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Short communication

Synthesis of 12-oxa, 12-aza and 12-thia cholanetriols

Malika Ibrahim-Ouali[a,*], Khalil Hamze[a], Luc Rocheblave[b]

[a] Institut des Sciences Moléculaires de Marseille UMR 6263 CNRS, Université d'Aix Marseille III, Faculté des Sciences et Techniques de Saint Jérôme, Avenue Escadrille Normandie Niémen, 13397 Marseille Cedex 20, France
[b] Université de Lyon, Université Lyon 1, ISPB-Faculté de Pharmacie, INSERM U863, IFR 62, F-69373 Lyon Cedex 08, France

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ABSTRACT

An efficient synthesis of 12-hetero steroids was achieved *via* a Baeyer–Villiger oxidation and a photolysis as the key steps. We set out to describe in this paper the first synthesis of 12-aza steroids. The characteristic ^{1}H and ^{13}C NMR spectroscopic features of the synthesized compounds are reported.

1. Introduction

The steroid system, selected by the evolutionary process to perform some of the most fundamental biological functions, has not only inspired biochemists and endocrinologists, but also become the basis of many important discoveries in organic chemistry [1].

Steroids can regulate a variety of biological processes and thus have the potential to be developed as drugs for the treatment of a large number of diseases including cardiovascular [2], autoimmune diseases [3], brain tumours, breast cancer, prostate cancer, osteoarthritis, etc. [4].

Modified steroids have attracted a great deal of attention these last years. Their preparation is a stimulating challenge to the organic chemist, often demanding the development of new and generally useful reactions [5–7]. Moreover, the biological properties of modified steroids have proved to be of interest [8–11].

The replacement of one or more carbon atoms of a steroid molecule by a heteroatom affects the chemical properties of a steroid and often results in useful alterations to its biological activity. The potential of heterosteroids in general, and azasteroids in particular, as novel drugs and the challenge of their synthesis prompted numerous research groups to undertake studies in this field. Particularly, the biological activity of azasteroids has been the subject of some reviews [12–14]. Those steroids are one of the best-known classes of xenobiotics [15], and several have been described as inhibitors of 5α-reductase [16,17].

Azasteroids are by far the most common heterocyclic steroids. This is probably due to the fact that a –NH– group has approximately the same size as a methylene group. Consequently, the insertion of a nitrogen atom into the steroid nucleus does not distort the shape of the molecule to any great extent.

We have recently reported a synthetic route to 3-hetero steroids from cholic acid [18,19]. We were now interested by the synthesis of 12-hetero derivatives of steroid and particularly of 12-aza steroids using the same starting material. Indeed, though many azasteroids have been reported, curiously and much to our surprise, 12-aza steroids have never been reported. So, in connection with our ongoing interest in the synthesis of heterosteroids, here we wish to report the extension of our method for the preparation of 12-heterosteroids (Scheme 1).

2. Experimental

All reactions were run under argon in oven-dried glassware. ^{1}H and ^{13}C NMR spectra were recorded at 200 or 400 and 50 and 100 MHz respectively, in $CDCl_3$ solutions. Chemical shifts (δ) are reported in ppm with tetramethylsilane as internal standard. IR spectra were recorded on a Perkin-Elmer 1600 spectrophotometer. Optical rotations were determined on a Perkin-Elmer 343 polarimeter. Flash chromatography was performed on silica gel (Merk 60 F254) and TLC on silica gel. Dichloromethane was distilled over calcium hydride and tetrahydrofuran (THF) over sodium/benzophenone. Triethylamine and pyridine were distilled

* Corresponding author. Tel.: +33 491288416; fax: +33 49128828345.
E-mail addresses: malika.ibrahim@univ-cezanne.fr, malika.ibrahim@univ.u-3mrs.fr (M. Ibrahim-Ouali), luc.rocheblave@univ-lyon1.fr (L. Rocheblave).

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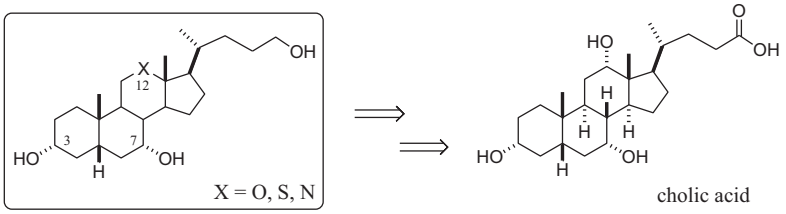


Scheme 1.

from potassium hydroxide and stored over KOH under argon. MW-promoted reactions were carried out in a Biotage Initiator MW. Temperature could be measured at the end of the reaction with a thermocouple thermometer.

Compound **2** was prepared according to the previously described procedure [20].

2.1. 3α,7α,24-Trimethoxy-5β-cholan-12α-ol (**3**)

To a stirred suspension of NaH (0.1 g, 4.4 mmol) in THF (10 mL) at 0 °C under argon was added a solution of tetrol **2** (0.5 g, 1.26 mmol) in 5 mL of THF. The reaction mixture was stirred for 15 min, and then iodomethane (274 μL, 4.4 mmol) was added dropwise. After 48 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 (3 × 10 mL) 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 **7** (0.45 g, 82%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): 0.86 (d, *J* = 6.5, 3H, H-21), 0.89 (s, 3H, H-19), 1.21 (s, 3H, H-18), 2.10 (bs, 1H, OH), 3.02 (m, 2H, H-24), 3.14 (m, 1H, H-12), 3.21 (s, 3H, OCH_3), 3.24 (m, 1H, H-3), 3.28 (s, 3H, OCH_3), 3.29 (s, 3H, OCH_3), 3.34 (m, 1H, H-7); ^{13}C NMR (75 MHz, $CDCl_3$): 10.6, 16.9, 20.1, 24.9, 27.6, 28.2, 28.5, 29.5, 32.1, 32.8, 34.7, 34.9, 35.9, 36.9, 37.4, 39.2, 40.5, 44.6, 47.8, 50.8, 57.4, 57.6, 59.8, 74.9, 79.4, 82.2, 88.9. HRMS (EI) for $C_{27}H_{48}O_4$ [M^+] calcd 436.3553 found 436.3558.

