Inhibition of Steroid 5α-Reductase by Unsaturated 3-Carboxysteroids

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A series of unsaturated steroids bearing a 3-carboxy substituent has been prepared and assayed in vitro as inhibitors of human and rat prostatic steroid 5α-reductase (EC 1.3.1.30). It is proposed that the observed tight binding of the 3-androstene-3-carboxylic acids is due to mimicry of a putative, high-energy, enzyme-bound enolate intermediate formed during the NADPH-dependent conjugate reduction of testosterone by steroid 5α-reductase. These compounds were prepared through palladium(0)-catalyzed carbomethoxylations of enol (trifluoromethyl)sulfonates derived from 3-keto precursors. Modification of A and B ring unsaturation and substitution at C-3, -4, -6, and -11 was explored. Mono- and dialkylcarboxamides were employed as 17β side chains to enhance inhibitory activity with the human enzyme.

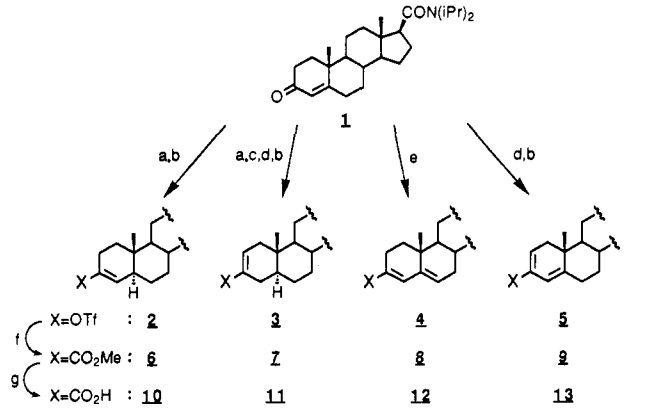
Benign prostatic hyperplasia (BPH) is an age-related, progressive disease which afflicts a high percentage of men over 50 years of age. Owing to the circumjacent relation of the prostate to urethra, glandular enlargement typically results in compromised urinary function calling for medical intervention. Currently, the front-line therapy is surgery.

It has long been established that prostatic growth is stimulated by androgens and more recent investigations suggest that this trophic support is provided by 5α-dihydrotestosterone (DHT) rather than by the classic testicular hormone testosterone (T).[1] Perhaps the most striking evidence for DHT involvement in BPH derives from the study of males who are genetically deficient in the enzyme which converts T to DHT, steroid 5α-reductase.[2] The study of these DHT-deficient individuals has provided a clinical model for discerning T-mediated versus DHT-mediated androgen responses. The affected males are born pseudohermaphroditic, lacking well-developed external genitalia, indicating a critical prenatal DHT requirement for normal male fetal development. At puberty, however, phallic growth is observed as well as the development of normal male sexual orientation, libido, and performance. Furthermore, these men show no hairline regression nor acne[3] and have small, often undectable prostates, and never develop the symptomatology of BPH.[4]

In keeping with this model, recent preclinical studies with steroid 5α-reductase inhibitors have demonstrated selective retardation of prostatic growth coincident with suppression of DHT biosynthesis.[5,6] Hence, it is now widely recognized that selective inhibition of steroid 5α-reductase offers potential treatment for the disease states of BPH, acne, and male pattern baldness, most importantly while maintaining T-supported functions and avoiding the feminizing side-effects associated with nonspecific androgen ablative therapy.

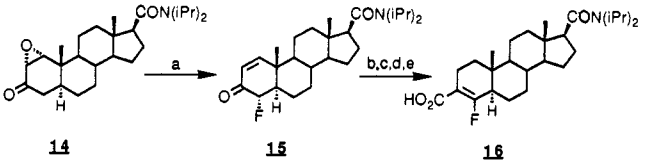
Recently we described a new class of 3-androstene-3-carboxylic acids which exhibit potent uncompetitive (vs T) inhibition of human prostatic steroid 5α-reductase. From the results of preliminary studies, we proposed a novel mode of inhibition for this enzyme involving an enzyme–$NADP^+$–inhibitor ternary complex.[7,8] Herein we

Scheme I[a]



[a] (a) Li/NH_3; (b) $PhNTf_2$; (c) H_3O^+; (d) $LiN(SiMe_3)_2$; (e) Tf_2O, base; (f) Pd^0, CO, MeOH; (g) K_2CO_3, H_2O, MeOH, reflux.

Scheme II[a]



[a] (a) HF–pyr; (b) $LiN(SiMe_3)_2$, $PhNTf_2$; (c) Pd^0, CO, MeOH; (d) H_2, Pd/C; (e) K_2CO_3, H_2O, MeOH.

(1) Anderson, K. K.; Liao, S. *Nature (London)* **1968**, *219*, 277–279. Bruchovsky, N.; Wilson, J. D. *J. Biol. Chem.* **1968**, *243*, 2012–2021. Baulieu, E. E.; Lasnitzki, I.; Robel, P. *Nature (London)* **1968**, *219*, 2155–2156. Lasnitzki, I.; Mizuno, T. *J. Endocr.* **1977**, *74*, 47–55.

(2) Imperato-McGinley, J.; Peterson, R. E.; Gautier, T.; Sturla, E. *J. Steroid Biochem.* **1979**, *11*, 637–648. Imperato-McGinley, J.; Gautier, T. *Trends Gen.* **1986**, 130–133. Peterson, R. E.; Imperato-McGinley, J.; Gautier, T.; Sturla, E. *Am. J. Med.* **1977**, *62*, 170–191.

(3) The sebaceous gland of the skin has been shown to be under DHT stimulation and elevated local concentrations of DHT in skin have been correlated with acne and male pattern baldness: Vermorken, A. J. M.; Goos, C. M. A. A.; Roelofs, H. M. J. *Br. J. Dermatol.* **1980**, *102*, 695–701. Takayasa, S.; Adachi, K. *Endocrinology* **1972**, *90*, 73. Sansone, G.; Reisner, R. M. *J. Invest. Dermatol.* **1971**, *56*, 366. Schweikert, H.; Wilson, J. *J. Clin. Endocrinol. Metab.* **1974**, *38*, 811.

(4) A correlation between DHT and human prostatic growth in BPH has been observed as well as increased 5α-reductase activity in hyperplastic tissue: Watanabe, H. *Ultrasound Med. Biol.* **1986**, *12*, 567–571. Bruchovsky, N.; Lieskovsky, G. *J. Endocr.* **1978**, *80*, 289–301.

(5) Brooks, J. R.; Berman, C.; Primka, R. L.; Reynolds, G. F.; Rasmusson, G. H. *Steroids* **1986**, *47*, 1–19. Blohm, T. R.; Laughlin, M. E.; Benson, H. D.; Johnston, J. O.; Wright, C. L.; Schatzman, G. L.; Weintraub, P. M. *Endocrinol.* **1986**, *119*, 959–966.

(6) One of these inhibitors, a 4-azasteroid, is in clinical trial: Stoner, E.; Gregg, H.; Otterbein, J. J.; Stein, E.; Lamkin, G.; Vlasses, P. H.; Danzeisen, J.; Tobert, J. A. *Clin. Res.* **1987**, *35* (*3*), 402A.

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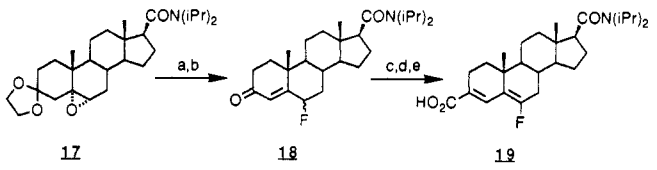
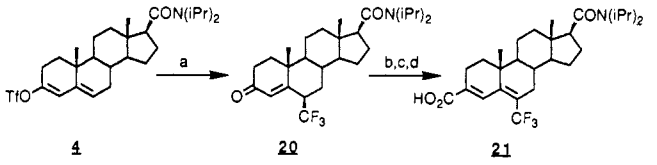
Scheme III[a]



[a] (a) BF_3–Et_2O; (b) HCl, HOAc; (c) Tf_2O, base: (d) Pd^0, CO, MeOH; (e) K_2CO_3, H_2O, MeOH.

