Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

SUPPORTING INFORMATION

Chemoenzymatic synthesis of (+)-isoagatholactone, (+)-spongian-16-one, and 3-deoxychavalone A via biocatalytic polyene cyclization

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I. Supplementary figures

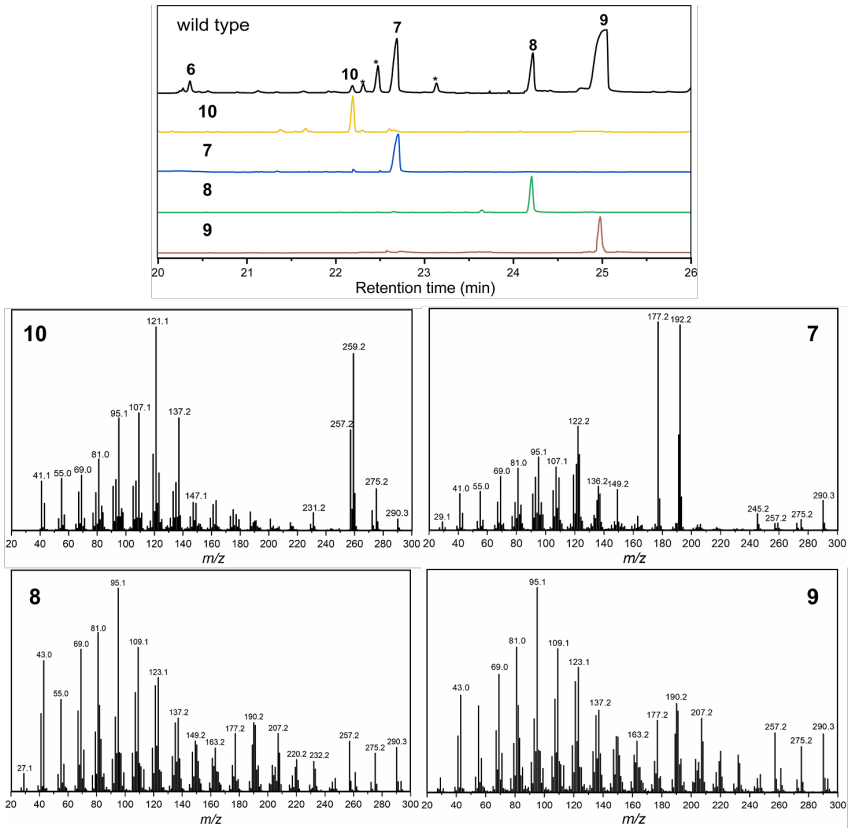

Figure S1. GC–MS analysis of the product profile of geranylgeraniol cyclization catalyzed by *Aac*SHC compared with corresponding pure products compound **7**, **8**, **9**, and **10**.

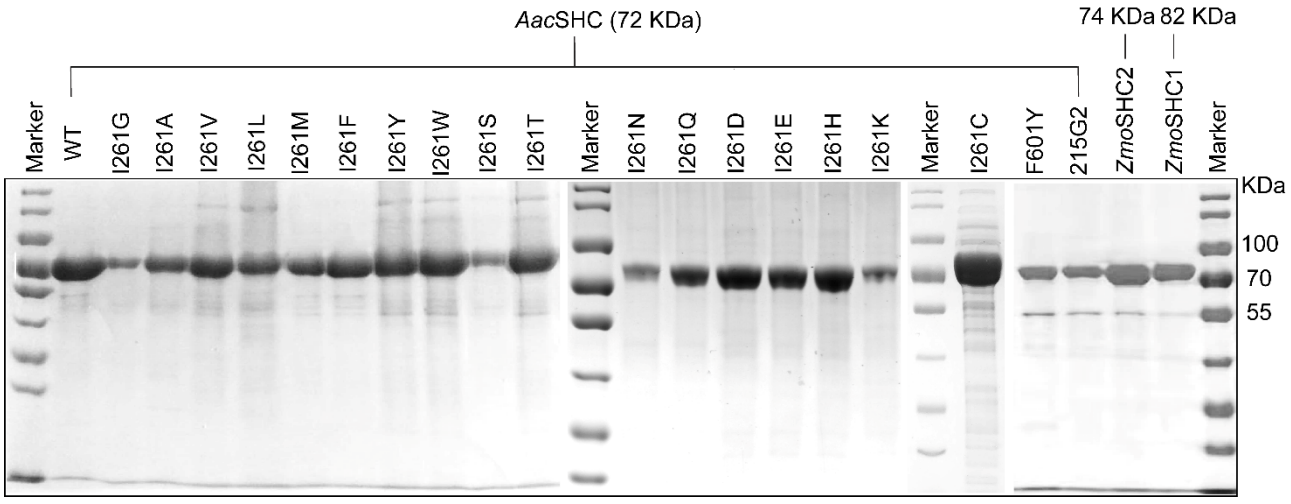

Figure S2. SDS-PAGE analysis of *Aac*SHCs (wild type and its variants), *Zmo*SHC 1 and *Zmo*SHC2. The purified SHCs samples were loaded into gel for characterization. Prestained Protein Ladder was used as marker (Genentech, R1001-010).

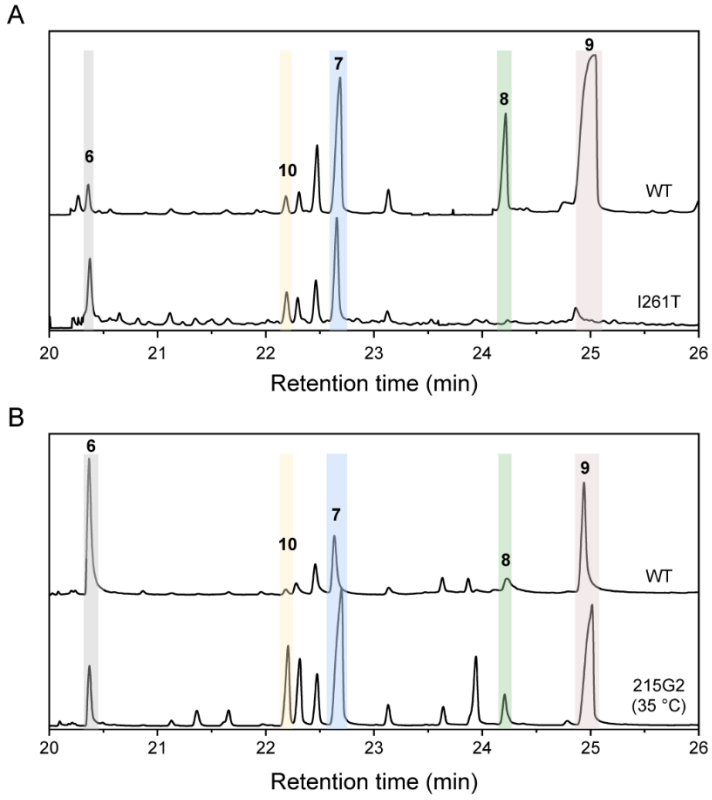

Figure S3. GC–MS analysis of the product profile of geranylgeraniol cyclization catalyzed by *Aac*SHC and selected varaints corresponding to Figure 3B-E.

Table S1. Reaction conditions for the amine-catalyzed condensation reactions of compound **13** and 3-hydroxypyrone (**5**).

Entry	Amino acid or amine salt	Solvent	Dehydrating agent	Temperature (°C)	Time
1	L-Proline	EtOAc	/	70	24 h
2	piperidine-acetic acid	EtOAc	/	80	4 days
3	*β*-alanine	EtOAc	$CaSO_4$	80	24 h
4	piperidine-acetic acid	EtOAc	Na_2SO_4	80	24 h
5	L-Proline	THF	/	65	24 h
6	*β*-alanine	toluene	$CaSO_4$	110	5 days
7	piperidine hydrochloride	EtOAc	$CaSO_4$	80	24 h
8	piperidine hydrochloride	toluene	$CaSO_4$	110	48 h

II. Biocatalytic polyene cyclization

Strains and plasmids

All the molecular biology manipulations were performed according to standard protocols. *Escherichia coli* DH5*α* cells were used for molecular cloning and site-directed mutagenesis. *E. coli* BL21(DE3) cells were used for protein expression.

The *AacSHC* and *ZmoSHC2* genes were codon-optimized and synthesized by Genewiz (Suzhou, China) and subcloned into pET28a(+) vector with NdeI/XhoI restriction site to afford the plasmid pET28a-*Aac*SHC and pET28a-*Zmo*SHC2, respectively. The *ZmoSHC1* gene was codon-optimized and synthesized by GenScript (Nanjing, China) and subcloned into pET28a(+) vector with NcoI/SacI restriction sites to afford the plasmid pET28a-*Zmo*SHC1.

Protein and nucleotide sequences of *Aac*SHC (631 aa, 1896 bp)

MAEQLVEAPAYARTLDRAVEYLLSCQKDEGYWWGPLLSNVTMEAEYVLLCHILDRVDRDRMEKIRRYLLHEQREDGTWALYPGGPPDL
DTTIEAYVALKYIGMSRDEEPMQKALRFIQSQGGIESSRVFTRMWLALVGEYPWEKVPMVPPEIMFLGKRMPLNIYEFGSWARATVVA
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Protein and nucleotide sequences of *Zmo*SHC1 (725 aa, 2180 bp)

MGIDRMNSLSRLLMKKIFGAEKTSYKPASDTIIGTDTLKRPNRRPEPTAKVDKTIFKTMGNSLNNTLVSACDWLIGQQKPDGHWVGAV
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Protein and nucleotide sequences of *Zmo*SHC2 (658 aa, 1977 bp)

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tgaagaaggccaaccagccgatcgtgcattatggtatgtaa

Site-directed mutagenesis

Site-directed mutagenesis was carried out using the overlap extension PCR and homologous recombination method following the standard protocol of Q5® Hot Start High-Fidelity 2× Master Mix (NEB) and ClonExpress® II (or MultiS) One Step Cloning Kit (Vazyme, Nanjing, China), or the Quik-Change method with the primers described in **Table S2**. The resulting mutant genes were verified by DNA sequencing.

Table S2. Primers used in this study.

Primer	Nucleotide Sequence (5'-3')
SHC-I261G-F	gcgatggtagctggggcggtGGCcagccgccgtgg
SHC-I261G-R	GCCaccgccccagctaccatcgcctgcttgacgt
SHC-I261A-F	gcgatggtagctggggcggtGCCcagccgccgtgg
SHC-I261A-R	GGCaccgccccagctaccatcgcctgcttgacgt
SHC-I261F-F	gcgatggtagctggggcggtTTTcagccgccgtgg
SHC-I261F-R	AAAaccgccccagctaccatcgcctgcttgacgt
SHC-I261W-F	gtagctggggcggtTGGcagccgccgtg
SHC-I261W-R	CCAaccgccccagctaccatcgcctgc
SHC-I261T-F	gcgatggtagctggggcggtaCCcagccgccgtgg
SHC-I261T-R	GGtaccgccccagctaccatcgcctgcttgacgt
SHC-I261S-F	gcgatggtagctggggcggtTCTcagccgccgtgg
SHC-I261S-R	AGAaccgccccagctaccatcgcctgcttgacgt
SHC-I261Y-F	gcgatggtagctggggcggtTATcagccgccgtgg
SHC-I261Y-R	ATAaccgccccagctaccatcgcctgcttgacgt
AacSHC-F	gtgccgcgcggcagccatA
AacSHC-R	gtggtggtggtggtgctc
SHC-I261V-F	gcgatggtagctggggcggtGTGcagccgccgtgg
SHC-I261V-R	aaccacggcggctgCACaccgccccag
SHC-I261L-F	gcgatggtagctggggcggtCTGcagccgccgtgg
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SHC-I261D-F	gcgatggtagctggggcggtGATcagccgccgtgg
SHC-I261D-R	aaccacggcggctgATCaccgccccag
SHC-I261E-F	gcgatggtagctggggcggtGAAcagccgccgtgg
SHC-I261E-R	aaccacggcggctgTTCaccgccccag
SHC-I261H-F	gcgatggtagctggggcggtCATcagccgccgtgg
SHC-I261H-R	aaccacggcggctgATGaccgccccag
SHC-I261K-R	aaccacggcggctgCTTaccgccccag
SHC-F601Y-F	acacaggcaccggttATccgggcgacttc
SHC-F601Y-R	ATaaccggtgcctgtgtagtaaggttc
215G2-R1	aaccagggctaaccaACGgcgggtaaacacgcggctactttc
215G2-F2	CGTtggttagccctggttggc
215G2-R2	ctggtaaccatgcagCACgcgatccagggcgtcaaag
215G2-F3	GTGctgcatggttaccagaagc

215G2-R3	gaaatcacagaacggGGTgtgattcggcagatcgctgg
215G2-F4	ACCccgttctgtgatttcggtg

Expression and purification

For protein expression, the plasmid harboring the *SHC* gene was transformed into *E. coli* BL21(DE3) competent cell. A single colony was inoculated and grown in 10 mL LB medium with kanamycin (50 mg/L) at 37 °C and 220 rpm overnight. The seed culture was added to 1 L TB medium with kanamycin (50 mg/L), and incubated at 37 °C and 220 rpm until OD_{600} of ~0.7 was reached, then cells were induced with 1 mM IPTG and incubated at 37 °C, 180 rpm for 20 h. Afterward, cells were harvested (8000*g*, 30 min, 4 °C), frozen in liquid nitrogen and stored at −80 °C. For fermentation in large-scale, 200 mL of seed culture was inoculate into 4 L ZYP-5052 medium (2 g/L lactose, 5 g/L glycerol, 0.5 g/L glucose, 3.3 g/L $(NH_4)_2SO_4$, 6.8 g/L KH_2PO_4, 7.1 g/L Na_2HPO_4, 0.24 g/L $MgSO_4$, 10 g/L tryptone, 5 g/L yeast extract) with kanamycin (50 mg/L) in a 5 L bioreactor, followed by auto-induced at 37 °C, 200 to 550 rpm for 16 h.

For protein purification, the frozen cell pellet was resuspended in lysis buffer (200 mM citrate, pH 6.0, 3 mL per gram pellet). After addition of phenylmethanesulfonylfluoride (PMSF, 1 mM), lysozyme (0.5 mg/mL) DNAseI (~10 μg/mL), and $MgCl_2$ (5 mM), the resulting suspension was lysed by high-pressure homogenizer (AH-NANO, ATS, 80 MPa). The suspension was centrifuged (38,700*g*, 30 min, 4 °C), and the pellet was washed with wash buffer (60 mM citrate, pH 6.0, 3 ml per gram pellet). After centrifugation (38,700*g*, 50 min, 4 °C), the pellet was resuspended in solubilization buffer (60 mM citrate, 1% (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate) (CHAPS), pH 6.0, 1 ml per gram pellet) and stirred gently overnight at 4 °C. After centrifugation (38,700*g*, 45 min, 4 °C), the pellet was discarded, the solution was subsequently heat shocked at 50 °C for 15 min, and precipitated host proteins were removed via centrifugation (38,700*g*, 50 min, 4 °C). The resulting semi-purified enzyme was used for biotransformation in preparative scale.

To obtain pure protein, the CHAPS-solubilized AacSHC (wild-type or variants) was further purified with Nickel–Nitrilotriacetic acid beads 6FF (Ni-NTA, Smart-life sciences), washed with buffer A (20 mM PBS buffer, 500 mM NaCl, 10% glycerol and 20 mM imidazole, pH 7.2), and eluted with buffer B (20 mM PBS buffer, 500 mM NaCl, 10% glycerol and 250 mM imidazole, pH 7.2). The purity of protein was analyzed by SDS-PAGE and the purified proteins were dialyzed against buffer (60 mM citrate, 10% glycerol, pH 6.0) overnight. After addition of CHAPS (final concentration 0.2%), the proteins were centrifuged (38,700*g*, 30 min, 4 °C) and concentrated (Millipore, 15 mL, 50 kDa). The concentration was determined with Bradford Ultra (Expedeon Powerwave XS2, Biotek) using

bovine serum albumin as standard.

In vitro enzymatic assay

The cyclization reaction of geranylgeraniol (**6**) catalyzed by *Aac*SHC (wild-type or I261 mutants) was performed using CHAPS as detergent in 500 μL reaction volume. Briefly, pure *Aac*SHC (final concentration 0.1 mM) was added into the reaction buffer (0.2% CHAPS, 60 mM citrate, pH 6.0) with 2 mM compound **6** (200 mM stock solution in DMSO). The reaction mixture was incubated at 50 °C and 120 rpm for 153 h. The reaction mixture without *Aac*SHCs or compound **6** were performed as negative controls. The reaction was terminated by the addition of 500 μL ethyl acetate with vigorous vortexing for 10 s. The aqueous layer was extracted with ethyl acetate (3 × 500 μL). The combined organic extracts were concentrated under a steady stream of N_2 and resuspended with 40 μL ethyl acetate before GC–MS analysis (**Figure S1**). All assays were performed in triplicate.

For comparison of *Aac*SHC, F601Y and 215G2 variant, as well as *Zmo*SHC1 and *Zmo*SHC2, the reaction was carried out 1.5 mol% catalyst and 1 mM geranylgeraniol (**6**) in reaction buffer (0.2% CHAPS, 60 mM citrate buffer pH 5.4). The reaction mixture (500 μL) was incubated at 50 °C (for *Aac*SHCs) and 30 °C (for *Zmo*SHCs) respectively, and 150 rpm for 20 h. All assays were performed in triplicate. The pro-treatment was similar to mentioned above.

GC–MS analysis

Gas chromatography with mass spectral detection (GC–MS) was carried on GCMS-QP2020 NX equipped with an SH-Rxi-5Sil column (30 m × 0.25 mm × 0.25 μm film thickness). Samples were injected in split-less mode by an AOC-20i autosampler with the injection port temperature set at 250 °C. The carrier gas was helium at a constant flow of 1 mL/min. The oven temperature initially started at 80 °C, and increased of 8 °C/min to 300 °C, which was held for 5 min. Quantification was determined by quotient $AREA_{product}/(AREA_{substrate}+AREA_{product})*100\%$ from the total ion chromatogram. Data are represented as mean ± s.d.

Molecular docking

The 2D structure of compound **6** were downloaded from the PubChem (http://pubchem.ncbi.nlm.nih.gov). The transformation from 2D to 3D was completed using the MM2 force field implemented on the software CS Chem3D. The result was saved in .pdb format. The 3D structure was visualized with the PyMOL program. Crystallographic structure of *Aac*SHC in complex with aza-squalene (PDB: 1UMP) were downloaded from the Brookhaven Protein

Data Bank (http://www.rcsb.org/). The macromolecule was separated from the ligand using PyMOL and saved in .pdb format. The MGLtools 1.5.6 software was used to optimize the 3D structure of *Aac*SHC and compound **6** including adding hydrogen atoms and repairing the charges by adding a Gasteiger charge. Both were saved as .pdbqt format and prepared for the docking process. Docking studies were performed using AutoDock Vina 1.2.2. The search space was included in a box of 40 × 40 × 40 Å, centered on the proposed binding site of the ligand. The protein-ligand visualization was processed using PyMOL.

