

# Residual Dipolar Couplings in NMR Spectroscopy Using Lanthanide Tags

Elias Akoury

**Abstract**—Nuclear Magnetic Resonance (NMR) spectroscopy is an indispensable technique used in structure determination of small and macromolecules to study their physical properties, elucidation of characteristic interactions, dynamics and thermodynamic processes. Quantum mechanics defines the theoretical description of NMR spectroscopy and treatment of the dynamics of nuclear spin systems. The phenomenon of residual dipolar coupling (RDCs) has become a routine tool for accurate structure determination by providing global orientation information of magnetic dipole-dipole interaction vectors within a common reference frame. This offers accessibility of distance-independent angular information and insights to local relaxation. The measurement of RDCs requires an anisotropic orientation medium for the molecules to partially align along the magnetic field. This can be achieved by introduction of liquid crystals or attaching a paramagnetic center. Although anisotropic paramagnetic tags continue to mark achievements in the biomolecular NMR of large proteins, its application in small organic molecules remains unspread. Here, we propose a strategy for the synthesis of a lanthanide tag and the measurement of RDCs in organic molecules using paramagnetic lanthanide complexes.

**Keywords**—Lanthanide Tags, NMR spectroscopy, residual dipolar coupling, quantum mechanics of spin dynamics.

## I. INTRODUCTION

RDCs have become a fundamental approach in structural determination of proteins, nucleic acids, and carbohydrates. RDCs are spectroscopic interactions that appear in high resolution NMR spectroscopy. Although it is originally discovered for small molecules in liquid crystal solvents [1], the RDC spectra were too complex for practical use in structure determination. However, the discovery of weak orienting media in aqueous solutions led to a boost in their application in biomolecular analysis. RDCs can provide long-range angular and distance restraints to solve structural ambiguities, an advantage over the conventional Nuclear Overhauser Effect (NOE) analysis, which is limited to 5 Å radius [2].

RDCs are orientation-dependent interactions that are observed in an anisotropic environment provided by suitable alignment media. This anisotropy allows extraction of angular information relative to an external reference, determination of conformations and configurations of molecules and distinguishing of enantiomers. While dipolar coupling interactions are dominant in solid-state NMR, they are averaged to zero in liquid-state NMR due to isotropic uniform distribution of the orientations based on the *Rotational*

*Brownian Diffusion*. Consequently, a wealth of structural insights is lost once the dipolar couplings vanish. Nevertheless, once displayed in a homogenous magnetic field together with an alignment media, the molecule adapts a preferred orientation that favors anisotropy. This allows the anisotropic magnetic interactions to become observable [3].

Using quantum mechanics, the dipolar interaction of nuclear spins rotating at the *Larmor frequency* is described in a simplified two-spin system. Spins *I* and *S* with a fixed distance  $R_{IS}$  are oriented at an internuclear angle  $\Theta_{IS}$  in a static magnetic field  $B_0$  (Fig. 1). Spin *I* induces a magnetic field added to the static magnetic field felt by spin *S* and causes a shift in its resonance frequency, the dipolar coupling [2]. Since spins parallel and anti-parallel to  $B_0$  are equally populated, splitting into the dipolar coupling  $2D_{IS}$  is observed and is directly proportional to  $3\cos^2(\Theta_{IS} - 1)$ . This RDC contains valuable structural information and covers a large portion of the NMR timescale allowing its application in small organic molecules and biomolecules.

The quantum mechanical approach of RDCs is based on the averaging process of the anisotropic tumbling motion and is described by the alignment tensor *A*. The dipolar coupling Hamiltonian  $H_D$  depicting the two spins *I* and *S* has the form:

$$H_D = 2\pi D \left\{ I_{zL}S_{zL} - \frac{1}{2}I_{xL}S_{xL} - \frac{1}{2}I_{yL}S_{yL} \right\} \quad (1)$$

If the spins *I* and *S* are heteronuclear,  $H_D$  is simplified to:

$$H_D = 2\pi D I_{zL}S_{zL} \quad (2)$$

and the dipolar coupling constant *D* is represented by the equation:

$$D = \frac{K}{R^3} \left( \cos^2\Theta_{IS} - \frac{1}{3} \right) \quad (3)$$

*K* is a term that gathers the physical constants of the spins:

$$K = -\frac{3}{8\pi^2} (\gamma_I \gamma_S \mu_0 \hbar) \quad (4)$$

where  $\gamma_I$  and  $\gamma_S$  are the gyromagnetic ratios of spins *I* and *S*, respectively,  $\mu_0$  is the vacuum permeability, and  $\hbar$  is Plank's constant. It is worth mentioning that *D* and  $H_D$  are both time-dependent, and therefore, the time-averaged RDC constant  $\bar{D}$  is represented as:

$$\bar{D} = \frac{K}{R^3} \left( \overline{\cos^2\Theta_{IS}} - \frac{1}{3} \right) \quad (5)$$

