Electronic Supplementary Material (ESI) for RSC Advances.

Antiviral Activity of Sulphated Specialized Metabolites from Sea Urchin *Clypeaster humilis*: *in vitro* and *in silico* studies

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Contents:

NMR data of known compunds:

2'-Deoxyuridine (3):

^{1}H-NMR spectrum (400 MHz, DMSO-d_6): δ_H 7.84 (1H, d, J = 8.0, H-4), 6.15 (1H, t, J = 6.4, H-1'), 5.61 (1H, d, J = 8.0, H-3), 4.22 (1H, m, H-3'), 3.77 (1H, m, H-4'), 3.55 (1H, m, H-5'), 2.07 (2H, m, H-2').

DEPT-Q spectrum (100 MHz, DMSO-d_6): δ_C 39.93 (C-2′), 61.26 (C-5′), 70.36 (C-3′), 84.04 (C-1′), 87.04 (C-4′), 101.62 (C-3), 140.56 (C-4), 150.57 (C-2), 163.37 (C-6).

Inosine (4):

^{1}H-NMR spectrum (400 MHz, DMSO-d_6): δ_H 8.32 (1H, s, H-3), 8.07 (1H, s, H-3), 5.86 (1H, d, J = 5.6, H-5), 3.0-5.0 (5H, ribose protons).

^{13}C-NMR spectrum (100 MHz, DMSO-d_6): δ_C 138.6 (C-1), 146.3 (C-2), 148.3 (C-3), 157.2 (C-4), 124.5 (C-5), 87.6 (C-1′), 70.4 (C-2′), 74.1 (C-3′), 85.7 (C-4′), 61.4 (C-5′).

Thymidine (5):

^{1}H-NMR spectrum (500 MHz, DMSO-d_6): δ_H 7.69 (1H, s, H-6), 6.16 (1H, t, J = 7.0, H-1′), 4.23 (1H, br.s, H-3′), 3.75 (1H, m, H-4′), 3.59 (1H, dd, J = 4.0, 12.0, H-5′a), 3.53 (1H, dd, J = 4.0, 12.0, H-5′b), 2.08 (2H, m, H-2′), 1.76 (3H, s, CH_3).

^{13}C-NMR spectrum (125 MHz, DMSO-d_6): δ_C 163.8 (C-4), 150.5 (C-2), 136.2 (C-6), 109.4 (C-5), 87.3 (C-4′), 83.8 (C-1′), 70.5 (C-3′), 61.4 (C-5′), 40.0 (C-2′), 12.3 (CH_3).

Uracil (6):

^{1}H-NMR spectrum (500 MHz, DMSO-d_6): δ_H 7.38 (1H, d, J = 8 Hz) and 5.44 (1H, d, J = 7.5 Hz).

^{13}C-NMR spectrum (125 MHz, DMSO-d_6): δ_C 164.4 (C-4), 151.6 (C-2), 142.3 (C-6) and 100.2 (C-5).

Glyceryl monopalmitate (7):

The negative HR-ESI-MS analysis showed a characteristic molecular ion peak $[M+HCOO]^-$ at *m/z* 375.2772 (calcd for $C_{20}H_{39}O_6$, 375.2747).

^{1}H-NMR spectrum (500 MHz, $CDCl_3$): δ_H 4.21 (1H, dd, J = 11.5, 4.5 Hz, H-1a), 4.15 (1H, dd, J = 12.0, 6.5 Hz, H-1b), 3.93 (1H, m, H-2), 3.69 (1H, dd, J = 11.5, 4.0 Hz, H-3a), 3.61 (1H, dd, J = 11.5, 6.0 Hz, H-3b), 2.36 (2H, t, J = 7.5 Hz, H-2′), 0.88 (3H, t, J = 7.0 Hz, H-16′).

^{13}C-NMR spectrum (125 MHz, $CDCl_3$): δ_C 174.5 (C-1), 34.3 (C-2), 32.1 (C-3), 29.8-22.8 (C-4~15), 14.3 (C-16), 65.3 (C-1′), 70.4 (C-2′), 63.5 (C-3′).

Benzyl benzoate (8):

^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ_H 8.10 (2H, d, J = 8, H-2'/H-6'), 8.08 (2H, d, J = 8, H-2/H-6), 7.58 (1H, t, J = 7.5, H-4'), 7.55 (1H, t, J = 7.5, H-4), 7.45 (2H, m, H-3'/H-5'), 7.40 (2H, m, H-3/H-5), 5.38 (s, 2H).

DEPT-Q spectrum (100 MHz, $CDCl_3$): δ_C 166.6 (C-7'), 136.3 (C-1), 133.2 (C-4'), 129.8 (C-1'), 128.7 (C-2'/C-6'), 128.5 (C-4), 128.4 (C-3'/C-5'),128.4 (C-2/C-6), 128.3 (C-3/C-5), 66.8 (C-7).

Methyl-(*E*)-nonadec-10-enoate (9):

The positive HR-ESI-MS analysis showed a characteristic molecular ion peak $[M+K]^+$ at *m/z* 349.1776 (calcd for $C_{20}H_{38}KO_2$, 349.2509).

^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ_H 5.33-5.36 (2H, m, H-11/H-10), 3.66 (3H, s, H-1'), 2.30 (2H, t, J = 8.0, H-2), 1.99 (4H, m, H-9/H-12), 1.62 (2H, m, H-3), 1.25-1.32 [22H, m, (H-4 to H-8) and (H-13 to H-18)], 0.88 (3H, t, J = 7.2, H-19).

DEPT-Q spectrum (100 MHz, $CDCl_3$): δ_C 174.29 (C-1), 130.14 (C-11), 129.90 (C-10), 51.60 (C-1'), 34.26 (C-2), 31.93 (C-17), 29.88-29.14 [(C-4) to (C-8) and (C-13) to (C-16)], 27.37 (C-12), 27.31 (C-9), 25.10 (C-3), 22.81 (C-18), 14.26 (C-19).

Cholesterol (10):

^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ_H 0.67 (3H, s, H-18), 0.86 (3H, d, J = 6.6, H-26), 0.84 (3H, d, J = 6.6, H-27), 0.91 (3H, d, J = 6.5, H-21), 1.02 (3H, s, H-19), 1.51 (2H, m, H-2), 1.99 (2H, m, H-7), 1.82 (2H, m, H-8), 2.26 (2H, m, H-4), 3.52 (1H, m, H-3), 5.34 (1H, br.d, J = 5.2, H-6).

DEPT-Q spectrum (100 MHz, $CDCl_3$): δ_C 12.01 (C-18), 18.86 (C-21), 19.55 (C-19), 21.23 (C-11), 22.71 (C-26), 22.98 (C-27), 23.97 (C-23), 24.44 (C-15), 28.16 (C-25), 28.38 (C-16), 31.76 (C-2), 32.02 (C-7), 32.05 (C-8), 35.94 (C-20), 36.33 (C-22), 37.39 (C-10), 39.65 (C-1), 39.66 (C-24), 39.92 (C-12), 42.40 (C-4), 42.41 (C-13), 50.26 (C-9), 56.28 (C-17), 56.90 (C-14), 71.95 (C-3), 121.86 (C-6), 140.86 (C-5).

1,2-Di-*O*-palmitoyl-3-*O*-(6'''-sulfo-*α*-D-quinovopyranosyl)-glycerol (11):

The negative HR-ESI-MS analysis showed a characteristic molecular ion peak $[M-H]^-$ at *m/z* 793.5311 (calcd for $C_{41}H_{77}O_{12}S$, 793.5136).

^{1}H-NMR spectrum (400 MHz, DMSO-d_6): δ_H 5.13 (1H, m, H-2), 4.57 (1H, d, J = 3.6, H-1'''), 4.34 (1H, dd, J = 2.8, 12.0, H-1a), 4.14 (1H, dd, J = 7.6, 12.0, H-1b), 3.88 (1H, dd, J = 6.0, 10.4, H-3a), 3.39 (1H, m, H-3b), 3.77 (1H, m, H-5'''), 3.34 (1H, m, H-3'''), 3.18 (1H, m, H-2'''), 2.92 (1H, m, H-4'''), 2.90 (1H, dd, J = 4.4, 14.0, H-6'''a), 2.55 (1H, dd, J = 6.8, 14.0, H-6'''a), 2.27 (2H, m, H-2'), 2.27 (2H, m, H-2''), 1.49 (2H, m, H-3'), 1.49 (2H, m, H-3''), 1.23 (24H, m, H-4' to H-15'), 1.23 (24H, m, H-4'' to H-15''), 0.85 (3H, t, J = 7.2, H-16'), 0.85 (3H, t, J = 7.2, H-16'').

DEPT-Q spectrum (100 MHz, DMSO-d_6): δ_C 172.55 (C-1'), 172.39 (C-1''), 98.29 (C-1'''), 74.29 (C-4'''), 72.91 (C-3'''), 71.63 (C-2'''), 69.73 (C-2), 68.56 (C-5'''), 64.61 (C-3), 62.65 (C-1), 54.57 (C-6'''), 33.58 (C-2'), 33.45 (C-2''), 31.33 (C-3'), 31.33 (C-3''), 29.11-22.13 (C-4') to (C-15'), 29.11-22.13 (C-4'') to (C-15''), 13.97 (C-16'), 13.97 (C-16'').

Boldine (12):

The positive HR-ESI-MS analysis (Figure S35) showed a characteristic molecular ion peak $[M+H]^+$ at *m/z* 328.1589 (calcd for $C_{19}H_{22}NO_4$, 328.1549).

^{1}H-NMR spectrum (400 MHz, CD_3OD): δ_H 2.76 (3H, s, N-CH3), 2.60-2.78 (3H, m, H-7b, H-4), 2.88-3.12 (4H, m, H-6a, H-5, H-7a), 3.58 (3H, s, 2-OCH3), 3.86 (3H, s, 10-OCH3), 6.59 (1H, s, H-3), 6.76 (1 H, s, H-8), 7.96 (1H, s, H-11).

DEPT-Q spectrum (100 MHz, CD_3OD): δ_C 28.05 (C-4), 33.74 (C-7), 42.64 (N-CH3), 53.92 (C-5), 56.57 (10- OCH3), 60.34 (1-OCH3), 63.77 (C-6a), 112.73 (C-11), 115.14 (C-3), 115.98 (C-8), 124.02 (C-11c), 124.38 (C-11b), 128.17 (C-11a), 128.96 (C-3a), 129.36 (C-7a), 144.77 (C-1), 147.39 (C-9), 148.06 (C-10), 151.41 (C-2).

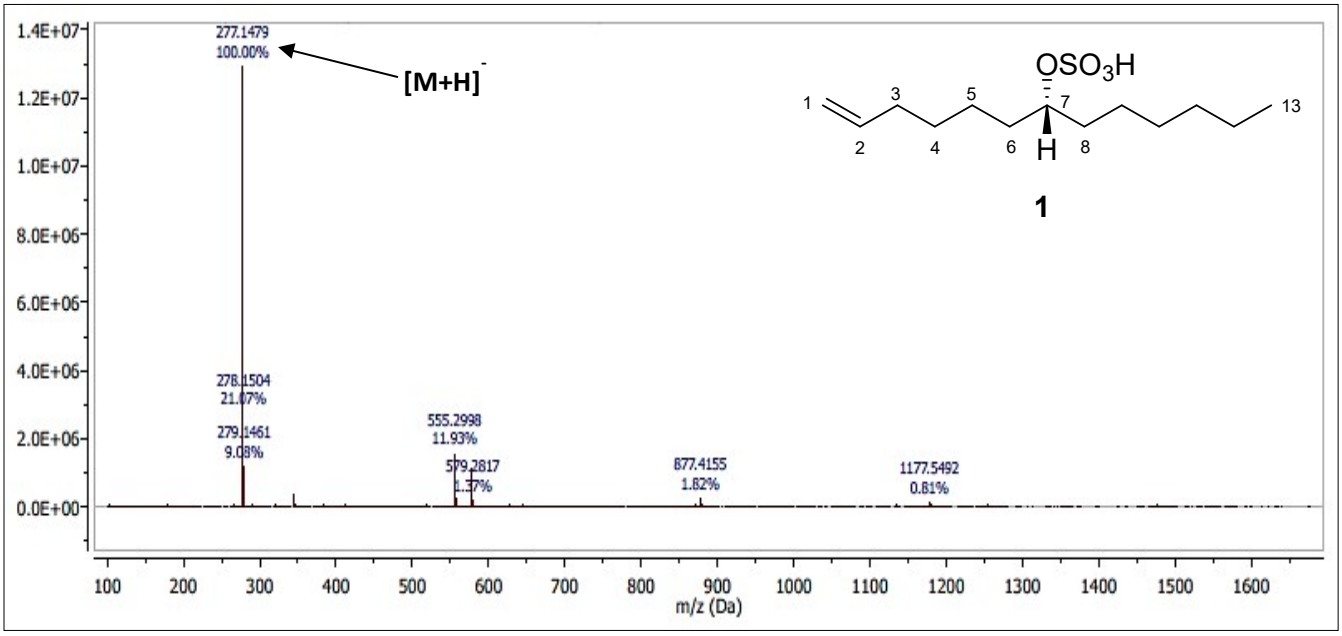

Figure S1. Negative HR-ESI-MS spectrum of compound **1**.

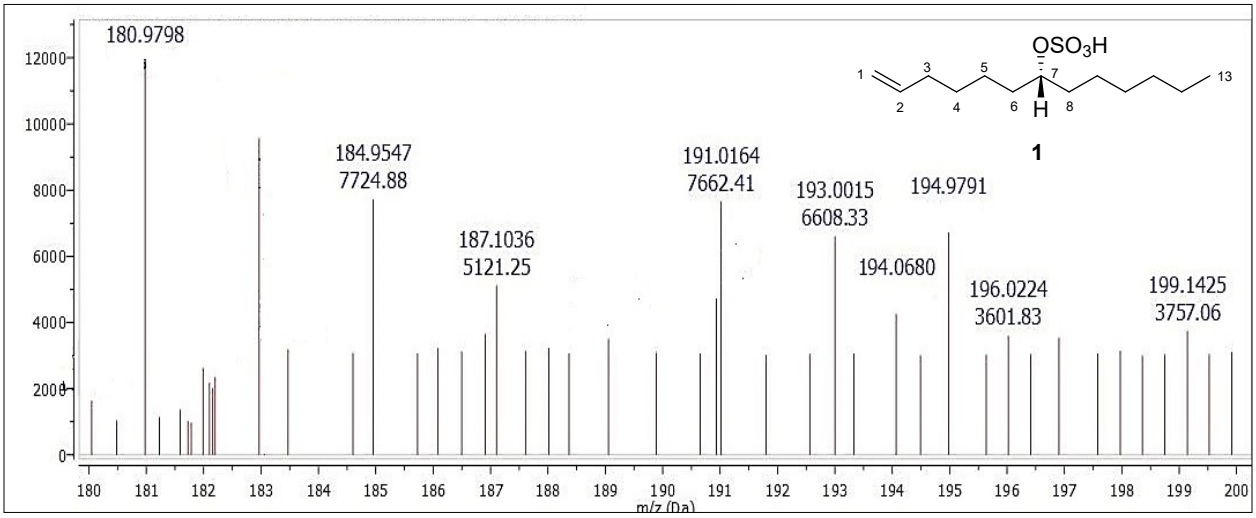

Figure S2. Expanded negative HR-ESI-MS spectrum of compound **1**.

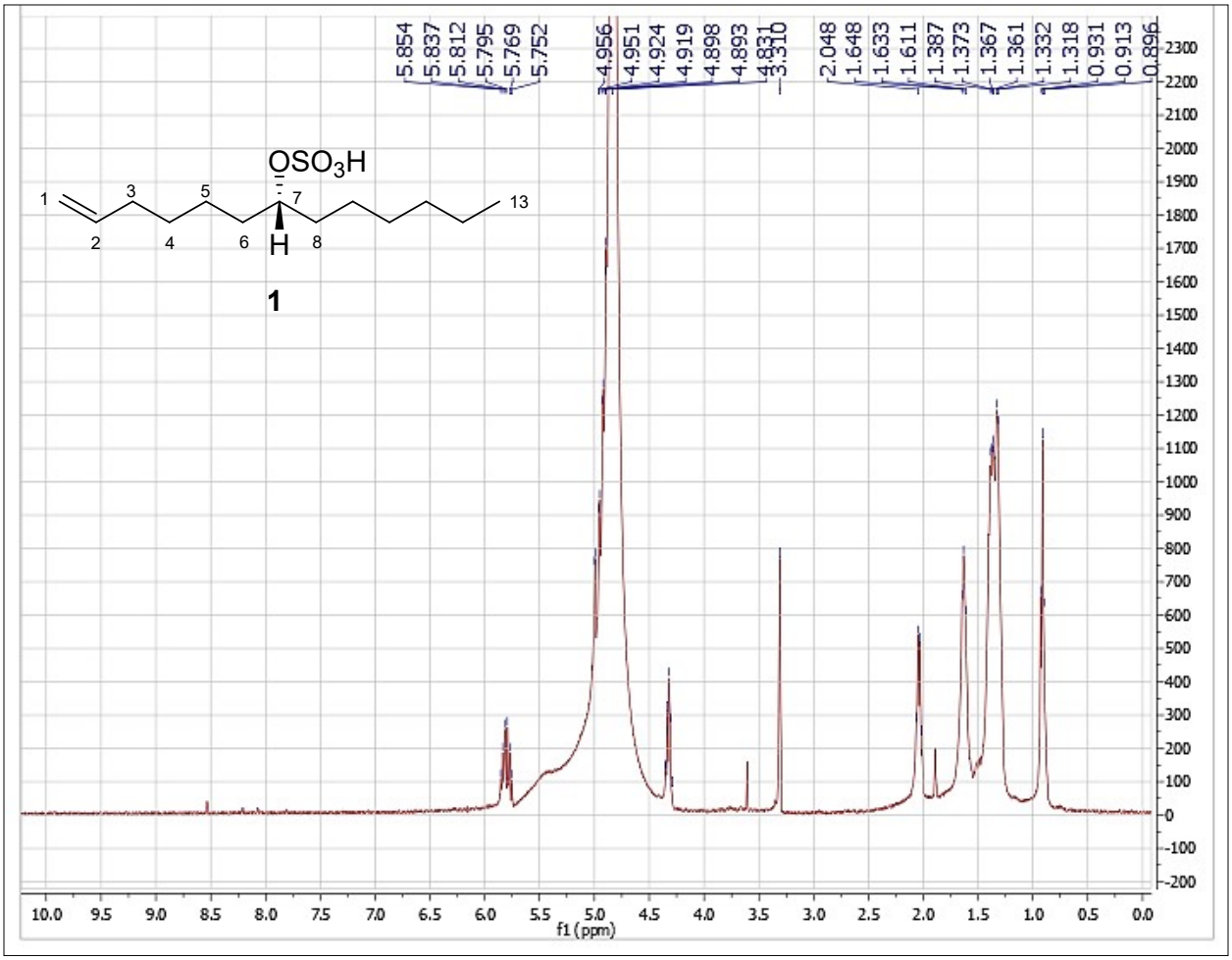

Figure S3. ^{1}H-NMR spectrum of compound **1** (400 MHz, CD_3OD).

