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Synthesis of Estrone-3-Sulfate Analogues Bearing Novel Non-Hydrolyzable Sulfate Mimetics

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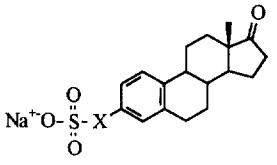
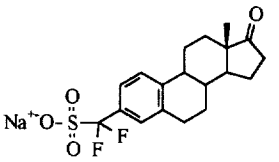
Abstract: Estrone sulfate analogues have been prepared in which the sulfate group has been replaced with an α,α-difluoromethylenesulfonate or α,α-difluoromethylenetetrazole group. The key step in these syntheses was the electrophilic fluorination of neopentylsulfonate ester and nitrile intermediates with N-fluorobenzenesulfonimide.

Keywords: steroids; halogenation; sulfonic acids and derivatives; tetrazoles

Estrogens and other steroids are essential for supporting the growth of approximately one-third of all breast tumours.[1] One possible source for estrogens in breast tumour cells is via hydrolysis of steroid sulfates via steroid sulfatases.[2] Thus, it is not surprising that in recent years there have been a number of reports describing the development of steroid sulfatase inhibitors.[3a-g] In a recent study conducted by Li and coworkers, a number of sulfonate analogues of steroid sulfates were examined as steroid sulfatase inhibitors.[3f,g] Among these analogues was compound **2** in which the labile sulfate ester oxygen of estrone sulfate (**1**) was replaced with a non-hydrolyzable methylene unit. Compound **2** was found to be a poor inhibitor of steroid sulfatase, exhibiting a K_i (140 μM) two orders of magnitude higher than the K_m for **1**.[3f] On the basis of these results and on studies with other sulfonate analogues, Li and coworkers concluded that an oxygen atom or an electronically and sterically similar link between the aryl moiety and the sulfur atom is essential for high affinity binding.[3f,g]



1, X = O; **2**, X = CH_2



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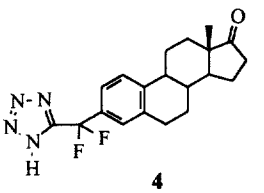

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As part of our program to develop steroid sulfatase inhibitors, we undertook a search for a link between the aryl moiety and the sulfur atom that would be electronically and sterically similar to oxygen. The difluoromethylene moiety has been used as an electronic and steric surrogate of labile oxygen atoms in phosphate esters ($R\text{-}CF_2\text{-}PO_3^{-2}$ vs. $R\text{-}O\text{-}PO_3^{-2}$). This group has been used extensively in the design of inhibitors of enzymes that hydrolyze or bind phosphate esters. The increased inhibitory potency of the α-fluorinated phosphonic acids compared to their non-fluorinated counterparts can be to attributed the lower pK_a values of the fluorinated phosphonic acids[4a-c] or to H-bonding interactions between the fluorines and specific residues in the active site.[4d,e]

