

Supplementary Material

Restoration of Chemosensitivity in P-glycoprotein-dependent Multidrug Resistant Cells by Dihydro- β -agarofuran Sesquiterpenes from *Celastrus vulcanicola*

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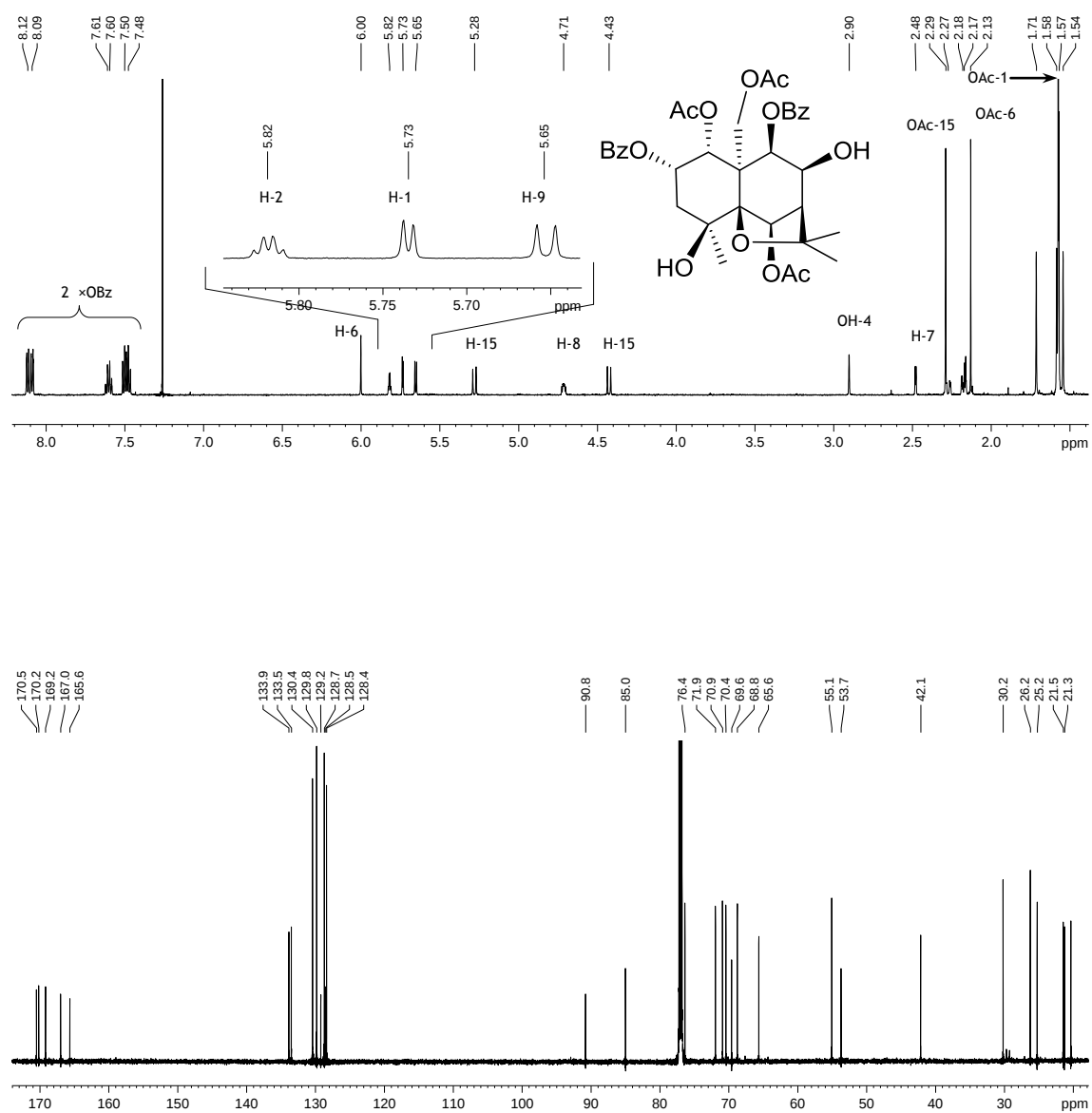
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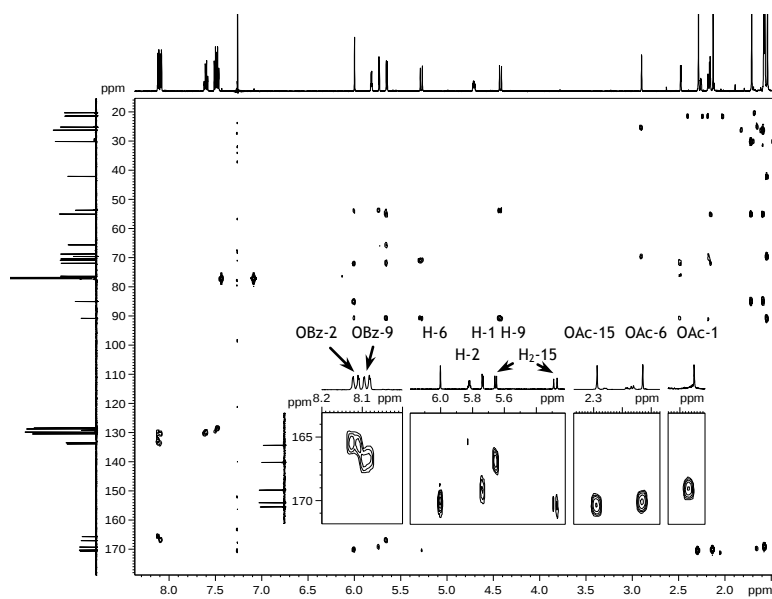
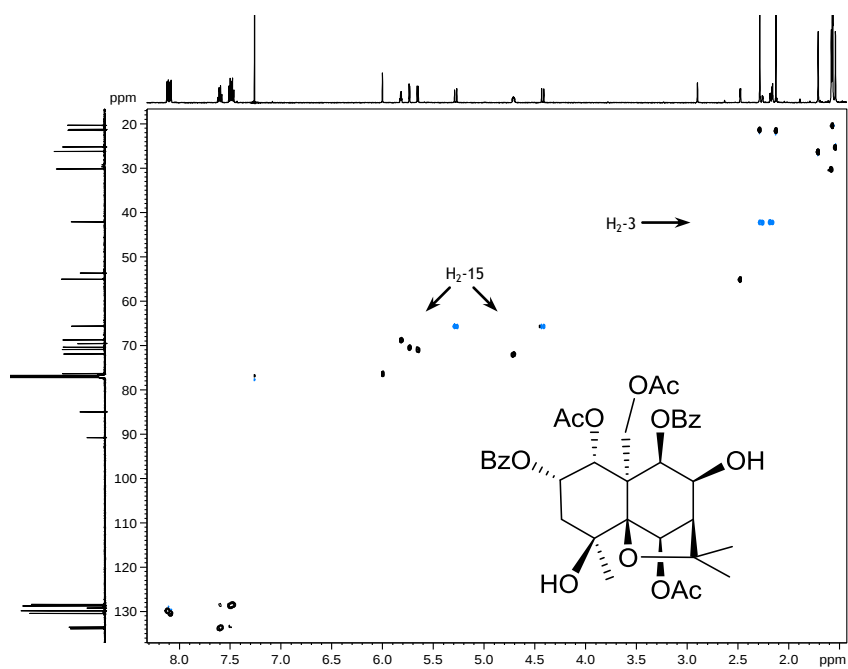
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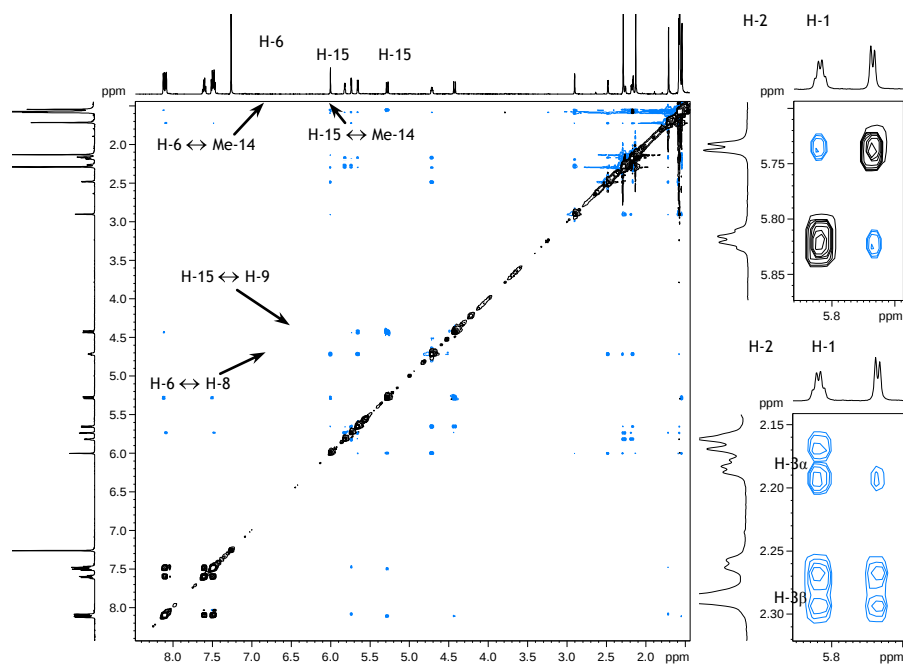
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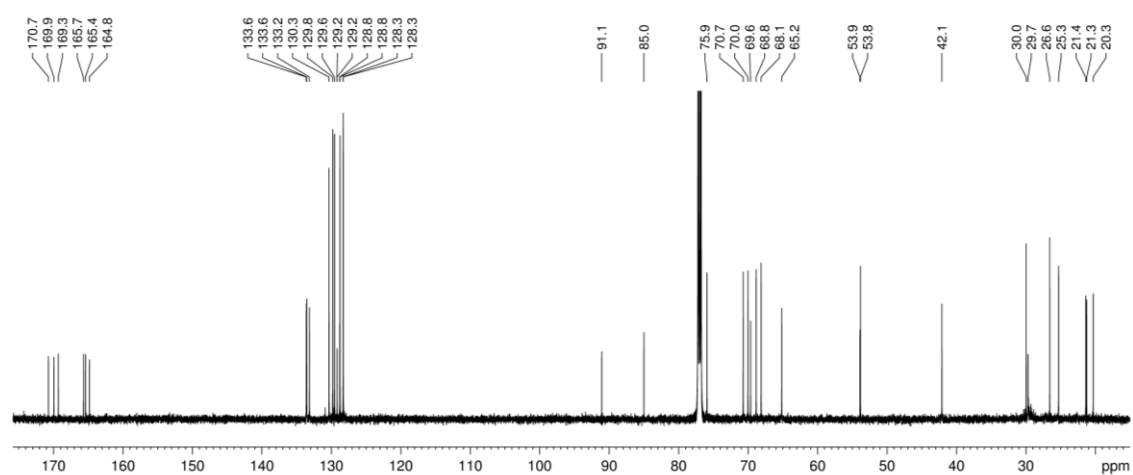
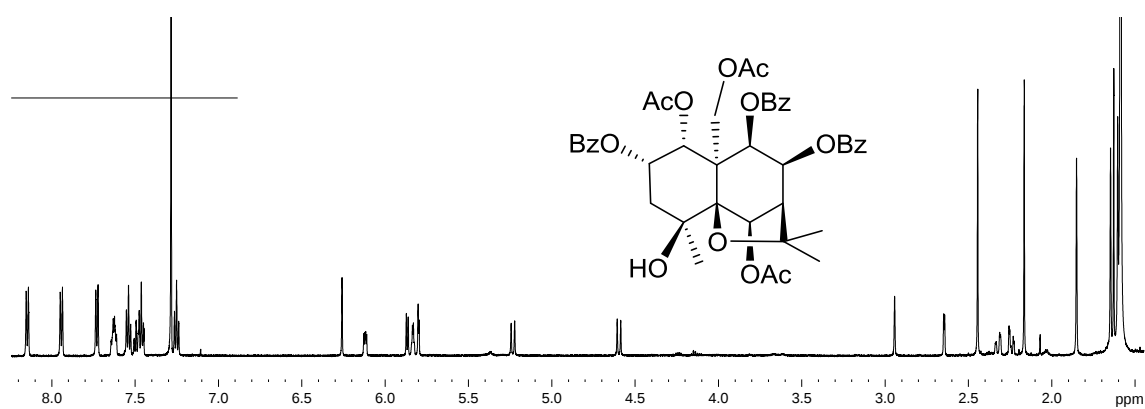
S3. ¹H and ¹³C NMR spectra of **1** in CDCl₃ (600 and 150 MHz, respectively).



