Tetrahedron 66 (2010) 2455–2462

Contents lists available at ScienceDirect

Tetrahedron

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

# Efficient trans-diaxial hydroxylation of $\Delta^5$-steroids

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ARTICLE INFO

*Article history:*
Received 6 November 2009
Received in revised form
19 January 2010
Accepted 26 January 2010
Available online 1 February 2010

ABSTRACT

A convenient, fast, and high-yielding process to synthesize 5α,6β-dihydroxysteroids directly from the correspondent $\Delta^5$-steroids is reported. The reaction protocol consists in the conjugation of a readily available and stable oxidant, magnesium bis(monoperoxyphthalate) hexahydrate, with the non-toxic bismuth(III) triflate in acetone to afford the trans-diaxial hydroxylation product in a stepwise manner and in excellent yields.



## 1. Introduction

Sterols comprise an important class of compounds as a consequence of their widespread presence in living organisms and, mostly, of their remarkable biological roles.[1,2] Oxysterols are characterized by the presence of additional oxygenated groups in the cholesterol template. These compounds have attracted increasing attention due to their biological activities, such as regulation of cell proliferation[3] and differentiation,[4] apoptosis induction,[5] cholesterol homeostasis,[6] inflammation,[7] and interference with membrane proteins.[8] The discovery of new biological roles, such as in morphogenesis through the Hedgehog signaling pathways and as photoxidation products in retina,[9] has given even more emphasis to these compounds.

On the other hand, natural polyhydroxylated steroids, from diverse sources, have been isolated, characterized, and biologically evaluated in multiple studies.[10,11] Marine organisms are natural suppliers of interesting polyhydroxysteroids, which are unique in having unusual functionalizations and may represent useful leads in the development of pharmaceutical agents.[12] Steroids from marine species have frequently oxygenated functions on rings A and B of the steroid nucleus.[13–15]

It is believed that the physiological actions of steroids are related to the presence of specific functional groups on the steroid template.[16,17] The 3β,5α,6β-hydroxylation pattern is found in several natural sources[13,18–20] and also in human tissues mainly as an oxidation product of cholesterol.[21] Indeed, cholestane-3β,5α,6β-triol is a widely studied compound and has been shown to be cytotoxic,[22–25] angiotoxic,[26] mutagenic,[27–29] and carcinogenic.[30] Moreover, natural steroids with such hydroxylation pattern have shown to possess interesting cytotoxic properties in different cancer cell lines.[31,32] Furthermore, the 5α,6β-dihydroxy pattern is useful for the synthesis of 6-oxa-steroids,[33] which are interesting molecules due to the biological activities of heterocyclic steroids.[34]

The oxidation of unsaturated steroids is a valuable strategy for the incorporation of new functional groups and the synthesis of polyoxygenated sterols. The dihydroxylation of alkenes can be achieved by a *syn* or *anti* approach and a great deal of work has been done in the field of steroids, mainly concerning the *syn*-dihydroxylation. In general, the *syn*-dihydroxylation of steroidal alkenes is achieved using osmium tetraoxide or alkaline potassium permanganate as oxidants, and a large review on the subject was written by Salvador et al.,[35] where different methodologies for several positions in the steroid template are described.

Trans-dihydroxylation can be achieved by treatment of the olefin with a peroxycarboxylic acid in a two-step procedure where an epoxide is produced and then converted into the *trans*-diaxial diol by ring opening through the anti-attack of the hydroxyl nucleophile.[36] Due to the increasing demand for environmental benign processes, a great effort has been done toward the development of one-pot reactions,[37] and a few direct trans-dihydroxylation processes from the respective olefins have been described recently in the literature.[38–40]

In the field of steroids, the use of formic acid and hydrogen peroxide has allowed the one-pot trans-dihydroxylation of cholesterol and sitosterol in low to moderate yields.[41,42]

The opening of 5,6-epoxides is stereoselective rendering 5α,6β-vicinal diols. In fact, the trans-diaxial hydroxylation is imposed by the substrate itself, since the stereochemistry is modulated by the steric hindrance from the two angular methyl groups, at C-10 and C-13 on the steroid nucleus, both in the epoxidation step as in the subsequent epoxide opening under acidic conditions.

In a previous study, we have described a very efficient epoxidation protocol to access 4,5- and 5,6-epoxysteroids using magnesium bis(monoperoxyphthalate) hexahydrate (MMPP) in acetonitrile under reflux.[43] MMPP is a solid oxygen donor,

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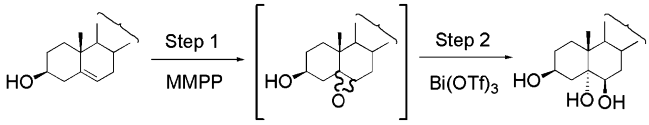
0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.01.089

inexpensive, with low toxicity, and safe to handle even in larger scale reactions.[44,45]

Herein we report a new method for the fast and efficient trans-diaxial hydroxylation of $\Delta^5$-steroids, using MMPP in combination with an acidic catalyst in a straightforward two-step procedure, without the need of an intermediary work-up and keeping the same reaction solvent in both steps (Scheme 1).




**Scheme 1.** Synthesis of trihydroxysteroids from 3β-hydroxy-$\Delta^5$-steroids.

## 2. Results and discussion

The typical procedure to obtain the 5α,6β-dihydroxy derivatives consists in the epoxidation of the $\Delta^5$-steroid followed by a second reaction for the acidic opening of the epoxide. This protocol is time and money consuming, involving two different work-ups. Due to the importance of 5α,6β-dihydroxysteroids and the need to produce them through a simple and fast procedure, we aimed for a one-pot process to perform the trans-diaxial hydroxylation using a stable oxidant and an environment friendly Lewis acid. Bismuth(III) compounds ($BiX_3$, X=Cl, Br, $NO_3$ and OTf, etc.) are moisture- and air-tolerant Lewis acids, which have been widely applied in organic synthesis in the past few years as green catalysts due to the non-toxic properties of bismuth.[46] Bismuth triflate ($Bi(OTf)_3$) is a readily available and cheap Lewis acid, which can be easily prepared[47,48] and has been widely explored for the epoxide opening reaction.[49–51] Moreover, it has been recently used in the field of terpenoid[52] and steroid chemistry.[53,54] Consequently, $Bi(OTf)_3$ was chosen as catalyst for the epoxide opening step.

High yielding and fast generation of steroid epoxides can be achieved by the oxidation of alkene steroids with MMPP in acetonitrile at reflux, with predominance of the α-epoxide.[43] In order to develop an efficient synthesis of 3β,5α,6β-trihydroxysteroids from the corresponding 3β-hydroxy-$\Delta^5$-steroids, we decided to study the combination of MMPP with $Bi(OTf)_3$.

