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Expeditious and convenient synthesis of pregnanes and its glycosides as potential anti-dyslipidemic and anti-oxidant agents

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Abstract—A series of new pregnane derivatives and its glycosides were synthesized in order to find new 'leads' against some important targets. The 3β-hydroxy-16α-(2-hydroxy ethoxy) pregn-5-en-20-one (**5**) was synthesized from 3β-hydroxy-5,16-pregnadiene-20-one (**2**) by adopting general modified procedure using BF_3:Et_2O as a catalyst. Reduction of **5**, with sodium borohydride yielded 3β,20β-dihydroxy-16α-(2-hydroxy ethoxy) pregn-5-en (**7**) as the major isolable product. O-alkylation of the C-20-oxime-pregnadiene (**9**) with 1,5-dibromopentane yielded 20-(*O*-5-bromopentyl)-oximino-3β-hydroxy-pregn-5,16-diene (**11**). Synthesis of C-16 substituted pregnane glycosides (**20**) and (**21**) were accomplished with the imidate method using BF_3:Et_2O. The synthesis of 4-chlorobenzoate (**3**) and 2-chlorobenzoate (**4**), derivatives of **2** were also accomplished. These compounds were evaluated for their anti-dyslipidemic and anti-oxidant activity and amongst them compounds **3** and **7** showed more lipid lowering and anti-oxidant activity.
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1. Introduction

Pregnanes, the C-21 steroid derivatives, are an important class of bioactive compounds having diverse pharmacological activity and which has provided interesting 'leads' for the development of new drugs. These potent pregnane derivatives have been found to posses cytotoxic,[1] antifeedant,[2] anti-inflammatory,[3,4] anti-asthamatic,[5] lipid lowering[6] and anti-viral[7] activities. They also find use as neurosteroids[8] and as inhibitors of testosterone 5α reductase[9] which helps in the treatment of androgen sensitive prostate cancer in men.[10] Besides this, a number of pregnanes are not only similar to cardiac glycosides with respect to molecular and cellular site of action, but they have enhanced cardiac contractibility.[11]

Pregnane glycosides because of their remarkable pharmacological activities are one of the major components of traditional Chinese medicine.[12] These biologically active compounds isolated chiefly from Asclepiadaceae[13] family plants have been found to posses anti-cancer,[14] anti-asthamatic,[15] anti-tumor[16] and anti-fungal activities.[17] The synthetic derivatives of this class of compounds are larger, more hydrophilic, have increased efficacy and reduced toxicity relative to the parent compound (aglycone)[18] and have primarily been used for the treatment of ulcerative colitis,[19] Crohn's disease[19] and cardiac contractibility.[20] Owing to the immense importance of these medicinally important pregnane derivatives, considerable attention is being devoted to the synthesis and biological evaluation of this important class of compounds.[21–27] In the search of newer derivatives, the synthesis of novel pregnane derivatives and its glycosides in excellent yields is herein reported.

2. Chemistry

During the course of our studies towards the synthesis of C-16-substituted pregnane derivatives we employed an earlier reported method[28] and carried out a number of modifications in order to increase the total yield of the desired product. The modified procedure involved the stirring of the compound 3β-hydroxy pregn-5,16-diene-20-one **2** with nucleophilic reagent ethylene glycol

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Keywords: Pregnanes; Glycosides; Oximo ether; Anti-dyslipidemic; Anti-oxidant.

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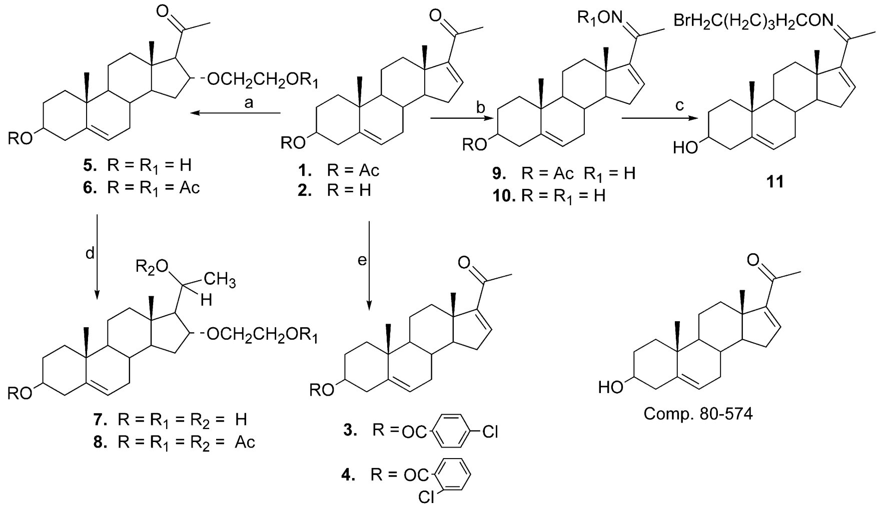
in the presence of $BF_3{:}Et_2O$ as a catalyst at different temperatures. The best results were obtained when the reaction mixture was stirred at 30 °C and that too for lesser period (55–60 h). Employing this modified procedure compound **5** was synthesized. The yield of the desired derivative was not only excellent (80%) but also free from any other position being substituted. The ^1H NMR spectrum not only conclusively proved the structure of compound **5** but it also helped in ascertaining the orientation of side chain at C-16. A well-defined doublet at δ 2.58 was attributed to the methine proton at C-17. The magnitude of the coupling constant of this doublet, $J = 6.4$ Hz due to $J_{16\beta H-17\alpha H}$ confirms the orientation of this side chain at C-16 to be α.[29] Reduction of **5** with sodium borohydride afforded **7** in good yield. The stereochemistry of the orientation of hydroxyl group at C-20, which was postulated to be β,[30] was confirmed by the presence of small NOE[31] between C-13 and H-20. Acetylation of **5** and **7** yielded their respective acetyl derivatives **6** and **8**. Guggulsterone and related compound 80–574 have been found to act as antagonists of the bile acid receptor[32] (BAR). But the compound 80–574 is very poorly absorbed through the intestines. In order to impart better absorption,[32b,2c] the synthesis of two newer benzoyl derivatives **3** and **4** was accomplished by treating compound **2** with 4-chlorobenzoyl chloride and 2-chlorobenzoyl chloride, respectively (Scheme 1).