E. Akoury is a research associate at the Faculty of Chemistry and Pharmacy at the Ludwig-Maximilians University LMU, Munich, Germany (corresponding author, e-mail: elias.akoury@lmu.de).

To calculate  $\bar{D}$  for any pair of spins, the alignment properties of a molecule in the molecular frame approach have to be first described. In this reference system, the orientation of the magnetic field is time-dependent, the inter-nuclear vector is set constant, and the *probability tensor*  $\mathbf{P}$  is introduced.  $\mathbf{P}$  is a second order approximation of the orientation probability distribution for the direction of the external magnetic field [4] and is represented by an ellipsoid with fixed orientation in the (x,y,z) molecular frame (Fig. 2). The three principal values of  $\mathbf{P}$  are the probabilities of finding the magnetic field along the principal axes of the ellipsoid such that

$$P_{\bar{x}} + P_{\bar{y}} + P_{\bar{z}} = 1 \quad (6)$$

This simplifies the calculation of the RDC constants and requires only knowledge of the three Cartesian components  $r_{\bar{x}}$ ,  $r_{\bar{y}}$  and  $r_{\bar{z}}$  of a given inter-nuclear unit vector  $\vec{r}$ :

$$\overline{\cos^2 \Theta_{IS}} = P_{\bar{x}} r_{\bar{x}}^2 + P_{\bar{y}} r_{\bar{y}}^2 + P_{\bar{z}} r_{\bar{z}}^2 \quad (7)$$

The alignment tensor  $\mathbf{A}$  of the anisotropic tumbling motion is then extracted from  $\mathbf{P}$  and fitted with the principal components:

$$\mathbf{A} = \mathbf{P} - \frac{1}{3} \quad (8)$$

$$A_{\bar{x}} + A_{\bar{y}} + A_{\bar{z}} = 0 \quad (9)$$

$$\left( \overline{\cos^2 \Theta_{IS}} - \frac{1}{3} \right) = A_{\bar{x}} r_{\bar{x}}^2 + A_{\bar{y}} r_{\bar{y}}^2 + A_{\bar{z}} r_{\bar{z}}^2 \quad (10)$$

The time-averaged RDC representing the direct dipole-dipole interaction for spins  $\mathbf{I}$  and  $\mathbf{S}$  is represented by the equation:

$$\bar{D}_{IS} = \frac{\gamma_I \gamma_S \mu_0 \hbar}{16\pi^2} \left\langle \frac{1}{r_{IS}^3} \left( 3 \overline{\cos^2 \Theta_{IS}} - 1 \right) \right\rangle \quad (11)$$

The observed anisotropic dipolar coupling  $\mathbf{D}_{IS}$  adds to the scalar  $\mathbf{J}$  coupling, and both contribute to the total coupling constant  $\mathbf{T}$ :

$$|T| = |J + 2D| \quad (12)$$

Therefore, two measurement series are required to determine the dipolar coupling  $\mathbf{D}$  for a spin system; an isotropic spectrum that determines  $\mathbf{J}$  scalar couplings, and another anisotropic spectrum to measure  $\mathbf{T}$  total couplings. The difference between the two spectra determines  $\mathbf{D}$  (Fig. 2).

The most common orienting media used for partially aligning a molecule are liquid crystals, strain-induced alignment gels (SAGs), and anisotropic paramagnetic tags. The application of liquid crystals is limited to small symmetric molecules since they induce high degree of order and consequently large dipolar couplings [3]. On the other hand, the alignment stretch of SAG is solely determined by mechanical stretching, and its use is limited due to the lengthy

preparation time for solutes to diffuse into the polymer gel [5].

