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A convenient new synthesis of quaternary ammonium glucuronides of drug molecules

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ABSTRACT

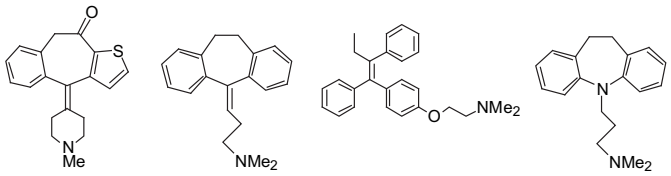
N-Glucuronides, of various structural types, are frequently encountered as drug metabolites. Efficient chemical synthesis of these compounds, both as analytical standards and for toxicological investigation, is therefore an important goal. Earlier syntheses of N^+- glucuronides of aliphatic tertiary amine drugs involved direct reaction of the drug molecule with a bromosugar, but yields were generally low and of poor reproducibility, with many by-products. In addition the final products were often of low stability, hindering effective isolation and purification. We now report that a stable, readily prepared glucuronic acid hemiacetal is a reliable precursor for metabolites of this type and give three pharmaceutically relevant examples. We report further on the stability of the final metabolites and the conditions required for their isolation and purification.

1. Introduction

Xenobiotic metabolism is a fundamental process by which the body is detoxified from drug molecules. In phase II metabolism,[1,2] a functional group on the drug such as OH, NR_2 or SH is conjugated to a polar unit, yielding a water-soluble metabolite which is excreted. Glucuronides,[3,4] including *O*, *N*, *S*, and *C*-types, are by far the most important class of phase II metabolites. In continuation of a long-standing interest in glucuronides and efficient methods for their synthesis,[3b,5] we now report on the synthesis of quaternary ammonium glucuronides of aliphatic tertiary amine-containing drugs. At present there are no satisfactory general methods for this important class.

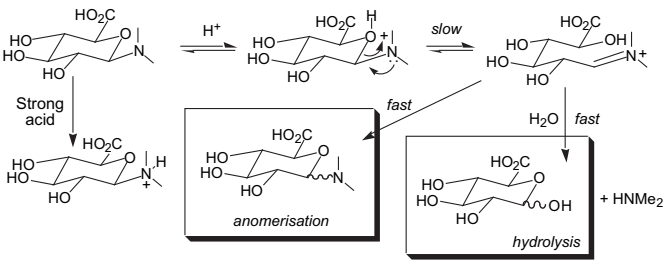
With the increasing number of *N*-heterocyclic and amine-containing drugs now marketed, *N*-glucuronides[6–8] are being reported more frequently. Neutral [9–11] and quaternary ammonium[12,13] *N*-glucuronides result from secondary and tertiary amines, respectively. Heterocyclic *N*-glucuronides[14] are also familiar, e.g. the pyridinium N^+-glucuronides[15,16] derived from nicotine and analogues.

N-Glucuronidation is common for drugs such as antipsychotics, antihistamines and tricyclic antidepressants, [17,18] accounting for up to 25% of the initial dose of ketotifen[19] **1** and amitriptyline[20] **2**, for example.[21] Further examples are the important anticancer agent tamoxifen **3**[22] (whose N^+ glucuronide is reported to be equiactive with the parent) and imipramine **4**, another antidepressant. It is important to note that quaternary ammonium glucuronides are generally specific human metabolites[23] and therefore not detected prior to clinical trials.



1 **2** **3** **4**

The stability of all classes of *N*-glucuronides is strongly pH dependent.[24,25] Thus the stability of neutral *N*-glucuronides is low in weakly acidic media due to the available nitrogen lone pair.[26] In these conditions, the pyranose ring oxygen becomes protonated and catalyses the formation of an iminium ion, which in turn readily hydrolyses (Scheme 1).



Scheme 1. Acid catalysed hydrolysis of neutral *N*-glucuronides.

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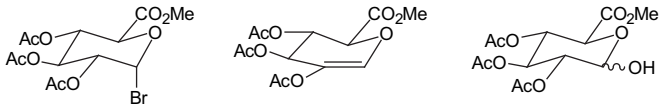
By contrast, under *strongly* acidic conditions *N*-glucuronides are generally more stable. Protonation on nitrogen forms a quaternary ammonium species, rendering the iminium ion inaccessible (Scheme 1). Likewise, quaternary ammonium (zwitterionic) N^+-glucuronides are generally more robust in acidic media but may hydrolyse under basic conditions to release the aglycone.[17] *N*-Glucuronidation is feasible for many, if not most, drugs, which contain an amine group, but due to the labile nature of their glycosidic linkages, such metabolites are probably unstable in the excretory media.[7] Thus it is likely that *N*-glucuronidation has been underestimated.