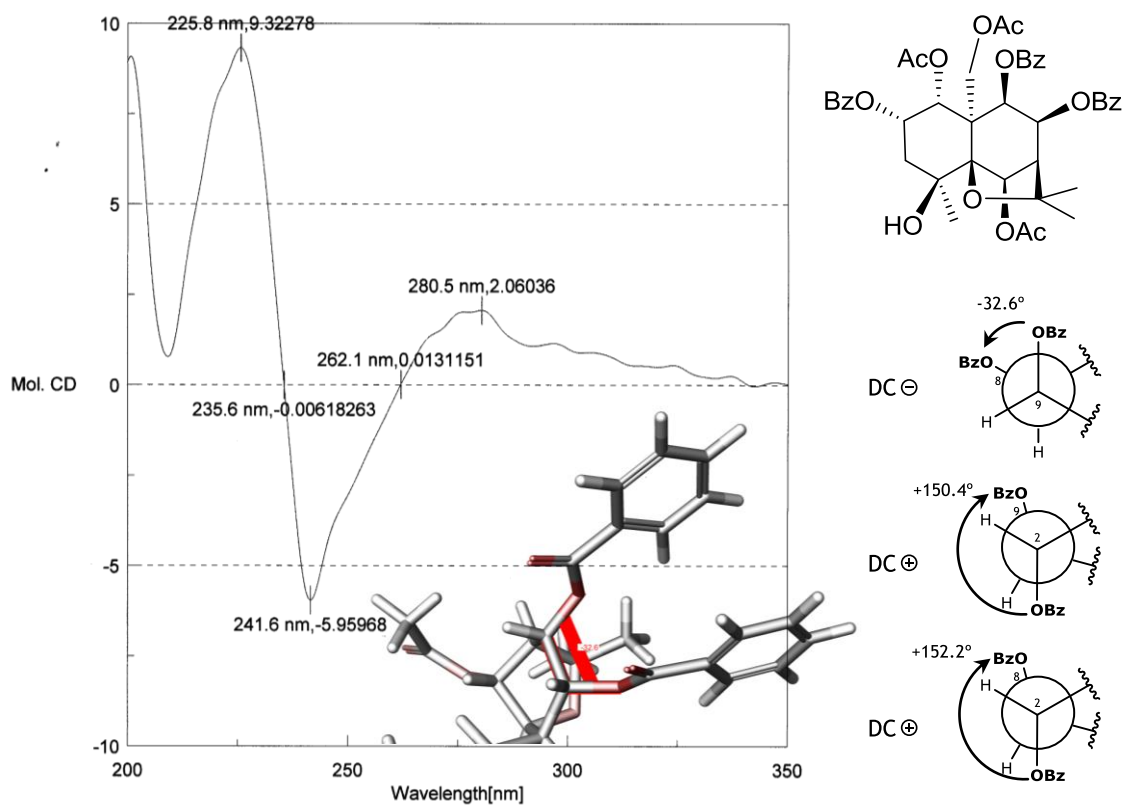
S4. HSQC and HMBC experiments (600 MHz, CDCl_3) of **1**.



