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Effects of Biradical Deuteration on the Performance of DNP: Towards Better Performing Polarizing Agents

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Abstract

We study the effects of the deuteration of biradical polarizing agents on the efficiency of dynamic nuclear polarization (DNP) via the cross-effect. To this end, we synthesized a series of bTbK and TOTAPol biradicals with systematically increased deuterium substitution. The deuteration increases the radical's relaxation time, thus contributing to a higher saturation factor and larger DNP enhancement, and reduces the pool of protons within the so-called spin diffusion barrier. Notably, we report that full or partial deuteration leads to improved DNP enhancement factors in standard samples, but also slows down the build-up of hyperpolarization. Improvements in DNP enhancements factors of up to 70% and time savings of up to 38% are obtained upon full deuteration. It is foreseen that this approach may be applied to other DNP polarizing agents thus enabling further sensitivity improvements.

Dynamic nuclear polarization (DNP) is the most widely applicable hyperpolarization technique to enhance the sensitivity of nuclear magnetic resonance (NMR) experiments. In most DNP solid-state (SS)NMR applications, a sample is placed in contact with a source for unpaired electrons (usually an exogenous radical polarization agent) and is irradiated, at low temperature (< 110 K), with high power microwaves near the electron Larmor frequency.[1] Electron polarization can then be transferred to the nuclei by a series of mechanisms, the cross-effect generally being the most efficient. The cross-effect is a process involving three coupled spins: two electrons and one nucleus.[24]

Large strides have been made in recent years towards the improvement of DNP for solids. Specifically, the development of stable high-power microwave sources (gyrotrons), and advances in probe technology for low temperature magic angle spinning (MAS) have enabled the application of DNP SSNMR to high magnetic field strengths (> 9.4 T).[512] Another important line of inquiry, of relevance here, concerns the development of polarization agents capable of generating larger NMR enhancement factors (ε) and operating at higher temperatures. A notable breakthrough was reached by Hu et al. who demonstrated that the cross-effect condition was easier to satisfy by tethering two nitroxide radicals together in a single biradical molecule.[13,14] Matsuki et al. then showed that further improvement could be obtained by using a rigid linker, thus fixing the relative orientation of the two electrons' g tensors.[15,16] Most recently, Zagdoun et al. demonstrated that even larger enhancements may be obtained by using a biradical with a high molecular weight.[1719] It was hypothesized that the lessened molecular motions in these compounds slow the electrons' relaxation, thus enabling a higher electron saturation factor, and a larger DNP enhancement.[17,20] These breakthroughs may be combined, thus multiplying the improvements available from each approach, as is the case for the TEKPol biradical.[19] Note that such efforts may not be beneficial for DNP experiments performed at liquid helium temperatures, where the electron relaxation times can become much longer.[21]

Instead of hindering the molecular motions that are responsible for the electrons' relaxation, the size of the interactions contributing to relaxation may also be lessened. Given that dipolar coupling to protons, particularly those in rapidly rotating methyl groups, is a leading cause of electron relaxation under the DNP conditions, these interactions may be reduced by a factor of 6.5 by simply perdeuterating the polarizing agent.[22,23] Perdeuteration also eliminates the ^{1}H spins that are nearest to the radical. These nuclei possess short relaxation times and thus become a polarization sink. Since spin diffusion from these nuclei to the bulk is also slowed by the presence of pseudocontact shifts, they are said to be within the so-called spin diffusion barrier.[24-26] As a result of deuteration, less electron polarization will be spent repolarizing these rapidly relaxing ^{1}H spins. It is important to note that the use of deuterated solvent, or sample, is also known to lead to an increase in DNP enhancement factor.[27-29]

We have prepared four versions of the benchmark organic-soluble polarizing agent bTbK[15] using reagents at natural abundance (bTbK-d0), a deuterated pentaerythritol linker (bTbK-d8), deuterated nitroxides (bTbK-d32), as well as perdeuterated reagents (bTbK-d40), see Fig. 1.

