

# Synthesis of 14- $\alpha$ -Aminomethyl substituted Lanosterol Derivatives; Inhibitors of Fungal Ergosterol Biosynthesis

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Several 14- $\alpha$ -aminomethyl-substituted lanosterol derivatives have been synthesized involving a complete  $\Delta^{7,8}$  to  $\Delta^{8,9}$  isomerization; these compounds are inhibitors of fungal ergosterol biosynthesis and are active against intact *Candida* and dermatophyte strains.

Ergosterol is an important constituent of the fungal cell membrane and has also been found in the mitochondria associated with mitochondrial enzymes.<sup>1,2</sup> An important step in the fungal biosynthesis of ergosterol is the cytochrome P<sub>450</sub> mediated 14-demethylation of lanosterol (**1**). This P<sub>450</sub> monooxygenase enzyme oxidatively removes the 14-methyl group by three O<sub>2</sub>-NADPH dependent steps.<sup>3</sup> Inhibitors of this enzyme are not only potentially useful as cholesterol lowering agents<sup>4</sup> but also as antifungal agents. Studies have shown that the imidazole (Im) and triazole antifungals inhibit this enzyme by the binding of the heterocyclic nitrogen atom (N-3 of imidazole and N-4 of triazole) to the protoheme iron atom. This results in the exclusion of oxygen that would normally take part in the reaction.<sup>5</sup> It is further thought that the non-heterocyclic portion of the antifungal molecule binds to the lipophilic sites of cytochrome P<sub>450</sub>.<sup>5</sup> Since the target of the enzyme is the 14-methyl position of lanosterol, a logical inhibitor could be lanosterol with a heme binding component (R = CH<sub>2</sub>Im) at the 14-methyl position. We, therefore, set out to synthesize several 14-aminomethyl substituted lanost-8-ene-3-ols and specifically 14-1H-imidazolylmethyl lanost-8-ene-3-ol where R = CH<sub>2</sub>-imidazole. An example of this approach is shown in the reported antifungal activity of the azasterol antibiotics,<sup>6</sup> and more recently, a series of 14-methyl-functionalized lanost-7-ene-3-ols were synthesized where R = CHF<sub>2</sub>, CH<sub>2</sub>CHF<sub>2</sub>, CH<sub>2</sub>CCH, CH=CH<sub>2</sub>, and CCH. These compounds were shown to be inhibitors of the cytochrome P<sub>450</sub>-14-demethylase enzyme.<sup>7</sup>

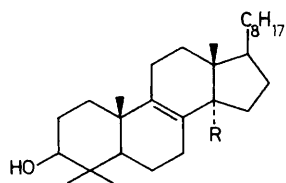
Typical methods of introducing a 1H-alkylimidazole group involve the nucleophilic displacement of alkyl halides or alkyl sulphonates with imidazole. Presumably a 14-methyltosylate or halide could be prepared from the known 14-hydroxymethyl lanosterol,<sup>8</sup> however displacement with a nucleophile such as imidazole adjacent to this neopentyl centre could prove extremely difficult. For this reason we introduced an amine at the 14-methyl position and built up the imidazole ring.

Our strategy (Scheme 1) utilized the photolysis of 3 $\beta$ -acetoxylanost-7 $\alpha$ -nitrite (**1**) to obtain the oxime (**2**).<sup>8</sup> Attempted reduction of the oxime with either lithium aluminium hydride (LAH), LAH-AlCl<sub>3</sub>, or Red-Al failed to give the desired 14-aminomethyl compound. However, hydrogenation using Pt catalysis with perchloric acid (1.1 equiv.) gave the desired compound in 25% yield. This low yield prompted us to

investigate the reduction of the 14-nitrile (**3**) obtained by dehydration of (**2**) with Ac<sub>2</sub>O-pyridine. LAH Reduction of (**3**) followed by aqueous workup gave the expected aldehyde.<sup>8</sup> Even under forced conditions of excess LAH and higher temperatures no aminomethyl compound was detected. However, treatment with 1:1 LAH-AlCl<sub>3</sub> gave the desired aminomethyl compound (**4**)† in 82% yield. Compound (**4**) was then dehydrated using HBr-HOAc to give a 4:1 mixture of alkenes (**5**) and (**6**), respectively as determined by the integration of the 7-alkenic proton at  $\delta$  5.23.

