www.sphinxsai.com

ChemTech

International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.1, pp 557-561, Jan-Mar 2010

An efficient Synthesis of amino-dihydrotestosterone derivative

***[1]Figueroa-Valverde L., [2]Díaz-cedillo F., [3]Camacho-Luis A., López Ramos M., Elodia García Cervera**

[1]Laboratorio de Investigación de la Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Campeche, Av. Agustín Melgar, Col Buenavista C.P.24039 Campeche Cam., México. lauro_1999@yahoo.com

[2]Laboratorio de Química Orgánica de la Esc. Nal. de Ciencias Biológicas del Instituto Politécnico Nacional. Prol. Carpio y Plan de Ayala s/n Col. Santo Tomas, México, D.F. C.P. 11340. stybium@yahoo.com

[3]Facultad de Medicina de la Universidad Autónoma del Estado de Durango, Dgo., México. loky001@hotmail.com.

Corres.author:lauro_1999@yahoo.com; Telefono: (981) 8119800 Ext. 73006; Fax (981) 8119800 Ext. 73099.

Abstract: In this work was synthesized an amino-steroid derivative (**4**); the route involved preparation of dihydrotestosterone-hemisuccinate (**2**) by the esterification of dihydrotestosterone (**1**) using succinic anhydride and pyridine (method A) or using succinic acid and 1,3 dicyclohexylcarbodiimide in presence of *p*-toluensulfonic acid (method B). After **2** was coupled to 4-hidroxy benzoic acid using 1,3 dicyclohexylcarbodiimide as catalyst to form **3**. Finally, **4** was obtained by the reaction between **3** and ethylendiamine hydrochloride in presence of 1-ethyl-3(3-dimethylamino-propyl)carbodiimide.
Keywords:amino-steroid, dihydrotestosterone-hemisuccinate, esterification.

Introduction

In the past decades, several amino steroid-derivatives were synthesized with a wide spectrum of biological actions, as antibacterial[1,2], antimalarial[3] as well as antiviral[4] drugs. For example, there are reports[5] which show the synthesis of 17β-[N-methyl-N-(aminoethyl)amino-5α-androsterone by reduction of oxime-androsterone derivative using $LiAlH_4$. Additionally, other reports[6] showed the synthesis of a series of 7-amino- and polyaminosterol analogues of squalamine and trodusquemine as catalyst. Another studies made by Walker and coworkers[7] reported the synthesis of several amino-steroids derivatives by the reaction between cholic acid methyl ester with polyamines (spermine, pentamine, or hexamine) using N-hydroxysuccinamide. Other studies made by Acs and coworkers[8] showed the synthesis of 11-carboxamido-androstan-4,9(11)-diene using via palladium as catalyst. In addition, another reports indicate the synthesis of 7α-[4′-(aminophenyl) thio]pregna-4-ene-3,20-dione by the reaction between

pregna-4,6-diene-3,20-dione with 4-aminothiophenol in presence of sodium hydroxide.

On the other hand, there are reports which show the synthesis of hemysuccinate-pregnenolone-ethyl endiamine conjugate by the reaction of hemysuccinate of pregnenolone with ethylendiamine using 1-ethyl-3(3-dimethylamino-propyl)- carbodiimide as catalyst.[9] All these experimental data show several protocols for synthesis of amino steroid-derivatives, nevertheless, the use of some reagents requires of special conditions. Therefore in this work our initial design included a facile synthesis of an amino steroid-derivative (**4**) that contains in the cyclopentene-ring of dihydro testosterone-derivative nucleus a spacer arm with ester functional group bound to aromatic ring which in addition has an arm with a free amino group.

Experimental

Dihydrotestosterone (5α-androstan-17β-ol-3-one) and the other compounds evaluated in this study were purchased from Sigma-Aldrich Co., Ltd. The melting points for the different compounds were determined on an Electrothermal (900 model). Ultraviolet spectroscopy (UV) was carried out in dry methanol on a Perkin-Elmer model 552 spectrophotometer and infrared spectra (IR) was recorded using KBr pellets on a Perkin Elmer Lambda 40 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300/5 FT NMR spectrometer at 300 and 75.4 MHz in $CDCl_3$ using TMS as internal standard. EIMS spectra were obtained with a Finnigan Trace GCPolaris Q. spectrometer. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/0 2400 elemental analyzer.

Synthesis of 5α-androstan-17β-ol-3-one 17-hemisuccinate (2)

Method A.

