Supplementary Material

(2R,4aS,6aS,12bR,14aS,14bR)-N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-10-hydroxy-2,4a,6a,9,12b,14a-hexamethyl-11-oxo-1,2,3,4,4a,5,6,6a,11,12b,13,14,14a,14b-tetradecahydropicene-2-carboxamide

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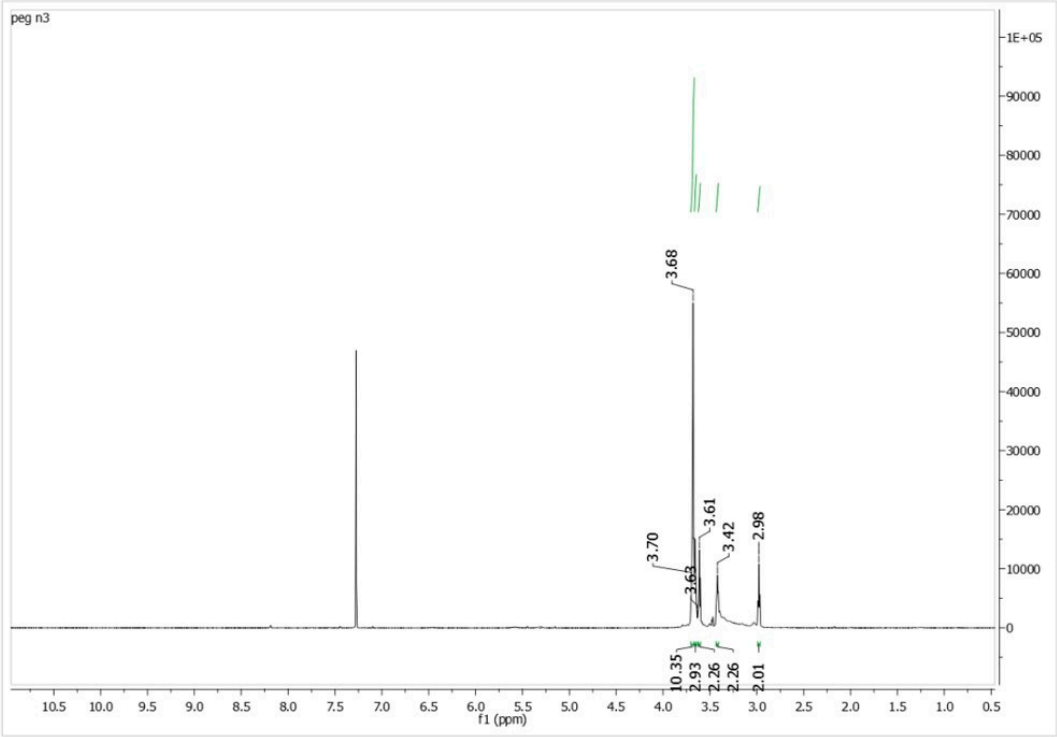
Contents

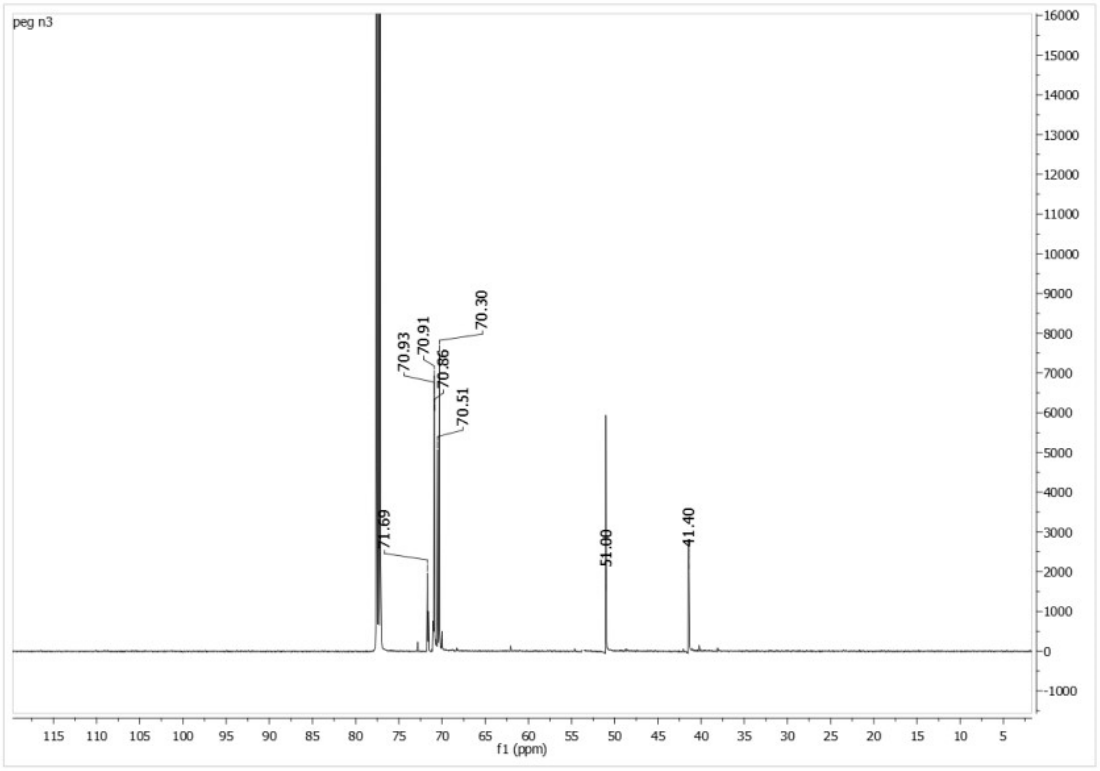
NMR spectrometry

Figure S1. ^{1}H-NMR spectrum ($CDCl_3$, 600 MHz) of compound **3**.



^{1}H-NMR (600 MHz $CDCl_3$) δ_H (600MHz, $CDCl_3$) 3.70-3.63 (10H, m, H3-H7), 3.61 (2H, t, H2), 3.42 (2H, m, H8), 2.98 (2H, t, H1)

Figure S2. ^{13}C-NMR spectrum ($CDCl_3$, 150 MHz) of compound **3**.



^{13}C-NMR (150 MHz $CDCl_3$) δ = 71.6 (C2), 70.9, 70.9, 70.8, 70.5, 70.3 (C3-C7), 51 (C8), 41.4 (C1).

Figure S3. DEPT-135 spectrum ($CDCl_3$, 150 MHz) of compound **3**.

