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A click chemistry approach to secosteroidal macrocycles

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ABSTRACT

A new synthetic pathway towards secosteroidal macrocycles was described *via* a reaction of cycloaddition as the key step. The characteristic ^{1}H and ^{13}C NMR spectroscopic features of the synthesized compounds are reported.

1. Introduction

Secosteroids have attracted considerable interest because of the broad range of biological activities of many naturally occurring representatives, such as vitamins D [1], with anolides [2], and marine steroids [3,4]. Apart from Vitamins D with their innumerable biological effects [5], secosteroids with cytotoxic [6–8], antihistamine [9], and anticancer [10] activity should be mentioned as compounds with great potential for drug development. The activity of seco analogs of normal steroidal hormones in humans and higher animals is a matter of scientific interest as well. Some of these compounds were prepared synthetically and showed hormonal or antihormonal activity [11–18]. It is evident that the higher conformational flexibility of seco steroids in comparison with normal steroids may result in novel, pharmaceutically useful compounds.

Moreover, [1,2,3]-triazoles are important class of five-membered nitrogen heterocycles. They have been reported to have important biological activities, including anti-HIV [19], anti-tumor [20], anti-bacterial [21], and anti-tuberculosis [22], and can also act as glycosidase [23–24], tyronase [25], and serine hydrolase [26] inhibitors.

The incidence of life-threatening fungal infections has tremendously increased in the last two decades due to greater use of immunosuppressive drugs, prolonged use of broad spectrum antibiotics, widespread use of indwelling catheters, and also in cancer and AIDS patients. The presently marketed antifungal and antibacterial drugs are either highly toxic or becoming ineffective due to the appearance of resistant strains. This necessitates continuing research into new classes of antimicrobial agents. 1,2,3-Triazole-containing molecules is one of these classes.

1,2,3-Triazole moieties are attractive connecting units because they are stable to metabolic degradation and capable of hydrogen bonding, which can be favorable in the binding of biomolecular targets and can improve the solubility [27,28]. The 1,2,3-triazole moiety does not occur in nature, although the synthetic molecules that contain 1,2,3-triazole units show diverse biological activities. The importance of triazolic compounds in medicinal chemistry is undeniable. Contrary to other azaheterocycles, the 1,2,3-triazole ring is not protonated at physiological pH because of its poor basicity.

Recently, Pore and co-workers [29,30] reported the synthesis of novel 1,2,3-triazole-linked β-lactam-bile acid conjugates **A** and some dimeric compounds **B** by 1,3-dipolar cycloaddition reaction of azido β-lactam and terminal alkyne of bile acids by using a click reaction (Scheme 1). Most of the compounds exhibited significant antifungal and moderate antibacterial activity against all the tested strains.

The unique chemistry behavior of this moiety aroused the chemist's interest, ranging from a synthetic point of view to the context of biological and pharmacological application.

So, for some years, we have been interested to develop new synthetic approaches to prepare secosteroidal molecules. Herein, we report a new and simple preparation of secocholanic steroids possessing a macrocycle and a triazole unit in their structure. Indeed, this combination of secocholanic skeleton with varied types of macrocycles, produces high levels of skeletal diversity and complexity. Additionally, there are no example, which describe the application of the copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction for the synthesis of secosteroids.

We report here the full details of these syntheses.

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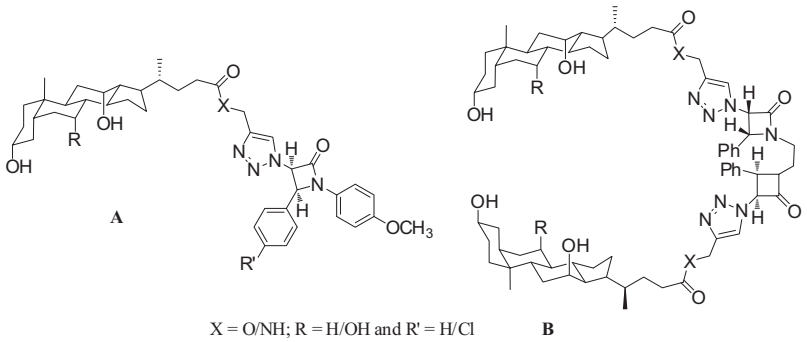


Scheme 1. Bile acid derivatives with biological activities.

2. Experimental section

All reactions were run under argon in oven-dried glassware. ^{1}H and ^{13}C NMR spectra are recorded at 200 or 400 and 50 and 100 MHz respectively, in $CDCl_3$ solutions. Chemical shift (δ) are reported in ppm with tetramethylsilane as internal standard. IR spectra were recorded on a Perkin–Elmer 1600 spectrophotometer. Flash chromatography was performed on silica gel (Merk 60 F_{254}) and TLC on silica gel. Dichloromethane was distilled from P_2O_5 and tetrahydrofuran (THF) over sodium/benzophenone.

Compounds **14** and **15** were prepared according to the previously described procedure [31]. The nomenclature used for the steroids is not the nomenclature used by Chemical Abstracts [32,33].

2.1. Propargyl 3α,7α,12α-trihydroxy-5β-cholan-24-oate (2)

A solution of cholic acid **1** (320 mg, 0.78 mmol), propargyl bromide (0.14 g, 1.63 mmol), *N,N*-dicyclohexylcarbodiimide (0.14 g, 0.70 mmol) and 4-dimethyl-aminopyridine (86 mg, 0.70 mmol) in dichloromethane (5 mL) was stirred at room temperature until the reaction was completed (about 12 h). The *N,N*-dicyclohexyl urea was filtered off and the filtrate was washed with water, 5% acetic acid solution and again water, dried over magnesium sulfate and the solvent was evaporated to afford propargyl cholate **2** (260 mg, 75%) as an oil. IR (neat) 3280, 1736, 1220, 1070 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 0.68 (s, 3H, H-18), 0.98 (d, J = 6.4 Hz, 3H, H-21), 1.18 (s, 3H, H-19), 2.48 (t, J = 2.4 Hz, 1H, H-27), 2.87 (m, 1H, H-7), 2.92 (m, 1H, H-3), 3.42 (m, 1H, H-12), 4.67 (d, J = 2.8 Hz, 1H, H-25); ^{13}C NMR (75 MHz, $CDCl_3$): 17.4, 20.4, 24.3, 27.7, 29.2, 29.8, 31.6, 34.7, 34.6, 35.0, 35.9, 37.4, 40.6, 45.4, 46.6, 49.6, 51.8, 53.5, 57.6, 63.4, 68.3, 74.8, 76.5, 77.9, 79.8, 79.9, 173.4. HRMS (EI) for $C_{27}H_{42}O_5$ [M$^+$] calcd 446.3032 found 446.3036.