A solution of dihydrotestosterone 200 mg (0.69 mmol), succinic anhydride 138 mg (1.38 mmol), 3 mL of pyridine in 10 mL of toluene was gently refluxed for 8 h, and then cooled to room temperature. The reaction mixture was evaporated to a smaller volume, diluted with water, and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol:water (3:1) yielding 60 % of product, mp 130-134 ^{o}C; UV (MeOH) λ_{max} (log ε) 214 (0.74), 288 (2.14) nm; IR V_{max} 3330, 1700 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H; 0.74 (s, 3H, 19-C**H**$_3$), 0.80 (m, 1H), 0.96 (s, 3H, 18-C**H**$_3$), 1.04-118 (m, 6H), 1.22-1.52 (m, 5H), 1.62-1.75 (m, 4H), 1.98-2.06 (m, 2H), 2.17-2.33 (m, 4H), 2.54 (s, 4H, broad), 4.51 (m, 1H), 10.02 (1H, br, CO_2**H**). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ_C: 12.07 (C-19), 17.00 (C-18), 20.75 (C-14), 23.5 (C-15), 27.05 (C-7), 27.60 (C-16), 28.80 (C-8), 29.45 (C-23), 29.48 (C-24), 35.21 (C-9), 35.34 (C-5), 36.92 (C-13), 37.51 (C-1), 37.86 (C-6), 42.72 (C-12), 43.45 (C-3), 45.41 (C-4), 47.22 (C-10), 50.60 (C-11), 82.43 (C-17), 171.85 (**C**O_2H), 173.38 (**C**O_2), 212.22 (**C**=O). EIMS [M+, 17] m/z 390.24, Anal. Calcd. for C_{23}, H_{34}, O_5; C, 70.74, H, 8.78, O, 20.49. Found: C, 70.62, H, 8.76.

Method B.

200 mg (0.69 mmol) of dihydrotestosterone was added to solution of succinic acid 162 mg (1.38 mmol), 1,3 dicyclohexylcarbodiimide 285 mg (1.38 mmol) in acetonitrile-water (15 mL, 3:1) and *p*-toluensulfonic acid monohydrate 197 mg (1.03 mmol) was added and the mixture was stirred at room temperature for 72 hours. After the solvent was removed under vacuum and the crude product was purified by crystallization from methanol:hexane:water (3:2:1) yielding 75 % of product; similar ^{1}H NMR and ^{13}C NMR data were obtained and compared with method A product.

4-[-(10,13-dimethyl-3-oxo-hexadecahydro-cyclopenta[a]phenanthren-17-yl)-4-oxo-pentanoyloxy]-benzoic acid (3)

A solution of **2** (100 mg, 0.26 mmol), 4-hydroxybenzoic acid (54 mg, 0.39 mmol), 1,3 dicyclohexylcarbodiimide (107 m, 0.52 mmol) and *p*-toluensulfonic acid monohydrate (74 mg, 0.39 mmol) in acetonitrile-water (15 mL, 3:1) was stirred at room temperature for 72 hours. After the solvent was removed under vacuum and the crude product was purified by crystallization from methanol:hexane:water (3:2:1) yielding 65 % of product; mp 168-170 ^{o}C; UV (MeOH) λ_{max} (log ε) 218 (3.24), 253 (3.68) nm; IR V_{max} 3332, 1602 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H; 0.80 (s, 3H, 19-C**H**$_3$), 0.85 (m, 1H), 0.98 (s, 3H, 18-C**H**$_3$), 1.03-1.22 (m, 7H), 1.32-1.36 (m, 2H), 1.50-1.55 (m, 3H), 1.73-1.91(m, 4H), 2.03-2.25 (m, 5H), 2.49 (s, 2H), 2.71 (s, 2H), 4.73 (m, 1H), 7.18 (d, 2H), 8.08 (d, 2H), 10.80 (s, (1H, br, CO_2**H**). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ_C; 12.07 (C-19), 17.01 (C-18), 20.70 (C-14), 23.05 (C-15), 27.05 (C-7), 27.60 (C-16), 28.80 (C-8), 29.02 (C-23), 30.02 (C-24), 35.20 (C-9), 35.30 (C-5), 36.90 (C-13), 37.50 (C-1), 37.82 (C-6), 43.04 (C-12), 43.45 (C-3), 45.40 (C-4), 47.20 (C-10), 50.65 (C-11), 81.52 (C-17), 123.77 (C-29, C-33), 128.08 (C-31), 131.30 (C-30, C-32), 156.19 (C-28), 164.25 (**C**O_2H), 167.46 (C-26), 173.08 (C-21), 212.40 (C=O). EI-MS, *m/s* 510.60 (M^+, 17), Anal. Calcd. for C_{30}, H_{38}, O_7; C, 70.57, H, 7.50, O, 21.93. Found: C, 70.51, H, 7.38.

