# 4,7β-Dimethyl-4-azacholestan-3-one (MK-386) and Related 4-Azasteroids as Selective Inhibitors of Human Type 1 5α-Reductase

Raman K. Bakshi,*,† Gool F. Patel,†
Gary H. Rasmusson,† Walter F. Baginsky,‡
George Cimis,‡ Kenneth Ellsworth,‡ Benedict Chang,‡
Herb Bull,‡ Richard L. Tolman,† and
Georgianna S. Harris‡

*Departments of Synthetic Chemical Research and Biochemistry, Merck Research Laboratories, Rahway, New Jersey 07065*

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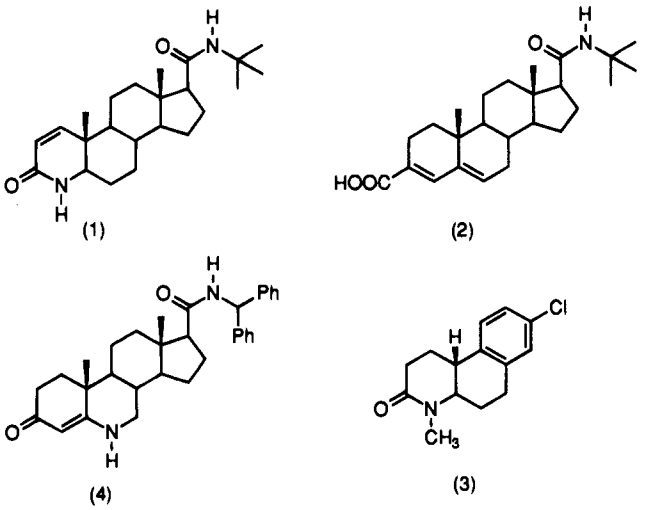
Steroid 5α-reductase catalyzes the stereoselective reduction of testosterone (T) to dihydrotestosterone (DHT), the androgen implicated in the development of benign prostatic hyperplasia (BPH),[1] acne,[2] hirsutism,[3] and male pattern baldness.[4] Early reports suggested the presence of more than one form of 5α-reductase in both human[5] and rat[6] tissues, although conclusions based on the analysis of steroid metabolites of a genetically deficient population implied that a single 5α-reductase[7] was present. It has now been shown that there are two genes encoding distinct 5α-reductases, types 1 and 2, in both the human and rat.[8] Workers from our laboratories have studied the biochemical properties of the enzyme present in scalp and have identified it as the type 1 isoenzyme.[9a] Immunoblotting studies have shown that type 1 enzyme is present in skin and liver whereas type 2 enzyme was identified in prostate, seminal vesicle, liver, and epididymis.[10]

Selective inhibitors of human type 2 enzyme, finasteride[11c,d] (**1**) and epristeride[12] (**2**) (Chart 1), have undergone successful clinical investigation to examine DHT lowering. In a multiple oral dose study for 7 days, finasteride at 1 and 10 mg doses reduced the serum DHT levels 71% and 73%, respectively,[13] whereas **2** after 8 days reduced DHT by 25–54% at 0.4–160 mg doses.[14] Much of the residual DHT in the above studies could be due to production by the type 1 enzyme which is not effectively inhibited by **1** or **2**. Inhibitors of type 1 enzyme in combination with **1** or **2** could theoretically lower residual DHT levels further and thus might afford a more effective clinical treatment of BPH. The type 1 inhibitors alone, because of the localization of the target enzyme in the skin,[9a] could potentially be used for treatment of acne, hirsutism, or male pattern baldness. Recently, a benzoquinolone,[15a,b] LY 191704 (**3**), was reported as a human type 1 selective inhibitor and the 6-azasteroid[15c] (**4**) was reported as a dual inhibitor of both human 5α-reductase isozymes.

In preliminary screening of compounds as inhibitors of 5α-reductases from human scalp and prostate, 4-aza-4-methyl-5α-cholestan-3-one (**12**, $R_7$ = H) appeared to show highly selective inhibition of the scalp (type 1) enzyme. An earlier qualitative study of the inhibition of uncharacterized 5α-reductase activity in plucked human hair follicles by azasteroids suggested that a 7β-methyl substituent might enhance potency.[11b] This led us to prepare various 7β-substituted azacholestan-3-ones as type 1 5α-reductase inhibitors. We report herein the stereoselective synthesis of a new series of 7β-substituted 4-azacholestan-3-ones and their *in vitro* activity against human type 1 and type 2 5α-reductase enzymes.

