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Design and synthesis of new water soluble forskolin derivatives for positive inotropic activity

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A new class of forskolin derivatives have been synthesized based on 2- hydroxy ethyl spacer and 2-dialkylaminomethyl chain had incorporated for promoting water solubility. The synthetic strategy involves the regiospecific epoxide ring opening reaction with HCl in acetonitrile or by secondary amine. None of the compounds shows interesting biological activity.

During our earlier structure activity relationship studies on forskolin derivatives, we had shown that positive inotropic activity of forskolin could be improved by hydroxyacetyl spacer at 6β- or 7β-position of forskolin[1] (**Figure 1**). Considering this idea we had developed highly potent water soluble orally active forskolin analogs[2]. However one of the major difficulty associated with these compounds was their unstability in aqueous solution.

To overcome this shortcoming we wanted to replace hydroxyacetyl group by far more stable hydroxy alkyloxy group as 'spacer'. Therefore, we adopted a strategy to synthesize new forskolin analogs where the 7β- acetyl group could be shifted away from the ring by the addition of 2-hydroxyethyloxy chain as shown by the bold line in **Figure 2**. To retain the earlier idea of having stability and water solubility, we had chosen aminoalkyl chain as one of the appendages as shown in dotted line in **Figure 2**. In this paper we would like to describe the synthetic strategy for such compounds and the influence of these changes on biological activity.

Chemistry

The suitable synthetic route for the synthesis of such compounds could be the ring opening reaction on epoxide **1**[3] as shown in the **Scheme 1**. Initially we had chosen the ring opening reaction with HCl in organic solvents to generate chlorohydrin[4]. The regioselectivity of this reaction is largely dependent upon the nature of the substituent present on epoxide[5–8]. However, when epoxide on terminal double bond is opened, the chlorine usally gets attached to the terminal position. Such observation could be found in the literature where ethyl 3,4-epoxybutyrate gave 3-chloro-2-hydroxybutyrate as the major product[9]. Since our system is close to that reported in the literature, we could expect a similar reaction to generate the key intermediate. Thus, when **1** was reacted with 12% aqueous HCl in acetonitrile we could get the required chlorohydrin **3** in a regioselective manner with concomitant loss of 1α-silyl group. The ^{1}H NMR spectrum indicated the correct regiochemistry of compound **3**. Still it was necessary to prove beyond doubt the correct isomer before continuing further. Therefore, we chose another substrate **2**[3] to get a less complex ^{1}H NMR spectrum. Thus, **2** had reacted with 12% aqueous HCl in acetonitrile as described earlier to give compound **4** which showed a doublet at δ 3.66 corresponding to C*H*$_2$Cl, a one-proton multiplet around 3.62-3.96 assigned to C*H*(OH) and two sets of multiplets at δ 3.98-4.23 integrating to two protons which were due to the protons of CH_2 attached to 7β-position. These signals indicate that chlorine got attached to the terminal position. This was further confirmed by acylation of the secondary hydroxyl group by reacting **4** with formic or acetic acid in the presence of DCC-DMAP (**Scheme I**) to yield **5a** and **5b** respectively. Analysis of ^{1}H NMR data of **5a** and **5b** showed a down-field shift of the proton due to attachment of electron-withdrawing formyl or acetyl group at 2′-CH(OH)- position. In **5a** it

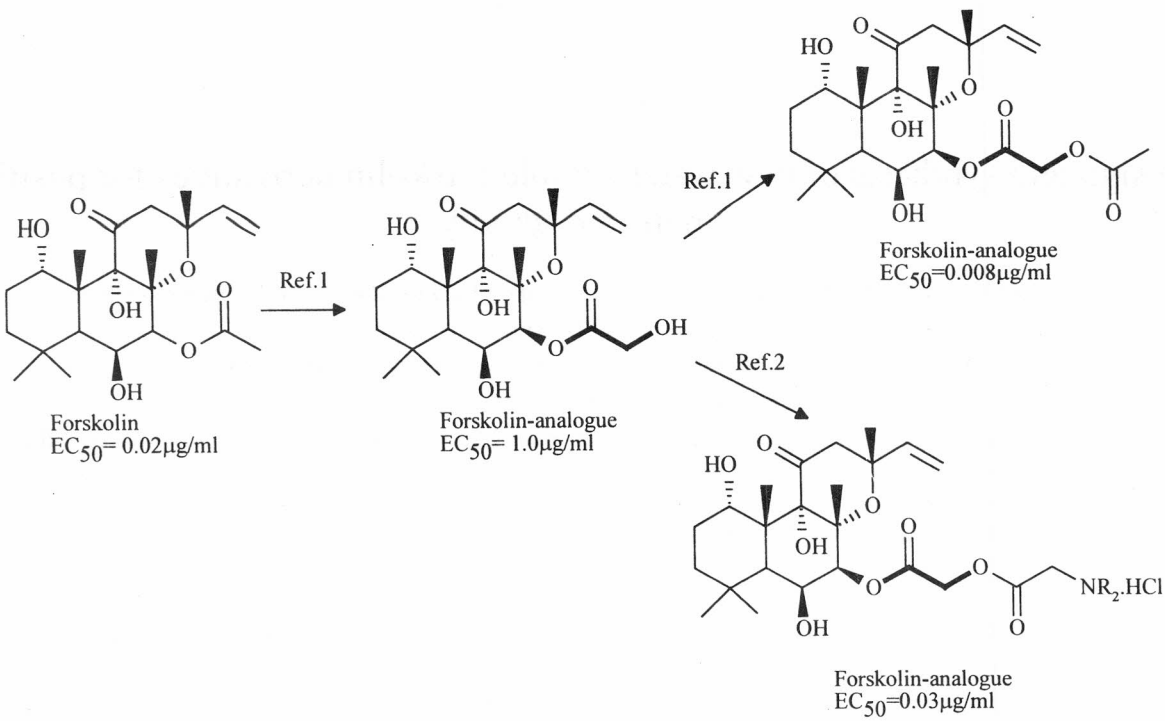


Fig 1: In vitro positive inotropic activity of forskolin and some of its analogs synthesized earlier based on 2-hydroxy acetyl "spacer"[1,2]

