

## DERIVATIZATION OF STEROIDS FOR OBTAINING CONJUGATES: USE AS VECTORS FOR DRUG DELIVERY

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### ABSTRACT

Mono-mode microwave assisted additions of  $\alpha$ -aminoacids (glycine / valine / phenylalanine) in the presence of  $K_2CO_3$  to 16 $\alpha$ , 17 $\alpha$ -epoxy-3 $\alpha$ -hydroxypregnenolone (**20**) afford corresponding 17 $\beta$ -hydroxy-16-*N*-carboxyalkylaminosteroids (**21a-c**) in good yield. Similar conjugate addition of aminoacids to 16-dehydropregnenolone-3 $\alpha$ -acetate (**15**), under identical conditions, affords 16-*N*-carboxyalkylamino steroidal derivatives (**22a-c**). All the products have been purified chromatographically and characterized spectroscopically. The obtained molecules can be conjugated with molecules/moieties for use as steroidal vectors.

**Keywords:** *steroids, conjugates; vectors; microwave; carboxyalkylaminosteroid*

### INTRODUCTION

Derivatization of steroids has been carried out for desired pharmacological applications. This includes preparation of more potent molecules,<sup>1-3</sup> antihormones,<sup>4,6</sup> steroidal drugs such as anticancer,<sup>7-11</sup> anti-inflammatory,<sup>12</sup> immunosuppressant,<sup>13</sup> antihyperglycemic,<sup>13</sup> antiobesity,<sup>14</sup> antidiabetic,<sup>15</sup> enzyme inhibitors,<sup>16-18</sup> muscle relaxant<sup>19</sup> and steroidal antibodies<sup>20</sup> etc. Transformations of steroids have also been carried out for mapping the distribution of steroid receptors<sup>21</sup> and their labeling for antibody production,<sup>22</sup> and recently for use as vectors for anticancer moieties/ molecules.

The use of steroids for treatment of certain types of tumors is well known and in the past few decades lot of effort has been devoted to utilize steroids for targeting anticancer moieties/ molecules. This approach is getting more importance due to the discovery of steroidal receptors in cancers of different organs e.g., Lung cancer,<sup>23</sup> meningiomas,<sup>24</sup> in addition to brain, kidney, and prostate cancers where the expression of receptor gene is well documented.<sup>7-11</sup> Some of the potential anticancer steroids and steroidal conjugates introduced in the recent past include estradiol and cholesterol derivatives (**1-11**). Among these, the compound (**7**) is a nitrogen mustard conjugate of a bile acid, whereas (**6**), an antiestrogenic aromatase inhibitor is used to treat hormone dependent tumors.<sup>25</sup> Similarly, RU 484 (**8**) is an anti-progestogen, which is used in the therapy of prostate cancer.<sup>25</sup> Useful anticancer properties are also displayed by androstane derivatives such as **10** and **11**.<sup>24,25</sup>

Recently, some amino substituted steroids such as (**12a,b**) have also been involved in glycolipids designed for targeting lipoproteins to hepatic asialoglycoprotein receptors as a part of antiatherosclerotic therapy.<sup>26</sup>

Steroids biotinylated through aminoalkyl tethers (**13, 14**) have been developed as chemical inducers of protein dimerization that efficiently dimerizes estrogen receptors and streptavidin proteins.<sup>27</sup>

