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Towards the synthesis of aryl glucuronides as potential heparanase probes. An interesting outcome in the glycosidation of glucuronic acid with 4-hydroxycinnamic acid

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Abstract—This work describes our preliminary efforts towards the development of aryl glucuronides as potential probes for heparanase. During the course of these initial investigations, attempted glycosidation of methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate trichloroacetimidate with 4-hydroxycinnamic acid gave a complex mixture of four different components. These were identified as the 1-cinnamyl glucuronate ester **13**, the cinnamyl linked disaccharide **14**, the glucuronate trichloroacetamide **15**, and the glucuronyl α-fluoride **16**. This paper rationalises the formation of each of these products, and reports our efforts in trying to optimise the formation of the α-fluoride **16**.

Keywords: Heparan sulfate; D-Glucuronic acid; Glycosyl fluoride; Glycoside formation; Heparanase

1. Introduction

Heparanase is an *endo*-β-D-glucuronidase that degrades heparan sulfate (HS), a major component of the extracellular matrix and basement membranes, and plays an important role in a variety of biological processes including inflammation, the metastatic potential of tumour cells and angiogenesis.[1,2] HS–glycosaminoglycans (HS–GAGs) are sulfated linear polysaccharides of up to 400 sugar residues, composed of D-glucuronic acid (GlcA) β-(1→4)-linked to *N*-acetyl-D-glucosamine (GlcNAc), with various structural modifications.[3] Heparanase hydrolyses the glucuronide linkage in HS–GAGs, but only at a few sites (e.g., as indicated in Fig. 1), yielding HS fragments of 10–20 saccharide units.[4,5]

Despite the important biological functions of heparanase, investigations of this enzyme have proven quite challenging due to the difficulties in isolation and, until recently, low yields of active recombinant protein.[6]

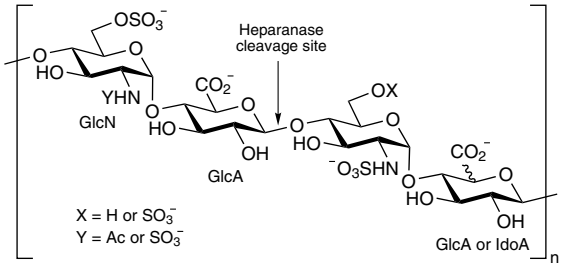

Figure 1. Schematic of heparan sulfate showing the heparanase cleavage site.

The lack of a convenient functional assay has also hampered progress, prompting our investigation into the possibility of preparing compounds such as **1** that could be used in a simple fluorometric or colourimetric assay. The general concept is shown in Figure 2, wherein heparanase cleavage of tagged compounds like **1** would result in a measurable response. The development of such an assay would be extremely beneficial in studies on heparanase, including the kinetic evaluation of

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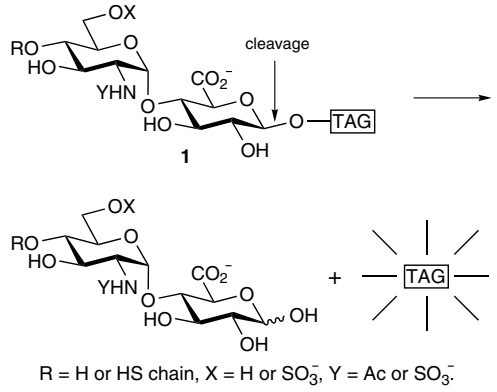
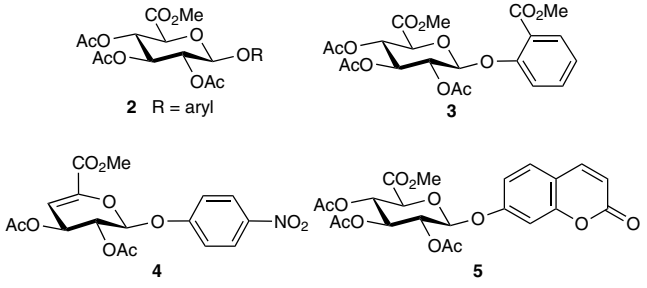


Figure 2. Heparanase cleavage of the chromogenic or fluorogenic tag from **1** generates a measurable response.

potential inhibitors and the correlation of heparanase activity with various disease states.

2. Results and discussion

Our initial approach towards compounds such as **1** involved the preparation of a series of aryl glucuronides of the general structure **2**, which could serve as potential substrates for heparanase after deprotection. Several methods are available for the synthesis of glycosides of glucuronic acids, and these can be placed into two broad categories involving either oxidation of the corresponding glucoside[7,8] or by carrying out the glycosidation on an activated glucuronic acid.[9–14] In relation to compounds like **2**, we were particularly interested in the previously reported methods for the synthesis of aryl glucuronides, including the 2′-methoxycarbonyl derivative **3**,[10,15] the *p*-nitrophenyl glycoside **4**[16] and the 7-hydroxycoumarin glucuronide **5**.[11]



In the work towards **5** described by Stachulski and co-workers,[11] the choice of anomeric activating group in the glucuronide is clearly important, since glucuronyl α-halides only gave low (~10%) yields of **5**. Bearing these issues in mind, our approach towards simple aryl glucuronides like **2** as probes for heparanase involved the acid-catalysed glycosidation between the trichloroacetimidate-activated glucuronic acid **6** and a variety of phenols (Fig. 3). There have been a number of approaches described for the synthesis of **6**, including using the corresponding α-bromo[11,14,17] or the anomeric acetate[11,18] glucuronic acid derivatives. In our hands, we found that the most efficient approach was from commercially available D-glucurono-6,3-lactone, wherein **6** could be obtained in ~30% overall yield over three steps. In some preliminary studies, the $BF_3{\cdot}OEt_2$-catalysed coupling of **6** with phenols produced consistently high yields (61–81%) of the desired aryl glucuronides **7** to **10** (Fig. 3). Whilst compounds **8**[13,14,19,20] and **10**[20,21] have been known for some time, they have previously only been reported with limited spectroscopic data.