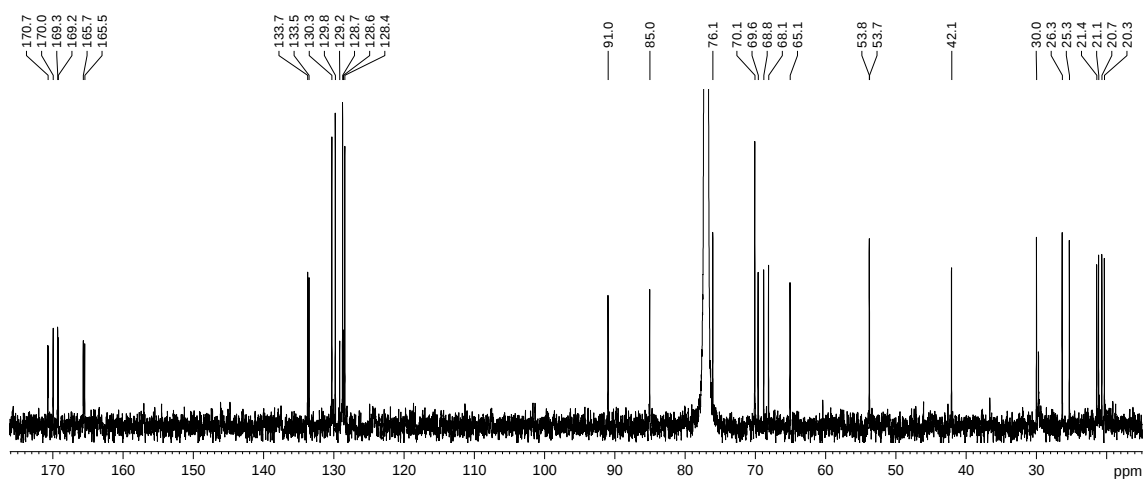
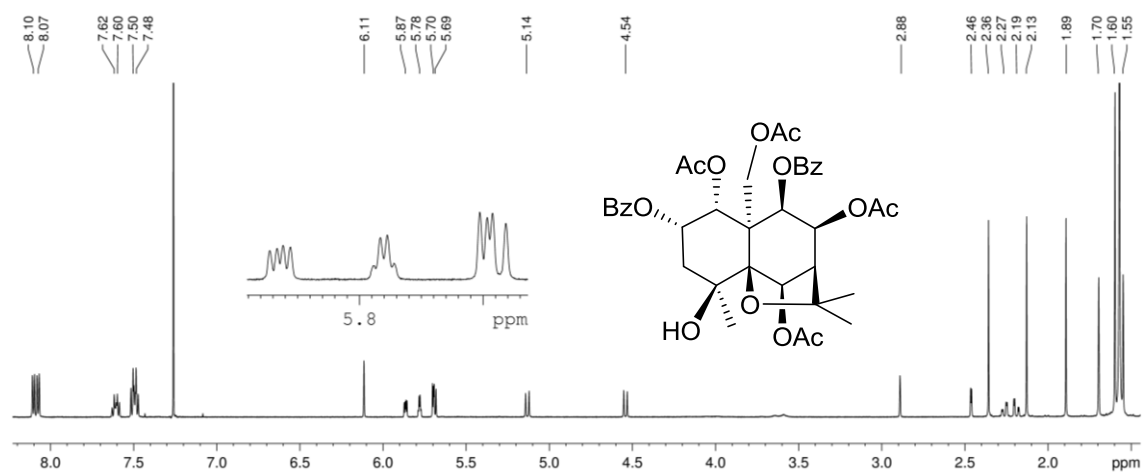
S5. ROESY experiment (600 MHz, CDCl_3) of **1**.



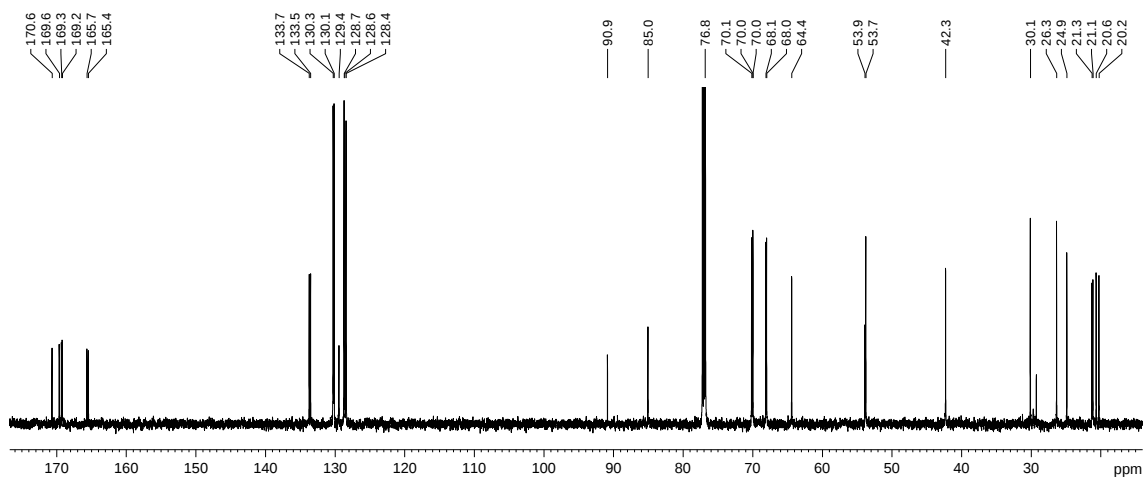
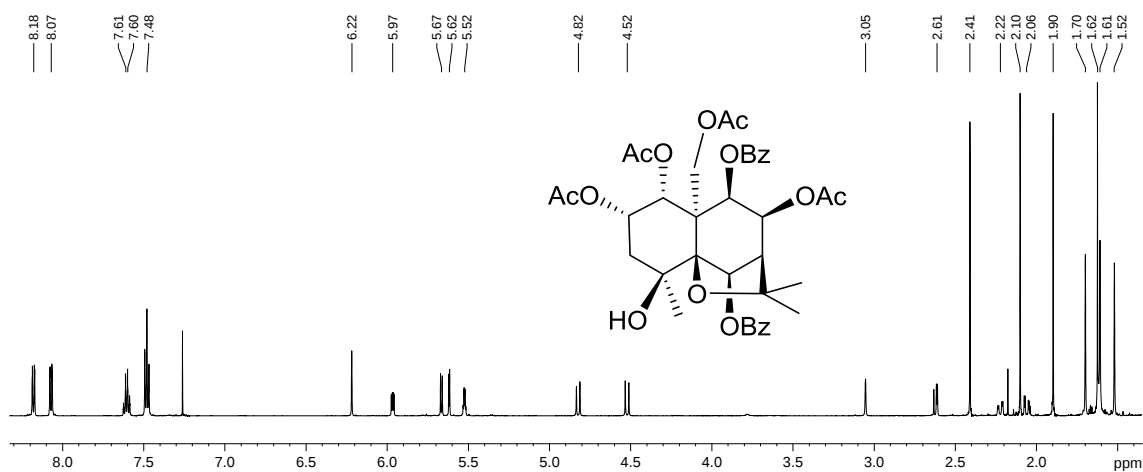
S6. ^1H and ^{13}C NMR spectra of derivative **1a** in CDCl_3 (600 and 150 MHz, respectively).