Using cholesterol (**1**) as model substrate, the epoxidation reaction was carried out in acetonitrile at reflux with 1.2 equiv of MMPP affording the α,β-epoxides **1a**,**b** (Table 1, entry 1). In the presence of a catalytic amount (10%) of $Bi(OTf)_3$, added at the beginning of the reaction, total substrate consumption was observed by TLC control, within 2 h, although the outcome was a complex mixture, from which 14% of triol **11** was separated (Table 1, entry 7). Another two products were separated together by flash chromatography and identified by $^1$H NMR analysis as being Ritter reaction products, the 6β-acetamido-5α-hydroxy and the 5α-acetamido-6β-hydroxy derivatives in 29% and 8% yields, respectively (Table 1, note *e*). Such results are consistent with the α- and β-epoxide formation promoted by MMPP in acetonitrile at reflux (*α*/*β* ratio 78:22, Table 1, entry 1).[43] The formation of the *vic*-acetamido-hydroxy derivatives is consistent with the results of Pinto et al., concerning the epoxide opening in the presence of catalytic $BiBr_3$ in acetonitrile.[53] Due to the presence of water in the reaction system, the acidic opening of the epoxide mixture in acetonitrile by $Bi(OTf)_3$ rendered also the triol **11**.

The formation of such undesired mixture prompted us to carry out a careful optimization of the reaction conditions. To avoid acetonitrile, several solvents were tested. Acetone and dioxane showed the best results for the epoxidation of cholesterol (**1**) and temperature had a strong effect on the reaction rate (Table 1, entries 2–6). Comparing with the epoxidation protocol in acetonitrile, previously described (Table 1, entry 1), the use of either acetone or dioxane at 57 °C provides longer reaction times, although the yields are quite satisfactory (Table 1, entries 3 and 6).

Next, the simultaneous addition of $Bi(OTf)_3$ with MMPP at 57 °C was tested for a one-pot epoxidation/*trans*-diaxial epoxide opening using the solvents screened before (Table 1, entries 8 and 9). Initially, a catalytic amount of the acid was added to the reaction system. However, no significant conversion into the triol **11** was observed by TLC, until a stoichiometric addition of $Bi(OTf)_3$ was reached. Using 1 equiv of $Bi(OTf)_3$, after 3 h the reactions showed no further progression, with a substantial amount of unreacted substrate and minor secondary products (TLC analysis). The reaction in acetone gave the best result, with the isolation of the desired product **11** in 27% (Table 1, entry 8). From the above results, the simultaneous addition of both reagents, oxidant and acid, fails to efficiently access the desired product.

Therefore, we attempted to develop a new protocol where a sequential addition of reagents was adopted. The epoxidation step was carried out in acetone at reflux using a higher amount of MMPP (1.5 mmol per mmol of substrate), followed by the epoxide hydrolysis at room temperature to avoid the formation of secondary products elicited by $Bi(OTf)_3$ at higher temperatures. Cholesterol (**1**) and dehydroepiandrosterone (**3**) were used as model substrates. Epoxidation with MMPP in acetone under reflux for 30 min ensured the majority of the substrate consumption. Then, the reactions were cooled to room temperature and the acidic catalyst was added. Reactions were monitored by TLC and stopped when no further progression was observed toward the formation of the triols. Isolated yields are summarized in Table 2.

**Table 1**
Studies on epoxidation and one-pot epoxidation/*trans*-diaxial epoxide opening, using cholesterol (**1**) as model substrate

| Entry | Substrate (mmol) | MMPP (mmol/mmol) | Acid (mL/mmol) | Solvent (mmol/mmol) | Temperature | Total time | Product | Yield (%)[a] |
|---|---|---|---|---|---|---|---|---|
| 1[b] | 0.150 | 1.1 | — | $CH_3CN$/15 | Reflux (82 °C) | 10 min | **1a**,**b**[c] | 83 (78:22, *α*:*β*)[d] |
| 2 | 0.300 | 1.2 | — | Acetone/38 | rt | 24 h | **1a**,**b** | 94 (77:23, *α*:*β*) |
| 3 | 0.200 | 1.2 | — | Acetone/38 | Reflux (57 °C) | 1 h | **1a**,**b** | 91 (75:25, *α*:*β*) |
| 4 | 0.200 | 1.2 | — | Dioxane/38 | rt | 24 h | **1a**,**b** | 85 (83:17, *α*:*β*) |
| 5 | 0.200 | 1.2 | — | Dioxane/38 | Reflux (102 °C) | 30 min | **1a**,**b** | 87 (79:21, *α*:*β*) |
| 6 | 0.200 | 1.2 | — | Dioxane/38 | 57 °C | 1 h | **1a**,**b** | 90 (82:18, *α*:*β*) |
| 7 | 0.129 | 1.1 | $Bi(OTf)_3$/0.1 | $CH_3CN$/23 | Reflux (82 °C) | 2 h | **11** | 14[e] |
| 8 | 0.300 | 1.2 | $Bi(OTf)_3$/1 | Acetone/38 | Reflux (57 °C) | 3 h | **11** | 27 |
| 9 | 0.300 | 1.2 | $Bi(OTf)_3$/1 | Dioxane/38 | 57 °C | 3 h | **11** | 12 |

[a] Isolated yield by flash chromatography.
[b] Published data: Ref. 41.
[c] 5α,6α- and 5β,6β-Epoxides (**1a**,**b**).
[d] *α*:*β* Ratio has been calculated by $^1$H NMR integration of 6-H of the isolated product.
[e] Flash chromatography afforded triol **11** (14%) and a mixture of 5α-acetamidocholestane-3β,6β-diol (8%) and 6β-acetamidocholestane-3β,5α-diol (29%) (calculated by $^1$H NMR integration of the N–H signals of the acetamido isomers and H-6 of the triol).

**Table 2**
Two-step conversion of $\Delta^5$-steroids to 3β,5α,6β-trihydroxysteroids[a]

| Entry | Substrate (mmol) | Acetone (mL/mmol) | Base[b] (mmol/mmol) | Acid (mmol/mmol) | Total time (h) | Product | Yield (%)[c] |
|---|---|---|---|---|---|---|---|
| 1 | **1**/0.259 | 38 | — | $Bi(OTf)_3$/0.75 | 9.5 | **11** | 20 |
| 2 | **1**/0.259 | 38 | — | $Bi(OTf)_3$/1.5 | 2 | **11** | 86 |
| 3 | **1**/0.194 | 38 | 0.15 | $Bi(OTf)_3$/1.5 | 2 | **11** | 87 |
| 4 | **1**/0.194 | 38 | 0.75 | $Bi(OTf)_3$/1.5 | 6.5 | **11** | 82 |
| 5 | **1**/0.388 | 38 | 1.5 | $Bi(OTf)_3$/1.5 | 9.5 | **11** | 52 |
| 6 | **1**/0.388 | 38 | 4.5 | $Bi(OTf)_3$/1.5 | 9.5 | **11** | 19 |
| 7 | **3**/0.150 | 14 | — | $Bi(OTf)_3$/1.5 | 1 | **13** | 91 |
| 8 | **1**/0.259 | 38 | — | $Yb(OTf)_3$/0.75 | 9.5 | **11** | 12 |
| 9 | **1**/0.259 | 38 | — | $Yb(OTf)_3$/1.5 | 8.5 | **11** | 77 |
| 10 | **1**/0.259 | 38 | 1.5 | $Yb(OTf)_3$/1.5 | 9.5 | **11** | 31 |
| 11 | **3**/0.277 | 14 | — | $Yb(OTf)_3$/1.5 | 5 | **13** | 74 |
| 12 | **1**/0.388 | 38 | — | $HClO_4$/0.75 | 24 | **11** | 14 |
| 13 | **1**/0.259 | 38 | — | $HClO_4$/1.5 | 2 | **11** | 86 |

[a] General conditions: Step 1: $\Delta^5$-steroid, 1.5 equiv MMPP, acetone, reflux (30 min.); Step 2: $Bi(OTf)_3$, rt.
[b] Proton scavenger: 2,6-di-*tert*-butyl-4-methylpyridine.
[c] Isolated yield by flash chromatography.