An alternative alignment method, the paramagnetic lanthanide tag, has recently implemented considerable advances in the field of biomolecular NMR of large proteins. Mainly, this involves a lanthanide ion that orients molecules by the anisotropy of the paramagnetic susceptibility. Paramagnetic centers have the advantage of providing long-range structural restraints and accordingly ingenious applications in structure determination of proteins, ligand-protein interactions [6], [7], and complex dynamics. So far, the use of lanthanide tags has not been applied to small organic molecules due to the broadening effects in spectral lines of neighboring nuclei covalently attached to the paramagnetic ions [2]. In this study, we describe the strategy of synthesizing and applying a dipicolinic acid known for its lanthanide tagging of proteins using a single cysteine residue [8]. The tag 4-mercaptomethyl-dipicolinic acid (4-MMDPA) coordinates metal ions in a non-chiral fashion and can be readily attached to a cysteine thiol group via disulfide bridging. Using thiol chemistry, we synthesized the 4-MMDPA tag and covalently attached it to naphthalene-2-thiol. Diamagnetic and paramagnetic lanthanide ions were then applied to induce RDC measurements in organic molecules.

## II. MATERIALS AND METHODS

### A. Materials

Benzotriazole, Dimethyl 2,6-pyridinedicarboxylate, 5,5'-dithiobis(2-nitrobenzoic acid), Naphthalene-2-thiol and pyridine dicarboxylic acid were purchased from Sigma Aldrich Chemie GmbH (Schnelldorf, Germany). All lanthanide compounds were purchased from Merck Chemicals GmbH (Darmstadt, Germany). Deuterated solvents were purchased from Deutero GmbH (Kastellaun, Germany). All NMR measurements were recorded at 25 °C on a BRUKER DRX 400 spectrometer and were referenced internally to TMS. The measured RDCs were obtained from the  $^{13}\text{C}$  dimension of the 2D  $^1\text{H}$ - $^{13}\text{C}$ -HSQC spectra recorded without carbon decoupling and with a digital resolution of 0.4 Hz/ data point.

### B. Methods

#### 1. Mono-Hydroxymethylation

Synthesis of 2,6-dimethoxy carbonyl -4-hydroxyl methylpyridine (**2**) – Compound (**1**) was first identified by  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.32 (d,  $J = 7.87$  Hz, 2H);  $\delta$  8.05 (t,  $J = 7.84$  Hz, 1H);  $\delta$  4.01 (s, 6H). (**1**) (2.226 g, 11.4 mmol) was added to sulfuric acid (30% v/v). A saturated aqueous solution of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  was added dropwise followed by  $\text{H}_2\text{O}_2$  solution (30% v/v, 131 mmol) with temperature control between 42–45 °C. The reaction was stirred for an additional hour at room temperature and the solution was pH 6.4. The suspension was filtered and extracted with ethyl acetate. The organic phase was dried, filtered, and evaporated to obtain product (**2**). After a column chromatography purification step ( $\text{SiO}_2$ , 100% ethyl acetate), product (**2**) (0.215 g, yield: 26 %) was identified by  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.31 (s, 2H);

$\delta$  4.91 (s, 2H);  $\delta$  4.01 (s, 6H). This reaction was repeated while varying the concentrations of  $\text{H}_2\text{O}_2$  and  $\text{H}_2\text{SO}_4$  and at

different temperatures. The optimal conditions are the ones mentioned above.