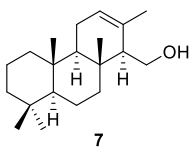
Preparative-scale cyclization reaction of geranylgeraniol catalyzed by *Aac*SHC and its variants

To determine the structure of the products and apply further to the chemoenzymatic synthesis of diterpene and meroterpenoid, large-scale biocatalytic polyene cyclization reactions were performed with semi-purified *Aac*SHCs. 80 g *E. coli* cell pellet (expressing wild-type *Aac*SHC) was used to generate ~23.3 mL semi-purified *Aac*SHC protein solution. For the reaction, 23.3 mL wild-type *Aac*SHC (~50.4 mg/mL, 0.70 mM, correspond to 2 mol% catalyst) and 237 mg geranylgeraniol (0.82 mmol, 4.1 mL, 200 mM stock solution in DMSO) were added in 391.6 mL reaction buffer (60 mM citrate, pH 6.0) and stirred in a round-bottom flasks at 50 °C for 15 days until geranylgeraniol was exhausted completely. Afterward, the reaction mixture was extracted with ethyl acetate (5 × 400 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The residue was purified using column chromatography (silica gel, hexane/ethyl acetate = 19/1 to 1/1) to give compound **7** (45 mg, 0.15 mmol, 19%), compound **8** (14.6 mg, 0.047 mmol, 5.8%), compound **9** (125.6 mg, 4.07 mmol, 53%), and the mixture of compounds **7** and **10** (15 mg, 0.052 mmol).

For *Aac*SHC-I261G, 240 g cell pellet was used to generate ~400 mL semi-purified *Aac*SHC-I261G protein solution. For the reaction, 400 mL *Aac*SHC-I261G (~14.2 mg/mL, 0.20 mM, correspond to 2.5 mol% catalyst) and 906.3 mg geranylgeraniol (3.12 mmol, 7.8 mL, 400 mM stock solution in DMSO) were added in 592.9 mL reaction buffer and stirred in a round-bottom flasks at 50 °C for 10 days. The pro-treatment was similar to mentioned above. After extraction and purification, compound **7** (340 mg, 1.17 mmol, 37%), compound **8** (46.8 mg, 0.15 mmol, 4.9%), and compound **9** (228.6 mg, 0.74 mmol, 23.8%) were obtained.

For *Aac*SHC-215G2, 83 g cell pellet was used to generate semi-purified protein solution with four times CHAPS-solubilization. For the reaction, up to 3.3 g geranylgeraniol (11.36 mmol, dissolved in 5% DMSO) were fed in the reaction buffer (0.2% CHAPS, 60 mM citrate buffer, pH 5.4) with the semi-purified *Aac*SHC-215G2 solution (98.7

mg, 1.31 μmol, correspond to 0.01 mol% catalyst) and incubated at 35 °C, 150 rpm for 22.5 days. With reaction time extension, the pH value of the reaction mixture increased continuously and was adjusted to 5.4 with citric acid during the process. The pro-treatment was similar to mentioned above. After extraction and purification, compound **7** (1.12 g, 3.86 mmol, 34%), compound **8** (98.1 mg, 0.32 mmol, 2.8%)，and compound **9** (736 mg, 2.39 mmol, 21%) were obtained.



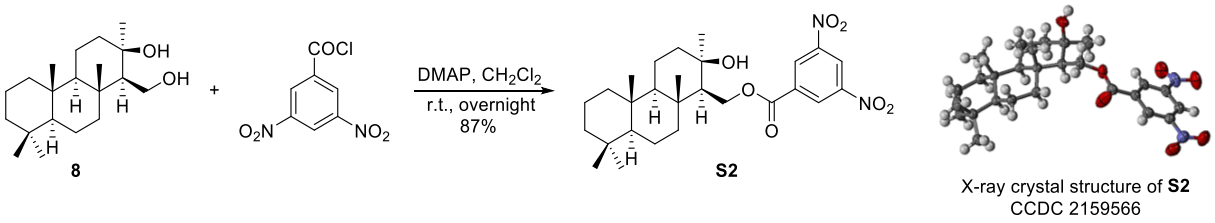
7

Compound 7. white solid. TLC: R_f = 0.45 (hexane/EtOAc = 9/1), PMA stain. $[\alpha]_D^{22.6}$ = –4.32 (c = 0.37, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.51 (s, 1H), 3.85 (dd, *J* = 11.2, 3.3 Hz, 1H), 3.73 (dd, *J* = 11.2, 5.1 Hz, 1H), 2.06 (dt, *J* = 12.8, 3.2 Hz, 1H), 1.94 – 1.83 (m, 3H), 1.78 (s, 3H), 1.63 (d, *J* = 12.4 Hz, 2H), 1.54 (d, *J* = 3.7 Hz, 1H), 1.43 – 1.32 (m, 3H), 1.27 – 1.08 (m, 4H), 0.89 (s, 3H), 0.86 (s, 3H), 0.83 (s, 3H), 0.82 (s, 3H), 0.78 (dd, *J* = 12.7, 3.7 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 132.8, 124.1, 61.0, 58.1, 56.4, 55.0, 42.1, 41.7, 40.1, 37.4, 36.4, 33.6, 33.3, 22.7, 22.0, 21.8, 18.9, 18.7, 16.0, 16.0. IR (KBr, cm^{-1}) 3361, 2921, 1041, 959, 839. HRMS-ESI (*m/z*): $[M+H]^+$ calculated for $C_{20}H_{35}O^+$, 291.2682; found, 291.2683.

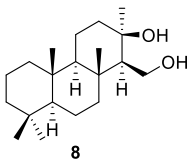
Comparison of NMR data for compound 7 to literature values ($CDCl_3$; δ, ppm)[1-2]

^{1}H NMR ($CDCl_3$)			^{13}C NMR ($CDCl_3$)		
Synthetic 500 MHz δ_H (ppm)	Literature[2] δ_H (ppm)	Δδ (ppm)	Synthetic 126 MHz δ_C (ppm)	Literature[1] δ_C (ppm)	Δδ (ppm)
5.51 (s, 1H)	5.50 (s, 1H)	0.01	132.8	132.9	–0.1
3.85 (dd, 11.2, 3.3, 1H)	3.88 – 3.82 (m, 1H)	/	124.1	124.1	0
3.73 (dd, 11.2, 5.1, 1H)	3.76 – 3.69 (m, 1H)	/	61.0	61.1	–0.1
2.06 (dt, 12.8, 3.2, 1H)	2.07 (dt, 3.2, 12.6, 1H)	–0.01	58.1	58.1	0
1.94 – 1.83 (m, 3H)	1.95 – 1.83 (m, 3H),	/	56.4	56.5	–0.1
1.78 (s, 3H)	1.78 (s, 3H)	0	55.0	55.1	–0.1
1.63 (d, 12.4, 2H)	1.67 – 1.57 (m, 2H)	/	42.1	42.1	0
1.54 (d, 3.7, 1H)	1.55 – 1.51 (m, 1H)	/	41.7	41.7	0
1.43 – 1.32 (m, 3H)	1.43 – 1.31 (m, 3H)	/	40.1	40.1	0
1.27 – 1.08 (m, 4H)	1.25 – 1.07 (m, 4H)	/	37.4	37.5	-0.1
0.89 (s, 3H)	0.89 (s, 3H)	0	36.4	36.4	0
0.86 (s, 3H)	0.86 (s, 3H)	0	33.6	33.6	0
0.83 (s, 3H)	0.84 (s, 3H)	–0.01	33.3	33.3	0
0.82 (s, 3H)	0.82 (s, 3H)	0	22.7	22.7	0
0.78 (dd, 12.7, 3.7, 1H)	0.81 – 0.78 (m, 1H)	/	22.0	22.0	0

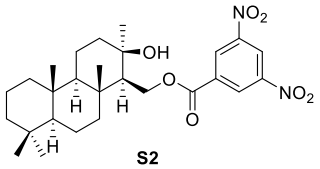
			21.8	21.9	–0.1
			18.9	18.9	0
			18.7	18.7	0
			16.0	16.0	0
			16.0	16.0	0



The structure of compound **8** was confirmed by the X-ray crystallography. To a solution of compound **8** (8 mg, 0.026 mmol, 1.0 equiv.) in dichloromethane (1 mL) was added 4-(dimethylamino)-pyridine (9.5 mg, 0.078 mmol, 3.0 equiv.) and 3,5-dinitrobenzoyl chloride (15.0 mg, 0.065 mmol, 2.5 equiv.). This reaction mixture was allowed to stir at room temperature for 24 h. Saturated aqueous Na_2CO_3 (3 mL) was added to the reaction mixture, and the aqueous layer was extracted with dichloromethane (3 × 3 mL). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/ethyl acetate = 50/1 to 20/1) to give compound **S2** (11.3 mg, 0.023 mmol, 87%) as a white solid. Solvent for growing crystal: acetone, ethanol and acetonitrile (1/1/1, v/v/v). CCDC 2159566 contains the supplementary crystallographic data for compound **S2**. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data-request/cif.

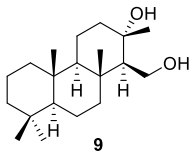


Compound 8. white solid. TLC: R_f = 0.63 (hexane/EtOAc = 1/1), PMA stain. $[\alpha]_D^{24.2}$ = –27.21 (c = 0.61, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.07 (dt, *J* = 11.6, 7.8 Hz, 2H), 2.55 (s, 2H), 1.99 (dt, *J* = 12.5, 3.3 Hz, 1H), 1.76 – 1.70 (m, 2H), 1.63 (d, *J* = 3.5 Hz, 2H), 1.55 – 1.37 (m, 6H), 1.33 (s, 3H), 1.23 (s, 3H), 1.13 (dd, *J* = 13.5, 4.1 Hz, 1H), 0.94 (dd, *J* = 18.0, 3.8 Hz, 2H), 0.86 (s, 3H), 0.84 (s, 3H), 0.81 (s, 3H), 0.77 (dd, *J* = 16.1, 6.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 74.0, 60.8, 60.2, 59.0, 56.8, 43.1, 42.3, 42.1, 40.3, 38.8, 37.8, 33.5, 33.4, 31.0, 21.5, 18.8, 18.4, 17.4, 16.52. IR (KBr, cm^{-1}) 3324, 3298, 1383, 1254, 1027. HRMS-ESI (*m/z*): $[M+Na]^+$ calculated for $C_{20}H_{36}ONa^+$, 331.2608; found, 331.2608.



S2

Compound S2. white solid. TLC: R_f = 0.54 (hexane/EtOAc = 9/1), PMA stain. $[\alpha]_D^{24.5}$ = –20.37 (c = 0.43, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.22 (t, *J* = 2.1 Hz, 1H), 9.13 (d, *J* = 2.1 Hz, 2H), 4.69 (d, *J* = 4.6 Hz, 2H), 2.01 (dt, *J* = 12.7, 3.2 Hz, 1H), 1.80 – 1.71 (m, 2H), 1.64 – 1.57 (m, 4H), 1.45 – 1.37 (m, 4H), 1.29 (s, 3H), 1.26 (d, *J* = 2.9 Hz, 1H), 1.24 – 1.08 (m, 4H), 1.04 (s, 3H), 0.92 – 0.88 (m, 1H), 0.87 (s, 3H), 0.85 (s, 3H), 0.82 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.5, 148.9, 134.4, 129.4, 122.4, 72.0, 64.9, 60.3, 58.3, 56.6, 42.9, 42.2, 41.6, 40.2, 38.6, 37.8, 33.5, 33.4, 31.3, 21.5, 18.8, 18.2, 17.4, 17.2, 16.49. IR (film) 3560, 3094, 2938, 2842, 1720, 1748, 1343, 1298, 1268, 1167, 914, 728. HRMS-ESI (*m/z*): $[M+Na]^+$ calculated for $C_{27}H_{38}N_2O_7Na^+$, 525.2571; found, 525.2572.



9

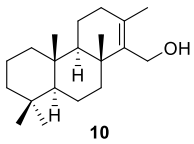
Compound 9. white solid. TLC: R_f = 0.51 (hexane/EtOAc = 1/1), PMA stain. $[\alpha]_D^{24.7}$ = –3.55 (c = 0.76, $CHCl_3$) (literature[3] for the enantiomer: $[\alpha]_D$ = +5.12, c = 1.11, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90 (d, *J* = 6.4 Hz, 2H), 2.27 (s, 2H), 1.84 (dd, *J* = 16.9, 12.8 Hz, 2H), 1.69 – 1.50 (m, 6H), 1.46 – 1.36 (m, 3H), 1.33 (s, 3H), 1.27 – 1.11 (m, 4H), 0.92 (d, *J* = 11.8 Hz, 1H), 0.84 (s, 3H), 0.81 (d, *J* = 2.1 Hz, 1H), 0.79 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 75.1, 61.2, 61.0, 60.7, 56.5, 44.6, 42.2, 41.9, 40.2, 38.1, 37.7, 33.4, 24.3, 21.5, 19.2, 18.7, 18.5, 17.4, 16.5. IR (KBr, cm^{-1}) 3340, 2937, 1384, 1139, 1028. HRMS-ESI (*m/z*): $[M+Na]^+$ calculated for $C_{20}H_{36}ONa^+$, 331.2608; found, 331.2607.

Comparison of NMR data for compound 9 to literature values ($CDCl_3$; δ, ppm)[4]

^{1}H NMR ($CDCl_3$)			^{13}C NMR ($CDCl_3$)		
Synthetic 500 MHz δ_H (ppm)	Literature δ_H (ppm)	Δδ (ppm)	Synthetic 126 MHz δ_C (ppm)	Literature δ_C (ppm)	Δδ (ppm)
3.90 (d, 6.4 Hz, 2H)	3.87 (d, 6, 2H)	0.03	75.1	75.1	0
2.27 (s, 2H),	/	/	61.2	61.1	0.1
1.84 (dd,16.9, 12.8 Hz, 2H)	/	/	61.0	60.9	0.1
1.69 – 1.50 (m, 6H)	/	/	60.7	60.6	0.1
1.46 – 1.36 (m, 3H)	/	/	56.5	56.4	0.1
1.33 (s, 3H)	1.35 (3 H, s)	–0.02	44.6	44.5	0.1
1.27 – 1.11 (m, 4H)	/	/	42.2	42.1	0.1
0.92 (d, 11.8 Hz, 1H)	/	/	41.9	41.8	0.1
0.84 (s, 3H)	0.87 (3 H, s)	–0.05	40.2	40.1	0.1
0.81 (d, 2.1 Hz, 1H)	/	/	38.1	38.0	0.1
0.79 (s, 9H)	0.82 (9 H, s)	–0.03	37.7	37.7	0

			33.4	33.4	0
			33.3	33.3	0
			24.3	24.3	0
			21.5	21.4	0.1
			19.2	19.1	0.1
			18.7	18.7	0
			18.5	18.4	0.1
			17.4	17.4	0
			16.5	16.5	0

The mixture of compound **7** and **10** (15 mg, 0.052 mmol) were mixed with the solution of *t*-BuOOH (18.5 μL, 0.102 mmol, 2 equiv., 5.5 M in decane) and SeO_2 (4.5 mg, 0.041 mmol, 0.8 equiv.) in dichloromethane. After stirring at 0 °C for 24 h and at rt for 5 h, pure **10** was obtained to identify its structure (compound **7** was oxidized with SeO_2, but compound **10** not).



10

Compound 10. white solid. TLC: R_f = 0.45 (hexane/EtOAc = 9/1), PMA stain. $[\alpha]_D^{22.7}$ = –21.50 (c = 1.44, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.18 (d, *J* = 11.5 Hz, 1H), 4.03 (d, *J* = 11.5 Hz, 1H), 2.05 – 2.00 (m, 2H), 1.70 (s, 3H), 1.63 (dd, *J* = 11.1, 6.5 Hz, 4H), 1.39 (ddd, *J* = 16.1, 6.0, 3.6 Hz, 6H), 1.13 (dd, *J* = 13.7, 4.5 Hz, 2H), 1.10 – 1.05 (m, 2H), 0.95 (s, 3H), 0.84 (s, 6H), 0.81 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.2, 132.4, 76.8, 58.3, 56.6, 56.5, 42.3, 39.8, 38.5, 38.5, 37.5, 34.2, 33.4, 21.9, 21.5, 19.3, 18.8, 18.8, 17.9, 16.5. IR (KBr, cm^{-1}) 3445, 3383, 2925, 2868, 1461, 1386, 1363, 1266, 1194, 995, 748. HRMS-ESI (*m/z*): $[M+Na]^+$calculated for $C_{20}H_{34}ONa^+$, 313.2502; found, 313.2488.

Comparison of NMR data for compound 10 to literature values ($CDCl_3$; δ, ppm)[5]

^{1}H NMR ($CDCl_3$)			^{13}C NMR ($CDCl_3$)		
Synthetic 400 MHz δ_H (ppm)	Literature δ_H (ppm)	Δδ (ppm)	Synthetic 101 MHz δ_C (ppm)	Literature δ_C (ppm)	Δδ (ppm)
4.18 (d, 11.5, 1H)	4.18 (d, 11.5, 1H)	0	141.16	140.97	0.19
4.03 (d, 11.5, 1H)	4.04 (d, 11.5, 1H)	–0.01	132.44	132.28	0.16
2.03 (dd, 10.3, 4.8, 2H)	2.04 (m, 2H)	–0.01	58.30	58.14	0.16
1.97 (dd, 9.4, 3.2, 1H)	1.97 (m, 1H)	0	56.60	56.42	0.18
1.70 (s, 3H)	1.71 (s, 3H)	–0.01	56.53	56.35	0.18
1.65 – 1.61 (m, 2H)	1.61 (m, 2H)	0.01	42.28	42.11	0.17
1.43 – 1.35 (m, 5H)	1.39 (m, 5H)	0	39.84	39.67	0.17
1.16 – 1.07 (m, 3H)	1.11 (m, 3H)	0.01	38.52	/	/
0.95 (s, 3H)	0.97 (s, 3H)	–0.02	38.46	38.28	0.18
0.84 (s, 3H)	0.85 (s, 6H)	–0.01	37.53	37.36	0.17

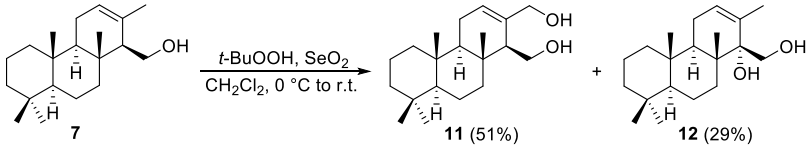
0.84 (s, 3H)	0.82 (s, 3H)	0.02	34.18	34.01	0.17
0.81 (s, 3H)	0.81 (s, 3H)	0	33.41	33.26	0.15
			21.90	21.73	0.17
			21.48	21.32	0.16
			19.32	19.16	0.16
			18.80	18.63	0.17
			18.75	18.59	0.16
			17.87	17.71	0.16
			16.52	16.37	0.15

III. Chemical synthesis

General information

Unless otherwise mentioned, all the reactions were carried out under an argon atmosphere with dry solvents. Reagents were used without further purification. Solvent purification was conducted according to *Purification of Laboratory Chemicals* (Peerrin, D. D.; Armarego, W. L. and Perrins, D. R., Pergamon Press: Oxford, 1980). Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Tsingdao silica gel plates (GF-254). Staining was performed with an ethanolic solution of phosphomolybdic acid (PMA), or by oxidative staining with an aqueous basic potassium permanganate ($KMnO_4$) solution and subsequent heating. Tsingdao silica gel (60, particle size 0.040-0.063 mm) was used for flash column chromatography. NMR spectra were recorded on Brüker Advance 500 (^{1}H: 500 MHz, ^{13}C: 126 MHz). Residual undeuterated solvent was used as an internal reference ($CDCl_3$: ^{1}H NMR δ_H = 7.26 ppm, ^{13}C NMR δ_C = 77.16 ppm). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High-resolution mass spectra (HRMS) were measured on Thermo Q Exactive Focus. The ionization method is ESI and the mass analyzer type of TOF. IR spectra were recorded on an IR Prestige-21 FTIR spectrometer with a KBr disc. Optical rotation values were recorded on a Rudolph Research Analytical Autopol I polarimeter (Rudolph Research Co.).