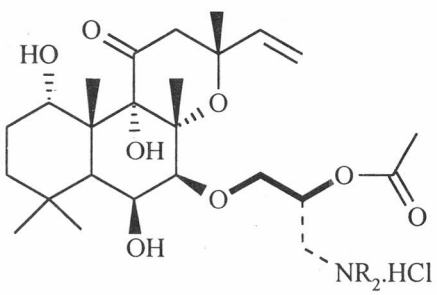
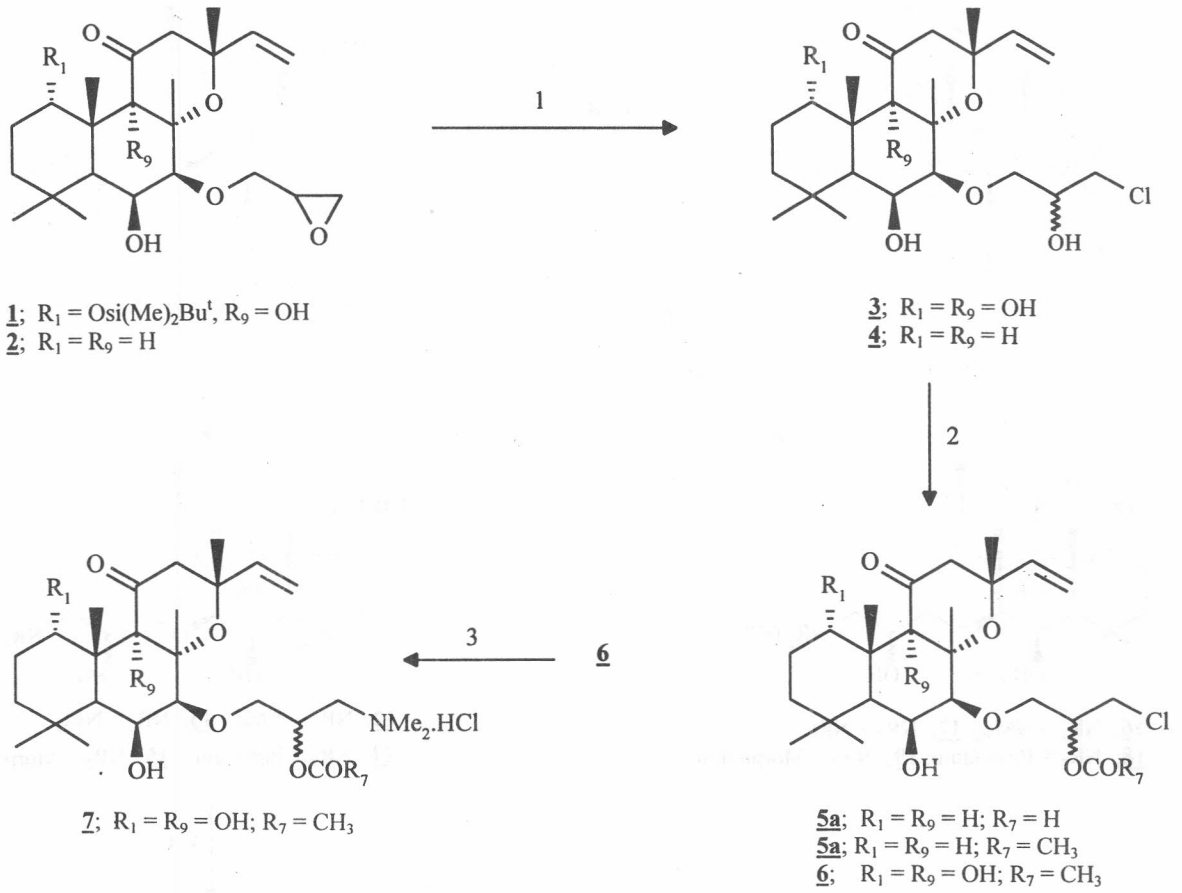


Fig. 2

appeared at δ 5.26-5.46 and at δ 5.06-5.34 in **5b** as multiplets corresponding to one proton. The doublet of CH_2Cl protons appeared at δ 3.82 for **5a** and at 3.83 for **5b** respectively. We have also observed well separated jeminal coupling for OCH_2 protons attached to 7β-position (see Experimental). Coming back to original substrate when **3** had reacted with AcOH in the presence of DCC-DMAP, product **6** was obtained as shown in **Scheme I**. The ^1H NMR of this compound also showed a substantial down-field shift of C*H*(OAc) proton at δ 5.11-5.31 in comparison to δ 3.71-4.13 for C*H*(OH) proton observed for compound **3**. The doublet for CH_2Cl appeared at δ 3.77 which was very close to the value observed for **3** (δ 3.61). These data clearly established the structure of **3**. The key intermediate **6** was then utilized to get the target compounds by replacing chlorine by different amines. Thus, **6** was reacted with dimethyl amine in toluene-dichloromethane at room temperature to get **7** in poor yield. The poor yield was due to side reactions including hydrolysis of the acetyl group. The other alternative strategy could be to replace Cl by amine in **3** followed by acetylation. This strategy was not pursued due to possibility of side reactions such as epoxidation as well as acetylation at 1α-position. The later reaction contributes significantly when the terminal position contains a bulky group (unpublished result). In the meantime we were looking for an alternative approach which involved opening of the epoxide ring with the required amine with 1α-protection intact, followed by acetylation. The final compound could be obtained by silyl deprotection. Thus, compound **1** was treated with different secondary amines in CH_2Cl_2 at room temperature to obtain **8** to **11** (**Scheme II**) in excellent yields. Compounds **8-11**-were converted to the acetyl derivatives **12-15** by the reaction of acetic acid in the presence of DCC-DMAP as described earlier.



1; $R_1 = Osi(Me)_2Bu^t$, $R_9 = OH$
2; $R_1 = R_9 = H$

3; $R_1 = R_9 = OH$
4; $R_1 = R_9 = H$

7; $R_1 = R_9 = OH$; $R_7 = CH_3$

5a; $R_1 = R_9 = H$; $R_7 = H$
5a; $R_1 = R_9 = H$; $R_7 = CH_3$
6; $R_1 = R_9 = OH$; $R_7 = CH_3$

Scheme I. 1. 12 % aq.HCl, CH_3CN, room temperature. 2. Acetic acid, DCC, DMAP, EtOAc, room temperature. 3. Me_2NH in toluene, CH_2Cl_2, room temperature.