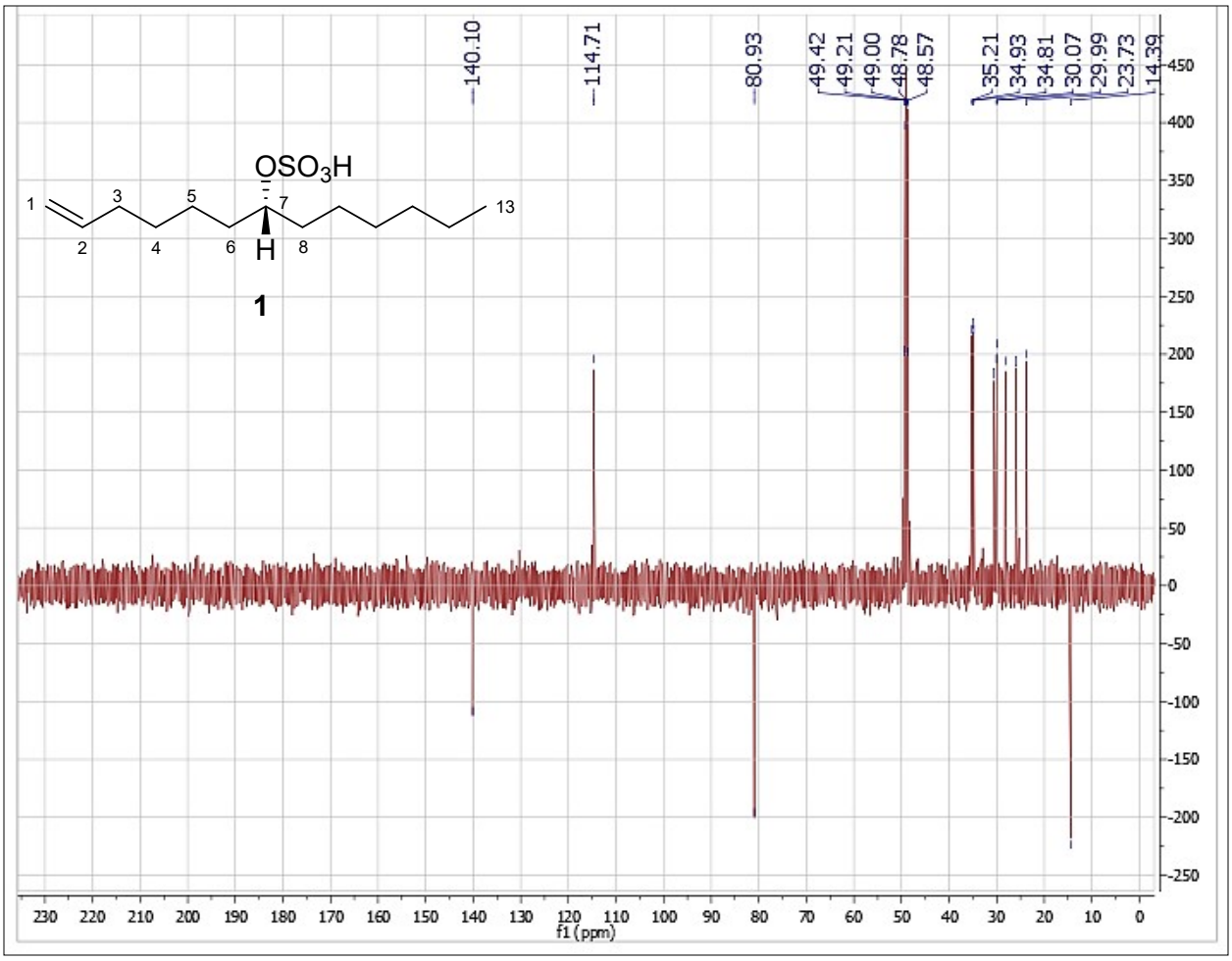

Figure S4. DEPT-Q spectrum of compound **1** (100 MHz, CD_3OD).

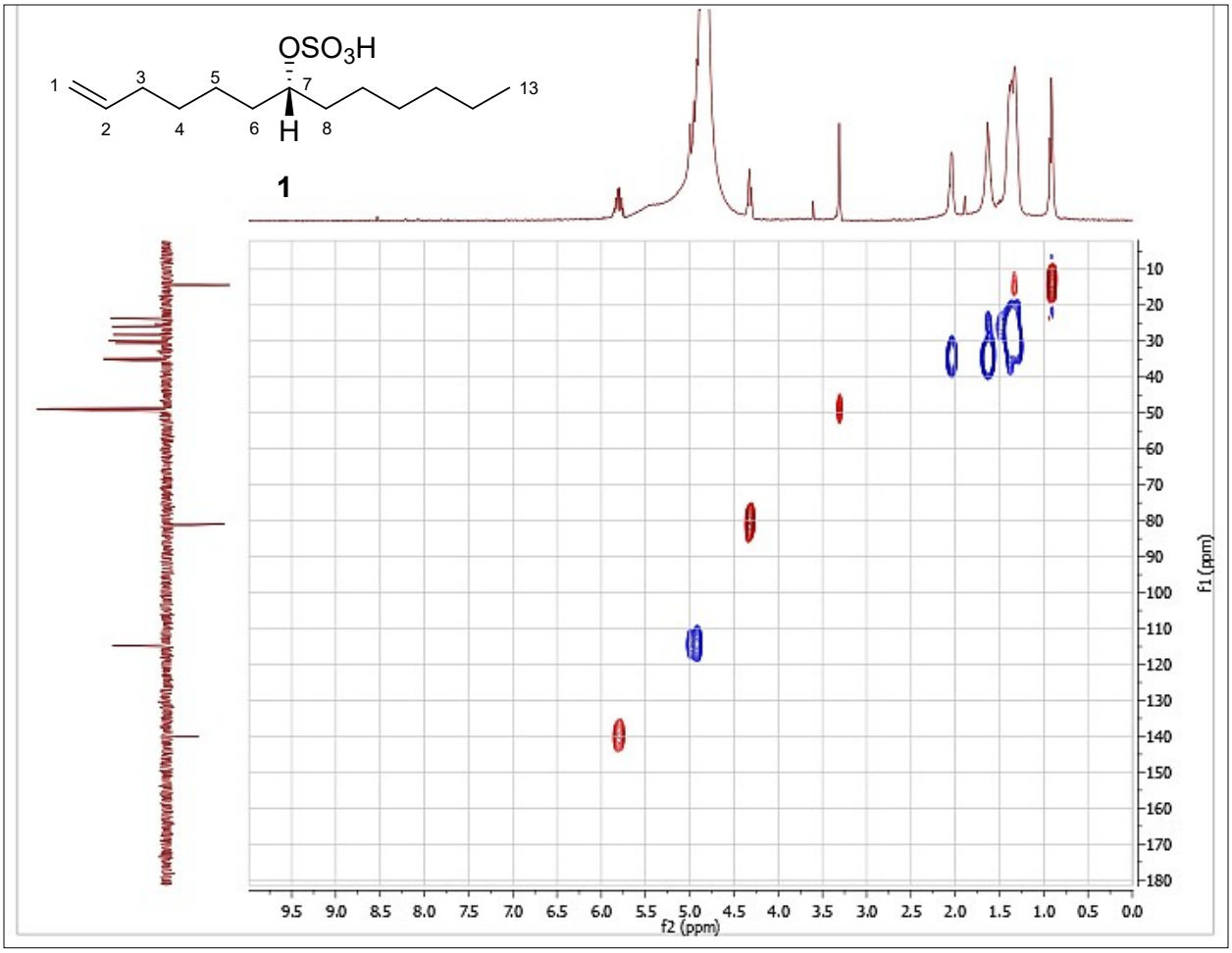

Figure S5. HSQC spectrum of compound **1** (CD_3OD).

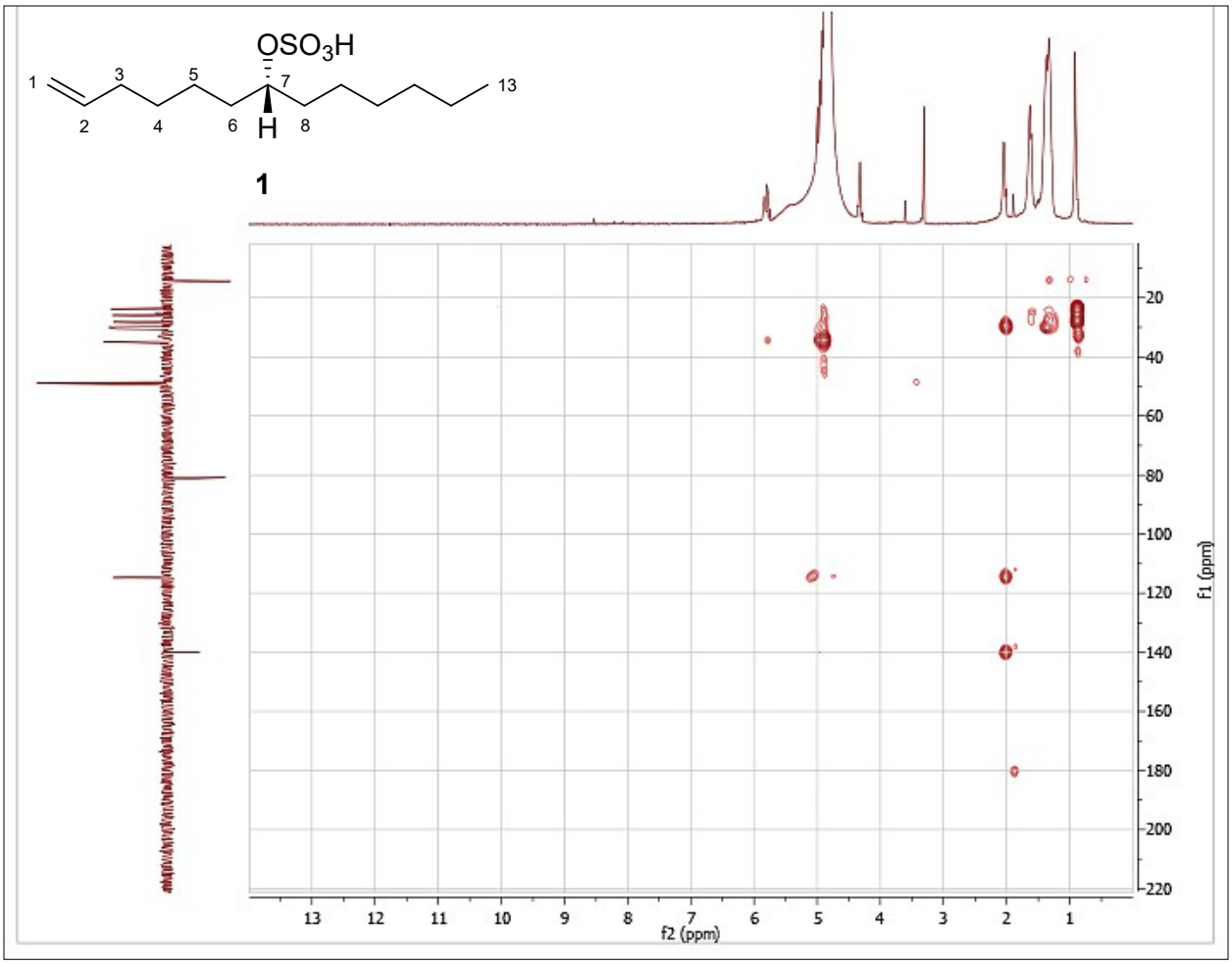

Figure S6. HMBC spectrum of compound **1** (CD_3OD).

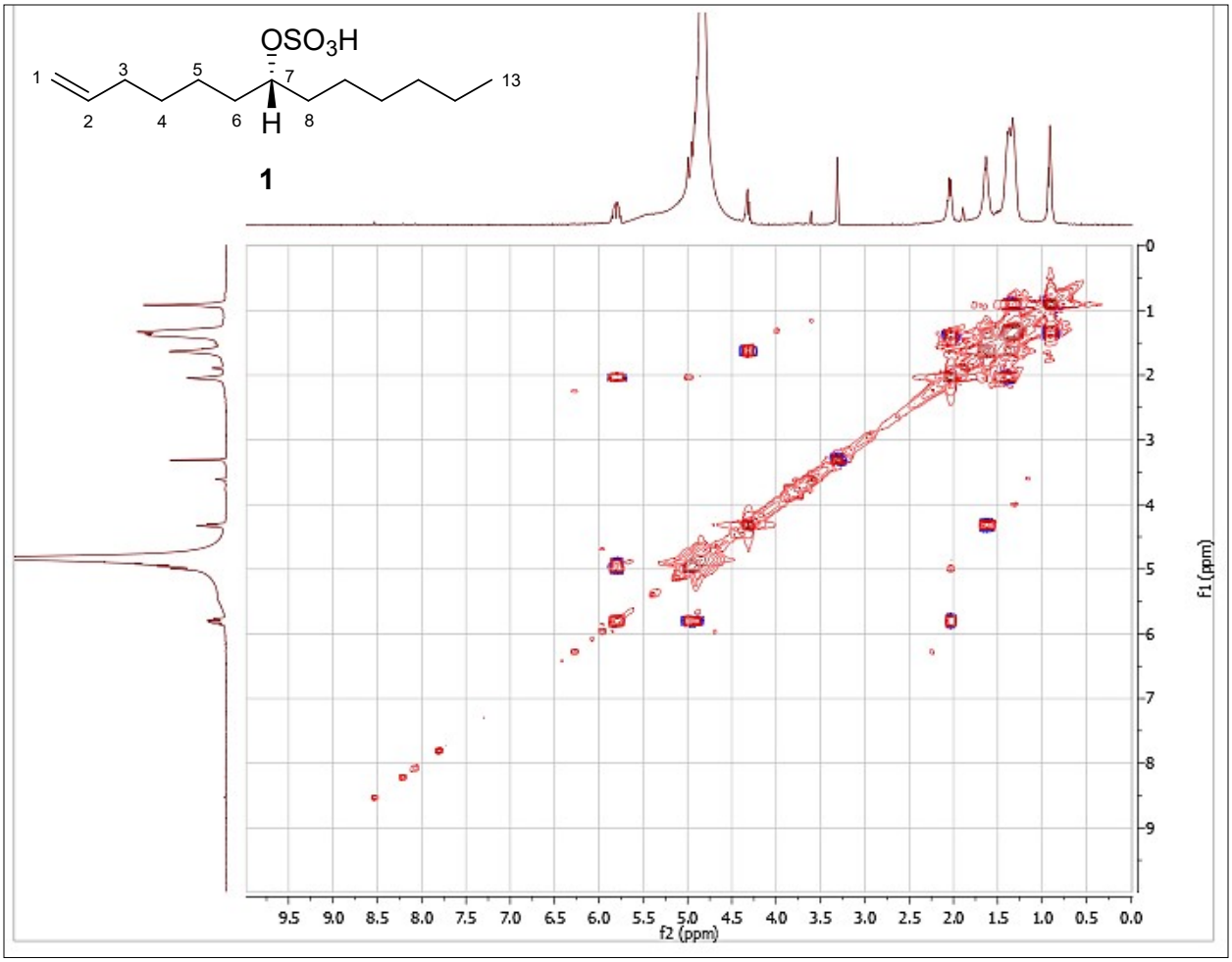

Figure S7. ^{1}H-^{1}H COSY spectrum of compound **1** (CD_3OD).

