DOI: 10.1002/ejoc.200800686

Residual Dipolar Couplings (RDCs) in Organic Structure Determination

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Keywords: NMR spectroscopy / Configuration determination / Conformation analysis / Residual dipolar couplings

After the huge impact that residual dipolar couplings (RDCs) have had on the structure determination of biological macromolecules by NMR spectroscopy, their utility in the structure determination of small/medium-sized organic compounds is more and more recognised. This microreview will provide a short overview on how to introduce the anisotropic environment necessary to measure RDCs, the corresponding measurement methods and the applications for the determination of the relative configurations and the assignment of diastereotopic moieties. Their application to rigid compounds will be described, as will their very promising application to more flexible compounds, which is an area in which RDCs are expected to yield information that is not accessible by any other NMR spectroscopic means.

1. Introduction

Out of the four NMR spectroscopic interactions, namely, chemical shift, scalar coupling, quadrupolar coupling and dipolar coupling, only two − the isotropic chemical shift and the scalar coupling − are directly observable in high-resolution NMR spectra of organic compounds in isotropic solution. The other two and the orientation-dependent (anisotropic) parts of the former are averaged by the fast tumbling motion in solution and are noticed only by their contribution to relaxation. Some of these relaxation phenomena are mainly considered to be a nuisance, whereas others can be used for structure determination, as is the case with dipolar relaxation, which gives rise to the nuclear Overhauser effect (NOE) and yields distance information[1] or cross-correlated relaxation of double- and zero-quantum coherences that yields angular information.[2]

It was recognised rather early in the history of NMR spectroscopy that anisotropic NMR interactions, dipolar coupling being one, become observable directly and, therefore, give direct access to important structural information if the compound in question is partially oriented in the magnetic field.[3,4] The orienting media, which were used almost exclusively up to the mid 1990s (mainly nematic liquid crystals) result in a rather high degree of order. Consequentially, dipolar couplings observed are large (kHz range) relative to scalar couplings (Hz range) and spectra in these anisotropic media are dominated by the many dipolar couplings and seem uninterpretable to the eye of a nonspecialist. Applications, therefore, have been limited to small and mostly highly symmetric compounds. The structural information obtained form these data (also by using sophisticated computational tools for spectra interpretation), however, is astonishing.[5–8]

Only with the advent of orienting media that induce a low degree of order ("weak alignment method")[9] did it become possible to access additional structural information contained in dipolar couplings for large compounds with little symmetry. In orienting media (also called alignment media) inducing a low degree of order (order parameter $S < 10^{-3}$) anisotropic NMR interactions are averaged significantly but not entirely. The size of the dipolar couplings is thus tremendously reduced, such that dipolar couplings are usually smaller than scalar couplings and the spectral quality of high-resolution NMR spectra is retained. That is why

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Christina Thiele studied chemistry at Dortmund University and King's College London. While pursuing her Masters degree in natural product synthesis she already felt a high affinity towards structure determination by NMR spectroscopy. This continued throughout her Ph.D. studies, which dealt with synthetic organometallic chemistry in combination with NMR spectroscopic structure determination. After receiving her Ph.D. in 2002 she moved deeper into NMR spectroscopy and started independent research first at Leipzig University (2002–2005) and then since 2005 at Darmstadt Technical University. Her current research interests are focused on the interface of NMR spectroscopy and organic chemistry, where she develops methods for organic structure determination, mainly by using residual dipolar couplings (RDCs). These methods are currently also applied for investigations concerning the functions of organic molecules and catalytically active species.

they are then called residual dipolar couplings (RDCs). As long as RDCs are smaller than scalar couplings the important consequence is that the dipolar coupling D is observed as an additional contribution to the line splitting. The line splitting in anisotropic media (usually called total coupling constant, T) is not caused by the scalar coupling J alone (as is the case in isotropic solution), but by the sum of scalar and (residual) dipolar coupling D [Equation (1)].[10]

$$|T| = |J + 2D| \quad (1)$$

The measurement of RDCs, therefore, has the prerequisite of performing two (series of) measurements: one in isotropic solution to obtain the scalar coupling constant J and one in an anisotropic environment to get access to the sum of the scalar and residual dipolar coupling. The dipolar coupling D is obtained from the difference of the former two. If a too-high degree of order is introduced or if RDCs other than those connecting directly bonded spins are considered, care concerning the determination of the sign has to be taken, as only the absolute value of coupling constants is observed in conventional spectra.[11,12] Hence, there are many arguments as to why it is crucial to obtain suitable alignment conditions such that the order induced results in RDCs in the right range. If this is achieved, modern NMR spectroscopic techniques can be used for the measurement of RDCs and thus access is given to novel structural information. The kind of structural information obtainable from RDCs and the theoretical background of their use are outlined first, and then, the variety of methods that can be used to induce order in solutes (alignment media as generic term) will be described. This will be followed by some brief words about measurement techniques and a detailed description of the applications of RDCs published so far.

2. Structural Information from RDCs

The direct dipole–dipole interaction can be defined for two isolated (heteronuclear) spins I and S by Equation (2).[13]

$$D_{IS} = -\frac{\mu_0 \gamma_I \gamma_S \hbar}{8\pi^2} \left\langle \frac{3\cos^2\theta_{IS} - 1}{2} \frac{1}{r_{IS}^3} \right\rangle \quad (2)$$

where μ_0 is the vacuum permeability and γ_I and γ_S are the magnetogyric ratios of the two spins I and S. The angle Θ_{IS} is the angle between the vector connecting I and S and the direction of the magnetic field; the symbols $\langle\ \rangle$ indicate a time average over all orientations sampled by the interspin vector and r_{IS} is the distance between the spins (= length of the vector $\mathbf{r_{IS}}$). If I and S are directly bonded heteronuclear spins (e.g., ^{1}H and ^{13}C or ^{1}H and ^{15}N) this distance is the bond length. For flexible compounds, r_{IS} and Θ_{IS} are additionally averaged by the internal motion of the compound (see below).[14]

2.1 Rigid Compounds

If the compound in question is rigid (or occurs in one almost exclusively populated conformation), all parts of the molecule are assumed to move as one rigid entity, so that the time averaging [as denoted by the brackets in Equation (2)] functions as a scaling factor, which is equal for all parts of the molecule. In this case, RDCs contain angular and distance information. These two kinds of information, however, cannot be separated easily. If the distance is known (e.g., $r_{C,H}$ for $^1D_{C,H}$), the size of the RDC only depends on the angle Θ_{IS} to the magnetic field. This is why most applications especially in biomolecular NMR[15] use RDCs as an *angular restraint* (Figure 1). It should be stated that in contrast to NOE and J couplings, which are local NMR parameters that define distances and orientations between neighbouring groups, RDCs function as global NMR restraints and angular relationships of very distant moieties can be obtained (at least for rigid compounds).

