Contents

1 Materials, methods, and instrumentation

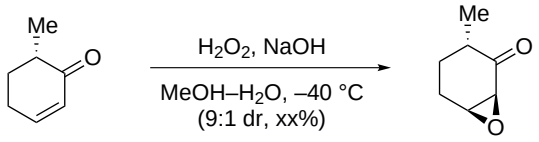
All reagents and solvents were commercial grade and purified prior to use when necessary. Acetonitrile (MeCN) and tetrahydrofuran (THF) were dried by passage through a column of activated alumina as described by Grubbs,[1] for microscale reactions, tetrahydrofuran was distilled from sodium–benzophenone ketyl still. Flame-dried (under vacuum) glassware was used for all reactions. Stainless steel syringes or cannulae were used to transfer air- and moisture-sensitive liquids. Anhydrous magnesium or sodium sulfate was used as a drying agent in extractions. (+)-(2*S*,5*S*)-*trans*-Dihydrocarvone,[2] (−)-(*S*)-6-methylcyclohex-2-en-1-one,[3] de-*A*,*B*-cholestan-8β-ol,[4] 9,9,14-trideuterio-de-*A*,*B*-cholestan-8-one[5] De-*A*,*B*-cholest-8-en-8-yl trifluoromethanesulfonate[5] were prepared according to the literature procedure.

Thin layer chromatography (TLC) was performed using glass-backed silica gel (250 µm) plates and flash chromatography utilized 230–400 mesh silica gel from EMD. UV light, and/or the use of potassium iodoplatinate, potassium permanganate or phosphomolybdic acid solutions were used to visualize products. Anhydrous magnesium or sodium sulfate was used as a drying agent in extractions

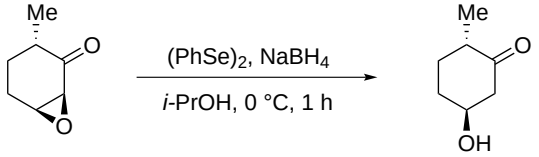
Normal phase HPLC was conducted at 15 mL/min on a Waters 600E system coupled with Waters 2487 dual wavelength absorbance detector using Dynamax Macro HPLC Si 250 mm × 21.4 mm column. Reverse phase HPLC was conducted on the same system using Supelco Discovery C18 569226-U 250 mm × 22.2 mm column. Photochemical reactions were performed in a quartz vessel using UV 450 W immersion mercury vapor lamp with a Vycor filter.

HPLC–MS was conducted on Waters Alliance 2695 3 µm 150 mm × 4.6 mm silica column (Phenomenex, Inc.); 10 % 2-propanol in hexanes; 1.0 mL/min coupled with Thermo Finnigan TSQ Quantum Ultra spectrometer: discharge current, 10 µA; sheath gas pressure, 20 mTorr; ion sweep gas pressure, 2 mTorr; auxiliary gas pressure, 15 mTorr; tube lens, 92 V; skimmer offset, 6 V; collision pressure, 1.50 mTorr; collision energy, 13 V; vaporizer temperature: 300 °C.

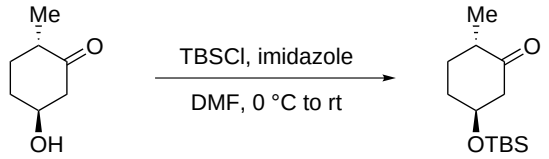
2 Experimental procedures



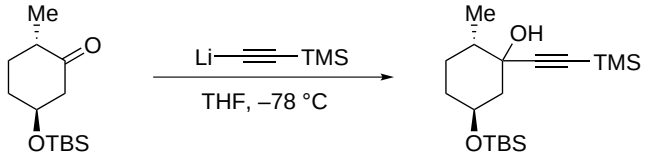
(1*S*,3*S*,6*S*)-3-methyl-7-oxabicyclo[4.1.0]heptan-2-one (**7**). To a solution of the enone (7.15 g, 65 mmol) in methanol–water (2:1, v/v, 200 mL) at −20 °C was added H_2O_2 (30 % in water, 14.7 g, 100 mmol) followed by satd aq K_2CO_3 (4.1 g, 30 mmol in 3.5 mL of water) and the solution was stirred at −20 °C for 1 h. The solution was cooled to −60 °C, quenched with HCl and the resulting yellow solution was poured into water (500 mL) and extracted with dichloromethane. The combined organic layers were washed with brine, dried, and concentrated. The resulting yellow oil was purified using flash chromatography (SiO_2, 20 % ethyl acetate in hexanes) to afford the epoxide as a yellow oil (4.0 g, 48 %). $[\alpha]_D^{20}$ −46.5 (*c* 2.51, $CHCl_3$); R_f = 0.33 (25 % Et_2O/hexanes); IR (film): 2963, 2926, 2868, 2854, 1713, 1600, 1457, 1380, 1353 1/cm; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.56 (dddd, J = 3.8, 3.8, 1.3, 1.2 Hz, 1H), 3.23 (dd, J = 3.9, 0.6 Hz, 1H), 2.77 (ddddd, J = 11.6, 6.8, 6.8, 6.8, 5.0 Hz, 1H), 2.18 (ddddd, J = 15.5, 9.7, 6.6, 0.9, 0.9 Hz, 1H), 2.01 (dddd, J = 15.3, 7.4, 3.8, 3.8 Hz, 1H), 1.97–1.87 (m, 1H), 1.53 (dddd, J = 13.7, 11.0, 10.0, 6.9 Hz, 1H), 0.97 (d, J = 3.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 209.6, 59.0, 55.3, 37.8, 31.5, 21.9, 14.5; HRMS (ESI): Exact mass calcd for $C_7H_{11}O_2$ $[M+H]^+$ 127.0754, found 127.0770.



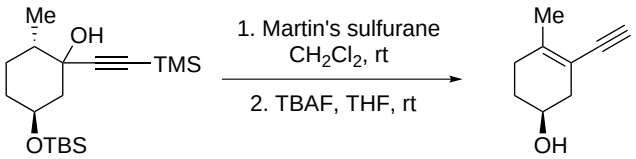
(2*S*,5*S*)-5-hydroxy-2-methylcyclohexan-1-one(**8**). To a solution of diphenyl diselenide (468 mg, 1.50 mmol) in 2-propanol (7 mL) at rt was added $NaBH_4$. After 10 min, AcOH (400 µL, 7.0 mmol) was added via syringe. The reaction was stirred at rt for 5 min and cooled to 0 °C. A solution of the epoxide (126.2 mg, 1.00 mmol) in 2-propanol (3 mL) was added, the reaction was stirred for 15 min at rt, diluted with ethyl acetate (30 mL) and then poured into brine. The layers were separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried, concentrated, and the resulting oil was purified using flash chromatography (SiO_2, 40 % ethyl acetate in hexanes) to afford the alcohol as a colorless liquid (112.7 mg, 88 %). $[\alpha]_D^{20}$ 1.3 (*c* 1.47, $CHCl_3$); R_f = 0.33 (50 % EtOAc/hexanes); IR (film): 3402, 2922, 2852, 1707, 1457 1/cm; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.91 (dddd, J = 11.0, 11.0, 4.6, 4.6 Hz, 1H), 2.75 (ddd, J = 13.0, 4.9, 2.2 Hz, 1H), 2.38 (ddd, J = 12.5, 11.1, 1.2 Hz, 1H), 2.31 (m, 1H), 2.16 (ddddd, J = 12.9, 3.7, 3.7, 3.7, 2.2 Hz, 1H), 2.03 (dddd, J = 13.8, 7.6, 3.9, 3.9 Hz, 1H), 1.71 (dddd, J = 12.9, 12.9, 10.7, 3.9 Hz, 1H), 1.30–1.15 (m, 2H), 1.02 (d, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 210.5, 70.4, 51.0, 43.9, 34.0, 29.5, 14.0; HRMS (ESI): Exact mass calcd for $C_7H_{13}O_2$ $[M+H]^+$ 129.0910, found 129.0910.



(2*S*,5*S*)-5-((*tert*-butyldimethylsilyl)oxy)-2-methylcyclohexan-1-one (**9**). To a solution of the alcohol (3.35 g, 26.1 mmol) in dimethylformamide (54 mL) at 0 °C was added TBSCl (6.2 g, 41 mmol) followed by imidazole (3.7 g, 54 mmol). The reaction was warmed to rt and stirred for 12 h. The reaction mixture was poured into water and extracted with diethyl ether. The combined organic layers were died, concentrated, and the resulting oil was purified using flash chromatography (SiO_2, 5–10 % ethyl acetate in hexanes) to afford the product as a pale yellow oil (6.07 g, 96 %). $[\alpha]_D^{20}$ −7.1 (*c* 1.47, $CHCl_3$); R_f = 0.25 (10 % EtOAc/hexanes); IR (film): 2956, 2933, 2894, 2858, 2166, 1462, 1378 1/cm; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.85 (dddd, J = 10.3, 10.3, 4.5, 4.5 Hz, 1H), 2.64 (ddd, J = 13.2, 4.8, 2.0 Hz, 1H), 2.38 (ddd, J = 13.1, 10.6, 1.3 Hz, 1H), 2.30 (m, 1H), 2.08–1.96 (m, 2H), 1.71 (m, 1H), 1.20 (ddd, J = 14.5, 14.5, 2.8 Hz, 1H), 1.03 (d, J = 6.6 Hz, 3H), 0.88 (s, 9H), 0.06 (d, J = 3.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 210.5, 71.2, 51.6, 43.9, 34.6, 29.4, 25.7, 18.0, 14.1, −4.82, −4.83; HRMS (ESI): Exact mass calcd for $C_{13}H_{26}NaO_2Si$ $[M+Na]^+$ 265.1594, found 265.1600.

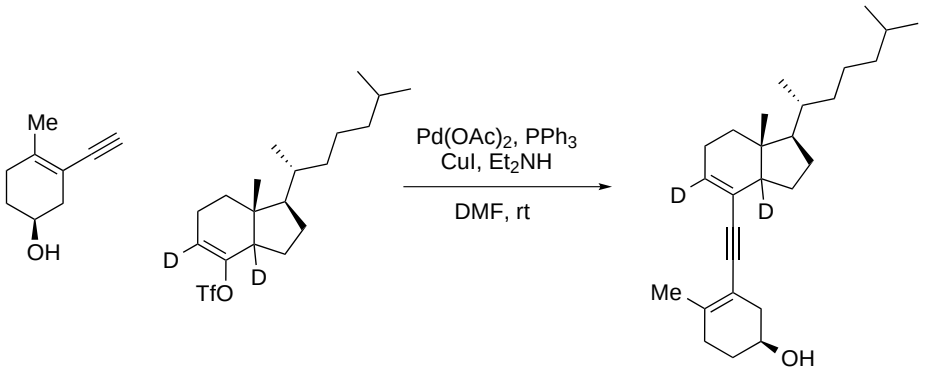


Alkyne 10 To a solution of trimethylsilylacetylene (2.3 mL, 16.5 mmol) in THF (8 mL) at −78 °C was added *n*-BuLi (2.5 M in hexanes, 6.0 mL, 15 mmol) and the solution was stirred for 30 min. A solution of the ketone (1.212 g, 5.0 mmol) in THF (3 mL) was added via cannula and the reaction was stirred at −78 °C for 3 h and then quenched with satd aq NH_4Cl. The reaction mixture was concentrated, extracted with diethylether, and the combined organic layers were dried and concentrated to give the tertiary alcohol as a colorless liquid which was used without further purification (1.7 g, 99 %). $[\alpha]_D^{20}$ −7.1 (*c* 1.47, $CHCl_3$); R_f = 0.25 (10 % EtOAc/hexanes); IR (film): 2952, 2927, 2856, 1716, 1461, 1375, 1255, 1092, 1062, 1008 1/cm; ^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.86 (dddd, J = 9.9, 9.9, 4.0, 4.0 Hz, 1H), 2.36 (br s, 1H), 2.18 (ddd, J = 11.9, 3.7, 1.9 Hz, 1H), 1.80 (m, 1H), 1.68–1.46 (m, 3H), 1.35–1.18 (m, 2H), 1.02 (d, J = 6.6 Hz, 3H), 0.89 (s, 9H), 0.17 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 106.5, 90.6, 72.3, 69.6, 41.4, 29.7, 25.9, 18.2, 15.4, −0.1, −4.8; HRMS (ESI): Exact mass calcd for $C_{18}H_{36}NaO_2Si_2$ $[M+Na]^+$ 363.2146, found 363.2139.

