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Synthesis of (20*S*)-[7,7,21,21-$^2H_4$]-3β-(*tert*-butyldimethylsilanyloxy)-20-methyl-pregn-5-en-21-ol, a useful intermediate for the preparation of deuterated isotopomers of sterols☆

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Abstract

(20*S*)-[7,7,21,21-$^2H_4$]-3β-(*tert*-Butyldimethylsilanyloxy)-20-methyl-pregn-5-en-21-ol, an intermediate for the preparation of deuterated isotopomers of sterols to be used as standards for biomedical studies, was prepared by reduction with dichloroaluminum deuteride of ethyl (20*S*)-3β-(*tert*-butyldimethylsilanyloxy)-7-oxo-pregn-5-en-20-carboxylate. Using controlled experimental conditions, it has also been shown that the dichloroaluminum hydride reduction of a 7-keto steroid affords the corresponding 7β-hydroxy derivative in a highly stereoselective manner.

Keywords: Deuterated isotopomers; Deuterated sterols; (20*S*)-[7,7,21,21-$^2H_4$]-3β-(*tert*-Butyldimethylsilanyloxy)-20-methyl-pregn-5-en-21-ol; Dichloroaluminum deuteride; 7β-Hydroxysterols

1. Introduction

The synthesis of multideuterated sterols is highly needed to achieve the most accurate gas chromatography–mass spectrometry (GC–MS) analysis of biologically relevant steroids. This can be useful, for example in bile acids biosynthesis for metabolites such as 24-, 25- or 27-hydroxycholesterols [1]. In connection with studies related to the evaluation of serum 27-hydroxycholesterol levels in patients with primary biliary cirrhosis [2], it became necessary to prepare deuterated 27-hydroxycholesterol for intravenous infusions aimed to evaluate the 27-hydroxylation pathway in bile acid production [3]. Syntheses of deuterated 27-hydroxycholesterol (**1**) from two isoprenoids, kryptogenin (**2**) and diosgenin (**3**), for biomedical use have been already described [4,5] (Fig. 1). However, these preparations suffer of several disadvantages, such as the very limited availability of kryptogenin (**2**) and yields from both **2** and **3** that are not reproducible or low in general. Furthermore, starting from **2**, the deuterium incorporation is not easy to control, since the labeled 27-hydroxycholesterol **1** prepared by Clemmensen reduction of **2** contained five to nine deuterium atoms [4].

We, therefore, designed a more versatile approach to the synthesis of deuterated isotopomers of 27-hydroxycholesterol, that could be extended to any other labeled sterol with a functionalised side chain. The synthesis should proceed via a deuterated C-22 steroid suitably protected at 3β position that becomes a versatile intermediate for the construction of any required side chain. (20*S*)-[7,7,21,21-$^2H_4$]-20-Methyl-pregn-5-en-3β,21-diol (**4a**) fulfills all the above requirements as one of the possible deuterated C-22 steroid intermediates for the synthesis of labeled C-27 sterols [6] (Fig. 2).

2. Experimental

2.1. General

Melting points were recorded on Stuart Scientific SMP3 instrument and are uncorrected. All reagents were purchased from Sigma Chemical Co. (USA). Tetrahydrofuran (THF) and diethyl ether were distilled from sodium/benzophenone. Cholenic acid (**5**) was purchased from Steraloids (USA).

☆ Dedicated to the memory of Dr. Eliahu Caspi, Principal Scientist at The Worcester Foundation for Experimental Biology (Mass., USA).

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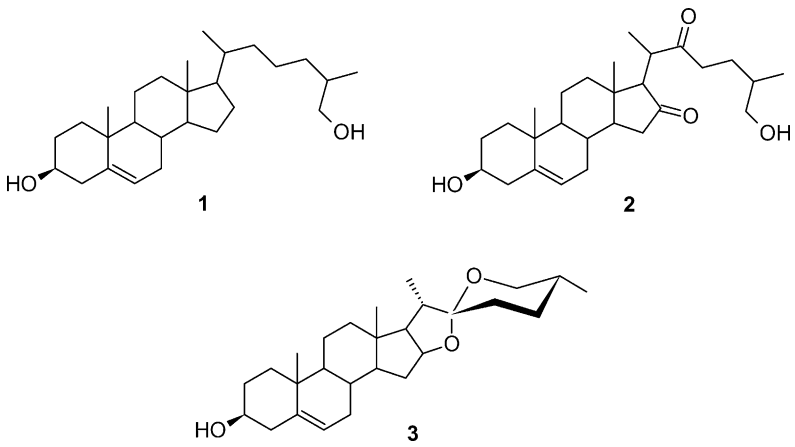


Fig. 1. Structure of 27-hydroxycholesterol **1**, kryptogenin **2** and diosgenin **3**.

