Deuterated polymer gels for measuring anisotropic NMR parameters with strongly reduced artefacts†

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Perdeuterated poly(styrene) is introduced as an almost artefact-free and arbitrarily scalable alignment medium for measuring residual dipolar couplings and other anisotropic NMR parameters; the spectral quality achievable in this new medium is demonstrated for HSQC spectra leading to the conformational analysis of staurosporine and homonuclear TOCSY-type experiments.

Residual dipolar couplings (RDCs) as anisotropic NMR-parameters play an important role for the structure determination of biomacromolecules[1] and small organic molecules in solution.[2] For the measurement of anisotropic parameters liquid crystalline phases or stretched polymer gels can be used for achieving the necessary partial alignment, with gels having the advantage of arbitrary scalability of the alignment strength.[3–5] However, alignment media generally introduce undesired NMR-signals which might severely overlap with signals of the molecule of interest and become especially annoying in polymer gels at low alignment strengths.[6]

One solution to the problem is the use of an alignment medium with few NMR signals, like poly(dimethylsiloxane) (PDMS) with only one ^{1}H NMR signal at 0.1 ppm.[7] The disadvantage of this approach is of course the strong limitation in the choice of alignment medium. A different, more generally applicable technique might be the perdeuteration of the alignment medium which has been previously applied to the liquid crystalline phase 4-η-pentyl-4′-cyanobiphenyl-d_{19}.[8] Our aim in the presented research was therefore to create a gel-based alignment medium with *per se* full scalability of alignment strength and practically no additional ^{1}H NMR signals *via* deuteration of the polymer.

A number of deuterated polymers have been synthesised and used in NMR studies, *e.g.* perdeuterated poly(ethylene) and PDMS, partially deuterated poly(styrene) (PS) or copolymers of perdeuterated PS with partially deuterated poly(methylmethacrylate), which have all been used to investigate the behaviour of the polymer chains *via* ^{2}H NMR spectroscopy.[9] Having the synthetic experience for cross linked PS already available in our laboratory[4,6] we decided to produce perdeuterated and cross linked poly(styrene) (dPS) out of commercially available styrene-d_8, divinylbenzene (DVB) and azobisisobutyronitrile (AIBN) to prove the applicability and usefulness of deuteration of gel-based alignment media (see ESI for synthesis details†).

After synthesising and swelling cylindrically shaped, cross linked PS and dPS sticks in $CDCl_3$ as described previously,[4,6] we recorded ^{1}H 1D-spectra for a first comparison (Fig. 1(a)). The reduction of polymer NMR signals is enormous with only small remaining signals for the dPS gel originating from residual protons of styrene-d_7 and non-deuterated DVB and AIBN. The most intense signal in the dPS spectrum is the residual solvent peak of $CDCl_3$ at 7.26 ppm. The dramatic improvement of spectral quality can easily be seen as soon as approximately 25 mM strychnine is diffused into the gel and corresponding 1D-spectra are recorded (Fig. 1(b)): While artefactual signals from PS dominate the spectrum in the non-deuterated case, they are hardly visible in the dPS sample.

In heteronuclear experiments for the extraction of one-bond ^{1}H,^{13}C-RDCs, signal overlap is usually reduced due to the additional spectral dimension. However, the CLIP-HSQC[10] of approximately 4 mM staurosporine diffused in a PS/$CDCl_3$ gel clearly demonstrates that overlap in the aromatic region still is a significant problem at low solute concentrations (Fig. 1(c)). The corresponding spectrum in a dPS/$CDCl_3$ gel shows no overlap with the very small residual polymer signals and allows the extraction of all aromatic RDCs (Fig. 1(d)). With all of the RDCs finally in hand, the configurational and conformational analysis of staurosporine, a natural product isolated from *Streptomyces staurosporeus*, is possible: The molecule consists of an extensive aromatic ring system and a six-membered tetrahydropyran ring attached *via* two nitrogen atoms. The tetrahydropyran ring in principle contains 4 stereogenic centers but cyclisation reduces the degree of freedom to 3, resulting in $2^3 = 8$ possible configurations (4 diastereomers *SRRR*, *SRSR*, *SSRR*, *SSSR*, and their enantiomers *RSSS*, *RSRS*, *RRSS*, *RRRS*). Since the NMR experiments are carried out in an achiral environment, enantiomers show identical spectra and only the 4 diastereomers have to be considered. Finally, in addition to the different configurations, chair or boat conformations can be assumed for the six-membered ring, resulting in a total of 8