2.2. 12-Oxo-3α,7α,24-trimethoxy-5β-cholane (**4**)

Alcohol **3** (200 mg, 0.4 mmol) was mixed in a mortar with pyridinium chlorochromate (PCC) (0.13 g, 0.6 mmol). The mixture was transferred to a pressure-resistant tube (pyrex) and irradiated with MW at 170 °C for 15 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 0.12 g (71% yield) of 12-oxo steroid **4** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): 0.85 (d, *J* = 6.5, 3H, H-21), 0.89 (s, 3H, H-19), 1.24 (s, 3H, H-18), 3.00 (m, 2H, H-24), 3.20 (s, 3H, OCH_3), 3.26 (m, 1H, H-3), 3.30 (s, 3H, OCH_3), 3.31 (s, 3H, OCH_3), 3.32 (m, 1H, H-7); ^{13}C NMR (75 MHz, $CDCl_3$): 11.5, 18.9, 22.4, 23.8, 26.4, 26.7, 27.6, 28.0, 29.3, 31.8, 34.7, 36.0, 37.5, 37.9, 39.4, 41.5, 46.6, 52.8, 53.8, 55.4, 56.0, 56.8, 58.5, 73.4, 76.9, 80.2, 214.3. HRMS (EI) for $C_{27}H_{46}O_4$ [M^+] calcd 434.3396 found 434.3401.

2.3. 3α,7α-Dimethoxy-13-oxa-C-homo-cholan-12-one (**5**)

To a solution of ketone **4** (0.26 g, 0.6 mmol) in dry dichloromethane (30 mL) containing *p*-toluenesulfonic acid (90 mg, 0.6 mmol) *m*-CPBA (12 mg) was added [19]. The solution was stirred for 12 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 0.25 g of pure lactone **5** (97%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): 0.86 (s, 3H, H-19), 1.05 (d, *J* = 6.4, 3H, H-21), 1.34 (s, 3H, H-18), 3.20 (s, 3H, OCH_3), 3.25 (m, 2H, H-3 and H-7), 3.31 (s, 3H, OCH_3), 3.32 (s, 3H, OCH_3), 3.35 (m, 1H, H-24); ^{13}C NMR (75 MHz, $CDCl_3$): 14.5, 15.3, 17.7, 22.3, 24.2, 25.2, 26.1, 26.5, 27.5, 31.6, 32.8, 34.6, 35.2, 35.9, 36.3, 41.2, 42.7, 50.1, 55.7, 58.5, 58.9, 65.9, 73.3, 76.9, 80.2, 86.9, 174.8. HRMS (EI) for $C_{27}H_{46}O_5$ [M^+] calcd 450.3345 found 450.3348.

2.4. 3α,7α-Dimethoxy-13-oxa-C-homo-5β-cholan-12-ol (**6**)

To a solution of lactone **5** (100 mg, 0.2 mmol) in dry dichloromethane (20 mL) at −78 °C diisobutyl-aluminium hydride (DIBAL) (1.0 M in hexane) (1.5 mL) [19] was added dropwise over the course of 10 min. The solution was stirred for 2 h at −78 °C and poured into ice water. After removal of the precipitates, the solution was washed with water (10 mL) and dried over anhydrous magnesium sulfate. Evaporation of the solvent gave lactol **6**, which was purified by chromatography on silica gel with CH_2Cl_2/MeOH (95/5), to yield an oily product **6** (95 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$: 0.87 (s, 3H, H-19), 1.06 (d, *J* = 6.6, 3H, H-21), 1.35 (s, 3H, H-18), 2.16 (m, 2H, H-11), 3.21 (s, 3H, OCH_3), 3.25 (m, 1H, H-3), 3.32 (s, 3H, OCH_3), 3.33 (s, 3H, OCH_3), 3.36 (m, 1H, H-7), 5.22 (m, 1H, H-12), 5.36 (m, 1H, H-12); ^{13}C NMR (75 MHz, $CDCl_3$): 14.5, 17.7, 22.3, 23.3, 24.3, 26.1, 27.5, 29.9, 31.6, 32.1, 32.8, 34.7, 35.9, 36.4, 42.2, 42.7, 50.1, 53.5, 55.4, 55.7, 58.5, 65.5, 73.3, 76.9, 80.3, 86.8, 93.3. HRMS (EI) for $C_{27}H_{48}O_5$ [M^+] calcd 452.3502 found 452.3506.

2.5. 11-Iodo-C-nor-11,12-seco-3α,7α,24-trimethoxy-cholan-5β-yl formate (**7**)

To the lactol **6** (200 mg, 0.4 mmol) in dry benzene (25 mL) containing pyridine (0.7 mL) mercury(II) oxide (214 mg) and iodine (251 mg) was added. The solution was irradiated in a Pyrex vessel with a 100-W high-pressure mercury arc (EIKOSHA, PIH-100), for 2 h under an argon atmosphere [21]. The solution was filtered and the filtrate was washed with saturated aqueous $NaHCO_3$ (20 mL). The organic layer was dried with $MgSO_4$, filtered and concentrated to give a crude oily product. This product was purified by flash chromatography on silica gel (diethyl ether/petroleum ether: 9/1) to afford 0.22 g of formate 7 (88%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): 1.06 (d, *J* = 6.5, 3H, H-21), 1.16 (s, 3H, H-19), 1.50 (s, 3H, H-18), 2.78 (m, 1H, H-3), 2.79 (m, 1H, H-7), 3.21 (m, 2H, CH_2-I), 3.24 (s, 3H, OCH_3), 3.25 (s, 3H, OCH_3), 3.37 (m, 2H, H-24), 8.04 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): 6.9, 13.8, 19.5, 19.7, 23.5, 24.4, 29.9, 30.8, 31.6, 32.0, 33.8, 34.1, 35.7, 38.3, 38.4, 41.5, 42.8, 46.2, 46.3, 57.1, 57.4, 59.3, 74.9, 82.4, 82.9, 89.2, 160.9. HRMS (EI) for $C_{27}H_{47}IO_5$ [M^+] calcd 578.2468 found 578.2475.