At this point, three different acids, $Bi(OTf)_3$, ytterbium triflate ($Yb(OTf)_3$), and perchloric acid ($HClO_4$), in increasing amounts, were tested for the epoxide hydrolytic step. The addition of 0.75 equiv of the acids led to incomplete reactions, even after long reaction periods, as shown in Table 2, entries 1, 8 and 12 for cholestane-3β,5α,6β-triol (**11**). However, when the addition of the acid (1.5 equiv) equals the amount of the oxidant, epoxide hydrolysis occurred much faster with $Bi(OTf)_3$ and $HClO_4$ in very clean and high-yielding reactions (Table 2, entries 2 and 13). Under $Yb(OTf)_3$ catalysis, the reaction was slow and incomplete (Table 2, entry 9).

Using $Bi(OTf)_3$ after the epoxidation step, for a more polar substrate, dehydroepiandrosterone (**3**), we were able to obtain 3β,5α,6β-trihydroxyandrostan-17-one (**13**) in 1 h with very good yield, 91% (Table 2, entry 7). With $Yb(OTf)_3$ the reaction was slower than with $Bi(OTf)_3$ (Table 3, entries 7 and 11). Moreover, dehydroepiandrosterone (**3**) was more reactive than cholesterol (**1**) either in the presence of $Bi(OTf)_3$ (Table 2, entries 2 and 7) or $Yb(OTf)_3$ (Table 2, entries 9 and 11).

Several authors have discussed for Lewis acid catalyzed reactions, the 'in situ' generation of Brønsted acids through hydrolysis of metal complexes and that, in fact, protons are the true catalytic species. Such observations have been reported for reactions catalyzed with bismuth(III)[52,55] and ytterbium(III)[55,56] salts. $Bi(OTf)_3$ is known to be easily hydrolysed in the presence of water[48] generating triflic acid (TfOH), which is also considered a superacid,[57] while $Yb(OTf)_3$, being more stable in water, will behave more as Lewis acid, and these different characteristics may explain the different behavior of both acids under our reaction conditions. Furthermore, $Bi(OTf)_3$ proved to be more efficient than the strong protic acid $HClO_4$, which only affords the desired triol **11** after overnight reaction (Table 3, entries 1 and 2), suggesting a synergistic effect of the Lewis acid with the TfOH.

In an outstanding review[58] Yamamoto presented the concept of 'designer acids' resulting from the combined acid catalysis where a Brønsted acid can synergistically affect the action of the Lewis acid and vice versa. There are also reports related to catalysis promoted by the conjugation of metal triflates with Brønsted acids.[59,60]

**Table 3**
Studies on the acidic epoxide opening conditions[a]

| Entry | Substrate[b] (mmol) | Acetone (mL/mmol) | Base[c] (mmol/mmol) | $MgCl_2 \cdot 6H_2O$[d] (mmol/mmol) | Acid (mmol/mmol) | Total time | Product | Yield (%)[e] |
|---|---|---|---|---|---|---|---|---|
| 1 | **1a**,**b**/0.248 | 38 | — | — | $HClO_4$/0.25 | Overnight | **11** | 90 |
| 2 | **1a**,**b**/0.248 | 38 | — | — | $Bi(OTf)_3$/0.1 | 0.5 h | **11** | 92 |
| 3 | **1a**,**b**/0.248 | 38 | 0.1 | — | $Bi(OTf)_3$/0.1 | 0.5 h | **11** | 91 |
| 4 | **1a**,**b**/0.248 | 38 | 0.3 | — | $Bi(OTf)_3$/0.1 | 9 h | **11** | 16 |
| 5 | **3a**,**b**/0.246 | 14 | — | — | $Bi(OTf)_3$/1.5 | 0.5 h | **13** | 90 |
| 6 | **3a**,**b**/0.246 | 14 | — | — | $Bi(OTf)_3$/0.1 | 0.5 h | **13** | 91 |
| 7 | **3a**/0.164 | 14 | — | 1 | $Bi(OTf)_3$/0.5 | 0.5 h | **13** | —[f] |
| 8 | **3a**/0.164 | 14 | — | 1 | $Bi(OTf)_3$/1 | 0.5 h | **13** | 58[g] |

[a] General conditions: epoxide, acid, acetone, rt.
[b] 5,6-Epoxycholestan-3β-ol (**1a**,**b**, 78:22, $\alpha$:$\beta$), 5,6-epoxy-3β-hydroxyandrostan-17-one (**3a**,**b**, 75:25, $\alpha$:$\beta$) and 5α,6α-epoxy-3β-hydroxyandrostan-17-one (**3a**).
[c] Proton scavenger: 2,6-di-*tert*-butyl-4-methylpyridine.
[d] Simultaneous addition of 3 mL of water/mmol of $MgCl_2 \cdot 6H_2O$.
[e] Isolated yield by flash chromatography.
[f] Only traces of product were visible by TLC.
[g] 6β-Chloro-3β,5α-dihydroxyandrostan-17-one was also isolated in 33% yield.

In order to further study the epoxide opening by $Bi(OTf)_3$ in acetone we have prepared the intermediary epoxides 5,6-epoxycholestan-3β-ol (**1a**,**b**, 78:22, $\alpha$:$\beta$), 5,6-epoxy-3β-hydroxyandrostan-17-one (**3a**,**b**, 75:25, $\alpha$:$\beta$) and 5α,6α-epoxy-3β-hydroxyandrostan-17-one (**3a**) using our recent oxidative protocol.[43] Then, we promoted the **3a**,**b** epoxide hydrolysis in acetone using 1.5 equiv of $Bi(OTf)_3$. This reaction was complete in half an hour with 90% yield of triol **13** (Table 3, entry 5). It is worth noting that, using a catalytic amount of the same triflate (10%), the isolated yield and reaction time were the same (Table 3, entry 6). A similar result was obtained with 5,6-epoxycholestan-3β-ol (**1a**,**b**), using 10% of $Bi(OTf)_3$ (Table 3, entry 2).

In order to fully understand the epoxide hydrolysis catalyzed by $Bi(OTf)_3$ in acetone, in the present conditions, a systematic set of experiments was performed using the Lewis acids in conjugation with 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP), a highly hindered organic base known to act as a proton scavenger. Such property is a consequence of the bulky *tert*-butyl substituents, which do not allow the interaction with the metal catalyst[61] and has been used to discriminate the nature of the acidic species.[62]

The epoxide opening reaction was not affected in the presence of equimolar amounts of $Bi(OTf)_3$ and base (Table 3, entries 2 and

3), while a threefold increase of the amount of base led to a drastic decrease in reactivity (<20% yield) (Table 3, entries 3 and 4).

