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Glucuronidation of steroidal alcohols using iodosugar and imidate donors

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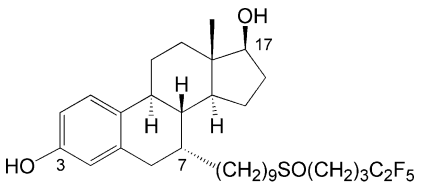
We report a study of the glucuronidation of a number of important steroidal secondary alcohols. The alcohols studied are androsterone **7**, epiandrosterone **8**, 17-acetoxy-androstane-3α,17β-diol **9**, 11α-hydroxyprogesterone **10**, and 3-benzoylestradiol **11**. These were first glucuronidated using the Schmidt trichloroacetimidate method with variations in acyl substituent (*viz.* derivatives **2** and **3**), Lewis acid catalyst and order of addition. The results are contrasted with those obtained using our recently described glycosyl iodide donor **4**, catalysed either by *N*-iodosuccinimide (NIS) or various metal salts.

Introduction

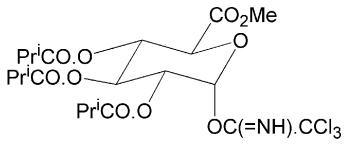
Glucuronides play a vital role in phase 2 metabolism, generally but not universally effecting detoxification.[1,2] Among the most important examples are the alkyl and aryl glucuronides of steroids, both hormonal and xenobiotic, which may be formed either directly from the parent steroid or from phase 1 hydroxylated metabolites. The efficient synthesis of steroidal glucuronides is therefore of importance, both to prepare analytical standards and for toxicological evaluation.

There has also been a resurgence of interest in steroid-based drugs, notably for breast cancer chemotherapy. In this context we synthesised some time ago putative metabolites of the antiestrogen, fulvestrant (ICI 182,780) **1**, including the 3- and 17-glucuronides.[3,4] During those studies we observed a significant order of addition effect in glucuronidation of the 17-hydroxy group of **1** which in that instance was crucial to an effective synthesis of the metabolite, and presented a few further examples of the 'inverse addition' method, which had previously[5] been noted for a fucosyl imidate donor.



1

We now present a detailed study of the glucuronidation of a set of steroidal secondary alcohols, employing the tri-isobutyryl **2** and tri-pivaloyl **3** trichloroacetimidates and our recently disclosed glycosyl iodide **4** as donors. As well as the intrinsic importance of the products as noted above, these reactions afford good tests of methodology. Glucuronates are well known to be poor glycosyl donors among carbohydrates[6] and the alcohols in question are of relatively low reactivity. The influences of acyl group, Lewis acid and order of addition on the reactivity of **2** and **3** are first discussed. Examples of the use of **4** are then presented.



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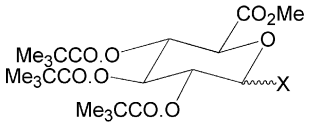

3 X = α–OC(=NH)CCl$_3$
4 X = α-I
5 X = β–O.OCCMe$_3$
6 X = α/β-OH

Discussion

The tri-isobutyryl imidate **2** was used extensively in our earlier studies[3,4] but at that time the corresponding tri-pivaloate **3** was unknown: subsequently two references to its use appeared[7,8] although without details of the preparation. We anticipated that the pivaloyl groups present in **3** should reduce still further the incidence of transacylation, which was a significant side reaction when using **2**[3,4] and was frequently the major reaction observed when using the corresponding tri-acetate.[9] No report on the use of **3** in steroidal glucuronidation had previously appeared.

Rather than proceed *via* the corresponding bromosugar[7] and incur an extra step, we treated the known tetra-pivaloate **5**[10] with hydrazinium acetate in DMF[11] and obtained clean, if slow, conversion to the hemiacetal **6**, which was isolated in 74% yield. In a recent report of the synthesis of a deuterated androsterone glucuronide,[12] treatment of **5** with dibutyl tin oxide[13] or tributyl tin methoxide,[14] *en route* to **6**, was reported to fail. Standard reaction of **6** with trichloroacetonitrile and K_2CO_3 in DCM[15] led to the desired imidate **3** in 87% yield as a single α-anomer.

Our earlier studies on **1** were arguably a special case in that **1** contains a sulfoxide group that binds Lewis acids such as BF_3 very strongly. Here we employed androsterone **7**, epiandrosterone **8**, 3-*O*-acetylandrostane-3α,17β-diol **9**, 11α-hydroxyprogesterone **10**, and 3-*O*-benzoylestradiol **11** as acceptors.

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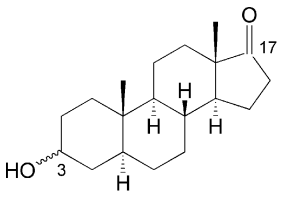
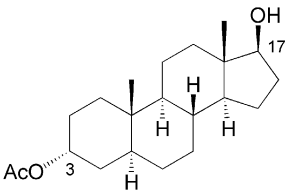


7 3α-OH
8 3β-OH



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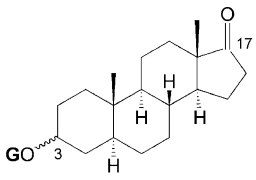
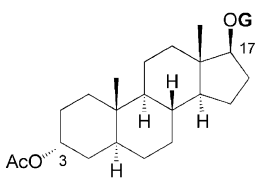


12, 22 3α-OH
13, 23 3β-OH



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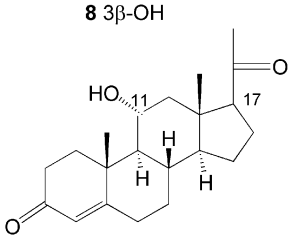

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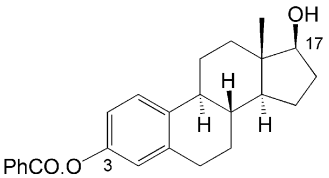

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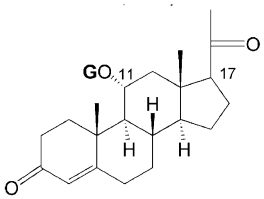
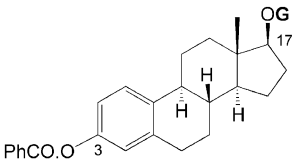
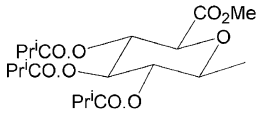


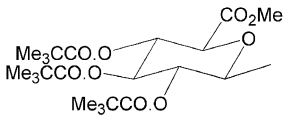
15, 24



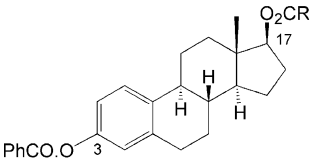
16, 17



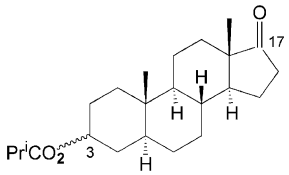
G (structures 12-16)



G (structures 17, 22 , 23 and 24)



18 R = Pr^i
19 R = Bu^t



20 3α- O_2CPr^i
21 3β- O_2CPr^i

These steroids contained at least one carbonyl oxygen atom capable of coordinating to Lewis acids. We first studied the reaction of tri-isobutyryl imidate **2** under both 'normal' (Method A; *viz.* adding Lewis acid catalyst to the mixture of alcohol and **2**) and 'inverse' conditions (Method B; *viz.* adding imidate to a mixture of alcohol and catalyst).