Scheme IV[a]



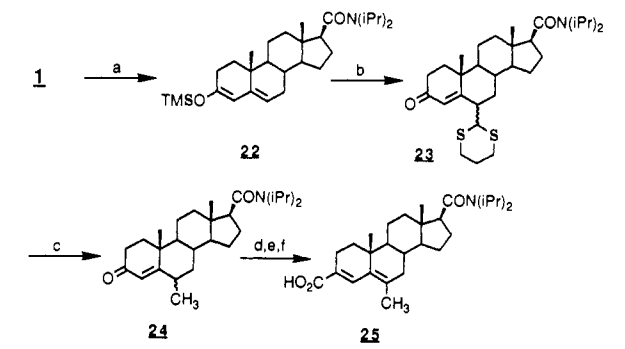
[a] (a) $h\nu$, pyr; (b) Tf_2O, base; (c) Pd^0, CO, MeOH; (d) K_2CO_3, H_2O, MeOH.

report the details of the syntheses of members of this class as well as the structure–activity relationships derived from in vitro study of these congeners.

Chemistry. The preparation of the steroidal 3-carboxylates was, in general, accomplished through a one-carbon homologation of the corresponding enol triflates effected via a palladium(0)-catalyzed carbomethoxylation. The requisite triflates were obtained either by trapping of the preformed lithium enolates with *N*-phenyltriflimide[9] or by treatment of the ketone directly with triflic anhydride.[10] Scheme I is illustrative of the versatility of this approach. Birch reduction of enone 1 using the conditions of Stork[11] provided stereo- and regiospecifically the kinetic 5α-Δ³-enolate which was trapped as the triflate (**2**) with *N*-phenyltriflimide. On the other hand, protonation of the Birch product yielded the 5α-3-keto steroid. Subsequent enolate generation with lithium bis(trimethylsilyl)amide followed by treatment with the triflimide reagent afforded predominately the thermodynamic enol triflate **3**. Treatment of enone 1 with triflic anhydride and a hindered pyridine base provided heteroannular dienol triflate **4**, whereas kinetic deprotonation and subsequent triflation afforded the homoannular dienol triflate **5**. Triflates **2**–**5** were then subjected to the palladium-catalyzed carbonylation conditions developed by Cacchi et al.[12] to afford methyl esters **6**–**9**. Basic hydrolysis then yielded acids **10**–**13**.

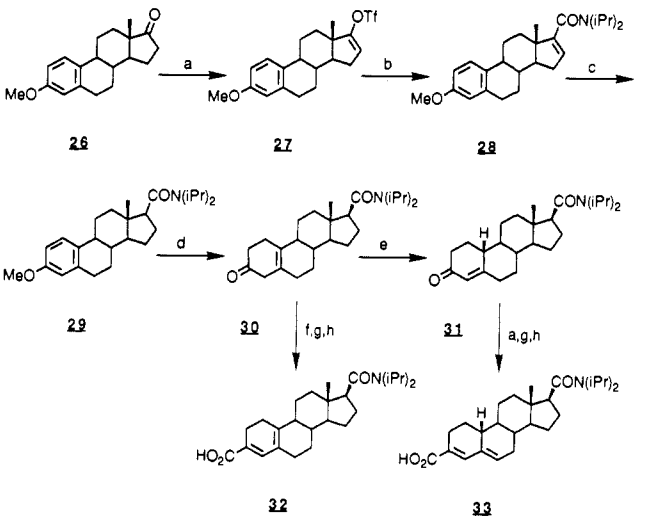
Unsaturated acids substituted at C-4 or C-6 with fluorine were prepared by acid-catalyzed openings of epoxides. Treatment of 1α,2α-epoxy ketone **14** (Scheme II) with pyridinium poly(hydrogen fluoride) resulted in S_N2' displacement (via the epoxy enol) and dehydration to afford 4α-fluoro Δ¹-3-ketone **15**.[13] Homologation and selective hydrogenation produced fluoro acid **16**. Similarly, as shown in Scheme III, boron trifluoride opening of 5α,6α-epoxy ketal **17** gave, after dehydrative β-elimination, 6-fluoro Δ⁴-3-ketone **18**,[14] which was homologated in the standard fashion to fluorodienyl acid **19**.

Scheme V[a]



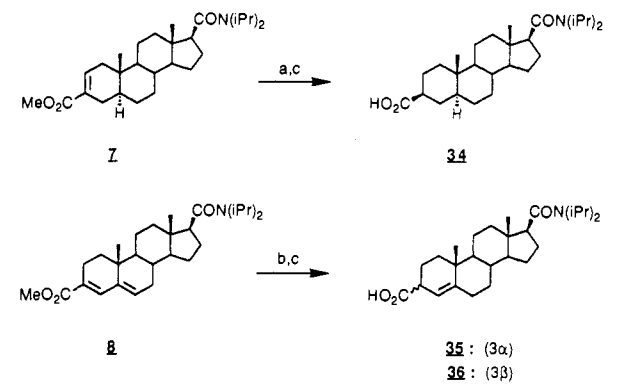
[a] (a) TMSCl, Et_3N; (b) 1,3-dithienium fluoroborate; (c) Raney Ni; (d) Tf_2O, base; (e) Pd^0, CO, MeOH; (f) K_2CO_3, H_2O, MeOH.

Scheme VI[a]



[a] (a) Tf_2O, base; (b) Pd^0, CO, iPr_2NH; (c) H_2, PtO_2; (d) Li, NH_3, tBuOH; (e) HCl; (f) $LiN(SiMe_3)_2$, $PhNTf_2$; (g) Pd^0, CO, MeOH; (h) K_2CO_3, H_2O, MeOH.

Scheme VII[a]



[a] (a) H_2, 10% Pd/C; (b) Mg, MeOH; (c) K_2CO_3, H_2O, MeOH.

Incorporation of a 6-trifluoromethyl substituent was accomplished via a novel photochemical rearrangement[15] of dienyl triflate **4** (Scheme IV) while the corresponding 6-methyl analogue was prepared by using the method of Paterson[16] (Scheme V).

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(8) Details of the enzyme mechanistic studies will be published elsewhere: Levy, M. A.; Brandt, M.; Holt, D. A.; Metcalf, B. W. *J. Steroid Biochem.*, in press.
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(11) Stork, G.; Rosen, P.; Goldman, N. L. *J. Am. Chem. Soc.* **1961**, *83*, 2965–2966.
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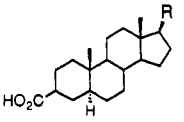
(13) Neeman, M.; O'Grodnick, J. S. *Can. J. Chem.* **1974**, *52*, 2941.
(14) Bowers, A.; Ringold, H. J. *Tetrahedron* **1958**, *3*, 14.
(15) Lan-Hargest, H.-Y.; Elliott, J. D.; Eggleston, D. S.; Metcalf, B. W. *Tetrahedron Lett.* **1987**, *28*, 6557–6560.