Analyzing the two-step conversion protocol of the olefin **1** into the triol **11** (Table 2) in the presence of MMPP and $Bi(OTf)_3$, we observed that an increasing amount of base corresponds to a decrease of the product yield (Table 2, entries 3–6). Moreover, the addition of equimolar amounts of DTBMP and $Bi(OTf)_3$ led to a significant decrease in yield and reactivity (Table 2, entry 5), although the same experiment performed on the acidic epoxide opening reaction (Table 3, entry 3) did not influence the triol formation, which indicates that $Bi(OTf)_3$ is deactivated not only by DTBMP but also by the MMPP products. Other works report that equimolar amounts of this base are enough to abolish the metal triflate reactivity.[55,56]

The catalysis promoted by $Yb(OTf)_3$ also involves TfOH, since a substantial decrease in triol **11** was observed after the addition of DTBMP (Table 2, entry 10).

These experiments clearly show that the formation of triol **11** is dependent on the amount of base added, which reinforces the 'in situ' generation of TfOH. On the other hand, the fact that a threefold addition of base did not prevent completely the reaction (Table 3, entry 4) suggests that $Bi(OTf)_3$ acts also as a Lewis acid. Therefore the strong reactivity observed should result from the synergistic effect of the metal triflate and the triflic acid generated 'in situ', supporting the hypothesis of a Lewis acid assisted Brønsted acid catalysis.

The fact that the epoxide opening reaction alone requires only 0.1 equiv of $Bi(OTf)_3$ (Table 3, entry 2) while the sequential oxidation/epoxide opening requires 1.5 equiv of the acidic catalyst (Table 2, entry 2) suggests that the insoluble products formed during the oxidation step deactivated the acid. Corroborating this conclusion, we observed complete dissolution of the MMPP resulting products only after the addition of the equimolar amount of the $Bi(OTf)_3$.

The insoluble products formed were removed by filtration after the epoxidation step and identified by mass spectrometry as magnesium biphthalate **c**, as expected. Moreover, phthalic acid **d** was found in the resulting acetone solution (Scheme 3). Therefore, the magnesium biphthalate salt **c**, in the presence of a strong acid, would behave as a base, interfering with the acidic potential of the $Bi(OTf)_3$. To confirm such results, two additional experiments of the $Bi(OTf)_3$ catalysed epoxide opening reaction were performed in the presence of another magnesium salt, the magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), known to be a weak Lewis acid.[63] As presented in Table 3, entries 7 and 8, using a half molar amount of $Bi(OTf)_3$ compared to $MgCl_2 \cdot 6H_2O$, only traces of the desired product were observed by TLC after 30 min of reaction, while using an equimolar amount of the $Bi(OTf)_3$, after 30 min all 5α,6α-epoxy-3β-hydroxyandrostan-17-one (**3a**) was consumed with the formation of 58% of triol **13**, and also of 33% of the chlorohydrin product, 6β-chloro-3β,5α-dihydroxyandrostan-17-one. These results confirm the deactivating properties of magnesium salts for the acidic epoxide opening catalysis.

Summarizing so far, we report a fast and efficient high-yielding preparation of 3β,5α,6β-trihydroxysteroids using $\Delta^5$-steroids as starting materials. This new synthetic protocol involves two steps: (i) formation of the epoxide from $\Delta^5$-steroids, using MMPP as oxidative agent in commercial acetone; followed by (ii) *trans*-diaxial epoxide opening with $Bi(OTf)_3$ in a sequential procedure. Although this is a straightforward and quantitative process to afford 3β,5α,6β-trihydroxysteroids from $\Delta^5$-steroids, the requirement of a large amount of $Bi(OTf)_3$ weakens the process, despite the green properties of $Bi(OTf)_3$.

In this way, in order to optimize the process, the introduction of an additional filtration step was tested. Thus, using cholesterol (**1**) as substrate, the trans-diaxial hydroxylation reaction was performed followed by filtration of the insoluble magnesium salts after the epoxidation step. Subsequent addition of 25% of $Bi(OTf)_3$ proved to be efficient for a fast and high-yielding synthesis of the desired triol **11** (Table 4, entry 1). The amount of triflate catalyst was further decreased to 5 and 10% affording successfully triol **11**, although 5% of catalyst resulted in a longer reaction time (Table 4, entries 2 and 3). Therefore, a simple filtration of the magnesium salts, taking advantage of their low solubility in acetone, allows the use of a catalytic amount of $Bi(OTf)_3$.

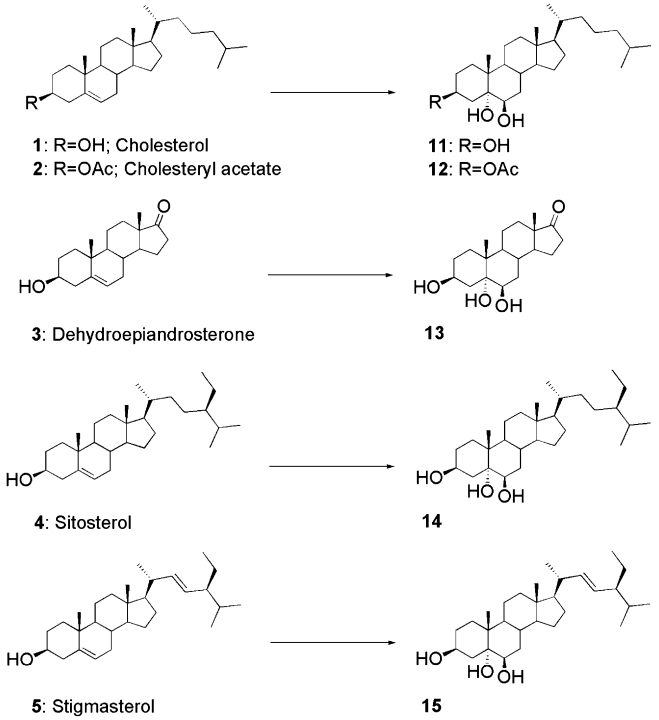
**Table 4**
Direct synthesis of 3β,5α,6β-triols from $\Delta^5$-steroids by sequential addition of reagents and intermediary filtration[a]

| Entry | Substrate (mmol) | Acetone (mL/mmol) | $Bi(OTf)_3$ (mmol/mmol) | Total time (h) | Product | Yield (%)[b] |
|---|---|---|---|---|---|---|
| 1 | **1**/0.259 | 38 | 0.25 | 1 | **11** | 84 |
| 2 | **1**/0.259 | 38 | 0.10 | 1 | **11** | 85 |
| 3 | **1**/0.517 | 38 | 0.05 | 1.5 | **11** | 84 |
| 4 | **1**/0.259 | 12 | 0.10 | 1 | **11** | 93 |
| 5 | **2**/0.467 | 12 | 0.10 | 1.5 | **12** | 92 |
| 6 | **3**/0.347 | 14 | 0.10 | 1 | **13** | 92 |
| 7 | **4**/0. 362 | 10 | 0.10 | 1 | **14** | 89 |
| 8 | **5**/0.363 | 10 | 0.10 | 1 | **15** | 86 |
| 9 | **6**/0.316 | 16 | 0.10 | 1 | **16** | 93 |
| 10 | **7**/0.277 | 20 | 0.10 | 1 | **17** | 85 |
| 11 | **8**/0.636 | 20 | 0.10 | 1 | **18** | 86 |
| 12 | **9**/0.450 | 31 | 0.10 | 1 | **19** | 73 |
| 13 | **10**/0.335 | 15 | 0.10 | 1 | **20** | 90 |

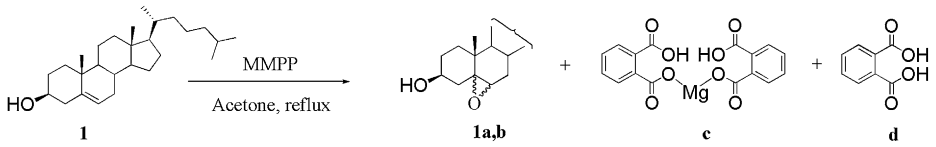
[a] Reaction conditions: Step 1: 1 mmol of $\Delta^5$-steroid, 1.5 mmol MMPP, acetone, reflux (30 min.), followed by filtration of insoluble products; Step 2: $Bi(OTf)_3$, rt.
[b] Isolated yield by flash chromatography.

