SUPPORTING INFORMATION

Fast Amide Bond Cleavage Assisted by a Secondary Amino and a Carboxyl Group – a Model for yet Unknown Peptidases?

Igor V. Komarov[1]*, Aleksandr Yu. Ishchenko[2], Aleksandr Hovtvianitsa[2], Viacheslav Stepanenko[2], Serhii Kharchenko[2], John E. Davies[3], Andrew D. Bond[3], and Anthony J. Kirby[3],*

[1] Taras Shevchenko National University of Kyiv, Institute of High Technologies, Vul. Volodymyrska 64/13, 01601 Kyiv, Ukraine; ik214@yahoo.com

[2] Enamine Ltd., Vul. Chervonotkatska 78, 02094 Kyiv, Ukraine; igor.komarov@enamine.net

[3] University Chemical Laboratory, University of Cambridge, Lensfield Road, CB2 1EW Cambridge, UK; ajk1@cam.ac.uk

* Correspondence: ajk1@cam.ac.uk; ik214@yahoo.com
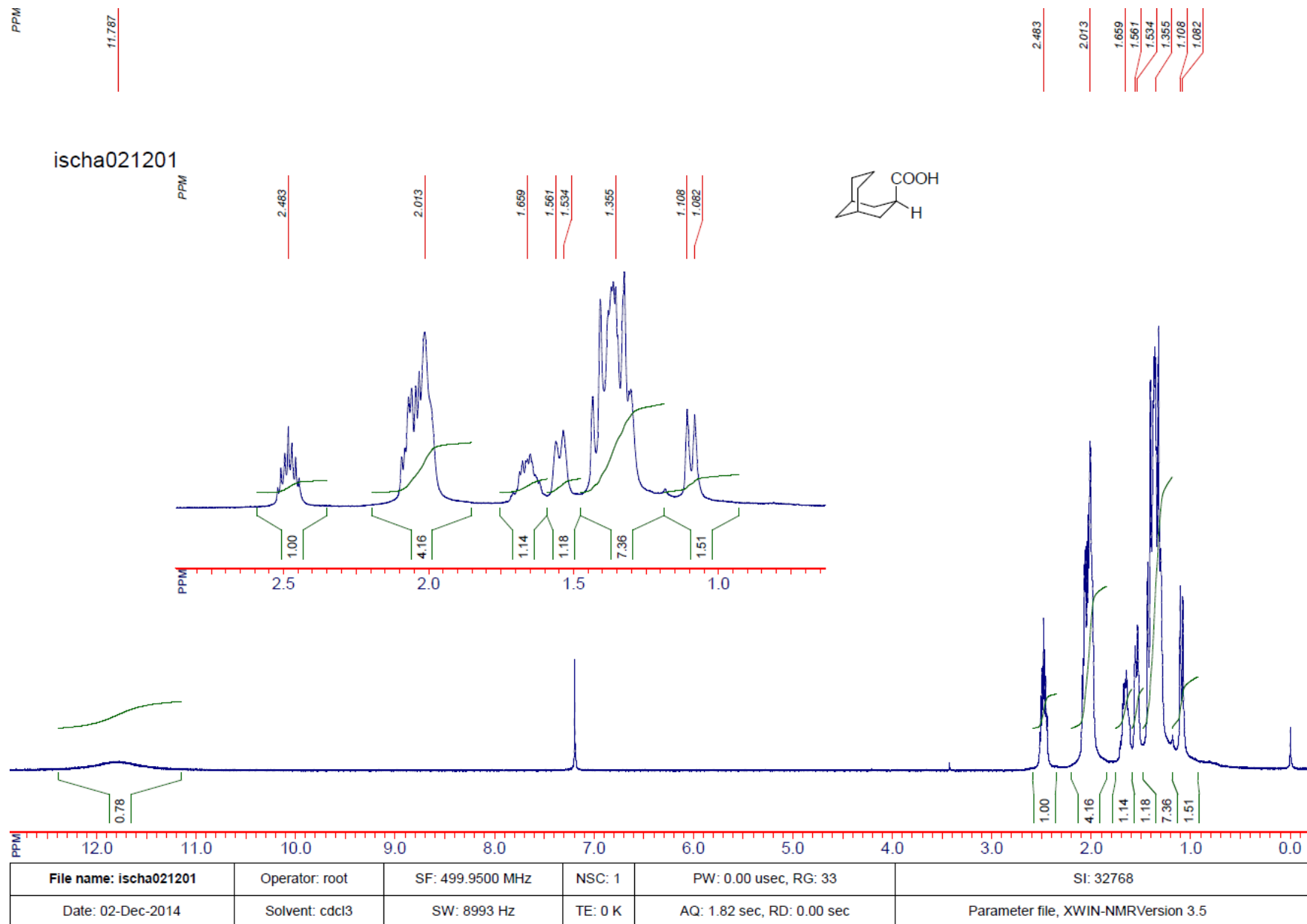
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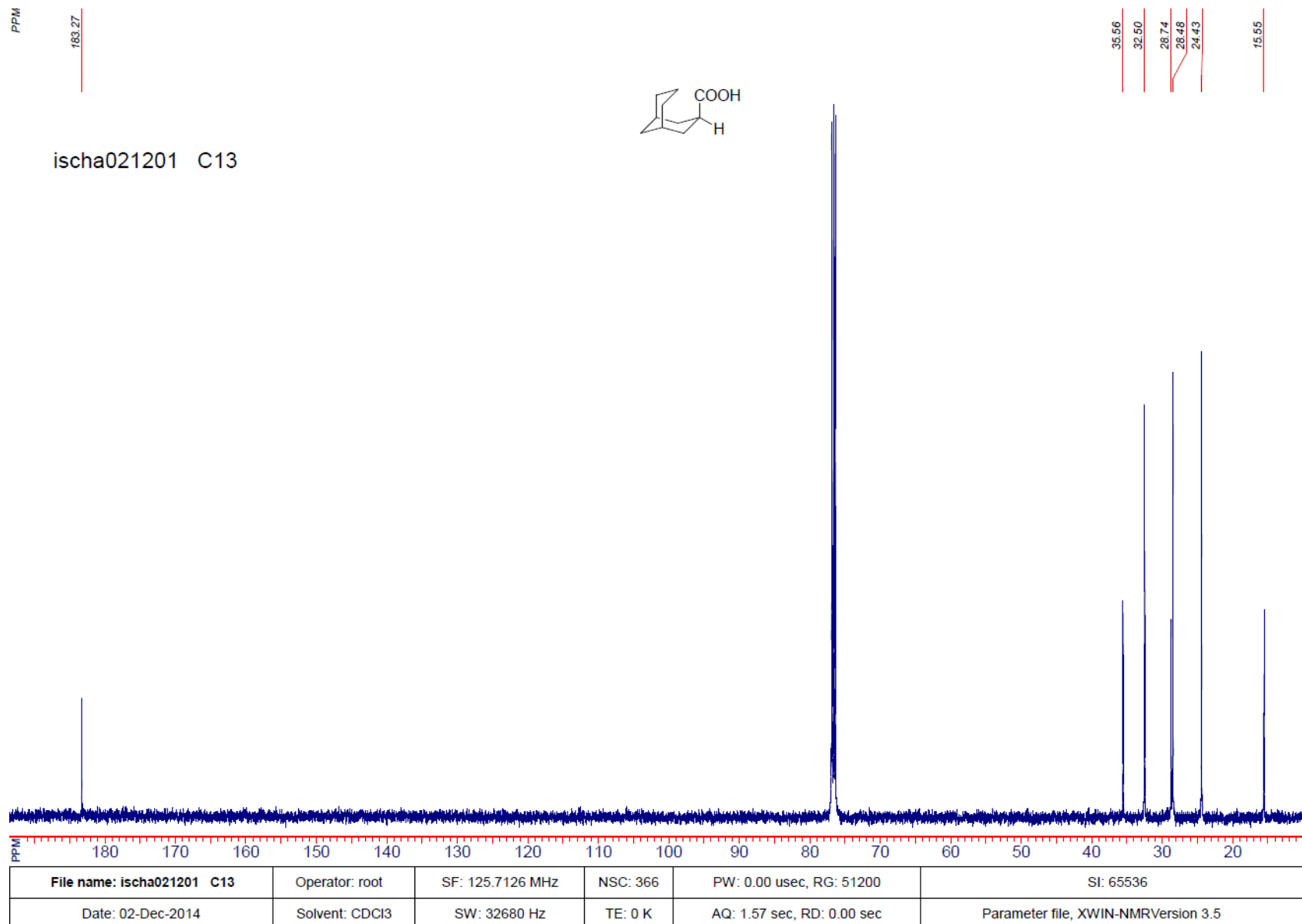**Figure S1**. ^{1}H-NMR spectrum of compound **20**.

Figure S2. ^{13}C-NMR spectrum of compound **20**.

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Sample    : CLN22363
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ALS Vial  : 42   Sample Multiplier: 1

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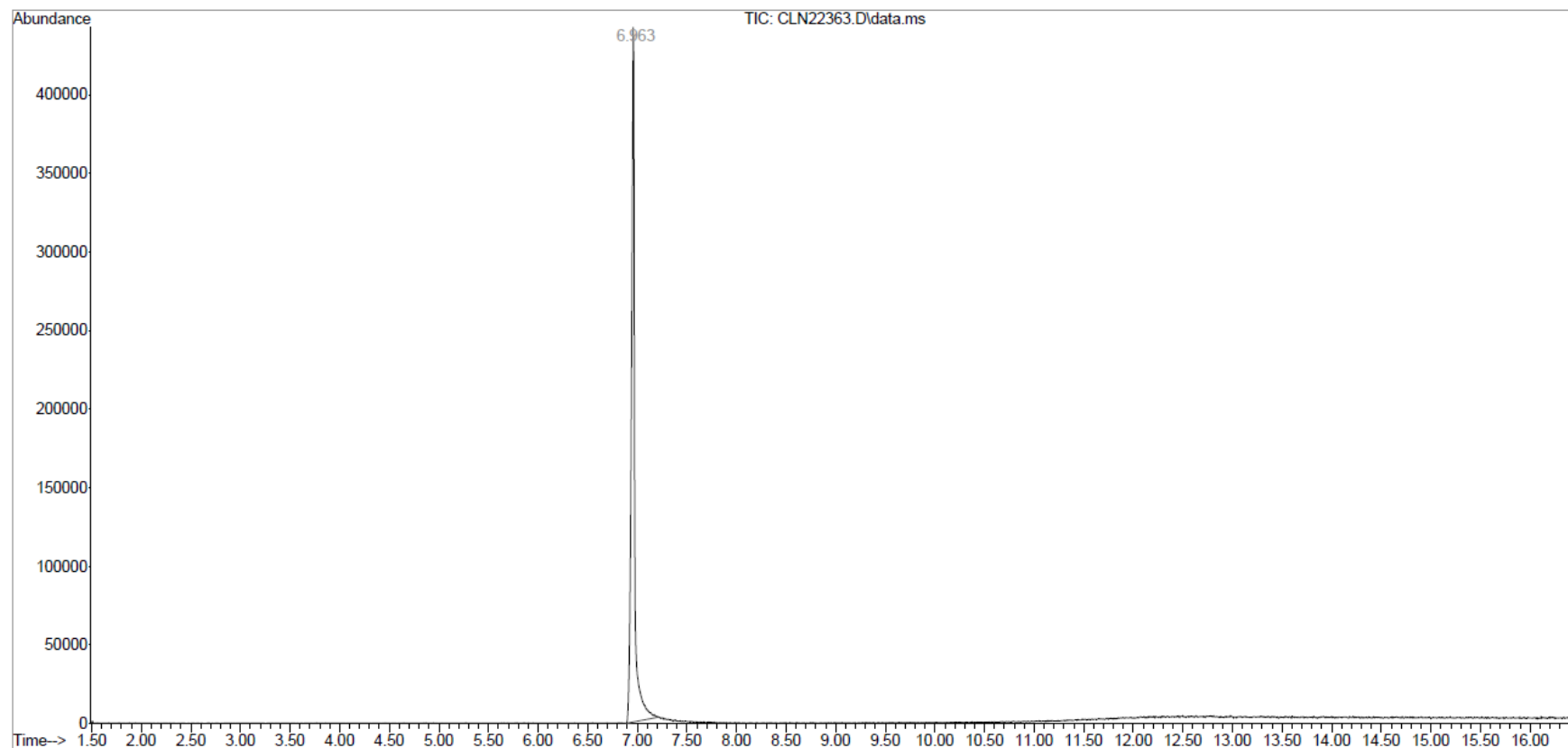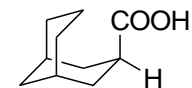
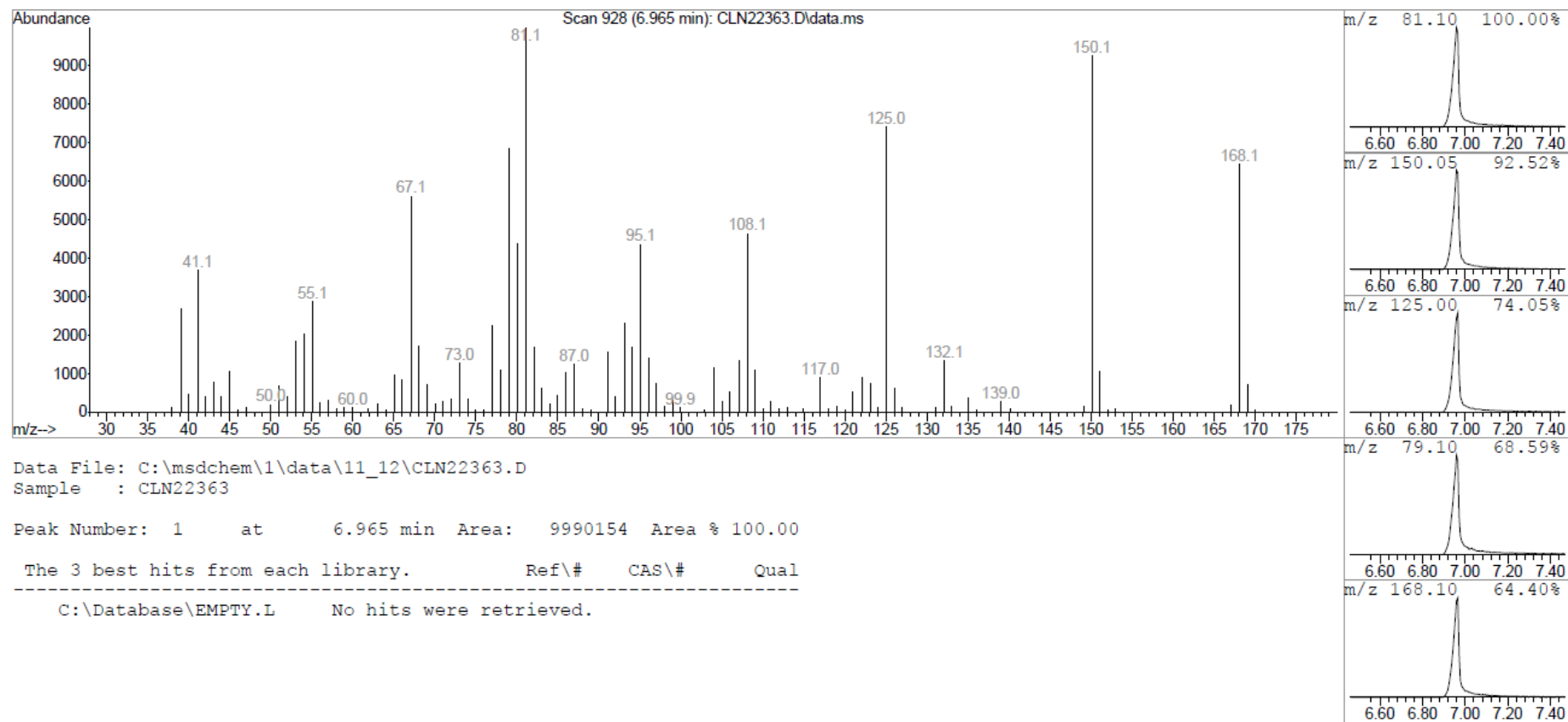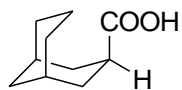**Figure S3**. GC-MS trace of compound **20** (continued overleaf).
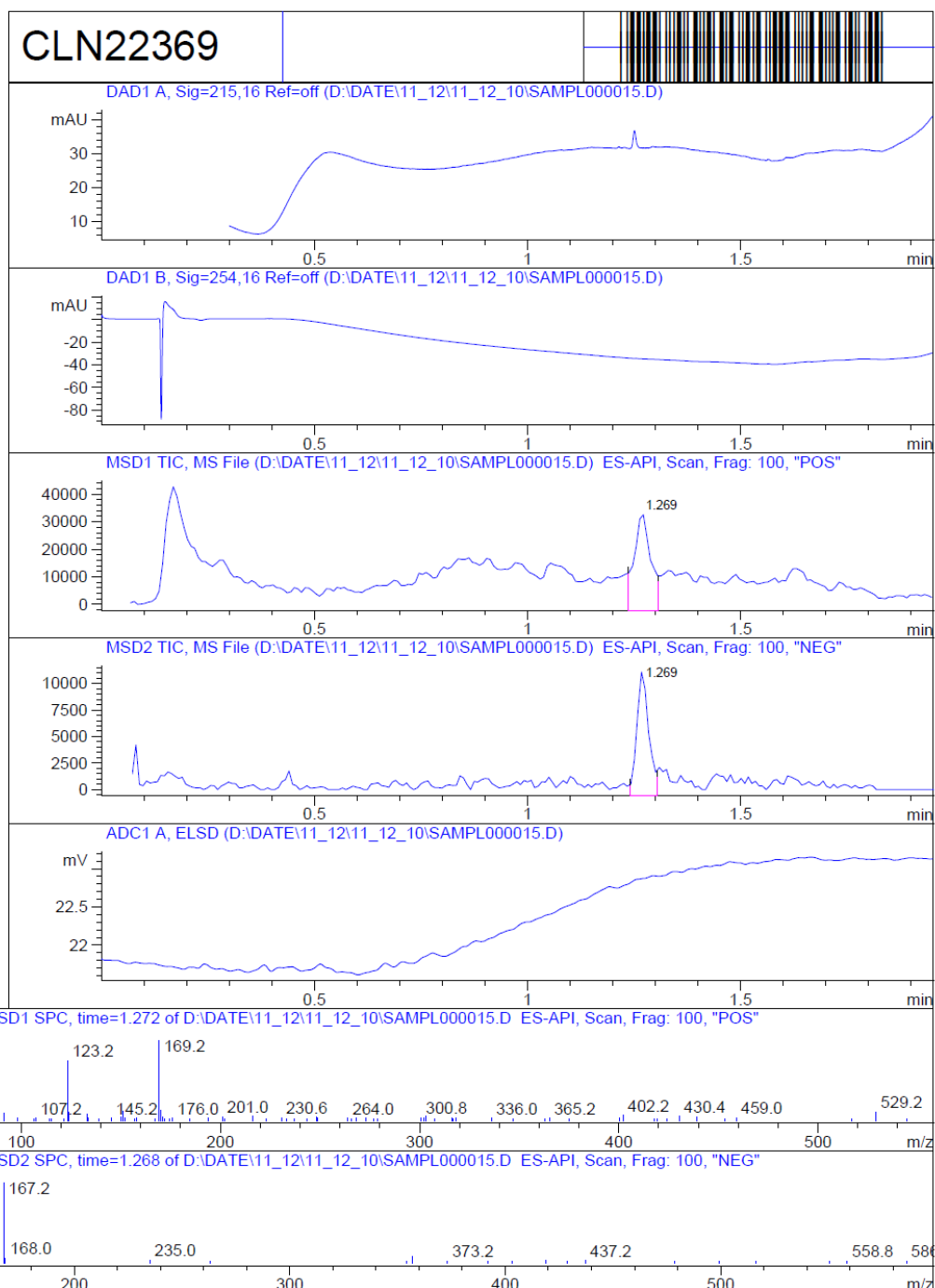
Figure S4. GC-MS trace of compound **20**.

CLN22369

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COOH

H

Mol Wt   **0**
Exact Mass

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RT 1.269

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RT 1.269

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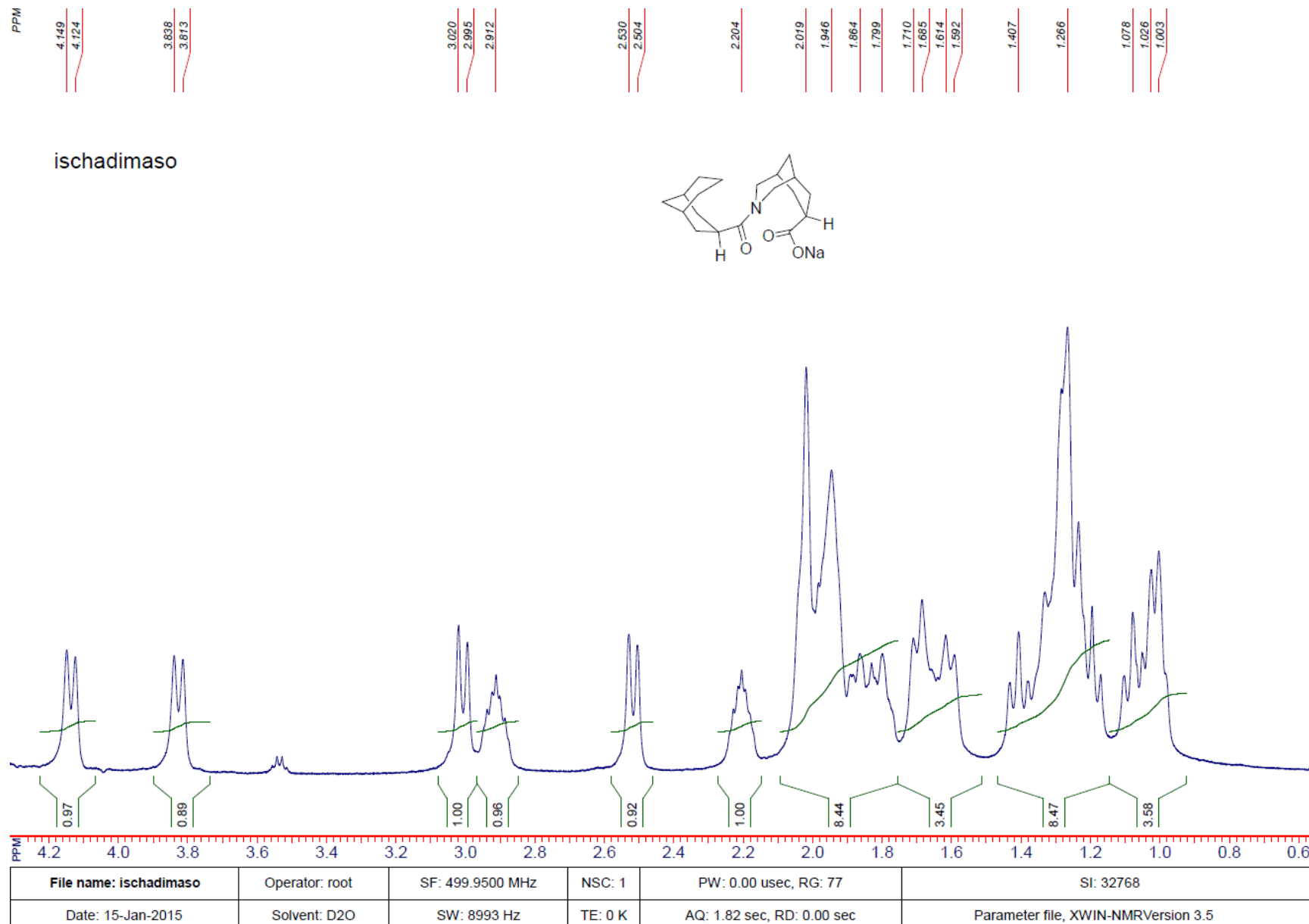**Figure S5**. LC-MS trace of compound **20**.

