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Hyperpolarization of amino acid precursors to neurotransmitters with parahydrogen induced polarization†

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Several important neurotransmitter precursors were hyperpolarized *via* homogeneous hydrogenation with parahydrogen. Polarization enhancement was achieved for ^{1}H and ^{13}C spins by several orders of magnitude compared to thermal spectra. Such large signal enhancements of these molecules could facilitate neurotransmitter studies.

Neurotransmitters are chemical messengers that transmit signals across synapses in the nervous system. These chemicals play an important role in cognitive processing (in particular dopamine, glutamate and GABA). It is believed that hypo- or hyperproduction of certain neurotransmitters is associated with disorders such as Alzheimer's and Parkinson's diseases,[1] and schizophrenia,[2] respectively. Limited imaging techniques exist to follow the formation of neurotransmitters from their biological precursors to detect neurotransmitter production.[3]

Hyperpolarization techniques such as dynamic nuclear polarization (DNP), optical pumping, and parahydrogen induced polarization (PHIP) have gained great interest in the past decade because they can provide sensitivity boosts by several orders of magnitude. PHIP is an economical and potentially high-throughput technique to yield hyperpolarized substrates, and it can be implemented either through a hydrogenation reaction using parahydrogen (p-H_2) or through reversible exchange (signal amplification by reversible exchange, SABRE).[4,5] Both approaches can provide hyperpolarized species that may serve as promising tools for studies of enzymatic transformations and associated pathology.

Here, we report the hyperpolarization of a collection of neurotransmitter precursors using PHIP. We chose to focus on amino acid biosynthetic precursors of the neurotransmitters dopamine (phenylalanine, tyrosine, and DOPA), serotonin (tryptophan) and histamine (histidine). The requisite *N*-acetyl dehydro-amino acids are easily prepared and their structures are well suited for obtaining both ^{1}H and ^{13}C PHIP signals. The hydrogenated amino acid products are capable of deacylation (*e.g.*, by aminoacylase I[6,7]) and could be readily transformed into neurotransmitters.[8] In addition, such amino acid analogs have been radiolabeled as tracers for tumor imaging using positron emission tomography (PET).[9,10] These agents therefore could also have the potential to become dual-modality probes for simultaneous PET-MRI.[11]

The hyperpolarization of some amino acids, such as phenylalanine and histidine, has been achieved at moderate enhancement factors using the SABRE method and detected at low magnetic fields.[12,13] A preliminary report indicated that γ-aminobutyric acid (GABA) was hyperpolarized by PHIP,[13] which typically provides large enhancements. This protocol is compatible with hydrogenation at high magnetic fields (PASADENA approach[14]). Therefore, we present here synthetic *N*-acetyl dehydro-amino acids, which upon hydrogenation, form amino acid neurotransmitter precursors. We investigated the polarization enhancement of the hyperpolarized precursors and transferred the hyperpolarization to ^{13}C to explore the possibility of exploiting the longer signal lifetimes.

Fig. 1 shows the nine *N*-acetyl dehydro-amino acids prepared by the Erlenmeyer synthesis.[15] Parahydrogen was generated using the procedure described previously.[16] Hydrogenation of *N*-acetyl dehydro-amino acids (Scheme 1) was initiated by bubbling p-H_2

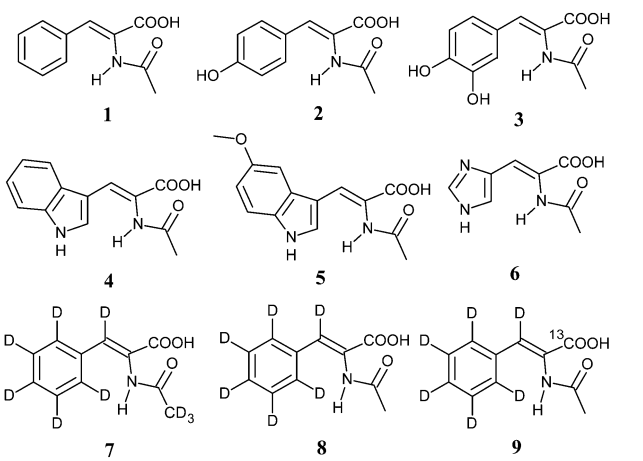

Fig. 1 Synthesized *N*-acetyl dehydro-amino acids.

