Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Desktop NMR for structure elucidation and identification of strychnine adulteration

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Abstract:

Elucidating the structure of complex molecules is difficult at low magnetic fields due to the overlap of different peak multiplets and second-order coupling effects. This is even more challenging for rigid molecules with small chemical shift differences and with prochiral centers. Since low-field NMR spectroscopy is sometimes presumed as restricted to the analysis of only small and simple molecules, this paper aims to counter this misconception: It demonstrates the use of low-field NMR spectroscopy in chemical forensics for identifying strychnine and its counterions by exploring the chemical shift as a signature in different 1D ^{1}H and ^{13}C experiments. Hereby the applied methodologies combined various 1D and 2D experiments such as 1D ^{1}H, ^{13}C, DEPT, and 2D COSY, HETCOR, HSQC, HMBC and *J*-resolved spectroscopy to elucidate the molecular structure and skeleton of strychnine at 1 Tesla. Strychnine was exemplified here, because it is a basic precursor in the chemistry of natural products and is employed as a chemical weapon and as a doping agent in sports such as the Olympics. In our study, the molecular structure of the compound could be identified either with a 1D experiment at high magnetic field or with HMBC and HSQC experiments at 1 T. In conclusion, low-field NMR spectroscopy enables the chemical elucidation of the strychnine structure

through a simple click of the computer mouse. In situations where a high-field NMR spectrometer is unavailable, compact NMR spectrometers can nevertheless generate knowledge of the structure, important for identifying the different chemical reaction mechanisms associated with the molecule. Desktop NMR is a cost-effective viable option in chemical forensics. It can prove adulteration and identify the origin of different strychnine salts, in particular, the strychnine free base, strychnine hemisulphate and strychnine hydrochloride. The chemical shift signatures help to look into the chemical structure of the molecules because of the impact of counterions in the chemical shift of the protons adjacent to the heteroatoms. This can serve as the methodology for structure elucidation of complex molecules at lower-magnetic fields.

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1. Experimental Procedures

Each sample of strychnine with its counterions was prepared using 0.5 ml of solvent $CDCl_3$, D_2O or CD_3OD. The amount of sample depended on the solubility of the sample in the particular solvent (Tab. S-1). In order to compare the results, each sample was measured with a 1Tesla instrument from Magritek (Fig. S-1) and with a Bruker 9.4Tesla spectrometer.

Fig. S-1. 1 Tesla Compact NMR spectrometer from Magritek, Wellington.

Tab. S-1. Solubility of strychnine, strychnine hydrochloride and strychnine hemisulphate in different solvents.

Solvent	Strychnine (mM)	Strychnine hydrochloride (mM)	Strychnine hemisulphate (mM)
$CDCl_3$	135	27	26
D_2O	Not Soluble	67	65
CD_3OD	30	67	65

Results and Discussion

2. 1D NMR spectroscopy of strychnine

2.1. 1D ^{1}H NMR spectrum of strychnine at 9.4 Tesla

A 135 mM solution of strychnine was prepared in $CDCl_3$ and measured at 9.4 Tesla with 16 scans and a repetition time of 5.6 seconds for obtaining a 1D ^{1}H NMR spectrum (Fig. S-2). All proton peaks were assigned according to the literature.[1] Since peaks 17a, 17b, 18a, 18b did not show any clear multiplet structure, it was not possible to find the coupling constants for these.

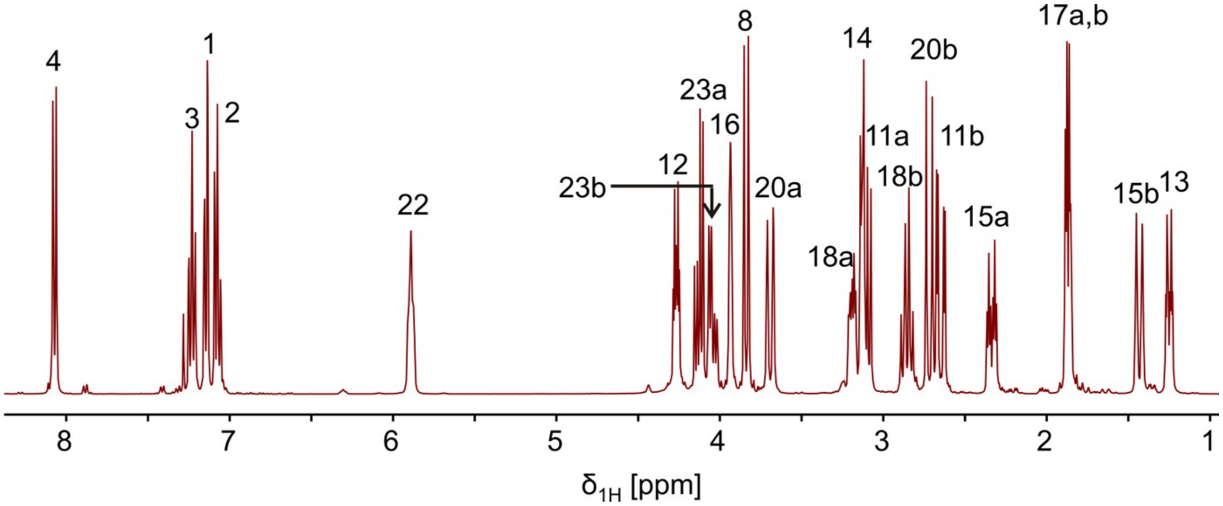

Fig. S-2. 1D ^{1}H NMR spectrum of 135 mM of strychnine in $CDCl_3$ obtained at 9.4 Tesla with 16 scans and a repetition time of 5.6 seconds.

2.2. 1D ^{13}C NMR spectrum of strychnine and strychnine hydrochloride at 9.4 Tesla

A 135 mM solution of strychnine and a 27 mM solution of strychnine hydrochloride were prepared in $CDCl_3$ and measured at 9.4 Tesla with 1024 scans and a repetition time of 1.75 seconds for obtaining a 1D ^{13}C NMR spectrum (Fig. S-3a). All peaks were assigned according to the literature.[1] Clear differences in the chemical shifts of the peaks belonging to carbons 3, 6, 10, 15, 16, 17, 21 and 22 (Tab. S-2) can be seen. These differences agree with those determined from the 1D ^{13}C spectrum obtained at 1 Tesla.

3. 2D NMR spectroscopy of strychnine

3.1. 2D HETCOR (Heteronuclear Correlation) spectroscopy

In the 2D ^{13}C-^{1}H HETCOR experiment, the transverse ^{1}H magnetization is generated and transferred to ^{13}C after the evolution time. Thus, ^{1}H-modulated ^{13}C magnetization is detected. This experiment unravels the resonances of a complex second-order 1D ^{1}H spectrum by correlating them with the directly attached ^{13}C nucleus. The 2D HETCOR spectrum (Fig. S-4) of strychnine (135 mM) in $CDCl_3$ was recorded at 1 Tesla with 128 scans for each of the 64 steps in 22.7 hours. Each carbon coupled to protons can be identified by cross peaks, simplifying the peak assignment in the 1D ^{1}H spectrum. The peaks of the aromatic protons 1, 2, 3, 4 can be easily correlated with their corresponding carbon atoms and visualized directly without cumbersome analysis of the 1D ^{1}H spectrum. The diastereomeric protons 11a and 11b can also be distinguished. The peaks 16 and 8 at the very close chemical shift of 3.94 and 3.86 ppm (Fig. S-2) can be distinguished although they slightly overlap in the 2D spectrum. Similarly, the other carbon resonances can be correlated with the corresponding resonances in the proton spectrum on the other axis.

3.2. 2D HSQC (Homo-nuclear Single Quantum Correlation) Spectroscopy

The signal intensity of the ^{13}C-^{1}H HETCOR spectrum is lower than that of the HSQC spectrum, because ^{1}H-modulated ^{13}C magnetization is detected in the HETCOR experiment whereas in the HSQC experiment, ^{13}C magnetization is transferred to ^{1}H and detected indirectly via ^{1}H. The 2D HSQC NMR spectrum of strychnine (Fig. S-5) was recorded at 9.4 T. The correlation between the different protons and carbons were observed which confirms the resonance of the nuclei in the 2D HSQC spectrum obtained at 1 T.

3.3. 2D HMBC (Homo-nuclear Multiple Bond Correlation) Spectroscopy

The 2D HMBC experiment provides long-range correlations with non-protonated carbon atoms. It has the benefit of better elucidating the structures of the chemical groups. One-bond correlations can provide useful structural information by proton assignments along with long-range ^{1}H-^{13}C correlations across carbon-carbon linkages, which define the molecular skeleton. The 2D HMBC NMR spectrum of strychnine (Fig. S-6) was recorded at 9.4 T. The correlation between the different protons and carbons confirms the resonances of the nuclei in the 2D HMBC spectrum obtained at 1 T.

3.4. 2D ^{1}H-^{1}H COSY (Correlation Spectroscopy)

In the homonuclear COSY spectrum, both frequency axes contain the same chemical shift information, and cross peaks arise between coupled spins. The ^{1}H-^{1}H COSY spectrum of strychnine (Fig. S-7) was measured at 1 Tesla within 38 minutes. The assignment of resonances based on cross peaks in the COSY spectrum of strychnine is challenging, generally due to resonance overlap but can be readily accomplished for the aromatic protons 1,2,3 and 4 because their chemical shifts are in the less crowded region between 8.5 and 7 ppm. The couplings of proton 22 are distinct because of the sp^2 hybridized carbon atom and its neighboring protons 23a and 23b. They are easy to identify by means of cross and diagonal peaks. The couplings of proton 12 with protons 11a and 11b were assigned with the aid of the HETCOR experiment in agreement with the ^{1}H-^{1}H COSY spectrum. Proton 12 has the highest chemical shift followed by proton 22, due to its neighboring electronegative oxygen atom, which deshields proton 12, shifting its resonance to higher ppm value. The chemical shifts of protons 16 and 8 are near to those of protons 23a and 23b because of the adjacent N atom. The spin-spin couplings of proton 16 with protons 15b and 15a lead to the assignment of the latter. Moreover, the coupling of protons 8 and 13 was found by the spin-spin correlation revealed in the COSY spectrum. Subsequently, the couplings of proton 15a and 15b with proton14 were identified. The couplings of protons 18a and 18b with protons 17a and 17b as well as of 20a with 20b were identified by means of HETCOR and HSQC spectra. The ^{1}H-^{1}H COSY spectrum recorded at 9.4 Tesla (Fig. S-8) correlates well with the COSY spectrum recorded at 1 Tesla. The combination of HETCOR and HSQC spectra gives the single-bond correlations, which can be further confirmed and identified with COSY experiments, whereas multiple bond correlations from the HMBC spectrum give the linkages of the molecular skeleton.

