Supporting information

Lycorine carbamate derivatives for reversing P-glycoprotein mediated multidrug resistance in human colon adenocarcinoma cancer cells.

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Table of contents

1. NMR data of parental compound 1

Lycorine (1)

Amorphous powder. $[\alpha]_D^{25}$ − 70.6 (*c* 0.31, MeOH); IR ν_{max} cm^{-1} (KBr): 3334, 1485, 744. ESI-MS (positive mode) m/z (rel. int) 288 $[M + H]^+$ (100). ^1H NMR (300 MHz, DMSO-d_6) δ 6.80 (1H, *s*, H-10), 6.67 (1H, *s*, H-7), 5.95 (2H, *dd*, *J* = 4.1, 0.9 Hz,O$\underline{CH_2}$O), 5.37 (1H, *bs*, H-3), 4.85 (1H, *d*, *J* = 6.2 Hz, 2-OH), 4.75 (1H, *d*, *J* = 4.2 Hz, 1-OH), 4.27 (1H *bs*, H-1), 4.04 (1H, *d*, *J* = 14.2 Hz, H-6β), 3.96 (1H, *bs*, H-2), 3.29 (1H, *d*, *J* = 14.2 Hz, H-6α), 3.18 (1H, *ddd*, *J* = 9.1, 7.2, 2.1 Hz, H12-β), 2.61 (1H, *d*, *J* = 10.4 Hz, H-10b), 2.51 (1H, *m*, H-4a), 2.47 (2H, *m*, H-11), 2.20 (1H, *q*, *J* = 8.5 Hz, H-12α) ppm. ^{13}C NMR (75 MHz, DMSO- d_6) δ 145.6 (C-9), 145.1(C-8), 141.6 (C-4), 129.7(C-6a), 129.5(C-10a), 118.4 (C-3), 106.9 (C-7), 105.0 (C-10), 100.5 (O$\underline{CH_2}$O), 71.7 (C-2), 70.2 (C-1), 60.7 (C-4a), 56.7 (C-6), 53.2 (C-12), 40.1(C-10b), 28.1 (C-11) ppm.

2. Representative ^{1}H and ^{13}C NMR spectra

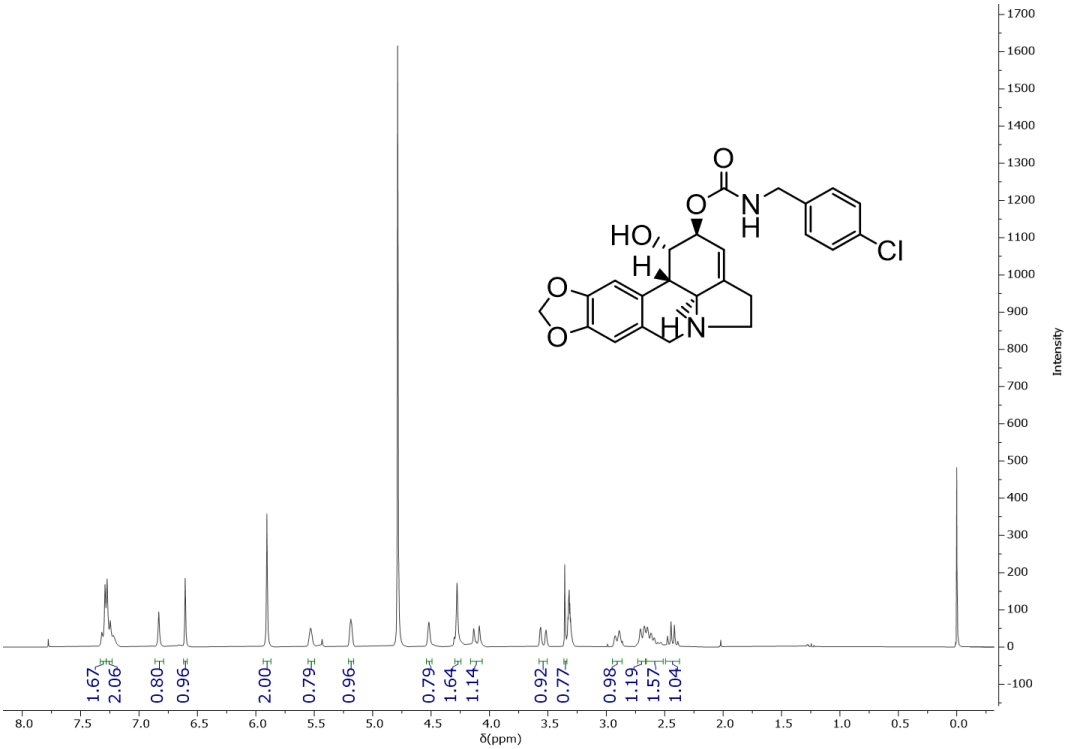


Figure S1: ^{1}H-NMR spectrum of compound **2** (300 MHz, $CDCl_3/CD_3OD$).

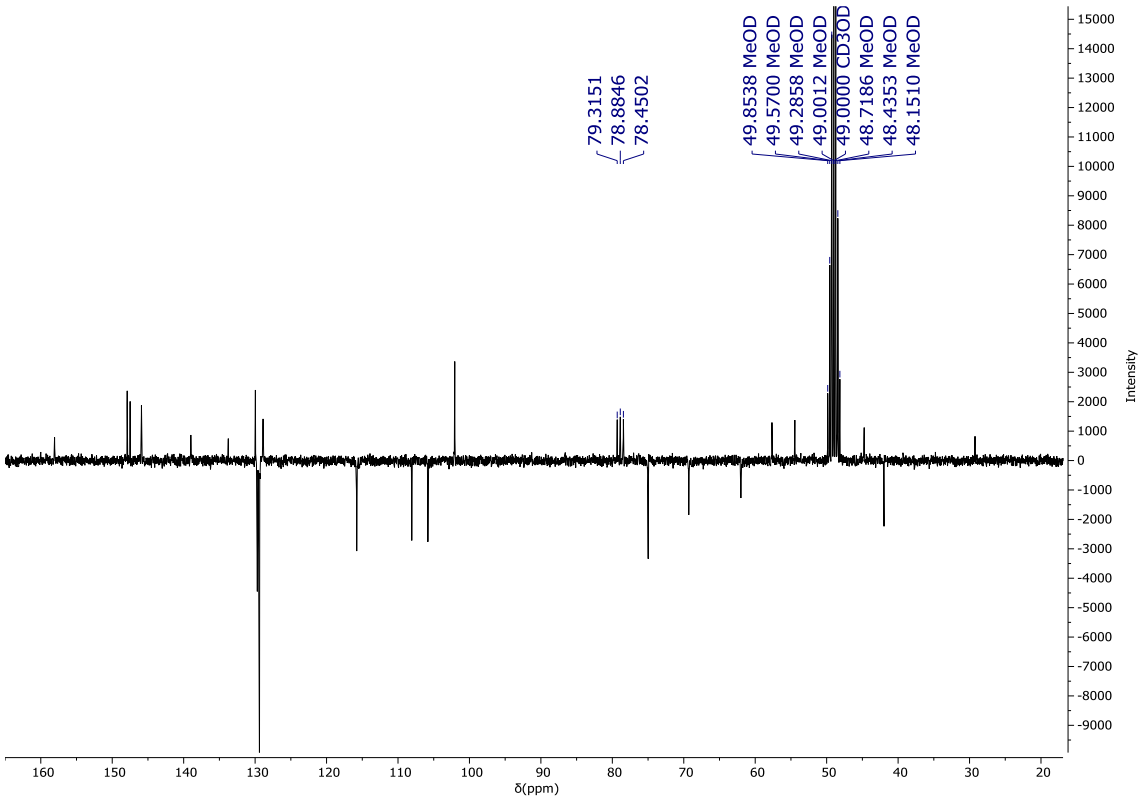


Figure S2: ^{13}C-APT NMR spectrum of compound **2** (75 MHz, $CDCl_3/CD_3OD$).

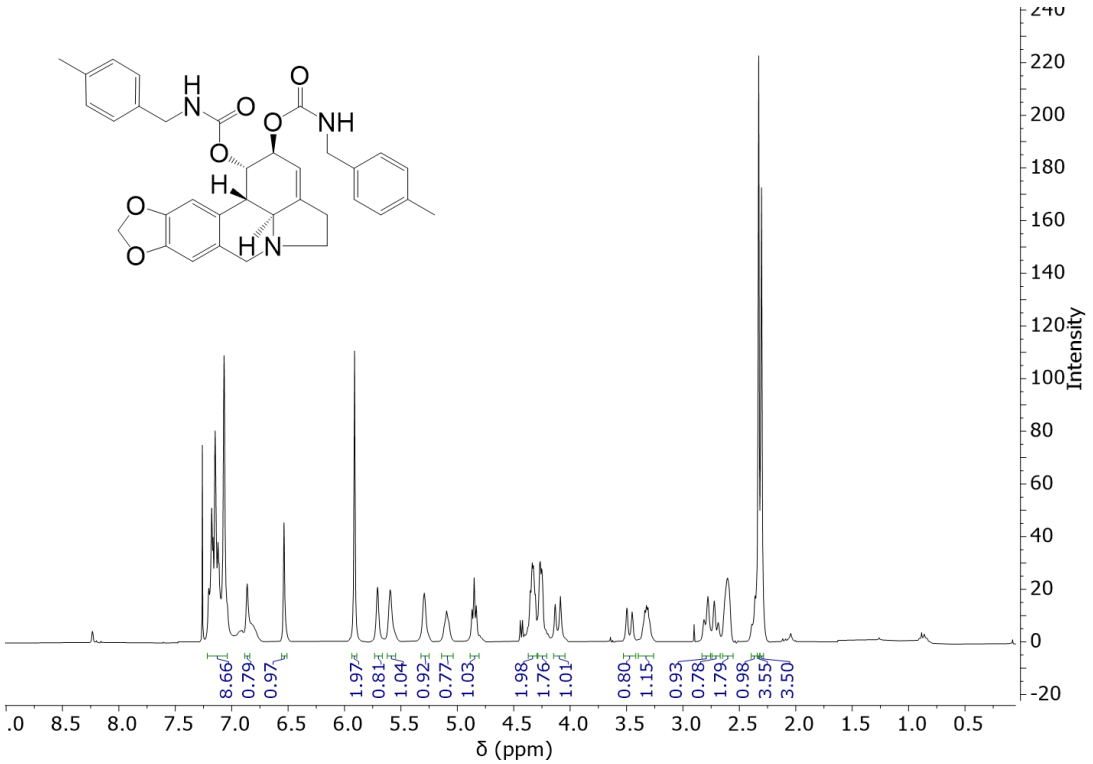


Figure S3: ^{1}H-NMR spectrum of compound **5** (300 MHz, $CDCl_3$).

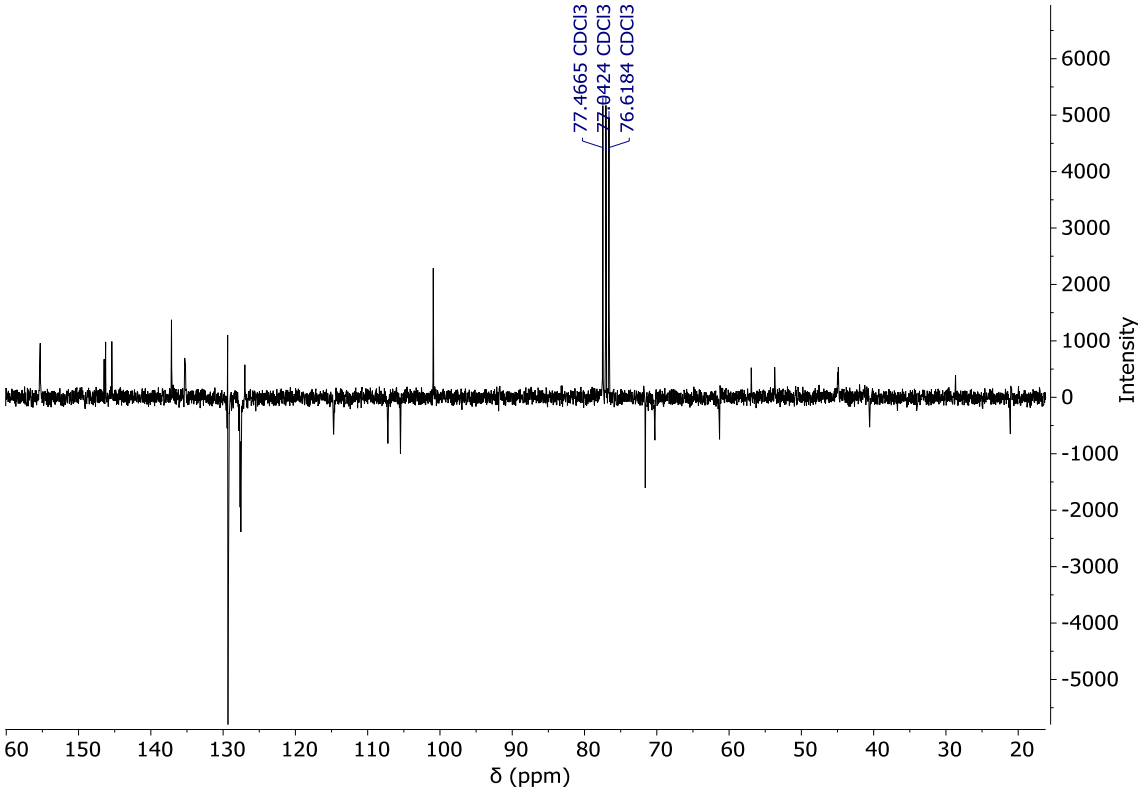


Figure S4: ^{13}C-APT NMR spectrum of compound **5** (75 MHz, $CDCl_3$).