The (**5,6**) mixture was then treated with both strong acid and base under both protic and aprotic conditions with no apparent change in the ratio of (**5**) to (**6**). However, when a solution of the (**5,6**) mixture in methanol was adjusted to pH 4.5 with 1 M HCl we observed a complete isomerization of the 7,8-ene to give the desired 8,9-ene compound (**6**)† as determined by the complete disappearance of the 7-alkenic proton at  $\delta$  5.23. Complete isomerization apparently took place only in the presence of an amine group at the 14-methyl position. Thus when the 14-hydroxymethyl (**13**), or 14-nitrile (**10**) compounds (Scheme 2) with the double bond at the 7,8-position were treated at pH 4.5 under similar conditions, no isomerization was observed. When lanosterol is treated with strong acid, an equilibrium mixture of the  $\Delta^{7,8}$  and  $\Delta^{8,9}$  compounds is obtained.<sup>9</sup> Presumably, a similar equilibrium is set up with the 14- $\alpha$ -aminomethyl compound to give a 4:1 ratio of the  $\Delta^{7,8}$  to  $\Delta^{8,9}$  compounds under similar strongly acidic conditions. Therefore a plausible mechanism for this  $\Delta^{7,8}$  to  $\Delta^{8,9}$  isomerization may involve the intramolecular protonation of the 7,8-double bond by the proximal 14-ammonium ion at pH 4.5.

An alternative to the reduction of (**3**) to obtain (**6**) was to dehydrate (**2**) with MsCl-collidine to give the 7,8-ene-14-nitrile followed by LAH-AlCl<sub>3</sub> reduction to give (**5**), albeit in a lower yield than the previous route. Again, treatment of (**5**)



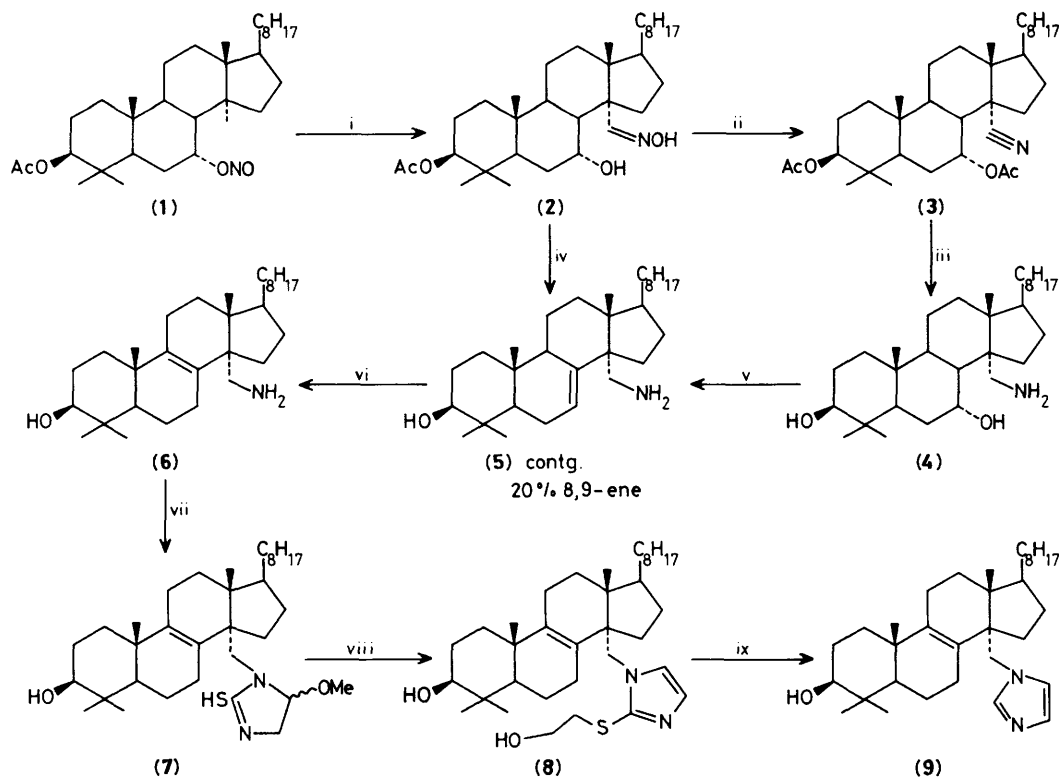
(1)

