SUPPLEMENTARY MATERIAL

Neogenkwanine I from the flower buds of *Daphne genkwa* with its stereostructure confirmation using quantum calculation profiles and antitumor evaluation

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ABSTRACT

Neogenkwanine I (**1**), a new daphnane-type diterpene with 4,7-ether group, along with four known ones (**2**–**5**), were isolated from *Daphne genkwa*. The structure including absolute configurations of **1** was established on the basis of NMR, ^{13}C-NMR and ECD calculations and CD exciton chirality analysis. ^{13}C-NMR and ECD calculations of daphnane-type diterpenes were reported here for the first time. All of the diterpenes were screened for their cytotoxic activities against MCF-7 and Hep3B cell lines. The cytotoxicity structure- activity relationship of compounds was illustrated with the absence of ortho- ester group of daphnane-type diterpenes.

KEYWORDS *Daphne genkwa*; daphnane-type diterpenes; ^{13}C-NMR and ECD calculations; cytotoxicity; structure-activity relationship

Table of Contents

14*R**) of **1**.

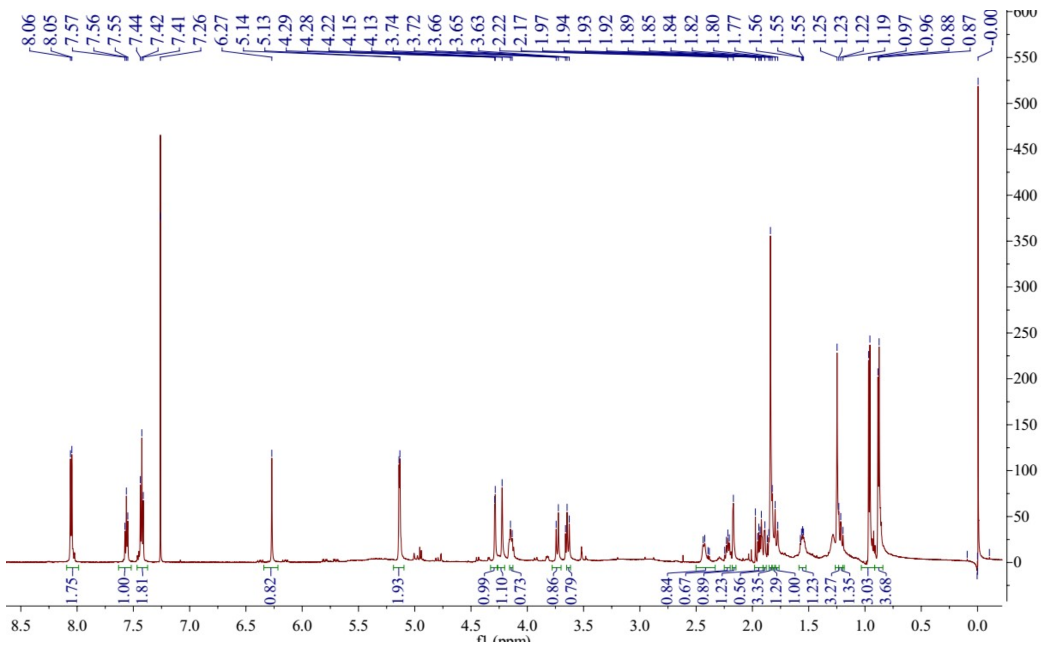


Figure S1. ^{1}H-NMR spectrum (600 MHz, Chloroform-*d*) of compound **1**

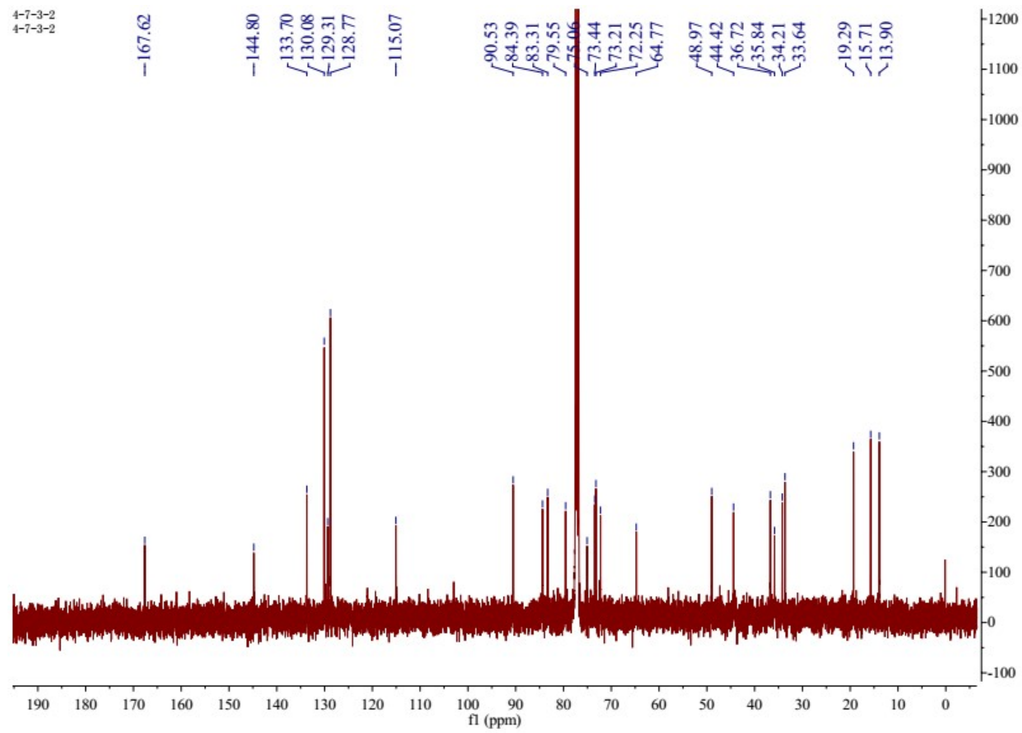


Figure S2. ^{13}C-NMR spectrum (100 MHz, Chloroform-*d*) of compound **1**

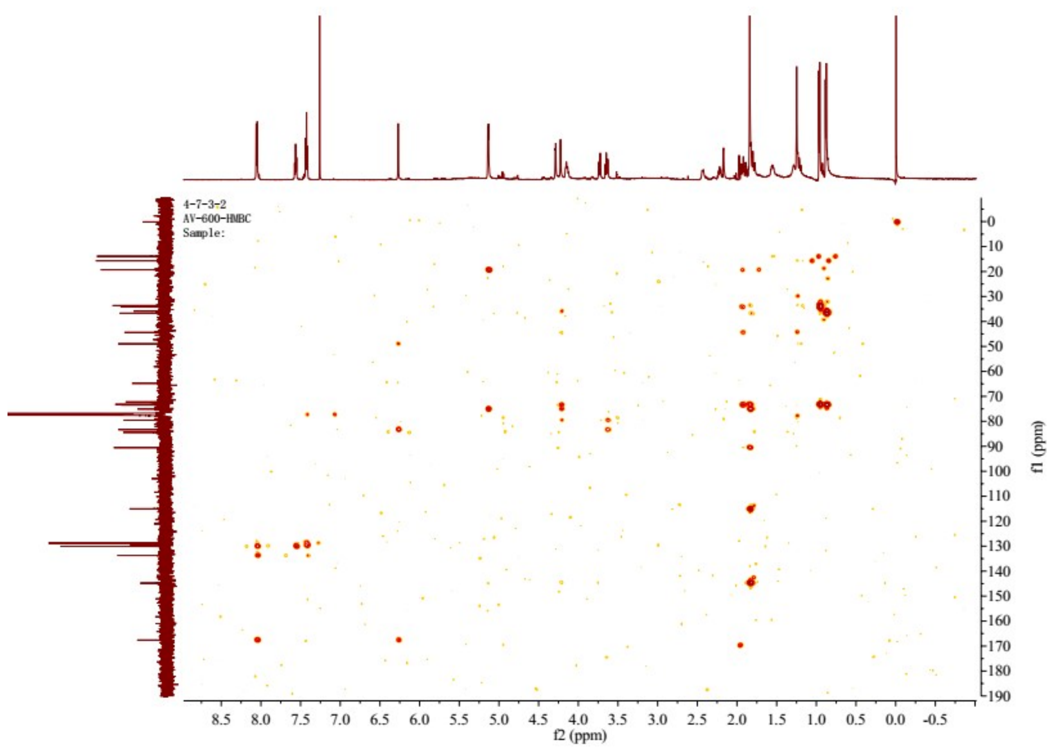


Figure S3. HMBC spectrum (600 MHz, Chloroform-*d*) of compound **1**

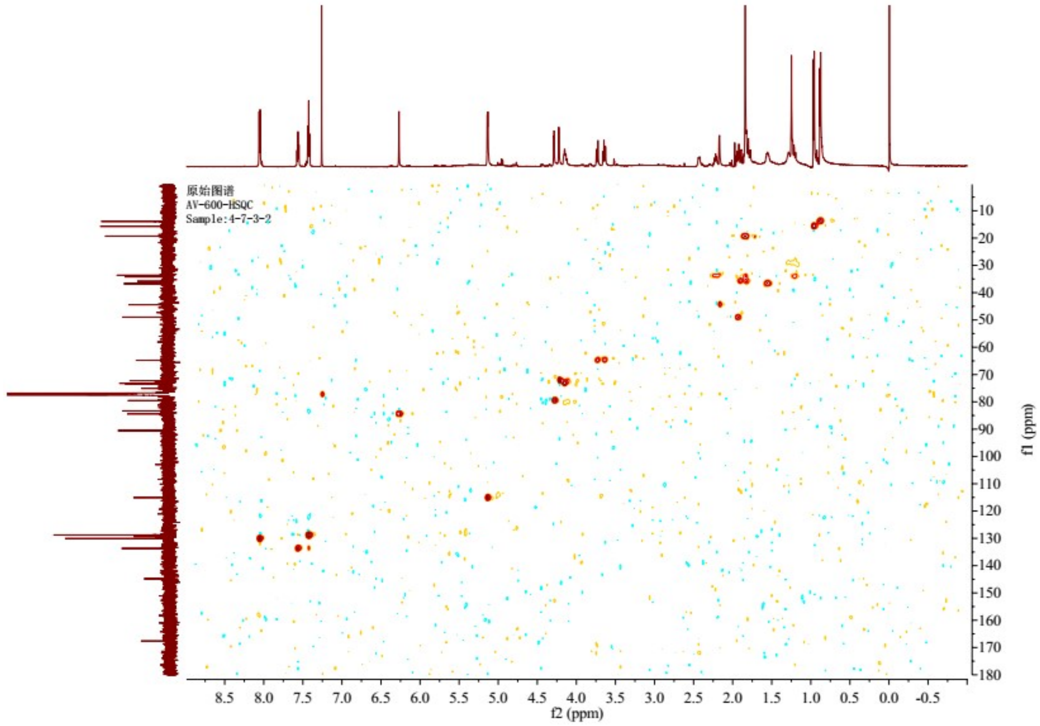


Figure S4. HSQC spectrum (600 MHz, Chloroform-*d*) of compound **1**

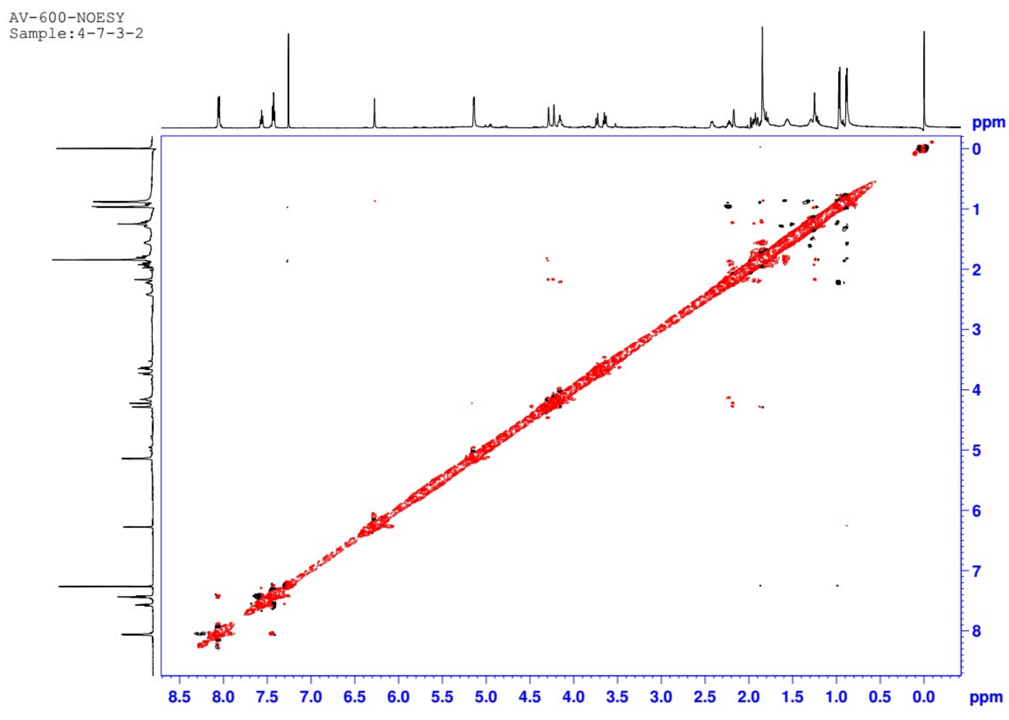
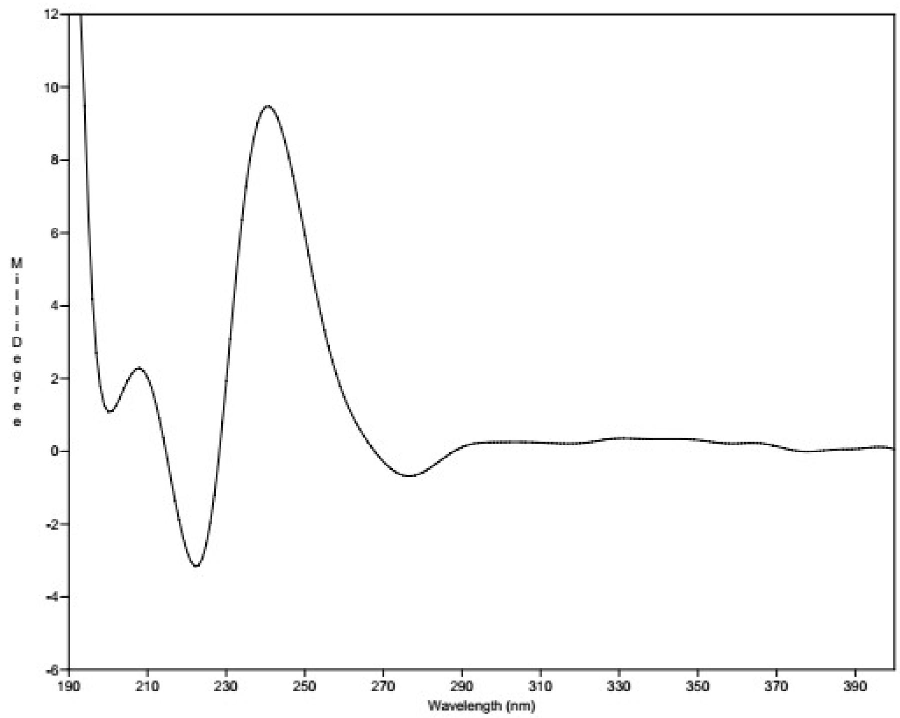


Figure S5. NOESY spectrum (600 MHz, Chloroform-*d*) of compound **1**



