CHAPTER 4

MW Energy in Ugi-4CR: Synthesis of Some Hetero-Steroid-Amino acid conjugates - Another Class of Novel Steroid Hybrid Molecules
4.1: Introduction

As discussed in the previous Chapters, it is seen that the structurally modified steroids have attracted a great deal of attention in recent years. Naturally occurring steroid nuclei have been modified in several ways with the aim of finding more active compounds, free from undesirable or harmful side effects and of recognizing the structural and stereochemical features required for the display of specific, selective physiological activity. Among the various known analogues of steroids, heterostereoids or heterocyclic steroids have received much attention in view of their diverse and interesting biological activities. Marked changes in the biological properties of steroid molecules are observed through isosteric replacement of carbon atoms of the steroid backbone by hetero atoms (O, S or N). The hetero steroids thus formed have been found to show localized changes in hydrogen bonding capacity and hydrophobicity with no significant changes in the overall conformation of the steroid nucleus or enhanced steric bulk. Among them, azasteroids have received much attention during the last decade due to their numerous biological activities. Thus hetero steroids emerge as an important area of research and enormous efforts have been made for their synthesis because of inherent biological activities. Considering the importance of heterostereoids, in this chapter we like to report the synthesis of Steroid-Peptide Conjugates based on some hetero-stereoids containing N, O and S coupling with N-(tert-butoxycarbonyl)-L-phenylalanine as the amino acid residue applying MW prompted Ugi-4CR. Hetero-steroids are synthesized from abundant steroids as per literature procedures and were subjected to Ugi-4CR to get a number of Hetero-Steroid-Peptide Conjugates- a class of
new Steroid-Peptide Conjugate so far not reported in literature and might possesses diverse biological activities.

**4.2: Results and Discussion**

The present study describes a microwave-prompted synthesis of a number of Hetero-Steroid-Amino Acid Conjugates utilizing Ugi-4CR as the coupling reaction with an amino acid residue in presence of benzyl isocyanide, paraformaldehyde and triethyl amine. The amino acid used included N-(tert-butoxycarbonyl)-L-phenylalanine, Hetero-Steroids with an amino group in them were synthesized from abundant steroid molecules, viz., 16-DPA and its relatives and epiendrosterone using literature procedure for their eventual subjecting to MW irradiated Ugi-4CR to get the desired Hetero-Steroid Peptide Conjugate. All the Hetero-Steroid Peptides Conjugates thus synthesized were fully characterised by their IR, NMR and Mass spectroscopy analysis. This new class of Steroid-Peptide Conjugates may possess diverse biological properties for their commercial exploitations.

**4.3: Synthesis of Hetero-Steroid Amino Acid Conjugates**

**4.3.1: Hetero-Steroid-Amino Acid Conjugate (4) based on D-ring fused hetero steroidal amine**

Scheme 4.1 describes the synthesis of Hetero-Steroid-Amino Acid conjugate (4) starting from epiendrosterone (1) to incorporated a hetero cyclic ring fused to its D-ring.

Epiendrosterone (1) was brominated by cupric bromide in refluxing methanol to furnish its 16β-bromo-epiendrosterone (2) as the sole product and was then treated
with thiourea in presence triethyl amine to get 2-amino-thiazolehydroepiandrosterone (3) in 65% yield. The spectral data of compounds (2) and (3) were compared with authentic samples \(^6\) to confirm their structures. The hetero steroid (3) with an amino group was then subjected to the conjugation process with N-(\textit{tert}-butoxycarbonyl)-L-phenylalanine, benzyl isocyanide, paraformaldehyde and triethylamine to get the desired hetero-steroid amino acid conjugate (4) in 78% yield. In its IR spectrum, the characteristic N-H bending absorption frequency appeared near 1650 cm\(^{-1}\). In its \(^1\)H NMR spectrum, the newly introduced ten aromatic protons appeared as multiplets in the region \(\delta\ 7.13-7.31\) ppm. In its \(^{13}\)C NMR spectrum, three carbonyl atoms appeared as separate peaks at \(\delta\ 158.1, 165.3\) and 171.5 ppm. All these data are in quite conformity with the structure as represented by (4).
**Scheme 4.1 Reagent and conditions**

i) CuBr$_2$, MeOH, reflux; ii) thiourea, Et$_3$N, MeOH, reflux; iii) Ugi-4CR: N-(tert-butoxycarbonyl)-L-phenylalanine, paraformaldehyde, triethyl amine, benzylisocyanide.

Under classical Ugi-4CR, however hetero-steroidal amine (3) could furnish only 25% yield of the desired Hetero-Steroid-Peptide Conjugate (4) in 48h with same amino acid residue.

