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Synthesis of sulfosucrose derivatives for evaluation as regulators of fibroblast growth factor activity

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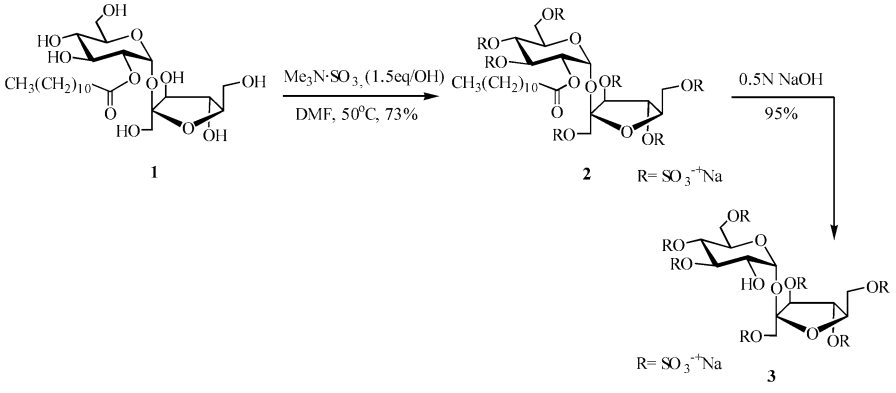
Abstract—Based on X-ray crystallographic studies on sucrose octasulfate in complex with fibroblast growth factor and its receptor, three analogs of sucrose octasulfate were regioselectively synthesized for biological evaluation as regulations of cell proliferation and differentiation.

Fibroblast growth factors (FGFs) play crucial roles in embryonic development, angiogenesis, wound healing, and malignant transformation.[1–3] FGFs exert their actions by binding and activating members of FGF receptor (FGFR) family (FGFR1-4).[4] FGFs have been implicated in the etiology of several human angiogenic pathologies such as diabetic retinopathy, rheumatoid arthritis, arteriosclerosis, and tumor neovascularization. Moreover, gene amplification and over-expression of FGFRs have been detected in human cancers.[5–8]

FGFs require specific heparan sulfate (an extracellular heparin-like polysaccharide) to bind, dimerize and activate FGFRs. Based on the 'two-end' model emerged from the FGF2-FGFR1-heparin crystal structure, heparin interacts concomitantly with the heparin binding sites of one FGF and one FGFR to form a stable 1:1:1 FGF-FGFR-heparin ternary complex. Two such stable ternary complexes then form a symmetric 2:2:2 FGF-FGFR-heparin dimer. The dimer is stabilized via interactions of FGF, FGFR and heparin from one ternary complex with the FGFR in the other ternary complex.[9]

Sucrose octasulfate (SOS) is used as the insoluble aluminum salt (Sucralfate) to treat ulcers.[5,10] The ulcer healing activity of SOS is believed to involve binding and stabilization of FGF in the GI tract. Indeed, the soluble sodium salt of SOS has been shown to bind into the heparin-binding site of FGF and stabilize FGF against various domains.[11,12] We have recently discov-



Scheme 1. Synthesis of 2-*O*-hydroxy-sucrose-heptasulfate (**3**).

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ered that SOS causes FGF-FGFR complexes to dimerize. The crystal structure of the FGF-FGFR-SOS dimer reveals that SOS dimerizes FGF-FGFR complex with a mode and stoichiometry reminiscent of the 'two-end' model.[13]

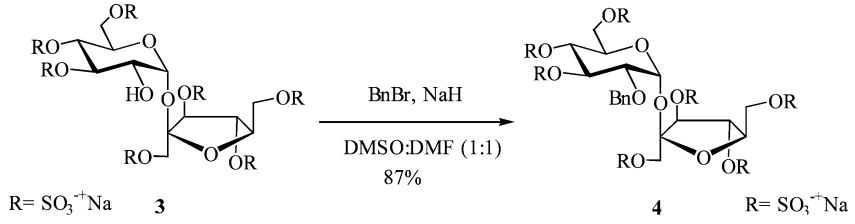
In this communication, we describe the regioselective synthesis of three SOS derivatives with alterations of functional groups predicted to be critical in promoting complex formation. The first lacks a 2-*O*-sulfo group and in the second the 2-*O*-sulfo group is replaced with a 2-*O*-benzyl group. The third SOS derivative lacks both the 4- and 6-*O*-sulfo groups.

