Carborane-Based Design of a Potent Vitamin D Receptor Agonist

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I. Docking calculations

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I. Docking Calculations

The receptor and the ligand were used as MOL2 files. Energy minimization was not performed on the protein. The compound **1** was built using the Builder module of the InsightII molecular modeling program.[1] The reported crystal of 1-CH_2OCH_3-2-COOH-1,2-*closo*-$C_2B_{10}H_{10}$ (Acta Crystallographic C code: LN1176[2]) was used for the carborane cage. The crystal structure of 1,25D, obtained from the complex of 1,25D-hVDR LBD (protein data bank code: 1DB1[3]), was used for the vitamin D part. The initial conformation of the ligand was energy-minimized using the Discover program and cvff force-field (5000-step, steepest descent, in vacuum at 300 K).[4] The boron "B" atoms were considered as carbon sp^3 atoms "C.3" only for the empirical potential energy function.[5] Finally, docking studies to predict the affinity of the new ligand for the VDR were carried out using the GOLD program (version Suite 5.2) using boron parameters. A modified crystal structure (addition of hydrogen, reconstituted gaps and corrected His tautomers) of the complex between 1,25D-hVDR LBD was chosen as protein (PBD code: 1DB1). The Ligand Binding Pocket of the mutant LBD was defined as Binding Site with the automatic active-site detection on, and the radius was set to 10 Å. Ligand was docked in 25 independent genetic algorithm (GA) runs, for each of which a maximum of 125000 GA operations were performed on a single population of 100 individuals. Operator weights for crossover, mutation, and migration in the entry box were used as default parameters (95, 95, and 10, respectively), as well as the hydrogen bonding (4.0 Å) and van der Waals (2.5 Å) parameters. The "flip ring corners" flag was switched off, while all the other flags were on. CHEMPLP was used as a scoring function and GoldScore as a re-scoring function. The best 3 solutions were obtained with an associated score. The values were 123.7 (corresponds with the structure shown in Supplementary Fig. 1), 123.3 and 124.1 with a rmsd of 0.73. These punctuations were compared with the 1,25D solutions. In all cases, the fitness scores for the new ligand were better than the 1,25D scores (108.0, 107.7 and 106.9).

II. Crystallization

Crystallization and Structure Determination. *c*DNA encoding *z*VDR LBD (156-453 AA) was cloned into pET28b vector to generate *N*-terminal His-tag fusion proteins. Purification was carried out as previously described, including metal affinity chromatography and gel filtration.[7] The protein was concentrated using Amicon ultra-30 (Millipore) to 3-7 mg/ml and incubated with a two-fold excess of ligand and a three-fold excess of the coactivator SRC-2 peptide (686-KHKILHRLLQDSS-698). Crystals were obtained in 50 mM Bis–Tris pH 6.5, 1.6 M lithium sulfate and 50 mM magnesium sulfate. Protein crystals were mounted in a fiber loop and flash-cooled under a nitrogen flux after cryo-protection with 20% glycerol. Data collection

from a single frozen crystal was performed at 100 K on the PX1 beamline at SOLEIL (France). The raw data were processed and scaled with the HKL2000 program suite.[8] The crystals belong to the space group $P6_522$, with one LBD complex per asymmetric unit. The structure was solved and refined using BUSTER[9] and iterative model building using COOT.[10] Crystallographic refinement statistics are presented in Supplementary Table 1. All structural figures were prepared using PyMOL (www.pymol.org/).

Supplementary Table S1: Data collection and refinement statistics.

	zVDR LBD - carborane 1
Data Processing	
X-ray source	Soleil PX1
Detector	Pilatus CBF
λ	1.07207 Å
Temperatue	100 K
Resolution (Å)	25.0-2.4 (2.49-2.40)
Crystal space group	$P6_522$
Cell parameters (Å)	a=b=66.56 ; c=262.077
Unique reflections	14509
Mean redundancy	8.2 (6.2)
Rsym (%)	8.8 (28.5)
Completeness (%)	97.3 (79.8)
Mean I/σ	24 (4.5)
Refinement	
Resolution (Å) (last Shell)	25-2.4 (2.59-2.4)
Number of non-hydrogen atoms	
Protein	1996
Ligand	38
Water molecules	94
Rcryst (%) (last Shell)	18.6 (20.3)
Rfree (%) (last Shell)	21.3 (24.8)
RMSD bond length (Å)	0.010
RMSD bond angles (°)	0.86
RMSD peptide omega torsion angles (°)	2.52
Ramachandran plot (%)	
Core	97.53
Allow	2.47

III. Biological Assays

MTT Metabolization. Cell proliferation experiments were carried out using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays, where MTT (Merck, Darmstadt, Germany) is reduced to purple formazan by the mitochondria of living cells. Increase in cell number is detected by increased MTT metabolization. MCF-7 cells were plated at a 1×10^5 cells per well in 24-well plates. Twenty-four hours later, the cells were treated with 1,25D, or carborane **1** at 10 or 100 nM during 48 h. Then, MTT (0.5 μg/μl) was added to each well, and the mixture was incubated for 1 h. The medium was removed, and DMSO (500 μl) was added to each well. Absorbance of samples was measured at 570 nm in a Mithras LB 940 from Berthold Technologies (Bad Wildbad, Germany).

Human VDR Binding Assay. Binding affinity to VDR was evaluated using PolarScreen Vitamin D receptor competitor assay kit (Invitrogen, Darmstadt, Germany). This kit is a fluorescence polarization (FP)-based competition assay. VDR is added to a fluorescent VDR ligand to form a receptor/tracer complex resulting in a high polarization value. This complex is then added to individual test compounds. Competitors will displace the tracer from the complex, causing the fluorescent ligand to tumble more rapidly during its fluorescence lifetime and resulting in a low polarization value. The polarized fluorescence was measured in a 384-well black plate during 200 ms/well using a Mithras LB 940 (Berthold Technologies). 1,25D or the carborane **1** were evaluated within the range from 10^{-11} to 10^{-6} M. IC_{50} values were calculated using average of measured values.

Transient Transfection and Luciferase Reporter Gene Assays. HEK293 EBNA cells plated into 24-well plates at 10^5 cells per well were cotransfected with 150 ng of the expression plasmid pSG5-hVDR wild-type and 150 ng of the reporter plasmid pLuc-MCS (Stratagene, La Jolla, USA) containing the proximal promoter region (-414 to -64) of the human *CYP24A1* gene, 3 ng of the pRL plasmid (Promega, Madison, USA) containing the Renilla luciferase gene (transfection and cell viability control), and 697 ng of the carrier plasmid pBluescript (Stratagene). Transfection was performed with jetPEI (PolyPlus Transfection, Illkrich, France) according to the manufacturer's instructions. Six hours after transfection, tested compounds were added. Cells were harvested after eighteen hours of incubation with ligands. The amounts of reporter gene product (firefly luciferase) and constitutively expressed Renilla luciferase produced in the cells were measured using Dual-Luciferase® Reporter Assay System (Promega) on a luminometer plate reader LB 96P (Berthold Technologies). Luminescence of firefly luciferase values were normalized to the Renilla luciferase activity. Luciferase activities are expressed as relative units of light intensity. Data points represent the mean of assays performed in triplicate for at least three independent experiments. For every triplicate, the mean and the standard deviation of the mean were calculated.

RNA Isolation and Real-Time PCR. MCF-7 cells were treated during 24 h with 1,25D and the carborane **1** at 10, 50, 100, and 200 nM as described above. Isolation of total RNA from MCF7 cells was performed using TRIzol reagent (Invitrogen, Barcelona, Spain). cDNA synthesis was carried out as previoulsly described.[10] CYP24A1 mRNA levels were quantified by real-time PCR (Eppendorf Master cycler ep realplex, Hamburg, Germany). The 25-μl amplification mixture contained 1 μl of RT reaction products plus each primer at 0.5 μM, and 12.5 μl of Luminaris Color HiGreenqPCR Master Mix (Thermo Scientific,

Waltham, USA). After initial denaturation at 94 °C for 30 sec, reactions were cycled 40 times as follows: denaturation at 95 °C for 2 sec, annealing at 58 °C for 10 sec, and extension at 72 °C for 15 sec. The amount of PCR products formed in each cycle was evaluated on the basis of SYBR Green fluorescence. At the end of each run, melting curve profiles were produced (cooling the sample to 68 °C and heating slowly to 95 °C, with continuous measurement of fluorescence) to confirm amplification of specific transcripts (data not shown). Cycle-to-cycle fluorescence emission readings were monitored and quantified. The CYP24A1 mRNA levels were normalized with respect to the 18S level in each sample.

Serum Calcium Quantitation. All animal studies were approved by the University of Santiago de Compostela Ethics Committee for Animal Experiments. Male Swiss cd-1 mice were obtained from Santiago de Compostela University animal facilities. Mice (5 per group) were injected intraperitoneally either with 1,25D or the carborane **1** (0.3 μg/kg weight, each) dissolved in sesame oil every other day for three weeks. Control group (n=5) was injected with sesame oil. Calcium in serum was determined using the QuantiChom Calcium Assay Kit (BioAssay Systems, Hayward, USA).

IV. Synthesis

General Materials and Methods

Reagents were obtained from Aldrich Chemical (www.sigma-aldrich.com) or Acros Organics (www.acros.com) and used without further purification. All dry solvents were distilled under Ar immediately prior to use. Tetrahydrofuran (THF), Et_2O and toluene were distilled from Na/benzophenone. CH_2Cl_2 was distilled from P_2O_5. Pyridine was distilled from CaH_2. *tert*-Butyl methyl ether (TMBE) was used as received. Solutions of *n*-butyllithium in hexanes, and *tert*-butyllithium in pentane were tritiated with *N*-benzylbenzamide prior to use.

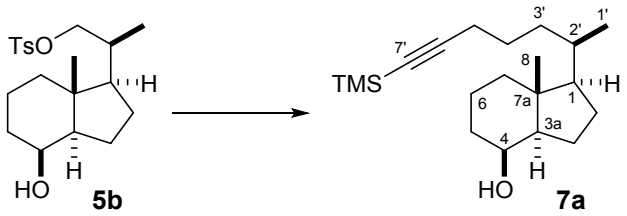
All reactions involving oxygen- or moisture-sensitive compounds were carried out under a dry Ar atmosphere using oven-dried or flame-dried glassware and standard syringe/septa techniques. Reaction temperatures refer to external bath temperatures. Liquid reagents or solutions of reagents were added by syringe or cannula. Organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated using a rotary evaporator at aspirator pressure (20-30 mm Hg).

Reactions were monitored by thin-layer chromatography (TLC) using aluminium-backed MERCK 60 silica gel plates (0.2 mm thickness). The chromatograms were visualized first with ultraviolet light (254 nm) and then by immersion in solutions of ceric ammonium molybdate or *p*-anisaldehyde followed by heating with a hot gun. Flash column chromatography was performed with Merck silica gel 60 (230-400 mesh).

