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Preparation of ORMOSIL Nanoparticles Conjugated with Vitamin D_3 Analogues and their Biological Evaluation

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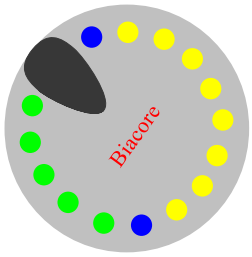
EXPERIMENTAL PROTOCOLS AND ANALITYCAL DATA

General. 2-(4-Chlorosulfonylphenyl)ethyltrimethoxysilane (50% solution in dichloromethane) was obtained by ACROS. High surface area hydrophobic Bio-Beads® were obtained from Bio-Rad and used according to instructions of the supplier. Water was purified using a Milli-Q® and water purification system. All the others chemical reagents were bought from Aldrich at highest commercial quality and used without further purification. SO_2 was generated adding dropwise concentrated sulphuric acid over a solution of Na_2SO_3 in water. All non-aqueous reactions were carried out under anhydrous conditions in dry, freshly distilled solvents. Reactions were monitored by TLC developed on 0.25 mm Merck silica gel plates (60 F_{254}) using UV light as visualizing agent and/or heating after spraying with a 5% aqueous sulphuric acid solution containing cerium (IV) sulphate (1%) and molybdophosphoric acid (2.5%). Flash chromatography was performed using silica gel 60 (230–400 mesh). IR spectra were recorded as thin films on NaCl plates or KBr pellets on an Infrared FT spectrophotometer. High-resolution mass spectra (HRMS) were recorded on a VG 7070 HS mass spectrometer under electron spray ionization (ESI) conditions using a micrOTOF-Q spectrometer. UV-Vis absorption spectra were measured in water on a Perkin-Elmer Lambda 45 UV-Vis spectrophotometer with 1 cm path length quartz cuvettes. Fluorescence spectra were measured in water on a Perkin-Elmer LS-50B fluorimeter with 1 cm path length quartz cuvettes. Ultrafiltration of the nanoparticles was carried out using a 75 ml Amicon Ultrafiltration Cell Millipore using a cellulose membrane with a cut-off of 10000 Da. 2D NMR (COSY, HMBC, HSQC) methods were employed to elucidate the structures of analogues and their intermediates. Spectra of the compounds were obtained using 300.13 or 400.13 MHz for ^1H, and 75.5 or 100.61 MHz for ^{13}C NMR instruments. ^1H NMR spectra of the nanoparticles were recorded on a Bruker Avance (250 MHz) or on a Bruker AC-300, operating at 300 MHz. A LEDBP sequence was used to acquire the diffusion-filtered spectra with a mixing time of 0.3 s. The particle size was measured by Dynamic Light Scattering (DLS) with a Malvern Zetasizer Nano-S with a HeNe laser (633 nm): temperature of 25 ºC, aqueous suspension, and viscosity of 0.8872 cP. Transmission electron microscopy (TEM) imagines was recorded on a Jeol 300PX instrument. One drop of sample was placed on the sample grid and the solvent was allowed to evaporate. Surface Plasmon Resonance (SPR) experiments were performed with a Biacore X100 instrument. A CM5 chip, research grade (cat. no. BR-1000-14, Biacore-GE Healthcare, Piscataway, NJ), was used. The immobilization of it involves activation of carboxymethyl groups on a dextran-coated chip by reaction with *N*-hydroxysuccinimide, followed by covalent bonding of the protein to the chip surface via amide linkages and blockage of excess activated carboxyls with ethanolamine. The sample preparation was performed as described below:



- Samples in 5% DMSO/buffer (10 mM Hepes, 150 mM NaCl, 3 mM EDTA, 0.005% Polisorbate 20) with different concentrations
- Blank: 5% DMSO/buffer
- Solvent correction: 1-8% DMSO/buffer

Synthesis and purification of (4), (5), and nanoparticles

They were described in our previous work.[1]

Synthesis of Vitamin D Analogues

Vitamin D_3-SO_2 Adduct (9).[2] Vitamin D_3 (200 mg, 0.53 mmol) was gently refluxed in liquid SO_2 (approx. 2.7 mL) for 20 min. Then, excess liquid SO_2 was removed under reduced pressure and the white solid obtained was sufficiently pure for direct use in the next step. Rf: 0.2 (30% EtOAc /hexane); IR (KBr): ν 3502, 2951, 2868, 1471, 1456, 1436, 1375, 1303 cm^{-1}; ^1H NMR (300.13 MHz, $CDCl_3$): Mixture of both isomers δ 0.55 (s, 3H, Me_{18}), 0.63 (s, 3H, Me_{18}), 0.84 (d, 6H, Me_{26} + Me_{27}, *J* 6.6 Hz), 0.86 (d, 6H, Me_{26} + Me_{27}, *J* 6.6 Hz), 0.93 (d, 6H, Me_{21}, *J* 6.6 Hz), 1.00 – 2.61 (several m, 52H, $4H_1$ + $4H_2$ + $2H_4$ + $4H_9$ + $4H_{11}$ + $4H_{12}$ + $2H_{14}$ + $4H_{15}$ + $4H_{16}$ + $2H_{17}$ + $2H_{20}$ + $4H_{22}$ + $4H_{23}$ + $4H_{24}$ + $2H_{25}$ + 2OH), 3.66 (m, 4H, H_{19}), 4.06 (m, 2H, H_3), 4.57 (d, 1H, H_6, *J* 9.3 Hz), 4.65 (d, 1H, H_6, *J* 9.8 Hz), 4.74 (t, 2H, H_7, *J* 9.4 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 11.9 (Me_{18}), 12.2 (Me_{18}), 19.0 (Me_{21}), 22.3 (CH_2), 22.7 and 22.9 (Me_{26} + Me_{27}), 23.7 (CH_2), 23.9 (CH_2), 24.0 (CH_2), 24.1 (CH_2), 24.5 (CH_2), 27.7 (CH_2), 28.1 (CH, C_{25}), 29.6 (CH_2), 30.0 (CH_2), 30.1 (CH_2), 30.5 (CH_2), 33.4 (CH_2), 33.9 (CH_2), 36.1 (CH, C_{20}), 36.2 (CH_2), 36.3 (CH_2), 39.6 (CH_2), 39.7 (CH_2), 40.2 (CH_2), 40.4 (CH_2), 45.7 (C, C_{13}), 46.3 (C, C_{13}), 56.2 (CH, C_{17}), 56.4 (CH, C_{17}), 56.6 (CH, C_{14}), 56.8 (CH, C_{14}), 58.1 (CH_2, C_{19}), 58.2 (CH_2, C_{19}), 65.9 (CH, C_3), 66.3 (CH, C_3), 67.0 (CH, C_6), 67,4 (CH, C_6), 109.4 (CH, C_7), 126.7 (C, C_{10}), 127.0 (C, C_{10}), 130.2 (C, C_8), 130.7 (C, C_8), 150.8 (C, C_5), 151.1 (C, C_5) ppm; MS (ESI^+, m/z): 471 [$(M+H)^+$, 100%].

3-*O*-(*tert*-Butyldimethylsilyl)vitamin D_3-SO_2 Adduct (10).[3] To a solution of **9** (136 mg, 0.30 mmol) in anhydrous CH_2Cl_2 (1.2 mL) at 0 ºC, were added imidazole (28 mg, 0.41 mmol) and *tert*-butyldimethylsilyl chloride (57 mg, 0.38 mmol). Afterwards, the reaction was stirred at room temperature overnight. Then, solvent was evaporated and the residue purified by column chromatography (15% EtOAc/hexane as eluent) to give protected alcohol **10** in 85% yield. Rf: 0.8 (30% EtOAc /hexane); IR (KBr): ν 2950, 2856, 1471, 1377, 1310 cm^{-1}; ^1H NMR (300.13 MHz, $CDCl_3$): Mixture of both isomers δ 0.04 (s, 3H, Si*Me)*, 0.05 (s, 3H,

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[1] I. M. Rio-Echevarria, F. Selvestrel, D. Segat, G. Guarino, R. Tavano, V. Causin, E. Reddi, E. Papini and F. Mancin, *J. Mat. Chem.*, 2010, **20**, 2780-2787.

[2] Previously described in S. Yamada, T. Suzuki and H. Takayama, *J. Org. Chem.*, 1983, **48**, 3483-3488.

[3] Previously described in J. A. Marshall, J. Grote and B. Shearer, *J. Org. Chem.*, 1986, **51**, 1635-1637.

Si*Me)*, 0.09 (s, 3H, Si*Me)*, 0.10 (s, 3H, Si*Me)*, 0.56 (s, 3H, Me_{18}), 0.64 (s, 3H, Me_{18}), 0.86 (d, 12H, Me_{26} + Me_{27}, *J* 6.8 Hz,), 0.87 (s, 18H, SiCMe_3), 0.92 (d, 6H, *J* 6.5 Hz, Me_{21}), 1.00–2.61 (several m, 50H, $4H_1$ + $4H_2$ + $2H_4$ + $4H_9$ + $4H_{11}$ + $4H_{12}$ + $2H_{14}$ + $4H_{15}$ + $4H_{16}$ + $2H_{17}$ + $2H_{20}$ + $4H_{22}$ + $4H_{23}$ + $4H_{24}$ + $2H_{25}$), 3.65 (m, 4H, H_{19}), 3.99 (m, 2H, H_3), 4.49-4.78 (several m, 4H, H_6 + H_7) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ -4.62 (Si*Me*), 11.9 (Me_{18}), 12.1 (Me_{18}), 18.17 (Si*C*), 18.23 (Si*C*), 18.9 (Me_{21}), 22.1 (CH_2), 22.2 (CH_2), 22.6 and 22.9 (Me_{26} + Me_{27}), 23.7 (CH_2), 23.9 (CH_2), 24.0 (CH_2), 24.4 (CH_2), 24.8 (CH_2), 25.9 (SiCMe_3), 27.6 (CH_2), 27.7 (CH_2), 28.1 (CH, C_{25}), 29.5 (CH_2), 30.0 (CH_2), 30.8 (CH_2), 31.1 (CH_2), 34.2 (CH_2), 34.4 (CH_2), 36.1 (CH_2), 36.2 (CH), 36.1 (CH_2), 39.6 (CH_2), 39.7 (CH_2), 40.2 (CH_2), 40.5 (CH_2), 45.6 (C), 46.3 (C), 56.2 (CH, C_{17}), 56.4 (CH, C_{17}), 56.5 (CH, C_{14}), 56.7 (CH, C_{14}), 58.1 (CH_2, C_{19}), 58.2 (CH_2, C_{19}), 66.7 (CH, C_3), 66.9 (CH, C_3), 67.0 (CH, C_6), 67,7 (CH, C_6), 109.7 (CH, C_7), 110.2 (CH, C_7), 126.5 (C, C_{10}), 126.1 (C, C_{10}), 130.6 (C, C_8), 130.9 (C, C_8), 150.1 (C, C_5), 150.7 (C, C_5) ppm; MS (ESI^+, m/z): 563 [$(M+H)^+$, 100%].

(5*E*)-3-*O*-(*tert*-Butyldimethylsilyl)vitamin D_3 (11).[3] A mixture of **10** (200 mg, 0.35 mmol) obtained above, $NaHCO_3$ (298 mg, 3.55 mmol) and EtOH (1.8 mL) was refluxed for 3 h and cooled to room temperature. The solvent was removed and then the residue was filtered over Celite®. The organic extract was washed with brine, dried ($MgSO_4$), and evaporated to dryness to afford **11** in a 98% yield. Rf: 0.7 (5% EtOAc/hexane); IR (KBr): ν 2952, 2857, 1471, 1463, 1377, 1253 cm^{-1}; ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 0.07 (s, 3H, Si*Me*), 0.08 (s, 3H, Si*Me*), 0.56 (s, 3H, Me_{18}), 0.86 (2d, *J* 6.7 Hz, 6H, Me_{26} + Me_{27}), 0.88 (s, 9H, SiCMe_3), 0.93 (d, 3H, Me_{21}, *J* 6.4Hz), 1.00 –2.52 (several m, 24H, H_{1ax} + $2H_2$ + H_{4ax} + $2H_9$ + $2H_{11}$ + $2H_{12}$ + H_{14} + $2H_{15}$ + $2H_{16}$ + H_{17} + H_{20} + $2H_{22}$ + $2H_{23}$ + $2H_{24}$ + H_{25}), 2.66 (dd, 1H, H_{4eq}, *J* 13.8 + 3.1Hz), 2.86 (d, 1H, H_{1eq}, *J* 12.1 Hz), 3.83 (m, 1H, H_3), 4.64 (s, 1H, H_{19}), 4.93 (s, 1H, H_{19}), 5.86 (d, 1H, H_7, *J* 11.4Hz), 6.48 (d, 1H, H_6, *J* 11.4Hz) ppm;^{13}C NMR (75.5 MHz, $CDCl_3$): δ -4.6 (Si*Me*), -4.5 (Si*Me*), 12.2 (Me_{18}), 18.3 (Si*C*), 19.0 (Me_{21}), 22.3 (CH_2), 22.7 and 23.0 (Me_{26} + Me_{27}), 23.7 (CH_2), 24.0 (CH_2), 26.0 (SiCMe_3), 27.8 (CH_2), 28.2 (CH, C_{25}), 29.2 (CH_2), 31.3 (CH_2), 35.3 (CH_2), 36.2 (CH_2), 36.3 (CH_2), 37.7 (CH_2), 39.7 (CH_2), 40.7 (CH_2), 46.0 (C), 56.7 and 56.9 (2CH, C_{14} + C_{17}), 69.6 (CH, C_3), 107.7 (CH_2, C_{19}), 116.2 (CH, C_7), 120.2 (CH, C_6), 136.3 (C, C_{10}), 143.9 (C, C_8), 150.1 (C, C_5) ppm; MS ($APCI^+$, m/z): 499 [$(M+H)^+$, 100%].

(5*E*)-3-*O*-(*tert*-Butyldimethylsilyl)-1α/β-hydroxyvitamin D_3 (12/13).[3] To a solution of **11** (1.22 g, 2.45 mmol) in anhydrous CH_2Cl_2 (12.3 mL) was added *N*-methylmorpholine oxide (1.15 g, 9.79 mmol), and the mixture was refluxed for 5 min. To this solution was added a mixture of selenium oxide (325 mg, 2.93 mmol), which was sonicated in MeOH (12.3 mL) at room temperature. The whole mixture was refluxed for 2 h and cooled to room temperature, and then poured into ice water. The mixture was extracted with EtOAc and the organic extract was washed brine, and dried ($MgSO_4$). The solvent was removed by reduced pressure and the residue was

purified by chromatography on silica gel. The column was eluted with 2-5% EtOAc–hexane to yield a mixture of **12** and its isomer with 1β-hydroxyl group **13** (Relation between both isomers 90:10) Rf: 0.3 (10% EtOAc/hexane); IR (KBr): ν 3430, 2953, 2860, 1471, 1463, 1377, 1253 cm^{-1}. ^1H NMR (300.13 MHz, $CDCl_3$): <u>1α-hydroxy isomer **12**</u>: δ 0.07 (s, 6H, Si*Me*), 0.55 (s, 3H, Me_{18}), 0.87 (s, 9H, SiCMe_3), 0.86 (d, 6H, Me_{26} + Me_{27}, *J* 6.4 Hz), 0.93 (d, 3H, Me_{21}, *J* 6.4 Hz), 1.00 –2.92 (several m, 25H, $2H_2$ + $2H_4$ + $2H_9$ + $2H_{11}$ + $2H_{12}$ + H_{14} + $2H_{15}$ + $2H_{16}$ + H_{17} + H_{20} + $2H_{22}$ + $2H_{23}$ + $2H_{24}$ +H_{25} + OH), 4.19 (m, 1H, H_3), 4.50 (m, 1H, H_1), 4.96 (s, 1H, H_{19}), 5.07 (s, 1H, H_{19}), 5.85 (d, 1H, H_7, *J* 11.4Hz), 6.51 (d, 1H, H_6, *J* 11.4Hz) ppm.; <u>1β-hydroxy isomer **13**</u>: δ 0.11 (CH_3, 6H, Si*Me*), 0.54 (s, 3H, Me_{18}), 0.86 (s, 9H, SiCMe_3), 0.86 (d, 6H, Me_{26} + Me_{27}, *J* 6.4 Hz), 0.93 (d, 3H, Me_{21}, *J* 6.4Hz), 1.00 –2.92 (several m, 25H, $2H_2$ + $2H_4$ + $2H_9$ + $2H_{11}$ + $2H_{12}$ + H_{14} + $2H_{15}$ + $2H_{16}$ + H_{17} + H_{20} + $2H_{22}$ + $2H_{23}$ + $2H_{24}$ + H_{25} + OH), 4.26 (m, 1H, H_3), 4.50 (m, 1H, H_1), 4.91 (s, 1H, H_{19}), 5.04 (s, 1H, H_{19}), 5.85 (d, 1H, H_7, *J* 11.4Hz), 6.65 (d, 1H, H_6, *J* 11.5Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ -4.6 (Si*Me*), -4.5 (Si*Me*), 12.2 (Me_{18}), 18.2 (Si*C*), 19.0 (Me_{21}), 22.4 (CH_2), 22.7 y 23.0 (Me_{26} + Me_{27}), 23.7 (CH_2), 24.0 (CH_2), 25.9 (SiCMe_3), 26.0 (SiCMe_3), 27.8 (CH_2), 28.2 (CH, C_{25}), 29.2 (CH_2), 36.3 (CH_2 + CH), 37.1 (CH_2), 39.7 (CH_2), 40.7 (CH_2), 43.0 (CH_2), 46.1 (C), 56.7 and 56.8 (2CH, C_{14} + C_{17}), 66.9 (CH, C_3), 70.8 (CH, C_1), 107.9 (CH_2, C_{19}), 116.2 (CH, C_7), 122.6 (CH, C_6), 134.3 (C, C_{10}), 144.6 (C, C_8), 153.2 (C, C_5) ppm.; MS ($APCI^+$, *m/z*): 515 [$(M+H)^+$, 100%].

3-*O*-(*tert*-Butyldimethylsilyl)-1α-hydroxyvitamin D_3 (14).[3] To a solution of **12** y **13** (Mixture of both isomers 10:1, 195 mg, 0.38 mmol) in toluene (15 mL) in a quartz tube were added triethylamine (100 μL, 0.72 mmol) and anthracene (25 mg, 0.13 mmol). A cold finger was introduced inside of it and UV radiation was applied for 3 h. Photochemical isomerization favoured the enrichment of the mixture of isomers into isomer **14**, showing just traces of the minor isomer. After this time, the solvent was evaporated under reduced pressure. The resulting crude was purified by flash chromatography (5% EtOAc/hexane). Rf: 0.4 (10% EtOAc/hexane); IR (KBr): ν 3416, 2954, 2933, 2867, 1469, 1377, 1255 cm^{-1}; ^1H NMR (300.13 MHz, $CDCl_3$): 0.07 (s, 6H, Si*Me*), 0.54 (s, 3H, Me_{18}), 0.86 (2d, 6H, , Me_{26} + Me_{27}, *J* 5.7 Hz), 0.87 (s, 9H, SiCMe_3), 0.92 (d, 3H, Me_{21}, *J* 6.4Hz), 1.00–2.92 (several m, 25H, $2H_2$ + $2H_4$ + $2H_9$ + $2H_{11}$ + $2H_{12}$ + H_{14} + $2H_{15}$ + $2H_{16}$ + H_{17} + H_{20} + $2H_{22}$ + $2H_{23}$ + $2H_{24}$ + H_{25} + OH), 4.19 (m, 1H, H_3), 4.40 (m, 1H, H_1), 4.96 (s, 1H, H_{19}), 5.30 (s, 1H, H_{19}), 6.00 (d, 1H, H_7, *J* 11.3Hz), 6.32 (d, 1H, H_6, *J* 11.3Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ -4.6 (Si*Me*), -4.5 (Si*Me*), 12.2 (Me_{18}), 18.3 (Si*C*), 19.0 (Me_{21}), 22.4 (CH_2), 22.7 and 23.0 (Me_{26} + Me_{27}), 23.7 (CH_2), 24.0 (CH_2), 26.0 (CH_3, SiCMe_3), 27.8 (CH_2), 28.2 (CH, C_{25}), 29.1 (CH_2), 36.3 (CH + CH_2), 39.7 (CH_2), 40.7 (CH_2), 43.6 (CH_2), 46.0 (C), 46.3 (CH_2), 56.5 (CH, C_{17}), 56.7 (CH, C_{14}), 67.4 (CH, C_3), 72.1 (CH, C_1), 112.4 (CH_2, C_{19}), 117.4 (CH, C_7), 124.6 (CH, C_6), 133.7 (C, C_{10}), 142.7 (C, C_8), 147.8 (C, C_5), ppm; MS (ESI^+, m/z): 515 [$(M+H)^+$, 100%].

3-*O*-(*tert*-Butyldimethylsilyl)-25-ethoxymethyloxyvitamin D_3 (21).[4] To a solution of phosphine oxide **19** (173 mg, 0.38 mmol) in anhydrous THF (1.5 mL) at -78 ºC in darkness was added dropwise ^{n}BuLi (213 μL, 1.6 M in hexane, 0.341 mmol) resulting in a deep red colour solution. After 1 h at this temperature, ketone **20** (68 mg, 0.201 mmol) dissolved in THF (2.2 mL) was added slowly and the reaction mixture was stirred for 3 h at -78 ºC and then warmed to -40 ºC and stirred for 2 h. The reaction was quenched by the addition of H_2O (4 mL) and the mixture was then poured into a separatory funnel and extracted with Et_2O. The residue was purified by column chromatography (gradient eluent 1.5–40% EtOAc/hexane) to give **21** in 94% yield and unreacted phosphine oxide (53 mg). Rf: 0.7 (10% EtOAc/hexane); IR (NaCl): ν 2935, 2854, 1471, 1440, 1379 cm^{-1}; ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 0.067 (s, 3H, Si*Me*), 0.073 (s, 3H, Si*Me*), 0.54 (s, 3H, Me_{18}), 0.88 (s, 9H, SiCMe_3), 0.92 (d, 3H, Me_{21}, *J* 6.2 Hz), 1.20 (t, 3H, Me_3, *J* 7.0 Hz), 1.22 (s, 6H, Me_{26} + Me_{27}), 0.98-2.50 (several m), 2.83 (d, 1H, *J* 11.6 Hz), 3.61 (q, 2H, $H_{2'}$, *J* 7.1 Hz), 3.82 (m, 1H, H_3), 4.75 (s, 2H, $H_{1'}$), 4.78 (s, 1H, H_{19}), 5.01 (s, 1H, H_{19}), 6.01 and 6.17 (2d, 2H, H_6 + H_7, *J* 11.2 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ -4.5 (Si*Me*), -4.4 (Si*Me*), 12.2 (Me_{18}), 15.4 ($Me_{3'}$), 18.3 (Si*C*), 19.0 (Me_{21}), 20.7 (CH_2), 22.4 (CH_2), 23.6 (CH_2), 26.0 (SiCMe_3), 26.5 and 26.6 (Me_{26} + Me_{27}), 27.9 (CH_2), 29.1 (CH_2), 32.9 (CH_2), 36.3 (CH, C_{21}), 36.5 (CH_2), 36.6 (CH_2), 40.7 (CH_2), 42.4 (CH_2), 45.9 (C, C_{13}), 47.0 (CH_2), 56.5 and 56.7 (2CH, C_{14} + C_{17}), 63.1 (CH_2, $C_{2'}$), 70.7 (CH, C_3), 76.3 (C, C_{25}), 89.6 (CH_2, $C_{1'}$), 112.3 (CH_2), 118.0 (CH, C_7), 121.5 (CH, C_6), 136.4 (C, C_{10}), 141.6 (C, C_8), 145.5 (C, C_5) ppm; MS (ESI+, m/z): 595 [(M+Na)+, 100%].

25-Ethoxymethyloxyvitamin D_3 (22).[4] TBAF (1.2 mL, 1 M in THF, 1.15 mmol) was added dropwise to a solution of silyl ether **21** (264 mg, 0.46 mmol) in anhydrous THF (4.6 mL) at 0 ºC in darkness. The reaction mixture was followed by TLC (25% EtOAc/hexane) until complete consumption of starting material (5–7 h). Then, solvent was evaporated and the residue purified by column chromatography (eluent 20% EtOAc/hexane) to give **22** in 91% yield. Rf: 0.4 (40% EtOAc/hexane); IR (NaCl): ν 3383, 2937, 2874, 1645, 1472, 1442 cm^{-1}; ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 0.54 (s, 3H, Me_{18}), 0.92 (d, 3H, Me_{21}, *J* 6.3 Hz), 1.19 (t, 3H, $Me_{3'}$, *J* 7.1 Hz), 1.21 (s, 6H, Me_{26} + Me_{27}), 0.87-2.50 (several m), 2.57 (dd, 1H, H_{4eq}, *J* 13.0, 3.5 Hz), 2.82 (dd, 1H, H_{4eq}, *J* 11.5, 3.5 Hz), 3.62 (q, 2H, $H_{2'}$, *J* 7.1 Hz), 3.82 (m, 1H, H_3), 4.75 (s, 2H, $H_{1'}$), 4.82 (d, 1H, H_{19}, *J* 2.5 Hz), 5.04 (d, 1H, H_{19}, *J* 2.4 Hz), 6.03 (d, 1H, H_7, *J* 11.2 Hz), 6.23 (d, H, H_6, *J* 11.2 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 12.2 (Me_{18}), 15.3 ($Me_{3'}$), 19.0 (Me_{21}), 20.7 (CH_2), 22.4 (CH_2), 23.7 (CH_2), 26.5 and 26.6 (Me_{26} + Me_{27}), 27.8 (CH_2), 29.2 (CH_2), 32.1 (CH_2), 35.3 (CH_2), 36.3 (CH, C_{20}), 36.6 (CH_2), 40.7 (CH_2), 42.4 (CH_2), 46.0 (CH_2), 46.1 (C, C_{13}), 56.5 and 56.8 (2CH, C_{14} + C_{17}),

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[4] Previously described in: a) J. W. Blunt and H. F. DeLuca, *Biochemistry*, 1969, **8**, 671-675. b) M. M. Kabat, J. Kiegiel, N. Cohen, K. Toth, P. M. Wovkulich and M. R. Uskokovic, *J. Org. Chem.*, 1996, **61**, 118-124.

63.1 (CH_2, $C_{2'}$), 69.4 (CH, C_3), 76.4 (C, C_{25}), 89.6 (CH_2, $C_{1'}$), 112.6 (CH_2, C_{19}), 117.7 (CH, C_7), 122.6 (CH, C_6), 135.2 (C, C_{10}), 142.4 (C, C_8), 145.2 (C, C_5) ppm; MS (ESI^+, m/z): 481 [$(M+Na)^+$, 100%].

25-Hydroxyvitamin D_3 (24).[4] (-)-CSA (235 mg, 1.01 mmol) was added to a solution of **23** (116 mg, 0.20 mmol) in anhydrous MeOH (1.3 mL) and the mixture was stirred at r.t. for 3 h. Then, the solvent was removed and the mixture was extracted with CH_2Cl_2. The residue was purified by column chromatography (15% EtOAc/hexane). to give **24** in 65% yield as a white powder. Rf: 0.2 (25% EtOAc/hexane); mp 106–107 °C; ^1H NMR (400.13 MHz, $CDCl_3$): δ 0.54 (s, 3H, Me_{18}), 0.93 (d, 3H, Me_{21}, *J* 6.4 Hz), 1.21 (s, 6H, Me_{26} + Me_{27}), 0.98-2.45 (several m, $2H_2$ + H_{4ax} + H_{1ax} + $2H_9$ + $2H_{11}$ + $2H_{12}$ + H_{14} + $2H_{15}$ + $2H_{16}$ + H_{17} + H_{20} + $2H_{22}$ + $2H_{23}$ + $2H_{24}$ + 2OH), 2.57 (1H, dd, H_{4eq}, *J* 12.6, 2.6 Hz), 2.82 (dd, 1H, H_{1eq}, *J* 11.9, 3.5 Hz), 3.94 (m, 1H, H_3), 4.82 (s, 1H, H_{19}), 5.05 (s, 1H, H_{19}), 6.03 (d, 2H, H_7, *J* 11.0 Hz), 6.23 (d, 2H, H_6, *J* 11.0 Hz) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 12.1 (Me_{18}), 19.0 (Me_{21}), 21.0 (CH_2), 22.4 (CH_2), 23.7 (CH_2), 27.8 (CH_2), 29.2 (CH_2), 29.4 and 29.5 (Me_{26} + Me_{27}), 32.1 (CH_2, C_1), 35.3 (CH_2), 36.3 (CH, C_{20}), 36.6 (CH_2), 40.7 (CH_2, C_{12}), 44.6 (CH_2), 46.0 (C, C_{13}), 46.1 (CH_2), 56.5 and 56.7 (2CH, C_{17} + C_{14}), 69.3 (CH, C_3), 71.2 (C, C_{25}), 112.6 (CH_2, C_{19}), 117.7 (CH, C_7), 122.6 (CH, C_6), 135.2 (C, C_{10}), 142.4 (C, C_8), 145.2 (C, C_5) ppm. MS ($APCI^+$, m/z): 401 [$(M+H)^+$, 100%].

3-*O*-(*tert*-Butyldimethylsilyl)-25-hydroxivitamin D_3 (25).[5] To a solution of **24** (14 mg, 0.04 mmol) in anhydrous CH_2Cl_2 (232 μL) at 0 ºC, were added imidazole (4 mg, 0.05 mmol) and *tert*-butyldimethylsilyl chloride (6 mg, 0.04 mmol). Afterwards, the reaction was stirred at room temperature for 6 h. Reaction was stopped by addition of water followed by extraction with CH_2Cl_2. The residue was purified by column chromatography (5% EtOAc/hexane as eluent) to give protected alcohol **25** in 89% yield. Rf: 0.4 (10% EtOAc/hexane); ^1H NMR (300.13 MHz, $CDCl_3$): δ 0.06 (s, 3H, Si*Me*), 0.07 (s, 3H, Si*Me*), 0.55 (s, 3H, Me_{18}), 0.88 (s, 9H, SiCMe_3), 0.93 (d, 3H, Me_{21}, *J* 6.3 Hz), 1.21 (s, 6H, Me_{26} + Me_{27}), 0.98-2.54 (several m, $2H_2$ + $2H_4$ + H_{1ax} + $2H_9$ + $2H_{11}$ + $2H_{12}$ + H_{14} + $2H_{15}$ + $2H_{16}$ + H_{17} + H_{20} + $2H_{22}$ + $2H_{23}$ + $2H_{24}$ + OH), 2.83 (d, 1H, H_{1eq}, *J* 15.4 Hz), 3.82 (m, 1H, H_3), 4.78 (s, 1H, H_{19}), 5.01 (s, 1H, H_{19}), 6.01 (d, 1H, H_7, *J* 11.2 Hz), 6.16 (2d, 1H, H_6, *J* 11.2 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ -4.5 (Si*Me*), -4.4 (Si*Me*), 12.2 (Me_{18}), 18.3 (Si*C*), 19.0 (Me_{21}), 21.0 (CH_2), 22.4 (CH_2), 23.6 (CH_2), 26.0 (CH_3, SiCMe_3), 27.9 (CH_2), 29.1 (CH_2), 29.4 y 29.5 (Me_{26} + Me_{27}), 32.9 (CH_2, C_1), 36.3 (CH, C_{20}), 36.55 (CH_2), 36.58 (CH_2), 40.7 (CH_2, C_{12}), 44.6 (CH_2), 45.9 (C, C_{13}), 47.0 (CH_2), 56.5 and 56.7 (2CH, C_{14} + C_{17}), 70.7 (CH, C_3), 71.2 (C, C_{25}), 112.3 (CH_2, C_{19}), 118.0 (CH, C_7), 121.5 (CH, C_6), 136.5 (C, C_{10}), 141.6 (C, C_8), 145.5 (C, C_5) ppm; MS ($APCI^+$, m/z): 515 [$(M + H)^+$, 100%].

