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Note

Synthesis of urine drug metabolites: glucuronic acid glycosides of phenol intermediates

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Abstract—The investigation of drug metabolism requires substantial amount of metabolites. Isolation from urine is tedious, therefore, the material obtained by synthesis is preferred. Substantial amounts of three tentative drug metabolites, phenolic glucuronides, have been prepared using easily available glycosyl donors. The final products [3(2-*N*-methyl-*N*-isopropylaminoethoxy)phenyl] β-D-glucopyranosiduronic acid, 4-amino-3,5-dimethylphenyl β-D-glucopyranosiduronic acid and [2(*S*)-propanoyl-6-*O*-naphthyl] β-D-glucopyranuronic acid are useful as, for example, reference material in metabolite investigations.

Keywords: Metabolites; Glycosylation; Glucuronic acid donor; Phenolic glucuronide

One of the most common ways to rid the body of lipid metabolites is by oxidation or hydrolysis producing additional hydroxyl- or carboxyl-groups, these derivatives[1] are subsequently used forming a glucuronic acid derivative, either the glycoside or the anomeric ester, both β-linked. To investigate drug metabolism, identification of these glucuronic acid derivatives is important, as is testing of all aspects of their biological activity. Since often only minor amounts can be extracted from the urine, identification is much simplified by comparison with a synthetic derivative of known structure. Also, activity studies require substantial amount of metabolites, preferably obtained by synthesis. Although several glucuronic acid donor/promoter systems have been developed,[2,3] still there is no universal method found for the synthesis of glucuronides (Scheme 2). Herein we report the synthesis of three phenolic glucuronides, all tentative urine drug metabolites: Glucuronide **14** is a possible metabolite of a novel local anaesthetic and glucuronide **17** is likely to be a metabolite of lidocaine, also a local anaesthetic. The third glucuronide **19** is a tentative metabolite of the anti-inflammatory substance naproxen.

Since the synthesis of glucuronides has to be elaborated for each individual case,[4] a number of glucuronic acid donors, **1**–**7** (Chart 1) were tried with acceptor **10**, which was prepared in an acceptable yield from *O*-2-hydroxyethylresorcinol (Scheme 1). A silver triflate-promoted coupling using the acetylated donor **1**[5] at 0 °C

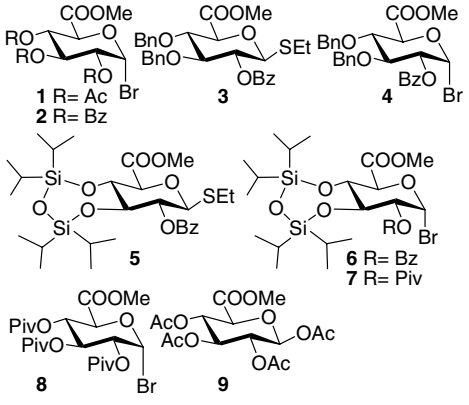

Chart 1. Glucuronic acid donors.

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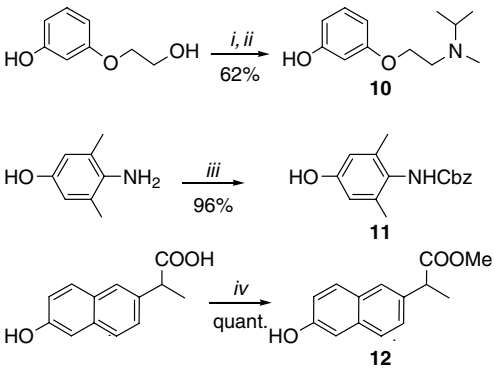


