

## Instructions for using the Excel file to compute the *J*-DP4 probabilities

- In order to simplify the calculation of the *J*-DP4 probabilities, an Excel file is provided from the authors at:

sarotti-NMR.weebly.com  
iquir-conicet.gov.ar/Sarotti-NMR  
Digital CSIC (<http://dx.doi.org/10.20350/digitalCSIC/8634>)

- The file contains 4 sheets: "Main", "Unscaled", "Scaled" and "Errors" (Figure S1).

- The "Main" sheet contains the enabled cells to introduce the experimental and calculated NMR data, and provides the results of the *J*-DP4 calculations. It is divided in 5 different regions: A, B, C, D and E.

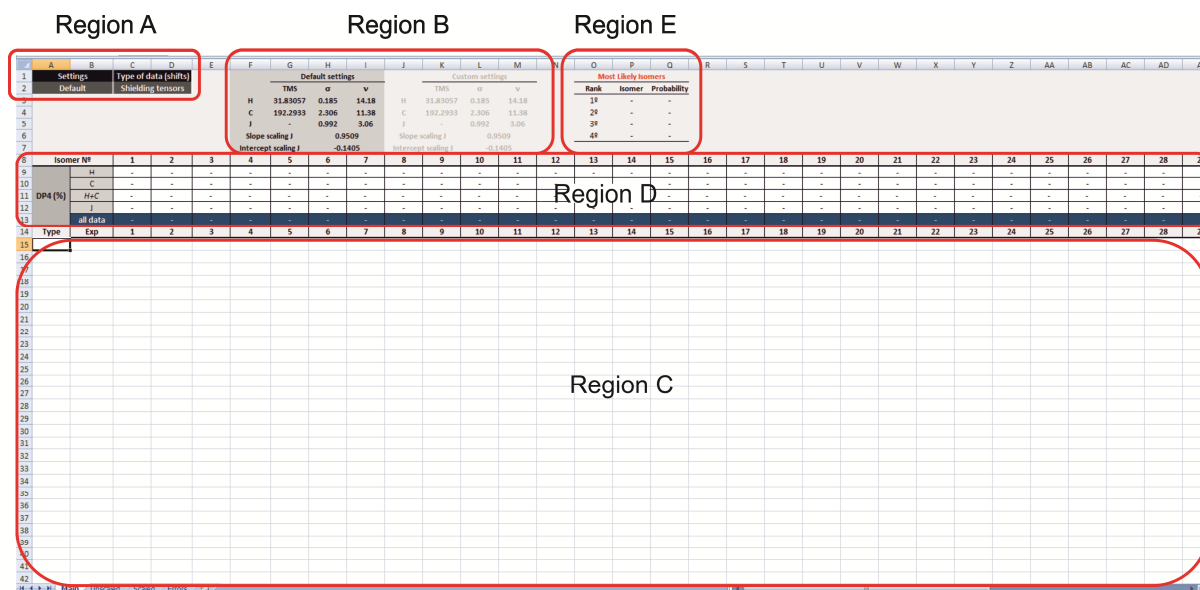


Figure S1

### Region A: user selection (drop down lists):

- *Settings*: indicates the settings to be employed to compute the *J*-DP4 probability (Default or Custom). The choice will be reflected in Region B.
- *Type of Data*: indicates the type of calculated NMR data entered in Region C (Shielding Tensors, Unscaled Shifts or Scaled Shifts).

### Region B: settings to compute the *J*-DP4 probability

The *J*-DP4 probability is built with the standard deviation and degrees of freedom [ $\sigma, \nu$ ] of the shifts and *J* errors series, and the scaling parameters of *J* as well. These values depend upon the level of theory employed during the calculations.

If the user selects "Settings>Default" in Region A, the Default settings will be highlighted in Region B. These are the settings used in this work and were obtained at the B3LYP/6-31G\*\*//MMFF level, and are fixed.

