**, *105*, 1741-1761.
[25] a) J. Choay, J.-C. Lormeau, M. Petitou, P. Sinay, J. Fareed, *Ann N. Y. Acad. Sci.* **1981**, *370*, 644-649; b) L. Thunberg, G. Backstrom, U. Lindahl, *Carbohydr. Res.* **1981**, *100*, 393-410; c) M. Petitou, P. Duchaussoy, I. Lederman, J. Choay, P. Sinay, J. C. Jacquinet, G. Torri, *Carbohydr. Res.* **1986**, *147*, 221-236.
[26] S. Peterson, J. Liu, *J. Biol. Chem.* **2012**, *287*, 34836-34843.
[27] M. Ni, S. Elli, A. Naggi, M. Guerrini, G. Torri, M. Petitou, *Molecules* **2016**, *21*, 1602.
[28] L. Lázár, M. Herczeg, A. Fekete, A. Borbás, A. Lipták, S. Antus, *Tetrahedron Lett.* **2010**, *51*, 6711-6714.
[29] L. Lázár, E. Mező, M. Herczeg, A. Lipták, S. Antus, A. Borbás, *Tetrahedron* **2012**, *68*, 7386-7399.
[30] M. Herczeg, L. Lázár, A. Borbás, A. Lipták, S. Antus, *Org. Lett.* **2009**, *11*, 2619-2622.
[31] a) E. Mező, M. Herczeg, D. Eszenyi, A. Borbás, *Carbohydr. Res.* **2014**, *388*, 19-29; b) M. Herczeg, A. Mező, D. Eszenyi, L. Lázár, M. Csávás, I. Bereczki, S. Antus, A. Borbás, *Eur. J. Org. Chem.* **2013**, 5570-5573.
[32] E. Mező, D. Eszenyi, E. Varga, M. Herczeg, A. Borbás, *Molecules* **2016**, *21*, 1497.
[33] M. Ek, P. J. Garegg, H. Hultberg, S. Oscarson, *J. Carbohydr. Chem.* **1983**, *2*, 331-336.
[34] G. Despras, C. Bernard, A. Perrot, L. Cattiaux, A. Prochiantz, H. Lortat-Jacob, J.-M. Mallet, *Chem. Eur. J.* **2013**, *19*, 531-540.
[35] O. Tacar, P. Sriamornsak, C. R. Dass, *J. Pharm. Pharmacol.* **2013**, *65*, 157-170.
[36] M. Petitou, C. A. A. van Boeckel, *Angew. Chem. Int. Ed.* **2004**, *43*, 3118-3133; *Angew. Chem.* **2004**, *116*, 3180-3196.
[37] N. S. Gandhi, R. L. Mancera, *Drug Discovery Today* **2010**, *15*, 1058-1069.
[38] M. Szántó, A Oláh, A. G. Szöllősi, K. F. Tóth, E. Páyer, N. Czakó, Á. Pór, I. Kovács, C. C. Zouboulis, L. Kemény, T. Bíró, B. I. Tóth, *J. Invest. Dermatol.* **2019**, *139*, 250-253.
[39] B. I. Tóth, N. Dobrosi, A. Dajnoki, G. Czifra, A. Oláh, A. G. Szöllősi, I. Juhász, K. Sugawara, R. Paus, T. Bíró, *J. Invest. Dermatol.* **2011**, *131*, 1095-1104.
[40] Á. Szappanos, A. Mándi, K. Gulácsi, E. Lisztes, B. I. Tóth, T. Bíró, S. Antus, T. Kurtán, *Org. Biomol. Chem.* **2020**, *18*, 2148-2162.

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