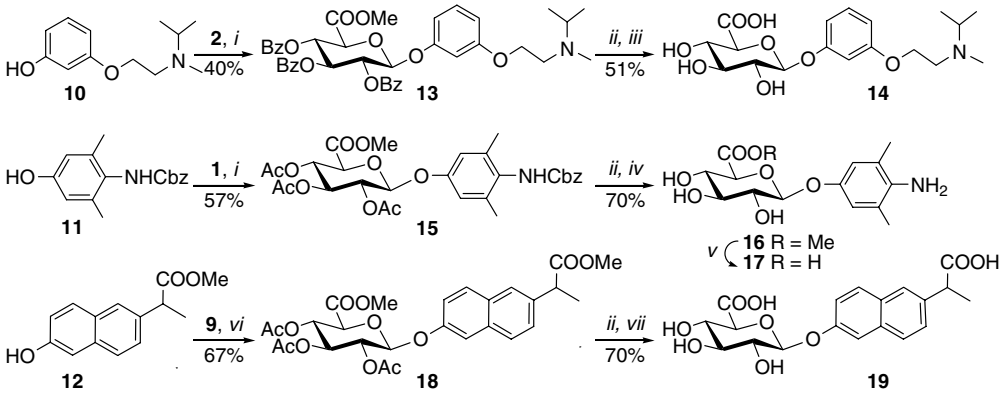
Scheme 1. Synthesis of the acceptor molecules. Reagents and conditions: (i) (1) MsCl, Et_3N, *t*-BuOMe; (2) *i*-PrMeNH; (ii) NaOH, *t*-$BuHSO_4$, 1,4-dioxane; (iii) CbzCl, NaOAc/water; (iv) TMSCl, MeOH.

gave an α/β-mixture (1:3) in 40% yield, whereas the benzoylated donor **2**[6] produced a mixture of β-glycoside **13** and the corresponding orthoester in similar yield. Exclusive formation of β-glycoside **13**, albeit still only in a moderate yield (40%), was obtained when the coupling between donor **2** and acceptor **10** was carried out at ambient temperature. The use of more elaborated donors[7,8] (**4**–**7**) did not improve the yield. Further it was found that the use of thioglycoside donors **3** and **5** in combination with thiophilic promoters, that is, DMTST and NIS/TfOH, was not compatible with acceptor **10**. DMTST as promoter produced a number of unidentified products, while NIS/TfOH gave iodinated phenyl derivatives (as identified by MS), products of possible side reactions with the activated aromatic acceptor. However, taking into account the accessibility of donor **2** and acceptor **10**, the yield in the glycosylation was sufficient to synthesise enough material of the desired glucuronide **13** (Scheme 2). Conventional deprotection afforded in two steps target compound **14** (51%).

The preparation of glucuronide **17** (Scheme 2) was first tried with the unprotected aminophenol as acceptor under various conditions. Since no significant formation of coupling product was observed (MALDI-TOF), we decided to protect the amino group. Trifluoroacetylation gave an almost insoluble compound and the phthalimido protected acceptor produced only small amounts of the desired glucuronide under Koenigs-Knorr conditions both with donors **1**, **2** and **8**.[9] The best result (approx. 40%) with this acceptor was obtained using donor **9**[5] and BF_3–etherate, but the product was difficult to purify. The benzyloxycarbonyl protected acceptor **11** (Scheme 1) did not react with donor **9** under these conditions but gave a β-glucuronide using donor **2**. Depending on reaction conditions, yields between 20% and 50% were isolated but both orthoester formation and transesterification were frequent side reactions. The major drawback, however, was the severe competition of the elimination reaction during the debenzoylation step. Fortunately, the acetylated donor **1**[5] gave glucuronide **15** in a good yield (57%, Scheme 2). Deprotection afforded target derivative **17** (38%), which was found to be rather labile and therefore characterised and stored as the hydrochloride salt of methyl ester **16**.

In case of the naproxen derivative **12**, which was prepared in two steps from commercial (*S*)-2-(6-methoxy-2-naphthyl)propanoic acid (naproxen, Scheme 1), a silver promoted coupling was not feasible because the acceptor is easily oxidised by silver (Ag^+). However, using the 1-*O*-acetyl derivative **9** as donor together with BF_3–etherate gave **18** in 67% yield. One-pot conventional deprotection then afforded the third target structure **19** in 70% yield.