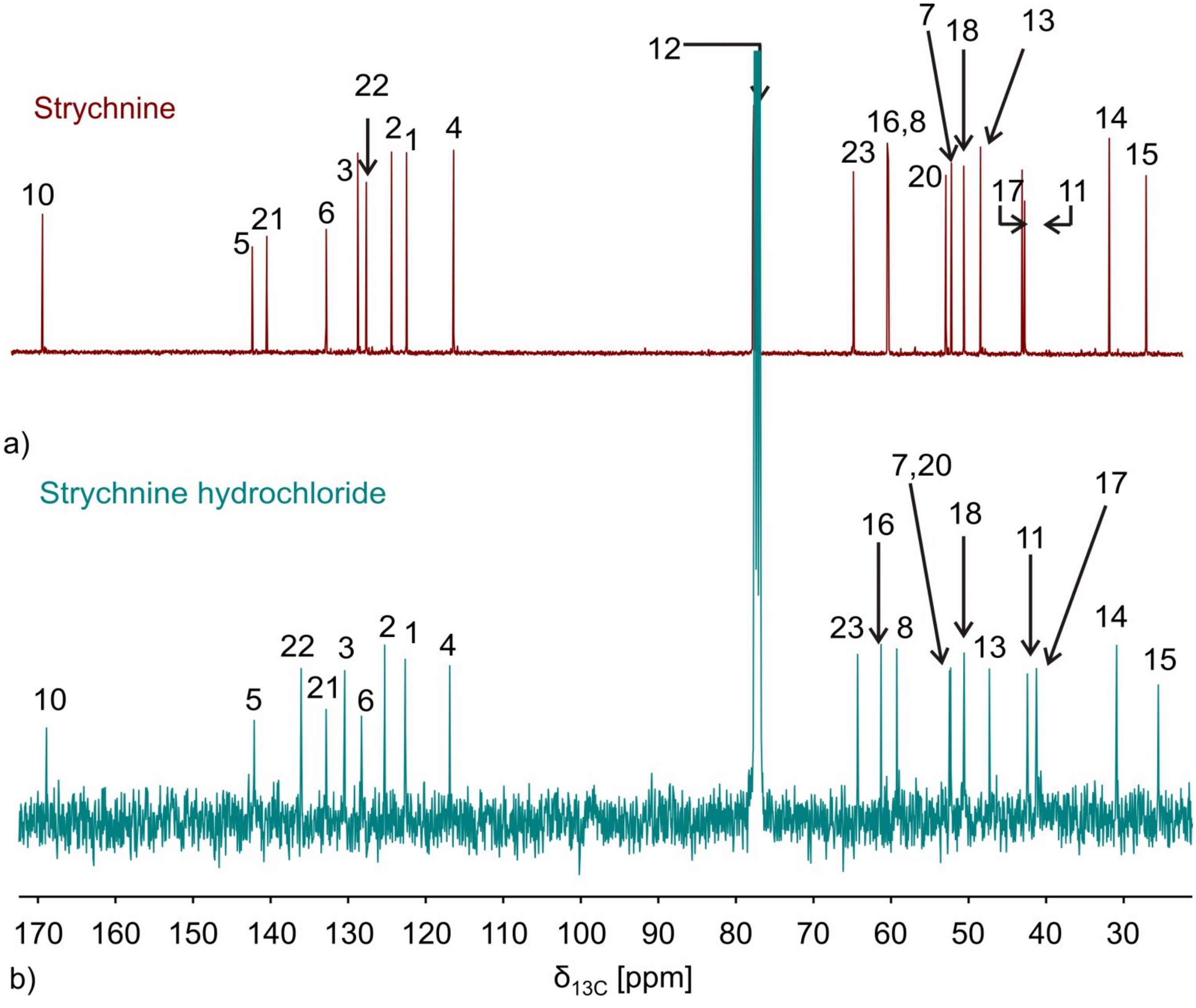

Fig. S-3. a) 1D ^{13}C NMR spectrum of a) Strychnine (135 mM) b) Strychnine hydrochloride (27 mM) in $CDCl_3$ obtained at 9.4 Tesla with 1024 scans and a repetition time of 1.75 seconds.

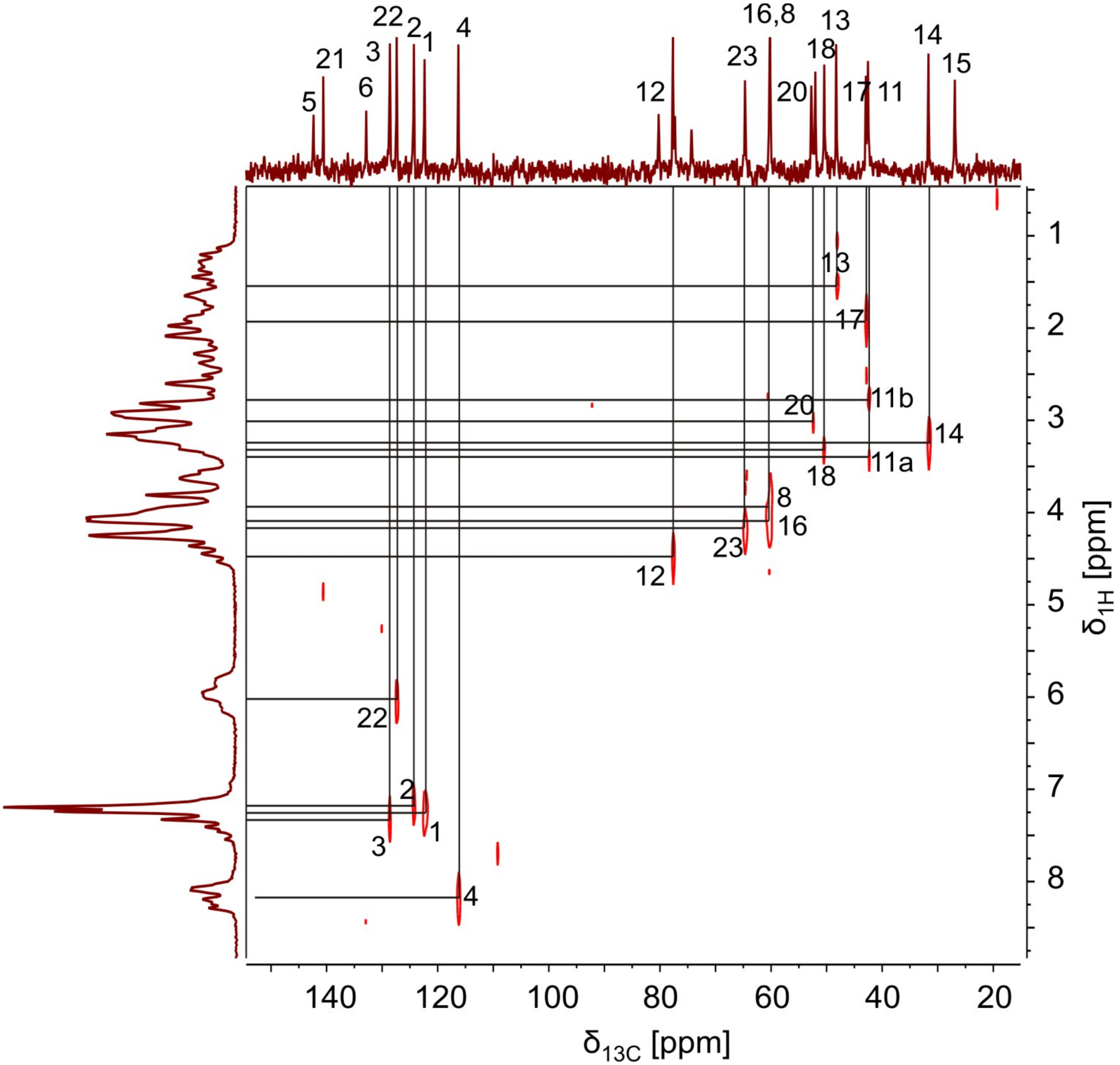

Fig. S-4. 2D ^{13}C-^{1}H HETCOR spectrum of strychnine recorded at 1 Tesla with 128 scans in 22.7 hours.