Imidate reactions

Using firstly $BF_3{\cdot}Et_2O$ (1.1 equiv.) as catalyst, and employing 1.0 equiv. of imidate **2** in each case, glucuronide esters **12**–**16** were obtained with the results shown in Table 1. The results agree with our earlier observations[3,4] that superior yields were obtained using the inverse addition procedure, although the absolute yields of glucuronide conjugate were not always impressive. Transacylation was observed in these experiments but always as a *minor* pathway in the 'inverse' mode. Clearly 3-*O*-benzoylestradiol **11** is the exception here – for reasons that are unclear.

We then proceeded to study the influence of two other significant variables, namely the Lewis acid used and the acyl substituent present in the imidate, on steroidal glucuronidation. In these further experiments (Table 2) the yields of β-glucuronide and transacylated product were both monitored, while studying initially just one substrate, namely 3-*O*-benzoylestradiol **11**. As

Table 1 Yields (%) from glucuronidation of alcohols **7**–**11** using tri-isobutyryl imidate **2** (1.0 equiv.) with 1.1 equiv. catalyst. Method A, normal mode; Method B, inverse mode (see text)

Steroidal alcohol	Imidate	Catalyst	Glucuronide	Yield by Method A	Yield by Method B
7	**2**	$BF_3{\cdot}Et_2O$	**12**	16	41
8	**2**	$BF_3{\cdot}Et_2O$	**13**	34	54
9	**2**	$BF_3{\cdot}Et_2O$	**14**	13	23
10	**2**[a]	$BF_3{\cdot}Et_2O$	**15**	31	77
11	**2**	$BF_3{\cdot}Et_2O$	**16**	29	28

[a] Using 1.5 equiv. of **2**, ref. 4.

Table 2 Yields (%) from glucuronidation of alcohols **7**, **8** and **11**: Lewis acid and imidate variation. Method A, normal mode; Method B, inverse mode (see text). Some data from Table 1 are included

	Imidate **2**					Imidate **3**				
		$BF_3{\cdot}Et_2O$[a]		TMSOTf[b]			$BF_3{\cdot}Et_2O$[a]		TMSOTf[b]	
Alcohol	Product	A	B	A	B	Product	A	B	A	B
11	Glucuronide ester **16**	29	28	19	11	Glucuronide ester **17**	45	44	65	52
	Transacyl product **18**			60	49	Transacyl product **19**	19	2	15	13
7	Glucuronide ester **12**	16	41	33	34					
	Transacyl product **20**			40[c]	40					
8	Glucuronide ester **13**	34	54	62	52					
	Transacyl product **21**			23	11					

[a] Optimum amount of $BF_3{\cdot}Et_2O$ is 1.1 equiv. for **2**, 0.5 equiv. for **3**. [b] 0.25 equiv. used. [c] Approx. yield, some additional carbohydrate residue present.

noted above, this particular substrate showed little variability between the 'normal' and 'inverse' modes of addition.

The use of TMSOTf in conjunction with imidate **2** was not impressive in this case, and transacylation, giving the 17-isobutyrate **18**, was the major pathway in both normal and inverse modes, especially the former. As anticipated, the tripivaloyl imidate **3** gave a much better product profile, and using either $BF_3{\cdot}Et_2O$ or TMSOTf the β-glucuronide ester **17** was easily the major product, with <20% transacylation. Minimal transacylation (*ca.* 2%) was observed in the $BF_3{\cdot}Et_2O$ 'inverse' mode, though the yield of **17** was no better than in the 'normal' mode. The best yield of **17** was obtained with TMSOTf in the normal mode, though here the yield of **19** was higher.

Interestingly, glucuronidation : transacylation ratios also varied with the *stereochemistry* of the alcohol used, other things being equal. The axial/equatorial alcohol pair, androsterone **7** and epiandrosterone **8**, illustrate this point clearly (Table 2). With the axial alcohol **7**, transacylation was the major pathway when using **2** with TMSOTf though the desired conjugate **12** could be obtained in acceptable yield. In contrast, **8** gave a very satisfactory yield of **13** using the TMSOTf 'normal' or 'inverse' procedure with **2**, transacylation being a minor pathway, especially in the 'inverse' mode – a striking contrast to the behaviour of **11**. Possibly, the different behaviour of $BF_3{\cdot}Et_2O$ and TMSOTf depends on the relatively weaker binding of the latter by the substrate. In the inverse addition mode, this could lead to the concentration of 'free' catalyst being less affected for TMSOTf.

Finally, removal of trichloroacetamide from the glucuronide products may be a practical problem when using chromatographic separation, as these steroidal derivatives are fairly lipophilic. On a larger scale, where crystallisation becomes feasible, this should not be a concern.

Glycosyl iodide reactions

We recently reported the preparation and crystal structure[16] of glycosyl iodide **4** and noted that it was a good, versatile donor for the glucuronidation of a wide range of alcohols including carbohydrate acceptors and two steroidal alcohols.[17] Treatment with NIS/I_2 followed by TMSOTf was shown to be a reliable procedure. Thus (Table 3) when epiandrosterone **8** was treated with glycosyl iodide **4** (1.1 equiv.), NIS (1.1 equiv.) and I_2 (0.25 equiv.), complete reaction of **4** was observed in 4 h; addition of TMSOTf (0.5 equiv.) at 0 °C followed by warming to 20 °C over 2 h, to rearrange some orthoester initially formed, led to a good yield (65%) of the desired glucuronide **23**, entry 1. This was very similar to the yield obtained using the imidate **2** with TMSOTf in this case, Table 2. Glucuronidation of 3-*O*-benzoylestradiol **11** by the same procedure afforded the desired conjugate **17** in good yield (70%), entry 4, again very similar to the imidate method. It is important to note, however, that the use of **4** leads to negligible transacylation.

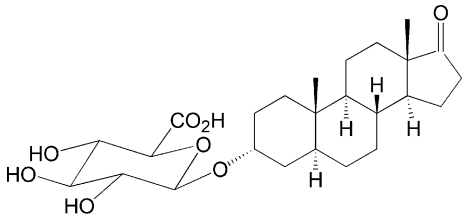
Careful examination of the ^1H NMR spectra of the above products revealed that the products were contaminated with small amounts of α-anomers (β : α = *ca.* 25 : 1). The mechanism is not wholly clear: we suggest that small amounts of the β-glycosyl iodide may be formed by iodide exchange on **4** followed by S_N2 reaction of the alcohol from the α-face. There is no evidence that the glucuronide products can equilibrate under the reaction conditions. Preparatively, such small amounts of the α-anomers are not a concern as they are easily removed by recrystallisation.