All NMR spectra were measured with solutions in $CDCl_3$ in a Bruker DPX-250 (250 MHz), a Varian Inova 400 (400 MHz), Varian Inova 500 (500 MHz) and Bruker DRX-500 (500 MHz) spectrometers. Chemical shifts are reported on the δ scale (ppm) downfield from tetramethylsilane (δ = 0.0 ppm) using the residual solvent signal at δ = 7.26 ppm (^{1}H) and δ = 77.0 ppm (^{13}C) as internal standards and δ = 0.00 ppm (^{11}B) as external reference ($BF_3.OEt_2$ in $CDCl_3$). Coupling constants (*J*) are reported in Hz. Distortionless Enhancement by Polarization Transfer (DEPT) was used to assign carbon types. NMR signals have been assigned using steroidal numbering. High resolution mass spectra (HRMS) were performed in a Micromass Instruments Autospec, a Thermo Finningan MAT95XP and an Applied Biosystems QSTAR Elite spectrometers. IR spectra were recorded on a silicon disc on a Bruker IFS-66V and VECTOR 22 FT-IR spectrometers. UV spectra were registered in a Hewlett Packard 8452A spectrometer. Optical rotations were measured on a Jasco DIP-370 polarimeter in a 1 dm cell. [α] and *c* are given in deg $cm^3g^{-1}dm^{-1}$ and g cm^{-3} respectively. HPLC purifications were performed on a Shimadzu preparative liquid chromatograph model LC-8A equipped with a TSP UV-1 absorbance detector, using HPLC Phenomenex Luna column (SiO_2, Ø 250 mm x 10 mm). Yields refer to chromatographically purified compounds, unless otherwise stated.

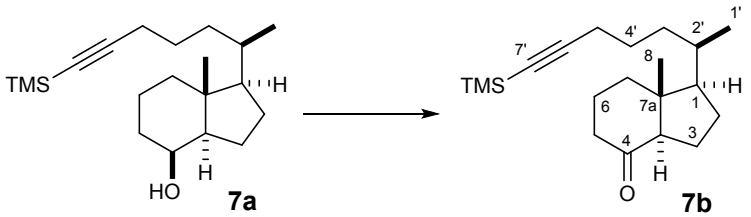
Experimental procedures

1. (1*R*,3a*R*,4*S*,7a*R*)-7a-Methyl-1-[(*R*)-7'-(trimethylsilyl)hept-6'-yn-2'-yl]octahydro-1H-inden-4-ol (7a).



A solution of [4-(trimethylsilyl)but-3-ynyl]magnesium bromide[11] (14.76 mmol, 4 equiv) in Et_2O/THF (20 mL, 1:1) was added dropwise via cannula to a solution of tosylate **5b**[12] (1.35 g, 3.69 mmol) in Et_2O/THF (20 mL, 1:1) at -78 ºC. A solution of Li_2CuCl_4 (0.36 mL, 0.1 M, 0.036 mmol, 0.1 equiv) in THF was added. The cooling bath was removed and the reaction mixture was allowed to reach room temperature overnight. The mixture was filtered through a pad of Celite and poured into aqueous HCl (100 mL, 10%). The aqueous phase was extracted with Et_2O (3x50 mL). The combined organic phases were dried, filtered and concentrated. The residue was purified by flash chromatography (SiO_2, Ø 3.5x10 cm, Hexanes:EtOAc, 92:8) to afford **7a** [1.13 g, 3.54 mmol, 96%, colorless oil, R_f = 0.65 (Hexanes:EtOAc, 8:2), $[\alpha]_D^{25}$ = 36.8 (*c* = 1.5, $CHCl_3$)]. ^{1}H-NMR (250 MHz, $CDCl_3$): δ 4.07 (br s, 1H, H-4), 2.17 (td, J_1 = 6.5, J_2 = 3.2, 2H, CH_2-5'), 1.99 (br d, *J* = 14, 1H, H-5), 0.92 (s, 3H, CH_3-8), 0.90 (d, *J* = 6.7, 3H, CH_3-1'), 0.14 (s, 9H, Me_3Si); ^{13}C-NMR (63 MHz, $CDCl_3$): δ 107.5 (C, C-6'), 84.0 (C, C-7'), 68.9 (CH, C-4, 56.3 (CH, C-1), 52.5 (CH, C-3a), 41.6 (C, C-7a), 40.3 (CH_2, C-7), 34.6 (CH_2, C-5), 34.5 (CH, C-2'), 33.5 (CH_2, C-3), 27.0 (CH_2, C-2), 24.9 (CH_2, C-5'), 22.5 (CH_2, C-4'), 19.9 (CH_2, C-3'), 18.4 (CH_3, C-1'), 17.3 (CH_2, C-6), 13.3 (CH_3, C-8), 0.0 (3xCH_3, Me_3Si); IR (film, cm^{-1}): 3413 (ν_{O-H}), 2944 (ν_{C-H}), 2867 (ν_{C-H}), 2173 ($\nu_{C\equiv C}$); EI-MS (m/z, %): 320 ([M]$^+$, 0.5), 305 ([M-Me]$^+$, 8), 302 ([M-H_2O]$^+$, 0.5), 111 (100); EI-HRMS [M-Me]$^+$ Calcd. for $C_{19}H_{33}OSi$: 305.2295, Found 305.2295.

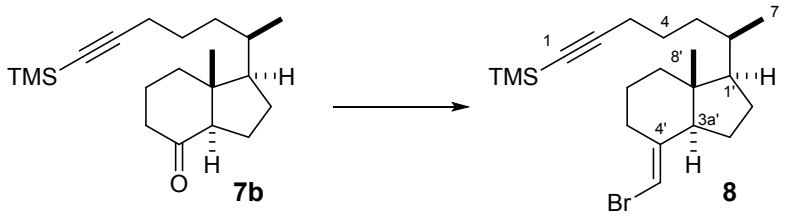
2. (1*R*,3a*R*)-7a-Methyl-1-((*R*)-7'-(trimethylsilyl)hept-6'-yn-2'-yl)hexahydro-1H-inden-4(2H)-one (7b).



Pyridinium dichromate (3.22 g, 8.56 mmol, 3 equiv) was added to a stirred solution of alcohol **7a** (0.91 g, 2.85 mmol) in dry CH_2Cl_2 (45 mL). The mixture was stirred in the dark at room temperature for 5 h. The resulting black mixture was diluted with TBME (50 mL), filtered through a pad of Celite/silicagel and the solids were washed with TBME (3x20 mL). The solution was concentrated and the brown oil was purified by flash chromatography (SiO_2, Ø 2.5x5 cm, Hexanes:EtOAc, 98:2) to afford **7b** [0.89 g, 2.79 mmol, 98%, colorless oil, R_f = 0.75 (Hexanes:EtOAc, 8:2), $[\alpha]_D^{25}$ = 23.3 (*c* = 1.1, $CHCl_3$)]. ^{1}H-NMR (250 MHz, $CDCl_3$): δ 2.37 (dd, J_1 = 10.6, J_2 = 7.5, 1H, H-5), 0.88 (d, *J* = 5.2, 3H, CH_3-1'), 0.56 (s, 3H, CH_3-8), 0.06 (s, 9H,

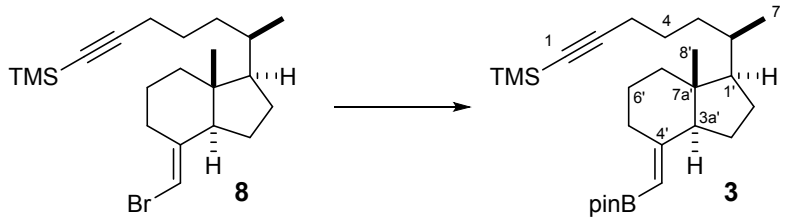
Me_3Si); ^{13}C-NMR (63 MHz, $CDCl_3$): δ 211.7 (C, C-4), 107.3 (C, C-7'), 84.3 (C, C-6'), 61.8 (CH, C-3a), 56.2 (CH, C-1), 49.7 (C, C-7a), 40.8 (CH_2, C-7), 38.8 (CH_2, C-5), 34.7 (CH, C-2'), 34.5 (CH_2, C-3), 27.2 (CH_2, C-2), 24.8 (CH_2, C-5'), 23.9 (CH_2, C-4'), 19.9 (CH_2, C-3'), 18.9 (CH_2, C-6), 18.5 (CH_3, C-1'), 12.3 (CH_3, C-8), 0.0 (3xCH_3, Me_3Si); IR (film, cm^{-1}): 2945 (ν_{C-H}), 2890 (ν_{C-H}), 2178 ($\nu_{C\equiv C}$), 1713 ($\nu_{C=O}$); EI-MS (m/z, %): 318 ([M]$^+$, 2), 303 ([M-Me]$^+$, 16), 73 (100); EI-HRMS [M-Me]$^+$ Calcd. for $C_{19}H_{31}OSi$: 303.2139. Found 303.2141.

3. [(6*R*)-6-((1'*R*,3a'*R*,7a'*R*,*E*)-4'-(Bromomethylene)-7a'-methyloctahydro-1H-inden-1-yl) hept-1-ynyl] trimethylsilane (8).



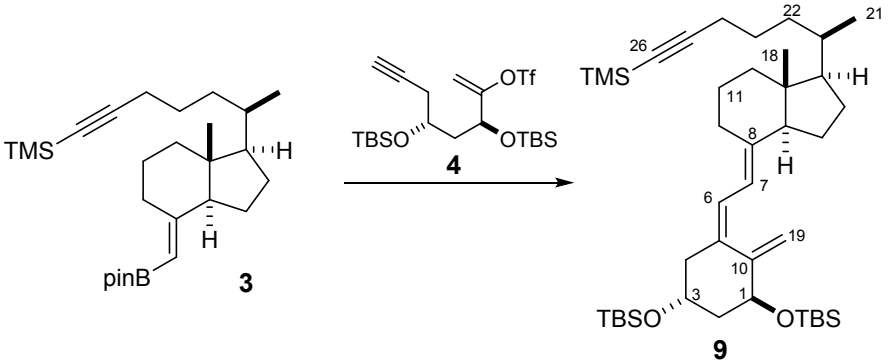
A suspension of $(Ph_3PCH_2Br)Br$ (3.93 g, 9.02 mmol, 8 equiv) in dry toluene (30 mL) was sonicated for 30 min (twice for 15 min). The suspension was cooled to -15 ºC and a solution of KO*t*-Bu in THF (5.25 mL, 1.7 M, 7.9 equiv) was added dropwise. The mixture was stirred for 2 h at -15 ºC, then warmed to 0 ºC (30 min), cooled to -15 ºC and stirred for 15 min. A 0 ºC cooled solution of ketone **7b** (0.36 g, 1.13 mmol) in dry THF (4 mL) was transferred via cannula to the -15 ºC cooled suspension of the ylide. The mixture was stirred at -15 ºC for 1.5 h, and at 0 ºC for 0.5 h. The cooling bath was removed and the reaction mixture was stirred for 1 h at room temperature. The reaction was quenched by the addition of a saturated aqueous NH_4Cl (1 mL). The mixture was filtered through a layer of silica gel. The solids were washed with Et_2O (3x20 mL) and the resulting filtrate was concentrated. The residue was purified by flash chromatography (SiO_2, Ø 3x7 cm, hexanes) to afford **8** [0.35 g, 0.90 mmol, 79%, yellow oil, R_f = 0.80 (Hexanes:EtOAc, 99:1), $[\alpha]_D^{25}$ = 58.2 (*c* = 1.6, $CHCl_3$)]. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.64 (s, 1H, HCBr), 2.86 (m, 1H, H-5'), 2.20 (d, *J* = 6.2, 1H, H-3), 2.17 (d, *J* = 6.6, 1H, H-3), 0.93 (d, *J* = 6.2, 3H, CH_3-7), 0.56 (s, 3H, CH_3-8'), 0.14 (s, 9H, Me_3Si); ^{13}C-NMR (63 MHz, $CDCl_3$): δ 144.9 (C, C-4'), 107.5 (C, C-1), 97.4 (CH, CHBr), 84.3 (C, C-2), 55.9 (CH, C-3a'), 55.5 (CH, C-1'), 45.4 (C, C-7a'), 39.8 (CH_2, C-7'), 35.4 (CH, C-6), 34.8 (CH_2, C-5'), 31.0 (CH_2, C-3'), 27.4 (CH_2, C-2'), 25.0 (CH_2, C-3), 22.5 (CH_2, C-4), 22.0 (CH_2, C-5), 20.1 (CH_2, C-6'), 18.7 (CH_3, C-7), 11.8 (CH_3, C-8'), 0.14 (3xCH_3, Me_3Si); IR (film, cm^{-1}): 2950 (ν_{C-H}), 2870 (ν_{C-H}), 2174 ($\nu_{C\equiv C}$); EI-MS (m/z, %): 393 ([M-Br]$^+$, 10), 315 (80), 227 (80), 73 (100); EI-HRMS [M-Br]$^+$ Calcd. for $C_{21}H_{35}Si$: 315.2503, Found 315.2503.