The recently issued FDA guidelines[27] have emphasised the importance of determining the safety of drug metabolites that are either identified only in humans or are present at disproportionately higher levels (viz those accounting for >10% of parent systemic exposure, as measured by AUC) in humans than in any animal species used in testing. In some cases it may be necessary to carry out non-clinical testing of N^+-glucuronide metabolites: this has added impetus to the search for effective syntheses. Luo et al.[18] reported a two phase system for the synthesis of these metabolites, but this procedure has proved low-yielding or difficult to repeat.[19,28] We now report an efficient new approach to N^+-glucuronide synthesis of some generality, illustrated by three examples.

2. Results and discussion

2.1. Synthesis of N^+-glucuronides via the bromosugar

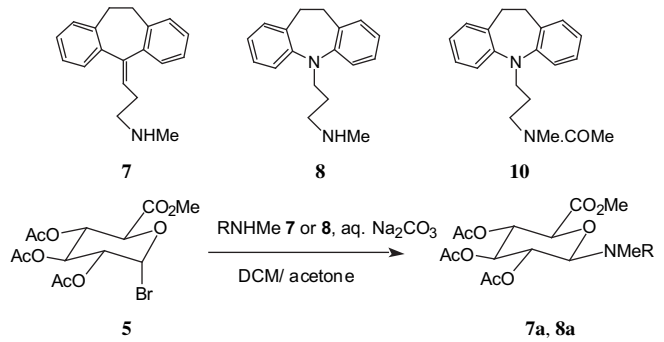
We first studied the method of Luo et al.[18] reacting bromosugar **5** and amitriptyline **2** in a two phase toluene/aq $NaHCO_3$ mixture. The major organic product from this reaction was the glucal **6**, formed by E2 elimination owing to the basic action of **2**. Direct substitution of **5** by heating with tertiary amines has also been employed, e.g., for the N^+-glucuronide of tamoxifen **3**.[22] The relative success of this reaction (though still in<10% yield) can be explained by the lower pK_a of tamoxifen **3**, a β-alkoxy amine, compared to amitriptyline **2**.



5 **6** **9**

We also employed secondary amines in the direct substitution reaction, intending to quaternise later: we recently showed this to be a good approach to N^+-glucosides.[29] Frequently tertiary amino drugs are commercially available as their des-methyl derivatives, or demethylation can be achieved using the Olofson reaction[30]: we used nortryptiline **7** and desipramine **8**, derived from **2** and **4** respectively.

The reaction of nortriptyline **7** with **5** (Scheme 2) under basic conditions led to modest yields of **7a**, up to 26%. The basic conditions in this reaction, as well as removing in situ generated HBr, probably lead to generation of hemiacetal **9**, which could react independently with **7**. Less glucal **6** was formed in this reaction than with tertiary amines. Reaction of desipramine **8** with **5** gave similar yields of **8a** (15–17%), but transacylation giving amide **10** was then significant. The use of isobutyrate ester protecting groups[31,32] reduced transacylation, but yields were not greatly improved.



7 **8** **10**

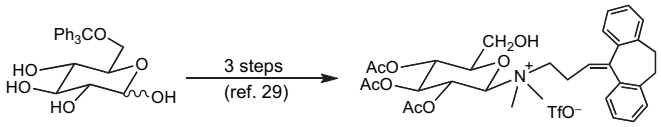
5 **7a, 8a**

Scheme 2. Base-catalysed reaction of a bromosugar with secondary amines.

Quaternisation of intermediates **7a**/**8a**, as in the glucose series,[29] required methyl triflate (MeI was insufficiently reactive), then the products could be deprotected to give the desired free glucuronides as described below. However, in view of the poor yields and lack of generality of this approach we sought an improved synthesis.

2.2. Synthesis by oxidation of N^+-glucosides

We recently showed the value of (6-*O*-trityl)glucose **11** in N^+-glucoside synthesis[29]: both the relatively lipophilic amine and the sugar are made soluble in organic solvent. Reaction of **7** with **11**, followed by acetylation of free OH groups and quaternisation with MeOTf, afforded the intermediate compound **12** with a free 6-OH in acceptable yield (30% overall), Scheme 3.



11 **12**

Scheme 3. N^+-Glucuronide synthesis via the corresponding glucoside.