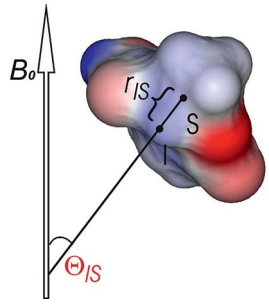

Figure 1. Two (heteronuclear) spins I and S within one rigid compound (or rigid fragment within one compound). D_{IS} depends on the distance r_{IS} (bond length for $^1D_{IS}$) and the angle Θ_{IS} the interspin vector (bond orientation for $^1D_{IS}$) takes with the magnetic field on average.

The conceptually simplest way to obtain structural information from RDCs involves the investigation of whether bonds are parallel or not. In rigid compounds, the averaging process is equal for all parts of the compound; thus, RDCs of the same size are observed for parallel bonds/interspin vectors (of the same kind), as they enclose the same angle with the magnetic field (at all times) and therefore exhibit RDCs of the same size. This can, for example, be used to distinguish axial from equatorial protons in six-membered rings.[16,17] All C–H bonds to axial protons are parallel, such that the values of $^1D_{C,H}$ are the same size for all axial protons.[18]

For most applications, however, it is necessary to know the key points of how the averaging process, which is due to the molecular tumbling motion, is treated mathematically. Usually the averaging process and the resulting preferred orientation of the compound is described by the use of what is called the alignment tensor **A** (other terms used are: orienting tensor, Saupe order matrix, etc.).[19] Tensor **A** is a symmetric and traceless 3×3 matrix and therefore contains five independent elements. It can be determined as soon as five linearly independent RDCs are obtained.[20] As soon as more than five (linearly independent) RDCs are used, structural information is obtained (in addition to the information on the averaging process and the resulting preferred orientation described by **A**).

The most popular way to obtain structural information from RDCs does not use the properties of **A** itself, but performs a fit of observed RDCs to the structure. Several structural proposals, for example, different diastereoisomers in the case of unknown relative configuration, are fit to the RDCs and the best fit (best solution for **A**) is searched for. As a result of the above-described properties of **A**, an excellent fit is obtained for all structural proposals if only five RDCs are used (or if some of the RDCs are redundant); thus, no structural information is obtained in such cases. In well-defined cases (use of at least six linearly independent RDCs), however, there will be a significant difference for different structural proposals (e.g., the different diastereoisomers). The correct structure will lead to an excellent fit in the determination of **A**, whereas incorrect structures will lead to significantly worse fits. It should be mentioned at this stage, however, that the precision of RDCs significantly influences the quality of the fit and the corresponding quality factors. The smaller the errors in the RDCs used in the fitting procedure (leading to narrow confidence limits), the larger the difference in the quality of the fit between different structural proposals. A distinction of different structural proposals is only possible if the RDCs used are able to distinguish between the structural proposals; these usually differ in some intramolecular angles, and therefore, RDCs of different sizes (e.g., for $^1D_{C,H}$) should be obtained.[18] The differences in distances of different structural proposals will only be detectable by using RDCs if couplings other than ^1D are used.

From the determination of **A** the RDCs that are to be expected for the proposed structure are obtained automatically; these are usually called back-calculated RDCs (D_{calc}), which are then usually shown in comparison with the observed ones (D_{obs}) to illustrate the quality of the fit. An example is given in Figure 2, where the correct diastereoisomer (*trans*-configured **1**) gives a significantly better fit of observed versus back-calculated RDCs (D_{obs} vs. D_{calc}) than the wrong diastereoisomer (*cis*-configured **2**). Care, however, must be taken so that all possible structures (diastereoisomers and conformers!) are entered into the selection process, as this fitting routine only selects the best-fitting structure entered. This is especially important when intramolecular flexibility comes into play (see below). Figure 2 therefore serves only for illustration purposes, as compound **1** is not considered to be rigid.[12,21]

This approach is in a way unsatisfying, as one might remain suspicious whether all possible structures have been entered into the selection process and whether the best-fitting one is the best representation of the "true" structure. To alleviate this problem, a third approach can be used for rigid compounds, which automatically functions as a cross check for the data. It uses the fact that in rigid compounds all parts of the compound experience the same averaging process such that the average orientation of the whole compound (known and unknown parts) can be determined from the RDCs of the known part(s). The different structures in question (diastereoisomers, etc.) again differ in some diagnostic angles within the unknown part of the

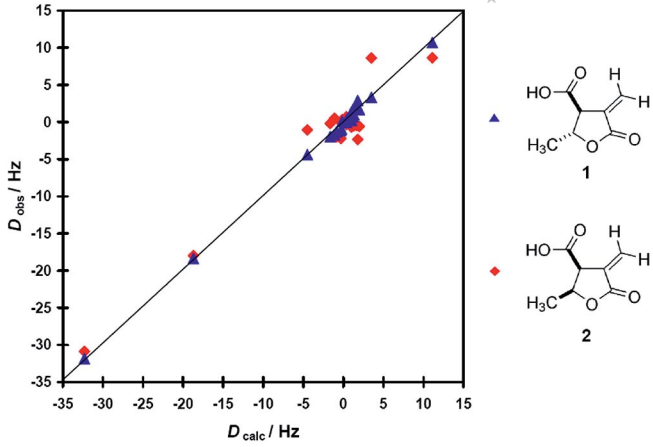

Figure 2. Comparison of fits (of back-calculated RDCs D_{calc} vs. observed RDCs D_{obs} in the determination of **A**) for the two diastereoisomers of α-methylene-γ-butyrolactone (average conformation assumed to be correct representation). *trans*-Configured **1** (blue triangles) shows a significantly better fit than *cis*-configured **2** (red diamonds); thus, the relative configuration can in this case be safely assigned to *trans*.[12,21]

compound. The internuclear vectors (e.g., C–H bonds) in the unknown part will point into different directions for different structural proposals. These will therefore give rise to different sizes of RDCs (except in unfavourable cases[18]). In this approach, **A** is calculated from RDCs belonging to known parts of the compound (again through a best-fit routine) giving the preferred (averaged) orientation of the compound with respect to the magnetic field (as described by **A**). In a second (set of) calculation(s), in which the orientation of the compound is kept fixed (at **A**, which was obtained from the known parts of the compound) RDCs for the unknown part can be predicted. These predicted RDCs are then compared with the ones obtained from the measurement. If the predicted RDCs resemble the measured ones the structural proposal is correct. If the predicted RDCs (of all structural proposals) do not fit the measured RDCs, this means that either the correct structure is not among the structural proposals (used for the prediction) or that, for example, unexpected conformational equilibria are operative. We have shown this approach to work for the (simultaneous) assignment of all diastereotopic protons in strychnine.[22] It is to be expected that it also leads to conclusive solutions for the determination of the relative configurations in rigid compounds.