All reactions were monitored by TLC on Silica Gel 60 F_{254} precoated plates with a fluorescent indicator (Merck).

Flash chromatography [7] was performed using Silica Gel 60 (230–400 mesh, Merck).

Mass spectra were recorded on a particle beam quadrupolar mass spectrometer Hewlett-Packard 5988A spectrometer equipped with an interface PB 5998A and a low pressure HPLC HP1050. The mass spectrometric analysis was performed in positive ion chemical ionization (PICI) with CH_4 as chemical reactant gas at an electron energy of 240 eV and with a source temperature of 250 °C. The particle beam desolvation chamber temperature was 50 °C, the helium pressure was 50 psi and the source pressure 0.9 Torr. Mass spectra were acquired over 50–540 mass unit ranges at 0.78 scan s^{-1}. Mass spectral data are given as *m*/*z* (relative abundance).

2.2. NMR experiments

All NMR spectra were recorded on a Bruker AM-500 spectrometer operating at 500.13 and 125.76 MHz for ^1H and ^{13}C, respectively, in $CDCl_3$ solutions. The sample temperature was 303 K. Chemical shifts are reported as δ (ppm) relative to $CHCl_3$ fixed at 7.24 ppm for ^1H-NMR spectra and relative to $CDCl_3$ fixed at 77.00 ppm for ^{13}C-NMR. Coupling constants (*J*) are given in Hz and the ^1H signals were assigned by ^1H-homodecoupling and COSY experiments.

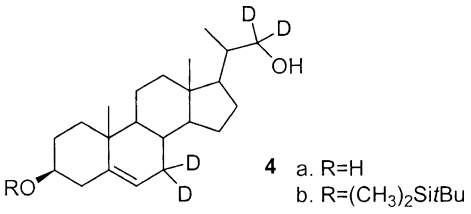


Fig. 2. Structure of (20*S*)-[7,7,21,21-$^2H_4$]-20-methyl-pregn-5-en-3β,21-diol (**4a**) and (20*S*)-[7,7,21,21-$^2H_4$]-3β-(*tert*-butyldimethylsilanyloxy)-20-methyl-pregn-5-en-21-ol (**4b**).

The multiplicity of signals as doublets is indicated in the text as d. The complete ^{13}C signal assignments were made using DEPT experiments for the unequivocal identification of primary, secondary, tertiary and quaternary carbon atoms and using two-dimensional techniques, such as HSQC.

2.3. Ethyl (20S)-3β-(tert-butyldimethylsilanyloxy)-pregn-5-en-20-carboxylate (6)

A mixture of **5** (2 g, 5.7 mmol), absolute ethanol (100 ml) and 96% sulphuric acid (5 ml) was refluxed for 10 h. After cooling, the mixture was partially evaporated, neutralized with a saturated solution of $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 50 ml). The organic layer was dried (Na_2SO_4) and evaporated. The residue (2.08 g, 98%) was sufficiently pure by TLC (hexane/EtOAc 6:4) to be used in the next step without purification.

