

SUPPORTING INFORMATION

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Title: Hidden Flexibility of Strychnine

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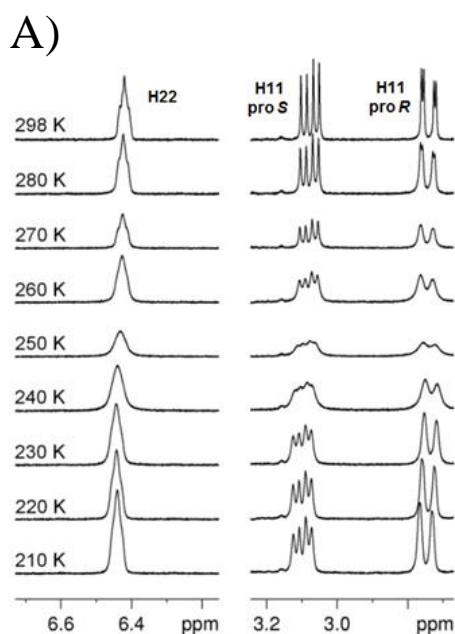
NMR

^1H and ^{13}C NMR spectra were recorded on a Bruker DRX 500 instrument (BBO probe) at 500.13 and 125.76 MHz, respectively, in steps of 10 K between 210 and 280 K (15 mg of the base form in 600 μL CDCl_3), between 180 and 280 K (10 mg of the hydrochloride in 600 μL $[\text{d}_4]\text{methanol}$), and 298 K. Temperature calibration was done with a methanol reference sample. ^1H NMR spectra were acquired using a spectral width of 12 kHz, an acquisition time of 5.5 s and 16 scans, zerofilled to 128 k datapoints (0.09 Hz per point) and processed with a 1 Hz exponential decay. ^{13}C NMR spectra were acquired using a spectral width of 30 kHz, an acquisition time of 1.1 s and 2048 scans in about 1h, zerofilled to 128 k datapoints (0.23 Hz per point) and also processed without apodization. Peaks were fitted to a Lorentzian lineshape using MestreNova 8.0.0 (MestreLab Research S.L.) To increase the signal-to-noise ratio for peaks broadened beyond 20 Hz, the ^{13}C NMR spectra were reprocessed with a 5 Hz exponential decay. ^1H -EXSY spectra were recorded on a TBI inverse probe at 210 K (both samples), 200 and 190 K (hydrochloride in methanol- d_4). The spectra were acquired using a spectral width of 4 kHz, 1024 x 512 complex time domain datapoints, a mixing period of 50 ms (additionally 200 ms at 190 K) and 8 scans in about 4.5 h. The spectra were zerofilled to 2048 x 2048 datapoints and processed with a shifted square sine bell apodization in both dimensions. Populations and exchange rates were obtained from diagonal- and crosspeak integrals using EXSYCalc (MestreLab Research S.L.).

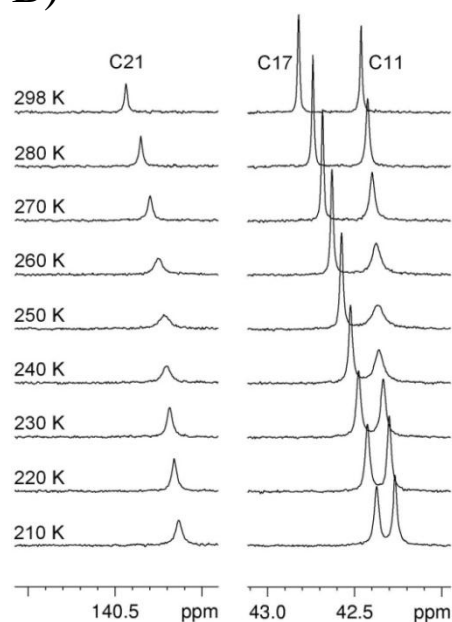
Low-Temperature NMR: Line broadening

In Figures S1A and S1B the broadening and subsequent sharpening of signals with decreasing temperature clearly indicated chemical exchange with a coalescence temperature for proton H22 around 250 K (strychnine HCl proton spectrum: Figure S1A; carbon spectrum strychnine base: Figure S1B).

Figures S1A and B. Variable-temperature ^1H and ^{13}C NMR spectra of strychnine. A) H22 and H11 proR/proS resonances of strychnine HCl in $[\text{D}_4]\text{methanol}$. B) C22, C17 and C11 resonances of strychnine base in CDCl_3 .



B)

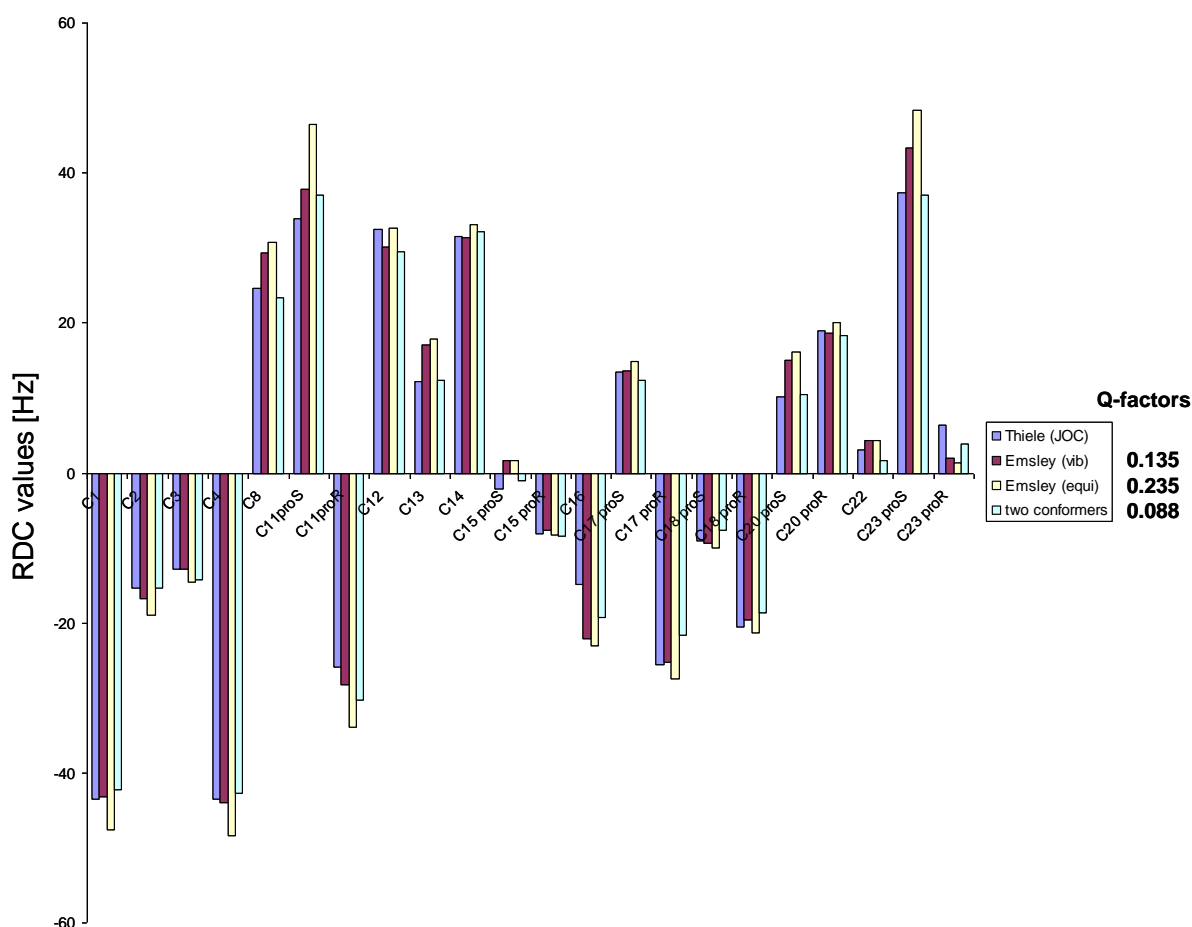


Residual dipolar coupling (RDC) analysis of strychnine base in chloroform

Based on the two conformers a detailed RDC analysis was performed. Although chemical exchange using RDCs has been characterized for N,N-dimethyltrichloroacetamide by the Palmer group (Igumenova et al., 2007), strychnine is decisively different: the population of the minor conformer is too low to be detected directly.

