Notes

Potential Organ or Tumor Imaging Agents. 16. Fluorinated Androstanes and the Prostate

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A series of substituted 5α-androstan-17β-ols was synthesized and evaluated for their potential use in the development of a prostate imaging agent. The ability of the synthesized compounds to compete with [^{3}H]-5α-dihydrotestosterone for rat prostate androgen receptor protein served as the screening assay. For 3-substituted derivatives, the order of binding to the androgen receptor protein was =O > -OH > H ≃ F. 3β-Fluoro-5α-androstan-17β-ol was found to have approximately 5% the androgenic activity of testosterone propionate in the castrated rat. The low biological activity for the 3β-fluoro derivatives, coupled with the synthetic obstacles associated with introducing fluorine-18, has led us to search for more suitable halo steroids as potential radiodiagnostics.

Satisfactory agents for the diagnosis and treatment of prostate disease are still unavailable. Over the past several years, efforts in our laboratory have been aimed at developing a radiodiagnostic suitable for imaging the prostate and associated tumors.

One of our approaches to this problem has been based on the following knowledge: (1) prostatic disease in general requires a functioning testis,[1] (2) a specific androgen receptor protein has been identified in prostatic tissue from various species,[2-5] and (3) high specific activity tritium-labeled 5α-dihydrotestosterone (5α-DHT) is selectively taken up by rodent prostate and other androgen target tissues.[6,7] Although it is not clear at this time whether the last two findings can be extended to man,[8-10] synthesis of 5α-DHT analogues labeled with γ-emitting nuclides appears to offer an attractive approach to a prostate imaging agent.

Previous structure–activity studies with analogues of 5α-DHT have shown that the C-3 ketone may not be essential for androgenic activity.[11] For example, Nutting and co-workers[12] showed that 17α-methyl-5α-androstan-17β-ol (11) possessed approximately 50% the androgenic activity of 17α-methyltestosterone when administered orally to rats. Wolff and Kasuya[13] subsequently pointed out, however, that this androgenic activity may result from the in vivo conversion of these 3-deoxy compounds to C-3 oxygenated metabolites. Indeed, they found that 11 underwent extensive oxygenation at C-3 in a rabbit liver homogenate.

Accordingly, our interest in 3-fluorinated androstanes was twofold. First, the availability of fluorine-18 for radiopharmaceutical synthesis[14] meant that androgens suitably labeled with this nuclide would have potential diagnostic value in prostatic disease. Secondly, such 3-fluorinated androstanes would not be expected to readily give rise to C-3 oxygenated derivatives in vivo. Thus a comparison of the receptor binding capacity of the 3-deoxy and 3-fluorinated androgens with the reported biological activities of these compounds would provide additional evidence regarding the necessity of a 3-oxygenated species for androgenic activity.

The important intermediate, 3β-fluoroandrost-5-en-17-one was prepared according to the procedure of Ayer[15] and involved treatment of dehydroepiandrosterone with *N*-(2-chloro-1,1,2-trifluoroethyl)diethylamine (FAR).[16] Subsequent reduction and alkylation of this intermediate led to the 3β-fluoro-5α-androstanes listed in Table I.

The ability of these fluorinated analogues to compete with [^{3}H]-5α-DHT in the receptor assay is also illustrated in Table I. The corresponding 3-unsubstituted and 3β-hydroxy analogues were included for comparison. The 3β-hydroxy-5α-androstanes displayed the greatest affinity for the receptor protein. This result is in accord with the finding that 5α-androstane-3β,17β-diol inhibits uptake of [^{3}H]-DHT in myometrial and mammary cancer tissue in vivo.[17] The 3-deoxy and 3β-fluoro analogues were very similar in their binding affinity, which was approximately 50–65% of that found for the 3β-hydroxy-5α-androstanes. Introduction of unsaturation at C-5,6 reduced binding affinity in all cases.

The essential equivalency in the binding affinity of the 3-deoxy and 3-fluorinated androstanes is in accordance with previous biological studies. Syndor[18] found that 3β-fluoro-5α-androstan-17β-ol (**3**) was equivalent to the 3-unsubstituted analogue **5** in promoting growth in hypophysectomized rats. On the bases of these findings then, it appears that C-3 oxygenated androstanes are not obligatory for androgenic activity in the rat.

In the castrated rat, **3** was found to have approximately 5% of the androgenicity of testosterone propionate following intramuscular administration. Thus, despite the apparent ability of **3** to bind to androgen receptor protein in vitro, its weak in vivo activity, coupled with the synthetic obstacles associated with introducing the short-lived ($t_{1/2}$ = 110 min) fluorine-18, has led us to search for more suitable halo steroids as potential radiodiagnostics.

