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Sugar conjugates of fulvestrant (ICI 182,780): efficient general procedures for glycosylation of the fulvestrant core

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Abstract—We have prepared glucose and cellobiose conjugates at the phenolic 3- and hydroxylic 17-positions of the pure anti-estrogenic compound fulvestrant (ICI 182,780), which has recently been approved in the USA for the treatment of advanced postmenopausal breast cancer. Glycosylation at the 17-position was achieved most effectively using pivaloyl protection of the sugar imidates employed, which we found suppressed the competing transacylation reaction and led to improved yields of the product glycosides.

1. Introduction

The 7α-substituted estradiol derivative fulvestrant (ICI 182,780) (7α-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)-nonyl]estra-1,3,5,(10)-triene-3,17β-diol) **1** is a pure receptor antagonist of estrogen[1] and is an effective treatment for advanced postmenopausal estrogen-dependent breast cancer.[2] As part of an investigation into the oral bioavailability of **1** we required access to the 3-glucosyl **2**, 17-glucosyl **3**, 3,17-bisglucosyl **4**, 3-cellobiosyl **5**, and 17-cellobiosyl **6** conjugates (Fig. 1). Fulvestrant is currently an injectable product with low oral bioavailability. These compounds were expected to enhance oral bioavailability and also improve water solubility.

Preparation of the phenolic 3-glycosides **2** and **5** proved relatively straightforward. 2,3,4,6-Tetra-*O*-acetylglucose-1-α-trichloroacetimidate **7** was prepared from pentaacetylglucose via selective anomeric deacylation using benzylamine[3] (90%) followed by cesium carbonate-mediated reaction with trichloroacetonitrile[4] (80%). Similarly, 2,3,6,2′,3′,4′,6′-hepta-*O*-acetylcellobiose-1-α-trichloroacetimidate **8** was prepared from octaacetylcellobiose via anomeric deacylation using hydrazine acetate[5] (69%) followed by imidate formation (94%). The known imidates so obtained were condensed with 17-acetylfulvestrant **9**[6] using $BF_3{\cdot}OEt_2$ as catalyst by means of the inverse addition method.[6] A solution of imidate was added dropwise over 5 min to a premixed solution of the catalyst and alcohol in 1,2-dichloroethane (1,2-DCE) at −20 °C (Scheme 1). The peracetylated 3-glycosides **10** and **11** so obtained were deprotected using NaOH in MeOH–water, providing the 3-glucosyl **2** and 3-cellobiosyl **5** conjugates in good overall yield after purification by reverse-phase chromatography.

Previous exploration[6] of the 17-glucuronidation of **1** found significant advantage in the use of the inverse addition technique and also established that use of the more hindered isobutyryl protecting group for the glycosyl donor reduced the amount of transacylation, the major reaction when using acetyl protection. We sought to investigate these findings further by exploring the reaction of 3-acetylestradiol[7] with a range of differently protected glucose imidates (Table 1) and found pivaloyl protection to be the favored choice.

Despite its apparent usefulness we found little precedent[8] for the use of the promising intermediate, 2,3,4,6-tetra-*O*-pivaloylglucose-1-α-trichloroacetimidate[9] **12** in