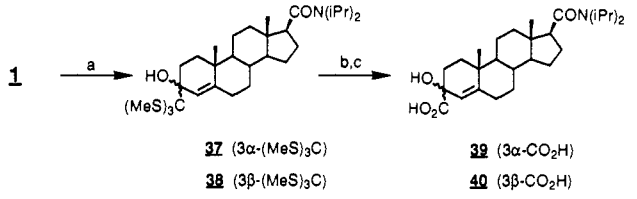
Table I. Steroid 5α-Reductase in Vitro Inhibitory Activities of 3-Carboxysteroids



no.	unsatn	substn	R	$K_{i,app}$, nM human	$K_{i,app}$, nM rat
10	3–4		$CON(iPr)_2$	30	70
50	3–4		CONH(tBu)	110	11
12	3–4,5–6		$CON(iPr)_2$	7–18	35–50
57	3–4,5–6		CONH(tBu)	30–36	20–30
11	2–3		$CON(iPr)_2$	85	110
34	–	(3β)	$CON(iPr)_2$	2200	340
13	2–3,4–5		$CON(iPr)_2$	52	120
35	4–5	(3α)	$CON(iPr)_2$	570	>10000
36	4–5	(3β)	$CON(iPr)_2$	200	2000
39	4–5	3β-OH	$CON(iPr)_2$	220	>10000
40	4–5	3α-OH	$CON(iPr)_2$	320	>10000
65	1–2,3–4		$CON(iPr)_2$	62	65
69	1–2,3–4,5–6		$COO(iPr)_2$	60	600
101	2–3,4–5,6–7		$CON(iPr)_2$	7–12	300
45	3–4,5–6,11–12		$CON(iPr)_2$	7	47
16	3–4	4-F	$CON(iPr)_2$	26	35
19	3–4,5–6	6-F	$CON(iPr)_2$	32	33
21	3–4,5–6	6-CF_3	$CON(iPr)_2$	700–900 (IC_{50})	1000
82	3–4,5–6	4-CH_3	$CON(iPr)_2$	35	70
25	3–4,5–6	6-CH_3	$CON(iPr)_2$	170	200
32	3–4,5–10	19-nor	$CON(iPr)_2$	110	290
33	3–4,5–6	19-nor	$CON(iPr)_2$	50	140
54	3–4		20(S)-CH_3CHCH_2OH	5000	155
102[a]	3–4,5–6		CN	790	140

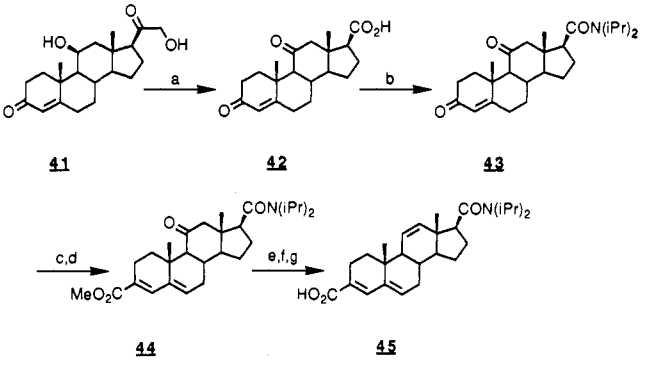
[a] Reference 30.

Scheme VIII[a]



[a] (a) $(MeS)_3CLi$; (b) HgO, $HgCl_2$, H_2O, MeOH; (c) K_2CO_3, H_2O, MeOH.

Scheme IX[a]



[a] (a) Jones; (b) $(COCl)_2$, iPr_2NH; (c) Tf_2O, base; (d) Pd^0, CO, MeOH; (e) $LiN(SiMe_3)_2$, $PhNTf_2$; (f) Pd^0, HCO_2H; (g) K_2CO_3, H_2O, MeOH.

The 19-nor congeners **32** and **33** were derived from estrone methyl ether (**26**) as depicted in Scheme VI. The 17-ketone was transformed in three steps to the 17β-amide. Palladium-catalyzed carbonylation of enol triflate **27**, in this case utilizing diisopropylamine in place of methanol, provided α,β-unsaturated amide **28**. Catalytic hydrogenation produced the desired 17β-amine **29**. Birch reduction of the A ring allowed for the selective preparation of either $\Delta^{5(10)}$-3-ketone **30** or Δ^4-3-ketone **31**, which were then homologated to **32** and **33**, respectively.

Catalytic hydrogenation of Δ^2-ester **7** followed by hydrolysis provided saturated acid **34** (Scheme VII). Reduction of dienyl ester **8** with magnesium in methanol[17] yielded a 1:1 mixture of Δ^4-3α and -3β esters (**35** and **36**) as major products. Lithium in ammonia reduction of **8** resulted in a 9:1 ratio of **36**:**35** in a yield comparable to that of the magnesium reduction.

Diastereometric Δ^4-3-hydroxy 3-carboxylates **39** and **40** (Scheme VIII) were prepared via [tris(methylthio)-methyl]lithium addition to enone 1 followed by sequential hydrolytic steps.[18] Stereochemical assignments (C-3) were based on X-ray crystallographic structure determination of an intermediate ester.[19]

The 3-carboxysteroids functionalized at C-11 (Scheme IX) were derived from corticosterone (**41**). Oxidative cleavage of the side chain followed by amidation afforded diketo amide **43**, which was homologated to ester **44** without involvement of the C-11 carbonyl. Triflation of the 11-ketone followed by palladium-catalyzed reduction[20] led to Δ^{11}-acid **45**.

Structure–Activity Relationships. Androst-3-ene-3-carboxylic acid **10** was designed as a mimic of the putative enolate intermediate of steroid 5α-reductase. Incorporated were both the 5α-ring fusion and the C-3 double

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(17) Hudlicky, T.; Sinai-Zingde, G.; Natchus, M. G. *Tetrahedron Lett.* **1987**, *28*, 5287–5290.
(18) Damon, R. E.; Schlessinger, R. H. *Tetrahedron Lett.* **1976**, *17*, 1561–1564; Dailey, O. D.; Fuchs, P. L. *J. Org. Chem.* **1980**, *45*, 216–236.
(19) Eggleston, D. S., unpublished results.
(20) Cacchi, S.; Morera, E.; Ortar, G. *Tetrahedron Lett.* **1984**, *25*, 4821–4824.

bond of the enolate and, most importantly, an acidic, potentially anionic carboxylate moiety serving as a replacement of the enolate oxyanion.[7]

Inhibition data (Table I) for congeners with varied sites and degrees of unsaturation suggest that the Δ^3 unsaturation is preferred but not essential for in vitro potency with human 5α-reductase (e.g. compounds 11 and 13). Trigonalization of C-5 results in no substantial loss of activity (2-fold or less) and in the case of the $\Delta^{3,5}$-acid 12 an apparent 2–3-fold increase in activity is observed.

Kamata and co-workers have recently reported enhanced bindings of Δ^{11}-androgens and progestins to their respective receptors and attribute this to the A-ring conformational differrence.[21] Molecular mechanics calculations suggest a significant change in conformation of $\Delta^{3,5}$-dienyl acids containing a C-11 double bond.[22] Nevertheless, no significant change in the in vitro activity of 45 relative to 12 was observed with the human or rat enzymes.

Substitution of fluorine or trifluoromethyl groups at C-4 or C-6 of the dienyl acid was expected to lower the pK_a of the acid by as much as 1 pK_a unit[23] and provide potential acceptor sites for hydrogen-bonding interactions. The 4-fluoro (16) and 6-fluoro (19) compounds maintained activity on the human enzyme while the 6-trifluoromethyl compound (21) exhibited a dramatic loss of activity. The decreased affinity of the 6-methyl compound (25) as well suggests an unfavorable steric interaction in the C-6 region.

Removal of the C-19 angular methyl group (32 and 33) resulted in a 3–5-fold decrease in potency.

C-17 side chain variations in 5α-reductase inhibitors have been examined extensively.[24] From these studies, 17β-carboxamides appear to exert the greatest positive effect on steroid binding to human 5α-reductase. The enzymes from human, rat, and dog show significant differences in C-17 specificity with the rat enzyme being particularly more permissive of side chain variants than that of the human.[25] These findings as well as earlier studies in our labs (e.g., compounds 54, 102, and unpublished data) led us to limit our study of side-chain variations primarily to two carboxamides. In the 3-carboxy series of compounds, the 17β-diisopropylamides display slightly greater affinity for human 5α-reductase than the *tert*-butylamide-containing analogues by a factor of 2–3.

Summary and Conclusions. Potent in vitro inhibition of human and rat prostatic steroid 5α-reductase has been observed with a series of 17β-carbamoyl-3-androstene-3-carboxylic acids. Activity is enhanced in analogues possessing an additional Δ^5-olefin and diminished in analogues lacking the Δ^3-olefin. We propose that these unsaturated stereoidal acids function as mimics of the putative enolate intermediate of the enzymatic process. Oral activity in rats and primates by inhibitors of this class will be the subject of future publications.