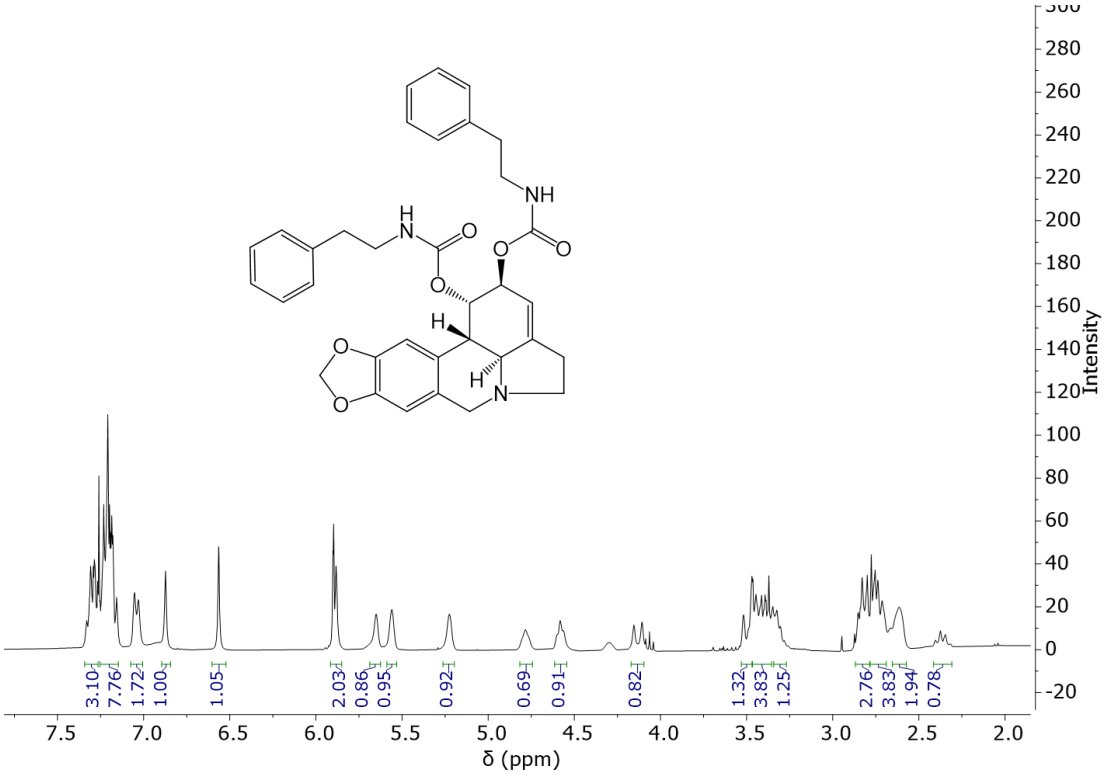


Figure S5: ^{1}H-NMR spectrum of compound **9** (300 MHz, $CDCl_3$).

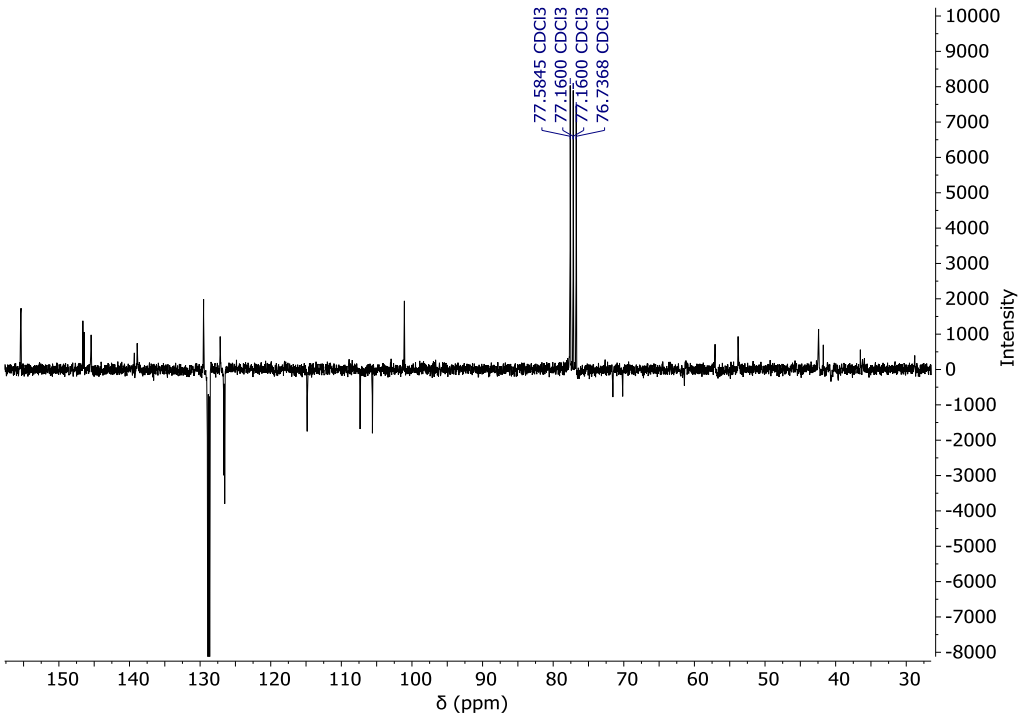


Figure S6: ^{13}C-APT NMR spectrum of compound **9** (75 MHz, $CDCl_3$).

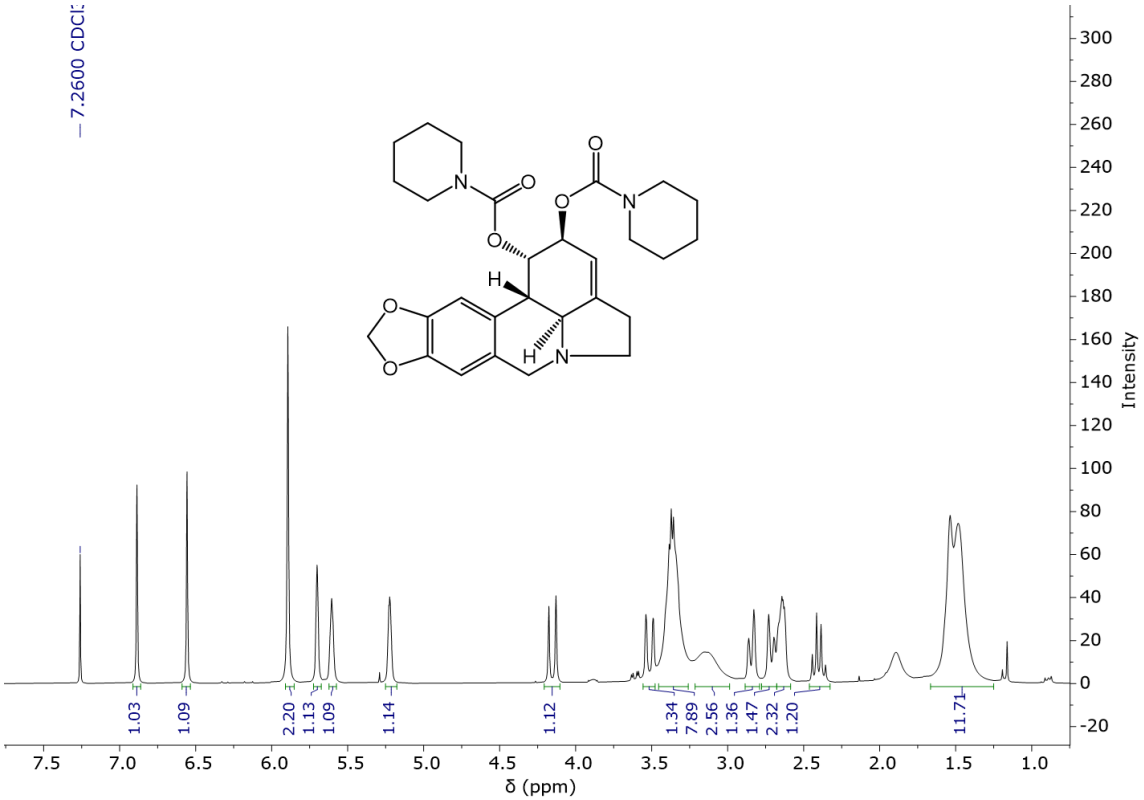


Figure S7: ^{1}H-NMR spectrum of compound **16** (300 MHz, $CDCl_3$).

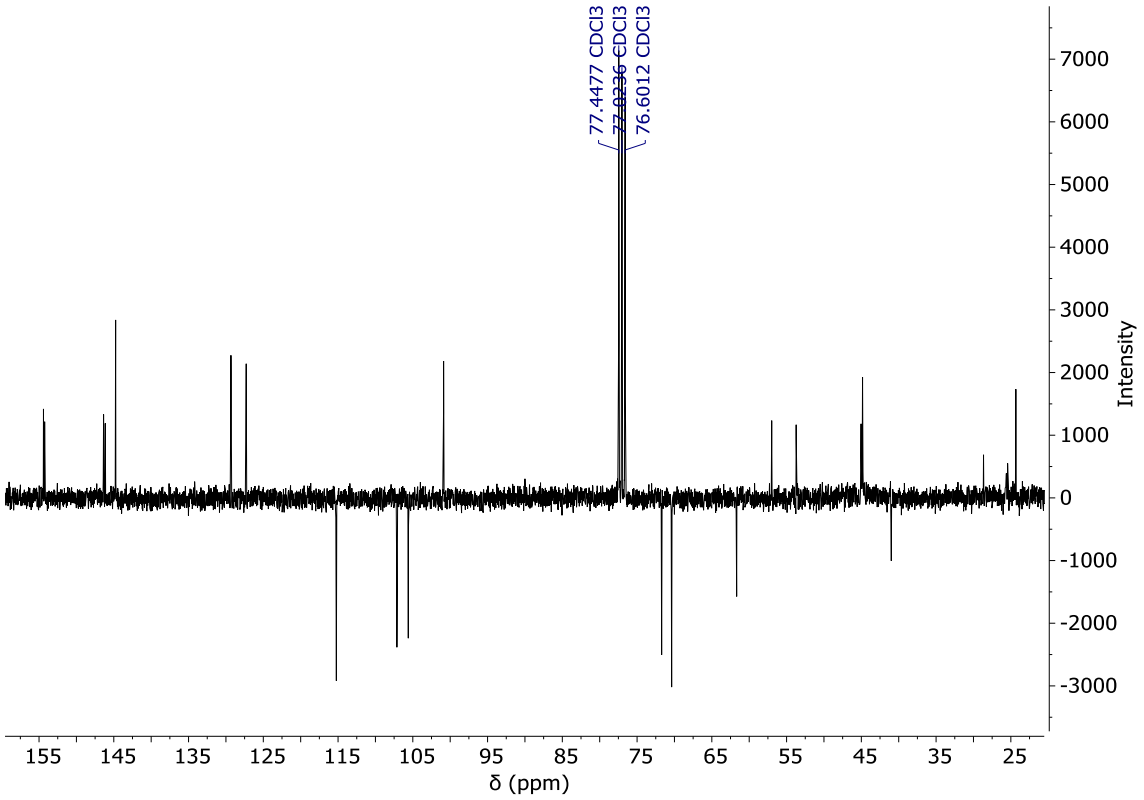


Figure S8: ^{13}**C**-APT NMR spectrum of compound **16** (75 MHz, $CDCl_3$).

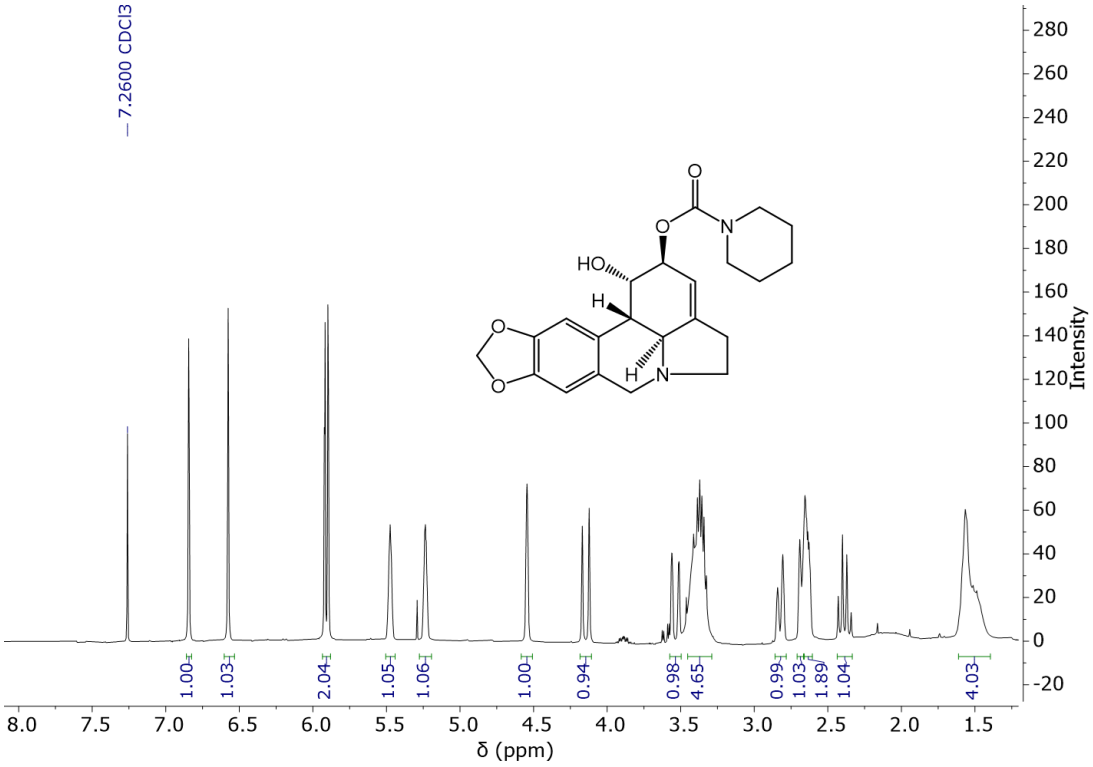


Figure S9: ^{1}H-NMR spectrum of compound **17** (300 MHz, $CDCl_3$).

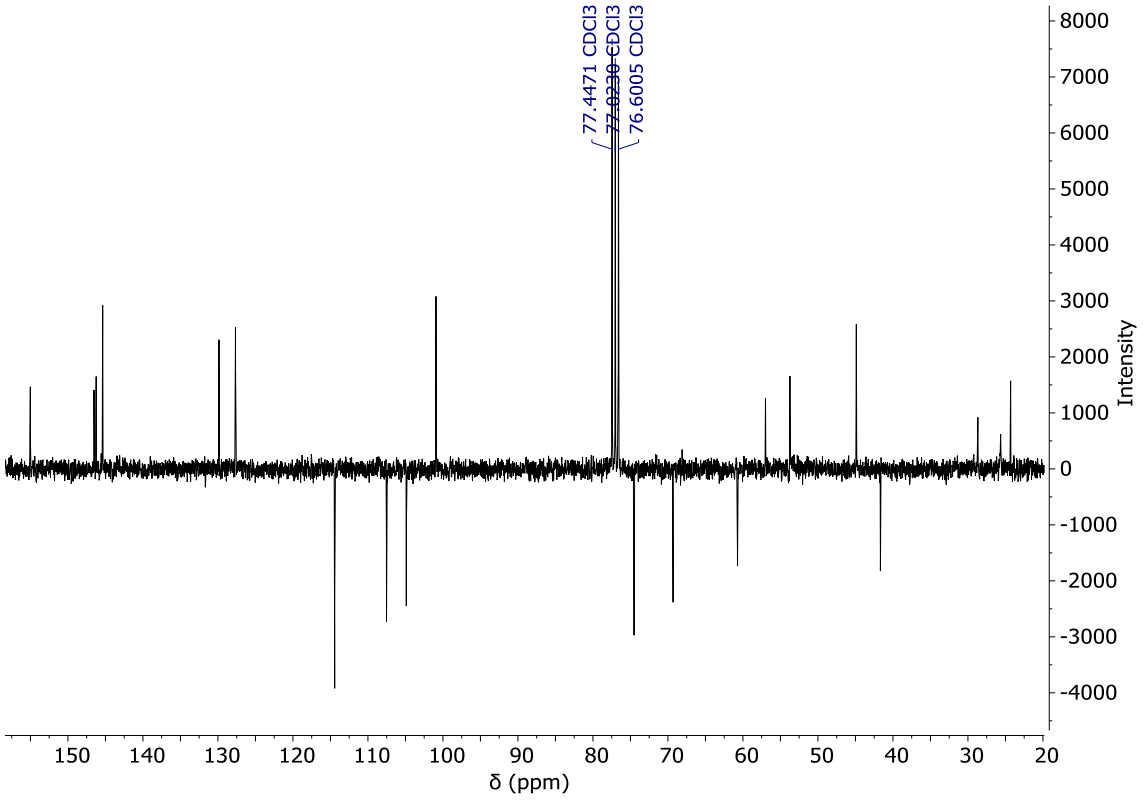


Figure S10: ^{13}C-APT NMR spectrum of compound **17** (75 MHz, $CDCl_3$).