**Chart 1**




The synthesis of the azasteroids is shown in Scheme 1. The 7β-substituted enones **6a-d** were prepared stereoselectively following chemical methodology recently described.[16] These were converted to the corresponding α,β-unsaturated ketones **7a-d** on reaction with DBU in THF. Oxidative cleavage of the A ring of **7a-d** with $KMnO_4/NaIO_4$ furnished seco acids **8a-d**, which were cyclized to azasteroids **9a-d** by refluxing with a solution of ammonium acetate in acetic acid. Catalytic hydrogenation of **9a-d** with platinum in acetic acid afforded the 5α-reduced derivatives **10a-d**. N-Alkylations of **9a-d** and **10a-d** were carried out using standard conditions.[11a] Conversion of **10** and **10a** to the $\Delta^1$-analogs **13** and **13a** was performed using DDQ chemistry (Scheme 2).[17] Methylation of **13a** with methyl iodide in the presence of NaH/DMF gave **14a**. The 7-unsubstituted azasteroids were prepared by using chemistry developed earlier in these laboratories.[11a]

The rank potencies of 4-azacholestane derivatives as inhibitors of the recombinant human type 1 and type 2 isozymes are indicated in Table 1. Two features of these data deserve mention: many compounds exhibit extensive nonspecific binding, and some are also slow-binding inhibitors.[18] Assay conditions include a preincubation of cofactor and inhibitor with enzyme to allow equilibration of the system.

The extremely hydrophobic nature of many of these compounds makes their potency appear to depend on the purity of the enzyme. Initially these inhibitors were evaluated using crude homogenates of native enzyme from human scalp and prostate preparations, respectively.[9a] In contrast, the present data were obtained using recombinant enzyme preparations[9b,9c] which have specific activities 1000-fold greater than in native tissue. In this situation compounds display some 10–30-fold increased potency against both isozymes. Despite this increase in apparent potency, the relative selectivity for the type 1 over the type 2 isozyme remains unchanged. Control experiments, including enriching the native enzyme with the recombinant one, indicate the effect

† Department of Synthetic Chemical Research.
‡ Department of Biochemistry.

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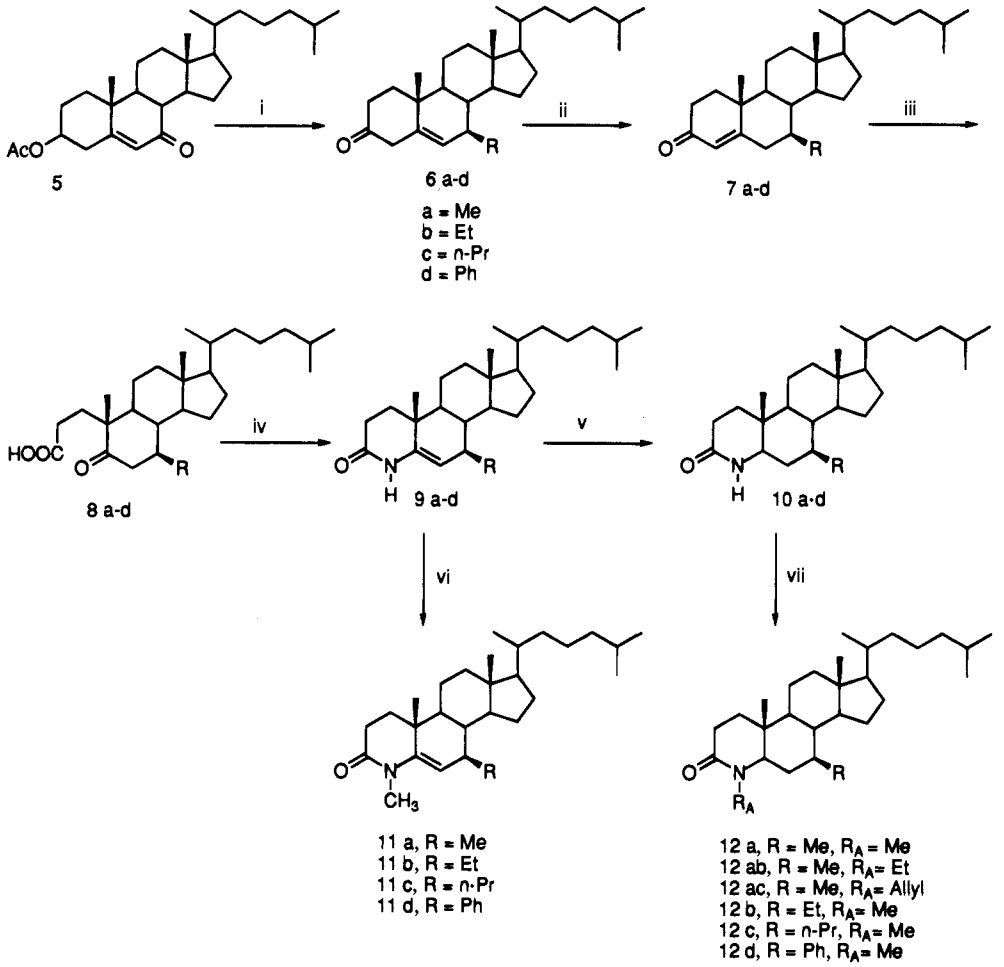
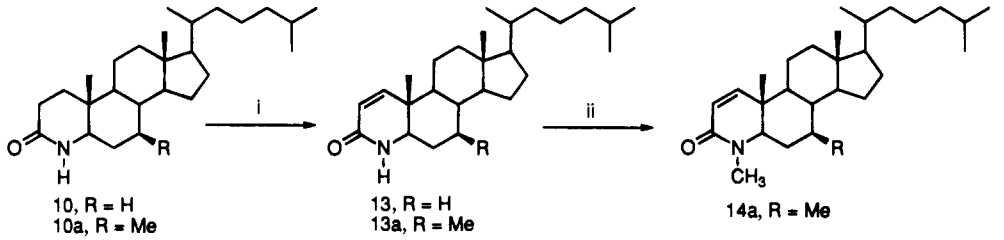
**Scheme 1**[a]




