Supplementary Informations

The stereochemistry of two monoterpenoid diastereomers from *Ferula dissecta*

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List of supplementary informations

Contents:	Page:
General experimental procedures	S2
Fig. S1 CD spectrum of **1**	S2
Fig. S2 IR spectrum of **1**	S2
Fig. S3 ^{1}H-NMR spectrum of **1**	S3
Fig. S4 ^{13}C-NMR spectrum of **1**	S3
Fig. S5 HR-EI-MS spectrum of **1**	S4
Fig. S6 HMBC spectrum of **1**	S4
Fig. S7 NOSEY spectrum of **1**	S5
Fig. S8 Enlarged NOSEY spectra of **1** (part 1)	S6
Fig. S9 Enlarged NOSEY spectra of **1** (part 2)	S7
Fig. S10 UV of **2**	S8
Fig. S11 CD spectrum of **2**	S8
Fig. S12 IR spectrum of **2**	S8
Fig. S13 ^{1}H-NMR spectrum of **2**	S9
Fig. S14 ^{13}C-NMR spectrum of **2**	S9
Fig. S15 HR-EI-MS spectrum of **2**	S10
Fig. S16 NOSEY spectrum of **2**	S11
Fig. S17 Enlarged NOSEY spectrum of **2**	S12
Fig. S18 Enlarged NOSEY spectrum of **2**	S13
Fig. S19 CD spectrum of **5**	S14
Fig. S20 ^{1}H-NMR of **5**	S14
Fig. S21 CD spectrum of **6**	S15
Fig. S22 ^{1}H-NMR of **6**	S15
Fig. S23 Calculated ECD of **1** and **2** using different basic sets	S16
Fig. S24 The HOMO, LUMO, HOMO-1 and LUMO + 1 orbitals of **1**	S16
Synthesis of **5** and **6**	S16
Cytotoxity assay and computational details	S17
Table S1. ^{1}H NMR, ^{13}C NMR and NOESY spectral data of compound **1**	S18
Table S2. ^{1}H NMR, ^{13}C NMR and NOESY spectral data of compound **2**	S19

General experimental procedures

Column chromatography (CC): Silica gel (200–300 mesh; Qingdao Marine Chemical Group, Co.); ODS (30-50 μm; YMC CO. Ltd. Japan). Preparative HPLC: Waters 600E pump, Waters 2489 UV spectrophotometric detector at 220 nm, Waters Sunfire Prep ODS reversed phase column (10 μm, 10×150 mm, flow rate: 2.0 mL/min); NMR spectra were recorded on Bruker AV-300 spectrometer, TMS as internal standard, δ in ppm, J in Hz; HR-ESI-MS were obtained on Waters LCT Premier XE time-of-flying mass spectrometer; CD spectrum were gotten on Biologic MOS-450 CD spectrometer.

All the chemicals and solvents used in the synthesis were obtained from commercial suppliers. (1*S*，2*R*，4*S*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol (**3**) and (1*S*，2*S*，4*S*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol (**4**)were obtained from Sigma Chemical (St. Louis, MO, USA). Solvents were dried without further purification except when otherwise noted. Reactions were monitored by TLC, which were visualized by UV inspection and stained with a 10% H_2SO_4 solution.