Synthesis of (+)-isoagatholactone (1) and (+)-spongian-16-one (2)

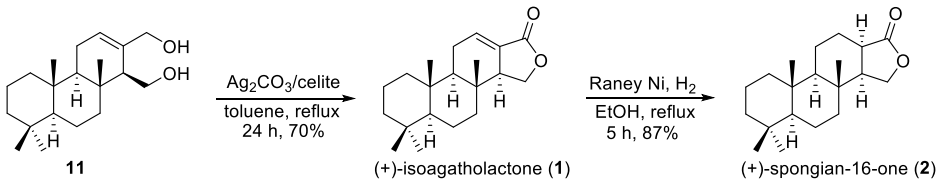


Compound 11. To the suspension of SeO_2 (37.3 mg, 0.34 mmol, 0.8 equiv.) in CH_2Cl_2 (2 mL) was added a solution

of *t*-BuOOH (153 μL, 0.84 mmol, 2 equiv., ca. 5.5 M in decane) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C, and then treated with a solution of compound **7** (122 mg, 0.42 mmol, 1 equiv.) in CH_2Cl_2 (10 mL). After stirring at 0 °C for 24 h, the reaction mixture was stirred at rt for another 5 h. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ (10 mL) and extracted with ethyl acetate (3 × 3 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/ethyl acetate = 8/1 to 5/2) to give compound **11** (65.1 mg, 0.21 mmol, 51%) and compound **12** (37.7 mg, 0.12 mmol, 29%).

Compound 11. white solid. TLC: R_f = 0.24 (hexane/EtOAc = 5/2), PMA stain. $[\alpha]_D^{23.0}$ = –7.71 (*c* = 1.46, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.81 – 5.73 (m, 1H), 4.34 (d, *J* = 12.1 Hz, 1H), 3.98 (d, *J* = 12.1 Hz, 1H), 3.90 (dd, *J* = 10.8, 2.1 Hz, 1H), 3.68 (dd, *J* = 10.8, 8.4 Hz, 1H), 2.14 (s, 1H), 2.07 – 2.01 (m, 2H), 1.91 (dd, *J* = 14.1, 2.1 Hz, 1H), 1.61 – 1.56 (m, 2H), 1.41 – 1.10 (m, 8H), 0.87 (s, 3H), 0.87 (s, 3H), 0.82 (s, 3H), 0.73 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.8, 127.6, 67.6, 61.6, 56.3, 55.2, 54.4, 42.0, 41.1, 40.0, 37.4, 35.9, 33.5, 33.3, 22.8, 21.8, 19.0, 18.6, 15.8, 15.6. IR (KBr, cm^{-1}) 3318, 2994, 2926, 2847, 1673, 1384, 1266, 990, 741. HRMS-ESI (*m/z*): $[M+Na]^+$ calculated for $C_{20}H_{34}O_2Na^+$, 329.2451; found, 329.2452.

Compound 12. white solid. TLC: R_f = 0.51 (hexane/EtOAc = 5/2), PMA stain. $[\alpha]_D^{23.5}$ = –17.14 (c = 2.45, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.55 (s, 1H), 3.76 (d, *J* = 11.1 Hz, 1H), 3.59 (d, *J* = 11.1 Hz, 1H), 1.94 – 1.89 (m, 1H), 1.80 (s, 3H), 1.62 – 1.52 (m, 7H), 1.37 (dd, *J* = 15.5, 6.9 Hz, 5H), 1.17 – 1.13 (m, 1H), 0.93 (s, 3H), 0.87 (s, 3H), 0.82 (s, 3H), 0.81 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 135.2, 127.5, 75.8, 62.4, 55.5, 48.0, 42.0, 41.0, 40.3, 37.4, 33.5, 33.2, 33.1, 23.2, 21.8, 20.3, 18.7, 16.4, 16.00. IR (KBr, cm^{-1}) 3478, 2924, 2867, 2846, 1730, 1458, 1388, 1369, 1044, 743. HRMS-ESI (*m/z*): $[M+Na]^+$ calculated for $C_{20}H_{34}O_2Na^+$, 329.2451; found, 329.2453.



(+)-isoagatholactone (1). To the mixture of compound **11** (18 mg, 0.059 mmol, 1 equiv.) in toluene (2 mL) was added Ag_2CO_3/celite[8] (0.88 g, 1.17 mmol, 20 equiv.). The reaction mixture was refluxed for 24 h, and then cooled to room temperature. The solid phase was filtered off, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, hexane/ethyl acetate = 7/1) to give (+)-

isoagatholactone (12.5 mg, 0.041 mmol, 70%) as white solid. TLC: R_f = 0.67 (hexane/EtOAc = 3/1), $KMnO_4$ stain. $[\alpha]_D^{24.0}$ = +1.71 (c = 0.2, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 6.86 (q, *J* = 3.4 Hz, 1H), 4.37 (t, *J* = 9.2 Hz, 1H), 4.04 (t, *J* = 9.1 Hz, 1H), 2.86 – 2.74 (m, 1H), 2.34 (ddd, *J* = 20.3, 9.3, 3.9 Hz, 1H), 2.16 – 2.04 (m, 1H), 1.69 (dd, *J* = 9.6, 2.9 Hz, 1H), 1.67 – 1.50 (m, 4H), 1.46 – 1.36 (m, 3H), 1.35 – 1.25 (m, 3H), 1.20 – 1.11 (m, 1H), 0.92 (s, 3H), 0.87 (s, 3H), 0.83 (s, 3H), 0.77 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.38, 136.61, 127.10, 67.40, 56.85, 54.59, 51.35, 41.87, 40.92, 39.92, 37.44, 34.63, 33.54, 33.35, 24.34, 21.76, 18.44, 15.45, 14.27. IR (KBr, cm^{-1}) 1731,1670, 1462, 1251, 1158, 1102, 969,738. HRMS-ESI (*m/z*): $[M+Na]^+$ calculated for $C_{20}H_{30}ONa^+$, 325.2138; found, 325.2139.

Comparison of ^1H NMR data for (+)-isoagatholactone (1) to literature values ($CDCl_3$; δ, ppm)[9-10]

^1H NMR ($CDCl_3$)			
Synthetic 400 M δ_H (ppm)	Literature 1[9] δ_H (ppm)	Literature 2[10] δ_H (ppm)	Δδ2
6.86 (q, 3.4, 1H)	6.85 (q, 3.4, 1H)	6.86 (d, 3.4, 1 H)	0
4.37 (t, 9.2, 1H)	4.36 (dd, 9.1, 9.1 1H)	4.36 (t, 9.2, 1 H)	0.01
4.04 (t, 9.1, 1H)	4.02 (t, 9.1, 1H)	4.04 (t, 9.1, 1 H)	0
2.86 – 2.74 (m, 1H)	2.78 (m, 1H)	2.79 (dd, 8.3, 4.1, 1 H)	0
2.34 (ddd, 20.3, 9.3, 3.9, 1H)	2.32 (dddd, 20.3, 5.6, 3.4, 3.4, 1H)	2.33 (dd, 12.1, 8.1, 1 H)	0.01
2.16 – 2.04 (m, 1H)	2.08 (dddd, 20.3, 11.5, 4.9, 3.4)	2.16 – 2.02 (m, 1 H)	0
1.69 (dd, 9.6, 2.9, 1H)		1.68 (dd, 12.6, 3.0, 1 H)	0.01
1.67 – 1.50 (m, 4H)		1.65 – 1.53 (m, 4 H)	0
1.46 – 1.36 (m, 3H)		1.46 – 1.24 (m, 6 H)	0
1.35 – 1.25 (m, 3H)			0
1.20 – 1.11 (m, 1H)		1.15 (dt, 13.7, 7.3, 1 H)	0
0.92 (s, 3H)	0.90 (s, 3H)	0.92 (s, 3 H)	0
0.87 (s, 3H)	0.87 (s, 3H)	0.87 (s, 3 H)	0
0.83 (s, 3H)	0.81 (s, 3H)	0.83 (s, 3 H)	0
0.77 (s, 3H)	0.75 (s, 3H)	0.77 (s, 3 H)	0

Comparison of ^{13}C NMR data for (+)-isoagatholactone (1) to literature values ($CDCl_3$; δ, ppm)[9-10]

^{13}C NMR ($CDCl_3$)				
Synthetic 101 M δ_H (ppm)	Literature 1[9] δ_H (ppm)	Literature 2[10] δ_H (ppm)	Δδ1 = Synthetic-Literature 1 (ppm)	Δδ2= Synthetic-Literature 2 (ppm)
170.38	170.17	170.38	0.21	0
136.61	136.4	136.6	0.21	0.01

127.1	126.88	127.1	0.22	0
67.4	67.2	67.4	0.2	0
56.85	56.65	56.9	0.2	–0.05
54.59	54.39	54.6	0.2	–0.01
51.35	51.14	51.4	0.21	–0.05
41.87	41.66	41.9	0.21	–0.03
40.92	40.71	40.9	0.21	0.02
39.92	39.72	39.9	0.2	0.02
37.44	37.23	37.5	0.21	–0.06
34.63	34.44	34.7	0.19	–0.07
33.54	33.34	33.6	0.2	–0.06
33.35	33.15	33.4	0.2	–0.05
24.34	24.14	24.4	0.2	–0.06
21.76	21.56	21.8	0.2	–0.04
18.44	18.24	18.5	0.2	–0.06
15.45	15.26	15.5	0.19	–0.05
14.27	14.08	14.3	0.19	–0.03

(+)-spongian-16-one (2). To the mixture of (+)-isoagatholactone (10 mg, 0.033 mmol) in ethanol (1 mL) was carefully added Raney Ni (0.2 mL, in water). The reaction mixture was refluxed for 5 h under H_2 atmosphere, and then cooled to room temperature. The reaction was quenched with aqueous HCl (1 mL, 2 mol/L) and stirred for 15 min at room temperature. The mixture was filtered. Saturated aqueous $NaHCO_3$ was added to the filtrate and then the mixture was extracted with CH_2Cl_2 (3 × 3 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated to dryness under reduced pressure. The residue was purified by flash chromatography (silica gel, hexane/ethyl acetate = 9/1) to give (+)-spongian-16-one (8.8 mg, 0.029 mmol, 87%). TLC: R_f = 0.56 (hexane/EtOAc = 5/1), PMA stain. $[\alpha]_D^{24.6}$ = +13.75 (c = 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21 (d, *J* = 9.7 Hz, 1H), 4.10 (dd, *J* = 9.7, 5.3 Hz, 1H), 2.53 (t, *J* = 7.8 Hz, 1H), 2.34 – 2.26 (m, 1H), 2.08 (dd, *J* = 7.9, 5.4 Hz, 1H), 1.82 (dt, *J* = 12.7, 3.1 Hz, 1H), 1.73 (d, *J* = 12.3 Hz, 1H), 1.62 (d, *J* = 5.4 Hz, 2H), 1.52 (d, *J* = 3.5 Hz, 1H), 1.51 – 1.47 (m, 1H), 1.46 – 1.39 (m, 1H), 1.38 (dd, *J* = 6.1, 3.1 Hz, 1H), 1.36 – 1.32 (m, 1H), 1.30 (d, *J* = 8.9 Hz, 1H), 1.18 – 1.08 (m, 1H), 1.03 (td, *J* = 13.0, 4.0 Hz, 1H), 0.86 (s, 3H), 0.85 (s, 3H), 0.82 (s, 3H), 0.80 (s, 3H), 0.79 (s, 1H), 0.78 – 0.75 (m, 1H), 0.74 (t, *J* = 3.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.2, 67.8, 56.8, 56.6, 50.7, 42.3, 42.1, 40.2, 37.6, 37.5, 35.9, 33.5, 22.5, 21.7, 18.7, 18.1, 17.4, 16.5, 15.6. IR (KBr, cm^{-1}) 1769, 1696, 1449, 1195, 1141, 961. HRMS-ESI (*m/z*): $[M+Na]^+$ calculated for $C_{20}H_{32}ONa^+$, 327.2295; found, 327.2295.

Comparison of ^{1}H NMR data for (+)-spongian-16-one (2) to literature values ($CDCl_3$; δ, ppm)[11]

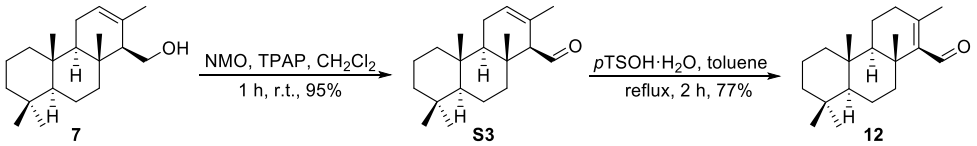
^{1}H NMR ($CDCl_3$)		
Synthetic δ_H (ppm)	Literature δ_H (ppm)	Δδ (ppm)
4.21 (d, 9.7, 1H),	4.21 (d, 9.8, 1H)	0
4.10 (dd, 9.7, 5.3, 1H)	4.10 (dd, 9.8, 5.4, 1H)	0
2.53 (t, 7.8, 1H)	2.53 (ddd, 7.9, 7.9, 1.1, 1H)	0
2.34 – 2.26 (m, 1H)	2.30 (dddd, 14.2, 5.0, 1.8, 1.3,1H)	0
2.08 (dd, 7.9, 5.4, 1H)	2.08 (dd, 7.9, 5.4, 1H)	0
1.82 (dt, 12.7, 3.1, 1H)	1.82 (ddd, 12.8, 2.2, 2.2, 1H)	0
1.73 (d, = 12.3, 1H)	1.73 (dddd, 12.7, 3.4, 3.4, 1.5, 1H)	0
1.62 (m, 2H)	1.60 (m) 1.61 (m, 1H)	0.01
1.52 (d, 3.5, 1H)	1.53 (m, 1H)	–0.01
1.51 – 1.47 (m, 1H)	1.52 (m, 1H)	–0.02
1.46 – 1.39 (m, 1H)	1.41 (ddddd, 14.1, 3.8, 3.8, 3.3, 3.3, 1H)	0.03
1.38 (dd, 6.1, 3.1, 1H)	1.37 (ddd, 13.1, 3.5, 1.5, 1H)	0.01
1.36 – 1.32 (m, 1H)	1.34 (dddd, 13.6, 13.6, 13.6, 3.2, 1H)	0
1.30 (d, 8.9, 1H)	1.27 (dddd, 13.4, 13.4, 13.4, 5.1, 1H)	0.03
1.18 – 1.08 (m, 1H)	1.13 (ddd, 13.5, 13.5, 4.0, 1H)	0
1.03 (td,13.0, 4.0, 1H)	1.03 (ddd, 12.8, 12.8, 3.3, 1H)	0
0.86 (s, 3H)	0.86 (s, 3H)	0
0.85 (s, 3H)	0.85 (d, 0.6, 3H)	0
0.82 (s, 3H)	0.82 (d, 0.8, 3H)	0
0.80 (s, 3H)	0.81 (m, 3H)	–0.01
0.79 (s, 1H)	0.80 (s, 1H)	–0.01
0.78 – 0.75 (m, 1H)	0.78 (ddd, 12.7, 12.7, 3.9, 1H)	–0.02
0.74 (t, 3.9, 1H)	0.76 (dd, 12.3, 2.2, 1H)	–0.02

Comparison of ^{13}C NMR data for (+)-spongian-16-one (2) to literature values ($CDCl_3$; δ, ppm)[11]

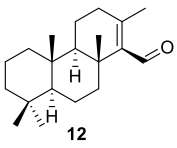
^{13}C NMR ($CDCl_3$)		
Synthetic δ_C(ppm)	Literature δ_C (ppm)	Δδ (ppm)
179.2	179.0	0.2
67.8	67.6	0.2
56.8	56.7	0.1
56.6	56.4	0.2
50.7	50.5	0.2
42.3	42.2	0.1
42.1	41.9	0.2
40.2	40.0	0.2
37.6	37.4	0.2

37.5	37.3	0.2
35.9	35.7	0.2
33.5	33.3	0.2
22.5	22.4	0.1
21.7	21.5	0.2
18.7	18.5	0.2
18.1	17.9	0.2
17.4	17.2	0.2
16.5	16.3	0.2
15.6	15.5	0.1

Synthesis of deoxychevalone A (4)

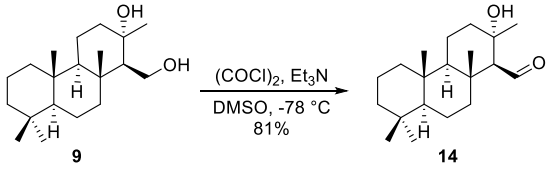


Compound S3. To a mixture of **7** (70 mg, 0.24 mmol, 1 equiv.), N-methylmorpholine N-oxide (NMO) (50.5 mg, 0.43 mmol, 1.8 equiv.) and molecular sieves (120 mg, 500 mg/mmol) in anhydrous CH_2Cl_2 (8 mL) under argon and at room temperature, TPAP (8.4 mg, 0.024 mmol, 0.1 equiv.) was added. The reaction mixture was stirred for 1 h and then filtered through a short pad of silica gel and celite, eluting with EtOAc. Evaporation of the solvent yielded the aldehyde **S3** (65.8 mg, 0.23 mmol, 95%). TLC: R_f = 0.74 (hexane/EtOAc = 9/1), PMA stain. $[\alpha]_D^{25.3}$ = –12.34 (c = 0.43, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.69 (d, J = 5.1 Hz, 1H), 5.67 – 5.62 (m, 1H), 2.59 (s, 1H), 2.02 – 1.96 (m, 2H), 1.75 (dd, J = 9.8, 2.9 Hz, 1H), 1.61 (s, 3H), 1.58 – 1.52 (m, 2H), 1.38 (dddd, J = 14.4, 12.9, 10.0, 4.0 Hz, 6H), 1.16 – 1.06 (m, 3H), 1.04 (s, 3H), 0.91 (s, 3H), 0.85 (s, 3H), 0.81 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.9, 127.8, 125.4, 68.2, 56.6, 54.1, 42.0, 40.0, 37.6, 37.4, 33.5, 33.3, 22.8, 21.8, 21.6, 18.5, 18.44, 16.7, 16.04. IR (KBr, cm^{-1}) 2997, 2879, 2849, 2714, 1789, 1437, 1392, 1194, 1067, 816. HRMS-ESI (m/z): $[M+H]^+$ calculated for $C_{20}H_{33}O^+$, 289.2526; found, 289.2526.

