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NUCLEOPHILIC VINYLIC "ADDITION-ELIMINATION" SUBSTITUTION REACTION OF 3β-ACETOXY-17-CHLORO-16-FORMYLANDROSTA-5,16-DIENE: A NOVEL AND GENERAL ROUTE TO 17-SUBSTITUTED STEROIDS. PART 1 - SYNTHESIS OF NOVEL 17-AZOLYL-Δ^{16} STEROIDS; INHIBITORS OF 17α-HYDROXYLASE/17,20-LYASE (17α-LYASE)

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Abstract: We have discovered that chlorine in 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene (**1**) can be smoothly displaced by nitrogen heterocyclic nucleophiles (het$^-$) to give heretofore unknown 17-substituted-Δ^{16} steroids in high yields (73-92%). This enabled us to synthesize novel 3β-hydroxy-17-(1*H*-1,2,4-triazol-1-yl)androsta-5,16-diene (**4**) and 3β-hydroxy-17-(1*H*-imidazol-1-yl)androsta-5,16-diene (**7**), both of which are potent inhibitors of rat testicular 17α-lyase. Spectroscopic studies with a modified form of human 17α-lyase indicates that the inhibition process involves coordination of steroidal azole nitrogen to the heme-iron of the enzyme.

The enzyme 17α-hydroxylase/17,20-lyase (17α-lyase) is a cytochrome *P*-450 monooxygenase complex that catalyzes the conversion of progesterone and pregnenolone into androgens, androstenedione, and dehydroepiandrosterone, respectively;[2] the direct precursors of estrogens and testosterone. Prostate cancer is the second leading cause of cancer-related mortality in men in the U. S. A. and Europe. It is estimated that about 80% of patients respond to androgen deprivation, reflecting a requirement for circulating testosterone for their tumor growth. Compounds that inhibit 17α-lyase enzyme and reduce production of androgens may become useful drugs for treatment of prostate cancer.[3]

We and others have reported several categories of steroidal[4] and nonsteroidal[5] inhibitors of 17α-lyase that have been designed for this purpose. Given the significance of some azole (imidazole and triazole) groupings of many drugs,[3,6] which are *P*-450 enzyme inhibitors including aromatase, we reasoned that introducing azole grouping at C-17 together with 16-double bond of androstane skeleton should also yield potent 17α-lyase inhibitors. It should be noted that these compounds are expected to inhibit the enzyme (in part) by the binding of the heterocyclic nitrogen atom (N-3 of imidazole and N-4 of triazole) to the prosthetic heme iron atom. This should result in the exclusion of oxygen that would normally take part in the enzyme's catalytic reaction.

Although a few Δ^{16}-17-azole-androstane steroids[7] in which the azole is attached to the steroid nucleus

through a carbon atom of the heterocycle are known, the isomeric compounds in which the azole group is attached to the steroid nucleus through a nitrogen of the azole appear to constitute a class of steroids not hitherto reported. In addition, steroidal Δ^{16}-17-triazole and tetrazole compounds are reported here for the first time.

In our research we have now discovered that chlorine in the 17 position together with a 16,17-double bond and an activating 16-formyl group can be smoothly displaced by various nitrogen heterocyclic nucleophiles (het^-). Thus, treatment of 3β-acetoxy-17-chloro-16-formylandrosta-15,16-diene (1)[8] with a variety of azole nucleophiles in dimethylformamide (DMF) at 75-80 °C yielded Δ^{16}-azole steroids in high yields (73-92%). Two of the latter compounds served as key intermediates in the synthesis of our target compounds (Scheme 1). The representative results to these 17-substituted-Δ^{16}-androstane steroids are listed in the Table.

