# Functionalization at C-12 of 1α,25-Dihydroxyvitamin $D_3$ Strongly Modulates the Affinity for the Vitamin D Receptor (VDR)

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## GENERAL METHODS AND PROCEDURES

All reactions involving oxigen- or moisture-sensitive compounds were carried out in oven- or flame-dried glassware under a dry argon (L-50) atmosphere.

Solvents used for reactions were purified according to Brown.[1] All dry solvents were distilled from an appropriate drying agent immediatly prior to use under an argon atmosphere. Tetrahydrofuran (THF) and ether ($Et_2O$) were distilled from Na/benzophenone; absolute methanol (MeOH) from $Mg/I_2$; dichloromethane ($CH_2Cl_2$) from $P_2O_5$; hexamethylphosporamide (HMPA), dietylamine ($Et_2NH$), triethylamine ($Et_3N$), diisopropylamine ($^iPr_2NH$) and were distilled from $CaH_2$.

Comercially available solutions of $^n$BuLi (solution in hexanes, Aldrich), $^n$HexLi (solution in hexanes, Aldrich) and MeLi (solution in ether, Aldrich) were used after tritation.

Pyridinium dicromate (PDC) was prepared following Corey's procedure.[2] Other reagents such as *m*-chloroperbenzoic acid (*m*-CPBA), were purified according to Perrin's indications.[3]

Acetone-dry ice baths were used for reactions at low temperature. Alternatively, acetone baths were cooled with a refrigerating apparatus CRYOCOOL-Inmersion Cooler CC-100 II by NESLAB, provided with a temperature regulator. The indicated temperatures refer to external bath tempertures.

Additions were performed under argon either *via* syringe or cannula. Unless otherwise indicated, reactions were carried out with magnetical stirring.

Organic extracts were dried over $Na_2SO_4$, filtered, and concentrated using a rotatory evaporator at reduced pressure (20-30 mmHg).

Disposable plastic (Discardit) or teflon (Hamilton) syringes provided with Llorach-Luer needles were used for solvent additions.

Glassware for reactions was dried in an oven at 150 °C for at least 12 h and then flame-dried under an argon flow.

Reactions were monitored by thin-layer chromatography (TLC) using aluminum-coated Merck GF-254 plates. After visualization under ultraviolet light at 254 nm, the plates were developped by inmersion in a solution containing either a mixture of *p*-anisaldehyde (2.5%), acetic acid (1%) and sulfuric acid (3.4%) in 95% ethanol or a mixture of phosphomolibdic acid (5%) in methanol.

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1 Brown H. C. *Organic Synthesis via Boranes*. John Wiley & Sons. New York, 1975.

2 Corey E. J.; Schmidt, G. "Useful Procedures for the Oxidation of Alcohols Involving Pyridinium Dichromate in Aprotic Media." *Tetrahedron Lett*. **1979**, 399.

3 Perrin D. D.; Amarego W. L. F. *Purification of Laboratory Chemicals*. *Pergamon Press*. Oxford, 1988.

Flash column chromatography was performed with Merck 60 (230-400 mesh) silica gel following Still's indications.[4] Solvents for flash chromatography (hexanes and ethyl acetate) were distilled and dried ($Na_2SO_4$) prior to use. Reverse phase chromatography was carried out using C-18 coated Merck silica gel and eluted with $H_2O$/MeOH mixtures.

NMR data were collected in Bruker spectrometers WM-250 MHz, WM-300 MHz, and DRX-500 belonging to the NMR service of the University of Santiago de Compostela. All spectra were performed in $CDCl_3$ unless otherwise indicated. Chemical shifts are reported on the $\delta$ scale (ppm) downfield from tetramethylsilane ($\delta$=0.0 ppm) using the residual solvent signal as an internal reference: 7.27 ppm ($^1$H) and 77.0 ppm ($^{13}$C, triplet). For NMR experiments carried out in MeOH-$d_4$ the internal reference was 3.31 ppm ($^1$H, quintuplet) and 49.0 ppm ($^{13}$C, heptaplet).

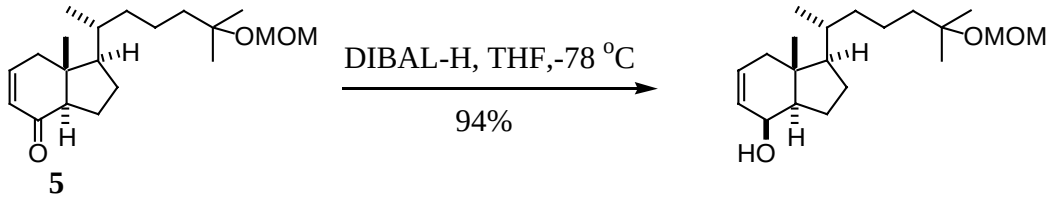
Melting points were measured in a Büchi apparatus and are uncorrected.

CD-ring intermediates were named following the steroidal nomenclature.[5] Vitamin D analogs are named as vitamin D derivatives. IUPAC rules were used for the other compounds.

[4] Still W. C.; Kahn, M.; Mitra, A. "Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution." *Journal of Organic Chemistry* **1978**, *43*, 2923.

[5] (a) Okamura W. H. Vitamin D Nomenclature: A Chemist's Viewpoint. *Vitamin D: Basic Research and its Clinical Application*. Norman AW, Schaefer K, Herrath DV. *Walter de Gruyter*. Berlín, 1979; p 1231. (b) Coffey, S. *Rodd's Chemistry of Carbon Compounds. Vol II. Steroids. Elsevier*. London, 1970; p 422. (c) Definitive Rules for Nomenclature of Steroids. *Pure & Applicated Chem*istry **1972**, *31*, 283-322.

**1. 25-Methoxymethyloxy-de-*A*,*B*-cholest-9(11)-en-8β-ol.**



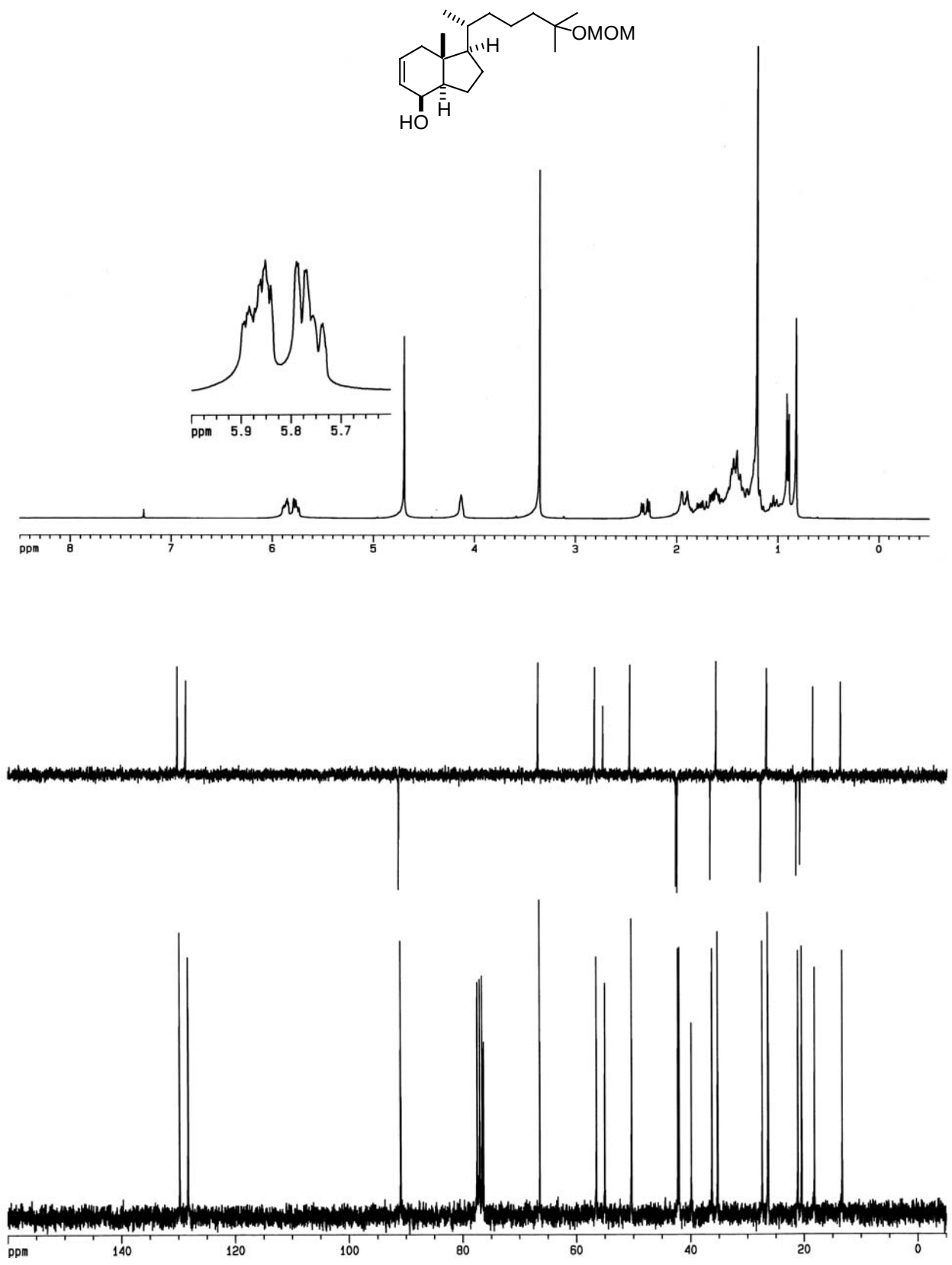

**5**

Diisobutylaluminium hydride (25 mL, 25 mmol, 1 M solution in hexanes) was added dropwise to a solution of **5** (5.37 g, 16.69 mmol) in dry THF (100 mL) at –78 °C. After stirring for 30 min the reaction was quenched by the successive addition of $H_2O$ (50 mL) and aqueous HCl (100 mL, 5%). The mixture was extracted with $Et_2O$ (3x100 mL) and the combined organic solution was washed with saturated aqueous $NaHCO_3$ (100 mL), dried, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (13% EtOAc/hexanes), to give the desired alcohol [5.08 g, 15.69 mmol, 94%, $R_f$ = 0.35 (30% EtOAc/hexanes), colorless oil].

**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 5.89-5.73 (2H, m, H-9 and H-11), 4.69 (2H, s, $OCH_2O$), 4.13 (1H, broad s, H-8), 3.35 (3H, s, $OCH_3$), 1.20 (6H, s, Me-26 and Me-27), 0.90 (3H, d, $J$ = 6.4 Hz, Me-21), 0.82 (3H, s, Me-18).

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 129.73 (CH), 128.27 (CH), 90.91 ($CH_2$), 76.29 (C), 66.44 (CH), 56.50 (CH), 55.01 ($CH_3$), 50.34 (CH), 42.19 ($CH_2$), 41.98 ($CH_2$), 35.85 (C), 36.22 ($CH_2$), 35.20 (CH), 27.35 ($CH_2$), 26.35 ($CH_3$), 26.27 ($CH_3$), 21.09 ($CH_2$), 20.44 ($CH_2$), 18.17 ($CH_3$), 13.31 ($CH_3$).

**MS:** m/z ($[CI]^+$, %): 263 ($[M\text{-}OMOM]^+$, 29), 245 ($[M\text{-}H_2O]^+$, 100). **HRMS:**calculated for $[C_{18}H_{31}O]^+$ ($[M\text{-}OMOM]^+$): 263.2375; found: 263.2383.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HO
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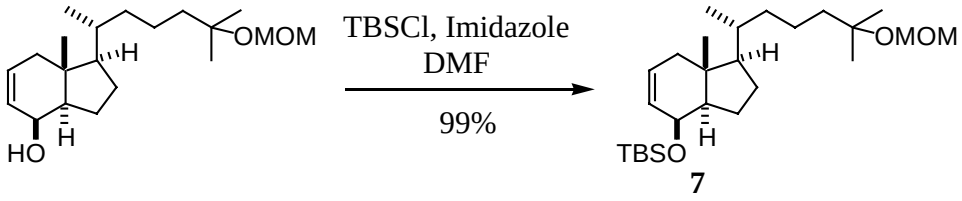
**2. 8β-*tert*-Butyldimethylsilyloxy-25-methoxymethyloxy-de-*A*,*B*-cholest-9(11)-ene.**



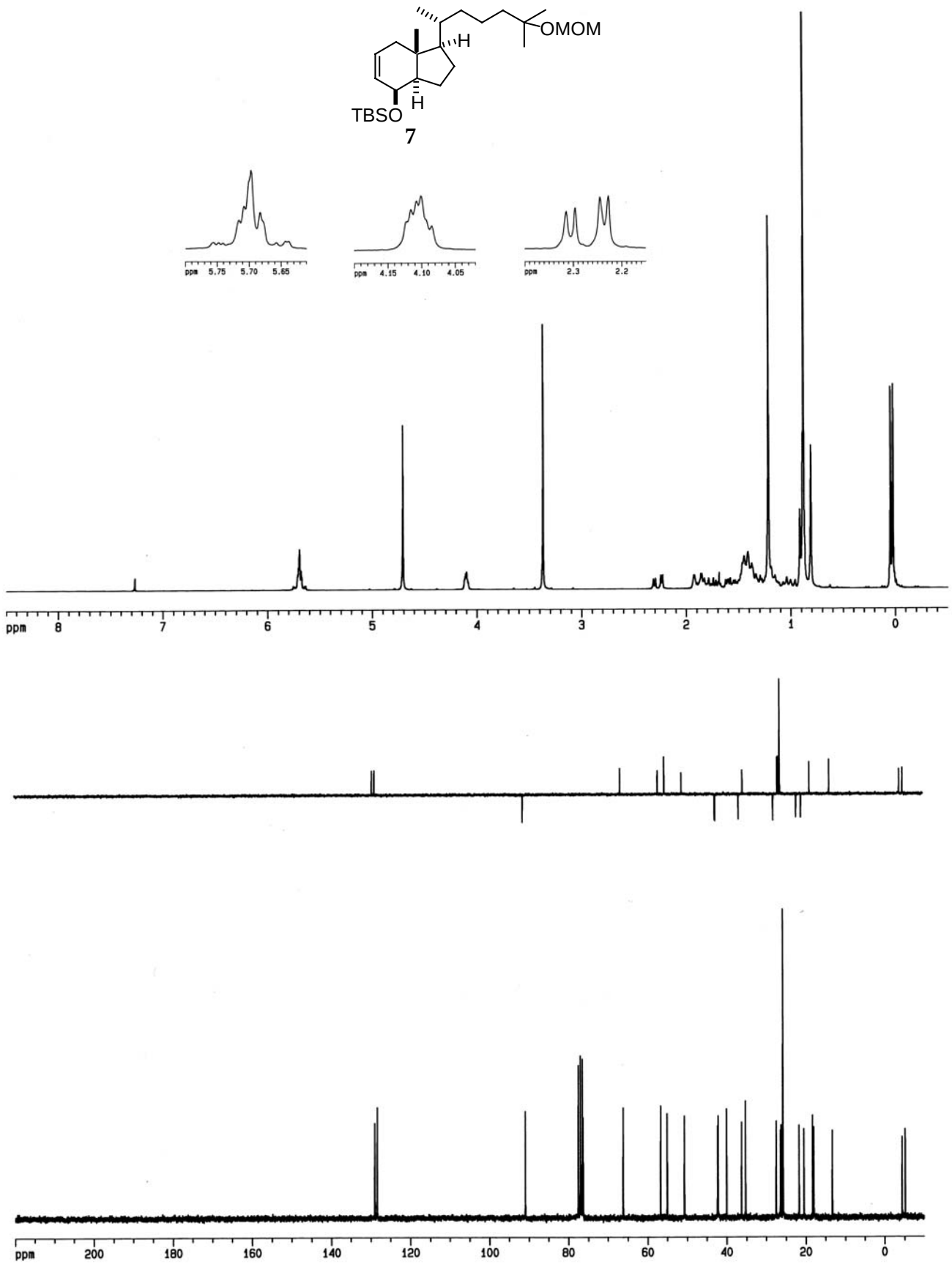

**7**

Imidazole (5.13 g, 75.3 mmol) and *tert*-butyldimethylsilyl chloride (9.08 g, 60.3 mmol) were successively added to a solution of the unprotected alcohol (4.88 g, 15.05 mmol) in dry DMF. The reaction mixture was stirred overnight at rt. Brine (150 mL) was added and the aqueous layer was extracted with hexanes (3x150 mL). The combined organic solution was dried, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (4% EtOAc/hexanes) to afford **7** [6.54 g, 14.89 mmol, 99%, $R_f$ = 0.7 (20% EtOAc/hexanes), colorless oil].

**$^{1}$H-RMN** ($CDCl_3$, 250 MHz, δ): 5.70 (2H, m, H-9 and H-11), 4.70 (2H, s, $OCH_2O$), 4.10 (1H, m, H-8), 3.37 (3H, s, $OCH_3$), 1.21(6H, s, Me-26 and Me-27), 0.90 (3H, d, $J$ = 6.6 Hz, Me-21), 0.88 (9H, s, $^{t}$BuSi), 0.81 (3H, s, Me-18), 0.05 (3H, s, MeSi), 0.02 (3H, s, $Me_2Si$).

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 129.03 (CH), 128.39 (CH), 90.95 ($CH_2$), 76.34 (C), 66.23 (CH), 56.75 (CH), 55.06 ($CH_3$), 50.71 (CH), 42.33 ($CH_2$), 42.20 ($CH_2$), 40.08 (C), 36.29 ($CH_2$), 35.28 (CH), 27.52 ($CH_2$), 26.41 ($CH_3$), 26.32 ($CH_3$), 25.80 ($CH_3$), 21.73 ($CH_2$), 20.49 ($CH_2$), 18.31 ($CH_3$), 18.03 (C), 13.32 ($CH_3$), –4.37 ($CH_3$), –5.16 ($CH_3$).

**MS:** m/z ($[CI]^+$, %): 377 ($[M\text{-}OMOM]^+$, 87), 245 ($[M\text{-}OMOM\text{-}TBSOH]^+$, 100). **HRMS:** calculated for $[C_{24}H_{45}OSi]^+$ ($[M\text{-}OMOM]^+$): 377.3240; found: 377.3223.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H
TBSO
7
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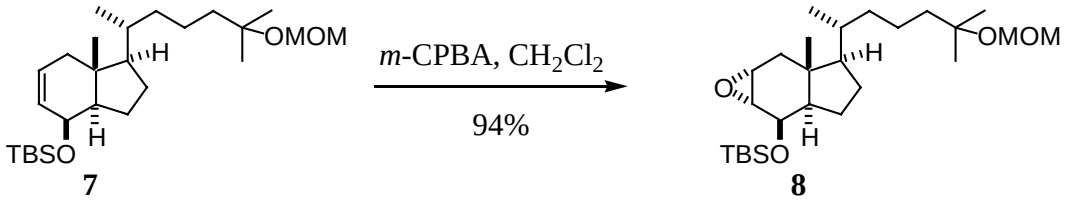
ppm 4.15 4.10 4.05
ppm 2.3 2.2
ppm 8 7 6 5 4 3 2 1 0
ppm 200 180 160 140 120 100 80 60 40 20 0

**3. 8β-*tert*-Butyldimethylsilyloxy-25-methoxymethyloxy-de-*A,B*-9α,11α-epoxycholestane.**



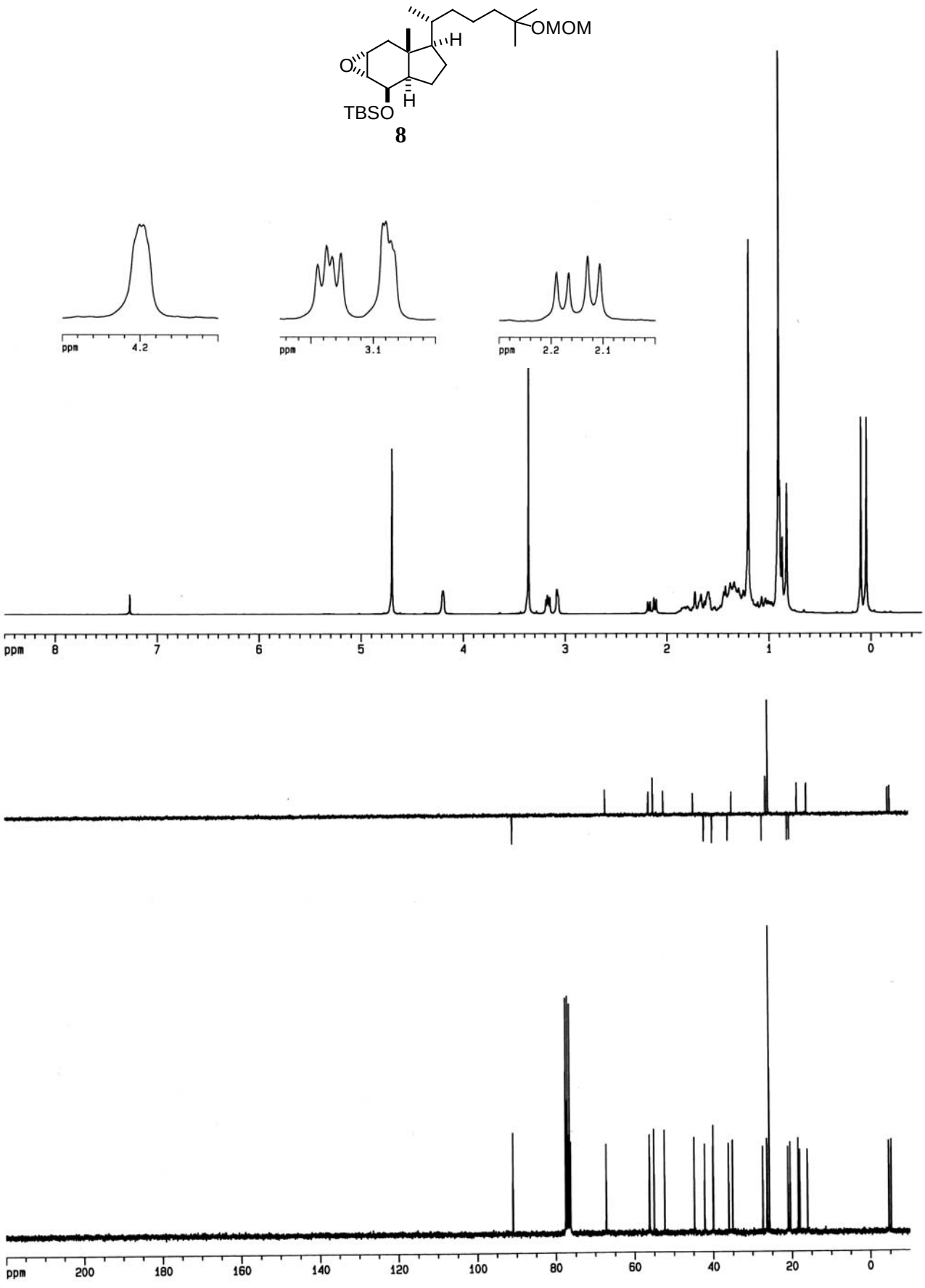

**7** **8**

3-Chloroperoxybenzoic acid (5.26 g, 30.5 mmol) was added in portions to a solution of **7** (5.35 g, 12.2 mmol) in dry $CH_2Cl_2$ (130 mL) at 0 °C . The mixture was stirred in the dark for 2 h at 0 °C and then for 3 h at rt. The reaction was quenched by the addition of saturated aqueous $Na_2S_2O_4$ (80 mL). The resulting mixture was vigorously shaken and the aqueous layer was extracted with $CH_2Cl_2$ (3x50 mL). The combined organic solution was dried, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (4% EtOAc/hexanes) to afford **8** [5.21 g, 11.5 mmol, 94%, $R_f$ = 0.5 (10% EtOAc/hexanes), white solid (mp 48 °C)].