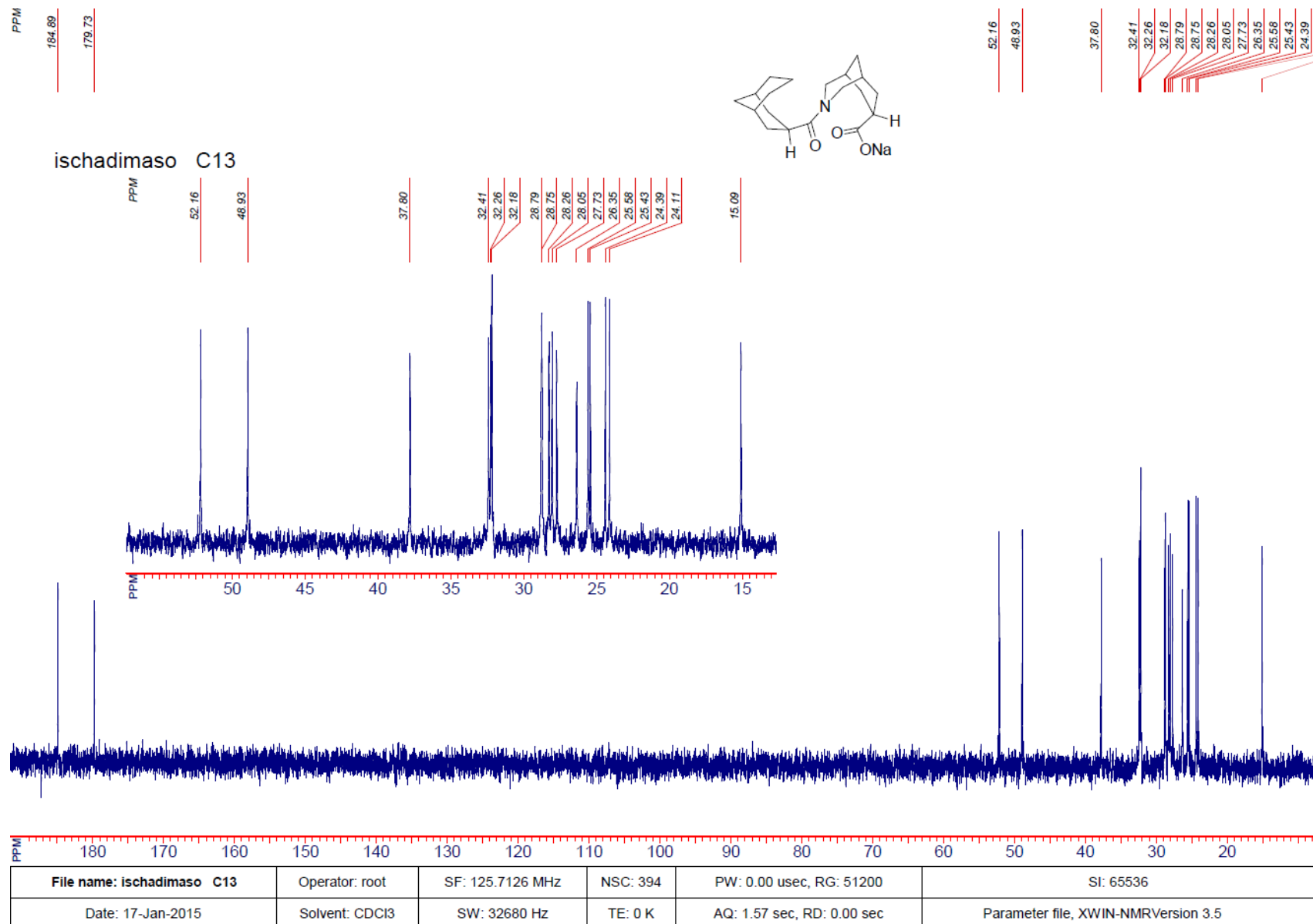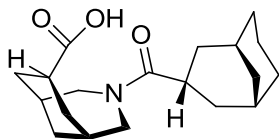**Figure S6**. ¹H-NMR spectrum of sodium salt of compound **13**.

Figure S7. ^{13}C-NMR spectrum of sodium salt of compound **13**.

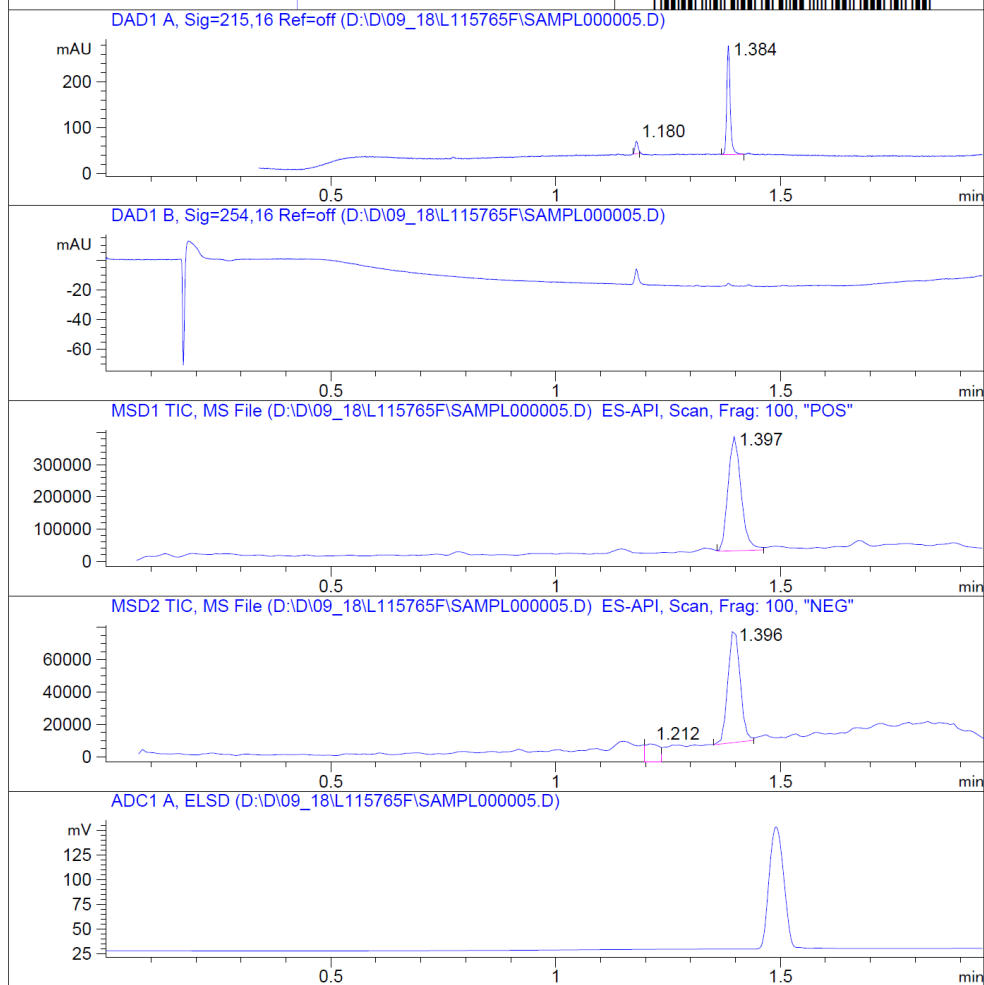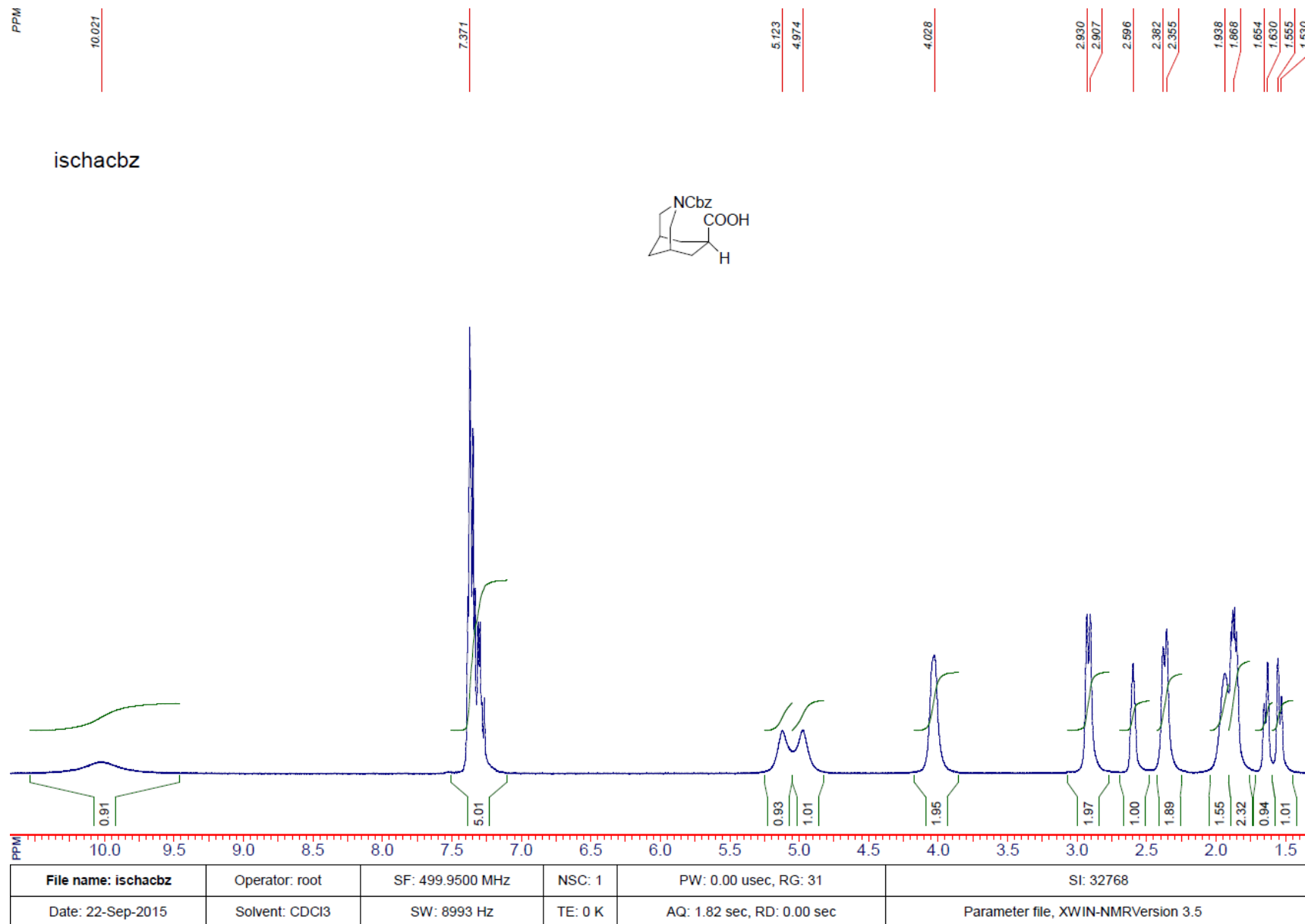**Figure S8**. LC-MS trace of compound **13**.

ischacbz

Figure S9. ^{1}H-NMR spectrum of compound **21**.
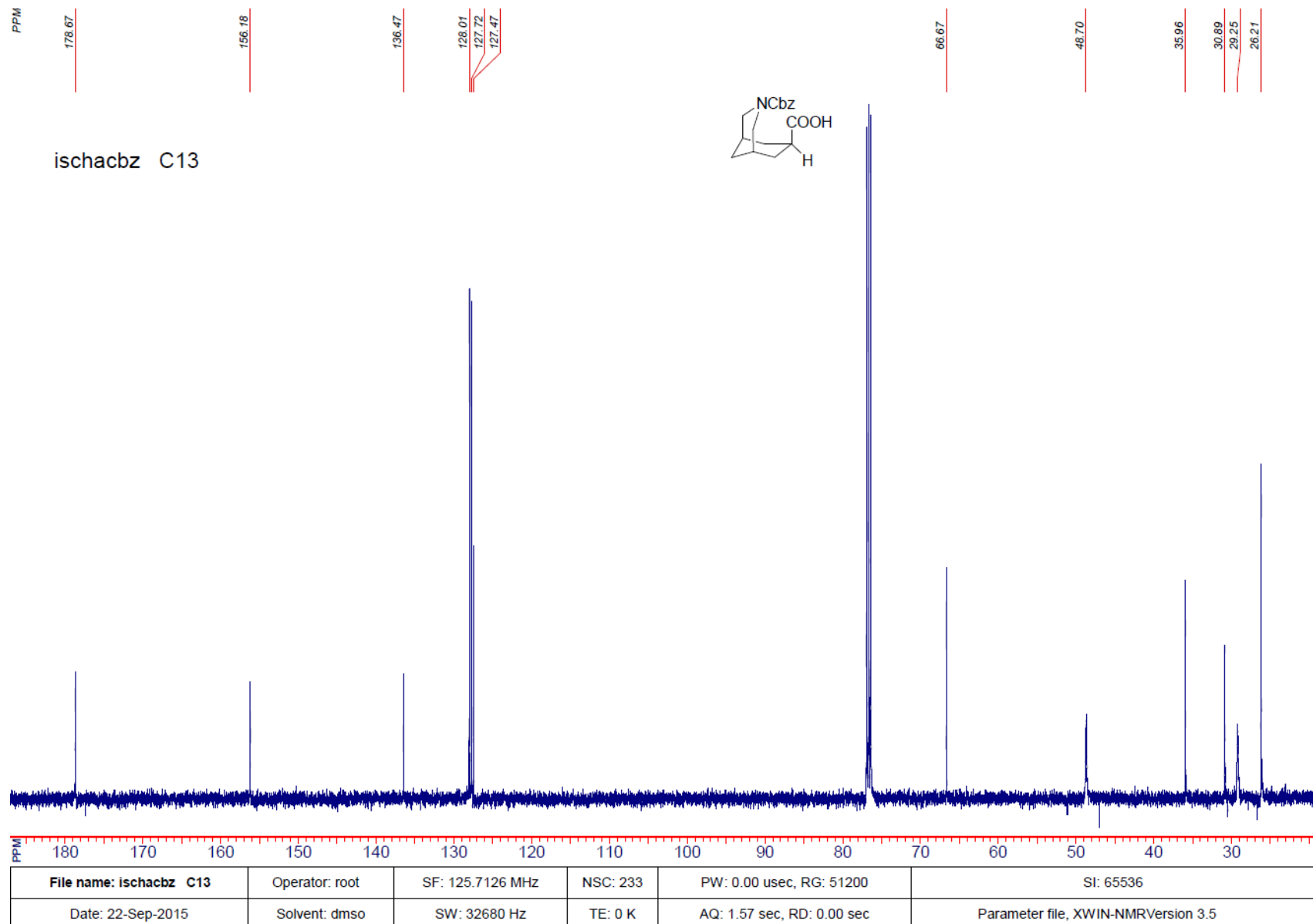
Figure S10. ^{13}C-NMR spectrum of compound **21**.

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CLN48435

NCbz
COOH
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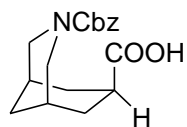
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Exact Mass

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mAU
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-10
-20
0   0.5   1   1.5   min

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400000
300000
200000
100000
1.252
0   0.5   1   1.5   min

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100000
50000
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30
20
0   0.5   1   1.5   min

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RT 1.252

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304.2
157.1   260.2   326.3
100   200   300   400   500   m/z

*MSD2 SPC, time=1.252 of D:\DATE\04_15\04_15_30\SAMPL006.D , Scan, Frag: 120, "Neg"

RT 1.253

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302.3
303.1
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Figure S11. LC-MS trace of compound **21**.
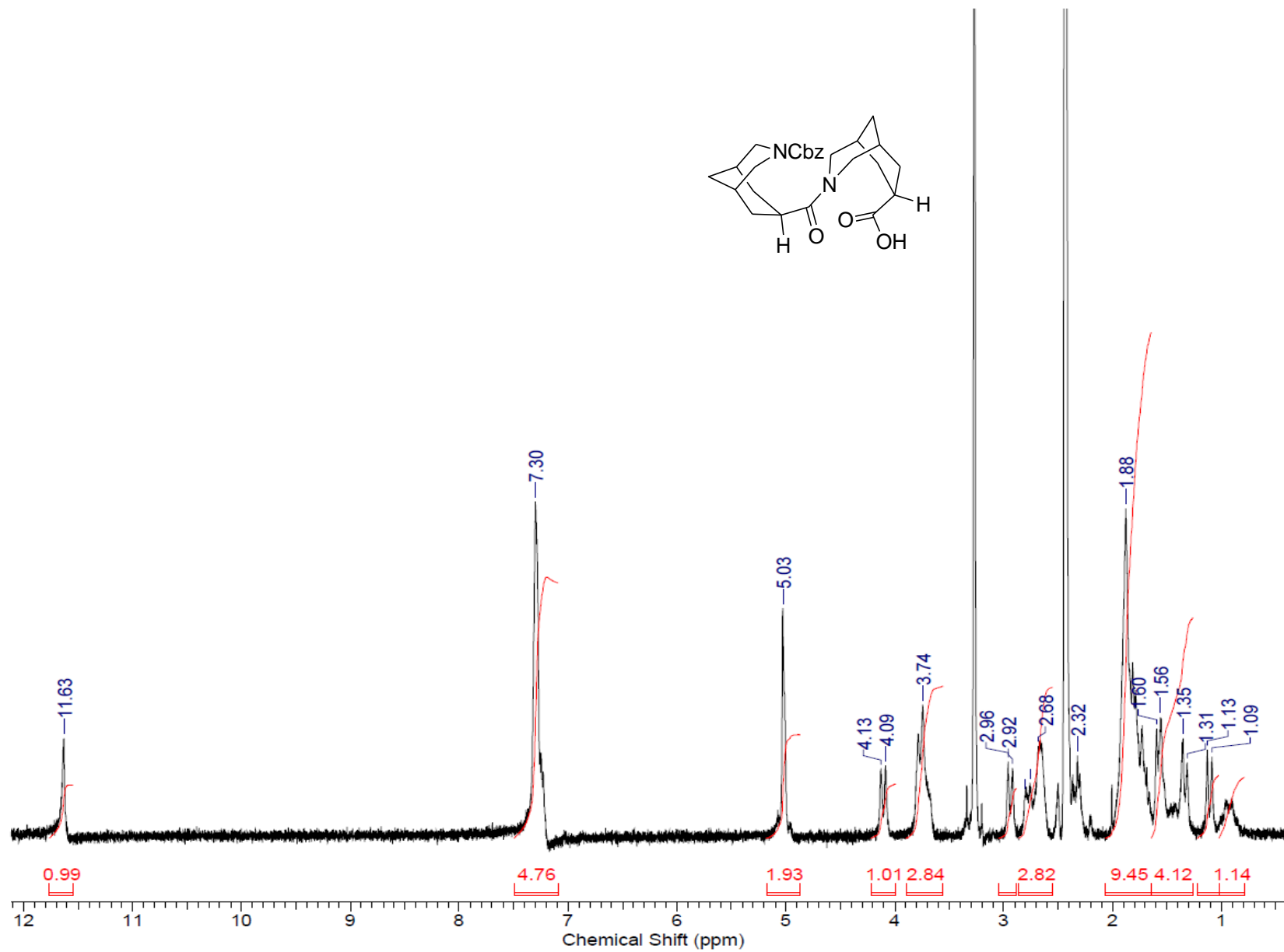
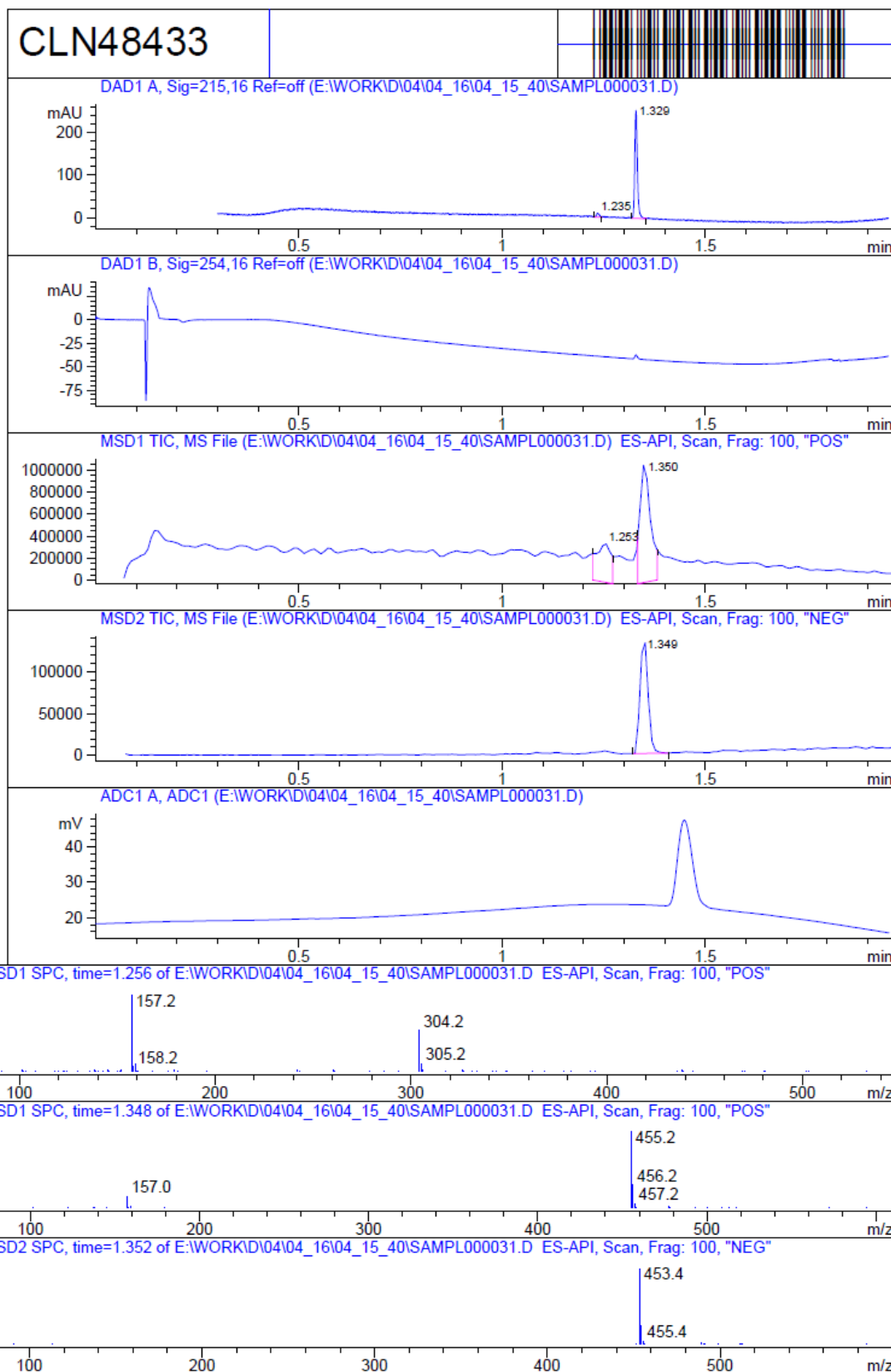**Figure S12**. ¹H-NMR spectrum of compound **22**.

