Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 339 (2004) 1873–1887
Carbohydrate RESEARCH

Synthesis of a glucuronic acid and glucose conjugate library and evaluation of effects on endothelial cell growth ☆

Nigel Pitt,[a] Rhona M. Duane,[b] Alan O' Brien,[a] Helena Bradley,[a] Stephen J. Wilson,[b] Kathy M. O' Boyle[b,*] and Paul V. Murphy[a,*]

[a]*Centre for Synthesis and Chemical Biology, Department of Chemistry, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland*
[b]*Department of Pharmacology, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland*

Received 23 April 2004; accepted 25 May 2004
Available online 25 June 2004

Abstract—Compounds that alter endothelial cell growth are of interest in the development of angiogenesis modulators. A structurally diverse series of saccharide derivatives (glycosylamide conjugates) have been synthesized and evaluated for their effects on bovine aortic endothelial cell (BAEC) growth. Heparin-albumin (HA) reduced BAEC growth by 32% at 10 μg/mL and a number of the novel saccharide conjugates from the library were found to mimic the effect of HA as they also inhibit endothelial cell survival under identical conditions. Two thiophene conjugates, thioglucamide (24% inhibition at 35 μM) and a related glucuronide (26% inhibition at 33 μM) were the most potent inhibitors of BAEC growth, as determined using a methylthiazol tetrazolium (MTT) assay. The effects of thioglucamide and HA on absolute cell number were also studied using cell counting experiments; thioglucamide (47% after 24 h) was more potent than indicated by the MTT assay and initially reduced the BAEC number to a greater extent than HA (30% after 24 h); however, its actions were over more rapidly than were HA's as cell growth had returned to levels of the control after 72 h where HA still caused 25% inhibition. The binding of the monosaccharide conjugates to fibroblast growth factor (FGF-2) in competition with heparin-albumin by ELISA was investigated to establish the possible mechanism by which glycoconjugates could alter growth but there was no general correlation between reduction in viable cell population and binding to FGF-2. No glycoconjugate reduced the proliferation of mouse mammary epithelial cells, nor did any alter gross cell morphology, supporting a proposal that the reduction in BAEC survival by monosaccharide conjugates such as thioglucamide is a result of the inhibition of cell proliferation rather than being an induction of cytotoxicity. These studies indicate that cell biological studies to determine the mechanism of action of the simple monosaccharide conjugates may be worthwhile.

Keywords: Signal transduction; Angiogenesis; Glycoconjugates; Endothelial cell growth; Carbohydrate libraries

1. Introduction

The signal transduction processes that modulate cellular behaviour are important biological events. For example, angiogenesis[1] provides new blood vessels to growing and developing tissue and it relies on the up-regulation of endothelial cell proliferation. In adults, angiogenesis is tightly regulated and occurs usually only during pregnancy or in wound healing. However, up-regulated angiogenesis is characteristic in rheumatoid arthritis, diabetic retinopathy, during tumour growth and metastasis.[2] Consequently inhibitors or, in some cases, promoters of endothelial cell survival and proliferation are of interest as potential therapeutics. A number of strategies[3] being considered for the development of anti-angiogenic agents include synthesis of inhibitors of the recognition of extracellular matrix proteins to integrin

☆ Supplementary data associated with this article can be found in the online version, at 10.1016/j.carres.2004.05.024

* Corresponding authors. Tel.: +353-1-7162504; fax: +353-1-7162501; e-mail: paul.v.murphy@ucd.ie

0008-6215/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2004.05.024

receptors,[4] matrix metalloprotease inhibitors[5] as well as natural products,[6] collagenase inhibitors, COX inhibitors, kinase inhibitors, aminopeptidase inhibitors[7] and inhibitors of growth factors and their receptors. Several growth factors, such as FGF-2, VEGF and PDGF are involved in the stimulation of angiogenesis, and so the blockage of one factor alone might not be sufficient to inhibit angiogenesis for a prolonged period, as the other factors might be up-regulated to compensate. Thus, it is likely that in chemotherapy more than one agent will be needed for treatments and that the continuing development of such agents will be necessary. Aside from their angiogenic properties, FGFs display a range of activities such as cell growth, differentiation and wound healing. The cellular receptors for FGFs are tyrosine kinases (FGFR) and these are activated by ligand-induced dimerization, requiring heparin or heparan sulfate proteoglycans (HSPG's) as co-receptors (**1**, **2**).[8] The consequences of exposing cells to growth factors in the presence of heparin/HSPG's can be cell movement, differentiation, proliferation or protection from death. It is widely believed that highly sulfated oligosaccharides containing at least six saccharide units comprised of iduronic acid are required to promote signaling.[8] A report by Ornitz et al. showed that non-sulfated disaccharides and trisaccharides (e.g., **3**, **4**), with structural features found in heparin, were active in a number of FGF-2 dependent assays:[9] they displayed binding to FGF-2 in competition with ^{125}I heparin, enhanced the binding of FGF-2 to its receptor and, in the presence of FGF-2, promoted proliferation of cell lines expressing this receptor. These workers determined 3D structures of their oligosaccharides binding to FGF-2 and also performed cross-linking experiments; suggesting that the oligosaccharides could promote the formation of FGF-2 clusters or aggregates that are responsible for the observed activity (Chart 1). The 3D structure showed occupation of other binding sites, not normally associated with heparin binding that may be physiologically relevant.[10] Some of the compounds were potent stimulators of F32 cell proliferation in the presence of FGF-2 even though the binding affinity of the oligosaccharides, measured in competition with heparin for FGF-2, was moderate. Some oligosaccharides did not show affinity in the binding assay but were also able to stimulate proliferation of these cells. We were interested to explore an approach in trying to identify compounds reduced in carbohydrate character (monosaccharides) that might be mimetics of disaccharides **3** or **4** or identify compounds that would inhibit cell growth. Herein, we describe the synthesis and biological evaluation of a library of monosaccharide derivatives, which has led to identification of novel saccharide conjugates for inhibition of endothelial cell growth and are thus of interest as potential angiogenesis or signal transduction inhibitors.

2. Results and discussion

2.1. Design and synthesis of the saccharide conjugate library

Recent studies by Hindsgaul and co-workers (*carbohybrids* targeting oligosaccharide receptors)[11] and Wong and co-workers (targeting RNA)[12] and by a number of researchers in the selectin area[13] have shown that it is possible to replace parts of oligosaccharide-like structures by non-carbohydrate groups and retain biological activity giving rise to glycomimetics reduced in carbohydrate character that are still biologically active. With a view to discovery of *carbohybrids* (Chart 2) which possibly would display inhibition or promotion of FGF-2 mediated processes, we synthesized a collection of structurally diverse glycoconjugates (Chart 3) where D-glucuronic acid and D-glucopyranoside are attached to a diverse range of non-carbohydrate groups. Such com-

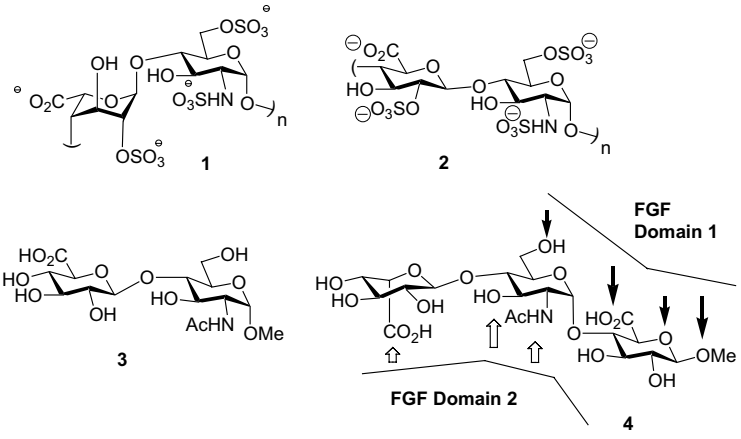


Chart 1. Structures of **1**–**4**. Evidence from 3D structures determined by crystallography and cross-linking experiments suggests that **3** and **4** induce dimers/oligomers of FGF that can activate signal transduction.

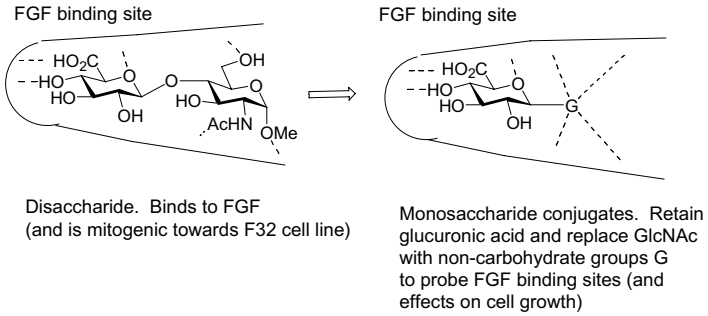


Chart 2. Basis for choosing monosaccharide conjugates for synthesis and biological evaluation.

pounds were selected for synthesis based on a hypothesis that they might bind in regions where the disaccharides **3** and **4** have previously been shown to bind (Chart 2).

The synthetic sequences used for preparation of **5**–**29** were based on methods described in the literature (Scheme 1). The diversity of *O*-glycosides that could be prepared by glycosidation reactions from the imidate **41** or bromide **42** was limited due to low reactivity of these donors.[14] However, the synthesis of the glycosyl amides (e.g., **7**–**29**) could be achieved more easily and from a wide range of carboxylic acids. The glucuronic acid conjugate precursors (Scheme 1) were condensed with acid chlorides (from **43** and promoted by PPh_3) or carboxylic acids (from **44** using amide coupling reagents, e.g., DCC) and the protecting groups were then removed to give the β-glucuronic acid conjugates. The carboxylic acid derivatives used for conjugation to the glucuronic acid were selected to have maximum diversity based on their partial atomic charge, atomic σ-charge, atomic π-charge, hydrogen bonding, topological characteristics and on the 'Lipinksi rule of 5' (Chart 2).[15]

In addition glucose conjugates were prepared; for example, **31** was prepared from the azide **45** (Scheme 2) by reduction to the amine and subsequent coupling to thiophene-2-carboxylic acid followed by saponification. The related glucoside **36** (not shown in Scheme 2) was obtained by reaction of **45** with benzoyl chloride and PPh_3 and subsequent deacetylation. The tertiary amide **34** was prepared via the reaction of D-glucose with methylamine in MeOH followed by reaction of the glycosylamine product with thiophene-2-carbonyl chloride in MeOH in the presence of Na_2CO_3. The reaction of azide **43** with DBU gave **46**, which after coupling with thiophene-2-carbonyl chloride using triphenylphosphine and deprotection gave **32** as a mixture of anomers (Scheme 3). The synthesis of the divalent glucuronic acid conjugate **30** has been described previously.[16] The syntheses of other divalent conjugates are shown in Scheme 4. Thus EDC–HOBt promoted reaction of **44** with isophthalic acid gave **48**, which is converted to **38** by coupling with pentane-1,5-diamine followed by deprotection. The coupling of **44** with 1,6-dioxaoctanedioic acid and removal of protecting groups gave **39**.

