Carbohydrate Research 344 (2009) 2394–2398

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Note

Synthesis of a heparan sulfate mimetic disaccharide with a conformationally locked residue from a common intermediate

Jon K. Fairweather [†], Tomislav Karoli, Ligong Liu [‡], Ian Bytheway, Vito Ferro [*,§]

Drug Design Group, Progen Pharmaceuticals Limited, Brisbane, Qld 4066, Australia

ARTICLE INFO

Article history:
Received 6 July 2009
Received in revised form 11 September 2009
Accepted 14 September 2009
Available online 19 September 2009

Keywords:
Heparan sulfate mimetics
Disaccharides
Fibroblast growth factors

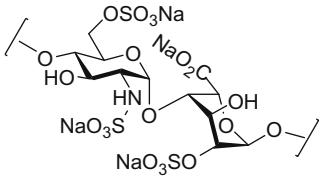
ABSTRACT

A simple mimetic of a heparan sulfate disaccharide sequence that binds to the growth factors FGF-1 and FGF-2 was synthesized by coupling a 2-azido-2-deoxy-D-glucopyranosyl trichloroacetimidate donor with a 1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose acceptor. Both the donor and acceptor were obtained from a common intermediate readily obtained from D-glucal. Molecular docking calculations showed that the predicted locations of the disaccharide sulfo groups in the binding site of FGF-1 and FGF-2 are similar to the positions observed for co-crystallized heparin-derived oligosaccharides obtained from published crystal structures.

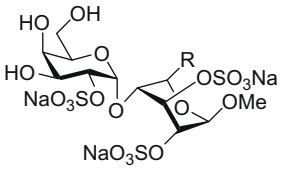
The fibroblast growth factors FGF-1 and FGF-2 are proteins that play important roles in tumor angiogenesis.[1] They initiate this process by binding with their receptors (FGFRs) and heparan sulfate (HS) to form a ternary complex which leads to receptor dimerization/activation and subsequent cell signaling.[2] Inhibiting the formation of the HS–FGF–FGFR complex by antagonizing HS–FGF binding with HS mimetics is thus a viable strategy for antiangiogenic therapies.[3–5]

Various groups have reported the synthesis of HS and HS-like oligosaccharides designed to interact with FGF-1 or FGF-2.[6–8] These studies have provided valuable information about the structural requirements for oligosaccharide-FGF binding and activation, however, the syntheses of such oligosaccharides are difficult and laborious. This has lead to the pursuit of less synthetically challenging oligosaccharide mimetics as FGF antagonists.[9–12] As part of a program aimed at developing antiangiogenic compounds, we recently described[12] the preparation of simple disaccharides such as **2** and **3** which mimic the HS disaccharide GlcN(2*S*, 6*S*)-IdoA(2*S*) (**1**, Fig. 1), which has been postulated from X-ray crystallographic analyses as a minimal heparin/HS consensus sequence for FGF binding.[13] As well as maintaining the α-(1→4) linkage between the two monosaccharide units and the spatial orientation of the two key sulfo groups [GlcN(2*S*) and IdoA(2*S*)], the compounds were designed to mimic the conformational flexibility[14,15] of the IdoA residue. Docking calculations showed that the predicted locations of disaccharide sulfo groups in the binding site of FGF-1 were consistent with the positions observed for co-crystallized heparin-derived oligosaccharides. Docking scores correlated with experimental K_d values (22 μM to 1.4 mM) obtained from binding assays.[12] The docking score for a model HS disaccharide binding to FGF-1 was similar.[12]

In crystal structures of heparin oligosaccharides bound to FGF, IdoA is found in the $^1C_4$ conformation when bound only to the protein[16,17] or in a skew-boat (^2S_O) conformation when part of a ternary complex.[18,19] NMR studies also indicate that FGF-1 can bind both conformations of IdoA in a bioactive hexasaccharide.[20] These observations led us to consider the synthesis of simple disaccharides in



1



2 R = CH_2OSO_3Na
3 R = H

Figure 1. Structures of the GlcN(2*S*, 6*S*)-IdoA(2*S*) disaccharide sequence **1**, which represents a minimal consensus sequence for FGF–HS binding,[13] and two conformationally flexible mimetics **2** and **3**.[12]

* Corresponding author. Tel.: +617 3138 1108; fax: +617 3138 1804.
E-mail addresses: vito.ferro@qut.edu.au, v.ferro@griffith.edu.au (V. Ferro).
† Present address: Chemical Analysis Pty Ltd, Bulleen, Vic. 3105, Australia.
‡ Present address: Centre for Drug Design and Development, Institute for Molecular Bioscience, University of Queensland, Brisbane, Qld 4072, Australia.
§ Present addresses: School of Physical and Chemical Sciences, Queensland University of Technology, GPO Box 2434, Brisbane, Qld 4001, Australia; Institute for Glycomics, Griffith University, Gold Coast Campus, Qld 4222, Australia.