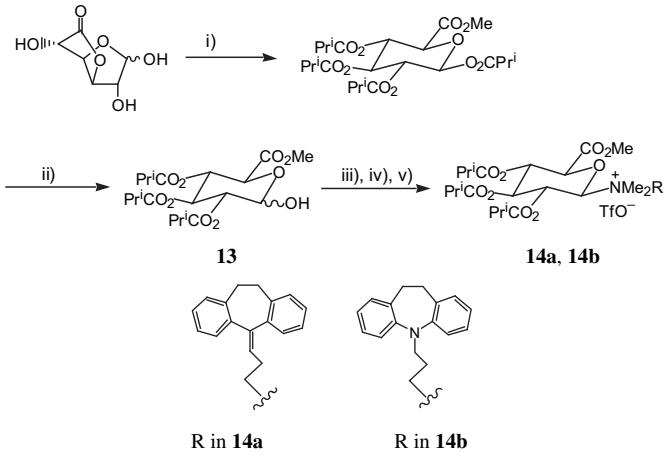
We hoped to achieve our goal by oxidation of **12**. However, under a variety of conditions (using PDC, $RuCl_3$–$NaIO_4$ or TEMPO in conjunction with NaBr/NaOCl or trichloroisocyanuric acid) the desired carboxylic acid could not be obtained: generally aglycone cleavage resulted. The use of TEMPO[33] with $PhI(OAc)_2$ as terminal oxidant in MeCN did give some of the desired glucuronide (these conditions generate acetic acid as a by-product, which our product and intermediate will tolerate) but the product was not pure by LCMS analysis. As well as oxidation of the primary alcohol, oxidation occurred at another site, probably the alkene double bond, both as well as and in competition with the desired reaction. Nevertheless, this should be a viable approach for N^+-glucuronides of non-functional aglycones, specifically those without other oxidisable sites.

2.3. Synthesis via a glucuronate ester hemiacetal

Instead a glucuronate hemiacetal (cf. note to Scheme 2, vide supra) proved to be a robust precursor: in this way no oxidation step is needed. We illustrate this method with syntheses of the N^+-glucuronides of both amitriptyline **2** and imipramine **4**, tricyclic antidepressants in regular use. In another example, we have prepared a model N^+-glucuronide bearing a different structural feature, namely a pyrrolidine unit.

In contrast to our N^+-glucosidation studies,[29] here the amine is reacted successfully with an *acylated* hemiacetal: to reduce the risk of transacylation, triisobutyrate **13**[31] (cf. **9**) is preferred. This derivative, a stable crystalline solid at 20 °C, is available in two steps from glucuronolactone (Scheme 4). Monoesters such as allyl or benzyl glucuronate, which we used very successfully in acyl glucuronide synthesis,[34] were ineffective here: on treatment with primary and secondary amines they readily formed amides.

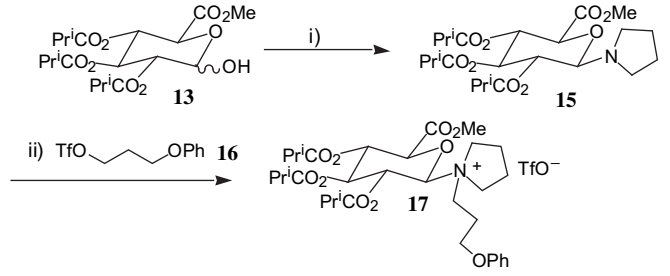
Nortriptyline **7** and desipramine **8** react readily with **13**, giving selectively β-glycosylamines. This reaction cannot be monitored by TLC because of the acid instability of the products, but by evaporation of aliquots and NMR analysis the conversion to the



Scheme 4. N^+-Glucuronide synthesis via the hemiacetal. i) MeOH, NEt_3 (0.1 equiv), trace AcOH, then evap., Pr^iCOCl, py, DCM, 40 °C, 59%; ii) $N_2H_4{\cdot}H_2O$, AcOH, DMF, 85%; iii) **7** or **8**, PhMe, 50 °C; iv) pyridine, Ac_2O, 0 °C; v) MeOTf, DCM, 15–20% (when isolated, see text).

glycosylamines was shown to be 80–90%. Typically these intermediates show a peak around δ 4.20 (1H, d, J ca. 8 Hz) for the anomeric proton and only the β-anomer is seen. Prior to quaternisation with MeOTf, unreacted OH and NH_2 groups were acetylated using Ac_2O/pyridine (otherwise release of TfOH led to hydrolysis of the adducts); then satisfactory yields of quaternised products were obtained. The quaternisation is accompanied by a characteristic downfield shift of 0.5–1 ppm in the anomeric proton. Purification on silica was usually possible at this stage, though with significant losses.[29] Thus intermediates **14a** and **14b** were fully characterised, but sometimes it was simpler to triturate with ether after the MeOTf step, then after removing the non-polar material, hydrolyse the residue directly (vide infra).

