Antiandrogenic Steroidal Sulfonylpyrazoles

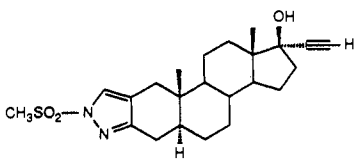
Robert G. Christiansen, Malcolm R. Bell,* Thomas E. D'Ambra, John P. Mallamo, John L. Herrmann, James H. Ackerman, Chester J. Opalka, Rudolph K. Kullnig, Richard C. Winneker, Ben W. Snyder, Frederick H. Batzold, and H. Phillip Schane

Departments of Chemistry and Pharmacology, Sterling Research Group, Rensselaer, New York 12144.
Received December 18, 1989

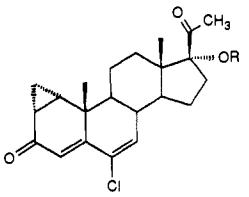
The steroidal sulfonylpyrazole 1 bound to the rat ventral prostate androgen receptor in vitro; it inhibited testosterone propionate induced increases in ventral prostate weight in vivo in the castrated, immature male rat with an ED_{50} of 15 mg/kg po. Compound 1 lacked androgenic activity in vivo in contrast to the parent steroidal pyrazole **5**, which was both androgenic and antiandrogenic. The 2'- and 5'-methylsulfonyl isomers **6** and **6a** did not bind to the androgen receptor. Introduction of an alkylsulfonyl at the N-1'-position has served, therefore, to isolate the intrinsic antiandrogenic properties of the steroidal heterocycle free of apparent hormone agonist properties. Structure–activity relationship studies revealed that a methylsulfonyl group at N-1' together with a C-17α-substituent were the optimal combination for in vitro androgen receptor binding, in vivo antiandrogenic potency, and a lack of androgenic activity.

Introduction

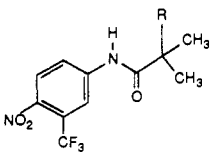
Benign prostatic hyperplasia, prostatic cancer, acne, and hirsutism are clinical conditions where androgen receptor antagonism can provide effective therapy.[1] Both steroidal and nonsteroidal antiandrogens have been identified. Many, however, have properties that reduce their clinical utility.[2,3] The most thoroughly characterized steroidal antiandrogen is cyproterone acetate (**2b**), which, in addition to its antihormonal properties, is both progestational and weakly androgenic.[4] In contrast to steroidal antiandrogens, there are a growing number of examples of nonsteroidal antiandrogens which lack hormone agonist activity. Flutamide (**3a**),[5] its active metabolite hydroxyflutamide (**3b**),[6] and the recently reported arylsulfonyl derivative ICI-176334 (**4**)[7,8] are important examples. Since there are no well-characterized nonsteroidal androgens, it is of theoretical interest that some relatives of these nonsteroidal antiandrogens have been reported to cause an elevation of the weight of the ventral prostate of the immature male rate, an androgen-specific event.[9]



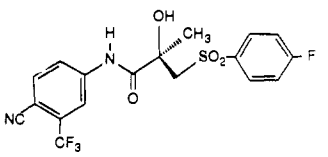
1



2a R=H ; Cyproterone
2b R=OAc; Cyproterone Acetate

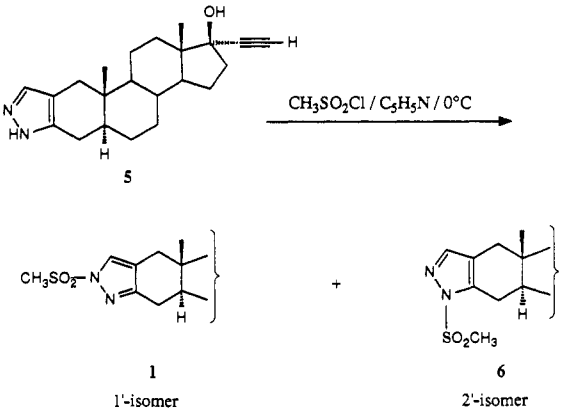


3a Flutamide R=H
3b Hydroxyflutamide R=OH

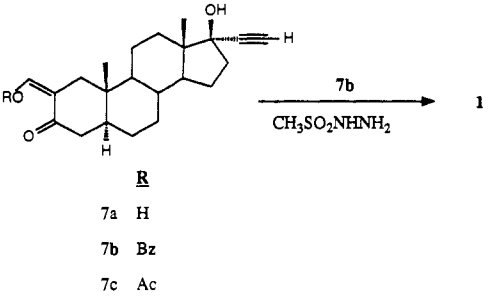


4 ICI 176334

Scheme I



Scheme II

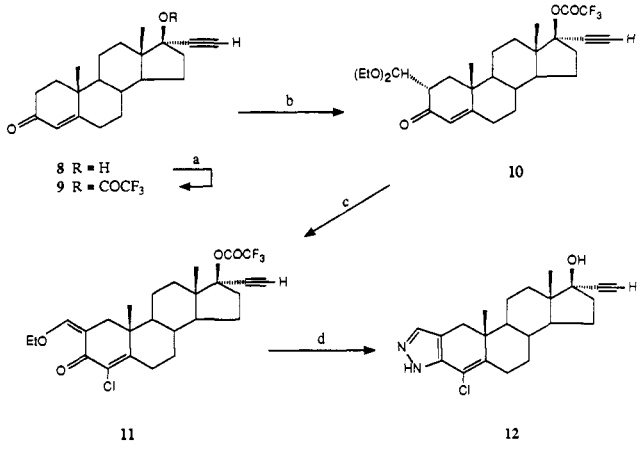


During an investigation of structure–activity relationships among steroidal [3,2-*c*]pyrazoles we prepared a number of 1'-alkylsulfonyl derivatives, some of which displayed antiandrogenic activity. Pyrazole 1 bound to the rat ventral prostate androgen receptor in vitro and inhibited androgen-induced responses in the rat and lacked androgen agonist activity in vivo.[10,11] It has been chosen from this series of compounds for advanced evaluation.

(1) Neri, R.; Kassem, N. In Hormone Antagonists; Agarwal, M. K., Ed.; Walter de Gruyter: New York, NY, 1982; pp 247–268.
(2) Neuman, F.; Topert, M. *J. Steroid Biochem.* **1986**, *5B*, 885.
(3) Knuth, U. A.; Hano, R.; Nieschlag, E. *J. Clin. Endocrinol. Metab.* **1984**, *59*, 963.
(4) Neri, R.; Monahan, M. D.; Meyer, J. G.; Afonso, B. A.; Tabachnik, I. A. *Eur. J. Pharmacol.* **1967**, *1*, 438.
(5) Neri, R.; Florance, K.; Koziol, P.; Van Cleave, S. *Endocrincology* **1972**, *91*, 427.
(6) Neri, R.; Peets, E.; Watnick, A. *Biochem. Soc. Trans.* **1979**, *7*, 565.
(7) Tucker, H.; Crook, J. W.; Chesterton, G. J. *J. Med. Chem.* **1988**, *31*, 954.
(8) Tucker, H.; Chesterton, G. J. *J. Med. Chem.* **1988**, *31*, 885.
(9) Glen, A. T.; Hughes, L. R.; Morris, J. J.; Taylor, P. J., *Spec. Publ. R. Soc. Chem.* **1986**, *55* (SCI-RSC Med. Chem. Symp., 3rd., 1985), 345–361.
(10) Winneker, R. C.; Snyder, B. W.; Batzold, F. H.; Reel, J. R., *Abstracts of Papers*; The Annual Laurentian Hormone Conference, Quebec, Canada, August 28–September 2, 1988; p 19.
(11) Synder, B. W.; Winneker, R. C.; Batzold, F. H. *J. Steroid Biochem.*, in press.