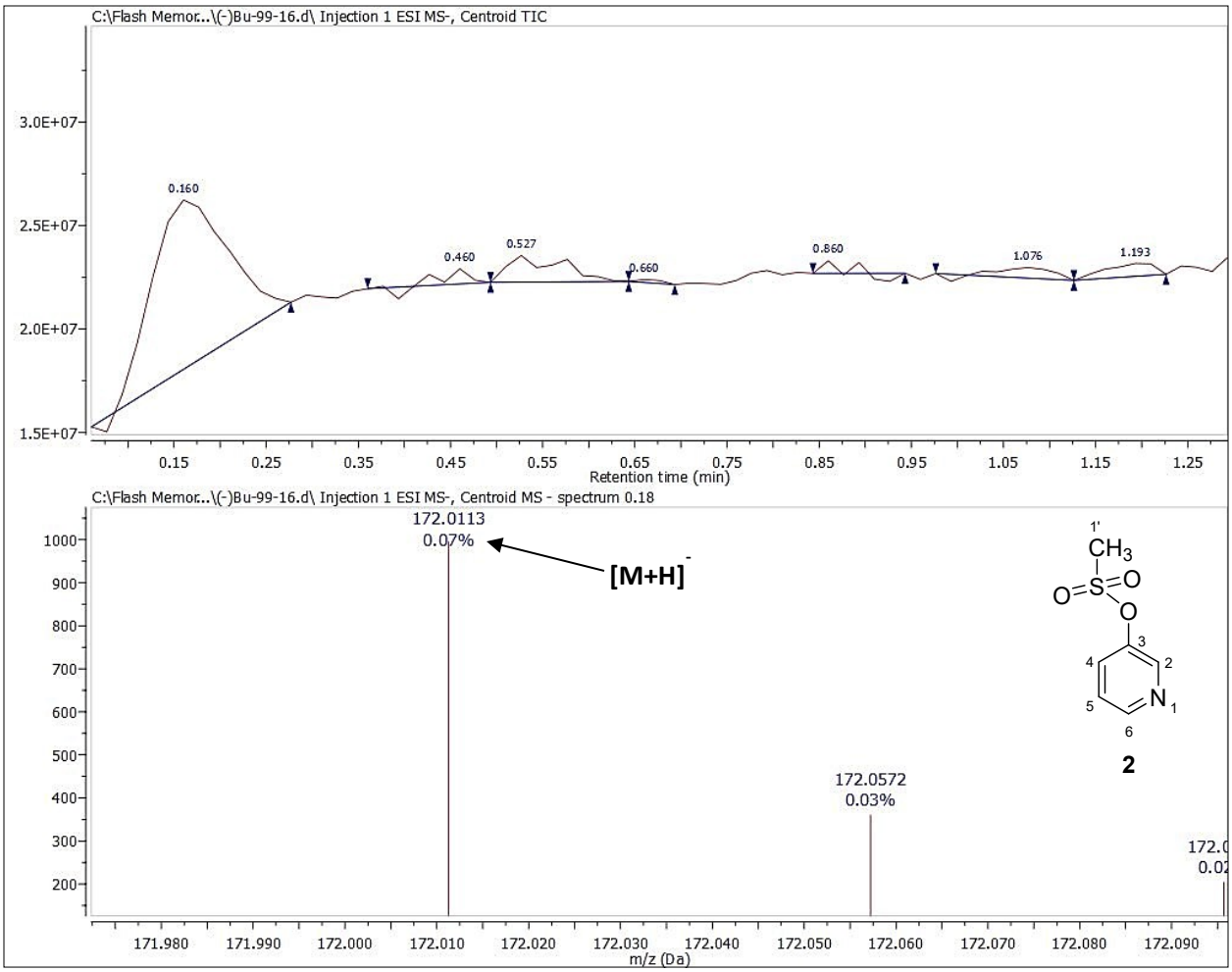

Figure S8. Negative HR-ESI-MS spectrum of compound **2**.

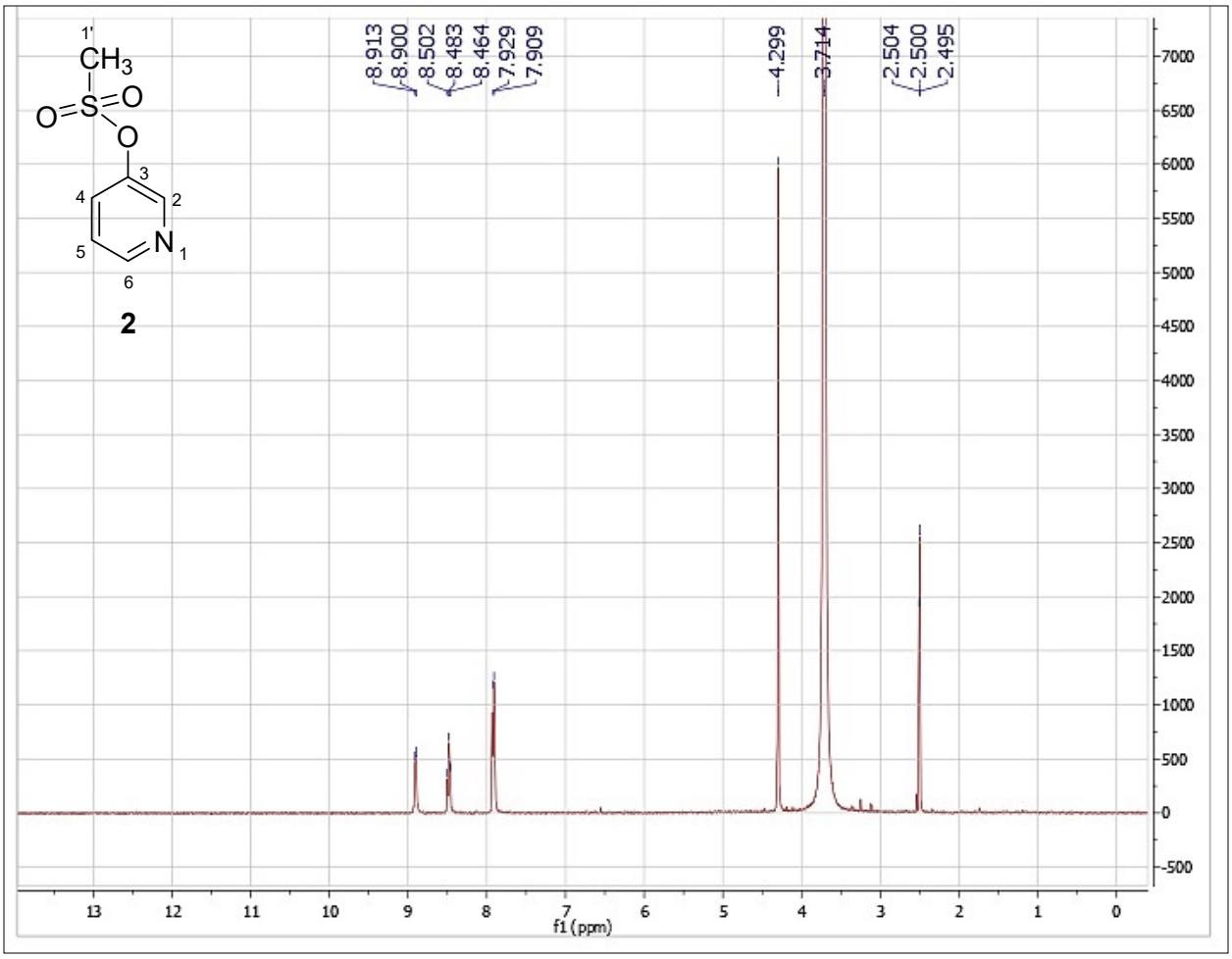

Figure S9. ^{1}H-NMR spectrum of compound **2** (400 MHz, DMSO-d_6).

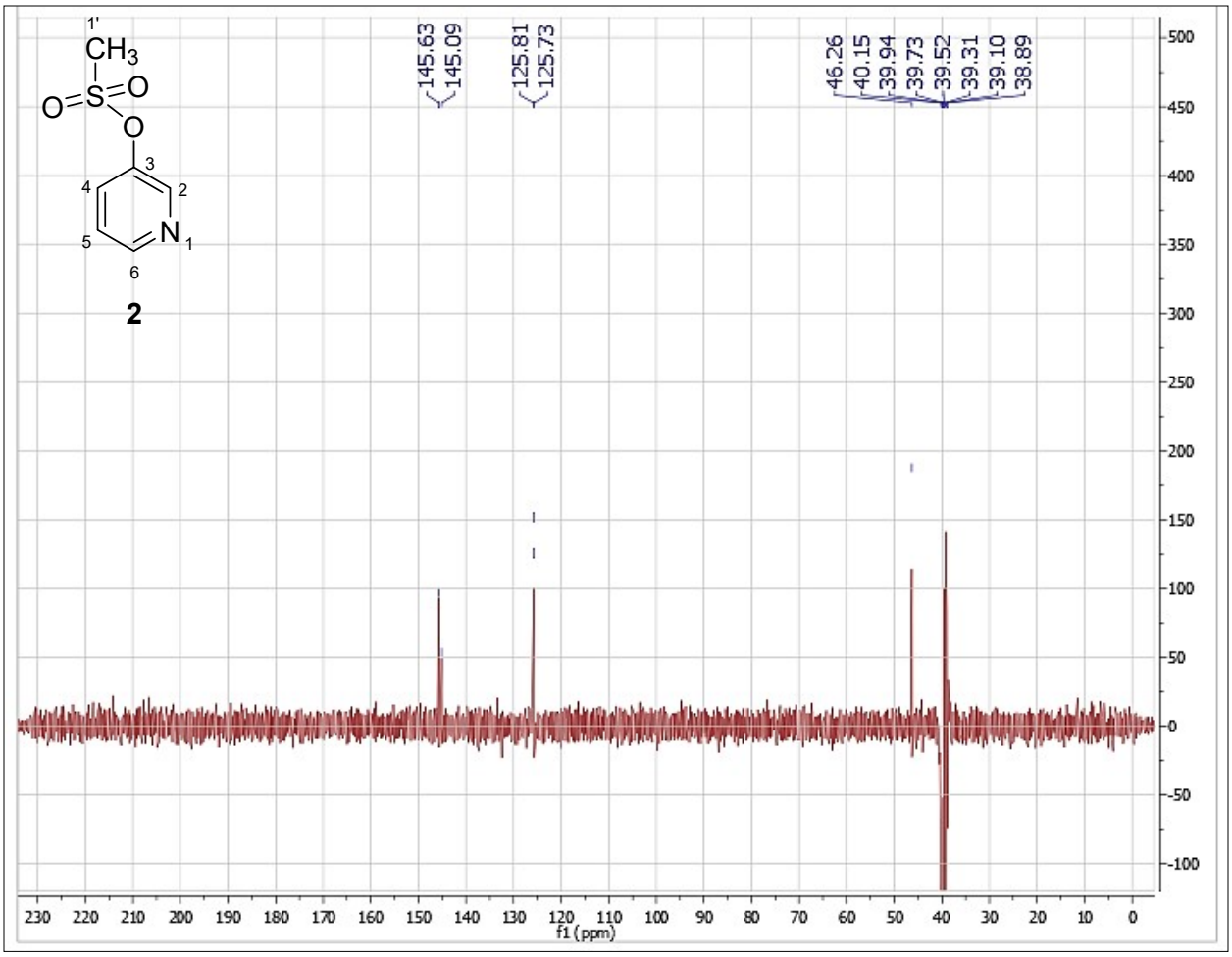

Figure S10. DEPT-Q spectrum of compound **2** (100 MHz, DMSO-d_6).

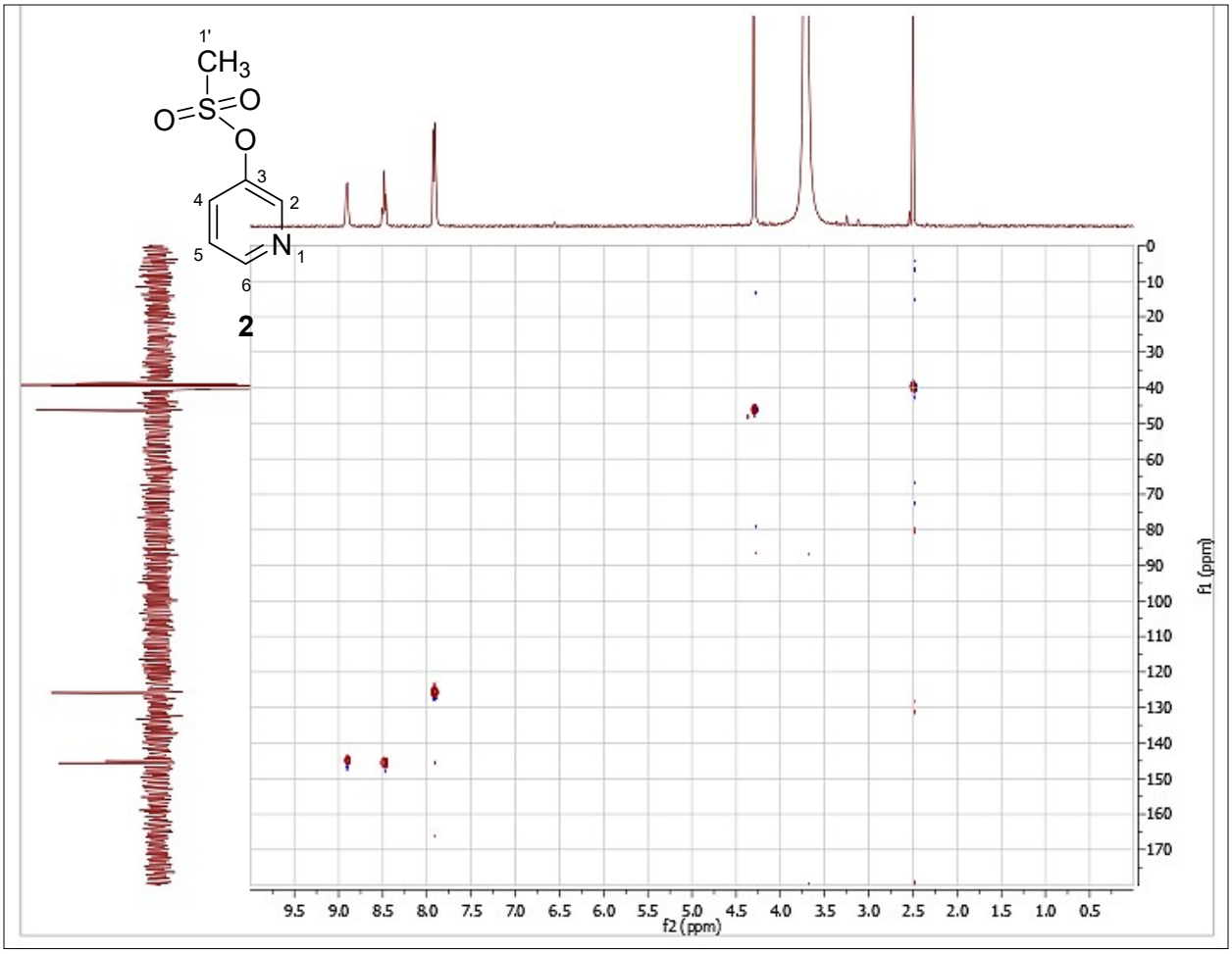

Figure S11. HSQC spectrum of compound **2** (DMSO-d_6).

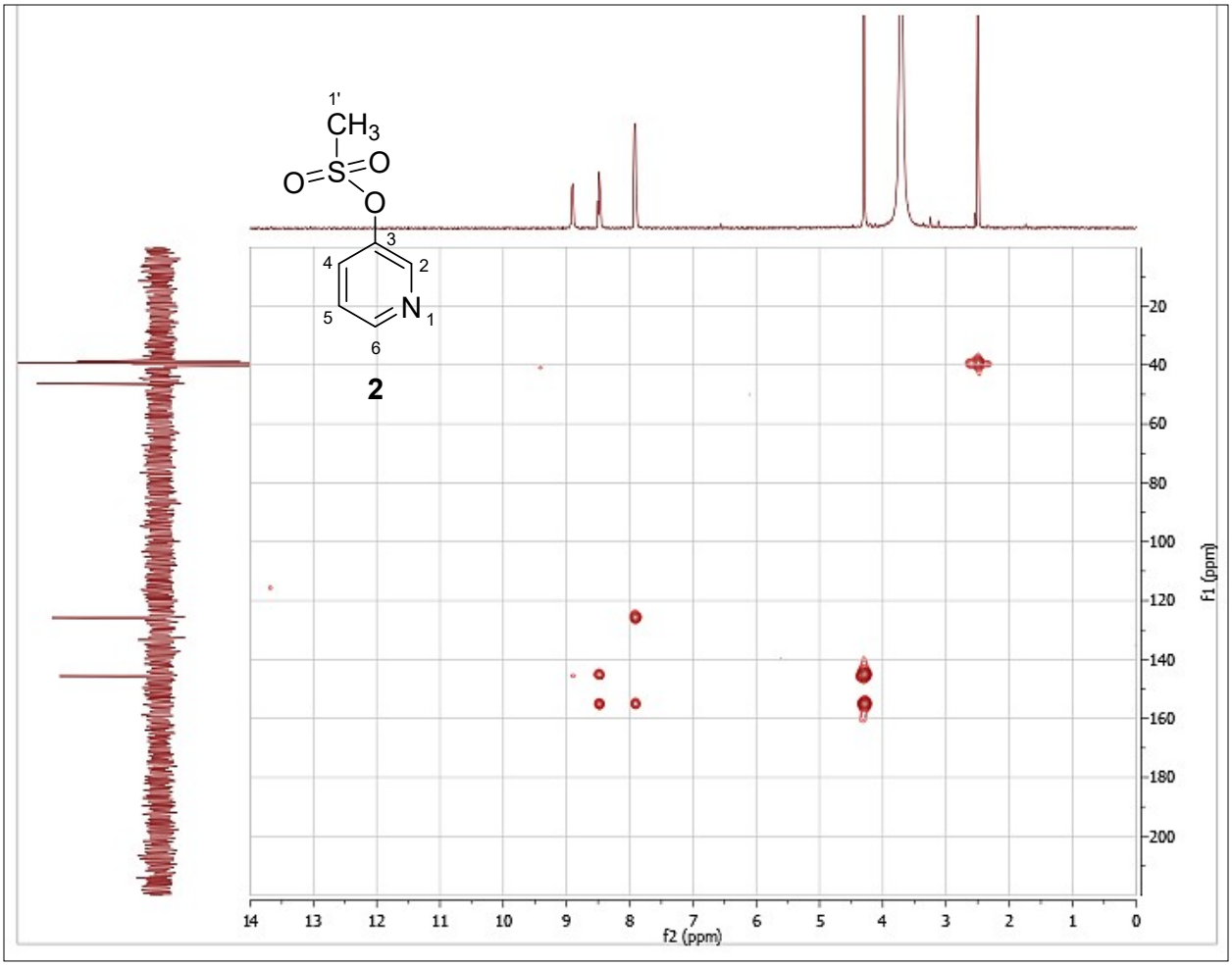

Figure S12. HMBC spectrum of compound **2** (DMSO-d_6).

