Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

~~Superior~~ Enhanced Antibacterial Activity of New "Composite" Biocides with both *N*-chloramine and Quaternary Ammonium Moieties

Chenxi Ning‡,[1] Lingdong Li‡,[2] Sarvesh Logsetty,[3] Sadegh Ghanbar,[4] Melinda Guo,[5] Werner Ens,[6] and Song Liu*,[1,4,7,8]

*Correspondence: Song Liu, Department of Textile Sciences, Faculty of Human Ecology; Department of Biosystems Engineering, Faculty of Engineering, University of Manitoba, Winnipeg, Canada R3T 2N2, 204-474-9616 (phone); 204-474-7593 (fax); Song.Liu@umanitoba.ca

Table of Contents

1. ^{1}H and ^{13}C NMR of compounds # 1-3, 5-9 and 10-12

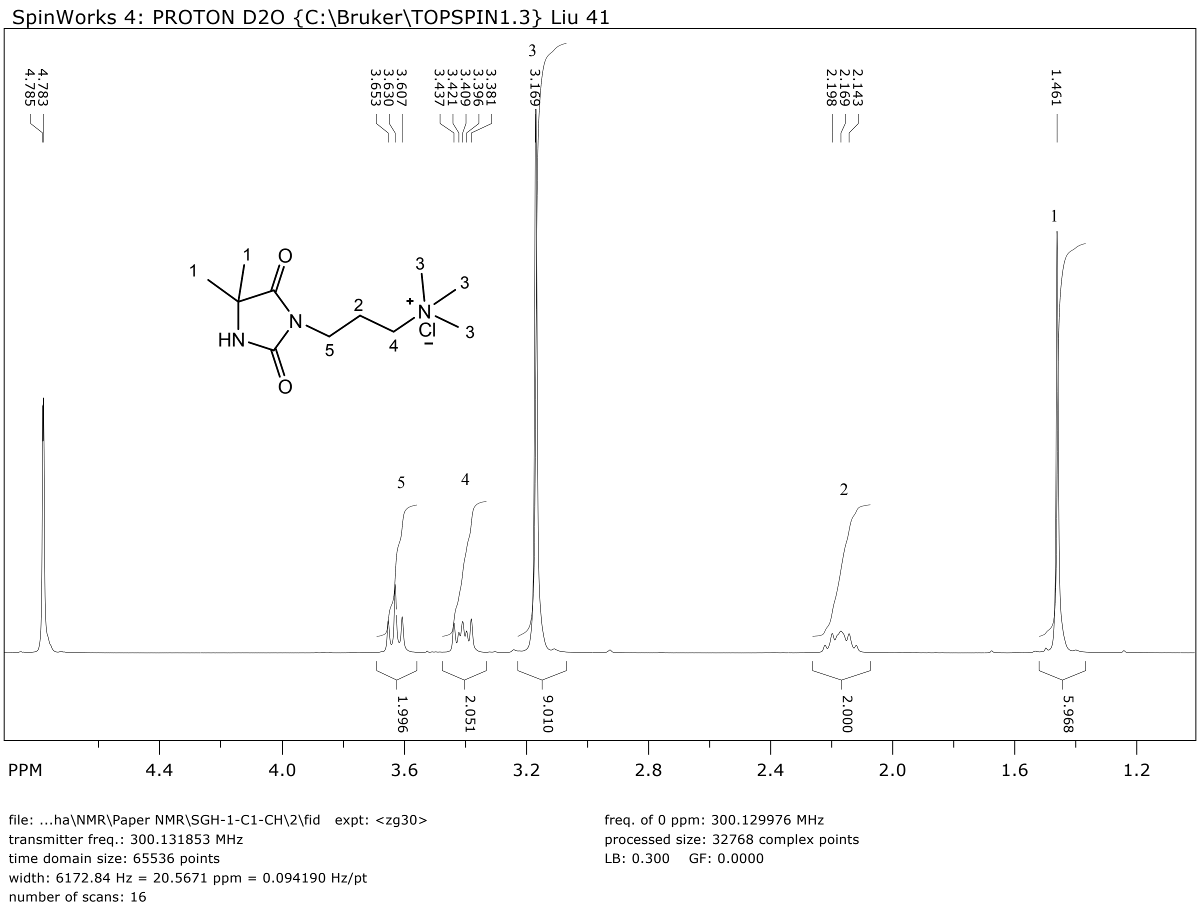


Figure S1. ^{1}H-NMR spectrum of **compound#1** (D_2O).

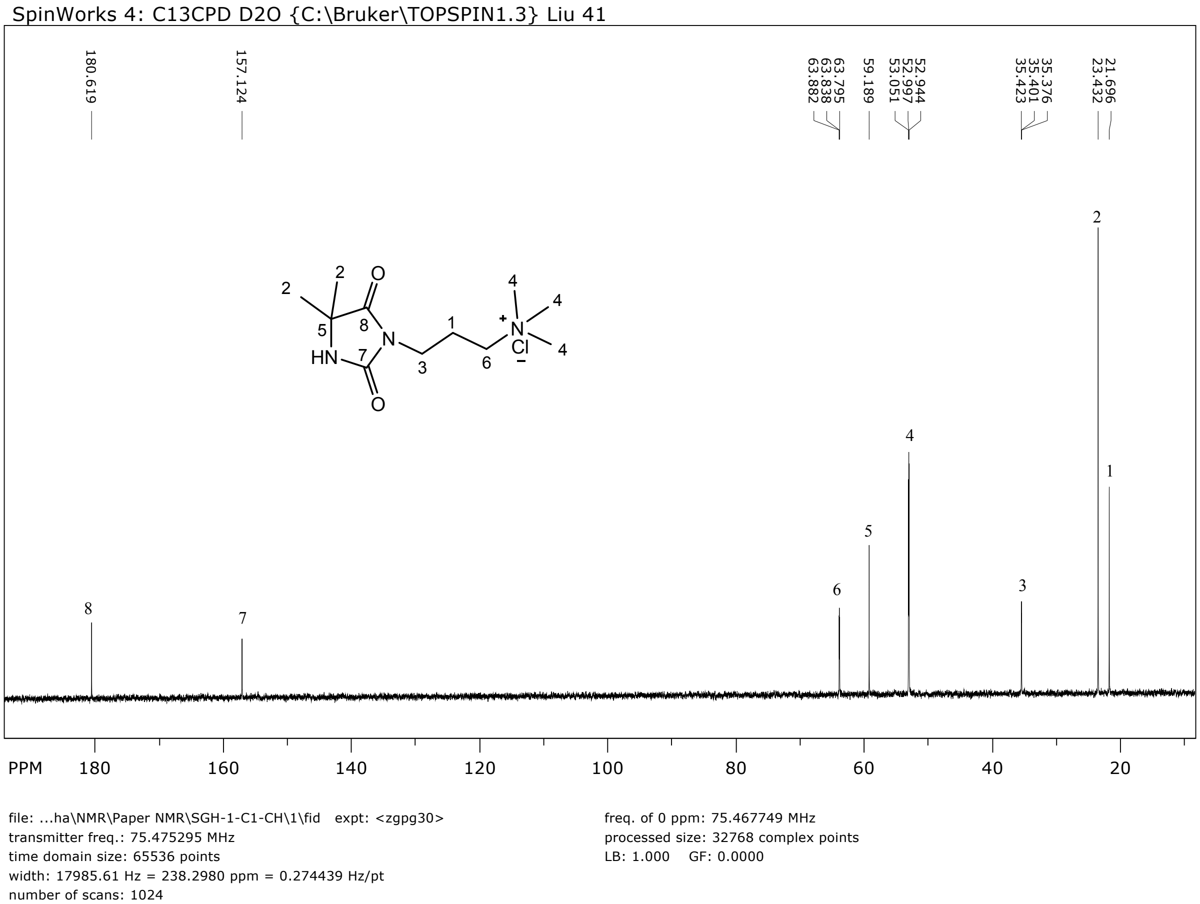


Figure S2. ^{13}C NMR spectrum of **compound#1** (D_2O).

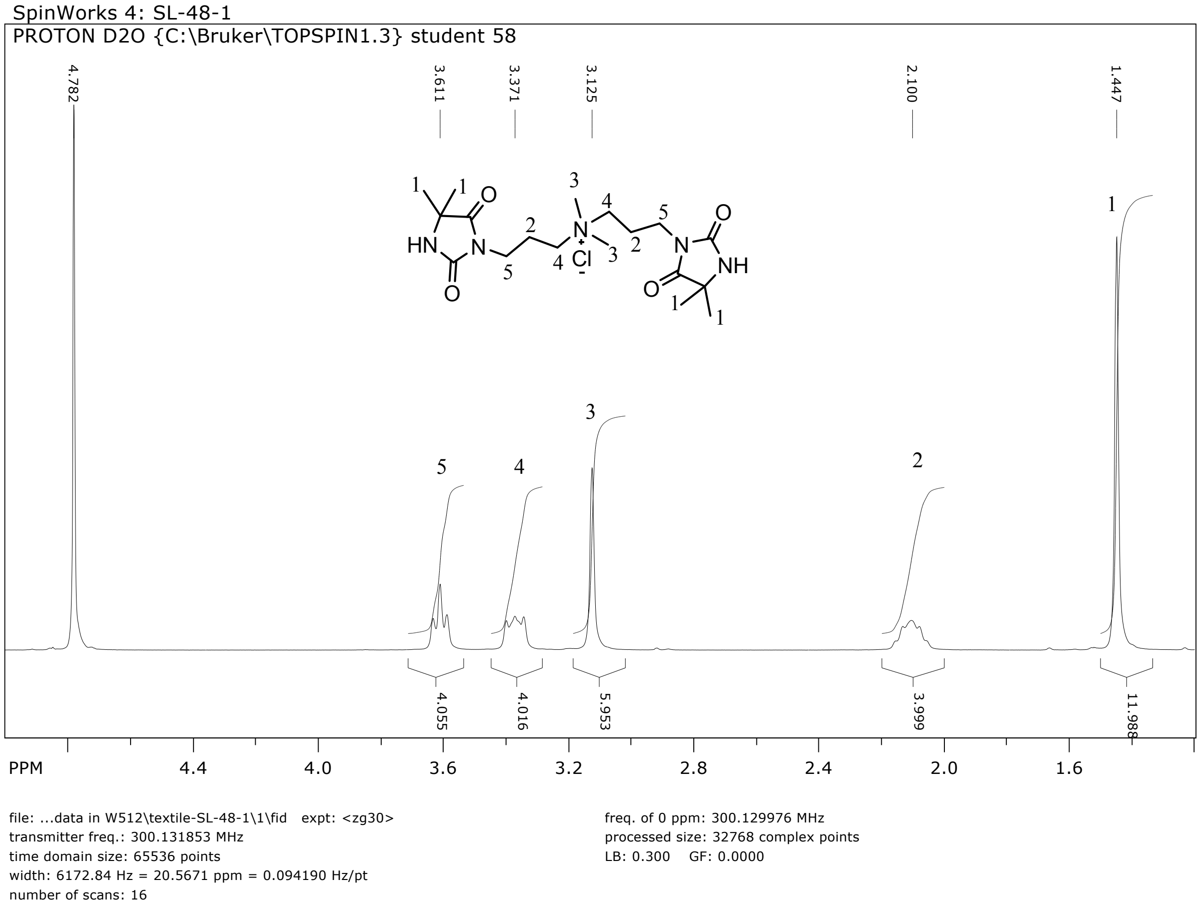


Figure S3. ^{1}H-NMR spectrum of **compound#2** (D_2O).

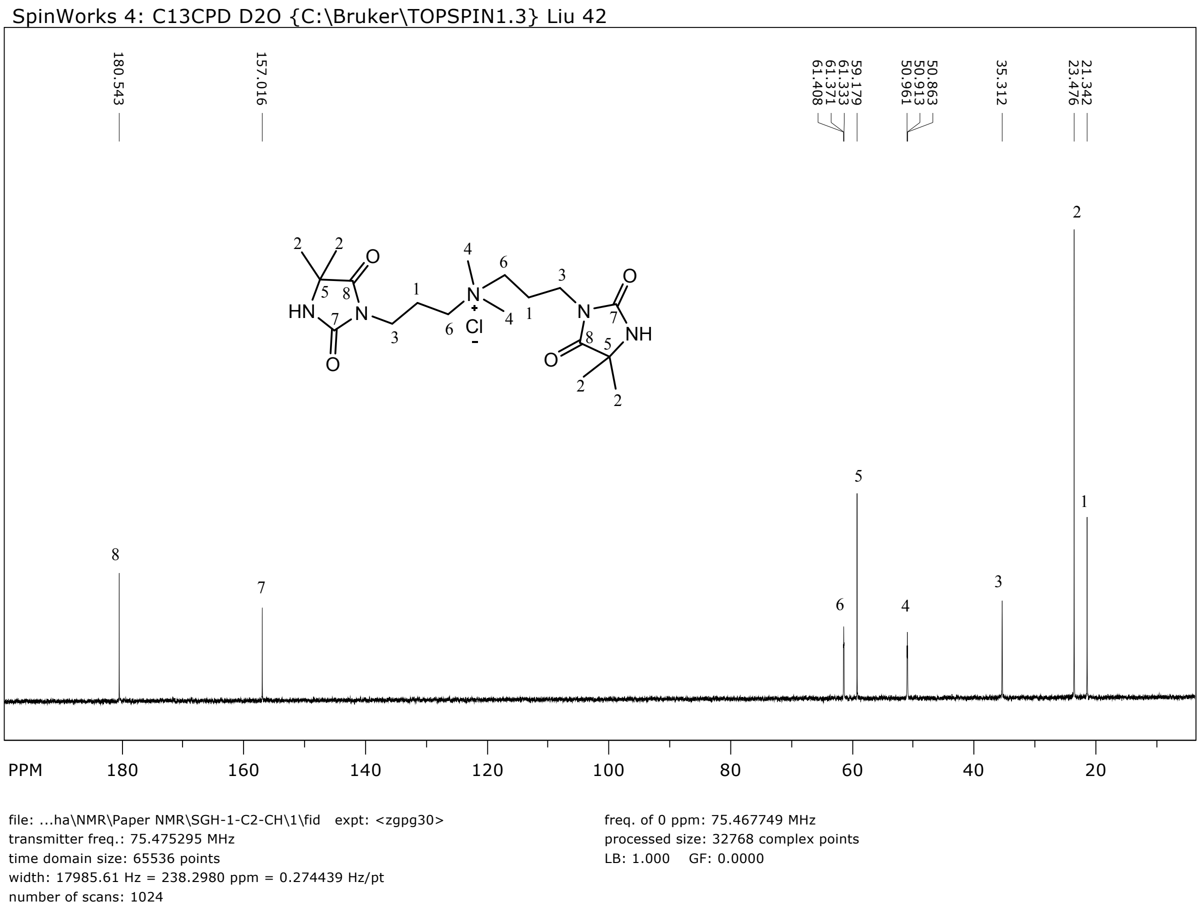


Figure S4. ^{13}C NMR spectrum of **compound#2** (D_2O).

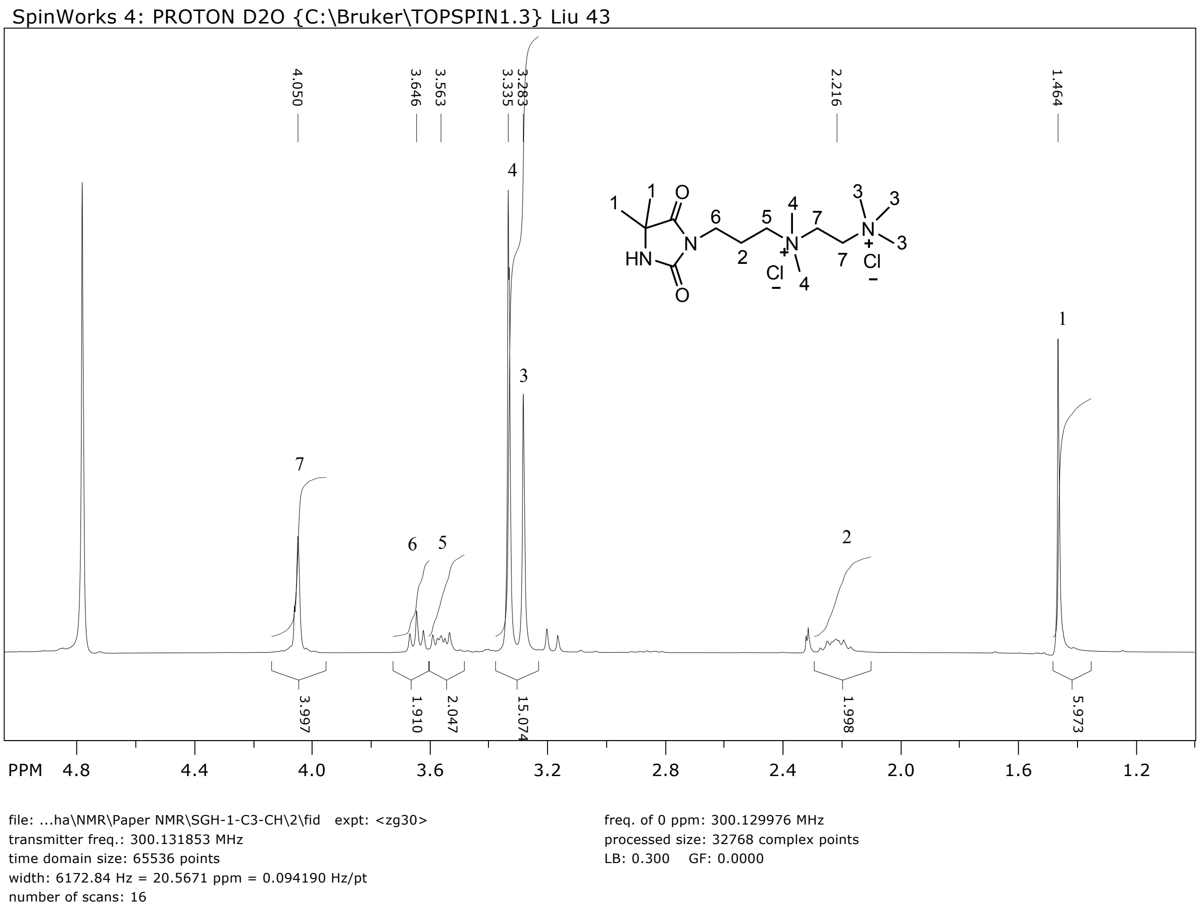
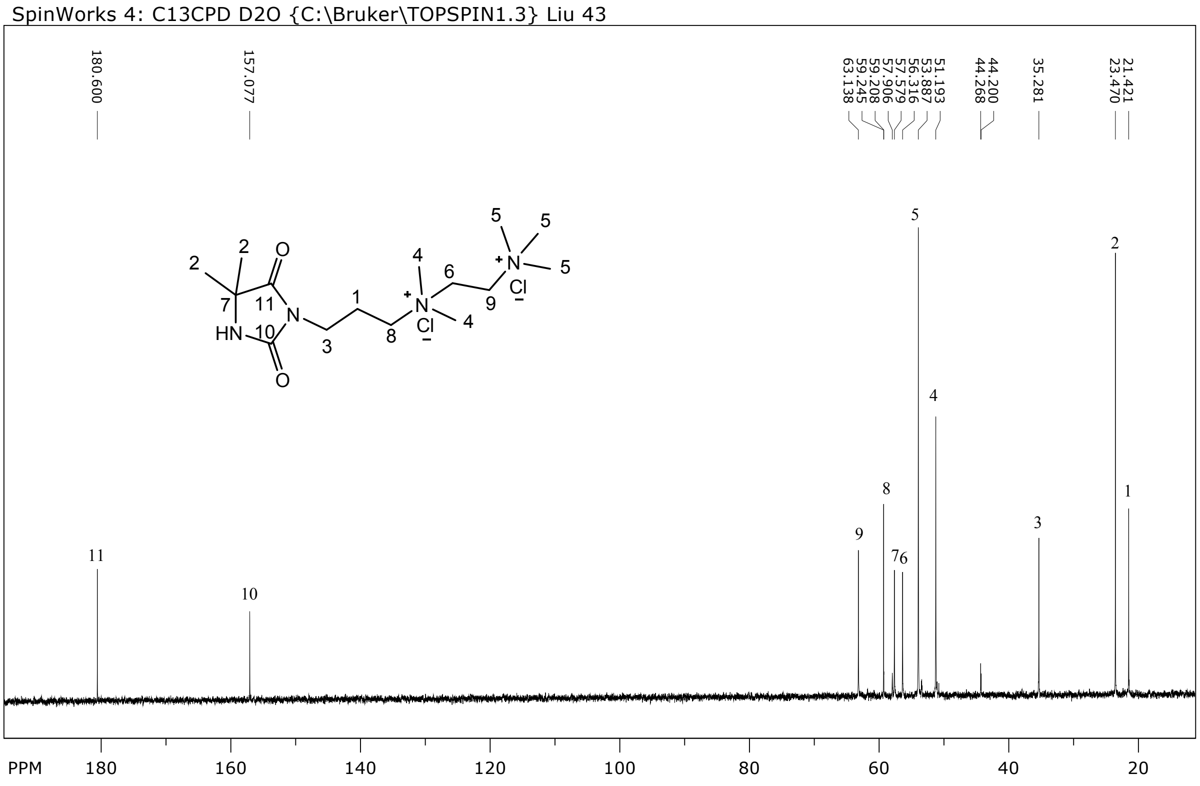