Interestingly, the attempted $BF_3{\cdot}OEt_2$-catalysed glycosidation of **6** with 4-hydroxycinnamic acid (**11**, 1–3 equiv) did not give the desired glycoside **12**, but instead gave a complex mixture from which the four products **13**–**16** (Fig. 4) were identified, with yields for each product shown in parentheses.

The formation of **13** and **14** can be rationalised as shown in Figure 5. The isolation of both **13** and **14** from attempted glycosidation between **6** and **11** suggests that, due to the resonance structures of **11**, 4-hydroxycinnamic acid (**11**) preferentially reacts initially through the carboxylate group to give **13**, which then subsequently reacts with another molecule of **6** to give **14**. The enhanced nucleophilicity of the carboxylate group in other phenolic carboxylic acid species has been noted previously.[22] The formation of the trichloroacetamide byproduct **15** during attempted coupling of **6** with **11** arises from the acid-catalysed rearrangement of **6**. The acid-catalysed rearrangement of trichloroacetimidate-activated glucose and galactose derivatives to the corresponding trichloroacetamides has been noted previously during their attempted coupling with relatively unreactive acceptors such as 2-thio-glucosides or enol silyl ethers.[23–25]

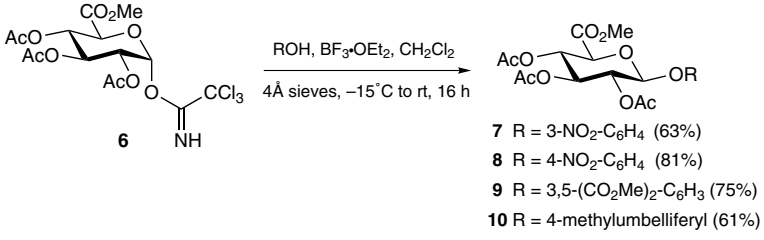


Figure 3. Synthesis of some aryl glucuronides.

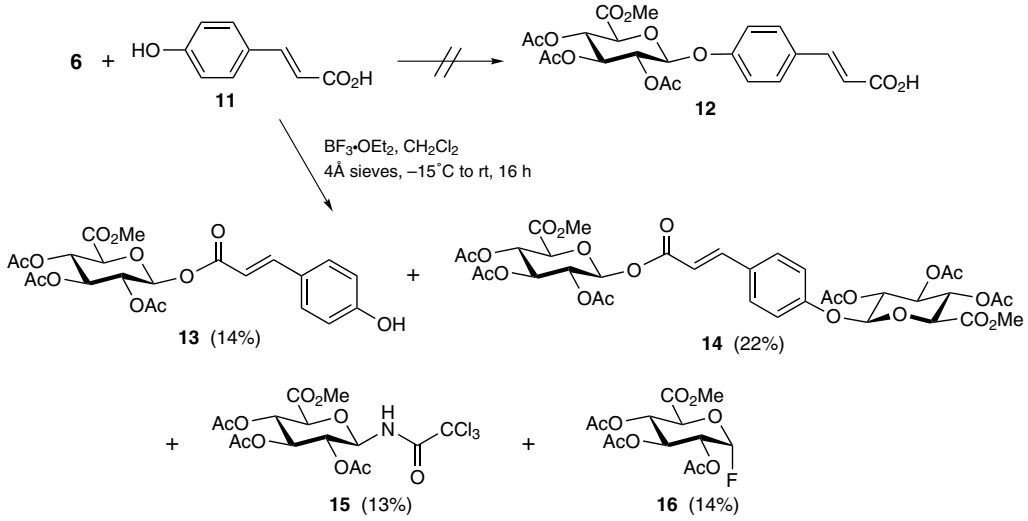


Figure 4. Attempted glycosidation of the donor **6** with 4-hydroxycinnamic acid (**11**).

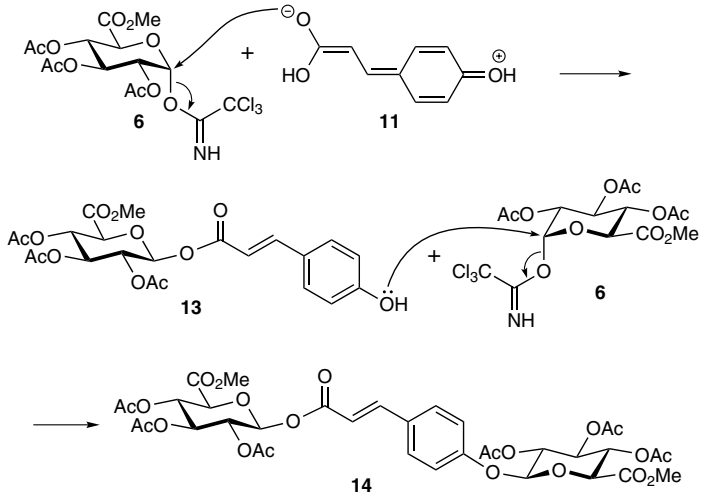


Figure 5. Proposed mechanism for the formation of **13** and **14**.

The isolation of the glucuronosyl ester **13** from the attempted glycosidation of **6** with **11**, albeit as a minor component of a complex mixture of products, prompted us to investigate this reaction further, perhaps as an avenue into novel glucuronosyl ester derivatives. Reaction of **6** with **11** in the presence of TMSOTf under conditions analogous to those employed above using $BF_3{\cdot}OEt_2$, gave the glucuronosyl ester **13** (38%), together with the reducing sugar **17**[14] (27%) (Fig. 6). It is noteworthy that the dimeric product **14** was only formed in trace amounts (<10%) in this instance, compared with its being obtained as the major product (22%) when $BF_3{\cdot}OEt_2$ was used as the Lewis acid (Fig. 4). Stirring **6** in the presence of **11** for 16 h without the addition of a Lewis acid catalyst failed to furnish any of the product **13**, returning predominantly unreacted **6** together with a small amount (~20%) of the reducing sugar **17**. This suggests that **11** is not acidic enough to catalyse its own reaction with **6**. Interestingly, treatment of **6** with the known protected cinnamic acid derivative **18**[26] in the presence of $BF_3{\cdot}OEt_2$ gave the glucuronosyl ester **19** in 54% yield (Fig. 6), together with small amounts of the reducing sugar **17** (~20%) as well as the glucuronosyl fluoride **16** (~12%).

