Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Research 341 (2006) 1370–1390
Carbohydrate RESEARCH

N-Glycosyl-thiophene-2-carboxamides: synthesis, structure and effects on the growth of diverse cell types

Sarah L. Rawe,[a] Dearbhla Doyle,[a] Violeta Zaric,[b] Isabel Rozas,[d] Kevin McMahon,[c] Manuela Tosin,[a] Helge Müller Bunz,[a] Evelyn P. Murphy,[c] Kathy M. O' Boyle[b] and Paul V. Murphy[a,*]

[a]*Centre for Synthesis and Chemical Biology and UCD School of Chemistry and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland*
[b]*UCD School Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland*
[c]*UCD School of Agriculture and Veterinary Medicine, UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland*
[d]*Centre for Synthesis and Chemical Biology, School of Chemistry, Trinity College Dublin, Dublin 2, Ireland*

Received 27 January 2006; received in revised form 7 April 2006; accepted 14 April 2006

Abstract—A range of *N*-glycosyl-thiophene-2-carboxamides, including a 6*H*-thieno[2,3-*c*]pyridin-7-one and a bivalent compound, have been synthesised and assayed for their effects on DNA synthesis in bovine aortic endothelial cells or on the growth of synoviocytes. Per-O-acetylated analogues of the glycoconjugates were significantly more effective inhibitors when compared to their corresponding non-acetylated analogues, indicating that the lower potency observed for hydroxylated derivatives is due to less efficient transport of these compounds across the cell membrane. Thiophene-2-carboxamide was inactive as an inhibitor of bFGF induced proliferation, confirming the requirement of the carbohydrate residue for the observed biological properties. Glucose, mannose, galactose and 2-amino-2-deoxy-glucose analogues were active as were a variety of substituted thiophene derivatives; the 6*H*-thieno[2,3-*c*]pyridin-7-one conjugate was inactive. Conformational analysis of the title compounds was investigated. X-ray crystal structural analysis of four *N*-glucosyl-thiophene-2-carboxamides showed that the pyranose rings adopted the expected $^4C_1$ conformations and that *Z-anti* structures were predominant (H1–C1–N–H anomeric torsion angle varied from −168.2° to −175.0°) and that the carbonyl oxygen and sulfur of the thiophene adopted an s-cis conformation in three of the isomers. In a crystal structure of a 3-alkynyl derivative, the hydrogen atom of the NH group was directed toward the acetylene group. The distance between the hydrogen atom and acetylene carbons and angles between nitrogen, hydrogen and carbon atoms were consistent with hydrogen bonding and this was supported by IR and NMR spectroscopic studies. The geometries of thiophene-2-carboxamides were explored by density functional theory (DFT) and Møller-Plesset (MP2) calculations and the s-cis conformer of thiophene-2-carboxamide was found to be more stable than its s-trans isomer by 0.83 kcal mol^{-1}. The s-cis conformer of 3-ethynyl-thiophene-2-carboxamide was 5.32 kcal mol^{-1} more stable than the s-trans isomer. The larger stabilisation for the s-cis conformer in the 3-alkynyl derivatives is explained to be due to a moderate hydrogen bonding interaction between the alkyne and NH group.

Keywords: Signal transduction; Angiogenesis; Rheumatoid arthritis; Glycosyl amides; Thiophene-2-carboxamides; Endothelial cells; Synoviocytes; Inhibitors; Proliferation; Growth; DNA synthesis

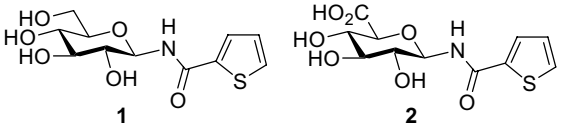
1. Introduction

The signal transduction processes that modulate cellular behaviour are important biological events. For example, angiogenesis[1] provides new blood vessels to growing and developing tissues including tumours, and it relies on the up-regulation of endothelial cell proliferation. Up-regulated angiogenesis is characteristic in rheumatoid arthritis and diabetic retinopathy during tumour growth and metastasis.[2] The tumour angiogenesis process results from the production of the pro-angiogenic factors basic

* Corresponding author. Fax: +353 1 7162501; e-mail: Paul.V.Murphy@ucd.ie

0008-6215/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2006.04.041

fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) in a signalling cascade, and the down-regulation of negative modulators, like angiostatin, in tissues with a quiescent vasculature. Angiogenesis is also a major factor affecting the metastatic spread of malignant cells. Thus the development of angiogenic inhibitors may allow a new therapeutic strategy against malignant tumours. Consequently, inhibitors of endothelial cell proliferation and growth are of interest and a number of strategies are being considered for the development of anti-proliferative agents.[3] Additionally, signalling pathways up-regulated by bFGF are also important in arthritis. The normal synovium is a delicate tissue lining the joint capsule; however, in inflammatory joint diseases including rheumatoid arthritis (RA), the synovium transforms into an aggressive, tumour-like structure called pannus. Synoviocyte cells in the pannus tissue are targeted by many signals including cytokines (IL-1β and TNF-α) and growth factors (bFGF and TGB-β) to promote proliferative and invasive capacity, increased cell adhesion molecule expression and guided migration.[4] Previously, efforts to discover compounds reduced in carbohydrate character (monosaccharide conjugates) that had potential as modulators of bFGF induced endothelial cell growth[5] led to the identification of *N*-(β-D-glucopyranosyl)-thiophene-2-carboxamide **1** and the glucuronic acid analogue **2** as inhibitors of bovine aortic endothelial cell (BAEC) growth.[6] The development of more potent analogues than **1** could ultimately be helpful for the determination of the biological mechanism of these glycoconjugates, which is unknown and could lead to the identification of novel targets in signalling pathways. The synthesis of novel analogues of **1**, the evaluation of their effects on the proliferation and growth of both endothelial and synovial cells[7] and a structural study of these bioactive compounds is described herein.

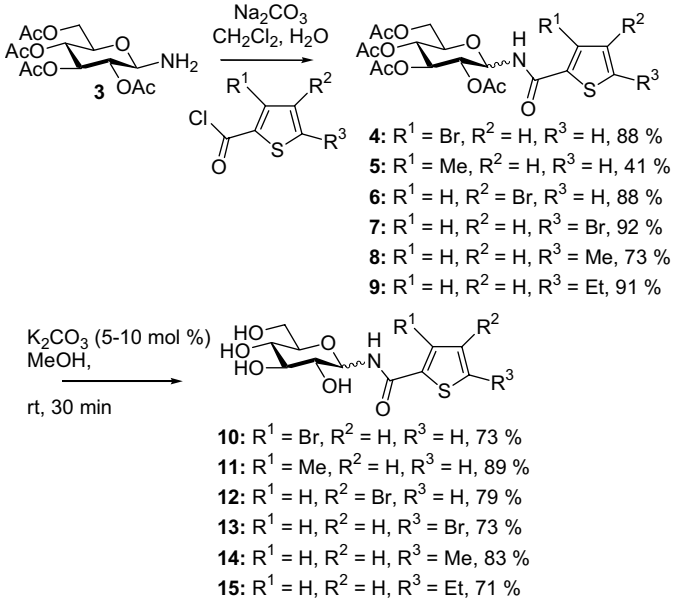


2. Results and discussion

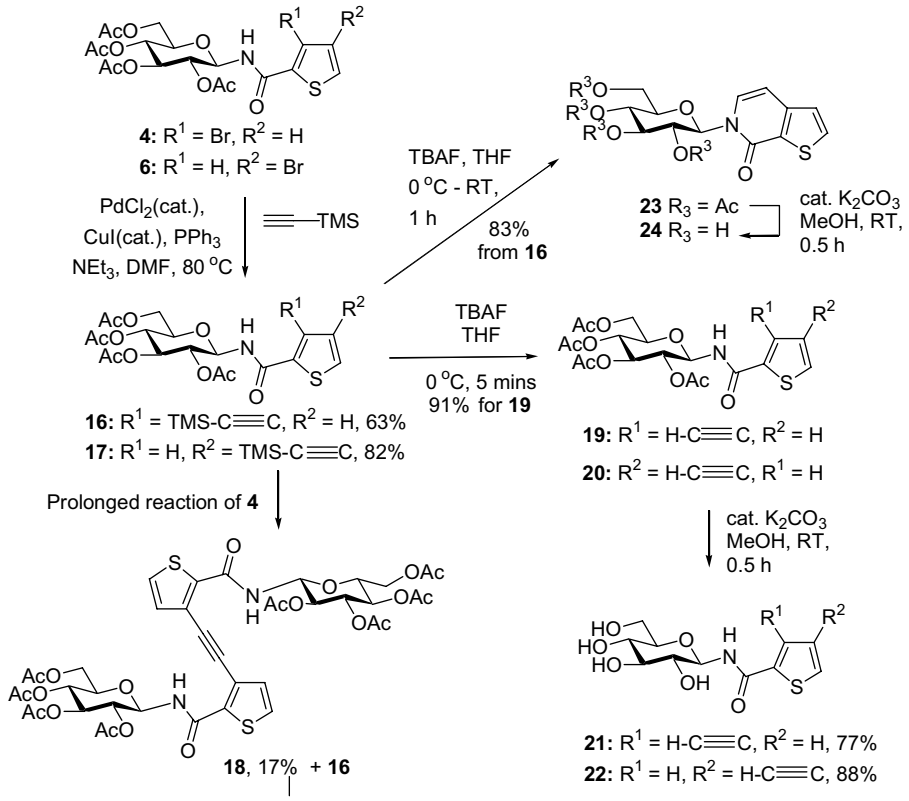
2.1. Synthesis of *N*-glycosyl-thiophene-2-carboxamides

N-Glucosyl-thiophene-2-carboxamides were prepared from the protected glucopyranosylamine **3** (Scheme 1). The reactions of **3**, in the presence of sodium carbonate, with acyl chlorides derived from thiophene-2-carboxylic acids afforded **4–9**. Anomerisation occurred during the coupling reactions and led to mixtures of anomers (α:β = 1:18–1:16 by ^{1}H NMR). The reaction of acid chlorides with **3** in the presence of pyridine in dichloromethane or coupling of the appropriate thiophene-2-carboxylic acid using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) with **3** also resulted in mixtures (α:β = 1:16–1:3 by ^{1}H NMR). Deacetylation of per-O-acetylated derivatives **4–9** gave the unprotected *N*-glucosyl-thiophene-2-carboxamides **10–15**. Mixtures of anomers were separated by reverse phase HPLC to obtain pure β-anomers for biological evaluation.

The acetylene derivatives **16/17** were prepared from **4/6** by a Sonogashira coupling with ethynyltrimethylsilane (Scheme 2), which was carried out in a sealed reaction



Scheme 1. Synthesis of *N*-glycosyl-thiophene-2-carboxamides.



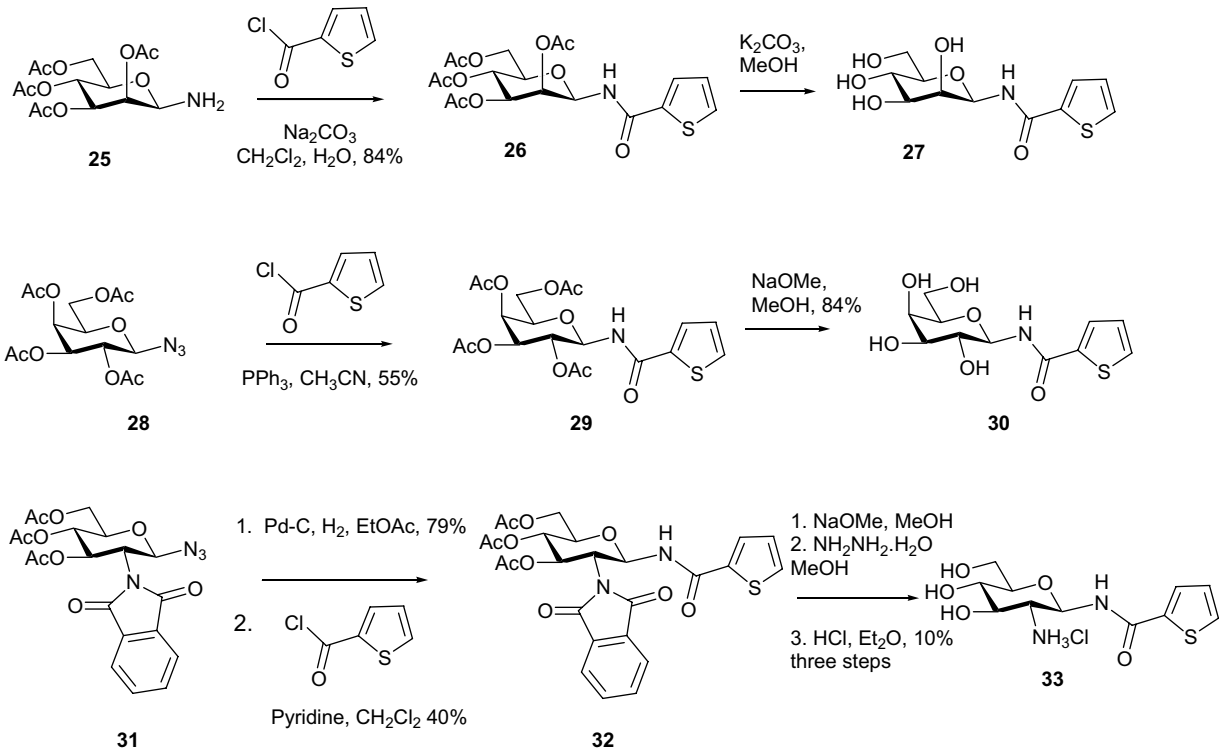
Scheme 2. Synthesis of *N*-glycosyl-thiophene-2-carboxamides.

vessel at 80 °C. The formation of the dimeric N-glucoside **18** was also observed from **4**, under conditions where the concentration of ethynyltrimethylsilane in solution was allowed to decrease and where removal of the TMS group of **6** became competitive. The resulting intermediate underwent a second Sonogashira coupling with the bromide **4** and gave **18**. The removal of the terminal TMS group from **16/17** was achieved by their reaction with *n*-Bu_4NF in THF at 0 °C for 5 min giving **19/20**. Subsequent removal of the acetyl protecting groups gave **21/22** in high yields. The reaction of **16**, employing one molar equivalent of *n*-Bu_4NF in THF at 0 °C for 1 h gave the cyclised pyridone **23** in good yield (Scheme 2). This was attributed to the basicity of the fluoride anion which, in addition to removing the TMS group, promoted nucleophilic attack at the terminal carbon atom of the alkyne by the amide nitrogen atom. The pyridone **23** results from a regioselective 6-*endo-dig* cyclisation with none of the 5-*exo-dig* product being observed. Base-induced cyclisations of nucleophiles onto acetylenes are known but have been most often reported for alkynylphenyl derivatives and have typically resulted in a mixture of *exo* and *endo* products.[8] Deacetylation of **19** and **23** gave **21** and **24**, respectively. Also, **16** could be converted to **24** by a concomitant cyclisation and deprotection reaction promoted by sodium methoxide in hot ethanol.

The mannose derivative **26** was prepared from amine **25**; removal of the protecting groups from **26** gave **27** (Scheme 3). The β-configuration assigned to **27** was confirmed by measurement of H1–C1 coupling constant (J 156.5 Hz)[9] using HMQC. The galactopyranosyl amide was prepared by the phosphine promoted coupling of azide **28** with thiophene-2-carbonyl chloride to give **29**; subsequent removal of the protecting groups from **29** gave **30**. The glucosamine derivative **33** was prepared from the azide **31**.[10] The azide group of **31** was first reduced by catalytic hydrogenation and the resulting amine was coupled with thiophene-2-carbonyl chloride in the presence of pyridine to give **32**. The sequential removal of acetate protecting groups and the phthalimide protecting group from **32** gave the amino sugar **33**.

2.2. Effect of *N*-glycosyl-thiophene-2-carboxamides on bovine aortic endothelial cell (BAEC) proliferation

The 3-bromothiophene conjugate **10** showed a dose-dependent inhibition of [^{3}H]thymidine incorporation into DNA of serum-stimulated BAEC after 24 h exposure; 10 reduced incorporation of [^{3}H]thymidine by $25 \pm 3\%$ at 500 μM, by $17.6 \pm 2.2\%$ at 250 μM and by $10 \pm 3\%$ at 100 μM. The 3-methyl derivative **11** reduced [^{3}H]thymidine incorporation by $16 \pm 3\%$ at 500 μM and



Scheme 3. Synthesis of *N*-glycosyl-thiophene-2-carboxamides.

also showed a dose-dependent inhibition of DNA synthesis (Fig. 1).

The effect of a wider range of glycoconjugates on [^{3}H]thymidine incorporation after both 24 and 48 h exposure is shown in Table 1. Most glycoconjugates were found, at a concentration of 500 μM, to consistently produce a maximal suppressive response after 24 h exposure. The inhibitory effect was reduced for all glycoconjugates over 48 h, in contrast with heparin–albumin (HA).

