Structure Elucidation

DOI: 10.1002/anie.201101561

Constitutional, Configurational, and Conformational Analysis of Small Organic Molecules on the Basis of NMR Residual Dipolar Couplings

*Roberto R. Gil**

configuration determination · conformation analysis · NMR spectroscopy · residual dipolar couplings · structure elucidation

Dedicated to Professor Aksel Bothner-By on the occasion of his 90th birthday

NMR spectroscopy is arguably one of the most powerful tools available to chemists for the structural analysis of molecules. NMR experiments based on the nuclear Overhauser enhancement (NOE)[1] and on the analysis of ^3J coupling constants[2] have been used intensively in the configurational and conformational analysis of small organic molecules. However, as these two NMR parameters provide only localized structural information, they fail to provide information on the relative configuration of remotely located molecular fragments.

Unlike NOEs and ^3J coupling constants, the direct spin–spin interaction known as dipolar coupling (D_{ij}; Figure 1) can provide very powerful structural information of a nonlocal character.[3] The value of D_{ij} between two nuclei i and j (which can either be bonded to one another or not directly connected) depends not only on the distance between them (r), but also on the angle of the internuclear vector (r_{ij}) with respect to the direction of the magnetic field (B_0), which acts as a global axis of reference. Hence, from the equation in Figure 1, it can be inferred that a unique combination of dipolar-coupled pairs of spins can produce a unique set of dipolar couplings, which in turn can enable unambiguous determination of the relative spatial arrangement of atoms in a molecule. This spatial arrangement reflects the constitution, configuration, and preferred conformation of the molecule.

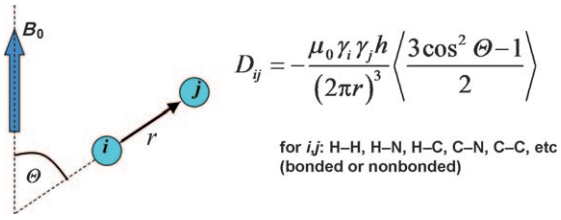


Figure 1. Dependence of the dipolar coupling (D_{ij}) of a pair of magnetically active nuclei i and j (bonded or nonbonded) on the internuclear distance (r) and on the angle (Θ) of the internuclear vector (r_{ij}) and the direction of the magnetic field (B_0). In the equation, μ_0 is the permeability of a vacuum, γ is the gyromagnetic constant, and h is the Planck constant; the brackets around the angular term represent averaging in solution.[3c]

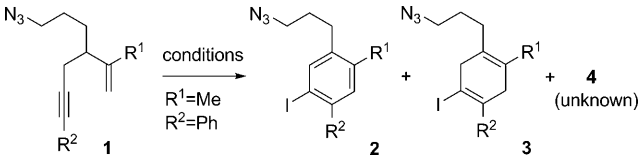
When the concept is explained in this way, the use of dipolar couplings for the analysis of small molecules sounds very simple and straightforward. However, the size of dipolar couplings is on the order of kilohertz. In solid-state powders, all possible spatial orientations adopted by molecules lead to extremely broad lines in the NMR spectrum, which makes the extraction of individual dipolar couplings impossible. In solution, dipolar couplings average out as a result of isotropic molecular tumbling and are not observable in the NMR spectrum (isotropic conditions). However, if molecules are forced to adopt a minor degree of alignment in solution, a measurable fraction of the original dipolar coupling, known as residual dipolar coupling (RDC), is observed in the NMR spectrum. RDCs conserve the same structural information provided by the original dipolar couplings, since they are scaled down proportionally. From a practical standpoint, RDCs can be measured relatively easily provided that the degree of alignment is weak (0.01–0.1% of the maximum dipolar-coupling value). If the alignment is too strong, the RDCs produced are very difficult to analyze. In theory, any sufficiently proximate pair of magnetically active nuclei will yield a RDC value; however, one-bond C–H RDCs ($^1D_{CH}$) and two-bond H–H RDCs ($^2D_{HH}$) are relatively easy to measure and are commonly used in the structural analysis of small molecules.

The first small molecule to be aligned was benzene in 1963;[4] however, its alignment in organic liquid crystals was so strong that RDCs were difficult to analyze. RDCs have been used extensively in the analysis of biomacromolecules since 1997.[3b,d,5] The first application of RDCs to the configurational analysis of small organic molecules was proposed almost simultaneously by Mangoni et al.[6] and Yan et al.[7] in 2003, although their methods were restricted to the use of water-compatible alignment media. Soon afterwards, the development of alignment media compatible with organic solvents began with the introduction of lyotropic liquid-crystalline phases formed by homopolypeptides dissolved in

[*] Prof. R. R. Gil
Department of Chemistry, Carnegie Mellon University
4400 Fifth Ave, Pittsburgh, PA 15213 (USA)
Fax: (+1) 412-268-1061
E-mail: rgil@andrew.cmu.edu
Homepage: http://www.chem.cmu.edu/faculty/profiles/gil.html