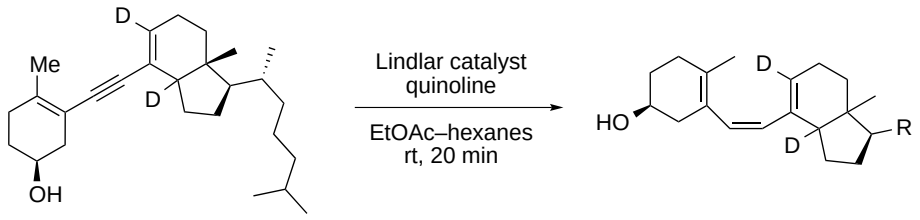


Enynol 2 To a solution of the alcohol (2.5 g, 7.4 mmol) in dichloromethane (100 mL) at rt was added Martin's sulfurane (5 g, 7.4 mmol) and the solution was stirred at rt for 2 h. The reaction mixture was concentrated and

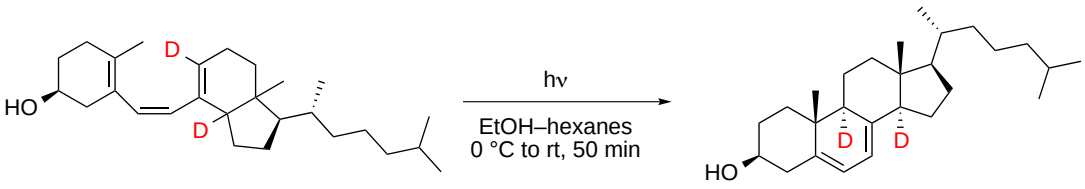
purified using flash chromatography (SiO_2, dichloromethane) to give mixture of enyne isomers (4.5:1 ratio, 2.0 g, 82 %). The mixture was dissolved in tetrahydrofuran (12 mL) and cooled to 0 °C. To this solution was added TBAF (1 M in THF 18 mL, 18 mmol) via syringe. The reaction was warmed to rt and stirred at rt for 6 h. The reaction mixture was concentrated, poured into water and extracted with diethyl ether. The combined organic layers were washed with satd aq NH_4Cl, dried and concentrated. Purification by preparative normal phase HPLC (30 % EtOAc, 70 % hexanes, t_r = 0 min) afforded enynol as a clear oil (563 mg, 56 %). $[\alpha]_D^{20}$ −56.4 (c 3.57, $CHCl_3$); R_f = 0.00 (0 % EtOAc/hexanes); IR (film): 3296, 2927, 2855, 2080, 1730, 1441 1/cm; ^{1}H NMR (600 MHz, $CDCl_3$, δ): 3.92 (m, 1H), 3.00 (s, 1H), 2.44 (m, 1H), 2.25–2.04 (m, 4H), 1.87 (s, 3H), 1.84–1.76 (m, 1H), 1.66–1.56 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 142.9, 110.6, 83.9, 78.7, 65.9, 38.1, 30.2, 29.0, 21.5; HRMS (ESI): Exact mass calcd for $C_9H_{13}O$ $[M+H]^+$ 137.0961, found 137.0958.



Dienynol-d2 12. To a solution of $Pd(OAc)_2$ (76.1 mg, 339 µmol) and Ph_3P (200 mg, 750 µmol) in DMF (6 mL) and diethylamine (6 mL) was added CuI (323 mg, 1.13 mmol). The solution was stirred at rt for 30 min and then degassed (freeze-pump-thaw, 3 cycles). A degassed solution of the triflate[5] (900 mg, 2.26 mmol) and the enynol (500 mg, 1.62 µmol) in DMF (6 mL) and diethylamine (6 mL) was added via cannula. The reaction was stirred in the dark at rt for 1 h. The reaction mixture was poured into water and extracted with diethyl ether. The combined organic layers were washed with water, satd aq NH_4Cl and brine. The extract was dried, concentrated, and purified using flash chromatography (SiO_2, 25 % ethyl acetate in hexanes) to afford the dienynol as a yellow oil. Further purification by preparative normal phase HPLC (30 % EtOAc, 70 % hexanes, t_r = 10.85 min–12.80 min, fractions collected at −78 °C) afforded dienynol-d_2 as a colorless oil which was used immediately in the next step. $[\alpha]_D^{20}$ 1.2 (c 0.41, $CHCl_3$); R_f = 0.375 (20 % EtOAc/hexanes); IR (film): 3367, 2951, 2930, 2870, 1463, 1442, 1372, 1157 1/cm; ^{1}H NMR (600 MHz, $CDCl_3$, δ): 3.95 (m, 1H), 2.48 (dddd, J = 14.8, 4.7, 1.7, 1.7 Hz, 1H), 2.26–2.09 (m, 5H), 1.99 (ddd, J = 13.0, 5.0, 3.2 Hz, 1H), 1.92 (m, 1H), 1.87 (s, 3H), 1.86–1.76 (m, 2H), 1.64 (m, 1H), 1.55–1.46 (m, 2H), 1.46–1.38 (m, 3H), 1.38–1.29 (m, 3H), 1.28–1.06 (m, 4H), 1.00 (m, 1H), 0.93 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.7 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 139.8, 132.4 (t, J = 24 Hz), 122.5, 111.9, 91.6, 88.2, 66.4, 54.8, 49.6 (t, J = 19 Hz), 41.7, 39.5, 38.7, 36.2, 39.1, 35.9, 30.5, 29.1, 28.0, 27.97, 25.0, 24.1, 23.9, 22.8, 22.5, 21.6, 18.7, 11.0; HRMS (ESI): Exact mass calcd for $C_{27}H_{39}D_2O_2$ $[M+O_2-H_2O+H]^+$ 399.3227, found 399.3258.



Previtamin D_3-d_2 (1-d2). To the solution of dienynol (ca. 200 mL, 30 % EtOAc, 70 % hexanes) was added Lindlar catalyst (500 g) and quinoline (250 µL). The reaction vessel was evacuated, a H_2 baloon was placed, and the progress was monitored by TLC. After 20 min the reaction was filtered through Celite and concentrated. Purification by preparative normal phase HPLC (30 % EtOAc, 70 % hexanes, t_r = 8.0 min–9.5 min, fractions collected at −78 °C) afforded previtamin D_3-d_2 as a colorless oil (450 mg, 52 % from **2**). $[\alpha]_D^{20}$ 25.5 (*c* 0.20, $CHCl_3$); R_f = 0.35 (20 % EtOAc/hexanes); IR (film): 3307, 2948, 2870, 1642, 1462, 1373 1/cm; ^{1}H NMR (600 MHz, $CDCl_3$, δ): 5.94 (d, J = 12.4 Hz, 1H), 5.69 (ddd, J = 12.1, 2.0, 2.0 Hz, 1H), 3.90 (m, 1 H), 2.41 (d, J = 16.0 Hz, 1H), 2.22–2.04 (m, 5H), 1.98 (ddd, J = 12.8, 6.6, 1.2 Hz, 1H), 1.90 (m, 1H), 1.86–1.80 (m, 1H), 1.70–1.57 (m, 5H), 1.57–1.45 (m, 2H), 1.45–1.22 (m, 6H), 1.22–1.06 (m, 4H), 1.06–0.96 (m, 1H), 0.94 (d, J = 6.7 Hz, 3H), 0.87 (d, J = 6.7 Hz, 3H), 0.86 (d, J = 6.7 Hz, 3H), 0.94 (s, 3H) ^{13}C NMR (150 MHz, $CDCl_3$, δ): 136.4, 129.5, 128.9, 128.6, 125.9, 124.3 (t, J = 23 Hz), 124.1, 67.5, 54.5, 50.3 (t, J = 19 Hz), 42.0, 39.5, 37.6, 36.16, 36.13, 36.1, 31.1, 29.7, 28.3, 28.0, 24.8, 23.9, 23.3, 22.8, 22.5, 19.7, 18.8, 11.2; HRMS (ESI): Exact mass calcd for $C_{27}H_{42}D_2OAg$ $[M+Ag]^+$ 493.2569, found 493.2571.



7-Dehydrocholesterol-d_2. A solution of Previtamin D_3-d_2 (450 mg, 1.16 mmol) in degassed hexane–ethanol (80/20 v/v, 15 mL) under argon was placed next to medium pressure Hg lamp arc for 50 min. The reaction mixture was concentrated and purified by preparative normal phase HPLC (2 % 2-propanol , 98 % hexanes, t_r = 21.5 min) afforded 7-DHC-d_2 as a white solid. Minor impurities were removed by reversed phase HPLC (methanol, t_r = 18.5 min) and afforded 7-DHC-d_2 as a white solid (16.2 mg, 3.6 %). $[\alpha]_D^{20}$ −84.3 (*c* 0.14, $CHCl_3$); R_f = 0.22 (20 % EtOAc/hexanes); IR (film): 3366, 2933, 2869, 1646, 1463, 1375 1/cm; ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.62 (s, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H), 0.94 (d, J = 5.9 Hz, 3H), 0.95 (s, 3H), 1.01 (m, 1H), 1.18–1.06 (m, 3H), 1.43–1.19 (m, 8H), 1.55–1.46 (m, 2H), 1.63–1.55 (m, 2H), 1.69 (m, 2H), 1.95–1.84 (m, 3H), 2.08 (ddd, J = 12.9, 4.5, 2.7 Hz, 1H), 2.28 (ddd, J = 14.0, 11.7, 2.6, 2.6 Hz, 1H), 2.46 (ddd, J = 14.1, 6.8, 2.1 Hz, 1H), 3.63 (dddd, J = 11.0, 11.0, 4.2, 4.2 Hz, 1H), 5.38 (d, J = 5.7 Hz, 1H), 5.57 (dd, J = 5.7, 2.5 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, δ): 141.3, 139.7, 119.6, 116.3, 70.5, 55.9, 54.0 (t, J = 18.5 Hz), 45.7 (t, J = 18.4 Hz), 42.8, 40.8, 39.5, 39.1, 38.3, 36.9, 36.13, 36.10, 32.0, 28.07, 28.00, 23.9, 22.9, 22.8, 22.5, 21.0, 18.8, 16.3, 11.8; MS (ESI): Mass calcd for $C_{27}H_{41}D_2$ $[M-H_2O+H]^+$ 369.3485, found 369.3477.

2.1 Mosher ester analysis of the ketone *R*)-8

Our initial attempt to synthesize enynol **2** from hydroxyketone **8**, following the procedure published by Solladie and Hutt[6] gave the enantiomer of **2**. After specific rotation analysis of enynol **2** and Mosher ester analysis of hydroxyketone **8** we deduced that stereochemistry of **8** assigned by Solladie and Hutt was incorrect. Below we present the Mosher ester analysis of (2*R*,5*R*)-hydroxyketone *R*)-**8** derived from (*R*)-(+)-6-methylcyclohex-2-en-1-one (*R*)-**6**). Using representations of the conformations of isomers[7] and chemical shift differences (Table S1), protons with positive $\Delta\delta^{SR}$ were assigned to R^1 and protons with negative values were assigned to R^2. Absolute stereochemistry at C-5 was assigned as *R*, which is opposite to the desired enantiomer needed for the synthesis of previtamin D_3.

(*R*)-MTPA ester of 2*R*,5*R*-5-hydroxy-2-methylcyclohexan-1-one. To a solution of the alcohol (22 mg, 170 µmol), *(R)*-MTPA (124 mg, 530 µmol), and EDC (102 mg, 530 µmol) in dichloromethane (2 mL) was added DMAP (65 mg, 530 µmol) in one portion. The reaction was stirred at rt for 6 h, poured into water, and extracted with dichloromethane. The combined organic layers were washed with HCl, satd aq $NaHCO_3$, and brine. The extract was dried, concentrated and purified using flash chromatography (SiO_2, 0 % ethyl acetate in hexanes) to afford the ester as a colorless oil (31.5 mg, 54 %). The (*S*)-MTPA ester was prepared using the same procedure.