Experimental Section

All melting points were taken on a Thomas-Hoover apparatus and are uncorrected. Microanalyses were performed by Midwest Microlab Inc., Indianapolis, Ind., and were within ±0.4% of the calculated values. IR spectra were determined on a Perkin-Elmer 281 spectrophotometer; mass spectra were performed on a Du Pont 21-490 mass spectrometer. 5α-Androst-2-en-17β-ol and 5α-androst-2-en-17-one were a gift from Searle Laboratories.

5α-Androstan-17β-ol (5). 5α-Androst-2-en-17β-ol (1 g, 3.65 mmol) was hydrogenated over palladium on charcoal (500 mg).

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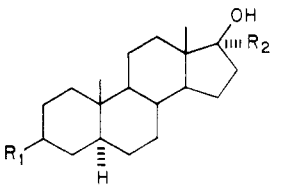
Table I. Percent Inhibition of [^{3}H]-5α-DHT Binding to Rat Prostate Androgen Receptor Protein in Vitro



Compd	Source[a]	R_1	R_2	% inhibn[b] Nucleus	% inhibn[b] Supernatant
5α-DHT				88.1	88.6
1	22	OH	H	72.7	77.4
2	23	OH	H 5-dehydro	53.0	53.5
3	24	F	H	48.8	54.4
4	24	F	H 5-dehydro	23.7	10.3
5		H	H	47.0	50.4
6	27	H	H 5-dehydro	10.2	18.0
7	25	OH	CH_3	47.5	43.8
8	22	OH	CH_3 5-dehydro	–[c]	19.5
9		F	CH_3	23.5	13.1
10	26	F	CH_3 5-dehydro	8.2	11.3
11		H	CH_3	11.2	15.5
12		H	CH_3 5-dehydro	–	30.2

[a] Compounds were prepared according to procedures described in the references cited. [b] See the Experimental Section for details of the assay. [c] The dash indicates that no inhibition was observed.

Recrystallization of the crude product from MeOH–H_2O afforded **5** (875 mg, 87%): mp 166–167 °C (lit.[19] mp 164–166 °C).

3β-Fluoro-17α-methyl-5α-androstan-17β-ol (9). A solution of 3β-fluoro-5α-androstan-17-one[24] (146 mg, 0.5 mmol) in ether (10 mL) was added dropwise to an ether solution of MeMgI prepared from magnesium (123 mg, 5.0 mmol). The reaction mixture was refluxed for 1 h and hydrolyzed with dilute NH_4Cl solution. The aqueous phase was extracted with ether, the ether extract dried (Na_2SO_4), and the solvent removed under reduced pressure. The residue was purified by preparative TLC [silica gel, benzene–ethyl acetate (10:1)]. Recrystallization of the product from MeOH–H_2O afforded pure **9** (108 mg, 70%): mp 153–156 °C; IR (KBr) 3385 cm^{-1}; EIMS *m/e* 308 (m).

17α-Methyl-5α-androstan-17β-ol (11). This compound was prepared in two steps from 5α-androst-2-en-17-one (1 g, 3.68 mmol). Hydrogenation over palladium on charcoal (500 mg) and subsequent treatment of the crude product with MeMgI furnished crude **11**. Crystallization from ether–hexane afforded pure **11** (547 mg, 51%): mp 163.0–163.5 °C (lit.[20] mp 162–164 °C).

17α-Methylandrost-5-en-17β-ol (12). A solution of androst-5-en-17β-ol (**6**) (100 mg, 0.36 mmol) in acetone (100 mL) was treated with Jones reagent. Filtration of the reaction mixture after dilution with H_2O gave a white solid showing a single spot on TLC. Without further purification, an ether solution of this solid was added to a solution of MeMgI prepared from magnesium (500 mg, 20.6 mmol). The resulting mixture was refluxed for 1 h and hydrolyzed with dilute NH_4Cl solution. The product was extracted with CH_2Cl_2 and crystallized from MeOH–H_2O to give **12** (78 mg, 74%): mp 152–153 °C; EIMS *m/e* 288 (m). Anal. ($C_{20}H_{32}O$) C, H.

Androgen Receptor Assay. Details describing this assay have been previously published.[21] The concentrations of [^{3}H]-5α-DHT and the test steroids that were added to the incubation media were 1.67×10^{-8} and 1.67×10^{-6} M, respectively. The inhibitory values listed in Table I were produced by comparing values obtained for the test steroids with control values in individual experiments, on a basis of dpm/mg of protein in the dialyzed 100 000g supernatant and dpm/μg of DNA in the nuclei. Values listed represent duplicate samples with less than 5.0% variance between samples.