4. [(6*R*)-6-((1'*R*,3a'*S*,7a'*R*,*E*)-7a'-Methyl-4'-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene) octahydro-1H-inden-1-yl)hept-1-ynyl]trimethylsilane (3).



A solution of **8** (0.21 g, 0.54 mmol) in dry THF/toluene (9 mL, 1:3) was cooled to -78 ºC and stirred for 30 min. Then a solution of *t-B*uLi in pentane (1.46 M, 0.78 mL, 2.1 equiv) was added dropwise to the yellow solution. The mixture was stirred for 1 h. B(O*i*-Pr)$_3$ (0.19 mL, 0.81 mmol, 1.5 equiv) was added dropwise. The resulting reaction mixture was stirred at -78 ºC for 1.5 h. Pinacol (0.083 g, 0.70 mmol, 1.3 equiv) was added. The mixture was allowed to reach room temperature for 4 h. H_2O (10 mL) and TBME (10 mL) were successively added. The aqueous phase was extracted with TBME (3x30 mL). The combined organic phases were dried, filtered and concentrated. The residue was purified by flash chromatography (SiO_2, Ø 3x9 cm, Hexanes:EtOAc, 97:3) to give **3** [0.21 g, 0.47 mmol, 88%, yellow oil, R_f = 0.72 (Hexanes:EtOAc, 8:2), $[\alpha]_D^{25}$ = 79.1 (*c* = 1.5, $CHCl_3$)]. ^{1}H-NMR (250 MHz, $CDCl_3$): δ 4.90 (s, 1H, HCB), 3.16 (dd, J_1 = 11.6, J_2 = 2.9, 1H, H-5'), 2.17 (td, J_1 = 6.6, J_2 = 2, 2H, CH_2-3), 1.26 (s, 12H, 4xMeCOB), 1.17-1.04 (m, 1H), 0.91 (d, J = 5.8, 3H, CH_3-7), 0.54 (s, 3H, CH_3-8'), 0.12 (s, 9H, Me_3Si); ^{13}C-NMR (63 MHz, $CDCl_3$): δ 166.2 (C, C-4'), 107.8 (C, C-1), 84.3 (C, C-2), 82.5 (2xC, 2xMeCOB), 58.0 (CH, C-3a'), 56.5 (CH, C-1'), 46.2 (C, C-7a'), 40.4 (CH_2, C-7'), 35.4 (CH, C-6), 34.9 (CH_2, C-5'), 33.2 (CH_2, C-3'), 27.3 (CH_2, C-2'), 25.1 (CH_2, C-3), 24.9 (2xCH_3, 2xMeCOB), 24.8 (2xCH_3, 2xMeCOB), 24.3 (CH_2, C-4), 22.2 (CH_2, C-5), 20.1 (CH_2, C-6'), 18.8 (CH_3, C-7), 12.1 (CH_3, C-8'), 0.16 (3xCH_3, Me_3Si); IR (film, cm^{-1}): 2950 (ν_{C-H}), 2870 (ν_{C-H}), 2174 ($\nu_{C\equiv C}$), 1638 (ν_{C-B}); ESI-MS (m/z, %): 443 ([M+H]$^+$, 100); ESI-HRMS [M+H]$^+$ Calcd. for $C_{27}H_{48}O_2BSi$: 443.3511, Found 443.3512.

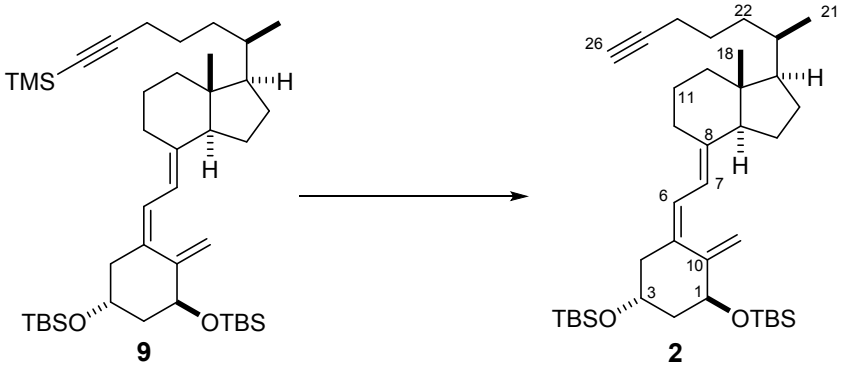
5. 1,3-bis(*tert*-Butyldimethylsilyl)-26-trimethylsilyl-27-nor-25-yne-1α-hydroxyvitamin D$_3$ (9).



An aqueous solution of K_3PO_4 (2.41 mL, 2 M, 27 equiv) was added to a solution of **3** (0.08 g, 0.18 mmol, 1 equiv) and **4** (0.09 g, 0.18 mmol, 1 equiv) in dry THF (5 mL). Then $PdCl_2(PPh_3)_2$ (6.2 mg, 0.009 mmol, 0.05 equiv) was added. The reaction mixture was vigorously stirred for 2 h in the dark. The reaction was quenched by the addition of H_2O (10 mL) and Et_2O (10 mL). The aqueous phase was extracted with Et_2O

(3x10 mL). The combined organic phases were dried, filtered and concentrated. The residue was purified by flash chromatography (SiO_2, Ø 2x3 cm, hexanes) to give **9** [0.12 g, 0.17 mmol, 98%, white foam, R_f = 0.85 (Hexanes:EtOAc, 95:5), $[\alpha]_D^{25}$ = 44.7 (*c* = 0.6, $CHCl_3$)]. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.24 and 6.02 (AB system, *J* = 11.2, 2H, H-6 and H-7), 5.18 (d, *J* = 2.5, 1H, H-19), 4.87 (d, *J* = 2.5, 1H, H-19), 4.37 (dd, J_1 = 6.7, J_2 = 3.6, 1H, H-1), 4.19 (dt, J_1 = 7.4, J_2 = 3.6, 1H, H-3), 2.82 (br d, *J* = 10.8, 1H), 2.45 (dd, J_1 = 13.2, J_2 = 4.0, 1H), 0.93 (d, *J* = 6.3, 3H, CH_3-21), 0.88 (s, 18H, 2xMe_3CSi), 0.54 (s, 3H, CH_3-18), 0.15 (s, 9H, Me_3Si), 0.07 (s, 6H, 2xMeSi), 0.06 (s, 6H, 2xMeSi); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 148.3 (C, C-10), 141.0 (C, C-8), 134.9 (C, C-5), 123.2 (CH, C-6), 117.9 (CH, C-7), 111.3 (CH_2, C-19), 107.8 (C, C-26), 84.3 (C, C-25), 72.1 (CH, C-1), 67.5 (CH, C-3), 56.4 (2xCH, C-17 and C-14), 46.1 (C, C-13), 45.8 (CH_2, C-4), 44.8 (CH_2, C-2), 40.6 (CH_2, C-12), 35.5 (CH, C-20), 35.0 (CH_2, C-9), 28.9 (CH_2, C-15), 27.6 (CH_2, C-16), 25.9 (3xCH_3, Me_3CSi), 25.8 (3xCH_3, Me_3CSi), 25.2 (CH_2, C-24), 23.5 (CH_2, C-23), 22.1 (CH_2, C-22), 20.1 (CH_2, C-11), 18.8 (CH_3, C-21), 18.2 (C, Me_3CSi), 18.1 (C, Me_3CSi), 12.0 (CH_3, C-18), 0.17 (3xCH_3, Me_3Si), -4.7 (2xCH_3, 2xMeSi), -4.8 (CH_3, MeSi), -5.1 (CH_3, MeSi); IR ($CHCl_3$, cm^{-1}): 2950 (ν_{C-H}), 2927 (ν_{C-H}), 2855 (ν_{C-H}), 2175 ($\nu_{C\equiv C}$); ESI-MS (m/z, %): 683 ([M+H]$^+$, 100); ESI-HRMS [M+H]$^+$ Calcd. for $C_{41}H_{75}O_2Si_3$: 683.5069, Found 683.5081.

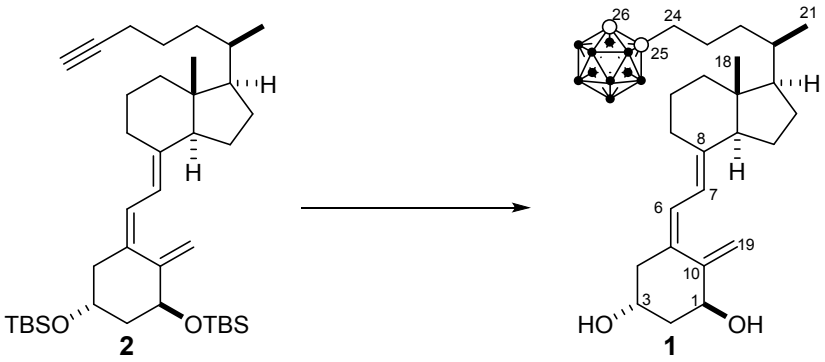
6. 1,3-bis(*tert*-Butyldimethylsilyl)-27-nor-25-yne-1α-hydroxyvitamin D_3 (2).