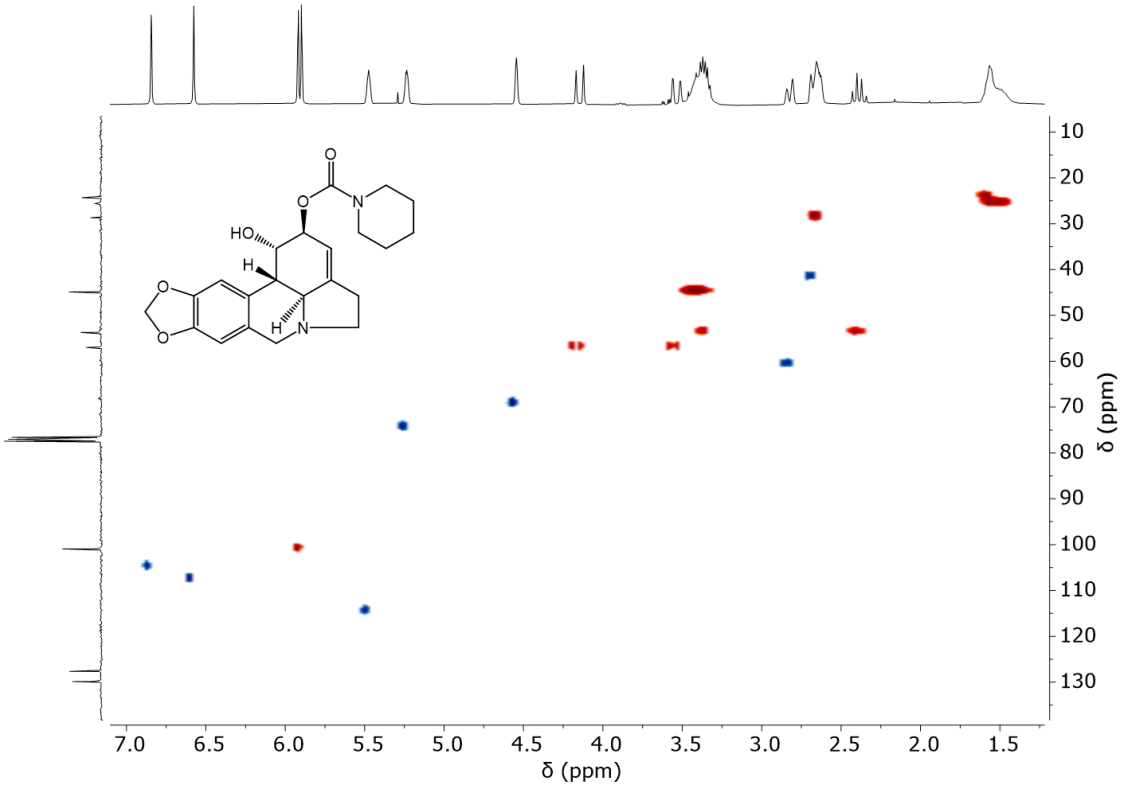


Figure S11: HSQC spectrum of compound **17.**

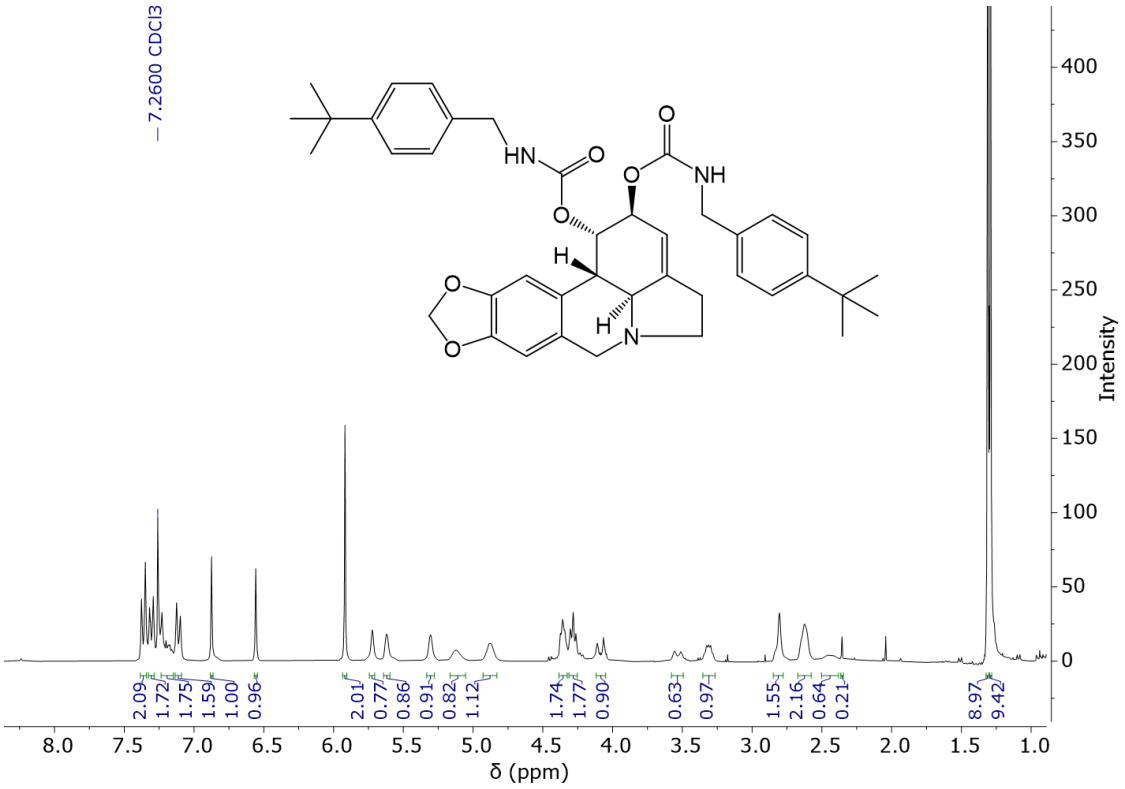


Figure S12: ^{1}H-NMR spectrum of compound **18** (300 MHz, $CDCl_3$).

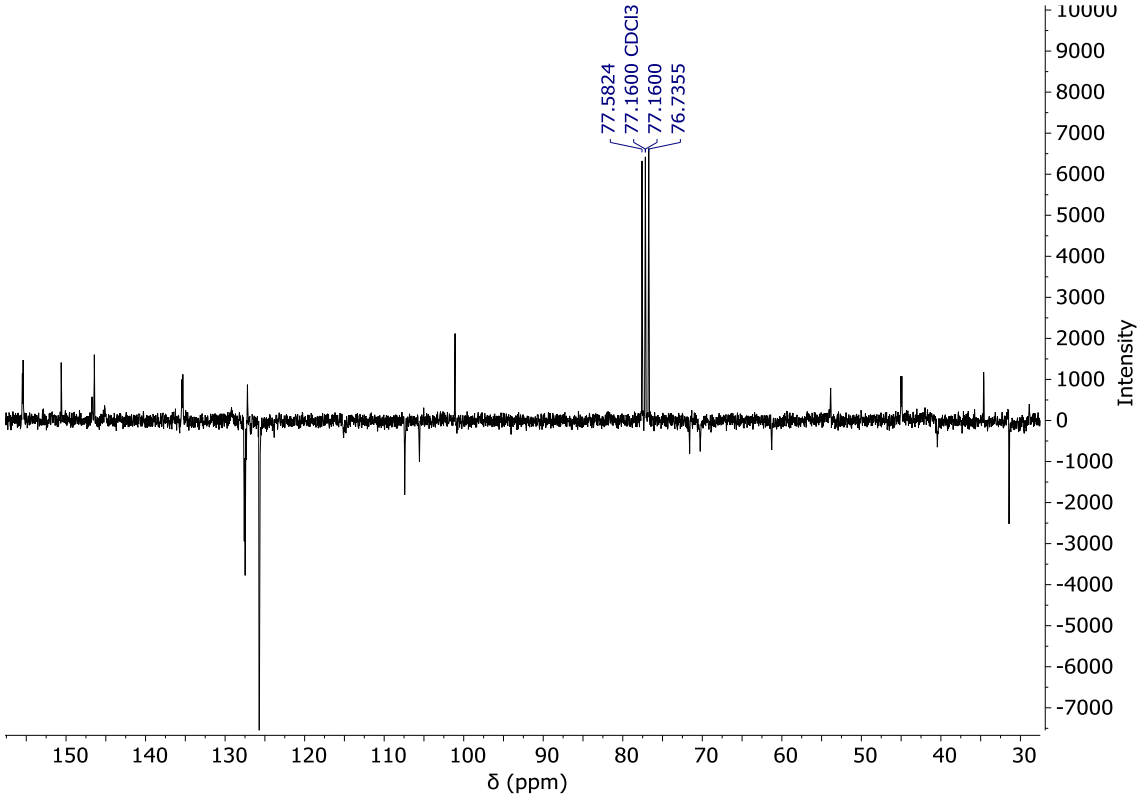


Figure S13: ^{13}C-APT NMR spectrum of compound **18** (75 MHz, $CDCl_3$).

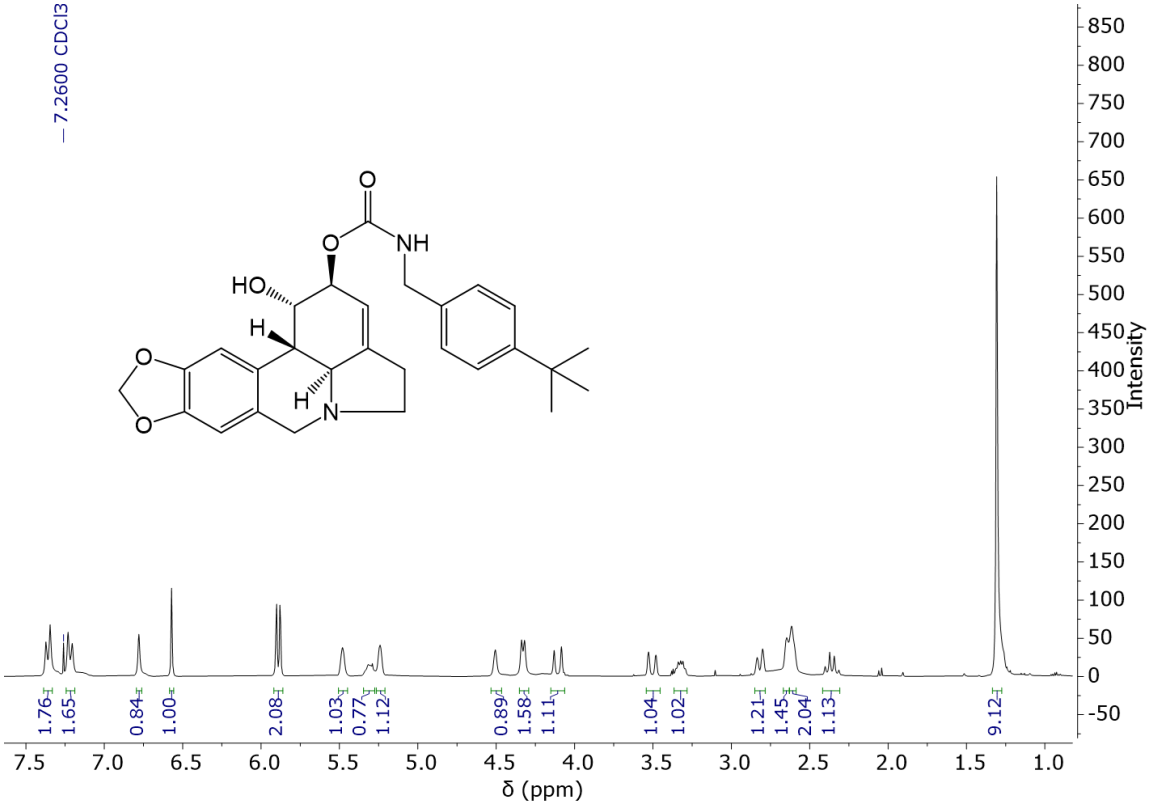


Figure S14: ^{1}H-NMR spectrum of compound **19** (300 MHz, $CDCl_3$).

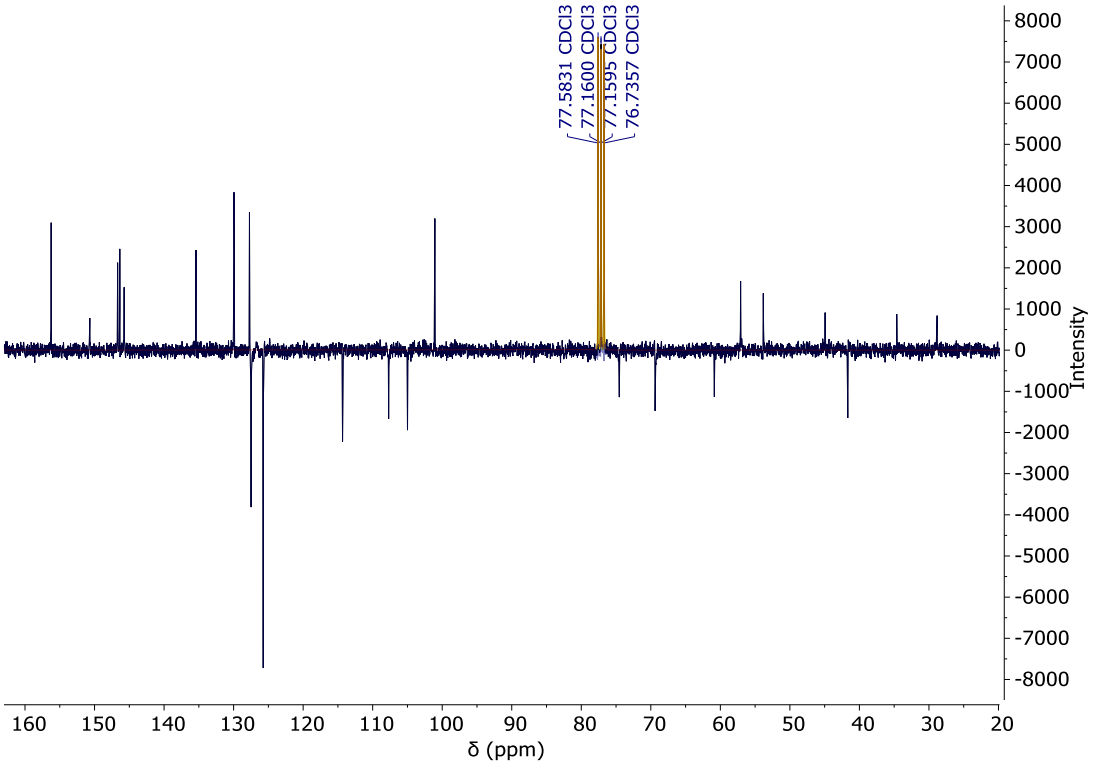


Figure S15: ^{13}C-APT NMR spectrum of compound **19** (75 MHz, $CDCl_3$).