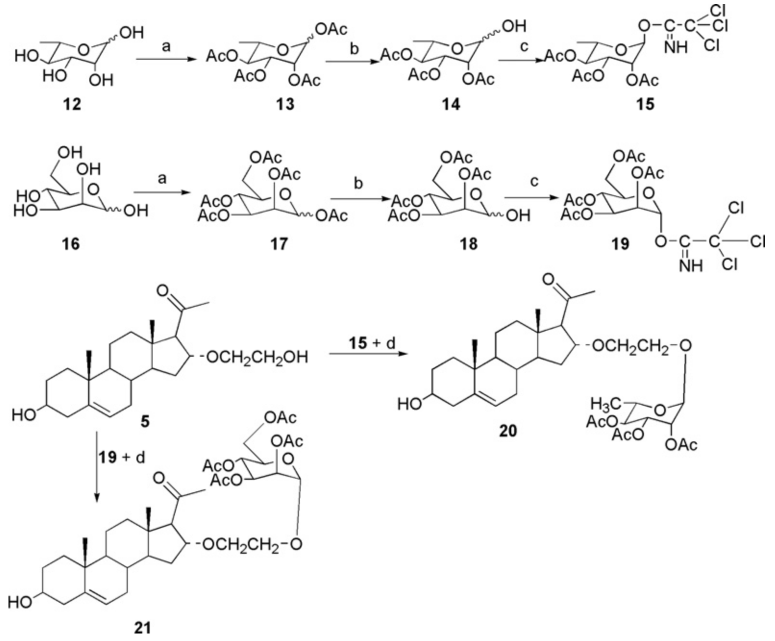
Steroidal oximino ether derivatives which posses contragestational activity,[33a] anti-androgenic activity,[33b] neuromuscular blocking activity[33c] have also been used for the treatment of breast cancer in women[34–36] by functioning as inhibitors of enzyme aromatase cytochrome P_{450}. A novel steroidal oximino ether derivative **11** was synthesized by treating **9** with 1,5-dibromopentane in dry DMF in presence of base NaH (Scheme 1).

L-Rhamnose containing oligosaccharides are widely distributed in nature as triterpenoid glycosides,[37] K-antigens[38] and phenolic glycolipids from mycobacteria.[39] It is also well recognized that L-rhamnose containing serogroups of mycobacterium are closely related to opportunistic pulmonary infection[40] and AIDS disseminate infections.[39b] A number of naturally occurring D-mannose related glycosides have also shown marked biological activities.[41]

In our effort to prepare these biologically important L-rhamnose/D-mannose-related pregnane glycosides, trichloroimidate method[42] was adopted in which 2,3,4-*tri*-*O*-acetyl-α-L-rhamnopyranosyl trichloroacetimidate **15**/ 2,3,4,6-*tetra*-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate **19**, derivatives of L-rhamnose and D-mannose were treated with pregnane genin **5** in presence of Lewis acid catalyst $BF_3{:}Et_2O$ affording glycosides **20** and **21**, respectively[43] (Scheme 2). The ^1H NMR spectrum not only confirmed the structure but also helped in ascertaining the configuration of the glycosidic linkage in **20**. The anomeric proton was observed as a singlet at δ 4.76. The negligible diequatorial coupling confirmed the glycosidic linkage to be α. Besides this, the downfield shifting of the methylene protons of the side chain at C-16 by ca. 0.3 ppm suggested that the sugar is glycosidically linked to the hydroxyl group of the side chain at C-16 and not to C-3 hydroxyl group. In the FAB MS of **20**, fragments at *m*/*z* 544 (Retro Diels Alder rearrangement at C-2 and C-3 of sugar), *m*/*z* 510 (Retro Diels Alder rearrangement at C-5 and C-6 of aglycone) and at *m*/*z* 429 (due to C-2 C-3 bond cleavage of sugar followed by loss of C-3 OH of aglycone as water molecule)[44] further confirm that the sugar is glycosidically linked to the hydroxyl group of the side chain and not to the C-3 hydroxyl group. Similarly in the ^1H NMR spectrum of **21** the anomeric proton was also observed as a singlet at δ 5.02, suggesting the linkage to be α. In the FAB MS, fragments at *m*/*z* 483 (Retro Diels Alder rearrangement at C-5 and C-6 of aglycone) and *m*/*z* 357 (Retro Diels Alder rearrangement at C-2 and C-3 of sugar) further supported the glycosidic nature of compound **21**.



