

# Putative metabolites of fulvestrant, an estrogen receptor downregulator. Improved glucuronidation using trichloroacetimidates

John R. Ferguson,<sup>a</sup> John R. Harding,<sup>b</sup> David A. Killick,<sup>b</sup> Keith W. Lumbard,<sup>a</sup> Feodor Scheinmann<sup>a</sup> and Andrew V. Stachulski<sup>\*†a</sup>

<sup>a</sup> Ultrafine UFC Ltd., Synergy House, Guildhall Close, Manchester Science Park, Manchester, UK M15 6SY

<sup>b</sup> AstraZeneca UK Ltd., Drug Metabolism and Pharmacokinetics Department, Mereside, Alderley Park, Macclesfield, Cheshire, UK SK10 4TG

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Following regioselective protection of the estrogen receptor downregulator fulvestrant (ICI 182,780) **1** as its 17-acetate **2** or 3-benzoate **4**, the 3-sulfate **5** and 3- and 17-glucuronide conjugates **8** and **12** were prepared. Satisfactory preparation of **12** required use of the tri-*O*-isobutyryl imidate derivative **10** in conjunction with an inverse-addition technique not previously employed in glucuronidation. The value of this method for simpler aglycones is discussed together with a study of variations in donor acyl substituent and catalyst. Another putative metabolite, the 17-ketone **19**, was prepared by direct oxidation of **1**.

## Introduction

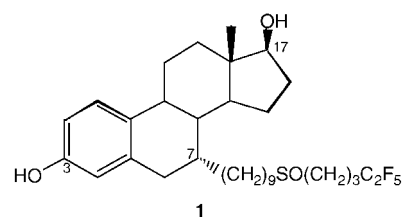
Effective chemotherapy for estrogen-dependent conditions, whether non-malignant or life-threatening such as breast cancer, is an important medicinal chemical goal. The 7 $\alpha$ -substituted class of estradiol derivatives shows promise in this area, and the compound Fulvestrant {ICI 182,780; 7 $\alpha$ -[9-(4,4,5,5,5-pentafluoropentylsulfinyl)nonyl]estra-1,3,5(10)-triene-3,17 $\beta$ -diol} **1** appears a very effective example.

Fulvestrant<sup>1,2</sup> is the first agent in a new class of antiestrogens that downregulate the estrogen receptor (ER) and is thus described by the term Estrogen Receptor Downregulator. Fulvestrant is the first nonagonist ('pure') antiestrogen that completely blocks the trophic action of estrogens and of the partial agonist antiestrogens (*e.g.*, tamoxifen). Fulvestrant has a novel mode of action that induces a rapid loss of ER protein from breast cancer cells.<sup>3</sup> This novel mode of action distinguishes fulvestrant from all other antiestrogens in current clinical use (*e.g.*, tamoxifen, toremifene and raloxifene) which are collectively described as Selective Estrogen Receptor Modulators (SERMs). Preclinical studies showed that fulvestrant is effective against models of tamoxifen-resistant breast cancer and predicted that fulvestrant would be an effective treatment for postmenopausal women with locally advanced or metastatic breast cancer who have been previously treated with endocrine therapy.

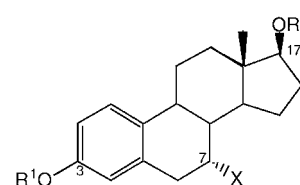
As part of a continuing programme to define the metabolism and distribution of **1** in man, a number of putative metabolites, namely the 3-sulfate, 3- and 17-glucuronides and 17-ketone, were required as metabolic standards. We now report detailed syntheses of these derivatives together with a wider study of order of addition, acyl substitution and catalysis effects in glucuronidation. A preliminary communication of this work has appeared.<sup>4</sup>

## Discussion

Initial experiments showed that regioselective synthesis of the conjugates of **1** could not be achieved without protection



of one or other hydroxy group. Selective 17-esterification of estradiol requires acid catalysis. Estradiol 17 $\beta$ -acetate itself has been obtained<sup>5</sup> by reaction of estradiol with acetic anhydride and BF<sub>3</sub>·Et<sub>2</sub>O, but when applied to **1** these conditions gave incomplete reaction even with excess of reagent and some decomposition. Instead, acid-catalysed transesterification<sup>6</sup> of **1** (ethyl acetate, tosic acid, distil) afforded the 17 $\beta$ -acetate **2** in excellent yield. The 17 $\beta$ -formate **3** was similarly obtained using ethyl formate. Protection of the phenol was readily achieved by Schotten–Baumann reaction of **1** with benzoyl chloride in a basic two-phase system, giving an excellent yield of **4**.