A greater than sixfold improvement for the per-O-acetylated derivatives was observed when they were compared to the corresponding deacetylated analogues (Table 1). The per-O-acetylated β-glucopyranoside **9** and per-O-acetylated β-mannopyranoside **26** applied at the highest concentration of 80 μM, were found to reduce cell proliferation by 41% and 46%, respectively. The corresponding deacetylated glucopyranoside **15** and the deacetylated mannopyranoside **27** were inactive at 80 μM (data not shown). The acetylated bivalent

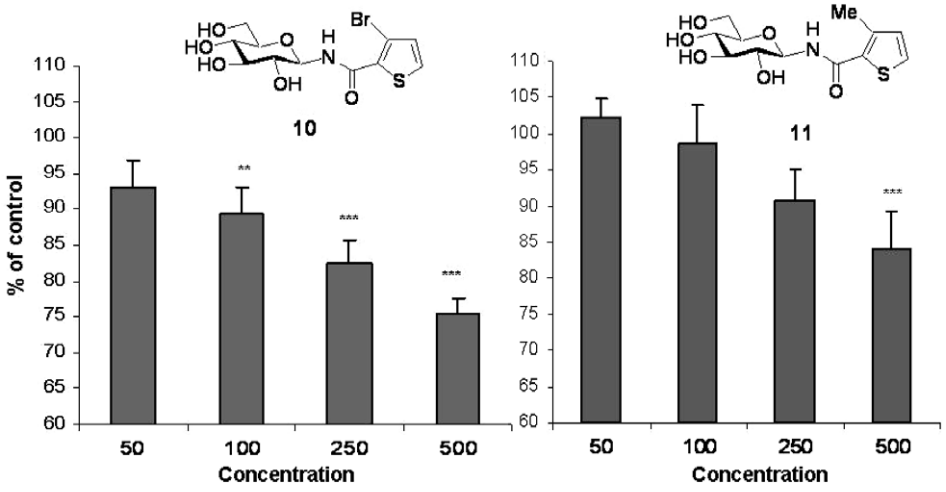


Figure 1. Concentration dependent effects of **10** and **11** on BAEC proliferation determined by [^{3}H]thymidine incorporation, after 24 h exposure with compounds. Each bar is the mean of triplicate values from three separate experiments. ***($p < 0.001$) versus control, **($p < 0.01$) versus control.

Table 1. Effect of *N*-glycosyl-thiophene-2-carboxamides and HA (10 μg/mL) on BAEC proliferation

Compound	24 h (% vs control)	*P* versus control	48 h (% vs control)	*P* versus control
HA	63 ± 2.5	0.001	42 ± 2.5	0.001
1	Not active	—	—	—
9	59.3 ± 1.4	0.001	72.56 ± 2	0.001
10	75.4 ± 1.3	0.001	Not active	—
11	84 ± 3	0.001	94.5 ± 2	0.05
13	65 ± 5.3	0.001	Not active	—
14	83 ± 1.8	0.001	91 ± 3.6	0.05
15	80.3 ± 2.5	0.001	88.6 ± 2.4	0.001
18	66 ± 3	0.001	Not determined	—
21	79.75 ± 2.7	0.001	90 ± 3.4	0.01
22	71 ± 3.3	0.001	77.3 ± 4.4	0.001
24	Not active	—	—	—
26	53.6 ± 2.3	0.001	Not determined	—
27	74.2 ± 1.9	0.001	88.8 ± 1.5	0.001
30	78.2 ± 3.3	0.001	94 ± 2	0.05
33	77 ± 6	0.01	Not determined	—

Proliferation was measured using a [^{3}H]thymidine incorporation assay. The cells were cultivated for 24 and 48 h with HA (10 μg/mL), deacetylated compounds (500 μM) and acetylated compounds (80 μM). The results are expressed as percentage of the values obtained for the control treated-cells (100%) and means ± SEM of three independent experiments are reported.

compound **18** also inhibited DNA synthesis of BAECs in a dose dependent manner (14 ± 1.5% at 16 μM and 34% at 80 μM). The per-O-acetylated derivative **9** (80 μM) also inhibited the bFGF induced thymidine uptake to control levels, whereas the corresponding deacetylated analogue **15** had no effect (80 μM) under identical conditions. Thiophene-2-carboxamide was inactive as an inhibitor of bFGF induced proliferation at concentrations 3.3–80 μM, confirming that the carbohydrate residue makes an essential contribution to the structure–activity relationship.

2.3. Effect of *N*-glucosyl-thiophene-2-carboxamides on synoviocyte growth

A normal human synoviocyte cell line (K4 IM) has been established and well characterised. The K4 IM cell line represents a valuable and unique tool to study mechanisms that induce or impede synoviocyte activation. The effects of **1** and the acetylated analogue **34** on the growth of K4 IM cells were explored. The thiophene conjugate **1** did not inhibit growth of K4 IM cells grown in full medium or under serum free conditions. Under serum free conditions, bFGF (12.5 ng/mL) stimulated K4 IM cells have significantly increased growth rates compared to untreated (control) cells over 24 (31%) and 48 h (40%). In the presence of **1** (138 μM), significant inhibition of bFGF-induced K4 IM cell growth was observed; growth was reduced to 15% after 24 h and further reduced to 6% after 48 h. Significant inhibition to below control levels of bFGF-induced K4 IM cell growth was observed in the presence of per-O-acetylated analogue **34** (33 μM) over both 24 and 48 h (Fig. 2).

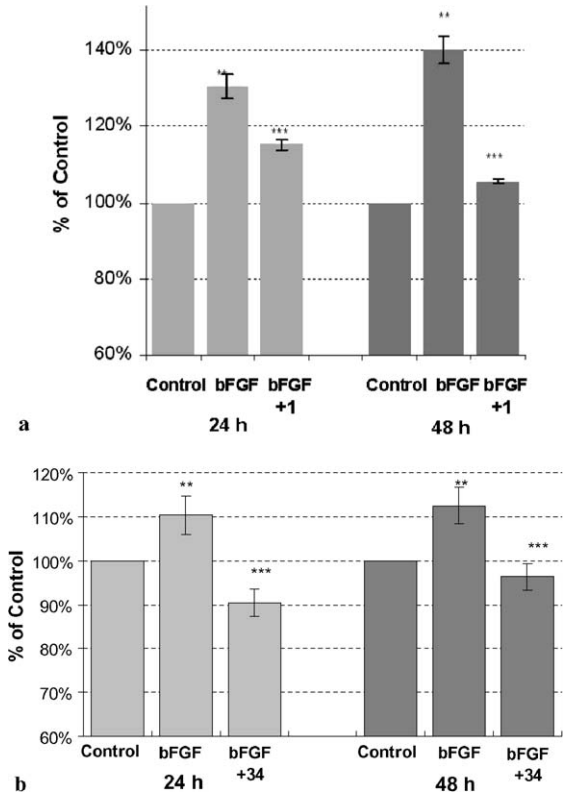
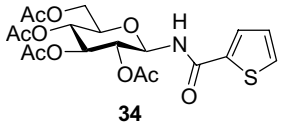


Figure 2. Effect of the glucose conjugates on bFGF induced growth of human synoviocyte (K4 IM) cells: (a) Under serum free conditions, bFGF (12.5 ng/mL) stimulated K4 IM cells display significant increased growth rates compared to untreated (control) cells over 24 and 48 h ($^{**}p < 0.05$). In the presence of **1** (138 μM), significant inhibition of bFGF-induced K4 IM cell growth was observed ($^{***}p < 0.05$). (b) Under serum free conditions, bFGF (12.5 ng/mL) stimulated K4 IM cells display significant increased growth rates compared to untreated (control) cells over 24 and 48 h ($^{**}p < 0.05$). In the presence of **34** (33 μM), significant inhibition of bFGF-induced K4 IM cell growth was observed ($^{***}p < 0.05$). Each bar is the mean and SD of triplicate values from at least three separate experiments.

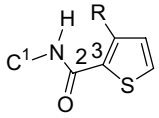


34

2.4. X-ray crystal structures of *N*-glucosyl-thiophene-2-carboxamides

Single crystals suitable for the X-ray crystal structure determination of **4**, **8**, **16** and **34** were obtained by vapour diffusion from ethyl acetate and cyclohexane. Selective crystallographic data for these compounds are provided in Table 2 and in Section 3. Structural dia-

Table 2. Selected bond lengths from crystal structures



Compound	Bond lengths (Å)				
	C1–N	N–C2	C2–C3	C2–O	N–H
4[a]	1.424(4)	1.360(4)	1.497	1.219(4)	0.88(00)
8	1.4311(16)	1.3741(17)	1.4717(18)	1.2265(17)	0.85(2)
16	1.440	1.372(2)	1.491	1.224(4)	0.88(2)
34	1.4310	1.369(2)	1.477(2)	1.2206(19)	0.86(2)

[a] Data for s-cis conformer.

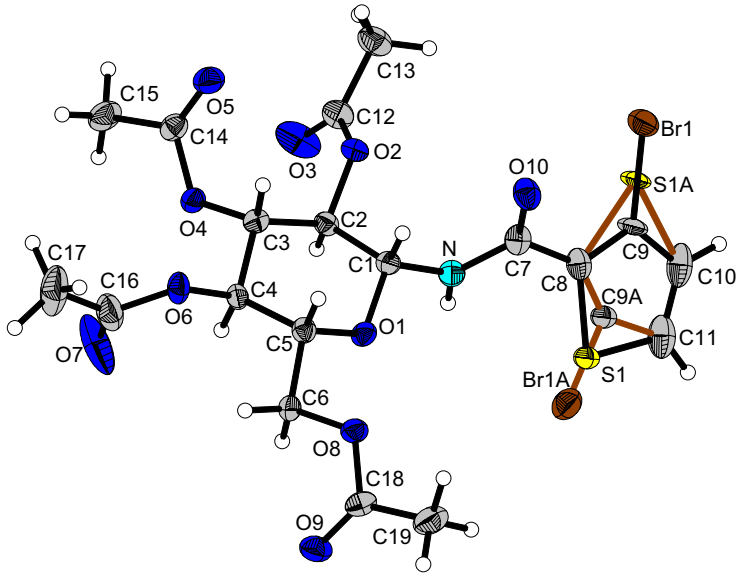


Figure 3. X-ray crystal structure of **4**. Thermal ellipsoids are drawn on the 50% probability level; the minor component of the disordered thiophene group is shown with the brown bonds.

grams are shown for **4** (Fig. 3) and **16** (Fig. 4). The amide adopted the *Z-anti* structure[†] (Fig. 5) in all crystal structures, the H1–C1–N–H torsion angle varying from −168.2° to −175.0° consistent with the anti-periplanar arrangement of the two hydrogen atoms. This structural preference is consistent with that observed for related benzamides.[11] The thiophene rings were, as expected, co-planar with the amide group for thiophenes **8**, **16** and **34** and the carbonyl oxygen and sulfur of the thiophene adopted the s-cis conformation (Fig. 5) in these three cases. X-ray crystal structure determination has shown that the s-cis conformation was observed in the solid state for thiophene-2-carbonyl derivatives previously[12] and both experimental and theoretical studies have shown that the s-cis isomer is generally preferred relative to the s-trans form in such compounds.[13] An exception in this case was for the 3-bromo derivative **4** in which there was disorder in the solid state and both s-cis (minor) and s-trans (major) conformers being observed (Figs. 4 and 5). In both conformers of **4**, the thiophene and amide groups were no longer co-planar (torsion S–C–C–O = −43.2° for s-cis; torsion S–C–C–O = 140.5° for s-trans).

For the 3-acetylenic derivative **16**, the hydrogen atom of the NH group was directed towards the acetylene group. The distance between the hydrogen atom and acetylene carbon atom bonded to the thiophene was 2.32 Å; the distance between the hydrogen atom and the acetylene carbon bonded to the TMS group was 2.61 Å. Both C···H distances are within the calculated sum of van der Waal radii of 2.80 Å.[14] The angle between the nitrogen, hydrogen and carbon atoms closest to hydrogen was 134° and the angle between

[†] The *Z-anti* nomenclature refers to amide configuration and conformation. For the *Z*-amide, the two groups of highest priority according to Cahn Ingold Prelog rules (i.e., the pyranose ring and oxygen atom) are on same side of the bond with double bond character (amide bond). The *anti* nomenclature refers to the torsion angle defined by H1–C1–N–H = 180 ± 90°.

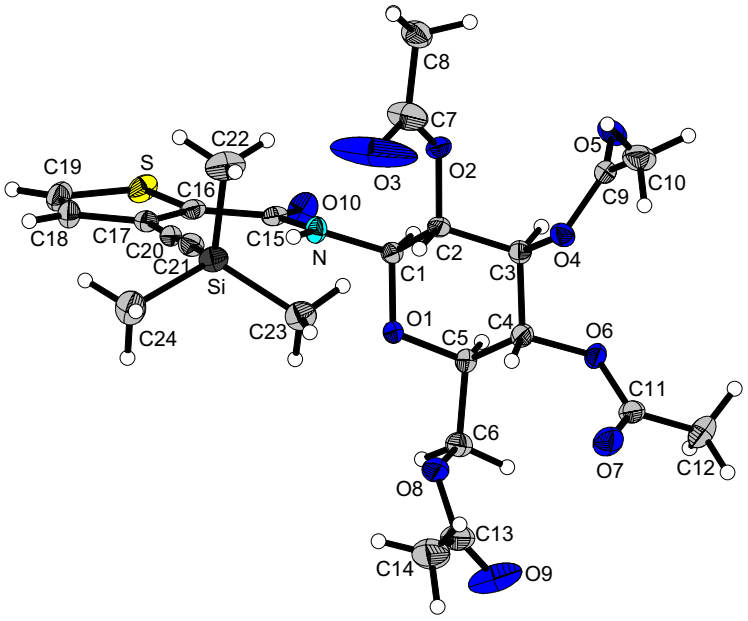


Figure 4. X-ray crystal structure of **16**. Thermal ellipsoids are drawn on the 50% probability level.

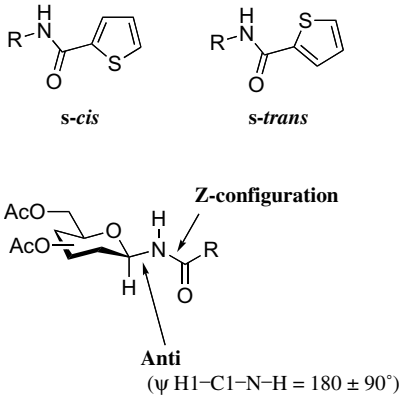


Figure 5. Definition of structure and nomenclature.

nitrogen, hydrogen and carbon of the acetylene group furthest away is 162°. This data falls within definitions of hydrogen bonding.[15] The pyranose groups in crystal structures had the expected $^4C_1$ conformations in each case and the arrangement of acetate groups in these structures was similar for all compounds.

2.5. NMR studies of *N*-glucosyl-thiophene-2-carboxamides

The NMR spectra showed one set of signals for the β-anomers of all *N*-glycosyl-thiophene-2-carboxamides, including mannose and galactose conjugates, in both the ^{1}H NMR and ^{13}C NMR spectra recorded in $CDCl_3$, D_2O, DMSO-d_6 or CH_3OD-d_4. The coupling constant between the pyranose H1 and NH proton, for acetylated compounds, was between 8.7 and 9.7 Hz ($CDCl_3$ and DMSO-d_6, Table 3); this is consistent with the molecules adopting a preferred *Z-anti* structure, as is observed in the solid state and consistent with NMR data reported for related benzamides.[11] The ^{1}H NMR spectra of non-acetylated compounds **14** (pH = 3.0) and **21**

Table 3. Selected ^{1}H NMR data

Compound	$CDCl_3$			DMSO-d_6		
	δ_{NH} (ppm)	$J_{NH,H1}$ (Hz)	$\Delta\delta/\Delta T$ (ppb)	δ_{NH} (ppm)	$J_{NH,H1}$ (Hz)	$\Delta\delta/\Delta T$ (ppb)
4	7.66	9.0	−3.7	nd	nd	nd
5	6.65	8.7	−3.0	8.72	9.3	−6.1
8	6.86	9.3	nd	nd	nd	nd
16	8.27	9.6	−2.4	8.30	9.6	−2.1
18	7.73	9.1	−2.1	nd	nd	nd
19	8.06	9.0	−2.6	8.67	9.3	−5.0
26	6.70	9.3	nd	nd	nd	nd
29	6.95	8.8	nd	nd	nd	nd
34	6.92	9.1	−4.7	9.23	9.3	−5.1

(pH = 5.0) showed signals for NH at $\delta = 8.90$ ppm ($J = 8.8$ Hz) and δ 8.75 ppm ($J = 8.6$ Hz), respectively; these results indicate that the *Z-anti* conformer is also preferred in water for these thiophene derived glycosyl amides.