To a solution of the above ester in THF (60 ml), imidazole (990 mg; 14.5 mmol) and *tert*-butyldimethylchlorosilane (1.0 g; 6.6 mmol) were sequentially added. After 12 h under continuous stirring at room temperature, the mixture was poured into water and extracted with CH_2Cl_2 (3 × 50 ml). The organic phase was dried over Na_2SO_4 and evaporated at reduced pressure. The product was purified by flash chromatography (hexane/EtOAc 95:5) to give compound **6** as a white solid (2.5 g, 95%). Mp 129–130 °C (from hexane); selected ^1H-NMR signals: δ 0.03 (6H, s, $Si(CH_3)_2$), 0.66 (3H, s, 18-CH_3), 0.86 (9H, s, $SiC(CH_3)_3$), 0.97 (3H, s, 19-CH_3), 1.15 (3H, d, J = 7.0 Hz, 21-CH_3), 1.22 (3H, t, J = 7.0 Hz, $COOCH_2CH_3$), 2.13 (1H, ddd, J = 2.8, 4.9 and 13.3 Hz, 4α-H), 2.23 (1H, ddddd, J = 2.1, 2.1, 2.1, 11.2 and 13.3 Hz, 4β-H), 2.37 (1H, dq, J = 7.0 and 9.8 Hz, 20-H), 3.45 (1H, dddd, J = 4.9, 4.9, 11.2 and 11.2 Hz, 3α-H), 4.08 (2H, q, J = 7.0 Hz, $COOCH_2CH_3$), 5.28 (1H, ddd, J = 2.1, 2.1 and 4.9 Hz, 6-H). ^{13}C-NMR: δ −3.92 ($Si(CH_3)_2$), 12.71 (CH_2CH_3), 14.95 (C-18), 17.80 (C-21), 18.93 ($SiC(CH_3)_3$), 20.09 (C-19), 21.68 (C-11), 25.04 (C-15), 26.61 ($SiC(CH_3)_3$), 27.82 (C-16), 30.38 (C-2), 32.54 (C-7), 32.75 (C-8), 37.24 (C-10), 38.02 (C-1), 40.26

(C-12), 43.08 (C-13), 43.33 (C-20), 43.48 (C-4), 50.83 (C-9), 53.52 (C-17), 57.02 (C-14), 60.63 (CH_2CH_3), 73.27 (C-3), 121.71 (C-6), 142.21 (C-5), 177.56 (*C*OOEt). MS: 489 ($M^+ + 1$, 78), 473 (39), 431 (35), 357 (100). Analysis calculated for $C_{30}H_{52}O_3Si$ (488.37): C, 73.71; H, 10.72. Found: C, 73.59; H, 10.86.

2.4. Ethyl (20S)-3β-(tert-butyldimethylsilanyloxy)-7-oxo-pregn-5-en-20-carboxylate (7)

To a solution of compound **6** (2.5 g, 5.2 mmol) in benzene (250 ml, dried over sodium) pyridinium chlorochromate (PCC, 25 g, 116 mmol) and molecular sieves (1.5 g, type 4 Å) were added. The reaction mixture was refluxed under nitrogen for 24 h, cooled and filtered through a pad of celite that was washed with hexane/EtOAc 1:1. The filtrate was dried over Na_2SO_4 and evaporated to dryness. The resulting crude reaction mixture was purified by flash chromatography (hexane/EtOAc 9:1) to give compound **7** as a white solid (1.7 g; 65%). Mp 173–174 °C; selected ^{1}H-NMR signals: δ 0.04 (6H, s, Si(CH_3)$_2$), 0.58 (3H, s, 18-CH_3), 0.77 (9H, s, SiC(CH_3)$_3$), 1.06 (3H, s, 19-CH_3), 1.08 (3H, d, $J = 7.0$ Hz, 21-CH_3), 1.13 (3H, t, $J = 7.0$ Hz, COOCH$_2$CH_3), 3.50 (1H, dddd, $J = 4.9$, 4.9, 11.2 and 11.2 Hz, 3α-H), 4.00 (2H, q, $J = 7.0$ Hz, COOCH_2CH$_3$), 5.56 (1H, d, $J = 2.1$ Hz, 6-H). ^{13}C-NMR: δ −3.94 (Si(*C*H$_3$)$_2$), 12.77 (CH$_2$*C*H$_3$), 14.88 (C-18), 17.85 (C-21), 17.91 (C-19), 18.72 (Si*C*(CH$_3$)$_3$), 21.75 (C-11), 26.45 (SiC(*C*H$_3$)$_3$), 26.96 (C-15), 27.97 (C-16), 32.33 (C-2), 36.99 (C-1), 38.92 (C-10), 39.13 (C-12), 43.06 (C-20), 43.15 (C-4), 43.74 (C-13), 45.89 (C-8), 50.23 (C-9), 50.50 (C-17), 52.31 (C-14), 60.59 (*C*H$_2$CH$_3$), 71.86 (C-3), 126.32 (C-6), 166.59 (C-5), 177.23 (*C*OOEt), 202.39 (C-7). MS: 503 ($M^+ + 1$, 100), 487 (12), 445 (14), 371 (23). Analysis calculated for $C_{30}H_{50}O_4Si$ (502.80): C, 71.66; H, 10.02. Found: C, 71.45; H, 10.12.

