Supporting Information

A novel vitamin K derived anticoagulant tolerant to genetic variations of vitamin K epoxide reductase

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Supporting Information Part 1: Synthesis

Abbreviations

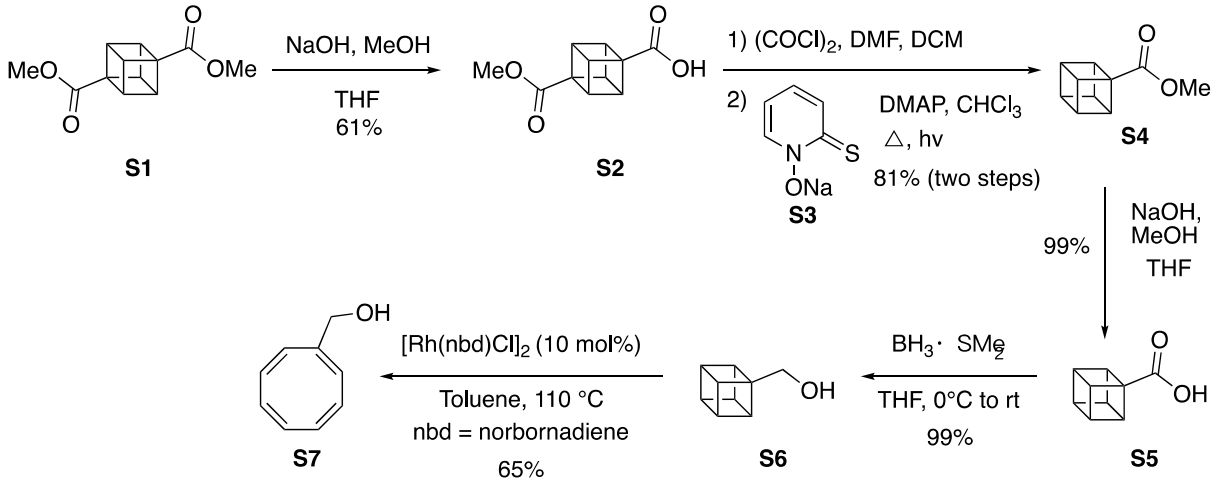
℃	degrees Celsius	Hz	Hertz
Δ	heat/reflux	*In vacuo*	in a vacuum
Δ	chemical shift	*J*	coupling constant
CAN	ceric ammonium nitrate	M	molar (moles per liter)
$CDCl_3$	deuterated chloroform	MHz	Mega-hertz
DCM	dichloromethane	m.p.	Melting point
DMAP	*N,N*-dimethyl-4- aminopyridine	*m/z*	Mass to charge ratio
DMF	*N,N*-dimethylformamide	NBS	*N*-Bromosuccinimide
DMSO	dimethyl sulfoxide	NMO	*N*-Methylmorpholine *N*-oxide
DMSO-d_6	deuterated dimethyl sulfoxide	NMR	nuclear magnetic resonance
et al	et alii / et aliae (and others)	ppm	part per million
EtOAc	Ethyl acetate	rt	room temperature
ESI	electrospray ionisation	THF	tetrahydrofuran
GCMS	gas chromatography mass spectrometry	TLC	Thin layer chromatography
HCl	hydrochloride acid	UV	ultraviolet
HRMS	High resolution mass spectrometry		

General Experimental

Unless stated otherwise commercially available chemicals were used without further purification. Moisture sensitive reactions were carried out with oven (160°C) dried or flame dried glassware under an argon atmosphere. Argon was dried by passing through a drying tube with 3Å molecular sieves and Drierite™. Tetrahydrofuran (THF) and 1,4-dioxane were distilled over elemental sodium/benzophenone under an argon atmosphere. Dichloromethane (DCM), acetonitrile and *N,N*-dimethylformamide (DMF) were distilled over calcium hydride under an argon atmosphere.[1] Analytical thin layer chromatography (TLC) was used to monitor reactions (silica-60 F254 plates). The plate was observed with ultraviolet (UV, 254nm) light, and/or developed by potassium permanganate, vanillin, or phosphomolybdic acid stains. Powdered 4Å molecular sieves were dried under vacuum (6 mm Hg) for 24 hours at 200 ◦C and stored under argon. Distilled solvents and silica gel (230-400 mesh) were used for flash column chromatography. NMR spectra were recorded using either a Bruker AS500 (500 MHz, 125 MHz), AV500 (500 MHz, 125 MHz), or a Bruker AV300 (300 MHz, 75 MHz) instrument. Chemical shifts (δ) are reported in parts per million (ppm), and internally referenced to solvent ($CDCl_3$ - 7.26 ppm for ^1H NMR and 77.16 ppm for ^{13}C NMR; DMSO- d_6 - 2.49 ppm for ^1H NMR and 39.52 ppm for ^{13}C NMR). Coupling constants (*J*) are given in hertz (Hz). Multiplicity was reported as follows: s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, quin = quintuplet, sext = sextuplet, spt = septet, m = multiplet. Low resolution electrospray ionisation mass spectrometry measurements (LRESIMS) were measured on a Bruker HCT 3D Ion Trap spectrometer with a Bruker ESI source and recorded in positive ion mode. High resolution electrospray ionisation (HRESIMS) accurate mass measurements were measured a Bruker MicroOTOF-Q spectrometer with a Bruker ESI source and recorded in positive ion mode. Melting points were measured by Digimelt MPA161 SRS apparatus. Compound **S13**, **S17**, **S18**, **S19, S21**were purified by High performance liquid chromatography (HPLC) before submitted for biological evaluation. High performance liquid chromatography (HPLC) was conducted with a Shimadzu LC-20AD pump, SPDM20A UV detector and ELSD-LT II light detector with a Phenomenex normal-phase (NP) Silica Luna 10 µm 100 Å column (250 × 10 mm) for analysis and purification. The column was maintained at 40 °C with a column oven. The purity was calculated from the surface integral of each peak.

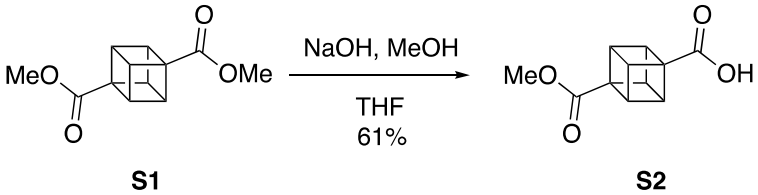
Experimental procedure

Synthesis of cubane derivatives



Scheme S1. Synthesis of cubane and cyclooctatetraene derivatives.

4-(Methoxycarbonyl)cubane-1-carboxylic acid (**S2**)



Following the procedure of Eaton *et al*: [2] dimethyl cubane-1,4-dicarboxylate (**S1**) (2.20 g, 10.0 mmol) was dissolved in THF (90 mL). A methanolic solution of sodium hydroxide (2.5 M, 5.2 mL) was then added slowly making the solution cloudy. The mixture was then stirred at room temperature for 16 hours, before evaporation of THF in vacuo. The solid residual was suspended in water (45 mL), and washed with dichloromethane (DCM) (3 x 30 mL). The resulting aqueous phase was carefully acidified to pH 2 with aqueous hydrochloric acid (1.0 M) solution before being extracted with DCM (1 x 40 mL, then 2 x 25 mL). The combined organic phases were dried over sodium sulfate, and evaporated *in vacuo* to give the titled compound **S2** (1.31 g, 61 %) as a white solid, which was directly used without further purification.

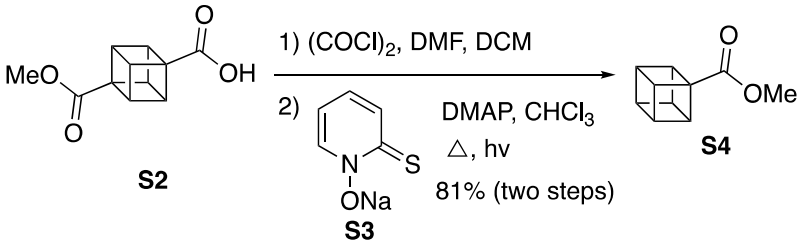
m.p. 176.8-177.6℃ (Lit.[3] 181-183℃ ref);

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 4.28 (s,6H), 3.72 (s,3H);

^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 176.2, 172.0, 56.0, 55.5, 51.8, 47.3, 47.2;

LRMS (ESI): *m/z* $[C_{11}H_{10}O_4]^+$ (M^+): 206.

Methyl cubane-1-carboxylate (**S4**)



Following the procedure of Ko *et al*:[4] 4-methoxycarbonylcubane-1-carboxylic acid (**S2**) (1.03 g, 5.00 mmol) was dissolved in anhydrous DCM (50 mL) under an argon atmosphere. Oxalyl chloride (500 μL, 6 mmol) and anhydrous DMF (30 μL) were added. After stirring the mixture at room temperature under an argon atmosphere for 1 hour, the DCM was removed in vacuo and placed under high vacuum (1 hour). The yellow solid that formed was then dissolved in anhydrous chloroform (50 mL). Meanwhile, freshly ground 2-mercaptopyridine *N*-oxide sodium salt (**S3**, 1.12 g, 7.52 mmol) and 4-(*N,N*-dimethylamino)pyridine (DMAP) (6 mg, mmol) were suspended in anhydrous chloroform (50 mL) under an argon atmosphere before being heated to reflux while irradiating with a 500-W tungsten lamp. [Note: chloroform was washed with water to remove ethanol and subsequently placed over 3Å molecular sieves for 16h]. The freshly formed acid chloride solution was then dissolved in anhydrous chloroform and added dropwise to the refluxing mixture over 30 minutes. The suspension was refluxed for 4h before cooling to room temperature and quenching with water (2 x 25 mL). The organic phase was partitioned and dried over sodium sulfate. The solution was concentrated *in vacuo* to give a brown oil which was purified by column chromatography (8% ethyl acetate/n-hexane v/v) to afford the titled compound **S4** (635 mg, 81%) as a white sweet smelling solid.

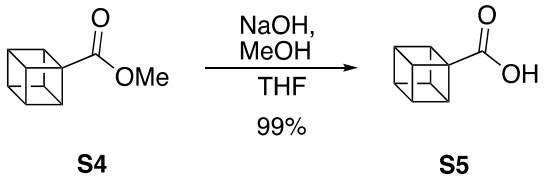
m.p. 54.8-55.5℃ (Lit.[4] 51.2-52.9℃);

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 4.27–4.23 (m, 3H), 4.04–3.98 (m, 4H), 3.70 (s, 3H);

^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 173.0, 55.8, 51.6, 49.6, 48.0, 45.3;

LRMS (ESI): *m/z* $[C_{10}H_{10}O_2]^+$ (M^+): 162.

Cubane-1-carboxylic acid (**S5**)



Following the procedure of Wlochal *et al*:[5] methyl cubane-1-carboxylate (**S4**) (620 mg, 3.83 mmol) was dissolved in THF (20 mL). A methanolic solution of sodium hydroxide (4.0 M, 1.1 mL, 4.40 mmol)

was added slowly, giving a cloudy solution. After stirring the mixture at room temperature for 16 hours, the THF was removed under vacuum, and the residual solid was suspended in water 20 mL. The suspension was washed with DCM (3 x 10 mL) and the aqueous phase was carefully acidified to pH 2 with hydrochloric acid (1.0 M). The acidified aqueous layer was extracted with DCM (1 x 20 mL, 2 x 15 mL). The combined organic phases were dried over sodium sulfate, and the solvent was removed under vacuum to give the titled compound **S5** (570 mg, 99 %) as a white solid, which was directly used without further purification.

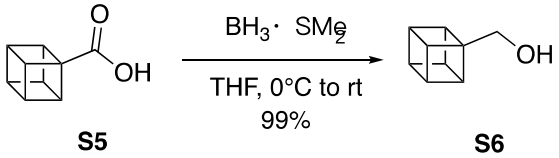
m.p. 123.5-125.4℃ (Lit.[6] 124-125℃);

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 4.32–4.27 (m, 3H), 4.06–3.99 (m, 4H);

^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 178.1, 55.5, 49.6, 48.0, 45.4;

LRMS (ESI): *m/z* $[C_9H_8O_2]^+$ (M^+): 148; ($M+H^+$): 149.

Cubylmethanol (**S6**)



S5 **S6**

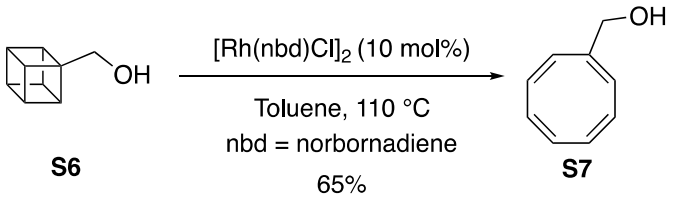
Following the procedure of Priefer *et al*:[6] borane dimethylsulfide complex (5 M in diethyl ether, 1.20 mL, 6.00 mmol) was slowly added to a solution of 4-methoxycarbonylcubane-1-carboxylic acid (**S5**) (300 mg, 2.03 mmol) in anhydrous THF (25 mL) at 0 °C under an argon atmosphere. The solution was stirred at 0 °C for 30 minutes, warmed to room temperature over 30 mins, and then cautiously quenched by addition of water (1 mL). The reaction mixture was extracted with diethyl ether (3 × 20 mL), and the combined organic phases were washed with water (25 mL), saturated sodium bicarbonate solution (20 mL) and brine (20 mL). The organic phase was dried over sodium sulfate, concentrated in vacuo to obtain the titled compound **S6** (266 mg, 99%) of as white solid.

m.p 62.6-64.2℃ (Lit.[6] 59-63℃);

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 4.06–4.01 (m, 1H), 3.93–3.87 (m, 6H), 3.75 (s, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 64.1, 58.5, 48.8, 46.9, 44.6;

LRMS (ESI): *m/z* $[C_9H_{10}O]^+$ (M^+): 134.

(Cyclooctatetraenyl)methanol (**S7**)

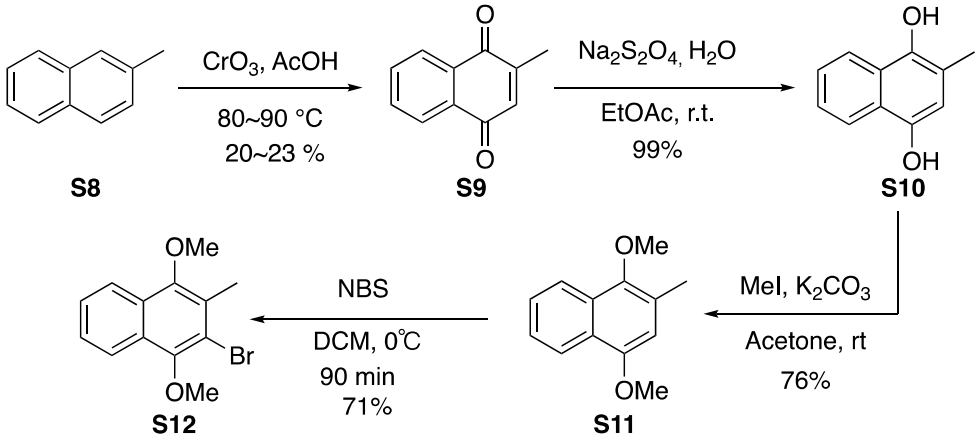


Following the procedure of Houston *et al*:[7] cubylmethanol (**S6**) (26 mg, 0.20 mmol) and bicyclo[2.2.1]heptan-2,5-diene-rhodium(I) chloride dimer (9 mg, 0.02 mmol) were suspended in anhydrous toluene and heated at 110℃ under an argon atmosphere. The reaction was monitored by ^{1}H NMR. When the reaction was completed (18 hours), the toluene was removed under vacuum and the residue was purified by column chromatography (10% ethyl acetate/n-hexane v/v) to give the titled compound **S7** (17 mg, 65 %) as a yellow oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 5.99-5.82 (m, 7H),4.07 (s, 3H);

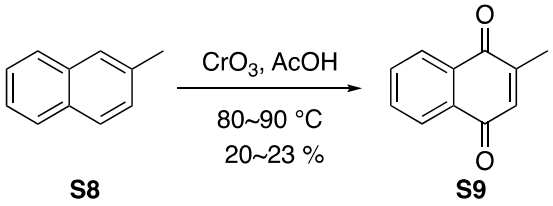
^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 143.7, 133.5, 132.2, 131.9, 131.7, 131.6, 127.4, 66.5; LRMS ESI: LRMS (ESI): *m/z* $[C_9H_{10}ONa]^+$ $(M+Na)^+$: 157.

Synthesis of 2-bromo-1,4-dimethoxy-3-methylnaphthalene



Scheme S2. Synthesis of 2-bromo-1,4-dimethoxy-3-methylnaphthalene.

2-Methyl-1 ,4-Naphthoquinone (**S9**)



Following the procedure of Fieser *et al:*[8] *β*-methylnaphthalene (**S8**) (4.26 g, 30.0 mmoL) was dissolved in glacial acetic acid (45 mL). Chromic anhydride (15.0 g, 150 mmol) in water (10 mL) was

diluted with glacial acetic acid (10 mL), which was added to the β-methylnaphthalene solution dropwise. The inner temperature of the reaction vessel was maintained at 60℃ with an external ice-water bath. When the temperature rapidly decreased, the ice-water bath was removed and the mixture was stirred at 80-90 ℃ (internal) for 1 hour then the reaction was allowed to cool to room temperature. Water (150 mL) was added and after stirring for several minutes, a precipitation formed, which was collected and thoroughly washed with water. The crude was recrystallized from methanol to give the titled compound **S9** (1.23 g, 23%) as yellow crystals.

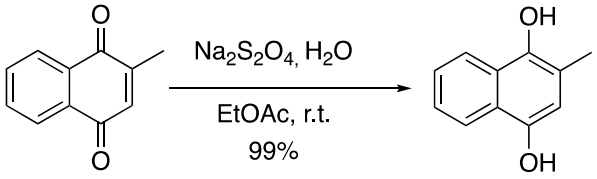
m.p 103.7-104.6℃ (Lit.[8] 105-106℃);

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 8.12-8.04 (m, 2H), 7.74-7.71 (m, 2H), 6.84 (q, *J* = 1.54 Hz, 1H), 2.20 (d, *J* = 1.54 Hz, 3H), ;

^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 185.7, 185.1, 148.3, 135.8, 133.8, 133.7, 132.4, 132.3, 126.7, 126.2, 16.6;

LRMS (ESI): *m/z* $[C_{11}H_{10}O_2]^+$ $(M+H)^+$: 173.

1,4-Dihydroxy-2-methylnaphthalene (**S10**)



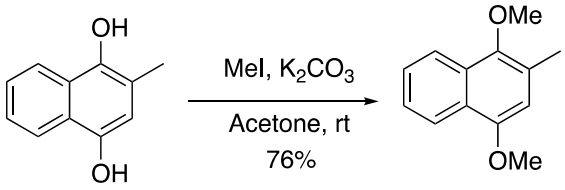
S9 **S10**

Following the procedure of Fujii *et al*:[9] To a solution of 2-methyl-1,4-naphthoquinone (**S9**) (400mg, 2.33 mmol) in ethyl acetate (4.6 mL) was added an aqueous solution of $Na_2S_2O_4$ (881 mg, 50.6 mmol, in 5mL water) at 0℃. The mixture was stirred at room temperature for 3 hours under an argon atmosphere. After diluting the mixture with a saturated NH_4Cl aqueous solution, ethyl acetate (3 x 20 mL) was used to extract the reaction mixture and the organic phase was washed with brine before being dried over sodium sulfate. The solvent was then removed under vacuum to give the titled compound **S10** (400 mg, 99 %) as a purple coloured solid, which was immediately used without further purification.