2.6. 3α,7α,24-Trimethoxy-12-oxa-5β-cholane (**8**)

A solution of the formate **7** (270 mg, 0.4 mmol) in dry THF (20 mL) was cooled at −78 °C. To this solution methyllithium in

diethyl ether (1 M, solution) (1.1 mL) was added dropwise while stirring [19]. After the solution was stirred for 3 h at −78 °C, the temperature of the solution rose to room temperature. Evaporation of the solvent left a residue which was dissolved in diethyl ether (15 mL). The organic layer was washed with water (15 mL) and extracted with diethyl ether (3 × 15 mL). The extracts were dried over $MgSO_4$, filtered and then concentrated under vacuum to yield a crude oily product. This product was purified by flash chromatography on silica gel (diethyl ether/petroleum ether: 1/9) to afford pure 12-oxa steroid 8 (0.15 g, 75%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): 1.06 (d, *J* = 6.4, 3H, H-21), 1.16 (s, 3H, H-19), 1.31 (s, 3H, H-18), 2.16 (m, 2H, H-11), 2.78 (m, 1H, H-3), 2.79 (m, 1H, H-7), 3.21 (s, 3H, OCH_3), 3.24 (s, 3H, OCH_3), 3.25 (s, 3H, OCH_3), 3.37 (m, 2H, H-24), 3.43 (m, 1H, H-11), 3.68 (m, 1H, H-11); ^{13}C NMR (75 MHz, $CDCl_3$): 19.4, 20.7, 20.8, 23.5, 24.2, 27.6, 29.9, 32.0, 32.1, 32.2, 32.8, 33.8, 35.7, 37.5, 41.6, 43.5, 50.7, 57.1, 57.4, 58.5, 59.3, 61.8, 74.9, 80.6, 82.9, 90.4. HRMS (EI) for $C_{26}H_{46}O_4$ [M^+] calcd 422.3396 found 422.3401.

2.7. Reduction of iodo formate **7**

To a solution of iodo formate **7** (540 mg, 0.8 mmol) in dry toluene (20 mL) DIBAL (1.0 M in hexane, 7 mL) [19] was added at −78 °C under a nitrogen atmosphere. The solution was stirred for 30 min at −78 °C and an additional 6 h at room temperature. The solvent was then evaporated and the product extracted with diethyl ether (3 × 20 mL). The solution was washed with water and dried over anhydrous magnesium sulfate. Evaporation of the solvent gave an oily product which was purified by chromatography on silica gel (petroleum ether/ethyl acetate: 1/9), to yield iodo alcohol 9 (0.36 g, 82%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): 0.92 (s, 3H, H-18), 0.98 (d, *J* = 7.4, 3H, H-21), 1.02 (s, 3H, H-19), 2.0 (bs, 1H, OH), 2.78 (m, 2H, H-3 and H-7), 3.05 (t, *J* = 6.8 Hz, 2H, CH_2I), 3.20 (s, 3H, OCH_3), 3.22 (s, 3H, OCH_3), 3.25 (s, 3H, OCH_3), 3.42 (m, 2H, H-24); ^{13}C NMR (75 MHz, $CDCl_3$): 6.9, 19.3, 19.6, 23.3, 24.1, 24.2, 27.6, 29.8, 30.9, 31.2, 31.9, 32.4, 33.8, 33.9, 35.8, 38.7, 46.3, 49.5, 57.1, 57.4, 59.6, 61.2, 74.9, 75.6, 82.6, 89.7. HRMS calcd for $C_{26}H_{47}IO_4$ 550.2519, found 550.2523.

2.8. 3α,7α,24-Trimethoxy-12-thia-5β-cholane (**11**)

To a solution of iodo alcohol **9** (300 mg, 0.6 mmol) in pyridine (20 mL) methanesulfonyl chloride (1.2 mL, 1.8 g, 15 mmol) [19] was added at 0 °C under a nitrogen atmosphere. The solution was stirred for 24 h at room temperature; the solvent was then evaporated and the product extracted with diethyl ether (3 × 15 mL). The combined extracts were dried over $MgSO_4$, filtered and then concentrated under vacuum to give the crude mesylate **10** (0.28 g, 68%). The latter was then used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): 0.92 (s, 3H, H-18), 0.96 (d, *J* = 6.8, 3H, H-21), 1.02 (s, 3H, H-19), 2.77 (m, 2H, H-3 and H-7), 2.94 (s, 3H, CH_3SO_2), 3.10 (m, 2H, CH_2I), 3.21 (s, 3H, OCH_3), 3.24 (s, 3H, OCH_3), 3.26 (s, 3H, OCH_3), 3.48 (m, 2H, H-24).

