ARTICLES

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Glossary of Terms in Computational Drug Design

Computational drug design is a rapidly growing field which is now an important component in the discipline of medicinal chemistry. At the same time many medicinal chemists lack significant formal training in this field and may not have a clear understanding of some of the terminology used; however they need to grasp concepts, follow research results, define problems, and utilize the findings. In this context IUPAC felt it would be useful to develop a glossary of terms used in computational drug design for easy reference purposes. Accordingly a working party of seven experts in the field have constructed a glossary of some 108 terms. Concise but explanatory definitions have been formulated based on a variety of literature sources and selected key references provided.

Copies of the text may be obtained from Dr Alan McNaught, The Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge CB4 4WF. IUPAC would welcome comments (by **31 May 1996**) before preparation of a final draft for publication in *Pure Appl. Chem.*

Regiospecific synthesis of 4-fluoro-Δ^4,3-keto steroids using caesium fluoroxysulfate

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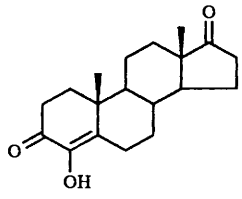
The regiospecific synthesis of two A-ring fluorinated steroids is described. Treatment of a 4-trimethylstannyl Δ^4,3-keto steroid with caesium fluoroxysulfate gave the corresponding fluorinated steroid in good yield; use of the *N*-fluorodiazabicyclic reagent, Selectfluor, as the fluorine source provides the same compounds in lower yield. The fluorination was found to be sufficiently rapid to be potentially useful in the synthesis of ^{18}F labelled steroids for PETT applications.

Introduction

The synthesis of modified steroids has been extensively studied over the last few decades, mainly with regard to the search for chemotherapeutic reagents. Many developmental and physiological processes in organisms ranging from fungi to humans are regulated by a small number of steroid hormones, all of which are biosynthesised from cholesterol. These are small, hydrophobic molecules that cross cell membranes easily and they exert much of their influence at receptors that regulate gene transcription. One of the most important of aspects of steroid research has been the discovery that analogues of some endogenous steroids can be used to regulate the synthesis and modification of some of the steroids involved in the proliferation of malignant tumours. For example, although breast cancer is one of the most common malignancies in women today, it is a well understood disease and surgery is gradually being replaced by drug treatment. The incidence of breast cancer has been reliably linked to elevated levels of the steroid estrone, which is synthesised from androgens. Suppression of peripheral estrogen production, by drug-mediated inhibition of estrone aromatase (the enzyme that converts androgens to estrogens) is one way of achieving clinical benefit.[1]

Consideration of the mechanism involved in the enzyme catalysed conversion of androgens into estrogens led several groups to suggest the investigation of 4-substituted derivatives of Δ^4,3-keto steroids as mechanism-based ('suicide') inhibitors of the enzymes involved, in order to develop chemotherapeutic agents.[2] Several 4-substituted derivatives of Δ^4,3-keto steroids have been evaluated as potential therapeutic compounds, for example in the treatment of prostatic carcinoma[3] and estrogen-dependent mammary tumours.[4] Covey proposed a mechanism for the time-dependent inhibition of aromatase by 4-hydroxyandrostene-3,17-dione **1** that involved formation of a covalent bond between the enzyme and the 4-position of the steroid nucleus.[5] The fluoro analogue of **1**, 4-fluoroandrostene-3,17-dione **2** has also been synthesised and was found to inhibit aromatase with a potency similar to **1**.[4] The therapeutic usefulness of **1** is limited by its poor bio-availability and rapid glucuronidation, but it might be envisaged that the fluoro analogue would overcome this problem as it is more lipophilic and lacks the conjugate-forming hydroxy group.

