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ORIGINAL PAPER

# An improved and efficient synthesis for IPL576,092 and its analogues

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**Abstract** An improved and efficient preparation of IPL576,092 was developed. The synthetic route involves selective allylic oxidation of $\Delta^5$-sterols and subsequent hydroboration–oxidation as the key steps. In addition, two analogues were readily synthesized from commercially available steroidal compounds in two steps in the same way.

**Keywords** IPL576,092 · Allylic oxidation · Hydroborations · α,β-unsaturated ketones · Steroids

## Introduction

IPL576,092 (**1**), a potent anti-inflammatory agent, was identified from a novel group of polyhydroxylated sterols based on the marine natural product contignasterol [1]. Currently in phase II human clinical trials, IPL576,092 displays a biological activity profile which justifies the continuation of its evaluation as a potential new treatment for asthma (Fig. 1) [2–4].

However, to the best of our knowledge, there have been only a few reports concerning the synthesis of IPL576,092 (**1**) and its analogues. In 2002, Burgoyne et al. [2] first reported the structure and preparation of IPL576,092 from dehydroepiandrosterone (DHEA) in nine steps in 25 % overall yield. In 2003, Filippo et al. [5] also accomplished the synthesis of IPL576,092 used for the research of a transmembrane ionophore by a similar method in 11 steps in 22 % overall yield. Almost the only route available for the preparation of these trihydroxysteroids (e.g., 5α-androstan-17-one-3β,6α,7β-triol **4**) starts from DHEA. First the 3β-hydroxyl group of **5** has to be protected (usually as the acetate or TBDMS ether) otherwise it would be oxidized to a ketone group during the subsequent reaction. In the second and third step, allylic oxidation at C7 afforded the α,β-unsaturated steroid **7**, which was reduced under Luche conditions to give the 7β-alcohol **8** as a single detectable isomer. In the fourth step, a highly stereoselective hydroboration–oxidation gave the 6α,7β-diol **9**. Finally, **4** was obtained by protection group removal with aqueous AcOH (Fig. 2a). Preparation of IPL576,092 (**1**) from **4** needs another three-step reaction (Scheme 1).

In 2004, Burgoyne et al. [6] studied synthesis and biological activities of analogues of IPL576,092. Compound **2** was synthesized from cholesterol acetate in four steps by a traditional method and compound **3** was obtained by catalytic hydrogenation of the C17–C20 double bond of compound **1**. The study showed that 5α-cholestane-3β,6α,7β-triol (**2**) and 5α-pregnane-3β,6α,7β-triol (**3**) have good anti-inflammatory activity (Scheme 2).

We wish to report herein an improved and efficient route for the synthesis of structural analogues of IPL576,092 (**1**). The synthetic route developed involves selective allylic oxidation of $\Delta^5$-sterols and subsequent hydroboration–oxidation as the key steps (Fig. 2b).

## Results and discussion

The procedures for the successful synthesis of 7-keto-$\Delta^5$-cholesterol from cholesterol **12** in acceptable yields by using $CrO_3$/*N*-hydroxyphthalimide (NHPI)-activated clay oxidizing system, in which the 3β-hydroxyl group remained

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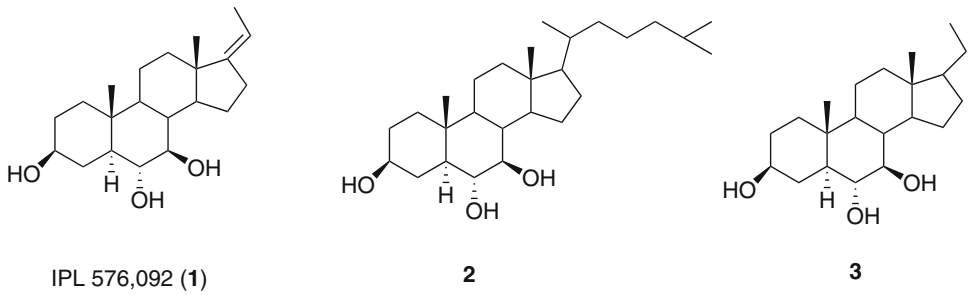