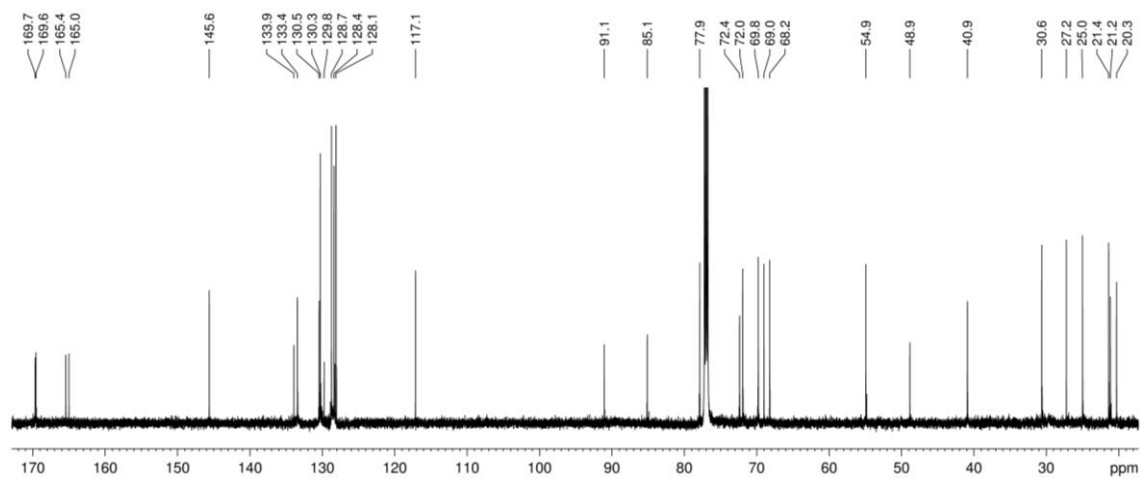
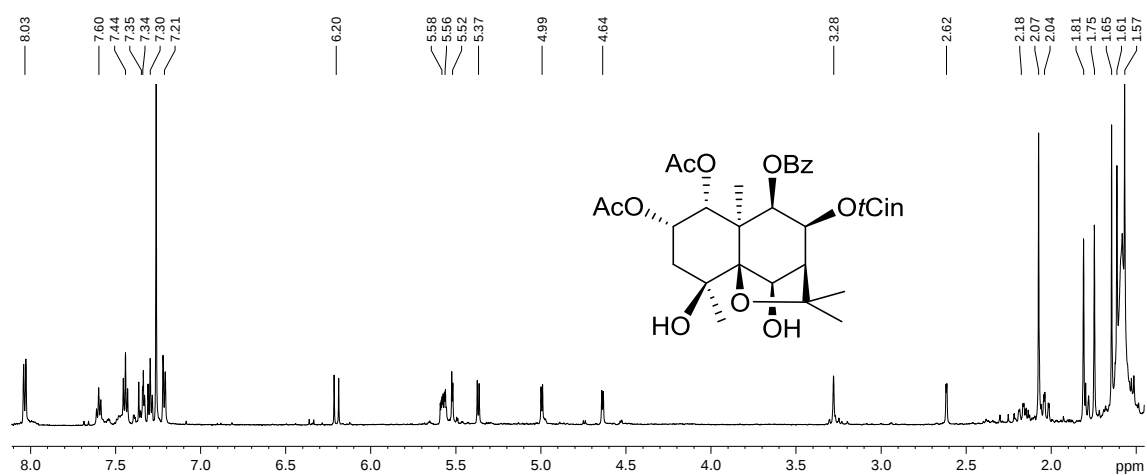
S7. CD spectrum of derivative **1a**.



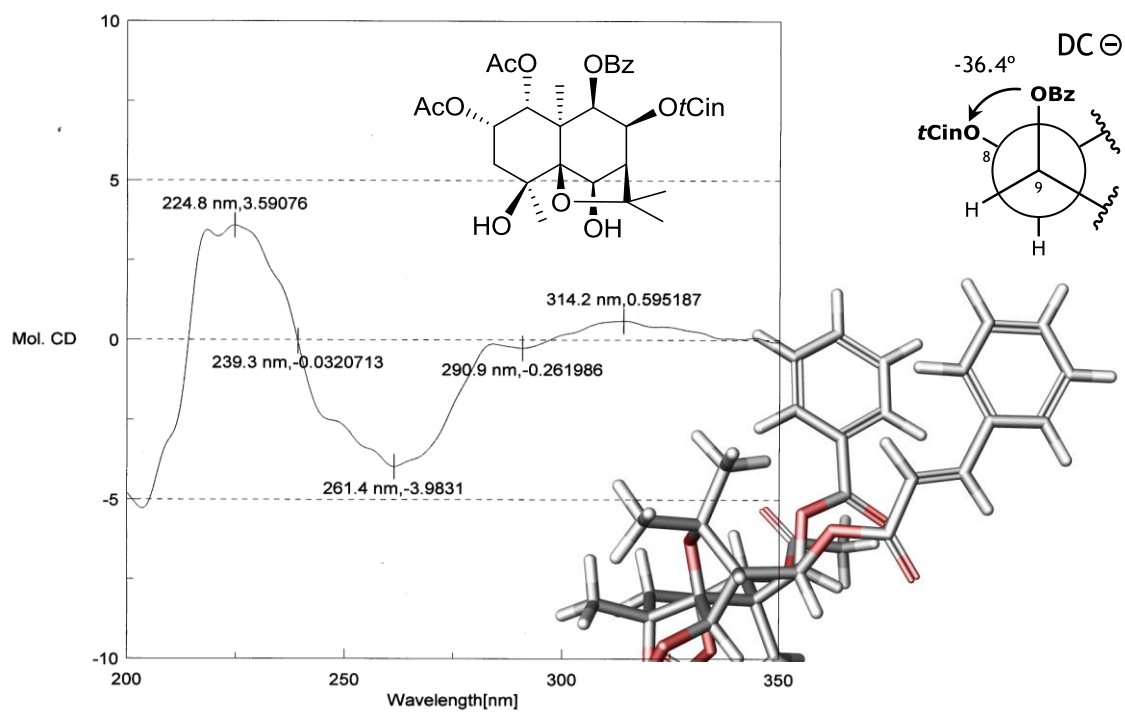
S8. ¹H and ¹³C NMR spectra of **2** in CDCl₃ (600 and 150 MHz, respectively).