Figure S5. ^{1}H-NMR spectrum of **compound#3** (D_2O).



file: ...ha\NMR\Paper NMR\SGH-1-C3-CH\1\fid expt: <zgpg30>
transmitter freq.: 75.475295 MHz
time domain size: 65536 points
width: 17985.61 Hz = 238.2980 ppm = 0.274439 Hz/pt
number of scans: 1024

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000

Figure S6. ^{13}C NMR spectrum of **compound#3** (D_2O).

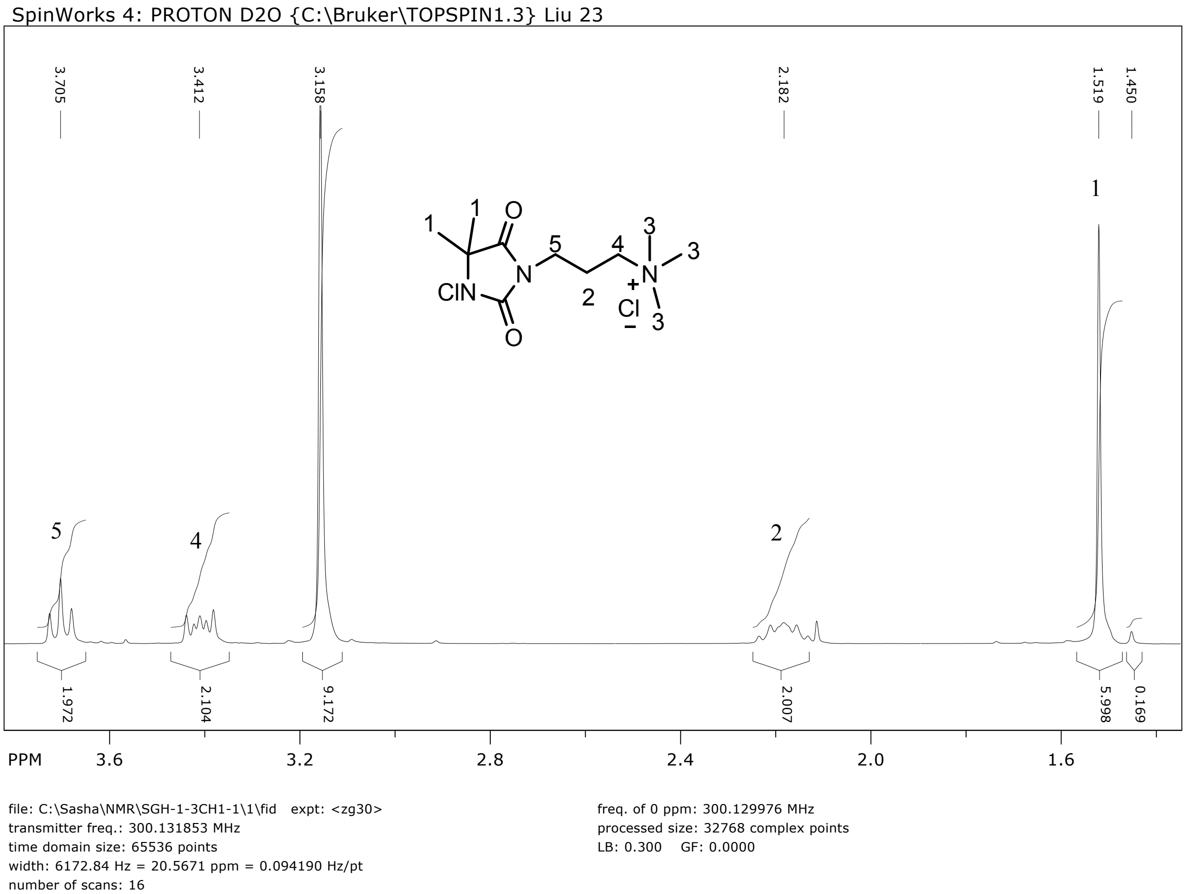
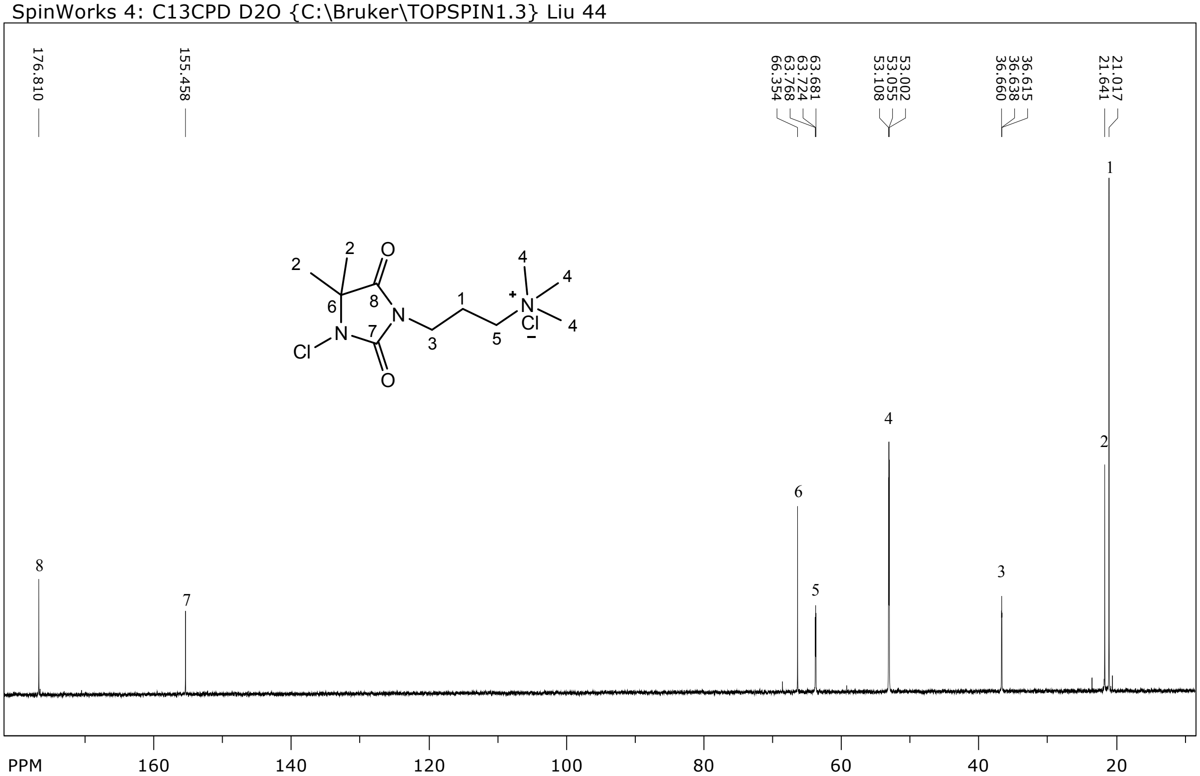


Figure S7. ^{1}H-NMR spectrum of **compound#4** (D_2O).

SpinWorks 4: C13CPD D2O {C:\Bruker\TOPSPIN1.3} Liu 44



file: ...ha\NMR\Paper NMR\SGH-1-C4-CH\1\fid expt: <zgpg30>
transmitter freq.: 75.475295 MHz
time domain size: 65536 points
width: 17985.61 Hz = 238.2980 ppm = 0.274439 Hz/pt
number of scans: 1024

freq. of 0 ppm: 75.467749 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000

Figure S8. ^{13}C NMR spectrum of **compound#4** (D_2O).

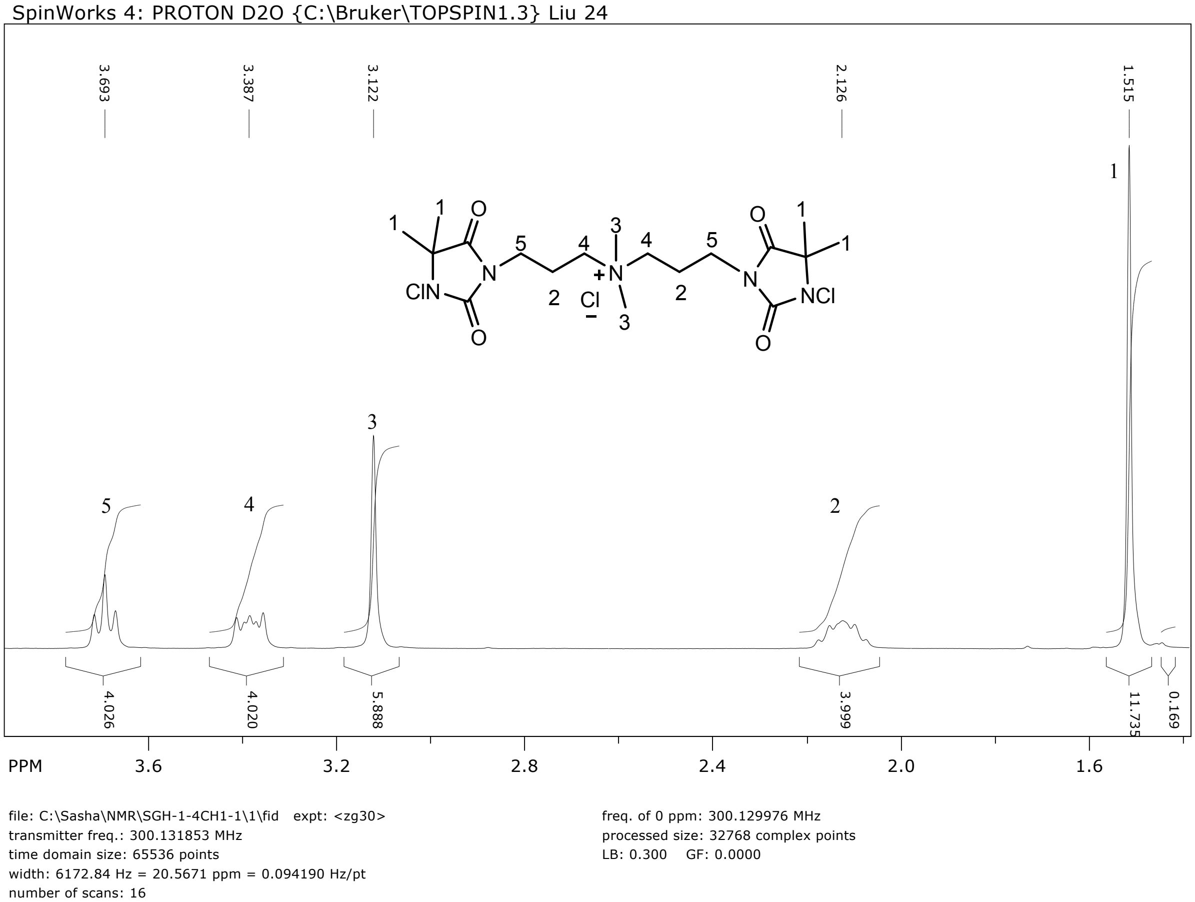


Figure S9. ^{1}H-NMR spectrum of **compound#5** (D_2O).

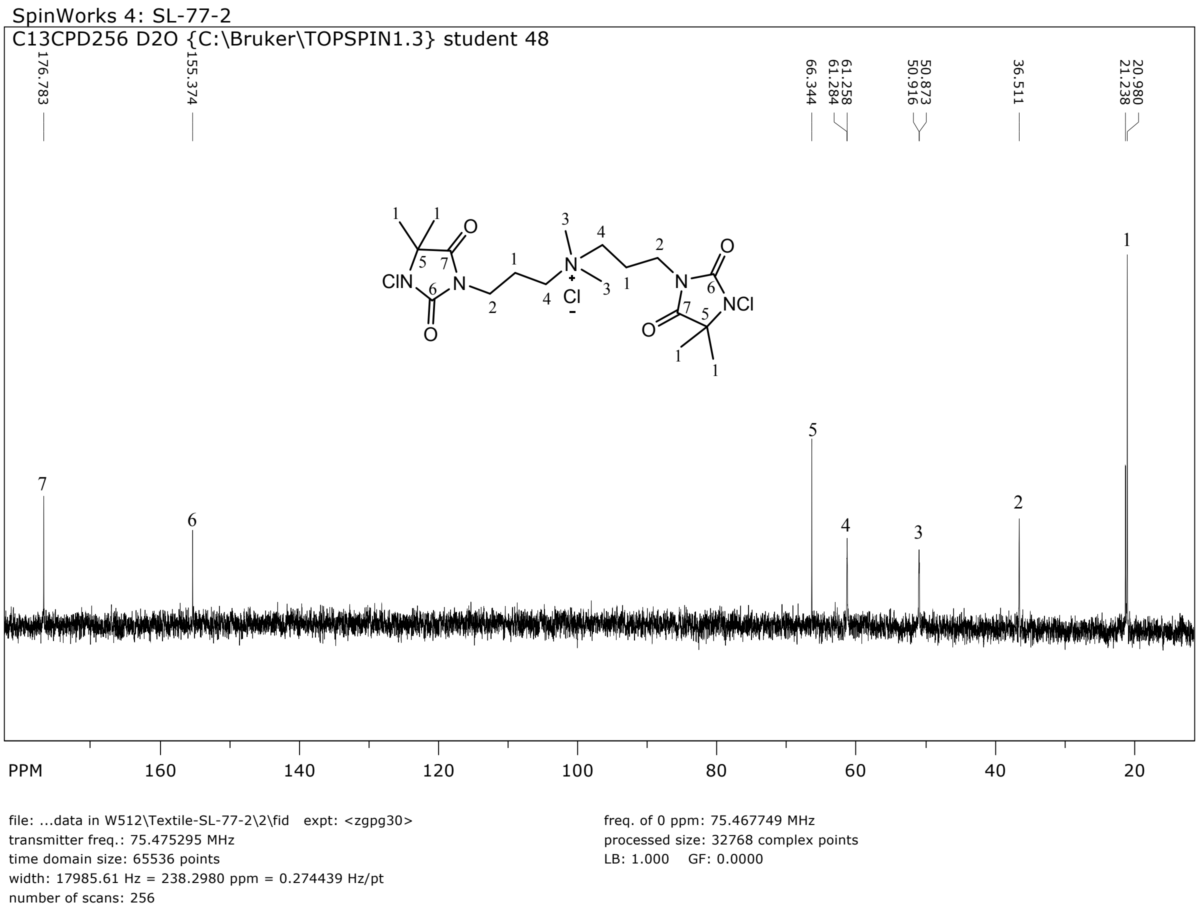


Figure S10. ^{13}C NMR spectrum of **compound#5** (D_2O).