[a] (i) (a) RLi or RMgX/THF, (b) $Al(OiPr)_3$/toluene/$C_6H_{10}O$, (c) Li/THF/$NH_3$; (ii) DBU/THF/reflux; (iii) $KMnO_4/NaIO_4$/t-BuOH/$H_2O$/70 °C; (iv) $NH_4OAc$/AcOH/reflux; (v) $PtO_2$/AcOH/$H_2$; (vi) MeI/NaH/DMF; (vii) MeI or EI or allyl bromide/NaH/DMF.

**Scheme 2**[a]




[a] (i) (a) DDQ/BSTFA/TfOH/toluene/23 °C/24 h, (b) methyl acetoacetate/reflux/24 h; (ii) NaH/MeI/DMF.

derives from extensive partitioning of these inhibitors into the membrane component of these enzyme preparations.

Some of these compounds have been found to be slow-binding inhibitors and are far more potent than the fixed-time assay results in Table 1 would imply. Data defining the time-dependent inhibition of the type 1 isozyme by one of these compounds, MK-386 (**12a** in the table), are shown in Figure 1. These data indicate this inhibitor forms a preliminary complex with $K_i = 3$ nM, which then rearranges with a half-life of 5 min ($k = 2 \times 10^{-3}$ $s^{-1}$) to a high-affinity complex. The second-order rate constant for formation of the high-affinity complex is $7 \times 10^5$ $M^{-1}$ $s^{-1}$. The absence of detectable activity at infinite time implies that the dissociation constant of the final complex is <100 pM, and that the half-life of this complex is >3 h ($k < 7 \times 10^{-5}$ $s^{-1}$). A detailed evaluation of the mechanism of inhibition of the type 1 isozyme by this compound, and other high-affinity inhibitors, is in progress.