Others have shown that the $BF_3{\cdot}OEt_2$-catalysed coupling of **18** with peracetylated glucosyl fluoride gives the corresponding β-glucosyl ester in 67% yield.[27] This same work also attempts to define the difference in reactivity between the phenol group and the carboxylic acid group in cinnamic acid derivatives.[27] Thus, treatment of a 1:1 mixture of **18** and the methyl ester of **11** with $BF_3{\cdot}OEt_2$ in the presence of peracetylated glucosyl fluoride gave a 73% yield of the glycoside **20** and a 10% yield of the glucosyl ester **21** (Fig. 7).[27] Our isolation of both **13** and **14** from coupling between **6** and **11**, together with our failure to observe the formation

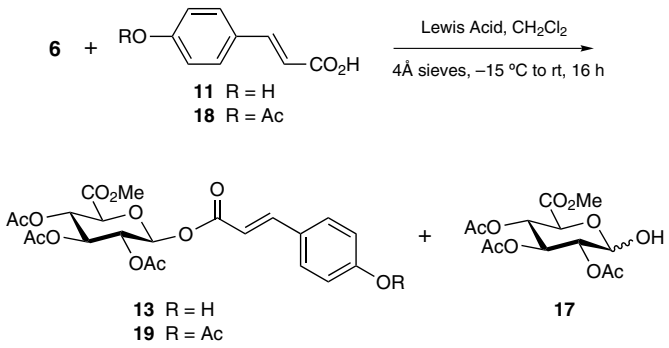


Figure 6. Alternative approach towards glucuronosyl esters like **13** and **19**.

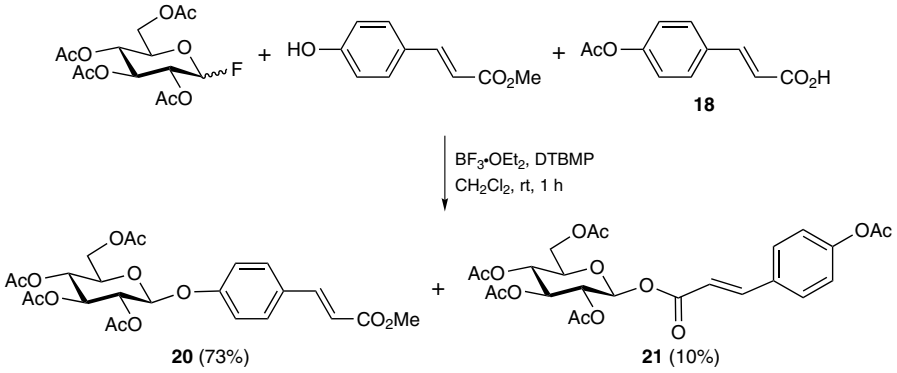


Figure 7. Reported competitive reactivity of phenols versus carboxylic acids.[27]

of any of the glycoside **12** (Fig. 4), suggests that in our system a different reactivity preference is operating.

Turning our attention back to the original $BF_3{\cdot}OEt_2$-catalysed attempted glycosidation of **6** with **11** shown in Figure 4, we were also interested in the isolation of the glucuronosyl fluoride **16**, obtained exclusively as the α-anomer. That the product obtained was indeed the α-fluoride was evident from examination of the ^1H NMR spectrum of **16**. Most notably, the signal due to H-1 (δ 5.81) was present as a doublet of doublets, due to coupling to H-2 ($J = 2.7$ Hz) and to fluorine ($J = 52.5$ Hz).[28] The magnitude of the coupling between H-1 and H-2 in **16** (2.7 Hz) is clearly consistent with an equatorial–axial relationship between H-1 and H-2 and supports the fluoride in **16** being in the α-configuration. To the best of our knowledge, this is the first time that exclusively the α-anomer of **16** has been reported. Others have reported the formation of exclusively the β-glucuronosyl fluoride, from the corresponding hemiacetal, by treatment with hydrogen fluoride–pyridine[29] or diethyl-aminosulfur trifluoride (DAST).[30,31] The synthesis of a 1:9 α:β-mixture of **16** has also been described by treatment of the hemiacetal with α-fluoroenamine, although there is limited spectroscopic data reported for the α-fluoride **16** in this mixture.[28]

The isolation of the α-fluoride **16** from coupling between **6** and **11** was somewhat surprising, since the only source of fluoride in the glycosidation reaction was $BF_3{\cdot}OEt_2$, and this was present in up to 0.5 molar equiv. Glycosyl fluorides have been used for over 20 years as anomeric activating groups in glycosidations.[28,29,32–35] Whilst reagents like HF or HF–pyridine, DAST, and metal fluorides (e.g., AgF_2) are commonly used to prepare glycosyl fluorides,[35] to the best of our knowledge glycosyl fluorides have never been prepared previously using $BF_3{\cdot}OEt_2$ as the source of fluoride. Perhaps the closest example is a modification of the Mitsunobu reaction wherein the mannosyl fluoride **22** is generated by exposure of **23** to DEAD and triethyloxonium tetrafluoroborate, which is believed to proceed through the stabilised carbonium ion **24** (Fig. 8).[36]

Although not completely clear, we suggest that in our case it is possible that reaction of the trichloroacetimidate **6** with $BF_3{\cdot}OEt_2$ initially forms the stabilised glucuronosyl cation intermediate **25** (Fig. 9). Presumably the fluoride that is required to form the product **16** arises from the boron adduct $[(F_3B{-}OR)^-]$ generated during the initial formation of the intermediate **25**.