To a solution of mesylate **10** (280 mg, 0.44 mmol) in acetonitrile (20 mL) sodium sulfide nonahydrate (865 mg) was added. The solution was heated under reflux for 3 days. The solvent was evaporated and the product dissolved in diethyl ether. The solution was washed with water and dried over anhydrous magnesium sulfate. Evaporation of the solvent gave a crude product, which was purified by chromatography on silica gel (petroleum ether/ethyl acetate: 1/9), to give thia steroid **11** (70 mg, 36%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): 1.03 (d, *J* = 6.4, 3H, H-21), 1.16 (s, 3H, H-19), 1.38 (s, 3H, H-18), 2.36 (m, 1H, H-11), 2.62 (m, 1H, H-11), 2.76 (m, 2H, H-3 and H-7), 3.21 (s, 3H, OCH_3), 3.23 (s, 3H, OCH_3), 3.24 (s, 3H, OCH_3), 3.42 (m, 2H, H-24); ^{13}C NMR (75 MHz, $CDCl_3$): 18.8, 19.9, 22.6, 24.6, 26.2, 26.8, 27.6, 28.3, 29.9, 31.3, 31.4, 34.7, 35.7, 38.2, 39.2, 42.4, 43.2, 44.3, 48.6, 50.2, 57.1, 57.3, 59.3, 74.8, 82.6, 89.9. HRMS (EI) for $C_{26}H_{46}O_3S$ [M^+] calcd 438.3168 found 438.3172.

2.9. 12-Thia-3α,7α,24-trimethoxy-5β-cholan-12-oxide (**12**)

Thia steroid **11** (100 mg, 0.2 mmol) was dissolved in CH_2Cl_2 (20 mL), under argon. The solution was cooled at 0 °C and *m*-CPBA (36 mg, 0.2 mmol) was added [19]. After stirring at this temperature for 4 h, the mixture was hydrolysed with a saturated solution of $NaHCO_3$ (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The organic phase was dried with $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate, 9/1 to 5/5) to give an inseparable 1/3 mixture of two diastereoisomers **12**α/**12**β (60 mg, 66%) as an oil. Major isomer: ^{1}H NMR (300 MHz, $CDCl_3$): 0.76 (s, 3H, H-18), 0.88 (d, *J* = 6.4, 3H, H-21), 0.95 (s, 3H, H-19), 2.56 (m, 2H, CH_2–S=O), 3.02 (m, 2H, H-3 and H-7), 3.21 (s, 3H, OCH_3), 3.22 (s, 3H, OCH_3), 3.26 (s, 3H, OCH_3), 3.36 (m, 2H, H-24); ^{13}C NMR (75 MHz, $CDCl_3$): 19.6, 20.4, 20.6, 26.4, 26.9, 27.4, 29.9, 32.1, 32.4, 33.6, 33.8, 34.5, 34.9, 35.6, 38.2, 38.4, 44.2, 45.8, 48.4, 57.2, 57.6, 59.3, 67.8, 74.2, 82.9, 89.1. HRMS (EI) for $C_{26}H_{46}O_4S$ [M^+] calcd 454.3117 found 454.3122.

2.10. 12-Thia-3α,7α,24-trimethoxy-5β-cholan-12,12-dioxide (**13**)

Thia steroid **11** (100 mg, 0.2 mmol) was dissolved in CH_2Cl_2 (15 mL), under argon. The solution was cooled at 0 °C and *m*-CPBA (70 mg, 0.4 mmol) was added [19]. After stirring at room temperature for 24 h, the mixture was hydrolysed with a saturated solution of $NaHCO_3$ (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The organic phase was dried with $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH: 100/0 to 95/5) to afford 80 mg (85% yield) of sulfone **13** as colorless needles. Mp = 161–162 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 0.82 (s, 3H, H-18), 0.88 (d, *J* = 6.5, 3H, H-21), 0.97 (s, 3H, H-19), 2.78 (m, 2H, H-3 and H-7), 3.20 (s, 3H, OCH_3), 3.21 (s, 3H, OCH_3), 3.24 (s, 3H, OCH_3), 3.26–3.48 (m, 4H, H-24 and CH_2–SO_2); ^{13}C NMR (75 MHz, $CDCl_3$): 14.8, 19.2, 20.8, 25.6, 26.4, 27.2, 28.4, 28.9, 30.9, 31.7, 32.0, 32.2, 33.4, 34.2, 35.7, 37.8, 39.4, 43.5, 52.2, 57.1, 57.3, 59.0, 64.2, 74.5, 82.2, 89.8. HRMS (EI) for $C_{26}H_{46}O_5S$ [M^+] calcd 470.3066 found 470.3068.

2.11. 11,13-Diiodo-C-nor-11,12-seco-7α,7α,24-trimethoxy-5β-cholane (**14**)

To a solution of iodo formate **7** (290 mg, 0.5 mmol) in dry carbon tetrachloride (5 mL) iodotrimethylsilane (213 μL, 1.5 mmol) was added dropwise over a period of 5 min under an argon atmosphere. The solution was heated at 60–70 °C for 2 days and diethyl ether was added (15 mL). The organic layer was washed with 5% aqueous sodium hydrogen carbonate, 5% aqueous sodium thiosulfate, and saturated brine successively and then dried over anhydrous sodium sulfate. Evaporation of the solvent *in vacuo* left a red oil, which was passed through a short silica gel column (petroleum ether) to give pure diiodide 14 (0.3 g, 92%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): 1.06 (d, *J* = 6.4, 3H, H-21), 1.12 (s, 3H, H-19), 2.10 (s, 3H, H-18), 2.76 (m, 2H, H-3 and H-7), 2.96 (m, 1H, H-11), 3.19 (m, 1H, H-11), 3.21 (s, 3H, OCH_3), 3.23 (s, 3H, OCH_3), 3.24 (s, 3H, OCH_3), 3.38 (m, 2H, H-24); ^{13}C NMR (75 MHz, $CDCl_3$): 6.7, 18.1, 19.2, 27.6, 28.0, 28.7, 29.9, 30.5, 30.7, 31.2, 31.4, 33.5, 35.7, 36.5, 37.2, 38.6, 41.0, 46.3, 48.8, 52.0, 57.1, 57.4, 59.1, 74.8, 82.9, 88.3. HRMS (EI) for $C_{26}H_{46}I_2O_3$ [M^+] calcd 660.1536 found 660.1541.