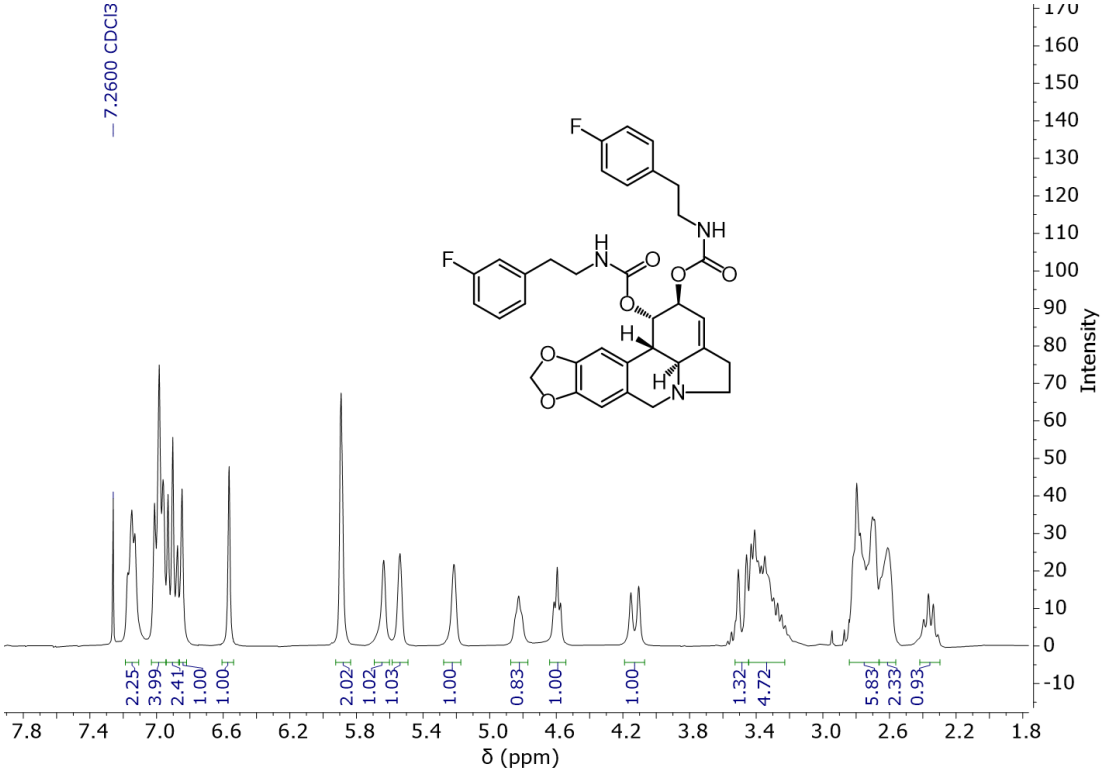


Figure S16: ^{1}H-NMR spectrum of compound **23** (300 MHz, $CDCl_3$).

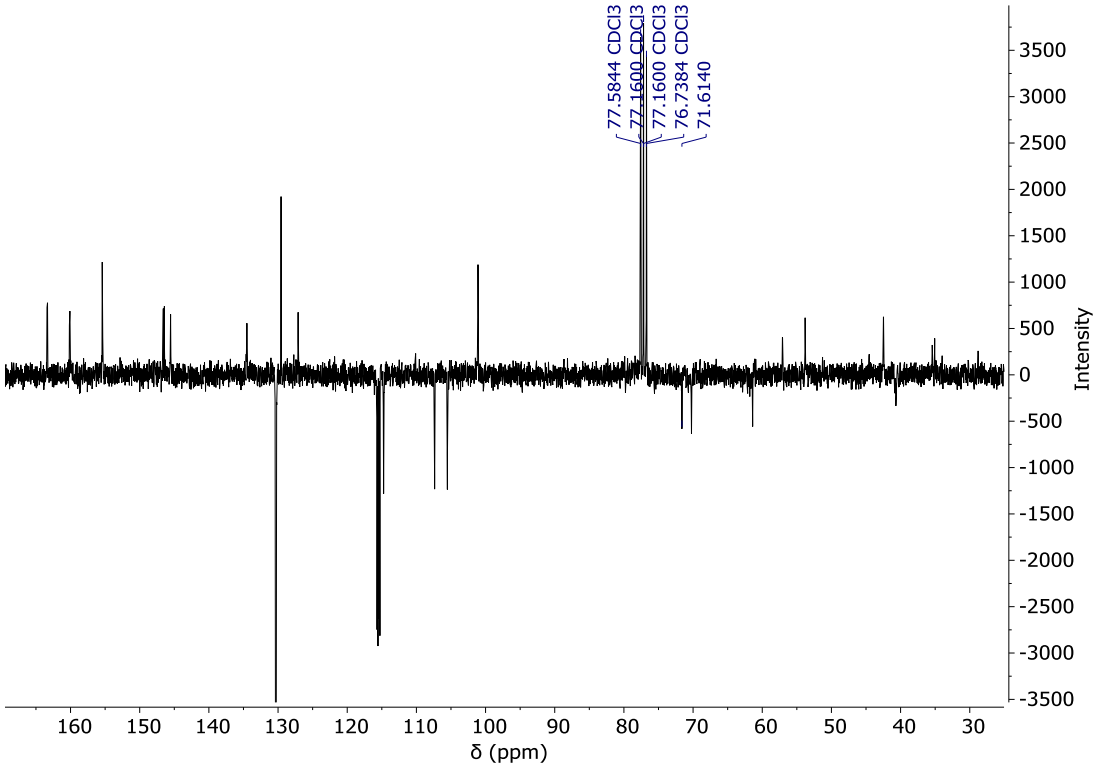


Figure S17: ^{13}C-APT NMR spectrum of compound **23** (75 MHz, $CDCl_3$).

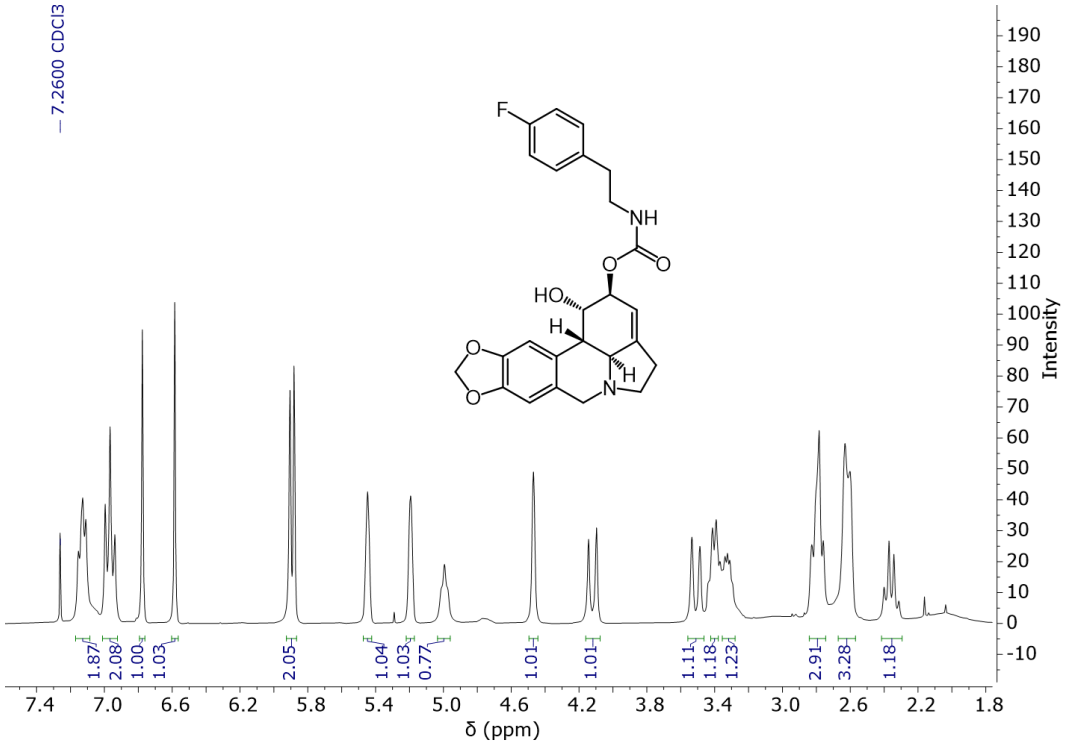


Figure S18: ^{1}H-NMR spectrum of compound **24** (300 MHz, $CDCl_3$).

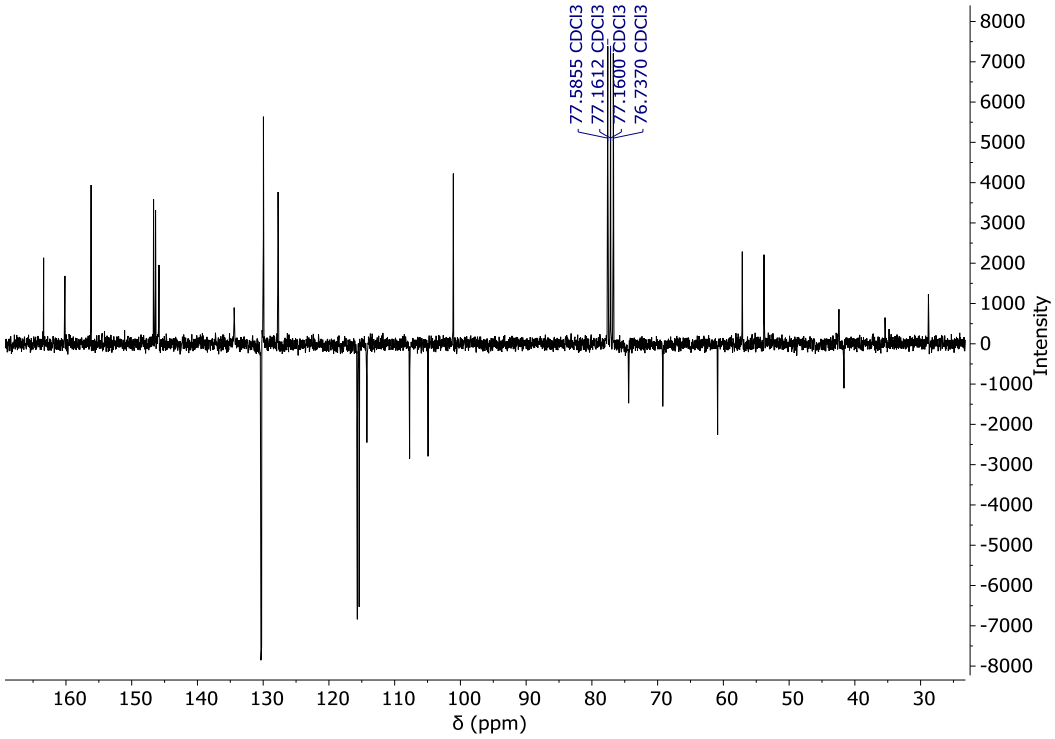


Figure S19: ^{13}C-APT NMR spectrum of compound **24** (75 MHz, $CDCl_3$).

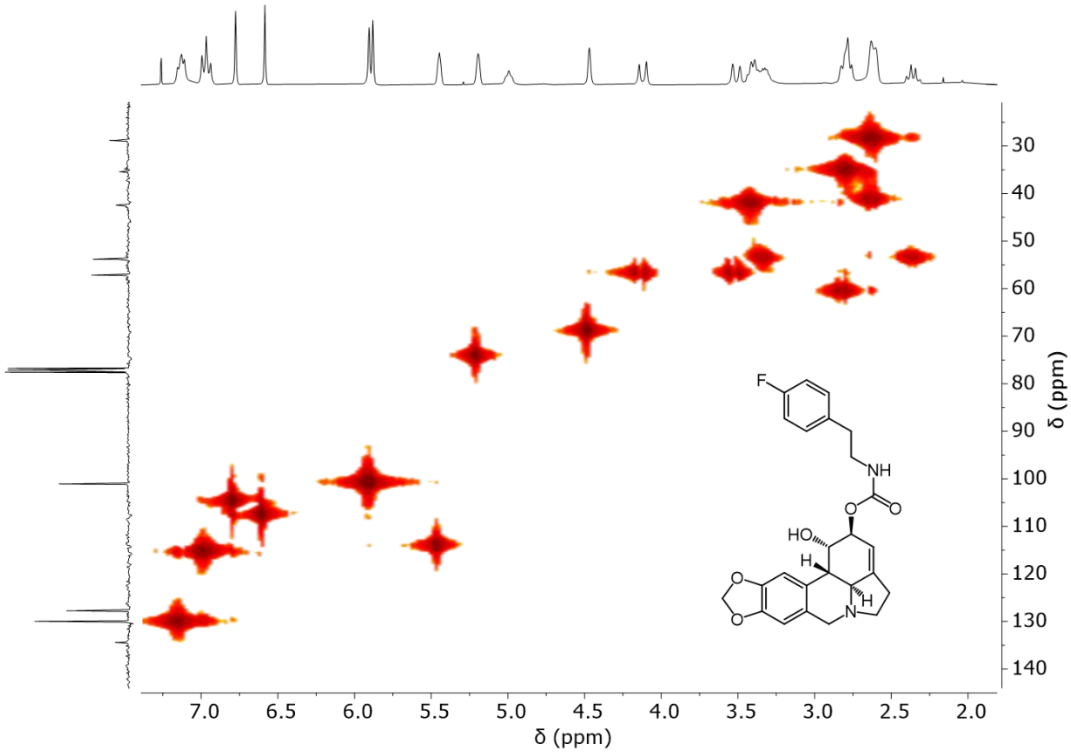


Figure S20: HMQC spectrum of compound **24**.