**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 4.69 (2H, s, $OCH_2O$), 4.20 (1H, broad singlet, H-8), 3.36 (3H, s, $OCH_3$), 3.17 (1H, dd, *J* = 5.7 and 3.5 Hz, H-11), 3.08 (1H, m, H-9), 1.20 (6H, s, Me-26 and Me-27), 0.91 (9H, s, $^t$BuSi), 0.84 (3H, d, *J* = 6.6 Hz, Me-21), 0.82 (3H, s, Me-18), 0.10 (3H, s, MeSi), 0.04 (3H, s, $Me_2Si$).

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 90.94 ($CH_2$), 76.30 (C), 67.24 (CH), 56.26 (CH), 56.17 (CH), 55.05 ($CH_3$), 52.36 (CH), 44.82 (CH), 42.18 ($CH_2$), 40.05 ($CH_2$), 39.98 (C), 36.06 ($CH_2$), 35.07 (CH), 27.41 ($CH_2$), 26.36 ($CH_3$), 26.29 ($CH_3$), 25.75 ($CH_3$), 20.96 ($CH_2$), 20.42 ($CH_2$), 18.39 ($CH_3$), 17.96 (C), 16.00 ($CH_3$), –4.69 ($CH_3$), –5.24 ($CH_3$).

**MS:** m/z ([CI]$^+$, %): 394 ([M-OMOM]$^+$, 76), 335 ([M-OMOM-*t*-Bu]$^+$, 100), 262 ([M-OMOM-TBSOH, 71]$^+$. **HRMS:** calculated for $[C_{24}H_{45}O_2Si]^+$ ([M-OMOM]$^+$): 393.3189; found: 393.3176.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TBSO
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3.1
ppm
2.2
2.1
ppm
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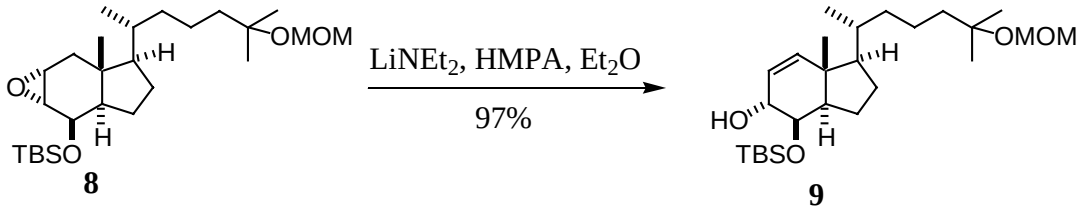
7
6
5
4
3
2
1
0
ppm
200
180
160
140
120
100
80
60
40
20
0

**8**

**4. 8β-*tert*-Butyldimethylsilyloxy-25-methoxymethyloxy-de-*A,B*-cholest-11-en-9α-ol.**




**8** **9**

A solution of lithium diethylamide was prepared by the addition of $^{n}$BuLi (28.9 mL, 67.1 mmol, 2.32 M solution in hexanes) to neat $Et_2NH$ (8.33 mL, 80.57 mmol) at –78 ºC. The cooling bath was removed and the temperature was allowed to warm until formation of a semisolid slurry. Dry $Et_2O$ (35 mL) was added and the resulting solution was stirred at rt for 15 min.

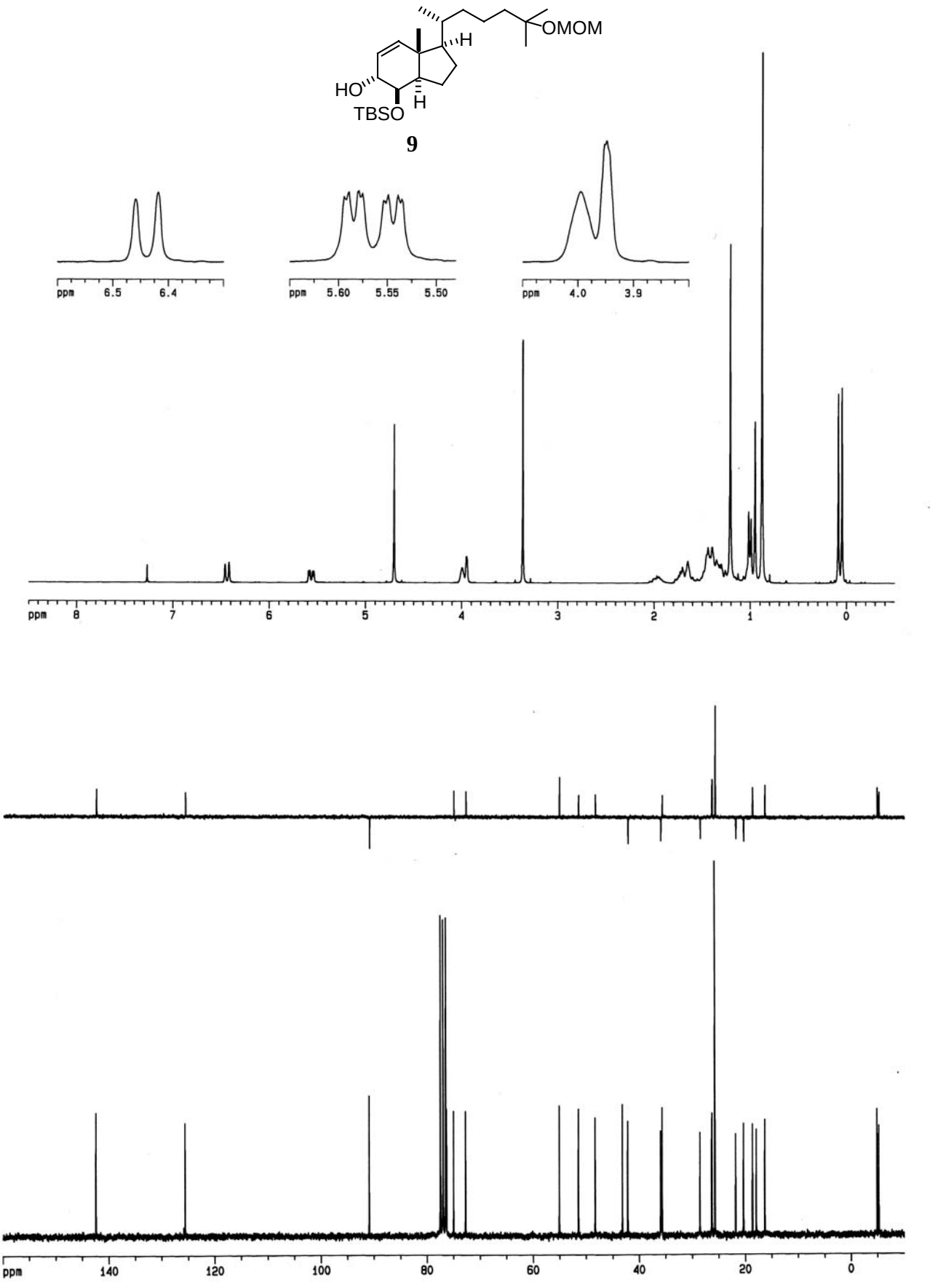
**8** (6.11 g, 13.4 mmol) was dissolved in a mixture of dry $Et_2O$ (35 mL) and HMPA (14.0 mL, 80.5 mmol) and added dropwise to the above solution of $LiNEt_2$. The resulting mixture was stirred overnight at rt.The reaction was quenched by the successive addition of a few drops of aqueous $NH_4Cl$ and HCl (100 mL, 2%). The mixture was extracted with $Et_2O$ (3x100 mL) and the combined organic solution was dried, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (10% EtOAc/hexanes) to give **9** [5.95 g, 13.0 mmol, 97%, $R_f$ = 0.5 (25% EtOAc/hexanes), white solid (mp 54 $^{o}$C)].

**$^{1}$H-RMN** ($CDCl_3$, 250 MHz, δ): 6.44 (1H, d, *J* = 10.2 Hz, H-12), 5.58 (1H, ddd, *J* = 10.2, 3.6 and 1.1 Hz, H-11), 4.70 (2H, s, $OCH_2O$), 3.99 (1H, broad singlet, H-8), 3.95 (1H, d, *J* = 1.1 Hz, H-9), 3.36 (3H, s, $OCH_3$), 1.21 (6H, s, Me-26 and Me-27), 1.01 (3H, d, *J* = 6.4 Hz, Me-21), 0.95 (3H, s, Me-18), 0.88 (9H, s, $^{t}$BuSi), 0.08 (3H, s, MeSi), 0.05 (3H, s, MeSi).

**NOESY** ($CDCl_3$, 500 MHz) experiments show a clear space proximity between H-9 and $Me_2Si$, thus confirming the stereochemistry of compound **9**.

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 142.46 (CH), 125.63 (CH), 90.92 ($CH_2$), 76.34 (C), 75.04 (CH), 72.74 (CH), 55.08 ($CH_3$), 51.48 (CH), 48.33 (CH), 43.19 (C), 42.19 ($CH_2$), 35.97 ($CH_2$), 35.72 (CH), 28.58 ($CH_2$), 26.36 ($CH_3$), 26.30 ($CH_3$), 25.75 ($CH_3$), 21.84 ($CH_2$), 20.37 ($CH_2$), 18.66 ($CH_3$), 17.96 (C), 16.34 ($CH_3$), –4.87 ($CH_3$), –5.20 ($CH_3$).

**MS:** m/z ([CI] $^{+}$, %): 438 ([M-OH]$^{+}$, 19), 394 ([M-OMOM]$^{+}$, 9), 376 ([M-OMOM-$H_2O$, 34]$^{+}$, 244 ([M-OMOM-$H_2O$-TBSOH]$^{+}$, 100). **HRMS:** calculated for $[C_{24}H_{45}O_2Si]^{+}$ ([M-OMOM]$^{+}$): 393.3189; found: 393.3183.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TBSO
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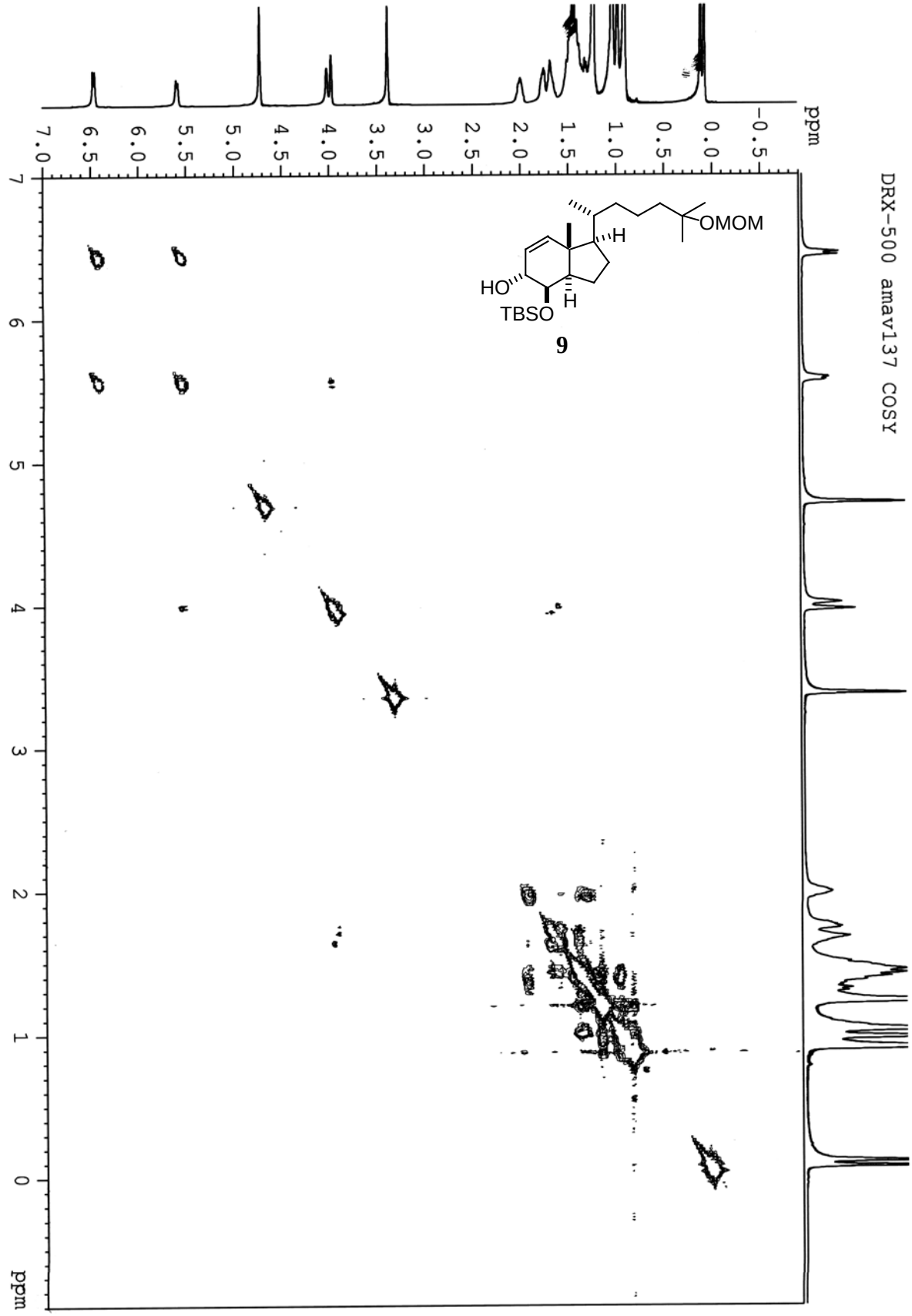
**9**

**COSY**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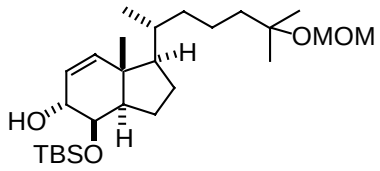
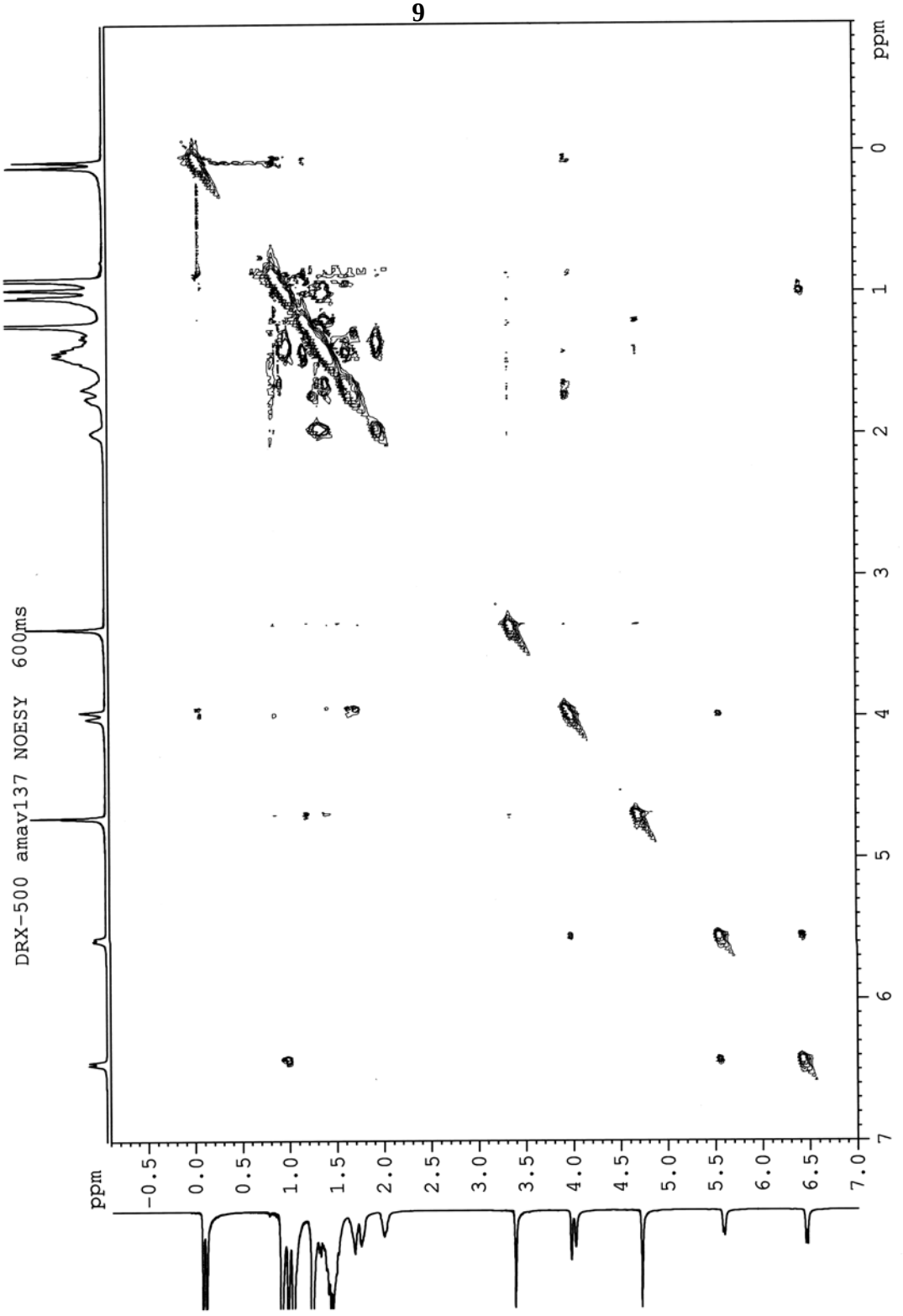
0.5
0.0
−0.5
OMOM
H
HO
H
TBSO
DRX-500 amav137 COSY
ppm
7
6
5
4
3
2
1
0

**9**

**NOESY**


OMOM
H
HO
H
TBSO

**9**


DRX-500 amav137 NOESY 600ms
ppm
ppm
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0.5
1.0
1.5
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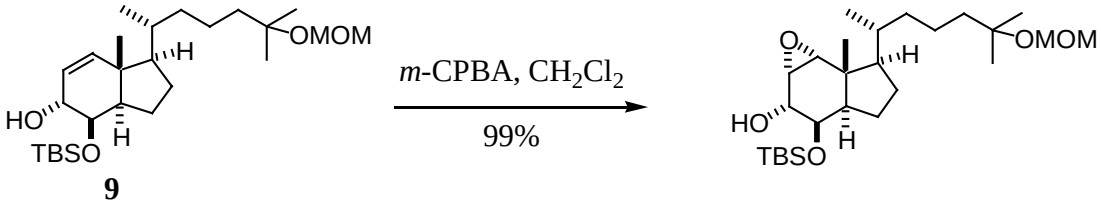
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
0
1
2
3
4
5
6
7

**5. 8β-*tert*-Butyldimethylsilyloxy-25-methoxymethyloxy-de-*A*,*B*-11α, 12α-epoxycholestan-9α-ol.**



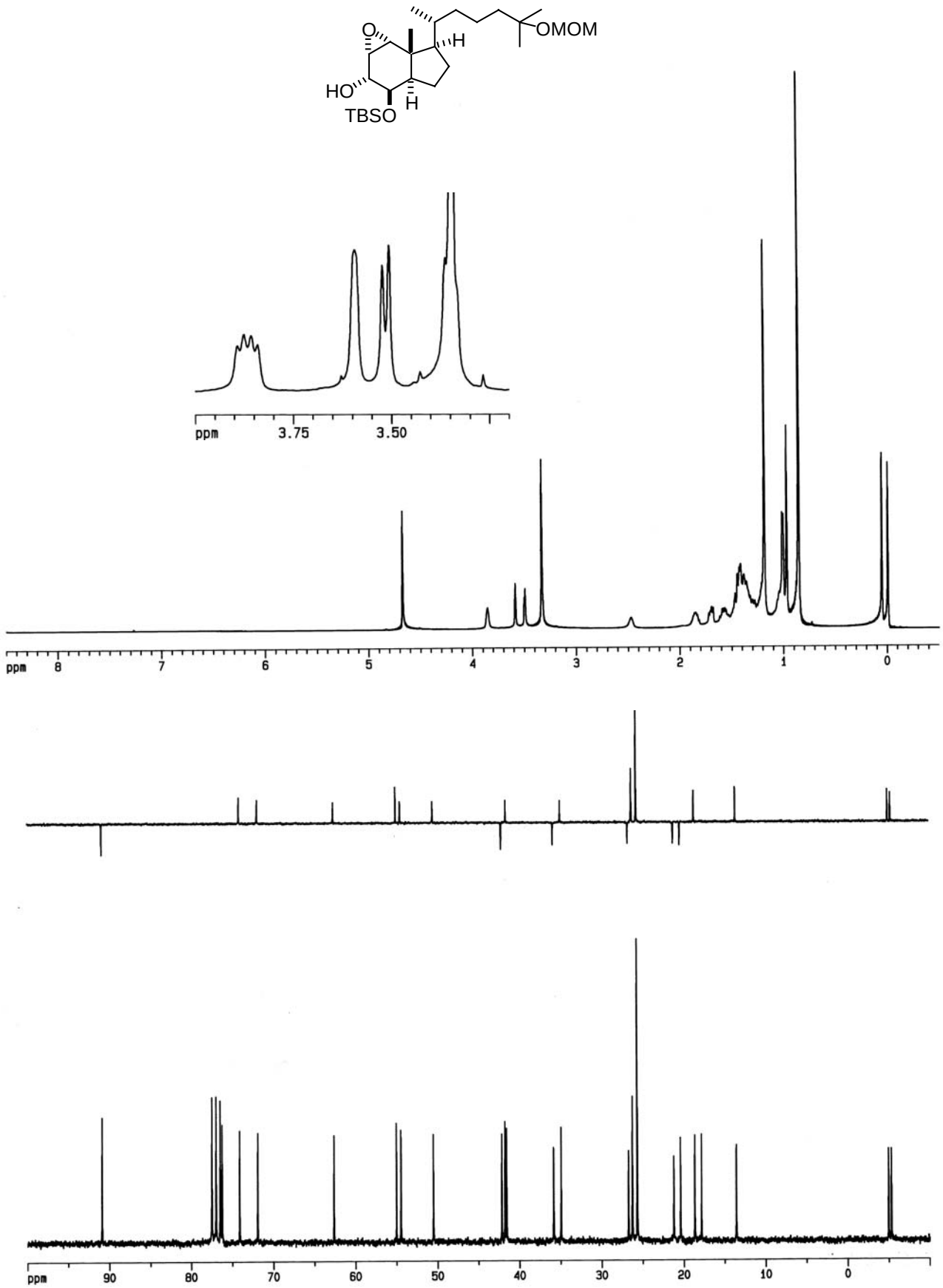

3-Chloroperoxybenzoic acid (1.67 g, 9.70 mmol) was added in portions to a solution of **9** (2.21 g, 4.85 mmol) in dry $CH_2Cl_2$ (60 mL) at 0 $^oC$ . The mixture was allowed to reach rt overnight while stirring in the dark. The reaction was quenched by the addition of aqueous $Na_2S_2O_4$ (40 mL). The resulting mixture was vigorously shaken and the aqueous layer was extracted with $CH_2Cl_2$ (2x25 mL). The combined organic solution was washed with aqueous $NaHCO_3$ (40 mL), dried, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (11% EtOAc/hexanes) to give the desired epoxialcohol [2.209 g, 4.80 mmol, 99%, $R_f$ = 0.45 (25% EtOAc/hexanes), (mp 81 $^oC$) ].

**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 4.68 (2H, s, $OCH_2O$), 3.86 (1H, dd, *J* = 8.7 and 4.2 Hz, H-9), 3.59 (1H, s, H-8), 3.51 (1H, d, *J* = 4.1 Hz, H-12), 3.35 (4H, overlapped signals, $OCH_3$ and H-11), 1.19 (6H, s, Me-26 and Me-27), 1.01 (3H, d, *J* = 5.5 Hz, Me-21), 0.98 (3H, s, Me-18), 0.86 (9H, s, $^t$BuSi), 0.06 (3H, s, MeSi), 0.00 (3H, s, $Me_2Si$).

The absolute configuration at C-9, C-11 and C-12 was assigned by **NOESY** ($CDCl_3$, 500 MHz). H-11 showed NOE with H-9 and H-12, thus confirming the predicted stereochemistry.