0008-6215/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2009.09.012

which the IdoA mimic is locked in the $^1C_4$ conformation. A similar approach has been successfully used to probe the active conformations of the ATIII-binding heparin pentasaccharide.[21,22]

It has been demonstrated that an *O*-sulfo group can substitute for an *N*-sulfo group in a heparin oligosaccharide without loss of binding affinity.[23] The known 1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose **8** was thus identified as a suitable IdoA mimic precursor because it is locked in the required $^1C_4$ conformation, can be selectively sulfonated at the C-2 position and is readily available in four steps from D-glucal (**4**) via intermediates **5**–**7** (Fig. 2).[24] The precursor to **8** in this sequence of transformations can, in fact, be easily converted, via intermediates **9**–**11**, into useful glycosyl donors such as the imidate **12** for use in the synthesis of heparin/HS oligosaccharides.[24–26] It was therefore decided to prepare both **8** and **12** and use them to synthesize a disaccharide (**17**) with the desired features.

Following literature precedent,[27] TBDMSOTf was selected as the promoter for the glycosylation of the alcohol **8** with the imidate **12**. The reaction proceeded well in dichloromethane at −20 °C; however, a chromatographically inseparable mixture of anomers resulted (α/β = 3.8:1). The product was thus converted into the tribenzoate via Zémplen deacetylation followed by benzoylation with benzoyl chloride and pyridine, and the desired α-linked disaccharide **13** was isolated by flash chromatography in good overall yield (46%, three steps, Scheme 1), along with 12% of the β-linked disaccharide **13b**. The azide groups of **13** were then reduced via transfer hydrogenation with ammonium formate over $Pd(OH)_2$ catalyst and the resulting diamine **14** was sulfonated with SO_3·trimethylamine complex and debenzoylated (1 M NaOH) to give the benzyl ether **16** in moderate overall yield (28%, three steps). Hydrogenolysis over $Pd(OH)_2$ at 50 psi then furnished the target disaccharide **17** in excellent yield (98%).

Molecular docking calculations were performed using the GLIDE program[28] to examine the binding modes of **16** and **17** with FGF-1 and FGF-2. Compound **16** was examined in order to probe the effects of an extra hydrophobic group on FGF binding as it has been shown that some heparin derivatives with lipophilic modifications can bind to FGF-1 with similar or greater affinity than unmodified heparin.[29,30] The poses of **16** and **17** with the best GlideScores for binding to FGF-1 and FGF-2 are shown in Figure 3. Also shown are the van der Waals surfaces of the central sulfo groups of cocrystallized, heparin-derived hexa- and tetrasaccharide ligands from the crystal structures (pdb accession codes 2AXM[16] for FGF-1 and 1BFB[17] for FGF-2, respectively).

The preferred mode of binding of **16** and **17** to FGF-1 in Figure 3a shows congruence between the ligand sulfo groups with those observed crystallographically. The FGF-2 binding mode of **16**, shown in Figure 3b, also shows the same congruence; however, the preferred mode for **17**, involves ionic hydrogen bonding interactions with the positively charged residues LYS130 and LYS120 of FGF-2. These residues are not involved in binding to the cocrystallized heparin tetrasaccharide fragment, although their proximity to the binding site region and their inherent flexibility suggests that their involvement in ligand binding is reasonable. In the absence