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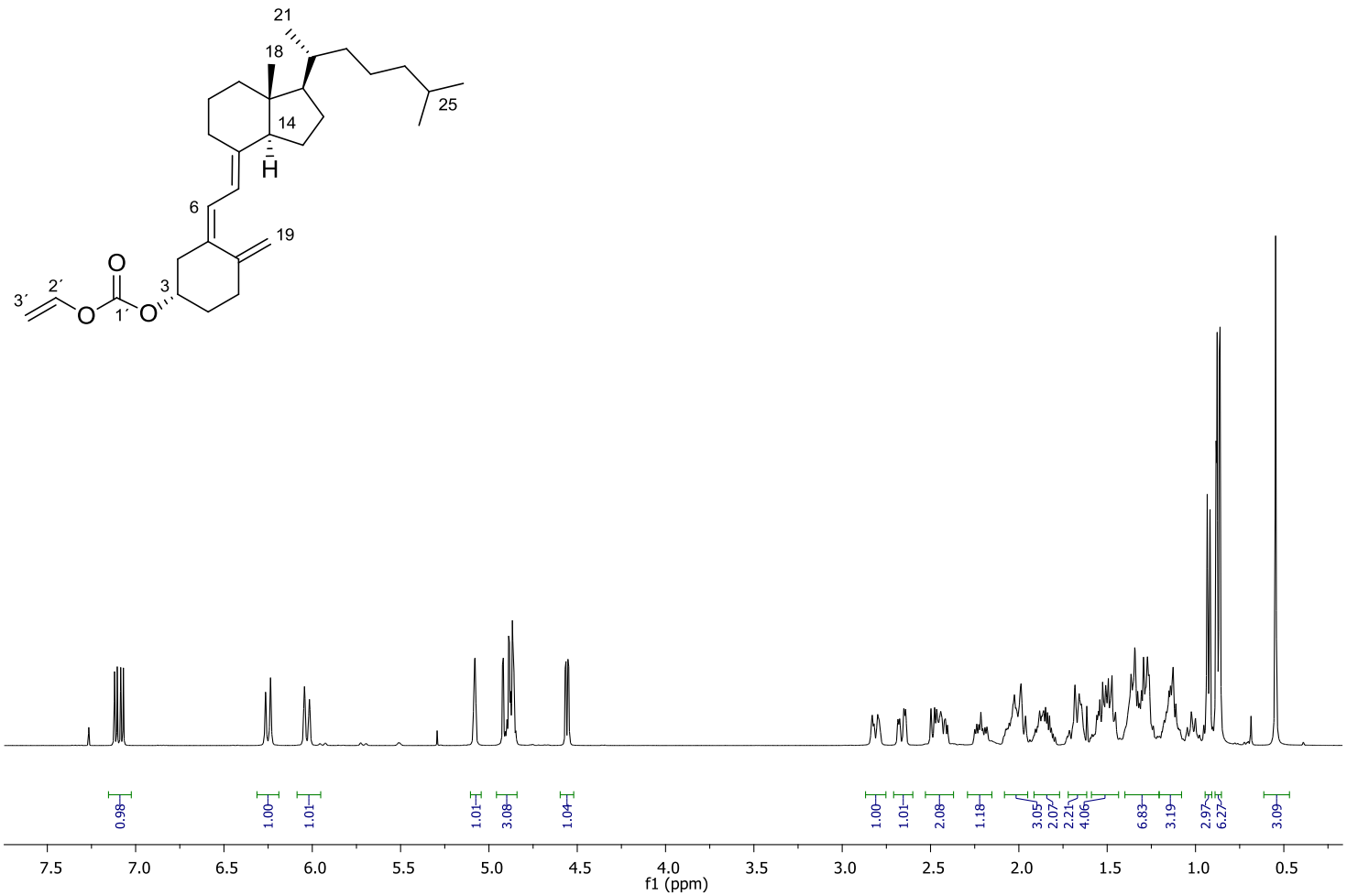
[5] Previously described in R. Ray, D. Vicchio, A. Yergey and M. F. Holick, *Steroids,* 1992, **57**, 142-146.

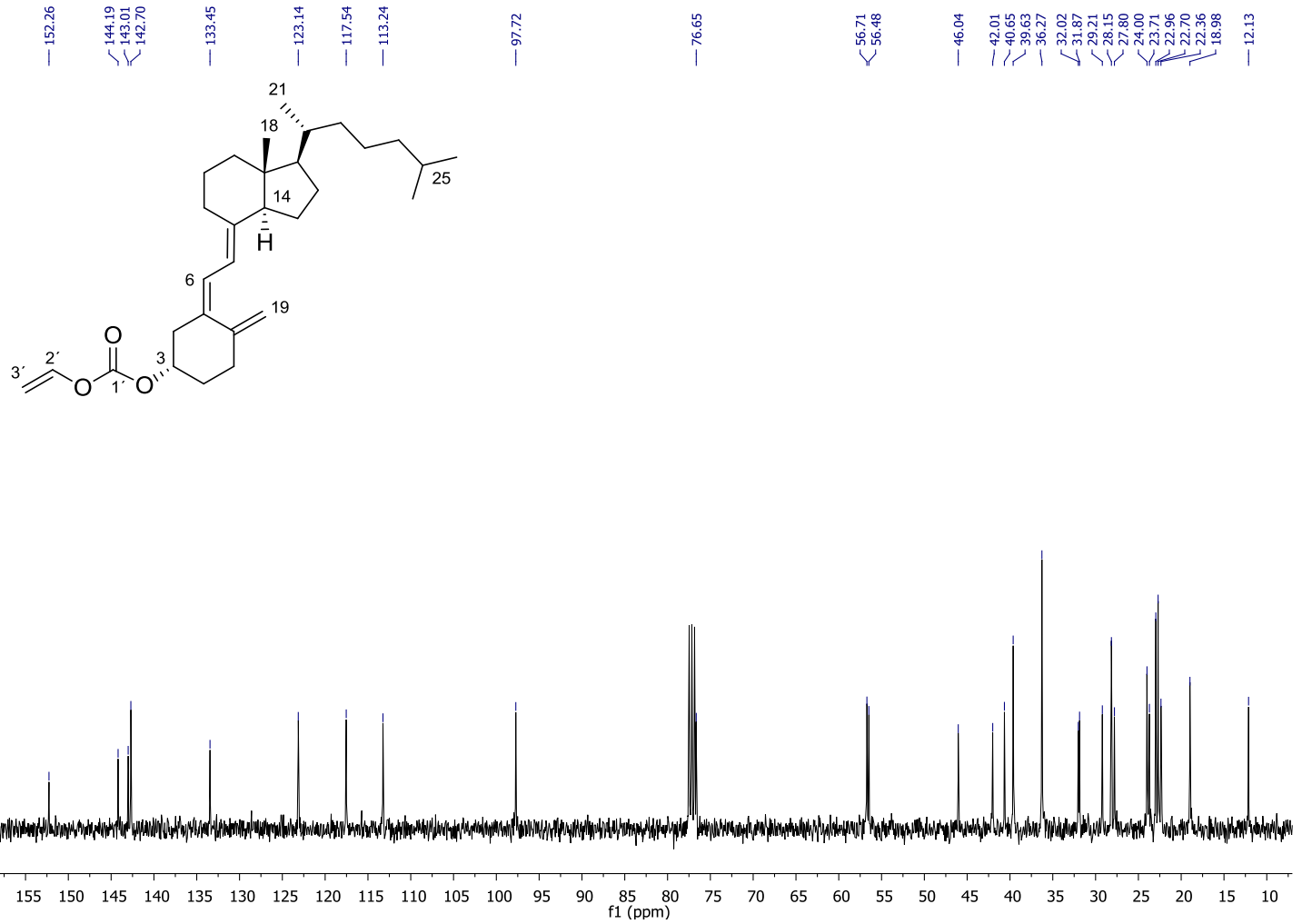
3-*O*-(Vinyloxycarbonyl)vitamin D_3 (8)

^{1}H-RMN (300.13 MHz, $CDCl_3$)



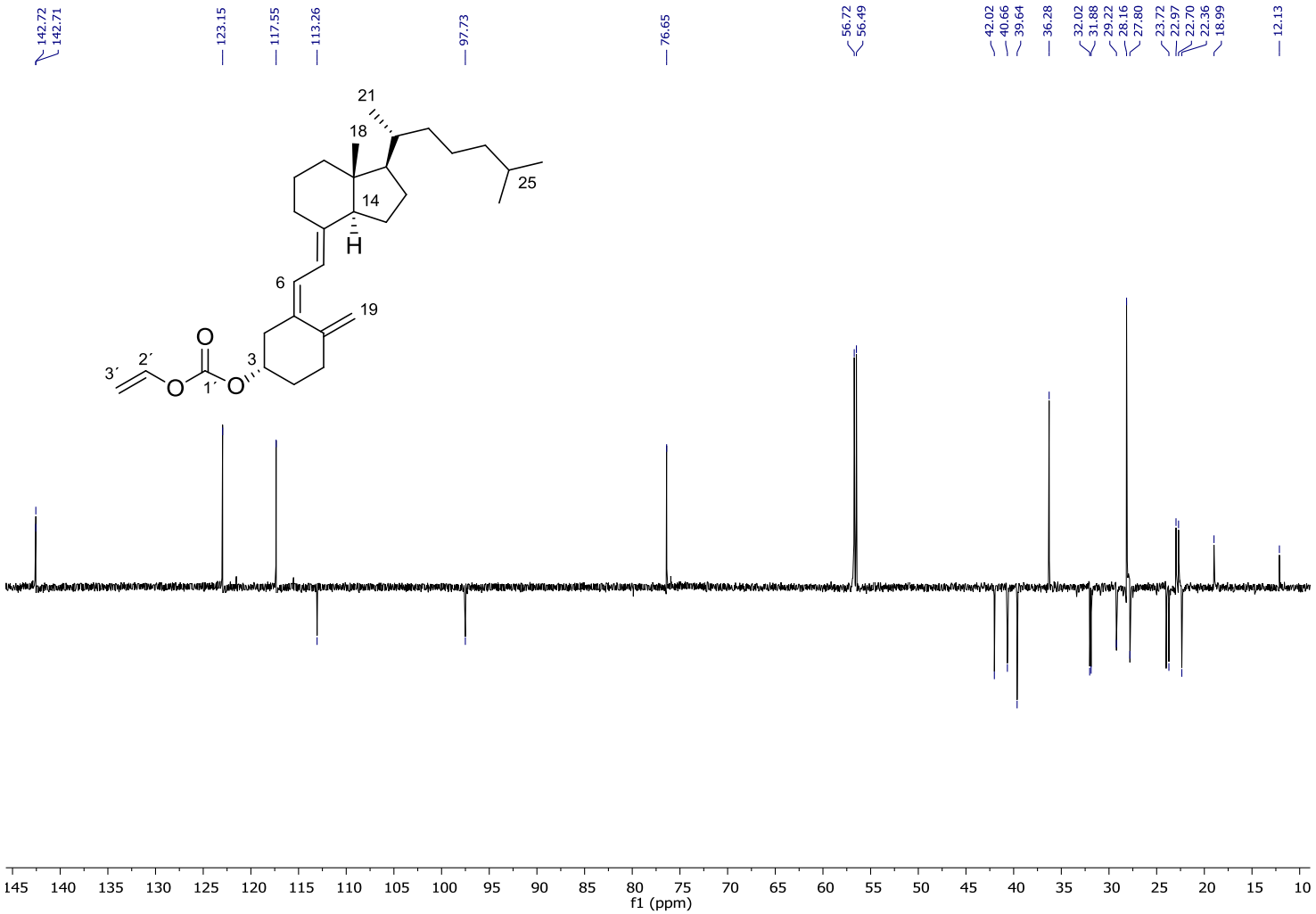
3-*O*-(Vinyloxycarbonyl)vitamin D_3 (8)

^{13}C-RMN (75.5 MHz, $CDCl_3$)



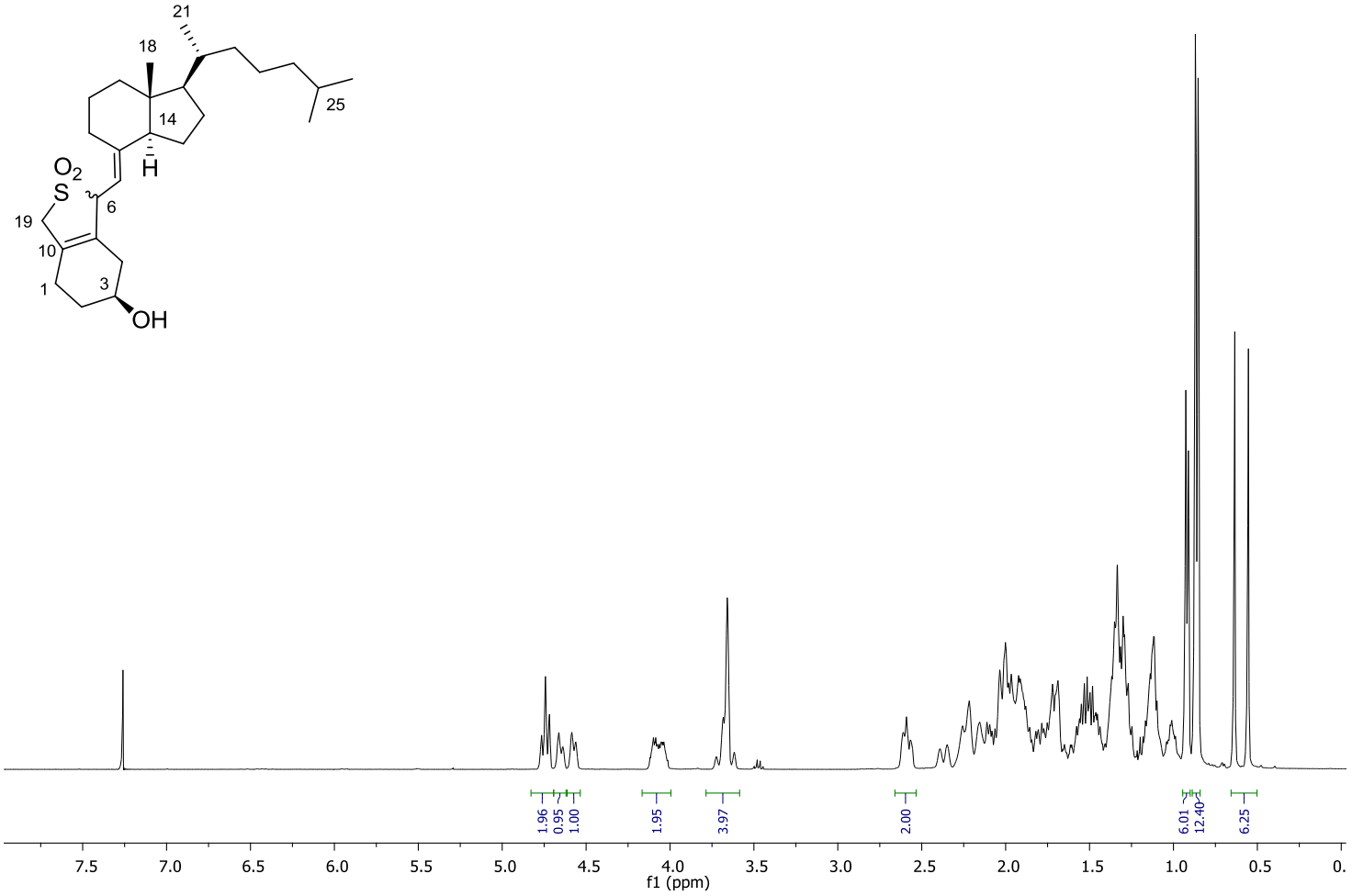
3-*O*-(Vinyloxycarbonyl)vitamin D_3 (8)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$)



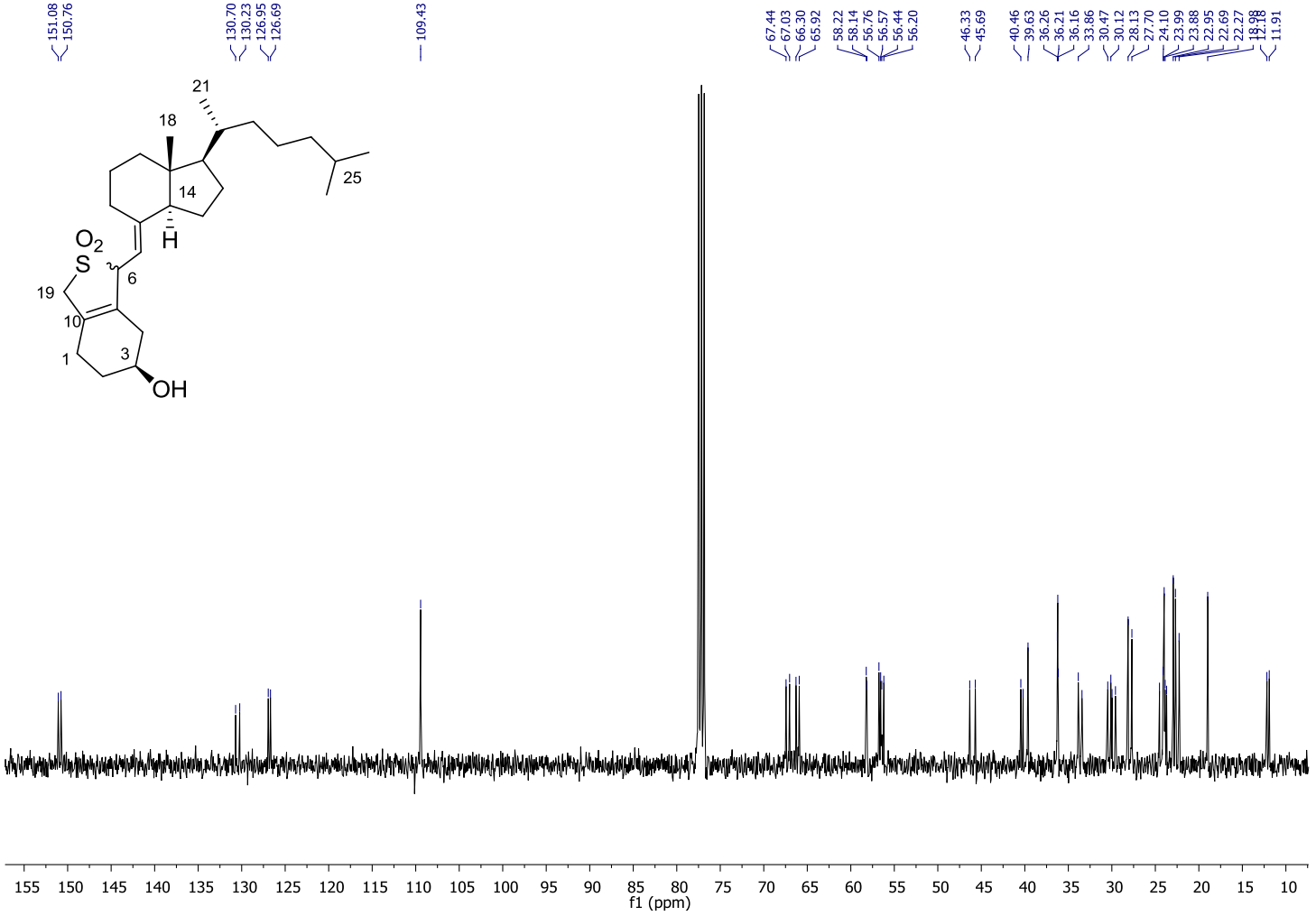
Vitamin D_3-SO_2 Adduct (9)

^{1}H-RMN (300.13 MHz, $CDCl_3$)



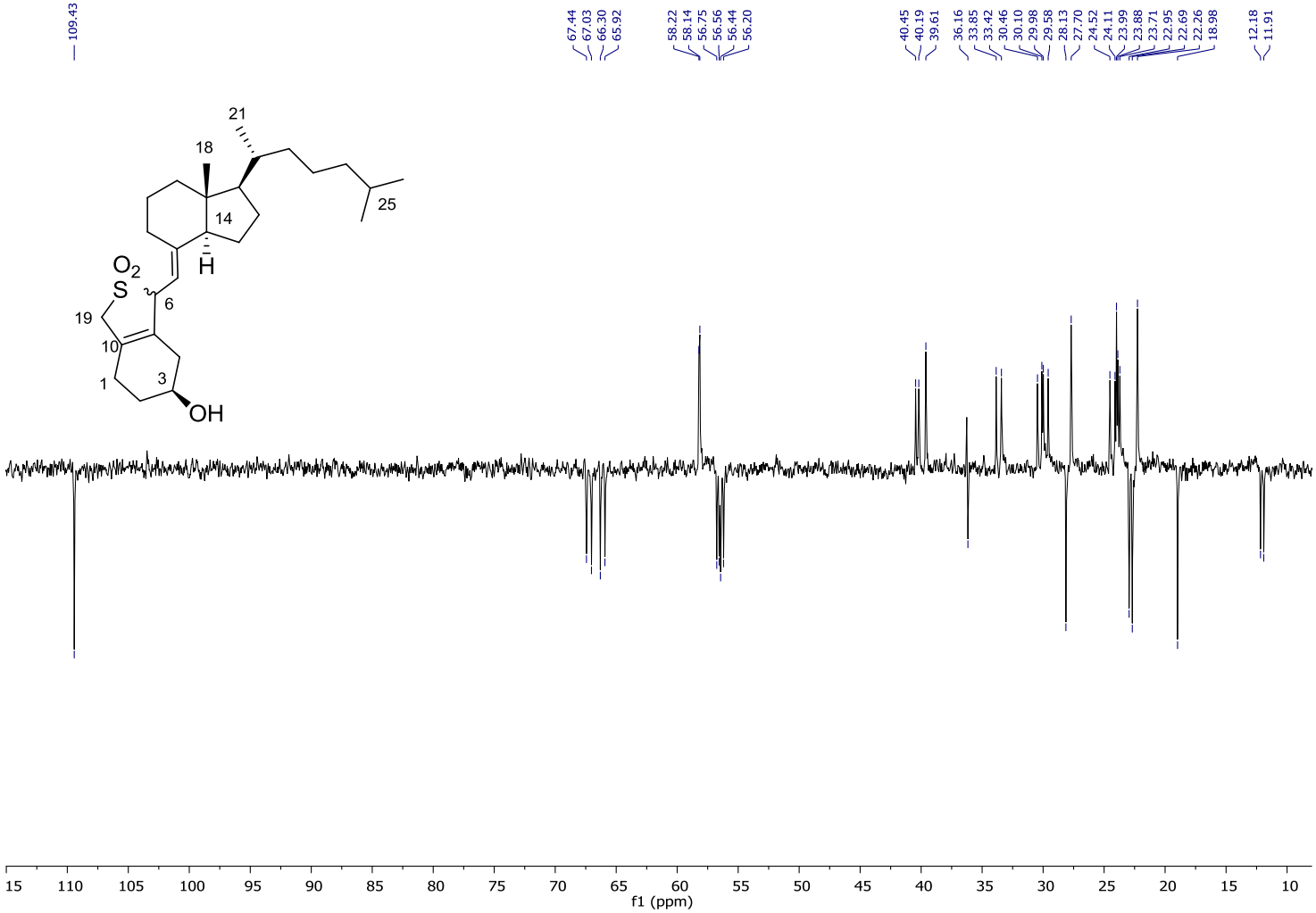
Vitamin D_3-SO_2 Adduct (9)

^{13}C-RMN (75.5 MHz, $CDCl_3$)

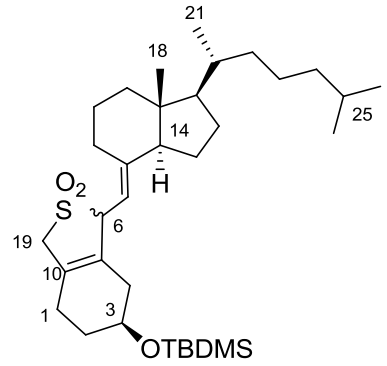


Vitamin D_3-SO_2 Adduct (9)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$)



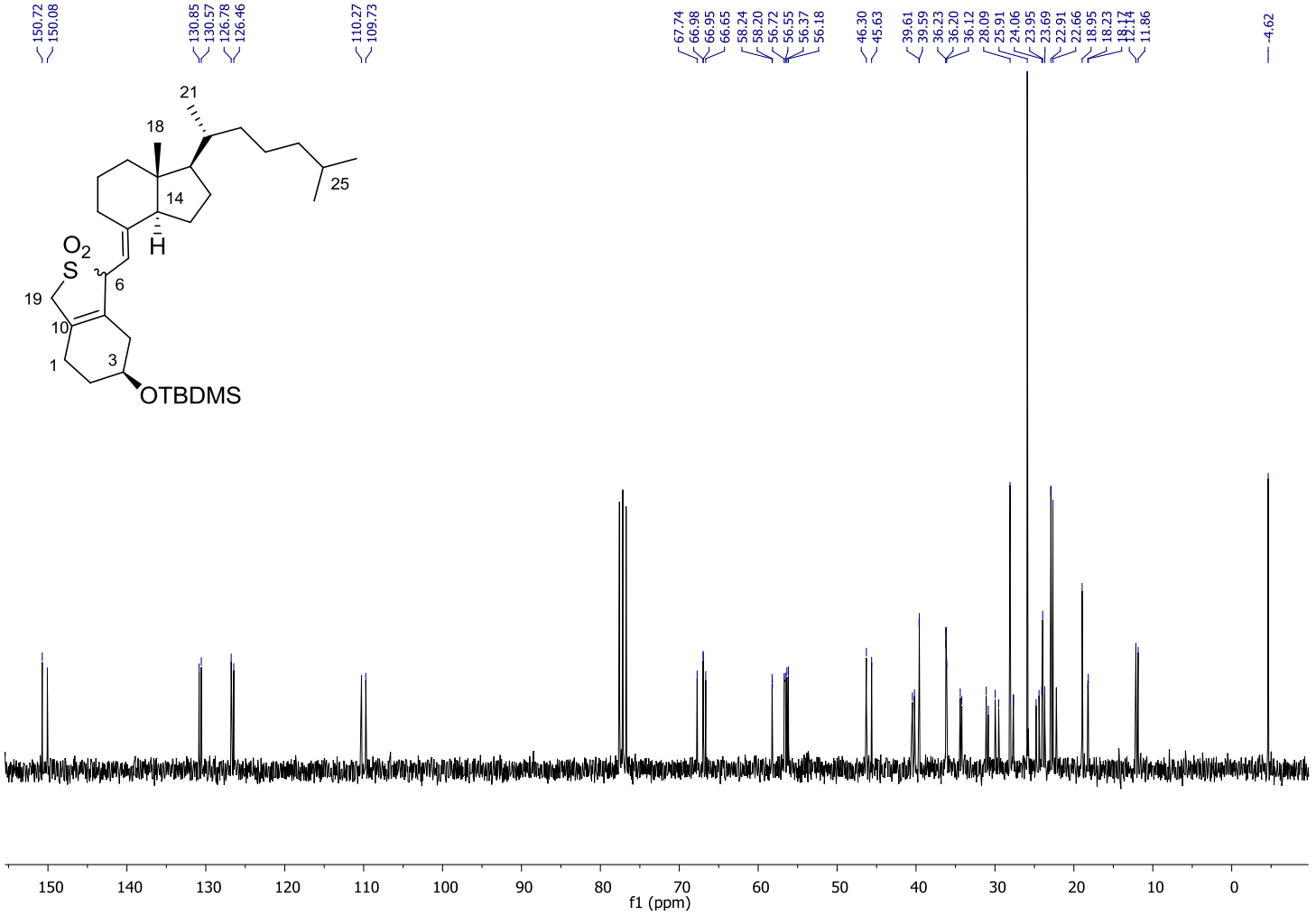
3-*O*-(*tert*-Butyldimethylsilyl)vitamin D_3-SO_2 Adduct (10)



^{1}H-RMN (300.13 MHz, $CDCl_3$)

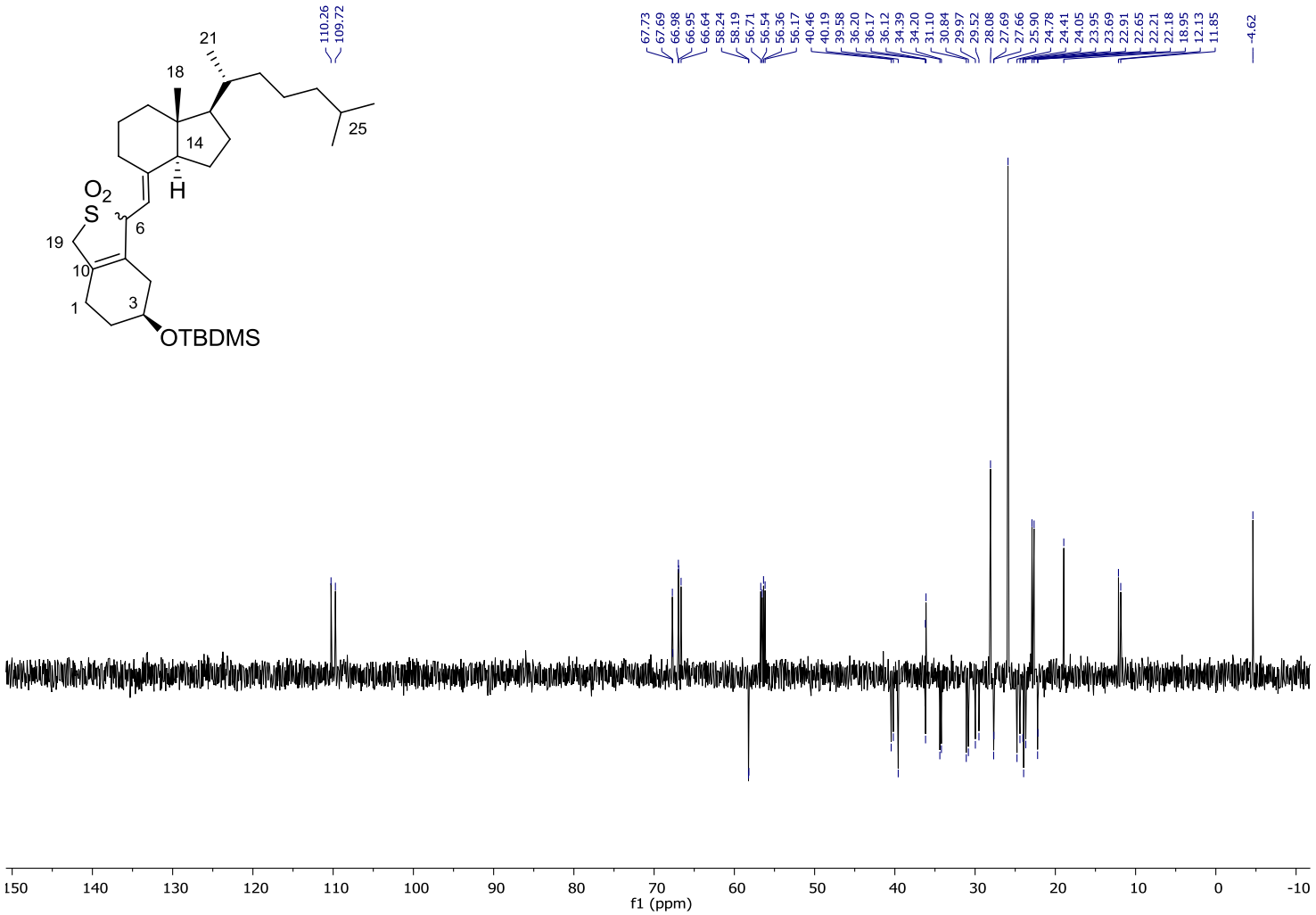
3-*O*-(*tert*-Butyldimethylsilyl)vitamin D_3-SO_2 Adduct (10)

^{13}C-RMN (75.5 MHz, $CDCl_3$)

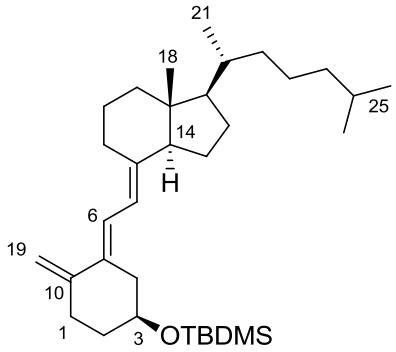


3-*O*-(*tert*-Butyldimethylsilyl)vitamin D_3-SO_2 Adduct (10)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$)



