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SYNTHESIS OF 2'-(3α-BENZYLOXY-24-NORCHOLAN-23-YL)-2',4',4'-TRIMETHYL-4',5'-DIHYDROOXAZOLINE-N-OXYL - A NEW POTENTIAL SPIN PROBE FOR BIOMEMBRANES

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ABSTRACT : The synthesis of a new steroidal nitroxide, viz. 2'-(3α-benzyloxy-24-norcholan-23-yl)-2',4',4'-trimethyl-4',5'-dihydrooxazoline-N-oxyl 7, a potential spin probe for biomembranes is described. The title compound in improved yield could be obtained by a direct Grignard reaction on the oxaziridine 4. This effected a one-step reduction in the overall synthetic sequence. The methodology of the Grignard reaction on the oxaziridine to yield the nitroxide is reported for the first time. In dilute solutions, the isotropic ESR parameters for 7 have been measured.

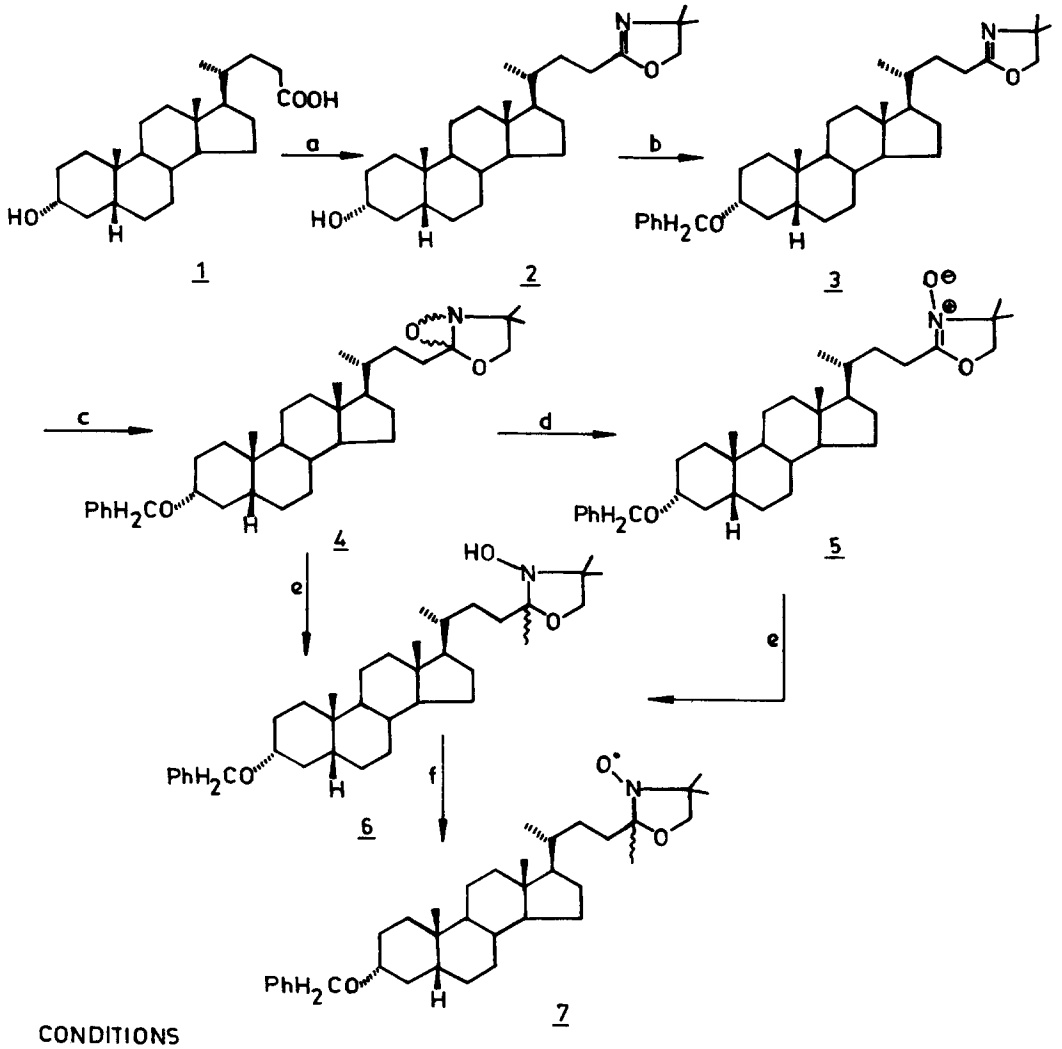
INTRODUCTION

Several rigid doxyl (4,4-dimethyloxazolidine-N-oxyl) nitroxides of steroids have been synthesized[1-3] where the nitroxide moiety is attached to the main ring and these have been used extensively as spin probes for biomembranes.[4-7] Unlike rigidly attached doxyl nitroxides which are largely used for oriented multibilayer studies[4], the steroidal nitroxideswith the nitroxide in the side chain would have higher mobility owing to the free rotation and are expected to exhibit greater versatility as spin probes for biomembranes. To this end, it was felt pertinent to synthesize steroidal nitroxides with the nitroxide in the side chain. In this paper, we report the synthesis of a new steroidal doxyl nitroxide, 2'-(3α-benzyloxy-24-norcholan-23-yl)-4',4',2'-trimethyloxazolidine-N-oxyl, 7 as a potential spin labelled compound.