K_2CO_3 (0.052 g, 0.38 mmol, 2.2 equiv) was added to a solution of **9** (0.12 g, 0.17 mmol) in MeOH (2 mL). The resulting suspension was stirred at room temperature for 14 h. The reaction was quenched with saturated NH_4Cl (5 mL) and H_2O (10 mL). The mixture was concentrated to a small volume and extracted with TBME (3x10 mL). The combined organic phases were dried, filtered and concentrated. The residue was purified by flash chromatography (SiO_2, Ø 2x4 cm, hexanes) to give **2** [0.09 g, 0.16 mmol, 92%, white foam, Rf = 0.35 (Hexanes:EtOAc, 98:2), $[\alpha]_D^{25}$ = 42.5 (*c* = 2.0, $CHCl_3$)]. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.24 and 6.02 (AB system, *J* = 11.2, 2H, H-6 and H-7), 5.18 (d, *J* = 2.6, 1H, H-19), 4.87 (d, *J* = 2.6, 1H, H-19), 4.37 (dd, J_1 = 6.7, J_2 = 3.7, 1H, H-1), 4.19 (dt, J_1 = 7.4, J_2 = 3.6, 1H, H-3), 2.82 (br d, *J* = 13.2, 1H), 2.45 (br d, *J* = 13.3, 1H), 1.94 (t, *J* = 2.6, 1H, H-27), 0.93 (d, *J* = 6.6, 3H, CH_3-21), 0.87 (s, 18H, 2xMe_3CSi), 0.54 (s, 3H, CH_3-18), 0.06 (s, 12H, 2xMe_2Si); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 148.3 (C, C-10), 141.0 (C, C-8), 134.9 (C, C-5), 123.1 (CH, C-6), 117.9 (CH, C-7), 111.2 (CH_2, C-19), 84.8 (C, C-25), 72.0 (CH, C-1), 68.1 (CH, C-26), 67.5 (CH, C-3), 56.4 and 56.3 (CH, C-17 and C-14), 46.0 (CH_2, -C4), 45.8 (C, C-13), 44.8 (CH_2, C-2), 40.6 (CH_2, C-12), 35.7 (CH, C-20), 35.1 (CH_2, C-9), 29.7 (CH_2, C-11), 28.8 (CH_2, C-15), 27.7 (CH_2, C-16), 25.9 (3xCH_3, Me_3CSi), 25.8 (3xCH_3, Me_3CSi), 25.2 (CH_2, C-24), 23.5 (CH_2,

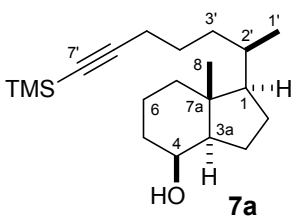
C-23), 22.1 (CH_2, C-22), 18.8 (CH_3, C-21), 18.2 (C, Me_3CSi), 18.1 (C, Me_3CSi), 12.0 (CH_3, C-18), -4.7 (2xCH_3, 2xMeSi), -4.8 (CH_3, MeSi), -5.1 (CH_3, MeSi); IR ($CHCl_3$, cm^{-1}): 2926 (ν_{C-H}), 2855 (ν_{C-H}); EI-MS (m/z, %): 610 ([M]$^+$, 16), 595 ([M-Me]$^+$, 4), 553 ([M-*t*Bu]$^+$, 4), 248 (100); EI-HRMS [M]$^+$ Calcd. for $C_{38}H_{66}O_2Si_2$: 610.4601. Found 610.4596.

7. 1α-Hydroxy-27-nor-25-*o*-carboranyl-vitamin D_3 (1)



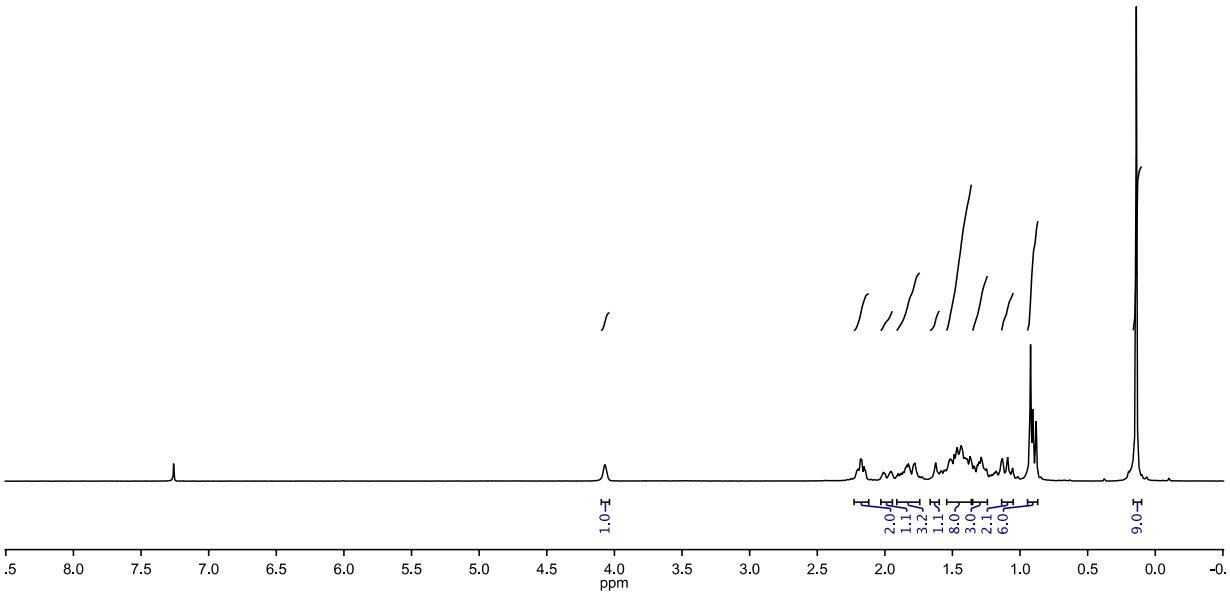
$PhNMe_2$ (0.14 mL, 1.05 mmol, 6.5 equiv) was added to a solution of **2** (0.099 g, 0.16 mmol) in dry toluene (5 ml) at room temperature. Decaborane (0.059 g, 0.49 mmol, 3 equiv) was added. The solution was heated with vigorous stirring in dark at 110 ºC for 1 h. The mixture was concentrated. The residue was purified by flash chromatography (SiO_2, Ø 2.5x4 cm, Hexanes:EtOAc, 98:2) to give the protected analog [0.05 g, 0.070 mmol, 43%, R_f = 0.35 (Hexanes:EtOAc, 98:2)]. A solution of protected triene (0.05 g, 0.07 mmol, 1 equiv) in CH_2Cl_2/CH_3CN (8 mL, 1:3) was treated with HF (0.15 mL, 48% aqueous solution). The mixture was stirred at room temperature in the dark for 12 h and then poured onto saturated $NaHCO_3$ (50 mL) (CAUTION! gas evolution!). The mixture was extracted with EtOAc (3x25 mL). The combined organic phase was washed with saturated NaCl (20 mL), dried, filtered and concentrated at room temperature. The residue was purified by flash chromatography (SiO_2, Ø 2x4 cm, Hexanes:EtOAc, 6:4) to give **1** [0.03 g, 0.16 mmol, 93%, white foam, R_f = 0.15 (Hexanes:EtOAc, 6:4)], $[\alpha]_D^{25}$ = 6.8 (*c* = 0.5, EtOH 96%)]. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.36 and 6.00 (AB system, *J* = 11.2, 2H, H-6 and H-7), 5.32 (s, 1H, H-19), 4.99 (s, 1H, H-19), 4.42 (m, 1H, H-1), 4.22 (m, 1H, H-3), 3.57 (s, 1H, H-26), 3.05-1.18 (broad band, 10H, BH), 2.82 (br d, *J* = 12.9, 1H), 2.58 (br d, *J* = 13.3, 1H), 2.30 (dd, J_1 = 13.4, J_2 = 6.5, 1H), 1.04-0.97 (m, 1H), 0.91 (d, *J* = 6.4, 3H, CH_3-21), 0.53 (s, 3H, CH_3-18); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 147.5 (C, C-10), 142.8 (C, C-8), 133.0 (C, C-5), 124.9 (CH, C-6), 117.1 (CH, C-7), 111.8 (CH_2, C-19), 75.5 (C, C-25), 70.7 (CH, C-1), 66.8 (CH, C-3), 61.0 (CH, C-26), 56.3 and 56.2 (CH, C-14 and C-17), 45.8 (C, C-13), 45.2 (CH_2, C-4), 42.8 (CH_2, C-2), 40.4 (CH_2, C-12), 38.5 (CH_2, C-24), 35.8 (CH, C-20), 35.1 (CH_2, C-9), 29.0 (CH_2, C-15), 27.6 (CH_2, C-16), 26.1 (CH_2, C-11), 23.5 (CH_2, C-23), 22.2 (CH_2, C-22), 18.7 (CH_3, C-21), 12.0 (CH_3, C-18). ^{11}B-NMR (160.46 MHz, $CDCl_3$): δ -0.68, -4.04, -7.54, -9.41, -10.12, -11.15; IR ($CHCl_3$, cm^{-1}): 2922 (ν_{C-H}), 2852 (ν_{C-H}); UV (EtOH 96%): λ_{max} = 254 nm (ε = 30.500), λ = 276 nm (ε = 26.000); ESI-MS (m/z, %): 524 ([M+Na]$^+$, 4), 303 (100); ESI-HRMS [M+Na]$^+$ Calcd. for $C_{26}H_{48}B_{10}O_2Na^+$: 525.4477, Found 525.4515.

C. NMR spectra

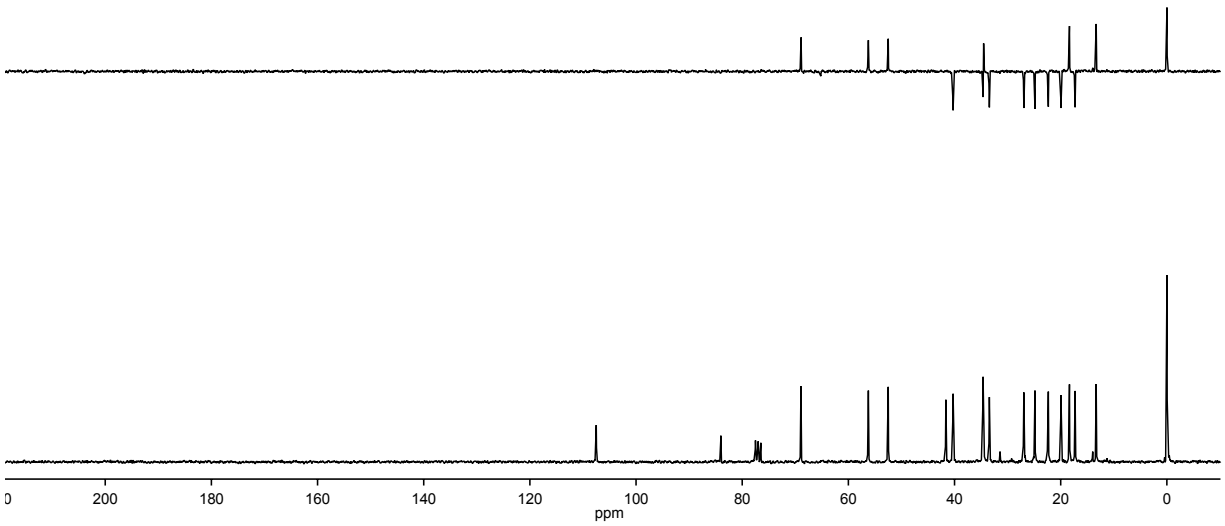


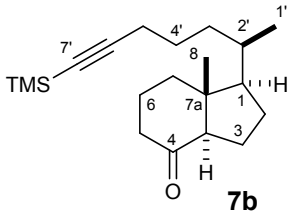
7a

^{1}H-NMR (250 MHz, $CDCl_3$): δ 4.07 (br s, 1H, H-4), 2.17 (td, J_1 = 6.5, J_2 = 3.2, 2H, CH_2-5'), 1.99 (br d, J = 14, 1H, H-5), 0.92 (s, 3H, CH_3-8), 0.90 (d, J = 6.7, 3H, CH_3-1'), 0.14 (s, 9H, Me_3Si).