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† Electronic supplementary information (ESI) available: Detailed instruction for dPS polymerisation and gel preparation; details concerning the assignment, measured RDCs, and structural interpretation of data using PALES and GROMACS for staurosporine; ^{2}H 1D-spectrum of a dPS gel; example TOCSY spectra including the MOCCA-XY16 mixing sequence. See DOI: 10.1039/b812905c

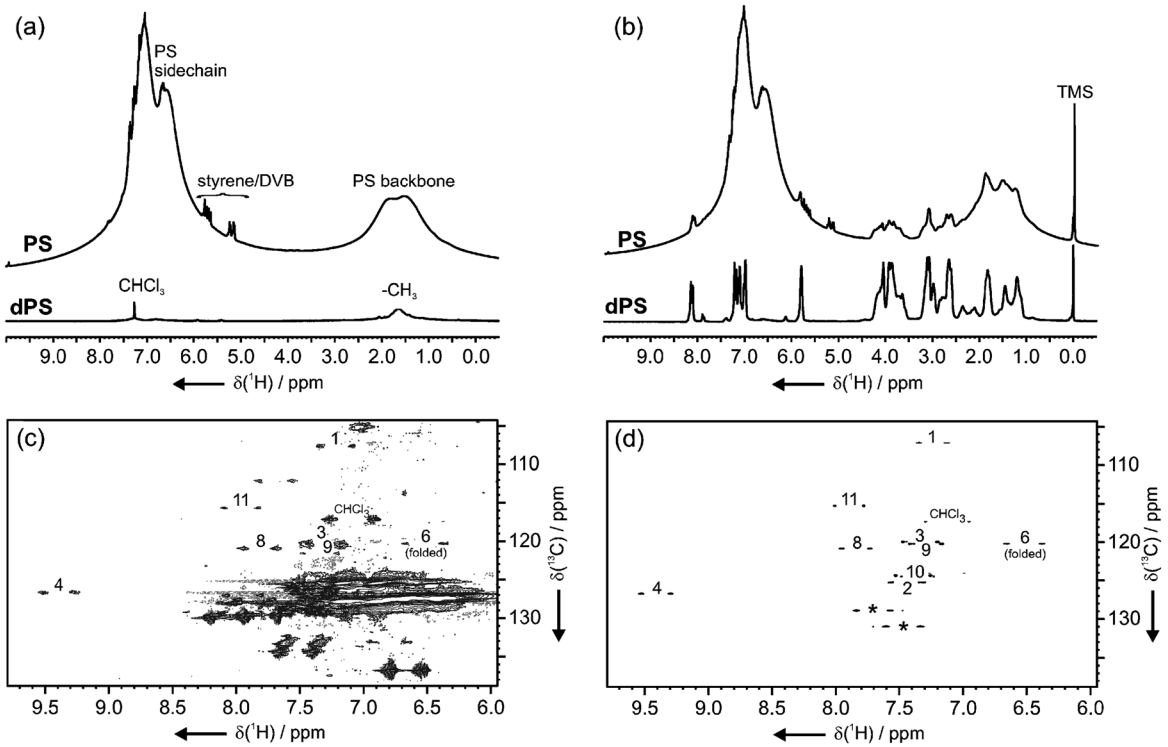

Fig. 1 Comparison of spectra acquired on various stretched PS/$CDCl_3$ and correspondingly deuterated and stretched dPS/$CDCl_3$ gels. (a) Comparison of ^{1}H 1D-spectra of the polymer gels with assignment of selected signals; (b) the corresponding 1D-spectra of the stretched gels with $\approx$25 mM strychnine diffused into them; (c),(d) aromatic region of CLIP-HSQC spectra[10] acquired on the corresponding non-deuterated PS (c) and deuterated dPS (d) gels containing $\approx$4 mM staurosporine. Contour levels were adjusted to allow for the identification of staurosporine signals as far as possible. Cross peaks are assigned wherever possible. Residual unidentified signals originating from the alignment medium are marked with an asterisk.

distinguishable structures. The experimentally obtained 13 one-bond ^{1}H,^{13}C-RDCs were fitted against the potential structures using the program PALES with the bestFit option[11] and the squares of the corresponding correlation factors for the fits, R^2, are shown in Fig. 2(a). The best correlation (R^2 = 0.996) clearly results for the *SRRR* configuration in the chair conformation which supports the structure reported for the free base of staurosporine.[12] The results are also corroborated by RDC-restrained molecular dynamics simulations where the best match of measured and back calculated RDCs is obtained for the same structure (see ESI†).

