NMR Signal Enhancement

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Continuous ^{1}H and ^{13}C Signal Enhancement in NMR Spectroscopy and MRI Using Parahydrogen and Hollow-Fiber Membranes**

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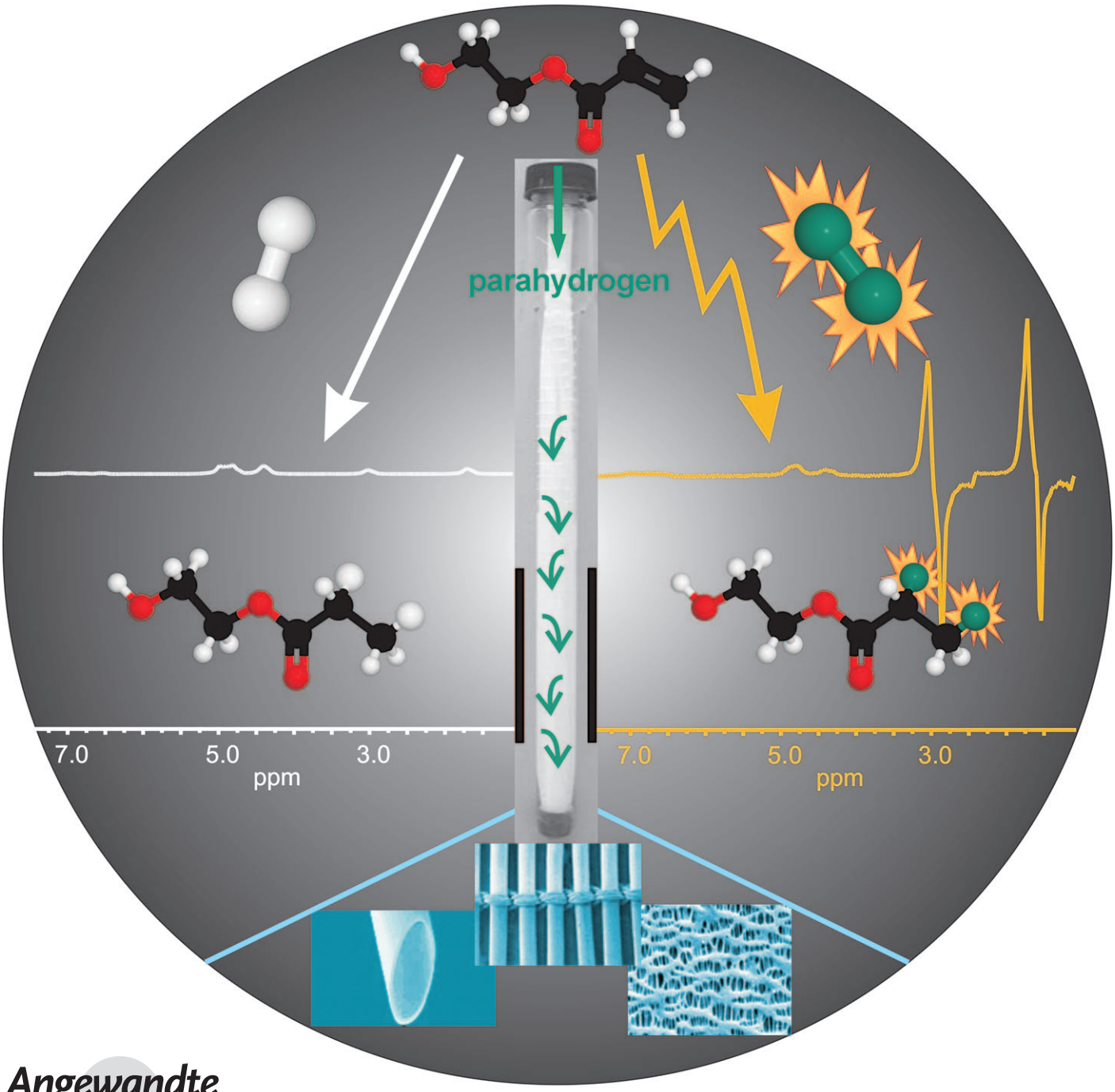