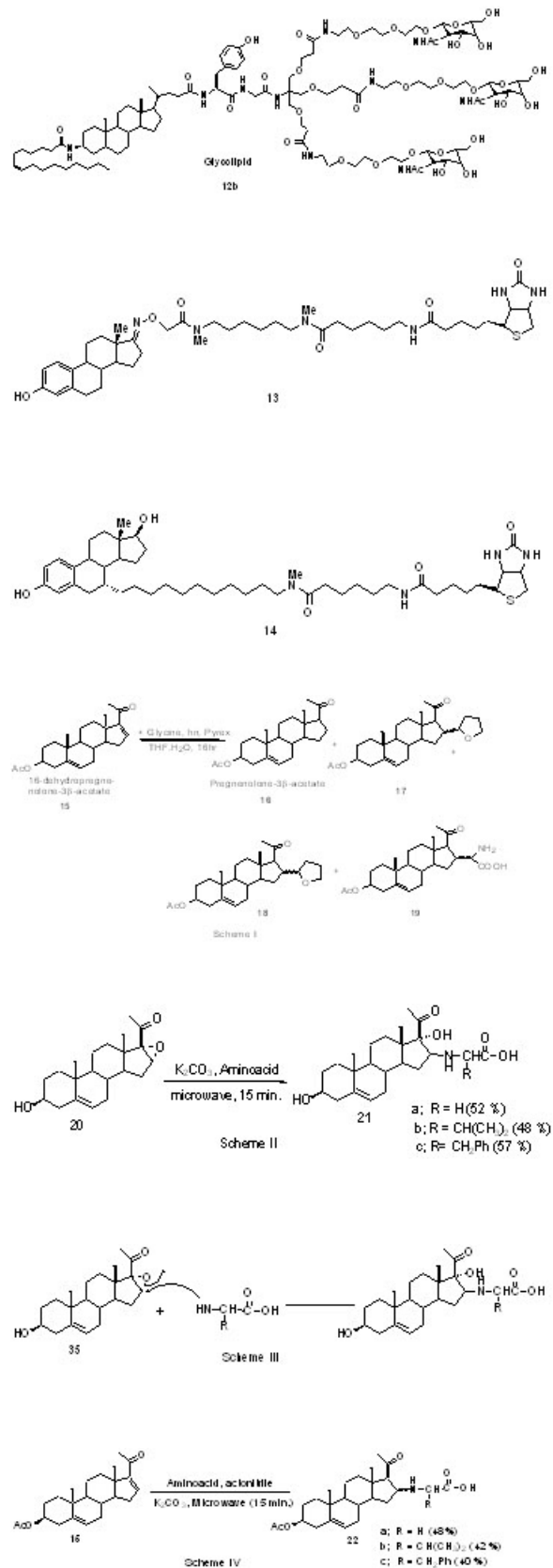
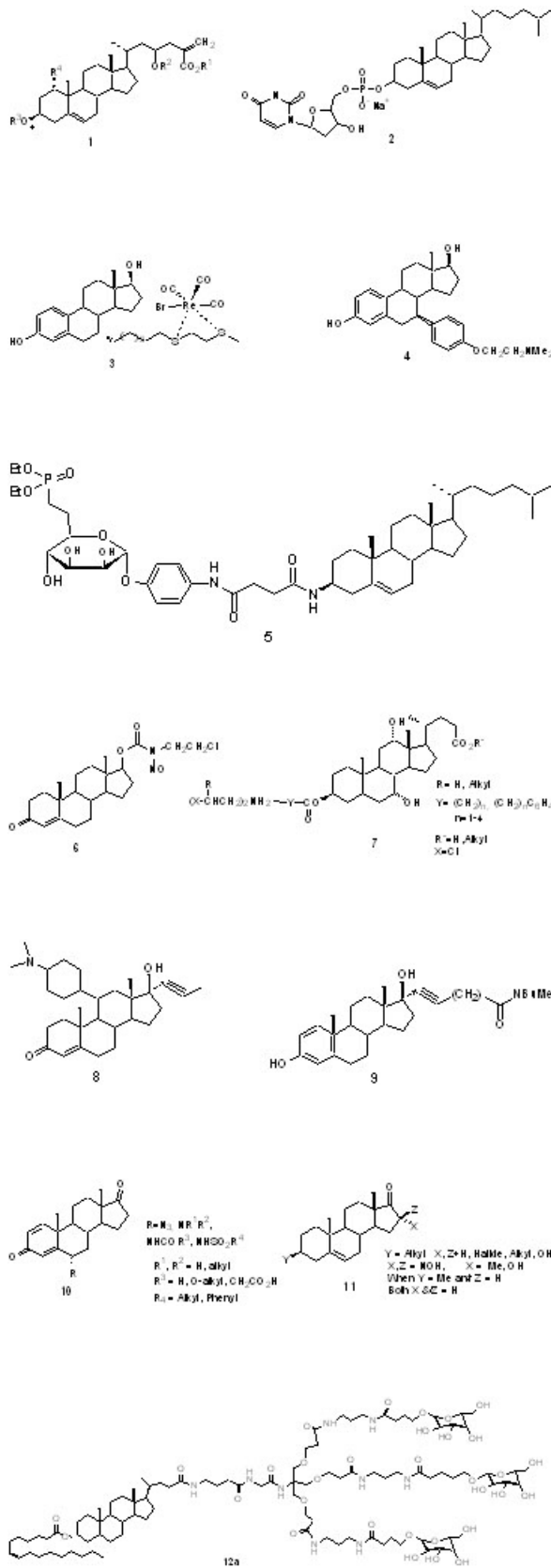
Amino substituted steroids have also been exploited as polyfunctionalized well defined scaffolds with potential medicinal, supramolecular and combinatorial applications.<sup>28</sup>