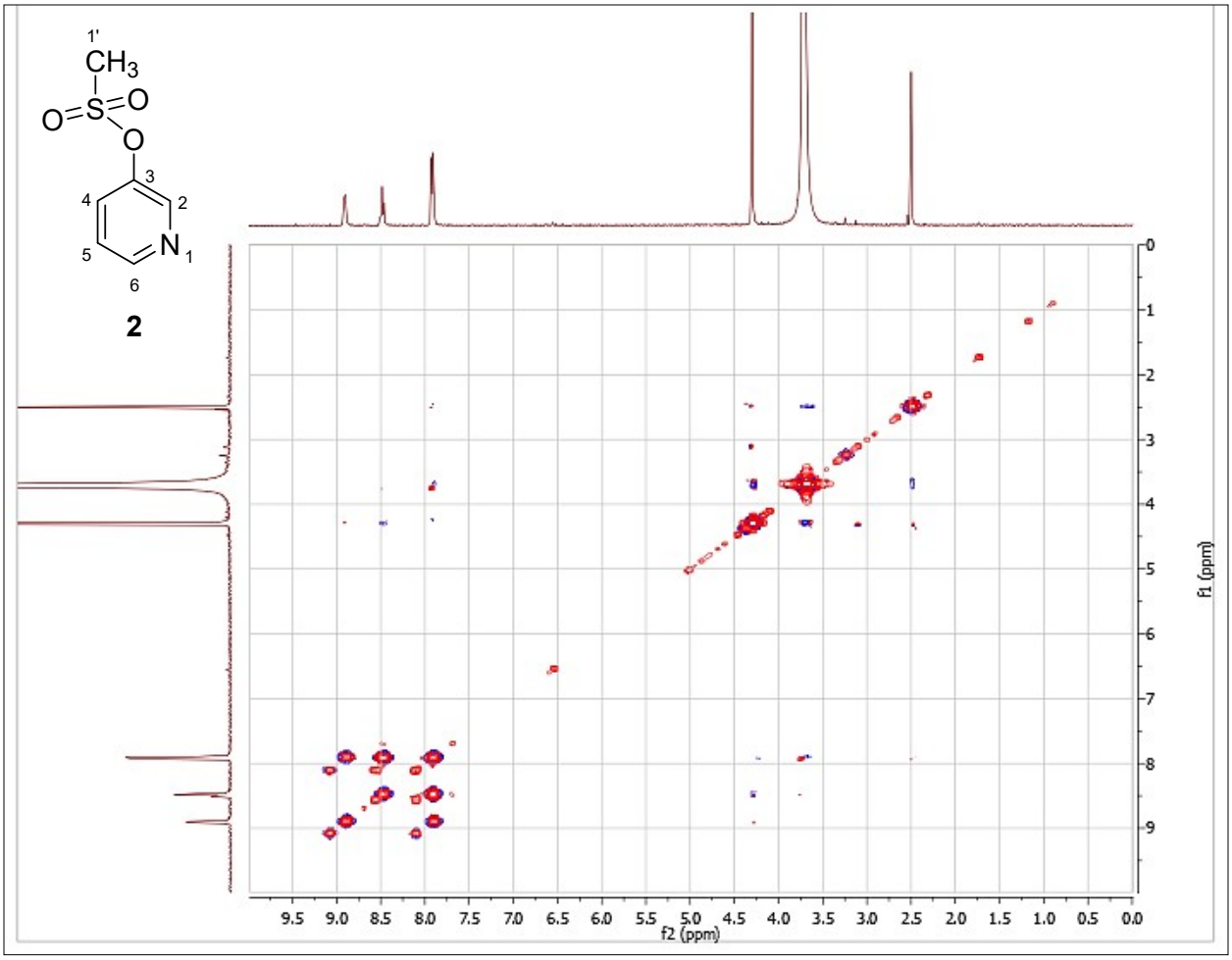

Figure S13. ^{1}H-^{1}H COSY spectrum of compound **2** (DMSO-d_6).

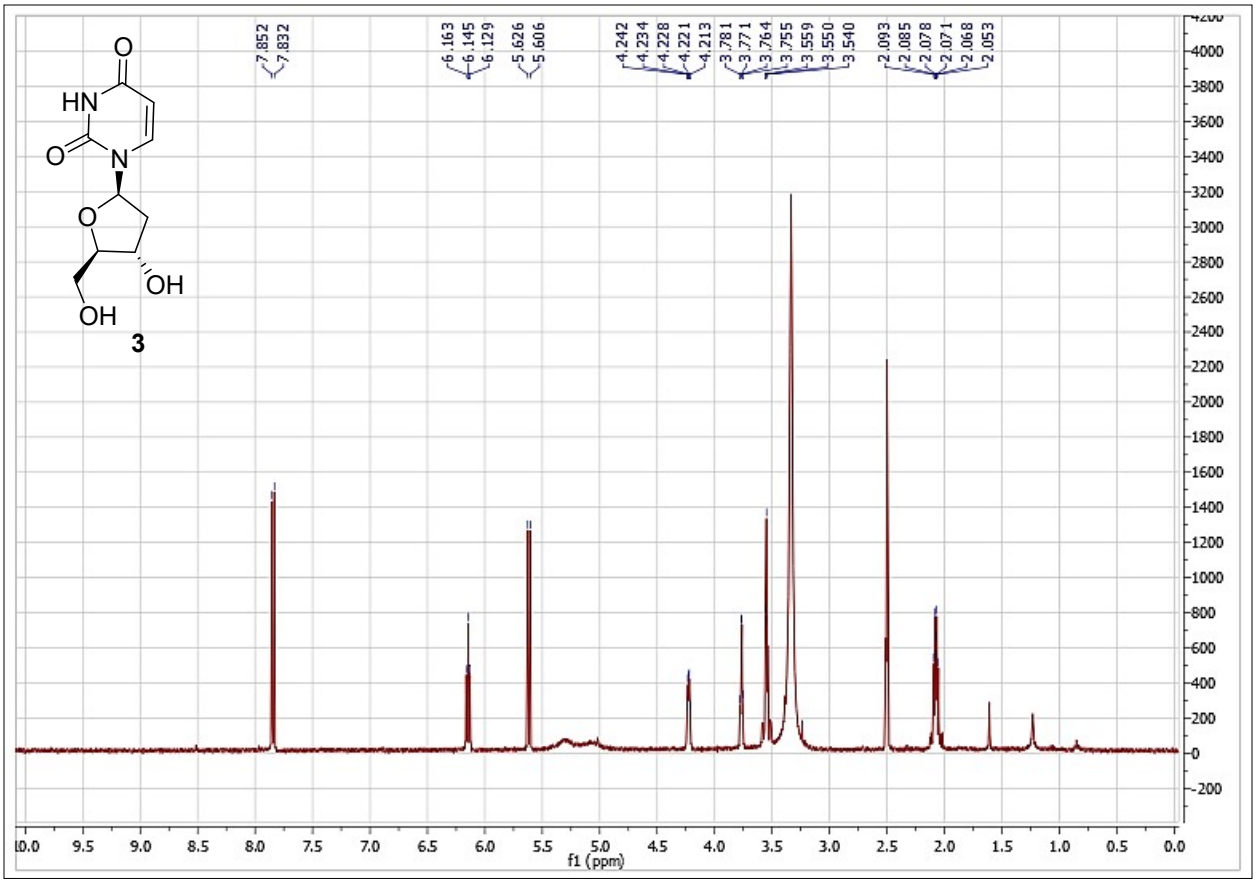

Figure S14. ^{1}H-NMR spectrum of compound **3** (400 MHz, DMSO-d_6).

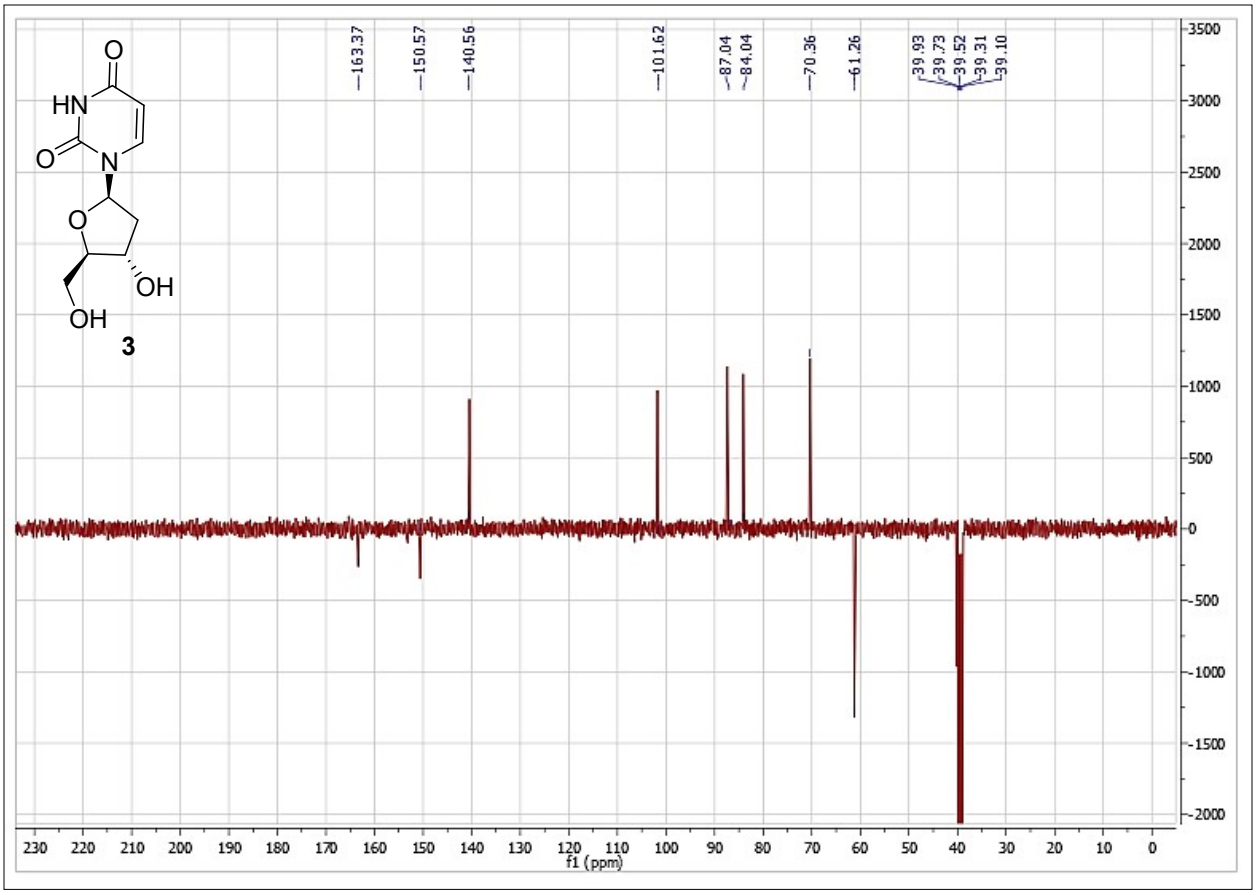

Figure S15. DEPT-Q spectrum of compound **3** (100 MHz, DMSO-d_6).

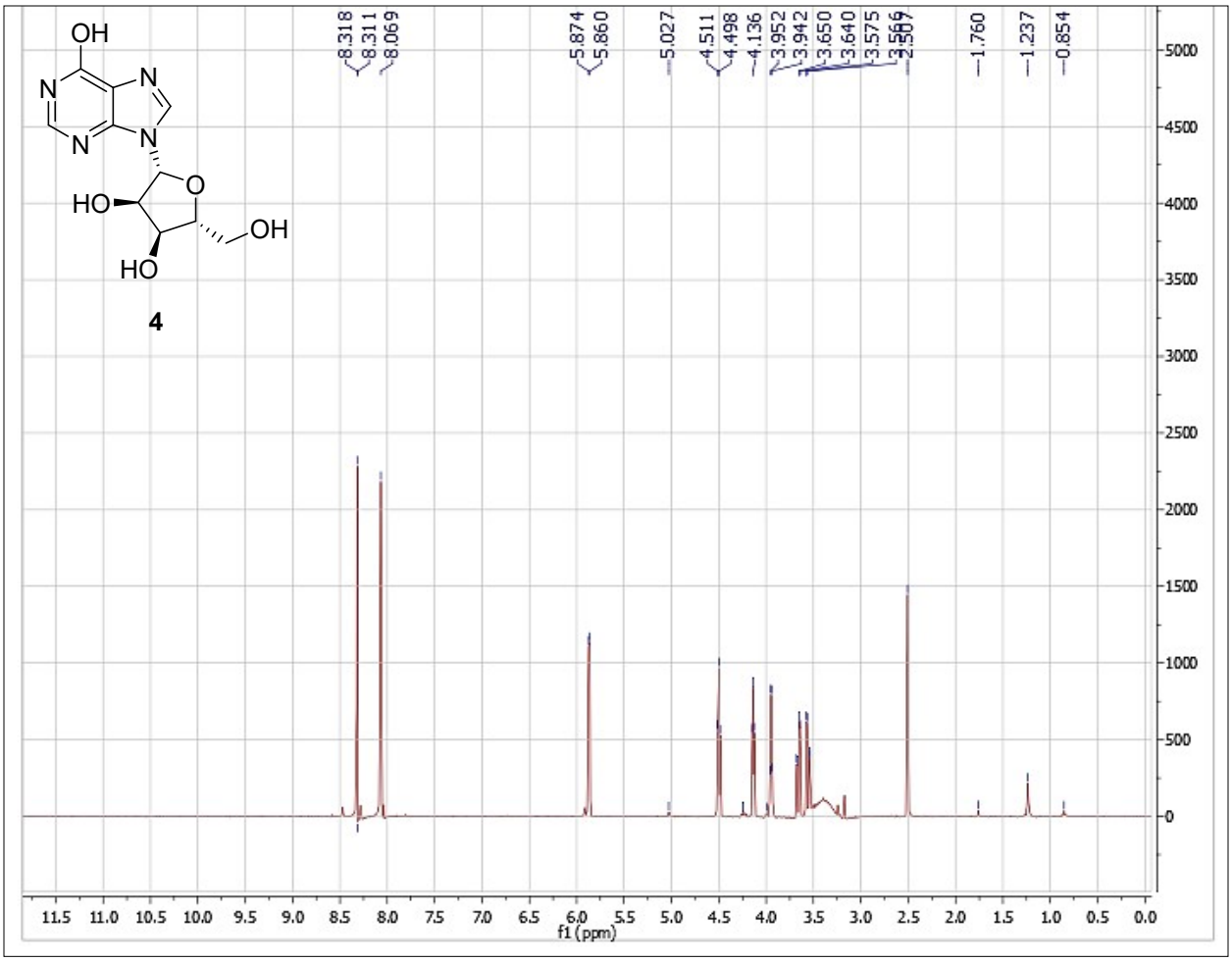

Figure S16. ^{1}H-NMR spectrum of compound **4** (400 MHz, DMSO-d_6).

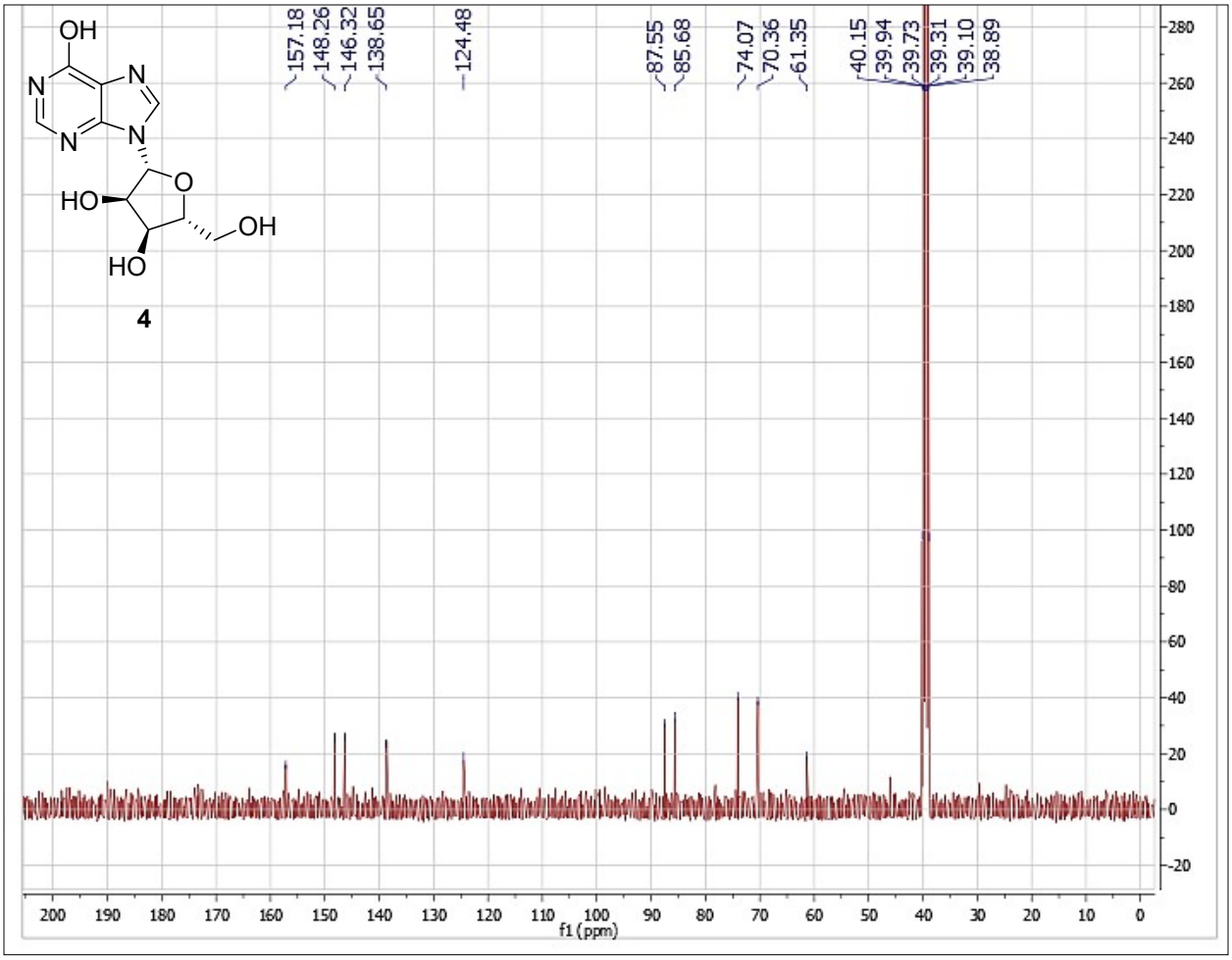

Figure S17. ^{13}C-NMR spectrum of compound **4** (100 MHz, DMSO-d_6).