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 90.90 ($CH_2$), 76.25 (C), 74.13 (CH), 71.93 (CH), 62.65 (CH), 55.01 ($CH_3$), 54.48 (CH), 50.51 (CH), 42.18 ($CH_2$), 41.81 (C), 41.60 (CH), 35.87 ($CH_2$), 34.97 (CH), 26.57 ($CH_2$), 26.29 ($CH_3$), 25.70 ($CH_3$), 21.22 ($CH_2$), 20.41 ($CH_2$), 18.68 ($CH_3$), 17.87 (C), 13.61 ($CH_3$), –4.97 ($CH_3$), –5.31 ($CH_3$).

**MS:** m/z ([CI] $^+$, %): 409 ([M-OMOM]$^+$, 32), 391 ([M-OMOM-$H_2O$, 35]$^+$, 259 ([M-OMOM-$H_2O$-TBSOH]$^+$, 100). **HRMS:** calculated for [$C_{24}H_{45}O_3Si$]$^+$ ([M-OMOM]$^+$): 409.3138; found: 409.3134.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TBSO
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ppm
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7
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5
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2
1
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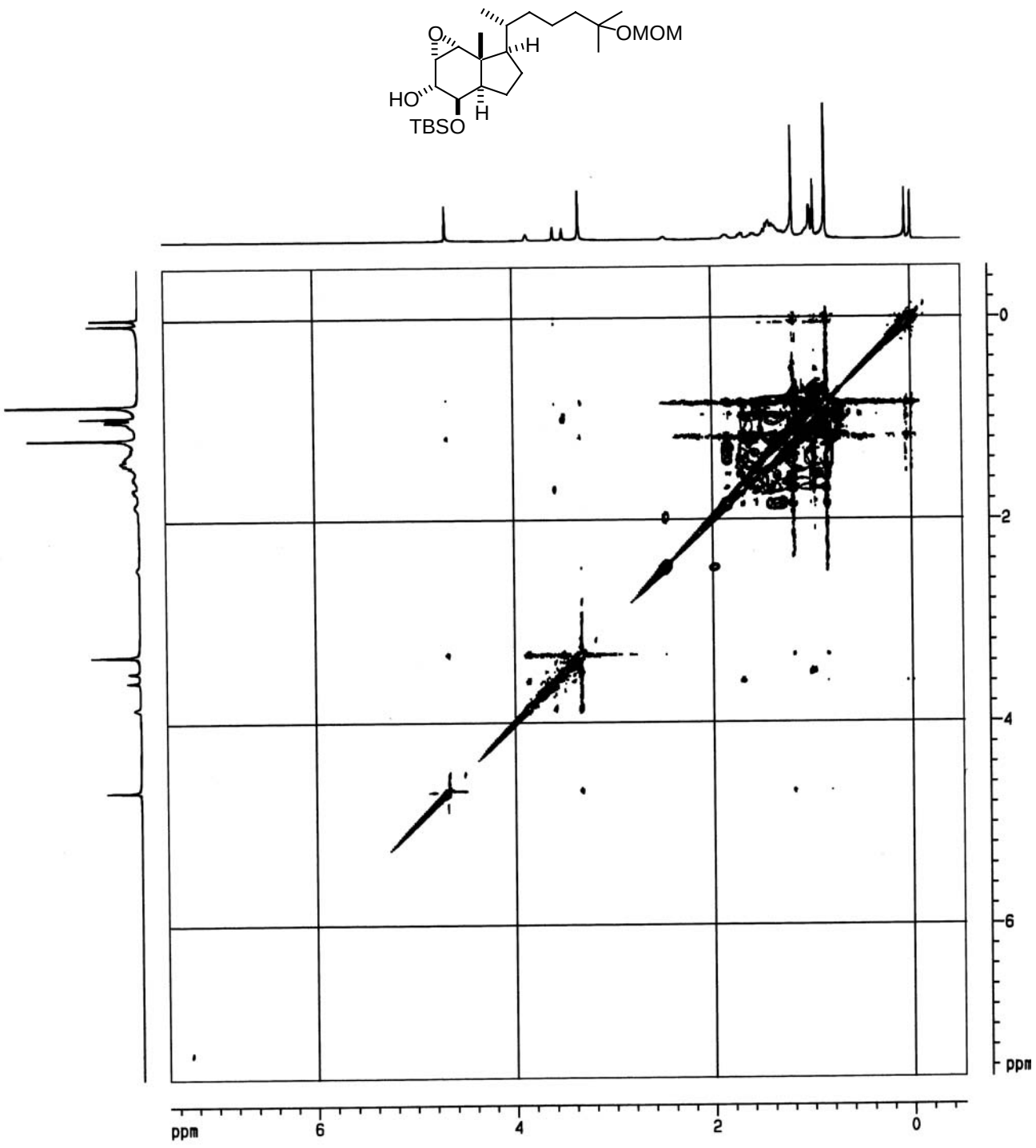
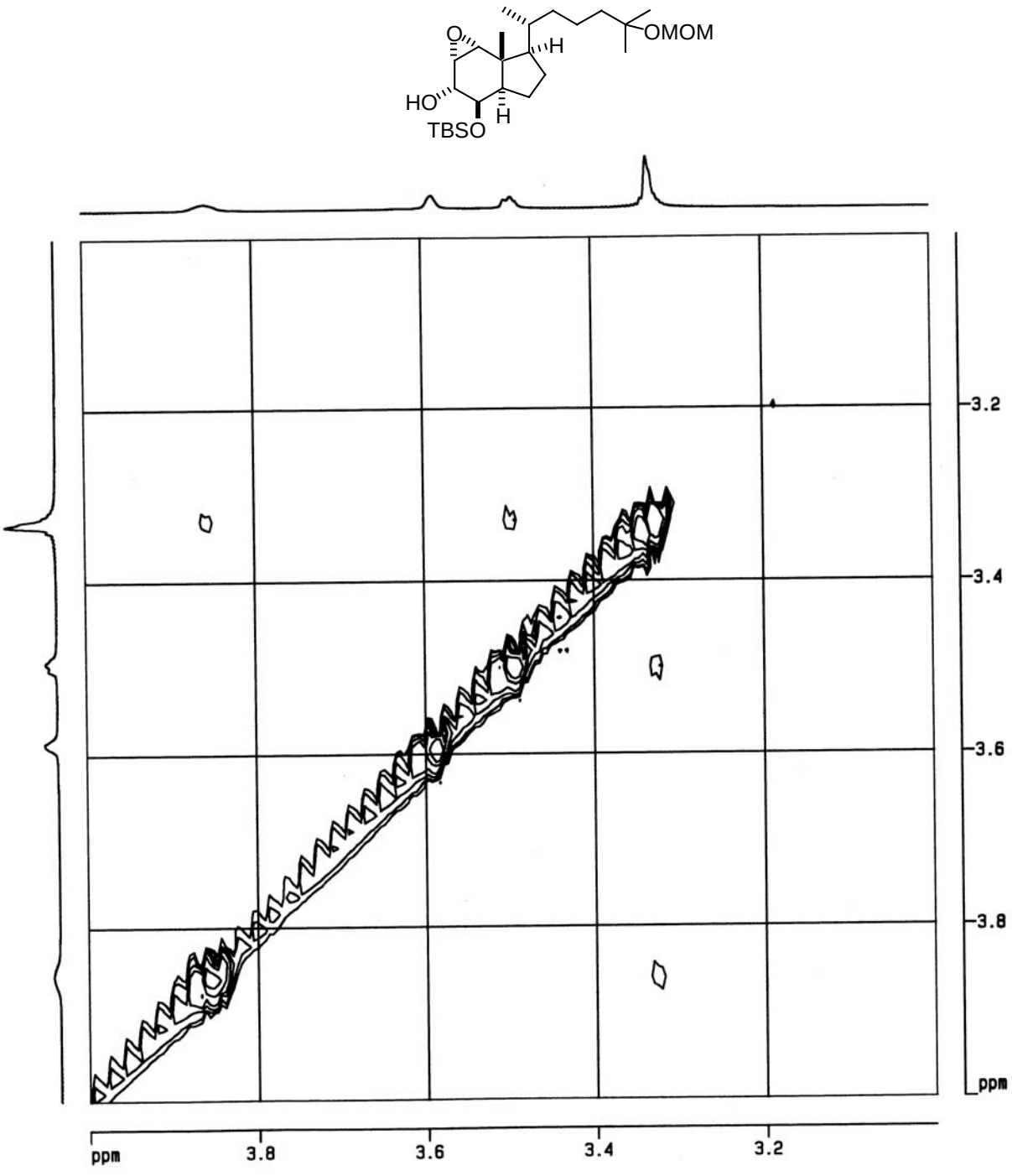
ppm
90
80
70
60
50
40
30
20
10
0

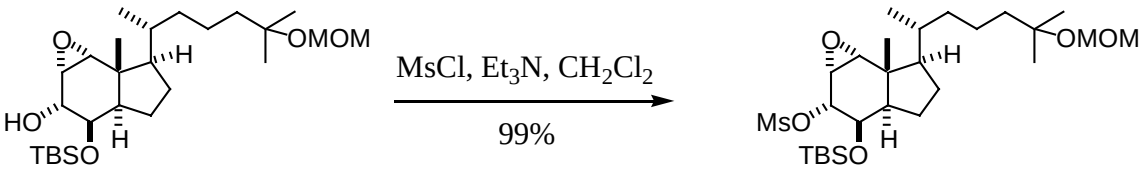
**NOESY**



**NOESY (Detail)**



**6. 8β-*tert*-Butyldimethylsilyloxy-25-methoxymethyloxy-de-*A,B*-11α, 12α-epoxycholestan-9α-yl-methanesulfonate.**

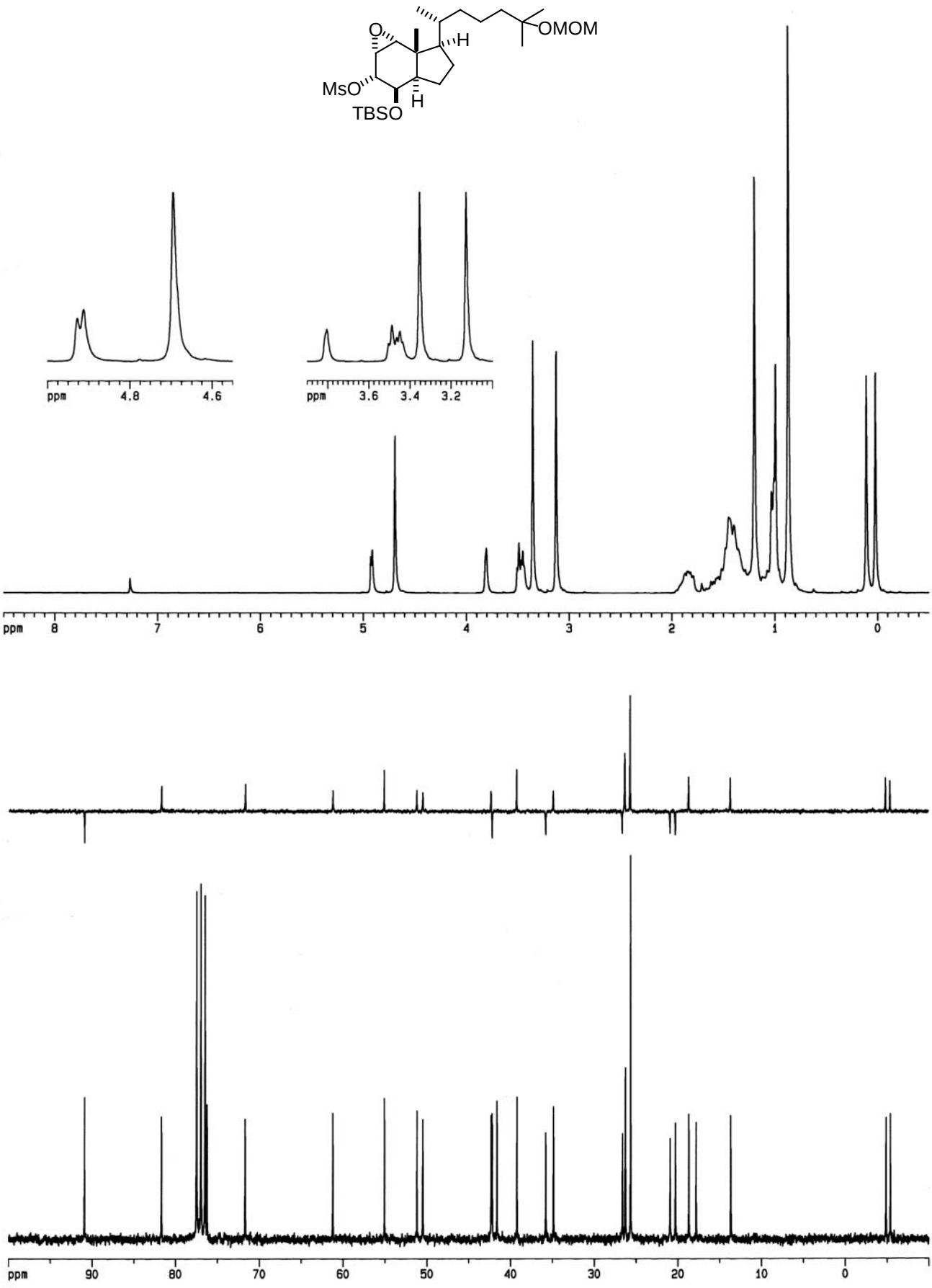



Dry $Et_3N$ (0.85 mL, 6.1mmol) and dry methanesulfonyl chloride (0.44 mL, 5.6 mmol) were successively added to a solution of the starting epoxialcohol (2.21 g, 4.68 mmol) in dry $CH_2Cl_2$ (15 mL) at -20 $^oC$. The mixture was stirred for 3 h and quenched with water (20 mL). The aqueous layer was extracted with $CH_2Cl_2$ (2x15 mL). The combined organic solution was dried, filtered and concentrated in vacuo. The residue was purified by flash column chromatography 10% EtOAc/hexanes) to give the desired product [2.209 g, 4.63 mmol, 99%, $R_f$ = 0.5 (20% EtOAc/hexanes), same $R_f$ as for **28**, white solid (mp 45 $^oC$)].

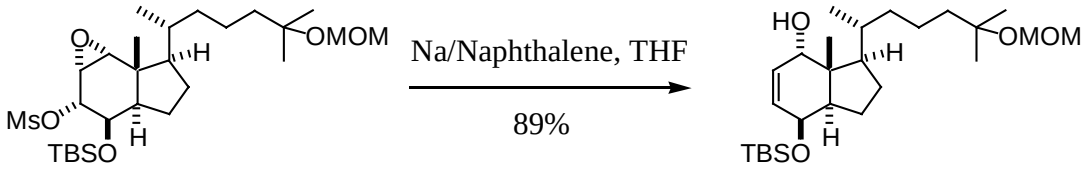
**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 4.92 (1H, d, *J* = 3.7 Hz, H-9), 4.69 (2H, s, $OCH_2O$), 3.80 (1H, s, H-8), 3.51-3.43 (2H, overlapped signals, H-11 and H-12), 3.35 (3H, s, $OCH_3$), 3.13 (3H, s, S-Me),1.20 (6H, s, Me-26 and Me-27), 1.07-1.00 (6H, overlapped signals, Me-21 and Me-18), 0.87 (9H, s, $^t$BuSi), 0.11 (3H, s, MeSi), 0.02 (3H, s, $Me_2Si$).

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 90.93 ($CH_2$), 81.70 (CH), 76.23 (C), 71.70 (CH), 61.23 (CH), 55.06 ($CH_3$), 51.19 (CH), 50.49 (CH), 42.33 (CH), 42.21 ($CH_2$), 41.63 (C), 39.24 ($SCH_3$), 35.80 ($CH_2$), 34.89 (CH), 26.63 ($CH_2$), 26.30 ($CH_3$), 25.67 ($CH_3$), 20.92 ($CH_2$), 20.29 ($CH_2$), 18.69 ($CH_3$), 17.82 (C), 13.69 ($CH_3$), –4.86 ($CH_3$), –5.40 ($CH_3$).

**MS:** m/z ([CI] $^+$, %): 487 ([M-OMOM]$^+$, 18), 391 ([M-OMOM-MsOH, 67]$^+$, 259 ([M-OMOM-MsOH-TBSOH]$^+$, 100). **HRMS:** calculated for $[C_{25}H_{47}O_5SSi]^+$ ([M-OMOM]$^+$): 487.2913; found: 487.2893.


O
H
OMOM
MsO
H
TBSO
ppm
4.8
4.6
ppm
3.6
3.4
3.2
ppm
8
7
6
5
4
3
2
1
0
ppm
90
80
70
60
50
40
30
20
10
0

**7. 8β-*tert*-Butyldimethylsilyloxy-25-methoxymethyloxy-de-*A*,*B*-cholest-9(11)-en-12α-ol.**



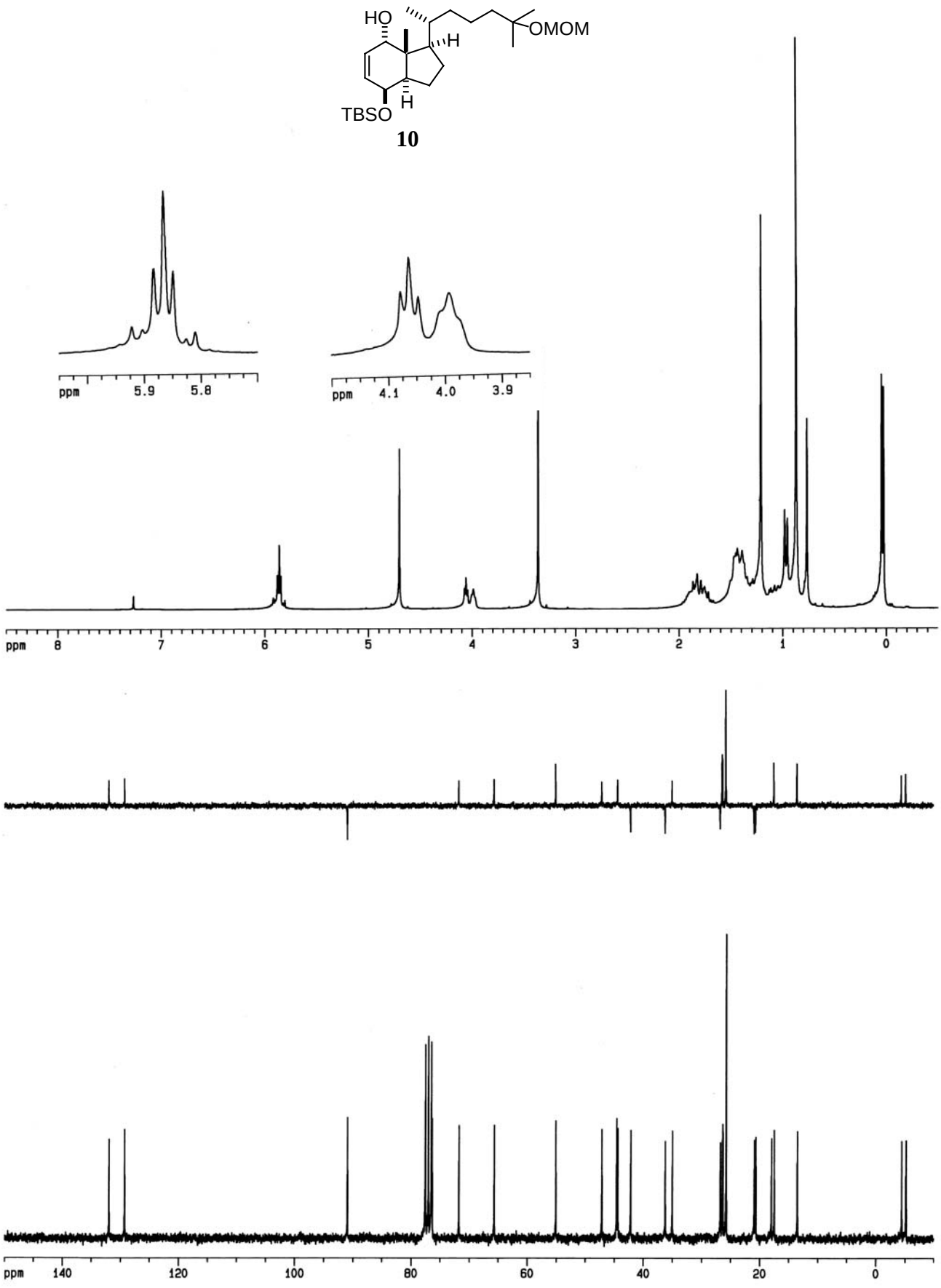

**10**

A 3 M sodium naphthalenide solution was prepared by the addition of a solution of naphthalene (3.84 g, 30 mmol) in dry THF (10 mL) to small pieces of Na (0.69 g, 30 mmol) at rt. After stirring overnight, the deep blue solution was added in 3 mL portions every 30 min to a solution of the starting mesylate (2.29 g, 4.18 mmol) in dry THF (20 mL) at rt. The reaction was monitored by TLC (20% AcOEt/hexanes) and quenched with water (80 mL) at –78 ºC. The mixture was extracted with EtOAc (3x60 mL) and the combined organic solution was dried, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (9% EtOc/hexanes) to afford **10** [1.69 g, 26.7 mmol, 89%, $R_f$ = 0.45 (20% EtOAc/hexanes), colorless oil].

**$^{1}$H-RMN** ($CDCl_3$, 250 MHz, δ): 5.86 (2H, overlapped signals, H-9 and H-11), 4.70 (2H, s, $OCH_2O$), 4.06 (1H, t, *J* = 3.9 Hz, H-12), 3.99 (1H, broad triplet, H-8), 3.36 (3H, s, $OCH_3$), 1.21 (6H, s, Me-26 and Me-27), 0.97 (3H, d, *J* = 6.5 Hz, Me-21), 0.87 (9H, s, $^{t}$BuSi), 0.76 (3H, s, Me-18), 0.05 (3H, s, MeSi), 0.03 (3H, s, $Me_2Si$).

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 131.98 (CH), 129.28 (CH), 90.92 ($CH_2$), 76.33 (C), 71.74 (CH), 65.69 (CH), 55.05 ($CH_3$), 47.11 (CH), 44.58 (C), 44.36(CH), 42.17 ($CH_2$), 36.21 ($CH_2$), 35.00 (CH), 26.73 ($CH_2$), 26.36 ($CH_3$), 26.32 ($CH_3$), 25.74 ($CH_3$), 20.89 ($CH_2$), 20.61 ($CH_2$), 17.97 (C), 17.46 ($CH_3$), 13.49 ($CH_3$), –4.47 ($CH_3$), –5.20 ($CH_3$).

**MS:** m/z ([CI] $^{+}$, %): 437 ($[M\text{-}OH]^+$, 17), 393 ($[M\text{-}OMOM]^+$, 24), 375 ($[M\text{-}OMOM\text{-}H_2O]^+$, 81), 243 ($[M\text{-}OMOM\text{-}H_2O\text{-}TBSOH]^+$, 100). **HRMS:** calculated for $[C_{24}H_{45}O_2Si]^+$ ($[M\text{-}OMOM]^+$): 393.3189; found: 393.3190.