2.2. Biological evaluation of saccharides and discussion

The effects of compounds **5**–**40** on bovine aortic endothelial cell (BAEC) growth were investigated. The BAECs used in the assays express both the FGFR and heparan sulfate proteoglycans, and they release FGF-2. This drives growth or survival, and potently suppresses apoptotic cell death; evidence for this has been reported previously; FGF-2 activity can be inhibited using a neutralizing anti-FGF-2 antibody, which resulted in increased apoptosis.[17] The BAECs are thus suitable as models for identifying compounds that may act by inhibiting or promoting FGF-2 induced signaling pathways or that may alter cell growth and survival pathways by other mechanisms. The effect of heparin on endothelial cell growth in culture is complex, with both proliferative[18] and anti-proliferative[19] effects being observed. The conditions that were used in this case, where the cells were grown in the presence of serum, favoured anti-proliferative actions of heparin. Thus heparin-albumin (HA) reduced BAEC growth by 32% at 10 μg/mL. A number of the novel saccharide conjugates were found to mimic the effect of heparin as they inhibited endothelial cell survival under identical conditions, with thiophenes **20** and **31** (thioglucamide) the most active; these two compounds had similar potency, respectively, inhibiting the growth of endothelial cells by 26% at 33 μM and 24% at 35 μM. The MTT assay developed by Mosmann was used to determine cell populations during the first assays that were carried out. The effects of the growth inhibitor **31** and heparin-albumin (HA) on absolute cell number were subsequently studied using cell counting experiments; more specifically the number of cells present in BAEC cultures after 24–72 h exposure to thioglucamide **31** or HA was established and the results are shown in Figure 1. Thioglucamide **31** initially reduced the BAEC number to a greater extent than HA; however, its actions were over more rapidly than were HA's. Thus, after 24 h, thioglucamide **31** reduced BAEC number by 47% compared to a 30% reduction by HA. By 72 h, HA still caused a 25% reduction in cell number whereas the number of cells in the presence of **31** had returned to control levels. The cell counting experiments

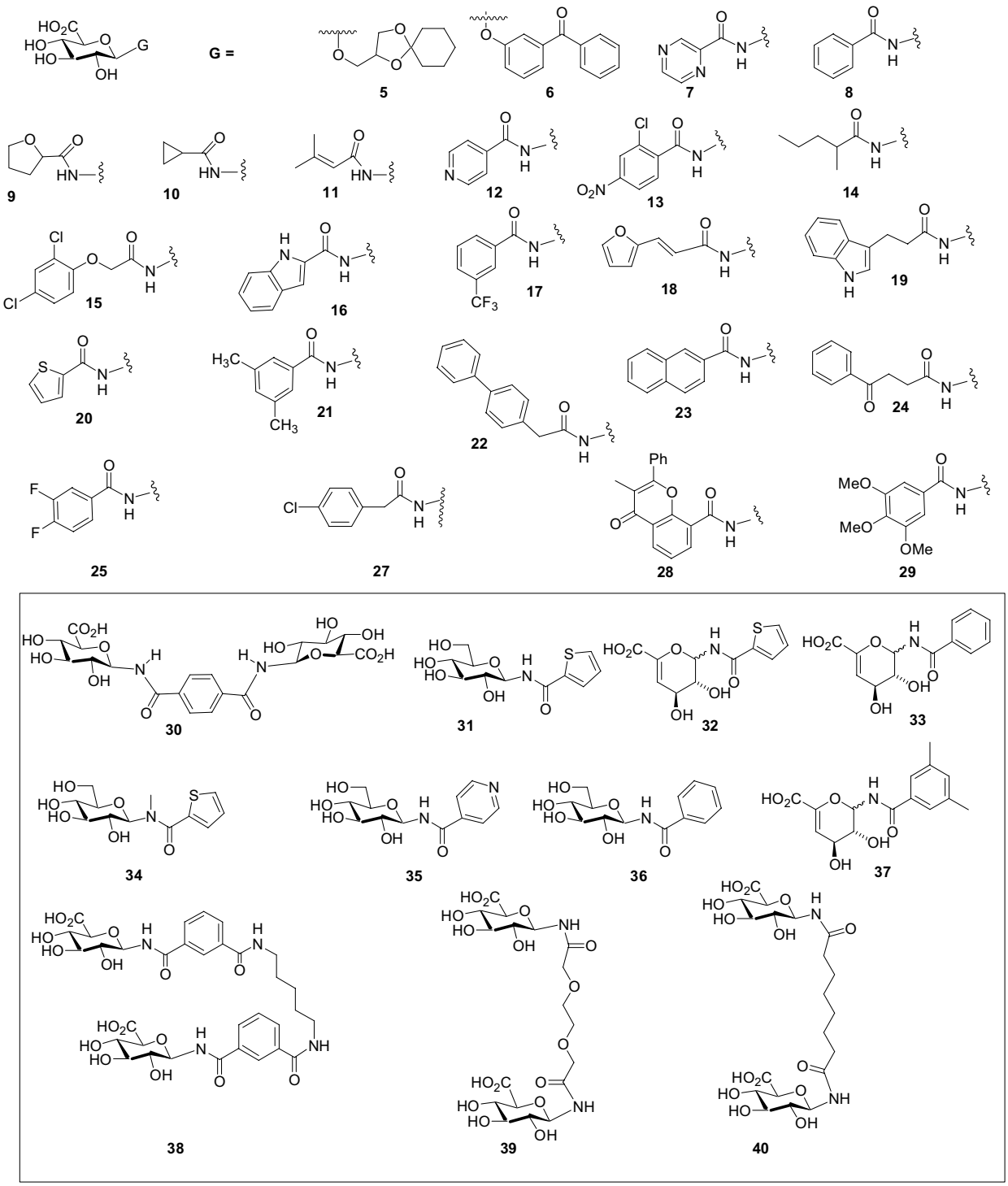
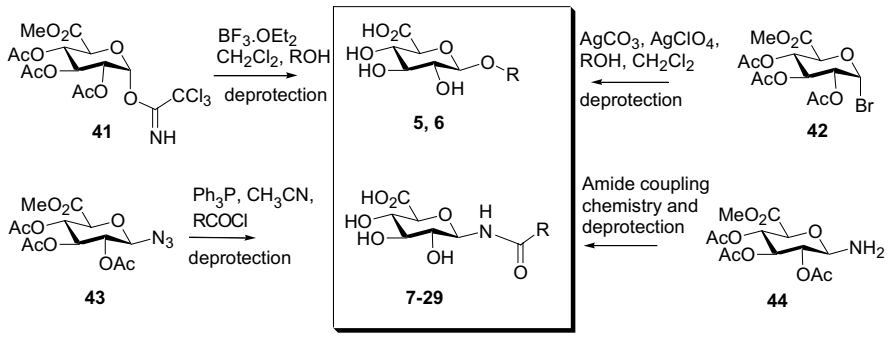


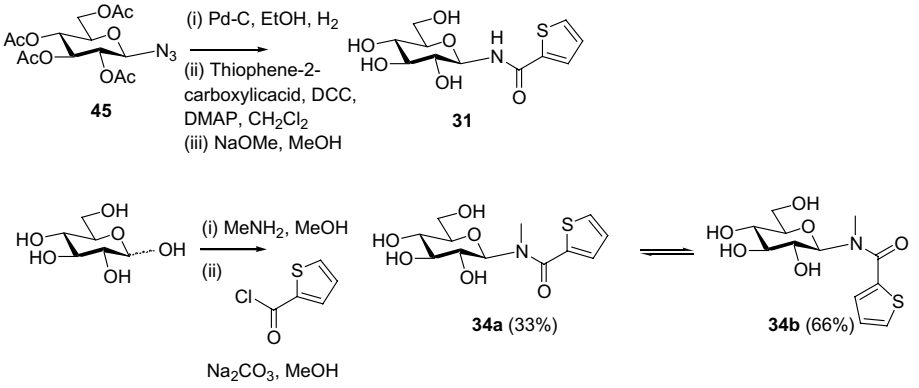
Chart 3. Glucuronic acid and other saccharide conjugates synthesized and evaluated in BAEC growth assays.

did indicate that **31** is more potent after 24 h than indicated by the MTT assay; this could imply that other compounds in the saccharide library may be more potent than they appear from MTT assay. The results are indicative that another cell survival pathway is up-regulated to compensate for the inhibitory effects displayed by **31**.

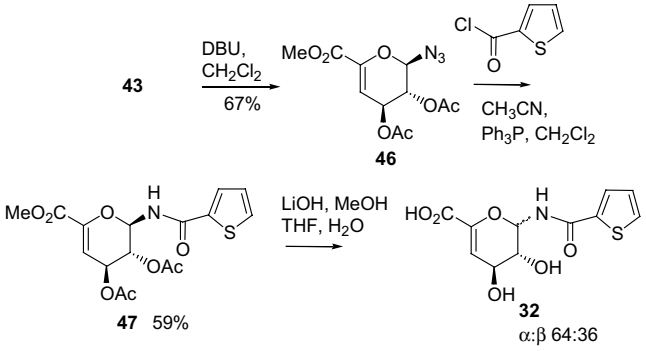
The compounds listed in Table 1 were also evaluated for their effects on growth of a mouse mammary epithelial cell line (NMuMG) in order to establish whether the inhibition of endothelial cell growth was merely a nonspecific toxic action. Heparin-albumin was found to reduce the number of viable epithelial cells by 12.2% after 24 h whereas no saccharide conjugate reduced the



Scheme 1. Synthesis of glucuronides **5**–**29**.



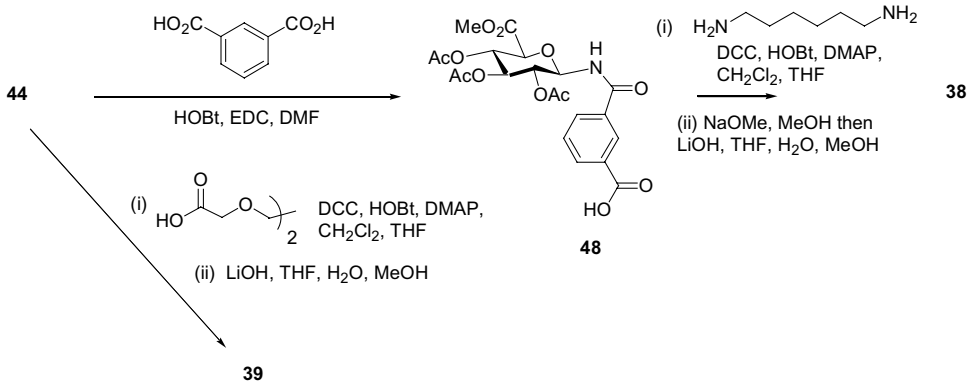
Scheme 2. Synthesis of glucosides.



Scheme 3. Synthesis of **32**.

proliferation of epithelial cells, nor did any alter gross cell morphology. Dimethylsulfoxide, a known cytotoxic agent, inhibited cell growth by >95%. These results support the proposal that the reduction in BAEC growth by the saccharide conjugates is a result of inhibition of cell proliferation or survival rather than being an induction of cytotoxicity.