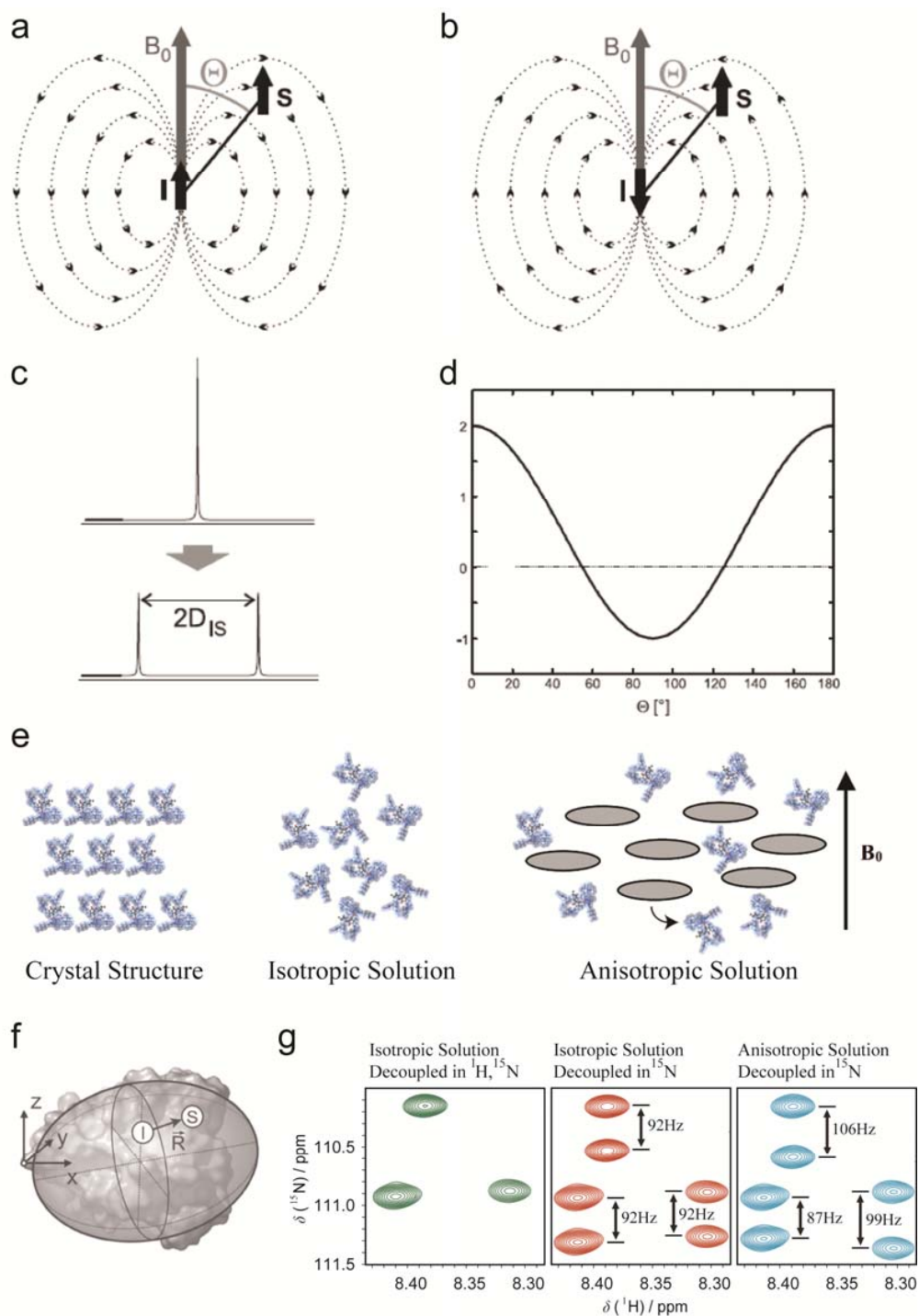


Fig. 1 Dipolar interaction between spins  $I$  and  $S$  (a) parallel and (b) anti-parallel to the magnetic field  $B_0$ . (c) Dipolar coupling splitting  $2D_{IS}$  and (d) the relation of angular component relative to  $B_0$  [2]. (e) The behavior of a molecule with  $B_0$  in crystal form, in isotropic solution and in anisotropic solution with a partially oriented medium. (f) Representation of the probability tensor  $P$  in the molecular frame reference system. (g) 2D  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra decoupled in one or both dimensions, isotropic or anisotropic

## 2. Tosylation

Synthesis of 2,6-dimethoxy carbonyl –4-tosyl oxymethylpyridine (**3**) – A solution of *p*-toluene sulfonyl chloride (0.4 g, 1.51 mmol) and dichloromethane was added dropwise to a cold solution containing product (**2**) (0.215 g, 0.96 mmol) in dichloromethane. Under stirring at 0 °C, triethylamine (0.8 ml) was added dropwise in three portions at 30 minutes interval. The mixture was diluted with ethyl acetate and the organic phase was extracted with water and 3M hydrochloric acid. The tosylated product (**3**) (0.186 g, yield: 86.5 %) was identified by <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 8.17 (s, 2H); δ 7.81 (d, *J* = 8.31 Hz, 2H); δ 7.36 (d, *J* = 8.24 Hz, 2H); δ 5.19 (s, 2H); δ 4.01 (s, 6H); δ 2.45 (s, 3H).

## 3. Bromination

Synthesis of 2,6-Dimethoxy carbonyl –4-bromo methylpyridine (**4**) – Lithium bromide (0.400 g, 4.60 mmol) was added to a solution containing product (**3**) (0.186 g, 0.492 mmol). The solution was stirred at room temperature for 3 hours and the suspension filtered, concentrated under vacuum and the residues triturated with chloroform. The product was dissolved in ethyl acetate and purified by preparative thin layer chromatography. The brominated compound (**4**) (0.240 g, yield: 65%) was obtained. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 8.32 (s, 2H); δ 4.50 (s, 2H); δ 4.03 (s, 6H).