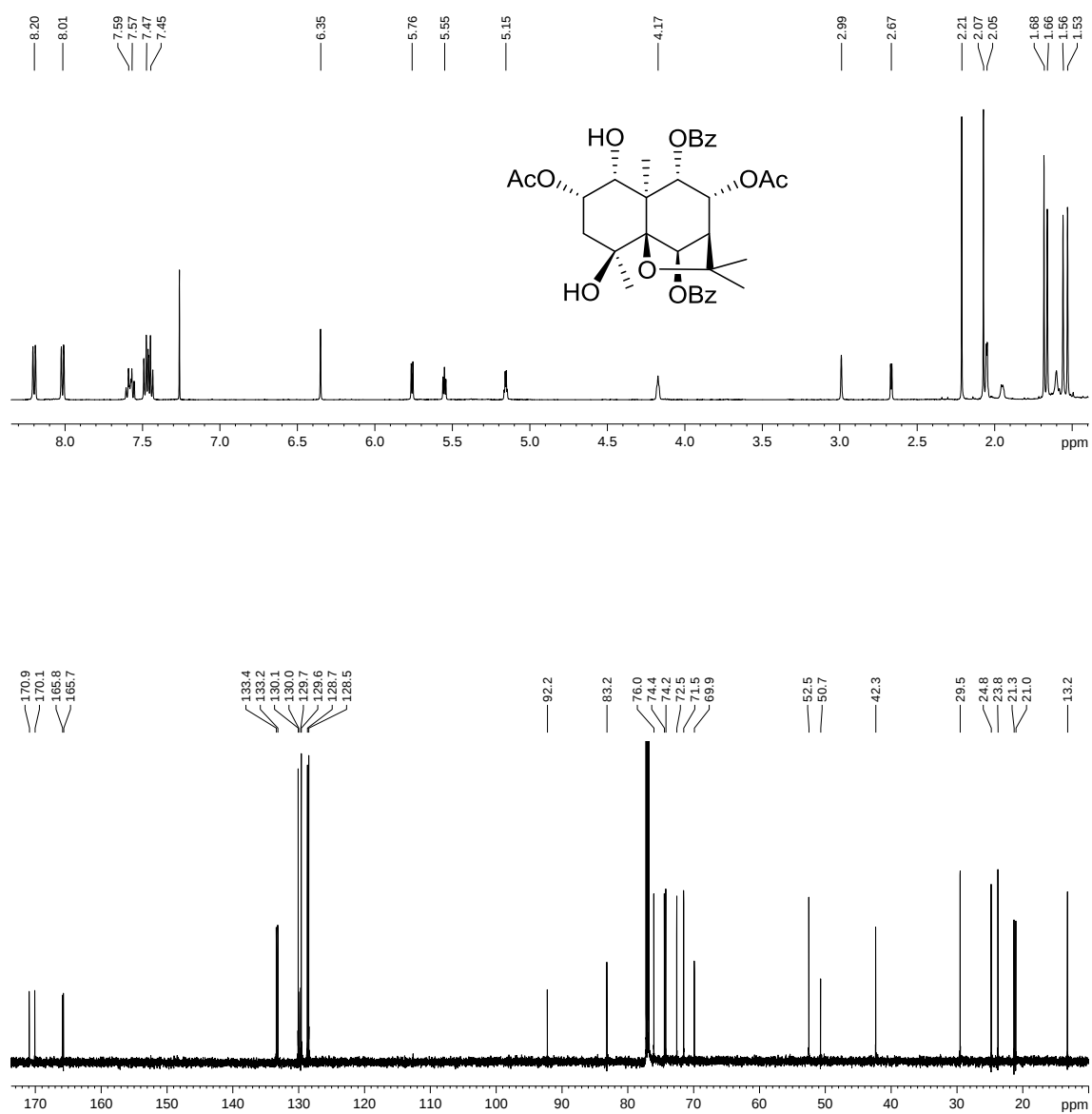
S9. ¹H and ¹³C NMR spectra of **3** in CDCl₃ (600 and 150 MHz, respectively).



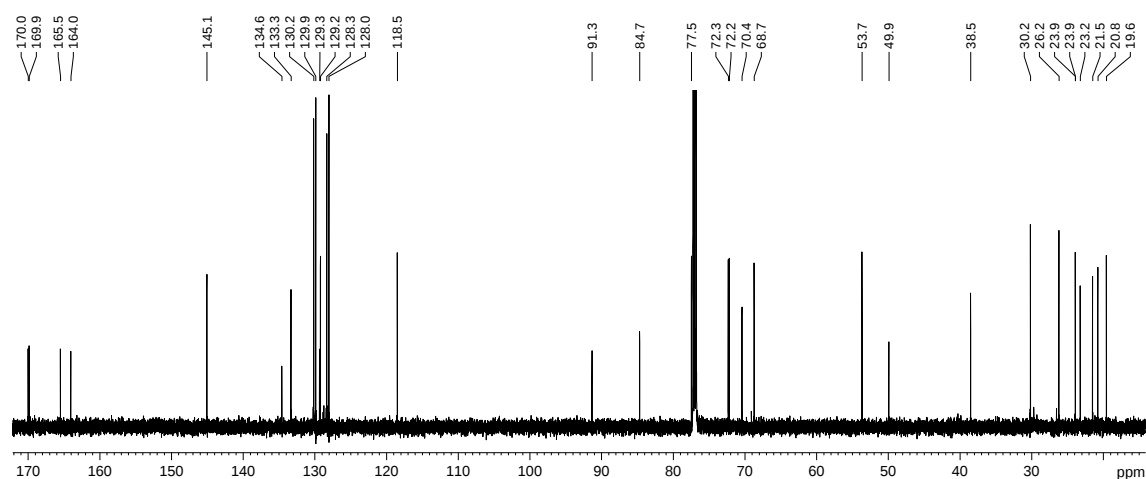
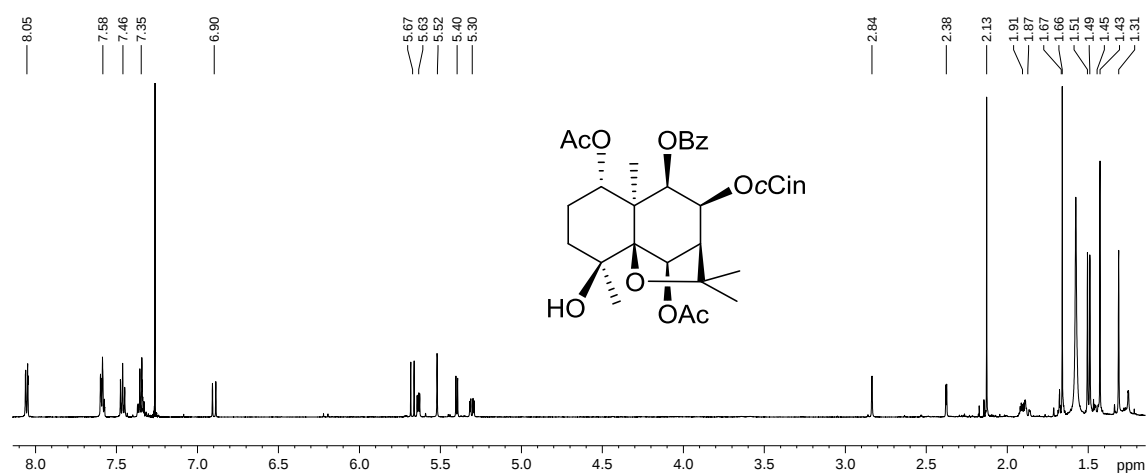
S9. ^1H and ^{13}C NMR spectra of **4** in CDCl_3 (600 and 150 MHz, respectively).



