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INHIBITION OF STEROID 5α-REDUCTASE BY "INVERTED", COMPETITIVE INHIBITORS

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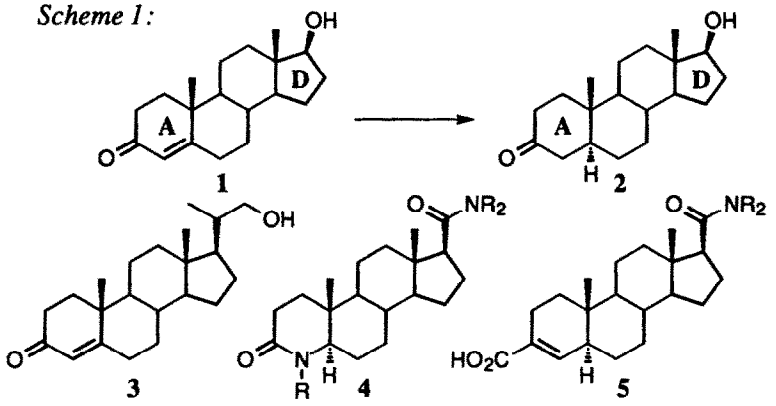
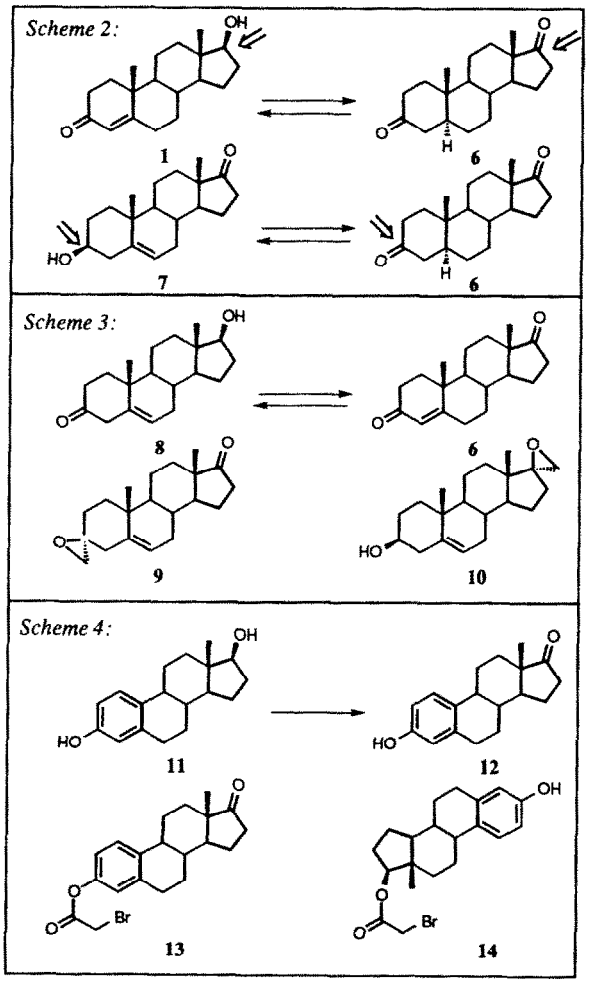
Abstract: Modified steroids, in which the D-ring has been converted to a six-membered ring lactam, were designed as inverted (or backward-binding), competitive inhibitors of steroid 5α-reductase.

Steroids play key physiological roles in the well-being of most forms of life. Their hormonal action, receptor binding properties and transcriptional regulatory properties continue to attract a great deal of scientific interest.[1] For many years, steroid-based compounds have been extremely important drugs for the treatment of many inflammatory disorders such as dermatitis, inflamed muscles, arthritis and asthma. In recent years, however, steroids have been implicated in a number of non-inflammatory diseases, in particular in hormone-dependent cancers, such as breast and prostate cancer.[1,2] A great deal of effort has been directed towards the design of inhibitors of prostatic steroid 5α-reductase to block the conversion of testosterone (**1**) to the more potent androgen, 5α-dihydrotestosterone (**2**) (Scheme 1) which is believed to be involved in the etiology of prostatic cancer.[3-5] In each of the major classes of 5α-reductase inhibitors (**3**, **4**, **5**), the molecules bind in the active site of the enzyme such that the steroid A-ring mimics the A-ring functionality of testosterone or some intermediate along the reaction pathway; the potency and selectivity is determined in part by appropriate substitution of the D-ring. We hypothesized that it should be possible to design inhibitors of steroid 5α-reductase in which correctly functionalized D- and A-rings of the putative inhibitor mimic the A-and D-rings, respectively, of testosterone or 5α-dihydrotestosterone. In other words, we wished to design 5α-reductase inhibitors based on the concept of "inverted" or "backward-binding" steroids.

