Residual Dipolar Couplings (RDCs) Analysis of Small Molecules Made Easy: Fast and Tuneable Alignment by Reversible Compression/Relaxation of Reusable PMMA Gels**

Chakicherla Gayathri,[a] Nicolay V. Tsarevsky,[b] and Roberto R. Gil*[a]

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Experimental Procedures.

Materials
The monomers, methyl methacrylate (MMA, 99 %, Aldrich) and ethylene glycol
dimethacrylate (EGDMA, 98 %, Aldrich) were purified prior to the experiments by passing the
neat liquids through a short column filled with basic alumina in order to remove the
polymerization inhibitor. The radical initiator, V-70 (2,2’-azobis(2,4-dimethyl-4-
methoxyvaleronitrile)) was purchased from Wako, and acetone-$d_6$ and chloroform-$d$ (99.9 % of
D atoms) were purchased from Cambridge Isotope Laboratories. Authentic sample of 10-epi-8-deoxycumambrin B (I) was reisolated from Stevia yaconensis var. subeglandulosa$^{[33]}$ by Dr.
Viviana E. Nicotra from Córdoba National University, Córdoba, Argentina. The benzazepines
samples C-F (see below) used for the example shown in Figure 2 were kindly provided by Dr.
Magdalena Cid Fernández from the University of Vigo, Vigo, Spain.

\[
\begin{align*}
\text{C} & \quad \text{D} & \quad \text{E} & \quad \text{F}
\end{align*}
\]

Preparation of Crosslinked PMMA of Gels

A solution containing MMA (10 mL), V-70 (0.0030 g), and acetone-$d_6$ (2 mL) was first
prepared, and 10 mL were taken (corresponding to 8.33 mL or 7.79x$10^{-2}$ mol of MMA) and
mixed with EGDMA (40 µL, 2.12x$10^{-4}$ mol). The fraction of crosslinker in the polymeriz-
ing 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 50°C. 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.

NMR experiments

All NMR experiments were collected on a Bruker Avance DMX-500 NMR instrument
operating at 500.13 MHz for $^1$H, 125.77 MHz for $^{13}$C and 76.73 MHz for $^2$H, equipped with a
broad band inverse (BBI) probe with only Z gradients and a 2H-TX board to perform $^2$H and $^1$H
3D gradshimming, and $^2$H NMR experiments. $^2$H 1D, $^1$H 1D, $^1$H 1D CPMG, HSQC experiments
were collected using standard Bruker software.

Alignment of Compound 1 Using Reversible Compression / Relaxation of PMMA Gels. A
PMMA gel stick of 2 mm in diameter and 25 mm long and crosslink density of 0.27 mol % (See
above) was inserted in a Wilmad-507-pp-7 5 mm NMR tube. The whole volume around the
polymer stick was filled with CDCl$_3$ and a Shigemi plunger was inserted into the NMR tube to
prevent swelling in the vertical direction. The position of the plunger was secured by wrapping
Teflon tape around the top of the NMR tube. After a period of 24-48 hr the gel has swollen
radially until it touched the walls of the NMR tube. A 1D $^2$H NMR has exhibited a quadrupolar
splitting ($\Delta \nu_Q$) of the solvent signal of 53 Hz since this polymer stick is longer. The plunger was released and the gel was allowed to relax. At this point, the $\Delta \nu_Q$ collapsed to a null value. The gel was washed 5 times with aliquots of 200 µL of CDCl$_3$ each time by gently compressing and relaxing the gel. A 20 ms 1D $^1$H CPMG NMR spectrum was collected between washes to monitor the cleaning process, as shown in Figure S1.