Though, a variety of strategies have been exploited for functionalization of steroidal nucleus,<sup>1-28</sup> recently Ishar *et. al.*, with the objective of developing substituted steroids as vector for conjugation with anticancer moieties, have investigated photochemical irradiation of  $\alpha, \beta$ -unsaturated steroidal ketones (**15**) in aqueous organic solvent, in the presence of amino acids for one step addition of amino acid residues to steroidal nucleus;<sup>29</sup> a variety of solvent and aminoacid addition products have been isolated (**16-19**, Scheme I).<sup>29</sup>

Consequently, development of efficient routes to suitably functionalized steroids and preparation of their conjugates with biologically active molecules is of considerable interest. In recent times another non-conventional method, which has shown tremendous potential in organic synthetic methodology is the microwave irradiation.<sup>30</sup> It was decided to carry out microwave assisted addition of aminoacid to steroidal epoxide and  $\alpha, \beta$ -unsaturated steroids to obtain valuable aminoacid substituted steroids. Thus, under present investigation following reactions were carried out:

- Microwave assisted reaction of 16 $\alpha$ , 17 $\alpha$ -epoxy-3 $\alpha$ -hydroxypregnenolone (**20**) with aminoacids
- Microwave assisted reaction of 16-dehydropregnenolone-3 $\alpha$ -acetate (**15**) with aminoacids

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### RESULTS AND DISCUSSION

Epoxide (**20**) was reacted with aminoacids under microwave irradiation conditions. It (**20**) was dissolved in tetrahydrofuran and mixed with aqueous solution of aminoacid (glycine/valine/ phenylalanine) followed by the addition of  $K_2CO_3$  (200 mg) to the solution. The resulting mixture was subjected to vacuum and warming till a dry solid mass was obtained. This solid mass was suspended in acetonitrile in a reaction vessel, and subjected to microwave irradiation in a mono-mode microwave reactor (CEM-Discover) for 15 minutes. Solvent was again evaporated and the residue stirred with chloroform. The chloroform extract was concentrated to a solid, which was resolved column chromatographically over silica gel to obtain unreacted (**20**) along with other components characterized spectroscopically (IR,  $^1H$  NMR,  $^{13}C$  NMR and mass) as 21a-c (Scheme II).

The assigned structures are based on detailed spectroscopic analysis. The incorporation of aminoacid residue was established by mass spectra (ESI). The IR spectrum of **21b** revealed besides, broad bands due to NH/OH moieties, a band at  $1755\text{ cm}^{-1}$  (-COOH) and another carbonyl band at  $1718\text{ cm}^{-1}$ . The incorporation of valine in 21b was established by deuterium exchangeable resonances at  $\delta$  10.51 and  $\delta$  4.35. The  $\alpha$ -proton of aminoacid residue appeared at  $\delta$  3.96, the characteristic steroidal proton multiplets in the upfield part of the  $^1H$  NMR spectrum revealed a doublet due to methyls of the valine moiety at  $\delta$  0.98, besides singlets due to steroidal methyls. The  $^{13}C$  NMR spectrum provided most conclusive evidence about the assigned structure. It revealed a carbonyl carbon resonance (COOH) at  $\delta$  175.4 and an oxygen linked quaternary carbon resonance at  $\delta$  106.8 attributed to  $C_{17}$ ; other oxygen linked carbon resonance ( $C_3$ ) appeared at  $\delta$  70.9. Resonances at  $\delta$  67.5 and 57.4 could be attributed to  $\alpha$ -carbon of aminoacid residue and  $C_{16}$ . The stereochemical assignment at  $C_{16}$  though not established unequivocally, is based primarily on mechanistic considerations and spectroscopic data alluded to the presence of a single isomer in each case. Structural assignments of 21a and 21c have also been similarly achieved.

Mechanistically, the reaction can be described as simple nucleophilic opening of the epoxide ring under basic conditions ( $K_2CO_3$ ) and microwave irradiation (Scheme-III).