The solvent volume also influences the reaction outcome, as shown in Table 4 (entries 2 and 4) since decreasing the reaction volume resulted in a higher yield of triol **11**. This fact resulted from the increased concentration of the oxidant, in a threefold less solvent volume, which led to complete consumption of cholesterol (**1**) after 30 min of epoxidation, as proved by TLC analysis.

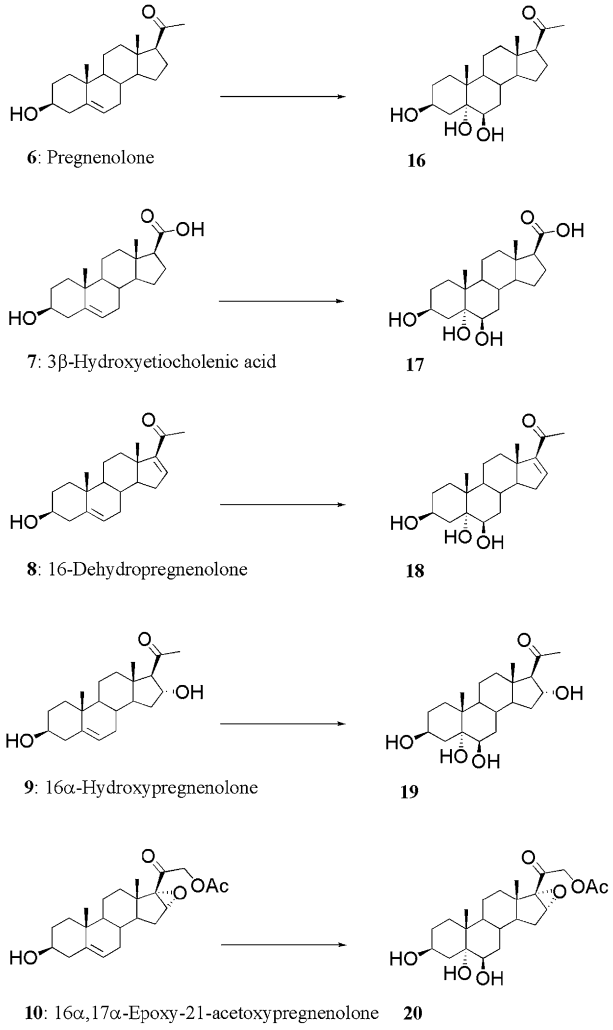
Having reached optimized reaction conditions, we studied the scope of this methodology in a variety of $\Delta^5$-steroids (Schemes 2 and 4), as outlined in Table 4. The present protocol revealed to be fast and clean, to afford good to excellent yields for the compounds




**Scheme 2.** Trans-diaxial hydroxylation of $\Delta^5$-steroids.




**Scheme 3.** Identified product from the epoxidation reaction of cholesterol with MMPP.




**Scheme 4.** Synthesis of trihydroxysteroids.

tested. Among the polar substrates, reactions proceeded very fast and efficiently for all substrates tested. Concerning 3β-hydroxyetiocholenic acid (**7**) and 16α-hydroxypregnenolone (**9**), precautions should be taken in the work-up due to the high solubility of the corresponding triols **17** and **19** in water. 16-Dehydropregnenolone (**8**), 16α-hydroxypregnenolone (**9**), and 16α,17α-epoxy-21-acetoxypregnenolone (**10**), with sensitive functionalities in the steroid framework, were efficiently converted (Table 4, entries 11–13). To the best of our knowledge, two of the molecules synthesized, **17**, **20**, are new compounds and compound **19**[64] has been further characterized.

## 3. Conclusions

In summary, we report herein a fast and efficient high-yielding sequential approach to the preparation of 5α,6β-dihydroxysteroids using $\Delta^5$-steroids as raw materials. This new synthetic protocol involves two steps: (i) formation of the epoxide from $\Delta^5$-steroids, using MMPP as oxidative agent; and (ii) *trans*-diaxial epoxide opening with $Bi(OTf)_3$ in commercial acetone. A catalytic opening of the epoxide was achieved when a filtration was performed to remove the insoluble salts formed in the first step.

With this methodology we have easily obtained a library of 5α,6β-dihydroxysteroids from readily available $\Delta^5$-steroids. The comprehensive study undertaken led to an optimized methodology with large application in steroid chemistry.

## 4. Experimental

### 4.1. General

Cholesterol, cholesteryl acetate, sitosterol, stigmasterol, dehydroepiandrosterone, pregnenolone, 16-dehydropregnenolone, 3β-hydroxyetiocholenic acid, and 16α,17α-epoxy-21-acetoxypregnenolone were purchased. 16α-Hydroxypregnenolone,[65] 5,6-epoxy-3β-hydroxyandrostan-17-one,[43] and 5,6-epoxycholestan-3β-ol[43] were prepared as described in literature. All commercial available starting materials were used without purification, except sitosterol, which was crystallized and recrystallized from ethanol. Solvents were used as purchased. Reactions were controlled by TLC using silica gel 60 $F_{254}$ aluminum sheets. Filtration was performed through a filter funnel with porosity 4 (10–16 μm). Reaction yields correspond to compounds isolated by flash column chromatography, performed in an automated system using a borosilikat 3.3 column and silica gel 60 (230–400 mesh ASTM). Melting points were obtained in open capillary tubes and are uncorrected. Optical rotations were measured in MeOH solutions on a Perkin–Elmer 343 polarimeter. IR analysis was carried out on a FTIR spectrometer in the range of 600–4000 $cm^{-1}$, and compound measurements realized as films on sodium chloride plates. $^1H$, $^{13}C$, and DEPT NMR spectra were recorded in instruments operating at 300, 400, and in a 500 MHz. Sample solutions were prepared in $CDCl_3$, acetone-$d_6$, DMSO-$d_6$ alone or in combination DMSO-$d_6$/$CDCl_3$. Chemical shifts ($\delta$) are given in parts per million (ppm) and coupling constants ($J$) in Hertz. Deuterated solvents were used as internal reference (7.26 and 77.0 for $CDCl_3$, 2.05 for acetone-$d_6$, and 2.5 and 39.4 for DMSO-$d_6$). Mass spectra were recorded on a QIT-MS spectrometer and were obtained using the ESI method. HRMS was recorded on an FTICR-MS Apex Ultra 7 Tesla equipment.