Introduction of a 7β-methyl group (**12a**, $IC_{50}$ 0.9nM) into the parent compound (**12**, $IC_{50}$ 1.7 nM) increases the potency of type 1 inhibition by 2-fold. However, a further increase in the size of the alkyl group at the 7β-position (**12b, 12c**) leads to a decrease in potency compared to the methyl compound (**12a**). A phenyl group at 7β-position (**12d**) results in significant loss of inhibitory activity. It is apparent from Table 1 that of all A-ring variants presented the $N^4$-methyl azasteroids are the most potent type 1 5α-reductase inhibitors. The $N^4$-H azasteroids (**10a**, **10b**, and **10c**) are generally less active than corresponding $N^4$-methyl compounds (**12a**, **12b**, and **12c**) and increases of $N^4$-alkyl chain length from methyl (**12a**) to ethyl (**12ab**) lead to a slight decrease in activity whereas a further increase in chain length to allyl (**12ac**) results in a drastic drop in

**Table 1.** Inhibition of Recombinant Type 1 and Type 2 5α-Reductases[a] by 4-Azacholestan-3-ones

| | | | | $IC_{50}$ (nM)[a] | |
|---|---|---|---|---|---|
| no. | 4-$NR_A$ | 7β-R | others | type 1 | type 2 |
| **9** | H | H | $\Delta^5$ | 19.1 | nd[b] |
| **9a** | H | $CH_3$ | $\Delta^5$ | 2.5 | 859 |
| **9b** | H | ethyl | $\Delta^5$ | 8.6 | 1050 |
| **9c** | H | *n*-propyl | $\Delta^5$ | 58 | 713 |
| **9d** | H | phenyl | $\Delta^5$ | >1000 | 1340 |
| **10** | H | H | | 2.6 | 218 |
| **10a** | H | $CH_3$ | | 2.6 | 280 |
| **10b** | H | ethyl | | 2.7 | 280 |
| **10c** | H | *n*-propyl | | 9.5 | 1100 |
| **10d** | H | phenyl | | >1000 | 443 |
| **12** | $CH_3$ | H | | 1.7 | 218 |
| **12a** | $CH_3$ | $CH_3$ | | 0.9[c] | 154 |
| **12ab** | ethyl | $CH_3$ | | 3.3 | 1390 |
| **12ac** | allyl | $CH_3$ | | 42 | 1300 |
| **12b** | $CH_3$ | ethyl | | 5.7 | 330 |
| **12c** | $CH_3$ | *n*-propyl | | 20 | 580 |
| **12d** | $CH_3$ | phenyl | | 134 | 428 |
| **11** | $CH_3$ | H | $\Delta^5$ | 4.3 | nd[b] |
| **11a** | $CH_3$ | $CH_3$ | $\Delta^5$ | 0.6 | 147 |
| **11b** | $CH_3$ | ethyl | $\Delta^5$ | 9.5 | 2120 |
| **11c** | $CH_3$ | *n*-propyl | $\Delta^5$ | 8.4 | 5.4 |
| **11d** | $CH_3$ | phenyl | $\Delta^5$ | >1000 | 2170 |
| **13** | H | H | $\Delta^1$ | 5.0 | nd[b] |
| **13a** | H | $CH_3$ | $\Delta^1$ | 1.6 | 298 |
| **14a** | $CH_3$ | $CH_3$ | $\Delta^1$ | 2.0 | 125 |
| **1** | finasteride | | | 52 | <0.1 |
| **2** | epristeride | | | >1000 | 0.6 |
| **3** | LY191704 | | | 8.6 | 1750 |
| **4** | 6-azasteroid | | | 186 | <0.1 |

[a] Each value represents the average of at least three determinations. The error in the determinations was estimated at 50%. For $IC_{50}$ determinations, the inhibitors were dissolved in ethanol and serially diluted to the appropriate concentration. The baculovirus-expressed recombinant type 1 5α-reductase was preincubated with inhibitor (0.1−1000 nM) in 40 mM sodium phosphate, pH 7.0, 500 μM NADPH, 1 mM DTT, and 1 mg/mL BSA for 18 h at 4 °C. The reaction was initiated by the addition of [7-$^3$H]T (NEN, 20 Ci/mmol) and NADPH to a final concentration of 0.3 μM and NADPH and incubated at 37 °C for 90 min. Similarly, baculovirus-expressed type 2 5α-reductase was preincubated with inhibitor (1−10 000 nM) in 40 mM sodium citrate, pH 5.5, 500 μM NADPH, 1 mM DTT, and 1 mg/mL BSA for 18 h at 4 °C. The reaction was initiated by the addition of [7-$^3$H]T (NEN, 20 Ci/mmol) and NADPH to a final concentration of 0.3 and 500 μM, respectively. The conversion of T to DHT was monitored using a radioflow detector following separation by reverse-phase HPLC (Whatman RACII C18 column, 1 mL/min 0.1% TFA in water:methanol (42:58); retention times T, 6.3 min, DHT, 9.7 min). [b] Not done. [c] $K_i$ value was determined only for this compound. A detailed kinetic study will be published elsewhere.[18]