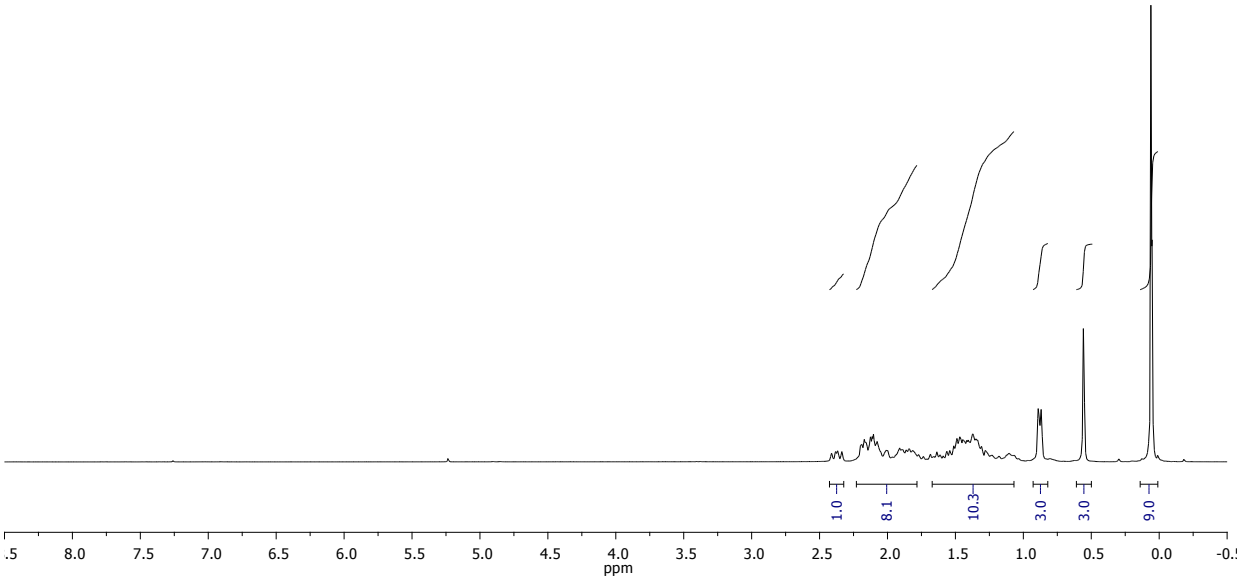
^{13}C-NMR (63 MHz, $CDCl_3$): δ 107.5 (C, C-6'), 84.0 (C, C-7'), 68.9 (CH, C-4, 56.3 (CH, C-1), 52.5 (CH, C-3a), 41.6 (C, C-7a), 40.3 (CH_2, C-7), 34.6 (CH_2, C-5), 34.5 (CH, C-2'), 33.5 (CH_2, C-3), 27.0 (CH_2, C-2), 24.9 (CH_2, C-5'), 22.5 (CH_2, C-4'), 19.9 (CH_2, C-3'), 18.4 (CH_3, C-1'), 17.3 (CH_2, C-6), 13.3 (CH_3, C-8), 0.0 (3xCH_3, Me_3Si).



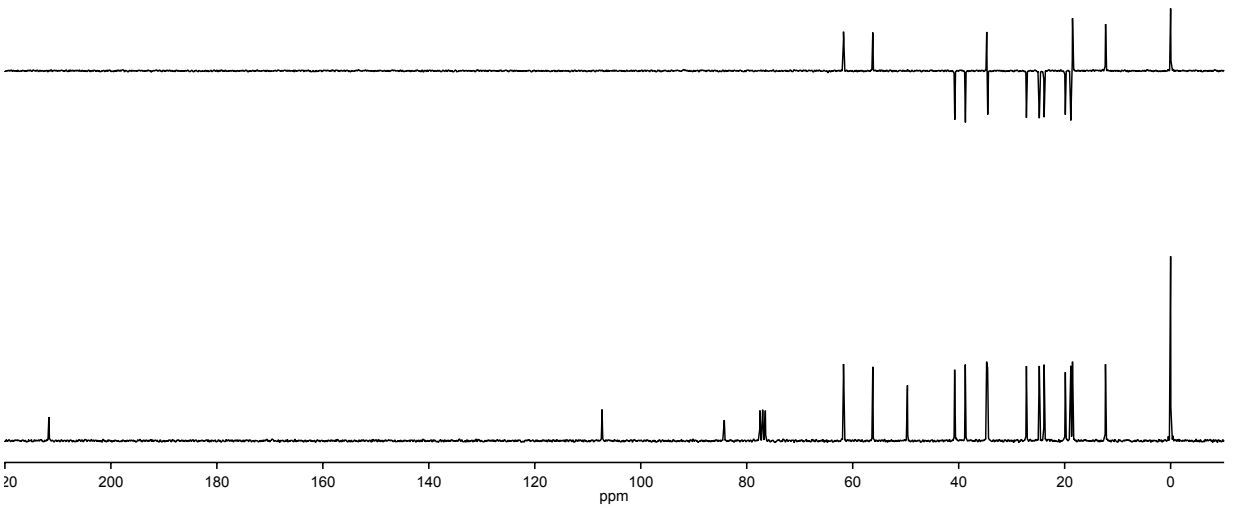


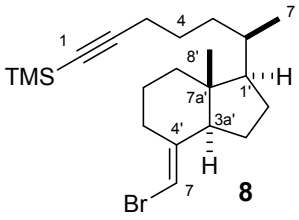
7b

^{1}H-NMR (250 MHz, $CDCl_3$): δ 2.37 (dd, J_1 = 10.6, J_2 = 7.5, 1H, H-5), 0.88 (d, J = 5.2, 3H, CH_3-1'), 0.56 (s, 3H, CH_3-8), 0.06 (s, 9H, Me_3Si).

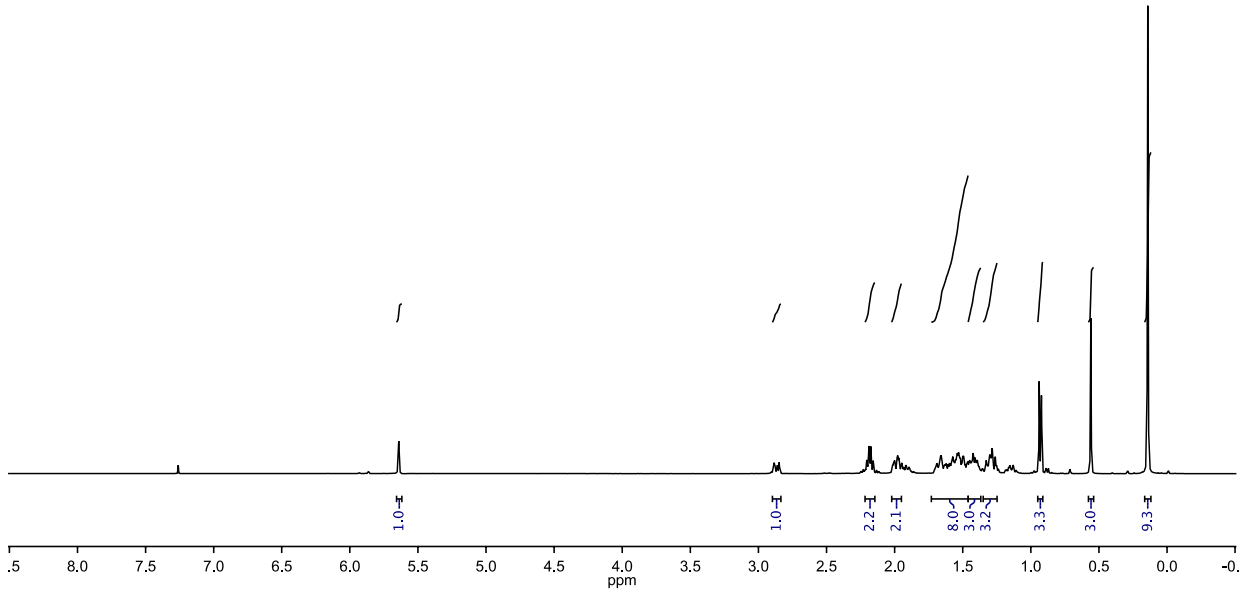


^{13}C-NMR ($CDCl_3$, 63 MHz, $CDCl_3$): δ 211.7 (C, C-4), 107.3 (C, C-7'), 84.3 (C, C-6'), 61.8 (CH, C-3a), 56.2 (CH, C-1), 49.7 (C, C-7a), 40.8 (CH_2, C-7), 38.8 (CH_2, C-5), 34.7 (CH, C-2'), 34.5 (CH_2, C-3), 27.2 (CH_2, C-2), 24.8 (CH_2, C-5'), 23.9 (CH_2, C-4'), 19.9 (CH_2, C-3'), 18.9 (CH_2, C-6), 18.5 (CH_3, C-1'), 12.3 (CH_3, C-8), 0.0 (3xCH_3, Me_3Si).

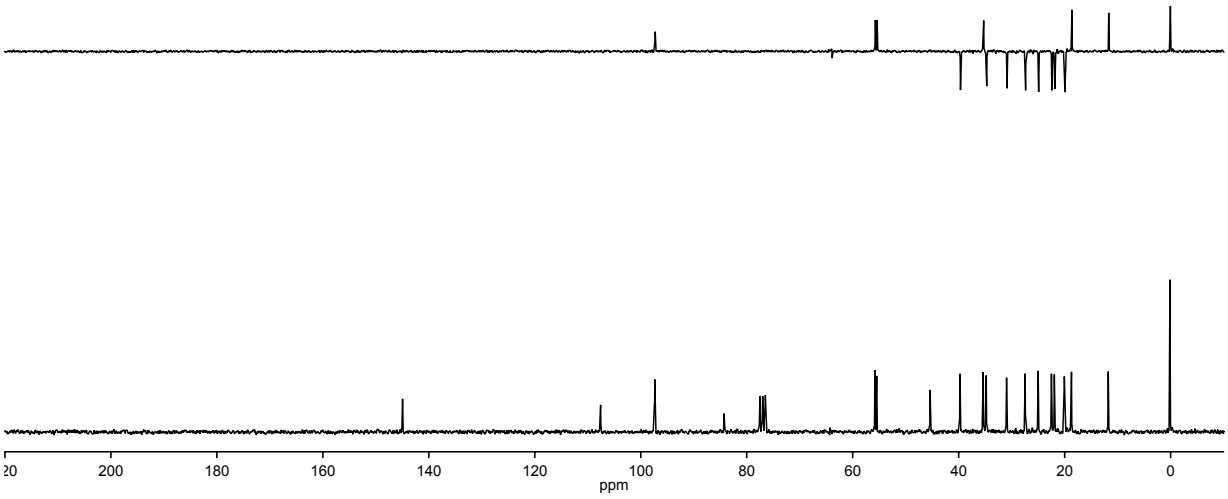


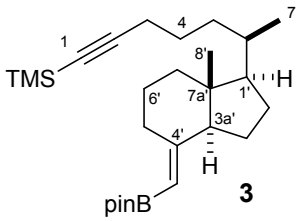


^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.64 (s, 1H, HCBr), 2.86 (m, 1H, H-5'), 2.20 (d, *J* = 6.2, 1H, H-3), 2.17 (d, *J* = 6.6, 1H, H-3), 0.93 (d, *J* = 6.2, 3H, CH_3-7), 0.56 (s, 3H, CH_3-8'), 0.14 (s, 9H, Me_3Si).