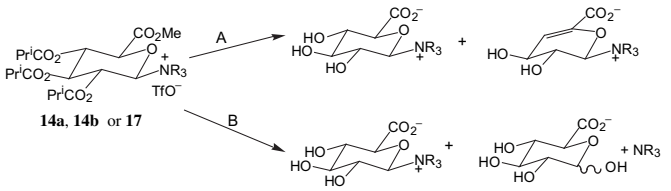
Hemiacetal **13** also reacts efficiently with cyclic secondary amines such as pyrrolidine. The resulting glycosylamine **15** (Scheme 5) was reacted with primary triflate **16** (prepared from the corresponding alcohol and used immediately),[35] giving adduct **17**, thus extending the scope of our quaternisations beyond methylation. The conversion to **17** was good and this compound was fully characterised, but the product was best hydrolysed directly without purification, cf. above.



Scheme 5. Synthesis of a pyrrolidinium N^+-glucuronide. i) Pyrrolidine (1.4 equiv), toluene, 50 °C, 100% yield; ii) **16**, DCE, 50 °C, 19% (chromatographed).

2.4. Hydrolysis

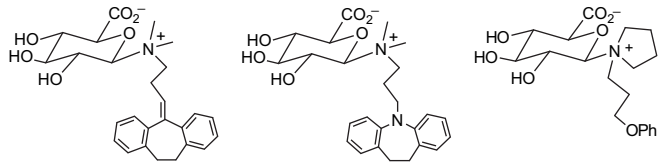
Finally, careful base hydrolysis released the zwitterionic N^+-glucuronides (Scheme 6). The final products are sufficiently base-stable to tolerate these conditions for a short time, but the optimum conditions depend on the aglycone. Two side reactions are possible: elimination of the 4-isobutyrate can occur (pathway A) or the aglycone can be liberated (pathway B). The elimination reaction was known from early work on degradation of glucuronic acid polymers and may also be observed in *O*-glucuronide synthesis.[36,37]



Scheme 6. Hydrolysis conditions. A: 10% w/v Na_2CO_3, MeOH; B: 1 M LiOH or NaOH, MeOH, −10 °C.

For amitriptyline and imipramine intermediates **14a** and **14b**, either 1 M NaOH or 1 M LiOH was satisfactory: some loss of the aglycone (Scheme 6, pathway B), but no elimination reaction, was observed under these conditions. When we used 10% w/v Na_2CO_3 we found that the elimination by-product was significant (Scheme 6, pathway A) and this proved more difficult to remove from the final product. The elimination reaction is a feature of the isobutyrates[36b] and is negligible when acetates are used; a characteristic alkene ^{1}H NMR signal (δ ca. 6.0, H-4) is observed for this by-product.

Conditions B could not be used for **17** owing to the more base-labile nature of the glycosidic bond: up to 80% loss of aglycone was observed. Instead, conditions A were satisfactory: up to 20% elimination was observed, but loss of aglycone was completely avoided. Finally the desired glucuronides **18** (16%), **19** (57%) and **20** (11% in three steps from **13**) were obtained in high purity.



Preparative reverse phase HPLC (aq MeCN, neutral conditions) was necessary to obtain highly pure product **20**, where elimination occurred: silica chromatography was acceptable for **18** and **19**, though losses are observed in either case. Nevertheless the method described will conveniently deliver pure product in 50–100 mg amounts.

3. Conclusions

In summary, we have described a promising new method for the synthesis of quaternary ammonium glucuronides from a stable, readily available hemiacetal sugar. The inherently low stability of this class of metabolites poses difficulties in both synthesis and pure product isolation. Glycosylamine formation using secondary amines, followed by quaternisation with a primary triflate, affords the protected precursors in good yields although there are stability issues with the intermediates. Successful hydrolysis can be achieved for both linear and cyclic quaternary esters: optimum conditions depend on the aglycone. The final products can be obtained in high purity using preparative HPLC. We are continuing to refine the hydrolysis and purification steps, but we believe the method described will be of great value in the pharmaceutical industry as a practical synthesis of quaternary ammonium glucuronide metabolites.