Synthesis of 5-(10,13-dimethyl-3-oxo-hexadeca hydro-cyclopenta[a]phenanthren-17-yl)-4-oxo-pentanoic acid-4-(2-amino-ethylcarbamoyl)phenyl ester (4)

A solution of **3** (200 mg, 0.39 mmol), ethylendiamine hydrochloride (140 mg 0.78 mmol), 1-ethyl-3(3-dimethylamino-propyl)carbodiimide (149 mg, 0.78 mmol) in 15 ml of acetonitrile:water (3:1) was stirred at room temperature for 72 hours. After the solvent was removed under vacuum and the crude product was purified by crystallization from methanol:hexane:water (3:2:1); yielding 60 % of product, mp 223-225 ^{o}C; UV (MeOH) λ_{max} (log ε) 217 (3.08) nm; IR V_{max} 3338, 1610, 1685 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H; 0.80 (s, 3H, 19-C**H**$_3$), 0.85 (m, 1H), 0.98 (s, 3H, 18-C**H**$_3$), 1.03-1.22 (m, 7H), 1.32-1.36 (m, 2H), 1.50-1.55 (m, 3H), 1.73-1.91(m, 4H), 2.03-2.25 (m, 5H), 2.49 (s, 2H), 2.71 (s, 2H), 3.02 (s, 2H), 3.50 (s, 2H), 4.50 (m, 3H, N**H**- and –N**H**$_2$), 4.65 (m, 1H), 7.10 (m, 2H, Ar-H), 7.80 (m, 2H, Ar-H). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ_C; 12.07 (C-19), 17.01 (C-18), 20.70 (C-14), 23.05 (C-15), 27.05 (C-7), 27.60 (C-16), 28.80 (C-8), 29.02 (C-23), 30.02 (C-24), 35.20 (C-9), 35.30 (C-5), 36.90 (C-13), 37.50 (C-1), 37.82 (C-6), 43.04 (C-12), 43.30 (C-38), 43.45 (C-3), 43.70 (C-39), 45.40 (C-4), 47.20 (C-10), 50.65 (C-11), 81.52 (C-17), 120.40 (C-29, C-33), 127.60 (C-30, C-32), 131.08 (C-31), 159.70 (C-28), 169.00 (-CO-NH), 170.02 (C-26, CO_2), 173.08 (C-2, CO_2), 212.40 (C=O). EI-MS, *m/s* 552.32 (M^+, 17), Anal. Calcd. for C_{32}, H_{44}, N_2, O_6: C, 69.54; H, 8.02; N, 5.07;O, 17.37. Found: C, 69.50, H, 8.04.

Results and Discussion

In this study, a straightforward route is reported for the synthesis of amino- dihydrotestosterone derivative (Figs. 1-3). The first step involves the esterification of the hydroxyl group of dihydrotestosterone (**1**) to form dihydrotestosterone-hemisuccinate (**2**) (scheme 1, see) using a modification of the method reported by Erlanger and coworkers[10] for esterification of steroids. Additionally, also it is important to mention that there are diverse reagents to produce esters derivatives[11,12], nevertheless; most of the conventional methods have found only a limited use for this purpose Therefore, in this work two methods were used; in method A, the **2** compound was synthesized by the reaction of **1** with succinic anhydride in presence of pyridine using toluene to avoid hydrolysis in the new arm formed in cyclopentene ring of **2**; which has characteristic of an arm with free carboxyl group. In the method B the compound **1** and *succinic acid* were made react using 1,3 dicyclohexylcarbodiimide as catalyst for the formation of **2** with different conditions (aceto nitrile:water). Nevertheless, it is important to mention that when 1,3 dicyclohexylcarbodiimide compound is used along as condensing agent in esters synthesis, the yield of esters is often unsatisfactory due to formation of N-acylurea derivative as byproduct. Some reports showed that addition of a catalytic amount of a strong acid in the esterification reaction in presence of dicyclohexylcarbodiimide considerably increases the yield of esters and decreases the formation of N-acylurea compound[13]. In this sense, the esterification of the hydroxyl group of **1** with succinic acid in presence of dicyclohexylcarbodiimide and *p*-toluensulfonic acid was used to increase the yielding of **2**.