† All compounds gave elemental analyses consistent with structural assignments. All n.m.r. data obtained at 200 MHz in CDCl<sub>3</sub> unless otherwise noted. (**4**): m.p. 258–260 °C; <sup>1</sup>H n.m.r.  $\delta$  0.75–1.9 (envelope), 2.88 (2H, apparent s, 14- $\alpha$ -CH<sub>2</sub>N), 3.33 (1H, dd, J 10 and 4 Hz, 3- $\alpha$ -H), 3.95 (1H, br.s, 7- $\beta$ -H); electron impact (e.i.) m.s. *m/z* (rel. intensity) 462 (*M* + 1, 4), 461 (*M*, 11), 364 (41), 299 (100).

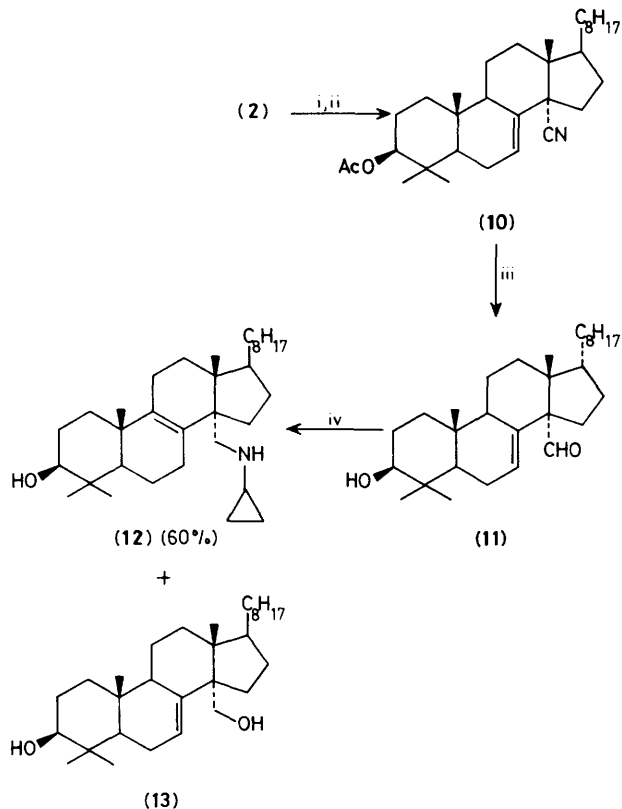
(**6**): m.p. 167–168 °C; <sup>1</sup>H n.m.r. [5% (CD<sub>3</sub>)<sub>2</sub>SO]  $\delta$  0.8–2.0 (envelope), 2.73 (2H, br.s, 14- $\alpha$ -CH<sub>2</sub>N) superimposed on 2.5–2.8 (2 exchangeable H), 3.23 (1H, dd, J 10 and 4 Hz, 3- $\alpha$ -H); fast atom bombardment (f.a.b.) *m/z* (rel. intensity) 444 (*M* + 1, 100), 427 (54), 426 (53), 409 (57).

(**9**): <sup>1</sup>H n.m.r.  $\delta$  0.8–2.2 (envelope), 2.82–2.98 (1H, m), 3.31 (1H, dd, J 10 and 4 Hz, 3- $\alpha$ -H), 3.92 (2H, dd, J 8 and 4 Hz, 14- $\alpha$ -CH<sub>2</sub>-Im), 6.89 (1H, s), 7.06 (1H, s), 7.45 (1H, s); e.i.m.s. *m/z* (rel. intensity) 494 (*M*, 17), 413 (25), 395 (35), 135 (27), 82 (100).

(**12**): <sup>1</sup>H n.m.r.  $\delta$  0.22–0.5 (4H, m, cyclopropyl), 0.7–2.1 (envelope), 2.48 (2H, apparent br.s), 3.25 (1H, dd, J 10 and 4 Hz); e.i.m.s. *m/z* (rel. intensity) 483 (*M*, 12), 414 (15), 413 (17), 412 (23), 323 (37), 225 (44), 197 (49), 70 (100).