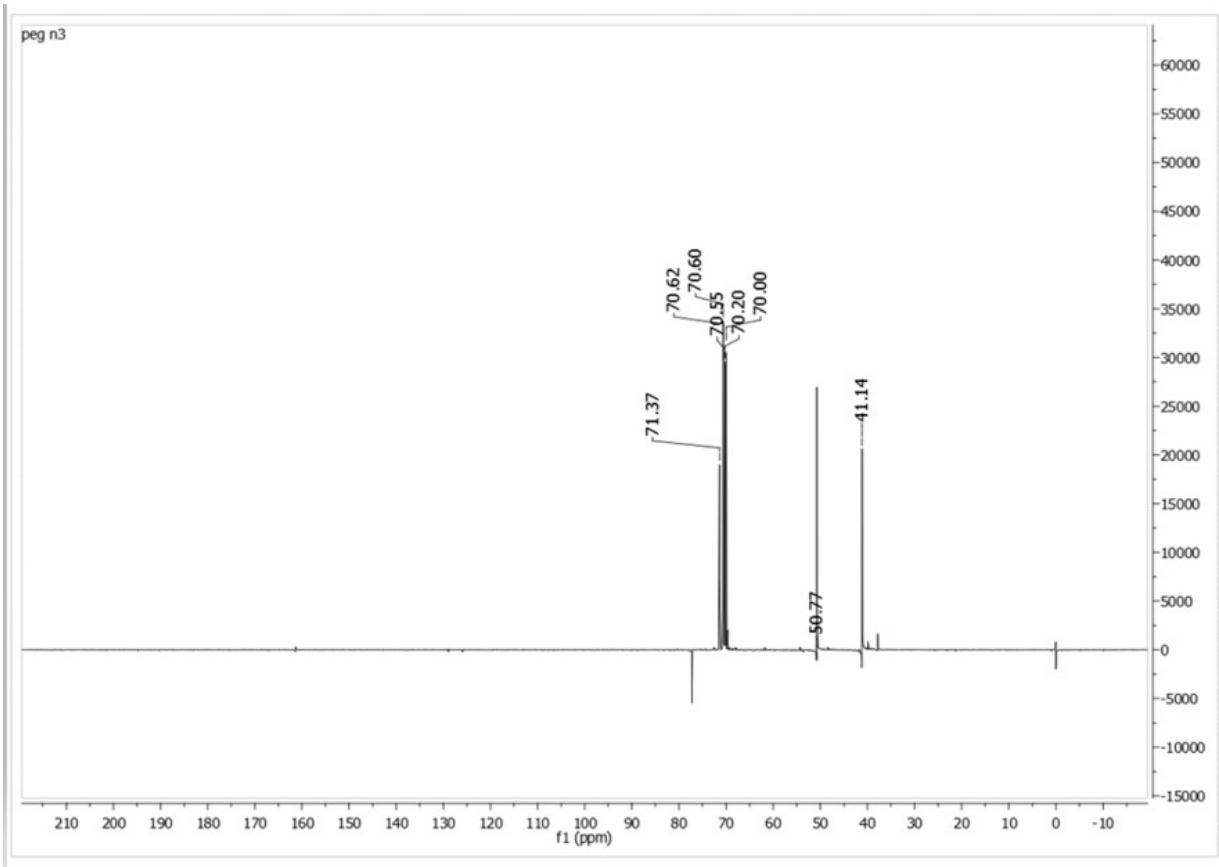
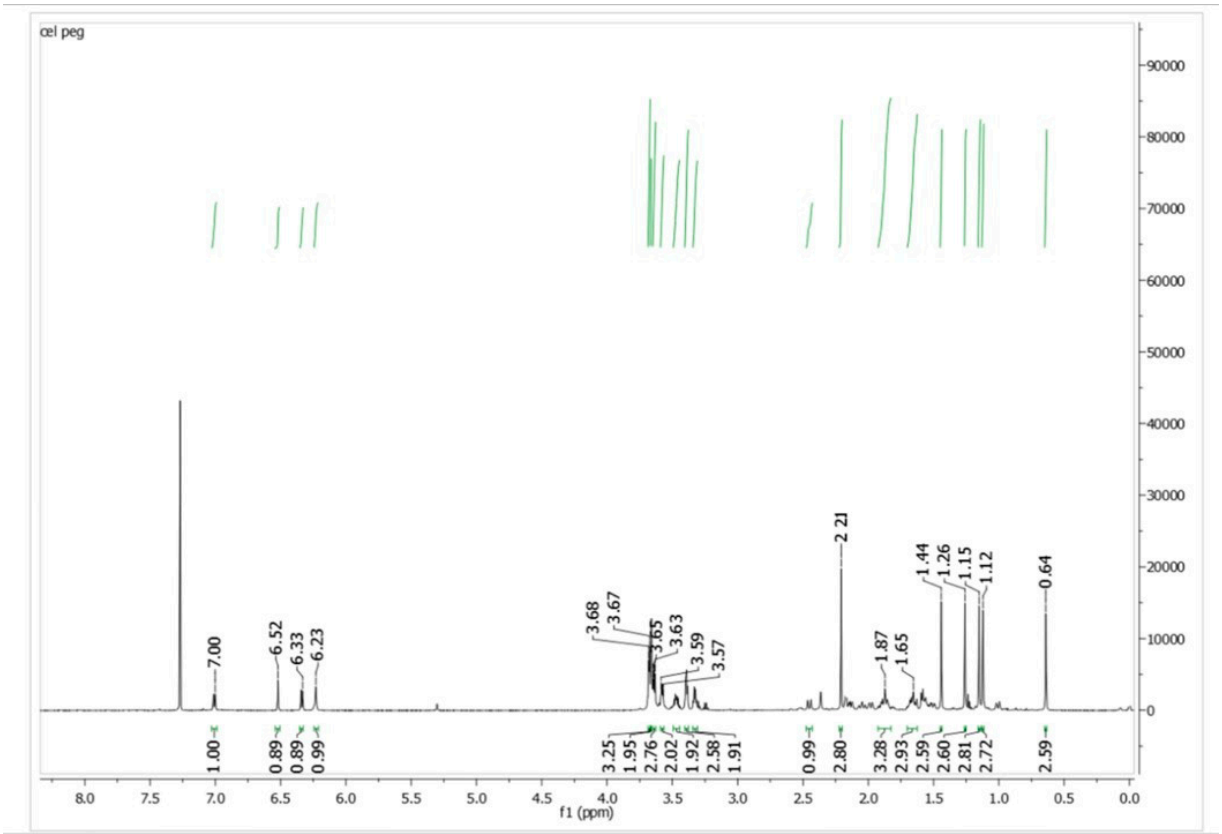
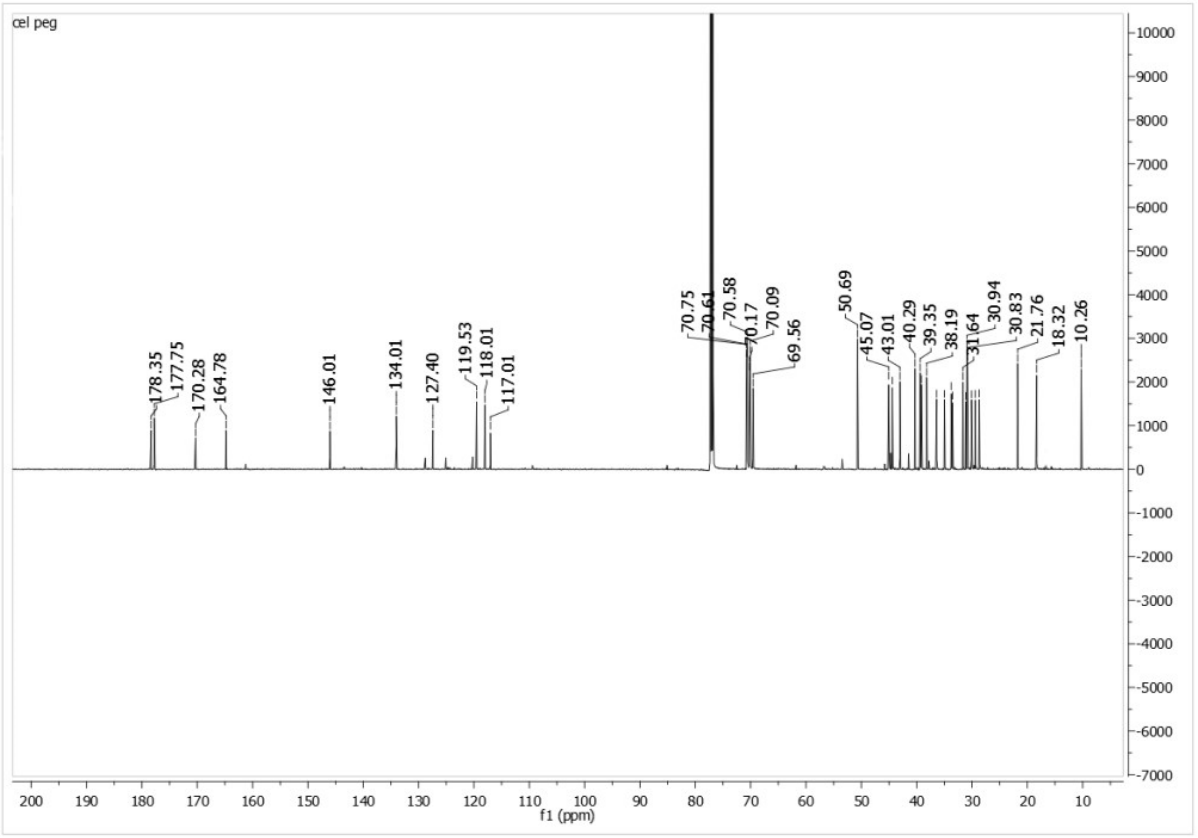


Figure S4. ^{1}H-NMR spectrum ($CDCl_3$, 600 MHz) of compound **4**.



