Steroids 77 (2012) 521–527

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Synthesis of [1,2,4]-triazolo-annulated 3-aza-A-homocholestanes—A novel class of pentacyclic compounds

Hexiang Bai, Shijing Xia, Zhiming Li, Jingmei Wang, Quanrui Wang *

Department of Chemistry, Fudan University, Shanghai 200433, PR China

ARTICLE INFO

Article history:
Received 10 November 2011
Received in revised form 18 January 2012
Accepted 22 January 2012
Available online 31 January 2012

Keywords:
5α-Cholestan-3-one
3-Aza-A-homocholestanes
1,3-Dipolar cycloaddition
Ring expansion

ABSTRACT

This study was performed to investigate the reactivity of azocarbenium salts derived from 5α-cholestan-3-one towards 1,3-dipolar cycloaddition reactions with inverse electron-demand to produce unprecedented steroidal heterocyclic derivatives, i.e. [1,2,4]-triazolo-annulated 3-aza-A-homocholestanes **8** and **11** and picrates **12**. The synthetic steps were comprised of oxidizing hydrazones **3** with *tert*-butyl hypochlorite to germinal chloroazo compounds **4**, generation of the 1-aza-2-azoniaallene cations **5** by action with equimolar antimony pentachloride and interception with nitrile and alkyne molecules by cycloaddition to the triple bond followed by ring enlargement. The structure of the compounds was principally established on the basis of the analytical and spectral data along with the previously published X-ray diffraction analysis on **8a**.

1. Introduction

Steroid heterocycles have interesting biological activities [1–3]. The replacement of one or more carbon atoms in a steroid molecule by a heteroatom, especially nitrogen often results in useful alterations of its biological activity [4]. On the other hand, azepine compounds with an additional triazolo ring fused on the seven-membered ring possess a wide spectrum of important biological properties, such as anti-tumor agents [5], selective inhibition of 11β-hydroxysteroid dehydrogenase [6], the reduction of beta-amyloid protein production [7], and even herbicidal activity [8]. In general, the fusion of a triazolo ring plays a vital role in enhancing the affinities for receptors. To the best of our awareness, available methods for achieving the expansion of the steroidal A-ring to an azepine ring seem to be scarce. The most exploited strategies are the Schmidt reaction [9,10] and the Beckmann rearrangement [11,12].

In recent years, we have been engaged in a program toward synthesizing novel triazolo annulated heterocycles. We have established a reliable synthetic pathway to [1,2,4]triazolo[3,2-*d*][1,5]benzoxazepines and their chalcogen analogs starting from chroman-4-ones and thiochroman-4-ones [13,14]. We have also successfully applied the protocol to the synthesis of novel thieno[2,3-*f*][1,2,4]triazolo[1,5-*a*]azepines, furo[3,2-*c*][1,2,4]triazolo[1,5-*a*]azepinium salts and furo[2,3-*f*][1,2,4]triazolo[1,5-*a*]azepinum picrates by starting from the appropriate bicyclic ketones [15,16]. Quite recently, we have also successfully employed this strategy to achieve a hitherto unknown 1,2,4-triazolo-fused steroidal azepine compound from 5α-cholestan-3-one [17].

In continuation of our long-standing interests on this topic, we report herein the detailed study on the synthesis of this class of novel 1,2,4-triazolo-fused steroidal azepine compounds. The synthetic genre shares a common mechanistic scenario: the key step employs the cycloaddition of 1-aza-2-azoniaallenium ions, positively charged four-electron, three-center 1,3-dipoles, to the triple bond of unsaturated compounds followed by ring enlargement and insertion of a nitrogen atom to furnish the pentacyclic products.

2. Experimental

2.1. General remarks

All commercial materials were used without further purification. Solvents were purified and dried by standard methods prior to use. Reactions were monitored by thin layer chromatography (TLC) on Silica Gel 60 F_{254} (Fluka). Infrared spectra were recorded as KBr disks on a Nicolet-360 IR spectrometer. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ solutions on a JEOL ECA 400 or a Bruker AMX 500 spectrometer using TMS as an internal reference and reported in ppm (δ). Coupling constant (J) values are given in Hz. Multiplicity are expressed as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Elemental analysis for C, H, N was performed on CAPLO ERBA1106 elemental analyzer. High resolution mass spectra (HRMS) were recorded on a SHIMADZU LCMS-IT-TOF mass spectrometer with ESI ionization. Melting points were determined in open capillary tubes and are uncorrected.

* Corresponding author. Fax: +86 21 65641740.
E-mail address: qrwang@fudan.edu.cn (Q. Wang).

0039-128X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2012.01.016

2.2. Organic synthesis

2.2.1. General experimental procedure for the synthesis of 5α-cholestan-3-one hydrazones

Equimolar amounts of 5α-cholestan-3-one **1** (1.93 g, 5 mmol) and hydrazine **2a** or hydrazide **2b** (5 mmol) in EtOH (20 mL) containing HOAc (0.1 mL) were heated under reflux for 3 h and then left to cool. The solid product **3** formed upon cooling at room temperature was collected by filtration and crystallized from hot EtOH (95%).

2.2.2. 5α-Cholestan-3-one (2,4,6-trichlorophenyl)hydrazone (**3a**)

The physical data and spectral data for **3a** were published previously (Ref. [17]).

2.2.3. 5α-Cholestan-3-one (N-ethoxycarbonyl)hydrazone (**3b**)

From 5α-cholestan-3-one **1** and ethyl carbazate **2b**. Yield 2.20 g (93%); white solid; mp 99–100 °C. IR (KBr): ν 3280, 1715, 1557, 1250 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 0.66–2.52 (m, 49H, steroidal H & $CO_2CH_2CH_3$), 4.27 (q, J = 6.1 Hz, 2H, $CO_2CH_2CH_3$), 7.62 (s, 1H, NH). *Anal.* required for $C_{30}H_{52}N_2O_2$ (%): C, 76.22; H, 11.09; N, 5.93. Found: C, 76.33; H, 10.95; N, 5.95.