X = as in **1**

**2** R<sup>1</sup> = H, R<sup>2</sup> = CH<sub>3</sub>CO

**3** R<sup>1</sup> = H, R<sup>2</sup> = CHO

**4** R<sup>1</sup> = PhCO, R<sup>2</sup> = H

**5** R<sup>1</sup> = NaOSO<sub>2</sub>, R<sup>2</sup> = H

Various methods of phenolic *O*-sulfation have been described, including carbodiimide coupling,<sup>7</sup> but amine complexes of SO<sub>3</sub> are most frequently used:<sup>8</sup> recently the DMF–SO<sub>3</sub> complex has been employed for tyrosine.<sup>9</sup> In this case SO<sub>3</sub>·Et<sub>3</sub>N proved more effective than SO<sub>3</sub>·Me<sub>3</sub>N, and when the former complex reacted with **2** a solid triethylammonium salt was isolated in 85%

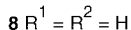
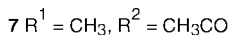
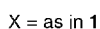
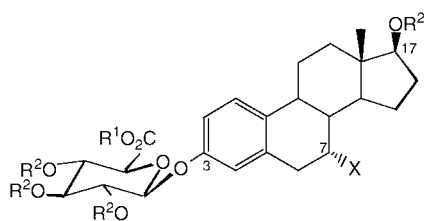
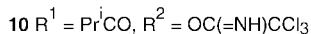
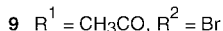
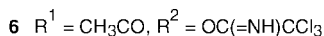
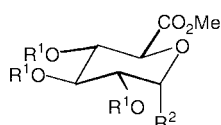
<sup>†</sup> Present address: Department of Chemistry, The Robert Robinson Laboratories, University of Liverpool, Liverpool, UK L69 3BX.

yield. Treatment with aq. NaOH achieved both ion exchange and cleavage of the 17-acetate, yielding the desired sulfate **5** in excellent yield and purity.

For the synthesis of the 3-glucuronide, the tri-*O*-acetyl imidate **6**<sup>10,11</sup> proved the reagent of choice, as is often the case for phenols.<sup>12</sup> Thus the reaction of 17-acetate **2** with 1.5 equiv. of **6** and the same excess of BF<sub>3</sub>·Et<sub>2</sub>O gave conjugate **7** in 85% yield. Such a large excess of catalyst is not normally needed for imidate glucuronidation: it may well be that the first equivalent complexes strongly to the sulfoxide.

Hydrolysis of **7** using aq. Na<sub>2</sub>CO<sub>3</sub> in methanol<sup>13</sup> left the 17β-acetate intact, and further treatment with NaOH was necessary to complete the hydrolysis. After acidification to pH 6.2 the 3-glucuronide **8** was conveniently isolated as its sodium salt and purified by reversed-phase silica chromatography. We subsequently found that the 17β-formate **3** gave a conjugate corresponding to **7** in virtually identical yield and in this case hydrolysis with aq. Na<sub>2</sub>CO<sub>3</sub> in methanol gave **8** directly.

Synthesis of the 17-glucuronide from **4** proved much more difficult. Reaction of **4** and **6** under standard conditions [1,2-dichloroethane (DCE), BF<sub>3</sub>·Et<sub>2</sub>O, 0 °C] gave only transacylation with no detectable glucuronide. It was reported<sup>14</sup> that a 3-protected androstane-3,17-diol derivative gave only an 8% yield of glucuronide on reaction with **6**. In that series, however, a Koenigs–Knorr reaction with bromo sugar **9** and Ag<sub>2</sub>CO<sub>3</sub> gave the desired conjugate in 34% yield. We obtained no glucuronide on reaction of **4** with **9** and Ag<sub>2</sub>CO<sub>3</sub>; when CdCO<sub>3</sub><sup>15</sup> was used as catalyst, an orthoester was produced in 75% yield [ $\delta_{\text{H}}$ , *inter alia*, 1.8 (3 H, s) and 5.9 (1 H, d)]. Attempted acid-catalysed rearrangement of the orthoester led only to decomposition.

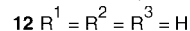
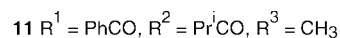
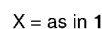
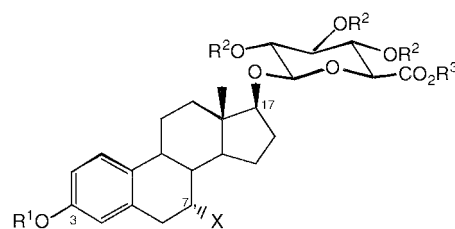


We have previously demonstrated<sup>16,17</sup> the advantages of the tri-*O*-isobutyryl imidate **10** as a donor. Initial results with this intermediate were not encouraging, however: under directly comparable conditions to those used for **6**, transacylation was still the major outcome, with just 7% of the conjugate **11** isolated by chromatography.

At this point, we discovered that the reaction was subject to a profound order-of-addition effect. Thus, when **10** was added slowly at -15 °C to a mixture of **4** and BF<sub>3</sub>·Et<sub>2</sub>O in DCE, rather than adding BF<sub>3</sub>·Et<sub>2</sub>O to premixed **4** and **10** in DCE, the conjugate **11** was isolated in 50% yield after chromatography, with only 15% of transacylated product. Schmidt and Toepfer noted<sup>18</sup> a similarly advantageous inverse addition when using a fucosyl imidate, which, being a 6-deoxy sugar, is a much more reactive donor.