^{1}H-NMR (300 MHz, DMSO-d_6): δ (ppm) 9.33 (s, 1H), 8.24 (s, 1H), 8.07-7.97 (m, 2H), 7.42-7.30 (m, 2H), 6.61 (s, 1H), 2.27 (s, 3H);

^{13}C-NMR (75 MHz, DMSO-d_6): δ (ppm) 145.8, 141.7, 126.5, 124.9, 123.8, 123.5, 121.9, 121.7, 118.7, 111.1, 16.6;

1,4-Dimethoxy-2-methylnaphthalene (**S11**)



S10 **S11**

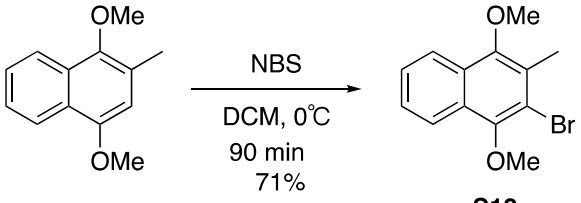
Following the procedure of Fujii *et al*:[9] To a 0 °C solution of 1,4-dihydroxy-2-methylnaphthalene (**S10**) (108 mg, 1.52 mmol) in anhydrous acetone (5.6 mL) was added iodomethane (0.56 mL, 9 mmol) and potassium carbonate (K_2CO_3) (0.690 g, 5.00 mmol). The mixture was then stirred at room temperature for 16 hours under an argon atmosphere. After diluting the reaction with diethyl ether (10 mL) and water (20mL), it was extracted with ethyl acetate (3 x 20 mL). The combined organic phases were washed with brine before being dried over sodium sulfate. The solvent was then removed under vacuum and the residue was purified by column chromatography (5% ethyl acetate/n-hexane v/v) to give the titled compound **S11** (308 mg, 76 %) as a colourless oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 8.21 (d, 1H, J=8.4Hz), 8.03 (d, 1H, J=8.4Hz), 7.51 (ddd, 1H, J=7.6, 7.1, 1.6 Hz), 7.42 (ddd, 1H, J = 7.6, 7.1, 1.6 Hz), 6.61 (s, 1H), 3.97 (s, 3H), 3.87 (s, 3H), 2.45 (s, 3H) ;

^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm)151.7, 147.1, 128.8, 126.6, 125.7, 125.4, 124.70, 122.3, 121.6, 107.0, 61.4, 55.8, 16.4;

LRMS (ESI): *m/z* $[C_{13}H_{14}O_2]^+$ (M^+): 202; $[C_{13}H_{15}O_2]^+$ $(M+H)^+$: 203.

2-Bromo-1,4-dimethoxy-3-methylnaphthalene (**S12**)



S11 **S12**

Following the procedure of Fujii *et al*:[9] To a solution of 1,4-dimethoxy-2-methylnaphthalene (**S11**) (308 mg, 1.52 mmol) in anhydrous DCM (2.2 mL) was added *N*-bromosuccinimide (271 mL, 1.52 mmol) at 0 °C. The mixture was stirred at 0 °C for 1.5 hours under an argon atmosphere. After quenching the reaction with 5% aqueous $Na_2S_2O_3$ solution, it was extracted with ethyl acetate (3 × 20 mL). The combined organic phases were washed with brine before being dried over sodium sulfate. The solvent was removed under vacuum and the residue was purified by column chromatography (3% ethyl acetate/n-hexane v/v) to give the titled compound **S12** (303 mg, 71 %) as a white solid.

m.p 82.6 - 83.6℃(Lit.[10] 84-85℃)

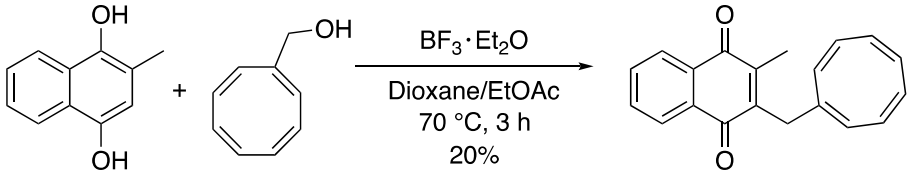
^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 8.10–8.04 (m, 2H) 7.56–7.47 (m, 2H), 3.98 (s, 3H), 3.88 (s, 3H), 2.54 (s, 3H);

^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 150.6, 150.0, 128.0, 127.7, 127.4, 126.7, 126.3, 122.6, 122.5, 117.4, 61.8, 61.4, 16.8;

LRMS (ESI): *m/z* $[C_{13}H_{13}O_2{}^{79}Br]^+$ $(M)^+$: 280; $[C_{13}H_{13}O_2{}^{81}Br]^+$ $(M)^+$: 282.

Synthesis of vitamin K3 analogues

2-(Cyclooctatetraen-1-ylmethyl)-3-methylnaphthalene-1,4-dione (**S13**)



S10 **S7** **S13**

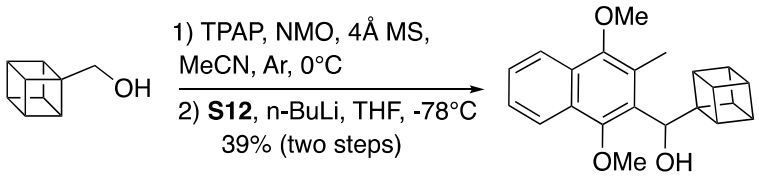
Adapted from the procedure of Suhara *et al*:[11] freshly prepared anhydrous menadiol (**S10**) (52 mg, 0.30 mmol) was dissolved in anhydrous 1,4-dioxane and ethyl acetate (1:1, 1mL). To this solution was added COT **S7** (90 mg, 0.6 mmol), followed by dropwise addition of boron trifluoride etherate (50 µL, 57 mg, 0.4 mmol). The mixture was stirred at 70℃ for 3 hours under an argon atmosphere before being cooled to room temperature. The reaction was then quenched with ice cooled water (10 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic phases were washed with water (20 mL), brine (20 mL), and dried over sodium sulfate. After removing the solvent *in vacuo*, the residue was purified by column chromatography (3% ethyl acetate/n-hexane v/v) to give the titled compound **S13** (16 mg, 20 %) as a yellow oil. A sample was further purified by HPLC (flow rate: 2.5 mL/min, 5 % propan-2-ol/n-hexane to 15% i-PrOH/n-Hexane in 10 min followed by 15% i-PrOH/n-Hexane to 5% i-PrOH/n-Hexane 2 min; Then, 5% i-PrOH/n-Hexane hold for 8 min; retention time 6.8 min).

^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 8.11–8.07 (m, 2H), 7.72–7.68 (m, 2H), 5.81–5.55 (m, 7H), 2.25 (br s, 2H), 2.25 (s, 3H);

^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 185.4, 184.4, 145.4, 144.4, 139.8, 133.8, 133.6, 133.5, 132.3, 132.2, 132.1, 132.0, 131.8, 131.7, 131.4, 127.9, 126.7, 126.4, 33.9, 13.4.

HRMS (ESI): *m/z* calculated for: $[C_{20}H_{16}O_2Na]^+$ $(M+Na)^+$: 311.1043; found: 311.1043. $[C_{20}H_{17}O_2]^+$ $(M+H)^+$: 289.1224; found: 289.1223.

(Cuban-1-yl)(1,4-dimethoxy-3-methylnaphthalen-2-yl)methanol (**S14**)



S6 **S14**

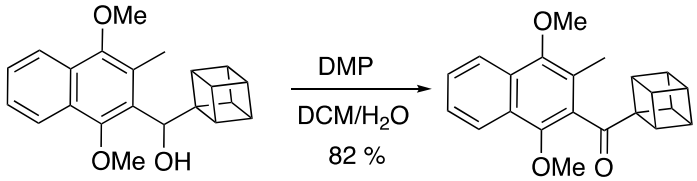
Adapted from the combined procedures of Moore *et al*[12] and Sidorov *et al*[13]. Cubylmethanol (**S6**) (120 mg, 0.896 mmol), *N*-Methylmorpholine *N*-oxide (NMO) (183 mg, 1.56 mmol) and 4Å molecular sieves were suspended in anhydrous acetonitrile (3 mL) at 0 ℃ under an argon atmosphere. The mixture was stirred for 1hour and tetrapropylammonium perruthenate (TPAP) (15 mg, 0.050 mmol) was added. The mixture was then warmed to room temperature and left to stir for 15 minutes until the complete consumption of cubane **S6** (monitored by TLC). Water (10 mL) was then added and the mixture was quickly extracted with low boiling petroleum ether (3 x 10 mL). The combined organic phases were dried over sodium sulfate and then concentrated *in vacuo* to around 10 mL.The aldehyde was then diluted with anhydrous THF (5 mL) and further dried by 3Å molecular sieves under argon for 1 hour before being cooled to -78℃. To a solution of bromide **S12** (252 mg, 0.900 mmol) in anhydrous THF (5 mL) was added n-BuLi (1.6 M in n-hexane solution, 630 µL, 1.00 mmol) dropwise. The mixture was stirred at -78℃ under argon for 30 minutes before addition of the pre-cooled aldehyde was added to the above solution dropwise via cannular transfer. The mixture became immediately reddish in colour, and was stirred at -78℃ for 40 minutes until complete consumption of the aldehyde (monitored by TLC) had occurred. On warming the reaction to the room temperature, hydrochloric acid (1.0 M) solution was carefully added. The reaction was then extracted with diethyl ether (3 × 15 mL), and the combined organic phases were washed with aqueous saturated sodium hydrogencarbonate ($NaHCO_3$) solution (30 mL), brine (30 mL), and dried over sodium sulfate. After removing the solvent *in vacuo*, the residue was purified by column chromatography (20% ethyl acetate/n-hexane v/v) to give the titled compound **S14** (118 mg, 39 %) as a white semi-solid.

^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 8.07 (m, 1H), 8.01 (m, 1H),7.51-7.45 (m, 2H), 5.35 (s, 1H), 3.97-3.94 (m, 3H), 3.94 (s, 3H), 3.85 (s, 3H), 3.84-3.81 (m, 4H), 2.39 (s, 3H);

^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 150.7, 150.4, 129.1, 128.3, 127.2, 126.1, 125.6, 122.5, 122.4, 72.5, 63.6, 61.9, 61.5, 48.4, 48.3, 44.1, 13.4;

HRMS (ESI): *m/z* calculated for: $[C_{22}H_{22}O_3Na]^+$ $(M+Na)^+$: 357.1462; found: 357.1461.

(Cuban-1-yl)(1,4-dimethoxy-3-methylnaphthalen-2-yl)methanone (**S15**)



S14 **S15**

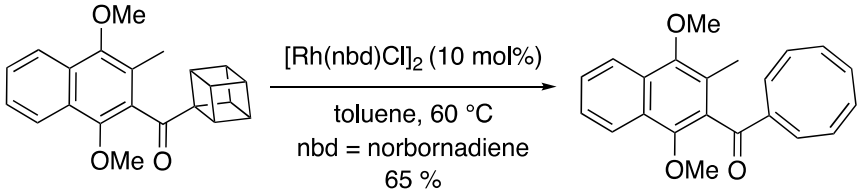
Adapted from the procedure of Meyer *et al*.[14] To a solution of **S14** (113 mg, 0.338 mmol) in anhydrous DCM (2 mL) and DMP (144 mg, 0.339 mmol) was added wet DCM (6 mL) dropwise. The solution was stirred vigorously at room temperature until complete consumption of **S14** (15 min) had occurred as monitored by TLC. The reaction mixture was then poured onto a 1:1 by volume solution of 10% $Na_2S_2O_3$ and saturated $NaHCO_3$ (20 mL), and the subsequent organic phase was washed with water (20 mL), brine (20 mL) and dried over sodium sulfate. After removing the solvent *in vacuo*, the residue was purified by column chromatography (15% ethyl acetate/n-hexane v/v) to give the titled compound **S15** (93 mg, 82%)of as a yellow oil.

^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 8.09-8.05 (m, 2H), 7.57-7.49 (m, 2H), 4.38-4.34 (m, 3H), 4.01-3.97 (m, 4H), 3.88 (s, 3H), 3.85 (s, 3H), 2.28 (s, 3H);

^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 207.2, 150.6, 149.2, 131.3, 129.3, 127.3, 127.1, 126.0, 123.2, 122.7, 122.5, 64.3, 63.9, 61.5, 51.5, 48.1, 45.1, 13.1;

HRMS (ESI): *m/z* calculated for: $[C_{22}H_{20}O_3Na]^+$ $(M+Na)^+$: 355.1305; found: 355.1305. $[C_{22}H_{21}O_3]^+$ $(M+H)^+$: 333.1486; found: 333.1485.

(Cyclooctatetraen-1-yl)(1,4-dimethoxy-3-methylnaphthalen-2-yl)methanone (**S16**)



S15 **S16**

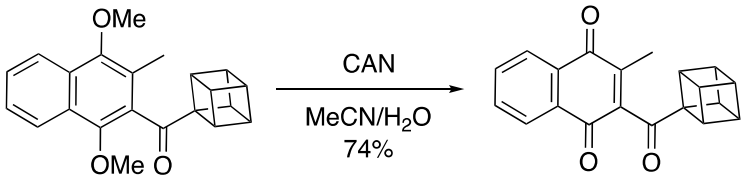
Adapted from the procedure of Houston *et al*.[7] Compound **S15** (20 mg, 0.060 mmol) and bicyclo[2.2.1]heptan-2,5-diene-rhodium(I) chloride dimer (5 mg, 0.01 mmol) were dissolved in anhydrous toluene and heated at 60℃ under an argon atmosphere. After 4 hours the reaction mixture was cooled and the toluene removed under vacuum. The residue was purified by column chromatography (20:5:75 DCM/ethyl acetate/n-hexane v/v) to give the titled compound **S16** (13 mg, 65 %) as a yellow oil.

^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 8.10-8.05 (m, 2H), 7.57-7.49 (m, 2H), 6.57 (br s, 1H), 6.27-5.79 (m, 6H), 3.90 (s, 3H), 3.89 (s, 3H), 2.30 (s, 3H);

^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm)197.4, 150.3, 149.0, 147.3, 143.1, 134.4, 133.8, 132.6, 131.3, 131.2, 130.2, 129.6, 129.3, 127.2, 127.1, 126.1, 123.7, 122.7, 122.5, 63.8, 61.7, 12.9.

HRMS (ESI): *m/z* calculated for: $[C_{22}H_{20}O_3Na]^+$ $(M+Na)^+$: 355.1305; found: 355.1287.

(Cuban-1-yl)(3-methylnaphthalen-2-yl)methanone (**S17**)



S15 **S17**

Adapted from the procedure of Annadi and Wee *et al*.[15] To a suspension of compound **S15** (50 mg, 0.15 mmol) in acetonitrile (2 mL) was added an aqueous CAN solution (1.0 M, 230 mg 0.420 mmol) in one portion. The mixture was stirred at room temperature for 30 min. The suspension was diluted with ethyl acetate (15 mL) and poured onto a saturated solution of $NaHCO_3$ (20 mL). The white precipitate which formed in the aqueous layer was vacuum filtered through a pad of celite®. After washing the precipitate with ethyl acetate several times, the organic phase was separated. The aqueous layer was back extracted with ethyl acetate (15 x 2 mL) and the combined organic phases were washed with brine (30 mL) and dried over sodium sulfate. After removing the solvent *in vacuo*, the residue was purified by column chromatography (8% ethyl acetate/n-hexane v/v) to give the titled compound **S17** (31 mg, 74 %) as a brown solid. A sample was further purified by HPLC (flow rate: 2.5 mL/min, 10 % propan-2-ol/n-hexane isocratic, retention time 7.2 min).

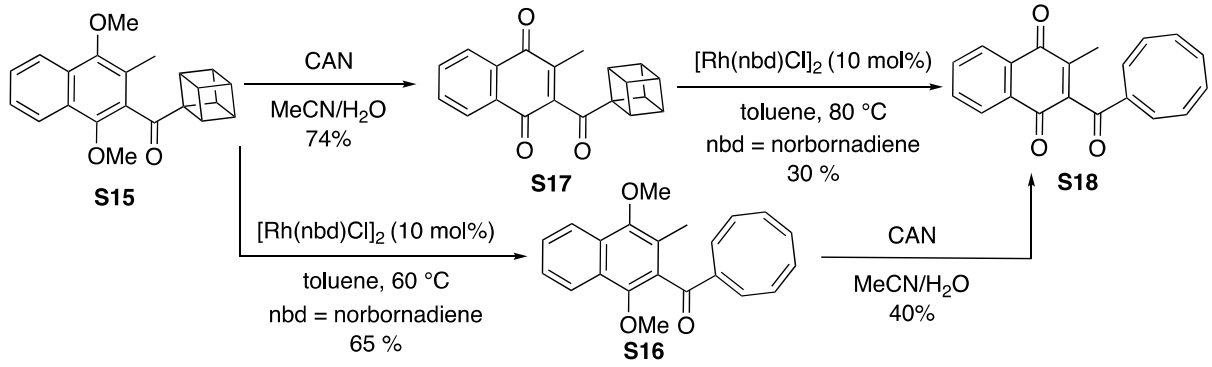
m.p 121.4-122.5℃;

^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 8.14-8.10 (m, 1H), 8.08-8.04 (m, 1H),7.78-7.74 (m, 2H), 4.40-4.35 (m, 3H), 4.05-3.97 (m, 4H), 2.11 (s, 3H);

^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 202.6, 185.3, 183.8, 144.5, 143.7, 134.5, 131.9, 131.6, 126.8, 126.4, 63.4, 50.8, 48.1, 45.3, 13.9.

HRMS (ESI): *m/z* calculated for: $[C_{20}H_{14}O_3Na]^+$ $(M+Na)^+$: 325.0836; found: 325.0830. $[C_{20}H_{15}O_3]^+$ $(M+H)^+$: 303.1016; found: 303.1011.

2-(Cyclooctatetraene-1-carbonyl)-3-methylnaphthalene-1,4-dione (**S18**)



Method A: Adapted from the procedure of Houston *et al*:[7] Compound **S17** (15 mg, 0.050 mmol) and bicyclo[2.2.1]heptan-2,5-diene-rhodium(I) chloride dimer (4.6 mg, 0.010 mmol) were suspended in anhydrous toluene and heated at 80℃ under an argon atmosphere. When the reaction was completed (3 hours), the solvent was removed under vacuum and the residue was purified by column chromatography (5% ethyl acetate/n-hexane v/v) to give the titled compound **S18** (3 mg, 30 %) as a yellow oil.

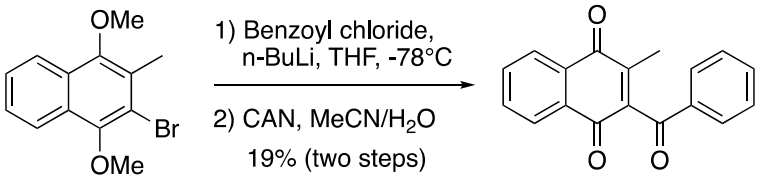
Method B: Adapted from the procedure of Annadi and Wee *et al*:[15] To a solution of compound **S16** (20 mg, 0.066 mmol) in acetonitrile (1 mL) was added an aqueous solution of CAN (1.0 M, 100 mg 0.182 mmol) in one portion. The mixture was stirred at room temperature for 30 minutes. The suspension was diluted with ethyl acetate (10 mL) and poured onto a saturated solution of $NaHCO_3$ (15 mL). The white precipitate which formed within aqueous layer was vacuum filtered through a pad of celite®. After washing the precipitate with ethyl acetate several times, the organic phase was separated. The aqueous layer was back extracted with ethyl acetate (10 x 2 mL) and the combined organic phases were washed with brine (20 mL) and dried over sodium sulfate. After removing the solvent *in vacuo*, the residue was purified by column chromatography (45% DCM/n-hexane v/v) to give the titled compound **68** (7 mg, 40 %) as a yellow oil. A sample was further purified by HPLC (flow rate: 2.5 mL/min, 10 % propan-2-ol/n-hexane isocratic, retention time 7.5 min).

^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 8.14-8.11 (m, 1H), 8.09-8.05 (m, 1H), 7.78-7.74 (m, 2H), 6.78 (br s, 1H), 6.15-5.80 (m, 6H), 2.12 (s, 3H);

^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 193.4, 184.9, 183.3, 147.3, 144.8, 143.8, 142.1, 135.1, 134.7, 134.3, 134.2, 132.6, 132.0, 131.7, 131.2, 129.7, 128.5, 126.8, 126.5, 13.7.

HRMS (ESI): *m/z* calculated for: $[C_{20}H_{14}O_3Na]^+$ $(M+Na)^+$: 325.0836; found: 325.0830. $[C_{20}H_{15}O_3]^+$ $(M+H)^+$: 303.1016; found: 303.1010.