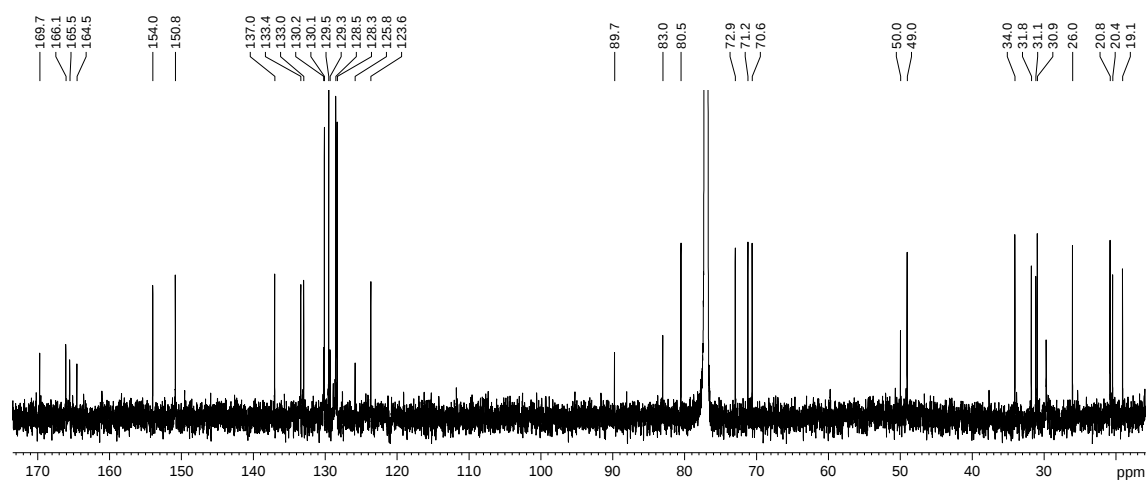
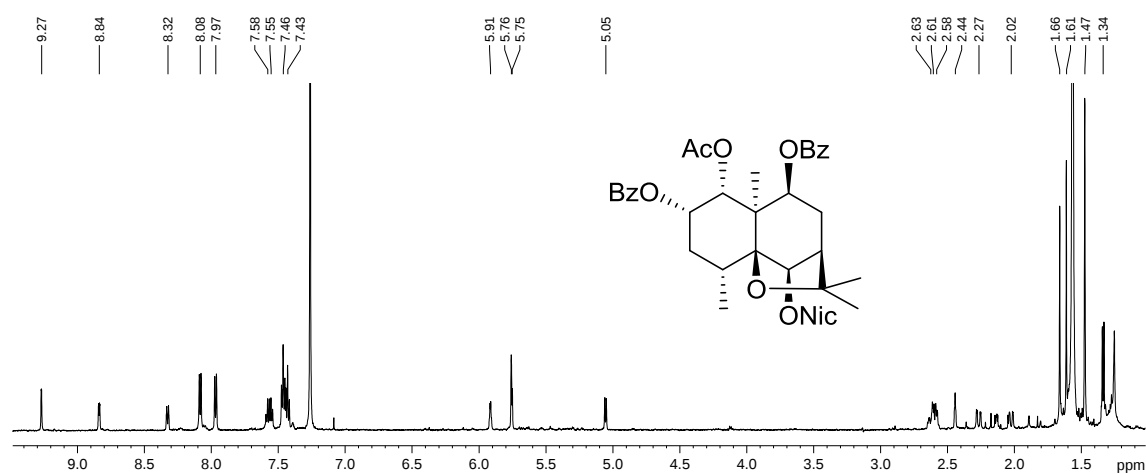
S11. CD spectrum of **4**.



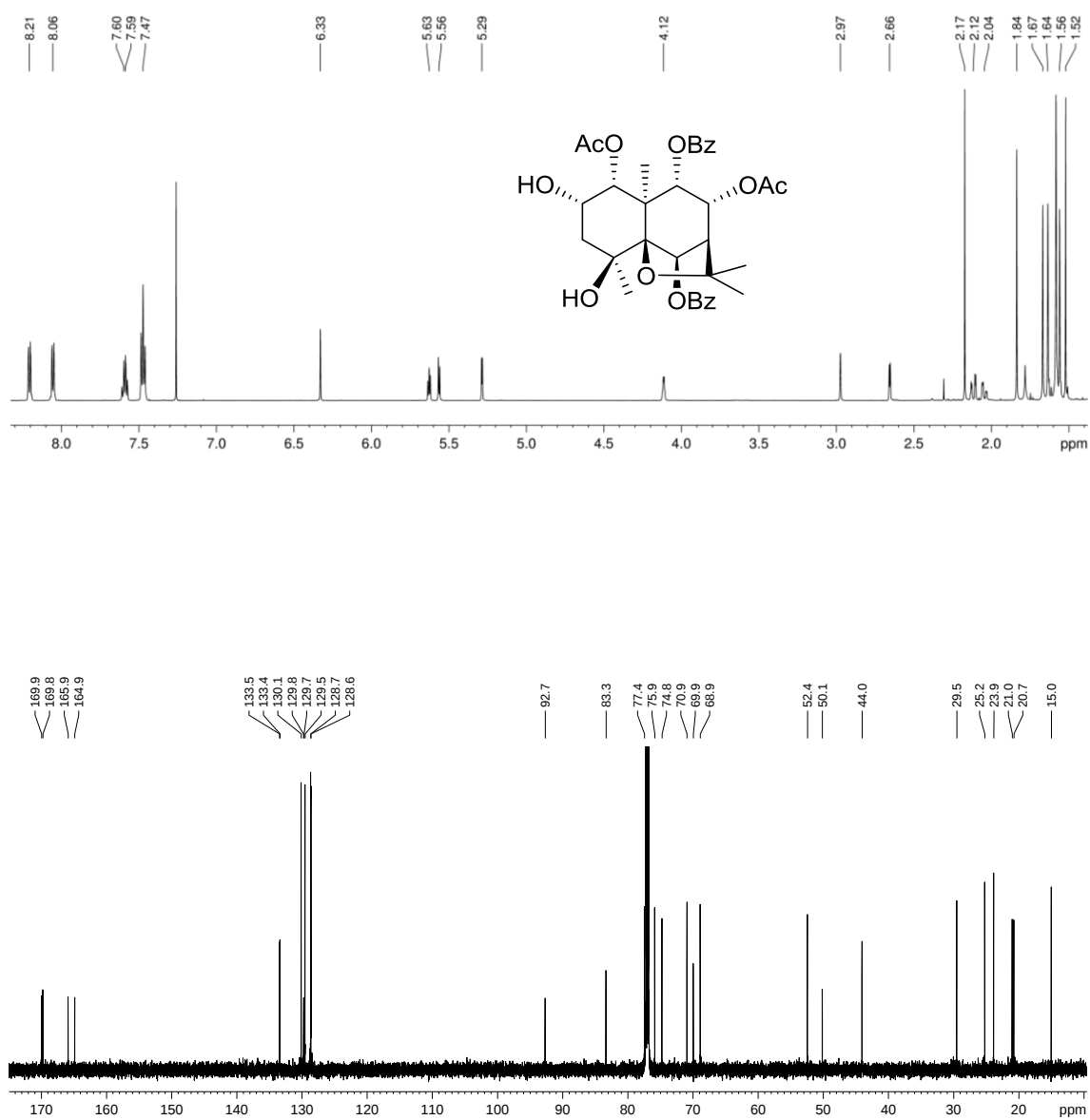
S12. ^1H and ^{13}C NMR spectra of **5** in CDCl_3 (500 and 150 MHz, respectively).