Many published analyses used the RDC data with a single tensor assuming strychnine as conformationally rigid (Thiele et al., 2003; Thiele, 2004; Luy, 2009; Emsley, 2010).

Figure S2: experimental (Thiele, J. Org. Chem. 2004) and back-calculated RDCs for strychnine base in chloroform



The vibrationally corrected back calculated RDCs increased the match with experiment (Thiele, 2004) compared to the equilibrium geometry (Emsley, 2010, Figure S2). However, the fitting using the two conformers gave the lowest Q factor indicating a contribution of the minor conformer to the experimental values. The best fit to the experimental RDCs is obtained with a mixture of 87:13 of conformer 1 and 2. Obviously, the RDC analysis overestimates the population of the minor conformer. However, it indicates that conformational averaging is taking place. One reason for the quantitative discrepancy could be that the alignment medium alters the conformational equilibrium. However, there is only one case reported for this to happen (oligosaccharides: Berthault et al. 2003). Another reason could be strong coupling effects. However, the $^1J_{CH}$ isotropic couplings could be predicted reliably by DFT calculations ruling out strong coupling effects on the isotropic values.

General DFT calculations

Calculations were performed with Gaussian09 on the mpw1pw91/cc-pvdz level of theory for geometry optimization (see following chapters for details), frequency/energy calculations, $^3J_{\text{HH}}$, $^2J_{\text{CH}}$, $^3J_{\text{CH}}$ couplings and chemical shifts. $^1J_{\text{CH}}$ and $^1J_{\text{CC}}$ couplings were calculated on the B3LYP/6-311+g(d,p) level of theory, the latter with the keyword NMR=mixed (Gaussian09). In all calculations the Polarizable Continuum Model (PCM) using the integral equation formalism variant (IEFPCM) was used as solvent model with chloroform or methanol as solvent.

Geometry optimization

A force field method (UFF), a semi-empirical method (PM6), and DFT methods with small basis sets such as 6-31G(d) failed to correctly model the bond lengths in the protonated piperidine moiety. Finally, we managed to obtain an optimized structure representing a minimum (no imaginary frequency) that matches with the x-ray structure.

The x-ray analysis of Ghosh et al. (1989) revealed that the N-C bond length of the aliphatic nitrogen atom are elongated in the protonated form compared to the base:

The sesquihydrate of strychnine HCl

N19-C16: 1.535(7), 1.536(7) [molecule A and B]

N19-C18: 1.517(7), 1.513(8)

N19-C20: 1.516(7), 1.507(8)

The strychnine base (Mostad et al., 1985)

N19-C16: 1.491(2)

N19-C18: 1.480(2)

N19-C20: 1.474(2)

The distances of protonated strychnine calculated at the mpw1pw91/cc-pvdz (iefpcm solvent model, methanol as solvent) level of theory reproduced the experimental values very well:

N19-C16: 1.540(4)

N19-C18: 1.509(9)

N19-C20: 1.507(0)

The mpw1pw91 functional was developed to show an improved long-range behaviour (Adamo and Barone, 1998).

Other functionals performed worse, e.g. B3LYP, where no bond between N19 and C16 was indicated after optimization which is of course due to the accepted normal length (Allen et al., 1987; (Csp³)-NH⁺: 1.502 Å, standard deviation 0.015, upper quartile: 1.512, number of observations: 509). This means that the experimental (and mpw1pw91 calculated) value for N19-C16 is within 3 sigma, whereas the B3LYP functional delivered a bond length of 1.56 Å which is very unlikely to represent a C-N bond.

The distances calculated at the B3LYP/6-31g(d) level of theory showed large differences to the experimental values:

N19-C16: 1.482(4) Å

N19-C18: 1.536(2) Å

N19-C20: 1.596(7) Å

Cartesian coordinates of the optimized structures of strychnine base in chloroform and protonated strychnine in methanol (Tables S3A-S3D), and the two corresponding transition structures (Tables S3E-S3F)

Table S3A: Cartesian coordinates of strychnine base in chloroform (conformer 1)

C	2.34376700	0.43792700	0.17369000
C	1.99786600	-0.91107800	0.02344300
C	3.65550600	0.87835900	0.00046100
C	2.97098500	-1.84216400	-0.31660300
C	4.61970700	-0.07118400	-0.34650000
C	4.28883300	-1.41639200	-0.50871400
N	1.19765800	1.19092500	0.48419800
C	-0.00254600	0.33456500	0.40937100
C	0.54439800	-1.12064400	0.36399700
C	-1.72927800	-0.24645700	-1.38482900
C	-0.27091800	-2.06918700	-0.54996500
C	1.07190500	2.54703600	0.30836200
C	-0.35557000	3.05659500	0.29895300
C	-0.80904300	0.82858400	-0.79421800
C	-1.41148300	2.18768300	-0.41641300
C	-2.80276300	-0.76586200	-0.44043800
C	-3.69776100	0.03830200	0.14565300
C	-3.68631500	1.53072700	-0.02636100
O	-2.50136900	2.12528800	0.48697300
C	-2.70510900	-2.24168400	-0.15409900
N	-1.36213000	-2.60313500	0.29932800
C	-1.10257000	-2.23847900	1.69525700
C	0.35932800	-1.82214900	1.72318400
O	2.03123700	3.29696100	0.19642700
H	-0.61115600	0.47848400	1.31426400
H	2.71390900	-2.89653300	-0.42990000
H	5.05918900	-2.13896600	-0.77930500
H	5.65109900	0.25281200	-0.49259700
H	3.90075700	1.92894000	0.12333400
H	-0.68117700	3.13197400	1.34856700
H	-0.32195400	4.07737500	-0.09622300
H	-1.74645400	2.69034900	-1.34266200
H	-4.51443700	1.98686800	0.53132600
H	-0.07864500	1.05148900	-1.59188500
H	-2.95199200	-2.82843900	-1.05339200
H	-3.42682500	-2.54216100	0.61706000
H	1.01547600	-2.70444500	1.75980200
H	0.61336500	-1.17735400	2.57533400
H	-1.31008600	-3.08247800	2.37131100
H	-1.74527400	-1.39604500	2.01752200
H	-3.81337200	1.81128000	-1.08972300
H	-4.44251200	-0.39197500	0.82104500
H	-2.22059900	0.18127100	-2.27573300
H	0.39368000	-2.90050300	-0.84228800
C	-0.81734300	-1.40318300	-1.80439100
H	-1.35583000	-2.13873500	-2.41709900
H	0.01562500	-1.03362700	-2.41968200

Table S3B: Cartesian coordinates of strychnine base in chloroform (conformer 2)

