# SYNTHESIS AND SELECTIVE CATALYTIC OXIDATION OF NEW DIMERIC STEROIDS

**Khaled Q. Shawakfeh, Ahmad M. Al-Ajlouni and Abdelatif Ibdah**
*Department of Applied Chemical Science, Jordan University of Science and Technology, P. O. Box 3030, Irbid 22110, Jordan*
*Phone: 962-2-7201000; Fax: 962-2-7201014; E-mail: shawakfa@just.edu.jo*

*Received 03-04-2002*

## Abstract

New dimeric steroids were synthesized by reductive amination of the aldehyde of 3-oxopregn-4-ene-20β-carboxaldehyde and the ketone of stigmasterol and cholesterol, with 1,3-diaminopropane, 1,4-diaminobutane and 1,6-diaminohexane using sodium triacetoxyborohydride. The catalytic oxidation of the double bond in these dimeric steroids by $CH_3ReO_3/H_2O_2$ system was carried out. In the case of stigmasterol dimer **6**, only the internal double bond was oxidized.

## Introduction

Dimeric and oligomeric steroids posses interesting micellular, detergent, and liquid crystal properties.[1] Steroidal dimers have been used as catalysts for different types of reactions and many lead to enhance pharmacological activity.[2,3] For example, the dimeric steroids cephalostatins are among the most potent natural cytotoxins.[4] This exceptional activity of cephalostatins spurred the interest in their synthesis as well as for their analogues as potential anti-tumor agents.[5] It has been suggested that polyamine dimeric steroid binds to DNA due to the presence of two parts, one hydrophilic (positively charged nitrogen) and the other is hydrophobic steroid skeleton.[6]

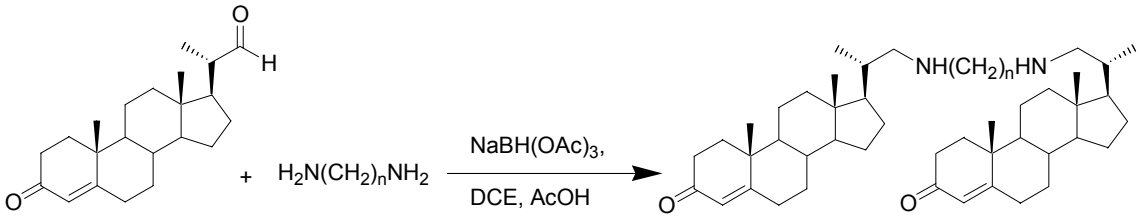
The dimeric steroidal alkaloids ritterazines are closely related to cephalostatin.[7] Seketsu and co-workers[7] have examined the cytotoxicity of ritterazines (A-D) derivatives. Their studies showed that ritterazine A which contains the highest number of hydroxyl groups (five hydroxyl group) is the most potent. The activity decreases with decreasing the number of hydroxyl groups. The study showed that the presence of hydroxyl groups is important for the enhancement of the biological activity.[8]

Here we are presenting new dimeric steroids that were prepared by reductive amination using alkyl diamines and selective reducing agent. The oxidation of some of these dimers was carried out by catalytic system (MTO/ $H_2O_2$) [9-12] that led finally to the 1,2-diol products.

## Results and Discussion

We prepared dimeric steroids using 3-oxopregn-4-ene-20β-carboxaldehyde (ketobisnoraldehde) (**1)** as starting material with 1,3-diaminopropane and 1,6-diaminohexane. Reductive amination of ketobisnoraldehyde (**1)** in the presence of 1,3-diaminopropane in dichoroethane (DCE) was carried out using $NaBH(OAc)_3$ and gave the dimer **2** in 65% yield (Scheme 1). The choice of $NaBH(OAc)_3$ as a reducing agent was crucial due to its selectivity in reducing imines intermediate.[13] Following the same procedure, ketobisnoraldehyde dimer **3** was synthesized by reductive amination of ketobisnoraldehyde with 1,6-diaminohexane in 64% yield. The $^1$H-NMR data showed a singlet at 5.7 ppm characteristic for C4-H, and a multiplet at 2.3 and 2.6 ppm characteristic for C22-$H_2$ and C23-$H_2$, respectively.