ELISA assays were developed for determination of binding of the monosaccharide conjugates to FGF-2 in competition with a heparin-albumin conjugate (HA) as it was possible that the compounds that altered endothelial cell growth were also inhibitors of heparin or HS binding to FGF-2 (see Table 1); in a preliminary communication, we provided some evidence that this may have been the case.[20] Heparin and HS were evaluated as standards in the binding assay; heparin showed a maximum inhibition (I_{max}) of 95% with 47.5% inhibition observed at 1.6 ng/mL whereas HS was less potent in the assay had a lower I_{max} (70%) and displayed 35% inhibition at 136.5 ng/mL. The average weight of the oligosaccharide fraction on HS that binds one FGF-2 molecule is estimated to be ~1100;[21] this would imply that the concentration of the oligosaccharide fraction required to inhibit FGF-2 binding to HS by 35% is thus 125 nM. The thiophene derivatives **31** and **32** (I_{max} 62% @ 346 μM and 65% @ 350 μM, respectively) and alkyl derivative **14** (I_{max} 51% @ 353 μM) were amongst the more potent compounds in the binding assay. Some compounds showed an apparent stimulation of the binding of heparin to FGF-2 (e.g., **6** and **8**) as increased amounts of FGF-2 were bound to the 96 well plates coated with HA in their presence. Experiments suggest that the increased amounts of FGF-2 binding to plates in presence of compounds is due to promotion of the interaction between the 1° antibody, used in the assay, and FGF-2, rather than the compound producing an enhancement of heparin/FGF-2 binding. The hypothesis



Scheme 4. Synthesis of divalent glucuronic acid conjugates.

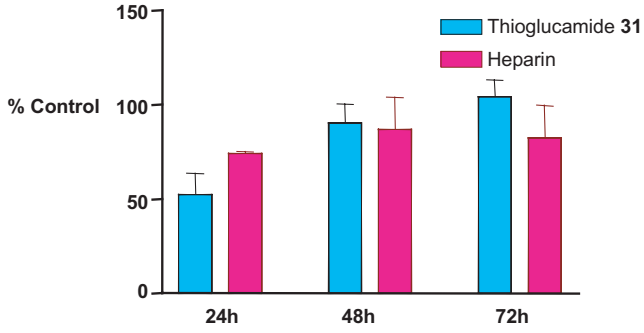


Figure 1. The effect of thioglucamide **31** (35 μM) and heparin-albumin (10 μg/mL) on the growth of BAECs as determined by cell counting experiments.

Table 1. Biological data for bioactive glycoconjugates and heparin/heparan sulfate[a]

Compound	FGF-ELISA		BAEC survival[b]	
	I_{max}%	Concn (μM)	%	Concn (μM)
8	50[c]	34	Inactive	34
12	45	340	15	34
14	51	350	Inactive	35
15	24	250	Inactive	25
20	40	330	26	33
24	20	280	Inactive	28
25	50	300	8	30
27	35	290	Inactive	29
28	25	220	Inactive	22
29	23	260	15	26
31	62	350	24.5	35
32	65	350	Inactive	35
Heparin-albumin	97.5	0.1 mg/mL	32	10 μg/mL
Heparin (Fluka)	95	0.1 mg/mL	n.d.	—
Heparan sulfate (Sigma)	70	0.1 mg/mL	n.d.	—

[a]Compounds not included in the table were inactive.
[b]The number of viable cells was estimated using the MTT assay.
[c]Stimulates binding.

that there is generally a correlation between inhibition of BAEC survival and inhibition of FGF-2 binding to HA is not supported by the results obtained; **14** and **32** were inactive and **31** was active in the endothelial cell assay; each of these compounds would have been expected to inhibit BAEC growth if their mechanism was due to the inhibition of action of FGF-2. Furthermore the synthesis of the disaccharide **3**, which is the simplest disaccharide component of heparin, has recently been completed within our group; it is inactive in the BAEC assay and did not inhibit FGF-2 binding to heparin in the ELISA; this clearly shows that **20** and **31** are not mimics of simple heparin disaccharides.[22] These observations, based on evaluation of a wider range of compounds than that reported in the preliminary communication and on the biological evaluation of **3** suggest thus that although the saccharide conjugates were designed as putative FGF-2 inhibitors and putative mimetics of **3**, their ability to cause a reduction in viable cell populations is not through their inhibition of heparin/FGF-2 binding interactions; it may be through the alteration of another step in the FGFR-mediated signal transduction pathway, or through the alteration of an entirely different signal transduction pathway.

Galactose amide conjugates that are structurally related to **31** and **36** are weak inhibitors of an α-galactosidase (mM range). Indeed other compounds structurally related to the aromatic-saccharide conjugates described herein have been synthesized for evaluation as glycosidase inhibitors.[23] We considered the possibility that a possible mode of action for **31** might include glycosidase inhibition; however 1-deoxynojirimycin was found to be inactive as an inhibitor of BAEC growth.[24] It is known that glycosidase inhibitor castanospermine is an angiogenesis inhibitor but neither it nor *N*-methyl-1-deoxynojirimycin altered the proliferation of cultured endothelial cells when evaluated previously.[25] These observations rule out the possibility that **20** and **31** are acting as glycosidase inhibitors.

The order of activity in BAEC assay of **31** = **20** > **32** may be suggesting that the compounds are inhibiting a glucose or glucuronide receptor, although this would need to be investigated in greater detail by evaluation of, for example, the galactose and mannose conjugates. Also the orientation of the thiophene with respect to the pyranose appears to be important for biological activity. The formal N-methylation of **31** to give **34** would alter aromatic group presentation with respect to the pyranose: secondary amides such as **31** would be expected to adopt the *Z*-anti structure (*trans* arrangement of the amide N–H and C=O and H_1–C_1–N–H dihedral angle approaching 180°), whereas the N-methylated compound would be expected to adopt preferentially *E*-anti structures; these are based on comparisons with related benzamides.[26] The NMR spectra of **34** revealed, as expected, that there were two signal sets;[25] these correspond with the interconverting amide rotamers **34a** (*Z*-anti, 33%) and **34b** (*E*-anti, 66%), the relative proportions being quantified by integration.[25] The lack of activity in the cell growth assay of **34** is thus explained by the *E*-anti isomer **34b** adopting preferentially the orthogonal arrangement of the thiophene and pyranose groups and the aromatic group of the *Z*-anti isomer **34a** being forced out of conjugation with the carbonyl group due to the steric interaction between the *N*-methyl group and aromatic protons.

2.3. Summary

The strategy to synthesize a collection of structurally diverse monosaccharide conjugates has provided novel compounds that alter BAEC proliferation. Such biological activities for such simple glycoconjugates have not been previously observed, to the best of our knowledge. The active compounds have low molecular weights and the synthesis of derivatives for further biological evaluation would be straightforward. The selectivity displayed by compounds in inhibiting endothelial cell proliferation but not epithelial cell proliferation seems significant and worthy of further investigation. The determination of the details of the biological mode of action of the saccharide conjugates could be important as it may yield new targets for modulating signal transduction pathways. Such mechanistic studies are underway as well as attempts to improve the potency of the thiophene derivatives and the results will be reported in due course.

3. Experimental section

3.1. General

Optical rotations were determined with a Perkin–Elmer 241 model polarimeter at the sodium D line at 23 °C. NMR spectra were recorded with JEOL JNM-GX270, Varian Inova 300 and 500, and Bruker DRX-600 MHz spectrometers. Chemical shifts are reported relative to internal Me_4Si in $CDCl_3$ (δ 0.0) or HOD for D_2O (δ 4.80) for ^1H and (δ 77.0) for ^{13}C. ^{13}C signals were assigned with the aid of DEPT-135. ^1H signals were assigned with the aid of COSY. Coupling constants are reported in Hertz. IR spectra were recorded with a Mattson Galaxy Series FTIR 3000 using either thin film between NaCl plates or KBr discs, as specified. Melting points were measured on a Gallenkamp melting point apparatus. Elemental analysis was performed on an Exeter Analytical CE440 elemental analyzer. Low and high-resolution mass spectra were measured at the University of York, UK or on a Micromass LCT KC420 or Micromass Quattro. TLC was performed on aluminium sheets precoated with Silica Gel 60 (HF254, E. Merck) and spots visualized by UV and charring with 1:20 H_2SO_4–EtOH. Flash column chromatography was carried out with Silica Gel 60 (0.040–0.630 mm, E. Merck) and employed a stepwise solvent polarity gradient correlated with the TLC mobility. Chromatography solvents used were EtOAc, MeOH, acetone and CH_2Cl_2 (Riedel-de Haen), petroleum ether (bp 40–60 °C, BDH laboratory supplies) and toluene (Aldrich). Analytical HPLC separations were performed using a Waters 600E pump and Waters 486 tuneable absorbance detector or Shimadzu LC10AT pump and Shimadzu SPD10A tuneable absorbance detector. All compounds were purified by semi-prep HPLC using a Waters 600E pump before biological evaluation (MeCN–water mixtures were used as eluant with a flow rate 10 mL/min). The semi-preparative columns used were YMC-Pack C-4 rev phase (S-10μm, 250 × 20 mm). Wavelength for both analytical and semi-preparative HPLC was 220 nm. Reaction solvents were dried and distilled where stated. The carboxylic acids used for the library synthesis are sold as DiversiChem® Building Block Kits by NovaBiochem. Heparan sulfate was obtained from Sigma (cat no. H9902, av MW 14,200) and heparin from Fluka (cat. no. 51536).

3.2. Preparation of *O*-glycosides

3.2.1. A soln of bromide **42**[27] (1.0 equiv) in CH_2Cl_2 was added to a stirred soln of the appropriate alcohol (0.66 equiv), silver carbonate (2.25 equiv), silver perchlorate (0.1 equiv) in CH_2Cl_2 over activated 4 Å molecular sieves, under nitrogen. After 12 h the reaction was diluted with CH_2Cl_2, filtered through Celite, washed with water, dried (sodium sulfate anhyd) and the solvent removed under diminished pressure. The residue was purified by chromatography.

3.2.2. The alcohol (1.0 equiv) and trichloroacetimidate **41**[28] (1.0 equiv) in anhyd CH_2Cl_2 over activated 4 Å molecular sieves was placed under a nitrogen atmosphere and cooled to −15 °C. Boron trifluoride diethyl

etherate (0.5 equiv in CH_2Cl_2) was added and the mixture stirred for 12 h at rt. The reaction mixture was then diluted with CH_2Cl_2, washed with Na_2CO_3 (aq), the organic phase was dried (sodium sulfate anhyd), the solvent removed under diminished pressure and the residue purified by chromatography.

3.3. Preparation of acid chlorides

Thionyl chloride (2 equiv) was added to the carboxylic acid (1 equiv) in anhyd toluene at 0 °C. The reaction mixture was then heated at 70 °C for 3 h. The acid chlorides were purified by distillation.

3.4. Synthesis of amides from azide 43[29]

To azide **43**[30] (1 equiv) and the acid chloride (2 equiv), dissolved in anhyd MeCN (4 mL), was added dropwise a soln of PPh_3 (1.3 equiv) in anhyd CH_2Cl_2 (1 mL) at rt. The reaction was allowed to stir for 12 h, diluted with CH_2Cl_2 (20 mL), washed with satd $NaHCO_3$ (10 mL) and then with water (10 mL). The organic phase was dried (sodium sulfate anhyd), the solvent removed under diminished pressure and the residue purified by chromatography.