HO
H
OMOM
H
TBSO
ppm
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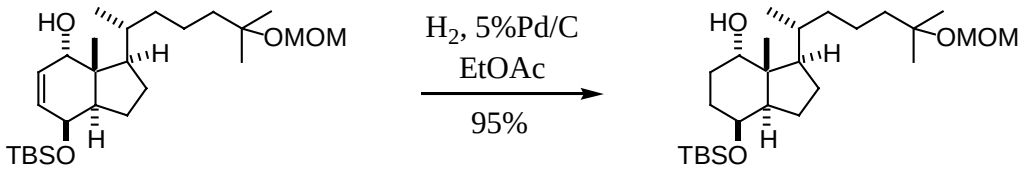
5.8
ppm
4.1
4.0
3.9
ppm
8
7
6
5
4
3
2
1
0
ppm
140
120
100
80
60
40
20
0

**10**

**8. 8β-*tert*-Butyldimethylsilyloxy-25-methoxymethyloxy-de-*A*,*B*-cholestan-12α-ol.**



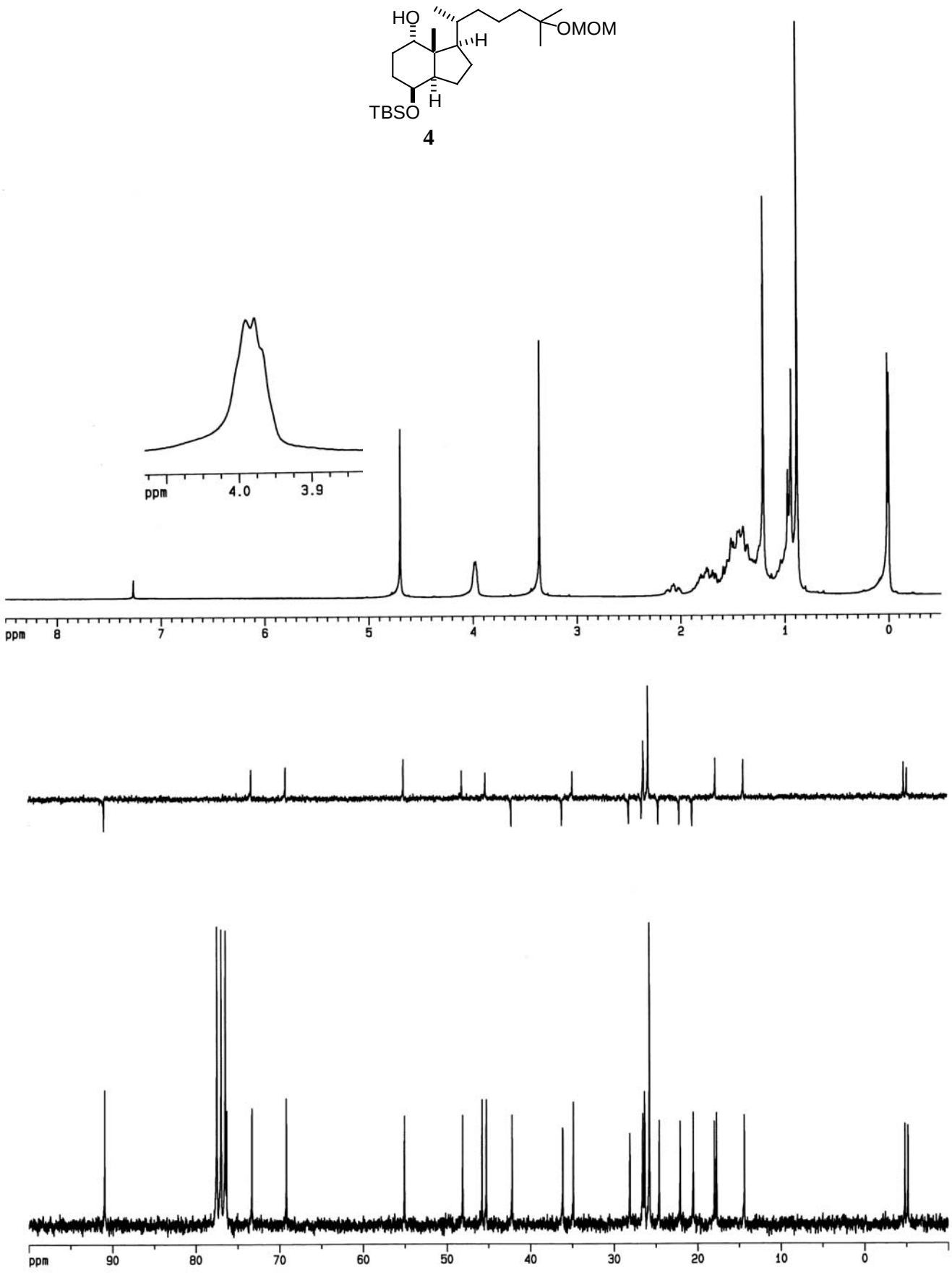

**10** **4**

A catalytic amount of palladium on activated carbon (5% Pd/C, 50 mg) was added to a solution of **10** (1.69 g, 3.71 mmol) in EtOAc (50 mL). The mixture was hydrogenated under balloon pressure for 2 h at rt. The solids were removed by filtration through a short layer of silica gel and the product was eluted with $Et_2O$. After concentration in vacuo, the residue was purified by flash column chromatography (9% EtOAc/hexanes) to afford **4** [1.61 g, 3.52 mmol, 95%, $R_f$ = 0.5 (20% EtOAc/hexanes), white solid (mp 35 $^oC$)].

**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 4.70 (2H, s, $OCH_2O$), 3.98 (2H, overlapped signals, H-12 and H-8), 3.36 (3H, s, $OCH_3$), 1.21 (6H, s, Me-26 and Me-27), 0.97-0.93 (6H, overlapped signals, Me-18 and Me-21), 0.88 (9H, s, $^t$BuSi), 0.01 (3H, s, MeSi), 0.00 (3H, s, $Me_2Si$).

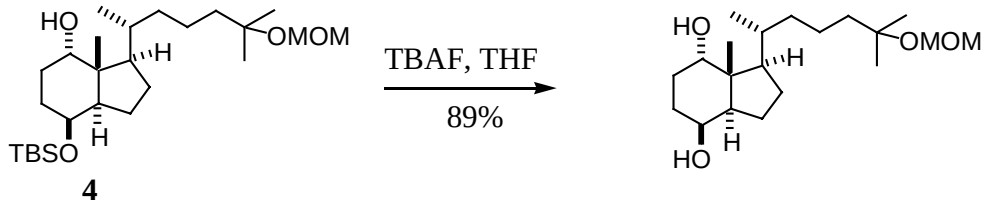
**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 90.94 ($CH_2$), 76.33 (C), 73.31 (CH), 69.19 (CH), 55.06 ($CH_3$), 48.10 (CH), 44.79 (C), 45.28 (CH), 42.19 ($CH_2$), 36.14 ($CH_2$), 34.88 (CH), 28.12 ($CH_2$), 26.59 ($CH_2$), 26.36 ($CH_3$), 26.32 ($CH_3$), 25.78 ($CH_3$), 24.60 ($CH_2$), 22.09 ($CH_2$), 20.51 ($CH_2$), 17.98 (C), 17.72 ($CH_3$), 14.40 ($CH_3$), –4.82 ($CH_3$), –5.19 ($CH_3$).

**MS:** m/z ([CI] $^+$, %): 439 ([M-OH]$^+$, 8), 395 ([M-OMOM]$^+$, 25), 377 ([M-OMOM-$H_2O$]$^+$, 41), 245 ([M-OMOM-$H_2O$-TBSOH]$^+$, 100). **HRMS:** calculated for [$C_{24}H_{47}O_2Si$]$^+$ ([M-OMOM]$^+$): 395.3345; found: 395.3334.


HO
H
OMOM
H
TBSO
ppm
4.0
3.9
ppm
8
7
6
5
4
3
2
1
0
ppm
90
80
70
60
50
40
30
20
10
0

**4**

**9 25-Methoxymethyloxy-de-*A*,*B*-cholestane-8β,12α-diol.**



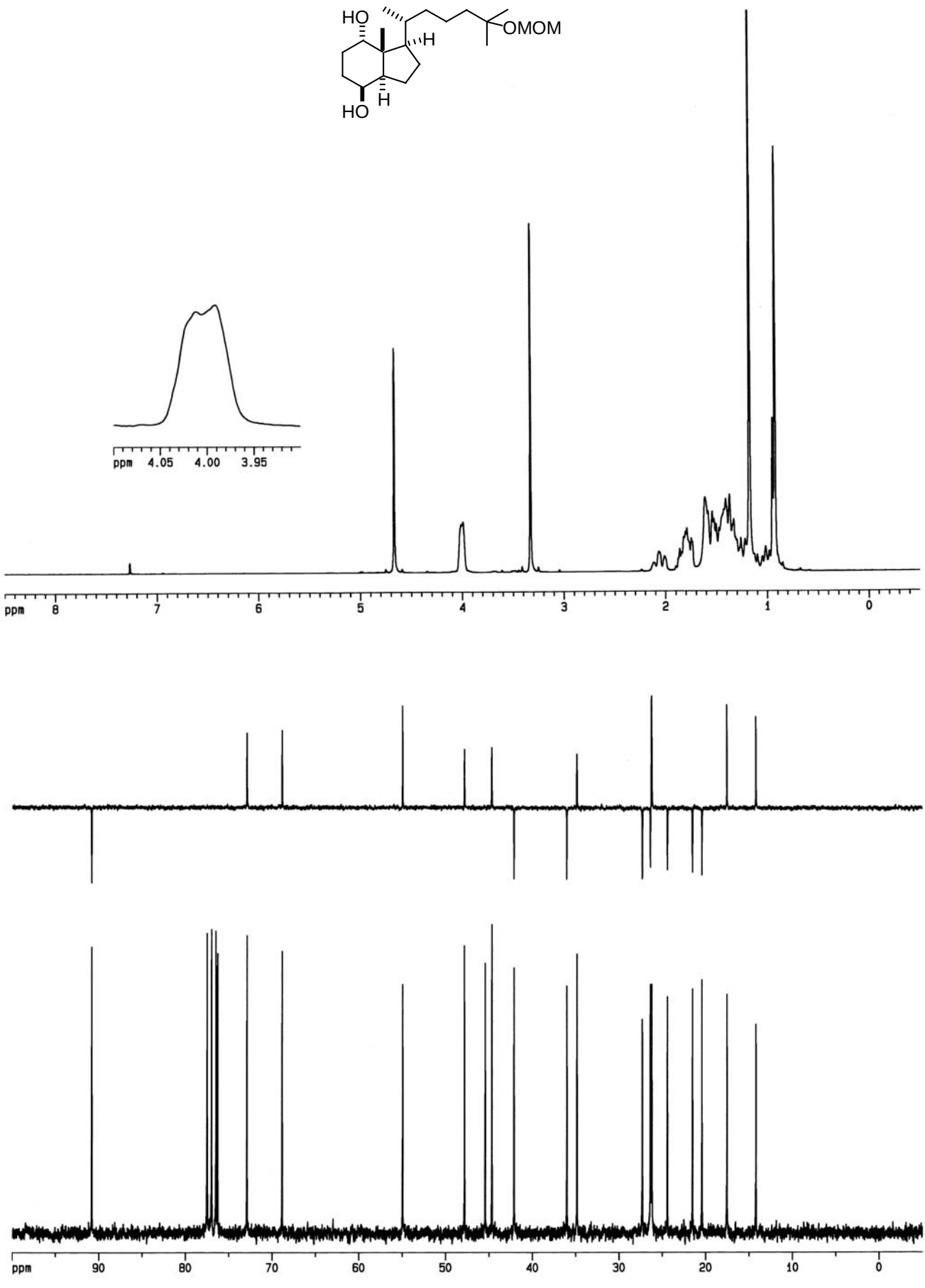

**4**

Tetrabutylamonium fluoride trihydrate (1.83 g, 5.81 mmol) was added to a solution of **4** (0.266 g, 0.581 mmol) in dry THF (6 mL). The mixture was refluxed for 48 h under stirring. Water (50 mL) was added and the aqueous layer was extracted with $Et_2O$ (3x30 mL). The combined organic solution was dried, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (30% EtOAc/$CH_2Cl_2$) to give the desired product [0.177 g, 0.517 mmol, 89%, white semisolid].

**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 4.67 (2H, s, $OCH_2O$), 4.00 (2H, overlapped signals, H-12 and H-8), 3.33 (3H, s, $OCH_3$), 1.18 (6H, s, Me-26 and Me-27), 0.95-0.92 (6H, overlapped signals, Me-18 and Me-21).

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 90.84 ($CH_2$), 76.30 (C), 72.92 (CH), 68.88 (CH), 54.98 ($CH_3$), 47.85 (CH), 45.46 (C), 44.70 (CH), 42.13 ($CH_2$), 36.05 ($CH_2$), 34.86 (CH), 27.33 ($CH_2$), 26.40 ($CH_2$), 26.27 ($CH_3$), 26.23 ($CH_3$), 24.43 ($CH_2$), 21.54 ($CH_2$), 20.46 ($CH_2$), 17.56 ($CH_3$), 14.22 ($CH_3$).

**MS:** m/z ([CI]$^+$, %): 281 ([M-OMOM]$^+$, 14), 263 ([M-OMOM-$H_2O$]$^+$, 100), 245 ([M-OMOM-2$H_2O$]$^+$, 92). **HRMS:** calculated for [$C_{18}H_{33}O_2$]$^+$ ([M-OMOM]$^+$): 281.2481; found: 281.2481.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OMOM
H
HO
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ppm 8 7 6 5 4 3 2 1 0
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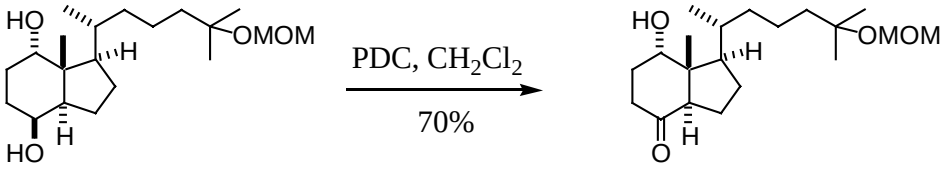
**10. 12α-Hydroxy-5-methoxymethyloxy-de-*A*,*B*-cholestan-8-one.**



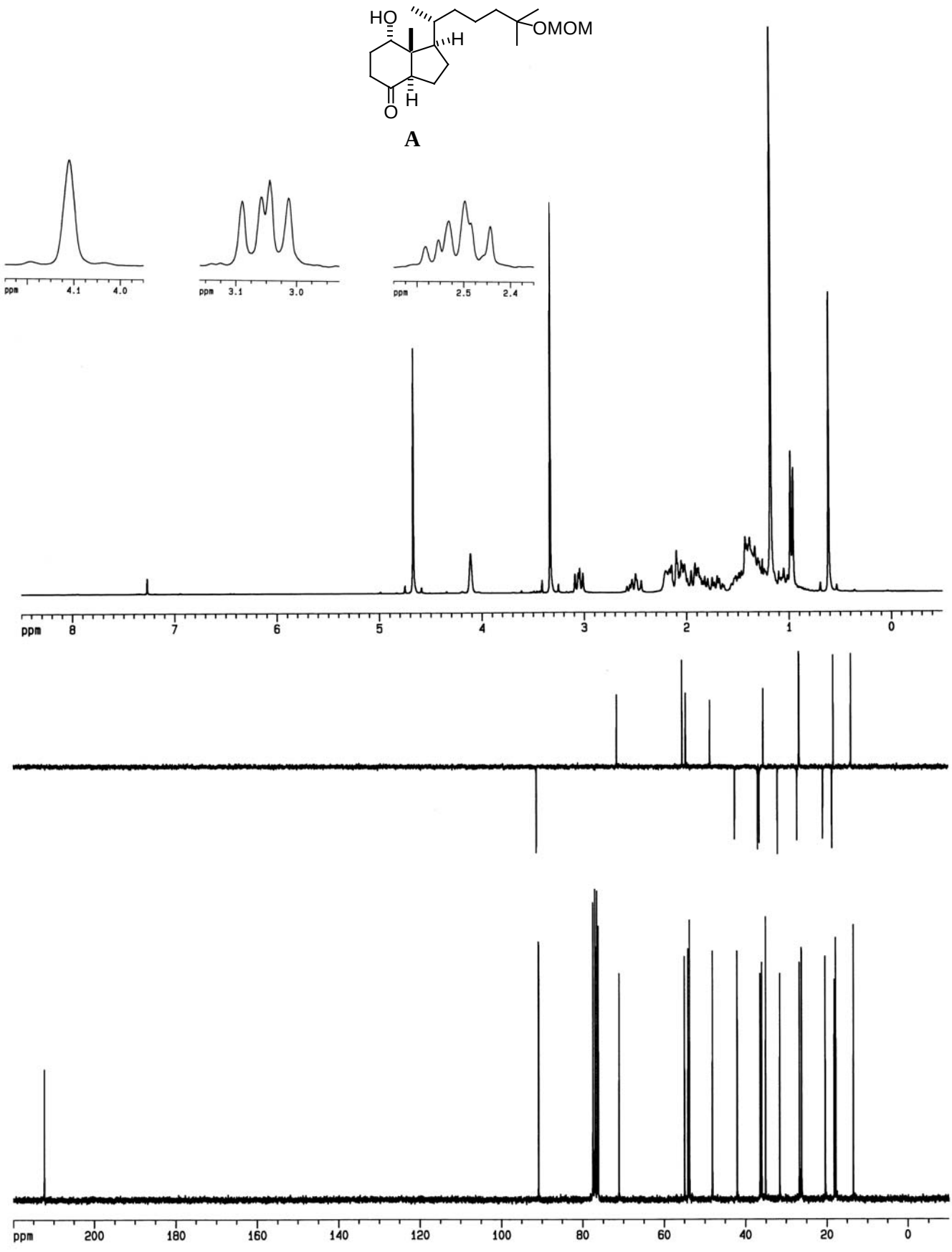

**A**

Pyridinium dicromaate (0.090 g, 0.240 mmol) was added to a stirred solution of the starting diol (0.066 g, 0.192 mmol) in $CH_2Cl_2$ (2 mL) at rt. Stirring was continued until the complete oxidation at C-8 (8 h at rt) as shown by TLC (40% EtOAc/hexanes). Solids were removed by filtration through a layer of silica gel and washed with $Et_2O$. The solution was concentrated in vacuo and the resulting residue was purified by flash column chromatography (20-25% EtOAc/hexanes) to give the desired ketone **A** [0.046 g, 0.135mmol, 70%, $R_f$ = 0.35 (40% EtOAc/hexanes), white solid (mp 59 $^oC$)], and a less polar product [$R_f$ = 0.55 (40% EtOAc/hexanes), presumably the diketone product]. The structure of ketone **A** was determined by comparison of the NMR spectra ($^1H$ and $^{13}C$) with those of ketone **B** (see next experiment).

**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 4.67 (2H, s, $OCH_2O$), 4.11 (1H, s, H-12), 3.33 (3H, s, $OCH_3$), 3.05 (1H, dd, *J* = 11.3 and 7.8 Hz), 2.5 (1H, m), 1.18 (6H, s, Me-26 and Me-27), 0.97 (3 H, d, *J* = 6.4 Hz, Me-21), 0.61 (6H, s, Me-18).

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 212.34 (CO), 90.87 ($CH_2$), 76.21 (C), 71.13 (CH), 55.00 ($CH_3$), 54.14 (CH), 53.76 (C), 48.16 (CH), 42.09 ($CH_2$), 36.43 ($CH_2$), 36.06 ($CH_2$), 35.10 (CH), 31.61 ($CH_2$), 26.80 ($CH_2$), 26.28 ($CH_3$), 26.23 ($CH_3$), 20.42 ($CH_2$), 18.20 ($CH_2$), 17.84 ($CH_3$), 13.52 ($CH_3$).

**MS:** m/z ([CI] $^+$, %): 279 ([M-OMOM]$^+$, 71), 261 ([M-OMOM-$H_2O$]$^+$, 51). **HRMS:** calculated for [$C_{18}H_{31}O_2$]$^+$ ([M-OMOM]$^+$): 279.2324; found: 279.2337.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OMOM
H
O
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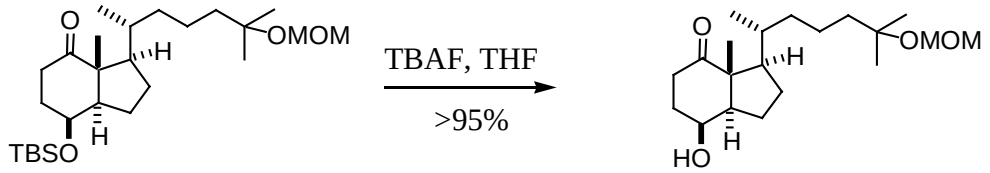
ppm 3.1 3.0
ppm 2.5 2.4
ppm 8 7 6 5 4 3 2 1 0
ppm 200 180 160 140 120 100 80 60 40 20 0

**A**

**11. 25-Methoxymethyloxy-de-*A*,*B*-cholestan-8β-ol-12-one.**

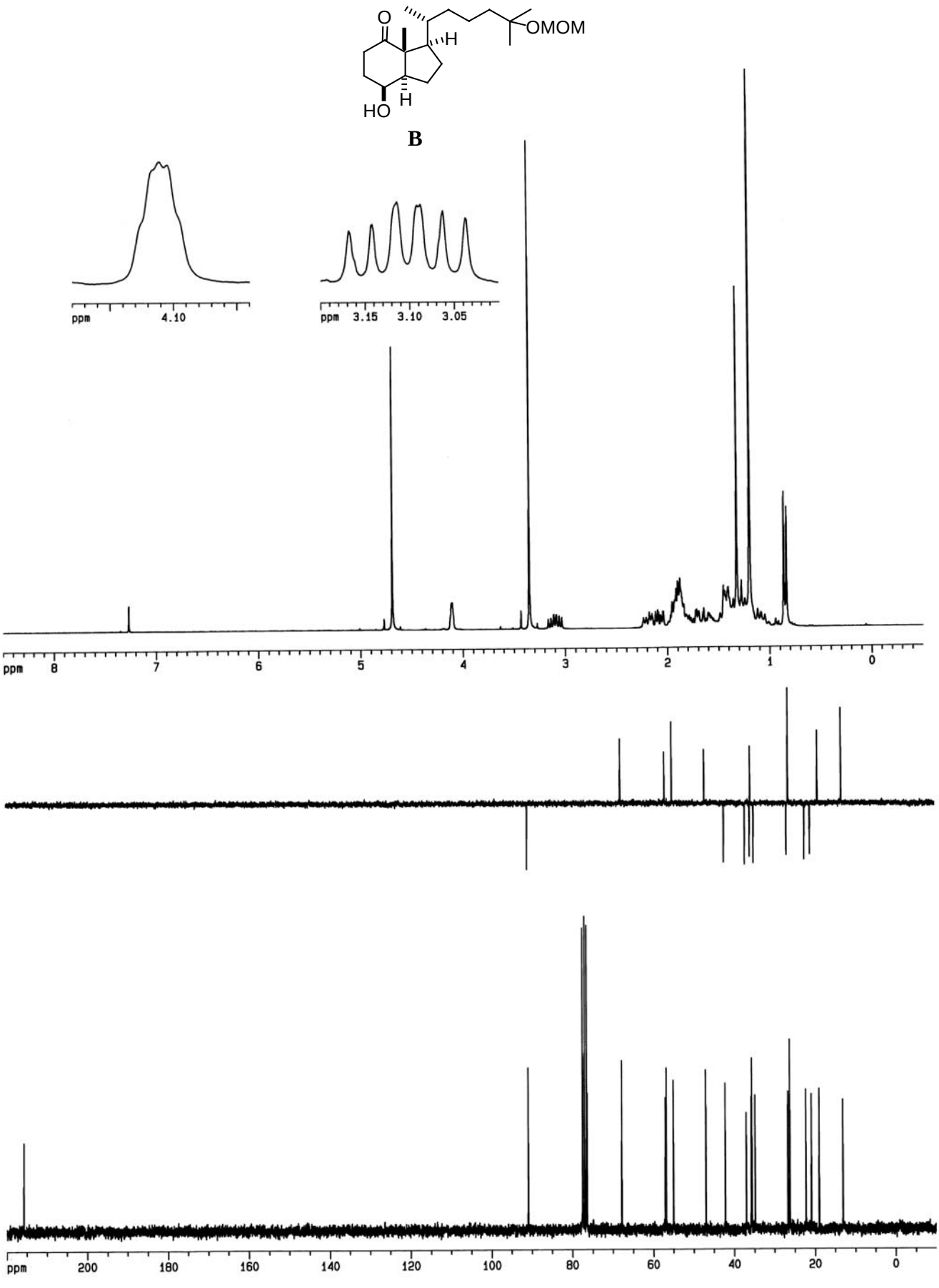



**B**

Tetrabutylamonium fluoride (1.02 mL, 0.878 mmol, 0.86 M solution in THF) was added to a solution of **28** (0.040 g, 0.088 mmol) in dry THF (3 mL). The stirred mixture was refluxed for 16 h. Water (25 mL) was added and the aqueous layer was extracted with $Et_2O$ (3x15 mL). The combined organic solution was dried, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (30% EtOAc/$CH_2Cl_2$) to give **46** [0.030 g, 0.088 mmol, >95%, $R_f$ = 0.35 (40% EtOAc/hexanes), colorless oil].