2.5. Reduction of 3β-(tert-butyldimethylsilanyloxy)-cholest-5-en-7-one (9) to 3β-(tert-butyldimethylsilanyloxy)-cholest-5-en-7β-ol (10)

3β-(*tert*-Butyldimethylsilanyloxy)-cholest-5-en-7-one (**9**) was obtained by allylic oxidation of 3β-(*tert*-butyldimethylsilanyloxy)-cholesterol (830 mg, 1.66 mmol) with PCC. The reaction was carried out and worked-up as described for compound **7**. Chromatographic purification of the crude mixture (hexane/EtOAc 9:1) afforded compound **9** as a white solid (530 mg, 62%). Mp 215–216 °C (from methanol); selected ^{1}H-NMR signals: δ 0.03 (6H, s, Si(CH_3)$_2$), 0.65 (3H, s, 18-CH_3), 0.83 (3H, d, $J = 6.3$ Hz, 26-CH_3), 0.84 (3H, d, $J = 6.3$ Hz, 27-CH_3), 0.86 (9H, s, SiC(CH_3)$_3$), 0.89 (3H, d, $J = 6.3$ Hz, 21-CH_3), 1.16 (3H, s, 19-CH_3), 3.50 (1H, dddd, $J = 4.9$, 4.9, 11.2 and 11.2 Hz, 3α-H), 5.63 (1H, d, $J = 2.1$ Hz, 6-H). ^{13}C-NMR: δ −3.97 (Si(*C*H$_3$)$_2$), 12.64 (C-18), 17.91 (C-19), 17.97 (C-21), 18.85 (Si*C*(CH$_3$)$_3$), 21.88 (C-11), 23.24 (C-26), 23.49 (C-27), 24.49 (C-23), 26.51 (SiC(*C*H$_3$)$_3$), 26.99 (C-15), 28.67 (C-25), 29.24 (C-16), 32.42 (C-2), 36.38 (C-20), 36.87 (C-22), 37.09 (C-1), 39.03 (C-10), 39.41 (C-12), 40.16 (C-4), 43.22 (C-24), 43.75 (C-13), 46.08 (C-8), 50.63 (C-9), 50.68 (C-17), 55.17 (C-14), 72.01 (C-3), 126.49 (C-6), 166.53 (C-5), 201.14 (C-7). MS: 515 ($M^+ + 1$, 100), 499 (33), 457 (22), 383 (45). Analysis calculated for $C_{33}H_{58}O_2Si$ (514.42): C, 76.98; H, 11.35. Found: C, 76.73; H, 11.22.

Lithium aluminum hydride ($LiAlH_4$, 105 mg, 2.75 mmol) was cautiously added to a stirred mixture of anhydrous aluminum chloride (1.05 g; 7.75 mmol) in dry ether (12 ml) at 0 °C under nitrogen. The mixture was refluxed for 30 min, cooled to room temperature, then a solution of compound **9** (500 mg, 0.95 mmol) in dry THF (5 ml) was added dropwise. The reaction mixture was stirred at room temperature for 5 min and than poured into ice. The aqueous layer was extracted with CH_2Cl_2 (3 × 15 ml) and the organic layer was washed with brine (6 ml), dried over Na_2SO_4, evaporated and chromatographed (hexane/EtOAc 9:1) to give compound **10** (480 mg, 98%). Mp 125–126 °C (from hexane); selected ^{1}H-NMR signals: δ 0.03 (6H, s, Si(CH_3)$_2$), 0.66 (3H, s, 18-CH_3), 0.83 (3H, d, $J = 6.3$ Hz, 26-CH_3), 0.835 (3H, d, $J = 6.3$ Hz, 27-CH_3), 0.86 (9H, s, SiC(CH_3)$_3$), 0.90 (3H, d, $J = 6.3$ Hz, 21-CH_3), 1.01 (3H, s, 19-CH_3), 2.17 (1H, ddd, $J = 2.8$, 4.9 and 13.3 Hz, 4α-H), 2.26 (1H, dddd, $J = 2.1$, 2.1, 11.2 and 13.3 Hz, 4β-H), 3.47 (1H, dddd, $J = 4.9$, 4.9, 11.2 and 11.2 Hz, 3α-H), 3.80 (1H, ddd, $J = 2.1$, 2.1 and 7.7 Hz, 7α-H), 5.23 (1H, dd, $J = 2.1$ and 2.1 Hz, 6-H). ^{13}C-NMR: δ −3.92 (Si(*C*H$_3$)$_2$), 12.50 (C-18), 18.92 (Si*C*(CH$_3$)$_3$), 19.47 (C-19), 19.87 (C-21), 21.74 (C-11), 23.25 (C-26), 23.50 (C-27), 24.51 (C-23), 26.60 (SiC(*C*H$_3$)$_3$), 27.07 (C-15), 28.70 (C-25), 29.24 (C-16), 32.73 (C-2), 36.41 (C-20), 36.89 (C-22), 37.19 (C-10), 37.75 (C-1), 40.19 (C-12), 40.28 (C-8), 41.63 (C-4), 42.97 (C-24), 43.62 (C-13), 49.02 (C-9), 56.15 (C-17), 56.69 (C-14), 72.96 (C-3), 74.13 (C-7), 125.71 (C-6), 144.92 (C-5). MS: 515 ($M^+ - 1$, 18), 499 (38), 385 (40), 367 (100), 325 (52). Analysis calculated for $C_{33}H_{60}O_2Si$ (516.44): C, 76.68; H, 11.70. Found: C, 76.52; H, 11.66.