2.2.4. Preparation of the α-chloroazo substrates (**4a**,**b**): general procedure

The reaction was carried out in the dark with exclusion of moisture. A solution of *t*-BuOCl (0.19 g, 1.5 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise to an ice-water cooled solution of hydrazone **3** (1 mmol) in dry CH_2Cl_2 (10 mL) over 10 min. The mixture was stirred for 15–30 min, and the reaction was monitored by TLC. After the reaction went to completion, anhydrous $CaCl_2$ was added to the resultant yellow solution. Substrates **4** could not be purified without partial decomposition, and therefore the solution containing **4** was used for next reaction. The solution can be stored at <4 °C in the dark for <24 h.

2.2.5. General procedure for the synthesis of the triazolo-fused 3-aza-A-homocholestanes **8a**–**f** and pyrazolo-fused analog **8g**

The reaction was carried out in a nitrogen atmosphere. The solution containing the chloride **4** was filtered. To the filtrate was added dropwise the appropriate triple bond compound **6** (nitrile or 1-ethynylbenzene, 1.5 mmol). The reaction mixture was cooled between −70 and −60 °C. A solution of $SbCl_5$ (0.37 g, 1.2 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise to the mixture over a period of 0.5 h. After being stirred for 2 h between −60 and −30 °C, the mixture was allowed gradually to warm to 30 °C (bath temperature) and stirred for additional 1.5 h. Different solvent or solvent combinations should be used depending on solubility of individual product (see below). This afforded five-membered ring annulated 3-aza-A-homocholestanes **8a**–**g**. Yields were calculated based on the employed hydrazone **3**.

2.2.6. [1R-[1α(R*),3aβ,3bα,5aβ,12aα,12bβ,14aα]]-1-(1,5-dimethylhexyl)-1,2,3,3a,3b,4,5,11,12,12a,12b,13,14,14a-tetradecahydro-8,12a,14a-trimethyl-9-(2,4,6-trichlorophenyl)-cyclopenta[5,6]naphtho[2,1-d][1,2,4]triazolo[1,5-a]azepinium hexachloroantimonate (**8a**)

The physical data and spectral data for **8a** were published previously (Ref. [17]).

2.2.7. [1R-[1α(R*),3aβ,3bα,5aβ,12aα,12bβ,14aα]]-1,2,3,3a,3b,4,5,11,12,12a,12b,13,14,14a-tetradecahydro-12a,14a-dimethyl-1-(1,5-dimethylhexyl)- 8-propyl-9-(2,4,6-trichlorophenyl)-cyclopenta[5,6]naphtho[2,1-d][1,2,4]triazolo[1,5-a]azepinium hexachloroantimonate (**8b**)

From hydrazone **3a** and butyronitrile. Upon completion of the reaction, all volatiles were removed by distillation under high vacuum, the green residue was recrystallized from MeOH and then from MeOH–MeCN (v/v 5:1) twice to afford pure **8b**. Yield 0.29 g (30%); brown powder; mp 170–172 °C. IR (KBr): ν 2933, 2868, 1560, 1556, 1457, 1386 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.65–2.50 (m, 47H, steroidal H & $CH_2CH_2CH_3$), 2.55 (t, J = 7.1 Hz, 2H, $CH_2CH_2CH_3$), 3.00 (m, 1H, H_c), 3.33 (m, 1H, H_d), 4.10 (m, 1H, H_a), 4.37 (m, 1H, H_b), 7.73 (s, 2H, Ar–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.9, 12.1, 18.7, 21.3, 22.6, 22.8, 23.82, 24.09, 28.0, 28.2, 28.4, 28.5, 30.3, 31.1, 31.5, 34.6, 34.8, 35.7, 36.1, 37.4, 39.5, 39.6, 39.7, 42.2, 43.7, 45.1, 46.2, 52.6, 56.2, 56.3 (steroidal & *n*-Pr), 122.4, 130.8, 131.1, 135.6, 136.6, 143.0 (Ar)), 162.6, 163.9 (C=N). HRMS (ESI): m/z calcd. for the cation $[C_{37}H_{55}Cl_3N_3]^+$: 646.3462; found: 646.3440.

2.2.8. [1R-[1α (R*),3aβ,3bα,5aβ,12aα,12bβ,14aα]]-8-benzyl-1-(1,5-dimethylhexyl)-1,2,3,3a,3b,4,5,11,12,12a,12b,13,14,14a-tetradecahydro-12a,14a-dimethyl-9-(2,4,6-trichlorophenyl)-cyclopenta[5,6]naphtho[2,1-d][1,2,4]triazolo[1,5-a]azepinium hexachloroantimonate (**8c**)

From hydrazone **3a** and phenylacetonitrile. Work-up as described above furnished **8c**. Yield 0.34 g (33%); brown powder; mp 140–142 °C. IR (KBr): ν 2929, 1627, 1562, 1449, 1385, 1106 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.66–2.50 (m, 42H, steroidal H), 3.02 (m, 1H, H_c), 3.31 (m, 1H, H_d), 4.05 (s, 2H, Ph-CH_2), 4.11 (m, 1H, H_a), 4.30 (m, 1H, H_b), 7.00–7.29 (m, 5H, Ph), 7.65 (s, 2H, $Cl_3C_6H_2$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.0, 18.6, 21.4, 22.5, 22.8, 23.8, 24.0, 24.1, 28.0, 28.2, 29.7, 30.3, 31.5, 33.9, 34.2, 34.6, 34.8, 35.7, 36.1, 39.5, 39.8, 42.2, 43.9, 52.8, 56.2, 56.3 (steroidal & $PhCH_2$), 122.6, 128.6, 129.2, 129.3, 130.3, 130.8, 131.4, 135.9 (Ar), 160.6, 163.3 (C=N). HRMS (ESI): m/z calcd. for the cation $[C_{41}H_{55}Cl_3N_3]^+$: 694.3462; found: 694.3480.