Figure S13. LC-MS trace of compound **22**.
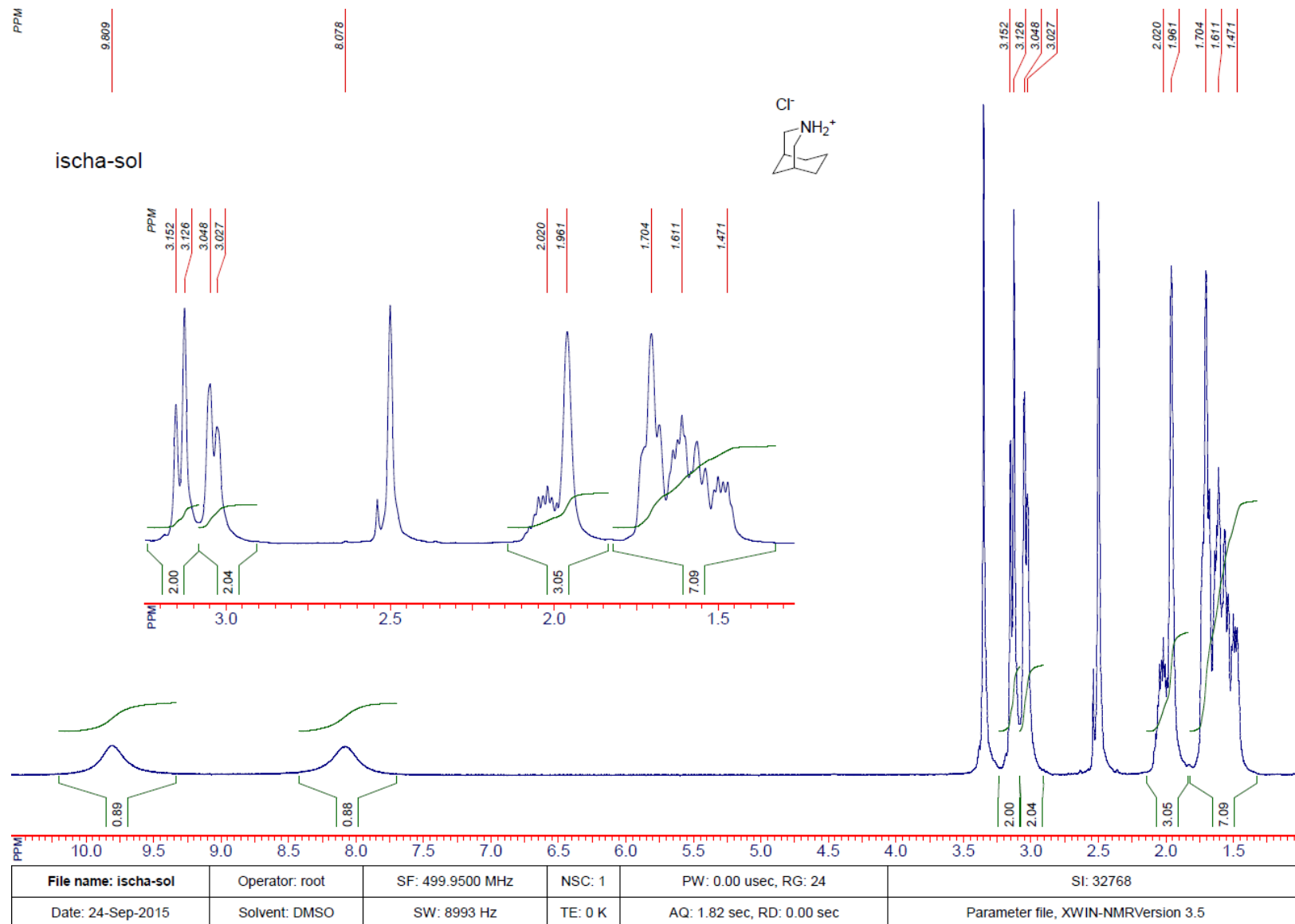
Figure S14. ^{1}H-NMR spectrum of compound **25** (hydrochloride).

ischa-sol_C13

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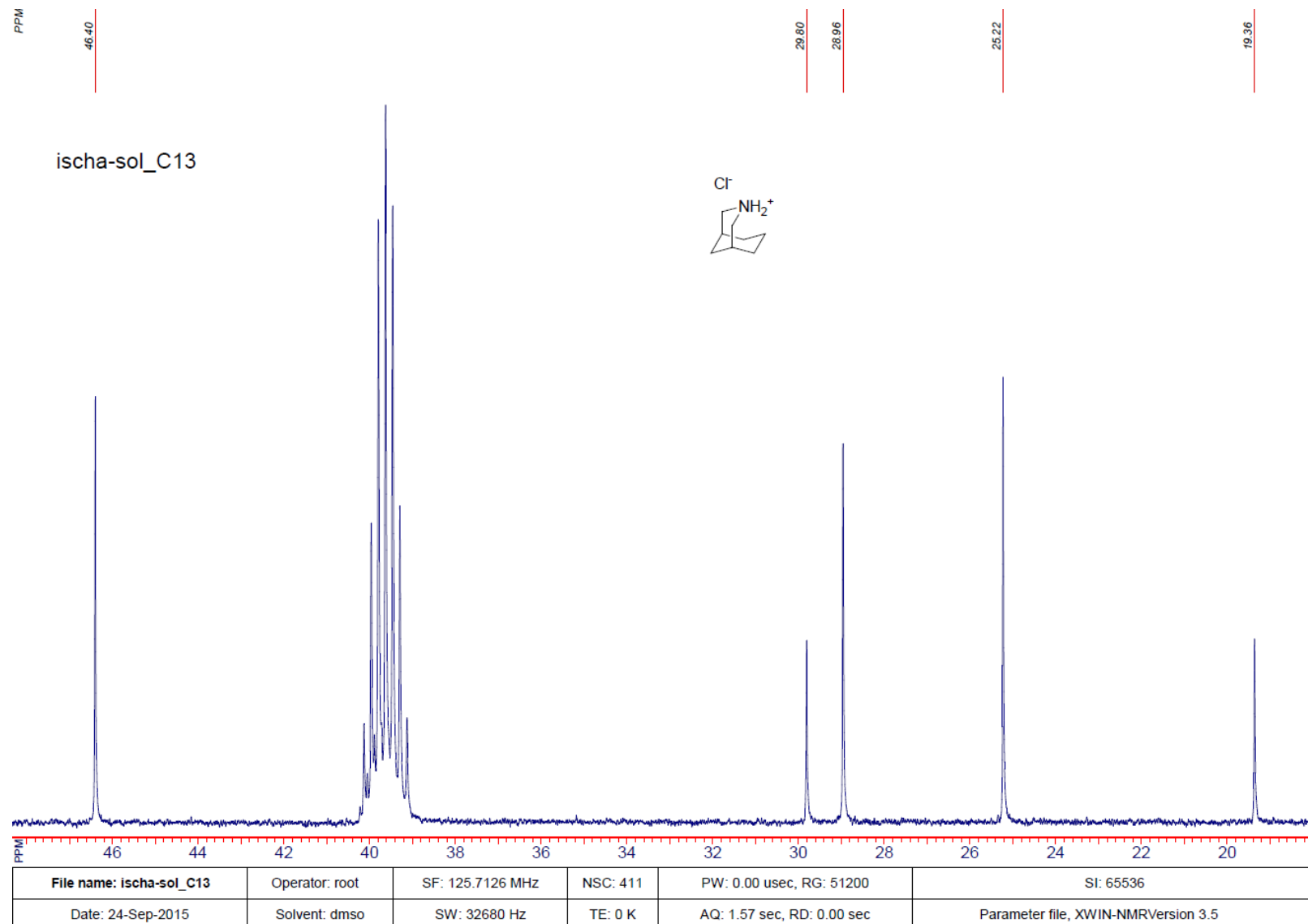
Figure S15. ^{13}C-NMR spectrum of compound **25** (hydrochloride).

Figure S16. ¹H-NMR spectrum of compound **26**.
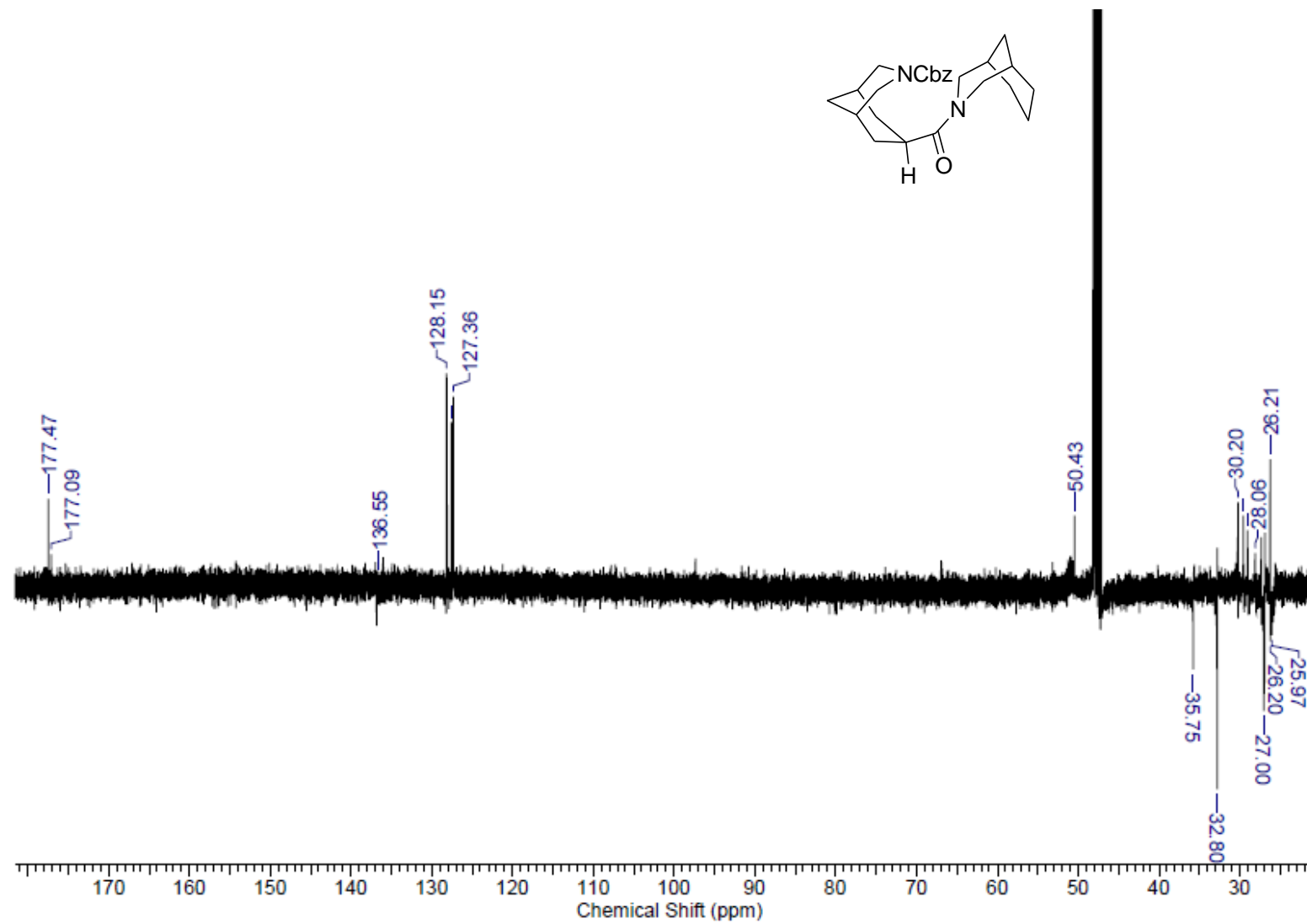
Figure S17. ¹³C-NMR spectrum (INEPT) of compound **26**.
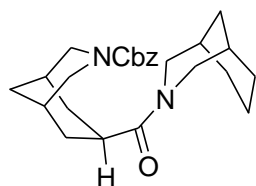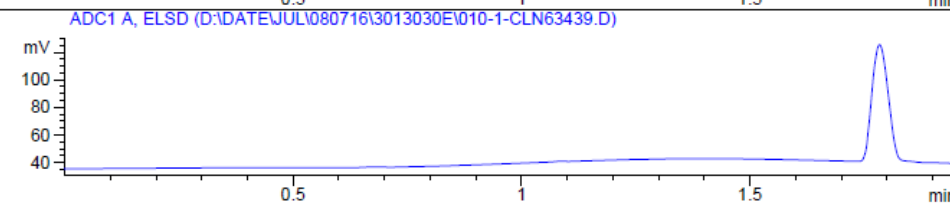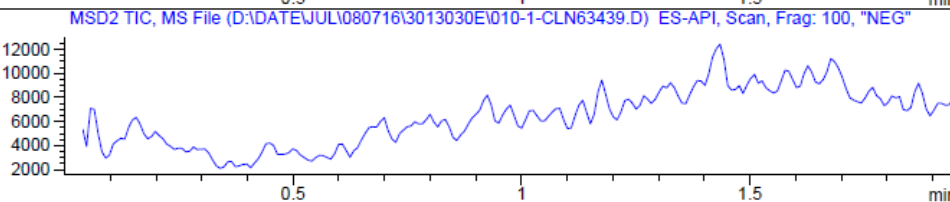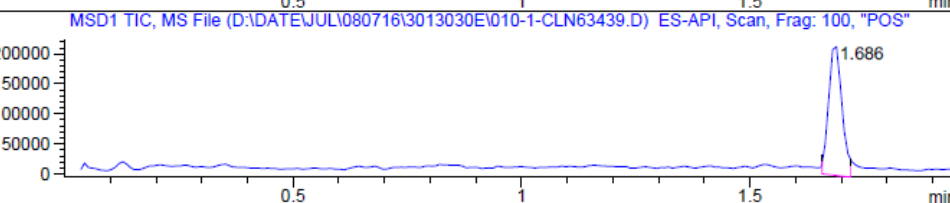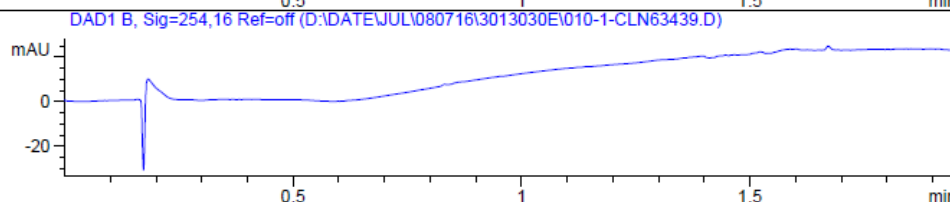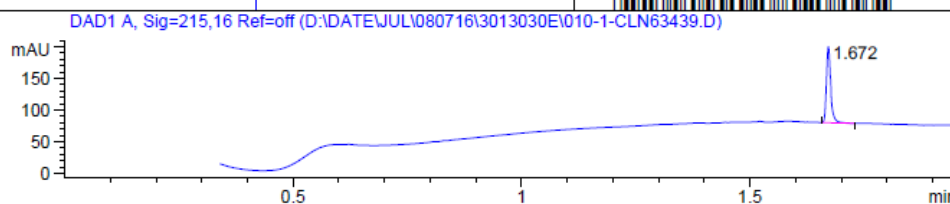
Figure S18. LC-MS trace of compound **26**.

Figure S19. ¹H-NMR spectrum of compound **14**.
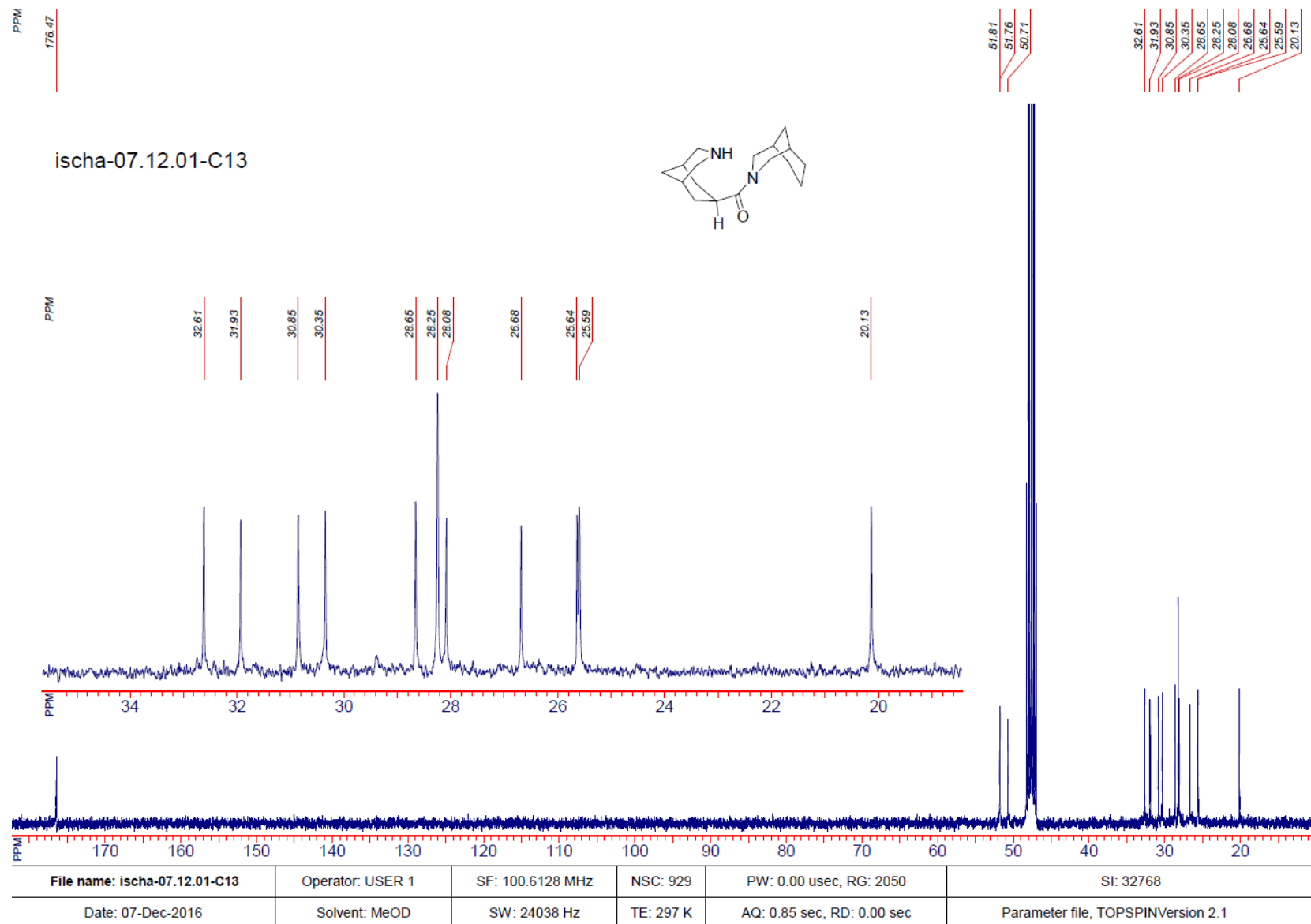
Figure S20. ^{13}C-NMR spectrum of compound **14**.

