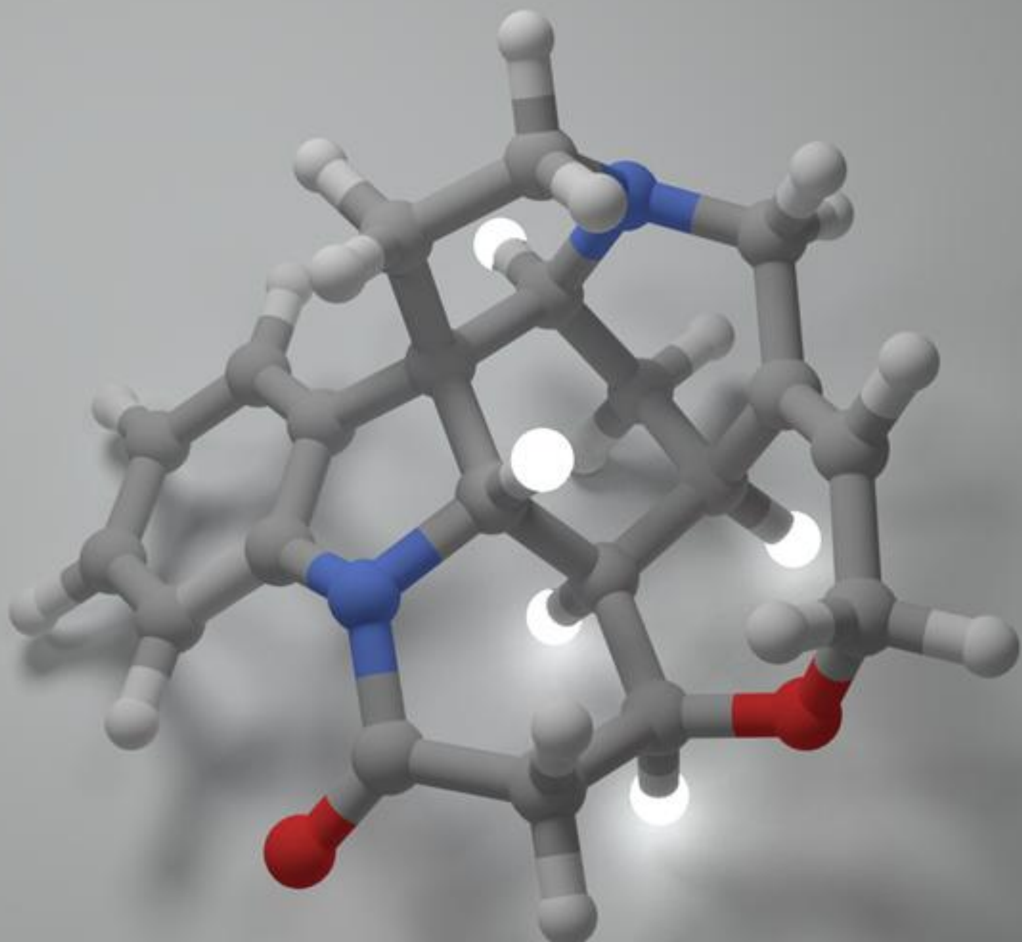
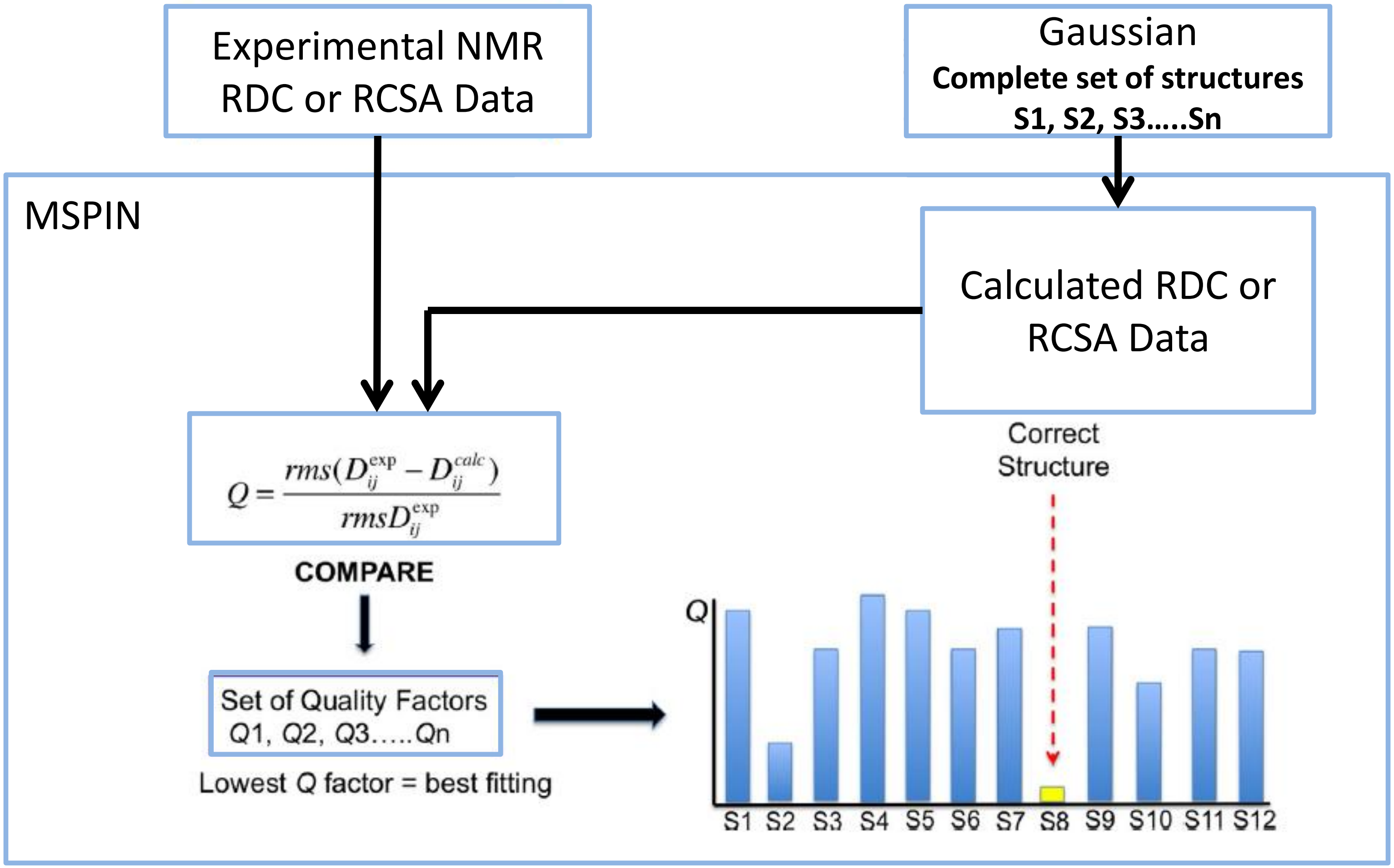
Relative Stereochemistry of Complex Molecules by Anisotropic NMR

Ken Conover
Princeton University



The Big Picture



RDC = Residual Dipolar Coupling
RCSA = Residual Chemical Shift Anisotropy

RR Gil, RDC in Small-Molecule NMR, 2017

Solution NMR

- <u>Isotropic Solution NMR</u> – Is solution state NMR where the solute freely rotates in the solvent.

- <u>Anisotropic Solution NMR</u> – Is solution state NMR where the solute's rotation is partially restricted. As a consequence, the solute is slightly aligned by the partially ordered media with the external magnetic field.

Solution State NMR Interactions

	Isotropic	Anisotropic
Chemical Shift	✓	✓
J-coupling	✓	✓
NOE	✓	✓
Dipolar Coupling	✗	✓
Chemical Shift Anisotropy	✗	✓

✓ Detectable

✗ NOT Detectable

Pros & Cons of Anisotropic NMR Analysis

The Good…

- Determine the relative configuration of stereocenters regardless of the distance between them.

- Removes investigator bias.

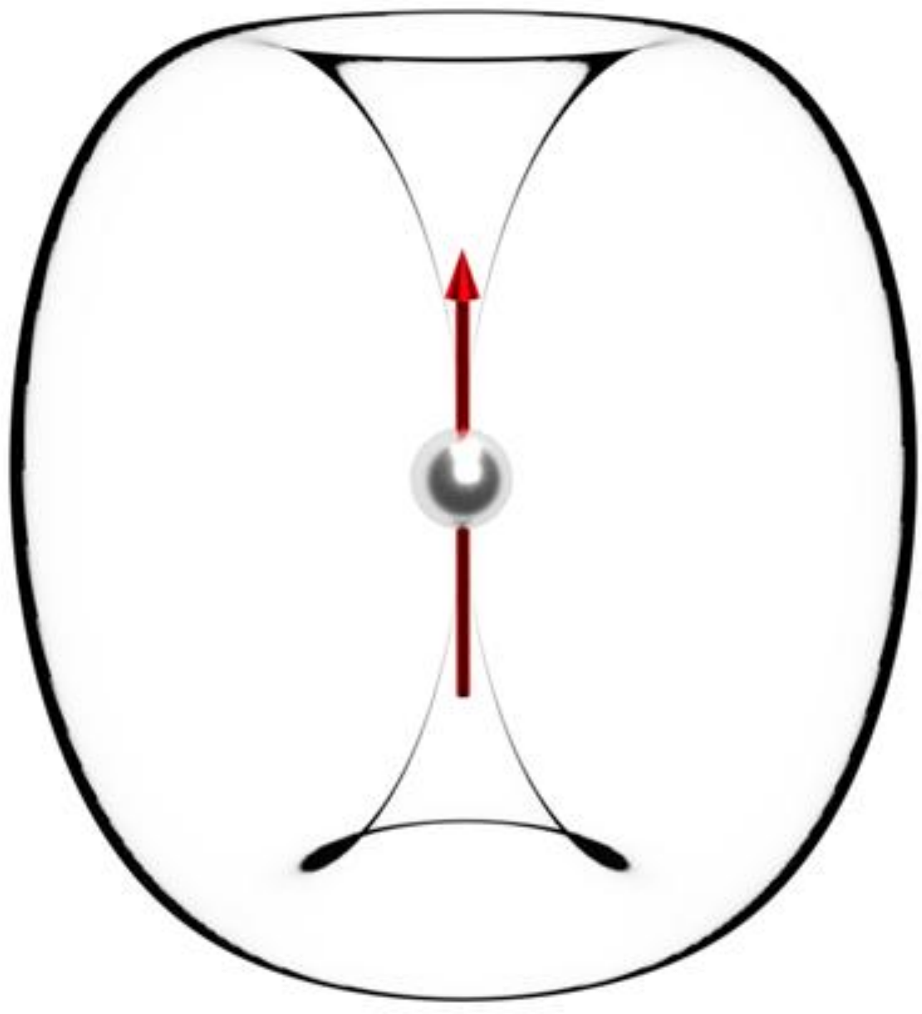
- Provides an orthogonal check for a structure solved by isotropic NMR.