Whilst anomerically pure glycosyl fluorides are preferred, many of the common fluorination methods lead to anomeric mixtures.[35,–40] Based upon our rationale above (Fig. 9), we wondered if we could promote the formation of the α-fluoride **16** by reacting **6** with $BF_3{\cdot}OEt_2$ in the absence of an aglycon. Unfortunately,

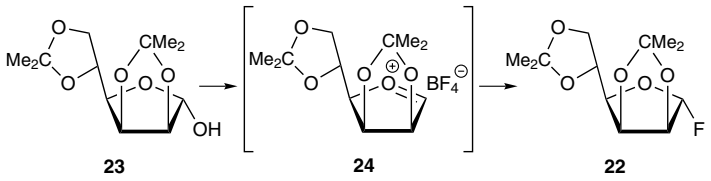


Figure 8. Reported synthesis of mannosyl fluoride **22**.[36]

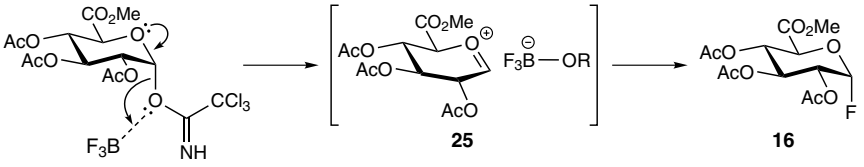


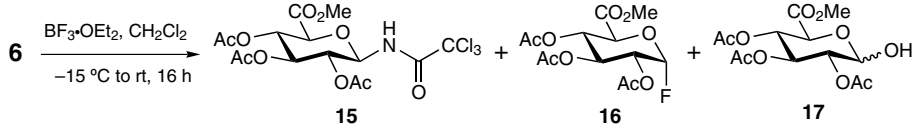
Figure 9. Possible rationale for the formation of the α-fluoride **16**.

as can be seen by the results shown in Table 1, an increase in $BF_3{\cdot}OEt_2$ from 0.3 (entries 1–3) to 1.0 molar equiv (entry 4) failed to increase the yield of **16**, which remained generally around 24%. Indeed, the increase in $BF_3{\cdot}OEt_2$ appeared to have little effect on the overall outcome of the reaction, with products **15** and **17** also being isolated in relatively consistent yields. Similarly, the use of either commercially available $BF_3{\cdot}OEt_2$ (entries 1 and 2) or freshly distilled $BF_3{\cdot}OEt_2$ (entries 3–5) had no discernible effect on the yields or respective ratios of products obtained (Table 1). Increasing the amount of $BF_3{\cdot}OEt_2$ to greater than 5 molar equiv generally resulted in substantial decomposition of **6**, with the formation of **15** and the methyl glucuronide (α:β ~2:1) as minor products upon quenching the reaction with methanol. At this stage we are yet to determine why increasing the amount of $BF_3{\cdot}OEt_2$ fails to improve the yield of the α-fluoride **16**. As expected, exposure of **6** to TMSOTf without any aglycon present afforded a mixture of the reducing sugar **17** and the rearranged trichloroacetamide derivative **15**.

During the course of this work, it was also noted that the workup procedure had a significant effect on the isolation of the α-fluoride **16**. Initially, the workup consisted of simply adding methanol to the reaction mixture and then filtering prior to concentration in vacuo (Table 1, entries 1–4). However, when an aqueous workup was employed (dilution of the reaction mixture with CH_2Cl_2, filtration to remove the molecular sieves, and washing with aqueous Na_2CO_3 prior to concentration in vacuo) the yield of the α-fluoride **16** was significantly lower (Table 1, entry 5).

The desired cinnamyl glycoside of glucuronic acid was ultimately obtained by use of the known[41] methyl ester **26** as the glycosyl acceptor. Compound **26** was conveniently synthesised in excellent yield (96%) by esterification of **11** with methanolic HCl [prepared in situ by the addition of trimethyl orthoformate and thionyl chloride to anhydrous methanol. (Potter, J. J.; von Itzstein, M. unpublished work.)] The glycosidation of **6** with **26** using $BF_3{\cdot}OEt_2$ as catalyst provided the fully protected glycoside **27** in 83% yield (Fig. 10). Interestingly, ^1H

Table 1. Attempted formation of the α-fluoride **16**



Entry	Scale **6** (mg)	$BF_3{\cdot}OEt_2$ (equiv)	Products isolated[a]		
			15 (%)	**16** (%)	**17** (%)
1	35	0.3[b]	6	16	57
2	381	0.3[b]	15	26	20
3	234	0.3[c]	11	24	ND
4	238	1.0[c]	15	24	31
5[d]	294	0.5[c]	12	6	45

[a] ND = not determined.
[b] Commercially available $BF_3{\cdot}OEt_2$.
[c] Distilled $BF_3{\cdot}OEt_2$.
[d] Alternative workup procedure.

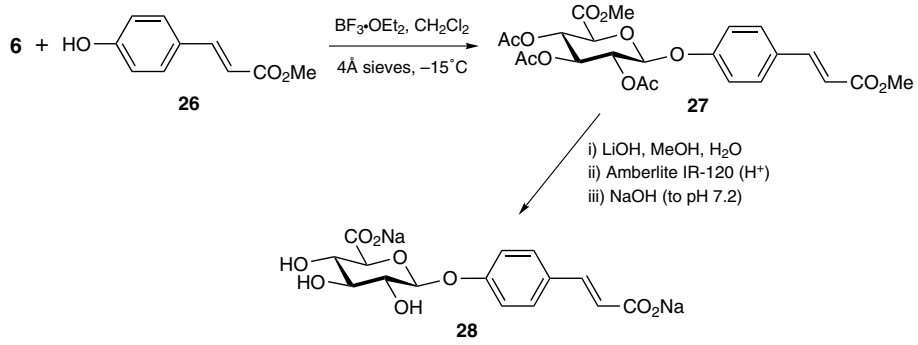


Figure 10. Synthesis of target compound **28** via glucuronide **27**.

NMR examination of the product **27** in $CDCl_3$ showed several overlapped peaks, which resulted in a complex, second-order spin system for H-5 of the glucuronic acid portion.[14] Changing the NMR solvent to either C_6D_6 or CD_3CN (see Section 3.6) resolved many of the overlapping signals and also simplified the second-order spin systems (in the case of CD_3CN).

