STEROIDS 73 (2008) 1270–1276

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Synthesis of 4-azasteroids by an intramolecular Ugi reaction

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ARTICLE INFO

Article history:
Received 18 April 2008
Accepted 12 June 2008
Published on line 21 June 2008

Keywords:
Azasterols
4-Azacholestanes
Multicomponent reaction
Ugi reaction

ABSTRACT

In this paper we report the use of an intramolecular Ugi reaction to synthesize new 4-azacholestanes diversely substituted both at N-4 and C-5.

Both the scope and the stereochemical outcome of this approach were studied by varying the nature of the components necessary for this multicomponent reaction.

In sight of our results we concluded that this methodology can be applied to obtain 4-azasteroids targeted to find new biologically active compounds.

1. Introduction

The replacement of one or more carbon atoms of a steroid molecule by a heteroatom affects its chemical properties and often results in useful alterations to its biological activity. Azasteroids specially feature numerous biological activities [1]. Among the large and heterogeneous group of azasteroids, 4-azasteroids have attracted much interest, as many 4-azalactams exhibit strong inhibition of human steroid 5α-reductase, making them potential drugs for the treatment of benign prostatic hyperplasia [2]. Finasteride (**1**) and turosteride (**2**) are two commercial drugs belonging to this class (Fig. 1).

In addition, some 4-azasteroids, such as the cholestane derivative **3** (Fig. 1), have been shown to have interesting antifungal and antibacterial properties that are strongly dependent on the structural features of the studied compounds [3].

Taking the broad spectrum of biological properties of 4-azasteroids into account, our group started a research program devoted to the development of new synthetic strategies to achieve them. This strategy should be based on a simple and fast generation of new compounds having a high structural diversity. Multicomponent reactions are best suited to achieve this goal.

Multicomponent reactions (MCRs) are generally defined as reactions where more than two starting materials react to form a product, incorporating essentially all of the atoms of the educts. The structure of the reaction product can be easily diversified by a systematic variation of the starting materials [4].

One of the most versatile MCRs is the Ugi four-component reaction (U-4CR), which is based on the exceptional reactivity of the isocyanide functional group [5].

U-4CR constitutes a homogeneous group of reactions in which an amino component, an acid, a carbonyl compound and an isocyanide react together to give an α-aminoacylamide [4,5].

Intramolecular versions of the U-4CR, where two of the four functional groups involved belong to the same molecule, are particularly interesting for their ability to generate various heterocycles [6,7]. In this paper we report a new synthetic

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0039-128X/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2008.06.002

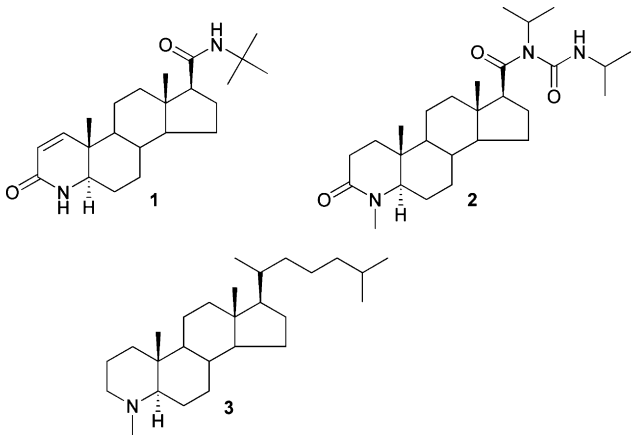


Fig. 1 – Examples of bioactive 4-azasteroids.

procedure to obtain 4-azasteroids, substituted at C-5, based on the intramolecular U-4CR between a bifunctional steroidal oxoacid and several amines and isocyanides.

2. Experimental

2.1. Synthesis

2.1.1. General

All the reagents were purchased from Sigma–Aldrich Chemical Co. EI-MS were measured either in a VG Trio-2 or in a Shimadzu QP-5000 mass spectrometer at 70 eV by direct inlet. Melting points were determined on a Fisher Johns apparatus and are uncorrected. All NMR spectra were recorded in $CDCl_3$ on a Bruker AM-500 (500 MHz for ^1H and 125.1 MHz for ^{13}C). Chemical shifts (δ) are given in ppm downfield from TMS as the internal standard. Coupling constant (J) values are in Hz. All solvents and reagents were of analytical grade.

All new compounds gave satisfactory NMR and mass spectral/combustion analysis data.

2.1.2. General synthetic procedure

The oxoacid **4** (5-oxo-A-nor-3,5-secocholestan-3-oic acid [8], 50 mg) was dissolved in methanol or ethanol and 1 equivalent of the corresponding amine was added. The mixture was stirred for 15 min at room temperature and 1 equivalent of the isonitrile was added. The reaction was refluxed for 16 h. The solvent was evaporated under reduced pressure and the residue was taken in EtOAc and washed with NaOH (5% aq). The mixture of epimers was separated by silica gel column chromatography (hexane/EtOAc gradient).

2.1.3. 4-Benzyl-5-N-(methoxycarbonylmethylen)-carboxamido-4-aza-5α-cholestan-3-one (**5a**)

M.p.: 177–178 °C. MS *m/z* (%): 606 (M^+, 0.1), 577 (0.5), 476 (97.2), 200 (20.9), 91 (100). ^1H NMR: 0.62 (18-H, 3H, s); 0.85 and 0.86 (26-H and 27-H, 3H, d, J = 2.3 Hz); 0.88 (H-21, 3H, d, J = 6.4 Hz); 0.92 (19-H, 3H, s); 2.62 (2-H, 2H, m); 3.68 and 3.87 ($NHCH_2COOCH_3$, 2H, dd, J = 5.7 and 17.7 Hz); 3.69 ($NHCH_2COOCH_3$, 3H, s); 4.66 and 4.91 (CH_2Ph, 2H, d, J = 14.8 Hz); 6.27 ($NHCH_2COOCH_3$, 1H, t, J = 5.7 Hz); 7.30 (CH_2Ph, 5H, m). ^{13}C NMR: 12.0 (C18); 16.0 (C19); 18.6 (C21); 22.0; 22.5 and 22.8 (C26 and C27); 23.8; 23.9; 26.7; 27.9; 28.2; 29.1; 29.3 (C2); 29.6; 33.6; 35.7; 36.1; 39.4; 39.7; 41.1 ($NHCH_2COOCH_3$); 41.4 (C10); 42.6 (C13); 43.9; 45.6 (CH_2Ph); 45.7; 52.2 ($NHCH_2COOCH_3$); 55.5; 56.0; 70.9 (C5); 127.3; 128.4; 128.7; 139.1; 169.9 ($CONHCH_2COOCH_3$); 173.7 (C3); 173.8 ($CONHCH_2COOCH_3$). Anal. Calcd. for $C_{38}H_{58}N_2O_4$: C, 75.21; H, 9.63; N, 4.62. Found: C, 75.33; H, 9.62; N, 4.59.