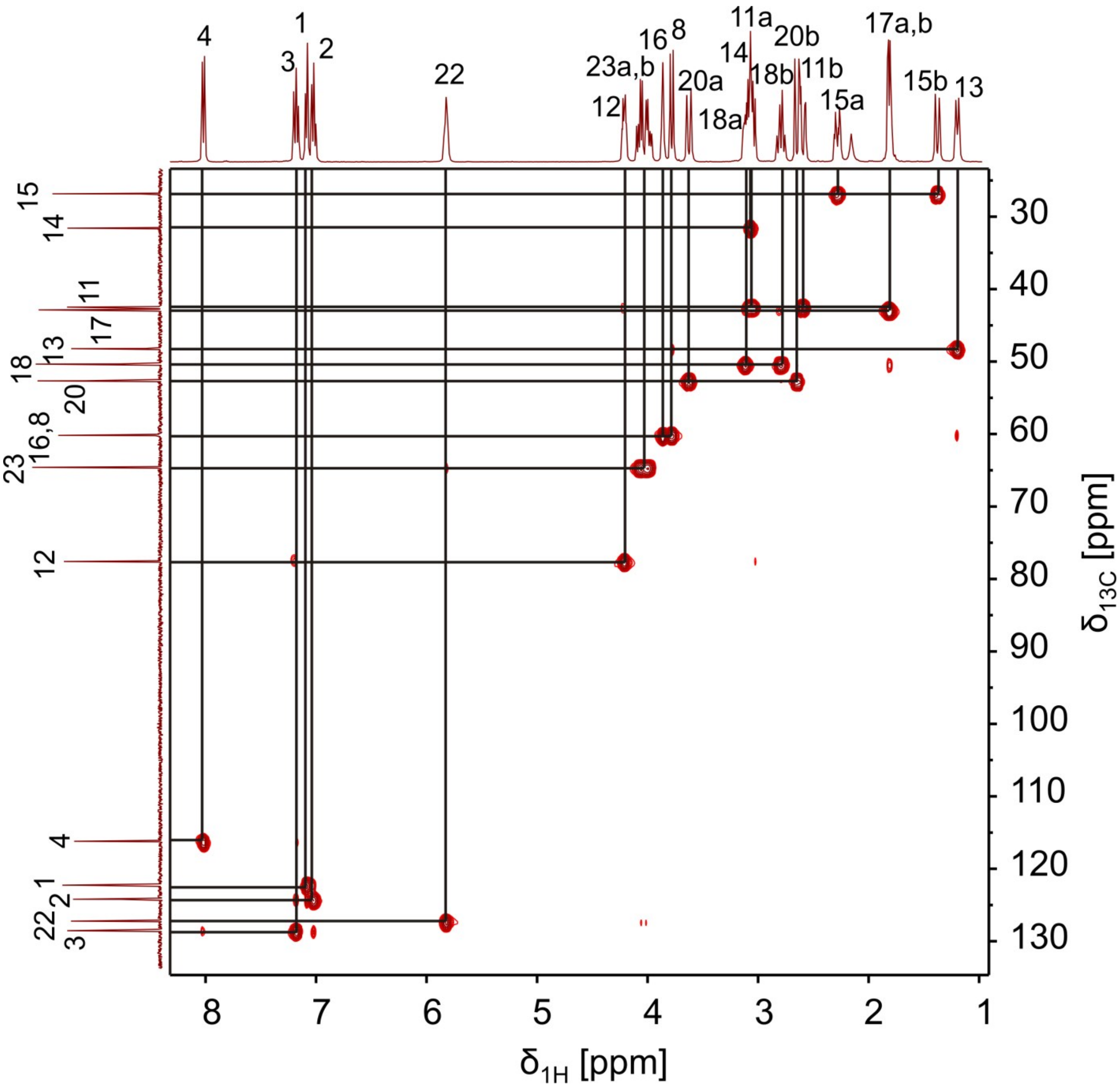

Fig. S-5. 2D HSQC spectrum of strychnine (135 mM) dissolved in $CDCl_3$ recorded at 9.4 Tesla

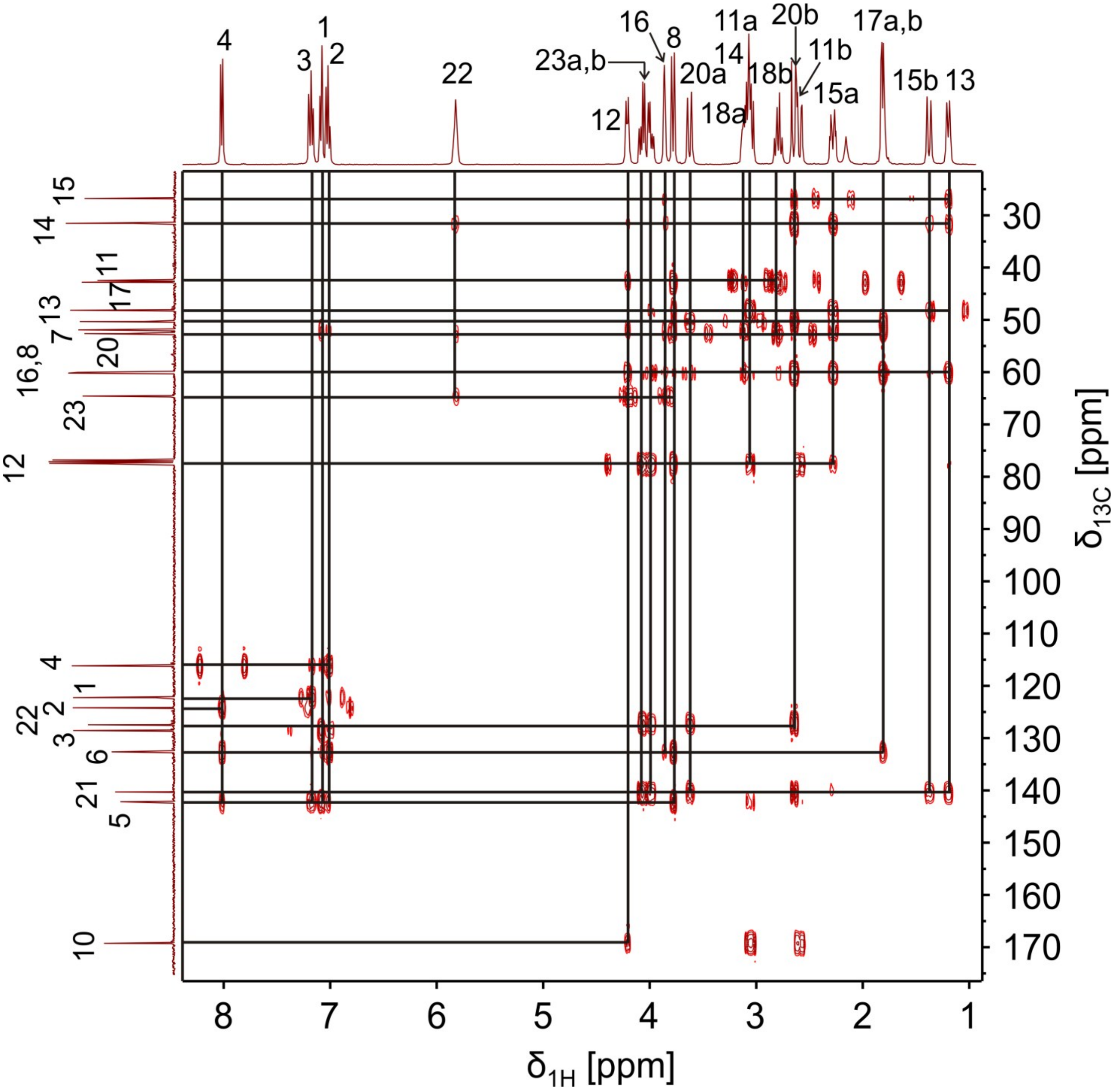

Fig. S-6. 2D HMBC NMR spectrum of strychnine measured at 9.4 Tesla.

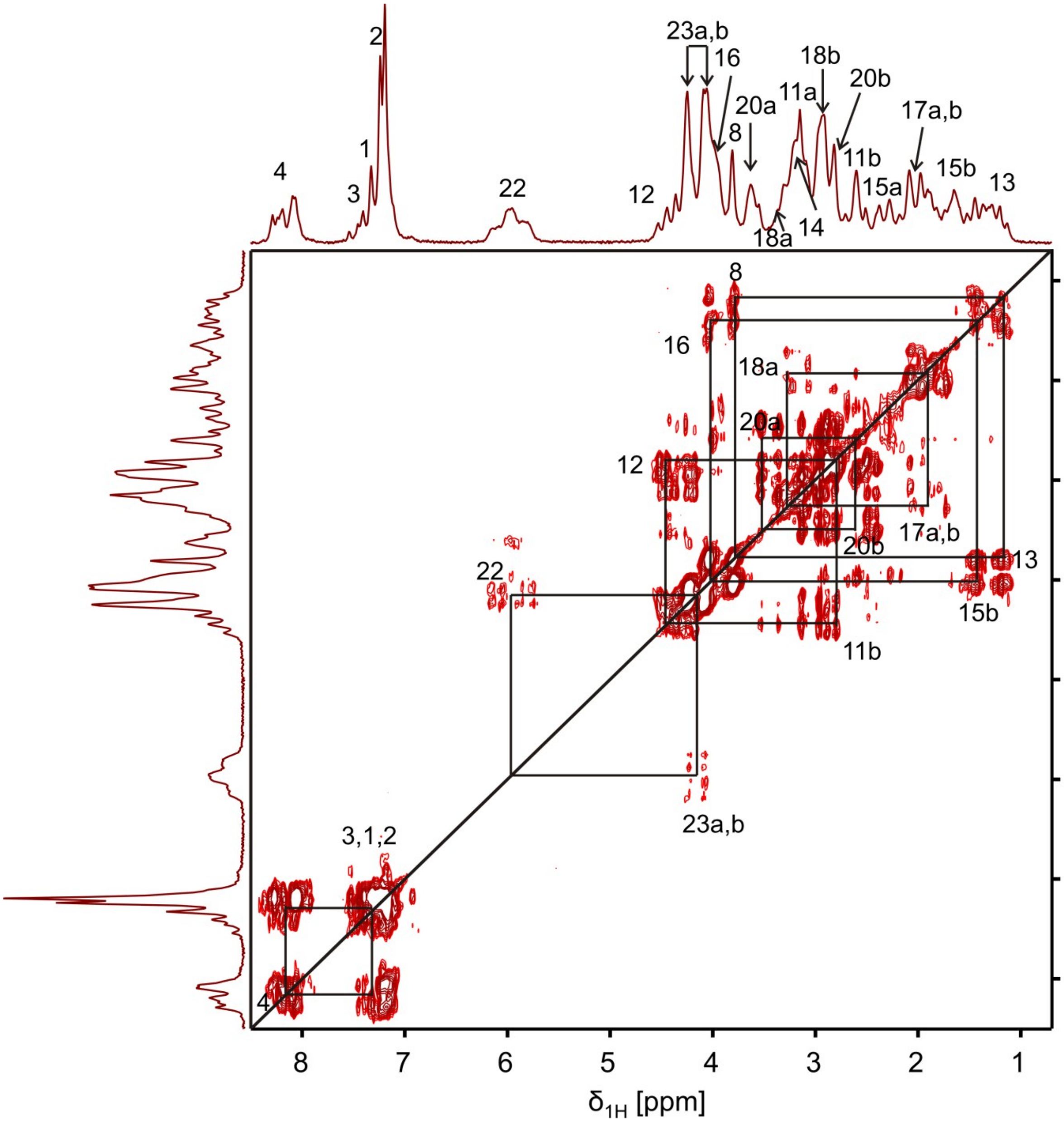

Fig. S-7. 2D ^{1}H-^{1}H COSY spectrum of strychnine measured in 38 minutes at 1 Tesla.