2.2. Propargyl 3α,7α-dimethoxy-12α-hydroxy-5β-cholan-24-oate (3)

To a stirred suspension of NaH (0.62 g, 26 mmol) in THF (10 mL) at 0 °C under argon was added a solution of triol **2** (5 g, 11.8 mmol) in 5 mL of THF. The reaction mixture was stirred for 15 min, and then iodomethane (367 μL, 5.9 mmol) was added dropwise. After 24 h at room temperature, the reaction was diluted with 10 mL of Et_2O and quenched by the slow addition of 10 mL of H_2O. The combined organic extracts were washed with 30 mL of brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (Et_2O: 100%) to give **3** (4.6 g, 86%) as a white solid. mp = 132 °C; ^{1}H NMR (300 MHz, $CDCl_3$): 0.62 (s, 3H, H-18), 0.83 (d, J = 6.5, 3H, H-21), 0.86 (s, 3H, H-19), 2.46 (t, J = 2.4 Hz, 1H, H-27), 2.94 (m, 1H, H-3), 3.14 (s, 3H, OCH_3), 3.18 (s, 3H, OCH_3), 3.21 (m, 1H, H-7), 3.29 (m, 1H, H-12), 4.76 (d, J = 2.8 Hz, 1H, H-25); ^{13}C NMR (75 MHz, $CDCl_3$): 17.7, 19.9, 24.8, 27.6, 28.8, 29.2, 31.4, 34.2, 34.7, 35.6, 36.9, 37.1, 40.2, 44.4, 45.6, 49.1, 52.0, 53.4, 56.9, 57.2, 58.0, 63.1, 67.9, 73.6, 76.2, 77.8, 79.1, 82.3, 173.1. HRMS (EI) for $C_{29}H_{46}O_5$ [M$^+$] calcd 474.3345 found 474.3348.

2.3. Propionyl ether of 3α,7α-dimethoxy-12α-hydroxy-5β-cholane (4)

A flask equipped with a magnetic stirring bar, an argon outlet and a condenser was charged with $NaBH_4$ (90 mg, 0.40 mmol) and anhydr. THF (7 mL)–diglyme (3 mL) under argon. The solution was cooled at 0 °C and then a solution composed of boron trifluoride etherate (0.42 g, 3 mmol), ester **3** (0.18 mmol) and anhydr. THF (5 mL) was added. After completion of the reaction (TLC), it was quenched by addition of 2 N hydrochloric acid (1 mL) and water (10 mL), the product was extracted with ether (3 × 20 mL). The extracts were dried over $MgSO_4$, filtered and then concentrated under vacuum. The residue was chromatographed on silica gel (Et_2O–petroleum ether 1:1). Yield 46 mg (56%). Oil IR (neat) 3280, 1220, 1070 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 0.66 (s, 3H, H-18), 0.81 (d, J = 6.5, 3H, H-21), 0.92 (s, 3H, H-19), 2.42 (t, J = 2.4 Hz, 1H, H-27), 2.89 (m, 1H, H-3), 3.16 (s, 3H, OCH_3), 3.18 (s, 3H, OCH_3), 3.24 (m, 1H, H-7), 3.32 (m, 1H, H-12), 4.27 (d, J = 2.6 Hz, 1H, H-25); ^{13}C NMR (75 MHz, $CDCl_3$): 17.4, 20.1, 24.7, 27.9, 29.1, 30.6, 32.7, 33.9, 34.6, 35.8, 37.1, 37.4, 40.8, 43.4, 45.1, 49.3, 51.4, 52.8, 57.1, 57.4, 59.0, 62.7, 67.4, 70.2, 72.6, 74.4, 76.9, 78.1, 80.3. HRMS (EI) for $C_{29}H_{48}O_4$ [M$^+$] calcd 460.3553 found 460.3559.

2.4. Propionyl ether of 3α,7α-dimethoxy-12-oxo-5β-cholane (5)

Alcohol **4** (1 g, 2.17 mmol) was mixed in a mortar with pyridinium chlorochromate (PCC) (0.57 g, 2.66 mmol). The mixture was transferred to a pressure-resistant tube (Pyrex) and irradiated with MW at 170 °C for 5 min. The reaction mixture was filtered through a Celite pad and the filtrate and washings (CH_2Cl_2, 3 ∗ 10 mL) were combined and evaporated under reduced pressure. The residue was chromatographed on silica gel (diethyl ether/petroleum ether: 7/3), to afford 0.68 g (68% yield) of 12-oxo steroid **5** as an oil. IR (neat) 3236, 1511 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 0.72 (s, 3H, H-18), 0.86 (d, J = 6.4, 3H, H-21), 0.91 (s, 3H, H-19), 2.47 (t, J = 2.1 Hz, 1H, H-27), 2.86 (m, 1H, H-3), 3.17 (s, 3H, OCH_3), 3.18 (s, 3H, OCH_3), 3.26 (m, 1H, H-7), 4.16 (d, J = 2.3 Hz, 1H, H-25); ^{13}C NMR (75 MHz, $CDCl_3$): 16.9, 20.3, 23.6, 27.4, 29.3, 30.1, 32.6, 33.4, 34.1, 36.2, 37.3, 38.4, 41.5, 43.6, 44.9, 49.6, 51.7, 52.1, 56.8, 57.9, 59.2, 61.9, 66.4, 69.9, 72.3, 75.7, 79.0, 81.1, 214.9. HRMS (EI) for $C_{29}H_{46}O_4$ [M$^+$] calcd 458.3396 found 458.3400.

2.5. 3α,7α-Dimethoxy-13-oxa-C-homo-cholan-12-one (**6**)

To a solution of ketone **5** (0.5 g, 1.09 mmol) in dry dichloromethane (30 mL) containing *p*-toluenesulfonic acid (167 mg, 1.09 mmol) *m*-CPBA (12 mg) was added. The solution was stirred for 24 h at room temperature. The solution was then diluted with water and extracted with dichloromethane (3 ∗ 15 mL). The solution was washed successively with a 5% $Na_2S_2O_3$ solution, saturated brine, and water and was dried over anhydrous magnesium sulfate. The oily product, obtained by evaporation of the solvent, was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH: 95/5) to afford 0.5 g of pure lactone **6** (96%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): 0.68 (s, 3H, H-18), 0.89 (d, J = 6.4, 3H, H-21), 0.99 (s, 3H, H-19), 2.44 (t, J = 2.1 Hz, 1H, H-27), 2.78 (m, 1H, H-3), 3.16 (s, 3H, OCH_3), 3.19 (s, 3H, OCH_3), 3.24 (m, 1H, H-7), 4.16 (d, J = 2.3 Hz, 1H, H-25); ^{13}C NMR (75 MHz, $CDCl_3$): 17.1, 21.3, 24.2, 27.8, 29.1, 30.9, 32.7, 33.6, 35.5, 36.7, 37.4, 37.9, 41.7, 42.8, 43.7, 49.2, 50.9, 52.3, 56.6, 57.5, 58.8, 67.4, 69.1, 71.9, 74.6, 77.6, 79.2, 80.6, 174.3. HRMS (EI) for $C_{29}H_{46}O_5$ [M^+] calcd 474.3345 found 474.3348.

2.6. 3α,7α-Dimethoxy-11,12-seco-5β-cholan-12,13α-diol (**7**)

A solution of propargyl ether **6** (1 g, 2.11 mmol) in dry ether (10 mL) was added in one portion to a suspension of $LiAlH_4$ (0.25 g, 6.46 mmol) in dry ether (20 mL) at room temperature. After 1 h the reaction was quenched with H_2O and EtOAc. The aqueous layer was acidified to pH 2 with diluted HCl, and layers were separated. The aqueous layer was further extracted with EtOAc (3 × 50 mL), and the combined organic layers were dried over anhydrous $MgSO_4$ and evaporated to dryness. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH: 9/1) to afford 0.86 g of pure triol **7** (85%) as an oil. IR (neat) 2950, 1606, 1089 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 0.76 (s, 3H, H-18), 0.92 (d, J = 6.4, 3H, H-21), 1.02 (s, 3H, H-19), 2.46 (t, J = 2.3 Hz, 1H, H-27), 2.81 (m, 1H, H-3), 3.17 (s, 3H, OCH_3), 3.19 (s, 3H, OCH_3), 3.29 (m, 1H, H-7), 4.23 (d, J = 2.4 Hz, 1H, H-25); ^{13}C NMR (75 MHz, $CDCl_3$): 19.8, 21.6, 24.9, 27.4, 28.8, 31.4, 32.5, 33.1, 36.3, 36.6, 37.7, 38.4, 40.7, 41.6, 42.9, 49.7, 51.1, 52.7, 57.9, 59.1, 59.8, 61.7, 66.9, 69.3, 71.2, 73.6, 76.5, 78.6, 82.1. HRMS (EI) for $C_{29}H_{50}O_5$ [M^+] calcd 478.3658 found 478.3661.