**Scheme 1. Reductive amination of (1)**

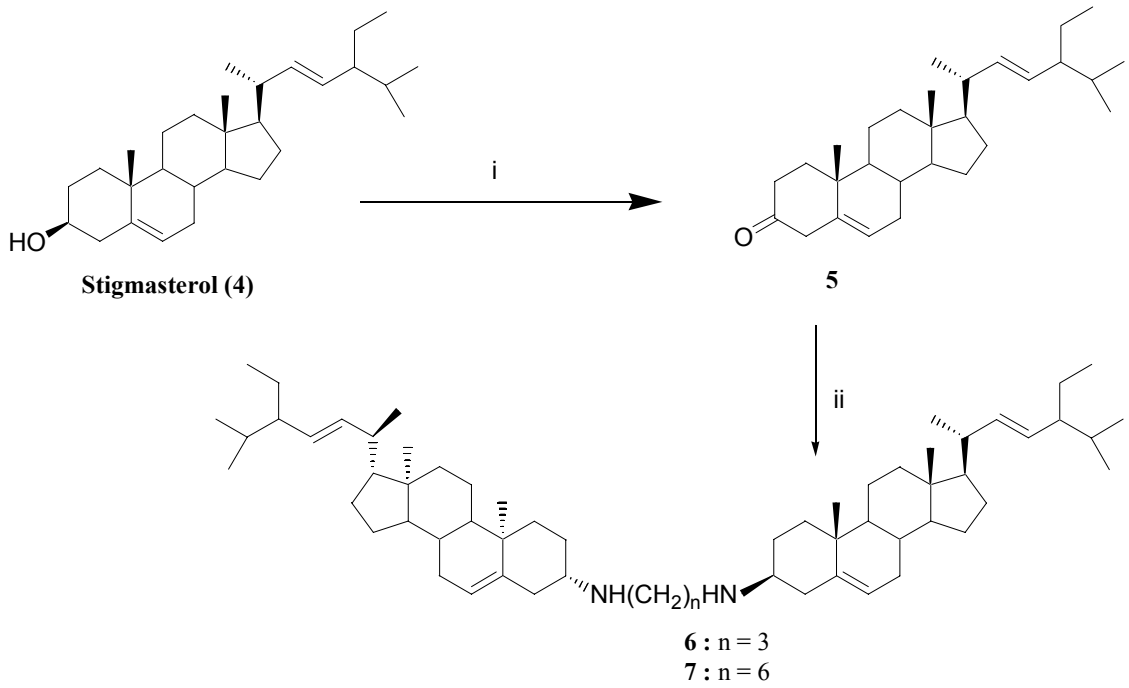



**1**

**2 :** n = 3
**3 :** n = 6

In order to study the structure activity relationships, new dimers were synthesized starting from cheap, readily available steroids such as stigmasterol and cholesterol. Starting from stigmasterol (**4)**, and using a modified method of Parish,[14] the desired ketone **5** was obtained in 82 % yield. Reductive amination of ketone **5** in the presence of 1,3-diaminopropane or 1,6-diaminohexane in DCE using $NaBH(OAc)_3$, gave the dimers **6** and **7** in 60% and 53% yield, respectively (Scheme 2). The $^1$H-NMR data for compounds **6** and **7** showed a singlet at 5.3 ppm characteristics for the hydrogen on C6, and a multiplet at 4.9-5.1 ppm characteristic for C22-H and C23-H. In addition, a triplet at 2.7 ppm indicates the presence of a methylene unit attached to NH (C30). The $^{13}$C-NMR data proved the disappearance of the carbonyl peak and the presence of an $sp^3$ carbon attached to NH (C3) at 54 ppm.