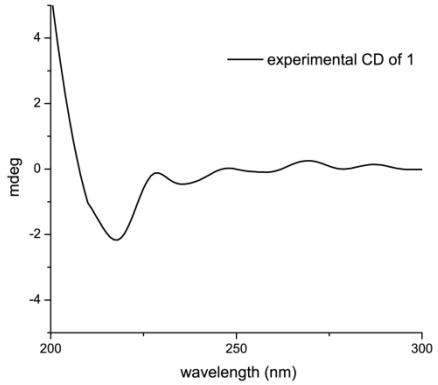


Fig. S1 CD spectrum of 1

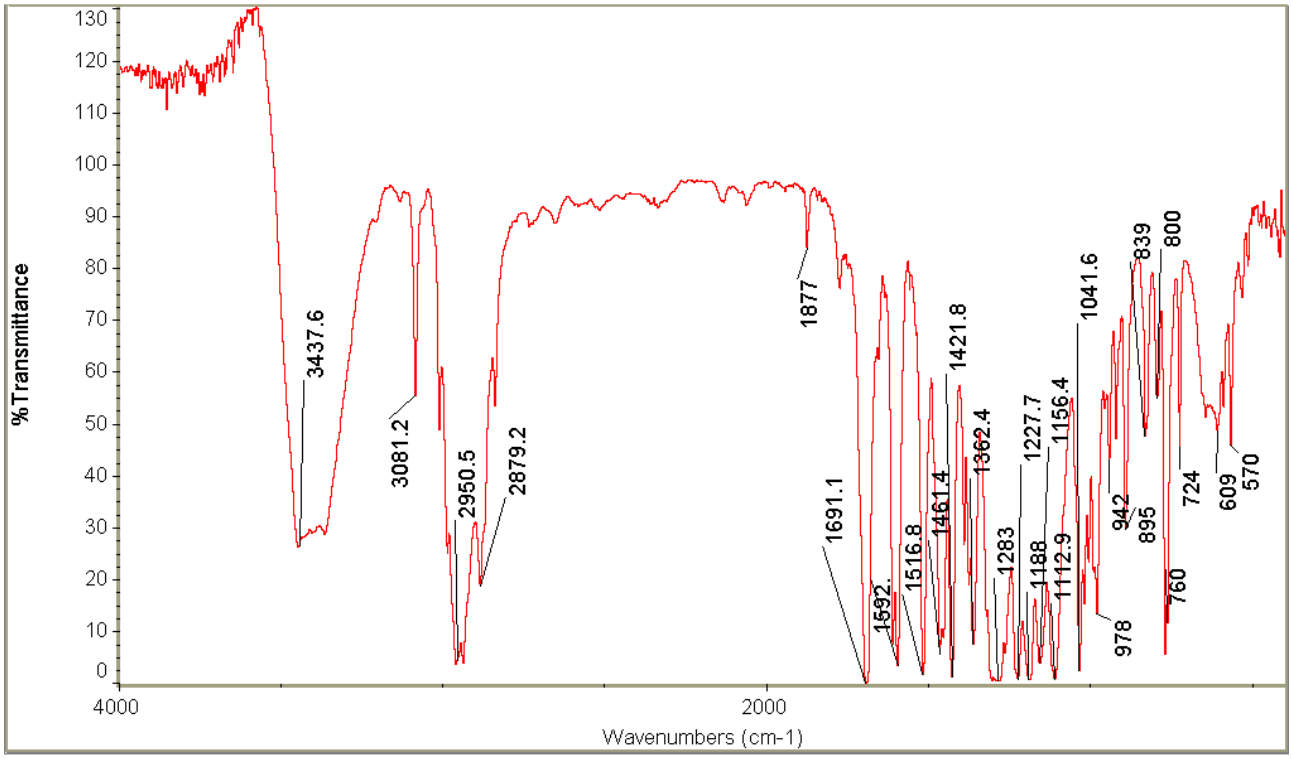


Fig. S2 IR spectra of **1**

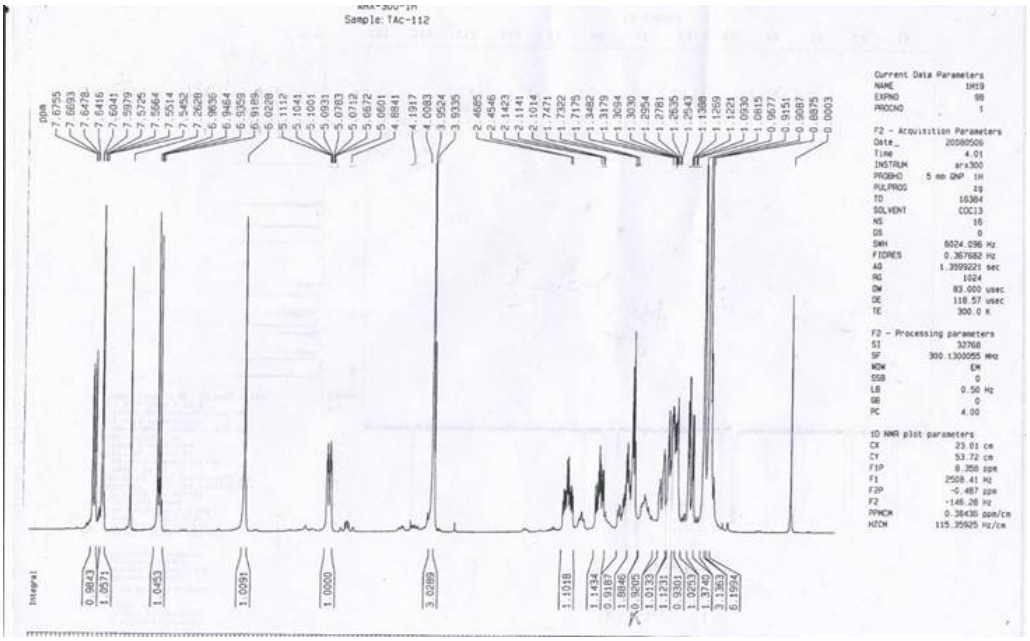


Fig. S3 ^{1}H-NMR spectrum of **1**

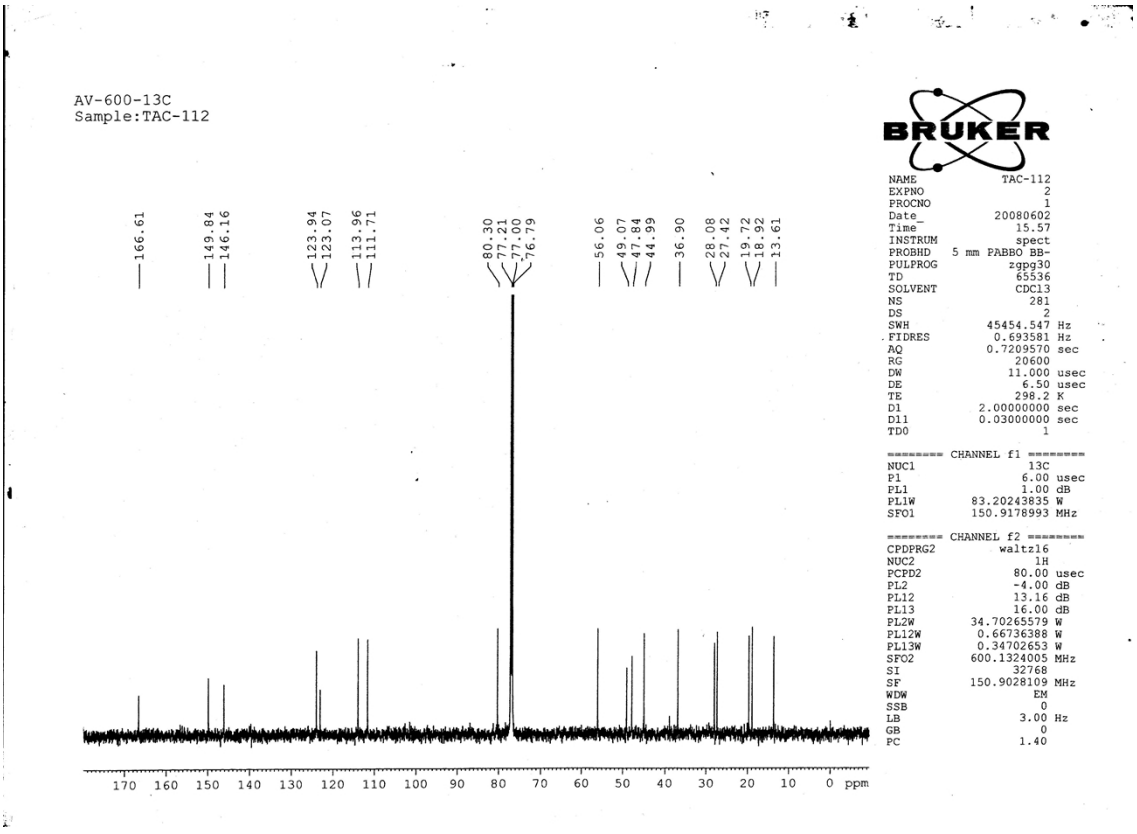
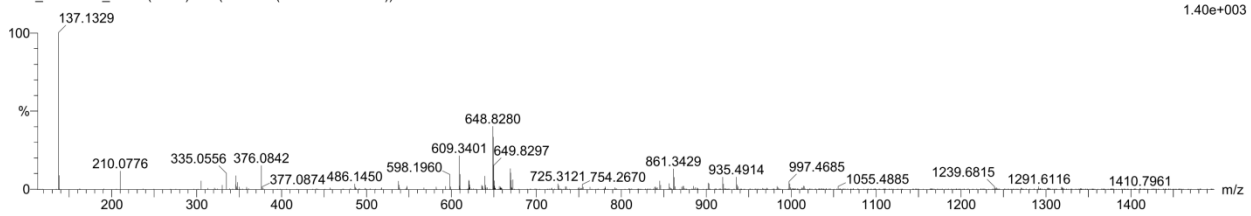


Fig. S4 ^{13}C-NMR spectrum of **1**

Monoisotopic Mass, Even Electron Ions
101 formula(e) evaluated with 7 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-150 O: 0-40 Na: 0-1
TAW-76-5
TAW_20091221_7 169 (3.819) Cm (169:170-(149:164+176:189))

1: TOF MS ES+
1.40e+003



Minimum:				-1.5		
Maximum:		20.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
305.1750	305.1753	-0.3	-1.0	6.5	n/a	C18 H25 O4
	305.1729	2.1	6.9	3.5	n/a	C16 H26 O4 Na
	305.1940	-19.0	-62.3	-1.5	n/a	C13 H30 O6 Na
	305.1600	15.0	49.2	2.5	n/a	C14 H25 O7
	305.1576	17.4	57.0	-0.5	n/a	C12 H26 O7 Na
	305.1905	-15.5	-50.8	10.5	n/a	C22 H25 O
	305.1881	-13.1	-42.9	7.5	n/a	C20 H26 O Na