The ESR spectra of the title compound 7 in different protic and aprotic solvents were studied. In dilute solutions, owing to the rapid tumbling of the spin labels, the isotropic g-values (g_o) and isotropic hyperfine splitting constants (a_o) are obtained. The variations in these spectral parameters were taken up with a view to extrapolating our findings in probing the microenvironment of the new spin label in phospholipid vesicles. The results of such studies will be reported elsewhere. In this paper, we present the variation of g_o and a_o values of the new spin label in different solvents.

RESULTS AND DISCUSSION

Two basic approaches for the synthesis of steroidal nitroxides have been reported. The first approach involves the covalent attachment of nitroxide containing molecule with the substrate molecule[8,9], while the second approach utilizes the construction of nitroxide moiety in a molecule by a series of chemical transformations.[10,11] Several doxyl nitroxides of cholestane and androstane derivatives have been reported.[1,2] However, in our case the substrate of choice was lithocholic acid 1. Apart from being readily available, it has the advantages of being a constituent of bile acid and hence exhibiting high membrane affinity. The presence of a single hydroxy group, remote from the desired site of attachment of the nitroxide group, renders it ideal as a hydrophilic membrane probe compared to the hydrophobic 3-doxyl cholestane.[12] Moreover, contrary to few literature reports where the nitroxide is introduced by derivatizing the functional group in the side chain, we have synthesized the nitroxide from a steroidal oxazole 2 as a key intermediate obtained from lithocholic acid 1 in high yield. To the best of our knowledge, this is the first report of synthesis of a doxyl nitroxide in steroid side chain by this approach.