2.6. (20S)-[7,7,21,21-$^2H_4$]-3β-(tert-Butyldimethylsilanyloxy)-20-methyl-pregn-5-en-21-ol (4b)

Anhydrous aluminum chloride (3.5 g, 25.8 mmol) was poured into dry ether (25 ml) at 0 °C and lithium aluminum deuteride ($LiAlD_4$, 350 mg; 8.3 mmol) was added cautiously keeping the temperature at 0 °C under nitrogen. The mixture was refluxed for 30 min, cooled to room temperature and **7** (1.7 g, 3.4 mmol) in dry THF (10 ml) was added dropwise to the mixture. After refluxing for 90 min, the mixture was poured into ice, the aqueous solution was extracted with CH_2Cl_2 (3 × 30 ml). The organic layer was washed with brine (10 ml), dried over anhydrous Na_2SO_4, evaporated and chromatographed (hexane/EtOAc 8:2) to give compound **4b** (987 mg, 72%). Mp 155–156 °C (from

hexane) ([8], 153.5–155.5 °C); selected ^{1}H-NMR signals: δ 0.03 (6H, s, Si(CH_3)$_2$), 0.67 (3H, s, 18-CH_3), 0.86 (9H, s, SiC(CH_3)$_3$), 0.97 (3H, s, 19-CH_3), 1.03 (3H, d, J = 7.0 Hz, 21-CH_3), 2.14 (1H, ddd, J = 2.8, 4.9 and 13.3 Hz, 4α-H), 2.24 (1H, dd, J = 2.1, 11.2 and 13.3 Hz, 4β-H), 3.45 (1H, dddd, J = 4.9, 4.9, 11.2 and 11.2 Hz, 3α-H), 5.28 (1H, d, J = 2.1 Hz, 6-H). ^{13}C-NMR: δ −3.99 (Si(*C*H$_3$)$_2$), 12.57 (C-18), 17.36 (C-21), 18.92 (Si*C*(CH$_3$)$_3$), 20.09 (C-19), 21.71 (C-11), 25.05 (C-15), 26.61 (SiC(*C*H$_3$)$_3$), 28.36 (C-16), 32.41 (C-2), 32.74 (C-8), 37.24 (C-10), 38.05 (C-1), 39.22 (C-20), 40.32 (C-12), 43.09 (C-13), 43.49 (C-4), 50.82 (C-9), 53.06 (C-17), 57.17 (C-14), 73.31 (C-3), 121.65 (C-6), 142.39 (C-5). The compound **4b** was >99.0% isotopically pure (^{1}H-NMR, ^{13}C-NMR and MS). MS: 449 ($M^+ - 1$, 13), 435 (29), 393 (22), 319 (70), 301 (100). Analysis calculated for $C_{28}H_{46}D_4O_2Si$ (450.81): C, 74.60; H, 12.07. Found: C, 74.43; H, 11.96.