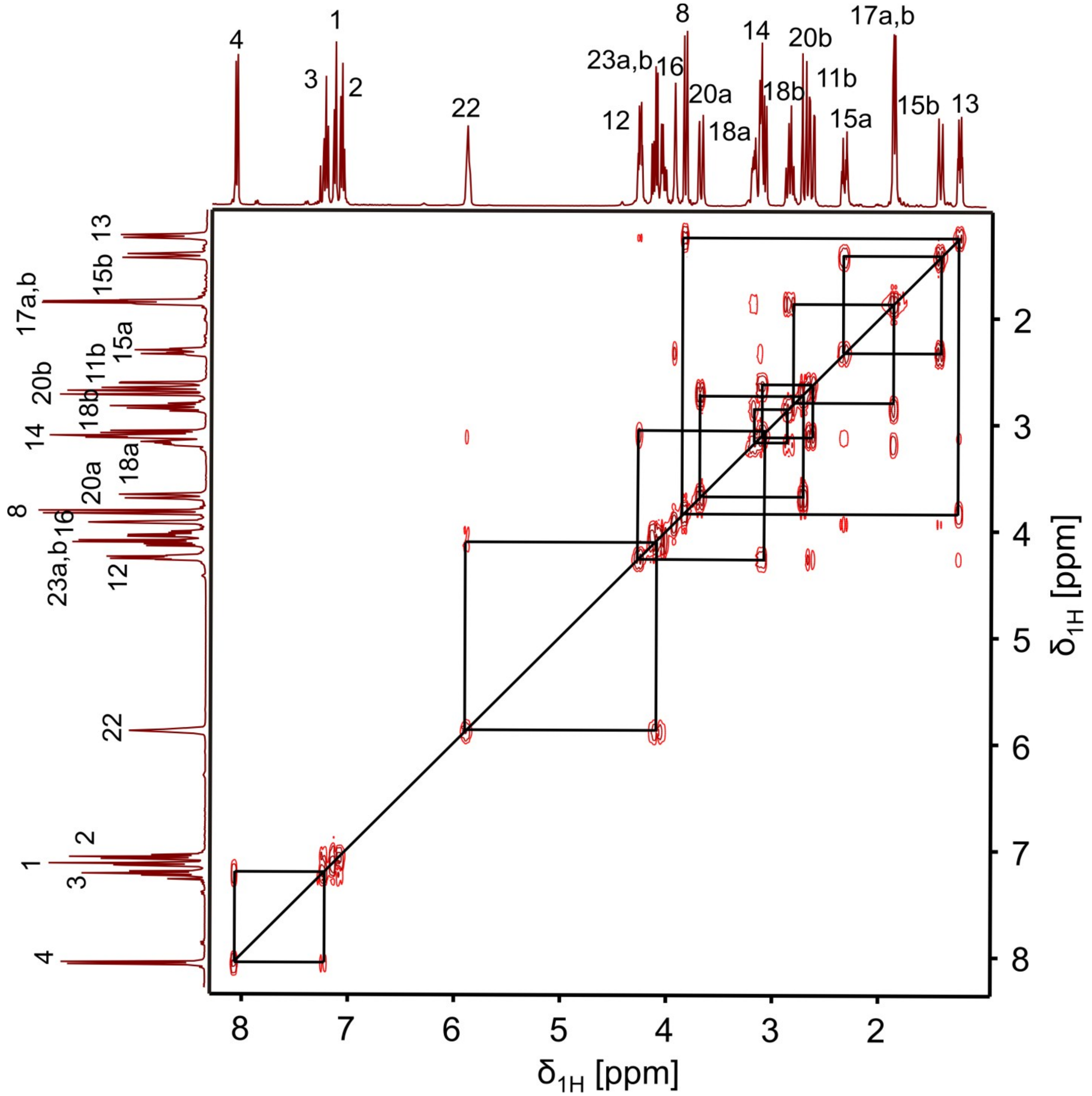

Fig. S-8. 2D ^{1}H-^{1}H COSY spectrum of strychnine measured at 9.4 Tesla.

4. Counterion identification of strychnine with chemical shift as a signature

4.1. Distinction of strychnine and its counterions in $CDCl_3$

4.1.1. 1D ^{1}H NMR spectroscopy of strychnine and its salts

^{1}H NMR spectra of strychnine, strychnine hydrochloride and strychnine hemisulphate recorded at 1 (Fig. S-9) and 9.4 Tesla (Fig. S-10). Solutions of strychnine free base and of its two salts, strychnine hemisulphate and strychnine hydrochloride, were prepared by dissolving 135 mM of strychnine and approximately 26 mM concentrations of the other two salts in 0.5 ml of $CDCl_3$.

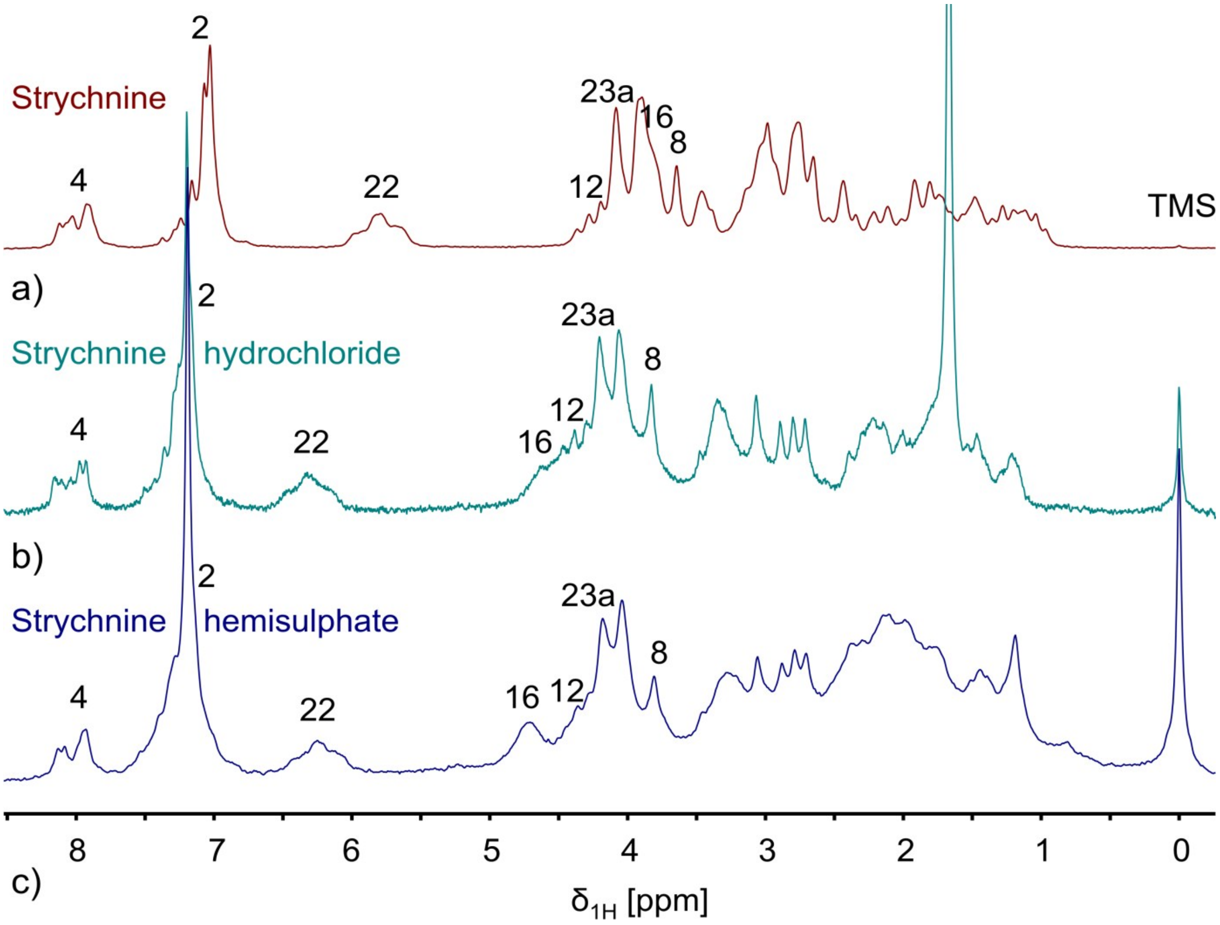

Fig. S-9. 1D ^{1}H NMR spectra of the different samples in $CDCl_3$ obtained at 1 Tesla. a) Strychnine (135 mM), 16 scans. b) Strychnine hydrochloride (26 mM), 256 scans. c) Strychnine hemisulphate (26 mM), 256 scans.