The Bad…

- Will not work on flexible molecules

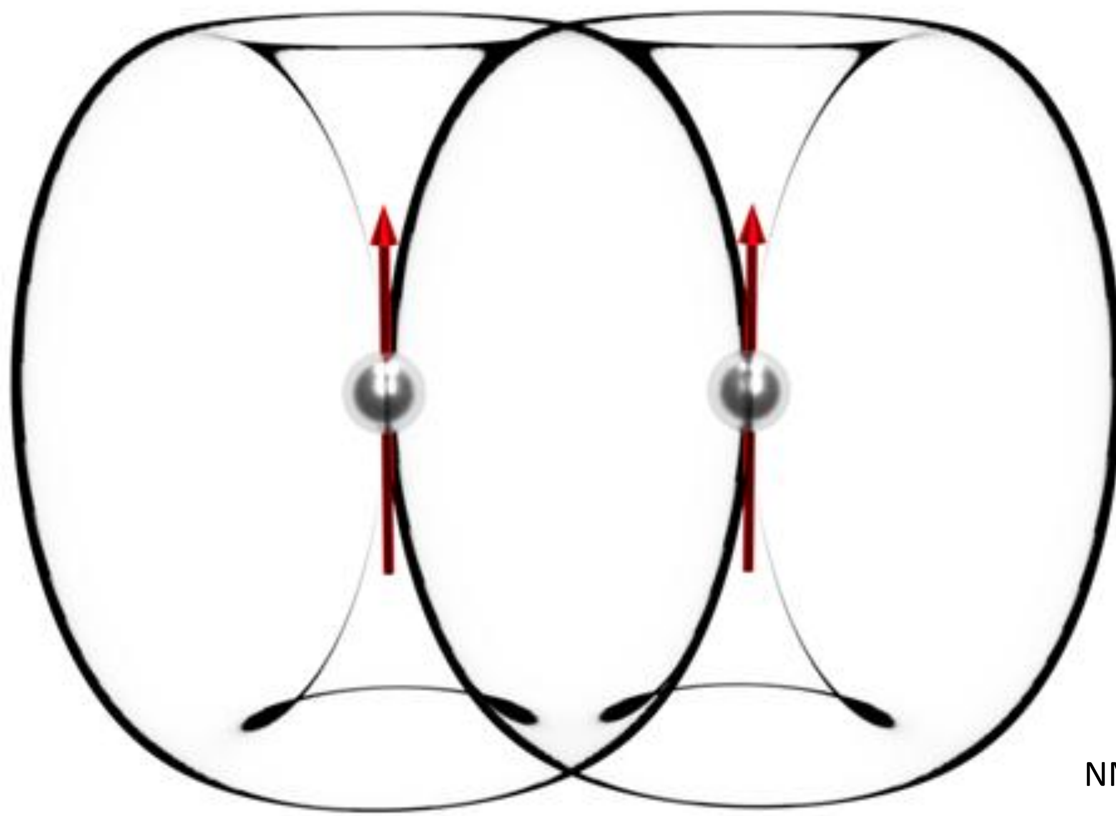
Nuclear Dipole

Spin ½ nuclei are dipoles



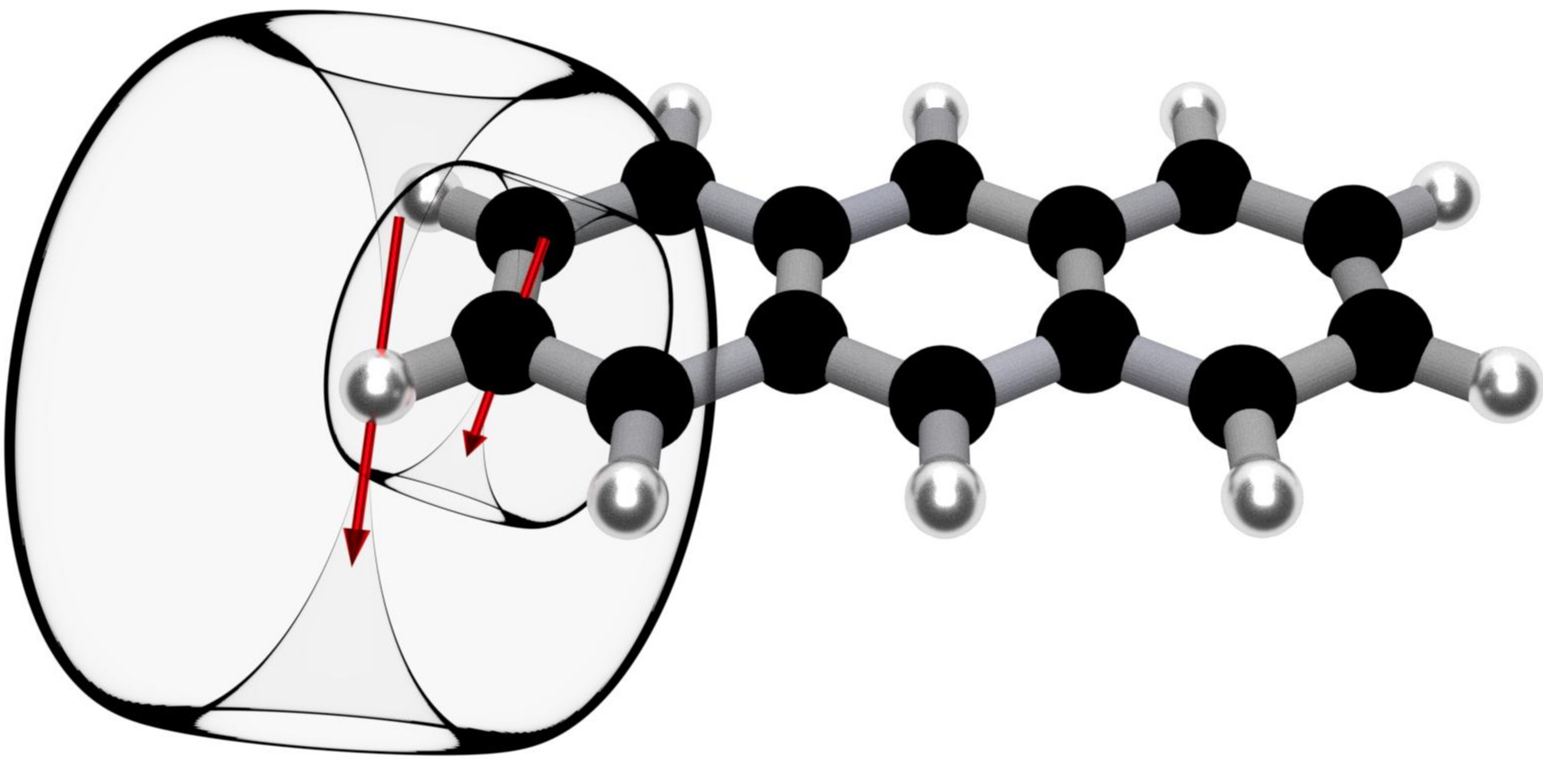
NMR Active Nucleus

Dipolar Coupling



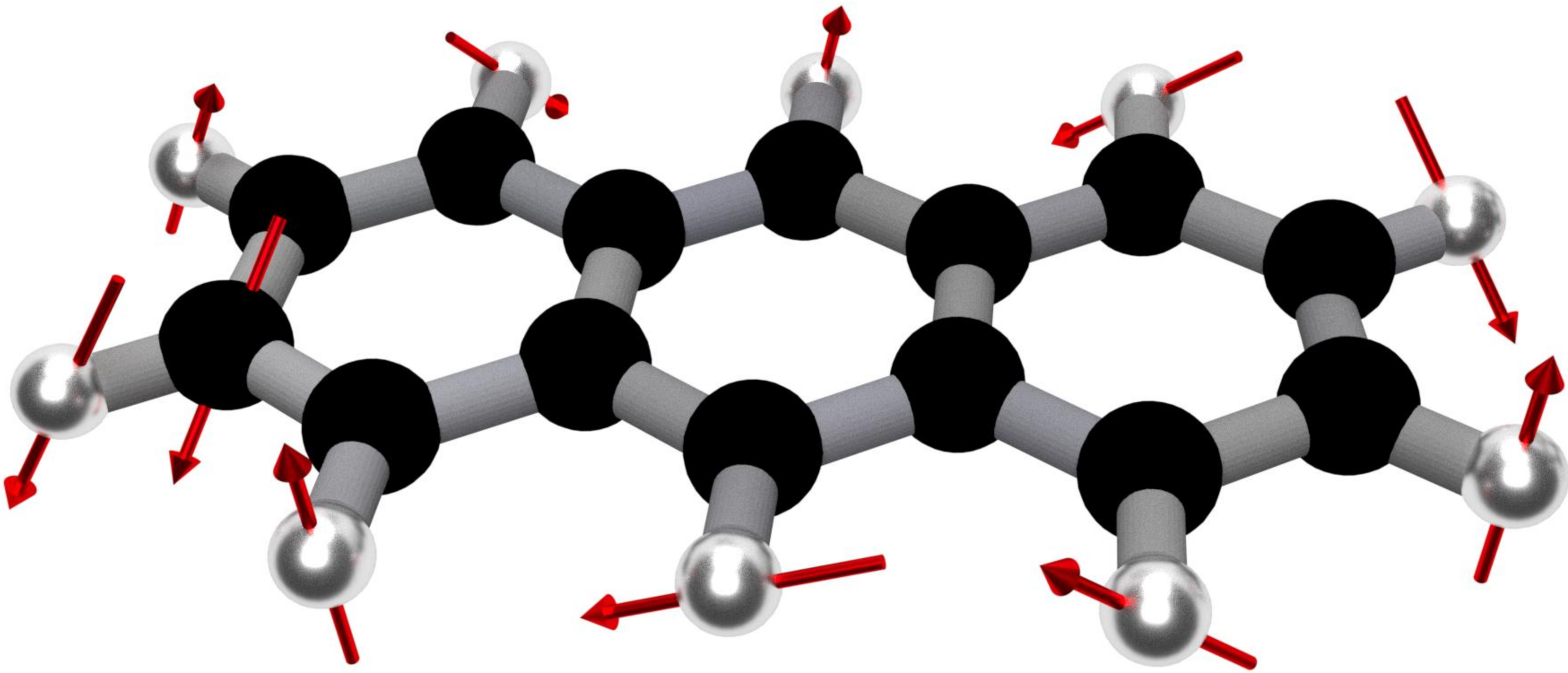
NMR Active Nuclei

Anthracene



Dipolar Coupling of Two Bonded NMR Active Nuclei

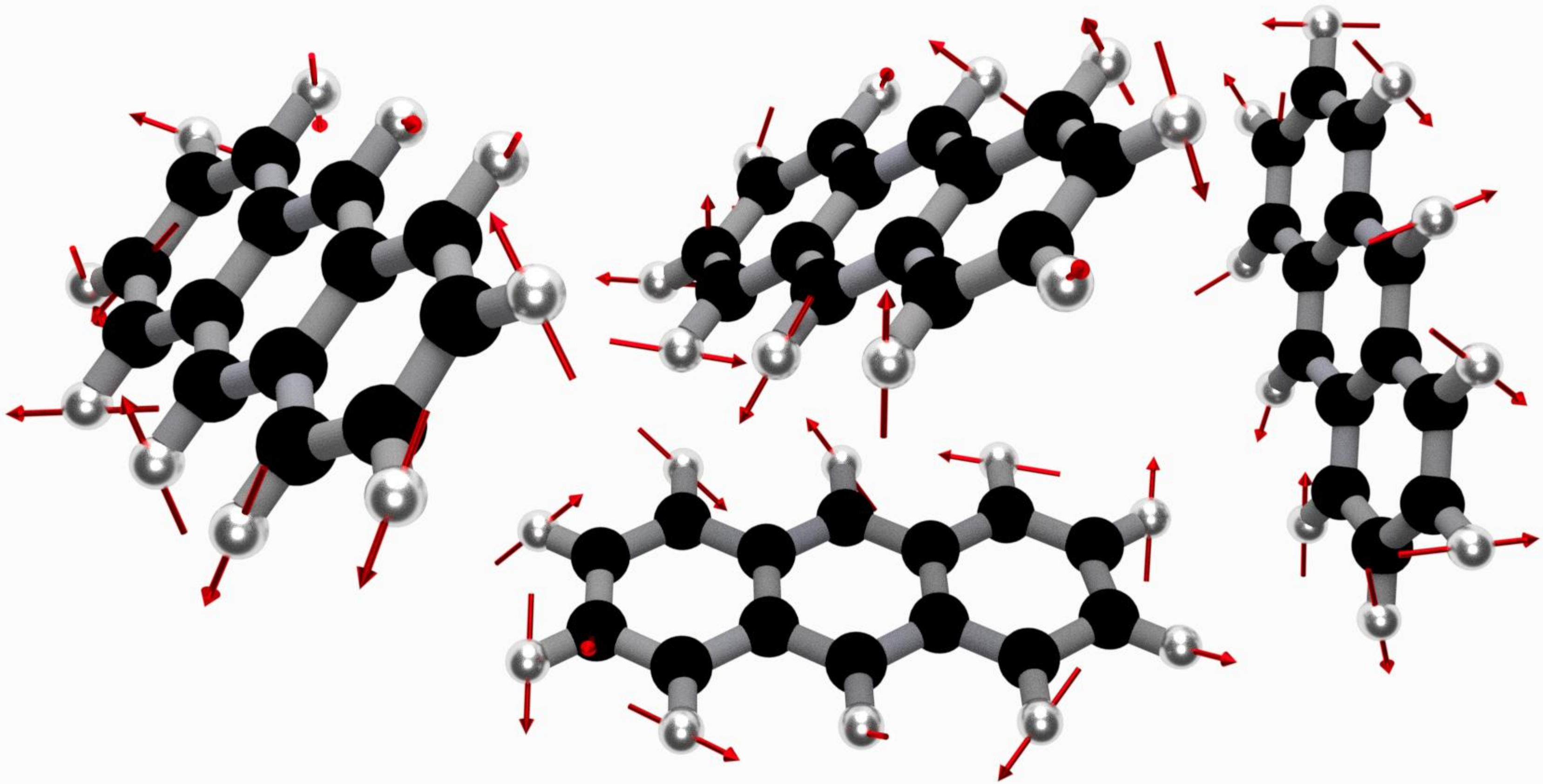
Anthracene



No Magnetic Field

Isotropic Solution, No Magnet

Anthracene in solution - nuclear spin magnetic vectors point in all directions
- molecular motion is random (isotropic)