**Fig. 1** IPL576,092 and its analogues

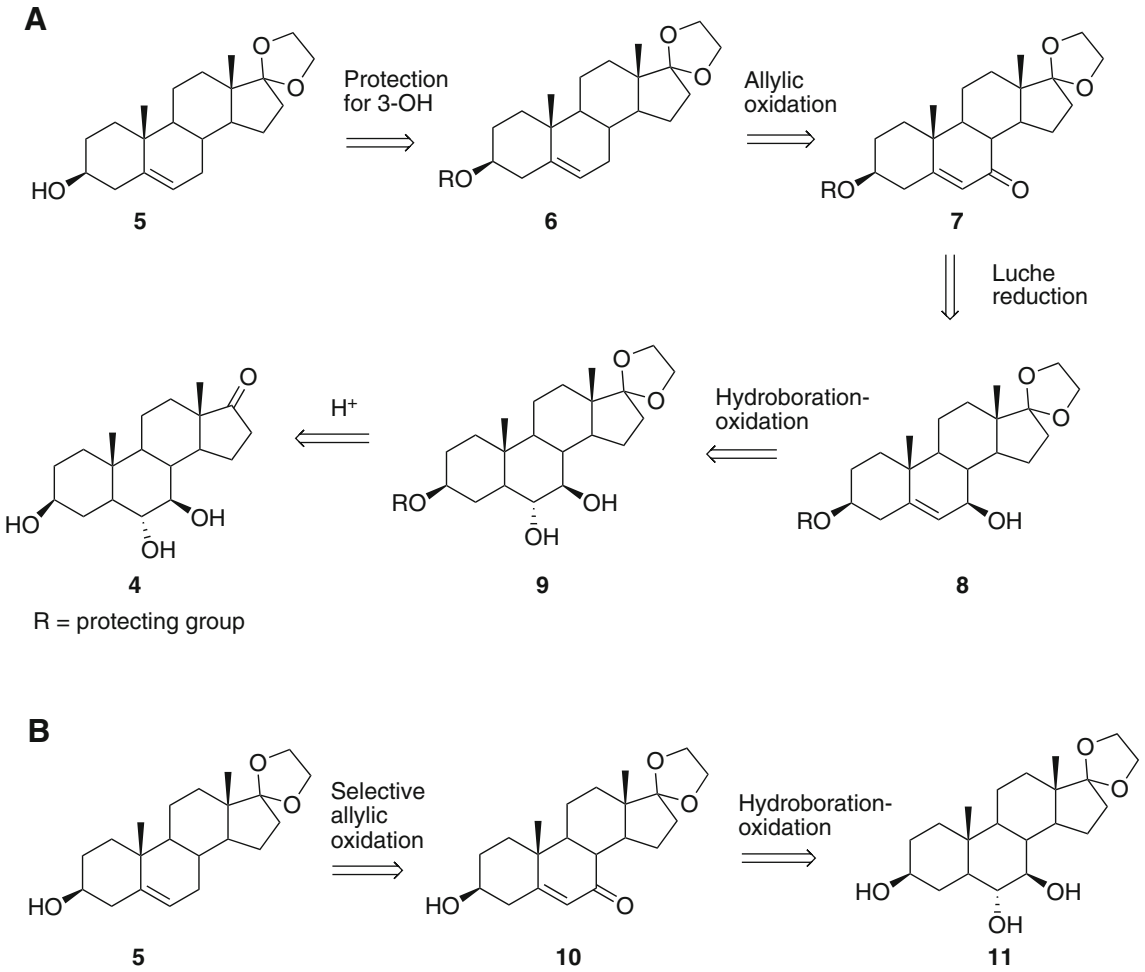
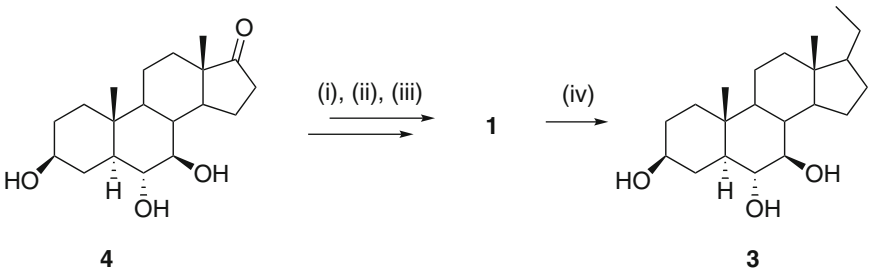