One of the first reports detailing a modification of the biological properties of a steroid by chemical transformation into a substituted analogue was made by Fried in the 1950s.[6] This work related to the anti-inflammatory properties of 9α-fluorohydrocortisone acetate and was also one of the first



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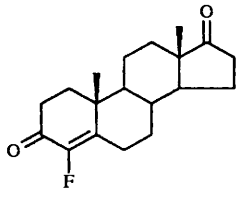

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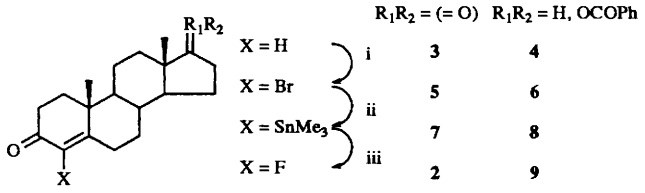
reported examples of the use of a fluorine substituent to modify the activity of a potential therapeutic agent. In recent years the use of fluorine as a tool for manipulating the properties of a biologically active compound has become extremely common, for reasons that are well documented,[7] and consequently much work has been dedicated to the development of mild, selective methods for the incorporation of this moiety. In addition, the development of Positron Emission Transaxial Tomography (PETT scanning) as a non-invasive technique for the visualisation of neuronal function and also for the imaging of receptor-positive tumours,[8] has led to interest in the synthesis of ^{18}F labelled reagents; ^{18}F is a positron emitter with a half-life of around 110 min. Current techniques for the synthesis of fluorine labelled probes are often based on exhaustive fluorination of the parent molecule with ^{18}F, followed by a time-consuming purification process to isolate the small amount of the desired compound. The time taken for the isolation of the desired material is obviously critical because of the relatively short half-life of the label. Therefore there is a clear need for a rapid method for the synthesis of ^{18}F labelled compounds, in a process that generates the minimum number of by-products, allowing rapid isolation of the desired product. The synthesis of ^{18}F labelled steroids has received a lot of attention because of the potential use of such compounds for the imaging of tumours on the basis of their steroid-receptor content.[9]

We have recently reported the application of a mild fluorodemetallation approach to the regioselective synthesis of fluoroalkenes and fluoroindoles,[10] using the electrophillic fluorinating reagent caesium fluoroxysulfate (CFS). This process is characterised by a fast and clean fluorination step allowing rapid isolation of the fluorinated product, essential features for the synthesis of a radio-labelled compound. It therefore appeared likely that if a 4-trimethylstannyl Δ^4,3-keto steroid could be prepared then this would provide a regioselective route to compounds such as **2** *via* a fluorodestannylation process. In addition, such a process



Scheme 1 *Reagents and conditions:* i, Br_2, AcOH, ethylene oxide; ii, $Pd(PPh_3)_4$, toluene, $(Me_3Sn)_2$, reflux; iii, CFS, CH_2Cl_2, MeOH

should be sufficiently rapid to allow its use in the synthesis of ^{18}F labelled steroids simply by employing radio-labelled fluorine in the synthesis of the caesium fluoroxysulfate.

Results and discussion

As the synthesis of alkenyltrialkylstannanes has been achieved by palladium catalysed coupling of alkenyl triflates,[11] and alkenyl halides,[12] it was considered that a potentially useful route to the trimethylstannyl precursor of **2** might be *via* bromination of the parent non-fluorinated steroid, followed by coupling with hexamethylditin.

Bromination at the 4-position of Δ^4,3-keto steroids with bromine (in acetic acid and ethylene oxide) has been reported.[13] Application of this methodology provided the 4-bromo derivative of androstenedione **5**, as well as that of a related steroid, *O*-benzoyltestosterone **6**, in 61 and 76% yields respectively. The 4-bromo steroids underwent coupling with hexamethylditin in toluene, catalysed by tetrakis(triphenylphosphine)palladium(0), to give the desired stannanes **7** and **8**, both in high yield. Fluorination of these stannanes with CFS under standard conditions (0 °C in dichloromethane–methanol) provided the fluoro derivatives **2** and **9** in 64–80% yield. Conversion of the vinylstannane into the vinyl fluoride was almost complete within 45 min, as judged by HPLC. The only other product detected in the reaction mixture in each case was the parent, protio-destannylated steroid.