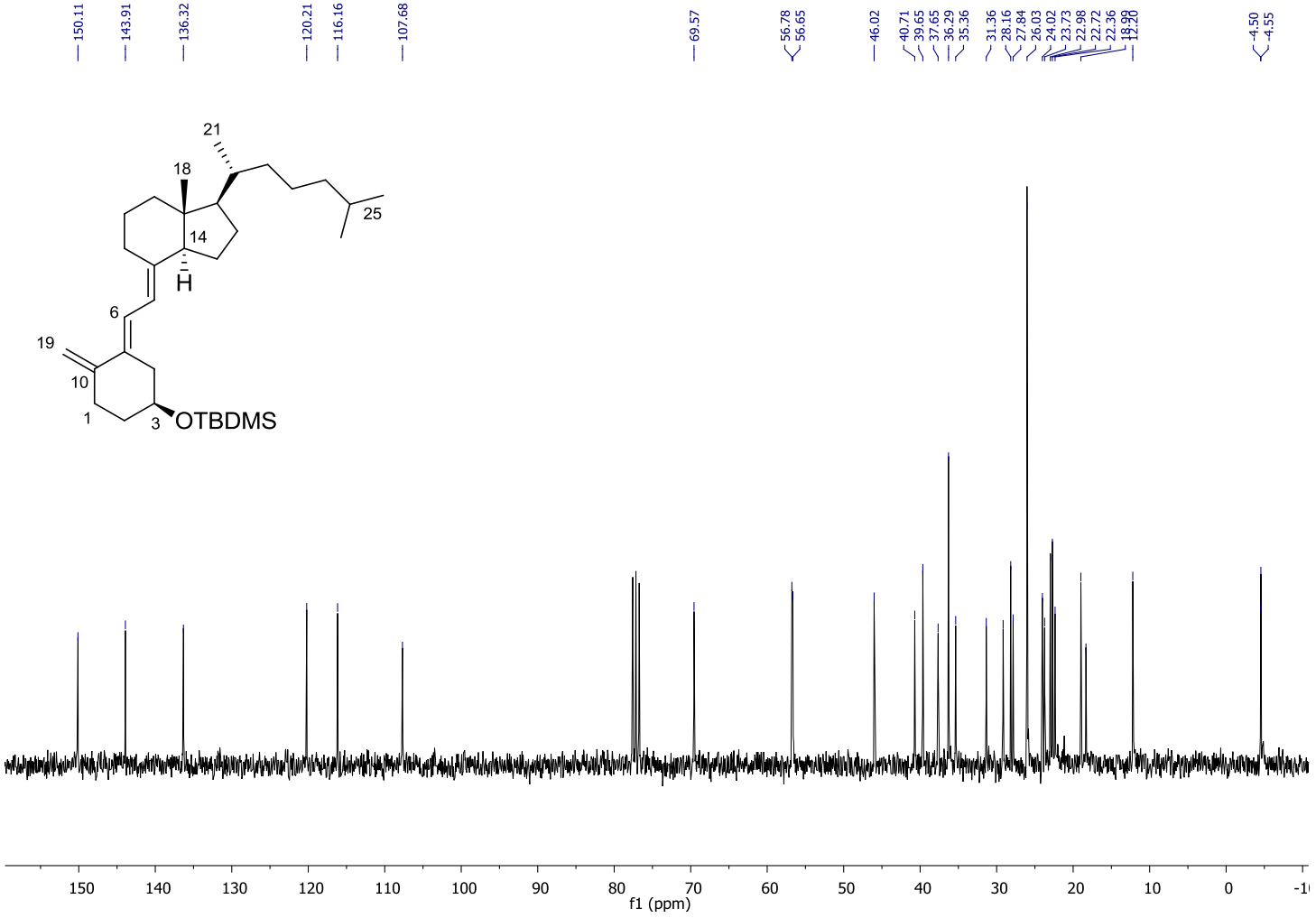
(5*E*)-3-*O*-(*tert*-Butyldimethylsilyl)vitamin D_3 (11)



^{1}H-RMN (300.13 MHz, $CDCl_3$)

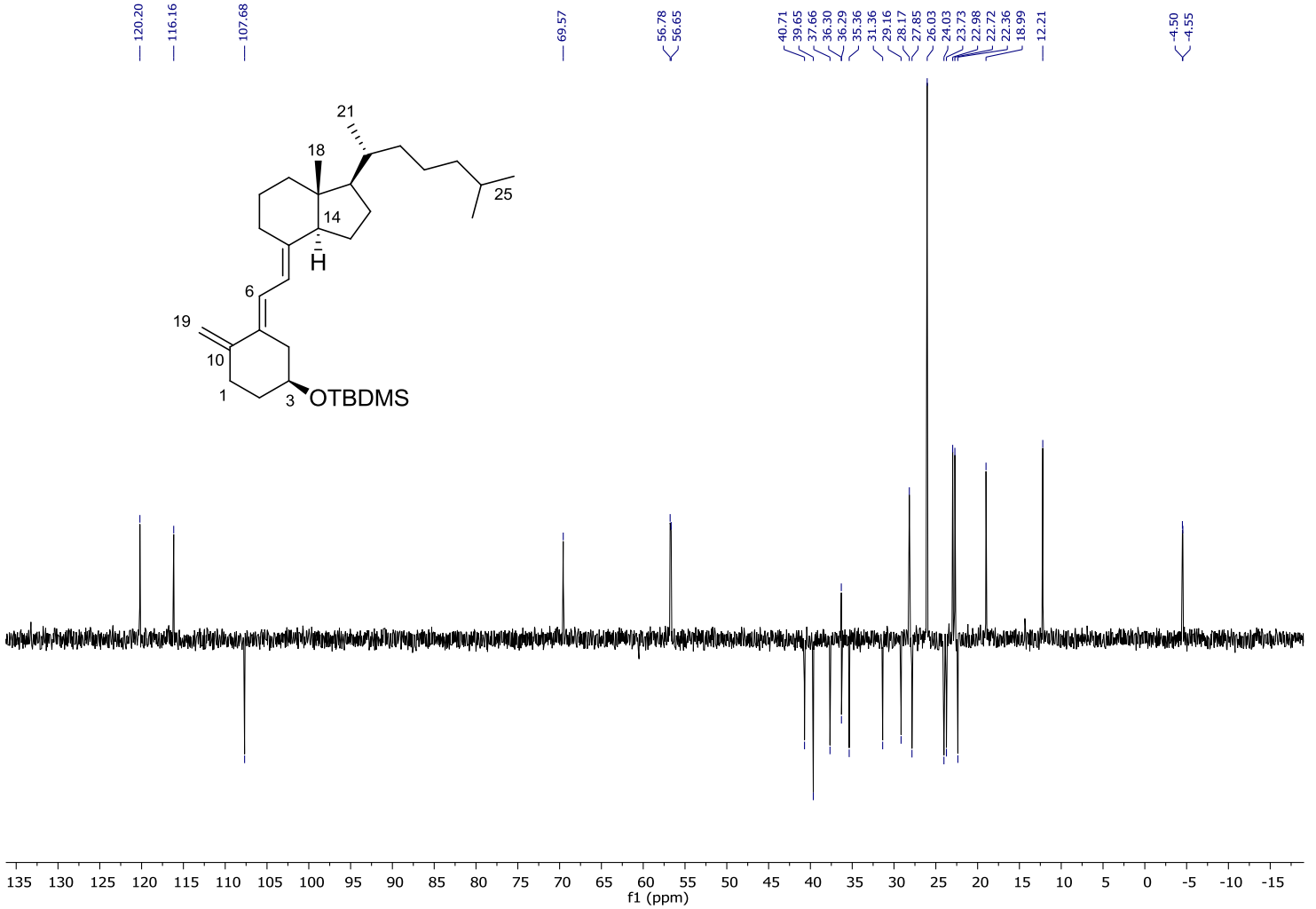
(5*E*)-3-*O*-(*tert*-Butyldimethylsilyl)vitamin D_3 (11)

^{13}C-RMN (75.5 MHz, $CDCl_3$)



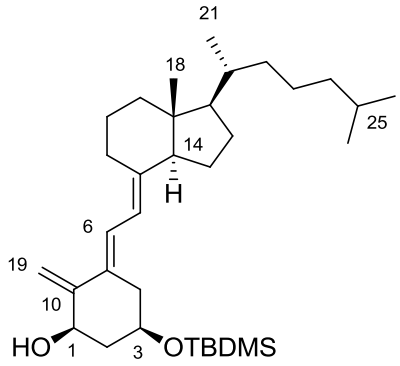
(5*E*)-3-*O*-(*tert*-Butyldimethylsilyl)vitamin D_3 (11)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$)

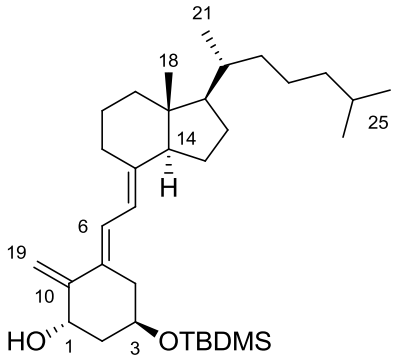


(5*E*)-3-*O*-(*tert*-Butyldimethylsilyl)-1α-hydroxyvitamin D_3 (12) and (5*E*)-3-*O*-(*tert*-Butyldimethylsilyl)-1β-hydroxyvitamin D_3 (13)

^{1}H-RMN (300.13 MHz, $CDCl_3$)



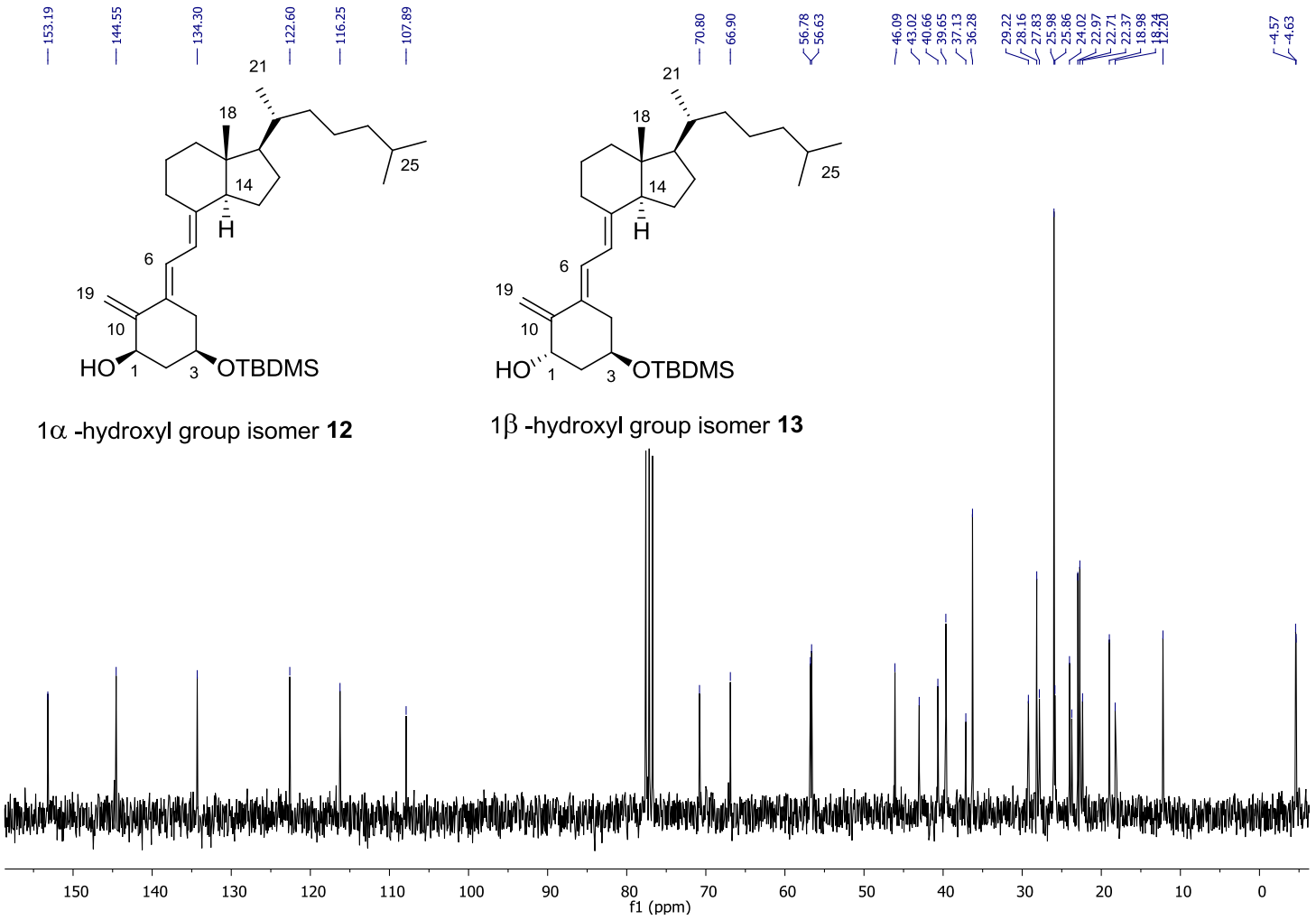
1α -hydroxyl group isomer **12**



1β -hydroxyl group isomer **13**

(5*E*)-3-*O*-(*tert*-Butyldimethylsilyl)-1α-hydroxyvitamin D_3 (12) and (5*E*)-3-*O*-(*tert*-Butyldimethylsilyl)-1β-hydroxyvitamin D_3 (13)

^{13}C-RMN (75.5 MHz, $CDCl_3$)

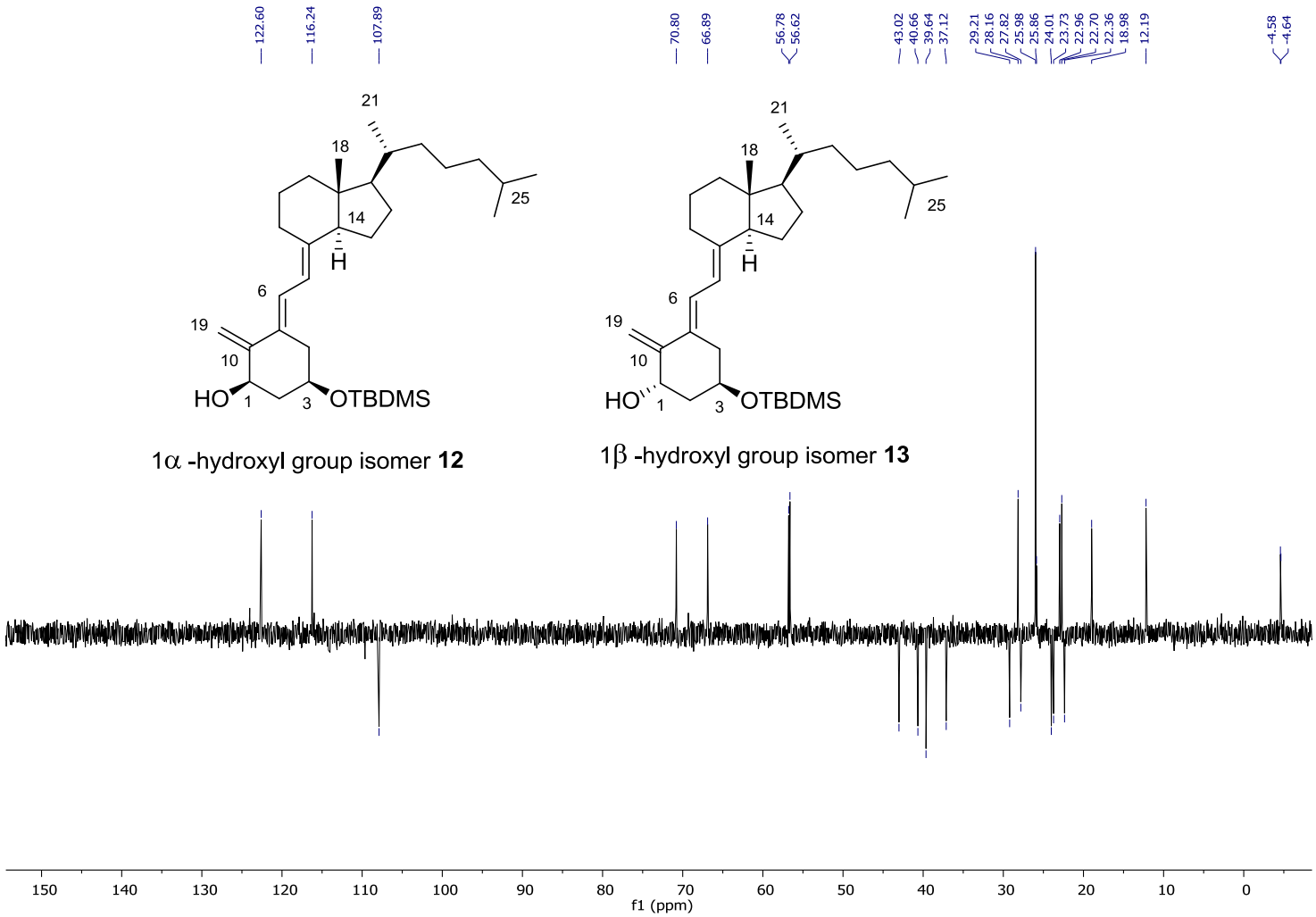


1α -hydroxyl group isomer **12**

1β -hydroxyl group isomer **13**

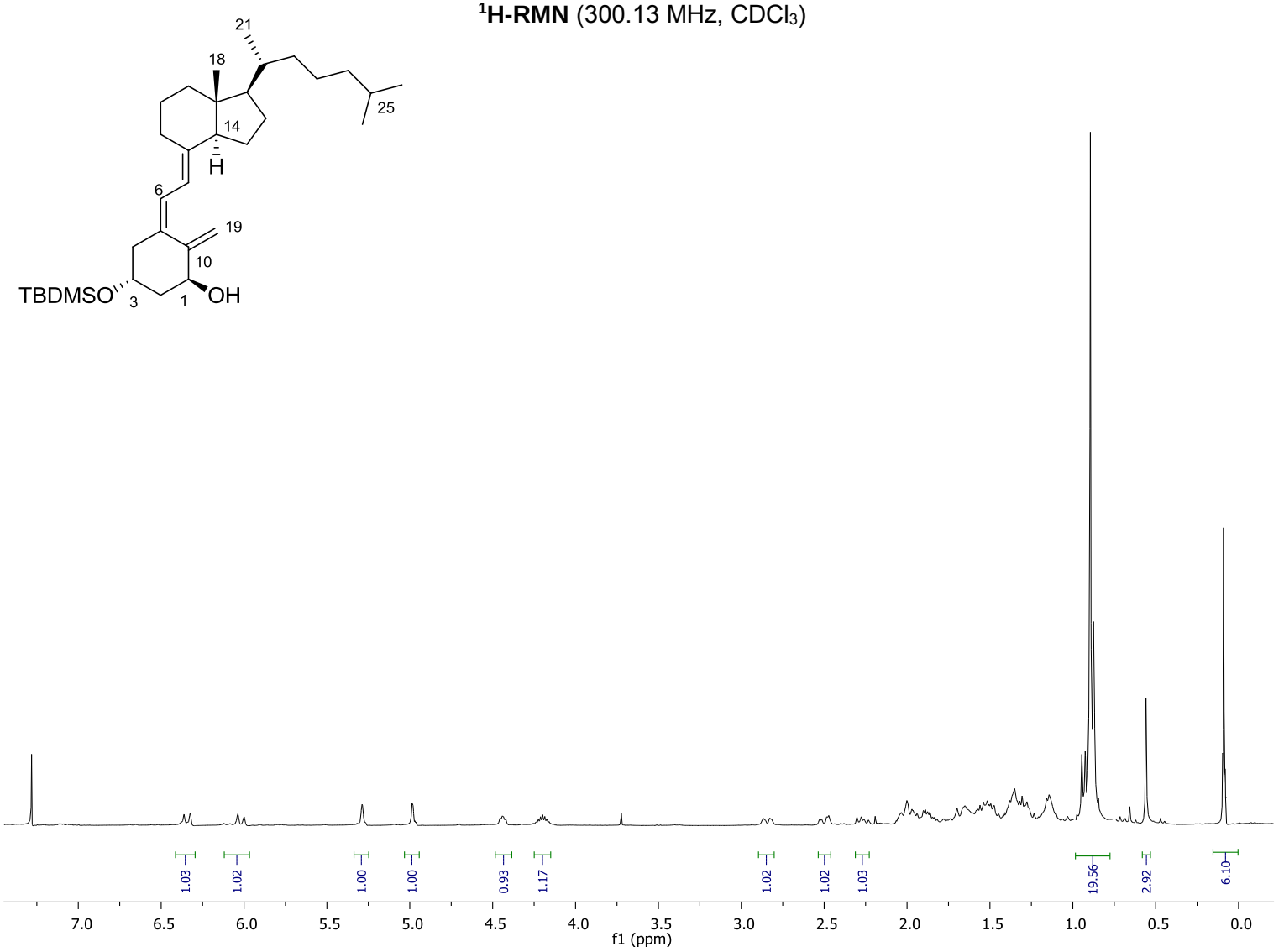
(5*E*)-3-*O*-(*tert*-Butyldimethylsilyl)-1α-hydroxyvitamin D_3 (12) and (5*E*)-3-*O*-(*tert*-Butyldimethylsilyl)-1β-hydroxyvitamin D_3 (13)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$)



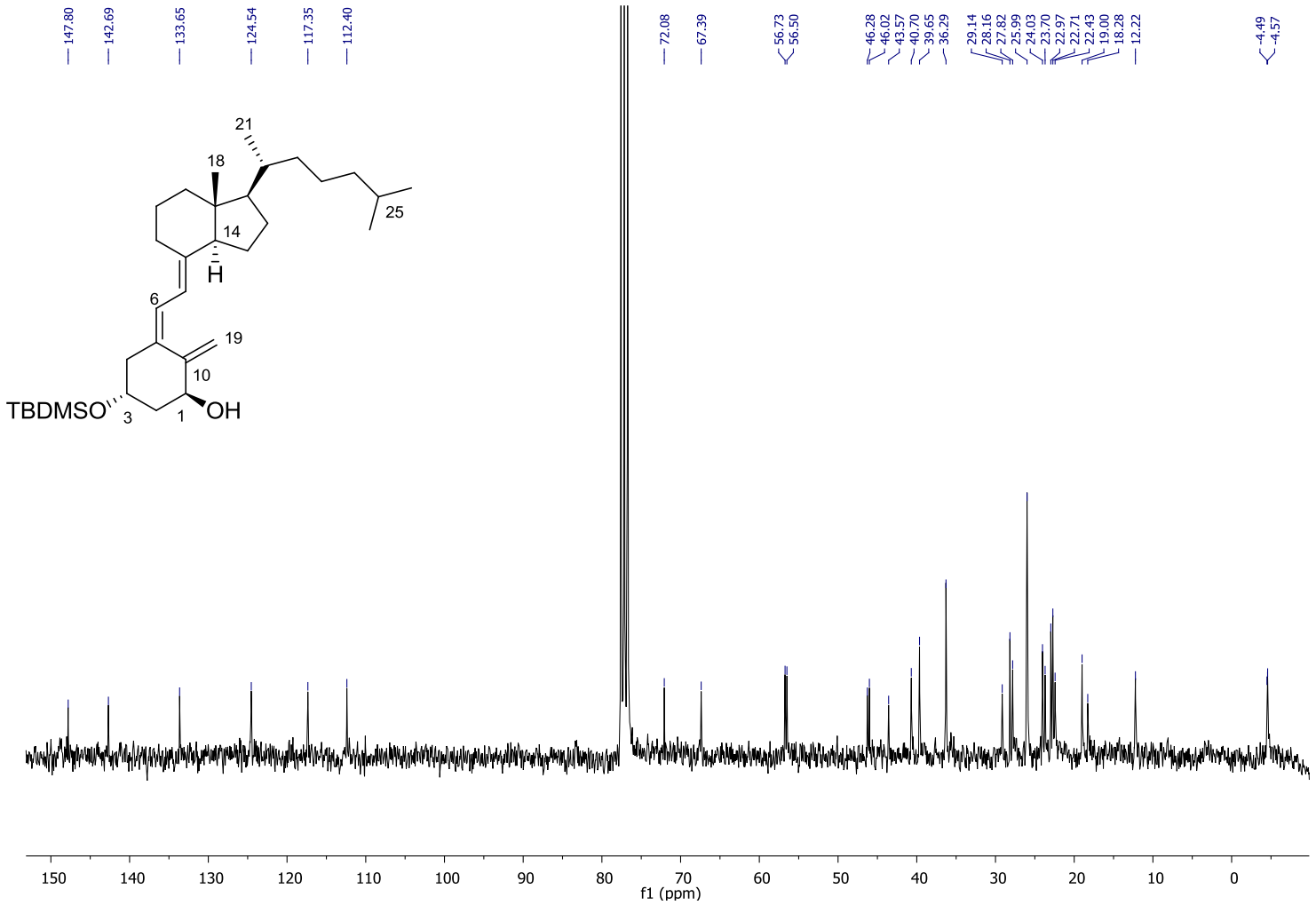
3-*O*-(*tert*-Butyldimethylsilyl)-1α-hydroxyvitamin D_3 (14)

^{1}H-RMN (300.13 MHz, $CDCl_3$)



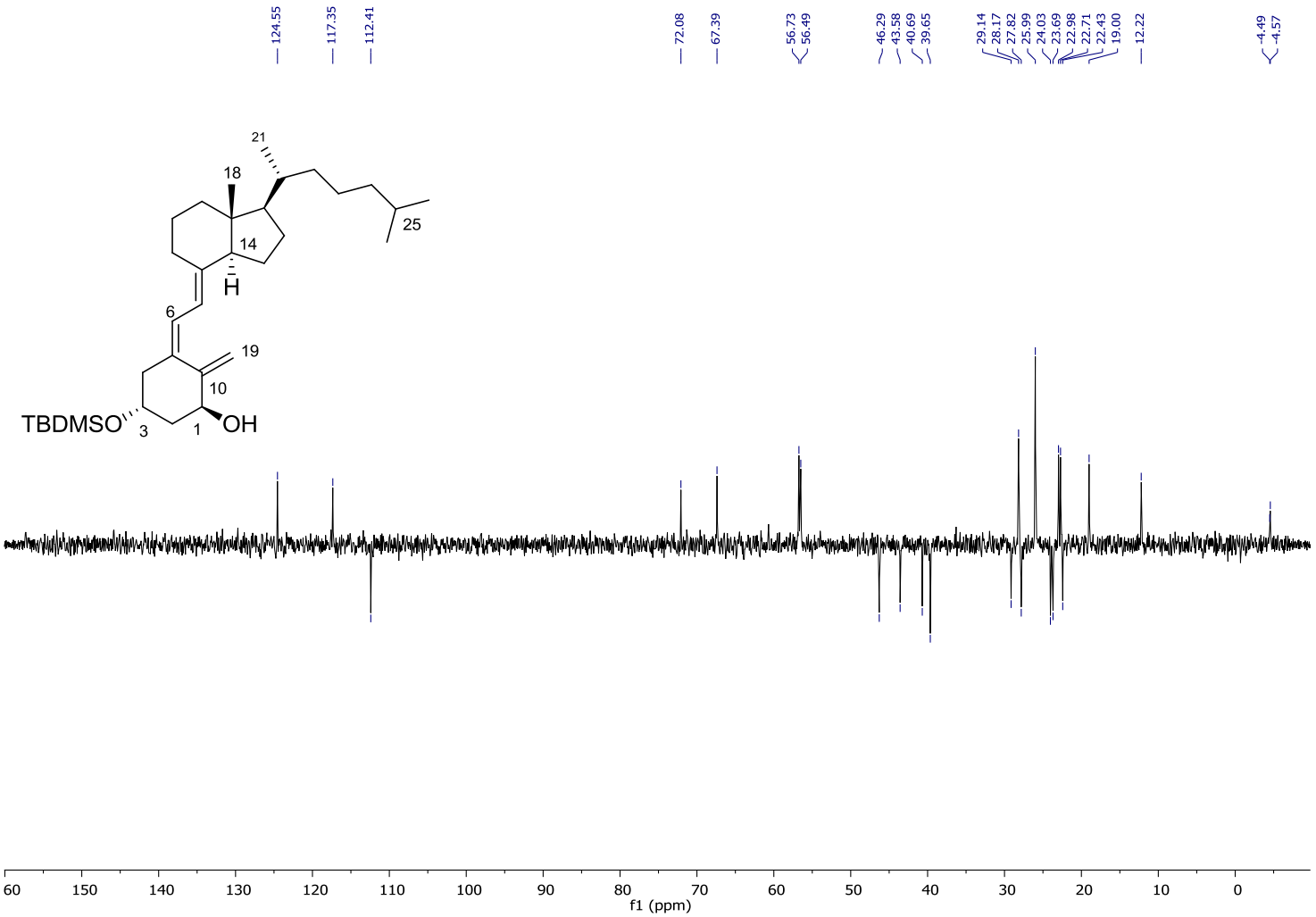
3-*O*-(*tert*-Butyldimethylsilyl)-1α-hydroxyvitamin D_3 (14)

^{13}C-RMN (75.5 MHz, $CDCl_3$)



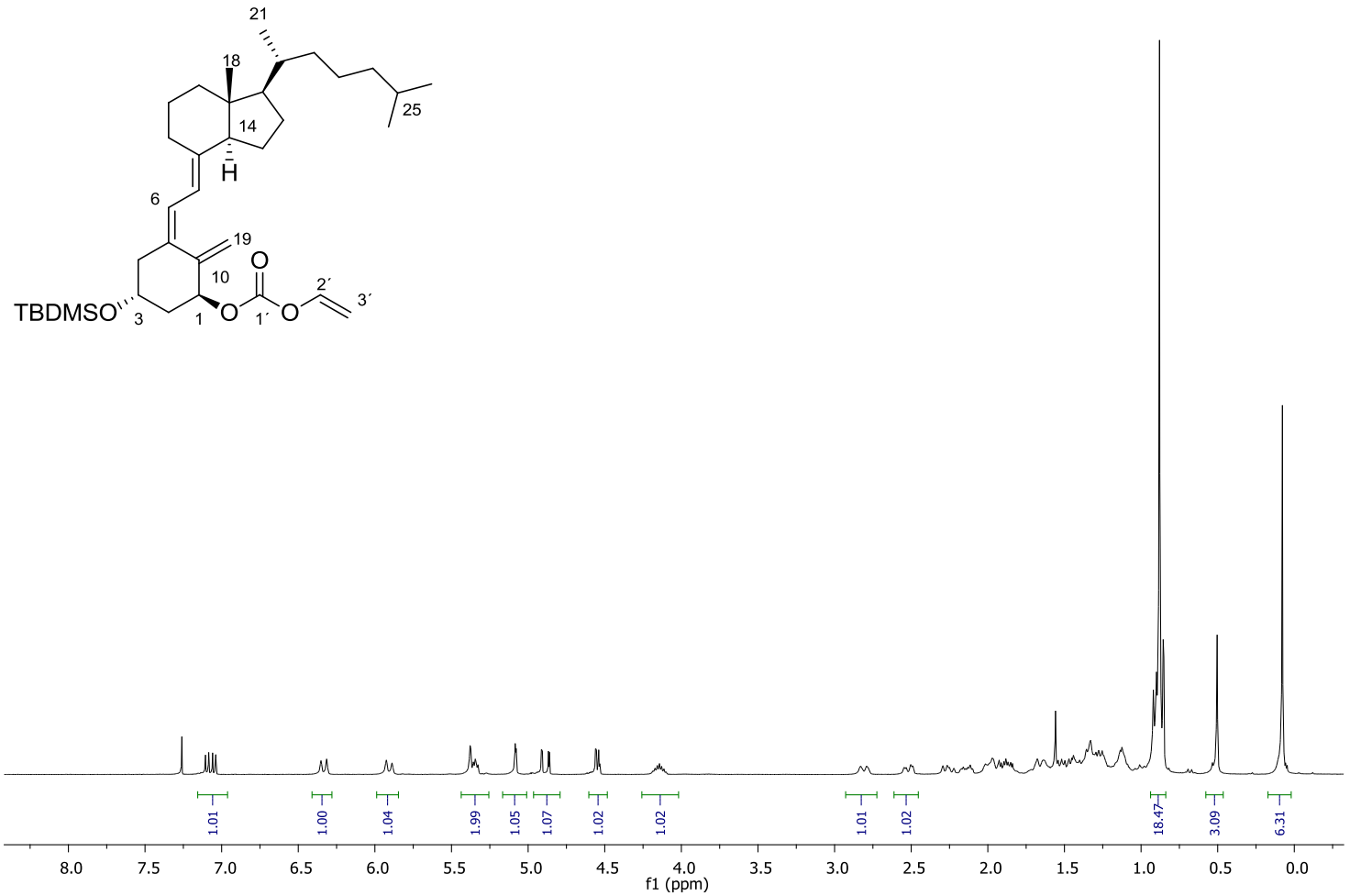
3-*O*-(*tert*-Butyldimethylsilyl)-1α-hydroxyvitamin D_3 (14)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$)