CONDITIONS

a) 2-amino-2-methyl propan-1-ol, H_3BO_3, xylene, reflux, 48h

b) NaH/THF, TBAI, $PhCH_2Br$, reflux, 3h

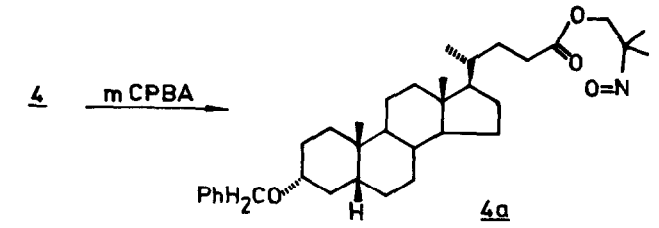
c) mCPBA, Et_2O, 10°C, 48h

d) Rearrangement on silica gel

e) CH_3MgI, Et_2O, rt

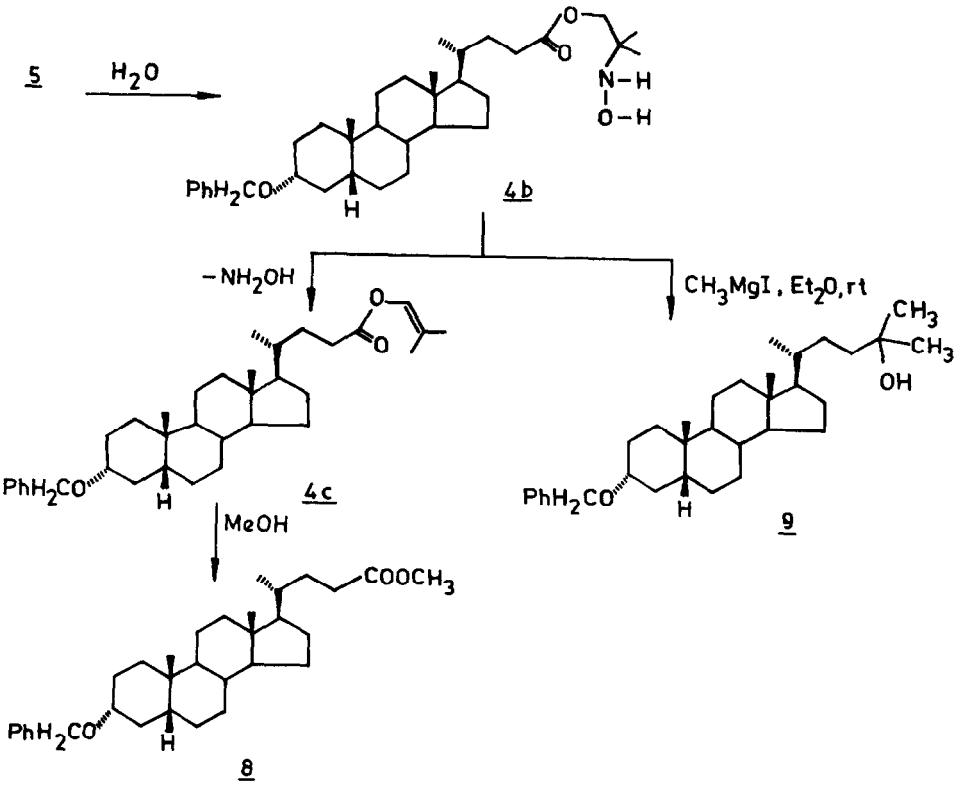
f) $Cu(OAc)_2 \cdot H_2O$, MeOH, 1h

Scheme-1



4a

Scheme - 2



Scheme - 3

The first step in the envisaged strategy involved the construction of the oxazoline moiety in the side chain of lithocholic acid 1. The oxazoline 2 was obtained in 96% yield by the method of Barton et al[13]. The next step of protecting the 3α-hydroxy group of 2, however, posed several problems. Of different protecting groups tried, the benzyl ether group was found to be the most suitable[14]. The desired benzylation was however achieved (yield 68%) only under modified conditions employing sodium hydride-benzyl bromide and catalytic amount of tetrabutyl ammonium iodide (TBAI) in refluxing THF.

The benzylated oxazoline 3 was then epoxidized with m-chloroperbenzoic acid. The IR spectrum of the product showed absence of the band at 1665 cm^{-1} (C=N). The ^1H NMR spectrum showed a pair of doublets at δ 3.58 and δ 3.45 with J = 8 Hz, corresponding to the two geminal protons at C-5 of the oxazoline ring. The geminal methyls of the oxazoline ring also appeared as two three-proton singlets at δ 1.36 and δ 1.13 in place of the six-proton singlet at δ 1.25, observed for the geminal methyls of 3. Alongwith the desired oxaziridine 4, a minor compound was formed. This was found to be the ring opened nitroso ester 4a. The presence of 4a was indicated in the IR spectrum by the bands at 1740 cm^{-1} (ester) and 1550 cm^{-1} (N=O). The ^1H NMR spectrum of the product exhibited the presence of a singlet at δ 3.84 corresponding to the $-CH_2OCO$ group of 4a. The mass spectrum of the product also revealed the presence of a low intensity peak at m/z 551 corresponding to the molecular ion peak of 4a. Such products resulting from the over-oxidation of oxaziridines by m-chloroperbenzoic acid (mCPBA) are well documented[15] (Scheme-2).

In order to effect the conversion of the oxaziridine to the nitrone, the crude product was placed on top of a dry silica gel column and the protocol[16] for rearrangement was followed. The desired nitrone 5, alongwith a small amount of the N-hydroxy ester 4b, was obtained in

the methanol eluate as a yellow viscous syrup in 66% yield. The nitrone exhibited a characteristic UV absorption[17] at 242.2 nm. The N-hydroxy ester 4b, formed by the ring opening of the nitrone, was detected in the ^{1}H NMR spectrum of the product. Purification of the reaction mixture at this stage was not attempted owing to the inherent instability of the nitrone.

The yellow viscous syrup was immediately subjected to Grignard reaction using excess methyl magnesium iodide at room temperature. The product 6 showed IR absorption at 3350 cm^{-1} (N-OH). Introduction of the methyl group as a result of Grignard reaction was confirmed by the signal at δ 1.28 in the 300 MHz ^{1}H NMR spectrum. In the mass spectrum, the molecular ion peak was not observed. Instead, two peaks at m/z 537 ($M^+ + 1 - CH_3$) and 521 (M^+-NO) were observed.