In addition to heteronuclear experiments the perdeuteration of the alignment medium also allows the acquisition of clean homonuclear correlation experiments. Fig. 3 shows the aliphatic region of two TOCSY spectra recorded on strychnine in a dPS/$CDCl_3$ gel. In a PS/$CDCl_3$ gel the spectra are limited by a reduced dynamic range due to the strong polymer signals and overlap with the polymer backbone signals (see Fig. 1) make the interpretation of the spectra more difficult. The two TOCSY experiments shown differ in the multiple pulse sequence used in the mixing period: While the so-called J-ONLY-TOCSY (Fig. 3(b)) with its JESTER-1 sequence shows only correlations *via* scalar couplings,[13] the DIPSI-2 sequence (Fig. 3(c)) leads to positive scalar and negative dipolar polarization transfer and combinations of them.[14] While the J-ONLY-TOCSY can be used in partially aligned samples to reassign scalar coupled spin systems with shifted chemical shifts,[13] the additional cross peaks present in the

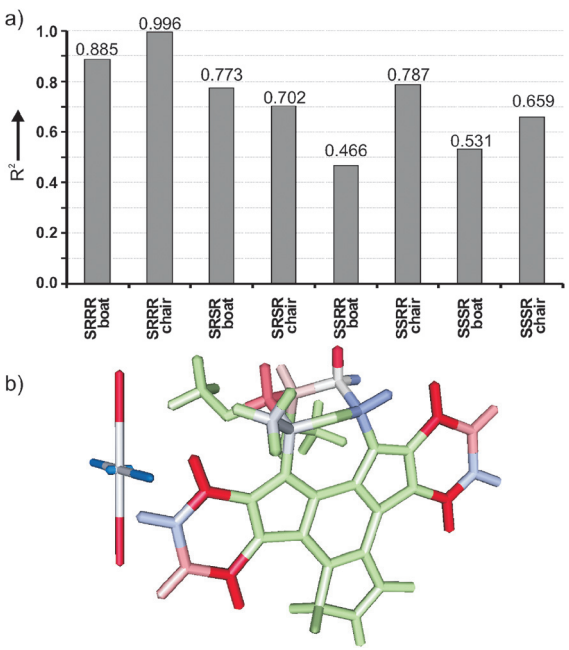

Fig. 2 Correlation between measured RDCs and potential staurosporine structures. The square of the correlation factor R^2 from the PALES fits for the different configurations and conformations (a) supports the *SRRR* chair structure shown in (b). The structure is shown with colour-coded bonds representing negative (red) and positive (blue) ^{1}H,^{13}C-RDCs and the axes of the corresponding alignment tensor drawn next to it.