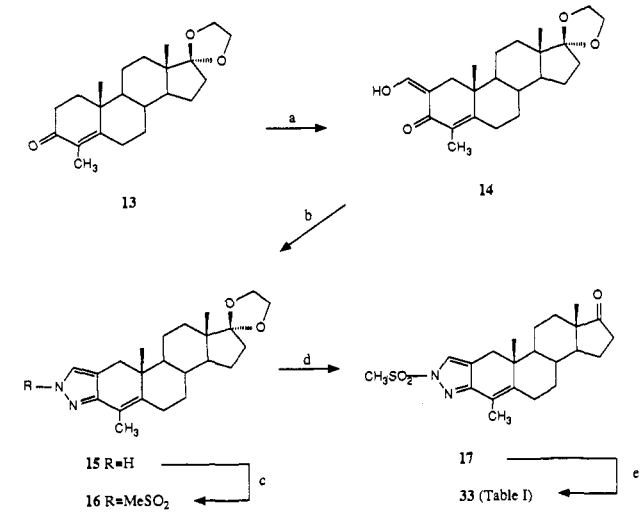
0022-2623/90/1833-2094$02.50/0

Scheme III[a]



[a] (a) $(F_3CCO)_2O/C_5H_5N$; (b) $(EtO)_3CH/BF_3{\cdot}Et_2O/(i\text{-}Pr)_2NEt$; (c) SO_2Cl_2; (d) N_2H_4.

Scheme IV[a]

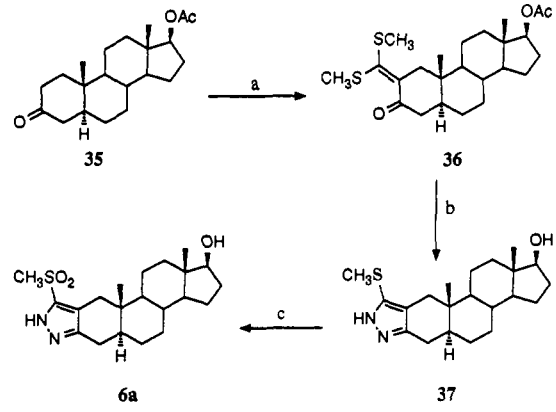


[a] (a) $MeOCHO/NaOCH_3$; (b) N_2H_4; (c) $MeSO_2Cl/C_5H_5N$; (d) $AcOH/H_2O$; (e) $HC{\equiv}CMgBr$.

Chemistry

The alkylsulfonyl group was introduced at the N-1′-position by reaction of the known, parent pyrazoles[12,13] with the appropriate alkylsulfonyl chloride in pyridine. The preparation of 1 and byproduct **6** in an approximate 4:1 ratio is illustrated in Scheme I. Mixtures of isomers were always obtained under electrophilic sulfonylation conditions provided C-4 was unsubstituted. Because of the poor regioselectivity of this sulfonylation, a regiospecific synthesis applicable to kilogram-scale preparations was developed (Scheme II). The success of this process is based upon the condensation of methanesulfonic acid hydrazide with the C-3 carbonyl group of the benzoate-protected hydroxymethylene steroid **7b** followed by pyrazole formation. Benzoate protection is required since treatment of the hydroxymethylene parent **7a** or the acetoxy derivative **7c** under identical conditions afforded mixtures of 1 and **6**. Treatment of the 4-substituted Δ^4-pyrazoles with methanesulfonyl chloride gave the 1′-methylsulfonyl adducts as the only isolated product. The greater regioselectivity observed in the Δ^4-4-substituted cases is attributed to the bulk of the C-4 substituent which sterically hinders formation of the 2′-isomer.

Scheme V[a]



[a] (a) $BuLi/CS_2/CH_3I/Et_2O$; (b) $N_2H_4/EtOH$; (c) MCPBA/CH_2Cl_2.

Synthesis of the 4-chloro- and 4-methyl-substituted-Δ^4 steroidal pyrazoles are depicted in Schemes III and IV. Preparation of the 4-chloro-Δ^4-steroidal pyrazole **12** (Scheme III) consisted of formation of acetal **10** by reaction of **9** with the $BF_3{\cdot}Et_2O$ complex of triethyl orthoformate[14] followed by chlorination of **10** exclusively at C-4 with sulfuryl chloride. Pyrazole formation and sulfonylation proceeded as described above for the Δ^4-4-substituted compounds.

The preparation of a 5′-(methylsulfonyl)pyrazole **6a** is shown in Scheme V. Base-promoted condensation of androstanolone acetate (**35**) with carbon disulfide followed by alkylation with methyl iodide yielded ketene dithioacetal **36**.[15] Reaction of **36** with hydrazine and oxidation of the resulting sulfide **37** afforded **6a**.

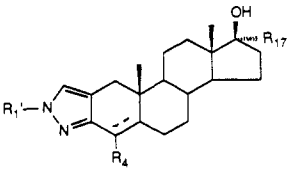
Assignment of regiochemistry to the alkylsulfonyl group was achieved through analysis of ^{13}C NMR chemical shifts. In the spectrum of 1, the 5′-carbon appeared at δ 127.8 ppm, whereas in **6** the 5′-carbon appeared at δ 143.9 ppm. This characteristic difference was used to assign structures to the major, N-1′, and minor, N-2′, isomers of the other pyrazoles.[16,17] The structure of 1 was unambiguously established from X-ray diffraction. Another reliable method for distinguishing the 1′- and 2′-isomers in the 4,5-dihydro-5α series was by comparison of their UV spectra. Although both isomers exhibited a UV maximum at about 235 nm, the N-1′-isomers typically possessed about double the extinction coefficient of the N-2′-isomer. For example, the extinction coefficient for 1 was 10 921 while the value for **6** was 5371. In the Δ^4-series the UV spectra were more complicated but again the N-1′-isomer was more strongly absorbing than the N-2′-isomer.

Biological Results and Discussion

Androgen receptor relative binding affinity (RBA) and in vivo antiandrogenic and androgenic activity are presented in Table I. Receptor affinity was determined following incubation periods with rat ventral prostate cytosol. Values were obtained following 1 h and approximately 18 h since it is characteristic of most androgen antagonists

(12) Clinton, R. O.; Manson, A. J.; Stonner, F. W.; Neumann, H. C.; Christiansen, R. G.; Clarke, R. L.; Ackerman, J. H.; Page, D. F.; Dean, J. W.; Dickinson, W. B.; Carabateas, C. *J. Am. Chem. Soc.* **1961**, *83*, 1478.
(13) Clinton, R. O. U.S. Patent 3 704 295, 1972; *Chem. Abstr.* **1968**, *68*, 69198s.
(14) Mock, W. L.; Tsou, H.-R. *J. Org. Chem.* **1981**, *46*, 2557.
(15) Corey, E. J.; Chen, R. K. H. *Tetrahedron Lett.* **1973**, 3817.
(16) Agnew, M. N.; Bauer, V. J.; Effland, R. C. *J. Heterocycl. Chem.* **1980**, *17*, 117.
(17) Dolmazon, R.; Gelin, S. *J. Heterocycl. Chem.* **1982**, *19*, 117.