2.7. Halogenated precursor (**8**)

Compound **7** (100 mg, 0.2 mmol) was dissolved in toluene (10 mL). To the homogenous solution CaH_2 (82.3 mg, 1.96 mmol), benzyltriethylammonium chloride (4.5 mg, 0.02 mmol) and chloroacetic chloride (0.054 mL, 0.67 mmol) were added. The suspension was refluxed for 3 h. Then it was cooled to room temperature and filtered. The filtrate was diluted with toluene (30 mL) and washed with water solution of $NaHCO_3$ (5%, 3 × 15 mL), brine (3 × 15 mL), water (2 × 20 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and obtained crude product was purified by column chromatography (hexane/AcOEt: 6/1) to give secosteroidal derivative **8** (80 mg, 70%) as a yellow oil. IR (neat) 3419, 3289, 2937, 2129, 1717 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 0.72 (s, 3H, H-18), 0.96 (d, J = 6.4, 3H, H-21), 1.04 (s, 3H, H-19), 2.43 (t, J = 2.2 Hz, 1H, H-27), 2.83 (m, 1H, H-3), 3.16 (s, 3H, OCH_3), 3.18 (s, 3H, OCH_3), 3.33 (m, 1H, H-7), 4.08 (m, 2H, H-12), 4.17 (d, J = 2.4 Hz, 1H, H-25), 4.46 (s, 2H, CH_2Cl); ^{13}C NMR (75 MHz, $CDCl_3$): 18.8, 20.9, 23.3, 27.6, 29.4, 31.7, 32.2, 32.9, 35.7, 36.3, 37.9, 39.3, 40.6, 40.9, 41.7, 43.0, 48.7, 50.9, 52.6, 56.3, 58.4, 59.2, 61.1, 67.3, 68.6, 70.2, 72.9, 76.3, 78.4, 81.7, 167.3. HRMS (EI) for $C_{31}H_{51}ClO_6$ [M^+] calcd 554.3374 found 554.3379.

2.8. Azido precursor (**9**)

Chloro derivative **8** (288 mg, 0.52 mmol) was dissolved in dry DMF (8 mL). To the solution, sodium azide (201.5 mg, 3.1 mmol) was added. The reaction mixture was heated at 60 °C for 24 h. Then the mixture was poured onto crushed ice and extracted with AcOEt. Organic layer was washed with water solution of $NaHCO_3$ (5%, 3 × 15 mL), brine (3 × 15 mL), water (2 × 20 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and obtained crude product was purified by column chromatography (hexane/AcOEt: 5/1) to give secosteroidal derivative **9** (180 mg, 62%) as a colourless oil. IR (neat) 3286, 2935, 2123, 1721 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 0.76 (s, 3H, H-18), 0.92 (d, J = 6.4, 3H, H-21), 1.06 (s, 3H, H-19), 2.49 (t, J = 2.2 Hz, 1H, H-27), 2.86 (m, 1H, H-3), 3.17 (s, 3H, OCH_3), 3.18 (s, 3H, OCH_3), 3.32 (m, 1H, H-7), 3.51 (s, 2H, CH_2N_3); 4.09 (m, 2H, H-12), 4.21 (d, J = 2.3 Hz, 1H, H-25); ^{13}C NMR (75 MHz, $CDCl_3$): 17.9, 21.3, 24.2, 27.8, 30.4, 31.6, 32.7, 33.1, 35.9, 36.6, 37.4, 39.8, 40.7, 41.6, 42.5, 47.9, 51.6, 52.3, 54.6, 56.7, 57.9, 59.7, 61.6, 66.8, 69.1, 70.4, 72.7, 76.4, 78.6, 81.2, 169.9. HRMS (EI) for $C_{31}H_{51}N_3O_6$ [M^+] calcd 561.3778 found 561.3780.

2.9. Macrocycle (**10**)

A mixture of the steroidal azide (115 mg, 0.2 mmol), $CuSO_4{\cdot}5H_2O$ (0.03 mmol, 7.5 mg), sodium ascorbate (0.076 mmol, 15 mg), DMF (2 mL) and water (2 mL) was stirred under argon at room temperature for 12 h. Then, brine (3 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 5 mL). The organic layers were combined, washed with brine (3 mL) and dried over $MgSO_4$. The solvent was removed under vacuum and the product was purified by column chromatography (hexane/AcOEt: 1/1) to afford the desired secosteroidal macrocycle (75 mg, 66%) as an oil. IR (neat) 3231, 1704 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 0.74 (s, 3H, H-18), 0.98 (d, J = 6.4, 3H, H-21), 1.04 (s, 3H, H-19), 2.81 (m, 1H, H-3), 3.16 (s, 3H, OCH_3), 3.17 (s, 3H, OCH_3), 3.26 (m, 1H, H-7), 4.13 (m, 2H, H-12), 4.66 (s, 2H, H-25), 4.72 (s, 2H, CH_2N), 7.56 (s, 1H, CH=); ^{13}C NMR (75 MHz, $CDCl_3$): 18.2, 21.9, 24.6, 27.4, 30.9, 32.3, 33.8, 34.1, 35.7, 36.9, 37.2, 39.3, 40.6, 41.5, 42.9, 46.3, 50.9, 52.7, 56.3, 56.1, 57.4, 61.7, 62.5, 66.1, 68.4, 69.7, 72.3, 77.4, 121.6, 142.8, 170.7. HRMS (EI) for $C_{31}H_{51}N_3O_6$ [M^+] calcd 561.3778 found 561.3782.

2.10. Macrocycle (**11**)

A flask equipped with a magnetic stirring bar, an argon outlet and a condenser was charged with $NaBH_4$ (90 mg, 0.40 mmol) and anhydr. THF (7 mL)–diglyme (3 mL) under argon. The solution was cooled at 0 °C and then a solution composed of boron trifluoride etherate (0.42 g, 3 mmol), macrocycle **10** (0.18 mmol) and anhydr. THF (5 mL) was added. After completion of the reaction (TLC), it was quenched by addition of 2 N hydrochloric acid (1 mL) and water (10 mL), the product was extracted with ether (3 × 20 mL). The extracts were dried over $MgSO_4$, filtered and then concentrated under vacuum. The residue was chromatographed on silica gel (Et_2O–petroleum ether 1:1), to give macrocycle **11** (43 mg, 44%). IR (neat) 2951, 1175, 1096 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 0.72 (s, 3H, H-18), 0.99 (d, J = 6.4, 3H, H-21), 1.06 (s, 3H, H-19), 2.83 (m, 1H, H-3), 3.16 (s, 3H, OCH_3), 3.18 (s, 3H, OCH_3), 3.32 (m, 1H, H-7), 4.17 (m, 2H, H-12), 4.38 (m, 4H, OCH_2CH_2N), 4.69 (s, 2H, H-25), 7.63 (s, 1H, CH=); ^{13}C NMR (75 MHz, $CDCl_3$): 17.9, 22.3, 24.4, 27.7, 31.2, 32.6, 33.4, 34.6, 35.1, 36.6, 37.3, 39.6, 40.1, 41.7, 43.5, 47.6, 51.2, 52.6, 56.9, 57.1, 57.7, 61.6, 62.3, 66.8, 67.9, 68.1, 69.3, 72.6, 78.1, 121.4, 142.0. HRMS (EI) for $C_{31}H_{53}N_3O_5$ [M^+] calcd 547.3985 found 547.3989.