2-*O*-Lauryl sucrose (**1**) was synthesized in 70% yield following the procedure of Plusquellec and coworkers[14] (Scheme 1), by reacting sucrose with 3-*O*-lauryl-thiazolidine-2-thione in the presence of NaH at room temperature. Sulfonation of 2-*O*-lauryl sucrose (**1**) using $NMe_3{\cdot}SO_3$ in DMF at 50°C afforded 2-*O*-lauryl-sucrose-heptasulfate (**2**) in 73% yield after purification by chromatography on Sephadex LH-20. The structure of 2-*O*-lauryl-sucrose-heptasulfate (**2**) was confirmed by 1D-NMR (^1H and ^{13}C) and 2D-COSY. Deacylation of **2** was achieved under basic conditions using 0.5 N NaOH and 2-*O*-hydroxy-sucrose-heptasulfate (**3**) was obtained in 95% yield. From NMR spectroscopy[15] (**3**) showed the chemical shift of H-2 proton significantly upfield compared to the H-2 proton in **2** (H-2 $\Delta\delta = 5.01$ ppm in **2** and $\Delta\delta = 3.70$ in **3**), consistent with the structure of **3**.

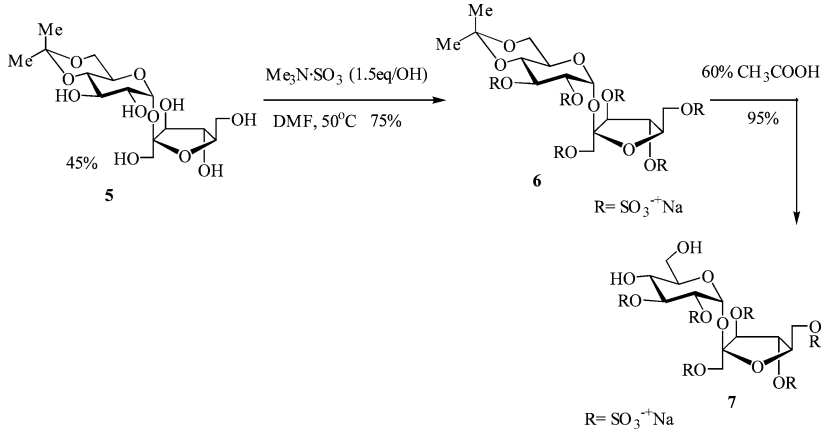
Next, we installed a bulky substituent at the 2-*O*-hydroxyl group in **3** to sterically interfere with FGF-FGFR dimerization. Benzylation of 2-*O*-hydroxy-sucrose-heptasulfate (**3**) was first attempted using standard benzylation conditions using NaH and BnBr in DMF, but **3** was not completely soluble in DMF. When the same reaction was performed in 1:1 DMF:DMSO 2-*O*-benzyl-sucrose-heptasulfate[14] (**4**) was obtained in 87% yield after purification (Scheme 2). To our knowledge benzylation of an *O*-sulfo sugar has not been previously reported.

Next, the synthesis of the 4,6-dihydroxy derivative was undertaken from the previously reported 4,6-mono-*O*-isopropylidene sucrose (**5**).[16] Sulfonation of **5** using $NMe_3{\cdot}SO_3$ in DMF at 50°C afforded 4,6-mono-*O*-isopropylidene-sucrose-hexasulfate (**6**) in 75% yield after purification on Sephadex LH-20. The structure of 4,6-mono-*O*-isopropylidenyl-sucrose-hexasulfate (**6**) again was confirmed by 1D-NMR[15] (^1H and ^{13}C) and 2D-COSY. Deacetalation of **6** using 60% acetic acid afforded 4,6-*O*-dihydroxy-sucrose-hexasulfate (**7**) in 95% yield (Scheme 3).