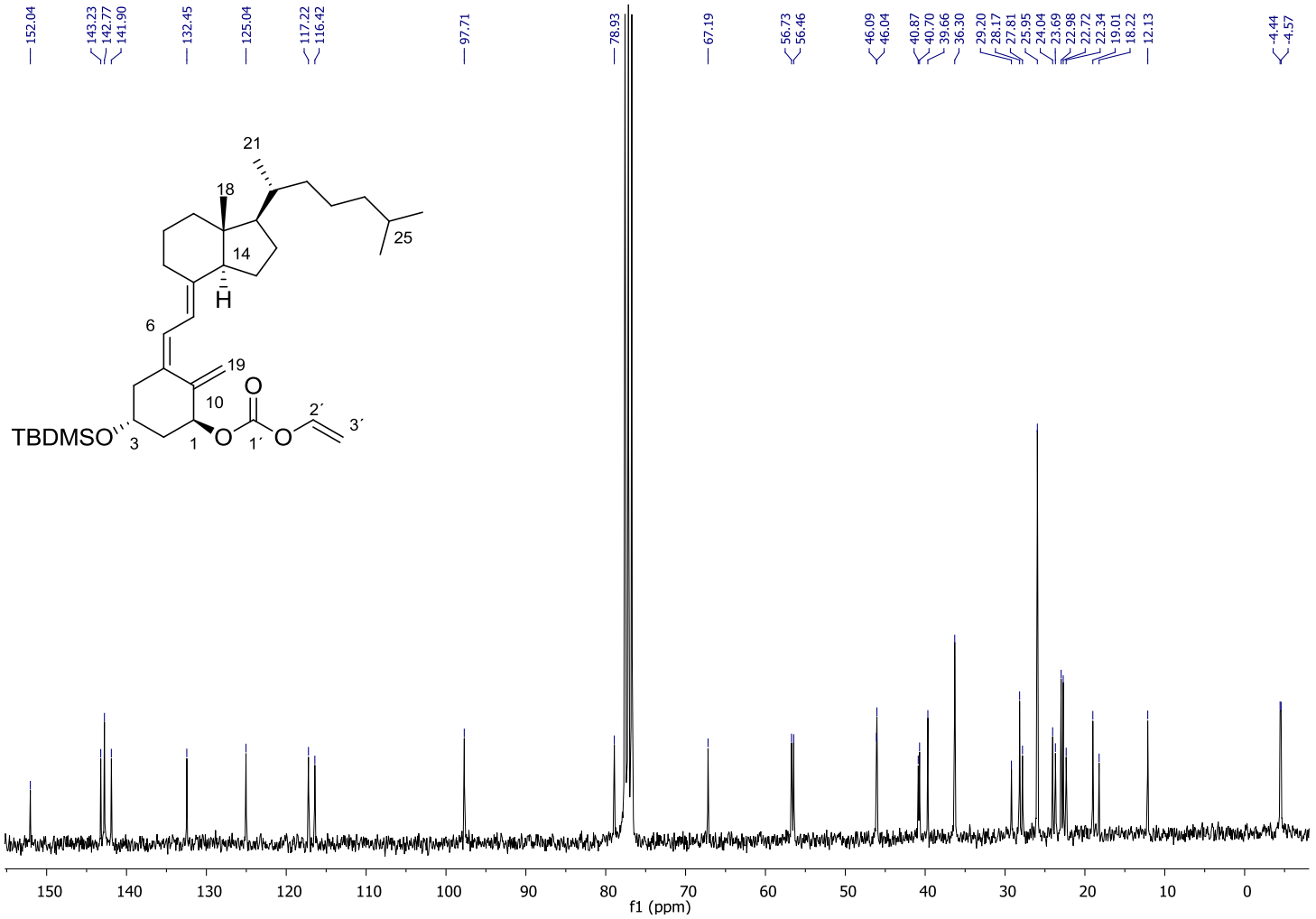
3-*O*-(*tert*-Butyldimethylsilyl)-1α-(vinyloxycarbonyloxy)vitamin D_3 (15)

^{1}H-RMN (300.13 MHz, $CDCl_3$)



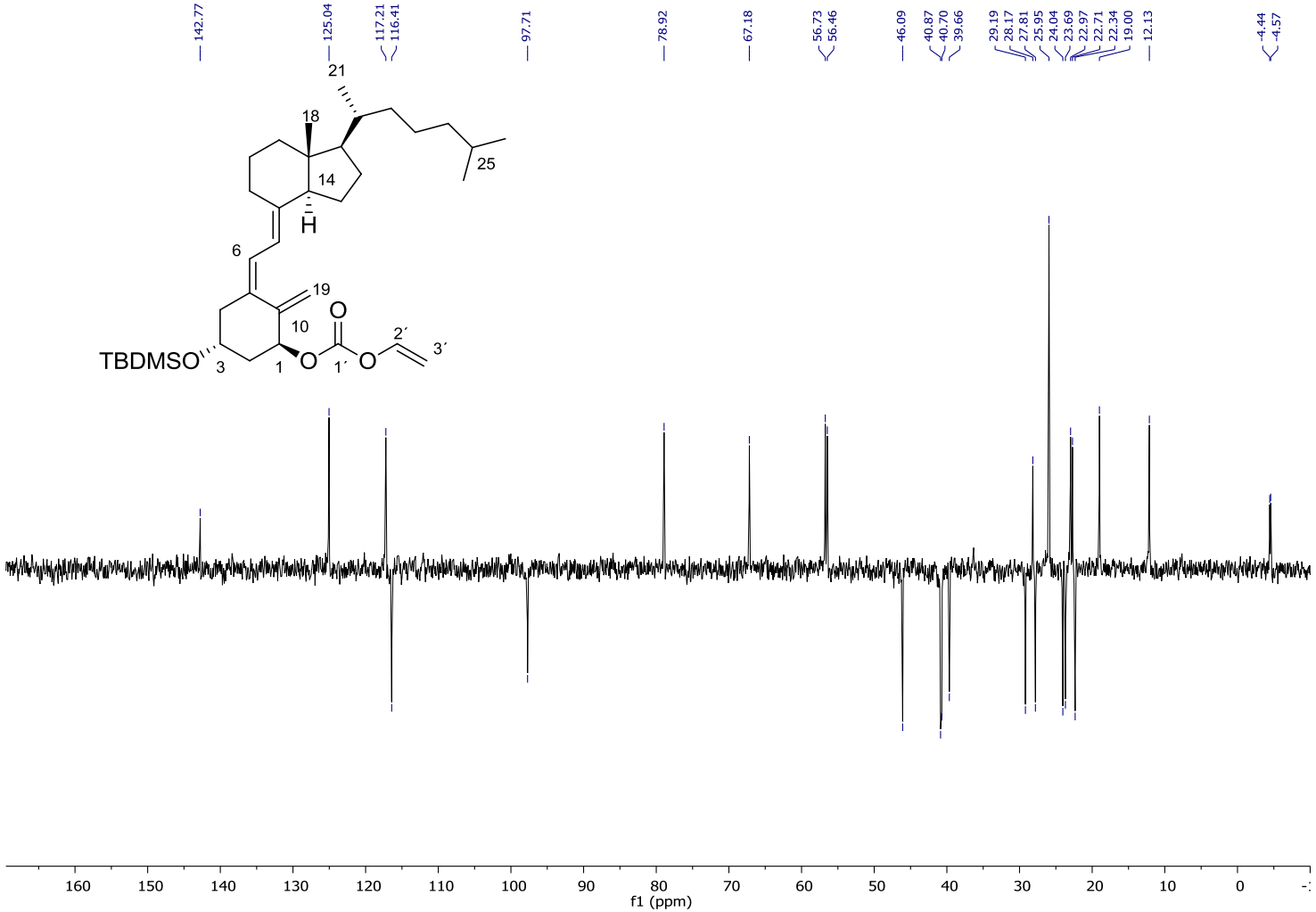
3-*O*-(*tert*-Butyldimethylsilyl)-1α-(vinyloxycarbonyloxy)vitamin D_3 (15)

^{13}C-RMN (75.5 MHz, $CDCl_3$)



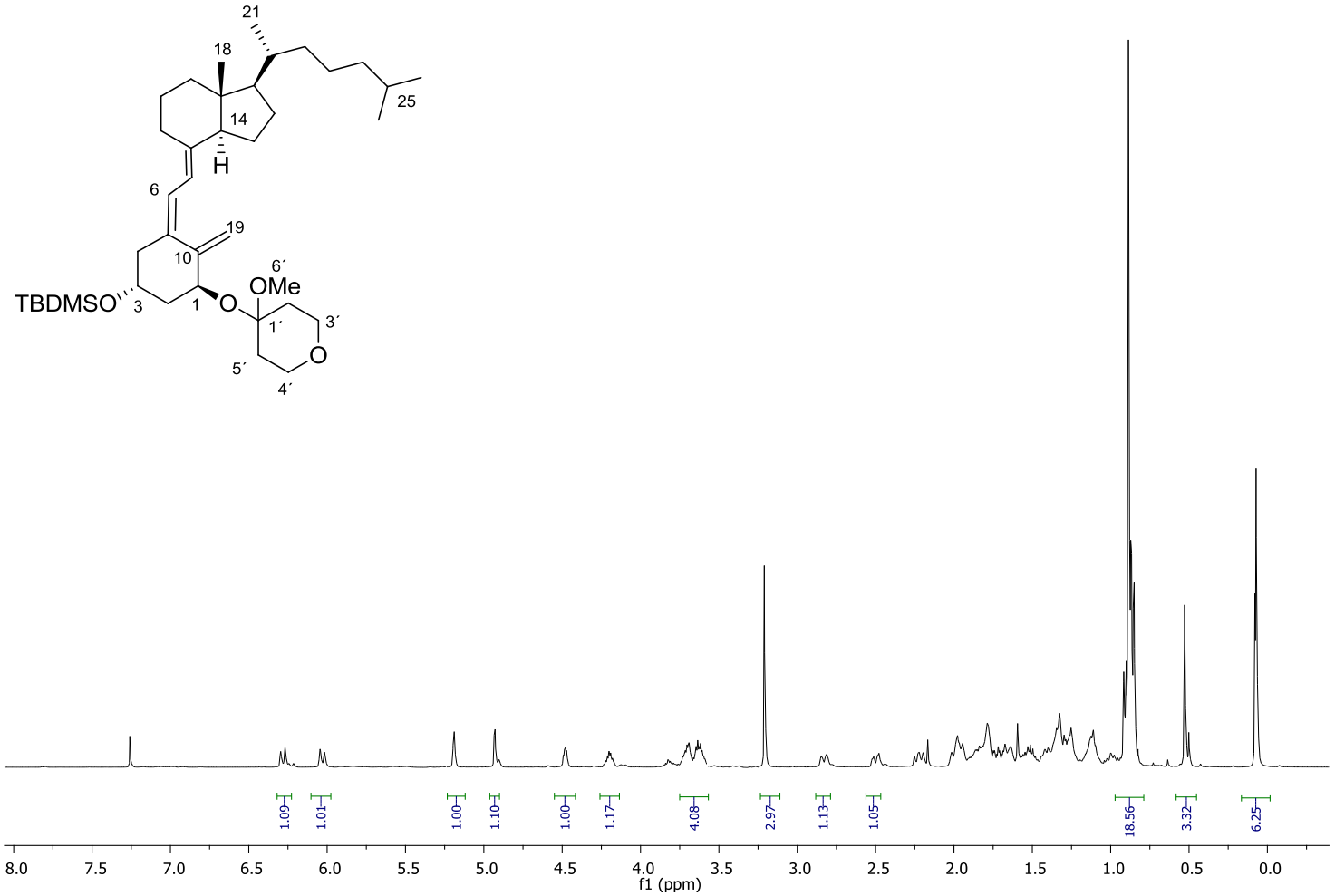
3-*O*-(*tert*-Butyldimethylsilyl)-1α-(vinyloxycarbonyloxy)vitamin D_3 (15)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$)



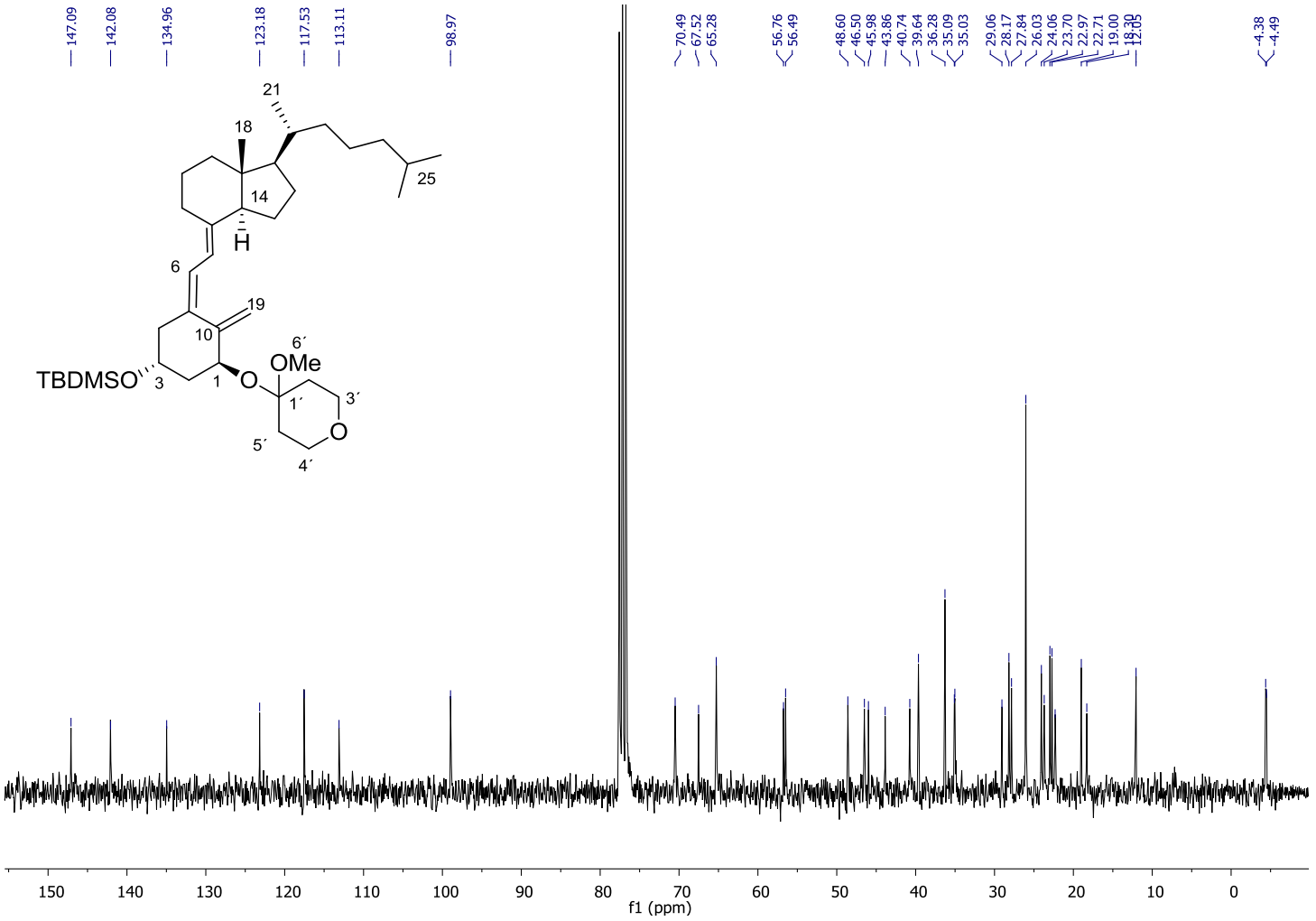
3-*O*-(*tert*-Butyldimethylsilyl)-1α-[(4-methoxytetrahydro-2*H*-pyran-4-yl)oxy]vitamin D_3 (16)

^{1}H-RMN (300.13 MHz, $CDCl_3$)



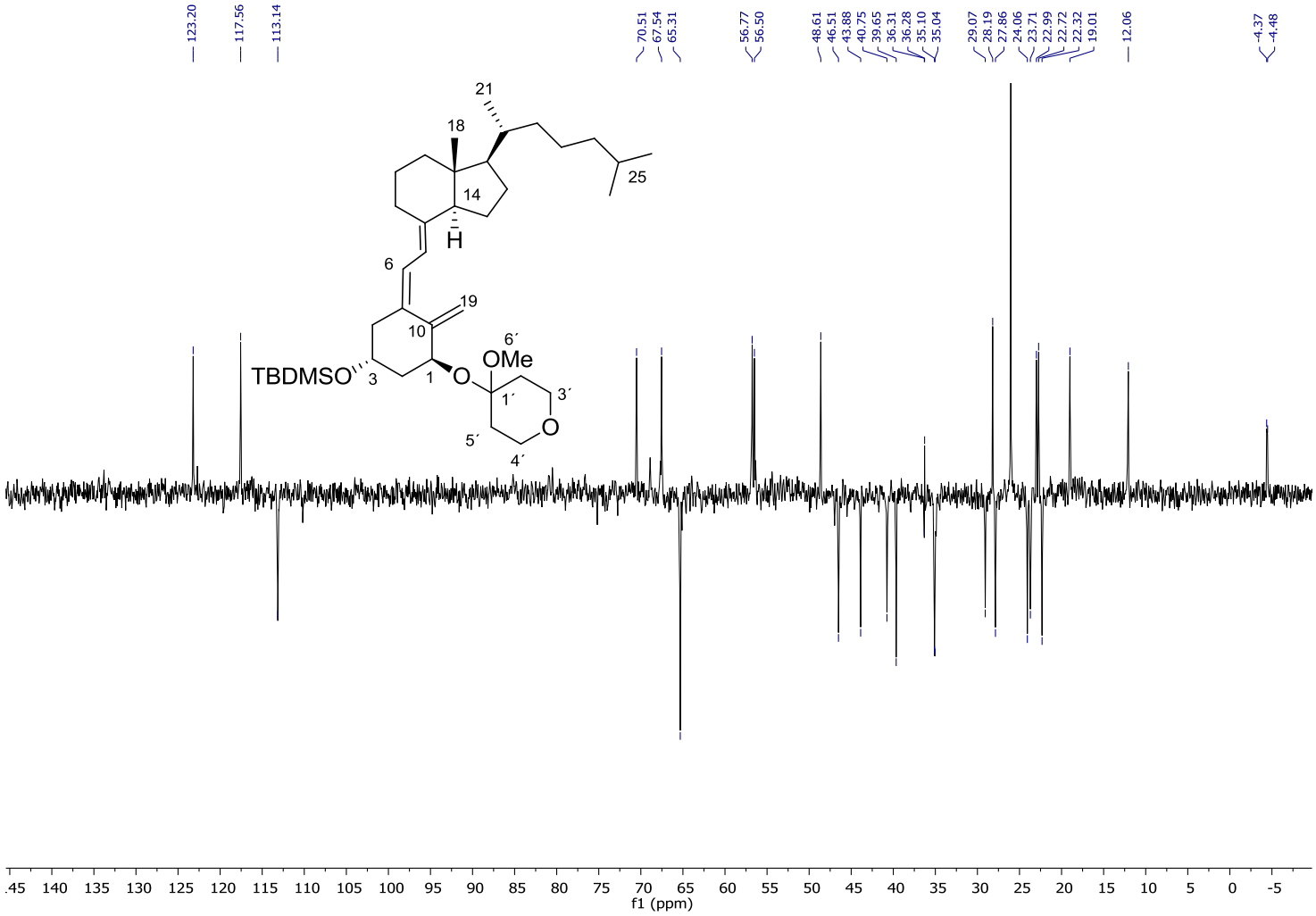
3-*O*-(*tert*-Butyldimethylsilyl)-1α-[(4-methoxytetrahydro-2*H*-pyran-4-yl)oxy]vitamin D_3 (16)

^{13}C-RMN (75.5 MHz, $CDCl_3$)



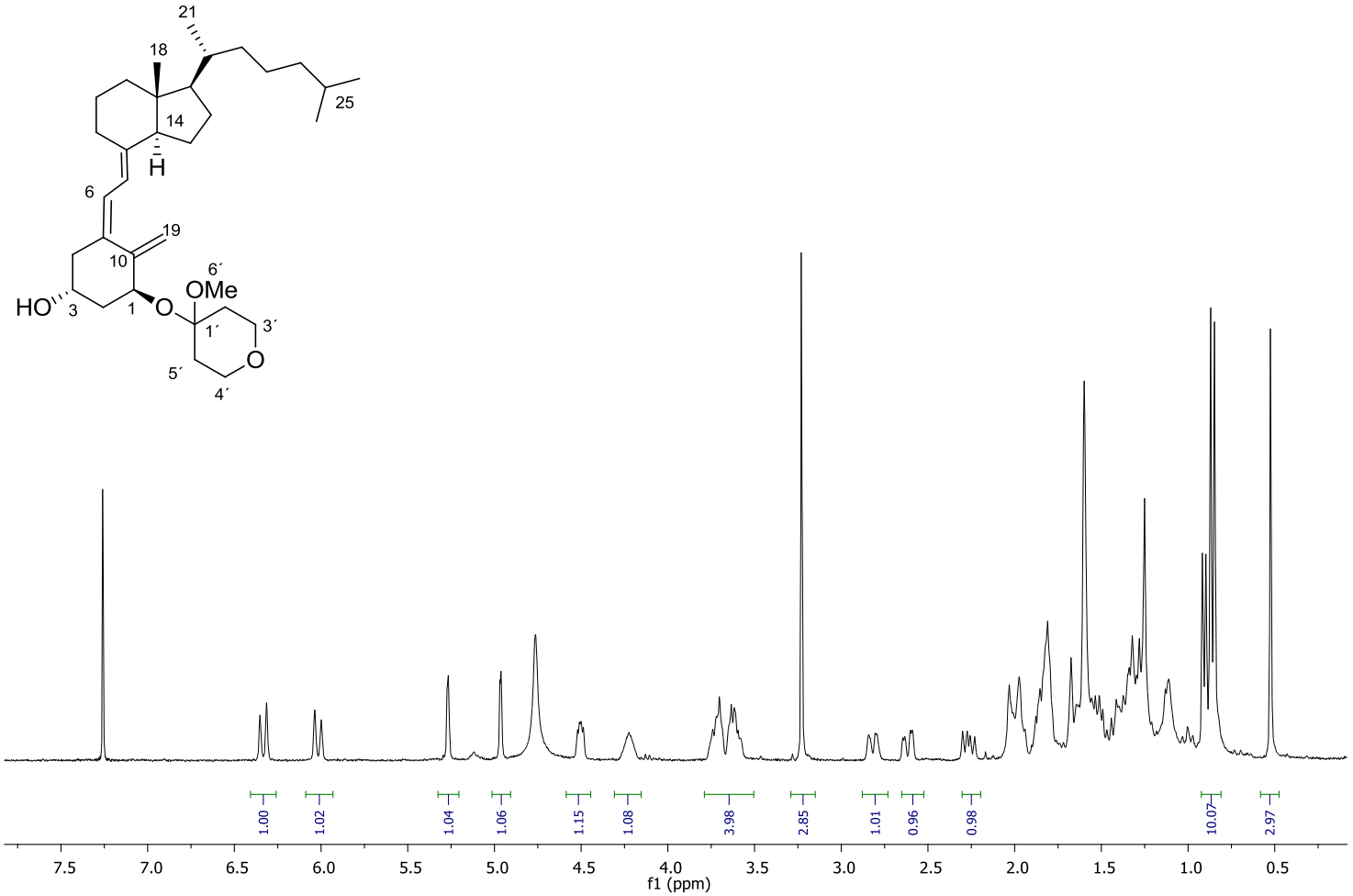
3-*O*-(*tert*-Butyldimethylsilyl)-1α-[(4-methoxytetrahydro-2*H*-pyran-4-yl)oxy]vitamin D_3 (16)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$)



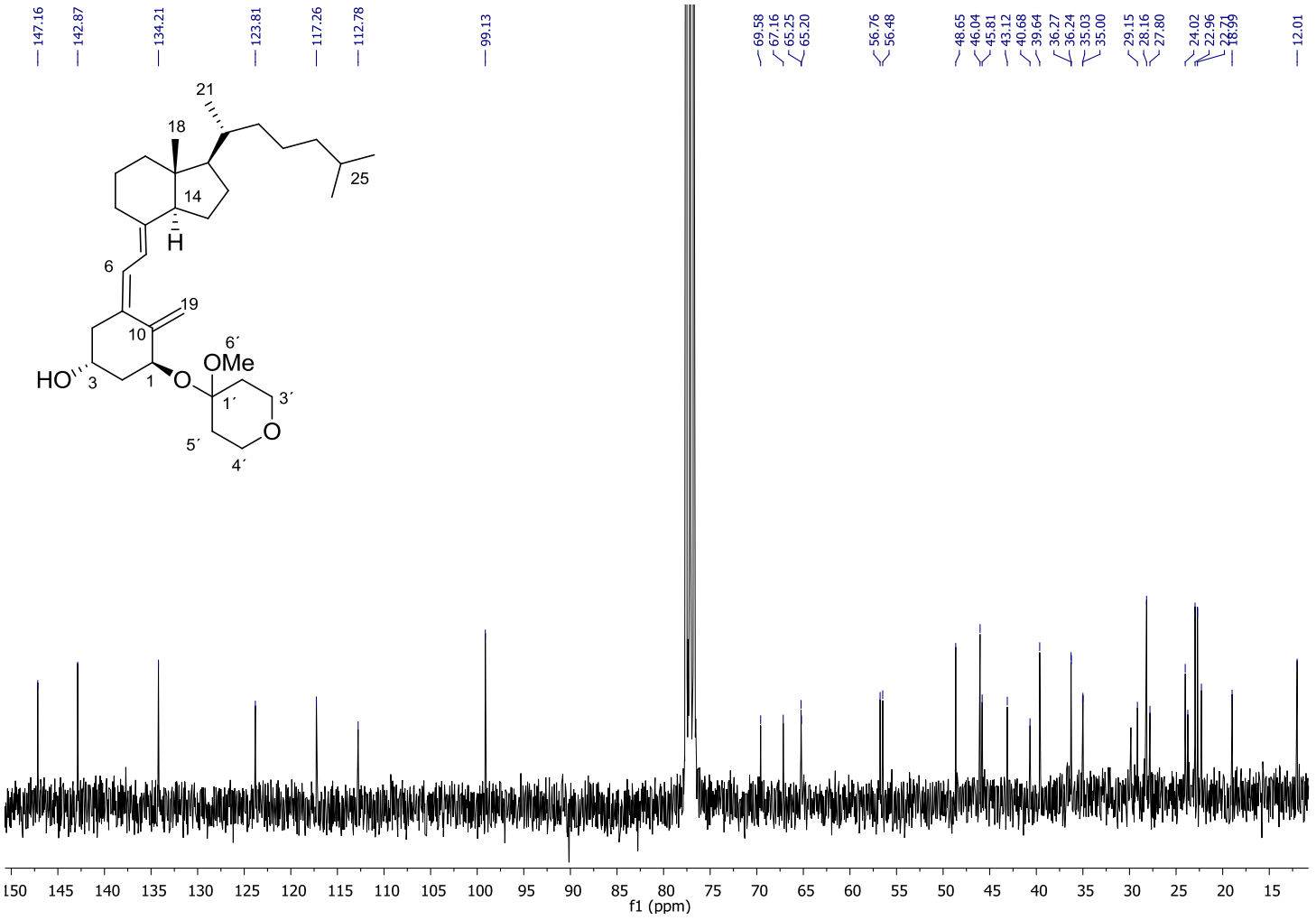
1α-[(4-Methoxytetrahydro-2*H*-pyran-4-yl)oxy]vitamin D_3 (17)

^{1}H-RMN (300.13 MHz, $CDCl_3$)



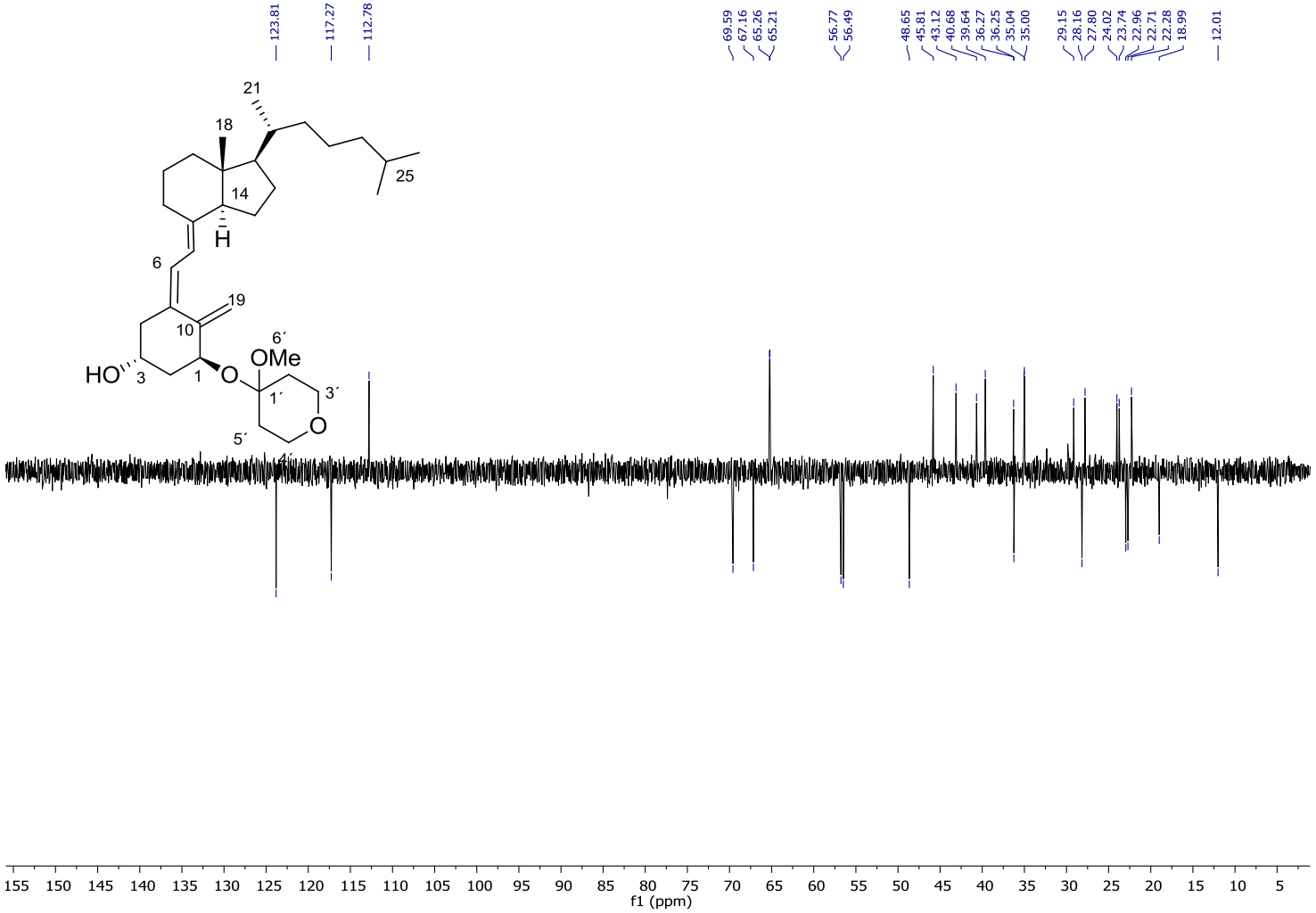
1α-[(4-Methoxytetrahydro-2*H*-pyran-4-yl)oxy]vitamin D_3 (17)

^{13}C-RMN (75.5 MHz, $CDCl_3$)



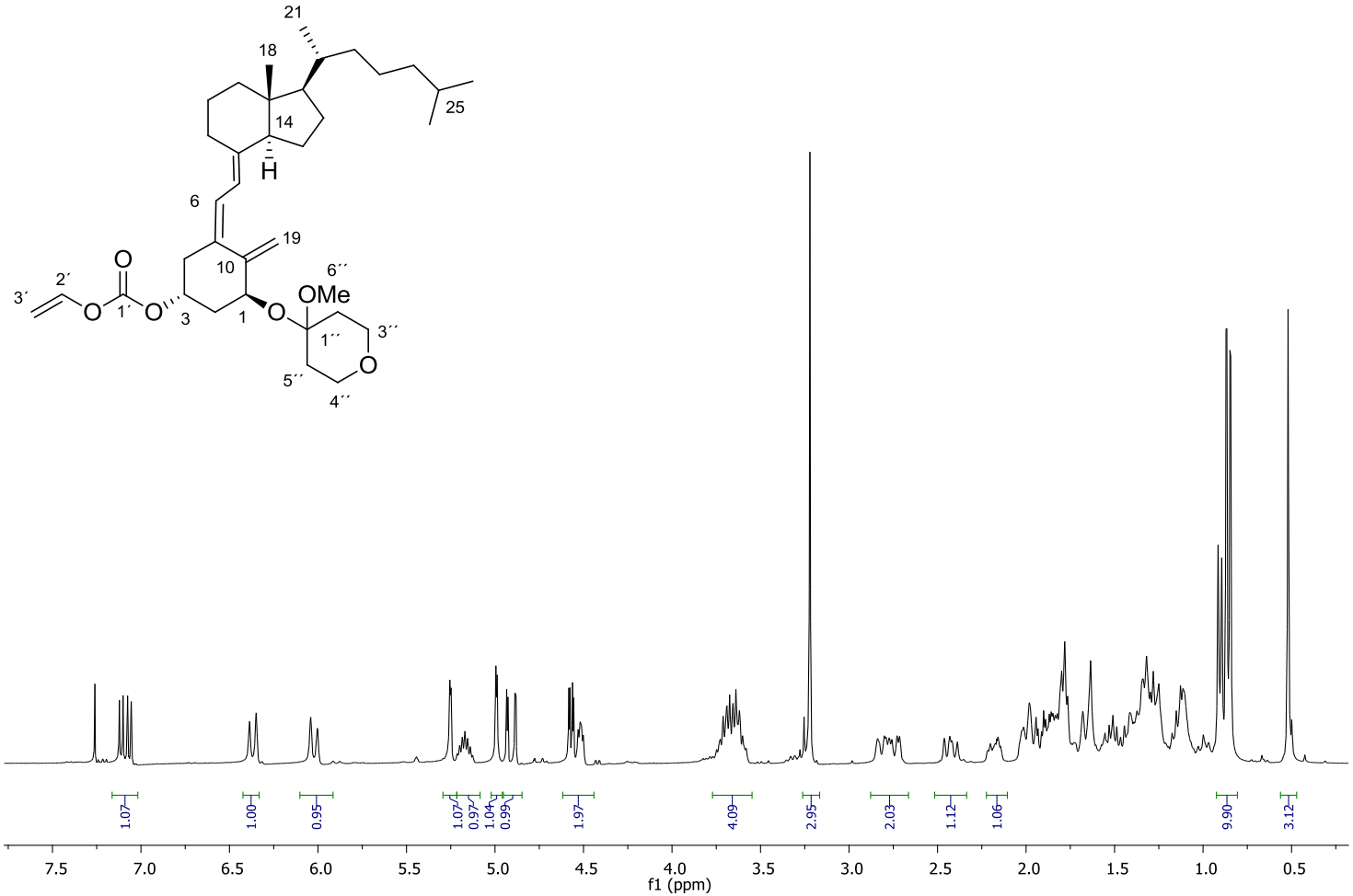
1α-[(4-Methoxytetrahydro-2*H*-pyran-4-yl)oxy]vitamin D_3 (17)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$)