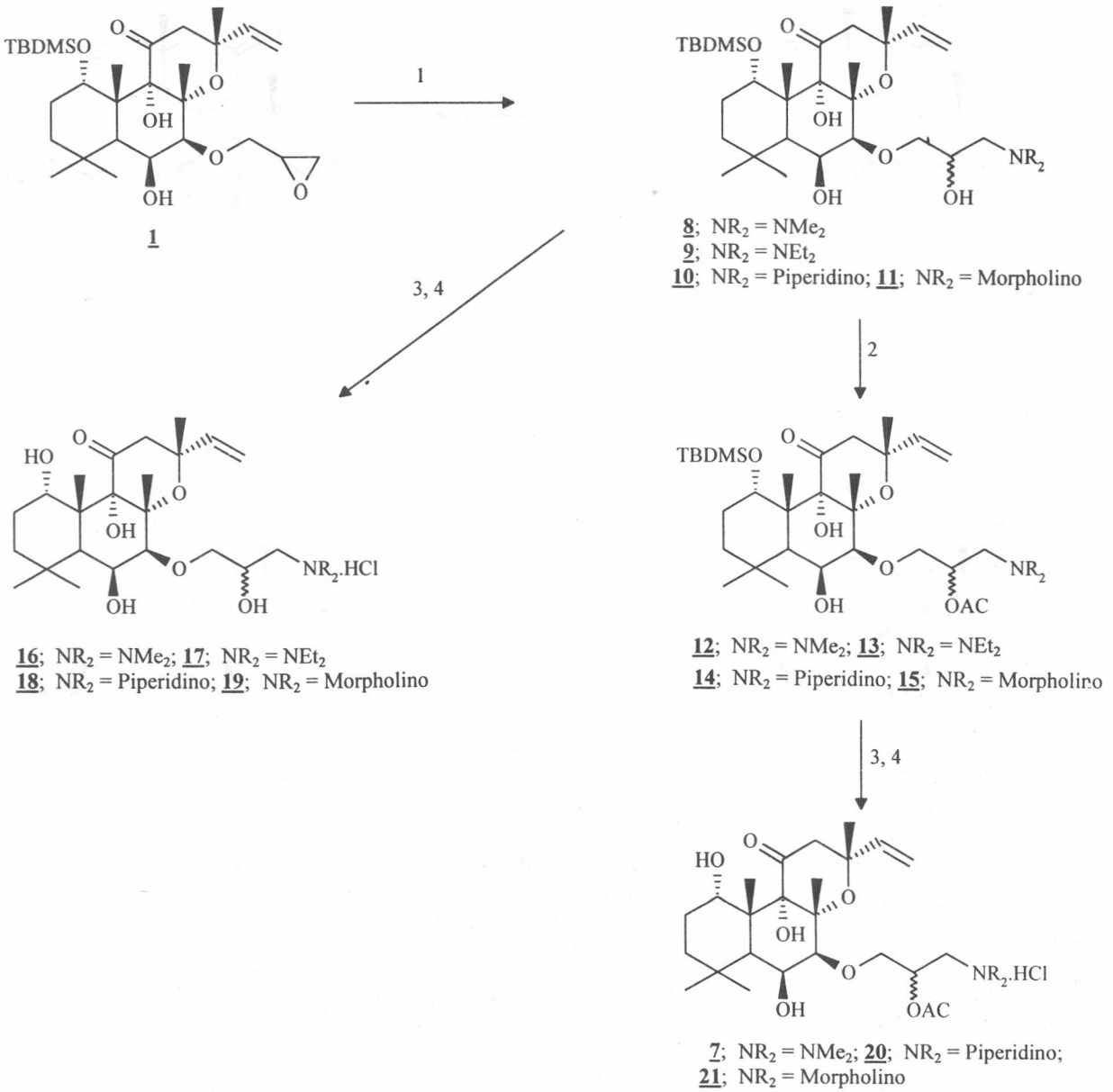
Once again the regiospecific ring opening could be established by the down-field shift of proton attached to the secondary alcohol after acetylation (see ^1H NMR values in Experimental Section).The deprotection of silyl group[10] from **8-11** gave the final compounds **16-19** with free hydroxy at 7β side chain and **12**, **14**, **15** were converted to **7**, **20** and **21** respectively as shown in **Scheme II**.

All the compounds were found to be readily soluble in water. The aqueous solution did not show any decomposition after keeping at room temperature for 24 hr. All these compounds, having free hydroxy or acetoxy group at 7β-side chain, were tested for positive inotropic and blood pressure lowering activity. None of the compounds however, showed any interesting biological activity. This clearly demonstrated that for retaining biological activity, one needs an acyl (OCOR) functionality directly attached to the 7β-position. Conversion of acyl to alkyl chain almost demolishes the biological activity.

In conclusion, we have completed the synthesis of a new class of water soluble forskolin analogs with 2-hydroxyethyloxy 'spacer'. This was achieved through a regioselective epoxide ring opening. This modification carried out on forskolin however failed to retain the biological activity of forskolin.

Experimental Section

General. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. IR spectra were determined on a Perkin-Elmer 157 spectrophotometer as KBr film unless otherwise mentioned. ^1H NMR spectra were recorded in $CDCl_3$ unless otherwise mentioned on a JEOL FT-90 spectrometer with TMS as internal standard (chemical shifts in δ, ppm and coupling constant



Scheme II : 1. R_2NH, CH_2Cl_2, r.t. 2. AcOH, DCC, DMAP, EtOAc, r.t. 3. 1M $nBu_4N^+F^-$, THF, r.t. 4. HCl-ether, EtOAc.

values in Hz). Petroleum ether refers to the fraction of bp 60-80 °C. For flash column chromatography silica gel (finer than 0.08 mm particle size) was used. Precoated (silica gel 60 F_{254}) TLC plates were used for checking purity of the compounds. Vaniline-50% orthophosphoric acid or anisaldehide-H_2SO_4 spray reagent was used and the plates were heated at 110°C for visualization. All compounds were homogeneous on TLC and gave proper spectral characteristics.

7β-(3-Chloro-2-hydroxypropyloxy)-8,13-epoxy-1α,6β,9α-trihydroxy-labd-14-en-11-one 3. Compound **1** (1.4 g; 2.6 mmol) was dissolved in CH_3CN (55 ml) and 12% aqueous HCl (5.5 mL) was added. The reaction mixture was stirred at room temperature for 2 h. Solvent was removed after neutralizing excess HCl by adding $NaHCO_3$ solution. The residue was extracted with EtOAc (3×10 mL). The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed and the residue purified by flash chromatography using 10%

CH_3CN in $CHCl_3$ as eluents yield, 1.08 g (83.5%), semisolid; IR (KBr): 3500-3400 (br), 2950 (br), 1720 cm^{-1}; ^1H NMR: 1.09, 1.29, 1.41, 1.44, 1.67 (5×s, 15H, 5×C*H*$_3$), 2.07 (d, 1H, *J*=2.53, 5-H), 2.48, 2.49 (2xd*, 1H, J_{gem}=16.2, 12-βH), 3.18, 3.19 (2×d*, 1H, J_{gem}=16.2, 12-αH), 3.61 (t, 2H, *J*=5.06, C*H*$_2$Cl), 3.71-4.13 [m, 4*H*, 7*H* and OC*H*$_2$C*H*(OH)CH$_2$Cl], 4.43 (dd, 1H, *J*=3.04, 2.53, 6-H), 4.61 (br, 1H, 1-H), 4.99 (dd, 1H, J_{cis}=11.14, J_{gem}=1.62, 15-H$_{cis}$), 5.16 (dd, 1H, J_{trans}=17.6, J_{gem}=1.62, 15-*H*$_{trans}$), 6.12, 6.14 (2×dd*, 1H, J_{trans}=17.6, J_{cis}=11.14, 14-H), 6.56,6.69 (2×br, 2H, exchangable, 2×*OH*). Anal. Calcd for $C_{23}H_{37}O_7Cl$: C, 59.92; H, 8.09; Cl, 7.69. Found: C, 59.79; H, 7.93; Cl, 7.58% (* due to diastereoisomers).

7β-(3-Chloro-2-hydroxypropyloxy)-8,13-epoxy-6β-hydroxy-labd-14-en-11-one 4. This compound was prepared according to the method described above starting from **2**. The crude product was purified by flash chromatography using 5% CH_3CN in $CHCl_3$ as eluent, yield 85.8%, semisolid; IR (KBr): 3500 (br), 3420 (br), 2980 (br), 1720 cm^{-1}; ^1H NMR: 0.79 (d, 1H, *J*=2.03, 5-H), 1.01, 1.24, 1.36, 1.41, 1.57 (5×s, 15H, 5×CH$_3$), 2.63 (s, 1H, 9-H), 2.69 (s, 2H, 12-H$_2$), 3.45 (d, 1H, *J*=4.05, 7-H), 3.66 (d, 2H, *J*=5.06, CH$_2$Cl), 3.62-3.96 [m, 1H, –C*H*(OH)], 3.98-4.23 [2×m, 2H, OC*H*$_2$CH(OH)], 4.36 (dd, 1H, *J*=4.05, 2.03, 6-H), 5.06 (dd, 1H, J_{cis}=11.14, J_{gem}=1.02, 15-H$_{cis}$), 5.13 (dd, 1H, J_{trans}=17.2, J_{gem}=1.02, 15-H$_{trans}$), 5.96 (dd, 1H, J_{trans}=17.2, J_{cis}=11.14, 14-H); Anal. Calcd for $C_{23}H_{37}O_5Cl$: C, 64.39; H, 8.69; Cl, 8.26. Found: C, 64.31; H, 8.65; Cl, 8.12%.

