

## Measurement of Long-Range Interatomic Distances by Solid-State Tritium-NMR Spectroscopy

Alexander K. L. Yuen,<sup>†</sup> Olivier Lafon,<sup>§</sup> Thibault Charpentier,<sup>\*,§</sup> Myriam Roy,<sup>†</sup> Francine Brunet,<sup>§</sup> Patrick Berthault,<sup>§</sup> Dimitrios Sakellariou,<sup>§</sup> Bruno Robert,<sup>‡</sup> Sylvie Rimsky,<sup>||</sup> Florence Pillon,<sup>†</sup> Jean-Christophe Cintrat,<sup>†</sup> and Bernard Rousseau<sup>\*,†</sup>

CEA, iBiTecS, Service de Chimie Bioorganique et de Marquage, F-91191 Gif sur Yvette, France, CEA, iBiTecS, Service de Bioénergétique, Biologie Structurale et Mécanismes, F-91191 Gif sur Yvette, France, CEA, IRAMIS, SIS2M, Laboratoire Structure et Dynamique par Résonance Magnétique, F-91191 Gif sur Yvette, France, and LBPA, CNRS, ENS de Cachan, 61, avenue du Président Wilson, F-94235 Cachan, France

Received January 29, 2009; Revised Manuscript Received October 19, 2009; E-mail: thibault.charpentier@cea.fr; bernard.rousseau@cea.fr

Solid-state NMR has clearly proven to be valuable for probing molecular structure, owing to its ability to measure accurate interatomic distances.<sup>1</sup> In the absence of high-resolution X-ray data, such measurements are mandatory for determining the conformation of a small molecule bound to an insoluble or membrane protein. Knowledge of this conformation is of key importance in drug design.<sup>2</sup> Long-range distances can be determined using either beacons such as <sup>19</sup>F (distances up to 8 Å)<sup>3</sup> or paramagnetic spin labels (up to 20 Å).<sup>4</sup> In both of these cases, however, modification of the ligand structure is an unwanted consequence. Isotopic labeling using <sup>13</sup>C—<sup>13</sup>C or <sup>13</sup>C—<sup>15</sup>N does not lead to structural modifications, but only short distances can be measured (5–6 Å).<sup>1</sup> Most of the conformational changes when a ligand binds its receptor involve variation of long-range interatomic distances. For instance, the bioactive conformation of paclitaxel requires the determination of a key distance greater than 10 Å.<sup>5</sup> To gain access to such distances without structural modifications, we speculated that tritium would be an attractive label owing to the fact that it has a spin of 1/2 and the highest gyromagnetic ratio of any nucleus (1.07 times that of proton). As the natural abundance of tritium is negligible (3 × 10<sup>-16</sup>%), no background signal would be observed. Furthermore, modern synthetic protocols can furnish a variety of selectively labeled materials with tritium atoms at precise locations.<sup>6</sup> Tritiated compounds can be purchased from commercial companies at a price in the same range as that for a <sup>13</sup>C- or <sup>15</sup>N-labeled compound. Their safe handling is well-known, and countless biochemistry and biology laboratories routinely use them. In addition, tritium has already proven useful in liquid-state NMR where it has helped elucidate stereochemical and mechanistic aspects of small-molecule chemistry over the past few decades.<sup>7</sup>

In this Communication, we show that solid-state <sup>3</sup>H NMR is a powerful tool for accurately determining long-range interatomic distances without modification of molecular structure. The largest NMR distance ever measured between two nuclei was obtained using a model compound.

To assess the potential of solid-state tritium-NMR, we designed small molecules rigid enough to obtain nonfluctuating <sup>3</sup>H—<sup>3</sup>H distances. Compounds 1–4 (Figure 1) incorporating two tritium atoms separated by 4.31, 6.0, 9.4, and 13.8 Å, respectively, were synthesized by catalytic hydrogenolysis of their brominated precursors using tritium gas and Pd/C.<sup>8</sup> For each NMR analysis, a powder

mixture of 50 mCi (~ 0.15 to 0.3 mg) of the tritiated compound, 40 mg of its unlabeled form, and 0.2 wt % of paramagnetic salt Cu(NO<sub>3</sub>)<sub>2</sub> was prepared (the latter being added to shorten relaxation times).