In conclusion, the synthesis of three possible drug metabolite phenolic glucuronides has been accomplished. The synthetic pathways utilise easily available glycosyl donors and allow preparation of substantial amounts of the target glucuronides. The use of more complex donors developed for oligosaccharide synthesis[7,8] was not an advantage with these phenolic aglycons. Furthermore, activated phenol aglycons were not compatible with thiophilic promoters, especially iodonium reagents.



Scheme 2. Synthesis of phenyl glucuronides. (i) AgOTf, CH_2Cl_2; (ii) NaOMe, MeOH; (iii) NaOH, MeOH; (iv) H_2, Pd/C, MeOH; (v) LiOH, MeOH; (vi) BF_3–Et_2O, CH_2Cl_2; (vii) LiOH, water.

1. Experimental

1.1. General methods

All organic solvents were distilled before use, except Et_2O, which was stored over Na. Organic solutions were dried over $MgSO_4$ before concentration, which was performed under diminished pressure at <40 °C (bath temperature). NMR spectra were recorded at 300 or 400 MHz (Varian/JEOL) (^1H) or at 75 or 100 MHz (^{13}C), respectively, in $CDCl_3$, D_2O or CD_3OD. Except for D_2O (δ 4.80), TMS was used as internal standard (δ 0) for ^1H spectra. ^{13}C Spectra were referred to the $CHCl_3$ signal (δ 77.17) or MeOH signal (δ 49.15). Silica Gel E. Merck 60 (0.040–0.063) was used for flash chromatography. TLC was performed on Silica Gel 60 (E. Merck) glass plates with detection by UV-light and/or charring with 8% sulfuric acid. MALDI-TOF spectra were recorded on a Bruker Biflex III using PEG 600, PEG 1000 and PEG 1500 as calibration reference and 2′,4′,6′-trihydroxy-acetophenone monohydrate (THAP) as matrix. High resolutions mass spectra were recorded on a Micromass Q-TOF Micro (ESI) with 10 mM NH_4OOCH at pH 3.2 as a buffer.

1.2. 3-(2-*N*-Methyl-*N*-isopropylaminoethoxy)phenol (10)

O-2-Hydroxyethyl resorcinol (5 g, 32.43 mmol) was dissolved in *tert*-butyl methyl ether (250 mL), flushed with nitrogen and cooled to 0 °C. Triethylamine (10.9 mL, 77.8 mmol) was added and then mesyl chloride (5.6 mL, 71.3 mmol) during a period of 10 min. The ice bath was removed after 1 h and stirring was continued for another hour, when TLC (4:1 toluene–EtOAc) showed complete reaction. The reaction mixture was diluted with Et_2O, washed with saturated sodium hydrogen carbonate twice, concentrated and co-evaporated with toluene. The residue was dissolved in 1:4 EtOAc–toluene (250 mL), then *N*-methyl-*N*-isopropylamine (60 mL, 575 mmol) was added and the soln was heated and left at 70 °C overnight. Another 1.5 mL (14 mmol) of *N*-methyl-*N*-isopropylamine was added and the reaction was complete within 4 h. The reaction mixture was diluted with toluene, washed with water three times, dried, filtered and concentrated. The residue was purified by flash-chromatography (10:1:0.1 EtOAc–MeOH–Et_3N) to produce 3-(2-*N*-methyl-*N*-isopropylaminoethoxy)-1-mesyl resorcinol (7.6 g, 26.3 mmol, 82%). NMR (CD_3OD): ^1H, δ 1.05 (d, 6H, *J* 6.6 Hz, CH–*Me*), 2.34 (s, 3H, NMe), 2.80 (dd, 2H, *J* 6.2 Hz, NCH_2), 2.90 (m, 1H, NCH), 3.13 (s, 3H, SCH_3), 4.05 (dd, 2H, *J* 6.2 Hz, OCH_2), 6.86 (3H, m, Ph*H*), 7.30 (t, 1H, Ph*H*); ^{13}C, δ 18.0 (2 × CH*Me*), 37.4, 38.5 (SCH_3 and NCH_3), 51.7, 54.4 (NCH and NCH_2), 67.3 (OCH_2), 108.8, 113.8, 114.0, 130.4, 150.2, 160.2 (Ph). 3-(2-*N*-Methyl-*N*-isopropylaminoethoxy)-1-mesyl resorcinol (7.5 g, 26.1 mmol) was dissolved in dioxane (100 mL) and saturated sodium hydroxide (45 mL) and *tert*-butyl hydrogensulfate (0.8 g, 2.4 mmol) was added. Then the reaction mixture was heated to 105 °C overnight and left at room temperature for two days (TLC: 9:1 MeCN–Et_3N). The reaction mixture was diluted with CH_2Cl_2, and the water phase (pH ~ 12), containing most of the desired product, collected. The water phase was adjusted to pH = 8 with HCl (2 M) and then extracted with CH_2Cl_2 twice. The combined organic phases were dried with sodium sulfate, filtered and evaporated to give **10** (4.1 g, 19.5 mmol, 75%). NMR (CD_3OD, Ref. δ 3.31): ^1H, δ 1.14 (d, 6H, *J* 6.6 Hz, CH–*Me*), 2.45 (s, 3H, NMe), 2.98 (dd, 3H, *J* 5.6 Hz, NCH_2), 3.08 (m, 1H, NCH), 4.09 (dd, 2H, *J* 5.6 Hz, OCH_2), 6.41 (m, 3H, Ph*H*), 7.06 (t, 3H, Ph*H*); ^{13}C δ 17.7 (2 × CH*Me*), 38.3 (NCH_3), 53.0 (NCH_2), 56.4 (NCH), 66.4 (OCH_2), 103.1, 106.8, 109.4, 131.1, 159.9, 161.3 (Ph). HRMS: Calcd for $C_{12}H_{19}NO_2$ 210.1494 $[M+H]^+$. Found: 210.1485 $[M+H]^+$.