![Figure S1. Series of 20 ms 1D $^1$H CPMG NMR spectra following the monomer washing process of the PMMP gel inside the NMR tube.](image)

After 5 washes, the Shigemi plunger was removed and 2.8 mg of compound I dissolved in CDCl$_3$ (200 µL) was placed into the NMR tube containing the clean and fully relaxed swollen PMMA gel stick. The Shigemi plunger was inserted again and the gel was compressed and relaxed by gently pumping it several times with the Shigemi plunger. With the gel in the fully relaxed stage, the tube was inserted in the NMR probe to verify by $^1$H NMR that compound I was already inside the gel and ready to collect a series of proton-coupled $^1$H-$^{13}$C HSQC experiments at different compression stages. At each compression stage, the position of the plunger was secured by wrapping Teflon tape around the top of the NMR tube. $^2$H NMR experiments were collected before and after each HSQC experiment to measure the $\Delta \nu_Q$ of the CDCl$_3$ in order to verify that the plunger did not change its position during the experiment. Experiments were collected to up to a $\Delta \nu_Q$ values of 31 Hz. Methylenes RDCs were not measured in this experiments due to strong overlapping of the corresponding submultiplets of within each CH$_2$ $^1T_{CH}$ doublets. The data extracted from this series of experiments is summarized below in Table S1.
Table S1. Summary of the NMR Data Collected during the Variable PMMA Gel Compression / Relaxation Experiments for Compound 1 in CDCl₃.

<table>
<thead>
<tr>
<th>Bond</th>
<th>(^1T_{CH} = ^1J_{CH} + ^1D_{CH}) (Hz)¹</th>
<th>Slopes² (R(^2))</th>
<th>(Y_0) (Hz)³</th>
<th>(^1J_{CH}) (Hz)⁴</th>
<th>II-(^1J_{CH}) Error (%)⁵</th>
<th>RDCs (Hz)⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1-H1</td>
<td>123.6 126.5 127.8 127.8 128.6 128.3</td>
<td>-0.1850 (0.94)</td>
<td>129.67</td>
<td>128.8</td>
<td>0.87 0.68</td>
<td>-5.2</td>
</tr>
<tr>
<td>C3-H3</td>
<td>170.4 168.7 166.2 165.1 163.6 162.2</td>
<td>0.3049 (0.92)</td>
<td>161.81</td>
<td>160.4</td>
<td>1.41 0.88</td>
<td>10.0</td>
</tr>
<tr>
<td>C5-H5</td>
<td>112.9 119.4 120.7 123.3 125.3 127.9</td>
<td>-0.5361 (0.99)</td>
<td>129.02</td>
<td>129.3</td>
<td>0.28 0.22</td>
<td>-16.4</td>
</tr>
<tr>
<td>C6-H6</td>
<td>132.6 139 142.7 145.4 146.6 150.8</td>
<td>-0.6480 (0.97)</td>
<td>151.84</td>
<td>154.9</td>
<td>3.06 1.98</td>
<td>-22.3</td>
</tr>
<tr>
<td>C7-H7</td>
<td>107.5 118.7 120 119.2 123.9 125.8</td>
<td>-0.6422 (0.96)</td>
<td>128.09</td>
<td>128.0</td>
<td>0.09 0.07</td>
<td>-20.5</td>
</tr>
<tr>
<td>C13-H13a</td>
<td>180.4 178.7 172.1 171 167.7 164.8</td>
<td>0.5839 (0.87)</td>
<td>164.35</td>
<td>163.8</td>
<td>0.55 0.34</td>
<td>16.6</td>
</tr>
<tr>
<td>C13-H13b</td>
<td>165 161.2 160.4 160.6 160.8 160</td>
<td>0.1747 (0.85)</td>
<td>158.91</td>
<td>160.3</td>
<td>1.39 0.87</td>
<td>4.7</td>
</tr>
<tr>
<td>C14-H3</td>
<td>122.5 124 124.3 123.9 124.8 125.2</td>
<td>-0.0919 (0.91)</td>
<td>125.39</td>
<td>125.8</td>
<td>0.41 0.33</td>
<td>-3.3</td>
</tr>
<tr>
<td>C15-H3</td>
<td>127.5 126.7 126.9 126.4 126.5 126.7</td>
<td>0.0339 (0.71)</td>
<td>126.31</td>
<td>126.6</td>
<td>0.29 0.23</td>
<td>0.9</td>
</tr>
<tr>
<td>(\Delta\nu_Q) (Hz)⁷</td>
<td>31.0 17.5 13.6 10.6 7.0 3.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Correspond to the total splitting of the \(^1H-^{13}C\) cross peaks \(^1T_{CH}\) in the F2 dimension of the proton-coupled HSQC, where \(^1J_{CH}\) is the \(^1H-^{13}C\) scalar coupling in isotropic conditions and \(^1D_{CH}\) is the \(^1H-^{13}C\) Residual Dipolar Coupling.