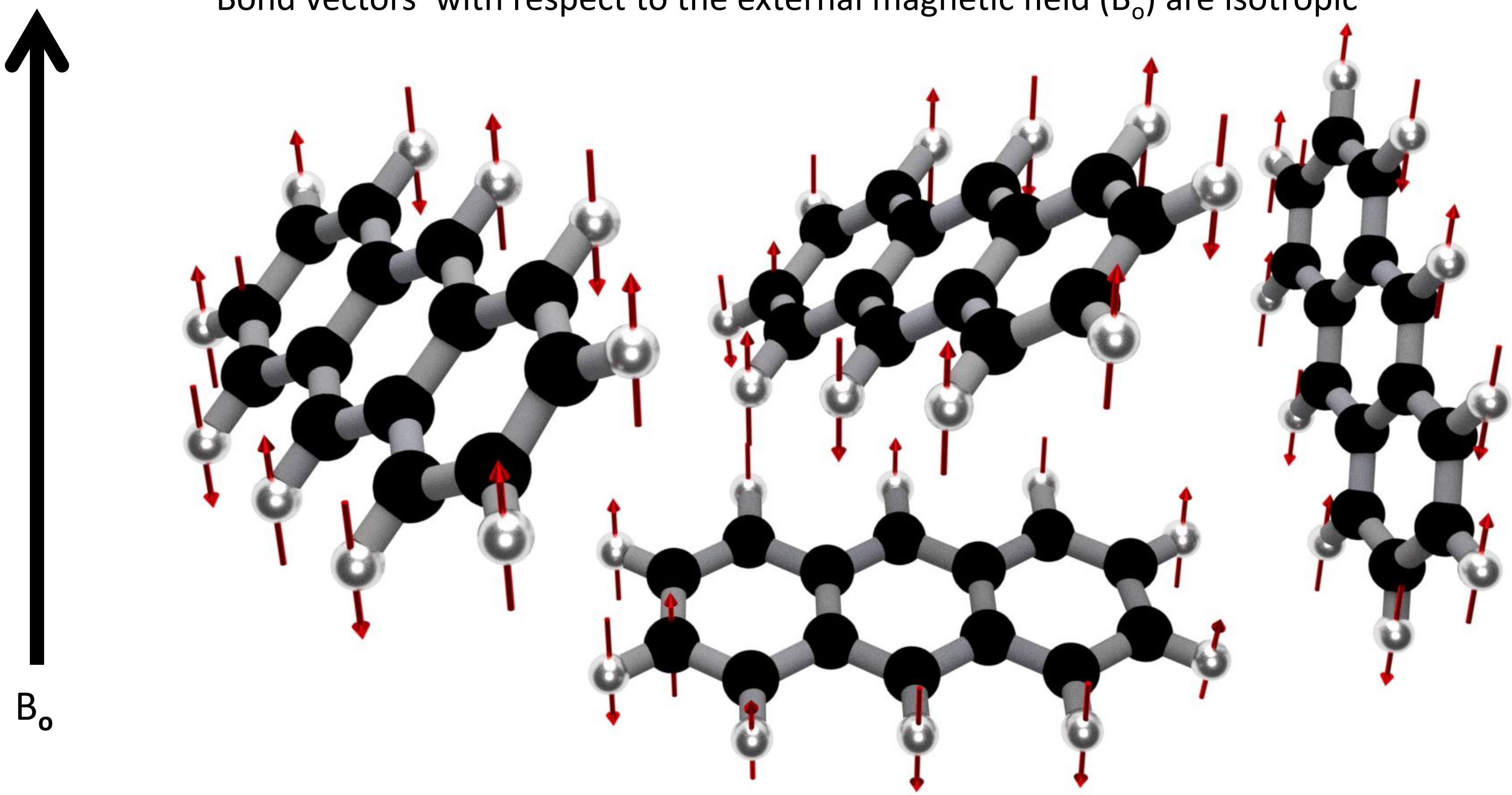


Solvent not shown

Isotropic Solution in a NMR

Anthracene in solution - nuclear spin magnet vectors align with magnetic field (B_o)

Bond vectors' with respect to the external magnetic field (B_o) are isotropic

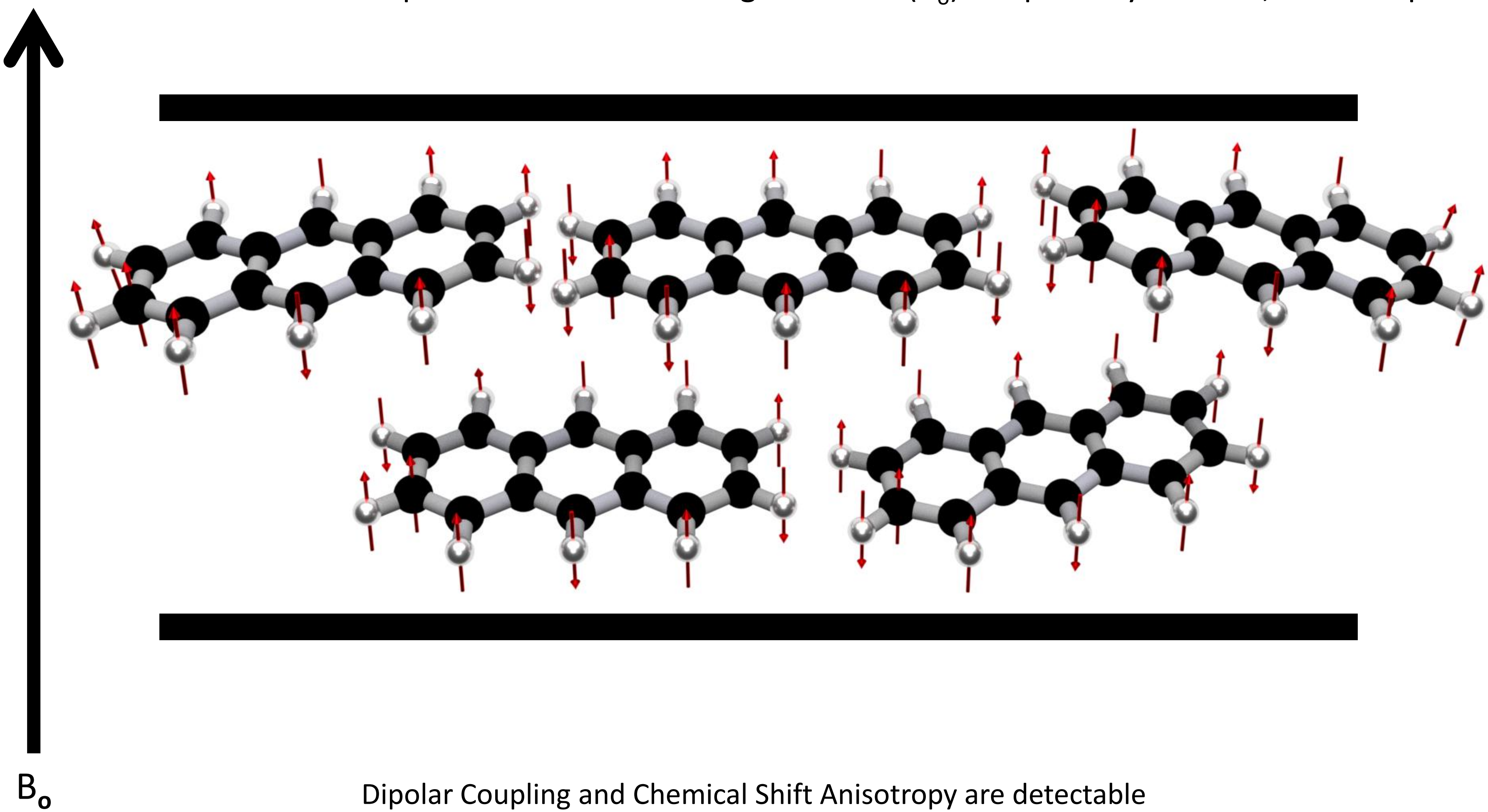


Dipolar Coupling and Chemical Shift Anisotropy not detectable

Anisotropic Solution in a NMR

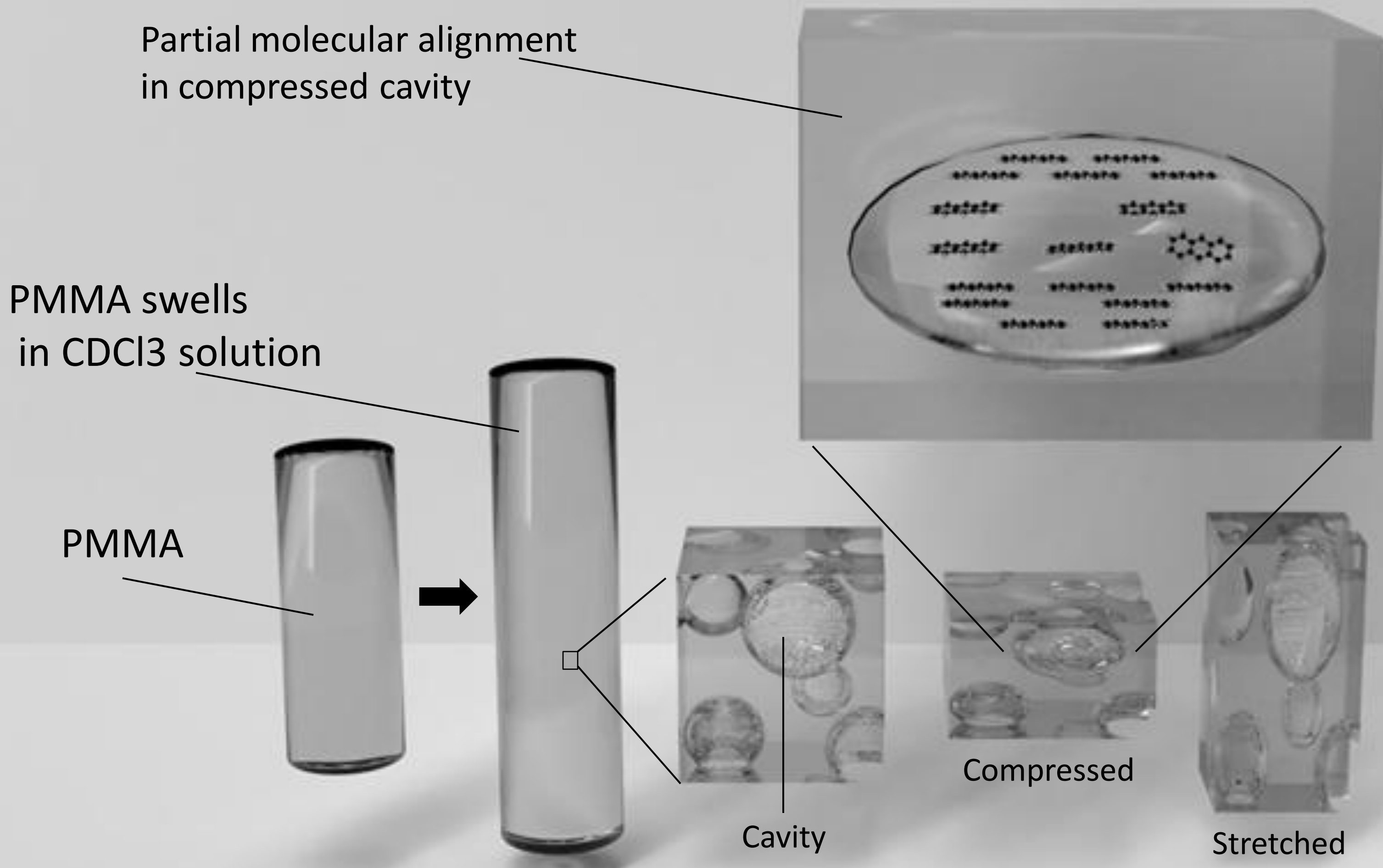
Anthracene in solution - nuclear spin magnet vectors align with the external magnetic field (B_o)

Bond vectors' with respect to the external magnetic field (B_o) are partially ordered, anisotropic



Dipolar Coupling and Chemical Shift Anisotropy are detectable

Alignment Medium



PMMA = Polymethyl Methacrylate

Gel Preparation

Preparation of Crosslinked PMMA of Gels

A solution containing MMA (10 mL), V-70 (0.0030 g), and acetone-d6 (2 mL) was first prepared, and 10 mL were taken (corresponding to 8.33 mL or 7.79×10-2 mol of MMA) and mixed with EGDMA (40 µL, 2.12×10-4 mol). The fraction of crosslinker in the polymerizing mixture was 0.27 mol %. The resulting solution was transferred to NMR tubes (d = 3 mm or 5 mm), which were then capped with rubber septa, and the septa were secured with tape. Each tube was evacuated for short time and back-filled with nitrogen. The cycle was repeated 3 times, and the NMR tubes were then inserted in an oil bath at 50oC. The polymerizations were carried out for 5 h, and then the tubes were taken out of the heating bath, the septa were removed and the gels were left to dry slowly at ambient conditions. Rods of 2 mm and 4 mm in diameter were obtained, respectively.

R.R. Gil et al, Residual Dipolar Couplings (RDCs) Analysis of Small Molecules Made Easy:
Fast and Tuneable Alignment by Reversible Compression/Relaxation of Reusable PMMA Gels,
Chem. Eur. J. 2010, 16, 3622 – 3626

Shopping List for Gels

- METHYL METHACRYLATE, M55909-500ML, Sigma
- ETHYLENE GLYCOL DIMETHACRYLATE, 335681-5ML, Sigma
- V70 free radical initiator, LB-V70-5g, Waco
- 3mm NMR tube, NE-H3-7, New Era
- 4mm NMR tube, NE-400-4-250, New Era

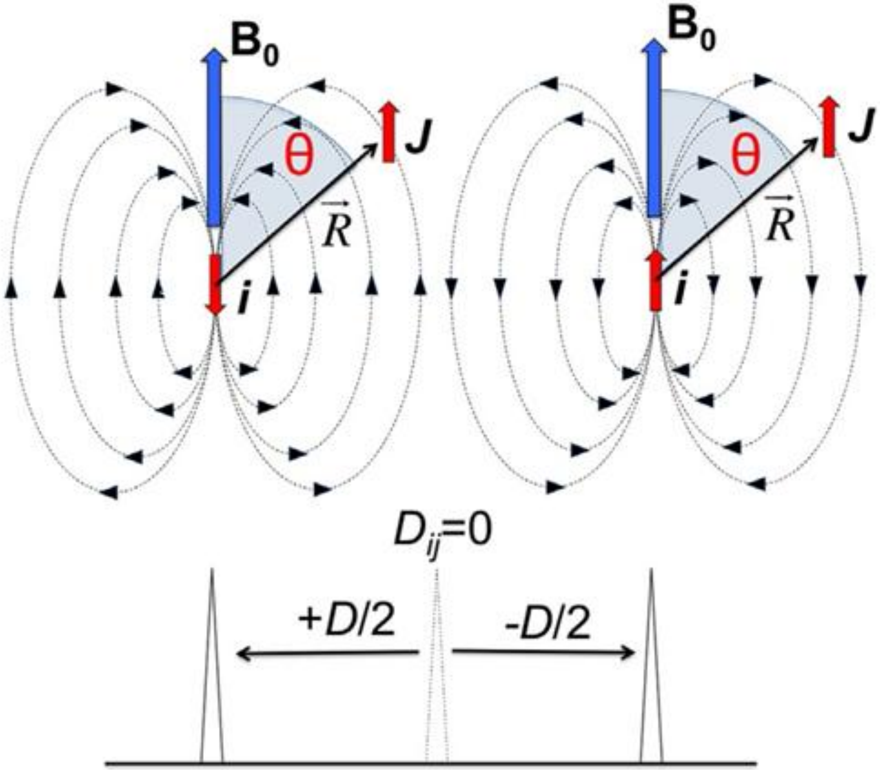
Wash Gels

Washing the gels removes excess monomers.