A downfield shift of the signal for NH protons of the acetylated acetylenic derivatives, **4** ($\Delta\delta = +0.74$ ppm), **16** ($\Delta\delta = +1.35$ ppm), **18** ($\Delta\delta = +0.81$ ppm) and **19** ($\Delta\delta = +1.14$ ppm) in $CDCl_3$ relative to the unsubstituted thiophene **34** (see Table 3) was observed. This contrasts with both **5** ($\Delta\delta = -0.27$ ppm) and **8** ($\Delta\delta = -0.06$ ppm) where upfield shifts for signals of the NH protons were observed when compared to **34**. The effect of the temperature on the NH chemical shift (δ) was investigated in both $CDCl_3$ for **4**, **5**, **16**, **18** and **19** and **34**. In every instance, a negative linear relationship was observed while $J_{H1,NH}$ remained constant. In $CDCl_3$, the smallest values of $\Delta\delta/\Delta T$ occurred for all the acetylene containing compounds ($\Delta\delta/\Delta T < -2.6$ ppb K^{-1}) whilst larger values (-3.0 to -4.7 ppb K^{-1}) were observed for the remaining thiophenes (Table 3). The effect of temperature on the NH chemical shift (δ) was also investigated in DMSO-d_6 for **5**, **16**, **19** and **34** and the values of $\Delta\delta/\Delta T$ were greater than -4 ppb K^{-1} with the exception of **16** where $\Delta\delta/\Delta T$ was -2.1 ppb K^{-1}.

The results of such variable temperature studies must be interpreted cautiously, but provided the protons are not involved in other temperature dependent interactions and no conformational changes are induced, large values of $\Delta\delta/\Delta T$ are indicative of protons exposed to the solvent or protons that are initially shielded but become transferred to solvated environment on increase in temperature while small values are indicative of protons shielded from the solvent.[16] This suggests shielding of the NH proton from the solvent for **5**, **16**, **19** and **34**, in $CDCl_3$ and for **16** in DMSO-d_6.[17] Downfield shifts of NH protons in $CDCl_3$ can be attributed to a stronger hydrogen bonding interaction of the NH proton with an acceptor other than the solvent.[18] They can also be attributed, in the case of **16** and **19**, to the magnetic anisotropy of the acetylene group; the NH proton lying in a region with reduced shielding.[19] In any case, the NMR studies indicate there is close contact of NH and acetylene groups for the 3-alkynyl-thiophene-2-carboxamides and that they adopt similar structures in solution as is observed in the crystal structure of **16**. For **16**, the conformation that displays this NH–acetylene interaction seems to be stable, even in DMSO-d_6, a solvent where intramolecular hydrogen bonding would be less important than for chloroform. The chemical shift for the NH of **16** is more upfield than for **34** in DMSO-d_6 indicating the acetylene group is shielding the NH from the solvent.

2.6. Infrared spectroscopic studies of *N*-glucosyl-thiophene-2-carboxamides

Infrared (IR) spectroscopy has been widely used for the investigation of hydrogen bonding. For secondary amides, a band at 3500–3400 cm^{-1} is considered to be typical of a non hydrogen bonding or free NH while a band between 3370 and 3250 cm^{-1} is usually attributed to hydrogen bonding NH.[20] The NH stretching frequencies for selected compounds are listed in Table 4. These were recorded in solution ($CDCl_3$, $CHCl_3$) and also in the solid state (diffuse reflectance (DR) and KBr disk methods). There was good agreement observed with data obtained by both DR and KBr in the solid state in most cases. A single NH band was observed for the 3-bromo derivative **4** by DR ($\nu_{NH} = 3409$ cm^{-1}) whereas there were two NH bands of approximately equal intensity ($\nu_{NH} = 3409$ and 3385 cm^{-1}) by the KBr method. For **34** there was evidence of hydrogen bonding in the solid state (by diffuse reflectance $\nu_{NH} = 3379$ cm^{-1}) but not in solution ($\nu_{NH} = 3429$ cm^{-1}). Examination of the close packing in the crystal structure of **34** shows that there are intermolecular hydrogen bonding interactions between the NH groups and pyranose oxygen, which explains the existence of the band consistent with hydrogen bonding in the solid state. The IR data in solution for **4**, **16**, **18** and **19**, is consistent with intramolecular hydrogen bonding (a red shift being observed) and correlates with the downfield shifts observed for the NH in the ^1H NMR spectra of each compound. For **16**, there

Table 4. Infrared spectroscopic data

Compound	ν_{NH} (cm^{-1})			$\nu_{C=O}$ (cm^{-1})		
	Solution	Solid state		Solution	Solid state	
	$CDCl_3$[a,b]	KBr	DR	$CDCl_3$	KBr	DR
4	3387	3409, 3385	3409	1662	1667	1671
5	3411	3302	3304	1674	1652	1658
16[c]	3352	3360	3361	1662	1674	1678
18	3382	nd	nd	1662	nd	nd
19	3373	3373	nd	1664	1667, 1653	nd
34	3429	3379	3379	1673	1671	1672

[a] The spectra were also recorded in $CHCl_3$ and agreed well with those obtained in $CDCl_3$.
[b] All solution spectra were recorded at a concentration of 20 mg mL^{-1} (4 to 6×10^{-2} M).
[c] The IR was also recorded in solution in DCM (at 40 and 20 mg mL^{-1}) and showed bands at 3354 cm^{-1} (NH) and 1663 cm^{-1} (C=O).

was evidence for hydrogen bonding by IR in the solid state ($\nu_{NH} = 3361\ cm^{-1}$) also, as indicated in the crystal structure. The shape of the peaks and the stretching frequency of the acetylene derivatives **16** and **19** in $CDCl_3$ solution was independent of the concentration, providing further evidence that the hydrogen bonding suggested for these acetylene derivatives is not intermolecular. Presumably for **5** there is intermolecular hydrogen bonding in the solid state ($\nu_{NH} = 3304\ cm^{-1}$) which does not exist in solution ($\nu_{NH} = 3411\ cm^{-1}$).[21]

2.7. Theoretical calculations on thiophene-2-carboxamides

The geometries of a series of thiophene-2-carboxamide derivatives were explored by calculations. Density functional theory (DFT) methods and Møller-Plesset (MP2) levels were used to investigate whether the geometries observed for the thiophene derivatives discussed herein would be expected. Interactions involving the electron donation from a lone pair to an empty d sulfur orbital (dative interaction) have been widely described in the literature.[13,22] As well, negative hyperconjugation has been found in aromatic systems with hypervalent centres such as 1,6-dioxa-6a-chalcapentalenes in which O→(S–C) interactions were observed and attributed to a donation of an electron pair from the highest π or n orbitals of the donor to the S–C antibonding MO σ* orbital. This $n_O \rightarrow \sigma^*_{S-C}$ hyperconjugation leads to the expansion of the valence shell of the S atom.[22f] Thus in the first instance, three pairs of s-cis/s-trans conformers of planar thiophene keto derivatives (2-formyl: **A**–**B**, 2-acetyl: **C**–**D** and 2-amido: **E**–**F**) were analysed to study a possible interaction between the lone pairs of the carbonyl O atom and the S atom of the thiophene ring considering the influence of three different adjacent groups (H, CH_3 and NH_2).

In the s-cis conformers **A**, **C** and **E**, the O atom is pointing towards the S atom, whereas in the s-trans conformers **B**, **D** and **F**, the O atom is pointing in the opposite direction. The structures optimised at the MP2/6-311+G** level of calculation are shown in Figure 6. In the case of derivatives **A**, **C** and **E**, the distance between the O atom of the carbonyl group and the S atom of the ring is the largest in the case of the 2-formyl derivative **A** (3.10 Å), it becomes shorter for the 2-acetyl derivative **C** (2.99 Å) and it is even shorter for the 2-amido derivative **E** (2.96 Å) as shown in Figure 6. This could be an indication of a certain type of interaction between both atoms. By looking at the energy of these three pairs of planar molecules, it is observed that in all the cases the s-cis conformer is the most stable (conformers **A**, **C** and **E**). In the case of the 2-formyl pair, s-cis conformer **A** is 1.5 kcal mol^{-1} more stable than **B**. As well, a difference in stability of 1.3 kcal mol^{-1} is observed between **C** and **D** and of 0.8 kcal mol^{-1} between **E** and **F**.

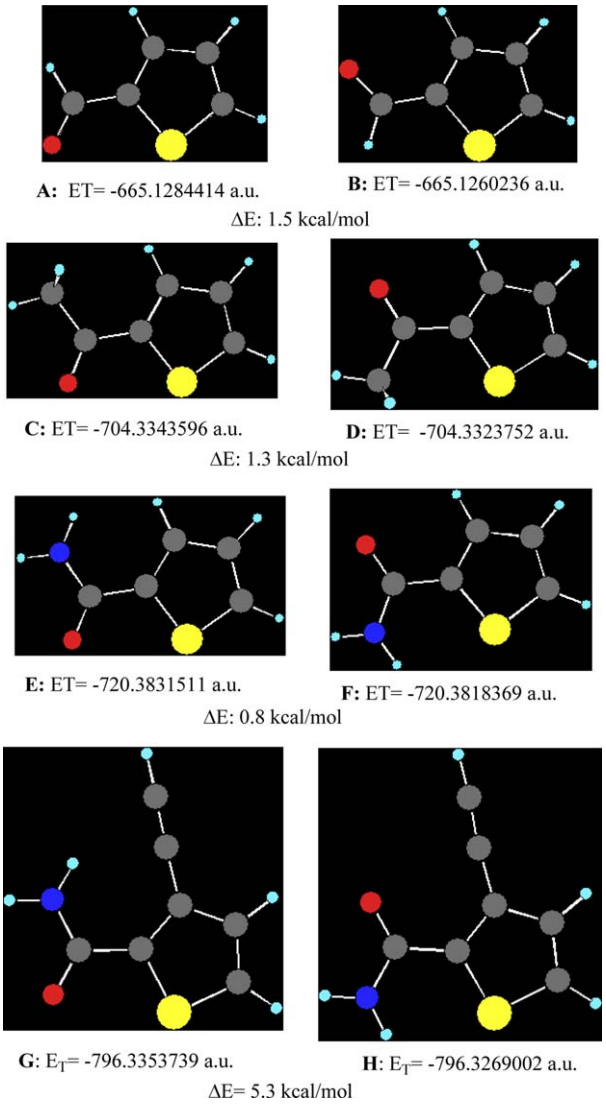


Figure 6. Structural isomers and energies of thiophene-2-carbonyl derivatives.

Those conformers in which a S···O interaction was suspected (**A**, **C** and **E**) were subjected to an analysis of the electron density topology by means of the AIM approach. The electron density between the S and O atoms was explored for each molecule, but no bond critical point was found discarding any possible interaction between those atoms. The analysis of the orbital interactions by means of the NBO approach provides an important indicator of the strength and nature of interactions established inter or intramolecularly. According to this analysis, at the B3LYP level, no interaction was observed between the empty d orbitals of the S atom and the O atoms in any of the three cases studied. By looking at the occupancy of the S atoms in the three pairs of conformers no critical differences were found between those conformers in which the S and O atoms were near and those conformers in which both atoms were apart. We did not find the chalcogen effect with

both the AIM and NBO methods used. However, the stability of the s-cis conformer was always higher than its s-trans isomer, as suggested by others previously, but we can neither confirm that the gain in stability is a consequence of dative interactions to the sulfur d orbitals or to negative hyperconjugation $n_O \rightarrow \sigma^*_{S-C}$ on the basis of calculations described herein.

Calculations of 3-alkynyl-thiophene-2-carboxamide conformers were next investigated. It has been described in the literature that hydrogen bonding between donors and π systems exist and are stabilising.[23] Thus model structures of the *N*-glycosyl-3-alkynyl-thiophene-2-carboxamides, as described herein, were investigated. The s-cis conformation **G** and the s-trans conformer **H** were calculated at the same level of theory as the previous structures **A**–**F** and the optimised geometry is shown in Figure 6. The difference of energy between **G** and **H** was 5.3 kcal mol^{-1} (much larger than those observed for **A**–**F**) with the s-cis conformer **G** being the more stable. The optimised structure of this conformer **G** shows a possible interaction between one of the H of the amido group and the π cloud of the triple bond.

The AIM analysis confirmed that a bond critical point (BCP) existed between this H atom and approximately the middle point of the triple bond. This BCP showed the following characteristics: electron density at the BCP, $\rho(\mathrm{BCP}) = 0.0158$ e a_0^{-3} and Laplacian $\nabla^2\rho(\mathrm{BCP}) = 0.0529$ e a_0^{-5}. Thus, the electron density at the BCP is around 10^{-2} and the Laplacian is positive which is in agreement with the formation of a hydrogen bond (HB) of medium strength. Consistent with the fact that this triple bond is not symmetrical, the BCP found between the H atom and the π system is not orientated towards the middle of the triple bond but is closer to the C atom directly connected to the thiophene ring, consistent with that observed in the crystal structure of **16**. As well, when comparing the interacting N–H bond of the 3-acetylene substituted derivative **G** (1.008 Å) with that of the unsubstituted analogue **E** (1.005 Å) or **H** (1.006 Å) a slight increment in the bond was observed when the HB is formed (see Table 5). Additionally, the amide C(O)–N bond in isomers **E** to **H** becomes shorter when the hydrogen bond has been established in isomer **G**, in agreement with the literature.[23] The stabilisation provided by the hydrogen bond found in **G** could account for the larger stabilisation observed for this s-cis conformer compared to the stabilisation computed for derivatives **A**–**F**, and the preference of the amido system to be in a s-cis disposition with respect to the thiophene ring, as has been observed by X-ray crystallography for the majority of the *N*-glycosyl-thiophene-2-carboxamides studied.

Table 5. Representative bond distances (Å) found for **A**–**H** computed at MP2/6-31+G* level of theory

	C(ring)–C(O)	C=O	C(O)–N	H–N/N–H	C≡C
A	1.465	1.221	—	—	—
B	1.470	1.220	—	—	—
C	1.477	1.226	—	—	—
D	1.485	1.224	—	—	—
E	1.485	1.225	1.368	1.008/1.005	—
F	1.490	1.224	1.369	1.008/1.006	—
G	1.489	1.228	1.362	1.008/1.008	1.224
H	1.491	1.221	1.372	1.008/1.006	1.223

2.8. Summary and conclusions

N-Glycosyl-thiophene-2-carboxamides (polyhydroxylated conjugates) showed ability to inhibit DNA synthesis in endothelial cells, induced by serum and bFGF. The importance of the carbohydrate residue for the observed biological activity was confirmed as thiophene-2-carboxamide which was inactive as an inhibitor of bFGF induced BAEC growth. Per-O-acetylated *N*-glycosyl-thiophene-2-carboxamides were found to be more effective inhibitors of thymidine uptake in endothelial cells than the polyhydroxylated compounds. Cytokine and growth factor modulation of K4 IM synoviocytes corresponds to primary rheumatoid arthritis synoviocyte responses.[24] The glycoconjugate **1** and its per-O-acetylated analogue **34** inhibited bFGF stimulated growth of synoviocytes, the per-O-acetylated analogue being more potent. A likely reason for the improved activity of acetylated conjugates for assays involving both cell types is that the compounds may be exerting their mechanism of action in an intracellular fashion. It is known that acetylated carbohydrates can have increased membrane permeability, compared to the polyhydroxylated analogues.[25] Esterases can hydrolyse the acetyl groups and generate the corresponding de-O-acetylated carbohydrate in the cytosol. Further investigations will explore the intracellular trafficking of acetylated analogues and the comparison of their cellular uptake versus non-acetylated compounds. Cell biological studies to determine their mechanism of action are underway. Investigations are also underway to demonstrate if there is specific modulation of bFGF induced signaling pathways by the carbohydrate derivatives. It is possible that the activity observed is in general non-specific inhibition. Finally, the structure of acetylated *N*-glycosyl-thiophene-2-carboxamides were investigated by X-ray crystallography, IR spectroscopy, NMR spectroscopy and theoretical calculations; there are clear preferences in the orientation of the thiophene and the amide groups relative to the sugar residue. Aside from these studies, the application of the structural principles described herein for constraining the geometrical orientation of groups on sugar scaffolding[26] could be envisaged.[21] In addition, the NH–alkyne interactions observed in 3-alkynyl-thiophene-2-carboxamides are relevant to studies on NH–π,[27] cation–π[28] and other weak interactions.[29]

3. Experimental

3.1. General

Low and high-resolution mass spectra were measured using a Micromass LCT KC420 or a Micromass Quattro. Chemical shifts are reported relative to internal $(CH_3)_4Si$ in $CDCl_3$ (0.0) for ^1H, and to $CDCl_3$ (77.16) for ^{13}C. Chemical shifts are reported relative to HOD in D_2O (4.79) for ^1H and to CH_3OD (49.86) for ^{13}C. ^{13}C Signals were assigned with the aid of DEPT-135. ^1H signals were assigned with the aid of COSY. Optical rotations were determined with a Perkin–Elmer 343 model polarimeter at the sodium D line at 23 °C. IR spectra were recorded with a Mattson Galaxy Series FTIR 3000 and/or Perkin–Elmer Spectrum One FTIR using either thin film between NaCl plates, as solutions in $CHCl_3$ or $CDCl_3$ or KBr discs, as specified. Melting points were measured with a Gallenkamp melting point apparatus. Elemental analysis was performed on an Exeter Analytical CE440 elemental analyser. TLC was performed on aluminium sheets pre-coated with Silica Gel 60 (HF254, E. Merck) and spots visualised by UV and charring with 1:20 H_2SO_4/EtOH or with 1:1 $KMnO_4$ (1% w/v solution)/$NaHCO_3$ (5% w/v solution). 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 (Fluka) and cyclohexane (Aldrich). CH_2Cl_2 was dried and distilled from CaH_2 before use. All chemicals and anhyd solvents (DMF, pyridine) were purchased from Sigma–Aldrich except 3-bromothiophene-2-carboxylic acid, which was obtained from Lancaster. 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 non-acetylated compounds were purified by semi-prep HPLC using a Waters 600E pump before biological evaluation (CH_3CN/H_2O 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-10 μm, 250 × 20 mm). Wavelength for both analytical and semi-preparative HPLC was 220 nm. Reaction solvents were dried and distilled where stated.