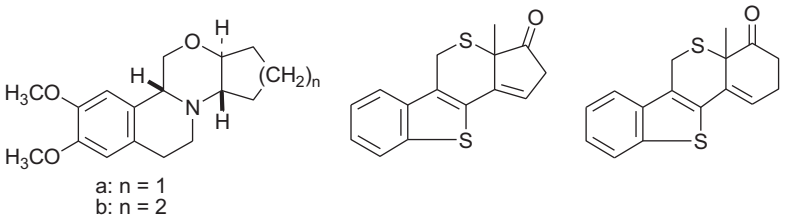


Fig. 1. Literature examples of 12-oxa [22] and 12-thia steroids [23].

2.12. N-Benzyl 3α,7α,24-trimethoxy-12-aza-5β-cholane (**15**)

To a solution of diiodide **14** (305 mg, 0.46 mmol) in dioxane (2 mL) benzylamine (125 μL, 122 mg, 1.15 mmol) was added. The solution was heated under reflux for 24 h and extracted with dichloromethane (3 × 20 mL). The extract was washed with water, saturated brine, dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate: 9/1), to afford aza steroid **15** (0.16 g, 68%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): 1.02 (d, J = 6.4, 3H, H-21), 1.12 (s, 3H, H-19), 1.16 (s, 3H, H-18), 2.16 (m, 2H, H-11), 2.78 (m, 2H, H-3 and H-7), 3.20 (s, 3H, OCH_3), 3.23 (s, 3H, OCH_3), 3.24 (s, 3H, OCH_3), 3.45 (m, 2H, H-24), 3.60 (s, 2H, NCH_2Ph), 7.20–7.35 (m, 5H, H aromatic); ^{13}C NMR (75 MHz, $CDCl_3$): 19.0, 19.5, 20.6, 25.0, 25.5, 26.6, 27.8, 28.3, 29.4, 29.9, 33.6, 34.6, 35.4, 35.8, 39.2, 42.6, 43.2, 44.3, 45.2, 49.8, 57.0, 57.3, 59.1, 63.6, 74.8, 76.9, 82.6, 127.3, 128.5, 128.9, 135.8. HRMS (EI) for $C_{33}H_{53}NO_3$ [M^+] calcd 511.4025 found 511.4029.

2.13. 12-Oxa-3α,7α-dihydroxy-5β-cholan-24-ol (**16**)

To a solution of 12-oxa steroid **8** (100 mg, 0.24 mmol) in chloroform (20 mL) trimethylsilyl iodide (0.2 mL) [19] was added. The solution was left overnight at room temperature. Methanol (1 mL) was then added to decompose any excess trimethylsilyl iodide. The solution was extracted with diethyl ether (3 × 10 mL), washed with water and saturated brine, and 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: 9/1), to give triol **16** (55 mg, 60%) as an oil. $\alpha_D^{20} = +52.55^\circ$ (c = 0.310, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): 0.86 (s, 3H, H-18), 1.06 (d, J = 6.4, 3H, H-21), 1.08 (s, 3H, H-19), 2.01 (bs, 1H, OH), 2.03 (bs, 1H, OH), 3.18 (m, 2H, H-3 and H-7), 3.40 (m, 1H, H-11) 3.52 (m, 2H, H-24), 3.62 (m, 1H, H-11); ^{13}C NMR (75 MHz, $CDCl_3$): 19.4, 20.4, 20.9, 23.2, 24.2, 30.4, 31.4, 31.8, 32.7, 32.8, 32.9, 36.6, 37.2, 38.2, 41.6, 46.2, 50.7, 58.5, 61.9, 63.2, 70.1, 71.4, 79.6. HRMS (EI) for $C_{23}H_{40}O_4$ [M^+] calcd 380.2927 found 380.2932.

2.14. 12-Thia-3α,7α-dihydroxy-5β-cholan-24-ol (**17**)

The same procedure described above was used [19]. Reaction of 200 mg (0.46 mmol) of **11** in chloroform (20 mL) and 0.4 mL of $ISi(CH_3)_3$ gave, after 24 h of reaction and after purification by flash chromatography on silica gel (CH_2Cl_2/MeOH: 9/1), 120 mg (66%) of **17** as an oil. $\alpha_D^{20} = +46.41^\circ$ (c = 0.540, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): 0.84 (s, 3H, H-18), 1.06 (d, J = 6.4, 3H, H-21), 1.12 (s, 3H, H-19), 2.01 (bs, 1H, OH), 2.32 (dd, J = 5.4 and 13.8, 1H, H-11), 2.58 (dd, J = 5.6 and 13.4, 1H, H-11), 3.16 (m, 2H, H-3 and H-7), 3.48 (m, 2H, H-24); ^{13}C NMR (75 MHz, $CDCl_3$): 18.6, 20.2, 23.2, 24.5, 26.8, 26.9, 30.4, 30.7, 31.2, 31.8, 32.5, 34.7, 34.8, 36.4, 37.9, 38.2, 42.1, 44.4, 48.6, 50.2, 63.4, 69.2, 69.8. HRMS (EI) for $C_{23}H_{40}O_3S$ [M^+] calcd 396.2698 found 396.2703.