The results indicate that ^{1}H NMR spectrum of **2** showed signals at 0.74 and 0.96 ppm corresponding to methyls presents in the steroid nucleus. In addition, another signal at 2.54 ppm for methylenes bound to carboxyl group was found. Finally, a signal at 10.02 ppm corresponding to the acidic hydrogen of C(=O)-OH was found.

The ^{13}C NMR spectra displays chemical shifts at 12.07 and 17.00 ppm for the carbons of methyls groups presents in the steroid nucleus. The chemical shift of the methylenes bound to carboxyl group is found out at 29.45-29.48 ppm. In addition, there are several signals (32.21-82.43 ppm) corresponding to carbons involved in the steroid nucleus and two signals at 171.85 ppm for **C**O_2H and at 173.38 ppm for ester group. Finally, a signal at 212.22 ppm corresponding to ketone group was found. Additionally, the presence of **2** was further confirmed from mass spectrum which showed a molecular ion at m/z 390.24.

The second step was achieved by the reaction of **2** with 4-hydroxybenzoic acid in presence of 1,3 dicyclohexylcarbodiimide and *p*-toluensulfonic acid in acetonitrile:water to form **3** (scheme 2, see). The ^{1}H NMR spectra of the **3** showed in addition of the characteristic chemical shifts of **2**, upfield chemical shifts at 0.80 and 0.98 ppm for methyls present in the steroid nucleus. Additionally, a signal at 2.49-2.71 ppm was found for methylenes involved in arm spacer between the steroid and aromatic ring. Finally, other signals at 10.80 ppm corresponding to the acidic hydrogen of C(=O)-OH was found.

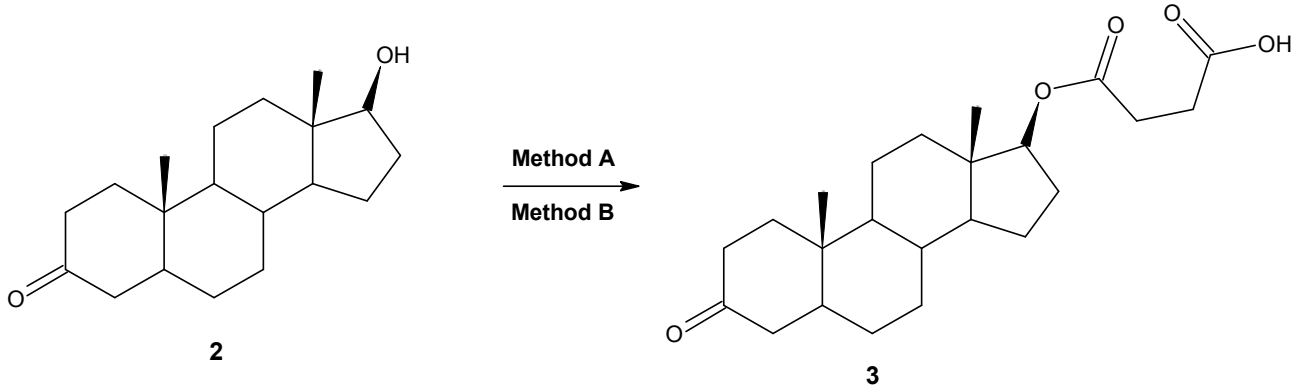
On the other hand, ^{13}C NMR spectra displays chemical shifts at 12.07 and 17.01 ppm for the carbons of methyls groups presents in the steroid nucleus of **3**. In addition, another chemical shifts at 29.02 ppm and 30.02 for carbons of methylenes involved in arm spacer between the steroid and aromatic ring were exhibited. Additionally, several signals at 123.77-156.19 ppm for carbons corresponding to aromatic ring were exhibited. Another chemical shifts at 164.25