Fig. S5 HR-EI-MS spectrum of **1**

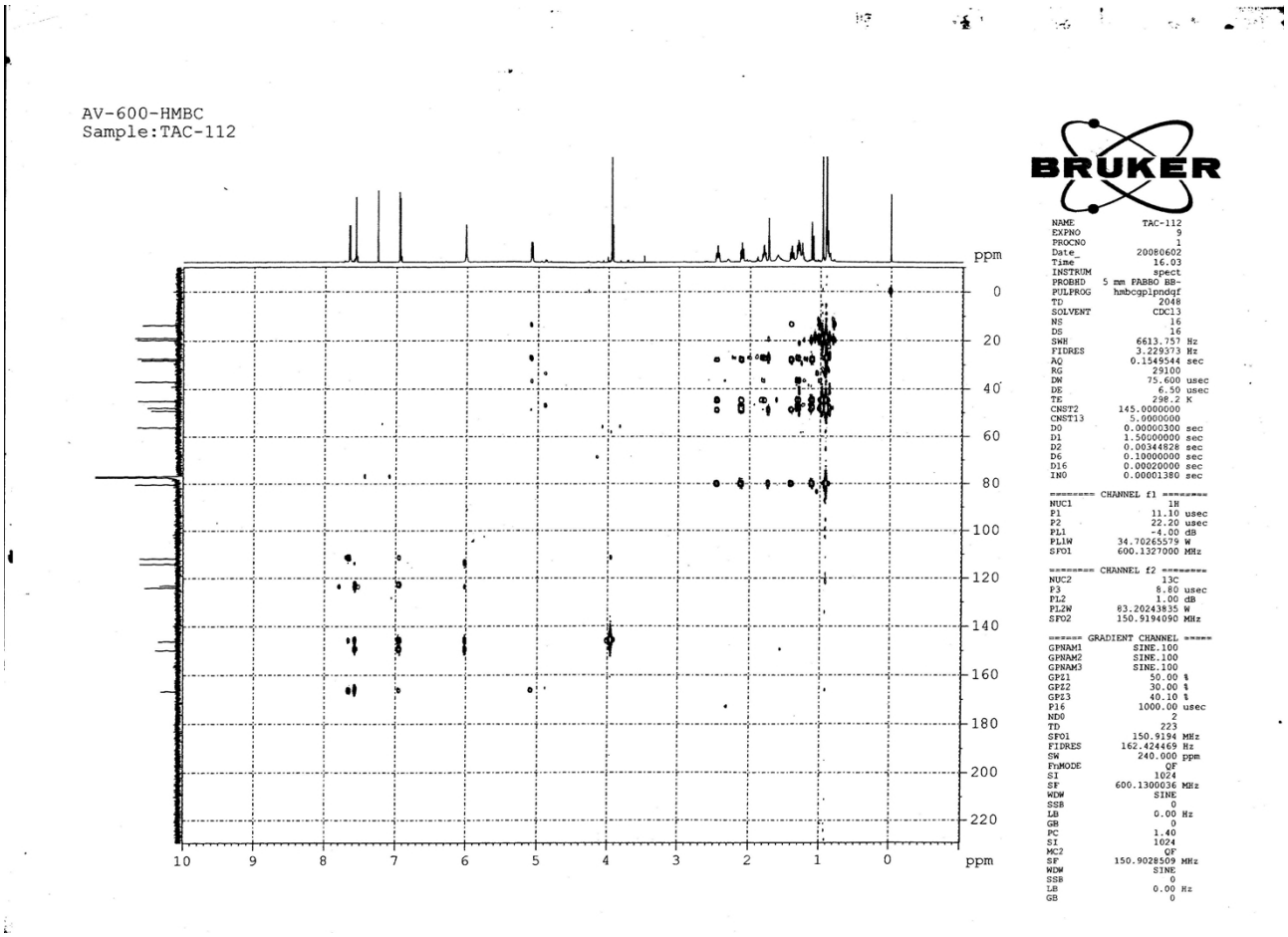


Fig. S6 HMBC spectrum of **1**

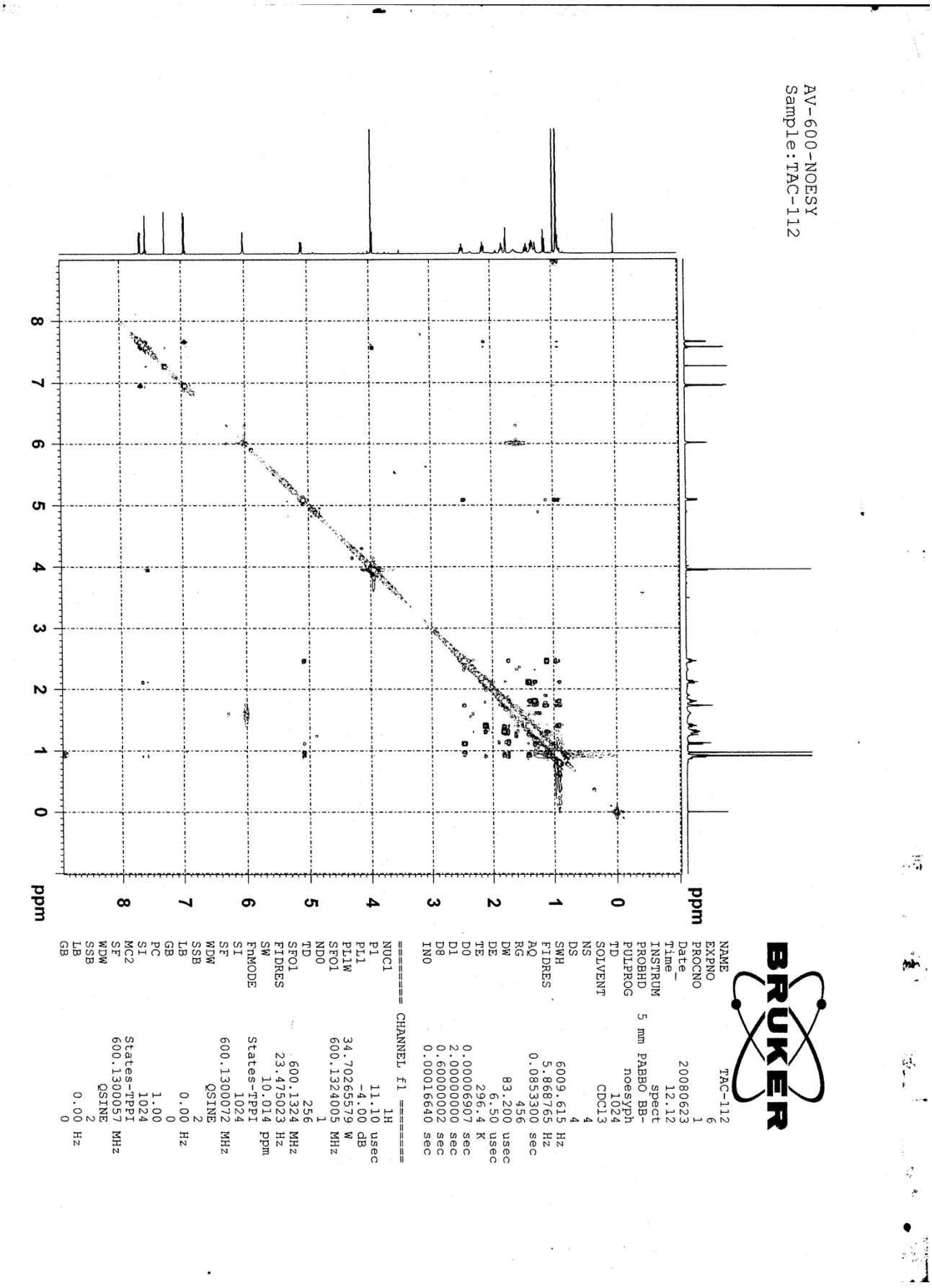