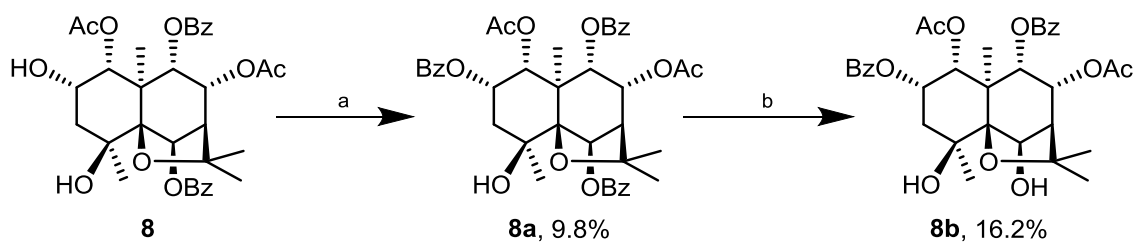
S13. ^1H and ^{13}C NMR spectra of **6** in CDCl_3 (600 and 150 MHz, respectively).



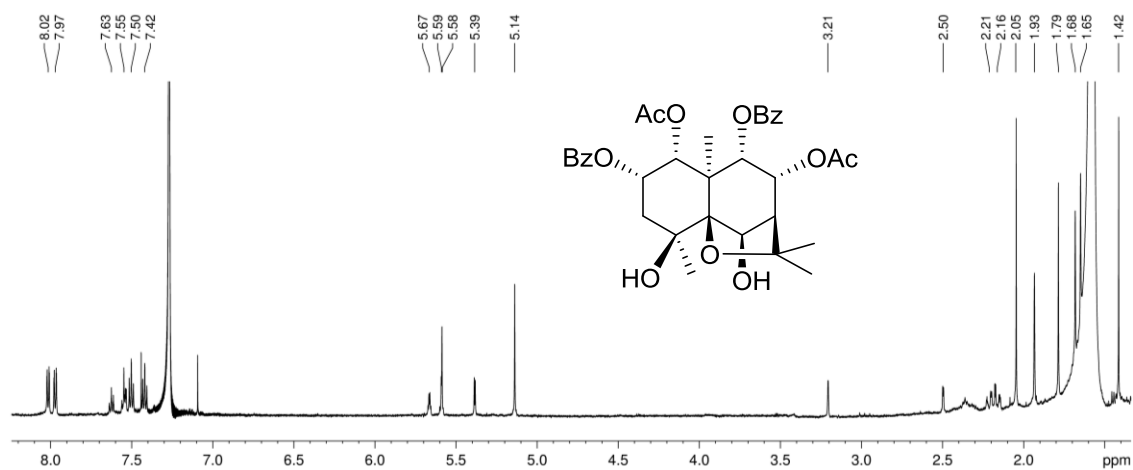
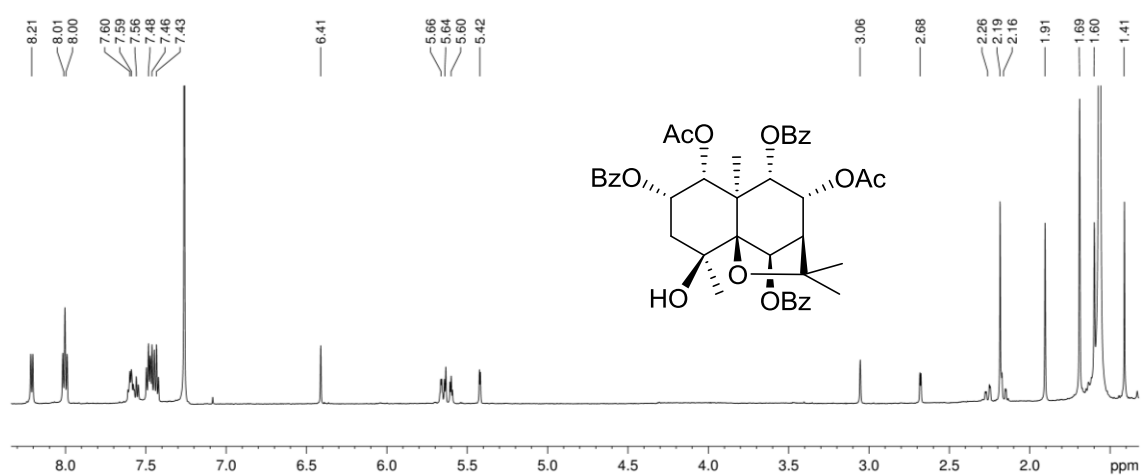
S14. ¹H and ¹³C NMR spectra of **7** in CDCl₃ (600 and 150 MHz, respectively).



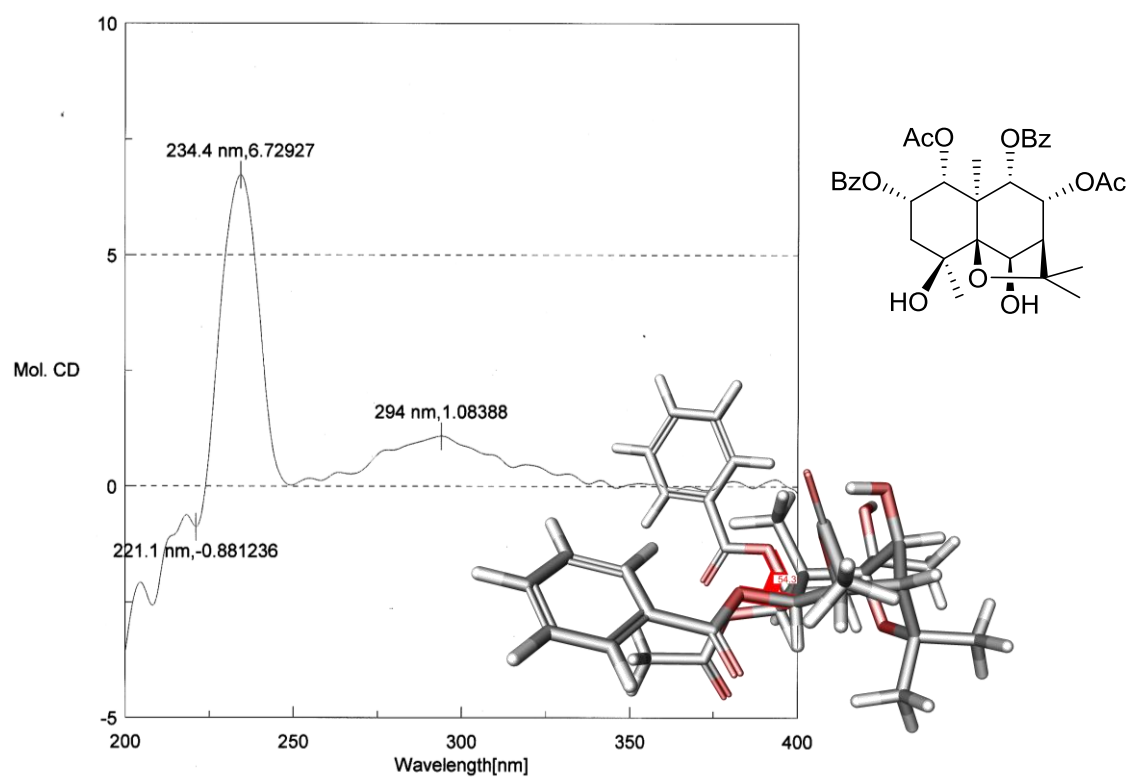
S15. ^1H and ^{13}C NMR spectra of **8** in CDCl_3 (500 and 150 MHz, respectively).