Scheme 1. Reagents: (a) $HOCH_2CH_2OH$, BF_3Et_2O; (b) $NH_2OH{\cdot}HCl$–pyridine; (c) $Br(CH_2)_5Br$, NaH, DMF; (d) $NaBH_4$; (e) 4/2 chloro benzoyl chloride.



Scheme 2. Reagents: (a) Ac_2O–pyridine; (b) *N*,*N*-DMF–NH_2NHAc; (c) Cl_3C–CN/K_2CO_3; (d) BF_3:Et_2O.

3. Biological activity

The present study has been undertaken to evaluate the antidyslipidemic activity of pregnane derivatives in triton model. The anti-oxidant activity of these derivatives was also evaluated by generating free radicals in vitro in the absence and presence of pregnane derivatives.

Animals used. Rats (Charles Foster strain, male, adult, body weight 200–225 g) were kept in a room with controlled temperature (25–26 °C), humidity (60–80%) and 12/12 h light/dark cycle (light on from 8.00 A.M. to 8.00 P.M.) under hygienic conditions. Animals, which were acclimatized for one week before starting the experiment, had free access to the normal diet and water.

Lipid lowering activity. Rats were divided into ten groups—control, triton induced, triton plus **3**, **4**, **5**, **6**, **7**, **8**, **11** and Gemfibrozil (100 mg/kg) treated groups containing six rats in each group. In this experiment of 18 h, hyperlipidemia was developed by administration of triton WR-1339 (Sigma chemical company, St. Louis, MO, USA) at a dose of 400 mg/kg. b.w. intraperitoneally to animals of all the groups except the control. Seven pregnane derivatives were macerated with gum acacia (0.2% w/v), suspended in water and fed simultaneously with triton with a dose of 250 mg/kg po to the animals of treated group and the diet being withdrawn. Animals of control and triton group without treatment with pregnane compounds were given same amount of gum acacia suspension (vehicle). After 18 h of treatment the animals were anaesthetized with thiopentone solution (50 mg/kg b.w.) prepared in normal saline and then 1 mL blood was withdrawn from retro-orbital sinus using glass capillary in EDTA coated Eppendorf tube (3.0 mg/ml blood). The blood was centrifuged (at 2500*g*) at 4 °C for 10 min and plasma was separated. Plasma was diluted with normal saline (ratio of 1:3) and used for analysis of total cholesterol (Tc), triglycerides (Tg) and phospholipids (Pl) by standard enzymatic methods[46–48] using Beckmann auto-analyzer and standard kits purchased from Beckmann Coulter International, USA.

Anti-oxidant activity (generation of free radicals). Superoxide anions (O^{-2}) were generated enzymatically[49] by xanthine (160 mM), xanthine oxidase (0.04 U) and nitroblue tetrazolium (320 μM) in absence or presence of compounds **3**, **4**, **5**, **6**, **7**, **8**, **11**, **20**, and **21** (400 μg/ml) in 100 mM phosphate buffer (pH 8.2). Fractions were sonicated well in phosphate buffer before use. The reaction mixtures were incubated at 37 °C and after 30 min the reaction was stopped by adding 0.5 mL glacial acetic acid. The amount of formazone formed was measured at 560 nm on a spectrophotometer. Percentage inhibition was calculated taking absorption coefficient of formazone as $7.2 \times 10^3\ M^{-1}\ cm^{-1}$. In another set of experiment, effects of compounds **3**, **4**, **5**, **6**, **7**, **8**, **11**, **20**, and **21** on generation of hydroxyl radicals ($OH^{\cdot}$) was also studied by non-enzymic reactants.[50] Briefly $OH^{\cdot}$ were generated in a non-enzymic system comprised of deoxy ribose (2.8 mM), $FeSO_4.7H_2O$ (2 mM), sodium ascorbate (2.0 mM) and H_2O_2 (2.8 mM) in 50 mM KH_2PO_4 buffer, pH 7.4 to a final volume of 2.5 mL. The above reaction mixtures in the absence or presence of compound **3**, **4**, **5**, **6**, **7**, **8**, **11**, **20**, and **21** (400 μg/ml) were incubated at 37 °C for 90 min. Reference tubes and reagent blanks were also run simultaneously.

Malondialdehyde (MDA) content in both experimental and reference tubes were estimated spectrophotometrically by thiobarbituric acid method as mentioned above.[51]

Statistical evaluation. Data were analyzed using Student's *t*-test. The hyperlipidemic groups were compared with control and **3**, **4**, **5**, **6**, **7**, **8**, and **11** treated groups. Similarly the generation of oxygen free radicals with different fractions of compounds **3**, **4**, **5**, **6**, **7**, **8**, **11**, **20**, and **21** were compared with that of their formation without fractions. $P < 0.05$ was considered to be significant.