Fig. S7. NOSEY spectrum of **1**

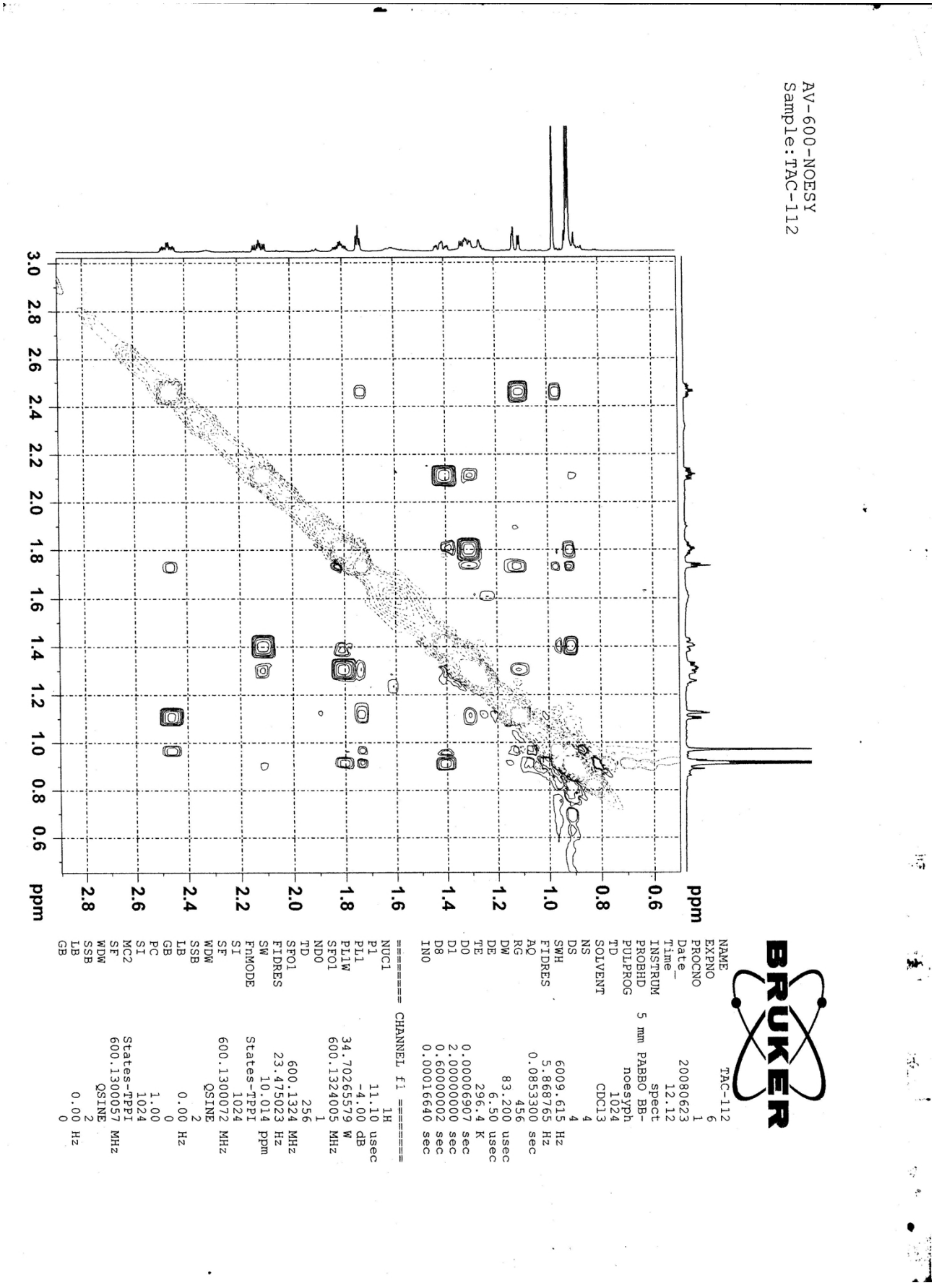
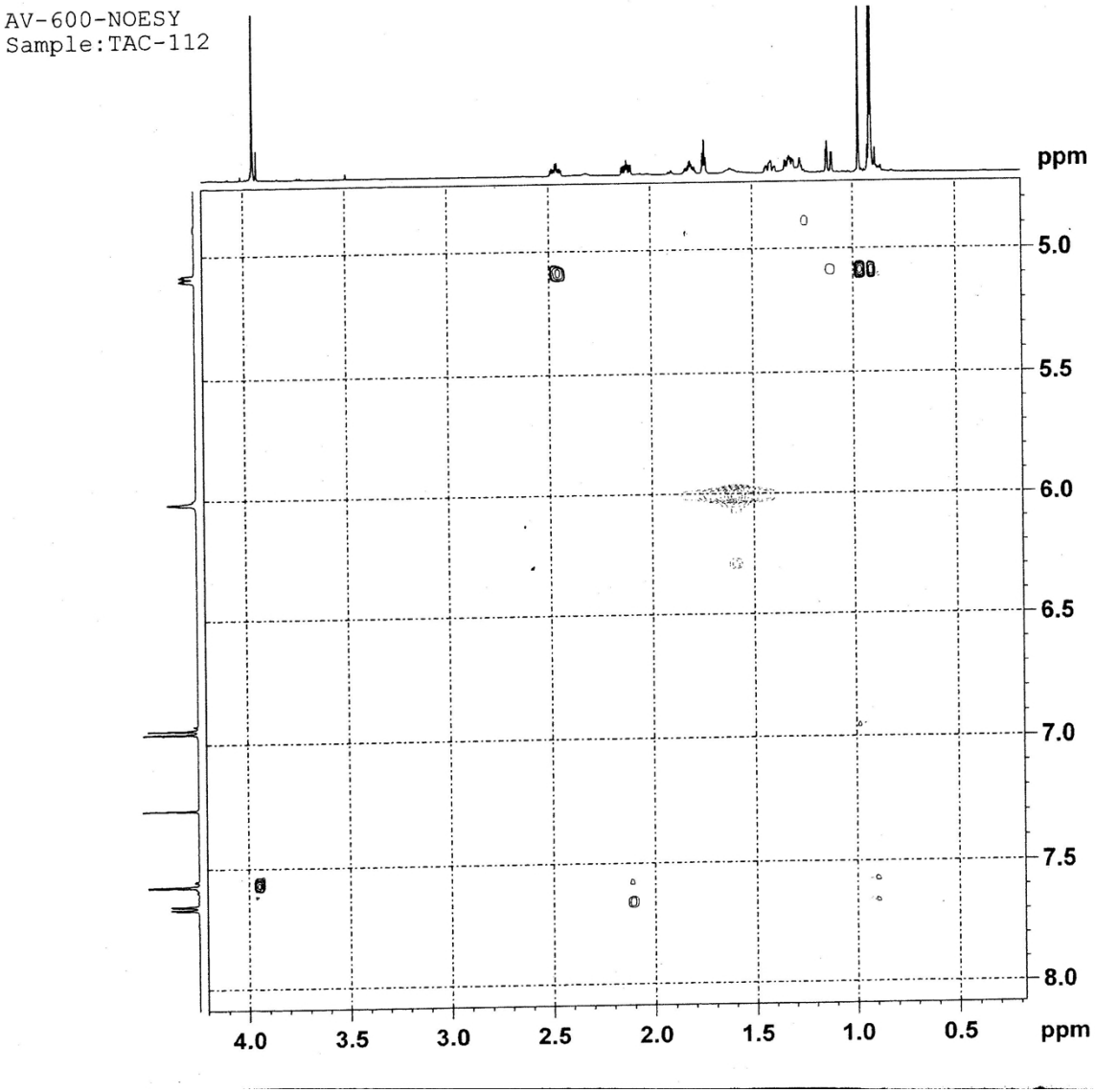