The investigations were further extended to 16-dehydropregnenolone-3 $\alpha$ -acetate (**15**) because the above results indicated that it may be possible to bring about microwave assisted Michael addition of aminoacids to  $\alpha$  - unsaturated ketone moiety in **15** to obtain aminoacid bearing pregnenolone derivatives.

16-Dehydropregnenolone-3 $\alpha$ -acetate (**15**) was dissolved in tetrahydrofuran and mixed with aqueous solution of aminoacid (glycine / valine / phenylalanine) in the presence of  $K_2CO_3$  and heated so that both aminoacid and steroid are solubilized. This mixture was

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then dried under reduced pressure to obtain a solid mass, which was resuspended in acetonitrile and in a 150 ml round bottom flask and subjected to microwave irradiation (CEM-Discover, focused monomode microwave apparatus; 2450 MHz, 300 W) at 20 power level for 15 minutes (1 min hold time and 14 minute running time). Reaction vessels were taken out and on evaporating acetonitrile under reduced pressure a solid separated out, water (~100 ml) was added and the contents were extracted with two 150 ml portions of chloroform; the chloroform extract was dried over anhydrous  $MgSO_4$ , filtered and concentrated under vacuum to obtain a solid residue. The residue obtained on evaporation of aqueous layer contained only amino acid. The chloroform residue obtained was resolved by column chromatograph over silica gel to obtain various components, some of which were further purified by preparative TLC and their spectroscopic data (IR,  $^1H$  NMR,  $^{13}C$  NMR and mass) was recorded. The major component in each case has been characterized as amino acid addition product (**22**, Scheme IV).

The assigned structures (**22a-c**) are based on detailed spectroscopic analysis. The formation of 1:1 aminoacid-steroid addition product was established by mass spectra (ESI). The IR spectrum of **22a** revealed carbonyl bonds at  $1758\text{ cm}^{-1}$  (-COOH),  $1732\text{ cm}^{-1}$  ( $CH_3COO^-$ ) and  $1716\text{ cm}^{-1}$  ( $CH_3CO$ ). Its proton NMR showed the absence of erstwhile  $C_{16}$ -H olefinic resonance, which appears in the  $^1H$  NMR spectra of 16-DPA (**27**) at  $\delta$  6.75. This together with the IR absorption band of  $C_{20}$  carbonyl ( $1716\text{ cm}^{-1}$ ) and the absence of erstwhile  $C_{16}$ - $C_{17}$  olefinic-C resonances in the  $^{13}C$  NMR spectrum of **22a**, and  $^{13}C$  NMR chemical shift of  $C_{20}$ , indicated the absence of  $C_{16}$ - $C_{17}$  double bond in **22a**. Other  $^1H$  NMR and  $^{13}C$  NMR assignments (vide-experimental) are in consonance with the assigned structure. Resonances of  $C_{17}$  and  $C_{16}$  in case of **22a** appeared at  $\delta$  63.7 and  $\delta$  56.9, indicating the linking of  $C_{16}$  with amino function of aminoacids. Structures of **22b** and **22c** have also been similarly assigned. The stereochemical disposition at  $C_{16}$  is not clear whereas stereochemical assignment at  $C_{17}$  is based on the chemical shift of  $C_{17}$ . The reaction can be described as Michael addition of aminoacid to  $\alpha$  -unsaturated carbonyl moiety of (**15**).

### CONCLUSIONS

Microwave irradiation of 16 $\alpha$ ,17 $\alpha$ -epoxy-3 $\alpha$ -hydroxypregn-5-ene-20-one (**20**) and 16-dehydropregnenolone-3 $\alpha$ -acetate (**15**) in the presence of  $K_2CO_3$  and aminoacid has furnished the valuable aminoacid addition products in moderate yields and within a very short reaction times. Possibly, their yield can be further improved by carrying out reactions under mild Lewis acid catalyzed conditions. The obtained molecule can be conjugated with biologically active moieties through carboxylic function. The steroid moiety can also be converted to  $\Delta^4$ -3-one system by oxidation. The resulting steroidal conjugates shall be valuable vectors for drug targeting.