C	2.27876300	0.48093700	0.21978700
C	1.99045400	-0.87017300	-0.03066100
C	3.56454300	0.99536200	0.06802300
C	3.00084700	-1.72416900	-0.45291800
C	4.56635000	0.12167700	-0.36367500
C	4.29422600	-1.22068300	-0.62703400
N	1.10148100	1.15521100	0.59551700
C	-0.04853800	0.26161600	0.38571800
C	0.55444000	-1.16392000	0.32940100
C	-1.70969300	-0.32238500	-1.41604600
C	-0.26732800	-2.14278900	-0.54983100
C	0.88868200	2.51341000	0.52285300
C	-0.58257300	2.87576600	0.43806100
C	-0.72943900	0.72800400	-0.90210000
C	-1.28852600	2.15431200	-0.73176800
C	-2.70828500	-0.73596500	-0.33963100
C	-3.39729900	0.12007800	0.42612700
C	-3.38322400	1.61869100	0.39475500
O	-2.70280800	2.22886900	-0.67967900
C	-2.69292100	-2.21749400	-0.05688500
N	-1.34547500	-2.65461700	0.32926800
C	-1.00958400	-2.34052200	1.72200400
C	0.43907800	-1.87078100	1.69397500
O	1.79092400	3.33648700	0.51680300
H	-0.73973300	0.34519400	1.23418200
H	2.79245300	-2.77786400	-0.64469900
H	5.09294900	-1.88239300	-0.96324600
H	5.57960100	0.50321100	-0.49681500
H	3.76281400	2.04396800	0.27163300
H	-1.05376100	2.58393200	1.38950500
H	-0.66330900	3.96364900	0.34683500
H	-1.06321300	2.71169900	-1.65041500
H	-3.01305100	1.99228900	1.37066700
H	0.07666800	0.80556700	-1.65126200
H	-3.02037300	-2.79275800	-0.93683900
H	-3.38426400	-2.47511300	0.75619600
H	1.13011800	-2.72656000	1.70785100
H	0.69946400	-1.21689500	2.53738400
H	-1.14771200	-3.21806000	2.37302100
H	-1.66266400	-1.53922200	2.11638400
H	-4.41845900	1.98793200	0.31986500
H	-4.05254700	-0.29187000	1.20221100
H	-2.24282200	0.07170700	-2.29423000
H	0.39766900	-2.98048200	-0.82311400
C	-0.85587300	-1.53181300	-1.81247500
H	-1.44215100	-2.28902100	-2.35029200
H	-0.04925500	-1.22087100	-2.49139000

Table S3C: Cartesian coordinates of protonated strychnine in methanol (conformer 1)

C	2.35147900	0.42310200	0.17850500
C	1.98377800	-0.91879600	0.02523100
C	3.67430700	0.83383800	0.01406200
C	2.93584800	-1.87381000	-0.30752000
C	4.61920200	-0.13636100	-0.32655600
C	4.26247200	-1.47523300	-0.49130900
N	1.21986800	1.19934300	0.48036500

C	0.00493000	0.37086900	0.39914400
C	0.52172800	-1.09687900	0.36401800
C	-1.72166700	-0.19004000	-1.39413800
C	-0.26398700	-2.02103500	-0.58927600
C	1.11931200	2.55907600	0.29967600
C	-0.29557700	3.10074500	0.26342400
C	-0.78537600	0.87365700	-0.81123100
C	-1.37245600	2.23643800	-0.42539900
C	-2.78062100	-0.69597200	-0.42846800
C	-3.66247000	0.10432100	0.18134900
C	-3.64513400	1.59866700	0.01980000
O	-2.43903900	2.16682100	0.50719000
C	-2.77237800	-2.17321100	-0.18576200
N	-1.40166600	-2.59912200	0.27355700
C	-1.12462100	-2.21941800	1.70846500
C	0.32989800	-1.80383900	1.72007800
O	2.09694000	3.28613200	0.20255500
H	-0.60576200	0.53287300	1.29905200
H	2.66119900	-2.92354400	-0.42251300
H	5.01863000	-2.21369500	-0.75718400
H	5.65794700	0.16546900	-0.46624200
H	3.94400100	1.87803000	0.13851900
H	-0.62003800	3.22851700	1.30824200
H	-0.23379100	4.10352300	-0.17198600
H	-1.72963600	2.73925200	-1.34106400
H	-4.45244500	2.05239000	0.60689800
H	-0.05530700	1.08614000	-1.61012700
H	-2.98791800	-2.74365500	-1.09745600
H	-3.48463600	-2.48113300	0.58671700
H	0.98871900	-2.68149900	1.77267800
H	0.56396300	-1.15401700	2.57180600
H	-1.37257900	-3.06685700	2.35456200
H	-1.79068600	-1.38205800	1.94805200
H	-3.80297800	1.88306900	-1.03701700
H	-4.40156200	-0.32919500	0.85937300
H	-2.22282200	0.23471100	-2.27868000
H	0.36376200	-2.87893700	-0.85333800
C	-0.83066500	-1.35773000	-1.82742600
H	-1.36846100	-2.08828500	-2.44455900
H	0.00923000	-0.99919500	-2.43712900
H	-1.35745700	-3.61741200	0.19598200

Table S3D: Cartesian coordinates of protonated strychnine in methanol (conformer 2)

C	-2.28803100	0.46836600	-0.22140900
C	-1.97801900	-0.87660100	0.02978600
C	-3.58499400	0.95500900	-0.07119900
C	-2.96845400	-1.75517000	0.44822700
C	-4.56844900	0.06010800	0.35684800
C	-4.27095800	-1.27761700	0.61943600
N	-1.12528300	1.16563700	-0.59529300
C	0.04045800	0.29864900	-0.38642400
C	-0.53370400	-1.14090500	-0.33492800
C	1.69983400	-0.26457800	1.42376400
C	0.25822900	-2.09266700	0.58645800
C	-0.93415900	2.52926600	-0.51937400
C	0.52977400	2.91885900	-0.43064200
C	0.70815000	0.77259700	0.90629200
C	1.25388000	2.20301700	0.73143300
C	2.68615100	-0.67275800	0.33591900
C	3.36431300	0.17806300	-0.44472800

C	3.34159900	1.67340800	-0.41298100
O	2.66772600	2.28012700	0.66475500
C	2.75509700	-2.14749500	0.08320800
N	1.38082800	-2.64780600	-0.30889400
C	1.02523800	-2.32380300	-1.74190200
C	-0.41536000	-1.85895500	-1.69396700
O	-1.85315300	3.33227100	-0.51772300
H	0.72651000	0.40209200	-1.23593200
H	-2.74316200	-2.80505400	0.64092800
H	-5.05636300	-1.95561500	0.95296700
H	-5.58930100	0.42064500	0.48836500
H	-3.80621000	1.99903400	-0.27349000
H	1.00282400	2.64881900	-1.38754500
H	0.58921700	4.00685500	-0.32904500
H	1.03573300	2.75505700	1.65402300
H	2.96474600	2.03226400	-1.39077100
H	-0.09914100	0.84361600	1.65320100
H	3.04827100	-2.71357800	0.97507300
H	3.43707700	-2.41313800	-0.73097100
H	-1.10461300	-2.71393400	-1.71593700
H	-0.66140700	-1.20459400	-2.53890900
H	1.20279500	-3.20954200	-2.35863100
H	1.70295400	-1.52647100	-2.06653900
H	4.37749800	2.04095800	-0.35206100
H	4.00953400	-0.23189500	-1.22857400
H	2.23700400	0.13260400	2.29626500
H	-0.37060900	-2.95520400	0.83260400
C	0.86387800	-1.48063800	1.83340500
H	1.44783400	-2.23155800	2.38023400
H	0.04896900	-1.17803700	2.50362200
H	1.38760900	-3.66426500	-0.19916800