4. Results and discussion

Effect of pregnane derivatives on hyperlipidemia: Administration of triton WR-1339 in rats induced marked hyperlipidemia as evidenced by increase in the plasma level of Tc (3.67-fold) Tg (3.39-fold) and Pl (3.97-fold) as compared to control (Table 1). Treatment of hyperlipidemic rats with pregnane derivatives at the dose of 400 mg/kg po reversed the plasma levels of lipid with varying extents. The effect of **3** causing decrease in plasma levels of Tc, Tg, and Pl by 27, 25, 27 and effect of **7** causing decrease in plasma levels of Tc, Tg, and Pl by 23, 26, and 25 and the effect of compound **4** showed Tc, Tg, Pl by 21, 14, 17%, respectively, while compound **6** and **8** showed mild lipid lowering activity as compared to triton which was more significant than other fractions. These data compared with standard drug Gemfibrozil at the dose of 100 mg/kg showed decrease in plasma levels of Tc, Tg, Pl by 37,32,36%, respectively. The order of lipid lowering activity by these fractions in above model was **3** > **7**(Table 1).

Effect of **3**, **4**, **5**, **6**, **7**, **8**, **11**, **20**, and **21** on oxygen free radical generation in vitro: the scavenging potential of pregnane derivatives at 400 μg/ml against formation of O_2^- and $OH^{\cdot}$ in non-enzymic systems were also studied (Table 2). The significant decrease in superoxide anions by **3** and **7** and hydroxyl radicals (17%, 40%, 39%, 18%, 8%, and 16%) by fraction **3** and **7** were found at concentration of 400 μg/ml. The **3** and **7** derivatives showed more anti-oxidant activity in above test system as compared to that of **4**, **5**, **6**, **8**, **11**, **20**, and **21**.

The involvement of hydroxyl free radicals ($OH^{\cdot}$) has been found to be a major causative factor for peroxidative damage to lipoproteins, which is responsible for initiation and progression of atherosclerosis in hyperlipidemic subjects.[52] Hyperlipidemia may also induce other abnormalities like oxidation of fatty acids, leading to the formation of ketone bodies as well as masking liver and muscle resistance to insulin which initiates the progress of diabetes in patients.[53] Furthermore, due to hyperglycemia, increase in non-enzymic glycosylation occurs, accompanied with glucose oxidation and these reactions being catalyzed by Cu^{2+} and Fe^{2+}, resulting in formation of $O_2^{\cdot}$ and $OH^{\cdot}$ radicals which further accelerates the risk of cardiac diseases in dyslipidemic patients.[54]

Table 2. Anti-oxidant activity of pregnane derivatives in-vitro

Treatment	Concentration	Formation of superoxide anions[a]	Formation of hydroxyl radicals[b]
3	None	15.01 ± 1.06	41.62 ± 1.24
	400	11.10 ± 0.96*	27.16 ± 1.54**
4	None	17.91 ± 1.44	40.40 ± 3.66
	400	14.33 ± 1.22	35.01 ± 2.82
5	None	14.27 ± 2.08	41.52 ± 1.73
	400	7.83 ± 1.60**	36.12 ± 2.92*
6	None	23.20 ± 2.44	44.70 ± 4.77
	400	21.50 ± 1.77	41.50 ± 3.72
7	None	14.03 ± 1.43	40.19 ± 2.32
	400	9.33 ± 1.10*	31.09 ± 3.12**
8	None	13.60 ± 1.12	35.12 ± 3.62
	400	12.63 ± 1.24	33.90 ± 3.00
11	None	16.13 ± 1.76	41.52 ± 2.12
	400	15.33 ± 3.00 NS	38.52 ± 3.12 NS
20	None	14.25 ± 0.49	42.13 ± 1.39
	400	12.8 ± 1.02 NS	39.02 ± 3.01 NS
21	None	15.32 ± 1.32	39.00 ± 2.12
	400	13.22 ± 2.30 NS	35.05 ± 1.12 NS

Each value is means ± SD of four separate observations.

NS, non significant as compared to the systems without drug treatment.

[a] nmol formazone formed/minute.

[b] nmole MDA/h.

* $P < 0.05$.

** $P < 0.001$.

Table 1. Effect of pregnane derivatives on biochemical parameters in hyperlipidemic rats

Treatment	Total cholesterol (Tc)	Triglyceride (Tg)	Phospholipids (Pl)
Control	86.44 ± 7.21	82.66 ± 6.77	71.49 ± 5.62
Triton treated	318.82 ± 26.71 (+3.6F)***	280.41 ± 22.22 (+3.39F)***	283.87 ± 24.00 (+3.97F)***
Triton + **3**	232.17 ± 15.66 (−27)***	210.00 ± 14.73 (−25)***	206.28 ± 12.93 (−27)***
Triton + **4**	251.77 ± 18.66 (−21)**	240.12 ± 21.11 (−14)*	236.12 ± 16.66 (−17)*
Triton + **5**	275.11 ± 23.44 (−13)*	250.55 ± 18.73 (−11) NS	260.44 ± 20.82 (−8) NS
Triton + **6**	276.41 ± 24.92 (−13)*	235.52 ± 20.22 (−16)*	248.12 ± 16.66 (−13)*
Triton + **7**	244.00 ± 16.44 (−23)**	208.33 ± 18.23 (−26)***	212.24 ± 15.55 (−25)***
Triton + **8**	260.10 ± 16.88 (−18)*	244.22 ± 19.92 (−13)*	244.44 ± 17.81 (−14)*
Triton + **11**	305.55 ± 27.7 (−4) NS	266.22 ± 22.00 (−5) NS	270.35 ± 20.66 (−5) NS
Triton + gemfibrozil (100 mg/Kg) standard drug	200.00 ± 13.22 (−37)***	190.01 ± 17.00 (−32)***	180.11 ± 12.44 (−36)***

Unit: mg/dl. Each value is means ± SD of six rats.