2.2.9. [1R-[1α(R*),3aβ,3bα,5aβ,12aα,12bβ,14aα]]-8-(2-(bromomethyl)phenyl)-1-(1,5-dimethylhexyl)-1,2,3,3a,3b,4,5,11,12,12a,12b,13,14,14a-tetradecahydro-12a,14a-dimethyl-9-(2,4,6-trichlorophenyl)-cyclopenta[5,6]naphtho[2,1-d][1,2,4]triazolo[1,5-a]azepinium hexachloroantimonate (**8d**)

From hydrazone **3a** and *o*-(bromomethyl)benzonitrile. Work-up as described above furnished **8d**. Yield 0.99 g (89%); pale-brown powder; mp 154–156 °C. IR (KBr): ν 2937, 2866, 1568, 1464, 1382 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.66–2.43 (m, 42H, steroidal H), 3.16 (m, 1H, H_c), 3.49 (m, 1H, H_d), 4.26 (m, 1H, H_a), 4.59 (m, 1H, H_b), 4.76–4.87 (m, 2H, BrCH_2), 7.10–7.73 (m, 6H, Ar–H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.0, 18.6, 21.3, 22.6, 22.8, 23.8, 24.1, 28.0, 29.3, 31.5, 34.8, 36.1, 39.5, 39.7, 42.2, 43.8, 52.8, 56.2 (steroidal & CH_2Br), 120.9, 123.7, 128.6, 129.2, 130.8, 131.2, 133.3, 134.0, 142.8 (C_6H_4, $Cl_3C_6H_2$), 158.8, 163.1 (C=N). HRMS (ESI): m/z calcd. for the cation $[C_{41}H_{54}BrCl_3N_3]^+$: 772.2567; found: 772.2579.

2.2.10. [1R-[1α(R*),3aβ,3bα,5aβ,12aα,12bβ,14aα]]-1-(1,5-dimethylhexyl)-1,2,3,3a,3b,4,5,11,12,12a,12b,13,14,14a-tetradecahydro-12a,14a-dimethyl-9-(2,4,6-trichlorophenyl)-8-vinyl-cyclopenta[5,6]naphtho[2,1-d][1,2,4]triazolo[1,5-a]azepinium hexachloroantimonate (**8e**)

From hydrazone **3a** and acrylonitrile. Work-up as described above furnished **8e**. Yield: 0.90 g (93%); pale-yellow powder; mp 192–194 °C. IR (KBr): ν 2934, 2866, 1569, 1560, 1477, 1460, 1390 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.66–2.43 (m, 42H, steroidal H), 3.00 (m, J = 16.5 Hz, 1H, H_c), 3.35 (m, J = 16.0 Hz, 1H, H_d),

4.14 (m, 1H, H_a), 4.40 (m, 1H, H_d), 6.19 (m, 2H, CH_2=CH), 6.91 (m, 1H, CH_2=CH), 7.74 (s, 2H, $Cl_3C_6H_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.4, 18.6, 21.2, 23.8, 24.1, 27.7, 28.2, 30.4, 31.2, 31.5, 34.7, 34.8, 35.8, 36.1, 37.5, 39.5, 39.7, 42.2, 43.7, 45.2, 52.3, 56.2, 56.3 (steroidal), 117.5, 121.9 (CH_2=CH), 131.1, 131.2, 135.3, 135.9, 136.9, 143.1 ($Cl_3C_6H_2$), 156.7, 163.9 (C=N). HRMS (ESI): *m/z* calcd. for the cation $[C_{36}H_{51}Cl_3N_3]^+$: 630.3149; found: 630.3166.

2.2.11. [1R-[1α(R),3aβ,3bα,5aβ,12aα,12bβ,14aα]]-1-(1,5-dimethylhexyl)-1,2,3,3a,3b,4,5,11,12,12a,12b,13,14,14a-tetradecahydro-12a,14a-dimethyl-8-phenyl-9-(2,4,6-trichlorophenyl)-cyclopenta[5,6]naphtho[2,1-d][1,2,4]triazolo[1,5-a]azepinium hexachloroantimonate (**8f**)*

From hydrazone **3a** and benzonitrile. Work-up as described above furnished **8f**. Yield: 0.91 g (89%); pale-brown solid; mp 207–208 °C. IR (KBr): *ν* 2932, 2865, 1574, 1560, 1482, 1460, 1380 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.66–2.53 (m, 42H, steroidal H), 3.10 (m, *J* = 16.3 Hz, 1H, H_c), 3.43 (m *J* = 13.7 Hz, 1H, H_d), 4.20 (m, *J* = 14.4 Hz, 1H, H_a), 4.49 (m, *J* = 13.1 Hz, 1H, H_b), 7.26–7.67 (m, 5H, Ph), 7.72 (s, 2H, $Cl_3C_6H_2$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.8, 12.0, 18.6, 21.2, 22.5, 22.8, 23.8, 24.1, 28.0, 30.4, 31.2, 31.5, 34.8, 52.8, 56.3 (steroidal), 122.6, 124.3, 128.5, 129.9, 130.8, 131.2, 134.3, 135.9, 136.9, 142.9 (Ar), 159.3, 164.3 (C=N). *Anal.* required for $C_{40}H_{53}Cl_9N_3Sb$ (%): C, 47.25; H, 5.25; N, 4.13. Found: C, 47.13; H, 5.26; N, 4.21.

2.2.12. [1R-[1α(R),3aβ,3bα,5aβ,12aα,12bβ,14aα]]-1-(1,5-dimethylhexyl)-1,2,3,3a,3b,4,5,11,12,12a,12b,13,14,14a-tetradecahydro-12a,14a-dimethyl-8-phenyl-9-(2,4,6-trichlorophenyl)-cyclopenta[5,6] naphtho[2,1-d][1,2,4]pyrazolo[1,5-a]azepinium hexachloroantimonate (**8g**)*

From hydrazone **3a** and phenylacetylene. After the reaction was complete, the mixture was evaporated under reduced pressure, and the residue was recrystallized from hot MeOH–MeCN (*v/v* 3:1). The crude product was subjected to a repeated recrystallization from hot MeOH–MeCN (*v/v* 5:1) to give pure **8g**. Yield: 0.54 g (53%); off-white powder; mp 204–206 °C. IR (KBr): 2935, 2866, 1562, 1467, 1382 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.66–2.43 (m, 42H, steroidal H), 2.88 (m, 1H, H_c), 3.29 (m, 1H, H_d), 3.99 (m, 1H, H_a), 4.46 (m, 1H, H_b), 6.94 (s, 1H, pyrazole-H), 7.28–7.70 (m, 7H, Ar–H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 11.9, 12.0, 18.6, 21.2, 22.5, 22.8, 23.8, 24.1, 28.0, 28.2, 30.2, 30.4, 35.7, 36.1, 38.1, 39.5, 39.7, 39.8, 42.2, 44.9, 45.9, 53.1, 56.2, 56.3, 56.9 (steroidal C), 109.3 (pyrazole C), 124.6, 126.0, 128.2, 128.3, 129.0, 129.6, 130.2, 130.4, 132.1, 136.5, 137.6, 141.4 (Ph, $Cl_3C_6H_2$), 151.4, 155.1 (C=N). HRMS (ESI): *m/z* calcd. for the cation $[C_{41}H_{54}Cl_3N_2]^+$: 679.3353; found: 679.3380.