### 4.2. General procedure for one-pot trans-diaxial hydroxylation of $\Delta^5$-steroids with sequential addition of MMPP and $Bi(OTf)_3$

To a solution of cholesterol (1) (100 mg, 0.259 mmol) in acetone (9.8 mL) at reflux temperature (57 °C), MMPP was added (192.2 mg, 0.388 mmol) and reaction mixture stirred for 30 min. Then it was cooled to room temperature, and addition of $Bi(OTf)_3$ was made (256.1 mg, 0.388 mmol). After 1 h 30 min, the reaction mixture was stopped by evaporation under vacuum. The white solid residue was dissolved in ethyl acetate (saturated in water), and the resulting organic phase was washed with $Na_2SO_3$ (10% aq soln) and water,

dried with anhydrous $Na_2SO_4$, filtered, and evaporated to yield a white crude product. Flash chromatography (petroleum ether/ethyl acetate 1:1) afforded pure cholestane-3β,5α,6β-triol (**11**) (93.7 mg, 86%). Mp 237–238 °C (EtOH); lit.,[66] 237–239 °C; $[\alpha]_D^{20}$ +3.3 (*c* 0.30); IR (film) 3394, 2936, 2867, 1454, 1373, 1041, 960, 874 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ ppm 0.68 (3H, s, 18-$CH_3$), 0.86 and 0.86 (each 3H, 2 d, *J*=6.6 Hz, 26-$CH_3$ and 27-$CH_3$), 0.90 (3H, d, *J*=6.5 Hz, 21-$CH_3$), 1.18 (3H, s, 19-$CH_3$), 3.54 (1H, t, *J*=3.2 Hz, 6α-H), 4.10 (1H, tt, *J*=11.2, 5.5 Hz, 3α-H); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ ppm 12.1, 16.9, 18.6, 21.2 ($CH_2$), 22.5, 22.8, 23.8 ($CH_2$), 24.1 ($CH_2$), 28.0, 28.2 ($CH_2$), 30.2, 30.8 ($CH_2$), 32.4 ($CH_2$), 34.5 ($CH_2$), 35.8, 36.1 ($CH_2$), 38.3 (C-10), 39.5 ($CH_2$), 39.9 ($CH_2$), 40.7 ($CH_2$), 42.7 (C-13), 45.9, 55.9, 56.2, 67.6, 76.1, 76.1 (C-5); MS [*m*/*z* (%)]: 421.6 (18) $[M+H]^+$, 418.1 (27), 382.6 (72), 372.4 (40), 358.8 (100), 356.3 (23), 345.7 (28), 340.4 (78), 327.2 (47), 298.3 (86), 283.3 (41), 240.8 (44), 234.3 (28), 158.1 (40).

### 4.3. General procedure for direct trans-diaxial hydroxylation of $\Delta^5$-steroids with sequential addition of MMPP and $Bi(OTf)_3$

To a solution of cholesteryl acetate (**2**) (200 mg, 0.467 mmol) in acetone (5.6 mL) at reflux temperature (57 °C), MMPP was added (346.5 mg, 0.700 mmol) and the reaction mixture stirred for 30 min. Then it was cooled to room temperature, filtered, and the filtrate washed with acetone (max 5.6 mL). To the resulting clear solution, $Bi(OTf)_3$ (30.8 mg, 0.047 mmol) was added. After 1 h of stirring at room temperature, the reaction mixture was stopped by evaporation under vacuum. The white solid residue was dissolved in ethyl acetate (saturated in water), and the resulting organic phase was washed with $NaHCO_3$ (satd aq soln) and water, dried with anhydrous $Na_2SO_4$, filtered, and evaporated to yield a white crude product. Flash chromatography (petroleum ether/ethyl acetate 1:1) afforded the pure 5α,6β-dihydroxycholestan-3β-yl acetate (**12**) (198.6 mg, 92%) as white solid. Mp 208–209 °C (EtOH); lit.,[66] 205–206 °C; $[\alpha]_D^{20}$ +16.3 (*c* 0.43); IR (film) 3456, 2936, 2867, 1716, 1460, 1373, 1268, 1030, 960, 874, 757 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ ppm 0.67 (3H, s, 18-$CH_3$), 0.86 and 0.86 (each 3H, 2 d, *J*=6.6 Hz, 26-$CH_3$ and 27-$CH_3$), 0.90 (3H, d, *J*=6.6 Hz, 21-$CH_3$), 1.18 (3H, s, 19-$CH_3$), 2.02 (3H, s, 3β-$CH_3COO$), 2.16 (1H, dd, *J*=12.9, 11.3 Hz), 3.52 (1H, t, *J*=2.5 Hz, 6α-H), 5.15 (1H, tt, *J*=11.3, 5.5 Hz, 3α-H); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ ppm 12.1, 16.7, 18.6, 21.0 ($CH_2$), 21.5, 22.5, 22.8, 23.9 ($CH_2$), 24.1 ($CH_2$), 26.6 ($CH_2$), 28.0, 28.2 ($CH_2$), 30.2, 32.1 ($CH_2$), 34.6 ($CH_2$), 35.8, 36.1 ($CH_2$), 36.9 ($CH_2$), 38.3 (C-10), 39.5 ($CH_2$), 39.8 ($CH_2$), 42.7 (C-13), 45.4, 55.8, 56.2, 71.2, 75.7 (C-5), 76.2, 170.9 ($CH_3COO$); MS *m*/*z* (%): 461.6 (100) $[M-H]^+$, 430.7 (28), 421.3 (53), 419.7 (62), 417.5 (41), 396.3 (26), 351.8 (74), 320.9 (25), 308.2 (63), 291.6 (41), 272.4 (46), 232.5 (50).

*4.3.1. 3β,5α,6β-Trihydroxyandrostan-17-one (**13**).* Flash chromatography (chloroform/ethanol 15:1) afforded a pure white solid. Mp 303–305 °C (EtOH); lit.,[67] 300–302 °C; $[\alpha]_D^{20}$ +46.8 (*c* 0.34); IR (film) 3442, 3348, 2942, 2861, 1723, 1471, 1373, 1077, 1047, 1030, 1001, 960, 874 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$) δ ppm 0.77 (3H, s, 18-$CH_3$), 1.04 (3H, s, 19-$CH_3$), 2.36 (1H, dd, *J*=19.0, 8.2 Hz), 3.35 (1H, m, 6α-H), 3.74 (1H, s, OH), 3.78 (1H, m, 3α-H), 4.22 (1H, d, *J*=5.8 Hz, OH), 4.51 (1H, d, *J*=4.3 Hz, OH); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) δ ppm 13.4, 16.2, 20.0, 21.4 ($CH_2$), 29.6, 31.0 ($CH_2$), 31.5 ($CH_2$), 32.0 ($CH_2$), 33.3 ($CH_2$), 35.3 ($CH_2$), 37.9 (C-10), 40.8 ($CH_2$), 44.8, 47.2 (C-13), 50.5, 65.6, 73.8, 74.3 (C-5), 220.0 (C-17); MS *m*/*z* (%): 321.3 (9) $[M-H]^+$, 293.2 (20), 280.4 (23), 265.5 (100), 250.2 (13), 90.3 (54).