NS, non-significant.

Hyperlipidemic group was compared with control, hyperlipidemic + pregnane derivatives treated with hyperlipidemic.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

In order to overcome these ailments, a drug having multifold properties such as anti-oxidant, anti-diabetic and lipid lowering activities is in great demand. In our present study, we have investigated these properties in different analogues of pregnanes. Both **3** and **7** caused significant decrease in the plasma level of lipid in triton models of hyperlipidemia. Triton WR-1339 acts as surfactant, suppresses the action of lipase and blocks the uptake of lipoproteins from the circulation by extrahepatic tissues resulting an increase in the levels of circulating lipid.[55] These test samples inhibited cholesterol biosynthesis and potentiated the activity of lipolytic enzymes to early clearance of lipids from circulation in triton-induced hyperlipidemia. We have successfully used this model for evaluation of lipid lowering activity of pregnane derivatives.

To access the anti-oxidant activity of pregnane derivatives, we have used in-vitro model of non-enzymic and enzymic superoxide and hydroxyl radicals generation. The properties of **3** and **7** as anti-oxidant and scavenger of oxygen free radicals appears to be mediated through activity like metal ion chaelators and xanthine oxidase inhibitors.

5. Experimental

IR spectra were recorded on Beckmann Aculab-10, Perkin-Elmer 881 and FTIR 8210 PC, Schimadzu spectrophotometers using KBr discs. Nuclear magnetic resonance (NMR) spectra were recorded on either Bruker advance DRX-300 MHz or Bruker DPX 200 FT spectrometers using TMS as an internal reference. FAB mass spectra were recorded on JEOL SX 102/DA 6000 whereas ESI was recorded on MICROMASS QUATTRO II triple quadrupole mass spectrometer. Optical rotations were recorded on ORIBA, SEPA-300 digital polarimeter. Chemical analysis was carried out on Carlo-Erba-1108 instrument. The melting points are recorded on an electrically heated melting point apparatus and are uncorrected.

5.1. General procedure for the synthesis of compounds

5.1.1. 3β-Acetoxy-5,16-pregnadiene-20-one (1). Diosgenin was converted into compound **1** by reported method.[45] It was identified[56] by its mp 166–170 °C, ^1H NMR and MS.

5.1.2. 3β-Hydroxy-5,16-pregnadiene-20-one (2). Deacetylation of compound **1** by zemplen method[57] yielded compound **2**, identified by its mp 212–214 °C (lit mp 216 °C),[58] ^1H NMR and MS.

5.2. General procedure for the synthesis of compounds 3 and 4

To a solution of **2** (0.5 g, 1.59 mmol) in dry pyridine (2.5 mL) at 0 °C was added 0.5 mL of 4/2-chloro benzoyl chlorides. The reaction mixture was stirred at room temperature for 2 h. Ice water (10 mL) was added and the mixture extracted with dichloromethane, washed with aqueous Na_2CO_3, water, dried over anhydrous sodium sulphate, filtered and concentrated. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate 99:1) to afford the pure compounds.

5.2.1. Pregn-5,16-diene-20-one 3β-yl-4-chlorobenzoate (3). White solid, yield: 90%; mp 180–182 °C; $[\alpha]_D^{25}$ −7.0 (*c* 0.50, MeOH); IR (KBr) ν_{max} 2921, 1722, 1592, 1278, 1096, 770 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm) 8.05–8.02 (m, 2H, Ar–H), 7.39–7.37 (m, 2H, Ar–H), 6.72 (m, 1H, H-16), 5.43 (m, 1H, H-6), 4.87 (m, 1H, H-3), 2.27 (s, 3H, CH_3-21), 1.107 (s, 3H, CH_3-19), 0.93 (s, 3H, CH_3-18). MS (*m*/*z*) 452 (M^+,Cl^{35}), 454 (M^+,Cl^{37}). Anal. Calcd for $C_{28}H_{33}ClO_3$: C, 74.24; H, 7.34; Cl, 7.83. Found: C, 74.12; H, 7.28; Cl, 7.78.

5.2.2. Pregn-5,16-diene-20-one 3β-yl-2-chlorobenzoate (4). White solid, yield: 90%; mp 192–195 °C; $[\alpha]_D^{25}$ −12.0 (*c* 0.25, MeOH); IR (KBr) ν_{max} 2919, 1716, 1536, 1230, 1096, 593 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm) 8.04–8.01 (m, 2H, Ar–H,) 7.46–7.37 (m, 2H, Ar–H), 6.71 (m, 1H, H-16), 5.5 (m, 1H, H-6), 4.82 (m, 1H, H-3), 2.27 (s, 3H, CH_3-21), 1.10 (s, 3H, CH_3-19), 0.88 (s, 3H, CH_3-18). MS (*m*/*z*) 452(M^+). Anal. Calcd for $C_{28}H_{33}ClO_3$: C, 74.24; H, 7.34; Cl, 7.83. Found: C, 74.14; H, 7.30; Cl, 7.76.