4. Experimental

4.1. General experimental methods

All organic solvents were anhydrous and of AR grade. Vacuum rotary evaporation was carried out at <30 °C. Analytical thin-layer chromatography was performed using Merck Kieselgel 60 F 254

silica plates; preparative column chromatography was performed on Merck 9385 silica gel. Optical rotations were measured on a Perkin Elmer model 343 polarimeter at 589 nm and 20 °C. Infrared spectra were obtained using a Jasco FT/IR-4200 instrument. Both ^{1}H and ^{13}C NMR spectra were recorded for the solvents noted using either Bruker 250 MHz or 400 MHz instruments (the latter operating at 100 MHz for ^{13}C observation) with tetramethylsilane as internal standard. Mass spectra in the chemical ionisation (CI) mode were obtained using a VG7070E mass spectrometer. Both low and high resolution electrospray mode (ES) mass spectra were obtained using a Micromass LCT mass spectrometer operating in the +ve or –ve ion mode as indicated. Elemental microanalysis was performed by Mr. Steve Apter (Liverpool). 3-[2,2,3,3-$^{2}H_4$] Trimethylsilyl propionate sodium salt (TSP), sodium dihydrogen phosphate and disodium hydrogen phosphate, were purchased from Sigma–Aldrich Company, Ltd (Gillingham, Dorset, UK). HPLC–NMR grade deuterium oxide ($^{2}H_2O$) was obtained from Goss Scientific Instruments (Essex, UK).

4.2. General method for the synthesis of protected quaternary ammonium glucuronides

To a solution of **13**[31] (0.50 g, 1.19 mmol) in toluene (5 mL) at 50 °C was added nortriptyline **7** or desipramine **8** (1.19 mmol) as a solution in toluene (5 mL). The reaction was heated to 50 °C under a nitrogen atmosphere for 24 h. The toluene was then removed in vacuo and the residue dissolved in pyridine (15 mL). The solution was cooled to 0 °C and placed under nitrogen. Acetic anhydride (0.24 g, 2.39 mmol) was then added slowly to the reaction mixture, which was left stirring at 0 °C for 4 h. The reaction was then quenched with satd aq $NaHCO_3$ until pH 7–8 was achieved: DCM (20 mL) was added and the organic layer was separated. The aqueous phase was then extracted with further DCM (3×50 mL) and the organic layers combined, washed with brine (50 mL) and water (2×50 mL) and dried over Na_2SO_4. Solvent was then removed in vacuo; pyridine residues were removed under high vacuum. The residue was then dissolved in dry DCM (8 mL) and evacuated with nitrogen: methyl triflate (0.39 g, 2.39 mmol) was then added to the stirred solution, which was left stirring for 17 h at 20 °C. The DCM was then removed in vacuo and the residue chromatographed using EtOAc, then 10:90 Pr^{i}OH:DCM.

4.2.1. Methyl(N-(2,3,4-tri-O-isobutyryl)-β-D-glucopyranuronato)-N,N-dimethyl-3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-1-propanammonium trifluoromethanesulfonate ***14a****.* Non-crystalline foam, yield 0.177 g (18%). Found: C, 57.8; H, 6.4; N, 1.4; $[M]^+$ *m/z* 678.3660; $C_{40}H_{52}NO_{12}SF_3$ requires C, 58.0; H, 6.3; N, 1.7%; $[C_{39}H_{52}NO_9]^+$ requires *m/z*, 678.3642; ^{1}H NMR [400 MHz, $(CD_3)_2SO$]: 1.00–1.06 [18H, m, 3×CH(*CH*$_3$)$_2$], 2.40–2.49 (3H, m, 3×*CH*(CH$_3$)$_2$), 2.50–2.58 (2H, br s, CHC*H*$_2$CH$_2$N$^+$), 2.73–2.92 (2H, br s, *CH*$_2$CH$_2$), 2.98–3.09 [6H, 4 s, N$^+$(*CH*$_3$)$_2$], 3.20–3.31 (4H, br s, *CH*$_2$CH$_2$), 3.34 (3H, s, CO_2CH_3), 3.62–3.71 (2H, m, CHCH$_2$*CH*$_2$N$^+$), 4.50–4.52 (1H, m, 5-H), 5.14–5.30 (1H, m, 3-H), 5.30–5.40 (1H, m, 4-H), 5.50–5.51 (1H, t, 2-H), 5.7–5.84 (2H, t, =*CH*CH$_2$CH$_2$N$^+$ and m, 1-H) and 7.08–7.30 (8H, m, Ar); ^{13}C NMR [100 MHz, $(CD_3)_2SO$]: 14.3, 18.3, 18.6, 18.7, 18.8, 18.9, 22.4, 23.1, 31.6, 33.4, 33.5, 33.6, 33.6, 48.9, 48.9, 53.2, 67.1, 67.8, 72.3, 72.8, 90.5, 126.3, 126.5, 127.6, 128.0, 128.4, 128.5, 128.6, 128.7, 130.5, 137.1, 139.1, 139.3, 140.4, 174.8, 175.1 and 175.4 (×2); *m/z* (ES +ve ion mode) 678 $[M]^+$ 100%.