Table S3E: Cartesian coordinates of transition structure of strychnine base in chloroform

C	2.3007	0.5232	0.1906
C	2.0357	-0.8313	-0.0573
C	3.5706	1.0662	0.0054
C	3.0508	-1.6632	-0.5109
C	4.5781	0.2155	-0.4577
C	4.3277	-1.1315	-0.7191
N	1.1205	1.1679	0.6021
C	-0.0238	0.2559	0.4295
C	0.6176	-1.1537	0.3405
C	-1.7015	-0.3584	-1.3691
C	-0.1945	-2.1438	-0.5257
C	0.8868	2.5225	0.5641
C	-0.5887	2.8487	0.5503
C	-0.7821	0.7291	-0.8183
C	-1.3610	2.1453	-0.5906
C	-2.6994	-0.8225	-0.3261
C	-3.5441	0.0166	0.2812
C	-3.7897	1.4567	-0.0571
O	-2.7409	2.3668	-0.3492
C	-2.6004	-2.2859	0.0183
N	-1.2393	-2.6850	0.3749
C	-0.8862	-2.3610	1.7614
C	0.5523	-1.8679	1.7041
O	1.7765	3.3596	0.5326
H	-0.6790	0.3162	1.3087
H	2.8596	-2.7205	-0.7008
H	5.1307	-1.7747	-1.0801

H	5.5789	0.6191	-0.6173
H	3.7522	2.1178	0.2083
H	-1.0323	2.5161	1.5017
H	-0.7051	3.9355	0.4893
H	-1.1682	2.7056	-1.5188
H	-4.3415	1.9138	0.7790
H	-0.0040	0.8808	-1.5857
H	-2.9381	-2.8874	-0.8422
H	-3.2688	-2.5427	0.8506
H	1.2578	-2.7117	1.6960
H	0.8214	-1.2129	2.5437
H	-0.9991	-3.2383	2.4174
H	-1.5456	-1.5690	2.1650
H	-4.4845	1.4760	-0.9209
H	-4.2274	-0.3885	1.0345
H	-2.2370	0.0332	-2.2500
H	0.4831	-2.9643	-0.8185
C	-0.8045	-1.5301	-1.7780
H	-1.3685	-2.2941	-2.3305
H	-0.0044	-1.1844	-2.4480

Table S3F: Cartesian coordinates of transition structure of protonated strychnine in methanol

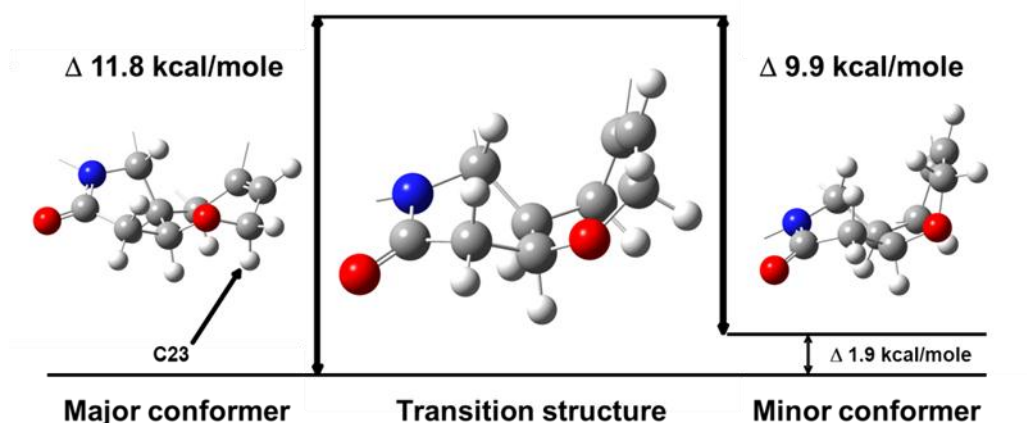
C	2.3168	0.5034	0.1973
C	2.0196	-0.8469	-0.0252
C	3.6070	1.0013	0.0213
C	3.0130	-1.7237	-0.4409
C	4.5942	0.1091	-0.4030
C	4.3081	-1.2364	-0.6376
N	1.1528	1.1925	0.5762
C	-0.0174	0.3132	0.4254
C	0.5825	-1.1200	0.3544
C	-1.6884	-0.2868	-1.3890
C	-0.1851	-2.0785	-0.5749
C	0.9596	2.5535	0.4911
C	-0.5024	2.9332	0.4807
C	-0.7701	0.7918	-0.8241
C	-1.3450	2.2034	-0.5930
C	-2.6826	-0.7651	-0.3512
C	-3.5159	0.0487	0.3039
C	-3.7436	1.5091	0.0753
O	-2.7024	2.4150	-0.2366
C	-2.6728	-2.2363	-0.0794
N	-1.2869	-2.6777	0.3174
C	-0.9559	-2.3284	1.7498
C	0.4776	-1.8458	1.7099
O	1.8783	3.3564	0.4362
H	-0.6602	0.4093	1.3104
H	2.7971	-2.7793	-0.6122
H	5.0962	-1.9122	-0.9690
H	5.6094	0.4781	-0.5541
H	3.8196	2.0508	0.2016
H	-0.9301	2.6677	1.4600
H	-0.5779	4.0194	0.3702
H	-1.2282	2.7388	-1.5475
H	-4.2202	1.9155	0.9800
H	0.0077	0.9516	-1.5890
H	-2.9393	-2.8131	-0.9736
H	-3.3515	-2.5264	0.7292
H	1.1779	-2.6919	1.7285
H	0.7133	-1.1920	2.5582

H	-1.1328	-3.2058	2.3787
H	-1.6480	-1.5325	2.0480
H	-4.5066	1.5836	-0.7253
H	-4.2016	-0.3912	1.0338
H	-2.2238	0.1082	-2.2662
H	0.4658	-2.9198	-0.8357
C	-0.7940	-1.4541	-1.8141
H	-1.3398	-2.2080	-2.3952
H	0.0233	-1.1013	-2.4568
H	-1.2499	-3.6942	0.2143

Transition structure calculations

In the transition structure of protonated strychnine the ring F is flattened compared to the two conformers with the oxygen acting as a junction between the chair and envelope arrangements (Figure S4). A similar transition structure was obtained for strychnine base (see supporting information). A detailed interpretation of the factors influencing the conformational change of rings F and G is out of scope since even for the simplest alkanes a controversy concerning the origin of the rotational barrier remains (Mo, 2011; Pophristic and Goodman, 2001; Schreiner, 2002).

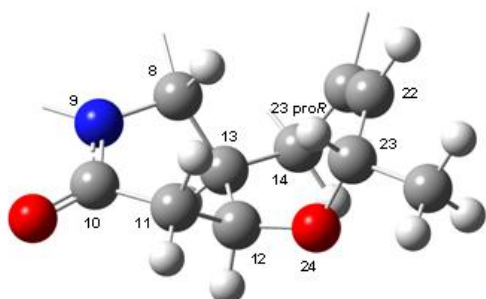
Figure S4. Calculated transition structure and the two conformers of protonated strychnine and associated energy differences (mpw1pw91/cc-pvdz, iefpcm solvent model with methanol as solvent).