## EXPERIMENTAL

**General:** Starting materials and reagents were purchased from commercial suppliers and used after further purification (crystallization/ distillation). Bruker AC-200 FT (200 MHz) and JEOL AL-300FT (300 MHz) spectrometers were used to record  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR (50 and 75 MHz) spectra. Chemical shifts ( $\delta$ ) are reported as down field displacements from TMS used as internal standards and coupling constant ( $J$ ) are reported in Hz. IR spectra were recorded with Shimadzu DR- 2001 FT-IR spectrophotometer on KBr pellets or in  $\text{CCl}_4$ . Mass Spectra, ESI- method, were recorded on Bruker Daltonics Esquire 300 mass spectrometer. The CEM- Discover Focused Monomode Microwave apparatus (2450 MHz, 300 W) was used for microwave irradiation. Column chromatography was conducted using silica gel (60-120 or 100-200 mesh). All melting points are uncorrected and measured in open glass capillaries on a Veego MP-D digital melting point apparatus. 16-Dehydropregnenolone acetate was kindly provided by Mehra Pharmaceuticals, Amritsar, India and 16 $\alpha$ , 17 $\alpha$ -epoxy-3 $\alpha$ -hydroxypregn-5-ene-20-one (**20**) was prepared from 16-dehydropregnenolone-3 $\alpha$ -acetate (**15**) by literature method.<sup>31</sup>

**General procedure for reaction of 16 $\alpha$ , 17 $\alpha$ -epoxy-3 $\alpha$ -hydroxypregnenolone (**20**) with aminoacids under microwave irradiation**

Epoxy-steroid (**20**, 100 mg) was solubilized in 20 ml of tetrahydrofuran (THF), and excess of aminoacid (valine/ glycine/ phenylalanine, ~ 10 equivalents) was dissolved in water (5 ml) in another conical flask. These two parts were then mixed in the presence of  $\text{K}_2\text{CO}_3$  (200 mg) and heated so that both aminoacid and steroid are solubilized. This mixture was then dried under reduced pressure to obtain a solid mass. The obtained solid mass was again resuspended in acetonitrile in a 150 ml round bottom flasks. It was fitted with a condenser with the lid and the contents were subjected to microwave irradiation in a CEM- Discover Focused Monomode Microwave apparatus (2450 MHz, 300 W) at 20 power level for 15 minutes (1 min hold time and 14 minute running time). The reaction vessels were taken out and acetonitrile was evaporated under reduced pressure to recover solid. Water (~100 ml) was added and the contents were extracted with chloroform (150 ml, twice); the residue obtained on evaporation of aqueous layer contained only amino acid. The chloroform extract was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and solvent was again removed under reduced pressure. The residue obtained was subjected to column chromatography over silica gel (60-120 mesh, column packed in Hexane and eluted with Hexane-EtOAc gradient and EtOAc, EtOAc-MeOH gradient) to obtain various components, some of which were further purified by preparative TLC (2 mm thick plates, developed with 0.5% MeOH in  $\text{CHCl}_3$  and components extracted with methanol) and their spectroscopic data was recorded.

**Microwave assisted reaction of 16 $\alpha$ , 17 $\alpha$ -epoxy-3 $\alpha$ -hydroxypregnenolone (**20**) in the presence of glycine**

Microwave assisted reaction of 16 $\alpha$ , 17 $\alpha$ -epoxy-3 $\alpha$ -hydroxypregnenolone (**20**, 100 mg) in the presence of glycine (200 mg.) under conditions described above, for 15 min and chromatographic resolution of the microlysate afforded *N*-(3 $\alpha$ -16 $\alpha$ -dihydroxypregnenolone-16 $\alpha$ -yl)glycine (**21a**), a light brown gummy material (63.0, mg):  $\nu_{\text{max}}$  ( $\text{CCl}_4$ ): 3320 (br, NH / OH), 3100 (br, NH /OH), 2970, 2965, 2945, 2925, 2900, 2840, 1750, 1716, 1654, 1601, 1563, 1484, 1462, 1422, 1390, 1264 and 1284  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 200  $\text{MHz}$ ):  $\delta$  10.7 (br, 1H), 6.25 (br d, NH), 5.34 (d, 1H,  $J$  4.30 Hz,  $\text{C}_6$ -H), 3.58-3.50 (m, 1H,  $\text{C}_3$ -H), 3.34 (br d, 2H), 2.90 (dd, 1H,  $J$  3.6, 6.2 Hz) 2.78-0.70 (overlapping multiplets, 31 H, with singlets at  $\delta$  2.16, 1.10, 0.70, methyls);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  210.7 ( $\text{C}_{20}$ ), 176.2 (-COOH), 139.8 ( $\text{C}_5$ ), 121.6 ( $\text{C}_6$ ), 107.4 ( $\text{C}_{17}$ ), 70.9 ( $\text{C}_3$ ), 57.4, 53.9, 52.4 50.6, 49.2, 44.1, 38.8, 38.8, 37.9, 36.8, 31.0, 31.2, , 27.7, 21.3, 20.6, 17.4, 14.7; Mass (ESI):  $m/z$  429.2, ( $\text{M} + \text{Na}+1$ ) $^+$ , 428.2 ( $\text{M} + \text{Na}$ ) $^+$ .