² Slopes from the linear fitting of \(^1T_{CH}\) vs. \(\Delta\nu_Q\). Plots shown in Figure S2 below.

³ \(Y_0\) intercept at \(\Delta\nu_Q = 0\) Hz from the linear regression analysis of the \(^1T_{CH}\) vs. \(\Delta\nu_Q\) plots shown in Figure S2. The \(Y_0\) value correspond theoretically to the isotropic \(^1J_{CH}\) value.

⁴ Isotropic \(^1J_{CH}\) measured from F2 slices of proton-coupled HSQC experiments of compound 1 dissolved in CDCl₃ (isotropic media)

⁵ Absolute value of the difference between the \(Y_0\) intercept (\(Y_0\)) and the isotropic \(^1J_{CH}\).

⁶ Error % of the difference between the \(Y_0\) intercept (\(Y_0\)) and the isotropic \(^1J_{CH}\).

⁷ RDCs (\(^1D_{CH}\)) values calculated from the difference between the \(^1T_{CH}\) values at the highest compression achieved for this experiment (\(\Delta\nu_Q = 31\) Hz) and the corresponding isotropic \(^1J_{CH}\) values.

⁸ Deuterium NMR quadrupolar splitting (\(\Delta\nu_Q\)) of the CDCl₃ signal at different compression stages. \(\Delta\nu_Q\) values of 31 and 3.5 Hz correspond to fully compressed and fully relaxed gel, respectively. Note that in the fully relaxed stage, the gel is not totally isotropic.
Figure S2. Tuneable alignment of compound 1 by variable compression / relaxation of a PMMA gel in CDCl₃. Plots of total one-bond $^1$H-$^{13}$C splitting $^1T_{CH}$ ($^1J_{CH}$+$^1D_{CH}$) vs. the $^2$H NMR quadrupolar splitting of the CDCl₃ signal at different compression stages for the CH bonds at carbons C1, C3, C5, C6, C7, C13, C14 and C15. $\Delta\nu_Q$ values of 31 and 3.5 Hz correspond to fully compressed and fully relaxed gel, respectively. Note that in the fully relaxed stage, the gel is not totally isotropic.
Alignment of Compound 1 in a 4 mm Stretched PMMA Gel. Compound 1 (3 mg) was aligned in a stretched PMMA gel using a previously described protocol. The following experimental conditions were used: a 4 mm x 15 mm crosslinked (0.27 mol % EGDMA) PMMA gel swollen in a 5 mm NMR tube using CDCl$_3$. The gel was stabilized in ~20 days and showed a $\Delta$ν$_Q$ = 77 Hz. The sample was diffused into the gel in 72 hours and a proton-coupled $^1$H-$^1$C HSQC was collected in order to measure the corresponding RDCs, as shown below in Table S2.

Table S2. $^1$J$_{CH}$, $^1$T$_{CH}$ and RDCs for compound 1 measured in a 4 mm stretched PMMA gel swollen in CDCl$_3$.