2-Benzoyl-3-methylnaphthalene-1,4-dione (**S19**)



S12 **S19**

Follow the procedure of Sidorov *et al*.[13] To the solution of bromide **S12** (70 mg, 0.25 mmol) in anhydrous THF (2 mL) was added n-BuLi (1.0 M in n-hexane solution, 280 µL, 0.28 mmol) dropwise. The mixture was stirred at -78℃ under an argon atmosphere for 30 minutes. Then, a solution of benzoyl chloride in anhydrous THF (1 mL) was added slowly to the above solution dropwise via cannular transfer. The mixture was stirred at -78℃ for 40 minutes until complete consumption of the acid chloride was observed. On warming the reaction to the room temperature, hydrochloric acid (1.0 M) solution was carefully added. Then the reaction mixture was extracted with diethyl ether (3 x 15 mL), and the combined organic phases were washed with a saturation aqueous solution of sodium hydrogencarbonate ($NaHCO_3$) (30 mL), brine (40 mL), and dried over sodium sulfate. After removing the solvent *in vacuo*, the residue was purified by column chromatography (40% DCM/n-Hexane v/v).

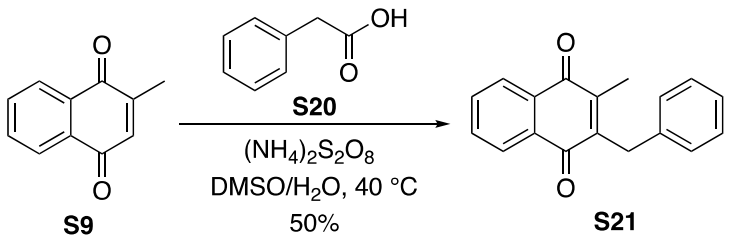
The desired benzoyl derivative (30 mg, 0.098 mmol)above was dissolved in acetonitrile (1.2 mL) and an aqueous solution of CAN (1.0 M, 225 mg, 0.41 mmol) was added. The reaction mixture was stirred at room temperature for 30 minutes. The suspension was diluted by ethyl acetate (10 mL) and poured onto a saturated solution of $NaHCO_3$. The white precipitate which formed within the aqueous layer was vacuum filtered through a pad of celite®. After washing the precipitate with ethyl acetate several times, the organic phase was separated. The aqueous layer was back extracted with ethyl acetate (10 x 2 mL) and the combined organic phases were washed with brine (20 mL) and dried over sodium sulfate. After removing the solvent *in vacuo*, the residue was purified by column chromatography (40% DCM/n-Hexane v/v) to give the titled compound **S19** (13 mg, 19% over two steps) as a yellow solid. A sample was further purified by HPLC (flow rate: 2.5 mL/min, 10 % Ethyl acetate/n-Hexane isocratic, retention time 21.2min).

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 8.21 – 8.14 (m, 1H), 8.10 – 8.04 (m, 1H), 7.95 – 7.89 (m, 2H), 7.83 – 7.74 (m, 2H), 7.68 – 7.60 (m, 1H), 7.54 – 7.46 (m, 2H), 2.07 (d, J = 0.3 Hz, 3H);

^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 193.8, 185.0, 183.5, 144.5, 144.1, 135.8, 134.7, 134.3, 134.3, 132.1, 131.7, 129.3, 129.2, 126.9, 126.6, 13.7;

LRMS (ESI): *m/z* $[C_{18}H_{13}O_3]^+$ $(M+H)^+$: 277.

2-Benzyl-3-methylnaphthalene-1,4-dione (**S21**)



Follow the procedure of Sutherland et al:[16] 2-Methyl-1,4-naphthoquinone (**S9**) (52mg. 0.3 mmol), phenylacetic acid (408mg, 3 mmol) and ammonium persulfate were added to a dry round bottom flask under an argon atmosphere. A solution of DMSO/H_2O (6 mL/10 µL; degassed by bubbling with argon for 15 mins) was then added, followed by stirring the reaction at 40 ℃ overnight. On completion the reaction was diluted with DCM (30 mL) and washed with saturated Na_2HCO_3 solution. The aqueous layer was extracted with DCM (15 x 2 mL). The combined organic phases were washed with brine (40 mL) and dried over sodium sulfate. After removing the solvent in vacuo, the residue was purified by column chromatography (2% ethyl acetate/n-hexane v/v) to give the titled compound **S21** (40 mg, 50 %) as a yellow solid. A sample was further purified by HPLC (flow rate: 2.5 mL/min, 5 % Ethyl acetate/n-Hexane isocratic, retention time 8.9min).

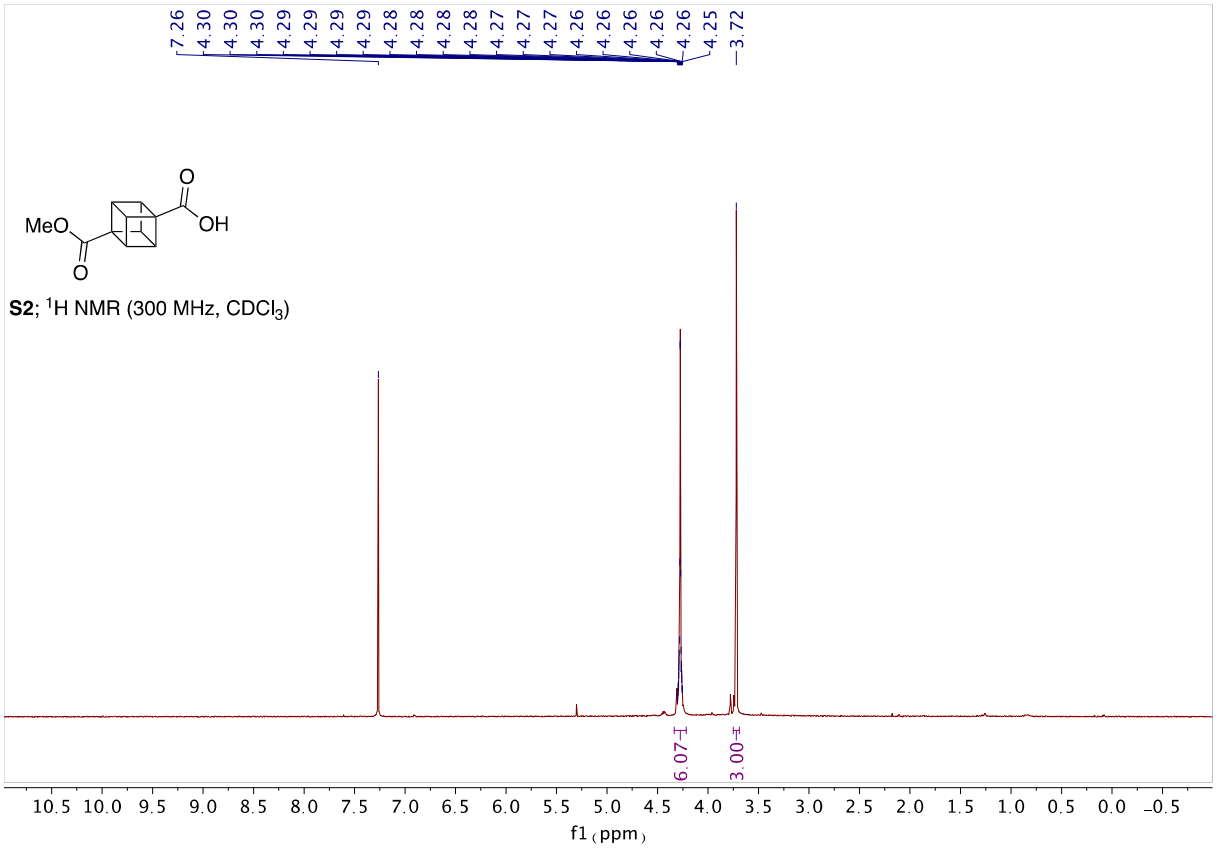
m.p 101.9-103.0℃ (Lit. [16] 106-108℃);

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 8.13 – 8.06 (m, 2H), 7.74 – 7.66 (m, 2H), 7.31 – 7.26 (m, 1H), 7.25 – 7.15 (m, 4H), 4.04 (s, 2H), 2.25 (s, 3H).;

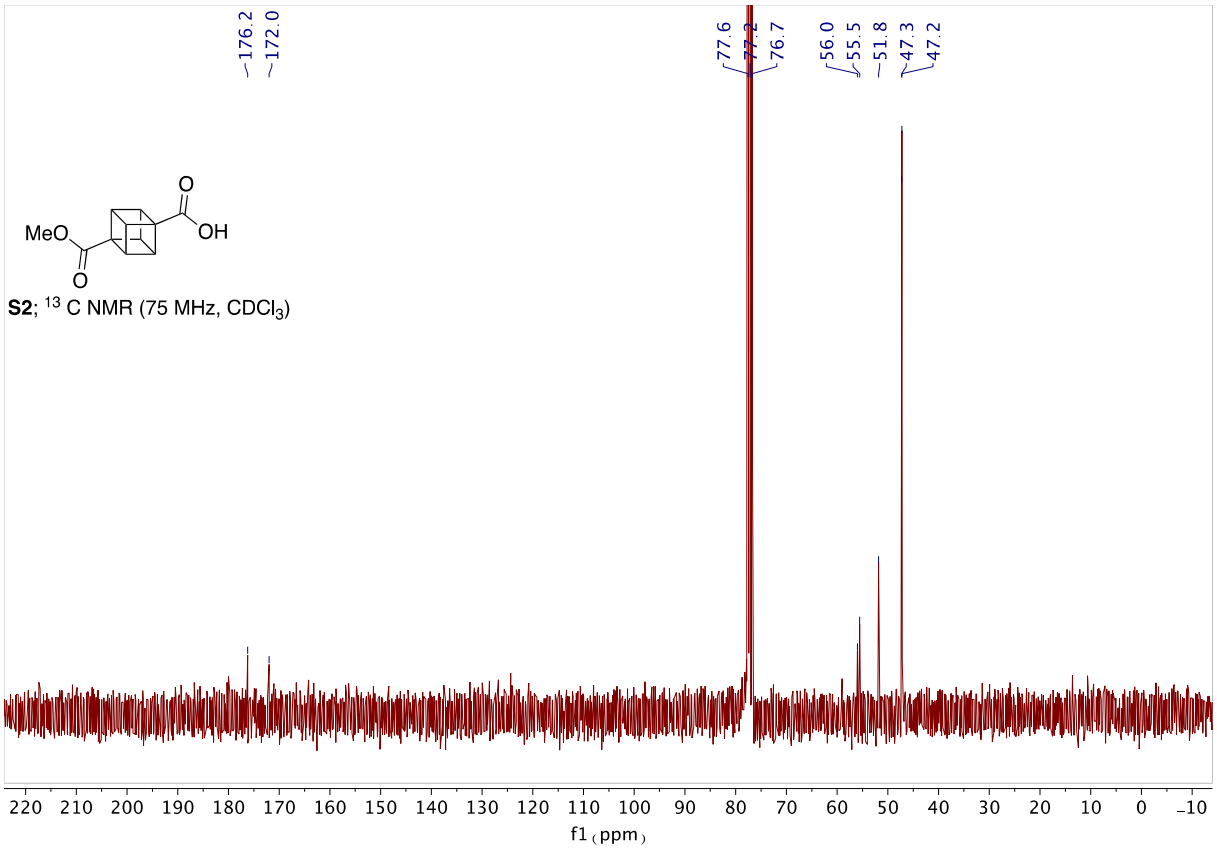
^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 185.5, 184.8, 145.5, 144.6, 138.2, 133.6, 133.6, 132.3, 132.2, 128.8, 128.7, 126.6, 126.6, 126.4, 32.6, 13.4;

LRMS (ESI): *m/z* $[C_{18}H_{15}O_2]^+$ $(M+H)^+$: 263.

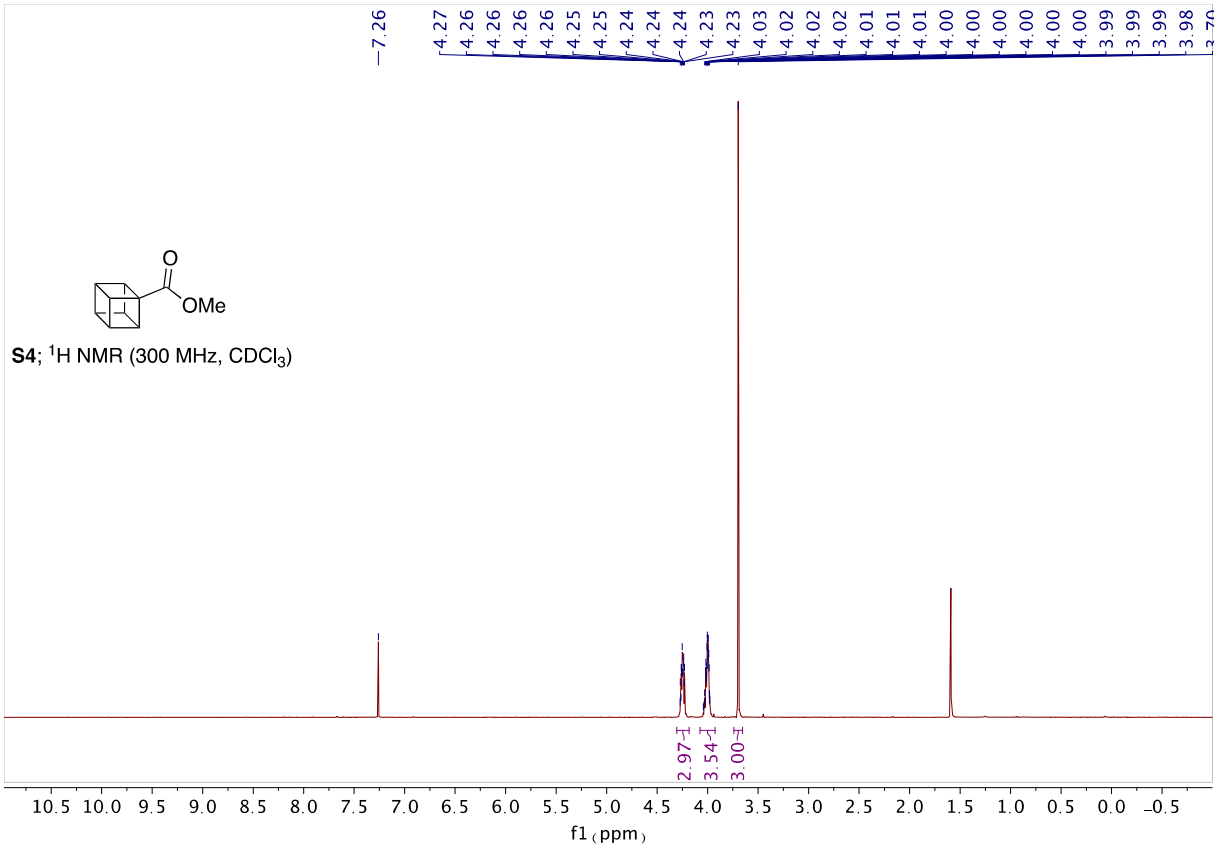
Supporting Information Part 2: ^{1}H and ^{13}C NMR Spectra



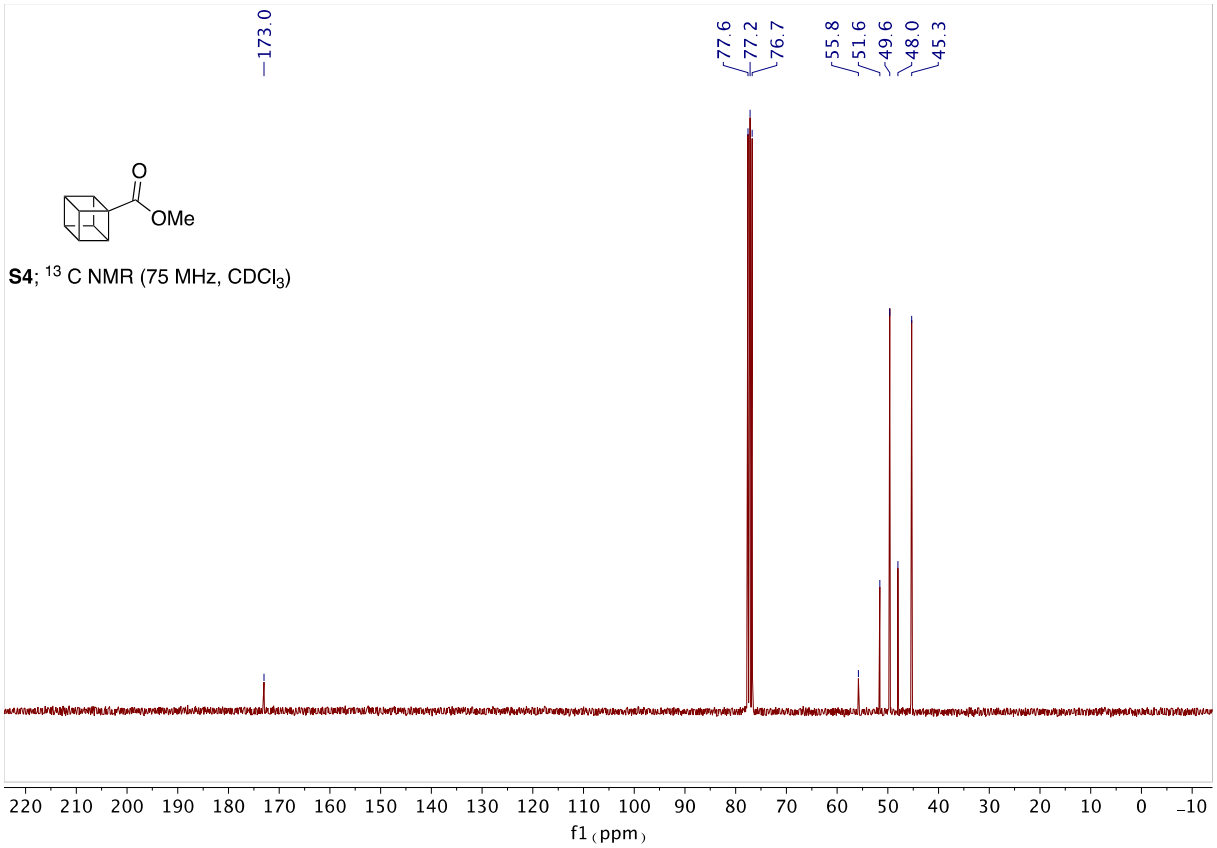
S2; ^{1}H NMR (300 MHz, $CDCl_3$)



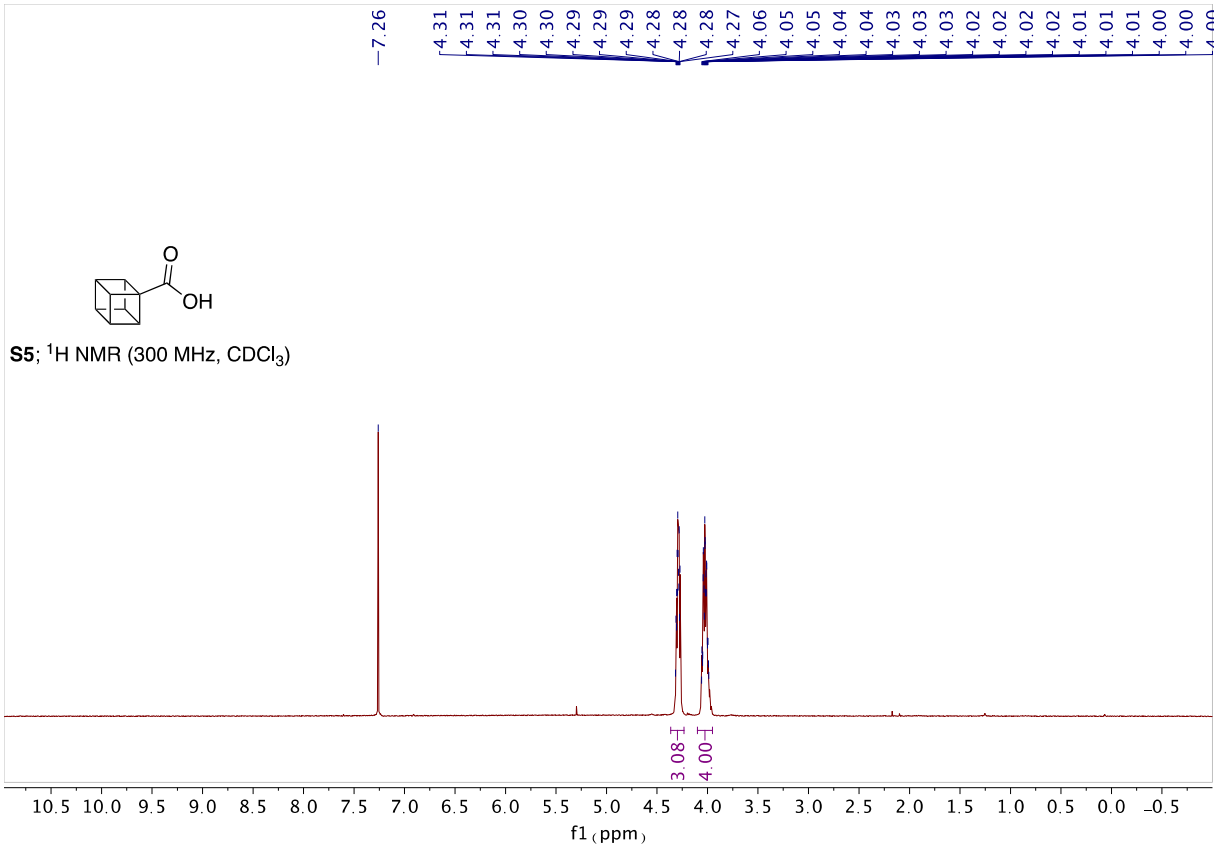
S2; ^{13}C NMR (75 MHz, $CDCl_3$)



