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# Hyperpolarization of $^{15}\text{N}$ in an amino acid derivative†

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Hyperpolarization is a nuclear magnetic resonance (NMR) technique which can be used to significantly enhance the signal in NMR experiments. In recent years, the possibility to enhance the NMR signal of heteronuclei by the use of *para*-hydrogen induced polarization (PHIP) has gained attention, especially in the area of possible applications in magnetic resonance imaging (MRI). Herein we introduce a way to synthesize a fully deuterated,  $^{15}\text{N}$  labelled amino acid derivative and the possibility to polarize the  $^{15}\text{N}$  by means of hydrogenation with *para*-hydrogen to a polarization level of 0.18%. The longevity of the polarization with a longitudinal relaxation time of more than a minute can allow for the observation of dynamic processes and metabolic imaging *in vivo*. In addition, we observe the phenomenon of proton–deuterium exchange with a homogeneous catalyst leading to signal enhanced allyl moieties in the precursor.

## Introduction

Nuclear magnetic resonance (NMR) is a versatile tool with possible applications ranging from chemical analysis over the observation of dynamic processes in *e.g.* biochemical research to medical diagnostics as magnetic resonance imaging (MRI). However, a major drawback, the inherently low sensitivity, still hampers the full potential of NMR. This is especially apparent in MRI, where mostly water is detected and the direct observation of other molecules is more difficult. A way to tackle this problem is hyperpolarization, which can increase the signal to noise by several orders of magnitude.<sup>1–9</sup> Several techniques have been explored to generate hyperpolarization of which some have already been shown to be a useful tool in the aforementioned fields of research. One technique, dissolution dynamic nuclear polarization (d-DNP) can be used to generate hyperpolarized contrast agents for MRI.<sup>7–12</sup> Another hyperpolarization technique with biomedical applications is spin-exchange optical pumping (SEOP), which can be used to polarize noble gases.<sup>13–16</sup> Another possibility to generate hyperpolarization, that has been gaining a lot of attention in recent years, is *para*-hydrogen induced polarization (PHIP), a technique which allows for the transfer of nuclear spin order from *para*-hydrogen to other nuclei and thus generates a signal enhancement by several orders of magnitude.<sup>17–28</sup> There are two different ways to

utilize *para*-hydrogen in PHIP. One of the possibilities is the signal amplification by reversible exchange (SABRE) which uses dissolved *para*-hydrogen and allows for magnetization transfer *via* an intermediate transition metal complex to a substrate.<sup>28–33</sup> Another way to make use of *para*-hydrogen is to hydrogenate an unsaturated bond in a substrate and subsequently transfer the magnetization to a heteronucleus in the same molecule.<sup>34–37</sup> Especially in the case of small molecules, attaching an unsaturated sidearm to the compound one desires to polarize can be a facile way to access polarized compounds. After reacting the modified compound with *para*-hydrogen, the magnetization is transferred to a heteronucleus in the compound and the sidearm can be cleaved off by, *e.g.* a change in pH to obtain the hyperpolarized compound. For this technique it is essential, that the longitudinal relaxation time ( $T_1$ ) of the precursor and the compound alike, is significantly longer than the time it takes to cleave the sidearm. This method is referred to as *para*-hydrogen induced polarization by sidearm hydrogenation (PHIP-SAH).<sup>38–40</sup> PHIP and PHIP-SAH can polarize metabolites in order to detect metabolic anomalies in medical diagnostics.<sup>25,27,41–46</sup> In all cases it is desired to store the magnetization over long periods of time in order to be able to monitor the compounds for a long time. To achieve this, the magnetization is usually transferred to heteronuclei such as  $^{13}\text{C}$  or  $^{15}\text{N}$  since they usually display long  $T_1$ . The use of  $^{15}\text{N}$  has been shown to be a promising approach, especially in quaternary ammonium compounds, which display long  $T_1$ .<sup>26,47</sup> One class of nitrogen-containing compounds which play a significant role in organisms are amino acids. Not only are they the building blocks of proteins but they also play a role in different metabolic cycles and therefore are an interesting target for hyperpolarization.<sup>48,49</sup> To that extend, several techniques have been applied.<sup>37,50</sup>

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Despite having hyperpolarized amino acids or their derivatives with PHIP by SABRE,<sup>51,52</sup> PHIP<sup>53–55</sup> and PHIP-SAH,<sup>50,56</sup> so far no attempts have been conducted to hyperpolarize the  $^{15}\text{N}$  of the amino function of perdeuterated,  $^{15}\text{N}$ -labelled amino acid derivatives with long  $T_1$ .