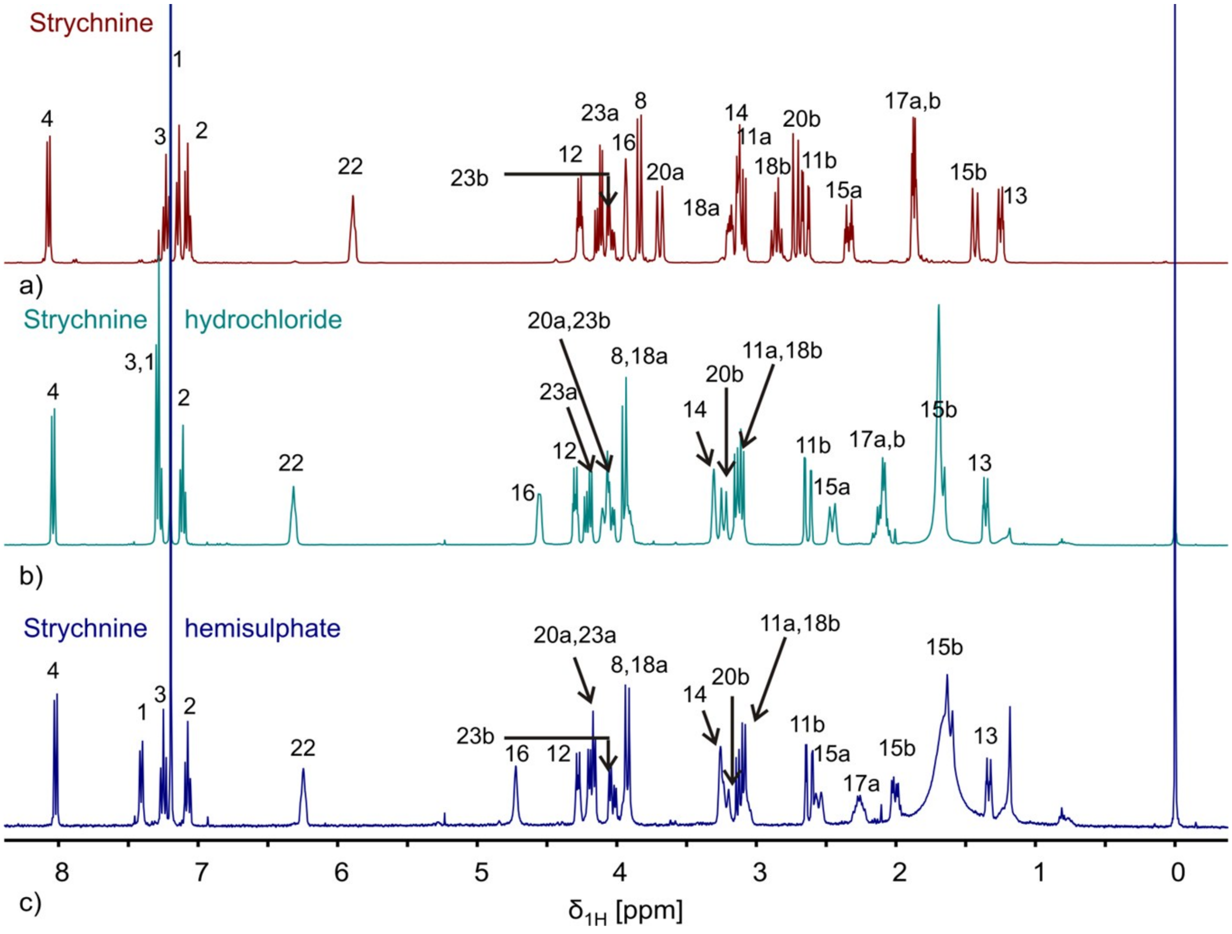

Fig. S-10. 1D ^{1}H NMR spectra of the different samples in $CDCl_3$ obtained at 9.4 Tesla. a) Strychnine (135 mM), 16 scans. b) Strychnine hydrochloride (26 mM), 256 scans. c) Strychnine hemisulphate (26 mM), 256 scans.

4.1.2. 1D ^{13}C NMR spectroscopy of strychnine and its salts

The same solutions which were used for obtaining the 1D ^{1}H NMR spectra were used for obtaining the 1D ^{13}C NMR spectra at 9.4 Tesla (Fig. S-3).

Tab. S-2. ^{13}C chemical shifts [ppm] of strychnine (135 mM) and strychnine hydrochloride (27 mM), dissolved in $CDCl_3$, measured at 1 Tesla. The red peaks show significant differences in the chemical shifts of both compounds.

Carbon	Strychnine	Strychnine Hydrochloride
1	122.50	122.84
2	124.43	125.48
3	128.76	130.65
4	116.43	117.14
5	142.47	142.61
6	133.00	129.85
7	52.16	52.51
8	60.41	59.46
10	169.50	166.68
11	42.68	42.56
12	77.42	77.94
13	48.41	47.52
14	31.80	31.10
15	27.05	25.70
16	60.41	61.46
17	43.08	41.40
18	50.55	50.75
20	52.88	52.51
21	140.73	133.09
22	127.52	136.24
23	64.82	64.46

Tab. S-3. ^{1}H chemical shifts [ppm] of strychnine (135 mM), strychnine hydrochloride (27 mM) and strychnine hemisulphate (26 mM) in $CDCl_3$ measured at 1 Tesla

Proton	Strychnine	Strychnine hydrochloride	Strychnine hemisulphate
22	5.82	6.34	6.25
16	3.93	4.65	4.74
8	3.65	3.83	3.81
2	7.08	7.16	7.13
12	4.28	4.39	4.39
23a	4.09	4.22	4.18

Tab. S-4. ^{1}H chemical shifts [ppm] of strychnine (135 mM), strychnine and strychnine hydrochloride (10 mM), and strychnine and strychnine hemisulphate (10 mM) in $CDCl_3$ measured at 1 Tesla

Proton	Strychnine	Strychnine + Strychnine hydrochloride	Strychnine + Strychnine hemisulphate
22	5.82	5.93	5.86
20a	3.48	3.62	3.54
20b	2.46	2.57	2.50
8	3.65	3.68	3.66
2	7.08	7.08	7.08
12	4.28	4.31	4.29
23a	4.09	4.11	4.10

4.2. Distinction of strychnine and its salts in CD_3OD

4.2.1. 1D ^{1}H NMR spectroscopy of strychnine and its salts

Solutions of the strychnine free base (30 mM) and the two salts, strychnine hemisulphate (65 mM) and strychnine hydrochloride (67 mM), were prepared in 0.5 ml of CD_3OD. Each salt was measured with 4 scans at 1 Tesla and the strychnine base with 64 scans. The 1D ^{1}H NMR spectra of strychnine (Fig. S-11) and its salts show the chemical differences in terms of chemical shift (Table S-5).The results were compared with the spectra for the same samples obtained at 9.4 T (Fig. S-12).

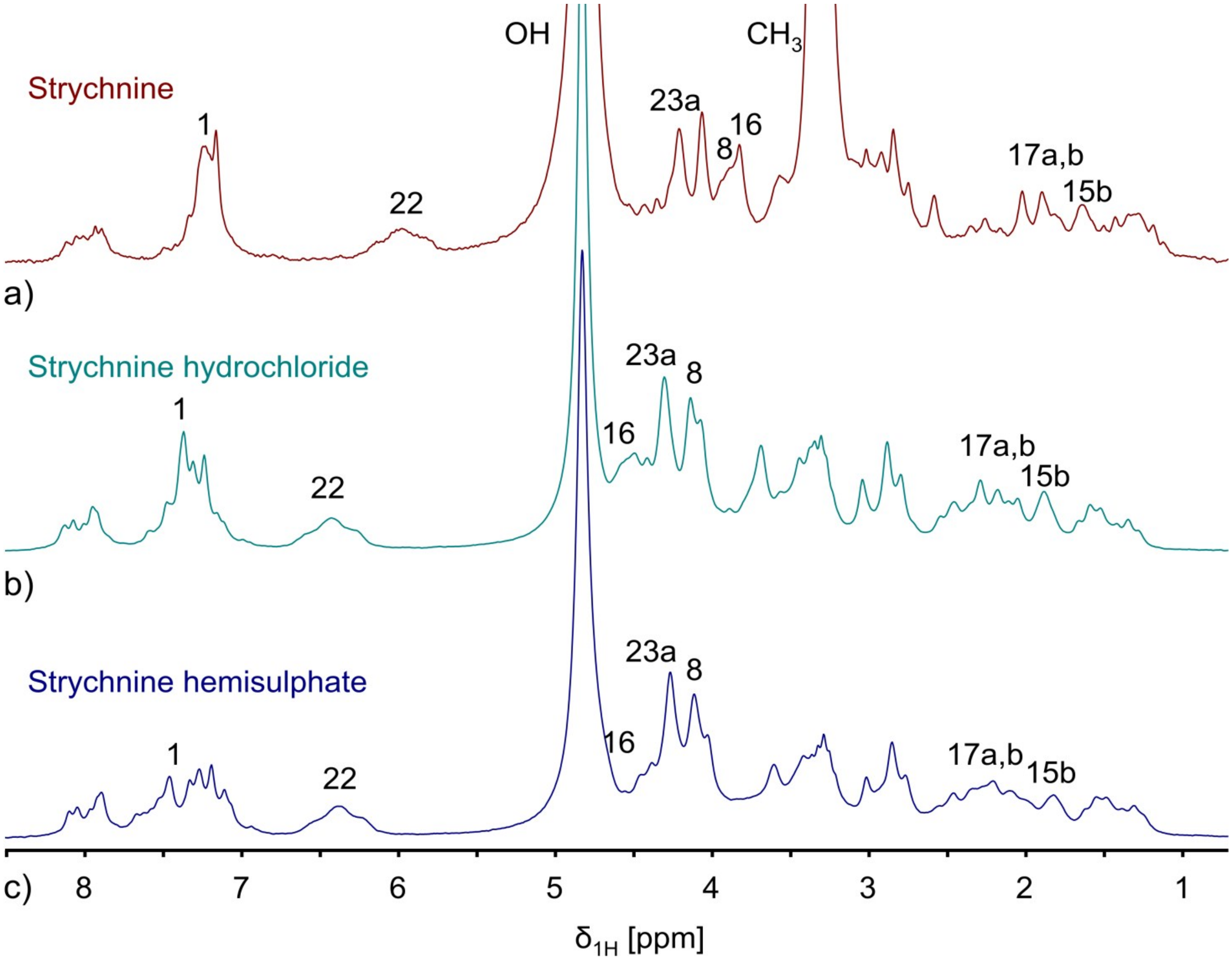

Fig. S-11. 1D ^{1}H NMR spectra obtained at 1 Tesla in 0.5 ml of CD$_3$OD. a) Strychnine (30 mM). b) Strychnine hydrochloride (67 mM), 4 scans c) Strychnine hemisulphate (65 mM), 4 scans