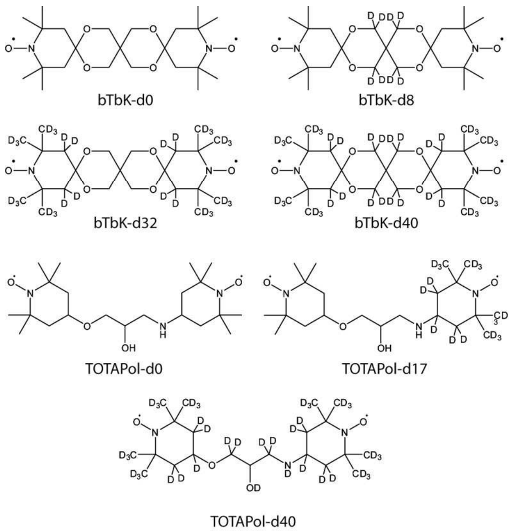

Fig. 1 Biradical polarizing agents used in this study.

All isotopologues of bTbK were dissolved in a 96:4 tetrachloroethane (TCE):methanol-d4 (CD_3OD) solvent mixture to form a 16 mM solution, and 20 μL of this solution was pipetted into 3.2-mm sapphire rotors. The solutions were degassed by repeatedly inserting and ejecting the sample, as previously described,[19,30] until the DNP enhancement reached a plateau. As shown in Fig. 2 and Table 1, the maximum achievable DNP enhancements at 109 K, using a constant microwave power near 30 W, progressively increase with the level of deuteration, from 53 in the protonated bTbK to 91 in the fully perdeuterated version. This corresponds to a 70% increase in DNP enhancement by simply deuterating the polarizing agent. Surprisingly, the deuteration of the linker, containing only 8 hydrogen atoms, was particularly important, leading to a comparable improvement in enhancement per atom than the deuteration of the TEMPO moiety (1.4-2.1% vs. 1.5-1.7% higher enhancement per hydrogen atom). As shown in Table 1, this increase in DNP enhancement is also accompanied by an increase in the DNP buildup time (T_{DNP}) from 3.5 s in the nonlabeled compound to 7.8 s in the perdeuterated compound. Because the signal to noise increases linearly as a function of the square of the number of scans, the relative sensitivity per unit of time (ε^2/T_{DNP}) is still 30% higher when using the perdeuterated radical. ^{13}C and ^{29}Si DNP-enhanced NMR experiments performed on 3-(3-phenylureido)propyl-functionalized mesoporous silica nanoparticles (PUP-MSNs) in fact demonstrate that larger enhancement factors are also be obtained on solid samples of interest (see Supporting Information), although in PUP-MSN the relative sensitivity per unit of time remains approximately the same. As can be seen in Figs. 2b and 3b, the extent of the signal loss, thought to be related to both blanking and MAS-induced depolarization, does not increase by a measurable amount for the deuterated biradicals.[31]