Experimental Section

General Methods. Melting points are uncorrected. ^1H NMR spectra were obtained in $CDCl_3$ solutions with Bruker AM-250 or Varian EM390 spectrometers and are reported in the supplemental material as ppm downfield from Me_4Si with multiplicity, coupling constants (Hz), and assignments indicated parenthetically. Mass spectra were obtained with a Finnigan-MAT quadrupole instrument generally with desorptive chemical ionization. Mass spectral data are reported as the $(M + H)^+$ parent followed by unassigned fragments (supplemental material). Chromatography refers to flash chromatography using Kieselgel 60, 230–400 mesh silica gel. Preparative HPLC was performed using a Sep Tech Lab 800B system with a 75 mm × 1 m column packed with 2.5 kg of Vydac Si HS 80A 15–20 μm silica. The 17β-substituted 4-en-3-ones were prepared as previously described.[24]

***N*,*N*-Diisopropyl-3-[[(trifluoromethyl)sulfonyl]oxy]-5α-androst-3-ene-17β-carboxamide (2).** Ammonia (200 mL) was distilled from lithium into a three-neck round-bottom flask equipped with a dry ice condenser and argon bubbler and cooled to −78 °C. Lithium wire (120 mg, 17.4 mmol) was dissolved in the ammonia. A solution of steroidal enone 1 (3.0 g, 7.5 mmol) and aniline (50 μL, 5.4 mmol) in THF (50 mL) was added dropwise to the Li/NH_3 solution. The resulting reaction mixture was stirred at −78 °C for 2 h and then quenched with dry isoprene until the blue color disappeared. The volatiles were removed by slow warming of the mixture under an argon flow initially and finally at reduced pressure. The residue was then dissolved in THF (50 mL) and cooled to 0 °C. A solution of *N*-phenyltriflimide (7.0 g, 20 mmol) in THF (10 mL) was added and the solution was allowed to stir at 4 °C overnight. The solvent was then evaporated and the residue was chromatographed (3% EtOAc in hexanes) to yield 2.9 g (72%) of enol triflate 2, mp 125–128 °C.

The following 5α-3-enol triflates were prepared in an analogous fashion from appropriate 4-en-3-ones: *N*-*tert*-butyl-3-[[(trifluoromethyl)sulfonyl]oxy]-5α-androst-3-ene-17β-carboxamide (48) and 21-[(*tert*-butyldimethylsilyl)oxy]-20(*S*)-methyl-5α-pregn-3-en-3-yl (trifluoromethyl)sulfonate (51).

***N*,*N*-Diisopropyl-3-[[(trifluoromethyl)sulfonyl]oxy]-androsta-3,5-diene-17β-carboxamide (4).** To a solution of enone 1 (5.0 g, 12.5 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (3.08 g, 17.0 mmol) in CH_2Cl_2 (50 mL) was added triflic anhydride (3.5 mL, 19 mmol) and the mixture was stirred for 30 min. The mixture was then diluted with CH_2Cl_2 (50 mL) and filtered. The filtrate was washed with 5% HCl (2 × 50 mL), saturated $NaHCO_3$ (50 mL), and brine, dried over Na_2SO_4, and evaporated. The crude triflate was purified by chromatography (20% EtOAc in hexanes) to yield 4 as a white foam (4.0 g, 61%).

Other triflates were prepared by a similar procedure: *N*-*tert*-butyl-3-[[(trifluoromethyl)sulfonyl]oxy]androsta-3,5-diene-17β-carboxamide (55), *N*,*N*-diisopropyl-3-[[(trifluoromethyl)-sulfonyl]oxy]-6-fluoroandrosta-3,5-diene-17β-carboxamide (75), *N*,*N*-diisopropyl-3-[[(trifluoromethyl)sulfonyl]oxy]-6-(trifluoromethyl)androsta-3,5-diene-17β-carboxamide (77), *N*,*N*-diisopropyl-3-[[(trifluoromethyl)sulfonyl]oxy]-4-methylandrosta-3,5-diene-17β-carboxamide (80), *N*,*N*-diisopropyl-3-[[(trifluoromethyl)sulfonyl]oxy]-6-methylandrosta-3,5-diene-17β-carboxamide (83), *N*,*N*-diisopropyl-3-[[(trifluoromethyl)sulfonyl]oxy]-10β-estra-3,5-diene-17β-carboxamide (91), and 3-[[(trifluoromethyl)sulfonyl]oxy]-17β-(*N*,*N*-diisopropylcarbamoyl)androsta-3,5-dien-11-one (96).

***N*,*N*-Diisopropyl-3-[[(trifluoromethyl)sulfonyl]oxy]-androsta-2,4-diene-17β-carboxamide (5).** To a solution of enone 1 (1.0 g, 2.5 mmol) in 10 mL of THF at −78 °C was added a solution of lithium bis(trimethylsilyl)amide (3.8 mL, 1 M in THF). After 2 h a solution of *N*-phenyltriflimide (2.0 g, 5.6 mmol) in 20 mL of THF was added. The resulting solution was stirred overnight at 4 °C and then evaporated to dryness. The residue was chromatographed (15% EtOAc in hexanes) to yield triflate 5 (1.13 g, 82%) as a white solid, mp 170–172 °C.

The following triflates were prepared in a similar fashion: *N*,*N*-diisopropyl-3-[[(trifluoromethyl)sulfonyl]oxy]-5α-androst-2-ene-17β-carboxamide (3), *N*,*N*-diispropyl-3-[[(trifluoro-

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(22) A relative movement of nearly 1 Å of the carboxylate carbon is observed when centers C-9, C-15, and C-17 of 12 and 45 are overlaid.

(23) See: Stewart, R. *The Proton: Applications to Organic Chemistry*; Academic: Orlando, 1985; Chapter 2. Brown, H. C.; McDaniel, D. H.; Hafliger, O. In *Determination of Organic Structures by Physical Methods*; Braude, E. A., Nachod. F. C., Eds.; Academic: New York, 1955; pp 567–655.

(24) Rasmusson, G. H.; Reynolds, G. F.; Utne, T.; Jobson, R. B.; Primka, R. L.; Berman, C.; Brooks, J. R. *J. Med. Chem.* **1984**, *27*, 1690–1701. 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. *J. Med. Chem.* **1986**, *29*, 2298–2315.

(25) Liang, T.; Cascieri, M. A.; Cheung, A. H.; Reynolds, G. F.; Rasmusson, G. H. *Endocrinology* **1985**, *117*, 571–579.

methyl)sulfonyl]oxy]-5α-androsta-1,3-diene-17β-carboxamide (**63**), *N,N*-diisopropyl-3-[[(trifluoromethyl)sulfonyl]oxy]androsta-1,3,5-triene-17β-carboxamide (**67**), *N,N*-diisopropyl-3-[[(trifluoromethyl)sulfonyl]oxy]-4-fluoro-5α-androsta-1,3-diene-17β-carboxamide (**70**), *N,N*-diisopropyl-3-[[(trifluoromethyl)-sulfonyl]oxy]estra-3,5(10)-diene-17β-carboxamide (**90**), and *N,N*-diisopropyl-3-[[(trifluoromethyl)sulfonyl]oxy]androsta-2,4,6-triene-17β-carboxamide (**99**).

Methyl 17β-(*N,N*-Diisopropylcarbamoyl)-5α-androst-3-ene-3-carboxylate (6). A mixture of enol triflate **2** (2.5 g, 4.7 mmol), bis(triphenylphosphine)palladium(II) acetate (105 mg, 0.14 mmol), triethylamine (1.3 mL, 9.4 mmol), methanol (10 mL), and DMF (10 mL) was stirred under 1 atm of carbon monoxide for 16 h. The resulting solution was diluted with EtOAc, washed with H_2O (3×), dried over Na_2SO_4, and concentrated. The residue was chromatographed (10% EtOAc in hexanes) to yield ester **6** as a white, crystalline solid (1.73 g, 83%), mp 157–159 °C.