The glycosyl iodide **4** will also react with alcohols under catalysis by various metal salts (other than traditional Koenigs–Knorr heavy metals, which were not used here). In our earlier communication[17] we identified $FeCl_3$–I_2 and CuCl–I_2 (using 1.1 equiv. of the metal salt in each case) as the most promising catalysts among a large number screened: neither metal salt was effective in the absence of iodine.[18] Our results using **4** with alcohols **7**, **8** and **11** in this mode are also shown in Table 3, entries 2, 3, 5, 6 and 7. The yields are at least comparable to those obtained by the imidate method, using **2** or **3**. In one case, entry 6, we used an excess of iodosugar **4** in order to react the steroid completely; this run afforded a 77% yield.

Complete anomeric stereocontrol is more difficult to achieve here, with significant amounts (up to 10%) of α-glucuronides being formed in some cases (entries 2 and 5). The results are in contrast to those seen with primary alcohol acceptors, where essentially complete β-selectivity could be achieved.[17] The metal salt does make a difference, and from results available to date using this mode it appears that CuCl may prove advantageous, *viz.* in the reactions of **7** and **8** (entries 7 and 3, respectively) where the β-selectivity for products **22** and **23** was excellent.

In the case of 11α-hydroxyprogesterone **10**, the iodosugar **4** was not an effective glucuronidating agent when activated by NIS–I_2 or MX_n–I_2, probably owing to reaction of the enone function. However, modifying a procedure of Gutman's[19] used for glucuronidation with a bromosugar, we heated **10** with **4** in the presence of $ZnCl_2$, obtaining a modest yield (15%) of the desired glucuronide **24** as the β-anomer only. We have not yet attempted to optimise this procedure: as noted earlier, **10** reacted efficiently with imidate **2**.

The only significant drawback to the use of **3** (or **4**) lies in the sluggish hydrolysis of the resulting pivaloates using conventional base hydrolysis (NaOH–aqueous alcohols); low solubility of the esters is also a problem. A more reliable procedure is to use $Et_4N^{+-}OH$,[20] which leads to a clear solution from the start and gives a workable rate of reaction at 20 °C. Following acidification by Amberlyst 120 (H^+) and brief reversed-phase chromatography, the free glucuronide could be isolated in good yield and excellent purity, *e.g.* androsterone glucuronide **25**,[21] whose preparation is given in detail (see the Experimental section).



25

Table 3 Glucuronidation of steroidal alcohols (1.5 equiv., except entry 6) using glycosyl iodide **4** under various conditions

Entry	Steroidal alcohol	Product	Conditions	Yield (%)	β : α Ratio
1	**8**	**23**	NIS, I_2, TMSOTf	65	96 : 4
2	**8**	**23**	$FeCl_3/I_2$	42	90 : 10
3	**8**	**23**	$CuCl/I_2$	64	β only
4	**11**	**17**	NIS, I_2, TMSOTf	70	96 : 4
5	**11**	**17**	$FeCl_3/I_2$	68	90 : 10
6[a]	**11**	**17**	$FeCl_3/I_2$	77[a]	95 : 5
7	**7**	**22**	$CuCl/I_2$	60	β only

[a] Using excess **4** (1.5 equiv.) rather than excess alcohol.

Conclusions

Re-examination of the 'inverse addition effect' previously reported[3,4] in the reaction of tri-*O*-isobutyryl imidate **2** with steroidal alcohols has shown that the situation is more complex than we first thought. Although the inverse method is generally superior when using $BF_3{\cdot}Et_2O$ catalysis, affording better yields and reduced transacylation, there is no advantage in yield with the inverse procedure when using TMSOTf. Nevertheless, the amount of transacylated product is consistently less when using the inverse procedure, with either catalyst.

The tri-isobutyryl imidate **2** gives satisfactory results with androsterone **7** and epiandrosterone **8**, but the tri-pivaloyl imidate **3** is the reagent of choice for 3-*O*-benzoylestradiol **11**: transacylation is significantly reduced compared to **2** with either catalyst.

Imidates **2** and **3** and iodide **4** are efficient donors for the β-glucuronidation of a range of steroidal secondary alcohols. Ketone and ester functional groups in the steroid are tolerated: yields of 60–70% are obtained *without* using large excesses of the donor. Traditional heavy metal catalysts are unnecessary when using **4** – this is a distinct advantage over the corresponding glycosyl bromide. Transacylation is low using **3** and negligible using **4**. While **3** gives pure β-products, it is difficult to exclude completely small amounts of α-products when using **4** but in both the NIS-I_2–TMSOTf and CuCl–I_2 modes they can be kept below 5%. On the other hand, **4** is accessible in fewer steps than **3**, namely one rather than two, from precursor **5**.

We believe that the above procedures offer reliable methods for glucuronidation of steroidal alcohols and will be of value to workers in that area. In a wider context, the glycosyl iodide **4** has shown clear potential in reacting efficiently with relatively poor acceptors and increases the range of stable, versatile glycosyl donors.

Experimental

Organic extracts were washed finally with saturated aqueous NaCl and dried over anhydrous Na_2SO_4 prior to rotary evaporation at <30 °C. Analytical thin-layer chromatography was performed using Merck Kieselgel 60 F 254 silica plates. Preparative column chromatography was performed on Merck 938S silica gel. Rotations were measured at 20 °C on an Optical Activity polarimeter operating at the wavelength of the sodium D-line. Infra-red spectra were recorded using a Perkin Elmer RX1 FTIR instrument, for the physical forms noted. Unless otherwise stated, ^{1}H and ^{13}C NMR spectra were recorded on $CDCl_3$ solutions using either Bruker 250 or 400 MHz (100 MHz for ^{13}C) instruments with tetramethylsilane as internal standard. Mass spectra in the chemical ionisation (CI) mode were obtained using a VG7070E mass spectrometer; for the electrospray (ES) mode, a Micromass LCT spectrometer was used for both low and high resolution spectra, operating in the +ve or –ve mode as stated.

Methyl 2,3,4-tri-*O*-pivaloyl-α,β-D-glucopyranuronate 6

A solution of tetra-pivaloate **5**[9] (3.30 g, 5.5 mmol) in DMF (25 mL) was stirred at −10 °C (ice–MeOH bath) and treated with acetic acid (0.46 g, 7.7 mmol) and hydrazine monohydrate (0.385 g, 7.7 mmol). The solution was allowed to warm to 20 °C, and after 74 h reaction appeared complete by TLC; 5% aqueous citric acid was added and the product was extracted with Et_2O (3 × 25 mL). The combined organic extracts were washed with water (2×) and brine, dried ($MgSO_4$) and evaporated to give an oil which was purified by chromatography on silica, eluting with 33% EtOAc–hexane. Appropriate fractions were evaporated to give **6** as white crystals (1.90 g, 75%), mp 114–116 °C. Found: C, 57.3; H, 8.0; MNa^+, 483.2230. $C_{22}H_{36}O_{10}$ requires C, 57.4; H, 7.9%; $C_{22}H_{36}O_{10}Na$ requires *m/z* 483.2206; $[\alpha]_D^{20}$ +50° (*c* = 2, $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 3450 (br), 1765 (sh), 1745, 1350 (sh) and 1280; δ_H (400 MHz), major (α-) anomer, 1.10–1.20 (27 H, 3 s, 3 × Me_3C), 3.73 (3 H, s, CH_3O), 4.59 (1 H, d, *J* = 10.2 Hz, 5-H), 4.89 (1 H, dd, *J* = 10.1 and 3.8 Hz, 2-H), 5.22 (1 H, t, *J* = 9.8 Hz, 4-H), 5.57 (1 H, approx. t, d, *J* = 3.5 Hz on D_2O exch., 1-H) and 5.67 (1 H, t, *J* = 9.7 Hz, 3-H); the minor (β-) anomer is distinguished principally by δ_H 3.74 (3 H, s, CH_3O), 3.94 (1 H, d, *J* = 10.0 Hz, 5-H), 4.96 (1 H, dd, *J* = 9.5 and 8.0 Hz, 2-H), 5.26 (1 H, t, *J* = 9.7 Hz, 4-H) and 5.42 (1 H, t, *J* = 9.5 Hz, 3-H); the 1-H of the β-anomer is obscured; δ_C (100 MHz, both anomers): 27.36, 27.40, 27.45, 27.51, 39.08, 39.20, 53.07, 53.19, 68.42, 69.18, 69.72, 71.37, 71.49, 73.17, 73.30, 90.48, 96.20, 167.94, 169.01, 177.04, 177.15, 177.30, 177.40, 178.00 and 178.40; *m/z* (ES +ve mode) 483 (MNa^+, 100%).