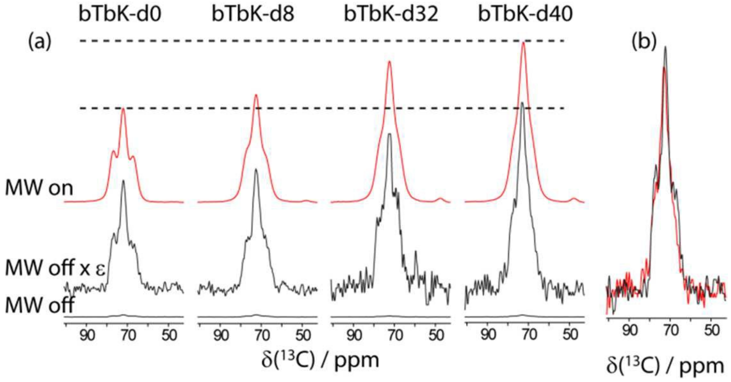

Fig. 2 (a) DNP enhancement of 16 mM 96:4 TCE:CD_3OD solutions of bTbK-dn radicals. The 'MW off' spectrum is shown on the bottom along with this same spectrum scaled by the enhancement factor listed in Table 1. The 'MW on' spectrum is shown on the top of the Figure and dashed lines highlight the relative intensities of the spectra acquired using bTbK-d0 and bTbK-d40. A comparison of 'MW off' spectra of equal-volume solutions of the non- (black) and per-deuterated (red) biradicals is shown in (b) highlighting the minute difference of depolarization for both radicals.

To confirm that this is indeed a generally applicable strategy for improving the effectiveness of other DNP polarization agents we have also synthesized and tested deuterated versions of the hydrophilic TOTAPol biradical.[1b] Natural abundance (TOTAPol-d0), perdeuterated (TOTAPol-d40), as well as a biradical with deuterium labeling on only the amine-terminated nitroxide (TOTAPol-d17) were prepared, see Fig. 1. These radicals were then dissolved in 60:30:10 glycerol-d8:D_2O:H_2O (the so-called 'DNP juice') at a concentration of 10 mM as well as in a deuterium-free 60:40 mixture of glycerol and H_2O.[32] The DNP enhancement measurements on these solutions were performed following the protocol described for the bTbK radicals.

As shown in Fig. 3a and Table 1, the DNP enhancement increases upon deuteration of TOTAPol from 67 to 85 for the d0 and d17 derivatives, respectively, when using DNP juice. The fully deuterated biradicals, TOTAPol-d40, yields the same enhancement as partly-deuterated TOTAPol-d17, within uncertainty in the estimates (±5%). Since the DNP build-up time increases from 8.5 to 11.0 and 23.5 s upon deuteration, the sensitivity per time is affected accordingly; the reason for this will be expanded later in the text. As was expected, however, the enhancement increases steadily from 39 to 51 and 59 for the d0, d17, and d40 derivatives, respectively, when using the fully protonated solvent. The enhancement factor for TOTAPol then increases by 50% upon perdeuteration and the sensitivity per unit of time increases by 38%.

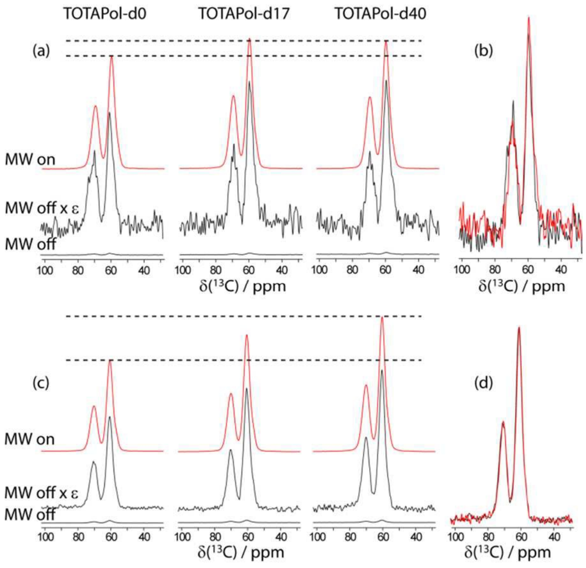

Fig. 3 DNP enhancement of 10 mM 60:30:10 glycerol-d8:D_2O:H_2O (a) and 60:40 glycerol:H_2O (c) solutions of various TOTAPol radicals. The 'MW off' spectrum is shown on the bottom along with this same spectrum scaled by the enhancement factor listed in Table 1. The 'MW on' spectrum is shown on the top of the Figure and dashed lines highlight the relative intensities of the spectra acquired using TOTAPol-d0 and TOTAPol-d40. A comparison of 'MW off' spectra of equal-volume solutions of the non- (black) and per-deuterated (red) biradicals is shown in (b,d) highlighting the minute difference of depolarization for both radicals.