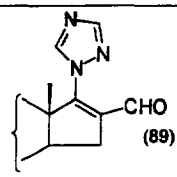
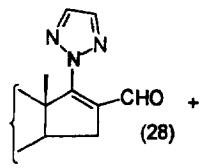
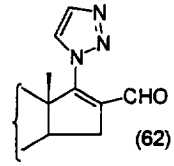
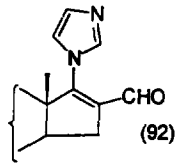
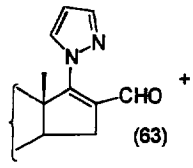
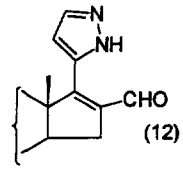
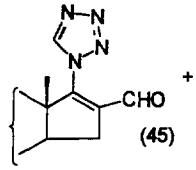
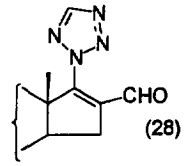
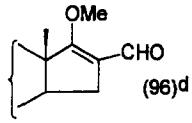
In a typical procedure (entry 1), a solution of 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene (**1**) (0.6 g, 1.6 mmol) and sodium triazolate (218 mg, 2.40 mmol, 1.5 equiv) in dry DMF (10 mL) under N_2 was stirred at 75-80 °C for 30 min. After cooling to room temperature, the reaction mixture was poured onto ice-water (250 mL), and the resulting white precipitate was filtered, washed with water, and dried to give a white solid. This was crystallized from hexane/EtOAc to give **2** (580 mg, 89%), mp 160-162 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (3H, s, 18-Me), 1.20 (3H, s, 19-Me), 2.04 (3H, s, 3β-OAc), 4.61 (1H, m, 3α-H), 5.42 (1H, d, J = 4.2 Hz, 6-H), 8.13 (1H, s, 3'-H), 8.42 (1H, s, 5'-H) and 10.12 (1H, s, 16-CHO). Anal calcd for $C_{24}H_{31}O_3N_3$: C, 70.37; H, 7.63; N, 10.27, Found: C, 70.28; H, 7.82; N, 10.21. HRMS calcd for $C_{24}H_{31}O_3N_3$ 409.2365, found 409.2348.

That the 16-formyl activating group was essential for the above nucleophilic vinylic substitution reaction to take place was revealed by the observation that 3β-acetoxy-17-chloroandrosta-5,16-diene when treated with sodium triazolate in DMF at 100 °C for 2 days, was completely unchanged. Nucleophilic vinylic substitution reactions are well documented.[9]

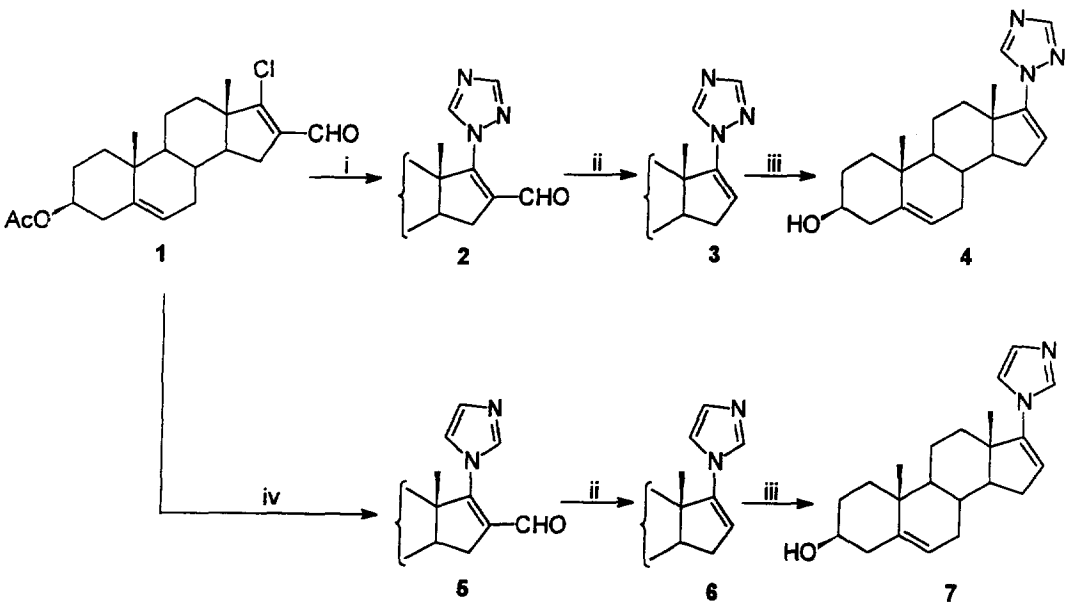
We also discovered that the 17-chlorine of compound **1** was easily displaced by methoxide ion (MeO^-) (entry 6) under mild reaction conditions. Furthermore, the formation of a minor product in which the pyrazole ring is attached to C-17 through a carbon atom indicates that this novel reaction could be successfully extended to carbon nucleophiles (entry 4). These together with the other examples of the Table demonstrates the generality of the process and we believe that similar results may be realized with a variety of nucleophiles under appropriate reaction conditions.