In conclusion, synthesis of SOS derivatives, 2-*O*-hydroxy-sucrose-heptasulfate (**3**), 2-*O*-benzyl-sucrose-heptasulfate (**4**), and 4,6-*O*-dihydroxy-sucrose-hexasulfate (**7**), is reported. Such compounds are currently viewed as potential therapeutic agents for the modulation of biological activities invoked by FGFR activation.[17]



Scheme 2. Synthesis of 2-*O*-benzyl-sucrose-heptasulfate (**4**).



Scheme 3. Synthesis of 4,6-*O*-dihydroxy-sucrose-hexasulfate (**7**).

Acknowledgements

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15. Compound **3**: ^{1}H NMR (500 MHz, D_2O): δ 3.88 (dd, 1 H, H-2), 4.23 (d, 1 H, H-1′a), 4.30–4.50 (m, 7 H, H-6a, H-1′b, H-5, H-6b, H-5′, H-4, H-6′a), 4.57 (dd, 1 H, H-6′a), 4.67 (t, 1 H, H-3), 4.95 (t, 1 H, H-4′), 5.15 (d, 1 H, H-3′), 5.66 (dd, 1 H, H-1); ^{13}C NMR (D_2O, 500 MHz): δ 68.9 (C-6), 69.9 (C-1′), 70.9 (C-6′), 71.9 (C-5), 72.6 (C-2), 76.2 (C-4), 80.7 (C-5′), 80.9 (C-4′), 81.7 (C-3 and C-3′), 94.4 (C-1), 104.9 (C-2′). Compound **4**: ^{1}H NMR (500 MHz, D_2O): δ 3.70 (dd, 1 H, H-2), 4.08 (d, 1 H, H-1′a), 4.25–4.41 (m, 6 H, H-6a, H-1′b, H-5, H-6b, H-5′, H-6′a), 4.42 (dd, 1 H, H-4), 4.55 (dd, 1 H, H-6′a), 4.67 (t, 1 H, H-3), 4.74 (d, 2 H, PhCH_2), 4.87 (t, 1 H, H-4′), 5.09 (d, 1 H, H-3′), 5.69 (d, 1 H, H-1), 7.34–7.50 (m, 5 H, Ph); ^{13}C NMR (D_2O, 500 MHz): δ 69.2 (C-6), 70.5 (C-1′), 71.1 (C-6′), 72.1 (C-5), 75.8 (*C*H_2) 76.9 (C-4), 79.0 (C-2), 80.6 (C-4′), 80.7 (C-5′), 81.1 (C-3), 81.6 (C-3′), 92.7 (C-1), 105.1 (C-2′), 131.1–140.4 (Ph). Compound **7**: ^{1}H NMR (500 MHz, D_2O): δ 3.34 (t, 1 H, H-4), 3.84 (dd, 2 H, H-6a, H-6b), 4.03 (m, 1 H, H-5), 4.15 (d, 1 H, H-1′a), 4.25–4.40 (m, 4 H, H-1′b, H-2, H-6′a, H-5′, H-6′a), 4.54 (m, 1 H, H-6′b), 4.60 (t, 1 H, H-3), 4.80 (t, 1 H, H-4′), 5.07 (d, 1 H, H-3′), 5.82 (d, 1 H, H-1); ^{13}C NMR (D_2O, 500 MHz): δ 62.5 (C-6), 69.8 (C-1′), 71.2 (C-4), 71.3 (C-6′), 74.9 (C-5), 77.3 (C-2), 80.6 (C-4′), 80.7 (C-5′), 80.9 (C-3′), 82.1 (C-3), 92.5 (C-1), 104.4 (C-2′).
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