Several recent reports have detailed the success of alternative electrophilic fluorine sources, such as xenon difluoride,[14] *N*-fluorosulfonamides[15,16] and the *N*-fluorodiazabicyclic reagent, Selectfluor {1-chloromethyl-4-fluoro-1,4-diazabicyclo[2.2.2]-octane bis(tetrafluoroborate), 'F-TEDA $(BF_4)_2$}™,[17] for the synthesis of fluoroalkenes *via* fluorodemetallation. We have evaluated one of these reagents, the commercially available Selectfluor (from Air Products PLC), for the fluorodestannylation described above. In this case Selectfluor provided slightly inferior results when compared to CFS, with **2** and **9** being isolated in 52 and 50% yield, respectively. This is broadly in agreement with previously reported observations of the reactivity of Selectfluor; this reagent tends to react more completely with stronger nucleophiles such as Grignard species.[17]

These results demonstrate the usefulness of fluorodestannylation as a general synthetic procedure for the preparation of interesting fluoro-organics, and, because of the fast and selective nature of the fluorination step, the potential for use in the synthesis of ^{18}F labelled compounds for PETT studies.

Experimental

Melting points were determined on a Gallenkamp MFB apparatus and are uncorrected. ^1H NMR were recorded on a Bruker AC (200 MHz) spectrometer and ^{19}F NMR on a Bruker AMX (600 MHz) spectrometer. Chemical shifts are reported in ppm downfield from tetramethylsilane (^1H NMR) or chlorotrifluoromethane (^{19}F NMR). Apparent *J* values are given in Hz and multiplicities are recorded as: s, singlet; d, doublet; t, triplet; q, quartet. Mass spectra are EI (Concept 32, Kratos) or FAB (MS50, Kratos). High resolution mass spectra were recorded on a Concept 32 using the EI technique. IR spectra were recorded on a Bruker IFS 66 spectrometer. Microanalyses were carried out in the Department of Physical Sciences, Wellcome Research Laboratories, Beckenham. All reactions involving the fluorinating reagents CFS and Selectfluor were carried out under an atmosphere of dry, oxygen-free nitrogen. All solvents were either purchased as anhydrous or distilled prior to use. Column chromatography was carried out using Silica gel 60 supplied by Merck Chemicals; elution was with mixtures of diethyl ether or ethyl acetate and cyclohexane. Other reagents were purified according to literature procedures.[18]

Selectfluor was a kind gift from Air Products and Chemicals PLC. Fluorine gas (5% in nitrogen) and handling equipment were purchased from Gas and Equipment PLC. All other starting materials were purchased from The Aldrich Chemical Co.

Caesium fluoroxysulfate

The method employed for the synthesis of CFS was an adaption of the method described by Appelman.[19] A solution of caesium sulfate (2 mol dm^{-3} in distilled and deionised water) was placed in a Teflon bottle and cooled in a salt–ice bath (bath temperature −10 °C). The solution was flushed with a slow stream of nitrogen for 10 min and then fluorine (5% in nitrogen) was bubbled slowly through the solution for 30 min. A fine white precipitate was removed by filtration through a sintered funnel and washed with a little cold deionised water. The combined filtrates were returned to the fluorination apparatus for a further 30 min and the process repeated. This procedure could be repeated up to 4 times without change in the quality of the CFS produced. Each batch of precipitated CFS was dried under nitrogen on the filter and then transferred to a Teflon bottle and further dried under vacuum over phosphorus pentoxide and then stored under nitrogen at −5 °C. Typical total yields were 2.5 g of CFS from 10 g caesium sulfate (36%).

The CFS synthesised by this method was usually used within 5 days and was always handled with a Teflon spatula. The quoted amounts of CFS employed in the fluorinations described below refer to CFS that has been standardised by the method described by Appelman. A weighed portion of CFS (100–150 mg) was placed in a conical flask and an excess (>4 equiv.) of aq. potassium iodide (0.1 mol dm^{-3}) and acetic acid (2–3 drops) were added. The resulting solution was titrated for I_3^- with aq. sodium thiosulfate (0.1 mol dm^{-3}) using starch as an endpoint indicator. Typical fluoroxysulfate titres were 80–85% for a fresh sample (1–5 days old) and 50–60% for a sample over 1 week old. It should be noted that this method of analysis fails to take into account the presence of peroxydisulfate, which can arise during decomposition of CFS, and would distort the measurement of the oxidising power of the batch of CFS.