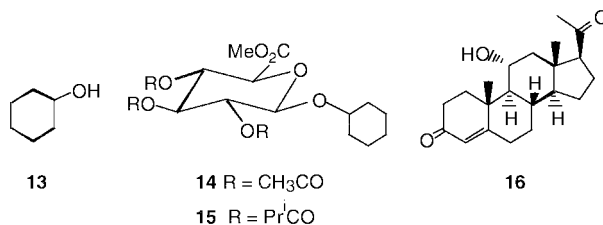
**Table 1** Comparison of imidate glucuronidation of some secondary alcohols using the 'normal' (method **A**) and 'inverse' (method **B**) procedures

Alcohol	Imidate	Method	%Yield of glucuronide
<b>4</b>	<b>10</b>	<b>A</b>	7
<b>4</b>	<b>10</b>	<b>B</b>	50
<b>13</b>	<b>6</b>	<b>A</b>	18
<b>13</b>	<b>6</b>	<b>B</b>	24
<b>13</b>	<b>10</b>	<b>A</b>	39
<b>13</b>	<b>10</b>	<b>B</b>	49
<b>16</b>	<b>6</b>	<b>A</b>	31
<b>16</b>	<b>10</b>	<b>B</b>	77



Intermediate **11** was cleanly hydrolysed using NaOH in aq. Pr<sup>i</sup>OH, and the free glucuronide **12** was isolated and purified as for **8**, giving material of >98% purity by analytical reversed-phase HPLC.

The value of the inverse-addition technique was further studied using other alcohols, in particular the secondary alcohol cyclohexanol **13** (Table 1). Reaction of either **6** or **10** with **13** using 0.5 eq. BF<sub>3</sub>·Et<sub>2</sub>O gave a slightly but consistently higher yield of conjugates **14** and **15** when using the inverse technique, though the improvement was not as dramatic as that seen with **4**. Using either addition method, **10** gave double the yield of **6**. Another steroidal aglycone, 11 $\alpha$ -hydroxyprogesterone **16**, gave a far better yield of conjugate **17** by the inverse method. It appears that the effect is most pronounced with aglycones bearing groups which can complex strongly to the Lewis acid (C=O, S=O): we note this was the case in Schmidt and Toepfer's example cited above.



**13**

**14** R = CH<sub>3</sub>CO

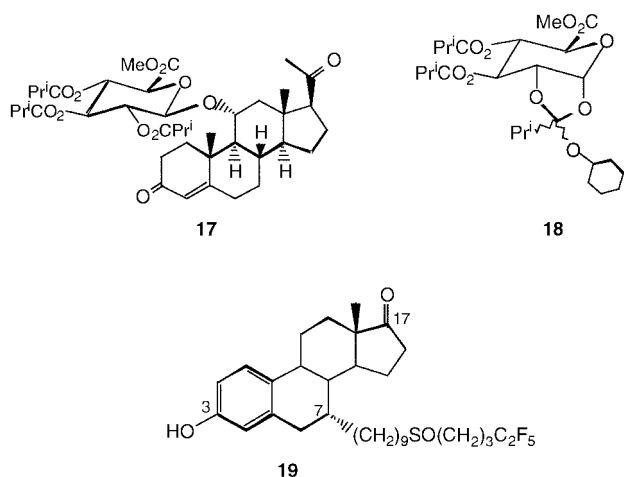
**15** R = Pr<sup>i</sup>CO

**16**

When just 0.25 eq. of BF<sub>3</sub>·Et<sub>2</sub>O was used in the reaction of **13** with **10**, significant quantities of the orthoester **18** resulted initially by technique **A** or **B**, in addition to conjugate **15**. Prolonged reaction (40 h) gave steady conversion of **18** to **15** in virtually the same yield (51%) as that seen before. The use of ZnCl<sub>2</sub> (0.5 eq.) as a catalyst<sup>19</sup> gave **18** as the main product in the early stages: prolonged reaction led again to a satisfactory yield (39%) of glucuronide. Using the stronger Lewis acid trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.25 eq.), 'inverse' reaction of **13** with **10** was complete in *ca.* 0.25 h at -10 °C and no orthoester was detected; **15** resulted in 52% yield.

#### Inverse addition: mechanistic discussion

In the paper cited above, Schmidt and Toepfer<sup>18</sup> considered that, in the inverse-addition mode, prior complexation of acceptor component and catalyst led to minimal contact between the imidate donor and the catalyst, thereby reducing



decomposition and side reactions of the imidate. There, as here, the acceptor contained functionality which could strongly bind the catalyst. Our observation that the beneficial effect of 'inverse addition' is much less with acceptors lacking such functionality (C=O, S=O, etc.) is consistent with this proposal. It is also interesting that the effect is pronounced with donors of such differing ability, from benzyl ether-protected fucosyl imidates (excellent donors) to acyl-protected glucuronate imidates (much poorer donors, requiring also considerably more Lewis acid).

It is noticeable that orthoester formation is not suppressed by the isobutyryl esters in **10** and becomes pronounced at lower concentrations of  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  even with the 'inverse' technique (**10** + **13**). There is a clear gradation across the three Lewis acids studied, with no orthoester seen (or transient?) using the more powerful TMSOTf even in lower concentration. Possibly two distinct glucuronidation mechanisms are operating, namely either via orthoester and rearrangement or via  $\text{S}_{\text{N}}2$ -like displacement of complexed imidate by acceptor without participation by the 2-ester, leading to  $\alpha$ -to- $\beta$  inversion. The 'inverse' mechanism, in which the imidate on addition meets an excess of acceptor, is more likely to adopt the latter pathway.

We conclude that the tri-*O*-isobutyryl imidate **12** is a versatile reagent for the glucuronidation of a range of alcohols. Order of addition, acyl substitution and catalyst are all important variables in glucuronidation, with inverse addition having advantages especially for complex aglycones.