3.5. Synthesis of amides from amine 44

Reaction of azide **43**[30] with Pd/C in anhyd THF at −5 °C under H_2 gave amine **44** and its α-anomer in a 13:1 ratio after filtration and removal of solvent; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.30 (t, 1H, J 9.6), 5.16 (t, 1H, J 9.6), 4.86 (t, 1H, J 9.6), 4.23 (d, 1H, J 8.6), 4.04 (d, 1H, J 10.0), 3.75–3.72 (overlapping signals, 5H), 2.07, 2.03, 2.02 (each s, each 3H). This freshly prepared amine **44** (1 equiv) was added to a soln of the carboxylic acid (1.2 equiv), HOBT (1.2 equiv), DMAP (1.2 equiv) and DCC (1.2 equiv) in anhyd THF at 0 °C. The reaction mixture was then stirred at rt for 12 h, the precipitated dicyclohexyurea was filtered off and the solvent removed under diminished pressure. The residue was dissolved in CH_2Cl_2, washed with $NaHCO_3$ (aq), brine and dried (sodium sulfate anhyd). The organic phase was then concentrated under diminished pressure, and the residue purified by chromatography.

3.6. Deprotection of glucuronic acid conjugates

A soln of 0.05 M LiOH in 2.5:1.0:0.5 MeOH–water–THF (6 equiv) was added to the glycosylamide or *O*-glycoside (1 equiv). The soln was stirred at 0 °C until complete deprotection (TLC, 3:1 EtOAc–MeOH). The soln was then diluted with water and the pH adjusted to 3.0 with Amberlite IR120 (H^+). The Amberlite was removed by filtration and the MeOH and THF were removed under diminished pressure. The resulting aq soln was freeze dried and the residue purified by flash chromatography and then semi-preparative C-4 RP HPLC (flow rate: 10 mL/min) to give, after freeze drying, the desired compound.

3.7. 1,4-Dioxaspirol[4,5]dec-2-yl-β-D-glucopyranosiduronic acid (5)

(1,4-Dioxaspirol[4,5]dec-2-yl)-2,3,4-tri-*O*-acetyl-β-D-glucopyranosiduronic acid, methyl ester (235 mg, 41%) was prepared as in Section 3.2.1. This intermediate (85 mg, 0.17 mmol) was deprotected as described in Section 3.6 to afford **5** as a gum (39 mg, 66%); $[\alpha]_D$ −22.7 (c 0.30, MeOH); IR (KBr) cm^{-1} 3424, 2928, 1617, 1420, 1284, 1054, 663; ^{1}H NMR (D_2O, 300 MHz): δ 4.56–4.53 (m, 2H), 4.51–3.37 (m, 7H), 3.46–3.36 (m, 1H), 1.80 (m, 10H); ^{13}C NMR (D_2O, 125 MHz): δ 178.6, 114.0, 113.9 (each s), 105.1, 105.0, 78.8, 78.1, 77.0, 76.6, 75.7, 75.6, 74.5 (each d), 73.4, 72.8, 67.7, 38.1, 38.0, 36.4, 27.2 (each t); ESIHRMS: m/z $[M+Na]^+$: calcd 371.1336, found 371.1318.

3.8. (3-Benzoylphenyl)-β-D-glucopyranosiduronic acid (6)

The reaction of 3-benzoylphenol with **42** gave the protected precursor (269 mg, 52%); a portion (153 mg, 0.29 mmol) was deprotected to give **6** as an off-white solid (50 mg, 46%); $[\alpha]_D$ −4.2 (c 0.26, MeOH); IR (KBr) cm^{-1} 3400, 2907, 1625, 1435, 1204, 1062; ^{1}H NMR (D_2O, 300 MHz): δ 7.68–7.38 (m, 9H), 5.05 (br s, 1H), 3.79 (br s, 1H), 3.69 (m, 3H); ^{13}C NMR (D_2O, 125 MHz): δ 202.6, 178.0 (each s, C=O), 159.0, 140.8, 139.1 (each s), 136.4, 133.0, 132.8, 131.3, 127.7, 124.5, 120.8 (each d), 102.5 (d, C-1), 78.9, 78.1, 75.4, 74.5 (each d); ESIHRMS: m/z $[M+Na]^+$: calcd 397.0899, found 397.0978.

3.9. 1-Deoxy-1-[pyrazinylcarbonylamino]-β-D-glucopyranuronic acid (7)

The protected precursor was prepared (250 mg, 39%) from amine **44** and a portion (70 mg, 0.16 mmol) deprotected to give **7** as an off-white solid (43 mg, 90%); $[\alpha]_D$ −11.6 (c 0.25, water); IR (KBr) cm^{-1} 3453, 3253, 2917, 1790, 1659, 1562, 1368, 1239, 1065, 1065, 1041; ^{1}H NMR (D_2O, 270 MHz): δ 7.95–7.40 (m, 3H), 5.25 (d, 1H, J 8.6), 3.88 (d, 1H, J 9.5), 3.66 (m, 3H); ^{13}C NMR (D_2O, 125 MHz): δ 175.4, 169.2 (each s), 150.5, 147.1 (each d), 146.8 (s), 146.3, 82.3, 79.2, 78.8, 74.2, 74.0 (each d); ESIHRMS: m/z $[M-H]^-$: calcd 298.0687, found 298.0687.

3.10. 1-(Benzoylamino)-1-deoxy-β-D-glucopyranuronic acid (8)

The protected intermediate (270 mg, 62%) was prepared from azide **43** and a portion deprotected (110 mg, 0.25 mmol) to give **8** as an off-white solid (54 mg, 72%); $[\alpha]_D^{20}$ +2.5 (c 0.62, water); IR (film) cm^{-1} 3399 (OH),

2900, 1649 (C=O), 1644 (C=O), 1258; ^{1}H NMR (D_2O, 270 MHz): δ 8.00 (m, 5H), 5.18 (d, 1H, J 8.2), 4.01 (d, 1H, J 9.0), 3.50 (m, 3H); ^{13}C NMR (D_2O, 125 MHz): δ 176.0, 174.6, 149.9 (each s), 135.6, 131.6, 130.3, 82.6, 79.6, 78.9, 74.2, 74.1 (each d); ESIHRMS: m/z $[M+Na]^+$: calcd 320.0746, found 320.0757.

3.11. 1-Deoxy-1-[[(tetrahydro-2-furanyl)carbonyl]amino]-β-D-glucopyranuronic acid (9)

The protected precursor (224 mg, 35%) was prepared from azide **44** and a portion (52 mg, 0.12 mmol) deprotected to give **9** as an off-white solid (23 mg, 68%); $[\alpha]_D$ −18.7 (c 0.08, MeOH); IR (KBr) cm^{-1} 3429, 2921, 2343, 1536, 1424, 1092, 668; ^{1}H NMR (D_2O, 300 MHz): δ 4.95 (d, 1H, J 8.0), 4.42 (d, 1H, J 8.3), 3.95 (dd, 1H, J 6.0, 14.0), 3.85 (dd, 1H, J 6.5, 14.0), 3.75 (d, 1H, J 9.0), 3.50 (m, 3H), 2.28 (m, 1H), 2.02–1.84 (m, 3H); ^{13}C NMR (D_2O, 125 MHz): δ 180.3, 178.5 (each s), 81.7, 80.5, 79.0, 74.5, 74.4, 72.7 (each d), 72.7, 33.0, 27.8 (each t); ESIHRMS: m/z $[M+Na]^+$: calcd 314.0852, found 314.0886.

3.12. 1-[Cyclopropylcarbonylamino]-1-deoxy-β-D-glucopyranuronic acid (10)

The protected precursor (910 mg, 48%) was prepared from azide **44** and a portion (95 mg, 0.23 mmol) deprotected to give **10** as an off-white solid (52 mg, 87%); $[\alpha]_D$ −20.4 (c 0.054, MeOH); IR (KBr) cm^{-1} 3374, 1569, 1415, 1069, 946, 649; ^{1}H NMR (D_2O, 270 MHz): δ 4.82–4.79 (d, 1H, J 9.0), 3.60 (d, 1H, J 9.5), 3.32–3.24 (m, 3H), 1.65–1.47 (m, 1H), 0.74 (d, 4H); ^{13}C NMR (D_2O, 125 MHz): δ 181.2, 178.5 (each s), 79.0, 77.6, 76.1, 71.6 (2s) (each d), 17.0 (t), 10.6, 10.3; ESIHRMS: m/z $[M+Na]^+$: calcd 284.0724, found 284.0746.

3.13. 1-Deoxy-1-[(3-methyl-1-oxo-2-butenyl)amino]-β-D-glucopyranuronic acid (11)

The protected precursor (273 mg, 33%) was prepared from azide **44** and a portion (100 mg, 0.24 mmol) deprotected to give **11** as an off-white solid (62 mg, 93%); $[\alpha]_D$ −45.6 (c 1.1, MeOH); IR (KBr) cm^{-1} 3423, 2922, 1627, 1419, 1266, 1683, 945, 655; ^{1}H NMR (D_2O, 300 MHz): δ 5.82 (s, 1H), 5.05 (d, 1H, J 9.0, 1H), 4.03 (d, 1H, J 9.0), 3.68–3.42 (m, 3H), 1.96 (s, 6H); ^{13}C NMR (D_2O, 125 MHz): δ 178.5, 173.4 (each s, C=O), 158.6 (s, *C*=CH), 119.4 (d, C=*C*H), 81.8, 80.6, 79.0, 74.4, 74.4 (each d), 29.2, 22.4 (each q); ESIHRMS: m/z $[M+Na]^+$: calcd 298.0903, found 298.0903.

3.14. 1-Deoxy-1-[(4-pyridinylcarbonyl)amino]-β-D-glucopyranuronic acid (12)

The protected precursor was prepared from amine **44** (310 mg, 52%) and a portion (268 mg, 0.61 mmol) deprotected to give **12** as an off-white solid (118 mg, 65%); $[\alpha]_D$ −1.3 (c 0.6, water); IR (KBr) cm^{-1} 3401 (OH), 2960, 1889, 1634, 1551, 1418, 1300, 1087, 1027; ^{1}H NMR (D_2O, 270 MHz): δ 8.63 (s, 2H), 7.71 (d, 2H) 5.19 (d, 1H, J 8.2), 3.83 (d, 1H, J 9.1), 3.55 (m, 3H); ^{13}C NMR (D_2O, 125 MHz): δ 178.4, 172.2 (each s), 152.3, 152.3 (each d), 144.1 (s), 124.7, 82.3, 81.0, 79.0, 74.5, 74.4 (each d); ESIHRMS: m/z $[M+H]^+$: calcd 299.0879, found 299.0861.

3.15. 1-Deoxy-1-[(2-methyl-1-oxopentyl)amino]-β-D-glucopyranuronic acid (14)

The precursor (54 mg, 42%) was prepared from azide **44** (mixture of diastereoisomers, 1:1.2) and a portion (100 mg, 0.23 mmol) deprotected to give **14** as an off-white solid (56 mg, 85%); $[\alpha]_D$ −16.6 (c 0.04, MeOH); IR (KBr) cm^{-1} 3605, 1555, 1418, 1077, 1021, 639; ^{1}H NMR (D_2O, 270 MHz): δ 4.79 (m, 1H), 3.62 (m, 1H), 3.42–3.19 (m, 3H), 2.28–2.03 (m, 5H), 0.94 (m, 3H), 0.70 (m, 3H); ^{13}C NMR (D_2O, 125 MHz): δ 181.8, 181.7, 181.2, 175.5 (each s), 79.0, 78.9, 77.6, 76.2, 76.2, 71.6, 71.5, 40.5, 40.4 (each d), 35.5, 35.4, 19.7 (each t), 16.8, 16.7, 13.1, 13.1 (each q); ESIHRMS: m/z $[M+Na]^+$: calcd 314.1225, found 314.1216.