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CLO05269

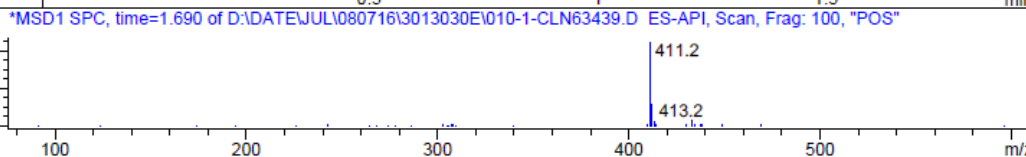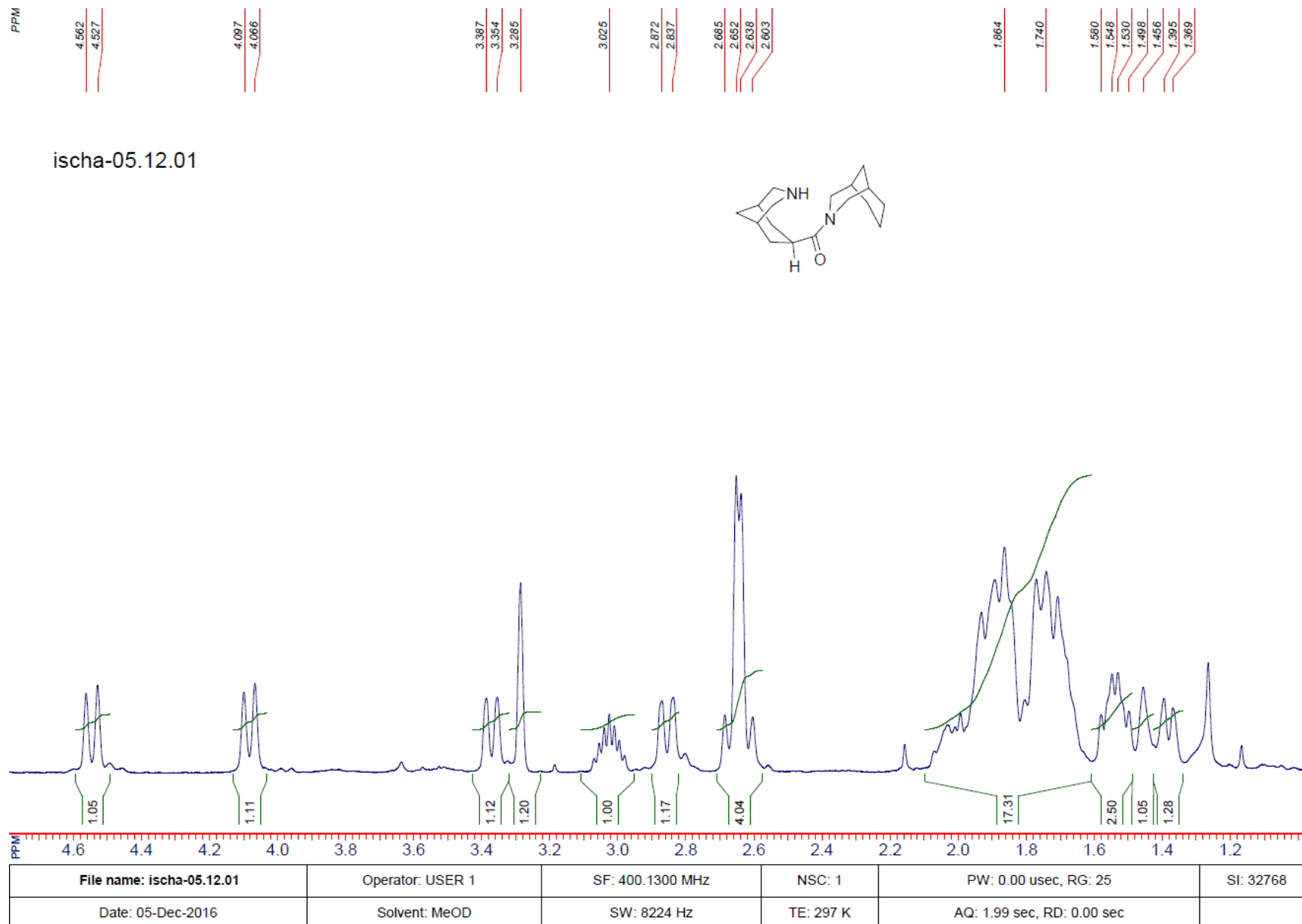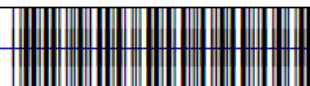DAD1 A, Sig=215,16 Ref=off (D:\DATE\SEPTEMBER\L020199D\043-1-CLO05269.D)

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mAU
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0
-20

0   0.5   1   1.5   min

Mol Wt   **0**
Exact Mass

#	Time	Area%
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600000
400000
200000
0

0.976

0   0.5   1   1.5   min

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2500
2000
1500
1000
500
0

0   0.5   1   1.5   min

ADC1 A, ELSD (D:\DATE\SEPTEMBER\L020199D\043-1-CLO05269.D)

mV
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60
40
20

0   0.5   1   1.5   min

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RT 0.976

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0

277.2

291.2

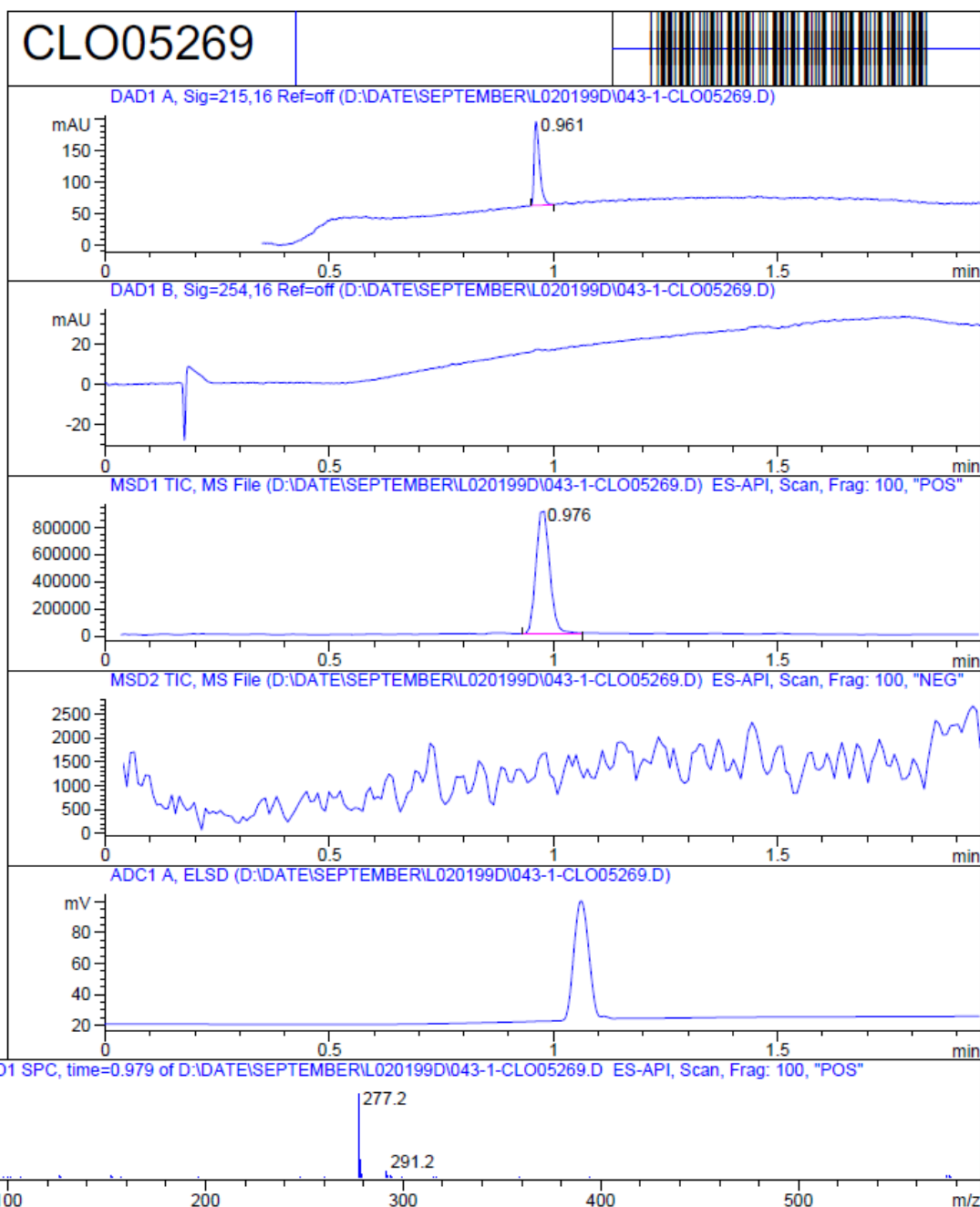100   200   300   400   500   m/z
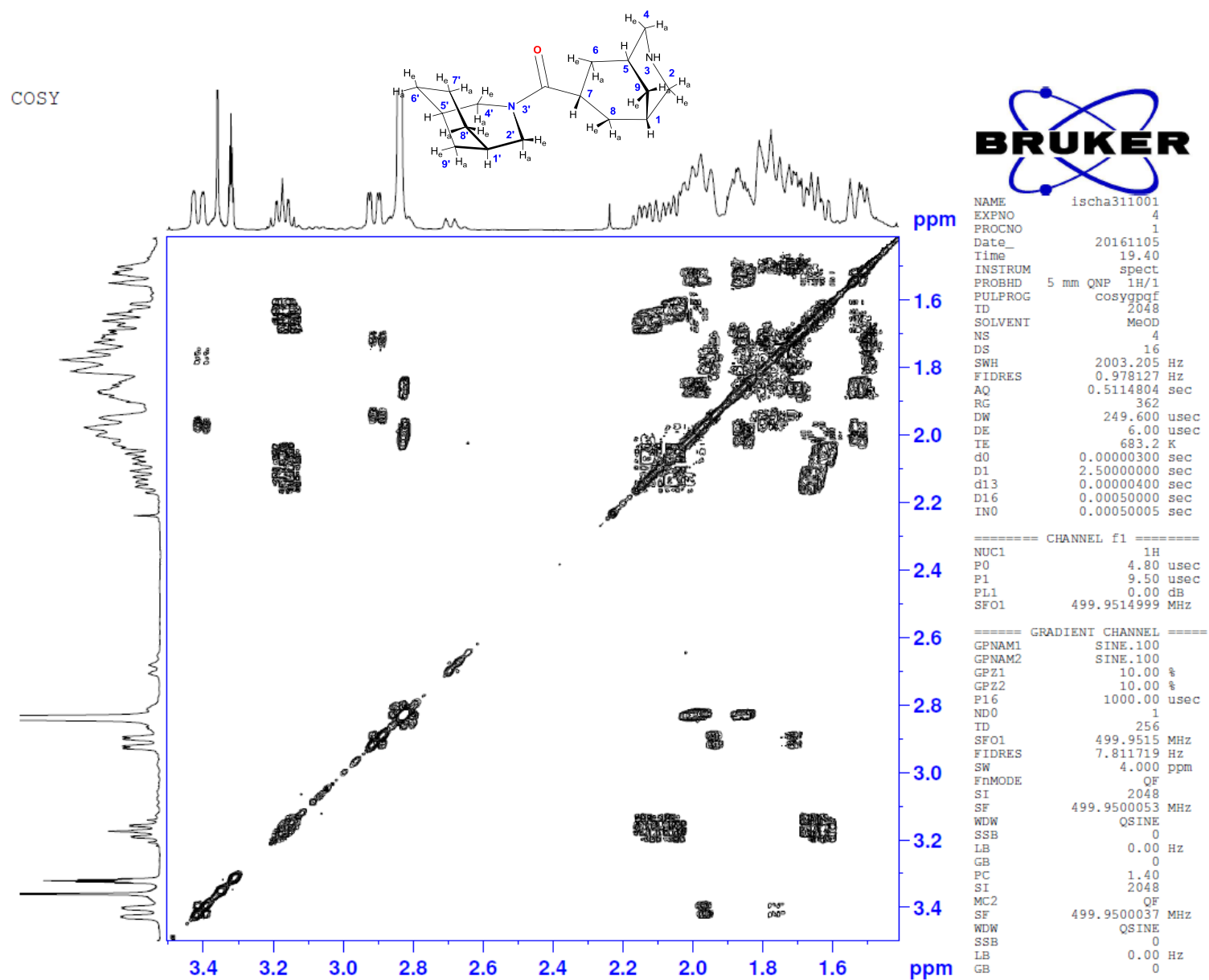
Figure S21. LC-MS trace of compound **14**.

Figure S22. H,H-COSY spectrum of compound **14**.
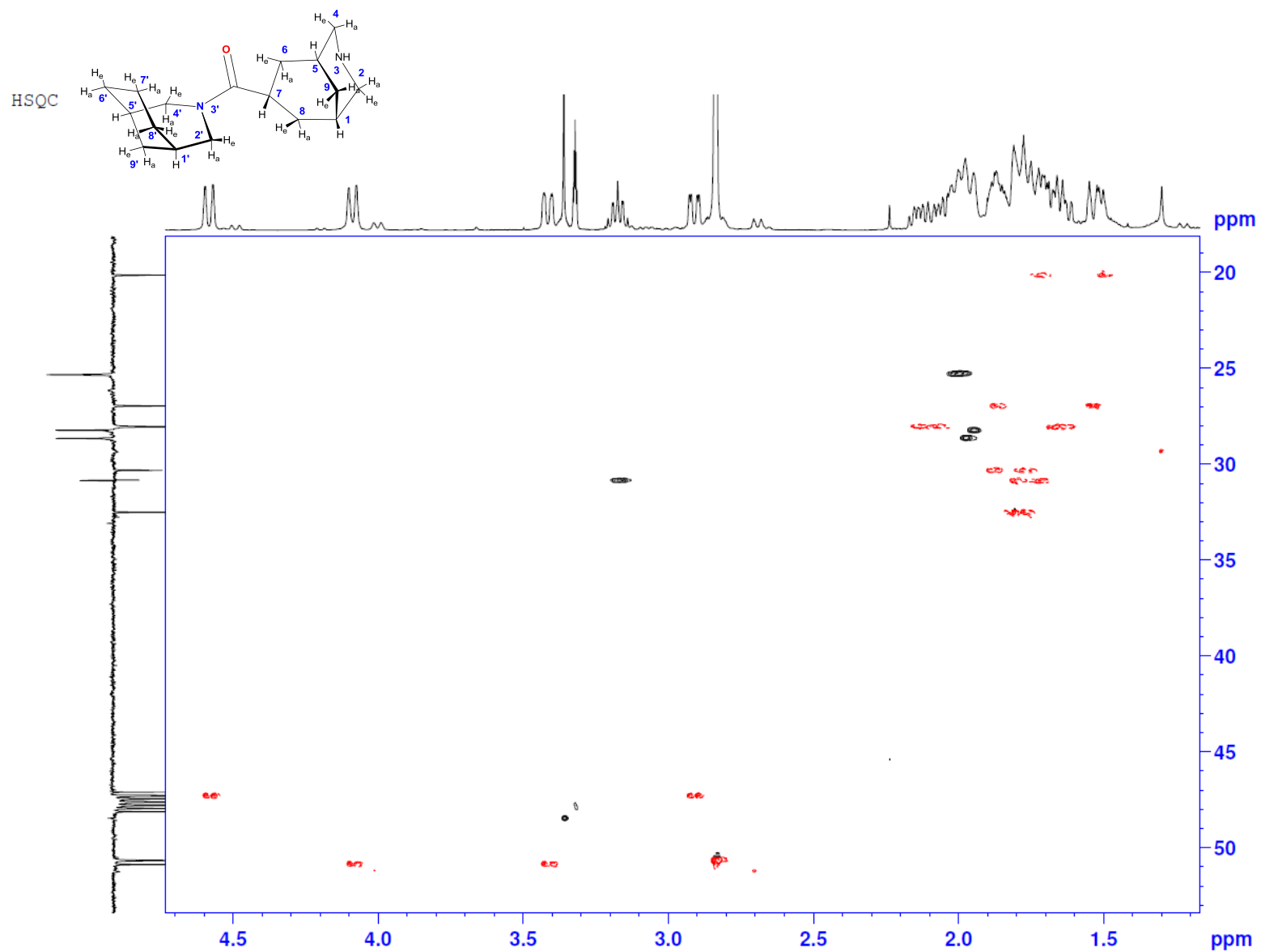
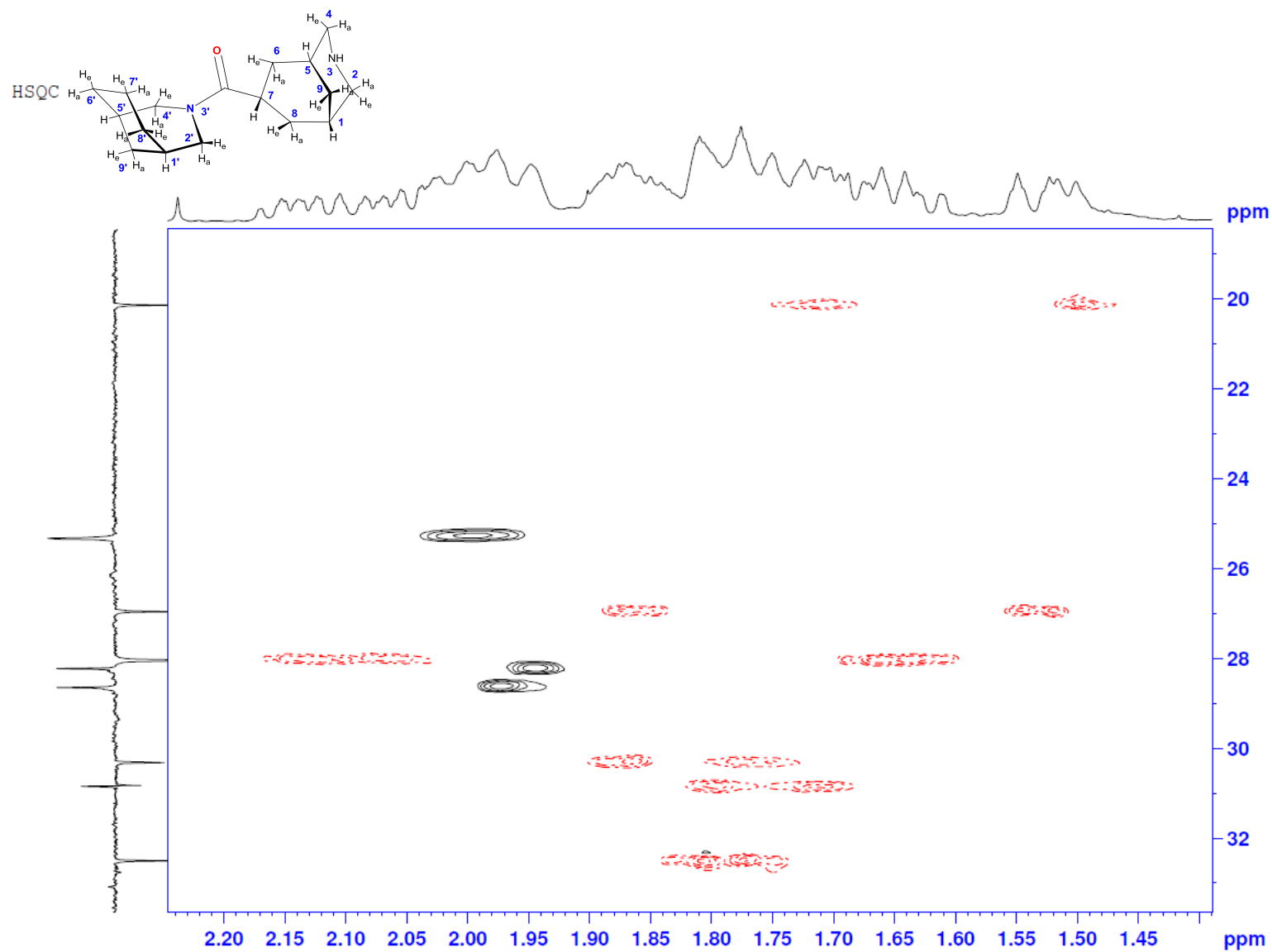**Figure S23**. C,H-HSQC spectrum of compound **14**.