2.11. Macrocycle (**12**)

To a solution of macrocycle **11** (50 mg, 0.10 mmol) in chloroform (20 mL) was added trimethylsilyl iodide (0.1 mL). The solution was left overnight at room temperature. Methanol was then added to decompose any excess trimethylsilyl iodide. The solution was extracted with diethyl ether, washed with water and saturated brine, dried over anhydrous magnesium sulfate. Evaporation of the solvent gave a crude product, which was purified by chromatography on silica gel (CH_2Cl_2/MeOH: 95/5), to give triol **12**. Yield 47 mg (92%). Oil. IR (neat) 3429, 2950, 1606, 1089 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 0.76 (s, 3H, H-18), 1.01 (d, J = 6.4, 3H, H-21), 1.07 (s, 3H, H-19), 2.84 (m, 1H, H-3), 3.29 (m, 1H, H-7), 4.15 (m, 2H, H-12), 4.29 (m, 4H, OCH_2CH_2N), 4.64 (s, 2H, H-25), 7.58 (s, 1H, CH=); ^{13}C NMR (75 MHz, $CDCl_3$): 18.3, 21.9, 24.6, 27.5, 30.8, 32.1, 33.7, 34.2, 36.3, 36.7, 37.1, 38.4, 39.8, 40.7, 43.9, 47.2, 51.6, 53.2, 57.2, 61.4, 62.6, 66.3, 67.1, 67.9, 69.8, 71.7, 78.2, 120.9, 142.6. HRMS (EI) for $C_{29}H_{49}N_3O_5$ [M^+] calcd 519.3672 found 519.3676.

2.12. Propionyl ether of 3α,7α,12α-trihydroxy-5β-cholane (**13**)

A flask equipped with a magnetic stirring bar, an argon outlet and a condenser was charged with $NaBH_4$ (90 mg, 0.40 mmol) and anhydr. THF (7 mL)–diglyme (3 mL) under argon. The solution was cooled at 0 °C and then a solution composed of boron trifluoride etherate (0.42 g, 3 mmol), ester **2** (0.18 mmol) and anhydr. THF (5 mL) was added. After completion of the reaction (TLC), it was quenched by addition of 2 N hydrochloric acid (1 mL) and water (10 mL), the product was extracted with ether (3 × 20 mL). The extracts were dried over $MgSO_4$, filtered and then concentrated under vacuum. The residue was chromatographed on silica gel (Et_2O–petroleum ether 1:1) to afford 40 mg (48%) of derivative **13** as an oil. IR (neat) 2981, 1370, 1048 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 0.72 (s, 3H, H-18), 1.01 (d, J = 6.4 Hz, 3H, H-21), 1.16 (s, 3H, H-19), 2.52 (t, J = 2.4 Hz, 1H, H-27), 2.84 (m, 1H, H-7), 2.89 (m, 1H, H-3), 3.38 (m, 1H, H-12), 3.52 (m, 1H, H-24), 4.13 (d, J = 2.2 Hz, 1H, H-25); ^{13}C NMR (75 MHz, $CDCl_3$): 17.6, 20.5, 24.7, 27.3, 29.6, 30.7, 31.4, 34.3, 34.6, 35.1, 36.3, 37.2, 41.6, 44.4, 46.8, 49.1, 50.7, 54.4, 57.5, 63.1, 68.6, 72.4, 74.7, 76.2, 77.4, 78.7, 79.2. HRMS (EI) for $C_{27}H_{44}O_4$ [M^+] calcd 432.324 found 432.3243.

2.13. Propionyl ether of 3α,12α-dihydroxy-7-oxo-5β-cholane (**14**)

Steroid **13** (3 g, 6.94 mmol) was added to a solution of $NaHCO_3$ (120 mL, 0.372 M) warmed at about 60 °C until the solid was completely dissolved. After cooling to room temperature, NBS (3.27 g, 18.37 mmol) was added and the resulting mixture was stirred for 17 h at room temperature and for 2 h at 80–85 °C, then allowed to cool to room temperature. The mixture was acidified with aqueous HCl (6 M, 100 mL): the yellow precipitate was isolated by filtration and washed with water, vigorously scratching. The product was dissolved in acetone and dried over anhydrous Na_2SO_4; the solvent was removed under reduced pressure affording the crude product **14** (2.85 g, 95%). IR (neat) 2961, 1145 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 0.68 (s, 3H, H-18), 0.99 (d, J = 6.4 Hz, 3H, H-21), 1.12 (s, 3H, H-19), 2.51 (t, J = 2.4 Hz, 1H, H-27), 2.92 (m, 1H, H-3), 3.46 (m, 1H, H-12), 3.52 (m, 1H, H-24), 4.24 (d, J = 2.3 Hz, 1H, H-25); ^{13}C NMR (75 MHz, $CDCl_3$): 16.9, 21.3, 24.4, 27.6, 29.1, 31.3, 32.4, 33.3, 34.9, 35.6, 36.7, 37.8, 42.3, 44.2, 46.6, 49.3, 51.2, 54.6, 56.9, 64.4, 67.4, 73.1, 74.6, 76.4, 77.1, 78.2, 78.9. HRMS (EI) for $C_{27}H_{42}O_4$ [M^+] calcd 430.3083 found 430.3087.

2.14. 3α,12α-Dihydroxy-7-oxo-8-oxa-B-homo-5β-cholane (**15**)

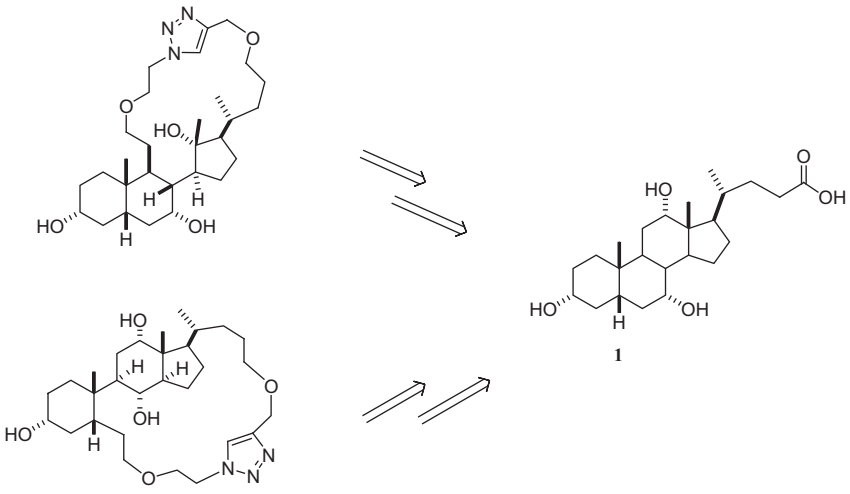
To a solution of ketone **14** (0.5 g, 1.11 mmol) in dry dichloromethane (30 mL) containing p-toluenesulfonic acid (167 mg, 1.11 mmol) m-CPBA (12 mg) was added. The solution was stirred for 24 h at room temperature. The solution was then diluted with water and extracted with dichloromethane (3 ∗ 15 mL). The solution was washed successively with a 5% $Na_2S_2O_3$ solution, saturated brine, and water and was dried over anhydrous magnesium sulfate. The oily product, obtained by evaporation of the solvent, was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH: 95/5) to afford 0.51 g of pure lactone **15** (98%) as an oil. ^1H NMR (300 MHz, $CDCl_3$): 0.66 (s, 3H, H-18), 0.89 (d, J = 6.4 Hz, 3H, H-21), 0.98 (s, 3H, H-19), 2.48 (t, J = 2.3 Hz, 1H, H-27), 2.96 (m, 1H, H-3), 3.42 (m, 1H, H-12), 3.86 (m, 1H, H-8), 3.49 (m, 1H, H-24), 4.19 (d, J = 2.3 Hz, 1H, H-25); ^{13}C NMR (75 MHz, $CDCl_3$): 16.1, 22.6, 24.7, 26.9, 29.8, 31.4, 32.6, 34.0, 34.7, 35.2, 36.9, 39.8, 42.1, 43.7, 46.5, 49.7, 52.0, 54.6, 56.3, 63.7, 67.1, 72.9, 73.2, 75.8, 76.3, 77.6, 78.8. HRMS (EI) for $C_{27}H_{42}O_5$ [M^+] calcd 430.3032 found 446.3036.