**Scheme 2. Synthesis of Dimers 6 , 7 from Stigmasterol**




**Stigmasterol (4)**

**5**

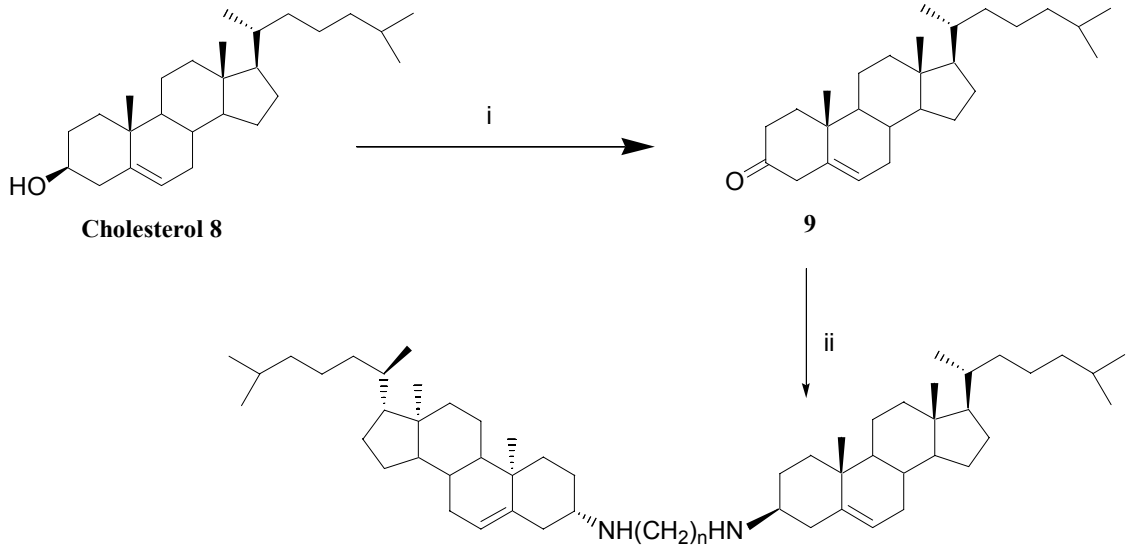
**6 :** n = 3
**7 :** n = 6

i) PCC, $CaCO_3$, $CH_2Cl_2$ ; ii) $H_2N(CH_2)_nNH_2$, $NaBH(OAc)_3$, DCE, AcOH

Starting from cholesterol **8**, and using the same oxidation method, the desired product β,γ-unsaturated ketone **9** was obtained in 89 % yield.[14] Reductive amination of the ketone **9** with 1,3-diaminopropane, 1,4-diaminobutane and 1,6-diaminohexane in DCE using $NaBH(OAc)_3$, gave the dimers **10, 11** and **12** in 60% yield, (Scheme 3). The $^1$H-NMR data for compounds **10, 11** and **12** showed a singlet at 5.3ppm characteristics for the hydrogen on C6-H, and a multiplet at 3.2-2.7 ppm characteristics for the hydrogen on C3-H. The $^{13}$C-NMR spectral data were also in good agreements with their chemical shift regions.[15-17]

The conversion of the olefinic bond of some of these new steroidal diamine dimers was carried out by oxidation with $H_2O_2/CH_3ReO_3$ (MTO) followed by hydration to trans-1,2-diols which is expected to be biologically active polyoxygenated steroids.[18]

**Scheme 3. Synthesis of Dimers 10 , 11 and 12 from Cholesterol**




**Cholesterol 8** **9**

**10 :** n = 3
**11 :** n = 4
**12 :** n = 6

i) PCC, $CaCO_3$, $CH_2Cl_2$ ; ii) $H_2N(CH_2)_nNH_2$, $NaBH(OAc)_3$, DCE, AcOH

The double bond of cholesterol dimer **11** was oxidized by $H_2O_2$/MTO to diol **13** in 60% yield ( (Scheme 4). The $^1$H-NMR spectral data indicate the presence of a broad peak at 3.4ppm charectaristic for C6-H.[19]The double bond protons of **11** at 5.3 ppm disappeared indicating total conversion.