5.3. General procedure for the synthesis of compounds 5

550 mg (1.75 mmol) of compound **2** was dissolved in 4.2 mL (67 mmol) of freshly distilled ethylene glycol and then 0.5 mL of boron trifluoride etherate was added to it. The reaction mixture was stirred at 30 °C for 55–60 h (reaction was monitored by t.l.c during this period). Ice-cold solution of sodium bicarbonate was added to light brown solution of reaction mixture and the aqueous phase was extracted with dichloromethane. The organic layer was washed with water and then dried over anhydrous sodium sulphate. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate 80:20) to afford the pure compound.

5.3.1. 3β-Hydorxy-16α-(2-hydroxy ethoxy)pregn-5-en-20-one (5). White solid, yield: 80%; mp 170–172 °C; $[\alpha]_D^{25}$ −26.6 (*c* 0.75, MeOH); IR (KBr) ν_{max} 3432, 1658, 1057 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm) 5.34 (1H, m, H-6), 4.53 (1H, m, H-16), 3.68–3.64 (2H, m, $OCH_2C\underline{H}_2OH$), 3.64–3.38 (3H, m, $OC\underline{H}_2CH_2OH$ and H-3), 2.58 (1H, d, H-17, *J* = 6.4 Hz), 2.18 (3H, s, CH_3-21), 0.85 (3H, s, CH_3-19), 0.63 (3H, s, CH_3-18); ^{13}C NMR (75 MHz, $CDCl_3$) δ 208.6 (C-20), 140.9 (C-5), 121.4 (C-6), 80.0 (C-16), 71.84 (C-3), 71.77 ($O\underline{C}H_2CH_2$), 70.77 ($OCH_2\underline{C}H_2OH$), 67.47 (C-17), 54.68 (C-14), 49.98 (C-9), 44.6 (C-13), 42.4 (C-4), 38.9 (C-12), 37.3 (C-1), 36.7 (C-10), 34.15 (C-15), 32.42 (C-8), 31.87 (C-7), 31.72 (C-2), 31.64 (C-21), 20.9 (C-11), 19.6 (C-19), 14.6 (C-18). MS (*m*/*z*) 377 (M^++1). Anal. Calcd for $C_{23}H_{36}O_4$: C, 73.37; H, 9.64. Found: C, 73.22; H, 9.56.

5.3.2. 3β-Acetoxy-16α-(2-acetoxy ethoxy)pregn-5-en-20-one (6). Conventional acetylation of compound **5** with acetic anhydride and pyridine yielded compound

6.White solid, yield: 90%; mp 154–156 °C; $[\alpha]_D^{25}$ −31.66 (*c* 0.12, $CHCl_3$); IR (KBr) ν_{max} 2946, 1730, 1662, 1248, 1037 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 5.36 (m, 1H, H-6), 5.005 (m, 1H, H-16), 4.64–4.44 (m, 3H, $OCH_2C\underline{H}_2OAc$ and H-3), 3.64–3.57 (m, 2H, $OC\underline{H}_2$-CH_2OAc), 2.26 (s, 3H, CH_3-21), 2.03 (s, 3H, oAc), 1.97 (s, 3H, OAc), 1.05 (s, 3H, CH_3-19), 0.88 (s, 3H, CH_3-18). MS (*m*/*z*) 460 (M^+). Anal. Calcd for $C_{27}H_{40}O_6$: C, 70.41; H, 8.75. Found: C, 70.34; H, 8.80.

5.4. General procedure for the synthesis of compound 7

Compound **5** (435 mg, 1.15 mmol) in dry tetrahydrofuran (7.1 mL) and dry methanol (2.1 mL) was treated with sodium borohydride (84 mg, 2.21 mmol) portionwise during 10 min at 5 °C; reaction mixture was stirred at the same temperature for 1 h, when t.l.c showed completition of reaction. Acetic acid was added dropwise until the reaction mixture became neutral. Water was added and the resulting mixture concentrated under reduced pressure. The aqueous phase was extracted with dichloromethane. The organic layer was washed with water and then dried over anhydrous sodium sulphate. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate 75:25) to afford the pure compound.

5.4.1. 3β,20β-Dihydorxy-16α-(2-hydroxy ethoxy)pregn-5-en (7). White solid, yield: 85%; mp 199–201 °C; $[\alpha]_D^{25}$ −38.0 (*c* 0.50, MeOH); IR (KBr) ν_{max} 3308, 2926, 1654, 1055 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm) 5.36 (m, 1H, H-6), 4.23 (m, 1H, H-16), 3.92 (q, 1H, H-20, *J* = 6.0 Hz), 3.70–3.65 (m, 2H, OCH_2-$C\underline{H}_2OH$), 3.61–3.43 (m, 3H,$OC\underline{H}_2CH_2OH$ and H-3), 1.29 (d, 3H, CH_3-21, *J* = 6.0 Hz) 1.01 (s, 3H,CH_3-19), 0.79 (3H, s, CH_3-18); ^{13}C NMR (75 MHz, MeOD) δ 142.42 (C-5), 122.34 (C-6), 84.80 (C-16), 72.47 (C-3), 71.81 ($O\underline{C}H_2CH_2$), 69.94 ($OCH_2\underline{C}H_2OH$), 66.29 (C-20), 62.48 (C-17), 54.86(C-14), 51.90 (C-9), 44.10 (C-13), 43.07 (C-4), 40.95 (C-12), 38.54 (C-1), 37.80 (C-10), 33.42 (C-15), 33.09 (C-8), 32.49 (C-7), 30.78 (C-2),23.88 (C-21), 21.80 (C-11), 19.93 (C-19), 14.16 (C-18). MS (*m*/*z*) 379 (M^++1). Anal. Calcd for $C_{23}H_{38}O_4$: C, 72.98; H, 10.12. Found: C, 70.92; H, 10.06.