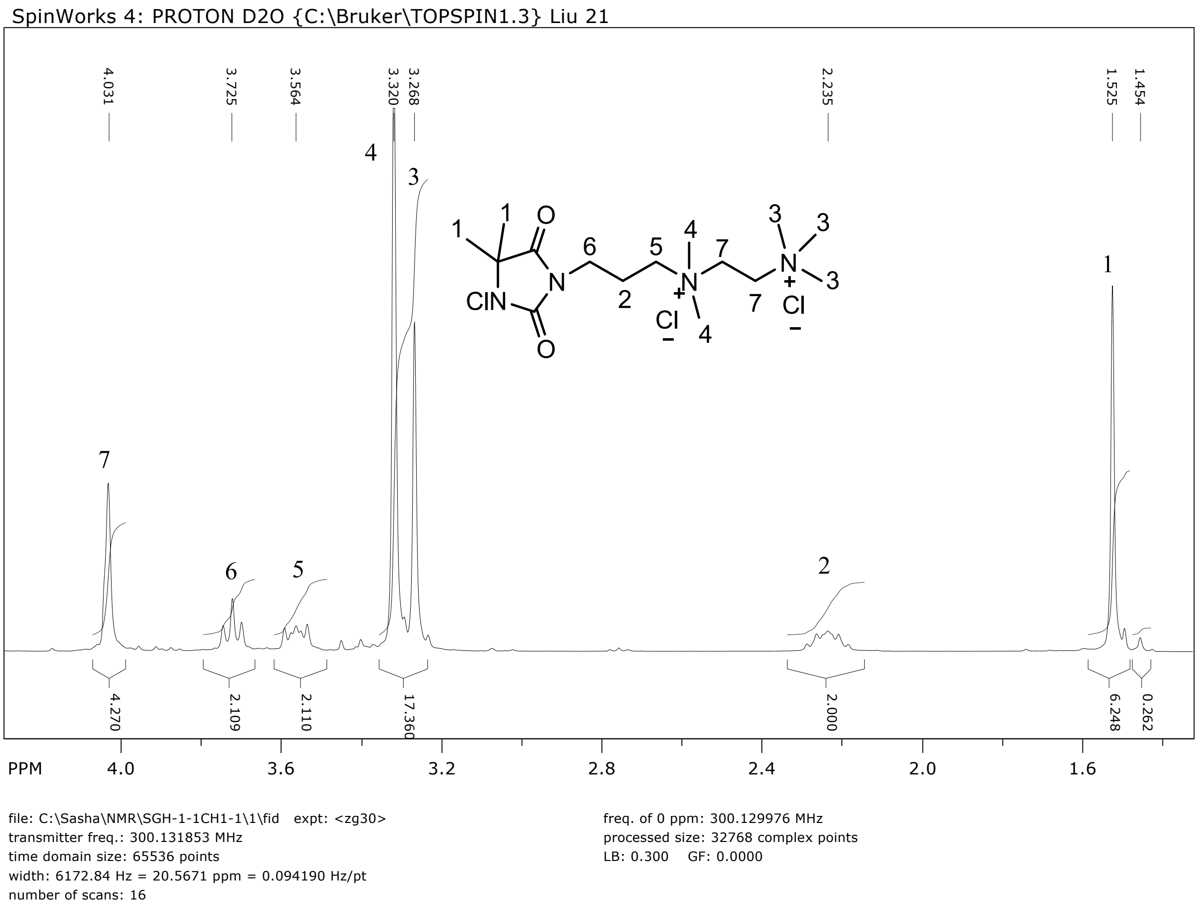


Figure S11. ^{1}H-NMR spectrum of **compound#6** (D_2O).

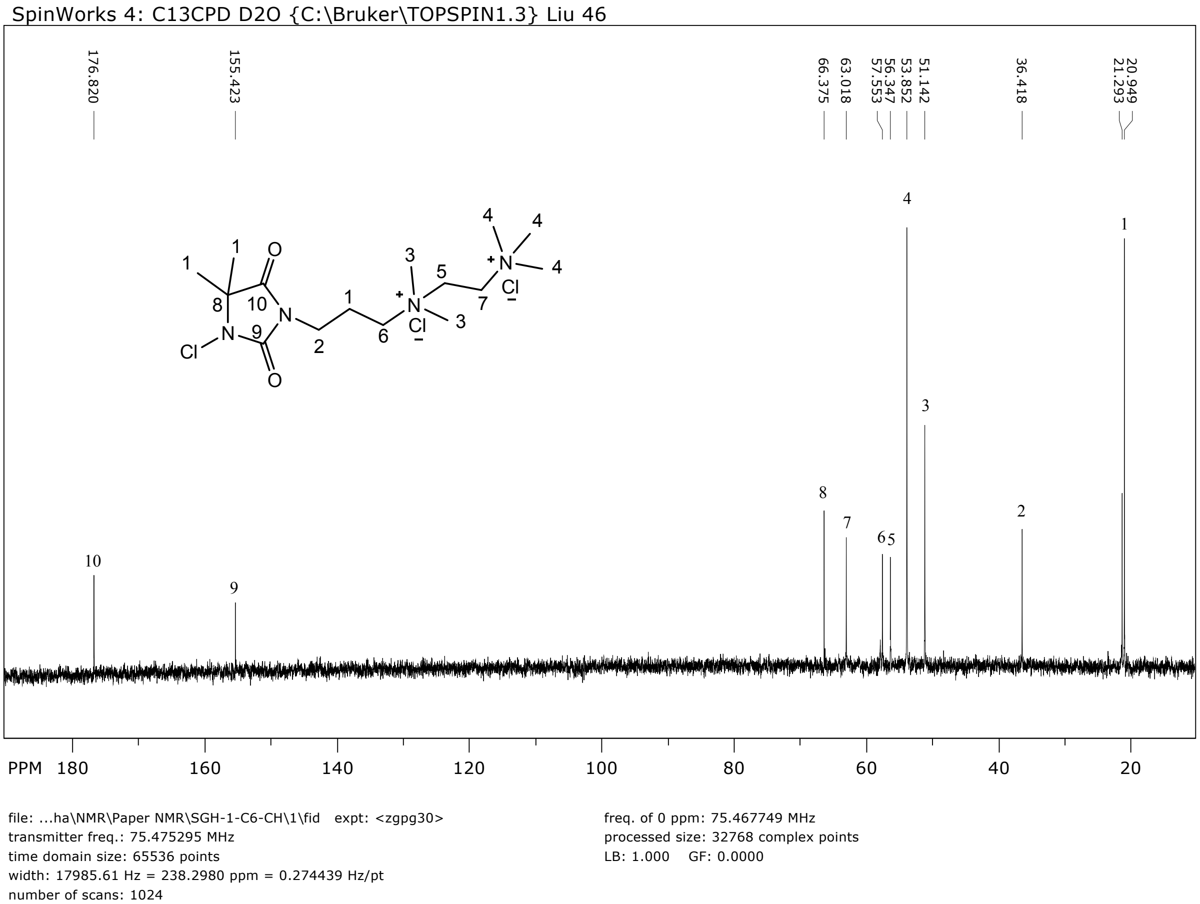


Figure S12. ^{13}C NMR spectrum of **compound#6** (D_2O).

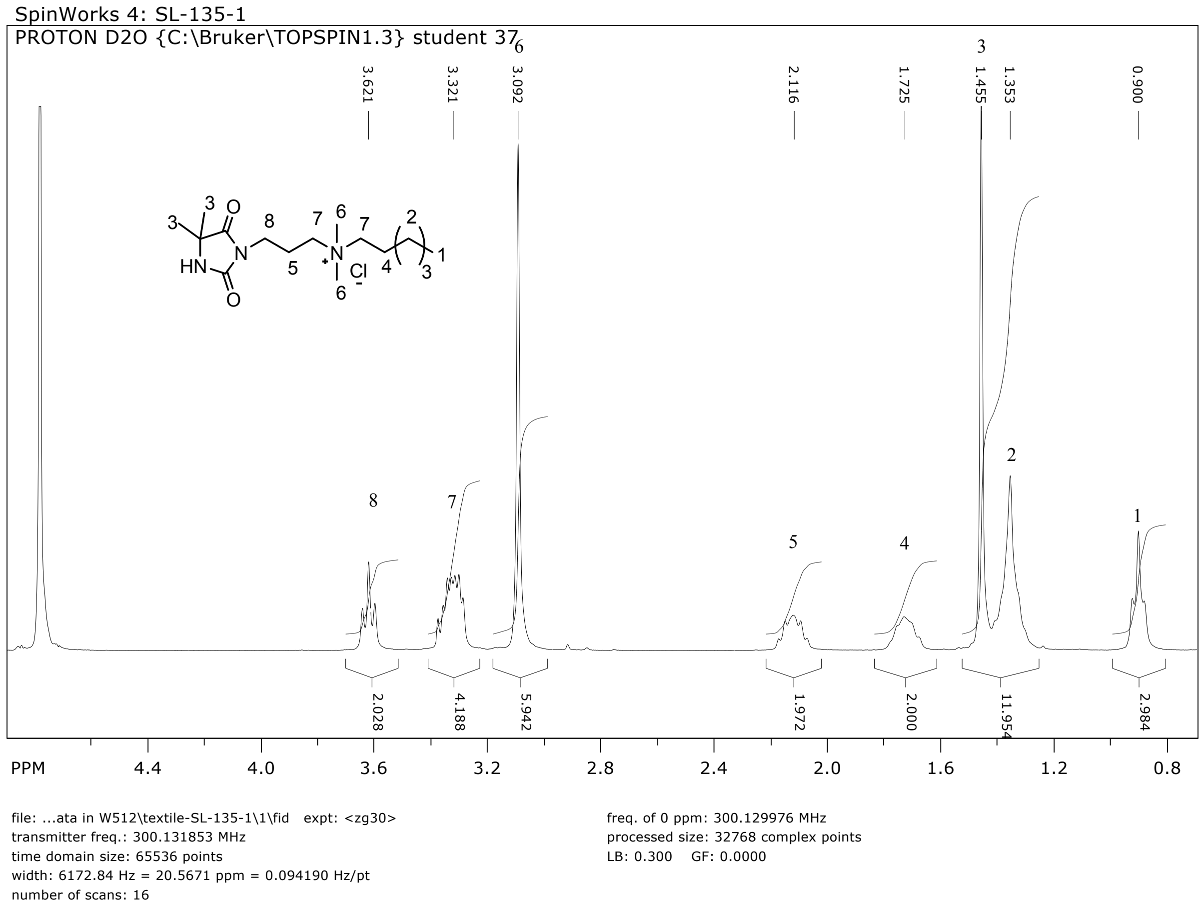


Figure S13. ^{1}H-NMR spectrum of **compound#7** (D_2O).

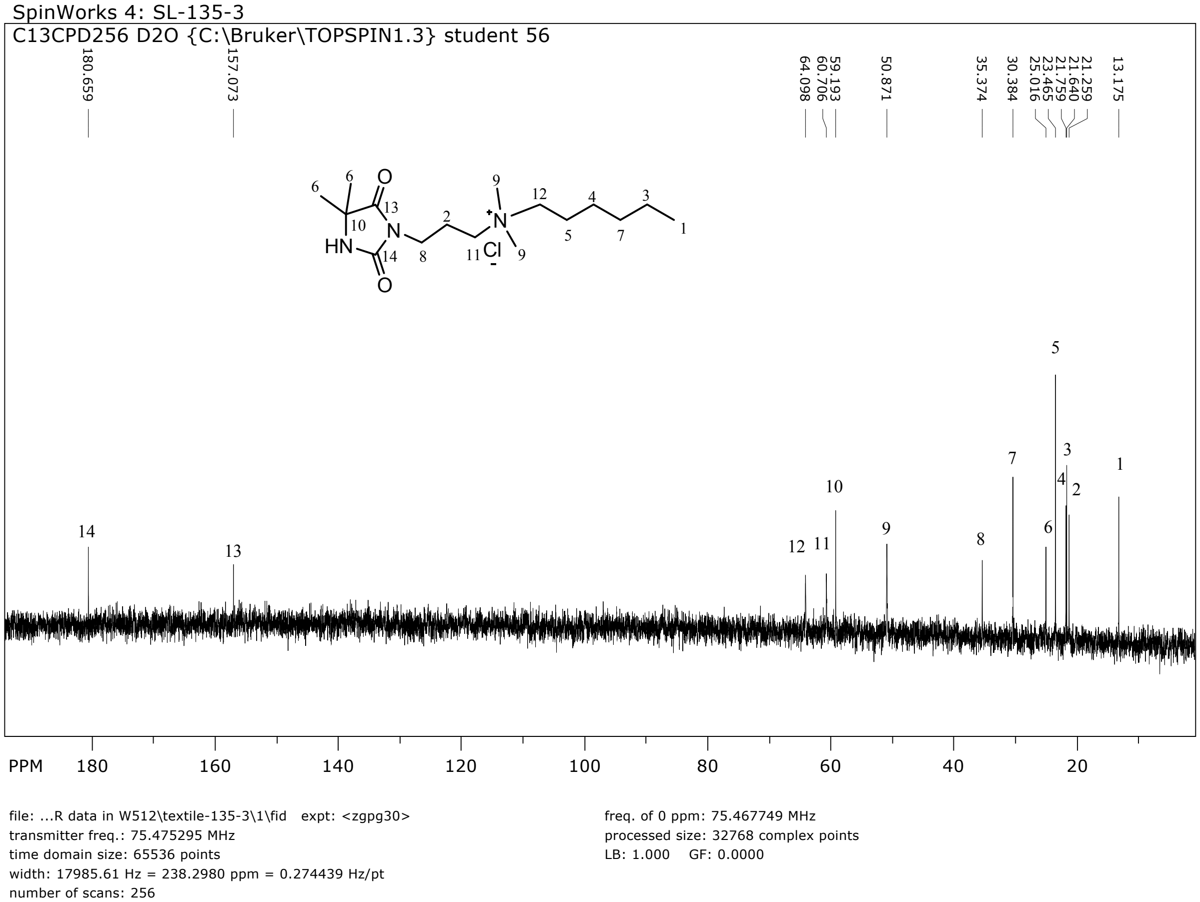
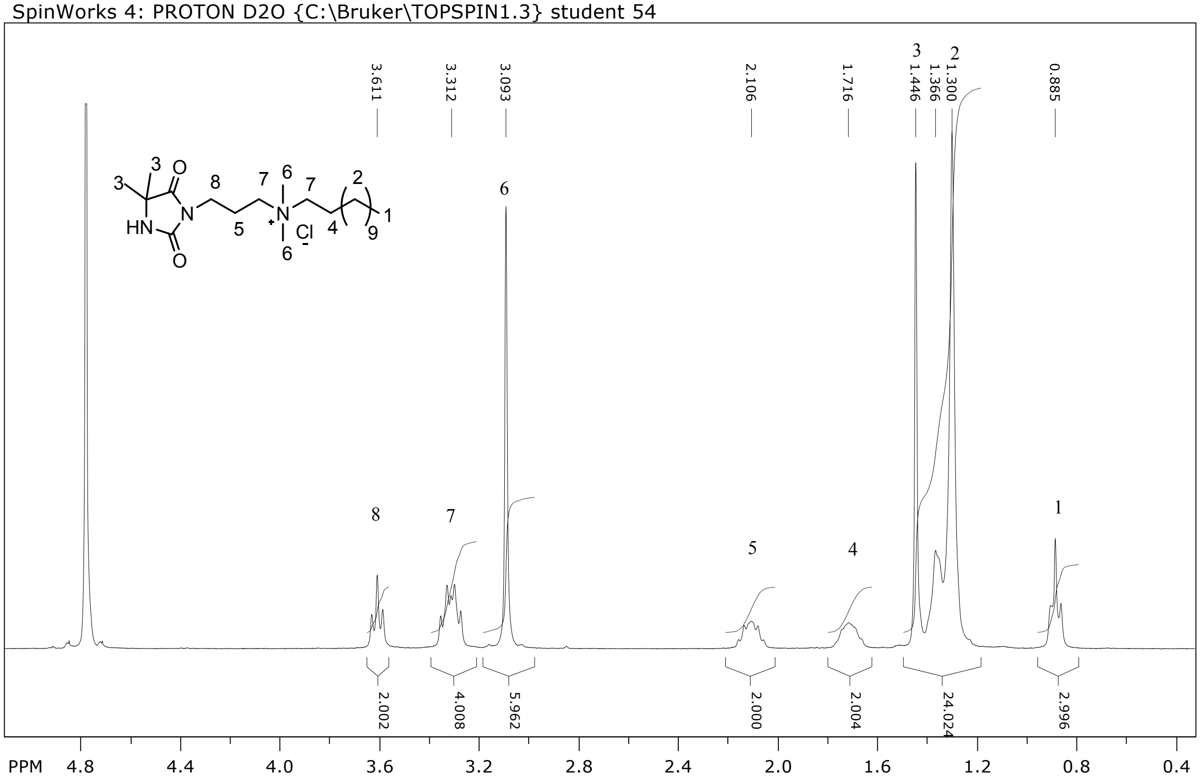


Figure S14. ^{13}C NMR spectrum of **compound#7** (D_2O).

SpinWorks 4: PROTON D2O {C:\Bruker\TOPSPIN1.3} student 54



file: ...a\NMR data in W512\sgh-138-4\1\fid expt: <zg30>
transmitter freq.: 300.131853 MHz
time domain size: 65536 points
width: 6172.84 Hz = 20.5671 ppm = 0.094190 Hz/pt
number of scans: 16

freq. of 0 ppm: 300.129976 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000

Figure S15. ^{1}H-NMR spectrum of **compound#8** (D_2O).