$CDCl_3$.[8] Later, in 2004, Luy et al. introduced the use of stretched polystyrene swollen in $CDCl_3$ as a novel alignment medium compatible with organic solvents.[9] Since then, a wide variety of polymer-gel/organic-solvent combinations have been developed,[3e–h] mainly by the Luy research group, as well as methods for the reversible stretching[10] and compression[11] of swollen polymer gels. Various research groups have taken advantage of the availability of this wide variety of alignment media compatible with organic solvents and used RDCs for the configurational and conformational analysis of several small molecules.[12] Owing to challenging structural problems, the use of NOEs and ^3J coupling constants for the structural analysis of some of these molecules failed to provide a unique solution.[12b,g,j] The methodology has proven very effective for rigid and semirigid small molecules. However, although successful in a few cases,[12c,d] its application to the conformational and configurational analysis of flexible molecules still needs further development.

Recently, Luy, Kirsch, and co-workers proposed the very elegant and original idea of expanding the use of RDCs to determine the constitution of a small organic molecule whose structure could not be determined by using a combination of conventional spectroscopic methods.[13] The electrophilic cyclization of azide-containing 1,5-enynes **1** was known to selectively produce either the corresponding arene **2** or cyclohexadiene **3**. However, under certain experimental conditions, the reaction produced a minor unknown product, **4** (Scheme 1). Attempts to determine the constitution of



Scheme 1. Electrophilic cyclization of 1,5-enynes **1** with the formation of an unknown compound **4**.[13]

compound **4** by using a combination of spectroscopic techniques that included MS, IR spectroscopy, and conventional 2D NMR spectroscopy (COSY, HSQC, HMBC) failed to provide a solution, even when ^{13}C–^{13}C correlations were collected in a 1,1-ADEQUATE experiment. From these experiments, it was only possible to identify the presence of 11 fragments. A total of 63 cross-peaks in the ^1H,^{13}C HMBC spectrum and 7 cross-peaks in the ^1H,^{15}N HMBC spectrum correlated almost every fragment with every other fragment and indicated the presence of a very compact structure. These ambiguous correlation patterns commonly hamper the analysis of small and compact structures by 2D NMR spectroscopy.

The authors used the 11 fragments as the pieces of a puzzle and generated a set of potential structures that agreed with the molecular formula obtained by high-resolution mass spectrometry and also made sense from a synthetic-chemistry standpoint. Additional chemically unlikely structural models were also added, and a set of 14 energy-minimized structures were generated. A total of 12 one-bond C–H RDCs ($^1D_{CH}$) and 5 geminal H–H RDCs ($^2D_{HH}$) were collected. Strikingly, only one structure from the whole pool gave a good fit to the experimental set of RDCs. Thus, the experiments led to the unambiguous determination of the constitution of compound **4**: an unexpected compact tricyclic compound; they even simultaneously provided the correct relative configuration (Figure 2). These results are not only relevant from a structural point of view but also provided new insight into the electrophilic cyclization of 1,5-enynes.

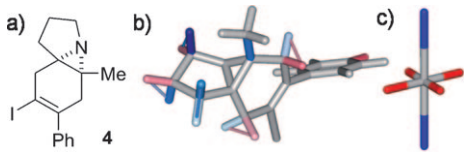


Figure 2. a) Structure of compound **4**, as determined by RDCs. b) The 3D structure of compound **4** with RDCs color-coded in the bonds (red: negative, blue: positive). c) Axis of the calculated alignment tensor.[13]

This approach proposed by Luy and co-workers is a significant breakthrough in the application of RDCs to the structural analysis of small organic molecules. It is just a matter of time before the use of RDCs will become an everyday laboratory technique for the routine determination of the constitution, configuration, and conformation of small organic molecules.