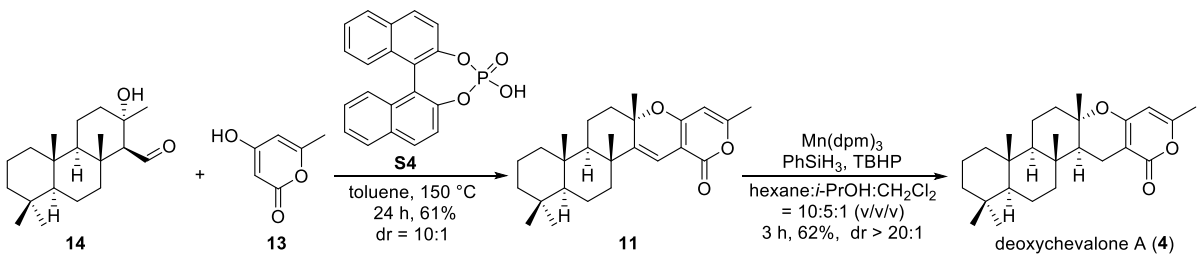


Compound 13. To a solution of aldehyde **S3** (40 mg, 0.14 mmol, 1 equiv.) in dry toluene (2 mL), *p*TsOH·H_2O (2.4 mg, 0.014 mmol, 0.1 equiv.) was added and the mixture was heated at reflux temperature for 2 h. Then, saturated aqueous $NaHCO_3$ was added. The organic phase was separated and the aqueous phase was extracted with ethyl acetate (3 × 3 mL). Extracts were washed with brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The obtained crude product

was purified by column chromatography (silica gel, hexane/ethyl acetate = 30/1) to give compound **12** (31 mg, 0.11 mmol, 77%). TLC: R_f = 0.77 (hexane/EtOAc = 15/1), PMA stain. $[\alpha]_D^{25.6}$ = –30.37 (c = 0.27, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 10.03 (s, 1H), 2.64 (dt, *J* = 13.0, 3.3 Hz, 1H), 2.25 (d, *J* = 5.5 Hz, 2H), 2.02 (s, 3H), 1.74 – 1.55 (m, 7H), 1.46 – 1.36 (m, 5H), 1.18 (s, 3H), 1.05 (dd, *J* = 7.2, 1.9 Hz, 1H), 0.86 (s, 3H), 0.84 (s, 3H), 0.81 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.8, 153.4, 143.9, 77.4, 77.2, 76.9, 56.7, 56.4, 42.2, 40.0, 38.2, 38.2, 37.8, 37.0, 33.4, 33.4, 21.4, 21.4, 19.3, 18.8, 18.7, 17.3, 16.6. IR (KBr, cm^{-1}) 2924, 2851, 1667, 1454, 1375, 1262, 1082, 969, 759. HRMS-ESI (*m/z*): $[M+H]^+$ calculated for $C_{20}H_{33}O^+$, 289.2526; found, 289.2524.



Compound 15. A solution of diol **9** (43 mg, 0.14 mmol, 1.0 equiv.) in CH_2Cl_2 (5 mL, plus a 2.5 mL rinse) was added dropwise to the swern reagent (prepared by adding a solution of oxalyl chloride (24 μL, 0.28 mmol, 2.0 equiv.) in CH_2Cl_2 (0.5 mL) to a solution of DMSO (44.7 μL, 0.63 mmol, 4.5 equiv.) in CH_2Cl_2 (1 mL) at –78 °C and stirring for 20 min) under argon at –78 °C, and stirred for 45 min at the same temperature. To the reaction mixture was then added Et_3N (97 μL, 0.7 mmol, 5.0 equiv.) dropwise. After 10 min, the reaction mixture was warmed to room temperature and stirred for 10 h. The reaction was quenched by water and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated to dryness under reduced pressure. The residue was purified by flash chromatography (silica gel, hexane/ethyl acetate = 8/1) to give compound **14** (35 mg, 0.114 mol, 81%). TLC: R_f = 0.55 (hexane/EtOAc = 5/2), PMA stain. $[\alpha]_D^{25.3}$ = –14.59 (c = 0.61, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 10.02 (d, *J* = 1.1 Hz, 1H), 2.10 – 2.08 (m, 1H), 1.80 (dt, *J* = 12.6, 3.3 Hz, 1H), 1.69 – 1.61 (m, 4H), 1.50 – 1.40 (m, 4H), 1.37 (s, 3H), 1.34 – 1.25 (m, 3H), 1.21 – 1.15 (m, 1H), 1.11 (s, 3H), 0.94 (dd, *J* = 12.0, 2.2 Hz, 1H), 0.90 (d, *J* = 2.4 Hz, 1H), 0.87 (s, 3H), 0.84 (s, 3H), 0.82 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 208.2, 72.6, 71.8, 59.6, 56.6, 48.4, 42.7, 42.0, 41.5, 39.9, 37.9, 37.8, 33.4, 25.2, 21.4, 19.0, 18.8, 18.6, 18.0, 16.2. IR (KBr, cm^{-1}) 3320, 2995, 2937, 2922, 2848, 1715, 1707, 1457, 1385, 1191, 1127, 745. HRMS-ESI (*m/z*): $[M+Na]^+$ calculated for $C_{20}H_{34}O_2Na^+$, 329.2451; found, 329.2450.



Compound 14. A dried Schlenk tube was charged with **14** (6 mg, 0.02 mmol, 1 equiv.) in toluene (0.2 mL), **13** (3.7 mg, 0.03 mmol, 1.5 equiv.), and phosphoric acid **S4** (2 mg, 0.006 mmol, 0.3 equiv.). The tube was sealed and placed in a pre-heated oil bath at 150 ˚C for 24 hours. After cooling to room temperature, the reaction mixture was concentrated in vacuo. The residue was purified by preparative thin-layer chromatography (hexane/EtOAc = 3/1) to yield **14** (4.1 mg，0.122 mmol, 61%, dr = 10:1) as a white solid. TLC: R_f = 0.63 (hexane/EtOAc = 3/1), UV & PMA stain. $[\alpha]_D^{26.5}$ = –25.85 (c = 0.53, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.18 (s, 1H), 5.77 (s, 1H), 2.20 (d, *J* = 0.6 Hz, 3H), 2.18 – 2.13 (m, 1H), 2.08 – 2.04 (m, 1H), 1.86 (dt, *J* = 13.5, 6.8 Hz, 1H), 1.74 (d, *J* = 20.0 Hz, 2H), 1.71 – 1.64 (m, 2H), 1.61 (dd, *J* = 17.3, 3.6 Hz, 2H), 1.46 (s, 2H), 1.45 (s, 3H), 1.41 (dd, *J* = 8.6, 3.9 Hz, 4H), 1.13 (s, 3H), 1.03 – 0.98 (m, 1H), 0.86 (s, 3H), 0.85 (s, 3H), 0.82 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.1, 162.6, 161.6, 147.1, 108.7, 100.3, 99.9, 82.1, 56.7, 56.3, 42.1, 41.3, 40.1, 39.9, 39.8, 38.0, 33.4, 33.4, 26.9, 25.1, 21.5, 20.3, 18.7, 18.2, 16.5. IR (KBr, cm^{-1}) 1719, 1668, 1570, 1457, 1387, 1239, 987, 810, 742. HRMS-ESI (*m/z*): $[M+Na]^+$ calculated for $C_{26}H_{36}O_3Na^+$, 419.2557; found, 419.2557.

3-Deoxychevalone A (4). A solution of **14** (4.7 mg, 0.011 mmol, 1 equiv.) in dry hexanes/*i*-PrOH/CH_2Cl_2 (10:5:1, 1.0 mL hexanes, 0.5 mL *i*-PrOH, 0.1 mL CH_2Cl_2) was treated with phenylsilane (4 μL, 0.033 mmol, 3 equiv.), and 5.5 M TBHP in decanes (6 μL, 0.033 mmol, 3 equiv.). The solution was degassed with argon via subsurface sparging for 10 minutes, then $Mn(dpm)_3$ (2.2 mg, 0.0036 mmol, 0.3 equiv.) was added and the reaction was degassed for a further 30 seconds. The solution was stirred for 3 hours, then concentrated in vacuo and purified by preparative thin-layer chromatography (hexane/EtOAc = 3/1) to yield deoxychevalone A (**4**) (2.7 mg, 0.0068 mmol, 62%) as a white solid. TLC: R_f = 0.46 (hexane/EtOAc = 3/1), UV & PMA stain. $[\alpha]_D^{25.8}$ = –12.22 (c = 0.27, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.68 (s, 1H), 2.43 (dd, *J* = 16.5, 4.7 Hz, 1H), 2.17 (s, 3H), 2.14 – 2.07 (m, 1H), 2.07 – 2.01 (m, 1H), 1.85 – 1.79 (m, 1H), 1.76 – 1.59 (m, 4H), 1.52 – 1.43 (m, 2H), 1.43 – 1.38 (m, 2H), 1.35 (dd, *J* = 12.6, 3.7 Hz, 2H), 1.30 (s, 2H), 1.19 (s, 3H), 1.08 (dd, *J* = 33.6, 11.1 Hz, 2H), 1.02 – 0.92 (m, 2H), 0.88 (s, 3H), 0.85 (s, 3H), 0.83 (s, 3H), 0.81 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.1, 162.6, 161.6, 147.1, 108.7, 100.3, 99.9, 82.1, 56.7, 56.3, 42.1, 41.3, 40.1, 39.9, 39.8, 38.0, 33.4, 33.4, 26.9, 25.1, 21.5, 20.3, 18.7, 18.2, 16.5.

IR (KBr, cm^{-1}) 2994, 2925, 2870, 2855, 1719, 1654, 1570, 1457, 1387, 1239, 987, 742. HRMS-ESI (*m/z*): $[M+H]^+$ calculated for $C_{26}H_{39}O_3^+$, 399.2894; found, 399.2894.

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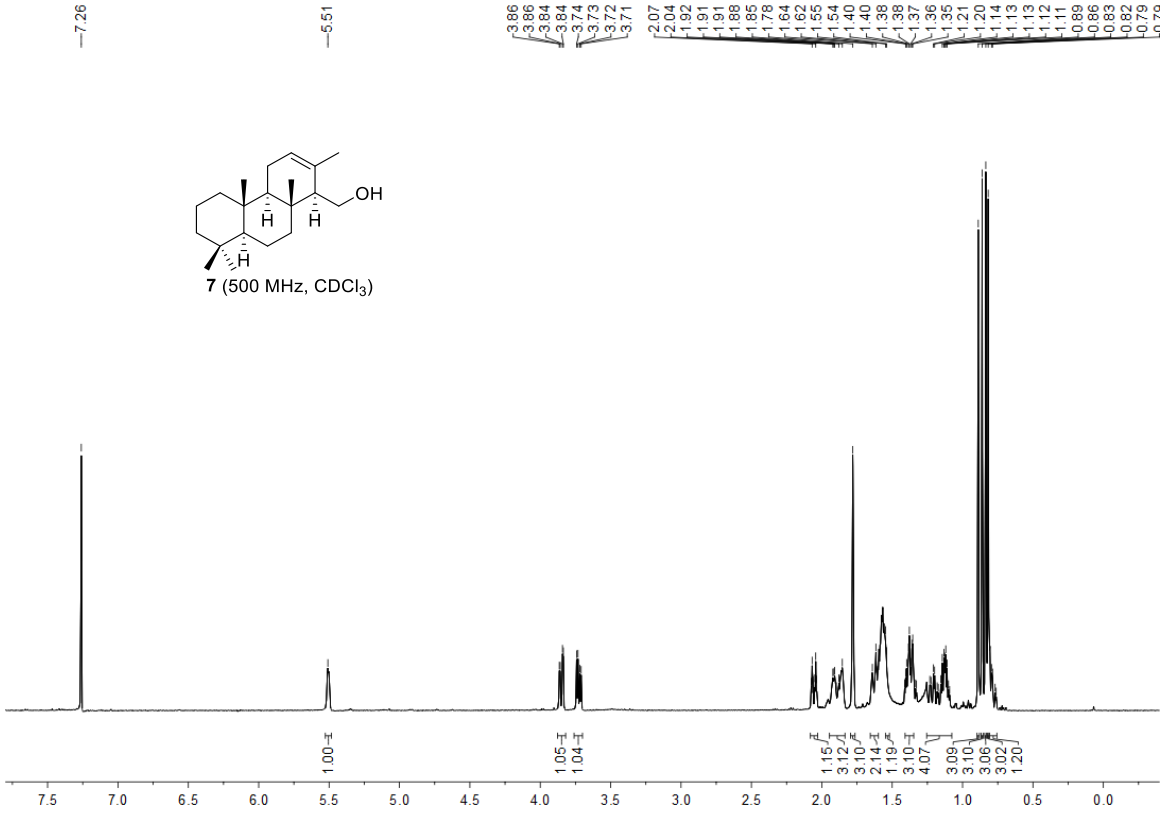
V. NMR spectra



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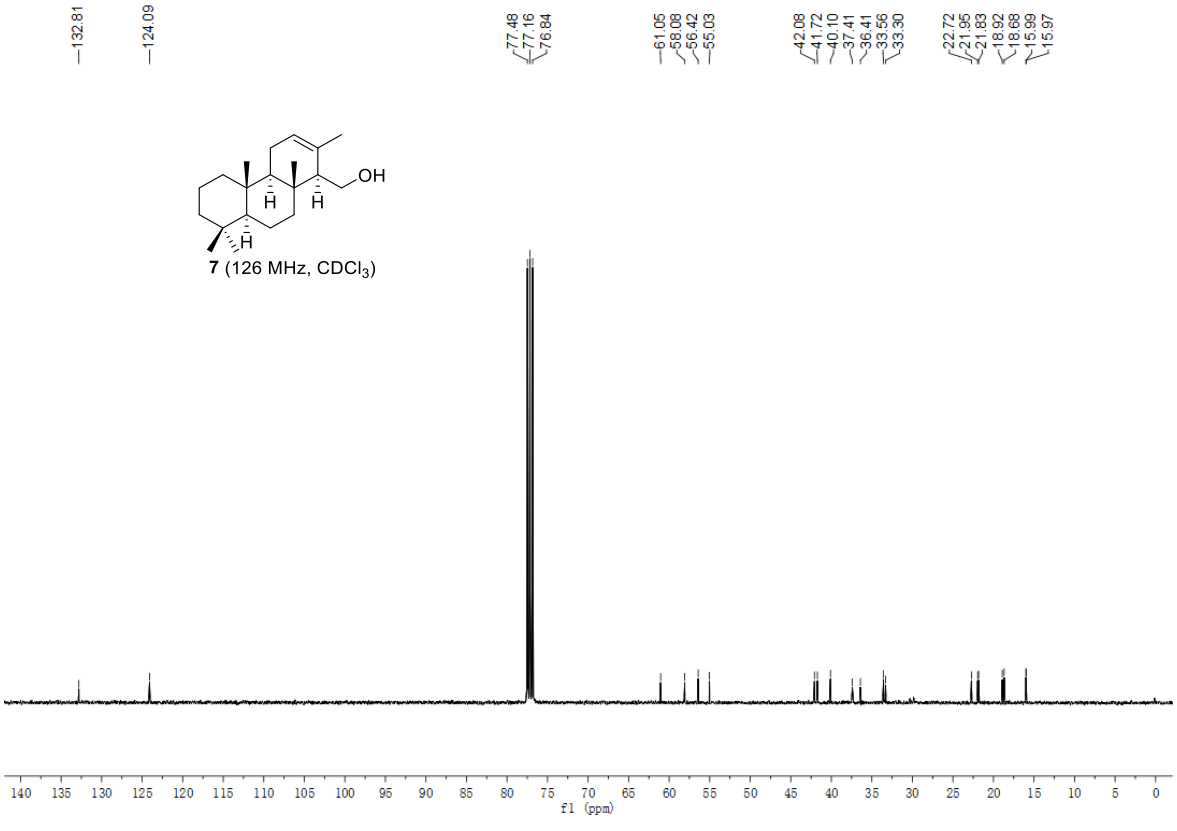

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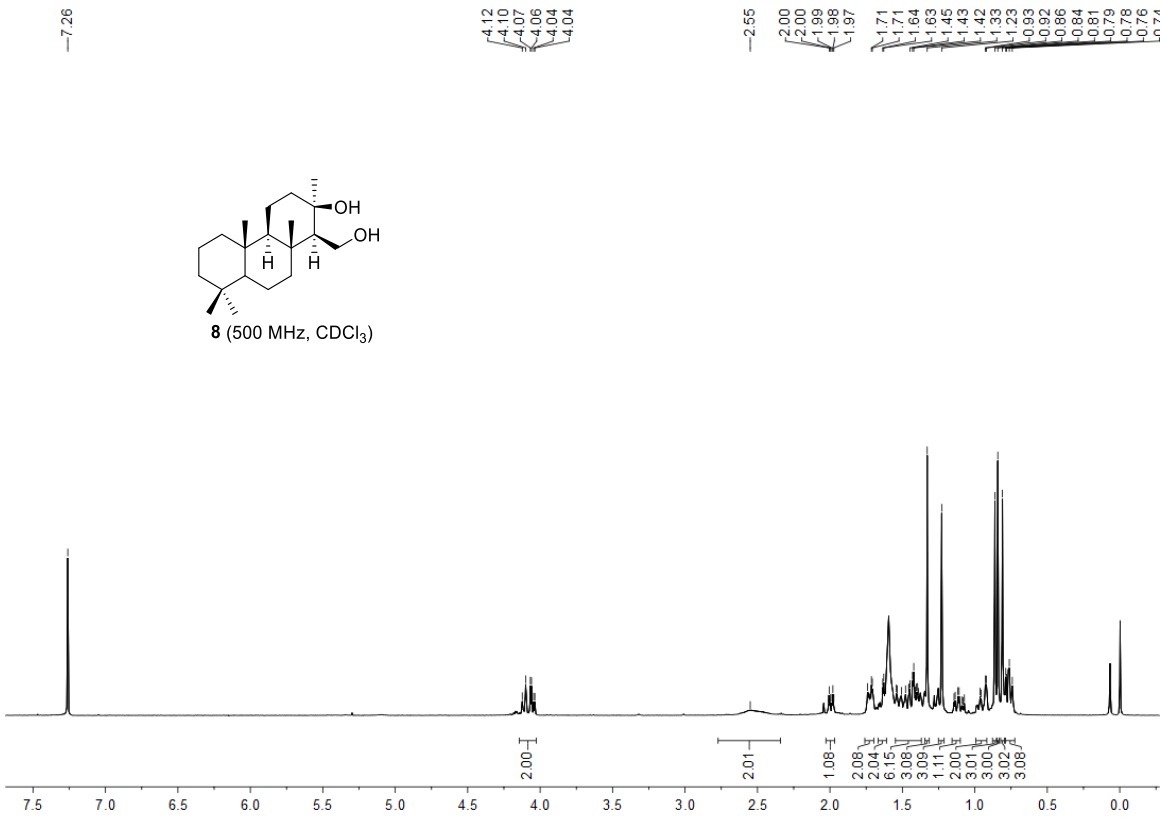