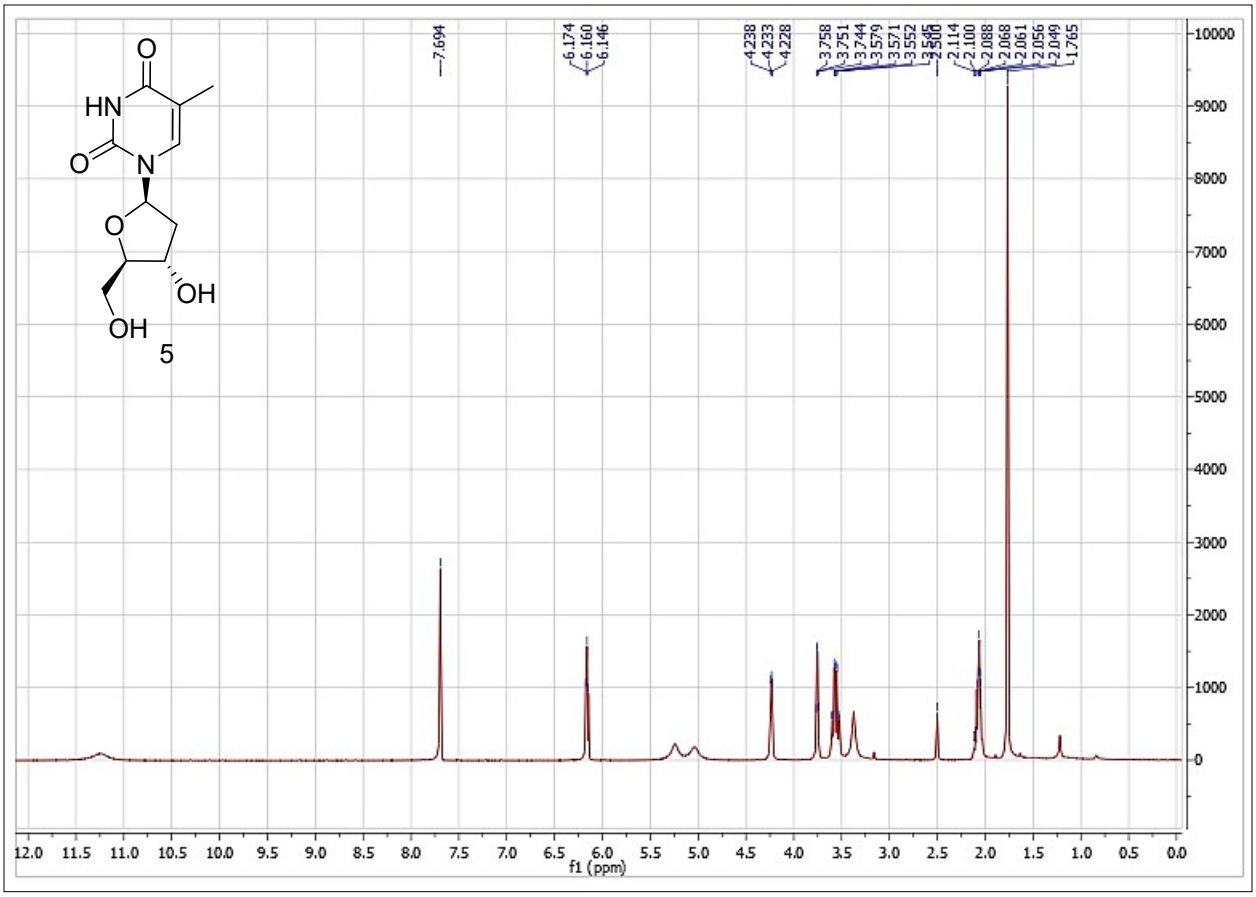

Figure S18. ^{1}H-NMR spectrum of compound **5** (500 MHz, DMSO-d_6).

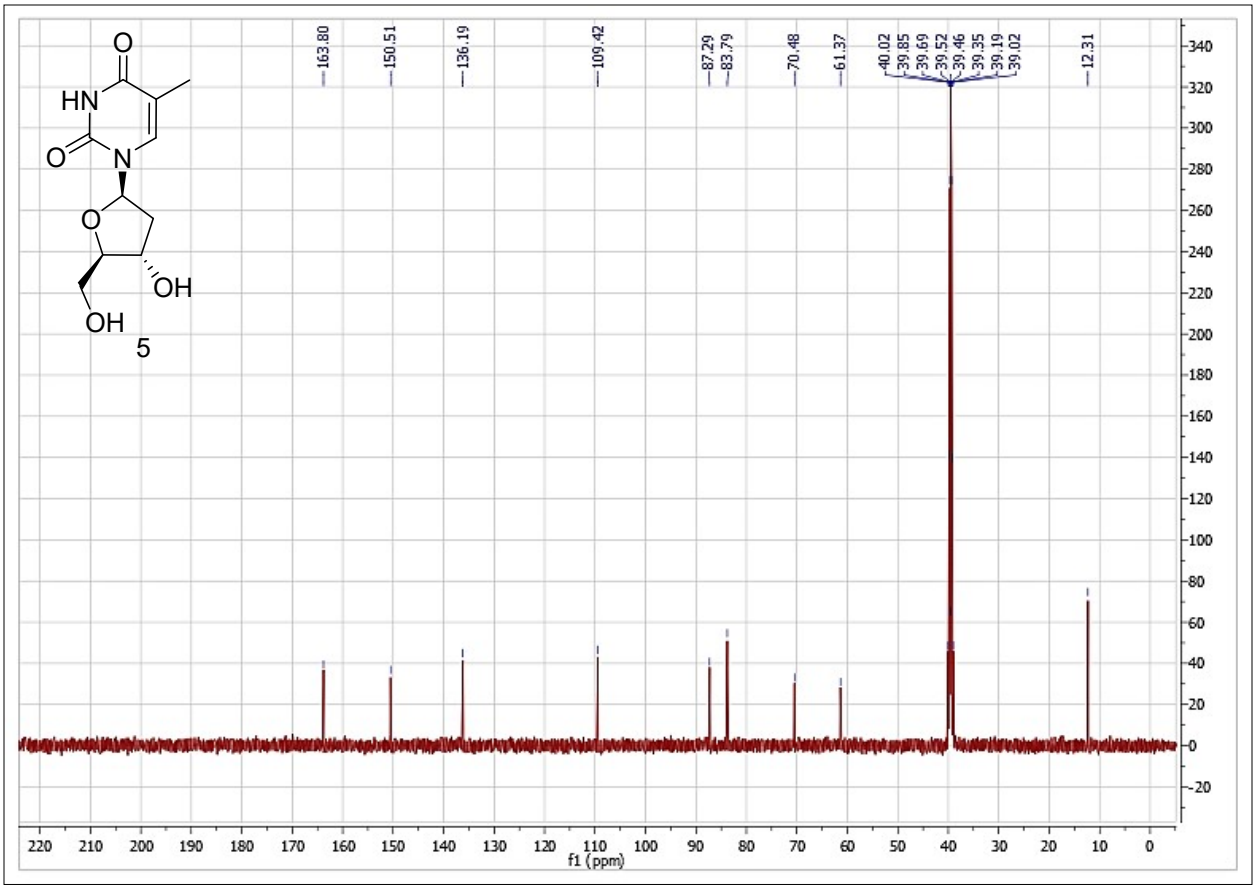

Figure S19. ^{13}C-NMR spectrum of compound **5** (125 MHz, DMSO-d_6).

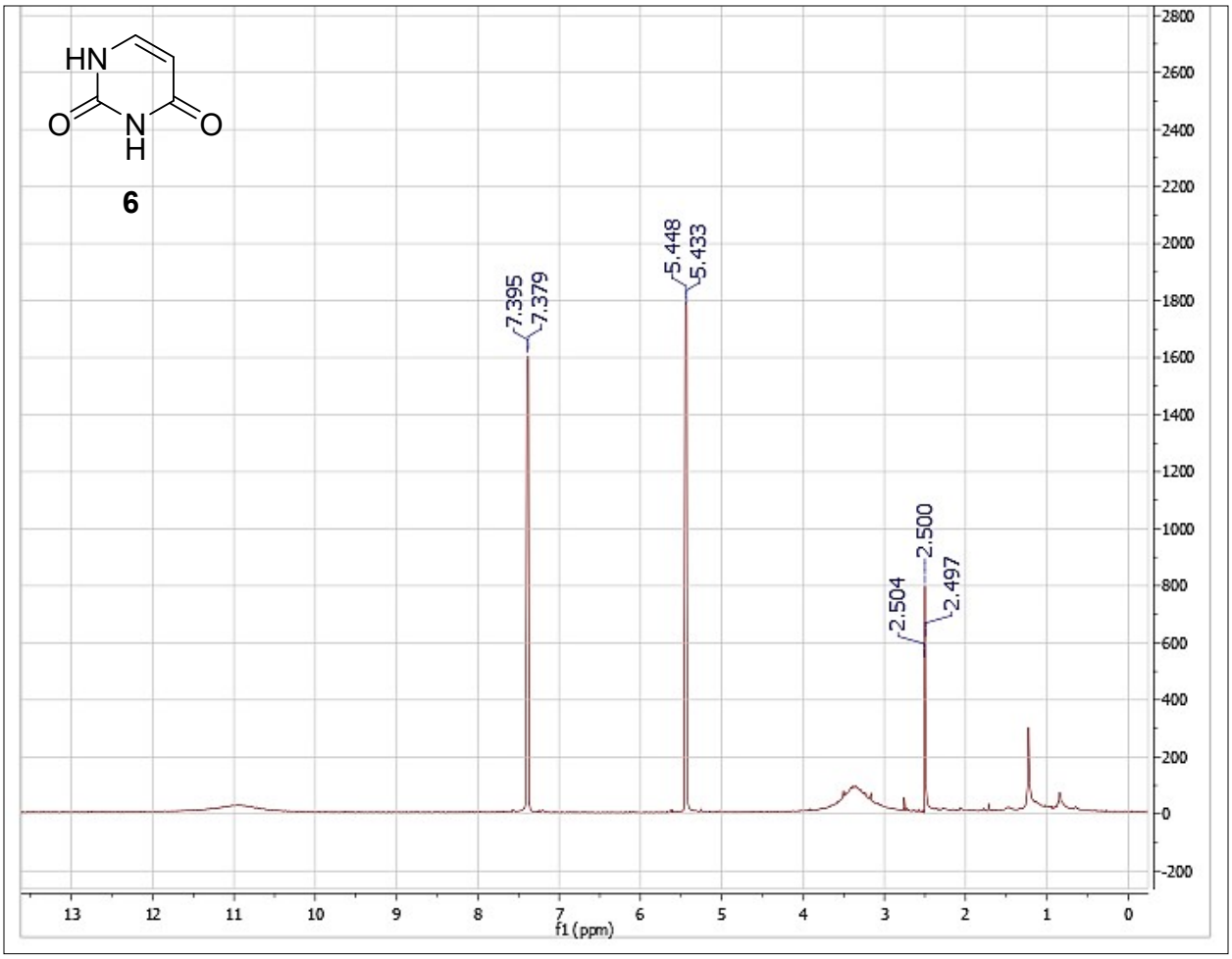

Figure S20. ^{1}H-NMR spectrum of compound **6** (500 MHz, DMSO-d_6).

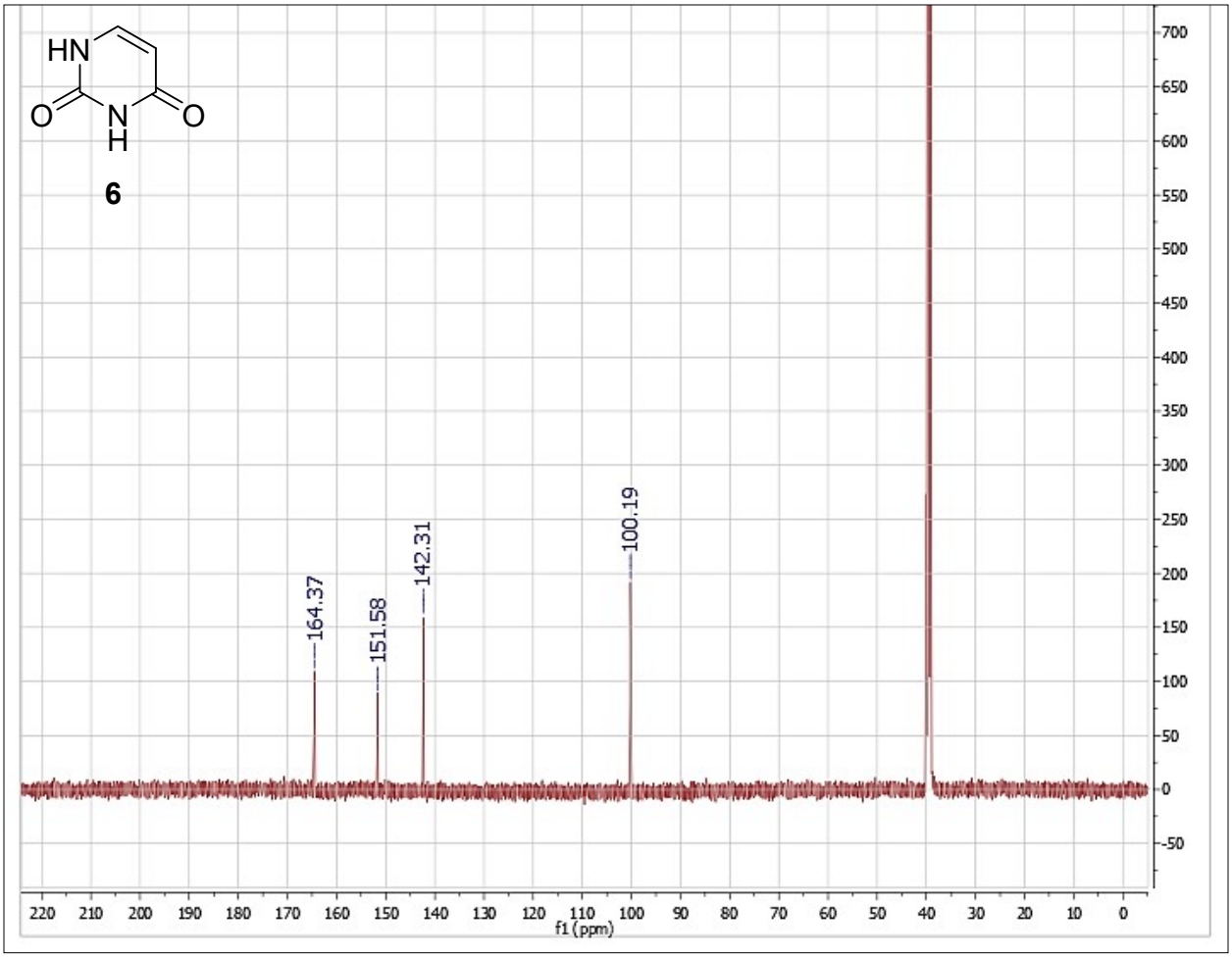

Figure S21. ^{13}C-NMR spectrum of compound **6** (125 MHz, DMSO-d_6).

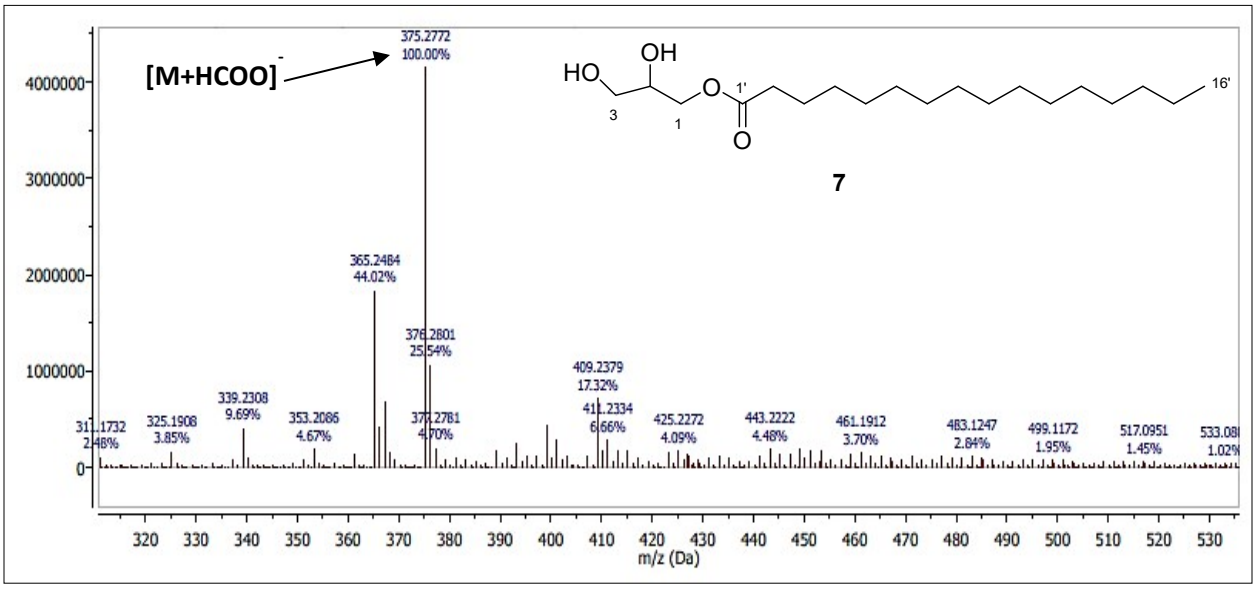

Figure S22. Negative HR-ESI-MS spectrum of compound **7**.