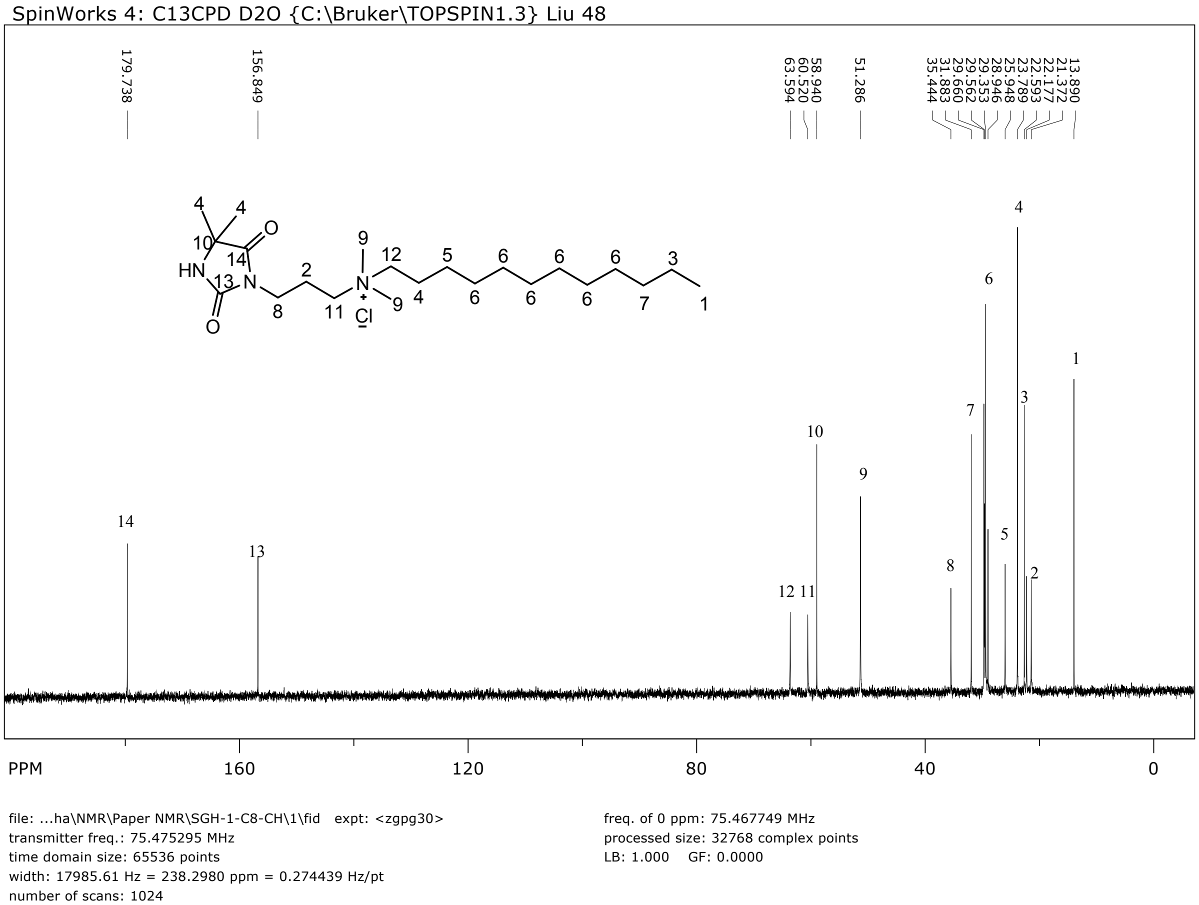


Figure S16. ^{13}C NMR spectrum of **compound#8** (D_2O).

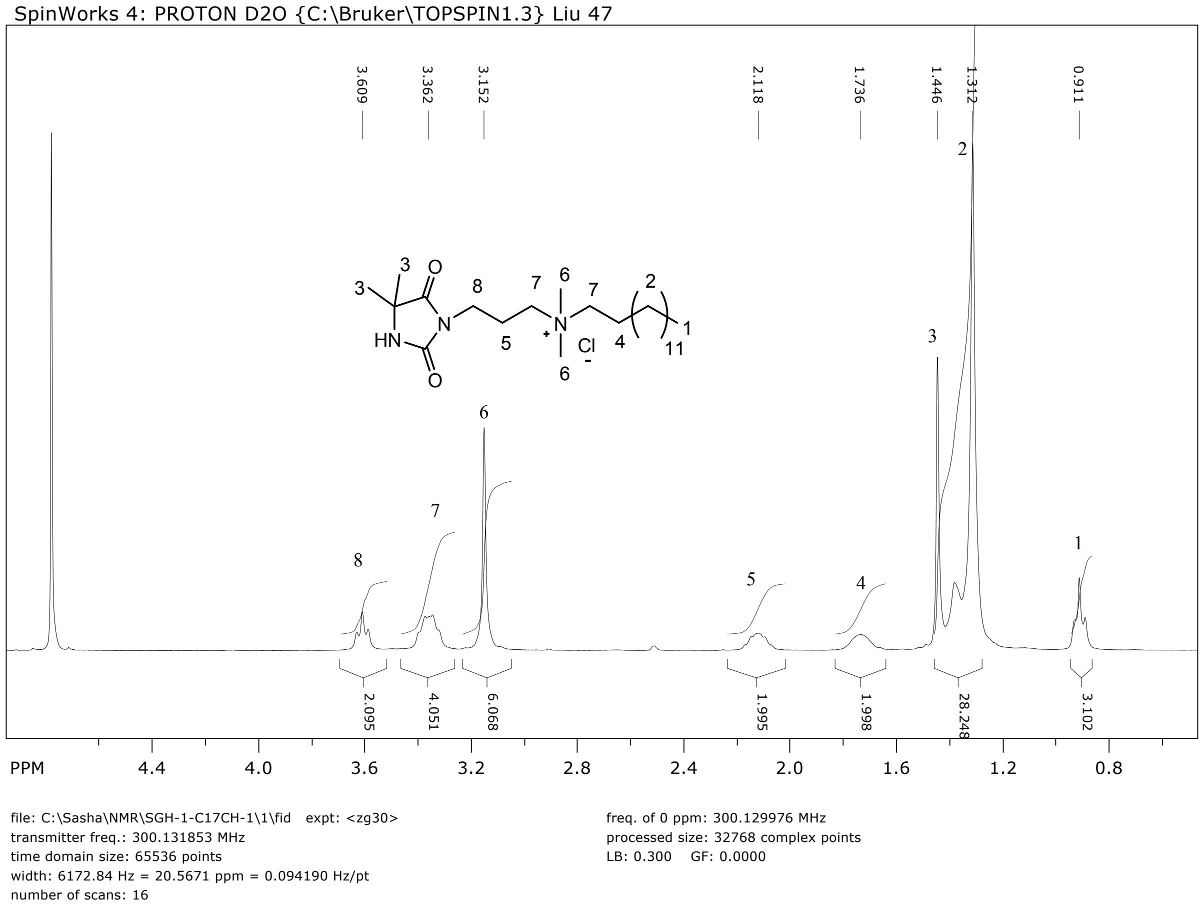


Figure S17. ^{1}H-NMR spectrum of **compound#9** (D_2O).

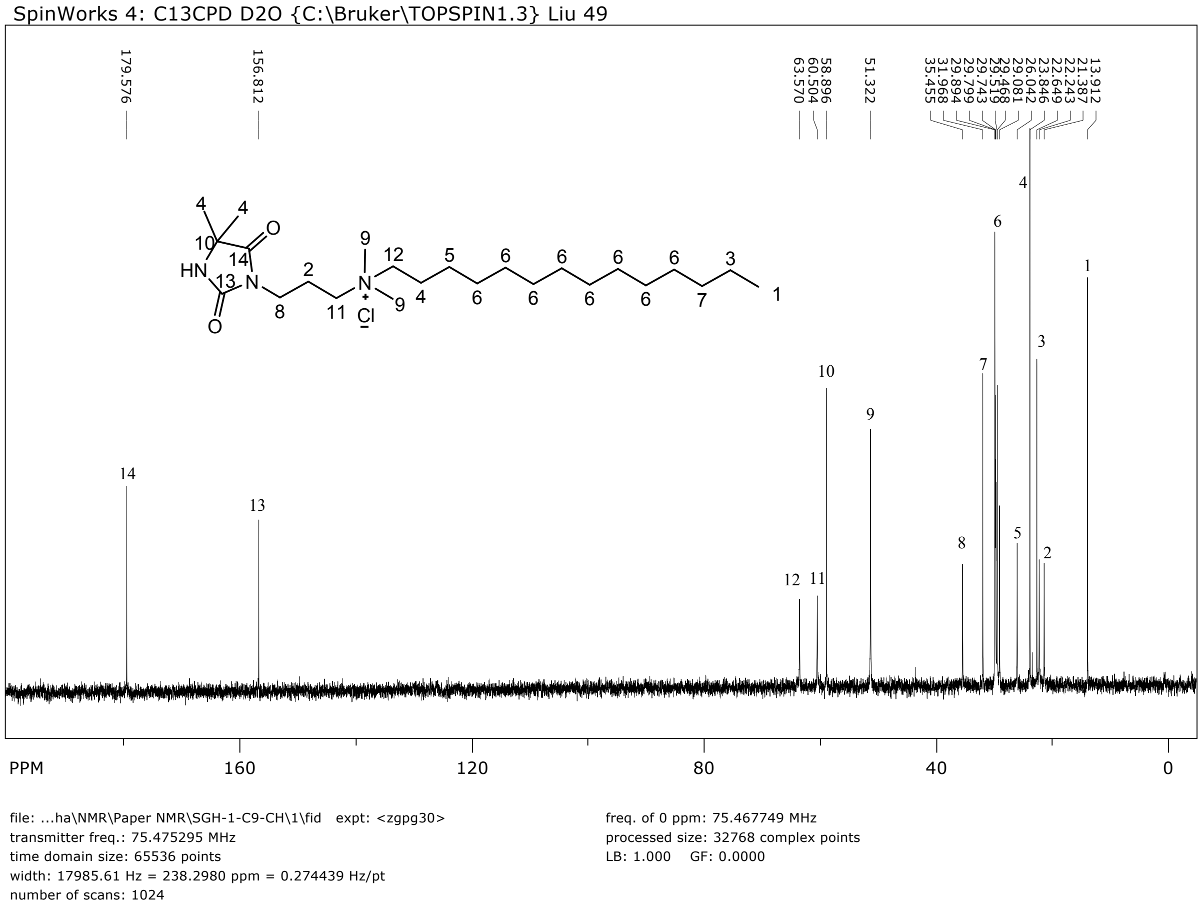


Figure S18. ^{13}C NMR spectrum of **compound#9** (D_2O).

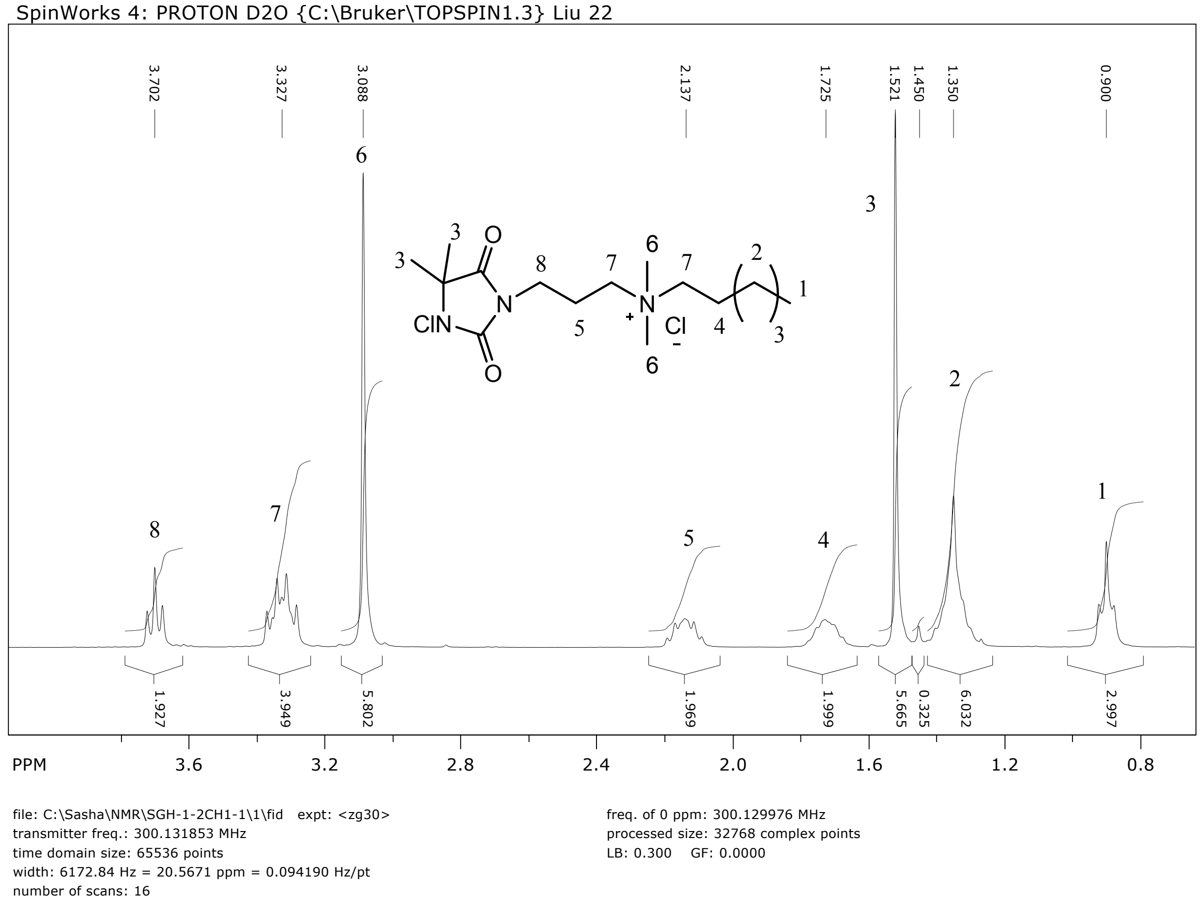


Figure S19. ^{1}H-NMR spectrum of **compound#10** (D_2O).

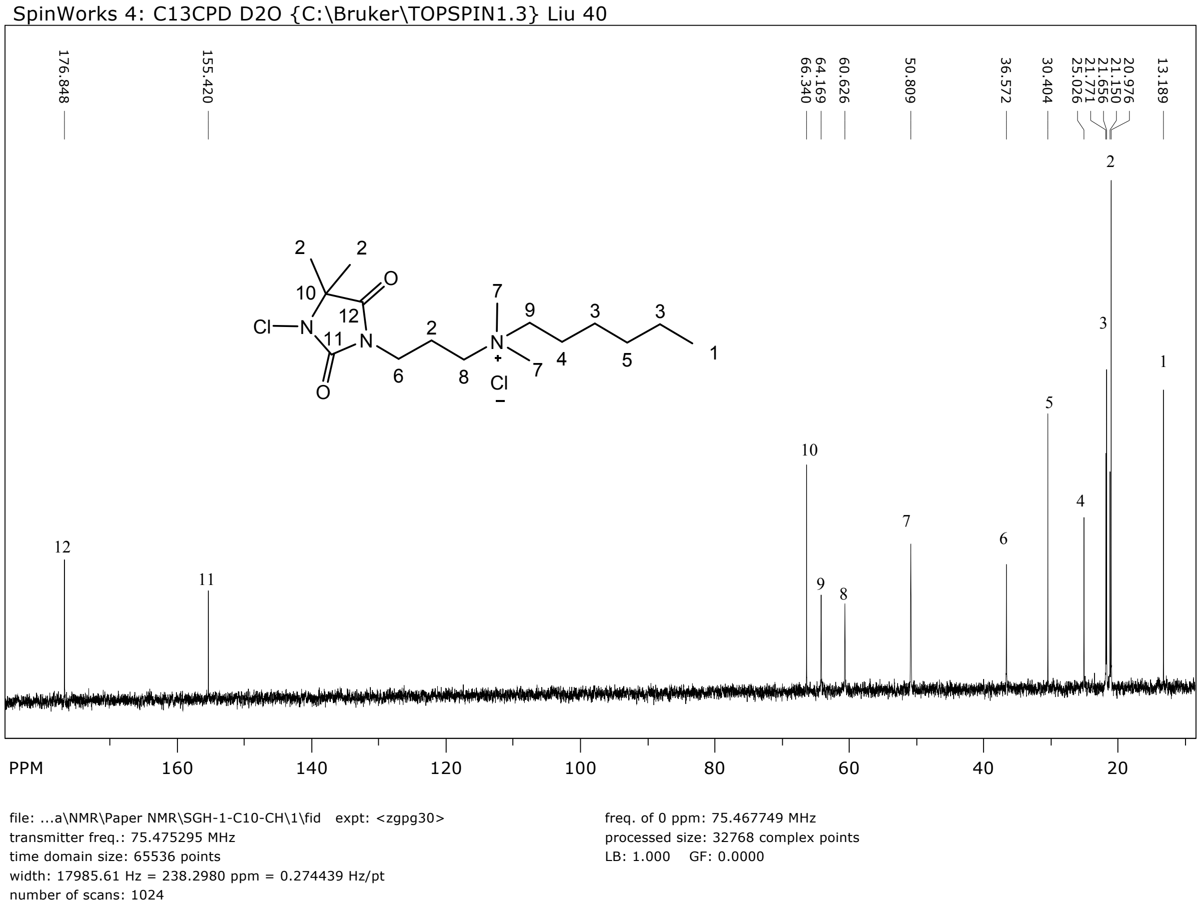


Figure S20. ^{13}C NMR spectrum of **compound#10** (D_2O).

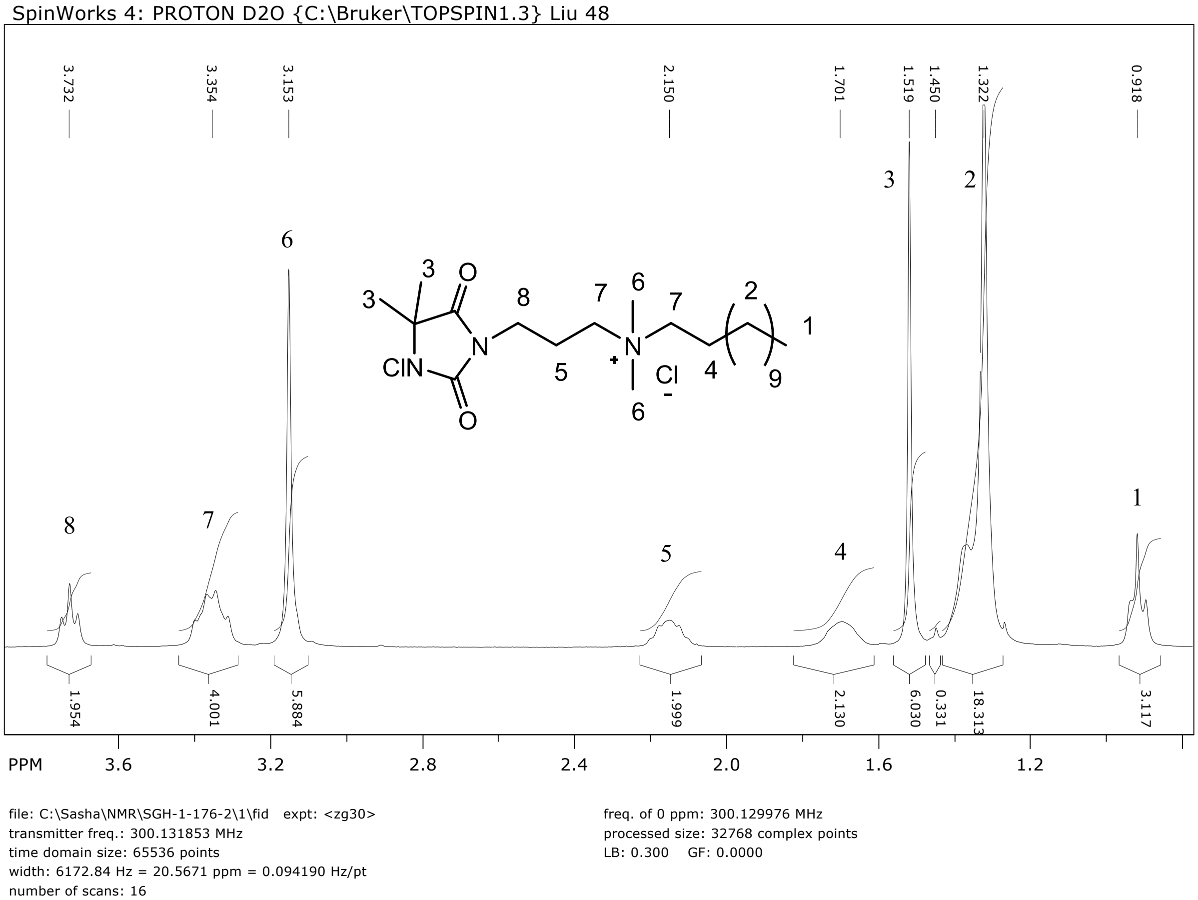


Figure S21. ^{1}H-NMR spectrum of **compound#11**(D_2O).