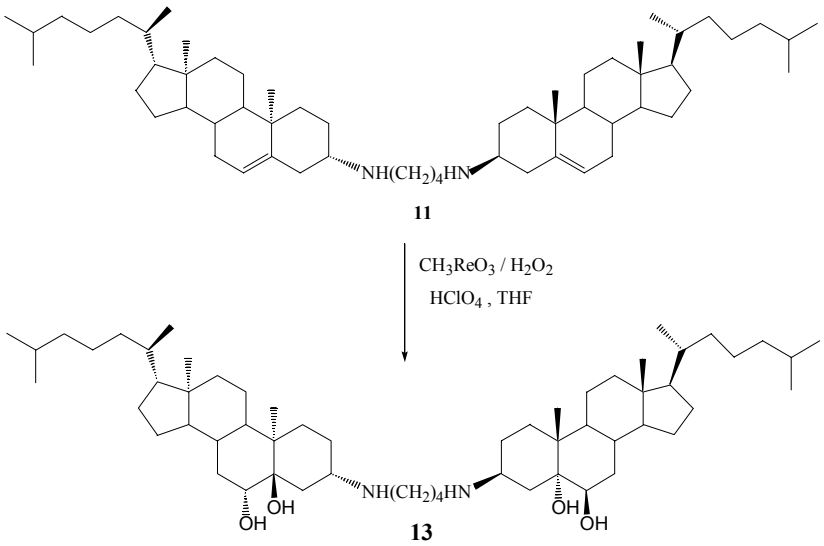
Under the same conditions used for cholesterol dimer **13**, the stigmasterol dimer **6** was epoxidized and the dimer **14** was produced in 67% yield (Scheme 5). Only the internal double bond (C5-C6) was oxidized. The $^1$H-NMR spectrum showed a multiplet at 5.2-4.9 ppm characteristics of C22-H and C23-H, and triplet at 3.4ppm characteristic for C6-H. The $^{13}$C-NMR spectrum showed only two olefinic carbons at 129.5 and 137.8 ppm characteristic for C-23 and C-22, respectively. In addition, two peaks at 76 and 67.6 ppm characteristic for C-6 and C-5 that have the hydroxyl groups.

## Conclusions

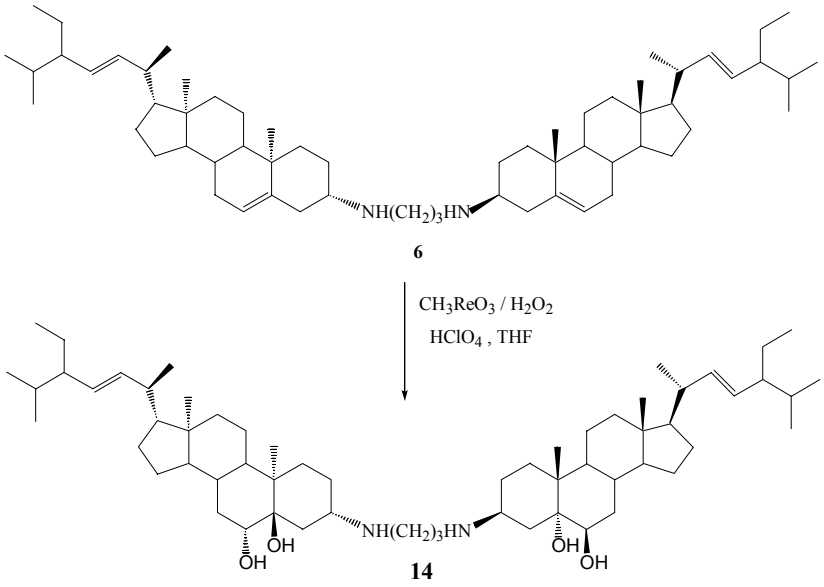
New dimeric steroids were synthesized starting from cheap and available steroids such as 3-oxopregn-4-ene-20β-carboxaldehyde, cholesterol and stigmasterol by reductively amination in the presence of 1,3-diaminopropane, 1,4-diaminobutane and

1,6-diaminohexane using sodium triacetoxyborohydride. The catalytic oxidation using $CH_3ReO_3/H_2O_2$ of stigmasterone dimer was selectively epoxidized on the internal double bond. The final products were characterized as the 1,2-diols.

**Scheme 4. Synthesis of Hydroxylated Steroid Dimer 13**




**Scheme 5. Synthesis of Hydroxylated Steroid Dimer 14**



## Experimental

### General

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet-Impact 410 FT-IR spectrometer. $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded using a 200 MHz or 300 MHz Brucker. Spectra were recorded in deuteriochloroform $CDCl_3$ with chemical shift values in ppm relative to the solvent peak (7.26 ppm). All reductive amination reactions were carried out under nitrogen atmosphere. Cholesterol (95% from Acros) was recrystallized from acetone before use. The following chemicals were used as received: Stigmasterol (Aldrich), ketobisnoraldehyde (donated from Temple University).