Table I. Androgen Receptor Binding and in Vivo Activity of the Sulfonylpyrazoles



no.		R_{17}	relative binding affinity[a] 1 h	relative binding affinity[a] 18 h	antiandrogenic[b] ED_{50}, mg/kg	androgenic activity[c] % increase
			4,5-Dihydro-5α Series (R_4 = 2 H)			
	R_1'					
5	H	C_2H	28.0	0.8	50	50
1	$MeSO_2$	C_2H	2.2	0.05	15	ns[d]
18	H	Me	164.0	0.8		
19	$MeSO_2$	Me	16.0	1.0	10	ns
6	2′-$MeSO_2$	C_2H	<0.01	<0.01	≫100	ns
6a	5′-$MeSO_2$	H	0.05	<0.01		
20	$EtSO_2$	C_2H	2.7	0.1	100	50
21	*n*-$PrSO_2$	C_2H	3.2	0.1	<100	80
22	*i*-$PrSO_2$	C_2H	<0.01	<0.01	>100	
23	*n*-$BuSO_2$	C_2H	0.2	<0.01	>100	
24	$MeSO_2$	H	30.0	2.0	>100	425 sc
25	$MeSO_2$	17-C=O	<0.01	<0.01	100	ns
26	$MeSO_2$	C_2H_5	3.0	0.1	41	ns
27	$MeSO_2$	C_2H_3	4.0	0.1	33	ns
28	$MeSO_2$	C_2H	0.6	0.01	40	ns (19-nor)
			Δ^4-Series (R_1' = $MeSO_2$)			
	R_4					
29	H	H	10.0	0.7	>100	
30	H	Me	12.0	0.9	16	ns
31	H	C_2H	0.4	<0.01	<100	ns
32	Me	Me	18.0	1.0	3	ns
33	Me	C_2H	7.0	0.05	14	ns
34	Cl	C_2H	1.7	0.01	>100	
			Reference Agents			
2a	cyproterone		1.3	0.1	87	
2b	2a-acetate		17.0	1.7	18	
3a	flutamide		0.1	0.01	5	ns
3b	hydroxy-3a		2.6	0.1	4	ns
4	ICI-176334		2.0	0.4	4	ns
testosterone			81.0	25.0		978
dihydrotestosterone			87.0	88.0		
19-nor-DHT			97.0	41.0		

[a] Values represent the mean of at least three separate determinations of rat ventral prostate androgen receptor binding affinity which is defined as ([R1881] at 50% binding inhibition/[competitor] at 50% binding inhibition) × 100. [b] Values represent graphically determined ED_{50} (dose required to inhibit testosterone propionate induced rat ventral prostate weight gain by 50%). [c] Values represent the percent increase in rat ventral prostate weight caused by a 100 mg/kg per day × 10 oral dose regime unless indicated otherwise. [d] Ns = not significant ($p < 0.01$, Dunnett's test).

that their affinity for the androgen receptor falls precipitously over this time period.[18,19] Antiandrogens which lack detectable androgenic activity in vivo usually bind weakly to the receptor compared to androgen agonists. The reference antiandrogens, hydroxyflutamide (**3b**) and ICI 176334 (**4**), are examples. In contrast most androgen agonists bind approximately equally well at both time points. Cyproterone acetate (**2b**) bound with greater affinity to the receptor than **3b** or **4** but was not androgenic at the doses tested. This agent, however, has been reported by others to be weakly androgenic.[4]

The parent pyrazole **5** bound strongly to the androgen receptor and displayed significant androgenic and antiandrogenic activity in vivo. Its 1′-methylsulfonyl derivative **1** bound with less affinity to the androgen receptor, was more potent than **5** in vivo as an antiandrogen, and lacked significant androgenic activity in vivo at the dose tested. Likewise, the known anabolic agent **18**[20] bound avidly to the androgen receptor and was a potent androgen in vivo. 1′-Methylsulfonyl derivative **19** bound with less affinity to the receptor, lacked significant androgenic activity in vivo, and was antiandrogenic. The 2′-(methylsulfonyl)pyrazoles, e.g. **6**, and the 5′-(methylsulfonyl)-pyrazole **6a** did not bind significantly to the androgen receptor and were not androgenic or antiandrogenic in vivo. Thus the location of the methylsulfonyl group on the pyrazole ring is critical for significant binding to the receptor and antiandrogenic activity. In a limited investigation of alkylsulfonyl groups, it was observed that the ethyl and propyl derivatives (**20** and **21**, respectively) bound to the androgen receptor with the same affinity as methyl derivative **1**, but the *i*-Pr and *n*-Bu derivatives **22** and **23**

(18) Raynaud, J. P.; Bouton, M. M.; Ojasoo, T. *Trends Pharmacol. Sci.* **1980**, *1*, 324.
(19) Raynaud, J. P.; Bouton, M. M.; Moguilewsky, M.; Ojasoo, T.; Philbert, D.; Beck, G.; Labrie, F.; Mornon, J. P. *J. Steroid Biochem.* **1980**, *12*, 143.
(20) Arnold, A. A.; Potts, G. O.; Beyler, A. L. *Endocrinology* **1963**, *72*, 408.

bound weakly or not at all, indicating an intolerance to larger alkyl groups. Although ED_{50}'s were not established for **20** or **21**, the inhibition observed at 100 mg/kg suggested that they were less antiandrogenic than **1**. For reasons not clear to us, **20** and **21** also displayed androgenic activity in vivo.

17-α-H derivative **24** bound to the androgen receptor and displayed antiandrogenic activity in vivo, but the inhibition did not reach 50% at the highest dose tested. 17-Ketone **25** did not bind to the receptor but displayed modest antiandrogenic activity in vivo. Metabolic conversion to **24** is a possible explanation for this activity. 17-α-Me derivative **19** bound to the receptor with nearly the same affinity as 17-α-H derivative **24** but was about 10 times more potent as an antiandrogen in vivo. It has been reported that a 17-α-Me group is compatible with strong androgen-receptor binding.[21]

In the Δ^4-series, the binding affinity of **29** and **30** was comparable to that of 4,5-dihydro-5α-derivatives **24** and **19**. 17-Acetylene derivative **31** had diminished androgen receptor affinity when compared to that of **1** but introduction of a methyl group at C-4 to give **33** resulted in an approximately 17-fold increase in binding affinity relative to that of **31** with in vivo potency comparable to that of **1**. Introduction of a methyl group at C-4 in **30** to give **32** caused an approximately 5-fold increase in in vivo potency. 4-Cl derivative **34** proved to be much less active than **33** both in binding affinity and in vivo. In contrast, 4-chlorocyproterone acetate is reported to be the only derivative of cyproterone acetate whose antiandrogenic potency approached that of the parent compound.[22]

Additional in vitro and in vivo studies supported the view that the antiandrogenic activity of **1** is a consequence of binding to the androgen receptor.[10,11] In vitro, **1** did not inhibit rat ventral prostate 5α-reductase but did inhibit the uptake of [^{3}H]testosterone and testosterone-induced nuclear accumulation of androgen receptor in the rat ventral prostate.