### 4.3.2: Hetero-Steroid-Amino Acid Conjugate (12) based on A ring hetero steroidal amine

**Scheme 4.2** was formulated to synthesize the Hetero-Steroid-Amino Acid conjugate (11) starting from easily available progesterone (5) after incorporating a hetero atom in its ring A. The compound (5) on oxidizing with NaIO$_4$ and KMnO$_4$ in presence of Na$_2$CO$_3$ furnished 5-oxo-A-nor-3,5-seco-steroid-3-oic acid (6) in excellent yield (84%). The structure of the compound (6) was confirmed by direct comparison with the authentic sample. The seco-steroid acid derivative (6) was then subjected to MW irradiation with urea in presence of Lewis acid catalyst BF$_3$.etherate to furnish 3-oxo-4-azasteroid (7) in good yield. The compound (7) was directly compared with the authentic material where its IR, $^1$H NMR and $^{13}$C NMR and mass spectra were in conformity with the proposed structure. The compound (7) on reduction with NaBH$_4$ at 0°C yielded 20β-hydroxy-3-oxo-4-azasteroid (8) as the major product. The compound (8) on chlorination with thionyl chloride furnished 20β-chloro-3-oxo-4-azasteroid (9), which on azidation with NaN$_3$ in DMF furnished corresponding 20α-azido-3-oxo-4-azasteroid (10). In its IR spectrum a strong absorption band at 2087 cm$^{-1}$ confirmed the
presence of an azide group. The compound (10) on reduction with H₂/Pd-C yielded 20α-amino-5α,6-dihydro-3-oxo-4-azasteroid (11) which was fully characterized by its IR, NMR and mass spectral data. The azasteroid (11) containing an amino group at C-20 was then subjected to MW assisted Ugi-4CR with N-(tert-butoxycarbonyl)-L-phenylalanine as described in general procedure to get the desired Hetero-Steroid-Amino Acid Conjugate (12) in 78% yield. In its IR spectrum, the characteristic N-H bending displayed absorption frequency near 1650 cm⁻¹. In its ¹H NMR spectrum, the newly introduced ten aromatic protons appeared as multiplets in the region δ 7.14-7.31 ppm. In its ¹³C NMR spectrum, four carbonyl carbon atoms appeared as separate peaks at δ 155.1, 169.3, 170.6, 171.5 ppm. All these data confirmed its structure to be as (12).
Scheme 4.2: Reagent and codition i) NaIO₄, KMnO₄, reflux; ii) Urea, BF₃·Et₂O, MW; iii) NaBH₄, 0°C; iv) SOCl₂, ether, 0°C; v) NaN₃, DMF; vi) H₂/Pd-C; vii) Ugi-4CR: N-(tert-butoxycarbonyl)-L-phenylalanine, paraformaldehyde, triethyl amine, benzylisocyanide.

However, under classical Ugi-4CR, hetero-amino steroid (11) could furnish only 20-35% yield of the desired Hetero-Steroid-Amino Acid Conjugate (12) in 48h, with same amino acid residue.

4.3.3: Hetero-Steroid-Amino Acid Conjugate (18) based on B-ring hetero steroidal amine

Scheme 4.3 was conceived to synthesize the Hetero-Steroid-Amino Acid Conjugate (18) from the starting material 3β-acetoxy-19-formyloxy-7-iodo-6-nor-5,7-seco-pregn-5,20-dione (14) synthesized as described in Chapter 3. This compound is the immediate precursor of a B-ring hetero steroid to incorporate an oxygen atom in ring B. Thus when this compound (14) was treated with NaBH₄, it furnished the desired oxygen containing B-ring hetero steroid 19, 20-dihydroxy-6-oxa-steroid (13) with simultaneous reduction of C-and formyl groups. The hetero steroid (13) was then subjected to chlorination with thionyl chloride in ether at 0°C to get 19, 20β-dichloro-6-oxa-steroid (15). The compound (15) was fully characterized by its IR, NMR, Mass and elemental analyses. The compound (15) on azidation with NaN₃ in DMF resulted the formation of 19, 20α-(bis)- azido-6-oxa-steroid (16) in 86% yield. The compound (16) exhibited a double strength band at 2087 cm⁻¹ showing the presence of two azide groups in its IR spectrum. The compound (16) on reduction with H₂/Pd-C resulted the 19, 20α-diamino-6-oxa-steroid in 80% yield. In its IR spectrum, disappearance of azide band
was observed with replacement of band for –NH₂ groups at 3400 cm⁻¹. The compound (17) was finally subjected to MW assisted Ugi-4CR with N-(tert-butoxycarbonyl)-L-phenylalanine as described in general procedure to get the desired Hetero-Steroid-Amino Acid Conjugate (18). In its IR spectrum, the characteristic N-H bending displayed absorption frequency near 1650 cm⁻¹. In its ¹H NMR spectra, the newly introduced ten aromatic protons appeared as multiplet in the region δ 7.14-7.31 ppm. In its ¹³C NMR spectrum, six carbonyl groups appeared as separate peaks at δ 155.9, 155.9, 169.5, 170.5, 171.1 and 171.2 ppm. The spectral data confirmed the structure of the Hetero-Steroid-Amino Acid Conjugate as represented by (18).
Scheme 4.3: i) NaBH₄; ii) SOCl₂, ether, 0°C; iii) NaN₃, DMF; iv) H₂/Pd-C; v) Ugi-4CR: N-\((\text{tert}-\text{butoxycarbonyl})\)-L-phenylalanine, paraformaldehyde, triethyl amine, benzylisocyanide.

Under classical Ugi-4CR, however, hetero-steroidal amine (17) could furnish only 22% yield of hetero-steroid-peptide conjugate (18) in 48h.