## Results and discussion

Herein we introduce an approach to obtain a perdeuterated amino acid derivative with residue attached to the amino function which allows for hydrogenation in order to hyperpolarize the  $^{15}\text{N}$ . We chose a perdeuterated allyl residue for that matter, which has been shown to be well suited for hydrogenation and consecutive magnetization transfer.<sup>47</sup> Furthermore we modified the amino acid in a way to generate a quaternary ammonium function to obtain long  $T_1$  for long time monitoring in a potential setting for metabolic studies. This was achieved by adding two deuterated methyl residues to the amine function, which hinders possible relaxation through proton exchange with the solvent and heightens the symmetry around the  $^{15}\text{N}$ . We could show that the attached perdeuterated allyl sidearm can be hydrogenated with *para*-hydrogen and the thus obtained magnetization transferred to the  $^{15}\text{N}$  using the ESO-THERIC pulse sequence (Fig. 1).<sup>44,45</sup>

At first we devised a synthetic approach to obtain the precursor molecule *N*-(2-(methoxy- $d_3$ )-2-oxoethyl-1,1- $d_2$ )-*N,N*-bis(methyl- $d_3$ )prop-2-ene-1-aminium- $d_5$ - $^{15}\text{N}$  bromide (**1**) in a multistep synthesis starting from glycine- $^{15}\text{N}$ . In a first reaction step we were able to exchange the glycine protons in a base catalyzed reaction over ruthenium on activated charcoal (Fig. 2a), which was reacted with formaldehyde- $d_2$  in an Eschweiler–Clarke analogueous reaction to form *N,N*-dimethylglycine- $^{15}\text{N}$ - $d_9$  (Fig. 2b). In the next steps, *N,N*-dimethylglycine- $^{15}\text{N}$ - $d_9$  was reacted with methanol- $d_4$  and thionyl chloride followed by transformation of the thus obtained chloride in basic conditions in  $\text{D}_2\text{O}$  to form the perdeuterated methyl ester (Fig. 2d). In a final step, the *N,N*-dimethylglycine methyl ester- $^{15}\text{N}$ - $d_{11}$  was reacted with allyl bromide- $d_5$  to form (**1**).

Polarization experiments of (**1**) have been conducted in methanol- $d_4$  with a homogeneous rhodium catalyst  $[\text{Rh}(\text{dppb})(\text{COD})][\text{BF}_4]$  (dppb: diphenylphosphino butane, COD: cyclooctadiene) at 320 K and a 7 T magnetic field. At first, proton-NMR-experiments were run in order to estimate the coupling constants needed for efficient polarization transfer. Hydrogen gas was enriched in the *para* spin isomer at 99% by

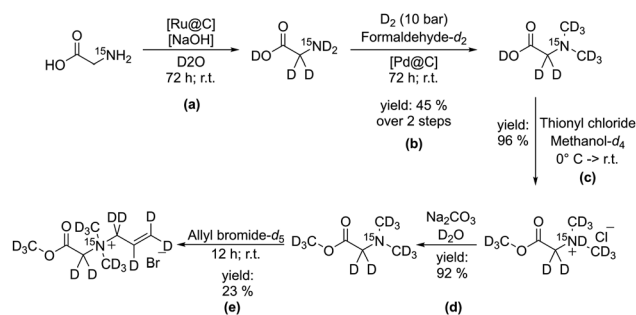


Fig. 2 Synthesis of (**1**) in a 5-step approach. Starting from glycine- $^{15}\text{N}$  a multistep synthesis to generate (**1**) has been developed. The steps include: (a) deuteration of glycine, (b) *N*-methylation, (c) esterification, (d) basic work up and (e) obtaining the quaternized amino acid derivative.