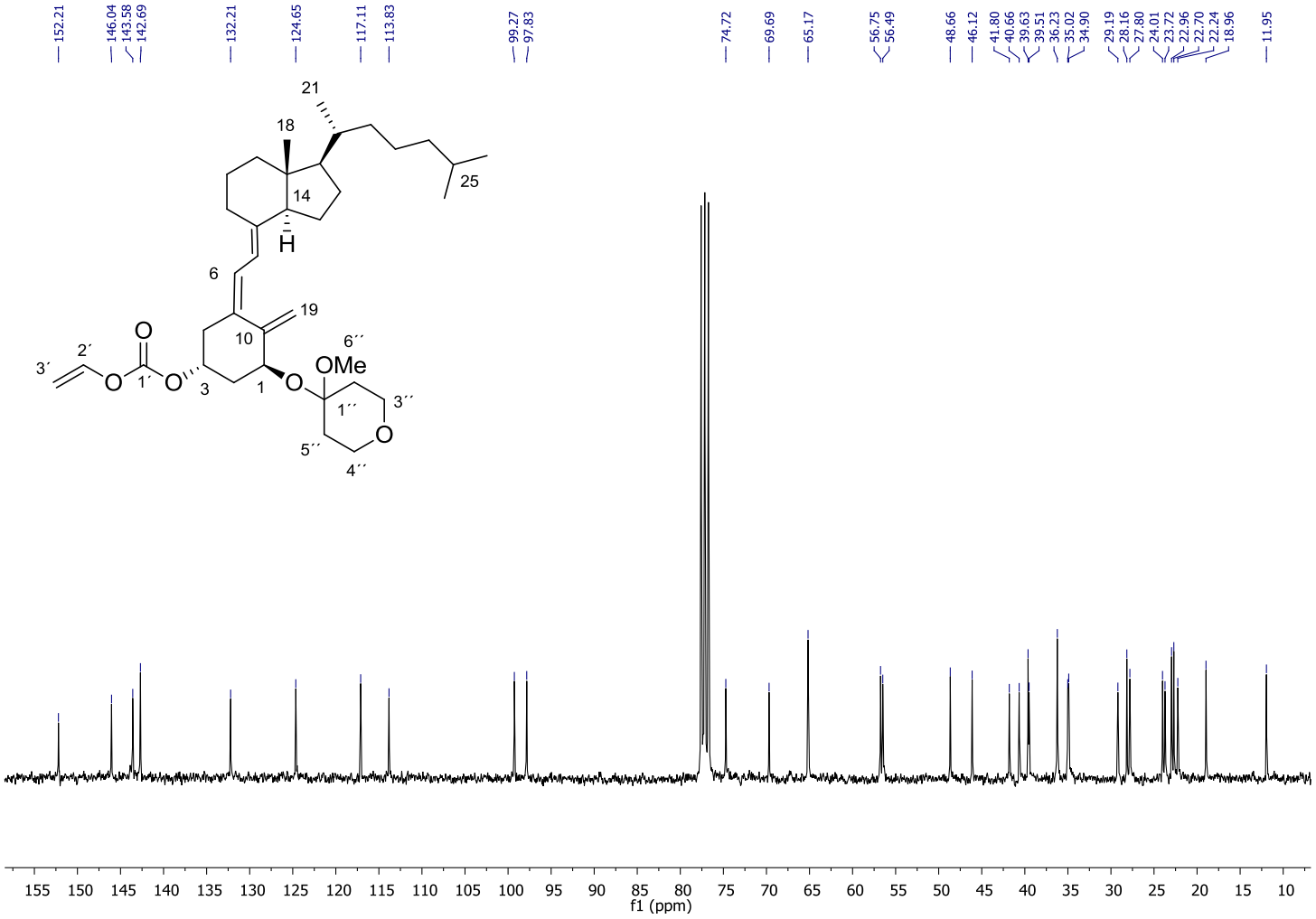
1α-(4-Methoxytetrahydro-2*H*-pyran-4-yl)oxy-3-*O*-(vinyloxycarbonyl)vitamin D_3 (18)

^{1}H-RMN (300.13 MHz, $CDCl_3$)



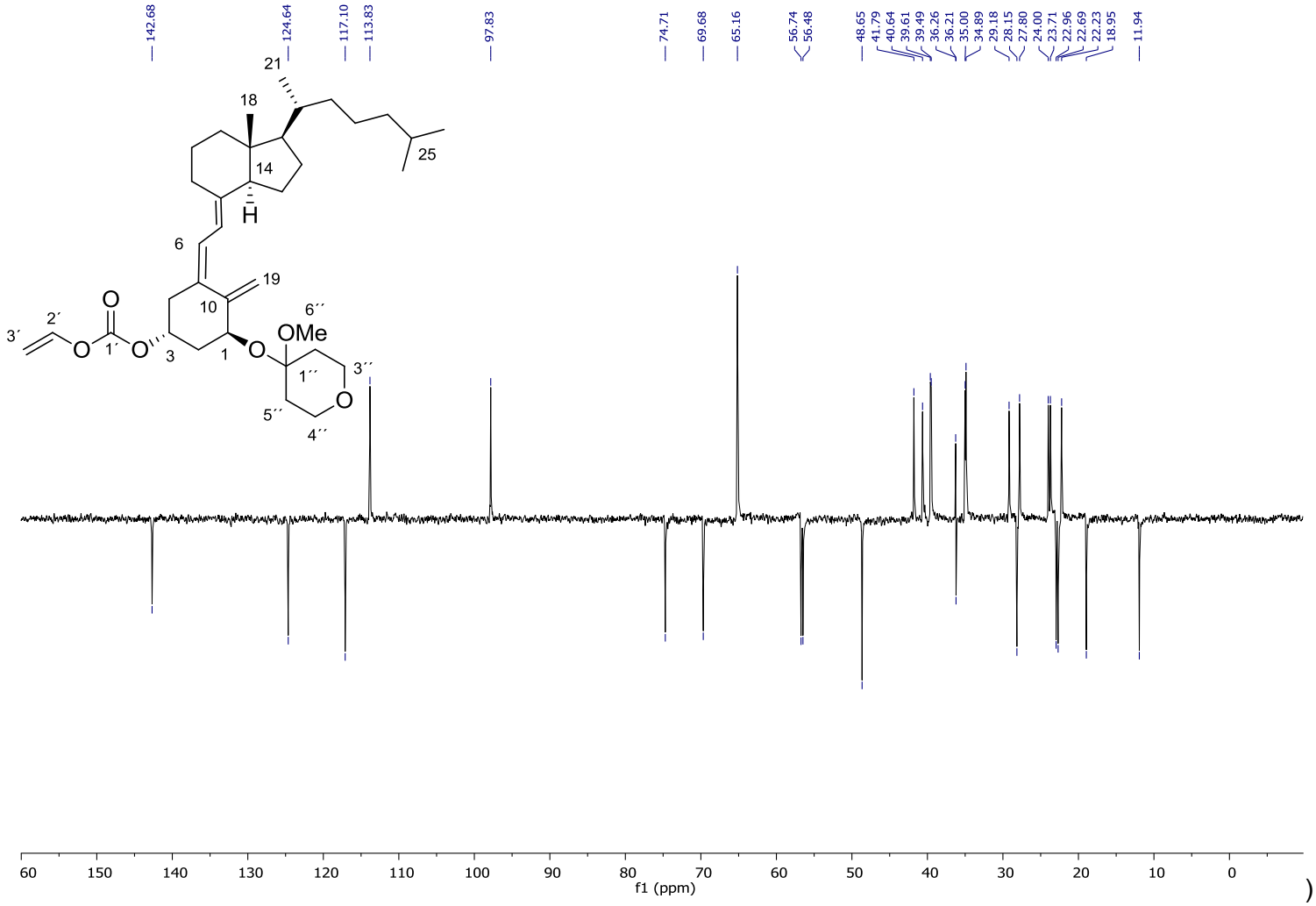
1α-(4-Methoxytetrahydro-2*H*-pyran-4-yl)oxy-3-*O*-(vinyloxycarbonyl)vitamin D_3 (18)

^{13}C-RMN (75.5 MHz, $CDCl_3$)



1α-(4-Methoxytetrahydro-2*H*-pyran-4-yl)oxy-3-*O*-(vinyloxycarbonyl)vitamin D_3 (18)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$)



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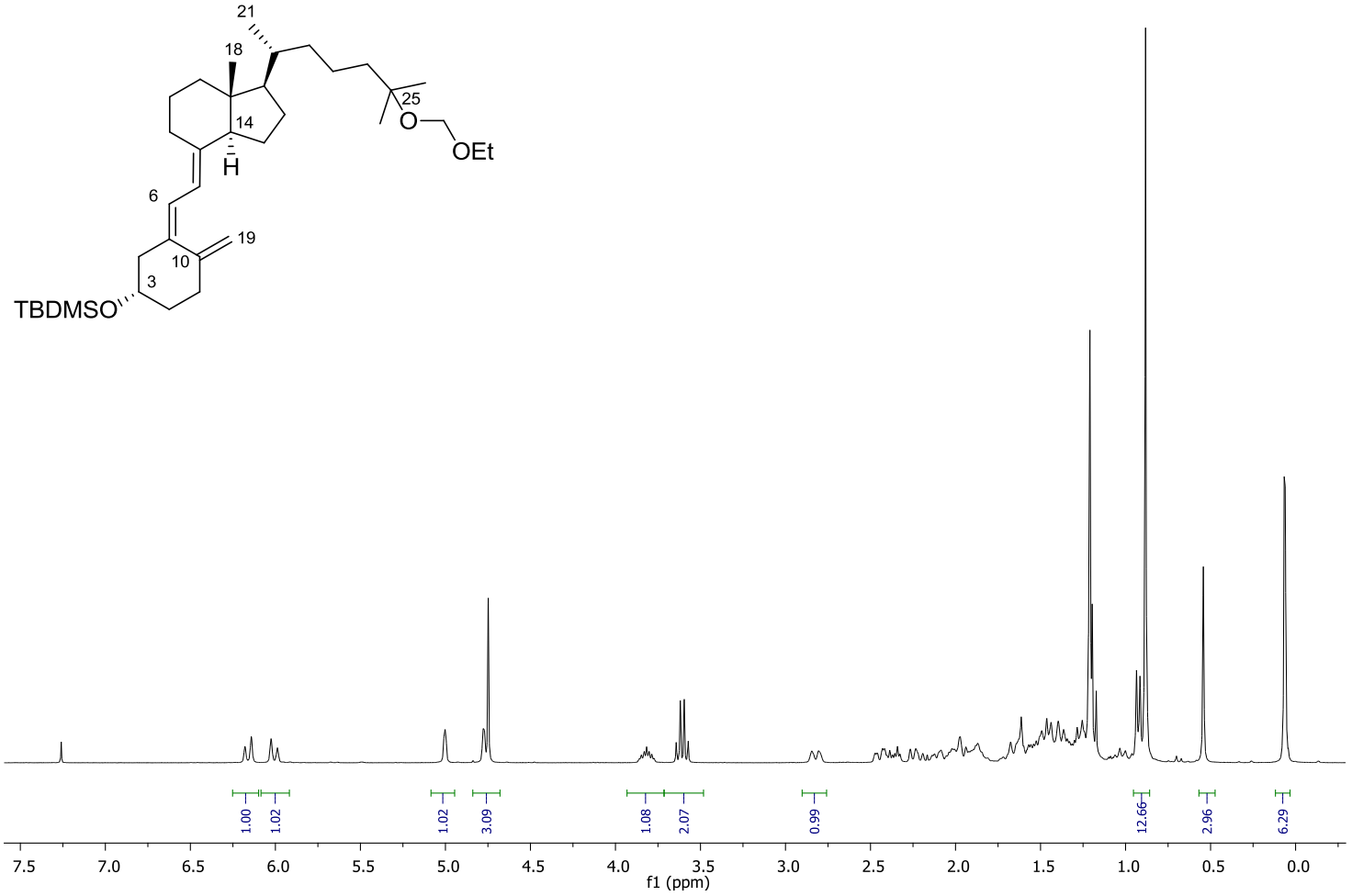
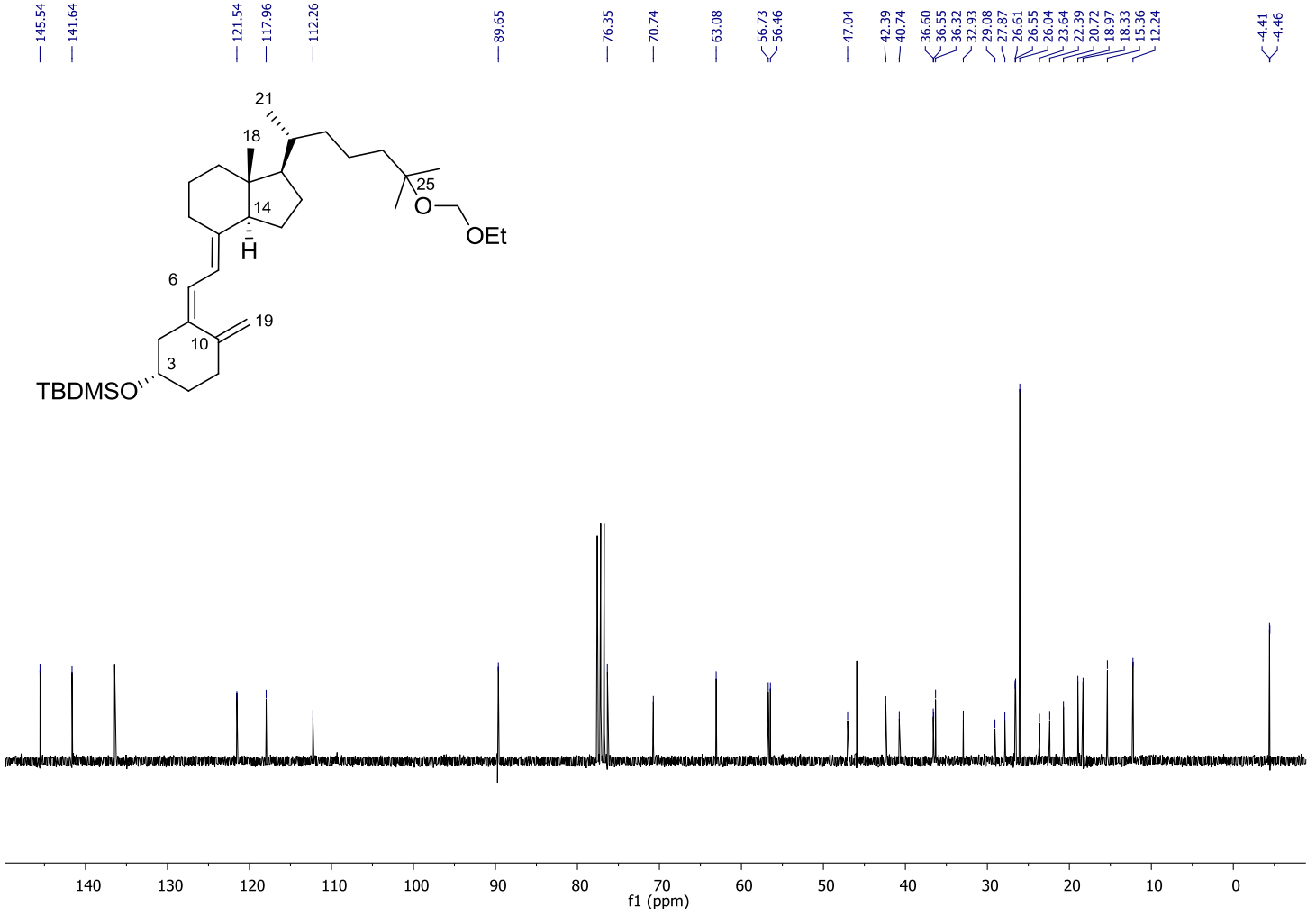
3-*O*-(*tert*-Butyldimethylsilyl)-25-ethoxymethyloxyvitamin D_3 (21)

^{1}H-RMN (300.13 MHz, $CDCl_3$)



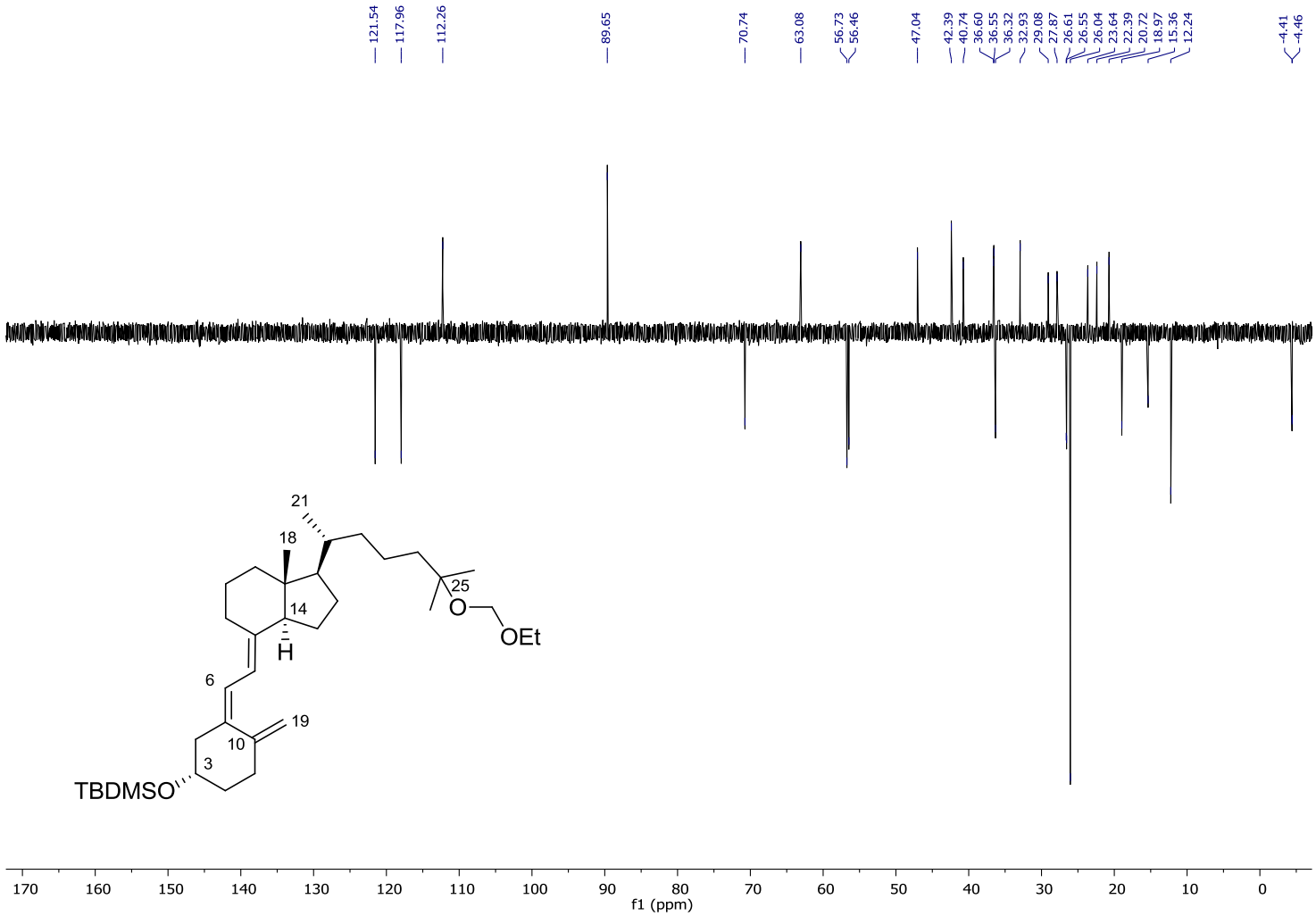
3-*O*-(*tert*-Butyldimethylsilyl)-25-ethoxymethyloxyvitamin D_3 (21)

^{13}C-RMN (75.5 MHz, $CDCl_3$)



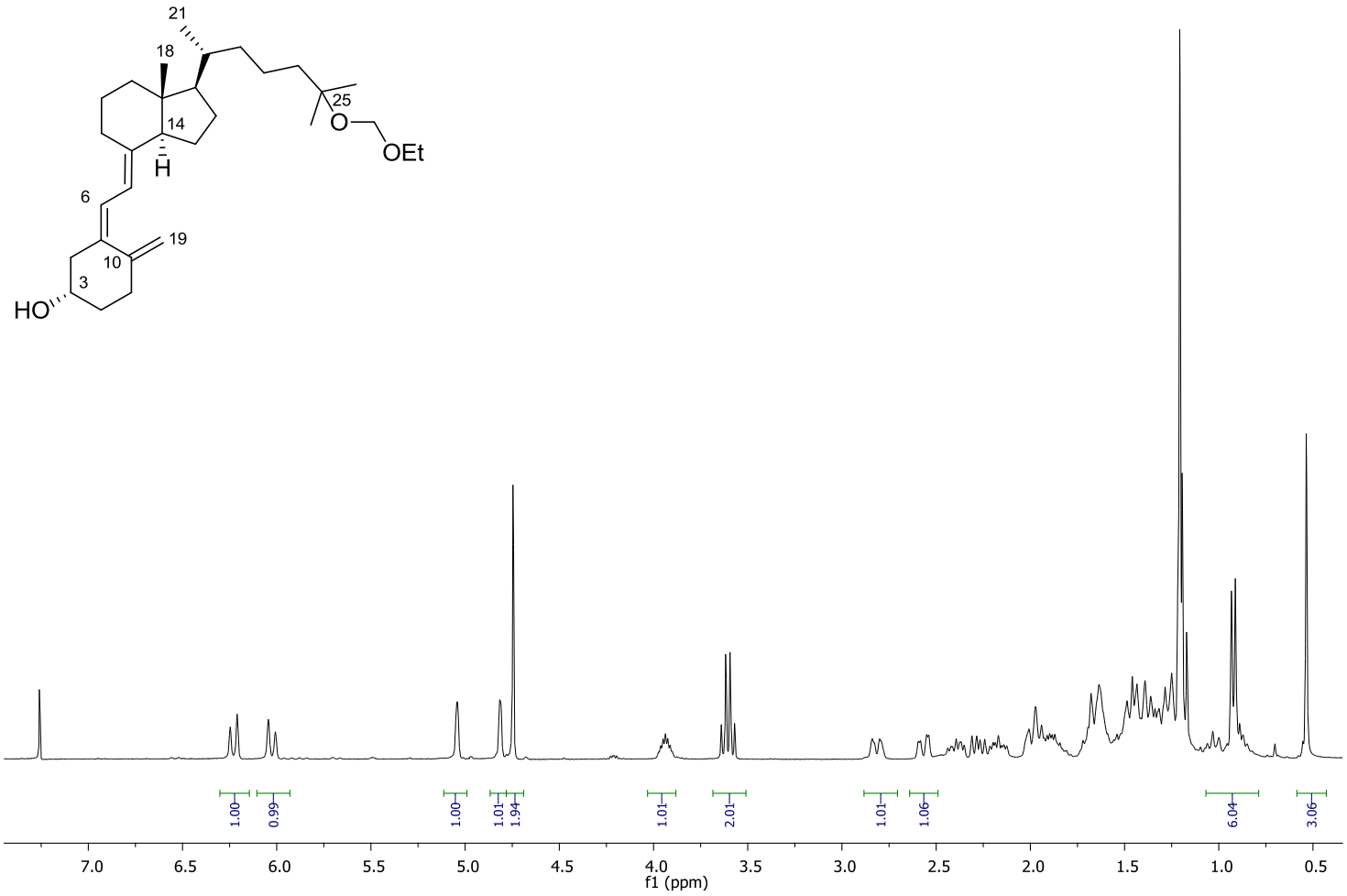
3-*O*-(*tert*-Butyldimethylsilyl)-25-ethoxymethyloxyvitamin D_3 (21)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$)



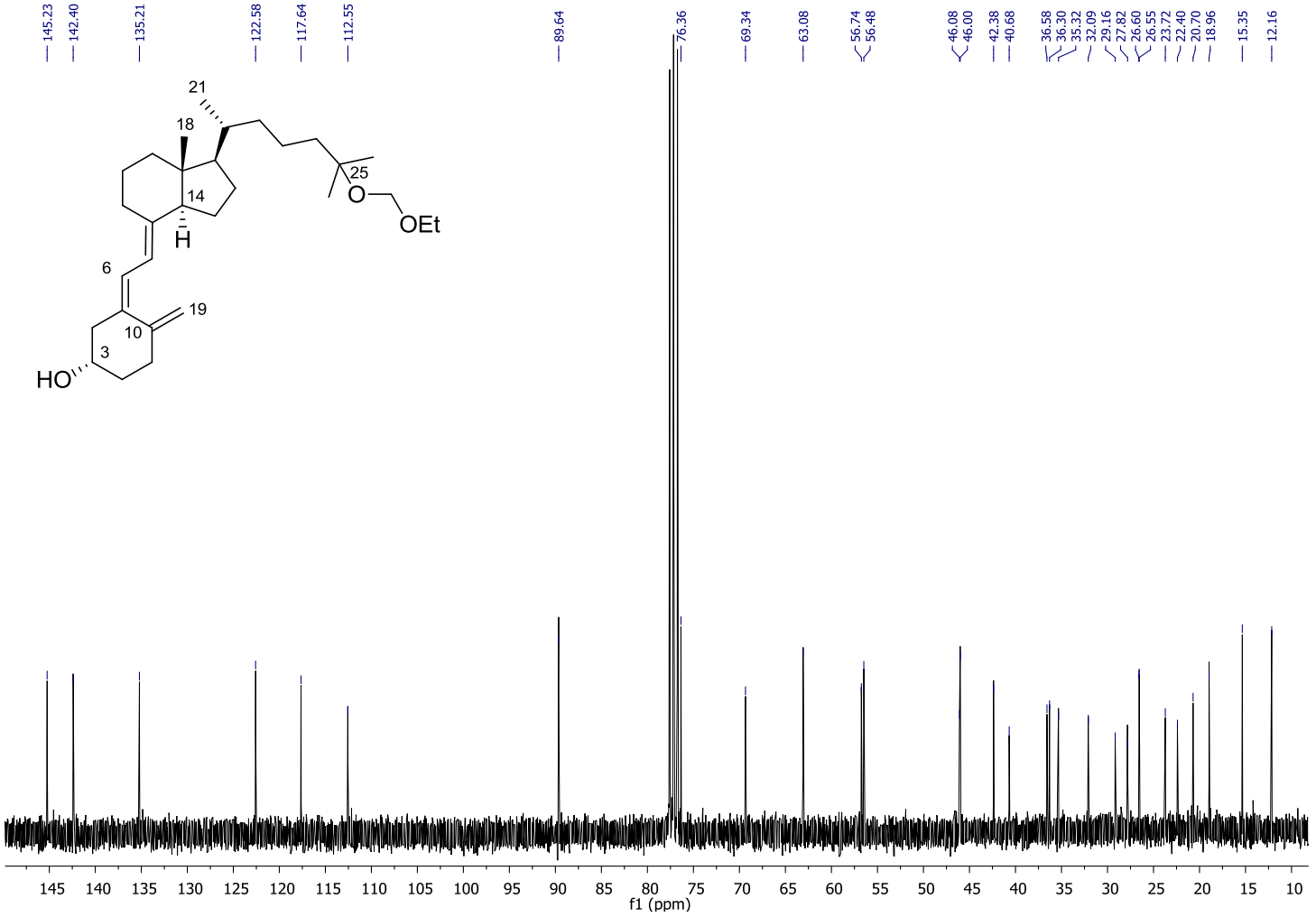
25-Ethoxymethyloxyvitamin D_3 (22)

^{1}H-RMN (300.13 MHz, $CDCl_3$)



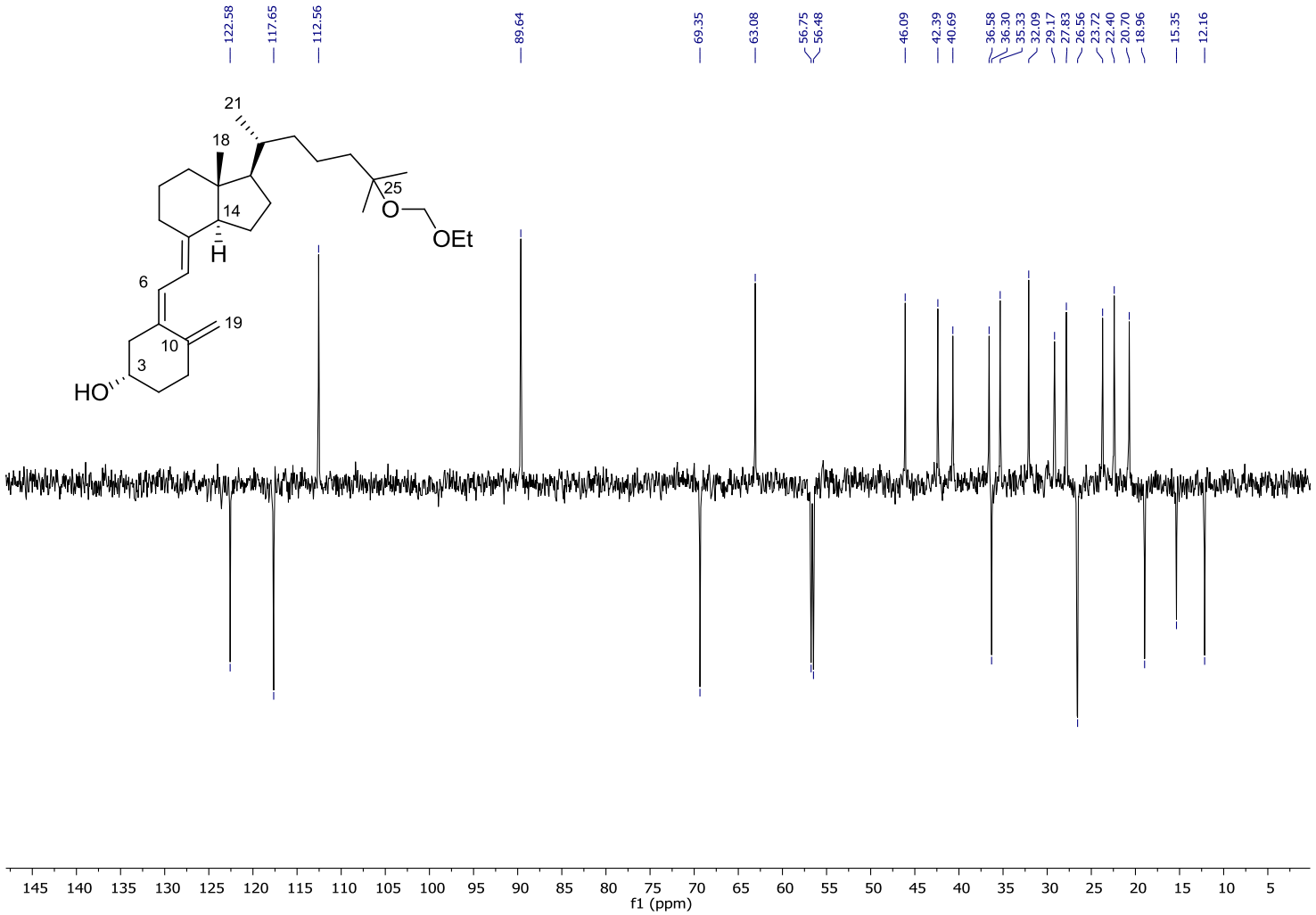
25-Ethoxymethyloxyvitamin D_3 (22)