Theoretical methylated strychnine

With a reliable structural model at hand, a theoretical 23-methyl derivative can be proposed, which should have a stabilized envelope conformation of ring F (Figure S5). By substitution of the 23-proS proton, an equatorial position of the methyl group is obtained resulting in a stabilized conformer (conformer populations based on calculated energy differences: 86 % / 14 %). For this derivative, we predict to have an inverted population ratio between chair and envelope of ring F, compared to the naturally occurring strychnine.

Figure S5. Major conformer of a 23-methyl derivative (proS proton substituted) of protonated strychnine showing a similar conformation of rings F and G of the minor conformer of protonated, unsubstituted strychnine.



NMR parameter calculations:

Strychnine Base

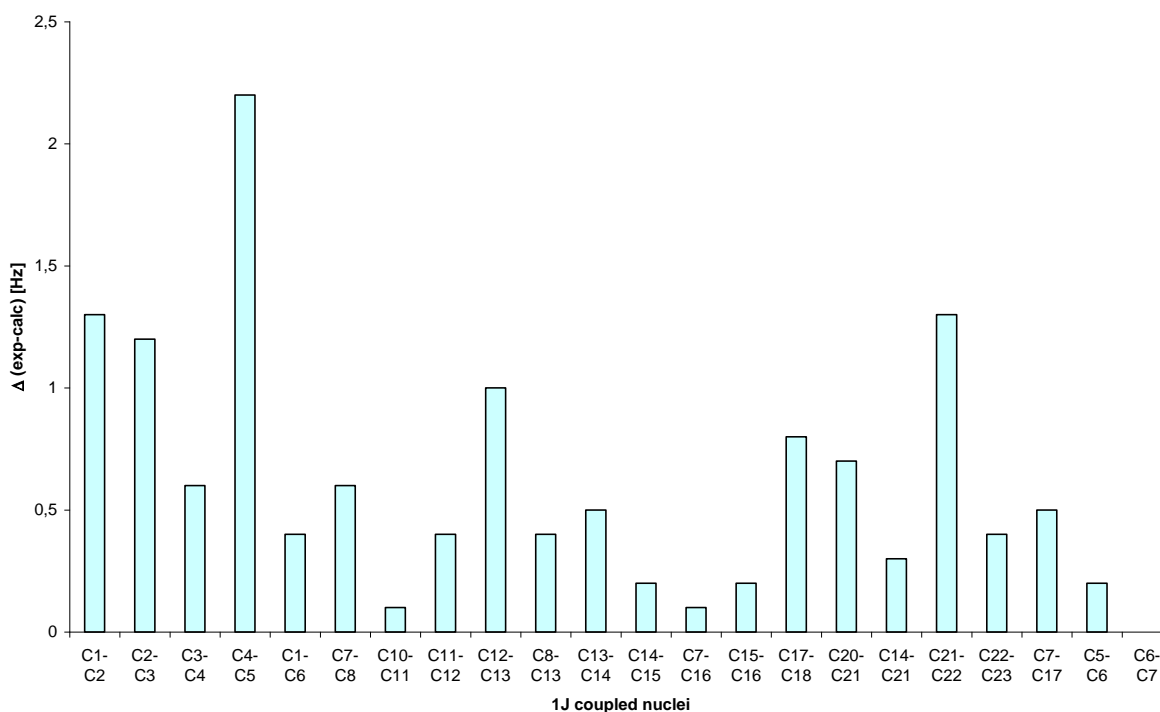
The addition of p-functions (G09 keyword: NMR=mixed) significantly improved the calculation of $^1J_{CC}$ couplings (Deng et al., 2006). To be consistent with literature we applied the B3LYP/6-311+g(d,p) level of theory (Bifulco et al., 2013). Using our optimized geometry we obtained a better fit to experimental $^1J_{CC}$ couplings (Table S6, Figure S7). The mean absolute error was 0.6 Hz and 1.14 Hz for conformer 1 and conformer 2, respectively. Obviously, the geometry optimization improved the match to experiment.

Table S6: Experimental and calculated $^1J_{CC}$ couplings (B3LYP/6-311+g(d,p); NMR=mixed) of strychnine base in chloroform, with the geometry optimized structures on the mpw1pw91/cc-pvdz (iefpcm as solvent model, chloroform as solvent) level of theory

$^1J_{CC}$	exp Bagnò	geometry: mpw1pw91/cc-pvdz	
		conf1	conf2
C1-C2	58,1	59,4	59,3
C2-C3	58,9	60,1	60,3
C3-C4	59,9	59,3	59,3
C4-C5	64,9	67,1	67,2
C1-C6	65,7	66,1	65,7
C7-C8	33,9	34,5	34
C10-C11	50,1	50,2	49,6
C11-C12	37,6	37,2	31,8

C12-C13	36,8	35,8	35
C8-C13	32,4	32,8	33
C13-C14	32,4	32,9	32,8
C14-C15	32,6	32,8	32,5
C7-C16	33,7	33,8	34
C15-C16	33,3	33,5	33,6
C17-C18	34	33,2	32,8
C20-C21	41,6	40,9	40,7
C14-C21	37,6	37,3	37,5
C21-C22	71,8	73,1	76,8
C22-C23	45,6	46	44,3
C7-C17	32,1	31,6	31,5
C5-C6	60,7	60,9	61
C6-C7	43,3	43,3	43,2

Figure S7: Differences between calculated and experimental $^1J_{CC}$ couplings of strychnine base in chloroform



$^1J_{CH}$ couplings as indicators of stereoelectronic effects can be used for conformational analysis of organic and bio-organic compounds (Fischer and Reinscheid, 2006). For the $^1J_{CH}$ couplings we obtained a good fit between experiment and calculation on the B3LYP/6-311+g(d,p) level of theory (Table S8). As a by-product, prochiral protons could be assigned in some cases based on the corresponding $^1J_{CH}$ coupling.

Table S8: Experimental and calculated $^1J_{CH}$ couplings (B3LYP/6-311+g(d,p) of strychnine base in chloroform, with the geometry optimized structures on the mpw1pw91/cc-pvdz (iefpcm as solvent model, chloroform as solvent) level of theory

Thiele (2004) atom	Exp. $^1J_{CH}$ (average)	descriptor	Calc. conf1	Calc. conf2
1	158,44		148,8	149,2
2	162,43		151,6	151,6

3	160,83		150,1	150,3
4	168,69		160,5	160
11	135,72	proR	129,8	133,2
11	125,44	proS	121,1	119,2
12	149,38		140,7	143,5
13	125,4		120,6	122,5
14	131,13		124,7	130,1
15	130,65	proS	125,1	124,9
15	130,25	proR	124,5	124,9
16	146,62		138,8	138,1
17	132,96	proR	124,7	125,2
17	132,96	proS	129,4	129
18	145,21	proS	137,4	137,5
18	132,24	proR	123,4	123,2
20	138,84	proR	131,2	132,1
20	138,63	proS	129,8	129,6
22	160,28		150,5	144,2
23	145,23	proR	137,1	129
23	137,12	proS	129	136,8

Calculated $^1J_{CH}$ couplings were also in very good agreement to experiment. After linear regression, the mean absolute error was 0.99 Hz for conformer 1, and 2.22 Hz for conformer 2. The standard deviation of conformer 1 (1.33 Hz) outperforms the best results for B3LYP among 20 functionals, with a standard deviation for aromatic C: 2.42 Hz, sp²-type: 2.97 Hz, sp³-type: 2.07 Hz (Maximoff et al. 2005).