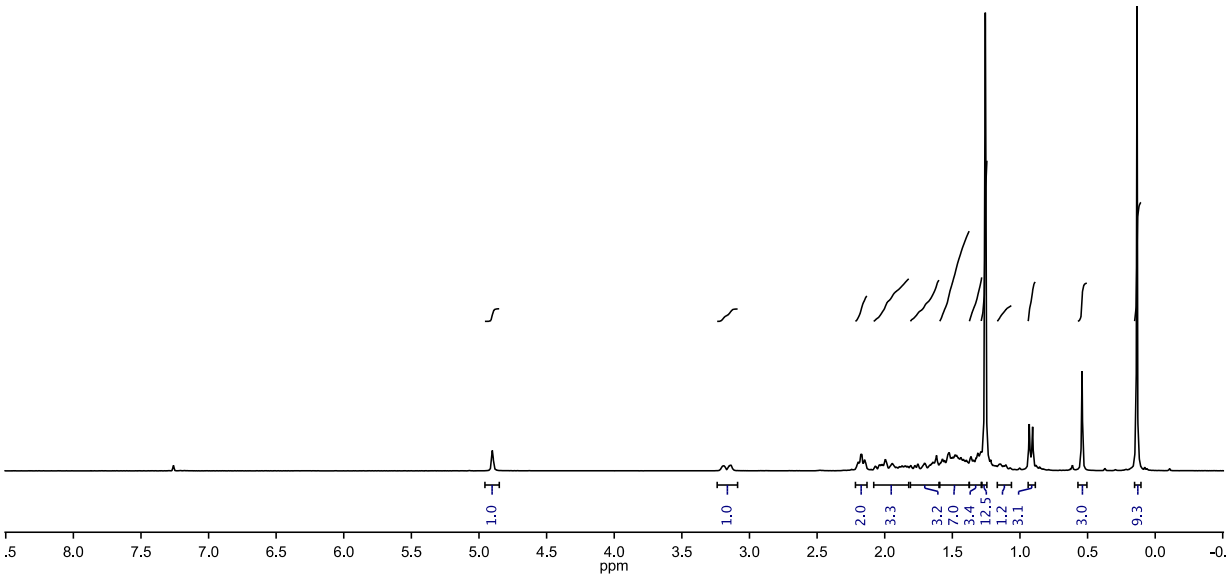
^{13}C-NMR (63 MHz, $CDCl_3$): δ 144.9 (C, C-4'), 107.5 (C, C-1), 97.4 (CH, CHBr), 84.3 (C, C-2), 55.9 (CH, C-3a'), 55.5 (CH, C-1'), 45.4 (C, C-7a'), 39.8 (CH_2, C-7'), 35.4 (CH, C-6), 34.8 (CH_2, C-5'), 31.0 (CH_2, C-3'), 27.4 (CH_2, C-2'), 25.0 (CH_2, C-3), 22.5 (CH_2, C-4), 22.0 (CH_2, C-5), 20.1 (CH_2, C-6'), 18.7 (CH_3, C-7), 11.8 (CH_3, C-8'), 0.14 (3xCH_3, Me_3Si).



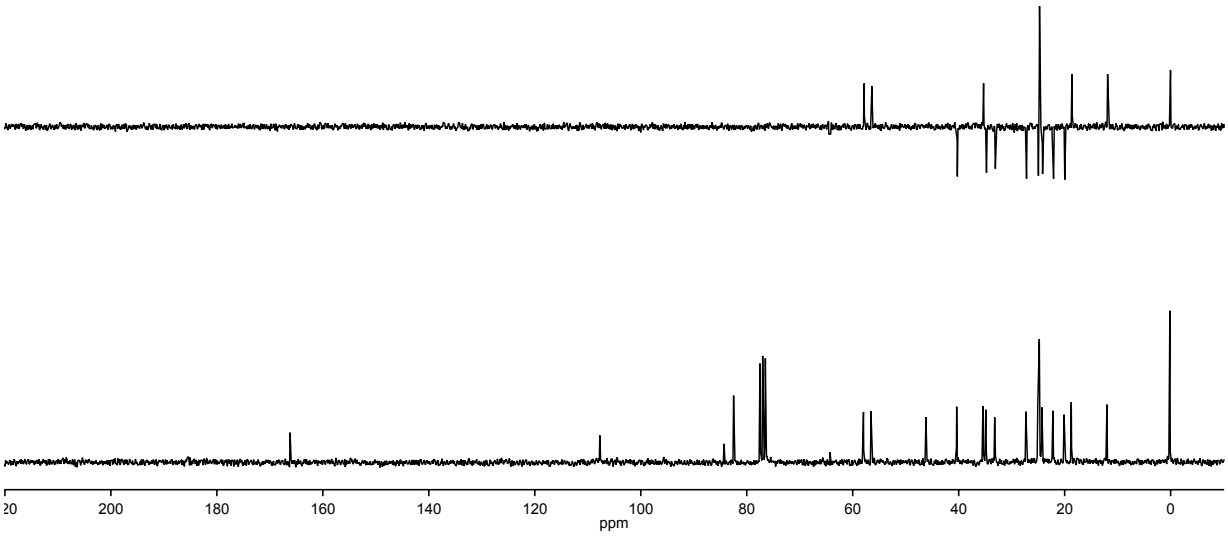


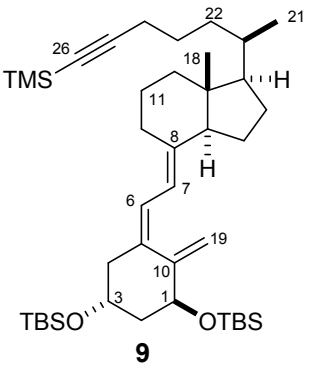
3

^{1}H-NMR (250 MHz, $CDCl_3$): δ 4.90 (s, 1H, HCB), 3.16 (dd, J_1 = 11.6, J_2 = 2.9, 1H, H-5'), 2.17 (td, J_1 = 6.6, J_2 = 2, 2H, CH_2-3), 1.26 (s, 12H, 4xMeCOB), 1.17-1.04 (m, 1H), 0.91 (d, J = 5.8, 3H, CH_3-7), 0.54 (s, 3H, CH_3-8'), 0.12 (s, 9H, Me_3Si).



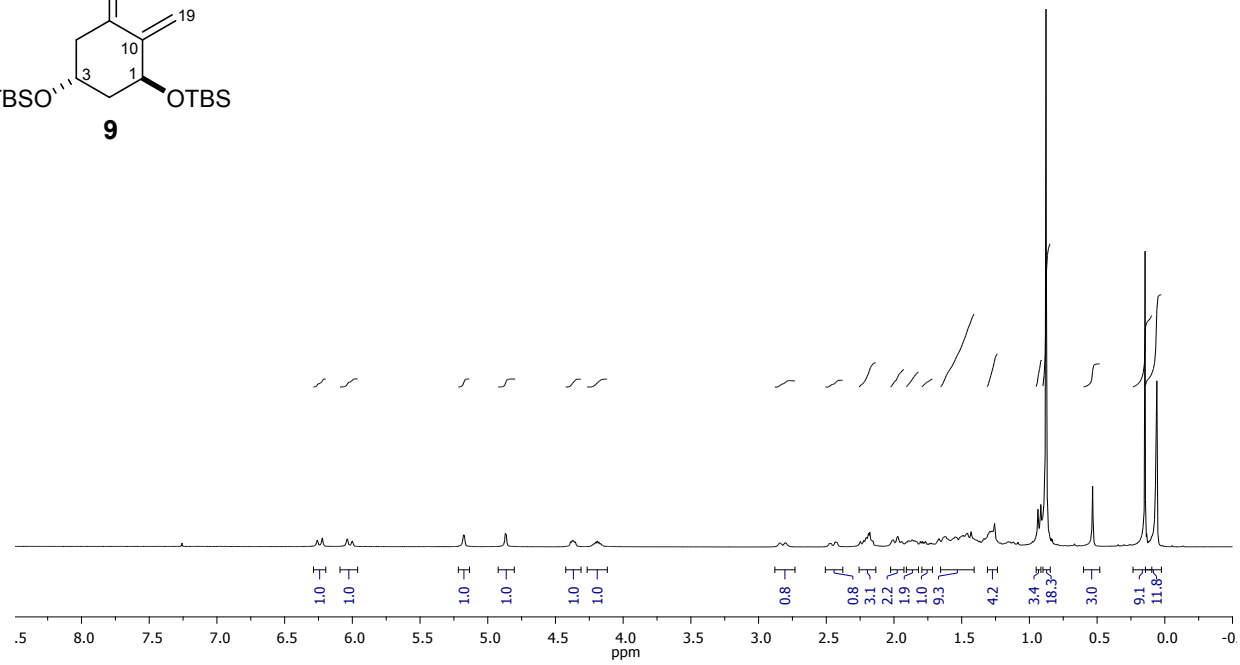
^{13}C-NMR (63 MHz, $CDCl_3$): δ 166.2 (C, C-4'), 107.8 (C, C-1), 84.3 (C, C-2), 82.5 (2xC, 2xMeCOB), 58.0 (CH, C-3a'), 56.5 (CH, C-1'), 46.2 (C, C-7a'), 40.4 (CH_2, C-7'), 35.4 (CH, C-6), 34.9 (CH_2, C-5'), 33.2 (CH_2, C-3'), 27.3 (CH_2, C-2'), 25.1 (CH_2, C-3), 24.9 (2xCH_3, 2xMeCOB), 24.8 (2xCH_3, 2xMeCOB), 24.3 (CH_2, C-4), 22.2 (CH_2, C-5), 20.1 (CH_2, C-6'), 18.8 (CH_3, C-7), 12.1 (CH_3, C-8'), 0.16 (3xCH_3, Me_3Si).