^{13}C-RMN (75.5 MHz, $CDCl_3$)



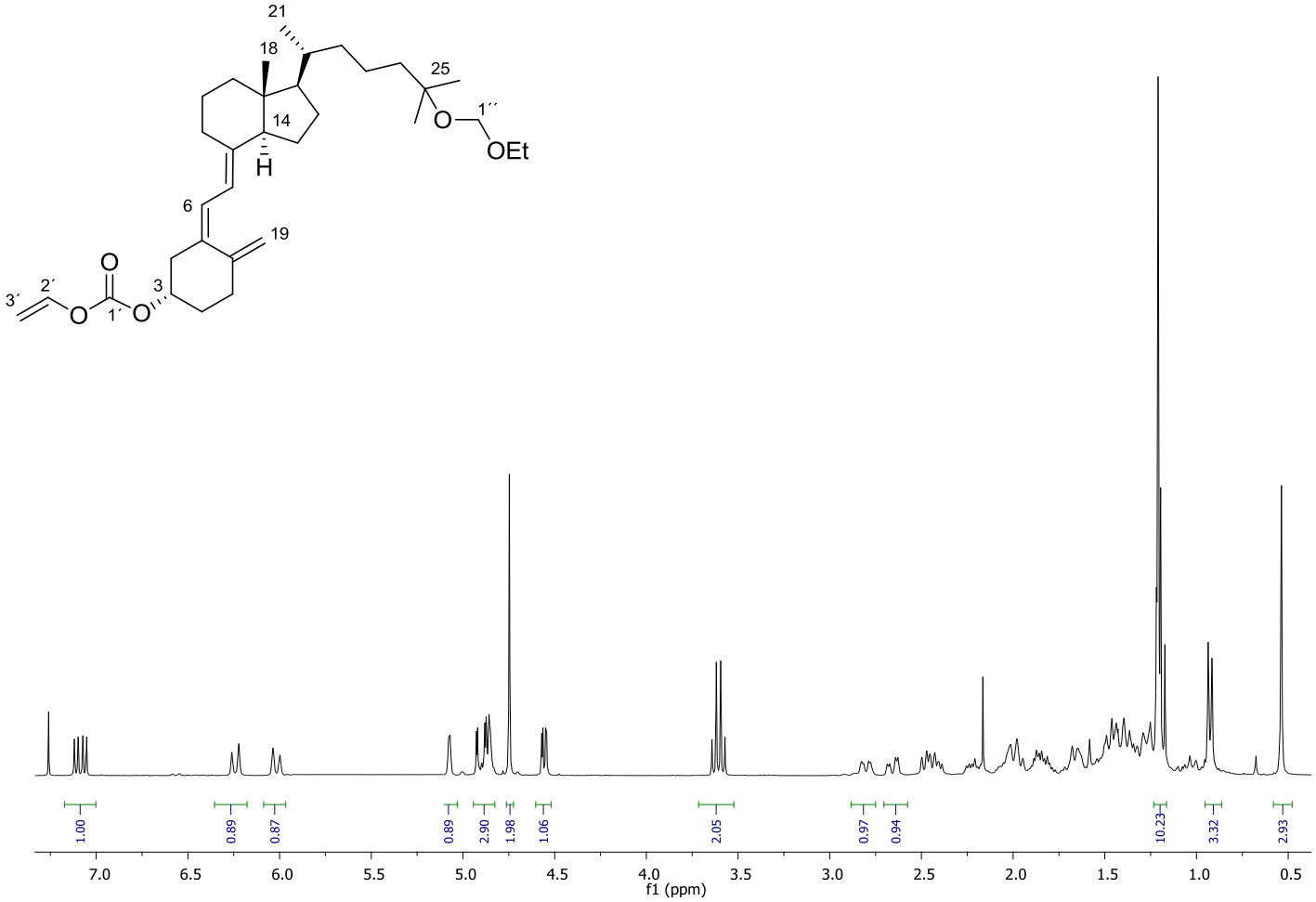
25-Ethoxymethyloxyvitamin D_3 (22)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$)



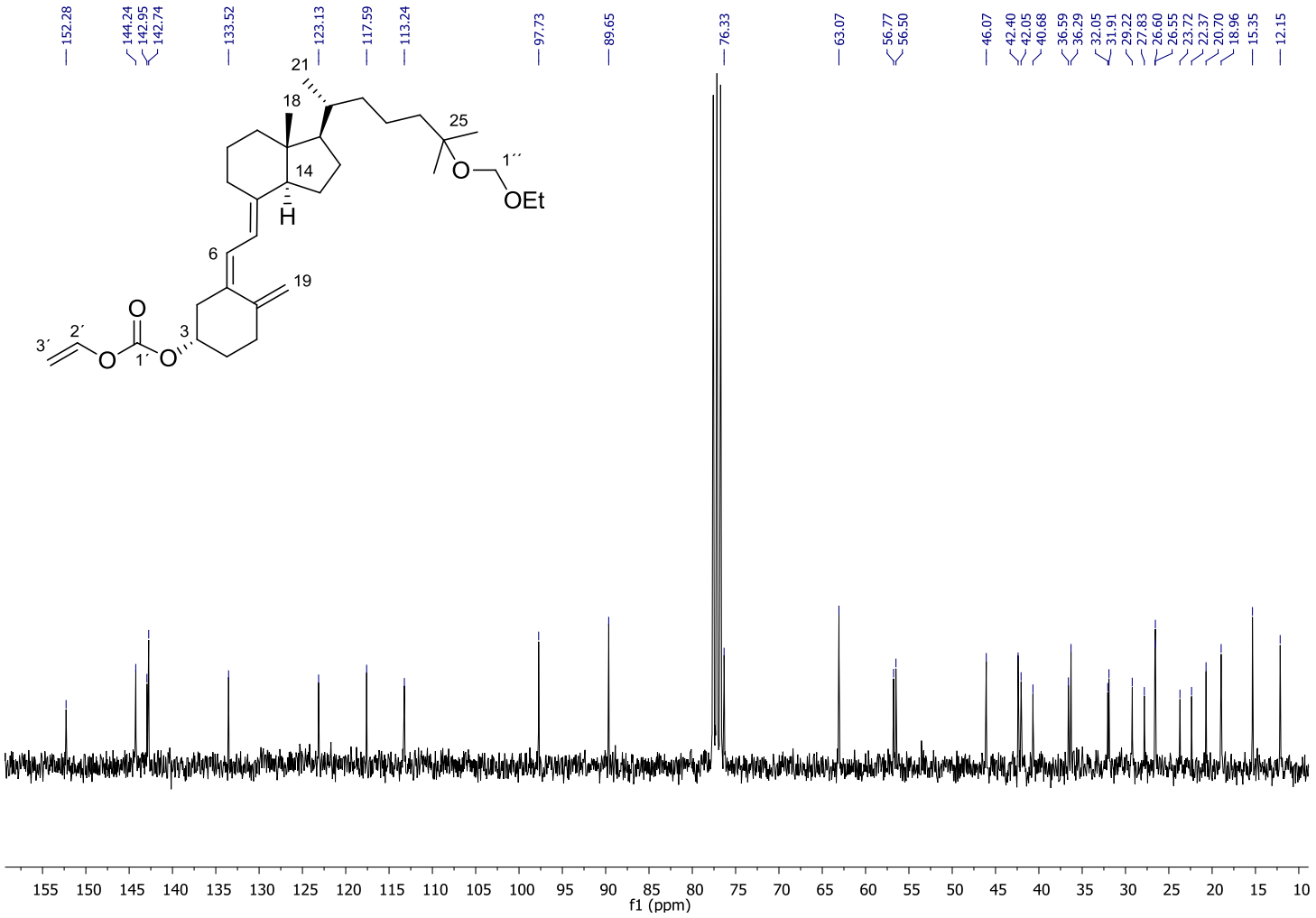
3-*O*-(Vinyloxy)carbonyl-25-ethoxymethyloxyvitamin D_3 (23)

^{1}H-RMN (300.13 MHz, $CDCl_3$)



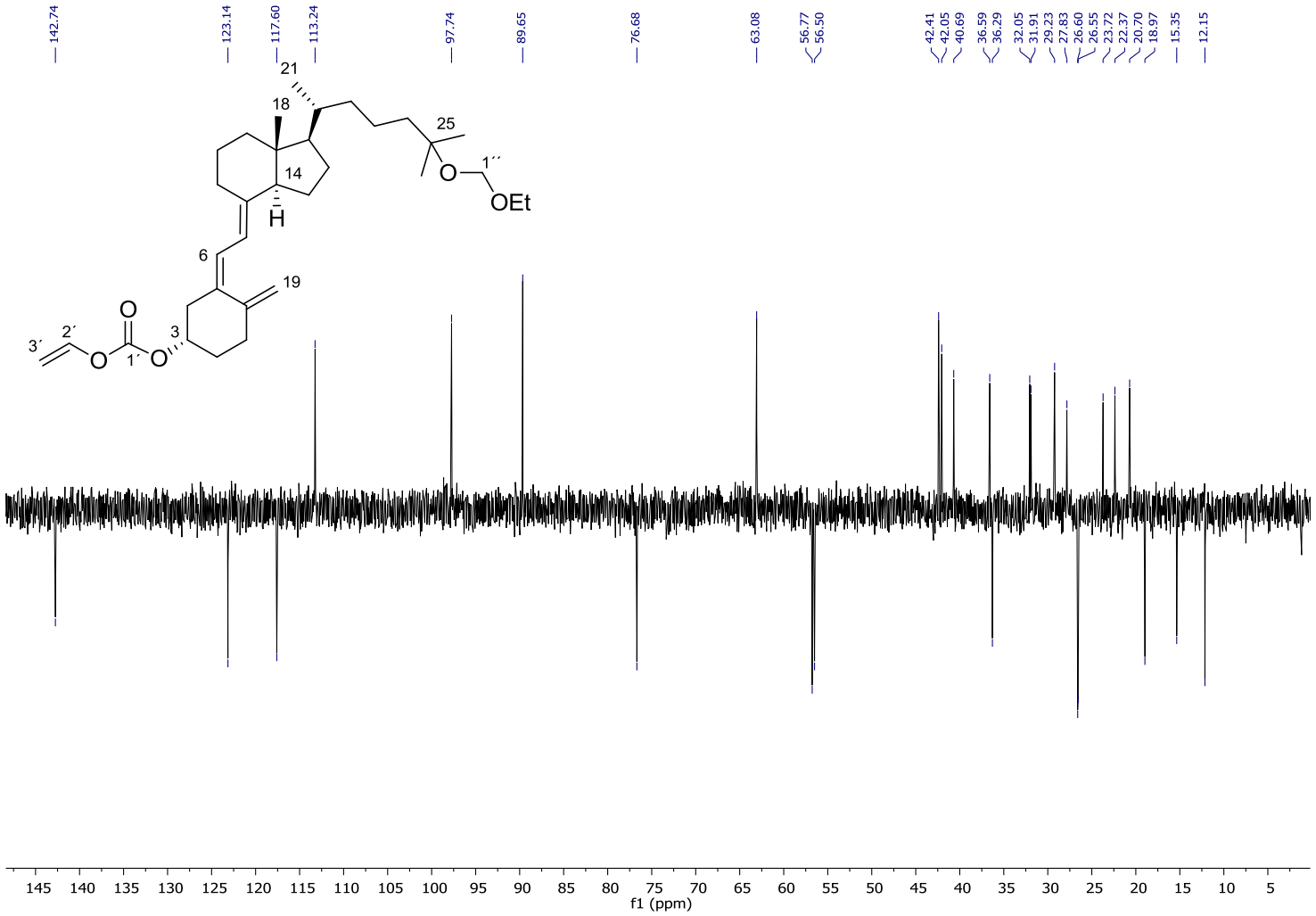
3-*O*-(Vinyloxy)carbonyl-25-ethoxymethyloxyvitamin D_3 (23)

^{13}C-RMN (75.5 MHz, $CDCl_3$)



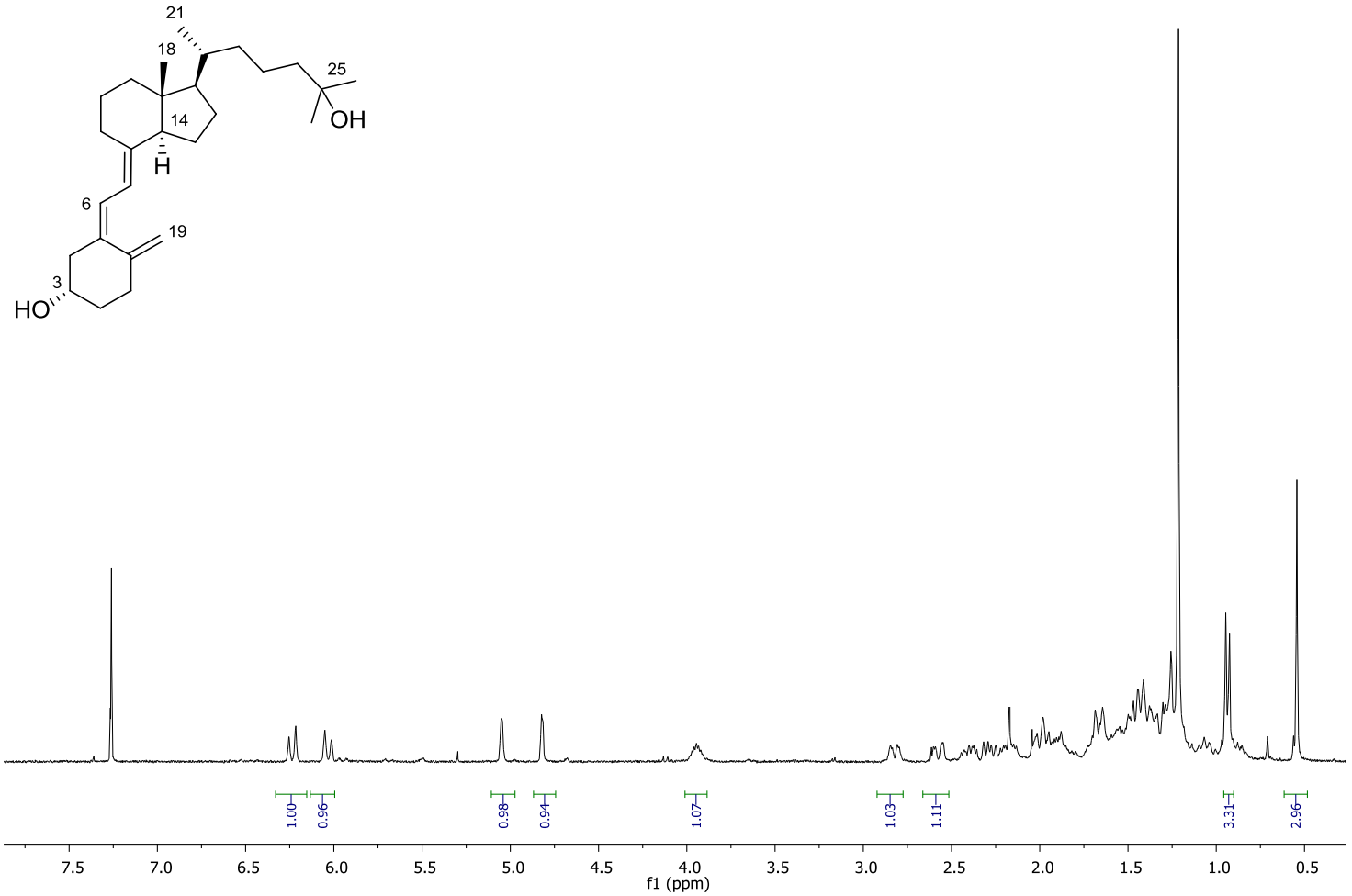
3-*O*-(Vinyloxy)carbonyl-25-ethoxymethyloxyvitamin D_3 (23)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$)



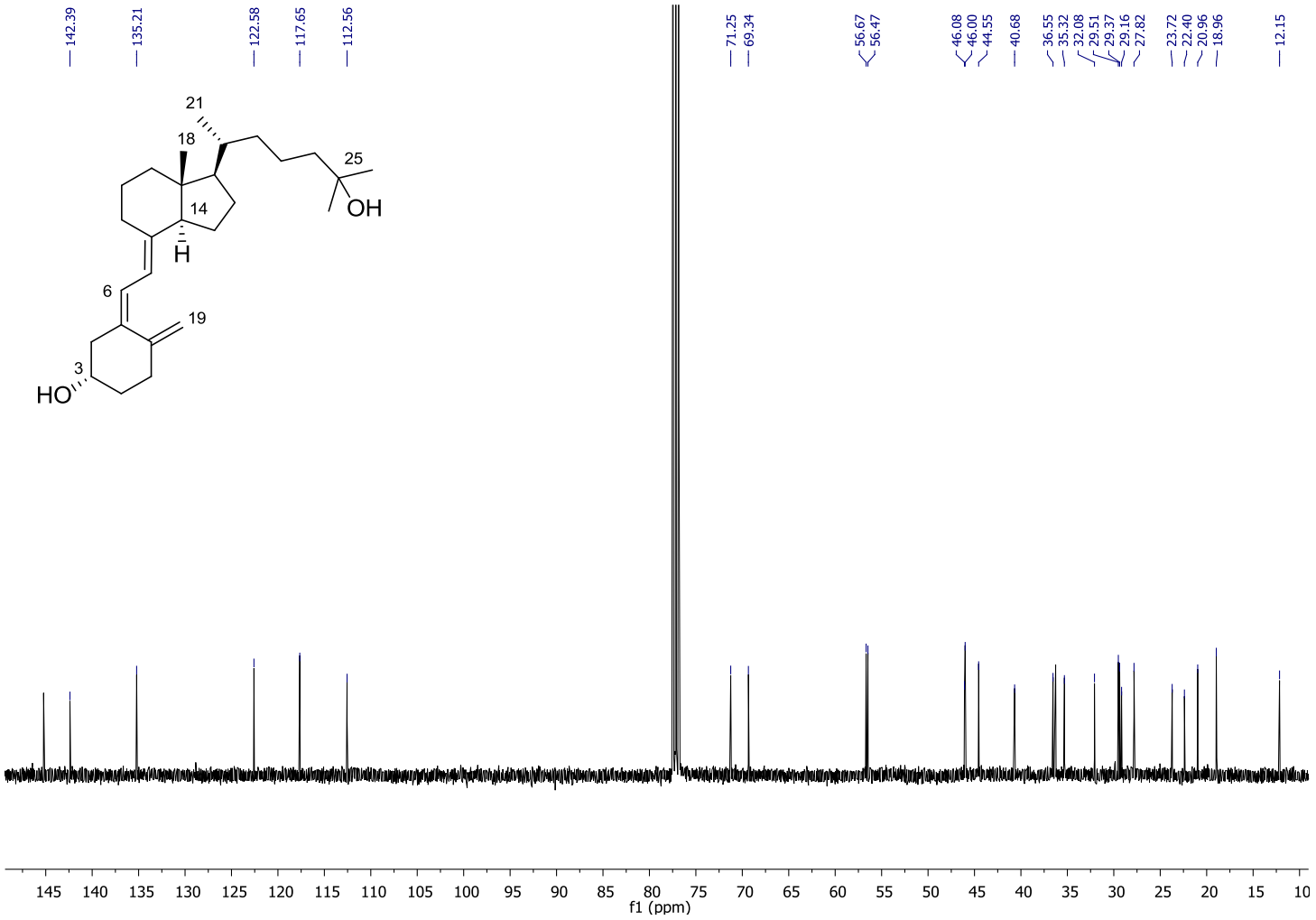
25-Hydroxyvitamin D_3 (24)

^{1}H-RMN (400.13 MHz, $CDCl_3$)



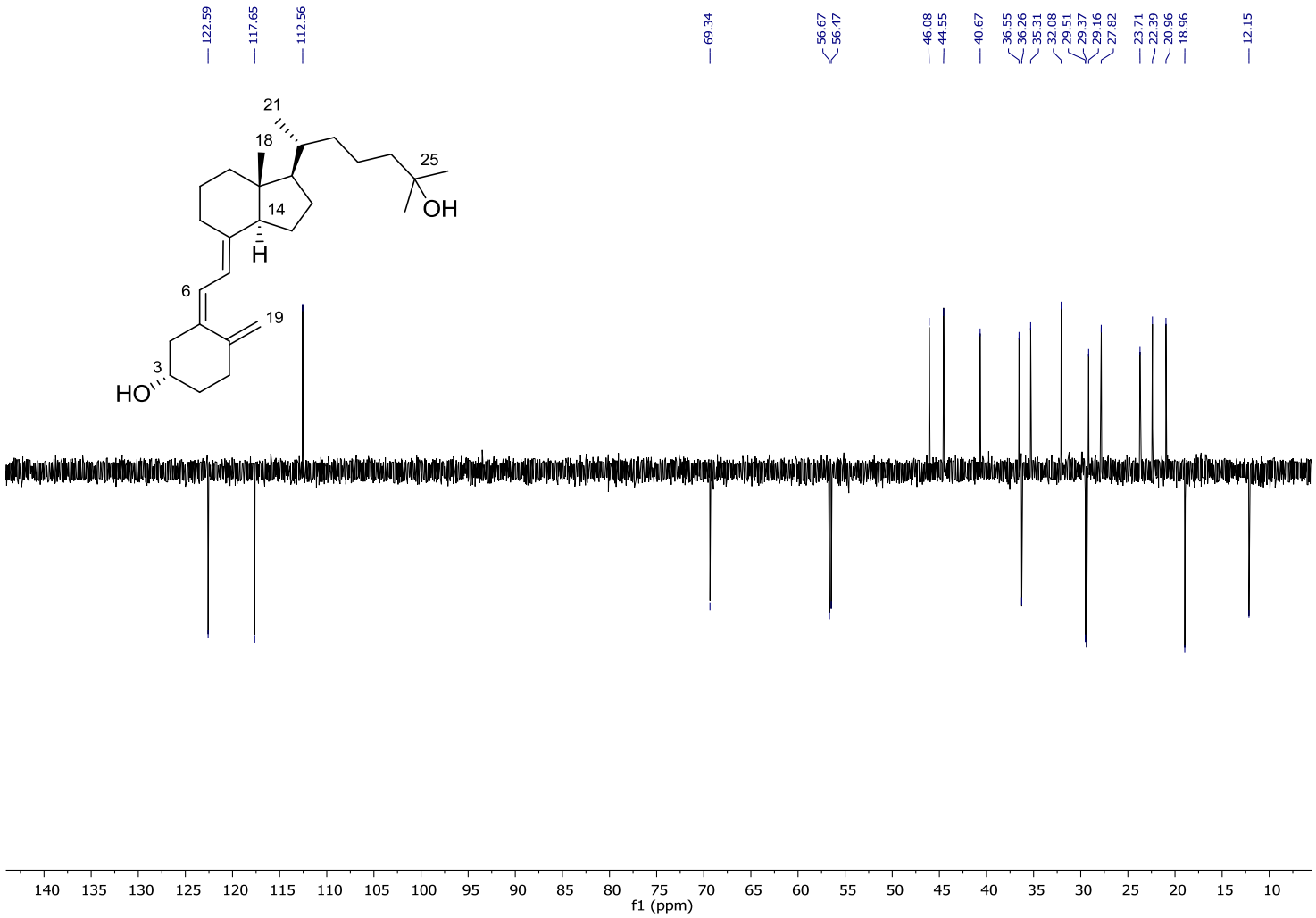
25-Hydroxyvitamin D_3 (24)

^{13}C-RMN (100.5 MHz, $CDCl_3$)



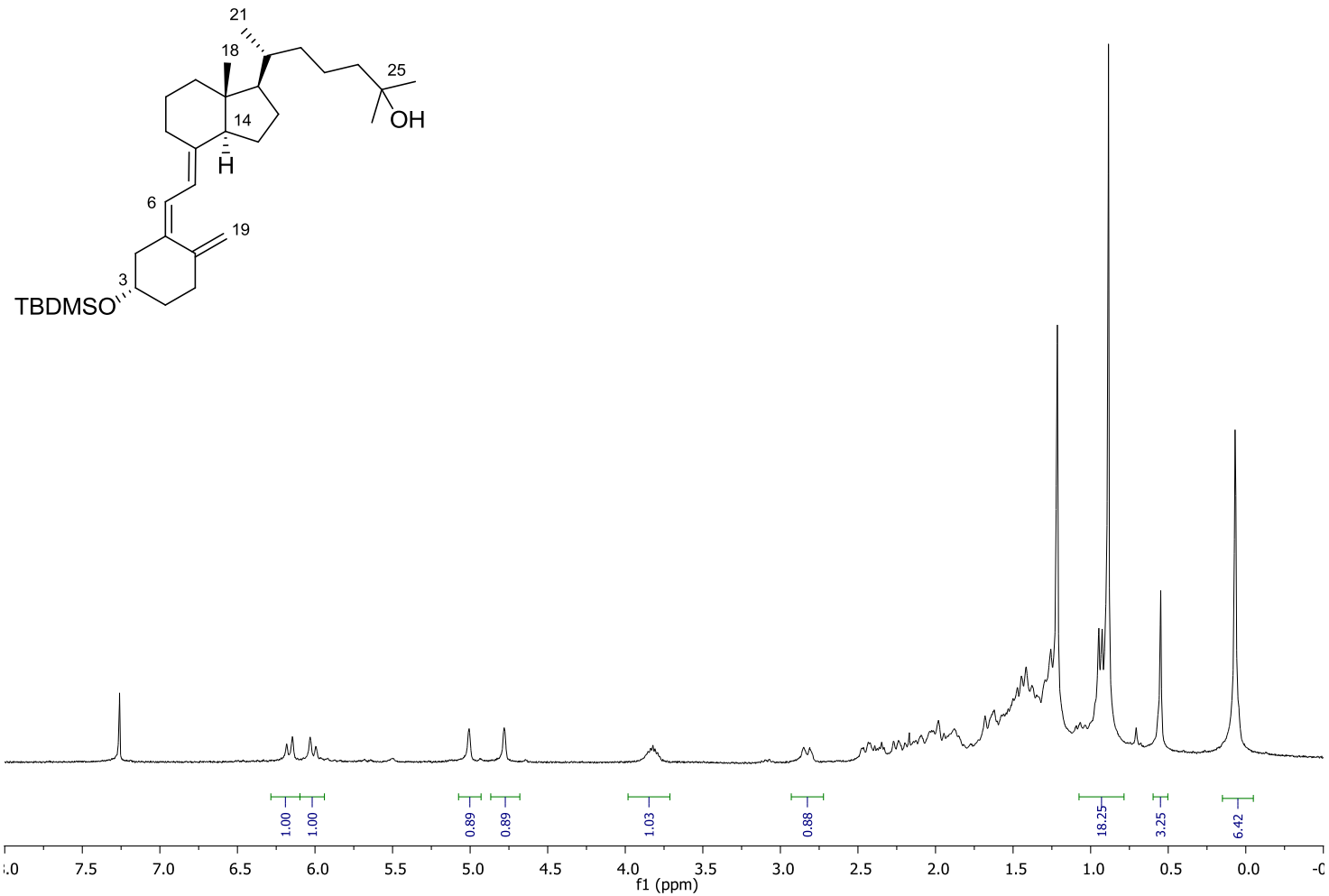
25-Hydroxyvitamin D_3 (24)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$)



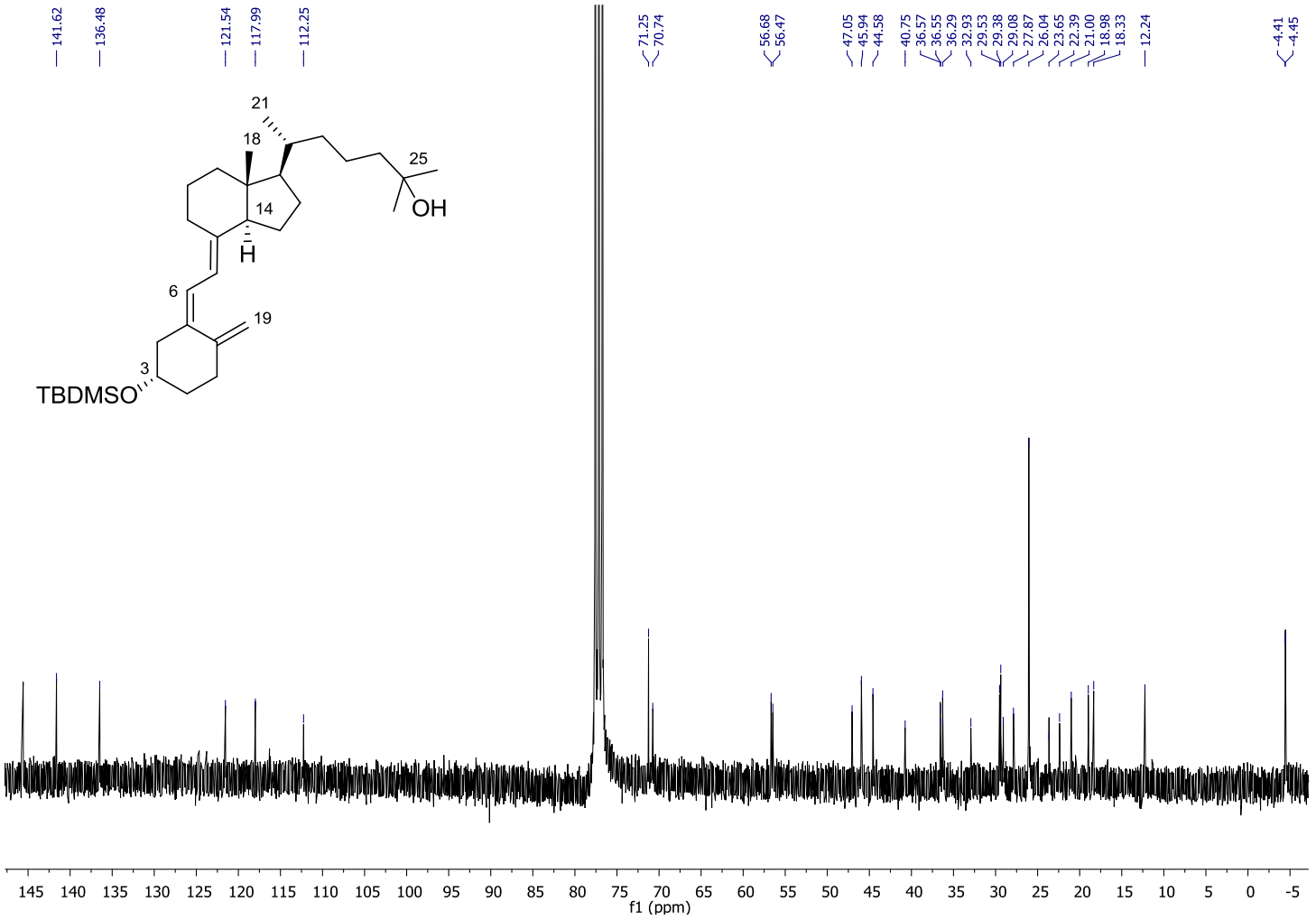
3-*O*-(*tert*-Butyldimethylsilyl)-25-hydroxyvitamin D_3 (25)

^{1}H-RMN (300.13 MHz, $CDCl_3$)