Interestingly, the glycosidation of **6** with **26** using TMSOTf as a Lewis acid catalyst furnished the desired glucuronide **27** in lower yield (~40%) as well as small amounts (~10%) of the known[42] 1-*O*-TMS glucuronide together with some of the reducing sugar **17**. Finally, deprotection of **27** (dil aq LiOH in MeOH) gave the desired compound **28** (Fig. 10). A series of glucuronic acid aryl glycosides like **1** and **28** are currently being evaluated as potential substrates for heparanase.

3. Experimental

3.1. General methods

All solvents and reagents were either freshly distilled or of analytical grade. Reactions were monitored by thin-layer chromatography (TLC) using Merck precoated silica gel GF_{254} aluminium-backed plates that were visualised under UV light (254 nm), followed by dipping in H_2SO_4 in EtOH (5% v/v) and heating to ~200 °C. Column chromatography was carried out following the procedure described,[43] using Scharlau Silica Gel 60 (230–400 mesh). ^1H and ^{13}C NMR spectra were recorded using a Bruker Avance 300 spectrometer, and low-resolution mass spectra were obtained in the electrospray ionisation mode on a Bruker Esquire 3000 mass spectrometer. High-resolution mass spectra (HRMS) were recorded by the mass spectrometry service at the Department of Chemistry (University of Queensland) or within the School of Science (Griffith University), in the positive-ion electrospray-ionisation mode. Combustion analyses were carried out by the microanalysis service of the Department of Chemistry, University of Queensland. Compound **6** was prepared from D-glucurono-6,3-lactone by a three-step procedure that involved the initial generation of methyl 1,2,3,4-tetra- *O*-acetyl-D-glucopyranosyluronate,[44] selective removal of the anomeric acetate using benzylamine[45] and subsequent introduction of the trichloroacetimidate group following the method described.[18]

3.2. General procedure for glycosidations

A suspension of **6** (1.0 equiv) and alcohol (1.1 equiv) in anhydrous CH_2Cl_2 under Ar over 4 Å sieves was stirred at rt for 60 min, then cooled to −15 °C before the addition of $BF_3{\cdot}OEt_2$ (0.5 equiv) in CH_2Cl_2. The reaction mixture was allowed to warm to rt and was stirred for 16 h at rt before being diluted with CH_2Cl_2, filtered through Celite® and washed with aq Na_2CO_3. The organic phase was dried over Na_2SO_4, the solvent was removed in vacuo, and the residue were purified by flash chromatography on silica gel. In this way the compounds listed in Sections 3.2.1, 3.2.2, 3.2.3 and 3.2.4 were prepared.

3.2.1. Methyl (3′-nitrophenyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (7). Yield 63%; R_f 0.2 in 2:1 hexanes–EtOAc. ^1H NMR (300 MHz, $CDCl_3$): δ 7.96 (ddd, 1H, $J_{4',5'}$ 8.2, $J_{4',2'}$ 2.2, $J_{4',6'}$ 0.9 Hz, H-4′); 7.86 (t, 1H, $J_{2',6'}$ 2.2 Hz, H-2′); 7.48 (t, 1H, $J_{5',6'}$ 8.2 Hz, H-5′); 7.34 (ddd, 1H, H-6′); 5.40–5.24 (m, 4H, H-1, H-2, H-3, H-4); 4.26 (AX m, 1H, H-5); 3.73 (s, 3H, CO_2Me); 2.08, 2.06, 2.05 (3 × s, 9H, 3 × OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.0, 169.3, 169.2 (3 × O*C*(O)CH_3); 166.6 (C-6); 156.8 (C-1′); 149.1 (C-3′); 130.3 (C-5′); 123.6 (C-6′); 118.4 (C-4′); 111.9 (C-2′); 98.7 (C-1); 72.6 (C-5); 71.6, 70.9, 68.8 (C-2, C-3, C-4); 53.1 (CO_2CH_3); 20.6, 20.6, 20.5 (3 × OC(O)CH_3). ESIMS (*m*/*z*): 477.8 [M+Na^+]. HRMS Calcd for $C_{19}H_{21}NNaO_{12}$ [M+Na^+] 478.0961. Found 478.0967.

3.2.2. Methyl (4′-nitrophenyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (8). Yield 81%; R_f 0.2 in 2:1 hexanes–EtOAc.[13,14] Recrystallisation of the column-purified product from hexanes–ethyl acetate gave **8** as

white needles: mp 155–157 °C (lit.[13] 153–154 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19 (AX m, 2H, H-3′, H-5′); 7.07 (AX m, 2H, H-2′, H-6′); 5.38–5.28 (m, 4H, H-1, H-2, H-3, H-4); 4.26 (AX m, 1H, H-5); 3.70 (s, 3H, CO_2Me); 2.06, 2.05, 2.04 ($3\times$s, 9H, $3\times$OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.0, 169.3, 169.1 ($3\times$O*C*(O)CH_3); 166.6 (C-6); 161.0 (C-1′); 143.3 (C-4′); 125.8 (C-3′, C-5′); 116.7 (C-2′, C-4′); 98.0 (C-1); 72.6 (C-5); 71.4, 70.8, 68.7 (C-2, C-3, C-4); 53.1 (CO_2CH_3); 20.6, 20.6, 20.5 ($3\times$OC(O)*C*H_3). ESIMS (*m*/*z*): 477.8 [M+Na$^+$].

3.2.3. Methyl (3′,5′-dimethoxycarbonyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (9). Yield 75%; R_f 0.2 in 2:1 hexanes–EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.37 (t, 1H, $J_{4',2'} = J_{4',6'}$ 1.45 Hz, H-4′); 7.82 (d, 2H, H-2′, H-6′); 5.39–5.26 (m, 4H, H-1, H-2, H-3, H-4); 4.26 (AX m, 1H, H-5); 3.92 (s, 6H, $2\times CO_2Me$); 3.70 (s, 3H, CO_2Me); 2.06, 2.04, 2.03 ($3\times$s, 9H, $3\times$OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.0, 169.3, 169.2 ($3\times$O*C*(O)CH_3); 166.7 (C-6); 165.6 ($2\times C O_2CH_3$); 156.5 (C-1′); 132.1 (C-3′, C-5′); 125.6 (C-4′); 122.1 (C-2′, C-6′); 98.5 (C-1); 72.6 (C-5); 71.6, 71.0, 68.9 (C-2, C-3, C-4); 53.0 (CO_2CH_3); 52.5 ($2\times CO_2CH_3$); 20.6, 20.6, 20.5 ($3\times$OC(O)*C*H_3). ESIMS (*m*/*z*): 548.9 [M+Na$^+$]. HRMS Calcd for $C_{23}H_{26}NaO_{14}$ [M+Na$^+$] 549.1220. Found 549.1220.