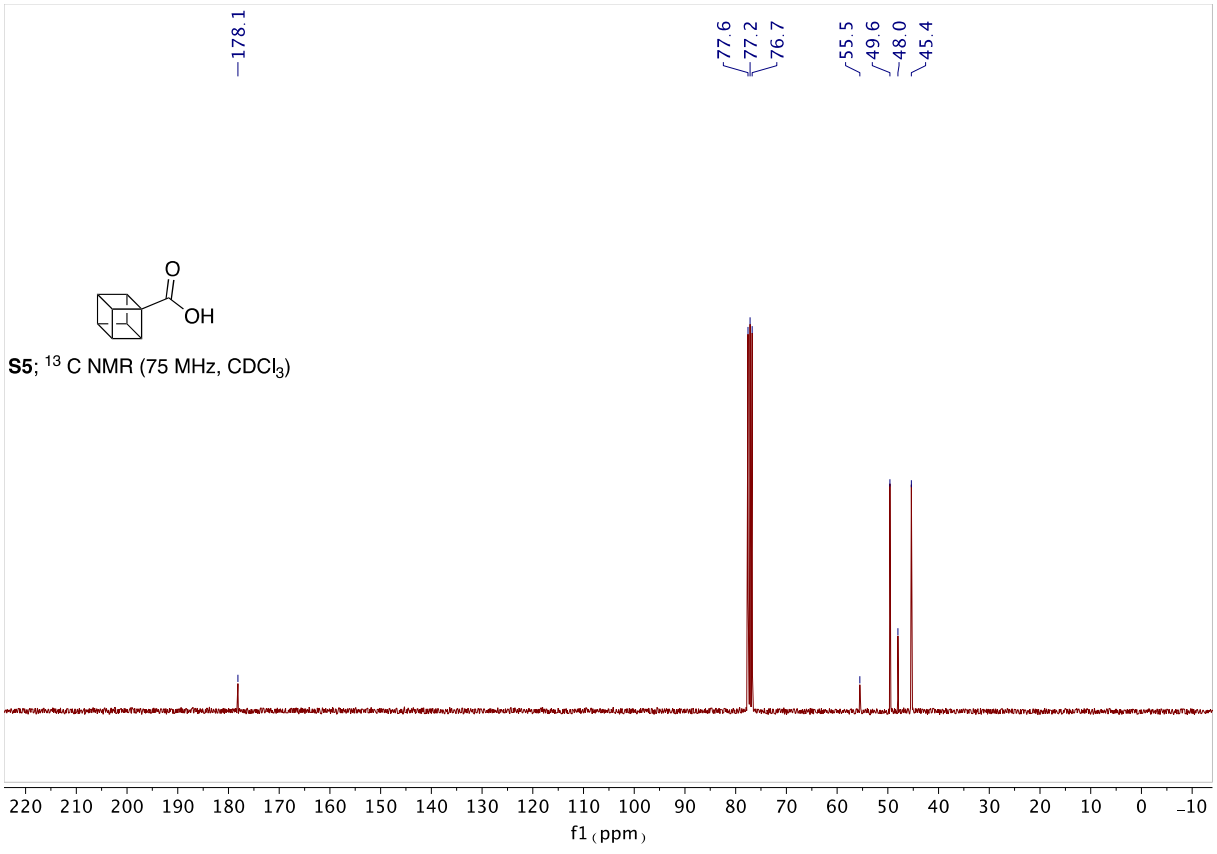
S4; ^{1}H NMR (300 MHz, $CDCl_3$)



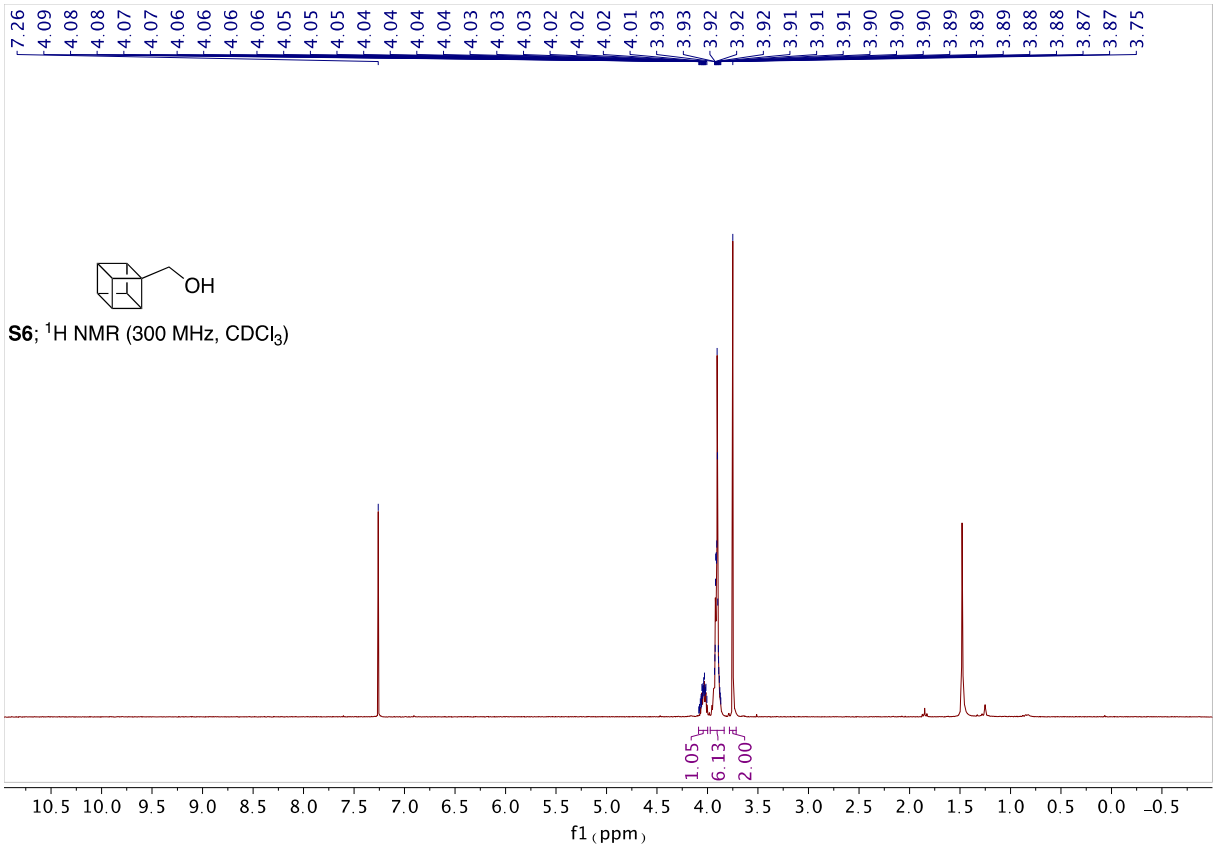
S4; ^{13}C NMR (75 MHz, $CDCl_3$)

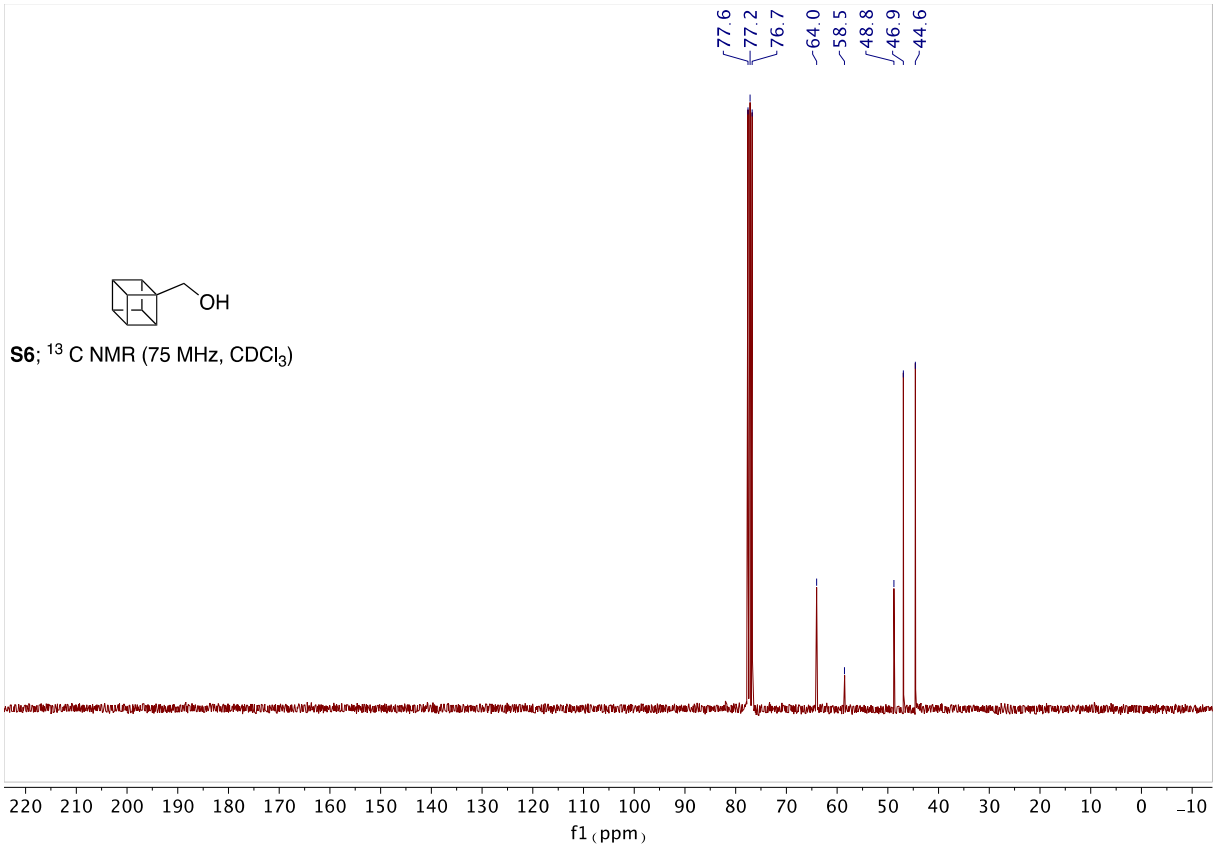


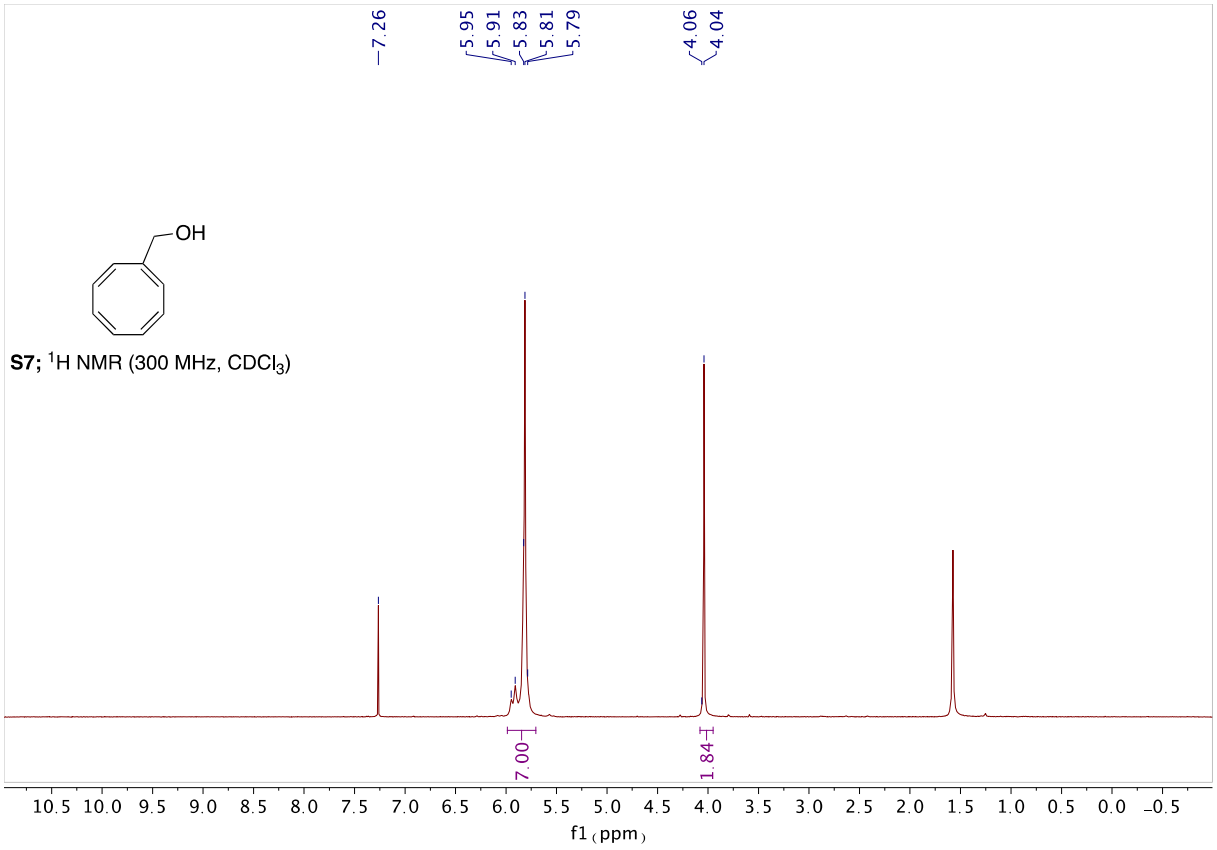
S5; ^{1}H NMR (300 MHz, $CDCl_3$)



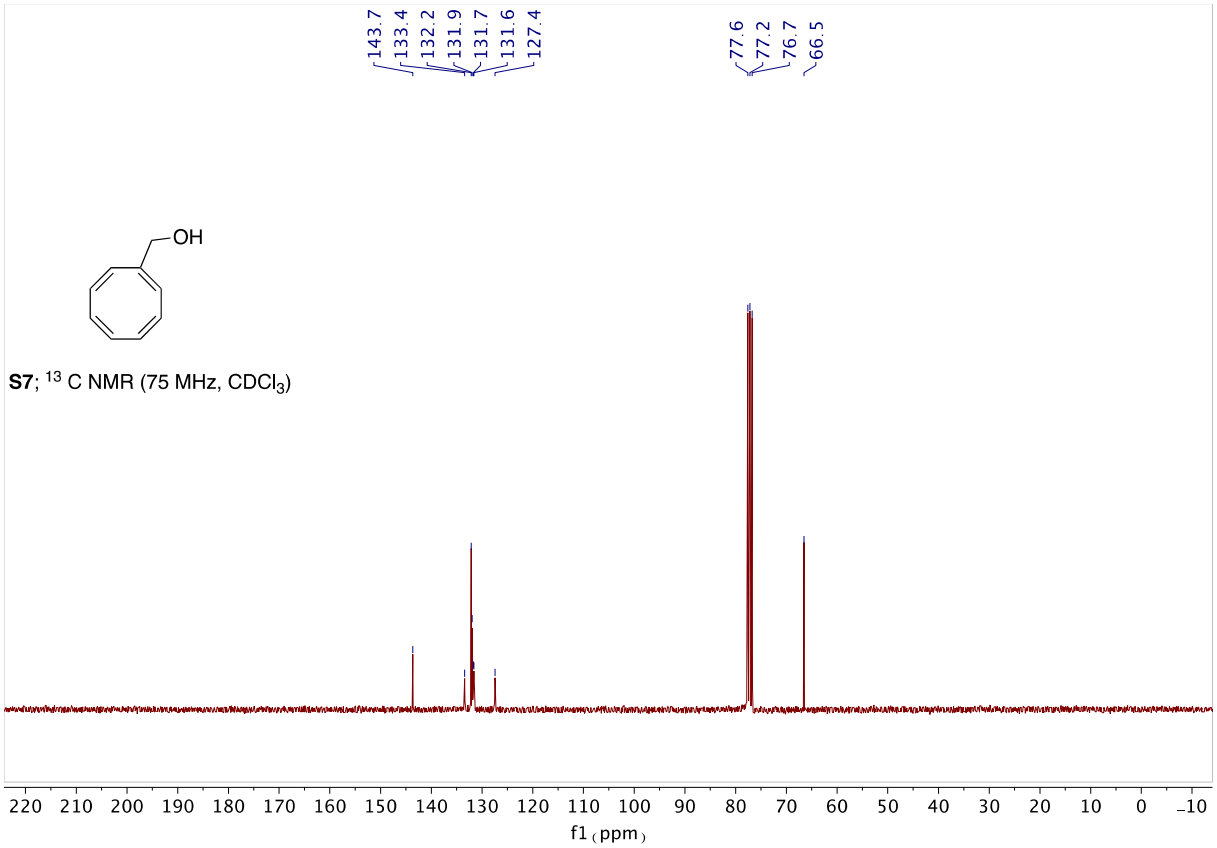
S5; ^{13}C NMR (75 MHz, $CDCl_3$)


OH
S6; 1H NMR (300 MHz, CDCl3)
7.26 4.09 4.08 4.08 4.07 4.07 4.06 4.06 4.06 4.06 4.05 4.05 4.05 4.04 4.04 4.04 4.04 4.03 4.03 4.02 4.02 4.02 4.01 3.93 3.93 3.92 3.92 3.92 3.91 3.91 3.91 3.90 3.90 3.90 3.89 3.89 3.89 3.88 3.88 3.87 3.87 3.75
1.05 6.13 2.00
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