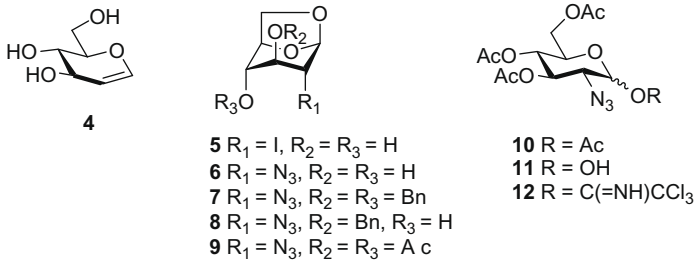
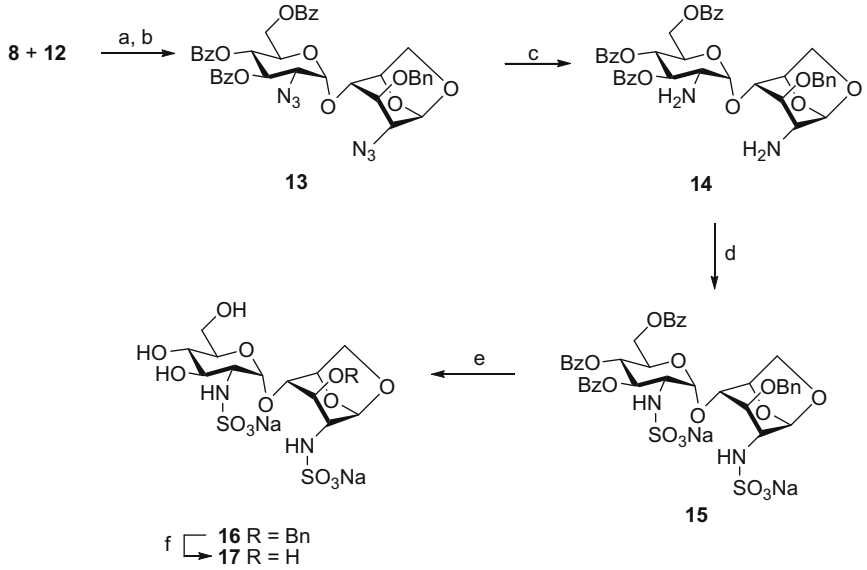


Figure 2. The structures of the glycosyl acceptor and glycosyl donor and their intermediates used in this study.



Scheme 1. Reagents and conditions: (a) TBDMSOTf, CH_2Cl_2, −20 °C; (b) (i) NaOMe, MeOH, (ii) BzCl, pyridine, 46%, three steps; (c) $Pd(OH)_2$, NH_4HCO_2, EtOAc–MeOH, 58%; (d) SO_3·Me_3N, DMF, 60 °C; (e) 1 M NaOH, 28%, three steps; (f) H_2, $Pd(OH)_2$, 98%.

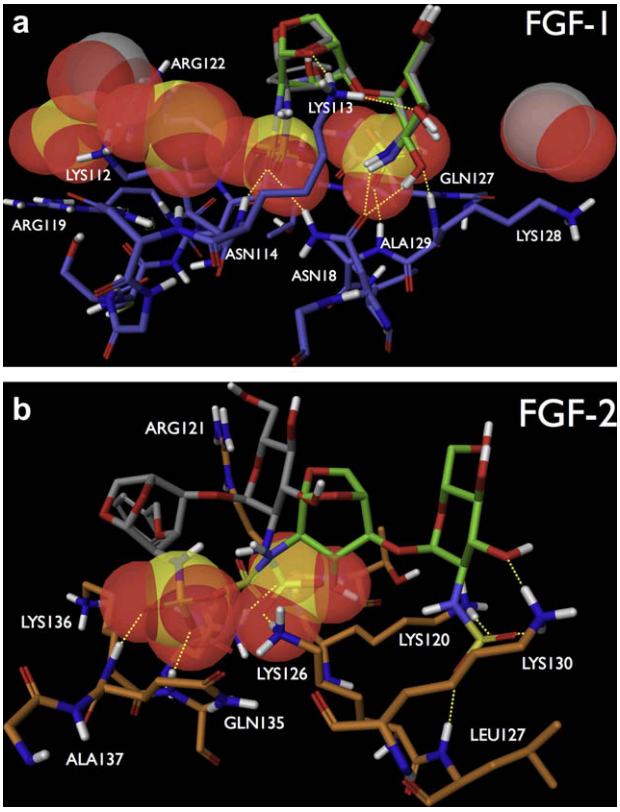


Figure 3. The best Glide poses for **16** (gray C atoms) and **17** (green C atoms) docked with FGF-1 (represented by a blue α-carbon backbone) and FGF-2 (represented by an orange α-carbon backbone). Also shown are the van der Waals surfaces of the sulfo groups of the heparin fragments cocrystallized with each growth factor (red O atoms and yellow S atoms). (a) The best poses for **16** (Gscore = −9.2 kcal/mol) and **17** (Gscore = −7.5 kcal/mol) docked with FGF-1; (b) The best poses for **16** (Gscore = −7.2 kcal/mol) and **17** (Gscore = −6.1 kcal/mol) docked with FGF-2. Hydrogen bonding interactions are depicted by the dotted yellow lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of confirmatory experimental binding data these results are not definitive. However, they do suggest that conformationally locked residues and hydrophobic substituents[29,30] should be further explored in HS mimetic design to target amino acid residues adjacent to the HS-binding site of FGF-1 and FGF-2.