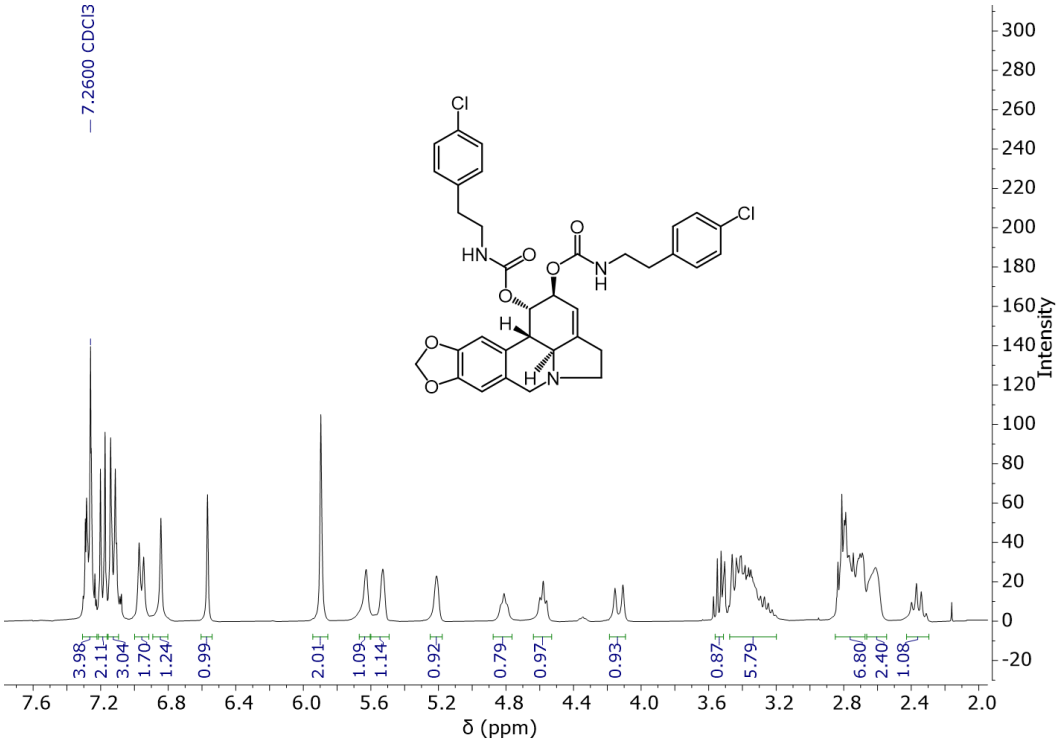


Figure S21: ^{1}H-NMR spectrum of compound **25** (300 MHz, $CDCl_3$).

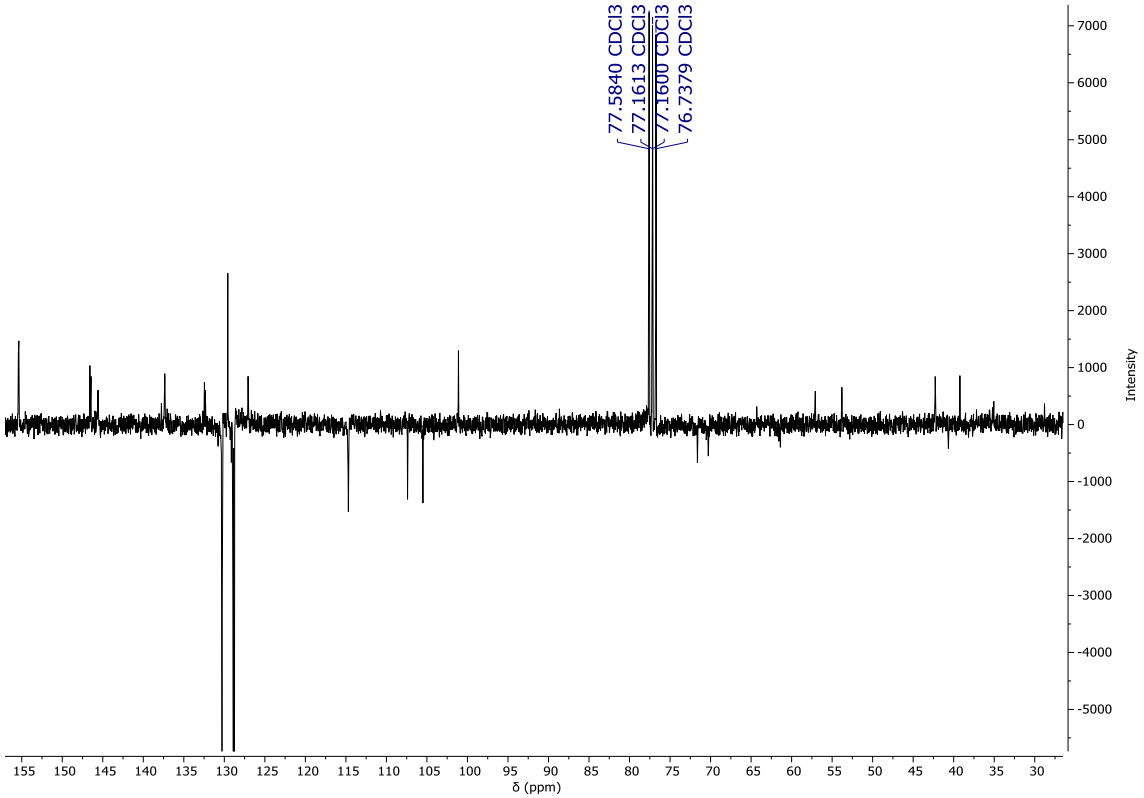


Figure S22: ^{13}C-APT NMR spectrum of compound **25** (75 MHz, $CDCl_3$).

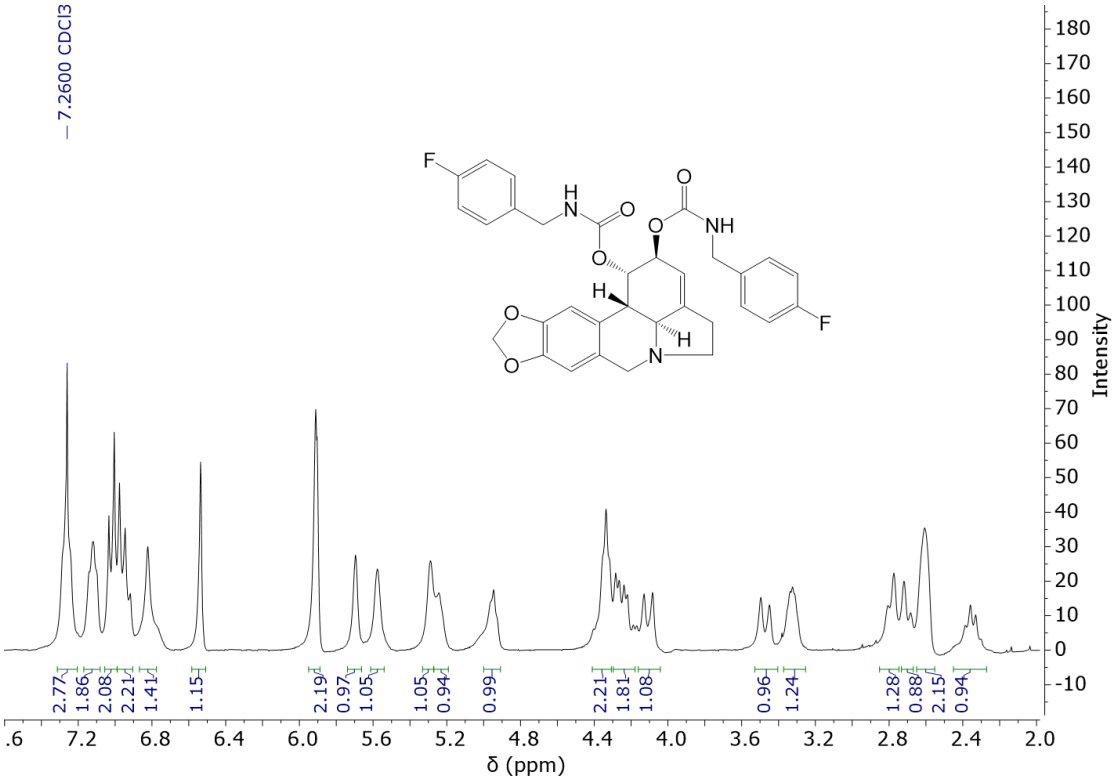


Figure S23: ^{1}H-NMR spectrum of compound **27** (300 MHz, $CDCl_3$).

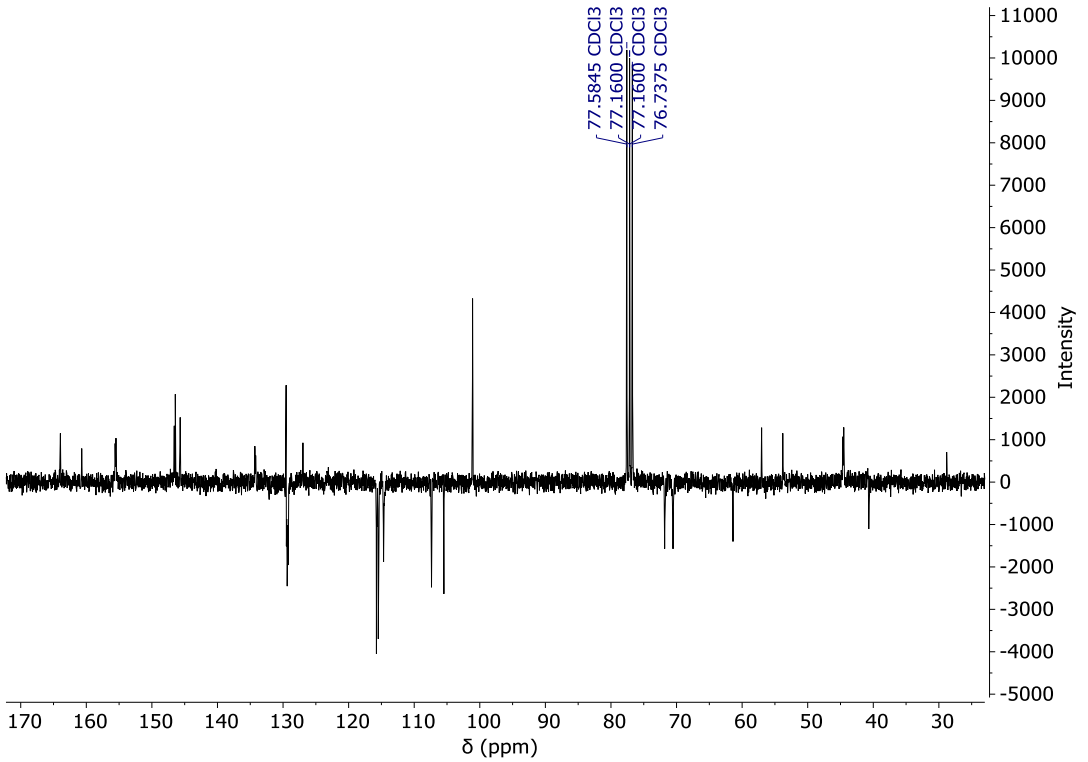


Figure S24: ^{13}C-APT NMR spectrum of compound **27** (75 MHz, $CDCl_3$).

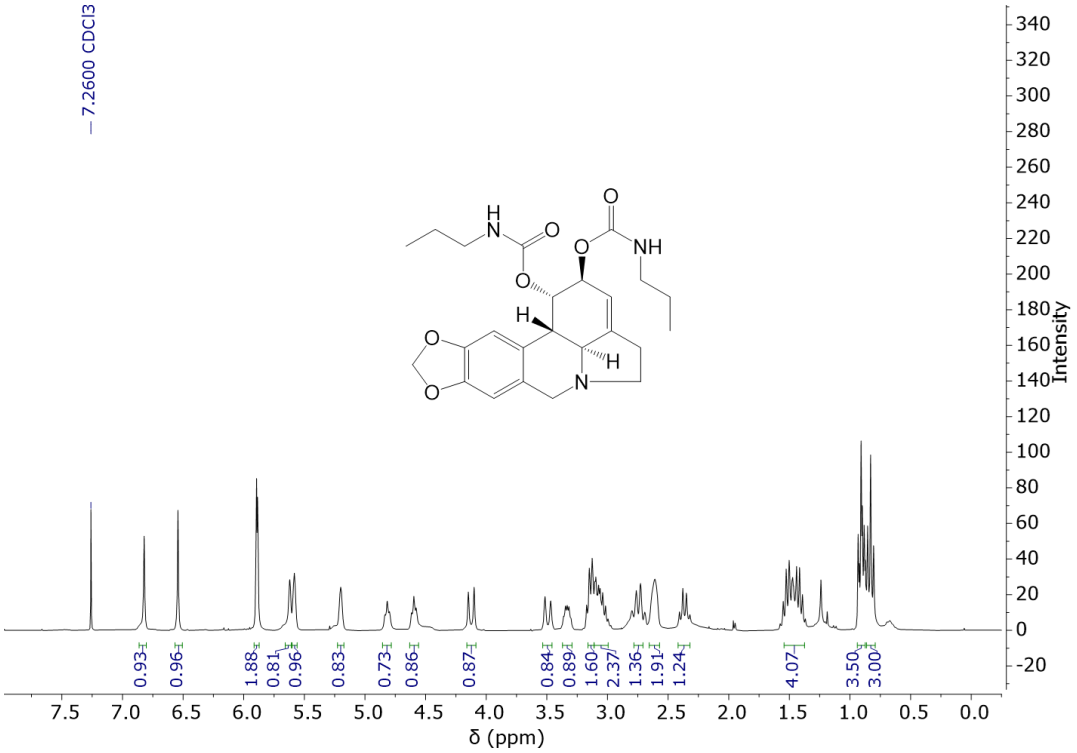


Figure S25: ^{1}H-NMR spectrum of compound **29** (300 MHz, $CDCl_3$).