3.2.4. Methyl (4′-methylumbelliferyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (10). Yield 61%; R_f 0.2 in 2:3 hexanes–EtOAc.[20,21] ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51 (d, 1H, $J_{5',6'}$ 9.5 Hz, H-5′); 6.94–6.90 (m, 2H, H-6′, H-8′); 6.18 (q, 1H, $J_{3',Me'}$ 1.2 Hz, H-3′); 5.37–5.23 (m, 4H, H-1, H-2, H-3, H-4); 4.24 (AX m, 1H, H-5); 3.73 (s, 3H, CO_2Me); 2.39 (d, 3H, Me′); 2.06, 2.05, 2.05 ($3\times$s, 9H, $3\times$OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.8, 169.3, 169.1 ($3\times$O*C*(O)Me); 166.7 (C-6); 160.6 (C-2′); 158.9 (C-7′); 154.5, 152.3 (C-4′, C-8a′); 125.7 (C-5′); 115.4 (C-4a′); 113.7 (C-6′); 112.9 (C-3′); 103.8 (C-8′); 97.8 (C-1); 72.2 (C-5); 71.5, 70.6, 68.8 (C-2, C-3, C-4); 52.9 (CO_2CH_3); 20.5, 20.5, 20.3 ($3\times$OC(O)*C*H_3); 18.5 (C-4′ *C*H_3). ESIMS (*m*/*z*): 515.0 [M+Na$^+$]. HRMS Calcd for $C_{23}H_{26}NaO_{14}$ [M+Na$^+$] 515.1165. Found 515.1172.

3.3. Attempted glycosidation of 6 with 11

To a stirred suspension of **6** (144 mg, 0.30 mmol) in anhyd CH_2Cl_2 (5 mL) under Ar over 4 Å sieves at −15 °C was added **11** (98 mg, 0.90 mmol). After 30 min at −15 °C, $BF_3{\cdot}OEt_2$ (20 μL, 0.16 mmol) was added, the reaction was allowed to warm to rt, and the mixture was stirred overnight. MeOH (0.5 mL) was added, and the mixture was filtered through cotton wool. The solvents were removed in vacuo, and the product was purified by flash chromatography (3:1 toluene–acetone, followed by 1:1 hexanes–EtOAc) to give a mixture of four products, **13**–**16** data for which are provided in Sections 3.3.1–3.3.4.

3.3.1. Methyl 1-*O*-[2′(*E*)-3′-(4″-hydroxyphenyl)-2′-propenoyl] 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate (13). Yield 14%; R_f 0.2 in 3:1 toluene–acetone. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60 (d, 1H, $J_{3',2'}$ 16.0 Hz, H-3′); 7.36 (d, 2H, $J_{2'',3''}$ 8.4 Hz, H-2″); 6.82, (d, 2H, H-3″); 6.13 (d, 1H, H-2′); 5.89 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1); 5.40–5.23 (m, 3H, H-2, H-3, H-4); 4.24, (d, 1H, $J_{5,4}$ 9.0 Hz, H-5); 3.74 (s, 3H, CO_2Me); 2.06, 2.05, 2.02 ($3\times$s, 9H, $3\times$OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.0, 169.7, 169.6 ($3\times$O*C*(O)Me); 167.5 (C-6); 165.1 (C-1′); 159.2 (C-4″); 147.6 (C-3′); 130.5 (C-2″); 126.0 (C-1″); 116.0 (C-3″); 112.5 (C-2′); 91.3 (C-1); 72.7 (C-5); 71.8, 70.1, 69.1 (C-2, C-3, C-4); 53.2 (CO_2CH_3); 20.6, 20.6, 20.5 ($3\times$OC(O)*C*H_3). ESIMS (*m*/*z*): 503.1 [M+Na$^+$]. Anal. Calcd for $C_{22}H_{24}O_{12}{\cdot}H_2O$: C, 53.01; H, 5.26. Found: C, 53.01; H, 4.92.

3.3.2. Methyl (4′-{3″-[methyl (2‴,3‴,4‴-tri-*O*-acetyl-β-D-glucopyranosyl)uronate prop-2″(*E*)-enoate]}phenyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate (14). Yield 22%; R_f 0.1 in 3:1 toluene–acetone. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.69 (d, 1H, $J_{3'',2''}$ 16.0 Hz, H-3″); 7.48 (d, 2H, $J_{3',2'}$ 8.8 Hz, H-3′); 7.00, (d, 2H, H-2′); 6.28 (d, 1H, H-2″); 5.89 (d, 1H, $J_{1''',2'''}$ 7.6 Hz, H-1‴); 5.39–5.19 (m, 7H, H-1, H-2, H-3, H-4, H-2‴, H-3‴, H-4‴); 4.24–4.20, (m, 2H, H-5, H-5‴); 3.72, 3.71 ($2\times$s, 6H, $2\times CO_2Me$); 2.05, 2.04, 2.04, 2.04, 2.03, 2.01 ($6\times$s, 18H, $6\times$OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.9, 169.8, 169.3, 169.2, 169.2, 169.1 ($6\times$O*C*(O)Me); 166.7, 166.7 (C-6, C-6‴); 164.4 (C-1″); 158.4 (C-1′); 146.5 (C-3″); 130.0 (C-3′); 129.0 (C-4′); 117.0 (C-2′); 114.9 (C-2″); 98.2 (C-1); 91.4 (C-1‴); 72.9, 72.5, 71.7, 71.6, 70.8, 70.0, 68.9, 68.8 (C-2, C-3, C-4, C-5, C-2‴, C-3‴, C-4‴, C-5‴); 52.9, 52.9 ($2\times CO_2CH_3$); 20.6, 20.5, 20.5, 20.4, 20.4, 20.3 ($6\times$OC(O)*C*H_3). ESIMS (*m*/*z*): 819.2 [M+Na$^+$]. Anal. Calcd for $C_{35}H_{40}O_{21}$: C, 52.77; H, 5.06. Found: C, 52.92; H, 5.08.