2.15. 12-Thia-3α,7α-dihydroxy-5β-cholan-12-oxide-24-ol (**18**)

The same procedure described above was used [19]. Reaction of 110 mg (0.24 mmol) of **12** in chloroform (20 mL) and 0.2 mL of $ISi(CH_3)_3$ gave, after 24 h of reaction and after purification by flash chromatography on silica gel (CH_2Cl_2/MeOH: 9/1), 65 mg (65%) of an inseparable 1/3 mixture of two diastereoisomers 18α/18β as an oil. Physical data for the mixture: $\alpha_D^{20} = +36.68^\circ$ (c = 0.892, $CHCl_3$) and HRMS (EI) for $C_{23}H_{40}O_4S$ [M^+] calcd 412.2647 found 412.2650; NMR data for the major isomer (from spectra of the isomer mixture): ^{1}H NMR (300 MHz, $CDCl_3$): 0.88 (s, 3H, H-18), 1.07 (d, J = 6.5, 3H, H-21), 1.16 (s, 3H, H-19), 2.02 (bs, 1H, OH), 2.41 (m, 1H, H-11), 2.64 (m, 1H, H-11), 3.18 (m, 2H, H-3 and H-7), 3.52 (m, 2H, H-24); ^{13}C NMR (75 MHz, $CDCl_3$): 19.6, 20.6, 20.9, 26.3, 26.8, 30.2, 31.6, 32.0, 32.4, 32.9, 34.6, 34.8, 36.2, 37.8, 38.2, 40.5, 44.3, 45.8, 48.2, 63.2, 67.6, 68.8, 69.9.

2.16. 12-Thia-3α,7α-dihydroxy-5β-cholan-12,12-dioxide-24-ol (**19**)

The same procedure described above was used [19]. Reaction of 100 mg (0.21 mmol) of **13** in chloroform (20 mL) and 0.2 mL of $ISi(CH_3)_3$ gave, after 24 h of reaction and after purification by flash chromatography on silica gel (CH_2Cl_2/MeOH: 9/1), 60 mg (67%) of **19** as an oil. $\alpha_D^{20} = +36.10^\circ$ (c = 0.563, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): 0.89 (s, 3H, H-18), 1.04 (d, J = 6.4, 3H, H-21), 1.12 (s, 3H, H-19), 2.01 (bs, 1H, OH), 3.16 (m, 2H, H-3 and H-7), 3.22 (m, 1H, H-11), 3.45 (m, 1H, H-11), 3.52 (m, 2H, H-24); ^{13}C NMR (75 MHz, $CDCl_3$): 15.4, 19.1, 20.4, 25.6, 26.4, 28.1, 30.2, 31.0, 31.4, 31.8, 32.2, 32.5, 34.4, 36.3, 38.4, 39.1, 40.6, 43.5, 52.4, 63.2, 64.1, 68.5, 69.7. HRMS (EI) for $C_{23}H_{40}O_5S$ [M^+] calcd 428.2596 found 428.2600.

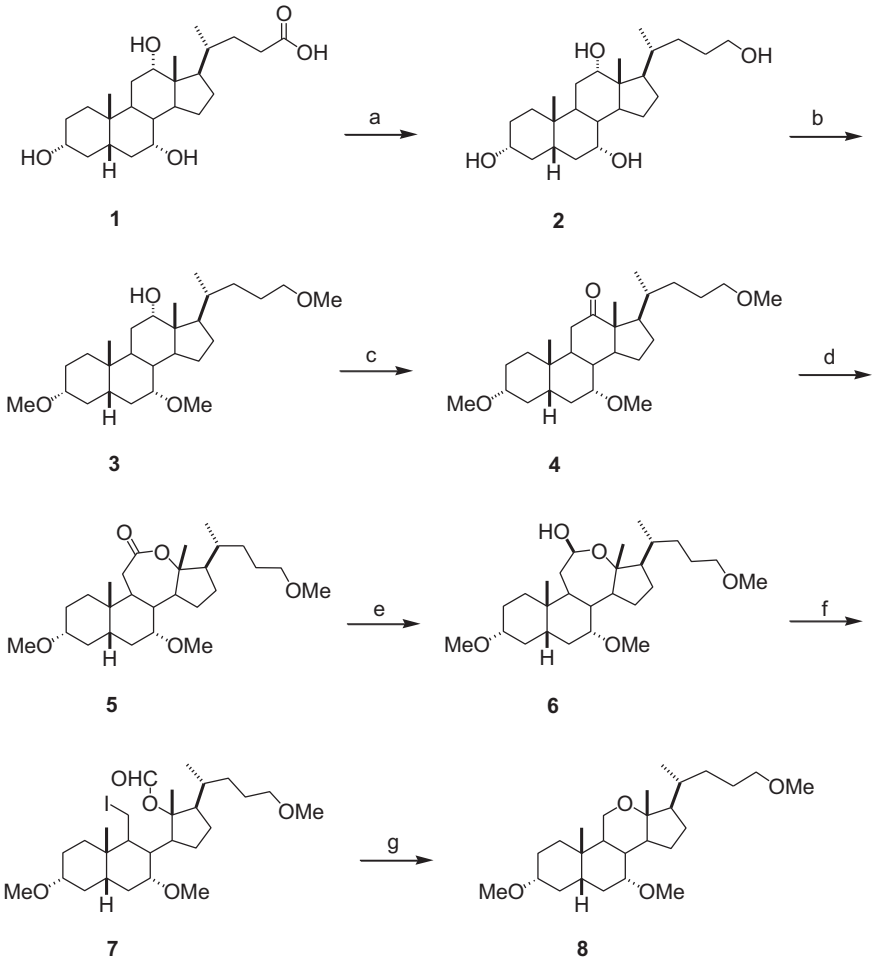
2.17. N-Benzyl 12-aza-3α,7α-dihydroxy-5β-cholan-24-ol (**20**)