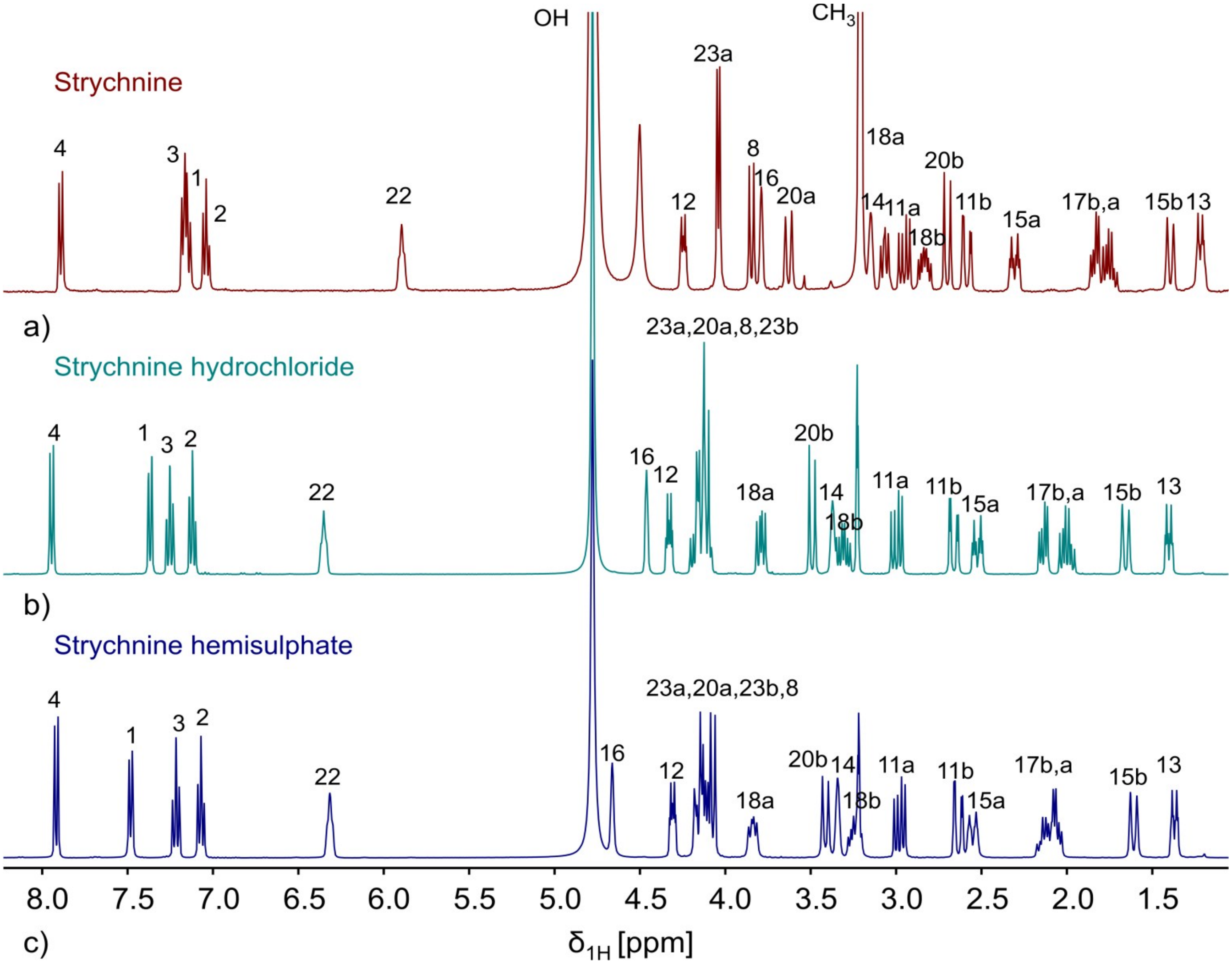

Fig. S-12. 1D ^{1}H NMR spectra of the different samples in CD$_3$OD obtained at 9.4 Tesla with 16 scans. a) Strychnine (27 mM). b) Strychnine hydrochloride (65 mM). c) Strychnine hemisulphate (65 mM).

4.2.2. 1D ^{13}C NMR spectroscopy of strychnine and its salts

The same solutions which were used for obtaining the 1D ^{1}H NMR spectra were used for obtaining the 1D ^{13}C NMR spectra at 1 (Fig. S-13) and 9.4 Tesla (Fig. S-14).The results showed the differences in the chemical shift of certain carbon atoms summarized in the Tab. S-6.

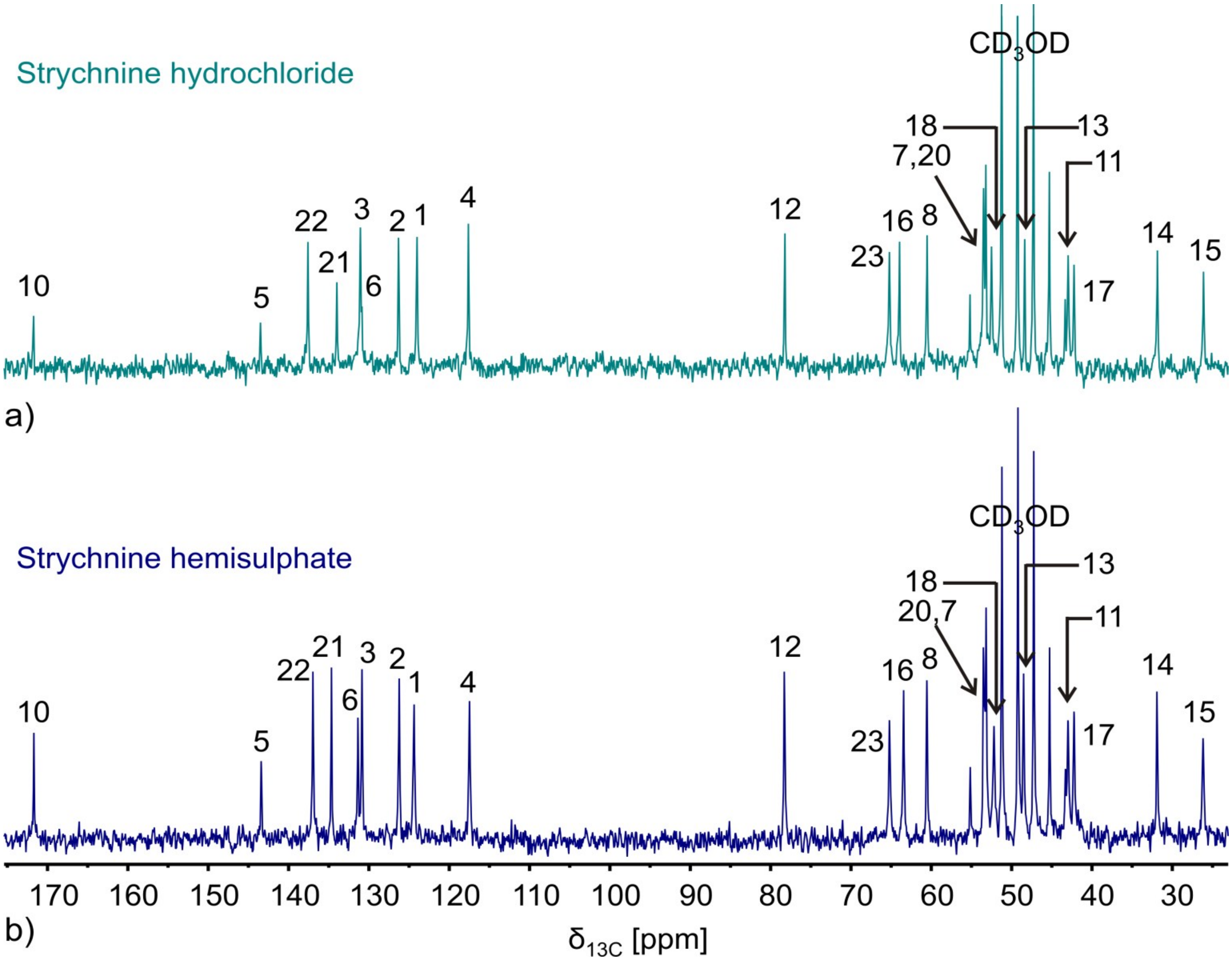

Fig. S-13. 1D ^{13}C NMR spectra obtained at 1 Tesla in 0.5 ml of CD$_3$OD. a) Strychnine hydrochloride (67 mM), 4096 scans. b) Strychnine hemisulphate (65 mM), 4096 scans.

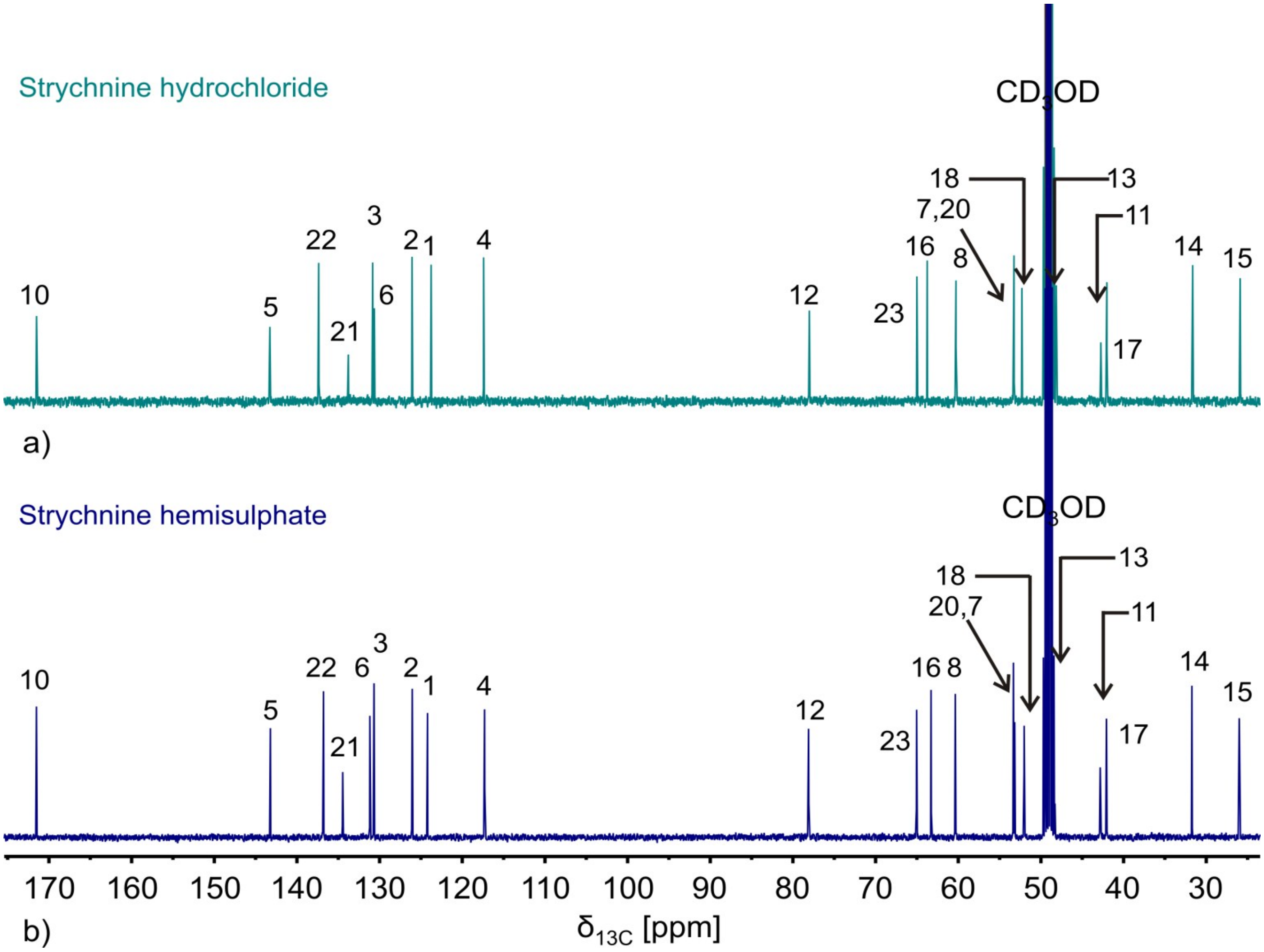

Fig. S-14. 1D ^{13}C NMR spectra of a) Strychnine hydrochloride (67 mM) and b) Strychnine hemisulpahte (65 mM) in 0.5 ml of CD_3OD obtained at 9.4 Tesla with 1024 scans and a repetition time of 1.75 seconds.