4.2.2. Methyl(N-(2,3,4-tri-O-isobutyryl)-β-D-glucopyranuronato)-N,N-dimethyl-3-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-1-propanammonium trifluoromethanesulfonate ***14b****.* Non-crystalline foam, yield 0.158 g (16%). Found: $[M]^+$ *m/z* 681.376, $C_{38}H_{53}O_9N_2$ requires *m/z* 681.375; ^{1}H NMR (400 MHz, $CDCl_3$): 1.05–1.08 [18H, m, 3xCH(*CH*$_3$)$_2$], 2.1–2.2 (2H, m, NCH$_2$*CH*$_2$CH$_2$), 2.35–2.6 [3H, m, 3×*CH*(CH$_3$)$_2$], 2.95 (3H, s, N$^+$*CH*$_3$), 3.08 (3H, s, N$^+$*CH*$_3$), 3.18 (4H, br s, *CH*$_2$*CH*$_2$), 3.4–3.5 (1H, m, N*CH*$_2$CH$_2$CH$_2$), 3.55–3.65 (1H, m, N*CH*$_2$CH$_2$CH$_2$), 3.76 (3H, s, CO_2CH_3), 3.8–3.98 (2H, m, NCH$_2$CH$_2$*CH*$_2$N$^+$), 4.5–4.53 (1H, d, *J*=9.5 Hz, 5-H), 5.09–5.18 (1H, t, 4-H), 5.30–5.50 (2H, 2×t, 2-H+3-H), 5.60–5.65 (1H, d, *J*=8.6 Hz, 1-H) and 6.90–7.23 (8H, m, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): 18.5, 18.8, 19.0, 21.1, 32.5, 34.1, 34.2, 34.3, 46.8, 48.3, 49.0, 53.4, 60.9, 67.4 (4-C), 68.2 (3-C), 72.8 (2-C), 74.1 (5-C), 93.6 (1-C), 120.0, 123.9, 127.3, 130.7, 134.7, 147.3, 166.3, 175.1 and 175.9 (×2); *m/z* (ES +ve ion mode) 681 $[M]^+$ 100%.

4.3. Methyl [*N*-(2,3,4-tri-*O*-isobutyryl)-β-D-glucopyranuronato]-pyrrolidine 15

To a solution of **13**[31] (1.09 g, 2.6 mmol) in toluene (8 mL) under a nitrogen atmosphere was added pyrrolidine (0.26 mL, 0.22 g, 3.12 mmol). The reaction was heated to 50 °C for 6 h, then solvent and excess reagent were removed in vacuo to give the product **15** as an oil, which was sufficiently pure to use directly (1.23 g, 100%). Found: $[M+H]^+$ *m/z*, 472.256; $C_{23}H_{38}O_9N$ requires *m/z*, 472.255; ^{1}H NMR (400 MHz, $CDCl_3$): 0.98–1.2 (18H, m, 3×CH(*CH*$_3$)$_2$), 1.60–1.61 (4H, m, *CH*$_2$*CH*$_2$), 2.28–2.42 (3H, m, 3×*CH*(CH$_3$)$_2$), 2.68–2.84 (4H, m, *CH*$_2$N*CH*$_2$), 3.67 (3H, s, CO_2CH_3), 3.90–3.93 (1H, d, *J*=10.0 Hz, 5-H), 4.28–4.31 (1H, d, *J*=9.3 Hz, 1-H), 5.03–5.14 (2H, 2×t, 2-H+4-H) and 5.20–5.26 (1H, t, 3-H); ^{13}C NMR (100 MHz, $CDCl_3$): 17.7, 17.9, 17.9, 23.5, 32.8, 32.9, 32.9, 45.7, 51.5, 67.9, 68.7, 71.7, 73.0, 89.2, 174.3, 174.5, 174.9 and 181.6; *m/z* (ES +ve ion mode) 472 $[M+H]^+$ 100%.