The final step involved the aerial oxidation of 6 in presence of cupric acetate as catalyst. The crude product was purified to yield the desired nitroxide 7, as a yellow crystalline solid. The ESR spectrum of 7 in chloroform was observed to be a characteristic triplet with a g_o value of 2.0054 and a hyperfine splitting (a_o) of 14.36 G. The molecular ion peak (m/z 550) was observed in the mass spectrum along with other characteristic fragments for the doxyl nitroxide[18]. The ^{1}H NMR spectrum of this compound however showed considerable line broadening as is expected of the paramagnetic nitroxide moiety present in this compound. A well resolved, informative ^{1}H NMR spectrum was obtained by reducing, *in situ*, the $CDCl_3$ solution of 7 with 1.5 equivalents of phenyl hydrazine[19]. The two singlets at δ 1.31 and δ 1.30 with intensity ratio of 1:1 corresponded to the methyls of the two diastereomers obtained via the Grignard addition to the nitrone in a ratio of 1:1. The diastereomeric mixture appeared as a single spot in TLC. All attempts to separate it by HPLC using various solvent systems were, however, unsuccessful.

Alongwith the desired nitroxide 7, two other minor compounds 8 and 9 were isolated as colourless, crystalline solids. Compound 8 was less polar than the nitroxide. The elemental analysis of 8 corresponded to $C_{32}H_{48}O_3$. The IR spectrum showed a band at 1735 cm^{-1} and the ^1H NMR spectrum exhibited a three-proton singlet at δ 3.66 for the $-COOCH_3$ group. The comparatively more polar compound 9 showed an IR band at 3420 cm^{-1}, indicating the presence of a hydroxy group. The ^1H NMR revealed the presence of a singlet at δ 1.19 integrating for six protons. Absence of any deshielded proton which could result from the attachment of a hydroxy group suggested that the -OH group was tertiary. This was further confirmed by the ^{13}C NMR spectrum of the compound which showed a deshielded carbon atom at δ 71.03 (C-24). In keeping with the assigned structure of 9, the signal at δ 71.03 (C-24) was not observed in the DEPT spectrum. The elemental analysis corresponded to the molecular formula of $C_{33}H_{32}O_2$. The mass spectrum however showed an ion peak at m/z 462. This was attributed to be the (M^+-H_2O) peak. The formation of compound 9 could be rationalized by the addition of Grignard reagent on the N-hydroxy ester 4b (Scheme 3) which invariably accompanied the nitrone 5. The formation of compound 8 could be explained by the methanolysis of the unstable vinyl ester 4c, formed, in turn, by the elimination[15] of hydroxylamine from 4b. The plausible mechanism is outlined in Scheme 3.

Though the Grignard addition to the nitrone 5 followed by oxidation yielded the nitroxide 7, it was felt that Grignard addition directly to the oxaziridine 4 could well lead to the desired intermediate 6, thereby reducing one step in the overall synthetic sequence. Hence, oxaziridine 4 was subjected to Grignard reaction using methyl magnesium iodide. As expected, the product obtained was the desired N-hydroxy compound 6 which on oxidation in presence of cupric acetate again yielded the diastereomeric (1:1) nitroxide 7. The overall yield of 7

from lithocholic acid 1 improved to 27.8% as compared to that of 16.3% obtained via the nitrone 5.

The ESR spectra of the nitroxide 7 were recorded in a series of solvents. The variations in the g_o values and nitrogen hyperfine coupling constants (a_o) are compiled in Table 1. The purpose of obtaining these ESR parameters was to determine the behaviour of the new nitroxide in different homogeneous environments of varying polarity. This was with a view to investigating the applicability of the nitroxide as a spin probe for biomembranes.

Table-1. Isotropic ESR parameters for nitroxide 7 in different solvents

Sr. No.	Solvent	a_o (Gauss)	g_o
1.	Hexane	13.83	2.0058
2.	Isooctane	13.87	2.0058
3.	Toluene	14.31	2.0055
4.	Chloroform	14.36	2.0054
5.	Ethyl acetate	14.37	2.0053
6.	Tetrahydrofuran	14.39	2.0052
7.	Acetone	14.56	2.0052
8.	Dichloromethane	14.81	2.0051
9.	Isopropanol	14.93	2.0051
10.	Ethanol	15.06	2.0050

The variation of a_o and g_o values with gradual increase in solvent polarity follows the expected trend. The studies on the nitroxide 7 as a spin probe for biomembranes will be reported elsewhere.