**Microwave assisted reaction of 16 $\alpha$ , 17 $\alpha$ -epoxy-3 $\alpha$ -hydroxypregnenolone (**20**) in the presence of valine**

Microwave assisted irradiation of 16 $\alpha$ , 17 $\alpha$ -epoxy-3 $\alpha$ -hydroxypregnenolone (**20**, 100 mg) in the presence of valine (200 mg.) under conditions described above, for 15 min and chromatographic resolution of the microlysate afforded *N*-(3 $\alpha$ -16 $\alpha$ -dihydroxypregnenolone-16 $\alpha$ -yl)valine (**21b**), a yellow solid material (65.0, mg); mp. 157-159  $^\circ\text{C}$ :  $\nu_{\text{max}}$  (KBr): 3315 (br, NH, OH), 3150 (br, NH, OH), 2978, 2945, 2925, 2900, 2880, 2840, 1755, 1718, 1650, 1605, 1560, 1480, 1465, 1425, 1390, 1260 and 1280  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300  $\text{MHz}$ ):  $\delta$  10.5 (br, 1H), 5.33 (d, 1H,  $J$  4.30 Hz,  $\text{C}_6$ -H), 4.35 (br d, NH), 3.96 (m, 1H), 3.58-3.50 (m, 1H,  $\text{C}_3$ -H), 2.92(dd, 1H,  $J$  4.2x 5.8 Hz) 2.78-0.70 [overlapping multiplets, 37 H, with singlets at  $\delta$  2.15, 1.15, 0.72 (methyls) and doublet at  $\delta$  0.98 (methyls)];  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  208.7 ( $\text{C}_{20}$ ), 175.4 (COOH), 140.1 ( $\text{C}_{15}$ ), 121.8 ( $\text{C}_6$ ), 106.8 ( $\text{C}_{17}$ ), 71.1 ( $\text{C}_3$ ), 67.5, 57.2, 54.1, 50.8, 49.1, 45.7, 43.9, 38.7, 37.7, 36.8, 31.9, 31.4, 30.1, 27.7, 21.3, 20.6, 19.3, 17.4, 14.7; Mass (ESI):  $m/z$  471.2, ( $\text{M} + \text{Na}+1$ ) $^+$ , 470.2 ( $\text{M} + \text{Na}$ ) $^+$ .

**Microwave assisted irradiation of 16 $\alpha$ , 17 $\alpha$ -epoxy-3 $\alpha$ -hydroxypregnenolone (**20**) in the presence of phenylalanine**

Microwave assisted irradiation of 16 $\alpha$ , 17 $\alpha$ -epoxy-3 $\alpha$ -hydroxypregnenolone (**20**, 100 mg) in the presence of glycine (200 mg.) under conditions described above, for 15 min and chromatographic resolution of the microlysate afforded *N*-(3 $\alpha$ -16 $\alpha$ -dihydroxypregnenolone-16 $\alpha$ -yl)phenylalanine (**21c**) a white solid material (85.0, mg); mp 169-171  $^\circ\text{C}$ :  $\nu_{\text{max}}$  (KBr): 3320 (br, NH / OH), 3160 (br, NH, extends up to 3000  $\text{cm}^{-1}$ , aromatic C-H, OH), 2972, 2963, 2945, 2927, 2905,