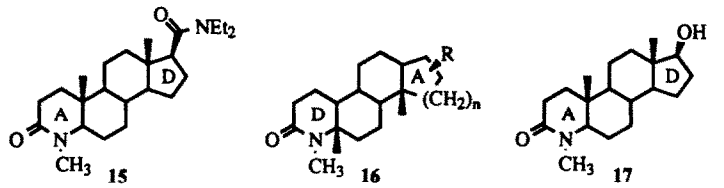
Scheme 1:



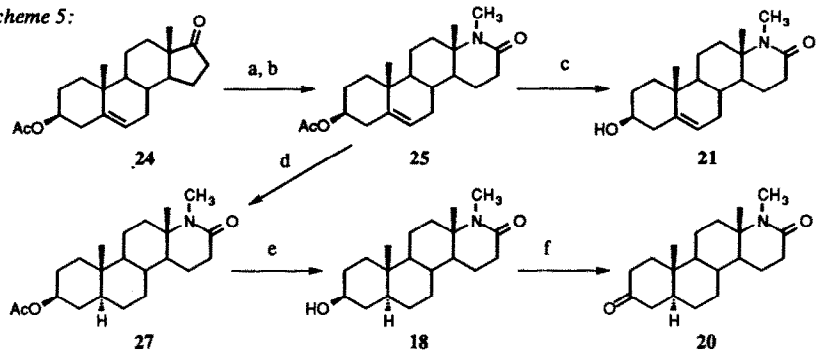
The idea that steroids have the potential to bind in two orientations in the active site of various metabolizing enzymes is not new. Marcus and Talalay[6] first reported that 3(17)β-hydroxysteroid dehydrogenase converts both testosterone (**1**) and dehydroepiandrosterone (**7**) to androst-4-ene-3,17-dione (**6**) (Scheme 2). More recently, two examples of enzyme inhibition by inverted steroids have appeared. The oxiranes **9** and **10** are active site-directed, irreversible inhibitors of 3-oxo-Δ^5-steroid isomerase (Scheme 3)[7] and the bromoacetates **13** and **14** act as affinity labels for estradiol 17β-dehydrogenase (Scheme 4).[8]



In this communication we wish to present some of our preliminary results which suggest it will be possible to design potent, backward-binding, competitive inhibitors of 5α–reductase, based initially upon the lactam steroids, such as **15**. The generalized backward-binding equivalent, therefore, would be **16**. Regarding the synthesis of **16**, we have prepared the azasteroids **18-23** and found encouraging inhibitory activity.

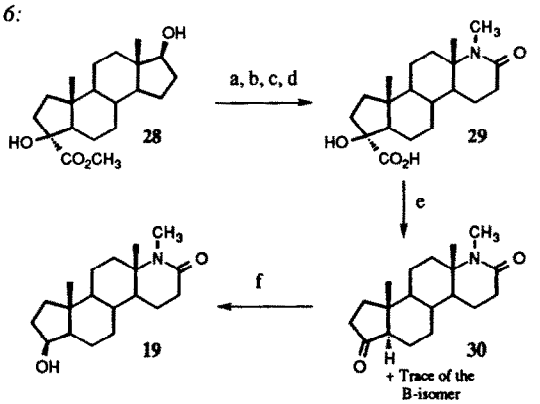


Scheme 5:



Conditions: a) $CH_3NHOH.HCl$, EtOH, $NaHCO_3$, RT, 16 h, 58% yield. b) TosCl, pyridine, H_2O, 0°C to RT, 1 h, 86%. c) K_2CO_3, CH_3OH, RT, 2 h, 86%. d) H_2, Pd/C, HOAc, 40 psi, 3 h, 67%. e) K_2CO_3, CH_3OH, RT, 2 h, 54%. f) Na_2CrO_7, HOAc, RT, 1 h, 48%.