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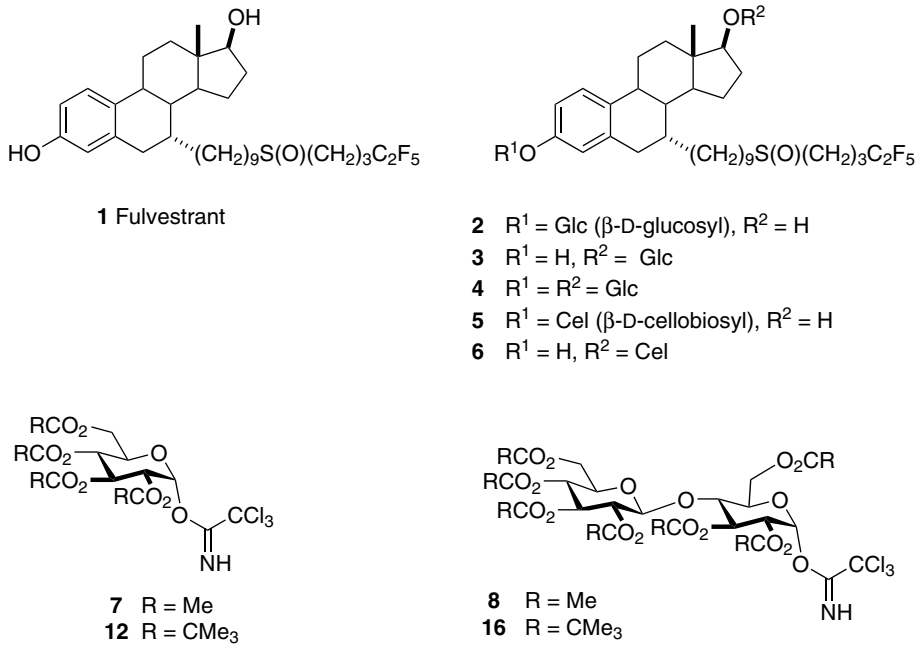
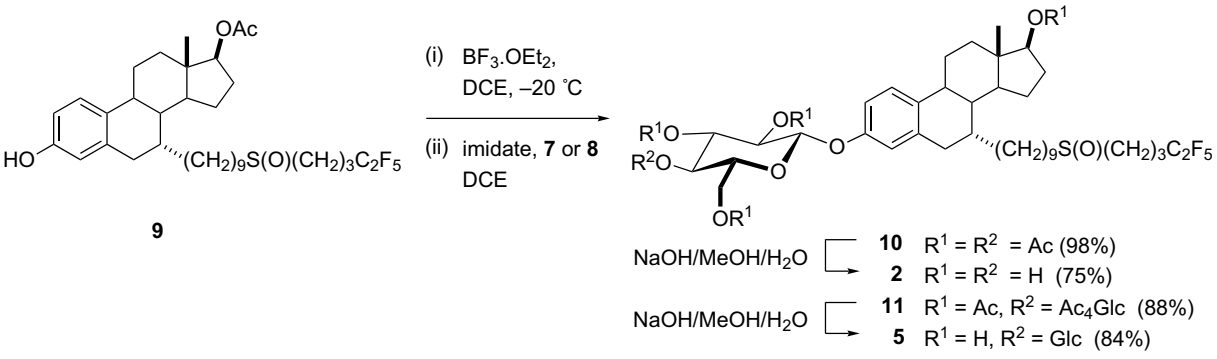


Figure 1. The structure of fulvestrant, imidates, and the glycoside derivatives prepared.



Scheme 1. Glycosylation at the phenolic 3-position of 17-acetylfulvestrant.

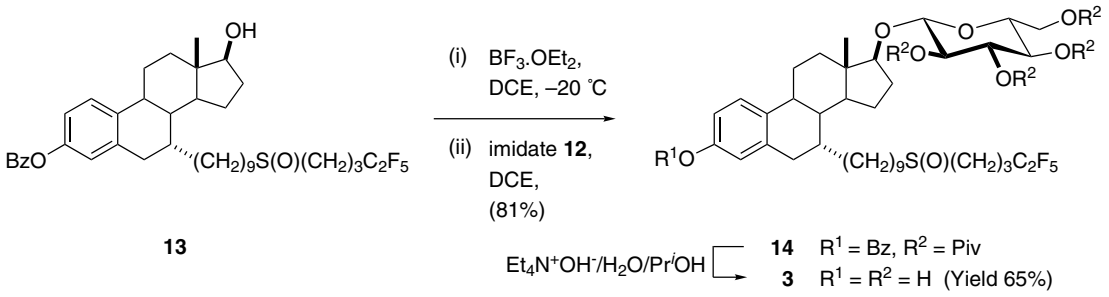
Table 1. Comparative yields for glucosylation of 3-acetylestradiol using differently protected glucose imidates (reactions were performed at −20 °C using 0.5 equiv $BF_3{\cdot}OEt_2$ and 1.5 equiv of imidate)