## 4. Thiolation

Synthesis of 4-Mercaptomethyl-2,6-pyridinecarboxylic acid (**6**) – Under nitrogen atmosphere, a solution of (**4**) (0.120 g, 0.418 mmol) and thiourea (0.060 g, 0.788 mmol) in methanol was refluxed overnight. The exchange of the bromine by the thiol functional group yielded product (**5**), which was then dissolved in deoxygenated water and sodium hydroxide. The mixture was refluxed overnight under nitrogen atmosphere. Product (**6**) was obtained (0.07 g, 58.3%) <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ 8.28 (s, 2H); δ 3.86 (s, 2H).

## 5. Disulfide Bridging

Synthesis of 4-(Naphthalen-2-yl-disulfanyl)-pyridine-2,6-dicarboxylic acid (**10**) – 5,5'-Dithio-Bis-(2-Nitrobenzoic acid) (DTNB) (**8**) was identified by <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) δ 7.97 (d, *J* = 8.76 Hz, 2H); δ 7.59 (d, *J* = 8.92 Hz, 2H); δ 7.53 (m, 2H). (**8**) (1 g, 2.53 mmol) was dissolved in 1:1 methanol/H<sub>2</sub>O solution. Naphthalene-2-thiol (**7**) (0.4 g, 2.49 mmol) was added dropwise into DTNB solution. (**6**) (0.02 g, 0.094 mmol) was poured over a solution containing (**7**) and (**8**). The resulting compound was concentrated, extracted with diethyl ether and identified by <sup>1</sup>H NMR spectroscopy as compound (**10**).

## 6. Incorporation of Lanthanide Ion

A solution of the tagged molecule (**10**) (1.67 mg) was dissolved in DMSO and different lanthanide ions were added as follows: LaCl<sub>3</sub>·7H<sub>2</sub>O (3.7 mg) in 1:1 ratio (10 mM); Eu(FOD)<sub>3</sub> (2.068 mg) in 0.2:1 ratio (2 mM); Eu<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> (1.184 mg) in 0.2:1 ratio (2 mM); Eu(FOD)<sub>3</sub> (5.17 mg) in 0.5:1 ratio (5 mM); Eu<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> (2.959 mg) in 0.5:1 ratio (5 mM);

Eu<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> (5.918 mg) in 1:1 ratio (10 mM); Tb(NO<sub>3</sub>)<sub>3</sub> (3.809 mg) in 0.5:1 ratio (10 mM).

## III. RESULTS AND DISCUSSION

The design of functional lanthanide-containing coordination compounds requires the precise control of the lanthanide inner coordination sphere [10]. We propose a strategy for measuring RDCs in organic compounds using paramagnetic lanthanide tags. The synthesis of 4-MMDPA (**6**) is outlined in Fig. 2 based on modified literature methods [8], [9]. The starting material Dimethyl 2,6-pyridinedicarboxylate (**1**) undergoes a direct nucleophilic aromatic substitution to incorporate a mono-hydroxymethyl group (**2**), which is then protected by tosylation (**3**). The tosyl group is by nature electron-withdrawing hence readily substituted by bromine. The resulting bromomethyl pyridine (**4**) undergoes thiolation and generates product (**5**) which upon the de-protection of its carboxylic moieties yields the desired product 4-MMDPA (**6**). All intermediate products were identified by 1D <sup>1</sup>H NMR (Fig. 2).

The majority of the methods developed for the synthesis of unsymmetrical disulfides involve nucleophilic substitution of a sulfonyl derivative by a thiol or its derivative. However, the preparation of the sulfonyl intermediate requires several steps and the use of toxic chlorinating agents such as SOCl<sub>2</sub> and Cl<sub>2</sub>. However, our attempt to prepare the unsymmetrical disulfide compound from the corresponding thiols, 4-MMDPA (**6**) and naphthalene-2-thiol (**7**), followed a modified approach with milder conditions that was adopted from disulfide bridging methods of protein chemistry. 5, 5'-Dithiobis (2-nitrobenzoic acid), DTNB, also referred to as Ellman's reagent, is a symmetrical disulfide commonly used in analytical biochemistry, particularly in labeling cysteine residues. Reports suggested that 4-MMDPA can be readily attached to a cysteine thiol group of a protein via a disulfide bridge using established DTNB chemistry [6], [7]. DTNB breaks its disulfide bridge to form the thiol nitrobenzoic acid, which forms a disulfide bond to a thiol-containing compound. We have therefore depicted this method to couple naphthalene-2-thiol (**7**) with 4-MMDPA (**6**) (Fig. 3). DTNB (**8**) reacts with naphthalene-2-thiol (**7**) to generate the disulfide intermediate product (**9**). 4-MMDPA (**6**) readily exchanges the TNB moiety of (**9**) to yield the desired asymmetrical disulfide product (**10**).