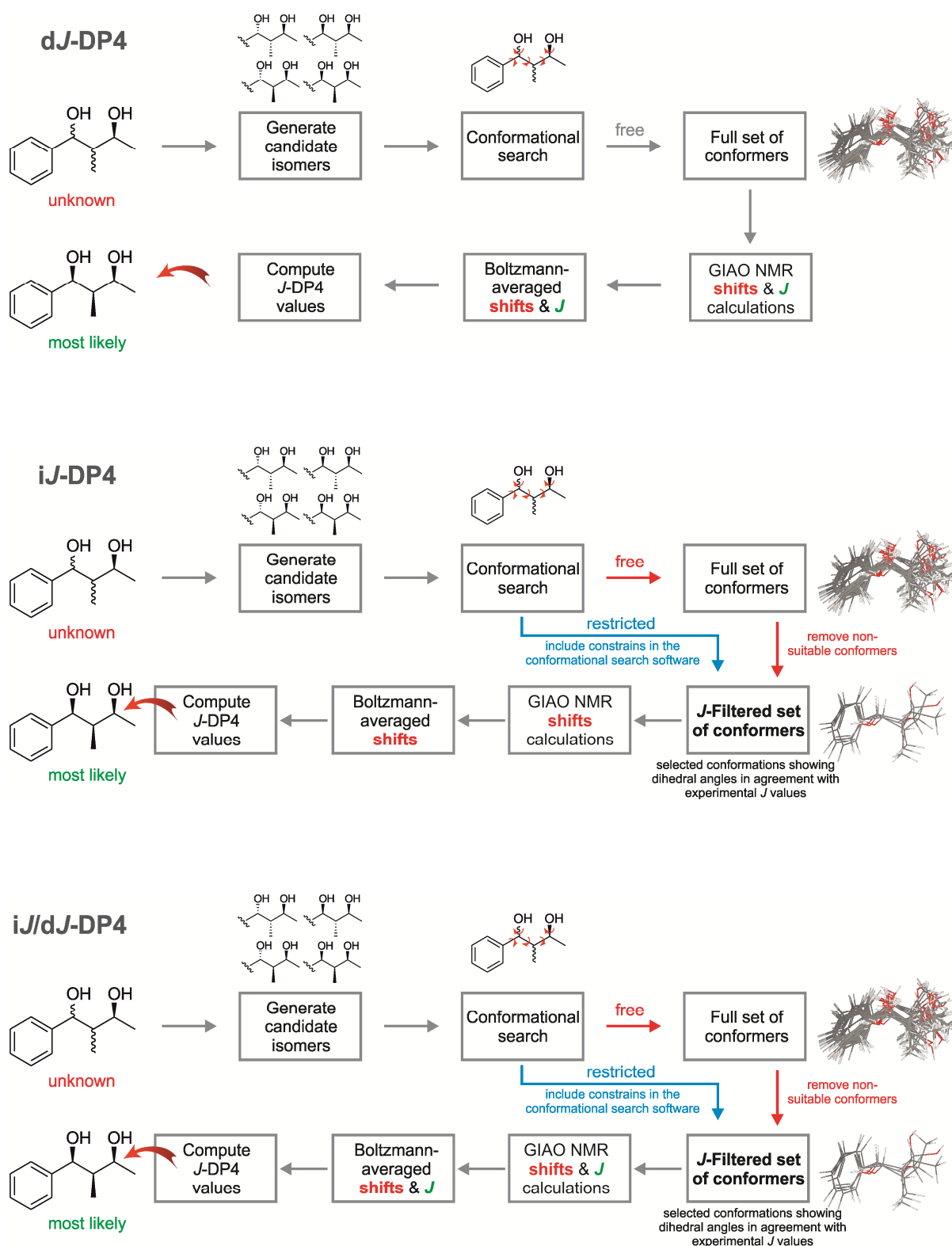
If the user selects "Settings>Custom" in Region A, the Custom settings will be highlighted in Region B. These settings can be changed freely depending on the user preferences (for example, those obtained by using other levels of theory). **Warning: to obtain meaningful results, the modified parameters must be estimated at the desired level of theory using a large set of known compounds.**

### Region C: enabled cells to enter the data:

- *Type*: enter the letter "C" (without quotes) for  $^{13}\text{C}$  data, the letter "H" (without quotes) for  $^1\text{H}$  data, and the letter "J" (without quotes) for *J* data.
- *Experimental*: enter the experimental  $^{13}\text{C}$  and/or  $^1\text{H}$  chemical shifts and/or *J* couplings.
- *Isomer 1, Isomer 2, etc*: enter the computed data for the candidate structures.