3.3.3. Methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate trichloroacetamide (15). Yield 13%; R_f 0.3 in 3:1 toluene–acetone. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.61 (br d, 1H, $J_{NH,1}$ 8.8 Hz, NH); 5.43 (dd, 1H, $J_{3,4}$ 9.5 Hz, $J_{3,2}$ 9.5 Hz, H-3); 5.25–5.15 (m, 2H, H-1, H-4); 5.09 (dd, 1H, $J_{2,1}$ 9.5 Hz, H-2); 4.20 (d, 1H, $J_{5,4}$ 9.7 Hz, H-5); 3.74 (s, 3H, CO_2Me); 2.06, 2.06, 2.04 ($3\times$s, 9H, $3\times$OAc). ^{1}H NMR (300 MHz, C_6D_6): δ 7.85 (br d, 1H, $J_{NH,1}$ 9.0 Hz, NH); 5.47 (dd, 1H, $J_{3,4}$ 9.5 Hz, $J_{3,2}$ 9.2 Hz, H-3); 5.40 (dd, 1H, $J_{4,5}$ 9.5 Hz, H-4); 5.27 (dd, 1H, $J_{1,2}$ 9.2 Hz, H-1); 5.15 (dd, 1H, H-2); 3.74 (d, 1H, H-5); 3.24 (s, 3H, CO_2Me); 1.82, 1.69, 1.58 ($3\times$s, 9H, $3\times$OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.0, 169.7, 169.5 ($3\times$O*C*(O)CH_3); 166.8 (C-6); 162.1

(NH*C*(O)CCl_3); 91.5 (*C*Cl_3); 79.8 (C-1); 74.2 (C-5); 71.3 (C-3); 70.0 (C-2); 69.5 (C-4); 53.1 (CO_2*C*H_3); 20.6, 20.5, 20.4 (3 × OC(O)*C*H_3). ^{13}C NMR (75 MHz, C_6D_6): δ170.7, 169.7, 169.4 (3 × O*C*(O)CH_3); 166.9 (C-6); 162.2 (NH*C*(O)CCl_3); 92.5 (*C*Cl_3); 80.0 (C-1); 74.2 (C-5); 72.1 (C-3); 70.8 (C-2); 69.7 (C-4); 52.2 (CO_2*C*H_3); 20.2, 20.1, 20.0 (3 × OC(O)*C*H_3). ESIMS (*m*/*z*): 501.7 [M+Na^+].

3.3.4. Methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate fluoride (16).

Yield 14%; R_f 0.4 in 3:1 toluene–acetone. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.80 (dd, 1H, $J_{1,F}$ 52.5 Hz, $J_{1,2}$ 2.7 Hz, H-1); 5.54 (dd, 1H, $J_{3,2}$ 9.9 Hz, $J_{3,4}$ 9.9 Hz, H-3); 5.22 (dd, 1H, $J_{4,5}$ 9.9 Hz, H-4); 4.96 (ddd, 1H, $J_{2,F}$ 24.0 Hz, H-2); 4.45 (d, 1H, H-5); 3.75 (s, 3H, CO_2Me); 2.09, 2.03, 2.03 (3 × OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.9, 169.7, 169.4 (3 × O*C*(O)Me); 166.9 (C-6); 103.5 (d, $J_{1,F}$ 230 Hz, C-1); 69.9 (d, $J_{5,F}$ 4 Hz, C-5); 69.9 (d, $J_{2,F}$ 24 Hz, C-2); 68.6 (C-4); 68.5 (C-3); 53.1 (CO_2*C*H_3); 20.6, 20.5, 20.4 (3 × OC(O)*C*H_3). ESIMS (*m*/*z*): 359.0 [M+Na^+].

3.4. Methyl 1-*O*-[2′(*E*)-3′-(4″-acetoxyphenyl)-2′-propenyl] 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate (19)

To a stirred suspension of **6** (171 mg, 0.36 mmol) in anhyd CH_2Cl_2 (5 mL) under Ar over 4 Å sieves at rt was added 4-acetoxycinnamic acid (**18**,[26] 102 mg, 0.50 mmol). After 60 min, the mixture was cooled to −15 °C, BF_3·OEt_2 (14 μL, 0.11 mmol) in CH_2Cl_2 (1 mL) was added, the reaction mixture was allowed to warm to rt, and the mixture was stirred overnight. MeOH (0.5 mL) was added, and the mixture was filtered through Celite®. The solvents were removed in vacuo, and the product was purified by flash chromatography (2:1 hexanes–EtOAc, R_f 0.2) to afford the title compound **19** (100 mg, 54%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71 (d, 1H, $J_{3',2'}$ 16.0 Hz, H-3′); 7.53 (d, 2H, $J_{2'',3''}$ 8.4 Hz, H-2″); 7.11 (d, 2H, H-3″); 6.34 (d, 1H, H-2′); 5.88 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1); 5.39–5.20 (m, 3H, H-2, H-3, H-4); 4.23 (d, 1H, $J_{5,4}$ 9.6 Hz, H-5); 3.71 (s, 3H, CO_2Me); 2.29 (s, 3H, PhOAc); 2.02, 2.02, 2.00 (3 × s, 9H, 3 × OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.9, 169.5, 169.3, 169.1 (4 × *O* *C*(O)Me); 166.8 (C-6); 164.4 (C-1′); 152.6 (C-4″); 140.5 (C-3′); 131.5 (C-1″); 129.6 (C-2″); 122.3 (C-3″); 116.2 (C-2′); 91.5 (C-1); 73.0 (C-5); 71.7 (C-3); 70.1 (C-2); 69.0 (C-4); 53.1 (CO_2*C*H_3); 21.1 (PhOC(O)*C*H_3); 20.6, 20.6, 20.5 (3 × OC(O)*C*H_3). ESIMS (*m*/*z*): 545.1 [M+Na^+]. HRMS Calcd for $C_{24}H_{26}NaO_{13}$ [M+Na^+] 545.12656. Found 545.12810.