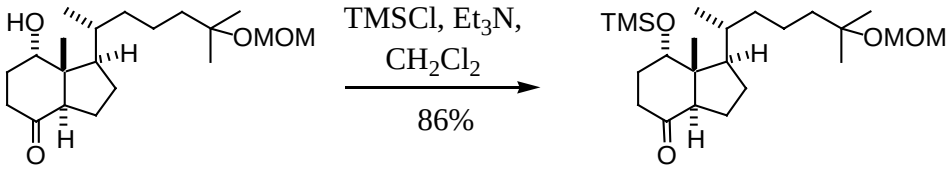
**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 4.69 (2H, s, $OCH_2O$), 4.11 (1H, d, $J$ = 1.6 Hz, H-8), 3.35 (3H, s, $OCH_3$), 3.10 (1H, m, H-11), 1.32 (6H, s, Me-18), 1.20 (6H, s, Me-26 and Me-27), 0.85 (3 H, d, $J$ = 6.5 Hz, Me-21).

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 215.74 (CO), 90.91 ($CH_2$), 76.35 (C), 67.82 (CH), 57.07 (C), 56.80 (CH), 55.04 ($CH_3$), 47.00 (CH), 42.22 ($CH_2$), 37.03 ($CH_2$), 35.84 ($CH_2$), 35.66 (CH), 34.83 ($CH_2$), 26.71 ($CH_2$), 26.28 (2x$CH_3$), 22.28 ($CH_2$), 20.91 ($CH_2$), 18.97 ($CH_3$), 13.12 ($CH_3$).


O
H
OMOM
HO
H
ppm 4.10
ppm 3.15 3.10 3.05
ppm 8 7 6 5 4 3 2 1 0
ppm 200 180 160 140 120 100 80 60 40 20 0

**B**

**12. 25-Methoxymethyloxy-12α-trimethylsilyloxy-de-*A*,*B*-cholestan-8-one.**



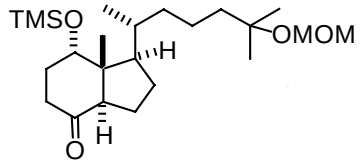

**3a**

A solution of chlorotrimethylsilane (0.50 M) and $Et_3N$ (0.63 M) in dry $CH_2Cl_2$ was prepared by dissolving dry TMSCl (0.148 mL, 1.17 mmol) and dry $Et_3N$ (0.175 g, 1.46 mmol) in dry $CH_2Cl_2$ (2 mL). This solution (1.15 mL) was added to a solution of the starting unprotected alcohol (0.046 g, 0.135 mmol) in dry $CH_2Cl_2$ (2 mL) at –20 ºC. The reaction mixture was allowed to warm to rt for 7 h and then stirred for further 2 h. The reaction was quenched by the addition of water (5 mL) and the aqueous layer was extracted with $CH_2Cl_2$ (3x5 mL). The combined organic solution was dried, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (20% $Et_2O$/EtOAc) to give **3a** [0.048 g, 0.116 mmol, 86%, $R_f$ = 0.5 (20% EtOAc/hexanes), colorless oil].

**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 4.70 (2H, s, $OCH_2O$), 4.11 (1H, s, H-12), 3.37 (3H, s, $OCH_3$), 3.10 (1H, dd, *J* = 11.4 and 7.8 Hz), 2.42 (1H, m), 1.21 (6H, s, Me-26 and Me-27), 0.96 (3 H, d, *J* = 6.5 Hz, Me-21), 0.61 (6H, s, Me-18), 0.10 (9H, s, TMS).

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 212.65 (CO), 90.85 ($CH_2$), 76.28 (C), 72.44 (CH), 55.06 ($CH_3$), 54.53 (CH), 54.20 (C), 47.30 (CH), 42.26 ($CH_2$), 36.40 ($CH_2$), 36.21 ($CH_2$), 35.49 (CH), 31.84 ($CH_2$), 27.00 ($CH_2$), 26.33 ($CH_3$), 26.27 ($CH_3$), 20.31 ($CH_2$), 18.43 ($CH_2$), 18.08 ($CH_3$), 13.42 ($CH_3$), 0.64 ($CH_3$).

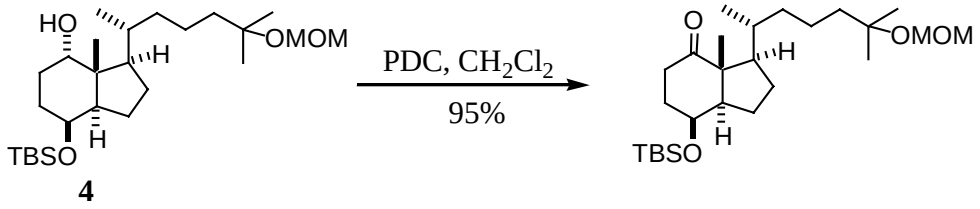
**MS:** m/z ([CI]$^+$, %): 351 ([M-OMOM]$^+$, 12), 261 ([M-OMOM-TMSOH]$^+$, 15). **HRMS:** calculated for $[C_{24}H_{47}O_2Si]^+$ ([M-OMOM]$^+$): 351.2719; found: 351.2736.


TMSO
H
OMOM
H
O

**3a**


ppm 4.15 4.10 4.05
ppm 3.15 3.10 3.05
ppm 2.5 2.4
ppm 8 7 6 5 4 3 2 1 0
ppm 200 180 160 140 120 100 80 60 40 20 0

**13. 8β-*tert*-Butyldimethylsilyloxy-25-methoxymethyloxy-de-*A,B*-cholestan-12-one.**



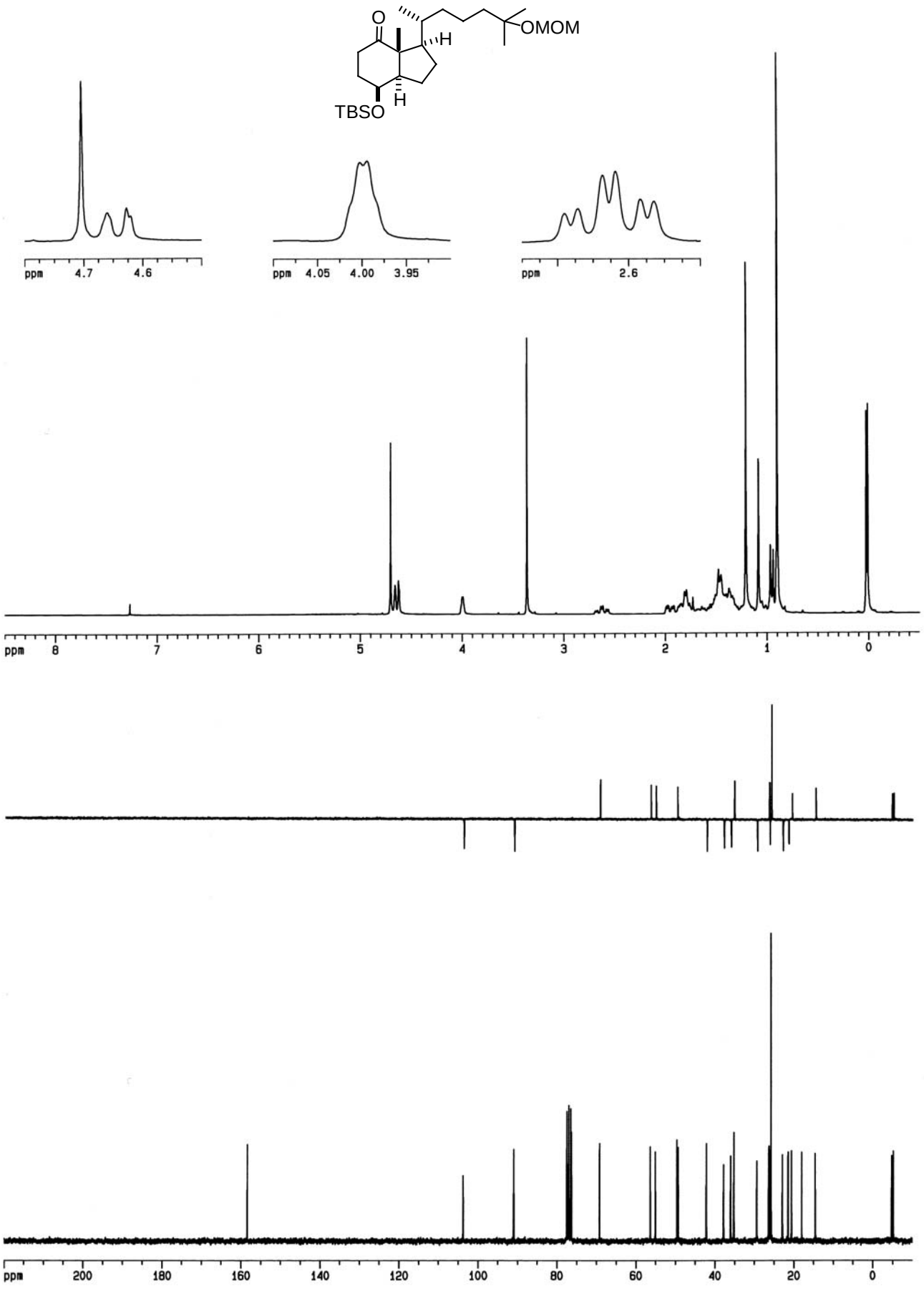

**4**

Pyridinium dicromate (2.84 g, 7.56 mmol) was added to a solution of **4** (1.38 g, 3.02 mmol) in dry $CH_2Cl_2$ (40 mL). The mixture was stirred for 24 h at rt and then filtered through a short layer of silica gel. The solids were washed with $Et_2O$. After concentration in vacuo, the residue was purified by flash column chromatography (5% EtOAc/hexanes) to afford the desired C-12 ketone [1.31 g, 7.18 mmol, 95%, $R_f$ = 0.5 (18% EtOAc/hexanes), colorless oil].

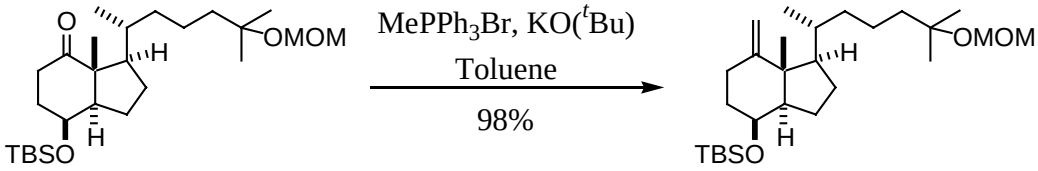
**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 4.69 (2H, s, $OCH_2O$), 4.01 (1H, s, H-8), 3.35 (3H, s, $OCH_3$), 3.06 (1H, td, *J* = 13.5 and 6.2 Hz, H-11α), 1.30 (3H, s, H-18), 1.19 (6H, s, Me-26 and Me-27), 0.83 (3H, d, *J* = 6.4 Hz, Me-21), 0.88 (9H, s, $^t$BuSi), 0.05 (3H, s, MeSi), 0.04 (3H, s, $Me_2Si$).

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 216.05 (C), 90.90 ($CH_2$), 76.30 (C), 68.20 (CH), 57.25 (CH), 55.02 ($CH_3$), 47.02 (CH), 42.18 ($CH_2$), 37.87 ($CH_2$), 35.83 ($CH_2$), 35.62 (CH), 34.88 ($CH_2$), 26.74 ($CH_2$), 26.29 ($CH_3$), 26.27 ($CH_3$), 25.72 ($CH_3$), 22.74 ($CH_2$), 20.90 ($CH_2$), 19.00 ($CH_3$),17.94 (C), 13.23 ($CH_3$), –4.82 ($CH_3$), –5.19 ($CH_3$).

**MS:** m/z ([CI] $^+$, %): 393 ([M-OMOM]$^+$, 100), 243 ([M-OMOM-$H_2O$-TBSOH]$^+$, 46). **HRMS:** calculated for [$C_{24}H_{47}O_2Si$]$^+$ ([M-OMOM]$^+$): 393.3189; found: 393.3200.


O
H
OMOM
H
TBSO
ppm 4.7 4.6
ppm 4.05 4.00 3.95
ppm 2.6
ppm 8 7 6 5 4 3 2 1 0
ppm 200 180 160 140 120 100 80 60 40 20 0

**14. 8β-*tert*-Butyldimethylsilyloxy-12-methylen-25-methoxymethyloxy-de-*A,B*-cholest-12-ene.**

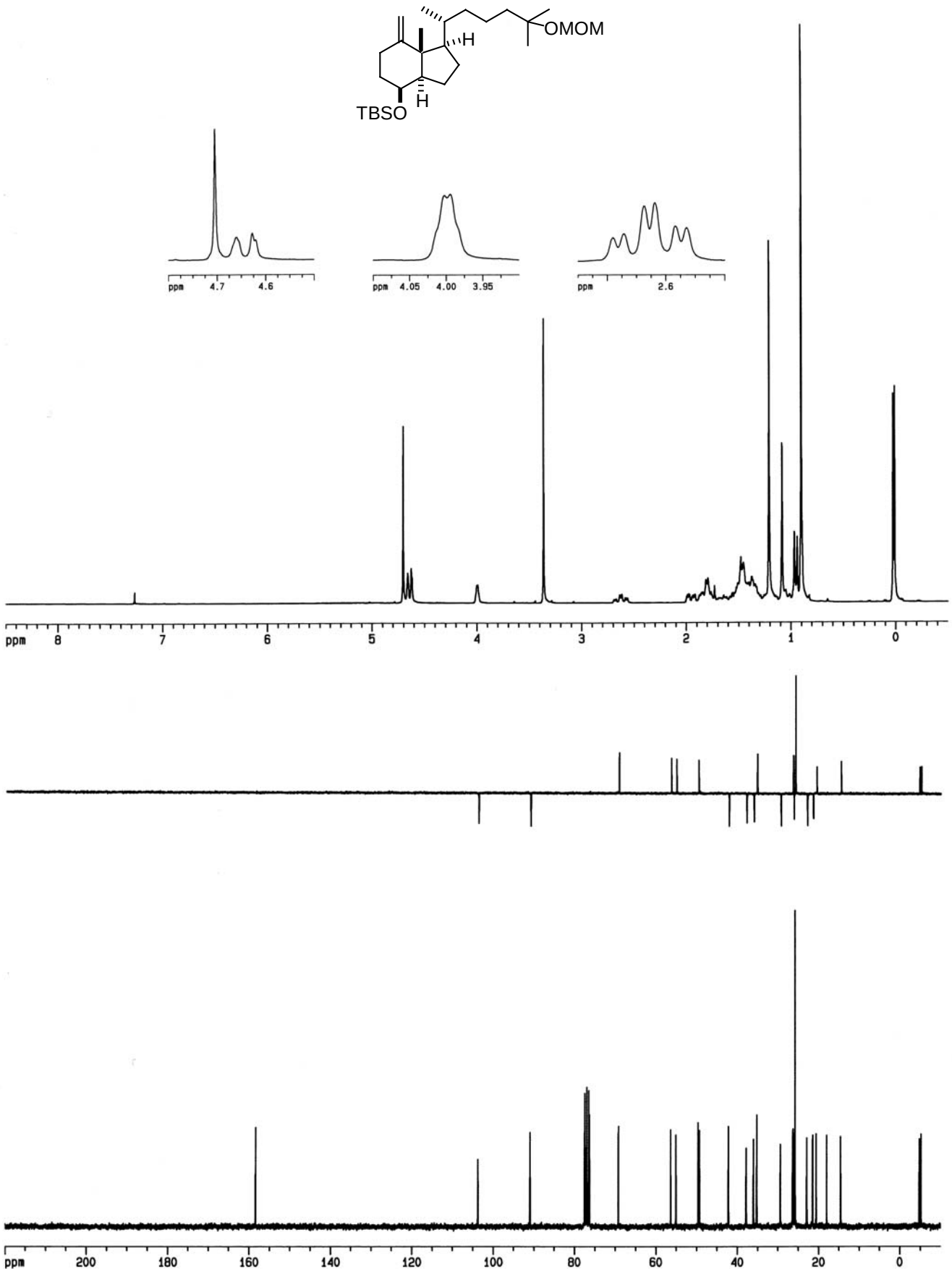



A mixture of dry methyltriphenylphosphonium bromide (2.23 g, 6.25 mmol), potasium *tert*-butoxide (0.651, 5.81 mmol) and dry toluene (40 mL) was heated with stirring at 90 ºC for 30 min. The resulting lemon-yellow solution was added dropwise to a solution of the starting ketone (0.407 g, 0.893 mmol) in dry toluene (20 mL). The mixture was stirred at 90 ºC for 3 h and then allowed to reach rt. Water (60 mL) was added and the mixture was extracted with hexanes (2x40 mL). The combined organic solution was dried, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (2% EtOAc/hexanes) to give the desired product [0.398 g, 0.875 mmol, 98%, $R_f$ = 0.75 (10% EtOAc/hexanes), colorless oil].

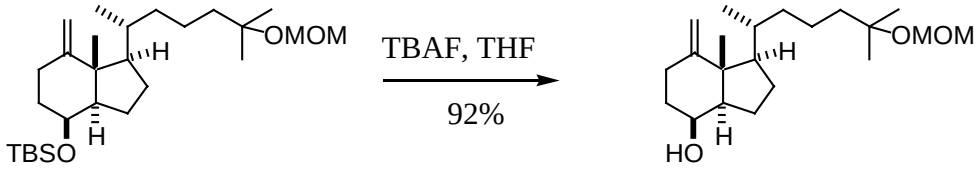
**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 4.70 (2H, s, $OCH_2O$), 4.66 and 4.62 (2H, broad signals, H-12$_Z$ and H-12$_E$), 4.00 (1H, broad signal, H-8), 3.36 (3H, s, $OCH_3$), 2.62 (1H, td, $J$ = 13.3 and 4.6 Hz, H-11α), 1.21 (6H, s, Me-26 and Me-27), 1.08 (3H, s, H-18), 0.95 (3H, d, $J$ = 6.8 Hz, Me-21), 0.90 (9H, s, $^t$BuSi), 0.01 (3H, s, MeSi), -0.05 (3H, s, $Me_2Si$).

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 158.42 (C), 103.76 ($CH_2$), 90.94 ($CH_2$), 76.32 (C), 69.23 (CH), 56.36 (CH), 55.05 ($CH_3$), 49.62 (CH), 49.30 (C), 42.18 ($CH_2$), 37.80 ($CH_2$), 36.04 ($CH_2$), 35.21 (CH), 29.39 ($CH_2$), 26.40 ($CH_3$), 26.29 ($CH_3$), 26.19 ($CH_2$), 25.79 ($CH_3$), 22.88 ($CH_2$), 21.45 ($CH_2$), 20.59 ($CH_3$), 17.99 (C), 14.55 ($CH_3$), –4.78 ($CH_3$), –5.18 ($CH_3$).

**MS:** m/z ([CI] $^+$, %): 453 ($[M+H]^+$, 6), 391 ($[M\text{-}OMOM]^+$, 63), 259 ($[M\text{-}OMOM\text{-}TBSOH]^+$, 100). **HRMS:** calculated for $[C_{25}H_{47}OSi]^+$ ($[M\text{-}OMOM]^+$): 391.3396; found: 391.3395.


OMOM
H
H
TBSO
ppm 4.7 4.6
ppm 4.05 4.00 3.95
ppm 2.6
ppm 8 7 6 5 4 3 2 1 0
ppm 200 180 160 140 120 100 80 60 40 20 0

**15. 25-Methoxymethyloxy-12-methylen-de-*A,B*-cholestan-8β-ol.**

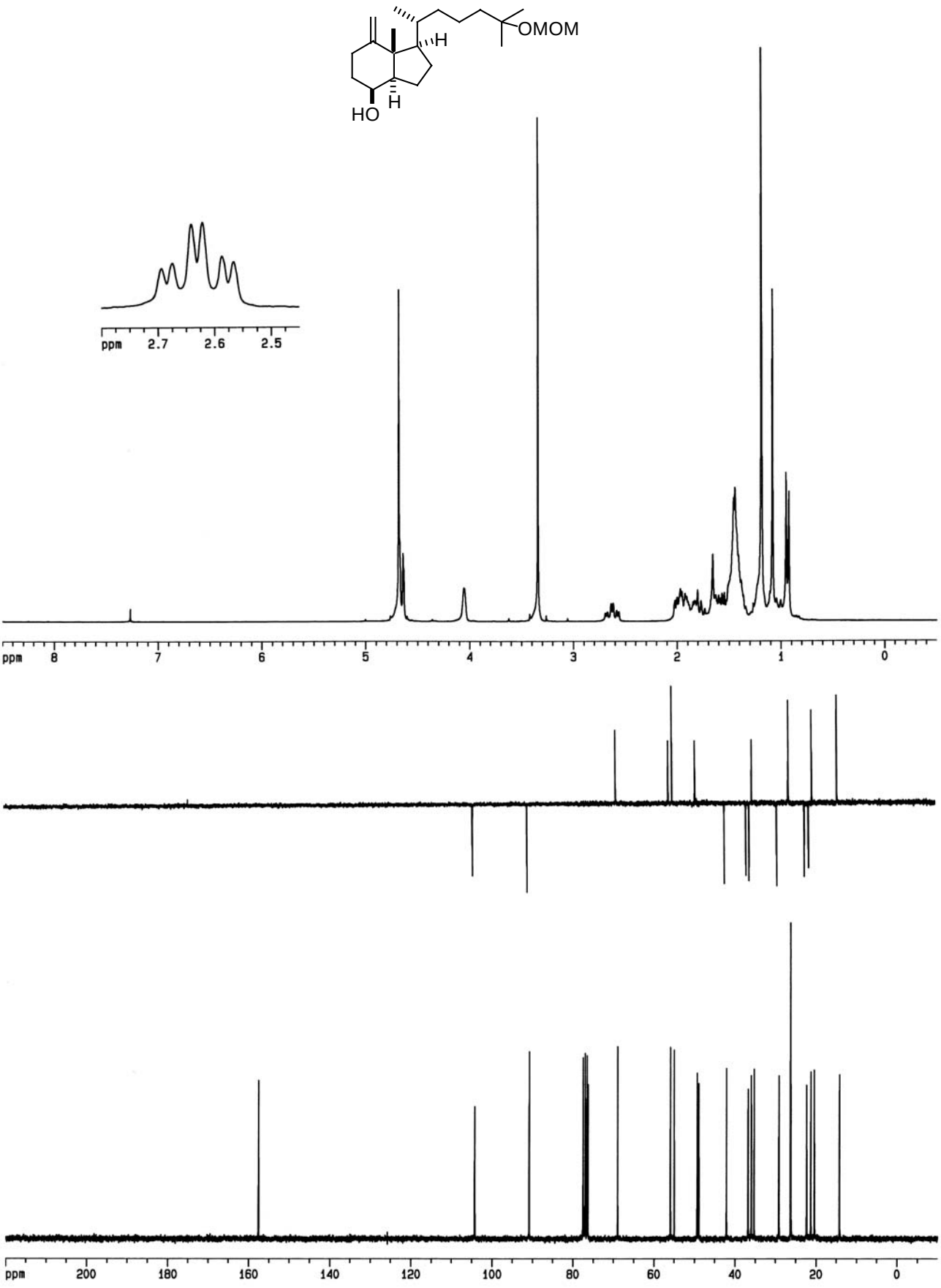



Tetrabutylamonium fluoride trihydrate (3.46 g, 11.0 mmol) was added to a solution of the protected starting alcohol (0.382 g, 0.846 mmol) in dry THF (5 mL). The stirred mixture was refluxed for 24 h. Water (15 mL) was added and the aqueous layer was extracted with $Et_2O$ (3x10 mL). The combined organic solution was dried, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (14% EtOAc/hexanes) to give the desired product [0.262 g, 0.778 mmol, 92%, $R_f$ = 0.45 (20% EtOAc/hexanes), colorless oil].