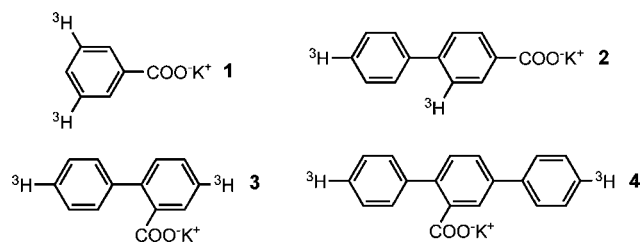


Figure 1. Model compounds for distance measurement.

The <sup>3</sup>H spectra of compound 1 obtained in 8 scans are displayed in Figure 2. They prove that a high signal-to-noise ratio can be obtained with small quantities of tritiated material. The use of <sup>1</sup>H—<sup>3</sup>H cross-polarization significantly increases the sensitivity, mostly as a consequence of the shorter proton relaxation time. For compounds 1–4 the proton T<sub>1</sub> was determined to be approximately 10 times shorter than that of tritium (e.g., T<sub>1</sub> > 360 s for <sup>3</sup>H and T<sub>1</sub> = 26 s for <sup>1</sup>H in compound 1).

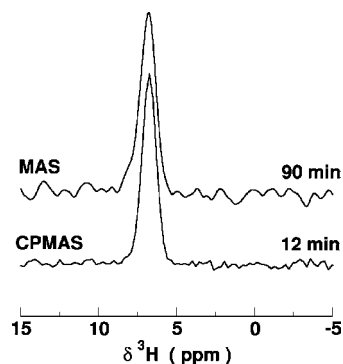


Figure 2. Comparison between <sup>3</sup>H spectra of compound 1 obtained by direct acquisition (top) and <sup>1</sup>H—<sup>3</sup>H cross-polarization (bottom) in eight scans. Total experiment times are indicated on the right; MAS = magic angle spinning; CP = cross-polarization.

For interatomic distance measurements, numerous NMR recoupling techniques have been reported in the literature and their improvement is still the subject of intensive research. Among the several common pulse sequences we investigated, the best results

<sup>†</sup> CEA, iBiTecS, Service de Chimie Bioorganique et de Marquage.

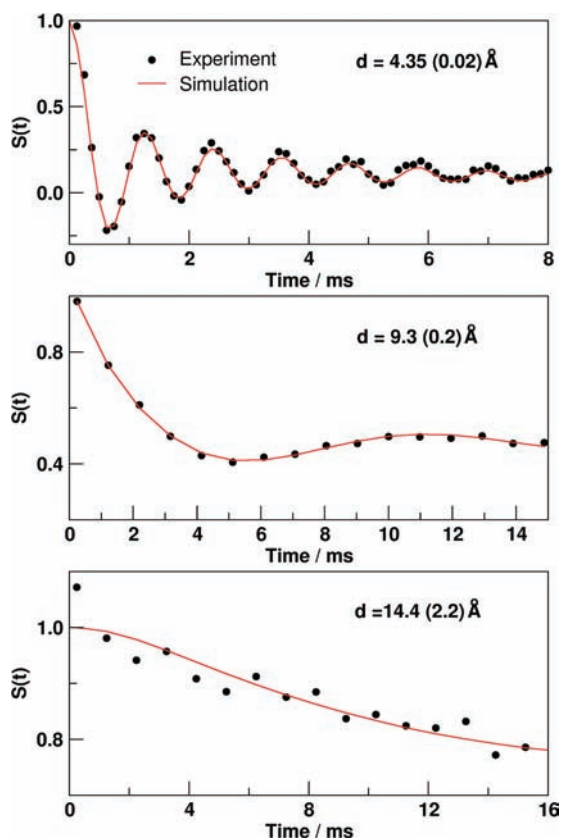
<sup>§</sup> CEA, IRAMIS.