3.16. 1-Deoxy-1-[[(2,4-dichlorophenoxy)acetyl]amino]-β-D-glucopyranuronic acid (15)

The protected precursor (250 mg, 12%) was prepared from **44** and a portion (160 mg, 0.3 mmol) deprotected to give **15** as an off-white solid (98 mg, 83%); $[\alpha]_D$ −7.3 (c 0.06, MeOH); IR (KBr) cm^{-1} 3452 (OH), 2386, 2569, 1417, 1238, 1078, 1019, 604; ^{1}H NMR (D_2O, 270 MHz): δ 7.38 (s, 1H), 7.15 (d, 1H, J 9.0), 6.86 (d, 1H, J 9.0), 4.91 (d, 1H, J 9.2), 3.65 (d, 1H, J 9.5), 3.41–3.32 (m, 3H); ^{13}C NMR (D_2O, 125 MHz): δ 181.9, 175.4, 151.7 (each s), 129.8, 128.0 (each d), 126.7, 123.1 (each s), 115.5, 78.7, 77.8, 76.1, 71.6, 71.5 (each d), 67.8 (t); ESIHRMS: m/z $[M-H]^-$: calcd 394.0096, found 394.0102.

3.17. 1-Deoxy-1-[(1*H*-indol-2-ylcarbonyl)amino]-β-D-glucopyranuronic acid (16)

The protected precursor was prepared from amine **44** (385 mg, 54%) and a portion (100 mg, 0.21 mmol) deprotected to give **16** as an off-white solid (5.6 mg, 10%). IR (KBr) cm^{-1} 3421, 2936, 2359, 1975, 1623, 1559, 1410, 1224, 1072, 882; $[\alpha]_D$ +5.0 (c 0.026, MeOH); ^{1}H NMR (D_2O, 300 MHz): δ 7.73 (m, 1H), 7.55 (m, 1H), 7.35 (m, 1H), 7.26 (s, 1H), 5.20 (d, 1H, J 8.9), 3.83 (d, 1H, J 9.3), 3.60 (m, 3H); ^{13}C NMR (D_2O, 125 MHz): δ 178.5, 167.2, 139.8, 132.6, 129.8 (each s), 128.0, 125.2, 123.6 (each d), 115.2 (s), 108.3, 82.3, 80.9, 79.1, 74.6, 74.5 (each d); ESIHRMS: m/z $[M+Na]^+$: calcd 359.0889, found 359.0855.

3.18. 1-Deoxy-1-[[3-(trifluoromethyl)benzoyl]amino]-β-D-glucopyranuronic acid (17)

The protected precursor was prepared from amine **44** (115 mg, 38%) and a portion (60 mg, 0.12 mmol) deprotected to give **17** as an off-white solid (30 mg, 83%); $[\alpha]_D$ −12.3 (*c* 0.39, water); IR (KBr) cm^{-1} 3403, 2903, 1723, 1661, 1551, 1330, 1128, 1074; ^1H NMR (D_2O, 270 MHz): δ 8.13–7.55 (m, 4H), 5.19 (d, 1H, *J* 8.4), 3.83 (d, 1H, *J* 9.2), 3.53 (m, 3H); ^{13}C NMR (D_2O, 125 MHz): δ 173.0, 170.5, 133.8, 131.4 (each s), 129.9, 129.5, 124.8, 124.7, 80.1, 76.6, 76.4, 71.7, 71.5 (each d); ESIHRMS: *m/z* $[M+Na]^+$: calcd 388.0620, found 388.1421.

3.19. 1-Deoxy-1-[[3-(2-furanyl)-1-oxo-2-propenyl]amino]-β-D-glucopyranuronic acid (18)

The protected precursor (82 mg, 40%) was prepared from azide **44** and a portion (150 mg, 0.33 mmol) deprotected to give **18** as an off-white solid (73 mg, 71%); $[\alpha]_D$ −7.5 (*c* 6.9, MeOH); IR (KBr) cm^{-1} 3422, 2925, 1618, 1566, 1419, 1283, 1073, 883, 749; ^1H NMR (D_2O, 300 MHz): δ 7.88 (br s, 1H), 7.68 (d, 1H, *J* 15.0), 7.25 (d, 1H, *J* 3.0), 6.83 (t, 1H, *J* 2.0), 6.70 (d, 1H, *J* 15.0), 5.28 (d, 1H, *J* 9.0), 4.01 (d, 1H, *J* 9.0), 3.86–3.65 (m, 3H); ^{13}C NMR (D_2O, 125 MHz): δ 178.5, 173.4, 172.3, 153.3, 152.8 (each s), 148.2, 147.3, 132.7, 129.0, 119.8, 119.1, 118.5, 118.0, 115.4, 115.0, 82.1, 81.9, 80.7, 79.0, 74.6, 74.5, 74.0 (each d); ESIHRMS: *m/z* $[M+H]^+$: calcd 314.0876, found 314.0867.

3.20. 1-Deoxy-1-[[3-(1*H*-indol-3-yl)-1-oxopropyl]amino]-β-D-glucopyranuronic acid (19)

The protected precursor (286 mg, 37%) was prepared from amine **45** and a portion (50 mg, 0.1 mmol) deprotected to give **19** as an off-white solid (29 mg, 81%); $[\alpha]_D$ −10.26 (*c* 1.49, water); IR (KBr) cm^{-1} 3420, 2920, 2358, 1578, 1417, 1076, 893, 512; ^1H NMR (D_2O, 300 MHz): δ 7.74 (d, 1H, *J* 7.3), 7.55 (d, 1H, *J* 9.2), 7.48–7.06 (m, 3H), 5.0 (d, 1H, *J* 9.0), 3.71 (d, 1H, *J* 9.0), 3.68–3.49 (m, 2H), 3.43 (t, 1H, *J* 9.0), 3.15 (t, 2H, *J* 7.3), 2.75 (t, 2H, *J* 7.5); ^{13}C NMR (D_2O, 125 MHz): δ 181.7, 177.8, 136.4, 126.8 (each s), 123.3, 122.1, 119.3, 118.8, 113.5 (each d), 112.2 (s), 79.3, 78.1, 76.5, 72.0, 71.9 (each d), 36.9, 20.8 (each t); ESIHRMS: *m/z* $[M+Na]^+$: calcd 387.1168, found 387.1188.

3.21. 1-Deoxy-1-[(2-thienylcarbonyl)amino]-β-D-glucopyranuronic acid (20)

The protected precursor was prepared from azide **44** (260 mg, 53%) and a portion (159 mg, 0.36 mmol) deprotected to give **20** as an off-white solid (90 mg, 83%); $[\alpha]_D$ −21.1 (*c* 0.21, water); IR (KBr) cm^{-1} 3400, 2928, 1913, 1729, 1655, 1614, 1545, 1419, 1296, 1089, 1024; ^1H NMR (D_2O, 270 MHz): δ 7.79–7.15 (m, 3H), 5.15 (d, 1H, *J* 8.4), 3.82 (d, 1H, *J* 9.0), 3.56 (m, 3H); ^{13}C NMR (D_2O, 125 MHz): δ 178.4, 168.0, 139.1 (each s), 135.4, 133.5, 131.0 (each d), 82.4 (d), 80.9, 78.9, 74.5, 74.4 (each d); ESIHRMS: *m/z* $[M+H]^+$: calcd 304.0491, found 304.0529.

3.22. 1-Deoxy-1-[(3,5-dimethylbenzoyl)amino]-β-D-glucopyranuronic acid (21)

The precursor was prepared from azide **44** (560 mg, 56%) and a portion (276 mg, 0.59 mmol) deprotected to give **21** as an off-white solid (162 mg, 85%); $[\alpha]_D$ −10.5 (*c* 0.2, water); IR (KBr) cm^{-1} 3431 (OH), 2940, 1723, 1661, 1535, 1312, 1245; ^1H NMR (D_2O, 270 MHz): δ 7.41–7.21 (m, 3H), 5.13 (d, 1H, *J* 8.6), 3.81 (d, 1H, *J* 9.1), 3.52 (m, 3H), 1.86 (s, 6H); ^{13}C NMR (D_2O, 125 MHz): δ 178.5, 174.5 (each s), 142.0, 136.4, 135.5, 128.9, 82.5 (d, C-1), 80.6, 79.0, 74.4 and 74.2 (each d), 33.3 (q); ESIHRMS: *m/z* $[M+Na]^+$: calcd 326.1240, found 326.1359.

3.23. 1-[([1,1′-Biphenyl]-4-ylacetyl)amino]-1-deoxy-β-D-glucopyranuronic acid (22)

The protected precursor (0.59 g, 32%) was prepared from azide **44** and a portion (52 mg, 0.1 mmol) deprotected to give **22** as an off-white solid (35 mg, 91%); $[\alpha]_D$ −76.3 (*c* 0.08, MeOH); IR (KBr) cm^{-1} 3365, 2912, 1849, 1658, 1449, 1603, 1449, 1024; ^1H NMR (D_2O, 300 MHz): δ 7.71–7.40 (m, 9H), 5.0 (d, 1H, *J* 9.0), 3.85 (d, 1H, *J* 9.0), 3.73 (s, 2H), 3.73–3.51 (m, 2H), 3.44 (t, 1H, *J* 9.0); ^{13}C NMR (D_2O, 125 MHz): δ 178.2, 177.2, 143.0, 142.3, 136.6 (each s), 132.6, 131.9, 130.4, 130.0, 129.6, 82.2, 80.0, 79.1, 74.5, 74.4 (each d), 44.7 (t); ESIHRMS: *m/z* $[M+Na]^+$: calcd 410.1196, found 410.1216.

3.24. 1-Deoxy-1-[(2-naphthalenylcarbonyl)amino]-β-D-glucopyranuronic acid (23)

The protected precursor (208 mg, 59%) was prepared from amine **44** and a portion (60 mg, 0.12 mmol) deprotected to give **23** as an off-white solid (31 mg, 76%); $[\alpha]_D$ 47.5 (*c* 0.18, water); IR (KBr) cm^{-1} 3447, 1658, 1596, 1422, 1077, 1024; ^1H NMR (D_2O, 300 MHz): δ 8.37 (s, 1H), 7.97 (m, 3H), 7.81 (m, 1H), 7.60 (m, 2H), 5.20 (d, 1H, *J* 8.8), 3.83 (d, 1H, *J* 9.5), 3.54 (m, 3H); ^{13}C NMR (D_2O, 75 MHz): δ 178.0, 174.5, 137.7, 135.0, 133.0, 131.9, 131.4, 131.4, 131.3, 130.6, 130.1, 126.5, 82.7, 80.6, 79.1, 74.5 (2s); ESIHRMS: *m/z* $[M+H]^+$: calcd 348.1083, found 348.1063.