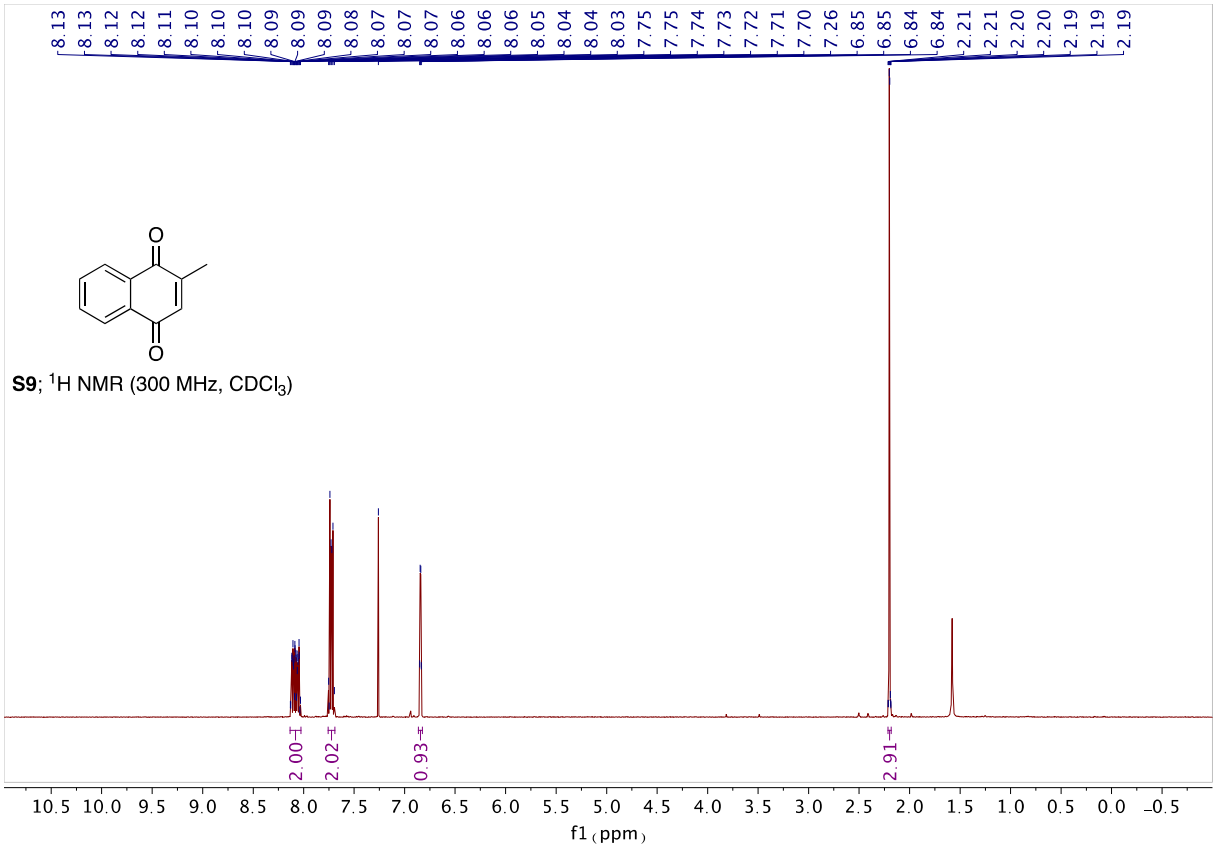

OH
S6; 13 C NMR (75 MHz, CDCl3)
77.6 77.2 76.7 64.0 58.5 48.8 46.9 44.6
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)



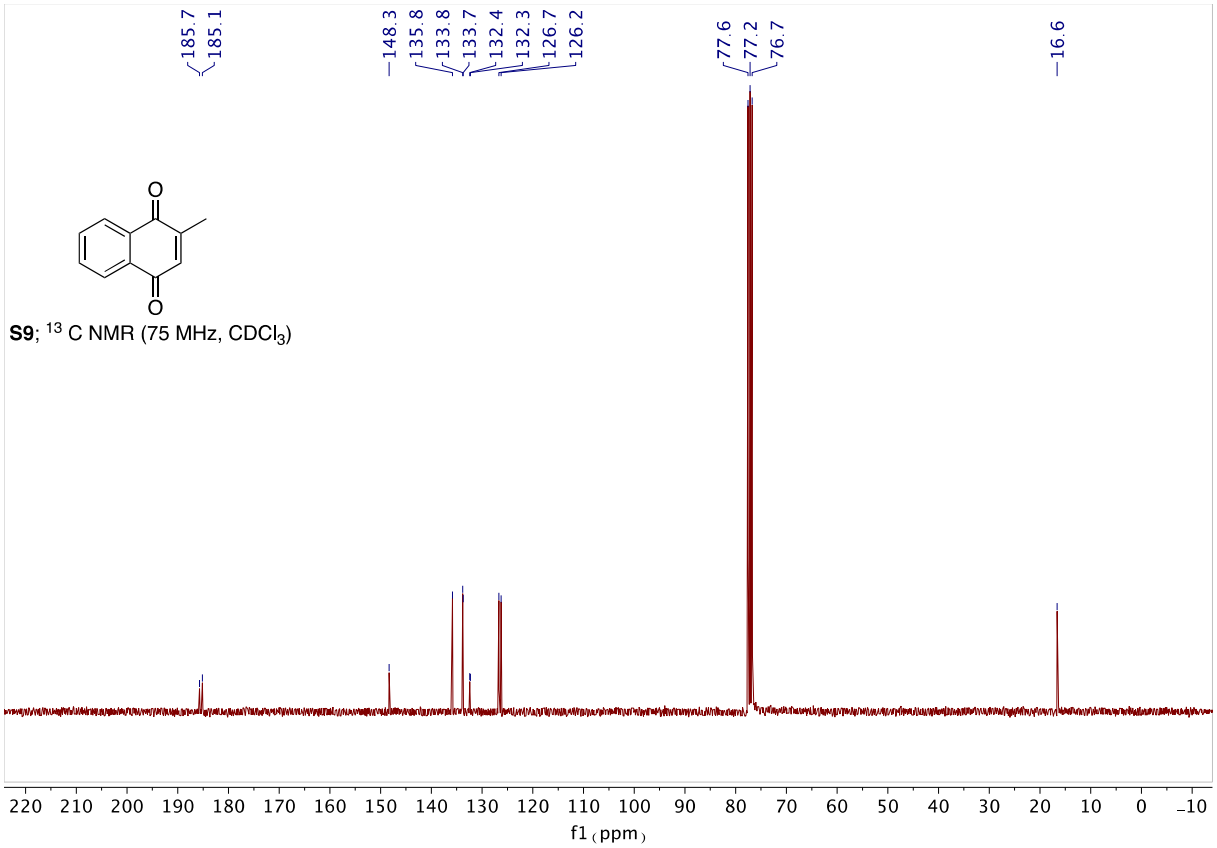
S7; ^{1}H NMR (300 MHz, $CDCl_3$)



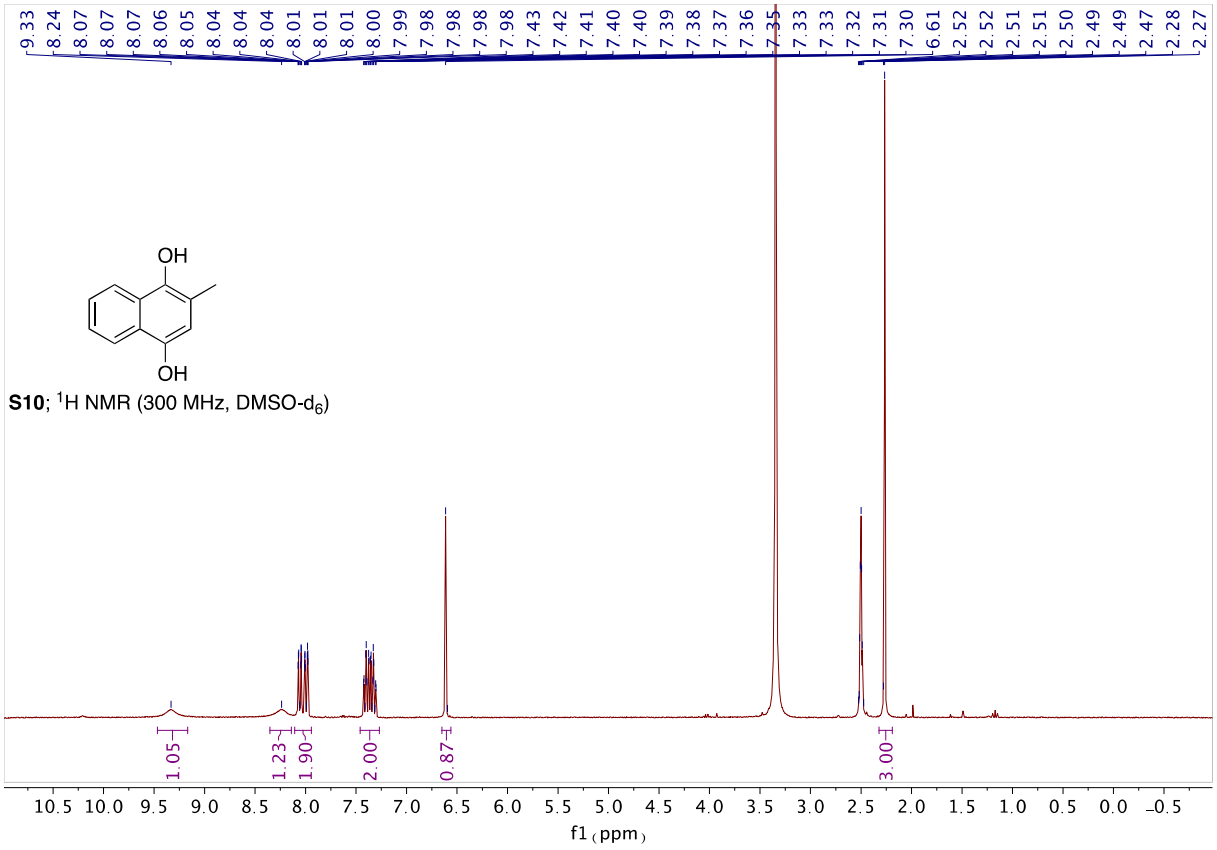
S7; ^{13} C NMR (75 MHz, $CDCl_3$)



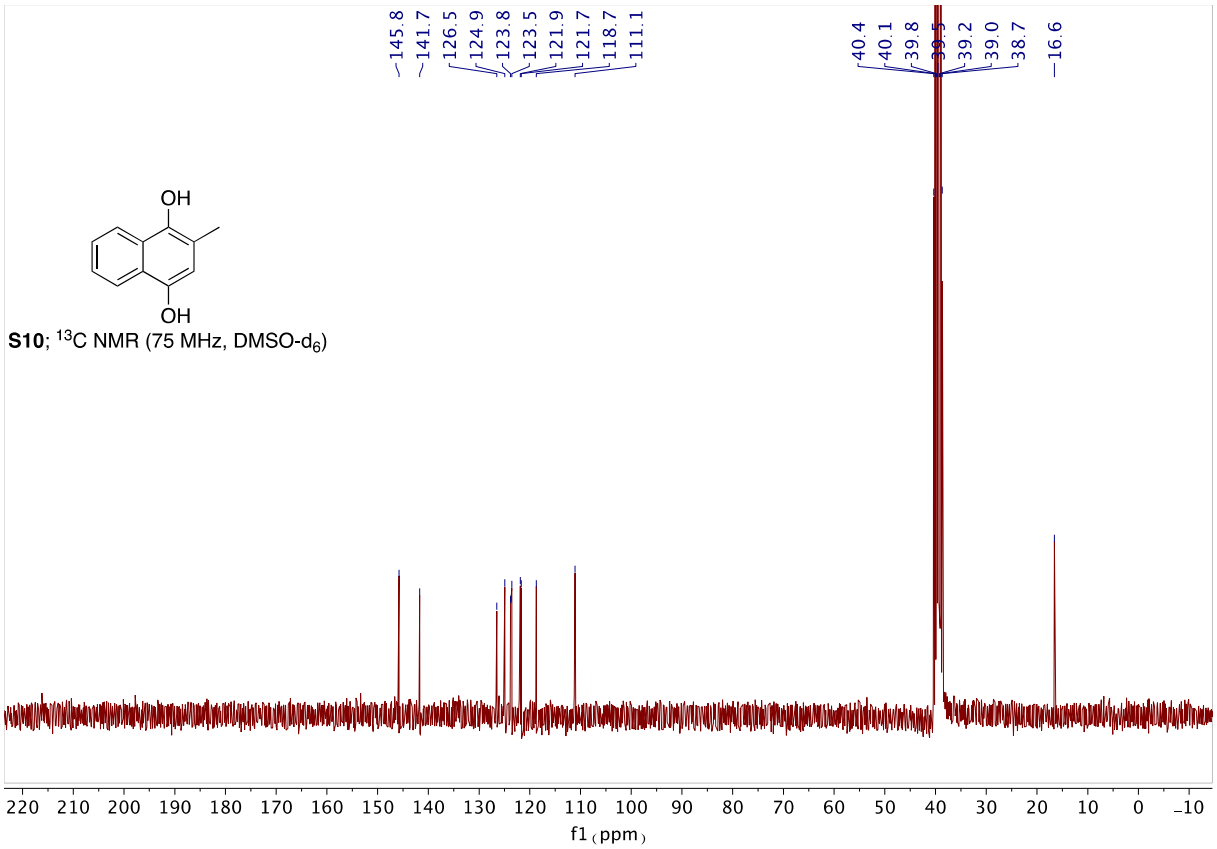
S9; ^{1}H NMR (300 MHz, $CDCl_3$)



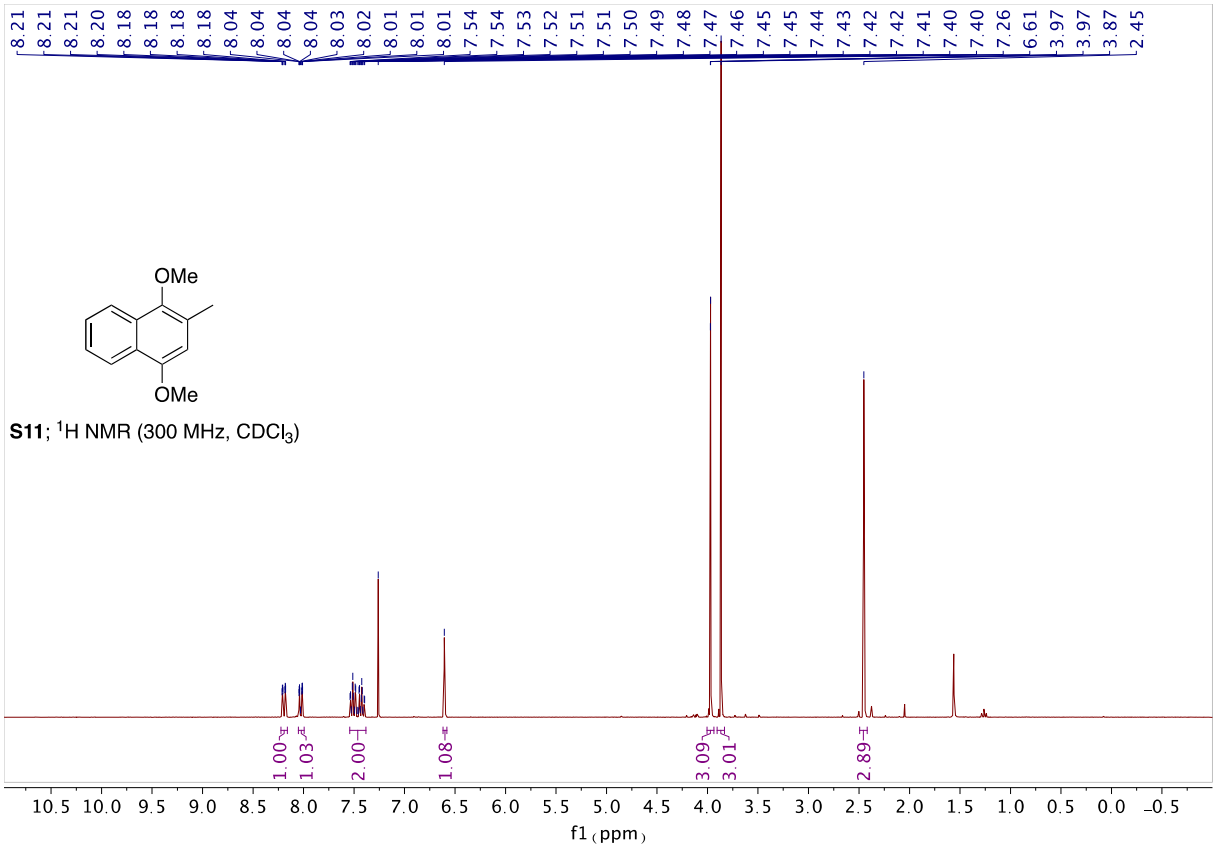
S9; ^{13}C NMR (75 MHz, $CDCl_3$)



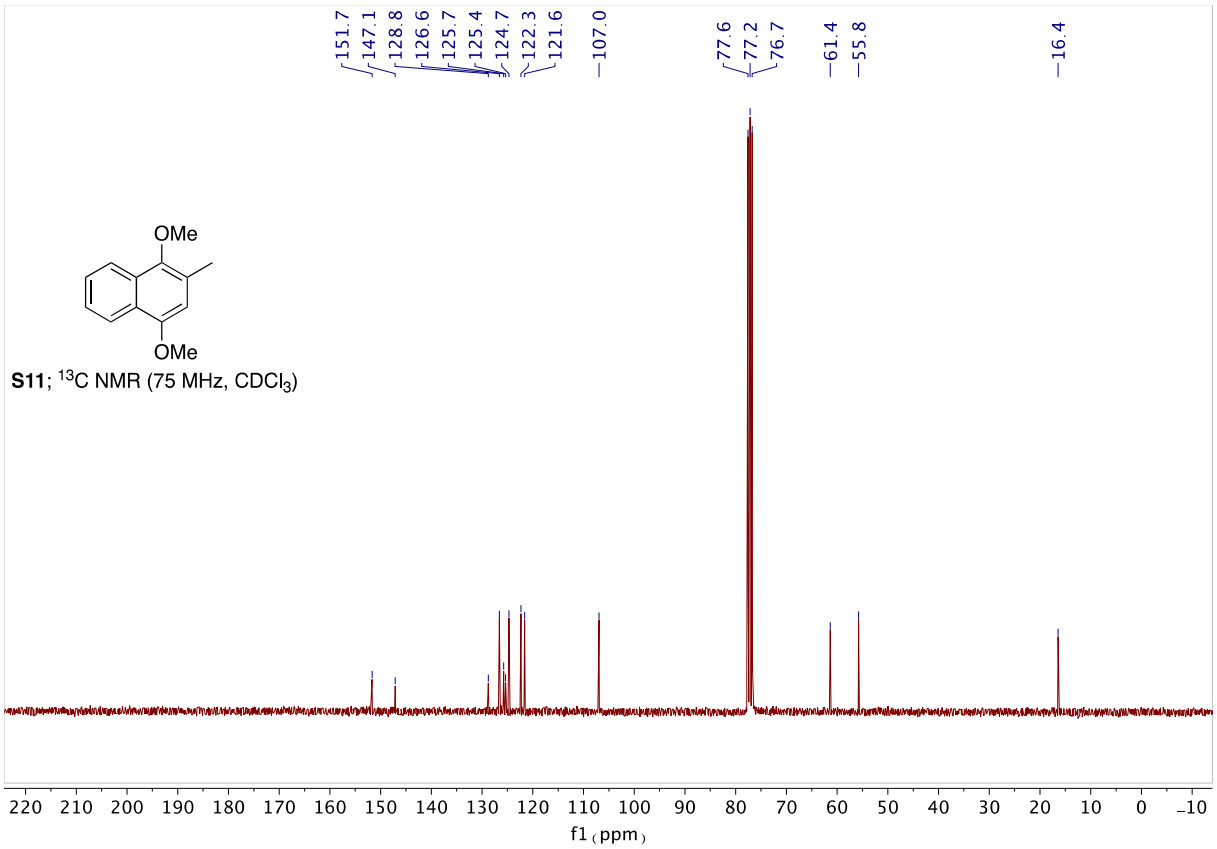
S10; ^{1}H NMR (300 MHz, DMSO-d_6)