Table 1. DNP performance of various deuterated biradicals

Polarizing agent[a]	solvent[b]	$\varepsilon_{C,CP}$[c]	T_{DNP} / s[d]	ε^2/T_{DNP} / s^{-1}	T_{1e} / μs[e]
bTbK	96:4 TCE : CD_3OD	53	3.5	800	35
bTbK-d8		59	3.9	890	62
bTbK-d32		78	5.8	1050	87
bTbK-d40		91	7.8	1060	121
TOTAPol-d0	60:30:10 glycerol-d8 : D_2O : H_2O	67	8.5	530	120
TOTAPol-d17		85	11.0	660	131
TOTAPol-d40		83	23.5	290	153
TOTAPol-d0	60:40 glycerol : H_2O	39	6.1	250	124
TOTAPol-d17		51	8.6	300	128
TOTAPol-d40		59	10.1	344	153

[a] Note that the true deuteration level of the TOTAPol biradicals in solution will differ as there are 2 exchangeable protons. [b] The concentrations used were of 16 mM and 10 mM for the bTbK and TOTAPol radicals, respectively. [c] To reduce unnecessary human errors, the enhancement factors were measured by integrating the ^{13}C CPMAS signals with the microwaves turned on and off. The uncertainties of the enhancement factors are then on the order of ±5%. [d] The proton T_1 values were approximately equal to T_{DNP}. [e] The T_{1e} values were measured at 9 GHz.

Using a model spin system consisting of two electrons and one nucleus, Thurber and Tycko have shown theoretically that long T_{1e} values prevent the loss of magnetization between the

MAS-induced frequency crossing events that lead to cross-effect polarization transfer.[26] The subsequent investigation of a series of functionalized nitroxide biradicals by Zagdoun et al.[19] confirmed that electron relaxation properties were highly correlated with the DNP enhancements. Thus, both theoretical and experimental studies of the cross effect under MAS demonstrate that longer T_{1e} relaxation times of the electrons generally lead to higher enhancement factors;[17,33,34] as was mentioned earlier, this was the main motivation for the synthesis of the perdeuterated biradicals by the present authors. The results of T_{1e} measurements performed using the very same solutions used to measure the DNP enhancements, as explained in the Supporting Information, are presented in Tables 1 and S1. As can be seen from the plots shown in Fig. 4, the T_{1e} and ε values indeed increase linearly as the biradical polarizing agents are progressively deuterated. The deuteration of the solvent, on the other hand, does not lead to an increase of T_{1e}.[35] We also note that, for bTbK, the observed increase in DNP enhancement is in agreement with the numerical calculations of Mance et al. for the increase in T_{1e} that we observed.[33] However, for TOTAPol, the modest increase in T_{1e} does not appear to fully explain the observed 50% increase in DNP enhancement factor.[33] This may be the result of the use of a much lower magnetic field for the EPR measurements (9 vs 263 GHz). Another plausible mechanism contributing to an increase in ε values would be that, as mentioned earlier, there is a diminished loss of polarization within the so-called spin diffusion barrier when perdeuterated radicals are used.[24] The elimination of ^1H spins within the spin diffusion barrier also, unfortunately, has the effect of increasing the ^1H DNP build-up time due to the reduced efficiency of DNP-assisted spin diffusion (see Table 1).[36] Hovav and coworkers demonstrated that the rate at which hyperpolarization is transferred to the bulk nuclei depends on the strength of the dipolar coupling between the core nuclei and the electron and depends very little on the dipolar coupling between the bulk nuclei. The deuteration of the radicals then reduces the dipolar coupling between the core nuclei and the electrons and essentially slows down the spin diffusion of hyperpolarization to the bulk. In extreme cases when deuterated solvents are used this can lead to particularly long T_{DNP} values and a loss of sensitivity.