### General Procedure for Reductive Amination of Ketobisnoaldehyde with Diamines:

To a solution of ketobisnoaldehyde (350 mg, 1 mmol) in DCE (20 ml) diamines (1 mmol) was added, the mixture was stirred at r.t. under nitrogen for 24 hrs. Sodium triacetoxyborohydride (430 mg, 2 mmol) and glacial acetic acid (0.5 ml) was added. The reaction mixture was stirred for 72 hrs. The reaction was neutralized with 1N NaOH and the product was extracted with chloroform (4x40 ml). The organic layer was washed twice with brine (25 ml), dried over $MgSO_4$, and filtered. The solvent was evaporated under vacuum to give a pale yellow solid that was recrystalized from EtOH.

**Product 2** ( Diamine is 1,3-diaminopropane): Yield = 65%. M.p = 140-142 $^{o}$C. IR (KBr): ν 3455, 2945, 1667 cm$^{-1}$. $^{1}$H NMR: δ 5.7 (brs, 1H, C4), 2.3 (m, 2H, C22), 2.6 (m, 2H, C23), 1.21 (s, 3H, C19), 0.85 (s, 3H, C18). $^{13}$C-NMR: δ 199.4 (C3), 170.9 (C5), 123.8 (C4), 56.04 (C14), 55.8 (C17), 53.75 (C22), 39.35 (C23), 17.1 (C19), 12.1 (C18). Anal. calc. for: $C_{47}H_{74}N_2O_2$: C, 80.75; H, 10.67; N, 4.01. Found: C, 80.45; H, 10.47; N, 3.94.

**Product 3** ( Diamine is 1,6-diaminohexane)**:** Yield =64%. M.p = 175-176 $^{o}$C. IR (KBr): ν 3414, 2940, 1673. $^{1}$H NMR: δ 5.7 (brs, 1H, C4), 2.8-3.0 (m, 4H, C22, C23), 2.5-2.7 (m, 2H, C2), 1.26 (s, 3H, C19), 0.85 (s, 3H, C18). $^{13}$C-NMR: δ 199 (C3), 170 (C5), 123 (C6), 54.5 (C22), 39 (C23), 17.8 (C19), 12.5 (C18). Anal. calc. for: $C_{50}H_{80}N_2O_2$: C, 81.02; H, 10.88; N, 3.78. Found: C, 80.56; H, 10.70; N, 3.68.

**General Procedure for Reductive Amination of Ketone 5 with Diamines:**

To a solution of **5** (410 mg, 1.0 mmol) in DCE (40 ml) was added diamines(1.0 mmol) and $NaBH(OAc)_3$ (850 mg, 4.0 mmol) at r.t.. The mixture was stirred under argon for 48 hrs, then glacial acetic acid (1.0 ml) was added and the mixture stirred for 88 hrs. Work up procedure similar to **2** afforded a solid which was recrystalized from EtOH.

**Product 6** ( Diamine is 1,3-diaminopropane): Yield = 60%. M.p = 170-172 C°; IR (KBr): ν 3450, 2956 $cm^{-1}$. $^1H$ NMR: δ 5.25 (brs, 1H, C6), 4.9-5.1(m, 2H, C22, C23), 3.1(b, 1H, C3), 2.6-2.9 (m, 2H, C30), 1.01 (s, 3H, C19), 0.82 (s, 3H, C18); $^{13}C$-NMR:δ 146 (C5), 125 (C6), 138 (C22), 129 (C23), 56.0 (C14), 55.8 (C17), 54.5 (C3), 39.67 (C30), 19.1 (C19), 12.4 (C18). Anal. calc. for: $C_{61}H_{102}N_2$: C, 84.85; H, 11.91; N, 3.24. Found: C, 84.78; H, 11.86; N, 3.21.

**Product 7**(Diamine is 1,6-diaminohexane)**:** Yield = 53%. M.p = 120-123 °C; IR (KBr):ν 3459, 2940 $cm^{-1}$. $^1H$ NMR: δ 5.3 (brs, 1H, C6), 4.9-5.1 (m, 2H, C22, C23), 2.6-2.9 (m, 2H, C30), 1.06 (s, 3H, C19), 0.77 (s, 3H, C18). $^{13}C$-NMR: δ 147 (C5), 123 (C6), 138 (C22), 129 (C23), 54.6 (C3), 39.3 (C30), 19.3 (C19), 12.1 (C18). Anal. Calcd. For: $C_{64}H_{108}N_2$: C, 84.89; H, 12.02; N, 3.09. Found: C, 84.48; H, 11.96; N, 3.00.