Figure S24. C,H-HSQC spectrum of compound **14** (expansion).
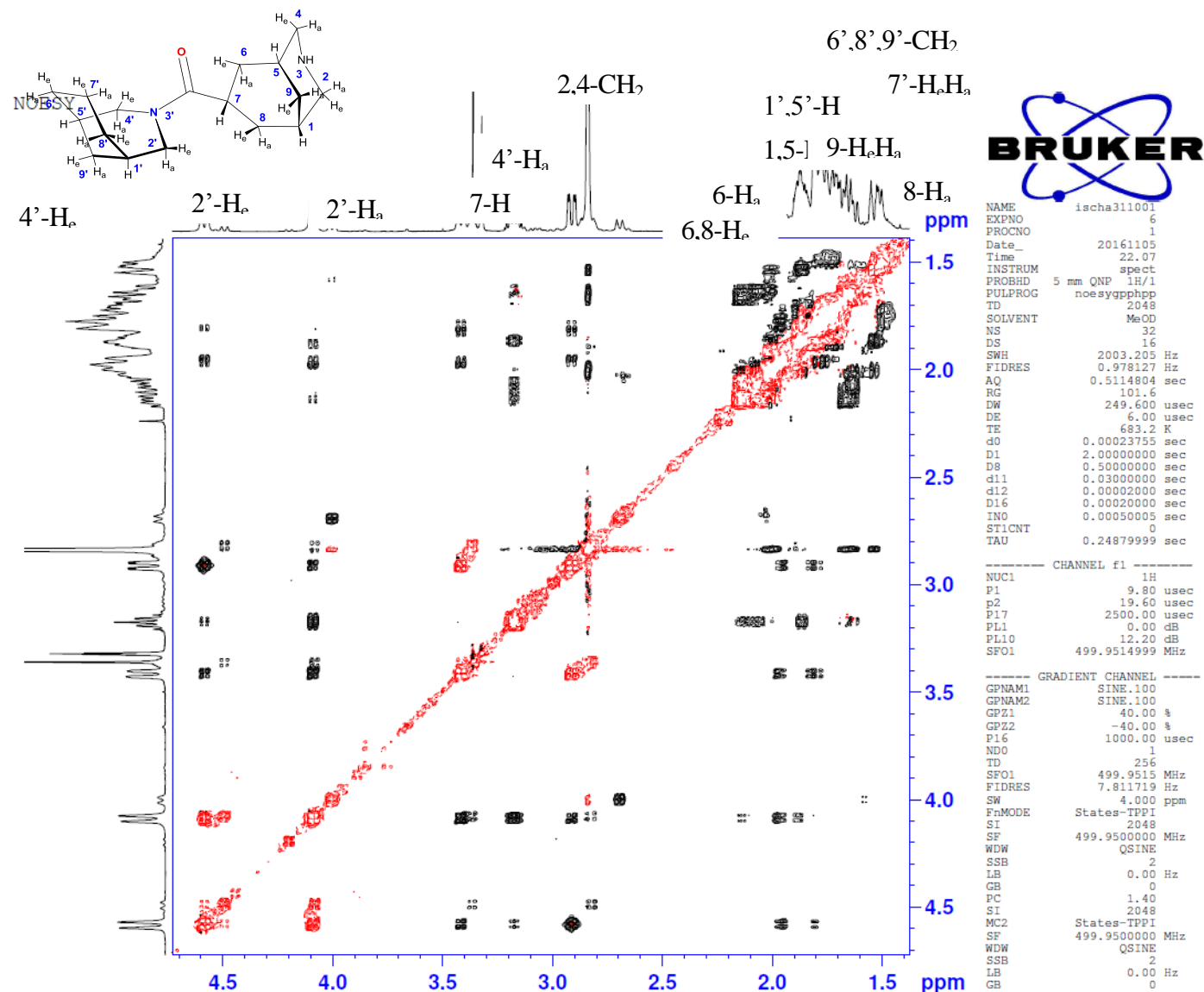
Figure S25. NOESY spectrum of compound **14**.
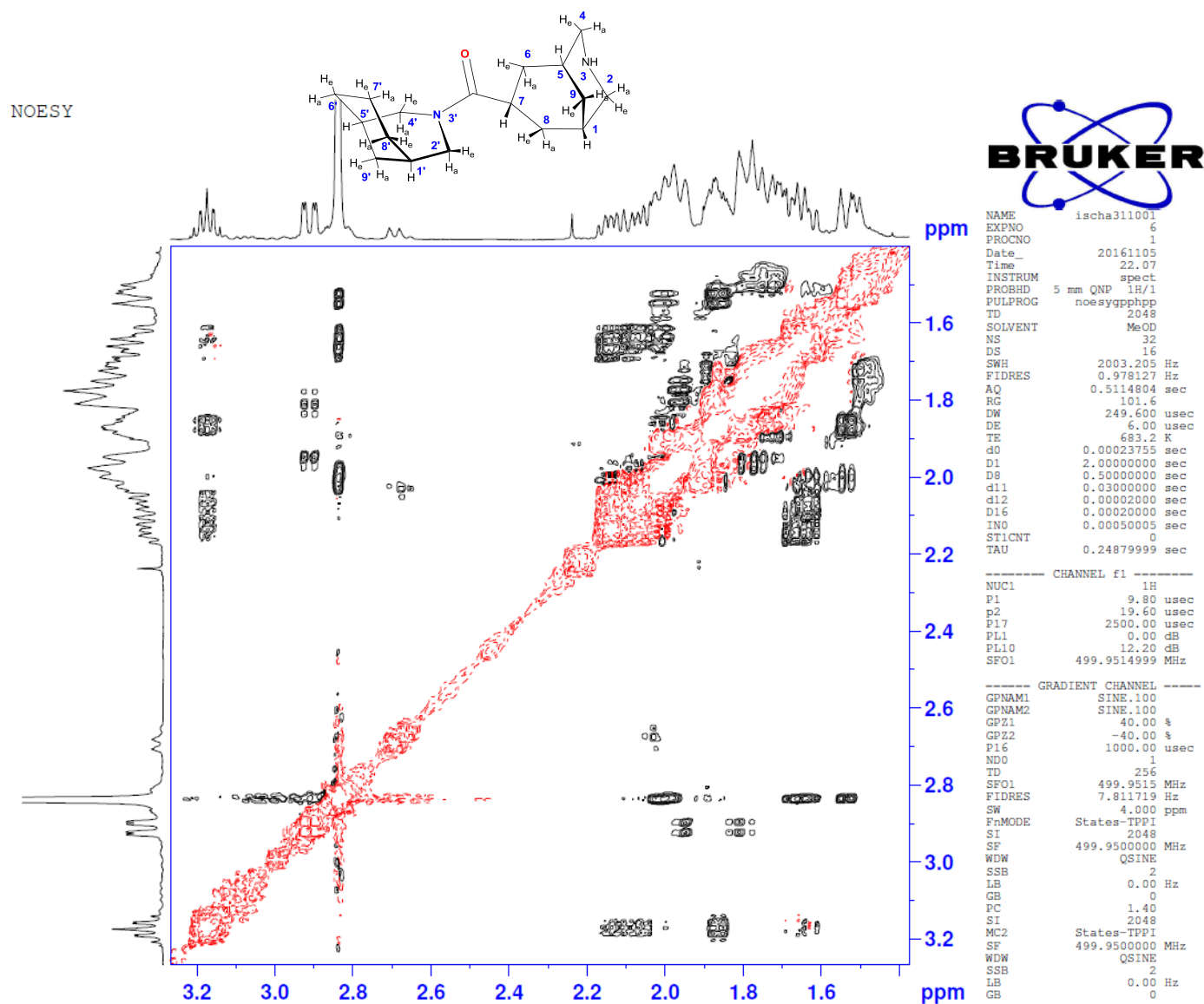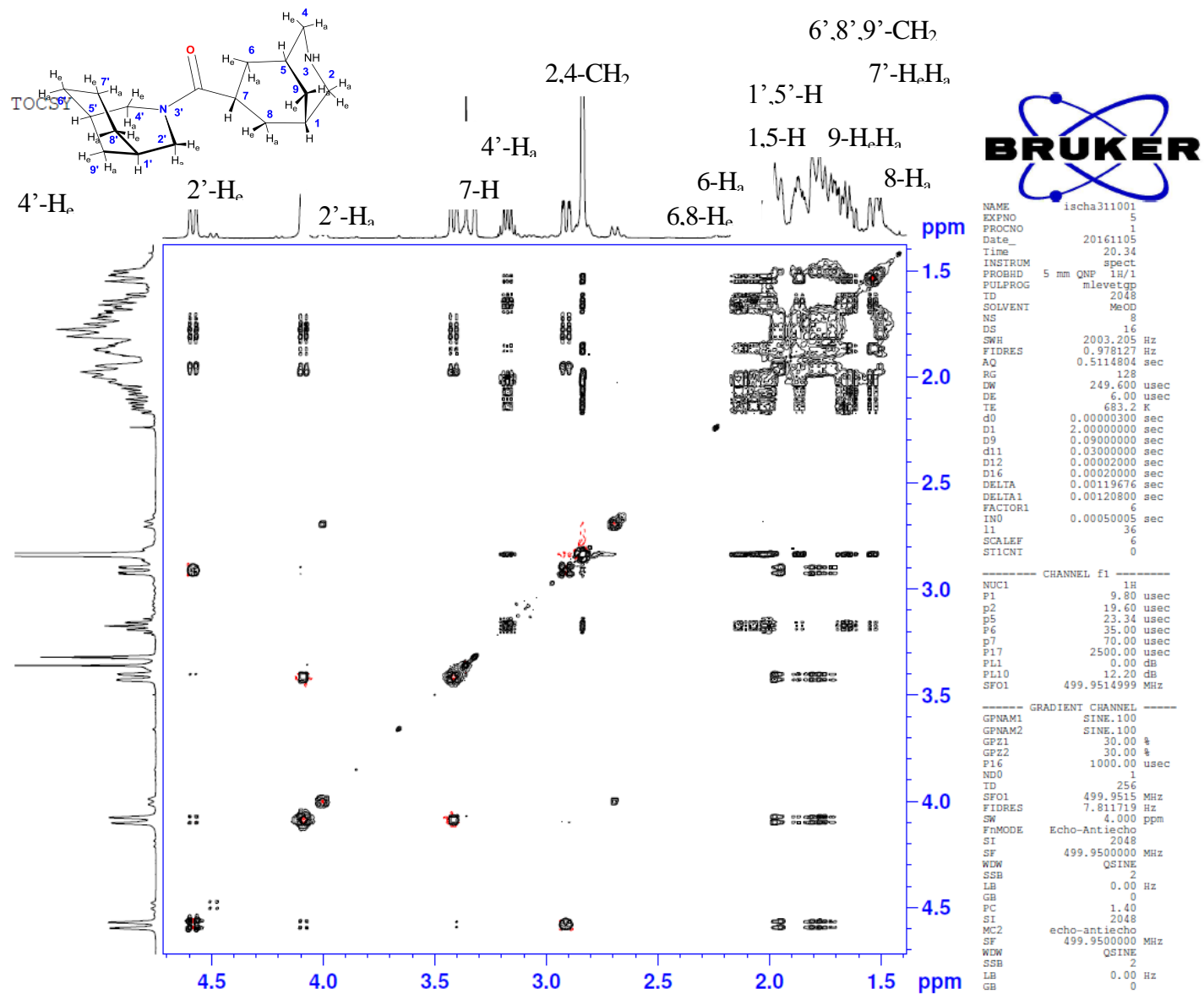
Figure S26. NOESY spectrum of compound **14** (expansion).
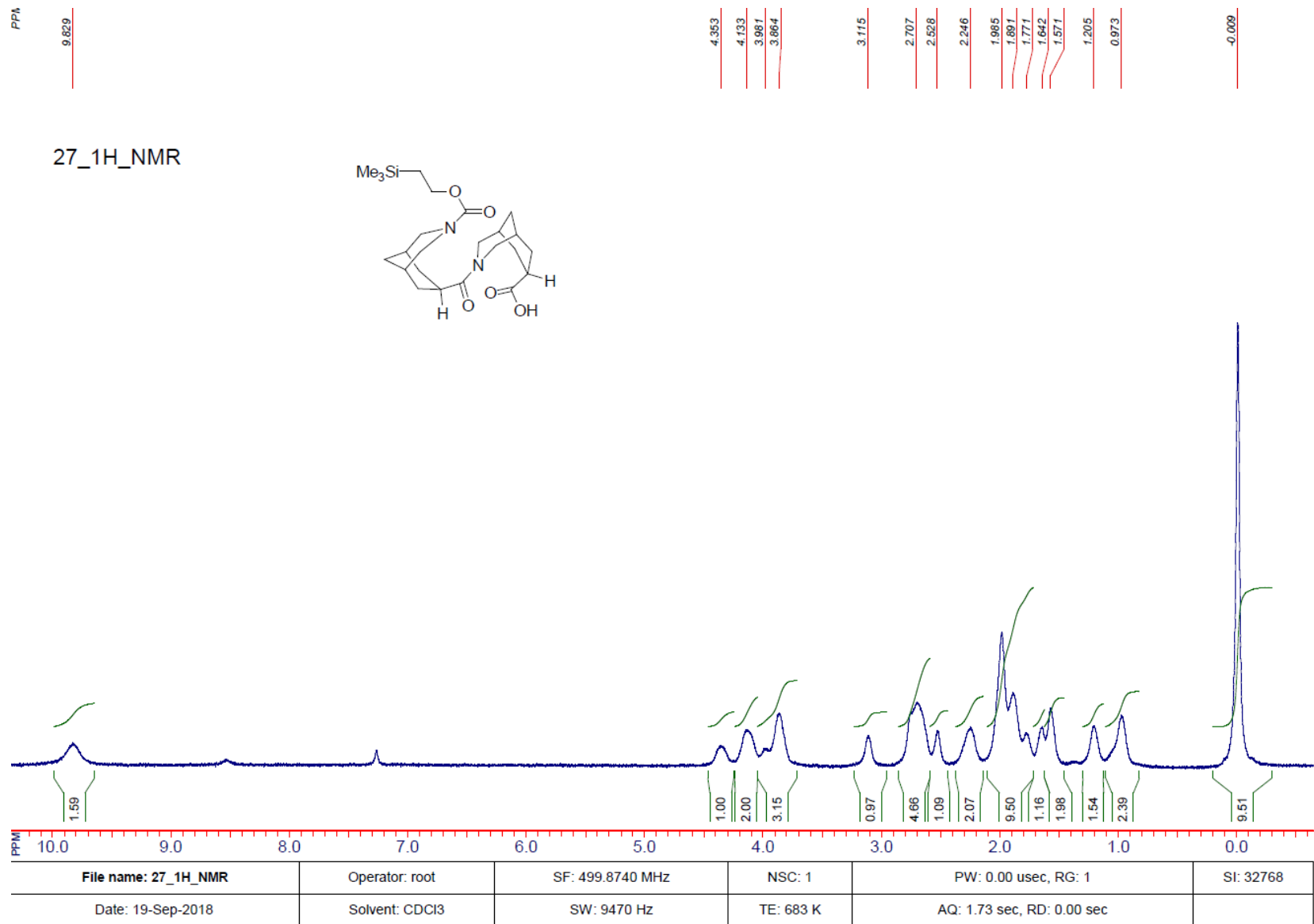
Figure S27. TOCSY spectrum of compound **14**.