Tab. S-5. ^{1}H chemical shifts [ppm] of strychnine (30 mM) and strychnine hydrochloride (67 mM) and strychnine hemisulphate (65 mM) in CD_3OD measured at 1 Tesla

Proton	Strychnine	Strychnine hydrochloride	Strychnine hemisulphate
22	5.98	6.44	6.39
8	3.84	4.20	4.20
16	3.80	4.56	4.56
23a	4.21	4.31	4.28

Tab. S-6. ^{13}C chemical shifts of strychnine hydrochloride and strychnine hemisulphate, dissolved in CD_3OD, measured at 1 Tesla. The red peaks depict significant differences in the chemical shifts of both compounds. The blue peaks show alternate chemical shifts in both compounds.

Carbon	Strychnine hydrochloride	Strychnine hemisulphate
1	123.96	124.39
2	126.26	126.23
3	131.03	130.87
4	117.57	117.49
5	143.47	143.40
6	130.84	131.36
7	53.17	53.36
8	60.48	60.55
10	171.70	171.70
11	42.93	42.99
12	78.19	78.29
13	48.34	48.51
14	31.81	31.91
15	26.08	26.19
16	63.92	63.46
17	42.21	42.23
18	52.47	52.19
20	53.45	53.18
21	133.95	134.66
22	137.55	136.97
23	65.19	65.22

4.3. Analysis of strychnine salts dissolved in D_2O

4.3.1. 1D ^{1}H NMR spectroscopy

As strychnine is insoluble in D_2O, solutions of the two salts strychnine hemisulphate (65 mM) and strychnine hydrochloride (67 mM) were prepared in 0.5 ml D_2O. For each sample ^{1}H spectrum was measured with 4 scans at 1 Tesla. The 1D ^{1}H NMR spectra

(Fig. S-15) do not show any differences. This was confirmed by recording the 1D ^{1}H (Fig. S-16) NMR spectra of strychnine hydrochloride and strychnine hemisulphate at 9.4 Tesla.

4.3.2. 1D ^{13}C NMR spectroscopy

The same samples studied by ^{1}H NMR spectroscopy were measured with 4096 scans to record their ^{13}C spectra at 1 Tesla (Fig. S-17). The ^{13}C spectra of both salts do not show significant differences (Tab. S-7) even at high field (Fig. S-18). This is due to the fact that the solvation of the cations and anions in the salts of strychnine by D_2O molecules reduces the electrostatic interactions of the cations with the anions.

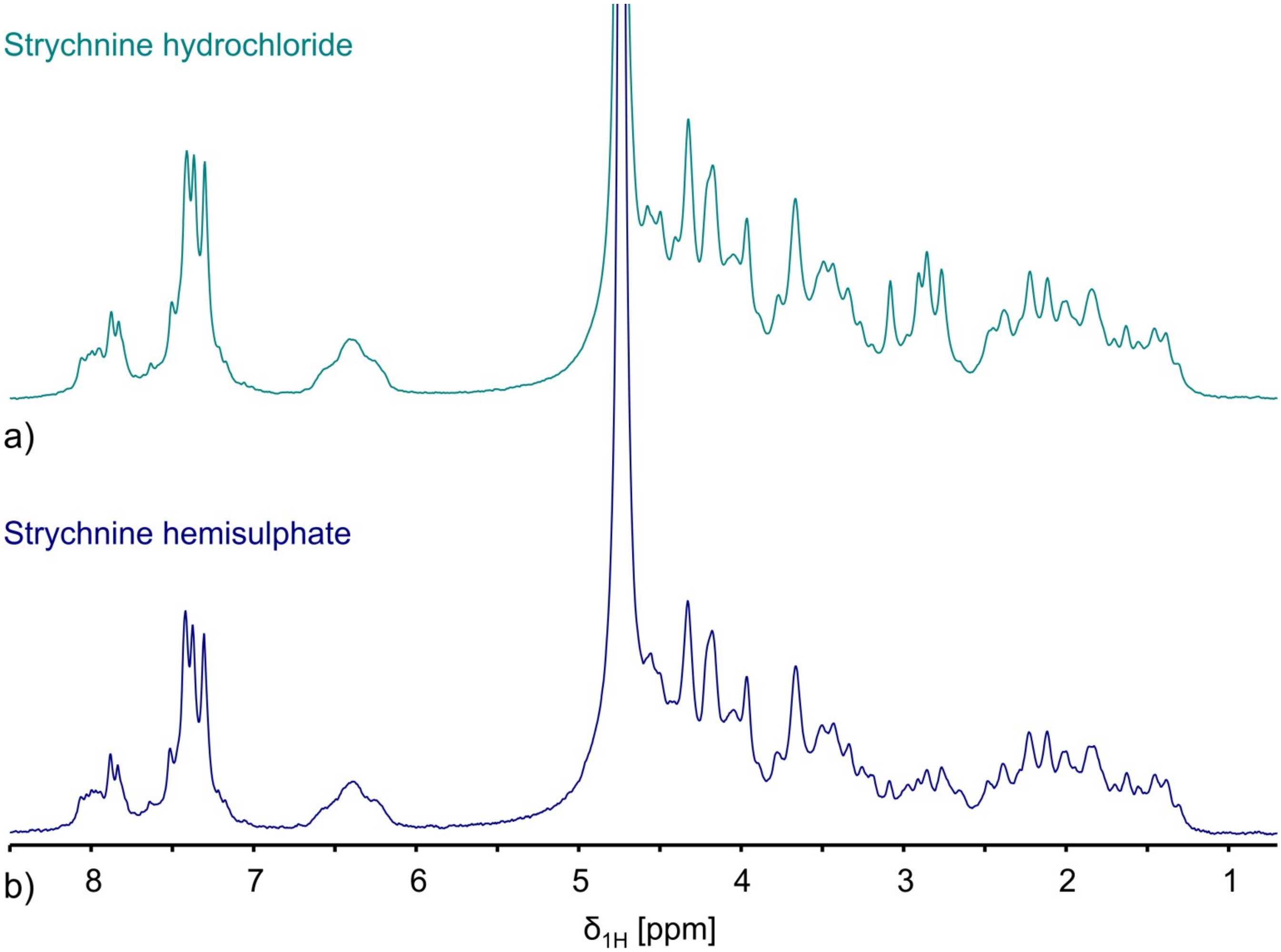

Fig. S-15. 1D ^{1}H NMR spectrum obtained at 1 Tesla in 0.5 ml of D_2O. a) Strychnine hydrochloride (67 mM). b) Strychnine hemisulphate (65 mM).

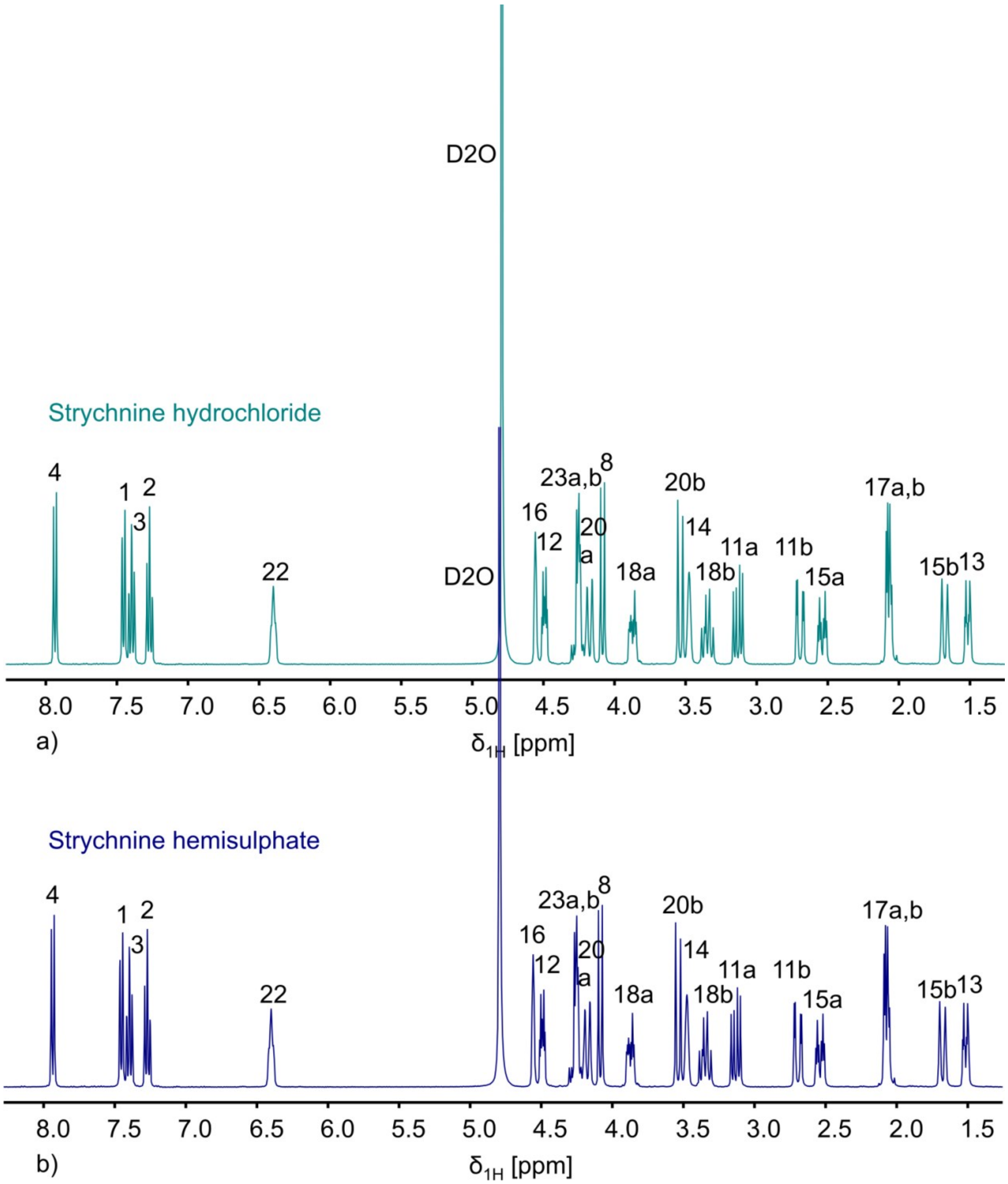

Fig. S-16. 1D ^{1}H NMR spectra of a) Strychnine hydrochloride (65 mM) and b) Strychnine hemisulphate (65 mM) obtained at 9.4 Tesla in 0.5 ml of D_2O.