In conclusion, a simple conformationally locked HS mimetic disaccharide was efficiently synthesized via the coupling of a 2-azido-2-deoxy-D-glucopyranosyl trichloroacetimidate donor with a 1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose acceptor. Both the donor and acceptor were obtained from a common intermediate readily obtained from D-glucal. Molecular docking calculations indicated that the predicted locations of the disaccharide sulfo groups in the binding site of FGF-1 and FGF-2 are similar to the positions observed for co-crystallized heparin-derived oligosaccharides. These results may aid in the design of potential inhibitors of FGF-mediated angiogenesis.

1. Experimental

1.1. General methods

General experimental details have been given previously.[31] Capillary electrophoresis (CE) was performed in reverse polarity mode with inverse UV detection, using 10 mM 5-sulfosalicylic acid (pH 3) as the background electrolyte as described previously.[32]

1.2. 3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-D-glucopyranosyl trichloroacetimidate (12)

(a) A mixture of D-glucal (1.95 g, 13.3 mmol), $(n\text{-}Bu_3Sn)_2O$ (5.44 mL, 10.6 mmol), and molecular sieves (4.0 g of 3 Å powder) in MeCN (50 mL) was heated under reflux (3 h). The mixture was cooled (rt) and I_2 (4.06 g, 16.0 mmol) was introduced with continuous stirring (1.5 h). The mixture was filtered and the filtrate was washed with hexane (3 × 100 mL) prior to evaporation of the solvent affording, presumably, the iodide **5** as a pale yellow oil. This was used directly in the next reaction without further purification or characterization. (b) A mixture of the crude iodide and NaN_3 (2.59 g, 39.9 mmol) in a mixture of DMF (45 mL) and H_2O (5 mL) was heated under reflux (o/n) and then evaporated and co-evaporated (toluene). The residue was treated with pyridine (10 mL), Ac_2O (5 mL), and DMAP (50 mg) and the combined mixture was stirred (rt, o/n). The mixture was then treated with ice-water (10 mL) and stirring continued (3 h) before being subjected to workup (EtOAc) and flash chromatography (10→40% EtOAc–hexane) to yield the diacetate **9** as a colorless oil (1.90 g), used in the next reaction without further purification or characterization. (c) Concd H_2SO_4 (50 μL) was added to a cooled (0 °C) solution of crude **9** (2.33 g, 8.53 mmol) and Ac_2O (5.00 mL, 49.0 mmol) in AcOH (10 mL) and the combined mixture was stirred (0 °C→rt, o/n). Sodium acetate (500 mg) was added portionwise until pH >5.0 and then the mixture was treated with MeOH (3 mL). The mixture was filtered and the solvent was evaporated and co-evaporated (toluene) prior to workup (EtOAc) to yield a colorless oil (3.04 g). This residue was co-evaporated (MeCN, 2 × 100 mL) and used in the next reaction without further purification or characterization. (d) Hydrazine acetate (921 mg, 10.0 mmol) was added to a stirred solution of the crude mixture from (c) (8.53 mmol, max.) in DMF (20 mL) and the combined mixture was heated (55 °C, 15 min). The mixture was poured onto saturated brine and extracted (EtOAc). The organic layer was evaporated and subjected to rapid silica filtration (10→40% EtOAc–hexane) to yield, presumably, the hemiacetal **11** as a colorless oil (1.89 g, 76%, two steps). This residue was co-evaporated (MeCN, 2 × 100 mL) and used in the next reaction without further purification or characterization. (e) K_2CO_3 (1.30 g, 9.5 mmol) was added to a solution of the hemiacetal **11** (1.85 g, 6.3 mmol) and trichloroacetonitrile (2.0 mL, 20 mmol) in 1,2-DCE (20 mL) and the combined mixture was stirred (0 °C→rt, 1 h). The mixture was filtered, the solvent was evaporated, and the residue was subjected to flash chromatography (10–30% EtOAc–hexane) to yield an anomeric mixture (α/β = 9:1) of the imidate **12** as a colorless oil (2.19 g, 80%). This mixture was separable by more careful chromatography and gave ^{1}H NMR data in good agreement with the literature.[33,34] α-Imidate: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02, 2.03, 2.08 (3s, 3H each; 3 × Ac), 3.74 (dd, 1H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 10.5 Hz; H-2); 4.06 (dd, 1H, $J_{5,6a}$ = 2.1, $J_{6a,6b}$ = 12.4 Hz; H-6a), 4.18 (ddd, 1H, $J_{4,5}$ = 10.3, $J_{5,6b}$ = 4.3 Hz; H-5), 4.21 (dd, 1H, H-6b), 5.12 (dd, 1H, $J_{3,4}$ = 9.4 Hz; H-4), 5.48 (dd, 1H, H-3), 6.46 (d, 1H, H-1), 8.80 (s, 1H; NH). β-Imidate: partial ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.68 (d, 1H, $J_{1,2}$ = 8.4 Hz; H-1).