Other α,β-unsaturated esters were prepared in a similar fashion: methyl 17β-(*N-tert*-butylcarbamoyl)-5α-androst-3-ene-3-carboxylate (**49**), methyl 21-[(trimethylsilyl)oxy]-20(*S*)-methyl-5α-pregn-3-ene-3-carboxylate (**52**), methyl 17β-(*N,N*-diisopropylcarbamoyl)androsta-3,5-diene-3-carboxylate (**8**), methyl 17β-(*N-tert*-butylcarbamoyl)androsta-3,5-diene-3-carboxylate (**56**), methyl 17β-(*N,N*-diisopropylcarbamoyl)-5α-androst-2-ene-3-carboxylate (**7**), methyl 17β-(*N,N*-diisopropylcarbamoyl)-androsta-2,4-diene-3-carboxylate (**9**), methyl 17β-(*N,N*-diisopropylcarbamoyl)-5α-androsta-1,3-diene-3-carboxylate (**64**), methyl 17β-(*N,N*-diisopropylcarbamoyl)androsta-1,3,5-triene-3-carboxylate (**68**), methyl 17β-((*N,N*-diisopropylcarbamoyl)-4-fluoro-5α-androsta-1,3-diene-3-carboxylate (**71**), ethyl 17β-(*N,N*-diispropylcarbamoyl)-6-fluoroandrosta-3,5-diene-3-carboxylate (**76**), ethyl 17β-(*N,N*-diisopropylcarbamoyl)-6-(trifluoromethyl)androsta-3,5-diene-3-carboxylate (**78**), methyl 17β-(*N,N*-diisopropylcarbamoyl)-4-methylandrosta-3,5-diene-3-carboxylate (**81**), methyl 17β-(*N,N*-diisopropylcarbamoyl)-6-methylandrosta-3,5-diene-3-carboxylate (**84**), methyl 17β-(*N,N*-diisopropylcarbamoyl)estra-3,5(10)-diene-3-carboxylate (**92**), methyl 17β-(*N,N*-diisopropylcarbamoyl)-10β-estra-3,5-diene-3-carboxylate (**93**), methyl 17β-(*N,N*-diisopropylcarbamoyl)androsta-2,4,6-triene-3-carboxylate (**100**), and methyl 17β-(*N,N*-diisopropylcarbamoyl)-11-oxoandrosta-3,5-diene-3-carboxylate (**44**).

17β-(*N,N*-Diisopropylcarbamoyl)-5α-androst-3-ene-3-carboxylic Acid (10). A mixture of ester **6** (1.59 g, 3.59 mmol), K_2CO_3 (1.0 g), H_2O (9 mL), and MeOH (90 mL) was heated at reflux overnight. The volatiles were removed by roto-evaporation and the residue was partitioned between 5% HCl and EtOAc. The aqueous layer was further extracted with CH_2Cl_2, and the combined organic layers were dried over Na_2SO_4 and concentrated to a white foam. Recrystallization from acetonitrile afforded acid **10** (1.15 g, 75%) as a white solid, mp 242–244 °C.

Other esters were hydrolyzed in a similar fashion to give the following acids: 17β-(*N-tert*-butylcarbamoyl)-5α-androst-3-ene-3-carboxylic acid (**50**), 21-hydroxy-20(*S*)-methyl-5α-pregn-3-ene-3-carboxylic acid (**54**), 17β-(*N,N*-diisopropylcarbamoyl)-androsta-3,5-diene-3-carboxylic acid (**12**), 17β-(*N-tert*-butylcarbamoyl)androsta-3,5-diene-3-carboxylic acid (**57**), 17β-(*N,N*-diisopropylcarbamoyl)-5α-androst-2-ene-3-carboxylic acid (**11**), 17β-(*N,N*-diisopropylcarbamoyl)androsta-2,4-diene-3-carboxylic acid (**13**); 17β-(*N,N*-diisopropylcarbamoyl)-5α-androstane-3β-carboxylic acid (**34**), 17β-(*N,N*-diisopropylcarbamoyl)androst-4-ene-3β-carboxylic acid (**36**), 17β-(*N,N*-diisopropylcarbamoyl)-androst-4-ene-3α-carboxylic acid (**35**), 17β-(*N,N*-diisopropylcarbamoyl)-5α-androsta-1,3-diene-3-carboxylic acid (**65**), 17β-(*N,N*-diisopropylcarbamoyl)androsta-1,3,5-triene-3-carboxylic acid (**69**), 17β-(*N,N*-diisopropylcarbamoyl)-4-fluoro-5α-androst-3-ene-3-carboxylic acid (**16**), 17β-(*N,N*-diisopropylcarbamoyl)-6-fluoroandrosta-3,5-diene-3-carboxylic acid (**19**), 17β-(*N,N*-diisopropylcarbamoyl)-6-(trifluoromethyl)androsta-3,5-diene-3-carboxylic acid (**21**), 17β-(*NN*-diisopropylcarbamoyl)-4-methylandrosta-3,5-diene-3-carboxylic acid (**82**), 17β-(*N,N*-diisopropylcarbamoyl)-6-methylandrosta-3,5-diene-3-carboxylic acid (**25**), 17β-(*N,N*-diisopropylcarbonyl)estra-3,5(10)-diene-3-carboxylic acid (**32**), 17β-(*N,N*-diisopropylcarbamoyl)-10β-estra-3,5-diene-3-carboxylic acid (**33**), 17β-(*N,N*-diisopropylcarbamoyl)-3α-hydroxyandrost-4-ene-3β-carboxylic acid (**40**), 17β-(*N,N*-diisopropylcarbamoyl)-3β-hydroxyandrost-4-ene-3α-carboxylic acid (**39**), 17β-(*N,N*-diisopropylcarbamoyl)androsta-3,5,11-triene-3-carboxylic acid (**45**), and 17β-(*N,N*-diisopropylcarbamoyl)androsta-2,4,6-triene-3-carboxylic acid (**101**).

Methyl 21-Hydroxy-20(*S*)-methyl-5α-pregn-3-ene-3-carboxylate (53). To a solution of silyl ether **52** (500 mg, 1.05 mmol) in THF (20 mL) was added a solution of tetrabutylammonium fluoride (2 mL, 1 M in THF). After 3.5 h the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The organic extract was dried over Na_2SO_4 and concentrated. Chromatography (20% EtOAc in hexanes) afforded 300 mg (78%) of **53** as a white solid.

17β-(*N,N*-Diisopropylcarbamoyl)-5α-androst-3-one (58). Ammonia (1500 mL) was distilled into a three-neck round-bottom flask equipped with a dry ice condenser, mechanical stirrer, and argon bubbler and cooled to −78 °C. Lithium wire was dissolved in the ammonia (ca. 2 g). A solution of steroidal enone **1** (20.0 g, 50.1 mmol) and aniline (5 mL) in THF (500 mL) was slowly added to the Li/NH_3 solution. The resulting reaction mixture was stirred at −78 °C for 2 h and then quenched with dry isoprene (until the blue color disappeared) followed by solid NH_4Cl. The ammonia was allowed to evaporate by slow warming. The remaining mixture was diluted with H_2O and thoroughly extracted with EtOAc. The organic extract was washed with 5% HCl and brine, dried over K_2CO_3, and concentrated. The residue was chromatographed (15% EtOAc in hexanes) to yield 16.1 g (80%) of 5α-3-ketone **58** as a white solid, mp 168–170 °C.

17β-(*N,N*-Diisopropylcarbamoyl)-5α-androst-1-en-3-one (62). To a solution of ketone **58** (2.3 g, 5.74 mmol) in EtOAc (100 mL) was added phenylselenyl chloride (1.1 g, 5.74 mmol) and the reaction mixture was stirred for 2 h. The reaction mixture was then washed with 5% sodium bicarbonate solution and brine. The EtOAc solution was cooled to 0 °C and 50 mL of THF was added. Hydrogen peroxide (6 mL of a 30% solution) was slowly added and the reaction mixture was then washed with 5% sodium bicarbonate solution and brine and evaporated to dryness. Purification by chromatography eluting with 20% ethyl acetate in hexanes afforded 1.3 g (56.5%) of enone **62**.