Fig. S8 Enlarged NOSEY of **1** (part 1)



BRUKER

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PROCNO	1
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PULPROG	noesyph
TD	1024
SOLVENT	CDCl3
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DS	4
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FIDRES	5.868765 Hz
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RG	456
DW	83.200 usec
DE	6.50 usec
TE	296.4 K
D0	0.00006907 sec
D1	2.00000000 sec
D8	0.60000002 sec
IN0	0.00016640 sec
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P1	11.10 usec
PL1	-4.00 dB
PL1W	34.70265579 W
SFO1	600.1324005 MHz
ND0	1
TD	256
SFO1	600.1324 MHz
FIDRES	23.475023 Hz
SW	10.014 ppm
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SF	600.1300072 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0
PC	1.00
SI	1024
MC2	States-TPPI
SF	600.1300057 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0

Fig. S9 Enlarged NOSEY of **1** (part 2)

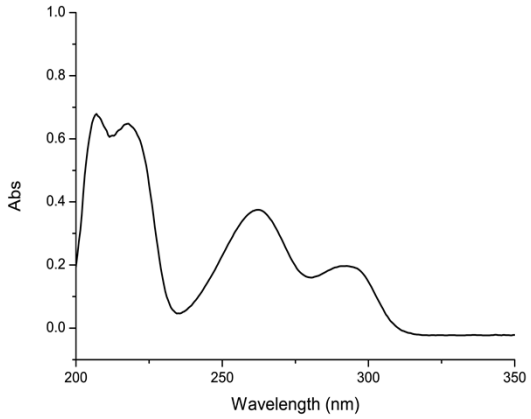


Fig. S10 UV of **2**

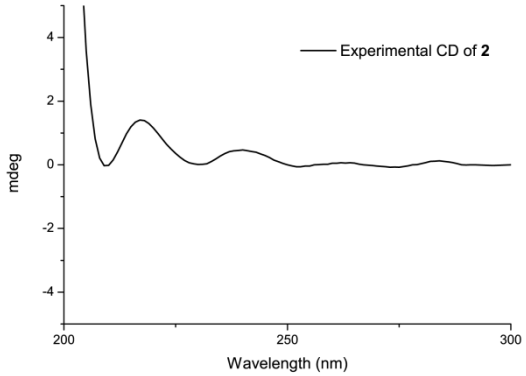


Fig. S11 CD of **2**

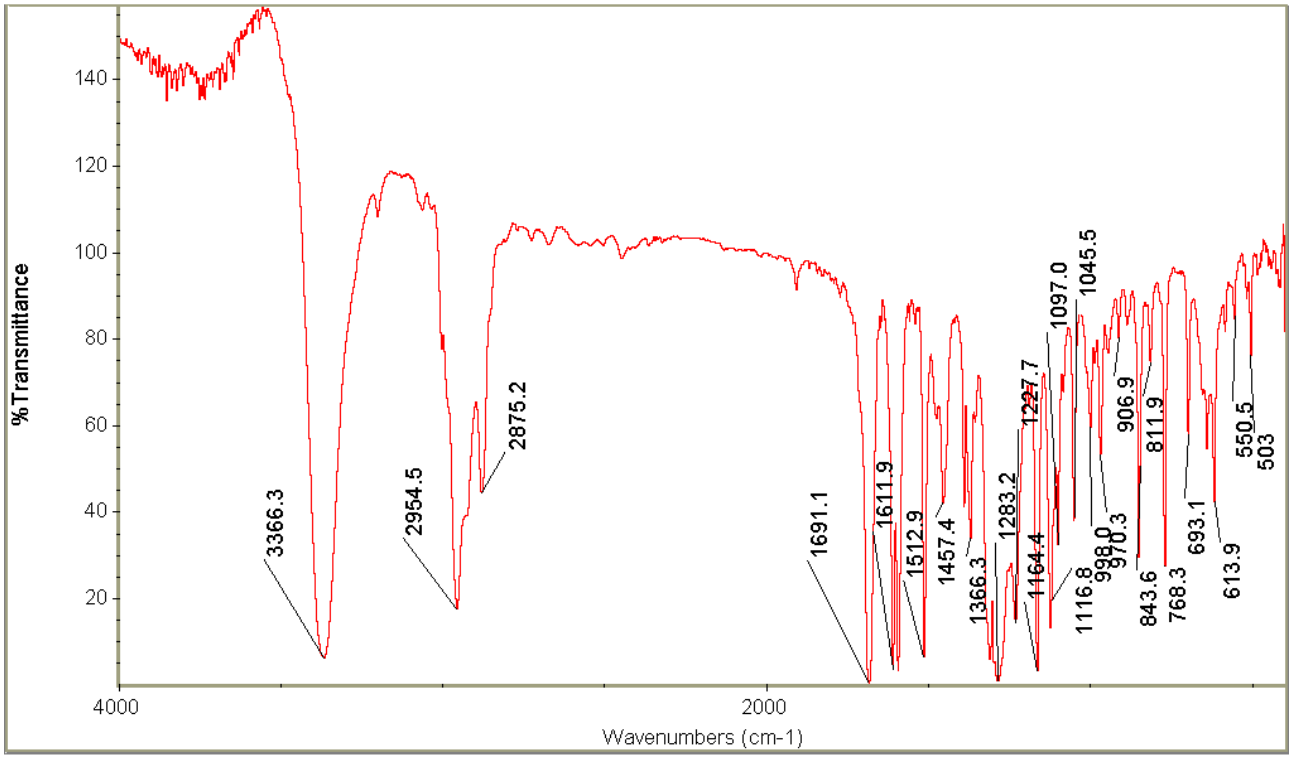


Fig. S12 IR spectrum of **2**

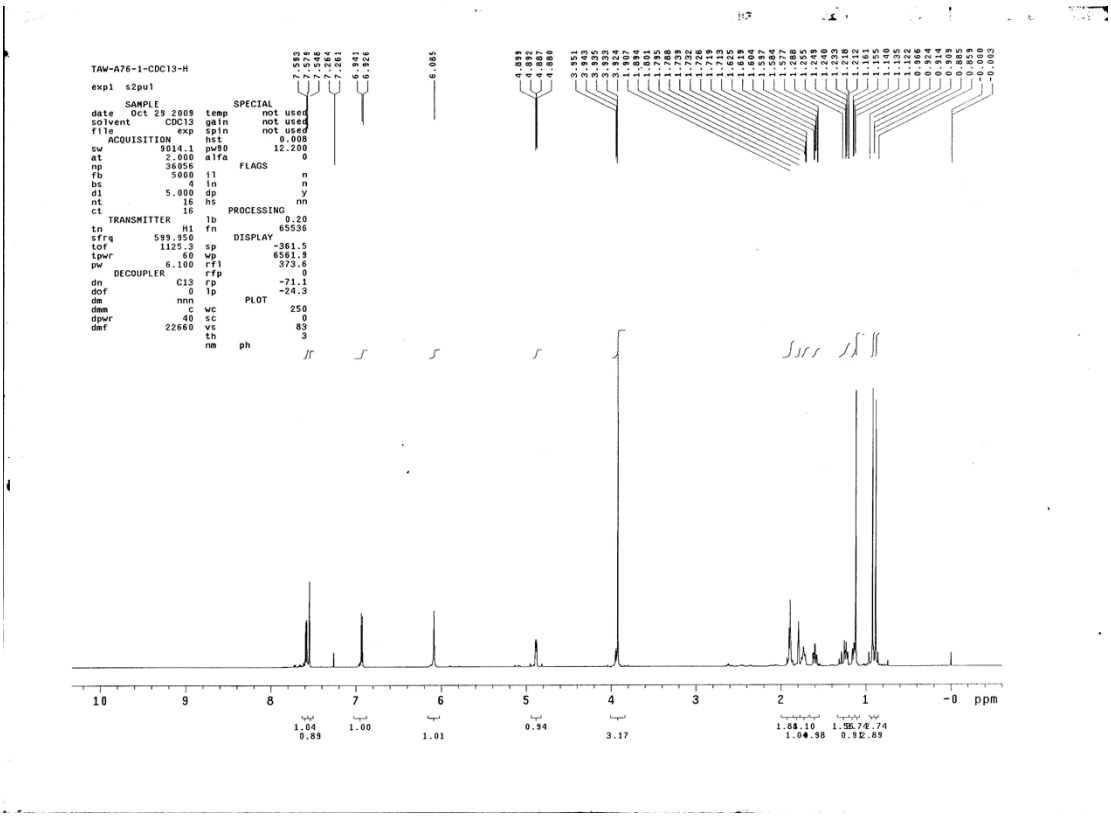


Fig. S13 ^{1}H-NMR of **2**

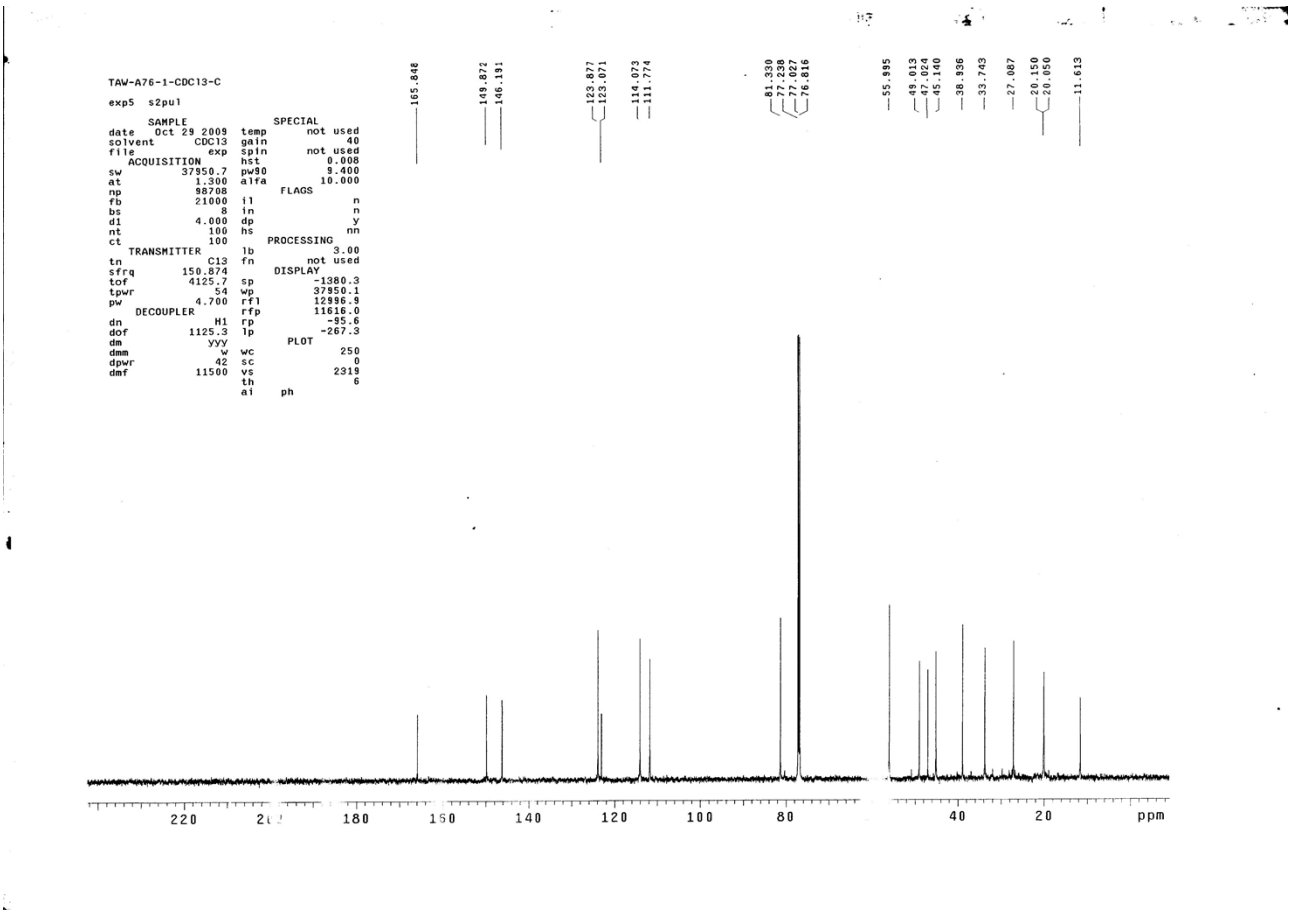
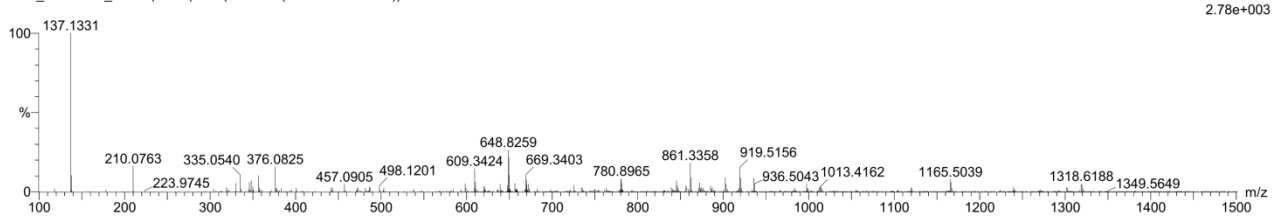


Fig. S14 ^{13}C-NMR of **2**

Monoisotopic Mass, Even Electron Ions
178 formula(e) evaluated with 7 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-150 O: 0-40
TAW-76-1
TAW_20091221_6 168 (3.774) Cm (167:170-(149:162+180:192))

1: TOF MS ES+
2.78e+003



Minimum:				-1.5		
Maximum:		20.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
609.3424	609.3427	-0.3	-0.5	12.5	6.6	C36 H49 O8
	609.3275	14.9	24.5	8.5	7.5	C32 H49 O11
	609.3369	5.5	9.0	21.5	7.8	C43 H45 O3
	609.3580	-15.6	-25.6	16.5	9.4	C40 H49 O5
	609.3486	-6.2	-10.2	3.5	10.5	C29 H53 O13
	609.3521	-9.7	-15.9	25.5	12.2	C47 H45
	609.3334	9.0	14.8	-0.5	13.8	C25 H53 O16