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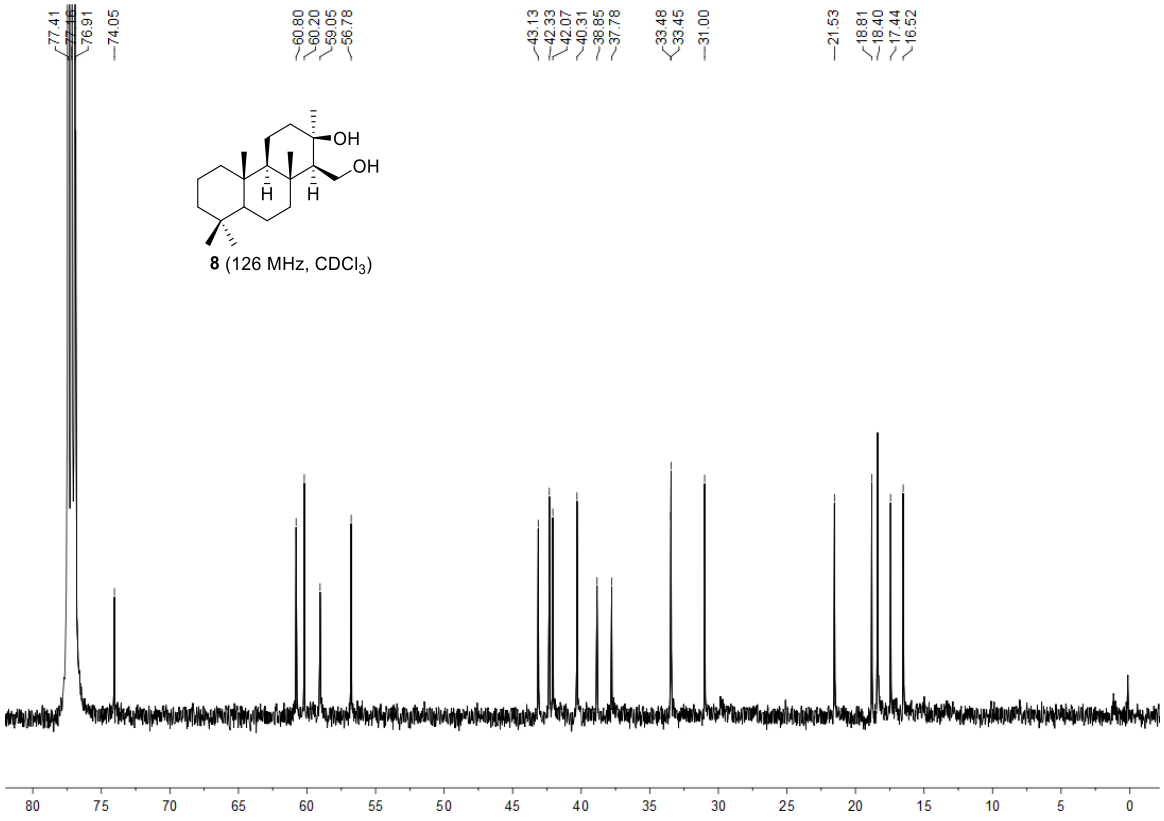

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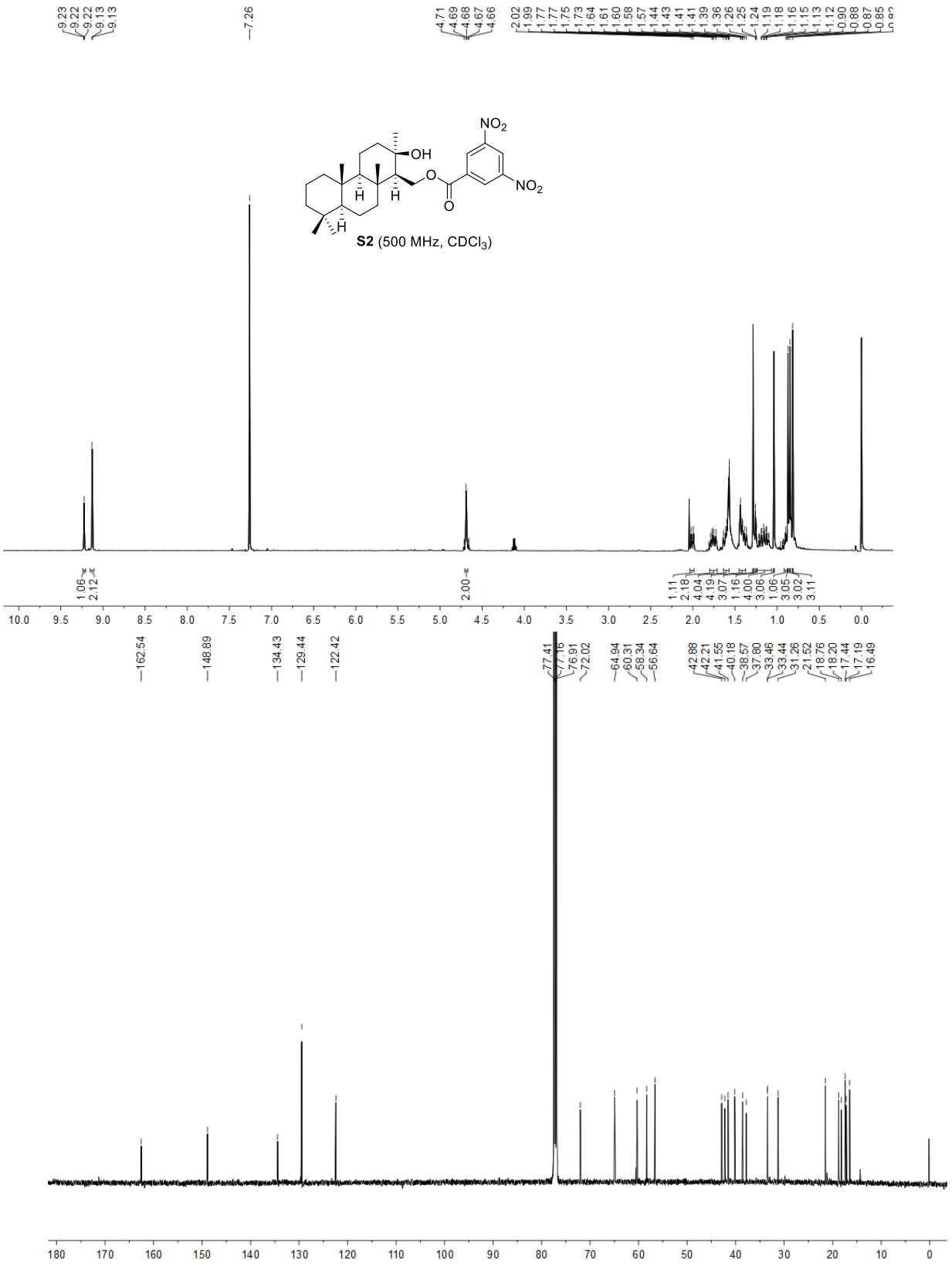
9.23
9.22
9.22
9.13
9.13
7.26
4.71
4.69
4.68
4.67
4.66
2.02
1.99
1.77
1.77
1.75
1.73
1.64
1.61
1.60
1.58
1.57
1.44
1.43
1.41
1.41
1.39
1.36
1.26
1.25
1.24
1.19
1.18
1.16
1.15
1.13
1.12
0.90
0.88
0.87
0.85
NO2
OH
O
NO2
H
H
H
O
S2 (500 MHz, CDCl3)
1.06
2.12
2.00
1.11
2.18
4.04
4.19
3.07
1.16
4.00
3.06
1.06
3.05
3.02
3.11
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
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0.0
162.54
148.89
134.43
129.44
122.42
77.41
77.16
76.91
72.02
64.94
60.31
58.34
56.64
42.88
42.21
41.55
40.18
38.57
37.80
33.46
33.44
31.26
21.52
18.76
18.20
17.44
17.19
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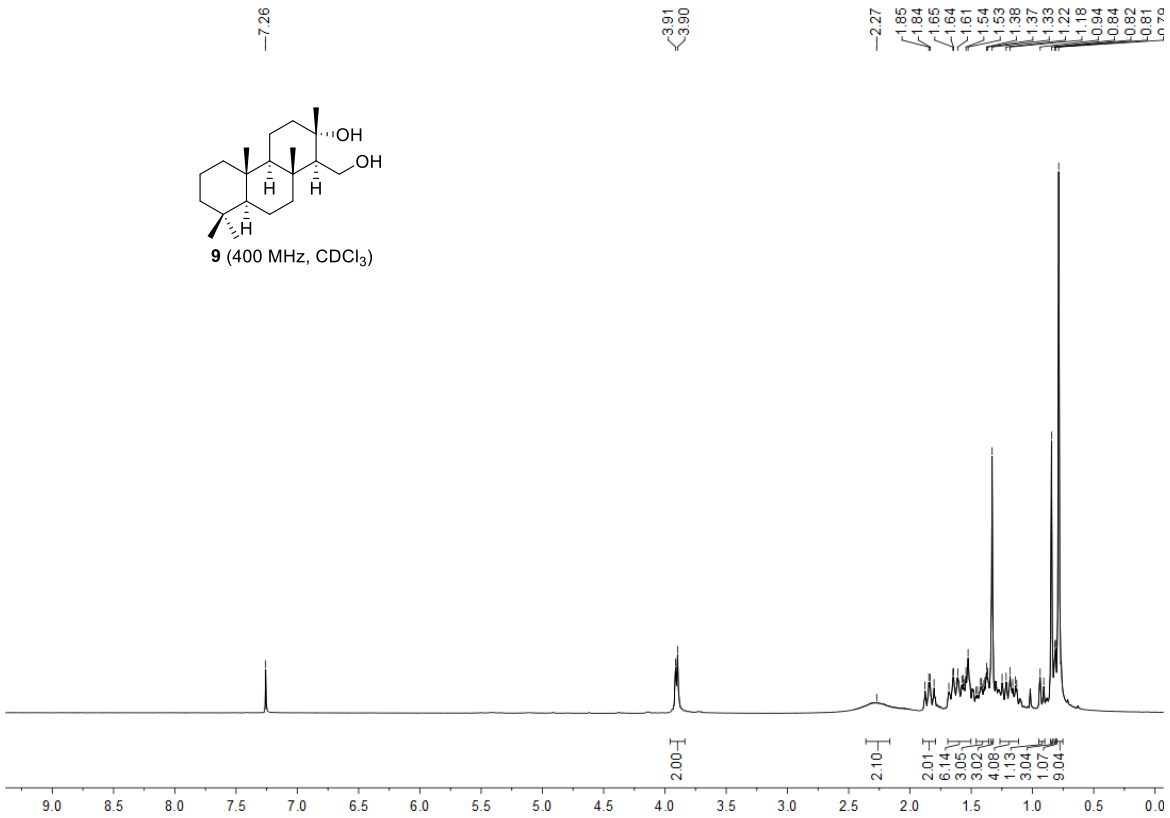

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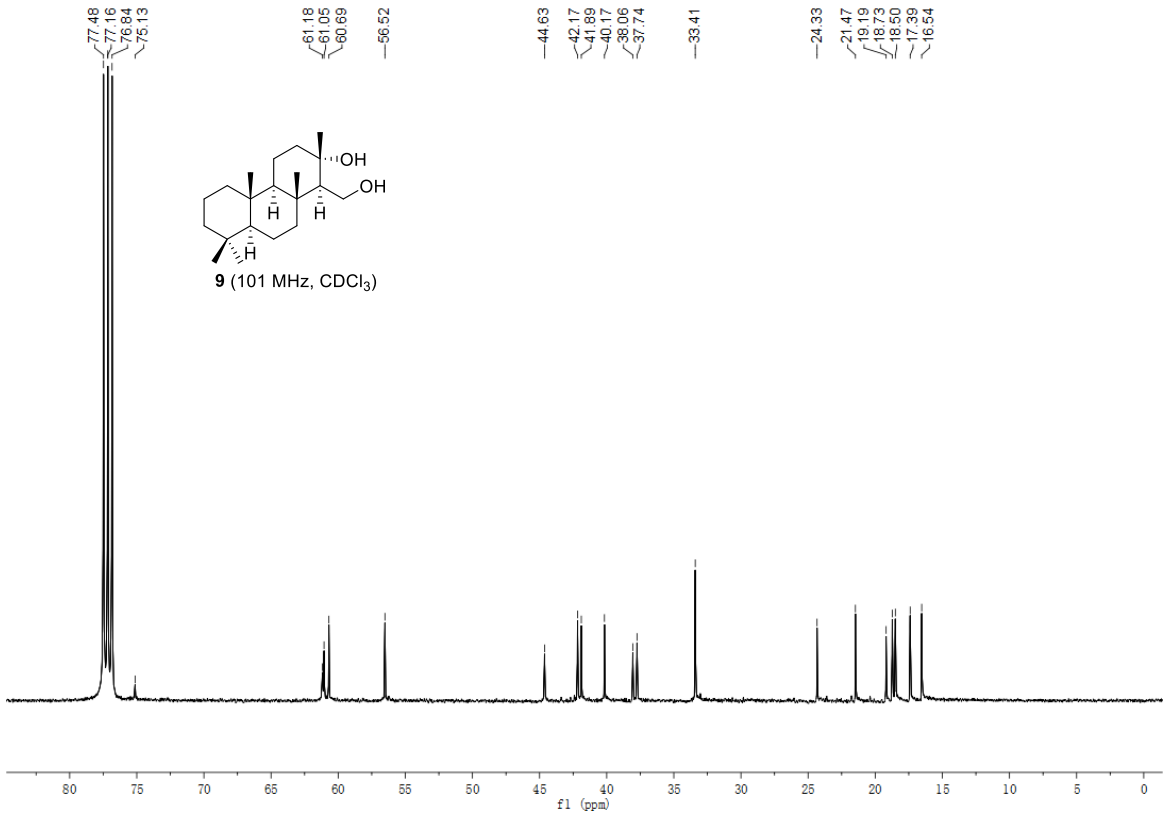

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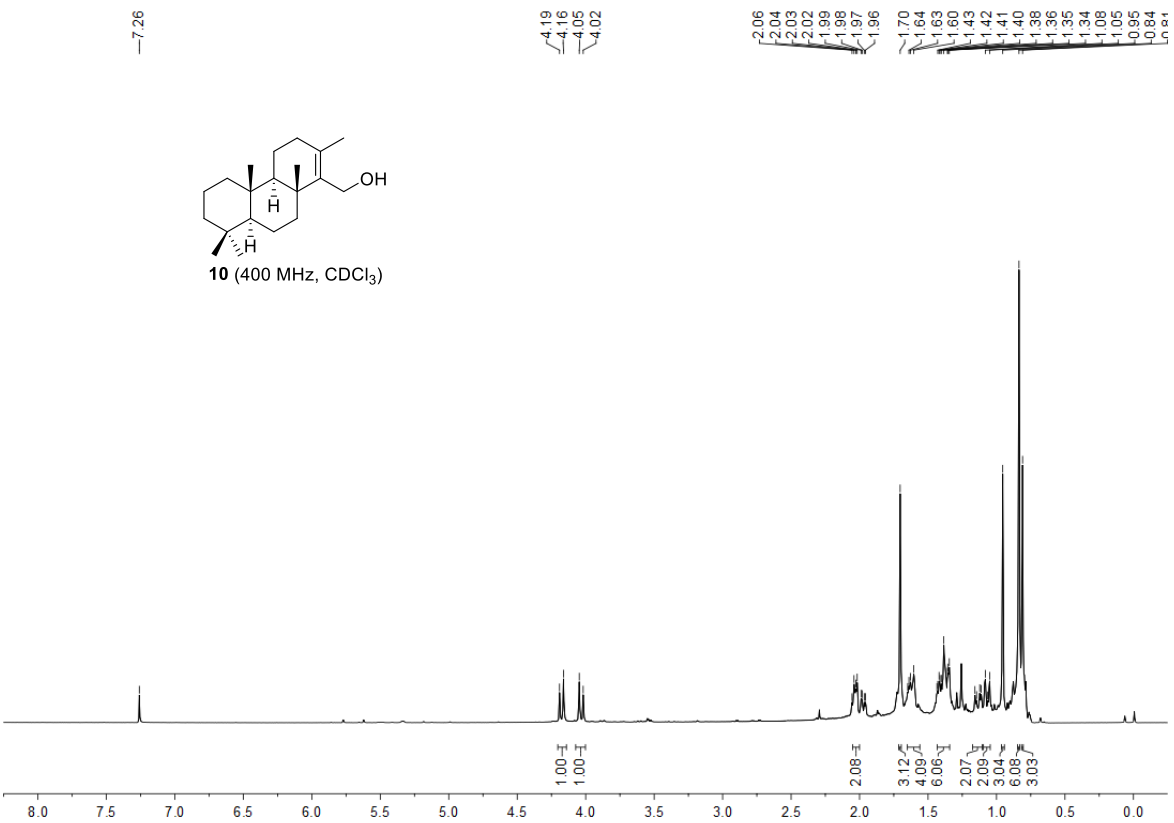