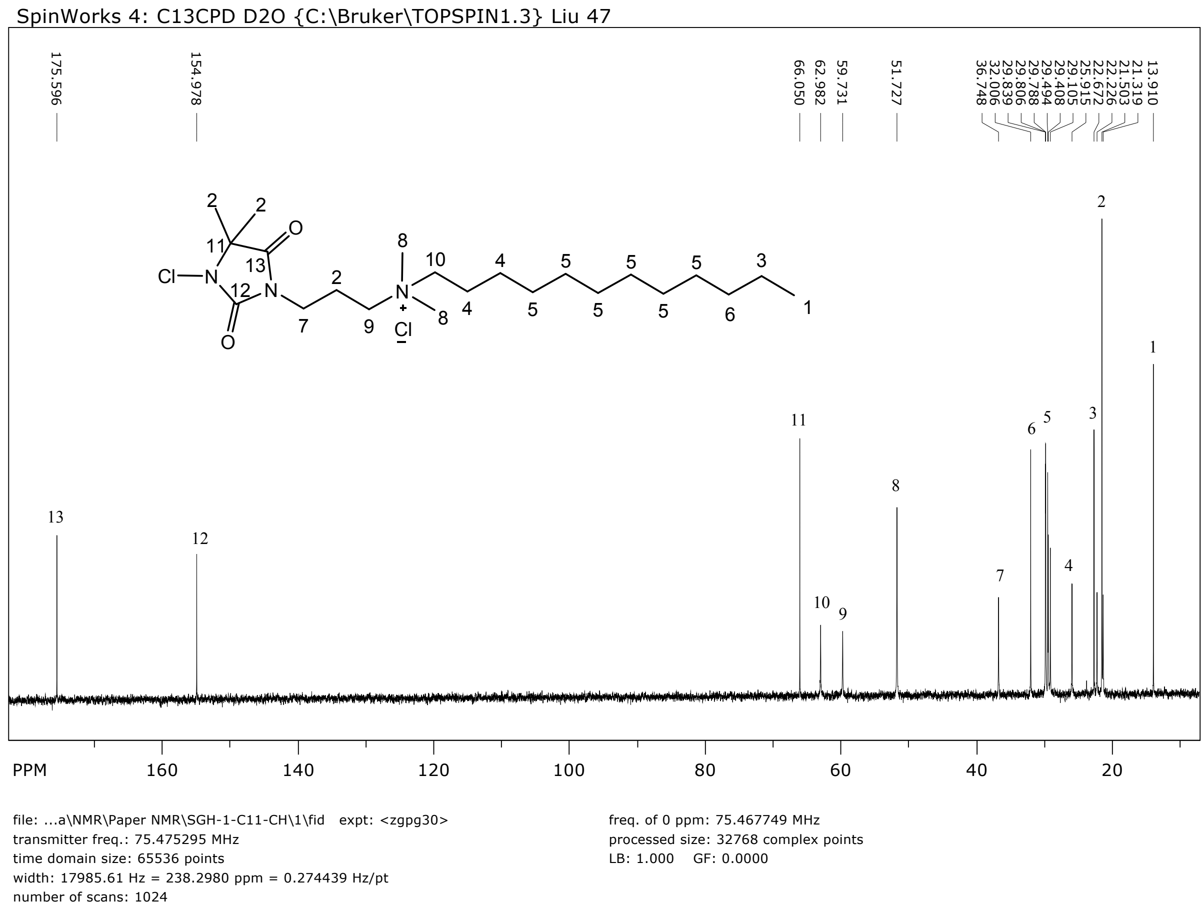


Figure S22. ^{13}C NMR spectrum of **compound#11**(D_2O).

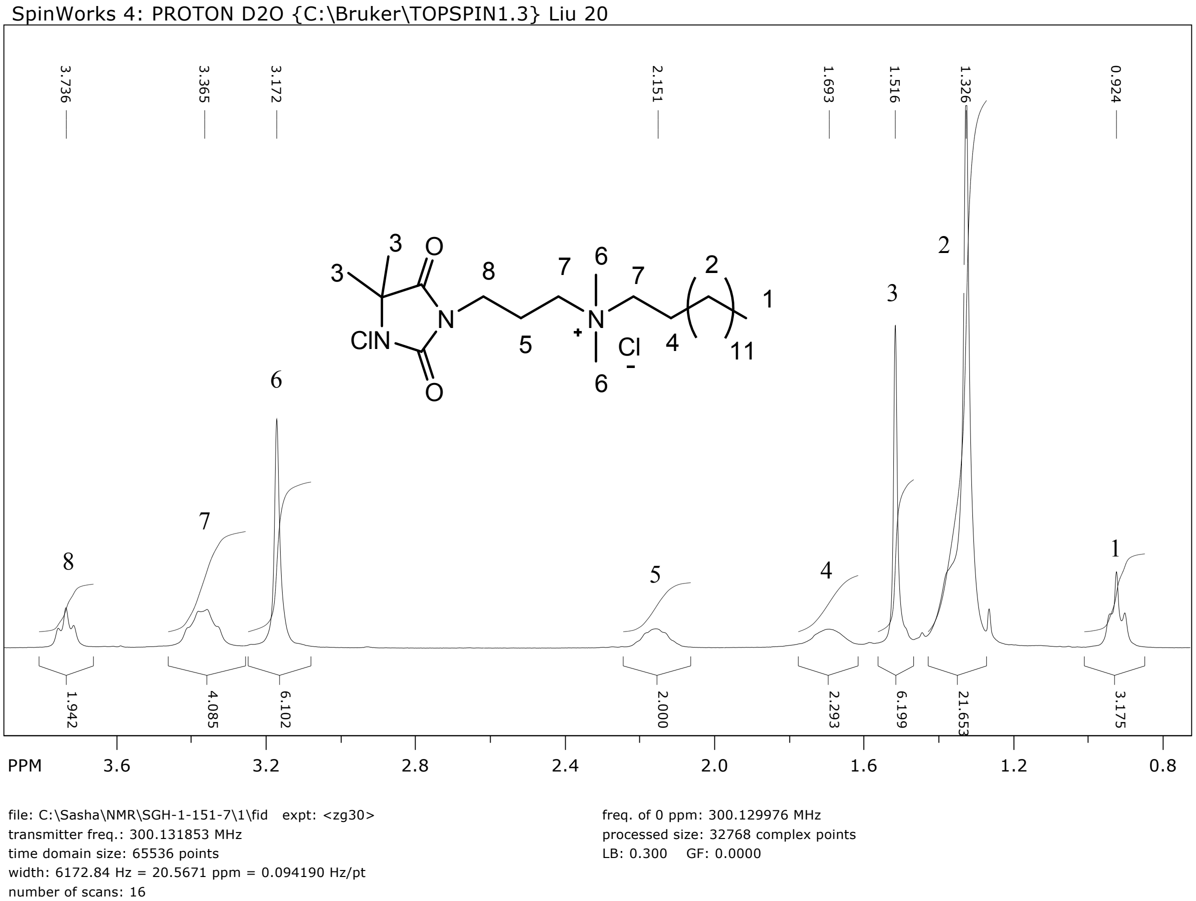


Figure S23. ^{1}H-NMR spectrum of **compound#12** (D_2O).

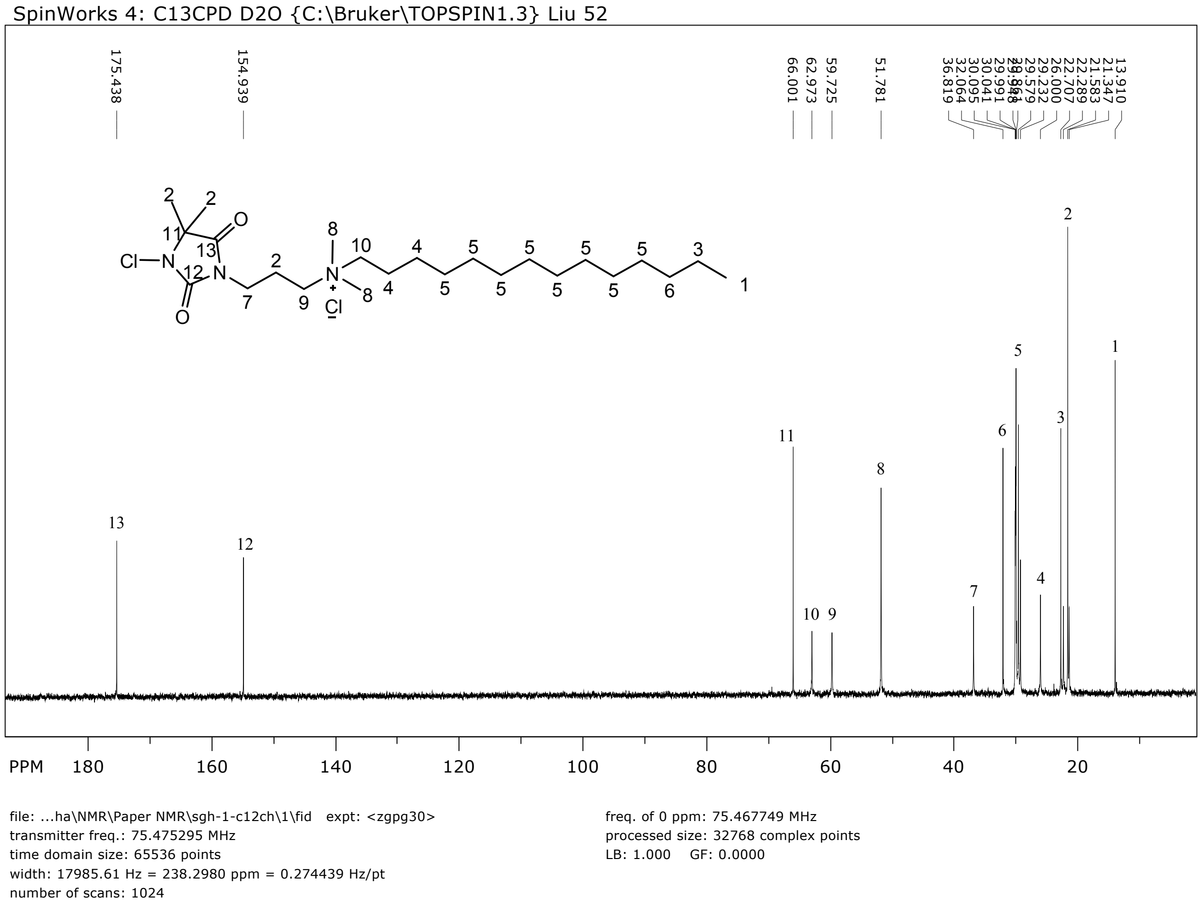


Figure S24. ^{13}C NMR spectrum of **compound#12** (D_2O).

2. HPLC-MS analysis of compounds # 1-3,7-9

Analytic HPLC was run on a Varian 212 HPLC instrument, equipped with X-Bridge™ BEH C18 2.5μm and Inertsil C8 3.3μm columns and interfaced with Varian 500 MS-Ion trap detector. Eluent system was gradient: acetonitrile: water (5:95, v/v, 0.1% of formic acid), a linear gradient was applied for 20 min, up to an acetonitrile: water ratio of 90:10 (v/v), after which elution with a acetonitrile: water (5:95, v/v) gradient was used. Flow: 0.4 ml min^{-1}. Compound #7-8 were analyzed using X-Bridge™ BEH C18 2.5μm and the rest were ran through Inertsil C8 3.3μm column.

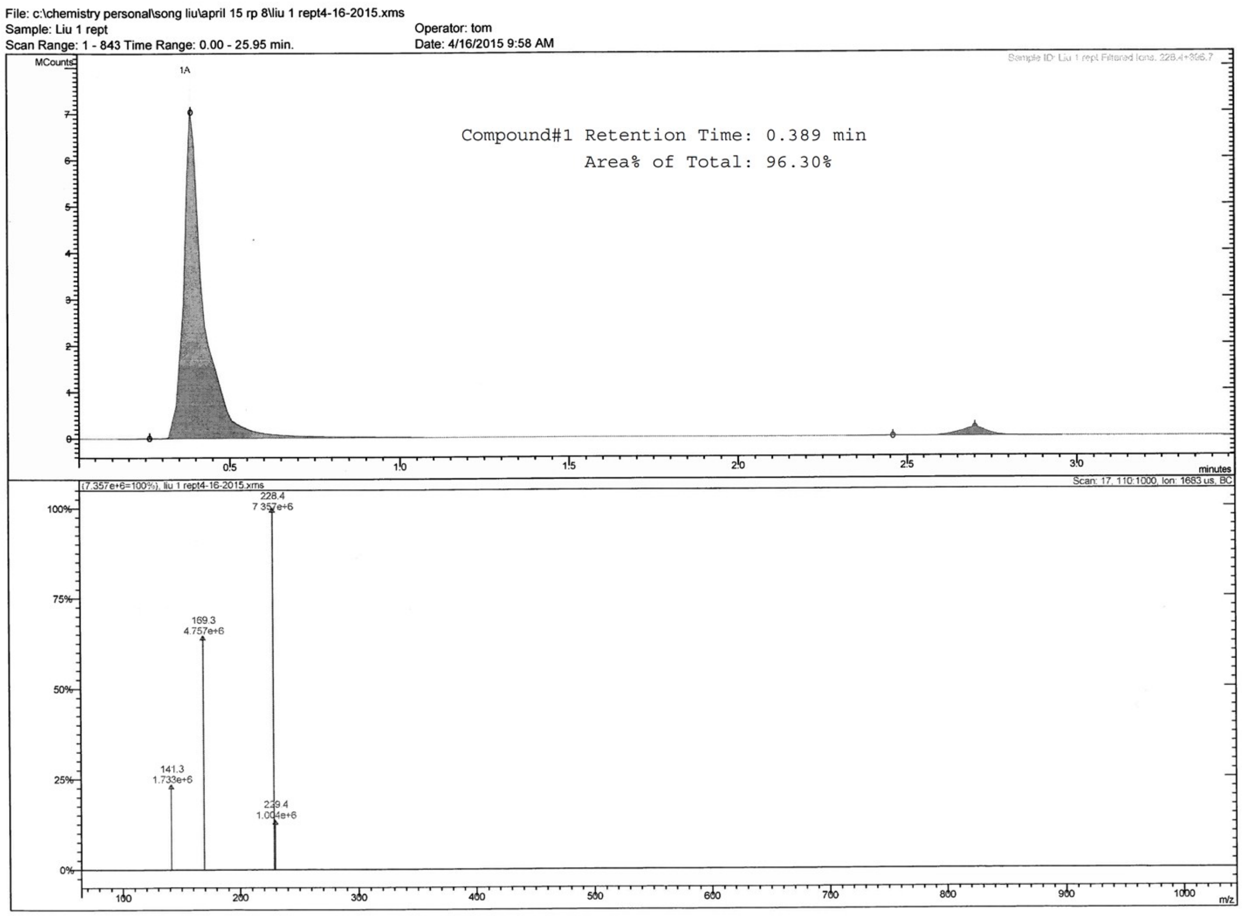


Figure S25. LCMS analysis of compound#**1**, purity: 96.30%

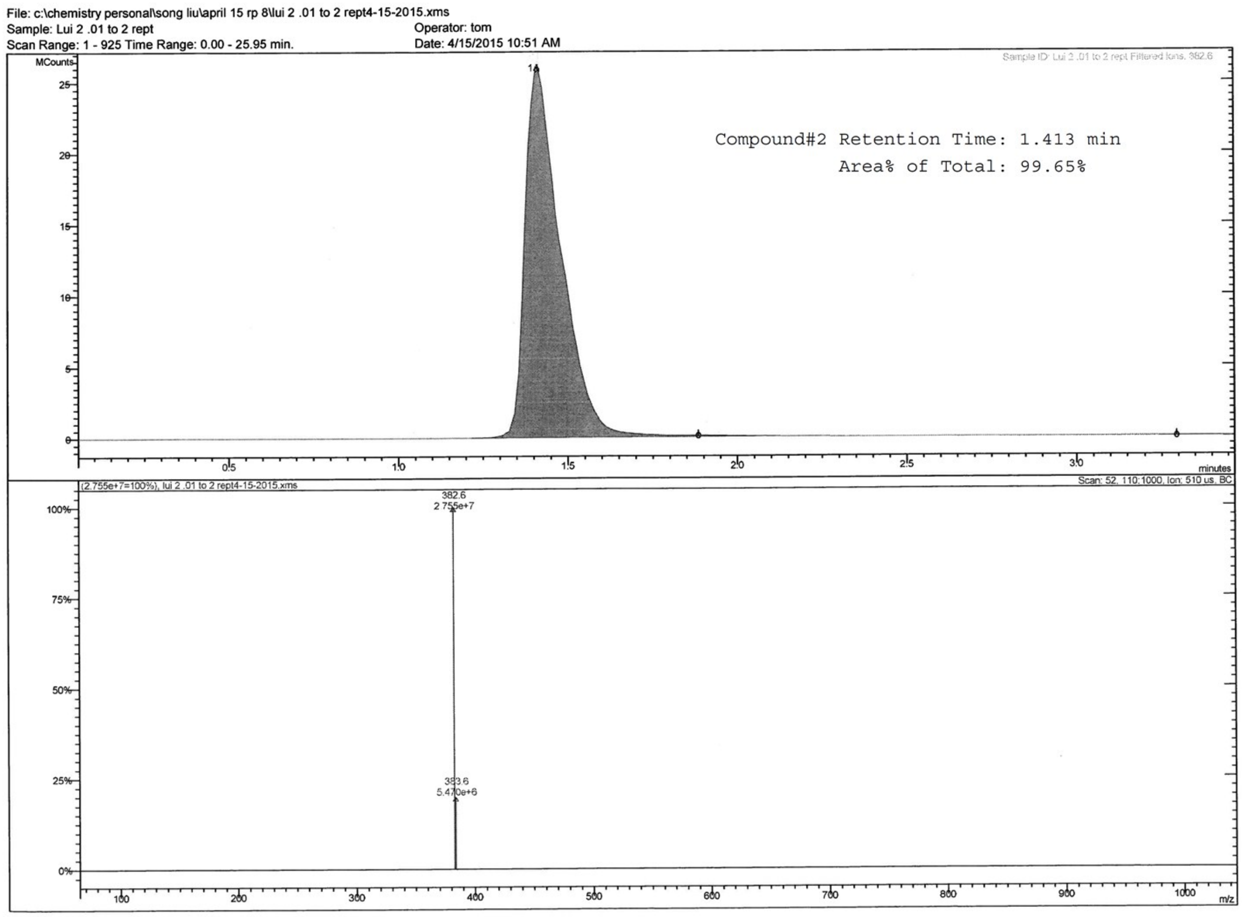


Figure S26. LCMS analysis of compound#**2**, purity: 99.65%.