2.7. (20S)-3β-(tert-Butyldimethylsilanyloxy)-20-methyl-pregn-5-en-21-ol (11)

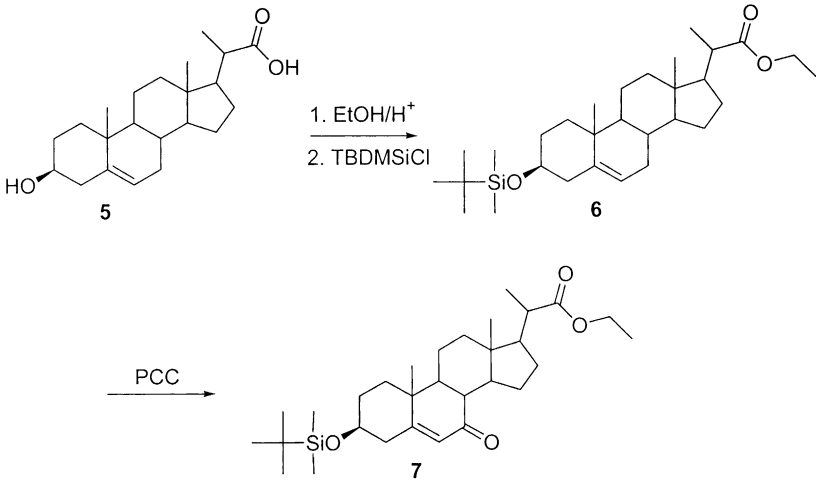
The reaction was carried out as described for the preparation of **4b** and, starting from ethyl (20*S*)-3β-(*tert*-butyldimethylsilanyloxy)-pregn-5-en-20-carboxylate (**6**), compound **11** was obtained. Mp 153–154 °C (from hexane) ([8], 153.5–155.5 °C); selected ^{1}H-NMR signals: δ 0.03 (6H, s, Si(CH_3)$_2$), 0.67 (3H, s, 18-CH_3), 0.86 (9H, s, SiC(CH_3)$_3$), 0.97 (3H, s, 19-CH_3), 1.03 (3H, d, J = 7.0 Hz, 21-CH_3), 1.48 (1H, m, 7α-H), 1.94 (1H, m, 7β-H), 2.14 (1H, ddd, J = 2.8, 4.9 and 13.3 Hz, 4α-H), 2.24 (1H, ddddd, J = 2.1, 2.1, 2.1, 11.2 and 13.3 Hz, 4β-H), 3.34 (1H, dd, J = 7.0 and 10.5 Hz, 22b-H), 3.45 (1H, dddd, J = 4.9, 4.9, 11.2 and 11.2 Hz, 3α-H), 3.61 (1H, dd, J = 3.5 and 10.5 Hz, 22a-H), 5.28 (1H, ddd, J = 2.1, 2.1 and 4.9 Hz, 6-H). ^{13}C-NMR: δ −3.99 (Si(*C*H$_3$)$_2$), 12.52 (C-18), 17.35 (C-21), 18.89 (Si*C*(CH$_3$)$_3$), 20.03 (C-19), 21.69 (C-11), 25.09 (C-15), 26.55 (SiC(*C*H$_3$)$_3$), 28.35 (C-16), 32.54 (C-7), 32.57 (C-2), 32.66 (C-8), 37.21 (C-10), 38.02 (C-1), 39.39 (C-20), 40.29 (C-12), 43.07 (C-13), 43.39 (C-4), 50.83 (C-9), 53.11 (C-17), 57.17 (C-14), 68.32 (C-22), 73.37 (C-3), 121.77 (C-6), 142.16 (C-5). MS: 445 ($M^+ - 1$, 12), 431 (20), 389 (16), 315 (79), 297 (100).