Received: March 3, 2011
Published online: June 29, 2011

[1] F. A. L. Anet, A. J. R. Bourn, *J. Am. Chem. Soc.* **1965**, *87*, 5250.
[2] a) M. Karplus, *J. Chem. Phys.* **1959**, *30*, 11; b) C. A. G. Haasnoot, F. Deleeuw, C. Altona, *Tetrahedron* **1980**, *36*, 2783.
[3] For selected articles and reviews on residual dipolar couplings, see: a) A. Bax, G. Kontaxis, N. Tjandra, *Methods Enzymol.* **2001**, *339*, 127; b) J. H. Prestegard, C. M. Bougault, A. I. Kishore, *Chem. Rev.* **2004**, *104*, 3519; c) F. Kramer, M. V. Deshmukh, H. Kessler, S. J. Glaser, *Concepts Magn. Reson. Part A* **2004**, *21A*, 10; d) M. Blackledge, *Prog. Nucl. Magn. Reson. Spectrosc.* **2005**, *46*, 23; e) C. M. Thiele, *Concepts Magn. Reson. Part A* **2007**, *30A*, 65; f) C. M. Thiele, *Eur. J. Org. Chem.* **2008**, 5673; g) G. Kummerlöwe, B. Luy, *TrAC Trends Anal. Chem.* **2009**, *28*, 483; h) "Residual Dipolar Couplings for the Configurational and Conformational Analysis of Organic Molecules": G. Kummerlöwe, B. Luy, A. W. Graham, *Annual Reports on NMR Spectroscopy, Vol. 68*, Elsevier Academic Press Inc., San Diego, **2009**, p. 193.
[4] A. Saupe, G. Englert, *Phys. Rev. Lett.* **1963**, *11*, 462.
[5] a) N. Tjandra, A. Bax, *Science* **1997**, *278*, 1111; b) J. R. Tolman, H. M. Al-Hashimi, L. E. Kay, J. H. Prestegard, *J. Am. Chem. Soc.* **2001**, *123*, 1416; c) W. Peti, J. Meiler, R. Bruschweiler, C. Griesinger, *J. Am. Chem. Soc.* **2002**, *124*, 5822; d) A. Bax, A. Grishaev, *Curr. Opin. Struct. Biol.* **2005**, *15*, 563.
[6] A. Mangoni, V. Esposito, A. Randazzo, *Chem. Commun.* **2003**, 154.
[7] J. L. Yan, A. D. Kline, H. P. Mo, M. J. Shapiro, E. R. Zartler, *J. Org. Chem.* **2003**, *68*, 1786.
[8] a) C. Aroulanda, V. Boucard, F. Guibe, J. Courtieu, D. Merlet, *Chem. Eur. J.* **2003**, *9*, 4536; b) C. M. Thiele, S. Berger, *Org. Lett.*

2003, *5*, 705; c) L. Verdier, P. Sakhaii, M. Zweckstetter, C. Griesinger, *J. Magn. Reson.* **2003**, *163*, 353.
[9] B. Luy, K. Kobzar, H. Kessler, *Angew. Chem.* **2004**, *116*, 1112; *Angew. Chem. Int. Ed.* **2004**, *43*, 1092.
[10] G. Kummerlöwe, E. F. McCord, S. F. Cheatham, S. Niss, R. W. Schnell, B. Luy, *Chem. Eur. J.* **2010**, *16*, 7087.
[11] C. Gayathri, N. V. Tsarevsky, R. R. Gil, *Chem. Eur. J.* **2010**, *16*, 3622.
[12] For selected examples of the configurational and/or conformational analysis of small molecules on the basis of RDCs, see: a) C. M. Thiele, *J. Org. Chem.* **2004**, *69*, 7403; b) M. E. García, S. Pagola, A. Navarro-Vázquez, D. D. Phillips, C. Gayathri, H. Krakauer, P. W. Stephens, V. E. Nicotra, R. R. Gil, *Angew. Chem.* **2009**, *121*, 5780; *Angew. Chem. Int. Ed.* **2009**, *48*, 5670; c) A. Schuetz, J. Junker, A. Leonov, O. F. Lange, T. F. Molinski, C. Griesinger, *J. Am. Chem. Soc.* **2007**, *129*, 15114; d) A. Schuetz, T. Murakami, N. Takada, J. Junker, M. Hashimoto, C. Griesinger, *Angew. Chem.* **2008**, *120*, 2062; *Angew. Chem. Int. Ed.* **2008**, *47*, 2032; e) C. Gayathri, M. C. de la Fuente, B. Luy, R. R. Gil, A. Navarro-Vázquez, *Chem. Commun.* **2010**, *46*, 5879; f) J. Klages, C. Neubauer, M. Coles, H. Kessler, B. Luy, *ChemBioChem* **2005**, *6*, 1672; g) R. R. Gil, C. Gayathri, N. V. Tsarevsky, K. Matyjaszewski, *J. Org. Chem.* **2008**, *73*, 840; h) C. J. Arnusch, J. H. Ippel, H. Kooijman, A. L. Spek, R. M. J. Liskamp, J. Kemmink, R. J. Pieters, *Eur. J. Org. Chem.* **2010**, 4501; i) C. Farès, J. Hassfeld, D. Menche, T. Carlomagno, *Angew. Chem.* **2008**, *120*, 3782; *Angew. Chem. Int. Ed.* **2008**, *47*, 3722; j) C. M. Thiele, A. Marx, R. Berger, J. Fischer, M. Biel, A. Giannis, *Angew. Chem.* **2006**, *118*, 4566; *Angew. Chem. Int. Ed.* **2006**, *45*, 4455.
[13] G. Kummerlöwe, B. Crone, M. Kretschmer, S. F. Kirsch, B. Luy, *Angew. Chem.* **2011**, *123*, 2693; *Angew. Chem. Int. Ed.* **2011**, *50*, 2643.