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† Electronic supplementary information (ESI) available: General methods, synthesis and characterization data of all compounds, and additional PHIP ^{1}H NMR spectra of **2a**–**6a** and **8a**. See DOI: 10.1039/c3cc40426a

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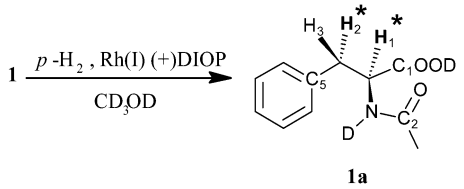

Scheme 1 Hydrogenation of *N*-acetyl dehydro-phenylalanine.

(enriched to 50%) for 15 s into the NMR tube containing the reaction solution, while catalyzed by $[Rh(cod)(+)(diop)]BF_4$ in deuterated methanol, except for **6**. Compound **6** was hydrogenated in deuterated water with sodium dodecylsulfate (SDS)[16] because of limited solubility in methanol.

The reaction clearly started at low field, but continued at high field, as shown using Only Parahydrogen SpectroscopY (OPSY) experiments[17] (Fig. S7, ESI†): the new signal was produced continuously for more than 60 s at high field. Therefore, the PASADENA mechanism can be assumed for the interpretation of the observed spectra. The enhancement factors (EFs), calculated by comparing the hyperpolarized ^{1}H NMR signals to those at thermal equilibrium, are summarized in Table S1 (ESI†). Compounds **1a**–**3a** yielded significantly larger EFs compared to **4a**–**6a**. It was observed that **1**–**3** hydrogenated faster than **4** and **5** by a factor of ten. This rate difference may derive from the faster reaction of electron-poor alkenes.[18] A faster reaction rate may translate into a higher EF, since more hyperpolarized hydrogenation products are produced at a given time.[5] Product **6a** has a smaller EF because hydrogenation is slower in the deuterated water–SDS system[19] compared to a homogeneous system in deuterated methanol.[20]

The ^{1}H spectrum of the product, **1a**, obtained from hydrogenation of **1** with p-H_2 is shown in Fig. 2a. A substantial sensitivity improvement (EF of 755 at 11.7 T) was achieved for H_2* at 3.25 ppm. The hyperpolarized signal of H_1* at 4.85 ppm showed a similar enhancement. Interestingly, another strong hyperpolarized signal was observed at 2.95 ppm, arising from H_3, the geminal hydrogen on the β-carbon atom. Evidently, this signal is a result of polarization transfer from the newly added proton, H_2*. The more distant aromatic protons at around 7.2 ppm were also hyperpolarized, but at weaker levels. These polarization transfers most likely result from transfers *via* the J-coupling network or the nuclear Overhauser effect (NOE) during or after hydrogenation.[21–23]

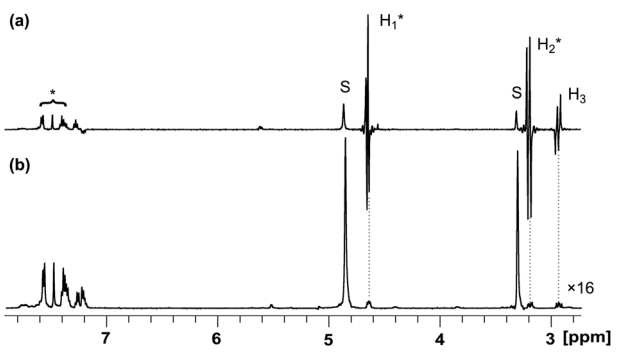

Fig. 2 (a) Single-scan ^{1}H NMR spectrum recorded immediately after hydrogenation of **1**; (b) reference spectrum of a mixture of **1** and **1a** after the hyperpolarization decay. ''*'' indicates starting material peaks, ''S'' indicates solvent peaks.