*2.2.13. General procedure for the synthesis of the triazolo-fused 3-aza-A-homocholestanes (**11**) or the picrates (**12**)*

The reaction was performed by employing hydrazone **3b** in place of **3a** under the condition as described for the synthesis of **8a–g**. Instead of precipitating the heterocycles by adding Et_2O upon completion of the reaction, the resulting mixture was cooled to 0 °C, and an aqueous solution of NaOH (2 N, 10 mL) was added dropwise with vigorous stirring. After further stirring for 20 min, the mixture was extracted with CH_2Cl_2 (3 × 20 mL), the combined extracts were washed with H_2O (2 × 20 mL) and dried over Na_2SO_4. Removal of the solvent under reduced pressure furnished the neutral free bases of pentacyclic compounds **11**, which was subjected to recrystallization or salt formation by addition of a saturated picric acid in MeOH to give the corresponding picrate **12**.

2.2.14. [1R-[1α(R),3aβ,3bα,5aβ,12aα,12bβ,14aα]]-1-(1,5-dimethylhexyl)-1,2,3,3a,3b,4,5,11,12,12a,12b,13,14,14a-tetradecahydro-8-ethy-12a,14a-dimethyl -cyclopenta[5,6]naphtho[2,1-d][1,2,4]triazolo[1,5-a]azepine (**11b**)*

From **3b** (0.48 g, 1 mmol) and propionitrile (0.11 g, 2 mmol). The crude product was recrystallized from methanol. Yield 0.30 g (67%); mp 220–222 °C. IR (KBr, cm^{-1}): 2934, 2869, 1602, 1507, 1520, 1467, 1376. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 0.67–2.14 (m, 45H, steroidal, CH_2CH_3), 2.90 (q, *J* = 6.0 Hz, 2H, CH_2CH_3), 2.97 (m, 1H, H_c), 3.60 (m, 1H, H_d), 4.03 (m, 1H, H_a), 4.21 (m, 1H, H_b). ^{13}C NMR (100 MHz, $CDCl_3$): 11.2, 11.8, 11.9, 18.5, 11.9, 18.5, 19.3, 21.2, 22.5, 22.7, 23.7, 27.9, 28.1, 31.2, 34.8, 35.7, 36.0, 39.4, 39.7, 42.1, 45.6, 52.5, 53.6, 56.1, 56.3 (steroidal, CH_2CH_3), 154.4, 156.1 (C = N). *Anal.* required for $C_{30}H_{51}N_3$ (%): C, 79.41; H, 11.33; N, 9.26. Found: C, 79.20; H, 11.64; N, 9.20.

2.2.15. [1R-[1α(R),3aβ,3bα,5aβ,12aα,12bβ,14aα]]-1-(1,5-dimethylhexyl)-1,2,3,3a,3b,4,5,11,12,12a,12b,13,14,14a-tetradecahydro-12a,14a-dimethyl-8-phenyl-cyclopenta[5,6]naphtho[2,1-d][1,2,4]triazolo[1,5-a]azepine (**11c**)*

From **3b** (0.48 g, l mmol) and benzonitrile (0.21 g, 2 mmol). The crude product was recrystallized from MeOH–MeCN (2:1). Yield 0.27 g (54%); mp 197–199 °C. IR (KBr, cm^{-1}): 2953, 2867, 1530, 1487, 1466, 1442, 1350 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68–2.11 (m, 42H, steroidal), 2.83–3.09 (m, 2H, H_c/H_d), 4.02–4.35(m, 2H, H_a/H_b), 7.26–8.04 (m, 5H, Ph). ^{13}C NMR (125 MHz, $CDCl_3$)): δ 11.7, 12.0, 18.6, 21.2, 21.7, 22.5, 22.8, 23.8, 24.1, 27.5, 28.0, 28.2, 31.5, 35.0, 36.1, 36.2, 40.0, 42.2, 46.4, 52.0, 53.0, 56.2, 56.4 (steroidal), 126.0, 128.4, 128.7, 131.1 (Ph), 157.4, 159.6 (C=N). *Anal.* Calcd. for $C_{34}H_{51}N_3$ (%): C, 81.38; H, 10.24; N, 8.37. Found: C, 81.02; H, 10.40; N, 7.92.

2.2.16. [1R-[1α(R),3aβ,3bα,5aβ,12aα,12bβ,14aα]]-1-(1,5-dimethylhexyl)-1,2,3,3a,3b,4,5,11,12,12a,12b,13,14,14a-tetradecahydro-8,12a,14a-trimethyl-cyclopenta[5,6]naphtho[2,1-d][1,2,4]triazolo[1,5-a]azepinium picrate (**12a**)*

From **3b** and acetonitrile. The crude free base **11a** was obtained as brown oil which was transformed to the picrate by addition of a 1.2 equiv. of picric acid in MeOH with vigorous stirring for 10 min. The solid formed was filtered and recrystallized from hot MeOH to furnish the pure picrate **12a**. Yield: 0.55 g (82%); yellow crystals; mp 156–158 °C. IR (KBr): *ν* 2936, 2866, 1629, 1568, 1364, 1317, 1272 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.66–2.43 (m, 42H, steroidal H), 2.57 (s, 3H, CH_3), 2.96 (m, 1H, H_c), 3.2–3.55 (m, 1H, H_d), 4.03–4.43 (m, 1H, H_a), 4.19–4.36 (m, 1H, H_b), 8.93 (s, 2H, Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.4, 11.7, 11.8, 12.0, 18.59, 18.60, 22.5, 22.8, 23.8, 24.1, 28.0, 28.2, 34.8, 34.9, 35.7, 36.1, 39.47, 39.48, 44.1, 52.6, 56.1, 56.2, 56.3 (steroidal & 3 CH_3), 126.4, 129.2, 141.5, 152.0, 154.4 ($C_6H_2N_3O_7$), 154.9, 161.3 (C=N). HRMS (ESI): *m/z* calcd. for the cation $[C_{29}H_{50}N_3]^+$: 440.4005; found: 440.4007.