**Region D**: displays the *J*-DP4 probabilities once the data is entered in Region C. **Region E**: displays the four most likely candidates (in descending order) depending on the values given in Region D.

## Workflow of *dJ*-DP4, *iJ*-DP4 and *iJ/dJ*-DP4 methods



## J-DP4 calculations tutorial

**Step 1:** perform a conformational search at the MMFF level using a suitable package (Spartan, MacroModel, etc). It is recommended to keep all conformations within 5 kcal/mol (~21 kJ/mol) from the global minimum.

- For **dJ-DP4**: free conformational search.
- For **iJ-DP4** and **iJ/dJ-DP4**: use experimental *J* information to constrain the conformational sampling. This can be done in two different ways:
  - Running full conformational searches and keeping only the suitable conformations (that is, those conformations showing dihedral angles in agreement with the experimental  $^3J_{\text{HH}}$  values).
  - Running constrained searches by freezing (within a safe range) the dihedral angle/s in agreement with the experimental  $^3J_{\text{HH}}$  values.

The agreement between a given dihedral angle and the experimental  $^3J_{\text{HH}}$  value can be easily estimated using the proper Karplus equation for that system. To avoid missing relevant conformations, the constrained sampling should be done within a safe range ( $\pm 2.5$  Hz from the experimental value). As a rough guide, if the experimental  $^3J_{\text{Ha-Hb}}$  value is low (<4 Hz) it should be recommended to keep the conformations showing Ha/Hb dihedral angles in the range 50-120° (absolute value). On the other hand, if the experimental  $^3J_{\text{Ha-Hb}}$  value is high (>8 Hz) the conformations showing Ha/Hb dihedral angles in the ranges 0-30° and/or 140-180° (absolute value) should be kept.

**Step 2:** perform the NMR GIAO calculations at the B3LYP/6-31G\*\* level of theory.

- For **dJ-DP4** and **iJ/dJ-DP4**: compute both the shielding tensors and coupling constants (FC only).
- For **iJ-DP4**: compute only the chemical shifts.

**Step 3:** compute the Boltzmann-averaged values for each compound.

- For **dJ-DP4** and **iJ/dJ-DP4**: both the shielding tensors and coupling constants (FC only).
- For **iJ-DP4**: only the shielding tensors.

**Step 4:** open the Excel file.

**Step 5:** select the Settings and Type of data (Region A).

**Recommended:** Settings>Default  
Type of data>Shielding tensors.

- If selected Settings>Custom: introduce the desired parameters in the enabled cells of Region B
- If selected Type of data>Unscaled shifts: compute the unscaled chemical shifts (in a different file) from the Boltzmann-averaged shielding tensors calculated for all isomers and the corresponding value of a suitable standard of reference (TMS, MSTD, etc) according to:

$$\delta_{\text{unscaled}} = \sigma_{\text{ref}} - \sigma_{\text{x}} + \delta_{\text{ref}}$$

- If selected Type of data>Scaled shifts: compute the scaled chemical shifts (in a different file) from the unscaled chemical shifts according to:

$$\delta_{\text{scaled}} = (\delta_{\text{unscaled}} - b) / m$$

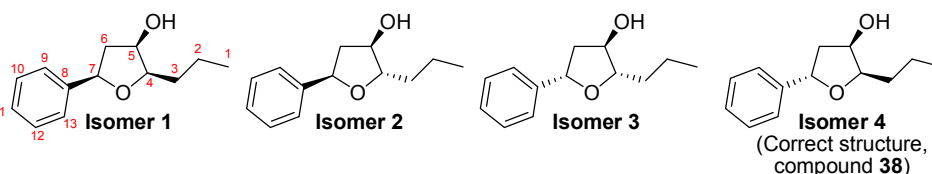
where *b* and *m* are the intercept and slope, respectively, obtained from a large dataset or from the plot of  $\delta_{\text{unscaled}}$  vs  $\delta_{\text{exp}}$ .