2.1.4. 4-Benzyl-5-N-(methoxycarbonylmethylen)-carboxamido-4-aza-5β-cholestan-3-one (**5b**)

M.p.: 155–156 °C. MS *m/z* (%): 606 (M^+, 0.2), 577 (0.5), 476 (95.2), 200 (22.9), 91 (100). ^1H NMR: 0.40 (7α-H, 1H, m); 0.62 (18-H, 3H, s); 0.86 and 0.87 (26-H and 27-H, 3H, d, J = 2.3 Hz); 0.89 (H-21, 3H, d, J = 6.4 Hz); 0.99 (19-H, 3H, s); 2.53 and 2.59 (2-H, 2H, m); 3.58 and 3.81 ($NHCH_2COOCH_3$, 2H, dd, J = 6.0 and 18.5 Hz); 3.67 ($NHCH_2COOCH_3$, 3H, s); 4.41 and 4.81 (CH_2Ph, 2H, d, J = 15.4 Hz); 6.33 ($NHCH_2COOCH_3$, 1H, t, J = 5.6 Hz); 7.26 (CH_2Ph, 5H, m). ^{13}C NMR: 11.9 (C18); 18.6 (C21); 19.4 (C19); 21.0; 22.5 and 22.8 (C26 and C27); 23.8; 26.2; 26.8; 27.4; 28.0; 28.2; 28.5 (C2); 34.0; 35.7; 36.1; 38.1 (C10); 39.5; 39.8; 41.5 ($NHCH_2COOCH_3$); 42.2; 42.3 (C13); 47.5 (CH_2Ph); 52.2 ($NHCH_2COOCH_3$); 56.1; 56.2; 73.8 (C5); 127.1; 128.2; 128.8; 138.5; 170.0 ($CONHCH_2COOCH_3$); 172.4 ($CONHCH_2COOCH_3$); 173.5 (C3). Anal. Calcd. for $C_{38}H_{58}N_2O_4$: C, 75.21; H, 9.63; N, 4.62. Found: C, 75.19; H, 9.70; N, 4.65.

2.1.5. 4-Benzyl-5-N-(diethoxyphosphorylmethylen)-carboxamido-4-aza-5α-cholestan-3-one (**6a**)

Colorless oil. MS *m/z* (%): 476 (M^+–$CONHPO(OEt)_2$, 0.3), 332 (1.2), 91 (8.4), 43 (100). ^1H NMR: 0.63 (18-H, 3H, s); 0.85 and 0.86 (26-H and 27-H, 3H, d, J = 2.5 Hz); 0.88 (H-21, 3H, d, J = 6.7 Hz); 0.92 (19-H, 3H, s); 1.33 and 1.37 ($NHCH_2PO(OCH_2CH_3)_2$, 6H, t, J = 7.1 Hz); 2.60 (2-H, 2H, m); 3.28 and 3.71 ($NHCH_2PO(OCH_2CH_3)_2$, 2H, ddd, J = 6.9, 11.6 and 15.8 Hz); 4.12 ($NHCH_2PO(OCH_2CH_3)_2$, 4H, m); 4.65 and 4.84 (CH_2Ph, 2H, d, J = 15.1 Hz); 6.07 ($NHCH_2PO(OCH_2CH_3)_2$, 1H, t, J = 5.7 Hz); 7.30 (CH_2Ph, 5H, m). ^{13}C NMR: 12.0 (C18); 16.0 (C19); 16.4 ($CONHCH_2PO(OCH_2CH_3)_2$, J = 6.0 Hz); 18.6 (C21); 22.1; 22.5 and 22.8 (C26 and C27); 23.8; 23.9; 26.7; 27.9; 28.2; 29.1; 29.2 (C2); 29.5; 33.6; 34.4 ($CONHCH_2PO(OCH_2CH_3)_2$, J = 154.6 Hz); 35.7; 36.1; 39.4; 39.7; 41.8 (C10); 42.6 (C13); 45.6 (CH_2Ph); 45.7; 55.6; 56.0; 62.3 and 62.5 ($CONHCH_2PO(OCH_2CH_3)_2$, J = 5.8 Hz); 71.0 (C5); 127.4; 128.3; 128.8; 139.0; 172.7 ($CONHCH_2PO(OCH_2CH_3)_2$, J = 3.1 Hz); 173.7 (C3). Anal. Calcd. for $C_{39}H_{63}N_2O_5P$: C, 69.82; H, 9.46; N, 4.18. Found: C, 69.95; H, 9.45; N, 4.20.

2.1.6. 4-Benzyl-5-N-(diethoxyphosphorylmethylen)-carboxamido-4-aza-5β-cholestan-3-one (**6b**)

Colorless oil. MS *m/z* (%): 476 (M^+–$CONHPO(OEt)_2$, 0.4), 332 (1.5), 91 (9.3), 43 (100). ^1H NMR: 0.45 (7α-H, 1H, m); 0.72 (18-H, 3H, s); 0.85 and 0.86 (26-H and 27-H, 3H, d, J = 2.4 Hz); 0.88 (H-21, 3H, d, J = 6.9 Hz); 0.99 (19-H, 3H, s); 1.31 and 1.35 ($NHCH_2PO(OCH_2CH_3)_2$, 3H, t, J = 7.0 Hz); 2.54 (2-H, 2H, m); 3.21 and 3.63 ($NHCH_2PO(OCH_2CH_3)_2$, 2H, ddd, J = 6.0, 13.0 and 17.1 Hz); 4.09 ($NHCH_2PO(OCH_2CH_3)_2$, 4H, m); 4.39 and 4.77 (CH_2Ph, 2H, d, J = 14.6 Hz); 6.11 ($NHCH_2PO(OCH_2CH_3)_2$, 1H, t, J = 6.0 Hz); 7.28 (CH_2Ph, 5H, m). ^{13}C NMR: 11.9 (C18); 16.4 ($CONHCH_2PO(OCH_2CH_3)_2$, J = 6.0 Hz); 18.6 (C21); 19.4 (C19); 21.0; 22.5 and 22.8 (C26 and C27); 23.8; 26.2; 26.8; 27.4; 28.0; 28.5 (C2); 34.0; 34.6 ($CONHCH_2PO(OCH_2CH_3)_2$, J = 150.0 Hz); 35.7; 36.1; 38.2 (C10); 39.5; 39.8; 42.3; 42.6 (C13); 47.4 (CH_2Ph); 56.0; 56.1; 62.3

and 62.5 ($CONHCH_2PO(OCH_2CH_3)_2$, $J = 5.8$ Hz); 73.9 (C5); 127.1; 128.3; 128.9; 138.4; 171.7 ($CONHCH_2PO(OCH_2CH_3)_2$, $J = 2.9$ Hz); 173.5 (C3). Anal. Calcd. for $C_{39}H_{63}N_2O_5P$: C, 69.82; H, 9.46; N, 4.18. Found: C, 69.83; H, 9.39; N, 4.25.