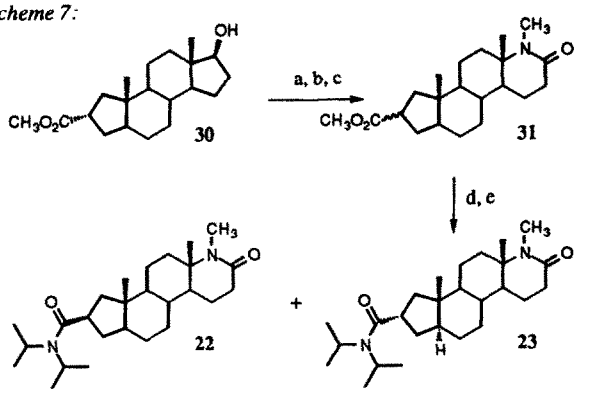
Scheme 6:



Conditions: a) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -50°C to RT, 1 h, 64% yield. b) $CH_3NHOH.HCl$, CH_3OH, $NaHCO_3$, RT, 72 h, 83%. c) TosCl, pyridine, H_2O, 5°C to RT, 1 h, 44%. d) LiOH, CH_3OH, H_2O, reflux, 2 h, then RT, 18 h, 99%. e) $NaBiO_3$, AcOH, H_2O, RT, 1 h, 99%. f) $NaBH_4$, EtOH, RT, 3 h, 47%.

The syntheses of **18**, **20**, and **21** is shown in Scheme 5.[9] Thus, Beckmann-type rearrangement of the nitrone derived from **24**[10] led to **25**[11] which was either hydrolysed to **21** or hydrogenated and further elaborated to **18** and **20**. Oxidation of the C-17 alcohol **28**[12] (Scheme 6) and further elaboration of the D-ring to the lactam proceeded well to give **29**. Decarboxylative oxidation[12] followed by hydride reduction led to **19**. The remaining

Scheme 7:



Conditions: a) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, RT, 72 h, 93%. b) $CH_3NHOH.HCl$, CH_3OH, $NaHCO_3$, RT, 72 h, 95%. c)TosCl, pyridine, H_2O, 0°C to RT, 1 h, 36%. d) LiOH, CH_3OH, H_2O, RT, 1 w, 88%. e) $(COCl)_2$, CH_2Cl_2, RT, 2h then iPr_2NH, CH_2Cl_2, RT, 2 h, ca. 37% of the mixture of 22 (3 parts) and 23 (1 part).

azasteroids, **22** and **23**, were prepared by the route shown in Scheme 7. Thus, conversion of known **30**[13] to the lactam **31** and functionalization of the acid group afforded a mixture of the amides **22** and **23**, which were separable by hplc.

These compounds were tested as inhibitors of human prostatic steroid 5α–reductase and compared to the azasteroid **17**[4] (Table). With the exception of **23**, the inverted azasteroids are approximately 10-100 less potent than the "normal" azasteroids. The compound **23**, which is α–substituted in the pseudo D-ring, was much less potent than its β-isomer **22**, which is in agreement with the observed stereochemistry in natural steroids. Clearly, however, these compounds are less potent than azasteroids such as **15** (IC_{50} = 8.6 nM)[4]. Molecular modeling studies, as well as chemical intuition, strongly suggest that other molecules of the generalized structure **16** will better fit the active site of the enzyme. We are pursuing synthetic chemistry towards these compounds.

Table. Inhibition of Human Prostatic Steroid 5α–Reductase[14]

Azasteroid	IC_{50} (μM)
17	>0.43[4]
18	4
19	12
20	15
21	32
22	40
23	>100

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9. Note: For ease of recognition, the steroids will be drawn in their well-recognized orientations in synthetic Schemes 4-6.

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