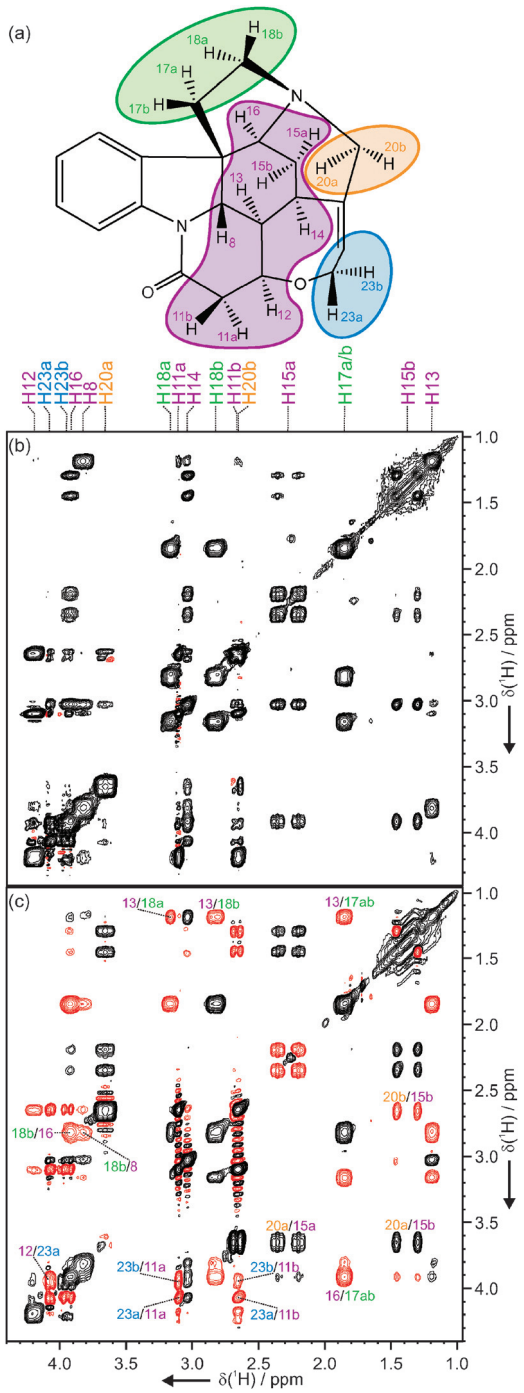

Fig. 3 Aliphatic region of TOCSY-type spectra recorded on strychnine diffused in a stretched dPS/$CDCl_3$ gel with the colour-coded assignment of spin systems shown in (a). The J-ONLY-TOCSY (b) shows only correlations within scalar coupled spin systems. Additional cross peaks appear in spectra acquired with the DIPSI-2 (c) mixing scheme due to coherence transfer *via* dipolar couplings through space. Positive and negative cross peaks are drawn in black and red contour lines, respectively.

DIPSI-2 experiment can be used to identify neighboring spin systems that are now coupled through space *via* $^{1}H,^{1}H$-RDCs[15] (see colour-coded assignments in Fig. 3). The MOCCA-XY16 TOCSY optimised for maximum combined scalar and dipolar transfer[16] is shown in the ESI.†

In summary, one can conclude from the dPS example that perdeuterated polymer gels significantly improve the quality of spectra measured on samples partially aligned in the stretched gel. The almost signal free and scalable alignment medium enables the measurement of RDCs at very low concentrations and allows the acquisition of any kind of homonuclear correlation experiment. Small residual polymer signals of the dPS presented here originate from the non-deuterated cross linker DVB and radical starter AIBN, which could be further reduced by using the corresponding deuterated educts or substituting the radical starter with a protonless one. So far we only synthesised deuterated poly(styrene) suitable for apolar solvents, but the approach shown here can most certainly be extended to other polymer gels like *e.g.* poly(acrylonitrile)[17] or poly(acrylamide),[3] enabling then the acquisition of high quality spectra with scalable alignment for the most common NMR solvents.

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