5.4.2. 3β,20β-Diacetoxy-16α-(2-acetoxy ethoxy)pregn-5-en (8). Conventional acetylation of compound **7** with acetic anhydride and pyridine yielded compound **8**. White solid, yield: 90%; mp 144–146 °C; $[\alpha]_D^{25}$ −66 (*c* 0.1,$CHCl_3$); IR (KBr) ν_{max} 2932, 1729, 1250, 1044 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 5.37 (m, 1H, H-6), 5.005 (m, 1H, H-20), 4.60 (m, 1H, H-16), 4.18–4.10 (m, 3H, $OCH_2C\underline{H}_2OAc$ and H-3), 3.65–3.54 (m, 2H, $OC\underline{H}_2$-CH_2OAc), 2.33 (s, 3H, OAc); 2.03 (s, 6H, OAc), 1.25 (d, 3H, CH_3-21, *J* = 6.4 Hz), 1.00 (s, 3H, CH_3-19), 0.65 (3H, s, CH_3-18). MS (*m*/*z*) 505 (M^++1). Anal. Calcd for $C_{29}H_{44}O_7$: C, 69.02; H, 08.79. Found: C, 68.95; H, 8.74.

5.5. General procedure for the synthesis of compound 9

5.5.1. 3β-Acetoxy-5,16-pregnadiene-20-one oxime (9). Compound **1** was converted into compound **9** by reported method.[59] It was identified by its mp 225–228 °C, ^1H NMR and MS.

5.5.2. 3β-Hydroxy-5,16-pregnadiene-20-one oxime (10). Deacetylation of compound **9** by zemplen method[57] yielded compound **10**.[59b] White solid, mp 210–214 °C; IR (KBr) ν_{max} 3422, 2927, 1654, 1560, 1111 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm) 6.05 (m, 1H, H-16), 5.36 (m, 1H, H-6), 3.53 (m, 1H, H-3), 1.99 (s, 3H, CH_3-21), 1.04 (s, 3H, CH_3-19), 0.94 (s, 3H, CH_3-18). MS (*m*/*z*) 330(M^++1). Anal. Calcd for $C_{21}H_{31}NO_2$: C, 76.55; H, 9.48; N, 4.25. Found: C, 76.41; H, 9.60; N, 4.09.

5.6. General procedure for the synthesis of compound 11[60]

Compound **9** (500 mg, 1.40 mmol), NaH (96 mg, 4.01 mmol) in dry DMF (25 mL) was stirred at 0 °C for 30 min. To the reaction mixture 1,5-dibromopentane (0.5 mL, 2.2 mmol) was added and the reaction mixture further stirred at room temperature for 3 h. DMF was evaporated under reduced pressure and the solid residue obtained was extracted with chloroform, washed with water, dried over anhydrous sodium sulphate and concentrated in vaccuo. Column chromatography of the resultant residue (hexane/ethyl acetate, 99:1)afforded compound **11**.

5.6.1. 20-(*O*-5-Bromopentyl)-oximino-3β-hydorxy-pregn-5,16-diene (11). Light brownish solid, yield: 60%; mp 148–152 °C; $[\alpha]_D^{25}$ −4.0 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3417, 1594, 1065 cm^{-1}; ^1H NMR($CDCl_3$, 200 MHz) δ (ppm) 6.0 (m, 1H, H-16), 5.36 (m,1H, H-6); 4.08 (t, 2H, $N{-}O{-}C\underline{H}_2CH_2$, *J* = 6.2 Hz), 3.54–3.49 (m,1H, H-3), 3.41 (t, 2H, $CH_2C\underline{H}_2Br$), 1.98 (s, 3H, CH_3-21), 1.69–1.39 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2Br$), 1.05 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18): ^{13}C (75 MHz, $CDCl_3$): 166.1 (C-20), 152.6 (C-17), 145.6 (C-16), 140.8 (C-5), 121.4 (C-6), 72.8 ($=N{-}O\underline{C}H_2$), 71.8 (C-3), 56.63 (C-14), 50.46 (C-9), 44.7 (C-13), 42.29 (C-4), 38.6 (C-12), 37.11 (C-1), 36.8 (C-10), 35.7 ($=N{-}OCH_2CH_2CH_2\underline{C}H_2$), 33.8 ($=N{-}OCH_2CH_2CH_2\underline{C}H_2Br$), 32.58 (C-15), 31.8 (C-8), 31.61 (C-7), 30.28 (C-2), 28.7 ($=N{-}OCH_2\underline{C}H_2$), 24.7 ($=N{-}OCH_2CH_2\underline{C}H_2$), 20.93 (C-11), 19.3 (C-19), 15.88 (C-21), 14.3 (C-18). MS (*m*/*z*) 478 (M^++1, Br^{79}), 480 (M^++1, Br^{81}). Anal. Calcd for $C_{26}H_{40}BrNO_2$: C, 65.26; H, 8.43; Br, 16.70; N, 2.93. Found: C, 65.06; H, 8.46; Br, 16.56; N, 2.91.