7β-(3-Chloro-2-formyloxypropyloxy)-8,13-epoxy-6β-hydroxy-labd-14-en-11-one 5a. Compound **4** (0.215 g, 0.5 mmol) and DCC (0.138 g, 0.67 mmol) were dissolved in EtOAc (10 mL). Formic acid (0.023 mL, 0.61 mmol) was added to the vigorously stirred solution at room tempetature followed by DMAP (0.1 g; 0.82 mmol). After stirring for 3 h at room temperature, the reaction mixture was kept in the freezer overnight. The DCU was filtered off and the filtrate washed with brine. It was dried over anhydrous Na_2SO_4.The residue after removing solvent was purified by flash chromatography using 15% EtOAc in light petroleum as eluent. It was crystallized from EtOAc-light petroleum, yield 0.19 g (83%); mp, 95-98 °C; IR (KBr): 3530 (br), 2940 (br), 1735, 1710 cm^{-1}; ^1H NMR: 0.91 (d, 1H, *J*=2.03, 5-H), 1.0, 1.23, 1.30, 1.40, 1.51 (5×s, 15H, 5×CH$_3$), 1.97 (s, 1H, exchangable, OH), 2.57 (s, 1H, 9-H), 2.61 (s, 2H, 12-H$_2$), 3.39 (d, 1H, *J*=4.05, 7-H), 3.82 (d, 2H, *J*=5.05, CH$_2$Cl), 3.99, 4.19 [2×t, 2H, J_{gem}=11.14, *J*=5.05, OC*H*$_2$CH(OCHO)], 4.34 (dd, 1H, *J*=4.05, 2.03, 6-H), 5.03 (dd, 1H, J_{cis}=10.13, J_{gem}=1.82, 15-H$_{cis}$), 5.14 (dd, 1H, J_{trans}=17.2, J_{gem}=1.82, 15-H$_{trans}$), 5.26-5.46 [m, 1H, C*H*(OCHO)], 5.95 (dd, 1H, J_{trans}=17.2, J_{cis}=11.13, 14H), 8.11 [s, 1H, CH(OC*H*O)]; Anal. Calcd for $C_{24}H_{37}O_6Cl$: C, 63.07; H, 8.16; Cl, 7.76. Found: C, 62.88; H, 8.16; Cl, 7.93%.

7β-(2-Acetoxy-3-chloropropyloxy)-8,13-epoxy-6β-hydroxy-labd-14-en-11-one 5b..This compound was prepared from **4** by the same method as described above using AcOH in place of formic acid, yield 85.7%; mp, 99-101 °C (EtOAc-light petroleum); IR (KBr): 3500 (br), 2970 (br), 2940, 1750, 1730 (br) cm^{-1}; ^1H NMR: 0.79 (d, 1H, *J*=2.53, 5-H), 1.0, 1.23, 1.31, 1.41, 1.51 (5×s, 15H, 5×CH$_3$), 2.14 (s, 3H, COC*H*$_3$), 2.56 (s, 1H, 9-H), 2.63 (s, 2H, 12-H$_2$), 3.39 (d, 1H, *J*=4.05, 7-H), 3.83 (d, 2H, *J*=5.06, CH$_2$Cl), 3.93, 4.14 [2×t, 2H, J_{gem}=10.13, *J*=5.67, OC*H*$_2$CH(OAc)], 4.33 (dd, 1H, *J*=4.05, 2.53, 6-H), 5.02 (dd, 1H, J_{cis}=11.14, J_{gem}=1.82, 15-H$_{cis}$), 5.15 (dd, 1H, J_{trans}=17.2, J_{gem}=1.82, 15-H$_{trans}$), 5.06-5.34 [m, 1H, C*H*(OAc)], 5.96 (dd, 1H, J_{trans}=17.2, J_{cis}=11.14, 14-H). Anal. Calcd for $C_{25}H_{39}O_6Cl$: C, 63.75; H, 8.35; Cl, 7.53. Found: C, 62.84; H, 8.47; Cl, 7.62%.

7β-(2-Acetoxy-3-chloropropyloxy)-8,13-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-one 6. This compound was prepared from **3** and AcOH by the method described for the synthesis of compound **4**. The crude product was purified by flash chromatography using 8% CH_3CN in $CHCl_3$, yield 93.25%, semisolid; IR (KBr): 3500-3400 (br), 2955 (br), 1745, 1725 cm^{-1}; ^1H NMR: 1.07, 1.29, 1.43 (6H), 1.63 (4×s, 15H, 5×CH$_3$), 2.07 (br, 1H, 5-H), 2.13 (s, 3H, COC*H*$_3$), 2.44 (d, 1H, J_{gem}=16.2, 12-βH), 3.11 (d, 1H, J_{gem}=16.2, 12-αH), 3.77 (m, 3-H, 7-H and C*H*$_2$Cl), 3.86-4.03 [m, 2H, OC*H*$_2$CH(OAc)-], 4.41 (t, 1H, *J*=3.04, 6-H), 4.54 (br, 1H, 1-H), 4.96 (dd, 1H, J_{cis}=11.14, J_{gem}=1.82, 15-H$_{cis}$), 5.16 (dd, 1H, J_{trans}=17.21, J_{gem}=1.82, 15-H$_{trans}$), 5.11-5.31 [m, 1H, C*H*(OAc)], 6.04 (dd, 1H, J_{trans}=17.21, J_{cis}=11.14, 14-H), 6.33 (br, 1H, exchangable, OH). Anal. Calcd for $C_{25}H_{39}O_8Cl$: C, 59.69; H, 7.81; Cl, 7.05. Found: C, 59.51; H, 7.78; Cl, 7.15%.

7β-(2-Acetoxy-3-dimethylaminopropyloxy)-8,13-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-one 7. Compound **6** (0.15 g, 0.3 mmol) was dissolved in CH_2Cl_2 (2 mL) and a saturated solution of dimethyl amine in toluene (0.2 mL) was added to it. The reaction mixture was stirred at room temperature for 12 h. Solvent was removed and the residue purified by flash chromatography using 5% MeOH in $CHCl_3$ as eluent. The pure product was converted to hydrochloride salt and recrystallized from MeOH-dry ether, yield 0.045 g (29.4%), mp, 147-52 °C; IR (KBr): 3400-3300 (br), 2950 (br), 1755 (br), 1718 cm^{-1}; ^1H NMR: 1.07, 1.29, 1.43 (6-H), 1.64 (4×s, 15H, 5×CH_3), 2.04 (d, 1H, J=2.1, 5-H), 2.24 (s, 3H, $COCH_3$), 2.38 (d, 1H, J_{gem}=16.2, 12-βH), 2.89, 2.93 [2×s, 6H, $N(CH_3)_2$], 3.24 (d, 1H, J_{gem}=16.2, 12-αH), 3.44 (m, 2H, $-CH_2Cl$), 3.79 (d, 1H, J=4.05, 7-H), 3.91 [m, 2H, $OCH_2CH(OAc)$], 4.47 (br, 2H, 1-H and 6-H), 4.94 (dd, 1H, J_{cis}=11.1, J_{gem}=1.2, 15-H_{cis}), 5.13 (dd, 1H, J_{trans}=17.2, J_{gem}=1.8, 15-H_{trans}), 5.33 [m, 1H, $-CH(OAc)$], 6.09 (dd, 1H, J_{trans}=17.2, J_{cis}=11.1, 14-H). Anal. Calcd for $C_{27}H_{46}NO_8Cl$. 0.5 H_2O: C, 58.21; H, 8.49; N, 2.51; Cl, 6.36. Found: C, 58.08; H, 8.66; N, 2.39; Cl, 6.44%.