3.5. Methyl 4-hydroxycinnamate (26)[41]

A solution of 4-hydroxycinnamic acid (1.00 g, 6.09 mmol) in dry MeOH (20 mL) containing $CH(OMe)_3$ (840 μL, 7.64 mmol) and $SOCl_2$ (20 μL, 0.27 mmol) was stirred at rt under N_2 for 24 h. Additional portions of $CH(OMe)_3$ (840 μL) and $SOCl_2$ (20 μL) were added, and the mixture stirred for a further 24 h. The solvent was removed in vacuo, and the residue azeotroped with toluene to give a pale purple solid. The product was purified by flash chromatography (3:2 hexanes–EtOAc, R_f 0.3) to afford **21** as a white solid (1.05 g, 96%), which exhibited spectroscopic data in agreement with those in the literature.[46]

3.6. Methyl {4′-[3″-(methyl prop-2″(*E*)-enoate)]phenyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid}uronate (27)

Compound **22** was obtained in 83% yield by the coupling of **6** and **26** using the general procedure for glycosidation (R_f 0.2 in 3:2 hexanes–EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.63 (d, 1H, $J_{3'',2''}$ 16.0 Hz, H-3″); 7.47 (d, 2H, $J_{3',2'}$ 8.7 Hz, H-3′); 6.99, (d, 2H, H-2′); 6.33 (d, 1H, H-2″); 5.38–5.25 (m, 3H, H-2, H-3, H-4); 5.19 (d, 1H, $J_{1,2}$ 6.9 Hz, H-1); 4.20 (AX m, 1H, H-5); 3.79, 3.71 (2 × s, 6H, CO_2Me, CO_2Me′); 2.05, 2.05, 2.04 (3 × s, 9H, 3 × OAc). ^{1}H NMR (300 MHz, CD_3CN): δ 7.62 (d, 1H, $J_{3'',2''}$ 16.0 Hz, H-3″); 7.58 (d, 2H, $J_{3',2'}$ 8.7 Hz, H-3′); 7.04, (d, 2H, H-2′); 6.43 (d, 1H, H-2″); 5.41 (dd, 1H, $J_{3,2}$ 9.6 Hz, $J_{3,4}$ 9.6 Hz, H-3); 5.40 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1); 5.22 (dd, 1H, H-2); 5.19 (dd, 1H, $J_{4,5}$ 9.8 Hz, H-4); 4.38 (d, 1H, H-5); 3.72, 3.65 (2 × s, 6H, CO_2Me, CO_2Me′); 1.99, 1.99, 1.98 (3 × s, 9H, 3 × OAc). ^{1}H NMR (300 MHz, C_6D_6): δ 7.73 (d, 1H, $J_{3'',2''}$ 16.0 Hz, H-3″); 6.96 (d, 2H, $J_{3',2'}$ 8.7 Hz, H-3′); 6.75, (d, 2H, H-2′); 6.32 (d, 1H, H-2″); 5.59–5.48 (m, 3H, H-2, H-3, H-4); 4.83 (AX m, 1H, H-1); 3.87 (d, 1H, $J_{5,4}$ 9.2 Hz, H-5); 3.49, 3.23 (2 × s, 6H, CO_2Me, CO_2Me); 1.69, 1.68, 1.68 (3 × s, 9H, 3 × OAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.9, 169.2, 169.1 (3 × O*C*(O)Me); 167.3, 166.7 (C-6, *C*O_2Me); 157.9 (C-1′); 143.8 (C-3′); 129.6 (C-4′); 129.5 (C-3′); 117.0 (C-2′); 116.7 (C-2″); 98.4 (C-1); 72.5, 71.6, 70.9, 68.9, (C-2, C-3, C-4, C-5); 52.9, 51.6 (2 × CO_2*C*H_3); 20.5, 20.5, 20.4, (3 × OC(O)*C*H_3). ESIMS (*m*/*z*): 517.0 [M+Na^+]. HRMS Calcd for $C_{23}H_{26}NaO_{12}$ [M+Na^+] 517.1322. Found 517.1317.

3.7. Sodium {4′-[3″-(sodium prop-2″(*E*)-enoate)]phenyl β-D-glucopyranosid}uronate (28)

A solution of 0.1 M LiOH (6 equiv) in MeOH–H_2O was added to **27** (239 mg, 0.48 mmol), and the mixture was stirred at 0 °C for 20 h. The solution was then diluted with H_2O, and the pH was adjusted to 4.0 with Amberlite IR120 (H^+). The resin was removed by filtration, the solution was neutralised to pH 7.2 with dilute NaOH and the solvents were removed in vacuo. The residue was then purified by flash chromatography (7:2:1 EtOAc–MeOH–H_2O) to give **23** (130 mg, 70%) after lyophilisation. ^{1}H NMR (300 MHz, D_2O): δ 7.59 (d,

2H, $J_{3',2'}$ 8.8 Hz, H-3′); 7.35 (d, 1H, $J_{3'',2''}$ 16.1 Hz, H-3″); 7.14 (d, 2H, H-2′); 6.43 (d, 1H, H-2″); 5.15 (d, 1H, $J_{1,2}$ 7.4 Hz, H-1); 3.91 (d, 1H, $J_{5,4}$ 9.3 Hz, H-5); 3.63 (m, 3H, H-2, H-3, H-4). ^{13}C NMR (75 MHz, D_2O): δ 175.2, 175.1 (C-6, CO_2Na); 157.6 (C-1′); 141.1 (C-3″); 129.8 (C-4′); 129.4 (C-3′), 122.7 (C-2″), 116.7 (C-2′); 99.7 (C-1), 76.2 (C-5), 75.2, 72.6, 71.6 (C-2, C-3, C-4). ESIMS (m/z): 406.8 [M+Na^+].

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Supplementary data

Supplementary data (NMR spectra for compounds **7**, **9**, **10**, **16** and **19)** associated with this article can be found, in the online version, at doi:10.1016/j.carres.2005.06.029.

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