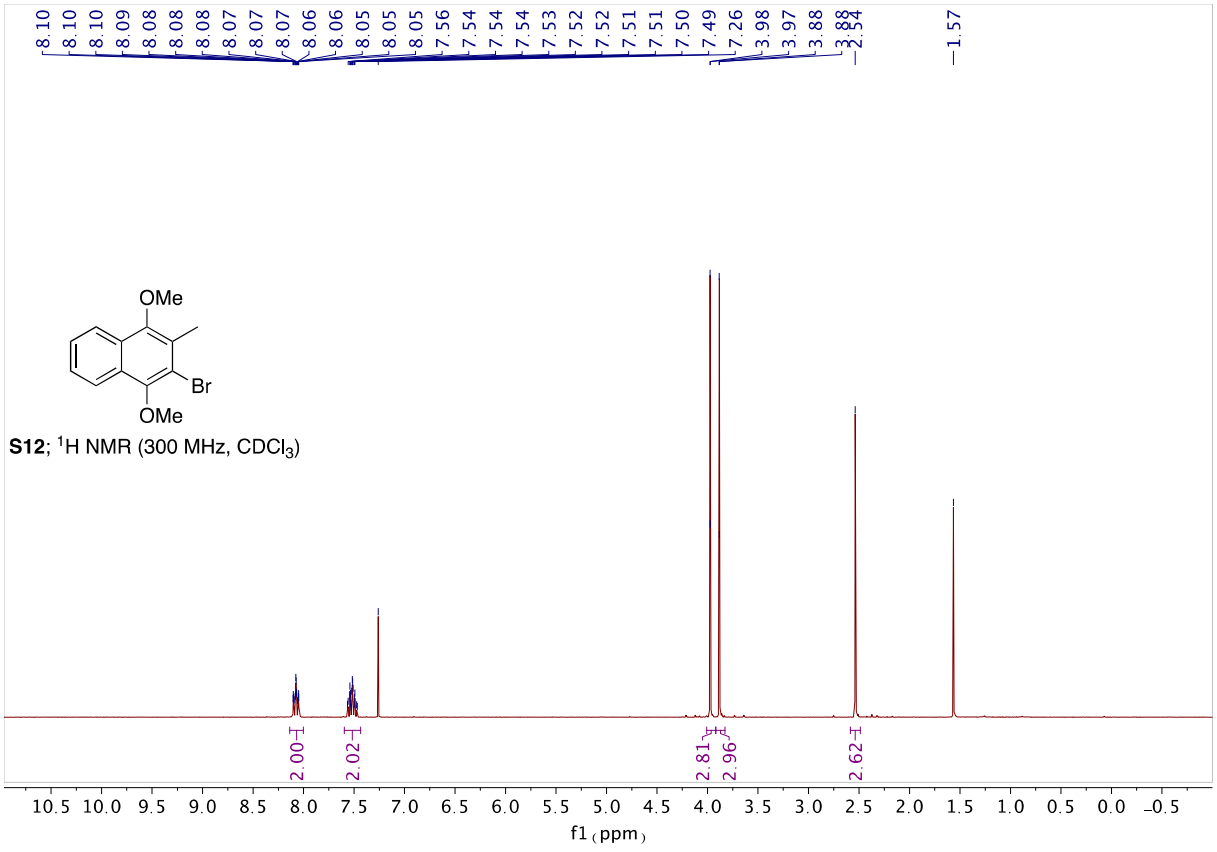
S10; ^{13}C NMR (75 MHz, DMSO-d_6)



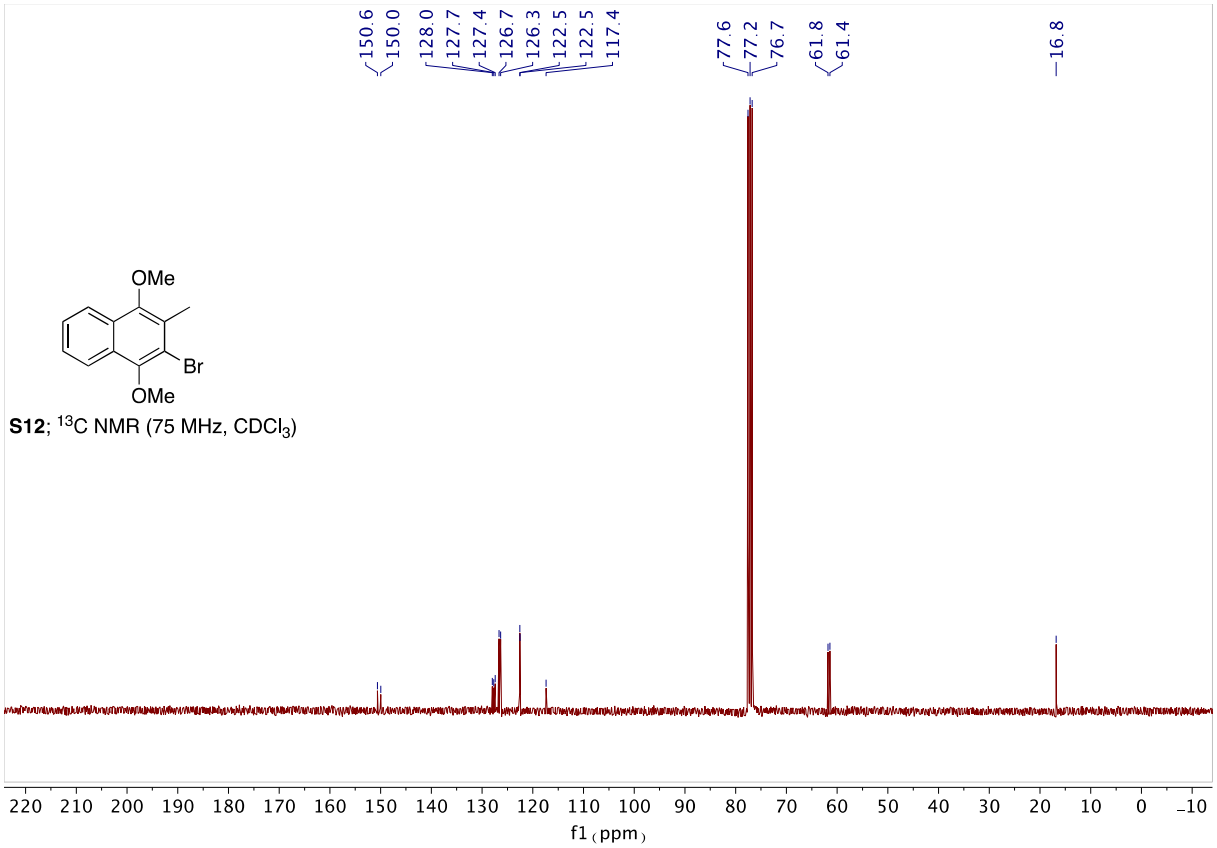
S11; ^{1}H NMR (300 MHz, $CDCl_3$)



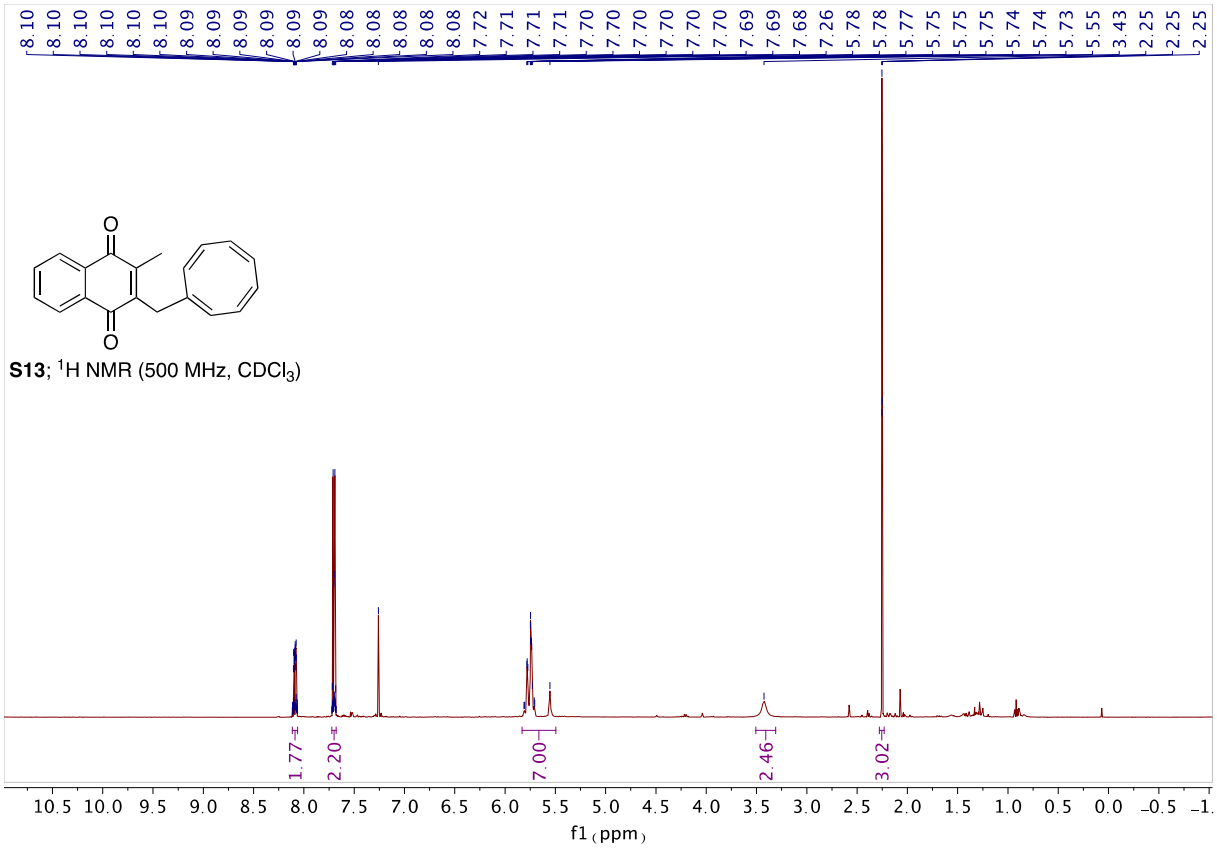
S11; ^{13}C NMR (75 MHz, $CDCl_3$)



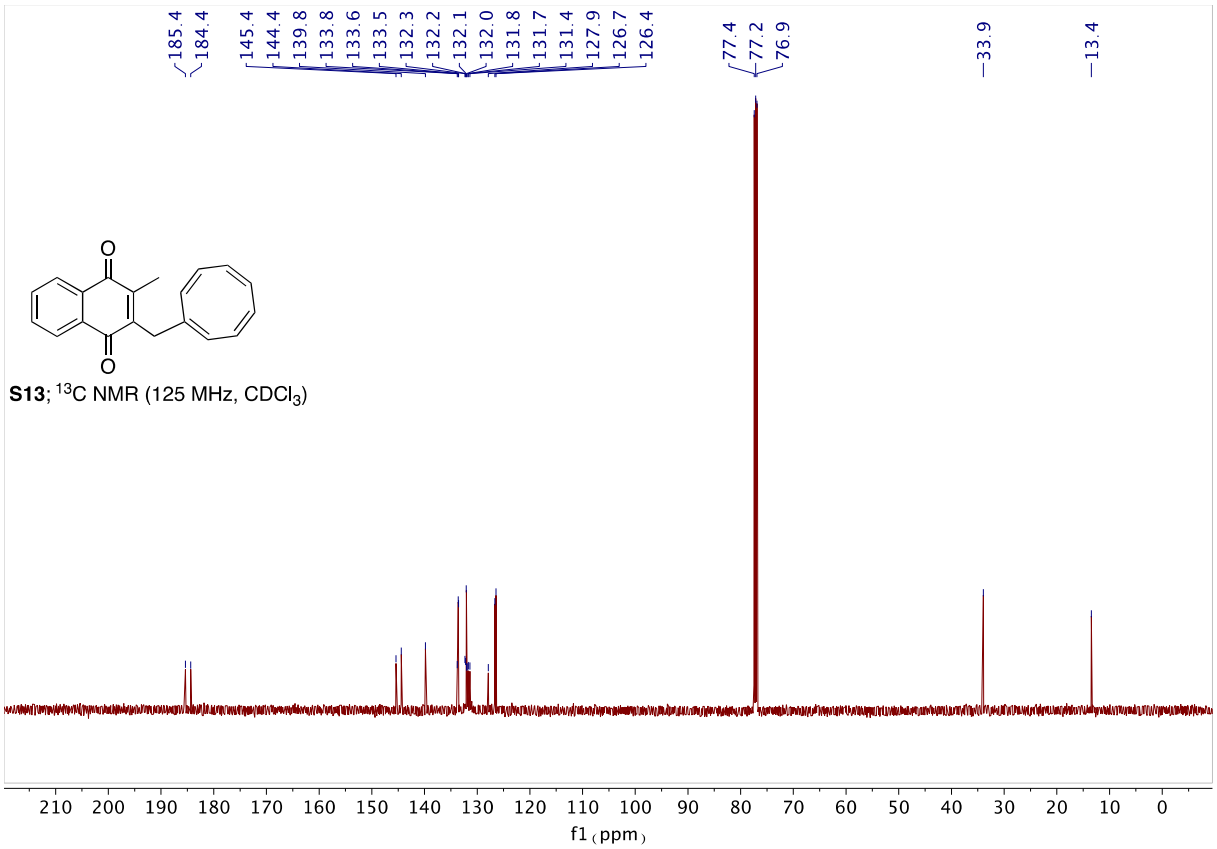
S12; ^{1}H NMR (300 MHz, $CDCl_3$)



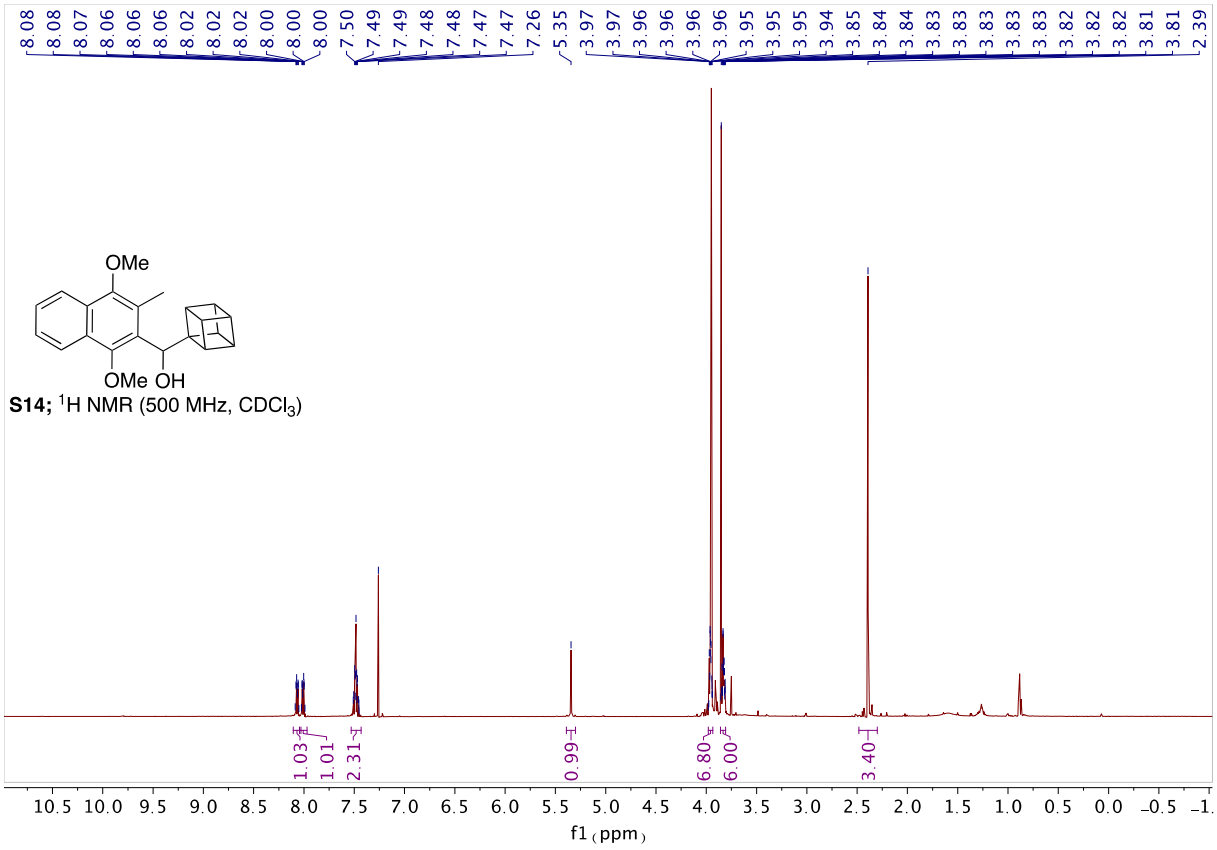
S12; ^{13}C NMR (75 MHz, $CDCl_3$)



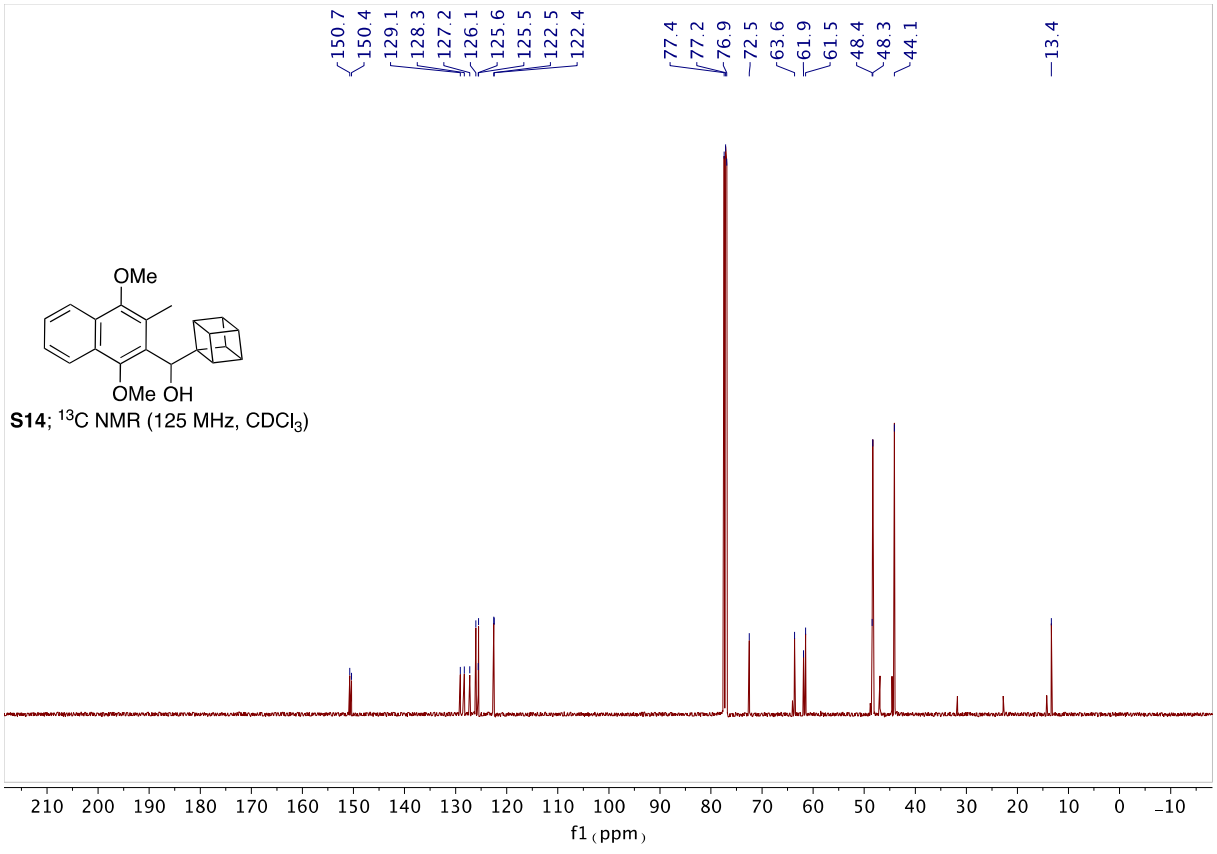
S13; ^{1}H NMR (500 MHz, $CDCl_3$)