δ_H (600MHz, $CDCl_3$) 0.64 (3H, s, CH_3), 0.99 (1H, d, H – 22), 1.12 (3H, s, CH_3), 1.15 (3H, s, CH_3), 1.26 (3H, s, CH_3), 1.44 (3H, s, CH_3), 1.49 – 1.70 (7H, m), 1.82 – 1.92 (4H, m), 1.94 – 2.15 (3H, m), 2.21 (3H, s, CH_3), 2.44 (1H, t, H – 19), 3.29 – 3.34 (2H, m), 3.39 (2H, t, CH_2), 3.44 – 3.50 (2H, m, CH_2), 3.57 – 3.59 (2H, m, CH_2), 3.63 – 3.68 (8H, m, CH_2), 6.23 (1H, br, NH), 6.33 (1H, d, H-7), 6.52 (1H, s, H-1), 7.00 (1H, d, H-6) ppm.

Figure S5. ^{13}C-NMR spectrum ($CDCl_3$, 600 MHz) of compound **4**.



δ_C (150MHz, $CDCl_3$) 10.2 (CH_3), 18.3 (CH_3), 21.7 (CH_3), 28.6, 29.4, 30.0, 30.9, 31.1, 31.6, 33.5, 33.7, 34.9, 36.4, 38.1, 39.1, 39.3, 40.2, 43.0, 44.4, 45.0, 50.6, 69.5, 70.0, 70.1, 70.5, 70.6, 70.7, 117.0, 118.0, 119.5, 127.4, 134.0 146.0, 164.7, 170.2, 177.7, 178.3 ppm).

Impurity CH_2Cl_2 52 ppm; acetone 30.8 ppm.

Figure S6a. HSQC spectrum ($CDCl_3$, 600 MHz) of compound **4**.

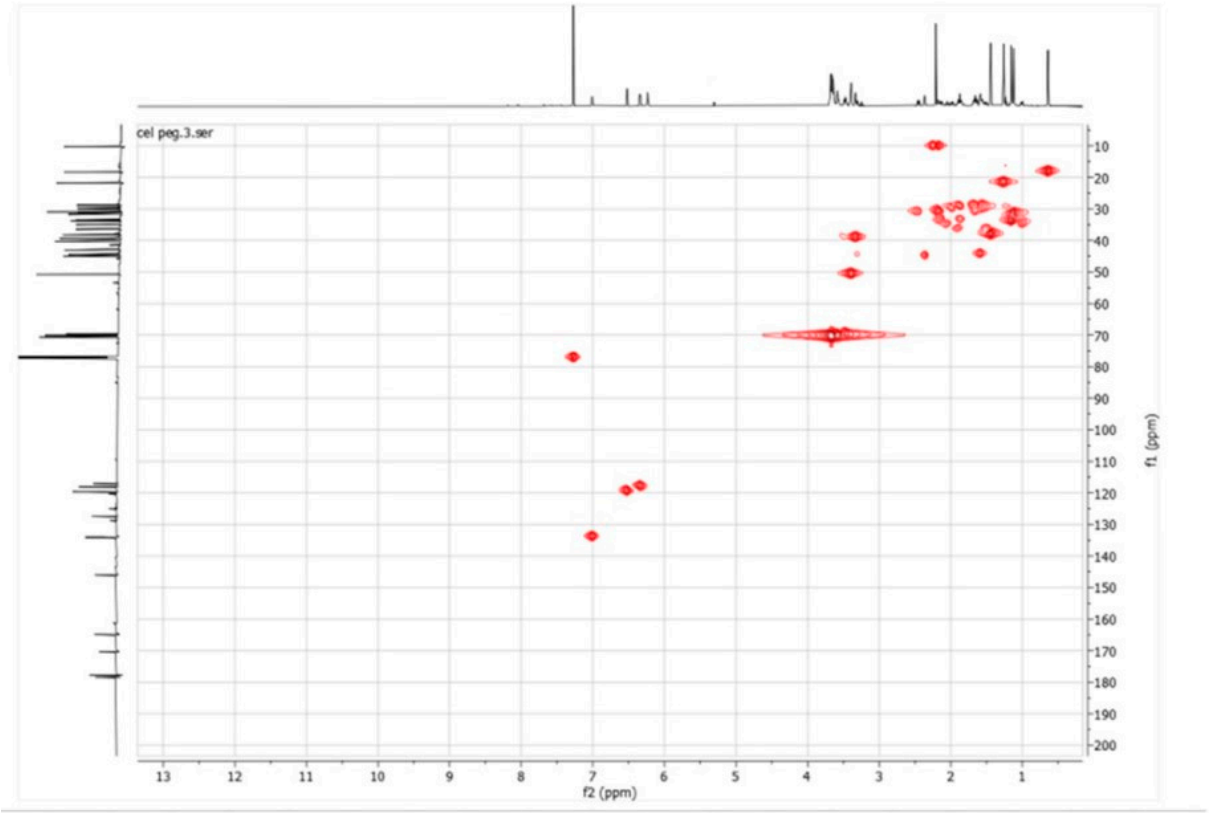


Figure S6b. An expanded view of HSQC spectrum ($CDCl_3$, 600 MHz) of compound **4**.