**General Procedure for Reductive Amination of the Ketone 9 with Diamines:**

To a solution of **9** (380 mg, 1.0 mmol) in DCE (40 mL) was added diamines (1.35 mmol) and $NaBH(OAc)_3$ (439 mg, 2.0 mmol) at r.t. The mixture was stirred under argon for 40 hrs, then glacial HOAc (1.0 ml) was added and the mixture stirred for 36 hrs. Work up procedure similar to **2** afforded a yellow solid which was washed with ether.

**Product 10** ( Diamine is 1,3-diaminopropane): Yield = 60%. M.p.= 178- 180 °C; IR (KBr): ν 3450, 2970 $cm^{-1}$. $^1H$ NMR: δ 5.25 (brs, 1H, C6), 2.7-2.4 (b, 3H, C3, C28), 1.10 (s, 3H, C19), 0.70 (s, 3H, C18); .$^{13}C$-NMR:δ 150 (C5), 116 (C6), 55.9 (C14), 54.6 (C17), 54.1 (C3), 39.5 (C28), 18.8 (C19), 11.9 (C18). Anal. calc. for: $C_{57}H_{98}N_2$ : C, 84.37; H, 12.17 ; N, 3.45. Found: C, 84.18; H, 11.96; N, 3.27.

**Product 11** ( Diamine is 1,4-diaminobutane): Yield = 60%. M.p.= 174 –176 °C; IR (KBr): ν 3465, 2976 $cm^{-1}$. $^{1}H$ NMR: δ 5.3 (brs, 1H, C6), 3.2 (b, 1H, C3), 2.7-2.9 (b, 2H, C28), 1.12 (s, 3H, C19), 0.73 (s, 3H, C18); .$^{13}C$-NMR: δ 148 (C5), 120 (C6), 54.3 (C3), 39.5 (C28), 18.6 (C19), 11.8 (C18). Anal. Calcd. For: $C_{58}H_{100}N_2$: C, 84.40; H, 12.21; N, 3.39. Found: C, 84; H, 12.16; N, 3.10.

**Product 12** ( Diamine is 1,6-diaminohexane): Yield = 60%. M.p.= 134 -136 °C; IR (KBr): ν 3459, 2980 $cm^{-1}$. $^{1}H$ NMR: δ 5.4 (brs, 1H, C6), 3.1-2.7 (b, 3H, C3, C28), 1.11 (s, 3H, C19), 0.71 (s, 3H, C18); .$^{13}C$-NMR: δ 152 (C5), 114 (C6), 55.9 (C14), 54.9 (C17), 54.1 (C3), 39.3 (C28), 19.0 (C19), 12.1 (C18). Anal. Calcd. For: $C_{60}H_{104}N_2$: C, 84.44; H, 12.28; N, 3.29. Found: C, 84.25; H, 12.09; N, 3.21.

**General Procedure for Epoxidation with MTO/$H_2O_2$:**

To a mixture of MTO (10 mg, 0.04 mmol), 1mL 30% $H_2O_2$ and 1mL $HClO_4$ (1.0 M) in 15 mL THF, steroid dimers (0.15 mmol) were added. The reaction mixture was stirred and protected from light for 24 h. The reaction mixture was neutralized with 1N NaOH, and the product was extracted with chloroform. The organic layer was washed with brine. Dried over $MgSO_4$, and filtered. The solvent was evaporated under vacuum. The crude product was recrystalized from $CHCl_3$.

**Product 13:** Yield = 60%. M.p.= 185-187 °C. IR (KBr): ν 3489, 2970. $^{1}H$ NMR: δ 3.4 (brs, 1H, C6), 2.8-3.2(brs, 3H, C3, C28), 1.1 (s, 3H, C19), 0.75 (s, 3H, C18). $^{13}C$-NMR: δ 75.1 (C6), 67.5 (C5), 54.5 (C3), 19.9 (C19), 13.1 (C18). Anal. calc. or: $C_{58}H_{104}N_2O_4$: C, 77.97; H, 11.73; N, 3.14. Found: C, 77.58; H, 11.66; N, 3.09.