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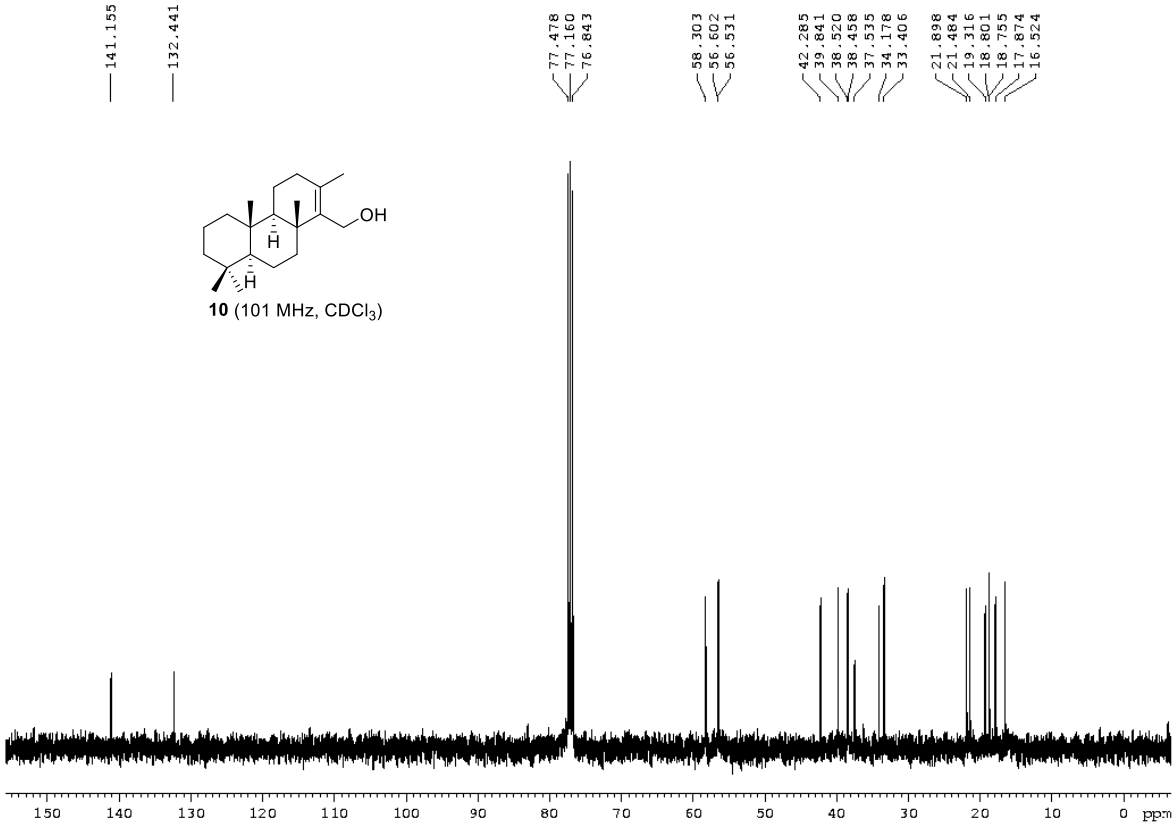

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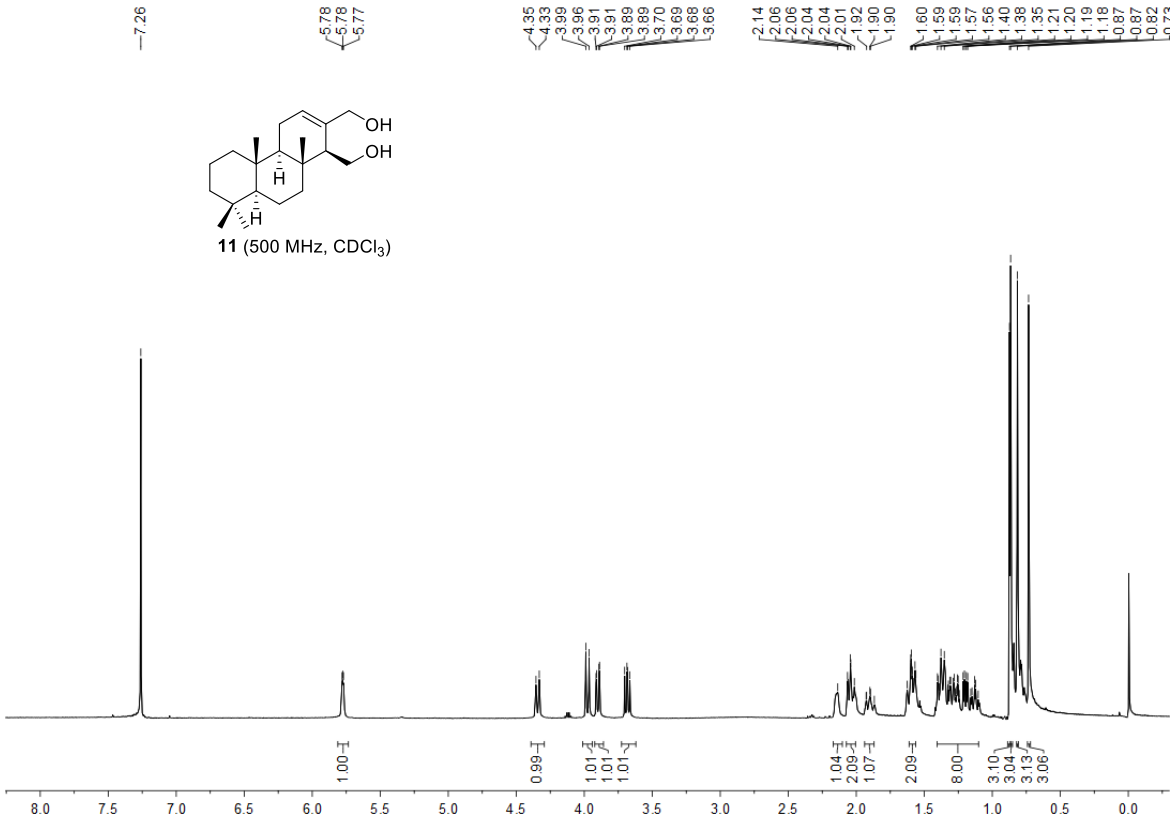

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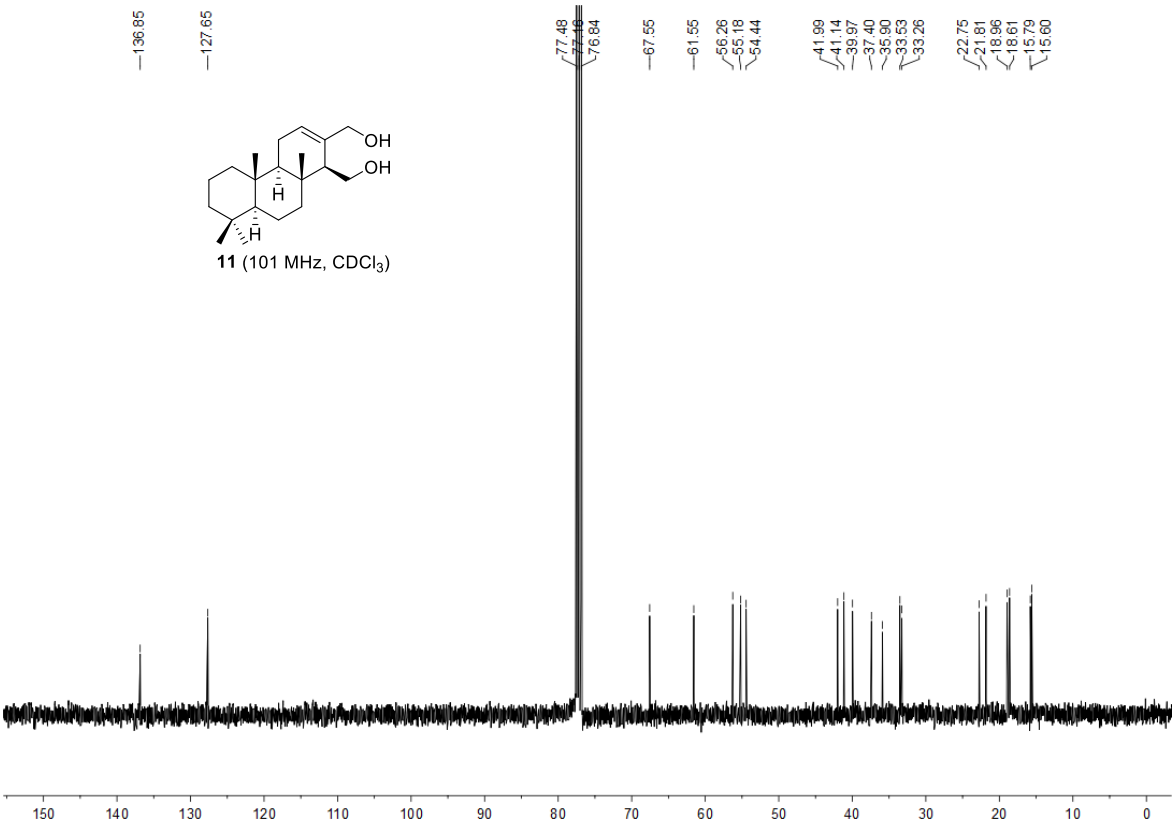

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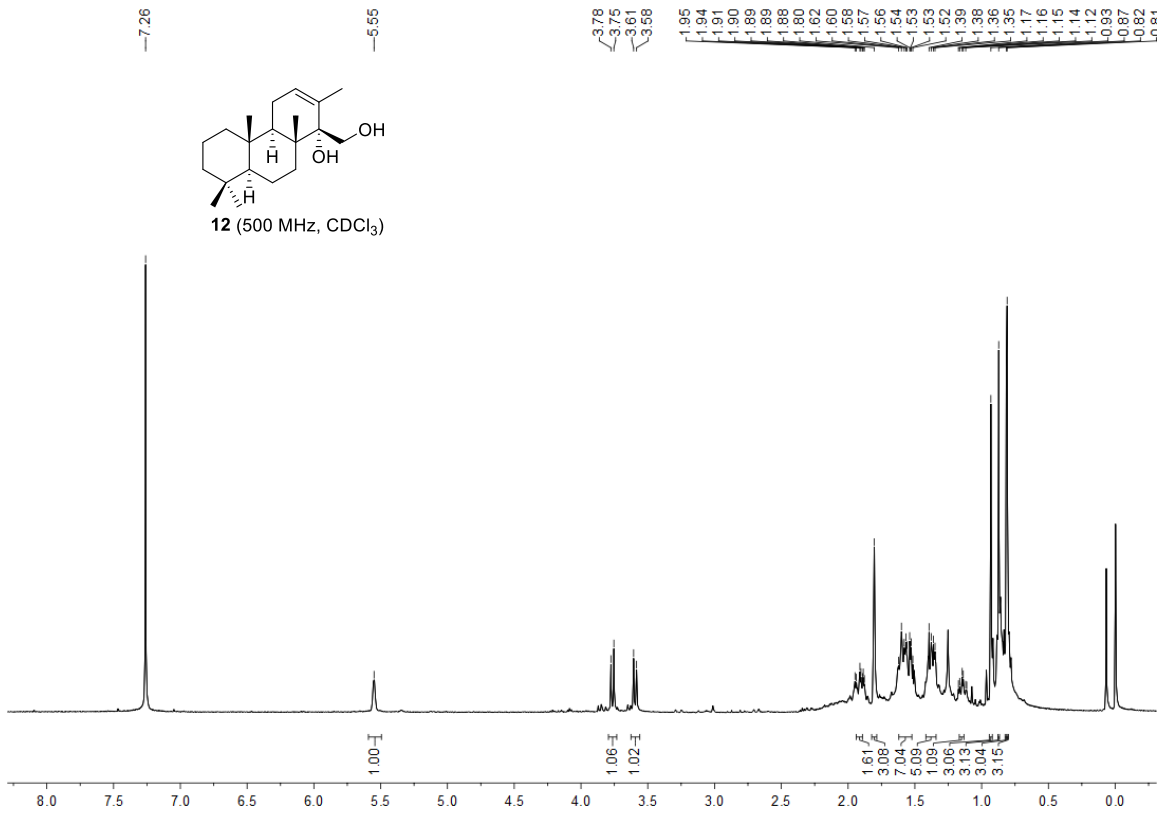

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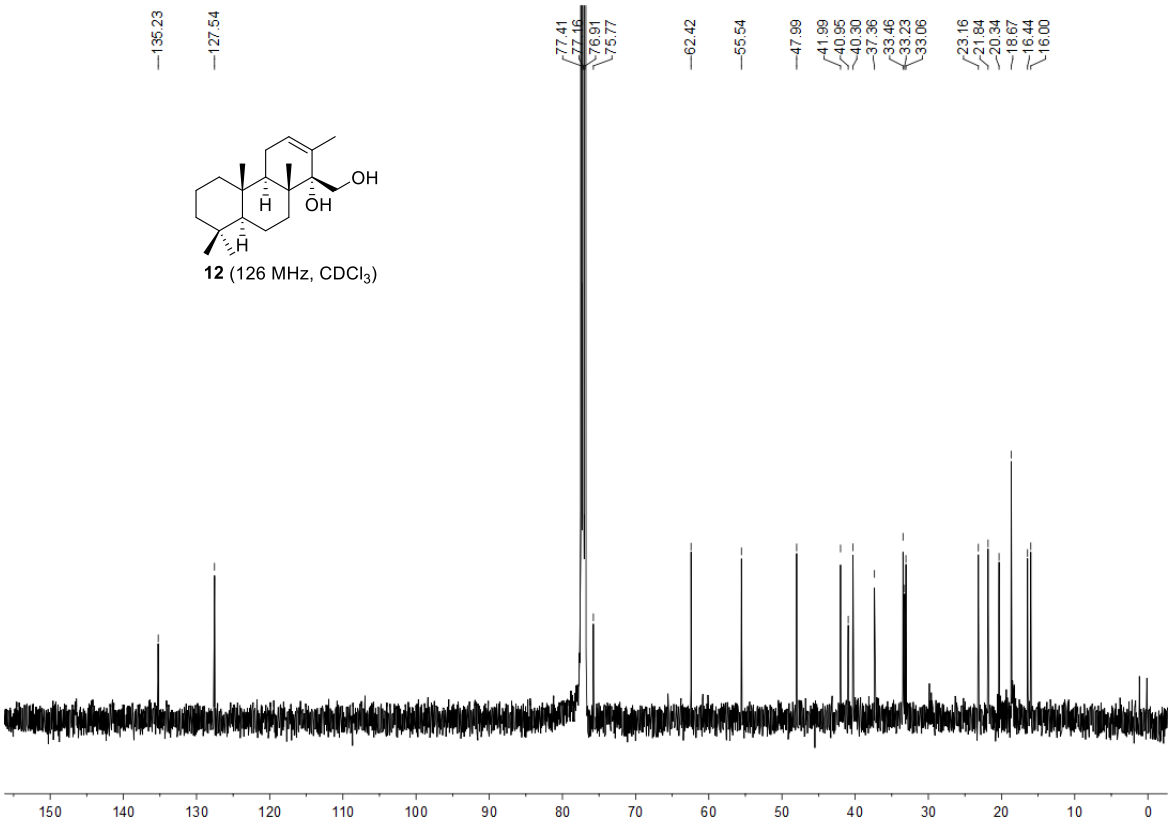