2.2 Flexible Compounds

As soon as conformational flexibility (meaning large-amplitude, low-frequency torsional motion) is present, all NMR parameters used for structure determination are averaged, which leads to average angles and distances in the structures determined from these data. This is not only true for dihedral angles from ^3J couplings and distances from NOEs,[23] but of course also for RDCs. This averaging of NMR parameters leads to serious consequences in terms of

interpretation of data and is one of the most serious problems in NMR spectroscopic structure determination. The development of methods to make RDCs also applicable to flexible compounds is, however, currently still in its infancy. RDCs will certainly provide solutions to this problem in the future.

Most of the approaches used for structure determination described for rigid compounds (see Section 2.1) are inapplicable to flexible compounds. This is due to the fact that two averaging processes are observed simultaneously: the averaging process due to the tumbling motion of the compound (as for rigid compounds) and the averaging process due to conformational flexibility. On the timescale of an NMR experiment this leads to averaging of distances and angles and therefore also of RDCs. The extent to which RDCs are averaged also depends on the timescale of these motions, such that evidence can be obtained on different degrees of flexibility for different moieties. This will certainly be very useful in the future.

The straightforward use of intramolecular angles and distances, as is possible for rigid compounds, however, is almost impossible for flexible compounds (Figure 3). RDCs of different size are, for example, observed for parallel bonds/vectors, etc.

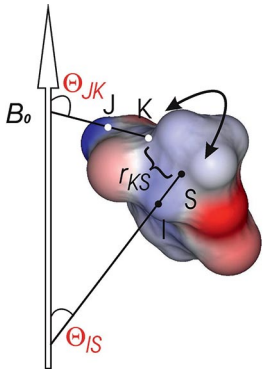

Figure 3. Two (heteronuclear) spin pairs I–S and J–K within one flexible compound. Two averaging processes are observed simultaneously: the averaging process due to the tumbling motion of the compound (as for rigid compounds) and the averaging process due to conformational flexibility. This leads to the averaging of distances (as shown for r_{KS}) and to differential averaging for the angles Θ_{JK} and Θ_{IS}, which makes the use of intramolecular angles and distances much more complicated.

The problems due to these two different kinds of averaging processes have been known almost as long as dipolar couplings.[4,24,25] If flexible systems are to be described exactly, a huge amount of experimental data (RDCs) would be necessary to describe all degrees of freedom. Thus, systems are usually seriously underdetermined and more or less physically sound assumptions concerning the rotational/torsional potential are taken, like in the rotational isomeric state (RIS) approximation[26] or in the additive potential (AP) theory.[27] A different approach uses the maximum entropy (ME) method,[28] which gives the least biased solution in underdetermined systems. Recently, a combination of both methods, called additive potential maximum entropy (APME) method,[29] entered the field. For a more-detailed description and comparison of these methods, the reader is referred to the literature.[4,29]

One has to be aware that the more conformational flexibility that the compound exhibits the less determined the system will be and the more assumptions have to be made with our current means of data interpretation. Therefore, the chance that stereochemical questions (conformation AND configuration determination) can be solved exactly/correctly decreases with increasing flexibility of the compounds. Thus, there is a lot of room for improvement, which is what we and other groups are currently working on.

The few applications of RDCs on configuration determination of flexible compounds that have appeared so far (refs.[12,30,31,32] see also application paragraph) all assume a restricted number of conformers to be present (as known from NOE and J coupling data) and perform a fitting of RDCs to structure protocol (as is also the most popular method for rigid compounds, see above). The best-fitting structure/ensemble of structures is then assumed to represent the correct diastereoisomer.

The main difference between these investigations is the way conformational flexibility is treated. Either an average structure is assumed to be the correct representation (see Figure 2) or an ensemble of conformers is constructed. If one would want to solve this problem exactly, by taking into account all possible conformers, it would be necessary to calculate one alignment tensor per conformer, which requires five linearly independent RDCs per conformer. This is certainly feasible only when the number of conformers is very small, as in the case of our investigation on α-methylene-γ-butyrolactone (**1**), where only two ring conformers are possible per diastereoisomer. We were able to fit an ensemble of conformers (two in this case) to one set of data by using an alignment tensor each.[12] The more approximate approach that was taken by the other groups is the assumption that one alignment tensor can be fit to all conformers jointly, which significantly reduces the amount of linearly independent RDCs needed.[30,31,32] Thus, it will be seen in the future which assumptions can be made safely and what is to be paid attention to when treating conformationally flexible compounds.

3. Orienting Media

All anisotropic NMR parameters, RDCs only being one kind, can be measured only if the dissolved compound forfeits is isotropic tumbling motion and is partially oriented within the magnetic field. The size of the observed (R)DCs is directly proportional to the degree of order induced. In order to stay in the "weak alignment" region, where $D < J$ and the quality of the high-resolution NMR spectra of liquids is maintained, it is essential to control the order induced by the anisotropic medium. For a very detailed discussion concerning the proper choice of alignment conditions see ref.[33]

As RDCs have first been used in biomolecular NMR spectroscopy,[15,34,35] it is not surprising that there is a rather large amount of orienting media inducing a weak degree of orientation for water soluble compounds.[15b] These induce order with the use of using three main approaches: (1) liquid crystals as orienting media, (2) strain-induced alignment in a gel (SAG) and (3) orientation using the anisotropy of the paramagnetic susceptibility (paramagnetic tags).

When we and others[11,16,17,36–38] started our projects concerning configuration determination of organic compounds six years ago, there were hardly any orienting media for solvents other than water. Most organic compounds, however, are insoluble in water.[39] The only available alignment media based on organic solvents at that time where the chiral liquid crystalline phases of homopolypeptides, which were used very successfully by the group of Courtieu for enantiodiscrimination purposes.[40,41] The situation has improved significantly during the last years owing to the developments in SAG media[42–49] and also in the area of liquid crystals (LC).[50,51]

Nematic liquid crystals were the first orienting media employed.[3] These, however, usually introduce too large a degree of order (except when working very close to the clearing point[52]). The liquid crystalline orienting media used nowadays are usually lyotropic liquid crystalline phases, which are composed, in contrast to thermotropic systems, of at least two components: a "liquid crystal builder", which is responsible for the anisotropic behaviour, and a solvent (see Figure 4). This has several advantages. First, the order induced can be manipulated within certain borders and the main component of the mixture is usually the solvent, such that signals originating from the "liquid crystal builder" are not dominant in the spectra and the signals of the compound in question can be observed without problems.