The synthesis was completed as indicated in Scheme 1, where the 17-azole intermediates **2** and **5** were each converted separately into the desired 3β-hydroxy-17-(1*H*-1,2,4-triazol-1-yl)-androsta-5,16-diene (**4**) and 3β-hydroxy-17-(1*H*-imidazol-1-yl)-androsta-5,16-diene (**7**) by decarbonylation[10] at C-16 [Wilkinson's catalyst in refluxing toluene] to give the 17-triazole (**3**) and 17-imidazole (**6**), respectively, followed by cleavage of the 3β-acetate groups. All compounds gave satisfactory analytical and spectroscopic data.[11]

Table. Nucleophilic Vinylic Substitution reaction of Compound 1.[a]

Entry	Nucleophile[b] (het⁻)	Reaction time (h)	Product(s)[c] (isolated yield, %)
1	1*H*-1,2,4-sodium triazolate	0.5	 (89)
2	1*H*-1,2,3-triazole + K_2CO_3	1.5	 (28) +  (62)
3	1*H*-imidazole + K_2CO_3	1.5	 (92)
4	1*H*-pyrazole + K_2CO_3	1.0	 (63) +  (12)
5	1*H*-tetrazole + Li_2CO_3	4.0	 (45) +  (28)
6	MeO^-	1.0	 (96)[d]

[a]All reactions were carried out at 75-80°C in DMF under N_2 with a molar ratio of compound 1 : het⁻ = 1:1.5, unless otherwise specified. Reactions were monitored by TLC. [b]Ratio of azole : base = 1:2; MeO^- was in excess (10% methanolic KOH, N_2, rt). [c]All these new compounds were fully characterized by ^{1}H NMR and elemental analyses. [d]The 3β-hydroxy compound was obtained.



Scheme 1. *Reagents and conditions*: i, 1*H*-1,2,4-sodium triazolate, DMF, N_2, 75-80 °C; ii, $RhCl(PPh_3)_3$, toluene, N_2, reflux; iii, 10% methanolic KOH, N_2, rt; iv, 1*H*-imidazole, K_2CO_3, DMF, N_2, 75-80 °C.

The IC_{50} values of compounds 4 and 7 were determined[12] to be 56 and 256 nM, respectively, towards rat testicular 17α-lyase, which classifies them amongst the most potent inhibitors of this enzyme.[2] Difference spectra obtained when 4 and 7 were added to a modified human 17α-lyase enzyme preparation[13] indicated that each compound gave a type II difference spectrum. Figure 1 shows the spectrum for the imidazole 7; with a peak at 426 nm and trough at 390 nm. These type II spectra indicate the coordination of a nitrogen (probably N-4 of the triazole ring and N-3 of the imidazole ring) to the heme-iron of the cytochrome *P*-450 enzyme (Figure 2).

In conclusion, we have developed a method of introducing azoles at a vinylic carbon using a novel nucleophilic displacement. This eanbled us to synthesize the Δ^{16}-17-1*H*-triazole 4 and Δ^{16}-17-1*H*-imidazole 7 which proved to be powerful inhibitors of rat testicular 17α-lyase enzyme. With the addition of these novel azoles to the collection of potent 17α-lyase inhibitors, new advances in the therapeutic area are possible.

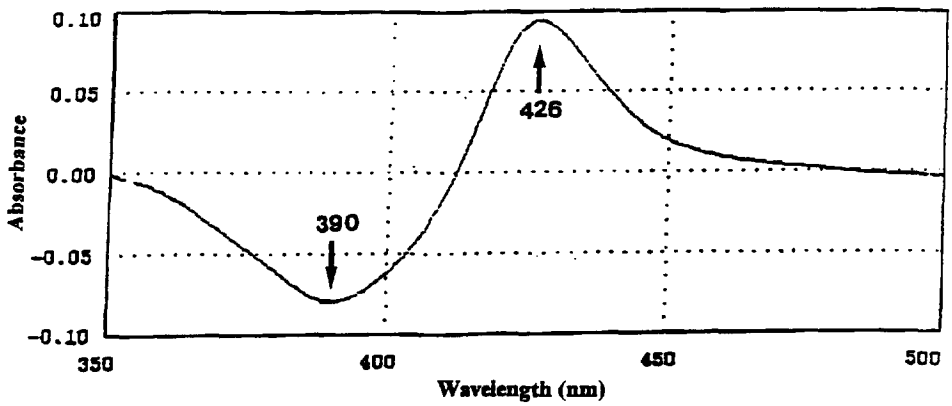


Figure 1. Difference spectrum. The sample and reference cuvettes contained a modified form of human 17α-lyase (*P*-450 concentration 1.8 μM). Spectrum shows the effect of addition of 20 μM Δ^{16}-17-imidazole (**7**).