Despite its wide applicability in chemistry, biology, and medicine, NMR spectroscopy still suffers from its inherently low sensitivity. Exploiting the large signal enhancements associated with hyperpolarization techniques, such as dynamic nuclear polarization (DNP)[1,2] or parahydrogen-induced polarization (PHIP),[3–5] however, NMR or MRI qualify for monitoring dynamic processes in real time in biological,[6] chemical,[7] and medical[8] investigations. Unfortunately, most current hyperpolarization techniques are limited by the short duration of the hyperpolarized state. Efficient relaxation processes restrict the hyperpolarization in liquids to typically last from seconds to at best a few minutes. Moreover, the complex pulse sequences of multidimensional NMR experiments partly destroy the hyperpolarized signal depending on the flip angles used. In contrast to the fast decay of the hyperpolarization, its build up can be quite slow. Therefore, most hyperpolarization procedures are realized in batch processes; that is, one hyperpolarized sample is produced at a time and used immediately thereafter using carefully designed NMR pulse sequences.[2,9] Some of these shortcomings are circumvented by stepwise use of the generated hyperpolarization applying small flip angles[10] or using specially designed sampling strategies.[11,12] A particularly intriguing concept stores the fast decaying hyperpolarization in slowly relaxing singlet states.[13,14]

Herein, we present a straightforward chemical approach by augmenting the PHIP method with a continuous delivery of parahydrogen through hollow fiber membranes. This provides a continuous supply of molecules with hyperpolarized ^{1}H and ^{13}C nuclei for several minutes that can be used in various NMR or MRI experiments. The range of applications of this polarization technique with its unique chemical selectivity is thereby enhanced significantly.

PHIP is a chemical hyperpolarization method that exploits the high symmetry of the nuclear spins in parahydrogen and converts their spin correlation into hyperpolarized hydrogenation products with exceptionally enhanced antiphase NMR signals.[3–5] The key feature of this technique is the pairwise transfer of the two hydrogen atoms of parahydrogen to a double or triple bond by a standard hydrogenation reaction. The homogeneous hydrogenation reaction can be carried out under different conditions in terms of the present magnetic field strength during the reaction. A distiction is made between polarization in high magnetic fields (in the spectrometer, PASADENA condition: parahydrogen and synthesis allow dramatically enhanced nuclear alignment[3a]) and in low magnetic fields (outside the spectrometer, ALTADENA condition: adiabatic longitudinal transport after dissociation engenders nuclear alignment[3b]). The standard PASADENA experiment is carried out by bubbling hydrogen through a thin capillary into the reaction mixture inside a NMR tube.[3a] To acquire a spectrum, it is necessary to stop the parahydrogen gas flow and wait a certain time period to let the solution calm down. Of course, this implies an interruption of the reaction and furthermore a loss of polarization during the waiting time as a result of spin–lattice relaxation. Another drawback of the standard PASADENA method is the diminutive amount of dissolved hydrogen in the solution owing to the small gas–liquid interface of the generated bubbles. To overcome this problem, we combine herein a membrane technique that was previously developed to optimize the dissolution of hyperpolarized Xe in liquids[15] with the PHIP technique. The use of such membranes, which exhibit a very large gas–liquid interface, provides the opportunity to bring a gas molecularly into solution without the problem of foaming and bubbles. This approach ensures the implementation of a continuous dissolution of parahydrogen in the reaction mixture, thus giving rise to high conversion rates whilst maintaining a high spectral resolution during the NMR acquisition. In contrast to batch techniques, for which acquisition is only possible during the T_1 time of the once-generated hyperpolarization, the continuous delivery of parahydrogen and the ongoing hydrogenation reaction generate a significantly high polarization of ^{1}H, which is stable for several minutes. The huge ^{1}H polarization can continuously be transferred to ^{13}C using an INEPT-derived pulse sequence, thereby enhancing the site selectivity dramatically.[16] Moreover, as demonstrated below, the constant polarization allows the implementation of more complicated NMR experiments as for example, recording of 2D NMR spectra that require multiple acquisitions with equal initial polarization.