3.2. *N*-(2,3,4,6-Tetra-*O*-acetyl-α/β-D-glucopyranosyl)-3-bromothiophene-2-carboxamide (4)

To a solution of 3-bromothiophene-2-carboxylic acid (0.60 g, 2.88 mmol) in anhyd CH_2Cl_2 (15 mL) was added oxalyl chloride (0.37 g, 0.25 mL, 2.88 mmol) followed by two drops of anhyd DMF and the solution was stirred at rt for 2 h. The solvent was removed under diminished pressure to afford the acid chloride as a yellow oil, which was used immediately without further purification. This oil was dissolved in CH_2Cl_2 (2 mL) and the solution was added to a biphasic mixture of **3**[11c,30] (1.0 g, 2.88 mmol) in CH_2Cl_2 (15 mL) and sodium carbonate (0.30 g, 2.88 mmol) in H_2O (15 mL) and stirring was continued overnight at rt. The mixture was then transferred to a separating funnel, the organic layer removed and the aq layer extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were washed (satd $NaHCO_3$, 20 mL), dried ($MgSO_4$) and the solvent was removed under diminished pressure. Chromatography (1:1, EtOAc/cyclohexane, $R_f = 0.20$) of the residue gave **4** as a colourless foam (1.36 g, 88%; α:β, 1:18). Analytical data for β anomer: mp 161–163 °C; IR (solution in $CHCl_3$): 3386 (NH), 2960, 2928, 2856 (CH), 1751 (ester C=O), 1663 (amide C=O), 1524, 1525, 1498, 1418, 1377, 1233, 1041 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.66 (1H, d, $J_{NH,H1} = 9.0$ Hz, NH), 7.51 (1H, d, $J = 5.1$ Hz, aromatic H), 7.05 (1H, d, $J = 5.1$ Hz, aromatic H), 5.46 (1H, t, $J = 9.0$ Hz, H-1), 5.35 (1H, t, $J = 9.0$ Hz, H-3), 5.16 (1H, t, $J = 9.0$ Hz, H-2), 5.13 (1H, t, $J = 9.0$ Hz, H-4), 4.33 (1H, dd, $J_{6a,6b} = -12.3$ Hz, $J_{6a,5} = 4.2$ Hz, H-6a), 4.11 (1H, dd, $J_{6b,6a} = -12.3$ Hz, $J_{6b,5} = 2.1$ Hz, H-6b), 3.89 (1H, ddd, $J_{5,4} = 10.2$ Hz, $J_{5,6a} = 4.5$ Hz, $J_{5,6b} = 2.1$ Hz, H-5), 2.08, 2.07, 2.04 (12H, each s, each CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7, 170.3, 169.9, 169.5 (each s, each ester C=O), 160.7 (s, amide C=O), 133.1 (s, aromatic C), 132.5, 131.5 (each d, aromatic CH), 110.6 (s, aromatic C), 78.3, 73.7, 72.9, 70.1, 68.1 (each d, C-2–5), 61.6 (t), 20.7, 20.6, 20.6 (each q); ESI-LRMS: found *m*/*z* 536 and 538 $[M+H]^+$, 478 and 476, 376 and 374, 331, 271, 211, 169; ESIMS *m*/*z* calcd for $C_{21}H_{23}BrNO_{10}S$ $[M+H]^+$: 536.0226. Found: 536.0215. Anal. Calcd for $C_{21}H_{22}BrNO_{10}S$: C, 42.55; H, 4.13; Br, 14.90; N, 2.61. Found: C, 42.54; H, 4.05; Br, 14.90; N, 2.47. Selected ^1H NMR data for α anomer: δ 7.57 (1H, d, $J = 5.1$ Hz, aromatic H), 7.12 (1H, d, $J = 5.1$ Hz, aromatic H), 6.03 (1H, dd, $J = 6.9$, 5.4 Hz, H1), 5.26 (1H, dd, $J = 10.2$, 5.4 Hz), 4.01 (1H, ddd, $J = 9.9$, 3.6, 2.4 Hz).

3.3. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3-methylthiophene-2-carboxamide (5)

The reaction of 3-methylthiophene-2-carboxylic acid (0.08 g, 0.58 mmol) as described for 3-bromothiophene-2-carboxylic acid gave **5** as a colourless solid (0.11 g, 41%; α:β, 1:16) following chromatography (1:1, EtOAc/cyclohexane, $R_f = 0.20$). Analytical data for β-anomer: mp 189–192 °C; IR (solution in $CHCl_3$): 3411 (NH), 2957, 2890 (CH), 1754 (ester C=O), 1674 (amide C=O), 1540, 1508, 1417, 1368, 1229, 1040 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.33 (1H, d, $J = 5.1$ Hz, aromatic H), 6.90 (1H, d, $J = 5.1$ Hz, aromatic H), 6.65 (1H, d, $J_{NH,H1} = 8.7$ Hz, NH), 5.37 (2H,

2 overlapping t, $J = 9.3$ Hz and $J = 9.6$ Hz, H-1,3), 5.11 (1H, t, $J = 9.6$ Hz, H-2), 5.05 (1H, t, $J = 9.6$ Hz, H-4), 4.35 (1H, dd, $J_{6a,6b} = -12.6$ Hz, $J_{6a,5} = 4.2$ Hz, H-6a), 4.11 (1H, dd, $J_{6b,6a} = -12.6$ Hz, $J_{6b,5} = 2.1$ Hz, H-6b), 3.88 (1H, ddd, $J_{5,4} = 9.9$ Hz, $J_{5,6a} = 4.2$ Hz, $J_{5,6b} = 2.1$ Hz, H-5), 2.53 (3H, s, CH_3), 2.09, 2.05, 2.04 (2s) (each 3H, each s, each CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.2, 170.6, 169.9, 169.9 (each s, each ester C=O), 162.8 (s, amide C=O), 143.6 (s, aromatic C), 132.4 (d, aromatic CH), 129.3 (s, aromatic C), 128.0 (d, aromatic CH), 78.8, 73.6, 72.7, 70.5, 68.2 (each d), 61.7 (t), 20.7, 20.6, 15.9 (each q); ESI-LRMS: found m/z 472 $[M+H]^+$, 457, 331, 310, 169; ESIMS m/z calcd for $C_{20}H_{26}NO_{10}S$ $[M+H]^+$: 472.1277. Found: 472.1277. Anal. Calcd for $C_{20}H_{25}NO_{10}S$: C, 50.95, H, 5.34, N, 2.97, S, 6.80. Found: C, 50.75; H, 5.41; N, 2.76; S, 6.38. Selected ^{1}H NMR data for the α-anomer: δ 7.37 (1H, d, $J = 4.8$ Hz, aromatic H), 6.96 (1H, d, $J = 5.1$ Hz, aromatic H), 6.45 (1H, d, $J_{NH,H1} = 7.2$ Hz, NH), 6.00 (1H, dd, $J_{H1,NH} = 6.9$ Hz, $J_{H1,H2} = 5.1$ Hz, H-1), 5.25 (1H, dd, $J = 5.4$, 10.2 Hz), 2.57 (3H, s, CH_3).

3.4. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-bromothiophene-2-carboxamide (6)

The reaction of 4-bromo-thiophene-2-carboxylic acid (0.60 g, 1.44 mmol) as described for 3-bromothiophene-2-carboxylic acid gave a mixture of anomers (colourless solid, α:β, 1:16). Recrystallisation from EtOAc and cyclohexane gave **6** as a crystalline solid (first crop, β-anomer 0.82 g, 53%); the second crop obtained from the mother liquor was a mixture of anomers (0.54 g, 35%). Analytical data for β-anomer: mp 132–135 °C; IR (solution in $CHCl_3$): 3425 (NH), 2956, 1760 (ester C=O), 1677 (amide C=O), 1530, 1503, 1368, 1251, 1042 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.45 (1H, d, $J = 1.5$ Hz, aromatic H), 7.37 (1H, d, $J = 1.5$ Hz, aromatic H), 6.90 (1H, d, $J_{NH,H1} = 9.0$ Hz, NH), 5.36 (2H, 2 overlapping t, $J = 9.6$, 10.2 Hz, H-1, 3), 5.10 (1H, t, $J = 9.6$ Hz, H-4), 5.01 (1H, t, $J = 9.6$ Hz, H-2), 4.35 (1H, dd, $J_{6a,6b} = -12.6$ Hz, $J_{6a-5} = 4.2$ Hz, H-6a), 4.10 (1H, dd, $J_{6b,6a} = -12.6$ Hz, $J_{6b,5} = 2.4$ Hz, H-6b), 3.88 (1H, ddd, $J_{5,4} = 10.2$ Hz, $J_{5,6a} = 4.2$ Hz, $J_{5,6b} = 2.1$ Hz, H-5), 2.08, 2.06, 2.05 (12H, each s, each OAc); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.6, 170.1, 169.9, 169.7 (each s, each ester C=O), 160.5 (s, amide C=O), 138.5 (s, aromatic C), 131.2 (d, aromatic CH), 129.1 (d, aromatic CH), 110.4 (s, aromatic C), 78.9, 73.7, 72.5, 70.8, 68.3 (each s), 61.7 (t, C-6), 20.7, 20.6 (each q, each OAc); ESI-LRMS: found m/z 536 and 538 $[M+H^+]$, 418, 403, 375, 331, 310, 271, 211, 169; ESIMS m/z calcd for $C_{21}H_{23}BrNO_{10}S$ $[M+H]^+$: 536.0226. Found: 536.0214. Anal. Calcd for $C_{21}H_{22}BrNO_{10}S$: C, 42.55; H, 4.13; Br, 14.90; N, 2.61; S, 5.98. Found: C, 42.35; H, 4.12; Br, 15.17; N, 2.61; S, 6.24. Selected ^{1}H NMR data for the α-anomer: δ 7.56 (1H, d, $J = 1.2$ Hz, aromatic H), 7.49 (1H, d, $J = 1.2$ Hz, aromatic H), 6.71 (1H, d, $J_{NH,H1} = 6.9$ Hz, NH), 6.01 (1H, dd, $J_{H1,NH} = 6.9$ Hz, $J_{H1,H2} = 5.7$ Hz, H-1).

3.5. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-bromothiophene-2-carboxamide (7)

To a solution of 5-bromothiophene-2-carboxylic acid (0.29 g, 1.38 mmol) in anhyd CH_2Cl_2 (7.5 mL) was added oxalyl chloride (0.17 g, 0.12 mL, 1.38 mmol) followed by anhyd DMF (two drop) and the solution was stirred at rt for 2 h. The solvent was removed under diminished pressure to afford the acid chloride as a yellow oil, which was used immediately without further purification. To a solution of the amine **3** (0.48 g, 1.38 mmol) in CH_2Cl_2 (7.5 mL) was added anhyd pyridine (0.11 g, 0.11 mL, 1.38 mmol) and a solution of the acid chloride in CH_2Cl_2 (1 mL) and stirring was continued overnight at rt. The solution was diluted with CH_2Cl_2 (20 mL), transferred to a separating funnel and washed with H_2O (20 mL) and satd $NaHCO_3$ solution (20 mL), dried ($MgSO_4$) and the solvent was removed under diminished pressure. Crystallisation of the residue from EtOAc/cyclohexane gave two crops; the first was β-anomer **7** as a crystalline solid (0.38 g, 51%) and the second crop was a mixture of anomers (0.30 g, 41%). Analytical data for β-anomer: mp 152–155 °C; $[\alpha]_D$ +10.4 (c 0.8, $CDCl_3$); IR (film on NaCl plates): 2956, 1752 (ester C=O), 1666 (amide C=O), 1541, 1516, 1420, 1370, 1229, 1039, 908, 810, 743, 599, 567 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.21 (1H, d, $J = 3.9$ Hz, aromatic H), 7.05 (1H, dd, $J = 3.6$, 0.9 Hz, aromatic H), 6.85 (1H, d, $J_{NH,H1} = 9.4$ Hz, NH), 5.32 (1H, t, $J = 9.3$ Hz, H-3), 5.10 (1H, t, $J = 9.6$ Hz, H-4), 5.03 (1H, t, $J = 9.6$ Hz, H-2), 4.35 (1H, dd, $J_{6a,6b} = -12.6$ Hz, $J_{6a,5} = 4.5$ Hz, H-6a), 4.10 (1H, dd, $J_{6b,6a} = -12.6$ Hz, $J_{6b,5} = 2.1$ Hz, H-6b), 3.88 (1H, ddd, $J_{5,4} = 10.2$ Hz, $J_{5,6a} = 4.2$ Hz, $J_{5,6b} = 2.1$ Hz, H-5), 2.51 (3H, s, CH_3), 2.08, 2.04 (12H, each s, each OAc); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.7, 170.6, 169.8, 169.6 (each s, each ester C=O), 160.6 (s, amide C=O), 138.9 (s, aromatic C), 130.9 (d, aromatic CH), 129.2 (s, aromatic C), 120.0 (d, aromatic CH), 79.0, 73.7, 72.5, 70.8, 68.2 (each d), 61.6 (t), 20.8, 20.6 (each q, each OAc); ESI-LRMS: found m/z 538 and 536 $[M+H]^+$, 374, 331, 271, 169; ESIMS m/z calcd for $C_{21}H_{23}BrNO_{10}S$ $[M+H]^+$: 536.0226. Found: 536.0247. Anal. Calcd for $C_{21}H_{23}BrNO_{10}S$: C, 42.55; H, 4.13; Br, 14.90; N, 2.61; S, 5.98. Found: C, 42.53; H, 4.01; Br, 15.33; N, 2.33; S, 6.30.

3.6. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-methylthiophene-2-carboxamide (8)

The reaction of 5-methylthiophene-2-carboxylic acid (0.07 g, 0.47 mmol) as for 5-bromothiophene-2-carboxylic

acid gave **8** as a pale yellow solid (0.16 g, 73%); mp 154–156 °C; IR (solution in $CDCl_3$): 3431 (NH), 2959, 2923 (CH), 1757 (ester C=O), 1671 (amide C=O), 1543, 1513, 1460, 1368, 1229, 1039 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.32 (1H, d, $J = 3.6$ Hz, aromatic H), 6.86 (1H, d, $J_{NH,H1} = 9.3$ Hz, NH), 6.75 (1H, dd, $J = 3.6$, 0.9 Hz, aromatic H), 5.36 (2H, t, $J = 9.3$ Hz, H-1,3), 5.10 (1H, t, $J = 9.5$ Hz, H-2), 5.03 (1H, t, $J = 9.6$ Hz, H-4), 4.34 (1H, dd, $J_{6a,6b} = -12.5$ Hz, $J_{6a,5} = 4.4$ Hz, H-6a), 4.11 (1H, dd, $J_{6b,6a} = -12.5$ Hz, $J_{6b,5} = 2.1$ Hz, H-6b), 3.88 (1H, ddd, $J_{5,4} = 10.2$ Hz, $J_{5,6a} = 4.2$ Hz, $J_{5,6b} = 2.1$ Hz, H-5), 2.51 (3H, s, CH_3), 2.08, 2.04 (2s), 2.03 (each 3H, each s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.5, 170.7, 169.9, 169.6 (s, each ester C=O), 161.7 (s, amide C=O), 147.3, 134.8 (each s, aromatic C), 129.7, 126.4 (each d, aromatic CH), 78.9, 73.6, 72.6, 70.7, 68.3 (each d), 61.7 (t), 20.6, 20.7, 15.7 (each q); ESI-LRMS: found m/z 472 $[M+H]^+$, 457, 399, 331, 310, 169; ESIMS m/z calcd for $C_{20}H_{26}NO_{10}S$ $[M+H]^+$: 472.1277. Found: 472.1271. Anal. Calcd for $C_{20}H_{25}NO_{10}S$: C, 50.95, H, 5.34, N, 2.97, S, 6.80. Found: C, 50.99, H, 5.44, N, 3.16, S, 7.04. Selected ^1H NMR data for the α-anomer: δ 7.49 (1H, d, $J = 3.6$ Hz, aromatic H), 6.79 (1H, d, $J = 3.9$ Hz, aromatic H), 6.02 (1H, dd, $J_{H1,NH} = 7.2$ Hz, $J_{H1,H2} = 5.7$ Hz, H1), 2.54 (3H, s, CH_3).