4.4. (3-Phenoxypropyl)trifluoromethane sulfonate 16[35]

To a solution of 3-phenoxypropan-1-ol (0.21 g, 1.4 mmol) in DCM (2 mL) at 0 °C under N_2 was added 2,3,6-collidine (0.254 g, 2.1 mmol) followed by triflic anhydride (0.258 mL, 0.434 g, 1.54 mmol). The reaction was left at 0 °C for 2 h and then the solvent removed in vacuo. The residue was partitioned between ether (50 mL) and water (30 mL) and the organic layer separated. The organic layer was then successively washed with 1 M HCl (2×20 mL), satd $NaHCO_3$ (20 mL), water (30 mL), dried over $MgSO_4$ and evaporated to afford crude product **16** (0.119 g, 30%) as a foam, which was used directly: ^{1}H NMR (250 MHz, $CDCl_3$): 2.22–2.40 (2H, q, CH$_2$*CH*$_2$CH$_2$), 4.0–4.18 (2H, t, CH$_2$*CH*$_2$OAr), 4.68–4.90 (2H, t, $CF_3SO_2OCH_2$), 6.82–7.10 (3H, m, ArH), 7.26–7.41 (2H, m, ArH).

4.5. [β-D-Glucopyranuronato-*N*-1-(3-phenoxypropyl)]-pyrrolidinium-1-yl 20

To a solution of **15** (0.74 g, 1.57 mmol) in dry DCE (4 mL) under nitrogen was added **16** (0.49 g, 1.72 mmol) in DCE (4 mL). The reaction was heated to 50 °C for 24 h. The DCE was then removed in vacuo and the residue triturated with diethyl ether. The residue containing intermediate **17** (0.47 mmol) was without purification dissolved in methanol (8 mL), cooled to −10 °C, stirred and treated with 10% aq Na_2CO_3 (2 mL, 1.89 mmol). After addition the reaction was stirred at −10 °C for 2 h, then Amberlite H^+ resin was added to give a pH of 6.5. Methanol was removed in vacuo and the residue partitioned between water (50 mL) and DCM (50 mL). The aqueous phase was further extracted with DCM (3×50 mL), then evaporated to dryness to give an amorphous solid (0.365 g, 61%); by NMR analysis this material was 4:1 product: eliminated by-product (see Scheme 6). HPLC using a gradient of MeCN in H_2O under neutral conditions was used to purify the material further: **20** was thus obtained as a colourless foam (0.066 g, 11%); $[\alpha]_D^{293K}=-11.47$ (0.8 g mL^{-1} in MeOH); Found: $[MH]^+$ *m/z* 382.1871; $C_{19}H_{28}O_7N$ requires *m/z* 382.1866; ν_{max} cm^{-1} 2500–3500 (br s), 2973, 2916, 1611, 1486, 1443, 1412 (m),1092 (vs), 883 (m) and 742; ^{1}H NMR (600 MHz, CD_3OD): 2.08–2.27 (4H, m, CH$_2$*CH*$_2$*CH*$_2$CH$_2$), 2.28–2.46 (2H, m, N$^+$CH$_2$*CH*$_2$CH$_2$O), 3.46–3.53 (2H, m, 3-H + 4-H), 3.61–3.69

(1H, dt, CH_2 N^+CH_2), 3.71–3.79 (4H, m, CH_2 N^+CH_2, $N^+CH_2CH_2CH_2O$ and 5-H), 3.79–3.83 (1 H, t, 2-H), 3.92–4.11 (4H, m, $N^+CH_2CH_2CH_2O$, CH_2 N^+CH_2), 4.67–4.71 (1H, d, J=8.9 Hz, 1-H), 6.89–6.93 (3H, m, Ar) and 7.22–7.28 (2H, m, Ar); ^{13}C NMR (150 MHz, CD_3OD): 23.6, 24.8, 25.6, 59.4, 62.4, 64.7, 66.2, 72.2, 73.3, 79.1, 79.8, 96.3, 116.0, 122.6, 131.0, 160.4 and 175.5; m/z (ES +ve ion mode) 382 $[MH]^+$ 100%. For the NMR of intermediate **17**, see Supplementary data.

4.6. [β-D-Glucopyranuronato-(*N*,*N*-dimethyl-3-(10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5-ylidene))-1-propanammonium 18