- Place gels in 1:1 solution of acetone and methanol for 5 hours.
- Place gels in chloroform overnight. Repeat the following day.
- Air dry gels and store.

Dipolar Coupling

The dipolar coupling is a direct interaction between two magnetically active nuclei (i and j), which can be bonded or nonbonded. D_{ij} is obtained experimentally , allowing the equation to be solved for the angle of the internuclear vector.



$$D_{ij} = -\frac{3\gamma_i\gamma_j\mu_0\hbar}{8\pi^2R^3}\left(\cos^2\theta - \frac{1}{3}\right)$$

D_{ij} Magnitude of dipolar coupling

γ Gyromagnetic ratio

μ_0 Permeability of a vacuum

$\hbar$ Planck constant divided by 2π

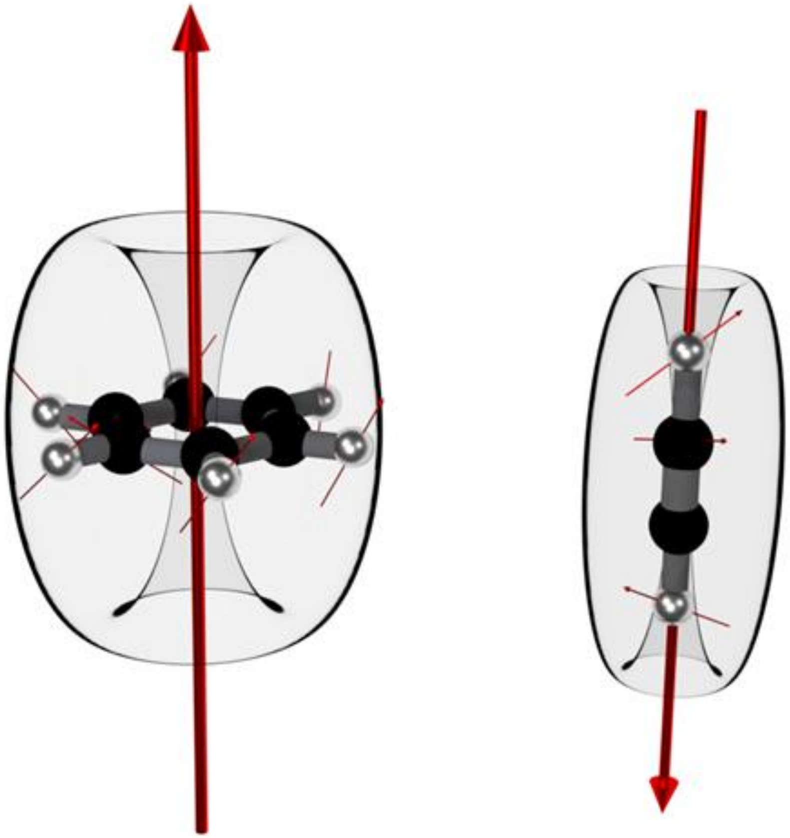
R Distance between *i* and *j*

B_0 External magnetic field

$\vec{R}$ Internuclear vector

RR Gil, RDC in Small-Molecule NMR, 2017

Chemical Shift Anisotropy



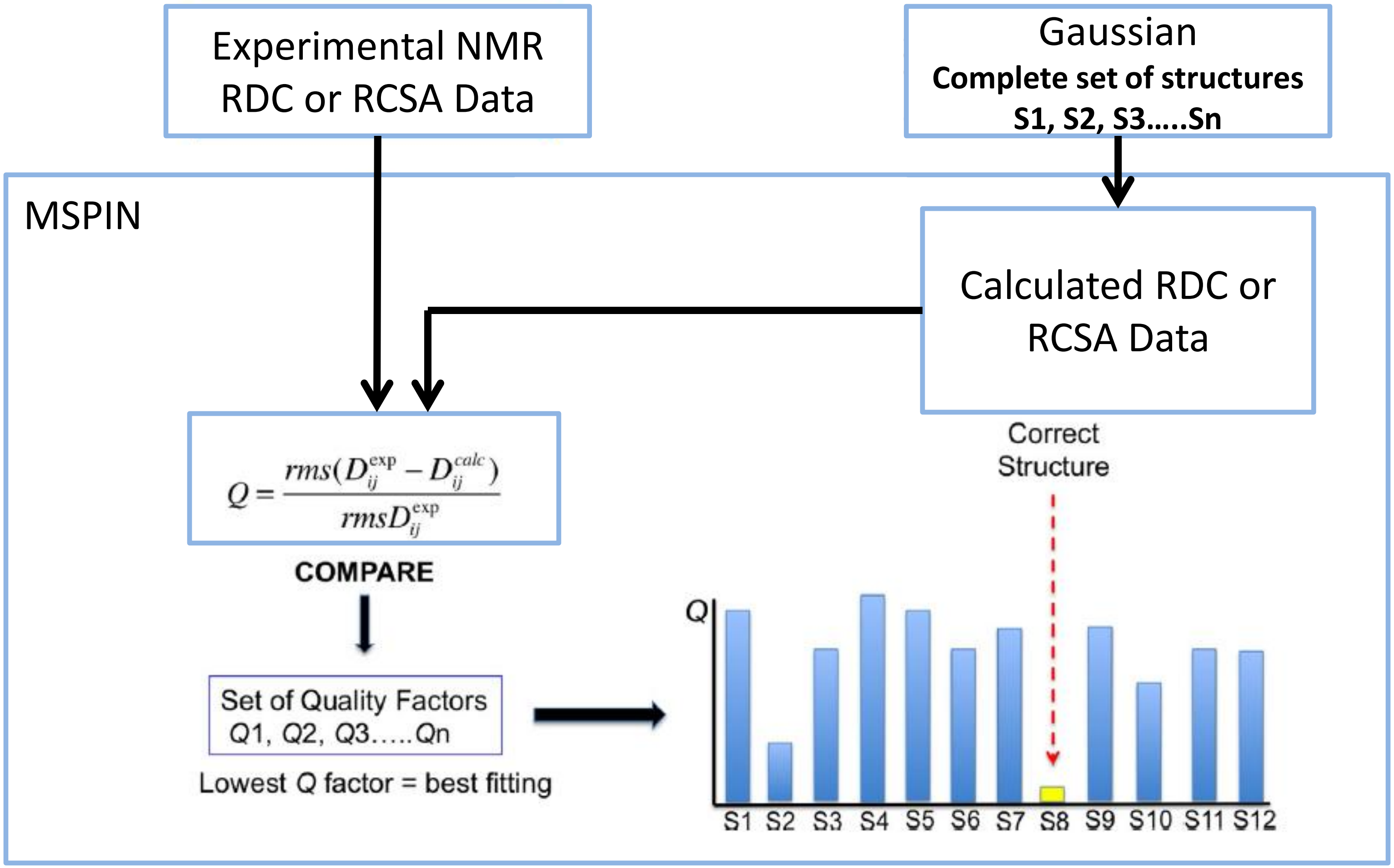
$$\sigma = \begin{bmatrix} \sigma_{xx} & \sigma_{xy} & \sigma_{xz} \\ \sigma_{yx} & \sigma_{yy} & \sigma_{yz} \\ \sigma_{zx} & \sigma_{zy} & \sigma_{zz} \end{bmatrix}$$

Chemical Shift Tensor

σ - Chemical Shift

The electronic environment around a nucleus is generally anisotropic. For this reason, the chemical shift is also anisotropic. In isotropic media the fast tumbling averages out the orientational difference, while in partially oriented media the averaging is incomplete leading to a different observed chemical shift.