Finally, the 17-keto metabolite **19** was accessible by direct, low-temperature Swern oxidation of **1**, affording the product in 65% yield after chromatography.

## Experimental

### General

Ether refers to diethyl ether. Organic extracts were finally washed with brine and dried over anhydrous sodium sulfate prior to rotary evaporation below 40 °C. Analytical TLC was performed on aluminium-backed Merck Kieselgel 60 plates; Merck silica gel 60 (art. 7729) was used for preparative chromatography. Mps were determined on a Kofler block and are uncorrected. NMR spectra were obtained on a Varian Inova instrument at 300 MHz or on a Bruker AC Spectrospin instrument at 250 MHz except where stated, using a deuterium lock, on  $\text{CDCl}_3$  solutions except where noted;  $\delta$ -values are  $\delta_{\text{H}}$  except where stated. Mass spectra were recorded on a Kratos MS 25 instrument working in the chemical ionisation (CI) or fast-atom bombardment (FAB) mode, or on a Waters ZMD instrument operating in the electrospray (ES) mode. Analytes were eluted with a gradient of acetonitrile (20%–35% over a period of 15 min; 35%–70% over a period of 10 min) in ammonium acetate (0.1 M; pH 6.9) at 0.9 ml min<sup>-1</sup>.

Abbreviations used for common solvents are AcOH (ethanoic acid), DCM (dichloromethane), DMF (*N,N*-dimethylformamide), EtOAc (ethyl ethanoate) and Pr<sup>i</sup>OH (propan-2-ol).

The trivial designation 'ICI 182,780' is used for the parent steroid **1**; the systematic name is 7 $\alpha$ -[9-(4,4,5,5,5-pentafluoronylsulfinyl)nonyl]estra-1,3,5(10)-triene-3,17 $\beta$ -diol.

### ICI 182,780-17 $\beta$ -acetate **2**

A solution of ICI 182,780 **1** (0.61 g, 1 mmol) and toluene-4-sulfonic acid monohydrate (0.050 g) in ethyl acetate (30 mL) was heated and distilled, with EtOAc added to maintain the volume of solution. Further toluene-4-sulfonic acid (0.20 g) was added after 2 h and the distillation was continued for a further 4 h, then the solution was cooled, washed sequentially with saturated aq.  $\text{NaHCO}_3$  and water (20 mL each) and evaporated to give a gum which on re-evaporation from ether (3  $\times$  5 mL) afforded acetate **2** as a crisp foam (0.63 g, 97%) (Found: *m/z*, 648.3267.  $\text{C}_{34}\text{H}_{49}\text{F}_5\text{O}_4\text{S}$  requires *M*, 648.3271);  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ )/cm<sup>-1</sup> 3600sh, 3500–3100br, 1725, 1610w, 1500w, 1045sh and 1025;  $\delta$  (Perkin-Elmer R12B, 220 MHz) *inter alia* 0.85 (3 H, s, 18-H<sub>3</sub>), 2.05 (3 H, s,  $\text{CH}_3\text{CO}$ ), 4.75 (1 H, m, 17-H), 6.65 (1 H, narrow d, 4-H), 6.75 (1 H, dd, 2-H) and 7.25 (1 H, d, 1-H); *m/z* (EI) 648 ( $\text{M}^+$ , 65%) and (CI,  $\text{NH}_3$ ) 649 ( $\text{MH}^+$ , 100%).

### ICI 182,780-17 $\beta$ -formate **3**

In a similar manner, ICI 182,780 **1** (1.82 g, 3.00 mmol) was allowed to react with ethyl formate (100 mL) using toluene-4-sulfonic acid monohydrate (0.80 g) as catalyst; reaction was noticeably quicker than for **2**. Work-up as for **2** gave formate **3** (1.90 g, quant.) as a foam (Found: *m/z*, 635.31964.  $\text{C}_{33}\text{H}_{48}\text{F}_5\text{O}_4\text{S}$  requires  $\text{MH}^+$ , 635.31935);  $\delta$  (400 MHz) *inter alia* 0.85 (3 H, s, 18-H<sub>3</sub>), 4.80 (1 H, m,  $\text{CHOCH}=\text{O}$ ), 6.56 (1 H, d, *J* 2.5 Hz, 4-H), 6.85 (1 H, dd, *J* 8.5 and 2.5 Hz, 2-H), 7.12 (1 H, d, *J* 8.5 Hz, 1-H) and 8.10 (1 H, s,  $\text{OCH}=\text{O}$ ); *m/z* (FAB +ve mode, 3-NOBA) 635 ( $\text{MH}^+$ , 20%) and 589 ( $[\text{M} - \text{HCO}_2]^+$ , 30).