Imidate protecting group	Yield of 3-acetylestradiol 17-glucoside, %	Yield of transacylation product, %
Benzoyl	22	45
Isobutyryl	23	37
Pivaloyl	68	Trace

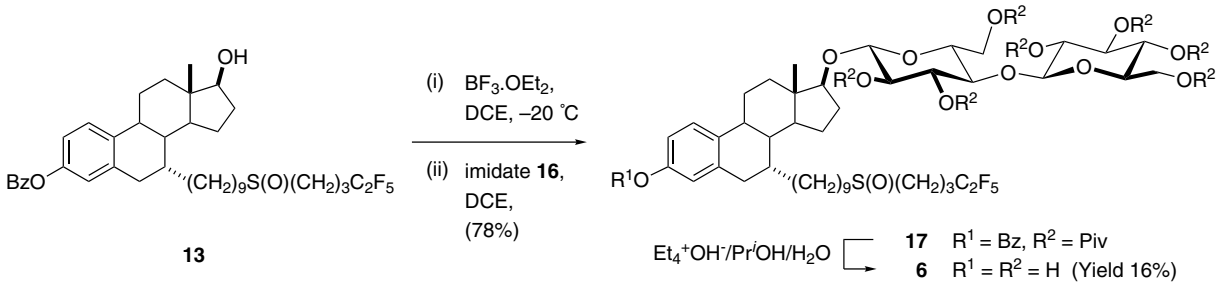
the glycosylation of hindered alcohols. We established that **12** could best be prepared by anomeric deprotection of pentapivaloylglucose[10] using hydrazine monohydrate in THF (50 °C, 7 d, 61%) and subsequent imidate formation by the usual method (81%).

Glycosylation of 3-benzoylfulvestrant[6] **13** using **12** was also found to be very efficient (81% yield). The peracylated glycoside **14** could best be deprotected using tetraethylammonium hydroxide in 2-propanol–water[11] (Scheme 2) giving conjugate **3**. In a similar fashion, direct glycosylation of fulvestrant (80%) using imidate **12** (3.5 equiv) and $BF_3{\cdot}OEt_2$ (2.5 equiv) to give intermediate **15** followed by deprotection of the pivaloyl groups (63%) provided the 3,17-bisglucoside **4**.

In order to access the 17-cellobiosyl conjugate **6**, the novel 2,3,6,2′,3′,4′,6′-hepta-*O*-pivaloyl-cellobiose-1-α-trichloroacetimidate **16** was prepared by peracylation of β-D-cellobiose with pivaloyl chloride (26%; a heptapivaloyl product was also separated chromatographically) followed by anomeric deprotection (67%) and imidate formation (88%). Glycosylation of 3-benzoylfulvestrant **13** using **16** proceeded in high yield (Scheme 3); however, in the deprotection of glycoside **17**, 17-cellobiosyl conjugate **6** was only obtained as a minor product (16%). The major product (54%) was established by NMR and MS to be a dipivaloyl derivative of **6** whose regiochemistry is unknown.[12] Optimization of this final step is therefore necessary to effect complete deprotection of **17**.



Scheme 2. Glycosylation at the hindered 17-position using a pivaloyl-protected imidate.



Scheme 3. Pivaloyl protection of the imidate of the disaccharide cellobiose also leads to effective glycosylation of the hindered 17-hydroxy group.