Methyl 1-*O*-trichloroacetimidoyl-2,3,4-tri-*O*-pivaloyl-α-D-glucopyranuronate 3

Trichloroacetonitrile (1.47 mL, 14.6 mmol) and anhydrous K_2CO_3 (2.018 g, 14.6 mmol) were added to a solution of hemiacetal **6** (1.00 g, 2.17 mmol) in anhydrous CH_2Cl_2 which was stirred at 20 °C. After 22 h, when reaction appeared complete by TLC, the reaction mixture was directly filtered through a pad of silica, eluting with hexane. Appropriate fractions were evaporated to give a pale yellow oil (1.213 g) which upon recrystallisation from hexane afforded **3** as white crystals (1.146 g, 87%), mp 104–106 °C. Found: C, 47.5; H, 6.0; N, 2.3; MNa^+, 626.1295. $C_{24}H_{36}Cl_3NO_{10}$ requires C, 47.6; H, 6.0; N, 2.3%; $C_{24}H_{36}Cl_3NO_{10}Na$ requires *m/z* 626.1302; $[\alpha]_D^{20}$ +70° (*c* = 2, $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 3340 (sharp), 1765, 1745, 1675, 1485 (sh), 1280, 1245 (w), 1206 (m) and 1130; δ_H (400 MHz), 1.13, 1.14 and 1.16 (27 H, 3 s, 3 × Me_3C), 3.72 (3 H, s, CH_3O), 4.50 (1 H, d, *J* = 10.3 Hz, 5-H), 5.21 (1 H, dd, *J* = 10.2 and 3.6 Hz, 2-H), 5.33 (1 H, t, *J* = 10.1 Hz, 4-H), 5.71 (1 H, t, *J* = 10.0 Hz, 3-H), 6.66 (1 H, d, *J* = 3.6 Hz, 1-H) and 8.72 (1 H, br s, NH); δ_C (100 MHz): 27.35, 27.44, 39.08, 53.25, 68.88, 69.06, 69.68, 71.02, 90.89, 92.77, 160.71, 167.57, 176.97, 177.13 and 177.40; *m/z* (ES +ve mode) 626 (MNa^+ for $^{35}Cl_3$, 100%).

Methyl 1-deoxy-1-iodo-2,3,4-tri-*O*-pivaloyl-α-D-glucopyranuronate 4

A solution of tetra-pivaloate **5**[9] (2.72 g, 5 mmol) in MeCN (5 mL) was heated under N_2 at 50 °C with iodotrimethylsilane (1.07 mL, 1.5 equiv.) for 2.75 h. Ester **5** had then disappeared by TLC, so the reaction mixture was cooled, diluted with EtOAc (25 mL), washed with 10% aqueous $Na_2S_2O_3$ (20 mL), saturated aqueous $NaHCO_3$, water and evaporated to a gum. Brief filtration through a pad of silica (5 g), eluting with hexane, afforded on evaporation the glycosyl iodide **4** as colourless crystals (2.59 g, 91%), mp 102–103 °C (from hexane). Found: C, 46.4; H, 6.25; MNa^+, 593.1230. $C_{22}H_{35}IO_9$ requires C, 46.3; H, 6.2%; $C_{22}H_{35}IO_9Na$ requires *m/z*, 593.1224; $[\alpha]_D^{20}$ +195° (*c* = 2.35, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3020, 2980, 1745 (vs), 1480 and 630; δ_H (400 MHz): 1.13, 1.18 and 1.20 (27 H, 3 s, 3 × Me_3C), 3.74 (3 H, s, CH_3O), 4.27 (1 H, dd, *J* = 9.8 and 4.4 Hz, 2-H), 4.37 (1 H, d, *J* = 10.4 Hz, 5-H), 5.31 (1 H, t, *J* = 10.0 Hz, 4-H), 5.61 (1 H, t, *J* = 9.6 Hz, 3-H) and 7.01 (1 H, d, *J* = 4.4 Hz, 1-H); δ_C (100 MHz): 27.05, 27.13, 38.57, 38.75, 38.80, 53.04, 67.88, 69.96, 70.46, 71.27, 75.04, 166.71, 176.63, 176.66 and 176.95; apparently one of the *t*-Bu methyl sets overlaps, though all the carbonyl Cs are distinguished; *m/z* (ES +ve mode) 593 (MNa^+, 100%).

Typical imidate glucuronidation

A. 'Normal' procedure. A solution of 3-*O*-benzoylestradiol **11** (0.190 g, 0.5 mmol) in 1,2-dichloroethane (DCE, 3 mL) was stirred over freshly activated 4 Å molecular sieves at 20 °C under N_2 for 1 h. A solution of imidate **3** (0.363 g, 0.6 mmol, 1.2 equiv.) in DCE (5 mL) was added, and after stirring for a further 1 h the solution was cooled to 0 °C and TMSOTf (0.023 mL, 0.0125 mmol, 0.25 equiv.) was added. After 1.5 h, and allowing

the mixture to warm to 20 °C, no imidate could be detected (TLC) and the reaction was quenched by addition of saturated aqueous $NaHCO_3$ and extracted with EtOAc (two portions). The combined organic extracts were washed with H_2O, brine, dried (Na_2SO_4) and evaporated to give a semi-solid that was chromatographed on silica, eluting with 25% EtOAc–hexane. Evaporation of appropriate fractions afforded the product **17** (0.272 g, 65%) identical to that obtained by the NIS-I_2–TMSOTf plus iodosugar procedure (characterisation below). Compound **16** was similarly prepared. Alternatively, $BF_3{\cdot}OEt_2$ (0.5 equiv.) was added instead of TMSOTf in the above procedure.

B. 'Inverse' procedure. A solution of **11** and the appropriate Lewis acid, $BF_3{\cdot}OEt_2$ or TMSOTf, was stirred in DCE at 20 °C under N_2 for 2 h, all quantities as above. After cooling to −10 °C a solution of the imidate **2** or **3** in DCE was added dropwise over 10 min with continued stirring. The reaction was allowed to warm to ambient temperature over 2 h: when no imidate could be seen it was worked up and the crude product chromatographed as above to give the pure glucuronide **16** or **17**.