1.3. 4-Benzyloxyamido-3,5-dimethylphenol (11)

Benzyl chloroformate (8.5 mL, 4 equiv) was added in two portions to a slurry of 4-amino-3,5-dimethylphenol[10,11] (2.0 g, 14.6 mmol) and NaOAc (50 mL, 2 M in water). After 10 min a mixture of EtOAc and toluene (50 mL, 1:1, v/v) was added and the stirring continued for further 30 min. The organic layer was then separated, dried and concentrated. The crude product was crystallised from toluene to obtain **11** (3.8 g, 14.0 mmol, 96%). Mp: 87–88 °C. NMR (CD_3OD): ^1H, δ 2.13 (s, 6H, Ph*Me*), 5.22 (s, 2H, *Cbz*), 6.30 (s, 2H, Ph*H*), 7.36–7.42 (m, 5H, Cbz); ^{13}C, δ 18.4 (Ph*Me*), 67.6 (*Cbz*), 115.4 (Ph*C*2, Ph*C*4), 125.2 (Ph*C*3, Ph*C*5), 128.3, 128.5, 128.8 (*Cbz*), 136.3 (Ph*C*4), 137.4 (*Cbz*), 155.4, 156.0 (*Cbz*, Ph*C*6).

Anal. Calcd for $C_{16}H_{17}NO_3$: C, 70.8; H, 6.3; N, 5.2; O, 17.7. Found: C, 70.8; H, 6.3; N, 5.2.

1.4. Methyl 2(*S*)-(6-hydroxy-2-naphthyl)propionate (12)

TMSCl (0.6 mL, 4.6 mmol) was added to a soln of 2(*S*)-(6-hydroxy-2-naphthyl)propionic acid[12] (0.50 g, 2.3 mmol) in MeOH (20 mL). TLC (45:4:1 $CHCl_3$–MeOH–AcOH) showed complete conversion after 2 h. Dry toluene (5 mL) was added and the mixture concentrated and coevaporated with dry toluene to give methyl 2(*S*)-(6-hydroxy-2-naphthyl)propionate **12** (0.53 g, 2.3 mmol, quant.) as a white solid. NMR ($CDCl_3$): ^1H, δ 1.59 (d, *J* 6.4 Hz, Me), 3.70 (s, 3H, OMe), 3.87 (m, 1H, CH), 7.05 (m, 2H, Ph*H*), 7.37 (d, 1H, Ph*H*), 7.65 (m, 3H, Ph*H*); ^{13}C (CD_3OD), δ 18.6 (Ph*Me*), 45.5 (CH), 52.4 (OMe), 109.4, 118.2, 126.1, 126.4, 127.0, 128.9, 129.8, 133.8, 153.7 (Ph), 175.0 (CO).