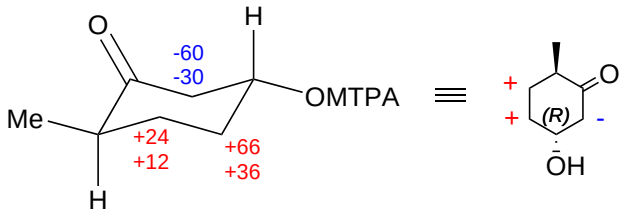


Table S1 Chemical shift comparison (^{1}H NMR 600 MHz, $CDCl_3$).

H	(*S*)-ester δ (ppm)	(*R*)-ester δ (ppm)	$\Delta\delta^{SR}$ (Hz)
4ax	1.90	1.79	+66
4eq	2.31	2.25	+36
3ax	1.37	1.35	+12
3eq	2.10	2.06	+24
2Me	1.06	1.06	0
2ax	2.32	2.33	−6
6ax	2.44	2.54	−60
6eq	2.81	2.86	−30
5ax	5.206	5.210	−2.4

3 Spectra

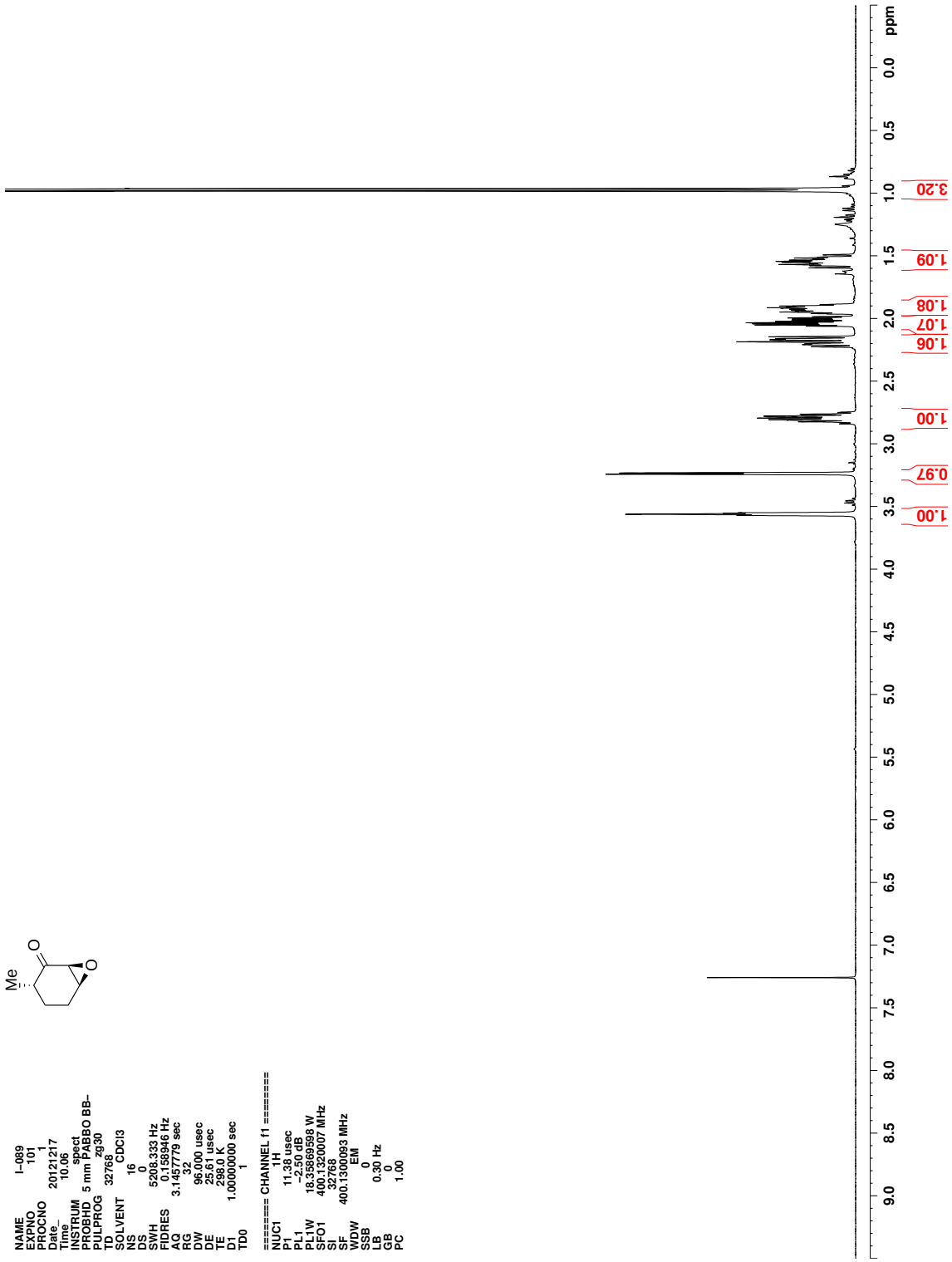


Figure S1 ^{1}H NMR ($CDCl_3$) of **7**

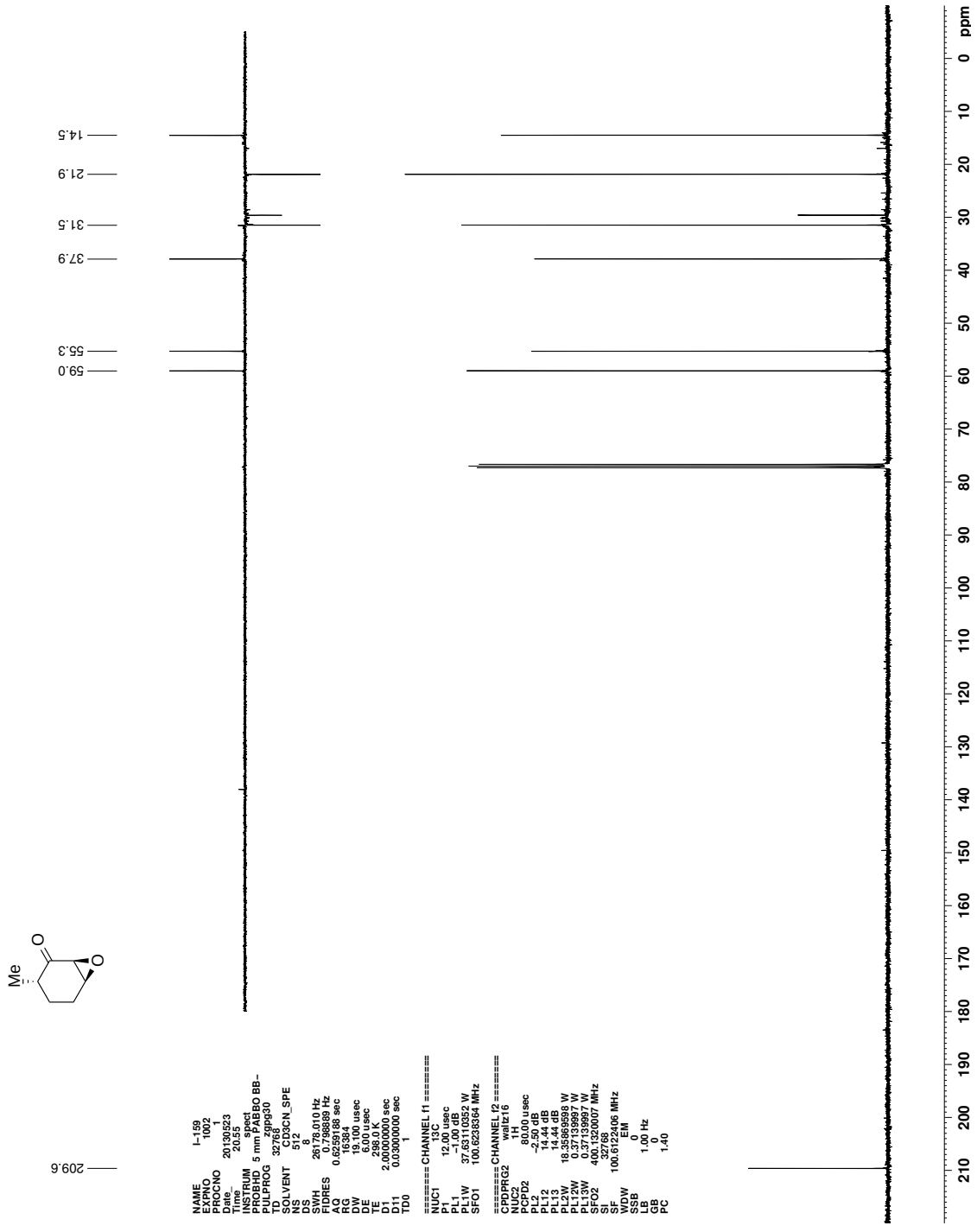


Figure S2 ^{13}C NMR ($CDCl_3$) of **7**

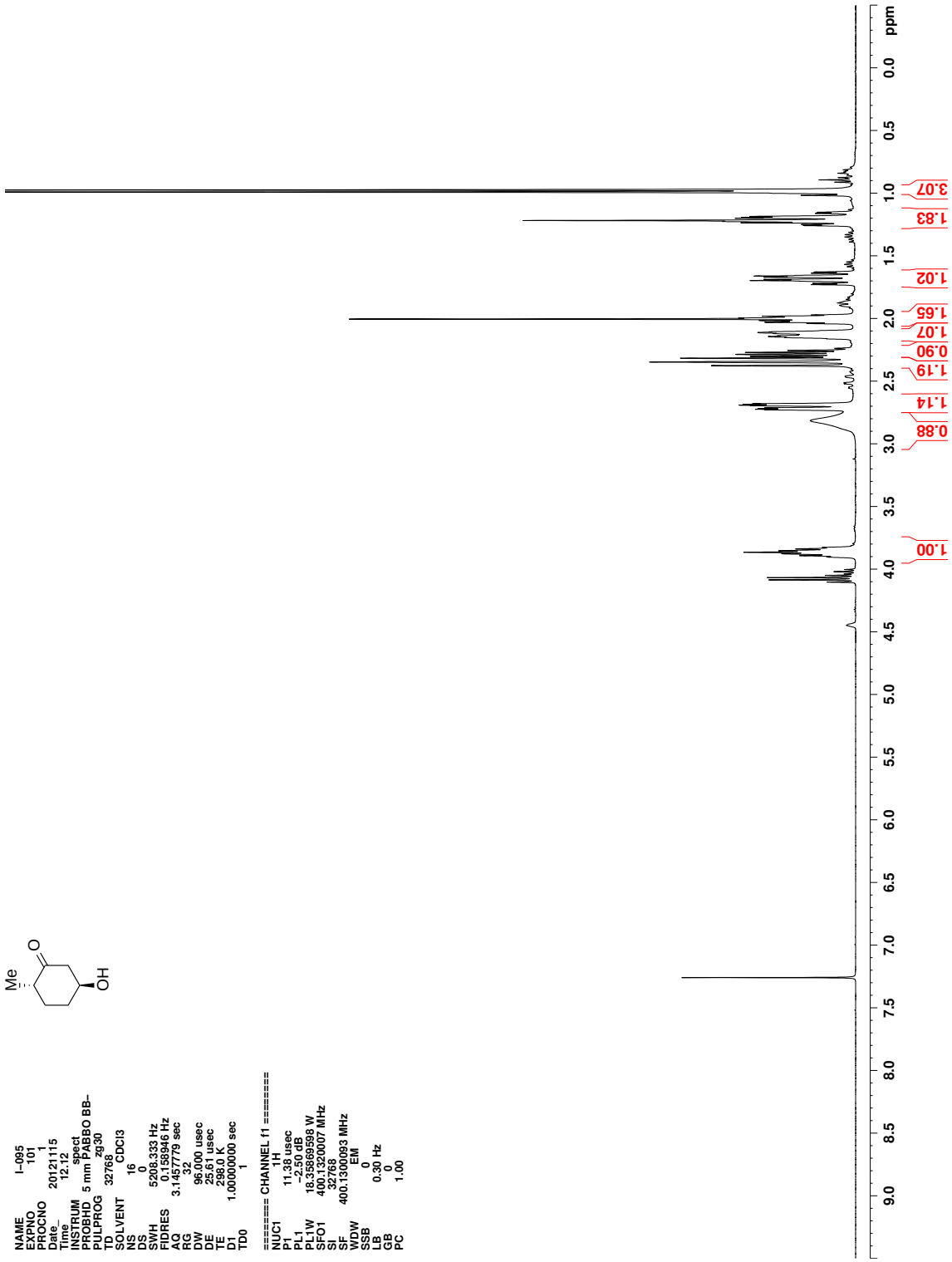


Figure S3 ^{1}H NMR ($CDCl_3$) of **8**

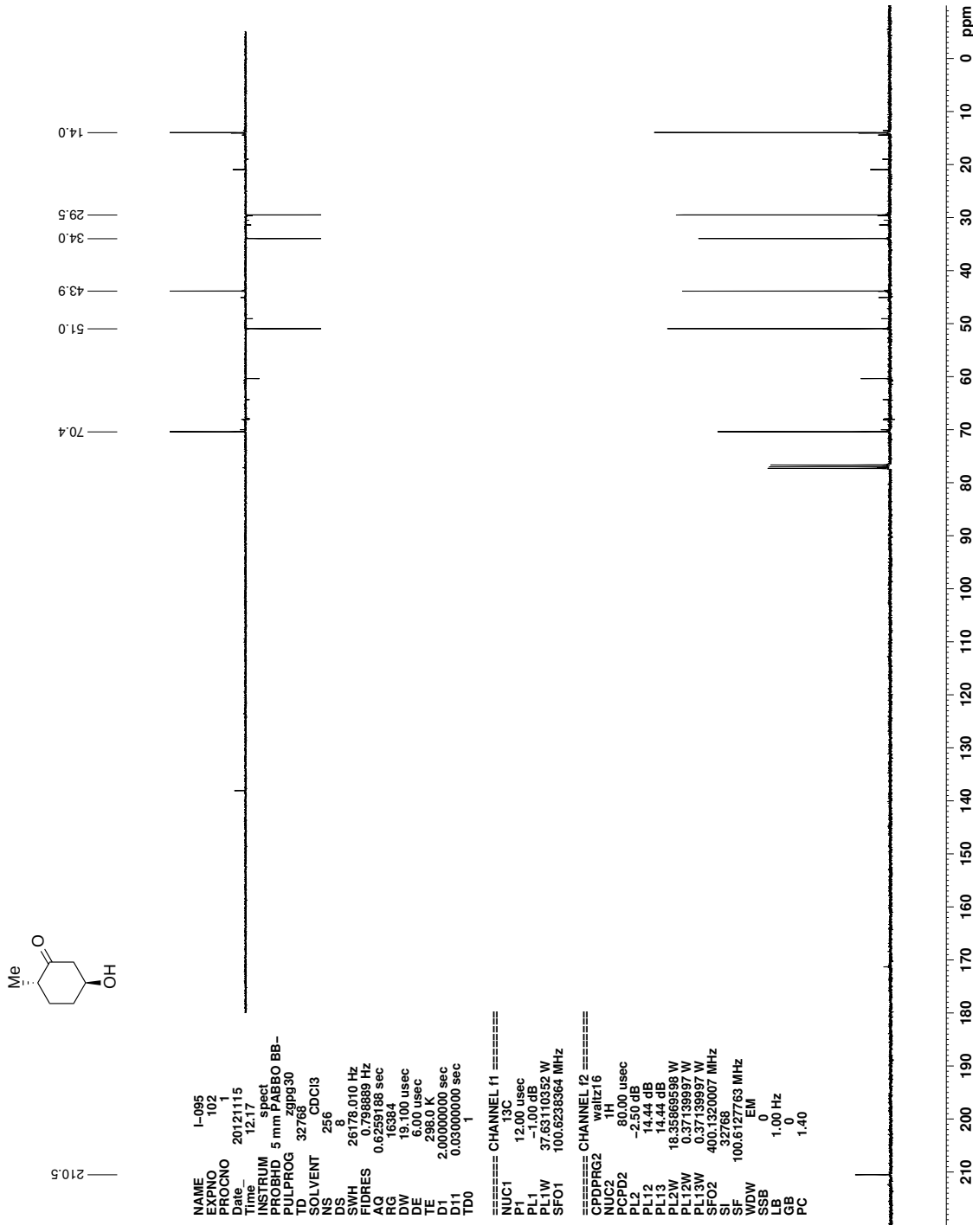


Figure S4 ^{13}C NMR ($CDCl_3$) of **8**

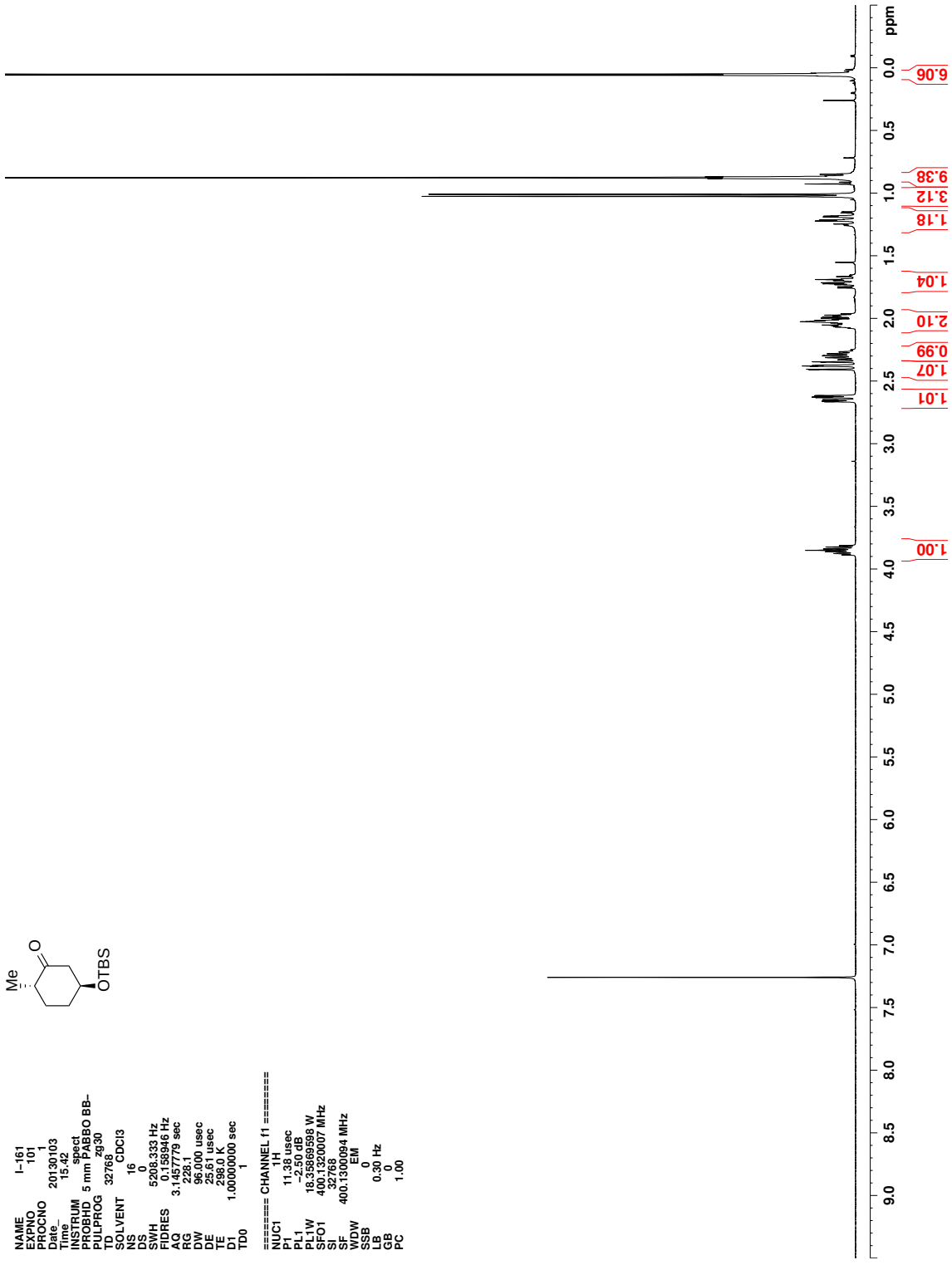


Figure S5 ^{1}H NMR ($CDCl_3$) of **9**

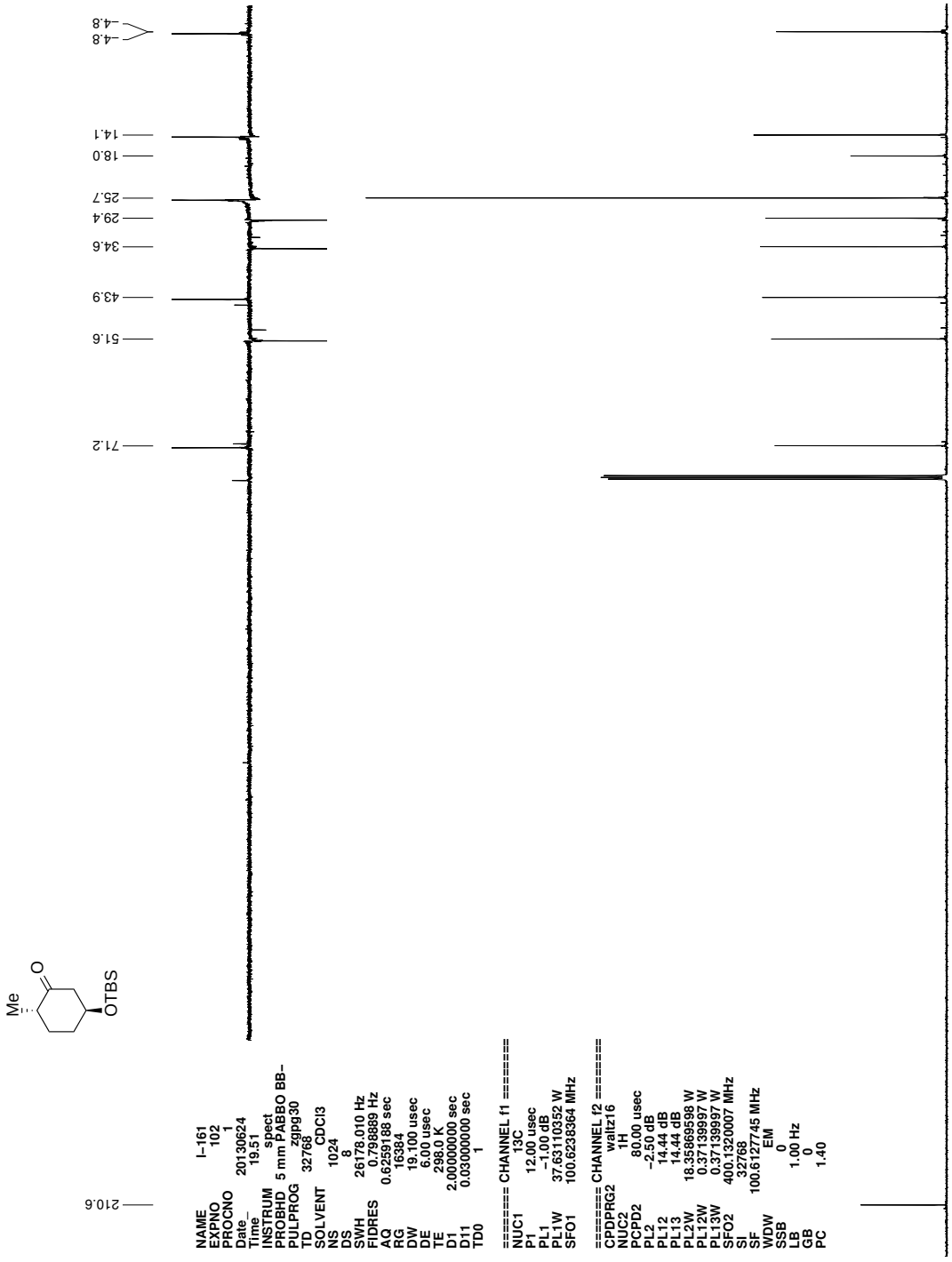


Figure S6 ^{13}C NMR ($CDCl_3$) of **9**

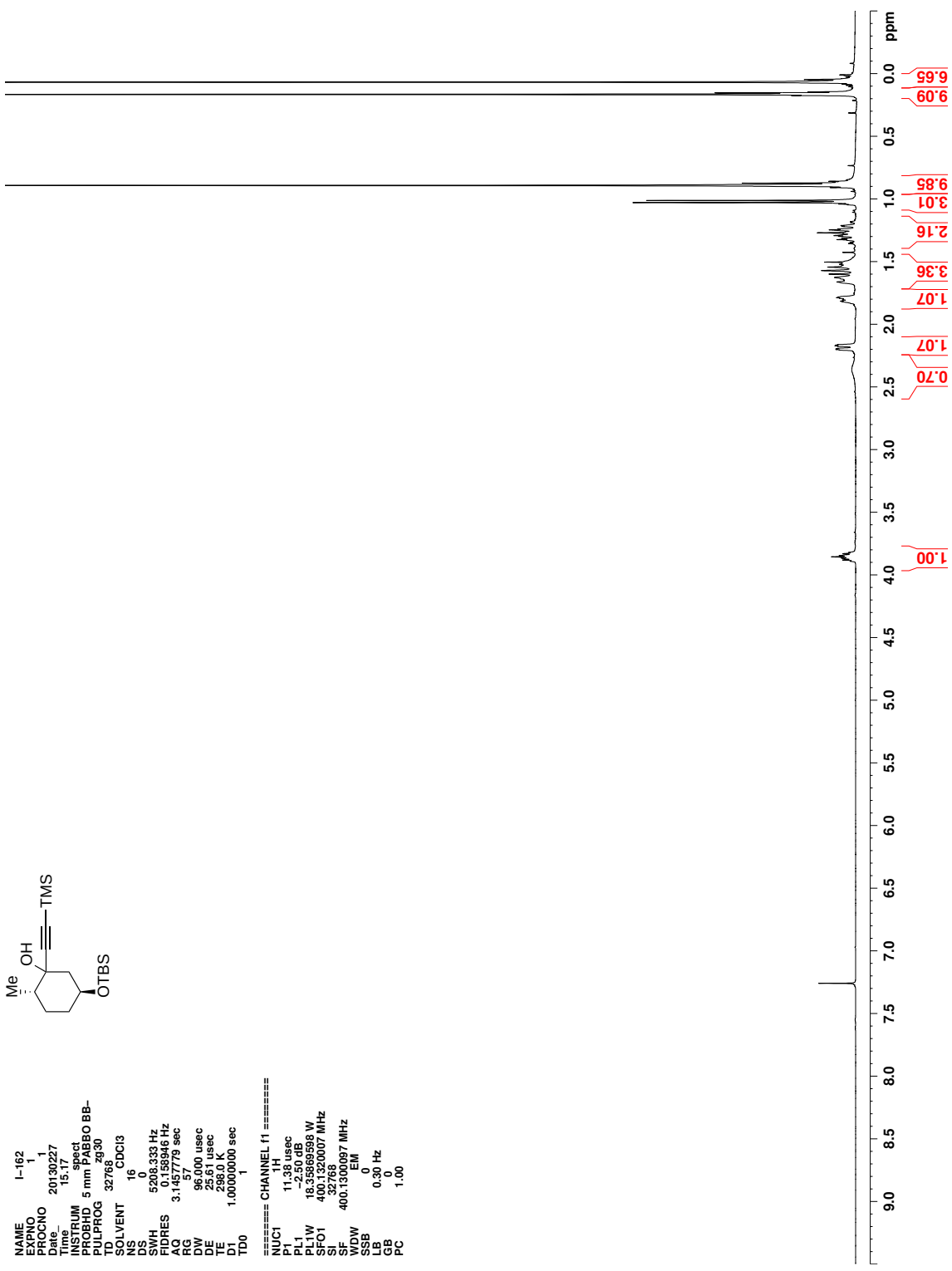


Figure S7 ^{1}H NMR ($CDCl_3$) of **10**