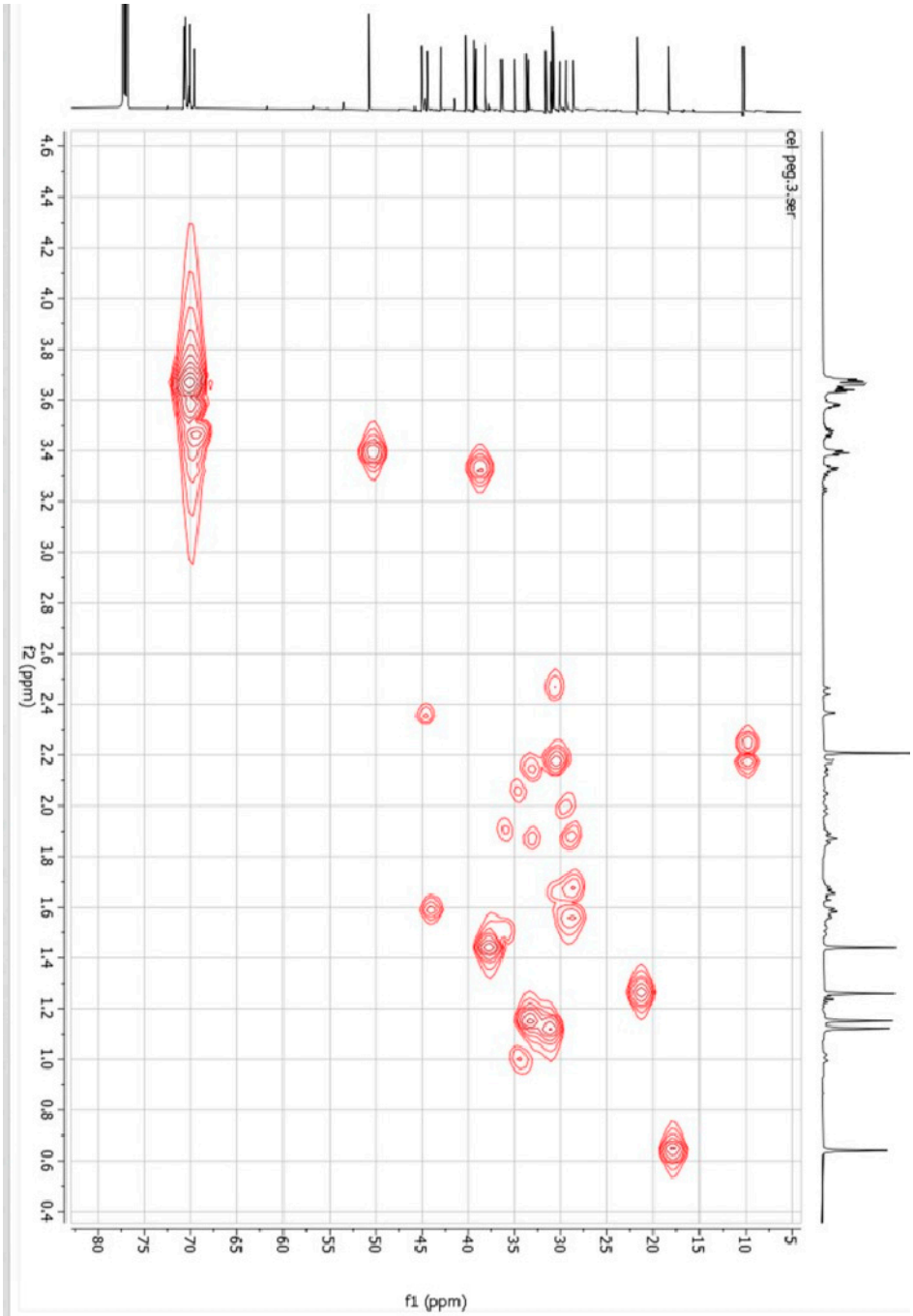


Figure S7. ^{1}H-COSY spectrum ($CDCl_3$, 600 MHz) of compound **4**.

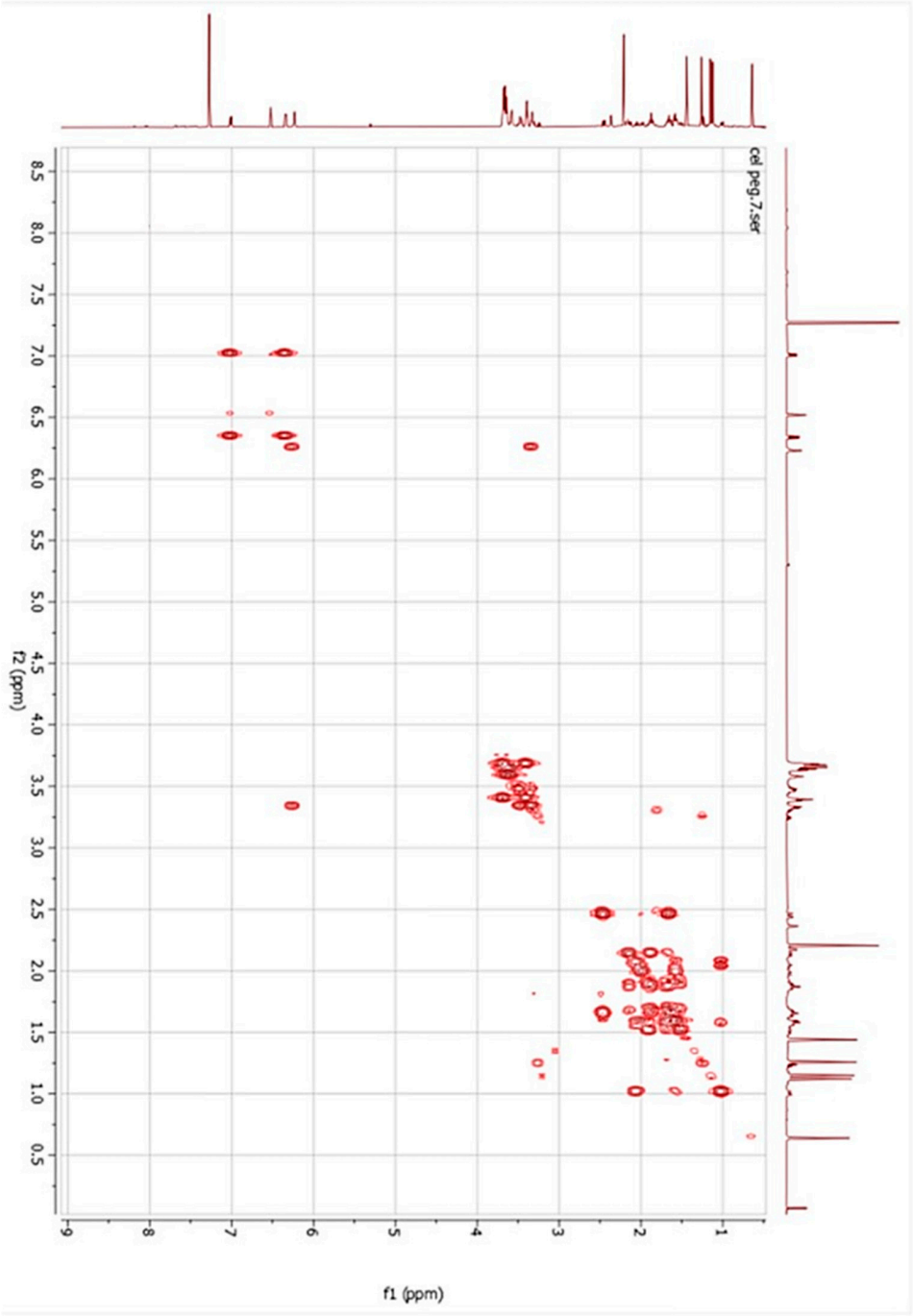
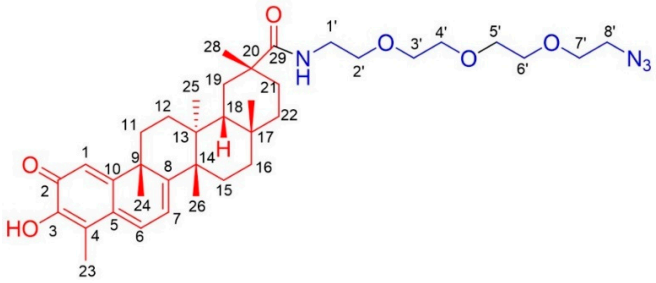


Table S1. ^{1}H and ^{13}C-NMR chemical shifts and 2D structure of **4**.



^{1}H Chemical Shift	^{13}C Chemical Shift	Assignment
2,21	10.26	(CH_3)
0.64	18.3	(CH_3)
1.12	31.6	(CH_3)
1.15	33.7	(CH_3)
1.26	21.7	(CH_3)
1.44	38.1	(CH_3)
1.87	28.6	(CH_2)
1.99	29.4	(CH_2)
2.16	30	(CH_2)
--	30.9	(C)
1.49-2.15(2H)	31.1	(CH_2)
0.99	33.5	C-22(CH_2)
1.49-2.15(2H)	34.9	(CH_2)
1.49-2.15(2H)	36.4	(CH_2)
3.29-3.34	39.1	(NCH_2)
--	39.3	(C)
--	40.2	(C)
--	43.0	(C)
1.58	44.4	C-18(CH)
--	45.0	(C)
3.39	50.6	(N_3CH_2)
6.23		NH
3,44-3.5	69.5	(OCH_2)
3.57-3.59	70	(OCH_2)
3.63-3.68 (8H)	70.1	(OCH_2)
	70.5	(OCH_2)
	70.6	(OCH_2)
	70.7	(OCH_2)
--	117	(C)
6.33	118	C-7 (CH)
6.52	119.5	C-1 (CH)
--	127.4	(C)
7.00	134.0	C-6(CH)
--	146.0	(C)
--	164.7	C-10(C)
--	170.2	C-8 (C)
--	177.7	C-2 (C)
--	178.3	C-29 (C)

Figure S8. DEPT-135 spectrum ($CDCl_3$, 150 MHz) of compound **4**.