17β-(*N,N*-Diisopropylcarbamoyl)androsta-1,4-dien-3-one (66). A mixture of enone **1** (3.0 g, 7.5 mmol) and DDQ (4.2 g, 18.5 mmol) in 300 mL of toluene was heated at reflux overnight. The cooled mixture was filtered. The filtrate was concentrated and chromatographed (20% EtOAc in hexanes) to yield dienone **66** as a white solid, which was recrystallized from methanol, mp 224–225 °C (1.25 g, 42%).

17β-(*N,N*-Diisopropylcarbamoyl)-4-methylandrost-4-en-3-one (79). To a boiling solution of potassium *tert*-butoxide (1.7 g, 15 mmol) in tBuOH (50 mL) was added a hot solution of enone **1** (4.0 g, 10 mmol) in tBuOH (25 mL). A solution of CH_3I (1.7 g, 12 mmol) in tBuOH (50 mL) was added over 1 h. Heating was continued for an additional 1 h, after which time the solution was cooled and carefully acidified with concentrated HCl. The volatiles were removed in vacuo, and the residue was partitioned between EtOAc and H_2O. The EtOAc layer was washed with H_2O and brine, dried, and concentrated. Chromatography (15% EtOAc in hexanes) yielded 4-methyl enone **79** as a white solid, mp 193–195 °C (1.6 g, 39%).

17β-(*N,N*-Diisopropylcarbamoyl)-1α,2α-epoxy-5α-androstan-3-one (14). To a chilled (ca. 15 °C) solution of enone **62** (4.6 g, 11.5 mmol) in CH_3OH (50 mL) was added 30% H_2O_2 (3.7 mL) followed by 10% NaOH (0.75 mL diluted with 8 mL of CH_3OH). The mixture was allowed to warm to ambient temperature and stirred for 1 h, after which time it was partitioned between ice–H_2O and CH_2Cl_2. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated to a white solid. Trituration with Et_2O–hexanes provided pure epoxy ketone **14**, mp 231–233 °C (4.78 g, 84%).

17β-(*N,N*-Diisopropylcarbamoyl)-4α-fluoro-5α-androst-1-en-3-one (15). To a solution of epoxy ketone **14** (1.1 g, 2.65 mmol) in THF (25 mL) cooled to −10 °C was slowly added hydrogen fluoride–pyridine (Aldrich, ca. 70% HF–30% pyr; 40 mL). The resulting solution was stirred at 0 °C for 15 min and then at ambient temperature. Once the solution became wine colored, it was poured into ice–H_2O and extracted with EtOAc. The organic extract was washed with H_2O, 5% $NaHCO_3$, and brine, dried, and concentrated. The residue was chromatographed (20% EtOAc in hexanes) to yield, after trituration with Et_2O, 0.98 g

(45%) of fluoroenone **15** as a white solid, mp 196 °C.

Methyl 17β-(*N*,*N*-Diisopropylcarbamoyl)-4-fluoro-5α-androst-3-ene-3-carboxylate (72). A solution of diene **71** (120 mg, 0.26 mmol) in 1:2 EtOAc–hexanes (15 mL) was rapidly stirred over 20 mg of 10% Pd on carbon under an atmosphere of hydrogen for 1 h. The mixture was filtered and the filtrate was concentrated. Chromatography (10% EtOAc in hexanes) afforded 70 mg (58%) of **72** which was recrystallized from MeOH–acetone; mp 171–172 °C.

3,3-(Ethylenedioxy)-*N*,*N*-diisopropylandrost-5-ene-17β-carboxamide (73). A solution of enone **1** (20.0 g, 50.1 mmol), $TsOH-H_2O$ (500 mg), and ethylene glycol (50 mL) in benzene (300 mL) was heated at reflux with azeotropic removal of water for 24 h. The solution was cooled and then stirred over $NaHCO_3$ for 1 h. The mixture was washed with water and brine, dried over K_2CO_3, and concentrated. The crude ketal was used without further purification for the next step.

An analytically pure sample of ketal **73** was obtained by chromatography (20% EtOAc in hexanes).

5,6-Epoxy-3,3-(ethylenedioxy)-*N*,*N*-diisopropylandrostane-17β-carboxamide (17 and 74). To a solution of crude ketal **73** in CH_2Cl_2 (100 mL) was slowly added a solution of mCPBA (15 g) in CH_2Cl_2 (150 mL), keeping the temperature below 25 °C with the use of a cool water bath. The solution was stirred an additional 1 h after completion of the addition and then washed successively with 10% Na_2SO_3, 10% $NaHCO_3$, and brine, dried, and concentrated. Chromatography (20% EtOAc in hexanes) yielded the first eluting 5,6β-epoxide **74** as a white solid (6.1 g after recrystallization from acetone; 27% from enone **1**) and the 5,6α-epoxide **17** as the second eluting product as a white solid (7.0 g; 30% from enone **1**).

17β-(*N*,*N*-Diisopropylcarbamoyl)-6ξ-fluoroandrost-4-en-3-one (18). To a solution of ketal epoxide **17** (2.5 g, 5.45 mmol) in benzene–ether (1:1, 100 mL) was added BF_3-Et_2O (Aldrich, 2.5 mL) and the resulting solution was stirred at ambient temperature for 4 h. The solution was then washed with 5% Na_2CO_3, H_2O, and brine, dried, and concentrated to a yellow oil. The unstable fluoroalcohol intermediate was immediately treated with an HCl-saturated solution of acetic acid (15 mL) at ambient temperature for 1.5 h. The reaction mixture was then diluted with EtOAc and washed with 5% $NaHCO_3$, H_2O, and brine, dried, and concentrated to a yellowish solid. Chromatography (20% EtOAc in hexanes) yielded the fluoroenone **18** as a mixture of C-6 epimers (1.7 g, 77%).

17β-(*N*,*N*-Diisopropylcarbamoyl)-6β-(trifluoromethyl)-androst-4-en-3-one (20). A solution of triflate **4** (1 g, 1.9 mmol) in pyridine (10 mL) was photolyzed with a Hanovia medium-pressure 450-W mercury-vapor lamp at room temperature for 18 h.[15] The reaction solution was then diluted with EtOAc, washed with 10% HCl and brine, dried, and concentrated. Chromatography (20% EtOAc in hexanes) afforded compound **20**.

***N*,*N*-Diisopropyl-3-[(trimethylsilyl)oxy]androsta-3,5-diene-17β-carboxamide (22).** To a solution of enone **1** (5.0 g, 12.5 mmol) in DMF (40 mL) was added TMSCl (2.7 g, 25 mmol) and Et_3N (3 g, 30 mmol). The resulting solution was heated at reflux for 5 h and then cooled and concentrated to dryness. The residue was thoroughly extracted with hexane. The extract was concentrated and chromatographed (10% EtOAc in hexanes) to yield 4.0 g (68%) of dienol ether **22**.

17β-(*N*,*N*-Diisopropylcarbamoyl)-6ξ-[[1,3-propanediylbis(thio)]methyl]androst-4-en-3-one (23). To a solution of dienol ether **22** (250 mg, 0.53 mmol) in CH_3NO_2 (1.5 mL) at −78 °C was added a solution of 1,3-dithienium fluoroborate (110 mg, 0.53 mmol) in CH_3NO_2 (0.5 mL). After stirring for 10 min at −78 °C, the mixture was partitioned between Et_2O and saturated $NaHCO_3$. The ethereal layer was washed with brine, dried, and concentrated. Chromatography (30% EtOAc in hexanes) yielded dithiane **23** (150 mg, 58%) as a white solid, mp 213–215 °C.

17β-(*N*,*N*-Diisopropylcarbamoyl)-6ξ-methylandrost-4-en-3-one (24). Dithiane **23** (1.6 g, 3.1 mmol) in acetone (400 mL) was treated with an excess of Raney Ni (Aldrich; washed with H_2O, MeOH, acetone) for 4 h. The acetone solution was then decanted from the Raney Ni and evaporated. The residue was redissolved in CH_2Cl_2, washed with H_2O, dried, and concentrated. Chromatography yielded the 6-methyl enone as a mixture of epimers (420 mg, 31%, mp 183–185 °C).