*2.1.7. 4-Benzyl-5-N-cyclohexylcarboxamido-4-aza-5α-cholestan-3-one (**7a**)*

M.p.: 160–160 °C. MS *m/z* (%): 602 (M^+, 0.2), 587 (0.5), 476 (50.1), 387 (3.2), 200 (16.8), 91 (100). ^{1}H NMR: 0.65 (18-H, 3H, s); 0.84 and 0.88 (26-H and 27-H, 3H, d, $J = 2.3$ Hz); 0.90 (H-21, 3H, d, $J = 6.5$ Hz); 0.91 (19-H, 3H, s); 2.47 (2-H, 2H, m); 3.49 ($NHCH(CH_2)_5$, 1H, m); 4.38 and 5.10 (NCH_2Ph, 2H, d, $J = 14.8$ Hz); 5.38 ($NHCH(CH_2)_5$, 1H, d, $J = 7.9$ Hz); 7.36 (Ph, 5H, m). ^{13}C NMR: 12.0 (C18); 16.6 (C19); 18.6 (C21); 22.4; 22.5 and 22.8 (C26 and C27); 23.8; 24.0; 24.8; 25.4; 26.8; 27.9; 28.2; 29.7 (C2); 29.9; 30.0; 31.5; 32.2; 32.5; 34.3; 35.8; 36.1; 39.4; 39.4; 39.9; 42.1 (C10); 42.7 (C13); 45.3 (NCH_2Ph); 48.1 ($NHCH(CH_2)_5$); 46.6; 55.5; 56.0; 70.5 (C5); 127.7; 129.0; 129.1; 139.2; 172.3 (C3); 173.8 ($CONHCH(CH_2)_5$). Anal. Calcd. for $C_{40}H_{62}N_2O_2$: C, 79.68; H, 10.36; N, 4.65. Found: C, 79.83; H, 9.99; N, 4.55.

*2.1.8. 4-Benzyl-5-N-cyclohexylcarboxamido-4-aza-5β-cholestan-3-one (**7b**)*

M.p.: 102–103 °C. MS *m/z* (%): 602 (M^+, 0.2), 587 (0.7), 476 (51.1), 387 (3.7), 200 (17.0), 91 (100). ^{1}H NMR: 0.43 (7α-H, 1H, m); 0.62 (18-H, 3H, s); 0.85 and 0.88 (26-H and 27-H, 3H, d, $J = 2.3$ Hz); 0.90 (H-21, 3H, d, $J = 6.5$ Hz); 0.99 (19-H, 3H, s); 2.55 (2-H, 2H, m); 3.57 ($NHCH(CH_2)_5$, 1H, m); 4.23 and 4.86 (NCH_2Ph, 2H, d, $J = 15.2$ Hz); 5.41 ($NHCH(CH_2)_5$, 1H, d, $J = 7.6$ Hz); 7.25 (Ph, 5H, m). ^{13}C NMR: 11.8 (C18); 18.6 (C21); 19.7 (C19); 21.0; 22.5 and 22.8 (C26 and C27); 23.8; 24.6; 25.4; 26.3; 27.1; 27.4; 28.0; 28.2; 28.5 (C2); 32.5; 32.9; 34.1; 35.7; 36.1; 38.0 (C10); 39.5; 39.7; 42.1; 42.2 (C13); 47.7 (NCH_2Ph); 48.8 ($NHCH(CH_2)_5$); 56.0; 56.2; 73.7 (C5); 127.0; 128.2; 128.9; 138.5; 170.6 (C3); 173.5 ($CONHCH(CH_2)_5$). Anal. Calcd. for $C_{40}H_{62}N_2O_2$: C, 79.68; H, 10.36; N, 4.65. Found: C, 79.54; H, 10.44; N, 4.72.

*2.1.9. 4-Butyl-5-N-cyclohexylcarboxamido-4-aza-5α-cholestan-3-one (**8a**)*

M.p.: 219–220 °C. MS *m/z* (%): 568 (M^+, 37.5), 553 (2.3), 444 (100), 166 (39.2), 83 (16.5). ^{1}H NMR: 0.66 (18-H, 3H, s); 0.86 and 0.87 (26-H and 27-H, 3H, d, $J = 2.3$ Hz); 0.89 (H-21, 3H, d, $J = 6.2$ Hz); 0.88 ($NCH_2(CH_2)_2CH_3$, 3H, t, $J = 7.2$ Hz); 0.93 (19-H, 3H, s); 2.42 (2-H, 2H, m); 3.19 and 3.62 ($NCH_2(CH_2)_2CH_3$, 2H, m, $J = 4.7$ and 13.2 Hz); 3.67 ($NHCH(CH_2)_5$, 1H, m); 5.67 ($NHCH(CH_2)_5$, 1H, d, $J = 7.7$ Hz). ^{13}C NMR: 12.1 (C18); 13.8 ($NCH_2(CH_2)_2CH_3$); 15.5 (C19); 18.6 (C21); 20.5; 22.1; 22.5 and 22.8 (C26 and C27); 23.9; 24.0; 24.6; 24.8; 25.4; 26.7; 28.0; 28.3; 29.0 (C2); 29.1; 29.2; 32.1; 32.6; 32.7; 33.6; 35.8; 36.1; 39.5; 39.8; 41.1 (C10); 42.0; 42.6 (C13); 45.5 ($NCH_2(CH_2)_2CH_3$); 48.2 ($NHCH(CH_2)_5$); 55.7; 56.1; 69.6 (C5); 172.6 (C3); 172.7 ($CONHCH(CH_2)_5$). Anal. Calcd. for $C_{37}H_{64}N_2O_2$: C, 78.11; H, 11.34; N, 4.92. Found: C, 78.31; H, 10.89; N, 4.82.