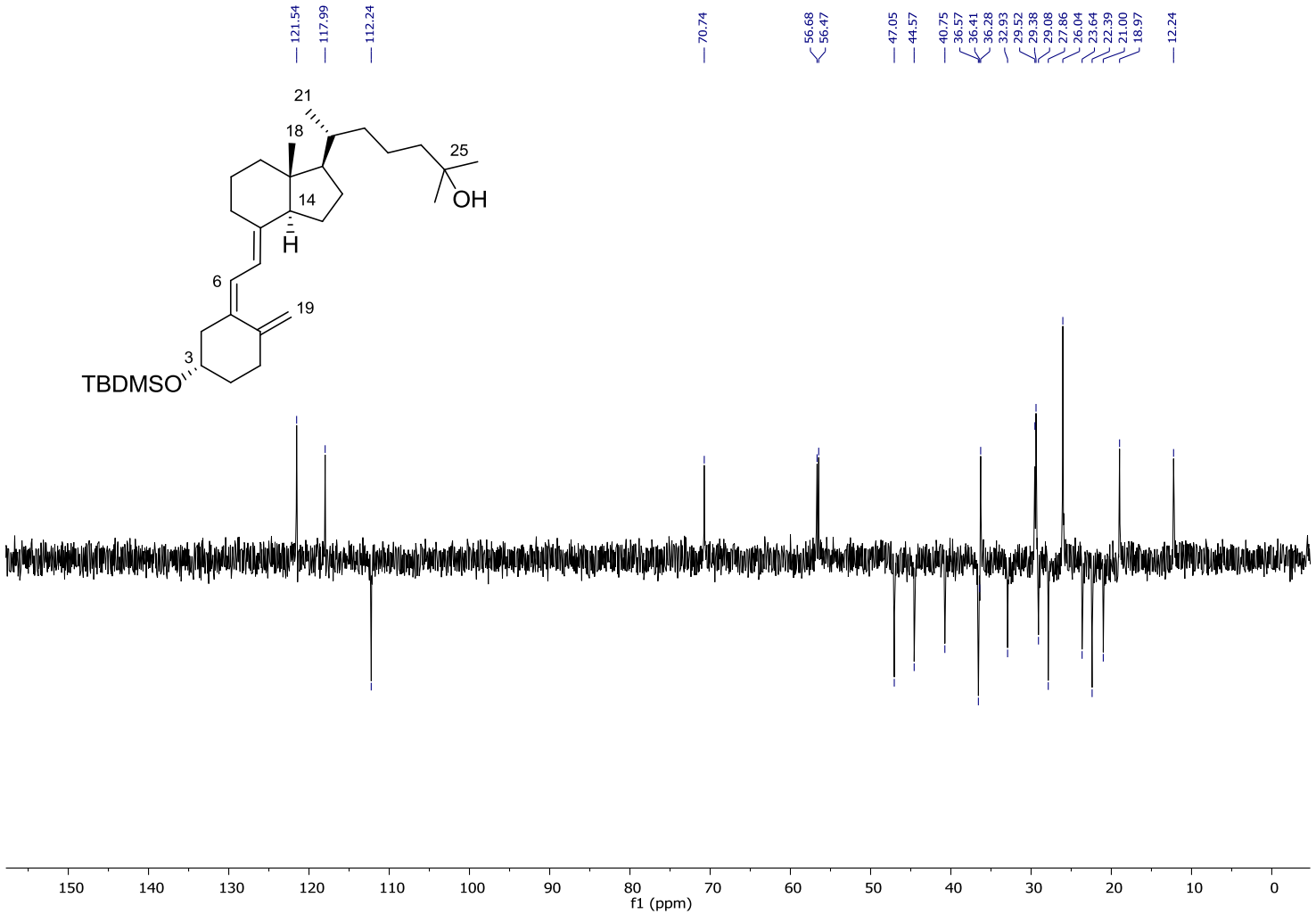
3-*O*-(*tert*-Butyldimethylsilyl)-25-hydroxyvitamin D_3 (25)

^{13}C-RMN (75.5 MHz, $CDCl_3$)



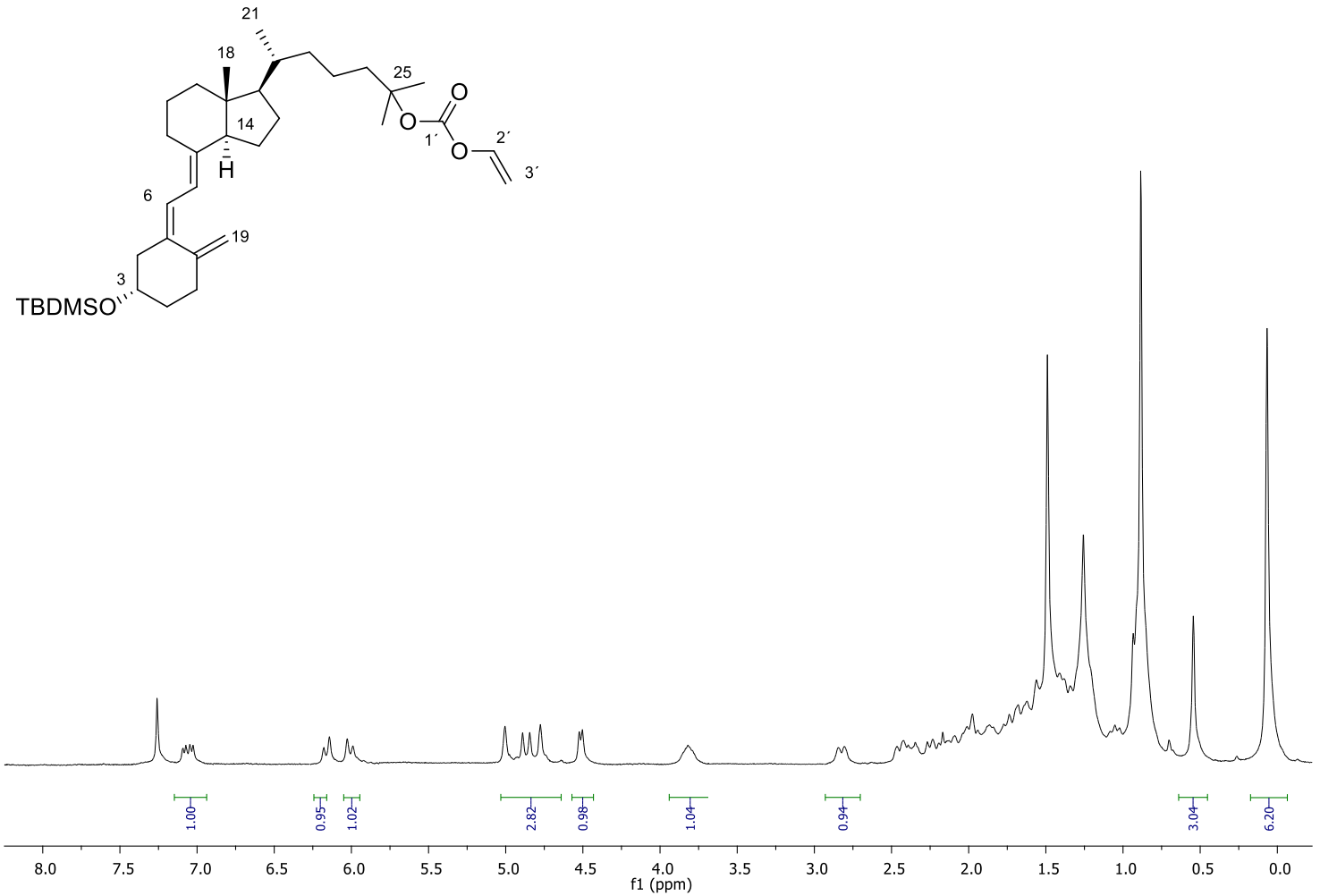
3-*O*-(*tert*-Butyldimethylsilyl)-25-hydroxyvitamin D_3 (25)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$, SW= 12820 Hz, O1= 6036 Hz)



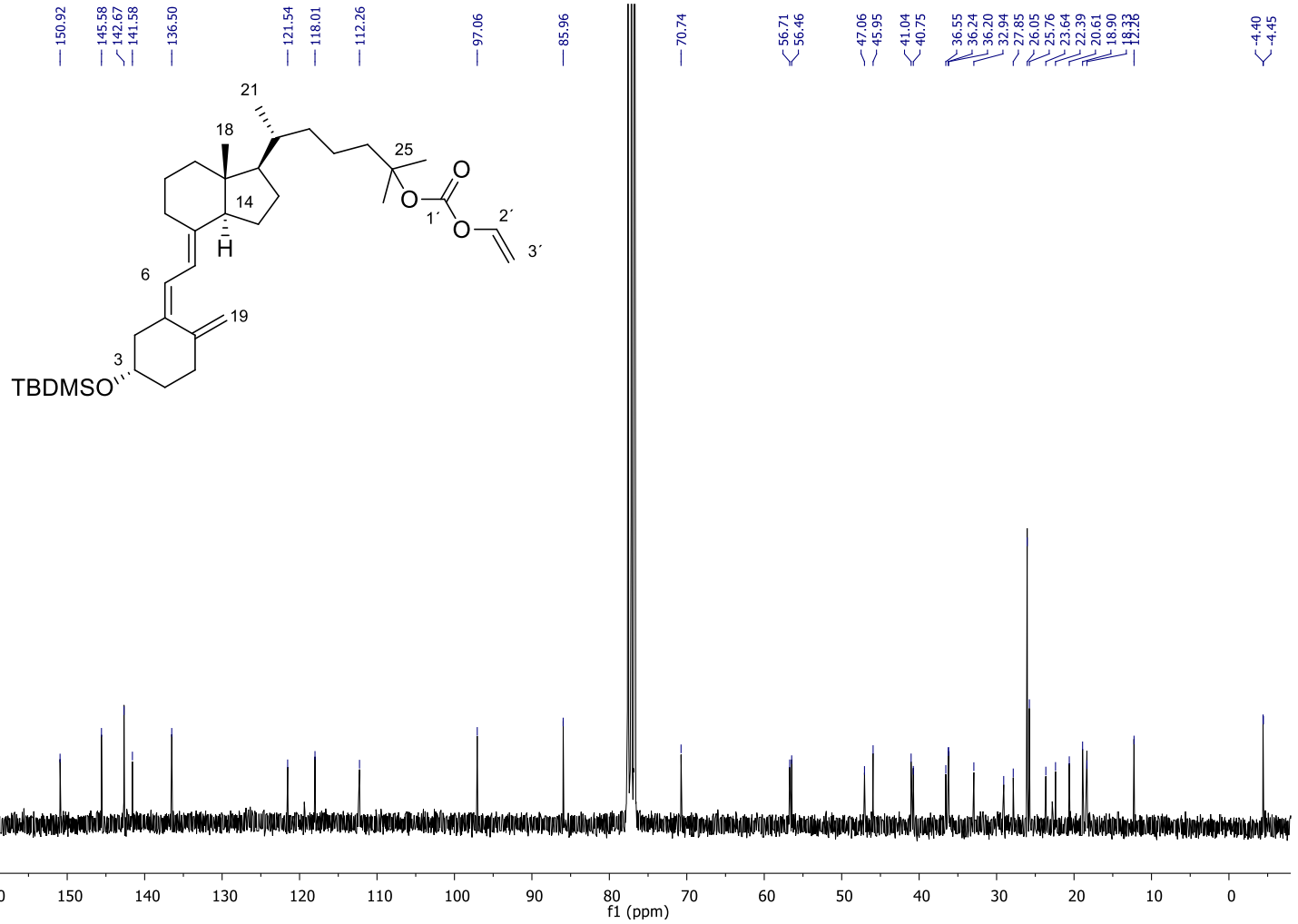
3-*O*-(*tert*-Butyldimethylsilyl)-25-(vinyloxycarbonyloxy)vitamin D_3 (26)

^{1}H-RMN (300.13 MHz, $CDCl_3$)



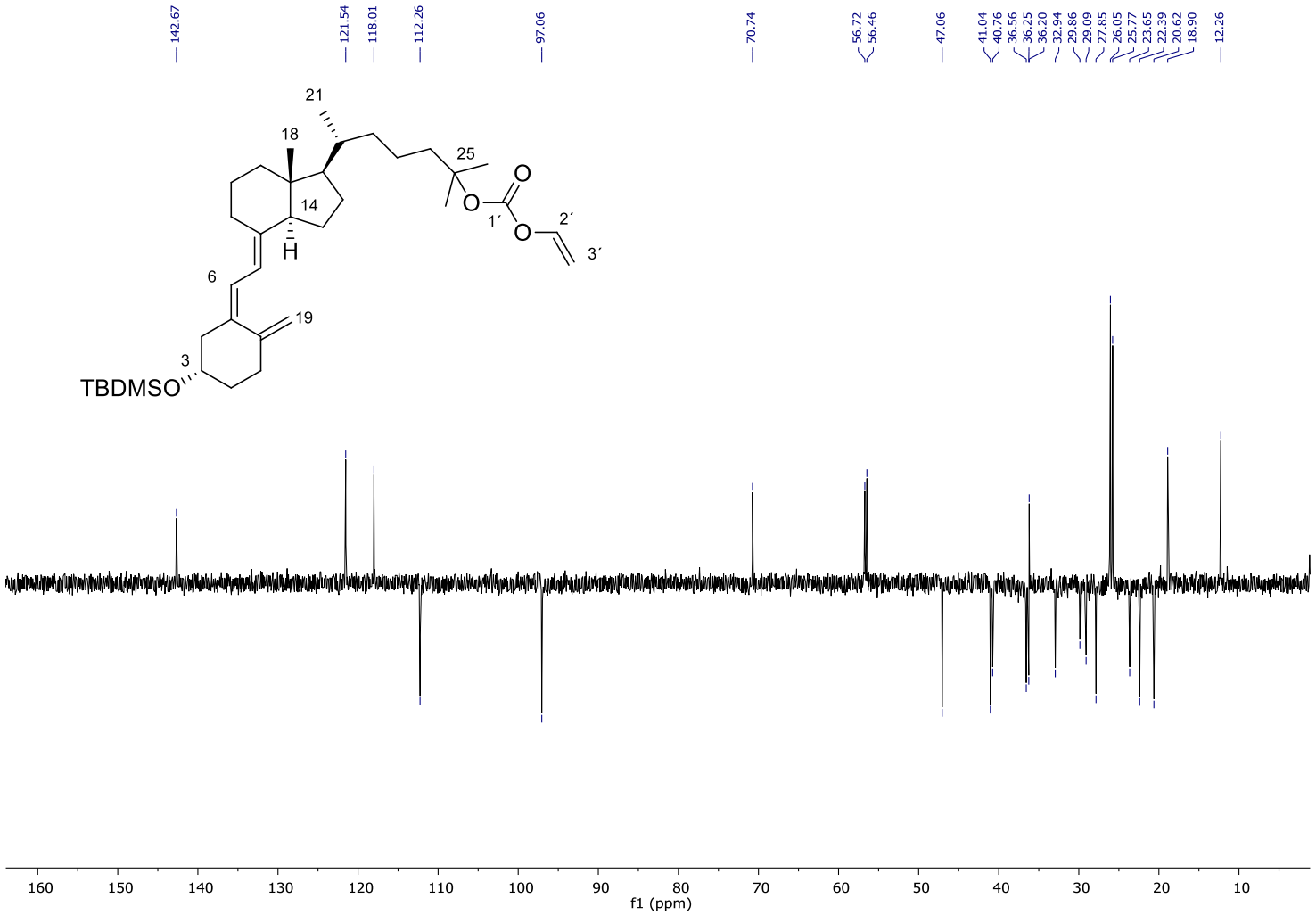
3-*O*-(*tert*-Butyldimethylsilyl)-25-(vinyloxycarbonyloxy)vitamin D_3 (26)

^{13}C-RMN (75.5 MHz, $CDCl_3$)



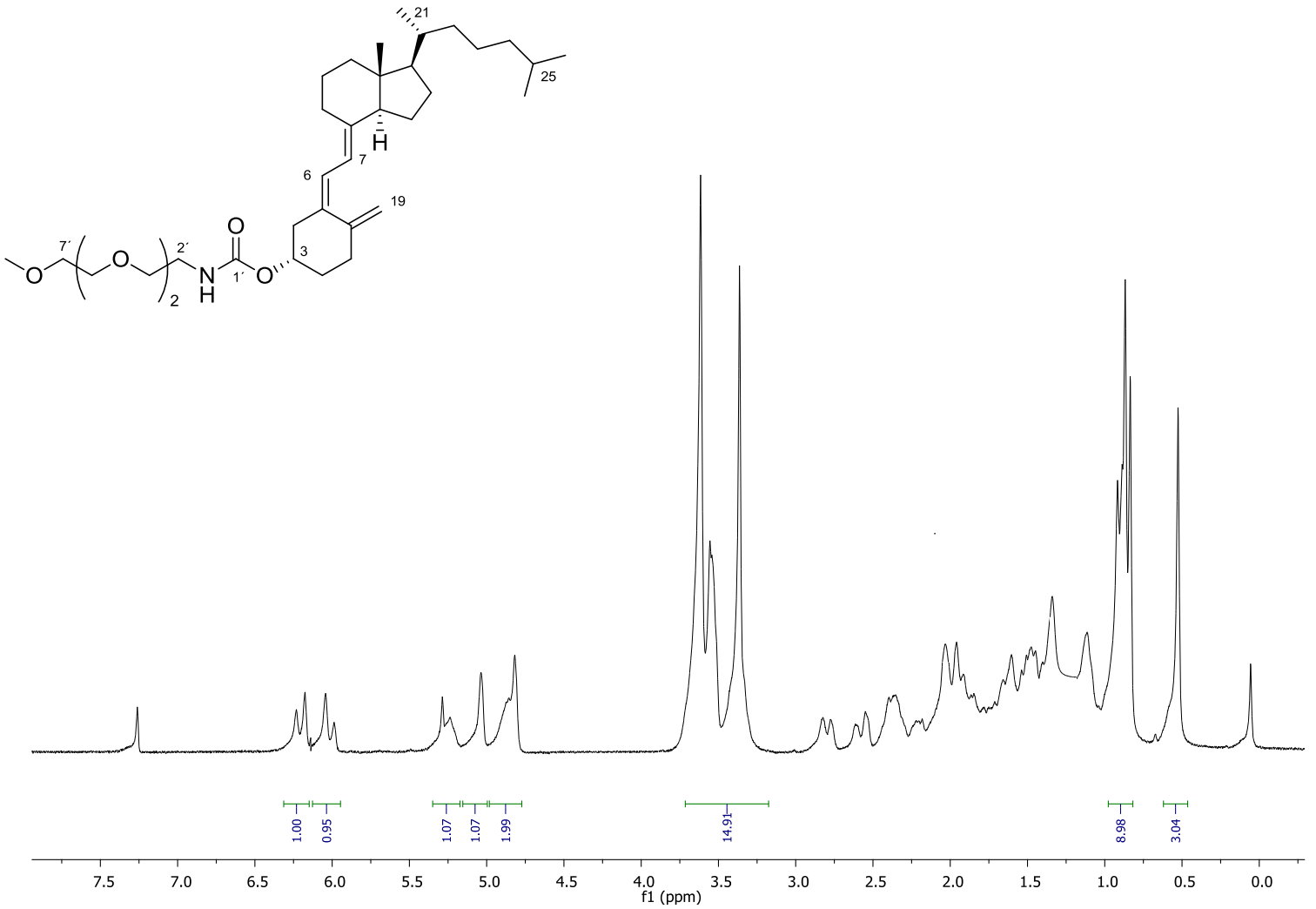
3-*O*-(*tert*-Butyldimethylsilyl)-25-(vinyloxycarbonyloxy)vitamin D_3 (26)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$, SW= 12820 Hz, O1= 6036 Hz)



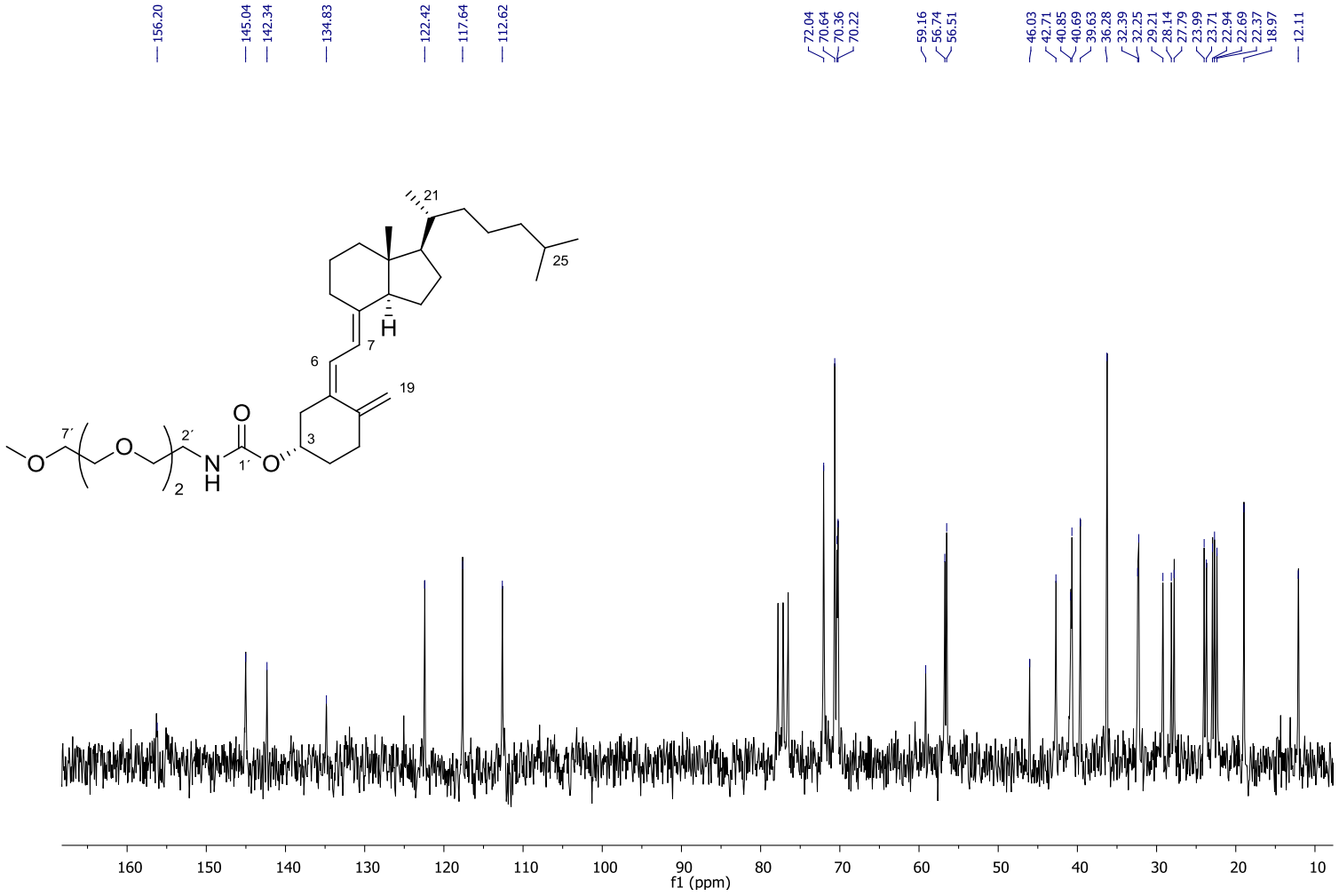
3-*O*-[*N*-(8-Methoxy-3,6-dioxaoctyl)carbamoyl]vitamin D_3 (28)

^{1}H-RMN (250.13 MHz, $CDCl_3$)



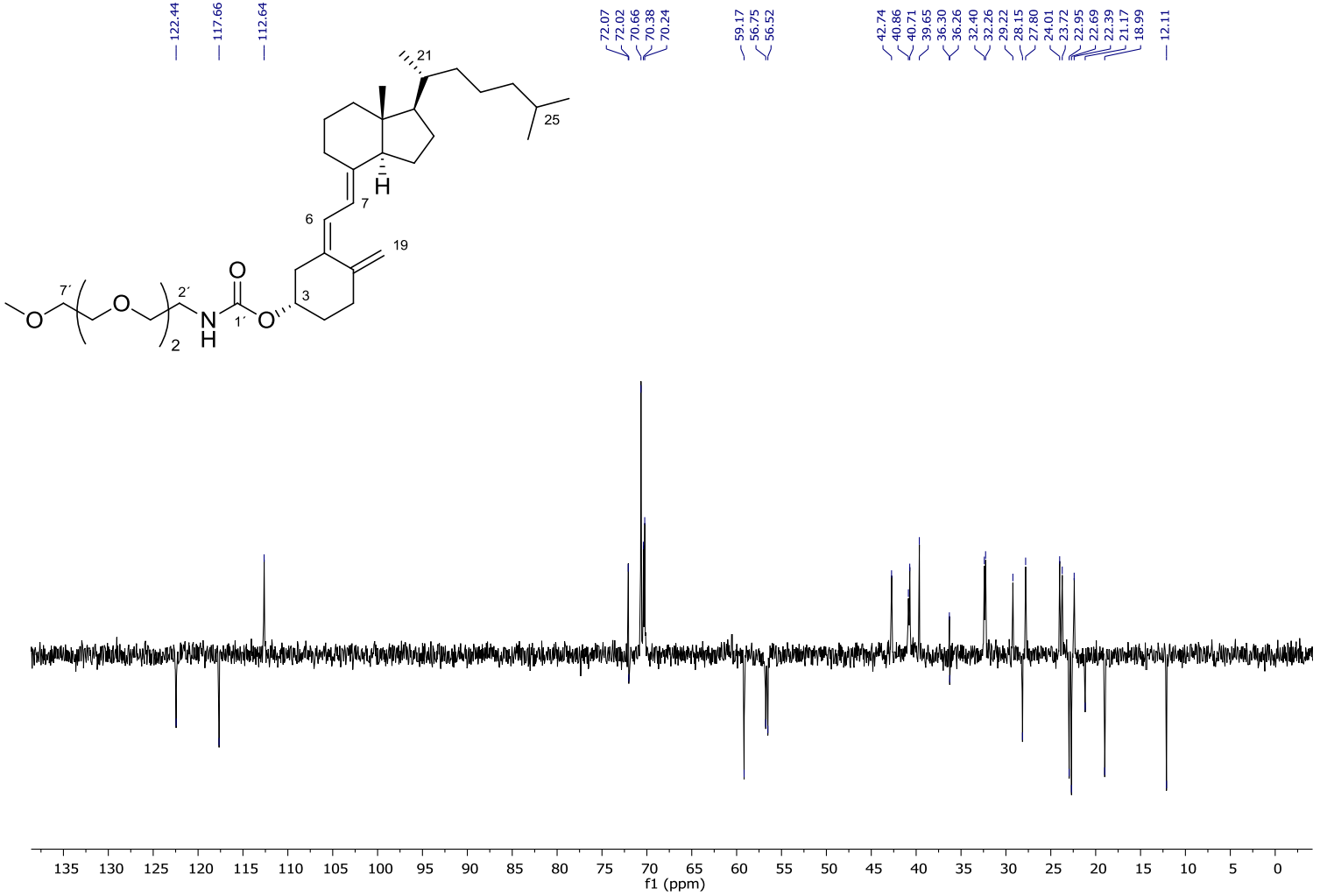
3-*O*-[*N*-(8-Methoxy-3,6-dioxaoctyl)carbamoyl]vitamin D_3 (28)

^{13}C-RMN (75.5 MHz, $CDCl_3$)



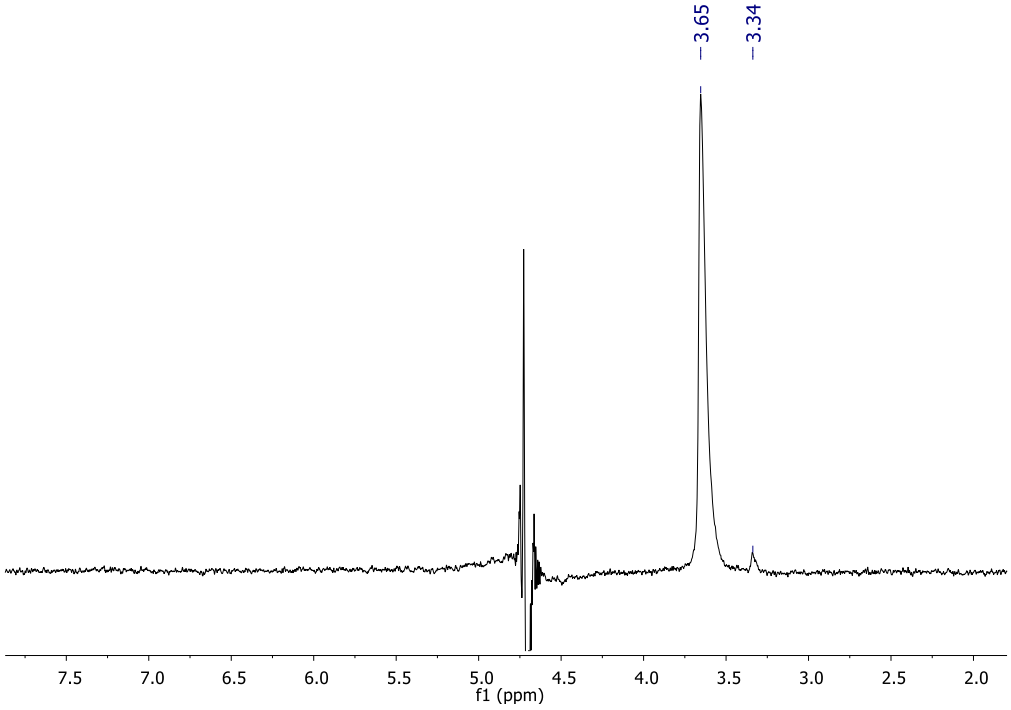
3-*O*-[*N*-(8-Methoxy-3,6-dioxaoctyl)carbamoyl]vitamin D_3 (28)

^{13}C-DEPT135-RMN (75.5 MHz, $CDCl_3$)



COPIES OF ^{1}H NMR SPECTRA OF NANOPARTICLES

a)



b)

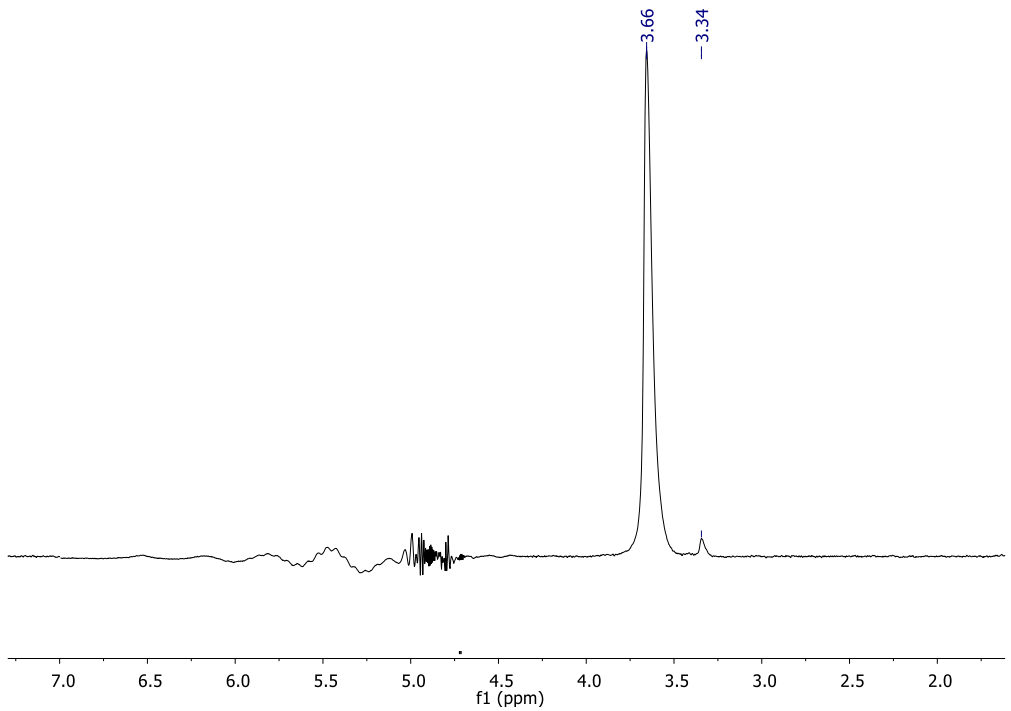


Figure S1. ^{1}**H** NMR experiments of the conjugated nanoparticles with two different pulse sequences: a) translational diffusion filtered spectrum; b) solvent suppression sequence spectrum. Both are identical and show only the presence of the signals relative to the PEG moiety: the methylene protons at 3.7 ppm and the terminal methyl at 3.3 ppm.