**Step 6:** Introduce the experimental data in Region C.

- Column A (Type of data): Identify the type of data (C for carbon shifts, H for proton shifts, J for coupling constants).
- Column B (Exp): Introduce the experimental values.
  - For **dJ-DP4** and **iJ/dJ-DP4**: both the chemical shifts and coupling constants
  - For **iJ-DP4**: only the chemical shifts

**Step 7:** Introduce the calculated data in Region C. All the DP4 values (proton data, carbon data, *J* data, all data) are automatically calculated and displayed in Region D for each isomer above the number of isomer. The most likely candidates are also shown in Region E. **Important: the NMR data must be assigned (know which shift corresponds to which nuclei). Using unassigned or misassigned NMR data can lead to erroneous results.**

Step-by-step example: assignment of compound 38 by J-DP4 (4 possible diastereoisomers).



Following the recommended procedure (see tutorial steps 1-3), and using a full conformational search (with no constrains) the Boltzmann averaged isotropic shielding values and coupling constants (FC only) of isomers 1-4 computed at the B3LYP/6-31G\*\*//MMFF level are:

Table S1

Atom	Exp	Calculated shielding tensors			
		Isomer 1	Isomer 2	Isomer 3	Isomer 4
C1	14.3	175.532	175.673	175.683	175.517
C2	19.7	168.959	168.608	168.763	168.326
C3	31.3	156.000	151.710	151.031	157.009
C4	83.1	109.040	105.543	105.599	109.155
C5	73.8	117.994	115.138	115.721	117.408
C6	44.7	148.007	148.477	147.884	147.635
C7	78.2	113.363	113.404	112.236	112.214
C8	143.8	48.873	50.084	51.154	51.130
C9 - C13	125.3	72.345	72.438	72.100	72.647
C10 - C12	128.3	68.274	68.482	69.081	68.955
C11	127.1	70.868	70.994	71.346	71.446
H1	0.94	30.749	30.775	30.774	30.737
H2a	1.40	30.191	30.305	30.036	30.275
H2b	1.48	30.191	30.040	30.275	30.008
H3a	1.58	30.128	30.335	30.407	29.983
H3b	1.66	29.993	30.416	30.287	30.432
H4	3.99	27.652	27.632	27.795	27.600
H5	4.30	27.831	27.763	27.660	27.713
H6a	2.39	29.296	29.253	29.557	29.341
H6b	2.02	29.508	29.696	29.928	30.091
H7	5.19	26.490	26.415	26.174	26.101
H9	7.26	24.230	24.252	24.295	24.314
H10	7.25	24.328	24.340	24.391	24.390
H11	7.18	24.518	24.525	24.557	24.556
J <sub>4,5</sub>	2.9	2.664	2.777	2.340	2.092
J <sub>5-6a</sub>	1.0	4.444	5.278	3.528	0.809
J <sub>5-6b</sub>	4.5	0.721	3.760	4.707	3.823
J <sub>6a-7</sub>	6.5	10.254	8.545	6.430	6.172
J <sub>6b,7</sub>	9.7	1.112	4.532	8.126	10.834

This information should be copy-pasted into the Excel file as shown in Figure S2. **Warning: do not cut-paste information in this Excel file since it modifies the cell references for the hidden equations).**

Figure S2

The DP4 probabilities computed are shown in Region D (Figure S3). Since the shifts and coupling constants were calculated after a full conformational search, the last row indicates the *dJ*-DP4 results.

Isomer N <sup>o</sup>		1	2	3	4
DP4 (%)	H	95.76	3.41	0.80	0.03
	C	35.58	0.03	0.02	64.37
	H+C	99.94	0.00	0.00	0.06
	J	0.00	0.02	10.63	89.35
	all data	0.19	0.00	0.10	99.70

Figure S3

In the sheet “Unscaled” (Figure S4) are shown the unscaled chemical shifts computed according to  $\delta_{\text{unscaled}} = \sigma_{\text{TMS}} - \sigma_x$  with the  $\sigma_{\text{TMS}}$  values indicated in the Region B of the main sheet.