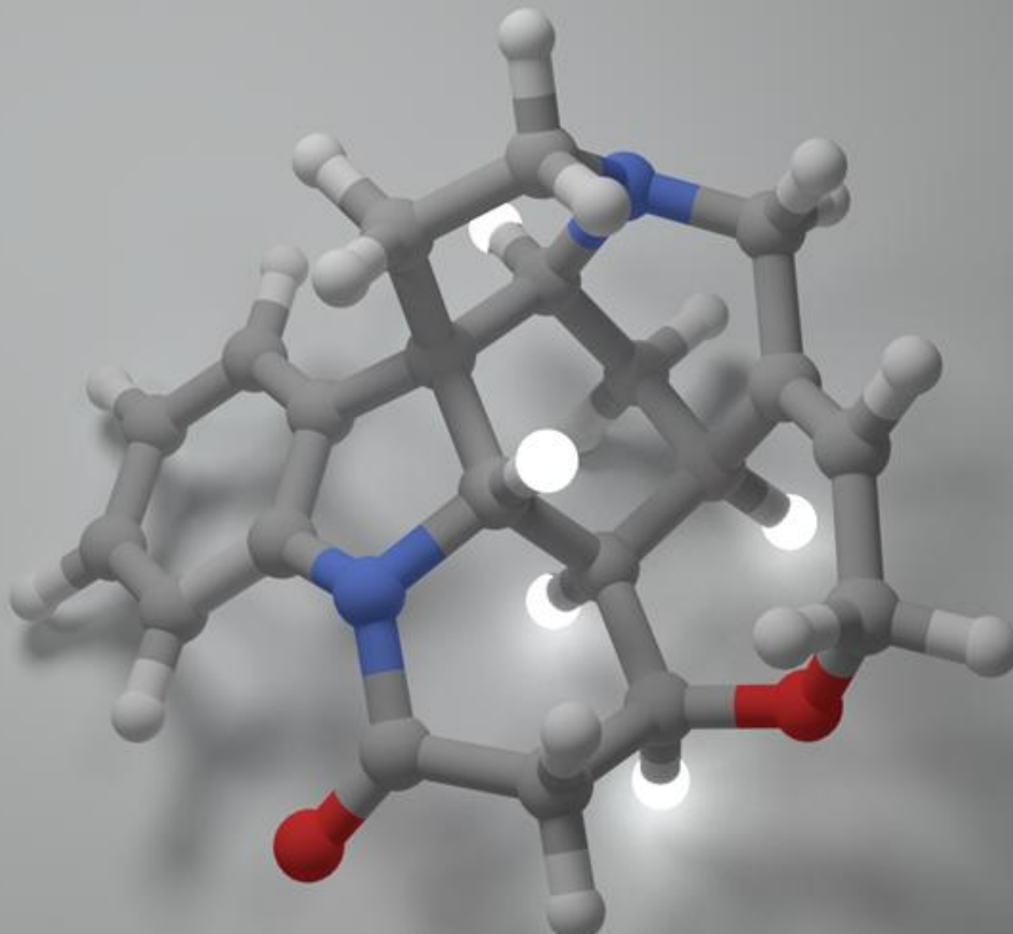
The Big Picture



RR Gil, RDC in Small-Molecule NMR, 2017

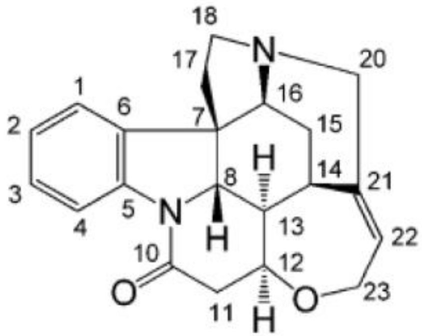
Our Test Molecule

Strychnine

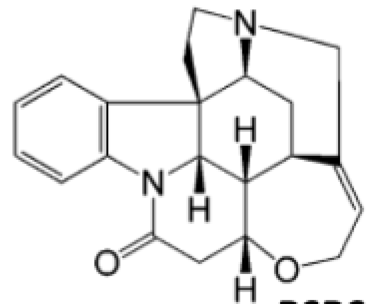


Gaussian

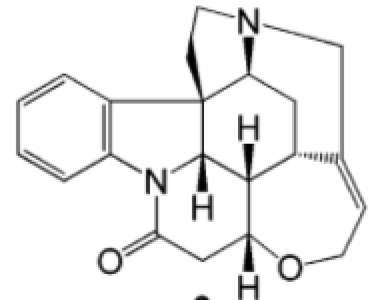
DFT-optimized Structures



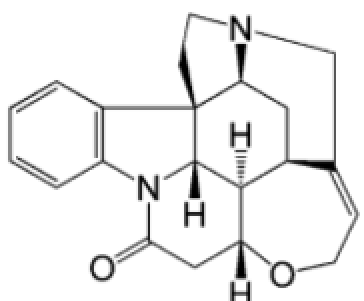
1, *RSSRRS*



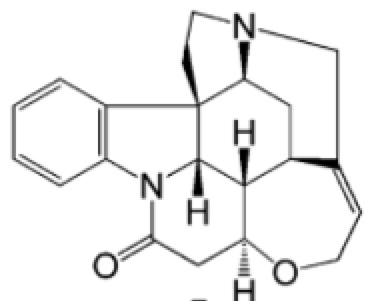
2 ***RSRSRS***



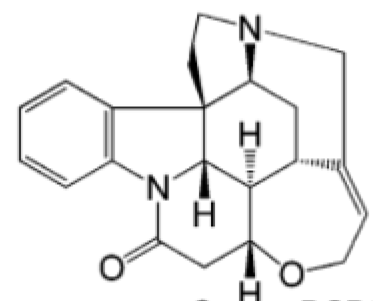
3 ***RSRSSS***



4 ***RSRRRS***



5 ***RSSSRS***

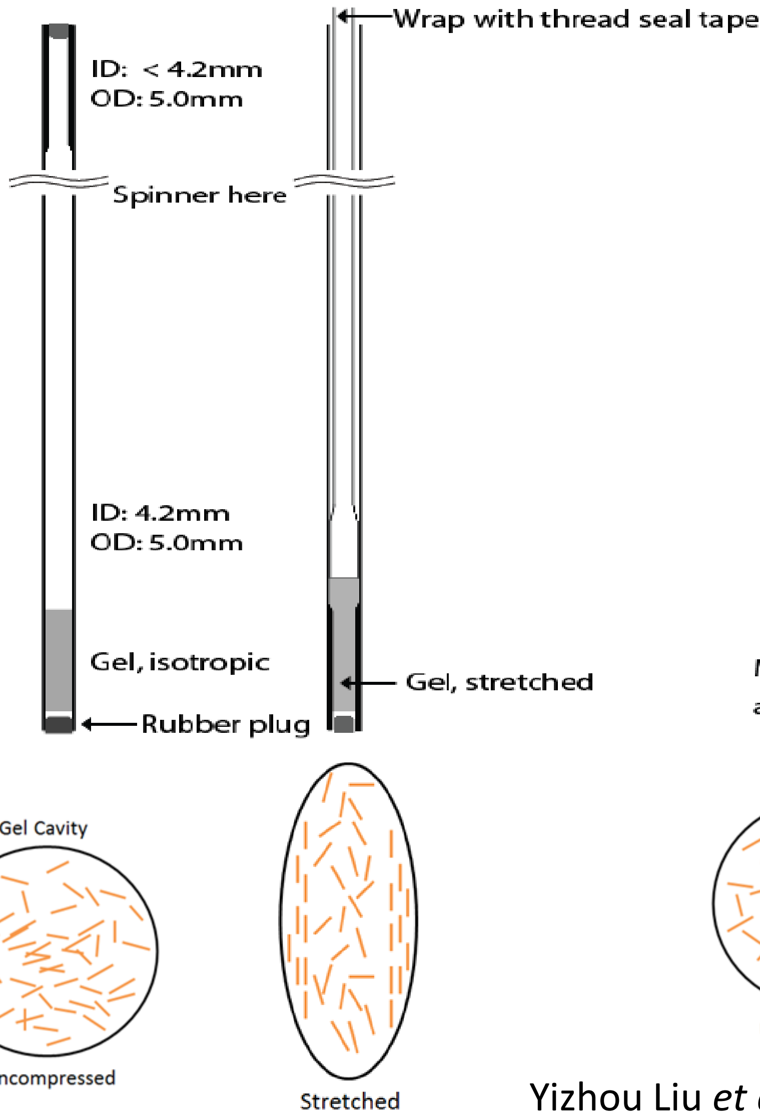


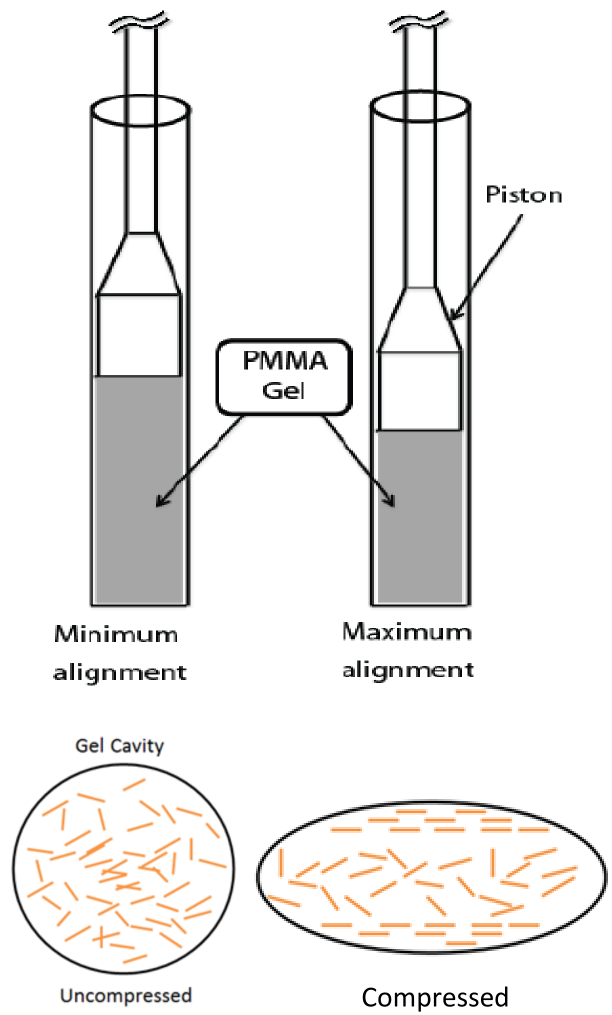
6 ***RSRRSS***

The configurations were labelled via the *R* or *S* configuration of carbons C7, C8, C13, C12, C14, and C16 respectively, for example *RSSRRS* for the true configuration.

Yizhou Liu *et al.* J. Am. Chem. Soc. 2016, 138, 9548−9556

NMR Tubes



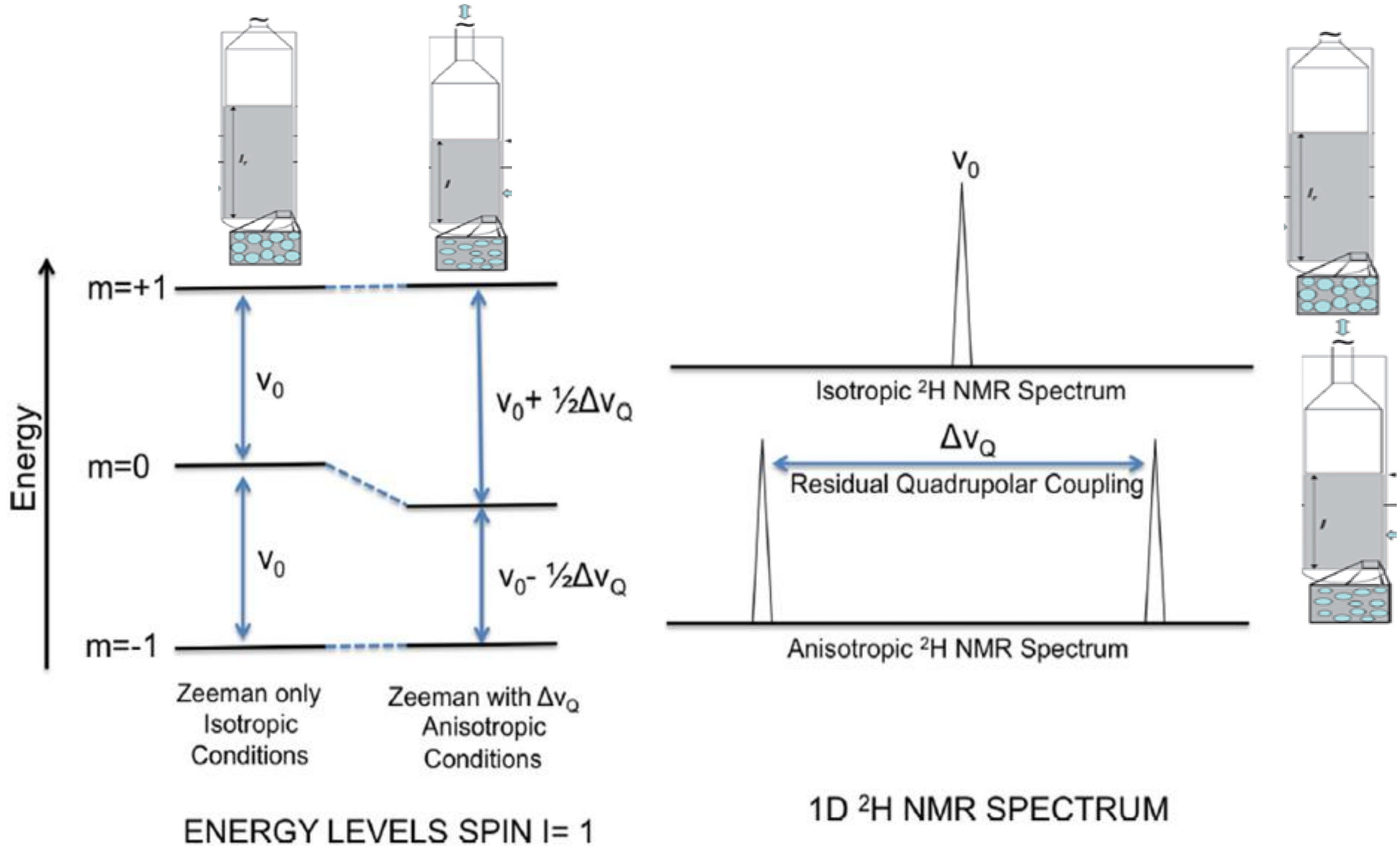


Yizhou Liu *et al.* J. Am. Chem. Soc. 2016, 138, 9548−9556

Shopping List for Devices

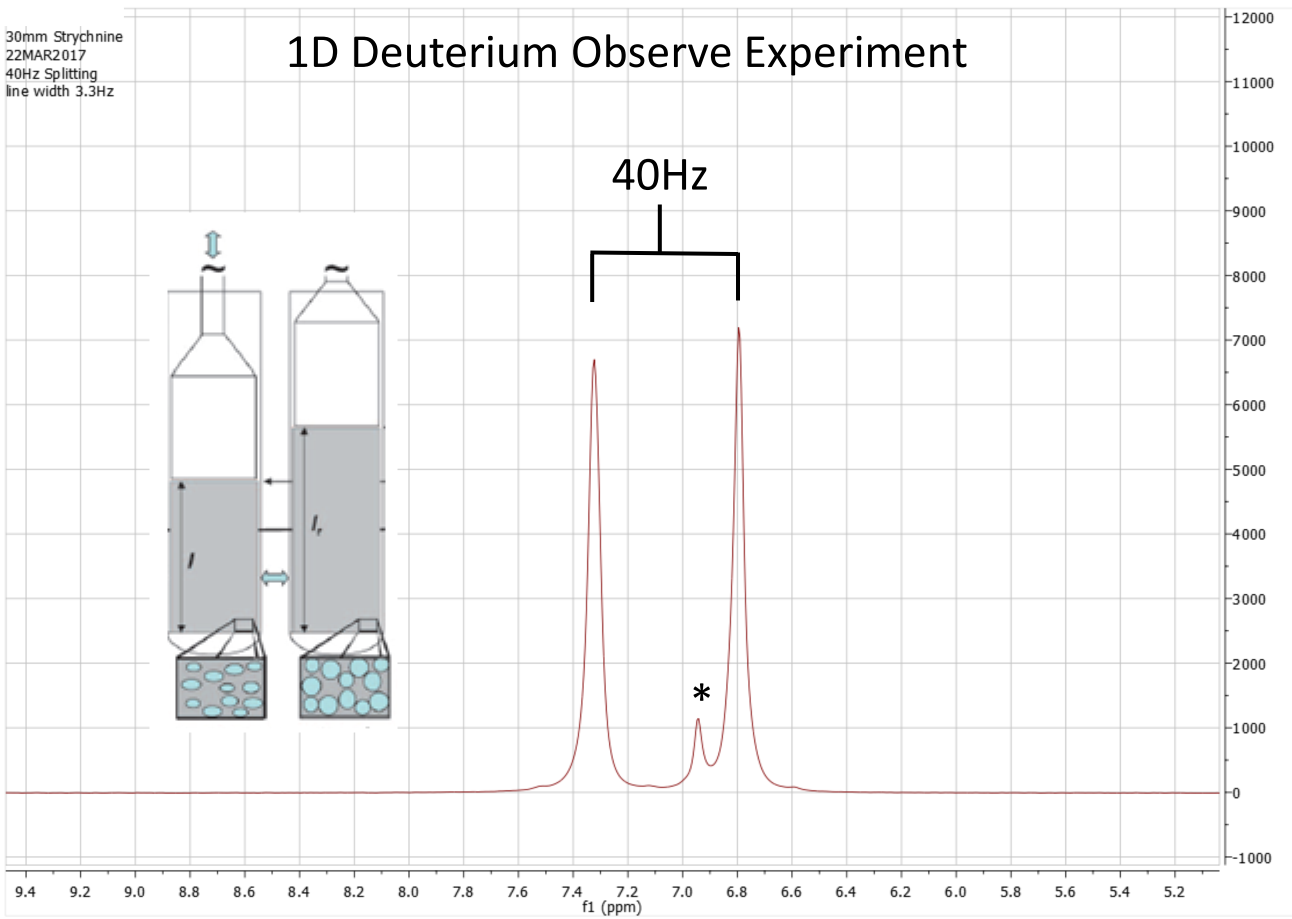
- Compression Gel Device, NE-375-5, New Era
- Stretched NMR Tube, NE-HP5-3.2-GT-7, New Era