The same procedure described above was used [19]. Reaction of 100 mg (0.19 mmol) of **15** and 0.2 mL of $ISi(CH_3)_3$ gave, after 24 h of reaction and after purification by flash chromatography on silica gel (CH_2Cl_2/MeOH: 9/1), 55 mg (62%) of **20** as an oil. $\alpha_D^{20} = +77.83^\circ$ (c = 0.415, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): 0.86 (s, 3H, H-18), 1.08 (d, J = 6.4, 3H, H-21), 1.12 (s, 3H, H-19), 2.01 (bs, 1H, OH), 2.12 (m, 1H, H-11), 2.34 (m, 1H, H-11), 3.10 (m, 2H, H-3 and H-7), 3.51 (m, 2H, H-24), 3.62 (s, 2H, NCH_2Ph), 7.21 (m, 5H, H aromatic); ^{13}C NMR (75 MHz, $CDCl_3$): 19.1, 19.6, 20.1, 25.0, 25.4, 30.0, 30.8, 31.4, 32.2, 33.6, 34.1, 35.6, 36.1, 38.1, 42.9, 48.9, 53.6, 56.6, 62.1, 62.9, 63.1, 69.8, 71.0, 71.4, 127.3, 127.9, 128.4, 134.9. HRMS (EI) for $C_{30}H_{47}O_3N$ [M^+] calcd 469.3556 found 469.3561.

3. Results and discussion

There are very few syntheses of 12-oxa [22] and 12-thia [23] steroids reported in the literature before (Fig. 1).

And firstly, we turned our attention to the synthesis of 12-oxa and 12-thia cholanols. The key reactions leading to these new heterosteroids are schematically depicted in Schemes 2 and 3. Taking into account the *cis* A/B ring junction of the final product, cholic acid

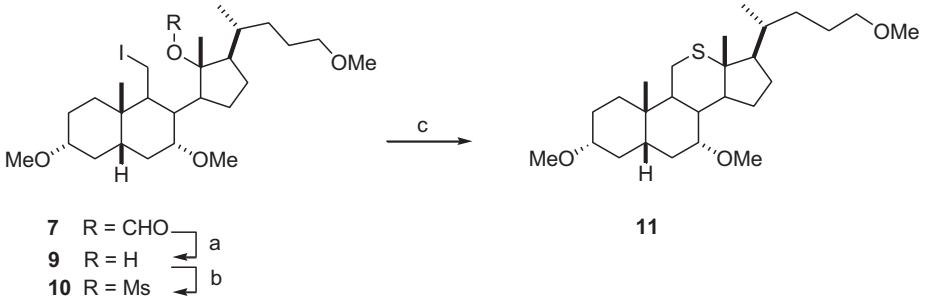


Reaction conditions : (a) $LiAlH_4$, THF, 0 °C, 87%; (b) CH_3I, NaH, THF, r.t., 82%; (c) PCC, MW, 15 min, 71%; (d) *m*-CPBA, PTSA, CH_2Cl_2, 97%; (e) DIBAL, CH_2Cl_2, 95%; (f) HgO-I_2, pyridine, C_6H_6, hν, 88%; (g) MeLi, THF, - 78 °C, 75%.

Scheme 2. Synthesis of a 12-oxa-5β-steroid **8** through a seven-step sequence.

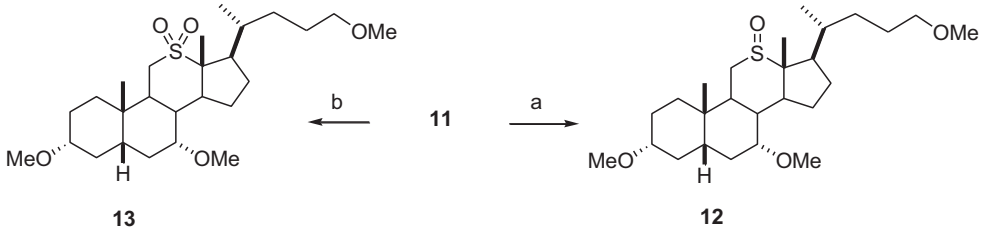
1, a commercial bile acid both inexpensive and readily available, was our choice of starting material. We adopted a more attractive way for the synthesis of 12-heterosteroids. Hydroxyls at different positions on the nucleus have different reactivities towards alkylating agents, generally in the order C-3 > C-7 > C-12 [19,24]. Since the photochemical cleavage of lactol **6** to the seco-steroid **7** does not tolerate free hydroxy groups, 3α,7α,12α-tetrol **2** was selectively tri-methoxylated leaving only the 12α-hydroxy group unprotected.

Thus, we proceeded firstly to an easy reduction [20] of the side chain acid function of **1** with $LiAlH_4$ in tetrahydrofuran at room temperature. Tetrol **2** was then isolated in good yield. This latter was selectively methylated [25] with methyl iodide-sodium hydride in THF, leading to the 3α,7α,24-trimethoxy cholan-12α-ol **3**, in 82% yield. Microwave (MW) [26] irradiation of **3** with pyridinium chlorochromate furnished ketone **4** very quickly, in a few minutes, and in 71% yield.



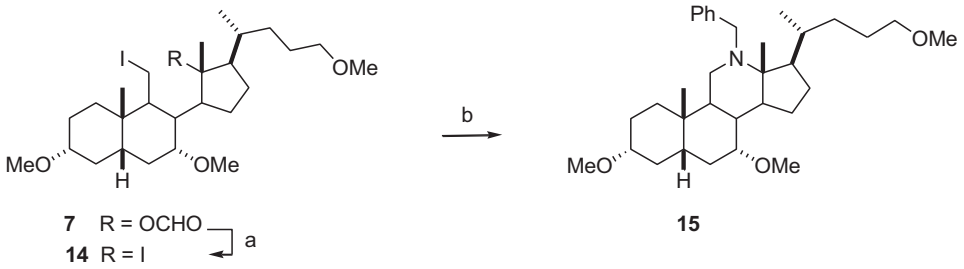
Reaction conditions : (a) DIBAL, CH_2Cl_2, - 78 °C to r.t., 12 h, 82%; (b) CH_3SO_2Cl, pyridine, r.t., 24 h, 68%; (c) $Na_2S.H_2O$, CH_3CN, Δ, 3 days, 36%.