Trifluoromethyl 3-Methoxyestra-1,3,5(10),16-tetraene-17-sulfonate (27). To a solution of estrone methyl ether (25 mg, 88 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (18.7 g, 92 mmol) in CH_2Cl_2 (650 mL) was added triflic anhydride (31.5 g, 110 mmol) and the resulting solution was stirred for 5 h. The reaction mixture was then filtered and the filtrate was washed with 5% $NaHCO_3$ and brine, dried, and concentrated. Chromatography (5% EtOAc in hexanes) yielded enol triflate **27** as a white solid (32.6 g, 87%).

***N*,*N*-Diisopropyl-3-methoxyestra-1,3,5(10),16-tetraene-17-carboxamide (28).** A mixture of triflate **27** (13.5 g, 33 mmol), $Pd(OAc)_2(PPh_3)_2$ (1.6 g, 3 mmol), Et_3N (9 mL), iPr_2NH (50 mL), and DMF (100 mL) was heated at 60 °C under an atmosphere of CO for 3 h. The reaction mixture was diluted with EtOAc and thoroughly washed with H_2O and brine, dried, and concentrated. Chromatography (10% EtOAc in hexanes) yielded amide **28** as a white solid (8.6 g, 67%).

***N*,*N*-Diisopropyl-3-methoxyestra-1,3,5(10)-triene-17β-carboxamide (29).** A mixture of unsaturated amide **28** (16.0 g, 15.2 mmol) and PtO_2 (5 g) in EtOAc–EtOH (3:1, 500 mL) was rapidly stirred under H_2 (1 atm) overnight. The mixture was filtered and the filtrate was concentrated to a white solid. Recrystallization from acetone afforded 14.25 g (89%) of 17β-amide **29**.

17β-(*N*,*N*-Diisopropylcarbamoyl)estr-5(10)-en-3-one (30). A solution of methyl ester **29** (4.5 g, 11.3 mmol) in THF (50 mL) and tBuOH (50 mL) was added to 100 mL of liquid NH_3. Li wire (1.5 g) was then added and the blue solution was stirred at −33 °C for 2 h, after which time the blue color had dissipated. An additional 0.3 g of Li was added and the solution was stirred for 1.5 h, after which time the blue color had again dissipated. Methanol (25 mL) was added to the solution, and the volatiles were allowed to evaporate overnight. The residue was partitioned between EtOAc and H_2O. The organic layer was washed with brine and concentrated. The residue was treated with oxalic acid (6 g) in 230 mL of MeOH and 30 mL of H_2O at ambient temperature for 4 h. The solution was then concentrated, diluted with EtOAc, washed with H_2O and brine, and concentrated. Chromatography (10% EtOAc in hexanes) yielded 1.4 g of enone **30** and 1.5 g of methyl ether starting material **29**.

17β-(*N*,*N*-Diisopropylcarbamoyl)-10β-estr-4-en-3-one (31). A mixture of enone **30** in MeOH–10% HCl (5:2) was heated at 65 °C for 1 h. After cooling, the mixture was diluted with H_2O and extracted with $CHCl_3$. The organic layer was dried over K_2CO_3 and concentrated to yield quantitatively enone **31** as a white solid.

Methyl 17β-(*N*,*N*-Diisopropylcarbamoyl)-5α-androstane-3β-carboxylate (59). Unsaturated ester **7** (87 mg) in a 10:1 mixture of EtOAc–HOAc was hydrogenated (1 atm) over a catalytic amount of 10% Pd on carbon. Filtration and concentration of the filtrate afforded saturated 3β-ester **59** (77 mg) as the only product (by ^1H NMR).

Methyl 17β-(*N*,*N*-Diisopropylcarbamoyl)androst-4-ene-3β-carboxylate (60) and Methyl 17β-(*N*,*N*-Diisopropylcarbamoyl)androst-4-ene-3α-carboxylate (61). To a solution of diene ester **8** (440 mg, 1 mmol) in MeOH (10 mL) was carefully added powdered magnesium (240 mg, 10 mmol, 50 mesh). The mixture was stirred for 3 h, after which time it was cautiously diluted with 10% HCl and extracted with CH_2Cl_2. The organic extract was washed with brine, dried, and concentrated to a white foam (420 mg). The product was nearly homogeneous by TLC (20% EtOAc in hexanes). NMR indicated a 1:1 mixture of isomers. Preparative HPLC (49:49:2 hexane–CH_2Cl_2–EtOAc) afforded pure samples of Δ^4-3α and Δ^4-3β esters **61** and **60**, respectively. Stereochemical assignments were based on differences in ^1H NMR coupling constants for H-3 in compounds **61** and **60** and their respective acids, **35** and **36**. A much larger coupling (ca. 12 Hz) was observed for the 3β-carboxy isomers, presumably a result of a pseudoaxial H-3α/axial H-2β interaction.

3-Hydroxy-*N*,*N*-diisopropyl-3-[tris(methylthio)methyl]-androst-4-ene-17β-carboxamide (37 and 38). To a −78 °C solution of tris(methylthio)methane (14.6 mL, 110 mmol) in THF (250 mL) was added a solution of *n*-BuLi (38.5 mL, 2.6 M in hexanes, 100 mmol). After 20 min, a solution of enone **1** (40 g, 100 mmol) in THF (250 mL) was slowly added. The resulting mixture was stirred at −78 °C for 3 h and then quenched with saturated aqueous NH_4Cl. The mixture was concentrated, diluted

with water, and thoroughly extracted with $CHCl_3$. The organic extract was concentrated and the residue was chromatographed (20% EtOAc in hexanes) to yield 21.1 g of β-hydroxy isomer **37** as white crystals and 14.0 g of a 1:1 mixture of α and β isomers, **38** and **37**, as a whitish solid.

Methyl 17β-(*N*,*N*-Diisopropylcarbamoyl)-3α-hydroxyandrost-4-ene-3β-carboxylate (94) and Methyl 17β-(*N*,*N*-Diisopropylcarbamoyl)-3β-hydroxyandrost-4-ene-3α-carboxylate (95). A mixture of thioorthoester **38** (11.6 g, 20 mmol), HgO (8.6 g, 40 mmol), $HgCl_2$ (27.1 g, 100 mmol), and 600 mL of MeOH-H_2O (12:1) was stirred at ambient temperature for 4 h. The mixture was filtered, and the residues were washed with CH_2Cl_2. The combined filtrate and washings were diluted with H_2O and extracted with CH_2Cl_2. The organic extract was then washed with saturated aqueous ammonium formate and saturated NH_4Cl, dried, and concentrated. Chromatography (30% EtOAc in hexanes) yielded 9.1 g (99%) of hydroxy ester **94** as a white solid.

The same hydrolysis conditions were applied to a 1:1 mixture of **37** and **38** to yield a mixture of hydroxy ester diastereomers **94** and **95**, which were separated by preparative HPLC (96:4 $CHCl_3$–EtOAc).

3,11-Dioxoandrost-4-ene-17β-carboxylic Acid (42). To a cooled (10 °C) solution of corticosterone (**41**, 10.0 g, 28.9 mmol) in 400 mL of acetone was added Jones reagent until the red color persisted. After stirring for an additional 1.5 h at room temperature, an excess of iPrOH was added to destroy the remaining Jones reagent. The volatiles were then removed under vacuum. The residue was partitioned between water and CH_2Cl_2. The organic layer was washed with water and brine, dried, and concentrated. Trituration with methanol afforded diketo acid **42** as a white solid (5.5 g, 58%), mp 265–268 °C.