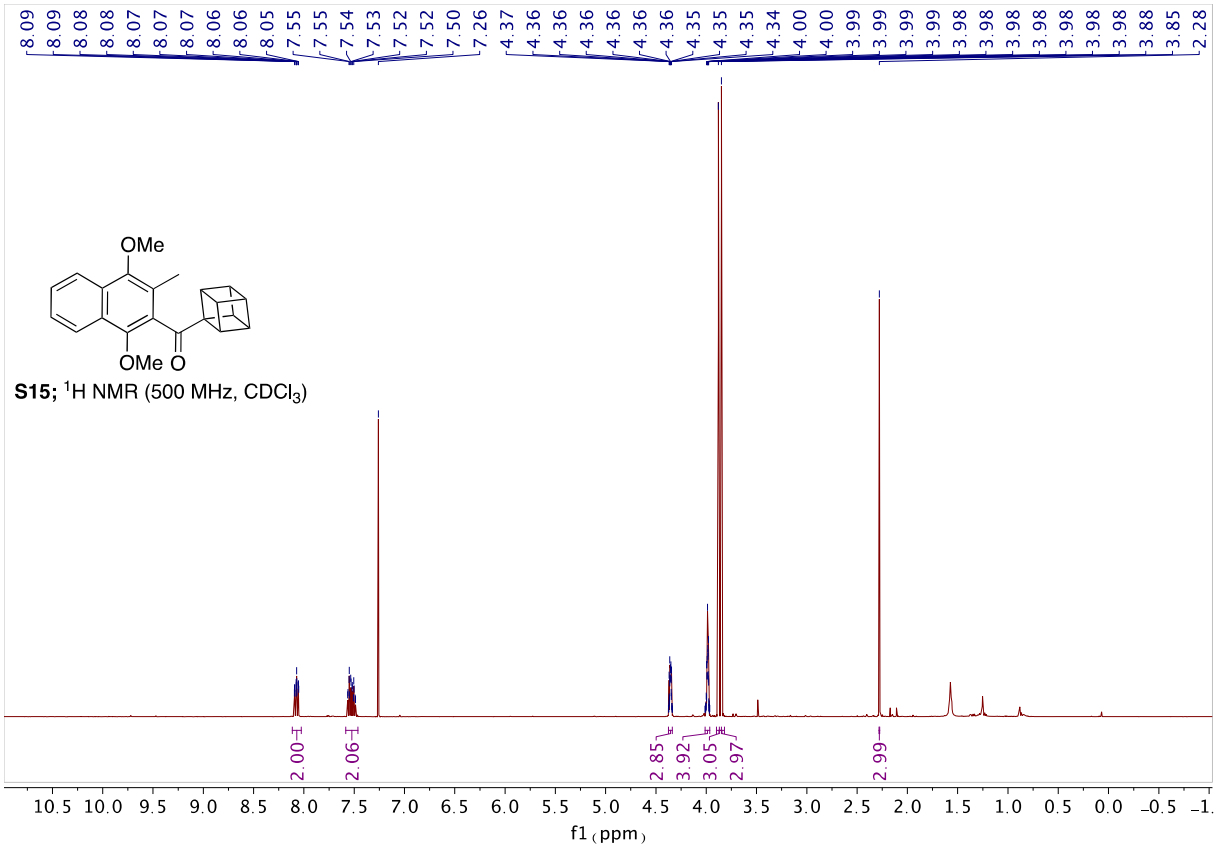
S13; ^{13}C NMR (125 MHz, $CDCl_3$)



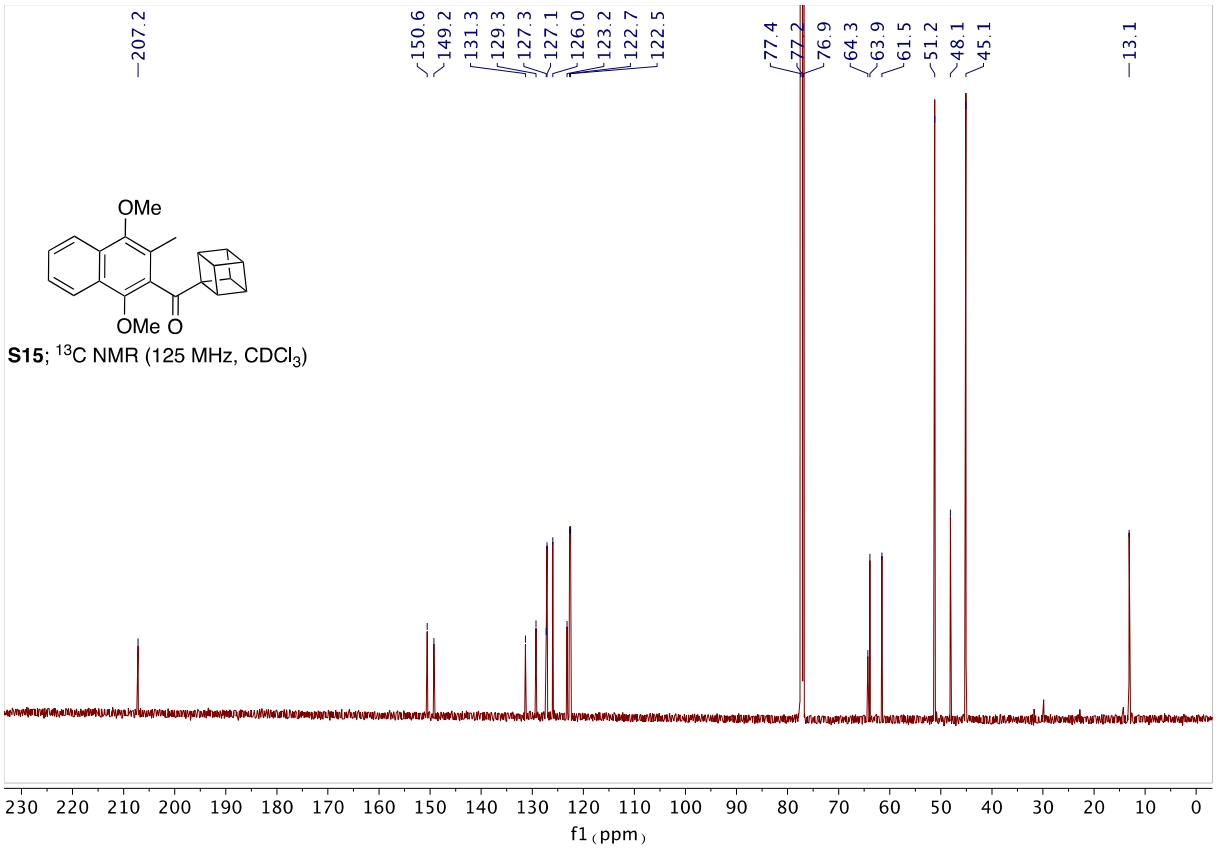
S14; ^{1}H NMR (500 MHz, $CDCl_3$)



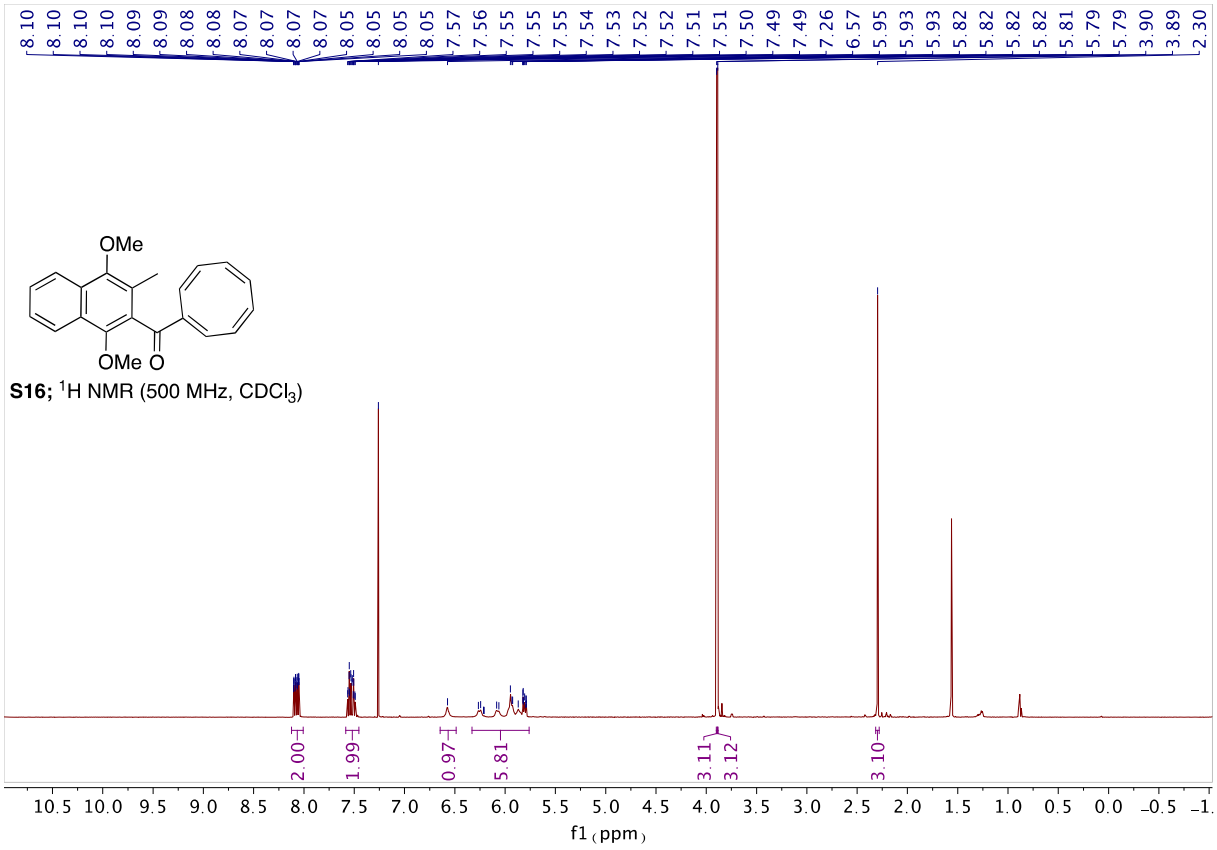
S14; ^{13}C NMR (125 MHz, $CDCl_3$)



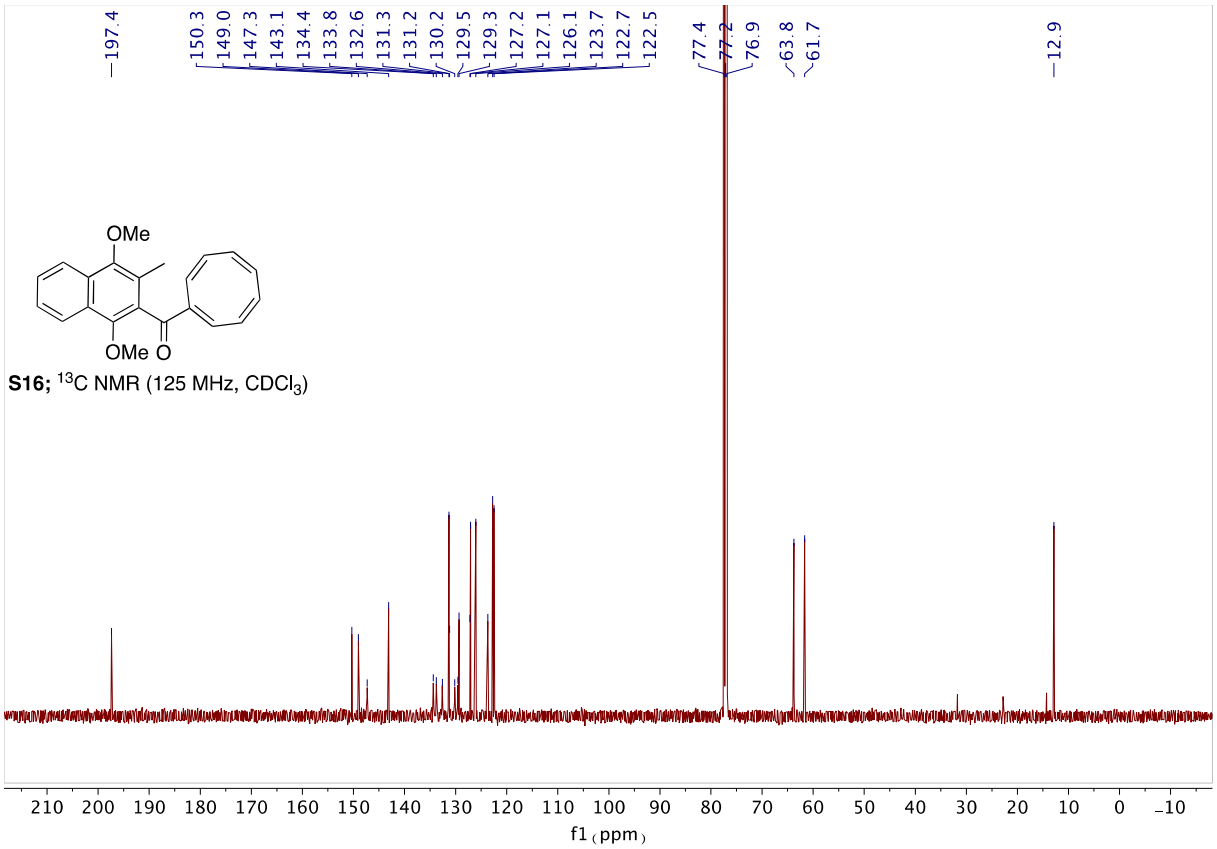
S15; ^{1}H NMR (500 MHz, $CDCl_3$)



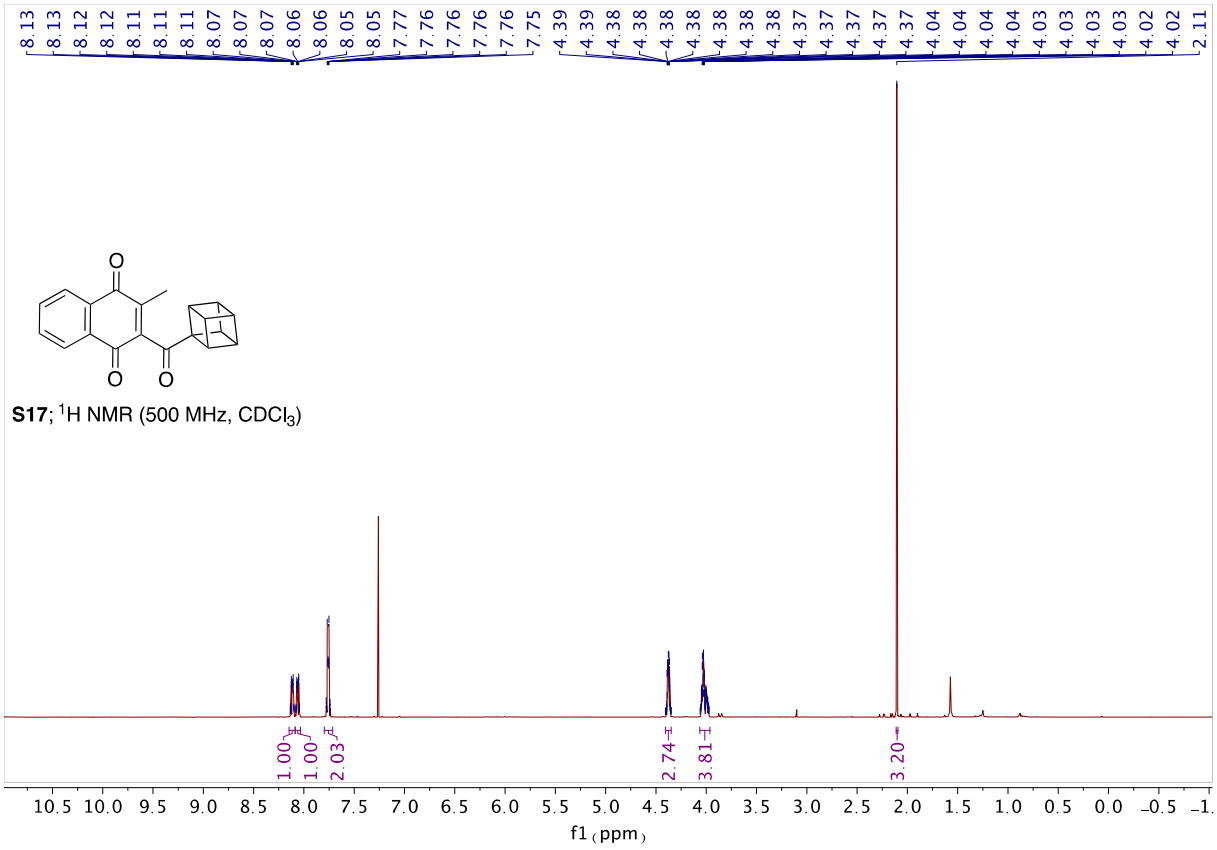
S15; ^{13}C NMR (125 MHz, $CDCl_3$)



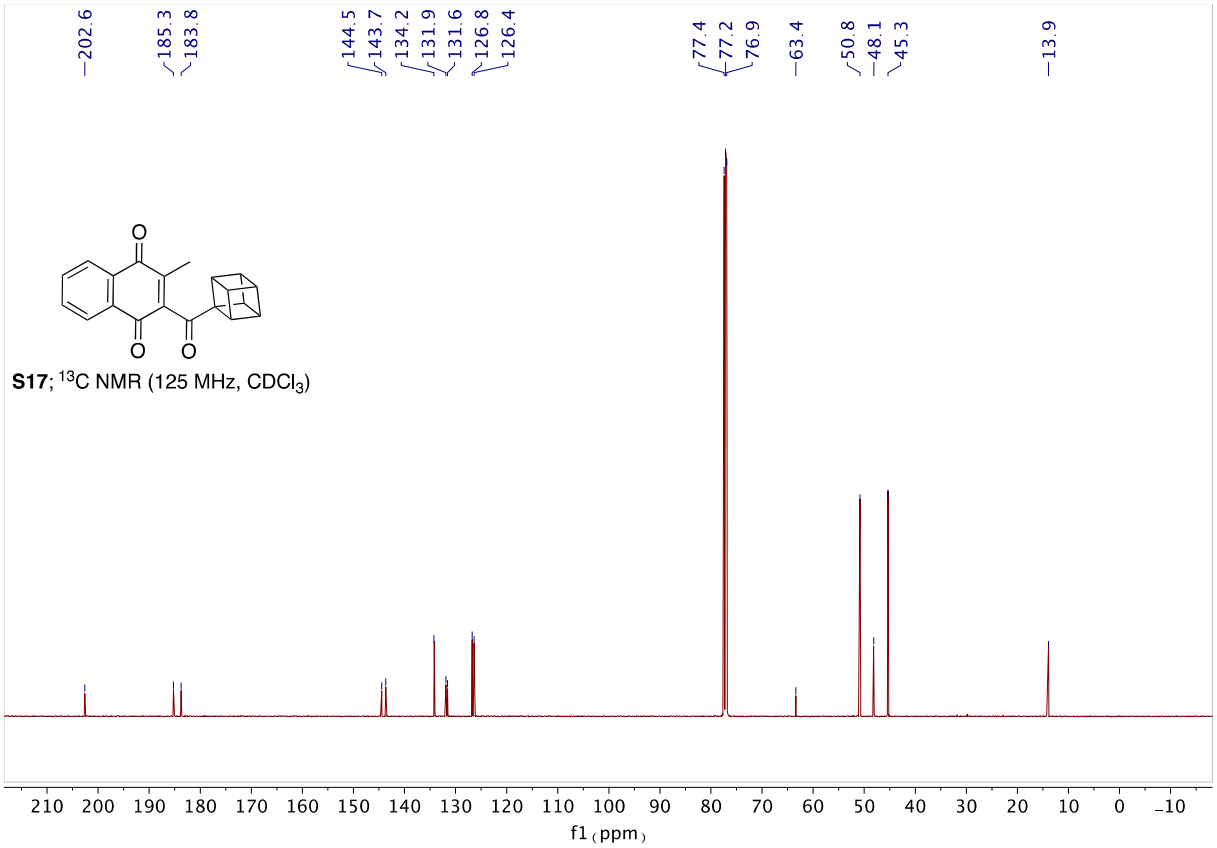
S16; ^{1}H NMR (500 MHz, $CDCl_3$)