*2.1.10. 4-Butyl-5-N-cyclohexylcarboxamido-4-aza-5β-cholestan-3-one (**8b**)*

M.p.: 186 °C. MS *m/z* (%): 568 (M^+, 35.4), 553 (2.9), 444 (100), 166 (38.1), 83 (17.0). ^{1}H NMR: 0.66 (18-H, 3H, s); 0.86 and 0.87 (26-H and 27-H, 3H, d, $J = 2.3$ Hz); 0.89 (H-21, 3H, d, $J = 6.2$ Hz); 0.90 ($NCH_2(CH_2)_2CH_3$, 3H, t, $J = 7.4$ Hz); 1.00 (19-H, 3H, s); 2.43 (2-H, 2H, m); 3.13 and 3.29 ($NCH_2(CH_2)_2CH_3$, 2H, m, $J = 4.5$ and 11.3 Hz); 3.79 ($NHCH(CH_2)_5$, 1H, m); 5.50 ($NHCH(CH_2)_5$, 1H, d, $J = 8.2$ Hz). ^{13}C NMR: 11.9 (C18); 13.7 ($NCH_2(CH_2)_2CH_3$); 18.6 (C21); 20.8; 19.7 (C19); 21.0; 22.5 and 22.8 (C26 and C27); 23.8; 24.0; 24.6; 24.8; 25.4; 27.1; 27.5; 28.0; 28.2; 28.4 (C2); 30.8; 32.7; 33.2; 34.4; 35.7; 36.1; 37.7 (C10); 39.5; 39.8; 42.2; 42.3 (C13); 45.0 ($NCH_2(CH_2)_2CH_3$); 48.8 ($NHCH(CH_2)_5$); 56.2; 56.1; 73.1 (C5); 171.0 (C3); 172.5 ($CONHCH(CH_2)_5$). Anal. Calcd. for $C_{37}H_{64}N_2O_2$: C, 78.11; H, 11.34; N, 4.92. Found: C, 78.02; H, 11.21; N, 4.97.

*2.1.11. 4-(2′-Phenylethyl)-5-N-cyclohexylcarboxamido-4-aza-5α-cholestan-3-one (**9a**)*

M.p.: 140–141 °C. MS *m/z* (%): 617 (M^+, 0.7), 602 (0.5), 491 (100), 387 (5.9), 214 (8.5), 105 (15.5). ^{1}H NMR: 0.68 (18-H, 3H, s); 0.86 and 0.87 (26-H and 27-H, 3H, d, $J = 2.3$ Hz); 0.90 (H-21, 3H, d, $J = 6.5$ Hz); 0.99 (19-H, 3H, s); 2.56 and 2.40 (2-H, 2H, m); 2.70 and 3.07 (NCH_2CH_2Ph, 2H, m, $J = 5.0$ and 12.2 Hz); 3.32 and 3.88 (NCH_2CH_2Ph, 2H, m, $J = 4.9$, 18.2 and 12.0 Hz); 3.68 ($NHCH(CH_2)_5$, 1H, m); 5.60 ($NHCH(CH_2)_5$, 1H, d, $J = 7.9$ Hz); 7.28 (Ph, 5H, m). ^{13}C NMR: 12.1 (C18); 15.5 (C19); 18.6 (C21); 22.1; 22.5 and 22.8 (C26 and C27); 23.9; 24.0; 24.6; 24.7; 25.4; 26.8; 28.0; 28.3; 28.9; 29.0; 29.2 (C2); 32.6; 32.8; 33.5; 35.8; 36.0 (NCH_2CH_2Ph); 36.1; 39.4; 39.8; 41.1 (C10); 44.2 (NCH_2CH_2Ph); 48.3 ($NHCH(CH_2)_5$); 42.6 (C13); 45.4; 55.7; 56.1; 69.7 (C5); 126.6; 128.6; 128.7; 138.8; 172.5 (C3); 172.7 ($CONHCH(CH_2)_5$). Anal. Calcd. for $C_{41}H_{64}N_2O_2$: C, 79.82; H, 10.46; N, 4.54. Found: C, 79.77; H, 10.39; N, 4.32.

*2.1.12. 4-(2′-Phenylethyl)-5-N-cyclohexylcarboxamido-4-aza-5β-cholestan-3-one (**9b**)*

M.p.: 225–228 °C. MS *m/z* (%): 617 (M^+, 0.6), 602 (0.6), 491 (100), 387 (6.2), 214 (8.2), 105 (14.0). ^{1}H NMR: 0.67 (18-H, 3H, s); 0.85 and 0.87 (26-H and 27-H, 3H, d, $J = 2.3$ Hz); 0.90 (H-21, 3H, d, $J = 6.5$ Hz); 1.02 (19-H, 3H, s); 2.03 and 2.51 (2-H, 2H, m); 2.79 and 2.92 (NCH_2CH_2Ph, 2H, m, $J = 4.5$ and 12.0 Hz); 3.35 and 3.42 (NCH_2CH_2Ph, 2H, m, $J = 4.9$, 12.1 and 20.0 Hz); 3.77 ($NHCH(CH_2)_5$, 1H, m); 5.50 ($NHCH(CH_2)_5$, 1H, d, $J = 8.2$ Hz); 7.25 (Ph, 5H, m). ^{13}C NMR: 11.8 (C18); 18.6 (C21); 19.7 (C19); 21.0; 22.5 and 22.8 (C26 and C27); 23.8; 24.0; 24.8; 25.4; 27.1; 27.2 (C2); 27.5; 28.0; 28.2; 28.5; 32.8; 33.2; 34.4; 34.9 (NCH_2CH_2Ph); 35.7; 36.1; 37.8 (C10); 39.5; 39.8; 42.2; 42.3 (C13); 46.9 (NCH_2CH_2Ph); 48.9 ($NHCH(CH_2)_5$); 56.0; 56.1; 73.1 (C5); 126.3; 128.5; 128.7; 139.4; 170.9 (C3); 172.8 ($CONHCH(CH_2)_5$). Anal. Calcd. for $C_{41}H_{64}N_2O_2$: C, 79.82; H, 10.46; N, 4.54. Found: C, 79.90; H, 10.35; N, 4.61.

*2.1.13. 4-(2′-(1H-benzo[b]azol-3-yl)ethyl)-5-N-cyclohexylcarboxamido-4-aza-5α-cholestan-3-one (**10a**)*

Colorless oil. MS *m/z* (%): 656 (M^+, 0.3), 529 (4.2), 476 (51.1), 386 (16.4), 143 (100), 83 (9.2). ^{1}H NMR: 0.68 (18-H, 3H, s); 0.85 and 0.86 (26-H and 27-H, 3H, d, $J = 2.3$ Hz); 0.91 (H-21, 3H, d, $J = 6.5$ Hz); 1.01 (19-H, 3H, s); 2.45 (2-H, 2H, m); 2.89 and 3.52 (NCH_2CH_2Ar, 2H, m, $J = 5.0$ and 12.1 Hz); 3.61 ($NHCH(CH_2)_5$, 1H, m); 3.22 and 3.94 (NCH_2CH_2Ar, 2H, m, $J = 4.0$ and 13.0 Hz); 5.7 ($NHCH(CH_2)_5$, 1H, d $J = 8.1$ Hz); 7.2 (Ar, 3H, m); 7.37 (Ar, 1H, m); 7.77 (Ar, 1H, m); 8.27 (Ar, 1H, s). ^{13}C NMR: 12.1 (C18); 15.7 (C19); 18.6 (C21); 22.2; 22.5 and 22.8 (C26 and C27); 23.9; 24.0; 24.7; 24.8; 25.3; 25.8; 26.8; 28.0; 28.3; 29.0; 29.1 (C2); 29.3; 32.5; 32.6; 33.6; 35.8; 36.1; 39.4; 39.8; 41.3 (C10); 42.6 (C13); 43.0; 45.6; 48.3

(NHCH(CH_2)$_5$); 55.7; 56.1; 69.7 (C5); 111.2; 112.7; 119.0; 119.5; 122.0; 122.1; 127.3; 136.3; 172.6 (CONHCH(CH_2)$_5$) 173.1 (C3). Anal. Calcd. for $C_{43}H_{65}N_3O_2$: C, 78.73; H, 9.99; N, 6.41. Found: C, 78.69; H, 10.09; N, 6.62.