The X-ray crystallographic representations of **1**, 5α-dihydrotestosterone (DHT),[23] and a superposition of **1** and DHT are presented in Figures 1 and 2.[24] With alignment of the D rings of **1** and DHT (carbons 14, 16, 17, and 12), a major departure in conformations at the A-ring locus is observed. Antiandrogen **1** is clearly "flatter" and larger[25] than DHT and the difference in VDW volume is shown in Figure 2. The progestin and estrogen receptors have been shown to bind steroids which are significantly larger at selected positions than their natural ligand agonists. Recent examples include introduction of an 11-aryl moiety to yield antiprogestins[26] and incorporation of a large alkyl appendage at C-7 to yield antiestrogens.[27] Our results indicate that the androgen receptor can accommodate ligands attached to the A ring fused heterocycle, which are larger than the previously reported A ring fused heterocyclic androstanes[12,18] and that this new ligand–receptor relationship results in the expression of significant antiandrogenic activity.

Experimental Section

Elemental analyses were performed by Galbraith Laboratories of Knoxville, TN, or Instranal Laboratories of Rensselaer, NY. Melting points are not corrected. Proton (^{1}H) NMR spectra were measured at 100 MHz on a Varian HA-100 instrument or at 200 MHz on an IBM instrument using $CDCl_3$ as solvent. Carbon (^{13}C) NMR spectra were measured at 67.8 MHz on a JEOL FX instrument. Assignment of carbon resonances was made with DEPT[28] experiments. Infrared spectra were measured on a Nicolet 20 SX FT IR or on a Perkin-Elmer Model 467 instrument. Ultraviolet spectra were measured on a Gilford Response UV–vis spectrophotometer. Mass spectra were measured on a JEOL JMS-01SC. All structures were consistent with NMR, IR, MS, UV, and TLC.

Analytical thin-layer chromatography (TLC) was performed on E. Merck 5 × 20 cm Kieselgel 60 F-254 plates. Column chromatography was performed with Whatman LPS2 (37–53 μM) SiO_2 or Kieselgel 60 (230–400 mesh). Preparative HPLC was performed on a Waters Prep 500 instrument using two SiO_2 cartridges. Analytical HPLC was performed on a Waters 6000A instrument using an Alltech C_{18} column (10 μm, 4.6 mm by 25 cm).

(5α,17α)-1′-(Methylsulfonyl)-1′*H*-pregn-20-yno[3,2-*c*]-pyrazol-17-ol (1) and 2′-Isomer 6. Method A. Sulfonylation of 5. Methanesulfonyl chloride (7.53 g, 0.0658 mol) was added with stirring and cooling at ice-bath temperatures to a solution of 14.6 g (0.0432 mol) of **5** in 75 mL of pyridine. The temperature of the mixture rose to 20 °C after the addition, then returned to 0–5 °C. TLC analysis (40% Et_2O in CH_2Cl_2) indicated the reaction was complete after 1 h. Water (125 mL) was added whereupon an oil separated which crystallized. The crystals were collected and dried to afford 15.5 g. The remaining product (1.1 g) was isolated by extraction of the filtrate with CH_2Cl_2. The reaction was repeated with 18.36 g (0.0542 mol) of **5** and 9.3 g (0.081 mol) of CH_3SO_2Cl. The combined product mixtures were chromatographed on 500 g of SiO_2, eluting with CH_2Cl_2–Et_2O (99:1) and collecting 400-mL fractions. Fractions 6–14 afforded 23.3 g (57%) of **1**. This was recrystallized from MeCN to give 18.6 g of analytically pure **1** (46%): mp 200–202 °C; ^{1}H NMR (200 MHz) δ 0.8 (s, 3 H, CH_3), 0.90 (s, 3 H, CH_3), 2.60 (s, 1 H, C≡CH), 3.25 (s, 3 H, CH_3SO_2), 7.70 (s, 1 H, CH=N); ^{13}C NMR δ 11.47 (CH_3), 12.60 (CH_3), 20.72 (CH_2), 23.04 (CH_2), 27.46 (CH_2), 29.03 (CH_2), 31.02 (CH_2), 32.51 (CH_2), 34.29 (CH_2), 35.99 (C), 36.21 (CH), 38.82 (CH_2), 41.11 (CH_3), 41.89 (CH), 46.54 (C), 50.15 (CH), 53.12 (CH), 73.84 (C), 79.67 (C), 87.49 (CH), 119.25 (C), 127.83 (CH, 5′-C), 155.40 (C); UV λ_{MAX} (EtOH) 233.5 (ϵ 10921); IR (KBr, cm^{-1}) 3389, 3289, 3012, 2970, 2931, 1477, 1371, 1173; MS (DCI, CH_4, *m/e*) 417 (M + 1). Anal. ($C_{23}H_{32}N_2O_3S$) C, H, N, S.

Further elution of the column and preparative HPLC purification of the mixed fractions (5% Et_2O in CH_2Cl_2) from a third experiment [36.52 g (0.108 mol) of **5** and 18.65 g (0.163 mol) of CH_3SO_2Cl] yielded 5.36 g (12%) of 2′-isomer **6**: mp 179–180 °C, after recrystallization from MeCN; ^{1}H NMR (200 MHz) δ 0.70 (s, 3 H, CH_3), 0.85 (s, 3 H, CH_3), 2.60 (s, 1 H, C≡CH), 3.25 (s, 3 H, CH_3SO_2), 7.55 (s, 1 H, CH=N); ^{13}C NMR δ 11.38 (CH_3), 12.59 (CH_3), 20.66 (CH_2), 23.01 (CH_2), 27.44 (CH_2), 28.60 (CH_2), 31.02 (CH_2), 32.51 (CH_2), 34.67 (CH_2), 35.70 (C), 36.13 (CH), 38.82 (CH_2), 41.47 (CH_3), 41.90 (CH), 46.59 (C), 50.10 (CH), 53.12 (CH), 73.82 (C), 79.67 (C), 87.52 (CH), 118.98 (C), 140.92 (C), 143.88 (CH, 5′-C); UV λ_{MAX} (EtOH) 233.5 (ϵ 5371); IR (KBr, cm^{-1}) 3397, 3270, 2999, 2967, 2929, 1466, 1373, 1207, 1176; MS (DCI, CH_4, *m/e*) 417 (M