**Fig. 2** Preparation of trihydroxysteroids. **a** Traditional route; **b** improved route

unaffected, are described in our previous work [7]. Considering that 7-keto-$\Delta^5$-cholesterol **13** possesses a flat and rigid molecular structure, we explored the hydroboration of the $\alpha,\beta$-unsaturated ketone of ring B of this compound and subsequent oxidation with $NaBO_3$. In this way, compound **5** was obtained directly in a one-step process (Scheme 2). Structural identification revealed that the complex resonance at $\delta = 3.58$ ppm in the $^1$H NMR spectrum assigned to the hydrogen attached to C3 is consistent with the 3$\beta$-OH configuration. The coupling constants for the resonances in the $^1$H NMR spectrum of compound **2** assigned to H6 ($\delta = 3.25$ ppm, dd, $J = 8.9$, 10.4 Hz) and H7 (3.11 ppm, dd, $J = 9.1$, 8.8 Hz) are consistent with *trans* diaxial relationships between H5 and H6, H6 and H7, and H7 and H8. Thus, the configuration of the hydroxyl groups at C6 and C7 can be assigned as $\alpha$ and $\beta$, respectively.

Therefore, we have found an efficient and short route for the preparation of structural analogues of IPL576,092. The synthesis of compounds **2** and **3** share a common route, as detailed in Scheme 2. Cholesterol **12** and

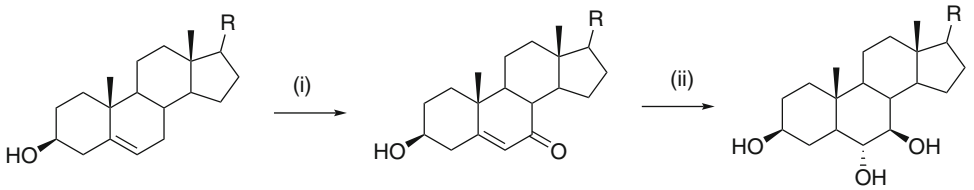
**Scheme 1**

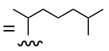



**4** **3**

(i) 2,2-Dimethoxypropane, TsOH, RT ; (ii) $EtPPh_3Br$, *t*-BuOK, toluene; (iii) HOAc, $H_2O$; (iv) Pd/C

**Scheme 2**




**12** R = 

**14** R= $C_2H_5$

**13** **15**

**2** **3**

(i) $CrO_3$/activated clay/$CH_2Cl_2$, RT; (ii) $NaBH_4/BF_3{\cdot}OEt_2$-$NaBO_3$, 0 °C

5-pregnene-3$\beta$-ol (**14**, prepared from pregnenolone) underwent selective allylic oxidation with the $CrO_3$/NHPI-activated clay system [7] to afford compound **13** (45 % yield) and **15** (52 % yield), respectively (it is noteworthy that oxidation of compound **6** under these conditions cleaved the acetal protecting group). Hydroboration of the cyclic $\alpha,\beta$-unsaturated ketone of compound **13** and subsequent oxidation with $NaBO_3$ yielded 5$\alpha$-cholestane-3$\beta$,6$\alpha$,7$\beta$-triol (**2**, 69 % yield). 5$\alpha$-Pregnane-3$\beta$,6$\alpha$,7$\beta$-triol (**3**, 71 % yield) was synthesized in the same way from compound **15**. The coupling constant between H-6 and H-7 in the $^1$H NMR spectrum of **3** also indicated that these hydrogens have a *trans* diaxal relationship and compound **3** has a 6$\alpha$,7$\beta$-diol configuration which is consistent with that in compound **2**.

IPL576,092 (**1**) was also prepared by the improved method. 5-Androsten-3$\beta$-ol-17-one ethyleneketal (**5**) was easily obtained by treating DHEA with ethylene glycol in the presence of *p*-toluenesulfonic acid. Selective allylic oxidation of compound **5**, i.e., in the presence of the sensitive 3$\beta$-hydroxyl group, by the NHPI/$O_2$/$Bz_2O_2$-$CuCl_2$ system [8] afforded the corresponding 7-keto-$\Delta^5$-steroid **10** in 67 % yield. Hydroboration–oxidation of **10** with $BF_3{\cdot}OEt_2$/$NaBH_4$-$NaBO_3$ system resulted in **11** and then treatment with 80 % acetic acid over 3 h gave key intermediate **4** in 67 % yield over two steps. IPL576,092 (**1**) was synthesized according to literature from **12** in three steps (Fig. 2b; Scheme 1).