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Polynomial Order=3
Derivative=0

Figure S6. CD spectrum of compound **1**

Mass Spectrum SmartFormula Report

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Comment			

Acquisition Parameter

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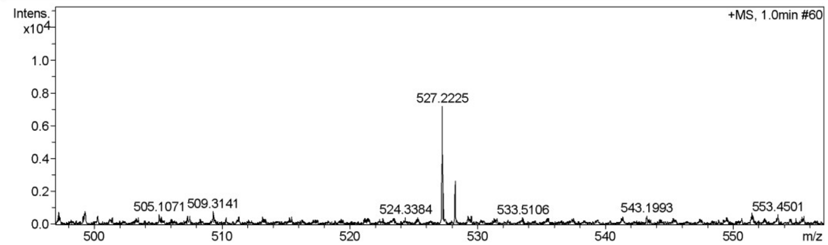
Scan End	1500 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source



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527.2225														
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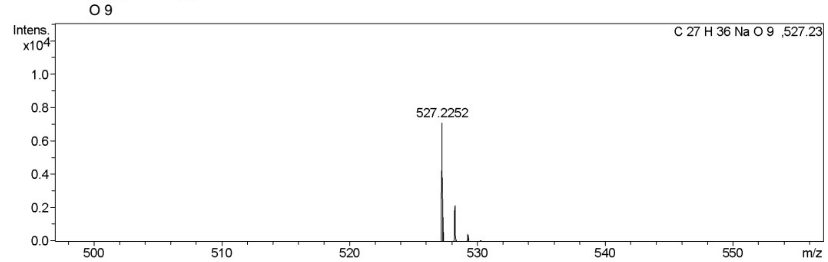


Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev

Figure S7. HRESIMS spectrum of compound **1**

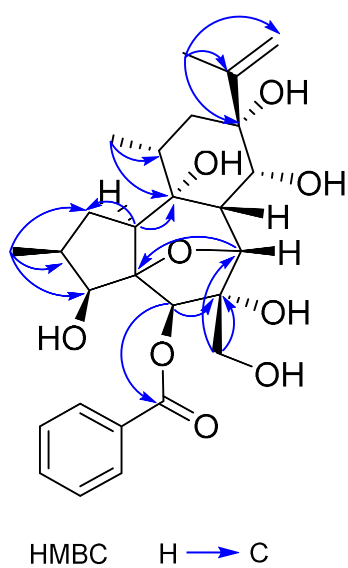


Figure S8. Key HMBC correlations of **1**.

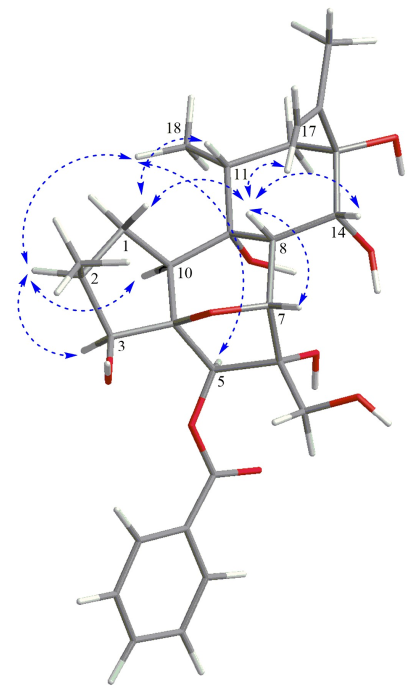


Figure S9. Key NOESY correlations of **1**(<-->).