(21) Ojasoo, T.; Delettre, J.; Mornon, J. P.; Turpin-VanDycke, C.; Raynaud, J. P. *J. Steroid Biochem.* **1987**, *27*, 255.
(22) Neumann, F. In *Pharmacology and Clinical Uses of Inhibitors Of Hormone Secretion and Action*; Furr, B. J. A.; Wakeling, A. E., Ed.; Bailliere Tindall: London, 1987; p 132.
(23) Duax, N. L.; Narton, D. A. *Atlas of Steroid Structures*; Plenum: New York, 1970.
(24) X-ray coordinates were imported into the molecular modeling software package CHEMX [Chemical Design Ltd., July 1988] running on a VAX 11/785 under VMS version 5.1. Standard facilities were used unmodified. Molecular "fitting" was performed in the CHEMX "FLY" facility in a rigid manner; the crystal-derived geometry was not altered. Surface maps were calculated by using the "SET MAP" facility and Boolean operations were performed in the standard manner.
(25) Compound **1** was calculated to occupy 78 Å^3 more volume than DHT.
(26) Neef, G. In *Trends in Medicinal Chemistry*; Proceedings of the Ninth International Symposium on Medicinal Chemistry, Berlin, 1986; Mutschler, E., Winterfeldt, E.; Weinheim-Federal Republic of Germany: VCH, New York, 1987.
(27) Wakeling, E.: Bowler, J. *J. Endocr.* **1987**, *112*, R7.
(28) Bendall, M. R.; Pegg, D. T.; Doddrell, D. M.; Johns, S. R.; Willing, R. I. *J. Chem. Soc., Chem. Commun.* **1982**, 1138. (b) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323.

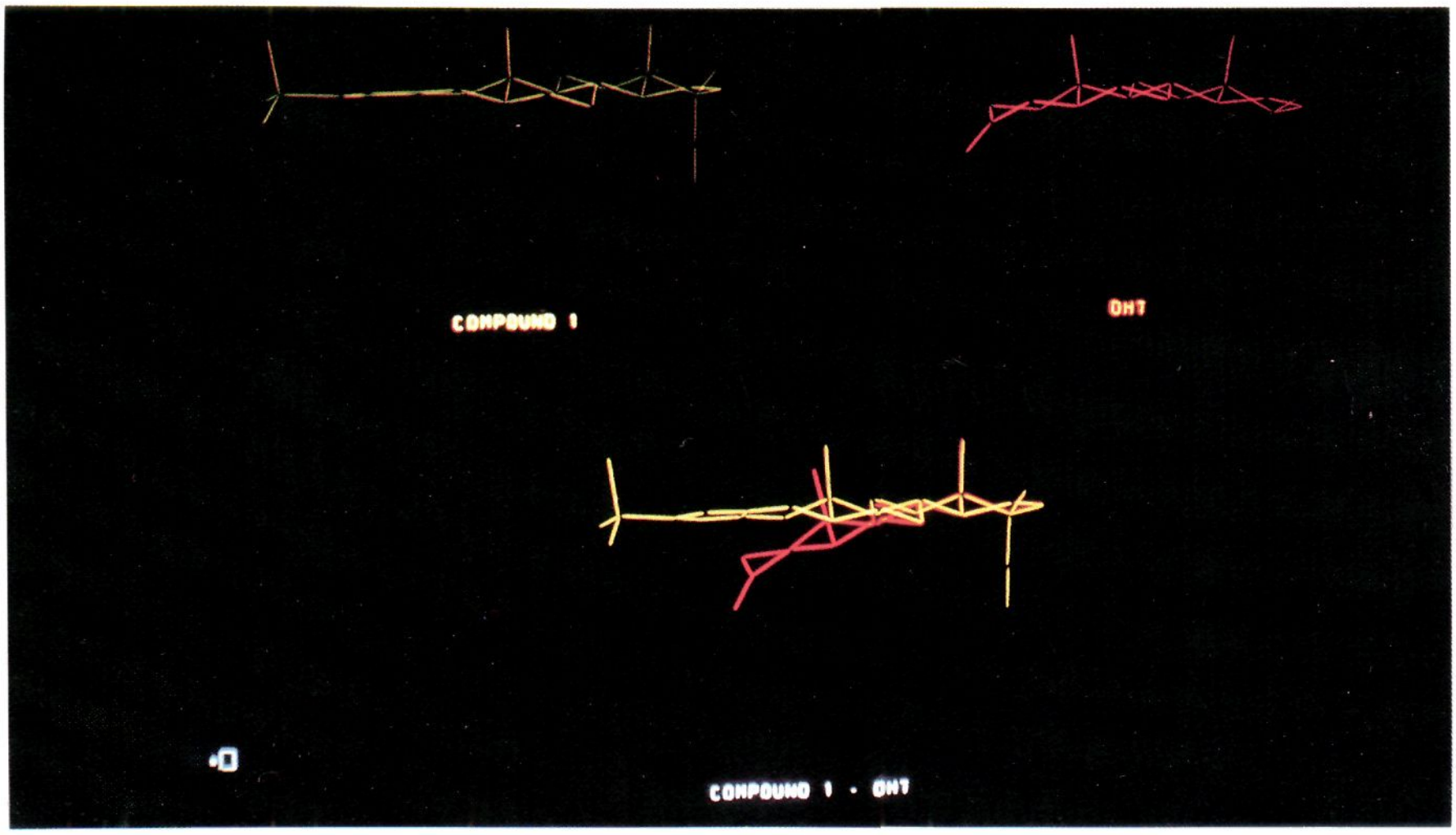


Figure 1. Stick diagrams of compound **1** and DHT (X-ray coordinates) and an overlay of these structures illustrating the divergence of conformation.