UV SPECTRA

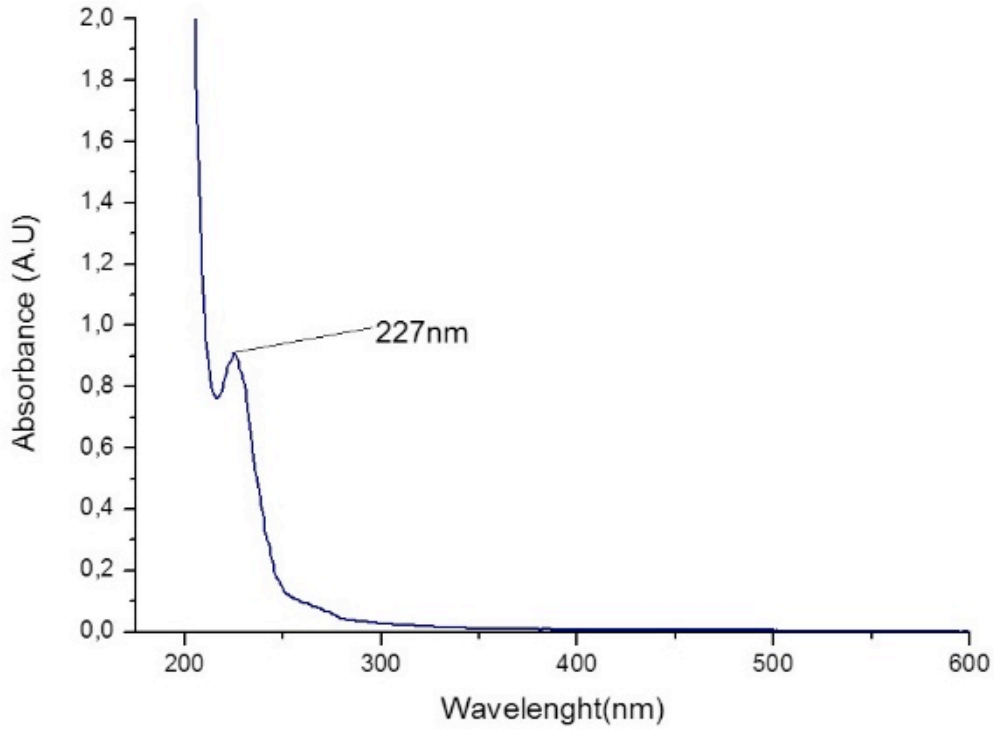
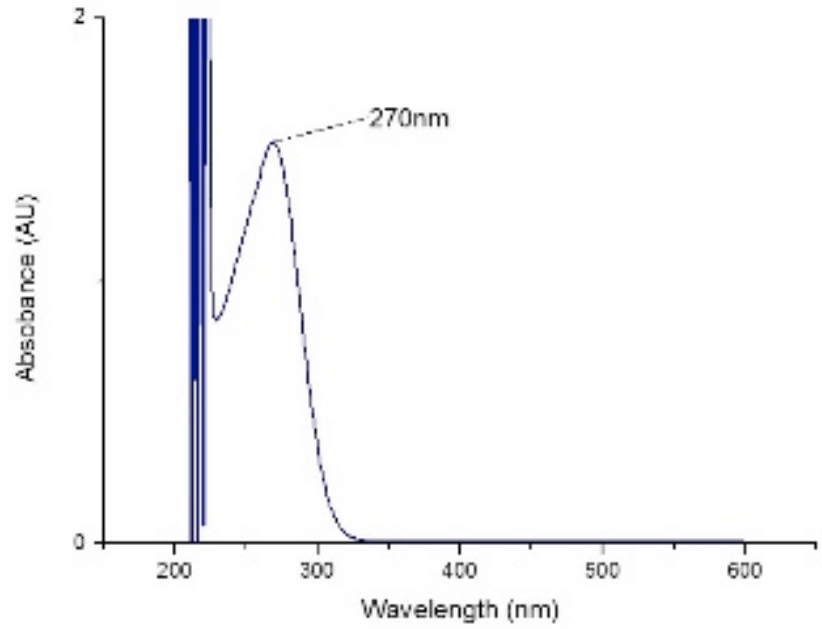


Figure S2. UV spectrum of the unconjugated nanoparticles.

a) Free analogue **8**



b) ORMOSIL-**8**

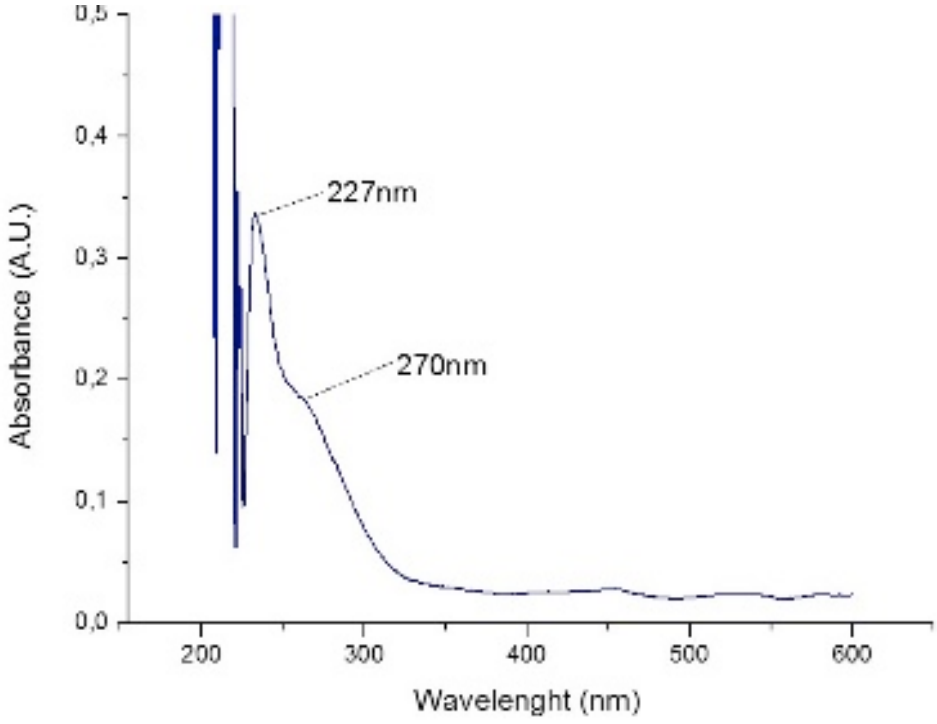
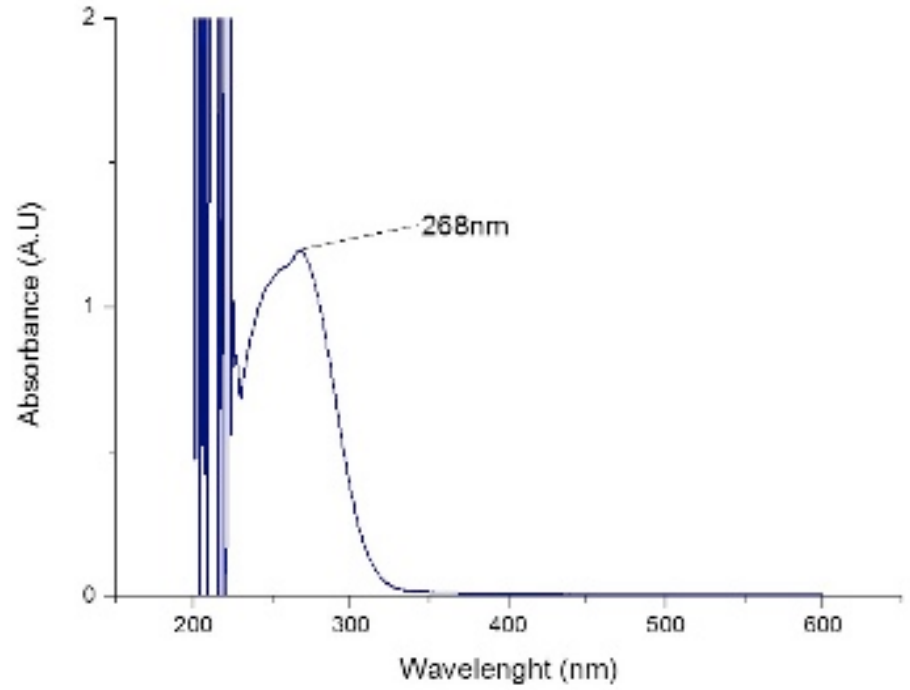


Figure S3. UV spectra of the free analogue **8** and conjugated nanoparticles ORMOSIL-**8**.

a) Free analogue **15**



b) ORMOSIL-**15**

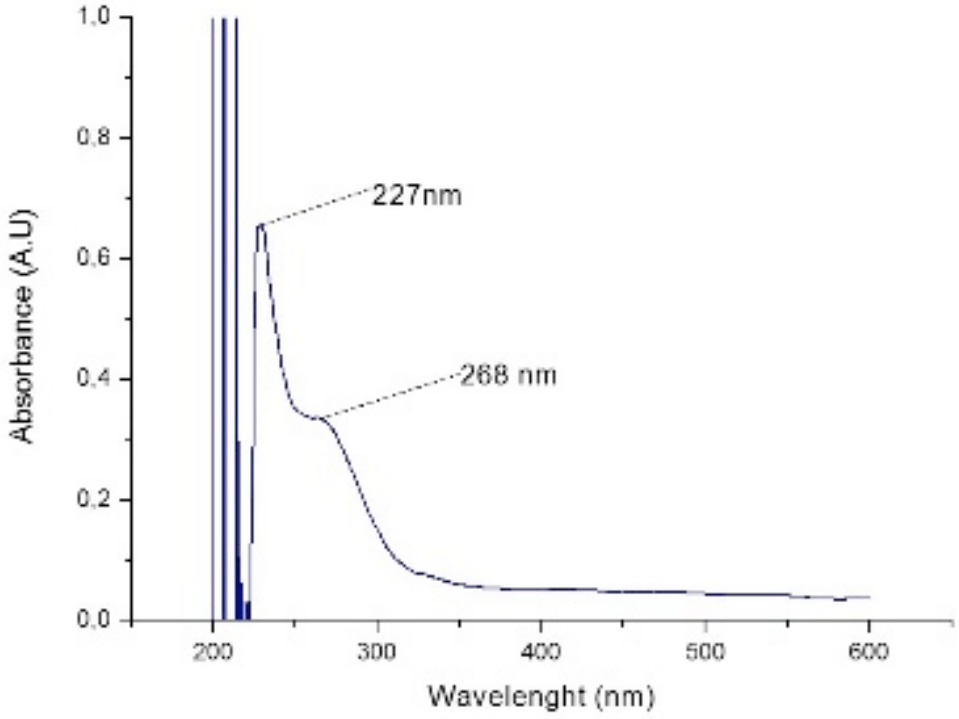
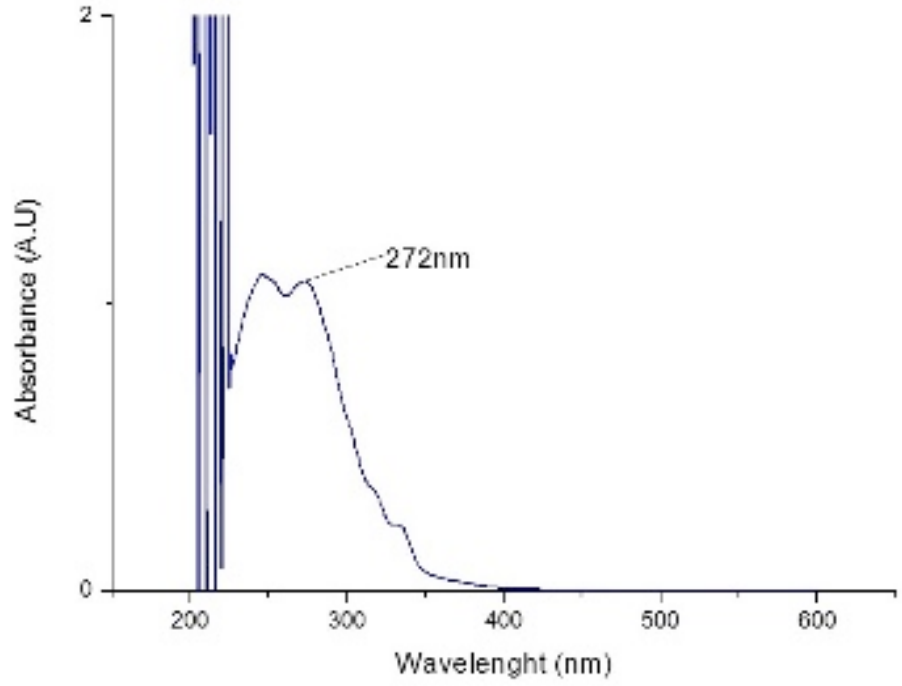


Figure S4. UV spectra of the free analogue **15** and conjugated nanoparticles ORMOSIL-**15**.

a) Free analogue **18**



b) ORMOSIL-**18**

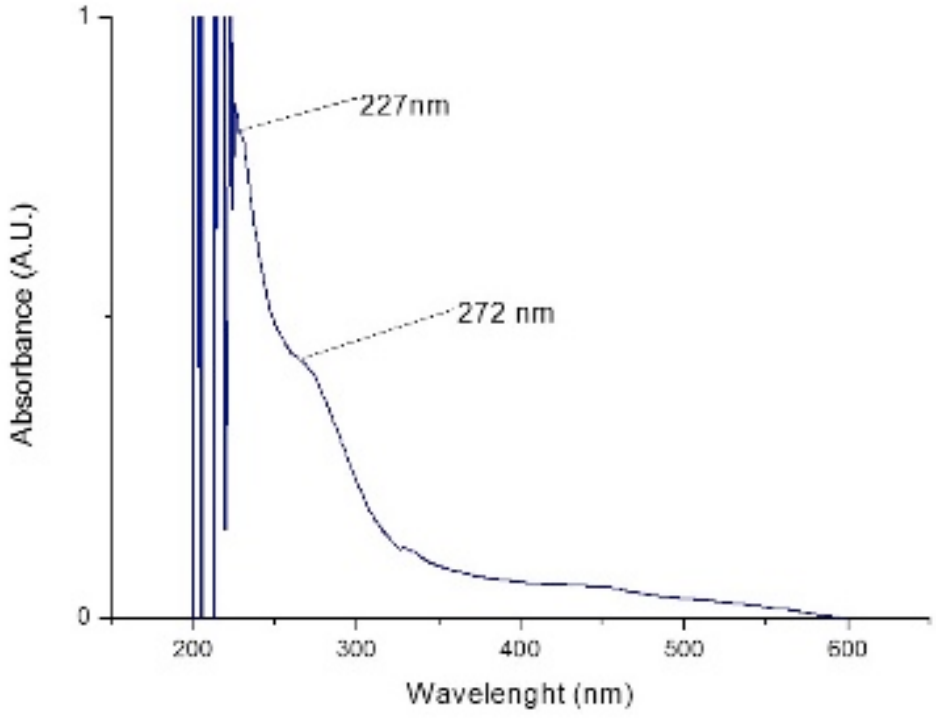


Figure S5. UV spectra of the free analogue **18** and conjugated nanoparticles ORMOSIL-**18**.

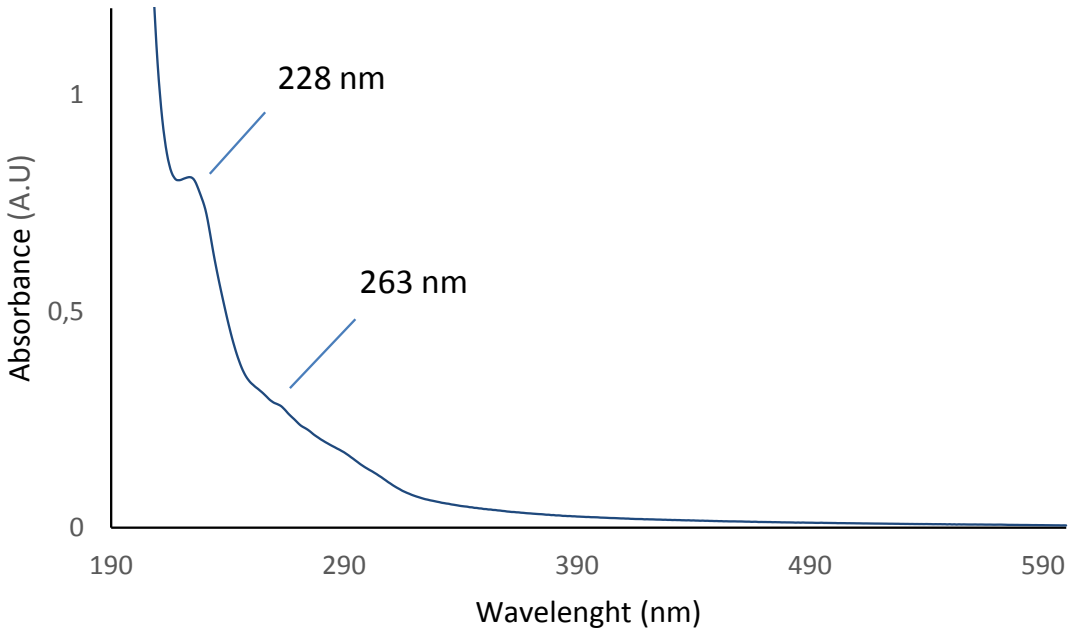


Figure S6. UV spectrum of conjugated nanoparticle ORMOSIL-**23.**

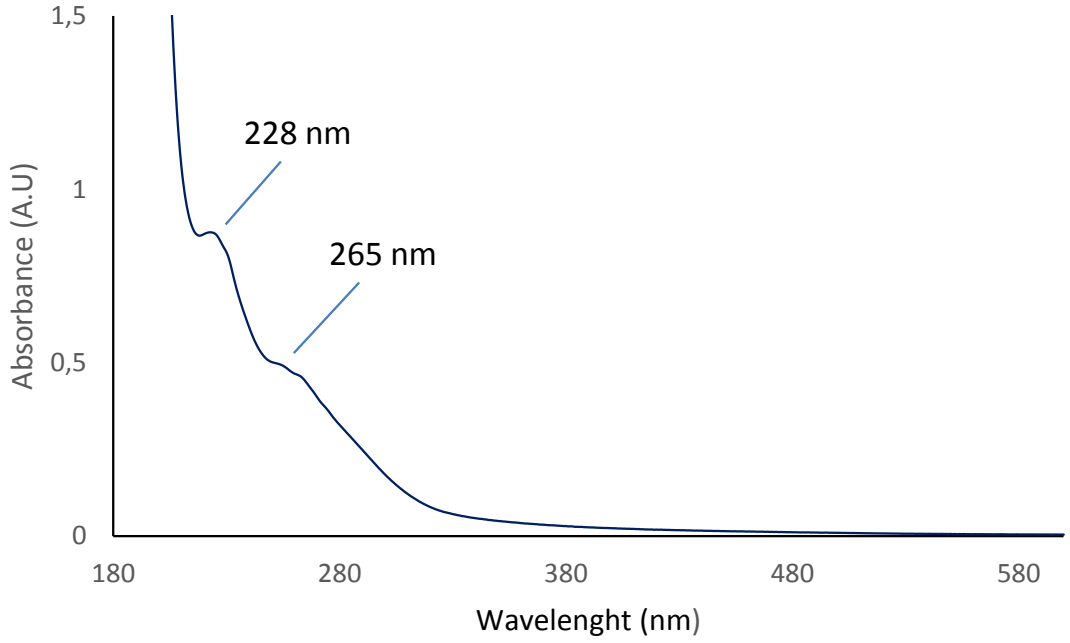


Figure S7. UV spectrum of conjugated nanoparticle ORMOSIL-**26.**

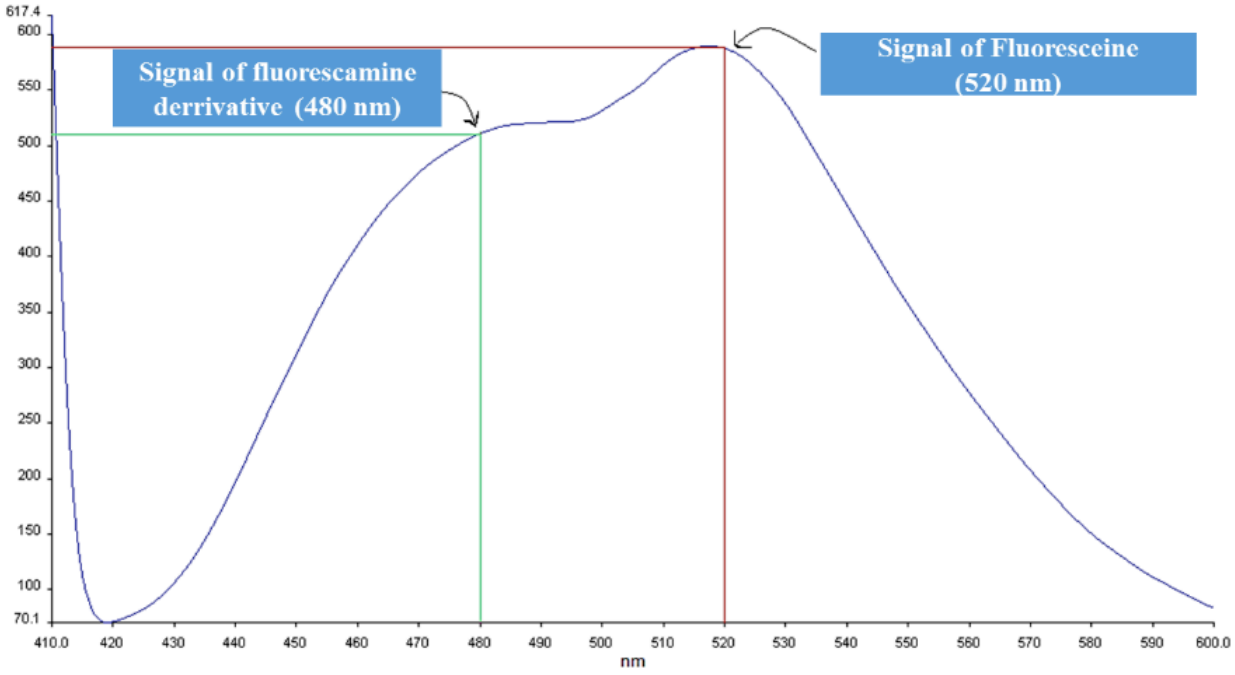


Figure S8. Fluorescence spectrum of nanoparticle with fluorescamine.

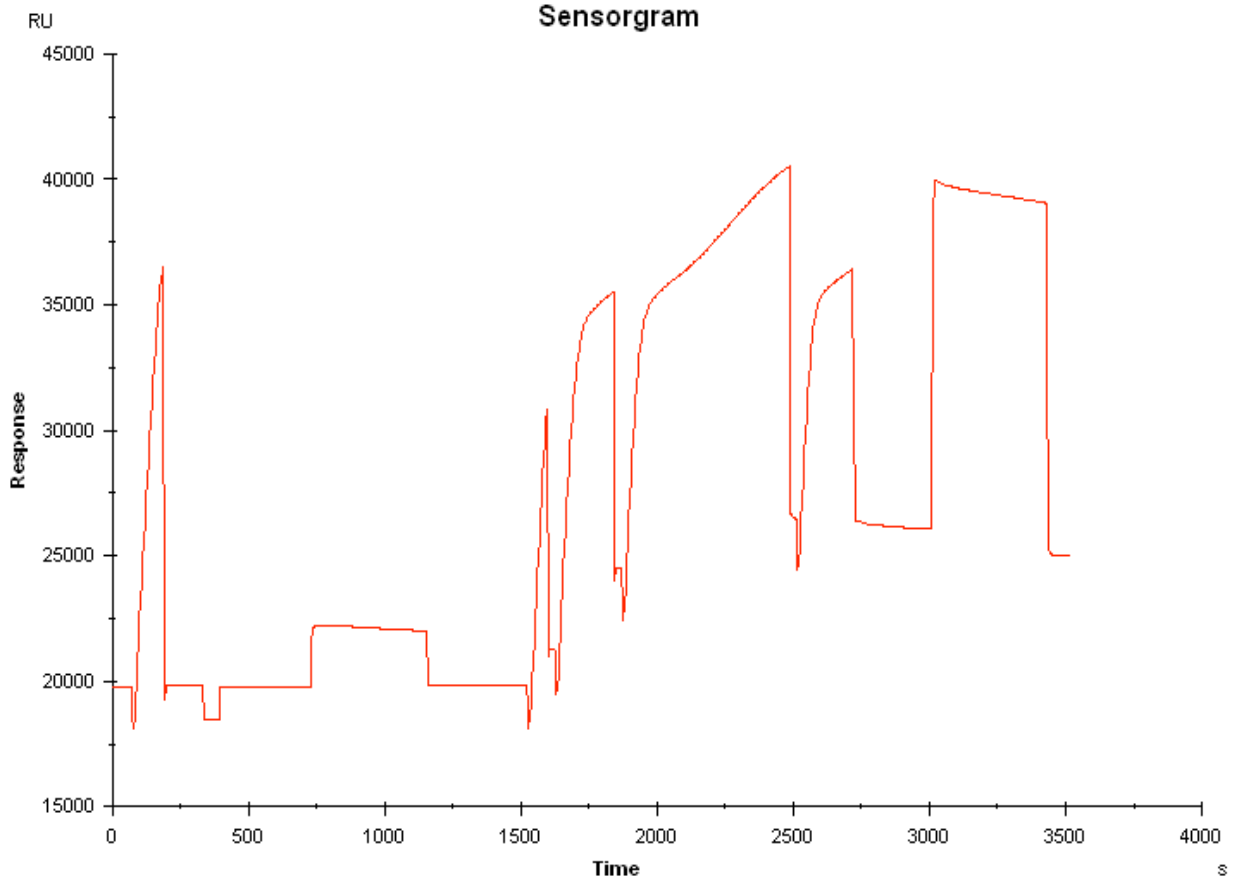
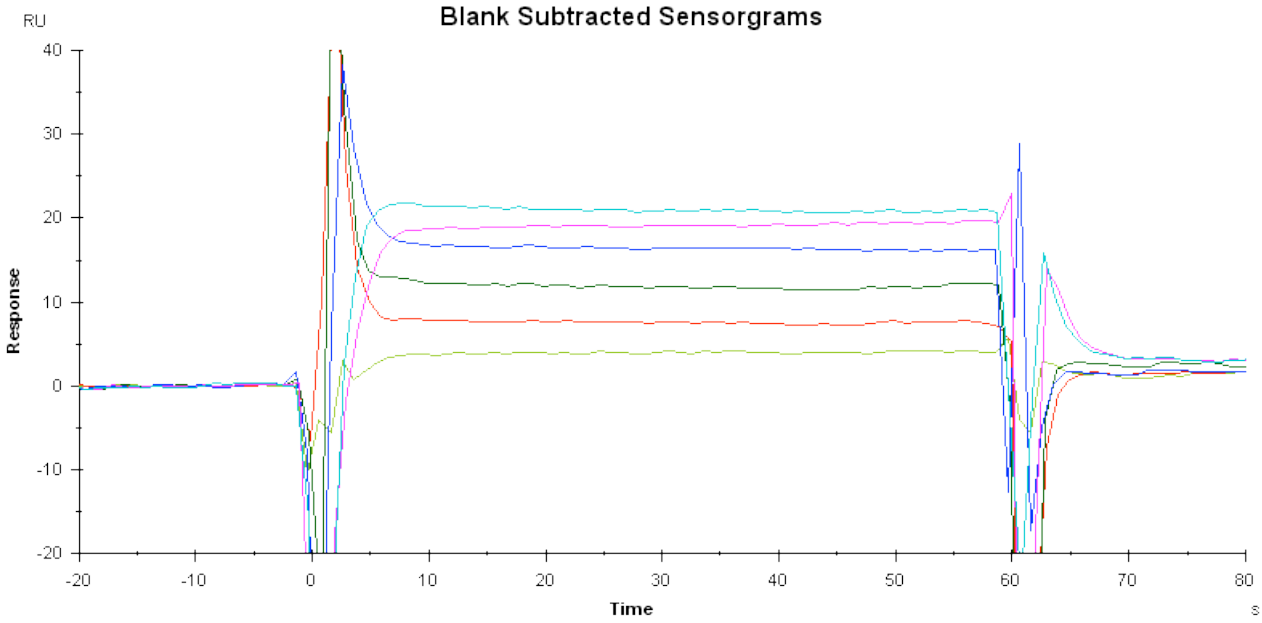


Figure S9. SPR sensorgram of the HSA-sensor chip surface binding.

a)



b)

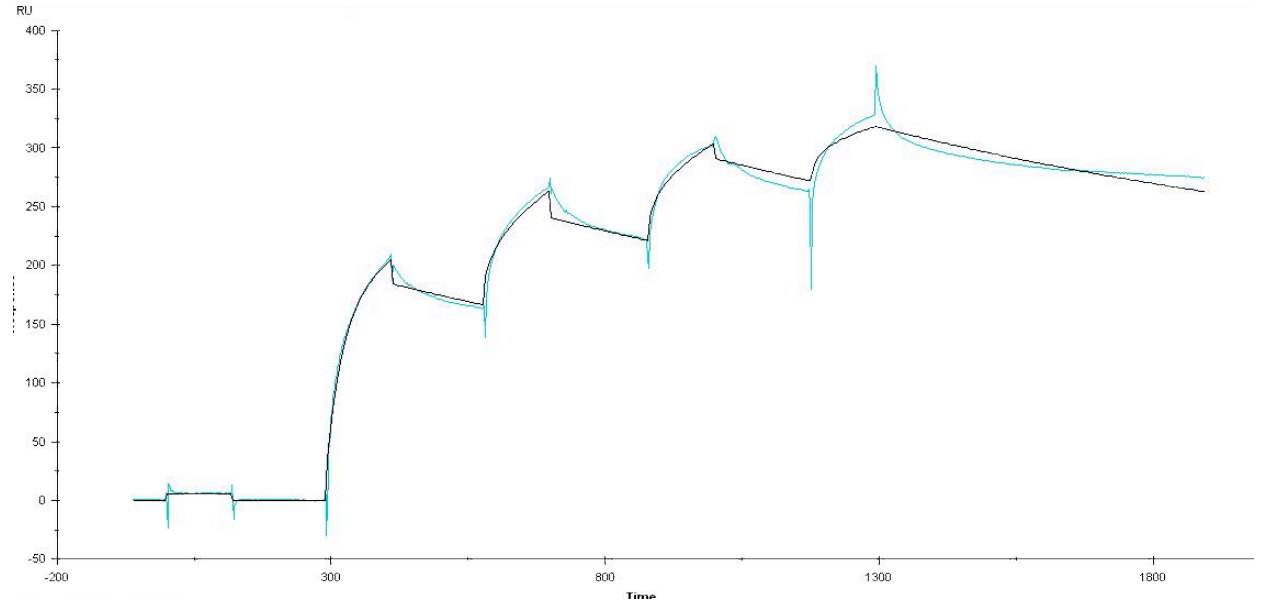
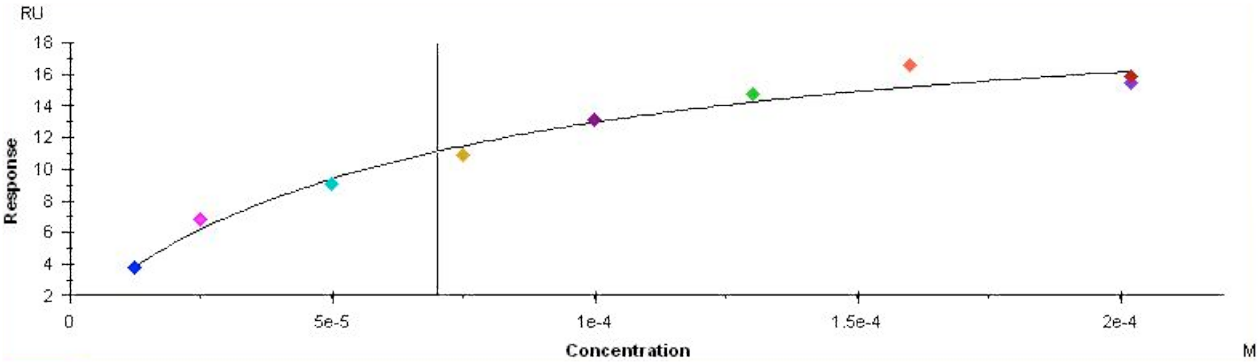


Figure S10. SPR sensorgram of: a) molecular ligands, as vitamin D_3 and **28** (multi cycle modality), they indicated a fast binding and release of the molecule from the chip, colors represent different concentrations; b) conjugated nanoparticles (single cycle modality), in this case, association and dissociation processes resulted slower.

a)



b)

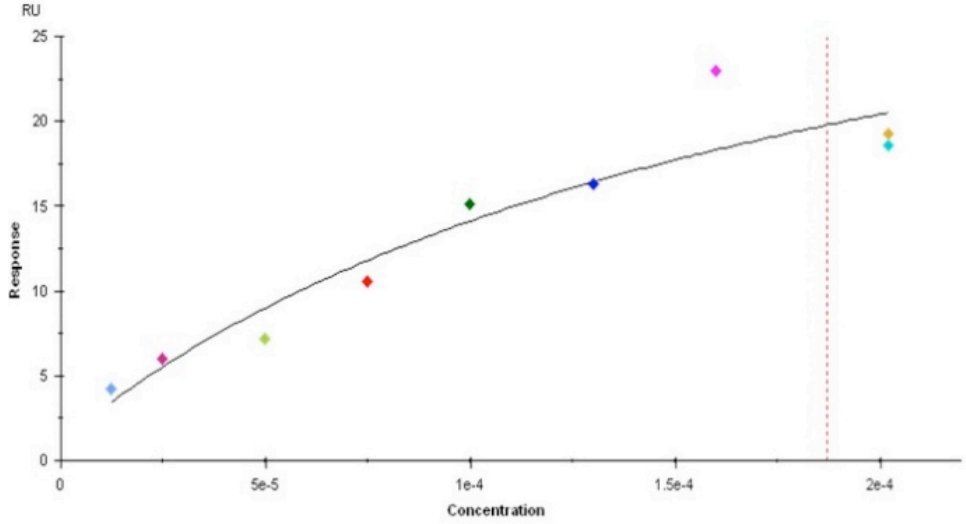


Figure S11. Affinity curves of: a) vitamin D_3-HSA; b) derivative **28**-HSA.