<sup>‡</sup> CEA, iBiTecS, Service de Bioénergétique, Biologie Structurale et Mécanismes.

<sup>||</sup> LBPA, CNRS.

were obtained with HORROR.<sup>9</sup> This sequence offered the advantage of requiring only a moderate <sup>3</sup>H rf-field amplitude (matched at half the spinning frequency) so that a high <sup>1</sup>H-decoupling rf-field (here 80 kHz) could be applied, keeping the total power reasonable on two channels very close in frequency.

As shown in Figure 3, remarkably long dipolar oscillations are observed for compound **1**, allowing precise <sup>3</sup>H–<sup>3</sup>H distance measurement. From these oscillations, the interatomic distance was extracted, accounting for the damped signal arising from the simultaneous response of the <sup>3</sup>H–<sup>3</sup>H spin pair and that of isolated <sup>3</sup>H spins. For **1**, the discrepancy between the measured and the expected values is less than 1% (Table 1). Even for longer distances such as those found in compounds **2** and **3**, the accuracy remained excellent, with a discrepancy in the range of 2%. To the best of our knowledge, this constitutes unprecedented accuracy for such long-range interatomic distances.



**Figure 3.** Variation of the <sup>3</sup>H-signal intensity  $S(t)$  (in arbitrary units) for compounds **1**, **3**, and **4** (from top to bottom) with the <sup>3</sup>H rf-irradiation time under HORROR conditions (here 4 kHz). The experimental data were fitted using an advanced theoretical framework to perform the spin pair dynamics simulations.<sup>10</sup> Experimental details in Supporting Information.

The case of compound **4** revealed that, even in the absence of an observable dipolar oscillation, it was possible to extract a 14.4 Å distance with a precision of approximately  $\pm 15\%$ . Such results were obtained due to high tritium dilution (less than 1 tritium for every 1000 hydrogen atoms), avoiding problems associated with the dipolar truncation effect.<sup>11</sup> Furthermore, the use of <sup>1</sup>H–<sup>3</sup>H

decoupling, which is more efficient than <sup>1</sup>H–<sup>1</sup>H decoupling, contributes to the precision of the method.<sup>12</sup> Finally, this method relies upon direct measurement of the dipolar oscillation, which is more precise than using diffusion measurements.

**Table 1.** Predicted vs Measured <sup>3</sup>H–<sup>3</sup>H Distances in Å for Compounds **1–4**

| compound                        | 1               | 2             | 3             | 4              |
|---------------------------------|-----------------|---------------|---------------|----------------|
| predicted distance <sup>a</sup> | 4.31            | 6.0           | 9.4           | 13.8           |
| measured distance               | 4.35 $\pm$ 0.02 | 5.9 $\pm$ 0.1 | 9.3 $\pm$ 0.2 | 14.4 $\pm$ 2.2 |

<sup>a</sup> Distances predicted for molecules **1–4** using DFT relaxed molecular structures with the PWSCF package.<sup>13</sup>

In summary, we have described a simple, sensitive, and accurate approach for the determination of short- to long-range interatomic distances using standard probe electronics and sample holders and have successfully applied it to model compounds. The measured distance of 14.4  $\pm$  2.2 Å reported here is the highest ever obtained using NMR. To generalize this approach to samples with a larger <sup>3</sup>H chemical shift distribution or shorter  $T_{1\rho}$  values, modifications of the experimental conditions such as use of higher spinning frequency, sample volume reduction, or design of new pulse sequences would be beneficial.

This novel method will allow the study of drugs at their binding sites in insoluble or membrane proteins. Application of this approach to the study of biological questions such as the bioactive conformation of microtubule-bound paclitaxel is underway in our laboratories.

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**Supporting Information Available:** Experimental details, spectroscopic data, and safety procedures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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