The parahydrogenation of the model compound 2-hydroxyethyl acrylate was carried out by continuously dissolving parahydrogen with the use of hollow fiber membranes into an aqueous solution (Figure 1). The hereby generated hyperpolarized product 2-hydroxyethyl propionate led to enhanced ^{1}H antiphase signals in the recorded spectra during the following minutes (Figure 2).

The top trace of the ^{1}H spectra shown in Figure 2a is a reference spectrum of the thermally polarized product acquired upon full conversion after 60 minutes. In the beginning of the parahydrogenation reaction, a buildup of hyperpolarized signal is observed, which is caused by the increasing conversion of the hydrogenation once the parahydrogen flow is switched on and the sample tube thermally equilibrates after insertion into the probe of the spectrometer heated to 80 °C. Towards the end of the reaction the intensity of the hyperpolarized signals decreases owing to lower amounts of starting material in the reaction mixture. However, during a timeframe of seven minutes, which is much longer than the T_1 time of the protons ($T_1(H_c) = 5.8$ s;

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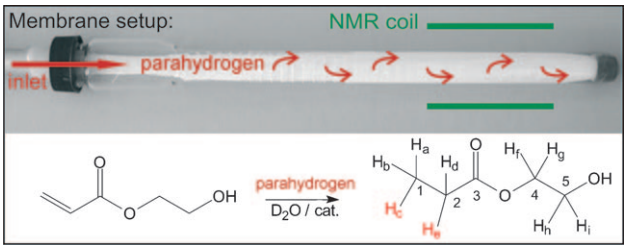

Figure 1. Implementation of the hollow-fiber membrane setup for parahydrogenation of 2-hydroxyethyl acrylate in D_2O leading to the hyperpolarized product 2-hydroxyethyl propionate. (See the Experimental Section for catalyst.)

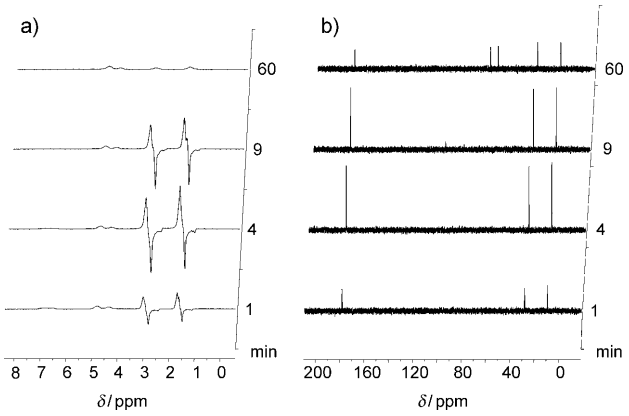

Figure 2. a) ^{1}H NMR spectra (reference: 1 scan; top trace) and b) ^{13}C NMR spectra (reference: 64 scans; top trace) during a time period of 60 min after starting the hydrogenation reaction. (See Supporting Information, Figure S1 for the complete time traces.)

$T_1(H_e) = 5.0$ s), the achieved signal enhancements remain rather constant, ranging between 1500 and 2000.