EXPERIMENTAL

Melting points are reported uncorrected. Laboratory solvents were predried before use according to standard procedures. Lithocholic acid was purchased from Aldrich and used as such. IR spectra were recorded on Perkin Elmer 688 spectrometer. The 300 MHz ^1H NMR spectra were recorded on Varian VXR 300S spectrometer as solutions in $CDCl_3$ at ambient temperature with TMS as internal standard. Mass spectra were obtained on Shimadzu QP1000 spectrometer. Elemental analyses were performed on CEST MOD 110 analyser. UV spectra were recorded on Shimadzu UV 260 spectrometer. ESR spectra were recorded at room temperature on Varian E-112 spectrometer operating in the X-band with tetracyanoethylene as the internal standard. All solvents used for ESR measurements were deoxygenated. The concentration of the nitroxide 7 used for ESR experiment was 10^{-5} (M).

2'-(3α-Hydroxy-24-norcholan-23-yl)-4',4'-dimethyl-4',5'-dihydro oxazole (2)

A mixture of lithocholic acid 1 (5.0 g, 13 mmol), 2-methyl-2-amino-propan-1-ol (1.72 mL, 18 mmol) and boric acid (296 mg, 4 mmol) was dissolved in anhydrous xylene (96 mL) and the solution refluxed with azeotropic removal of water for 48 h. The solvent was removed by vacuum distillation and the residue dissolved in hot methanol (10 mL). To this solution, 5% aqueous K_2CO_3 (105 mL) was added and the mixture was boiled for 1 h, cooled and extracted with ether (4 x 25 mL). The organic layer was worked up as usual and the residue so obtained was purified by column chromatography on silica gel using 5% methanol in benzene as eluant to give a white solid (5.46 g, 96%). M.pt.: 152°C. IR (nujol) $\bar{\nu}$ = 3230 (broad, OH), 1670 (C=N), 1395, 1375 ($-\dot{C}(CH_3)_2$) cm^{-1}; ^1H NMR ($CDCl_3$) : δ 3.89 (s, 2H, $-OCH_2$), 3.66-3.57 (m, 1H, 3β-H), 1.63 (broad, D_2O exchangeable, -OH), 1.25 (s, 6H, $-\dot{C}(CH_3)_2$), 0.93 (d, J = 6.41 Hz, 3H, 21-H_3), 0.91 (s, 3H, 19-H_3), 0.63 (s, 3H, 18-H_3);

MS:m/z (rel int) : 430 (M^++1, 4.4), 414 (2.2), 372 (4.0), 127 (12.9), 126 (97.6), 114 (12.7), 113 (100).

Anal.

Calcd. for $C_{28}H_{47}NO_2$: C, 78.27; H, 11.03; N, 3.26.
Found : C, 78.04; H, 10.98; N, 3.10.

2'-(3α-Benzyloxy-24-norcholan-23-yl)-4',4'-dimethyl-4',5'-dihydro oxazole (3)

To a suspension of NaH (55% in petroleum wax, 0.21 g, 4.4 mmol) in anhydrous THF (100 mL) was added dropwise a solution of 2 (1.90 g, 4 mmol) under nitrogen. After stirring for 15 min, benzyl bromide (0.52 mL, 4 mmol) was added dropwise, followed by catalytic amount of TBAI (14.7 mg, 0.04 mmol). The resultant solution was refluxed for 4 h. On cooling, saturated aqueous NH_4Cl solution was added dropwise and the reaction mixture extracted with CH_2Cl_2 (4 x 25 mL). The organic layer was worked up as usual and the residue so obtained was purified by column chromatography on silica gel (15% Ethyl acetate-petroleum ether b.pt. 60-80°C) to provide a thick viscous product (1.56 g, 68%).
IR ($CHCl_3$) $\bar{\nu}$ = 3100, 3070 (aromatic), 1670 (C=N), 1370, 1360 ($-\dot{C}(CH_3)_2$) cm^{-1}; ^1H NMR ($CDCl_3$) : δ 7.37-7.26 (m, 5H, Ar-H), 4.55 (s, 2H, $-OCH_2Ph$), 3.89 (s, 2H, $-OCH_2$), 3.38-3.33 (m, 1H, 3β-H), 1.25 (s, 6H, $-\dot{C}(CH_3)_2$), 0.93 (d, J = 6.25 Hz, 3H, 21-H_3), 0.91 (s, 3H, 19-H_3), 0.63 (s, 3H, 18-H_3); MS : m/z (rel int) : 521 (M^++2, 28.4), 519 (M^+, 2.2), 504 (14.2), 462 (32.8), 428 (6.5), 154 (29.3), 127 (87.5), 126 (100), 114 (100), 113 (100), 107 (27.8), 98 (29.2), 91 (100).