17β-(*N*,*N*-Diisopropylcarbamoyl)androst-4-ene-3,11-dione (43). Diketo acid **42** (4.7 g, 14 mmol) was suspended in 550 mL of toluene and azetropically dried until 100 mL of distillate had been collected. The mixture was cooled to 0 °C and pyridine (1.7 mL) was added followed by oxalyl chloride (2 mL). The reaction mixture was stirred at ambient temperature for 2 h, after which time excess oxalyl chloride was removed by roto-evaporation of 100 mL of solvent. The reaction mixture was then recooled to 0 °C and diisopropylamine (25 mL) was slowly added such that the temperature did not rise above 40 °C. The resulting mixture was allowed to stir overnight. The volatiles were removed, and the residue was redissolved in CH_2Cl_2 and washed with water, dilute HCl, and brine, dried, and concentrated. The residue was triturated with methanol and filtered. The filtrate was chromatographed (25% EtOAc in hexanes) to yield dione amide **43** as a white solid (3.28 g, 56%), mp 213–215 °C.

Methyl 11-[[(Trifluoromethyl)sulfonyl]oxy]-17β-(*N*,*N*-diisopropylcarbamoyl)androsta-3,5,11-triene-3-carboxylate (97). To a solution of keto ester **44** (1.2 g, 2.6 mmol) in THF (15 mL) at −78 °C was added a solution of lithium bis(trimethylsilyl)amide (6.4 mL, 1.0 M in THF, 6.4 mmol). After 2 h, a solution of *N*-phenyltriflimide (1.2 g, 3.4 mmol) in THF (15 mL) was added. The resulting solution was stirred at 4 °C overnight and then evaporated to dryness. Chromatography (15% EtOAc in hexanes) afforded triflate **97** as a white solid (1.28 g, 83%).

Methyl 17β-(*N*,*N*-Diisopropylcarbamoyl)androsta-3,5,11-triene-3-carboxylate (98). A mixture of triflate **97** (1.28 g, 2.1 mmol), bis(triphenylphosphine)palladium(II) acetate (64 mg, 0.09 mmol), formic acid (97%, 0.36 mL), tri-*n*-butylamine (1.53 mL), and DMF (5 mL) was heated at 60–70 °C for 5 h. The cooled reaction mixture was diluted with EtOAc, washed with water and brine, dried, and concentrated. Chromatography (15% EtOAc in hexanes) provided triene ester **98** as a white foam (0.7 g, 73%).

Inhibitor Evaluation. Assays for steroid 5α-reductase were performed with microsomal-associated enzyme activity from surgically derived benign hyperplastic human prostatic tissue and whole rat ventral prostates. Prostatic microsomes were prepared as previously described for the rat[26] and human[27] tissues. Enzyme activity was determined by measuring the conversion of T to total 5α-reduced metabolites, represented by the sum of DHT and 5α-androstanediol (ADIOL).[28] Briefly, [^{14}C]T (55 mCi/mmol, Amersham) and inhibitors in ethanol were deposited in test tubes and the solvent was removed to dryness. Following addition of incubation buffer to the tubes, the solutions were equilibrated to 37 °C. A 20-μL aliquot of freshly prepared 10 mM NADPH solution was added to each tube immediately before initiation of the reaction with enzyme. The final concentration of cofactor in the 0.5-mL incubation was 400 μM. The rat enzyme incubation buffer consisted of 20 mM sodium phosphate, pH 6.6; that for human microsomes was 50 mM sodium citrate, pH 5.0. Following 20–30-min incubations, the reactions were quenched with 4 mL of ethyl acetate containing 0.15 μmol each of T, DHT, androstenedione, and ADIOL. The mixture was vortexed and centrifuged to separate the solvent layers, and the organic layer was removed. Upon evaporation of solvent in vacuo, the residue was dissolved in 40 μL of 1:1 methanol–chloroform. Substrate and products were separated by TLC on silica gel plates (Baker, Si250F-PA) developed twice with 1:9 acetone–chloroform and evaporated with a Bioscan imaging scanner (Washington, DC). The relative amounts of radiolabel in substrate and products were used to calculate enzyme activity. Assays were conducted such that no more than 20% of initial T concentration was consumed in the reaction. Typically, the Michaelis constants for T with the rat and human prostatic enzymes were determined to be 0.9 and 4.5 μM, respectively.

Experiments to determine the potency of potential inhibitors were conducted at 400 μM NADPH, 1.2 μM T, and 0–10 μM of test compound. Apparent inhibition constants ($K_{i,app}$) were determined for compounds that followed a linear response by Dixon analysis.[29] For compounds that demonstrated nonlinear (hyperbolic) inhibition according to the Dixon plots, the concentration required to decrease enzyme activity to 50% of that of the control (IC_{50}) was determined. For compounds that showed linear kinetic behavior, values of $K_{i,app}$ and IC_{50} differed by less than 30% for the same experiment. Compounds were tested as inhibitors of the rat and human enzymes over several years with different preparations of microsomes; over this period of time, variability of inhibition potency was observed with some compounds. Consequently, a potency range of inhibition is presented for those compounds that were examined with more than one enzyme preparation. As convention, an inhibition potency of >10 000 has been used for compounds demonstrating less than 50% inhibition at the highest concentration tested.

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Novel [(Diazomethyl)carbonyl]-1,2,3,4-tetrahydronaphthalene Derivatives as Potential Photoaffinity Ligands for the 5-HT_{1A} Receptor

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The photolabile (diazomethyl)carbonyl function was introduced into the 8-position of 2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene in three ways, resulting in the ether 8-[[(diazomethyl)carbonyl]methoxy]-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (**2**), the ester 8-(diazoacetoxy)-2-(N,N-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (**3**), and the ketone 8-[(diazomethyl)carbonyl]-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (**4**). Specific binding of these compounds at the 5-hydroxytryptamine$_{1A}$ sites in rat brain membranes labeled with 1 nM [^{3}H]-8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (8-OH-DPAT) showed IC_{50} values of ca. 75, 125, and 25 nM, respectively, for the three compounds. Photolysis of methanolic solutions of **2**-**4** in the absence of receptor proteins lead in each case to an abundance of Wolff-rearranged products. In the case of ether **2**, subsequent β-elimination to 8-OH-DPAT removed this compound from serious consideration as a photoaffinity ligand. Ester **3** and ketone **4** were photolysed in vitro. Whereas ester **3** was ineffective in decreasing the specific binding of [^{3}H]-8-OH-DPAT, ketone **4** decreased 40% of the specific binding of [^{3}H]-8-OH-DPAT in the presence (but not the absence) of ultraviolet light. Thus this ketone emerges from these studies as a good candidate for a photoaffinity label for the 5-hydroxytryptamine$_{1A}$ receptor.

Among the many proteins that respond to 5-hydroxytryptamine (5-HT, serotonin), the 5-HT_{1A} receptor is distinguished by both ligand-binding and functional characteristics. The selectivity and nanomolar affinity of the agonist [^{3}H]-8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (8-OH-DPAT, **1**) for this receptor has made this the ligand of choice to label the receptor.[1] Unlabeled 8-OH-DPAT has been used to assign a number of in vivo physiological functions to the 5-HT_{1A} receptor, including some aspects of the "serotonin behavioral syndrome", anxiety, depression, feeding behavior, cardiovascular effects, and thermoregulation.[2] The cellular responses to 5-HT_{1A} agonists, opening of a Ca^{2+}-insensitive K^+ channel[3] and regulation of adenylyl cyclase,[4] are mediated via coupling to signal-transducing G-proteins, although exactly which G-protein(s) remains to be determined. It is also problematical whether agonist-occupied 5-HT_{1A} receptors in vivo can both stimulate[5,6] and inhibit the forskolin-stimulated[4,7] adenylyl cyclase as is observed in vitro. Receptor signal transduction mechanisms have in the last decade been addressed most successfully in experiments using the purified receptors, G-proteins, and effectors. More recently, chimeric receptors have been constructed and used to delineate ligand binding from signal-transducing domains.[8]

The direct characterization of the receptor protein(s) represents an approach to receptor taxonomy and function that is complementary to classical pharmacological methods using high-affinity reversible ligands. Technical advances in molecular biology and protein chemistry have made available both the DNA clone for the 5-HT_{1A} receptor and its expression in cell cultures.[9] Thus, the 5-HT_{1A} receptor can be expected to join the growing number of transmembrane proteins whose topology and function may be extensively mapped. A reliable, selective photoaffinity probe will be one of the tools required for these investigations, and we are developing novel reagent ligands for this purpose.

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