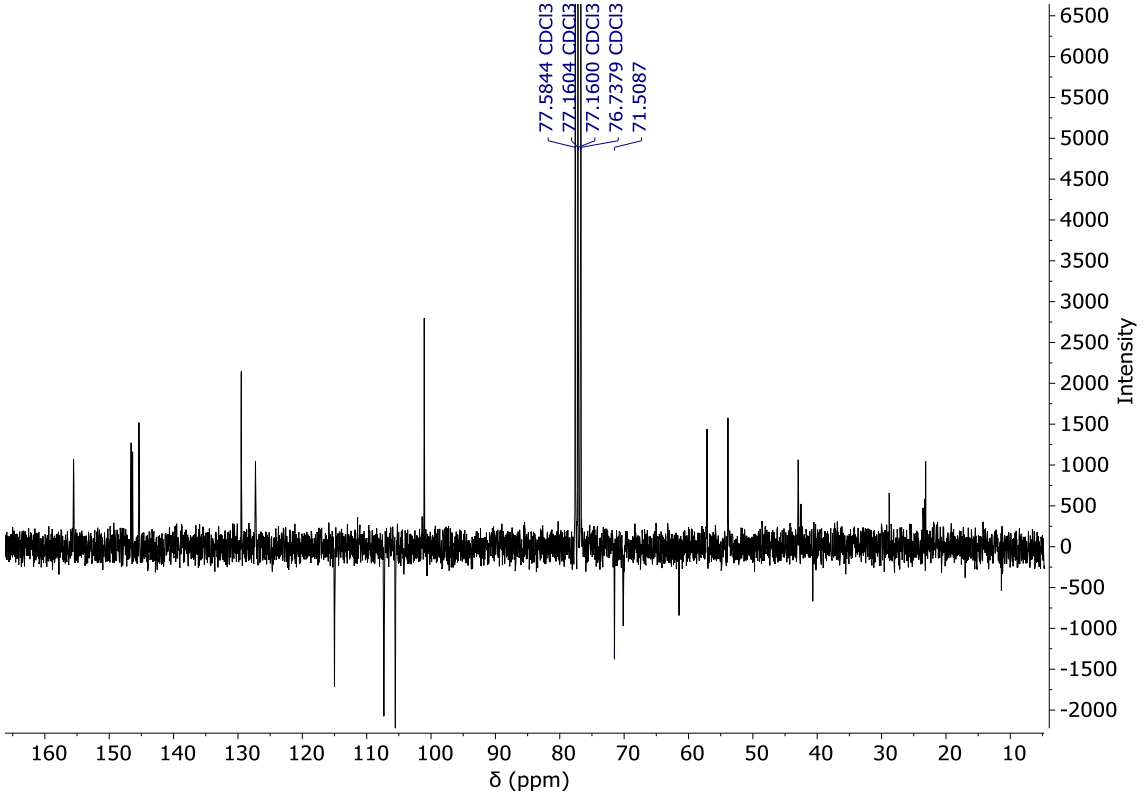


Figure S26: ^{13}C-APT NMR spectrum of compound **29** (75 MHz, $CDCl_3$).

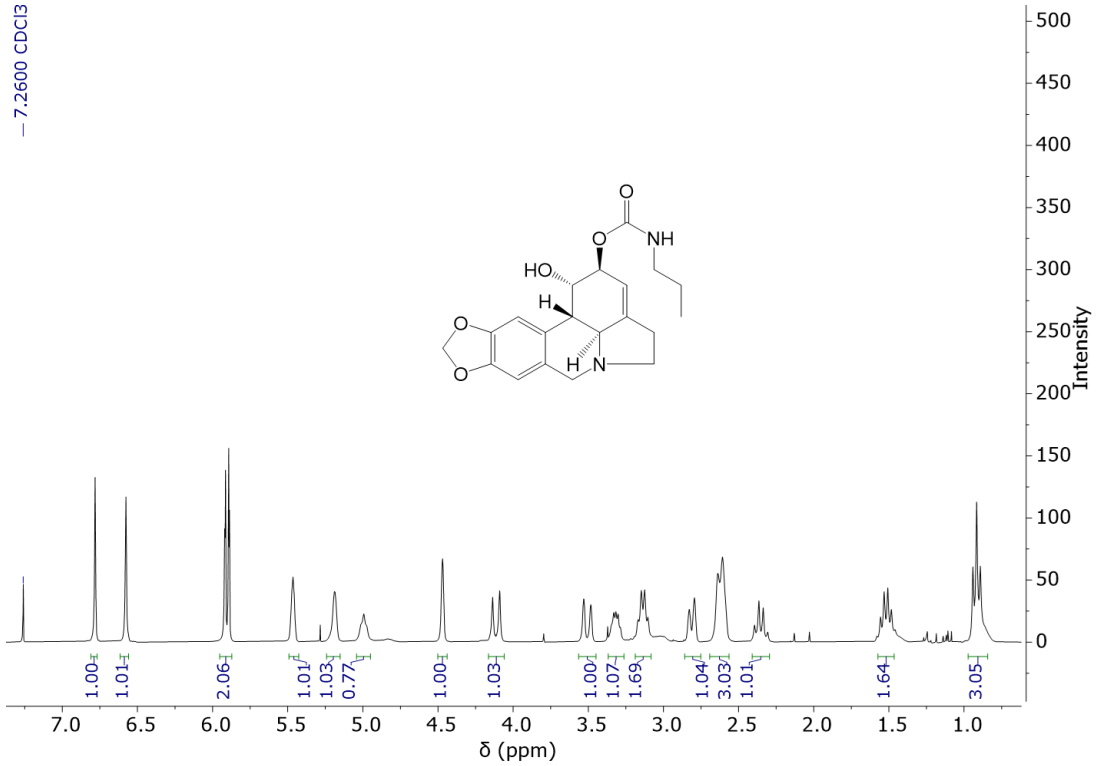


Figure S27: ^{1}H-NMR spectrum of compound **27** (300 MHz, $CDCl_3$).

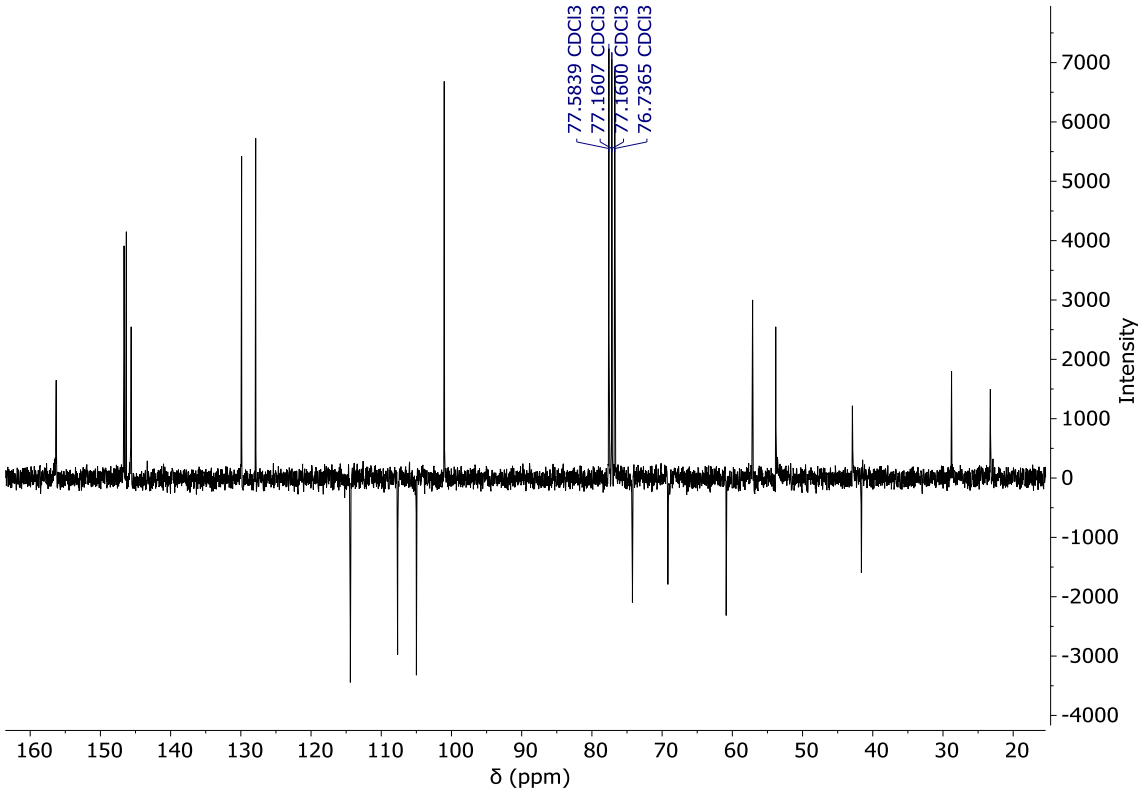


Figure S28: ^{13}**C**-APT NMR spectrum of compound **29** (75 MHz, $CDCl_3$).

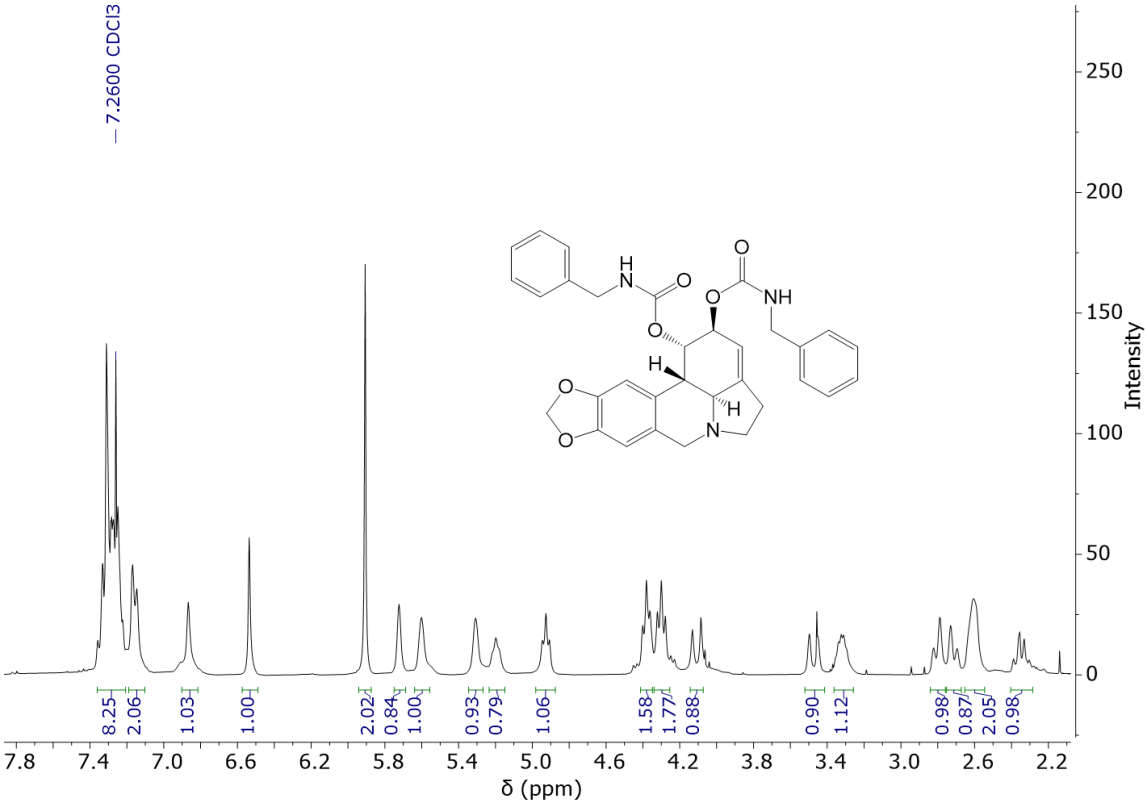


Figure S29: ^{1}H-NMR spectrum of compound **32** (300 MHz, $CDCl_3$).

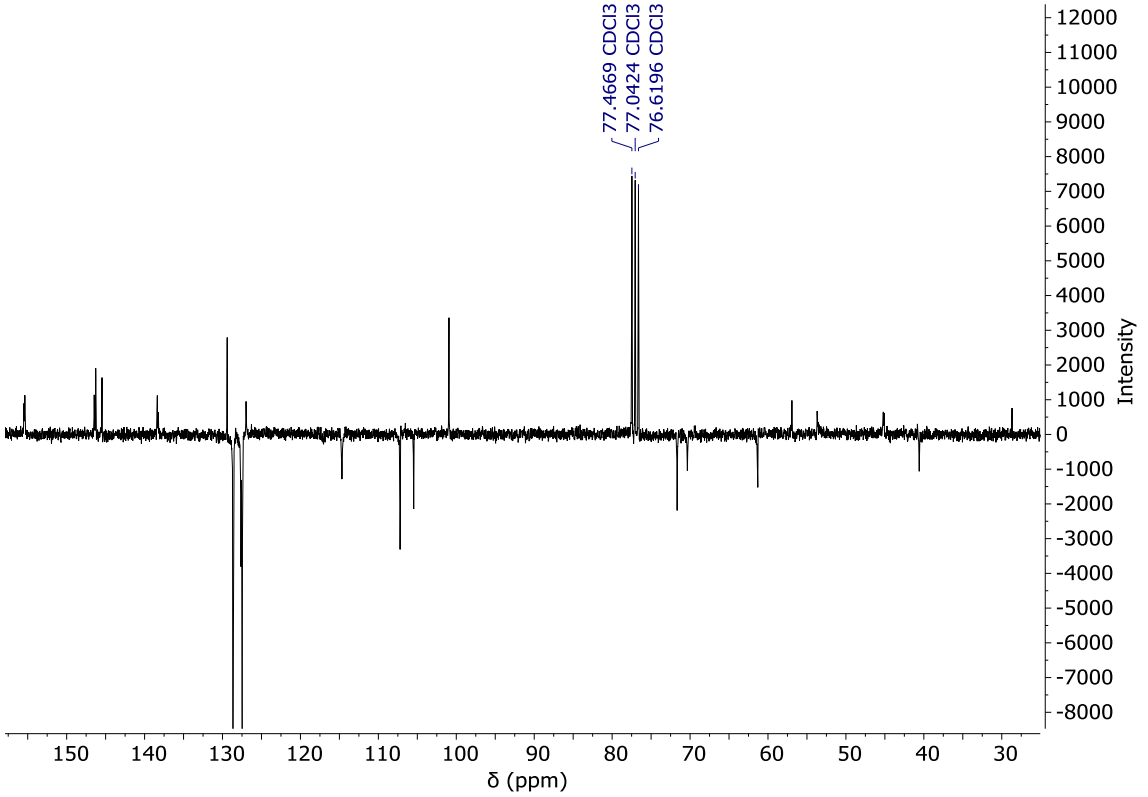
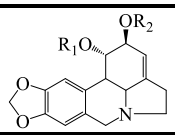


Figure S30: ^{13}C-NMR spectrum of compound **29** (75 MHz, $CDCl_3$).

3. Rhodamine-123 accumulation assay (compounds 1 – 32)

Table S1: P-pg inhibitory activity of compounds **1** – **32** on multidrug resistant human adenocarcinoma cells Colo320.