6β,9α-Dihydroxy-7β-(3-dimethylamino-2-hydroxypropyloxy)-8,13-epoxy-1α-terti-arybutyldimethylsilyloxylabd-14-en-11-one 8. Compound **1** (1.07 g; 2 mmol) was dissolved in CH_2Cl_2 (10 mL) and a saturated solution of dimethyl amine in toluene (2.5 mL) was added to it. The reaction mixture was stirred at room temperature for 12 hr. Solvent was removed and the residue purified by flash chromatography using 5% MeOH in $CHCl_3$ as eluent. The pure product was crystallized from EtOAc-light petroleum, yield 0.86 g (73.8%), mp, 155-57 °C; IR (KBr): 3520, 3360, 2980, 1725 (br), cm^{-1}; ^1H NMR: 0.05, 0.16 [2×s, 6H, $Si(CH_3)_2$], 0.97 [s, 9H, $SiC(CH_3)_3$], 1.07, 1.28, 1.44 (6H), 1.61 (4×s, 15H, 5×CH_3), 2.07 (d, 1H, J=3.04, 5H), 2.39 (d, 1H, J_{gem}=16.2, 12-βH), 2.44 [s, 6H, $N(CH_3)_2$], 2.43-2.63 (m, 2H, CH_2Cl), 3.21 (d, 1H, J_{gem}=16.2, 12-αH), 3.66, 3.94 (2×dd, 1H, J_{gem}=10.2, J=5.06, $OCH_2CH(OH)$-], 3.80-4.11 [m, 2H, 7-H and $-CH(OH)$], 4.46 (t, 1H, J=3.04, 6-H), 4.60 (br, 1H, 1-H), 4.93 (dd, 1H, J_{cis}=10.13, J_{gem}=1.6, 15-H_{cis}), 5.09 (dd, 1H, J_{trans}=17.2, J_{gem}=1.6, 15-H_{trans}), 6.18 (dd, 1H, J_{trans}=17.2, J_{cis}=10.13, 14-H). Anal. Calcd for $C_{31}H_{57}NO_7Si$, H_2O: C, 61.84; H, 9.88; N, 2.33. Found: C, 61.71; H, 9.59; N, 2.25%.

Compounds **9-11** were prepared by the same method using **1** and the appropriate amine in CH_2Cl_2 at room temperature.

6β,9α-Dihydroxy-7β- (3-diethylamino-2-hydroxypropyloxy) -8,13-epoxy-1α-tertiary-butyldimethylsilyloxy-labd-14-en-11-one 9. Compound **1** was treated with diethyl amine as described for **8**. The crude product was purified by flash chromatography using 5% MeOH in $CHCl_3$ as eluent. The pure product was crystallized from EtOAc-light petroleum, yield 96.6%; mp, 157-60 °C; IR (KBr): 3340 (br), 2980, 2950, 1722 cm^{-1}; ^1H NMR: 0.03, 0.13 [2×s, 6H, $Si(CH_3)_2$], 0.89 [s, 9H, $SiC(CH_3)_3$], 1.06, 1.27 (2×s, 6H, 2×CH_3), 1.28 (t, 6H, J=8.6, 2×NCH_2CH_3), 1.43 (s, 6H, 2×CH_3), 1.60 (s, 3H, CH_3), 2.34 (d, 1H, J=3.04, 5-H), 2.36 (d, 1H, J_{gem}=16.2, 12-βH), 3.07 (m, 6H, 2×NCH_2CH_3 and $-CH_2NEt_2$), 3.20 (d, 1H, J_{gem}=16.2, 12-αH), 3.51-4.17 [m, 4H, 7-H, $-OCH_2CH(OH)-$], 4.43 (t, 1H, J=3.04, 6-H), 4.57 (br, 1H, 1-H), 4.91 (dd, 1H, J_{cis}=10.13, J_{gem}=1.6, 15-H_{cis}), 5.06 (dd, 1H, J_{trans}=17.2, J_{gem}=1.6, 15-H_{trans}), 6.13 (dd, 1H, J_{trans}=17.2, J_{cis}=10.13, 14-H). Anal. Calcd for $C_{33}H_{61}NO_7Si$: C, 64.77; H, 10.05; N, 2.29. Found: C, 64.61; H, 9.95; N, 2.12%.

6β,9α-Dihydroxy-8,13-epoxy-7β-(3-piperidino-2-hydroxypropyloxy) -1α-tertiarybutyldimethylsilyloxylabd-14-en-11-one 10. Compound **1** was reacted with piperidine in dichloromethane as described for the synthesis of **8**. The crude product was purified by flash chromatography using 5% MeOH in $CHCl_3$ as eluent. The pure product was crystallized from EtOAc-light petroleum.Yield 90.9%; mp, 205-208 °C; IR (KBr): 3330 (br), 2960, 1732 cm^{-1}; ^1H NMR: 0.01, 0.14 [2×s, 6H, $Si(CH_3)_2$], 0.86 [s, 9H, $SiC(CH_3)_3$], 1.06, 1.26, 1.40 (6H), 1.60 (4×s, 15H, 5×CH_3), 2.05 (d, 1H, J=2.5, 5-H), 2.37 (d, 1H, J_{gem}=16.2, 12-βH), 2.70-2.94 (m, 4H, $-CH_2NCH_2-$), 3.18 (d, 1H, J_{gem}=16.2, 12-αH), 3.34-4.23 [m, 6H, 7-H and $-OCH_2CH(OH)-CH_2N$], 4.43 (t, 1H, J=2.5, 6-H), 4.59 (br, 1H, 1-H), 4.91 (dd, 1H, J_{cis}=11.14, J_{gem}=1.6, 15-H_{cis}), 5.07 (dd, 1H, J_{trans}=17.2, J_{gem}=1.6, 15-H_{trans}), 6.14 (dd, 1H, J_{trans}=17.2, J_{cis}=11.14, 14-H). Anal. Calcd for $C_{34}H_{61}NO_7Si$: C, 65.45; H, 9.86; N, 2.24. Found: C, 65.29; H, 9.69; N, 2.12%.