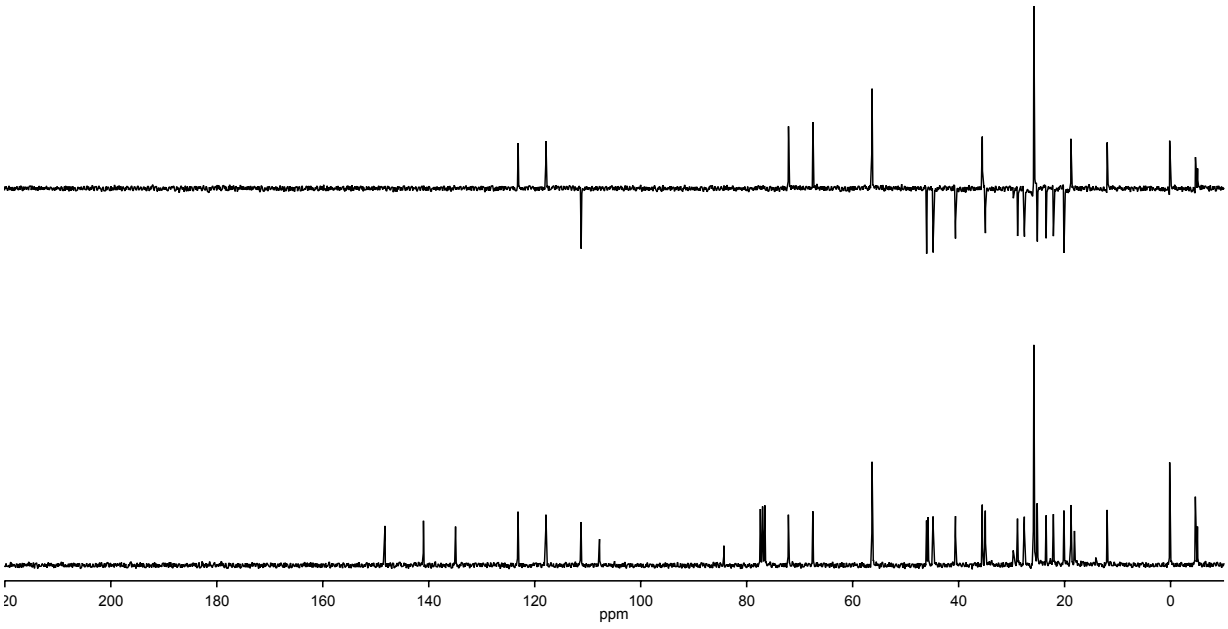


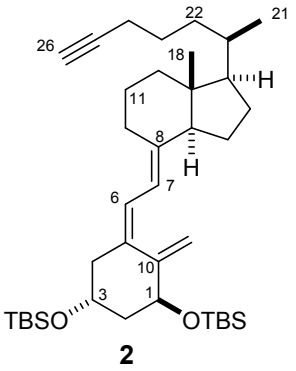
9

^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.24 and 6.02 (AB system, *J* = 11.2, 2H, H-6 and H-7), 5.18 (d, *J* = 2.5, 1H, H-19), 4.87 (d, *J* = 2.5, 1H, H-19), 4.37 (dd, J_1 = 6.7, J_2 = 3.6, 1H, H-1), 4.19 (dt, J_1 = 7.4, J_2 = 3.6, 1H, H-3), 2.82 (br d, *J* = 10.8, 1H), 2.45 (dd, J_1 = 13.2, J_2 = 4.0, 1H), 0.93 (d, *J* = 6.3, 3H, CH_3-21), 0.88 (s, 18H, 2xMe_3CSi), 0.54 (s, 3H, CH_3-18), 0.15 (s, 9H, Me_3Si), 0.07 (s, 6H, 2xMeSi), 0.06 (s, 6H, 2xMeSi).



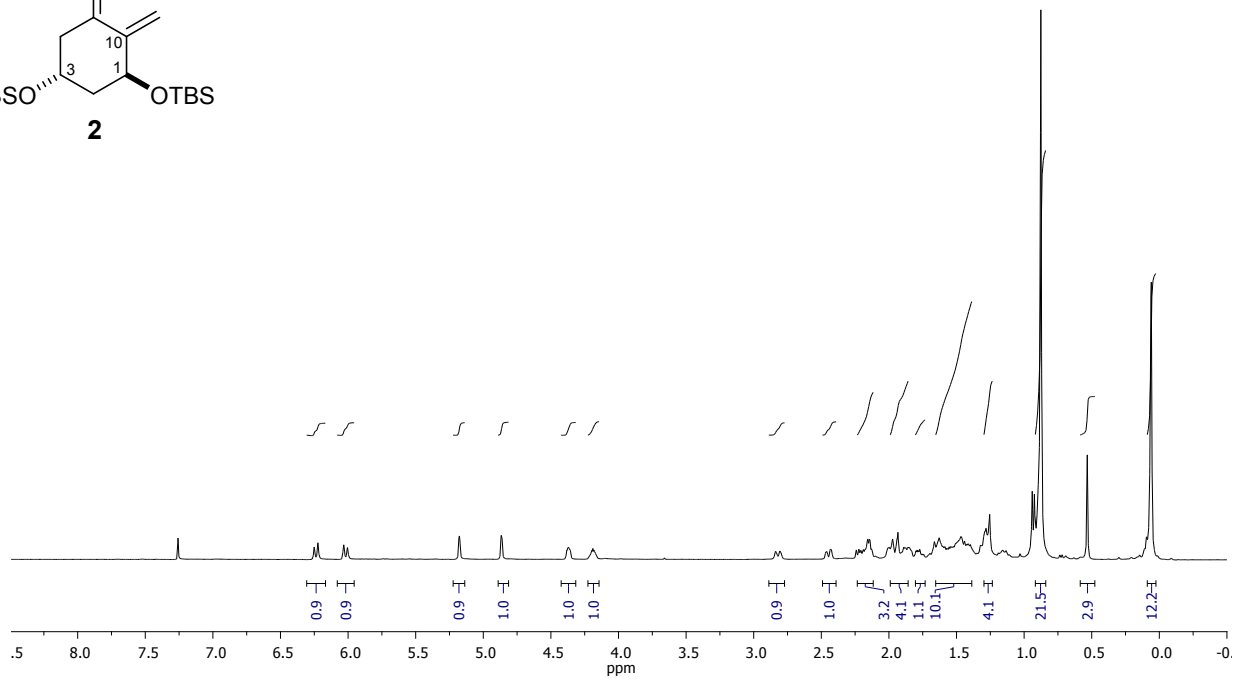
^{13}C-NMR (100 MHz, $CDCl_3$): δ 148.3 (C, C-10), 141.0 (C, C-8), 134.9 (C, C-5), 123.2 (CH, C-6), 117.9 (CH, C-7), 111.3 (CH_2, C-19), 107.8 (C, C-26), 84.3 (C, C-25), 72.1 (CH, C-1), 67.5 (CH, C-3), 56.4 (2xCH, C-17 and C-14), 46.1 (C, C-13), 45.8 (CH_2, C-4), 44.8 (CH_2, C-2), 40.6 (CH_2, C-12), 35.5 (CH, C-20), 35.0 (CH_2, C-9), 28.9 (CH_2, C-15), 27.6 (CH_2, C-16), 25.9 (3xCH_3, Me_3CSi), 25.8 (3xCH_3, Me_3CSi), 25.2 (CH_2, C-24), 23.5 (CH_2, C-23), 22.1 (CH_2, C-22), 20.1 (CH_2, C-11), 18.8 (CH_3, C-21), 18.2 (C, Me_3CSi), 18.1 (C, Me_3CSi), 12.0 (CH_3, C-18), 0.17 (3xCH_3, Me_3Si), -4.7 (2xCH_3, Me_2Si),-4.8 (CH_3, Me_3Si), -5.1 (CH_3, Me_3Si).



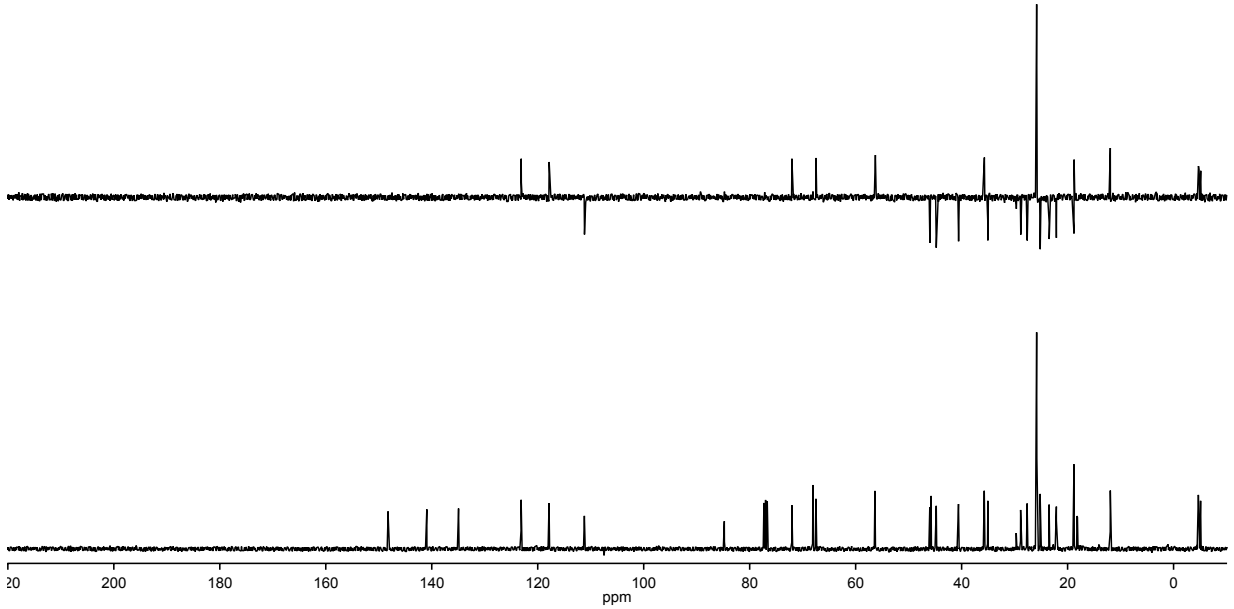


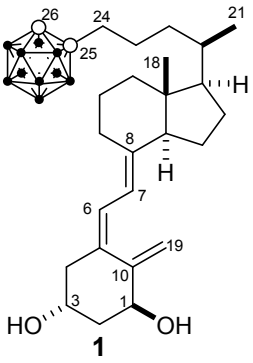
2

^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.24 and 6.02 (AB system, *J* = 11.2, 2H, H-6 and H-7), 5.18 (d, *J* = 2.6, 1H, H-19), 4.87 (d, *J* = 2.6, 1H, H-19), 4.37 (dd, J_1 = 6.7, J_2 = 3.7, 1H, H-1), 4.19 (dt, J_1 = 7.4, J_2 = 3.6, 1H, H-3), 2.82 (br d, *J* = 13.2, 1H), 2.45 (br d, *J* = 13.3, 1H), 1.94 (t, *J* = 2.6, 1H, H-27), 0.93 (d, *J* = 6.6, 3H, CH_3-21), 0.87 (s, 18H, 2xMe_3CSi), 0.54 (s, 3H, CH_3-18), 0.06 (s, 12H, 4xMeSi).