4-Bromoandrost-4-ene-3,17-dione 5

A dark glass bottle was cooled to −35 °C and filled with ethylene oxide (25 cm^3) under nitrogen. Androst-4-ene-3,17-dione (1.43 g, 5 mmol) was added in one portion, followed by dropwise addition of a solution of bromine (0.32 cm^3, 6 mmol) in acetic acid (5 cm^3) over 30 min. The mixture was stirred at −30 °C for 10 h after which it was stored in a freezer overnight. The dark orange solution was slowly poured into stirred, saturated aq. sodium hydrogen carbonate and diethyl ether was then added. The organic phase was separated, washed with water, dried and evaporated. The residue was purified by column chromatography on silica to give the *title product* (1.12 g, 61%) as a white powder, softens 51 °C, mp 80 °C (Found: C, 62.2; H, 6.9. $C_{19}H_{25}BrO_2$ requires C, 62.47; H, 6.85%);

ν_{max}(KBr)/cm^{-1} 2951, 2908, 1736, 1682, 1572, 1190 and 800; δ_H($CDCl_3$) 0.9–2.7 (19 H, m, aliphatics), 0.92 (3 H, s, 18-H), 1.24 (3 H, s, 19-H) and 3.35 (1 H, dt, *J* 12 and 4, 6α-H); *m/z* (EI) 364 and 366 (M^+) and 285.

17-*O*-Benzoyl-4-bromotestosterone 6

Ethylene oxide (20 cm^3) was cooled to −25 °C under nitrogen and 17-*O*-benzoyltestosterone (1.0 g, 2.54 mmol) added. A solution of bromine (0.16 cm^3, 3.05 mmol) in acetic acid (4 cm^3) was added dropwise to the mixture, and stirring continued at −25 °C for 24 h with protection from light. The solution was stored at −15 °C for 48 h after which it was filtered through basic alumina, evaporated and purified by column chromatography on silica to give the *title product* (910 mg, 76%) as a white powder, mp 175 °C (Pr^iOH) (Found: C, 66.2; H, 6.7. $C_{26}H_{31}BrO_3$ requires C, 66.24; H, 6.58%); ν_{max}(KBr)/cm^{-1} 2947, 1715, 1680, 1576, 1450, 1275, 1117 and 717; δ_H($CDCl_3$) 0.95–2.70 (18 H, m, aliphatics), 0.98 (3 H, s, 18-H), 1.25 (3 H, s, 19-H), 3.32 (1 H, dt, *J* 10 and 3, 6α-H), 4.87 (1 H, t, *J* 6, 17-H), 7.40–7.60 (3 H, m, Ar) and 8.00–8.10 (2 H, m, Ar); *m/z* (EI) 470 and 472 (M^+).

4-Trimethylstannylandrost-4-ene-3,17-dione 7

A solution of 4-bromoandrost-4-ene-3,17-dione (300 mg, 0.82 mmol) in dry toluene (20 cm^3) was stirred under nitrogen and tetrakis(triphenylphosphine)palladium(0) (100 mg, 12 mol%) was added followed by hexamethylditin (300 mg, 0.9 mmol). The resulting solution was heated at reflux overnight and then cooled and filtered. Evaporation of the filtrate gave a residue, which was chromatographed on silica to give the *title product* (310 mg, 84%), as a white solid, mp 152–154 °C (Found: C, 58.9; H, 7.4. $C_{22}H_{34}O_2Sn$ requires C, 58.84; H, 7.58%); δ_H($CDCl_3$) 0.20 (9 H, m, $SnMe_3$), 0.92 (3 H, s, 18-H), 1.22 (3 H, s, 19-H) and 1.25–2.55 (19 H, m, aliphatics); *m/z* (EI) 450 (M^+), 435 and 405.

17-*O*-Benzoyl-4-trimethylstannyltestosterone 8

A solution of 17-*O*-benzoyl-4-bromotestosterone (200 mg, 0.42 mmol) in dry toluene (20 cm^3) was stirred under nitrogen and hexamethylditin (160 mg, 0.51 mmol) was added followed by tetrakis(triphenylphosphine)palladium(0) (50 mg, 10 mol%). The initially pale green solution was heated to reflux for 10 h to give a dark solution which was cooled and filtered. Evaporation of the filtrate gave a residue, which was purified by column chromatography on silica to give the *title product* (145 mg, 62%) as a white powder, mp 165 °C (Found: C, 62.5; H, 7.4. $C_{29}H_{40}O_3Sn$ requires C, 62.72; H, 7.21%); ν_{max}(KBr)/cm^{-1} 2949, 2873, 1711, 1655, 1574, 1450, 1282, 1115, 781 and 715; δ_H($CDCl_3$) 0.25 (9 H, m, $SnMe_3$), 0.90–2.50 (18 H, m, aliphatics), 1.00 (3 H, s, 18-H), 1.22 (3 H, s, 19-H), 4.85 (1 H, t, *J* 6, 17-H), 7.35–7.60 (3 H, m, Ar) and 8.00–8.10 (2 H, m, Ar); *m/z* (EI) 556 (M^+), 541, 511 and 391.