1.5. [3(2-*N*-Methyl-*N*-isopropylaminoethoxy)phenyl] β-D-glucopyranosiduronic acid (14)

To a stirred soln of **10** (69 mg, 0.33 mmol) and **2** (307 mg, 0.53 mmol) in dry CH_2Cl_2 (3 mL) containing 4 Å molecular sieves (0.5 g), silver triflate (203 mg, 0.79 mmol) dissolved in dry toluene (1.5 mL) was added in small portions. After 4 h at room temperature under darkness the mixture was diluted with CH_2Cl_2 and filtered through Celite, concentrated, and the residue was purified by silica gel chromatography (24:1:0.025 CH_2Cl_2–MeOH–TEA) to give **13** (94 mg, 0.13 mmol, 40%). ^{1}H NMR ($CDCl_3$): δ 1.08 (dd, 6H, CH(*Me*)$_2$), 2.32 (s, 3H, NMe), 2.76 (t, 2H, NCH_2), 2.88 (m, 1H, NCH), 3.65 (s, 3H, OMe), 3.97 (t, 2H, OCH_2), 4.50 (d, $J_{4,5}$ 9.0 Hz, 1H, H5), 5.45 (d, $J_{1,2}$ 7.0 Hz, 1H, H1), 5.74 (dd, $J_{1,2}$ 7.4 Hz, $J_{2,3}$ 9.2 Hz, 1H, H2), 5.82 (t, $J_{3,4}$ 9.2 Hz, $J_{4,5}$ 9.2 Hz, 1H, H4), 5.96 (t, $J_{2,3}$ 9.2 Hz, $J_{3,4}$ 9.2 Hz, 1H, H3), 6.60 (m, 3H), 7.17 (t, 1H), 7.30–7.40 (m, 7H), 7.44–7.52 (m, 2H), 7.90–7.98 (m, 6H). Thermospray mass spectra from a Finnigan SSQ 7000 showed [M+H] = 712.

A soln of **13** (70 mg, 0.10 mmol) in MeOH (4 mL) was treated with sodium methoxide (4.4 mL, 0.1 M, 0.44 mmol) and stirred for 3 h. pH was adjusted to 6 with Dowex H^+ ion exchange resin, filtered and concentrated to give crude **14** (28 mg, 0.07 mmol, 70%). No further purification was performed. Thermospray mass spectra from a Finnigan SSQ 7000 showed [M+H] = 400. The crude product (10 mg, 0.025 mmol) was dissolved in MeOH (0.5 mL) before NaOH (40 μL, 0.038 mmol, 1 M) was added. After 4 h the mixture was neutralised with H^+-ion exchange resin, filtered and concentrated. The residue was purified by gel filtration on a Bio-Gel® P-2 (Fine) column and then freeze-dried to give **14** (7 mg, 0.018 mmol, 73%). NMR (CD_3OD): ^{1}H, δ 1.34 (dd, 6H, CH(*Me*)$_2$), 2.81 (s, 3H, NCH_3), 3.41 (m, 2H, NCH_2), 3.51 (m, 3H, H2–H5), 3.63 (m, 1H, NCH), 3.76 (m, 1H, H2–H5), 4.26 (m, 2H, OCH_2), 4.90 (d, $J_{1,2}$ 7.2 Hz, 1H, H1), 6.64 (dd, $J_{4,5}$ 8.4 Hz, $J_{2,4}$ 2.3 Hz, 1H, Ph*H*4), 6.75 (dd, $J_{5,6}$ 8.1 Hz, $J_{2,6}$ 1.8 Hz, 1H, Ph*H*6), 6.85 (t, $J_{2,4}$ 2.3 Hz, $J_{2,6}$ 2.2 Hz, 1H, Ph*H*2), 7.20 (t, $J_{4,5}$ 8.2 Hz, $J_{5,6}$ 8.2 Hz, 1H, Ph*H*5). ^{13}C, δ 16.6–16.7 (CH(*Me*)$_2$), 37.1 (NCH_3), 52.9 (NCH_2), 59.1 (NCH), 63.8 (OCH_2), 73.7, 74.8, 76.7, 78.0 (C2–C5), 102.5 (C1), 105.0 (Ph*C*2), 109.9 (Ph*C*4), 112.0 (Ph*C*6), 131.2 (Ph*C*5), 160.2, 160.4 (Ph*C*1, Ph*C*3), 176.4 (*COOH*). Q-TOF HRMS: Calcd for $C_{18}H_{28}NO_8$ 386.1815 $[M+H]^+$. Found: 386.1810 $[M+H]^+$.