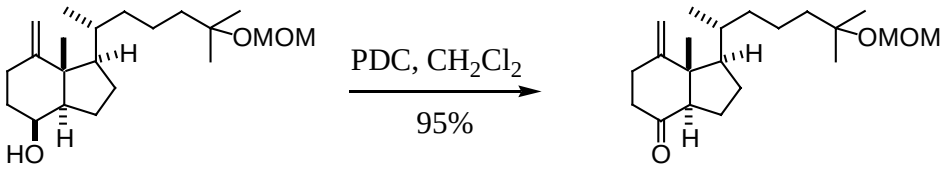
**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 4.68 (4H) and 4.64 (1H) (overlapped signals for $OCH_2O$, H-12$_Z$ and H-12$_E$), 4.06 (1H, broad s, H-8), 3.34 (3H, s, $OCH_3$), 2.63 (1H, td, $J$ = 13.4 and 4.9 Hz, H-11α), 1.19 (6H, s, Me-26 and Me-27), 1.08 (3H, s, H-18), 0.94 (3H, d, $J$ = 6.8 Hz, Me-21).

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 157.58 (C), 104.29 ($CH_2$), 90.86 ($CH_2$), 76.31 (C), 68.98 (CH), 55.93 (CH), 55.01 ($CH_3$), 49.34 (CH), 48.94 (C), 42.12 ($CH_2$), 36.82 ($CH_2$), 36.02 ($CH_2$), 35.34 (CH), 29.18 ($CH_2$), 26.32 (overlapped $CH_3$ and CH2), 26.22 ($CH_3$), 22.30 ($CH_2$), 21.31 ($CH_2$), 20.42 ($CH_3$), 14.24 ($CH_3$).

**MS:** m/z ([CI] $^+$, %): 321 ([M-$H_2O$]$^+$, 7), 277 ([M-OMOM]$^+$, 77), 259 ([M-OMOM-$H_2O$]$^+$, 100). **HRMS:** calculated for [$C_{19}H_{33}O$]$^+$ ([M-OMOM]$^+$): 277.2531; found: 277.2544.


OMOM
H
H
HO
ppm 2.7 2.6 2.5
ppm 8 7 6 5 4 3 2 1 0
ppm 200 180 160 140 120 100 80 60 40 20 0

**16. 25-Methoxymethyloxy-12-methylen-de-*A*,*B*-cholestan-8-one.**



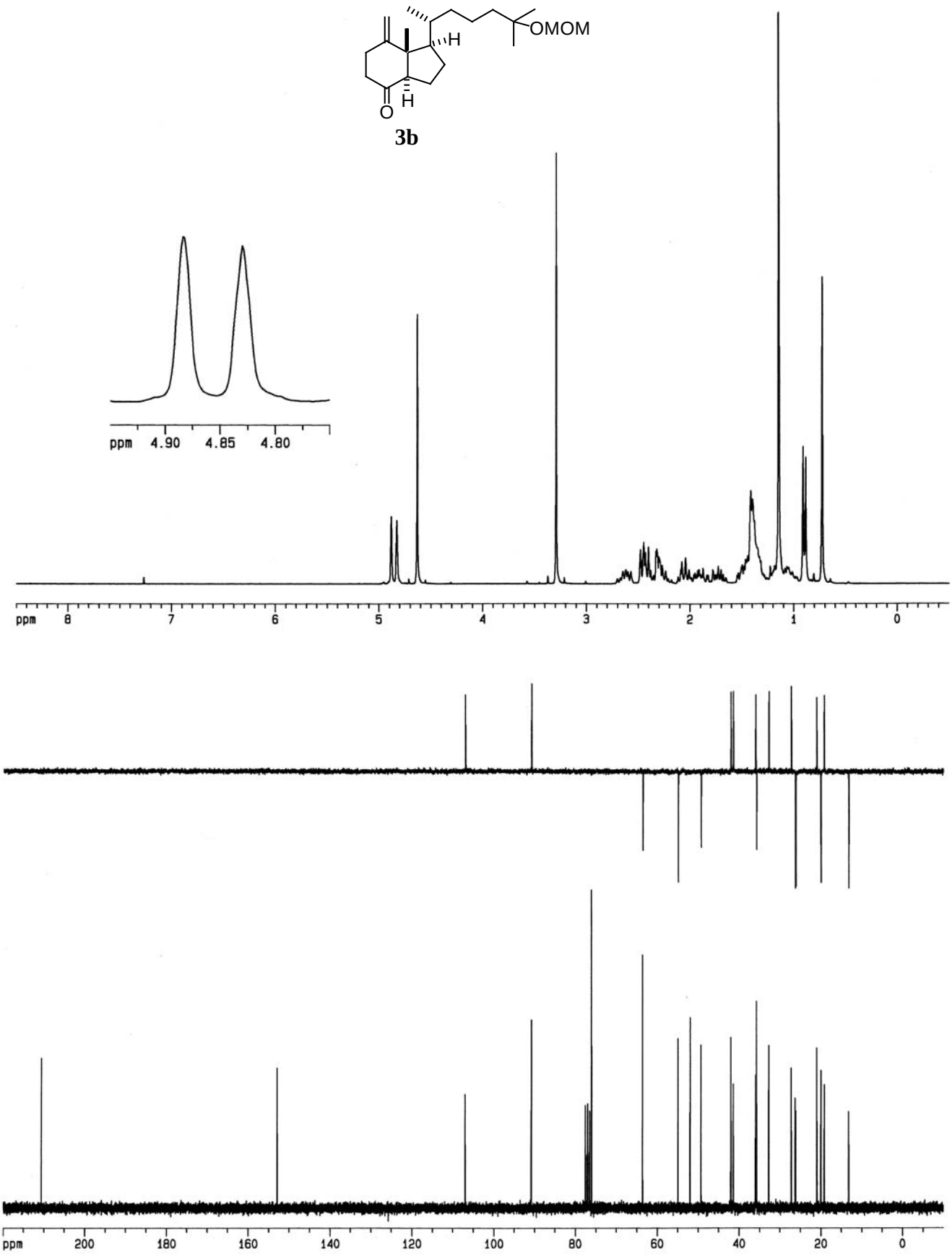

**3b**

Pyridinium dicromate (0.694 g, 0.738 mmol) was added to a solution of the starting alcohol (0.250 g, 0.738 mmol) in dry $CH_2Cl_2$ (15 mL). The mixture was stirred for 6 h at rt and then filtered through a short layer of silica gel. The solids were washed with $Et_2O$. After solvent evaporation in vacuo the residue was purified by flash column chromatography (9% EtOAc/hexanes) to give **3b** [0.237 g, 0.701 mmol, 95%, $R_f$ = 0.65 (20% EtOAc/hexanes), colorless oil].

**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 4.88 (1H, s) and 4.83 (1H, s) (H-12$_Z$ and H-12$_E$), 4.63 (2H, s, $OCH_2O$), 3.29 (3H, s, $OCH_3$), 1.15 (6H, s, Me-26 and Me-27), 0.90 (3H, d, $J$ = 6.7 Hz, Me-21), 0.73 (3H, s, H-18).

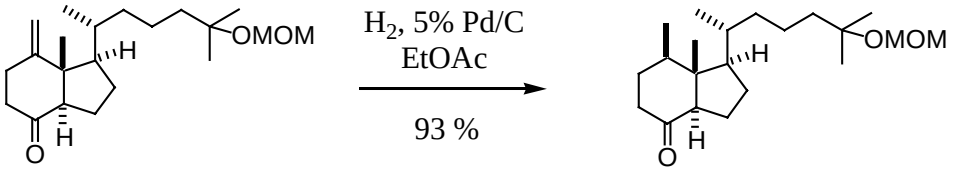
**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 210.59 (CO), 152.87 (C), 106.94 ($CH_2$), 90.77 ($CH_2$), 76.05 (C), 63.57(CH), 52.90 ($CH_3$), 51.93 (C), 49.29 (CH), 42.01 ($CH_2$), 41.42 ($CH_2$), 35.91 ($CH_2$), 35.73 (CH), 32.70 ($CH_2$), 27.20 ($CH_2$), 26.22 ($CH_3$), 26.14 ($CH_3$), 20.95 ($CH_2$), 19.95 ($CH_3$), 19.15 ($CH_2$), 13.18 ($CH_3$).

**MS:** m/z ([CI]$^+$, %): 321 ([M-$H_2O$]$^+$, 7), 275 ([M-OMOM]$^+$, 100), 257 ([M-OMOM-$H_2O$]$^+$, 44). **HRMS:** calculated for [$C_{19}H_{31}O$]$^+$ ([M-OMOM]$^+$): 275.2375; found: 275.2370.


OMOM
H
H
O
ppm 4.90 4.85 4.80
ppm 8 7 6 5 4 3 2 1 0
ppm 200 180 160 140 120 100 80 60 40 20 0

**3b**

**17. 25-Methoxymethyloxy-12β-methyl-de-*A,B*-cholestan-8-one.**



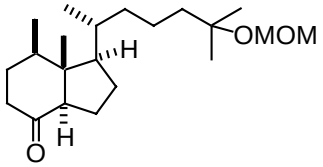

**3b** **3c**

A catalytic amount of palladium on activated carbon (5% Pd/C, 10 mg) was added to a solution of **3b** (0.154 g, 0.457 mmol) in EtOAc (6 mL). The mixture was hydrogenated at balloon pressure for 1 h at rt. The reaction was monitored by TLC (20% EtOAc/hexanes, 2.5% *p*-anisaldehyde, 1% acetic acid and 3.4% sulfuric acid in 95% ethanol). The solids were removed by filtration through a layer of silica gel and the compound was eluted with $Et_2O$. After concentration in vacuo, the residue was purified by flash column chromatography (9% EtOAc/hexanes) to afford **3c** [0.144 g, 0.425 mmol, 93%, $R_f$ = 0.64 (20% EtOAc/hexanes), colorless oil].

**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 4.58 (2H, s, $OCH_2O$), 3.24 (3H, s, $OCH_3$), 1.10 (6H, s, Me-26 and Me-27), 0.95 (3H, d, *J* = 6.4 Hz, Me-12β), 0.85 (1H, d, *J* = 6.9 Hz, Me-21), 0.47 (3H, s, H-18).
The absolute configuration at C-12 was assigned by **NOESY** ($CDCl_3$, 500 MHz). NOE was observed between Me-12β and Me-18 showing that both are in the β face of the molecule.

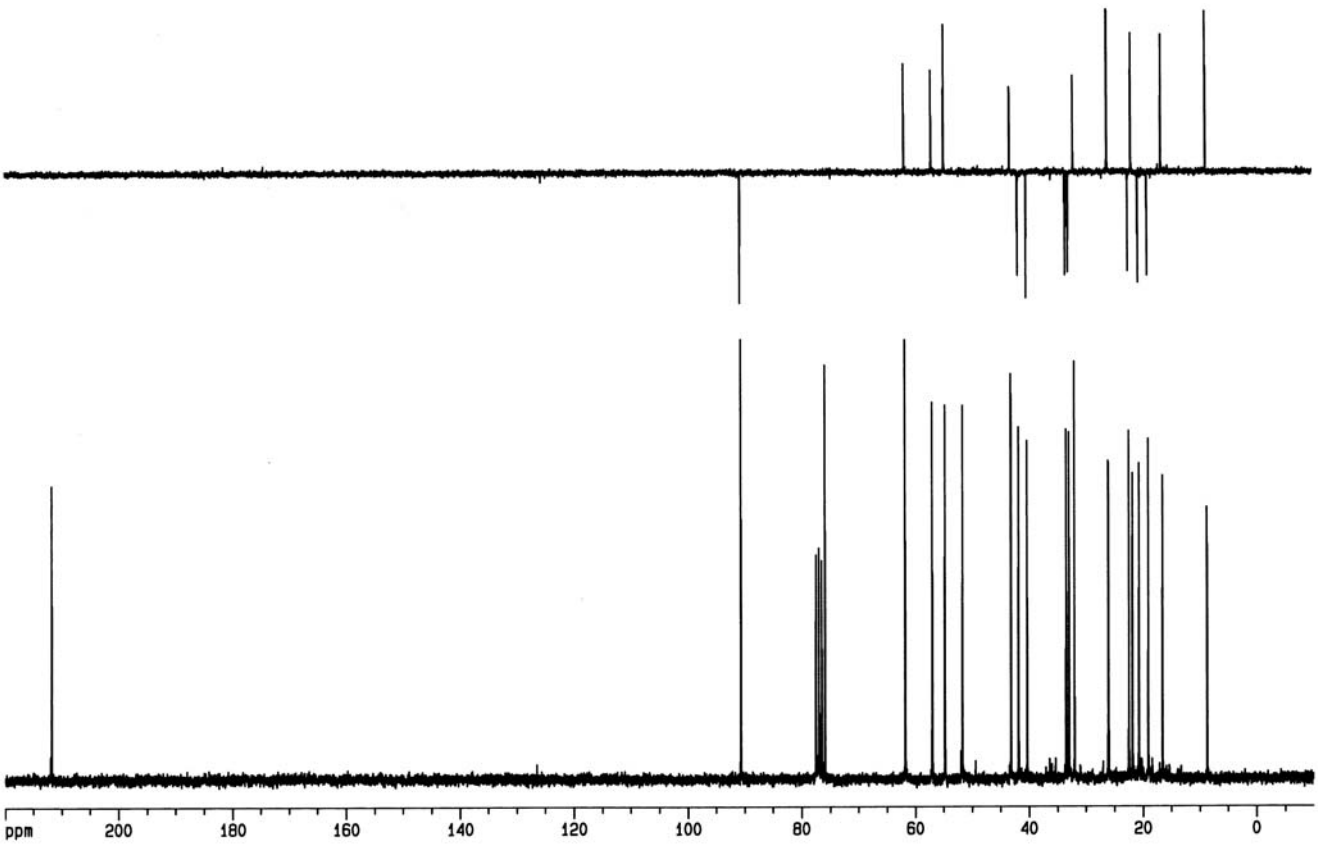
**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 211.85 (CO), 90.68 ($CH_2$), 75.92 (C), 61.80 (CH), 57.01 (CH), 54.79 ($CH_3$), 51.72 (C), 43.22 (CH), 41.90 ($CH_2$), 40.39 ($CH_2$), 33.51 ($CH_2$), 32.99 ($CH_2$), 32.05(CH), 26.12 ($CH_3$), 26.07 ($CH_3$), 22.50 ($CH_2$), 21.85 ($CH_3$), 20.74 ($CH_2$), 19.09 ($CH_2$), 16.58 ($CH_3$), 8.73 ($CH_3$).

**MS:** m/z ([CI] $^+$, %): 277 ([M-OMOM]$^+$, 32), 259 ([M-OMOM-$H_2O$]$^+$, 47). **HRMS:** calculated for [$C_{19}H_{33}O$]$^+$ ([M-OMOM]$^+$): 277.2531; found: 277.2528.

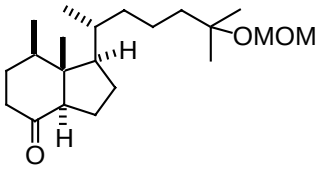



**3c**

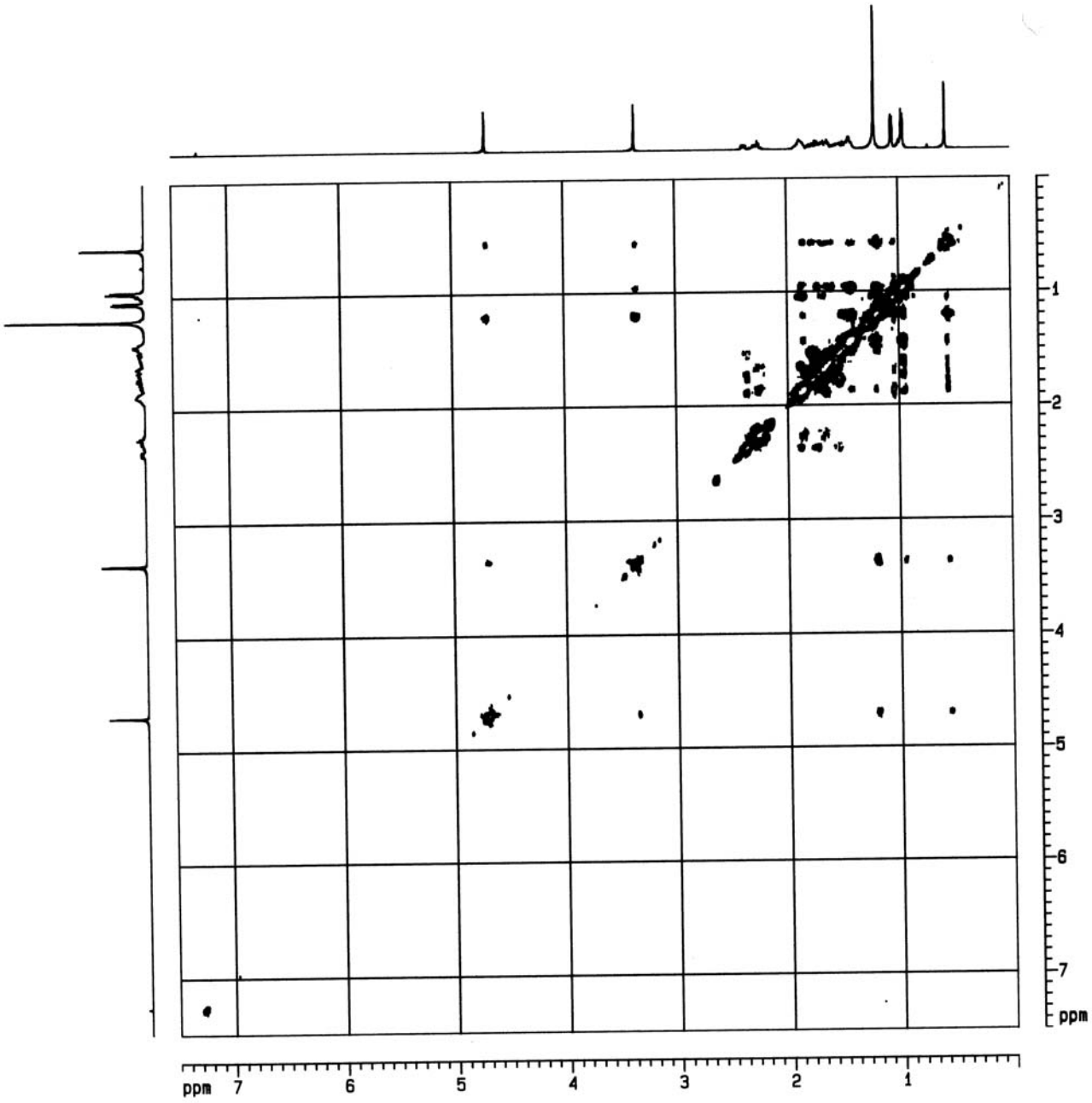


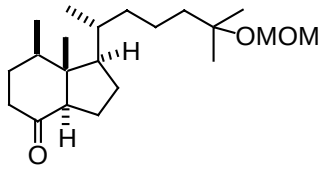
**NOESY**



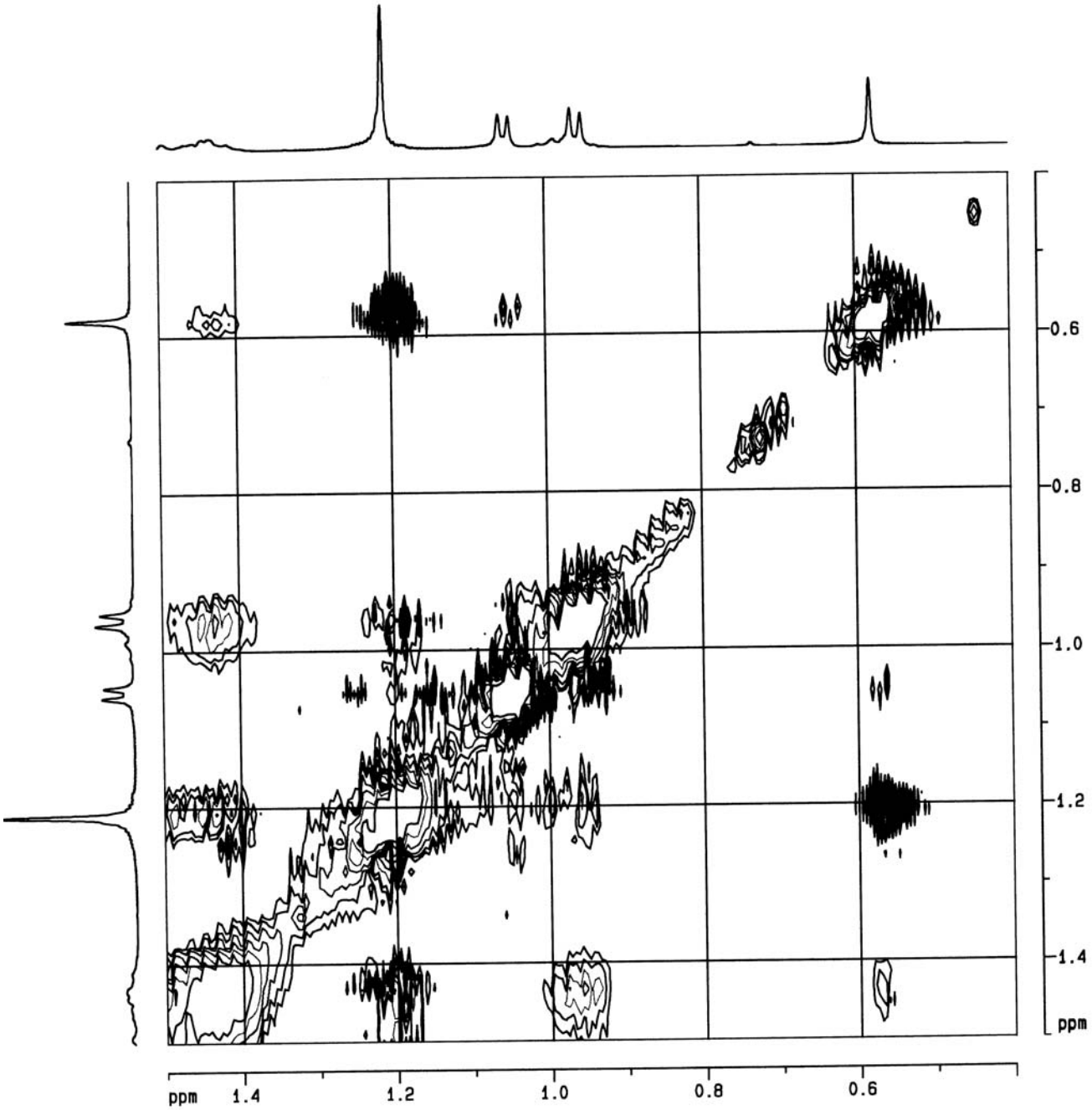

**3c**



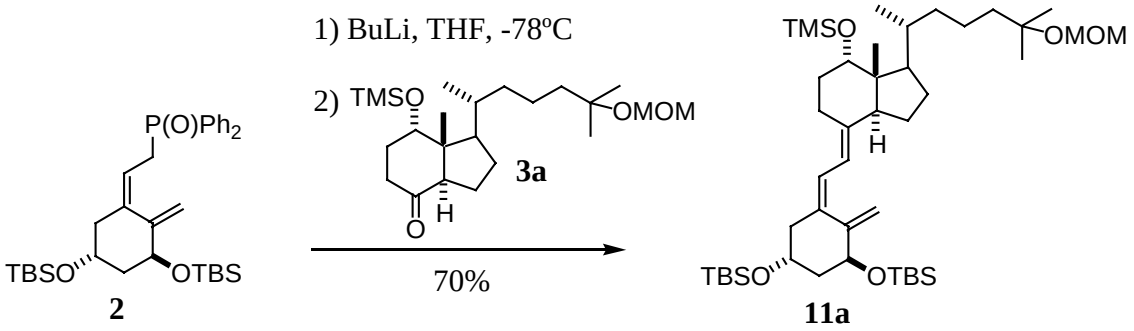
**NOESY (detail)**




**3c**



**18. 1α-*tert*-Butyldimethylsilyloxy-25-methoxymethyloxy-12α-trimethylsilyloxy-vitamin $D_3$ *tert*-butyldimethylsilyl ether.**