*2.1.14. 4-(2′-(1H-benzo[b]azol-3-yl)ethyl)-5-N-cyclohexylcarboxamido-4-aza-5β-cholestan-3-one (**10b**)*

M.p.: 132–134 °C. MS *m*/z (%): 656 (M^+, 0.3), 529 (4.5), 476 (51.9), 386 (15.6), 143 (100), 83 (9.5). ^{1}H NMR: 0.67 (18-H, 3H, s); 0.84 and 0.88 (26-H and 27-H, 3H, d, *J* = 2.3 Hz); 0.92 (H-21, 3H, d, *J* = 6.5 Hz); 1.04 (19-H, 3H, s); 2.49 (2-H, 2H, m); 2.95 and 3.40 (NCH_2CH_2Ar, 2H, m, *J* = 5.0 and 12.0 Hz); 3.73 (NHC*H*(CH_2)$_5$, 1H, m); 3.12 and 3.63 (NCH_2CH_2Ar, 2H, m, *J* = 4.4 and 12.2 Hz); 5.5 (N*H*CH(CH_2)$_5$, 1H, d *J* = 7.8 Hz); 7.16 (Ar, 3H, m); 7.35 (Ar, 1H, m); 7.80 (Ar, 1H, m); 8.09 (Ar, 1H, s). ^{13}C NMR: 11.9 (C18); 18.6 (C21);; 19.8 (C19); 21.1; 22.5 and 22.8 (C26 and C27); 23.8; 24.0; 24.6; 24.7; 24.8; 25.4; 27.12; 27.4; 27.6; 28.0; 28.2; 28.5 (C2); 32.8; 33.2; 34.4; 35.7; 36.1; 37.8; 39.4; 39.8 (C10); 42.3 (C13); 42.3; 46.1; 48.9 (NHCH(CH_2)$_5$); 56.0; 56.1; 73.1 (C5); 111.0; 113.6; 119.3; 119.4; 121.8; 122.0; 127.4; 136.2; 171.0 (C3); 172.8 (CONHCH(CH_2)$_5$). Anal. Calcd. for $C_{43}H_{65}N_3O_2$: C, 78.73; H, 9.99; N, 6.41. Found: C, 78.85; H, 10.12; N, 6.35.

*2.1.15. 4-(3′-Azidopropyl)-5-N-(ethoxycarbonylmethylen)-carboxamido-4-aza-5α-cholestan-3-one (**11a**)*

Colorless oil. MS *m*/z (%): 600 (M^+, 0.2), 469 (7.2), 386 (5.2), 258 (2.7), 43 (100). ^{1}H NMR: 0.66 (18-H, 3H, s); 0.85 and 0.86 (26-H and 27-H, 3H, d, *J* = 2.3 Hz); 0.89 (H-21, 3H, d, *J* = 6.5 Hz); 0.89 (19-H, 3H, s); 1.27 (NHCH$_2$COOCH$_2$C*H*$_3$, 3H, t, *J* = 7.0 Hz); 2.03 and 1.72 (NCH$_2$C*H*$_2$CH$_2$N$_3$, 2H, m); 2.51 (2-H, 2H, m); 3.28 and 3.83 (NC*H*$_2$CH$_2$CH$_2$N$_3$, 2H, m, *J* = 5.3, 10.1 and 13.8 Hz); 3.35 and 3.42 (NCH$_2$CH$_2$C*H*$_2$N$_3$, 2H, m); 3.89 and 3.98 (NHC*H*$_2$COOCH$_2$CH$_3$, 2H, dd, *J* = 5.5 and 17.7 Hz); 4.19 (NHCH$_2$COOC*H*$_2$CH$_3$, 2H, q, *J* = 7.0 Hz); 6.41 (N*H*CH$_2$COOCH$_2$CH$_3$, 1H, t, *J* = 5.6 Hz). ^{13}C NMR: 12.1 (C18); 14.1 (NHCH$_2$COOCH$_2$CH$_3$); 15.6 (C19); 18.6 (C21); 22.0; 22.5 and 22.8 (C26 and C27); 23.8; 24.0; 26.7; 28.0; 28.2; 28.8; 28.9 (NCH$_2$CH$_2$CH$_2$N$_3$); 29.0 (C2); 33.4; 35.8; 36.1; 39.4; 39.7; 39.9 (NCH$_2$CH$_2$CH$_2$N$_3$); 41.1 (C10); 41.5 (NHCH$_2$COOCH$_2$CH$_3$); 42.6 (C13); 45.4; 49.6 (NCH$_2$CH$_2$CH$_2$N$_3$); 55.6; 56.0; 61.5 (NHCH$_2$COOCH$_2$CH$_3$); 70.0 (C5); 169.6 (CONHCH$_2$COOCH$_2$CH$_3$); 173.2 (C3); 173.9 (CONHCH$_2$COOCH$_2$CH$_3$). Anal. Calcd. for $C_{34}H_{57}N_5O_4$: C, 68.08; H, 9.58; N, 11.68. Found: C, 68.25; H, 9.42; N, 11.40.

*2.1.16. 4-(3′-Azidopropyl)-5-N-(ethoxycarbonylmethylen)-carboxamido-4-aza-5β-cholestan-3-one (**11b**)*