inhibition potency. These results suggest that type 1 enzyme binding is enhanced by a small hydrophobic group at $N^4$-position. Azasteroids unsaturated at the 5,6-position ($\Delta^5$ compounds: **11**, **11a**, **11b**, and **11c**) were generally less active compared to the corresponding saturated compounds. Introduction of a 1,2-double bond in $N^4$-methyl series (**14a**) results in only a slight decrease in potency compared to parent compound (**12a**), whereas the $\Delta^1$-$N^4$-H azasteroids (**13**, **13a**) have comparable activity to the corresponding $N^4$-H compounds (**10**, **10a**). Under preincubation conditions, azasteroids have type 2 inhibitory activity with $IC_{50}$s ranging from 147 to 2170 nM with no clear cut feature contributing to selectivity or potency against this isozyme. The azasteroid **12a** (L-733692, MK-386) has been chosen for additional studies including human clinical trials.

In summary, 7β-substituted 4-azacholestan-3-ones are potent and selective type 1 steroid 5α-reductase inhibitors. The introduction of a 7β-methyl group increases the potency of azasteroids as type 1 enzyme inhibitors.

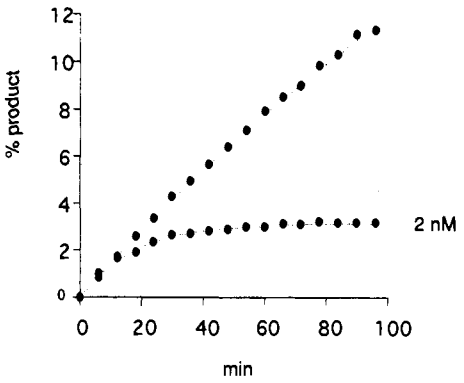



**Figure 1.** Slow-binding inhibition of the type 1 isozyme by MK-386. The plot shows product [$^3$H]dihydrotestosterone formation with time in a standard assay in the absence or presence of 2 nM inhibitor. The assays contained 25 nM [7-$^3$H]-testosterone and 500 μM NADPH in a buffer consisting of 0.1 M 3-(*N*-morpholino)propanesulfonic acid, 0.1% bovine serum albumin, and 1 mM ethylenediaminetetraacetic acid, at 37 °C and pH 7.20. The enzyme was the recombinant type 1 isozyme, obtained from a baculovirus expression system, and was employed at 230 pM in the control run and double this level in the inhibited case. The solid lines are theoretical and were determined by nonlinear regression to an integrated first-order rate equation, as reviewed by Morrison and Walsh,[19] with the kinetic constants given in the text.

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**Supplementary Material Available:** Details of experimental procedures and data (4 pages). Ordering information is given on any current masthead page.

## References

(1) (a) Monda, J. M.; Osterling, J. E. Medical Treatment of benign prostatic hyperplasia: 5α-reductase inhibitors and α-adrenergic antagonists. *Mayo Clin. Proc.* **1993**, *68*, 670−679. (b) Geller, J. Nonsurgical treatment of prostatic hyperplasia. *Cancer* **1992**, *70*, 339−345. (c) Rasmusson, G. H.; Chemical control of androgen action. *Annu. Rep. Med. Chem.* **1986**, *22*, 179−188.

(2) (a) Smith, L. S.; Tegeler, J. J. Advances in dermatology. *Annu. Rep. Med. Chem.* **1989**, *24*, 1177−186. (b) Sansone, G. L.; Reisner, R. M.; Differential rates of conversion of testosterone to dihydrotestosterone in acne and normal human skin - a possible pathogenic factor in acne. *J. Invest. Dermatol.* **1971**, *56*, 366−372.

(3) (a) Brooks, J. R. Treatment of hirsutism with 5α-reductase inhibitors. *Clin. Endocrinol. Metab.* **1986**, *15*, 391−405. (b) Serafini, P.; Lobo, R. A.; Increased 5α-reductase activity in idiopathic hirsutism. *Fertil. Steril.* **1985**, *43*, 74−78.