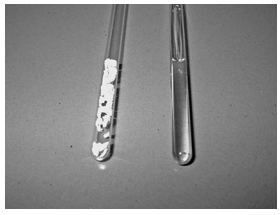

Figure 4. NMR sample tubes (5 mm) containing the solid "liquid crystal builder" poly-γ-benzyl-L-glutamate (PBLG, left-hand side) and the lyotropic liquid crystalline phase made out of PBLG and $CDCl_3$ (7% w/w PBLG in $CDCl_3$[51]) ready for use as orienting medium (right hand side).

One drawback of lyotropic liquid crystalline systems, such as PBLG, is that there is a critical concentration below which the liquid crystalline phase is disrupted and it then becomes an isotropic solution of the "liquid crystal builder" in the solvent. This reduces the scalability of RDCs in these solvents and a physical trick, namely, variable angle sample spinning (VASS),[53,54] needs to be applied if the order induced is still too large at the critical concentration. We are currently working on methods to improve orienting properties of homopolypeptides by chemical means,[51] as they are so far the only class of chiral orienting media compatible with organic solvents. Their chirality will certainly be a significant factor in the future.

A conceptually different approach of inducing order is the SAG method.[55–57] SAG samples are prepared by allowing a polymer gel to swell confined within certain borders (in this case the glass walls of the NMR sample tube), which are smaller than the equilibrium diameter of the gel in the swollen state (Figure 5). From this, a uniaxially anisotropic strain is introduced, such that the solvent and compounds dissolved in the solvent loose their isotropic tumbling motion. The order induced is, in contrary to liquid crystalline orienting media, independent of the strength of the magnetic field and its direction and can be modulated by choosing different degrees of cross linking of the gel[42,46] and by mechanically compressing or stretching the gel within the NMR sample tube.[47]

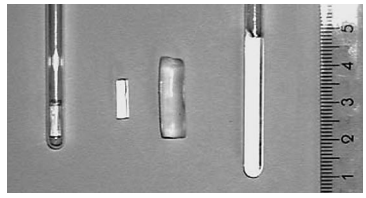

Figure 5. Demonstration of the SAG method by using cross-linked PS: 5-mm NMR sample tube containing dry polymer gel (left hand side), dry polymer stick (second from left) and polymer gel swollen to its equilibrium diameter (centre). If allowed to swell within an NMR sample tube, the gel is compressed, which introduces orientational order in the solvent and solute.[42] Reproduced with permission from Wiley-VCH.

The main advantage of SAG media in comparison to liquid crystalline media is that there is no lower border of the induced orientation and RDCs can be scaled at will by choosing the degree of cross linking before sample preparation or by stretching or compressing the readily prepared gel.[47,58,59] This is so far possible for gels in highly polar solvents (gelatine/D_2O, PAA/D_2O, PAN/DMSO). The corresponding device for gels in apolar solvents is currently being developed.[60] There is to date, however, no chiral SAG medium based on organic solvents. For a chiral water-based SAG medium see ref.[61]

The third approach, which is very successfully used in biomacromolecular NMR, namely, the use of lanthanide ions to orient proteins by the anisotropy of the paramagnetic susceptibility, has so far not been applied to organic compounds.[62–64]

So far, there are no rules as to which compound will give the best results in which alignment medium. When choosing alignment conditions (see Table 1), it must first be determined into which solvent the compound can be dissolved and to determine whether it is necessary to use a chiral phase or if a chiral phase would have adverse effects (as could be the case if racemic compounds are investigated). If the moieties that need to be distinguished/assigned give rise to RDCs of the same size in one orienting medium, it might be worth trying a medium that induces a different

Table 1. Currently available orienting media and their solvent compatibilities and properties (for water-based media, see ref.[15b]).[65]

	$CDCl_3$/ CD_2Cl_2	C_6D_6	*n*-hexane	THF	Dioxane	DMF	CD_3OD	CD_3CN	Acetone	DMSO	D_2O	chiral	Refs.
Homopolypeptides[a]	✓	✓	×	✓	✓	✓	n.s.[b]	n.s.	×	×	×	✓	[40,41,51]
PS gel[c]	✓	✓	×	✓	✓	n.s.	n.s.	×	×	×	×	×	[42,43]
PDMS gel[d]	✓	✓	✓	✓	✓	n.s.	n.s.	n.s.	n.s.	×	×	×	[44]
PMMA gel[e]	✓	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	×	[49]
PVAc gel[f]	✓	✓	n.s.	✓	✓	n.s.	✓	✓	✓	✓	×	×	[45]
PH / PAA gel[g]	n.s.	n.s.	n.s.	n.s.	n.s.	✓	n.s.	n.s.	n.s.	✓	✓	×	[48,56,57]
PAN gel[h]	×	n.s.	n.s.	n.s.	n.s.	×	×	n.s.	n.s.	✓	n.s.	×	[46]
Gelatine[i]	×	×	×	×	×	×	×	×	×	×	✓	✓	[61]
$C_{12}E_5$/*n*-hexanol[j]	×	×	×	×	×	×	×	×	×	✓[k]	✓	×	[50,65]

[a] Lyotropic liquid crystalline phases of PBLG, PELG and PCBLL in the above-stated solvent. [b] n.s.: not stated by the authors. [c] Cross-linked polystyrene. [d] Cross-linked poly(dimethylsiloxane)gel. [e] Cross-linked poly(methyl methacrylate) gel. [f] Cross-linked poly(vinylacetate). [g] Cross-linked poly(acrylamide). [g] Cross-linked poly(acrylonitrile). [i] Polymer gel, no cross-linking necessary due to hydrogen bonding in gelatine. [j] Lyotropic liquid crystalline phases of mixtures of $C_{12}E_5$ with *n*-hexanol in the above-stated solvent. [k] Only in mixtures of DMSO with D_2O.

orientation with respect to the magnetic field. Because of the different orientations, induced RDCs that are the same size by coincidence in one medium may differ in another medium.