4.4: Conclusion

In conclusion, MW promoted Ugi-4CR towards the synthesis of this new class of hybrid molecules, viz., Hetero-Steroid-Amino Acid Conjugates based on hetero-steroidal amines might be a useful addition to synthetic organic chemistry. The method would undoubtedly find a significant place in organic synthesis. Besides, Hetero-Steroid-Amino Acid Conjugates might possess diverse biological properties. Further, MW promoted Ugi-4CR would contribute in promoting green technology in organic synthesis.

4.5: Experimental and spectral data

4.5.1: Typical procedure for Ugi-4CR

4.5.2: Under MW irradiation

A mixture of Hetero-Steroidal Amine (741mg, 1mmol), N-\((\text{tert}-\text{butoxycarbonyl})\)-L-phenylalanine (196mg, 1mmol), paraformaldehyde (43mg, 1mmol), triethylamine (0.17mL, 1mmol) and benzylisocyanide (0.15mL, 1mmol) in MeOH (30mL) was irradiated in a closed vessel in a Synthos 3000 microwave reactor at 400 W, 100 °C and 15 bar for 15 min. The reaction mixture was then allowed to cool to rt. The solution was
concentrated under reduced pressure and then poured into water and extracted with CHCl₃ (3x100mL). The organic extract was dried over anhyd. Na₂SO₄ and evaporated under reduced pressure to get a residue which was purified by preparative TLC or column chromatography using combination of ethyl acetate and petroleum ether as eluent to get pure Hetero-Steroid-Amino Acid Conjugates.

4.5.3: Under classical

A solution of N-(tert-butoxycarbonyl)-L-phenylalanine (196mg, 1mmol), paraformaldehyde (43mg, 1mmol) and triethylamine (0.17mL, 1mmol) in MeOH (30mL) were stirred at rt for 1h to accomplish the formation of the corresponding imine. Steroidal amine (741mg, 1mmol) and benzylisocyanide (0.15mL, 1mmol) were then added and the reaction mixture was stirred for 48 h at room temperature. The solution was concentrated under reduced pressure and then poured into water and extracted with CHCl₃ (3x100mL). The organic extract was dried over anhyd. Na₂SO₄ and evaporated under reduced pressure to give a residue which was purified by preparative TLC or column chromatography using combination of ethyl acetate and petroleum ether as eluent to get pure hetero-steroid-amino acid conjugates.

4.6: Experimental and Characterization of Hetero-Steroid-Amino Acid Conjugate based on D-ring fused hetero-steroidal amine

4.6.1: 16β-bromo-epiendrosterone (2)
To a solution of epiandrosterone (1) (1g, 3.3mmol) in methanol (33mL), cupric bromide (1.53g, 6.8mmol) and the solution was reflux for 10 h. After completion of the reaction, the reaction mixture was poured into cold water (200mL) and was extracted with DCM (3×150mL). The organic extract was dried over anhyd. Na₂SO₄ and evaporated under reduced pressure to get a crude product which after purification by preparative TLC (EtOAc: PE::1:15) furnished pure 16β-bromo-epiandrosterone (2) as white solid (850mg, 66%).

mp 170-182°C; IR (CHCl₃): 3369, 1760, 1449 cm⁻¹; ¹H NMR (CDCl₃): δ 0.94 (s, 3H, Me), 1.0 1(s, 3H, Me), 3.6 (m, 1H, 3α-H), 4.54 (t, 1H, 16H); ¹³C NMR: δ 13.7, 14.1, 20.3, 28.1, 30.6, 31.4, 33.3, 34.1, 34.9, 36.8, 37.8, 43.7, 44.7, 46.3, 47.8, 49.2, 54.3, 71.0, 213.4; MS (ESI): m/z 369 (M⁺); Anal. Calcd. For C₁₉H₂₉BrO₂: C, 61.78; H, 7.85; Found: C, 61.79; H, 7.91.

4.6.2: 2-Amino-thiazole-epiandrosterone (3)

To a solution of 16β-bromo-epiandrosterone (2) (200mg, 0.54mmol) in ethanol (10mL), thiourea (198mg, 2.6mmol) and triethylamine (196.7mg, 1.94mmol) were added. The reaction mixture was refluxed for about 24 h during which a pale yellow solid crystal emerged. After completion of the reaction, the mixture was kept in a refrigerator over night to effect further crystallization. The crystals were filtered and
washed by EtOH twice to get the pure 2-amino-thiazole-epiendrosterone (3) as white solid (157mg, 84%).

mp 230°C; IR (CHCl₃): 3450, 1628, 1444 cm⁻¹; ¹H NMR (500 MHz, DMSO) δ 0.81 (s, 3H, 19-Me), 1.00 (s, 3H, 18-Me), 3.26 (m, 1H, 3a-H), 4.64 (D₂O exchangeable, d, 1H, J = 4.64 Hz, 3-OH), 6.75 (D₂O exchangeable, s, 2H, –NH₂); ¹³C NMR (125 MHz, DMSO), 17.0, 19.5, 20.6, 27.7, 30.4, 31.1, 31.9, 34.6, 36.8, 37.2, 39.9, 42.3, 42.7, 50.7, 59.4, 70.4, 117.0, 120.5, 142.1, 171.8; MS (ESI): m/z 346 (M⁺); Anal. Calcd. For C₂₀H₃₀N₂SO: C, 69.3; H, 8.67; N, 8.09; Found: C, 69.32; H, 8.73; N, 8.08.