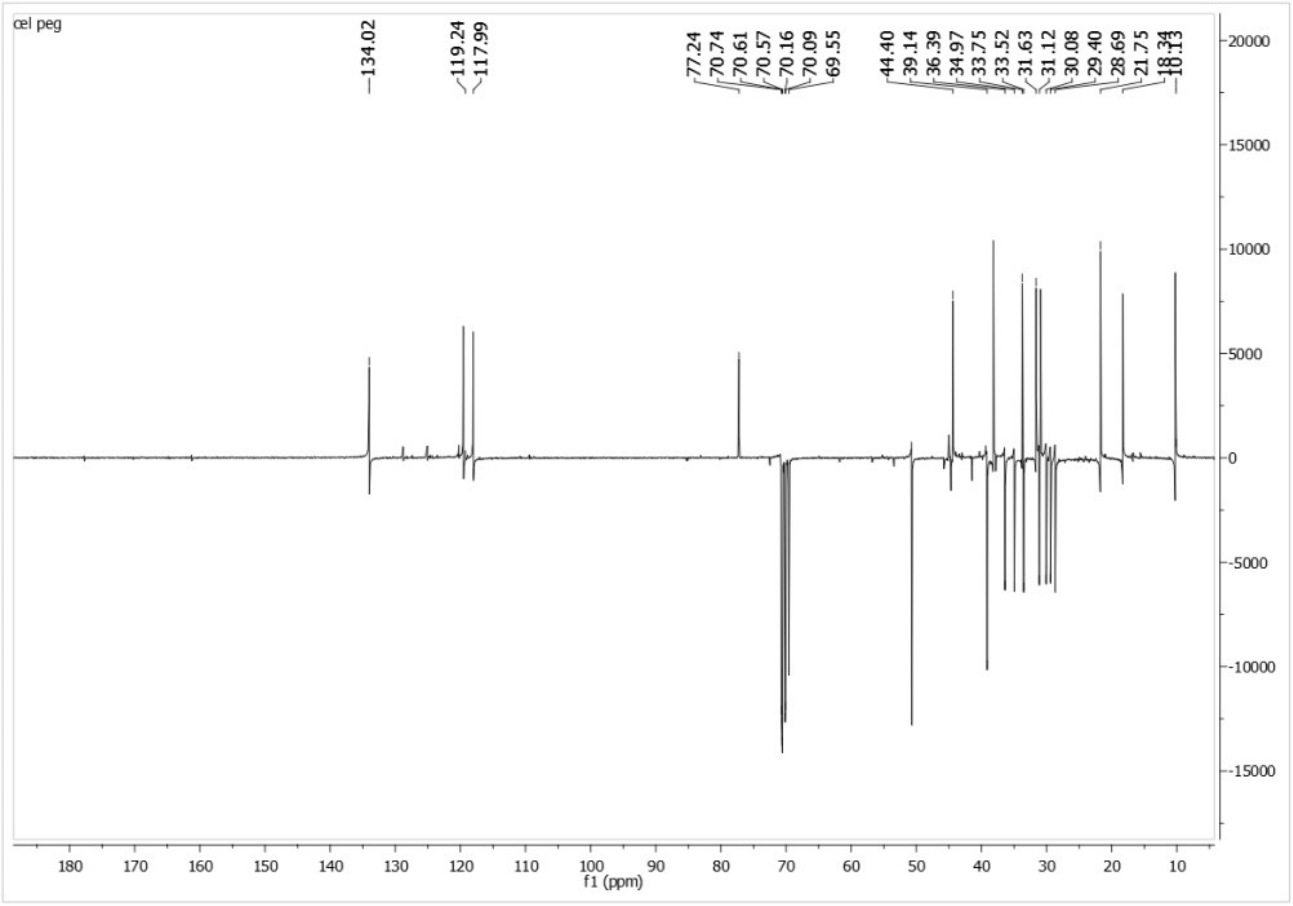


Figure S9. DEPT-90 spectrum ($CDCl_3$, 150 MHz) of compound **4**.

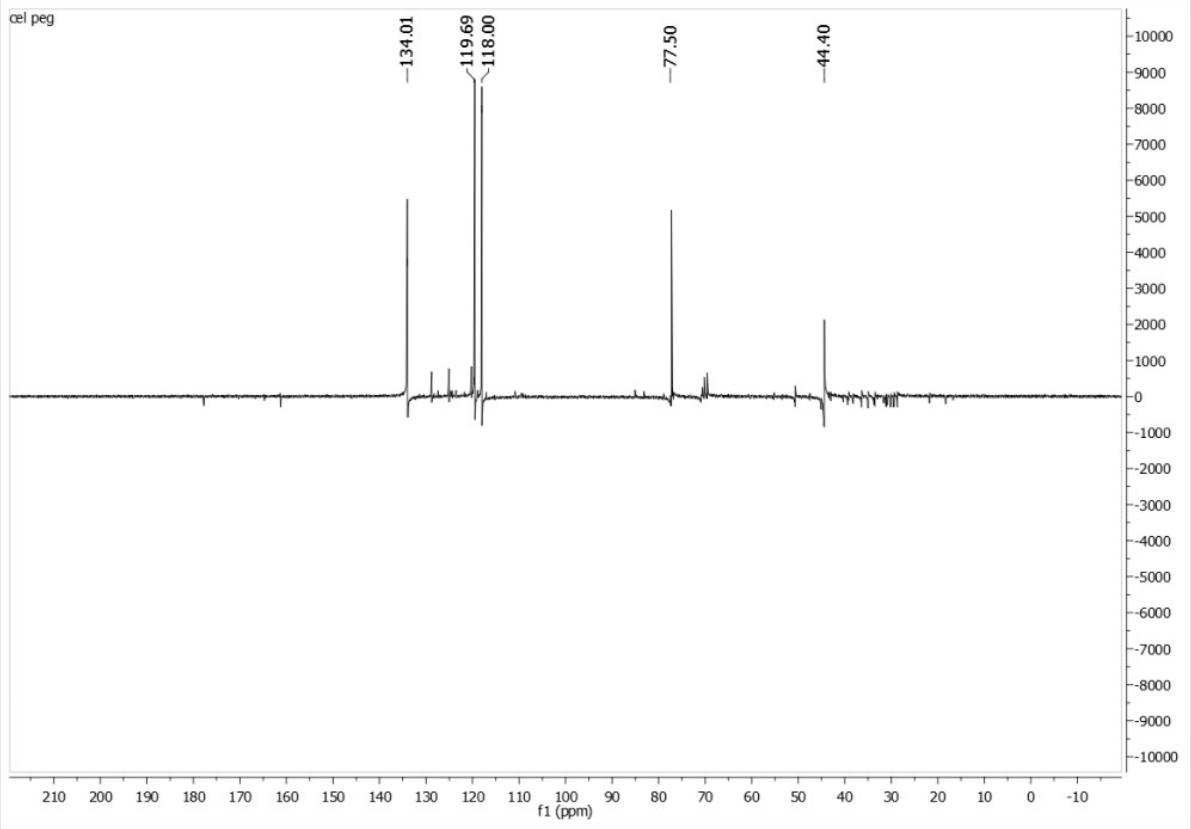
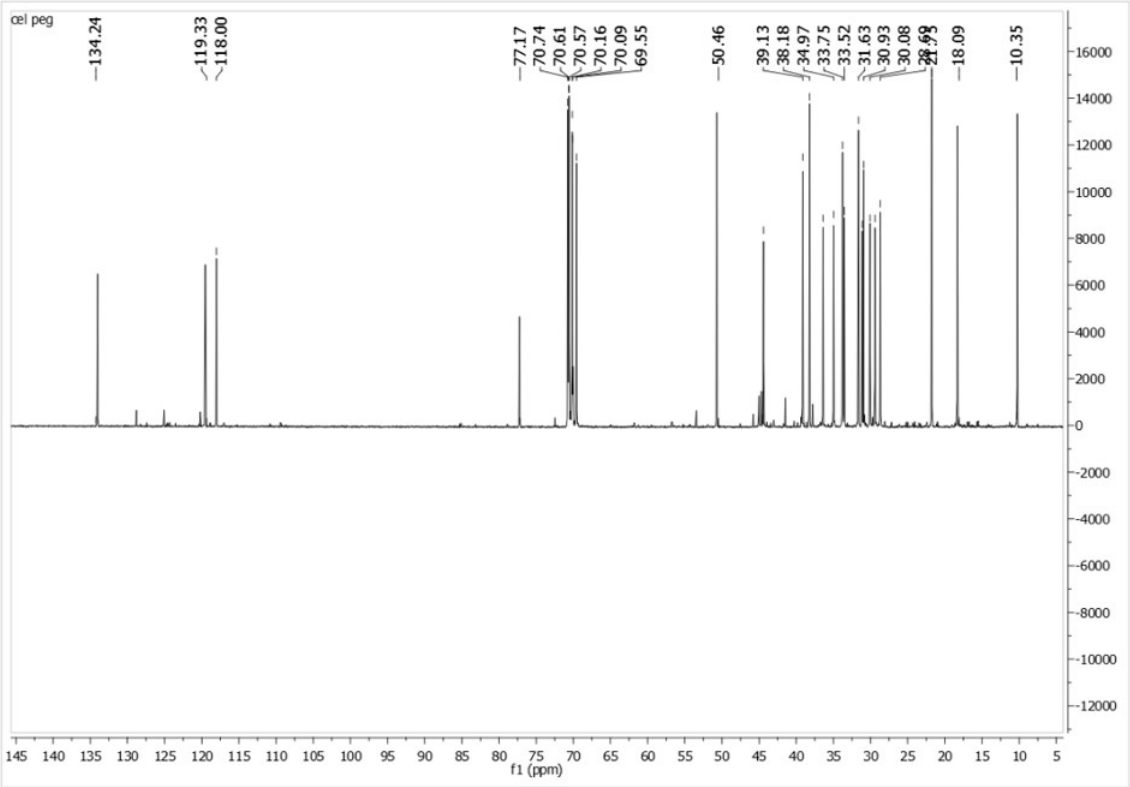