Anisotropic Check



RR Gil, Encyclopedia Spect. 2017 946-955

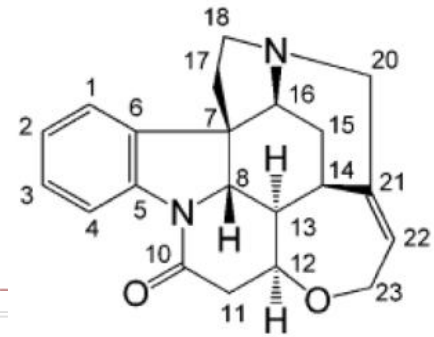
2H RQC Chloroform

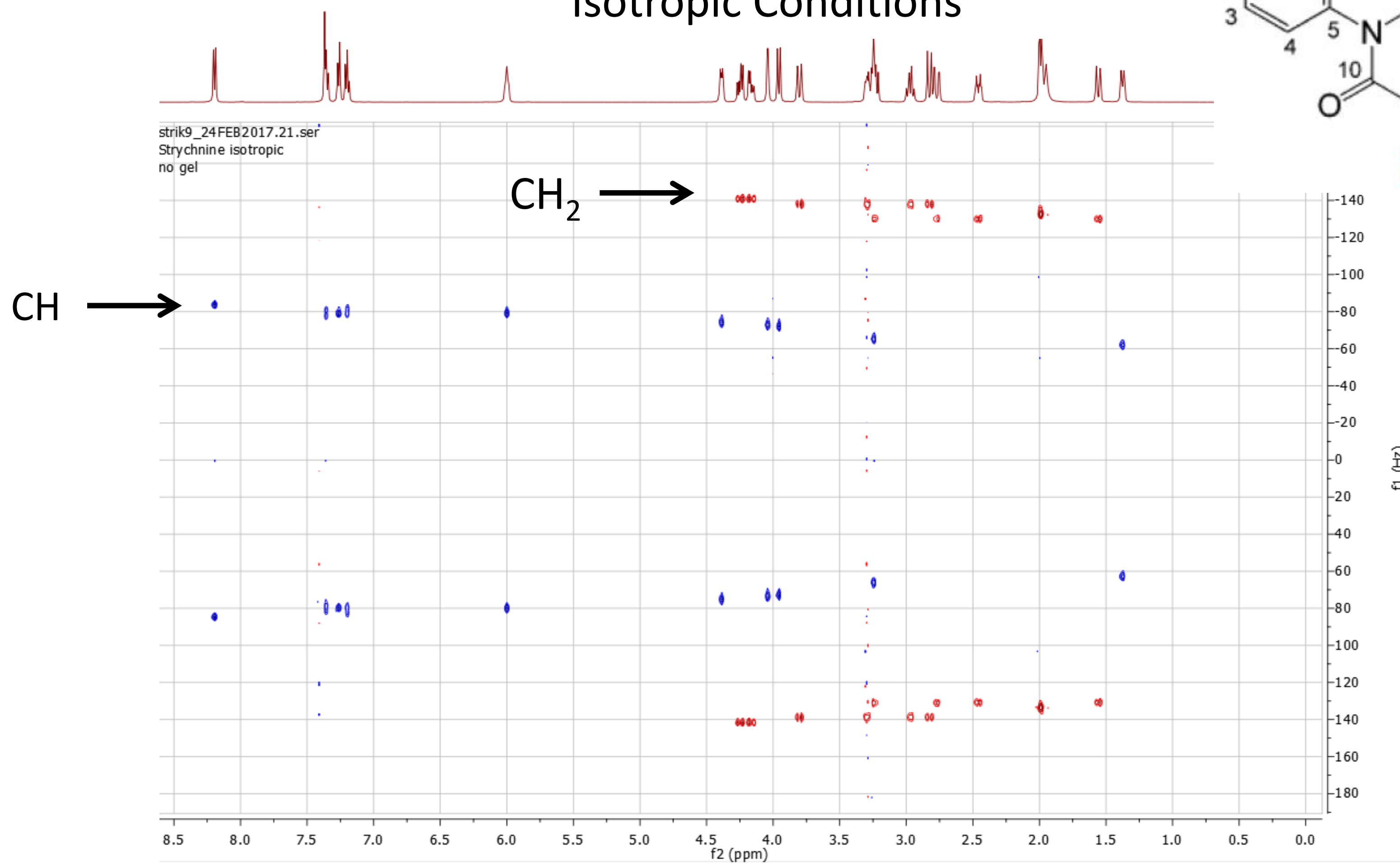


* Isotropic contribution – some of the solution remains outside the gel

The RDC Experiment

Isotropic Conditions



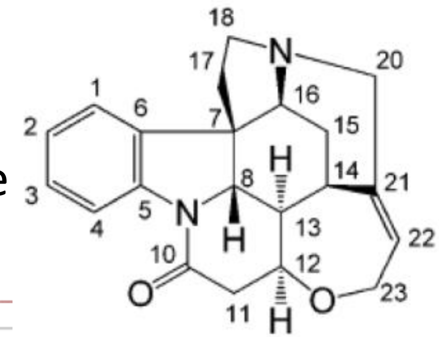


Homodecoupled J-resolved HSQC Experiment

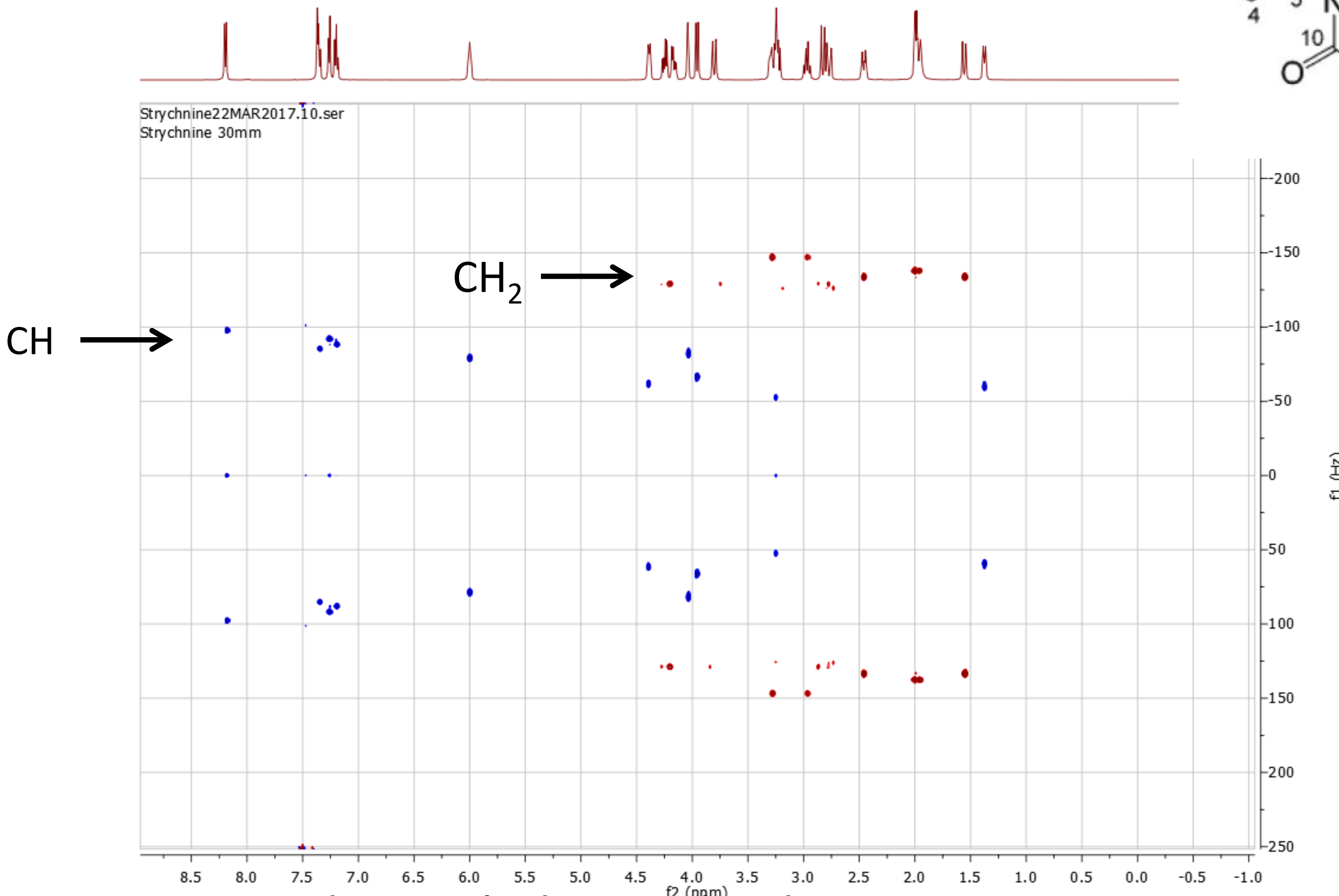
One Bond J-coupling between Proton and Carbon

The RDC Experiment

Anisotropic Conditions using a Compressed Ge



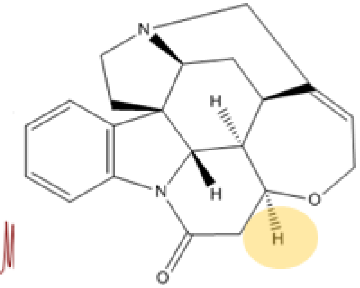
1, *RSSRRS*

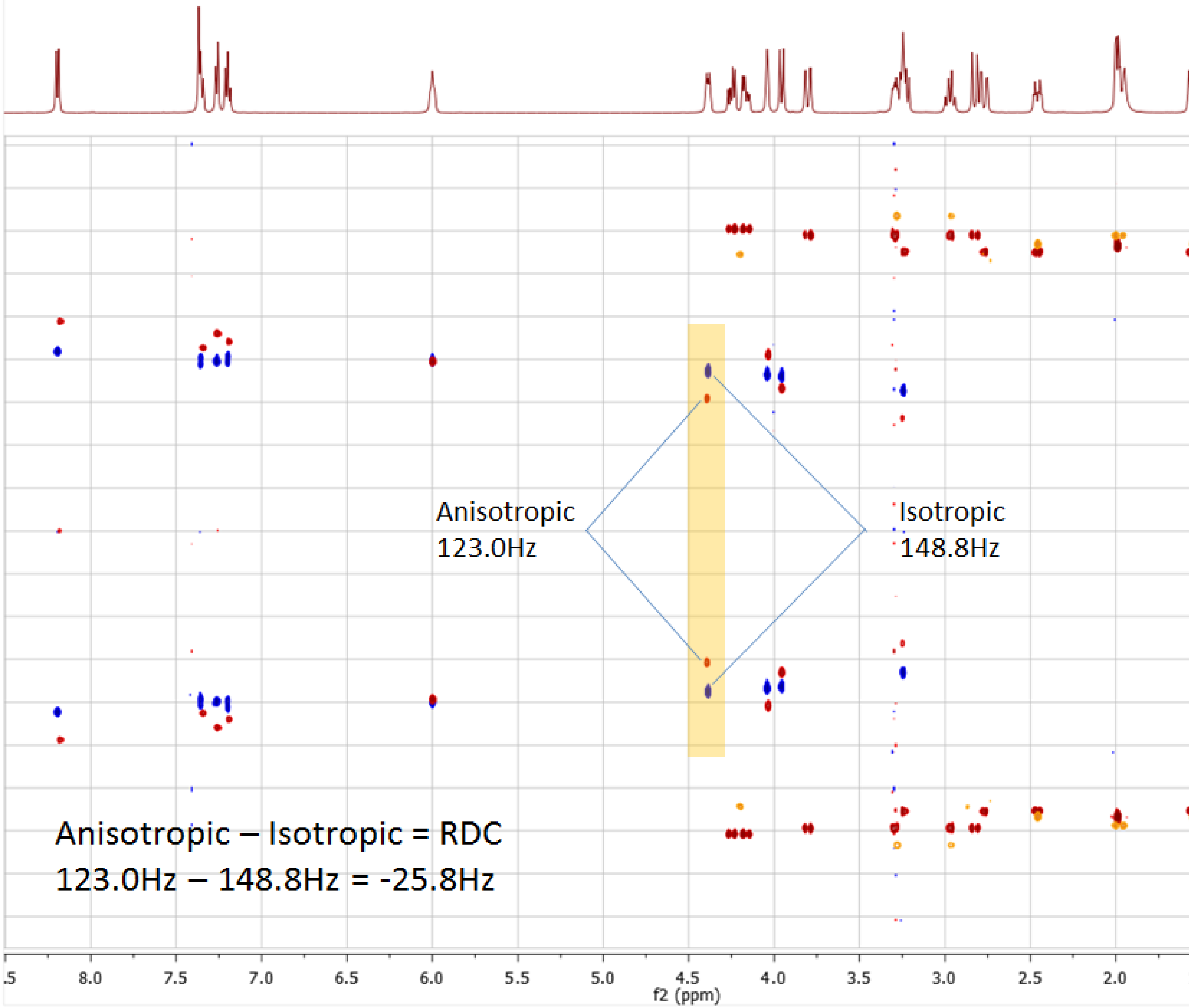


Homodecoupled J-resolved HSQC Experiment

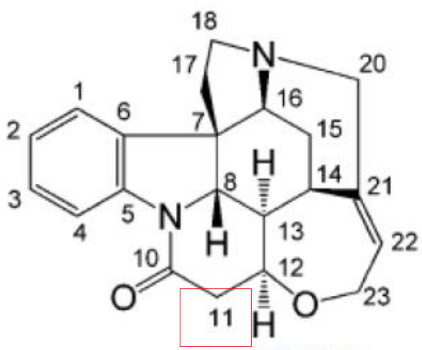
One Bond J-coupling between Proton and Carbon