2.15. 3α,7,8α,12α-Tetrahydroxy-7,8-seco-5β-cholan-24-oxyprop-1-yne (**16**)

A solution of propargyl ether **15** (1 g, 2.24 mmol) in dry ether (10 mL) was added in one portion to a suspension of $LiAlH_4$ (0.25 g, 6.73 mmol) in dry ether (20 mL) at room temperature. After 1 h the reaction was quenched with H_2O and EtOAc. The aqueous layer was acidified to pH 2 with diluted HCl, and layers were separated. The aqueous layer was further extracted with EtOAc (3 × 50 mL), and the combined organic layers were dried over anhydrous $MgSO_4$ and evaporated to dryness. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH: 9/1) to afford 0.78 g of pure tetraol **16** (78%) as an oil. IR (neat) 3418, 2976, 1606 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 0.62 (s, 3H, H-18), 0.84 (d, J = 6.4 Hz, 3H, H-21), 0.92 (s, 3H, H-19), 2.62 (t, J = 2.3 Hz, 1H, H-27), 3.34 (m, 1H, H-8), 3.52 (m, 2H, H-3 and H-12), 3.76 (m, 1H, H-7), 3.85 (m, 2H, H-24), 4.58 (d, J = 2.4 Hz, 1H, H-25); ^{13}C NMR (75 MHz, $CDCl_3$): 15.9, 21.7, 22.4, 27.6, 29.3, 30.8, 31.3, 32.4, 33.6, 35.1, 36.9, 37.3, 38.2, 38.7, 42.1, 49.9, 53.1, 54.6, 56.3, 64.6, 68.4, 71.5, 72.0, 73.0, 73.6, 76.7, 78.9. HRMS (EI) for $C_{27}H_{46}O_5$ [M^+] calcd 450.3345 found 446.3349.

2.16. Halogenated precursor (**17**)

Compound **16** (90 mg, 0.2 mmol) was dissolved in toluene (10 mL). To the homogenous solution CaH_2 (82.3 mg, 1.96 mmol), benzyltriethylammonium chloride (4.5 mg, 0.02 mmol) and chloroacetic chloride (0.054 mL, 0.67 mmol) were added. The suspension was refluxed for 3 h. Then it was cooled to room temperature and filtered. The filtrate was diluted with toluene (30 mL) and washed with water solution of $NaHCO_3$ (5%, 3 × 15 mL), brine (3 × 15 mL), water (2 × 20 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and obtained crude product was purified by column chromatography (hexane/AcOEt 6:1) to give secosteroidal derivative **17** (75 mg, 72%) as a yellow oil. IR (neat) 3416, 3290, 2940, 2125, 1718 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 0.64 (s, 3H, H-18), 0.88 (d, J = 6.4 Hz, 3H, H-21), 1.12 (s, 3H, H-19), 2.54 (t, J = 2.2 Hz, 1H, H-27), 3.59 (m, 1H, H-8), 3.72 (m, 2H, H-3 and H-12), 3.87 (m, 1H, H-7), 3.93 (m, 2H, H-24), 4.36 (d, J = 2.3 Hz, 1H, H-25), 4.48 (s, 2H, CH_2Cl); ^{13}C NMR (75 MHz, $CDCl_3$): 14.2, 19.8, 21.5, 22.7, 27.4, 29.5, 30.7, 31.9, 33.8, 35.4, 36.3, 37.4, 38.7, 39.8, 40.3, 40.8, 43.4, 48.6, 50.9, 52.0, 62.6, 65.6, 69.7, 69.9, 73.1, 73.7, 76.6, 78.9, 166.0. HRMS (EI) for $C_{29}H_{47}ClO_6$ [M^+] calcd 526.3061 found 526.3065.



Scheme 2. Retrosynthetic pathway.

2.17. Azido precursor (**18**)

Chloro derivative **17** (274 mg, 0.52 mmol) was dissolved in dry DMF (8 mL). To the solution sodium azide (201.5 mg, 3.1 mmol) was added. The reaction mixture was heated at 60 °C for 24 h. Then the mixture was poured onto crushed ice and extracted with AcOEt. Organic layer was washed with water solution of $NaHCO_3$ (5%, 3 × 15 mL), brine (3 × 15 mL), water (2 × 20 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and obtained crude product was purified by column chromatography (hexane/AcOEt 5:1) to give secosteroidal derivative **18** (188 mg, 68%) as a colourless oil. IR (neat) 3288, 2935, 2126, 1720 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 0.66 (s, 3H, H-18), 0.83 (d, J = 6.4 Hz, 3H, H-21), 1.08 (s, 3H, H-19), 2.59 (t, J = 2.1 Hz, 1H, H-27), 3.49 (m, 1H, CH_2N_3), 3.54 (m, 1H, H-8), 3.68 (m, 2H, H-3 and H-12), 3.82 (m, 1H, H-7), 3.97 (m, 2H, H-24), 4.28 (d, J = 2.3 Hz, 1H, H-25); ^{13}C NMR (75 MHz, $CDCl_3$): 14.6, 18.9, 21.3, 23.1, 26.7, 29.3, 29.8, 30.4, 31.6, 35.7, 36.0, 36.4, 38.9, 39.6, 40.7, 41.6, 43.7, 49.6, 51.2, 52.7, 63.0, 65.1, 69.6, 70.1, 72.9, 73.4, 76.2, 78.6, 169.8. HRMS (EI) for $C_{29}H_{47}N_3O_6$ [M^+] calcd 533.3465 found 533.3468.

2.18. Macrocyle (**19**)

A mixture of the steroidal azide (105 mg, 0.2 mmol), $CuSO_4{\cdot}5H_2O$ (0.03 mmol, 7.5 mg), sodium ascorbate (0.076 mmol, 15 mg), DMF (2 mL) and water (2 mL) was stirred under argon at room temperature for 12 h. Then, brine (3 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 5 mL). The organic layers were combined, washed with brine (3 mL) and dried over $MgSO_4$. The solvent was removed under vacuum and the product was purified by column chromatography (hexane/AcOEt: 1/1) to afford the desired secosteroidal macrocycle (65 mg, 62%) as an oil. IR (neat) 3302, 1738 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 0.78 (s, 3H, H-18), 1.01 (d, J = 6.4 Hz, 3H, H-21), 1.16 (s, 3H, H-19), 3.48 (m, 1H, H-8), 3.51 (m, 1H, H-3), 3.82 (m, 1H, H-12), 3.88 (m, 2H, H-24), 4.15 (m, 2H, H-7), 5.12 (s, 2H, H-25), 5.18 (s, 2H, CH_2N), 7.62 (s, 1H, CH=); ^{13}C NMR (75 MHz, $CDCl_3$): 13.7, 18.8, 21.7, 22.4, 26.6, 29.1, 29.9, 30.1, 31.7, 34.3, 36.3, 37.4, 38.8, 39.2, 40.1, 42.5, 43.7, 47.4, 52.0, 54.1, 65.4, 70.1, 70.6, 73.2, 74.7, 76.3, 121.8, 143.6, 170.2. HRMS (EI) for $C_{29}H_{47}N_3O_6$ [M^+] calcd 533.3465 found 533.3468.