Scheme 3. Synthesis of 12-thia-5β-steroid **11**.



Reaction conditions : (a) *m*-CPBA, 1 equiv, CH_2Cl_2, 0 °C, 1 h, 66%;
(b) *m*-CPBA, 2 equiv, CH_2Cl_2, r.t., 24 h, 85%.

Scheme 4. Synthesis of sulfoxide **12** (1/3 mixture of **12**α and **12**β diastereomers) and sulfone **13**.



Reaction conditions : (a) Me_3SiI-CCl_4, r.t., 48 h, 92%; (b) $PhCH_2NH_2$, dioxane, Δ, 24 h, 68%.

Scheme 5. First synthesis of an 12-aza steroid **15**.

In the next step, we undertook the formation of the lactone **5** from ketone **4** [27,28]. The Baeyer–Villiger [29] oxidation of the latter, was done using the experimental conditions applied recently [19], and 13-oxa-C-homo-5β-cholestan-12-one 5 was obtained as a single product in excellent yield (97%). The reduction of lactone **5** with DIBAL in toluene at −78 °C for 4 h readily gave lactol **6**, which was subjected to the hypoiodide photolysis under the conditions described by Suginome and Yamada [21], to give formate 7 in 88% yield. The cyclization of formate **7** with methyllithium in THF afforded a novel 12-oxa steroid **8** in an optimized yield of 75%.

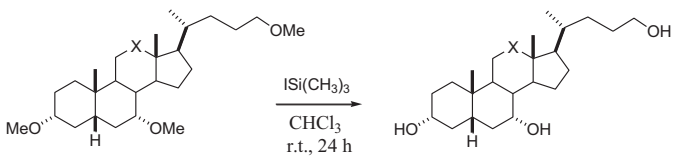
We turned then our attention to the introduction of a sulfur atom at the same position of the steroidal skeleton. The synthesis of 12-thia-5β-steroid **11** was achieved in three steps from iodo formate **7** (Scheme 3). Treatment of **7** with DIBAL in toluene at −78 °C gave iodo alcohol **9** in 82% yield. Its mesylation with mesylchloride/pyridine to the corresponding mesylate **10**, followed by treatment of the latter with sodium sulfide in acetonitrile gave 12-thia steroid **11** in 36% yield. The structure of **11** was determined by a series of ^{1}D NMR, COSY and NOESY experiments (400 MHz) and confirmed by the NOE effects. The NOE contacts observed in the NOESY spectra showed cross-peaks between the 7α-methoxy protons and the protons 14α, 6α and 11α. The α-geometry of all, which was derived from some peaks, indicates a spatial proximity between the 18-methyl protons and the protons at positions 11β, 5β, 16β. These interactions suggested a β stereochemistry for the 18-methyl protons.

Compounds containing a sulfide, sulfoxide or sulfone moiety are not only of synthetic interest but also are some of them of medicinal relevance [30,31]. So, we envisaged to oxidize this latter chemically [32,33]. And (Scheme 3), oxidation of **11** with 1 equiv. of 3-chloroperbenzoic acid in dichloromethane led to a 1/3 mixture of the corresponding sulfoxide **12**α and **12**β in good yield. The equatorial sulfoxide is the major isomer in accord with the known behaviour of peroxy acids with cyclic sulfides [34]. Indeed, the configuration at sulfur was allocated on the basis of the tendency of thianes to be oxidized predominantly to equatorial sulfoxides by peroxy acids. Thus, the ^{13}C NMR spectrum of the compound **12** shows shifts for the major peaks corresponding to the α-configurated sulfoxide.

In order to obtain the sulfone as the sole product, oxidation on compound **11** was carried out with 2 equiv. of *m*-CPBA in CH_2Cl_2 at room temperature. The corresponding steroid **13** bearing a sulfone moiety was isolated in 85% yield (Scheme 4).

Surprisingly and to the best of our knowledge, no previous successful formation of 12-aza steroids has been reported in the literature. We turned then our attention to the introduction of a nitrogen atom at the C-12 position of the steroidal skeleton (Scheme 5). Iodo formate **7** was converted into its diiodo derivative **14**, by treatment with trimethylsilyl iodide [35,36] in carbon tetrachloride, in 92% yield. This latter was treated with benzylamine in dioxane under reflux for 48 h to afford *N*-benzyl 12-aza-5β-steroid **19** in good yield. The structure of **15** was confirmed by spectroscopic methods. It is worth pointing out that here too, we observed retention of configuration at C-13.

Finally, in order to obtain the free alcohols we undertook different attempts of deprotection on steroids **8**, **11**–**13** and **15**. Thus, methoxy groups of compounds **8**, **11**–**13** and **15** were removed easily by using trimethylsilyl iodide. These demethylation reactions were carried out in chloroform at room temperature for 24 h, and afforded the expected deprotected hetero steroids **16**–**20** in satisfactory yields as indicated in Scheme 6.



8 X = O
11 X = S
12 X = SO
13 X = SO_2
15 X = NCH_2Ph

16 X = O; 60%
17 X = S; 66%
18 X = SO; 58%
19 X = SO_2; 67%
20 X = NCH_2Ph; 62%

Scheme 6. Final step of deprotection.

In conclusion, no report has so far been published on the synthesis of 12-aza steroids, and the present synthesis of the 12-aza-5β-steroid **20** is, as far as we know, the first successful one. Thus, this synthesis was achieved *via* 9 steps, in 16% overall yield, starting from commercially available cholic acid **1**. Tests to assess the biological activity of the steroids **16**–**20** are being conducted by our group.

Acknowledgements

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