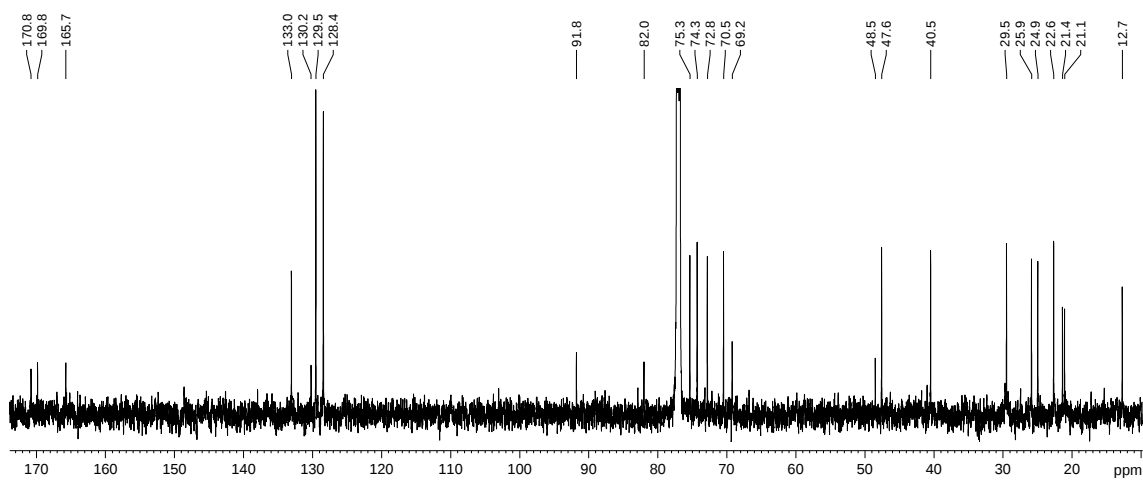
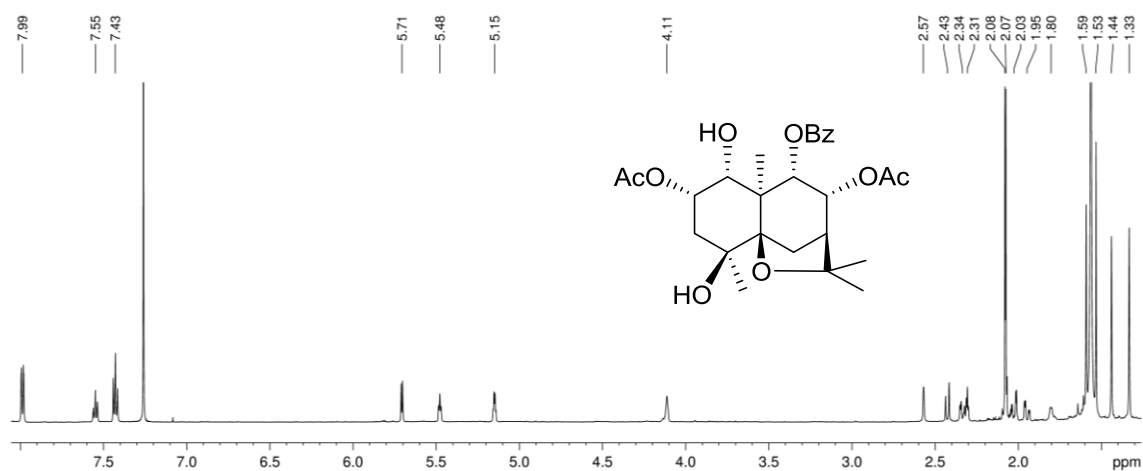
Reaction conditions: (a) BzCl, DMAP, Et₃N, DCM, 45 h, Ar. (b) NaHCO₃, MeOH/acetone (3:2), 40 °C, 47 h.



S16. ¹H NMR spectra of derivatives **8a** and **8b** in CDCl₃ (600 MHz).



S17. CD spectra of derivative **8b**.



S18. ^1H and ^{13}C NMR spectra of **9** in CDCl_3 (600 and 150 MHz, respectively).

S19. Inhibition of Pgp-mediated DNM transport across the cell membrane in intact MDR1 cells.^a

Compound	%DNM accumulation $\pm$ SD
1	73.2 $\pm$ 5.2
2	73.8 $\pm$ 1.4
3	79.7 $\pm$ 2.5
4	85.1 $\pm$ 8.1
5	73.1 $\pm$ 2.4
6	82.2 $\pm$ 3.3
7	80.1 $\pm$ 3.7
8	73.2 $\pm$ 1.5
9	37.0 $\pm$ 1.8
10	69.9 $\pm$ 2.4
11	73.6 $\pm$ 4.1
12	72.4 $\pm$ 2.5
13	68.2 $\pm$ 0.9
C	13.2 $\pm$ 1.1
V	60.3 $\pm$ 3.8

^a The cells were co-incubated with 2 μ M DNM and 10 μ M of sesquiterpenes, the DNM retention was determined by flow cytometry analysis as described in Biological Assays. DNM fluorescence intensity is expressed as relative fluorescence compared to a control (5 mM ortho-vanadate), representing 100% inhibition of Pgp. C = MDR1 cells incubated with DNM and without compounds. V = 10 μ M verapamil, a classical Pgp reversal agent used for comparison. Data are de mean $\pm$ S.D. of three independent experiments performed in triplicate.

S20. Drug resistance reversal ability of sesquiterpenes **3**, **4**, **6** and **7** in MDR1 cells^a

Compound	Reversion Index with DNM ^b			Reversion Index with VNB ^c		
	1 μ M	3 μ M	10 μ M	1 μ M	3 μ M	10 μ M
3	3.9 $\pm$ 0.7	12.7 $\pm$ 2.8	25.6 $\pm$ 4.3	16.4 $\pm$ 3.0	22.4 $\pm$ 2.5	70.3 $\pm$ 8.7
4	6.0 $\pm$ 1.6	16.4 $\pm$ 2.5	20.4 $\pm$ 5.5	12.8 $\pm$ 0.5	24.1 $\pm$ 4.2	87.1 $\pm$ 9.5
6	7.0 $\pm$ 1.8	24.0 $\pm$ 6.4	30.3 $\pm$ 6.5	12.1 $\pm$ 1.9	19.7 $\pm$ 3.1	77.0 $\pm$ 8.8
7	6.9 $\pm$ 2.6	13.1 $\pm$ 1.4	17.3 $\pm$ 2.8	21.7 $\pm$ 8.4	33.6 $\pm$ 5.4	54.9 $\pm$ 9.9
Verapamil	2.8 $\pm$ 0.2	6.2 $\pm$ 2.4	10.8 $\pm$ 2.1	8.7 $\pm$ 1.1	27.8 $\pm$ 2.6	129.9 $\pm$ 4.2

^a The reversal index is defined as the ratio between the IC₅₀ of cells without sesquiterpene and the IC₅₀ with sesquiterpene. IC₅₀ values were determined as described in the biological assays section. Results are expressed as the mean $\pm$ S.D. (p <0.05) of two independent experiments performed in triplicate. ^b The maximum reversal index with DNM (ratio between IC₅₀ for MDR1 and parental drug-sensitive cells) was 22. ^c The maximum reversal index with VNB (ratio between IC₅₀ for MDR1 and parental drug-sensitive cells) was 152.

S21. Intrinsic toxicity^a of sesquiterpenes **3**, **4**, **6** and **7** in parental and MDR1 cells.

Compound	MDR1 cells	Parental cells
3	71.4 ± 5.4	64.4 ± 5.2
4	78.4 ± 1.3	69.0 ± 2.0
6	64.3 ± 5.9	51.4 ± 4.4
7	70.2 ± 3.1	75.4 ± 6.4
Verapamil	80.8 ± 1.1	96.0 ± 3.6

^a Toxicity was determined using 10 μ M of compounds. Results are expressed as percentage mean of cell growth $\pm$ S.D. with respect to the control without compounds. Data are the mean of three independent experiments performed in triplicate.