### ICI 182,780-3-benzoate **4**

Benzoyl chloride (0.70 mL, 0.84 g, 6 mmol) was added to a well-stirred mixture of **1** (2.42 g, 4.0 mmol) in DCM (15 mL) and a solution of KOH (0.90 g, 16 mmol) in water (15 mL) at 0 °C. After 2 h, when the temperature had been allowed to rise to 20 °C, further benzoyl chloride (0.35 mL, 3 mmol) was added. After a further 0.5 h, the mixture was diluted with both water (50 mL) and ethyl acetate (100 mL), then the organic phase was separated, washed successively with saturated aq.  $\text{NaHCO}_3$  and water (20 mL each), and evaporated to leave a gum. Re-evaporation from ether (3  $\times$  10 mL) gave the benzoate **4** as a crisp foam (2.83 g, 99%) (Found:  $\text{M}^+$ , 710.3428.  $\text{C}_{39}\text{H}_{51}\text{F}_5\text{O}_4\text{S}$  requires *M*, 710.3435);  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ )/cm<sup>-1</sup> 1730, 1605w, 1585sh and 1495w;  $\delta$  (Perkin-Elmer R12B, 220 MHz) *inter alia* 0.80 (3 H, s, 18-H<sub>3</sub>), 3.75 (1 H, t, 17-H), 6.95 (1 H, narrow d, 4-H), 7.00 (1 H, dd, 2-H), 7.35 (1 H, d, 1-H), 7.55 (2 H, m, 3'-H + 5'-H), 7.65 (1 H, m, 4'-H) and 8.25 (2 H, approx. d, 2'-H + 6'-H); *m/z* (CI) 711 ( $\text{MH}^+$ , 100%); (EI) 710 ( $\text{M}^+$ , 15%). This product contained traces of a high-*R*<sub>f</sub> impurity, removable by chromatography, but was quite suitable for progression.

### ICI 182,780-3-Sulfate, sodium salt **5**

Sulfur trioxide–triethylamine complex ( $\text{SO}_3 \cdot \text{Et}_3\text{N}$ ) (1.5 g, 8.28 mmol) was added to a solution of the acetate **2** (5.0 g, 7.71 mmol) in anhydrous pyridine (20 mL) being stirred at 20 °C. After 1 h, further  $\text{SO}_3 \cdot \text{Et}_3\text{N}$  (1.2 g, 6.62 mmol) was added, and after 16 h the solution was diluted with ether (200 mL); after a few minutes the supernatant was decanted and the solid residue washed with further ether (2  $\times$  200 mL), then dried *in vacuo*. Chromatography of the crude product, eluting with 5% methanol–DCM, afforded a semi-solid (5.52 g) whose NMR

spectrum was consistent with the 17-acetate of the triethylammonium salt of **5**. A solution of this material in methanol (48 mL) was treated with 5 M aq. NaOH (11.5 mL) and stirred at 20 °C for 1 h, then glacial acetic acid was added to give a pH of 9.0. The solution was evaporated, and the residue was redissolved in water containing a little methanol and chromatographed on reversed-phase silica (LiChroprep RP-18), eluting initially with water, then with increasing percentages of up to 60% methanol in water. Evaporation of appropriate fractions afforded sodium salt **5** (3.75 g, 85%) (Found:  $m/z$ , 731.2459.  $C_{32}H_{46}F_5NaO_6S_2$  requires  $MNa^+$ , 731.2452);  $\nu_{max}(\text{Nujol})/\text{cm}^{-1}$  3700–3100br, 1610sh, 1270, 1190s and 1050;  $\delta$  ( $CD_3OD$ ) 0.82 (3 H, s, 18- $H_3$ ), 3.73 (1 H, m, 17-H), 7.04 (1 H, narrow d, 4-H), 7.08 (1 H, dd, 2-H) and 7.30 (1 H, d, 1-H);  $m/z$  (FAB +ve ion, 3-nitrobenzyl alcohol) 731 ( $MNa^+$ , 100%).

#### Methyl 2,3,4-tri-*O*-acetyl- $\beta$ -D-glucopyranuronate derivative 7

$BF_3 \cdot Et_2O$  (0.080 mL, 0.089 g, 0.625 mmol) was added at –10 °C to a solution of acetate **2** (0.32 g, 0.5 mmol) and imidate **6**<sup>12</sup> (0.36 g, 0.75 mmol) in anhydrous DCM (3 mL) which had been stirred over freshly activated 4 Å molecular sieves under argon for 0.75 h. The temperature was kept below 0 °C, and after 1 h, when reaction appeared complete by TLC, the solution was diluted with EtOAc (25 mL), washed successively with saturated aq.  $NaHCO_3$  and water (20 mL each), and evaporated to dryness, giving a gum (0.70 g), which was chromatographed on silica (*ca.* 15 g), and eluting with from 25 to 70% EtOAc in hexane in steps. Appropriate fractions (UV-absorbing, claret on resorcinol stain, more polar than starting material) were pooled and evaporated; addition of ether (5 mL) and re-evaporator gave glucuronide ester **7** as a crisp foam (0.402 g, 84%) [Found (FAB):  $m/z$ , 965.4144.  $C_{47}H_{66}F_5O_{13}S$  requires  $MH^+$ , 965.4141];  $\nu_{max}(\text{CHCl}_3)/\text{cm}^{-1}$  1755vs, 1725sh, 1610w, 1600w, 1075sh and 1040vs;  $\delta$  ( $CDCl_3$ ) *inter alia* 0.86 (3 H, s, 18- $H_3$ ), 2.05–2.10 (12 H, 4 s, 4  $\times$   $CH_3CO$ ), 3.78 (3 H, s,  $CH_3O$ ), 4.21 (1 H, m, 5'-H), 4.72 (1 H, t, 17-H), 5.14 (1 H, d,  $J$  8 Hz, 1'-H), 5.25–5.40 (3 H, m, 2'-H + 3'-H + 4'-H), 6.73 (1 H, narrow d, 4-H), 6.81 (1 H, dd, 2-H) and 7.23 (1 H, d, 1-H);  $m/z$  (ES +ve mode) 964 ( $M^+$ , 25%) and 987 ( $MNa^+$ , 100%).