6β,9α-Dihydroxy-8,13-epoxy-7β-(3-morpholino-2-hydroxypropyloxy)-1α-tertiarybutyldimethylsilyloxylabd-14-en-11-one 11. This was prepared by reacting **1** with morpholine. The crude product was

purified by flash chromatography using 5% MeOH in $CHCl_3$ as eluent, yield 94.8% (semisolid); IR (KBr): 3450 (br), 3330 (br), 2970, 1730 cm^{-1}; ^1H NMR: 0.04, 0.13 [2×s, 6H, $Si(CH_3)_2$], 0.89 [s, 9H, $SiC(CH_3)_3$], 1.06, 1.26, 1.41 (6H), 1.60 (4×s, 15H, 5×CH_3), 2.07 (d, 1H, J=3.04, 5-H), 2.38 (d, 1H, J_{gem}=16.2, 12-βH), 2.41-2.69 [m, 6H, –CH(OH)CH_2N– and CH_2NCH_2], 3.21 (d, 1H, J_{gem}=16.2, 12-αH), 3.54-4.0 [m, 8H, 7-H, –OCH_2CH(OH) and CH_2OCH_2], 4.44 (t, 1H, J=3.04, 6-H), 4.59 (br, 1H, H-1), 4.91 (dd, 1H, J_{cis}=10.13, J_{gem}=1.6, 15-H_{cis}), 5.07 (dd, 1H, J_{trans}=17.2, J_{gem}=1.6, 15-H_{trans}), 6.14 (dd, 1H, J_{trans}=17.2, J_{cis}=10.13, 14-H). Anal. Calcd for $C_{33}H_{59}NO_8Si$: C, 63.32; H, 9.50; N, 2.24; Found: C, 63.23; H, 9.69; N, 2.09%.

7β-(2-Acetoxy-3-dimethylaminopropyloxy)-6β,9α-dihydroxy-8,13-epoxy-1α-tertiarybutyldimethylsilyloxylabd-14-en-11-one 12. The compound **8** (0.86 g; 1.47 mmol) and DCC (0.72 g, 3.5 mmol) were dissolved in EtOAc (10 mL) and AcOH (0.17 mL; 2.97 mmol) was added under vigorous stirring at room temperature. After 5 min. DMAP (0.363 g, 3 mmol) was added and stirring continued for 4 h. DCU was filtered off and the filtrate washed with brine. The EtOAc layer was dried over anhydrous Na_2SO_4 and solvent removed. The residue was purified by flash chromatography using 20% CH_3CN in $CHCl_3$ as eluent, yield 0.822 g (89.2%), (semisolid); IR (KBr): 3330 (br), 2965, 2945, 1750 (br) 1725 cm^{-1}; ^1H NMR: 0.01, 0.14 [2×s, 6H, $Si(CH_3)_2$], 0.89 [s, 9H, $SiC(CH_3)_3$], 1.06, 1.26, 1.39, 1.43, 1.57 (5×s, 15H, 5×CH_3), 2.08 (br, 1H, J=3.04, 5-H), 2.09 (s, 3H, COCH_3), 2.31 [s, 6H, $N(CH_3)_2$], 2.33 (d, 1H, J_{gem}=16.2, 12-βH), 2.57 [m, 2H, –CH_2NMe_2), 3.26 (d, 1H, J_{gem}=16.2, 12-αH), 3.66-4.14 [m, 3H, 7-H, –OCH_2CH(OH)], 4.43 (m, 1H, 6-H), 4.56 (br, 1H, 1-H), 4.90 (dd, 1H, J_{cis}=10.13, J_{gem}=1.7, 15-H_{cis}), 5.09 (dd, 1H, J_{trans}=17.2, J_{gem}=1.7, 15-H_{trans}), 5.0-5.29 [m, 1H, CH(OAc)], 6.09 (dd, 1H, J_{trans}=17.2, J_{cis}=10.13, 14-H). Anal. Calcd for $C_{33}H_{59}NO_8Si$: C, 63.32; H, 9.50; N, 2.24. Found: C, 63.16; H, 9.61; N, 2.06%.

Compounds **13-15** were prepared according to the procedure described above using the starting materials **9-11**.

7β-(2-Acetoxy-3-diethylaminopropyloxy)-6β,9α-dihydroxy-8,13-epoxy-1α-tertiarybutyldimethylsilyloxylabd-14-en-11-one 13. This compound was prepared by reacting **9** with AcOH by DCC-DMAP method described for the synthesis of compound **12**. The residue was purified by flash chromatography using 20% CH_3CN in $CHCl_3$ as eluent, yield 88% (EtOAc-light petroleum), mp, 76-78 °C; IR (KBr): 3340 (br), 2955, 1753 (br), 1725 cm^{-1}; ^1H NMR: 0.06, 0.11 [2×s, 6H, $Si(CH_3)_2$], 0.90 [s, 9H, $SiC(CH_3)_3$], 1.02 (t, 9H, J=7.09, 2×CH_2CH_3 and CH_3), 1.29, 1.40, 1.44, 1.57 (4×s, 12H, 4×CH_3), 2.09 (s, 3H, COCH_3), 2.10 (br, 1H, 5-H), 2.33 (d, 1H, J_{gem}=16.2, 12-βH), 2.59 (quartet, 4H, J=7.09, 2×NC$H_2$$CH_3$), 2.60 (br, 2H, –C$H_2NEt_2$), 3.27 (d, 1H, J_{gem}=16.2, 12-αH), 3.69, 3.99 [2xdd, 2H, J_{gem}=9.1, J=5.06 –OCH_2CH(OH)], 3.76 (d, 1H, J=4.05, 7-H), 4.41 (dd, 1H, J=4.05 and 3.04, 6-H), 4.56 (br, 1H, 1-H), 4.89 (dd, 1H, J_{cis}=11.1, J_{gem}=1.7, 15-H_{cis}), 5.09 (dd, 1H, J_{trans}=17.2, J_{gem}=1.7, 15-H_{trans}), 4.99-5.16 [m, 1H, –CH(OAc)], 6.07 (dd, 1H, J_{trans}=17.2, J_{cis}=11.1, 14-H). Anal. Calcd for $C_{35}H_{63}NO_8Si$: C, 66.09; H, 9.71; N, 2.14. Found: C, 65.89; H, 9.97; N, 1.95%.

7β-(2-Acetoxy-3-piperidinopropyloxy) -6β,9α-dihydroxy-8,13-epoxy-1α-tertiarybutyldimethylsilyloxylabd-14-en-11-one 14. Compound **14** was prepared by reacting **10** with AcOH by DCC-DMAP method described for the synthesis of compound **12.** The residue was purified by flash chromatography using 20% CH_3CN in $CHCl_3$ as eluent, yield 94%, semisolid; IR (Neat): 3330 (br), 2950, 1745, 1720 (br) cm^{-1}; ^1H NMR: 0.01, 0.13 [2×s, 6H, $Si(CH_3)_2$], 0.86 [s, 9H, $SiC(CH_3)_3$], 1.04, 1.26, 1.39, 1.43, 1.56 (5×s, 15H, 5×CH_3), 1.20-2.0 [m, 10H, 2-CH_2, 3-CH_2, $NCH_2(CH_2)_3$-], 2.07 (br, 4H, COCH_3 and 5-H), 2.31 (d, 1H, J_{gem}=16.2, 12-βH), 2.31-2.63 [m, 6H, –CH(OAc)CH_2N– and –CH_2NCH_2–], 3.24 (d, 1H, J_{gem}=16.2, 12-αH), 3.63, 3.94 (m, 3H, 7-H and –OCH_2CH(OH)], 4.44 (br, 1H, 6-H), 4.53 (br, 1H, 1-H), 4.89 (dd, 1H, J_{cis}=11.1, J_{gem}=1.8, 15-H_{cis}), 5.06 (dd, 1H, J_{trans}=17.2, J_{gem}=1.8, 15-H_{trans}), 5.18 [m, 1H, –CH(OAc)], 6.10 (dd, 1H, J_{trans}=17.2, J_{cis}=11.1, 14-H). Anal. Calcd for $C_{36}H_{63}NO_8Si$: C, 64.92; H, 9.54; N, 2.10. Found: C, 64.97; H, 9.70; N, 1.99%.