*4.3.2. Sitostane-3β,5α,6β-triol (**14**).* Flash chromatography (chloroform/ethanol 20:1) afforded a pure white solid. Mp 244–245 °C (EtOH); lit.,[68] 242–245 °C; $[\alpha]_D^{20}$ +10.3 (*c* 0.39); IR (film) 3348, 2933, 2868, 1456, 1377, 1046, 956, 878 $cm^{-1}$; $^1H$ NMR (500 MHz, DMSO-$d_6$/$CDCl_3$) δ ppm 0.61 (3H, s, 18-$CH_3$), 0.78 and 0.80 (each 3H, 2 d, *J*=6.9 Hz, 26-$CH_3$ and 27-$CH_3$), 0.80 (3H, t, *J*=7.1, 7.1 Hz, $24^2$-$CH_3$), 0.87 (3H, d, *J*=6.5 Hz, 21-$CH_3$), 1.01 (3H, s, 19-$CH_3$), 3.29 (1H, m, 6α-H), 3.79 (1H, m, 3α-H); $^{13}C$ NMR (126 MHz, DMSO-$d_6$/$CDCl_3$) δ ppm 11.8, 12.0, 16.4, 18.6, 19.0, 19.8, 20.8($CH_2$), 22.7 ($CH_2$), 24.0 ($CH_2$), 25.6 ($CH_2$), 28.0 ($CH_2$), 28.8, 30.1, 31.1 ($CH_2$), 32.1 ($CH_2$), 33.4 ($CH_2$), 34.5 ($CH_2$), 35.7, 37.8 (C-10), 39.8 ($CH_2$), 40.9 ($CH_2$), 42.3 (C-13), 44.6, 45.2, 55.7, 55.9, 65.9, 74.2, 74.4 (C-5); MS *m*/*z* (%): 449.8 (100) $[M+H]^+$, 438.2 (39), 420.2 (20), 391.0 (36), 380.0 (69), 319.6 (33), 313.2 (26), 239.9 (27).

*4.3.3. Stigmast-22-ene-3β,5α,6β-triol (**15**).* Flash chromatography (chloroform/ethanol 20:1 to 10:1) afforded a pure white solid. Mp 252–253 °C (EtOH); lit.,[69] 256–257 °C; $[\alpha]_D^{20}$ −13.3 (*c* 0.30); IR (film) 3404, 2939, 2867, 1458, 1385, 1286, 1040, 962 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$/$CDCl_3$) δ ppm 0.63 (3H, s, 18-$CH_3$), 0.76 (3H, t, *J*=7.3 Hz, $24^2$-$CH_3$), 0.76 and 0.81 (each 3H, 2 d, *J*=6.5 Hz, 26-$CH_3$ and 27-$CH_3$), 0.97 (3H, d, *J*=6.5 Hz, 21-$CH_3$), 1.02 (3H, s, 19-$CH_3$), 3.29 (1H, m, 6α-H), 3.80 (1H, m, 3α-H), 4.98 and 5.12 (each 1H, 2 dd, *J*=15.1, 8.5 Hz, 22-H and 23-H); $^{13}C$ NMR (75 MHz, DMSO-$d_6$/$CDCl_3$) δ ppm 12.0 (2C), 16.2, 18.7, 20.6 ($CH_2$), 20.8, 20.9, 23.8 ($CH_2$), 24.8 ($CH_2$), 28.5 ($CH_2$), 29.9, 30.9 ($CH_2$), 31.3, 31.9 ($CH_2$), 34.3 ($CH_2$), 37.7 (C-10), 39.6 ($CH_2$), 40.0, 40.8 ($CH_2$), 42.0 (C-13), 44.5, 50.5, 55.4, 55.8, 65.7, 74.1, 74.3 (C-5), 128.5 and 138.1 (C-22 and C-23); MS *m*/*z* (%): 447.7 (42) $[M+H]^+$, 389.8 (77), 332.6 (32), 310.6 (100), 241.5 (51), 194.0 (45), 101.9 (62), 69.4 (59).

*4.3.4. 3β,5α,6β-Trihydroxypregnan-20-one (**16**).* Flash chromatography (chloroform/ethanol 10:1) afforded a pure white solid. Mp 259–260 °C (EtOH); lit.[70], 256–258 °C; $[\alpha]_D^{20}$ +46.2 (*c* 0.26); IR (film) 3386, 2937, 2872, 1693, 1471, 1356, 1147, 1072, 1039, 966, 966, 874, 751 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ ppm 0.51 (3H, s, 18-$CH_3$), 1.02 (3H, s, 19-$CH_3$), 2.04 (3H, s, 21-$CH_3$), 2.55 (1H, t, *J*=8.8 Hz, 17α-H), 3.31 (1H, m, 6α-H), 3.63 (1H, s, OH), 3.81 (1H, m, 3α-H), 4.18 (1H, d, *J*=5.5 Hz, OH), 4.38 (d, *J*=4.1 Hz, OH); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) δ ppm 13.2, 16.3, 20.8 ($CH_2$), 22.2 ($CH_2$), 24.1 ($CH_2$), 30.1, 31.1 ($CH_2$), 31.2, 32.1 ($CH_2$), 34.5 ($CH_2$), 37.8 (C-10), 38.5 ($CH_2$), 40.9 ($CH_2$), 43.7 (C-13), 44.5, 55.8, 62.8, 65.7, 74.0, 74.3 (C-5), 208.7 (C-20); MS *m*/*z* (%): 351.4 (64) $[M+H]^+$, 332.3 (30), 304.4 (94), 288.5 (100), 274.7 (37), 252.9 (63), 241.5 (68), 149.4 (37), 102.2 (57).

*4.3.5. 3β,5α,6β-Trihydroxyandrostane-17β-carboxylic acid (**17**).* Flash chromatography (chloroform/ethanol 9:1 to 5:1) afforded a pure white solid. Mp 247–248 °C (EtOH, thermal decomposition); $[\alpha]_D^{20}$ +7.1 (*c* 0.28); IR (film) 3395, 2938, 2872, 1703, 1292, 1070, 1040, 966, 874 $cm^{-1}$; $^1H$ NMR (400 MHz, acetone-$d_6$) δ ppm 0.72 (3H, s, 18-$CH_3$), 1.19 (3H, s, 19-$CH_3$), 2.37 (1H, t, *J*=9.3, 9.3 Hz, 17α-H), 2.89 (1H, s, OH), 3.27 (1H, br s, 6α-H), 3.53 (1H, br s, OH), 3.65 (1H, br s, OH), 4.02 (1H, m, 3α-H); $^1H$ NMR (400 MHz, DMSO-$d_6$) δ ppm 0.61 (3H, s, 18-$CH_3$), 1.02 (3H, s, 19-$CH_3$), 2.25 (1H, t, *J*=9.3 Hz, 17α-H), 3.66 (1H, s, OH), 3.80 (1H, m, 3α-H), 4.16 (1H, d, *J*=5.3 Hz, OH), 4.41 (1H, d, *J*=3.9 Hz, OH); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) δ ppm 13.3, 16.3, 20.6 ($CH_2$), 23.3 ($CH_2$), 24.2 ($CH_2$), 30.2, 31.0 ($CH_2$), 32.0 ($CH_2$), 34.5 ($CH_2$), 37.8 (C-10), 38.2 ($CH_2$), 40.8 ($CH_2$), 43.4 (C-13), 44.6, 54.8, 55.2, 65.7, 74.0, 74.3 (C-5), 175.1 (C-20); MS *m*/*z* (%): 353.9 (30) $[M+H]^+$, 303.3 (21), 268.7 (15), 241.0 (15), 164.4 (19), 149.6 (29), 145.7 (18), 102.7 (100), 100.6 (26); HRMS (ESI), positive mode, *m*/*z* $[M+Na]^+$ calcd for $C_{20}H_{32}NaO_5$: 375.2142, found: 375.2145.