3.7. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-ethylthiophene-2-carboxamide (9)

The reaction of 5-ethylthiophene-2-carboxylic acid (0.23 g, 1.44 mmol) as for 3-bromothiophene-2-carboxylic acid gave a mixture of anomers (0.64 g, 91% yield, α:β, 1:16). The residue was recrystallised from EtOAc and cyclohexane to afford β-anomer **9** as a colourless crystalline solid (0.42 g, 51%) and as an adduct with EtOAc (1:1); mp 64–66 °C; $[\alpha]_D$ +12 (c 8.0, $CDCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.33 (1H, d, $J = 3.9$ Hz, aromatic H), 6.84 (1H, d, $J_{NH,H1} = 9.3$ Hz, NH), 6.77 (1H, dd, $J = 3.9$, 0.9 Hz, aromatic H), 5.37 (2H, 2 × overlapping t, $J = 9.3$ Hz, H-1,3), 5.10 (1H, t, $J = 9.3$, H-4), 5.03 (1H, t, $J = 9.3$ Hz, H-2), 4.34 (1H, dd, $J_{6a,6b} = -12.5$ Hz, $J_{6a,5} = 4.2$ Hz, H-6a), 4.09 (1H, dd, $J_{6b,6a} = -12.5$ Hz, $J_{6b,5} = 2.1$ Hz, H-6b), 3.88 (1H, ddd, $J_{5,4} = 9.9$ Hz, $J_{5,6a} = 4.2$ Hz, $J_{5,6b} = 2.1$ Hz, H-5), 2.86 (2H, q, $J = 7.5$ Hz, CH_2CH_3), 2.08, 2.04 (2s), 2.03 (each 3H, each s, each CH_3), 1.32 (3H, t, $J = 7.5$ Hz, CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.5, 170.7, 169.9, 169.6 (each s, each ester C=O), 161.8 (s, amide C=O), 154.9, 134.3 (each s, each aromatic C), 129.5, 124.6 (each d, each aromatic CH), 78.9, 73.6, 72.6, 70.7, 68.3 (each d), 61.7 (t), 23.8 (t, CH_2CH_3), 20.6, 20.7 (each q, each OAc), 15.7 (q, CH_2CH_3); ESI-LRMS: found m/z 486 $[M+H]^+$, 324, 271, 169; ESIMS m/z calcd for $C_{21}H_{28}NO_{10}S$ $[M+H]^+$: 486.1434. Found: 486.1448. Anal. Calcd for $C_{25}H_{35}NO_{12}S$ (EtOAc adduct): C, 52.35; H, 6.15; N, 2.44; S, 5.59. Found: C, 52.22; H, 6.09; N, 2.49; S, 5.92.

3.8. General procedure for removal of acetate groups

To a solution of per-O-acetylated compound in CH_3OH (0.2 M) was added K_2CO_3 (5–10 mol %) and the mixture was stirred at rt (30 min) and the solvent was then removed under diminished pressure. The residue was immediately purified by flash chromatography on (1:9, CH_3OH–CH_3CN, R_f of product ~0.2–0.4) to afford the de-O-acetylated compounds as yellow syrups and as mixtures of anomers. The syrups were purified by reverse phase HPLC to obtain pure β-anomers for biological evaluation.

3.9. *N*-(β-D-Glucopyranosyl)-3-bromo-thiophene-2-carboxamide (10)

Removal of acetate protecting groups from **4** and purification by reverse phase HPLC gave **10** (0.10 g, 73%); $[\alpha]_D$ −18.1 (c 0.8, H_2O); ^1H NMR (300 MHz, D_2O): δ 7.60 (1H, d, $J = 4.2$ Hz, aromatic H), 7.24 (1H, d, $J = 4.2$ Hz, aromatic H), 5.15 (1H, d, $J_{1,2} = 8.7$ Hz, H-1), 3.91 (1H, dd, $J_{6a,6b} = -12.5$ Hz, $J_{6a,5} = 1.8$ Hz, H-6a), 3.77 (1H, dd, $J_{6b,6a} = -12.5$ Hz, $J_{6b,5} = 7.2$ Hz, H-6b), 3.64–3.51 (4H, overlapping signals, H-2–5); ^{13}C NMR (75 MHz, CD_3OD): δ 163.6 (s, C=O amide), 133.8 (s, aromatic C), 133.4, 131.7 (each d, aromatic CH), 112.6 (s, aromatic C), 81.7, 79.9, 79.0, 74.0, 71.3 (each d), 62.6 (t); ESI-LRMS: found m/z 368 and 366 $[M-H]^-$, 248, 246; ESIMS m/z calcd for $C_{11}H_{13}BrNO_6S$ $[M-H]^-$: 365.9647. Found: 365.9662.

3.10. *N*-(β-D-Glucopyranosyl)-3-methylthiophene-2-carboxamide (11)

Removal of acetate protecting groups from **5** and purification by reverse phase HPLC gave **11** (108 mg, 89%); $[\alpha]_D$ −7.0 (c 1.0, CH_3OH); IR (KBr): 3335 (br, OH), 2926, 2852 (CH), 1628 (C=O), 1540, 1521, 1415, 1379, 1286, 1078, 1036, 767, 731 cm^{-1}; ^1H NMR (300 MHz, D_2O): δ 7.64 (1H, d, $J = 5.1$ Hz, aromatic H), 7.11 (1H, d, $J = 4.8$ Hz, aromatic H), 5.21 (1H, d, $J_{1,2} = 8.7$ Hz, H-1), 3.98 (1H, dd, $J_{6a,6b} = -12.3$ Hz, $J_{6a,5} = 2.1$ Hz, H-6a), 3.83 (1H, dd, $J_{6b,6a} = -12.3$ Hz, $J_{6b,5} = 5.1$ Hz, H-6b), 3.70–3.50 (4H, overlapping signals, H-2–5); ^{13}C NMR (75 MHz, CD_3OD): δ 165.1 (s, amide C=O), 142.4 (s, aromatic C), 131.4, 127.5 (each d, each aromatic CH), 80.3, 78.4, 77.7, 72.3, 71.0 (each d), 61.3 (t), 14.5 (q); ESI-LRMS: found m/z 302 $[M-H]^-$, 182; ESIMS m/z calcd for $C_{12}H_{16}NO_6S$ $[M-H]^-$: 302.0698. Found: 302.0699.

3.11. *N*-(β-D-Glucopyranosyl)-4-bromothiophene-2-carboxamide (12)

Removal of acetate protecting groups from **6** and purification by reverse phase HPLC gave **12** (0.16 g, 79%): $[\alpha]_D$ −6.5 (*c* 0.8, H_2O); ^{1}H NMR (300 MHz, D_2O): δ 7.76 (1H, s, aromatic H), 7.75 (1H, s, aromatic H), 5.17 (1H, d, $J_{1,2} = 8.7$ Hz, H-1), 3.90 (1H, dd, $J_{6a,6b} = -12.3$ Hz, $J_{6a,5} = 2.1$ Hz, H-6a), 3.77 (1H, dd, $J_{6b,6a} = -12.3$ Hz, $J_{6b,5} = 4.8$ Hz, H-6b), 3.61–3.45 (4H, overlapping signals, H-2–5); ^{13}C NMR (75 MHz, CD_3OD): δ 162.3 (s, amide C=O), 139.7, 131.2 (each s, each aromatic C), 128.9, 109.5 (each d, each aromatic CH), 80.3, 78.4, 77.6, 72.3, 70.0 (each d), 61.3 (t); ESIMS *m*/*z* calcd for $C_{11}H_{13}BrNO_6S$ $[M-H]^-$: 365.9647. Found: 365.9665.

3.12. *N*-(β-D-Glucopyranosyl)-5-bromothiophene-2-carboxamide (13)

Removal of acetate protecting groups from **7** and purification by reverse phase HPLC gave **13** as a colourless solid (0.10 g, 73%); $[\alpha]_D$ −18.1 (*c* 0.8, H_2O); IR (KBr disk): 3380 (br, OH), 2923, 1643 (amide C=O), 1538, 1416, 1285, 1079, 1043, 881 cm^{-1}; ^{1}H NMR (300 MHz, D_2O): δ 7.53 (1H, d, $J = 4.2$ Hz, aromatic H), 7.18 (1H, d, $J = 4.2$ Hz, aromatic H), 5.09 (1H, d, $J_{1,2} = 8.7$ Hz, H-1), 3.84 (1H, dd, $J_{6a,6b} = -12.5$ Hz, $J_{6a,5} = 1.8$ Hz, H-6a), 3.71 (1H, dd, $J_{6b,6a} = -12.5$ Hz, $J_{6b,5} = 7.2$ Hz, H-6b), 3.58–3.39 (4H, overlapping signals, H-2–5); ^{13}C NMR (75 MHz, CD_3OD): δ 163.8 (s, C=O amide), 141.8 (s, aromatic C), 132.4, 131.0 (each d, aromatic CH), 119.9 (s, aromatic C), 81.7, 79.8, 79.1, 73.7, 71.5 (each d), 62.7 (t); ESIMS *m*/*z* calcd for $C_{11}H_{13}BrNO_6S$ $[M-H]^-$: 365.9647. Found: 365.9662.

3.13. *N*-(β-D-Glucopyranosyl)-5-methylthiophene-2-carboxamide (14)

Removal of acetate protecting groups from **8** and purification by reverse phase HPLC gave **14** (0.15 g, 83%); $[\alpha]_D$ −25.1 (*c* 0.6, CH_3OH); IR (KBr): 3361 (br, OH), 2923, 1638 (C=O), 1549, 1529, 1460, 1300, 1078, 1040, 894, 816, 747 cm^{-1}; ^{1}H NMR (300 MHz, D_2O): δ 7.70 (1H, d, $J = 3.9$ Hz, aromatic H), 6.98 (1H, d, $J = 3.6$ Hz, aromatic H), 5.21 (1H, d, $J_{1,2} = 8.7$ Hz, H-1), 3.97 (1H, dd, $J_{6a,6b} = -12.3$ Hz, $J_{6a,5} = 2.1$ Hz, H-6a), 3.82 (1H, dd, $J_{6b,6a} = -12.3$ Hz, $J_{6b,5} = 4.8$ Hz, H-6b), 3.70–3.51 (4H, overlapping signals, H-2–5), 2.46 (3H, s, CH_3); ^{13}C NMR (75 MHz, D_2O): δ 165.3 (s, amide C=O), 148.8 (s, aromatic C), 131.3, 126.9 (each d, aromatic CH), 79.9, 77.6, 76.5, 71.7, 69.2 (each d), 60.5 (t), 14.8 (q); ESI-LRMS: found *m*/*z* 302 $[M-H]^-$, 182, 119; ESIMS *m*/*z* calcd for $C_{12}H_{16}NO_6S$ $[M-H]^-$: 302.0698. Found: 302.0709.

3.14. *N*-(β-D-Glucopyranosyl)-5-ethylthiophene-2-carboxamide (15)

Removal of acetate protecting groups from **9** and purification by reverse phase HPLC gave **15** (0.14 g, 71%); $[\alpha]_D$ −16.3 (*c* 0.5, CH_3OH); IR (KBr): 3362 (br, OH), 2968, 2932, 1648 (amide C=O), 1551, 1530, 1464, 1365, 1207, 1171, 1079, 1040, 982, 889, 815, 745 cm^{-1}; ^{1}H NMR (300 MHz, D_2O): δ 7.56 (1H, d, $J = 3.6$ Hz, aromatic H), 6.84 (1H, d, $J = 3.6$ Hz, aromatic H), 5.07 (1H, d, $J_{1,2} = 8.7$ Hz, H-1), 3.83 (1H, dd, $J_{6a,6b} = -12.3$ Hz, $J_{6a,5} = 2.1$ Hz, H-6a), 3.68 (1H, dd, $J_{6b,6a} = -12.3$ Hz, $J_{6b,5} = 4.8$ Hz, H-6b), 3.56–3.37 (4H, overlapping signals, H-2–5), 2.79 (2H, q, $J = 7.8$ Hz, CH_2CH_3), 1.20 (3H, t, $J = 7.8$ Hz, CH_2CH_3); ^{13}C NMR (75 MHz, D_2O): δ 165.3 (s, amide C=O), 156.3 (s, aromatic C), 131.2, 125.1 (each d, aromatic CH), 79.9, 77.6, 76.5, 71.7, 69.3 (each d), 60.5 (t), 23.2 (t), 15.0 (q); ESI-LRMS: found *m*/*z* 316 $[M-H]^-$, 196, 119; ESIMS *m*/*z* calcd for $C_{13}H_{18}NO_6S$ $[M-H]^-$: 316.0855. Found: 316.0840.

3.15. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3-trimethylsilylethynyl-thiophene-2-carboxamide (16)

To a solution of **4** (0.40 g, 0.75 mmol) in degassed DMF (1.6 mL) in a 5 mL round-bottom flask were added palladium(II) chloride (14.1 mg, 0.08 mmol), Ph_3P (43.1 mg, 0.16 mmol), copper iodide (15.2 mg, 0.08 mmol) and Et_3N (0.15 g, 0.20 mL, 1.50 mmol). The reaction mixture was allowed to equilibrate for 20 min at 80 °C under an atmosphere of nitrogen. Ethynyltrimethylsilane (0.44 g, 0.64 mL, 4.50 mmol) was then added rapidly and the flask sealed. The mixture was heated at 80 °C for 96 h with further additions of ethynyltrimethylsilane (0.32 mL) every 24 h. The reaction mixture was allowed to cool, diluted with EtOAc (20 mL), washed with NH_4Cl (satd, 10 mL), H_2O (10 mL) and then dried ($MgSO_4$). The volatile components were removed under diminished pressure and chromatography (3:7, EtOAc/cyclohexane, $R_f = 0.17$) of the residue (a dark brown oil) gave the title compound as an orange/brown foam (0.26 g, 63%); mp 145–147 °C; $[\alpha]_D$ −48.6 (*c* 1.2, $CDCl_3$); IR (solution in $CHCl_3$): 3352 (NH), 2961, 2902 (CH), 2151 (C≡C), 1758 (ester C=O), 1662 (amide C=O), 1554, 1411, 1373, 1252, 1227, 1220, 1042 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.27 (1H, d, $J_{NH,H1} = 9.6$ Hz, NH), 7.45 (1H, d, $J = 5.1$ Hz, aromatic H), 7.12 (1H, d, $J = 5.1$ Hz, aromatic H), 5.56 (1H, t, $J = 9.6$ Hz, H-1), 5.35 (1H, t, $J = 9.6$ Hz, H-3), 5.07 (1H, t, $J = 9.6$ Hz, H-2), 5.01 (1H, t, $J = 9.3$ Hz, H-4), 4.27 (1H, dd, $J_{6a,6b} = -12.6$ Hz, $J_{6a,5} = 4.5$ Hz, H-6a), 4.12 (1H, dd, $J_{6b,6a} = -12.6$ Hz, $J_{6b,5} = 1.8$ Hz, H-6b), 3.88 (1H, ddd, $J_{5,4} = 10.2$ Hz, $J_{5,6a} = 4.5$ Hz, $J_{5,6b} = 1.8$ Hz, H-5), 2.07, 2.05, 2.03, 2.00 (12H, each s, each CH_3); ^{13}C NMR (75 MHz,

$CDCl_3$): δ 170.5, 169.91, 169.86, 169.5 (each s, each ester C=O), 161.0 (s, amide C=O), 140.1 (s, aromatic C), 132.0, 130.4 (each d, aromatic CH), 121.6 (s, aromatic C), 104.1, 98.2 (each s, C≡C), 78.1, 73.8, 73.0, 70.5, 68.2 (each d), 61.8 (t), 20.6 (3s), 20.5 (each q, each OAc), −0.45 (q, $(CH_3)_3Si$); ESI-LRMS: found *m/z* 554 $[M+H]^+$, 577, 331, 271, 169; ESIMS *m/z* calcd for $C_{24}H_{32}NO_{10}SSi$ $[M+H]^+$: 554.1516. Found: 554.1489. Anal. Calcd for $C_{24}H_{31}NO_{10}SSi$: C, 52.06; H, 5.64; N, 2.53. Found: C, 51.93; H, 5.50; N, 2.55.