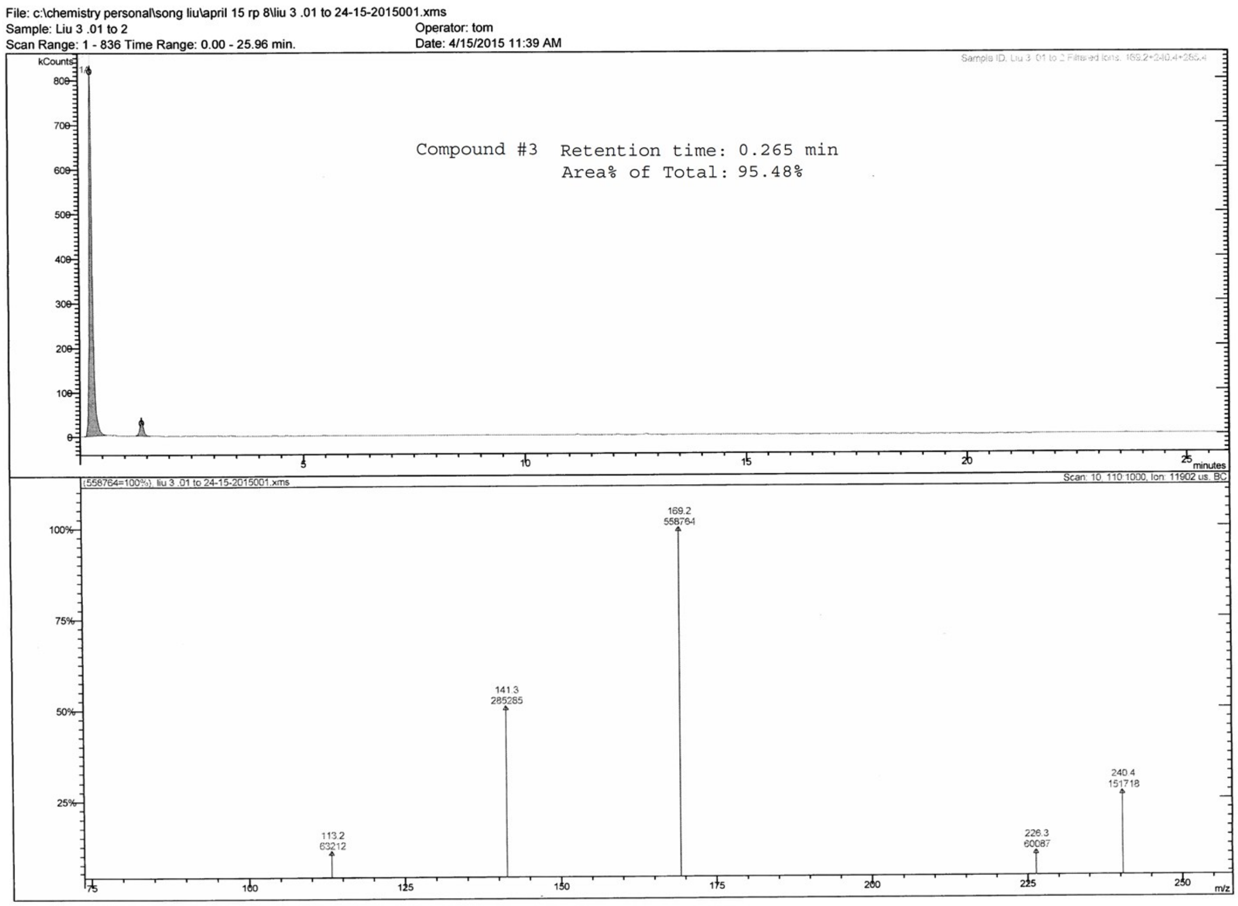


Figure S27. LCMS analysis of compound#**3**, purity: 95.48%.

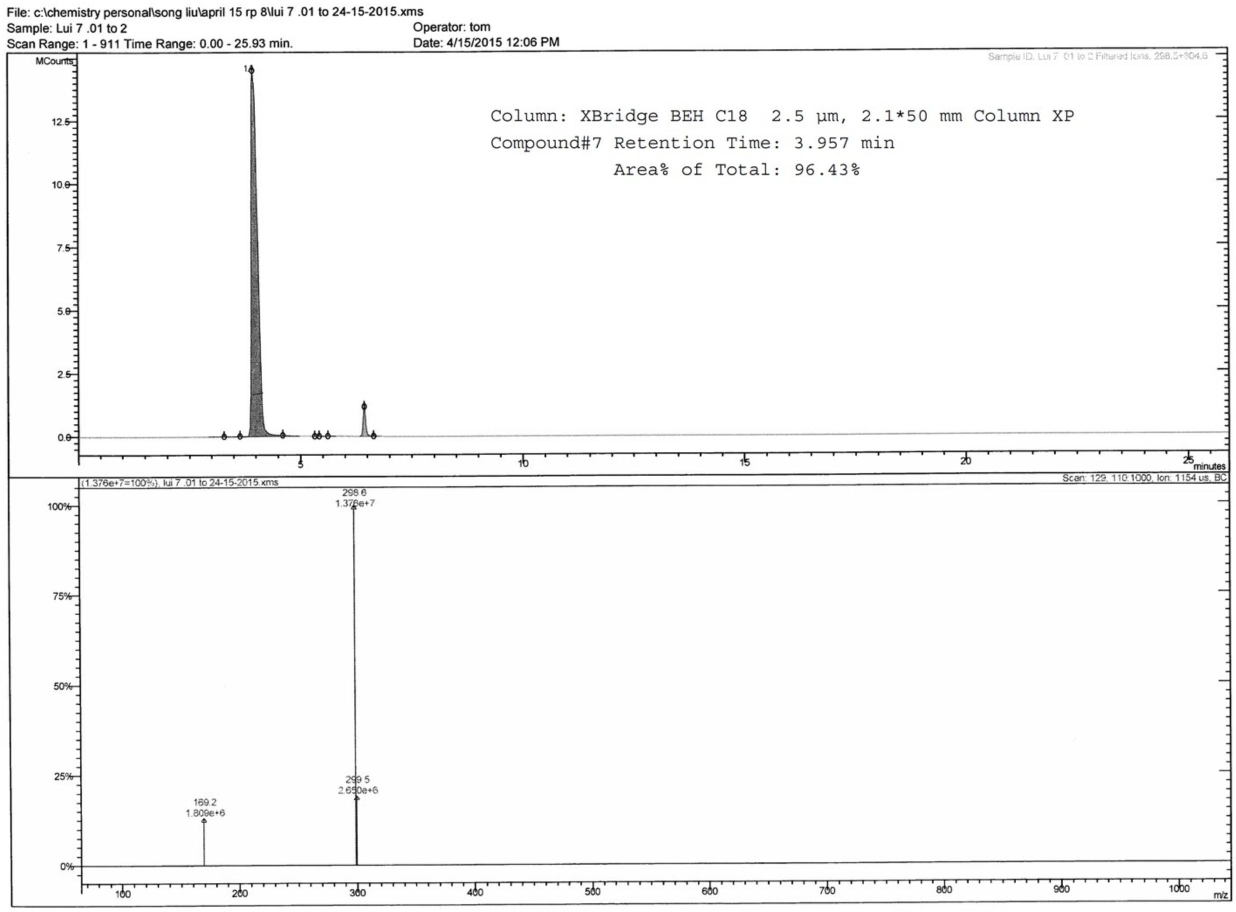


Figure S28. LCMS analysis of compound#**7**, purity: 96.43%.

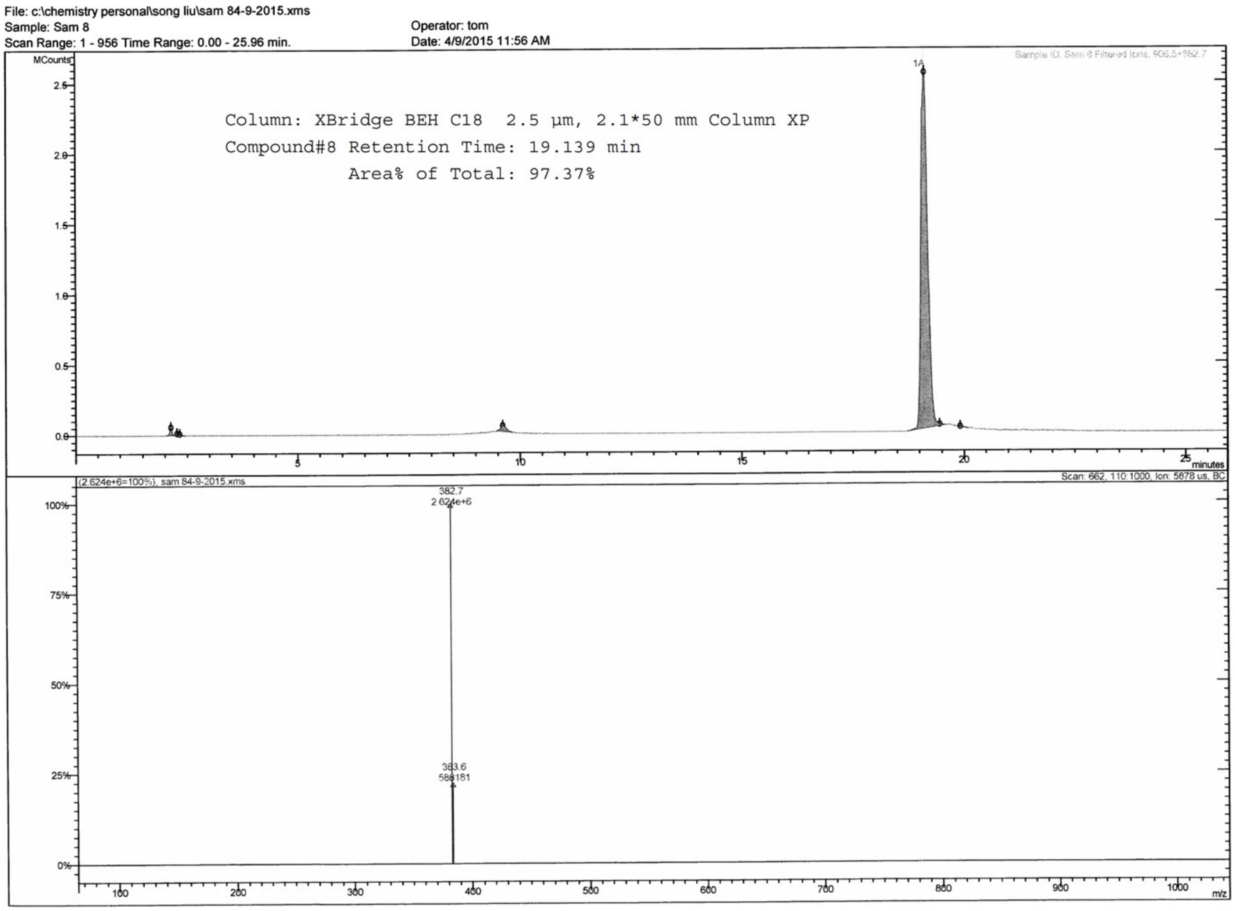


Figure S29. LCMS analysis of compound#**8**, purity: 97.37%.

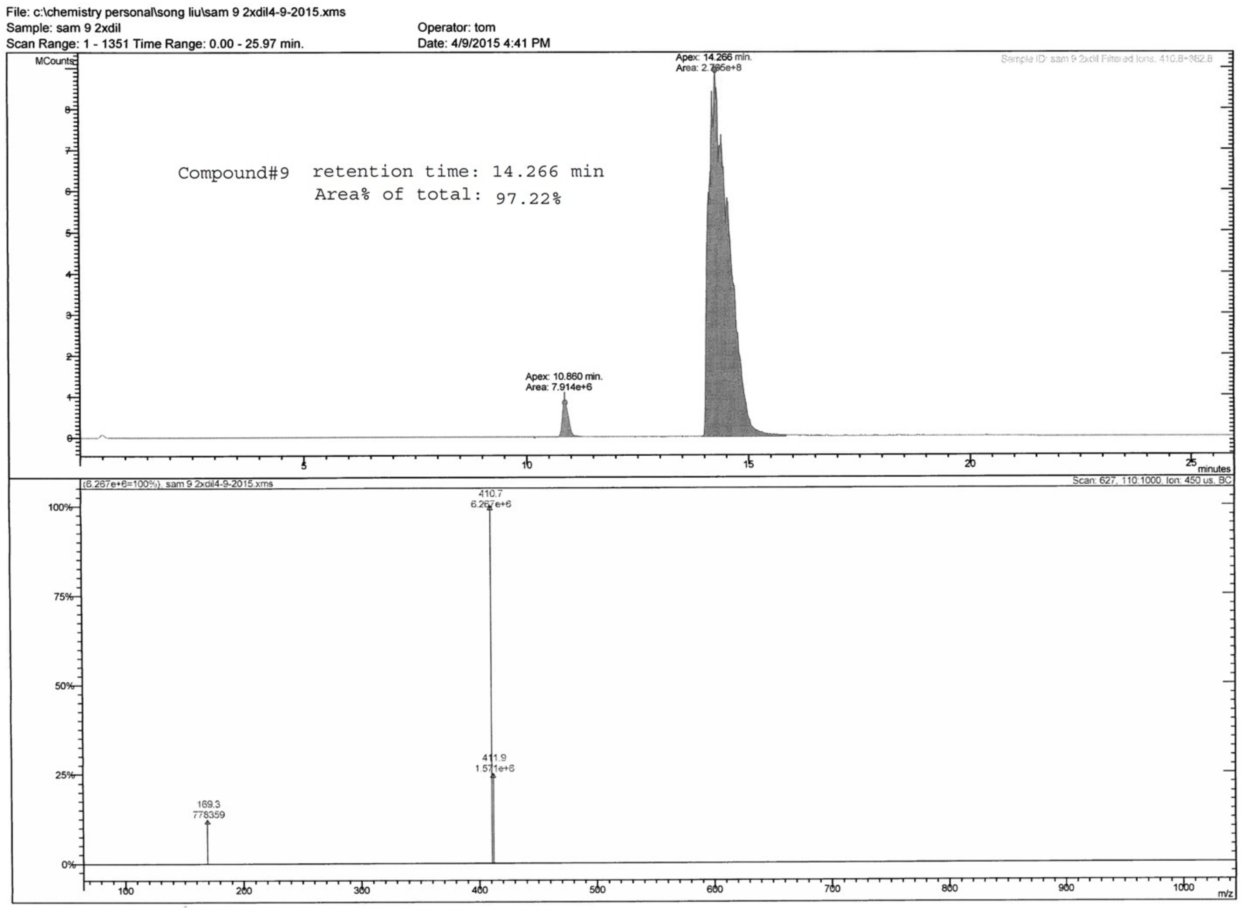


Figure S30. LCMS analysis of compound#**9**, purity: 97.22%.