*4.3.6. 3β,5α,6β-Trihydroxypregn-16-en-20-one (**18**).* Flash chromatography (chloroform/ethanol 10:1 to 6:1) afforded a pure white solid. Mp 243–245 °C (EtOH, thermal decomposition); lit.,[71] 248–250 °C; $[\alpha]_D^{20}$ +15 (*c* 0.40); IR (film) 3427, 3053, 2937, 1649, 1582, 1372, 1235, 1152, 1077, 1035, 959, 874 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$) δ ppm 0.80 (3H, s, 18-$CH_3$), 1.05 (3H, s, 19-$CH_3$), 2.20 (3H, s, 21-$CH_3$), 3.33 (1H, m, 6α-H), 3.70 (1H, s, OH), 3.80 (1H, m, 3α-H), 4.19 (1H, d, *J*=5.8 Hz, OH), 4.49 (1H, d, *J*=4.3 Hz, OH), 6.86 (1H, dd,

$J$=3.3, 1.8 Hz, 16-H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ ppm 15.8, 16.0, 20.4 ($CH_2$), 27.0, 28.3, 31.0 ($CH_2$), 31.8 ($CH_2$), 31.8 ($CH_2$), 34.3 ($CH_2$), 34.6 ($CH_2$), 38.0 (C-10), 40.8 ($CH_2$), 44.9, 45.8 (C-13), 55.7, 65.6, 74.0, 74.4, 145.3 (C-16), 154.4 (C-17), 196.2 (C-20); MS $m/z$ (%): 349.4 (15) $[M+H]^+$, 302.9 (96), 239.0 (54), 216.9 (42), 156.0 (45), 102.2 (100).

*4.3.7. 3β,5α,6β,16α-Tetrahydroxypregnan-20-one (**19**).* Flash chromatography (chloroform/ethanol 9:1 to 3:1) afforded a white solid. Mp 244–246 °C (AcOEt/EtOH, thermal decomposition); lit.,[64] 232–235 °C; $[\alpha]_D^{20}$ +20 ($c$ 0.25); IR (film) 3384, 2938, 2867, 1695, 1157, 1086, 1042, 1018, 961, 875 cm$^{-1}$; $^1$H NMR (400 MHz, acetone-$d_6$) $\delta$ ppm 0.62 (3H, s, 18-$CH_3$), 0.70 (1H, t, $J$=38.5, 38.5 Hz), 1.17 (3H, s, 19-$CH_3$), 2.10 (3H, s, 21-$CH_3$), 2.53 (1H, d, $J$=6.3 Hz, 17α-H), 2.92 (1H, s, OH), 3.28 (1H, d, $J$=4.3 Hz, OH), 3.54 (1H, m, 6α-H), 3.66 (1H, d, $J$=4.1 Hz, OH), 3.73 (1H, d, $J$=4.8 Hz, OH), 4.03 (1H, m, 3α-H), 4.68 (1H, m, 16β-H); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 0.51 (3H, s, 18-$CH_3$), 1.01 (3H, s, 19-$CH_3$), 2.07 (3H, s, 21-$CH_3$), 2.43 (1H, d, $J$=6.3 Hz, 17α-H), 3.31 (1H, m, 6α-H), 3.69 (1H, s, OH), 3.80 (1H, m, 3α-H), 4.18 (1H, d, $J$=4.2 Hz, OH), 4.42 (1H, d, $J$=4.3 Hz, OH), 4.50 (1H, t, $J$=6.3 Hz, 16β-H); 4.63 (1H, d, $J$=5.0 Hz, OH); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 14.3, 16.1, 20.3 ($CH_2$), 29.5, 31.0 ($CH_2$), 31.7, 31.8 ($CH_2$), 34.3 ($CH_2$), 35.6 ($CH_2$), 37.8 (C-10), 38.5 ($CH_2$), 40.8 ($CH_2$), 44.5, 44.6 (C-13), 53.4, 65.6, 70.4, 73.2, 73.9, 74.2 (C-5), 208.1 (C=O); MS $m/z$ (%): 365.5 (4) $[M-H]^+$, 293.5 (4), 265.8 (10), 165.2 (100), 121.2 (13), 111.2 (13); HRMS (ESI), positive mode, $m/z$ $[M+Na]^+$ calcd for $C_{21}H_{34}NaO_5$: 389.2298, found: 389.2305.

*4.3.8. 16α,17α-Epoxy-20-oxo-3β,5α,6β-trihydroxypregnan-21-yl acetate (**20**).* Flash chromatography (chloroform/ethanol 10:1 to 5:1) afforded a pure white solid. Mp 214–216 °C (EtOH); $[\alpha]_D^{20}$ +28.6 ($c$ 0.28); IR (film) 3399, 2940, 2867, 1745, 1719, 1381, 1231, 1152, 1053, 1007, 959, 874, 832 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 0.98 (3H, s, 18-$CH_3$), 1.03 (3H, s, 19-$CH_3$), 2.07 (3H, s, $CH_3COO$), 3.31 (1H, m, 6α-H), 3.69 (1H, s, OH), 3.79 (1H, m, 3α-H), 4.04 (1H, s, 16β-H), 4.17 (1H, d, $J$=5.7 Hz, OH), 4.46 (d, $J$=4.1 Hz, OH), 4.64 and 4.76 (each 1H, 2 d, $J$=17.4 Hz, 21-Ha and 21-Hb); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ ppm 14.9, 16.0, 20.0 ($CH_2$), 20.2, 27.0 ($CH_2$), 27.4, 30.9 ($CH_2$), 31.3 ($CH_2$), 31.8 ($CH_2$), 34.0 ($CH_2$), 37.9 (C-10), 40.7 ($CH_2$), 42.0 (C-13), 44.0, 44.9, 60.9, 65.5 ($CH_2$), 65.6, 69.6 (C), 73.9, 74.3 (C), 169.6 ($CH_3COO$), 199.3 (C=O); MS $m/z$ (%): 421.6 (6) $[M-H]^+$, 388.9 (8), 353.7 (7), 339.4 (6), 325.8 (7), 311.9 (7), 307.7 (7), 293.6 (47), 265.9 (97), 149.5 (100), 146.8 (12); HRMS (ESI), positive mode, $m/z$ $[M+Na]^+$ calcd for $C_{23}H_{34}NaO_7$: 445.2197, found: 445.2196.

## Acknowledgements

We thank Fundação para a Ciência e Tecnologia (FCT), Portugal, through FEDER/POCI 2010 for financial support. We acknowledge the 'Laboratório de Espectrometria de Massa do Centro de Estudos Farmacêuticos da Universidade de Coimbra', Portugal, for providing data from the LC-MS$^n$ (QIT-MS), integrated in the Portuguese National Mass Spectrometry Network (RNEM). J.F.S.C. also thanks Fundação para a Ciência e Tecnologia (FCT), Portugal, for a grant (SFRH/BD/18263/2004). Thanks are due to Fátima Nunes, from the Mass Spectrometry Lab, University of Coimbra (LEM at CEF-UC).

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2010.01.089.

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