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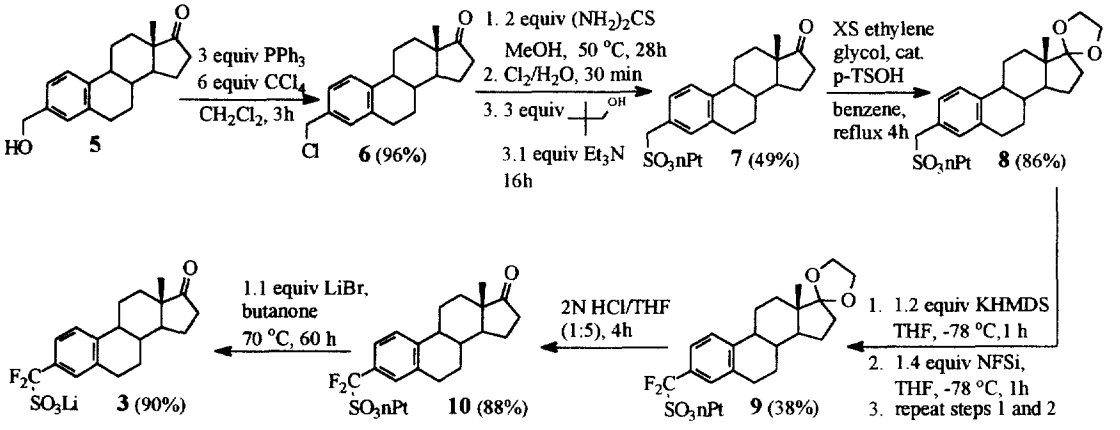
In the case of steroid sulfatases, it is unlikely that the poor binding of **2** compared to **1** is a result of the difference in pK_a values of the sulfonate and sulfate moieties since both the sulfate and sulfonate groups should be completely ionized at the pH under which the studies were performed. It is more likely that the poor inhibitory effect of **2** is a result of loss of an interaction (such as an H-bond) between the ester oxygen of estrone sulfate and the enzyme. Thus, we decided to examine whether the fluoromethylene moiety would be an effective replacement for the labile oxygen in steroidal sulfates in anticipation that the fluorines would interact with specific active site residues in a manner similar to that found with difluoromethylenephosphonic acid inhibitors of certain protein tyrosine phosphatases.[4d,e] In addition to finding a suitable non-hydrolyzable replacement for the labile ester oxygen of steroidal sulfates, we also were interested in developing inhibitors that did not bear a sulfate moiety. Recently, Tilley *et al* reported that the tetrazole group is an effective sulfate biostere for sulfotyrosine.[5] Consequently, we envisioned that the tetrazole group may be used as a sulfate surrogate in steroidal sulfates.

In this report we describe the synthesis of two novel estrone-3-sulfate analogues, **3** and **4**, in which the sulfate group is replaced with an α,α-difluorosulfonate (CF_2-SO_3^-) or α,α-difluorotetrazole (CF_2-tetrazole) group The key step in these syntheses was the electrophilic fluorination of an intermediate sulfonate ester and nitrile using the electrophilic fluorinating agent N-fluorobenzenesulfonimide (NFSi).

We have recently reported that neopentyl esters of simple benzylic α,α-difluorosulfonates can be obtained via electrophilic fluorination of the corresponding α-carbanions with NFSi.[6] Consequently, we reasoned that this methodology could be used for the preparation of more complex α-fluorinated sulfonates such as **3**. Our approach is outlined in Scheme 1. First, 3-hydroxymethylestra-1,3,5-(10)-triene-17-one[3f] (**5**) was converted into the alkyl chloride **6** in 96% yield using PPh_3/CCl_4. Using the procedure of Sprague and Johnson[7] for the synthesis of sulfonyl chlorides, **6** was reacted with thiourea in MeOH to give the