Anal.

Calcd. for $C_{35}H_{53}NO_2$: C, 80.87; H, 10.27; N, 2.69.
Found : C, 81.02; H, 10.17; N, 2.62.

5'-(3α-Benzyloxy-24-norcholan-23-yl)-1'-aza-4',6'-dioxabicyclo-[3.1.0]-hexane (4)

To a stirred solution of 3 (4.63 g, 8.9 mmol) in 70 mL dry ether, at

-10°C was added mCPBA (85%, 1.81 g) dissolved in 50 mL of dry ether under nitrogen atmosphere. After the addition was complete, the reaction mixture was kept stirring at 10°C for 48 h. The resultant pale blue solution was washed thoroughly with 10% Na_2CO_3 solution and dried over anhydrous K_2CO_3. The solvent was evaporated in vacuo to yield a pale blue viscous compound (4.72 g, 98.9%). IR ($CHCl_3$) $\bar{\nu}$ = 3060, 3020 (aromatic), 1480 (oxaziridine), 1370, 1360 ($-\overset{|}{C}(CH_3)_2$), 1740 ($-COOCH_2-$ of compound 4a), 1550 (N=O of 4a) cm^{-1}. ^1H NMR ($CDCl_3$) : δ 7.35-7.26 (m, 5H, Ar-H), 4.55 (s, 2H, $-OCH_2Ph$), 3.58 (d, J = 8.05 Hz, 1-H, $-OC\underline{H}H'$), 3.45 (d, J = 8.05 Hz, $-OCH\underline{H}'$), 3.38-3.33 (m, 1H, 3β-H), 1.36 (s, 3H, gem-methyl), 1.13 (s, 3H, gem-methyl), 0.92 (d, J = 7.7 Hz, 3H, 21-H_3), 0.90 (s, 3H, 19-H_3), 0.63 (s, 3H, 18-H_3). MS : m/z (rel int) : 536 (M^++1, 13.0), 535 (M^+, 7.6), 521 (7.6), 505 (4.3), 463 (10.8), 131 (19.1), 127 (36.7), 126 (100), 114 (64.0), 113 (100), 91 (100). A peak of very low intensity at m/z 551 was observed corresponding to the molecular ion of 4a.

2'-(3α-Benzyloxy-24-norcholan-23-yl)-4',4'-dimethyl-4',5'-dihydro oxazoline-N-oxide (5)

A solution of crude 4 (4.0 g, 7.47 mmol) in 50 mL dry $CHCl_3$ was allowed to stand for 45 mins on top of a dry silica gel column. The column was next eluted successively with dry chloroform (150 mL), acetone (150 mL) and finally with dry methanol (300 mL). Anhydrous condition was maintained all throughout. Concentration of the methanol fraction afforded the nitrone 5 as a yellow syrup (2.64 g, 66%). IR (neat) $\bar{\nu}$ = 3100, 3080 (aromatic), 1540 cm^{-1} ($-\overset{|}{C}=\overset{+}{N}-\overset{-}{O}$). UV (MeOH) : 242.2 nm (ε 4235). ^1H NMR ($CDCl_3$) : δ 7.35-7.26 (m, 5H, Ar-H), 4.55 (s, 2H, $-OCH_2Ph$), 4.11 (ABq, J = 14.28 Hz, 2H, $-OCH_AH_B$) , 3.4-3.2 (m, 1H, 3β-H), 1.27 (s, 3H, gem-methyl), 1.24 (s, 3H, gem-methyl), 0.90 (s, 3H, 19-H_3), 0.88 (d, J = 7.5 Hz, 3H, 21-H_3), 0.62 (s, 3H, 18-H_3). MS : m/z (rel

int) : 537 (M^++2, 0.2), 535 (M^+, 0.2), 522 (7.9), 521 (19.7), 505 (0.2), 463 (8.1), 413 (4.1), 358 (5.4), 357 (5.6), 144 (11.8), 131 (19.3), 127 (13.0), 126 (100), 114 (27.2), 113 (72.4), 91 (44.8).