The same experiment was repeated to achieve constant ^{13}C polarization by transferring the high proton polarization to the carbon nuclei in the molecule. For this purpose a PH-INEPT+ sequence[16] with a delay of 15 ms was applied to the parahydrogenation reaction every minute. Selected spectra featuring hyperpolarized ^{13}C signals recorded during the same time frame as the protons are depicted in Figure 2b. Furthermore, a reference spectrum was recorded of the fully converted sample after 1 hour with 64 scans (top trace). As can be seen in Figure 2, the implementation of the PH-INEPT+ sequence resulted in polarization transfer from the two introduced protons H_c and H_e to the carbons C_1, C_2 and most efficiently to carbonyl carbon C_3 of the hydrogenation product. The time evolution of the ^{13}C spectra follows that of the ^{1}H spectra and features almost constant ^{13}C polarization for several minutes. For better comparison, the time dependence of the measured signal enhancements of both the hyperpolarized protons and carbons is given in Figure 3.

As can be clearly seen, the observed signal enhancements of the former parahydrogen protons H_c and H_e (●, □) are similar in height over the observed time period and rather constant between minute 3 and 10 of the parahydrogenation reaction. The signal enhancements of the carbon atoms

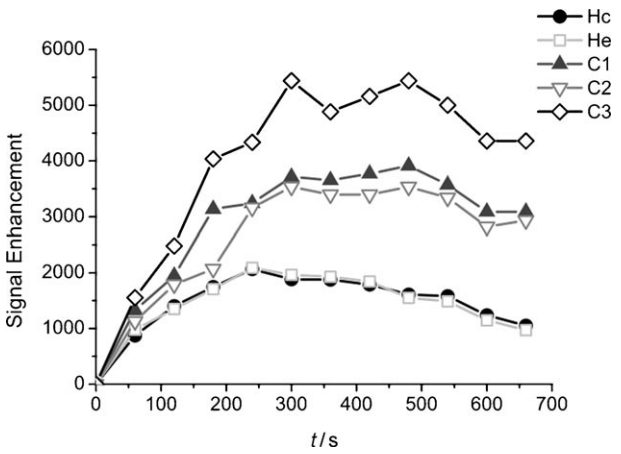

Figure 3. Time evolution of the ^{1}H and ^{13}C NMR signal enhancements during the parahydrogenation of 2-hydroxyethyl acrylate.

belonging to the generated single bond C_1 and C_2 (▲, ▽) are also comparable, ranging from 3000 to 4000. They show the same time evolution as the proton signal and are stable for seven minutes. Specifically, the carbonyl group in the product molecule C_3 (◇) showed significant signal enhancements between 4000 and 5500 after the conversion rate of reaction stabilized around the third minute. The reason for the higher signal enhancement of the carbonyl group might be that the 15 ms time delay chosen for the PH-INEPT+ sequence is most effective for the *J* couplings between the hyperpolarized protons and C_3, resulting in more efficient polarization transfer than to C_1 and C_2. This statement is supported by experiments applying different delays in the PH-INEPT+ sequence ranging from 10 to 25 ms (Supporting Information).

More complicated NMR experiments that require multiple excitations and longer acquisition times, such as recording 2D NMR spectra, provide rigorous tests to check whether or not constant hyperpolarization is indeed achieved using the membrane setup. In particular, removal of substrate by the membranes can be excluded in this way. Thus, a ^{1}H–^{1}H COSY experiment was performed during the parahydrogenation of 2-hydroxyethyl acrylate using the membrane setup. To ensure an optimal excitation of the PASADENA spin state, the first 90° pulse in the COSY sequence was replaced by a 45° pulse.[17] Due to the prior observation that the generated proton polarization was nearly constant for approximately 7 minutes, the PHIP ^{1}H–^{1}H COSY experiment was implemented by applying only one scan to stay within the timeframe of constant hyperpolarization. As a reference, a thermal ^{1}H–^{1}H COSY spectrum after total conversion was recorded with 8 scans, which required almost an hour. Both 2D NMR spectra are shown in Figure 4.