using a custom ordered Sumitomo generator operating at 20 K. *Para*-hydrogen was bubbled for 10 s at 7 bar directly into the NMR tube containing 2 mM of the catalyst and 1 mM of substrate *via* an automated console controlled delivery system.<sup>44</sup>

It was apparent, that several different hyperpolarized species were present in the sample after polarization (see Fig. 3). The observation that hyperpolarized anti-phase signal can be observed for the allyl protons, leads us to attribute this to a previously described hydrogenation–dehydrogenation mechanism.<sup>57</sup> It should be noted that the mechanism described was observed on surfaces whereby here, a homogeneous catalyst is used. In this proposed model, the unsaturated bond can be hydrogenated and thus hyperpolarized. In another step, the hydrogenated compound can interact with the used catalyst again and be dehydrogenated. The then again unsaturated compound can be hydrogenated again, and so forth. By this mechanism, a proton–deuterium exchange can take place at the allyl function and species with different deuteration degrees can be present in the sample.

Mass spectrometry measurements have been performed on the sample solution before and after hydrogenation gain additional information on the nature of the observed species. Although masses could be detected for (**2**) as well as the labelled *N,N*-dimethyl glycine methyl ester for the sample after hydrogenation, the same was true for the sample before hydrogenation, making it rather difficult to definitely tell whether parts of the sample already degrade during the hydrogenation or this effect is solely attributable to the ionization process during the mass spectroscopy measurements. What was apparent though, was that the mass detected for the quaternary ammonium compound were the same before and after hydrogenation, which gives a further indication that the previously discussed hydrogenation–dehydrogenation mechanism takes place, since the molecular mass of (**1**) is the same than the molecular mass of (**2**) in which two additional deuterons have been exchanged for protons. The respective mass spectra are shown in the ESI.†

Despite the manifold of signals, we were able to identify the signals of hydrogenated (**1**) and determine the coupling constants for efficient magnetization transfer from the deuterium decoupled hyperpolarized proton spectrum. The coupling constants have been determined to be  $^3J_{\text{H,H}} = 7.4$  Hz with

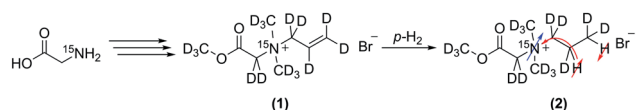


Fig. 1 The amino acid derivative *N*-(2-(methoxy- $d_3$ )-2-oxoethyl-1,1- $d_2$ )-*N,N*-bis(methyl- $d_3$ )prop-2-ene-1-aminium- $d_5$ - $^{15}\text{N}$  bromide (**1**) can be hydrogenated with *para*-hydrogen to form *N*-(2-(methoxy- $d_3$ )-2-oxoethyl-1,1- $d_2$ )-*N,N*-bis(methyl- $d_3$ )propane-1-aminium-1,1,2,3,3,3- $d_5$ - $^{15}\text{N}$  bromide (**2**). The thus obtained proton spin order is consecutively transferred to the  $^{15}\text{N}$  to generate  $^{15}\text{N}$  hyperpolarization.