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2844, 1752, 1717, 1645, 1605, 1563, 1520, 1500, 1484, 1462, 1422, 1390, 1264 and 1284  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 300  $\text{MHz}$ ):  $\delta$  10.5 (br, 1H), 7.25(m, 2H), 7.16 (d 2H, J 6.8 Hz), 7.10 (t, 1H, J 7.2 Hz) 5.85 (br d, NH), 5.34 (d, 1H, J 4.30 Hz,  $\text{C}_6\text{-H}$ ), 3.90 (br d, J, 5.64), 3.56-3.50 (m, 1H,  $\text{C}_3\text{-H}$ ), 2.88 (dd 1H, J 3.65, 6.24 Hz), 2.78-0.70 (overlapping multiplets, 32 H, with singlets at  $\delta$  2.14, 1.05, 0.72, methyls);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  209.4 ( $\text{C}_{20}$ ), 174.2 (COOH), 148.2, 140.1 ( $\text{C}_5$ ), 132.2, 130.7, 129.3, 120.4 ( $\text{C}_6$ ), 106.2 ( $\text{C}_{17}$ ), 71.2 ( $\text{C}_3$ ), 65.2, 56.1, 52.6, 51.4, 49.1, 48.2, 43.4, 37.5, 37.8, 36.6, 35.4, 32.9, 30.1, 26.1, 20.9, 19.8, 16.1, 13.8; Mass (ESI):  $m/z$  519.6 (M + Na) $^+$ , 518.6 (M + Na) $^+$ .

### General procedure for reaction of 16-dehydropregnenolone-3 $\beta$ -acetate(15) with aminoacids under microwave irradiation

Steroid (15, 100 mg) was solubilized in 20 ml of tetrahydrofuran (THF) and excess of aminoacid (valine / glycine / phenylalanine, ~ 10 equivalents) was dissolved in water (5 ml) in another conical flask. The two solutions were then mixed in the presence of  $\text{K}_2\text{CO}_3$  (200 mg) and heated so that both aminoacid and steroid are solubilized. This mixture was then dried under reduced pressure to form the solid mass. This solid mass was again resuspended in acetonitrile and subjected to microwave irradiation (CEM-Discover Focused Monomode Microwave apparatus, 2450 MHz, 300 W) at 20 power level for 15 minutes. After the completion of reaction, contents were taken out and acetonitrile was evaporated under reduced pressure and a solid mass separated out. Water (~100 ml) was added and the contents were extracted with chloroform (150 ml, twice); the residue obtained on evaporation of aqueous layer contained only amino acid. The chloroform extract was dried over anhydrous  $\text{MgSO}_4$ , filtered and solvent was again removed under reduced pressure. The residue obtained was column chromatograph on silica gel (60-120 mesh, column packed in hexane and eluted with Hexane-EtOAc gradient, EtOAc, EtOAc-MeOH gradient) to obtain various components, some of which were further purified by preparative TLC (as discussed earlier) and their spectroscopic data was collected.

### Microwave assisted reaction of 16-dehydropregnenolone-3 $\beta$ -acetate (15) in the presence of glycine

Reaction of 16-dehydropregnenolone-3 $\beta$ -acetate (15, 100 mg) in the presence of glycine (200 mg) under conditions described above, for 15 min and chromatographic resolution of the microlysate afforded *N*-(3 $\beta$ -hydroxypregnenolone-16-yl)glycine (22a), a dark brown gummy material (58.0, mg):  $\nu_{\text{max}}$  ( $\text{CCl}_4$ ): 3315(broad d, NH, OH), 3130 (broad d, NH, OH), 2967, 2964 2944, 2922, 2998, 2835, 1745, 1724, 1665, 1599, 1559, 1485, 1458, 1424, 1397, 1261 and 1288  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 300  $\text{MHz}$ ):  $\delta$  9.98 (broad d, 1H), 5.98 (broad d, NH), 5.56 (d, 1H, J 4.30 Hz,  $\text{C}_6\text{-H}$ ), 3.45 (broad

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d, 2H), 2.89 (dd, 1H, J 3.7, 6.2 Hz) 2.56-0.77 [overlapping multiplets, 31 H, with singlets at  $\delta$  2.18, 1.12, 0.69, methyls];  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  211.7 ( $\text{C}_{20}$ ), 178.1 (COOH), 172.3 ( $\text{CH}_3\text{COO-}$ ), 138.7 ( $\text{C}_5$ ), 122.3 ( $\text{C}_6$ ), 71.1 ( $\text{C}_3$ ), 56.7, 52.3, 51.1, 50.7, 48.5, 43.8, 37.4, 37.0, 36.8, 35.5, 31.2, 30.4, 26.2, 21.2, 16.1, 15.4; Mass (ESI):  $m/z$  454.2 (M + Na) $^+$ .