![Chemical structure](image)

### 4.6.3: Hetero-Steroid-Amino Acid Conjugate (4)

**Ugi-4CR: MW**

741mg (1mmol) of 2-Amino-thiazole-epiendrosterone (3) when subjected to MW assisted Ugi-4CR using N-((tert-butoxycarbonyl)-L-phenylalanine as the amino acid residue as per general procedure furnished pure Hetero-Steroid-Amino-Acid conjugate (4) as yellowish gum (290mg, 84%).

**Ugi-4CR: Classical**

741mg (1mmol) of 2-Amino-thiazole-epiendrosterone (3) when subjected to classical Ugi-4CR using N-((tert-butoxycarbonyl)-L-phenylalanine as the amino acid residue as
per general procedure furnished pure Hetero-Steroid-Amino-Acid conjugate (4) as yellowish gum (75mg, 22%).

\[
\begin{align*}
\text{HO} & \quad \text{S} \\
\text{N} & \quad \text{N} \\
\text{H} & \quad \text{O} \\
\text{HN} & \quad \text{Boc}
\end{align*}
\]

\[\alpha_d^{(-)} 44.44 \text{ (c1, CHCl}_3\text{)}; \text{ IR (CHCl}_3\text{): 3402, 1650, 1437cm}^{-1}; \text{ }^1\text{H NMR (500 MHz, CHCl}_3\text{)} \delta 0.89 \text{ (s, 3H, 19-Me), 1.34 (s, 3H, 18-Me), 1.42 (s, 12H, 3Me); 3.1 (m, 1H, 3\alpha-}
\text{H), 3.7 (m, 2H, NCH), 4.56 ( m, 2H, N-CH}_2\text{), 4.91-5.0 (m, 4H, 2N-NCH}_2\text{), 7.14-7.31 (m, 10H, Ph); }^1\text{C NMR (125 MHz, CDCl}_3\text{): }\delta 12.1, 17.3, 20.3, 27.1, 28.2, 28.3, 28.3, 30.1, 31.0, 31.0, 33.2, 34.3, 36.1, 37.1, 37.2, 37.2, 43.1, 43.2, 45.2, 53.1, 55.3, 55.3, 55.3, 56.2, 71.9, 79.4, 119.3, 125.4, 126.2, 126.2, 126.3, 127.1, 127.3, 128.2, 128.3, 128.3, 136.4, 137.4, 146.3, 155.2, 158.2, 165.2, 171.0; \text{ MS (ESI): m/z 741(M}^+\text{); Anal. Calcd. For C}_{43}\text{H}_{56}\text{N}_4\text{O}_5\text{S: C, 69.63; H, 7.55; N, 9.44; Found: C, 69.70; H, 7.62; N, 7.56.}
\]

4.7: Preparation and Characterization of Hetero-Steroid-Amino Acid Conjugate based on ring A hetero-steroidal amine

4.7.1: 5-oxo-A-nor-3, 5-seco-steroid-3-oic acid (6)

To a solution of progesterone (1g, 3.1mmol) in isopropanol (13mL) was added a solution of Na$_2$CO$_3$ (0.492g, 4.65mmol) in water (2mL). The mixture was brought to reflux and a solution of NaIO$_4$ (4.7g, 21.7mmol) and KMnO$_4$ (0.032g, 0.2mmol) in
warm water (75°C) was added gradually (1h) while reflux temperature was maintained. The reaction was cooled to 30 °C, and after 15 min the solid formed was removed by filtration. The solid was washed with water and the combined filtrates were concentrated under reduced pressure to remove most of the isopropanol. The reaction mixture was cooled and acidified (pH 3) with concentrated HCl solution and extracted with DCM. The organic extract was washed with water and dried over anhyd. Na$_2$SO$_4$. Removal of the solvent under reduced pressure afforded 5-oxo-A-nor-3, 5-seco-steroid-3-oic acid (6) as white solid (0.92g, 87%). The structure of the compound was confirmed through direct comparison of its spectral data with the authentic.  

4.7.2: 4-Aza-3-oxo-pregn-5-ene (7) 

A mixture of 5-oxo-A-nor-3, 5-seco-steroid-3-oic acid (6) (200mg, 0.598mmol), urea (107.8mg, 1.7mmol) and BF$_3$·Et$_2$O (0.5mL, 0.717mmol) was mixed intimately in a mortar and was irradiated in a closed vessel in a Synthos 3000 Microwave Reactor at 300W, 140 °C and 15 bar for 3 min. The reaction mixture was then cooled and poured into cold water (50mL) and extracted with DCM (3× 30mL). The organic extract was washed with water, dried over anhydrous Na$_2$SO$_4$ and the solvent was removed under reduced pressure to obtain a crude product. Column chromatography separation using EtOAc:P.E::1:1 as eluant over silica gel afforded 4-Aza-3-oxo-pregn-5-ene (7) in pure
form (190mg, 95%). The structure of the compound was confirmed by direct comparison of its spectral data with the authentic. \(^7\)