2.2.17. [1R-[1α(R),3aβ,3bα,5aβ,12aα,12bβ,14aα]]-1-(1,5-dimethylhexyl)-1,2,3,3a,3b,4,5,11,12,12a,12b,13,14,14a-tetradecahydro-12a,14a-dimethyl-8-propyl-cyclopenta[5,6]naphtho[2,1-d][1,2,4]triazolo[1,5-a]azepinium picrate (**12c**)*

From **3b** and butyronitrile. The crude oily product **11c** was transformed to the picrate by stirring with 1.2 equiv. of saturated picric acid in MeOH-MeCN–CH_2Cl_2 (*v/v/v* 5:5:1) overnight. The solid product was filtered off and recrystallized several times to give pure **12c**. Yield: 0.06 g (9%); yellow crystals; mp 122–124 °C. IR (KBr): *ν* 2934, 2869, 1602, 1507, 1520, 1467, 1376 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.66–2.25 (m, 47H, steroidal & $CH_3CH_2CH_2$), 2.85 (t, 2H, *J* = 7.4 Hz, $CH_3CH_2CH_2$), 2.97 (m, 1H, H_c), 3.23–3.55 (m, 1H, H_d), 4.06–4.40 (m, 1H, H_a), 4.23–4.26 (m, 1H, H_b), 8.91

(s, 2H, Ar–H). ^{13}C NMR (500 MHz, $CDCl_3$) : δ 11.6, 11.7, 11.9, 13.4, 18.6, 20.5, 20.6, 22.5, 23.8, 27.3, 27.9, 28.2, 35.7, 36.0, 39.1, 39.4, 42.2, 52.5, 56.1, 56.2, 56.3 (steroidal & Pr), 126.3, 128.8, 141.5, 154.1, 154.6, 155.36 (Ar), 155.39, 161.4 (C=N). HRMS (ESI): *m/z* calcd. for the cation $[C_{31}H_{54}N_3]^+$: 468.4318; found: 468.4337.

2.2.18. [1R-[1α(R),3aβ,3bα,5aβ,12aα,12bβ,14aα]]-1-(1,5-dimethylhexyl)-1,2,3,3a,3b,4,5,11,12,12a,12b,13,14,14a-tetradecahydro-12a,14a-dimethyl-8-vinyl-cyclopenta[5,6]naphtho[2,1-d][1,2,4]triazolo[1,5-a]azepine (**12e**)*

From **3b** and acrylonitrile. The crude oily product **11e** was transformed to the picrate **12e** as described for **11c**. The purification procedure described for **11c** gave **11e** as a light yellow. Yield: 0.16 g (23%); light-yellow crystals; mp 98–100 °C. IR (KBr): ν 2942, 2866, 1630, 1567, 1364, 1318, 1271 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.66–2.43 (m, 42H, steroidal), 2.89 (m, 1H, H_c), 3.35 (m, 1H, H_d), 4.04 (m, 1H, H_a), 4.18 (m, 1H, H_b), 5.70 (d, J = 11.3 Hz, 1H, H_e), 6.33 (d, J = 17.7 Hz, 1H, H_f), 6.64 (dd, J_1 = 17.7, J_2 = 11.3 Hz, 1H, H_g), 8.98 (s, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.0, 12.1, 18.7, 21.5, 22.5, 22.8, 23.8, 24.2, 28.0, 28.2, 29.0, 31.7, 35.4, 35.8, 36.1, 36.2, 38.6, 39.5, 39.9, 44.7, 46.7, 52.7, 53.9, 56.2, 56.3 (steroidal), 121.6, 124.8 (vinyl), 126.3, 130.5, 140.9 (Ar), 153.0, 155.5 (C=N). HRMS (ESI): *m/z* calcd. for the cation $[C_{30}H_{50}N_3]^+$: 452.4005; found: 452.4009.

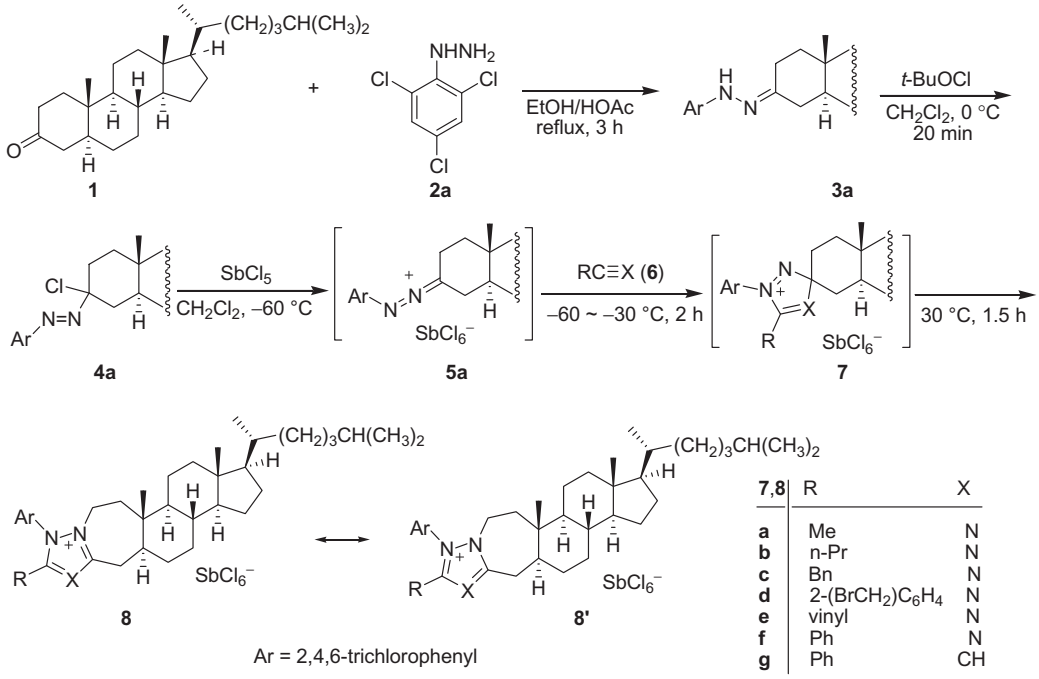
3. Results and discussion

The required starting material 5α-cholestan-3-one **1** was readily prepared in high yield according to the reported method [18,19]. The steroidal ketone **1** has been employed as a substrate for preparation of thiazolyl and thieno cholestane derivatives with potent anti-inflammatory effects [20], as well as 3-aza-A-homocholestanes and 3-aza-A-homo-5α-cholestano[3,4-*d*]tetrazole analogs [21,22]. In light of our success in the cationic [3 + 2$^+$] cycloaddition reactions between azocarbenium ions with nitriles, we decided to employ ketone **1** in the construction of unprecedented steroidal heterocycles.