**Product 14:** Yield = 67%. M.p.= 199-201 °C. IR (KBr): ν 3510, 2990. $^{1}H$ NMR: δ 4.9-5.2 (m, 2H, C22, C23), 3.05-3.4 (brs, 2H, C3 and C6), 1.4-2.2 (m, 6H, C-31, 32, 35), 1.0 (s, 3H, C-19), 0.71 (s, 3H, C-18). $^{13}C$-NMR: δ 129.5 (C23), 137.8 (C22), 76.0 (C-6), 67.6 (C5), 55.7 (C3), 19.6 (C19), 12.8 (C18). Anal. calc. for: $C_{61}H_{106}N_2O_4$: C, 78.65; H, 11.47; N, 3.01. Found: C, 78.20; H, 11.37; N, 2.94.

## Acknowledgements

The authors thank the Deanship of Research at Jordan University of Science and Technology for financial support, Grant No. 108/99.

## References and Notes

1. Yuexian, Li; Dias, J. R., *Chem. Rev*. **1997**, 97, 283 – 304.
2. Aiello, A.; Fattorusso, E.; Menna, M., *Steroids* **1999**, 64, 687-714.
3. Guthrie, J. P.; Cossar, J.; Dawson, B. A., *Can. J. Chem.*, **1986**, 64, 2456-2469.
4. Heathcock, C. H.; Smith, S. C., *J. Org. Chem.* **1994**, 59, 6828-6839.
5. Petit. G. R.**,** *J. Am. Chem. Soc.* **1988**, 110, 2006- 2007.
6. Pang. H. J., ***J.*** *Am. Chem. Soc*. **1994**, 116, 2077-2078.
7. Seketsu, F.; Shigeki, M.; Nobuhiro, F., *J. Org. Chem.* **1995**, 60, 608-614.
8. Seketsu, F.; Shigeki, M.; Nobuhiro, F., *J. Org. Chem.* **1997**, 62, 4484-4491.
9. Owens, G. S.; Arias, J.; Abu-Omar, M. M., *Catalysis Today* **2000**, 55, 317- 363.
10. (a) Adam, W.; Mitchell, C. M.; Saha-Moller, C. R.; Weichold, O., *Structure and Bonding (Hrsg.:B.Meunier)* **2000,** 97, 211. (b) Espenson, J. H.; Abu-Omar, M.M., *ACS. Adv. Chem.* **1997**, 253, 3507. (c) Romao, C.C.; Kuhn, F. E.; Hermann, W. A., *Chem. Rev.* ***1997***, 97, 3197.
11. (a) Al-Ajlouni, A. M.; Espenson, J. H., *J.Org. Chem.* **1996**, 61, 3969-3976. (b) Al-Ajlouni, A. M.; Espenson, J. H., *J. Am. Chem. Soc.* **1995**, 117, 9243-9250.
12. Zhu, Z., Espenson, J. H., *J. Org. Chem.* ***1995***, 60, 1326-1332.
13. Abdel-Magid, A.; Carson, K.; Harris, B.; Maryanoff, C.; Shah, R., *J. Org. Chem.* ***1996***, 61, 3840-3862.
14. Parish, E. J., *Synthetic Communication* **1992**, 19, 2839-2847.
15. Agrawal, P. K.; Jain, D. C.; Gupta, R. K.; Thakur, R. S., *Phytochemistry* **1985**, 24(11), 2479-2496.
16. Wehrli, S. L.; Moore, K. S.; Roder, H.; Dorell, S.; Zasloff, M., *Steroids* **1993**, 58, 370-378.
17. (a) Williams, J. R.; Shawakfeh, K. *Tetetrahedron*, submited. (b) Shawakfeh, K. *The Synthesis, Characterization, and Biological Testing of New Squalamine Analogs*, Ph.D thesis, Temple University, 1998.
18. Casapullo, A.; Minale, L.; Zollo, F., *Tett. Lett.* **1995**, 36 (15), 2669-2672.
19. Notaro, G.; Piccialli, V.; Sica, D., *J. Nat. Prod.* **1991**, 54 (6), 1570-1575.

## Povzetek

Z reduktivnim aminiranjem smo sintetizirali nove dimerne steroide. S katalitsko oksidacijo z $CH_3ReO_3/H_2O_2$ smo oksidirali dvojno vez.