\[
\text{mp 274–276 ◦C; IR (cm}^{-1}\text{): 3061, 2927, 1702, 1682, 1663, 1446, 1398, 1220; } \^1\text{H NMR (CDCl}_3, 300 MHz): } \delta 8.80 (1\text{H, bs, –NH}), 4.94 (1\text{H, s, 6-H}), 2.14 (3\text{H, s, 20-CH}_3), 1.09 (3\text{H, s, 19-CH}_3), 0.66 (3\text{H, s, 18-CH}_3), 2.82–0.62 (18\text{H, m, alkane protons}; } \^1\text{C NMR (CDCl}_3, 300 MHz): 209.98, 170.60, 140.10, 104.03, 63.90, 56.98, 45.92, 44.42, 38.85, 34.37, 33.31, 31.98, 31.86, 29.98, 28.74, 24.77, 23.18, 21.07, 19.12, 13.73; MS (ESI): m/z 316 (M\(^+\)); \text{Anal. Calcd. For C}_{20}\text{H}_{29}\text{NO}_2: C, 76.15; H, 9.27; N, 4.44; Found: C, 75.94; H, 9.17; N, 4.43}

4.7.3: 20β-hydroxy-3-oxo-4-azasteroid (8)

To a solution of 4-aza-3-oxo-pregn-5-ene (7) (300mg, 0.95mmol) in dry MeOH, NaBH\(_4\) (36mg, 0.95mmol) was added portionwise at 0°C under N\(_2\). The mixture was then allowed to reach rt and stirred for 2 h to be followed on TLC. The reaction mixture was then quenched with water and extracted with EtOAc. The organic extract was washed with brine, dried over anhyd. Na\(_2\)SO\(_4\) and evaporated under reduced pressure to get the crude residue which was purified by column chromatography over silica gel using EtOAc:PE::1:3 as the eluent to furnish the 20β-hydroxy-3-oxo-4-azasteroid (8) in pure form white solid (253mg, 84%).
mp 134.3 ºC; IR (CHCl₃): 3193, 3050, 2950, 1683, 1670, 1227 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz): δ 0.87 (s, 3H, 18-Me), 1.08 (s, 3H, 19-Me), 1.18 (s, 3H, 20-Me), 3.6-3.7 (m, 1H, 20-H), 5.04 (t, 1H, 6H), 8.59 (1H, bs, -NH); ¹³C NMR (CDCl₃, 125 MHz): δ 15.1, 18.3, 20.3, 23.3, 24.3, 26.4, 28.1, 29.3, 31.2, 31.2, 37.1, 39.2, 42.3, 48.2, 56.3, 58.4, 69.0, 104.1, 139.4, 169.2; MS (ESI): m/z 317 (M)⁺; Anal. calcd. for C₂₀H₃₁NO₂: C, 75.70; H, 9.77; N, 4.41; Found: C, 75.67; H, 9.84; N, 4.41.

**4.7.4: 20β-chloro-3-oxo-4-azasteroid (9)**

To a solution of compound (8) (0.2g, 0.63mmol) in dry diethyl ether (10mL) was added thionyl chloride (134mg, 1.134mmol) and stirred at 0ºC for 3h. After completion of the reaction, the reaction mixture was treated with a saturated aq. NaHCO₃ solution, more water added and extracted with DCM. The organic extract was dried over anhyd. Na₂SO₄. The crude product obtained after removal of the solvent was purified by silica gel column chromatography using EtOAc:PE::1:10 as the eluent to afford pure 20β-chloro-3-oxo-4-azasteroid (9) as gum (174 mg, 74%).
IR (CHCl₃): 3193, 3050, 2950, 1683, 1227 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 0.87 (s, 3H, 18-Me), 1.08 (s, 3H, 19-Me), 1.1 (s, 3H, 20-Me), 3.34 (m, 1H, 6H), 5.02 (t, 1H, 6H), 8.59 (1H, bs, -NH); ¹³C NMR (CDCl₃, 75 MHz): 14.1, 18.3, 20.3, 23.3, 24.3, 26.4, 28.1, 29.3, 31.2, 31.2, 37.1, 39.2, 42.3, 48.2, 56.3, 58.4, 69.0, 104.1, 139.4, 169.2; MS (ESI): m/z 381 (M⁺); Anal. calcd. for C₂₀H₃₀CNO: C, 71.51; H, 9.00, N, 4.76; Found: C, 71.64; H, 8.95, N, 4.17.

4.7.5: 20α-azido-3-oxo-4-azasteroid (10)

To a stirring solution of sodium azide (76.7mg, 1.18mmol) in dry DMF (15mL), 20β-chloro-3-oxo-4-azasteroid (9) (200mg, 0.59mmol) was added at 90°C. The reaction mixture was then stirred for 24 hours at room temperature and after completion of the reaction, as indicated by TLC, water was added to it and the reaction mixture was then extracted with ether and the aq. phase was further extracted with ethyl acetate. Both the organic phase were mixed and dried over anhyd. Na₂SO₄. The crude product obtained after removal of the solvent under reduced pressure was purified by silica gel column chromatography using EtOAc:PE::1:20 as the eluent to afford 20α-azido-3-oxo-4-azasteroid (10) as white solid (156mg, 86%).