(4) (a) Gadwood, J. R.; Fiedler V. C. Pathogenesis and treatment of alopecias. *Annu. Rep. Med. Chem.* **1989**, *24*, 187−196. (b) Diani, A. R.; Mulholland, M. J.; Shull, K. L.; Kubicek, M. F.; Johnson, G. A.; Schostarez, H. J.; Brunden, M. N.; Buhl, A. E. Hair growth effects of oral adminstration of finasteride, a steroid 5α-reductase inhibitor alone and in combination with topical minoxidil in the balding stumptail macaque. *J. Clin. Endocrinol. Metab.* **1992**, *74*, 345−350.

(5) (a) Moore, R. J.; Wilson, J. D. Steroid 5α-reductase in cultured human fibroblasts: biochemical and genetic evidence for two distinct enzyme activities. *J. Biol. Chem.* **1976**, *251*, 5895−5900. (b) Hudson, R. W.; Comparison of nuclear 5α-reductase activities in stromal and epithelial fractions of human prostatic tissue. *J. Steroid Biochem.* **1987**, *26*, 349−353. (c) Bruchovsky, N.; Rennie, P. S.; Batzold, F. H.; Goldenberg, S. L.; Fletcher, T.; McLoughlin, M. G. Kinetic parameters of 5α-reductase activity in stroma and epithelium of normal, hyperplastic, and carcinomatous human prostate. *J. Clin. Endocrinol. Metab.* **1988**, *67*, 806−816. (d) McGuire, J. S., Jr.; Tomkins, G. M. The heterogeneity of $\Delta^4$-3-keto-steroids reductases. *J. Biol. Chem.* **1960**, *235*, 1634−1638.