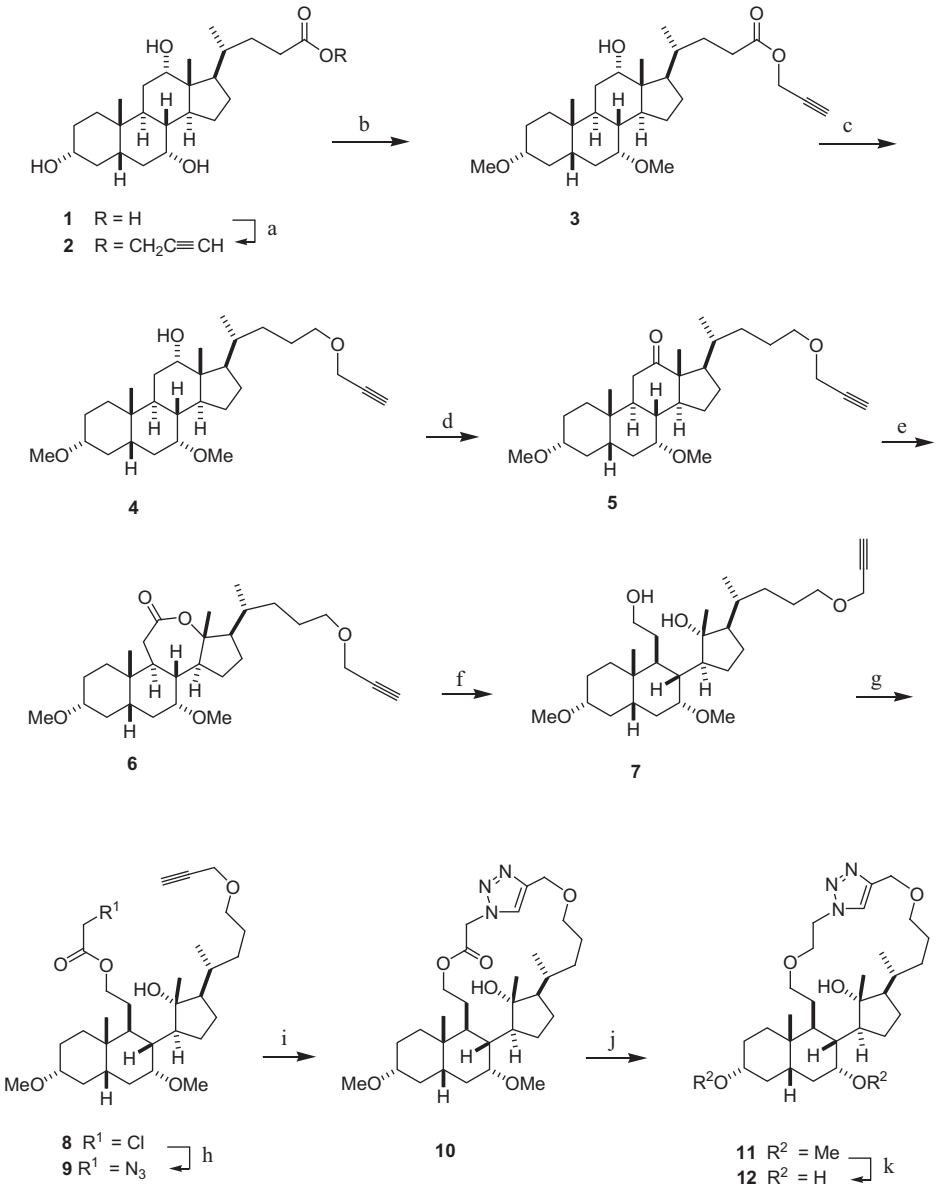
2.19. Macrocyle (**20**)

A flask equipped with a magnetic stirring bar, an argon outlet and a condenser was charged with NaBH4 (90 mg, 0.40 mmol) and anhydr. THF (7 mL)–diglyme (3 mL) under argon. The solution was cooled at 0 °C and then a solution composed of boron trifluoride etherate (0.42 g, 3 mmol), macrocycle **10** (0.18 mmol) and anhydr. THF (5 mL) was added. After completion of the reaction (TLC), it was quenched by addition of 2 N hydrochloric acid (1 mL) and water (10 mL), the product was extracted with ether (3 × 20 mL). The extracts were dried over $MgSO_4$, filtered and then concentrated under vacuum. The residue was chromatographed on silica gel (Et_2O–petroleum ether 1:1). IR (neat) 2951, 1175, 1096 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 0.76 (s, 3H, H-18), 1.04 (d, J = 6.4 Hz, 3H, H-21), 1.12 (s, 3H, H-19), 3.46 (m, 1H, H-8), 3.58 (m, 2H, H-3 and H-7), 3.77 (m, 1H, H-12), 3.86 (m, 2H, H-24), 4.02 (m, 4H, OCH_2–CH_2N), 5.06 (s, 2H, H-25), 7.54 (s, 1H, CH=); ^{13}C NMR (75 MHz, $CDCl_3$): 14.5, 19.2, 21.9, 23.1, 27.3, 29.4, 30.4, 30.7, 32.1, 33.9, 36.7, 37.5, 38.4, 39.5, 41.3, 42.7, 44.1, 47.9, 51.7, 54.6, 65.2, 67.4, 70.3, 71.7, 73.6, 74.2, 76.8, 121.4, 142.9. HRMS (EI) for $C_{29}H_{49}N_3O_5$ [M^+] calcd 519.3672 found 519.3676.

3. Results and discussion

Our interest in secosteroidal molecules prompted us to look into the possibilities of functionalizing the secosteroidal structures with azides and alkynes for synthesizing macrocycles with 1,2,3-triazole modifications. Our approach relies on a sequential ring-expansion/ring-opening and on a 'click reaction' that allows the construction of triazoles. Indeed, the copper(I)-catalysis for the regioselective cycloaddition of terminal alkynes and azides originally developed by Sharpless and co-workers [34] and Meldal and co-workers [35] has become the most useful, mild an efficient process to produce exclusively the 1,4-disubstituted triazoles. These latter are very stable to hydrolysis, reductive and oxidative conditions and metabolic degradation. Moreover, 1,2,3-triazoles show the ability to participate in hydrogen bonds and dipole interactions [36–38].

Two syntheses have been envisaged (Schemes 2 and 3) and in the two cases, cholic acid **1**, a commercial bile acid both inexpensive and readily available, was chosen as starting material.