1.6. 4-Amino-3,5-dimethylphenyl β-D-glucopyranosiduronic acid (17)

Silver triflate (1.0 g, 3.9 mmol) was added at room temperature to a stirred soln of **1** (1.6 g, 4.0 mmol) and **11** (0.81 g, 3.0 mmol) in CH_2Cl_2 (50 mL) containing 4 Å molecular sieves. After 3 h, Et_3N (1 mL) was added and the stirring was continued for 15 min. The mixture was diluted with CH_2Cl_2 and filtered through Celite, concentrated, and the residue was purified by silica gel chromatography (toluene → 6:1 toluene–EtOAc) to give **15** (15 g, 1.7 mmol, 57%). $[\alpha]_D$ −14.8 (*c* 1.0, CH_2Cl_2); ^{13}C NMR ($CDCl_3$): δ 18.7 (Ph*Me*), 20.6, 20.7 (CO*CH*$_3$), 53.1 (OMe), 67.2 (Cbz), 69.3, 71.2, 72.0, 72.8 (C2, C3, C4, C5), 99.2 (C1), 116.6 (Ph*C*2, Ph*C*4), 125.4 (Ph*C*3, Ph*C*5), 128.3, 128.7, 129.2 (Cbz), 137.8, 138.0 (Ph*C*4, Cbz), 155.3, 155.8 (Cbz, Ph*C*6), 167.0 (*CO*OMe), 169.4, 169.5, 170.2 (CO). A soln of **15** (900 mg, 1.53 mmol) in MeOH (100 mL) was treated with a catalytic amount of sodium in MeOH. After 30 min an additional amount of sodium in MeOH was added. The stirring was continued for 2 h. Although some starting material was left according to TLC (5:1 $CHCl_3$–MeOH), the reaction was quenched by addition of Dowex 50 (H^+) ion exchange resin, because the formation of elimination product started to occur. After filtration and concentration, the crude product was purified by flash chromatography ($CHCl_3$–MeOH 20:1 → $CHCl_3$–MeOH 10:1) giving methyl [(4-benzyloxyamido-3,5-dimethylphenyl) β-D-glucopyranosid]uronate (500 mg, 1.07 mmol, 71%). $[\alpha]_D$ −62.0 (*c* 1.03, MeOH); ^{1}H NMR (MeOD): δ 2.20 (s, 6H, Ph*Me*), 3.48 (m, 2H), 3.62 (t, *J* 9.7 Hz, 1H), 3.76 (s, 3H, OMe), 4.03 (d, *J* 9.7 Hz, H-1), 4.95 (d, *J* 7.0 Hz, 1H), 5.18 (s, 2H, Cbz), 6.80 (s, 2H, Ph*H*), 7.36 (m, 5H, Cbz). ^{13}C NMR (MeOD): δ 18.7 (Ph*Me*), 53.0 (OMe), 67.8 (Cbz), 73.1, 74.7, 76.8, 77.2 (C2–C5), 102.6 (C1), 117.4 (Ph*C*2, Ph*C*4), 126.0 (Ph*C*3, Ph*C*5), 128.9, 129.2, 129.6 (Cbz), 138.4, 138.9 (Ph*C*4, Cbz), 156.0, 157.3 (Ph*C*6, Cbz), 171.8 (CO). The above obtained compound (856 mg, 1.85 mmol) was dissolved in MeOH (100 mL) and 10% Pd/C (50 mg) was added. Hydrogenolysis was carried out under H_2 at atmospheric pressure overnight. The suspension was filtered through a plug of Celite and RP-C18 gel, which was washed with MeOH (100 mL). The combined solutions were titrated with HCl (1 N, ~1.8 mL) and concentrated to approx. 5 mL. The residual soln was diluted with water (50 mL) and washed with Et_2O. After freeze-drying, **16** (665 mg, 1.83 mmol, 99%) was obtained. $[\alpha]_D$ −78.0 (*c* 2.0, MeOH); ^{1}H NMR (MeOD): δ 2.14 (s, 6H, Ph*Me*), 3.45 (m, 2H), 3.61 (t, *J* 9.4 Hz, 1H), 3.77 (s, 3H, OMe), 3.94 (d, *J* 9.8 Hz, H-1), 4.78 (d, *J* 7.6 Hz, 1H), 6.68 (s, 2H, Ph*H*). ^{13}C NMR (CD_3OD): δ 18.1 (Ph*Me*), 53.0 (OMe), 73.2, 74.8, 76.8, 77.3 (C2–C5), 104.1 (C1), 118.6 (Ph*C*2, Ph*C*4), 124.9 (Ph*C*3, Ph*C*5), 139.6 (Ph*C*4), 151.3 (Ph*C*6), 171.2 (*CO*). Q-TOF HRMS: Calcd for $C_{15}H_{22}NO_7$ 328.1396 $[M+H]^+$, 350.1216 $[M+Na]^+$. Found: 328.1391 $[M+H]^+$, 350.1216 $[M+Na]^+$. The free amino compound **16** (42 mg, 128 μmol) was dissolved in a methanolic LiOH soln