7β-(2-Acetoxy-3-morpholinopropyloxy)-6β,9α-dihydroxy-8,13-epoxy-1α-tertiarybutyldimethylsilyloxylabd-14-en-11-one 15. Compound **15** was prepared from **11** using the same method as described for the synthesis of **12**. The residue was purified by flash chromatography using 10% CH_3CN in $CHCl_3$ as eluent, yield 89.8%, semisolid; IR (Neat): 3430 (br), 2950, 1750, 1725

(br) cm^{-1}; ^1H NMR: 0.01, 0.13 [2×s, 6H, $Si(CH_3)_2$], 0.86 [s, 9H, $SiC(CH_3)_3$], 1.04, 1.26, 1.37, 1.41, 1.54 (5×s, 15H, 5×CH_3), 2.06 (br, 4H, $COCH_3$ and 5-H), 2.31 (d, 1H, J_{gem}=16.2, 12-βH), 2.23-2.63 [m, 6H, –CH(OAc)CH_2– and –CH_2NCH_2–], 3.26 (d, 1H, J_{gem}=16.2, 12-αH), 3.61-4.14 (m, 7H, 7-H, –OCH_2CH(OH), and –CH_2OCH_2–], 4.43 (br, 1H, 6-H), 4.54 (br, 1H, 1-H), 4.89 (dd, 1H, J_{cis}=10.13, J_{gem}=1.6, 15-H_{cis}), 5.05 (dd, 1H, J_{trans}=17.2, J_{gem}=1.6, 15-H_{trans}), 5.17 [m, 1H, –CH(OAc)], 6.09 (dd, 1H, J_{trans}=17.2, J_{cis}=10.13, 14-H). Anal. Calcd for $C_{35}H_{61}NO_9Si$: C, 62.89; H, 9.21; N, 2.09. Found: C, 62.73; H, 9.11; N, 1.96%.

7β-(3-Dimethylaminopropyloxy-2-hydroxy)-8,13-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-one 16. The compound **8** (0.511 g, 0.86 mmol) was dissolved in THF (30 mL) and 1*M* $nBu_4N^+F^-$ in THF (1 mL, 1 mmol) added to it. The reaction mixture was stirred for 5 min. Solvent was removed and the residue purified by flash chromatography using 10% MeOH in $CHCl_3$ as eluent. The pure product was dissolved in EtOAc, and HCl in dry ether was added to it. The precipitated white solid was filtered off and washed with dry ether. It was finally crystallized from dry MeOH-dry ether, yield 0.28 g (63%), mp, 280-82 °C (d); IR (KBr): 3520 (br), 2960 (br), 1720, 1725 (br) cm^{-1}; ^1H NMR($CDCl_3$+ CD_3OD): 1.07, 1.30, 1.44 (6H), 1.66 (4×s, 15H, 5×CH_3), 2.06 (d, 1H, *J*=3.04, 5-H), 2.38 (d, 1H, J_{gem}=16.2, 12-βH), 2.96, 2.99 (2×s, 6H, 2xNCH_3), 3.29 (d, 1H, J_{gem}=16.2, 12-αH), 3.20-3.39 [m, 3H, CH_2NMe_2 and –CH(OH)], 3.81 (m, 3H, 7-H, and OCH_2CH(OH)–], 4.07-4.51 (m, 2H, 1-H and 6-H), 4.94 (dd, 1H, J_{cis}=10.13, J_{gem}=1.6, 15-H_{cis}), 5.14 (dd, 1H, J_{trans}=17.2, J_{gem}=1.6, 15-H_{trans}), 6.14, 6.15 (2×dd*, 1H, J_{trans}=17.2, J_{cis}=10.13, 14-H, *due to diastereomers). Anal. Calcd for $C_{25}H_{44}NO_7Cl$: C, 59.33; H, 8.76; N, 2.77; Cl, 7.01. Found: C, 59.23; H, 8.71; N, 2.80; Cl, 7.18%.

7β-(3-Diethylaminopropyloxy-2-hydroxy)-8,13-epoxy-1α,6β,9α-trihydroxy-labd-14-en-11-one 17. Compound **17** was prepared from **9** using the same method as described for the synthesis of **16**. The crude product was purified by flash chromatography using 8% CH_3CN in $CHCl_3$ as eluent. The pure product was converted to hydrochloride salt. It was finally crystallized from dry MeOH-dry ether, yield 61.9%, mp, 207-12° C (dry MeOH-dry ether); IR (KBr): 3500-3200 (br), 2950 (br), 1720, (br) cm^{-1}, ^1H NMR: 1.23, 1.31 (2×s, 6H, 2×CH_3), 1.41 (t, 6H, *J*=7.1, 2×CH_2CH_3), 1.43 (6H), 1.67 (2×s, 9H, 3×CH_3), 2.05 (d, 1H, *J*=2.5, 5-H), 2.41 (d, 1H, J_{gem}=16.2, 12-βH), 3.16-3.34 (m, 7H, 12-αH, 2×NCH_2CH_3 and CH_2NEt_2), 3.81 (m, 3H, 7-H and OCH_2CH(OH)–], 4.20-4.54 [m, 3H, 1H, 6-H and CH(OH)–], 4.94 (dd, 1H, J_{cis}=11.1, J_{gem}=1.2, 15-H_{cis}), 5.13 (dd, 1H, J_{trans}=17.2, J_{gem}=1.2, 15-H_{trans}), 6.10, 6.13 (2×dd*, 1H, J_{trans}=17.2, J_{cis}=11.1, 14-H, *due to diastereomers). Anal. Calcd for $C_{27}H_{48}NO_7Cl$: C, 60.71; H, 9.06; N, 2.62; Cl, 6.64. Found: C, 60.55; H, 9.16; N, 2.32; Cl, 6.73%.