In the PHIP ^{1}H–^{1}H COSY spectrum, only the cross-peaks of the hyperpolarized protons at around 1.5 ppm and 3.0 ppm are visible owing to the enhanced signal of the corresponding protons and the special excitation of the PHIP spin state. Furthermore, the acquisition of the 2D spectrum during the ongoing reaction led to the observation of small signals between 6 and 7 ppm stemming from the double bond of the starting material. These peaks cannot be observed in the

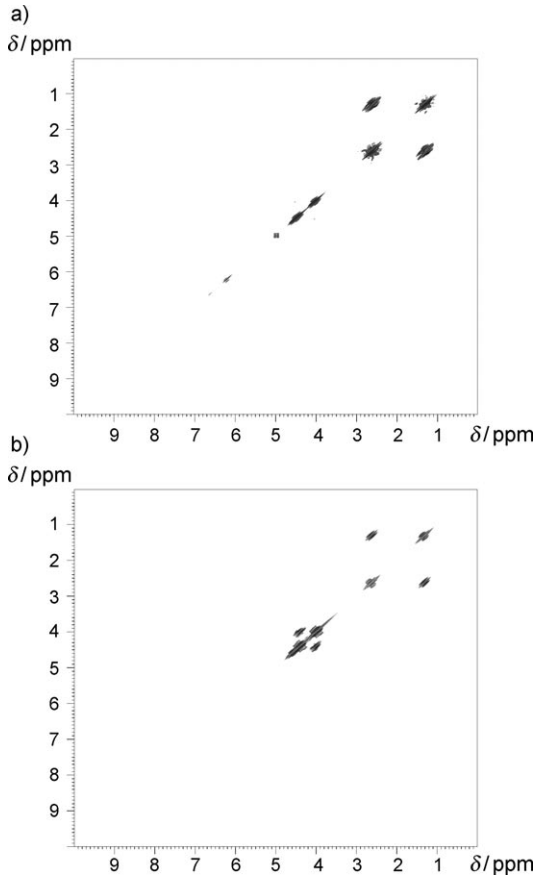

Figure 4. a) PHIP ^{1}H–^{1}H COSY NMR spectrum acquired during parahydrogenation of 2-hydroxyethyl acrylate in D_2O, single scan, experiment time: 7 min, 18 s. b) ^{1}H–^{1}H COSY reference spectrum of thermally polarized 2-hydroxyethyl propionate, 8 scans, experiment time: 57 min, 45 s.

reference ^{1}H–^{1}H COSY because the latter was acquired with the fully converted sample. As a consequence of the acquisition of the PHIP ^{1}H–^{1}H COSY without phase cycling, a small artifact can be recognized in the middle of the spectrum at 5 ppm that is not present in the reference COSY experiment where full-phase cycling was applied. PHIP 2D NMR spectroscopy was previously used by Duckett et al. to obtain structural information of metal dihydride complexes.[17] In comparison to the experiments presented herein, they were able to perform much faster 2D experiments with full-phase cycling (e.g. ^{1}H–^{1}H PHIP COSY in 2.6 min), because in their system, hyperpolarization is achieved without a chemical reaction. This allows short recycle delays (typically 20–100 ms) enabled by the fast refreshment of hyperpolarized signal due to the fast exchange of gaseous and bound parahydrogen in metal dihydride complexes. The 2D experiment demonstrated herein acquired during an ongoing chemical reaction had to be performed by applying a recycle time of 2 s, which is a trade-off of the time of constant proton polarization, signal enhancement owing to conversion of the parahydrogenation, and the T_1 relaxation time of the PHIP protons.

To conclude, we demonstrated that with the membrane technique it is possible to record a reliable 2D spectrum with chemical selectivity in much shorter time than by measuring a sample with thermal polarization only. Therefore, other 2D experiments should also be possible and useful for exploring the structure of reaction products resulting in significant time saving compared to the use of thermally polarized samples. Furthermore, the presented setup opens up the possibility to investigate otherwise elusive reaction intermediates because of the possibility of accumulating several scans during the ongoing reaction. Thus, the full site selectivity provided by PHIP can be exploited. The membrane technique can be easily extended to produce a continuous flow of a hyperpolarized liquid, as we have recently demonstrated by applying a continuous flow of Xe-enriched liquids for MRI applications.[18] This method, especially when combined with an important technique recently developed by Adams et al.,[19] which overcomes the restriction of the PHIP technique to unsaturated molecules, would allow for the continuous production of hyperpolarized molecules allowing for new applications in chemistry, biology, or medicine.