3.25. 1-[(1,4-Dioxo-4-phenylbutyl)amino]1-deoxy-β-D-glucopyranuronic acid (24)

The protected precursor (200 mg, 35%) was prepared from amine **44** and a portion (60 mg, 0.12 mmol) depro-

tected to give **24** as an off-white solid (25 mg, 76%); $[\alpha]_D$ −18.0 (*c* 0.05, water); IR (KBr) cm^{-1} 3441, 2910, 2343, 1647, 1448, 1211, 1038, 595; ^1H NMR (D_2O, 300 MHz): δ 8.06 (d, 2H), 7.75 (t, 2H, *J* 7.5), 7.62 (t, 2H, *J* 7.5), 5.06 (d, 1H, *J* 9.0), 3.89 (d, *J* 9.0), 3.68 (m, 4H), 2.81 (t, 2H, *J* 6.7); ^{13}C NMR (D_2O, 125 MHz): δ 203.5, 176.7, 175.9, 136.3 (each s), 134.3, 129.6, 129.1, 128.4, 128.4, 79.4, 78.0, 76.5, 72.0, 72.0 (each d), 33.8, 30.0 (each t, CH_2); ESIHRMS: *m*/*z* $[M+Na]^+$ calcd 376.1008, found 376.0999.

3.26. 1-Deoxy-1-[(3,4-difluorobenzoyl)amino]-β-D-glucopyranuronic acid (25)

The protected precursor (323 mg, 62%) was prepared from amine **44** and portion (162 mg, 0.34 mmol) deprotected to give **25** as an off-white solid (99 mg, 87%); $[\alpha]_D$ −17.7 (*c* 0.62, MeOH); IR (KBr) cm^{-1} 3412, 2940, 1759, 1662, 1597, 1512, 1431, 1378, 1228, 1074; ^1H NMR (D_2O, 270 MHz): δ 7.76 (m, 1H), 7.65 (m, 1H), 7.34 (m, 1H), 5.16 (d, 1H, *J* 8.0), 3.82 (d, 1H, *J* 9.3), 3.56 (m, 3H); ^{13}C NMR (D_2O, 300 MHz): δ 177.6, 172.3, 156.6, 153.6, 132.9, 127.6, 120.7, 120.1, 82.1, 80.4, 79.1, 74.4, 74.4; ESIHRMS: *m*/*z* $[M-H]^-$: calcd 332.0582, found 332.0576.

3.27. 1-[[(4-Chlorophenyl)acetyl]amino]-1-deoxy-β-D-glucopyranuronic acid (27)

The protected precursor (99 mg, 51%) was prepared from azide **43** and a portion (92 mg, 0.19 mmol) deprotected to give **27** as an off-white solid (53 mg, 82%); $[\alpha]_D$ −19.6 (*c* 0.12, MeOH); IR (KBr) cm^{-1} 3141, 1650, 1567, 1494, 1418, 1302, 736; ^1H NMR (D_2O, 300 MHz): δ 7.54 (d, 2H, *J* 8.5), 7.25 (d, 2H, *J* 8.5), 5.18 (d, 1H, *J* 9.0), 3.99 (d, 1H, *J* 9.0), 3.91 (br s, 2H, CH_2), 3.78 (m, 2H), 3.68 (t, 1H, *J* 9.0); ^{13}C NMR (D_2O, 125 MHz): δ 175.8, 174.7 (each s, C=O), 134.0, 132.7 (each s), 131.5, 129.0, 79.7, 77.9, 76.7, 72.1, 72.1 (each d), 41.9 (t); ESIHRMS: *m*/*z* $[M+Na]^+$: calcd 346.0694, found 346.0700.

3.28. 1-Deoxy-1-[[(3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-yl)carbonyl]amino]-β-D-glucopyranuronic acid (28)

The protected precursor (466 mg, 49%) was prepared from amine **44** and a portion (80 mg, 0.13 mmol) deprotected to give **28** as an off-white solid (47 mg, 84%); $[\alpha]_D$ −7.6 (*c* 0.05, MeOH); IR (KBr) cm^{-1} 3435, 2921, 2490, 1727, 1619, 1443, 1216, 1033, 836, 720; ^1H NMR (D_2O, 300 MHz): δ 8.24 (d, 1H, *J* 6.5), 8.07 (d, 1H), 7.82–7.56 (m, 2H), 7.56–7.51 (m, 4H), 5.16 (d, 1H, *J* 9.0), 3.79 (d, 1H, *J* 9.5), 3.81–3.47 (m, 2H), 3.36 (t, 1H, *J* 9.0), 2.09 (s, 3H); ^{13}C NMR (D_2O, 125 MHz): δ 183.1, 170.5, 165.3, 155.5 (each s), 137.2 (d), 134.8 (s), 133.9, 131.9, 131.8, 131.4, 131.3, 131.3, 128.5 (each d), 126.8, 124.6, 120.2, 118.0 (each s), 82.5, 79.3, 79.0, 74.6, 74.0 (each d), 13.8 (q); ESIHRMS: *m*/*z* $[M-H]^-$: calcd 454.1138, found 454.1116.

3.29. 1-Deoxy-1-[(3,4,5-trimethoxybenzoyl)amino]-β-D-glucopyranuronic acid (29)

The protected precursor (620 mg, 96%) was prepared from azide **44** and a portion of this material (264 mg, 0.50 mmol) deprotected to give **29** as an off-white solid (118 mg, 62%); $[\alpha]_D$ −48.8 (*c* 0.29, water); IR (KBr) cm^{-1} 3409, 1524, 1585, 1416, 1238, 1128, 1077; ^1H NMR (D_2O, 270 MHz): δ 7.03 (s, 2H), 5.17 (d, 1H, *J* 8.6), 3.80 (d, 1H, *J* 10.4), 3.56 (m, 3H), 3.29 (s, 9H); ^{13}C NMR (D_2O, 125 MHz): δ 175.9, 170.6 (each s, each C=O), 140.5, 129.1 (each s), 106.9, 105.8, 80.1, 78.2, 76.6, 72.1, 71.9 (each d), 61.2, 56.5 (each q); ESIHRMS: *m*/*z* $[M+Na]^+$ calcd 410.1063, found 410.1149.

3.30. *N*-(β-D-Glucopyranosyl)-2-thiophenecarboxamide (31)

Thiophene-2-carboxylic acid (0.07 g, 0.58 mmol), DCC (0.14 g, 0.7 mmol) and DMAP (catalytic) were suspended in anhyd CH_2Cl_2 (20 mL) and the reaction mixture was allowed to stir at rt for 1 h. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosylamine[31] (0.2 g, 0.58 mmol) obtained from azide **45**[32] was then added and the reaction mixture was allowed to stir at rt; TLC analysis (EtOAc) showed that the reaction was complete after 24 h. The solvent was removed and the residue purified by chromatography (1:1 EtOAc–petroleum ether) to give the protected precursor as a white solid (0.1 g, 41%); ESIHRMS: *m*/*z* $[M+H]^+$: calcd 458.1119, found 458.1121. This amide (0.06 g, 0.13 mmol) was suspended in MeOH (5 mL) and NaOMe (0.1 mL of a 0.25 M soln) was added followed by another 0.1 mL portion of the NaOMe soln after 1.5 h. After 2 h Amberlite was added, the reaction mixture was filtered and the solvent removed to give **31** as white solid (20 mg, 50%), which was purified by HPLC. ^1H NMR (D_2O, 300 MHz): δ 7.89 (dd, 1H, *J* 1.1, *J* 3.9), 7.86 (dd, 1H, *J* 1.2, *J* 5.1), 7.29 (dd, 1H, *J* 3.8, *J* 5.1), 5.24 (d, 1H, *J* 8.5), 3.97 (dd, 1H, *J* 2.27, *J* 12.4), 3.83 (dd, 1H, *J* 5.0, *J* 12.4), 3.52–3.71 (m, 4H); ^{13}C NMR (D_2O, 125 MHz): δ 168.2, 139.2 (each s), 135.6, 133.7, 131.2, 82.8, 80.5, 79.4, 74.5, 72.1 (each d), 63.4 (t); FABHRMS *m*/*z* $[M+Na]^+$: calcd 318.0518, found 318.0519.

3.31. 1-Azido-2,3-di-*O*-acetyl-1,4-dideoxy-α-L-*threo*-hex-4-enopyranuronic acid, methyl ester (46)

Azide **43** (2.00 g, 5.5 mmol) was dissolved in CH_2Cl_2 (20 mL), cooled to 0 °C and DBU (0.9 mL, 6.1 mmol) added. The reaction mixture was stirred for 40 min at 0 °C and then applied directly to a silica-gel column,

which was eluted with an 1:3 EtOAc–petroleum ether and gave **46** as a colourless oil (1.10 g, 67%); $[\alpha]_D$ −69.3 (*c* 2.1, CH_2Cl_2); ^1H NMR ($CDCl_3$, 300 MHz): δ 6.24 (m, 1H), 5.58 (m, 1H), 5.26 (m, 1H), 5.04 (m, 1H), 3.86 (s, 1H), 2.12 and 2.11 (2×s, each 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.1, 169.5, 161.7, 143.0 (each s), 107.7, 85.3, 68.2 and 64.3 (each d), 53.0, 21.0 and 20.9 (each q); CIHRMS: *m/z* $[M+NH_4]^+$: calcd 317.1097, found 317.1098.

3.32. 2,3-Di-*O*-acetyl-1,4-dideoxy-1-[2-thienylcarbonyl-amino]-α-L-*threo*-hex-4-enopyranuronic acid, methyl ester (47)

Triphenylphosphine (0.37 g, 1.4 mmol) was added to a soln of **46** (0.34 g, 1.1 mmol) and 2-thiophenoyl chloride (0.32 g, 2.2 mmol) in anhyd MeCN (12 mL) and stirred at rt for 16 h under a nitrogen atmosphere. The reaction was diluted with CH_2Cl_2 (~10 mL) and washed with satd $NaHCO_3$ soln (2×25 mL) and water (2×25 mL). The organic layer was dried ($MgSO_4$) and the soln was concentrated under diminished pressure. The residue was purified by chromatography (1:3 EtOAc–petroleum ether) to give **47** as a clear oil (0.25 g, 59%); $[\alpha]_D$ +21.1 (*c* 0.33, $CHCl_3$); IR (film) cm^{-1} 3395, 2924, 1742, 1660, 1543, 1239 and 1112; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.55 (m, 2H), 7.25 (m, 1H), 7.10 (m, 1H), 6.24 (m, 2H), 5.60 and 5.20 (each m, each 1H), 3.80 (s, 3H), 2.20 and 2.40 (each s, each 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.0, 169.0, 162.0 and 161.0 (each s), 144.5 (s), 137.5 (s), 132.0, 129.9, 127.8, 106.0, 79.0, 69.0 (each d), 53.0 (q), 21.0 (2s) (q); CIHRMS: *m/z* $[M+NH_4]^+$: calcd 401.1019, found 401.1022.

3.33. 1,4-Dideoxy-1-[2-thienylcarbonylamino]-α/β-L-*threo*-hex-4-enopyranuronic acid (32)

Ester **47** (41.0 mg, 0.11 mmol) was treated with 0.05 M LiOH as described above to afford the title compound **32** as an off-white solid [24.5 mg, 79%, α (64%) and β (36%) isomeric mixture]; $[\alpha]_D$ −39.3 (*c* 0.08, MeOH); ^1H NMR (CD_3OD, 300 MHz): δ 7.87, 7.75 and 7.17 (m, 6H), 6.11 (d, 1H, *J* 4.8), 6.02 (d, 1H, *J* 3.4), 5.78 (m, 2H), 4.32 (m, 1H), 4.10 (dd, 1H, *J* 4.9, *J* 2.5), 3.84 (m, 1H), 3.80 (m, 1H); ^{13}C NMR (CD_3OD, 125 MHz): δ 164.2, 139.5, 132.9, 132.8, 130.6, 129.1, 128.9, 111.7, 108.6, 80.6, 77.3, 71.1, 70.9, 69.5, 66.7; ESIHRMS: *m/z* $[M+Na]^+$: calcd 308.0205, found 308.0294.