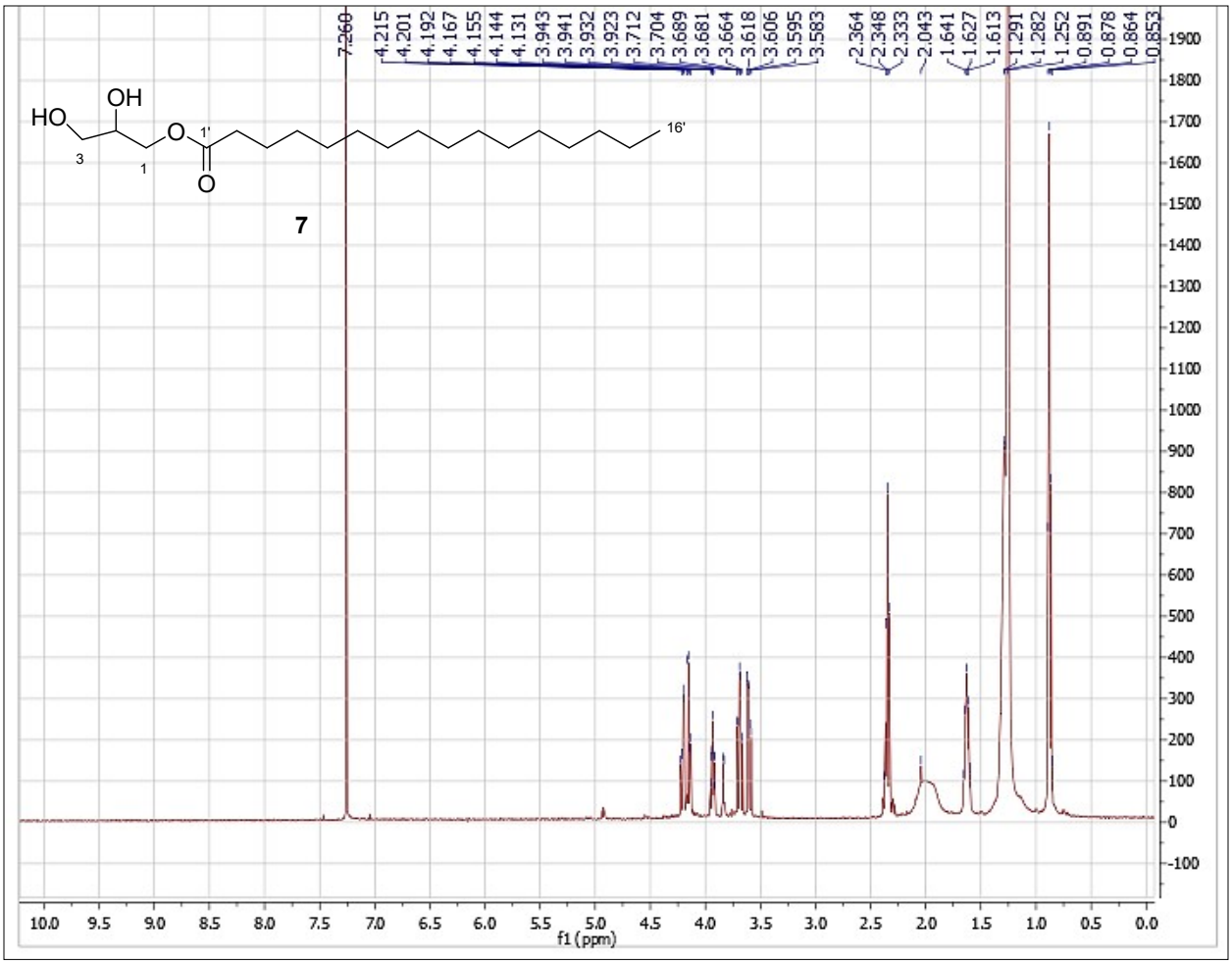

Figure S23. ^{1}H-NMR spectrum of compound **7** (500 MHz, $CDCl_3$).

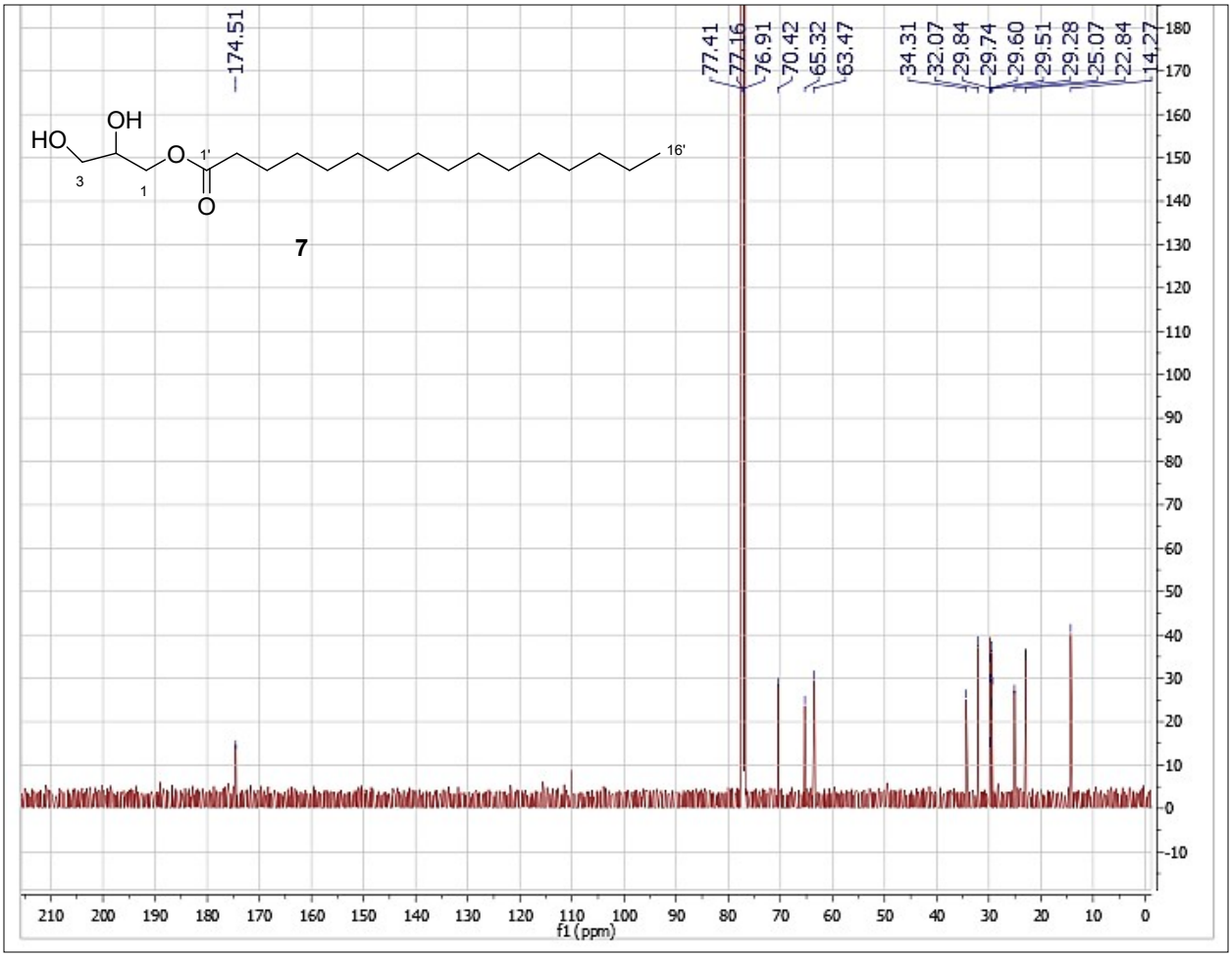

Figure S24. ^{13}C-NMR spectrum of compound **7** (125 MHz, $CDCl_3$).

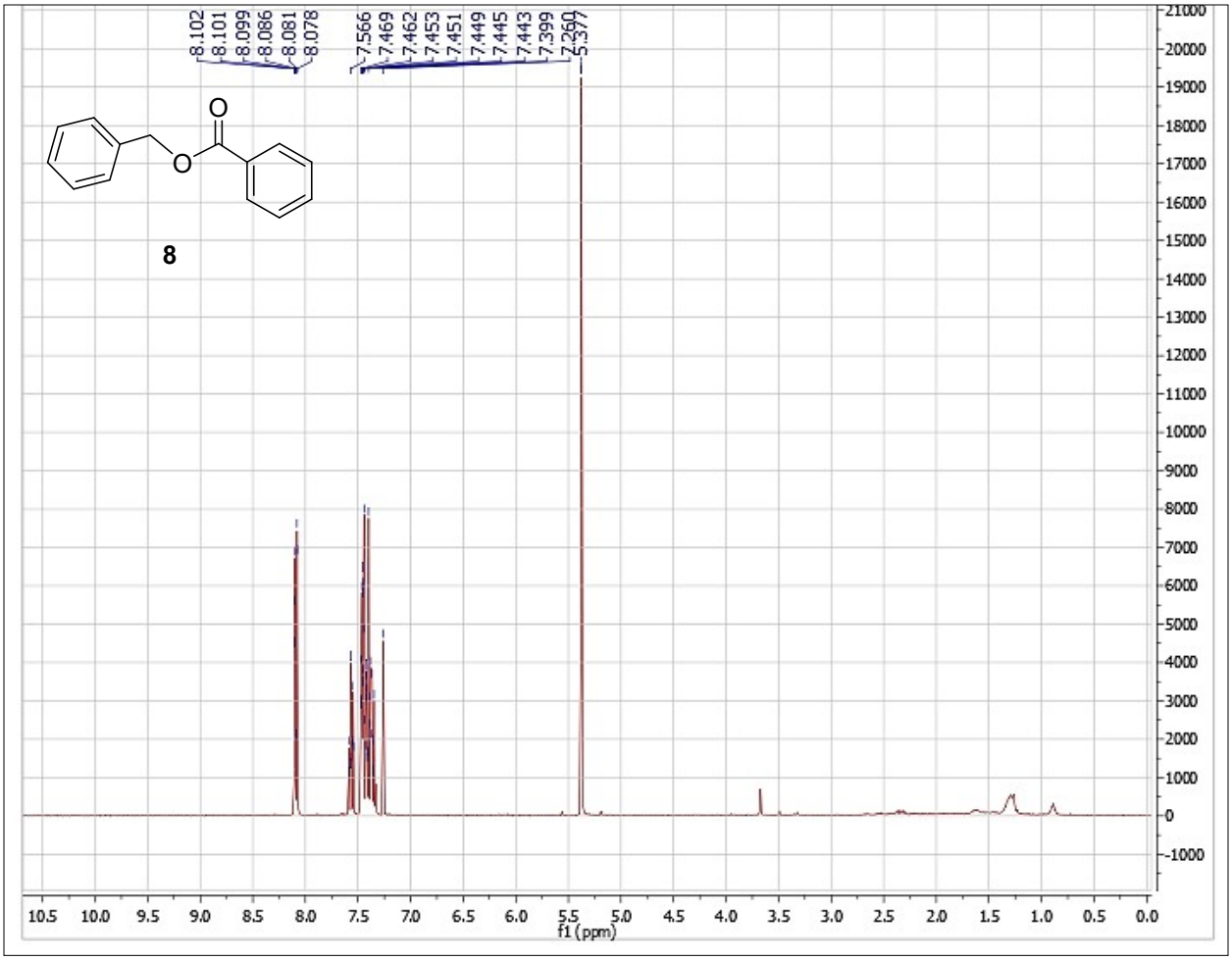

Figure S25. ^{1}H-NMR spectrum of compound **8** (400 MHz, $CDCl_3$).

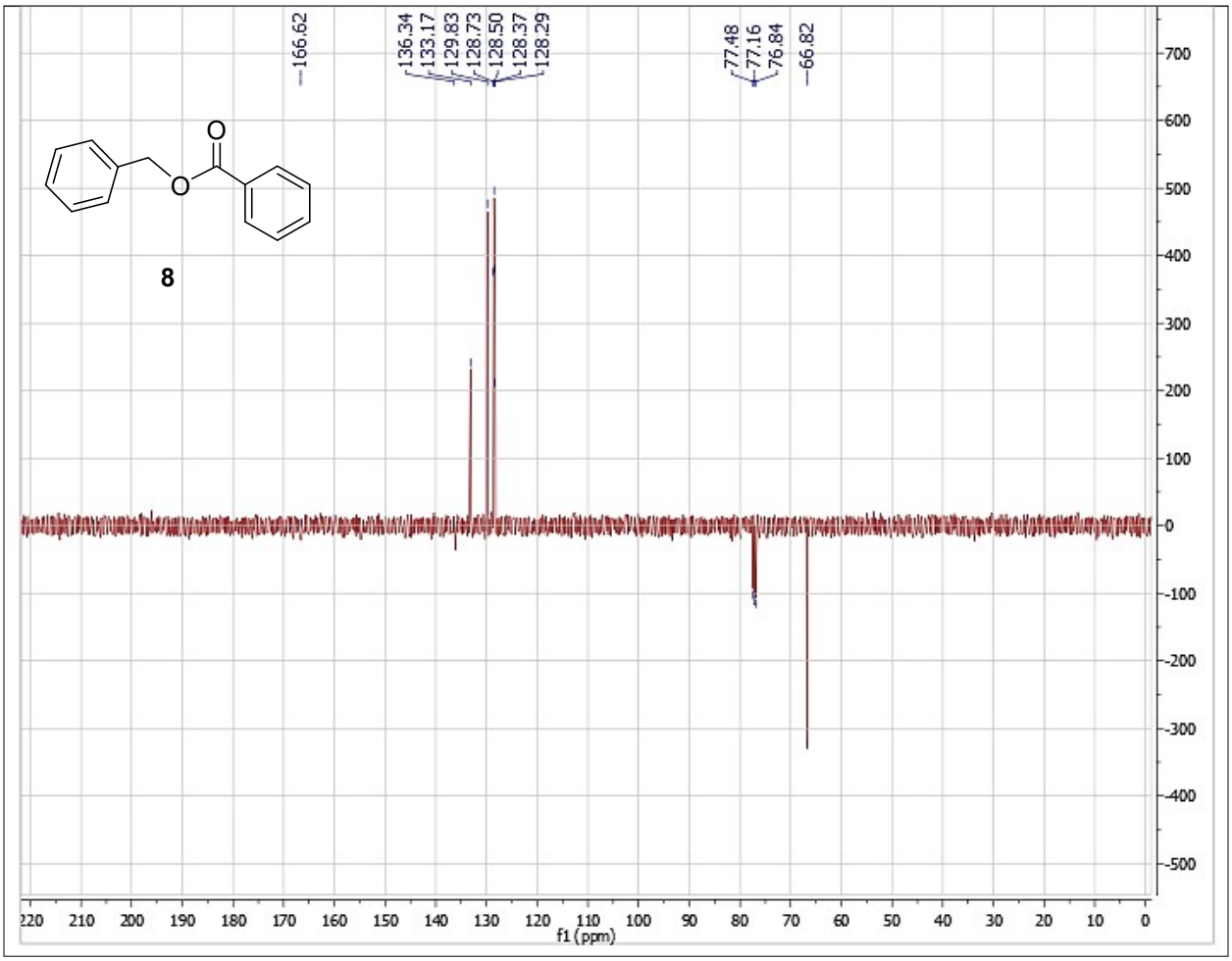

Figure S26. DEPT-Q spectrum of compound **8** (100 MHz, $CDCl_3$).

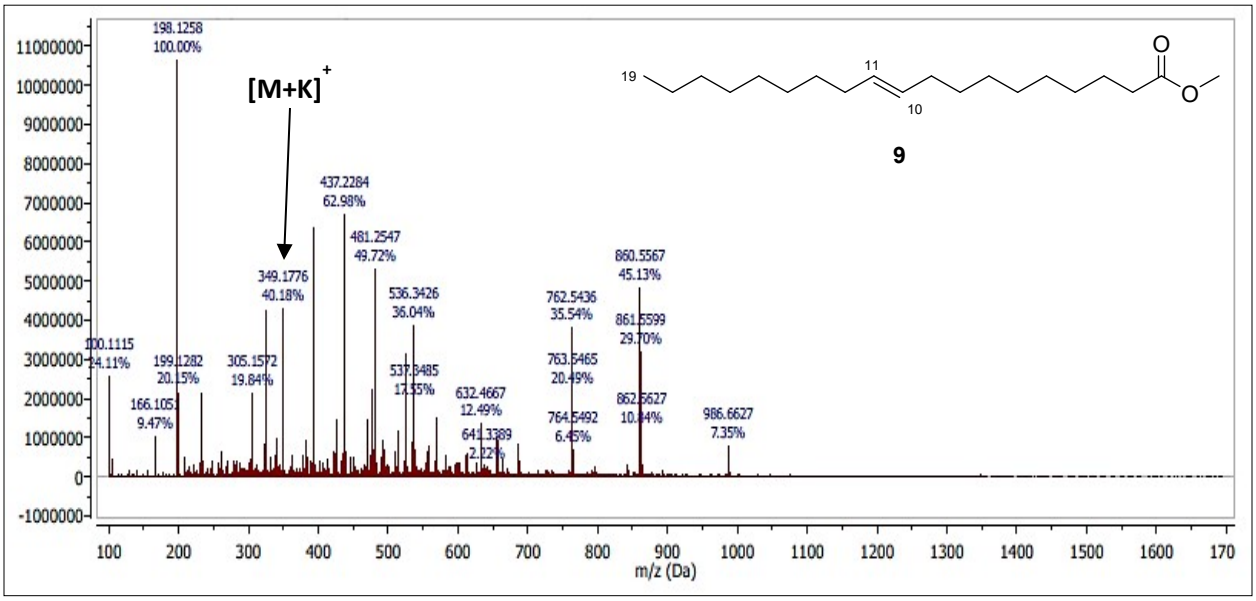

Figure S27. Positive HR-ESI-MS spectrum of compound **9**.