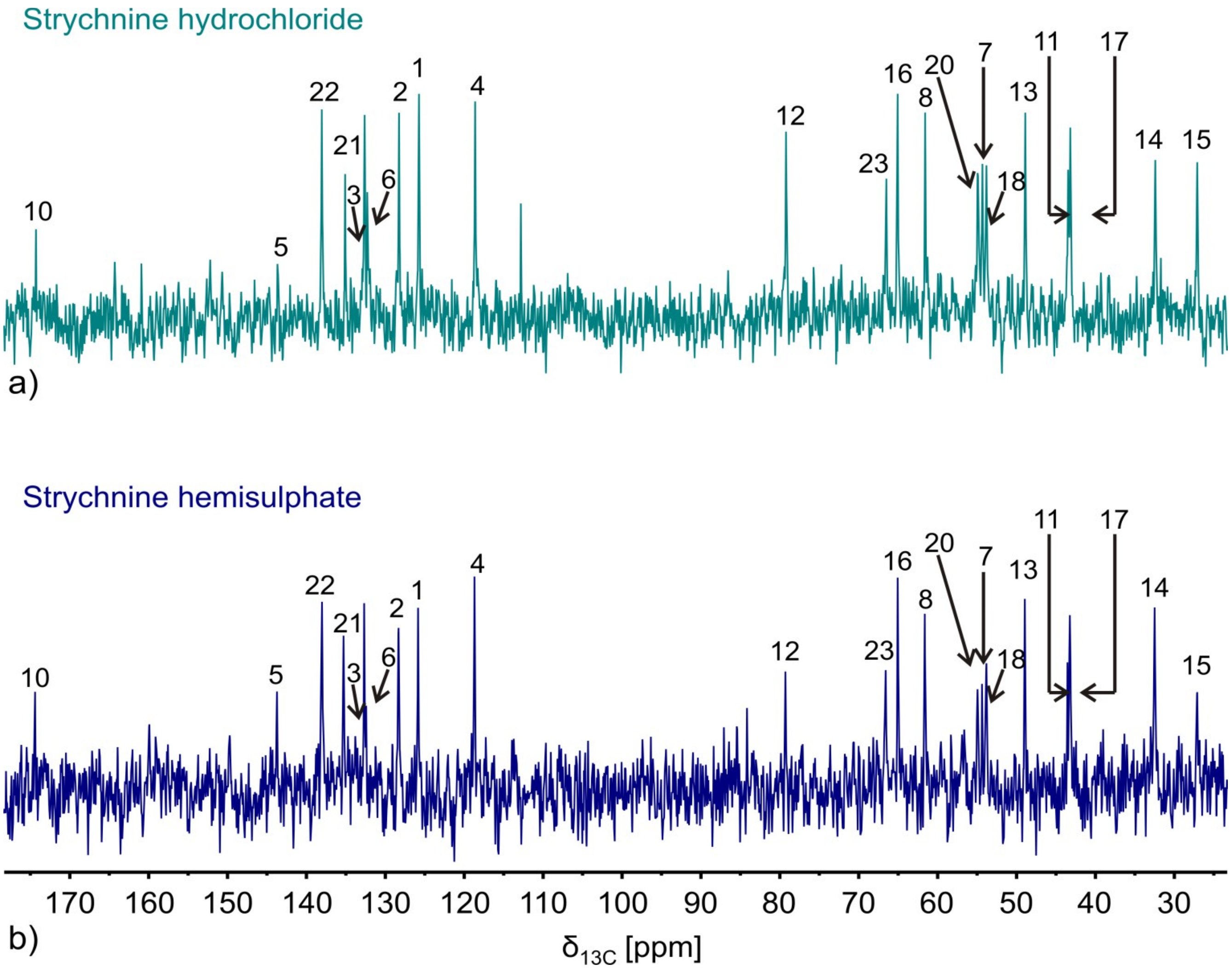

Fig. S-17. 1D ^{13}C NMR spectra of strychnine salts dissolved in D_2O and measured at 1 Tesla. a) Strychnine hydrochloride (67 mM), 4096 scans. b) Strychnine hemisulpahte (65 mM), 4096 scans.

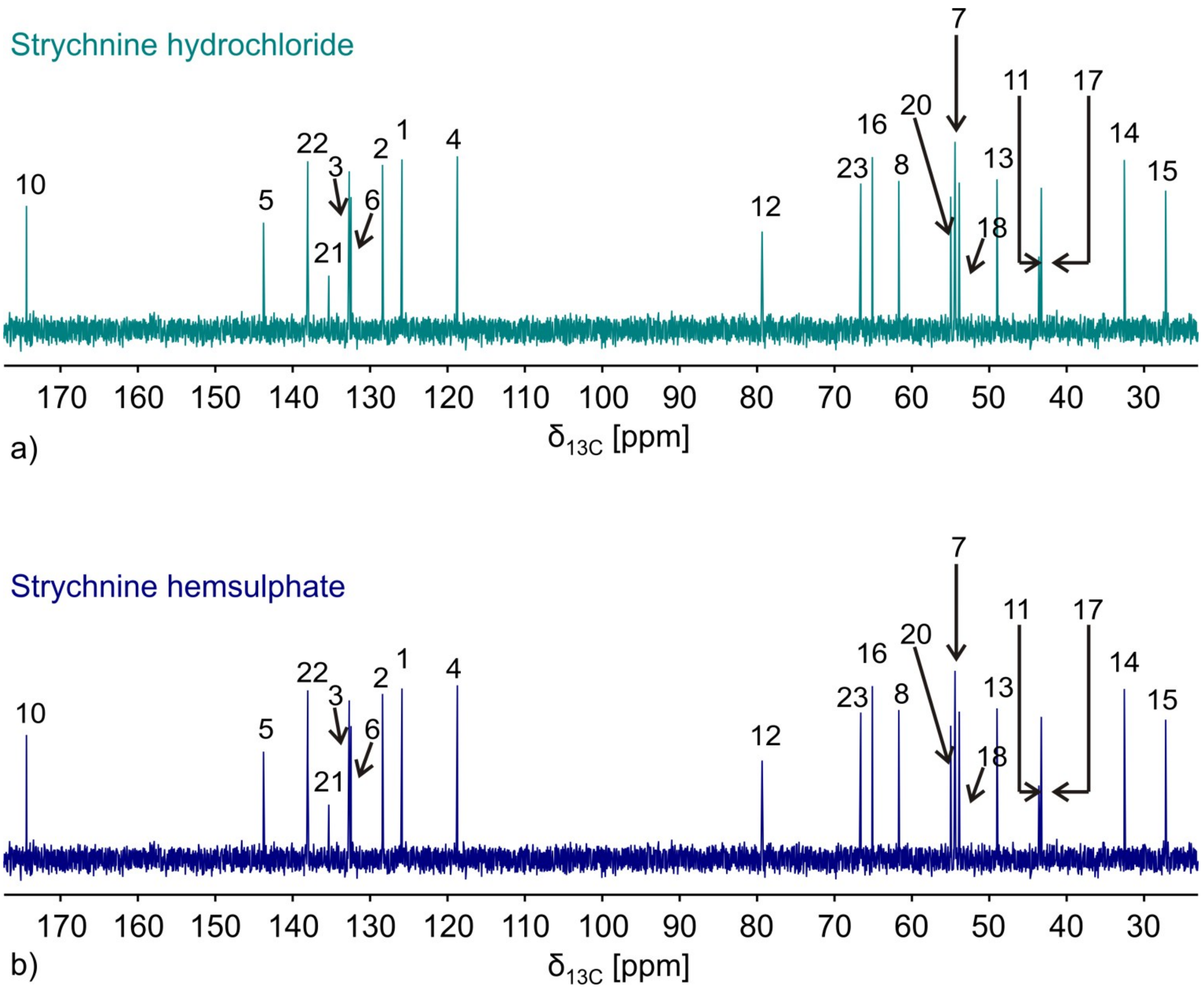

Fig. S-18.. 1D ^{13}C NMR spectra of a) Strychnine hydrochloride (65 mM) b) Strychnine hemisulphate (65 mM) obtained at 9.4 Tesla in 0.5 ml of D_2O.

Tab.S-7. ^{13}C chemical shifts measured at 1 Tesla for strychnine hydrochloride and strychnine hemisulphate in D_2O.

Carbon	Strychnine hydrochloride	Strychnine hemisulphate
1	125.72	125.75
2	128.26	128.24
3	132.62	132.58
4	118.61	118.60
5	143.59	143.64
6	132.27	132.35
7	54.31	54.27
8	61.57	61.54
10	174.27	174.29
11	43.45	43.45
12	79.20	79.20
13	48.88	48.86
14	32.41	32.40
15	27.09	27.03
16	65.07	64.97
17	43.18	43.14
18	53.82	53.73
20	54.90	54.87
21	135.09	135.20
22	138.04	137.95
23	66.51	66.49

References

1. A. E. Metaxas, J. R. Cort, *Magn. Reson. Chem.* 2013, **51**, 292-298.