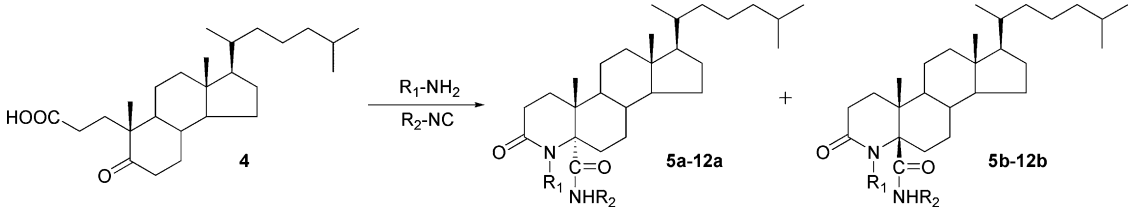
Colorless oil. MS *m*/z (%): 600 (M^+, 0.3), 469 (7.9), 386 (6.1), 258 (3.0), 43 (100). ^{1}H NMR: 0.67 (18-H, 3H, s); 0.86 and 0.87 (26-H and 27-H, 3H, d, *J* = 2.5 Hz); 0.90 (H-21, 3H, d, *J* = 6.5 Hz); 1.03 (19-H, 3H, s); 1.29 (NHCH$_2$COOCH$_2$C*H*$_3$, 3H, t, *J* = 7.1 Hz); 2.44 (2-H, 2H, m); 3.21 and 3.45 (NC*H*$_2$CH$_2$CH$_2$N$_3$, 2H, m, *J* = 5.3, 10.1 and 13.9 Hz); 3.34 (NCH$_2$CH$_2$CH$_2$N$_3$, 2H, m); 3.96 and 4.08 (NHC*H*$_2$COOCH$_2$CH$_3$, 2H, dd, *J* = 5.6 and 18.9 Hz); 4.21 (NHCH$_2$COOC*H*$_2$CH$_3$, 2H, q, *J* = 7.1 Hz); 6.25 (N*H*CH$_2$COOCH$_2$CH$_3$, 1H, t, *J* = 5.6 Hz). ^{13}C NMR: 11.9 (C18); 14.1 (NHCH$_2$COOCH$_2$CH$_3$); 18.6 (C21); 19.5 (C19); 21.0; 22.5 and 22.8 (C26 and C27); 23.8; 24.0; 26.7; 27.3; 27.5; 28.0; 27.9 (NCH$_2$CH$_2$CH$_2$N$_3$); 28.2; 28.5 (C2); 34.4; 35.7; 36.0; 39.5; 39.8 (NCH$_2$CH$_2$CH$_2$N$_3$); 38.0 (C10); 41.8 (NHCH$_2$COOCH$_2$CH$_3$); 42.2 (C13); 42.3; 42.7; 49.8 (NCH$_2$CH$_2$CH$_2$N$_3$); 56.1; 56.2; 61.7 (NHCH$_2$COOCH$_2$CH$_3$); 73.2 (C5); 169.6 (CONHCH$_2$COOCH$_2$CH$_3$); 173.2 (C3); 172.6 (CONHCH$_2$COOCH$_2$CH$_3$). Anal. Calcd. for $C_{34}H_{57}N_5O_4$: C, 68.08; H, 9.58; N, 11.68. Found: C, 68.12; H, 9.60; N, 11.67.

*2.1.17. 4-Propargyl-5-N-(ethoxycarbonylmethylen)-carboxamido-4-aza-5α-cholestan-3-one (**12a**)*

Colorless oil. MS *m*/z (%): 555 (M^+, 0.5), 540 (0.2), 424 (10.0), 102 (14.5), 43 (100). ^{1}H NMR: 0.66 (18-H, 3H, s); 0.85 and 0.87 (26-H and 27-H, 3H, d, *J* = 2.3 Hz); 0.89 (H-21, 3H, d, *J* = 6.6 Hz); 0.90 (19-H, 3H, s); 1.27 (NHCH$_2$COOCH$_2$C*H*$_3$, 3H, t, *J* = 7.2 Hz); 2.34 (CH$_2$C≡C*H*, 2H, t, *J* = 2.4 Hz); 2.54 (2-H, 2H, m); 3.89 and 3.97 (NHC*H*$_2$COOCH$_2$CH$_3$, 2H, dd, *J* = 5.7 and 17.8 Hz); 4.18 (NHCH$_2$COOC*H*$_2$CH$_3$, 2H, q, *J* = 7.2 Hz); 4.16 and 4.67 (C*H*$_2$C≡CH, 2H, dd, *J* = 17.8 and 2.4 Hz); 6.91 (N*H*CH$_2$COOCH$_2$CH$_3$, 1H, t, *J* = 5.6 Hz). ^{13}C NMR: 12.1 (C18); 14.1 (NHCH$_2$COOCH$_2$CH$_3$); 16.1 (C19); 18.6 (C21); 22.3; 22.5 and 22.8 (C26 and C27); 23.8; 24.0; 24.2; 26.7; 28.0; 28.9; 29.0 (C2); 29.4; 30.6 (CH$_2$C≡CH); 34.1; 35.8; 36.0; 39.5; 39.8; 41.5 (NHCH$_2$COOCH$_2$CH$_3$); 42.0 (C10); 42.7 (C13); 46.1; 55.5; 56.0; 61.4 (NHCH$_2$COOCH$_2$CH$_3$); 69.7 (C5); 72.7 (CH$_2$C≡CH); 80.1 (CH$_2$C≡CH); 169.5 (CONHCH$_2$COOCH$_2$CH$_3$); 172.8 (C3); 173.8 (CONHCH$_2$COOCH$_2$CH$_3$). Anal. Calcd. for $C_{34}H_{54}N_2O_4$: C, 73.61; H, 9.81; N, 5.05. Found: C, 73.81; H, 9.72; N, 5.25.

*2.1.18. 4-Propargyl-5-N-(ethoxycarbonylmethylen)-carboxamido-4-aza-5β-cholestan-3-one (**12b**)*

Colorless oil. MS *m*/z (%): 555 (M^+, 0.4), 540 (0.3), 424 (11.1), 102 (13.7), 43 (100). ^{1}H NMR: 0.67 (18-H, 3H, s); 0.86 and 0.87 (26-H and 27-H, 3H, d, *J* = 2.2 Hz); 0.89 (H-21, 3H, d, *J* = 6.5 Hz); 1.05 (19-H, 3H, s); 1.28 (NHCH$_2$COOCH$_2$C*H*$_3$, 3H, t, *J* = 7.1 Hz); 2.20 (CH$_2$C≡C*H*, 2H, t, *J* = 2.5 Hz); 2.48 (2-H, 2H, m); 3.90 and 4.10 (NHC*H*$_2$COOCH$_2$CH$_3$, 2H, dd, *J* = 5.8 and 17.9 Hz); 4.20 (NHCH$_2$COOC*H*$_2$CH$_3$, 2H, q, *J* = 7.1 Hz); 4.15 and 4.22 (C*H*$_2$C≡CH, 2H, dd, *J* = 17.6 and 2.4 Hz); 6.52 (N*H*CH$_2$COOCH$_2$CH$_3$, 1H, t, *J* = 5.4 Hz). ^{13}C NMR: 11.9 (C18); 14.2 (NHCH$_2$COOCH$_2$CH$_3$); 18.6 (C21); 19.4 (C19); 21.0; 22.5 and 22.8 (C26 and C27); 23.8; 24.0;



Scheme 1 – Synthesis of 4-azacholestanes using an intramolecular Ugi reaction.