Fig. S15 HR-EI-MS spectrum of **2**

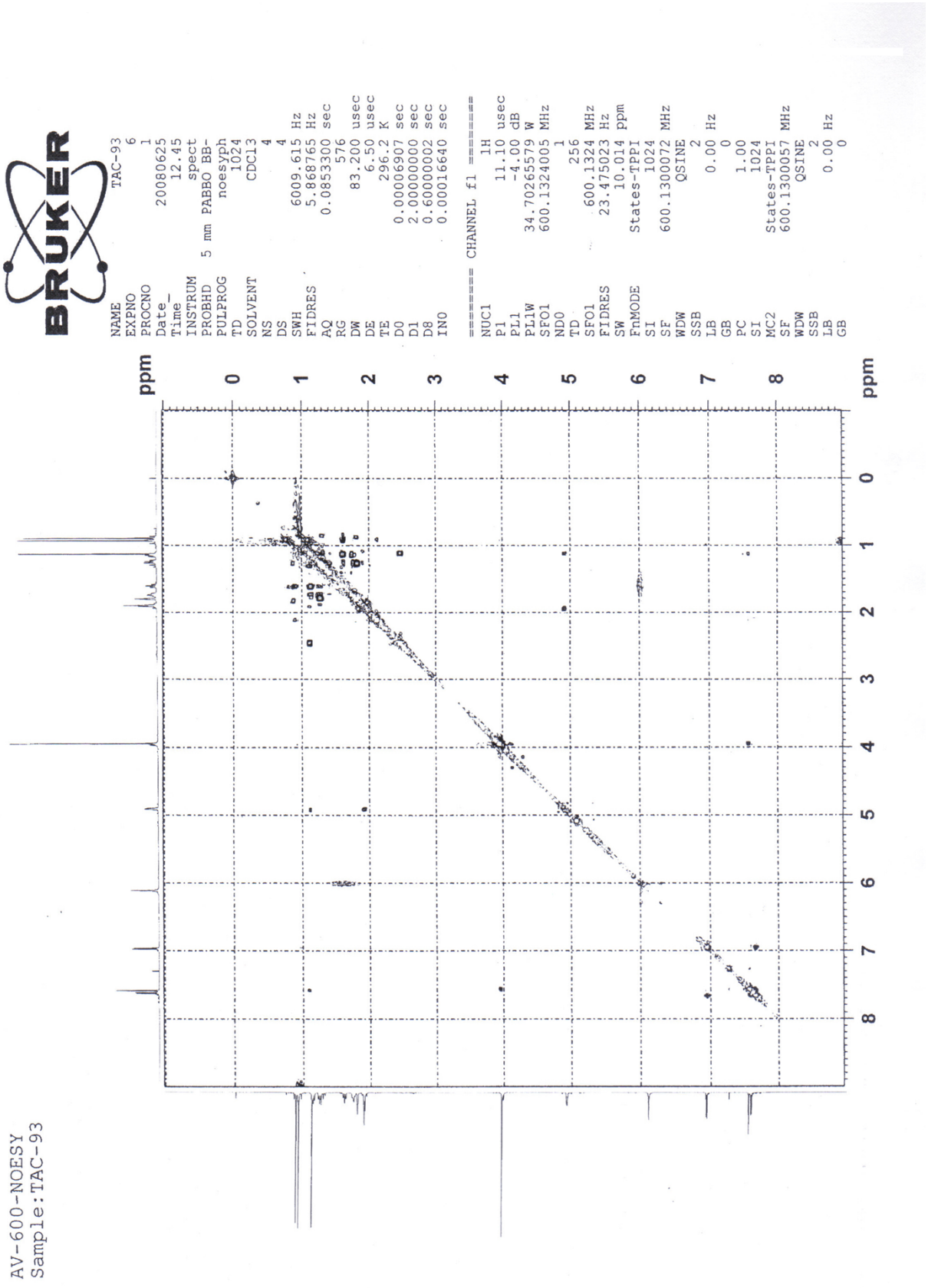


Fig.16 NOSEY spectrum of **2.**

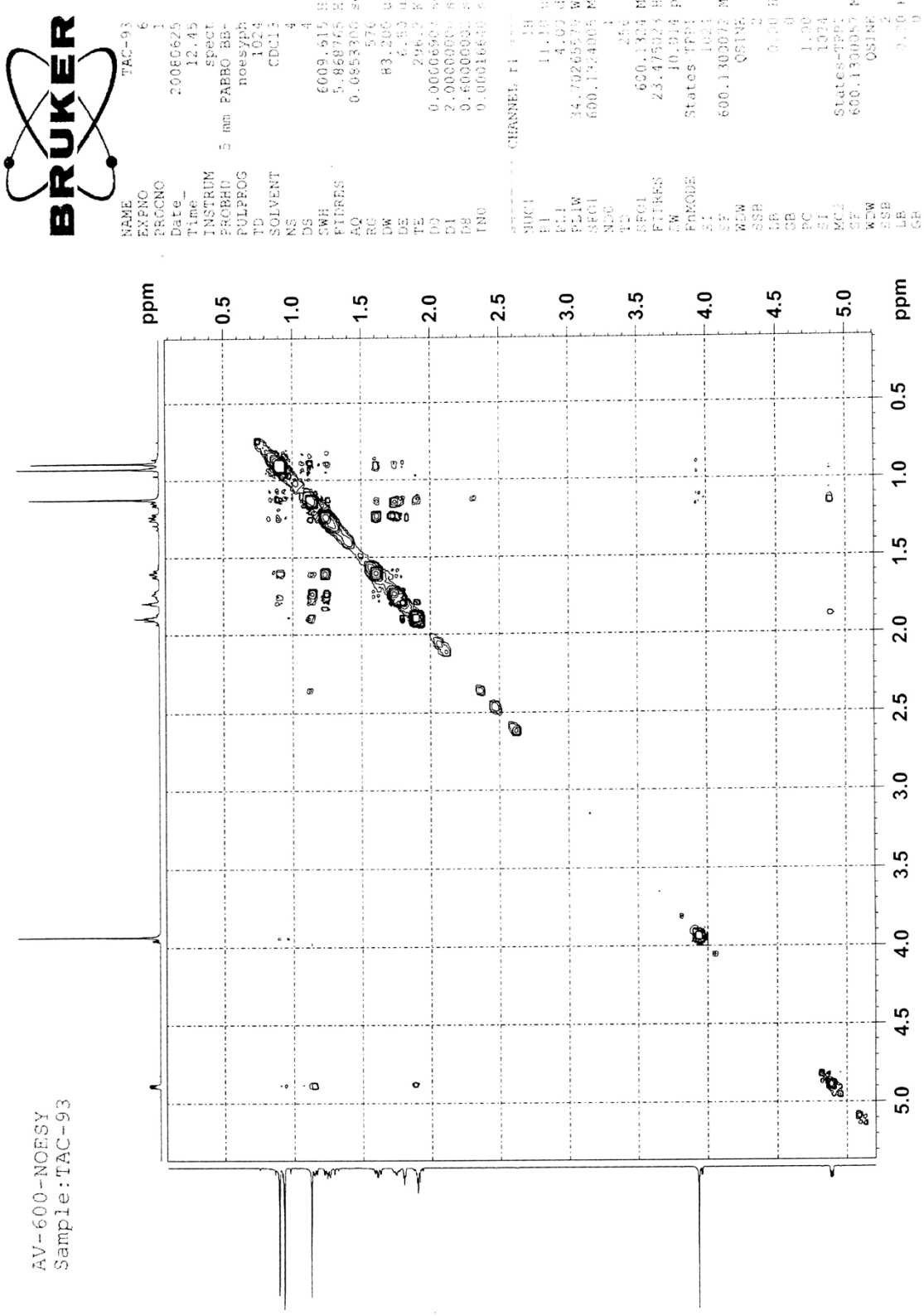


Fig.17 Enlarged NOSEY spectrum of **2.**

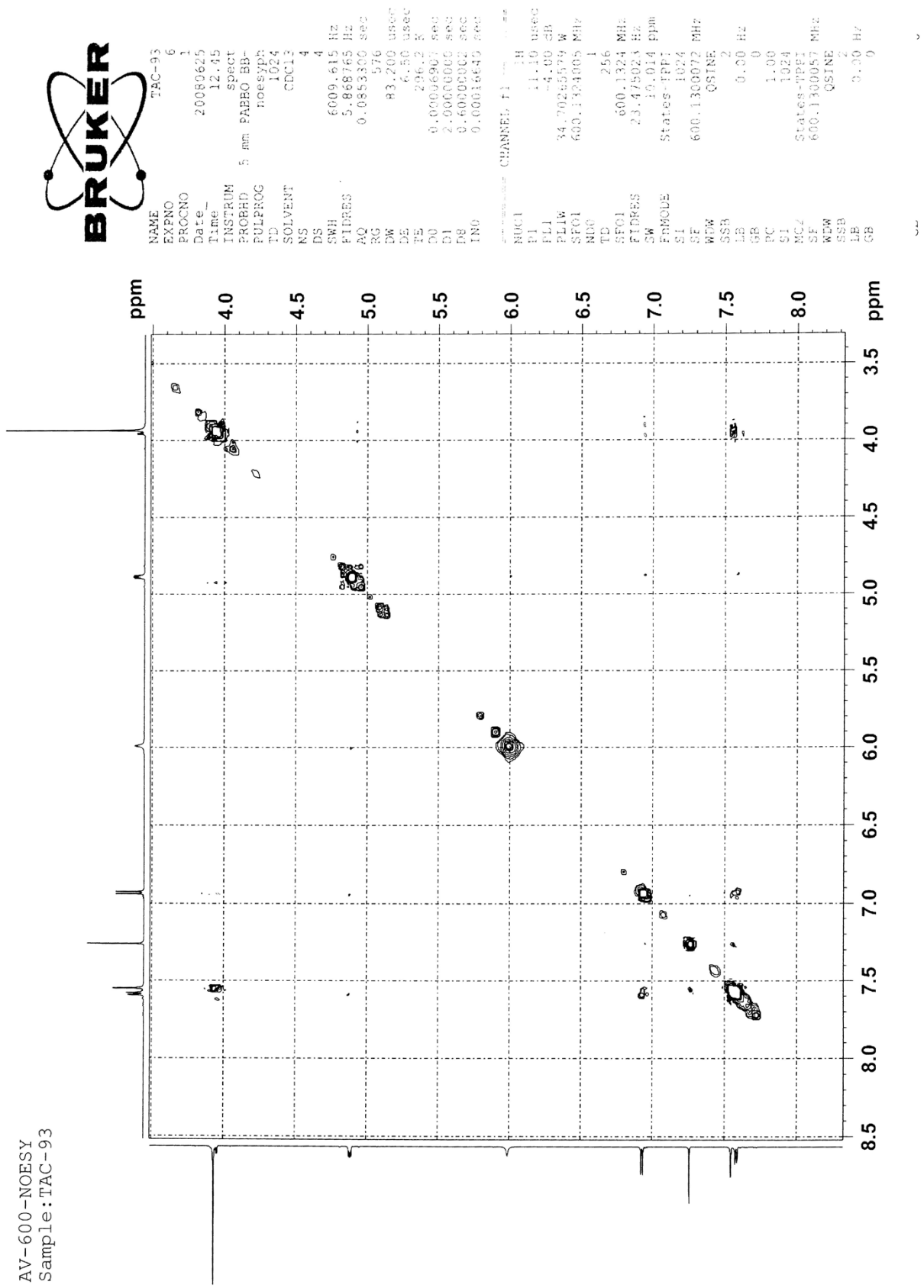


Fig.18 Enlarged NOSEY spectrum of **2.**

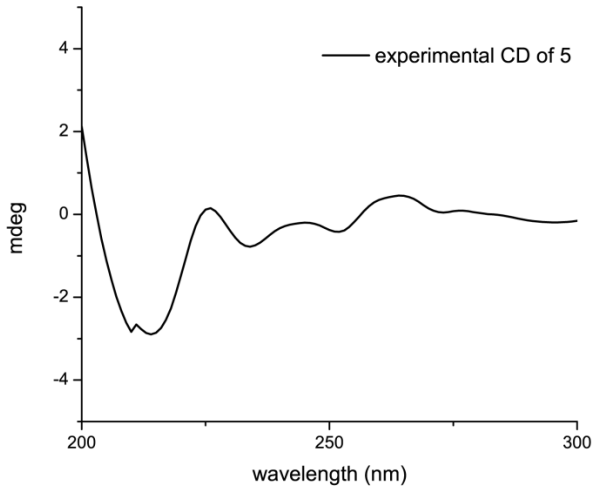


Fig. S19 CD spectrum of **5**

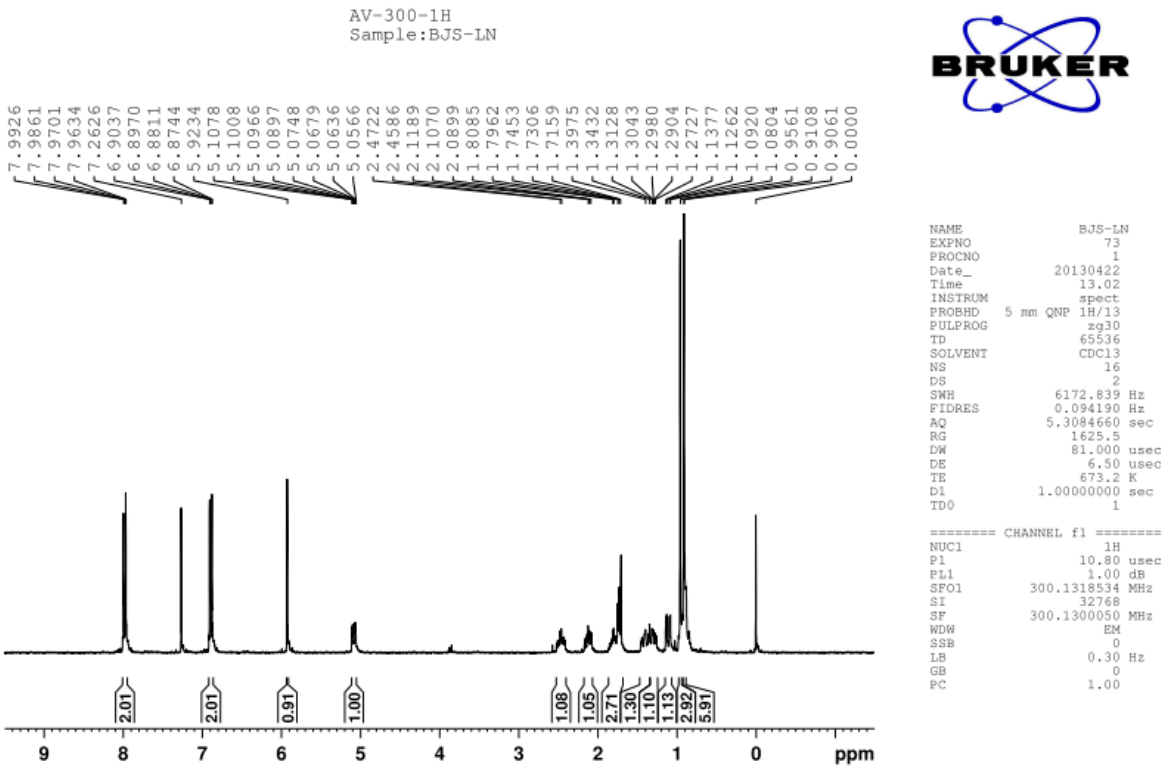


Fig. S20 ^{1}H-NMR of **5**