Reaction conditions : (a) propargyl bromide, CH_2Cl_2, DCC, DMAP, r.t.,12 h, 75%; (b) CH_3I, NaH, THF, r.t., 86%; (c) $NaBH_4$, $BF_3.Et_2O$, THF-diglyme, 0 °C, 4 h, 56%; (d) PCC, MW, 5 min, 68%; (e) *m*-CPBA, PTSA, CH_2Cl_2, r.t., 24 h, 96%; (f) $LiAlH_4$, THF, 0 °C to r.t., 12 h, 85%; (g) $ClCH_2COCl$, CaH_2, $BnEt_3N^+Cl^-$, toluene, Δ, 3 h, 70%; (h) NaN_3, DMF, 60 °C, 24 h, 62%; (i) $CuSO_4.5H_2O$, sodium ascorbate, DMF/H_2O, r.t., 12 h, 66%; (j) $NaBH_4$, $BF_3.Et_2O$, THF-diglyme, 0 °C, 4 h, 44%; (k) $ISi(CH_3)_3$, $CHCl_3$, r.t., 24 h, 90%.

Scheme 3. Synthesis of a steroidal macrocycle **12** from cholic acid through eleven steps sequence.

Firstly, we turned our attention to the synthesis of 12,13-secosteroidal macrocycles matching a *cis* A/B ring junction and a 1,2,3-triazole ring (Scheme 3). The key reactions leading to these new secosteroids are depicted in Scheme 1. First, simple esterification of cholic acid **1** led to propargyl cholate **2** [39], which was methylated with methyl iodide–sodium hydride in THF affording propargyl 3α,7α-dimethoxy cholate **3** in a yield of 86%. Simultaneous protection of the secondary hydroxyl groups at C-3 and C-7 was needed prior to the reductive opening of the lactone ring. In the following step, we reduced the ester function of the lateral chain of **3**, using sodium borohydride and boron trifluoride, in diglyme–tetrahydrofuran at 0 °C during 4 h. Thus, reduction according to the Pettit and Piatak procedure [40], afforded the expected ether derivative **4** in a satisfactory yield (56%).

Microwave (MW) [41] irradiation of **4** with pyridinium chlorochromate furnished ketone **5** very quickly, in a few minutes, and in 68% yield. Baeyer–Villiger oxidation of ketocholane **5** led to lactone **6** as the single regioisomer, as a result of a higher migration aptitude of the quaternary C-13 compared to the secondary C-11. Next, the reduction of the lactone moiety on ring C of **6** using lithium aluminium hydride (LAH) afforded the diprotected tetrahydroxysecocholane **7** in 85% yield.

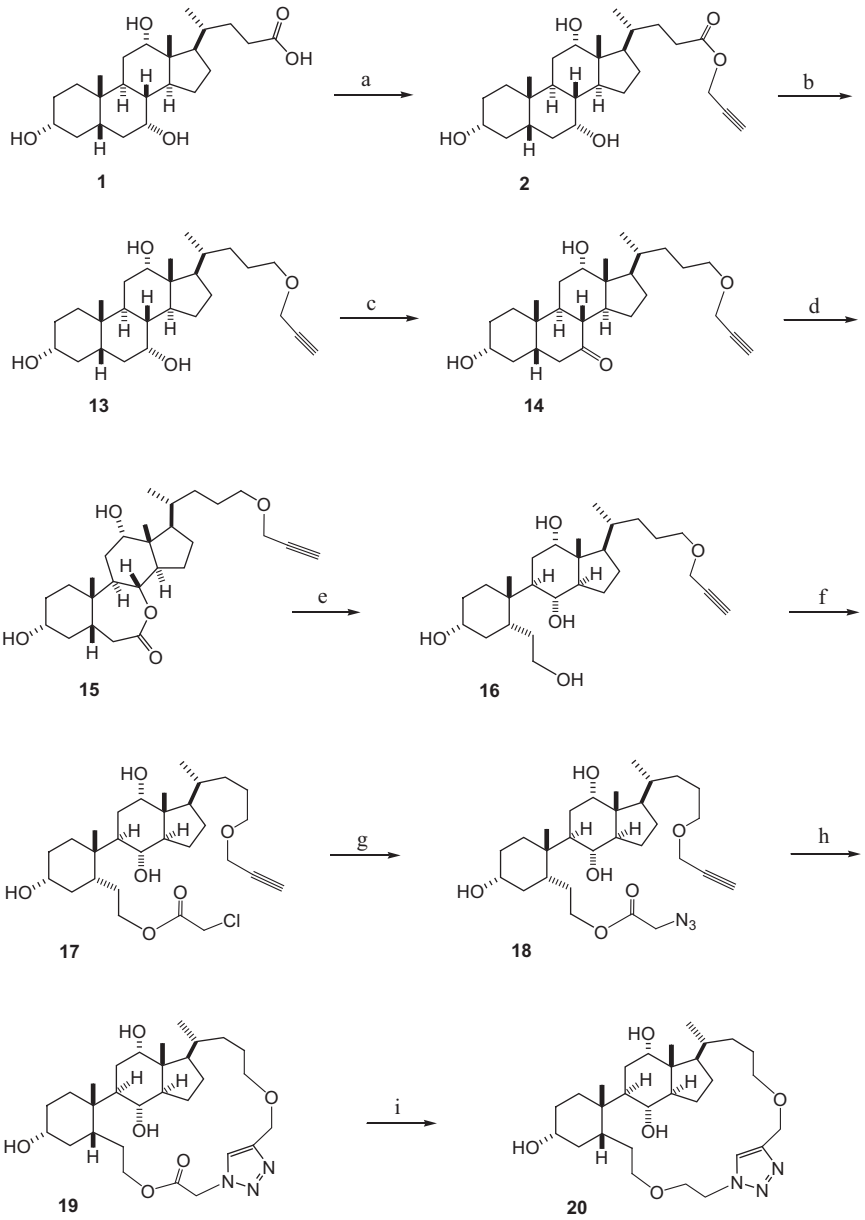
Table 1
CuAAC reaction of secosteroid **9** under different conditions.[a]

Entry	Catalyst	Additive	Solvent	Temp (°C)	**10**: Yield[c] (%)
1	$CuSO_4$	Na-ascorbate	*t*BuOH/H_2O	rt	18
2	$CuSO_4$	Na-ascorbate	*i*PrOH/H_2O	rt	23
3	$CuSO_4$	Na-ascorbate	CH_2Cl_2/H_2O	rt	52
4	$CuSO_4$	Na-ascorbate	DMF/H_2O	rt	66
5	$CuSO_4$	Na-ascorbate	DMSO/H_2O	rt	15
6	CuI	DIPEA[b]	CH_3CN	rt	26
7	CuI	DIPEA	THF	rt	19
8	CuI	DIPEA	DIPEA	rt	16

[a] Reaction conditions: **9**/Cu = 0.2 mmol/0.03 mmol in 4 mL solvent, 12 h.
[b] DIPEA: *N*,*N*-diisopropylethylamine.
[c] All reactions were carried out for 12 h. All yields are given for isolated products after column chromatography.

Next, we proceeded to the installation of the azide moiety required for the intramolecular 1,3-dipolar cycloaddition. Indeed, the key step of the synthesis is the construction of the macrocycle using the 'click reaction' [34]. Two steps were necessary: chloro derivative **8** was easily obtained from ester **7** in high yield by reaction with chloroacetyl chloride in the presence of CaH_2 and triethylbenzylammonium chloride. This latter was transformed into the corresponding azide **9** *via* a substitution reaction carried out in DMF in the presence of NaN_3. The ^1H NMR spectrum showed a signal at 3.51 ppm protons of the $–CH_2–N_3$ group.