In addition, the $^3J_{HH}$, $^2J_{HH}$ and $^3J_{CH}$ couplings were calculated and compared with experiment (Tables S9, S10 and S11).

Table S9: experimental $^3J_{HH}$ couplings from literature (Chazin and Colebrok, 1983), and calculated (mpw1pw91/cc-pvdz, iefpcm as solvent model, chloroform as solvent) for strychnine base

atoms	exp	Calc. Conf1	Calc conf2	assignment
1-2	7,4	5,2	5,2	
2-3	7,5	5	5	
3-4	8,1	5,7	5,7	
8-13	10,5	6,8	7,4	
11a-12	8,4	1,5	6,5	proR
11b-12	3,3	6,3	5	proS
12-13	3,1	2,6	6,6	
13-14	3,1	2,7	1,9	
15a-14	4	3,8	3,6	proS
15a-16	4,9	3,3	3	
15b-14	1	1,6	1,6	proR
15b-16	2	1,6	1,9	
17a-18a	8,4	5,3	5,6	proR/proS
17a-18b	11,7	8,7	8,4	
17b-18a	0,1	0,2	0,2	
17b-18b	6,9	4,2	4,7	
22-23a	5,7	5,2	2,6	proR
22-23b	6,9	4,2	3,3	proS

Table S10: experimental and calculated $^2J_{\text{HH}}$ couplings (mpw1pw91/cc-pvdz, iefpcm as solvent model, chloroform as solvent) for strychnine base

Proton numbers	exp	Calc conformer 1	Calc conformer 2
11a-11b	-17,4	-16,1	-13,2
15a-15b	-14,5	-13,7	-13,6
17a-17b	-15,5	-12,1	-12,2
18a-18b	-10,1	-9,5	-9,9
20a-20b	-14,7	-14,1	-13,7
23a-23b	-14,2	-13,1	-17,4

Table S11: $^3J_{\text{CH}}$ coupling constants (Edden and Keeler, 2004; mostly measured by method 1) of strychnine base in CDCl_3 ;
Calculated data only shown for CH pairs with differences > 0.5 Hz

H	C	method 1	Other experimental method indicated	Calc. Conformer 1	Calc. Conformer 2	
	13	15	3,5			
		14	4,6			
		8	6,3			
		12	0,6			
		21	7,8			
15b		14	3,2			
		13	3,3			
		16	2,7			
		21	6			
	17 7		3,3			
		16/8		5,6		
			6	1,9		
15a		14	1,8			
		13	8			
		7	7,2			
		16	4,5			
		12	0,9			
		21	0,9			
11b		12	7			
		10	7,9			
20b		14	5,4			
		18	3,5			
		16	6,9			
		12	4,5			
		21	2,4			
18b		17	5,6			

	20	7,1		
11a	13	3,4		
	12	2,5		
	10	5,8		
18a	17	2,7		
	7	4,6		
	16	4,3	method 3	
20a	18	9,3		
	22	6,1		
	21	4,9		
8	17	5,9		
	7	2,5		
	12	5,5		
	6	3,7		
	5	3,2		
23a	12	5,6	5,4	7,7
	22	3,8	2,2	7
	21	3,3	4,9	7,7
23b	12	8,6	6,8	2,3
	22	4		
	21	8,5	6,2	8,2
12	8	5,8		
	23	2,4	2,6	8,1
	10	5,8	method 2	
22	14	7,9		
	20	4,9		
	23	5,7		
4	2	7,5		
	6	5,5		
	5	0,9		

Procedure of calibration and prediction of chemical shifts

First, the DFT calculated data of the two conformers are collected for which the difference is smaller than a reasonable precision (rigid part), and for these data the averages for the two conformers are calculated. Second, this set of data is used for linear regression analysis (Scheme 1). Third, the slope and intercept are used to scale the calculated data for the two conformers. With this procedure we could predict the proton and carbon chemical shifts of the minor conformer very precisely (Tables S12A and B, S13A and B)

Scheme 1: calibration and prediction of chemical shifts of strychnine base in CDCl₃

Calibrations by linear regression were based on 12 proton and 10 carbon resonances.

	Proton CS	carbon CS	
Relative Standard deviations	2.2 %	1.3 %	For both data sets: R values > 0.99
Mean absolute error after calibration	0.10 ppm	1.3 ppm	
MAE =	$\frac{\sum \text{calc} - \text{ex} }{\text{number of data points}}$		

Predictions were done for 10 proton and 11 carbon resonances.

With this procedure we obtained a better fit between experiment and calculation for protons compared to the report of Bassarello et al. (2003; lowest MAE for the mpw1pw91 functional and a 6-311G(d,p) basis set: 0.16 ppm) (Scheme S1).

Table S12A: ¹³C chemical shifts/shieldings: calibration (diff < 1.0 ppm between the conformers) of strychnine base in CDCl₃

Carbon number	Exp (chem. shift, δ)	Shielding (σ) average of conf1 and conf2 (calc)
1	122,3	73,9
2	124,3	73,3
3	128,6	68,3
4	116,2	80,8
5	142,2	53,7
6	132,6	62,8
7	51,9	141,3
15	26,8	167,1
16	60,1	135,4
18	50,2	146,5

Table S12B: ¹³C chemical shifts: prediction of strychnine base in CDCl₃

Carbon number	exp	Calc Conformer	Calc Conformer
		1	2
8	60,1	60,0	62,4
10	169,4	167,6	169,3
11	42,3	42,1	32,1
12	77,5	77,0	70,5
13	48,2	48,5	50,6
14	31,5	33,1	30,9
17	42,8	43,5	42,1
20	52,7	50,7	52,5
21	140,3	144,5	136,4
22	127,7	129,0	126,5
23	64,6	63,3	61,1

Table S13A: ^1H chemical shifts (exp)/shieldings (calc): calibration (diff < 0.2 ppm between the conformers) of strychnine base in CDCl_3

proton number	Exp chemical shift (δ)	Shieldings (σ) average of conf1 and conf2 (calc)
1	7,15	24,23
2	7,08	24,33
3	7,23	24,17
4	8,09	23,37
8	3,85	27,83
14	3,13	28,39
16	3,92	27,81
17, pro-R	1,88	29,84
17, pro-S	1,89	29,7
18, pro-S	2,87	28,54
20, pro-R	3,7	27,85
20, pro-S	2,72	29,19

Table S13B: ^1H chemical shifts: prediction of strychnine base in CDCl_3

proton number	exp	Calc Conformer	Calc conformer
		1	2
11, pro-R	2,66	2,71	3,43
11, pro-S	3,11	3,03	2,31
12	4,27	4,07	4,32
13	1,25	1,03	1,48
15, pro-S	2,36	1,51	2,39
15, pro-R	1,46	2,40	1,53
18, pro-R	2,87	3,09	2,84
22	5,88	5,97	5,27
23, pro-R	4,05	4,10	4,69
23, pro-S	4,13	4,06	4,35