mp 123.6 °C; IR(CHCl₃) 3050, 2087, 1683, 1670, 1227 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 0.84 (s, 3H, 18-Me), 0.92 (s, 3H, 19-Me), 1.01(s, 3H, 20-Me), 2.3 (s, 3H, 21-
4.7.6: 20β-amino-3-oxo-4-azasteroid (11)

150 mg of 20α-azido-3-oxo-4-azasteroid (10) in 15 mL of ethanol was subjected to hydrogenation at 45 psi pressure adding 80 mg of 10% Pd/C for a period of 13 h. The reaction mixture was filtered and alcohol was distilled off under reduced pressure to furnish the crude hydrogenated product which was purified by column chromatography over silica gel using EtOAc:PE::1:1 as the eluent to furnish 20α-amino-3-oxo-4-azasteroid (11) in pure form as white solid (103 mg, 74%).

mp 156.6 °C; IR (CHCl₃): 3400, 1683, 1287 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 0.85 (s, 3H, 18-Me), 0.97 (s, 3H, 19-Me), 1.15 (s, 3H, Me), 2.3 (m, 3H, 21-h); ¹³C NMR (CDCl₃, 75 MHz): δ 14.1, 14.9, 18.7, 20.3, 23.3, 24.3, 26.2, 26.4, 27.3, 28.1, 29.3, 31.2, 31.2, 37.1, 39.2, 42.3, 48.2, 58.3, 58.4, 69.0, 169.2; MS (ESI): m/z 318 (M)+; Anal. calcd. for C₂₀H₃₄N₂O: C, 75.9; H, 10.69; N, 8.85; Found: C, 75.9; H, 10.62; N, 8.86.
4.7.7: Hetero-Steroid-Amino Acid Conjugate (12)

**Ugi-4CR: MW**

318mg (1mmol) of 20α-amino-3-oxo-4-azasteroid (11) when subjected to MW assisted Ugi-4CR using N-(tert-butoxycarbonyl)-L-phenylalanine as the amino acid residue as per general procedure to get the desired Hetero-Steroid-Amino Acid conjugate (12) as yellowish gum (170mg, 76%).

**Ugi-4CR: Classical**

318mg (1mmol) of 20α-amino-3-oxo-4-azasteroid (11) when subjected to classical Ugi-4CR using N-(tert-butoxycarbonyl)-L-phenylalanine as the amino acid residue as per general procedure furnished pure Hetero-Steroid-Amino-Acid conjugate (12) as yellowish gum (55mg, 25%).

\[
\begin{align*}
\text{O} & \text{N} \\
\text{NHBoc} & \text{NH} \\
\text{O} & \text{H} \\
\end{align*}
\]

\[\lbrack \alpha \rbrack^\text{D}_25:(-) 134.04 \text{ (c1,CHCl}_3\rbrack; \text{IR (CHCl}_3\rbrack: 3402, 1650, 1437, 1230 \text{ cm}^{-1}; \text{H NMR (500 MHz, CHCl}_3\rbrack: \delta 0.89 \text{ (s, 3H, 19-Me), 1.01 \text{ (s, 3H, 18-Me), 1.21 \text{ (s, 3H, 20-Me), 1.32 \text{ (s, 12H, 3Me); 3.7 \text{ (m, 2H, NCH), 4.56 \text{ (m, 2H, N-CH}_2\rbrack, 4.91-5.0 \text{ (m, 4H, 2N-NCH}_2\rbrack, 7.14-7.31 \text{ (m, 10H, Ph); 13C NMR (125 MHz, CDCl}_3\rbrack: \delta 14.2, 14.3, 18.3, 20.1, 26.2,}\]

**MW Energy in Ugi-4CR: Synthesis of Some Hetero-Steroid-Amino acid conjugates - Another Class of Novel Hybrid Molecules**
Anal. Calcd. For C_{43}H_{60}N_{4}O_{5}: C, 72.47; H, 8.42; N, 7.86; Found: C, 72.44; H, 8.48; N, 7.86.

4.8: Preparation and Characterization of Hetero-Steroid-Amino Acid Conjugate based on B-ring hetero-steroidal amine

4.8.1: 3β-acetoxy-19, 20β-dihydroxyl-6-oxa-5β-pregnane (13)

To a solution of 3β-acetoxy-19-formyloxy-7-iodo-6-nor-5,7-seco-pregnane-5,20-dione (14) (0.5g, 0.93mmol) in absolute ethanol (15mL) at 0°C, was added sodium borohydride (71mg, 1.86mmol). The solution was stirred at 0°C for 2h and then at room temperature for another 2h, acidified with hydrochloric acid (1mol dm^{-3}), and then neutralized with 10% aqueous sodium hydrogen carbonate. The reaction mixture was diluted with cold water and extracted with diethyl ether. The organic extract was washed with water, dried over anhyd. Na_{2}SO_{4} and then evaporated under reduced pressure to get a crude residue. The crude product on chromatography on silica gel with EtOAc:PE::1:10 furnished the desired 3β-acetoxy-19, 20β-dihydroxyl-6-oxa-5β-pregnane (13) (188mg, 53%).
IR (KBr): 3440, 1734, 1244, 1151, 1070, 1047, 1028 cm⁻¹; ¹H NMR (500 MHz, CHCl₃): δ 0.79 (s, 3H, 18-Me), 1.16 (d, 3H, J=6.0, 20-Me), 2.01 (s, acetate, 3H), 2.31 (1H, dd, J=13.2), 3.2 (1H, dd, J=12.5, 4-H), 3.25 (1H, t, J=11.5, 7-H), 3.51 (1H, d, J=11.0, 19-H), 3.57 (1H, dd, J=11.5, 7-H), 4.03 (1H, d, J=11, 19-H), 4.15 (1H, dd, J=12.5, 5-H), 4.83 (1H, m, 20-H), 5.16 (1H, br s, 3-H); ¹³C NMR (125 MHz, CDCl₃): δ 12.8, 19.9, 20.1, 21.3, 22.9, 23.3, 24.2, 25.7, 27.8, 34.6, 38.3, 39.2, 39.6, 42.6, 52.4, 54.6, 64.2, 66.7, 70.6, 71.4, 72.8, 170.5; MS (ESI): m/z 380 (M⁺); Anal. Calcd. For C₂₂H₃₆O₅: C, 69.47; H, 9.47; Found: C, 69.44; H, 9.54.