In the cycloaddition reaction, the catalytically active species is a Cu(I) complex. Although various Cu(I) salts and complexes can efficiently be used as catalyst, the simplest catalytic consists of $CuSO_4$ and sodium ascorbate. The catalytically active Cu(I) species



Reagents and conditions: (a) propargyl bromide, CH_2Cl_2, DCC, DMAP, r.t.,12 h, 75%; (b) $NaBH_4$, $BF_3.Et_2O$, THF-diglyme, 0 °C, 6 h, 48%; (c) NBS, H_2O, $NaHCO_3$, 12 h at rt then 2 h at 80-85 °C, 95%; (d) *m*-CPBA, PTSA, CH_2Cl_2, rt, 12 h, 76%; (e) $LiAlH_4$, Et_2O, 0 °C at rt, 12 h, 78%; (f) $ClCH_2COCl$, CaH_2, $BnEt_3N^+Cl^-$, toluene, Δ, 3 h, 72%; (g) NaN_3, DMF, r.t., 12 h, 68%; (h) $CuSO_4.5H_2O$, sodium ascorbate, DMF/H_2O, 60 °C, 24 h, 62%; (i) $NaBH_4$, THF-diglyme, $BF_3.Et_2O$, 0 °C at rt, 12 h, 52%.

Scheme 4. Synthesis of a steroidal macrocycle **20** from cholic acid through nine steps sequence.

Table 2
CuAAC reaction of secosteroid **18** under different conditions.[a]

Entry	Catalyst	Additive	Solvent	Temp (°C)	**19**: Yield[c] (%)
1	$CuSO_4$	Na-ascorbate	*t*BuOH/H_2O	rt	12
2	$CuSO_4$	Na-ascorbate	*i*PrOH/H_2O	rt	18
3	$CuSO_4$	Na-ascorbate	CH_2Cl_2/H_2O	rt	48
4	$CuSO_4$	Na-ascorbate	DMF/H_2O	rt	62
5	$CuSO_4$	Na-ascorbate	DMSO/H_2O	rt	21
6	CuI	DIPEA[b]	CH_3CN	rt	22
7	CuI	DIPEA	THF	rt	10
8	CuI	DIPEA	DIPEA	rt	14

[a] Reaction conditions: **18**/Cu = 0.2 mmol/0.03 mmol in 4 mL solvent, 12 h.
[b] DIPEA: *N*,*N*-diisopropylethylamine.
[c] All reactions were carried out for 12 h. All yields are given for isolated products after column chromatography.

is formed in situ from the Cu(II) salt in the presence of the ascorbate as the reducing agent. In this case, it is not necessary to use absolutely oxygen-free conditions and the reaction usually takes place smoothly at atmospheric pressure, at room temperature in an organic solvent/water mixture [34].

Firstly, the 'click reaction' of **9** was accomplished in the presence of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate, in a mixture *t*BuOH/H_2O. The desired macrocycle **10** was isolated as the sole product in a fair yield (18%). So, in order to get the best reaction conditions for the 1,3-dipolar cycloaddition, we undertook several attempts by changing the solvent and the catalyst. The results were reported in Table 1.

The activities of $CuSO_4$ + Na-ascorbate (entries 1–5) and CuI + base catalyst systems (entries 6–8), commonly used for similar reactions, were compared. The application of the Cu(I) precursor was found to be less efficient. Fair yields were observed for **10** (entries 6–8).

As reported in Table 1, the cycloaddition was found to be efficient in a solvent system of DMF/H_2O to produce the macrocycle **10** in 66% yield. The progress of the reaction was found to be slow and took 12 h to reach completion. Otherwise, the new triazole is formed in a completely regioselective manner.

Moreover, investigation of the temperature effects on the reaction revealed that raising the temperature had no significant influence on the yield although the reaction time could be decreased to six hours.

The structure of the macrocycle compound **10** was determined by a series of 1D NMR, COSY and NOESY experiments (400 MHz), and confirmed by the (+)-HRESI mass spectra of the speudomolecular ion $[M+H]^+$ at *m*/*z* 561.3782, corresponding to the molecular formula $C_{31}H_{51}N_3O_6$.

In the following step, in order to obtain ether-linked macrocycle **11** (Scheme 3), we reduced the ester function of **10**, using sodium borohydride and boron trifluoride, in diglyme-tetrahydrofuran at 0 °C during 4 h. Thus, the expected macrocycle derivative **11** was isolated in a satisfactory yield (44%).

Finally, removal of methoxy groups of macrocycle **11** was carried out with trimethylsilyl iodide [42,43] to afford the desired compound **12** in 90% yield.

With the success achieved in the introduction of the triazole moiety into the steroidal skeleton, we decided to explore the same strategy based on an intramolecular 'click reaction' to the preparation of 7,8-secosteroidal macrocycles. As reported in Scheme 4, cholic acid **1** was also the starting material which was converted into propargyl cholate **2** using propargyl alcohol, DCC and DMAP, in good yield. This latter was then reduced using sodium borohydride and boron trifluoride, in diglyme-tetrahydrofuran at 0 °C during 6 h. The resulting ether derivative **13** was obtained in satisfactory yield. The regioselective oxidation of the hydroxyl group at C-7 of **13** was performed with NBS [44]. The resulting 7-keto derivative **14** was subjected to the Baeyer–Villiger oxidation. As expected, this reaction furnished exclusively regioisomer **15**, as a result of the favoured migration of the tertiary C-8 compared to the secondary C-6. In the next step, the reductive opening of the lactone ring was done with lithium aluminium hydride in diethyl ether and led to the secocholane **16** in a 72% yield (over two steps). The chlorine in alkyne **16** was subsequently replaced by an azide group by treatment with sodium azide in DMF. The corresponding azide **17** was then isolated in 68% yield and confirmed by ^{1}H NMR spectroscopy which showed a characteristic signal at 3.58 ppm assigned to the $-CH_2-N_3$ group. Freshly obtained compound **17** was used as a substrate in the 'click reaction'.

Here too, in order to get the best reaction conditions for the 1,3-dipolar cycloaddition, we undertook several attempts by changing the solvent and the catalyst. The results were reported in Table 2.

It is worth pointing out similar results with the work we described above for the synthesis of 12,13-secosteroidal macrocycles. Indeed, the use of Cu (I) precursor was not very efficient (Table 2, entries 6–8), in the other hand the system $CuSO_4$ + Na-ascorbate used in DMF/H_2O as solvent at room temperature led to the cyclisation of **18** in a good yield (entry 4). And here too, the influence of the temperature on the reaction appeared to be much less important.

The new macrocycle **19** was completely characterized by NMR (the ^{1}H NMR spectrum of **19** showed a characteristic singlet at 7.62 ppm assigned to the triazole ring) and mass spectroscopic methods (HRMS calcd for $C_{29}H_4N_3O_6$ 533.3465, found 533.3468).

Finally, in order to obtain the ether-linked macrocycle **20**, the ester function of **19** was then reduced using sodium borohydride and boron trifluoride, in diglyme–tetrahydrofuran at 0 °C during 4 h. The expected ether derivative **20** was isolated in a satisfactory yield (48%).

In summary, we have developed a simple synthetic approach for the synthesis of a novel class of secosteroidal triazoles *via* intramolecular 1,3-dipolar cycloaddition. These syntheses of 7,8- and 12,13-secosteroidal macrocycles have been accomplished in eleven and nine steps respectively, starting from commercially available cholic acid. Interesting is to note that, in one step, a macrocycle and a new function were introduced efficiently on the steroidal skeleton. Further studies to broaden the scope towards the synthesis of novel biologically potential compounds are under investigation in our laboratory and will be reported in due course.

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