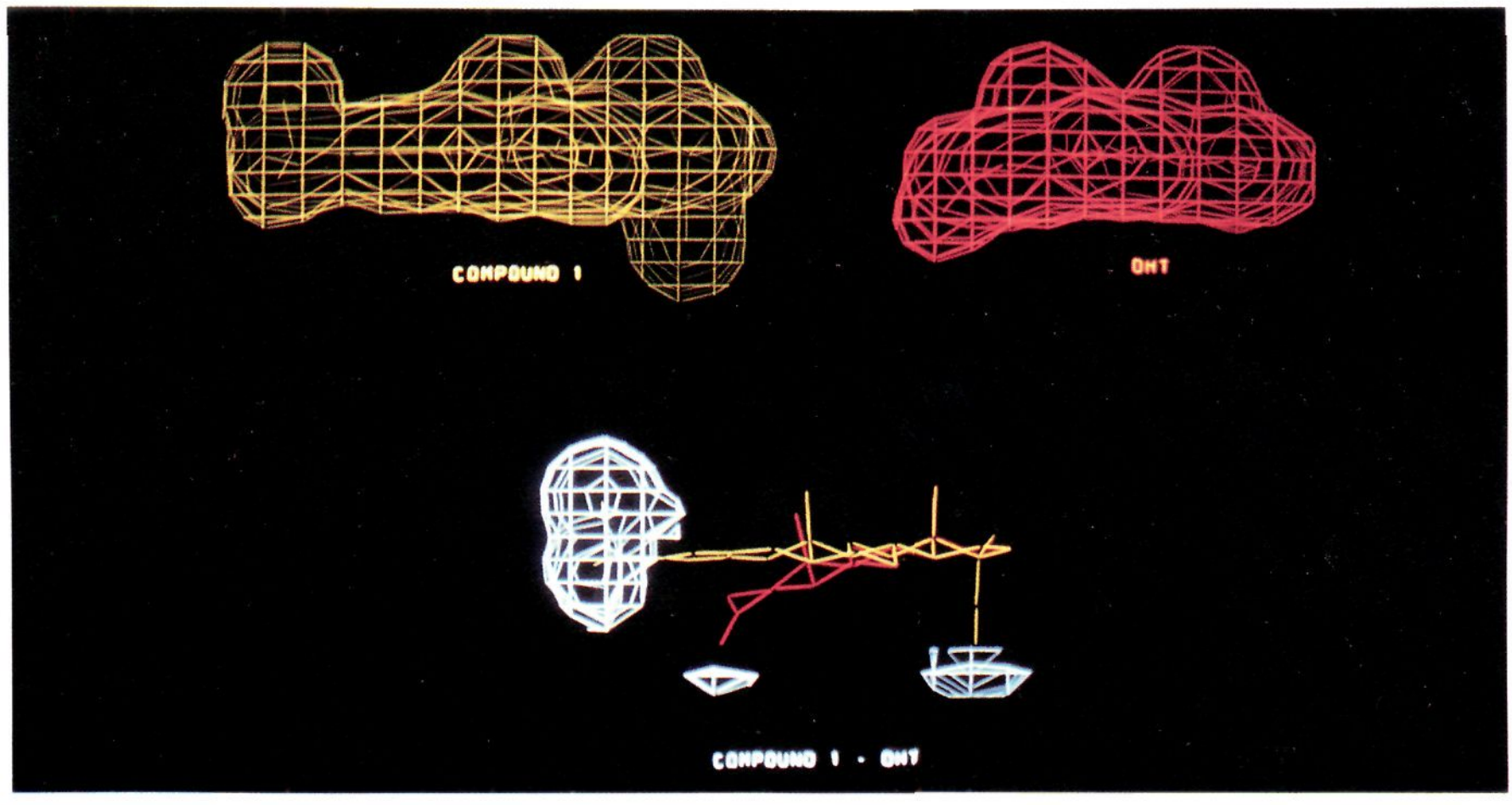


Figure 2. Overlayed, stick diagrams of compound 1 and DHT. This diagram also graphically illustrates the noncommon volume of these compounds with a (Boolean) difference map.

+ 1). Anal. ($C_{23}H_{32}N_2O_3S$) C, H, N.

Method B. Reaction of 7b with Methanesulfonic Acid Hydrazide. To a slurry of 100 g (0.29 mol) of (5α,17α)-2-(hydroxymethylene)-17-hydroxypreg-20-yn-3-one,[12,13] (**7a**) and 40.4 g (0.29 mol) of K_2CO_3 in 700 mL of Me_2CO was added 33.9 mL (0.29 mol) of benzoyl chloride dropwise over 30 min and the suspension stirred at room temperature for 18 h. The reaction mixture was filtered and the filtrate was concentrated to dryness under vacuum to give 126.6 g of a yellow solid. Recrystallization from 750 mL of toluene after charcoal treatment yielded 93.6 g (72%) of **7b** in two crops. An analytical sample was obtained by recrystallization from Me_2CO, mp 214–215 °C. Anal. ($C_{29}H_{34}O_4$) C, H.

To a stirred solution of 136 g (0.30 mol) of **7b** in 680 mL of CH_2Cl_2 and 175 mL of AcOH at 0 °C was added a solution of 51 g (0.45 mol) of methanesulfonic acid hydrazide in 175 mL of AcOH over 25 s. The mixture was stirred at 0 °C for 6 h and then at 12 °C for 17 h. After addition of 340 mL of CH_2Cl_2 and 680 mL of H_2O, the organic phase was washed twice with 680 mL of H_2O and neutralized with 300 mL of saturated aqueous $NaHCO_3$. The

Table II

no.	% yield[a]	solvent[b]	mp, °C	formula	anal.
16	73	CH_3CN	234–237	$C_{24}H_{34}N_2O_4S$	C,H,N,S
19	56	EtOH	189–190	$C_{22}H_{34}N_2O_3S$	C,H,N
20	47	CH_3OH	191–193	$C_{24}H_{34}N_2O_3S$	C,H,N
21	52	CH_3OH	182–183	$C_{25}H_{36}N_2O_3S$	C,H,N
22	56	CH_3CN	229–233	$C_{25}H_{36}N_2O_3S$	C,H,N,S
23	40	EtOAc/hexane	192–193	$C_{26}H_{38}N_2O_3S$	C,H,N
24	42	CH_2Cl_2/CH_3OH	176–178	$C_{21}H_{32}N_2O_3S$ $C_{21}H_{32.5}N_2O_{3.25}S$	C,H,N
28	32	EtOAc/hexane	176–178	$C_{21}H_{30}N_2O_3S$	C,H,N,S
29	36	$CHCl_3$/EtOH	227–229	$C_{20}H_{30}N_2O_3S$	C,H,N,S
30	63	CH_3CN	203–205	$C_{22}H_{32}N_2O_3S$	C,H,N
31	31	CH_3OH/H_2O	186–188	$C_{23}H_{36}N_2O_3S$	C,H,N
32	72	EtOH	219–220	$C_{23}H_{34}N_2O_3S$	C,H,N,S
34	60	CH_3CN	243–244	$C_{23}H_{29}ClN_2O_3S$	C,H,N,Cl

[a] For the sulfonylation step. [b] Used for recrystallization.

organic layer was dried over $MgSO_4$, treated with charcoal, filtered through Celite, and concentrated to afford 140 g of crude product. This was estimated to be 88% **1** by analytical HPLC (reverse phase, C_{18}, 70:30 CH_3OH/1% aqueous AcOH; 2 mL/min, 3200 psi). Recrystallization from MeOH and then MeCN yielded 98.1 g (77%) of **1** in two crops, each of which was >99% by HPLC.

The following sulfonylpyrazoles were prepared by a method analogous to the sulfonylation of **5**, Method A (Table II).

(17α)-17-[(Trifluoroacetyl)oxy]pregn-4-en-20-yn-3-one (9). A solution of 31.2 g (0.10 mol) of **8** in 100 mL of pyridine and 250 mL of CH_2Cl_2 at 5 °C was treated with 20 mL (0.14 mol) of trifluoroacetic anhydride over 15 min. The mixture was stirred for 45 min and then poured into a stirred mixture of ice and 12 N HCl. The aqueous layer was extracted with 3 portions of CH_2Cl_2. The combined organics were dried over $MgSO_4$, filtered through a plug of SiO_2, and concentrated to a white solid. This was recrystallized from cyclohexane to afford 37.0 g (91%) of **9**, mp 149–150 °C.

(2α,17α)-2-(Diethoxymethyl)-17-[(trifluoroacetyl)oxy]-pregn-4-en-20-yn-3-one (10). A solution of 3.25 mL (0.0195 mol) of triethyl orthoformate in 10 mL of CH_2Cl_2 at −30 °C was treated with 3 mL (0.0185 mol) of $BF_3{\cdot}Et_2O$ with stirring during 10 min. The mixture was then cooled to −78 °C, and 4.08 g (0.01 mol) of **9** in CH_2Cl_2 was added followed by 5 mL (0.0287 mol) of EtN(*i*-Pr)$_2$ during 15 min, and the temperature of the mixture was maintained below −60 °C. The reaction mixture was stirred for 30 min, while the temperature rose to 0 °C, and then poured into 200 mL of saturated aqueous $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2 two times. The combined organic phases were washed first with 1 N HCl then with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated. The resulting solid was recrystallized from hexane to give 3.5 g (69%) of **10**, mp 147–148 °C. Anal. ($C_{28}H_{37}F_3O_5$) C, H, F.