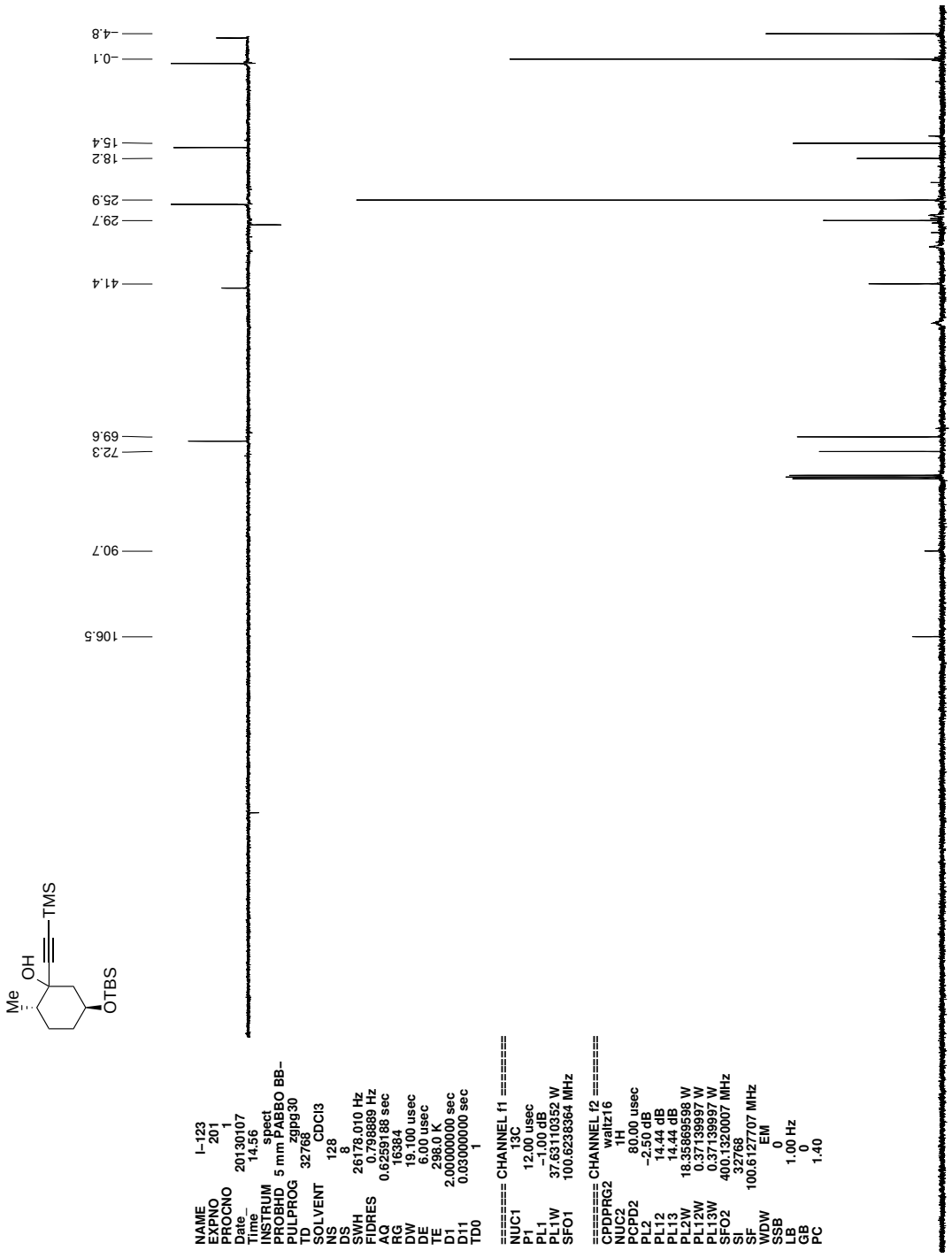


Figure S8 ^{13}C NMR ($CDCl_3$) of **10**

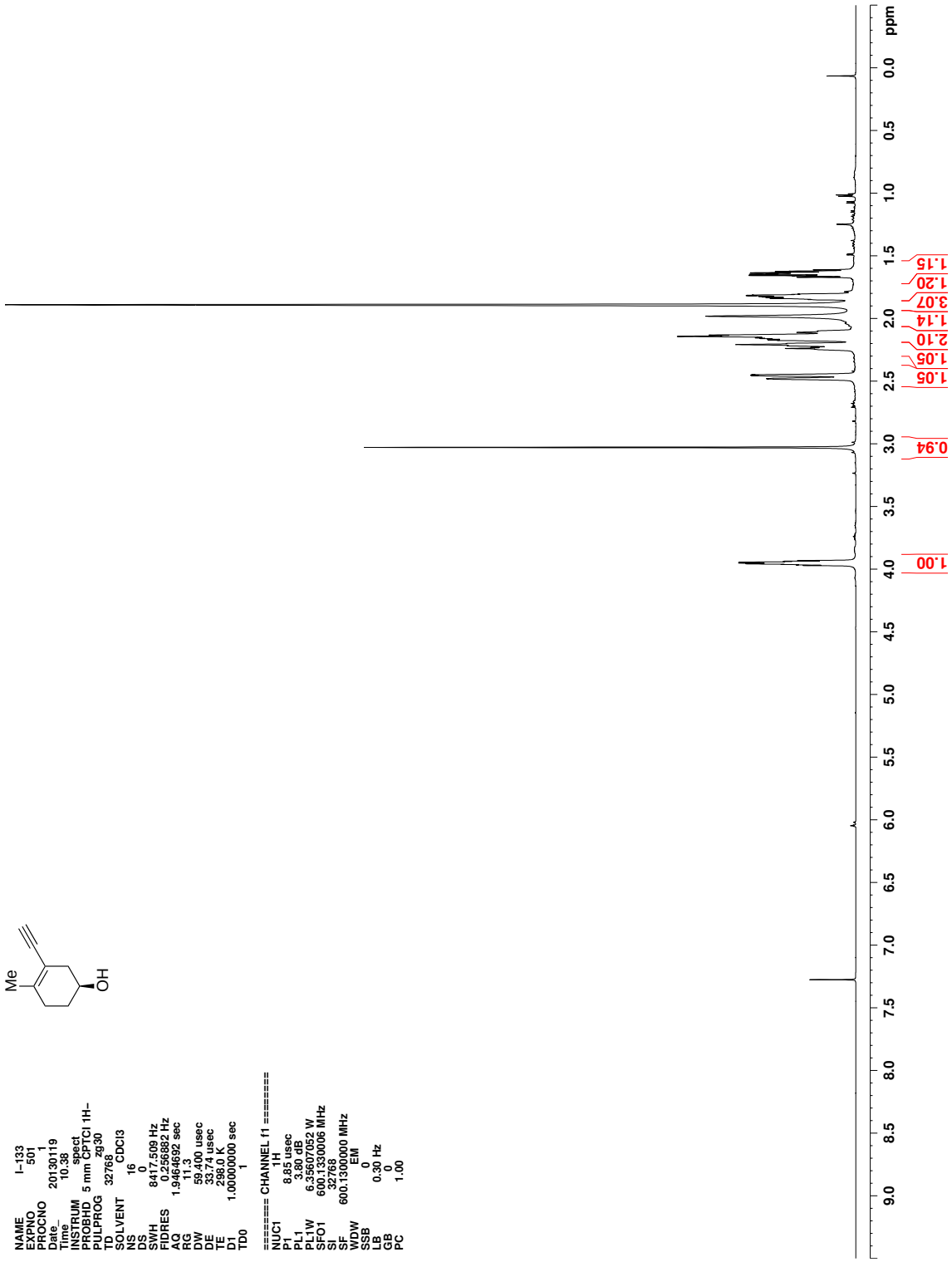


Figure S9 ^{1}H NMR ($CDCl_3$) of **2**

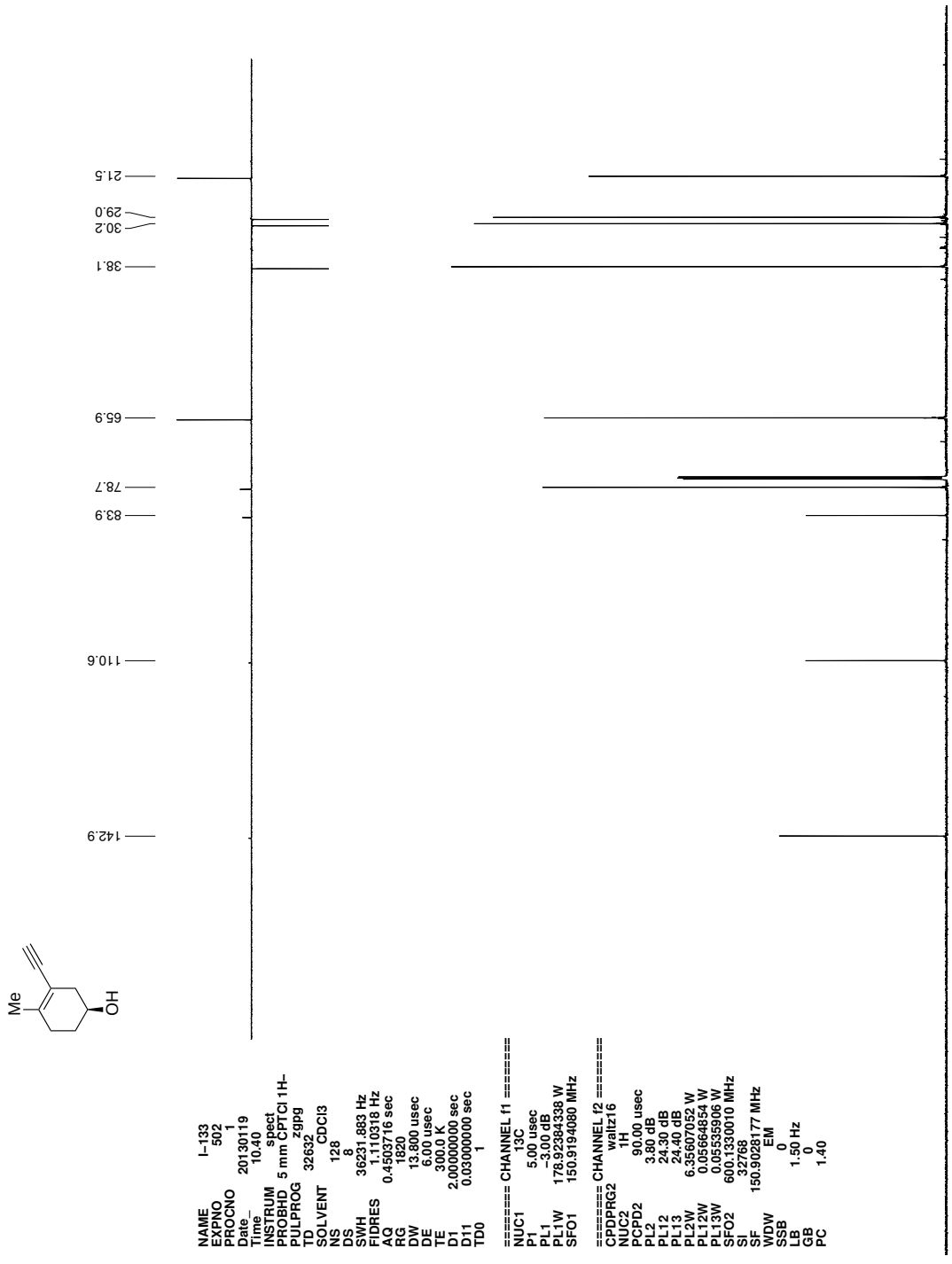


Figure S10 ^{13}C NMR ($CDCl_3$) of **2**

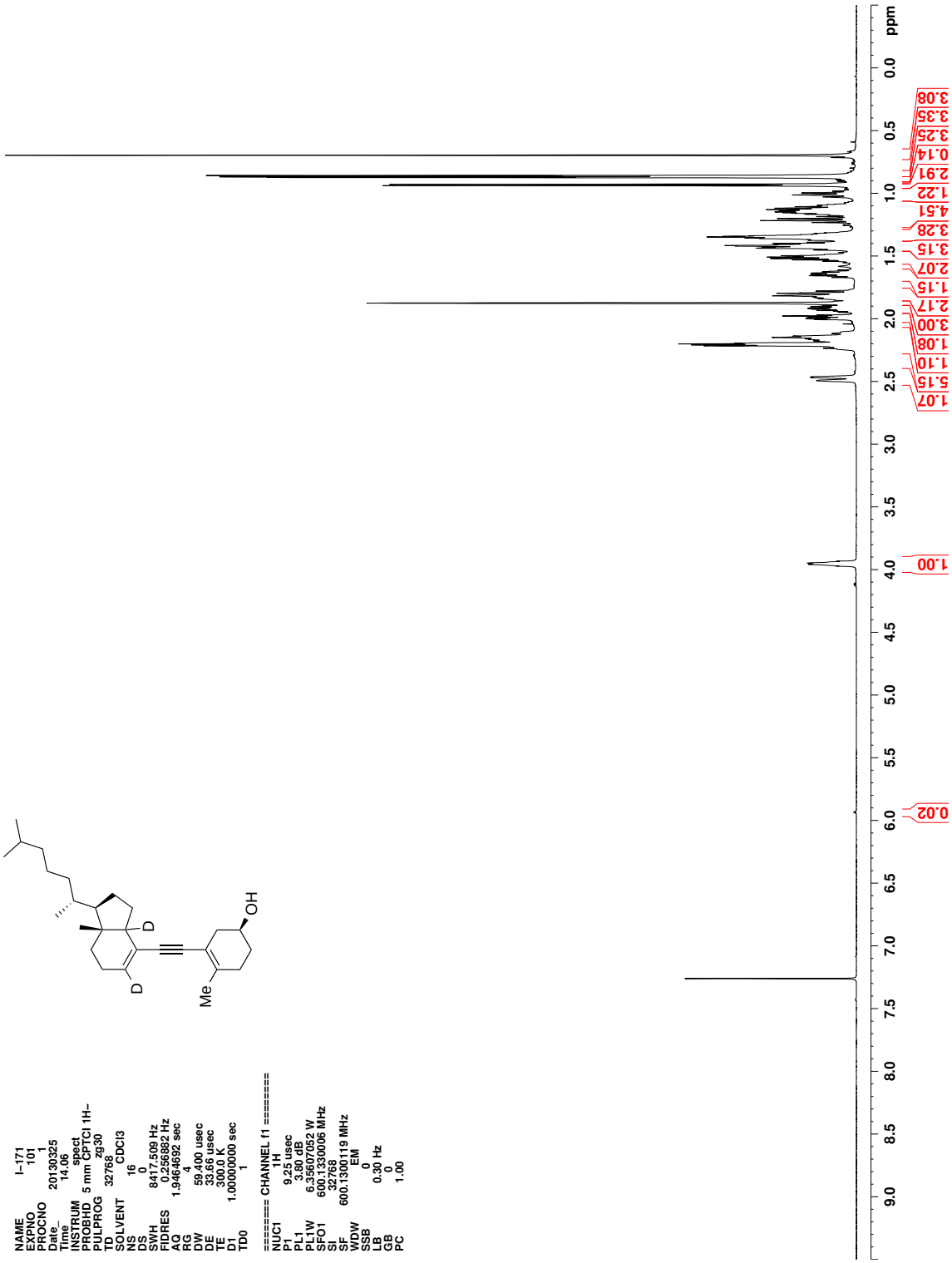


Figure S11 ^{1}H NMR ($CDCl_3$) of **12**

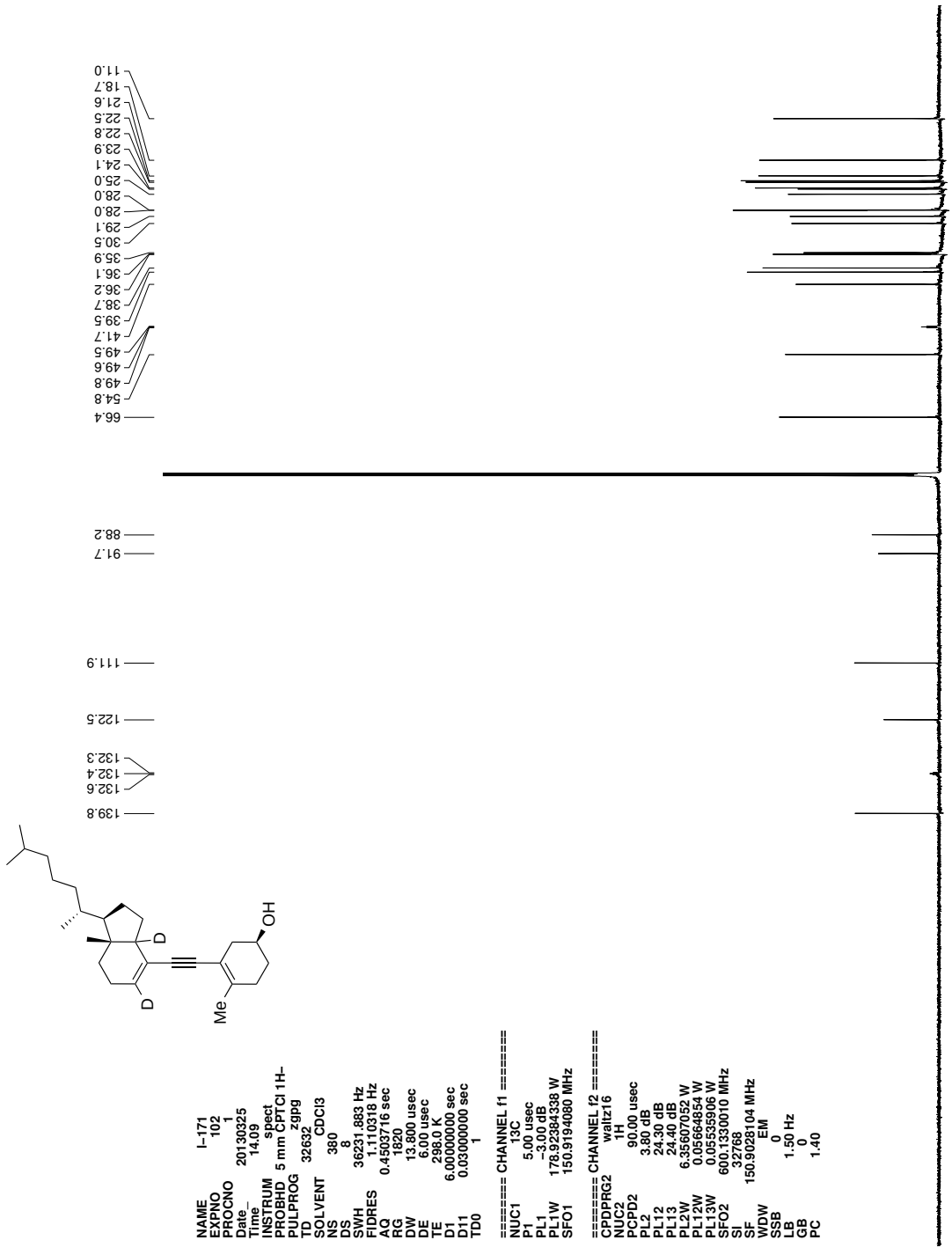


Figure S12 ^{13}C NMR ($CDCl_3$) of **12**

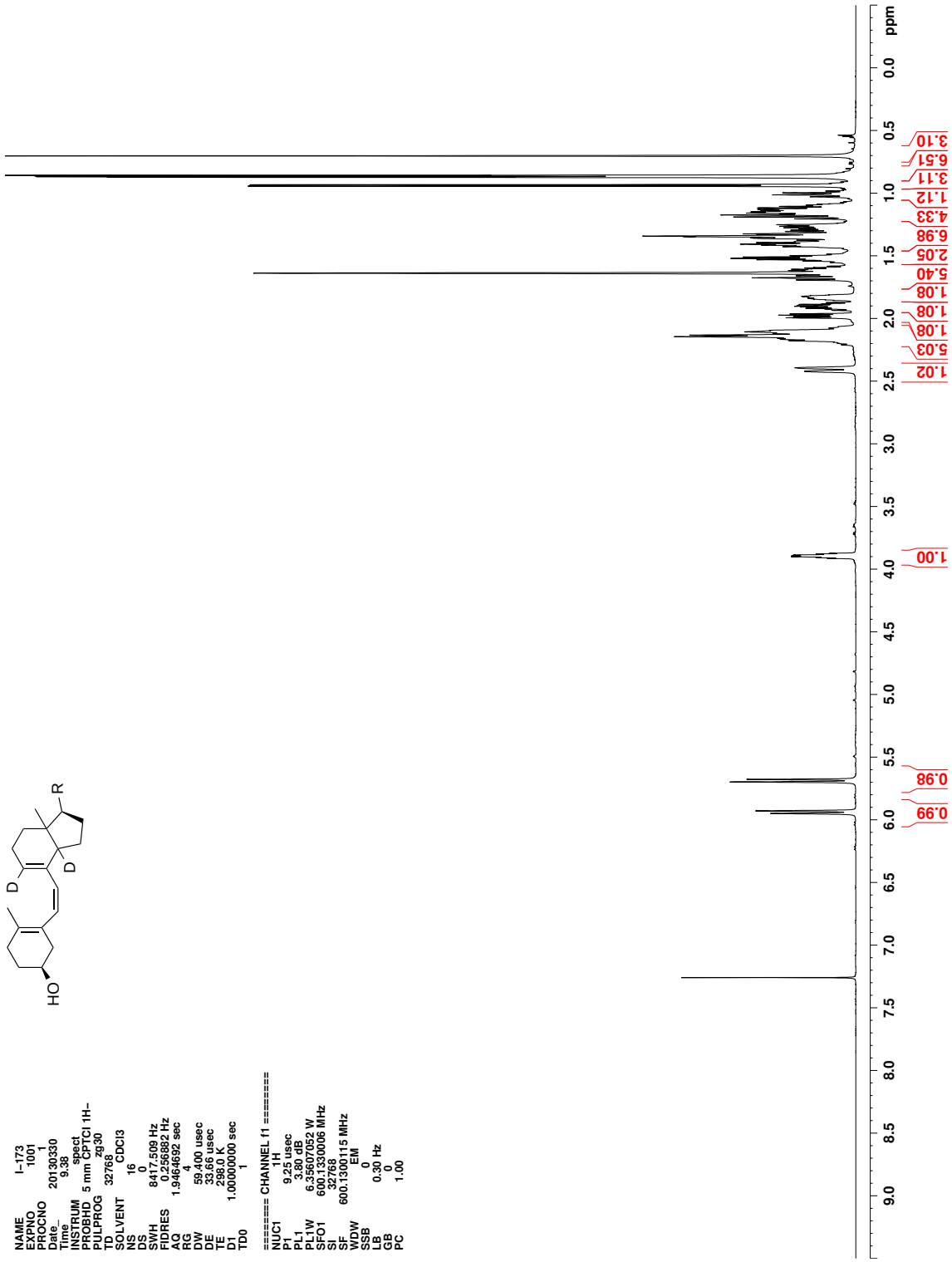


Figure S13 ^{1}H NMR ($CDCl_3$) of **1-d$_2$**

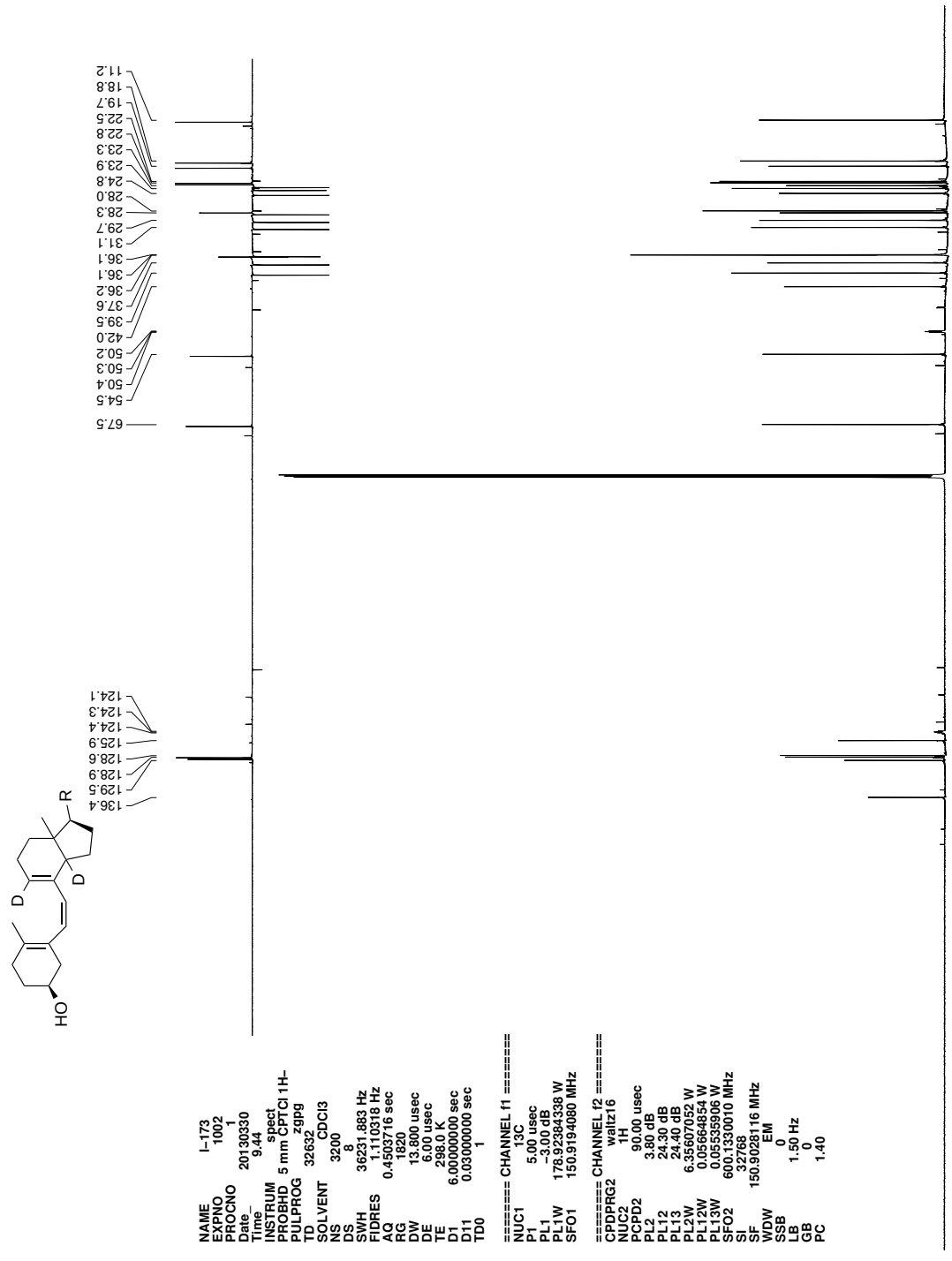


Figure S14 ^{13}C NMR ($CDCl_3$) of **1-d$_2$**

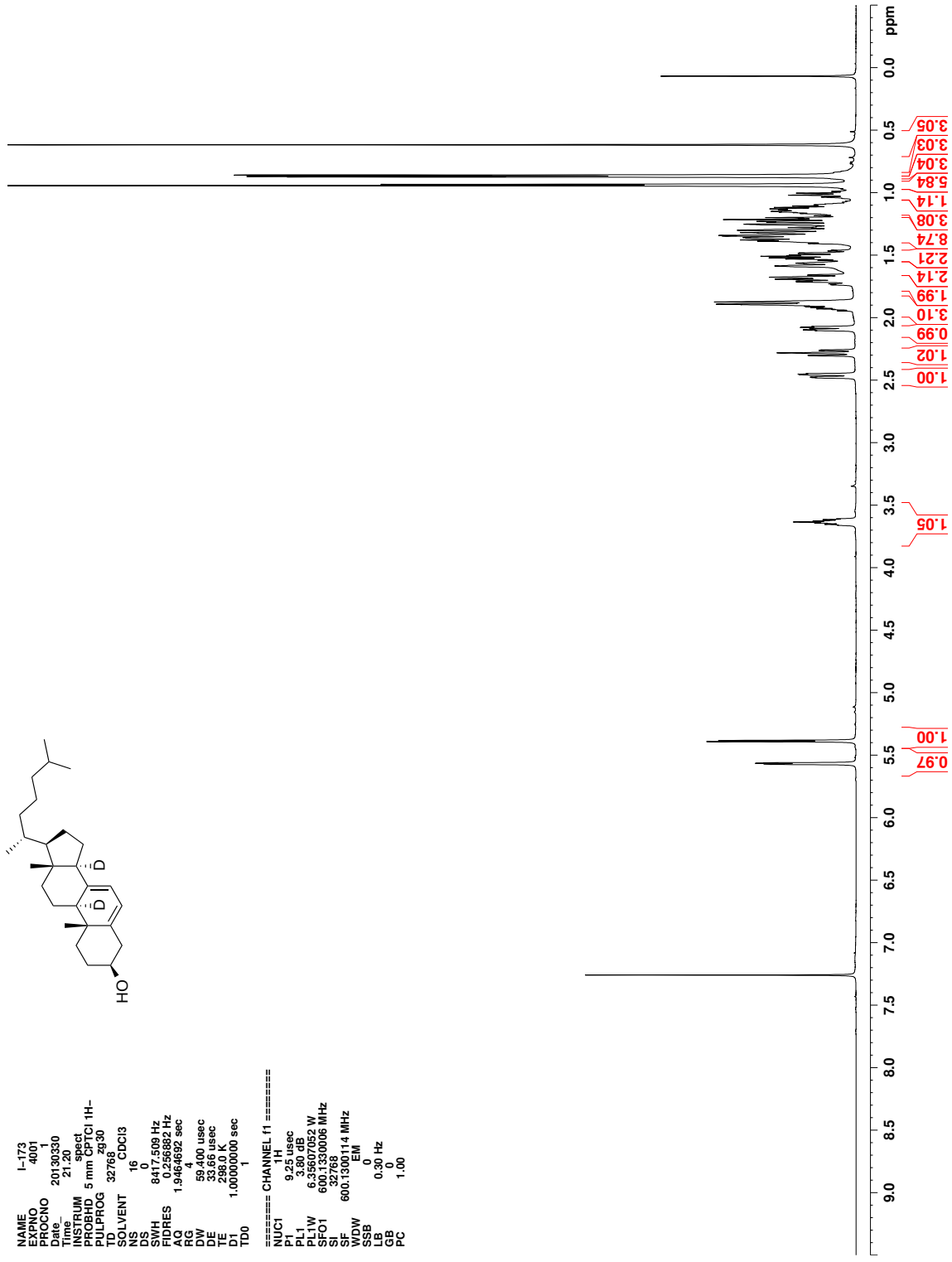


Figure S15 ^{1}H NMR ($CDCl_3$) of **7-DHC-d$_2$**

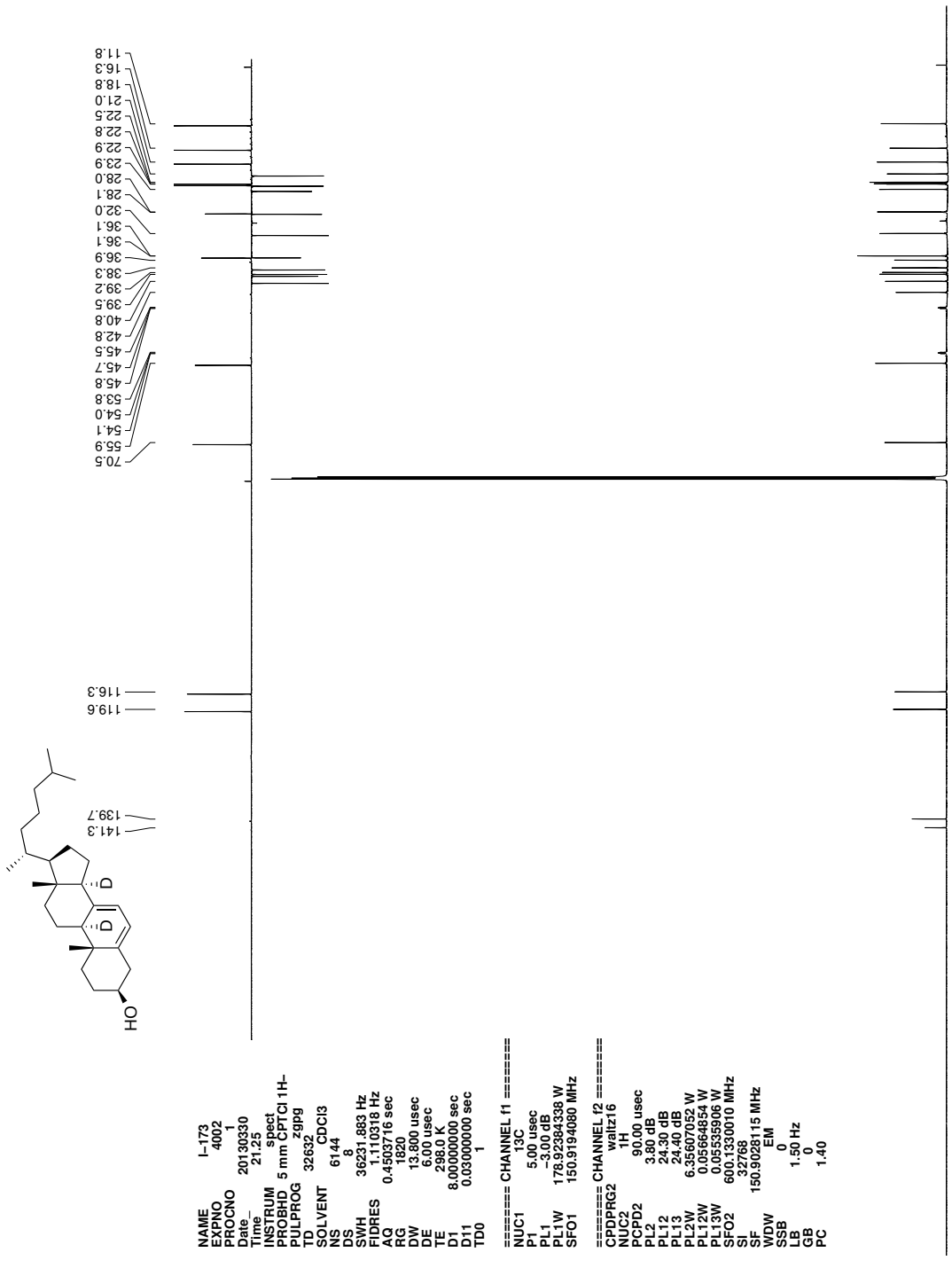


Figure S16 ^{13}C NMR ($CDCl_3$) of **7-DHC-d_2**

4 KIE determination

4.1 Sample Preparation