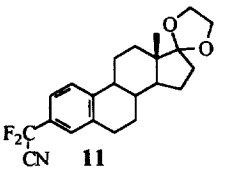


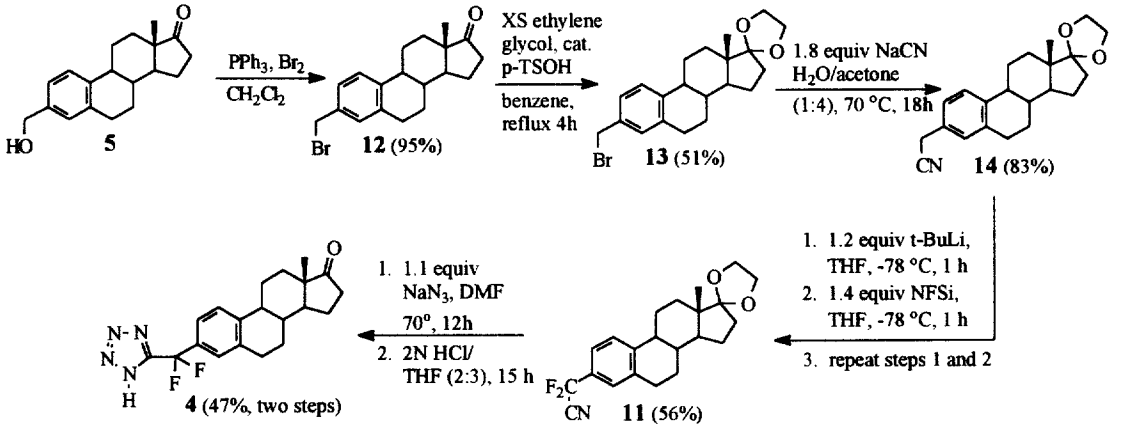
Scheme 1

hydrochloride salt of the isothiourea which was then converted into the sulfonyl chloride using Cl_2/H_2O. The crude sulfonyl chloride was reacted with neopentyl (nPt) alcohol to give the ester 7 (49%, 3 steps). The ketone moiety was protected as the cyclic ketal **8** in 86% yield by reacting 7 with excess ethylene glycol/cat. *p*-TSOH in refluxing benzene. We found that ester **8** could be difluorinated by reaction with 1.2 equiv of KHMDS[8] in THF at -78 °C for 1 hour, followed by the addition of a solution of 1.4 equiv NFSi in THF,

stirring for 1 hour and then repeating this process. After quenching the reaction with water followed by a standard work-up and chromatography, the fluorinated ester **9** was isolated in a 38% yield. Removal of the ketal protecting group in **9** using 2N HCl/THF (1:5) gave the ketone **10** in 88 % yield. The desired sulfonate **3** was obtained in 90% yield from **10** by heating **10** in a solution of LiBr in butanone for 60 hours.

Our approach to constructing **4** was to prepare the α,α-difluoronitrile precursor, **11**, and then convert this species into the tetrazole using NaN_3. The most common method for constructing α,α-difluoronitriles is by reacting difluorocarboxylic esters with ammonia and then dehydrating the resulting amide to the nitrile using P_2O_5.[9] However, we reasoned that applying this approach to the synthesis of **11** would require many steps. Recently, Hagele and Haas reported the synthesis of 2-phenyl-2,2-difluoroacetonitrile in 65% yield by converting benzoyl chloride to 2-phenyl-2-oxoacetonitrile followed by reaction with DAST in the presence of a catalytic amount of ZnI_2.[10] Although this methodology suggested a potentially effective route to **11**, we found that when performing this reaction on a model benzoyl chloride derivative, the yields were very low and products difficult to purify. We have recently reported that simple benzylic α,α-difluoronitriles can be obtained via electrophilic fluorination of the corresponding α-carbanions with NFSi.[6] Consequently, we reasoned that this approach may be used for preparing **11** from the non-fluoro analogue which we anticipated would be readily accessible.





Scheme 2

Our approach to **4** is outlined in Scheme 2. Compound **5** was converted into the alkyl bromide **12** in 95% yield using PPh_3/Br_2 (Scheme 2). The ketal moiety in **12** was protected as the cyclic ketal **13** in 51% yield using the same procedure described for **8**. Displacement of the bromide in **13** with cyanide gave the desired nitrile **14** (83%). As with the sulfonate derivative **8**, we have found that fluorines could be introduced alpha to the nitrile group in **14** using electrophilic fluorination. Thus, **14** was reacted with 1.2 equiv t-BuLi[11] in THF at -78 °C for 1 hour followed by the addition of 1.4 equiv NFSi in THF and this solution was stirred at -78 °C for 1 hour. After repeating this process followed by a standard workup and chromatography, the fluorinated nitrile **11** was isolated in a 56% yield. Compound **11** was converted into **4** by reacting it with NaN_3 in warm DMF to give the tetrazole followed by ketal hydrolysis with 2N HCl/THF (2:3) (47%, two steps).

In conclusion, estrone-3-sulfate analogues have been prepared in which the sulfate group is replaced with CF_2-sulfonates or CF_2-tetrazoles group. The key step in these syntheses was the electrophilic fluorination of neopentyl sulfonate ester **8** and nitrile **14** using NFSi. This synthesis demonstrates the utility of NFSi in the preparation of CF_2-sulfonates, CF_2-nitriles and CF_2-tetrazoles with potential biological applications. In addition, the CF_2-sulfonate and CF_2-tetrazole group may also find use in the development of inhibitors of other enzymes that bind or hydrolyze arylsulfates such as arylsulfatases A and B. The evaluation of **3** and **4** as inhibitors of steroid sulfatase is in progress and will be reported in due course.

Acknowledgement

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