NMR parameter calculations

Strychnine HCl/protonated strychnine

Table S14: experimental ^{13}C chemical shifts and calculated shielding constants of strychnine HCl in $[\text{d}_4]\text{methanol}$ (exp) and protonated strychnine (calc)

atoms	δ Exp.	σ Conf1	σ Conf2
1	123,8	73,6	73,2
2	126,0	72,2	71,1
3	130,8	66	66

4	117,3	80,2	80,5
5	143,2	53,9	54
6	130,6	67,8	68,2
7	53,2	141,3	141,3
8	60,2	136	133,7
10	171,4	29,1	27,7
11	42,7	153	162,7
12	78,0	119,1	125,4
13	48,1	147,8	144,9
14	31,6	162,9	165,2
15	25,9	169,7	169,3
16	63,7	129,5	130,5
17	42,0	153,8	155,4
18	52,2	143,5	144,3
20	53,2	142,2	140,8
21	133,7	63,3	69,5
22	137,3	53,6	54,2
23	65,0	132,5	133,5

Table S15: experimental ^1H chemical shifts and calculated shielding constants of strychnine HCl in [d4]methanol (exp) and protonated strychnine (calc)

atoms	δ exp	σ Conf1	σ Conf2
1	7,41	24,01	24,04
2	7,21	24,13	24,14
3	7,35	23,95	23,96
4	8,04	23,2	23,32
8	4,21	27,55	27,56
11, pro-R	3,09	28,44	29,18
11, pro-S	2,75	28,82	28,11
12	4,41	27,3	27,09
13	1,5	30,42	30,04
14	3,46	28,19	28,05
15, pro-S	2,6	28,95	28,99
15, pro-R	1,76	29,75	29,72
16	4,49	27,19	27,23
17, pro-S	2,25	29,25	29,25
17, pro-R	2,07	29,31	29,44
18, pro-S	3,85	27,69	27,71
18, pro-R	3,41	27,94	28,02
20, pro-R	4,23	27,24	27,27
20, pro-S	3,58	28,05	28,19
22	6,43	24,74	25,36
23, pro-R	4,27	27,24	26,74
23, pro-S	4,21	27,36	26,98

Figure S16: Experimental and calculated proton chemical shift differences between the two conformers of strychnine HCl (blue; exp) and protonated strychnine (green; calc) in methanol.

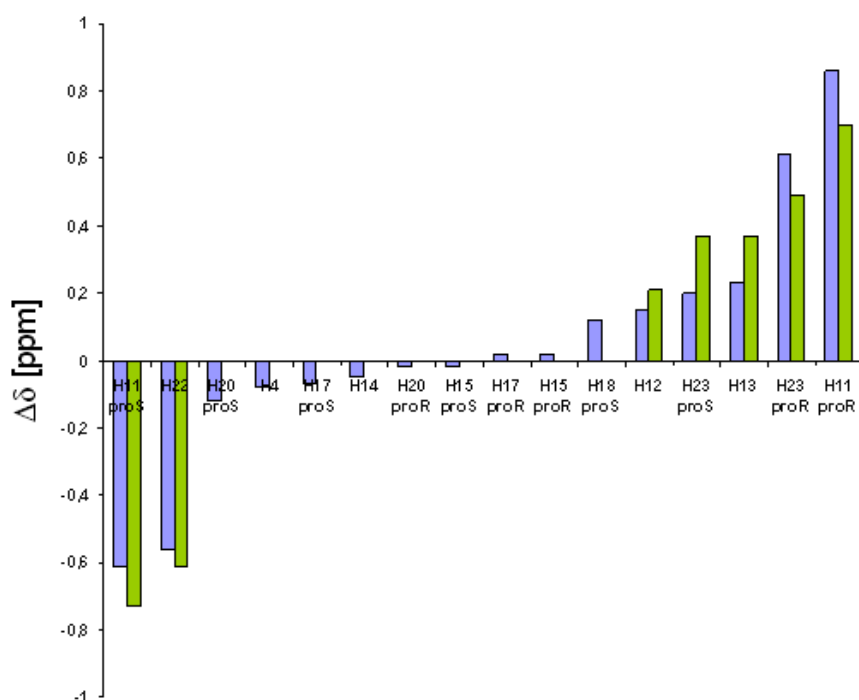


Table S17: experimental and calculated $^3J_{\text{HH}}$ couplings for strychnine HCl in [d₄]methanol (exp) and protonated strychnine (calc)

atoms	Exp [Hz]	Calc conf1 [Hz]	Calc conf2 [Hz]
1/2	7,7	5,3	5,3
2/3	7,7	5	5,1
3/4	8,2	5,8	5,7
8/13	10,8	6,9	7,5
11 pro-S/12	8,3	6,3	5,1
11 pro-R/12	3,2	1,1	6,1
12/13	3,1	2,3	6,5
15,proS/16	4,2	3,1	2,8
15 proS/14	4,2	3,7	3,5
15 proR/16	2,1	1,9	2,1
15 proR/14	2,1	1,7	1,6
17 proS/18	6,2	5,2	5,6
17 proR/18	7,7	9,7	9,3
23 proR/22	7,0	5,3	2,7
23 proS/22	5,9	4,2	3,5

Table S18: experimental and calculated $^1J_{\text{CH}}$ couplings for strychnine HCl in [d₄]methanol (exp) and protonated strychnine (calc)

atoms	$^1J_{\text{CH}}$ exp [Hz]	Conf1 [Hz]	Conf2 [Hz]
11,pro-R	126,4	121,6	120
11,pro-S	135,2	130,4	134,1
12	152,0	144	145,8

13	127,4	123,2	124,6
14	135,8	130,2	134,9
22	160,8	153,3	147,4
23,pro-R	147,2	140,4	131,3
23,pro-S	138,6	131,3	138,9
1	158,9	150	150,5
2	161,6	154	154
3	160,1	151,6	151,8
4	168,6	161,2	160,7
8	146,6	139,2	139,1
15,pro-R	133,8	128,1	128,9
15,pro-S	132,6	128,9	128,1
16	150,4	144,4	143,6
17,pro-S	133,7	128,8	129,5
17,pro-R	139,1	134,4	134,1
18,pro-R	150,4	138,5	138,8
18,pro-S	144,1	144,5	144
20,pro-R	144,8	139,4	139,7
20,pro-S	147,8	141	141

Geometry dependence of ^{13}C chemical shift calculations

The ^{13}C chemical shifts can be reliably predicted on the basis of a good structural model. Using the standard level of theory for geometry optimization (B3LYP/6-31g(d)) leads to large differences to experiment in the case of protonated strychnine, whereas the mpw1pw91/cc-pvdz level of theory leads to a very good match to experimental chemical shifts.

Figure S19A: B3LYP/6-31g(d) geometry, mpw1pw91/cc-pvdz calculated shielding constants of protonated strychnine (conformer 1) (IEFPCM with methanol as solvent) versus experimental ^{13}C chemical shifts; Deviation between experiment and predicted after linear regression ($R= 0.982$) for C16: 12 ppm, and for C20: 13 ppm

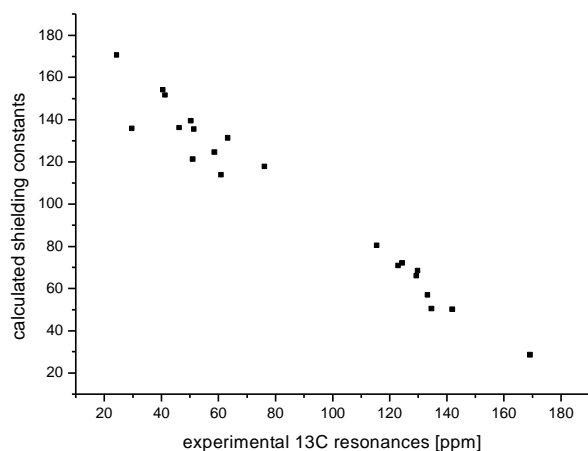
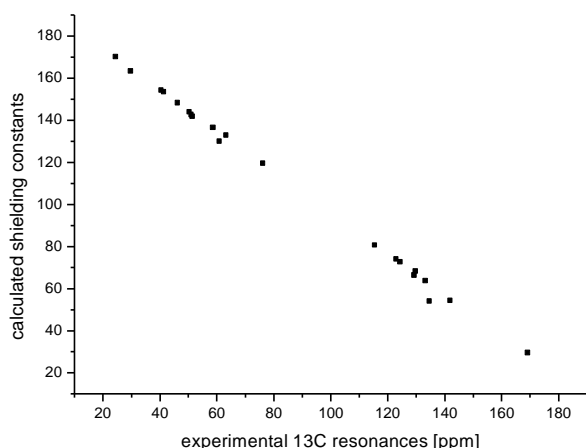