Figure S10. DEPT-45 spectrum ($CDCl_3$, 150 MHz) of compound **4**.



IR spectroscopy

Figure S11. IR spectrum (KBr) of compound **1**.

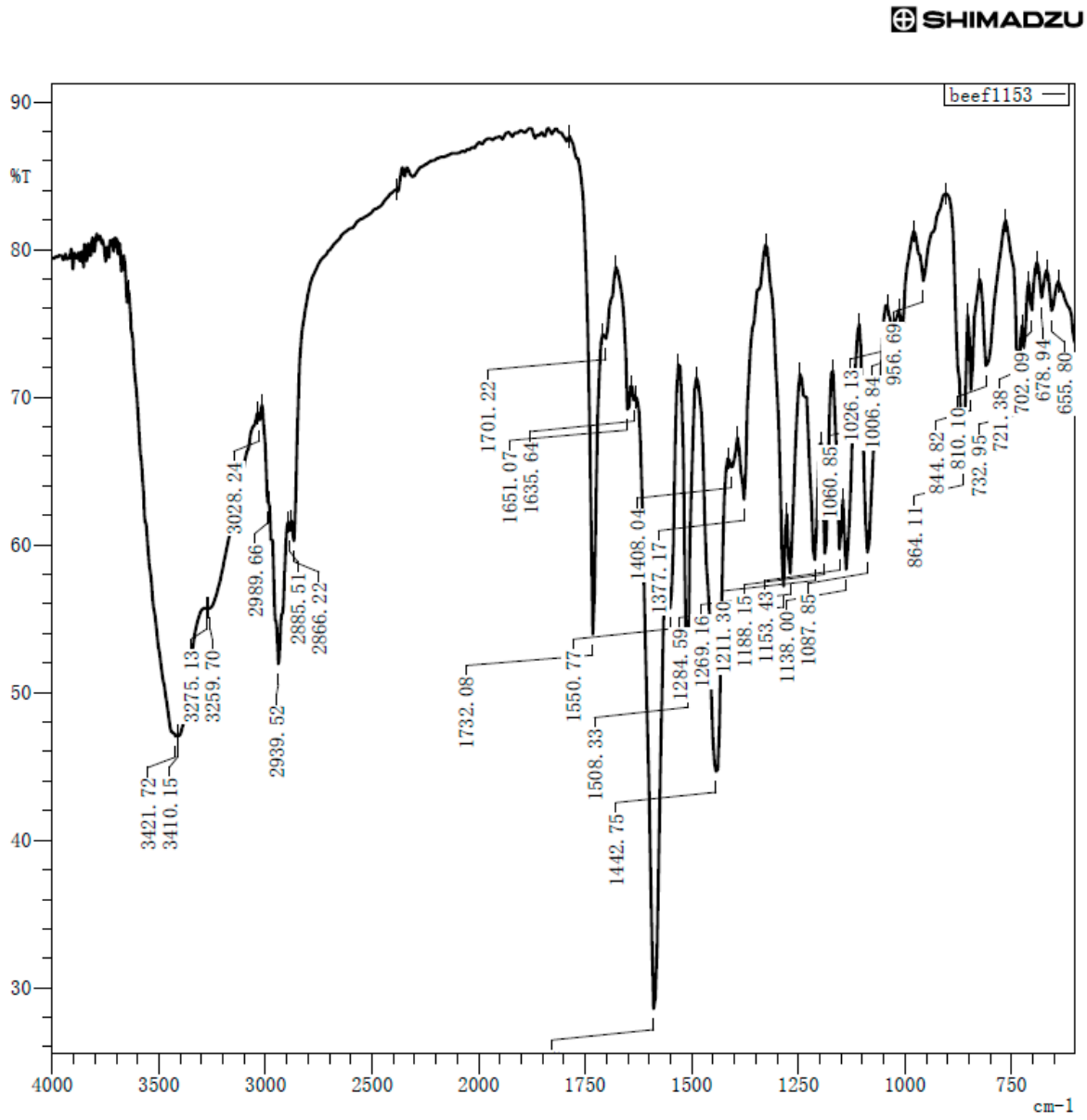
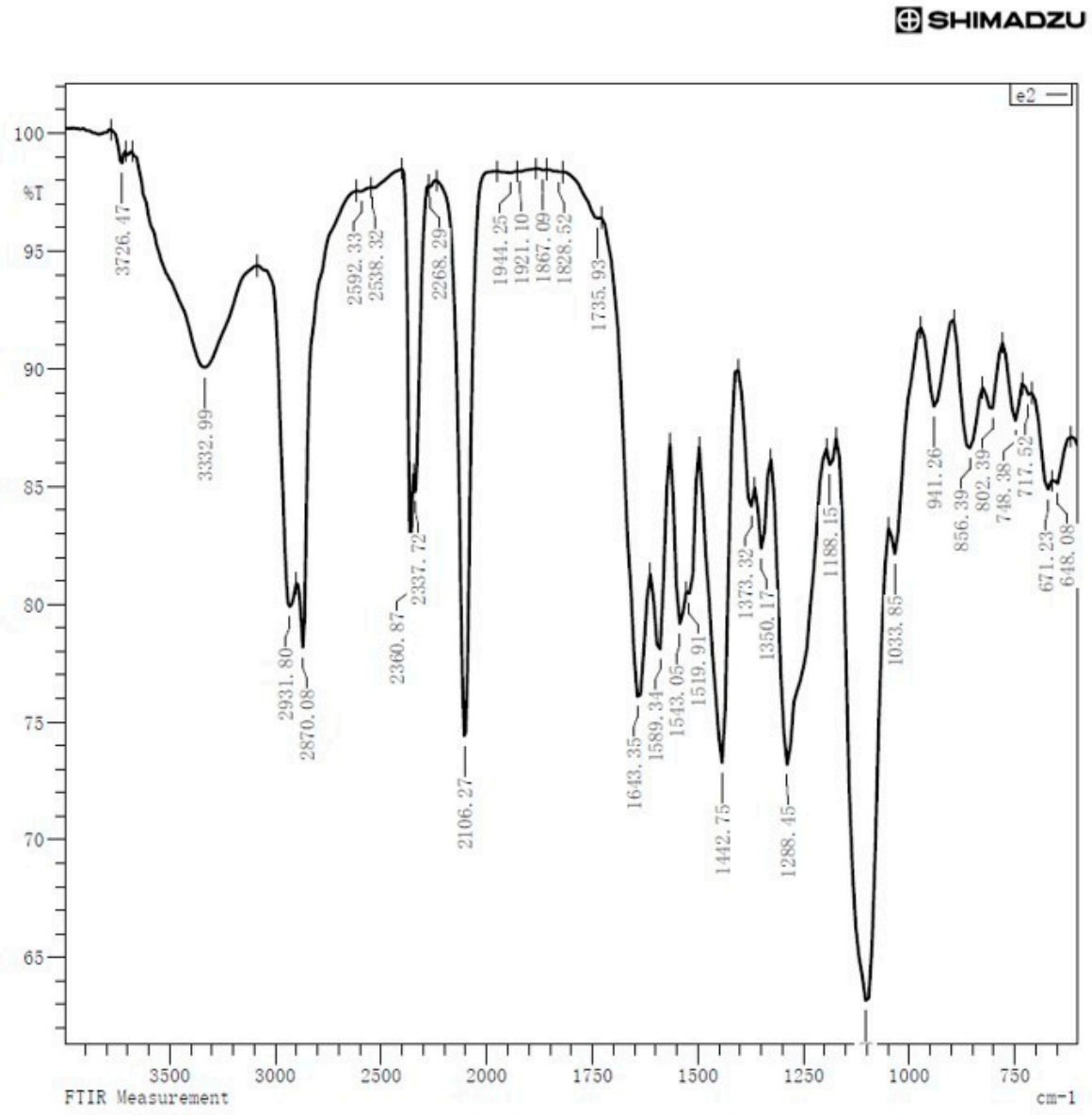