Figure S4

In the sheet “Scaled” (Figure S5) are shown the scaled chemical shifts and coupling constants. The chemical shifts are scaled according to  $\delta_{\text{scaled}} = (\delta_{\text{unscaled}} - b) / m$ , where *m* and *b* are the slope and intercept, respectively, obtained from the plot of  $\delta_{\text{unscaled}}$  vs  $\delta_{\text{exp}}$ , whereas the *J* values are scaled according to  $J_{\text{scaled}} = (J_{\text{unscaled}} - b) / m$ , where *m* and *b* are the slope and intercept scaling *J* values introduced in the Region B of the main sheet.

Figure S5

In the sheet "Errors" (Figure S6) are shown the differences between experimental and scaled values, which in turn are used to compute the different DP4 probabilities.

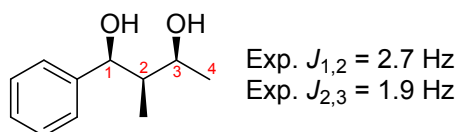
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	Type	Exp	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
2	C	14.3	-1.23	-3.20	-3.67	-1.73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	C	15.7	0.37	-0.96	-1.54	0.61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	C	31.3	2.57	3.69	0.15	1.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	C	85.1	0.78	3.77	3.77	0.92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	C	73.8	0.54	2.70	2.06	1.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	C	44.7	-2.32	-4.21	-3.83	-2.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	C	78.2	1.07	0.18	1.45	2.53	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	C	143.8	4.15	2.99	2.29	2.69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	C	125.3	-2.35	-2.66	-1.99	-1.97	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	C	128.3	-1.01	-1.39	-1.71	-1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	C	127.1	-2.58	-2.90	-2.97	-2.48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	H	0.94	-0.01	0.07	0.12	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	H	1.4	0.09	0.07	0.37	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	H	1.48	0.01	0.25	0.06	0.26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	H	1.38	-0.03	-0.14	-0.17	0.19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	H	1.66	0.03	-0.30	-0.13	-0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	H	3.99	0.03	0.09	-0.04	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	H	4.3	-0.46	-0.35	-0.22	-0.32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	H	2.98	-0.01	0.11	-0.15	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	H	2.02	0.15	0.04	-0.14	-0.36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	H	5.19	-0.01	0.07	0.33	0.37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	H	7.26	0.17	0.11	0.08	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	H	7.25	0.08	0.04	0.00	-0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	H	7.18	-0.04	-0.67	-0.10	-0.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	J	2.9	0.05	0.17	-0.29	-0.55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	J	1	2.82	4.70	2.86	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	J	4.5	-3.59	-0.40	0.60	-0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	J	6.5	4.43	2.63	0.41	0.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	J	9.7	-8.38	-4.79	-1.01	1.84	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Figure S6

In order to reduce the overall computational cost, an  $iJ/dJ$ -DP4 approach can be carried out. For example, using the experimental  $J_{5-6a}$  value of 1.0 Hz the number of suitable conformations for NMR calculations can be narrowed down. According to Karplus equation, the two protons (H-5 and H-6a should be almost perpendicular). Hence, the conformational sampling can be restricted by keeping all conformations showing dihedral angles within a safe range (between  $60^\circ$  and  $120^\circ$ ). The resulting conformations (21% of the total obtained with a full conformational search) are submitted to NMR calculations (both shifts and coupling), and the Boltzmann averaged values are placed in the Excel file as indicated above.

## Step-by-step example: constrained conformational search of compound 22

According to the experimental NMR data of compound **22**, both the  $J_{1,2}$  and  $J_{2,3}$  are small (2.7 and 1.9 Hz, respectively). Hence, according to Karplus equations, the H-1/H-2 and H-2/H-3 pairs should display a near perpendicular arrangement, or should be in equilibrium between two gauche conformers.