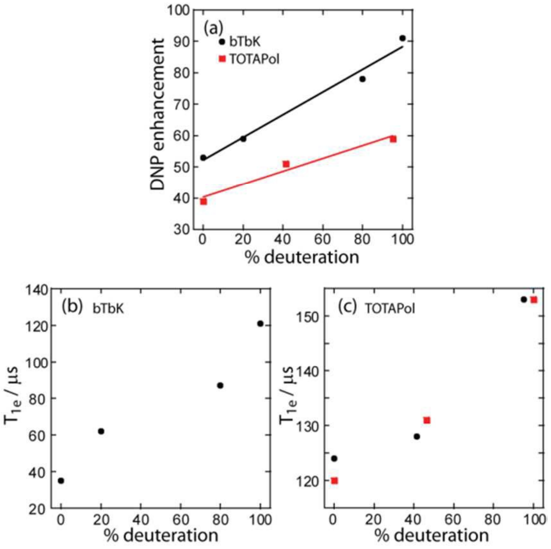

Fig. 4 (a) The DNP enhancement factors of various bTbK and TOTAPol radicals are plotted as a function of the deuteration level of the molecule. The linear fits correspond to $\varepsilon = 52.1 + 0.36{*}D$ for bTbK and $\varepsilon = 40.2 + 0.21{*}D$, where D corresponds to the % deuteration of the molecule. The correlation between the deuteration levels of a biradical and the T_{1e} values are plotted in (b) and (c) for bTbK and TOTAPol, respectively. In (c) the black circles correspond to the use of fully protonated solvent whereas the red squares correspond to the data acquired when using a 90% deuterated solvent.

In conclusion, we have explored a simple approach for improving the DNP enhancement afforded by biradical polarizing agents. Indeed, using the bTbK and TOTAPol biradicals, an increase in DNP enhancement factors of up to 70%, and an improved sensitivity per unit of time of up to 38% can be obtained by simply perdeuterating the polarizing agent. The deuteration of biradicals increases their T_{1e} values, thus improving the saturation factors upon continuous wave microwave irradiation, and consequently contributing to higher enhancement factors. The use of deuterated polarizing agents also reduces the number of ^{1}H spins within the spin diffusion barrier, which has the effects of reducing the polarization loss, but also leads to slower DNP-assisted spin diffusion and longer build-up times.[36] The perdeuteration, or partial deuteration, of the current state-of-the-art polarizing agents (AMUPol[18] and TEKPol[19]), which can already yield enhancement factors over 200 at 105 K, is likely to yield the next generation of biradicals giving enhancement factors well over 300; we are actively exploring this avenue. The results of this study may also help in understanding the mechanisms of the cross effect in DNP of solids, particularly with respect to the polarization of remote spins.

Notes

During the preparation of this manuscript a paper was published by Kubicki and co-workers describing the performance of three methyl-deuterated biradicals, PyPol-diMe, bTurea-diMe and bTbK, where increased enhancement factors and build-up times were also observed.[37]

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References

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[1] T. Maly, G. T. Debelouchina, V. S. Bajaj, K.-N. Hu, C.-G. Joo, M. L. Mak-Jurkauskas, J. R. Sirigiri, P. C. A. van der Wel, J. Herzfeld, R. J. Temkin, and R. G. Griffin, *J. Chem. Phys.* 2008, **128**, 052211.

[2] C. F. Hwang and D. A. Hill, *Phys. Rev. Lett.* 1967. **19**, 1011-1014.

[3] Ü. Akbey, W. T. Franks, A. Linden, M. Orwick-Rydmark, S. Lange, and H. Oschkinat, *Top. Curr. Chem.* 2013, **338**, 181-228.

[4] K. Michaelis, T.-C. Ong, M. K. Kiesewetter, D. K. Frantz, J. J. Walish, E. Ravera, C. Luchinat, T. M. Swager, and R. G. Griffin, *Isr. J. Chem.* 2014, **54**, 207-221.