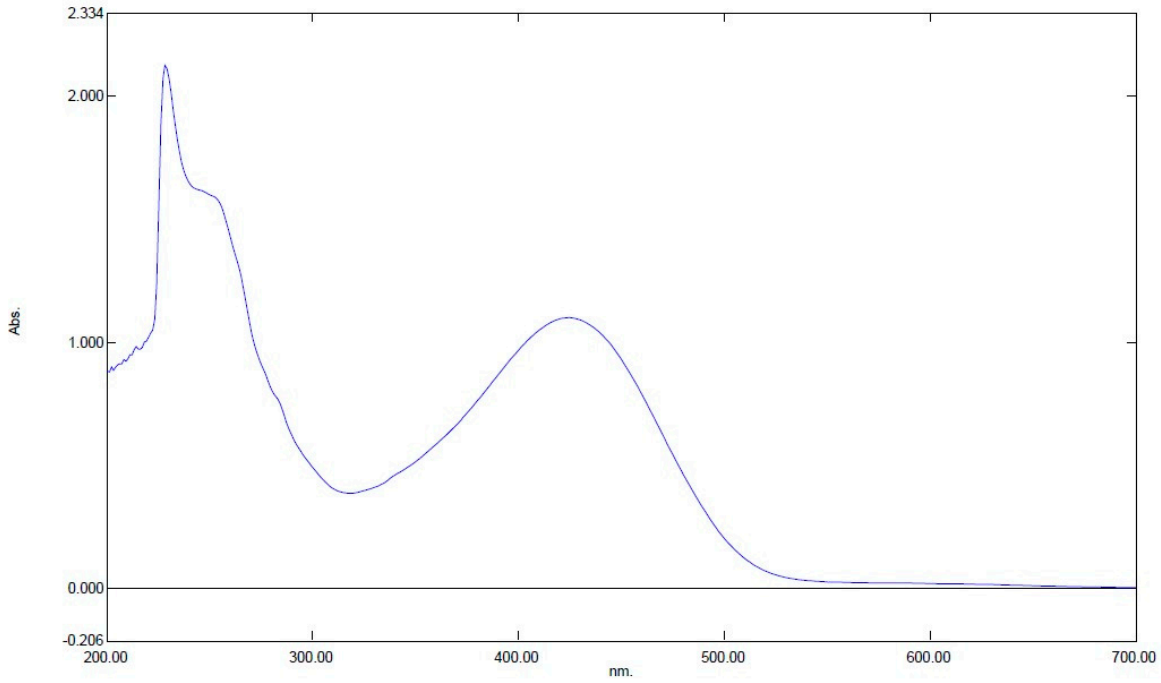
Figure S12. IR spectrum (FTIR) of compound **4**.



UV-VIS spectroscopy

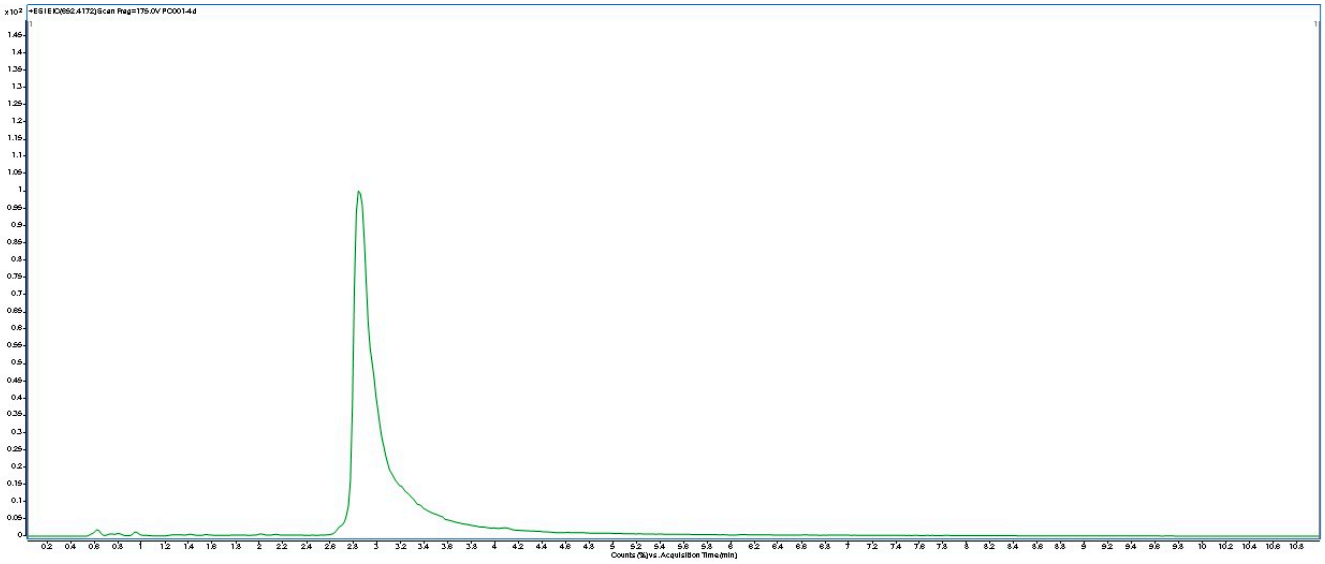
Figure S13. UV spectrum of compound **4** (range 200-500 nm in CH_2Cl_2)