In summary, we described an improved and efficient route for the synthesis of structural analogues of IPL576,092. The synthetic route developed involves selective allylic oxidation of $\Delta^5$-sterols and subsequent hydroboration–oxidation as the key steps. 5-Cholestane-3$\beta$,6$\alpha$,7$\beta$-triol (**2**) was synthesized from cholesterol in two steps in 40 % overall yield, 5$\alpha$-pregnane-3$\beta$,6$\alpha$,7$\beta$-triol (**3**) was synthesized from 5-pregnene-3$\beta$-ol in two steps in 39 % overall yield, and IPL576,092 (**1**) was synthesized from DHEA in seven steps in 27 % overall yields. This protocol provides an alternative method for application in combinatorial and parallel syntheses in drug discovery. In addition, to the best of our knowledge, $^1$H and $^{13}$C NMR data for compounds **2** and **3** have not been reported before.

## Experimental

$^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker DRX-500 spectrometer. IR spectra were recorded on Thermo Nicolet Avatar 360 FTIR. MS data were recorded on a Finnigan MAT 90 spectrometer at an ionization potential of 70 eV. Field-desorption (FD) mass spectra were recorded on a VG ZAB 2-SE-FPD spectrometer.

Analytical thin-layer chromatography was performed on Qingdao Haiyang silica gel GF254 plates, and flash chromatography was performed on 200–300 mesh silica gel. THF and toluene were dried by distillation over Na/benzophenone under a nitrogen atmosphere. Dehydroepiandrosterone and pregnenolone were obtained from Yongsheng Yinghua Phytochemical Industry Group Co, Ltd. Activated clay was obtained from Yunnan Jianshan Runtu Co, Ltd. Unless otherwise noted, all other starting materials and solvents were obtained from commercial suppliers and used without further purification.

*5-Androsten-7,17-dione-3β-ol 17-ethyleneketal* (**10**, $C_{21}H_{30}O_4$)

Compound **10** was prepared by a method based on a patent by Foricher et al. [8]. NHPI (3.0 g, 18.4 mmol) and 6.0 g 5-androsten-3$\beta$-ol-17-one ethyleneketal (**5**, 18.1 mmol; prepared from DHEA) were added to 200 $cm^3$ of a mixture of acetone and ethyl acetate (1:1). The mixture was stirred and warmed to 55 °C, and then 15 mg dibenzoyl peroxide ($Bz_2O_2$) was added and air was vigorously bubbled into the mixture at 55 °C for 48 h. After completion of the reaction (TLC control), 100 $cm^3$ cyclohexane was added, the mixture was cooled to RT, NHPI was filtered off, and the filtrate was washed with saturated aqueous $Na_2CO_3$ solution repeatedly until no orange color was observed. The organic layer was washed with saturated aqueous NaCl solution (3 × 100 $cm^3$), dried over $MgSO_4$, and the solvent was evaporated in vacuo. The resulting residue was added to 150 $cm^3$ pyridine, cooled to 0 °C, and $CuCl_2$ was added under stirring. Stirring was continued overnight and the mixture was then warmed to RT. The solvent was evaporated in vacuo and the residue was poured into 150 $cm^3$ $CH_2Cl_2$ and washed with saturated aqueous NaCl solution (3 × 100 $cm^3$). The organic layer was dried over $MgSO_4$ and the solvent was evaporated in vacuo. Purification of the product was done by column chromatography (eluent petroleum/ethyl acetate 3:1) to give enone **10** as a white solid (4.2 g, 67 % yield). M.p.: 198–200 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 5.69 (s, 1H, 6-H), 3.94–3.86 (m, 4H, –$OCH_2CH_2O$–), 3.52 (m, 1H, 3-H), 0.95 (s, 3H, 19-H), 0.86 (s, 3H, 18-H) ppm; $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 201.6 (C-7), 165.6 (C-17), 126.0 (C-6), 118.6 (C-5), 70.4 (C-3), 65.2, 64.5 (–$OCH_2CH_2O$–), 50.0, 46.2 (C-13), 45.4, 44.4, 41.9 (C-4), 38.3 (C-10), 36.4, 34.2, 31.2, 29.7, 25.1, 20.7, 17.4 (C-19), 14.4 (C-18) ppm; IR (KBr): $\overline{\nu}$ = 3,448, 1,666, 1,058 $cm^{-1}$; EI–MS (70 eV): *m/z* (%) = 346 ($M^+$, 5), 331 (7), 247 (6), 161 (4), 100 (52), 99 (100), 86 (15), 79 (5), 55 (4).