Table 2. Summary of yields obtained for the glycosylation step in the synthesis of compounds **2**–**6**

Substrate	Sugar	Imidate protecting group	Yield, % (product)
17-Ac Fulvestrant **9**	Glucose	Ac	98 (**10**)
17-Ac Fulvestrant **9**	Cellobiose	Ac	88 (**11**)
3-Bz Fulvestrant **13**	Glucose	Piv	81 (**14**)
3-Bz Fulvestrant **13**	Cellobiose	Piv	78 (**17**)
Fulvestrant **1**	Glucose	Piv	80 (**16**)

In conclusion, we have prepared five glycoside derivatives of fulvestrant and have achieved high glycosylation yields using the appropriate choice of protecting groups (Table 2). These results particularly establish the usefulness of pivaloyl-protected sugar imidates in the glycosylation of the hindered 17-position and show the advantage offered by this approach over more commonly employed protection strategies. Although they have not been widely used, we hope the present work will establish their value in the general glycosylation of hindered alcohols. The inverse addition method was found to be superior for glycosylation using both acetyl- and pivaloyl-protected imidates. Finally, only the β-glycosides were isolated in every case described (e.g., in **14**, $\delta_{H1'} = 4.62$, d, $^3J_{H1'-H2'} = 7.2$ Hz).

2. Experimental

2.1. Representative procedure for glycosylation

Boron trifluoride diethyl etherate (0.65 ml, 1.5 equiv) was added to a solution of 3-benzoylfulvestrant **13** (2.36 g, 3.32 mmol) in dry 1,2-DCE (16 ml) at −20 °C (ice–salt cooling) under Ar. After 45 min, a solution of 2,3,4,6-tetra-*O*-pivaloylglucose-1-α-trichloroacetimidate **12** (3.9 g, 1.75 equiv) in the same solvent (16 ml) was added dropwise over 5 min. The mixture was stirred for 4 h, by which time the temperature had risen to +10 °C. Solvent was evaporated and the residue taken up in DCM then washed with satd aq $NaHCO_3$ and then water. After evaporation of solvent, purification by silica column chromatography (20–25% EtOAc in toluene) gave pure glycoside **14** (3.27 g, 81%) as a white foam after re-evaporation from ether.

2.2. Representative deprotection procedure

Acylated glycoside **14** (1.20 g, 0.99 mmol) was dissolved in 2-propanol (19 ml) and the solution cooled to 0 °C then tetraethylammonium hydroxide (35% w/w aq soln, 2.9 ml, 7 equiv) was added dropwise. After stirring for 9 h, the reaction was complete as evidenced by TLC (10% MeOH–DCM). Aqueous methanol was added then the pH adjusted to 7.0 by addition of glacial AcOH. After evaporation, the residue was purified on a Lichroprep RP-18 column using a gradient of 50–95% MeOH in water to provide 17-β-glucosylfulvestrant **3** (0.50 g, 65%). δ_H (400 MHz, DMSO-d_6) 9.00 (br s, 1H), 7.05 (d, 1H, $J = 9.1$ Hz), 6.50 (dd, 1H, $J = 9.1$ Hz, 2.5 Hz), 6.41 (d, 1H, $J = 2.5$ Hz), 4.96–4.82 (br m, 3H), 4.50–4.44 (br t, 1H), 4.21 (d, 1H, $J = 7.5$ Hz), 3.75 (t, 1H, $J = 6.9$ Hz), 3.69–3.62 (m, 1H), 3.48–3.38 (m, 1H), 3.15–3.07 (m, 1H), 3.07–2.99 (m, 2H), 2.95–2.54 (m, 7H), 2.47–2.27 (m, 2H), 2.27–2.11 (m, 2H), 2.01–1.83 (m, 4H), 1.69–1.41 (m, 5H), 1.41–1.08 (m, 18H), 0.96–0.82 (br m, 1H), 0.78 (s, 3H). HRMS (FAB+), calcd for $C_{38}H_{58}O_8F_5S$ M+H 769.3773, found 769.3765 (MH^+). HPLC (Luna-ODS column; 1 ml min^{-1}; MeCN KCl_{aq} (30 mM), pH 2.2, 60:40; λ 275 nm), rt 2.88 min, purity 99.7%.

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12. Due to steric considerations, we believe this major product is most likely to be the 3,2′-dipivaloylcellobiosyl derivative.