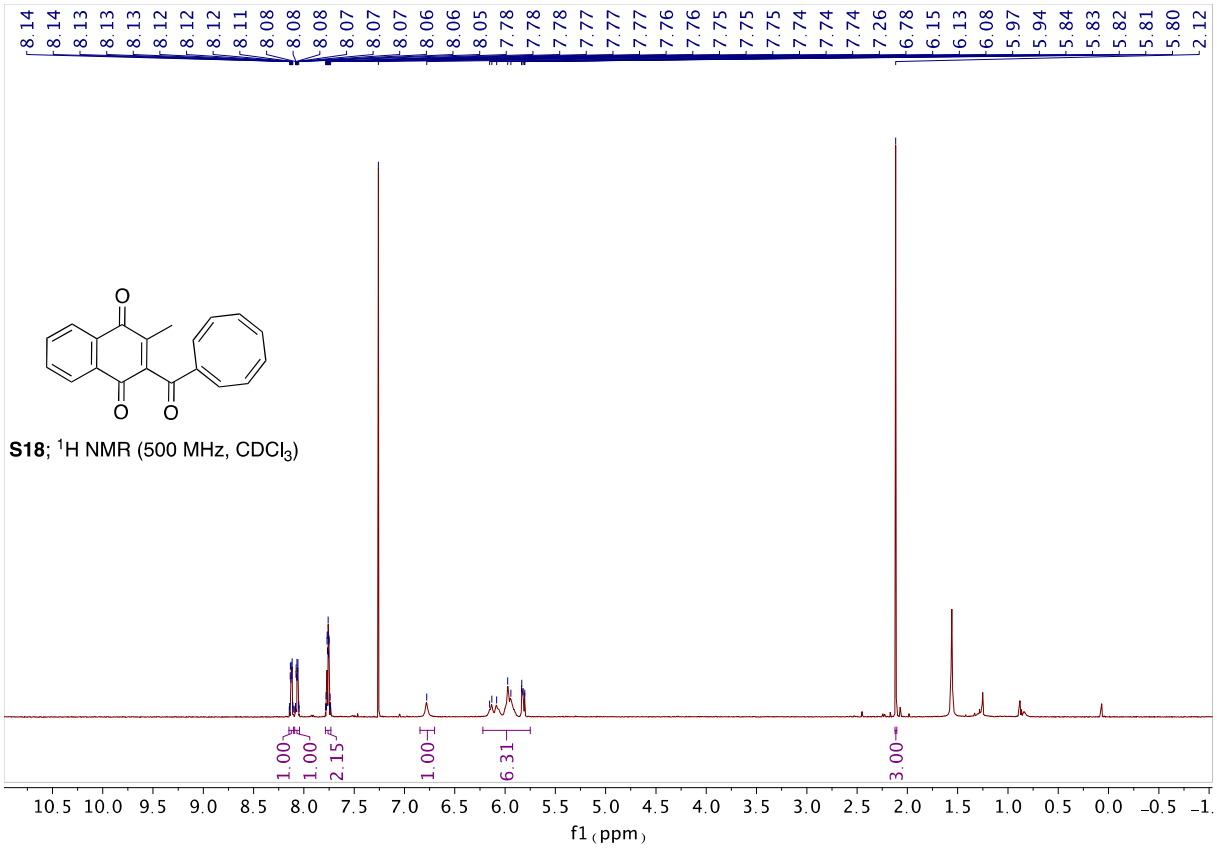
S16; ^{13}C NMR (125 MHz, $CDCl_3$)



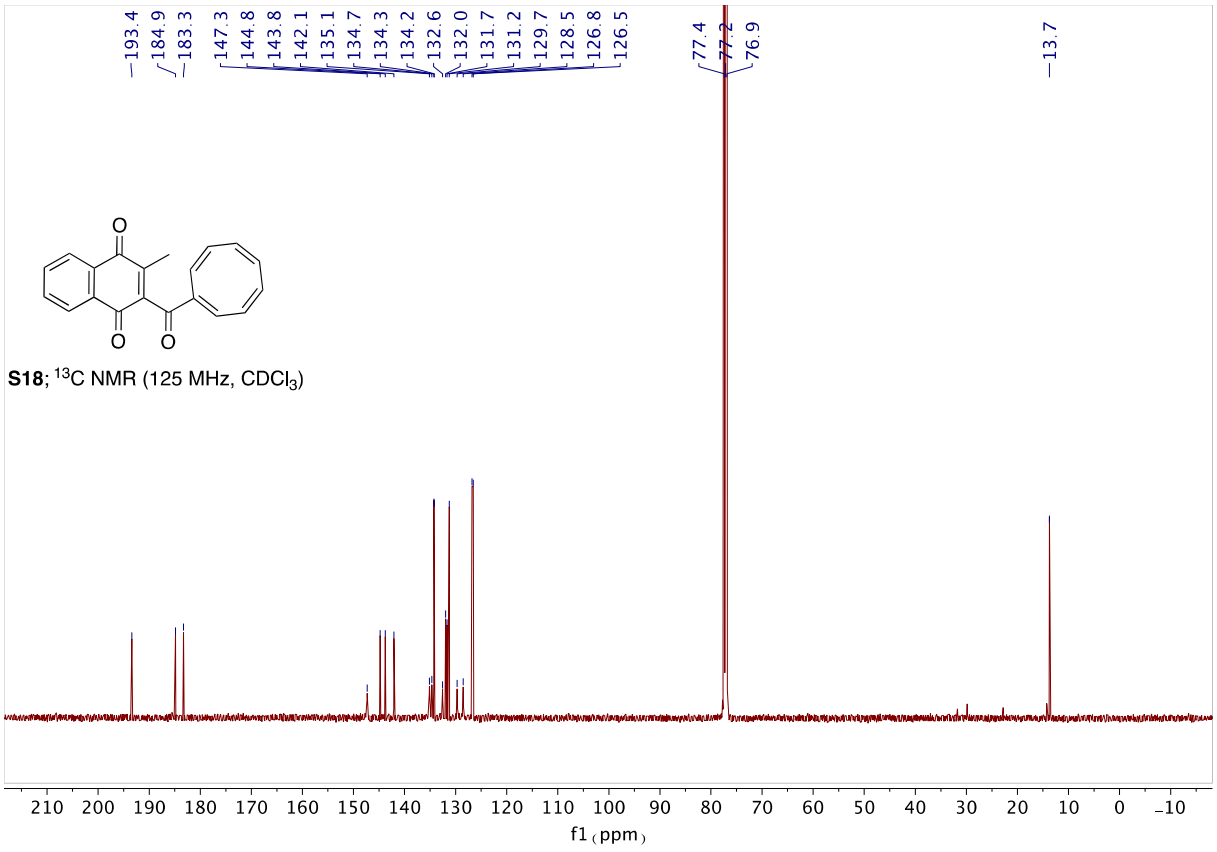
S17; ^{1}H NMR (500 MHz, $CDCl_3$)



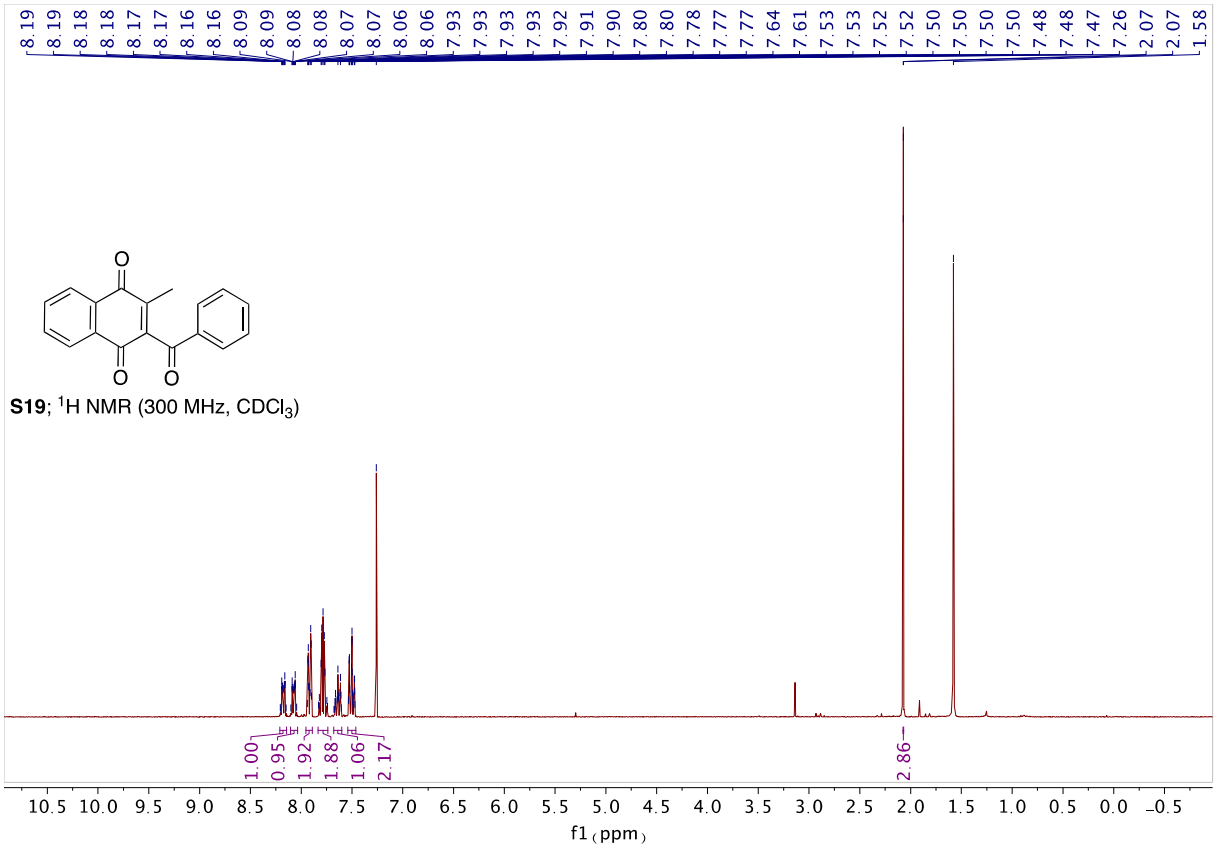
S17; ^{13}C NMR (125 MHz, $CDCl_3$)



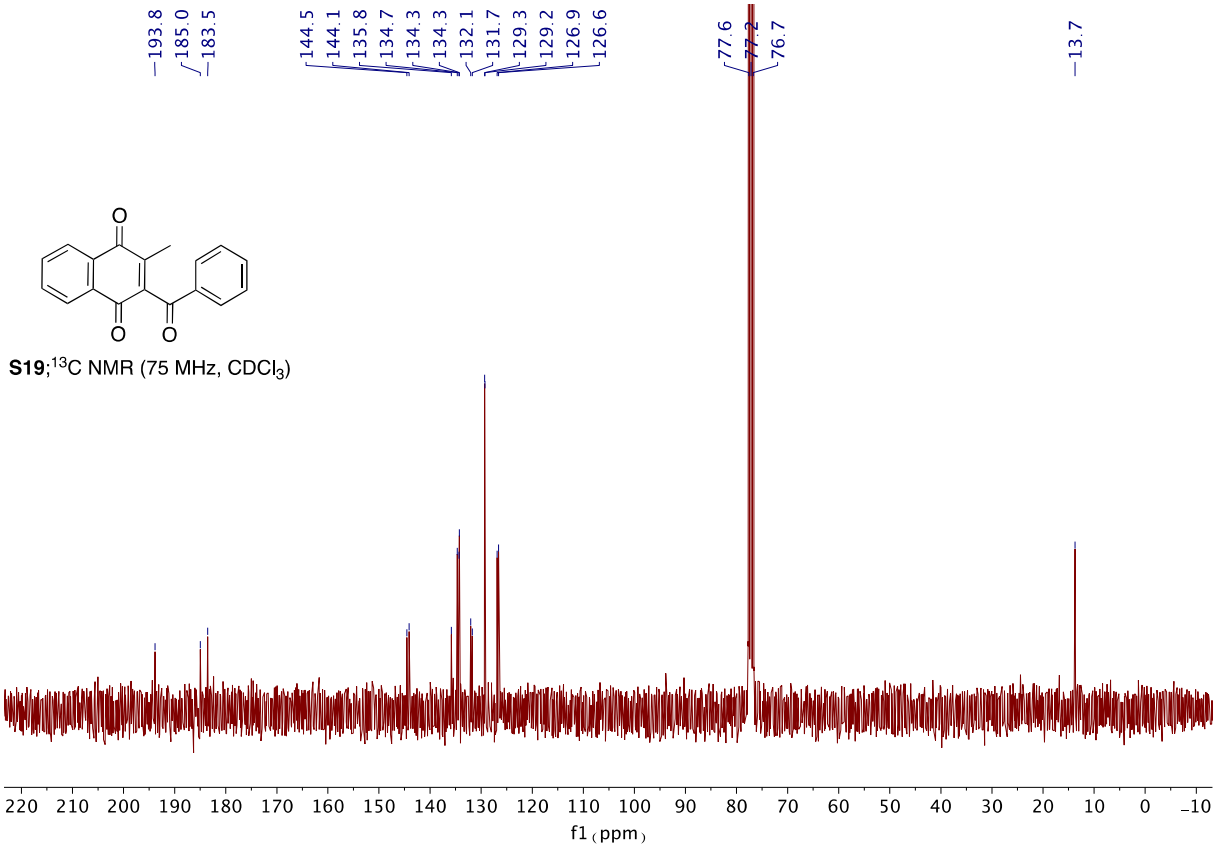
S18; ^{1}H NMR (500 MHz, $CDCl_3$)



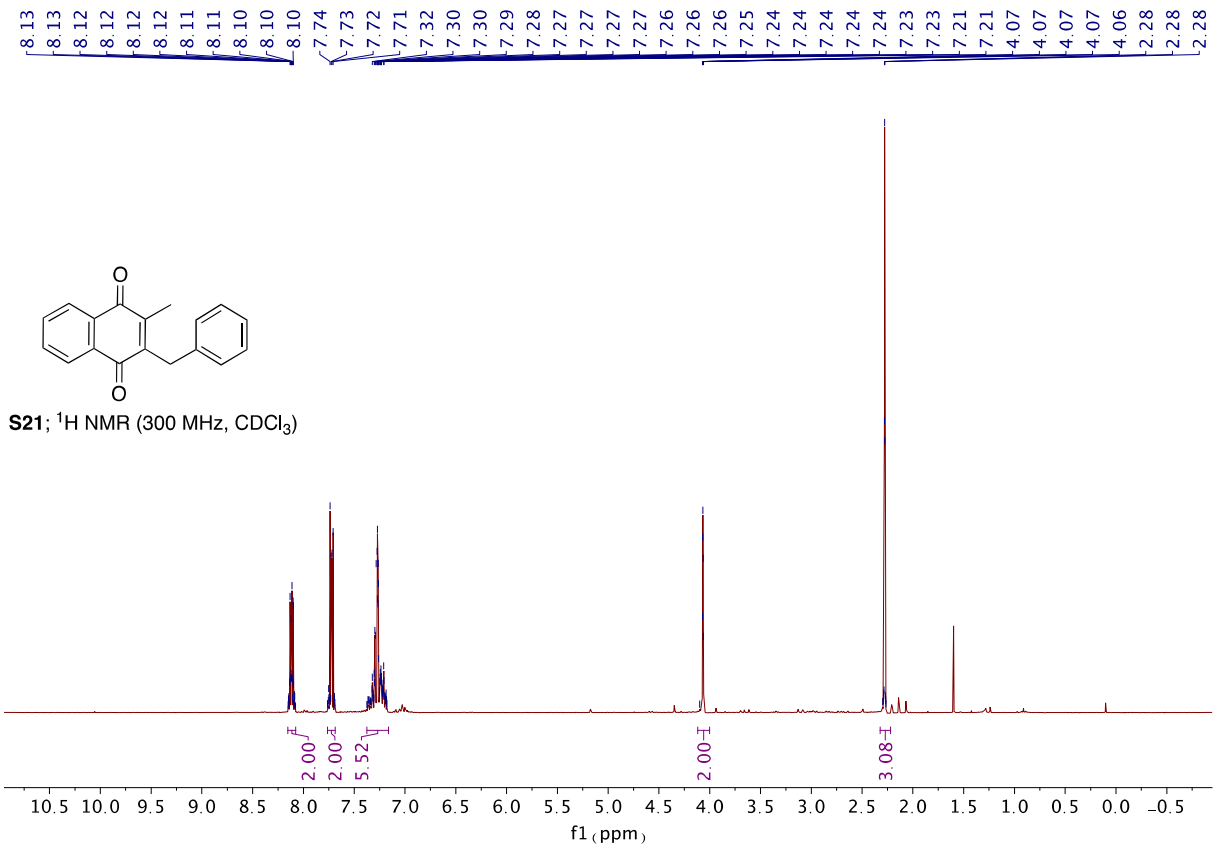
S18; ^{13}C NMR (125 MHz, $CDCl_3$)



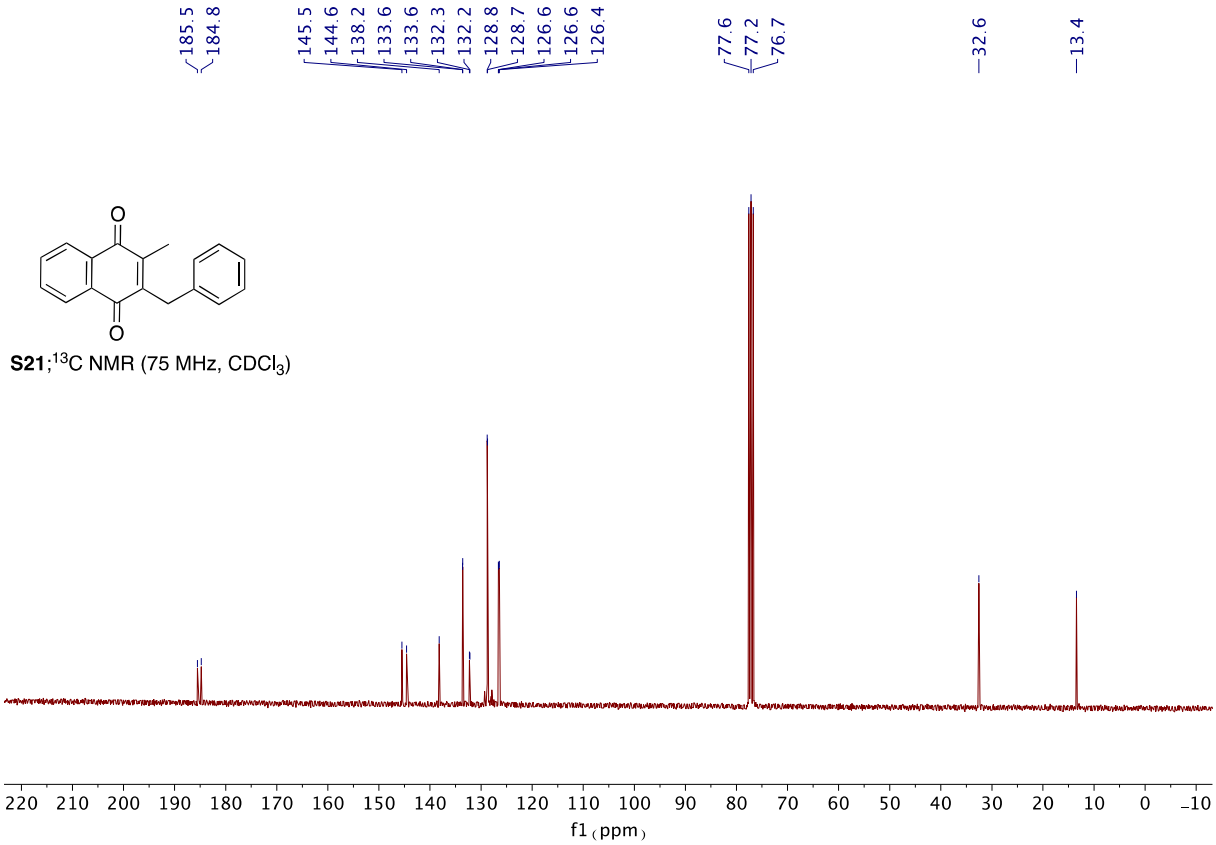
S19; ^{1}H NMR (300 MHz, $CDCl_3$)



S19;^{13}C NMR (75 MHz, $CDCl_3$)



S21; ^{1}H NMR (300 MHz, $CDCl_3$)



S21;^{13}C NMR (75 MHz, $CDCl_3$)

Supporting Information Part 3:

Supplemental Table 1 Cell-based and *in vitro* activity assays for vitamin K cycle enzymes

Enzyme	Assay	Activity	Cell line	Substrate	Note
VKOR	ELISA-based	KO reduction	FIXgla-PC/HEK293	KO	
VKOR	HPLC-based	KO reduction	GGCX knockout	KO	
VKR	ELISA-based	Vitamin K reduction	DGKO	Vitamin K_1	Coupled, vitamin K reduction and epoxidation
VKR	HPLC-based	Vitamin K reduction	DGKO	Vitamin K_1	Coupled, vitamin K reduction and epoxidation
GGCX	*in vitro*	Vitamin K epoxidation		KH_2	
UBIAD1	ELISA-based	VK analogue -> MK-4	UBIAD1 knockout	VK analogue	
UBIAD1	HPLC-based	VK analogue -> MK-4	HEK293	VK analogue	

VKOR: vitamin K epoxide reductase; VKR: vitamin K reductase; GGCX: gamma-glutamyl carboxylase; UBIAD1: UbiA prenyltransferase domain-containing protein 1

Supporting Information Part 4: Reference

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