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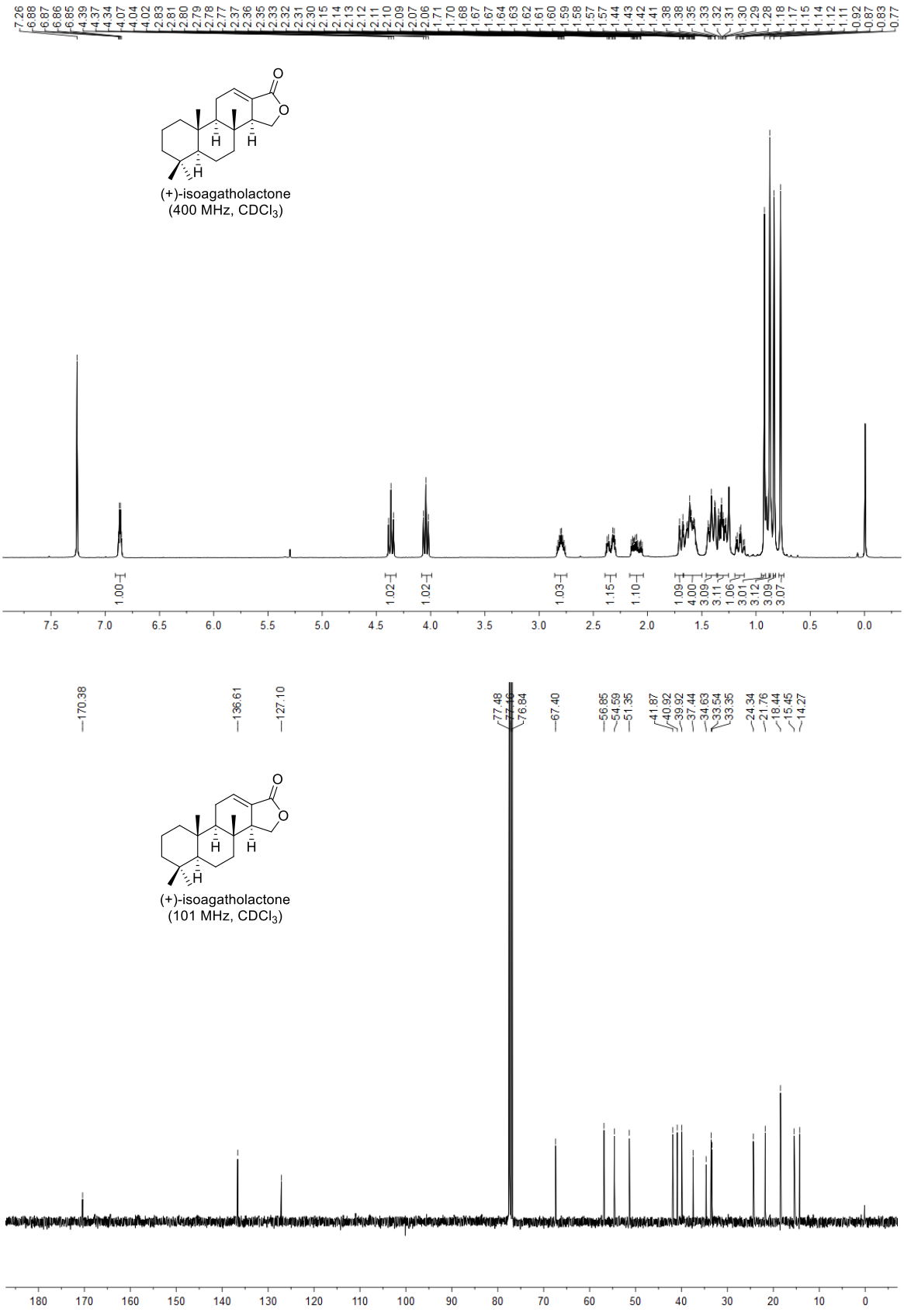
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O
O
H
H
H
(+)-isoagatholactone
(400 MHz, CDCl3)
1.00 1.02 1.02 1.03 1.15 1.10 1.09 4.00 3.09 3.11 1.06 3.01 3.12 3.09 3.07
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
170.38 136.61 127.10 77.48 77.16 76.84 67.40 56.85 54.59 51.35 41.87 40.92 39.92 37.44 34.63 33.54 33.35 24.34 21.76 18.44 15.45 14.27
O
O
H
H
H
(+)-isoagatholactone
(101 MHz, CDCl3)
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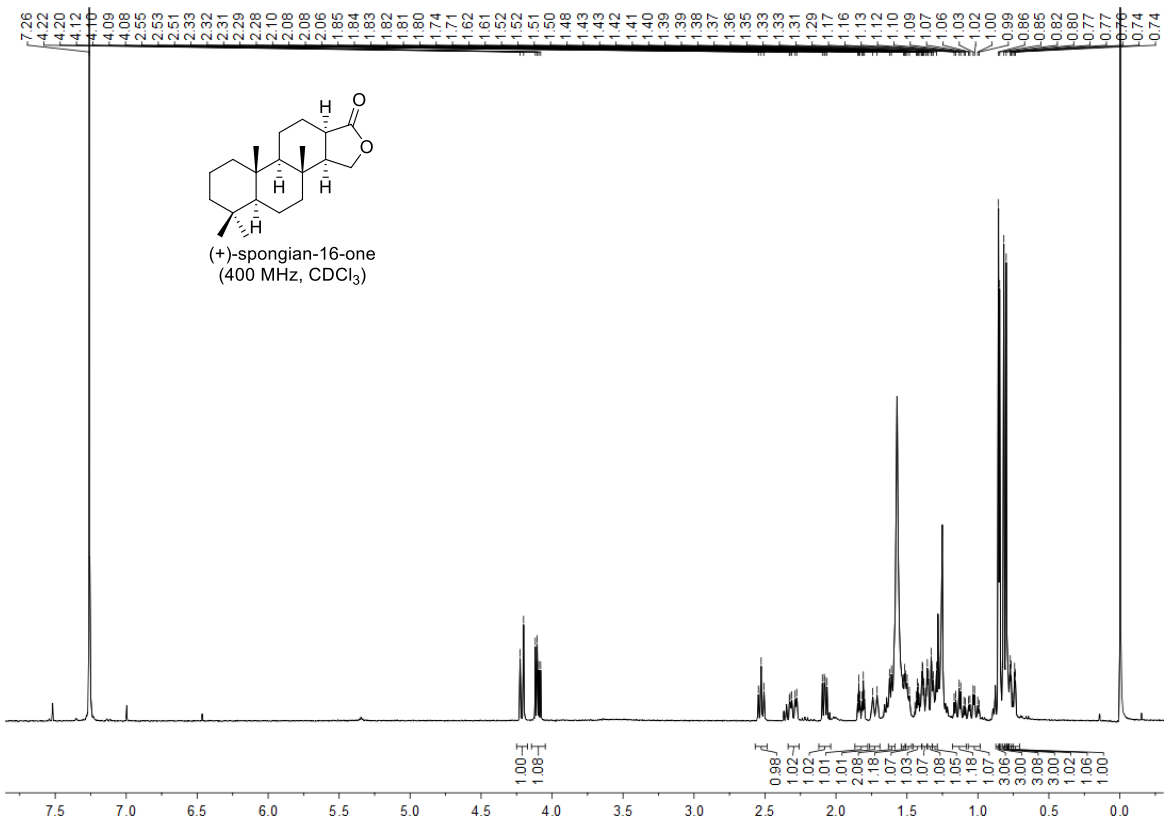
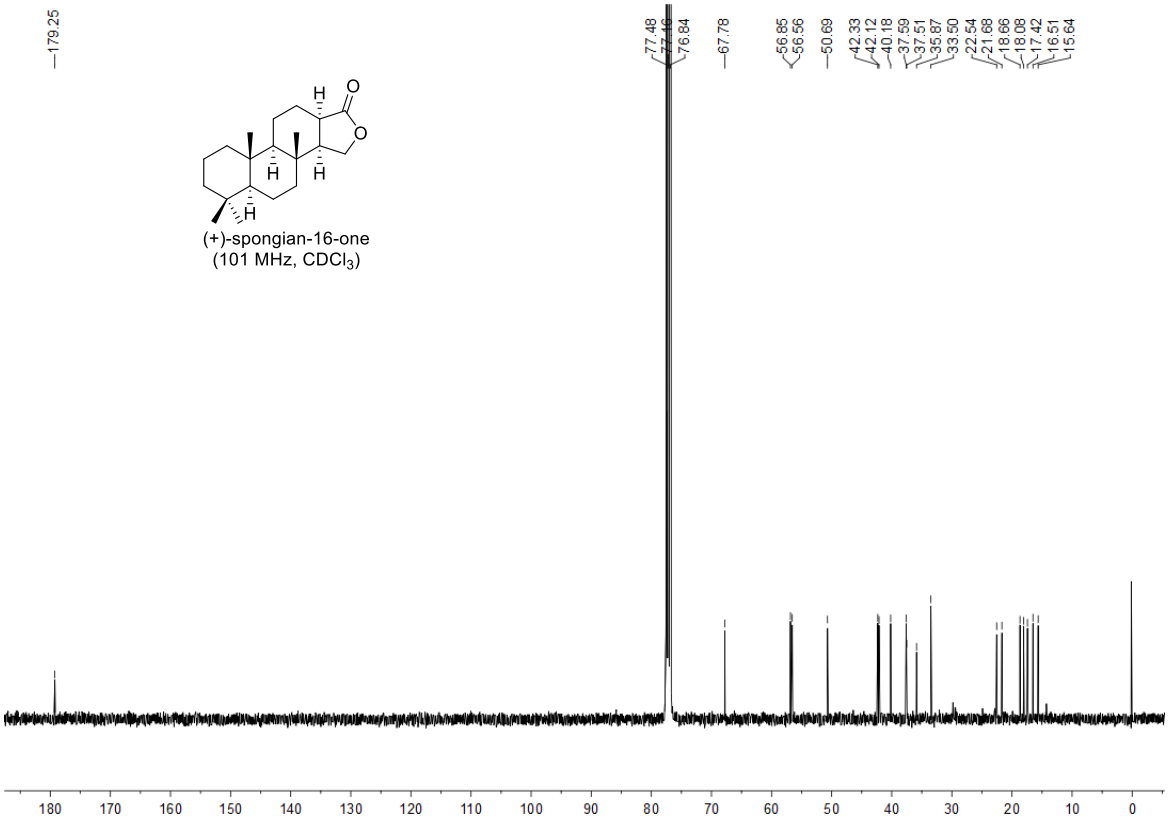
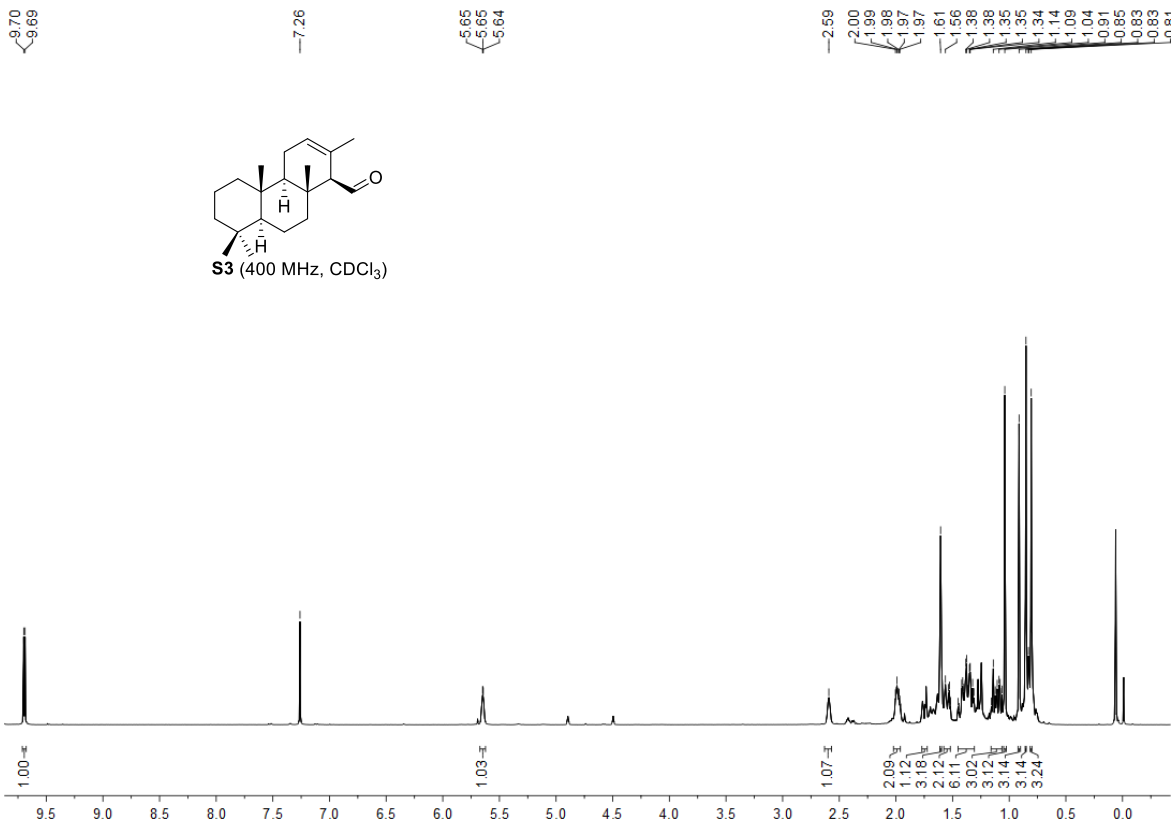


(+)-spongian-16-one
(400 MHz, $CDCl_3$)



(+)-spongian-16-one
(101 MHz, $CDCl_3$)



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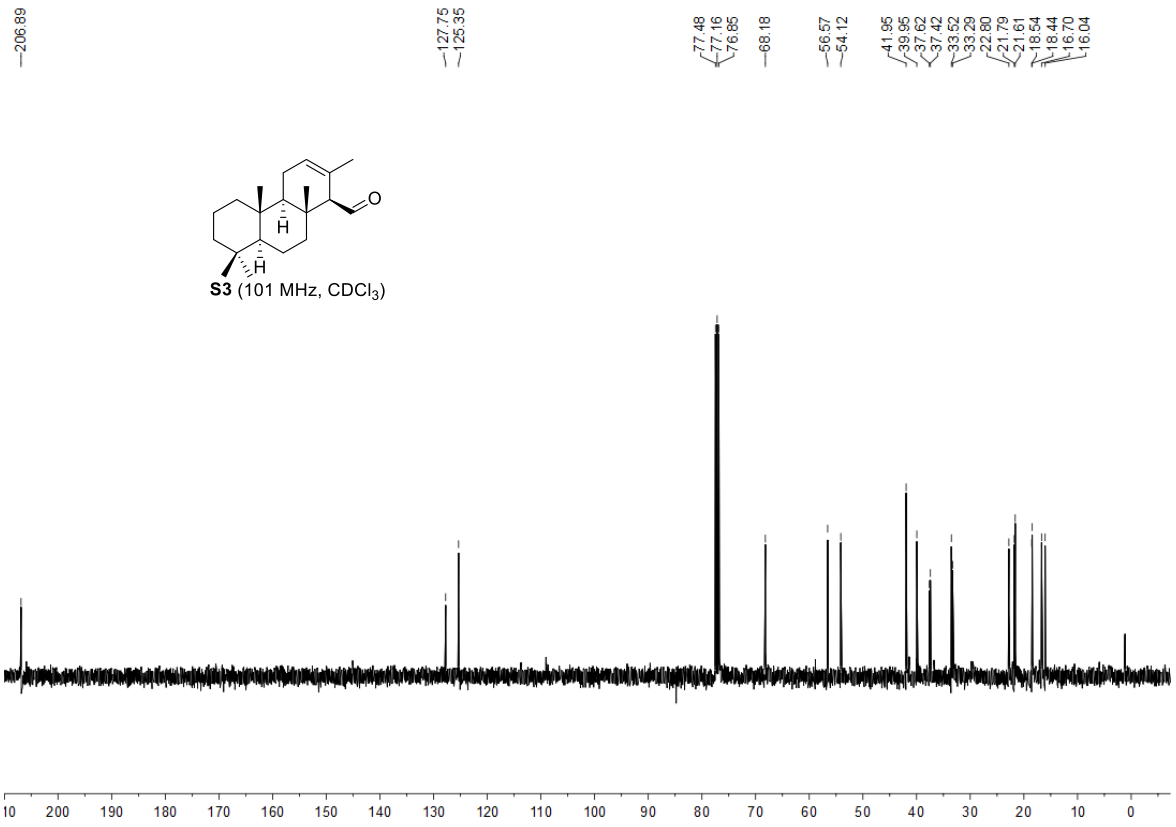

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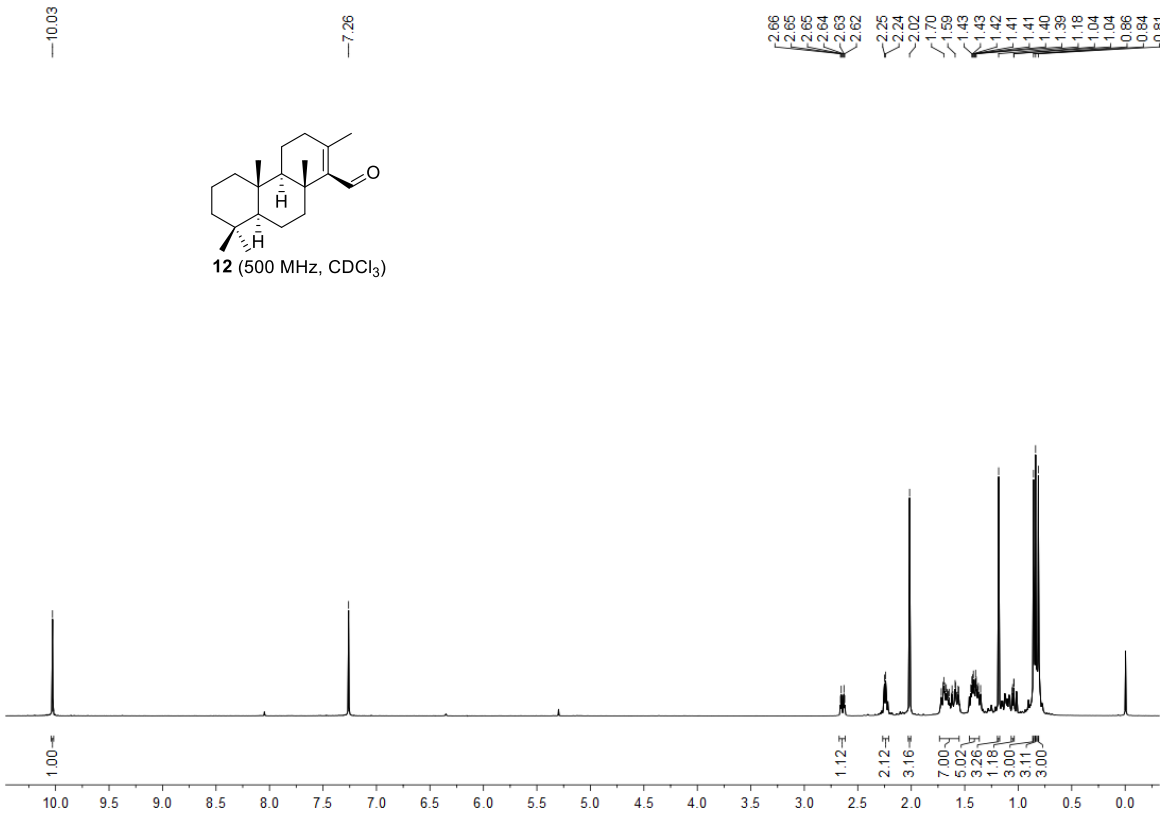

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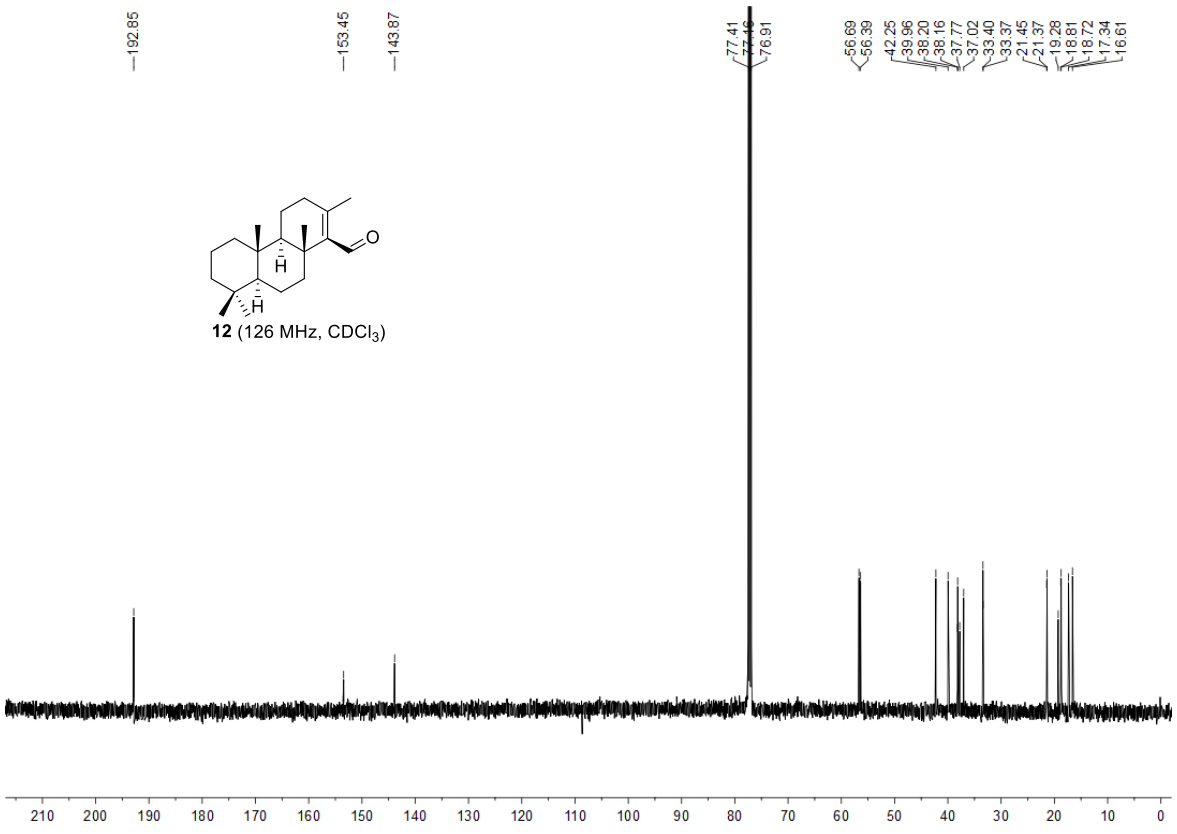