4. Measurement Methods

As already seen from Equation (1), two (series of) measurements have to be performed: one in isotropic solution yielding the scalar coupling constants J and one in anisotropic solution yielding the total coupling constants T. The value of D can then be obtained from the difference of the former two. This is, however, only true if the signs of J and T are known from measurement or other considerations ($^1J_{C,H}$ is known to be positive, $^1J_{N,H}$ is known to be negative) as only the absolute values of coupling constants are usually observed in spectra. For unknown signs of J and T, these lead to four possible values of D. This is very unsatisfactory, as structural information is to be obtained from D and the outcome might depend on knowing which of the four values is correct. So, it is strongly recommended to use methods that allow determination of the sign of the isotropic and total coupling constant. This is especially important when $^nD_{C,H}$ and $^nD_{H,H}$ are to be used. As a result of the mostly rather small values of $^nJ_{H,H}$ and $^nJ_{C,H}$, a zero crossing due to large D values and opposing sign of J and D can rather easily be overlooked. This review shall not provide a complete overview over measurement methods, but rather give some decision guidance. That is why special emphasis is laid on measurement methods that allow both the determination of the size and the sign of the scalar and/or total coupling constant. The pulse sequences that have proofed especially useful in biomolecular NMR are reviewed in refs.[15a,15b] up to 2004. The measurement methods described hereafter will not be classified by their mode of operation (frequency- or intensity-based methods) but by the kind of coupling extracted.

4.1. Measurement of $^1D_{C,H}$

As they are the easiest obtained, $^1D_{C,H}$ are the by far the most frequently used RDCs. One-bond heteronuclear scalar couplings are usually rather large ($^1J_{C,H}$ = 120–250 Hz) and if the degree of order is sufficiently small,[66] the values of ^1D are much smaller than ^1J, such that the determination of the sign and size of ^1D is straightforward. If T is larger than J, D is positive, and if T is smaller than J, D is negative (as $^1J_{C,H}$ is known to be positive). Thus, in principle, every method for the measurement of $^1J_{C,H}$ can be used to obtain $^1D_{C,H}$, even (not de-)coupled ^{13}C spectra.[67] This can, however, not be recommended, as measurement times are long, the achievable resolution is low, resonances are prone to overlap and the $^1J/D_{C,H}$ values of diastereotopic protons (within CH_2 groups) cannot be assigned to the corresponding ^1H chemical shift.

State-of-the-art methods are based on HSQC (heteronuclear single quantum coherence) experiments, from which couplings are extracted from frequency differences either in the direct (ω_2) or indirect dimension (ω_1) (see Figure 6).

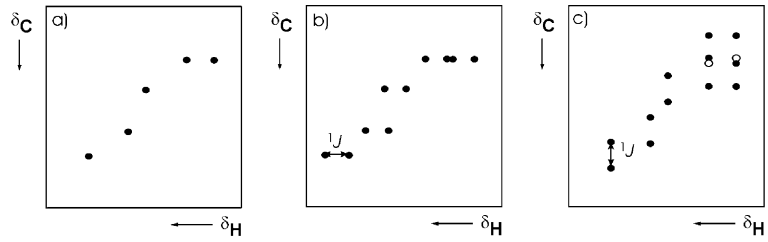

Figure 6. Pictorial representation of HSQC spectra. (a) Ordinary decoupled HSQC spectrum. (b) HSQC spectrum coupled in the direct dimension (other synonyms: ω_2 coupled, t_2 coupled, F2 coupled). The scalar or total coupling constant can be extracted from the splitting in the direct dimension. (c) HSQC spectrum coupled in the indirect dimension (ω_1 coupled, t_1 coupled, F1 coupled). The scalar or total coupling constant can be extracted from the splitting in the indirect dimension.

The arguments for using ω_2-coupled HSQCs (see Figure 6b) are numerous: experiment times are short, the achievable resolution is maximised and couplings to diastereotopic protons can be extracted easily, as long as signals do not overlap. There are, however, also some drawbacks of ω_2-coupled HSQCs when working with oriented samples. First of all, the proton–proton dipolar couplings also evolve during acquisition and are therefore observed in the direct dimension together with the $^1D_{C,H}$ couplings that are of interest, which complicates or even impedes extraction of the latter. This problem can be removed by using homonuclear dipolar decoupling during acquisition.[68] Another problem occurs due to the fact that D values can be either negative or positive, which leads to a rather large spread in total coupling constants. In all indirectly detected spectra, magnetisation transfer is optimum for an average coupling constant (which is usually set to 145 or 150 Hz). The large spread in the values of T leads to inevitably mismatched transfer delays, which lead to phase distortions in the spectra[16,22] (see Figure 7a). This problem can be avoided if the magnetisation is not converted back into in-phase magnetisation before detection, but is recorded starting from anti-phase magnetisation (leading to anti-phase signals, of course).[69,70a,71] The other possibility to get rid of these dispersive anti-phase distortions is to convert them into not-observable multiquantum magnetisation[69,70a] (see Figure 7b). Combining the so-called in-phase and anti-phase spectra even allows the determination of coupling constants in cases when the signals of diastereotopic protons are overlapping (Figure 6b, upper right corner).[70a]

As a result of the above-stated problems, especially the evolution of proton–proton dipolar couplings in the direct dimension, some groups prefer the extraction of coupling constants from ω_1-coupled HSQCs (especially if the orientation is not as small as would be desirable), in which the coupling is extracted from the splitting in the indirect dimension (Figure 6c). The obvious drawbacks of ω_1-coupled HSQCs are the much longer experiment times and the much lower resolution (due to the limited number of experiments that can be recorded in the indirect dimension). The extraction of couplings to diastereotopic protons is possible only if the difference in coupling constants is significant; otherwise, the anti-phase centre signals (Figure 6c, upper right corner) overlap. This overlapping leads to large errors in extracted coupling constants or even to the cancellation of signals. One less obvious drawback, namely, the evolution of long-range couplings, which broadens the signals, can be removed by modifying the HSQC pulse sequence with a G-BIRD module.[72]

This same module has also been used in the corresponding J-modulated experiments (intensity-based method), which allow a much more precise determination of coupling