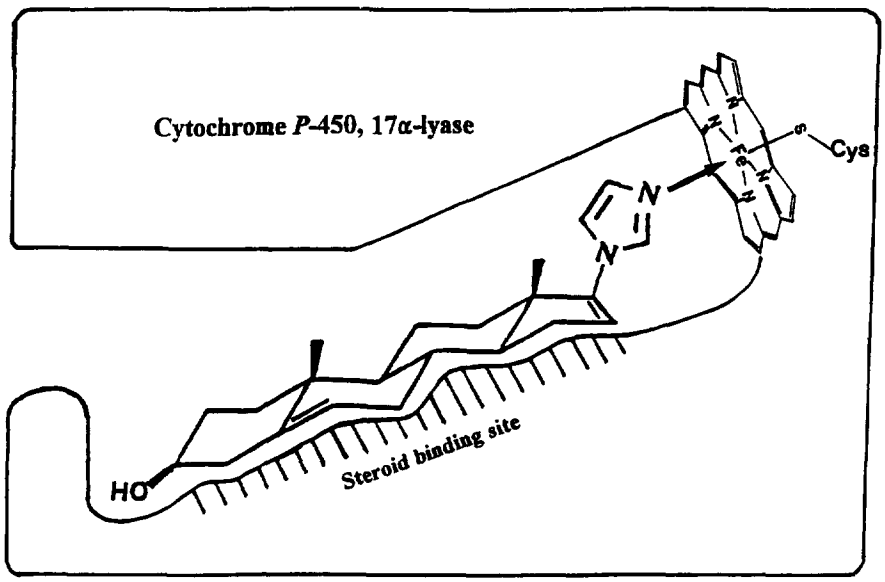


Figure 2. Proposed model for binding of Δ^{16}-17-imidazole (**7**) to cytochrome *P*-450 17α-lyase.