Overlay of Iso and Anisotropic conditions





RDC MSPIN Script

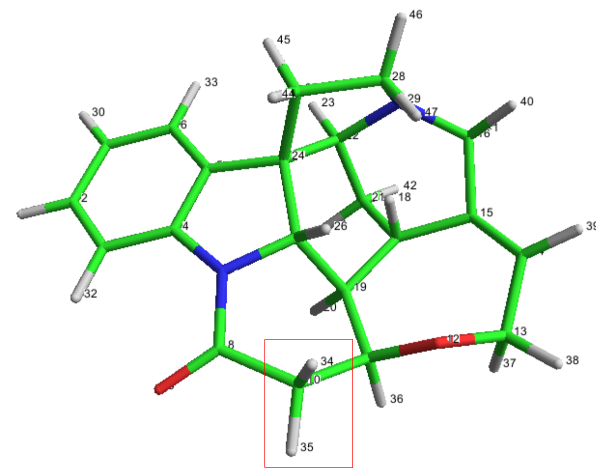


1, RSSRRS

Strychnine_RDC - Notepad

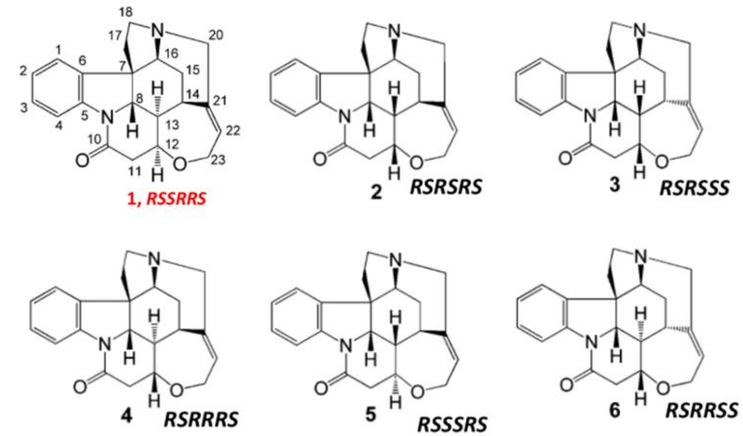
File Edit Format View Help

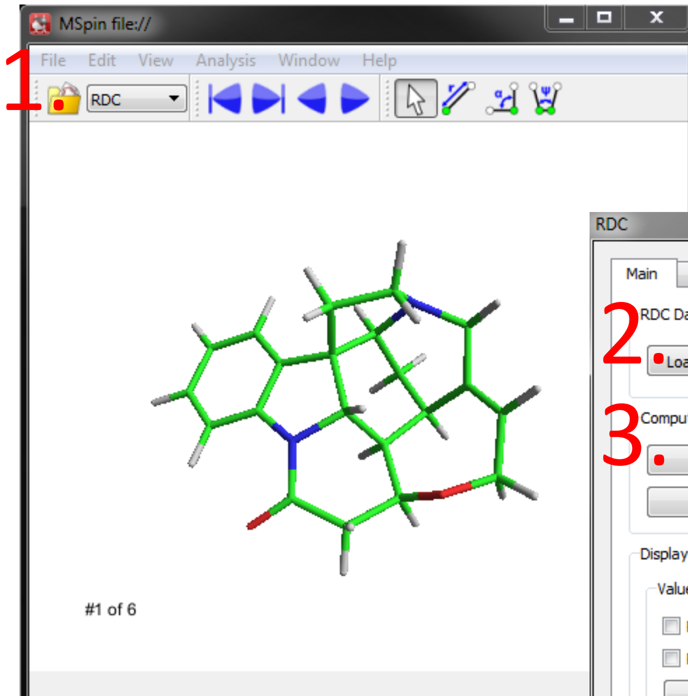
```
rdc_data {

#C1-H1
6 33 25.5
#C2-H2
1 30 18.1
#C3-H3
2 31 10.7
#C4-H4
3 32 26.8
#C8-H8
25 26 -12.9
#C12-H12
11 36 -26.6
#C13-H13
19 20 -6.3
#C14-H14
17 18 -26.3
#C16-H16
22 23 17.8
#C22-H22
14 39 -1.6
#C11-H11a*
10 34 -7.8
#C11-H11b
10 35 -10.2
#C18-H8a
28 46 17.2
#C18-H18b
28 47 16.7
#C20-H20a
16 40 -17.7
#C20-H20b
16 41 -19.4
#C23-H23a
13 37 -24
#C23-H23b
13 38 -24
}

permutations{
34 35
46 47
40 41
37 38
}
```

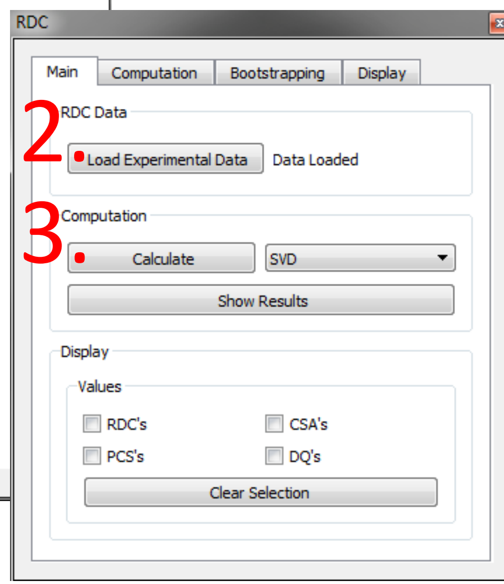


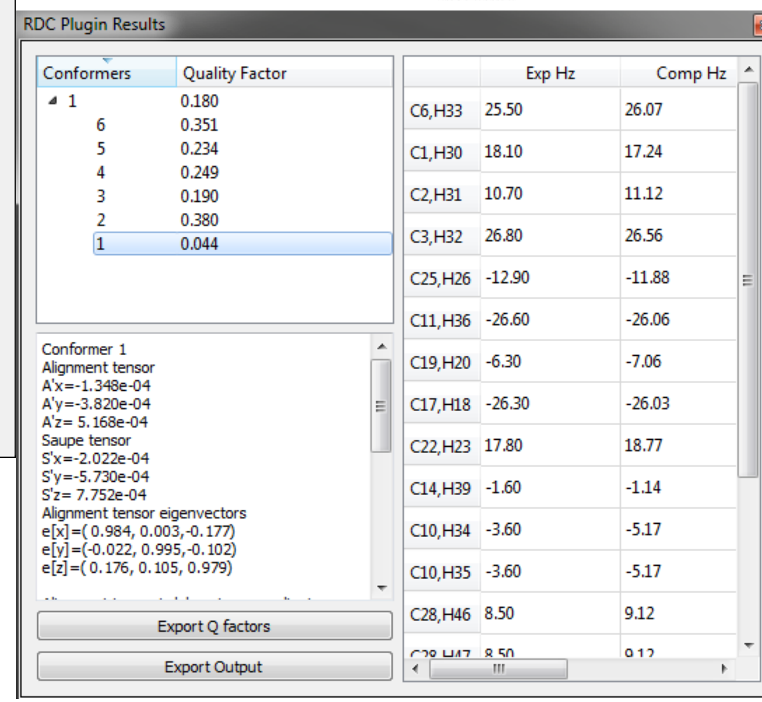
MSPIN RDC DATA

Strychnine





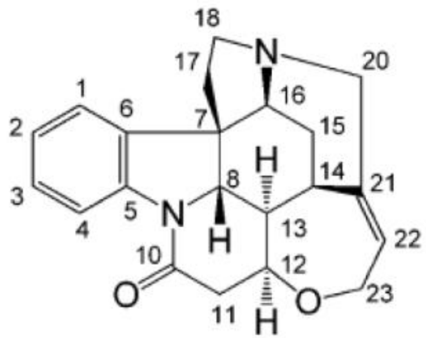




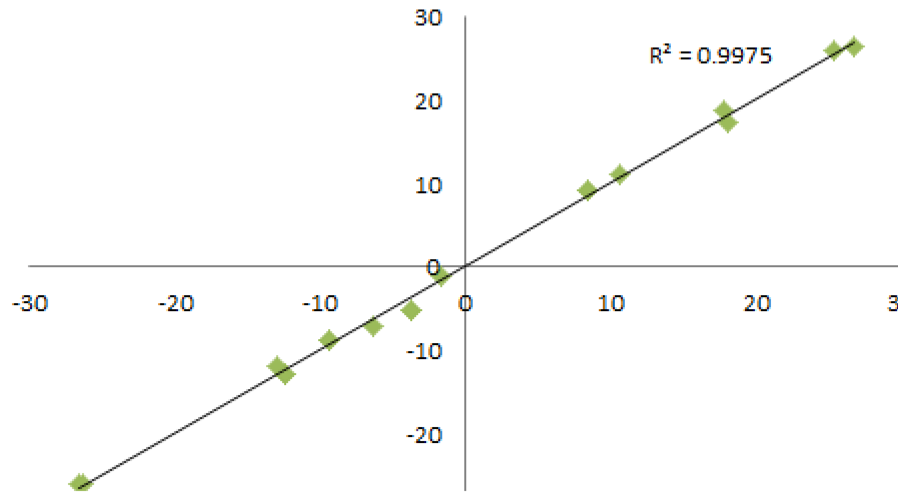
1. Load Structures
2. Load Experimental Data
3. Calculate Q factors

Computational Vs. Experimental

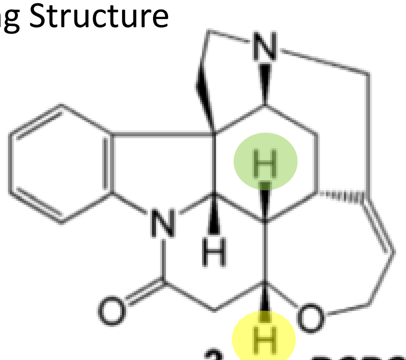
Correct Structure



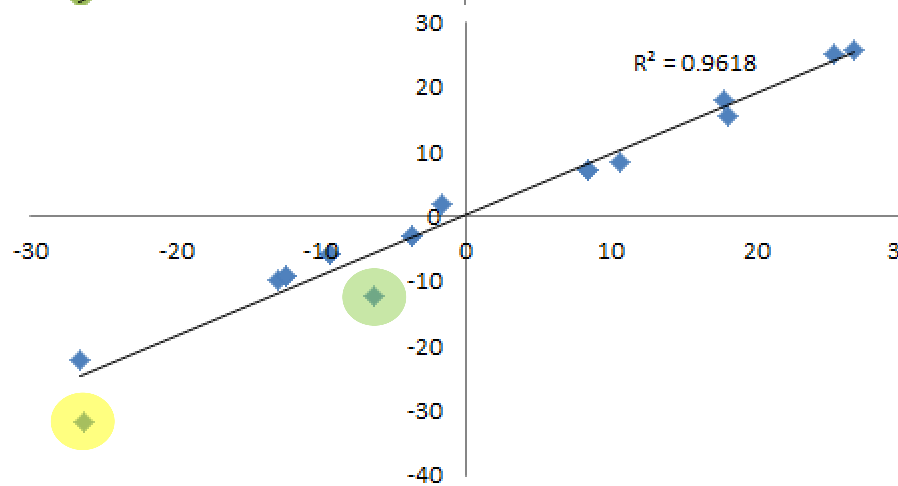
Q = 0.044 **1, *RSSRRS***



Wrong Structure



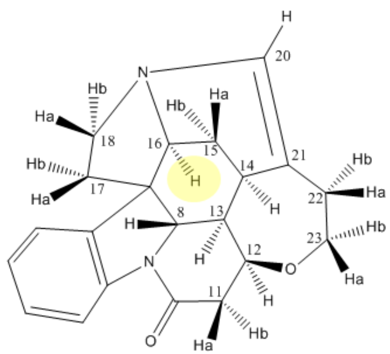
Q = 0.380 **3** ***RSRSSS***

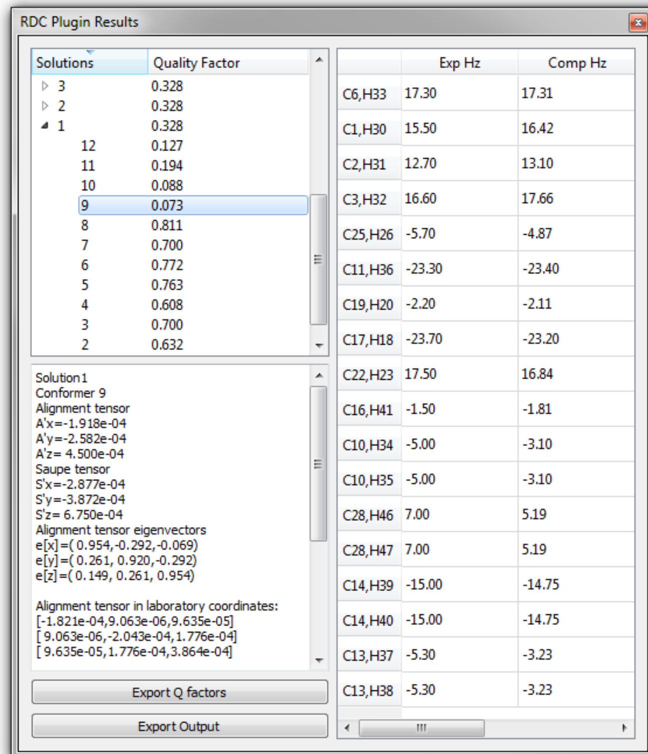


MSPIN RDC DATA

Isomer of Strychnine

Isomer 9 with H16 down is the correct isomer with a Q Factor of 0.073





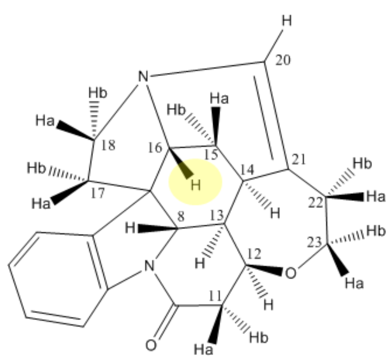
Solutions	Quality Factor
3	0.328
2	0.328
1	0.328
12	0.127
11	0.194
10	0.088
9	0.073
8	0.811
7	0.700
6	0.772
5	0.763
4	0.608
3	0.700
2	0.632