ppm for the acidic hydrogen [C(=O)-OH], at 167.46 and 173.08 ppm for ester groups. Finally, other signal at 212.40 ppm for ketone group. Additionally, the presence of **3** was further confirmed from mass spectrum which showed a molecular ion at m/z 510.60. Finally, **4** was obtained by the reaction between **3** and ethylendiamine hydrochloride using 1-ethyl-3(3-dimethylamino-propyl)carbodiimide as catalyst (scheme 3, see). It is important to mention that many procedures for the formation of amide groups are known in the literature, the most widely practiced method employs carboxylic acid chlorides as the electrophiles which react with the amine in the presence of an acid scavenger[14]. Despite its wide scope, this protocol suffers from several drawbacks; most notable are the limited stability of many acid chlorides and the need for hazardous reagents for their preparation (thionyl chloride)[15]. In this work, a derivate of carbodiimide was used as catalyzer[16] for amide bond formation in the new arm bound to aromatic ring. The ^{1}H NMR spectra of the **4** showed in addition of the characteristic chemical shifts of **3**, upfield chemical shifts at 0.80 and 0.98 ppm for methyls present in the steroid nucleus. In addition, a signal at 2.49-2.71 ppm was found for methylenes involved in arm spacer between the steroid and aromatic ring. Additionally, other signal at 4.50 ppm corresponding to both protons of amide and amino groups were found. It is important to mention that the ^{1}H NMR spectra of the secondary amides are usually more complex than the primary amides due to the presence of a substituent bonded to the amide nitrogen atom. These substituents produce a much wider range of chemical shifts for the amide proton which may, in addition, display coupling to aliphatic groups bonded to it. The chemical shifts of aliphatic groups bonded to the carbonyl group are similar to those observed for the primary amides, while those groups bonded to the-nitrogen resonate at slightly lower field than the corresponding amines[9].

On the other hand, the The ^{13}C NMR spectra displays chemical shifts at 12.07 and 17.01 for the carbons of methyls groups presents in the steroid nucleus of **4**. The chemical shift of the methylenes bound to carboxyl group is found out at 29.02-30.02 ppm. In addition, there are several signals (35.20-43.04 ppm) corresponding to carbons involved in the steroid nucleus and two signals at 43.30 and 43.70 for the methylenes bound to amine group. Additionally, other signals at 170.02 ppm and 173.08 for ester groups were found. Finally, a signal at 212.40 ppm corresponding to ketone group was found. In addition, the presence of **4** was further confirmed from mass spectrum which showed a molecular ion at m/z 552.32.

Conclusions

We report an easy methodology to synthesize amino-dihydrotestosterone derivative (**4**) that contains in the cyclopentene-ring of dihydrotestosterone-derivative nucleus a spacer arm with ester functional group bound to aromatic ring which in addition has an arm with a free amino group.



2 **3**

Figure 1. Synthesis of 5α-androstan-17β-ol-3-one 17-hemisuccinate (**2**). Method A: *dihydrotestosterone* (**1**), succinic anhydride, pyridine/toluene. Method B: *dihydrotestosterone* (**1**), succinic acid, 1,3 dicyclohexylcarbodiimide and *p*-toluensulfonic acid monohydrate in acetonitrile-water.

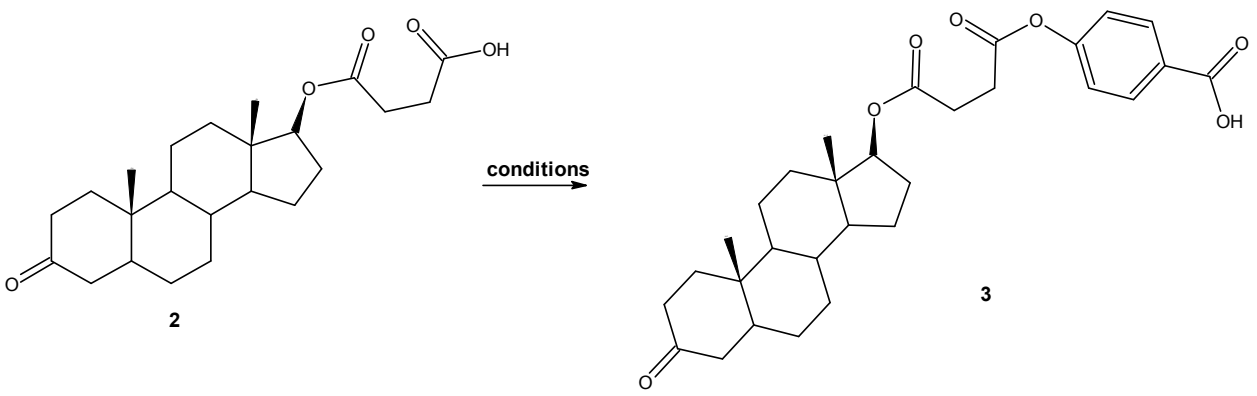


Figure 2. Synthesis of 4-[-(10,13-dimethyl-3-oxo-hexadecahydro-cyclopenta[a]phenanthren-17-yl)-4-oxo-pentanoyloxy]-benzoic acid (**3**). Conditions: 4-hydroxybenzoic acid, 1,3 dicyclohexylcarbodiimide and *p*-toluensulfonic acid monohydrate in acetonitrile-water.