4.8.2: 3β-acetoxy-19, 20β-dichloro-6-oxa-5β-pregnane (15)

To a solution of compound 13 (0.300 g, 0.78mmol) in diethyl ether (10mL) was added thionyl chloride (169mg, 1.42mmol) at 0°C for 3h. After completion of the reaction, the reaction mixture was then treated with saturated aqueous NaHCO₃ solution. The reaction mixture was then extracted with EA. The organic extract after drying over anhyd. Na₂SO₄ was evaporated under reduced pressure to get the crude product which was purified by column chromatography using EtOAc:PE::1:1 to get 3β-acetoxy-19,20β-dichloro-6-oxa-5β-pregnane (15) as gum (237mg, 72%).
IR (CHCl₃): 1734, 1244, 1151, 1070, 1028 cm⁻¹; ¹H NMR (500 MHz, CHCl₃): δ 0.80 (s, 3H, 18-Me), 1.52 (d, 3H, J=6.0, 20-Me), 2.01 (s, acetate, 3H), 2.31 (1H, dd, J=13.2, 4-H) 3.2 (1H, dd, J=12.5, 4-H), 3.25 (1H, t, J=11.5, 7-H), 3.51 (1H, d, J=11.0, 19-H), 3.57 (1H, dd, J=11.5, 7-H), 4.03 (1H, d, J=11, 19-H), 4.15 (1H, dd, J=12.5, 5-H), 4.83 (1H, m, 20-H), 5.16 (1H, br s, 3-H); ¹³C NMR (125 MHz, CDCl₃): δ 15.8, 19.9, 20.1, 21.3, 22.9, 23.3, 24.2, 25.7, 27.8, 34.6, 38.3, 39.2, 39.6, 42.6, 47.4, 54.6, 64.2, 66.7, 70.6, 71.4, 72.8, 170.5; MS (ESI): m/z 417(M⁺); Anal. Calcd. For C₂₂H₃₄Cl₂O₃: C, 63.30; H, 8.21; Found: C, 63.30; H, 8.15.

4.8.3: 3β-acetoxy-19, 20α-bis (azido)-6-oxa-5β-pregnane (16)

To a stirring solution of sodium azide (62mg, 0.95mmol) in dry DMF (15mL), 3β-acetoxy-19, 20β-dichloro-6-oxa-5β-pregnane (15) (200mg, 0.47mmol) was added at 90°C. The reaction mixture was then stirred for 24 hours at room temperature. After completion of the reaction, as indicated by TLC, water was added to it and the reaction mixture was then extracted with ether and the aqueous phase was washed with ethyl acetate. Both the organic phases were mixed and dried over anhyd. Na₂SO₄. The crude product obtained after removal of the solvent under reduced pressure was purified by
silica gel column chromatography using EtOAc:PE::1:20 as the eluent to afford 3β-acetoxy-19, 20α-bis(azido)-6-oxa-5β-pregnane azide (16) as white solid (173mg, 80%).

mp 149.6 °C; IR(CHCl₃) 2087, 1734, 1670, 1227cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 0.80 (s, 3H, 18-Me), 1.12 (d, 3H, J=5.0, 20-Me), 2.01 (s, acetate, 3H), 2.24 (1H, m, 20-H), 2.31 (1H, dd, J=13.2, 4-H), 3.2 (1H, dd, J=12.5, 4-H), 3.25 (1H, t, J=11.5, 7-H), 3.51 (1H, d, J=11.0, 19-H), 3.57 (1H, dd, J=11.5, 7-H), 4.03 (1H, d, J=11, 19-H), 4.15 (1H, dd, J=12.5, 5-H), 5.16 (1H, br s, 3-H); ¹³C NMR (125 MHz, CDCl₃): δ 15.8, 19.9, 20.1, 21.3, 22.9, 23.3, 24.2, 25.7, 27.8, 34.6, 38.3, 39.2, 39.6, 42.6, 48.4, 53.6, 64.2, 66.7, 79.6, 71.4, 72.8, 170.1; MS (ESI): m/z 430(M)⁺; Anal. calcd. for C₂₂H₃₄N₆O₃: C, 61.39; H, 7.9; N, 19.53; Found: C, 61.37; H, 7.96; N, 19.52.