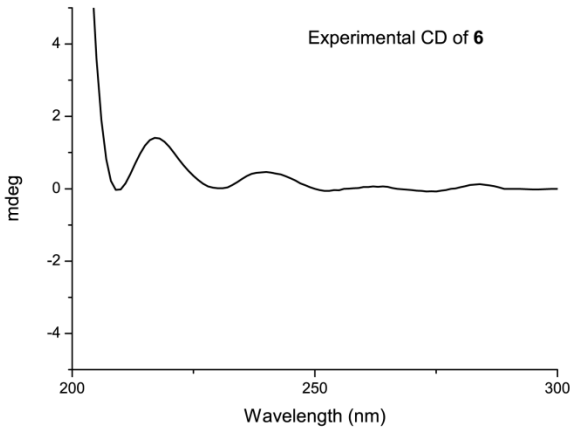


Fig. S 21 CD spectrum of **6**

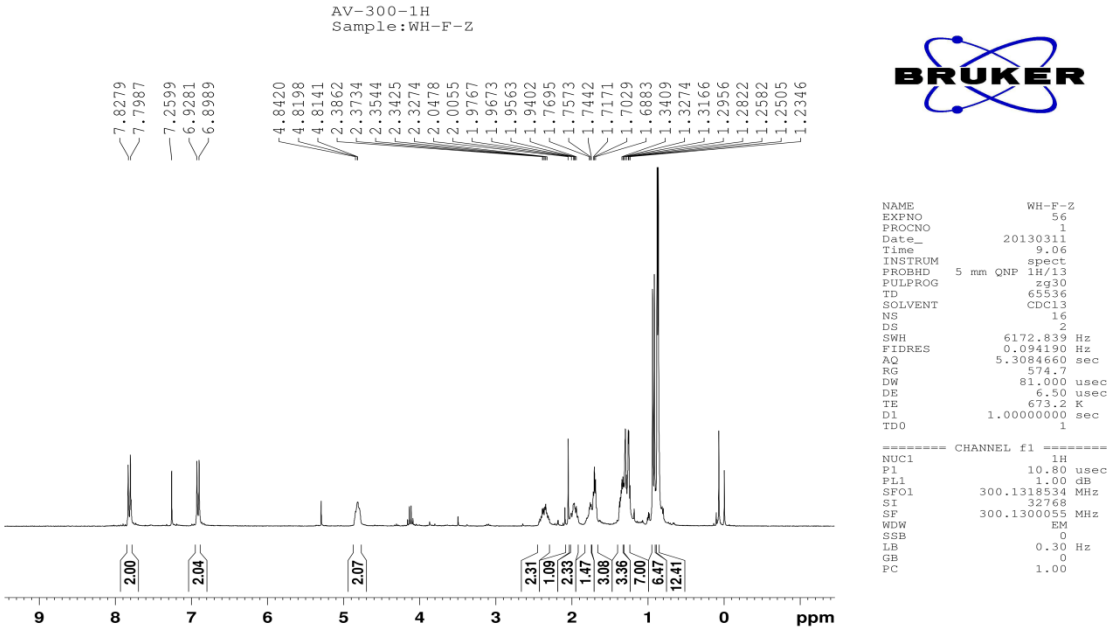


Fig. S 22 ^{1}H-NMR of **6**

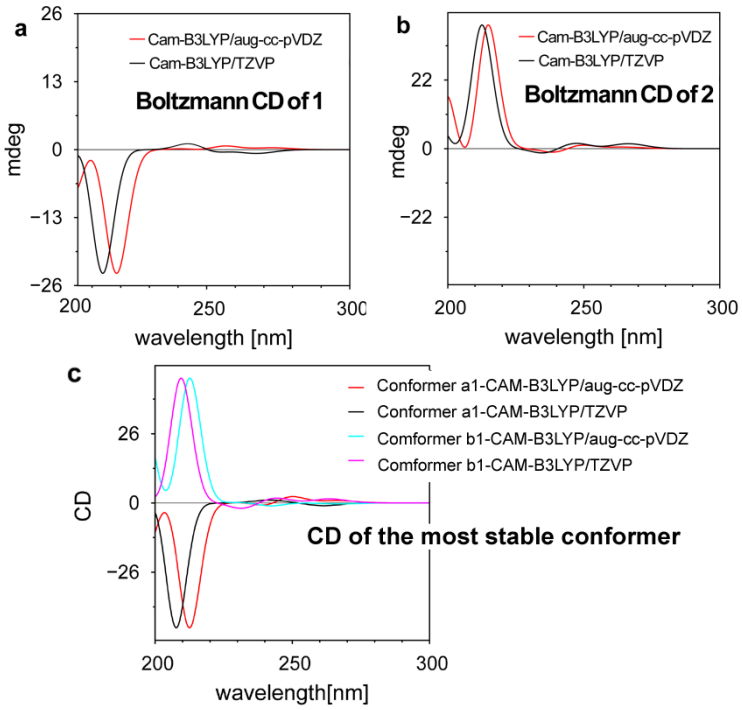


Fig. S23 Calculated ECDs of **1** and **2** using different basic sets

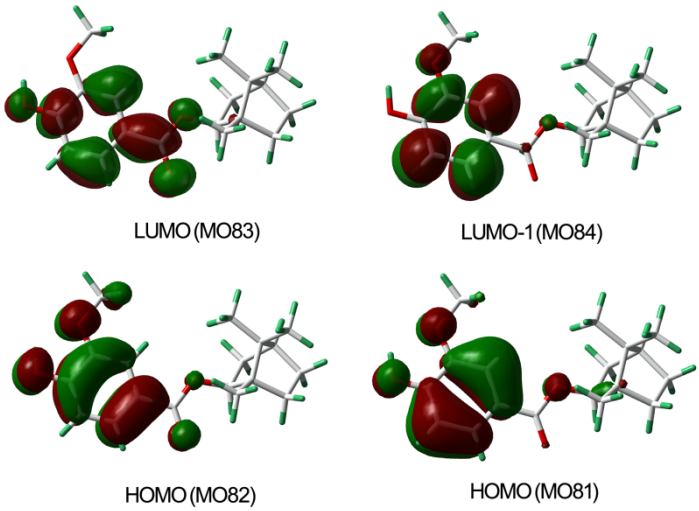


Fig. S24. The HOMO, LUMO, HOMO-1 and LUMO+1 orbitals of **1** generated by GaussView 5.0.8 program.