3.16. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-trimethylsilylethynyl-thiophene-2-carboxamide (17)

To a solution of **6** (0.70 g, 1.31 mmol) in degassed DMF (2.8 mL) in a 5 mL round-bottomed flask were added palladium(II) chloride (24.5 mg, 0.13 mmol), Ph_3P (68.0 mg, 0.26 mmol), copper iodide (24.8 mg, 0.13 mmol) and Et_3N (0.32 g, 0.44 mL, 3.14 mmol). The reaction mixture was allowed to equilibrate for 20 min at 80 °C under an atmosphere of nitrogen. Ethynyltrimethylsilane (0.67 mL, 0.32 g, 4.71 mmol) was then added rapidly and the flask sealed. The mixture was heated at 80 °C for 72 h with a further addition of ethynyltrimethylsilane after 48 h. The reaction mixture was allowed to cool, diluted with EtOAc (20 mL), washed with NH_4Cl (satd, 10 mL), H_2O (10 mL) and then dried ($MgSO_4$). The volatile components were removed under diminished pressure and chromatography (3:7, EtOAc/cyclohexane, $R_f = 0.17$) of the residue gave the title compound as an orange/brown foam (0.69 g, 95%); IR (film on NaCl plates): 2961, 2901, 2163 (C≡C), 1754 (ester C=O), 1674 (amide C=O), 1547, 1523, 1427, 1377, 1251, 1042, 966, 909, 857, 762, 737 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.63 (1H, s, aromatic H), 7.44 (1H, s, aromatic H), 6.85 (1H, d, $J_{NH,H1} = 9.0$ Hz, NH, 5), 5.37 (2H, overlapping t, $J = 9.3$, 9.0 Hz, H-1,3), 5.10 (1H, t, $J = 9.6$ Hz, H-4), 5.01 (1H, t, $J = 9.3$ Hz, H-2), 4.34 (1H, dd, $J_{6a,6b} = -12.3$ Hz, $J_{6a,5} = 4.2$ Hz, H-6a), 4.11 (1H, dd, $J_{6b,6a} = -12.3$ Hz, $J_{6b,5} = 1.9$ Hz, H-6b), 3.88 (1H, ddd, $J_{5,4} = 10.3$ Hz, $J_{5,6a} = 4.4$ Hz, $J_{5-6b} = 2.2$ Hz, H-5), 2.08, 2.05 (12H, each s, each OAc), 0.25 (9H, s, $(CH_3)_3Si$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.7, 170.8, 170.0, 169.8 (each s, each ester C=O), 161.2 (s, amide C=O), 137.6 (s, aromatic C), 135.2, 131.5 (each d, aromatic CH), 123.5 (s, aromatic C), 98.6, 95.2 (each s, each C≡C), 79.0, 73.8, 72.7, 70.9, 68.4 (each d), 61.8 (t), 20.9, 20.7 (each q, each OAc), 0.0 (q, $(CH_3)_3Si$); ESIMS *m/z* calcd for $C_{24}H_{32}NO_{10}SSi$ $[M+H^+]$: 554.1516. Found: 554.1506.

3.17. Dimeric conjugate (18)

Reaction of **4** (0.84 g, 1.57 mmol) as described for **16** above without replenishment of volatile ethynyltrimethylsilane gave after chromatography (gradient elution, 3:7–4:1, EtOAc/cyclohexane) **18** (0.14 g, 0.26 mmol, 17%) and **16** as an orange/brown foam (0.37 g, 60%). Analytical data for **18**: IR (solution in $CDCl_3$): 3382 (NH), 2960 (CH), 1730 (ester C=O), 1662 (amide C=O), 1522, 1423, 1378, 1230, 1043 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.73 (2H, d, $J = 9.3$ Hz, NH), 7.57 (2H, d, $J = 5.1$ Hz, aromatic H), 7.41 (2H, d, $J = 5.1$ Hz, aromatic H), 5.57 (2H, t, $J = 9.5$ Hz, H-1), 5.37 (2H, t, $J = 9.5$ Hz, H-3), 5.11–5.04 (2H, 2 overlapping t, $J = 9.9$ and $J = 9.6$ Hz, H-2,4), 4.30 (2H, dd, $J_{6a,6b} = -12.6$ Hz, $J_{6a,5} = 4.8$ Hz, H-6a), 4.12 (2H, dd, $J_{6b,6a} = -12.6$ Hz, $J_{6b,5}$ 2.1 Hz, H-6b), 3.94 (2H, ddd, $J_{5,4} = 10.2$ Hz, $J_{5,6a} = 4.5$ Hz, $J_{5,6b} = 2.1$ Hz, H-5), 2.05, 2.04, 2.02, 2.00 (each 6H, each s, each OAc); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5, 170.4, 169.8, 169.6 (each s, each ester C=O), 160.8 (s, amide C=O), 139.6 (s, aromatic C), 132.2, 130.4 (each d, each aromatic CH), 121.2 (s, aromatic C), 89.5 (s, C≡C), 78.5, 73.8, 73.0, 70.6, 68.3 (each d), 61.9 (t), 20.7, 20.8 (each q, each OAc); ESI-LRMS: found *m/z* 959 $[M+Na]^+$, 937 $[M+H]^+$, 482, 480, 331, 279, 169; ESIMS *m/z* calcd for $C_{40}H_{44}N_2NaO_{20}S_2$ $[M+Na]^+$: 959.1827. Found: 959.1808.

3.18. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3-ethynylthiophene-2-carboxamide (19)

To a solution of **16** (20.0 mg, 0.036 mmol) in THF at 0 °C was added *n*-Bu_4NF (36 μL of a 1.0 M solution in H_2O/THF 5:95, 0.036 mmol). The solution turned from pale brown to dark brown and was immediately diluted with Et_2O (10 mL) and poured onto satd NH_4Cl (5 mL). The layers were separated and the organic layer dried ($MgSO_4$) and the solvent was removed under diminished pressure. Chromatography of the residue (1:1, EtOAc/cyclohexane, $R_f = 0.27$) gave the title compound as a pale brown film (15.8 mg, 91%); $[\alpha]_D$ −17.8 (*c* 0.4, $CDCl_3$); IR (solution in $CHCl_3$): 3373 (NH), 3299 (C≡C–H), 2960, 2928, 2856 (CH), 2117 (C≡C), 1754 (ester C=O), 1664 (amide C=O), 1532, 1413, 1369, 1248, 1224, 1040 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.06 (1H, d, $J_{NH,H1} = 9.0$ Hz, NH), 7.48 (1H, d, $J = 5.1$ Hz, aromatic H), 7.15 (1H, d, $J = 5.1$ Hz, aromatic H), 5.47 (1H, t, $J = 9.5$ Hz, H-1), 5.33 (1H, t, $J = 9.5$ Hz, H-3), 5.14 (2H, t, $J = 9.5$ Hz, H-2,4), 4.34 (1H, dd, $J_{6a,6b} = -12.3$ Hz, $J_{6a,5} = 4.2$ Hz, H-6a), 4.11 (1H, dd, $J_{6b,6a} = -12.6$ Hz, $J_{6b,5} = 2.1$ Hz, H-6b), 3.89 (1H, ddd, $J_{5,4} = 10.2$ Hz, $J_{5,6a} = 4.5$ Hz, $J_{5,6b} = 2.1$ Hz, H-5), 3.70 (1H, s), 2.07, 2.05, 2.03, 2.00 (12H, each s, each CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7, 170.0, 169.9, 169.6 (each s, each ester C=O), 161.1 (s, amide C=O), 140.9 (s, aromatic C), 132.1, 130.7 (each d, each aromatic CH), 120.3 (s, aromatic C), 86.2 (s, C≡C), 78.2, 73.3, 69.9, 68.1 (each d), 61.7 (t), 20.7, 20.6 (each q, each OAc); ESI-LRMS: found

m/z 504 $[M+Na]^+$, 482 $[M+H]^+$, 233; ESIMS *m/z* calcd for $C_{21}H_{24}NO_{10}S$ $[M+H]^+$: 482.1121. Found: 482.1143.

3.19. *N*-(β-D-Glucopyranosyl)-3-ethynylthiophene-2-carboxamide (21)

Removal of acetate protecting groups from **19** and purification by reverse phase HPLC gave **21** as a white solid (63 mg, 77%). In this case excess K_2CO_3 (1.1 equiv) was used and the reaction was stirred for 3 h at rt; $[\alpha]_D$ −4.1 (*c* 0.7, CH_3OH); IR (KBr disk): 3496, 3348, 3278 (OH), 2933, 2108 (C≡C), 1662 (amide C=O), 1552, 1301, 1108, 1087, 1042, 776 cm^{-1}; ^1H NMR (300 MHz, D_2O): δ 7.80 (1H, d, $J = 5.4$ Hz, aromatic H), 7.37 (1H, d, $J = 5.1$ Hz, aromatic H), 5.26 (1H, d, $J_{1,2} = 8.7$ Hz, H-1), 3.98 (1H, dd, $J_{6a,6b} = -12.3$ Hz, $J_{6b,5} = 2.1$ Hz, H-6a), 3.69 (1H, dd, $J_{6b,6a} = -12.3$ Hz, $J_{6b,5} = 5.1$ Hz, H-6b), 3.69–3.49 (4H, overlapping signals, H-2–5); ESI-LRMS: found *m/z* 312 $[M-H]^-$, 192, 164, 150; ESIMS *m/z* calcd for $C_{13}H_{14}NO_6S$ $[M-H]^-$: 312.0542. Found: 312.0536.

3.20. *N*-(β-D-Glucopyranosyl)-4-ethynylthiophene-2-carboxamide (22)

Removal of TMS and acetate protecting groups from **17** and purification by reverse phase HPLC gave **22** as a white foam (75 mg, 88%); $[\alpha]_D$ −3.3 (*c* 0.6, CH_3OH); IR (KBr): 3356 (br, OH), 2927 (CH), 2111 (C≡C), 1655 (C=O), 1552, 1523, 1424, 1362, 1307, 1214, 1082, 1042, 982, 887, 855, 779 cm^{-1}; ^1H NMR (300 MHz, D_2O): δ 7.97 (1H, s, aromatic H), 7.84 (1H, s, aromatic H), 5.17 (1H, d, $J_{1,2} = 8.4$ Hz, H-1), 3.91 (1H, br d, $J_{6a,6b} = -12.3$ Hz, H-6a), 3.76 (1H, dd, $J_{6b,6a} = -12.3$ Hz, $J_{6b,5} = 4.8$ Hz, H-6b), 3.65–3.46 (4H, overlapping signals, H-2–5); ^{13}C NMR (75 MHz, CD_3OD): δ 162.8 (s, amide C=O), 138.8 (s, aromatic), 134.9 131.4 (each d, aromatic), 122.1 (s, aromatic), 80.3, 78.4, 77.6 (each d), 77.44 (s, C≡C), 77.39 (s, C≡C), 72.3, 70.0 (each d), 61.3 (t); ESI-LRMS *m/z* 312 $[M-H]^-$, 192; ESIMS *m/z* calcd for $C_{13}H_{14}NO_6S$ $[M-H]^-$: 312.0542. Found: 312.0544.

3.21. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-6*H*-thieno[2,3-*c*]pyridin-7-one (23)

To a solution of **16** (20.0 mg, 0.036 mmol) in THF at 0 °C was added *n*-Bu_4NF (36 μL of a 1.0 M solution in 5:95 H_2O/THF, 0.036 mmol). The reaction mixture immediately turned from pale brown to dark brown. The ice bath was removed and the reaction was allowed to attain rt and after 1 h was diluted with Et_2O (10 mL) and poured onto satd NH_4Cl (5 mL). The organic layer was separated, dried ($MgSO_4$) and the solvent removed under diminished pressure. Chromatography of the residue (1:1, EtOAc/cyclohexane, $R_f = 0.18$) gave **23** as a pale brown solid (14.2 mg, 83%); mp 82–84 °C; $[\alpha]_D$ +32.9 (*c* 1.3, $CDCl_3$); IR (film on NaCl plates): 2954, 2926, 2852 (CH), 1753 (ester C=O), 1659 (lactam C=O), 1598 (C=C), 1435, 1369, 1227, 1095, 1064, 1039, 899, 847, 789, 734, 661, 602 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.73 (1H, d, $J = 5.1$ Hz), 7.28 (1H, d, $J = 7.5$ Hz), 7.19 (1H, d, $J = 5.1$ Hz), 6.72 (1H, d, $J = 7.5$ Hz), 6.42 (1H, d, $J_{1,2} = 9.5$ Hz, H-1), 5.47 (1H, t, $J = 9.5$, H-3), 5.32 (1H, t, $J = 9.5$ Hz, H-2), 5.19 (1H, t, $J = 10.0$ Hz), 4.28 (1H, dd, $J_{6a,6b} = -12.6$ Hz, $J_{6a,5} = 5.1$ Hz, H-6a), 4.12 (1H, dd, $J_{6b,6a} = -12.6$ Hz, $J_{6b,5} = 2.1$ Hz, H-6b), 4.00 (1H, ddd, $J_{5,4} = 10.0$ Hz, $J_{5,6a} = 5.1$ Hz, $J_{5,6b} = 2.1$ Hz, H-5), 2.07, 2.06, 2.01, 1.86 (12H, each s, each OAc); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 169.8, 169.5, 169.3 (each s, each ester C=O), 158.0 (s, amide C=O), 145.1 (s, aromatic C) 134.5 (d, aromatic CH), 129.3 (s, aromatic C), 127.6, 124.6, 104.1, 79.5, 75.0, 73.1, 70.4, 68.2 (each d), 61.8 (t), 20.7, 20.56, 20.50, 20.3 (each q); ESI-LRMS: found *m/z* 504 $[M+Na]^+$, 482 $[M+H]^+$, 331, 271, 192, 169, 109; ESIMS *m/z* calcd for $C_{21}H_{24}NO_{10}S$ $[M+H]^+$: 482.1121. Found: 482.1103.

3.22. *N*-(β-D-Glucopyranosyl)-6*H*-thieno[2,3-*c*]pyridin-7-one (24)

Removal of acetate protecting groups from **23** and purification by reverse phase HPLC gave **24** (41.0 mg, 92%); IR (KBr): 3368 (br, OH), 2927, 2881, 1642 (amide C=O), 1575, 1079, 789 cm^{-1}; ^1H NMR (300 MHz, CD_3OD): δ 8.00 (1H, d, $J = 5.4$ Hz), 7.63 (1H, d, $J = 7.2$ Hz), 7.37 (1H, d, $J = 5.4$ Hz), 7.05 (1H, d, $J = 7.2$ Hz), 6.13 (1H, d, $J_{1,2} = 9.3$ Hz, H-1), 3.91–3.74 (4H, overlapping signals), 3.65 (1H, t, $J = 9.0$, 9.3 Hz); ^{13}C NMR (125 MHz, D_2O): δ 162.7 (s, amide C=O), 149.3 (s), 139.0 (d), 131.3 (s), 131.2, 127.8, 108.9, 85.1, 81.8, 79.1, 75.0, 72.0 (each d), 63.4 (t); ESI-LRMS: found *m/z* 312 $[M-H]^-$, 224, 150, 113; ESIMS *m/z* calcd for $C_{13}H_{14}NO_6S$ $[M-H]^-$: 312.0542. Found: 312.0556.