The principle states that when a paramagnetic lanthanide ion is introduced to a solution, a series of shifted resonances appears in the <sup>1</sup>H spectrum far outside the spectral range. But with an appropriate order of alignment, RDCs measurements are obtained within spectral range from the differences of the paramagnetic and diamagnetic states. We have investigated the effect of paramagnetic lanthanides by selecting four lanthanide reagents: (a) Lanthanum chloride heptahydrate (LaCl<sub>3</sub>·7H<sub>2</sub>O) as a diamagnetic reference; (b) Europium as a moderately paramagnetic lanthanide ion in its organic state, Eu(FOD)<sub>3</sub>, and inorganic state (Eu<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>) and (c) terbium (III) nitrate pentahydrate (Tb(NO<sub>3</sub>)<sub>3</sub>·5H<sub>2</sub>O) for its highly paramagnetic effect.

<sup>1</sup>H NMR and <sup>13</sup>C HSQC experiments were first recorded for

the diamagnetic reference (1:1 Tag– $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$  solution, 10 mM) The  $^1\text{H}$  NMR spectra (Fig. 4) showed chemical shifts and peak broadening. A difference in the  $J$  scalar coupling of the C–H bond of the naphthalene moiety of 3.53 Hz was observed in the  $^1\text{H}$ - $^{13}\text{C}$  HSQC set as  $J_{\text{dia}}$ , the scalar coupling from the diamagnetic reference.  $\text{Eu}(\text{FOD})_3$  (tris (6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato) europium consists of three bidentate acetylacetonato ligands bound to  $\text{Eu}^{3+}$  center. The paramagnetic compound induces shifts in the protons near the Lewis basic sites of the molecule. We measured 1D  $^1\text{H}$  and 2D  $^{13}\text{C}$  HSQC experiments on three samples of the lanthanide tag (10 mM) while varying  $\text{Eu}(\text{FOD})_3$  concentrations (2 mM, 5 mM and 10 mM). The proton  $^1\text{H}$  spectra (Fig. 4) showed broadening of the peaks and loss of resolution with increasing concentration accompanied with changes in the value of the *total coupling*  $T$ . The differences between  $T$  and  $J_{\text{dia}}$  correlate directly to the RDCs values. Indeed, the differences were significant (between 0.76 and 2.21 Hz). Similarly, we measured 1D  $^1\text{H}$  and 2D  $^{13}\text{C}$

HSQC experiments of the lanthanide tag (10 mM) while varying concentrations of  $\text{Eu}_2(\text{SO}_4)_3$  (2 mM, 5 mM, and 10 mM). The proton  $^1\text{H}$  spectra (Fig. 4) again showed dramatic peak broadening and loss of resolution as a function of lanthanide concentration. However, the broadening is more significant than in the  $\text{Eu}(\text{FOD})_3$  measurements since the sulfate ions behave differently in solution than the organic ligand, and the Eu ions are more readily available to induce paramagnetism. Similarly,  $^{13}\text{C}$  HSQC experiments (Fig. 4) showed again significant changes in the value of the total coupling  $T$ . The differences between  $T$  and  $J_{\text{dia}}$  in the case of  $\text{Eu}_2(\text{SO}_4)_3$  (3.93 and 5.37 Hz) were larger than the values obtained with  $\text{Eu}(\text{FOD})_3$ . Moreover, the highest lanthanide concentration induced a strong paramagnetic interaction which resulted in peak broadening beyond detection. The  $^1\text{H}$  NMR spectrum of the lanthanide tag in presence of  $\text{Tb}(\text{NO}_3)_3$  (10mM) proved the strong paramagnetic effect on peak broadening and dramatic loss in resolution.