<table>
<thead>
<tr>
<th>Bond</th>
<th>$^1$J$_{CH}$ (Hz)</th>
<th>$^1$T$_{CH}$ (Hz)</th>
<th>RDCs (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1-H1</td>
<td>128.8</td>
<td>160.0</td>
<td>31.2</td>
</tr>
<tr>
<td>C2-H2a</td>
<td>128.7</td>
<td>196.0</td>
<td>67.3</td>
</tr>
<tr>
<td>C2-H2b</td>
<td>130.2</td>
<td>100.0</td>
<td>-30.2</td>
</tr>
<tr>
<td>C3-H3</td>
<td>160.4</td>
<td>127.2</td>
<td>-33.2</td>
</tr>
<tr>
<td>C5-H5</td>
<td>129.3</td>
<td>196.0</td>
<td>66.7</td>
</tr>
<tr>
<td>C6-H6</td>
<td>154.9</td>
<td>240.0</td>
<td>85.1</td>
</tr>
<tr>
<td>C7-H7</td>
<td>128.0</td>
<td>205.0</td>
<td>77</td>
</tr>
<tr>
<td>C13-H13a</td>
<td>163.8</td>
<td>116.3</td>
<td>-47.5</td>
</tr>
<tr>
<td>C13-H13b</td>
<td>160.3</td>
<td>149.0</td>
<td>-11.3</td>
</tr>
<tr>
<td>C14-H14</td>
<td>125.8</td>
<td>142.0</td>
<td>16.2</td>
</tr>
<tr>
<td>C15-H15</td>
<td>126.6</td>
<td>130.5</td>
<td>3.9</td>
</tr>
<tr>
<td>$\Delta$ν$_Q$ (Hz)</td>
<td>77</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Structures Generation. The 3D structure of compounds 1 and 2 (Figure S3) were generated using the molecular modelling package HyperChem 7.5 from HyperCube Inc. (http://www.hyper.com/) and energy minimized using the semiempirical method AM1.

Figure S3. Side-by-side view of the 3D structures of the C-10 epimers 1 and 2.
Fitting RDCs to Structure using Singular Value Decomposition (SVD). SVD alignment tensor determination, back computation of RDC values and calculation of quality factors $Q$ were performed using the MSpin package.[38]

SVD fitting of slopes to the 3D structures of compounds 1 and 2. The slopes from Table S1 were SVD fitted to the structures of compounds 1 and 2 in MSpin. The slope corresponding to the methyl group at C4 (H$_3$-15) was excluded from the fitting since it shows a low $R^2$ values (0.71). Please note that the values of the component of the alignment tensor ($A_{xx}$, $A_{yy}$ and $A_{zz}$) do not represent the correct degree of alignment since we are using slopes and not RDCs values in the calculations. In addition, the GDO values are meaningless in this case. The following order parameters were obtained from MSpin:

**Compound 1**

$Q = 0.105$

Alignment vector

$A_{xx} = -3.64956e-06$

$A_{yy} = -9.46033e-06$

$A_{zz} = 1.31099e-05$

$(0.897855, 0.219591, -0.381624,)$

$(-0.0144918, 0.881021, 0.472855,)$

$(0.440053, -0.419025, 0.794211,)$

SVD condition number is 4.43941

Axial component $A_a=1.96648e-05$

Rhombic component $A_r=5.81077e-06$

Rhombicity $R=0.29549$

Asimmetry parameter $\theta=0.443235$

GDO=$2.37961e-05$ (Note: This parameter is meaningless when using slopes)$^{[2]}$

Euler Angles

Set 1

$(-27.8161, -26.1073, -0.9247)$

Set 2

$(152.184, 206.107, 179.075)$

**Compound 2**

$Q = 0.199$

Alignment vector

$A_{xx} = -3.91727e-06$

$A_{yy} = -9.64131e-06$

$A_{zz} = 1.35586e-05$

$(0.877393, 0.209959, -0.431391,)$

$(0.109285, 0.788059, 0.605822,)$

$(0.467159, -0.578689, 0.668492,)$

SVD condition number is 3.85678

Axial component $A_a=2.03379e-05$

Rhombic component $A_r=5.72404e-06$

Rhombicity $R=0.281447$
Asimmetry parameter etha=0.422171
GDO=2.45082e-05 (Note: This parameter is meaningless when using slopes)[2]

Euler Angles

Set 1
(-40.8815, -27.85, 7.09998)
Set 2
(139.119, 207.85, -172.9)

Order parameters when the RDC value corresponding to the methyl group C-14 is excluded from
the SVD fitting:

**Compound 1**

\( Q = 0.084 \)
Alignment vector

\( A_{xx} = -3.65009e-06 \)
\( A_{yy} = -9.46135e-06 \)
\( A_{zz} = 1.31114e-05 \)

(0.897883, 0.219695, -0.381498,)
(-0.0145931, 0.880956, 0.472974,)
(0.439993, -0.419108, 0.794201,)

SVD condition number is 4.43998
Axial component \( A_a = 1.96672e-05 \)
rhombic component \( A_r = 5.81126e-06 \)
rhombicity \( R = 0.29548 \)
Asimmetry parameter etha=0.443221
GDO=2.37988e-05

**Compound 2**

\( Q = 0.121 \)
Alignment vector

\( A_{xx} = -3.78479e-06 \)
\( A_{yy} = -9.61168e-06 \)
\( A_{zz} = 1.33965e-05 \)

(0.871051, 0.217146, -0.440588,)
(0.108716, 0.789501, 0.604044,)
(0.47901, -0.574052, 0.664088,)

SVD condition number is 3.84248
Axial component \( A_a = 2.00947e-05 \)
rhombic component \( A_r = 5.82689e-06 \)
rhombicity \( R = 0.289972 \)
Asimmetry parameter etha=0.434957
GDO=2.4276e-05
Euler Angles
Set 1
(-40.8408, -28.6208, 7.11432)
Set 2
(139.159, 208.621, -172.886)

SVD fitting of RDCs values from Table S1 (compressed PMMA gel) and Table S2 (4 mm stretched PMMA gel) to the 3D structure of compound 1 and calculation of the corresponding probability tensors P.

Order parameters from the SVD fitting to the structure of compound 1 using the RDCs data measured using the compressed PMMA gel (Table S1):

\[ Q = 0.127 \]
Alignment vector
\[ A_{xx} = -0.000111404 \]
\[ A_{yy} = -0.000292174 \]
\[ A_{zz} = 0.000403578 \]
(0.887699, 0.132136, -0.441056,)
(0.0539766, 0.921463, 0.384698,)
(0.457249, -0.365302, 0.81085,)
SVD condition number is 4.38035
Axial component \( A_a = 0.000605367 \)
Rhombic component \( A_r = 0.00018077 \)
Rhombicity \( R = 0.298612 \)
Asimmetry parameter \( \pi = 0.447918 \)
GDO = 0.000733241
Euler Angles
Set 1
(-24.2524, -27.2098, 3.47959)
Set 2
(155.748, 207.21, -176.52)

Probability Tensor \( P = A + 1/3 \mathbf{1} \) (Represented as a 3D probability ellipsoid in Figure 4):

\[ P_x = -0.000111404 + 1/3 = 0.333221929 \]
\[ P_y = -0.000292174 + 1/3 = 0.333041593 \]
\[ P_z = 0.000403578 + 1/3 = 0.333736911 \]

Order parameters from the SVD fitting to the structure of compound 1 using the RDCs data measured using the 4 mm stretched PMMA gel (Table S2):

\[ Q = 0.141 \]
Alignment vector
\[ A_{xx} = 0.000235527 \]
\[ A_{yy} = 0.00103524 \]
\[ A_{zz} = -0.00127076 \]
(0.74877, 0.03493, -0.661909,)
(0.141561, 0.967143, 0.211176,)
(0.647537, -0.251823, 0.719223,)
SVD condition number is 4.38035
Axial component \( A_a \) = -0.00190615
rhombic component \( A_r \) = -0.000799709
rhombicity \( R \) = 0.419542
Asimmetry parameter \( \alpha \) = 0.629314
GDO = 0.00240911
Euler Angles
Set 1
(-19.2967, -40.3561, 10.7059)
Set 2
(160.703, 220.356, -169.294)
Probability Tensor \((P = A + 1/3 \mathbf{1})[2]\) (Represented as a 3D probability ellipsoid in Figure 4):
\[ P_x = 0.000235527 + 1/3 = 0.333568860 \]
\[ P_y = 0.00103524 + 1/3 = 0.334368573 \]
\[ P_z = -0.00127076 + 1/3 = 0.332062573 \]

References