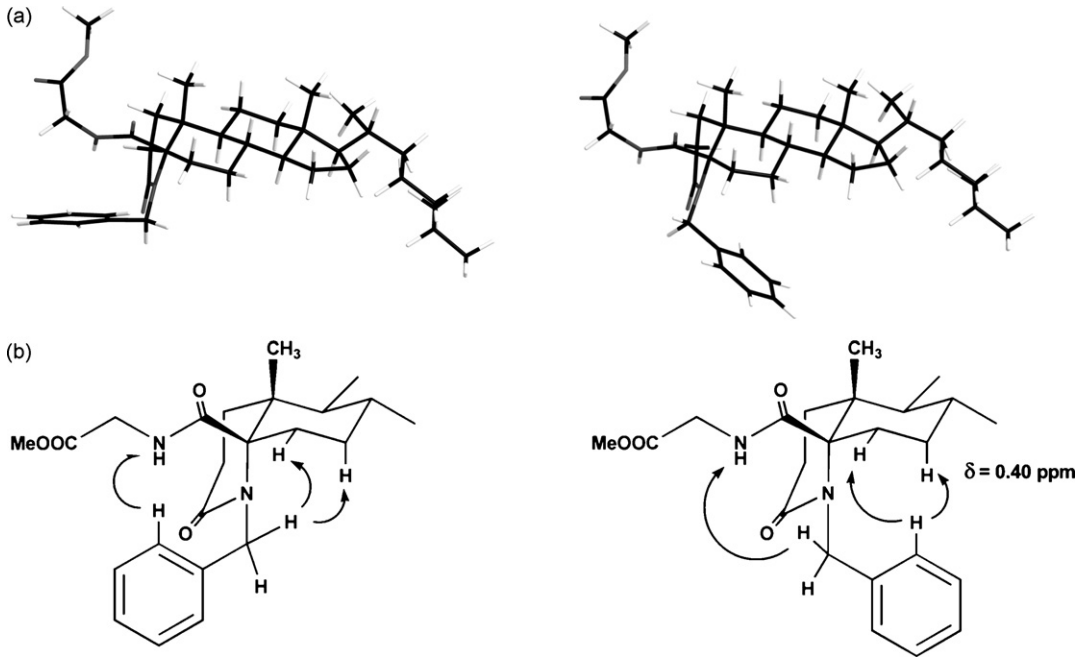


Fig. 2 – (a) Predicted lower energy conformations (B3LYP/6-31G (d,p)) for compound 5b. (b) Diagnostic nOe's for compound 5b.

26.7; 27.3; 27.6; 28.0; 28.2; 28.4 (C2); 32.8 ($CH_2C{\equiv}CH$); 34.3; 35.7; 36.1; 38.1 (C10); 39.4; 39.7; 41.8 ($NHCH_2COOCH_2CH_3$); 42.2; 42.3 (C13); 56.0; 56.1; 61.5 ($NHCH_2COOCH_2CH_3$); 72.0 (C5); 73.4 ($CH_2C{\equiv}CH$); 79.4 ($CH_2C{\equiv}CH$); 169.7 ($CONHCH_2COOCH_2CH_3$); 172.6 (C3); 172.1 ($CONHCH_2COOCH_2CH_3$). Anal. Calcd. for $C_{34}H_{54}N_2O_4$: C, 73.61; H, 9.81; N, 5.05. Found: C, 73.55; H, 9.84; N, 5.12.

2.1.19. *Molecular modeling*

A random conformational search of the lowest energy structures was performed for each model using the Tripos Molecular Field as implemented in the SYBYL 7.2 software [9]. The selected conformations were optimized *in vacuo* at the B3LYP/6-31G (d,p) level using Gaussian98 [10] and proved to be local minima via normal mode analysis.

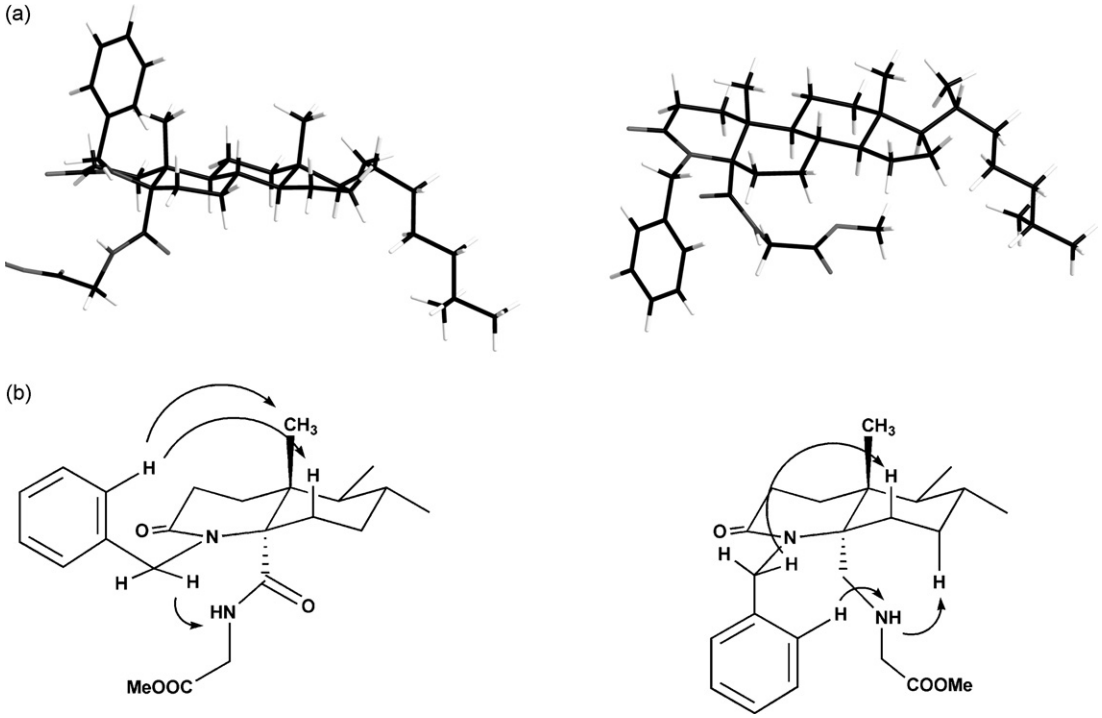
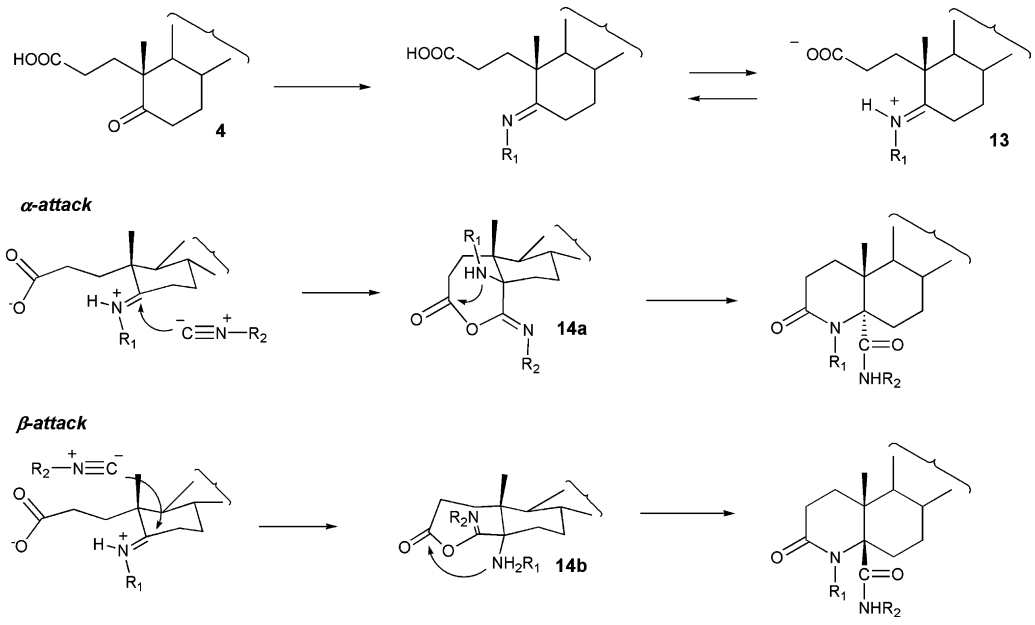