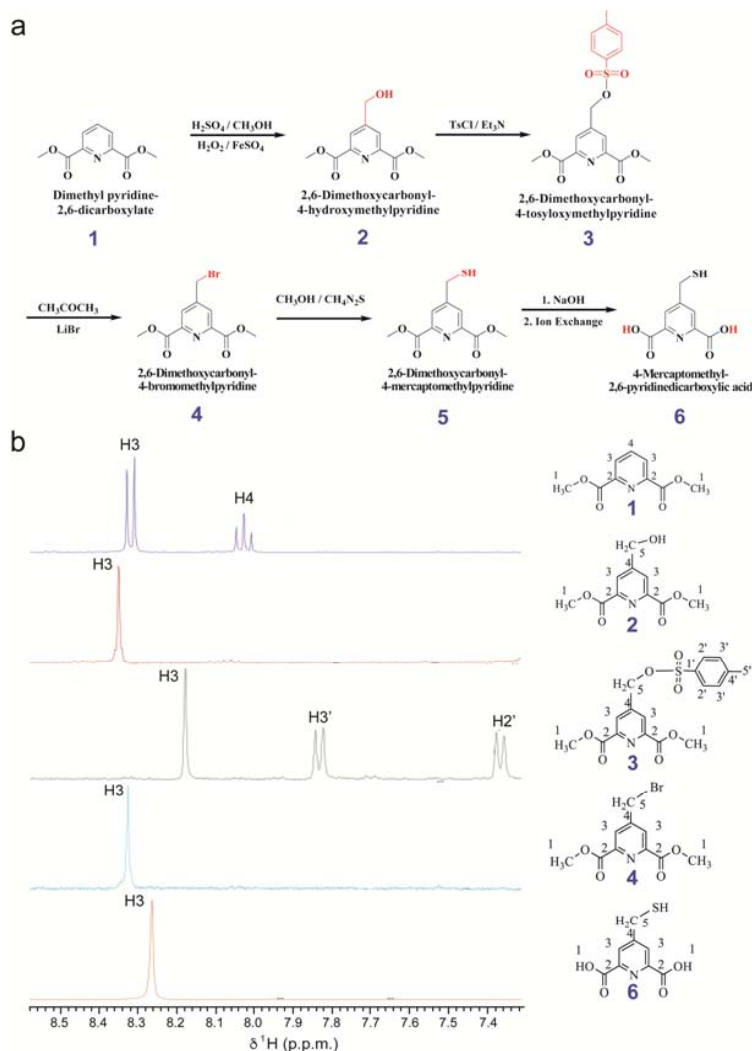


Fig. 2 Strategy for the synthesis of 4-MMDPA. (b) 1D  $^1\text{H}$  NMR spectra showing the aromatic region of the intermediates generated along the pathway of 4-MMDPA synthesis