1.3. 1,6-Anhydro-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (8)

The alcohol **8** was prepared according to the literature procedure[24,35] by selective debenzylation of the dibenzyl ether **7** with $TiCl_4$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96 (br s, 1H; OH), 3.48–3.49 (m, 1H; H-2), 3.56–3.58 (m, 1H; H-3), 3.64–3.66 (m, 1H; H-4), 3.75 (dd, 1H, $J_{5,6a}$ = 5.9, $J_{6a,6b}$ = 7.3 Hz; H-6a), 4.17 (dd, 1H, $J_{5,6b}$ = 1.1, H-6b), 4.50–4.52 (m, 1H; H-5), 4.58, 4.61 (AB, $J_{A,B}$ = 11.9 Hz; CH_2Ph), 5.42–5.43 (m, 1H; H-1), 7.26–7.36 (m, 5H; Ph).

1.4. 2-Azido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-glucopyranosyl-(1→4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranose (13)

(a) A solution of the imidate **12** (201 mg, 453 μmol) and alcohol **8** (84 mg, 302 μmol) in 1,2-DCE (5 mL) was stirred in the presence of activated mol. sieves (300 mg of 3 Å powder) under an atmosphere of argon (rt, 30 min). The mixture was cooled (−20 °C) with continuous stirring (10 min) and TBDMSOTf (21 μL, 91 μmol) was introduced dropwise and stirring was maintained (−20 °C, 10 min). Et_3N (100 μL) was introduced and the mixture was filtered and evaporated. The residue was subjected to workup (EtOAc) and flash chromatography (10→40% EtOAc–hexane) to yield a fraction presumed to contain the disaccharide product as a pale yellow oil (130 mg). This residue was co-evaporated (2 × 10 mL MeCN) and used in the next reaction without further purification or characterization. (b) A sliver of sodium was added to a stirred solution of the crude product from (a) (0.302 mmol, max.) in MeOH–THF (2:1, 3 mL) at 0 °C. The solution was allowed to warm to rt and stirred (3 h). The mixture was neutralized (Bio-Rad AG®-50W-X8 resin, H^+ form), filtered and the resin was washed with MeOH. The combined filtrate and washings were evaporated to yield the alcohol as a colorless oil (98 mg). This residue was co-evaporated (2 × 10 mL MeCN) and used in the next reaction without further purification or characterization. (c) The crude product (0.302 mmol, max.) was dissolved in CH_2Cl_2 (3 mL) and treated with benzoyl chloride (1.3 equiv per hydroxyl) and pyridine (1 mL). The mixture was stirred at rt overnight, washed with ice-chilled 0.5 M HCl and sat. $NaHCO_3$. The organic phase was dried ($MgSO_4$), filtered, and evaporated, and the residue was purified by flash chromatography (10→30% EtOAc–hexane) to give, firstly, the α-linked disaccharide **13** as a colorless foam (101 mg, 46%, three steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.11 (s, 1H; H-2^{I}), 3.41 (dd, 1H, $J_{1,2}$ = 3.7, $J_{2,3}$ = 10.7 Hz; H-6^{I}), 3.61 (s, 1H; H-3^{I}), 3.39 (s, 1H; H-4^{I}), 4.05 (d, 1H, $J_{6,6}$ = 7.3 Hz; H-6^{I}), 4.41–4.49 (m, 2H; H-6^{II}), 4.55, 4.68 (AB quartet, $J_{A,B}$ = 11.9 Hz; CH_2Ph), 4.79 (ddd, 1H, $J_{4,5}$ = 10.3, $J_{5,6}$ = 2.9, 5.9 Hz; H-5^{II}), 4.91 (br d, 1H, $J_{5,6}$ = 5.5 Hz; H-5^{I}), 5.08 (d, 1H, $J_{1,2}$ = 3.6 Hz; H-1^{II}), 5.51 (dd, 1H, $J_{3,4}$ = 9.5, $J_{4,5}$ = 10.2 Hz, H-4^{II}), 5.60 (s, 1H,; H-1^{I}), 6.10 (dd, 1H, $J_{2,3}$ = 10.7, $J_{3,4}$ = 9.3 Hz; H-3^{II}), 7.29–7.55, 7.89–8.03 (2m, 20H; ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 58.7, 61.5, 63.3, 64.8, 69.2, 69.5, 70.5, 73.2, 74.6, 78.1, 79.6, 100.7, 101.2, 128.1, 128.4, 128.5(8), 128.6(1), 128.6(4), 128.8, 128.9, 129.2, 129.8(6), 129.9(0), 130.1, 130.2, 133.4, 133.5, 133.7, 137.2, 165.6(1), 165.6(2), 166.3. Next, the β-anomer **13b** was obtained as a colorless oil (27 mg, 12%, three steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.19 (s, 1H; H-2^{I}), 3.74 (dd, 1H, $J_{5,6}$ = 6.2, $J_{6,6}$ = 7.1 Hz; H-6^{I}), 3.79–3.88 (m, 2H; H-2^{II}, H-3^{I}), 3.88 (ddd, $J_{4,5}$ = 9.2, $J_{5,6}$ = 3.1, 4.7 Hz; H-5^{II}), 3.95 (br s, 1H; H-4^{I}), 4.10 (d, 1H, $J_{6,6}$ = 7.3 Hz; H-6^{I}), 4.34 (dd, 1H; $J_{5,6}$ = .9, $J_{6,6}$ = 12.2 Hz; H-6^{II}), 4.50 (dd, 1H, $J_{5,6}$ = 3.1, $J_{6,6}$ = 12.3 Hz, H-6^{II}), 4.52, 4.59 (AB quartet, $J_{A,B}$ 12.0 = Hz; CH_2Ph), 4.65 (d, 1H, $J_{1,2}$ = 7.9 Hz; H-1^{II}), 4.69 (br d, 1H, $J_{5,6}$ = 5.5 Hz; H-5^{I}), 5.44 (t, 1H, $J_{2,3=3,4}$ = 9.7 Hz; H-3^{II}), 5.49 (br s, 1H; H-1^{I}), 5.51 (t, 1H, $J_{3,4=4,5}$ = 9.6 Hz; H-4^{II}), 7.23–7.50, 7.84–7.96 (2m, 20H; ArH).