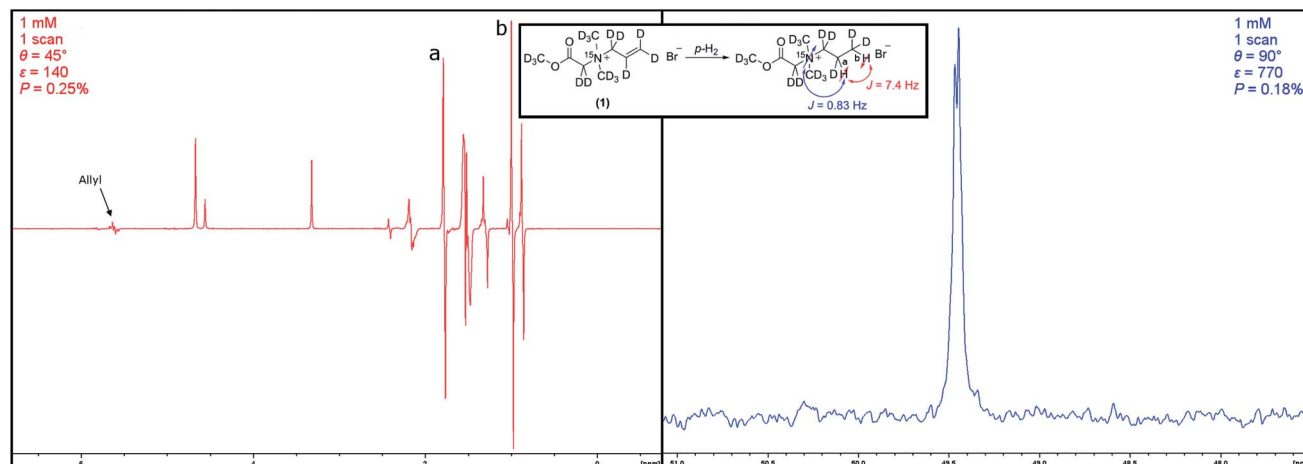


Fig. 3 Left: hyperpolarized  $^1\text{H}$ -NMR-spectrum of (2). Several peaks can be observed, including a hyperpolarized peak for the unsaturated allyl function. This indicates a hydrogenation–dehydrogenation cascade leading to various hyperpolarized species. The polarization  $P$  has been determined for the proton pair a and b in the hydrogenated substrate (2). Right: hyperpolarized  $^{15}\text{N}$ -NMR-spectrum of (2). Coupling constants used for the magnetization transfer are indicated in the insert and have been determined from the hyperpolarized proton spectrum.

Table 1 Summary of the obtained polarization values as well as the  $T_1$  of  $^{15}\text{N}$  in (2) at 320 K and a 7 T magnetic field

$\varepsilon^1\text{H}$	$P_{\text{H}} [\%]$	$\varepsilon^{15}\text{N}$	$P_{15\text{N}} [\%]$	$T_1(^{15}\text{N}) [\text{s}]$
140	0.28	770	0.18	$77 \pm 30$

a chemical shift difference of 239 Hz (0.8 ppm) and  $^3J_{\text{H,N}} = 0.84$  Hz. Hydrogenation with *para*-hydrogen then yielded a proton polarization of 0.28%, a signal enhancement of 140-fold at 7 T. Transfer of the magnetization to the  $^{15}\text{N}$  was successful with an efficiency of 72%, generating a polarization of 0.18%, corresponding to a signal enhancement of 770-fold at a 7 T field. The relatively low polarization can be attributed to a constant change in the hyperpolarized compounds, leading to a distribution of the polarization. To the same extent the polarization available for transfer to  $^{15}\text{N}$  is limited by the same effect. From hyperpolarized (2), the  $T_1$  of  $^{15}\text{N}$  has been determined by running several scans at low flip angles and was shown to be  $T_1 = 77 \pm 30$  s. An overview over the obtained data is shown in Table 1.

## Conclusion

In conclusion we were able to demonstrate the synthesis of a perdeuterated glycine derivative with a quaternary amino function by starting from glycine- $^{15}\text{N}$ . We could show that hydrogenation with *para*-hydrogen of the glycine derivative leads to hyperpolarized proton signals with a proton polarization of the hydrogenated compound of 0.28%. Interestingly, we also observed a proton–deuterium exchange with the homogeneous catalyst leading to enhanced allyl signals. The hyperpolarized protons on the alkyl moiety could be transferred efficiently to the  $^{15}\text{N}$  by using the ESOTHERIC pulse sequence. The relatively long  $T_1$  of the hydrogenated compound of  $T_1 = 77$  s gives rise to the possibility of using this and other

analogues of amino acids as potential signal enhanced contrast agents. With this publication, we are able to define a starting point for further investigation of the hyperpolarization of  $^{15}\text{N}$  in amino acids for the aforementioned application and even their use in a possible MRI setup.

## Conflicts of interest

There are no conflicts to declare.