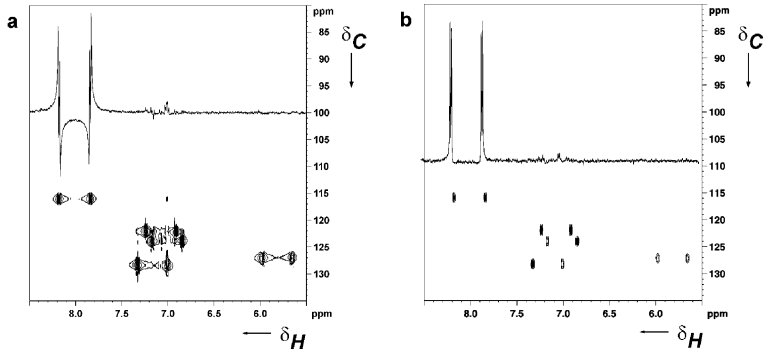

Figure 7. Example cross peaks of ω_2-coupled HSQC spectra of strychnine in $CDCl_3$. The magnetisation transfer delay is consciously mismatched by a duration corresponding to ca. 50 Hz to demonstrate the effect of a total coupling constant of 100 Hz. In (a) the phase distortions due to anti-phase magnetisation can be seen. In (b) the spectrum was recorded by using a slightly modified HSQC pulse sequence (CLIP-HSQC),[70a] in which the anti-phase component of the magnetisation and long-range interactions are removed, which leads to superior line shapes in the case of mismatched magnetisation transfer delays. Note that the residual phase distortion in (b) is due to pulse imperfections, which could be removed by using BEPOP/BIPOP pulses.[70]

constants, with the drawback of an enormous increase in experimental time as a series of spectra is recorded.[73] Intensity-based methods are best suited for extremely small value of D (little difference in J and T) and have so far been mostly applied for water-soluble compounds. J-modulated experiments have also been used for the very precise (but not sign-sensitive) measurement of very small proton–proton and proton–carbon long-range coupling constants.[74–76]

4.2 Measurement of $^nD_{H,H}$

All methods for the sign-sensitive determination of $^nJ_{H,H}/^nD_{H,H}$ rely on the E.COSY principle.[77–79] E.COSY (exclusive correlation spectroscopy) restricts coherence transfer to take place exclusively between connected transitions in the energy-level diagram, which reduces the complexity of multiplet patterns tremendously. It allows the extraction of very small coupling constants (without the problems associated with overlapping lines, see above) and the relative sign of coupling constants. The E.COSY pattern is, however, only observed for spin systems of three or more (nonequivalent) spins (in the original E.COSY experiment; see Figure 8a).

The pulse sequence that proved most useful in our hands is XLOC (X nucleus for long range couplings), which makes use of a directly bonded heteronucleus to obtain the E.COSY-type pattern.[80,81] This has the big advantage that the complexity of cross peaks is significantly reduced (see Figure 8b) and that the value and absolute sign of $^nJ_{H,H}$ can be obtained, as the displacement vector is spanned by $^nJ_{H,H}$ and $^1J_{C,H}$, the latter of which is known to be positive. The most serious drawback of XLOC also stems from the use of a directly bonded heteronucleus, which in organic compounds usually is ^{13}C in natural abundance. Therefore, this pulse sequence is rather insensitive relative to E.COSY.

Out of the huge amount of published pulse sequences (very few of which were designed for the measurement of RDCs) only two others are mentioned, which have been especially designed to measure sign-sensitive heteronuclear one-bond ($^1J/D_{C,H}$) and homonuclear two-bond couplings ($^2J/D_{H,H}$) within one spin system simultaneously, namely the SPITZE HSQC[82] (spin state selective zero overlap HSQC, for CH_2 groups only) and the P.E.HSQC[83] (for all multiplicities).

4.3 Measurement of $^nD_{C,H}$

There is a rather large amount of pulse sequences for the determination of long-range heteronuclear coupling constants (for an excellent review up to 2001, see ref.[84]; for a recent comparison of some pulse sequences for the determination of J and T, see ref.[85]). The determination of size and sign of coupling constants, however, is so far only possible by using pulse sequences based on the X (ω_1) half-filtered TOCSY called HETLOC (determination of heteronuclear long range couplings).[86,87] As can be seen from Figure 8c, a typical E.COSY-type pattern is observed, which allows convenient extraction of coupling constants together with their sign. HETLOC has the serious limitation that only couplings to nonquaternary carbon atoms can be obtained. This reduces the number of RDCs extractable by this method significantly. If RDCs to quaternary carbon atoms are needed, other methods need to be considered and the ambiguity due to the four possible values of D has to be dealt with.

4.4 Measurement of $^1D_{C,C}$ and $^nD_{C,C}$

Carbon–carbon RDCs (one bond and long range) would certainly be very useful additional restraints in the structure determination of organic compounds. There have been, however, very few reports of measured ^{13}C–^{13}C RDCs for organic compounds at natural abundance. All of these have used INADEQUATE to do so.[37,40] With the much more sensitive cryoprobes it seems at least to be possible to measure ^{13}C–^{13}C RDCs with a reasonable amount of sample. Apart from the classical INADEQUATE[88] there are several possible pulse sequences to do so, only two of which are mentioned here. The ^{13}C-detected IPAP-INADEQUATE was shown to allow the simultaneous measurement of one-bond and long-range scalar and residual dipolar

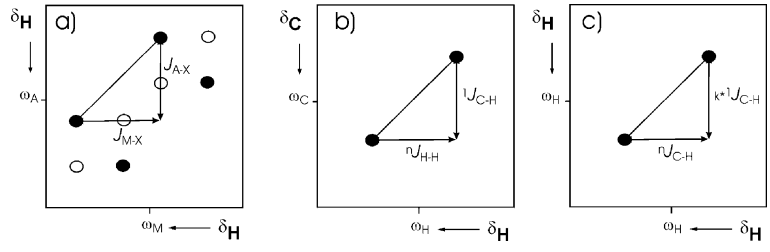

Figure 8. Pictorial representation of several measurement methods relying on the E.COSY principle. (a) AM cross peak in an E.COSY spectrum for an AMX spin system. The passive coupling constants ($J_{M,X}$ and $J_{A,X}$) can be extracted from the AM cross peak (two double anti-phase signals with active coupling constant $J_{A,M}$). However, only their relative sign is obtained from the slope of the displacement vector of the two double anti-phase squares ($J_{M,X}*J_{A,X} > 0$ in this case). (b) XLOC spectrum. The size of the $^nJ_{H,H}$ coupling constant is obtained together with its absolute sign from the displacement vector, as the sign of $^1J_{C,H}$ is known to be positive. $^nJ_{H,H}$ is also positive as can be seen from positive slope. (c) HETLOC spectrum. The size of the $^nJ_{C,H}$ coupling constant is obtained together with its absolute sign from the displacement vector, as the sign of $(k^*)^1J_{C,H}$ is known. $^nJ_{C,H}$ is also positive as can be seen from positive slope. All these sequences can of course not only be used for the extraction of scalar but also of total coupling constants.

couplings in a saccharide (in a water-based orienting medium).[89] Another possibility with the use of proton instead of carbon detection (which again reduces the number of RDCs accessible) is the *J*-modulated ADEQUATE experiment,[90,91] which also allows determination of $^{1}D_{C,C}$ and $^{n}D_{C,C}$. Two separate experiments need to be performed, however, by using different scaling factors in order to be able to do so. All the above-stated methods yield absolute values only, which is inconsequential for $^{1}D_{C,C}$, as is the case for $^{1}D_{C,H}$ ($^{1}J_{C,C}$ is known to be positive and is large relative to $^{1}D_{C,C}$).