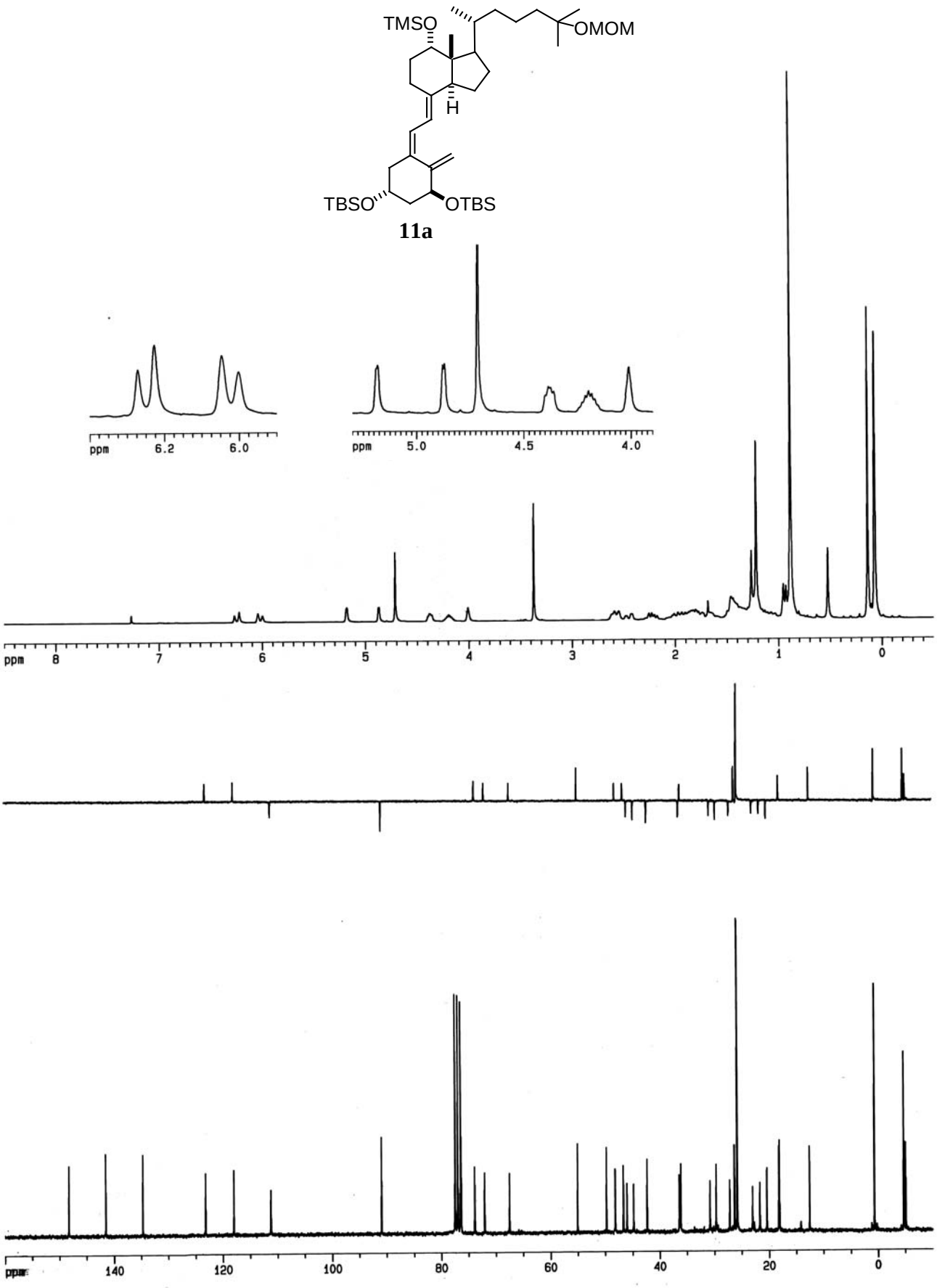



$^{n}$BuLi (0.115 mL, 0.282 mmol, 2.44 M solution) was added dropwise to a solution of the phosphine oxide **2** (0.175, 0.300 mmol) in THF (3 mL) at –78 ºC. The resulting deep red solution was stirred in the dark for 1 h followed by the addition of a solution of the ketone **3a** (0.025 g, 0.060 mmol) in THF (2 mL). The mixture was stirred in the dark for 3 h at –78 ºC and for 2 h at –40 ºC. The reaction was quenched with $H_2O$ (8 mL) and the aqueous layer was extracted with $Et_2O$ (3x7 mL). The combined organic solution was washed with brine (20 mL), dried, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (2-3% EtOAc/hexanes) to give **11a** [0.033 g, 0.042 mmol, 70%, $R_f$ = 0.65 (10% EtOAc/hexanes), colorless oil].

**$^{1}$H-RMN** ($CDCl_3$, 250 MHz, δ): 6.24 and 6.02 (2 H, AB, $J$ = 11.2 Hz, H-6 and H-7), 5.18 (1 H, broad singlet, H-19$_E$), 4.87 (1 H, d, $J$ = 2.1 Hz, H-19$_Z$), 4.71 (2H, s, $OCH_2O$), 4.37 (1 H, m, H-1), 4.19 (1 H, m, H-3), 4.01 (1H, broad singlet, H-12), 3.37 (3H, s, $OCH_3$), 1.22 (6H, s, Me-26 and Me-27), 0.94 (3H, d, $J$ = 4.4 Hz, Me-21), 0.88 (18 H, s, $^{t}$BuSi), 0.52 (3H, s, Me-18), 0.14 (9H, s, TMS), 0.07 (12H, s, MeSi).

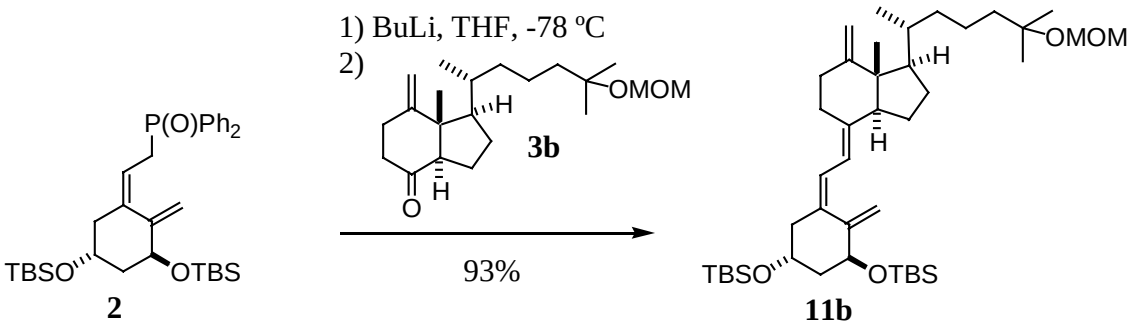
**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 148.25 (C), 141.50 (C), 134.71 (C), 123.17 (CH), 118.00 (CH), 111.24 ($CH_2$), 90.97 ($CH_2$), 76.37 (C), 73.90 (CH), 72.08 (CH), 67.51 (CH), 57.17 (CH), 55.05 ($CH_3$), 49.77 (C), 46.69 (CH), 46.02 ($CH_2$), 44.80 ($CH_2$), 42.32 ($CH_2$), 36.43 ($CH_2$), 36.17 (CH), 30.82 ($CH_2$), 29.70 ($CH_2$), 27.20 ($CH_2$), 26.36 ($CH_3$), 26.30 ($CH_3$), 25.85 ($CH_3$), 25.80 ($CH_3$), 23.02 ($CH_2$), 21.71 ($CH_2$), 20.39 ($CH_2$), 18.22 (C), 18.13 (C), 18.10 ($CH_3$), 12.55 ($CH_3$), 0.68 ($CH_3$), -4.68 ($CH_3$), -4.80 ($CH_3$), -5.08 ($CH_3$).

**MS:** m/z ([FAB] $^{+}$, %): 777 ([M+1]$^{+}$, 14), 776 ([M]$^{+}$, 11), 715 ([M-MOM]$^{+}$, 11), 625 ([M-MOM-TMSOH]$^{+}$, 28), 493 ([M-MOM-TMSOH-TBSOH]$^{+}$, 55). **HRMS:** calculated for $[C_{44}H_{85}O_5Si_3]^{+}$ ([M]$^{+}$): 777,5705; found: 777.5731.


TMSO
OMOM
H
TBSO
OTBS
ppm
6.2
6.0
ppm
5.0
4.5
4.0
ppm
8
7
6
5
4
3
2
1
0
ppm
140
120
100
80
60
40
20
0

**11a**

**19. 1α-*tert*-Butyldimethylsilyloxy-25-methoxymethyloxy-12-methylen-vitamin $D_3$ *tert*-butyldimethylsilyl ether.**

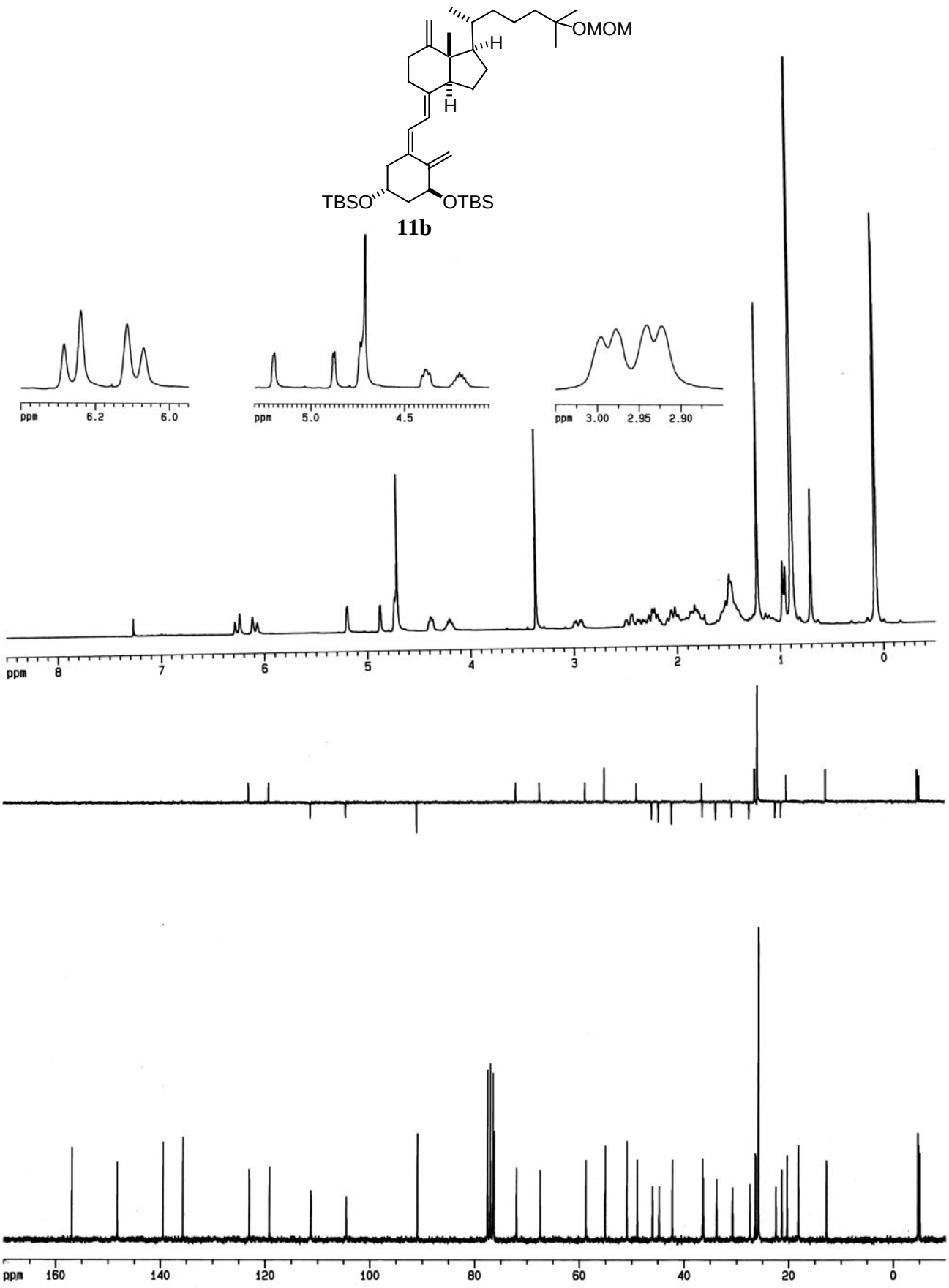



$^n$BuLi (0.253 mL, 0.557 mmol, 2.20 M solution in hexanes) was added dropwise to a stirred solution of the phosphine oxide **2** (0.360, 0.618 mmol) in dry THF (4 mL) at –78 ºC and the resulting deep red solution was stirred for 1 h. A solution of ketone **3b** (0.050 g, 0.148 mmol) in dry THF (3 mL) was slowly added and the resulting mixture was stirred in the dark for 3 h at –78 ºC and for 2 h at –40 ºC. The reaction was quenched with $H_2O$ (15 mL) and the aqueous layer was extracted with $Et_2O$ (3x10 mL). The combined organic solution was washed with brine (20 mL), dried, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (2-3% EtOAc/hexanes) to give **11b** [0.115 g, 0.138 mmol, 93%, $R_f$ = 0.7 (15% EtOAc/hexanes), colorless oil].

**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 6.26 and 6.09 (2 H, AB, *J* = 11.2 Hz, H-6 and H-7), 5.20 (1 H, broad singlet, H-19$_E$), 4.88 (1 H, d, *J* = 2.2 Hz, H-19$_Z$), 4.75-4.70 (4H, overlapped signals, $OCH_2O$, H-12$_Z$ and H-12$_E$), 4.38 (1 H, m, H-1), 4.21 (1 H, m, H-3), 3.37 (3H, s, $OCH_3$), 1.22 (6H, s, Me-26 and Me-27), 0.96 (3H, d, *J* = 6.5 Hz, Me-21), 0.89 and 0.88 (18 H, s, $^t$BuSi), 0.70 (3H, s, Me-18), 0.08 and 0.07 (12H, s, MeSi).

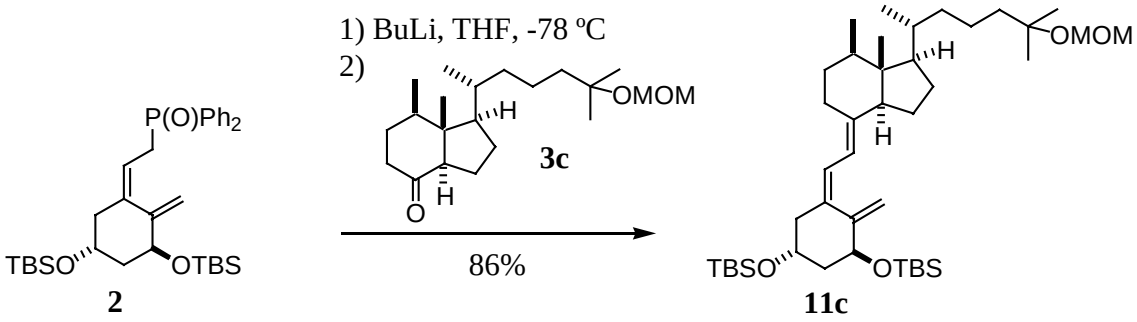
**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 156.95 (C), 148.25 (C), 139.51 (C), 135.72 (C), 123.02 (CH), 119.20 (CH), 111.48 ($CH_2$), 104.51 ($CH_2$), 90.95 ($CH_2$), 76.31 (C), 72.00 (CH), 67.47 (CH), 58.73 (CH), 55.04 ($CH_3$), 50.91 (C), 48.95 (CH), 46.01 ($CH_2$), 44.76 ($CH_2$), 42.21 ($CH_2$), 36.43 (CH), 36.30 ($CH_2$), 33.77 ($CH_2$), 30.71 ($CH_2$), 27.41 ($CH_2$), 26.38 ($CH_3$), 26.30 ($CH_3$), 25.83 ($CH_3$), 25.79 ($CH_3$), 22.45 ($CH_2$), 21.31 ($CH_2$), 20.30 ($CH_3$), 18.21 (C), 18.11 (C), 12.78 ($CH_3$), -4.69 ($CH_3$), -4.71 ($CH_3$), -4.81 ($CH_3$), -5.09 ($CH_3$).

**MS:** m/z ([CI] $^+$, %): 701 ($[M+1]^+$, 41), 639 ($[M\text{-}OMOM]^+$, 84), 507 ($[M\text{-}TBSOH\text{-}OMOM]^+$, 100). **HRMS:** calculated for $[C_{42}H_{77}O_4Si_2]^+$ ($[M+H]^+$): 701.5360; found: 701.5335.


OMOM
H
H
TBSO
OTBS
ppm 6.2 6.0
ppm 5.0 4.5
ppm 3.00 2.95 2.90
ppm 8 7 6 5 4 3 2 1 0
ppm 160 140 120 100 80 60 40 20 0

**11b**

**20. 1α-*tert*-Butyldimethylsilyloxy-25-methoxymethyloxy-12β-methyl-vitamin $D_3$ *tert*-butyldimethylsilyl ether.**

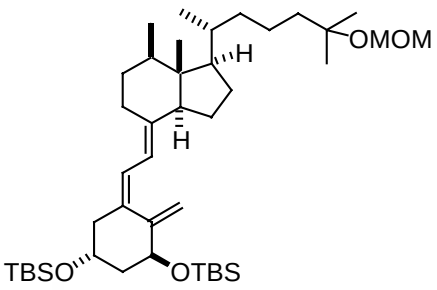



$^n$BuLi (0.245 mL, 0.54 mmol, 2.20 M solution in hexanes) was added dropwise to a stirred solution of the phosphine oxide **2** (0.350, 0.601 mmol) in THF (4 mL) at –78 ºC. After stirring the resulting deep red solution for 1 h, a solution of ketone **3c** (0.048 g, 0.141 mmol) in THF (3 mL) was added dropwise. The mixture was stirred in the dark for 3 h at –78 ºC and for 2 h at –40 ºC. The reaction was quenched with $H_2O$ (15 mL) and the aqueous layer was extracted with $Et_2O$ (3x10 mL). The combined organic solution was washed with brine (20 mL), dried, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (2-3% EtOAc/hexanes) to give **11c** [0.085 g, 0.121 mmol, 86%, $R_f$ = 0.7 (15% EtOAc/hexanes), colorless oil].

**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 6.22 and 6.02 (2 H, AB, *J* = 11.2 Hz, H-6 and H-7), 5.20 (1 H, broad singlet, H-19$_E$), 4.87 (1 H, d, *J* = 2.3 Hz, H-19$_Z$), 4.71 (2H, s, $OCH_2O$), 4.38 (1 H, m, H-1), 4.19 (1 H, m, H-3), 3.37 (3H, s, $OCH_3$), 1.22 (6H, s, Me-26 and Me-27), 0.96-0.88 (24 H, overlapped signals, Me-12β, Me-21 and $^t$BuSi), 0.44 (3H, s, Me-18), 0.07 and 0.00 (12H, s, MeSi).

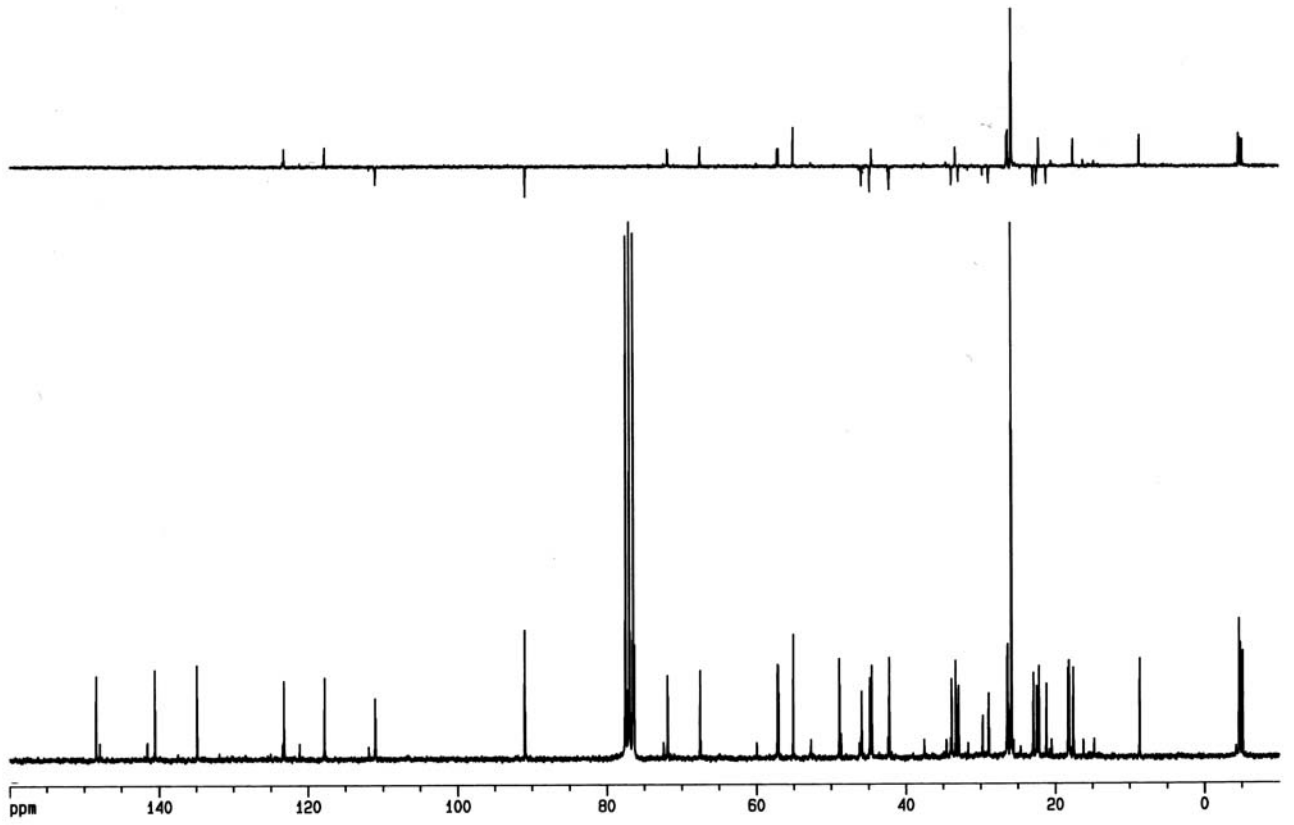
**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 148.38 (C), 140.54 (C), 134.89 (C), 123.22 (CH), 117.78 (CH), 111.03 ($CH_2$), 90.77 ($CH_2$), 76.34 (C), 71.86 (CH), 67.52 (CH), 57.17 (CH), 57.05 (CH), 55.07 ($CH_3$), 48.90 (C), 45.92 ($CH_2$), 44.79 ($CH_2$), 44.53 (CH), 42.21 ($CH_2$), 33.84 ($CH_2$), 33.31 (CH), 32.93 ($CH_2$), 28.89 ($CH_2$), 26.40 ($CH_3$), 26.32 ($CH_3$), 25.84 ($CH_3$), 22.91 ($CH_2$), 22.48 ($CH_2$), 22.15 ($CH_3$), 21.17 ($CH_2$), 18.25 (C), 18.14 (C), 17.57 ($CH_3$), 8.64 ($CH_3$), -4.65 ($CH_3$), -4.70 ($CH_3$), -4.81 ($CH_3$), -5.10 ($CH_3$).