λ max= 224 and 420 nM



Mass spectroscopy

Figure S14. UPLC-UV chromatogram (254 nm) and mass spectrum of compound **4**



Name: Binary Pump **Model:** G7120A

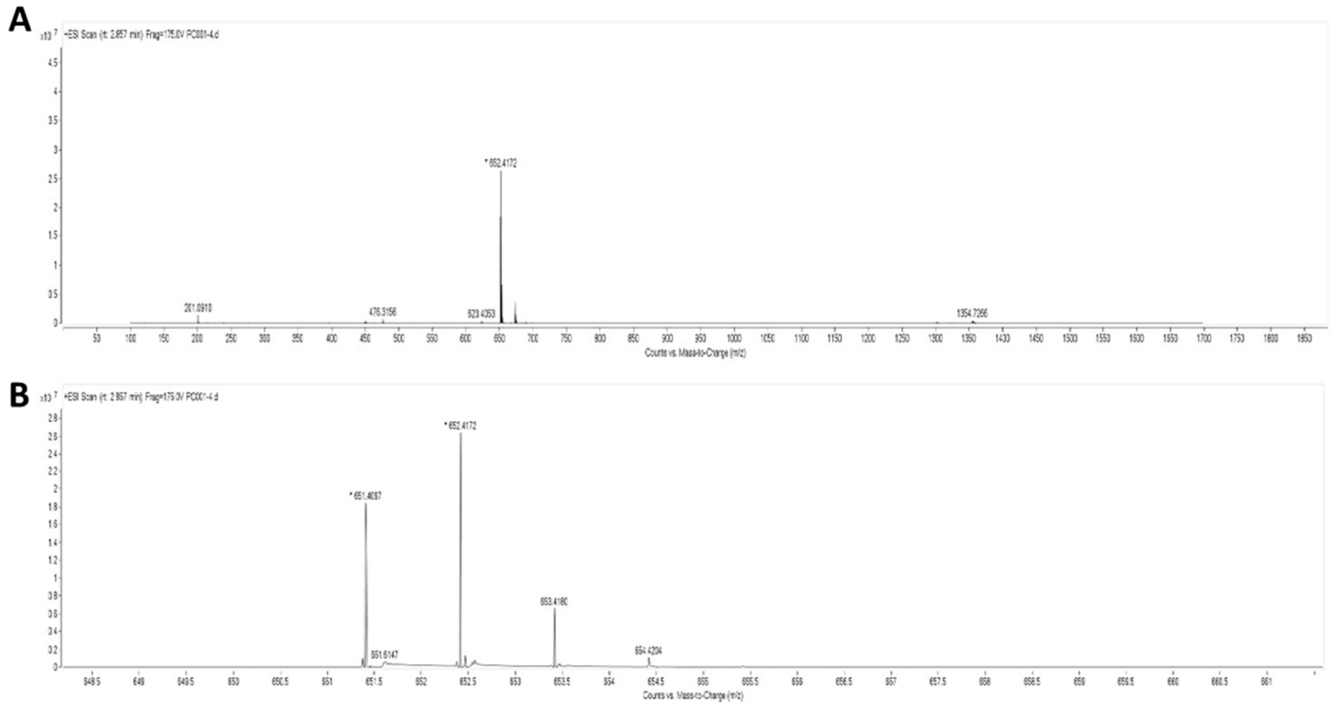
Flow	0.300 mL/min
Use Solvent Types	Yes
Stroke Mode	Synchronized
Low Pressure Limit	0.00 bar
High Pressure Limit	1000.00 bar
Max. Flow Ramp Up	100.000 mL/min²
Max. Flow Ramp Down	100.000 mL/min²
Expected Mixer	No check
Stroke A	
Automatic Stroke Calculation A	Yes
Stoptime	
Stoptime Mode	Time set
Stoptime	11.00 min
Posttime	
Posttime Mode	Time set
Posttime	2.00 min

Solvent Composition

	Channel	Ch. 1 Solv.	Name 1	Ch. 2 Solv.	Name 2	Selected	Used	Percent
1	A	100.0 % Water V.03	0.1% FA	100.0 % Water V.03		Ch. 1	Yes	25.00 %
2	B	100.0 % Acetonitrile V.03		100.0 % Acetonitrile V.03		Ch. 1	Yes	75.00 %

Timetable

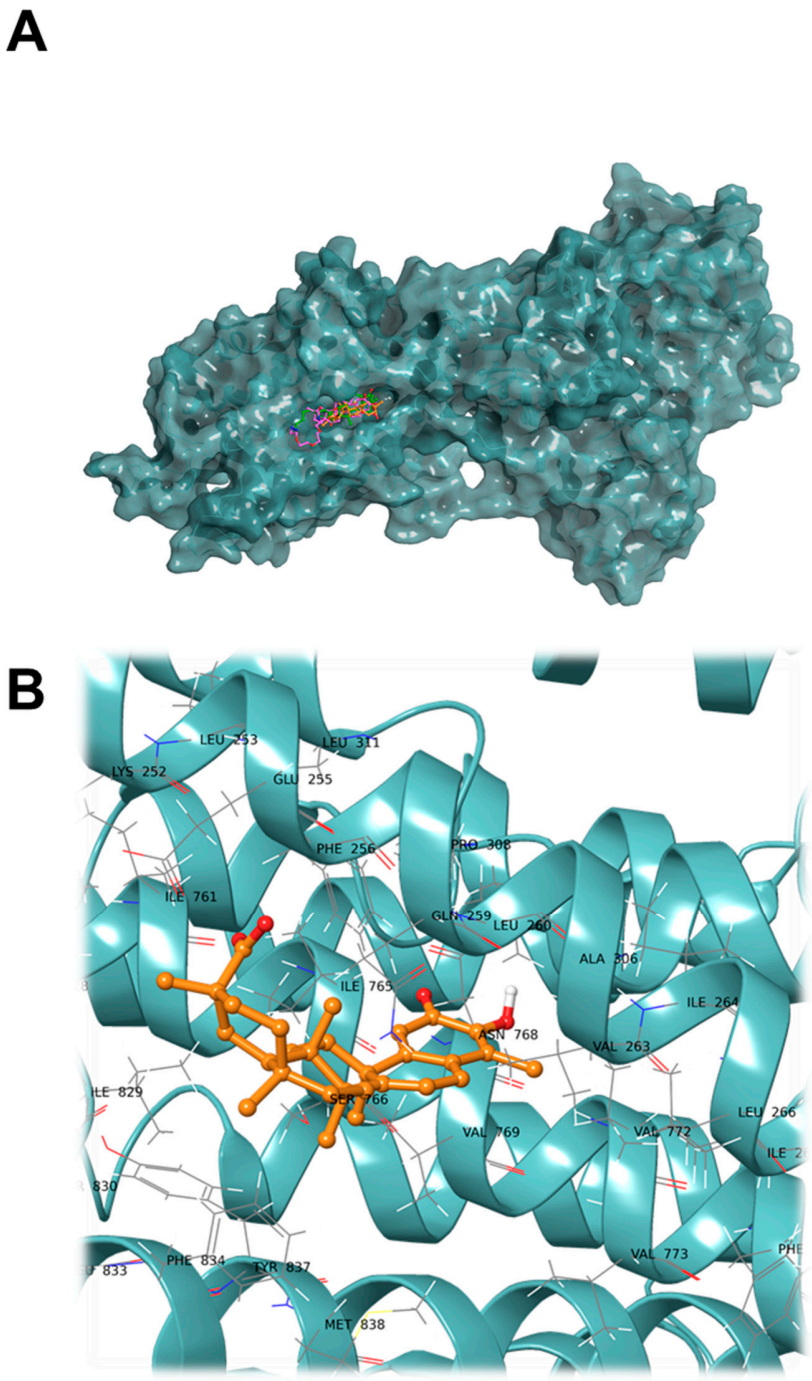
	Time	A	B	Flow	Pressure
1	Start. Cond. min	25.00 %	75.00 %	0.300 mL/min	1000.00 bar
2	10.00 min	5.00 %	95.00 %	--- mL/min	--- bar
3	11.00 min	25.00 %	75.00 %	--- mL/min	--- bar



A) ESI-MS m/z 651.40 $[M + H]^+$ (calcd. for $C_{37}H_{55}N_4O_6^+$, m/z 651.41). B) Magnification of the molecular ion peak.

Computational studies

Figure S15. Interaction of celastrol with SERCA.



Predicted interaction motif for compound **4** (pink), celastrol (orange) and co-crystallized pose of thapsigargin (green) within SERCA binding site (PDB ID: 1IWO) (A). Detailed view of best-

docked pose of celastrol (orange) in the binding site of SERCA. Residues located in a zone of 5 Å radius from the center of the ligand have been labeled (B).

Table S2. Physicochemical properties of compound **1** and **4** calculated by SwissADME [1]

Name	MW	MLogP	HBA	HBD	TPSA (Å^2)	nRtB	nviolations
Lipinsky's rule	≤500	<4.15	≤10	≤5	<75	≤10	**0**
1	450.62	5.06	4	2	74.60	1	**1**
4	650.85	2.18	9	2	143.84	14	**1**

MW: Molecular weight. HBA: Number of hydrogen-bond acceptors. HBD: Number of hydrogen-bond donors. TPSA: topological polar surface area; nRtB: Number of rotatable bonds. nviolations: Number of Lipinki's rule violation [1].

References

1. Daina, A., O. Michielin, and V. Zoete, *SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules.* Scientific Reports, 2017. **7**(1): p. 42717.