3. Results and discussion

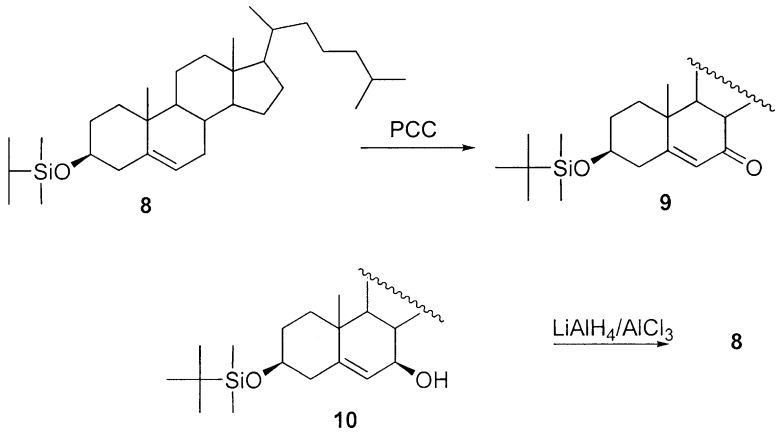
We have decided to synthesize (20*S*)-[7,7,21,21-$^{2}H_4$]-3β-(*tert*-butyldimethylsilanyloxy)-20-methyl-pregn-5-en-21-ol (**4b**), since a silyl ether is a protecting group stable under basic, slightly acidic, reducing or oxidizing conditions and is, therefore, compatible with most of chemical elaborations necessary for the construction of the side chain [9].

Commercially available cholenic acid **5** was transformed into the corresponding ethyl ester and protected at the 3β-hydroxy group as *tert*-butyldimethylsilyl ether (TBDMS) to afford compound **6**. Lithium aluminum deuteride reduction of ester **6** would introduce in the product only two deuterium atoms, but these are not sufficient for a deuterated sterol to be used as standard for GC–MS analysis. A tetradeuterated intermediate such as, for instance, compound **4b** would be more suitable for the above purpose. We planned the synthesis of the 7-keto derivative **7** that could be reduced with dichloroaluminum deuteride (generated from lithium aluminum deuteride and aluminum chloride in ether) to the corresponding [7-$^{2}H_2$]-steroid, according to a reported method [10] that has been used for the synthesis of 19-hydroxy-[7-$^{2}H_2$]-androstenedione for human metabolism studies [11]. Applied to compound **7**, the procedure could be particularly convenient since the simultaneous reduction of the ester and keto groups could allow the introduction of four deuterium atoms in one step.

The 7-keto compound **7** was obtained in 65% yield from compound **6** by an allylic oxidation with excess PCC [11] (Scheme 1).



Scheme 1.



Scheme 2.

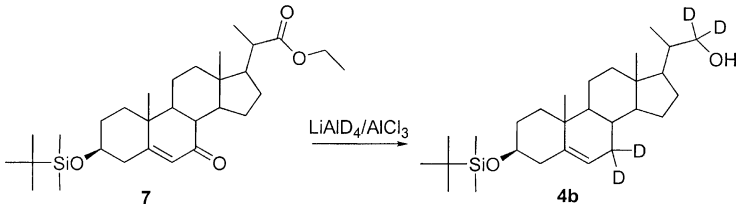
According to Blair et al. [10], the dichloroaluminum deuteride reduction of compound **7** should require conditions that could lead to undesired side reaction, mainly cleavage of the silyl-protecting group. Therefore, we decided to study the $LiAlH_4/AlCl_3$ reaction on a model silyl 7-keto steroid, 3β-(*tert*-butyldimethylsilanyloxy)-cholest-5-en-7-one **9**, that was prepared by allylic oxidation of 3β-(*tert*-butyldimethylsilanyloxy)-cholesterol **8** with PCC [12]. We found that the initial step of the reduction was the previously unreported conversion to the 7β-hydroxy compound **10**, that was obtained in 98% yield at room temperature (5 min), with no detectable amount of the 7α-isomer. The β-configuration of compound **10** was assigned by the analysis of the ^{1}H-NMR spectrum. Specifically, the value of 2.1 Hz, corresponding to the coupling constant between 6-H and 7-H, is consistent with a 6-H/7α-H system. A value of about 5 Hz would be expected for the 6-H/7β-H counterpart, that is characterized by a large dihedral angle between the two hydrogen atoms.

It should be reminded that previous methods for the stereoselective reduction of a 7-keto to a 7β-hydroxy steroid can be achieved at room temperature with $NaBH_4$–$CeCl_3$–MeOH [13] or at −78 °C with in situ generated $LiAlH(t\text{-}BuO)_3$ [14]. We propose, therefore, to add dichloroaluminum hydride to the reagents that can allow a highly stereoselective preparation of 7β-hydroxy steroids. The 7β-hydroxy compound **10** could then be reduced with dichloroaluminum hydride to the derivative **8** by additional reflux for 1.5 h (Scheme 2).