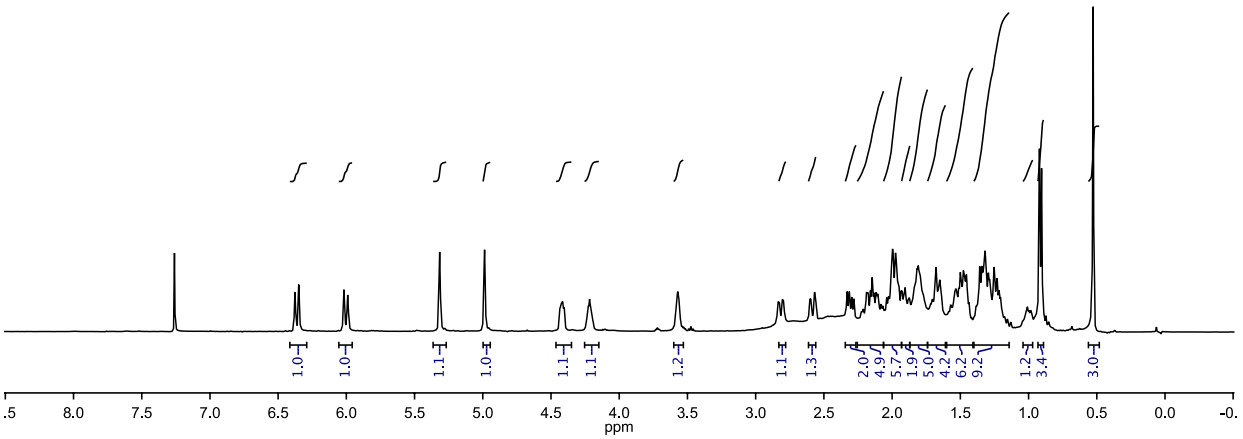
^{13}C-NMR (100 MHz, $CDCl_3$): δ 148.3 (C, C-10), 141.0 (C, C-8), 134.9 (C, C-5), 123.1 (CH, C-6), 117.9 (CH, C-7), 111.2 (CH_2, C-19), 84.8 (C, C-25), 72.0 (CH, C-1), 68.1 (CH, C-26), 67.5 (CH, C-3), 56.4 and 56.3 (CH, C-17 and C-14), 46.0 (CH_2, C-4), 45.8 (C, C-13), 44.8 (CH_2, C-2), 40.6 (CH_2, C-12), 35.7 (CH, C-20), 35.1 (CH_2, C-9), 29.7 (CH_2, C-11), 28.8 (CH_2, C-15), 27.7 (CH_2, C-16), 25.9 (3xCH_3, Me_3CSi), 25.8 (3xCH_3, Me_3CSi), 25.2 (CH_2, C-24), 23.5 (CH_2, C-23), 22.1 (CH_2, C-22), 18.8 (CH_3, C-21), 18.2 (C, Me_3CSi), 18.1 (C, Me_3CSi), 12.0 (CH_3, C-18), -4.7 (2xCH_3, 2xMeSi), -4.8 (CH_3, Me_3Si), -5.1 (CH_3, Me_3Si).



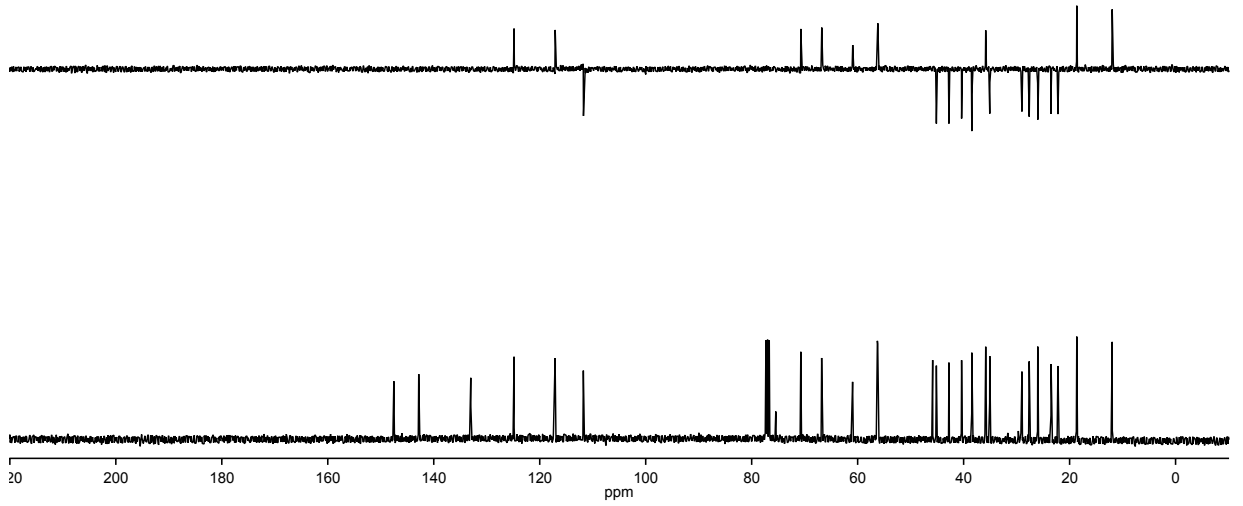


1

^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.36 and 6.00 (AB system, *J* = 11.2, 2H, H-6 and H-7), 5.32 (s, 1H, H-19), 4.99 (s, 1H, H-19), 4.42 (m, 1H, H-1), 4.22 (m, 1H, H-3), 3.57 (s, 1H, H-26), 3.05-1.18 (broad band, 10H, BH), 2.82 (br d, *J* = 12.9, 1H), 2.58 (br d, *J* = 13.3, 1H), 2.30 (dd, J_1 = 13.4, J_2 = 6.5, 2H), 1.04-0.97 (m, 1H), 0.91 (d, *J* = 6.4, 3H, CH_3-21), 0.53 (s, 3H, CH_3-18).



^{13}C-NMR (125 MHz, $CDCl_3$): δ 147.5 (C, C-10), 142.8 (C, C-8), 133.0 (C, C-5), 124.9 (CH, C-6), 117.1 (CH, C-7), 111.8 (CH_2, C-19), 75.5 (C, C-25), 70.7 (CH, C-1), 66.8 (CH, C-3), 61.0 (CH, C-26), 56.3 and 56.2 (CH, C-14 and C-17), 45.8 (C, C-13), 45.2 (CH_2, C-4), 42.8 (CH_2, C-2), 40.4 (CH_2, C-12), 38.5 (CH_2, C-24), 35.8 (CH, C-20), 35.1 (CH_2, C-9), 29.0 (CH_2, C-15), 27.6 (CH_2, C-16), 26.1 (CH_2, C-11), 23.5 (CH_2, C-23), 22.2 (CH_2, C-22), 18.7 (CH_3, C-21), 12.0 (CH_3, C-18).



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 Archive directory:

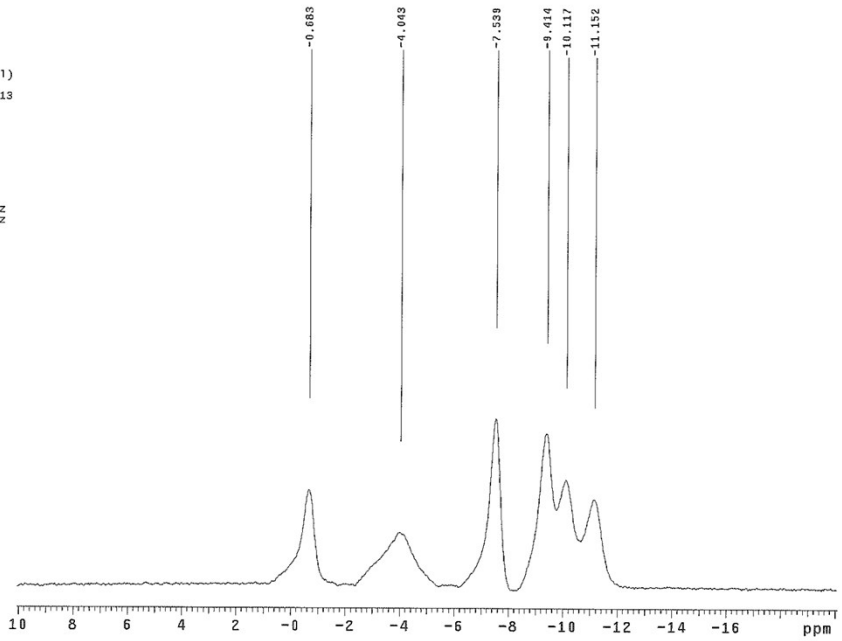
 Sample directory:

 FidFile: CARBON

Pulse Sequence: CARBON (s2pul)
Solvent: cdcl3
Data collected on: Feb 21 2013

 Temp. 25.0 C / 298.1 K
Operator: rgry

 Relax. delay 0.200 sec
 Pulse 37.5 degrees
 Acq. time 0.319 sec
 Width 12837.0 Hz
 256000 repetitions
OBSERVE  B11, 160.4614670 MHz
DECOUPLE  H1, 500.1321510 MHz
 Power 32 dB
 continuously on
 WALTZ-16 modulated
DATA PROCESSING
 Line broadening 5.0 Hz
FT size 8192
Total time 9 hr, 14 min
```

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am1roanalogoA  11B acop

 Sample Name:

 Data Collected on:
   Varian-NMR-inova500
 Archive directory:

 Sample directory:

 FidFile: CARBON

Pulse Sequence: CARBON (s2pul)
Solvent: cdcl3
Data collected on: Feb 21 2013

 Temp. 999.0 C / 1272.2 K
Operator: rgry

 Relax. delay 0.200 sec
 Pulse 37.5 degrees
 Acq. time 0.319 sec
 Width 12837.0 Hz
 256000 repetitions
OBSERVE  B11, 160.4614670 MHz
DECOUPLE  H1, 500.1321510 MHz
 Power 32 dB
 off during acquisition
 on during delay
 WALTZ-16 modulated
DATA PROCESSING
 Line broadening 5.0 Hz
FT size 8192
Total time 9 hr, 14 min
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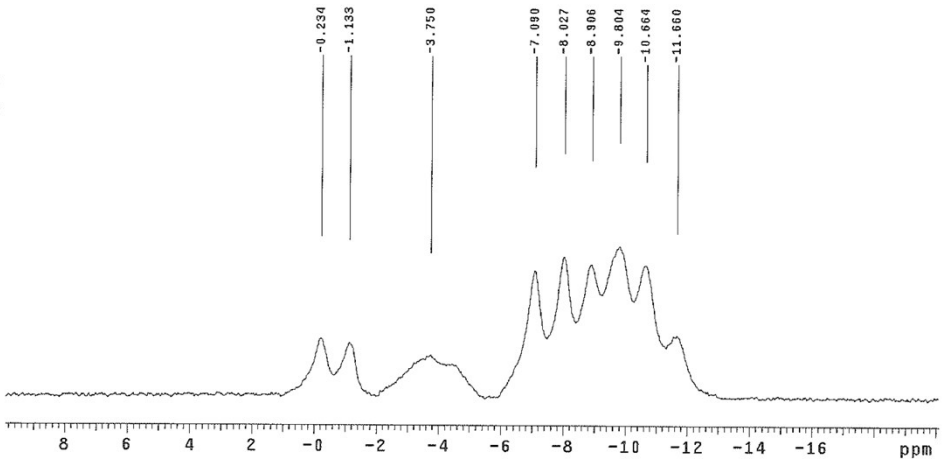

IV. References and Notes

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