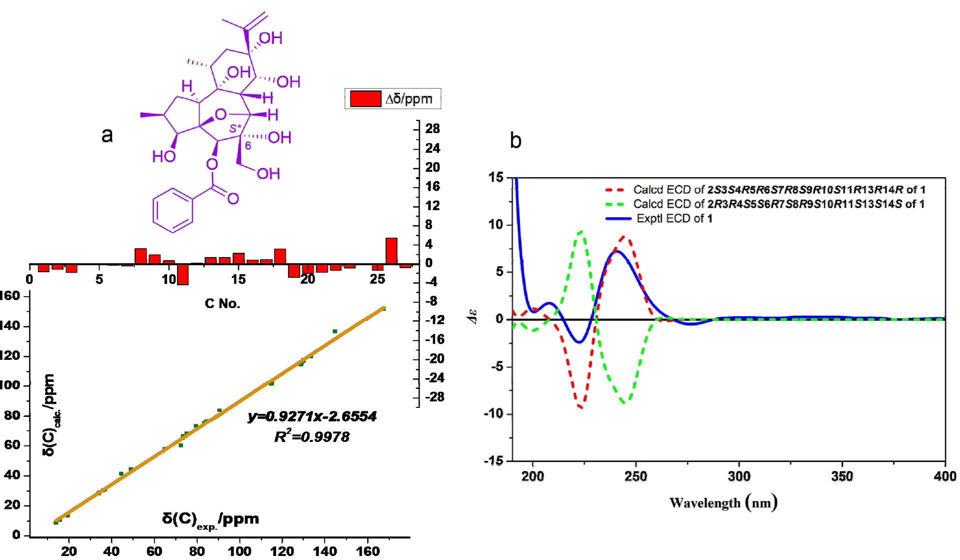
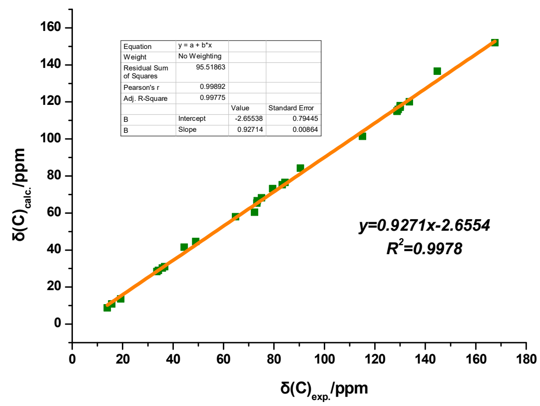


Figure S10. (**a**) Experimental and calculated ^{13}C chemical shifts of (2*S**,3*S**,4*R**,5*R**, 6*S* *, 7*R**, 8*S**,9*R**,10*S**, 11*R**,13*R**,14*R**)-**1**. Regression analysis of experimental versus calculated ^{13}C-NMR chemical shifts of (2*S**,3*S**,4*R**,5*R**,6*S**, 7*R**,8*S**,9*R**, 10*S**, 11*R**, 13*R**,14*R**)-**1** at the TMS B3LYP/6-311 + G(2d,p) GIAO level. Linear fitting is shown as a line. (**b**) Comparison of calculated ECD spectra with the experimental spectrum of **1**.

a



b

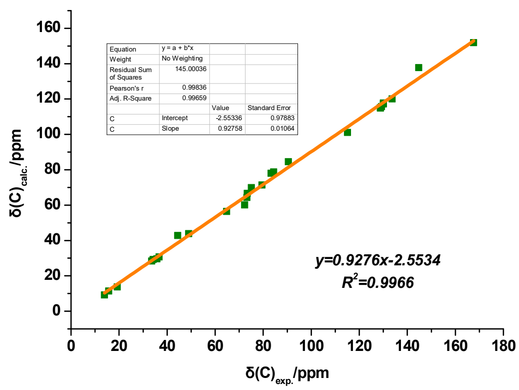


Figure S11. Correlation between experimental and calculated ^{13}C chemical shifts of (2*S**,3*S**,4*R**,5*R**,6*S**,7*R**,8*S**,9*R**,10*S**,11*R**,13*R**,14*R**)-**1** (**a**) and (2*S**,3*S**,4*R**,5*R**,6*R**,7*R**,8*S**,9*R**,10*S**,11*R**,13*R**,14*R**)-**1** (**b**)

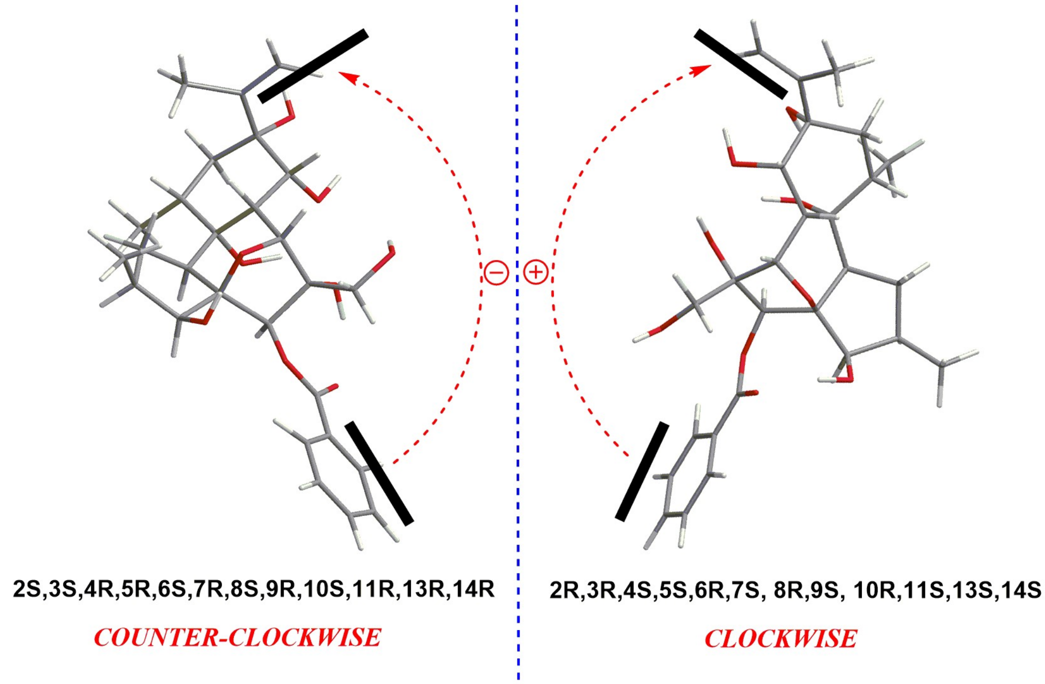


Figure S12. Stereoviews for 2*S*,3*S*,4*R*,5*R*,6S,*7R*,8*S*,9*R*,10*S*,11*R*,13*R*,14*R* and 2*R*,3*R*, 4*S*,5*S*,6*R*,7*S*,8*R*,9*S*, 10*R*,11*S*,13*S*,14*S* of compound **1**. Bold lines denote the electric transition dipole of the chromophores for compound **1**.