(2α,17α)-4-Chloro-2-ethoxymethylene-17-[(trifluoroacetyl)oxy]pregn-4-en-20-yn-3-one (11). There was added dropwise 20 mL (0.25 mol) of SO_2Cl_2 to a solution of 27.3 g (0.053 mol) of **10** in 250 mL of pyridine at 5 °C during 30 min, and the reaction mixture was stirred for 10 min. TLC analysis (2:1 hexane/EtOAc) showed the reaction to be complete. The mixture was poured into a mixture of cold, dilute HCl and ice and the aqueous layer was extracted with 5 portions of CH_2Cl_2. The combined, dried extracts were concentrated. The resulting solid was washed with Et_2O and then recrystallized from MeCN to afford 16.5 g (62%) of **11**. An analytical sample was prepared by recrystallization from EtOAc, mp 201–202 °C. Anal. ($C_{26}H_{30}ClF_3O_4$) C, H, Cl.

(17α)-4-Chloro-17-[(trifluoroacetyl)oxy]-1′*H*-pregn-4-en-20-yno[3,2-*c*]pyrazole (12). A suspension of 12.6 g (0.0253 mol) of **11** in refluxing EtOH was treated with 3 mL (0.062 mol) of 100% $N_2H_4{\cdot}H_2O$ in one portion. The source of the heat was removed. After 30 min the product crystallized from the reaction solution. The entire suspension was concentrated to dryness and recrystallized from MeOH to give 8.0 g (78%) of a monomethanol solvate, mp >300 °C. An analytical sample was prepared by recrystallization from THF/MeCN to give a white solid, mp >300 °C. Anal. ($C_{22}H_{27}ClN_2O$) C, H, N.

Compound 13. A solution of 120 g (0.40 mol) of 4-methylandrost-4-ene-3,17-dione,[29] 115 mL (2.06 mol) of ethylene glycol, and 1.0 g (0.005 mol) of *p*-toluenesulfonic acid monohydrate in 1.2 L of C_6H_6 was refluxed for 6 h with separation of H_2O in a Dean–Stark trap. The benzene layer was then washed with 2 portions of 120 mL of H_2O, once with saturated aqueous NaCl, dried, and concentrated to a solid. This was recrystallized from cyclohexane to afford 108.4 g (79%) of **13**, mp 164–166 °C. Anal. ($C_{22}H_{32}O_3$) C, H.

4-Methyl-1′*H*-androst-4-eno[3,2-*c*]pyrazol-17-one Cyclic 17-(1,2-Ethanediyl acetal) (15). To a solution of 106 g (0.31 mol) of ketal **13** in 900 mL of THF containing 50 g (0.93 mol) of NaOMe and 2 mL of MeOH was added 100 mL (1.62 mol) of methyl formate during 40 min with stirring. After 24 h the thick mixture was diluted with 200 mL of H_2O and concentrated under reduced pressure at 40 °C. The residue in 1 L of H_2O was acidified to pH 6 with 5.5 N HOAc at 5 °C to give 97.8 g of **14** (85%). An analytical sample was prepared by recrystallization from EtOAc, mp 135–140 °C. Anal. ($C_{23}H_{32}O_4$) C, H.

A solution of 3.72 g (0.01 mol) of **14** and 1 mL (0.021 mol) of 100% $N_2H_4{\cdot}H_2O$ was reacted analogously to the preparation of **12** to provide 3.8 g (98%) of **15** as a yellow foam, estimated to be 95% pure by TLC (1:1 hexane/EtOAc). An analytical sample was prepared by recrystallization from *i*-PrOH/H_2O, mp 212–215.5 °C. Anal. ($C_{23}H_{32}N_2O_2$) C, H, N.

4-Methyl-1′-(methylsulfonyl)-1′*H*-androst-4-eno[3,2-*c*]-pyrazol-17-one (17). A total of 64.2 g (0.144 mol) of **16** in 52 mL of 80% AcOH was heated on a steam bath. The product was filtered off and recrystallized from EtOAc to give 43.5 g (75%) of **17**. An analytical sample was prepared by a second recrystallization from EtOAc, mp 244–246 °C. Anal. ($C_{22}H_{30}N_2O_3S$) C, H, N.

(5α)-1′-(Methylsulfonyl)-1′*H*-androstano[3,2-*c*]pyrazol-17-one (25). To a stirred suspension of 44.84 g (0.208 mol) of pyridinium chlorochromate in 400 mL of CH_2Cl_2 was added a solution of 40.9 g (0.104 mol) of **24** in 300 mL of CH_2Cl_2. The mixture was stirred at room temperature overnight. The supernatant was decanted from the gummy residue, the residue washed with CH_2Cl_2, and the combined solutions were diluted with an equal volume of Et_2O and passed through a column of Florisil. Concentration of the eluant yielded 38.4 g of **25**, which was recrystallized from MeCN to provide 33.4 g (82%), mp 214–216 °C. Anal. ($C_{21}H_{30}N_2O_3S$) C, H, N, S.

(5α,17α)-1′-(Methylsulfonyl)-1′*H*-pregnano[3,2-*c*]-pyrazol-17-ol (26). A mixture of 13 g (0.031 mol) of **1**, 3 g (0.007 mol) of **27**, and 1.6 g of 10% Pd–C in 300 mL of EtOH was shaken with H_2 at room temperature at 40 psi until reduction was complete. Upon workup there was obtained 10.1 g (63%) of **26**, mp 194.5–195.5 °C, after recrystallization from EtOH. Anal. ($C_{23}H_{36}N_2O_3S$) C, H, N, S.

(5α,17α)-1′-(Methylsulfonyl)-1′*H*-pregn-20-eno[3,2-*c*]-pyrazol-17-ol (27). A solution of 19.4 g (0.0466 mol) of **1** in 300 mL of pyridine and 1.9 g of 2% Pd–$SrCO_3$ was shaken with H_2 at room temperature at 40 psi until reduction was complete. The catalyst was filtered off, and the volatiles were removed in vacuo to afford 14.2 g (73%) of **27**. An analytical sample (11.2 g, 58%) was prepared by recrystallization from EtOH, mp 171.5–173 °C. Anal. ($C_{23}H_{34}N_2O_3S$) C, H, N, S.