[5] L. R. Becerra, G. J. Gerfen, R. J. Temkin, D. J. Singel, and R. G. Griffin, *Phys. Rev. Lett.* 1993, **71**, 3561-3564.

[6] M. Rosay, J. C. Lansing, K. C. Haddad, W. W. Bachovchin, J. Herzfeld, R. J. Temkin, and R. G. Griffin, *J. Am. Chem. Soc.* 2003, **125**, 13626-13627.

[7] A. B. Barnes, M. L. Mak-Jurkauskas, Y. Matsuki, V. S. Bajaj, P. C. A. van der Wel, R. DeRocher, J. Bryant, J. R. Sirigiri, R. J. Temkin, J. Lugtenburg, J. Herzfeld, and R. G. Griffin, *J Magn. Reson.* 2009, **198**, 261-270.

[8] M. Rosay, L. Tometich, S. Pawsey, R. Bader, R. Schauwecker, M. Blank, P. M. Borchard, S. R. Cauffman, K. L. Felch, R. T. Weber, R. J. Temkin, R. G. Griffin, and W. E. Maas, *Phy. Chem. Chem. Phys.* 2010, **12**, 5850-5860.

[9] Y. Matsuki, H. Takahashi, K. Ueda, T. Idehara, I. Ogawa, M. Toda, H. Akutsu, and T. Fujiwara, *Phys. Chem. Chem. Phys.* 2010, **12**, 5799.

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[10] A. B. Barnes, E. Markhasin, E. Daviso, V. K. Michaelis, E. A. Nanni, S. K. Jawla, E. L. Mena, R. DeRocher, A. Thakkar, P. P. Woskov, J. Herzfeld, R. J. Temkin, and R. G. Griffin, *J. Magn. Reson.* 2012, **224**, 1.

[11] K. J. Pike, T. F. Kemp, H. Takahashi, R. Day, A. P. Howes, E. V. Kryukov, J. F. Macdonald, A. E. C. Collis, D. R. Bolton, R. J. Wylde, M. Orwick, K. Kosuga, A. J. Clark, T. Idehara, A. Watts, G. M. Smith, M. E. Newton, R. Dupree, and M. E. Smith, *J. Magn. Reson.* 2012, **215**, 1.

[12] T. Idehara, Y. Tatematsu, Y. Yamaguchi, E. M. Khutoryan, A. N. Kuleshov, K. Ueda, Y. Matsuki, and T. Fujiwara, *J. Infra. Millimeter and Terahertz Waves* 2015, **36**, 613.

[13] K.-N. Hu, H.-h. Yu, T. M. Swager, and R. G. Griffin, *J. Am. Chem. Soc.* 2004, **126**, 10844-10845.

[14] C. Song, K.-N. Hu, C.-G. Joo, T. M. Swager, and R. G. Griffin, *J. Am. Chem. Soc.* 2006, **128**, 11385-11390.

[15] Y. Matsuki, T. Maly, O. Ouari, H. Karoui, F. Le Moigne, E. Rizzato, S. Lyubenova, J. Herzfeld, T. Prisner, P. Tordo, and R. G. Griffin, *Angew. Chem. Int. Ed.* 2009, **48**, 4996-5000.

[16] M. K. Kiesewetter, B. Corzilius, A. A. Smith, R. G. Griffin, and T. M. Swager, *J. Am. Chem. Soc.* 2012, **134**, 4537-4540.

[17] A. Zagdoun, G. Casano, O. Ouari, G. Lapadula, A. J. Rossini, M. Lelli, M. Baffert, D. Gajan, L. Veyre, W. E. Maas, M. Rosay, R. T. Weber, C. Thieuleux, C. Coperet, A. Lesage, P. Tordo, and L. Emsley, *J. Am. Chem. Soc.* 2012, **134**, 2284-2291.

[18] C. Sauvée, M. Rosay, G. Casano, F. Aussenac, R. T. Weber, O. Ouari, and P. Tordo, *Angew. Chem. Int. Ed.* 2013, **52**, 10858-10861.