(0.5 mL, 0.1 M). The mixture was stirred at room temperature overnight. The TLC (12:3:3:1 EtOAc–AcOH–MeOH–water, $R_f \sim 0.5$) showed complete conversion to the uronic acid. Excess of base was removed by addition of Dowex 50 (H^+) ion exchange resin. Crude **17** (40 mg) was obtained after concentration. ^{1}H NMR (D_2O): δ 2.26 (s, 6H, Ph*Me*), 3.52 (m, 3H), 3.81 (d, *J* 7 Hz, 1H), 4.92 (d, *J* 9 Hz, 1H), 6.71 (s, 2H, Ph*H*). MALDI-TOF MS: Calcd for $C_{14}H_{19}NO_7$ 313.13 [M]; Found 335.98 $[M+Na]^+$, 352.00 $[M+K]^+$.

1.7. (2(*S*)-Propanoyl-6-*O*-naphthyl) β-D-glucopyranuronic acid (19)

A stirred soln of **9** (750 mg, 2.0 mmol) and **12** (460 mg, 2.0 mmol) in dry CH_2Cl_2 (15 mL) was cooled (0 °C) when $BF_3{\cdot}Et_2O$ (650 μL, 5 mmol) was added. The reaction mixture was allowed to attain room temperature and after additional 12 h, it was poured onto a slurry of ice water (100 mL). The organic phase was separated, diluted with CH_2Cl_2, washed with $NaHCO_3$ and brine and concentrated. The residue was applied onto a silica gel column and eluted (toluene → toluene–EtOAc 10:1) to give **18** (730 g, 1.34 mmol, 67%). $[\alpha]_D$ +19.5 (*c* 1.9, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 1.59 (d, *J* 7.2 Hz, CH*Me*), 2.02, 2.04, 2.05 (s, 9H, COMe), 3.66 (s, 3H, OMe), 3.73 (s, 3H, OMe), 3.87 (q, 1H, C*H*Me), 4.28 (d, *J* 9.5 Hz, 1H), 5.27–5.38 (m, 4H), 7.16 (dd, 1H, *J* 2.4 Hz, *J* 8.8 Hz, Ph*H*), 7.31 (d, 1H, *J* 2.2 Hz, Ph*H*), 7.41 (dd, 1H, *J* 1.0 Hz, *J* 8.5 Hz, Ph*H*), 7.65 (m, 3H, Ph*H*). ^{13}C NMR ($CDCl_3$): δ 18.7 (Ph*Me*), 20.7, 20.8 (CO*Me*), 45.5 (*C*H), 52.2, 53.2 (OMe), 69.3, 71.3, 72.0, 72.9 (C2–C5), 99.4 (C1), 111.7, 119.2, 126.1, 126.6, 127.8, 129.7, 130.4, 133.3, 137.1, 154.6 (Ph), 167.1, 169.4, 169.5, 170.2, 175.1 (CO, *CO*Me). NaOMe (1M, approx. 