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## Notes and references

- J. H. Ardenkjær-Larsen, B. Fridlund, A. Gram, G. Hansson, L. Hansson, M. H. Lerche, R. Servin, M. Thaning and K. Golman, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 10158–10163.
- K. Golman, R. in't Zandt and M. Thaning, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 11270–11275.
- S. E. Day, M. E. Kettunen, F. A. Gallagher, D.-E. Hu, M. Lerche, J. Wolber, K. Golman, J. H. Ardenkjær-Larsen and K. M. Brindle, *Nat. Med.*, 2007, **13**, 1382–1387.
- S. Jannin, A. Bornet, R. Melzi and G. Bodenhausen, *Chem. Phys. Lett.*, 2012, **549**, 99–102.
- X. Ji, A. Bornet, B. V. J. Milani, D. Gajan, A. J. Rossini, L. Emsley, G. Bodenhausen and S. Jannin, *Nat. Commun.*, 2017, **8**, 13975.



- 6 J. Milani, B. Vuichoud, A. Bornet, R. Melzi, S. Jannin and G. Bodenhausen, *Rev. Sci. Instrum.*, 2017, **88**, 015109.
- 7 F. A. Gallagher, M. I. Kettunen, S. E. Day, D.-E. Hu, J. H. Ardenkjær-Larsen, R. in't Zandt, P. E. Jensen, M. Karlsson, K. Golman, M. E. Lerche and K. M. Brindle, *Nature*, 2008, **453**, 940–943.
- 8 J. Kurhanewicz, D. B. Vigneron, K. M. Brindle, E. Y. Chekmenev, A. Comment, C. H. Cunningham, R. J. DeBernardinis, G. G. Green, M. O. Leach, S. S. Rajan, R. R. Rizi, B. D. Ross, W. S. Warren and C. R. Malloy, *Neoplasia*, 2011, **13**, 81–97.
- 9 S. J. Nelson, J. Kurhanewicz, D. B. Vigneron, P. E. Z. Larson, A. L. Harzstark, M. Ferrone, M. van Criekinge, J. W. Chang, R. Bok, I. Park, G. Reed, L. Carvajal, E. J. Small, P. Munster, V. K. Weinberg, J. H. Ardenkjær-Larsen, A. P. Chen, R. E. Hurd, L.-I. Odegaardstuen, F. J. Robb, J. Tropp and J. A. Murray, *Sci. Transl. Med.*, 2013, **5**, 198ra108.
- 10 K. Golman, R. in't Zandt, M. Lerche, R. Pehrson and J. H. Ardenkjær-Larsen, *Cancer Res.*, 2006, **66**, 10855–10860.
- 11 C. Gabellieri, S. Reynolds, A. Lavie, G. S. Payne, M. O. Leach and T. R. Eykyn, *J. Am. Chem. Soc.*, 2008, **130**, 4598–4599.
- 12 J. J. Miller, J. T. Grist, S. Serres, J. R. Larkin, A. Z. Lau, K. Ray, K. R. Fisher, E. Hansen, R. S. Tougaard, P. M. Nielsen, J. Lindhardt, C. Laustsen, F. A. Gallagher, D. J. Tyler and N. Sibson, *Sci. Rep.*, 2018, **8**, 15082.
- 13 T. G. Walker and W. Happer, *Rev. Mod. Phys.*, 1997, **69**, 629–642.
- 14 S. Appelt, A. B.-A. Baranga, C. J. Erickson, M. V. Romalis, A. R. Young and W. Happer, *Phys. Rev. A: At., Mol., Opt. Phys.*, 1998, **58**, 1412–1439.
- 15 H. E. Möller, X. J. Chen, B. Saam, K. D. Hagspiel, G. A. Johnson, T. A. Altes, E. E. de Lange and H.-U. Kauczor, *Magn. Reson. Med.*, 2002, **47**, 1029–1051.
- 16 L. Schröder, T. J. Lowery, C. Hilty, D. E. Wemmer and A. Pines, *Science*, 2006, **314**, 446–449.
- 17 C. R. Bowers and D. P. Weitekamp, *Phys. Rev. Lett.*, 1986, **57**, 2645–2648.
- 18 C. R. Bowers and D. P. Weitekamp, *J. Am. Chem. Soc.*, 1987, **109**, 5541–5542.
- 19 T. C. Eischenschmid, R. U. Kirss, P. P. Deutsch, S. I. Hommeltoft, R. Eisenberg, J. Bargon, R. G. Lawler and A. L. Balch, *J. Am. Chem. Soc.*, 1987, **109**, 8089–8091.
- 20 K. Golman, O. Axelsson, H. Jóhannesson, S. Måringnsson, C. Olofsson and J. Petersson, *Magn. Reson. Med.*, 2001, **46**, 1–5.
- 21 E. Y. Chekmenev, J. Hövener, V. A. Norton, K. Harris, L. S. Batchelder, P. Bhattacharya, B. D. Ross and D. P. Weitekamp, *J. Am. Chem. Soc.*, 2008, **130**, 4212–4213.