Figure S19B: mpw1pw91/cc-pvdz geometry, mpw1pw91/cc-pvdz calculated shielding constants of protonated strychnine (conformer 1) (IEFPCM with methanol as solvent) versus experimental ^{13}C chemical shifts; deviation between experiment and predicted after linear regression ($R= 0.999$) for C16: 3 ppm and for C20: 0.5 ppm



Population analysis using population weighted chemical shifts

This was done using experimental data from 190 K to 220 K, which were then extrapolated to 280 K. The difference to the experimental value at 280 K is given by the $p_{\text{conf}2}$ -weighted chemical shift difference ($\omega_{\text{conf}1} - \omega_{\text{exp}} = p_{\text{conf}2} * (\omega_{\text{conf}1} - \omega_{\text{conf}2})$), using calculated chemical shifts for $\omega_{\text{conf}1}$ and $\omega_{\text{conf}2}$. This was applied to the carbon resonances which showed the largest chemical shift differences between the two conformers (C11, C12, C13, C20).

The following differences Δ in ^{13}C chemical shifts ($\Delta\delta = \delta_{\text{conf}2} - \delta_{\text{conf}1}$) were determined experimentally for the two conformers of strychnine HCl in $[\text{d}_4]\text{methanol}$:

$\Delta \delta\text{C11}$: -8.1 ppm; $\Delta \delta\text{C12}$: -5.3 ppm; $\Delta \delta\text{C13}$: +3.1 ppm; $\Delta \delta\text{C20}$: +1.9 ppm

The temperature induced change in chemical shift was subtracted from the chemical shift value at 280 K. This was done using experimental data from 190 K to 220 K, which were then linearly extrapolated to 280 K. The difference to the experimental value at 280 K gives the population-weighted chemical shift ($\omega_{\text{exp}} = p_{\text{conf}1} * \omega_{\text{conf}1} + p_{\text{conf}2} * \omega_{\text{conf}2}$) using the relationship $p_{\text{conf}1} = 1 - p_{\text{conf}2}$ the population of conformer 2 at 280 K could be determined. Applying the Boltzmann equation finally yields the following populations of conformer 2 at 298 K based on resonances for C11, C12, C13, and C20, respectively:

5.0 %; 5.5 %; 3.3 %; 9.0 %

On average, the population of conformer 2 is determined as 5.7 %.

Optical rotation and electronic circular dichroism

Strychnine base was measured (20 mg/ml at 25°C) in chloroform which gave $[\alpha]_D = -141$. Strychnine HCl was measured at the same concentration and temperature to give $[\alpha]_D = -16$. Our experimental data match with literature values (strychnine base: 18 °C, c=1 in chloroform: $[\alpha]_D = -139$; strychnine sulfate, 20 mg/ml in water, $[\alpha]_D = -25$; Muthadi and Hifnawy, 1986).

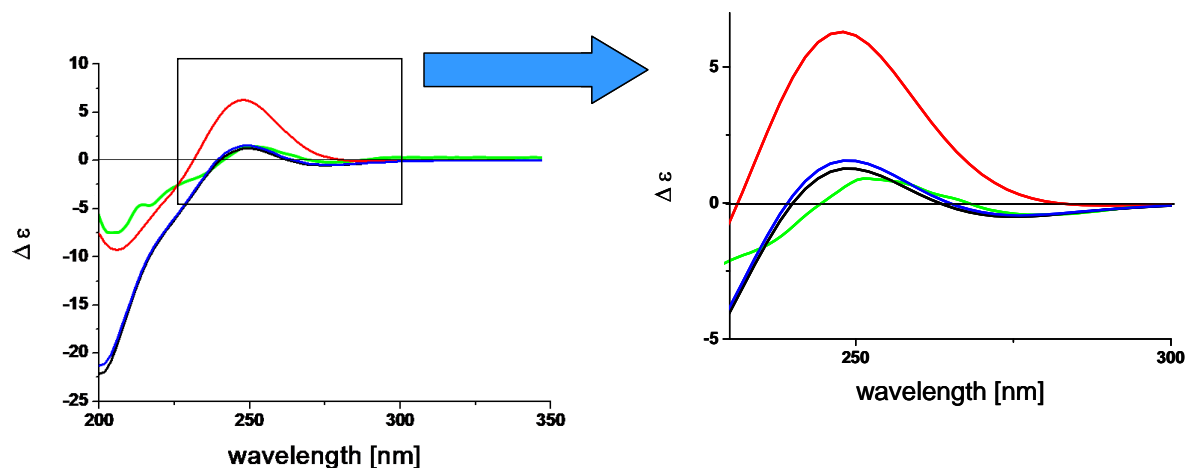
Both experimental values correspond very well to the calculated values for the base (mpw1pw91/aug-cc-pvdz; IEFPCM with chloroform as solvent): conformer 1: -88; conformer 2: -107; population mix (94.1:5.9): -89.

Likewise, for protonated strychnine (mpw1pw91/aug-cc-pvdz; IEFPCM with methanol as solvent): conformer 1: -25; conformer 2: +5; population mix (94.1:5.9): -23.

Note that the experimental values are adjusted to be comparable to the calculated values for the protonated strychnine, i.e. no ion-pair with Cl^- is assumed.

A good match between experimental and calculated ECD spectrum proves that the naturally occurring (-)-strychnine HCl is assigned 13*R* since this was the configuration of the structural model used (Figure S21). No wavelength shift was applied.

Figure S20: experimental (HCl) and calculated (protonated form) ECD of (-)-strychnine in methanol (mpw1pw91/cc-pvdz, iefpcm solvent model with methanol as solvent) (green: experiment; black: conformer 1; red: conformer 2; blue: population mix (94.1:5.9))



Sources of experimental data

Chemical shifts and couplings from literature (base in CDCl_3)

^{13}C chemical shifts: Chazin and Colebrok, 1983, Can. J. Chem., 61, 1749

^{13}C , ^1H and long-range CH couplings: Martin et al., 1999, Magn. Reson. Chem. 37, 517

^1H chemical shifts and $^3J_{\text{HH}}$ couplings: Cobas et al. 2005, *Magn. Reson. Chem.* 43, 843

Long-range CH couplings: Edden and Keeler 2004, *J. Magn. Reson.* 166, 53; Fäcke and Berger, *J. Magn. Reson. A* 1996, 119, 260; Marquez et al. 2001, *Magn. Reson. Chem.*, 39, 499; Espinosa et al., 2011, *Magn. Reson. Chem.* 49, 502; Miao and Freeman, 1995, *J. Magn. Reson. A*, 116, 273; Kupce et al. 1994, *J. Magn. Reson. A* 107, 246

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