3.23. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranosyl)-thiophene-2-carboxamide (26)

The reaction of thiophene-2-carboxylic acid (0.516 g, 4.03 mmol) with amine **25**[31] (0.56 g, 1.612 mmol) as described for 3-bromo-thiophene-2-carboxylic acid gave **26** as a white foam (0.62 g, 84%); $R_f = 0.36$ (1:1, cyclohexane/EtOAc); mp 204–206 °C; IR (KBr): 3426, 1746 (ester C=O), 1655 (amide C=O), 1534 1227, 1054 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.53 (dd, 1H, $J = 1.2$, 5.0 Hz, aromatic H), 7.50 (dd, 1H, $J = 1.2$, 3.8 Hz, aromatic H), 7.09 (dd, 1H, $J = 3.8$, 5.0 Hz, aromatic H), 6.70 (d, 1H, $J_{NH,1} = 9.3$ Hz, NH), 5.72 (dd, 1H, $J_{1,2} = 1.2$ Hz, $J_{1,NH} = 9.3$ Hz, H-1), 5.44 (dd, 1H, $J_{2,1} = 1.2$, $J_{2,3} = 3.3$ Hz, H-2), 5.31 (1H, t, $J = 9.9$ Hz, H-4), 5.19 (dd, 1H, $J_{3,2} = 3.3$ Hz,

$J_{3,4} = 10.0$ Hz, H-3), 4.37 (dd, 1H, $J_{6a,5} = 5.2$ Hz, $J_{6a,6b} = -12.6$ Hz, H-6a), 4.13 (dd, 1H, $J_{6b,5} = 2.1$ Hz, $J = 10.4$ Hz, $J_{6b,6a} = -12.6$ Hz, H-6b), 3.87 (ddd, 1H, $J_{5,6b}$ 2.12 Hz, $J_{5,6a} = 5.21$ Hz, $J_{5,4} = 9.9$ Hz, H-5), 2.28, 2.10, 2.06, 2.00 (each s, each 3H, each OAc); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 170.5, 169.8, 169.6, 160.8 (each s, each ester C=O), 137.1 (s, aromatic C), 131.4, 129.4, 127.8 (each d, each aromatic C), 74.2 (H-1_β, $J_{C1,H} = 156.5$ Hz), 71.5, 70.4, 65.2 (each d), 62.15 (t), 20.8, 20.7, 20.6, 20.5 (each q, each OAc); ESI-LRMS: found m/z 480.1 $[M+Na]^+$, 496.7 $[M+K]^+$; ESIMS m/z calcd for $C_{19}H_{24}NO_{10}S$ $[M+H]^+$: 458.1121. Found: 458.1126. Anal. Calcd for $C_{19}H_{23}NO_{10}S$: C, 49.89; H, 5.07; N, 3.06; S, 7.01. Found: C, 49.79; H, 5.00; N, 2.87; S, 7.38.

3.24. *N*-(β-D-Mannopyranosyl)-thiophene-2-carboxamide (27)

The amide **26** (0.15 g, 0.328 mmol) was added to CH_3OH (15 mL), followed by an addition of crushed K_2CO_3 (6 mg) and the solution was stirred at rt for 4 h. The reaction was acidified to pH 5, using solid CO_2 pieces. The solvent was then removed, the crushed K_2CO_3 was removed by filtration through a short column of silica gel (1:10, CH_3OH/EtOAc), the solvent was removed and the residual oil further free dried to give the product as a white foam (80 mg, 84%). The foam was further purified by reverse phase HPLC to give the material used for analytical purposes and biological testing; $R_f = 0.55$ (2:3, CH_3OH/EtOAc); $[\alpha]_D$ −7.86 (*c* 0.1, H_2O); IR (KBr disc): 3261, 3102, 2918, 1674, 1534, 1421, 1273, 1073 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.90 (dd, 1H, $J = 1.0$, 3.9 Hz, aromatic H), 7.86 (dd, 1H, $J = 1.1$, 5.2 Hz, aromatic H), 7.30 (dd, 1H, $J = 3.9$, 5.0 Hz, aromatic H), 5.49 (d, 1H, $J = 1.1$ Hz, H-1), 4.14 (dd, 1H, $J = 1.1$, 3.3 Hz, H-2), 3.81–4.01 (3H, m, overlapping signals, H-3,6a,6b), 3.73 (t, 1H, $J = 9.6$ Hz, H-4), 3.63 (ddd, 1H, $J = 2.2$, 5.8, 8.5 Hz, H-5); ^{13}C NMR (125 MHz, D_2O): δ 167.3 (s, amide C=O), 139.0 (s, aromatic C), 135.6, 133.7, 131.2 (each d, each aromatic CH), 81.1, 80.7, 76.1, 73.1, 69.3 (each d), 63.7 (t); ESI-LRMS: found m/z 312.0 $[M+Na]^+$, 308.1 $[M+H_2O+H]^+$, 290.0 $[M+H]^+$, 288.0 $[M-H]^-$; ESIMS m/z calcd for $C_{11}H_{14}NO_6S$ $[M-H]^-$: 288.0542. Found: 288.0536.

3.25. *N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-thiophene-2-carboxamide (29)

Ph_3P (1.81 g, 6.9 mmol) was added to a solution of galactopyranosyl azide **28** (2.00 g, 5.3 mmol) and thiophene-2-carbonyl chloride (2.13 mL, 10.6 mmol) in anhyd CH_3CN (50 mL). The solution was flushed with N_2, and stirred overnight at rt. The reaction was then diluted with CH_2Cl_2 (50 mL), and washed with satd $NaHCO_3$ (3 × 100 mL), H_2O (2 × 100 mL) and dried ($MgSO_4$). The solvent was removed under diminished pressure, and the residual brown oil was purified by chromatography (3:1, petroleum spirit/EtOAc), to give the title compound as a white powder (1.33 g, 55%; $R_f = 0.43$, 1:1, cyclohexane/EtOAc); mp181–183 °C; IR (KBr): 3372, 1748 (ester C=O), 1663 (amide C=O), 1538, 1237, 1053 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.53 (d, 1H, $J = 4.9$ Hz, aromatic H), 7.49 (d, 1H, $J = 2.9$ Hz, aromatic H), 7.08 (dd, 1H, $J = 2.9$, 4.9 Hz, aromatic H), 6.95 (1H, d, $J = 8.8$ Hz, NH), 5.47 (1H, d, $J = 1.6$ Hz, H-4), 5.37 (t, 1H, $J = 8.8$ Hz, H-1), 5.23–5.17 (m, 2H, H-2,3), 4.17–4.08 (m, 3H, H-5,6a,6b), 2.15, 2.02, 2.00 (each s, 12H, each OAc); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.8, 170.3, 170.0, 169.7 (each s, each ester C=O), 161.5 (s, amide C=O), 137.5 (s, aromatic C), 131.6, 129.1, 127.8 (each d, each aromatic CH), 79.1, 72.3, 70.7, 68.4, 67.2 (each d), 61.1 (t), 20.7, 20.6, 20.5, 20.5 (each q); ESI-LRMS: found m/z 458.1 $[M+H]^+$, 480.1 $[M+Na]^+$, 501.9 $[M+HCOO]^-$, 456.1 $[M-H]^-$; ESIMS m/z calcd for $C_{19}H_{23}NO_{10}S$ $[M+H]^+$: 458.1121. Found: 458.1124. Anal. Calcd for $C_{19}H_{23}NO_{10}S$: C, 49.89; H, 5.07; N, 3.06; S, 7.01. Found: C, 49.86; H, 4.91; N, 3.30; S, 7.09.

3.26. *N*-(β-D-Galactopyranosyl)-thiophene-2-carboxamide (30)

The amide **29** (0.43 g, 0.94 mmol) was added to CH_3OH (20 mL). A solution of sodium methoxide in CH_3OH (0.1 mL of 0.25 M) was then added and the reaction mixture was stirred at rt for 1 h. The reaction mixture was then acidified to pH 2 by stirring in the presence of Amberlite, then filtered, the solvent removed under diminished pressure and the residue purified by chromatography (1:10, CH_3OH/EtOAc) and reverse phase HPLC to give the product as a white foam (0.21 g, 79%); $R_f = 0.53$ (2:3, CH_3OH/EtOAc); $[\alpha]_D$ +5.71 (*c* 0.1, H_2O); IR (KBr): 3424, 2924, 2360, 1637 (amide C=O), 1547 cm^{-1}; ^{1}H NMR (500 MHz, D_2O): δ 7.92 (d, 1H, $J = 3.9$ Hz, aromatic H), 7.87 (d, 1H, $J = 4.9$ Hz, aromatic H), 7.30 (dd, 1H, $J = 3.9$, 4.9 Hz, aromatic H), 5.21 (d, 1H, $J = 8.8$ Hz, H-1), 4.10 (d, 1H, $J = 2.9$ Hz, H-4), 3.94–3.82 (5H, overlapping signals, H-2,3,5,6a,6b); ^{13}C NMR (125 MHz, D_2O): δ 168.3 (s), 139.2 (s, aromatic C), 135.5, 133.65, 131.2 (each d, each aromatic CH), 83.2, 79.7, 76.3, 72.1, 71.55 (each d), 63.8 (t); ESI-LRMS: found m/z 334.2 $[M+HCOO]^-$, 312.8 $[M+Na]^+$; ESIMS m/z calcd for $C_{11}H_{14}NO_6S$ $[M-H]^-$: 288.0542. Found: 288.0535.

3.27. *N*-(2-Deoxy-2-phthalimido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-thiophene-2-carboxamide 32

The azide **31**[32] (0.59 g, 1.28 mmol) was dissolved in anhyd EtOAc (25 mL). Activated Pd/C was added to

the solution whilst purging the flask with N_2. The reaction was then placed under an atmosphere of H_2 and the mixture stirred at rt for 2.5 h, then filtered through Celite, and the solvent was removed. Recrystallisation of the residue (EtOAc/petroleum ether) gave the desired amine intermediate (0.44 g, 79%); $R_f = 0.19$ (1:1, petroleum ether/EtOAc); IR (KBr): 3412, 1749, 1691, 1232, 1017 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.83–7.68 (m, 4H, aromatic H), 5.92 (t, 1H, $J = 9.6$ Hz, H-3), 5.14 (t, 1H, $J = 9.6$ Hz, H-4), 5.04 (d, 1H, $J = 9.3$ Hz, H-1), 4.31 (dd, 1H, $J_{6a,5} = 4.8$ Hz, $J_{6a,6b} = -12.3$ Hz, H-6a), 4.16 (overlapping signals, 2H, H-2, 6b), 3.89 (ddd, 1H, $J_{5,6b} = 2.2$ Hz, $J_{5,6a} = 4.8$ Hz, $J_{5,4} = 9.6$ Hz, H-5), 2.10, 2.02, 1.85 (each s, each 3H, each OAc); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7, 170.0, 169.6 (each s), 134.3, 134.1 (each d, each aromatic CH), 123.6 (s, aromatic C), 82.1, 72.7, 71.0, 69.2 (each d), 62.3 (t), 55.7 (d), 20.8, 20.6, 20.5 (each q); ESI-LRMS: found m/z 457.0 $[M+Na]^+$; 435.0 $[M+H]^+$; ESIMS m/z calcd for $C_{20}H_{23}N_2O_9$ $[M+H]^+$: 435.1404. Found: 435.1416. Anal. Calcd for $C_{20}H_{22}N_2O_9$: C, 55.30; H, 5.10; N, 6.45. Found: C, 55.01; H, 5.07; N, 6.15. Thiophene-2 carbonyl chloride was freshly prepared from thiophene-2-carboxylic acid (0.19 mL, 1.50 mmol) as described above and was dissolved in anhyd CH_2Cl_2 (5 mL) and a solution of the amine (0.58 g, 1.33 mmol) in anhyd CH_2Cl_2 (30 mL), followed by pyridine (0.1 mL, 1.33 mmol) were added. The resulting mixture was left stirring overnight at rt. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with satd $NaHCO_3$ (1×50 mL), satd $CuSO_4$ solution (2×50 mL) and H_2O (1×50 mL). The organic layer was dried ($MgSO_4$) and the solvent was removed, yielding an oil. Chromatography (3:1, cyclohexane/EtOAc) gave the title compound **32** (0.29 g, 40%); $R_f = 0.36$, (1:1, cyclohexane/EtOAc); mp 121–124 °C; IR (KBr) 3508, 1719, 1647, 1231, 1037 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.60 (m, 4H, aromatic H), 7.48 (d, 1H, $J = 4.9$ Hz, aromatic H), 7.43 (d, 1H, $J = 3.7$ Hz, aromatic H), 7.03 (t, 1H, $J = 3.7$, 4.9 Hz, aromatic H), 6.55 (d, 1H, $J_{NH,1} = 9.8$ Hz, NH), 6.19 (d, 1H, $J_{1,NH} = 9.8$ Hz, H-1), 6.13 (d, 1H, $J = 9.2$ Hz, H-3), 5.22 (t, 1H, $J = 9.7$ Hz, H-4), 4.45 (overlapping signals, 2H, H-2,6), 4.16 (overlapping signals, 2H, H-5,6), 2.11, 2.05, 1.88 (each s, each 3H, each OAc); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7, 169.8, 169.7, 161.5 (each s), 137.4 (s, aromatic C), 134.7, 134.2, 131.5, 129.1, 127.7, 124.1, 123.5 (each d, each aromatic CH), 76.5, 73.6, 70.4, 68.7 (each d), 61.9 (t), 54.1 (d), 26.9 20.7, 20.6, 20.4 (each q, each OAc); ESI-LRMS: found m/z 370.1 $[M+Na]^+$; 348.1 $[M+H]^+$, 346.1 $[M-H]^-$, ESIMS m/z calcd for $C_{25}H_{25}N_2O_{10}S$ $[M+H]^+$: 545.1230. Found: 545.1224. Anal. Calcd for $C_{25}H_{24}N_2O_{10}S$: C, 55.14; H, 4.44; N, 5.14; S, 5.89. Found: C, 54.87; H, 4.27; N, 4.88; S, 6.10.

3.28. *N*-(2-Amino-2-deoxy-β-D-glucopyranosyl)-thiophene-2-carboxamide (33)

A mixture of the amide **32** (0.11 g, 0.20 mmol) and $NaOCH_3$ (5 mg, 0.09 mmol) was stirred in CH_3OH (10 mL) for 2 h at rt. The pH of the reaction mixture was reduced to pH 5 by addition of CO_2 pellets. The solvent was then removed and the reside filtered through silica (10:1, EtOAc/CH_3OH). The oily substance obtained after evaporation of the solvent was dissolved in CH_3OH (15 mL), and hydrazine monohydrate (2 mL) was added. The mixture was heated at reflux (~ 90 °C) for 2 h. The solvent was removed to give a white powder. This material was filtered through silica (10:1, EtOAc/CH_3OH). The white powder that was obtained after removal of the solvent was dissolved in CH_3OH (15 mL), to which was added 1.0 M HCl in Et_2O (4 mL) and the volatile materials were then evaporated (0.17 g, yellow oil) and the oil obtained purified by reverse phase HPLC to give the material for biological assay (7 mg, 10%), $R_f = 0.17$ (2:3, CH_3OH/EtOAc); ^1H NMR (300 MHz, D_2O): δ 7.86 (d, 1H, $J = 3.8$ Hz, aromatic H), 7.83 (d, 1H, $J = 5.0$ Hz, aromatic H), 7.25 (t, 1H, $J = 4.1$, 4.8 Hz, aromatic H), 5.56 (d, 1H, $J = 9.8$ Hz, H-1), 3.93 (d, 1H, $J = 12.5$ Hz, H-6a), 3.84 (m, 2H, H-3,6b), 3.67 (m, 2H, H-4,5), 3.44 (t, 1H, $J =$ Hz, H-2); ^{13}C NMR (125 MHz, D_2O): δ 167.9 (s), 138.7 (s, aromatic C), 136.1, 134.1, 131.3 (each d, each aromatic CH), 80.8 (d, C-1), 79.7, 75.6, 72.1 (each d), 63.1 (t), 57.3 (d); ESI-LRMS: found m/z 357.1 $[M+CH_3OH+H]^+$, 347.3 $[M+Na]^+$, 325.2 $[M+H]^+$, 323.2 $[M-H]^-$; ESIMS m/z calcd for $C_{11}H_{15}N_2O_5SCl$ $[M-H]^-$: 323.0468. Found: 323.0477.

3.29. BAEC culture and evaluation of proliferation

Bovine aortic endothelial cells (BAEC) were obtained from the NIA Aging Cell Culture Repository (Coriell Institute for Medical Research, Camden, NJ, USA) and were cultured in RPMI supplemented with 10% foetal bovine serum (FBS), 50 μg/mL of gentamycin and 50 ng/mL amphotercin B. Cells were routinely used between the fifth and eight passages. BAE cells were seeded at 35,000 cells/well in 24-well plates in their respective culture medium. After a 24 h adhesion period, the compounds, diluted at the indicated concentrations in the culture medium, were added and cells were cultured for 24 and/or 48 h. In the last 4 h of treatment, 2 μCi of $[CH_3-^3H]$thymidine/well was added. Thereafter, the cells were washed two times with ice-cold PBS, and then incubated overnight at 4 °C in ice-cold 10% trichloroacetic acid. After one wash with ice-cold 10% trichloroacetic acid, the cells were solubilised with 0.1 N NaOH and transferred to counting vials containing 5 mL of liquid scintillant (ICN).

3.30. Synoviocyte cell culture and growth

The immortalised normal human K4 IM synoviocyte cell line was grown in RPMI 1640 at 37 °C in 5% CO_2 as previously described.[24,33] 1×10^5 synoviocytes cells were plated in 6×6 cm^2 dishes, allowed to attach and were grown in serum-free medium for 24 h before stimulation. Recombinant human FGF-2 (R&D Systems, Minneapolis, MN) was included as indicated. Following stimulation, cell number and viability were measured using the automated Coulter Vi-Cell XR Analyzer (Beckman Coulter, Buckinghamshire, UK). Data are presented as mean values ± SD. Comparisons between treatments were made using Student's *t*-test for paired samples. A p value below 0.05 is considered significant.