Typical glucuronidation using glycosyl iodide 4: NIS procedure

A solution of 3-*O*-benzoylestradiol **11** (0.107 g, 0.29 mmol) and iodosugar **4** (0.107 g, 0.19 mmol) in anhydrous DCE (1 mL) was stirred with freshly activated 3 Å molecular sieves (0.8 g) under N_2 at 20 °C for 1 h. After cooling to 0 °C, NIS (0.060 g, 0.27 mmol) and I_2 (0.018 g, 0.07 mmol) were added and the mixture was allowed to warm to 20 °C over 2 h. After 5 h, when no **4** could be detected by TLC, the mixture was cooled to −10 °C and TMSOTf (0.025 mL) was added. The solution was again allowed to warm to 20 °C and after a total of 6 h 10% aqueous $Na_2S_2O_3$ (10 mL) was added followed by extraction with EtOAc (2 × 10 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ and water, followed by evaporation to give a yellow gum (0.208 g). Chromatography, eluting with 10% EtOAc–hexane, then 15%, afforded the product **17** (0.109 g, 70%) with spectroscopic data as given below. About 4% of the α-glucuronide was also present, distinguished by signals at δ_H 4.38 (d, $J = 10.2$ Hz, 5′-H), 4.85 (dd, $J = 10.1$ and 3.8 Hz, 2′-H), 5.22 (t, $J = 9.8$ Hz, 4′-H) and 5.61 (t, $J = 9.7$ Hz, 3′-H); the 1-H of the α-anomer is hidden under another signal.

Typical glucuronidation using glycosyl iodide 4: metal salt procedure

Epiandrosterone **8** (0.084 g, 0.29 mmol) and CuCl (0.022 g, 0.22 mmol) were stirred together in anhydrous DCE (0.9 mL) over fresh 3 Å MS (0.8 g) under N_2 at 20 °C for 1 h. Iodosugar **4** (0.107 g, 0.19 mmol) was added and, after stirring for a further 1 h, the mixture was cooled to 0 °C and I_2 (0.076 g, 0.30 mmol) was added. The mixture was allowed to regain 20 °C slowly and stirred for a further 20 h, then partitioned between 10% aqueous $Na_2S_2O_3$ (10 mL) and EtOAc (2 × 10 mL). The combined organic extracts were washed with water and evaporated to give a white semi-solid (0.180 g). Chromatography, eluting with 15–25% EtOAc–hexane, afforded the product **23** (0.088 g, 64%) with spectroscopic data as given below.

Characterisation of new steroid glucuronic acid conjugates

Androsterone glucuronide ester (tri-isobutyrate) 12. Found: MNa^+, 713.3893. $C_{38}H_{58}O_{11}Na$ requires m/z, 713.3877; $[\alpha]_D^{20}$ +15° ($c = 2$, $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 1755 (vs), 1730 (sh), 1345 (w), 1191 and 1154; δ_H (400 MHz) 0.77, 0.84 (6 H, 2 s, 18- and 19-CH_3s), 0.95–1.05 (1 H, m), 1.05–1.12 [18 H, m, 3 × $CH(CH_3)_2$], 1.20–1.30 (6 H, m), 1.35–1.70 (10 H, m), 1.70–1.80 (2 H, m), 1.85–1.95 (1 H, m), 2.00–2.10 (1 H, m), 2.35–2.45 (1 H, m), 2.40–2.55 [3 H, m, 3 × $CH(CH_3)_2$], 3.72 (3 H, s, CH_3O), 3.94 (1 H, m, 3-H), 4.02 (1 H, d, $J = 9.75$ Hz, 5′-H), 4.62 (1 H, d, $J = 7.9$ Hz, 1′-H), 5.06 (1 H, dd, $J = 7.9$ and 9.5 Hz, 2′-H), 5.22 and 5.33 (2 H,2 t, $J = 9.5$ Hz, 3′-H + 4′-H); δ_C (100 MHz) 11.80, 14.20, 19.18, 19.44, 20.43, 22.11, 25.65, 28.34, 31.05, 31.97, 32.77, 34.19, 34.21, 34.30, 34.50, 35.40, 36.23, 39.67,48.18, 50.36, 51.88, 52.95, 53.07, 54.14, 54.72, 69.75, 71.40, 72.22, 73.07, 73.29, 74.74, 99.59, 120.55, 164.00, 167.77, 175.41, 175.68 and 176.38; m/z (ES +ve mode) 713 (MNa^+, 100%).

Epiandrosterone glucuronide ester (tri-isobutyrate) 13. Found: MNa^+, 713.3864. $C_{38}H_{58}O_{11}Na$ requires m/z, 713.3877; $[\alpha]_D^{20}$ +10° ($c = 2$, $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 1770, 1750, 1730, 1330 (w), 1205 and 1145; δ_H (400 MHz) 0.65–0.75 (1 H, m), 0.79, 0.86 (6 H, 2 s, 18- and 19-CH_3s), 1.05–1.15 [18 H, m, 3 × $CH(CH_3)_2$], 1.15–1.30 (7 H, m), 1.35–1.85 (10 H, m), 1.85–2.00 (2 H, m), 2.00–2.10 (1 H, m), 2.30–2.40 (1 H, m), 2.40–2.55 [3 H, m, 3 × $CH(CH_3)_2$], 3.55–3.65 (1 H, m, 3-H), 3.73 (3 H, s, CH_3O), 4.06 (1 H, d, $J = 9.8$ Hz, 5′-H),4.70 (1 H, d, $J = 7.9$ Hz, 1′-H), 5.00 (1 H, dd, $J = 7.9$ and 9.5 Hz, 2′-H), 5.21 and 5.32 (2 H, 2 t, $J = 9.5$ Hz, 3′-H + 4′-H); δ_C (100 MHz) 12.50, 14.10, 19.11, 19.14, 19.30, 20.83, 22.11, 28.83, 29.46, 31.21, 31.92, 34.14, 34.18, 34.27, 34.73, 35.39, 36.08, 36.17, 37.20, 44.99, 48.13, 51.77, 53.08, 54.81, 69.68, 71.33, 72.10, 73.06, 92.47, 99.69, 163.93, 167.76, 175.45, 175.63 and 176.34; m/z (ES +ve mode) 713 (MNa^+, 100%).