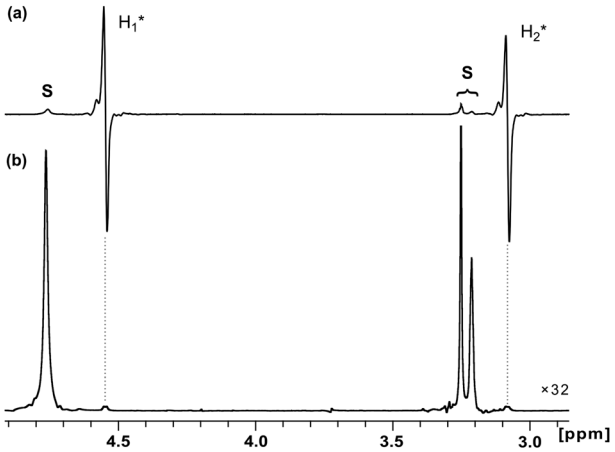

Fig. 3 (a) Single-scan ^{1}H NMR spectrum recorded immediately after hydrogenation of **8**; (b) reference spectrum of a mixture of **8** and **8a** after hyperpolarization decay. ''S'' indicates the solvent peaks.

The ability to generate large enhancement factors and to use the polarization in further experiments depends critically on the lifetime of the polarized states. Deuterating the substrates is a strategy to prolong signal lifetimes.[24,25] In order to minimize the relaxation caused by spin diffusion, and thereby boosting the enhancement of the hyperpolarized signals, deuterated substrates **7**–**9** were prepared. Compound **7** was obtained after hydrolyzing the azlactone that was condensed from d_6-benzaldehyde and d_3-*N*-acetyl glycine in d_6-acetic anhydride. Interestingly, when non-deuterated acetic anhydride was used, the CD_3 group of the *N*-acetyl moiety exchanges with the CH_3 group of acetic anhydride to yield **8**. The hydrogenation of **7**–**9** is stereospecific (Fig. S8, ESI†). The hyperpolarized ^{1}H NMR signals are shown in Fig. 3a. No signal is seen at 2.95 ppm, which is consistent with the fact that this site is deuterated. EFs of 1400 and 2000 (at 11.7 T) were obtained for **7a** and **8a**, respectively. These are much higher than the EF of **1a** because deuteration reduces dipolar coupling neighbors. We also observed longer relaxation times for the protons in the deuterated products: in **8a**, T_1 of H_1* and H_2* are 3.3 s and 3.4 s, respectively, while in **1a**, T_1 of H_1* and H_2* are 2.4 s and 1.1 s respectively.

For hyperpolarized contrast agents, it is desirable to transfer the polarization to carbon nuclei since they typically have much longer relaxation times than protons (for **9a**, T_1 of C_1 is ~12.6 s while T_1 of H_1* and H_2* are ~3 s at 11.7 T). The transfer was initially attempted *via* field cycling.[26] This approach failed, likely as a result of the weak J-couplings between ^{1}H and ^{13}C in question ($^2J_{CH}$ = 6.6 Hz). This value would make it necessary to perform adiabatic transport from zero field through 0.2 μT at a very slow pace, which would necessitate a high-precision field-cycling apparatus. Furthermore, the efficiency would be low because of relaxation during the slow passage, and the transfer would not be compatible with reactions at high field. Therefore, we chose the PH-INEPT+ pulse sequence[27] to effect the heteronuclear hyperpolarization transfer. The transfer to ^{13}C did not work for non-deuterated compounds, likely as a result of faster relaxation and/or dilution of polarization over several spins due to spin diffusion effects. For the deuterated substrates **7**–**9**, by contrast, large EFs were obtained. When **7** and **8** were hydrogenated with p-H_2, the hyperpolarization was transferred to the adjacent carbonyl carbon spin, C_1, as well as the carbonyl carbon spin of the *N*-acetyl moiety, C_2, and those of aromatic carbons in the phenyl