3.31. Methods for X-ray crystal structure determination

Crystal data were collected using a Bruker SMART APEX CCD area detector diffractometer. A full sphere of the reciprocal space was scanned by phi–omega scans. Semi-empirical absorption correction based on redundant reflections was performed by the program SADABS.[34] The structures were solved by direct methods using SHELXS-97[35] and refined by full matrix least-squares on F^2 for all data using SHELXL-97.[36] The hydrogen atom treatment varied depending on the crystal quality. For compounds **16** and **34**, hydrogens attached to nitrogen are refined, all others fixed. For compound **4**, all hydrogens are fixed. For **8**, all hydrogens are refined. Anisotropic temperature factors were used for all non-hydrogen atoms.

3.32. Crystal data for 4

Molecular formula, $C_{19}H_{22}NO_{10}SBr$, $M = 536.35$. Temperature, 100(2) K. Wavelength, 0.71073 Å. Crystal system, orthorhombic. Space group, $P2_12_12_1$ (#19). Unit cell dimensions: $a = 5.2341(9)$ Å, $\alpha = 90°$; $b = 16.232(3)$ Å, $\beta = 90°$; $c = 26.600(4)$ Å, $\gamma = 90°$. Volume, 2259.9(7) Å^3. Z, 4. Density (calculated), 1.576 Mg/m^3. Absorption coefficient, 1.966 mm^{-1}. $F(000)$, 1096. Crystal size, $0.60 \times 0.10 \times 0.10$ mm^3. Theta range for data collection, 1.53–25.00°. Index ranges, $-6 \leqslant h \leqslant 6$, $-19 \leqslant k \leqslant 19$, $-31 \leqslant l \leqslant 31$. Reflections collected, 16244. Independent reflections, 3980 [R(int) = 0.0427]. Completeness to theta = 25.00°, 99.9%. Absorption correction, semi-empiracle from equivalents. Max. and min. transmissions, 0.8276 and 0.7273. Refinement method, Full-matrix least-squares on F^2. Data/restraints/parameters, 3980/0/309. Goodness-of-fit on F^2, 1.083. Final R indices [$I > 2\sigma(I)$]. $R1 = 0.0414$, $wR2 = 0.0970$. R Indices (all data). $R1 = 0.0444$, $wR2 = 0.0992$. Absolute structure parameter, 0.012(9). Largest diff. peak and hole, 0.581 and −0.268 e Å^{-3}.

3.33. Crystal data for 8

Molecular formula, $C_{20}H_{25}NO_{10}S$ $M = 471.47$. Temperature, 100(2) K. Wavelength, 0.71073 Å. Crystal system, monoclinic. Space group, $C2$ (#5). Unit cell dimensions: $a = 16.2354(10)$ Å, $\alpha = 90°$; $b = 14.5561(9)$ Å, $\beta = 124.123(1)°$; $c = 11.2724(7)$ Å, $\gamma = 90°$. Volume, 2205.3(2) Å^3. Z, 4. Density (calculated), 1.420 Mg/m^3. Absorption coefficient, 0.204 mm^{-1}. $F(000)$, 992. Crystal size, $1.00 \times 0.80 \times 0.80$ mm^3. Theta range for data collection, 2.06–28.53°. Index ranges, $-21 \leqslant h \leqslant 20$, $-19 \leqslant k \leqslant 18$, $-14 \leqslant l \leqslant 15$. Reflections collected, 18,873. Independent reflections, 5163 [R(int) = 0.0178]. Completeness to theta = 28.53°, 94.6%. Absorption correction, semi-empiracle from equivalents. Max. and min. transmissions, 0.8541 and 0.7095. Refinement method, full-matrix least-squares on F^2. Data/restraints/parameters, 5163/1/389. Goodness-of-fit on F^2, 1.039. Final R indices [$I > 2\sigma(I)$]. $R1 = 0.0288$, $wR2 = 0.0732$. R indices (all data). $R1 = 0.0291$, $wR2 = 0.0734$. Absolute structure parameter, 0.02(4). Largest diff. peak and hole, 0.333 and −0.237 e Å^{-3}.

3.34. Crystal data for 16

Molecular formula, $C_{24}H_{31}NO_{10}SiS$ $M = 553.65$. Temperature, 100(2) K. Wavelength, 0.71073 Å. Crystal system, orthorhombic. Space group, $P2_12_12_1$ (#19). Unit cell dimensions: $a = 8.606(7)$ Å, $\alpha = 90°$; $b = 11.163(9)$ Å, $\beta = 90(10)°$; 30.42(2) Å, $\gamma = 90°$. Volume, 2923(4) Å^3. Z, 4. Density (calculated), 1.258 Mg/m^3. Absorption coefficient, 0.203 mm^{-1}. $F(000)$, 1168. Crystal size, $0.60 \times 0.40 \times 0.02$ mm^3. Theta range for data collection, 1.94–27.00°. Index ranges, $-10 \leqslant h \leqslant 10$, $-14 \leqslant k \leqslant 14$, $-38 \leqslant l \leqslant 38$. Reflections collected, 39,708. Independent reflections, 6351 [R(int) = 0.0369]. Completeness to theta = 27°, 100%. Absorption correction, semi-empirical from equivalents. Max. and min. transmissions, 0.9960 and 0.8969. Refinement method, full-matrix least-squares on F^2. Data/restraints/parameters, 6351/0/345. Goodness-of-fit on F^2, 1.070. Final R indices [$I > 2\sigma(I)$]. $R1 = 0.0447$, $wR2 = 0.1097$. R Indices (all data). $R1 = 0.0475$, $wR2 = 0.1114$. Absolute structure parameter, −0.00(8). Largest diff. peak and hole, 0.842 and −0.675 e Å^{-3}.

3.35. Crystal data for 34

Molecular formula, $C_{19}H_{23}NO_{10}S$ $M = 457.44$. Temperature, 100(2) K. Wavelength, 0.71073 Å. Crystal system, monoclinic. Space group, $C2$ (#5). Unit cell dimensions: $a = 16.2214(14)$ Å, $\alpha = 90°$; $b = 14.0868(12)$ Å, $\beta = 124.9450(10)°$; $c = 11.1702(10)$ Å, $\gamma = 90°$. Volume, 2092.3(3) Å^3. Z, 4. Density (calculated), 1.452 Mg/m^3. Absorption coefficient, 0.212 mm^{-1}. $F(000)$, 960. Crys-

tal size, $0.5 \times 0.5 \times 0.4$ mm^3. Theta range for data collection, 2.11–28.90°. Index ranges, $-21 \leqslant h \leqslant 21$, $-18 \leqslant k \leqslant 18$, $-14 \leqslant l \leqslant 14$. Reflections collected, 17,967. Independent reflections, 4940 [R(int) = 0.0249]. Completeness to theta = 28.59°, 94.6%. Absorption correction, semi-empirical from equivalents. Max. and min. transmissions, 0.9200 and 0.8206. Refinement method, full-matrix least-squares on F^2. Data/restraints/parameters, 4940/1/288. Goodness-of-fit on F^2, 1.070. Final R indices [$I > 2\sigma(I)$]. $R1 = 0.0337$, $wR2 = 0.0889$. R Indices (all data). $R1 = 0.0339$, $wR2 = 0.0891$. Absolute structure parameter, −0.05(5). Largest diff. peak and hole, 0.389 and −0.238 e Å^{-3}.

3.36. Computational methods

The geometries of all the thiophenes studied were first fully optimised with the program GAUSSIAN-98,[37] assuming *Cs* symmetry, using the hybrid B3LYP method[38] and the 6-311+G** basis set.[39] These optimised structures were then used as starting point in further optimisations with the MP2 method[40] using the 6-311+G** basis set. In all the cases, the nature of the complexes as a potential energy minimum was established at B3LYP/6-311+G** level, by verifying that all the corresponding frequencies were positive. The electron charge density was analysed using the atoms in molecules (AIM)[41] methodology. Using this formalism, we have tried to find bond critical points (i.e., points where the electron density function is minimum along the bond path and maximum in the other directions) because the formation of these critical points is a necessary condition for the formation of an interaction between atoms. The natural bond orbital (NBO)[42] analysis, implemented in GAUSSIAN-98 was used to determine the nature of the possible intramolecular interactions. This NBO framework allows the analysis of the bonding–antibonding mixing to verify the atoms involved in potential interactions.

Acknowledgements

The authors thank Geraldine Fitzpatrick for 500 MHz NMR spectra and Dilip Rai for HRMS. The research was funded by Science Foundation Ireland (03/IBN/B352).

Supplementary data

Crystallographic information files. Full crystallographic details, excluding structure features, have been deposited (Deposition Nos. CCDC 293634–293637) with the Cambridge Crystallographic Data Centre. These data may be obtained, on request, from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (tel. +44 1223 336408; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk). Extensive data (bond distances, angles, etc.) and the crystallographic information files have been provided as supplementary data files with this paper. Structural diagrams for **8** and **34**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.04.041.

References

1. (a) Liekens, S.; de Clercq, E.; Neyts, J. *Biochem. Pharm.* **2001**, *61*, 253–270; (b) Carmeliet, P.; Jain, R. K. *Nature* **2000**, *407*, 249–257.
2. Hanahan, D. *Nat. Med.* **1998**, *4*, 13–14.
3. Brower, V. *Nat. Biotech.* **1999**, *17*, 963–968.
4. Tak, P.; Bresnihan, B. *Arthritis Rheum.* **2000**, *43*, 2619–2633.
5. (a) 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; (b) Pitt, N.; Duane, R. M.; O. Brien, A.; Bradley, H.; Wilson, S. J.; O' Boyle, K. M.; Murphy, P. V. *Carbohydr. Res.* **2004**, *339*, 1873–1887.
6. O' Brien, A.; Lynch, C.; O' Boyle, K. M.; Murphy, P. V. *Carbohydr. Res.* **2004**, *339*, 2343–2354.
7. A preliminary account of this work has been published Rawe, S. L.; Zaric, V.; O' Boyle, K. M.; Murphy, P. V. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1316–1319.
8. Kundu, N. G.; Wahab Khan, M. *Tetrahedron* **2000**, *56*, 4777–4792.
9. Bock, K.; Pederson, C. *J. Chem. Soc., Perkin Trans. 2* **1983**, 293–297.
10. Szilagyi, L.; Gyoergydeak, Z. *Carbohydr. Res.* **1985**, *143*, 21–41.
11. (a) Avalos, M.; Babiano, R.; Carretero, M. J.; Cintas, P.; Higes, F. J.; Jiménez, J. L.; Palacios, J. C. *Tetrahedron* **1998**, *54*, 615–628; (b) Avalos, M.; Babiano, R.; Durán, C. J.; Jiménez, J. L.; Palacios, J. C. *J. Chem. Soc., Perkin Trans. 2* **1992**, 2205–2215; (c) Murphy, P. V.; Bradley, H.; Tosin, M.; Pitt, N.; Fitzpatrick, G. M.; Glass, W. K. *J. Org. Chem.* **2003**, *68*, 5693–5704.
12. Roques, B.; Combrisson, S.; Riche, C.; Pascard-Billy, C. *Tetrahedron* **1970**, *26*, 3555–3567.
13. (a) Nagata, S.; Yamabe, T.; Yoshikawa, K.; Kato, H. *Tetrahedron* **1973**, *29*, 2545–2552; (b) Kao, J.; Radom, L. *J. Am. Chem. Soc.* **1979**, *101*, 311–318.
14. Rowland, R. S.; Taylor, T. *J. Phys. Chem.* **1996**, *100*, 7384–7391.
15. Steiner, T. *Angew. Chem., Int. Ed.* **2002**, *41*, 48–76.
16. (a) Stevens, E. S.; Sugawara, N.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1980**, *102*, 7048–7050; (b) Vasella, A.; Witzig, C. *Helv. Chim. Acta* **1995**, *78*, 1971–1982.
17. Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512–523.
18. Llinás, M.; Klein, M. P. *J. Am. Chem. Soc.* **1975**, *97*, 4731–4737.
19. (a) Mallory, F. B.; Baker, M. B. *J. Org. Chem.* **1984**, *49*, 1323–1326; (b) Abraham, R. J.; Reid, M. *J. Chem. Soc., Perkin Trans. 2* **2001**, 1195–1204.

20. For examples see (a) Maxfield, F. R.; Leach, S. J.; Stimson, E. R.; Powers, S. R.; Scheraga, H. A.. *Biopolymers* **1979**, *18*, 2507–2521; (b) Tyler McQuade, D.; McKay, S. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1997**, *119*, 8528–8532.
21. Similar behaviour was observed for anilides of glucuronic acid Tosin, M.; O' Brien, C.; Fitzpatrick, G. M.; Müller-Bunz, H.; Glass, W. K.; Murphy, P. V. *J. Org. Chem.* **2005**, *70*, 4096–4106.
22. (a) Abraham, R. J.; Chadwick, D. J. *Tetrahedron* **1982**, *38*, 1485–1491; (b) Caccamese, S.; Montaudo, G.; Recca, A.; Fringuelli, F.; Taticchi, A. *Tetrahedron* **1974**, *30*, 4129–4135; (c) Sanz, P.; Yanez, M.; Mo, O. *J. Phys. Chem. A* **2002**, *106*, 4661–4668; (d) Minkin, V. I.; Minyaev, R. M. *Chem. Rev.* **2001**, *101*, 1247–1265.
23. Rozas, I.; Alkorta, I.; Elguero, J. *J. Phys. Chem. A* **1997**, *101*, 9457–9463.
24. McEvoy, A. N.; Murphy, E. A.; Ponnio, T.; Conneely, O. M.; Bresnihan, B.; FitzGerald, O.; Murphy, E. P. *J. Immunol.* **2002**, *168*, 2979–2987.
25. Luchansky, S. J.; Bertozzi, C. R. *ChemBioChem* **2004**, *5*, 1706–1709.
26. (a) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Salvino, J.; Leahy, E. M.; Sprengeler, P. A.; Furst, G.; Smith, A. B., III; Strader, C. D.; Cascieri, M. A.; Candelore, M. R.; Donaldson, C.; Vale, W.; Maechler, L. *J. Am. Chem. Soc.* **1992**, *114*, 9217–9218; (b) Wunberg, T.; Kallus, C.; Opatz, T.; Henke, S.; Schmidt, W.; Kunz, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 2503–2505; (c) Schweizer, F.; Hindsgaul, O. *Curr. Opin. Chem. Biol.* **1999**, *3*, 291–298; (d) Gruner, S. A. W.; Locardi, E.; Lohof, E.; Kessler, H. *Chem. Rev.* **2002**, *102*, 491–514; (e) Marcaurelle, L. A.; Seeberger, P. H. *Curr. Opin. Chem. Biol.* **2002**, *6*, 289–296.
27. Castellano, R. K.; Diederich, F.; Meyer, E. A. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210–1250.
28. Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303–1324.
29. Desiraju, G. R. *Chem. Commun.* **2005**, 2995–3001.
30. (a) Shiozaki, M.; Mochizuki, T.; Hanzawa, H.; Haruyama, H. *Carbohydr. Res.* **1996**, *288*, 99–108; (b) Perrin, C. L.; Armstrong, K. B. *J. Am. Chem. Soc.* **1993**, *115*, 6825–6834.
31. Ponpipom, M. M.; Bugianesi, R. L.; Shen, T. Y. *Carbohydr. Res.* **1980**, *82*, 135–140.
32. Ogawa, T.; Beppu, K. *Carbohydr. Res.* **1982**, *101*, 271–277.
33. Haas, C.; Aicher, W. K.; Dinkel, A.; Peter, H. H.; Eibel, H. *Rheumatol. Int.* **1997**, *16*, 241–247.
34. Sheldrick, G. M. *SADABS*; Bruker AXS: Madison, WI 53711, 2000.
35. Sheldrick, G. M. *SHELXS-97: Program for Crystal Structure Solution*; Göttingen 1997.
36. Sheldrick, G. M. *SHELXS-97: Program for Crystal Structure Refinement, Release 2*; Göttingen 1997.
37. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*; Gaussian: Pittsburgh, PA, 1998.
38. (a) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652; (b) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785–789.
39. (a) Ditcheld, R.; Hehre, W. J.; Pople, J. A. *J. Chem. Phys.* **1971**, *54*, 724; (b) Frisch, M. J.; Pople, J. A.; Krishnam, R.; Binkley, J. S. *J. Chem. Phys.* **1984**, *80*, 3265–3269.
40. Moller, C.; Plesset, M. S. *Phys. Rev.* **1934**, *46*, 618.
41. Bader, R. F. W. *Atoms in Molecules. A Quantum Theory*; Oxford University: New York, 1990.
42. Reed, A. E.; Curtiss, L. A.; Weinhold, F. *Chem. Rev.* **1988**, *88*, 899–926.