3-*O*-Acetylandrostane-3α,17β-diol glucuronide ester (tri-isobutyrate) 14. Found: C, 64.9; H, 8.4; MNa^+, 757.4159. $C_{40}H_{62}O_{12}$ requires C, 65.4; H, 8.5%; $C_{40}H_{62}O_{12}Na$ requires m/z, 757.4139; $[\alpha]_D^{20}$ +5° ($c = 2$, $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 1755, 1740, 1350 (w), 1270, 1240, 1190 and 1160; δ_H (400 MHz) 0.70, 0.79 (6 H, 2 s, 18- and 19-CH_3s), 0.85–1.00 (1 H, m), 1.05–1.15 [18 H, m, 3 × $CH(CH_3)_2$], 1.20–1.30 (7 H, m), 1.35–1.70 (10 H, m), 1.70–1.80 (2 H, m), 1.90–2.05 (2 H, m), 2.04 (3 H, s, CH_3CO), 2.45–2.50 [3 H, 3 m, 3 × $CH(CH_3)_2$], 3.56 (1 H, t, $J = 8.4$ Hz, 17-H), 3.73 (3 H, s, CH_3O), 4.01 (1 H, d, $J = 9.7$ Hz, 5′-H), 4.61 (1 H, d, $J = 7.9$ Hz, 1′-H), 4.96 (1 H, m, steroidal 3-H), 5.00–5.10 (1 H, dd, $J = 7.9$ and 9.3 Hz, 2′-H), 5.23 and 5.29 (2 H, 2 t, $J = 9.4$ Hz, 3′-H + 4′-H); δ_C (100 MHz) 11.39, 11.68, 19.14, 19.19, 19.47, 20.70, 21.90, 23.68, 26.48, 28.59, 29.11, 31.82, 33.28, 33.32, 34.19, 34.22, 34.31, 35.62, 36.26, 37.98, 40.47, 43.49, 51.24, 53.07, 54.72, 69.74, 70.46, 71.56, 72.23, 73.12, 90.32, 101.83, 167.75, 171.02, 175.35, 175.61 and 176.36; m/z (ES +ve mode) 757 (MNa^+, 100%) and 773 (MK^+, 15%).

3-*O*-Benzoylestradiol glucuronide ester (tri-isobutyrate) 16. Found: C, 67.9; H, 7.3; MNa^+, 799.3648. $C_{44}H_{56}O_{12}$ requires C, 68.0; H, 7.3%; $C_{44}H_{56}O_{12}Na$ requires m/z, 799.3669; $[\alpha]_D^{20}$ +13.3° ($c = 1.5$, $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 1750, 1730, 1600 (w),1580 (w), 1500 (sh), 1160 and 1080; δ_H (400 MHz) 0.76 (3 H, s, 18-CH_3), 1.07–1.17 [18 H, m, 3 × $CH(CH_3)_2$], 1.20–1.50 (6 H, m), 1.65–1.80 (2 H, m), 1.80–2.00 (2 H, m), 2.00–2.10 (1 H, m), 2.15–2.35 (2 H, m), 2.48–2.52 [3 H, 3 m, 3 × $CH(CH_3)_2$], 2.83 (2 H, m), 3.68 (1 H, m, 17-H), 3.75 (3 H, s, CH_3O), 4.04 (1 H, d, $J = 9.8$ Hz, 5′-H), 4.67 (1 H, d, $J = 7.8$ Hz, 1′-H), 5.10 (1 H, dd, $J = 7.8$ and 9.3 Hz, 2′-H), 5.25, 5.32 (2 H, 2 t, $J = 9.4$ Hz, 3′-H + 4′-H), 6.92 (1 H, d, $J = 2.3$ Hz, 4-H), 6.97 (1 H, dd, $J = 8.4$ and 2.3 Hz, 2-H), 7.32 (1 H, d, $J = 8.4$ Hz, 1-H), 7.50 (2 H, m, ArH), 7.62 (1 H, m, ArH) and 8.19 (2 H, m, ArH); δ_C (100 MHz) 11.62, 18.74, 18.79, 18.84, 19.12, 27.02, 28.82, 29.55, 33.86, 33.98, 37.51, 38.26, 43.37, 44.15, 49.89, 52.73, 69.38, 71.20, 71.88, 72.78, 89.78, 95.01, 96.20, 101.49, 118.70, 121.65, 126.40, 128.54, 129.81, 130.16, 133.47, 137.87, 138.32, 148.78, 165.44, 167.36, 175.02, 175.26 and 176.01; m/z (ES +ve mode) 799 (MNa^+, 100%) and 751 (10%).

3-*O*-Benzoylestradiol glucuronide ester (tri-pivaloate) 17. Mp 127–128 °C. Found: C, 69.1; H, 7.5; MNa^+, 841.4138. $C_{47}H_{62}O_{12}$ requires C, 68.9; H, 7.6%; $C_{47}H_{62}O_{12}Na^+$ requires m/z, 841.4139; $[\alpha]_D^{20}$ +10.5° ($c = 1.9$, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3020, 2975, 1745, 1605 (w), 1580 (w), 1495 and 1480; δ_H (400 MHz) 0.78 (3 H, s, 18-CH_3), 1.12, 1.14 and 1.19 (27 H, 3 s, 3 × Me_3C), 1.20–1.50 (6 H, m), 1.65–1.80 (2 H, m), 1.80–2.00 (2 H, m), 2.00–2.10 (1 H, m), 2.20–2.35 (2 H, m), 2.87 (2 H, m), 3.67

(1 H, t, $J = 8.5$ Hz, 17-H), 3.74 (3 H, s, CH_3O), 4.06 (1 H, d, $J = 9.9$ Hz, 5′-H), 4.69 (1 H, d, $J = 7.8$ Hz, 1′-H), 5.10 (1 H, dd, $J = 7.8$ and 9.3 Hz, 2′-H), 5.26, 5.34 (2 H, 2 t, $J = 9.5$ Hz, 3′-H + 4′-H), 6.91 (1 H, d, $J = 2.4$ Hz, 4-H), 6.96 (1 H, dd, $J = 8.4$ and 2.5 Hz, 2-H), 7.31 (1 H, d, $J = 8.6$ Hz, 1-H), 7.49 (2 H, m, ArH), 7.62 (1 H, m, ArH) and 8.18 (2 H, m, ArH); δ_C (100 MHz) 12.02, 23.51, 26.47, 27.38, 27.41, 27.53, 27.70, 29.38, 29.90, 38.60, 39.08, 39.13, 43.66, 44.49, 50.25, 53.04, 70.00, 71.85, 72.47, 73.11, 89.81, 101.61, 119.04, 121.98, 126.74, 128.89, 130.16, 130.50, 133.82, 138.25, 138.65, 149.13, 165.77, 167.80, 176.62, 176.88 and 177.49; *m/z* (ES +ve mode) 841 (MNa^+, 100%).

Androsterone glucuronide ester (tri-pivaloate) 22. Found: MNa^+, 755.4349. $C_{41}H_{64}O_{11}Na^+$ requires *m/z*, 755.4346; $[\alpha]_D^{20}$ +13.3° ($c = 1.5$, $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 1770 (sh), 1750, 1730 (sh), 1280 (w), 1230 (w), 1185 (sh) and 1145; δ_H (400 MHz) 0.78, 0.85 (6 H, 2 s, 18- and 19-CH_3s), 0.95–1.05 (1 H, m), 1.11, 1.12 and 1.16 (27 H, 3 s, 3 × Me_3C), 1.20–1.30 (6 H, m), 1.35–1.70 (10 H, m), 1.70–1.80 (2 H, m), 1.85–1.95 (1 H, m), 2.00–2.10 (1 H, m), 2.35–2.45 (1 H, m), 3.72 (3 H, s, CH_3O), 3.92 (1 H, m, steroid 3-H), 4.04 (1 H, d, $J = 9.9$ Hz, 5′-H), 4.64 (1 H, d, $J = 7.8$ Hz, 1′-H), 5.08 (1 H, dd, $J = 7.8$ and 9.3 Hz, 2′-H), 5.26 and 5.36 (2 H, 2 t, $J = 9.5$ Hz, 3′-H + 4′-H); δ_C (100 MHz) 11.41, 13.84, 20.07, 21.77, 25.66, 27.06, 27.15, 27.24, 28.03, 30.72, 30.89, 31.62, 32.55, 34.20, 35.07, 35.87, 35.92, 38.73, 38.76, 39.27, 47.81, 51.55, 52.63, 54.21, 69.61, 71.40, 72.11, 72.76, 74.73, 99.69, 167.43, 176.29, 176.53 and 177.13; *m/z* (time-of-flight ES +ve mode) 755 (MNa^+, 100%) and 771 (MK^+, 10%).