Experimental Section

Normal hydrogen with a purity of 5.0 was bought from Westfalen AG, Münster, Germany, and used as received. By cooling the thermal hydrogen with a closed-cycle cryostat setup (Advanced Research Systems, Macungie, PA, USA) down to 30 K, 98% enriched parahydrogen was generated and stored in transportable aluminum cylinders at up to 4 bar. The catalyst used for the parahydrogenation was a rhodium catalyst (1,4-Bis[sodium 3-(phenyl-3-propane sulfonate)phosphine] butane (2,5-norbonadiene) rhodium(I)-tetrafluoroborate), synthesized as described in the literature.[9] In contrast to the method used in Ref. [9], the catalyst was not only used as a freshly synthesized mixture in D_2O, but lyophilized and stored as a solid under argon atmosphere in the freezer. All other chemicals were purchased from Sigma–Aldrich or Merck and used without further purification. As hollow fiber membranes, the Celgard X50 (hydrophobic membranes composed of polypropylene for usage in aqueous systems) were acquired from Membrana GmbH. To our knowledge similar membranes suitable for organic solvents are currently being developed. The membranes were embedded into NMR pressure tubes by gluing them into a cap and sealing the NMR tube with this cap. Contrary to the membrane setup previously used for hyperpolarized Xe,[15] where an exchange of hyperpolarized and depolarized Xe was necessary, thereby requiring an inlet and outlet for the system, the experiments reported herein required only a parahydrogen inlet. As no indication for the conversion of parahydrogen into o-H_2 by the catalyst was observed (absence of the typical o-H_2 signal at 4 ppm) only the replacement of parahydrogen consumed by the hydrogenation has to be ensured. The 10 mm NMR tubes were filled with 5 mg of catalyst, 0.86 mmol of 2-hydroxyethyl acrylate, and 3 mL D_2O under argon atmosphere. The PASADENA experiment was carried out at elevated temperatures and pressures. Therefore, prior to the experiment, the reaction tube was pressurized with 3 bar of para-enriched H_2, and the pressure tightness of the module was controlled. Upon the dissolution of the parahydrogen through the membranes in the reaction mixture the NMR tube was carefully inserted into the spectrometer either with its associated lift or by hand, and the probe of the spectrometer was heated up to start the hydrogenation reaction. During the whole time, parahydrogen was delivered to the solution to ensure a constant reaction progress. The ^{1}H experiments were performed on a 300 MHz spectrometer with a Tecmag console, whereas the ^{13}C experiments and the 2D NMR experiments were performed on a Bruker AVANCE 300 MHz spectrometer. The polarization transfer from the protons to the

carbon atoms was achieved by applying the PH-INEPT+ sequence.[16] All reference spectra were measured using the same NMR parameters as for the PHIP spectra. Reference spectra of the corresponding reaction mixture were used to calculate the signal enhancements by comparing them with the recorded PHIP spectrum. Proton signal enhancements were calculated by comparing the absolute integrals, whereas for the carbon spectra the height of the peaks was chosen to calculate the signal enhancements. The PHIP ^{1}H–^{1}H COSY spectrum was acquired by replacing the 90° excitation pulse in a normal COSY sequence with a 45° pulse to excite the PASADENA spin system.[17] Using this sequence, the spectrum was recorded with 1 scan and 128 steps in the second dimension, a repetition time of 2 seconds, and a total acquisition time of 7 min, 18 seconds. Recording the reference ^{1}H–^{1}H COSY spectrum with a standard 90° excitation pulse and 8 scans needed a total acquisition time of 57 min, 45 seconds.

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