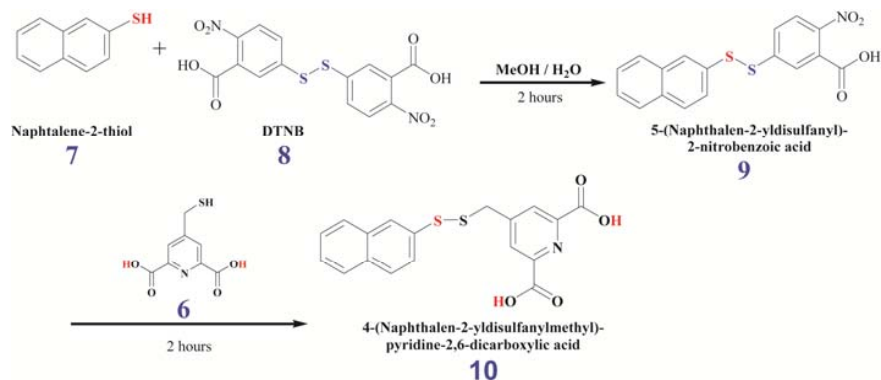
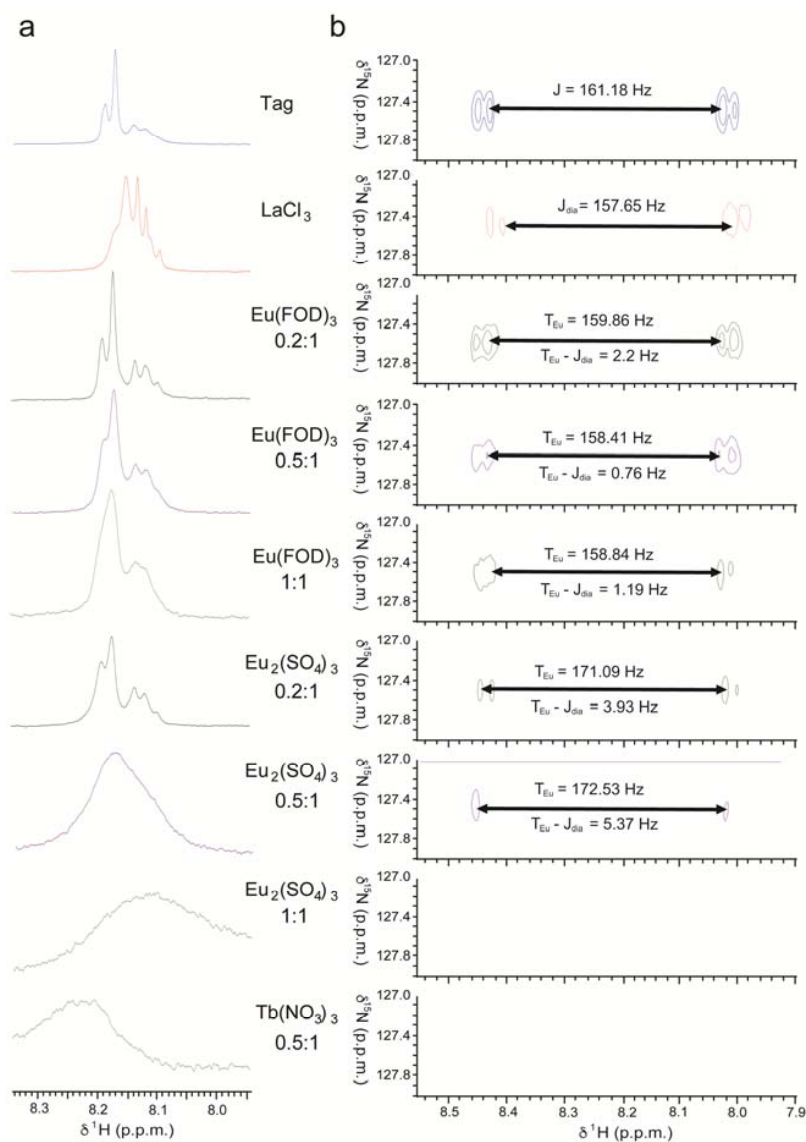


Fig. 3 Synthesis of the unsymmetrical disulfide between 4-MMDPA and naphthalene-2-thiol using DTNB

Fig. 4 (a) 1D  $^1\text{H}$  NMR and (b) 2D  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectra of the free tag, and in complex with the diamagnetic lanthanide  $\text{LaCl}_3$ , or the paramagnetic lanthanides  $\text{Eu}(\text{FOD})_3$ ,  $\text{Eu}_2(\text{SO}_4)_3$ ,  $\text{Tb}(\text{NO}_3)_3$

## IV. CONCLUSION

In this study, we report the strategy to measure RDCs in small organic molecules after attaching the 4-MMDPA thiol tag to the thiol organic molecule, naphthalene-2-thiol using disulfide chemistry. A diamagnetic reference ( $\text{LaCl}_3$ ) is compared with different paramagnetic lanthanides ( $\text{Eu}(\text{FOD})_3$ ,  $\text{Eu}_2(\text{SO}_4)_3$  and  $\text{Tb}(\text{NO}_3)_3$ ). Once a proper alignment within the magnetic field is achieved, the paramagnetic solution shows total coupling constants,  $T$ , and the differences with the scalar couplings from the diamagnetic solution,  $J$ , would mark the RDC constants,  $D$ . Paramagnetic broadening yields interactions between the NMR-active nuclei and the paramagnetic electrons. Consequently, this shortens the spin-lattice relaxation times of the nuclei and causes uncertainty broadening and loss of resolution. In our experiments, we have obtained RDCs values between 1.5 and 5.37 Hz that lie within the experimental digital resolution of 0.4 Hz/data point. This strategy broadens the application of paramagnetic lanthanide tags for the measurement of RDCs in organic molecules.

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E. Akoury designed the study, conducted the experiments, analyzed the data and wrote the manuscript. Dr. Stephenson is kindly acknowledged for the NMR discussions. Mrs. Silvana Fares is kindly acknowledged for her help in preparing the manuscript.

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