1.5. 2-Deoxy-2-sulfamino-α-D-glucopyranosyl-(1→4)-1,6-anhydro-2-deoxy-2-sulfamino-3-*O*-benzyl–D-glucopyranose, disodium salt (16)

The disaccharide **13** (95 mg, 127 μmol), Pearlman's catalyst (11 mg), and ammonium formate (300 mg) in 2:1 MeOH–EtOAc (7 mL) were heated (65 °C) under argon until complete by TLC (1 h). The mixture was cooled to rt, filtered (0.2 μm), and evaporated. The crude product was purified by SPE (300 mg C18 Waters cartridge, equilibrated with 5:95 MeOH–H_2O, gradient eluted 5:95→100:0 MeOH–H_2O) to yield the diamine **14** (53 mg, 58%). Without further purification, to the diamine were added DMF (5 mL), $SO_3{\cdot}Me_3N$ (41 mg, 295 μmol), and $NaHCO_3$ (40 mg, 475 μmol). The mixture was heated (60 °C) for 1 h then cooled to rt and the reaction was quenched with ice and Na_2CO_3 (satd aq). This suspension was stored at −18 °C overnight and the sample was filtered and evaporated. TLC indicated that partial hydrolysis of the benzoate groups had occurred (presumably in the workup procedure). Water (1 mL) and NaOH (250 μL, 1 M) were added and the solution was stirred overnight, then loaded directly onto a Bio-Gel P-2 (5 × 100 cm) column and was purified by size exclusion chromatography (0.1 M ammonium bicarbonate, flow rate = 2.8 mL/min). Fractions containing carbohydrate (determined by spotting onto silica gel plates and visualization by charring) were checked for purity by CE and those deemed to be free of salt were pooled and lyophilized to give disulfate **16** (22 mg, 28%, three steps) as an amorphous white solid. ^{1}H NMR (400 MHz, D_2O, solvent suppressed) δ 7.35–7.21 (m, 5H, Ph), 5.43 (br s, 1H, H-1^{I}), 5.18 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1^{II}), 4.72–4.69 (m, 1H, H-5^{I}), 4.54–4.52 (m, 2H, PhCH_2), 4.05 (d, 1H, J_{gem} = 7.9 Hz, H-6a^{I}), 3.85 (br s, 1H, H-3^{I}), 3.76–3.58 (m, 5H, H4^{I}, H6b^{I}, H5^{II}, H6a^{II}, 6b^{II}), 3.51 (dd, 1H, $J_{2\text{-}3}$ = 10.4, $J_{3\text{-}4}$ = 9.1, H-3^{II}), 3.34 (dd, 1H, $J_{3\text{-}4\sim4\text{-}5}$ = 9.2, H-4^{II}), 3.23 (br s, 1H, H-2^{I}), 3.12 (dd, 1H, H-2^{II}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.3, 124.8, 124.6, 124.4, 96.9, 95.1, 72.7, 71.5, 70.8, 68.3, 68.2, 67.2, 66.0, 61.0, 56.6, 54.0, 49.8. The purity was 99% by CE (t_m = 14.9 min).