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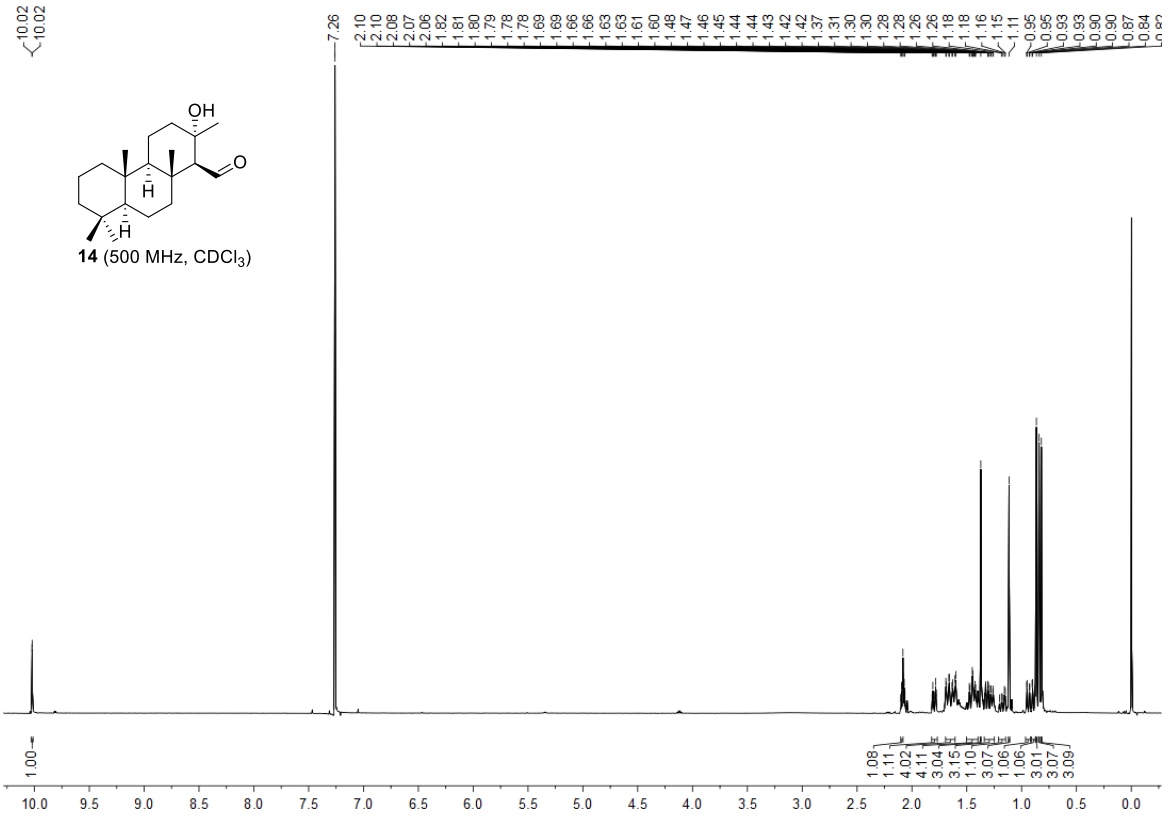

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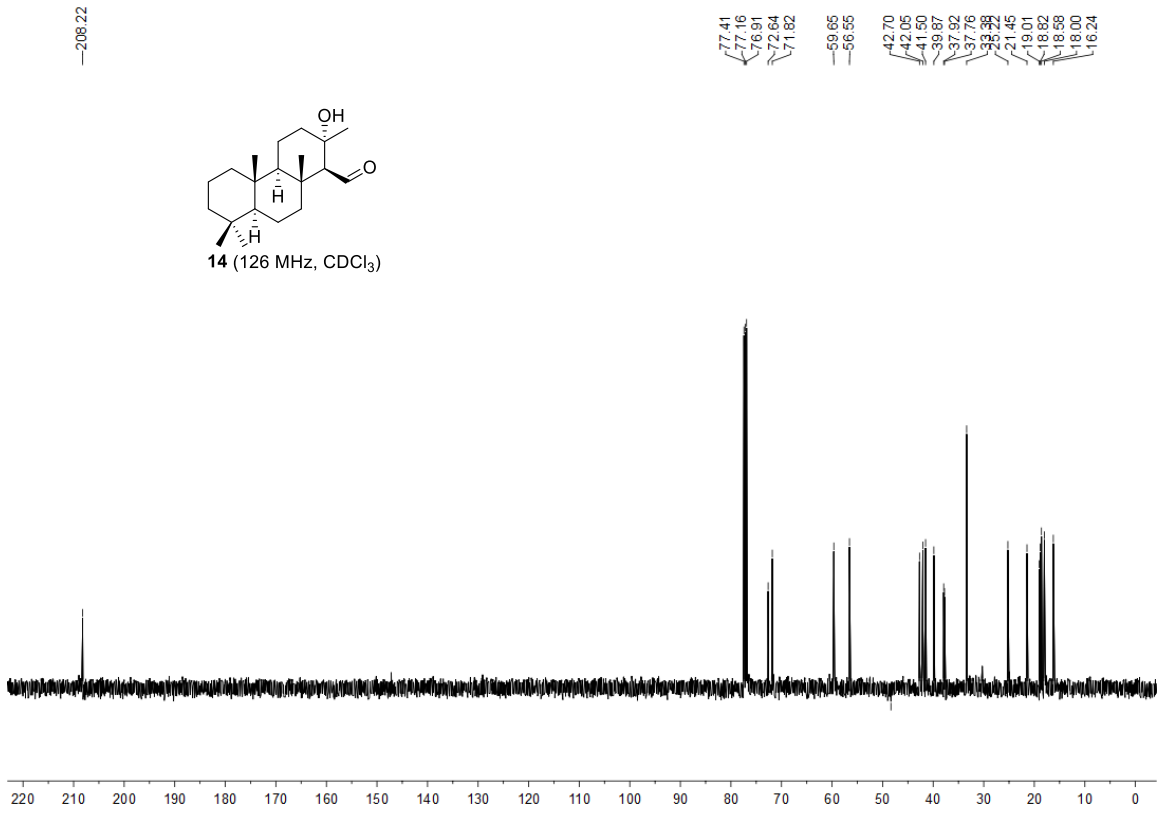

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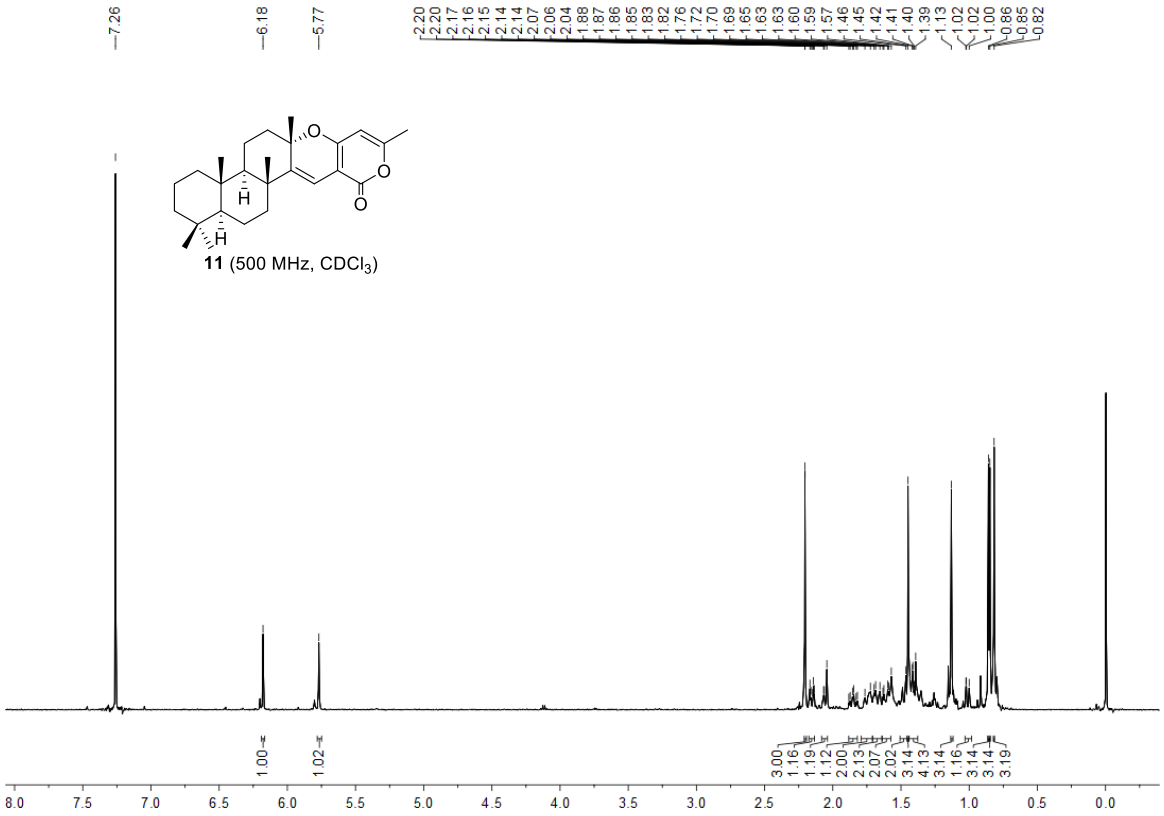

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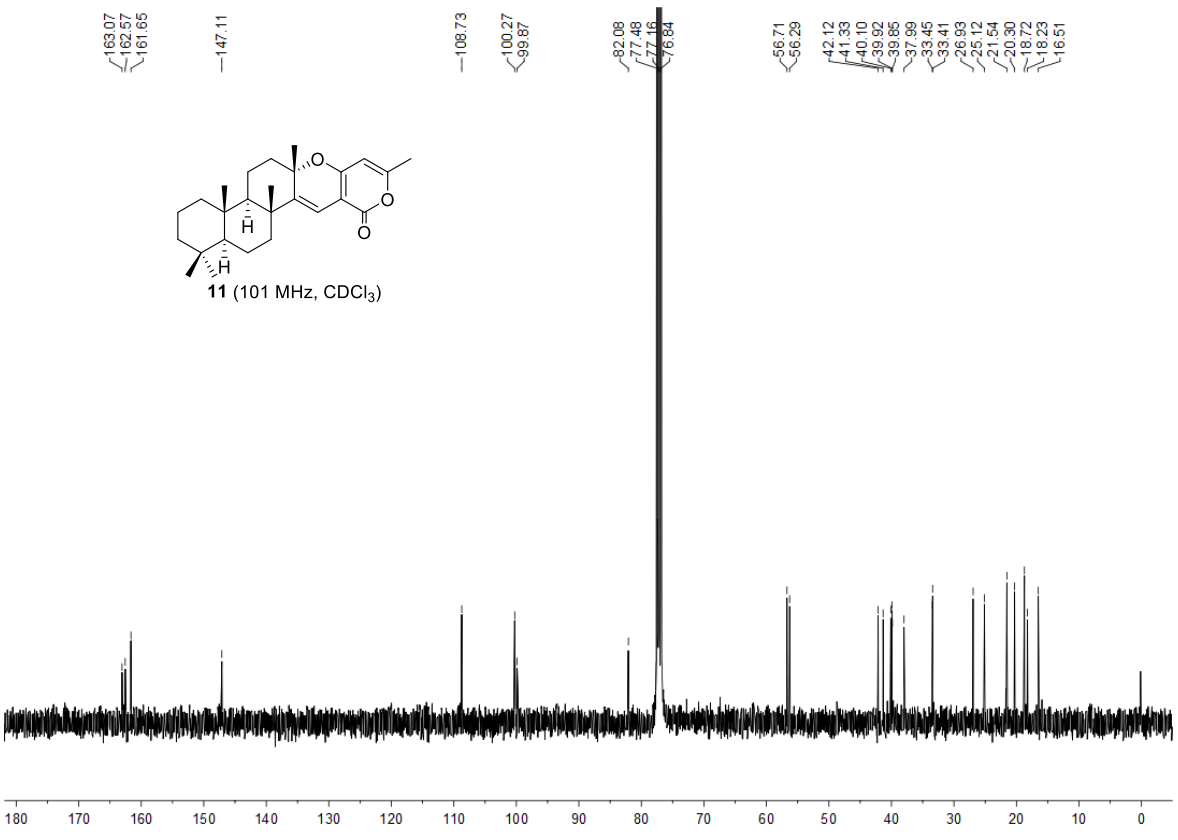

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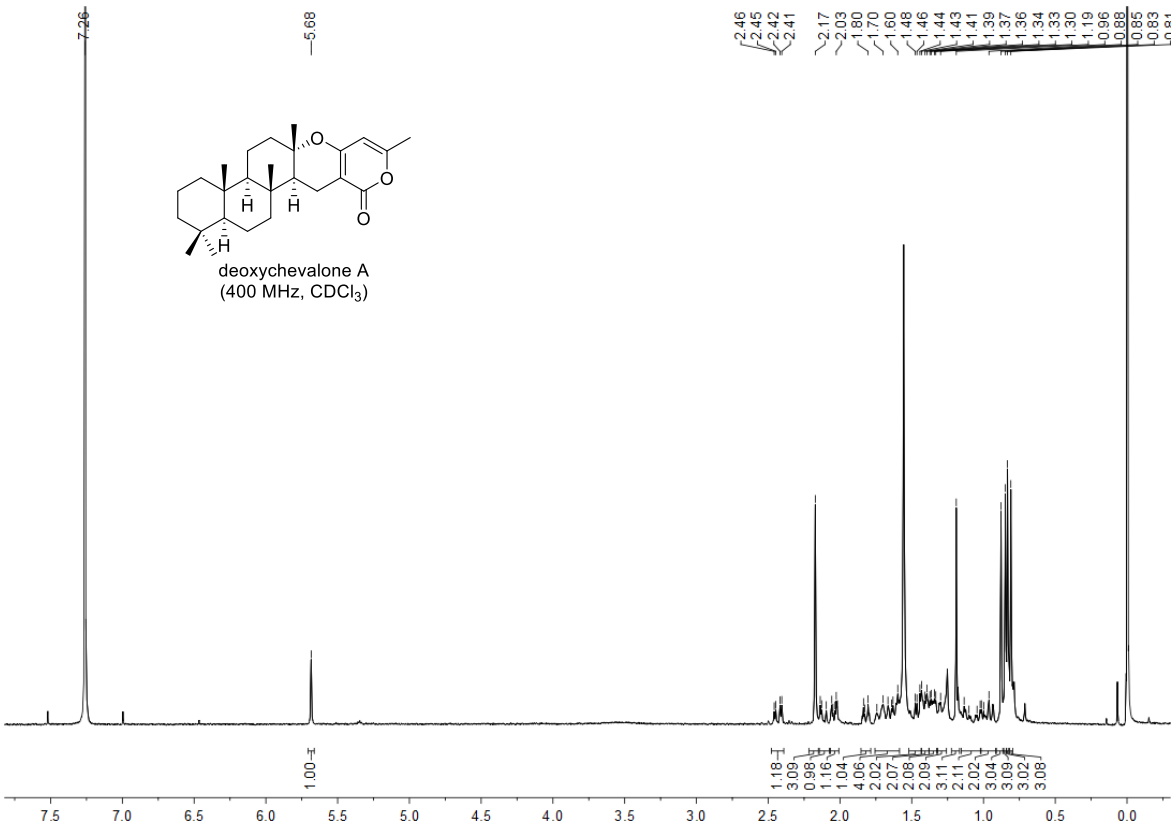
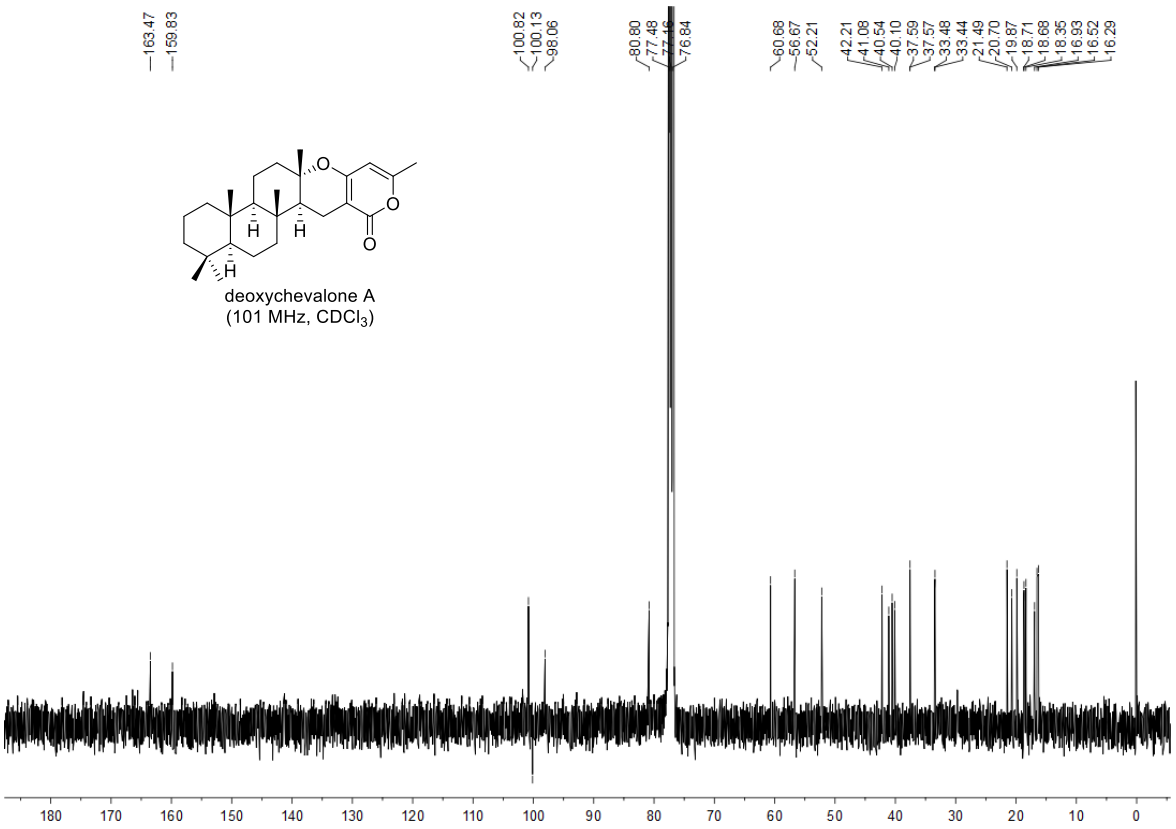


deoxychevalone A
(400 MHz, $CDCl_3$)



deoxychevalone A
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