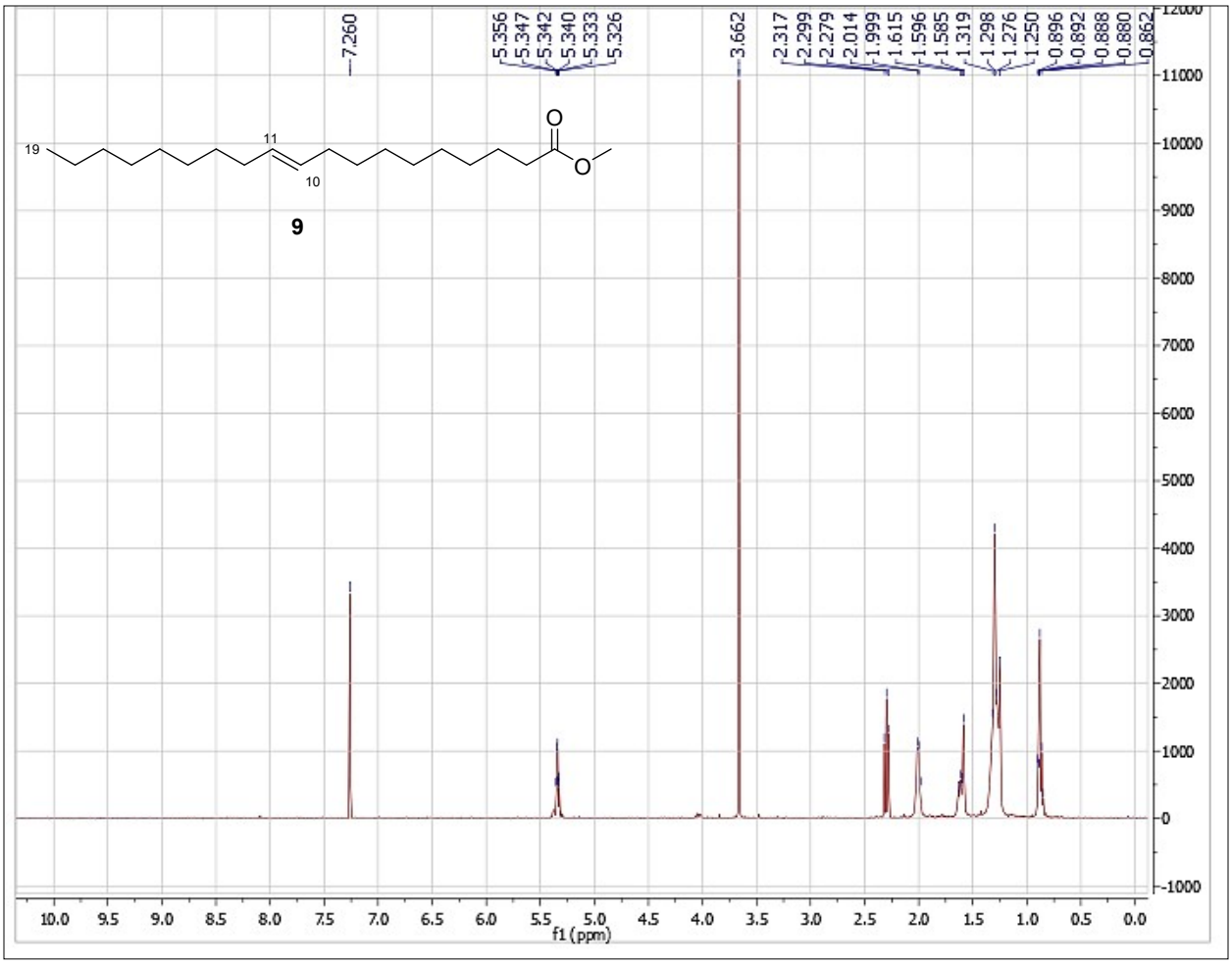

Figure S28. ^{1}H-NMR spectrum of compound **9** (400 MHz, $CDCl_3$).

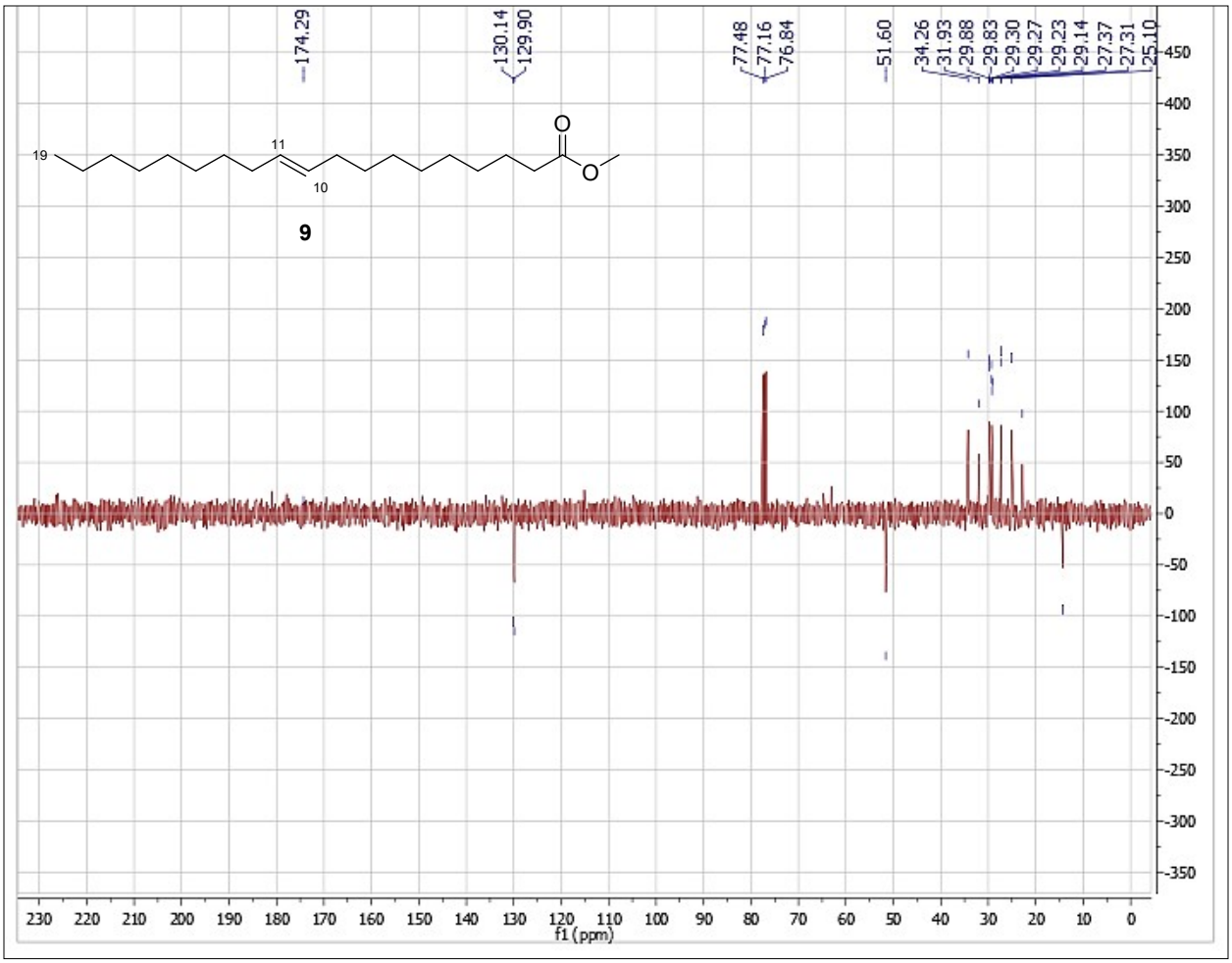

Figure S29. DEPT-Q spectrum of compound **9** (100 MHz, $CDCl_3$).

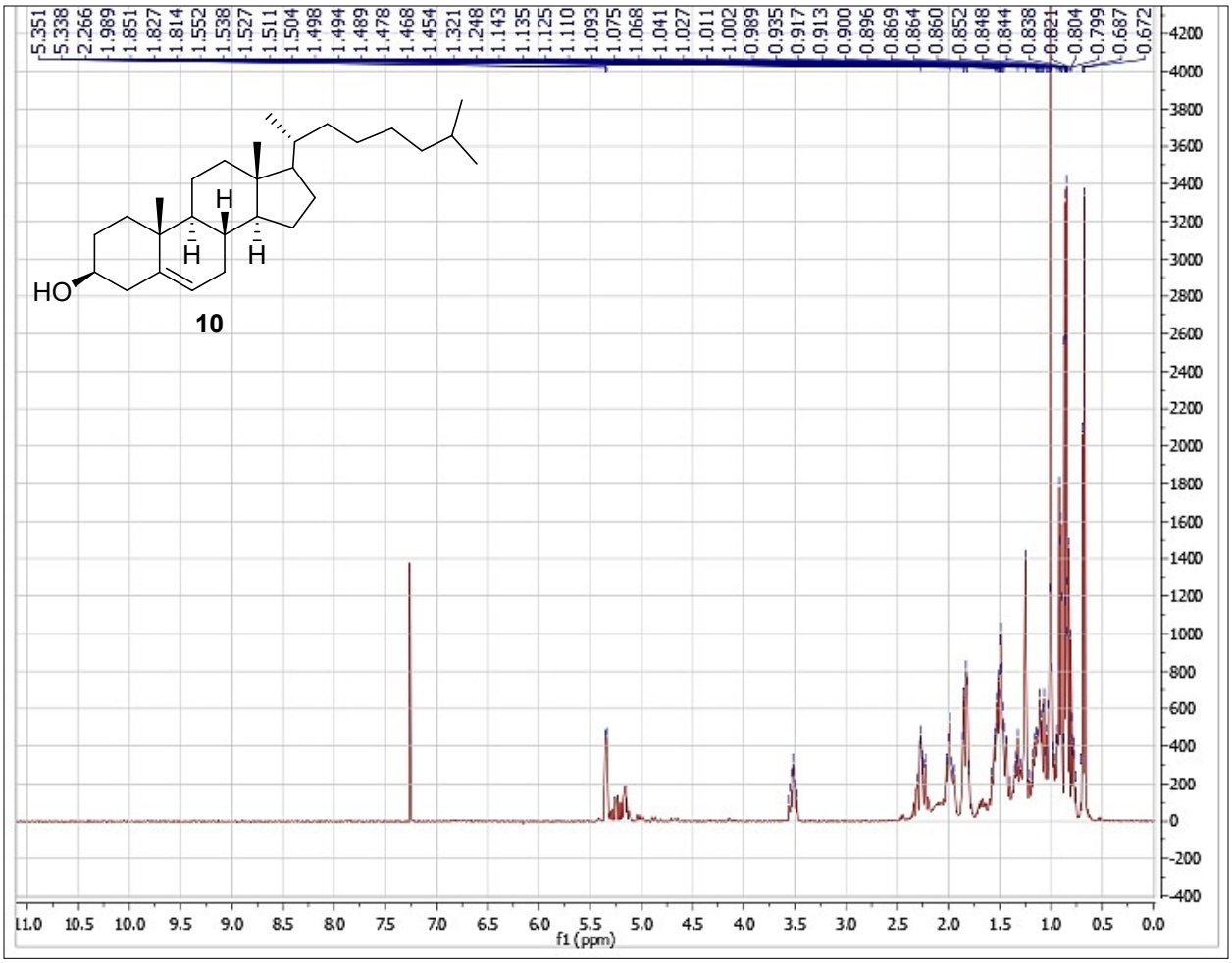

Figure S30. ^{1}H-NMR spectrum of compound **10** (400 MHz, $CDCl_3$).

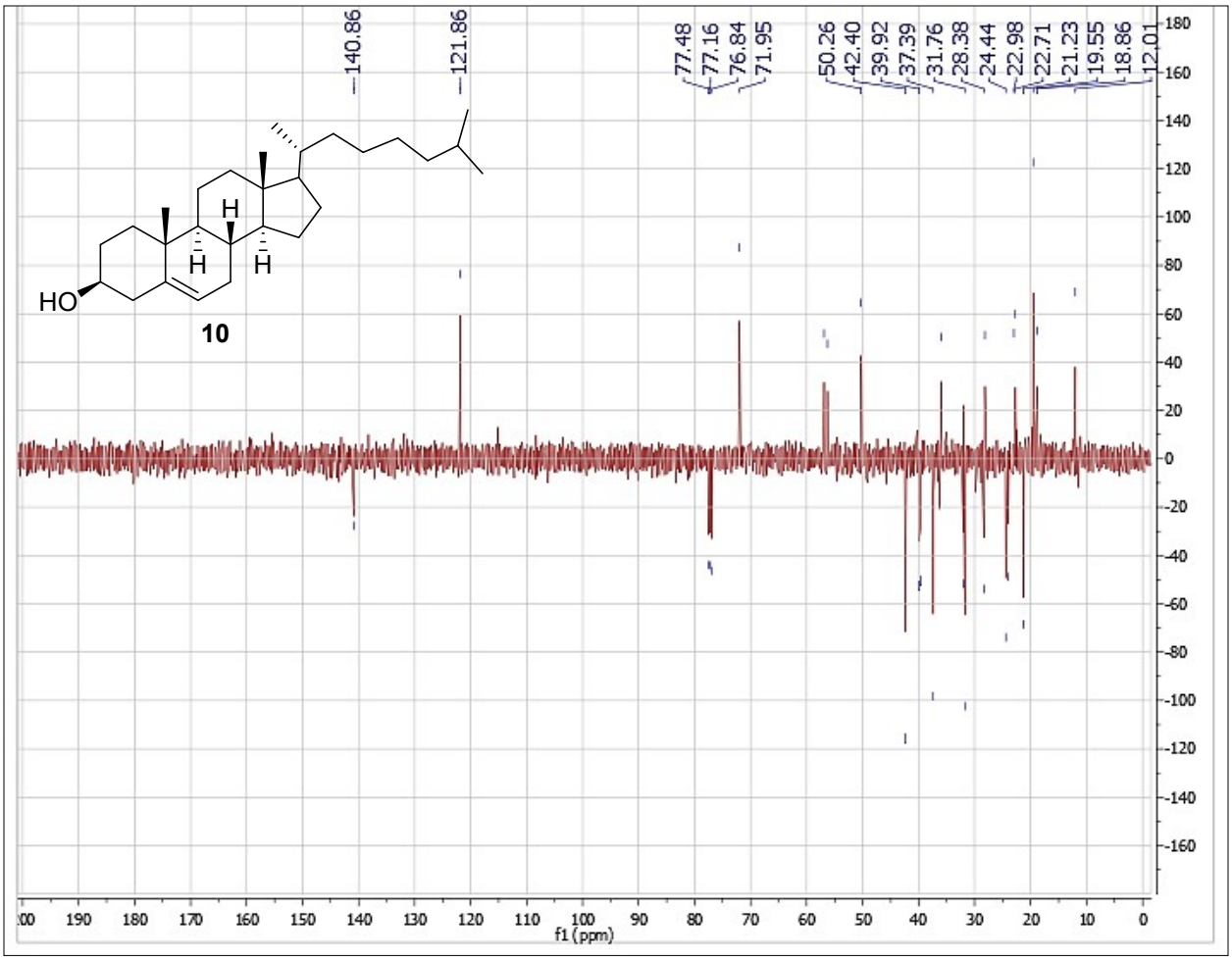

Figure S31. DEPT-Q spectrum of compound **10** (100 MHz, $CDCl_3$).

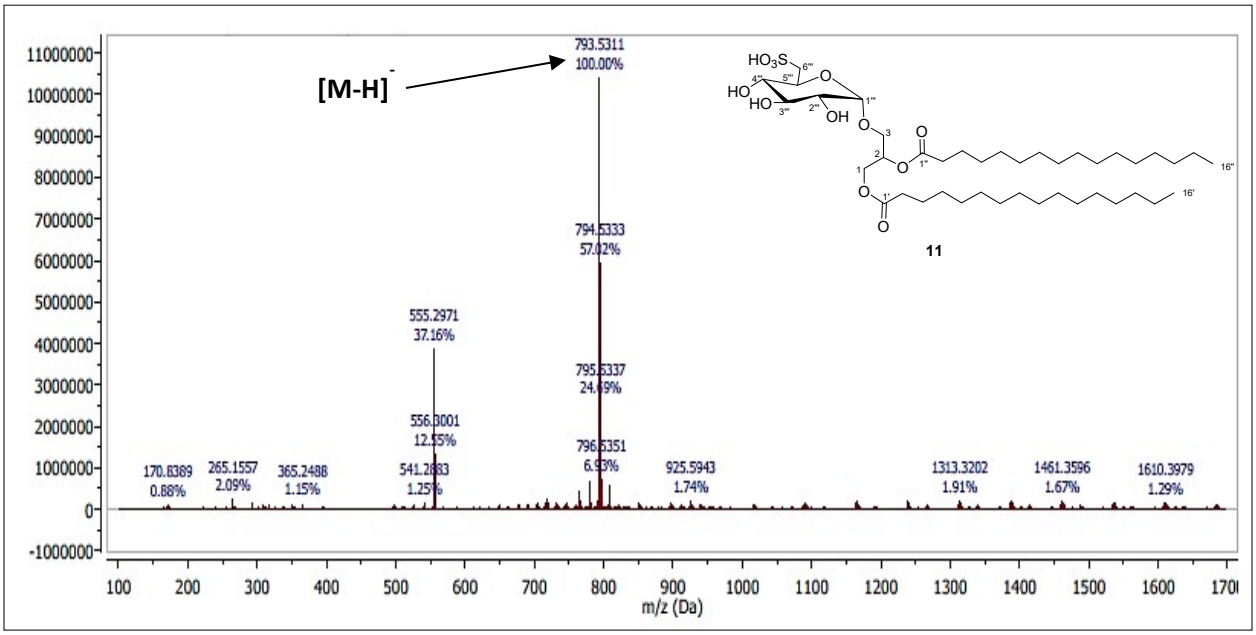

Figure S32. Negative HR-ESI-MS spectrum of compound **11**.