A mixture of 7-Dehydrocholesterol-d_2 and 7-dehydrocholesterol-d_7 (aprox. 10:1 ratio, aprox 10 mg) was purified by preparative reversed phase HPLC (methanol, t_r = 19 min). Fractions were collected at −78 °C into a flask containing α-tocopherol (250 mg) in benzene (2 mL). The solution was concentrated to a small volume and transfered to a 1 mL vial and dried to constant mass. Benzene (350 µL) was added and the solution (0.03 M in 7-DHC and 0.9 M in α-tocopherol) was divided into four vials (150 µL each) and the MeOAMVN initiator (0.03 M, 10 µL) was added. To the first vial (t_0) was immediately added PPh_3 (0.5 M in benzene, 20 µL) and BHT (0.5 M in benzene, 20 µL) and benzene (300 µL) and the vial was transferred to −80 °C freezer for storage. Three remaining vials were capped and incubated at 37 °C for 8 h and then was added PPh_3 (0.5 M in benzene, 20 µL) and BHT (0.5 M in benzene, 20 µL) and benzene (300 µL).

4.2 Determination of the 7-DHC-d_2 and 7-DHC-d_7

A small fraction of each reaction was directly injected into MS via syringe pump at a flow rate of 20 µL/min (with a make up flow of 1.0 mL/min by the HPLC) to obtain the ratio of the **7-DHC-d_2** and **7-DHC-d_7** by comparing the intensity of m/z at 369 and 374, respectively (Table S2).

Table S2 Determination of the ratio of **7-DHC-d_2** and **7-DHC-d_7**

Run	Area m/z 369	Area m/z 374	ratio
1	1.84E7	2.01E6	9.15
1	1.21E7	1.30E6	9.31
1	1.03E7	1.19E6	8.66
1	1.98E7	1.88E6	10.53
1	1.66E7	1.70E6	9.76
		average	**9.48**
		stdev	**0.71**

4.3 KIE measurement

HPLC-APCI-MS-MS analysis was carried out similarly to the previously reported method for 7-DHC-derived oxysterols with modification of the masses being monitored.[8–10] For example, for 7-DHC-d_7-derived oxysterols, masses with 7 additional mass units relative to the non-deuterated oxysterols were monitored; for 7-DHC-d_2-derived oxysterols (giving D1-oxysterols after