2'-(3α-Benzyloxy-24-norcholan-23-yl)-2',4',4'-trimethyl-4',5'-dihydro oxazoline-N-hydroxide (6)

To a solution of freshly prepared 5 (3.2 g, 5.86 mmol) in 100 mL of dry ether at room temperature was added dropwise with stirring under nitrogen atmosphere 3.5 equivalents of methyl magnesium iodide (3.4 g in 10.4 mL of dry ether). The reaction was exothermic and immediate precipitation was observed. It was kept stirring at room temperature for 10 h. The reaction mixture was quenched with saturated NH_4Cl solution. The ether layer was separated and the aqueous layer was extracted with ether (4 x 25 mL). The combined ether extracts were dried over anhydrous $MgSO_4$. Evaporation of solvent under vacuum afforded the semi-solid N-hydroxy compound 6 (2.50 g, 75.7%).
IR ($CHCl_3$) $\bar{\nu}$ = 3350 (N-OH), 3080 (aromatic), 1370, 1360 ($-\dot{C}(CH_3)_2$) cm^{-1}. The ^1H NMR spectrum of 6 was identical to that of the phenyl hydrazine reduced product of 7 and hence not reported here separately.
MS : m/z (rel int) : 537 (M^++1-CH_3, 4.5), 534 (4.5), 521 (6.8), 463 (5.3), 131 (4.8), 127 (12.9), 126 (100), 114 (43.1), 113 (75.7), 91 (47.0).

2'-(3α-Benzyloxy-24-norcholan-23-yl)-2',4',4'-trimethyl-4',5'-dihydro oxazoline-N-oxyl (7)

The crude N-hydroxy compound 6 (2.5 g, 4.5 mmol) was dissolved in methanol (50 mL). To it catalytic amount (30 mg) of $Cu(OAc)_2.H_2O$ was added and the mixture was stirred at room temperature, in presence of air, for 2 h. The resultant reaction mixture was thoroughly extracted with CH_2Cl_2 (5 x 20 mL) after saturation with brine. The organic layer was dried over $MgSO_4$ and solvent removed to give a greenish yellow semi-

solid product (2.3 g). The reaction mixture was purified by column chromatography over silica gel, using 2% ethylacetate-petroleum ether (b.pt. 60-80°C) as eluant. The first compound eluted was the methyl ester 8 (0.184 g) followed by the desired nitroxide 7 (1.26 g, 50.5%) and then compound 9 (0.276 g). Compounds 7 and 8 were recrystallized from chloroform, whereas compound 9 was recrystallized from chloroform-methanol (8:2).

2'-(3α-Benzyloxy-24-norcholan-23-yl)-2',4',4'-trimethyl-4',5'-dihydro oxazoline-N-oxyl (7)

M.pt. : 81°C. IR (KBr) $\overline{\nu}$ = 3100, 3080 (aromatic), 1383, 1375 ($-\overset{|}{C}(CH_3)_2$) 1360 (N-$\dot{O}$) cm^{-1}. ^1H NMR ($CDCl_3$) was recorded after reduction, in situ, by the addition of 1.5 equivalents phenylhydrazine. ^1H NMR ($CDCl_3$) : δ 7.35-7.26 (m, Ar-H), 4.55 (s, 2H, $-OCH_2Ph$), 3.63 (d, J = 8.3 Hz, 1H, $-OCH_AH_B$), 3.56 (d, J = 8.3 Hz, 1H, $-OCH_AH_B$), 3.47-3.33 (m, 1H, 3β-H), 1.31 and 1.30 (a pair of singlets, 3H, 24-H_3), 1.23 (s, 3H, gem-methyl), 1.18 (s, 3H, gem-methyl), 0.90 (s, 3H, 19-H_3), 0.89 (d, J = 5.2 Hz, 3H, 21-H_3), 0.63 (s, 3H, 18-H_3). UV ($CHCl_3$) : 244 nm (ε 1030), 365 (ε <5). MS : m/z (rel int): 551 (M^++1, 3.5), 550 (4.6), 521 (14.0), 520 (9.3), 463 (33.7), 413 (14.0), 412 (16.6), 411 (18.6), 356(9.3), 339 (11.8), 215 (14.1), 130 (11.8), 114 (24.7), 105 (44.7), 91 (100). ESR spectra (10^{-5} M in $CHCl_3$) : Symmetrical triplet with g_o = 2.0054, a_o = 14.36G.

Anal.

Calcd. for $C_{36}H_{56}NO_3$: C, 78.49; H, 10.24; N, 2.54.
Found : C, 78.35; H, 10.26; N, 2.52.