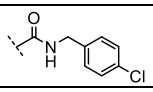
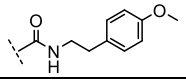
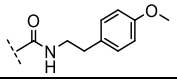
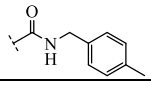
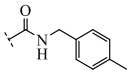
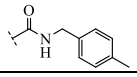
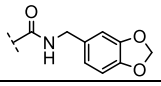
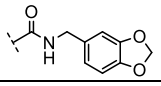
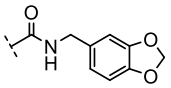
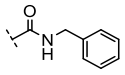
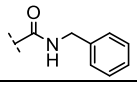
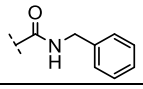
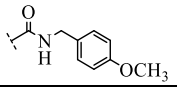
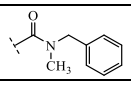
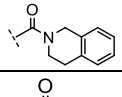
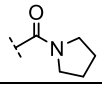
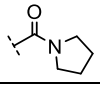
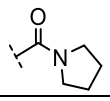
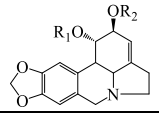
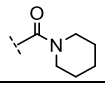
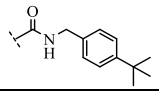
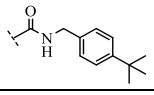
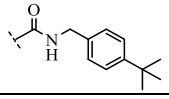
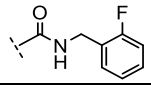
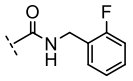
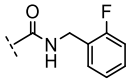
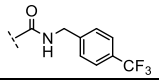
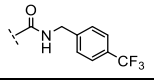
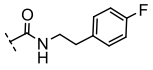
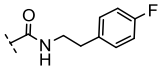
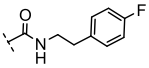
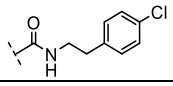
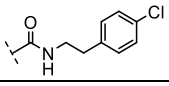
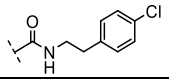
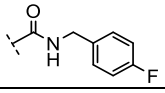
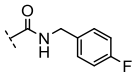
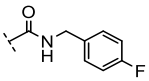
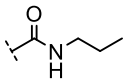
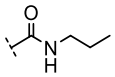
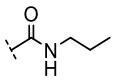
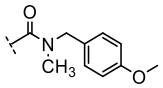
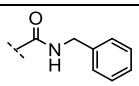
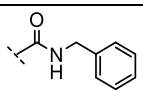
Compound	R_1	R_2	Conc (μM)	FAR[a]	FSC[b]	SSC[c]	FL-1[d]
Colo205	-	-	-	-	2141	758	107.0
Colo320	-	-	-	-	1993	1048	4.10
1	Lycorine		0.2	1.70	2056	1066	9.88
			2	1.74	2048	1074	7.75
			20	1.31	1991	1063	5.83
2	H		2	3.04	2000	1037	13.50
			20	7.76	1977	1041	34.40
3	H		2	1.58	1995	1039	7.01
			20	1.63	1965	1038	7.26
4			2	1.75	2009	1033	7.77
			20	3.20	1984	1064	14.20
5			2	9.93	1733	916	45.80
			20	16.41	1707	933	77.90
6	H		2	1.44	1745	920	7.25
			20	1.57	1742	328	11.6
7			2	4.40	1767	1048	17.60
			20	10.66	1727	1082	42.60
8	H		2	1.15	1732	899	5.77
			20	1.34	17.31	947	6.73
9			2	12.7	1933	1012	56.60
			20	17.92	1980	1053	73.60
10	H		2	1.24	1750	909	6.22
			20	1.65	1807	917	12.50
11	H		2	1.17	1760	919	5.90
			20	3.52	1748	934	17.70
12	H		2	1.26	1755	911	6.33
			20	4.70	1723	927	23.60
13	H		2	1.12	1758	943	5.66
			20	2.69	1716	952	13.50
14			2	1.11	1743	958	5.60
			20	1.35	17.33	965	6.80
15	H		2	0.94	1747	943	4.73
			20	0.84	1738	908	4.22
16			2	1.49	1979	1038	6.64
			20	3.77	1989	1070	16.70

Table S1: Continuation



Compound	R_1	R_2	Conc (μM)	FAR[a]	FSC[b]	SSC[c]	FL-1[d]
17	H		2	1.26	1759	930	6.73
			20	1.17	1711	940	5.89
18			2	3.45	1783	1077	13.80
			20	8.36	17.40	1099	33.40
19	H		2	1.83	1950	1037	12.70
			20	7.17	2004	1081	31.80
20			2	5.08	1773	1053	20.30
			20	13.01	1736	1012	52.00
21	H		2	1.16	1788	1079	4.60
			20	2.82	1739	1040	11.30
22			2	5.43	1760	1115	21.70
			20	13.04	1770	1119	52.10
23			2	6.93	1766	1091	27.70
			20	14.84	1753	1106	59.30
24	H		2	1.53	1787	1146	59.30
			20	4.33	1787	1146	6.15
25			2	15.84	1980	1034	64.90
			20	21.78	1944	1101	87.00
26	H		2	1.76	1992	1061	7.81
			20	6.50	2008	1085	28.80
27			2	4.70	1787	1068	18.80
			20	11.41	1754	1056	45.60
28	H		2	1.15	1783	1069	4.60
			20	1.20	1738	1049	4.82
29			2	1.03	1782	1077	4.15
			20	0.95	1699	1005	3.82
30	H		2	1.05	1780	1078	4.22
			20	0.77	1785	1114	3.09
31	H		2	0.85	1815	1114	3.43
			20	2.70	1774	1097	10.80
32			2	2.52	1773	1046	10.10
			20	9.48	1757	1066	37.90
Verapamil	-	-	20	7.78	1781	1074	31.10

[a]FAR (fluorescence activity ratio) values were determined by using the equation shown in section 3.4.4. Verapamil at 20μM was used as positive control. DMSO 2% (negative control) FAR = 0.89; [b]FSC: Forward scatter count of cells in the sample; [c]SSC: Side scatter count of cells in the sample; [d]FL-1: Mean fluorescence intensity of the cells.

4. Flow cytometry data

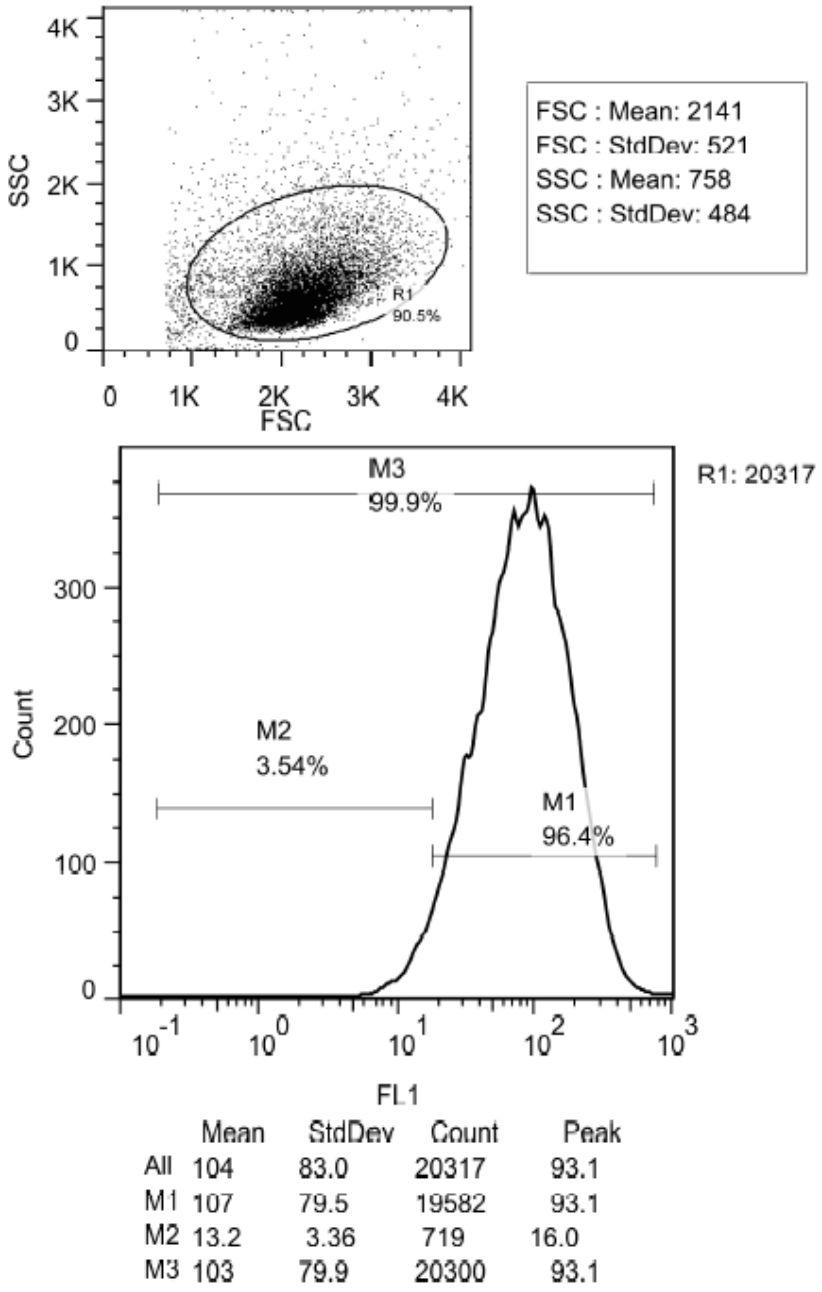


Figure S31: Flow cytometry data for sensitive human colon adenocarcinoma cells (Colo205).

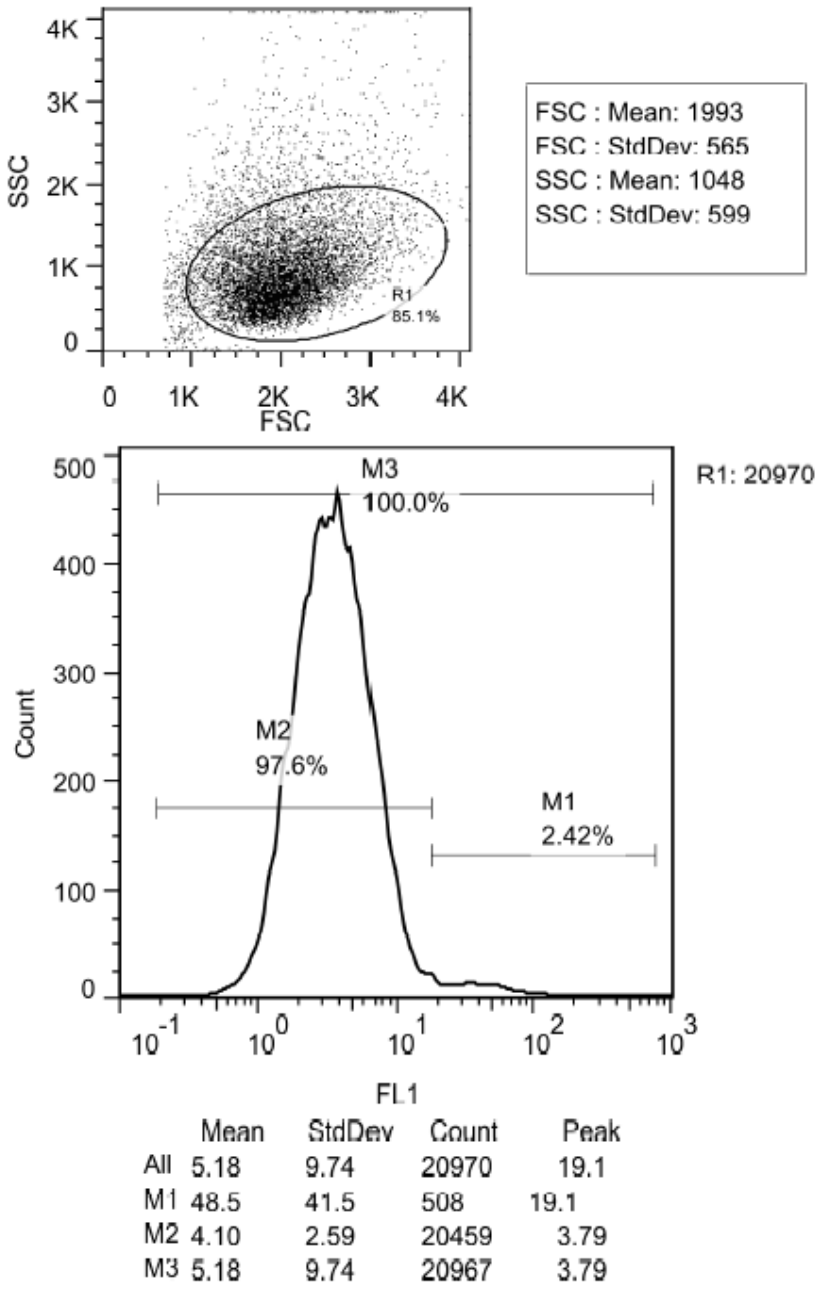


Figure S32: Flow cytometry data for resistant human colon adenocarcinoma cells (Colo320).

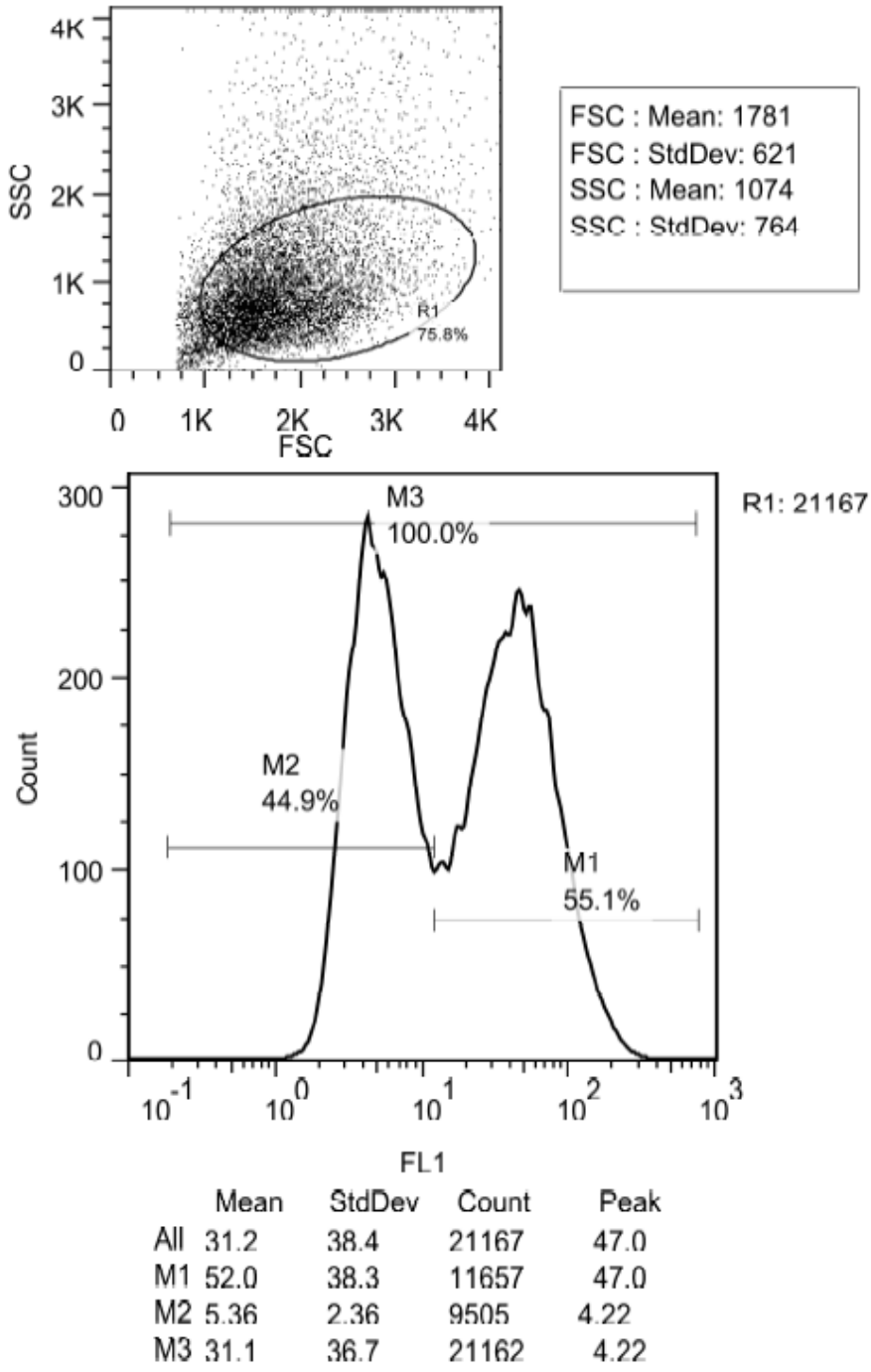


Figure S33: Flow cytometry data for verapamil (positive control) tested at 20 μM in resistant human colon adenocarcinoma cells (Colo 320).