Solution1
Conformer 9
Alignment tensor
A'x=-1.918e-04
A'y=-2.582e-04
A'z= 4.500e-04
Saupe tensor
S'x=-2.877e-04
S'y=-3.872e-04
S'z= 6.750e-04
Alignment tensor eigenvectors
e[x]=(0.954,-0.292,-0.069)
e[y]=(0.261, 0.920,-0.292)
e[z]=(0.149, 0.261, 0.954)

Alignment tensor in laboratory coordinates:
[-1.821e-04,9.063e-06,9.635e-05]
[9.063e-06,-2.043e-04,1.776e-04]
[9.635e-05,1.776e-04,3.864e-04]

	Exp Hz	Comp Hz
C6,H33	17.30	17.31
C1,H30	15.50	16.42
C2,H31	12.70	13.10
C3,H32	16.60	17.66
C25,H26	-5.70	-4.87
C11,H36	-23.30	-23.40
C19,H20	-2.20	-2.11
C17,H18	-23.70	-23.20
C22,H23	17.50	16.84
C16,H41	-1.50	-1.81
C10,H34	-5.00	-3.10
C10,H35	-5.00	-3.10
C28,H46	7.00	5.19
C28,H47	7.00	5.19
C14,H39	-15.00	-14.75
C14,H40	-15.00	-14.75
C13,H37	-5.30	-3.23
C13,H38	-5.30	-3.23

Isomer 10 with H16 up is incorrect with a Q Factor of 0.088

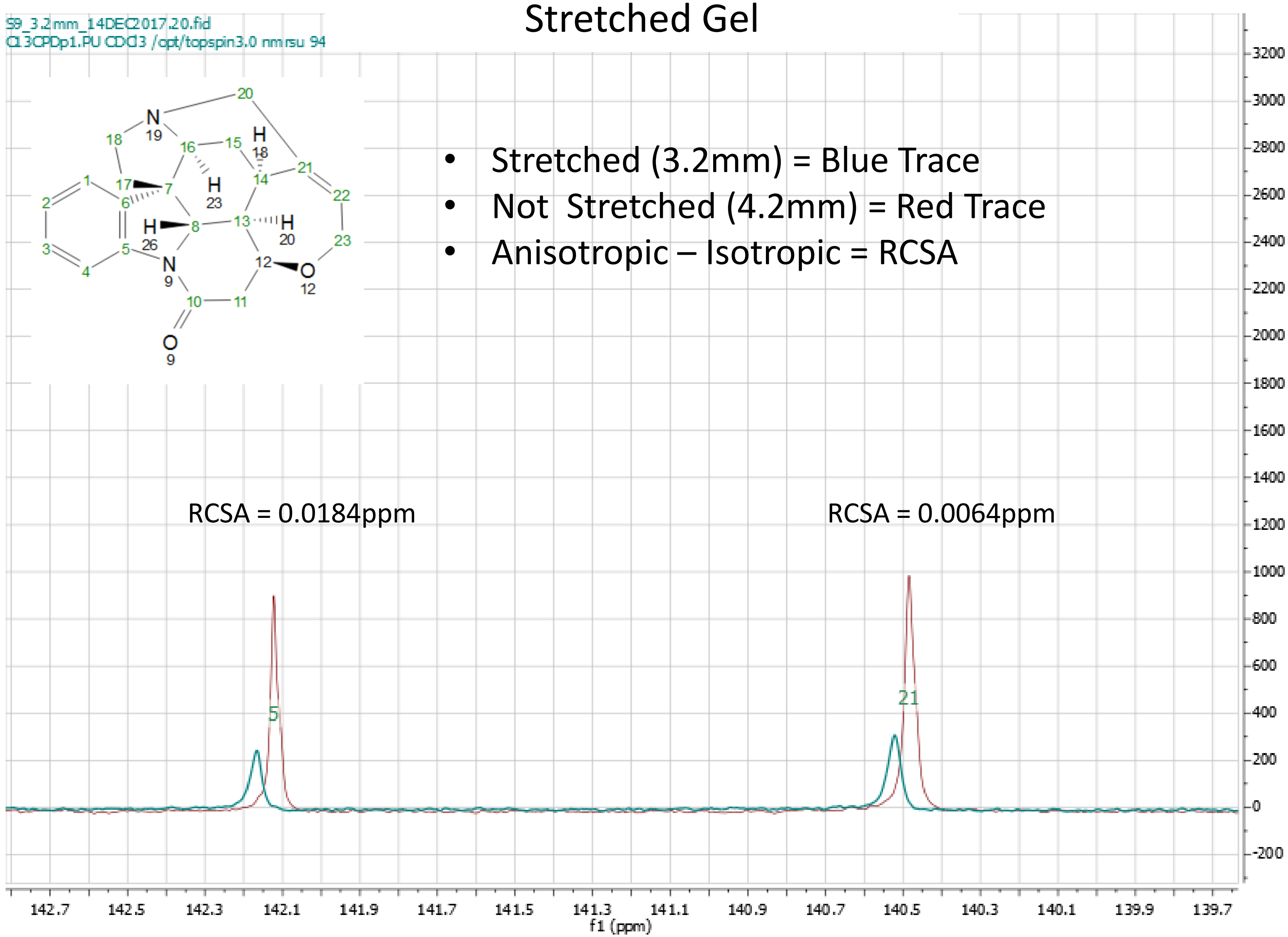


Mark Zhang

The RCSA Experiment

Stretched Gel

- Stretched (3.2mm) = Blue Trace
- Not Stretched (4.2mm) = Red Trace
- Anisotropic – Isotropic = RCSA



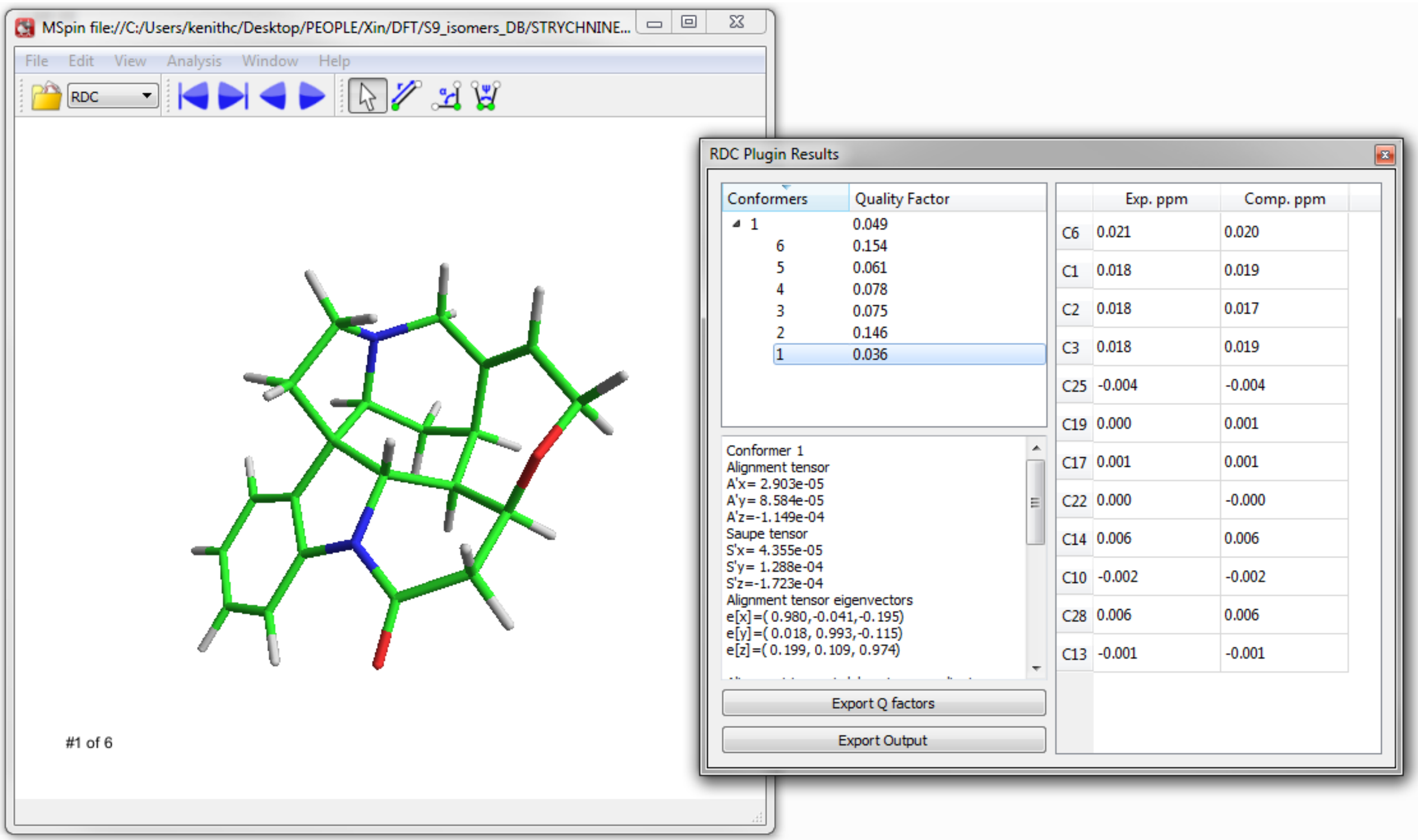
RCSA MSPIN Script

CSA_DATA_Script - Notepad

File Edit Format View Help

```
csa_data {

#C1
6 0.0210
#C2
1 0.0184
#C3
2 0.0178
#C4
3 0.0182
#C8
25 -0.0036
#C13
19 0.0004
#C14
17 0.0008
#C16
22 0.0004
#C22
14 0.0064
#C11
10 -0.0020
#C18
28 0.0056
#C23
13 -0.0010
}
csa_reference{
#c20
16
}
```

MPSIN RCSA DATA



RDC Experimental Parameters

Pulse program for Bruker spectrometers:

```
;J-resolved HSQC

;Shift Correlation version  s> cnst10= 0
;J-resolved version =>cnst10= 1

;F1-heterocoupled version==> cnst14= 0
;F1-heterodecoupled version==> cnst14= 1

;with J scaling in t1
;J-scaled in shift correlation experiments==> cnst12= 1-10
;J-scaled in J-resolved experiments==> cnst12= 1

;Conventional acquisition==> cnst15= 0
;Pure shift acquisition==> cnst15= 1

;phase sensitive using Echo/Antiecho-TPPI gradient selection
;with decoupling during acquisition
;using shaped pulses for inversion and refocussing on f2 -
channel
;using G_BIRD(r) to remove long range couplings in t1
```

DOI: 10.1021/acs.joc.6b02103 -- Click on link for supporting information

RCSA Experimental Parameters

C13CPDp1.PU – conventional carbon experiment

Gaussian RDC Calculation

%chk=S9_RSSRRS_DFT.chk

%mem=512MB

%nproc=2

opt freq b3lyp/6-31g(d) geom=connectivity

Gaussian RCSA Calculation

%chk=C:\Users\kenithc\Desktop\RDC\GIL\RCSA\Gaussian_RCSA\1_RSSRRS_DFT.chk

nmr=giao mpw1pw91/6-31g(d,p) nosymm geom=connectivity

References

Residual Dipolar Couplings in Small-Molecule NMR, RR Gil, 2017 Elsevier

Determination of Relative Configuration from Residual Chemical Shift Anisotropy, Nilamoni Nath, Manuel Schmidt, Roberto R. Gil, R. Thomas Williamson, Gary E. Martin, Armando Navarro-Vázquez, Christian Griesinger, and Yizhou Liu, J. Am. Chem. Soc. 2016, 138, 9548−9556

Residual Dipolar Couplings (RDCs) Analysis of Small Molecules Made Easy: Fast and Tuneable Alignment by Reversible Compression/Relaxation of Reusable PMMA Gels, Chakicherla Gayathri, Nicolay V. Tsarevsky, and Roberto R. Gil, Chem. Eur. J. 2010, 16, 3622 – 3626