3. Absolute quantitative [1]H NMR (qHNMR) of compounds# 4-6,10-12

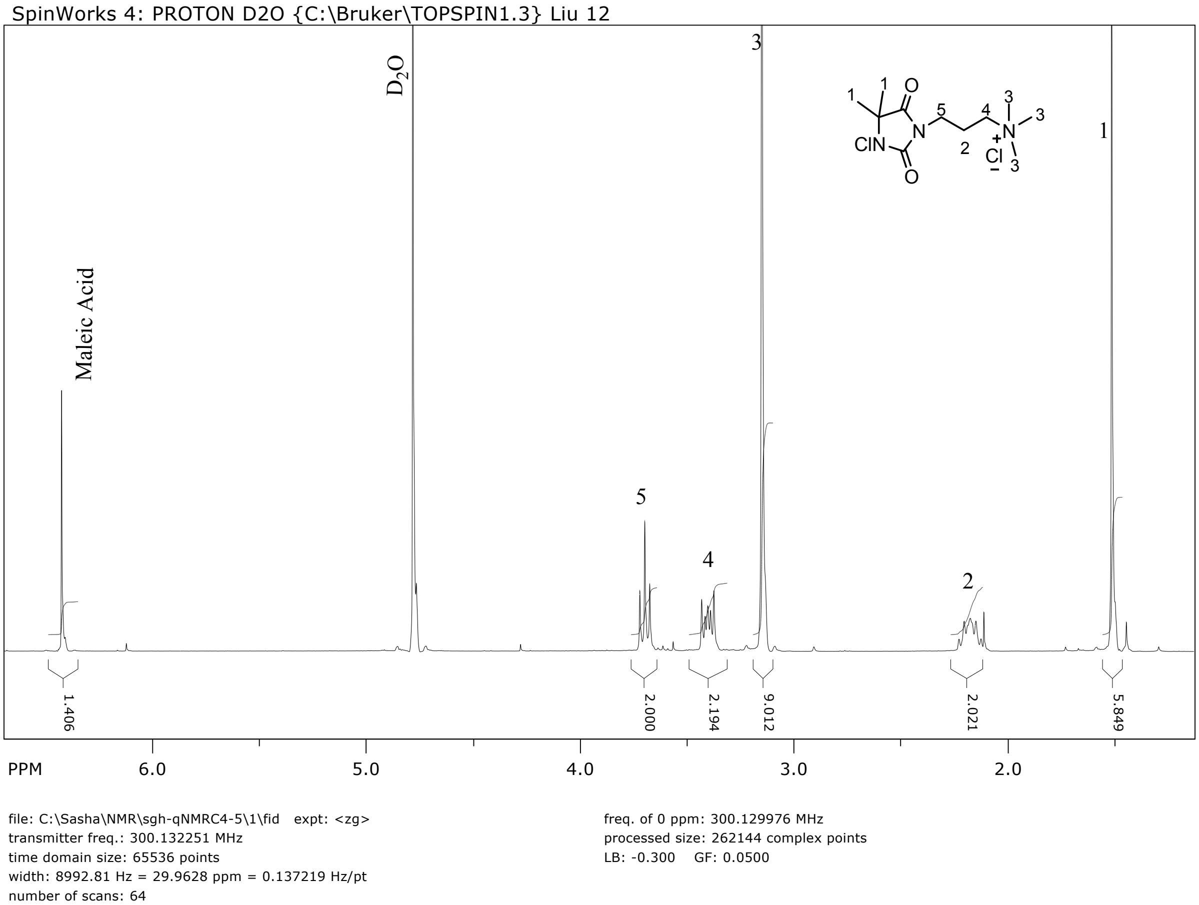


Figure S31. Quantitative [1]H NMR (qHNMR) spectrum of compound#**4**, purity: 99.30%.

$$P[\%] = \frac{n_{IC}.\,Int_t.MW_t.m_{IC}}{n_t.\,Int_{IC}.MW_{IC}.m_s}\,.\,P_{IC}$$

m_s= 4.995 mg, m_{IC}= 1.512 mg, P_{IC}=99.94,

Int_t= 17.586, n_t= 19, Int_{IC}= 1.449, n_{IC}=2,

MW_t= 298.21 g/mol, MW_{IC}=116.07 g/mol

Table S1. Absolute quantitative ^{1}H NMR (qHNMR) analysis of compound#**4** (4.995 mg/0.6 mL) in D_2O with the addition of maleic acid (99.94% pure) as internal calibrant.

	Peaks (ppm)	Integration	Number of porotons	Weigh (mg)	MWt (g/mol)
Compound 4	1.471-1.549	5.388	6	4.995	298.21
	3.108-3.205	8.341	9		
	3.301-3.472	2	2		
	3.640-3.785	1.857	2		
	Sum	17.586	19		
Internal St	6.285-6.493	1.449	2	1.512	116.07

$$P[\%] = \frac{2 * 17.586 * 298.21 * 1.512}{19 * 1.449 * 116.07 * 4.995} * 99.94 = \frac{15858.82}{15961.64} * 99.94 = 99.30\ \%$$

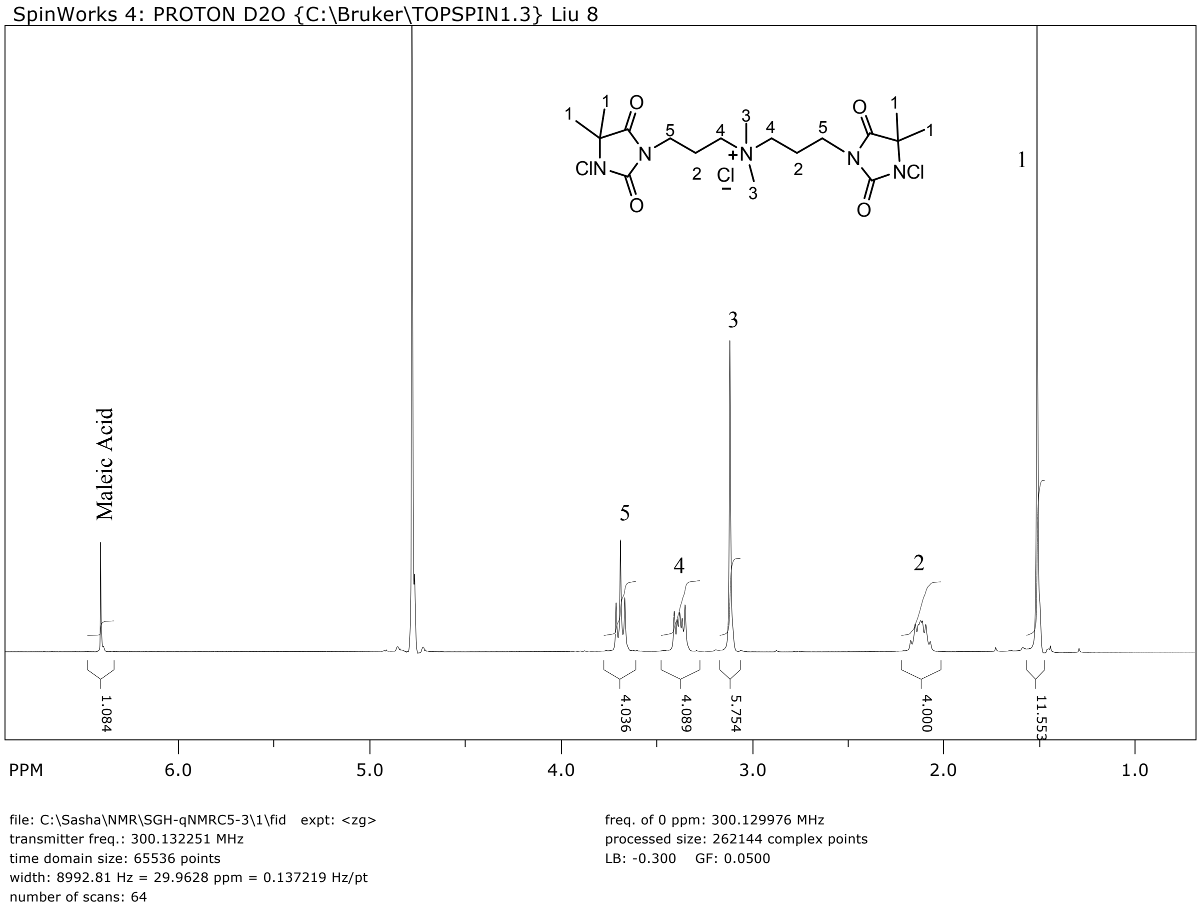


Figure S32. Quantitative ^{1}H NMR (qHNMR) spectrum of compound#**5**, purity: 96.55%.

$$P[\%] = \frac{n_{IC}.\, Int_t.MW_t.m_{IC}}{n_t.\, Int_{IC}.MW_{IC}.m_s}\,.\,P_{IC}$$

m_s= 4.66 mg, m_{IC}= 0.593 mg, P_{IC}=99.94,

Int_t= 29.433, n_t= 30, Int_{IC}= 1.084 , n_{IC}=2,

MW_t= 486.82 g/mol, MW_{IC}=116.07 g/mol

Table S2. Absolute quantitative ^{1}H NMR (qHNMR) analysis of compound#**5** (4.66 mg/0.6 mL) in D_2O with the addition of maleic acid (99.94% pure) as internal calibrant.

	Peaks (ppm)	Integration	Number of porotons	Weigh (mg)	MWt (g/mol)
Compound 5	1.472-1.567	11.553	12	4.66	486.82
	2.014-2.221	4	4		
	3.063-3.171	5.755	6		
	3.275 -3.478	4.089	4		
	3.610-3.777	4.036	4		
	Sum	29.433	30		
Internal St	6.338-6.478	1.084	2	0.593	116.07

$$P[\%] = \frac{2 * 29.433 * 486.82 * 0.593}{30 * 1.084 * 116.07 * 4.66} * 99.94 = \frac{16993.69}{17589.62} * 99.94 = 96.55\ \%$$

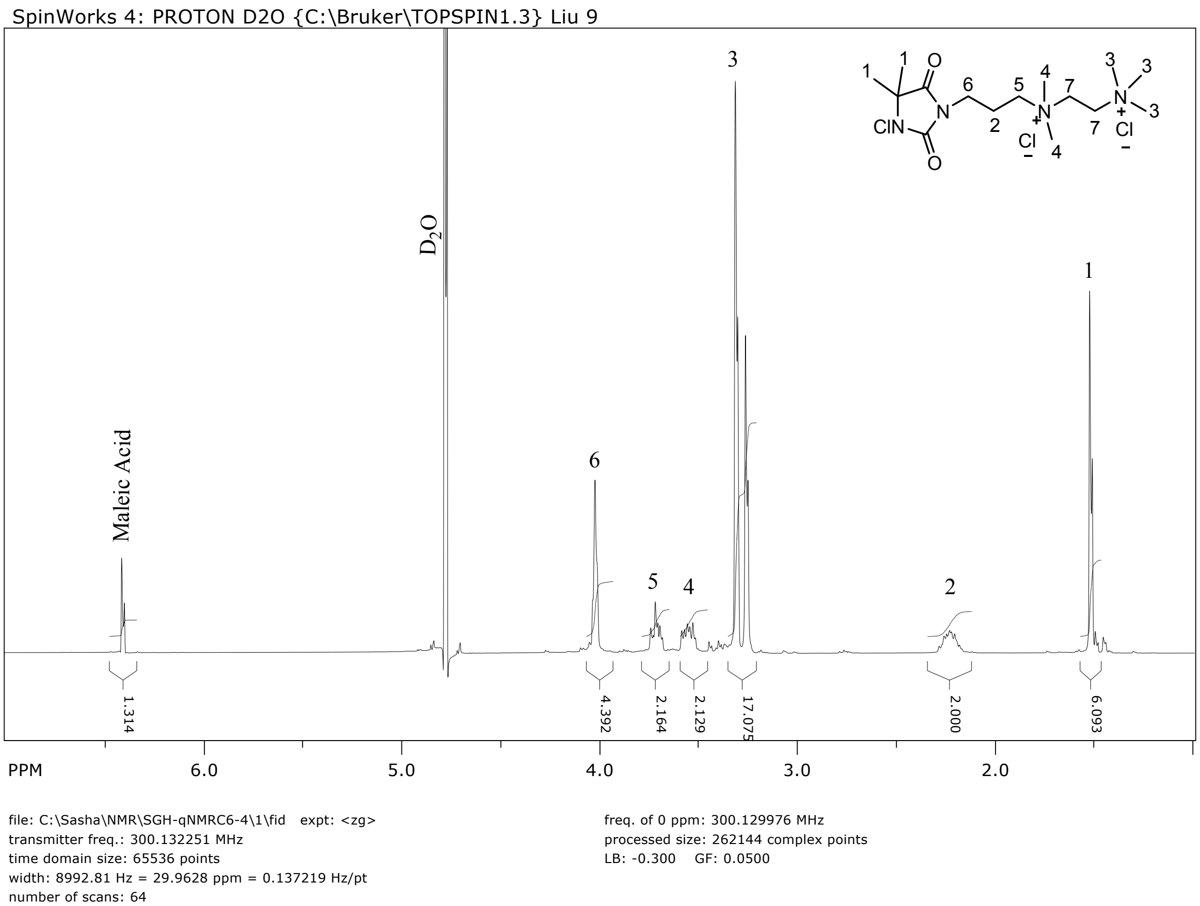


Figure S33. Quantitative ^{1}H NMR (qHNMR) spectrum of compound#**6**, purity: 97.02%.

$$P[\%] = \frac{n_{IC}.\, Int_t.MW_t.m_{IC}}{n_t.\, Int_{IC}.MW_{IC}.m_s}\,.\,P_{IC}$$

m_s= 6.123 mg, m_{IC}=1.023 mg, P_{IC}=99.94,

Int_t= 33.851, n_t=31, Int_{IC}=1.314, n_{IC}=2,

MW_t=405.79 g/mol, MW_{IC}=116.07 g/mol

Table S3. Absolute quantitative ^{1}H NMR (qHNMR) analysis of compound#**6** (6.123 mg/0.6 mL) in D_2O with the addition of maleic acid (99.94% pure) as internal calibrant.

	Peaks (ppm)	Integration	Number of porotons	Weigh (mg)	MWt (g/mol)
Compound 6	1.462-1.569	6.09	6	6.123	405.79
	2.118-2.342	2	2		
	3.207-3.351	17.076	15		
	3.453-3.593	2.129	2		
	3.649-3.788	2.164	2		
	3.933-4.067	4.392	4		
	Sum	33.851	31		
Internal St	6.342-6.482	1.314	2	1.023	116.07

$$P[\%] = \frac{2 * 33.851 * 405.79 * 1.023}{31 * 1.314 * 116.07 * 6.123} * 99.94 = \frac{28104.67}{28949.51} * 99.94 = 97.02\ \%$$

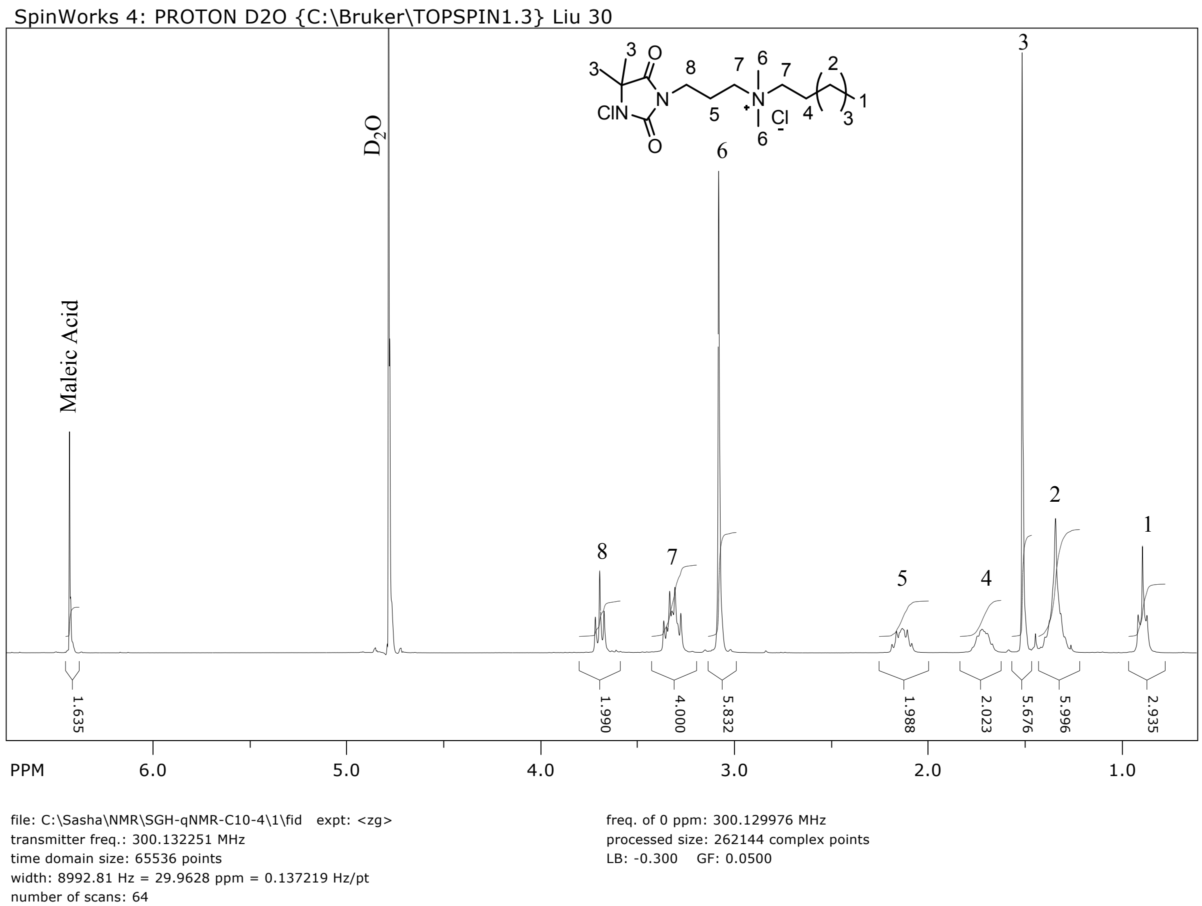


Figure S34. Quantitative [1]H NMR (qHNMR) spectrum of compound#**10**, purity: 95.54%.

$$P[\%] = \frac{n_{IC}.\, Int_t.MW_t.m_{IC}}{n_t.\, Int_{IC}.MW_{IC}.m_s} . P_{IC}$$

m_s= 7.794 mg, m_{IC}= 1.944 mg, P_{IC}=99.94,

Int_t= 30.441, n_t= 31, Int_{IC}= 1.635, n_{IC}=2,

MW_t= 370.36 g/mol, MW_{IC}=116.07 g/mol

Table S4. Absolute quantitative [1]H NMR (qHNMR) analysis of compound#**10** (7.794 mg/0.6 mL) in D_2O with the addition of maleic acid (99.94% pure) as internal calibrant.