3.34. 1-[Benzoylamino]-1,4-dideoxy-α/β-L-*threo*-hex-4-enopyranuronic acid (33)

Reaction of benzoyl chloride with azide **46** and deprotection as described above gave **33**; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.45, 7.43 and 7.30 (3s, 5H), 5.86 (m, 1H), 5.73 (d, 1H, *J* 3.1), 5.69 (d, 1H, *J* 8.2), 5.62 (d, 1H, *J* 1.3), 4.41 (m, 1H), 4.15 (m, 1H), 3.90 (m, 1H), 3.84 (m, 1H), 2.31 (s, 6H); ESIHRMS: *m/z* $[M-H]^-$: calcd 278.0665, found 278.0666.

3.35. *N*-(β-D-Glucopyranosyl)-*N*-methyl-2-thiophene-carboxamide (34)

To a soln of methylamine in MeOH (5.4 mL, 11.2 mmol) was added D-glucose (2.0 g, 11.2 mmol). The reaction mixture was heated to 60–65 °C for 30 min and then stirred at rt for 2 h. The solvent was removed under diminished pressure and the residue recrystallized from EtOH to yield *N*-methyl-β-D-glucopyranosylamine (off-white solid). Sodium carbonate (0.42 g, 4.03 mmol) was added to a soln of the amine (0.30 g, 1.55 mmol) in MeOH (15 mL) and this was cooled to 0 °C and thiophene-2-carbonyl chloride (0.45 mL, 3.10 mmol) was added dropwise over 5 min. The ice bath was then removed and the reaction mixture was allowed to stir at rt for 30 min. The solvent was removed and the residue purified by chromatography (1:4 MeOH–EtOAc) to give the title compound as a white solid (0.29 g, 63%); $[\alpha]_D$ −0.78 (*c* 0.9, MeOH); ^1H NMR (D_2O, 300 MHz; *Z*/*E*, 33:66): δ 7.62, 7.44, 7.07 (m, 6H), 5.40 (br s, 1H, H-1, *Z*-isomer), 5.01 (br s, 1H, H-1, *E*-isomer), 3.83–3.00 (m, 18H); ^{13}C NMR (D_2O): δ 171.6 (2×s, C=O, *E*- and Z-isomers), 137.5 (s), 133.8, 130.5 130.4 (each d), 90.4 (d, C-1, *E*-isomer), 86.0 (d, C-1, *Z*-isomer), 80.3, 79.9, 79.0, 78.9, 78.8, 72.2, 71.8, 71.4 (each d), 63.0 (t), 34.0 (q, *Z*-isomer), 31.3 (q, *E*-isomer); ESIHRMS: *m/z* $[M-H]^-$: calcd 302.0698, found 302.0690.

3.36. *N*-(β-D-Glucopyranosyl)-pyridine-4-carboxamide (35)

Isonicotinic acid (0.13 g, 1.09 mmol), EDC (0.21 g, 1.09 mmol) and DMAP (0.13 g, 1.09 mmol) were suspended in anhyd THF (10 mL) and the reaction mixture was stirred at 0 °C for 5 min. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosylamine[33] (0.38 g, 1.09 mmol) was then added and the reaction mixture stirred at rt. TLC analysis (EtOAc) showed that the reaction was complete after 12 h. The soln was diluted with EtOAc (20 mL) and washed with water (2×20 mL), brine and dried (sodium sulfate). The solvent was removed and the residue purified by chromatography (1:1 EtOAc–petroleum ether) to give the target compound as a white solid (0.21 g, 43%). This intermediate (70 mg, 0.15 mmol) was suspended in MeOH (5 mL) and NaOMe (0.1 mL of a 0.25 M soln) was added. Analysis by TLC (1:4 MeOH–EtOAc) showed that the reaction was complete after 2 h. The mixture was then filtered and the solvent removed to give 35 as a clear oil (61%). CIHRMS: *m/z* $[M+Na]^+$: calcd 307.0906, found 307.0898.

3.37. *N*-(β-D-Glucopyranosyl)benzamide (36)

The title compound (0.04 g, 100%) was obtained when benzoyl chloride was reacted with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide[34] and PPh_3 and subsequent removal of the protecting groups by reaction with catalytic NaOMe in MeOH as described above for related compounds; mp 218–220 °C [lit.[36] mp 230–232 °C]; R_f 0.18 (1:4 MeOH–EtOAc); $[\alpha]_D$ −45.0 (*c* 0.04, water) [lit.[35] $[\alpha]_D$ −11.6 (*c* 0.6, water)]; FABHRMS: *m*/*z* $[M+Na]^+$: calcd 306.0952, found 306.0954.

3.38. 1,4-Dideoxy-1-[(3,5-dimethyl)benzoylamino]-α/β-L-*threo*-hex-4-enopyranuronic acid (37)

The protected precursor (50 mg, 0.1 mmol), prepared as described for **47**, was reacted with 0.05 M LiOH as described above to give **37** as an off-white solid (10 mg, 33%; mixture of α (66%) and β (33%) anomers); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.45, 7.43 and 7.30 (3s, 3H), 5.86 (m, 1H), 5.73 (d, 1H, *J* 3.1), 5.69 (d, 1H, *J* 8.2), 5.62 (d, 1H, *J* 1.3), 4.41 (m, 1H), 4.15 (m, 1H), 3.90 (m, 1H), 3.84 (m, 1H), 2.31 (s, 6H) ; ESIHRMS: *m*/*z* $[M-H]^-$: calcd 306.0978, found 306.0990.

3.39. *N*-(Methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranuronosyl)amide-isophthalamic acid (48)

To a vigorously stirred soln of amine **44** (0.5 g, 1.5 mmol) in DMF (10 mL) at rt, was added isophthalic acid (0.5 g, 3.0 mmol), HOBt (0.2 g, 1.5 mmol), and EDC (0.3 g, 1.5 mmol) successively. Triethylamine (8 drops) was added and the mixture stirred and TLC analysis (EtOAc) showed the reaction was complete after 24 h. Excess solvent was removed and the residue was washed with water (3 × 50 mL), extracted with CH_2Cl_2 (3 × 50 mL), dried ($MgSO_4$), filtered and the solvent removed. Chromatography (1:2 EtOAc–petroleum ether) gave **48** as an off-white solid (0.29 g, 41%); $[\alpha]_D$ +40.0 (*c* 0.04, $CHCl_3$); IR (film) cm^{-1} 3070, 2988, 2331, 1757, 1675, 1439, 1376, 1265, 1223, 1039, 732; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.98 (d, 1H, *J* 9.5), 7.33–7.75 (m), 5.59 (t, 1H, *J* 9.5), 5.42 (t, 1H, *J* 9.5), 5.25 (t, 1H, *J* 9.5), 5.13 (t, 1H, *J* 9.5), 4.25 (d, 1H, *J* 9.5), 3.64 (s, 3H), 2.01, 1.97, 1.96 (each s, each 3H); ^{13}C NMR ($CDCl_3$): δ 171.5, 170.2, 169.8, 169.6, 167.6, 167.0 (each s, each C=O), 133.5, 130.2 (each s), 133.9, 133.1, 129.3, 129.0, 78.8, 74.2, 72.4, 70.9, 69.9 (each d), 53.1 (q), 20.9, 20.8, 20.7 (each q); ESIHRMS: *m*/*z* [M−H]: calcd 480.1142, found 480.1142.

3.40. Preparation of 38

1,5-Diaminopentane (0.1 mL, 0.11 mmol), HOBt (0.03 g, 0.21 mmol) and **48** (0.1 g, 0.21 mmol) were stirred in anhyd THF (15 mL) at 0 °C. DCC (0.21 mL, 0.21 mmol, of a 1.0 M soln in CH_2Cl_2) and DMAP (catalytic) were then added and the reaction mixture was allowed to stir at rt. TLC analysis (EtOAc) showed that the reaction was complete after 19 h. Excess solvent was removed and the residue extracted with CH_2Cl_2, washed with $NaHCO_3$ (3 × 50 mL) and water (3 × 50 mL), dried ($MgSO_4$), and the solvent removed. The residue was purified by chromatography (EtOAc) to give the protected precursor as an off-white foam [0.03 g, 28%, R_f 0.58 (1:1 EtOAc–petroleum ether)]. This intermediate was suspended in MeOH (10 mL), NaOMe (0.1 mL of a 0.25 M soln) was added and the mixture stirred at rt. After 40 min another 0.1 mL of NaOMe was added. To this soln was then added LiOH (1.6 mL of a 0.1 M soln in THF–water–MeOH). Analysis by TLC showed that the reaction was complete after a total of 3.5 h. The mixture was diluted with water (5 mL) and neutralized with Amberlite (H^+), filtered and the solvent removed to give the title compound as an orange oil (80 mg, quantitative); IR (film) cm^{-1} 3176, 3065, 2930, 2332, 1755, 1665, 1439, 1374, 1260, 1225, 1029, 732; ^1H NMR (D_2O, 300 MHz): δ 7.55–8.04 (m, 4H), 5.36 (br s, 1H), 3.60–4.31 (m, 4H), 3.50 (t, 2H, *J* 6.3), 1.49–1.78 (m, 4H); ^{13}C NMR (D_2O): δ 172.9, 172.3, 137.1, 135.7 (each s), 133.7, 133.3, 132.0, 128.9, 82.7, 79.0, 74.3, 74.1, 65.4 (each d), 42.2, 30.5, 25.6 (each t).

3.41. 3,6-Dioxaoctanedioic acid bis-*N*-(β-D-glucopyranuronosyl)-amide (39)

The protected precursor (0.1 g, 0.12 mmol) was prepared from amine **44** and then suspended in LiOH soln (2.87 mL, 1.44 mmol of a 0.1 M soln in 2.5:1.0:0.5 MeOH–water–THF) and the mixture was stirred at rt and TLC analysis (EtOAc) showed that the reaction to be complete after 1 h. The mixture was diluted with water (10 mL), neutralized with Amberlite (H^+), filtered and the solvent removed. The residue was purified by chromatography (MeOH) to yield the title compound **39** as an off-white solid (0.03 g, 52%); $[\alpha]_D$ −14.2 (*c* 0.04, water); IR (KBr) cm^{-1} 3433, 2924, 2853, 1691, 1606, 1420, 1275, 1072; ^1H NMR (D_2O, 300 MHz): δ 5.12 (d, 2H, $J_{1,2}$ 8.6), 4.28 (s, 4H), 3.90 (d, 1H, *J* 8.9), 3.89 (s, 2H), 3.56–3.69 (m, 3H); ^{13}C NMR (D_2O): δ 175.9, 174.0 (each s, each C=O), 79.0, 78.2, 76.5, 71.9 (2s) (each d), 70.6, 69.7 (each t); ESIHRMS: *m*/*z* $[M-H]^-$: calcd 527.1361, found 527.1384.