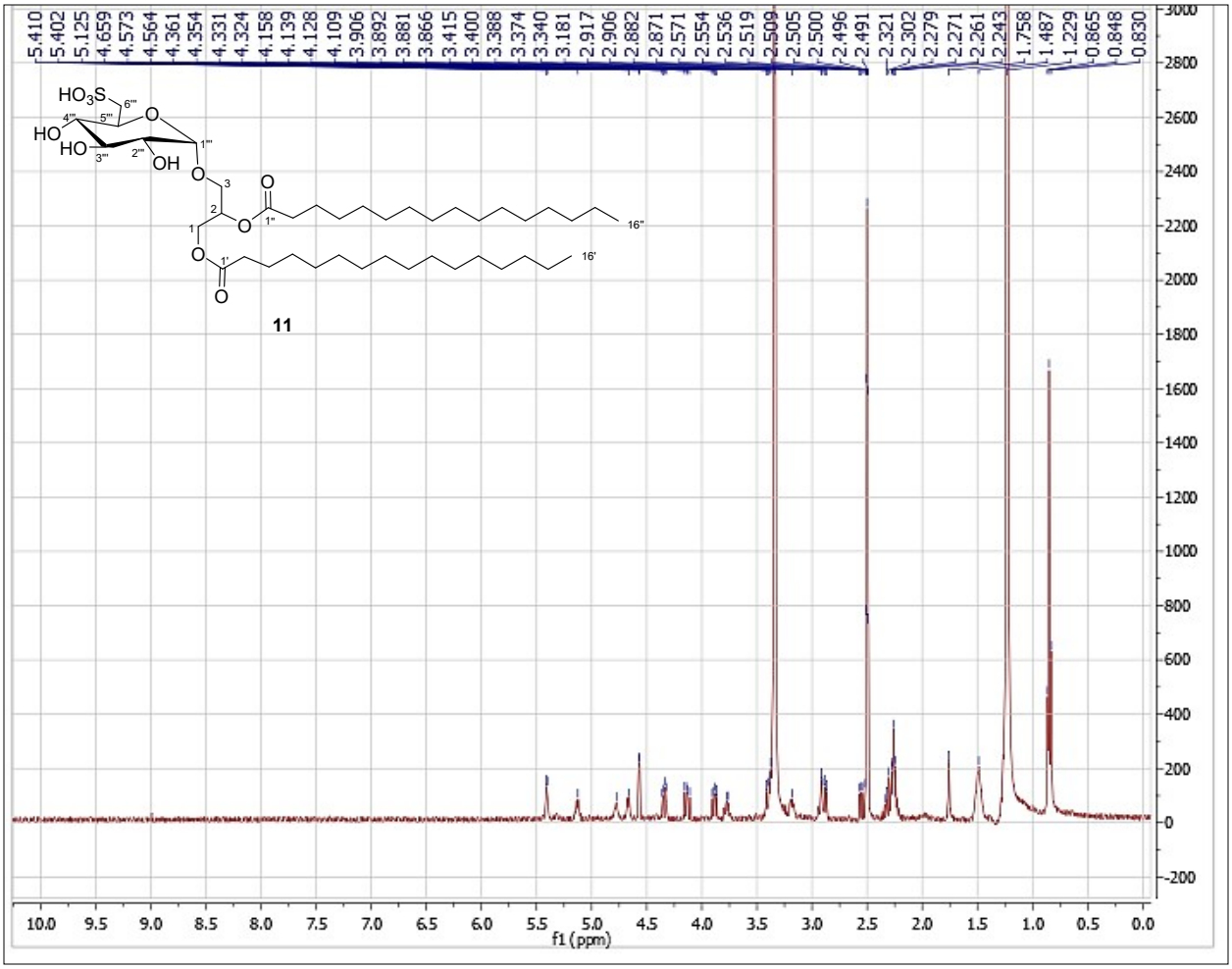

Figure S33. ^{1}H-NMR spectrum of compound **11** (400 MHz, DMSO-d_6).

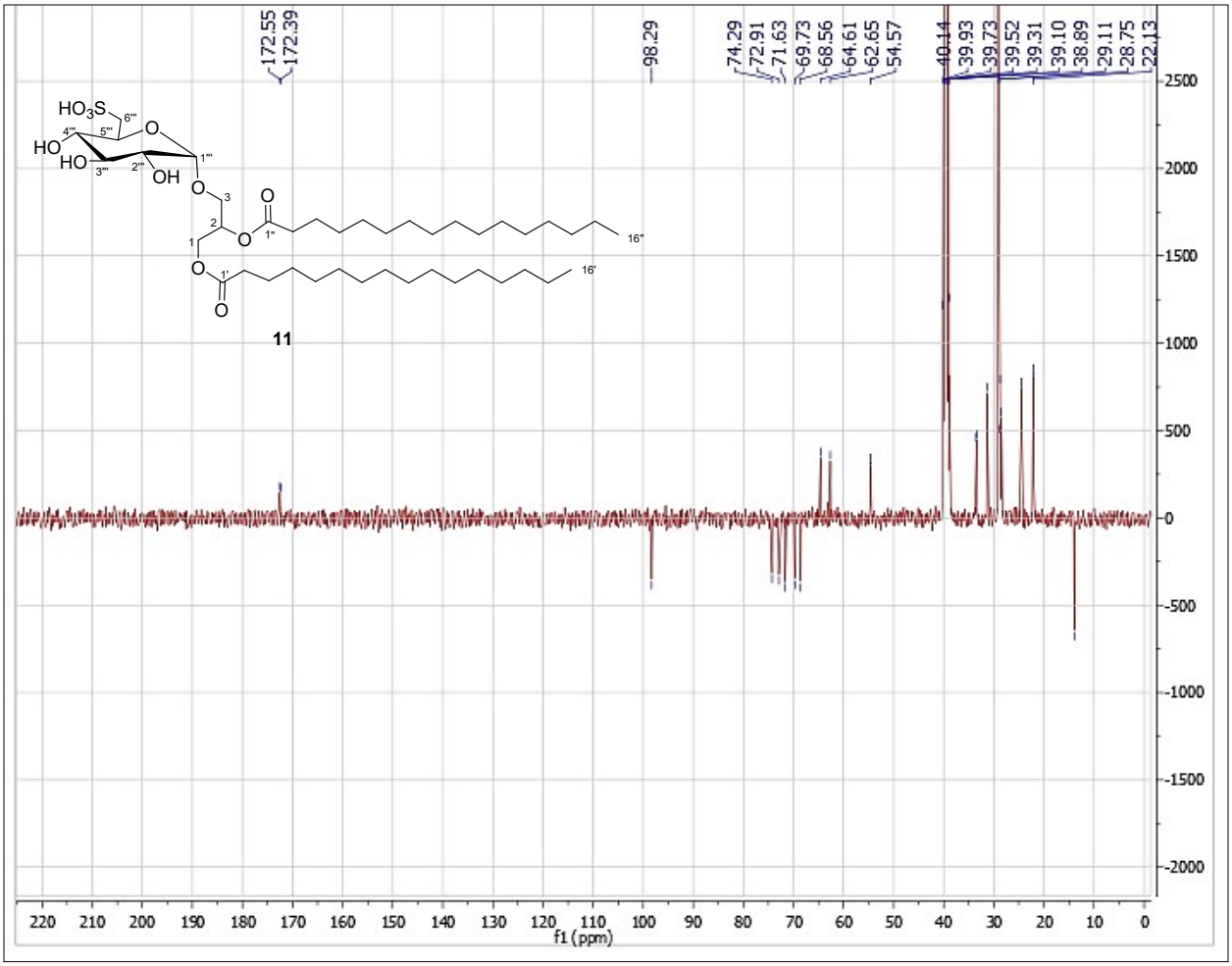

Figure S34. DEPT-Q spectrum of compound **11** (100 MHz, DMSO-d_6).

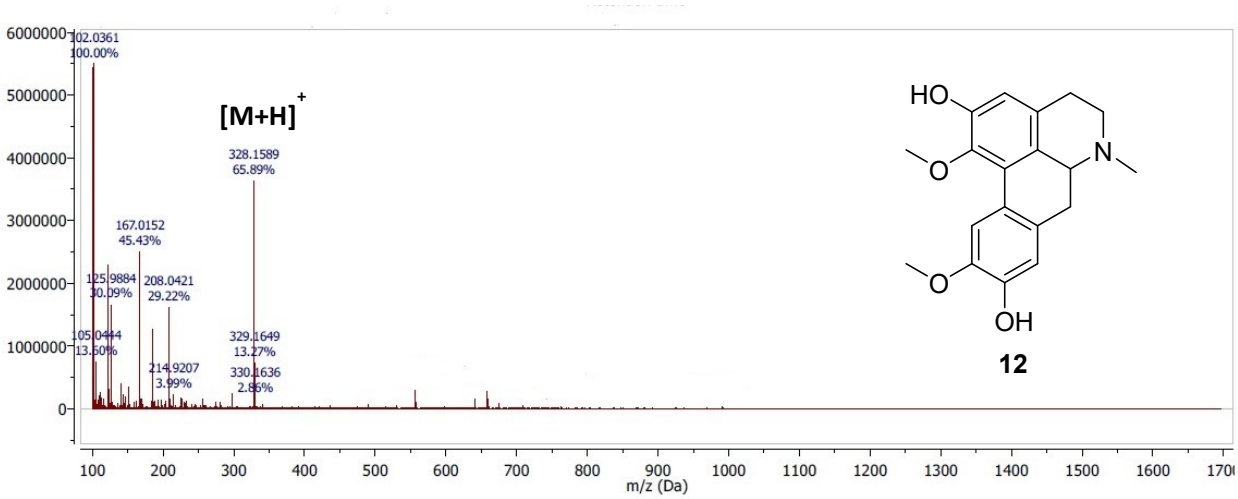

Figure S35. Positive HR-ESI-MS spectrum of compound **12**.

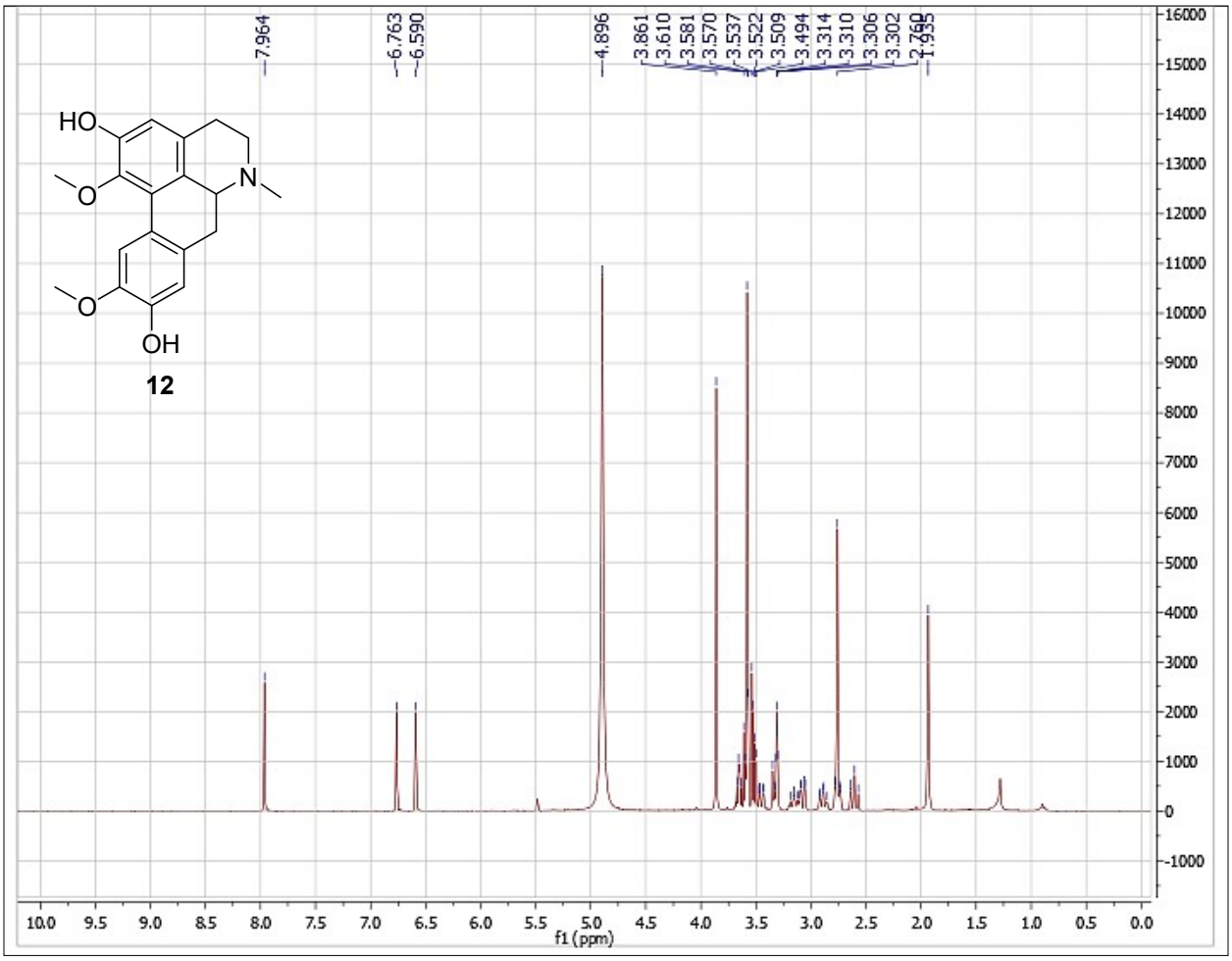

Figure S36. ^{1}H-NMR spectrum of compound **12** (400 MHz, CD_3OD).

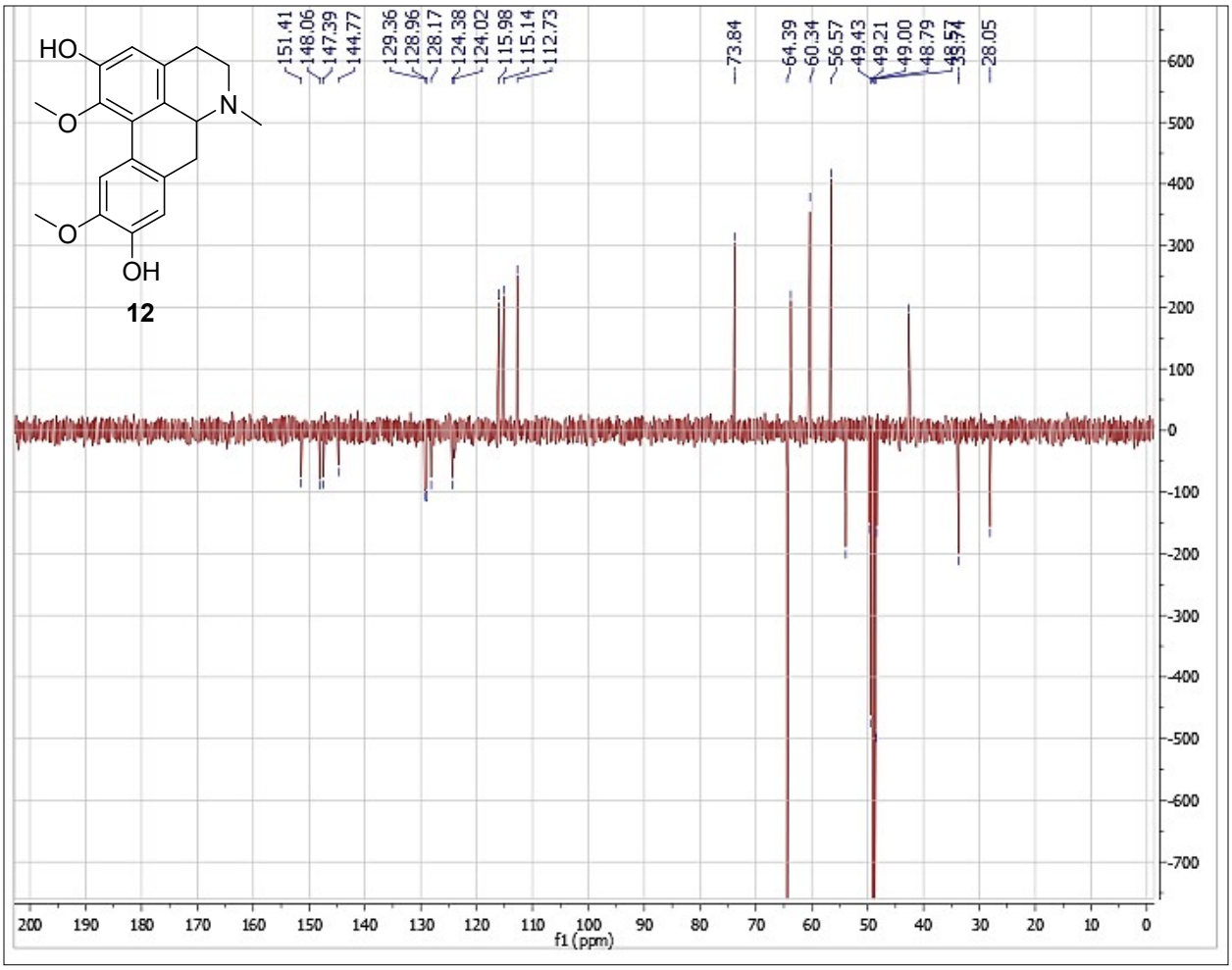

Figure S37. DEPT-Q spectrum of compound **12** (100 MHz, CD_3OD).

Table S1. Chemical quantum descriptors and their equations (1-8).

DFT parameter	Equations
Energy gap (ΔE)	$\Delta E = ELUMO - EHOMO$
Ionization potential (I)	$I = -EHOMO$
Hardness (η)	$\eta = (1 - A) / 2$
Softness (σ)	$\sigma = 1/2\eta$
Chemical potential (μ)	$\mu = -\chi \cong (EHOMO + ELUMO) / 2$
Electronegativity (χ)	$\chi = (I + A)/2$
Electron Affinity (A)	$A = -ELUMO$
Electrophilicity (ω)	$\omega = \mu 2/2\eta$

Table S2. Calculated quantum-chemical parameters of the compounds (**1**, **2** and **11**) using the B3LYP/6-311++G (2d,2p) method.

Compunds	ELUMO	EHOMO	ΔE	A	I	X	η	s	ω	ΔN max	ΔN
1	**-0.13963**	**-0.2043**	**0.06467**	**0.13963**	**0.2043**	**0.171965**	**0.032335**	**30.92624**	**0.91455**	**2.659116**	**105.5827**
2	**-0.15414**	**-0.3063**	**0.15216**	**0.15414**	**0.3063**	**0.23022**	**0.07608**	**13.14406**	**0.696652**	**1.513013**	**44.49119**
12	**-0.12027**	**-0.275**	**0.15473**	**0.12027**	**0.275**	**0.197635**	**0.077365**	**12.92574**	**0.504874**	**1.277289**	**43.96281**