The 17-formate **3** could equally well be used in this experiment.

#### $\beta$ -D-Glucopyranuronic acid derivative 8 (ICI 182,780-3- $\beta$ -D-glucuronide)

A solution of the glucuronate ester **7** (0.350 g, 0.36 mmol) in methanol (10 mL) was stirred at 0 °C with aq.  $Na_2CO_3$  (0.21 g, 2 mmol in 4 mL). The temperature was allowed to rise to 20 °C, when a solution was gradually obtained. After 4 h further aq.  $Na_2CO_3$  (0.03 g in 2 mL) was added: after 5 h, when reaction appeared complete by TLC (EtOAc- $Pr^iOH$ -water, 5 : 3 : 1), glacial AcOH was added to give a pH of 6.2, then the solution was evaporated to dryness and the residue azeotroped with ethanol (3  $\times$  5 mL). On trituration with cold water (2 mL), a white solid separated: the mother liquors were removed, then the solid was washed twice more with a little cold water. The material was then extremely hygroscopic, so it was re-evaporated from ethanol, then from ether (3  $\times$  5 mL) and dried *in vacuo*, under high vacuum to give a hard glass (0.306 g). NMR analysis (300 MHz;  $CD_3OD$ ) showed that this material contained the unhydrolysed 17-acetate.

The product was therefore redissolved in methanol (3 mL) and treated with 1 M NaOH (0.5 mL). After 16 h the solution was acidified to pH 7 with AcOH, then the product was isolated as above, except that the final sodium salt was too water-soluble for further washing after the first trituration. Instead the crude product (0.273 g) was rigorously dried *in vacuo*, dissolved in water (5 mL), and subjected to chromatography on reversed-phase silica (LiChroprep RP-18, 10 g) as described for compound **5**. On this occasion the column was eluted with up to

70% methanol in 10% steps. Product-rich fractions (UV, resorcinol) emerged at 60%; these were combined and evaporated to dryness, then trituated with ether to give the product **8** as its sodium salt as a flaky solid (0.199 g, 68%) (Found:  $m/z$ , 827.3204.  $C_{38}H_{54}F_5NaO_9S$  requires  $MNa^+$ , 827.3207);  $\delta$  [ $(CD_3)_2SO$  + few drops  $D_2O$ ] *inter alia* 0.65 (3 H, s, 18- $H_3$ ), 3.15–3.30 (3 H, m, 2'-H + 3'-H + 4'-H), 3.43 (1 H, d, 5'-H), 3.54 (1 H, t, 17-H), 4.74 (1 H, d,  $J$  8 Hz, 1'-H), 6.70 (1 H, narrow d, 4-H), 6.81 (1 H, dd, 2-H) and 7.18 (1 H, d, 1-H);  $m/z$  (ES –ve mode) 781 ( $M - Na^+$ , 100%); HPLC purity 97% (Spherisorb OSDS 2 column, methanol-aq.  $NH_4OAc$  mixtures), no aglycone.

#### Methyl 2,3,4-tri-*O*-isobutyryl- $\beta$ -D-glucopyranuronate derivative 11

A solution of imidate **10**<sup>15</sup> (0.42 g, 0.75 mmol) in anhydrous DCE (2 mL) was added dropwise over a period of 12 min at –15 °C to a solution of 3-benzoyl steroid **4** (0.355 g, 0.5 mmol) and  $BF_3 \cdot Et_2O$  (0.080 mL, 0.75 mmol) in DCE (3 mL) which had previously been stirred over 4 Å MS under argon for 0.75 h. The temperature was allowed to rise to +5 °C over a period of 4 h, then the reaction was worked up as described for compound **7**. The crude product (0.75 g) was chromatographed on silica (17 g), eluting with from 30 to 60% EtOAc-hexane in steps of 5%. Transacylated product (see text: bright red resorcinol stain) was eluted first, then the desired product (UV-absorbing, claret resorcinol stain, less polar than starting material). Appropriate fractions were combined, evaporated and azeotroped with ether to give glucuronide ester **11** (0.283 g, 51%) as a foam (Found:  $m/z$ , 1111.5243.  $C_{58}H_{80}F_5O_{13}S$  requires  $MH^+$ , 1111.5239);  $\nu_{max}(\text{Nujol})/\text{cm}^{-1}$  1745vs, 1605w, 1585w, 1085 and 1065m;  $\delta$  ( $CDCl_3$ ) *inter alia* 0.80 (3 H, s, 18- $H_3$ ), 3.75 (1 H, t, 17-H), 3.78 (3 H, s, MeO), 4.08 (1 H, d, 5'-H), 4.71 (1 H, d,  $J$  8 Hz, 1'-H), 5.10–5.40 (3 H, 3 t, 2'-H + 3'-H + 4'-H), 6.95 (1 H, br s, 4-H), 7.01 (1 H, dd, 2-H), 7.36 (1 H, d, 1-H), 7.55 (2 H, m, ArH), 7.67 (1 H, m, ArH) and 8.23 (2 H, d, ArH);  $m/z$  (CI) 1111 ( $MH^+$ , 20%) and 918 ( $[M - 193]^+$ ).