1.6. 2-Deoxy-2-sulfamino-α-D-glucopyranosyl-(1→4)-1,6-anhydro-2-deoxy-2-sulfamino–D-glucopyranoside, disodium salt (17)

A mixture of benzyl ether **16** (12.9 mg, 20.8 μmol) and Pearlman's catalyst (5 mg) in purified water (2 mL) was subjected to 50 psi H_2 overnight. The mixture was filtered and lyophilized to yield 10.7 mg (98%) of tetrol **17**. ^{1}H NMR (400 MHz, D_2O) δ 5.47 (br s, 1H, H-1^{I}), 5.20 (d, 1H, $J_{1\text{-}2}$ = 3.5, H-1^{II}), 4.68 (br d, 1H, $J_{5\text{-}4}$ = 5.5, H-5), 4.07 (d, 1H, J_{gem} = 7.6, H-6A^{I}), 3.98 (br s, 1H, H-3^{I}), 3.75–3.64 (m, 4H), 3.52 (t, 1H, $J_{2\text{-}3\sim3\text{-}4}$ = 9.3, H-3^{II}), 3.34 (t, 1H, $J_{3\text{-}4\sim4\text{-}5}$ = 9.3, H-4^{II}), 3.13 (obsd dd [partially obscured by H2^{I}], 1H, H-2^{II}), 3.11 (br s, 1H, H-2^{I}). Purity was 99% by CE (t_m = 12.6 min).

Acknowledgments

We thank Drs. Siska Cochran, Cai Ping Li, Robert Don, and Edward Hammond for useful discussions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2009.09.012.

References

1. Presta, M.; Dell'Era, P.; Mitola, S.; Moroni, E.; Ronca, R.; Rusnati, M. *Cytokine Growth Factor Rev.* **2005**, *16*, 159–178.
2. Eswarakumar, V. P.; Lax, I.; Schlessinger, J. *Cytokine Growth Factor Rev.* **2005**, *16*, 139–149.
3. Parish, C. R.; Freeman, C.; Brown, K. J.; Francis, D. J.; Cowden, W. B. *Cancer Res.* **1999**, *59*, 3433–3441.
4. Casu, B.; Guerrini, M.; Guglieri, S.; Naggi, A.; Perez, M.; Torri, G.; Cassinelli, G.; Ribatti, D.; Carminati, P.; Giannini, G.; Penco, S.; Pisano, C.; Belleri, M.; Rusnati, M.; Presta, M. *J. Med. Chem.* **2004**, *47*, 838–848.
5. Presta, M.; Leali, D.; Stabile, H.; Ronca, R.; Camozzi, M.; Coco, L.; Moroni, E.; Liekens, S.; Rusnati, M. *Curr. Pharm. Des.* **2003**, *9*, 553–566.
6. Angulo, J.; Ojeda, R.; de Paz, J. L.; Lucas, R.; Nieto, P. M.; Lozano, R. M.; Redondo-Horcajo, M.; Giménez-Gallego, G.; Martín-Lomas, M. *ChemBioChem* **2004**, *5*, 55–61.
7. Poletti, L.; Lay, L. *Eur. J. Org. Chem.* **2003**, 2999–3024.
8. O'Brien, A.; Lynch, C.; O'Boyle, K. M.; Murphy, P. V. *Carbohydr. Res.* **2004**, *339*, 2343–2354.