*5-Androsten-17-one-3β,6α,7β-triol ethyleneketal* (**11**, $C_{21}H_{34}O_5$)

5-Androsten-7,17-dione-3$\beta$-ol 17-ethyleneketal (**10**, 3.00 g, 9 mmol) and 2.40 g $NaBH_4$ (63 mmol) were dissolved in 100 $cm^3$ dry THF at RT under $N_2$. The slurry was cooled to 0 °C, and 4.6 $cm^3$ $BF_3{\cdot}OEt_2$ (36 mmol) in 10 $cm^3$ dry THF was slowly added, maintaining the temperature of the mixture below 0 °C for 1 h, then the mixture was warmed to RT. After 5 h 40 $cm^3$ $H_2O$ was slowly added, then 2.80 g $NaBO_3{\cdot}4H_2O$ (18 mmol) was added portionwise. The mixture was stirred at RT overnight. $CH_2Cl_2$ (100 $cm^3$) was added, the organic layer was separated and washed with saturated aqueous NaCl solution (3 × 100 $cm^3$), dried over $MgSO_4$, and the solvent was removed in vacuo. The obtained crude **11** (2.94 g) was used directly for the next step without further purification.

*5α-Androstan-17-one-3β,6α,7β-triol* (**4**, $C_{19}H_{30}O_4$)

Crude **11** (2.90 g) was treated with 120 $cm^3$ 80 % acetic acid over 3 h at RT. The mixture was then concentrated in vacuo and azeotroped to dryness with methanol (2 × 20 $cm^3$). Purification of the product was done by column chromatography (eluent ethyl acetate) to give triol **4** as a white solid (1.82 g). The yield for the conversion of **11** to **4** was 67 %. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 3.54 (m, 1H, 3-H), 3.38 (dd, $J$ = 8.6, 10.6 Hz, 1H, 6-H), 3.20 (dd, $J$ = 9.7, 9.2 Hz, 1H, 7-H), 2.47 (m, 1H), 0.97 (s, 3H, 19-H), 0.95 (s, 3H, 18-H) ppm; $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 224.7 (C-17), 81.5 (C-7), 76.3 (C-6), 72.2 (C-3), 54.0, 53.2, 49.3, 48.9 (C-13), 42.4, 39.0 (C-16), 37.3, 37.2 (C-10), 33.6, 33.2, 32.3, 26.2, 22.3, 14.9 (C-19), 14.3 (C-18) ppm.

*5α-Progest-17(20)-ene-3β,6α,7β-triol* (**1**)

This compound was prepared according to the literature procedure [2]; the overall yield for the conversion of **4** to **1** was 61 %. M.p.: 182–183 °C (Ref. [2] 183–184 °C). $^1$H NMR and $^{13}$C NMR spectra were found to be identical to those in the literature [2].

*5-Pregnene-7-one-3β-ol* (**15**, $C_{21}H_{32}O_2$)

To a solution of 1.50 g 5α-pregnene-3$\beta$-ol (**14**, 5 mmol; prepared from pregnenolone [9]) in 120 $cm^3$ $CH_2Cl_2$, 5.80 g of the $CrO_3$/NHPI-activated clay supported oxidant [7] ($CrO_3$ content 8 mmol) was added at RT under stirring. After 10–24 h, an additional amount of 5.80 g $CrO_3$/NHPI-activated clay supported oxidant ($CrO_3$ content 8 mmol) was added portionwise during a period of 10 h and stirring was continued until the reaction was completed (TLC control, about 24 h). The mixture was filtered through a bed of activated clay and washed with dichloromethane. The combined filtrate was washed repeatedly with saturated aqueous $Na_2CO_3$ solution until no orange color was observed in the organic layer. The organic layers were then washed with saturated aqueous NaCl solution (3 × 100 $cm^3$) and water (3 × 100 $cm^3$) and dried over $MgSO_4$; the solvent was removed in vacuo. Purification of the product was done by column chromatography (eluent

petroleum/ethyl acetate 3:1) to give **15** (0.87 g, 55 %). M.p.: 192–194 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 5.70$ (s, 1H, 6-H), 3.68 (m, 1H, 3-H), 1.20 (s, 3H, 19-H), 0.88 (m, 3H, 21-H), 0.57 (s, 3H, 18-H) ppm; $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta = 203.2$ (C-7), 166.3 (C-5), 127.0 (C-6), 71.4 (C-3), 52.7, 51.3, 50.4, 46.4 (C-17), 43.6 (C-13), 42.8, 39.3 (C-10), 37.8, 37.3, 32.1, 29.2, 27.6, 23.8, 22.0, 18.2 (C-21), 14.1 (C-19), 13.3 (C-18) ppm; IR (KBr): $\overline{v} = 3{,}430$, 1,673, 1,069 cm$^{-1}$; MS (TOF$^+$): $m/z = 339$ ($[M + Na]^+$).