- 22 S. Glöggler, A. M. Grunfeld, Y. N. Ertas, J. McCormick, S. Wagner, P. P. M. Schleker and L.-S. Bouchard, *Angew. Chem., Int. Ed.*, 2015, **54**, 2452–2456.
- 23 O. G. Salnikov, K. V. Kovtunov and I. V. Koptug, *Sci. Rep.*, 2015, **5**, 13930.
- 24 F. Reineri, T. Boi and S. Aime, *Nat. Commun.*, 2015, **6**, 5858.
- 25 A. B. Schmidt, S. Berner, W. Schimpf, C. Müller, T. Lickert, N. Schwaderlapp, S. Knecht, J. G. Skinner, A. Dost, P. Rovedo, J. Hennig, D. von Elverfeldt and J.-B. Hövener, *Nat. Commun.*, 2017, **8**, 14535.
- 26 J. McCormick, S. Korchak, S. Mamone, Y. N. Ertas, Z. Liu, L. Verlinsky, S. Wagner, S. Glöggler and L.-S. Bouchard, *Angew. Chem., Int. Ed.*, 2018, **57**, 10692–10696.
- 27 E. Cavallari, C. Carrera, M. Sorge, G. Bonne, A. Muchir, S. Aime and F. Reineri, *Sci. Rep.*, 2018, **8**, 8366.
- 28 J.-B. Hövener, A. N. Pravdivtsev, B. Kidd, C. R. Bowers, S. Glöggler, K. V. Kovtunov, M. Plaumann, R. Katz-Brull, K. Buckenmaier, A. Jerschow, F. Reineri, T. Theis, R. V. Shchepin, S. Wagner, P. Bhattacharya, N. M. Zacharias and E. Y. Chekmenev, *Angew. Chem., Int. Ed.*, 2018, **57**, 11140–11162.
- 29 R. W. Adams, J. A. Aguilar, K. D. Atkinson, M. J. Cowley, P. I. P. Elliott, S. B. Duckett, G. G. R. Green, I. G. Khazal, J. Lopez-Serrano and D. C. Williamson, *Science*, 2009, **323**, 1708–1711.
- 30 T. Theis, M. L. Truong, A. M. Coffey, R. V. Shchepin, K. W. Waddell, F. Shi, B. M. Goodson, W. S. Warren and E. Y. Chekmenev, *J. Am. Chem. Soc.*, 2015, **137**, 1404–1407.
- 31 T. Theis, G. X. Ortiz Jr, A. W. J. Logan, K. E. Claytor, Y. Feng, W. P. Huhn, V. Blum, S. J. Malcolmson, E. Y. Chekmenev, Q. Wang and W. S. Warren, *Sci. Adv.*, 2016, **2**, e1501438.
- 32 P. J. Rayner, M. J. Burns, A. M. Olaru, P. Norcott, M. Fekete, G. G. R. Green, L. A. R. Highton, R. E. Mewis and S. B. Duckett, *Proc. Natl. Acad. Sci. U. S. A.*, 2017, **114**, E3188–E3194.
- 33 M. Suefke, S. Lehmkuhl, A. Liebisch, B. Blümich and S. Appelt, *Nat. Phys.*, 2017, **13**, 568–572.
- 34 M. Haake, J. Natterer and J. Bargon, *J. Am. Chem. Soc.*, 1996, **118**, 8688–8691.
- 35 J. Natterer and J. Bargon, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1997, **31**, 293–315.
- 36 A. S. Kiryutin, G. Sauer, S. Hadjiali, A. V. Yurkovskaya, H. Breitzke and G. Buntkowsky, *J. Magn. Reson.*, 2017, **285**, 26–36.
- 37 A. N. Pravdivtsev, G. Buntkowsky, S. B. Duckett, I. V. Koptug and J.-B. Hövener, *Angew. Chem., Int. Ed.*, 2021, **60**, 23496–23507.
- 38 E. Cavallari, C. Carrera, T. Boi, S. Aime and F. Reineri, *J. Phys. Chem. B*, 2015, **31**, 10035–10041.
- 39 E. Cavallari, C. Carrera, S. Aime and F. Reineri, *J. Magn. Reson.*, 2018, **289**, 12–17.
- 40 S. Korchak, M. Emondts, S. Mamone, B. Blümich and S. Glöggler, *Phys. Chem. Chem. Phys.*, 2019, **21**, 22849–22856.
- 41 M. Goldman, H. Jóhannesson, O. Axelsson and M. Karlsson, *Compt. Rendus Chem.*, 2006, **9**, 357–363.
- 42 P. Bhattacharya, E. Y. Chekmenev, W. H. Perman, K. C. Harris, A. P. Lin, V. A. Norton, C. T. Tan, B. D. Ross and D. P. Weitekamp, *J. Magn. Reson.*, 2007, **186**, 150–155.
- 43 P. Bhattacharya, E. Y. Chekmenev, W. F. Reynolds, S. Wagner, N. Zacharias, H. R. Chan, R. Bünger and B. D. Ross, *NMR Biomed.*, 2011, **24**, 1023–1028.
- 44 S. Korchak, S. Yang, S. Mamone and S. Glöggler, *ChemistryOpen*, 2018, **7**, 344–348.
- 45 S. Korchak, S. Mamone and S. Glöggler, *ChemistryOpen*, 2018, **7**, 672–676.