Figure S28. ^{1}H-NMR spectrum of compound **27**.
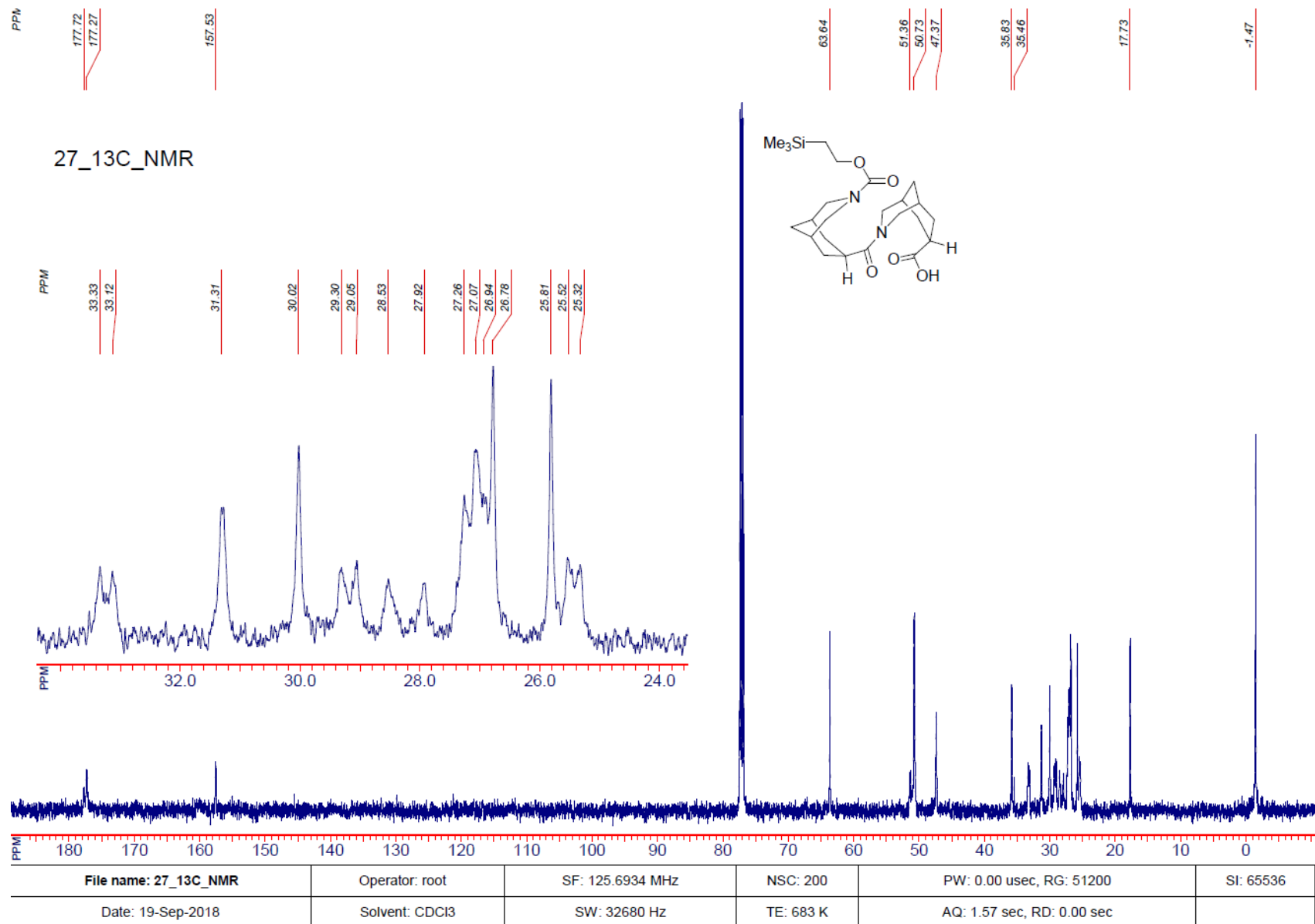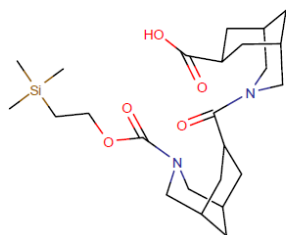
Figure S29. ^{13}C-NMR spectrum of compound **27**.
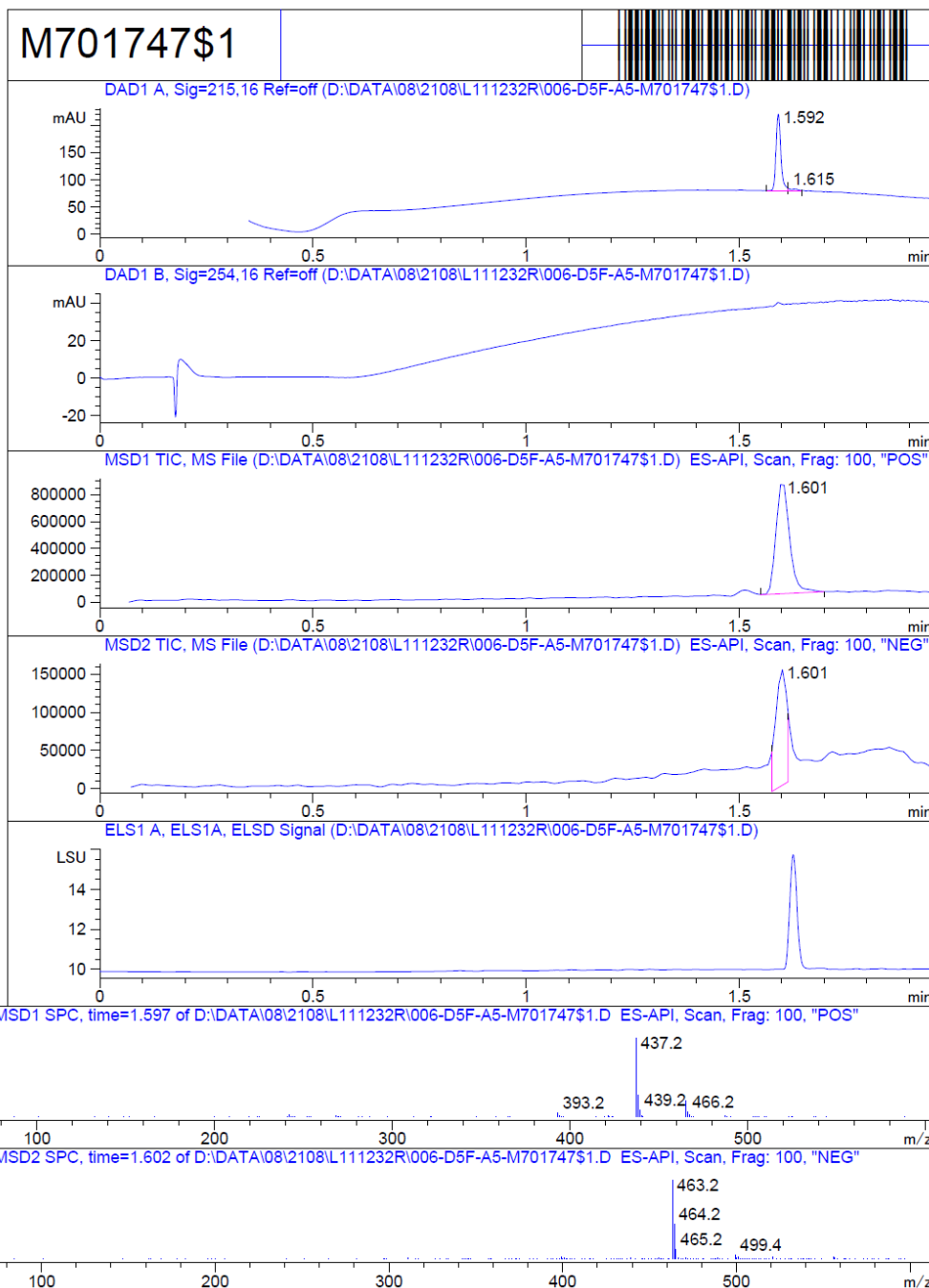
Figure S30. LC-MS trace of compound **27**.
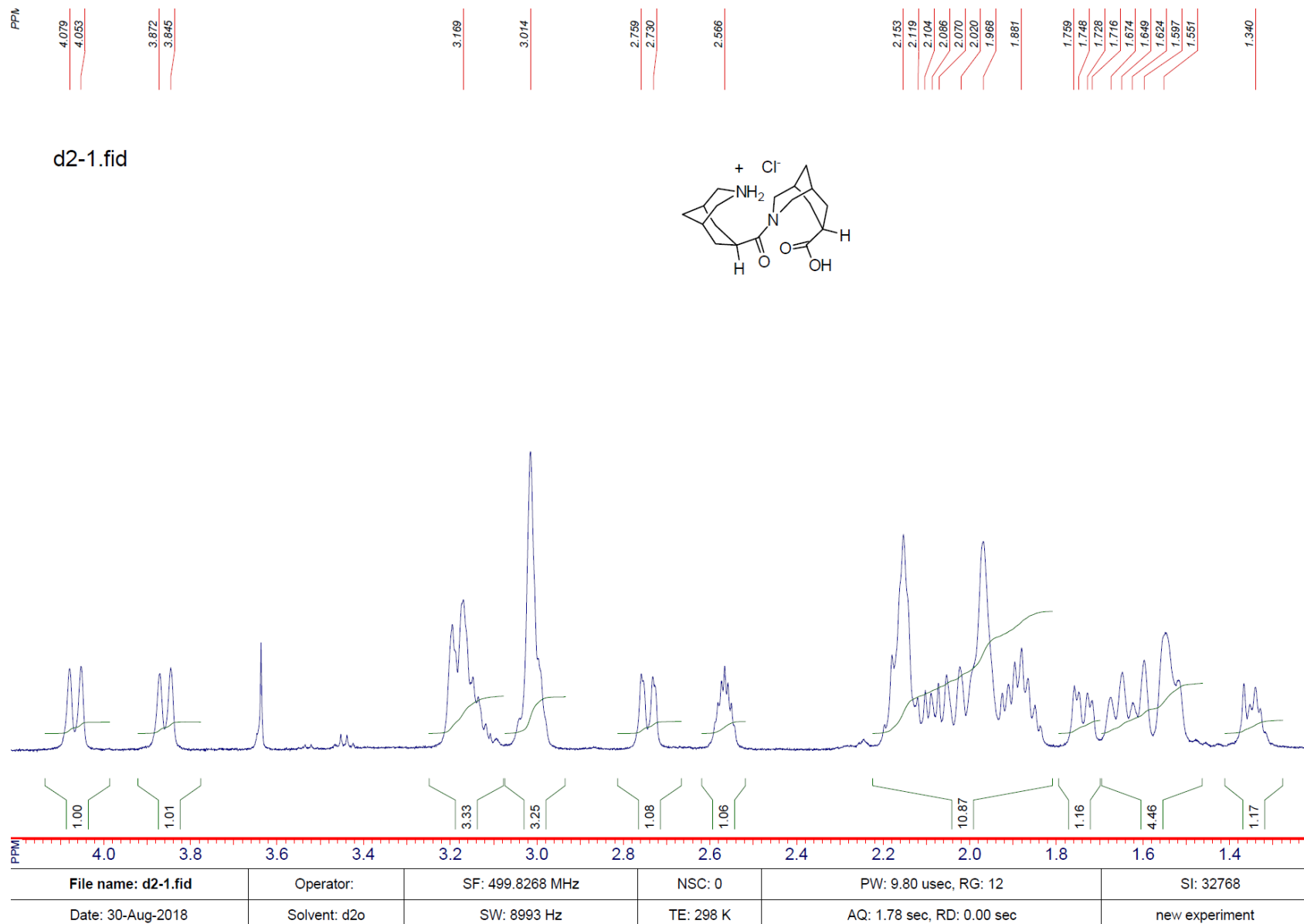
Figure S31. ^{1}H-NMR spectrum of compound **2**·HCl.
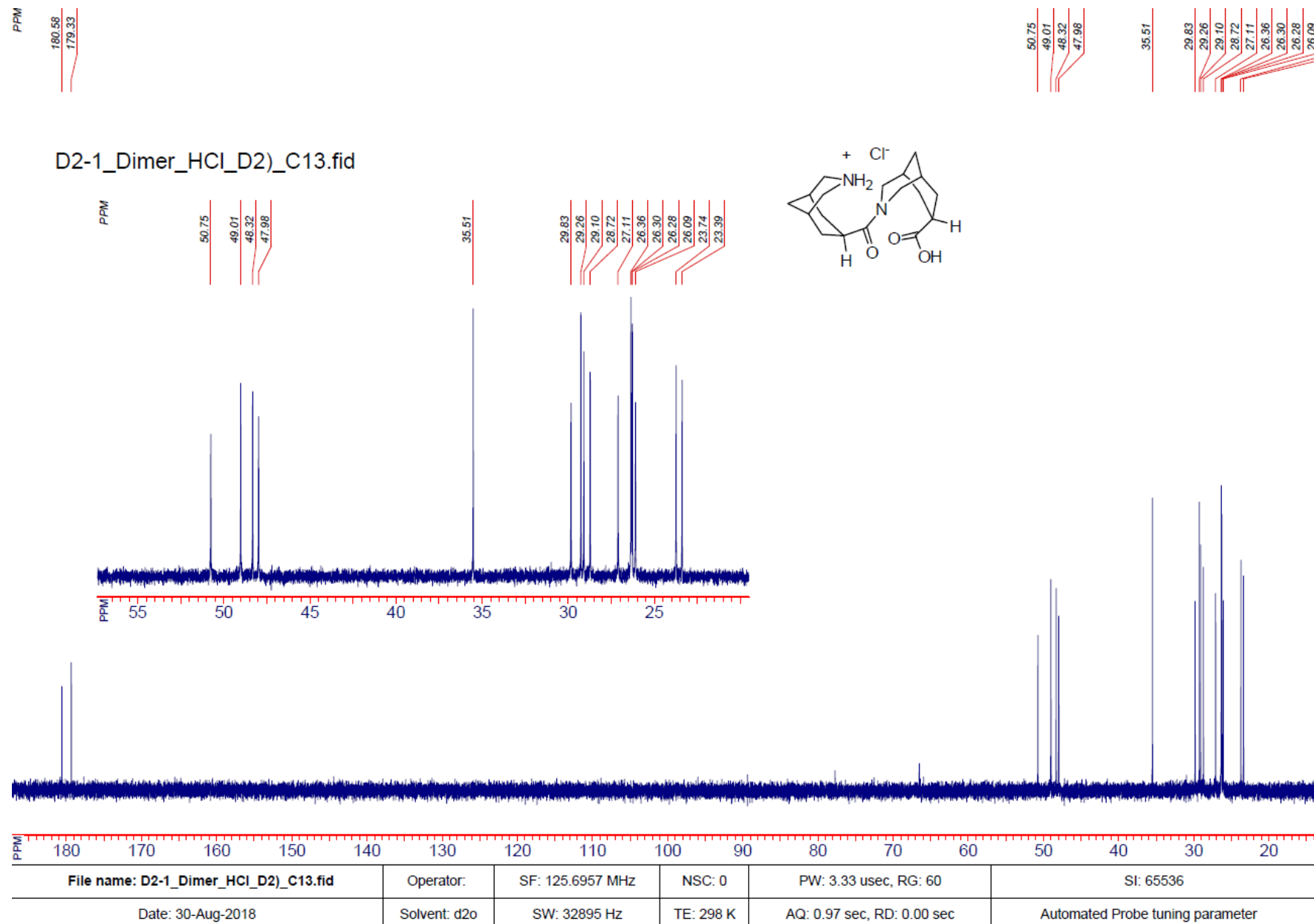
Figure S32. ^{13}C-NMR spectrum of compound **2**·HCl.
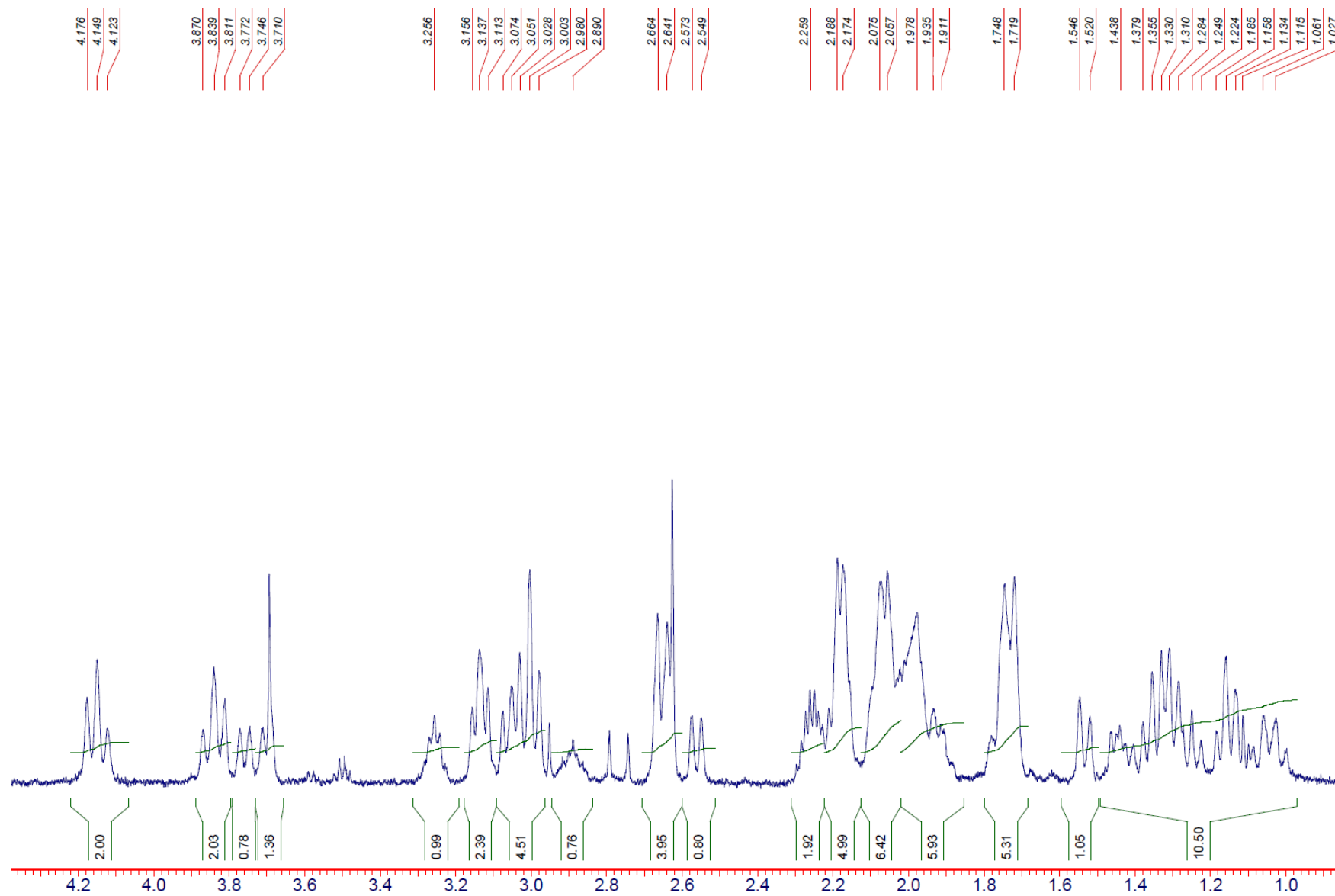
Figure S33. ^{1}H-NMR spectrum of the solution of **2** in carbonate D_2O buffer (pD 10.68) immediately after the preparation.
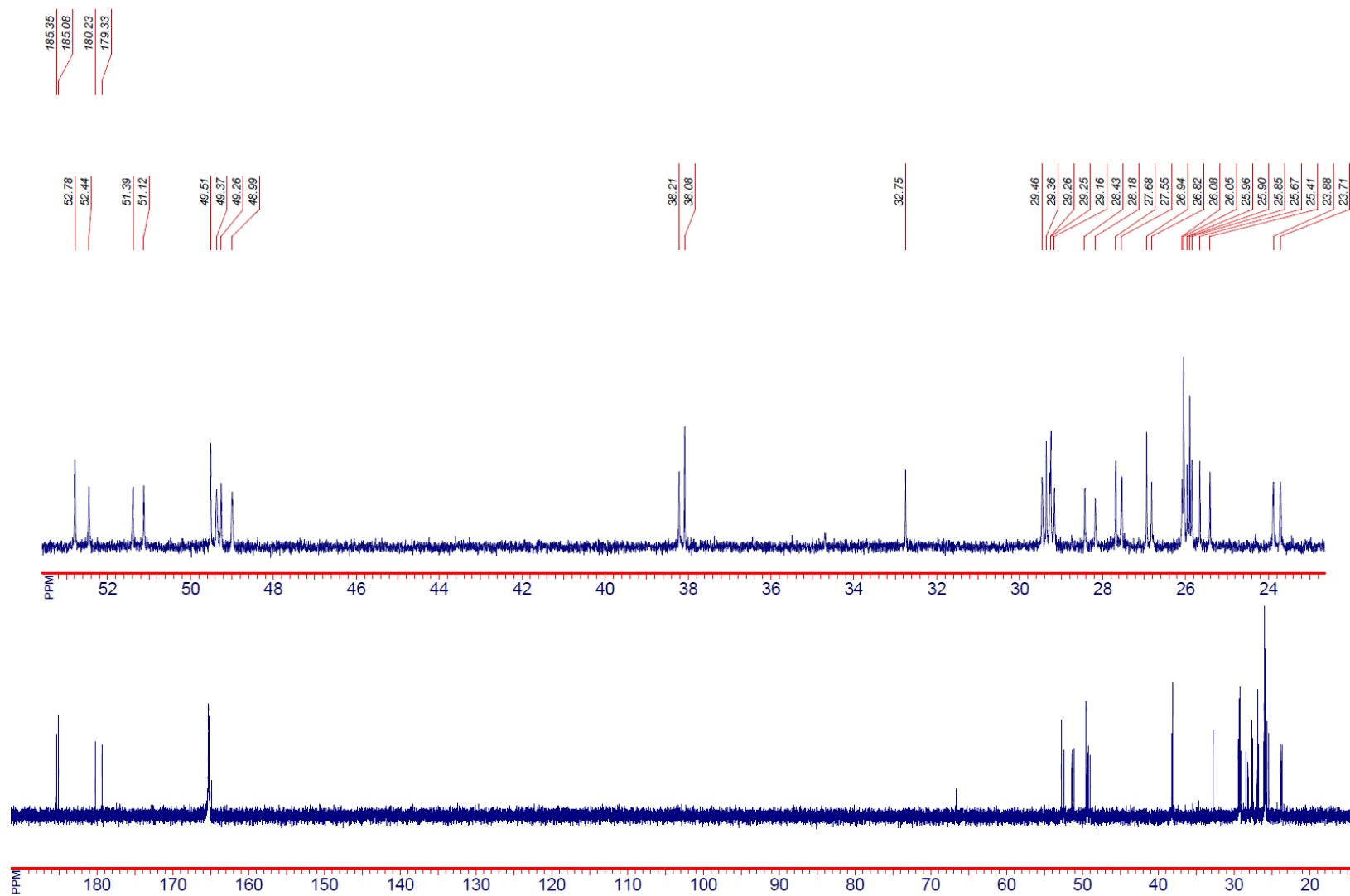
Figure S34. ^{13}C-NMR spectrum of the solution of **2** in carbonate D_2O buffer (pD 10.68) immediately after the preparation.
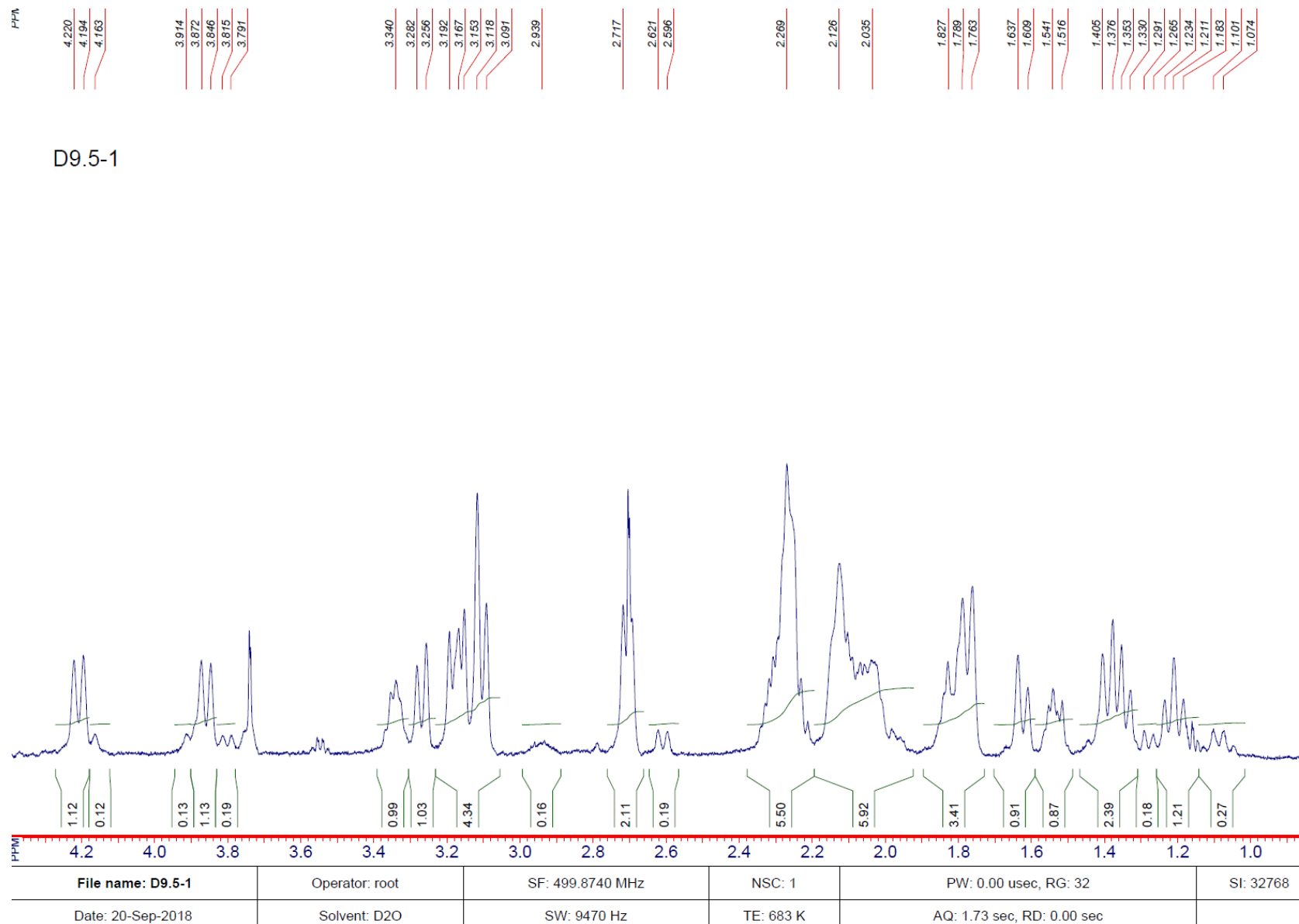
Figure S35. ^{1}H-NMR spectrum of the solution of **2** in carbonate D_2O buffer (pD9.45) immediately after the preparation.

Figure S36. ^{13}C-NMR spectrum of the solution of **2** in carbonate D$_2$O buffer (pD9.45) immediately after the preparation.

Kinetic data on the hydrolysis of 2·HCl in acetate D₂O buffer, pD 3.81.

Table S1. Molar percent of **2·**HCl vs time (min) in acetate buffer (pD 3.81, 23 °C)

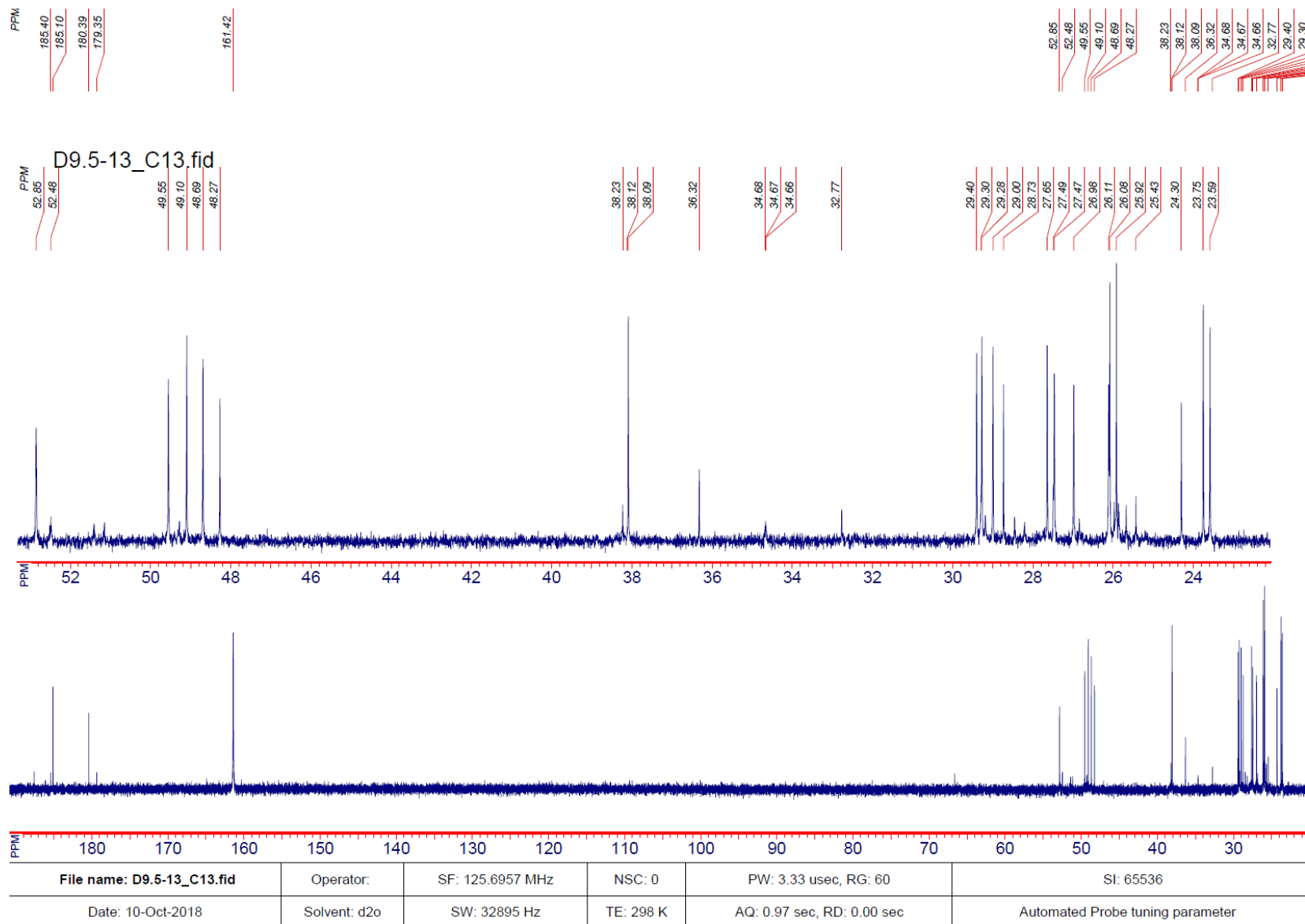| Time, min	0	273	3105	4606	7168	10950	15848	16127	17131
Molar % of **2·**HCl	100	97	81	79	74	61	57	51	49
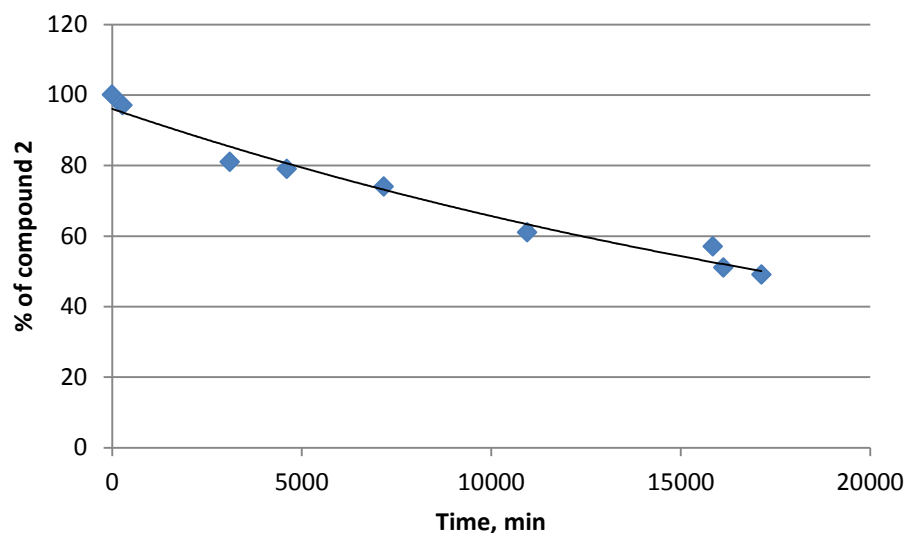
Figure S37. Exponential fit of the data (Table S1).