Methyl-3α-benzyloxy-5β-cholan-24-oate (8)

M.pt. : 152°C. IR (KBr) $\overline{\nu}$ = 3100, 3080, 3020 (aromatic), 1735 (-COOMe), 1380, 1370 ($-\overset{|}{C}(CH_3)_2$) cm^{-1}. ^1H NMR ($CDCl_3$) : δ 7.35-7.26 (m, 5H, Ar-H), 4.57 (s, 2H, $-OCH_2Ph$), 3.66 (s, 3H, $-COOCH_3$), 3.41-3.33 (m, 1H, 3β-H), 2.40-2.15 (m, 2H, 23-H_2), 0.907 (s, 3H, 19-H_3), 0.904 (d, J = 6.25 Hz,

3H, 21-H_3), 0.63 (s, 3H, 18-H_3). MS : m/z (rel int) : 481 (M^++1, 4.5), 447 (11.1), 416 (6.7), 390 (5.6), 373 (31.2), 372 (37.3), 358 (10.3), 257 (5.7), 215 (18.3), 105 (13.9), 91 (100).

Anal.

Calcd. for $C_{32}H_{48}O_3$: C, 79.95; H, 10.06.
Found : C, 79.83; H, 10.07.

2'-(3α-Benzyloxy-5β-norcholan)-propan-2'-ol (9)

M.pt. : 91°C. IR (KBr) $\bar{\nu}$ = 3400 (-OH), 3090, 3080, 3010 (aromatic), 1380, 1365 (-$\dot{C}(CH_3)_2$) cm^{-1}. ^1H NMR ($CDCl_3$) : δ 7.36-7.23 (m, 5H, Ar-H), 4.55 (s, 2H, -OCH_2Ph), 3.41-3.33 (m, 1H, 3β-H), 1.198 (s, 3H, gem-methyl), 1.193 (s, 3H, gem-methyl), 0.91 (d, J = 6.56 Hz, 3H, 21-H_3), 0.91 (s, 3H, 19-H_3), 0.64 (s, 3H, 18-H_3). MS : m/z (rel int): 462 (M^+-H_2O, 10.5), 447 (2.5), 406 (20.0), 391 (18.8), 371 (24.0), 355 (69.6), 339 (17.8), 299 (13.1), 298 (28.5), 255 (15.1), 215 (25.7), 107 (24.2), 105 (16.6), 91 (100). ^{13}C NMR ($CDCl_3$) : δ 139.20, 128.22, 127.46, 127.23, 78.65, 71.03, 69.82, 56.46, 55.98, 42.67, 42.17, 40.36, 40.16, 35.86, 35.39, 34.87, 33.26, 30.15, 29.30, 28.97, 28.16, 27.31, 27.23, 26.37, 24.16, 23.33, 20.80, 18.67, 11.99.

Anal.

Calcd. for $C_{33}H_{52}O_2$: C, 82.44; H, 10.90.
Found : C, 82.04; H, 11.00.

Grignard reaction on oxaziridine 4 and oxidation

To a stirred solution of 4 (0.168 g, 0.31 mmol) in 10 mL of dry ether was added dropwise, under nitrogen blanket, 3.5 equivalents of methyl magnesium iodide (0.182 g, in 2 mL of dry ether) at room temperature. The clear solution initially turned intense yellow and then colourless with white turbidity. The reaction was monitored by TLC. After 2.5 h of stirring at room temperature, the reaction mixture was quenched by dropwise addition of saturated NH_4Cl solution. The ether layer was

separated and the aqueous layer extracted with ether (3 x 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4. Concentration of the organic extract under vacuum afforded a semisolid product (0.128 g, 74%). This product was found to be identical with the product 6 obtained by the Grignard reaction of nitrone 5. The crude product was taken up in MeOH (5 mL) in presence of $Cu(OAc)_2.H_2O$ as catalyst. The solution was stirred for 2 h. The reaction mixture was extracted with CH_2Cl_2 (3 x 5 mL) after saturation with brine. The organic extracts were dried over anhydrous $MgSO_4$ and solvent removed to give a yellow semisolid product. Purification of the crude product by column chromatography on silica gel using 6% ethylacetate-petroleum ether (b.pt. 60-80°C) as eluant yielded yellow crystalline product, 7 (0.101 g, 58.4%). M.pt. : 81°C. The spectral data of this compound were identical to those reported earlier for 7.

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