Epiandrosterone glucuronide ester (tri-pivaloate) 23. Mp.236–238 °C. Found: C, 67.1; H, 9.1; MNa^+, 755.4373. $C_{41}H_{64}O_{11}$ requires C, 67.2; H, 8.8%; $C_{41}H_{64}O_{11}Na^+$ requires *m/z*, 755.4346; $[\alpha]_D^{20}$ +20° ($c = 2.0$, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3020, 2980, 1740, 1480, 1440 and 1280; δ_H (400 MHz) 0.65–0.75 (1 H, m), 0.79, 0.85 (6 H, 2 s, 18- and 19-CH_3s), 1.11, 1.13 and 1.16 (27 H, 3 s, 3 × Me_3C), 1.10–1.30 (7 H, m), 1.30–1.70 (10 H, m), 1.80–1.90 (2 H, m), 2.00–2.10 (1 H, m), 2.30–2.40 (1 H, m), 3.59 (1 H, m, 3-H), 3.73 (3 H, s, CH_3O), 4.05 (1 H, d, $J = 9.85$ Hz, 5′-H), 4.69 (1 H, d, $J = 8.1$ Hz, 1′-H), 5.02 (1 H, dd, $J = 8.1$ and 9.4 Hz, 2′-H), 5.23 and 5.35 (2 H, 2 t, $J = 9.5$ Hz, 3′-H + 4′-H); δ_C (100 MHz) 12.56, 14.20, 20.85, 22.3, 27.41, 27.51, 27.54, 27.70, 28.88, 29.52, 31.40, 32.10, 34.67, 35.40, 36.12, 36.15, 37.20, 39.07, 39.09, 44.98, 48.40, 51.79, 53.13, 54.81, 69.87, 71.35, 72.10, 73.27, 79.11, 99.58, 167.82, 176.64, 176.93, 177.54 and 222.80; *m/z* (ES +ve mode) 755 (MNa^+, 100%).

Methyl 1-*O*-(3,20-dioxopregn-4-en-11α-yl)-2,3,4-tri-*O*-pivaloyl-β-D-glucopyranuronate 24. 11α-Hydroxyprogesterone **10** (0.054 g, 0.16 mmol) and anhydrous $ZnCl_2$ (0.054 g, 0.40 mmol) were stirred together in anhydrous 1,2-dichloroethane (1 mL) over freshly activated 3 Å molecular sieves (0.5 g) under N_2 at 20 °C for 1 h, then glycosyl iodide **4** (0.062 g, 0.11 mmol) was added. No reaction was observed by TLC after several hours at 20 °C, so the reaction was heated at 80 °C for 2.5 h, leading to essentially complete consumption of **4**. The mixture was cooled and partitioned between EtOAc (2 × 10 mL) and water (15 mL), then the combined organic phases were washed with saturated $NaHCO_3$, water and evaporated to give crude product (0.091 g) which was chromatographed, eluting with a gradient from 15 to 33% EtOAc–hexane. Appropriate fractions were pooled and evaporated to give the product **24** (0.013 g, 15%); Found: MNa^+, 795.4276. $C_{43}H_{64}O_{12}Na$ requires *m/z*, 795.4295; $[\alpha]_D^{20}$ +54° ($c = 1.8$, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3016, 2973, 1745, 1705, 1670 and 1480; δ_H (400 MHz) 0.69 (3 H, s, 18-CH_3), 1.10, 1.13, 1.14 (27H, 3 s, 3 × Me_3C), 1.20–1.30 (6 H, m), 1.31 (3 H, s, 19-CH_3), 1.50–1.60 (1 H, m), 1.70–1.90 (3 H, m), 1.95–2.05 (1 H, m), 2.13 (3 H, s, CH_3CO), 2.20–2.55 (6 H, m), 2.70–2.75 (1 H, m), 3.72 (3 H, s, CH_3O), 4.05–4.15 (1 H, m, 11-H), 4.09 (1 H, d, $J = 10.0$ Hz, 5′-H), 4.74 (1 H, d, $J = 8.1$ Hz, 1′-H), 5.02 (1 H, dd, $J = 8.1$ and 9.6 Hz, 2′-H), 5.25, 5.41 (2 H, 2 t, $J = 9.6$ Hz, 3′-H + 4′-H) and 5.71 (1 H, s, 4-H); δ_C (100 MHz) 14.87, 17.91, 23.67, 24.64, 27.45, 27.53, 27.63, 31.72, 32.01, 33.91, 34.64, 35.31, 36.76, 39.09, 39.12, 39.14, 40.44, 43.82, 44.61, 53.04, 55.76, 57.09, 63.33, 69.75, 71.28, 72.04, 73.03, 74.25, 97.36, 125.11, 167.54, 170.76, 176.40, 177.00, 177.46, 200.92 and 208.91; *m/z* (ES +ve mode) 795 (MNa^+, 100%).

Transacylation products (Table 2)

3-*O*-Benzoyl, 17-*O*-isobutyrylestra-3,17β-diol 18. Mp 152–154 °C (from EtOH). Found: C, 78.35; H, 7.75; MNa^+, 469.2356. $C_{29}H_{34}O_4$ requires C, 78.0; H, 7.6%; $C_{29}H_{34}O_4Na$ requires *m/z*, 469.2355; $[\alpha]_D^{20}$ +28° ($c = 1.4$, $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 1736, 1599, 1586 and 1496; δ_H (250 MHz) 0.77 (3 H, s, 18-CH_3), 1.10 [6 H, 2 d, both Js = 6.9 Hz, (CH_3)$_2$CH), 1.20–1.50 (7 H, m), 1.60–1.75, 1.80–1.90 (3 H, 2 m), 2.10–2.30 (3 H, m), 2.48 [1 H, m, (CH_3)$_2$C*H*], 2.80 (2 H, m), 4.62 (1 H, m, 17-H), 6.85, 6.89 (2 H, 2 m, 2-H and 4-H), 7.24 (1 H, d, $J = 8.5$ Hz, 1-H), 7.42 (2 H, m, ArH), 7.54 (1 H, m, ArH) and 8.11 (2 H, approx. d, ArH); δ_C (100 MHz) 12.48, 19.39, 19.61, 23.70, 26.48, 27.46, 28.00, 29.95, 31.33, 34.64, 37.33, 38.64, 43.42, 44.42, 50.27, 82.62, 119.10, 122.02, 126.89, 128.93, 130.12, 130.54, 133.88, 138.33, 149.10, 165.85 and 177.59; *m/z* (ES +ve mode) 469 (MNa^+, 100%).