8,13-Epoxy-7β-(2-hydroxy-3-piperidinopropyl-oxy)-1α,6β,9α-trihydroxylabd-14-en-11-one 18. This was prepared from **10** using the same method as described for the synthesis of **16**. The crude product was purified by flash chromatography using 8% CH_3CN in $CHCl_3$ as eluent. The pure product was converted to hydrochloride salt. It was finally crystallized from dry MeOH-dry ether, yield 75.7%, mp, 225 °C (d) (dry MeOH-dry ether), IR (KBr): 3555, 3245 (br), 2950, 1695 (br) cm^{-1}; ^1H NMR ($CDCl_3$ + CD_3OD): 1.06 (br, 5H, CH_3 and 4′-CH_2 of piperidine), 1.27, 1.40 (6H), 1.64 (3×s, 12H, 4×CH_3), 2.03 (d, 1H, *J*=3.04, 5-H), 2.40 (d, 1H, J_{gem}=16.2, 12-βH), 3.17 (br, 4H, –CH_2NCH_2–), 3.23 (d, 1H, J_{gem}=16.2, 12-αH), 3.51-3.87 [m, 4H, 7-H and –CH(OH)CH_2N-], 4.21-4.53 [m, 4H, 1-H, 6-H and –OCH_2CH(OH)–], 4.94 (dd, 1H, J_{cis}=11.1, J_{gem}=1.8, 15-H_{cis}), 5.13 (dd, 1H, J_{trans}=17.2, J_{gem}=1.8, 15-H_{trans}), 6.10, 6.13 (2×dd*, 1H, J_{trans}=17.2, J_{cis}=11.1, 14-H, *due to diastereomers). Anal. Calcd for $C_{28}H_{48}NO_7Cl$: C, 61.58; H, 8.86; N, 2.57; Cl, 6.49. Found: C, 61.65; H, 8.79; N, 2.68; Cl, 6.55%.

8,13-Epoxy-7β-(2-hydroxy-3-morpholinopropyl-oxy)-1α,6β,9α-trihydroxy-labd-14-en-11-one 19. This was prepared from **11** using the same method as described for the synthesis of **16**. The crude product was purified by flash chromatography using 8% CH_3CN in $CHCl_3$ as eluent. The pure product was converted to hydrochloride salt. It was finally crystallized from dry MeOH-dry ether, yield 73.5%, mp, 240-43 °C (d) (dry MeOH-dry ether); IR (KBr): 3300 (br), 2960 (br), 1705 (br) cm^{-1}; ^1H NMR ($CDCl_3$+CD_3OD): 1.09 1.31, 1.46 (6H), 1.67 (4×s, 15H, 5×CH_3), 2.06 (d, 1H, *J*=2.7, 5-H), 2.38 (d, 1H, J_{gem}=16.2, 12-βH), 3.19-3.37 (m, 4H, –CH_2NCH_2–), 3.28 (d, 1H, J_{gem}=16.2, 12-αH),

3.44-3.83 [m, 4H, 7-H and $-CH(OH)CH_2N-$], 4.0 (br, 4H, $-CH_2OCH_2-$), 4.29-4.51 [m, 4H, 1-H, 6-H and $-OCH_2CH(OH)-$], 4.95 (dd, 1H, J_{cis}=11.1, J_{gem}=1.8, 15-H_{cis}), 5.15 (dd, 1H, J_{trans}=17.2, J_{gem}=1.8, 15-H_{trans}), 6.14, 6.15 (2×dd*, 1H, J_{trans}=17.2, J_{cis}=11.1, 14-H, *due to diastereomers). Anal. Calcd for $C_{28}H_{46}NO_8Cl$,0.5 H_2O: C, 58.21; H, 8.49; N, 2.51; Cl, 6.36. Found: C, 58.51; H, 8.48; N, 2.60; Cl, 6.47%.

7β-(2-Acetoxy-3-dimethylaminopropyloxy)-8,13-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-one 7. This compound was also prepared from **12** using the method described for the synthesis of **16**. The crude product was purified by flash chromatography using 20% CH_3CN in $CHCl_3$ as eluent. The pure product was converted to hydrochloride salt. It was finally crystallized from dry MeOH-dry ether, yield 71.6%, (mp, IR, ^{1}H NMR data and analyses have been given earlier).

7β-(2-Acetoxy-3-piperidinopropyloxy)-8,13-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-one 20. This was prepared from **14** according to the method as described for the synthesis of **16**. The crude product was purified by flash chromatography using 8% CH_3CN in $CHCl_3$ as eluent. The pure product was converted to hydrochloride salt. It was finally crystallized from dry MeOH-dry ether, yield 68%; mp, 158-60 °C (dry MeOH-dry ether); IR (KBr): 3500-3100 (br), 2960 (br), 1765, 1755 (br), 1725 cm^{-1}; ^{1}H NMR: 1.06 1.26, 1.40 (6H), 1.61 (4×s, 15H, 5×CH_3), 1.60-1.92 [m, 6H, 3-CH_2, 4-CH_2, 5-CH_2 of piperidine), 2.04 (d, 1H, J=2.7, 5-H), 2.17 (s, 3H, $COCH_3$), 2.39 (d, 1H, J_{gem}=16.2, 12-βH), 2.61-3.0 (m, 4H, CH_2NCH_2), 3.21 (d, 1H, J_{gem}=16.2, 12-αH), 3.34-4.0 [m, 5H, 7-H and $-CH_2CH(OAc)CH_2N-$], 4.0 (br, 4H, $-CH_2OCH_2-$), 4.53 (br, 2H, 1-H and 6-H), 4.92 (dd, 1H, J_{cis}=10.13, J_{gem}=2.0, 15-H_{cis}), 5.13 (dd, 1H, J_{trans}=17.2, J_{gem}=2.0, 15-H_{trans}), 5.43 [m, 1H, $-CH(OAc)$], 6.04, 6.07 (2×dd*, 1H, J_{trans}=17.2, J_{cis}=10.13, 14-H, *due to diastereomers). Anal. Calcd for $C_{30}H_{50}NO_8Cl$, 0.5 H_2O: C, 60.33; H, 8.62; N, 2.35; Cl, 5.94. Found: C, 60.32; H, 8.76; N, 2.30; Cl, 6.12%.

7β-(2-Acetoxy-3-morpholinopropyloxy)-8,13-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-one 21. This was prepared from **15** according to the method as described for the synthesis of **16**. The crude product was purified by flash chromatography using 8% CH_3CN in $CHCl_3$ as eluent. The pure product was converted to hydrochloride salt. It was finally crystallized from dry MeOH-dry ether, yield 69.9%, mp, 203-07 °C (dry MeOH-dry ether); IR (KBr): 3400-3225 (br), 2940 (br), 1755, 1710 cm^{-1}; ^{1}H NMR: 1.10 1.30, 1.46 (6H), 1.64 (4×s, 15H, 5×CH_3), 2.05 (d, 1H, J=3.04, 5-H), 2.24 (s, 3H, $COCH_3$), 2.41 (d, 1H, J_{gem}=16.2, 12-βH), 2.94 (m, 4H, CH_2NCH_2), 3.22 (d, 1H, J_{gem}=16.7, 12-αH), 3.43 [m, 2H, $-CH(OAc)CH_2N-$], 3.77-4.11 (m, 5-H, 7-H and CH_2OCH_2), 4.23 (m, 2H, OCH_2CH-], 4.57 (br, 2H, 1-H and 6-H), 4.95 (dd, 1H, J_{cis}=11.1, J_{gem}=1.8, 15-H_{cis}), 5.14 (dd, 1H, J_{trans}=17.2, J_{gem}=1.8, 15-H_{trans}), 5.47 [m, 1H, $-CH(OAc)$], 6.05, 6.10 (2xdd*, 1H, J_{trans}=17.2, J_{cis}=10.13, 14-H, *due to diastereomers). Anal. Calcd for $C_{29}H_{48}NO_9Cl$: C, 59.02; H, 8.19; N, 2.37; Cl, 6.01. Found: C, 58.83; H, 8.27; N, 2.30; Cl, 6.15%.

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