5.7. General procedure for the synthesis of compound 20[61] and 21[62]

Conventional acetylation of L-rhamnose (2 g, 12.2 mmol) with acetic anhydride (7.2 mL, 70.6 mmol) and pyridine (25 mL) yielded compound **13** (1,2,3,4 tetra-*O*-acetyl-L-rhamnose) (2.8 g). Compound **13** (2 g, 6.02 mmol) in *N*,*N*-DMF (20 mL) was stirred with hydrazine acetate (830 mg, 1.5 meq) at 50 °C for 2 h (reaction mointored by tlc) and then concentrated. Ethyl acetate (20 mL) was added and the organic layer was washed with water, dried and finally concentrated. Column chromatography of the residue gave **14** (1 g, 57.4%) (2,3,4-tri-*O*-acetyl-L-rhamnose) as a syrup. To a solution **14** (700 mg, 2.4 mmol) in dry dichloromethane (25 mL) was added anhydrous potassium carbonate (350 mg) and trichloroacetonitrile (2.4 mL, 24 mmol). The suspension was stirred for 48 h at room temperature under nitrogen atmosphere. The mixture was filtered over Celite, washed with dichloromethane (20 mL) and the filtrate concentrated under reduced pressure. The residue was purified to give **15** (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl trichloroacetimidate) as a yellow syrup. $[\alpha]_D^{25}$ −50.0 (*c* 1.0, $CHCl_3$). It was further identified by its ^1H NMR. To a stirred mixture of **5** (200 mg, 0.53 mmol) and 4 Å molecular seives in dichloromethane (15 mL) was added a solution of **15** (280 mg, 0.64 mmol) in dichloromethane (10 mL). The resulting mixture was stirred at −10 °C for 15 min after which $BF_3:ET_2O$ was added (0.088 mL). The reaction mixture was stirred for 60 minutes. Ice water (100 mL) was added to the reaction mixture and then extracted with dichloromethane. The organic phase was washed first with 5% aqueous Na_2CO_3 and then with water, dried over anhydrous sodium sulphate. The dried organic phase was concentrated, column chromatography of the residue ($CHCl_3$:MeOH, 95:5) afforded pure compound **20**.

5.7.1. 16α-[(2,3,4-*tri-O*-Acetyl-α-L-rhamnopyranosyl)oxy-ethoxy]3β-hydroxy pregn-5-en-20-one (20). Syrupy, $[\alpha]_D^{25}$ +66.6 (*c* 0.75, MeOH); IR (KBr) ν_{max} 3435, 2955, 1720, 1665 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm) 5.34–5.30 (2H, m, H-2′, H-3′), 5.28–5.27 (1H, m, H-6); 5.09 (1H, t, H-4′, $J = 9.2$Hz), 4.76 (1H, s, H-1′), 4.49–4.45 (1H, m, H-16); 3.95–3.88 (2H, m, $OCH_2C\underline{H}_2O$-sugar), 3.79–3.70 (1H, m, H-5′); 3.62–3.39 (3H, m, H-3 and $OC\underline{H}_2CH_2O$), 2.60 (1H, d, H-17, $J = 6.3$ Hz), 2.22 (3H, s, CH_3-21), 2.14 (3H, s, OAc), 2.04 (3H, s, OAc), 1.98 (3H, s, OAc), 1.23 (3H, d, CH_3-6′, $J = 6.3$ Hz), 1.05 (3H, s, CH_3-19), 0.63 (3H, s, CH_3-18). MS (*m*/*z*) 648 (M^+).

5.7.2. 16α-[(2,3,4,6-*tetra-O*-Acetyl-α-D-mannopyranosyl)oxy ethoxy]3β-hydroxy pregn-5-en-20-one (21). Syrupy, $[\alpha]_D^{25}$ +28.0 (*c* 0.50, MeOH); IR (KBr) ν_{max} 3440, 2990, 1710, 1645 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 5.35 (m, 1H, H-6), 5.33–5.11 (m, 2H, H-3′, H-4′), 5.02 (s, 1H, H-1′), 4.96 (m, d, H-2′, $J = 2$Hz), 4.62–4.57 (m, 1H, H-5′), 4.57–4.46 (m, 1H, H-16), 4.32–4.28 (m, 2H, H-6′), 4.24–4.06 (m, 4H, m, $OC\underline{H}_2$ $C\underline{H}_2O$), 3.59–3.45 (m, 1H, H-3), 2.59 (d, 1H, H-17, $J = 6.0$Hz), 2.18 (s, 3H, CH_3-21), 2.09 (s, 3H, OAc), 2.06 (3, 3H, OAc), 2.033 (s, 3H, OAc), 2.005 (s, 3H OAc), 1.01 (s, 3H CH_3-19), 0.64 (s, 3H, CH_3-18). MS (*m*/*z*) 707 (M^++1).

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