3-*O*-Benzoyl, 17-*O*-pivaloylestra-3,17β-diol 19. Mp 185–187 °C (from EtOH). Found: C, 78.5; H, 8.0; MNa^+, 483.2515. $C_{30}H_{36}O_4$ requires C, 78.3; H, 7.8%; $C_{30}H_{36}O_4Na$ requires *m/z*, 483.2511; $[\alpha]_D^{20}$ +39° ($c = 2.05$, $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 1732, 1599, 1585 and 1496; δ_H (250 MHz) 0.78 (3 H, s, 18-CH_3), 1.14 (9 H, s, Me_3C), 1.20–1.50 (7 H, m), 1.65–1.80 (1 H, m), 1.80–1.90 (2 H, m), 2.15–2.30 (3 H, m), 2.81 (2 H, m), 4.60 (1 H, m, 17-H), 6.85, 6.88 (2 H, 2 m, 2-H and 4-H), 7.25 (1 H, d, $J = 8.5$ Hz, 1-H), 7.42 (2 H, m, ArH), 7.55 (1 H, m, ArH) and 8.12 (2 H, approx. d, ArH); δ_C (100 MHz) 12.52, 23.73, 26.48, 27.46, 27.66, 28.00, 29.96, 37.35, 38.64, 39.31, 43.48, 44.42, 50.25, 82.66, 119.10, 122.03, 126.89, 128.93, 130.12, 130.55, 133.89, 138.34, 138.65, 149.10, 165.86 and 178.98; *m/z* (ES +ve mode) 483 (MNa^+, 100%), 515 (MNa^+ + MeOH, 7%).

3-*O*-Isobutyrylandrosterone 20. Mp 98–100 °C (from aqueous EtOH). Found: C, 76.45; H, 10.05; MH^+, 361.275. $C_{23}H_{36}O_3$ requires C, 76.65; H, 10.0%; $C_{23}H_{37}O_3$ requires *m/z*, 361.274; $[\alpha]_D^{20}$ +98° ($c = 0.8$, $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 1728 (br), 1411, 1388 and 1371; δ_H (250 MHz) 0.70 (1 H, m), 0.76, 0.80 (6 H, 2 s, 18- and 19-CH_3s), 0.90–1.00 (1 H, m), 1.10 [6 H, 2 d, both Js = 6.9 Hz, (CH_3)$_2$CH], 1.15–1.30 (7 H, m), 1.40–1.80 (10 H, m), 1.80–1.90 (1 H, m), 2.00–2.10 (1 H, m), 2.33 (1 H, m), 2.47 [1 H, m, (CH_3)$_2$C*H*] and 4.94 (1 H, br s, 3β-H); δ_C (100 MHz) 10.40, 12.81, 17.99, 18.13, 19.06, 20.56, 25.04, 27.08, 29.80, 30.53, 31.84, 31.93, 33.31, 34.01, 34.83, 34.95, 39.20, 46.79, 50.47, 53.41, 68.39, 175.53 and 220.36; *m/z* (CI, NH_3) 378 (MNH_4^+, 100%), 361 (MH^+, 15%) and 290 (60%).

3-*O*-Isobutyrylepiandrosterone 21. Mp 158–160 °C (from aqueous EtOH). Found: C, 76.45; H, 10.05; MH^+, 361.275. $C_{23}H_{36}O_3$ requires C, 76.65; H, 10.0%; $C_{23}H_{37}O_3$ requires *m/z*, 361.274; $[\alpha]_D^{20}$ +82° ($c = 1.0$, $CHCl_3$); ν_{max} (Nujol)/cm^{-1} 1741, 1718, 1377 and 1264; δ_H (250 MHz) 0.67 (1 H, m), 0.79 (6 H, s, 18- and 19-CH_3s), 0.90–1.02 (1 H, m), 1.07 [6 H, d, $J = 6.9$ Hz, (CH_3)$_2$CH], 1.10–1.30 (7 H, m), 1.40–1.80 (10 H, m), 1.80–1.90 (1 H, m), 1.95–2.05 (1 H, m), 2.40–2.50 [2 H, 2 m, including (CH_3)$_2$C*H*] and 4.61 (1 H, m, 3α-H); δ_C (100 MHz) 12.60, 14.00, 19.38, 19.41, 20.84, 22.15, 27.75, 28.66, 31.19, 31.90, 34.29, 34.54, 35.40, 36.20, 36.23, 37.08, 45.01, 48.16, 51.73, 54.68, 73.42, 177.15 and 221.66; *m/z* (CI, NH_3) 378 (MNH_4^+, 100%), 361 (MH^+, 20%) and 290 (33%).

(17-Oxo-5α-androstan-3α-yl)-β-D-glucuronic acid 25. A suspension of ester **22** (0.062 g, 0.083 mmol) in 2-propanol (2 mL) was treated with 20% w/w aqueous tetraethylammonium hydroxide (0.30 mL) and the mixture was stirred at 20 °C; a clear

solution soon resulted. Further portions of base (2 × 0.15 mL) were added after 1 d and 6 d; after 7 d in all, the base was neutralised by addition of Amberlyst IR-120 (H^+) (0.43 g @ 1.9 meq g^{-1}). The solution was filtered off, the resin was washed with water and methanol and the combined filtrate and washings were evaporated to dryness, then chromatographed on Merck 'Lichroprep' reversed-phase silica. Elution with a gradient from 10 to 80% of methanol in water afforded, on pooling and evaporation of appropriate fractions, the glucuronide **25** (0.029 g, 73%)[21] as a white amorphous solid. Found: $[M - H]^+$, 465.2503. $C_{25}H_{37}O_8$ requires *m/z*, 465.2488; δ_H (400 MHz, CD_3OD) 0.84, 0.85 (6 H, 2 s, 18- and 19-CH_3s), 1.00–1.10 (1 H, m), 1.15–1.35 (6 H, m), 1.35–1.45 (2 H, m), 1.45–1.60 (5 H, m), 1.60–1.90 (5 H, m), 1.90–2.00 (1 H, m), 2.00–2.10 (1 H, m), 2.35–2.50 (1 H, m), 3.22 (1 H, dd, $J = 7.9$ and 9.1 Hz, 2′-H), 3.36, 3.51 (2 H, 2 t, $J = 9.3$ Hz, 3′-H + 4′-H), 3.74 (1 H, d, $J = 9.7$ Hz, 5′-H), 3.94 (1 H, m, 3-H) and 4.36 (1 H, d, $J = 7.8$ Hz, 1′-H); δ_C (100 MHz, CD_3OD) 11.90, 14.27, 21.22, 22.75, 26.55, 29.39, 32.12, 32.94, 33.72, 35.36, 36.45, 36.75, 37.10, 40.53, 49.18, 53.01, 55.79, 73.27, 74.92, 75.73, 76.65, 77.75, 103.14, 172.90 and 224.23; *m/z* (ES −ve mode) 465 [$(M - H)^+$, 100%].

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