Table S1. ^{1}H and ^{13}C-NMR spectral data of compound **1**

Position		**1**[a]
No.	δ_C	δ_H (J in Hz)
1a	34.2	1.82 (1H, m)
1b		1.21 (1H, m)
2	33.6	2.23 (1H, m)
3	73.2	4.14 (1H, d, J = 9.0 Hz)
4	90.5	-
5	84.4	6.27 (1H, s)
6	83.3	-
7	79.5	4.29 (1H, d, J = 3.0 Hz)
8	44.4	2.17 (1H, brs)
9	73.4	-
10	49.0	1.94 (1H, m)
11	36.7	1.56 (1H, m)
12a	35.8	1.89 (1H, m)
12b		1.80 (1H, m)
13	75.1	-
14	72.3	4.22 (1H, brs)
15	144.8	-
16	115.1	5.14 (1H, s), 5.13 (1H, s)
17	19.3	1.85 (3H, s)
18	13.9	0.88 (3H, d, J = 6.4 Hz)
19	15.7	0.96 (3H, d, J = 7.2 Hz)
20a	64.8	3.64 (1H, d, J = 11.7 Hz)
20b		3.73 (1H, d, J = 11.7 Hz)
1'	167.6	-
2'	129.3	-
3',7'	130.1	8.04 (2H, d, J = 7.3 Hz)
4',6'	128.8	7.43 (2H, m)
5'	133.7	7.56 (1H, t, J = 7.4 Hz)

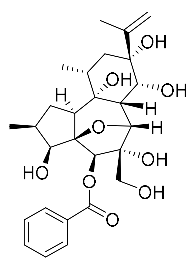
[a] ^{1}H (600 MHz) and ^{13}C (100 MHz) in $CDCl_3$.

Table S2. Cytotoxic activities of compounds **1-5** against the Hep3B and MCF-7 cell lines

Compound	IC_{50}(μM)	
	3B[a]	MCF-7[a]
1	>100	>100
2	38.55 ± 2.76	19.92 ± 0.33
3	42.24 ± 1.24	7.31 ± 0.48
4	>100	>100
5	8.86 ± 0.18	17.62 ± 0.75
5-fluorouracil	18.42 ± 1.01	39.83 ± 0.56

[a]All data were shown as means ± SD of three independent experiments.

Table S3. Conformations of (2*S**,3*S**,4*R**,5*R**,6*S**,7*R**,8*S**, 9*R**,10*S**,11*R**,13*R**, 14*R**)-**1** were obtained after the optimization.

(6*S**)1		
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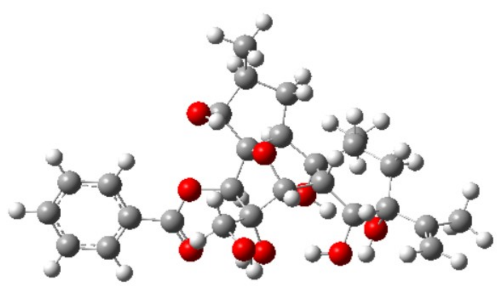
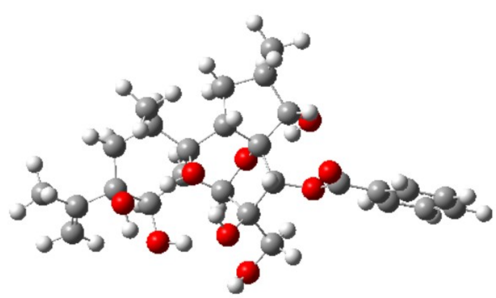
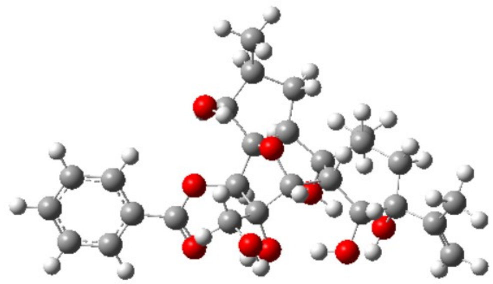
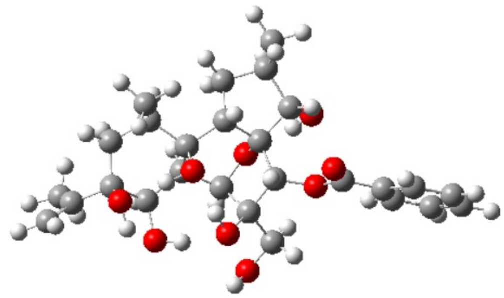
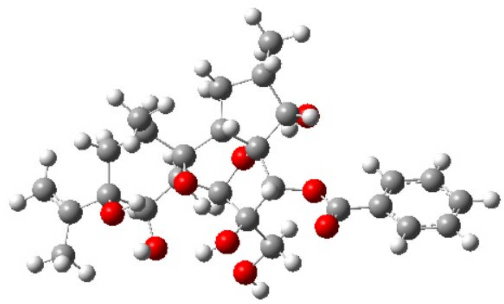
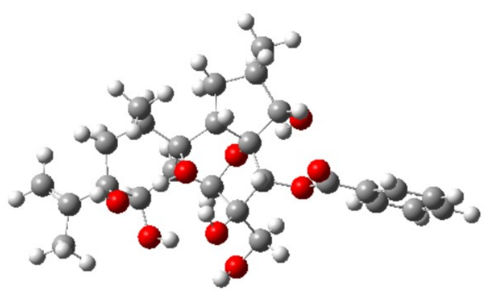
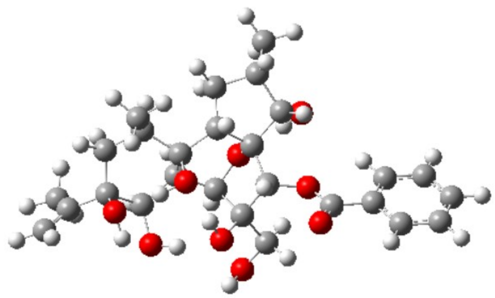
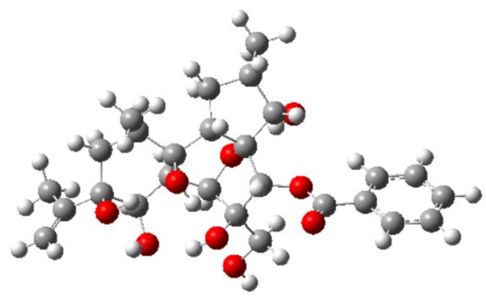
(6*S**)1-1		0.01
(6*S**)1-2		0.78