4.8.4: 3β-acetoxy-19, 20α-diamino-6-oxa-5β-pregnane (17)

150mg of the compound (16) in 15mL of ethanol was subjected to hydrogenation at 45 psi pressure adding 80mg of 10% Pd/C for a period of 13h. The reaction mixture was filtered and alcohol was distilled off under reduced pressure to furnish the crude hydrogenated product which was purified by column chromatography over silica gel using EtOAc:PE::1:1 as the eluent to furnish 3β-acetoxy-19,20α-diamino-6-oxa-5β-pregnane (17) in pure form as white solid (114mg, 87%).
mp 125.9 °C; IR(CHCl₃) 3400, 1734, 1670, 1227 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 0.89 (s, 3H, 18-Me), 1.12 (d, 3H, J=5.0, 20-Me), 2.01 (s, acetate, 3H), 2.31 (1H, dd, J=13.2, 4-H), 2.38 (1H, d, J=9.0, 19-H), 2.54 (1H, m, 20-H), 3.2 (1H, dd, J=12.5, 4-H), 3.25 (1H, t, J=11.5, 7-H), 2.68 (1H, d, J=9.0, 19-H), 3.57 (1H, dd, J=11.5, 7-H), 4.15 (1H, dd, J=12.5, 5-H), 5.16 (1H, br s, 3-H); ¹³C NMR (125 MHz, CDCl₃): δ 15.8, 19.9, 20.1, 21.3, 22.9, 23.3, 24.2, 25.7, 27.8, 34.6, 38.3, 39.2, 39.6, 42.6, 48.4, 58.6, 64.2, 66.7, 79.6, 71.4, 72.8, 170.1; MS (ESI): m/z 378 (M⁺); Anal. calcd. for C₂₂H₃₈N₂O₃: C, 69.84; H, 10.05; N, 7.40; Found: C, 69.8; H, 10.12; N, 7.40.

4.8.5: Hetero-Steroid-Amino Acid Conjugate (18)

Ugi-4CR: MW

378 mg (1 mmol) of 3β-acetoxy-19,20α-diamino-6-oxa-5β-pregnane (17) when subjected to MW assisted Ugi-4CR using N-(tert-butoxycarbonyl)-L-phenylalanine as the amino acid residue as per general procedure to get the desired Hetero-Steroid-Amino Acid conjugate (18) as yellowish gum (262 mg, 85%).

Ugi-4CR: Classical

378 mg (1 mmol) of 3β-acetoxy-19,20α-diamino-6-oxa-5β-pregnane (17) when subjected to Ugi-4CR using N-(tert-butoxycarbonyl)-L-phenylalanine as the amino acid residue as
per general procedure to get the desired Hetero-Steroid-Amino Acid conjugate (18) as yellowish gum (61mg, 20%).

\[ \alpha \]D 25:(-) 78.94 (c1, CHCl₃); IR (CHCl₃): 1734, 1650, 1437, 1230 cm⁻¹; \(^1\)H NMR (500 MHz, CHCl₃): δ 1.01 (s, 3H, 18-Me), 1.21 (s, 3H, 20-Me), 1.32 (s, 12H, 3Me), 1.26 (d, 3H, J=5.0, 20-Me), 2.01 (s, acetate, 3H), 2.31 (1H, dd, J=13.2, 4-H), 2.54 (1H, m, 20-H), 2.94 (1H, d, J=9.0, 19-H), 3.2 (1H, dd, J=12.5, 4-H), 3.25 (1H, t, J=11.5, 7-H), 2.68 (1H, d, J=9.0, 19-H), 3.57 (1H, dd, J=11.5, 7-H), 4.15 (1H, dd, J=12.5, 5-H), 5.16 (1H, br s, 3-H); 3.7 (m, 2H, NCH), 4.56 (m, 2H, N-CH₂), 4.91-5.0 (m, 4H, 2N-NCH₂), 5.63 (t, 1H, 6-H), 7.14-7.31 (m, 20H, Ph); \(^1^3\)C NMR (125 MHz, CDCl₃): δ 14.1, 14.5, 21.0, 21.3, 24.3, 25.3, 27.4, 28.6, 28.6, 28.6, 28.6, 28.6, 30.1, 32.3, 36.8, 34.7, 37.4, 37.8, 39.2, 41.2, 43.3, 43.3, 45.3, 50.3, 52.1, 52.3, 52.7, 53.4, 55.8, 56.1, 56.4, 66.2, 66.3, 79.5, 79.6, 80.2, 125.8, 125.8, 125.8, 126.4, 126.4, 126.4, 126.5, 126.9, 126.9, 127.3, 127.3, 127.3, 127.3, 128.4, 128.6, 128.6, 128.6, 128.6, 128.6, 128.6, 128.6, 128.6, 128.6, 128.6, 128.6, 128.6, 128.6, 128.6, 128.6, 136.3, 137.3, 155.4, 155.4, 169.8, 170.0, 171.0, 171.0; MS (ESI): m/z 1166 (M⁺); Anal. Calcd. For C₆₈H₉₀N₆O₁₁: C, 69.98; H, 7.71; N, 7.20; Found: C, 69.96; H, 7.77; N, 7.20.
4.9: References


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