**Scheme 1.** Reagents and conditions: i, hv, Ph; ii, Ac<sub>2</sub>O-Pyr (Pyr = pyridine), 93%; iii, LAH-AlCl<sub>3</sub>, 82%; iv, MsCl-Pyr followed by refluxing in collidine, followed by LAH-AlCl<sub>3</sub>, 32%; v, HBr-HOAc followed by NaOH, 83%; vi, pH 4.5/aq. MeOH, 100%; vii, (MeO)<sub>2</sub>CHCH<sub>2</sub>NCS, 75.8%; viii, (CH<sub>2</sub>OH)<sub>2</sub>/HCl, 92.5%; ix, Raney-Ni, 73%.



**Scheme 2.** Reagents and conditions: i, MsCl-Pyr; ii, collidine/reflux, 70%; iii, LAH, Et<sub>2</sub>O, 42%; iv, cyclopropylamine, NaCNBH<sub>3</sub>, pH 4.5.

**Table 1.** Biological activity of compounds (6) and (9).

Compound	MICs (mcg/ml, SDB)		Ergosterol inhibition IC <sub>50</sub> /μM
	<i>Candida</i>	Dermatophyte	
(6)	11.9	7.6	0.3
(9)	26.3	9.2	0.03
(12)	>95.1	9.2	1.0
KTZ	>28.8	12.1	0.03

in MeOH at pH 4.5 resulted in complete isomerization to give (6) (Scheme 1).

The imidazole ring was then prepared by the reaction of (6) with dimethoxyethylisothiocyanate to give (7).<sup>10</sup> Acid hydrolysis in ethylene glycol gave (8), which was then desulphurized with Raney-Ni to give (9)† in 51% overall yield from the aminomethyl compound (6).

*N*-Alkylated cyclopropylamines have been reported to be suicide inhibitors of P<sub>450</sub> mono-oxygenase enzymes.<sup>11</sup> Since theazole antibiotics are reversible inhibitors of cytochrome P<sub>450</sub> we prepared the 14-cyclopropylaminomethyl compound (12). We utilized the known 14-aldehyde (11) which was obtained from the 7α-hydroxy-14-oxime (2) (Scheme 2).<sup>8</sup> Upon reductive amination of (11) with cyclopropylamine at pH 4.5 with NaCNBH<sub>3</sub> we obtained the cyclopropylaminomethyl compound (12)† in 60% yield (with the double bond again isomerized completely to the 8,9 position) plus the 14-hydroxymethyl compound (13) (double bond still in the 7,8 position). This was further proof that an amine was required at the 14-methyl position for complete 7,8 to 8,9 double bond isomerization.

The biological results (Table 1) show that compounds (6)

and (9) were more active in minimum inhibitory concentration (MIC) tests than ketoconazole (KTZ); Dermatophyte strains appeared to be more sensitive to these compounds than *Candida* strains.‡ All compounds tested were ergosterol biosynthesis inhibitors as measured by a decreased level in (2-<sup>14</sup>C) mevalonic acid incorporation into ergosterol, and an accumulation of radioactive lanosterol in a cell free system.<sup>12</sup> It is noteworthy that the imidazole compound (9) was as effective an inhibitor as KTZ. Although all the compounds tested were inhibitors of ergosterol synthesis, there was no direct correlation between IC<sub>50</sub>s and MICs.

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- 12 The IC<sub>50</sub> is the concentration of the compound resulting in 50% reduction of incorporation of α(<sup>14</sup>C)mevalonic acid into ergosterol. A cell-free extract of *C. albicans* was obtained by disruption of the cells by shaking with glass beads. Ergosterol synthesis was measured by the procedure described by Mitropoulos, *Biochim. Biophys. Res. Commun.*, 1976, **71**, 892. The non-saponifiable fraction containing sterols was fractionated by h.p.l.c. as described, I. J. Sud and D. S. Feingold, *Antimicrob. Agents Chemother.*, 1983, **23**, 185.

‡ MICs were performed in sabauroud dextrose broth and are expressed as the geometric mean. *Candida* strains: 4, *C. albicans*; 1, *C. tropicalis*; 1, *Trichophyton mentagrophytes*, *T. rubrum*; *Microsporum canis*; *M. gypseum*, *Epidermophyton floccosum*.