### Microwave assisted reaction of 16-dehydropregnenolone-3 $\beta$ -acetate (15) in the presence of valine

Microwave assisted irradiation of 16-dehydropregnenolone-3 $\beta$ -acetate (15, 100 mg) in the presence of valine (200 mg) under conditions described above, for 15 min and chromatographic resolution of the microlysate afforded *N*-(3 $\beta$ -hydroxypregnenolone-16-yl)valine (22b), a light yellow semisolid material (55.0, mg);  $\nu_{\text{max}}$  ( $\text{CCl}_4$ ): 3350 (br, NH, OH), 3010 (br, NH, OH), 2969, 2951, 2930, 2905, 2880, 2844, 1758, 1732, 1717, 1649, 1610, 1565, 1489, 1456, 1431, 1385, 1266 and 1285  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 300  $\text{MHz}$ ):  $\delta$  9.06 (br, 1H), 5.32 (d, 1H, J 5.01 Hz,  $\text{C}_6\text{-H}$ ), 4.52 (m, 1H,  $\text{C}_3\text{-H}$ ), 4.38 (br d, NH), 3.96 (m, 1H), 2.82 (dd, 1H, J 4.4 & 5.9 Hz), 2.80-0.69 [overlapping multiplets, 40 H, with singlets at  $\delta$  2.35, 2.16, 1.05, 0.68 (methyls) and doublet at  $\delta$  0.92 (methyl)];  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  210.1 ( $\text{C}_{20}$ ), 177.2 (COOH), 170.2 ( $\text{CH}_3\text{COO-}$ ), 142.7 ( $\text{C}_5$ ), 122.2 ( $\text{C}_6$ ), 74.1 ( $\text{C}_3$ ), 66.1, 63.7, 56.9, 51.9, 50.2, 49.9, 44.6, 42.8, 37.4, 37.1, 35.9, 32.9, 30.3, 29.9, 26.3, 20.4, 20.0, 19.8, 19.4, 16.7, 15.3; Mass (ESI):  $m/z$  496.6 (M + Na) $^+$ .

### Microwave assisted irradiation of 16-dehydropregnenolone-3 $\beta$ -acetate (15) in the presence of phenylalanine

Microwave assisted irradiation of 16-dehydropregnenolone-3 $\beta$ -acetate (27, 100 mg) in the presence of phenylalanine (200 mg.) under conditions described above, for 15 min and chromatographic resolution of the microlysate afforded *N*-(3 $\beta$ -hydroxypregnenolone-16-yl) phenylalanine (22c), a white solid material (58.0, mg); mp 162-164  $^\circ\text{C}$ ;  $\nu_{\text{max}}$  (KBr): 3320 (br, NH, OH), 3160 (br, NH, extends up to 3000  $\text{cm}^{-1}$ , aromatic C-H, OH), 2972, 2963 2945, 2927, 2905, 2844, 1752, 1717, 1645, 1605, 1563, 1520, 1500, 1484, 1462, 1422, 1390, 1264 and 1284  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 300  $\text{MHz}$ ):  $\delta$  10.5 (br, 1H), 7.25(m, 2H), 7.16 (d 2H, J 6.8 Hz), 7.10 (t, 1H, J 7.2 Hz) 5.85 (br d, NH), 5.34 (d, 1H, J 4.30 Hz,  $\text{C}_6\text{-H}$ ), 3.90 (br d, J, 5.64), 2.88 (dd 1H, J 3.65, 6.24 Hz), 2.78-0.70 (overlapping multiplets, 32 H, with singlets at  $\delta$  2.14, 1.05, 0.72, methyls);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  209.4 ( $\text{C}_{20}$ ), 174.2 (COOH), 171.9 ( $\text{CH}_3\text{COO-}$ ), 148.2, 140.1 ( $\text{C}_5$ ), 132.2, 130.7, 129.3, 120.4 ( $\text{C}_6$ ), 79.3 ( $\text{C}_3$ ), 65.2, 56.1, 52.6, 51.4, 49.1, 48.2, 43.4, 37.5, 37.8, 36.6, 35.4, 32.9, 30.1, 26.1, 20.9, 19.8, 16.1, 13.8; Mass (ESI):  $m/z$  544.4 (M + Na) $^+$ .

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### ACKNOWLEDGEMENT

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