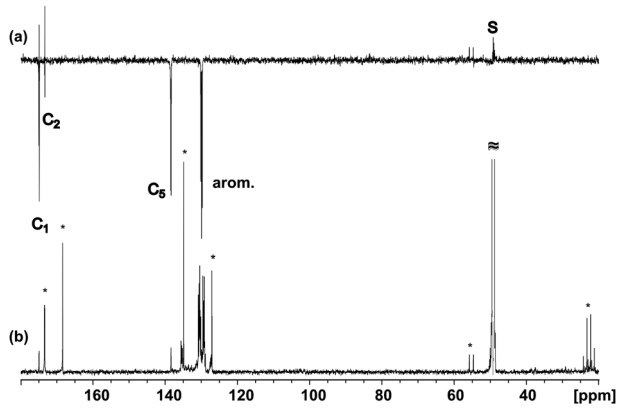

Fig. 4 ^{13}C NMR spectra of **8**: (a) a single-scan with the PH-INEPT+ sequence recorded immediately after hydrogenation. (b) The thermally polarized reference spectrum after hydrogenation (8888 scans). ''*'' indicates starting material peaks, ''S'' indicates the solvent peak.

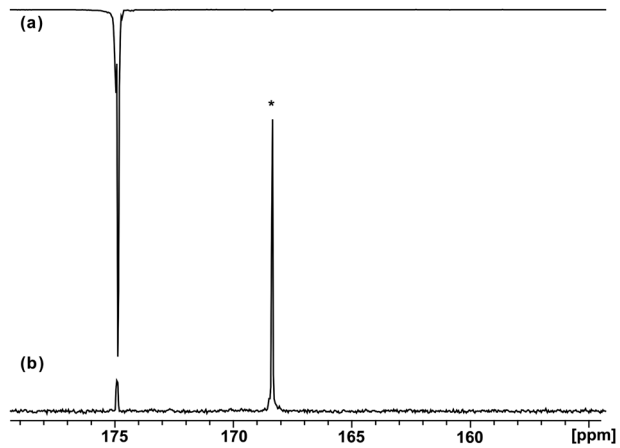

Fig. 5 ^{13}C NMR spectra of **9**: (a) a single-scan PH-INEPT+ sequence recorded immediately after hydrogenation. The enhancement factor is 1237 ± 212 at 11.7 T. (b) The thermally polarized reference spectrum after hydrogenation (16 scans). ''*'' indicates the starting material peak.

ring (Fig. 4). The signal-to-noise ratio of the hyperpolarized spectrum was much larger than that of a thermal spectrum obtained with 8888 scans (Fig. 4b). To maximize the signal for spectroscopic or imaging applications, the carbonyl carbon was enriched with ^{13}C (substrate **9**) and an average EF of 1237 ± 212 was achieved. This corresponds to ^{13}C polarization of 1.3% (at 11.7 T, 298 K). The hyperpolarized signals are shown in Fig. 5.

In summary, we demonstrated the hyperpolarization of several amino acids, known to be biosynthetic precursors to neurotransmitters, *via* PHIP and transferred the polarization to ^{13}C in deuterated and ^{13}C-enriched precursors (EF of 1200). With these large signal enhancement factors, this approach could be used to study *in vitro* enzymatic transformations involved in neurotransmitter biosynthesis. We are looking into adapting the production of hyperpolarized molecules in biocompatible media. Although signal lifetimes continue to be a limitation for many potential applications, it has been reported, for example, that L-DOPA is taken up in the brain and converted to dopamine within 15 s of injection.[28] The amount of dopamine that can be infused into the mouse brain is about 200 nmol,[29] and the single-scan detection limit of ^{13}C is about 20 μmol for a coil of 25 mm diameter and 30 mm length, hence an enhancement factor of 100 would be needed to detect this amount in spectroscopy. Starting from an EF of 1200, after 15 s we would have an EF of 365. There would likely be further loss in sensitivity due to shortened T_1 in tissue. Therefore, spectroscopy would be feasible, but imaging would remain relatively challenging.