(6*S**)1-3		0.09
(6*S**)1-4		5.13
(6*S**)1-5		0.19
(6*S**)1-6		0.01

(6*S**)1-7		19.42
(6*S**)1-8		0.9
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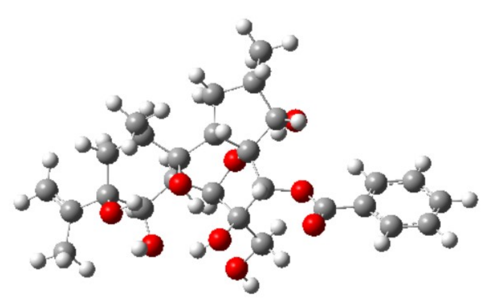
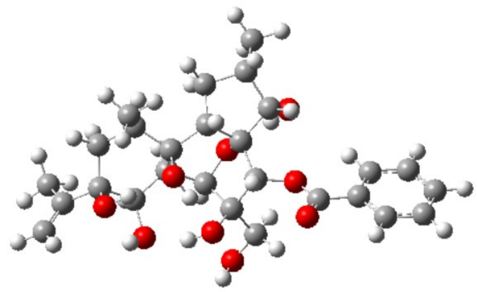
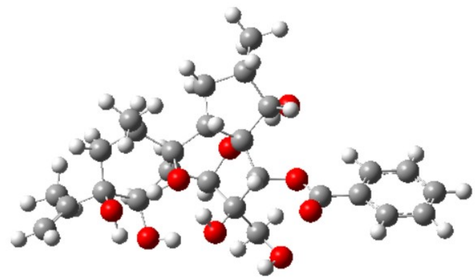
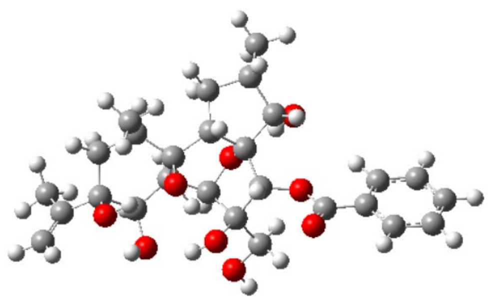
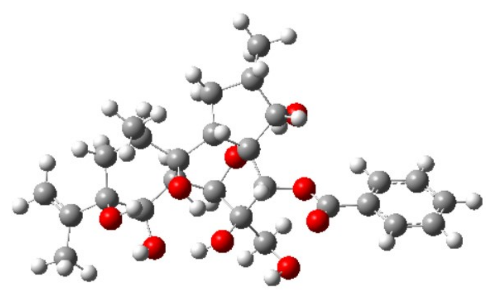
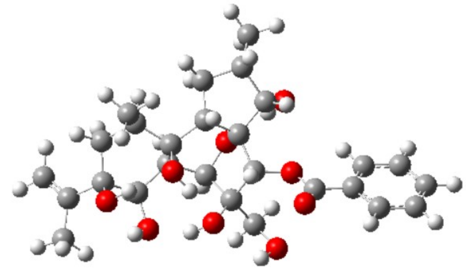
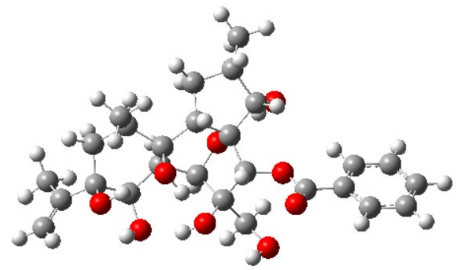
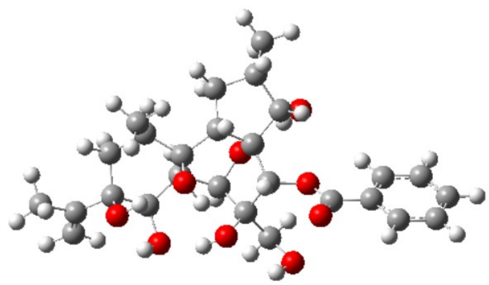
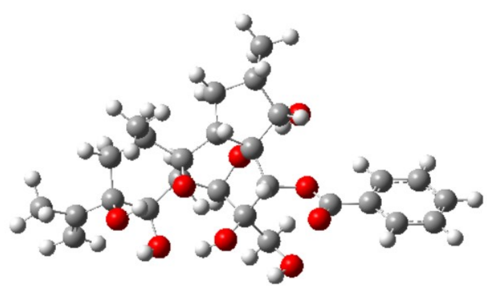
(6*S**)1-10		0.19

(6*S**)1-11		6.92
(6*S**)1-12		34.5
(6*S**)1-13		0.9
(6*S**)1-14		3.72

(6*S**)1-15		3.72
(6*S**)1-16		19.18
(6*S**)1-17		2.13
(6*S**)1-18		2.13

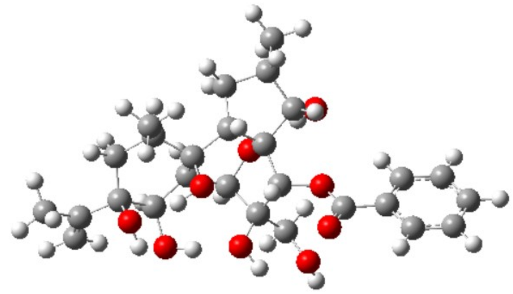
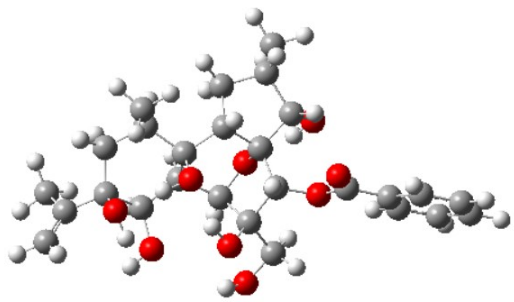
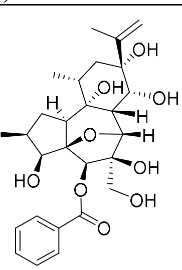
(6*S**)1-19		0
(6*S**)1-20		0

Table S4. Conformations of (2*S**,3*S**,4*R**,5*R**,6*R**,7*R**, 8*S**,9*R**,10*S**,11*R**, 13*R**,14*R**)-**1** were obtained after the optimization.