*5α-Pregnane-3β,6α,7β-triol* (**3**, $C_{21}H_{36}O_3$)

Compound **3** was prepared from **15** according to the procedure as described for **11**, yield 71 %. M.p.: 228–230 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 3.50$ (m, 1H, 3-H), 3.14 (dd, $J = 9.45, 10.0$ Hz, 1H, 6-H), 2.99 (dd, $J = 9.3$, 9.1 Hz, 1H, 7-H), 2.18 (m, 1H), 0.93 (m, 3H, 21-H), 0.90 (s, 3H, 19-H), 0.63 (s, 3H, 18-H) ppm; $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta = 82.1$ (C-7), 76.4 (C-6), 72.3 (C-3), 57.4, 54.4, 54.2, 49.4, 44.5 (C-13), 42.7, 39.7, 39.1, 37.2 (C-10), 33.7, 33.3, 29.9, 28.6, 24.6, 22.8, 14.4 (C-21), 14.2 (C-19), 13.6 (C-18) ppm; MS (TOF$^+$): $m/z = 359$ ($[M + Na]^+$).

*5-Cholesten-7-one-3β-ol* (**13**, $C_{27}H_{44}O_2$)

Compound **13** was prepared from cholesterol **12** according to the procedure as described for **15**, yield 58 %. M.p.: 166–167 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 5.69$ (s, 1H, 6-H), 3.68 (m, 1H, 3-H), 1.23 (s, 3H, 18-H), 0.88 (d, 3H, 21-H), 0.85 (m, 6H, 26-H, 27-H), 0.68 (s, 3H, 18-H) ppm; $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta = 202.6$ (C-7), 167.2 (C-5), 125.8 (C-6), 70.2 (C-3), 54.8, 50.0, 45.4, 43.1 (C-13), 41.9 (C-4), 39.5, 38.7, 38.3 (C-10), 36.4, 36.2, 35.7, 31.0, 28.5, 28.0, 26.3, 23.9, 22.8 (C-26), 22.6 (C-27), 21.2 (C-11), 18.9 (C-21), 17.3 (C-19), 12.0 (C-18) ppm; IR (KBr): $\overline{v} = 3{,}519$, 1,664, 1,061 cm$^{-1}$; EI–MS (70 eV): $m/z$ (%) = 400 (M$^+$, 59), 367 (30), 287 (25), 245 (16), 205 (31), 192 (86), 161 (100), 135 (80), 107 (48), 91 (42), 81 (32), 67 (19), 55 (20).

*5α-Cholestane-3β,6α,7β-triol* (**2**, $C_{27}H_{48}O_3$)

This compound was prepared from **13** according to the procedure as described for the synthesis of compound **11**, yield 69 %. M.p.: 224–225 °C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 3.58$ (m, 1H,3-H), 3.25 (dd, $J = 8.9$, 10.4 Hz, 1H,6-H), 3.11 (dd, $J = 9.1$, 8.8 Hz, 1H, 7-H), 2.18 (m, 1H), 2.02 (m, 1H), 0.96 (d, 3H, 21-H), 0.90 (m, 6H, 26-H, 27-H), 0.82 (s, 3H, 19-H), 0.71 (s, 3H, 18-H) ppm; $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta = 81.9$ (C-7), 75.6 (C-6), 72.0 (C-3), 56.8, 56.3, 53.2, 48.7, 44.5 (C-13), 42.2, 40.8, 40.4, 38.3, 37.2, 36.6, 36.5 (C-10), 33.3, 31.9, 29.4, 28.8, 27.7, 24.8, 23.5 (C-27), 23.3 (C-26), 22.2 (C-11), 19.7 (C-21), 14.5 (C-19), 13.0 (C-18); MS (TOF$^+$): $m/z = 443$ ($[M + Na]^+$).

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