#### $\beta$ -D-Glucopyranuronic acid derivative 12 (ICI 182,780-17- $\beta$ -D-glucuronide), sodium salt

Aq. NaOH (0.102 g, 2.55 mmol in 4 mL) was added to a suspension of ester **11** (0.380 g, 0.34 mmol) in  $Pr^iOH$  (4.5 mL) which was stirred at 0 °C. A solution gradually resulted and was allowed to attain ambient temperature. After 7 h, the solution was stored at 0 °C for 16 h, then restored to 20 °C. After a further 4 h, when reaction appeared complete by TLC (EtOAc- $Pr^iOH$ -water, 5 : 3 : 1), methanol (17 mL) was added, followed by addition of AcOH to pH 7 and evaporation to dryness, azeotroping with several portions of ethanol to counter frothing. The crude product (0.46 g) was suspended in water (20 mL) and applied to a column of LiChroprep RP-18 (7.5 g). Elution was performed as described for **5**, increasing the methanol percentage to 80% in 10% steps in this case. The product emerged in the range 60–80% methanol: appropriate fractions were pooled and evaporated, azeotroping with ethanol (6 $\times$ ) and eventually drying *in vacuo* in high vacuum to afford glucuronide **12** (0.204 g, 74%) as a hard glass (Found:  $m/z$ , 827.3191.  $C_{38}H_{54}F_5Na_2O_9S$  requires  $MNa^+$ , 827.3204);  $\delta$  ( $CD_3OD$ ) *inter alia* 0.88 (3 H, s, 18- $H_3$ ), 3.21, 3.36, 3.48 (3 H, 3 t, 2'-H + 3'-H + 4'-H), 3.65 (1 H, d, 5'-H), 3.84 (1 H, t, 17-H), 4.39 (1 H, d, 1'-H), 6.45 (1 H, narrow d, 4-H), 6.54 (1 H, dd, 2-H) and 7.08 (1 H, d, 1-H);  $m/z$  (ES) 781 ( $[M - Na]^+$ , 100%). A very weak  $[2 \times (M - Na) + H]^+$  peak was also seen,  $m/z$  1563; HPLC purity 98% (conditions as **8**), no aglycone.

#### Imidate glucuronidations: general procedures—A. 'Normal' addition

$BF_3 \cdot Et_2O$  was added to a mixture of the aglycone and imidate

(either **6** or **10**) which was stirred in DCE at the temperature stated.

**B. 'Inverse' addition.** A solution of imidate **6** or **10** in DCE was added dropwise over a period of 10 min at  $-10$  to  $-15$  °C to a stirred mixture of aglycone and  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  in DCE. Work-up of both methods **A** and **B** was performed as described for the conversion of **2** to **7**.

#### Methyl 1-*O*-cyclohexyl-2,3,4-tri-*O*-acetyl- $\beta$ -D-glucopyranuronate **14**

This compound, prepared from cyclohexanol **13** and imidate **6** by method **A** or **B**, had mp 137–140 °C (from ethanol) (lit.,<sup>20</sup> 137.5–139 °C);  $\delta$  (Perkin-Elmer R12B, 220 MHz;  $\text{CDCl}_3$ ) 1.15–1.95 (10 H, m,  $5 \times \text{CCH}_2$ ), 2.03, 2.05 (9 H, 3 s,  $3 \times \text{CH}_3\text{CO}$ ), 3.65–3.75 (1 H, m, cyclohexyl methine H), 3.78 (3 H, s,  $\text{OCH}_3$ ), 4.05 (1 H, d, 5-H), 4.68 (1 H, d, 1-H), 5.00 (1 H, m, 2-H) and 5.18–5.33 (2 H, m, 3-H + 4-H).

#### Methyl 1-*O*-cyclohexyl-2,3,4-tri-*O*-isobutyryl- $\beta$ -D-glucopyranuronate **15**

This compound was prepared from cyclohexanol **13** and imidate **10** using either method **A** or **B** with the results shown in Table 1 after purification by chromatography on silica, and eluting with 20% EtOAc–isohehexane, then 30% EtOAc–isohehexane. Compound **15** had mp 138–141 °C (from EtOAc–isohehexane) (Found: C, 60.2; H, 8.1.  $\text{C}_{25}\text{H}_{40}\text{O}_{10}$  requires C, 60.0; H, 8.0%);  $\delta$  (Perkin-Elmer R12B, 220 MHz;  $\text{CDCl}_3$ ) *inter alia* 1.05–1.22 [18 H, m,  $3 \times (\text{CH}_3)_2\text{CH}$ ], 2.40–2.60 [3 H, m,  $3 \times (\text{CH}_3)_2\text{CH}$ ], 3.60–3.72 (1 H, m, cyclohexyl methine H), 3.75 (3 H, s,  $\text{CH}_3\text{O}$ ), 4.06 (1 H, d, 5'-H), 4.68 (1 H, d, 1'-H), 5.05 (1 H, m, 2'-H) and 5.20–5.40 (2 H, m, 3'-H + 4'-H); *m/z* (CI) 518 ( $\text{MNH}_4^+$ , 100%).