(6*R**)1		
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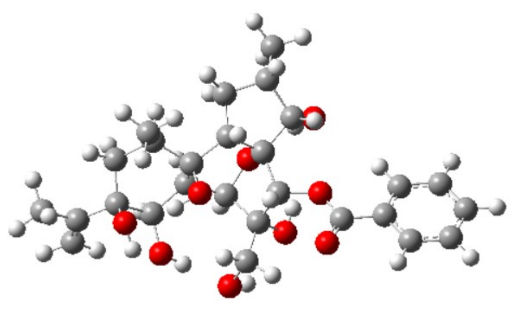
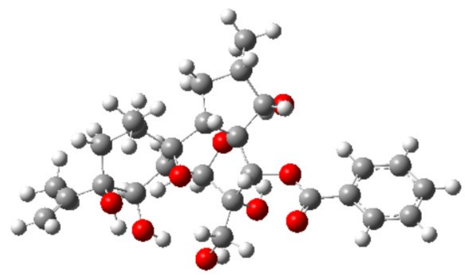
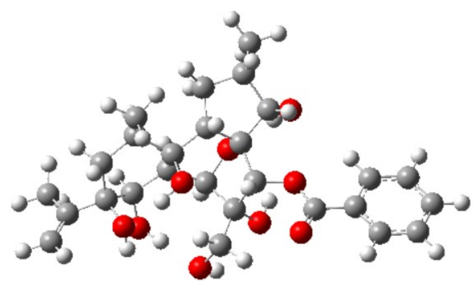
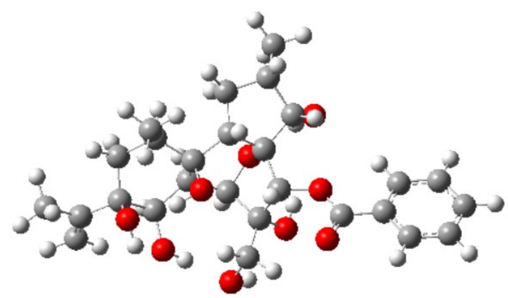
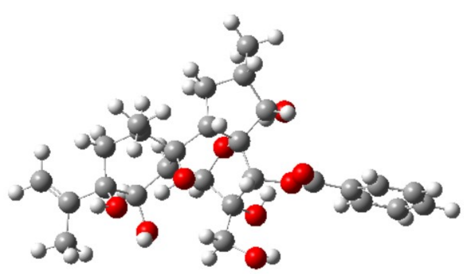
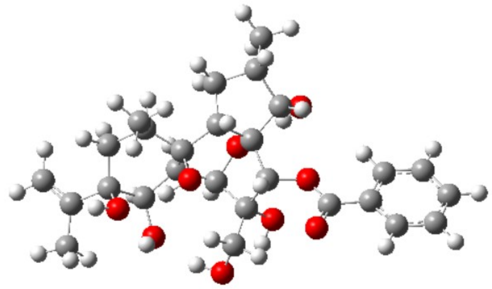
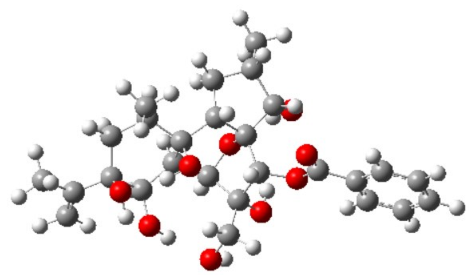
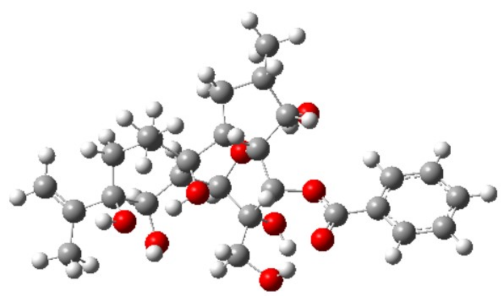
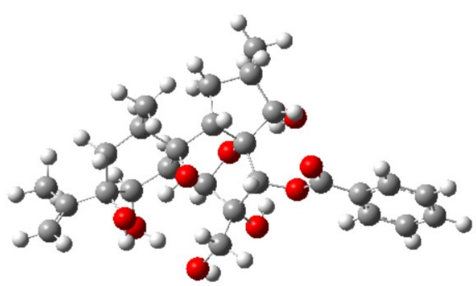
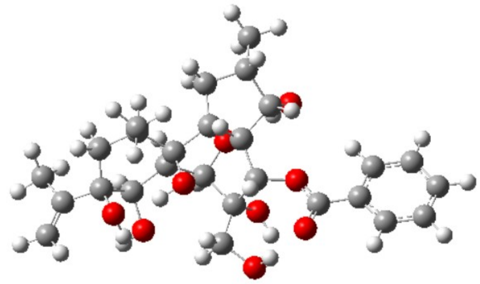
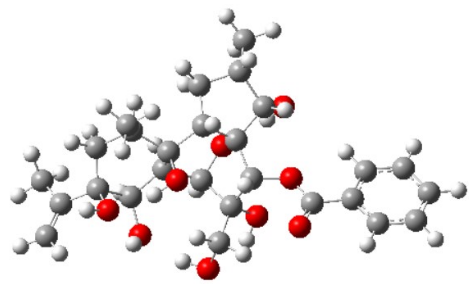
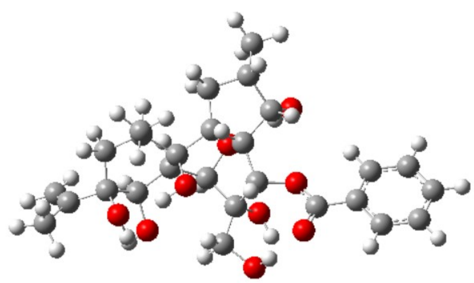
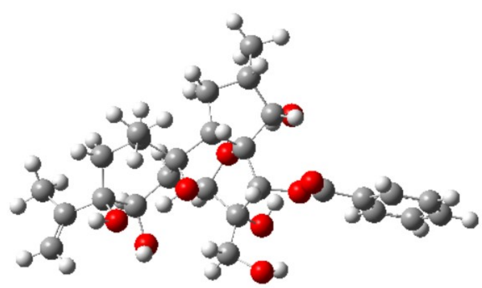
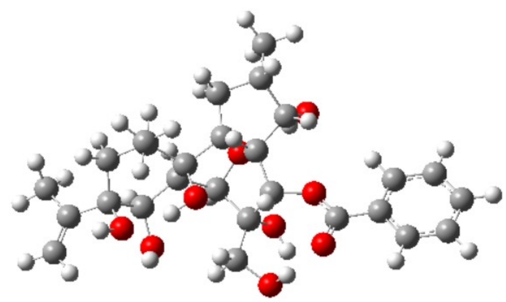
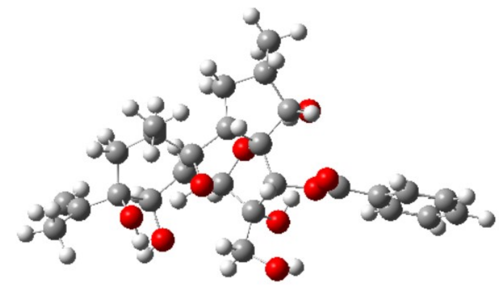
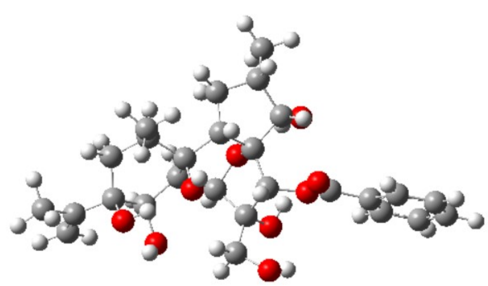
(6*R**)1-1		8.67