Fig. 3 – (a) Predicted lower energy conformations (B3LYP/6-31G (d,p)) for compound 5a. (b) Diagnostic nOe's for compound 5a.



Scheme 2 – Possible mechanism of the intramolecular Ugi reaction leading to 4-azasteroids.

3. Results and discussion

The synthesis of the novel 4-azasteroids is shown in Scheme 1. The oxoacid **4** was obtained from cholesterol following a standard procedure [8].

This intermediate compound contains both a carboxylic group and a keto group. Thus, the U-4CR with an amine and an isonitrile led to a mixture of the epimeric 4-azasteroids **5a–12a** and **5b–12b**. The reaction generally took place smoothly in boiling methanol or ethanol in good yields (45–77% of both isomers).

The stereochemistry at C-5 for each pair of isomers was established from both NOESY experiments and theoretical molecular models. Figs. 2 and 3 show the results obtained following this approach for compounds **5a** and **5b**.

The nOe's between diagnostic proton resonances, which were completely assigned using 1D and 2D NMR experiments, were determined.

On the other hand, the structures having the lower energies, found after a systematic conformational search, were optimized.

For example, the analysis of compound **5b** predicts the existence of several minima. Given the rigidity of the steroidal frame, these conformers differ mainly in the relative position of the substituents at N-4 and C-5. It is interesting to note that, in all of the predicted conformations, the benzyl moiety lies under the plane defined by the steroid rings. Fig. 2a depicts two of the most stable conformations. Fig. 2b shows a simplified model for each conformation in which the pairs of protons having correlations in the NOESY spectrum are indicated.

Furthermore, the ^{1}H NMR spectrum of compound **5b** has a resonance with a remarkable low chemical shift at 0.40 ppm, which can be assigned to H-7α. The proposed theoretical model shows that this proton might be affected by the anisotropic influence of the aromatic ring, confirming the C-5 stereochemistry (Fig. 2).

In the same way, Fig. 3a shows two of the predicted lower energy conformations for compound **5a**, which might account for the observed correlations in the NOESY spectrum (Fig. 2b).

There are several proposals on the U-4CR mechanism [11]. Scheme 2 depicts the possible mechanism that may be working in this case, and involves the formation of an iminium ion **13**, which gives an addition onto the carbenoid carbon of the isocyanide. The adduct (**14a** or **14b**) rearranges to the final products.

Table 1 shows that when aniline was used as the amino component, the reaction failed. It is thought that, although anilines react to give the imine readily (Scheme 2), the concentration of the corresponding iminium ion **13** is low, due to the electron withdrawing effect of the aromatic ring, thus preventing the reaction to proceed [11].

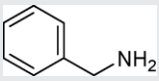
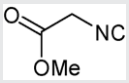
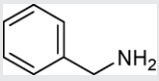
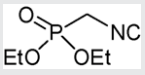
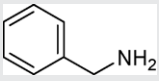
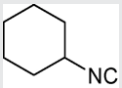
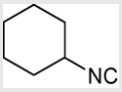
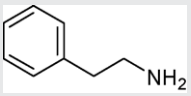
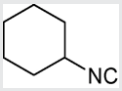
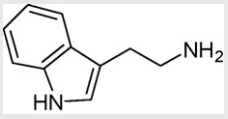
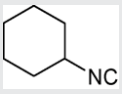
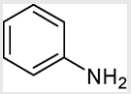
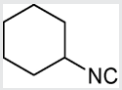
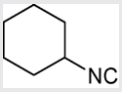
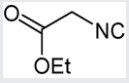
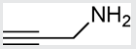
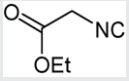
On the other hand, the low nucleophilicity of the *tert*-butylamine might explain that the U-4CR also failed when this amine was employed.

Except for the aforementioned components, the reaction worked well with a set of structurally diverse amines, which can also include additional functional groups (Table 1), generating a family of novel N-substituted 4-azasteroids.

The most notable feature of this synthetic approach is that it allows the facile introduction of a polyfunctionalized chain at C-5, an important task not reported previously. The structural variety of this side chain depends on the selected isonitrile.

As expected for the U-4CR, a low stereoselectivity was observed [12]. Nevertheless, when a bulky isonitrile was used, such as in the synthesis of compounds **6a** and **6b**, a high ratio

Table 1 – Yields of compounds 5–12

Amine R_1-NH_2	Isonitrile R_2-NC	Yield[a]
		63.3% (**5a/5b** 2.8:1)
		45.7% (**6a/6b** 20:1)
		70.2% (**7a/7b** 1.6:1)
		66.0% (**8a/8b** 1.5:1)
		62.7% (**9a/9b** 1.4:1)
		77.7% (**10a/10b** 1.7:1)
		No reaction
		No reaction
		68.3% (**11a/11b** 2.9:1)
		68.4% (**12a/12b** 1.9:1)

[a] Isolated yields.

of the product with a *trans* A/B ring junction was obtained, suggesting that in this case the attack on the α-face might be highly favored.

In order to explore the feasibility and scope of this synthetic methodology, in this work we used cholesterol as the starting material, and in sight of our results we suggest that this approach could be applied to other steroids with the convenient structures, targeted to find new biologically active compounds.

Further studies are under way both to expand this procedure to steroids other than cholestanes and to evaluate the biological properties of the new compounds.

Acknowledgements

This work was supported by grants from the Universidad de Buenos Aires (UBACyT X-190) and the Agencia Nacional de Promoción Científica y Técnica (ANPCyT PICT 38285/05). We are grateful to UMYMFOR (UBA-CONICET) for the analytical and spectroscopic determinations.

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