(17α)-4-Methyl-1′-(methylsulfonyl)-1′*H*-pregn-4-en-20-yno[3,2-*c*]pyrazol-17-ol (33). A solution of 55 mL (0.17 mol) of 3.1 M ethereal methylmagnesium bromide was added to 250 mL of THF during 1.7 h while acetylene was passed through the solution. At ice temperature a solution of 32.4 g (0.080 mol) of **17** in 380 mL of THF was added. The mixture was stirred at room temperature for 3.3 h and then quenched in 1.2 L of ammonium chloride. The aqueous layer was extracted with CH_2Cl_2, the combined organic layers were washed with NaCl solution, dried, and concentrated to afford 36 g as a yellow foam. This was chromatographed on 500 g of SiO_2, eluting initially with CH_2Cl_2/hexane and then with CH_2Cl_2/Et_2O mixtures, to give **33**. The reaction was repeated with 20 g (0.050 mol) of **17** and 35 mL (0.11 mol) of ethereal CH_3MgBr. The combined product (21.3 g) was recrystallized twice from toluene to give 12.7 g (23%) of analytically pure **33**, mp 221–224 °C. Anal. ($C_{24}H_{32}N_2O_3S$) C, H, N, S.

(29) Atwater, N. W. *J. Am. Chem. Soc.* **1960**, *82*, 2847.

(5α,17β)-2-[Bis(methylthio)methylene]-17-hydroxyandrostan-3-one 17-Acetate (36). To a solution of 30 g (0.136 mol) of 2,6-di-*tert*-butyl-4-methylphenol in 750 mL of ether under N_2 and cooled in an ice-salt bath was added slowly 56 mL (0.134 mol) of 2.4 M *n*-butyllithium in hexane. A white solid separated. The mixture was allowed to warm to room temperature, and 18 mL (22.8 g, 0.30 mol) of carbon disulfide and 19.5 g (0.0587 mol) of androstanolone 17-acetate **35** were added consecutively. After stirring overnight, 13 mL (29.6 g, 0.21 mol) of iodomethane was added over a 3-h period. The mixture was stirred for 5 h longer and then water was added carefully. The organic layer was washed with water and saturated NaCl and then evaporated under reduced pressure. The resulting orange oil (28.4 g) was dissolved in a mixture of hexane, ether, and benzene and chromatographed on 2 kg of silica gel, eluting with hexane and hexane/ether mixtures. This afforded 17.8 g (70%) of **36** as a yellow solid. An analytical sample was obtained by recrystallization from CH_3CN, mp 160.5–162 °C. Anal. ($C_{24}H_{36}O_3S_2$) C, H, S.

(5α,17β)-5′-(Methylthio)-1′*H*-androstano[3,2-*c*]pyrazol-17-ol (37). A solution of 30 mL of hydrazine hydrate, 250 mL of ethanol, and 13.3 g (0.030 mol) of **36** was refluxed for 15 min. The mixture was evaporated under reduced pressure. The solid residue was diluted with water and the solid was collected. This solid appeared to be a mixture of **37** and the 17-acetate of **37**. It was combined with 2.1 g (0.037 mol) of KOH and 160 mL of methanol, refluxed for 10 min, and evaporated under reduced pressure. The residue was taken up in water and the solid (10.9 g, 100%) was collected. After treatment with charcoal and recrystallization from ethyl acetate, there was obtained 7.0 g (65%) of pale cream colored, polymorphic 37, mp 189–191 °C and 205–207 °C. Anal. ($C_{21}H_{32}N_2OS$) C, H, N, S.

(5α,17β)-5′-(Methylsulfonyl)-1′*H*-androstano[3,2-*c*]pyrazol-17-ol (6a). A solution of 1.68 g (0.0047 mol) of methylthio steroid **37** in 70 mL of CH_2Cl_2 was cooled to −20 °C. A solution of 1.9 g (0.011 mol) of 3-chloroperoxybenzoic acid in 40 mL of CH_2Cl_2 was added dropwise. The mixture was stirred for 1 h at room temperature, washed with saturated $NaHCO_3$ and then with saturated NaCl, and evaporated under reduced pressure to afford 1.87 g (100%) of foam. Crystallization from acetonitrile gave 1.37 g (75%) of **6a**. Further recrystallization gave a white solid, mp 242–246 °C. Anal. ($C_{21}H_{32}N_2O_3S$) C, H, N, S.

Rat Prostate Androgen Receptor Competition Assay. Cytosol was prepared with ventral prostates from castrated adult rats weighing approximately 250 g. Tissues were homogenized in TMDG buffer (10 mM TRIS, 20 mM molybdate, 2.0 nM dithiothreitol, 10% glycerol, pH = 7.4) and centrifuged at the equivalent of 105000*g* for 1 h. Aliquots of the supernatant (cytosol) were incubated with [^{3}H]-R1881 (methyltrienolone, 5 nM final concentration) in either the absence or presence of increasing concentrations 10^{-9}–10^{-5} M) of R1881, reference, or test compounds for 1 h or overnight (approximately 18 h) at 4 °C. Because [^{3}H]-R1881 binds weakly to progesterone and glucocorticoid receptors (approximately 5% at 5 nM), cytosols were pretreated with 1 μM triamcinolone acetonide to block these interactions. After the 1- or 18-h incubation period, a suspension of dextran-coated charcoal (1% charcoal, 0.05% dextran T-70) was added to the ligand/cytosol mixture and incubated for 5 min. The charcoal-bound [^{3}H]-R1881, i.e. non-protein bound, was removed by centrifugation, and the supernatant (protein-bound [^{3}H]-R1881) was counted. Relative binding affinities (RBA; used to quantify receptor binding competition) were calculated as the ratio of the concentration required to inhibit [^{3}H]-R1881 specific binding by 50% (with R1881 arbitrarily set at 100). Compounds that did not inhibit binding by 50% at a competitor concentration of 10 μM were considered to be inactive (RBA < 0.01). The interassay coefficient of variation based on 50% inhibition by R1881 was 17.0%.

Androgenic/Antiandrogenic Activity in Castrated Immature Rats. Weanling Sprague–Dawley male rats were castrated and, beginning 1 week later, grouped by body weight and medicated orally with the test compound (vehicle was ethanol/cottonseed oil, 1:9 v/v) in the absence or presence of testosterone propionate (0.8 mg/kg/sc) for 10 consecutive days. The day following the last medication, the rats were weighed and sacrificed. The ventral prostate of each rat was removed, blotted, and weighed. Antiandrogenic activity is defined by a graphically determined ED_{50} defined as the dose required to inhibit testosterone propionate stimulated prostate weight gain by 50%. Compounds that did not inhibit prostate weight gain by 50% but demonstrated a significant inhibition ($p < 0.01$) at a dose of 100 mg/kg were assigned an ED_{50} of >100. Compounds that did not demonstrate a statistically significant inhibition ($p < 0.01$, Dunnett's test) of prostate weight were assigned an ED_{50} of ≫100. The interassay coefficient of variation based on the effect of 4.0 mg/kg per day × 10 po of flutamide was 14.2%. Androgenic activity was defined by the percent increase in ventral prostate weight at a defined dose.

Acknowledgment. The valuable assistance of the following people is gratefully acknowledged: George D. Beecham, III, Dr. Phillip M. Carabateas, Dr. Virendra Kumar, Dr. Stephen Clemens, Debra Cooke, John L. Houghtling, Christine K. Might, John F. Pochobradsky, Thomas J. Rodenmacher, Richard S. Tommell, and Margaret M. Wagner.

Supplementary Material Available: X-ray crystallographic data for compound 1 (7 pages). Ordering information is given on any current masthead page.