(6*R**)1-2		69.65
(6*R**)1-3		0.99
(6*R**)1-4		8.67
(6*R**)1-5		0

(6*R**)1-6		0.45
(6*R**)1-7		1
(6*R**)1-8		0.17
(6*R**)1-9		0.05

(6*R**)1-10		2.89
(6*R**)1-11		2.99
(6*R**)1-12		0.73
(6*R**)1-13		0

(6*R**)1-14		0.93
(6*R**)1-15		0
(6*R**)1-16		0
(6*R**)1-17		0

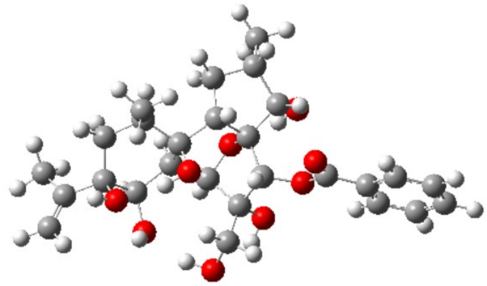
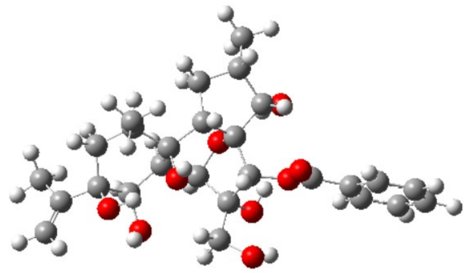
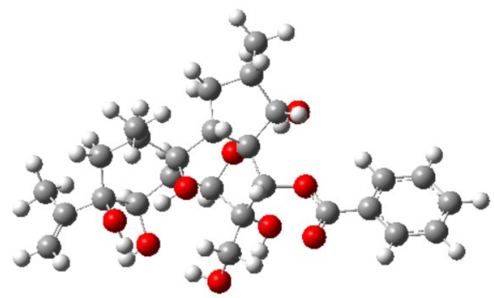
(6*R**)1-18		0.22
(6*R**)1-19		0
(6*R**)1-20		2.58

Table S5. Deviations between the calculated and experimental ^{13}C NMR chemical shifts for stereoisomers (2*S**,3*S**,4*R**,5*R**,6*S**,*7R**,8*S**,9*R**,10*S**,11*R**,13*R**,14*R** and 2*S**,3*S**,4*R**,5*R**,6*R**,7*R**,8*S**,9*R**,10*S**,11*R**,13*R**,14*R**) of **1**

EXL	*6S**				*6R**			
	calc.	scal.calc.	Δδ	|Δδ|	calc.	scal.calc.	Δδ	|Δδ|
13.9	8.7	12.3	-1.6	1.6	9.1	12.6	-1.3	1.3
15.7	10.9	14.6	-1.1	1.1	11.4	15.0	-0.7	0.7
19.3	13.6	17.5	-1.8	1.8	13.7	17.5	-1.8	1.8
33.6	28.3	33.4	-0.2	0.2	28.3	33.3	-0.3	0.3
34.2	29.0	34.2	0.0	0.0	29.2	34.2	0.0	0.0
35.8	30.2	35.5	-0.3	0.3	29.7	34.7	-1.1	1.1
36.7	31.0	36.3	-0.4	0.4	30.6	35.8	-0.9	0.9
44.4	41.5	47.6	3.2	3.2	42.8	48.9	4.5	4.5
49.0	44.5	50.9	1.9	1.9	43.9	50.1	1.1	1.1
64.8	58.0	65.4	0.6	0.6	56.4	63.6	-1.2	1.2
72.3	60.4	68.0	-4.3	4.3	60.1	67.5	-4.8	4.8
73.2	65.3	73.3	0.1	0.1	64.3	72.1	-1.1	1.1

73.4	66.6	74.7	1.3	1.3	66.7	74.6	1.2	1.2
75.1	68.2	76.4	1.3	1.3	69.9	78.1	3.0	3.0
79.5	73.2	81.8	2.3	2.3	71.4	79.7	0.2	0.2
83.3	75.3	84.1	0.8	0.8	78.0	86.8	3.5	3.5
84.4	76.5	85.3	0.9	0.9	78.7	87.6	3.2	3.2
90.5	84.2	93.7	3.2	3.2	84.5	93.8	3.3	3.3
115.1	101.4	112.2	-2.9	2.9	101.1	111.7	-3.4	3.4
128.8	114.8	126.7	-2.1	2.1	114.9	126.6	-2.2	2.2
128.8	115.1	127.0	-1.8	1.8	115.1	126.8	-2.0	2.0
129.3	116.0	127.9	-1.4	1.4	115.7	127.5	-1.8	1.8
130.1	117.1	129.2	-0.9	0.9	117.2	129.1	-1.0	1.0
130.1	117.9	130.0	-0.1	0.1	117.7	129.6	-0.5	0.5
133.7	120.0	132.3	-1.4	1.4	120.1	132.2	-1.5	1.5
144.8	136.6	150.2	5.4	5.4	137.8	151.3	6.5	6.5
167.6	151.9	166.8	-0.8	0.8	151.8	166.4	-1.2	1.2
			AveDev	1.6			AveDev	2.0
			MaxDev	5.4			MaxDev	6.5
			R^2	0.9978			R^2	0.9966