When **13** and **10** were allowed to react using  $\text{ZnCl}_2$  (0.5 eq.) as catalyst and the reaction was quenched before completion (saturated aq.  $\text{NaHCO}_3$  added, followed by extraction with EtOAc), a product less polar than **15** could be isolated as an oil which had spectral data corresponding to methyl 1,2-*O*-[1-(cyclohexyloxy)-2-methylpropylidene]-3,4-di-*O*-isobutyryl- $\alpha$ -D-glucopyranuronate **18** (Found: *m/z*, 501.2707.  $\text{C}_{25}\text{H}_{41}\text{O}_{10}$  requires  $\text{MH}^+$ , 501.2700);  $\delta$  (Bruker 250 MHz) *inter alia* 3.60 (1 H, m, cyclohexyl CHO), 3.77 (3 H, s,  $\text{CH}_3\text{O}$ ), 4.28 (1 H, m, 2-H), 4.51 (1 H, d,  $J$  7.25 Hz, 5-H), 5.15 (1 H, dd, 4-H), 5.27 (1 H, m, 3-H) and 5.89 (1 H, d,  $J$  5 Hz, 1-H); *m/z* (CI) 501 ( $\text{MH}^+$ , 10%) and 518 ( $\text{MNH}_4^+$ , 5). The assignments were confirmed by irradiation of the signal at  $\delta$  4.28, which caused the signal at  $\delta$  5.27 to become a doublet while that at  $\delta$  5.89 became a singlet, and irradiation of the signal at  $\delta$  4.51 which caused the signal at  $\delta$  5.15 to become a doublet. The shift values are typical for an orthoester in the glucuronic acid series.<sup>21</sup> When the  $\text{ZnCl}_2$ -catalysed reaction was allowed to proceed to completion, compound **15** was isolated in 39% yield.

#### Methyl 1-*O*-(3,20-dioxopregn-4-en-11 $\alpha$ -yl)-2,3,4-tri-*O*-isobutyryl- $\beta$ -D-glucopyranuronate **17**

11 $\alpha$ -Hydroxyprogesterone **16** was treated with imidate **10** using method **A** or **B** (Table 1). The crude product was dissolved in DCM and chromatographed, eluting with 5%, then 10%, EtOAc–isohehexane; appropriate fractions were pooled and evaporated to give pure **17**, yields as in Table 1, mp 205–208 °C (from ethanol) (Found: C, 64.0; H, 8.2.  $\text{C}_{40}\text{H}_{58}\text{O}_{12} \cdot 2\text{C}_2\text{H}_5\text{OH}$  requires C, 64.2; H, 8.5%);  $\nu_{\text{max}}$ (film)/ $\text{cm}^{-1}$  2973, 2940, 2880, 1751, 1704, 1670 and 1470;  $\delta$  ( $\text{CDCl}_3$ ) *inter alia* 0.74 (3 H, s, 19-H<sub>3</sub>), 1.08–1.22 [18 H, m,  $3 \times (\text{CH}_3)_2\text{CH}$ ], 1.35 (3 H, s, 18-H<sub>3</sub>), 2.18 (3 H, s, 21-H<sub>3</sub>), 3.75 (3 H, s,  $\text{CH}_3\text{O}$ ), 4.10 (1 H, d,  $J$  10 Hz, 5'-H), 4.75 (1 H, d,  $J$  8 Hz, 1'-H), 5.03 (1 H, dd, 2'-H), 5.27 (1 H, dd, 4'-H), 5.39 (1 H, t, 3'-H) and 5.75 (1 H, s, 4-H); *m/z* (FAB) 731 ( $\text{MH}^+$ ).

#### ICI 182,780-17-ketone **19**

Oxalyl dichloride (0.05 mL, 0.073 g, 0.58 mmol) was stirred in dry DCM (3 mL) at  $-70$  °C under nitrogen. A solution of dimethyl sulfoxide (0.1 mL, 0.1101 g, 1.41 mmol) in dry DCM was added dropwise over a period of 10–15 min, keeping the internal temperature below  $-60$  °C. The mixture was stirred for 5 min, then a solution of ICI 182,780 **1** (0.305 g, 0.5 mmol) in dry DCM (3 mL) was added dropwise over a period of 15 min, maintaining the same temperature. After stirring of the mixture at  $-70$  °C for 2 h, diisopropylamine (0.5 mL) was added over a period of 5 min and the mixture was stirred at  $-70$  °C for a further 30 min, then allowed to warm to room temperature. Water (5 mL) and DCM (5 mL) were successively added and the mixture was stirred for 25 min before the addition of further water (10 mL) and DCM (10 mL). The phases were separated and the organic phase was washed with water (30 mL), then dried over anhydrous  $\text{MgSO}_4$  for 16 h and evaporated to give an oil which solidified on storage. Chromatography, eluting with EtOAc–toluene (7 : 3 v/v), afforded on evaporation of appropriate fractions the product **19** as a clear oil (0.195 g, 65%) (Found:  $\text{M}^+$ , 604.2990.  $\text{C}_{32}\text{H}_{45}\text{F}_5\text{O}_3\text{S}$  requires  $M$ , 604.3010. Micromass GCT instrument, EI);  $\delta$  (400 MHz;  $\text{CDCl}_3$ ) *inter alia* 0.9 (3 H, s, 18-H<sub>3</sub>), 2.2 (2 H, m, 16-H<sub>2</sub>), 6.58 (1 H, narrow d, 4-H), 6.65 (1 H, dd, 2-H) and 7.13 (1 H, d, 1-H);  $\delta_{\text{C}}$  *inter alia* 220.5 (C-17); *m/z* (EI) 604 ( $\text{M}^+$ ).

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