---

[19] A. Zagdoun, G. Casano, O. Ouari, M. Schwarzwälder, A. J. Rossini, F. Aussenac, M. Yulikov, G. Jeschke, C. Copéret, A. Lesage, P. Tordo, and L. Emsley, *J. Am. Chem. Soc.* 2013, **135**, 12790-12797.

[20] V. Kathirvelu, C. Smith, C. Parks, M. A. Mannan, Y. Miura, K. Takeshita, S. S. Eaton, and G. R. Eaton, *Chem. Commun.* 2009, 454-456.

[21] L. Lumata, M. E. Merritt, C. R. Malloy, A. D. Sherry, and Z. Kovacs, *J. Phys. Chem. A* 2012, **116**, 5129-5138.

[22] B. van den Brandt, E. I. Bunyatova, P. Hautle, and J. A. Konter, *Nucl. Instrum. Methods Phys. Res. A* 2004, **526**, 53-55

[23] L. Lumata, A. K. Jindal, M. E. Merritt, C. R. Malloy, A. D. Sherry, and Z. Kovacs, *J. Am. Chem. Soc.* 2011, **133**, 8673-8680.

[24] C. Ramanathan, *Appl. Magn. Reson.* 2008, **34**, 409-421.

[25] A. A. Smith, B. Corzilius, A. B. Barnes, T. Maly, and R. G. Griffin, *J. Chem. Phys.* 2012, **136**, 015101.

[26] K. R. Thurber, R. Tycko, *J. Chem. Phys.* 2012, **137**, 084508.

[27] A. Kagawa, Y. Murokawa, K. Takeda, and M. Kitagawa, *J. Magn. Reson.* 2009, **197**, 9-13.

[28] Ü. Akbey, W. T. Franks, A. Linden, S. Lange, R. G. Griffin, B.-J. van Rossum, and H. Oschkinat, *Angew. Chem. Int. Ed.* 2010, **49**, 7803-7806.

[29] L. Lumata, M. E. Merritt, and Z. Kovacs, *Phys. Chem. Chem. Phys.* 2013, **15**, 7032.

[30] D. J. Kubicki, A. J. Rossini, A. Purea, A. Zagdoun, O. Ouari, P. Tordo, F. Engelke, A. Lesage, and L. Emsley, *J. Am. Chem. Soc.* 2014, **136**, 15711-15718.

[31] F. Mentink-Vigier, S. Paul, D. Lee, A. Feintuch, S. Hediger, S. Vega, and G. De Paëpe, *Phys. Chem. Chem. Phys.* 2015, **17**, 21824-21836.

---

[32] G. J. Gerfen, L. R. Becerra, D. A. Hall, R. G. Griffin, R. J. Temkin, and D. J. Singel, *J. Chem. Phys.* 1995, **102**, 9494-9497.

[33] D. Mance, P. Gast, M. Huber, M. Baldus, and K. L. Ivanov, *J. Chem. Phys.* 2015, **142**, 234201.

[34] F. Mentink-Vigier, Ü. Akbey, H. Oschkinat, S. Vega, and A. Feintuch, *J. Magn. Reson.* 2015, **258**, 102.

[35] A. Volkov, C. Codkter, T. Bund, H. Paulsen, and G. Jeschke, *Biophys. J.* 2009, **96**, 1124.

[36] Y. Hovav, A. Feintuch, and S. Vega, *J. Chem. Phys.* 2011, **134**, 074509.

[37] D. Kubicki, G. Casano, M. Schwarzwälder, S. Abel, C. Sauvée, K. Ganesan, M. Yulikov, A. H. Rossini, G. Jeschke, C. Copéret, A. Lesage, P. Tordo, O. Ouari, and L. Emsley, *Chem. Sci.* 2015, in press, DOI: 10.1039/C5SC02921J.