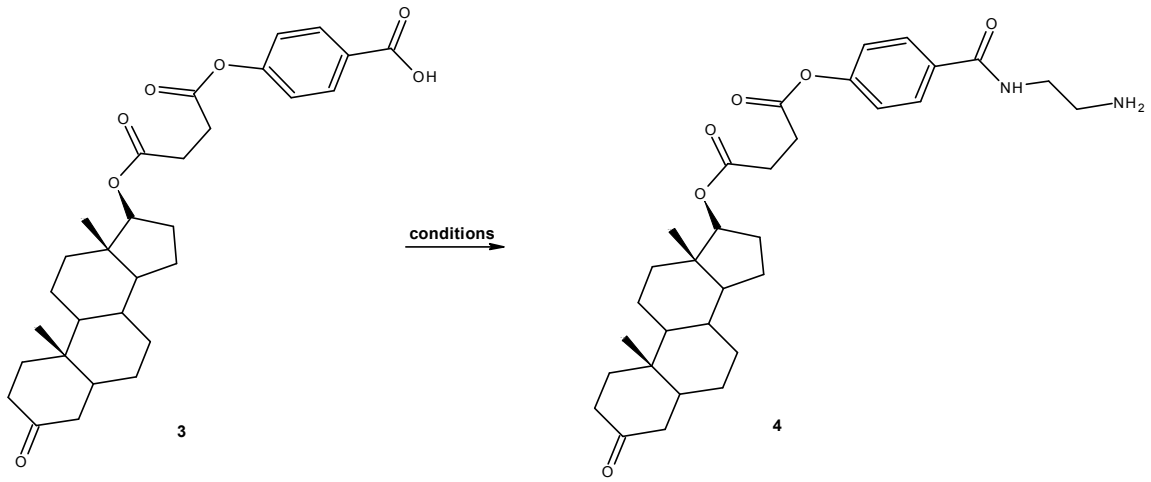


Figure 3. Synthesis of 5-(10,13-dimethyl-3-oxo-hexadecahydro-cyclopenta[a]phenanthren-17-yl)-4-oxo-pentanoic acid-4-(2-amino-ethylcarbamoyl)phenyl ester (**4**). Conditions: ethylendiamine hydrochloride and 1-ethyl-3(3-dimethylamino-propyl)carbodiimide in acetonitrile:water.

References

1. Smith RF, Shay DE, and Doorenbos NJ; J. Bacteriol. 1963, **85**, 1295.
2. Ding B, Taotofa U, Orsak T, Chadwell M, and Savage P. Org. Lett. 2004, **6**, 3433.
3. Sharma U, Srivastava K, Puri SK, and Singh C; Med. Chem. Res. 2008, **17**, 326.
4. Giner JL, and Faraldos JA; J. Org. Chem. 2002, **67**, 2717.
5. Merlani MI, Amiranashvili L,. Mulkidzhanyan K, and Kemertelidze E; Chem.Nat. Comp. 2006, **42**, 313.
6. Loncle C, Salmi C, Letourneux Y, and Brunel JM; Tetrahedron. 2007, **63**, 12968.
7. Walker S, Sofia MJ, Kakarla R, Kogan NA, Wierichs L, and Longley CB; Proc. Nat. Acad. Sci. USA. 1996, **93**, 1585.
8. Acs P, Jakab B, Takas A, and Kollar L; Steroids. 2007, **72**, 627.
9. Figueroa-Valverde L, Diaz Cedillo F, Tolosa L, Maldonado G, and Ceballos-Reyes G. J. Mex. Chem. Soc. 2006, **50**, 42.
10. Erlanger FB, Borek F, Beiser MS, and Lieberman S; J. Biol. Chem. 1957, **228**, 713.
11. Crossland RK, and Servis KL; J. Org. Chem. 1970, **35**, 3195.
12. Zhu HP, Yang F, Tang J, and He MY; Green Chem. 2003, **5**, 38.
13. Holmberg K, and Hansen B; Acta Chem. Scand. 1979, **B33**, 410.
14. Medvedeva A, Andreev M, Safronova L, and Sarapulova G; Arkivoc. 2001, **ix**, 143.
15. Levin D; Org. Process Res. Dev. 1997, **1**, 182.
16. DeSilva NS; Am. J. Respir. Cell Mol. Biol. 2003, **29**, 757.

*****