(6) Martini, L.; Zoppi, S.; Motta, M. Studies on possible existence of two 5α-reductases in the rat prostate. *J. Steroid Biochem.* **1986**, *24*, 177–182.
(7) Imperato-McGinley, J.; Gautier, T. Inherited 5α-reductase deficiency in man. *Trends Genet.* **1986**, *2*, 130–133.
(8) (a) Andersson, S.; Russell, D. W. Structural and biochemical properties of cloned and expressed human and rat steroid 5α-reductases. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3640–3644. (b) Andersson, S.; Berman, D. M.; Jenkins, E. P.; Russell, D. W. Deletion of steroid 5α-reductase 2 gene in male pseudohermaphroditism. *Nature* **1991**, *354*, 159–161. (c) Jenkin, E. P.; Andersson, S.; Imperato-McGinley, J.; Wilson, J. D.; Russell, D. W. Genetic evidence for more than one human steroid 5α-reductase. *J. Clin. Invest.* **1992**, *89*, 293–300.
(9) (a) Harris, G.; Azzolina, B.; Baginsky, W.; Cimis, G.; Rasmusson, G. H.; Tolman, R. L.; Raetz, C. R. H.; Ellsworth, K. Identification and selective inhibition of an isozyme of steroid 5α-reductase in human scalp. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 10787–10791. (b) Chan, H. K.; Geissler, W. M.; Andersson, S. Characterization of human steroid 5α-reductase type I and II expressed in the baculovirus system. In *Sex hormones and antihormones in endocrine dependent pathology: basic and clinical aspects*; Motta, M., Serio, M., Eds.; Elsevier Science B. V.: Amsterdam, 1994; in press. (c) Iehele, C.; Delos, S.; Martin, P. Baculovirus-directed expression of human prostatic steroid 5α-reductase 1 in an active form. *J. Steroid Biochem. Mol. Biol.* **1993**, *46*, 177–182.
(10) (a) Thigpen, A. E.; Silver, R. I.; Guileyardo, J. M.; Casey, M. L.; McConnell, J. D.; Russell D. W. Tissue distribution and ontogeny of steroid 5α-reductase isozyme expression. *J. Clin. Invest.* **1993**, *92*, 903–910. (b) Normington, K.; Russell, R. W. Tissue distribution and kinetic characteristics of rat steroid 5α-reductase isozyme. *J. Biol. Chem.* **1992**, *267*, 19548–19554.
(11) (a) Rasmusson, G. H.; Reynolds, G. F.; Steinberg, N. G.; Walton, E.; Patel, G. F.; Liang, T.; Cascieri, M. A.; Cheung, A. H.; Brooks, J. R.; Berman, C. Azasteroids: structure-activity relationship for inhibition of 5α-reductase and of androgen receptor binding. *J. Med. Chem.* **1986**, *29*, 2298–2315. (b) Mellin, T. N.; Busch, R. D.; Rasmusson, G. H. Azasteroids as inhibitors of testosterone 5α-reductase in mammalian skin. *J. Steroid Biochem. Mol. Biol.* **1993**, *44*, 121–131. (c) Steiner, J. F. Finasteride: a 5α-reductase inhibitor. *Clin. Pharm.* **1993**, *12*, 15–23. (d) Peters, D. H.; Sorkin, E. M. Finasteride. a review of its potential in the treatment of benign prostatic hyperplasia. *Drugs* **1993**, *46*, 177–208.
(12) Holt, D. A.; Levy, M. A.; Oh, H. J.; Erb, J. M.; Heaslip, J. I.; Brandt, M.; Lan-Hargest, H. Y.; Metcalf, B. W. Inhibition of steroid 5α-reductase by unsaturated 3-carboxysteroids. *J. Med. Chem.* **1990**, *33*, 943–950.
(13) Ohtawa, M.; Morikawa, H.; Shimazaki, J. Pharmacokinetics and biochemical efficacy after single and multiple oral administration of N-(2-methyl-2-propyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide, a new type of specific competitive inhibitor of testosterone 5α-reductase, in volunteers. *Eur. J. Drug Metab. Pharmacokinet.* **1991**, *16*, 15–21.
(14) (a) Audet, P.; Nurcombe, H.; Lamb, Y.; Jorkasky, D.; Lloyd-Davis, K.; Morris. Effect of multiple doses of epristeride(E) a steroid 5α-reductase inhibitor, on serum dihydrotestosterone (DHT) in older male subjects. *Clin. Pharmcol. Ther.* **1993**, *53*, 231 (PIII-99). (b) Chapelsky, M. C.; Nichols, A.; Jorkasky, D. K.; Lundberg, D. E.; Knox, S. H.; Audet, P. R. Pharmacodynamics of SK&F 105657 in healthy elderly male subjects. *Clin. Pharmcol. Ther.* **1992**, *51*, 154 (PII-32).
(15) (a) Jones, C. D.; Audia, J. E.; Lawhorn, D. E.; McQuaid, L. A.; Naubauer, B. L.; Pike, A. J.; Pennington, P. A.; Stamm, N. B.; Toomey, R. E.; Hirsch, K. S. Nonsteroidal inhibitors of human type I steroid 5α-reductase. *J. Med. Chem.* **1993**, *36*, 421–423. (b) Hirsch, K. S.; Jones, C. D.; Audia, J. E.; Anderson, S.; MaQuaid, L.; Stamm, N. B.; Neubauer, B. L.; Pennington, P.; Toomey, R. E. LY191704: A selective, nonsteroidal inhibitors of human steroid 5α-reductase type 1. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5277–5281. (c) Frye, S. V.; Haffer, C. D.; Maloney, P. R.; Mook, R. A.; Dorsey, G. F.; Hiner, R. N.; Batchelor, K. W.; Bramson, H. N.; Stuart, J. D.; Schweiker, S. L.; Arnold, J.; Bickett, D. M.; Moss, M. L.; Tian, G.; Unwalla, R. J.; Lee, F. W.; Tippen, T. K.; James, M. K.; Grizzel, M. K.; Long, J. E.; Schuster, S. V. 6-Azasteroids: Potent dual inhibitors of human type 1 and steroid 5α-reductase. *J. Med. Chem.* **1993**, *36*, 4313–4315.
(16) Bakshi, R. K.; Rasmusson, G. H. A stereoselective synthesis of 7β-phenyl and 7β-methylcholesterol. *Tetrahedron Lett.* **1993**, *34*, 2055–2058.
(17) Bhattacharya, A.; DiMichele, L. M.; Dolling, U. H.; Douglas, A. W. Silylation-mediated oxidation of 4-Aza-3-ketosteroids with DDQ proceeds via DDQ-substrate adducts. *J. Am. Chem. Soc.* **1988**, *110*, 3318.
(18) Harris, G; Bull, H. Manuscript in preparation.
(19) Morrison, J. F.; Walsh, C. T. The behavior and significance of slow-binding enzyme inhibitors. *Adv. Enzymol.* **1988**, *61*, 201–301.