Fluorinations with caesium fluoroxysulfate

4-Fluoroandrost-4-ene-3,17-dione 2. A solution of 4-trimethylstannylandrost-4-ene-3,17-dione **7** (200 mg, 0.44 mmol) in dichloromethane (10 cm^3) and methanol (5 cm^3) was cooled in an ice bath under nitrogen and caesium fluoroxysulfate (330 mg, 0.53 mmol) was added. The mixture was stirred and allowed to warm to room temperature overnight, after which it was washed with saturated aq. potassium fluoride (50 cm^3), filtered and evaporated to give a residue. Column chromatography on silica of the residue gave the product (119 mg, 88%) as a white solid, mp 177–178 °C (Found: C, 74.9; H, 8.6. $C_{19}H_{25}FO_2$ requires C, 75.0; H, 8.22%); ν_{max}(KBr)/cm^{-1} 2954, 2941, 1741, 1639, 1363, 1323, 1151, 1084 and 1020; δ_H($CDCl_3$) 0.90–2.58 (18 H, m, aliphatics), 0.93 (3 H, s, 18-H), 1.27 (3 H, s, 19-H) and 3.02 (1 H, dt, *J* 12 and 3, 6α-H); δ_F($CDCl_3$) −139 (s); *m/z* (FAB) 305 (MH^+).

17-*O*-Benzoyl-4-fluorotestosterone 9. A solution of 17-*O*-benzoyl-4-trimethylstannyltestosterone **8** (200 mg, 0.36 mmol) in dry dichloromethane (10 cm^3) and dry methanol (5 cm^3) was stirred under nitrogen and cooled in an ice bath. Caesium fluoroxysulfate (125 mg, 0.5 mmol) was added in one portion and the mixture stirred and allowed to warm to room temperature overnight. A small amount of white precipitate was filtered off and washed with dichloromethane. The combined filtrates were washed with saturated aq. potassium fluoride (50 cm^3) and then dried and evaporated to give a residue. Purification by column chromatography gave the *title product* (95 mg, 64%) as a white powder, mp 205–207 °C (Found: C, 75.9; H, 7.7. $C_{26}H_{31}FO_3$ requires C, 76.10; H, 7.76%); ν_{max}(KBr)/cm^{-1} 2949, 2937, 1715, 1694, 1279, 1115 and 719; δ_H($CDCl_3$) 0.99 (3 H, s, 18-H), 1.00–3.10 (19 H, m, aliphatics), 1.25 (3 H, s, 19-H), 3.00 (1 H, dt, *J* 13 and 2, 6α-H), 4.88 (1 H, t, *J* 6, 17-H), 7.35–7.55 (3 H, m, Ar) and 8.00–8.10 (2 H, m, Ar); δ_F($CDCl_3$) −139 (s); *m/z* (FAB) 411 (MH^+).

Fluorinations with Selectfluor

17-*O*-Benzoyl-4-fluorotestosterone 9. A solution of 17-*O*-benzoyl-4-trimethylstannyltestosterone **8** (100 mg, 0.18 mmol) in dry acetonitrile (20 cm^3) was treated with Selectfluor (100 mg, 0.23 mmol) and stirred at room temperature for 12 h. The resulting suspension was filtered, diluted with saturated aq. potassium fluoride (50 cm^3) and extracted with ethyl acetate. The combined extracts were dried and evaporated to give a residue. Chromatography on silica gave the title product (37 mg, 50%) as a white powder. Spectral characteristics were identical with those for the product prepared using CFS.

4-Fluoroandrost-4-ene-3,17-dione 2. Using the same method as above, the product was isolated as a white powder (7 mg, 52%). Spectral data were identical to those for the product prepared using CFS.

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