5. Applications

The conceptually different ways of using the structural information contained in RDCs and the approximations that have to be taken when dealing with flexible compounds were described thoroughly in Chapter 2 (see also ref.[33]). The applications for the determination of relative configurations and diastereotopic assignments will be treated jointly in this chapter, as the problem posed is conceptually identical and involves the determination of intramolecular angles (interspin vectors). The methodology used is well developed for rigid compounds and could be used by anyone. For flexible compounds, however, the developments are currently in full swing. The determination of conformation and configuration simultaneously from averaged NMR parameters is quite a challenge. We and other groups are currently working on methodologies for the proper treatment of conformationally flexible compounds, and it remains to be seen which approximations can be made safely.

5.1 Rigid Compounds

As described in Chapter 2 there are three different possibilities to use RDCs: (1) RDCs can be used to assess whether bonds are parallel or not. (2) The RDCs are fit to several structural proposals (differing in diastereotopic assignments or relative configurations). The structure for which the best solution is obtained contains the correct stereochemical relation. (3) The preferred orientation of the compound with respect to the magnetic field (alignment tensor) is calculated from known parts of the compound, and the RDCs are predicted for the unknown parts. Comparison of predicted and observed RDCs allows the assignment.

The first approach (parallel bonds exhibiting RDCs of equal size) is used for axial protons in six-membered chair-like rings. This can, for example, be used to assign α or β anomers in sugars, as was shown by Shapiro et al.[16] (see Figure 9, upper part). The distinction of α and β anomers in glucopyranose would already be possible from the angular dependence of the $^{3}J_{H,H}$ coupling. In the second example of this methodology, conventional NMR parameters fail due to the remoteness of the stereogenic centres. By using RDCs, however, it was easily possible to assign the relative configuration in both diastereoisomers of a dihydropyridone. Parallel vectors (C–H bonds) in the *trans*-configured compound exhibit RDCs of the same size; in the *cis*-configured compound different RDCs are observed.[17] (see Figure 9, lower part).

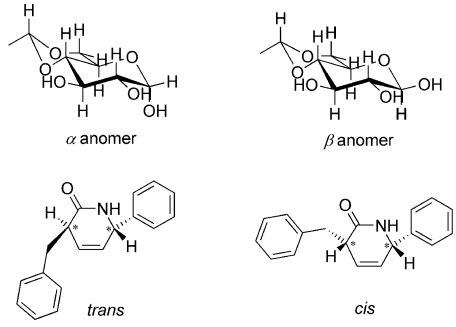

Figure 9. Assignment of relative configurations by using the property that parallel bonds lead to RDCs of the same size. In the upper part, the α and β anomers of a glucopyranose could be distinguished; in the lower part the relative configuration of a dihydropyridone was determined.

The second approach (fitting of RDCs to different structural proposals) is the by far the most used approach. It was used for the assignment of diastereotopic protons/groups in strychnine,[11,22] menthol,[37] norbornene,[92] sphaeropsidin A[45] and ludartin[48] (Figure 10). The first three of these examples (Figure 10a–c) were previously well characterised (including diastereotopic assignments) and have been used as "*proof of principle*" for the method of using RDCs in organic structure determination. For spharopsidin A in Figure 10d, however, several diastereotopic assignments were unknown before RDCs were used.

Furthermore it was possible to determine relative configurations in sodium cholate,[38] a spiroindene,[44] a bicyclic glutamate analogue,[93] and in ludartin[49] (Figure 10). Obviously, the relative configuration of sodium cholate was known before the study and served as a "*proof of principle*" (in this case for using an axially symmetric alignment tensor). The relative configurations of all five stereogenic centres of the bicyclic glutamate analogue (Figure 10f) and of the three stereogenic centres in the spiroindene (Figure 10e) were determined by using RDCs. These assignments were corroborated by X-ray crystallographic studies. For lutardin (Figure 10h), the assignment of the relative configuration involving the epoxide had to be obtained by chemical transformations and detailed NMR chemical shift analysis before the advent of RDCs, as the ^{3}J couplings and NOE data were not indicative for the configuration on the five-membered ring.

The third possibility to use RDCs, that is, the prediction of RDCs based on the calculation of the RDCs from a reduced data set, has to the best of our knowledge only been performed once. It was possible to assign all pairs of diastereotopic protons of strychnine simultaneously by using this strategy.[22]

Many of the above-mentioned examples show the very tedious procedure that is sometimes needed to be able to assign the configuration of natural products and how RDCs can facilitate it significantly.

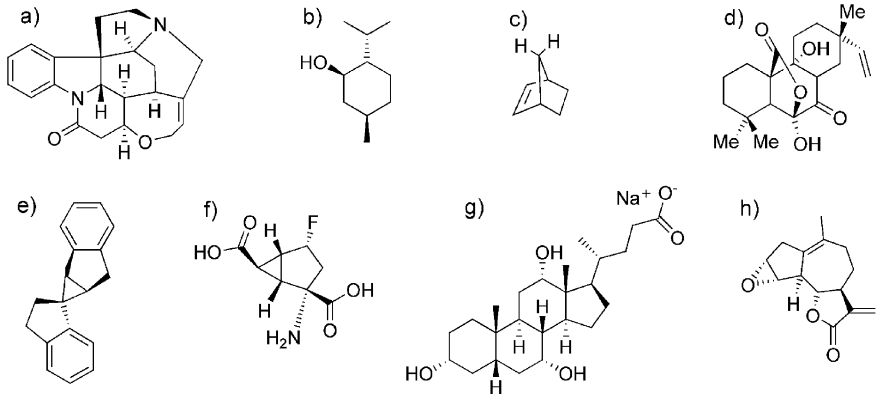

Figure 10. Assignment of diastereotopic groups and relative configurations of rigid compounds: (a) strychnine,[11,22] (b) menthol,[37] (c) norbornene,[94] (d) sphaeropsidin A,[45] (e) spiroindene,[44] (f) bicyclic glutamate analogue,[95] (g) sodium cholate[38] and (h) ludartin.[49] In compounds a, b, c, d and h diastereotopic protons or groups were assigned; in e, f, g and h the relative configuration was determined.

5.2 Flexible Compounds

Impressive reports on the use of RDCs for the investigation of the dynamics of proteins,[15d,15e] oligosaccharides[96–101] and peptides[102–104] can be found in the literature. The use of RDCs for the determination of configuration of organic compounds is still in its infancy, though. This can be attributed to the problem that not only the conformation (and the corresponding ensemble representation) needs to be determined, as is the case in biomolecular NMR, but that conformation *and* configuration are unknown.