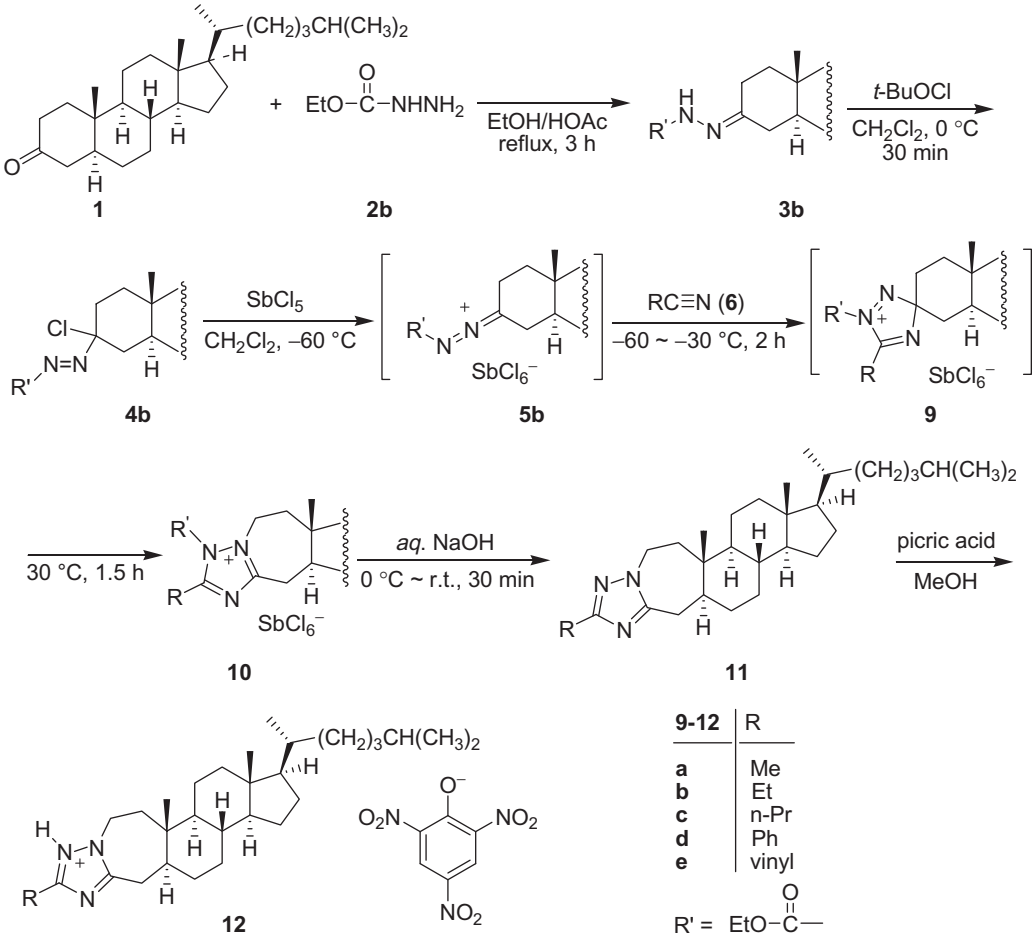
The initial study is outlined in Scheme 1. Cholestanone **1** was condensed with the aromatic hydrazine **2a** in refluxing ethanol to afford the corresponding hydrazone **3a** in 88% yield, which was then transformed to the α-chloro azo compound **4a** by reaction with *t*-BuOCl. Unlike the non-steroidal analogs, the oily compound **4a** proved to be extremely unstable under ambient conditions. Thus trials to purify this intermediate were unsuccessful. Nevertheless the intermediate solution can be stored in a refrigerator and used as obtained by evaporating the solvent. Upon addition of $SbCl_5$ as the Lewis acid at low temperature (−60 °C) is believed to generate the cationic 1,3-dipole intermediate **5a** by departure of the chloride ion of **4a**. In the presence of a slight excess of acetonitrile (**6a**), the highly reactive species **5a** underwent smooth cycloaddition to the triple bond to produce the 3-spiro-substituted triazolium salt intermediate **7a**. Intermediate **7a** underwent 1,2-shift upon warming to above room temperature (~30 °C) in the same vessel affording the novel pentacyclic 1,5-annulated-1,2,4-triazoloazepinium hexachloroantimonate **8a** in 93% isolated yield.

To extend the scope of the reaction, we employed different nitriles (**6**) with diverse electronic and steric characters for the above reaction sequence. We were pleased to find that the reaction is tolerant of the substituent on the cyano group. All reactions performed uniformly well to provide compounds **8b–f**, despite the low-yielding reactions with butyronitrile (30%) and phenylacetonitrile (33%). Furthermore, the carbon-carbon triple bond of alkynes also participated in the cationic [3 + 2] cycloaddition, as demonstrated by phenylacetylene which delivered the pyrazolo annulated counterpart **8g** in 53% yield under the same conditions.

We next considered extending this reaction to the synthesis of the N(1)-unsubstituted neutral steroidal triazoloazepine compounds (Scheme 2). Likewise, the (*N*-ethoxycarbonyl)hydrazone **3b** and the α-chloroazo compound **4b** were prepared from ketone **1** by sequential reactions with ethyl carbazate and *tert*-butyl hypochlorite. Departure of the α-chloro group was effected by treatment of **4b** with antimony pentachloride at low temperature (−60 °C), generating the azocarbenium ion **5b** as a reactive intermediate. In the presence of a nitrile molecule **6**, **5b** could be



Scheme 1. Synthetic pathway to triazolo-fused 3-aza-A-homocholestanes **8a–f** and pyrazolo-fused analog **8g**.