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Ferrocenyl Polyamines as Agents for the Chemoimmunotherapy of Cancer

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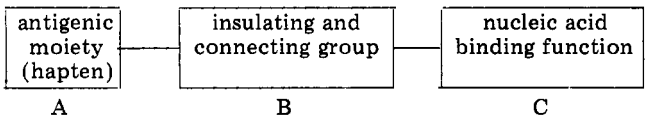
A series of ferrocenyl polyamines, compounds intended to bind to the tumor cell surface nucleic acid and elicit an immune response, was synthesized and screened for antitumor activity. Target ferrocenyl polyamines **1a,b**, **2**, and **3**, bearing 2-, 3-, and 4-amino groups, respectively, were readily obtained in yields of 31–58% from their corresponding ferrocenyl polyamides (**5a–d**) by reduction with diborane in THF; lithium aluminum hydride was not an effective reducing agent in this case. Although the target compounds failed to prolong the life of mice with P-388 lymphocytic leukemia, three of the intermediate amides (**5b–d**) did exhibit low but significant activity (T/C = 123, 132, and 120%, respectively).

The rationale and modus operandi for the development of chemoimmunotherapeutic agents has recently been described by Soloway et al.[1] Basically, the approach calls for the preparation of tagging haptens which could bind to the cell membrane of neoplastic cells, eliciting the generation of cytotoxic antibodies.[1] Chemoimmunotherapeutic agents, by selectively acting at the cell surface of the weakly antigenic tumor cells, would represent an advantage over the conventional antitumor agents which need to be delivered in high, and often toxic, levels to interfere with intracellular processes.

During the course of his work with the platinum–pyrimidine blues, Rosenberg uncovered evidence suggesting the presence of nucleic acids on the cell surface of tumor cells; none was found on normal cells.[2] In an attempt to exploit the apparent surface nucleic acid difference between tumor cells and normal cells, we have synthesized and evaluated the antitumor properties of a series of three-component compounds with the generalized structure



The intent was to produce materials that would interact strongly at site C with the tumor surface nucleic acid, thus anchoring the compounds to the cell. The hapten portion of the molecule, once conjugated with cell surface protein, would then, hopefully, stimulate antibody formation. Since the polyamines such as putrescine [$NH_2(CH_2)_4NH_2$], spermidine [$NH_2(CH_2)_3NH(CH_2)_4NH_2$], and spermine [$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$] are ubiquitous in nature, pose no specific toxicity problems, and are known to interact strongly with nucleic acids,[3] they were considered good candidates for the nucleic acid binding function. Of the various haptens available, ferrocene was chosen due to its demonstrated ability to elicit a strong antigenic response when conjugated to polypeptides.[4] As an insulating and connecting group, a methylene chain of at least four carbon atoms was used. The resulting ferrocenyl polyamine target compounds (**1a,b–3**) are shown in Chart I.

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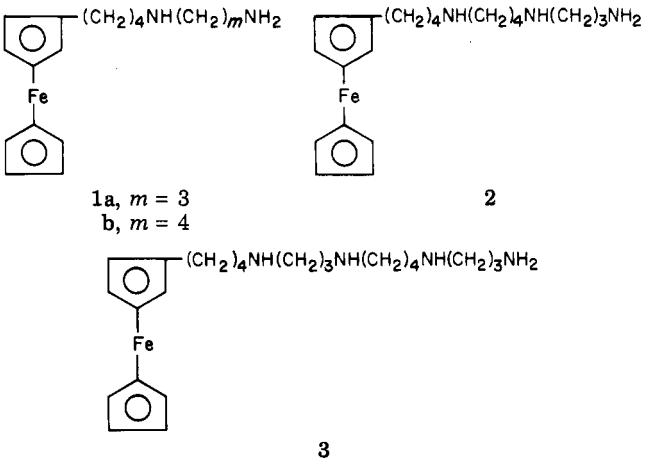
Chart I

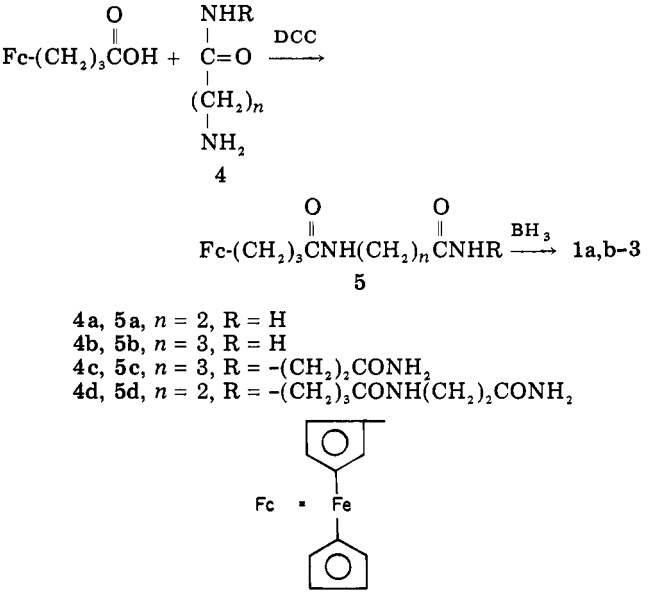


1a, $m = 3$
b, $m = 4$

2

3

Scheme I



Chemistry. Target polyamines **1a,b–3**, isolated as the hydrobromide salts, were readily obtained in moderate

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