**MS:** m/z ([CI] $^+$, %): 703 ([M+H]$^+$, 43), 641 ([M-OMOM]$^+$, 75), 509 ([M-TBSOH-OMOM]$^+$, 100). **HRMS (CI+)**: calculated for $[C_{42}H_{79}O_4Si_2]^+$ ([M+H]$^+$): 703.5517; found: 703.5495.

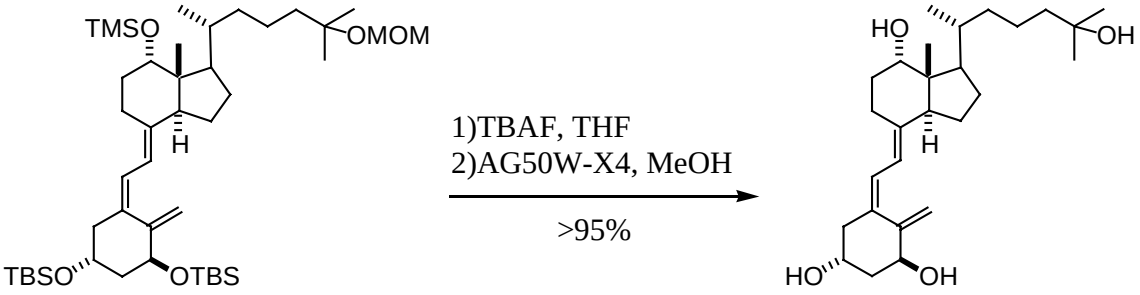



**11c**



**21. 1α,12α,25-Trihydroxyvitamin $D_3$.**



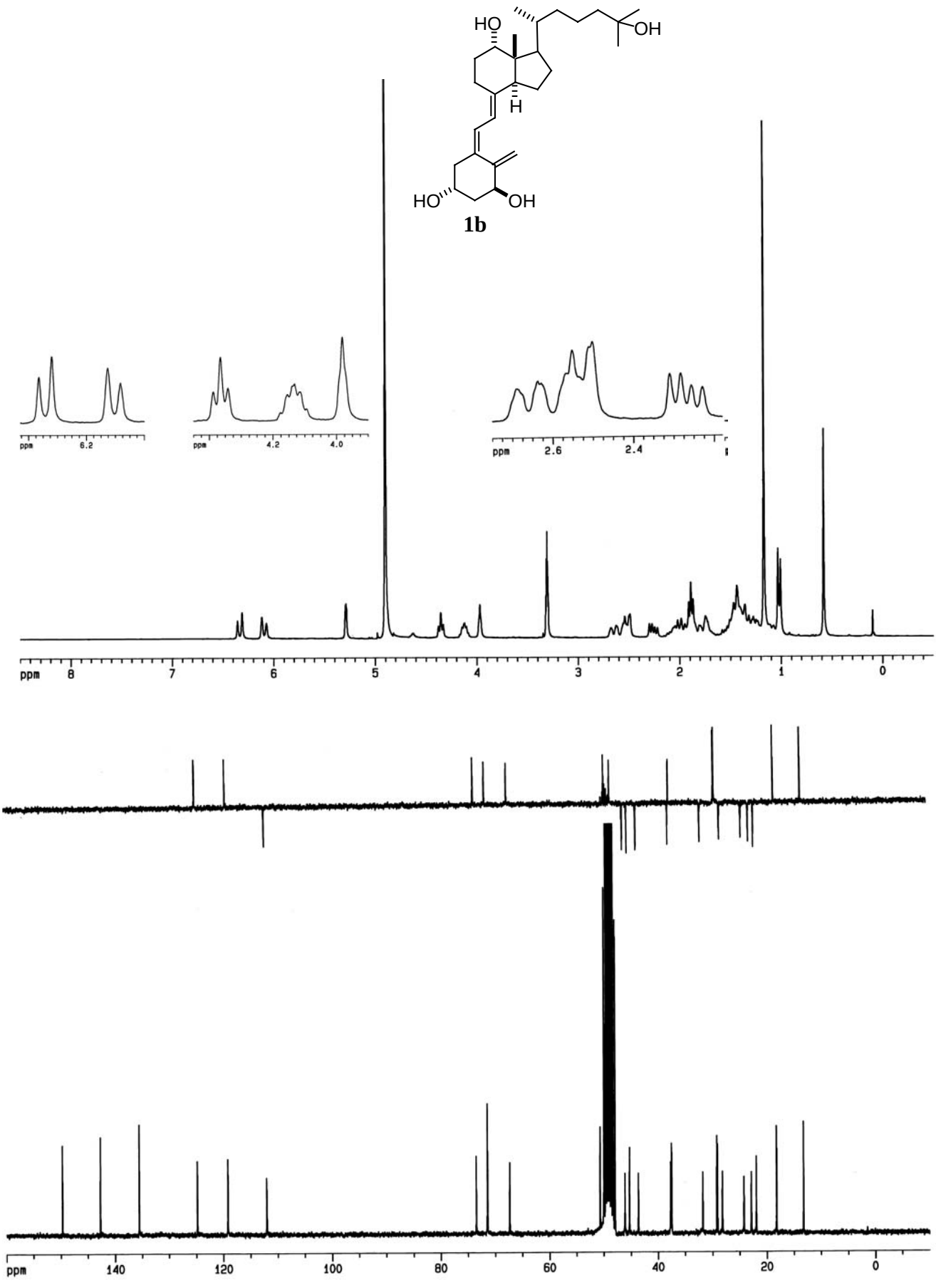

**11a** **1b**

Tetrabutyl amonium fluoride trihydrate (0.182 g, 0.579 mmol) was added to a solution of **11a** (0.045 g, 0.058 mmol) in dry THF (2 mL). The mixture was stirred in the dark for 16 h at rt. Aqueous $NH_4Cl$ (10 mL) was added and the aqueous layer was extracted with $Et_2O$ (3x8 mL). The combined organic solution was dried, filtered and concentrated in vacuo. The residue was dissolved in dry MeOH and treated with AG50W-X4 resin (0.4 g, freshly washed with dry methanol, wet weight). The suspension was stirred in the dark for 5 h at rt. The solids were removed by filtration and successively washed with MeOH and $Et_2O$. The solution was concentrated in vacuo and the residue was purified by flash column chromatography (65% EtOAc/hexanes) and by reverse phase (RP-18) column chromatography (25% $H_2O$/MeOH) to give **1b** [0.025 g, 0.057 mmol, >95% in two steps, $R_f$ = 0.5 (EtOAc), white solid].

**$^1$H-RMN** (MeOH-$d_4$, 250 MHz, δ): 6.33 and 6.10 (2 H, AB, $J$ = 11.2 Hz, H-6 and H-7), 5.29 (1 H, s, H-19$_E$), 4.90 ($H_2O$ superimposed over H-19$_Z$), 4.36 (1H, t, $J$ = 5.8 Hz, H-1), 4.12 (1 H, m, H-3), 3.97 (1 H, m, H-1), 1.17 (6H, s, Me-26 and Me-27), 1.02 (3H, d, $J$ = 6.5 Hz, Me-21), 0.58 (3H, s, Me-21).

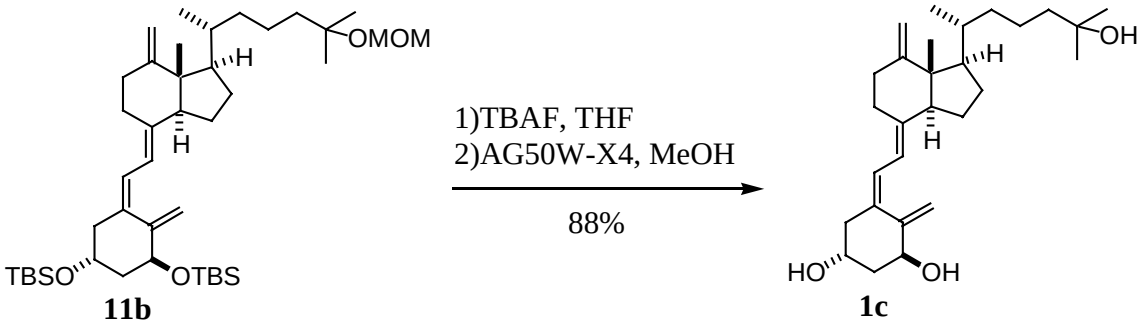
**$^{13}$C-RMN** (MeOH-$d_4$, 62.89 MHz, δ): 149.76 (C), 142.77 (C), 135.60 (C), 124.85 (CH), 119.23 (CH), 112.07 ($CH_2$), 73.53 (CH), 71.49 (C), 71.46 (CH), 67.37 (CH), 50.70 (C), 49.42 (CH), 48.38 (CH), 46.13 ($CH_2$), 45.31 ($CH_2$), 43.66 ($CH_2$), 37.74 ($CH_2$), 37.53 (CH), 31.80 ($CH_2$), 29.23 ($CH_3$), 29.11 ($CH_3$), 28.21 ($CH_2$), 24.24 ($CH_2$), 22.88 ($CH_2$), 21.96 ($CH_2$), 18.23 ($CH_3$), 13.25 ($CH_3$).

**MS:** m/z ([FAB]$^+$, %): 432 ([M]$^+$, 4), 415 ([M-OH]$^+$, 4), 397 ([M-OH-$H_2O$]$^+$, 4). **HRMS:** calculated for [$C_{27}H_{44}O_4$]$^+$ ([M]$^+$): 432.3240; found: 432.3237.




**1b**

**22. 1α,25-Dihydroxy-12-methylen-vitamin $D_3$.**



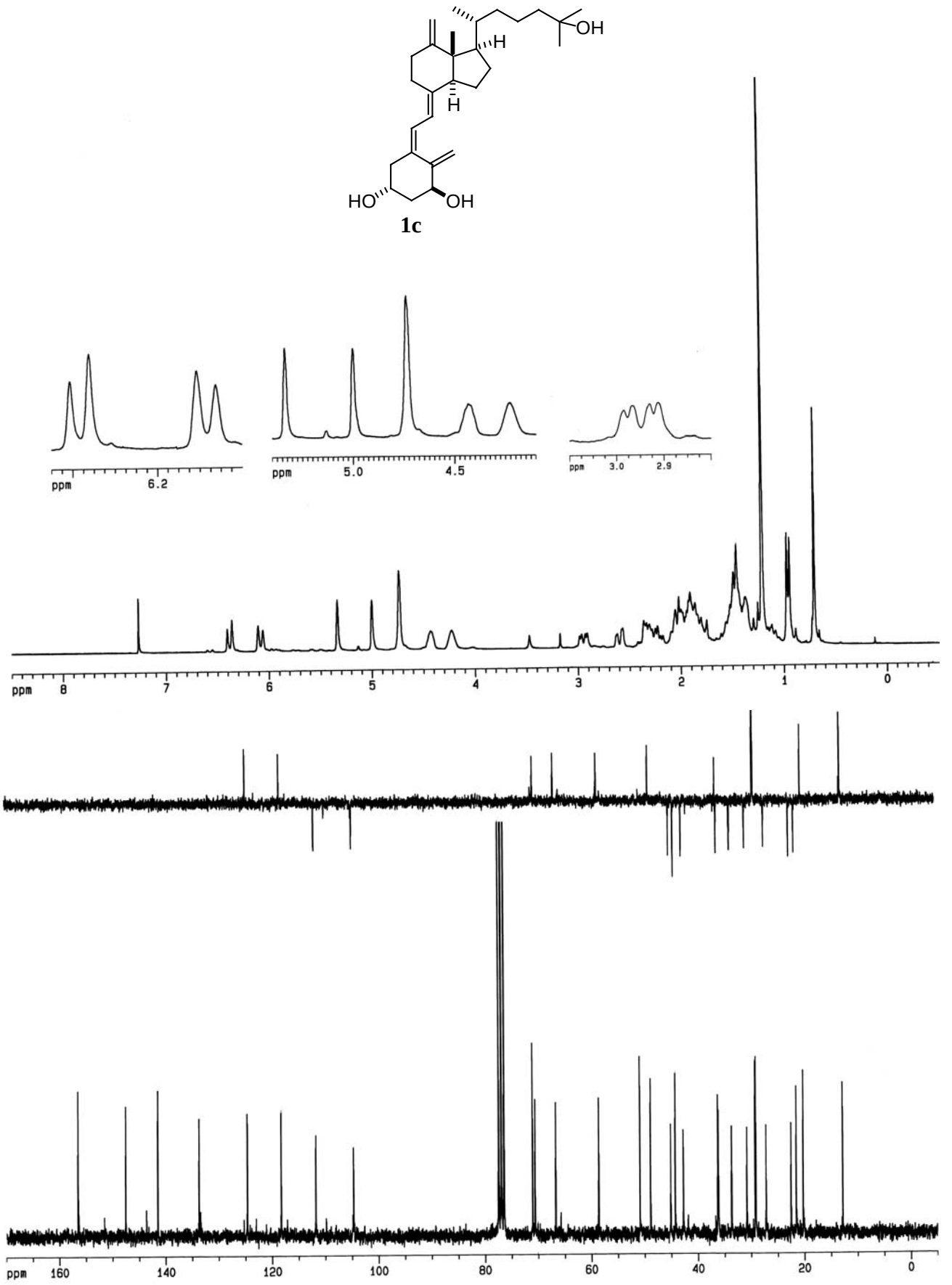

**11b** **1c**

Tetrabutylamonium fluoride (1.99 mL, 1.99 mmol, 1M solution in dry THF) was added to a solution of **11b** (0.060 g, 0.086 mmol) in dry THF (2 mL). The mixture was stirred in the dark for 16 h. Aqueous $NH_4Cl$ (8 mL) was added and the aqueous layer was extracted with $Et_2O$ (3x8 mL). The combined organic solution was dried, filtered and concentrated in vacuo. The residue was dissolved in dry MeOH and treated with AG50W-X4 resin (0.5 g, freshly washed with dry methanol, wet weight). The suspension was stirred in the dark for 5 h at rt. The resin was removed by filtration and successively washed with MeOH and $Et_2O$. The solution was evaporated in vacuo and the residue was purified by flash column chromatography (50-65% EtOAc/hexanes) to give **1c** [0.032 g, 0.076 mmol, 88% in two steps, $R_f$ = 0.5 (EtOAc), white solid].

**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 6.38 and 6.08 (2 H, AB, *J* = 11.2 Hz, H-6 and H-7), 5.34 (1 H, s, H-$19_E$), 5.00 (1 H, s, H-$19_Z$), 4.74 (2H, overlapped signals, H-$12_Z$ and H-$12_E$), 4.43 (1 H, m, H-1), 4.23 (1 H, m, H-3), 1.21 (6H, s, Me-26 and Me-27), 0.96 (3H, d, *J* = 6.7 Hz, Me-21), 0.71 (3H, s, Me-18).

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 156.52 (C), 147.59 (C), 141.54 (C), 133.76 (C), 124.70 (CH), 118.35 (CH), 111.84 ($CH_2$), 104.75 ($CH_2$), 71.11 (C), 70.65 (CH), 66.74 (CH), 58.65 (CH), 50.91 (C), 48.93 (CH), 45.15 ($CH_2$), 44.29 ($CH_2$), 42.78 ($CH_2$), 36.31 (CH), 36.18 ($CH_2$), 33.72 ($CH_2$), 30.84 ($CH_2$), 29.28 ($CH_3$), 29.14 ($CH_3$), 27.22 ($CH_2$), 22.56 ($CH_2$), 21.57 ($CH_2$), 20.27 ($CH_3$), 12.83 ($CH_3$).
**MS:** m/z ($[CI]^+$, %): 428 ($[M]^+$, 21), 411 ($[M\text{-}OH]^+$, 100), 393 ($[M\text{-}OH\text{-}H_2O]^+$, 84).

**HRMS:** calculated for $[C_{28}H_{44}O_3]^+$ ($[M]^+$): 428.3290; found: 428.3309.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ppm
8
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5
4
3
2
1
0
ppm
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140
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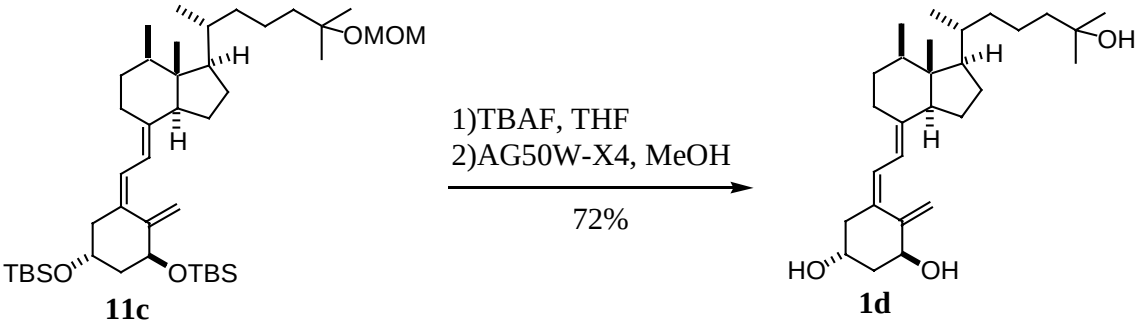
100
80
60
40
20
0

**1c**

**23. 1α,25-Dihydroxy-12β-methylvitamin $D_3$**



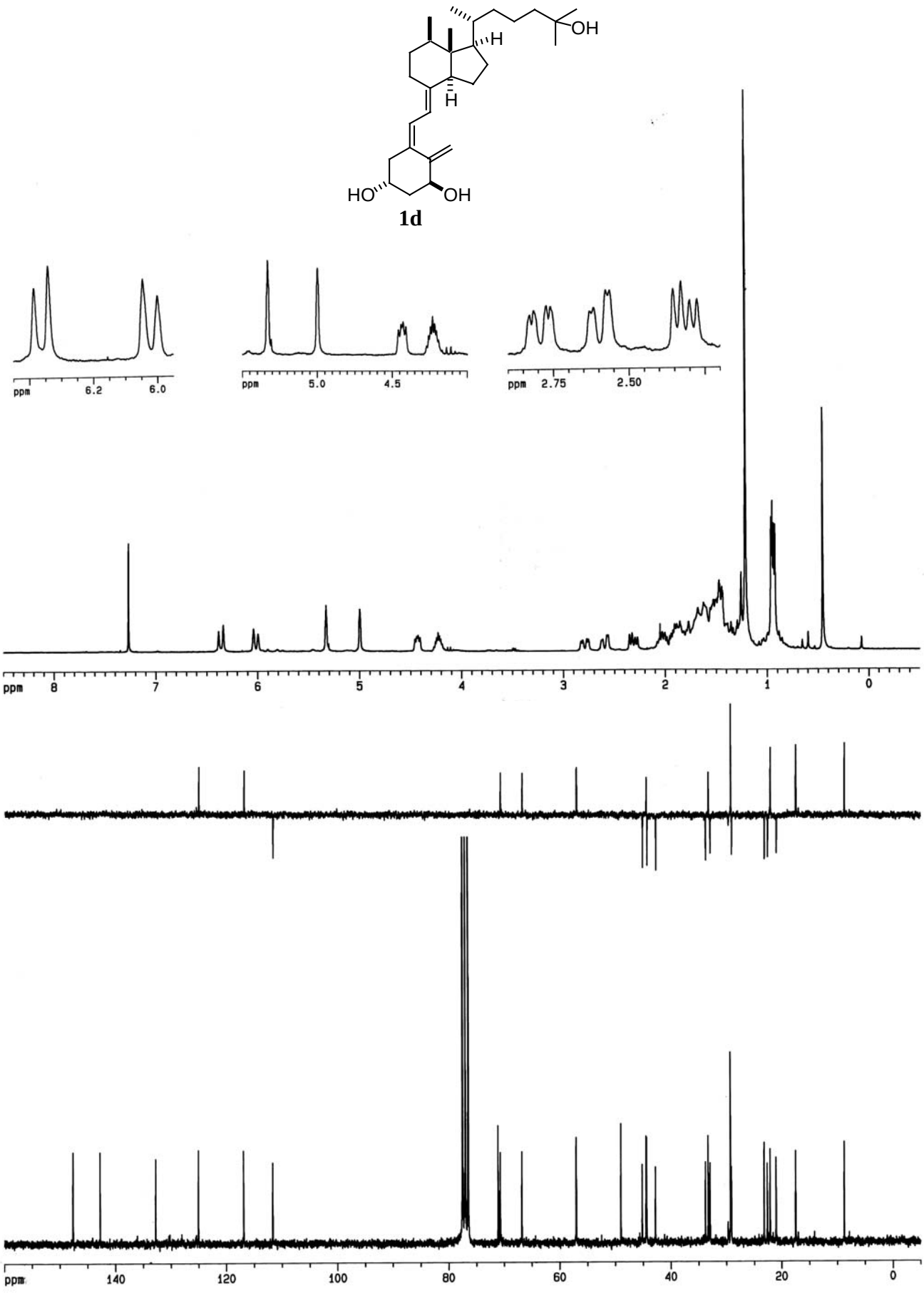

**11c** **1d**

Tetrabutylamonium fluoride (1.89 mL, 1.89 mmol, 1M solution in dry THF) was added to a solution of **11c** (0.057 g, 0.081 mmol) in dry THF (2 mL). The mixture was stirred in the dark for 16 h at rt. Aqueous $NH_4Cl$ (8 mL) was added and the aqueous layer was extracted with $Et_2O$ (3x8 mL). The combined organic solution was dried, filtered and concentrated in vacuo. The residue was dissolved with dry MeOH and treated with the resin AG50W-X4 (0.5 g, freshly washed with dry methanol, wet weight). The suspension was stirred in the dark for 5 h at rt. The solids were removed by filtration and successively washed with MeOH and $Et_2O$. After concentration in vacuo the resulting residue was purified by flash column chromatography (50-65% EtOAc/hexanes) to give **1d** [0.025 g,0.058 mmol, 72% in two steps, $R_f$ = 0.5 (EtOAc), white solid].

**$^1$H-RMN** ($CDCl_3$, 250 MHz, δ): 6.36 and 6.02 (2 H, AB, *J* = 11.2 Hz, H-6 and H-7), 5.32 (1 H, s, H-$19_E$), 5.00 (1 H, s, H-$19_Z$), 4.43 (1 H, m, H-1), 4.23 (1 H, m, H-3), 1.22 (6H, s, Me-26 and Me-27), 0.96-0.92 (6 H, overlapped signals, Me-12 and Me-21), 0.45 (3H, s, Me-18).

**$^{13}$C-RMN** ($CDCl_3$, 62.89 MHz, δ): 147.61 (C), 142.75 (C), 132.77 (C), 125.03 (CH), 116.88 (CH), 111.68 ($CH_2$), 71.10 (C), 70.71 (CH), 66.82 (CH), 57.07 (CH), 57.01 (CH), 49.00 (C), 45.16 ($CH_2$), 44.46 (CH), 44.33 ($CH_2$), 42.78($CH_2$), 33.76 ($CH_2$), 33.26 (CH), 32.94 ($CH_2$), 29.23 ($CH_3$, C26 and C27), 29.07 ($CH_2$), 24.18 ($CH_2$), 22.60 ($CH_2$), 22.11 ($CH_3$), 21.04 ($CH_2$), 17.51 ($CH_3$), 8.74 ($CH_3$).

**MS:** m/z ($[CI]^+$, %): 430 ($[M]^+$, 25), 413 ($[M\text{-}OH]^+$, 89), 509 ($[M\text{-}OH\text{-}H_2O]^+$, 100). **HRMS:** calculated for $[C_{28}H_{46}O_3]^+$ ($[M]^+$): 430.3447; found: 403.3436.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ppm 140 120 100 80 60 40 20 0

**1d**