Scheme 2. Synthesis of the N-unsubstituted triazolo-fused 3-aza-A-homocholestane picrates **12a**–**e**.

intercepted by the cationic [3⁺ + 2] cycloaddition to form the initial adducts **9a**–**e**, which gave rise to the ring expanded products **10a**–**e** by 1,2-shift when elevating the temperature to about 35 °C. Compounds **10a**–**e** were directly treated in the same reaction vial with an ice-cooled solution of aqueous sodium hydroxide to hydrolyze the ester group. The neutral products **11** were obtained after usual workup as brownish solids. Compounds **11** could be transformed to the corresponding picrates **12a**–**e** by action with picric acid (Scheme 2). It has been demonstrated that both aliphatic and aromatic nitriles can be applied to furnish acceptable to good yields of products.

The synthesized products were fully characterized by NMR spectra along with HRMS analyses. A representative numbering system is shown in Fig. 1. The ^{1}H or ^{13}C NMR spectra in the given ranges are very similar to the spectral patterns of the related triazoloazepine derivatives reported by us previously [13–16]. Thus, in the ^{1}H NMR spectra, the signals between 4.0 and 4.5 ppm were assigned to the methylene protons H_a/H_b connecting to the triazolo nitrogen atom. In addition, the corresponding absorption for compounds **8** was observed somewhat downfield as compared with **11** or picrates **12**. Signals from the methylene protons (H_c/H_d) were found between 3.0 and 3.5 ppm. These data verified that the migration took place exclusively with the steroidal C(2) side upon ring expansion (Scheme 3, route a).

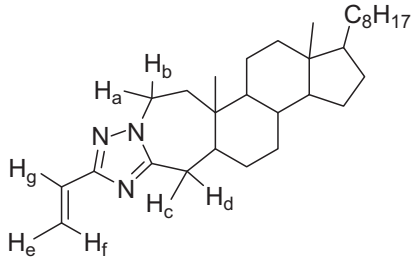
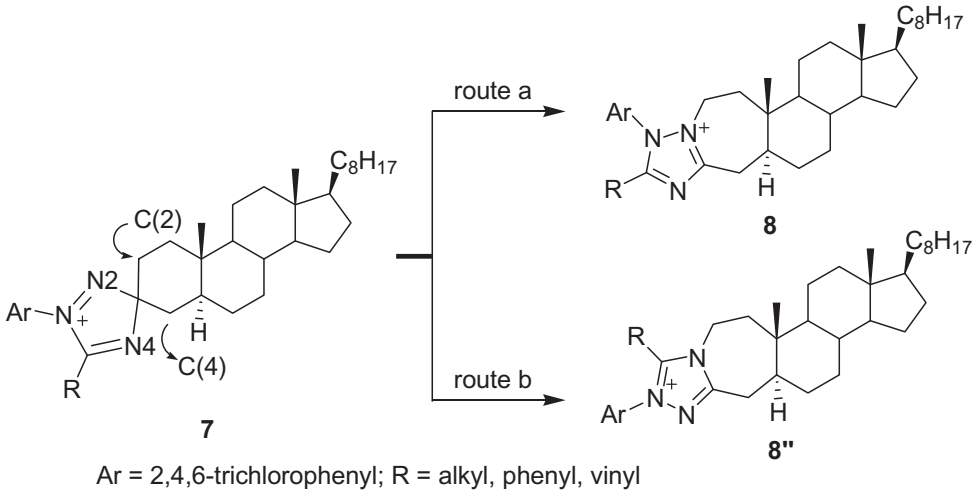


Fig. 1. A schematic illustration of the indicative protons.

We have recently reported the theoretical investigation on the parent cycloaddition reaction between azocarbenium ions and nitriles [23]. The results revealed that the reaction takes place via an initial formation of a 1:1 complex of the two reactants, followed by an asynchronous concerted cyclization forming the 3*H*-[1,2,4]-triazolium ions, which undergo [1,2]-shift to provide the 1*H*-[1,2,4]-triazolium ions. Mechanistically, the five-membered heterocyclic ring of the initial spiro-substituted adducts carries a diazenium function, in which the nitrogen atom N(2) exhibits latent nitrenium ion character. There are two possibilities for the subsequent 1,2-shift: the migration of the steroidal C(4) alkyl *vs* the C(2) residue. We have reported in the previous work [13–16] that the 1,2-rearrangement of carbon to electron-deficient nitrogen in 3*H*-1,2,4-triazolium ions like **7** and **9** occurs with complete migrant selectivity, and the migratory aptitude of the substituents tends to parallel their ability to accommodate the respective carbocation. In this context, the following migration sequence could be suggested: H > aryl > 3° alkyl > 2° alkyl > 1° alkyl > methyl ≈ cyclopropyl [13–16].



Scheme 3. Plausible 1,2-shift of the initially formed 3-spiro substituted 3*H*-1,2,4-triazolium salts **7**.

In the present cases, one would expect that the shift occurs with no or poor selectivity in view of the electronic similarities of the two competing migrants. However, we observed that the ring enlargement proceeded exclusively with migration of the C(2) aliphatic chain of the spiro compounds **7** to produce **8**, and no isomeric products **8** from the C(4) migration (Scheme 3, route b) were formed. The preferential migration of the C-2 vicinal methylene over the other one C-4 in the Baeyer–Villiger reaction of various 3-keto-5α-steroids has also been reported by Rivera et al. [24,25]. According to their theoretical studies, the better migrating aptitude of C-2 over C-4 in this process is controlled by conformational effects in the transition state of the Criegee rearrangement. Nevertheless, an earlier study by Suginome on the Baeyer–Villiger oxidation of 5α-cholestan-3-one with *m*-chloroperbenzoic acid in dichloromethane was found to be non-regioselective to produce a 1:1 mixture of 3-oxa-A-homo-5α-cholestan-4-one and 4-oxa-A-homo-5α-cholestan-3-one [26]. Factors that govern the migratory aptitude have to be further explored.

The previously published X-ray diffraction analysis for **8a** [17] confirmed the structure shown in Scheme 1 and eliminated the region isomer **8**″ shown in Scheme 3.

A fully completed Crystallographic Information File deposited with the CCDC is available (Deposition CCDC No. 754601).

4. Conclusion

In conclusion, we have developed a facile strategy for the synthesis of unprecedented steroidal triazoloazepine derivatives **8** and **11** through cycloaddition of the azocarbenium ions accompanied by a consecutive regioselective ring expansion. Our methodology provides a useful protocol for the synthesis of novel pentacyclic steroids with potential biological interests.