9. Murphy, P. V.; Pitt, N.; O'Brien, A.; Enright, P. M.; Dunne, A.; Wilson, S. J.; Duane, R. M.; O'Boyle, K. M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3287–3290.
10. Liu, L.; Li, C. P.; Cochran, S.; Ferro, V. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2221–2226.
11. Zhang, J.; Riverst, G.; Zhu, Y.; Jacobson, A.; Peyers, J.; Grundstrom, G.; Burch, P.; Hussein, S.; Marolewski, A.; Herlihy, W.; Rusche, J. *Bioorg. Med. Chem.* **2001**, *9*, 825–836.
12. Liu, L.; Bytheway, I.; Karoli, T.; Fairweather, J. K.; Cochran, S.; Li, C.; Ferro, V. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 344–349.
13. Pellegrini, L. *Curr. Opin. Struct. Biol.* **2001**, *11*, 629–634.
14. Ferro, D. R.; Provasoli, A.; Ragazzi, M.; Torri, G.; Casu, B.; Gatti, G.; Jacquinet, J. C.; Sinaÿ, P.; Petitou, M.; Choay, J. *J. Am. Chem. Soc.* **1986**, *108*, 6773–6778.
15. Ferro, D. R.; Provasoli, A.; Ragazzi, M.; Casu, B.; Torri, G.; Bossennec, V.; Perly, B.; Sinaÿ, P.; Petitou, M.; Choay, J. *Carbohydr. Res.* **1990**, *195*, 157–167.
16. DiGabriele, A. D.; Lax, I.; Chen, D. I.; Svahn, C. M.; Jaye, M.; Schlessinger, J.; Hendrickson, W. A. *Nature* **1998**, *393*, 812–817.
17. Faham, S.; Hileman, R. E.; Fromm, J. R.; Linhardt, R. J.; Rees, D. C. *Science* **1996**, *271*, 1116–1120.
18. Pellegrini, L.; Burke, D. F.; von Delft, F.; Mulloy, B.; Blundell, T. L. *Nature* **2000**, *407*, 1029–1034.
19. Schlessinger, J.; Plotnikov, A. N.; Ibrahimi, O. A.; Eliseenkova, A. V.; Yeh, B. K.; Yayon, A.; Linhardt, R. J.; Mohammadi, M. *Mol. Cell* **2000**, *6*, 743–750.
20. Canales, A.; Angulo, J.; Ojeda, R.; Bruix, M.; Fayos, R.; Lozano, R.; Gimenez-Gallego, G.; Martin-Lomas, M.; Nieto, P. M.; Jimenez-Barbero, J. *J. Am. Chem. Soc.* **2005**, *127*, 5778–5779.
21. Das, S. K.; Mallet, J. M.; Esnault, J.; Driguez, P. A.; Duchaussoy, P.; Sizun, P.; Hérault, J. P.; Herbert, J. M.; Petitou, M.; Sinaÿ, P. *Angew. Chem., Int. Ed.* **2001**, *40*, 1670–1673.
22. Das, S. K.; Mallet, J. M.; Esnault, J.; Driguez, P. A.; Duchaussoy, P.; Sizun, P.; Hérault, J. P.; Herbert, J. M.; Petitou, M.; Sinaÿ, P. *Chem. Eur. J.* **2001**, *7*, 4821–4834.
23. van Boeckel, C. A. A.; Petitou, M. *Angew. Chem., Int. Ed.* **1993**, *32*, 1671–1818.
24. Tailler, D.; Jacquinet, J. C.; Noirot, A. M.; Beau, J. M. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3163–3164.
25. de Paz, J. L.; Angulo, J.; Lassaletta, J. M.; Nieto, P. M.; Redondo-Horcajo, M.; Lozano, R. M.; Giménez-Gallego, G.; Martín-Lomas, M. *ChemBioChem* **2001**, *2*, 673–685.
26. Arndt, S.; Hsieh-Wilson, L. C. *Org. Lett.* **2003**, *5*, 4179–4182.
27. Orgueira, H. A.; Bartolozzi, A.; Schell, P.; Litjens, R. E. J. N.; Palmacci, E. R.; Seeberger, P. H. *Chem. Eur. J.* **2003**, *9*, 140–169.
28. Glide version 3.0, Scrödinger LLC, New York NY, 2005.
29. Huang, L.; Fernandez, C.; Kerns, R. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 419–423.
30. Huang, L.; Kerns, R. J. *Bioorg. Med. Chem.* **2006**, *14*, 2300–2313.
31. Fairweather, J. K.; Karoli, T.; Ferro, V. *Bioorg. Med. Chem.* **2004**, *12*, 6063–6075.
32. Yu, G.; Gunay, N. S.; Linhardt, R. J.; Toida, T.; Fareed, J.; Hoppensteadt, D. A.; Shadid, H.; Ferro, V.; Li, C.; Fewings, K.; Palermo, M. C.; Podger, D. *Eur. J. Med. Chem.* **2002**, *37*, 783–791.
33. Grundler, G.; Schmidt, R. R. *Liebigs Ann. Chem.* **1984**, 1826–1847.
34. Briner, K.; Vasella, A. *Helv. Chim. Acta* **1987**, *70*, 1341–1356.
35. Hori, H.; Nishida, Y.; Ohrui, H.; Meguro, H. *J. Org. Chem.* **1989**, *54*, 1346–1353.