Table S2. Fitting parameters and corresponding kinetic constants.

Equation	y0	A1	t1	R^2	Half-life, $t_{1/2}$, min	k_{obs}, min^{-1}	log(k_{obs})
y = A1·exp(-x/t1) + y0	34,43121± 11,0332	63,99936± 10,30033	12830,87499± 4149,08408	0,97726	18138	3.821·10^{-5}	-4.418

Figure S38. Representative spectral data set for hydrolysis of **2**·HCl in acetate D₂O buffer, pD 3.81, 23 °C.

Kinetic data on the hydrolysis of 2·HCl in acetate D₂O buffer, pD4.85.

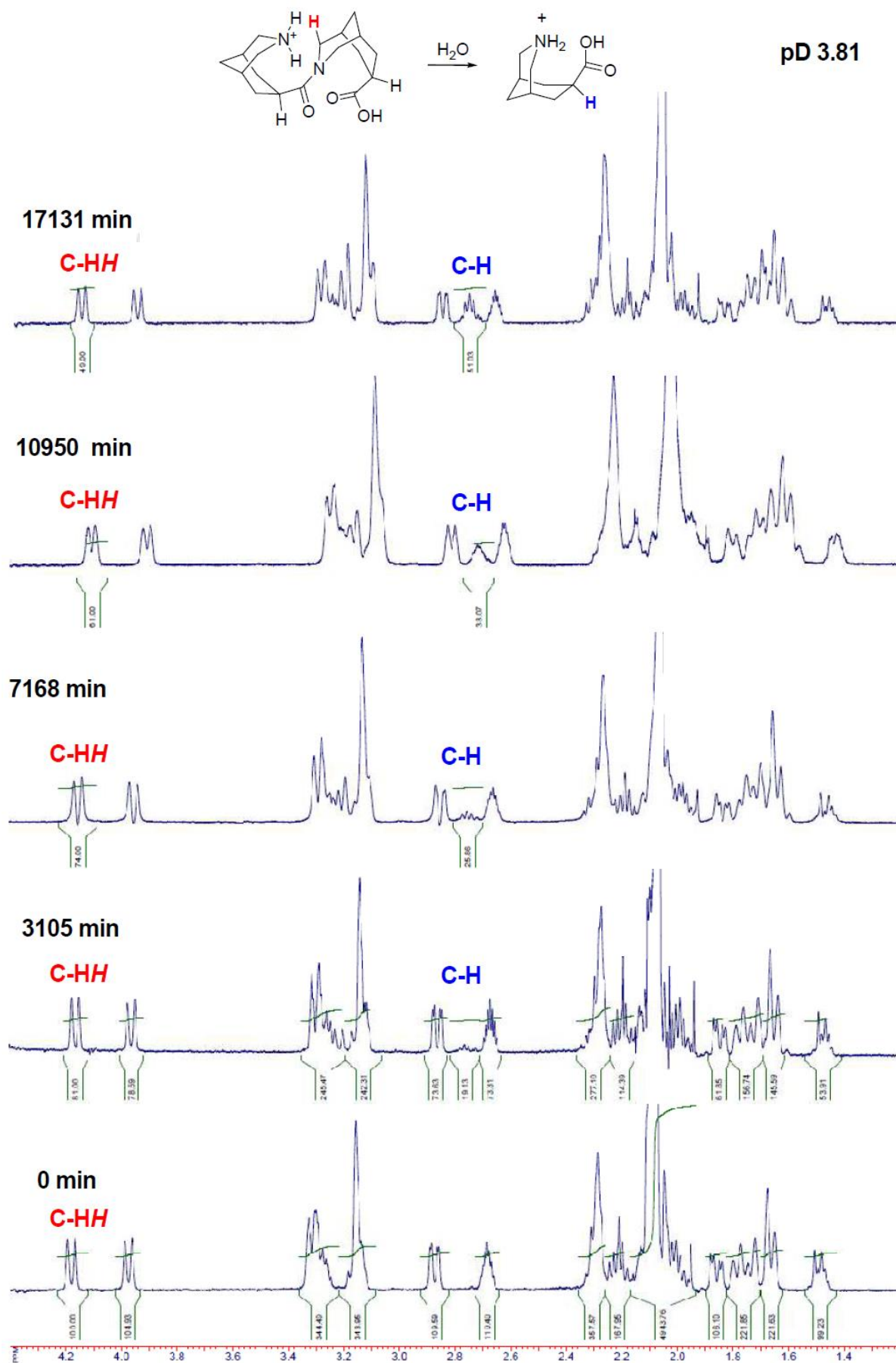**Table S3.** Molar percent of **2**·HCl vs time (min) in acetate buffer (pD 4.85, 23 °C)

Time, min	0	275	3110	4609	7150	10953	13148	16191	17136
Molar % of 2·HCl	100	96	62	45	39	23	20	11	5

Figure S39. Exponential fit of the data (Table S3).

Table S4. Fitting parameters and corresponding kinetic constants.

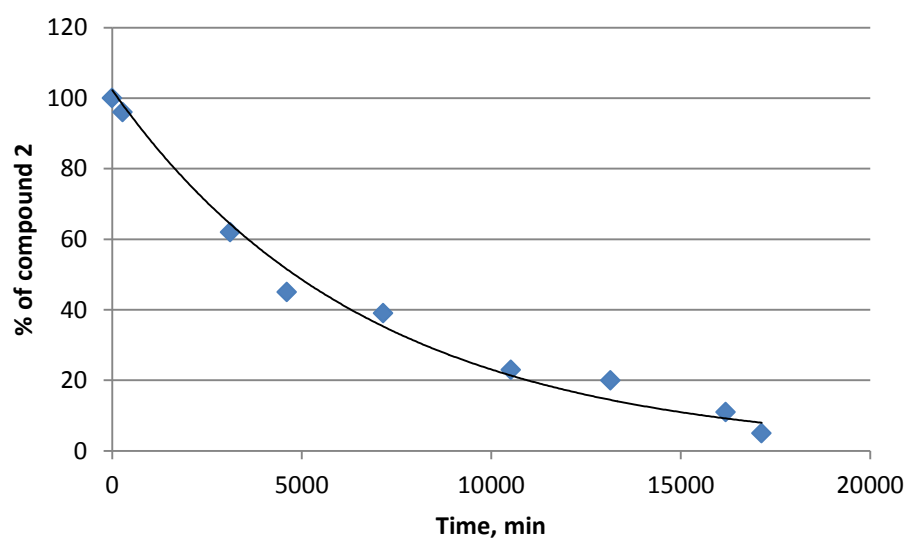| Equation	y0	A1	t1	R^2	Half-life, $t_{1/2}$, min	k_{obs}, min^{-1}	log(k_{obs})
y = A1·exp(-x/t1) + y0	2,69584± 4,7715	96,60028± 4,81107	6537,81499± 895,81784	0,98792	4668	$1.485·10^{-4}$	-3.828

Figure S40. Representative spectral data set for hydrolysis of **2**·HCl in acetate D₂O buffer, pD4.85, 23 °C.

Kinetic data on the hydrolysis of 2·HCl in phosphateD₂O buffer, pD6.68.

Table S5. Molar percent of **2**·HCl vs time (min) in phosphate buffer (pD6.68, 23 °C)

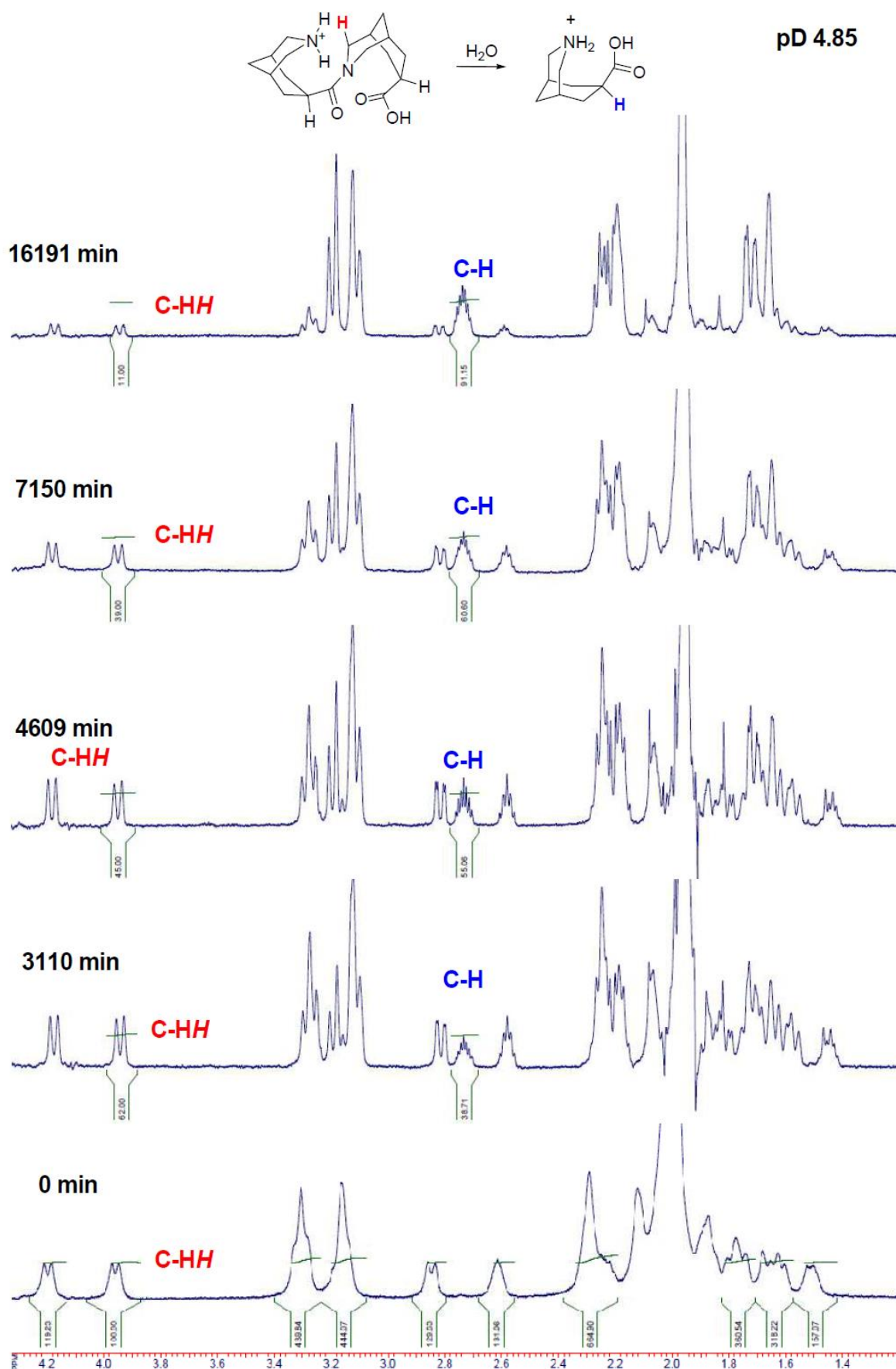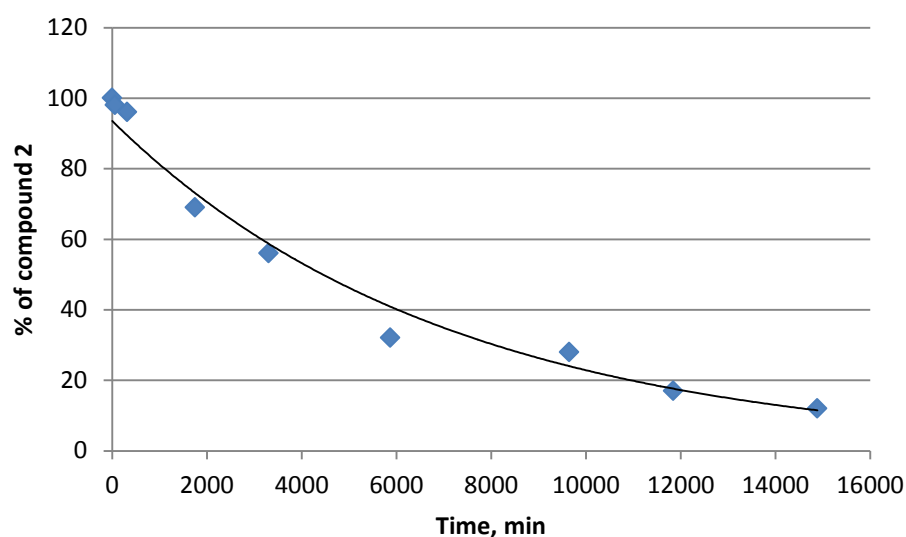| Time, min	0	60	313	1745	3302	5871	9645	11844	14884
Molar % of **2**·HCl	100	98	96	69	56	32	28	17	12

Figure S41. Exponential fit of the data (Table S5).

Table S6. Fitting parameters and corresponding kinetic constants.

Equation	y0	A1	t1	R^2	Half-life, $t_{1/2}$, min	k_{obs}, min^{-1}	log(k_{obs})
y = A1·exp(-x/t1) + y0	11,32544± 3,65066	88,61318± 3,75123	4520,24104± 565,27656	0,99053	3748	1.849·10^{-4}	-3.733

Figure S42. Representative spectral data set for hydrolysis of **2**·HCl in phosphate D_2O buffer, pD6.68, 23 °C.

Kinetic data on the hydrolysis of 2·HCl in phosphateD₂O buffer, pD7.95.

Table S7. Molar percent of **2** vs time (min) in phosphate buffer (pD7.95, 23 °C)
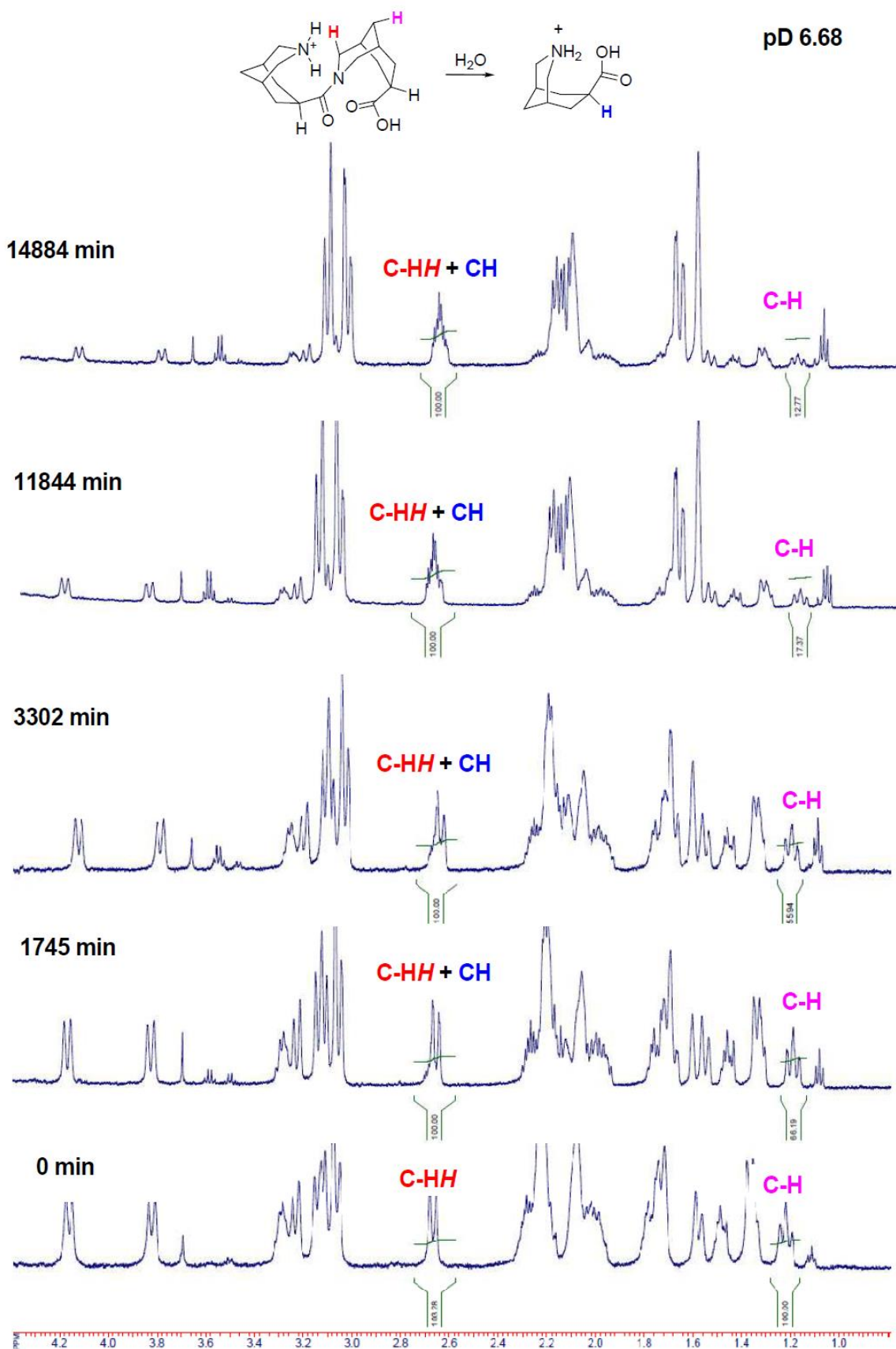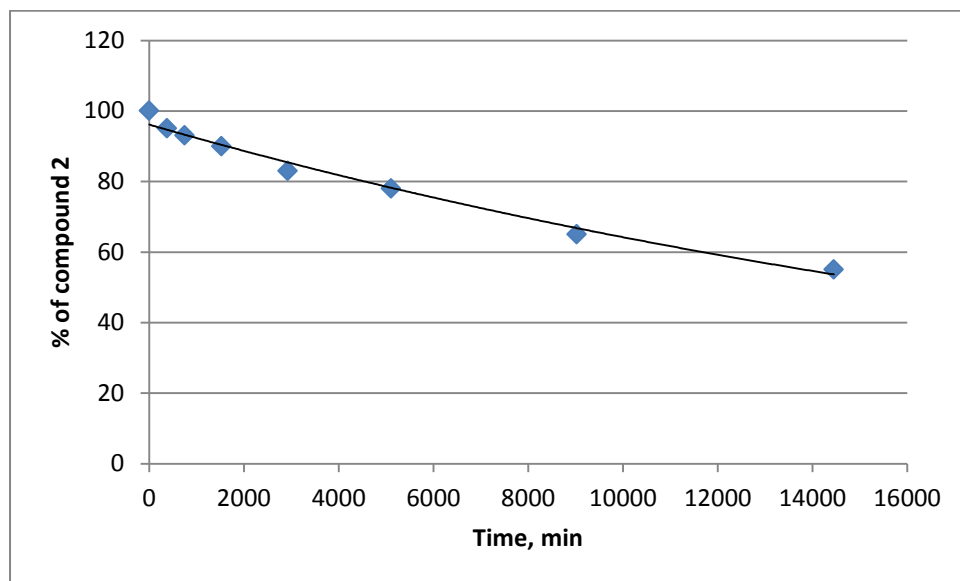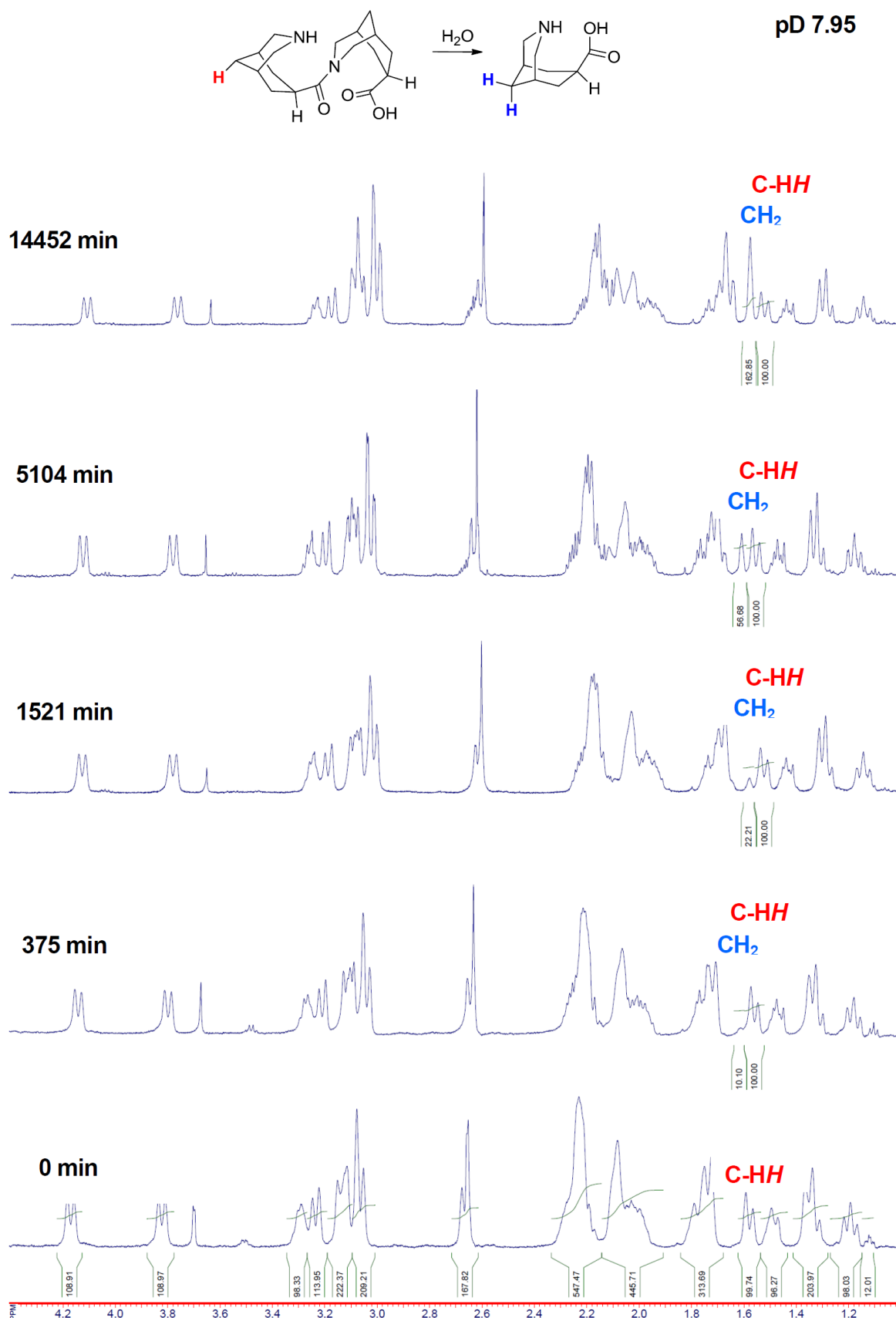
Time, min	0	375	746	1521	2924	5104	9022	14445
Molar % of **2**·HCl	100	95	93	90	83	78	65	55