Synthesis of **5** and **6**

A solution of (1*S*,2*R*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol (0.23 g, 1.5 mmol) and DBU (0.49 g, 3 mmol) in dried CH_2Cl_2 were stirred at 0 °C for 30 min before added to 4-methoxybenzoyl chloride (0.51 g, 3 mmol). The reaction mixture was then stirred at 0 °C for 1 h., acidified to pH 3-4 with 1 M HCl and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude product was purified by silica gel chromatography to

afford compound **5** as a white solid (0.33 g, 75.5%). The compound **6** (0.35 g, 80.1%) was synthetized with (1*S*,2*S*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol (0.23 g, 1.5 mmol) through the same process as the compound 5

Compound 5: White grease; ^{1}H NMR (300 MHz, CDCl3): δ_H 8.00 (2H, d, J = 9.0 Hz, H-3', H-7'), 6.92 (2H, d, J = 9.0 Hz, H-4', H-6'), 5.09 (1H, br d, J = 9.2 Hz , H-2), 3.86 (3H, s, 5'-OCH3), 2.47 (1H, ddd, J = 14.1, 9.2 and 4.2 Hz, H-3β), 2.12 (1H, ddd, J = 12.8, 9.0, 4.2 Hz, H-6β), 1.78 (1H, ddt, J = 13.9, 9.0, 4.2 Hz, H-5β), 1.74 (1H, t, J = 4.2 Hz, H-4), 1.41 (1H, ddd, J = 12.8, 10.2, 4.2 Hz, H-6α), 1.30 (1H, ddd, J = 13.9, 10.2, 4.2 Hz, H-5α), 1.12 (1H, dd, J = 14.1, 4.2 Hz, H-3α), 0.96 (3H, s, H-10), 0.91 (3H, s, H-9), 0.90 (3H, s, H-8). Compound 6: White grease; ^{1}H NMR (300 MHz, CDCl3): δ_H 7.93 (2H, d, J = 8.4 Hz, H-3', H-7'), 6.88 (2H, d, J = 8.4 Hz, H-4', H-6'), 4.88 (1H, dd, J = 6.4 and 4.6 Hz, H-2), 3.84 (3H, s, 5'-OCH3), 1.91 (1H, dd, J=13.2, 4.2 Hz, H-3β), 1.89 (1H, dd, J=13.2, 4.6 Hz, H-3α), 1.80 (1H, dq, J = 12.9, 4.2 Hz, H-5β), 1.74 (1H, br t, J = 4.2 Hz, H-4), 1.60 (1H, td, J = 13.8, 4.2 Hz, H-6β), 1.32 (1H, ddd, J = 12.9, 9.2, 4.2 Hz, H-5α), 1.13 (1H, dd, J = 13.8, 9.2 Hz, H-6α), 1.11 (3H, s, H-10), 0.92 (3H, s, H-8), 0.88 (3H, s, H-9).

Cytotoxity Assay

The human cervical cancer HeLa cell line was purchased from American Type Culture Collection (#HB-8065, ATCC, Manassas, VA, USA). The cells were cultured in RPMI 1640 medium supplemented with 10 % FCS and 0.03 % L-glutamine (Gibco), and maintained at 37 °C with 5 % CO_2 in a humidified atmosphere.

HeLa cells were incubated in 96-well plates (NUNC, Roskilde, Denmark) at a seeding density of 1 × 10^5 cells per well. The cells were incubated with the test compounds of different concentrations. Four hours before the end of incubation, 20 µL 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, St. Louis, MO, USA) solution (5.0 mg/L) was added to each well. The resulting crystals were dissolved in 100 µL DMSO. Absorbance was measured with a microplate reader (TECAN SPECTRA, Wetzlar, Germany). The cytotoxic effect was expressed as the relative percentage of cell growth inhibition as calculated below:

Cell growth inhibition (%) = [A_{570} (control) - A_{570} (compound)] /[A_{570} (control) - A_{570} (blank)]× 100

Computational details

The conformational search were performed using the Spartan 08 program with the MMFF94 molecular mechanics force field. Then all of the possible conformers were optimized at B3LYP level of theory using 6-311++G (d, p) and B3LYP/TZVP basis sets. Frequency calculations based on previously optimized geometries were performed in order to ensure the minimum energy of the structure. Relative population of each conformer was valued on the basis of Boltzmann weighting factor at 298 K .

After that the ab initio GIAO (gauge including atomic orbital) NMR chemical shift values were calculated at the level of B3LYP/6-311++G (d, p) using the Gaussian 09 software package. The theoretical chemical shifts were obtained by the equation:

$$\delta_{isoX} = \sigma_{isoTMSX} - \sigma_{isoX} \quad \text{(Eq.1)}$$

where δ_{isoX} is the isotropic chemical shift, $\sigma_{isoTMSX}$ is the absolute shielding of the standard (in this case, TMS), σ is the absolute shielding of our compounds. The calculated average value for ^{13}C isotropic magnetic shielding of TMS is 184.03 ppm and 31.97 ppm for ^{1}H.

Theoretical isotropic chemical shift depends on the Boltzmann distribution of each conformation:

$$\delta_{isoX}^{total} = \sum_{i=1}^{n} P_i \delta_{isoX_i} \quad \text{(Eq.2)}$$

Where δ_{isoXi} is the isotropic chemical shift of *i*th conformer, P_i is Boltzmann weighting factor of *i*th conformer, δ_{isoX}^{total} is the isotropic chemical shift.

Then the theoretical chemical shifts are empirically scaled according to the following equation:

$$\delta_{scaled} = \frac{\delta_{calc} - intercept}{slope} \quad \text{(Eq.3)}$$

Where slope and intercept are obtained from a plot of the calculated data against the experimental data to be assigned; the purpose of this approach is to remove systematic errors in the shift calculation. The correlation coefficients were calculated between the scaled theoretical chemical shifts and experimental chemical shifts.

The geometries used for the ECD calculation are optimized by DFT calculations at the B3LYP/TZVP levels. The ECD were then simulated by the time-dependent density functional theory (TDDFT) method at the level of CAM-B3LYP/TZVP. ECD curves were generated by Specdis using half bandwidth of 0.2 eV. To generate the final spectrum of ECD, all the simulated spectra of the lowest energy conformations were averaged according to the Boltzmann distribution theory in which their Gibbs free energy (G) was adopted.

Table S1. ^{1}H NMR, ^{13}C NMR and NOESY spectral data of compound **1** ($CDCl_3$) (^{1}H: 300 MHz, ^{13}C: 75 MHz, δ: ppm, *J*: Hz)

Position	δ(H) (*J* in Hz)	δ(C)	NOESY
1		49.1	
2	5.08 (1H, br d, *J*=9.6)	80.3	8, 9
3α	1.12 (1H, dd, *J*=14.4, 4.2)	36.9	5α, 6α
3β	2.51 (1H, ddd, *J*=14.4, 9.6, 3.6)		9
4	1.74 (1H, t, *J*=4.2)	45.0	
5α	1.32 (1H, ddd, *J*=14.4, 9.6, 4.2)	28.1	3α
5β	1.80 (1H, ddt, *J*=14.4, 10.8, 4.2)		10
6α	1.40 (1H, ddd, *J*=12.6, 9.6, 4.2)	27.4	3α
6β	2.11 (1H, ddd, *J*=12.6, 10.8, 4.2)		8, 10
7		47.8	
8	0.91 (3H, br. s)	13.6	2, 6β, 9
9	0.92 (3H, br. s)	19.7	2, 3β, 8
10	0.97 (3H, br. s)	18.9	5β, 6β
1′		166.6	
2′		123.1	
3′	7.60 (1H, d, *J*=1.8)	111.7	4′-CH_3O
4′		146.2	
5′		149.8	
6′	6.96 (1H, d, *J*=8.4)	113.9	
7′	7.64 (1H, dd, *J*=8.4, 1.8)	123.9	
4′-CH_3O	3.95 (3H, br. s)	56.1	3′
5′-OH	6.02 (1H, br. s)		

19. Table S2. ^{1}H NMR, ^{13}C NMR, and NOESY spectral data of compound **2** ($CDCl_3$) (^{1}H: 300 MHz, ^{13}C: 75 MHz, δ: ppm, J: Hz)

Position	δ(H) (J in Hz)	δ(C)	NOESY
1		49.0	
2	4.89 (1H, dd, *J*=6.6, 4.2)	81.3	3α, 6α
3α	1.91 (1H, dd, *J*=13.2, 6.6)	38.9	2, 5α
3β	1.93 (1H, dt, *J*=13.2, 4.2)		9
4	1.76 (1H, br t, *J*=4.2)	45.1	
5α	1.27 (1H, ddd, *J*=14.4, 9.6, 4.2)	27.1	3α
5β	1.80 (1H, dq, *J*=14.4, 4.2)		10
6α	1.16 (1H, ddd, *J*=12.6, 9.6, 4.2)	33.7	2
6β	1.60 (1H, td, *J*=12.6, 4.2)		8, 10
7		47.0	
8	0.91 (3H, br s)	11.6	6β, 9
9	0.88 (3H, br s)	20.0	3β, 8, 3’
10	1.12 (3H, br s)	20.1	5β, 6β
1′		165.8	
2′		123.1	
3′	7.55 (1H, br s)	111.8	9, 4′-CH_3O
4′		146.2	
5′		149.8	
6′	6.93 (1H, d, *J*=8.4)	114.1	
7′	7.59 (1H, br d, *J*=8.4)	123.9	
4′-CH_3O	3.93 (3H, s)	56.0	3′
5′-OH	6.08 (1H, br s)		

Table S3. Relative free energies and populations of the conformations of **1** and **2.**

Conf.	DFT/B3LYP/TZVP		DFT/B3LYP/cc-pVDZ	
	ΔG [a]	P% [b]	ΔG [a]	P% [b]
1a	0	80	0	74.7
1b	0.86	12.8	0.55	16.3
1c	1.02	7.2	0.62	9.0
2a	0	86.2	0	57.4
2b	0.93	7	0.58	22.5
2c	1.06	6.8	1.05	20.1

[a]The unit for ΔG is kcal mol^{-1}.

[b] Populations based on ΔG values.