Acknowledgements

The authors are indebted to the National Natural Science Foundation of China (Project 20372015) for financial support. We are also grateful to the anonymous referees for their thoughtful comments.

References

[1] Kwon T, Heiman AS, Oriaku ET, Yoon K, Lee HJ. New steroidal antiinflammatory antidrugs: steroidal [16α, 17α-*d*]-3′-carbethoxyisoxazolines. J Med Chem 1995;38:1048–53.
[2] Elmegeed GA, Wardakhan WW, Younis M, Louca NA. Synthesis and antimicrobial evaluation of some novel cholestane heterocyclic derivatives. Arch Pharm 2004;337:140–7.
[3] Abdelhamid AO, Abdelhalim MM, Elmegeed GA. New routes to steroidal heterocyclic derivatives: synthesis of biologically active pyrazolyl- and isoxazolylpregnene derivatives. J Heterocycl Chem 2007;44:7–11.
[4] Burbiel J, Bracher F. Azasteroids as antifungals. Steroids 2006;68:587–94.
[5] Al-Soud YA, Al-Masoudi NA, Ferwanah AERS. Synthesis and properties of new substituted 1,2,4-triazoles: potential antitumor agents. Bioorg Med Chem 2003;11:1701–8.
[6] Olson S, Aster SD, Brown K, Carbin L, Graham DW, Hermanowski-Vosatka A, et al. Adamantyl triazoles as selective inhibitors of 11β-hydroxysteroid dehydrogenase type 1. Bioorg Med Chem Lett 2005;15:4359–62.
[7] Marcin LR, Thompson III LA, Boy KM, Guernon JM, Higgins MA, Shi JL, et al. Preparation of bicyclic compounds, especially bicyclic triazoles, for the reduction of beta-amyloid protein production. PCT Int. Appl., 2010083141 [Chem Abstr 2009;53:204359].
[8] Wang YF, Liu WM, Zhu YQ, Zou XM, Hu FZ, Yang HZ. Design, synthesis and biological evaluation of novel 6,7,8,9-tetrahydro-2-(2-aryloxypyrimidin-4-yl)-2H-[1,2,4] triazolo[4,3-*a*]azepin-3(5*H*)-ones. J Heterocycl Chem 2006;43:1275–80.
[9] Desia P, Schildknegt K, Agrios KA, Mossman C, Milligan GL, Aube J. Reactions of alkyl azides and ketones as mediated by Lewis acids: Schmidt and Mannich reactions using azide precursors. J Am Chem Soc 2000;122:7226–32.
[10] Ahmad MS, Alam Z. The Schmidt reaction: synthesis of azasteroids. Ind J Chem, Sect B: Org Chem Include Med Chem 1988;27B:336–8.
[11] Craig JC, Naik AR. The Beckmann rearrangement of ketooximes: a new method. J Am Chem Soc 1962;84:3410.
[12] Doorenbos N, Wu MT. Steroids. III. Synthesis of some 3-aza-A-homocholestanes by the Beckmann and Schmidt rearrangements in polyphosphoric acid. J Org Chem 1961;26:2548–9.
[13] Liu XJ, Wang QR, Liu Y, Fan YB, Zing ZB. An expedient synthesis of novel [1,2,4]-triazolo[3,2-d][1,5]benzothiazepine derivatives. Synthesis 2000;435–41.
[14] Liu XJ, Liu Y, Wang QR, Zou JP. An efficient approach to the [1,2,4]-triazolo[3,2-d][1,5]benzoxazepine skeleton-a novel tricyclic ring system. Synth Commun 2000;30:119–30.
[15] Wang QR, Li Z, Yang HY, Li F, Ding ZB, Tao FG. Annulated 1,2,4-triazoles. A convenient synthesis of thieno[2,3-f][1,2,4]triazolo[1,5-a]azepines: a novel triheterocyclic ring system. Synthesis 2003;1231–5.
[16] Meng QQ, Bai HX, Wang QR, Tao FG. A general synthesis of furo[3,2-c][1,2,4]triazolo[1,5-a]azepine and furo[2,3-f][1,2,4]triazolo[1,5-a]azepine derivatives. Synthesis 2007;33–8.
[17] Bai HX, Wang JM, Xia SJ, Wang QR. Synthesis and crystal structure of [1,2,4]-triazolo-annulated 3-aza-A-homocholestane derivative: a novel pentaheterocyclic ring system. J Chem Crystallogr 2011;41:316–21.
[18] Bruce WF, Ralls JO. Dehydrocholesterol. Org Synth 1943;Coll Vol 2:191–4.
[19] Bruce WF. Cholestanone. Org Synth 1943;Coll Vol 2:139–40.
[20] Elmegeed GA, Wardakhan WW, Baiuomy AR. Synthesis of thiazolyl and thieno cholestane derivatives: a novel class of potent antiinflammatory steroid. Pharmazie 2005;60:328–33.
[21] Ahmad MS, Alam Z. Synthesis of steroidal tetrazoles: Schimidt reaction on steroidal ketones. Ind J Chem, Sect B: Org Chem Including Med Chem 1988;27B:1001–3.
[22] Mohammad M, Nadeem I. Schmidt reaction of 3-oxocholest-5-en-4α-yl acetate and 6β-chloro-5-hydroxy-5α-cholestan-3-one. J Chem Res-S 1986;254–5.

[23] Li ZM, Wang QR. A theoretical investigation on the cycloaddition reaction between azocarbenium ions and nitriles. Int J Quantum Chem 2008;108:1067–75.
[24] Rivera DG, Pando O, Suardiaz R, Coll F. Studies on the regioselectivity of the Baeyer–Villiger reaction of 3-keto steroids: conformational effects determine the migration aptitude. Steroids 2007;72:466–73.
[25] Rivera DG, Pando O, Coll F. Synthesis of peptidomimetic-spirostane hybrids via Ugi reaction: a versatile approach for the formation of peptide–steroid conjugates. Tetrahedron 2006;62:8327–34.
[26] Suginome H, Yamada S. Photoinduced transformation. 77. A four-step substitution of a carbonyl group of steroidal ketones by an oxygen atom. A new method for the synthesis of cyclic ethers. J Org Chem 1985;50:2489–94.