To a solution of **14a** (0.114 g, 0.14 mmol) in MeOH (2 mL) was added 1 M NaOH (0.42 mL) at −10 °C with stirring. After 3 h at this temperature, TLC (5:3:2 EtOAc/IPA/H_2O) showed complete reaction: Amberlite H^+ resin (0.42 mmol) was added to give a pH of 7. The resin was then filtered and washed with methanol, and the methanol removed in vacuo: the residue was partitioned between DCM (30 mL) and water (30 mL), the aqueous phase was separated and extracted with more DCM (3×30 mL) and water was removed in vacuo to yield the crude product. The residue was then purified using reverse phase C_{18} silica gel, eluting with 100% H_2O then 10–25% MeCN in H_2O to afford the product **18** (0.064 g, 16%) as an amorphous glass; $[\alpha]_D^{293K}$=−5.80° (0.5 mg/mL in MeOH). Found: $[MH]^+$ m/z, 454.2227; $C_{26}H_{32}O_6N$ $[MH]^+$ requires m/z, 454.2230; ν_{max} cm^{-1} 2500–3500 (br s), 2970, 2916, 1611, 1485, 1447, 1407, 1094 (vs), 885 (m) and 743; ^{1}H NMR (600 MHz, CD_3OD at 323 K): 2.57–2.72 (2H, m, NCH_2CH_2CH), 2.78–3.0 (2H, br m, CH_2CH_2), 3.15 (3H, s, NCH_3), 3.19 (3H, s, NCH_3), 3.40–3.47 (3H, m, 3-H, NCH_2, CH_2CH_2), 3.51–3.62 (2H, br s, NCH_2, CH_2CH_2), 3.69–3.74 (2H, m, 2-H, 4-H), 3.88–3.98 (1H, br s, 5-H), 4.3–4.51 (1H, br s, 1-H), 5.73–5.82 (1H, t, $=CH$ CH_2) and 7.04–7.31 (8H, m, Ar); ^{13}C NMR [150 MHz, CD_3OD at 323 K]: 24.4, 33.2, 34.9, 64.2, 64.8, 71.5, 71.6, 77.8, 78.7, 79.0, 79.1, 94.7, 95.2, 124.9, 125.2, 127.3, 127.7, 128.8, 131.1, 131.4, 138.5, 140.7, 140.8, 141.6, 148.5 and 175.1 m/z (ES +ve ion mode) 454 $[MH]^+$, 100%.

4.7. β-D-Glucopyranuronato-(*N*,*N*-dimethyl-3-(10,11-dihydro-5*H*-dibenzo[b,f]azepin-5-yl)-1-propanammonium) 19

To a solution of **14b** (0.115 g, 0.14 mmol) in MeOH (2 mL) was added 1 M LiOH (0.42 mL) with stirring at −10 °C. After 2 h at this temperature, TLC (100% EtOAc) showed completion. The reaction was quenched with Amberlite H^+ resin (0.42 mmol) to give a pH of 7. The resin was then filtered and washed with methanol, and the methanol removed in vacuo: the residue was partitioned between DCM (30 mL) and water (30 mL). The aqueous phase was separated and extracted with more DCM (3×30 mL), then the water was removed in vacuo to yield crude product, which was purified by preparative HPLC using MeCN/H_2O affording **19** (0.064 g, 57%) as an amorphous glass; $[\alpha]_D^{293K}$=−18.25 (2 g mL^{-1} in MeOH); Found: C, 61.0; H, 7.0; N, 5.4; $[MH]^+$ m/z, 457.2343. $C_{25}H_{32}O_6N_2.2H_2O$ requires C, 61.0; H, 7.4; N, 5.7%; $C_{25}H_{33}O_6N_2$ requires $[MH]^+$ m/z 457.2339; ν_{max} cm^{-1} 2500–3500 (br s), 2920, 2850,1610, 1486, 1447, 1403 (m), 1094 (vs), 883 (w) and 755; ^{1}H NMR (400 MHz, CD_3OD): 2.0–2.16 (2H, m, $N^+CH_2CH_2CH_2$), 3.05 (3H, s, N^+CH_3), 3.09 (3H, s, N^+CH_3), 3.18 (4H, s, CH_2CH_2), 3.37–3.54 (3H, m, 3-H and $N^+CH_2CH_2CH_2$), 3.69–3.71 (2H, m, 2-H+4-H), 3.73–3.84 (2H, m, 5-H and $N^+CH_2CH_2CH_2$), 3.88–3.98 (1H, m, $N^+CH_2CH_2CH_2$), 4.51–4.55 (1H, d, J=8.9 Hz, 1-H), 6.92–6.97 (2H, m, Ar) and 7.11–7.20 (6H, m, Ar); ^{13}C NMR (100 MHz, CD_3OD): 22.4, 33.6, 48.6, 49,6, 49.7, 63.8, 71.9, 73.1, 78.9, 79.5, 95.8, 121.1, 124.5, 128.1, 131.5, 136.0, 149.5 and 175.3; m/z (ES +ve ion mode) 457 $[MH]^+$ 100%.

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Supplementary data

The supplementary data associated with this article can be found in the on-line version at doi:10.1016/j.tet.2009.10.113.

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