	Peaks (ppm)	Integration	Number of porotons	Weigh (mg)	MWt (g/mol)
Compound 10	0.776-0.965	2.935	3	7.794	370.36
	1.217-1.430	5.997	6		
	1.465-1.568	5.676	6		
	1.623-1.836	2.023	2		
	1.997-2.253	1.988	2		
	2.990-3.136	5.832	6		
	3.195-3.427	4	4		
	3.589-3.801	1.99	2		
	Sum	30.441	31		
Internal St	6.382-6.453	1.635	2	1.944	116.07

$$P[\%] = \frac{2 * 30.441 * 370.36 * 1.944}{31 * 1.635 * 116.07 * 7.794} * 99.94 = \frac{43833.81}{45852.16} * 99.94 = 95.54\ \%$$

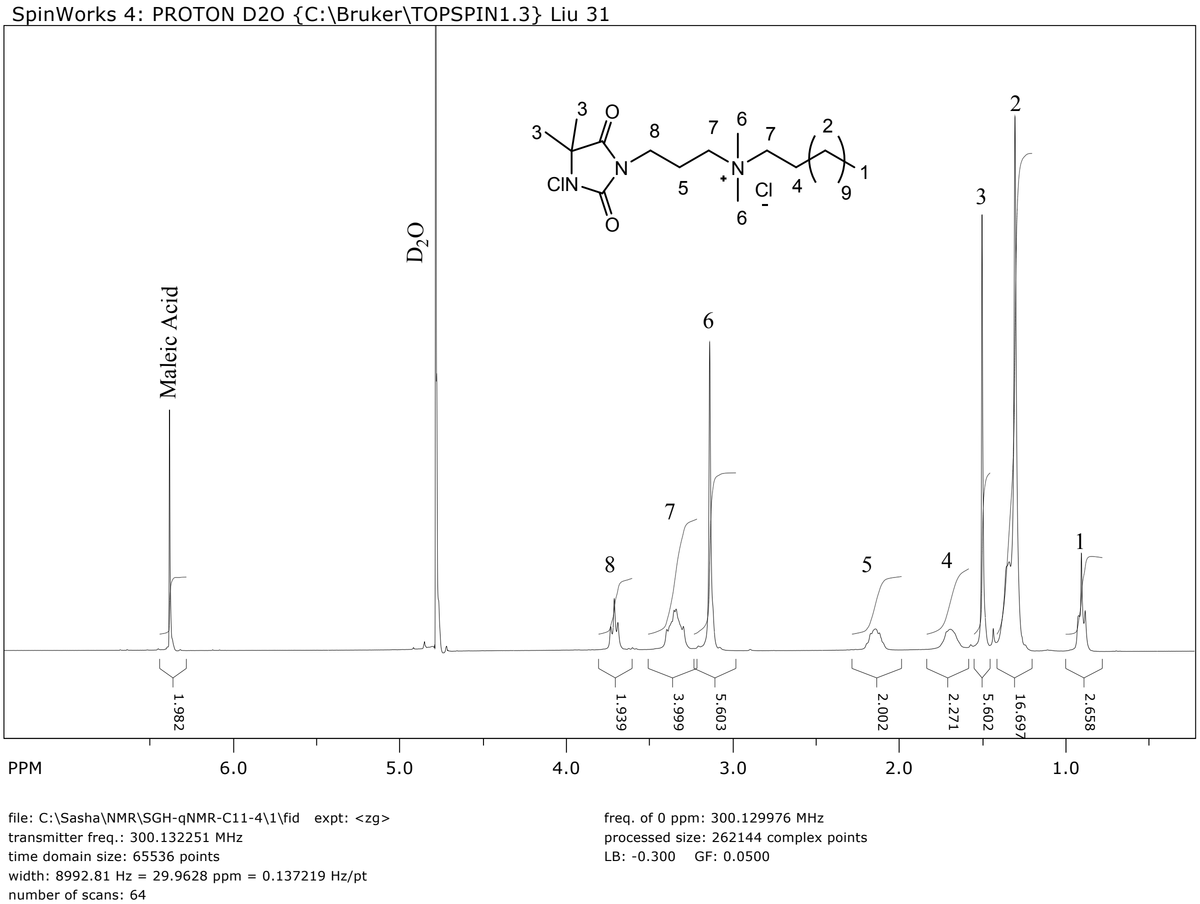


Figure S35. Quantitative ^{1}H NMR (qHNMR) spectrum of compound#**11**, purity: 95.76%.

$$P[\%] = \frac{n_{IC}.\, Int_t.MW_t.m_{IC}}{n_t.\, Int_{IC}.MW_{IC}.m_s} \,.\, P_{IC}$$

m_s= 7.498 mg, m_{IC}= 1.926 mg, P_{IC}=99.94,

Int_t= 40.773, n_t= 43, Int_{IC}= 1.982, n_{IC}=2,

MW_t= 452.5 g/mol, MW_{IC}=116.07 g/mol

Table S5. Absolute quantitative ^{1}H NMR (qHNMR) analysis of compound#**11** (7. 498 mg/0.6 mL) in D_2O with the addition of maleic acid (99.94% pure) as internal calibrant.

	Peaks (ppm)	Integration	Number of protons	Weigh (mg)	MWt (g/mol)
Compound 11	0.779-0.999	2.658	3	7.498	452.5
	1.201-1.412	16.697	18		
	1.453-1.55	5.602	6		
	1.582-1.834	2.272	2		
	1.986-2.284	2.002	2		
	2.981-3.233	5.603	6		
	3.215-3.509	4	4		
	3.605-3.807	1.939	2		
	Sum	40.773	43		
Internal St	6.284-6.445	1.982	2	1.926	116.07

$$P[\%] = \frac{2 * 40.773 * 452.5 * 1.926}{43 * 1.982 * 116.07 * 7.498} * 99.94 = \frac{71068.56}{74171.58} * 99.94 = 95.76\ \%$$

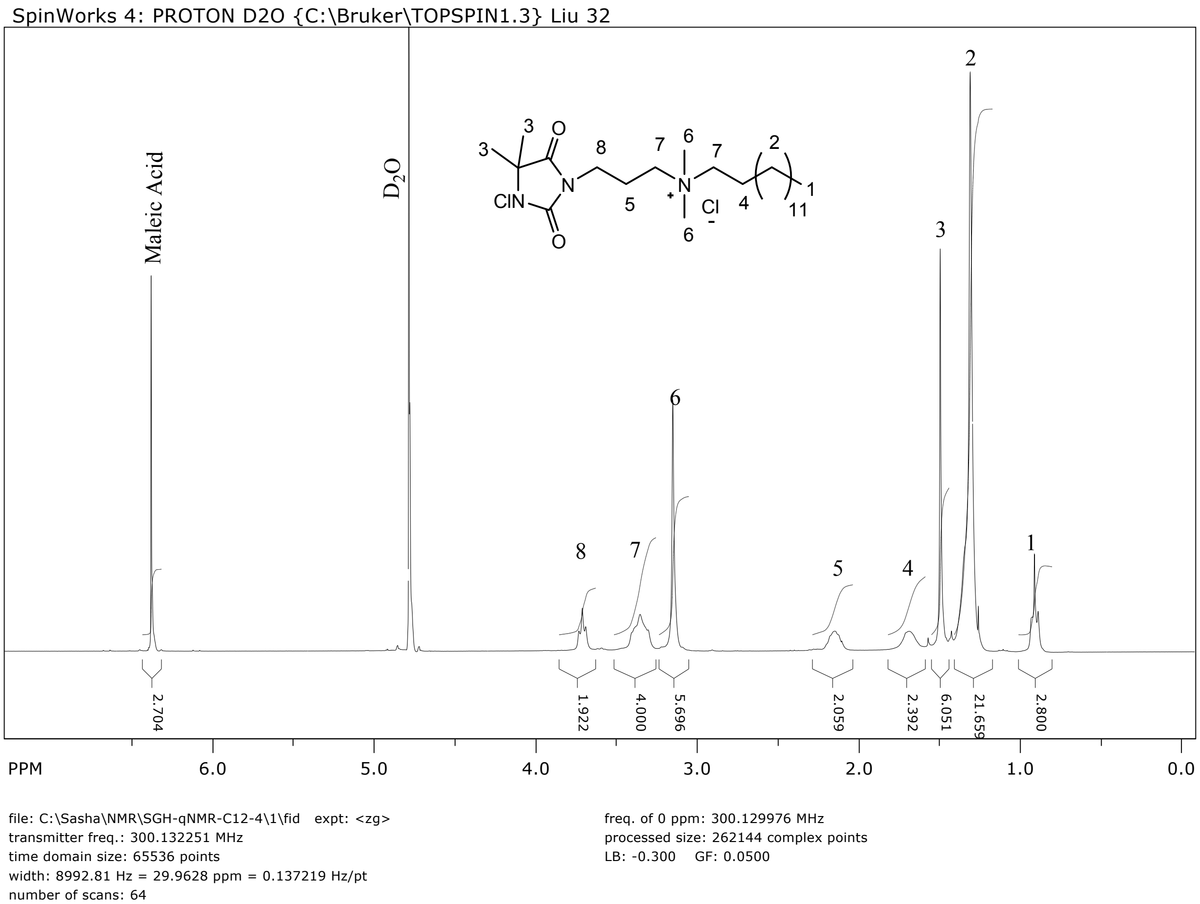


Figure S36. Quantitative ^{1}H NMR (qHNMR) spectrum of compound#**12**, purity: 95.65%.

$$P[\%] = \frac{n_{IC}.\,Int_t.MW_t.m_{IC}}{n_t.\,Int_{IC}.MW_{IC}.m_s}\,.\,P_{IC}$$

m_s= 8.887 mg, m_{IC}= 2.803 mg, P_{IC}=99.94,

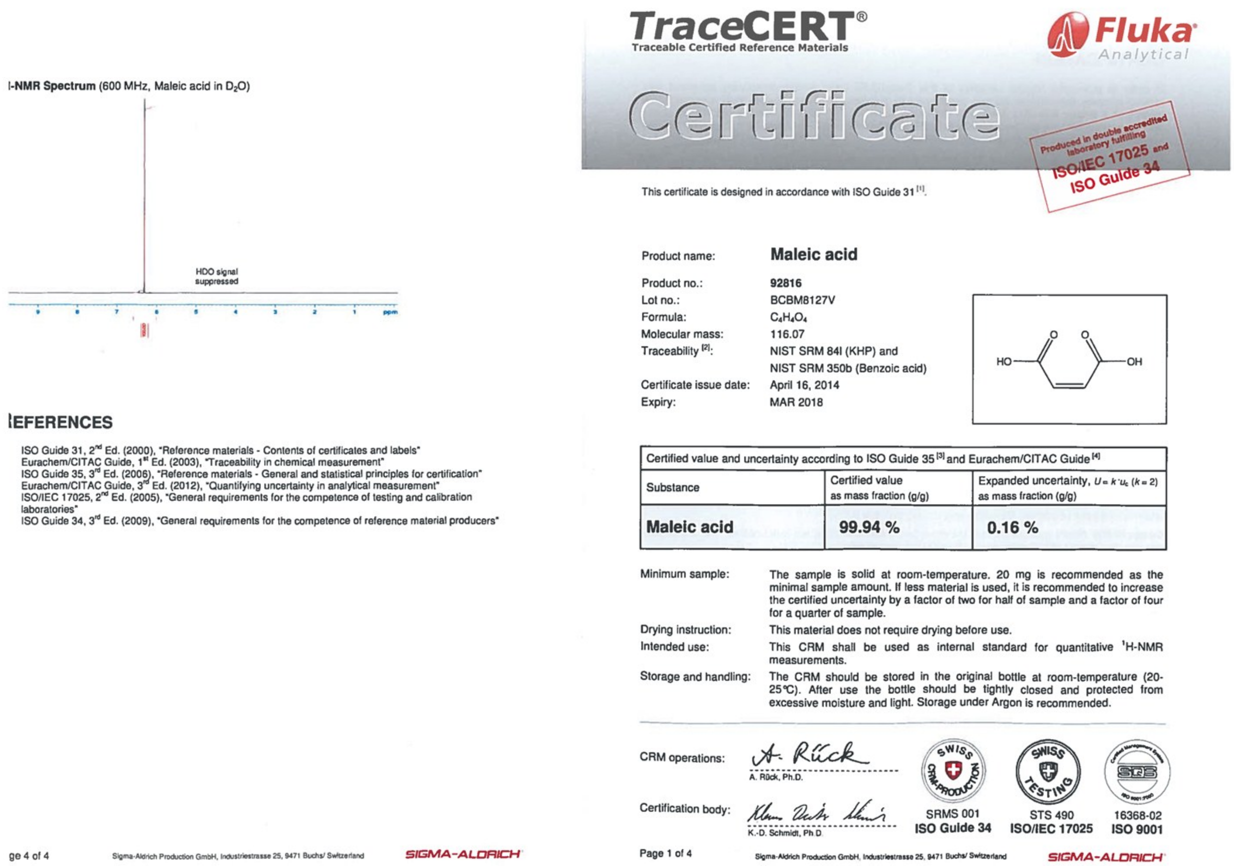
Int_t= 46.572, n_t= 47, Int_{IC}= 2.704, n_{IC}=2,

MW_t=480.55 g/mol, MW_{IC}=116.07 g/mol

Table S6. Absolute quantitative ^{1}H NMR (qHNMR) analysis of compound#**12** (8.887 mg/0.6 mL) in D_2O with the addition of maleic acid (99.94% pure) as internal calibrant.

	Peaks (ppm)	Integration	Number of porotons	Weigh (mg)	MWt (g/mol)
Compound 12	0.801-1.01	2.8	3	8.887	480.55
	1.17-1.407	21.65	22		
	1.44-1.549	6.051	6		
	1.586-1.818	2.393	2		
	2.035-2.286	2.059	2		
	3.052-3.236	5.696	6		
	3.255-3.515	4	4		
	3.629-3.856	1.923	2		
	Sum	46.572	47		
Internal St	6.319-6.437	2.704	2	2.803	116.07

$$P[\%] = \frac{2 * 46.572 * 480.55 * 2.803}{47 * 2.704 * 116.07 * 8.887} * 99.94 = \frac{125463.26}{131093.06} * 99.94 = 95.65\ \%$$



I-NMR Spectrum (600 MHz, Maleic acid in D_2O)

HDO signal suppressed

IEFERENCES

ISO Guide 31, 2[nd] Ed. (2000), "Reference materials - Contents of certificates and labels"
Eurachem/CITAC Guide, 1[st] Ed. (2003), "Traceability in chemical measurement"
ISO Guide 35, 3[rd] Ed. (2006), "Reference materials - General and statistical principles for certification"
Eurachem/CITAC Guide, 3[rd] Ed. (2012), "Quantifying uncertainty in analytical measurement"
ISO/IEC 17025, 2[nd] Ed. (2005), "General requirements for the competence of testing and calibration laboratories"
ISO Guide 34, 3[rd] Ed. (2009), "General requirements for the competence of reference material producers"

ge 4 of 4 Sigma-Aldrich Production GmbH, Industriestrasse 25, 9471 Buchs/ Switzerland SIGMA-ALDRICH

TraceCERT®
Traceable Certified Reference Materials

Fluka Analytical

Certificate

Produced in double accredited laboratory fulfilling ISO/IEC 17025 and ISO Guide 34

This certificate is designed in accordance with ISO Guide 31 [1].

Product name: **Maleic acid**

Product no.: **92816**
Lot no.: BCBM8127V
Formula: $C_4H_4O_4$
Molecular mass: 116.07
Traceability [2]: NIST SRM 84l (KHP) and NIST SRM 350b (Benzoic acid)
Certificate issue date: April 16, 2014
Expiry: MAR 2018

Certified value and uncertainty according to ISO Guide 35 [3] and Eurachem/CITAC Guide [4]		
Substance	Certified value as mass fraction (g/g)	Expanded uncertainty, $U = k \cdot u_c$ ($k = 2$) as mass fraction (g/g)
Maleic acid	**99.94 %**	**0.16 %**

Minimum sample: The sample is solid at room-temperature. 20 mg is recommended as the minimal sample amount. If less material is used, it is recommended to increase the certified uncertainty by a factor of two for half of sample and a factor of four for a quarter of sample.

Drying instruction: This material does not require drying before use.

Intended use: This CRM shall be used as internal standard for quantitative ^{1}H-NMR measurements.

Storage and handling: The CRM should be stored in the original bottle at room-temperature (20-25°C). After use the bottle should be tightly closed and protected from excessive moisture and light. Storage under Argon is recommended.

CRM operations: A. Rück, Ph.D.

Certification body: K.-D. Schmidt, Ph.D.

SRMS 001 ISO Guide 34 — STS 490 ISO/IEC 17025 — 16368-02 ISO 9001

Page 1 of 4 Sigma-Aldrich Production GmbH, Industriestrasse 25, 9471 Buchs/ Switzerland SIGMA-ALDRICH

Figure S37. Certificate of the internal calibrant maleic acid.

Table S7. Comparison of the antibacterial activity of tetradecyl # 9 and 12 with Benzyldodecyldimethylammonium bromide (C12) and Benzyltetradecyldimethylammonium chloride(C14) against 10^7 CFU/mL of MRSA and 10^6 CFU/mL MDR *P. aeruginosa*.

Synthetic compounds*		Bacterial Reduction at Various Contact Time (min)						
Gram-positive MRSA (10^7 CFU/mL)* **								
		1	**3**	**5**	**10**	**20**	**30**	**60**
9	%	94.23 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
	Log_{10}	1.24 ± 0.00	7.21 ± 0.09	7.21 ± 0.09	7.21 ± 0.09	7.21 ± 0.09	7.21 ± 0.09	7.21 ± 0.09
12	%	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
	Log_{10}	7.21 ± 0.09	7.21 ± 0.09	7.21 ± 0.09	7.21 ± 0.09	7.21 ± 0.09	7.21 ± 0.09	7.21 ± 0.09
C12	%	37.40 ± 52	58.96 ± 25	81.54± 16	99.64 ± 0.42	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
	Log_{10}	0.300 ± 0.42	0.430 ± 0.28	0.840 ± 0.45	2.69 ± 0.72	7.21 ± 0.09	7.21 ± 0.09	7.21 ± 0.09
C14	%	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
	Log_{10}	7.21 ± 0.09	7.21 ± 0.09	7.21 ± 0.09	7.21 ± 0.09	7.21 ± 0.09	7.21 ± 0.09	7.21 ± 0.09
Gram-negative MDR P. aeruginosa (10^6 CFU/mL)* **								
		1	**3**	**5**	**10**	**20**	**30**	**60**
9	%	85.60±18.2	99.98± 0.03	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
	Log_{10}	1.18± 0.88	4.73± 1.8	6.36 ± 0.45	6.36 ± 0.45	6.36 ± 0.45	6.36 ± 0.45	6.36 ± 0.45
12	%	98.94 ± 1.5	99.99 ± 0.01	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
	Log_{10}	3.85 ± 3.1	4.91 ± 1.6	6.36 ± 0.45	6.36 ± 0.45	6.36 ± 0.45	6.36 ± 0.45	6.36 ± 0.45
C12	%	97.09 ± 0.00	98.99± 1.4	99.44 ± 0.79	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
	Log_{10}	1.54 ± 0.00	2.8 ± 1.5	2.25 ± 0.04	6.36 ± 0.45	6.36 ± 0.45	6.36 ± 0.45	6.36 ± 0.45
C14	%	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
	Log_{10}	6.36 ± 0.45	6.36 ± 0.45	6.36 ± 0.45	6.36 ± 0.45	6.36 ± 0.45	6.36 ± 0.45	6.36 ± 0.45

*. Concentration of the compounds was set at 0.141 mM