15 drops) was slowly added to a soln of **18** (1.2 g, 2.2 mmol) in MeOH (50 mL) until the colour stopped changing (from bright yellow to orange-red). The reaction mixture was left overnight at room temperature to obtain complete removal of the acetate groups (TLC: 6:1 CH_2Cl_2–MeOH), neutralised with H^+ ion exchange resin, filtered and concentrated. The residue was purified by flash-chromatography (CH_2Cl_2 → CH_2Cl_2–MeOH 10:1) to produce methyl [2(*S*)-(methylpropanoyl)-6-*O*-naphthyl]-β-D-glucopyranosid]uronate (655 mg, 1.55 mmol, 70%). $[\alpha]_D$ −40.0 (*c* 1.0, MeOH); NMR ($CDCl_3$): ^{1}H, δ 1.56 (d, *J* 7.2 Hz, Me), 3.66 (s, 3H, OMe), 3.70 (s, 3H, OMe), 3.80–4.05 (m, 5H), 4.52 (sb, 1OH), 4.59 (sb, 1OH) 5.08 (d, *J* 7.2 Hz, 1H), 5.14 (sb, 1OH), 7.16 (dd, 1H, Ph*H*), 7.33 (m, 2H, Ph*H*), 7.59 (m, 3H, Ph*H*); ^{13}C, δ 18.7 (Ph*Me*), 45.5 (CH), 52.2, 53.1 (OMe), 71.4, 73.0, 74.6, 75.6 (C2–C5), 101.2 (C1), 111.8, 119.3, 126.0, 126.4, 127.8, 129.6, 130.2, 133.3, 136.8, 154.7 (Ph), 169.7, 175.1 (*CO*Me). $LiOH{\cdot}H_2O$ (130 mg, 3.1 mmol) was added to a soln of the obtained methyl ester derivate (630 mg, 1.5 mmol) in water (5 mL). After 2 h the mixture was neutralised with H^+ ion exchange resin, filtered and concentrated. The crude residue was dissolved in water (2 mL), filtered through a short RP C18 column (Sep-pack). Freeze drying gave **19** (600 mg, 1.50 mmol, quant.). NMR (D_2O): ^{1}H, δ 1.46 (d, *J* 7.2 Hz, *Me*), 3.63 (m, 3H), 3.75 (m, 1H), 3.92 (d, *J* 8.2 Hz, 1H), 5.19 (d, *J* 5.2 Hz, 1H), 7.28 (m, 2H, Ph*H*), 7.44 (m, 2H, Ph*H*), 7.71 (s, 1H, Ph*H*), 7.79 (m, 2H, Ph*H*); ^{13}C, δ 18.7 (Ph*Me*), 48.7 (CH), 72.2, 73.6, 76.1, 77.0 (C2–C5), 100.9 (C1), 111.3, 119.3, 126.0, 127.4, 128.0, 130.1, 130.2, 133.2, 139.9, 154.9 (Ph), 176.0, 183.9 (*CO*OH). Q-TOF HRMS: Calcd for $C_{19}H_{20}O_9$ 410.1451 $[M+NH_4]^+$, 415.1005 $[M+Na]^+$. Found: 410.1450 $[M+NH_4]^+$, 415.1007 $[M+Na]^+$.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.01.014.

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