Figure S43. Exponential fit of the data (Table S7).

Table S8. Fitting parameters and corresponding kinetic constants.

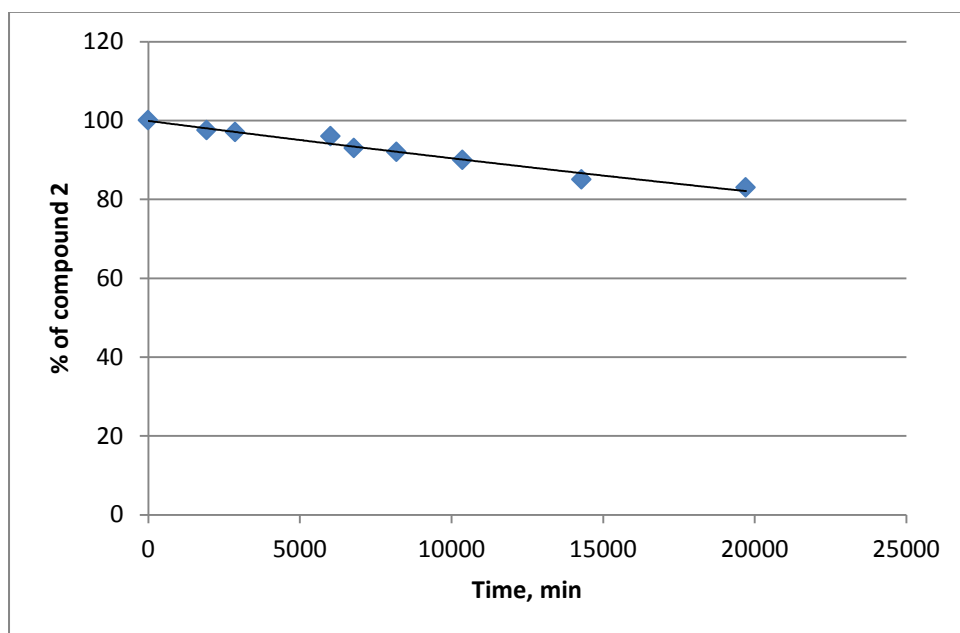| Equation	y0	A1	t1	R^2	Half-life, $t_{1/2}$, min	k_{obs}, min^{-1}	log(k_{obs})
$y = A1 \cdot \exp(-x/t1) + y0$	39,32703± 7,62041	58,542± 7,2248	11049,631± 2505,00153	0,99065	18802	3.686·10^{-5}	-4.433

Figure S44. Representative spectral data set for hydrolysis of **2**·HCl in phosphate D₂O buffer, pD7.95, 23 °C.

Kinetic data on the hydrolysis of 2·HCl in carbonate D_2O buffer, pD 9.45.

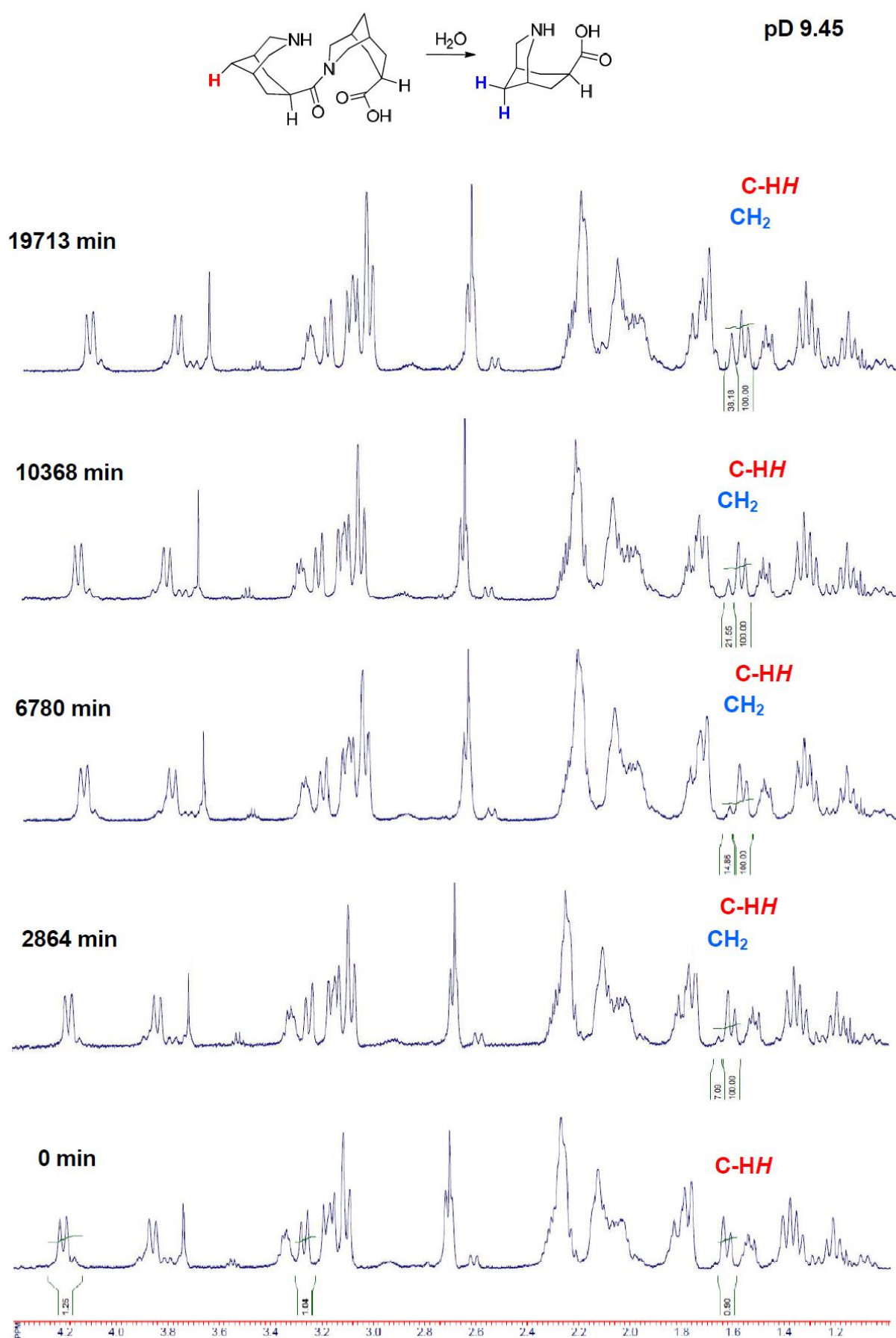**Table S9**. Molar percent of **2** vs time (min) in carbonate buffer (pD 9.45, 23 °C)

Time, min	0	1932	2864	6009	6780	8185	10368	14287	19713
Molar % of 2·HCl	100	97.5	97	96	93	92	90	85	83

Figure S45. Exponential fit of the data (Table S9).

Table S10. Fitting parameters and corresponding kinetic constants.

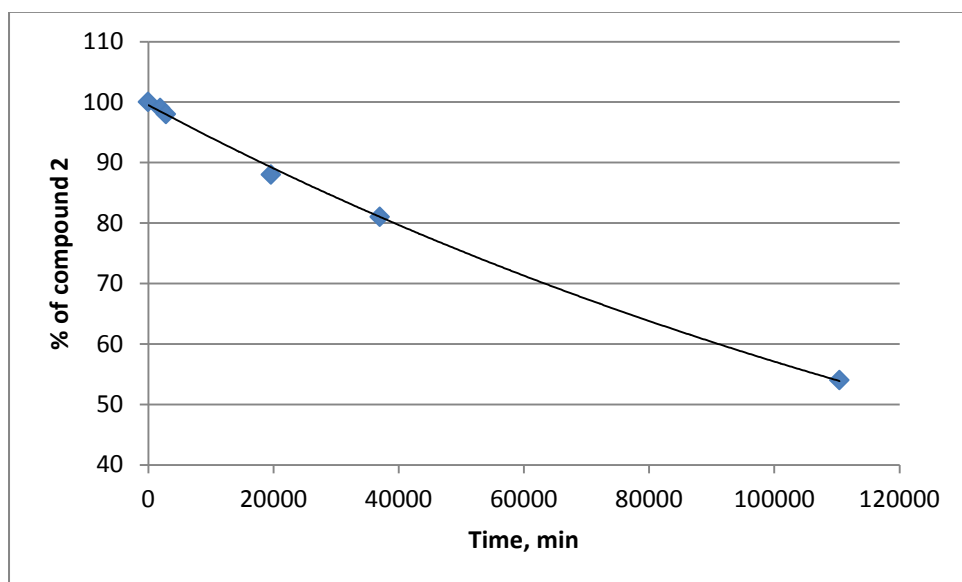| Equation	y0	A1	t1	R^2	Half-life, $t_{1/2}$, min	k_{obs}, min^{-1}	log(k_{obs})
$y = A1 \cdot exp(-x/t1) + y0$	37,98426± 69,45674	62,1325± 68,92624	58252,386± 76398,515	0,96279	91125	$7.605 \cdot 10^{-6}$	−5.119

Figure S46. Representative spectral data set for hydrolysis of **2**·HCl in phosphate D₂O buffer, pD 9.45, 23 °C.

Kinetic data on the hydrolysis of 2·HCl in carbonate D₂O buffer, pD 10.68.

Table S11. Molar percent of **2** vs time (min) in carbonate buffer (pD 10.68, 23 °C)

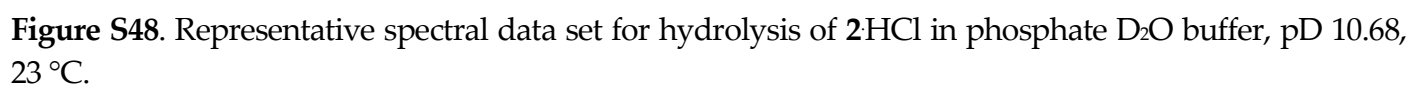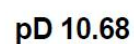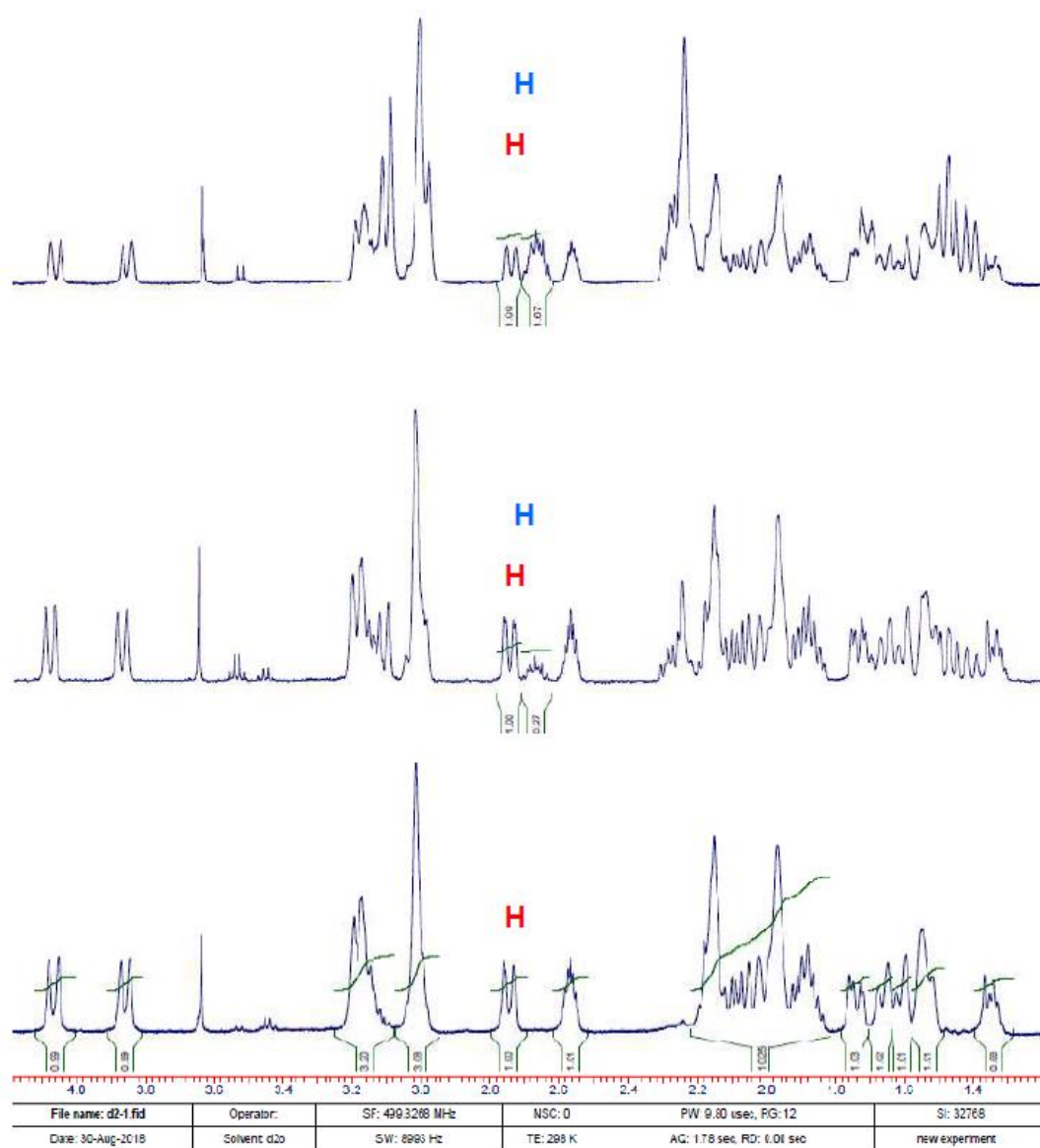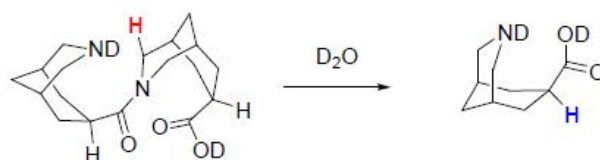| Time, min	0	1925	2863	19625
Molar % of 2·HCl	100	99	98	88

Figure S47. Exponential fit of the data (Table S11).

**Table S12**. Fitting parameters and corresponding kinetic constants.

Equation	y0	A1	t1	R^2	Half-life, $t_{1/2}$, min	k_{obs}, min⁻¹	log(k_{obs})
y = A1·exp(-x/t1) + y0	15,58528± 9,53246	84,25563± 9,34923	140851,4166± 23258,65209	0,9986	126114	~5.496·10⁻⁶	-5.260

Figure S48. Representative spectral data set for hydrolysis of **2**·HCl in phosphate D₂O buffer, pD 10.68, 23 °C.

Figure S49. Representative ^{1}H-NMR spectral data set for hydrolysis of **2**·HCl in D$_2$O (experiment run for estimation of the isotope kinetic effect), 23 °C.
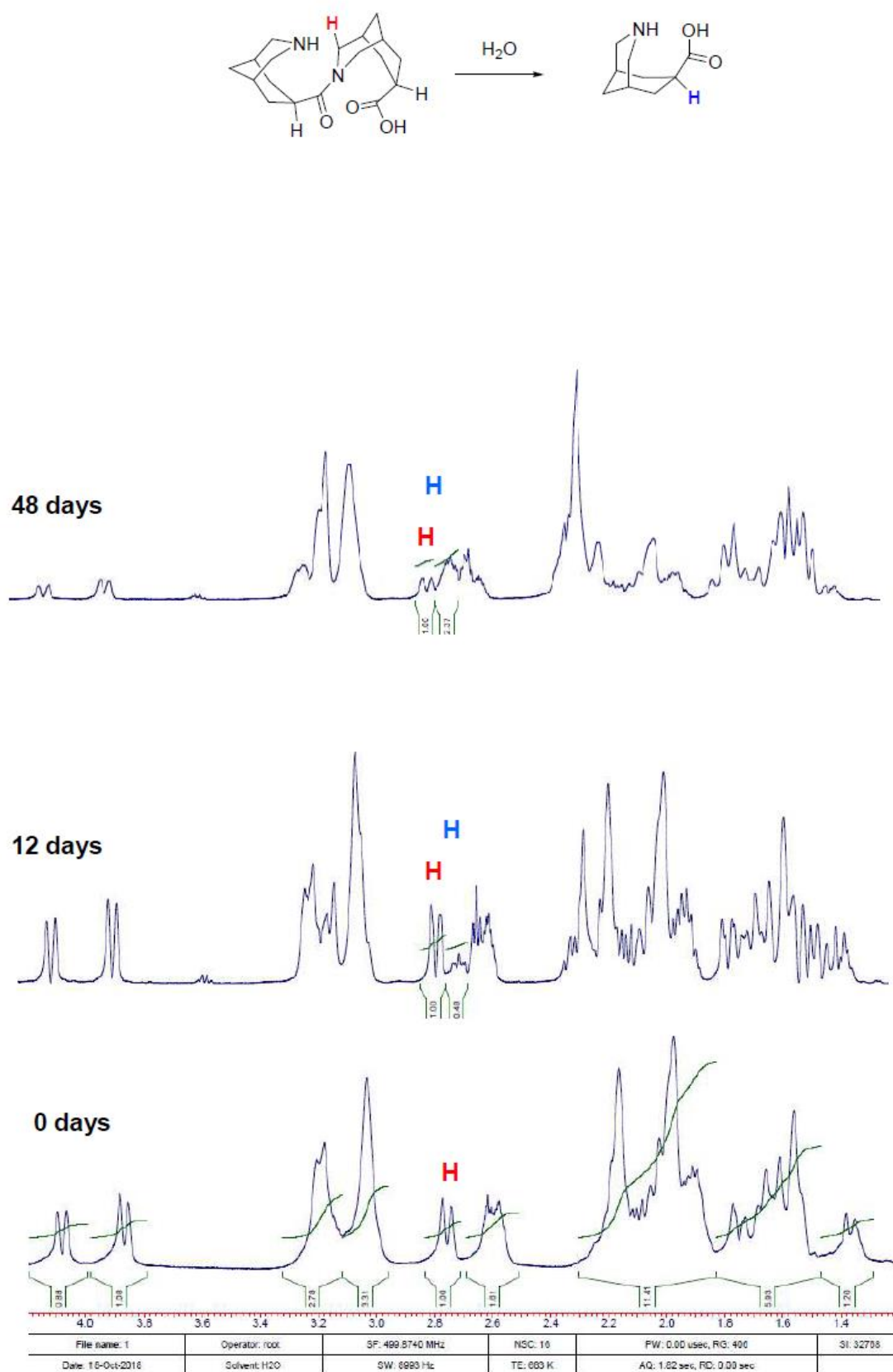
Figure S50. Representative ^{1}H-NMR spectral data set for hydrolysis of **2**·HCl in H$_2$O (experiment run for estimation of the isotope kinetic effect), 23 °C.