The direct reduction of the 7-keto derivative **9** to 3β-silyl derivative **8** was achieved in refluxing ether for 1.5 h and, therefore, these experimental conditions could be safely applied to the ketoester 7 bearing a silyl protecting group as well. The one-step preparation of the tetradeuterated compound **4b** from the ketoester 7 was indeed achieved in 72% yield (Scheme 3).

Comparison of the ^{1}H-NMR spectrum of **4b** and unlabeled **11**, confirmed the presence of four deuterium atoms at 22 position and 7, for the lack of two dd at 3.61 and 3.34 ppm indicative of the protons at C-22 and for the absence of signals corresponding to 7α-H (1.48 ppm) and 7β-H (1.94 ppm). Furthermore, a careful inspection of the spectra gave information about the position more affected by the deuterium substitution at C-7. In fact, the multiplicity of 6-H, a ddd at 5.28 ppm (J = 2.1, 2.1 and 4.9 Hz) for **11**, becomes d (J = 2.1 Hz) in **4b**, as a consequence of the disappearance of J with 7α-H (2.1 Hz) and 7β-H (4.9 Hz). The residual J = 2.1 Hz is due to the allylic coupling of 6-H with 4β-H. Moreover, the complex signal of 4β-H in 11, a ddddd at 2.24 ppm becomes a ddd for the absence of homoallylic coupling with 7α-H (J = 2.1 Hz) and 7β-H (J = 2.1 Hz). In addition, the 4α-H signal at 2.14 ppm is a ddd for the presence of a long range J with 2α-H (2.8 Hz), part of a sterically fixed "W" arrangement of atoms. Finally, in the ^{13}C-NMR spectrum of **4b** no signals were observed for C-7 (32.54 ppm) and C-22 (68.38 ppm) (Fig. 3).

The comparative study of mass spectra of **4b** and of non deuterated analogue **11** confirms the clean incorporation of four deuterium atoms in compound **4b**. The most abundant ions present in the spectrum of unlabeled 11 [445 (M − H), 431 (M − CH_3), 389 (M − C(CH_3)$_3$), 315



Scheme 3.

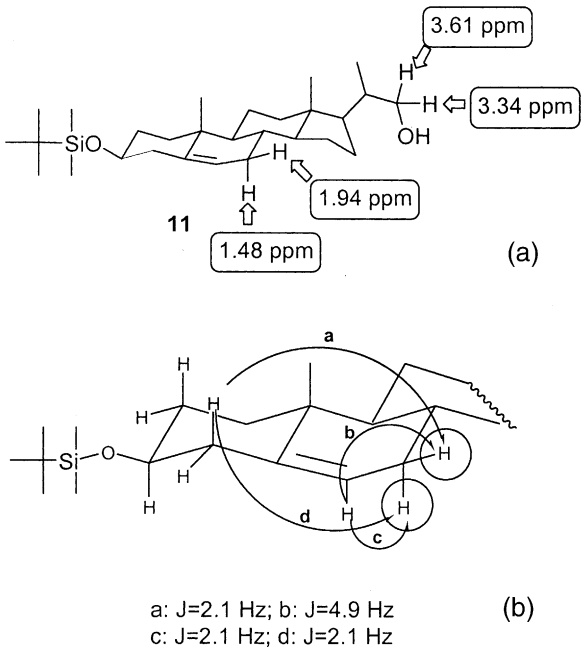


Fig. 3. (a) Resonance of 7α-H, 7β-H, 22a-H and 22b-H in unlabeled compound **11**. (b) Couplings of 6-H and 4β-H to 7β-H and 7α-H in **11** that disappear in **4b**.

(M − *tert*-butyldimethylsilyloxy), 297 (315 − H_2O)] became [449 (M − H), 435 (M − CH_3), 393 (M − $C(CH_3)_3$), 319 (M − *tert*-butyldimethylsilyloxy), 301 (319 − H_2O) in **4b** with nearly identical intensity of peaks (see Section 2). We, therefore, conclude that the deuterated compound **4b** can now be proposed as a valuable intermediate for the synthesis of deuterated isotopomers of biologically significant sterols.

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