- 46 S. Berner, A. B. Schmidt, M. Zimmermann, A. N. Pravdivtsev, S. Glöggler, J. Hennig, D. von Elverfeldt and J.-B. Hövener, *ChemistryOpen*, 2019, **8**, 728–736.
- 47 A. P. Jagtap, L. Kaltschnee and S. Glöggler, *Chem. Sci.*, 2019, **10**, 8577–8582.
- 48 M. Daugherty, B. Polanuyer, M. Farrel, M. Scholle, A. Lykidis, V. de Crécy-Lagard and A. Osterman, *J. Biol. Chem.*, 2002, **277**, 21431–21439.
- 49 W. Wang, Z. Wu, Z. Dai, Y. Yang, J. Wang and G. Wu, *Amino Acids*, 2013, **45**, 463–477.
- 50 L. Kaltschnee, A. P. Jagtap, J. McCormick, S. Wagner, L.-S. Bouchard, M. Utz, C. Griesinger and S. Glöggler, *Chem.–Eur. J.*, 2019, **25**, 11031–11035.
- 51 S. Glöggler, R. Müller, J. Colell, M. Emondts, M. Dabrowski, B. Blümich and S. Appelt, *Phys. Chem. Chem. Phys.*, 2011, **13**, 13759–13764.
- 52 L. Sellies, R. L. E. G. Aspers, M. C. Feiters, F. P. J. T. Rutjes and M. Tessari, *Angew. Chem., Int. Ed.*, 2021, **60**, 26954–26959.
- 53 P. C. Soon, X. Xu, B. Zhang, F. Gruppi, J. W. Canary and A. Jerschow, *Chem. Commun.*, 2013, **49**, 5304–5306.
- 54 T. Trantzsche, M. Plaumann, J. Bernarding, D. Lego, T. Ratajczyk, S. Dillenberger, G. Buntkowsky, J. Bargon and U. Bommerich, *Appl. Magn. Reson.*, 2013, **44**, 267–278.
- 55 J. A. Tang, F. Gruppi, R. Fleysher, D. K. Sodickson, J. W. Canary and A. Jerschow, *Chem. Commun.*, 2011, **47**, 958–960.
- 56 S. Glöggler, S. Wagnerb and L.-S. Bouchard, *Chem. Sci.*, 2015, **6**, 4261–4266.
- 57 O. G. Salnikov, L. M. Kovtunova, I. V. Skovpin, V. I. Bukhtiyarov, K. V. Kovtunov and I. V. Koptug, *ChemCatChem*, 2018, **10**, 1178–1183.