So far there are four publications dealing with the determination of conformation and configuration of flexible compounds (see Figure 11). For all of these compounds the determination of relative configuration was either not possible from ^3J coupling analysis and NOE data or there were conflicting assignments in the literature and could be solved by using RDCs as an additional NMR restraint. The relative configuration of the two stereogenic centres of α-methylene-γ-butyrolactone[12] (Figure 11a) was not assignable by using NOE and *J* couplings, as the ensembles of conformers for the two diastereoisomers fulfill the NMR spectroscopic data equally well. The conventional NMR restraints were also not sufficient to determine the relative configuration of sucro-neolambertellin.[31] For the six stereogenic centres of the hexahydroxy unit of Sagittamide A,[30] two conflicting assignments had been published.[105,106] The correct one was confirmed by total synthesis. For archazolide A, the correct structure had been determined (before RDCs became applicable) by using ^3J coupling, NOE and chemical derivatisation.[107]

These latter two examples illustrate the way in which the structure determination of natural products is usually conducted: as the conventional NMR restraints do not suffice to determine the configuration at all stereogenic centres unambiguously, chemical degradation, chemical derivatisation or even total synthesis of several diastereomers of the compounds needs to be performed. We believe that RDCs will certainly make this tedious (and invasive) procedure unnecessary in the future.

There are, however, still some developments necessary. As RDCs are observed as averaged NMR parameters, much effort has to be put into the investigation of how this averaging process can be treated with RDCs and how additional information about the conformational ensemble

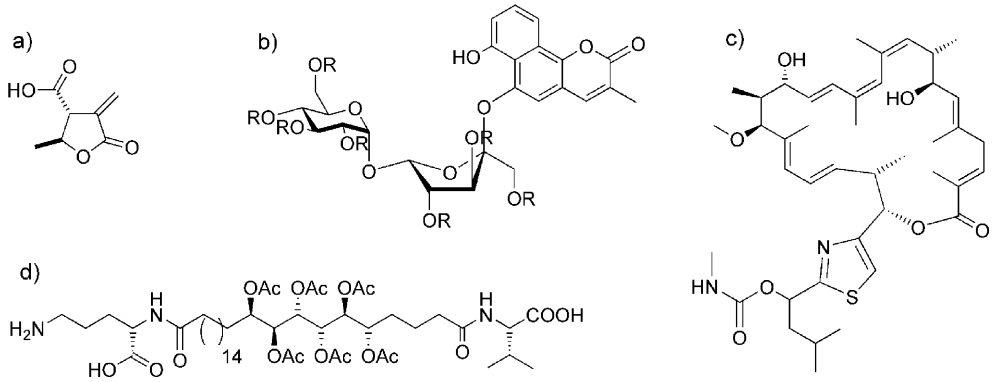

Figure 11. The four applications of RDCs to the relative configuration determination of flexible compounds published so far: (a) an α-methylene-γ-butyrolactone,[12] (b) sucro-neolambertellin,[31] (c) archazolide A[32] and (d) Sagittamide A.[30]

can be obtained. All of the above applications use NOE and ^3J couplings to either construct an average structure[12] (see Figure 2) or to construct ensembles of conformers for all possible diastereoisomers fulfilling NOE and ^3J couplings.[12,30,31,32] These are then subjected to RDC analysis and the ensemble of conformers that fits the data best represents the correct diastereoisomer. The main difference between these applications is whether one orienting tensor is fit to all conformers[30–32] or whether each conformer is represented by its own tensor.[12] The latter approach requires at least five linearly independent RDCs per conformer to be obtained and will certainly only be feasible with compounds exhibiting a small number of conformers. So there is a lot of room for improvement and many exciting developments are to be expected in this area in the future.

6. Conclusions and Outlook

If a compound is oriented with respect to the magnetic field, anisotropic NMR interactions can be obtained, the (residual) dipolar coupling being one example. Controlling the degree of order induced, however, is crucial in order to be able to reliably measure RDCs. The currently available orienting media belong to two groups, namely, compressed polymer gels (SAG) and lyotropic liquid crystalline phases, both of which were described in detail. As soon as the orientational order induced is in the right range, RDCs can be measured. The methods used for the measurement of different sorts of RDCs were described, with an emphasis laid on the determination of signs of coupling constants, as this is crucial for their application in structure determination.

RDCs contain distance and angular information. Their use as an angular restraint is not limited to local geometries (like NOE or *J* coupling), but RDCs can be used as a global restraint, which makes them highly complementary to the other NMR restraints.

The use of RDCs for the determination of the structure (mainly diastereotopic assignments and relative configuration determination) of rigid and flexible compounds has been described. The very few publications on flexible compounds that have appeared so far are very promising and already show that RDCs can revolutionise the way in which the structures of organic compounds are determined. Structure determination by using RDCs will hopefully supersede the tedious and invasive chemical derivatisation and total synthesis of several diastereoisomers of natural products.

Further developments, however, are desirable: there are hardly any chiral orienting media, which will hopefully allow absolute configuration determination in the future, and the treatment of intramolecular flexibility needs to be improved before RDCs are applicable to flexible compounds with remote stereogenic centres.

Acknowledgments

C. M. T. thanks Prof. M. Reggelin for his support and the Fonds der Chemischen Industrie and the Deutsche Forschungsgemeinschaft (DFG) (Emmy Noether programme) for funding.

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[13] The equation given here is modified relative to the one given in: M. H. Levitt, *Spin Dynamics: Basics of nuclear magnetic resonance*, Wiley, Chichester, **2001**, p. 425–431. The distance r_{IS} was moved into the "averaging bracket" to also be able to account for intramolecular flexibility.
[14] In principle, r_{IS} is also averaged by bond vibrations (fast, small-amplitude vibrational motion), which would require vibrational corrections. This is, however, usually neglected when dealing with RDCs. If exact structural information is to be obtained (see for example ref.[7]) this is indispensable.
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[20] There are several ways how to judge whether RDCs are linearly independent. One can examine the range of orientations within the compound that is covered by the interspin vectors, look at the distribution of values of RDCs (for RDCs of the same kind) or determine the condition number of the matrix.
[21] In this special case, the transition structures between the two possible conformers per diastereoisomer are an approximate representation of the conformational equilibrium and the treatment of the averaged structures as "rigid" is justified. For more details see ref.[12] and paragraph on flexible compounds.
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Received: July 10, 2008
Published Online: October 7, 2008