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References and Notes

1. Dr. Vincent C. O. Njar is on leave from Department of Chemistry, University of Ibadan, Ibadan, Nigeria.

2. (a) Nakajin, S.; Hall, P. F. *Biochemistry* **1981**, *20*, 4037. (b) Nakajin, S.; Hall, P. F.; Onada, M. J. *J. Biol Chem.* **1981**, *256*, 6134.
3. (a) Bossche, H. V.; Moereels, H. In *Design of enzyme Inhibitors as Drugs;* Sandler, M.; Smith, H. J., Eds.; Oxford University Press: Oxford, **1994**; Vol 2, pp 434-461. (b) De Coster, R.; Wouters, W.; Bruynseels, J. *J. Steroid Biochem. Molec. Biol.* **1996**, *42*, 313.
4. (a) Arth, G. E.; Patchett, A. A.; Jefopoulus, T.; Bugianesi, R. L.; Peterson, L. H.; Ham, E. A.; Kuehl, F. A., Jr.; Brink, N. G. *J. Med. Chem.* **1971**, *14*, 675. (b) Angelastro, M. R.; Laughlin, M. E.; Schatzman, G. L.; Bey, P.; Blohm, T. R. *Biochem. Biophys. Res. Commun.* **1989**, *162*, 1571. (c) Li, J.; Li, Y.; Son, C.; Banks, P.; Brodie, A. M. H. *J. Steroid Biochem. Molec. Biol.* **1992**, *42*, 313. (d) Li, J.; Li, Y.; Son, C.; Brodie, A. M. H. *The Prostate* **1995**, *26*, 140. (e) Potter, G. A.; Barrie, S. E.; Jarman, M.; Rowlands, M. G. *J. Med. Chem.* **1995**, *38*, 2463. (f) Njar, V. C. O.; Hector, M.; Hartmann, R. W. *Bioorg. Med. Chem.* **1996**, in press. (g) Li, J.; Li, Y.; Son, C. And Brodie, A. M. H. *J. Med. Chem.* **1996**, in press.
5. (a) Hall, F.; Eik-Nes, K. B.; Samuels, L. T. *Endocrinology*, **1963**, *73*, 547. (b) Neher, R.; Kahnt, F. W. *Experientia*, **1965**, *21*, 310. c) Ayub, M.; Levell, M. J. *J. Steroid Biochem.* **1987**, *28*, 521. (d) McCague, R.; Rowlands, M. G.; Barrie, S. E.; Houghton, J. *J. Med. Chem.* **1990**, *33*, 3050. (e) Sergejew, T.; Hartmann, R. W. *J. Enz. Inhib.* **1994**. *8*, 113. (f) Rowlands M. G.; Barrie, E. S.; Chan, F.; Houghton, J.; Jarman, M.; McCague, R.; Potter, G. A. *J. Med. Chem.* **1995**, *38*, 4191.
6. Gadher, P.; Mercer, E. I.; Baldwin, B. C.; Wiggins, T. E. *Pest. Biochem. and Physiol.* **1988**, *19*,1.
7. (a) Doorenbos, N. J.; Milewich, L. *J. Org. Chem.* **1966**, *31*, 3139. (b) Ling Y.; Li, J.; Klus, G. T.; Son, C.; Brodie, A. M. H. Personal communication (manuscript in preparation). It should be noted that some corresponding 16,17-dihydro-17β-azoles (imidazole, pyrazole and isoxazole) are well known. See ref 7a, Rorig, K. U. S. A. Patent No. 2,664,423, 1953; (*Chem. Abstr.* **1955**, *49*, 7608e). Nambara, T.; Shimada, K.; Nemota, T. *Chem. Pharm. Bull.* **1970**, *18*, 1658.
8. Siddiqui, A. U.; Maheshwar Rao, V. U.; Maimirani, M.; Siddiqui, A. H. *J. Heterocyclic Chem.* **1995**, *32*, 353.- Compound **1** was obtained in 76% yield following the Vilsmeier-Haack reaction of the readily available 3β-acetoxyandrost-5-en-17-one with phosphorous oxychloride and DMF.
9. For recent general references of nucleophilic vinylic substitution, see: Rappoport, Z. *Acc. Chem. Res.* **1981**, *14*, 7. Rappoport, Z. *Acc. Chem. Res.* **1992**, *25*, 474.
10. Walborsky, H. M.; Allen, L. E. *J. Amer. Chem. Soc.* **1971**, *93*, 5465.
11. Data for compound **4**: mp 185-188 °C (decomp., from EtOAc/MeOH).[1] H NMR (300 MHz, $CDCl_3$) δ 1.08 (3H, s, 18-Me), 1.10 (3H, s, 19-Me), 3.55 (1H, m, 3α-H), 5.39 (1H, d, J = 4.8 Hz, 6-H), 5.96 (1H, s, 16-H), 7.99 (1H, s, 3'-H), and 8.26 (1H, s, 5'-H). Anal calcd for $C_{21}H_{29}ON_3$: C, 74.29; H, 8.83; N, 12.38, Found: C, 74.20; H, 8.73; N, 12.38. HRMS calcd for $C_{21}H_{29}ON_3$ 339.2311, found 339.2297. For compound **7**: mp 220-223 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (3H, s, 18-Me), 1.06 (3H, s, 19-Me), 3.53 (1H, m, 3α-H), 5.39 (1H, d, J = 5 Hz, 6-H), 5.69 (1H, s, 16-H), 7.08 (2H, br. s, 4' and 5'-H), and 7.64 (1H, s, 2'-H). Anal calcd for $C_{22}H_{30}ON_2$: C, 78.05; H, 8.94; N, 8.28, Found: C, 78.02; H, 9.00; N, 8.22. HRMS calcd for $C_{22}H_{30}ON_2$ 338.2358, found 338.2361.
12. The lyase activity of the enzyme was measured by the release of [^{3}H]-acetic acid from [21-^{3} H]-17α-hydroxypregnenolone substrate as described in Akhtar, M.; Corina, D.; Miller, S.; Shyadehi, A. Z.; Wright, J. N. *Biochemistry* **1994**, *33*, 4410. IC_{50} refers to the inhibitor concentration which produced 50% inhibition of the enzyme activity. The correlation coefficents r > 95% for all tested compounds.
13. The enzyme used was a modified form of human 17α-lyase (CYP 17) which was expressed in *E. coli* and purified as descibed in Imai, T.; Globerman, H.; Gertner, J. M.; Kagawa, N.; Waterman, M. R. *J. Biol. Chem.* **1993**, *268*, 19681.

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