3.42. Octanedioic acid bis-(β-D-glucopyranuronosyl)-amide (40)

The protected precursor (0.1 g, 0.12 mmol) was prepared from amine **44** and then suspended in LiOH soln (2.87 mL, of a 0.1 M soln in 2.5:1.0:0.5 MeOH–water–THF, 1.44 mmol) and the mixture stirred at rt; TLC analysis (EtOAc) showed that the reaction was complete

after 1.5 h. The mixture was then diluted with water (10 mL), neutralized with Amberlite (H^+), filtered and the solvent removed. The residue was purified by chromatography (MeOH) to give **40** as a white solid (60 mg, 92%); $[\alpha]_D$ −78.8 (*c* 0.04, water); IR (KBr) cm^{-1} 3428, 3174, 2926, 1619, 1439, 1070, 1022; R_f 0.39 (EtOAc); ^1H NMR (D_2O, 300 MHz): δ 5.04 (d, 1H, *J* 9.1), 3.86 (d, 1H, *J* 9.4), 3.43–3.67 (m, 3H), 2.40 (t, 2H, *J* 7.4), 1.70 (t, 2H, *J* 7.0), 1.20 (br s, 2H); ^{13}C NMR (D_2O): δ 181.5 (s, CO_2H), 178.8, 176.0 (each s), 96.1, 92.4, 79.3, 76.5, 71.9 (2s) (each d), 35.9, 27.9, 25.0 (each t); ESIHRMS: *m/z* $[M-H]^-$: calcd 523.1775, found 523.1782.

3.43. Synthesis of heparin-albumin

Heparin [456 mg, 37.5 μmol; Fluka (cat. no. 51536)] and BSA [17 mg, 0.25 μmol; Fluka (cat. no. 05470)] were dissolved in 2.5 mL of 0.2 M potassium phosphate buffer, pH 8.0. Sodium cyanoborohydride (12.5 mg, 198.9 μmol) was then added and the mixture was incubated for 2 days at 37 °C. The mixture was dialyzed at rt against three changes of deionized water and freeze-dried to yield the heparin-albumin complex as a white solid (91 mg).

3.44. FGF-2 binding assay

A stock soln of heparin-albumin (5.0 mg/mL) was made up in distilled water and diluted to a final working concentration in a buffer containing 0.1 M Na_2CO_3 and 0.1 M $NaHCO_3$ and coated onto 96-well assay plates. Novel compounds, heparin-albumin and FGF-2 (100 ng/mL) were added to the wells in a 100 μL volume of distilled water and incubated for 4 h at 37 °C. Wells were then washed sequentially with PBS/0.05% T20 to remove any unbound protein and blot dried after each wash. Goat polyclonal IgG antibody was added 100 μL/well and incubated overnight at 37 °C. Wells were washed as before. The amount of bound protein retained in the wells was determined by ELISA using an alkaline phosphatase-conjugated rabbit anti-goat IgG heavy and light chain antibody. The ELISA absorbance readings were read at 405 nm. Results were analyzed using a nonlinear curve fitting programme (GraphPad PRISM).

3.45. Endothelial cell assay

BAEC were maintained in RPMI 1640 medium supplemented with 10% heat inactivated FCS, 25 mM glutamine, 75 U/mL penicillin and 75 μg/mL streptomycin. Cells were grown to confluency in 75 cm^2 tissue culture flasks and maintained at 37 °C in a humidified atmosphere containing 95% O_2 and 5% CO_2. Subcultures were created by passaging using a trypsin/EDTA (0.125%/0.05%) mixture in phosphate buffered saline (PBS), harvested by centrifugation (4 min at 210*g*) and seeded at the appropriate density. The methylthiazol tetrazolium (MTT) assay, adapted from that described in the literature,[36] was used to assess cell viability. Confluent monolayers of BAEC, grown in 24 well tissue culture plates were treated with test compounds (10 μg/mL) at the indicated concentration for 24 h at 37 °C. Following aspiration and washing with PBS, each well was incubated with MTT (0.45 mg/mL) in RPMI 1640 for 3 h at 37 °C. The overlying soln was then aspirated and the cells solubilized by the addition of 1 mL Me_2SO. Absorbance was measured at 590 nm and viability expressed as percentage of control (untreated) wells. Statistical significance of differences between group means was determined by ANOVA followed by a post-ANOVA Dunnett's test.

3.46. Epithelial cell assay

NmuMG cells were maintained in Dulbeccos modified Eagle medium (DMEM) supplemented with 10% foetal bovine serum, 4.5 g/L glucose and 10 μg/mL insulin. Cells were grown to confluency in 75 cm^2 tissue culture flasks and maintained at 37 °C in a humidified atmosphere containing 95% O_2 and 5% CO_2. Subcultures were created by passaging using a trypsin/EDTA (0.125%/0.05%) mixture in phosphate buffered saline (PBS), harvested by centrifugation (5 min at 1000 rpm) and seeded at the appropriate density. The methylthiazol tetrazolium (MTT) assay, was carried out as described above except that after incubation with MTT, cells were solubilized by the addition of 2.5 mL Me_2SO. Absorbance was measured at 590 nm and viability expressed as percentage of control (untreated) wells. Statistical significance of differences between group means was determined by ANOVA followed by a post-ANOVA Dunnett's test.

Acknowledgements

The authors are grateful to Enterprise Ireland for Basic Research Awards (SC/97/547 and SC/00/182), Health Research Board (Irl.)/Wellcome Trust for an equipment grant, BioResearch Ireland, IRCSET for a scholarship to R.M.D., Science Foundation Ireland for a Programme Investigator Award (P.V.M.) and Professor Paul Cahill, Dublin City University (Ireland) for the gift of BAEC cultures.

References

1. For a review, see: Liekens, S.; de Clercq, E.; Neyts, J. *Biochem. Pharm.* **2001**, *61*, 253–270.
2. Hanahan, D. *Nature Med.* **1998**, *4*, 13–14.
3. Brower, V. *Nature Biotech.* **1999**, *17*, 963–968.

4. (a) Lange, U. E. W.; Backfish, G.; Delzer, J.; Geneste, H.; Graef, C.; Hornberger, W.; Kling, A.; Lauterbach, A.; Subkowski, T.; Zechel, C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1379–1382, and references cited therein; (b) Hanahan, D. *Nature Med.* **1998**, *4*, 13–14.
5. Johnson, L. L.; Dyer, R.; Hupe, D. J. *Curr. Opin. Chem. Biol.* **1998**, *2*, 466–471.
6. Ingber, D.; Fujita, T.; Kishimoto, S.; Sudo, K.; Kanamaru, T.; Brem, H.; Folkman, J. *Nature* **1990**, *348*, 555–557.
7. Shim, J. S.; Kim, J. H.; Kim, H. Y.; Cho, Y. N.; Yum, S. H.; Kim, H.-J.; Shim, B. S.; Choi, S. H.; Kwon, H. J. *Chem. Biol.* **2003**, *10*, 695.
8. Linhardt, R. J. *J. Med. Chem.* **2003**, *46*, 2551.
9. Ornitz, D. M.; Herr, A. B.; Nilsson, M.; Westman, J.; Svahn, C.-M.; Waksman, G. *Science* **1995**, *268*, 432.
10. Moy, F. J.; Safran, M.; Seddon, A. P.; Kitchen, D.; Bohlen, P.; Aviezer, D.; Yayon, A.; Powers, R. *Biochemistry* **1997**, *36*, 4782–4791.
11. Nilsson, U. J.; Fournier, E. J.-L.; Hindsgaul, O. *Bioorg. Med. Chem.* **1998**, *6*, 1563–1575.
12. Park, W. K. C.; Auer, M.; Jaksche, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 10150–10155.
13. For a review, see: Simanek, E. E.; McGarvey, G. J.; Jablonski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833.
14. Mueller, T.; Schneider, R.; Schmidt, R. R. *Tetrahedron Lett.* **1994**, *35*, 4763.
15. (a) Anzali, S. et al. In *Perspectives in Drug Discovery and Design*; Kluwer Academic, 1998, p 273–299; (b) Barnickel, G., et al. *Nachr. Chem. Tech. Lab.* **1996**, *449*, 863–872.
16. Murphy, P. V.; Bradley, H.; Tosin, M.; Pitt, N.; Fitzpatrick, G. M.; Glass, W. K. *J. Org. Chem.* **2003**, *67*, 3733.
17. Inhibition of FGF-2 activity using a neutralizing anti-FGF-2 antibody results in increased apoptosis. See: Garcia, C. M.; D'Amore, P. A. *Invest. Opthalmol. Vis. Sci.* **1999**, *40*, 2945.
18. Tazawa, S.; Hayakawa, Y.; Ishikawa, T.-I.; Niiya, K.; Sakuragawa, N. *Thromb. Res.* **1993**, *72*, 439.
19. Rosenbaum, J.; Tobelem, G.; Molho, P.; Barzu, T.; Caen, J. P. *Cell. Biol. Int. Rep.* **1986**, *10*, 437.
20. 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.
21. Mach, H.; Volkin, D. B.; Burke, C. J.; Middaugh, C. R.; Linhardt, R. J.; Fromm, J. R.; Loganathan, D.; Mattsson, L. *Biochemistry* **1993**, *32*, 5480.
22. O' Brien, A.; Lynch, C.; O' Boyle, K. M.; Murphy, P. V. Unpublished results.
23. Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. *Chem. Rev.* **2002**, *102*, 515–553.
24. Savel'ev, A. N.; Ibatyllin, T. M.; Eneyskaya, E. V.; Kachurin, A. M.; Neustroev, K. N. *Carbohydr. Res.* **1996**, *296*, 261.
25. Pili, R.; Chang, J.; Partis, R. A.; Mueller, R. A.; Chrest, F. J.; Passaniti, A. *Cancer Res.* **1995**, *55*, 2920–2926.
26. See Ref. 16 and Avalos, M.; Babiano, R.; Carretero, M. J.; Cintas, P.; Higes, F. J.; Jiménez, J. L.; Palacios, J. C. *Tetrahedron* **1998**, *54*, 615.
27. Bollenback, G. N.; Long, J. W.; Benjamin, D. G.; Lindquist, J. A. *J. Am. Chem. Soc.* **1955**, *77*, 3310–3314.
28. Brown, R. T.; Scheinmann, F.; Stachulski, A. V. *J. Chem. Res.* **1997**, 370–371.
29. Boullanger, P.; Maunier, V.; Lafont, D. *Carbohydr. Res.* **2000**, *324*, 97.
30. (a) Györgydeák, Z.; Thiem, J. *Carbohydr. Res.* **1995**, *268*, 85; (b) Graf von Roedern, E.; Lohof, E.; Hessler, G.; Hoffmann, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156.
31. Bertho, A.; Maier, J. *Justus Liebigs Ann. Chem.* **1932**, *498*, 55.
32. Tropper, F. D.; Andersson, F. O.; Braun, S.; Roy, R. *Synthesis* **1992**, 618.
33. See Ref. 16 and Shiozaki, (a) M.; Mochizuki, T.; Hanzawa, H.; Haruyama, H. *Carbohydr. Res.* **1996**, *288*, 99; (b) Perrin, C. L.; Armstrong, K. B. *J. Am. Chem. Soc.* **1993**, *115*, 6825.
34. See Ref. 16 and Tropper, F. D.; Andersson, F. O.; Braun, S.; Roy, R. *Synthesis* **1992**, 618.
35. Cerezo, A. S.; Sproviero, J. F.; Deulofeu, V.; Delpy, S. *Carbohydr. Res.* **1968**, *7*, 395.
36. Mosmann, T. *J. Immunol. Meth.* **1983**, *65*, 55.