losing D-9 or D-14), masses with one additional mass unit were monitored. In general, selective reaction monitoring (SRM) was employed to monitor the dehydration process of the ion $[M+H]^+$ or $[M+H-H_2O]^+$ in the mass spectrometry.

A time course study of oxysterol formation from **7-DHC-d_2** and **7-DHC-d_7** (Figure S17) revealed that after 8 h of oxidation more products (>10 times) was present than at t=0 while the consumption of the starting sterols was still very low and these conditions were chosen for KIE study. Table S3 shows KIE analysis for a co-oxidation carried out for 8 hours at 37 °C and Figure S18 shows representative HPLC-MS chromatogram.

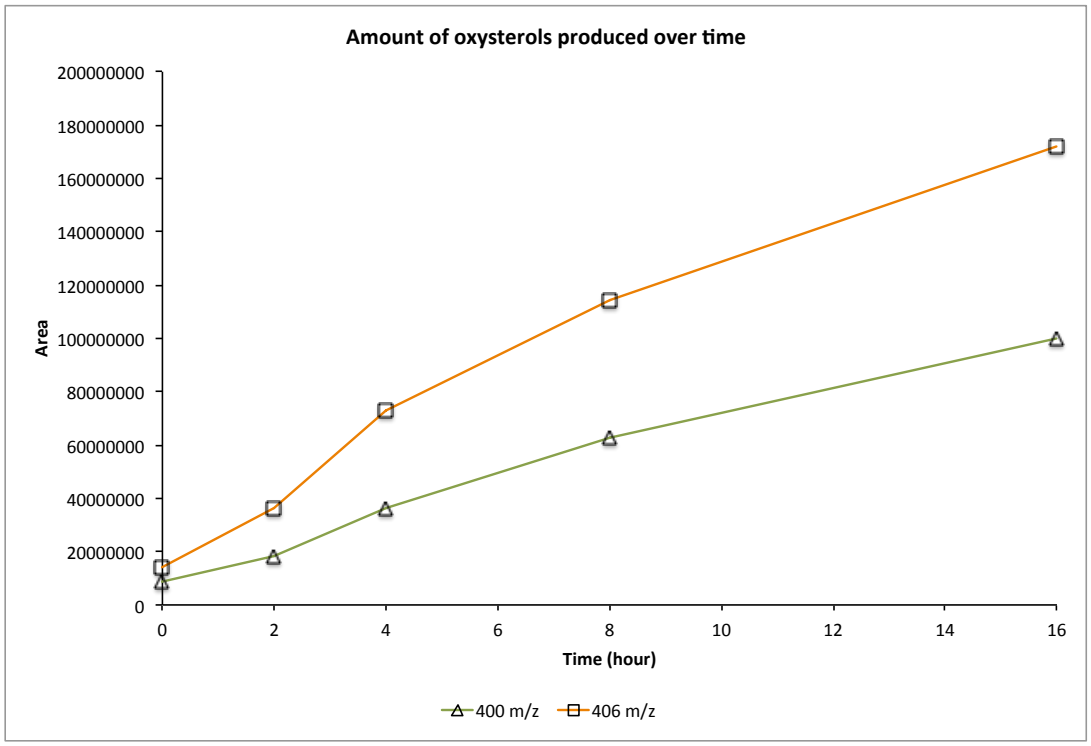


Figure S17 Oxysterol formation over time

Table S3 Determination of the KIE at for H-9 from 7-keto-8-DHC

Run	Area m/z 400→382	Area m/z 406→388	product ratio (d_7/d_1)	substrate ratio (d_7/d_2)	KIE