To compute  $iJ$ -DP4 or  $iJ/dJ$ -DP4 calculations, the conformational search of compound **22** can be constrained as follows:

**Step 1:** Run a full conformational search using MMFF, exploring all rotatable bonds. Using Spartan (6-fold sampling, systematic search) we found 39 unique conformations within the 5 kcal/mol window (similar results were obtained in Macromodel), but the number of conformers found might depend on the software and search options.

**Step 2:** Measure the H-1/H-2 and/or H-2/H-3 dihedral angles (Table S2).

**Step 3:** Remove the conformations showing dihedral angles in disagreement with the experimental  $J$  values. In this case, those with *syn* (dihedral angles in range 0-30°) or *anti* (dihedral angles in range 150-180°) relationships should be removed.

**Step 4:** The conformations kept (in this case, those with dihedral angles in the range 50-120°) must be employed for further GIAO NMR calculations at DFT level. Note that the filter can be done with  $J_{1,2}$ ,  $J_{2,3}$  or both. The number of remaining conformations will depend on the  $J$  employed to constrain the search (29 when filtering with  $J_{1,2}$ , 22 when filtering with  $J_{2,3}$ , and 15 when filtering with both  $J_{1,2}$  and  $J_{2,3}$ ).

Table S2

Conformation	MMFF Rel. Energy (kcal/mol)	Dihedral angles calculated at the MMFF level		Filter using exp. $J_{1,2}$ information	Filter using exp. $J_{2,3}$ information	Filter using exp. $J_{1,2}$ and $J_{2,3}$ information
		H-1/H-2	H-2/H-3			
c01	0.00	-71.26	60.50	keep	keep	keep
c02	0.12	174.99	61.78	remove	keep	remove
c03	0.44	-64.50	68.73	keep	keep	keep
c04	0.75	-69.15	63.19	keep	keep	keep
c05	0.87	179.11	63.62	remove	keep	remove
c06	1.50	178.44	63.06	remove	keep	remove
c07	1.82	-61.70	-170.78	keep	remove	remove
c08	2.05	-78.81	-62.81	keep	keep	keep
c09	2.09	-63.46	-173.66	keep	remove	remove
c10	2.22	-68.33	67.12	keep	keep	keep
c11	2.70	-63.27	-172.68	keep	remove	remove
c12	2.74	60.73	178.74	keep	remove	remove
c13	2.75	59.87	179.72	keep	remove	remove
c14	2.86	176.15	-70.49	remove	keep	remove
c15	2.90	62.40	81.74	keep	keep	keep
c16	2.98	-172.43	-58.61	remove	keep	remove
c17	3.03	-178.38	65.33	remove	keep	remove
c18	3.12	-81.88	-65.50	keep	keep	keep
c19	3.20	-64.89	-173.02	keep	remove	remove
c20	3.26	65.01	179.88	keep	remove	remove
c21	3.60	60.43	177.19	keep	remove	remove
c22	3.71	-172.97	-177.49	remove	remove	remove
c23	3.72	-73.70	-58.58	keep	keep	keep
c24	3.85	60.29	-45.65	keep	keep	keep
c25	3.94	64.75	80.63	keep	keep	keep
c26	3.95	-171.12	-174.67	remove	remove	remove
c27	3.98	-67.47	-176.39	keep	remove	remove
c28	4.12	58.02	-40.63	keep	keep	keep
c29	4.20	63.38	177.97	keep	remove	remove
c30	4.25	48.85	-58.29	keep	keep	keep
c31	4.31	-64.98	-173.47	keep	remove	remove
c32	4.34	177.71	-67.25	remove	keep	remove
c33	4.39	55.97	77.12	keep	keep	keep
c34	4.45	-62.82	-171.30	keep	remove	remove
c35	4.45	65.12	179.73	keep	remove	remove
c36	4.53	-169.85	-176.47	remove	remove	remove
c37	4.68	-66.93	-174.92	keep	remove	remove
c38	4.73	-82.50	-65.12	keep	keep	keep
c39	4.75	53.43	73.94	keep	keep	keep
			total kept	29	22	15
			total removed	10	17	24