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Notes and references

1 S.-C. Li and U. Lindenberger, in *Cognitive neuroscience of memory*, ed. L. G. N. H. J. Markowitsch, Hogrefe & Huber Publishers, Ashland, OH, US, 1999, pp. 103–146.
2 J. A. Lieberman, T. S. Stroup and D. O. Perkins, *The American Psychiatric Publishing textbook of schizophrenia*, American Psychiatric Pub., Washington, DC, 2006.
3 S. Guhlke, A. M. Verbruggen and S. Vallabhajosula, in *Clin. Nucl. Med.*, ed. H. J. Biersack and L. M. Freeman, Springer, Berlin; New York, 2007, pp. 34–73.
4 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.
5 S. B. Duckett and R. E. Mewis, *Acc. Chem. Res.*, 2012, **45**, 1247–1257.
6 Y. Endo, *Biochim. Biophys. Acta, Enzymol.*, 1978, **523**, 207–214.
7 S. M. Birnbaum, L. Levintow, R. B. Kingsley and J. P. Greenstein, *J. Biol. Chem.*, 1952, **194**, 455–470.
8 C. K. Mathews and K. E. Van Holde, *Biochemistry*, Benjamin/Cummings Pub. Co., Redwood City, Calif., 1990.
9 D. Hellwig, M. Menges, G. Schneider, M.-O. Moellers, B. F. Romeike, M. D. Menger, C.-M. Kirsch and S. Samnick, *Nucl. Med. Biol.*, 2005, **32**, 137–145.
10 P. L. Jager, W. Vaalburg, J. Pruim, E. G. E. de Vries, K.-J. Langen and D. A. Piers, *J. Nucl. Med.*, 2001, **42**, 432–445.
11 C. Catana, D. Procissi, Y. Wu, M. S. Judenhofer, J. Qi, B. J. Pichler, R. E. Jacobs and S. R. Cherry, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 3705–3710.
12 S. Gloggler, R. Muller, J. Colell, M. Emondts, M. Dabrowski, B. Blumich and S. Appelt, *Phys. Chem. Chem. Phys.*, 2011, **13**, 13759–13764.
13 J. Bargon, A. Koch, R. Rizi and J. Schmiedeskamp, *Proc. Intl. Soc. Mag. Reson. Med.*, Berlin, Germany, 2007.
14 C. R. Bowers and D. P. Weitekamp, *J. Am. Chem. Soc.*, 1987, **109**, 5541–5542.
15 H. N. C. Wong, Z. L. Xu, H. M. Chang and C. M. Lee, *Synthesis*, 1992, 793–797.
16 J. A. Tang, F. Gruppi, R. Fleysher, D. K. Sodickson, J. W. Canary and A. Jerschow, *Chem. Commun.*, 2011, **47**, 958–960.
17 J. A. Aguilar, P. I. P. Elliott, J. Lopez-Serrano, R. W. Adams and S. B. Duckett, *Chem. Commun.*, 2007, 1183–1185.
18 J. P. Candlin and A. R. Oldham, *Discuss. Faraday Soc.*, 1968, **46**, 60–71.
19 G. Oehme, E. Paetzold and R. Selke, *J. Mol. Catal.*, 1992, **71**, L1–L5.
20 R. Selke, C. Facklam, H. Foken and D. Heller, *Tetrahedron: Asymmetry*, 1993, **4**, 369–382.
21 L. Kuhn and J. Bargon, in *In situ NMR Methods in Catalysis*, ed. J. Bargon and L. Kuhn, Springer, Berlin, Heidelberg, 2007, pp. 25–68.
22 S. Aime, D. Canet, W. Dastrù\, R. Gobetto, F. Reineri and A. Viale, *J. Phys. Chem. A*, 2001, **105**, 6305–6310.
23 F. Gruppi, X. Xu, B. Zhang, J. A. Tang, A. Jerschow and J. W. Canary, *Angew. Chem., Int. Ed.*, 2012, **51**, 11787–11790.
24 H. Allouche-Arnon, M. H. Lerche, M. Karlsson, R. E. Lenkinski and R. Katz-Brull, *Contrast Media Mol. Imaging*, 2011, **6**, 499–506.
25 H. Allouche-Arnon, T. Wade, L. F. Waldner, V. N. Miller, J. M. Gomori, R. Katz-Brull and C. A. McKenzie, *Contrast Media Mol. Imaging*, 2013, **8**, 72–82.
26 H. Jóhannesson, O. Axelsson and M. Karlsson, *C. R. Phys.*, 2004, **5**, 315–324.
27 M. Haake, J. Natterer and J. Bargon, *J. Am. Chem. Soc.*, 1996, **118**, 8688–8691.
28 L. Wade and R. Katzman, *Am. J. Physiol.*, 1975, **228**, 352–359.
29 K. Golman and J. S. Petersson, *Acad. Radiol.*, 2006, **13**, 932–942.