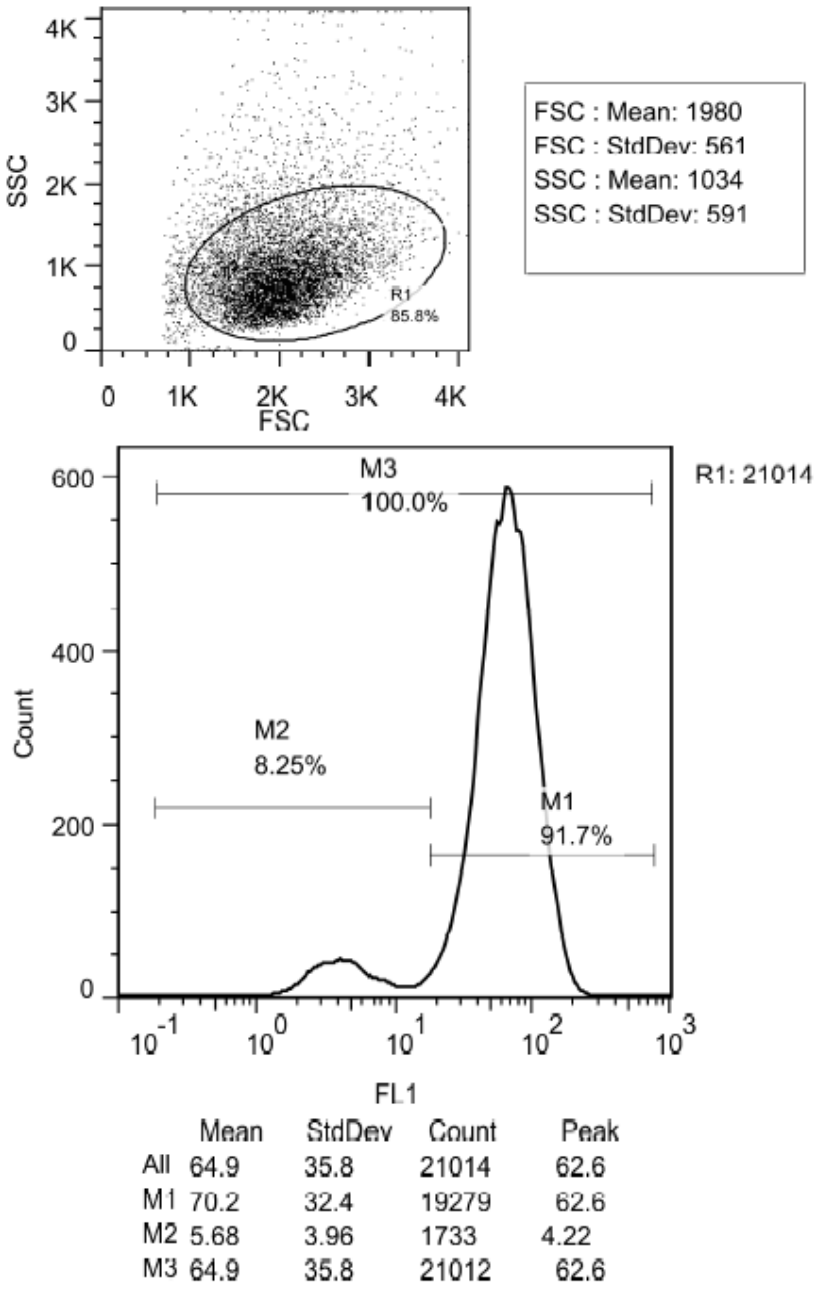


Figure S34: Flow cytometry data for compound **25** tested at 2 μM in resistant human colon adenocarcinoma cells (Colo320).

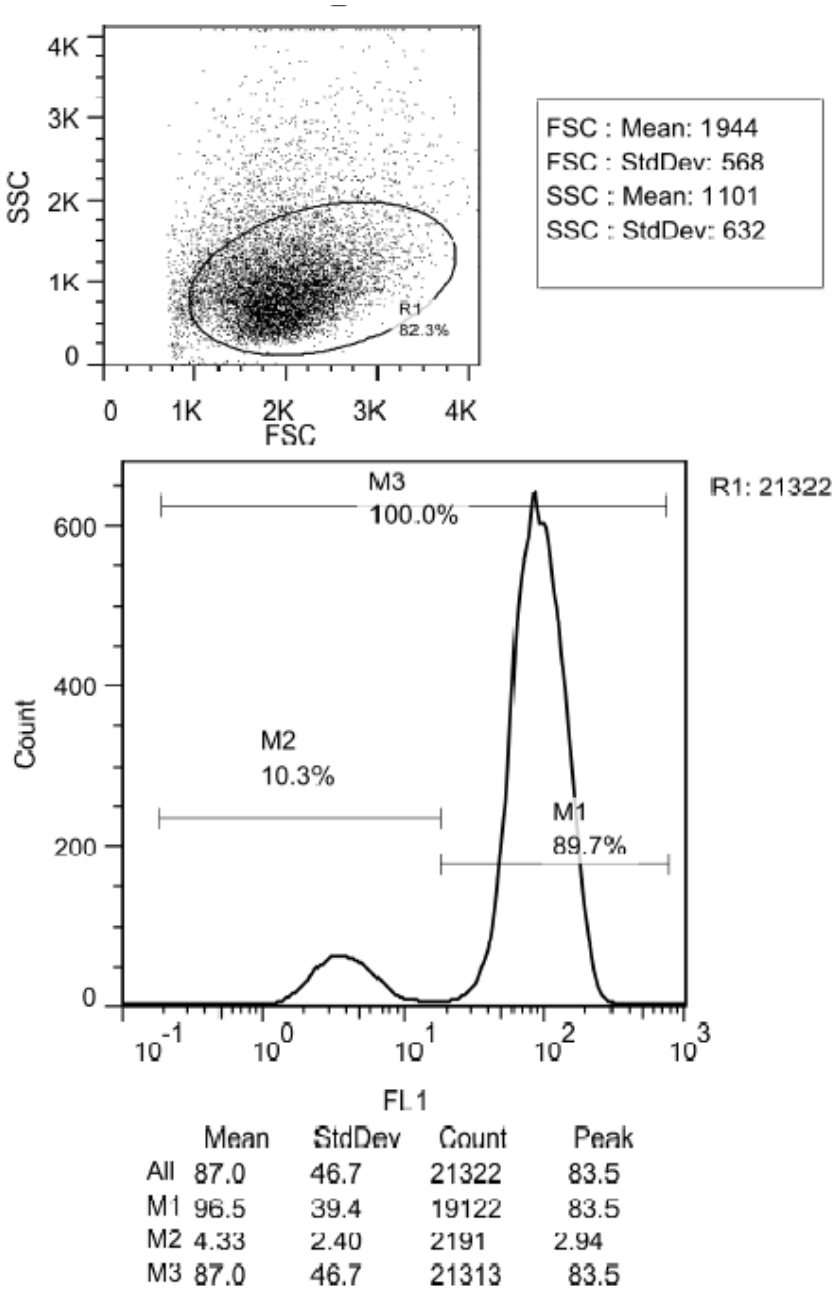


Figure S35: Flow cytometry data for compound **25** tested at 20 μM in resistant human colon adenocarcinoma cells (Colo320).

6. Combination chemotherapy results

Table S2: Effect of compounds **1**, **2**, **5**, **9**, **13**, **16**, **19**, **25**, and **26** in combination with doxorubicin on human adenocarcinoma cancer cell (Colo320).

Compound	Starting Conc. (μM)	Ratio*	CI at IC_{50}	SD	Type of interaction
1	2	1:1.68	1.33	0.19	Moderate antagonism
2	80	1:37.4	0.88	0.08	Slight synergism
5	65	1:60.7	0.13	0.014	Strong synergism
9	20	1:19	0.56	0.099	Synergism
13	156	1:289.6	0.69	0.089	Synergism
16	54	1:49.3	0.57	0.20	Synergism
19	15	1:28.3	0.88	0.20	Slight synergism
25	6	1:5.8	0.50	0.05	Synergism
26	76	1:35.1	0.88	0.18	Slight synergism

*Ratio: the applied combination and concentration of carbamates derivatives and doxorubicin. CI at IC_{50}: combination index (CI) at the 50 % growth inhibition dose (IC_{50}). CI < 0.1: very strong synergism; 0.1 < CI < 0.3: strong synergism; 0.3 < CI < 0.7: synergism; 0.7 < CI < 0.9: moderate to slight synergism; 0.9 < CI < 1.1: nearly additive; 1.10 < CI < 1.45: moderate antagonism; 1.45 < CI < 3.30: antagonism.

7. Physicochemical properties

Table S3: Physicochemical properties of compounds **1** – **32**.

Compounds	TPSA	MW	Log *P*	Nº H		MV
				Acceptors	Donnors	
1	62.16	287.31	0.74	5	2	249.40
2	80.27	454.91	3.33	6	2	383.50
3	80.71	400.43	1.22	7	1	347.48
4	116.84	641.72	4.71	9	2	575.22
5	98.37	581.67	5.23	7	2	523.65
6	80.27	434.49	2.99	6	2	386.52
7	135.31	641.63	4.07	11	2	538.38
8	98.73	464.47	2.41	8	2	393.89
9	98.37	581.16	4.70	7	2	524.13
10	80.27	434.49	2.72	6	2	386.76
11	98.50	450.49	2.69	7	2	395.51
12	71.48	434.49	3.02	6	1	386.91
13	71.48	446.50	2.95	6	1	393.11
14	80.79	481.54	3.22	7	0	427.60
15	71.48	384.43	1.98	6	1	338.50
16	80.79	509.60	4.0	7	0	461.20
17	71.48	398.45	2.37	6	1	355.30
18	98.37	665.83	7.21	7	2	622.90
19	80.27	476.57	3.98	6	2	436.15
20	98.37	589.59	4.89	7	2	500.39
21	80.27	438.45	2.82	6	2	374.89
22	98.37	689.60	6.65	7	2	553.12
23	98.37	617.64	4.98	7	2	533.99
24	80.27	452.48	2.86	6	2	391.70
25	98.37	650.55	6.00	7	2	551.20
26	80.27	468.93	3.37	6	2	400.30
27	98.37	589.59	4.89	7	2	500.39
28	80.27	438.45	2.82	6	2	374.89
29	98.37	457.52	3.03	7	2	414.44
30	80.27	372.42	1.89	6	2	331.92
31	80.71	464.51	3.03	7	1	412.45
32	98.37	4.61	4.62	7	2	490.53

TPSA, MW, and MV were determined by using Molispiration Cheminformatics (version September 2022, https://www.molinspiration.com/cgi-bin/properties).

Log *P* was determined by using pkCSM software (version September 2022, https://biosig.lab.uq.edu.au/pkcsm/prediction) [1].

8. Pharmacokinetic properties

Table S4: Calculated pharmacokinetic properties of verapamil and compounds **2** – **32.**

Compounds	Log *S* (mol/L)	Caco-2 Permeability (log Papp in 10^{-6} cm/s)	Intestinal absorption (%)	Fractional unbound (fu)	CNS permeability (log PS)	CYP3A4 inhibitor (Yes/No)
Verapamil	− 5.13	1.36	94.23	0	− 2.49	Yes
2	− 3.79	1.09	93.28	0.12	− 2.29	No
3	− 2.50	− 0.19	52.26	0.422	− 3.36	No
4	− 4.61	0.87	91.35	0.043	− 3.23	Yes
5	− 4.86	0.83	98.6	0.0	−2.94	Yes
6	− 3.65	1.13	94.73	0.13	−2.33	No
7	− 3.53	1.04	97.6	0.15	−3.47	Yes
8	− 3.52	0.96	93.51	0.15	−3.31	Yes
9	− 4.58	1.01	100	0.008	−2.79	Yes
10	− 3.78	0.99	95.18	0.089	−2.48	Yes
11	− 3.67	1.055	95.62	0.151	−3.18	Yes
12	− 3.72	0.99	94.45	0.10	−2.32	No
13	− 3.39	1.05	95.02	0.12	−2.17	No
14	− 3.20	1.19	95.05	0.33	−3.23	No
15	− 2.45	1.07	95.94	0.39	−2.51	No
16	− 3.48	1.09	94.27	0.30	−3.08	Yes
17	− 2.67	1.07	95.56	0.37	−2.42	No
18	− 4.45	0.96	100	0.046	−2.50	Yes
19	− 4.40	1.07	93.16	0.026	−2.09	Yes
20	− 4.58	1.01	100	0.008	−3.10	Yes
21	− 3.71	1.03	93.8	0.106	−3.10	No
22	− 4.65	0.84	95.2	0.0	−2.79	Yes
23	− 4.92	0.94	100	0.10	−3.10	Yes
24	−3.90	1.01	94.6	0.13	−3.13	Yes
25	− 4.92	0.82	100	0.0	−2.82	Yes
26	−3.98	1.09	93.80	0.10	−2.37	Yes
27	− 4.61	0.91	100	0.10	−3.09	Yes
28	− 3.71	0.98	94.13	0.16	−3.11	Yes
29	− 3.53	0.90	94.91	0.30	− 3.27	Yes
30	− 2.61	1.04	95.67	0.37	−2.69	No
31	− 3.78	1.07	95.41	0.14	−3.12	Yes
32	− 4.61	1.07	100	0	−2.95	Yes

Pharmacokinetic values were obtained using pkCSM software after conversion SMILES format, as described by Pires et al. [2]

9. References

1. Pires, D.E. V.; Blundell, T.L.; Ascher, D.B. PkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. *J. Med. Chem.* **2015**, *58*, 4066–4072.