t0	5 086 578	10 911 971	2.15	9.48	20.34
1	57 801 532	128 375 467	2.22	9.48	21.06
2	63 997 804	144 182 886	2.25	9.48	21.36
3	78 327 254	165 310 697	2.11	9.48	20.01
				average	**20.70**
				stdev	**0.70**

Table S4 Determination of the KIE at for H-9 from THCEO

Run	Area d_1	Area d_7	product ratio (d_7/d_1)	substrate ratio (d_7/d_2)	KIE
1	1 619 529	3 796 546	2.33	9.48	22.09
2	1 544 241	3 700 243	2.40	9.48	22.72
3	2 546 095	4 575 912	2.23	9.48	21.13
				average	**21.98**
				stdev	**0.80**

Table S5 Determination of the KIE at for H-9 from DHCDO

Run	Area d_1	Area d_7	product ratio (d_7/d_1)	substrate ratio (d_7/d_2)	KIE
1	453 388	1 579 456	3.48	9.48	33.02
2	493 871	1 366 763	2.75	9.48	26.08
3	832 646	2 325 038	2.79	9.48	26.47
				average	**28.52**
				stdev	**3.90**

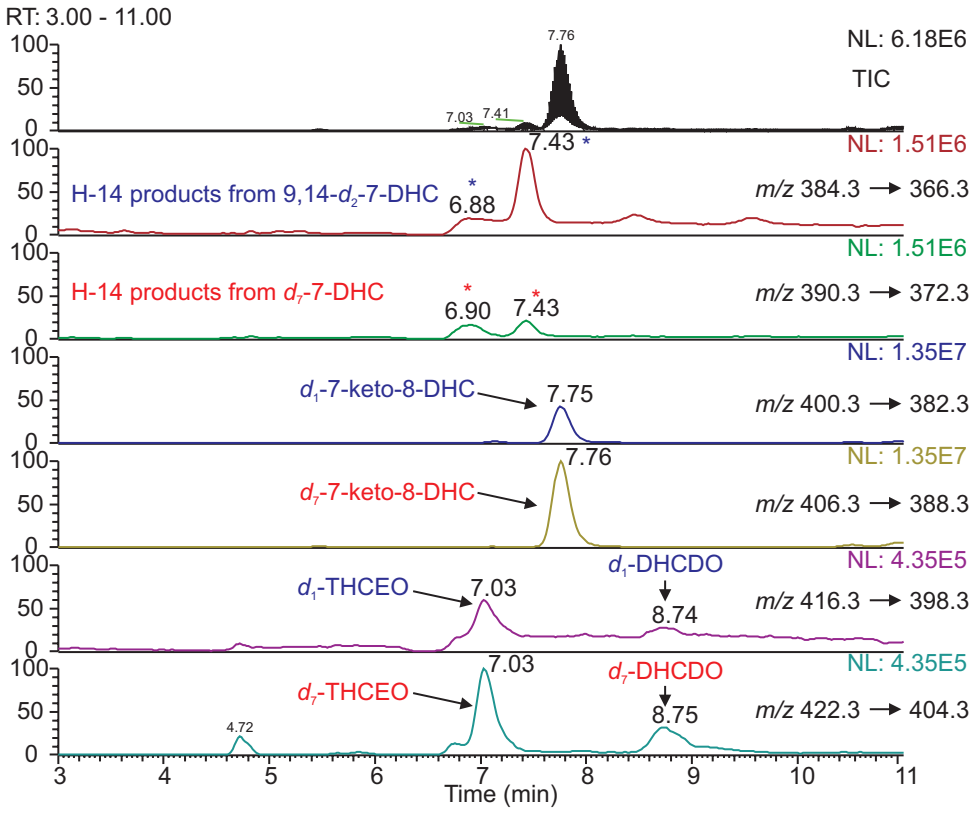


Figure S18 Representative HPLC-MS chromatogram for oxysterol detection

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