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Glycerol and glycerol carbonate as ultraviscous solvents for mixture analysis by NMR

Pedro Lameiras [a], Leslie Boudesocque [a], Zéphirin Mouloungui [b], Jean-Hugues Renault [a], Jean-Michel Wieruszeski [c], Guy Lippens [c], Jean-Marc Nuzillard [a,*]

[a] Université de Reims Champagne-Ardenne, Institut de Chimie Moléculaire de Reims, CNRS UMR 6229, IFR 53 Biomolécules, BP 1039, 51687 Reims Cedex 02, France
[b] Université de Toulouse, INRA, INP, Laboratoire de Chimie Agro-Industrielle, ENSIACET, 4 Allée Emile Monso, 31030 Toulouse, France
[c] Université des Sciences et Technologies de Lille 1, Unité de Glycobiologie Structurale et Fonctionnelle, CNRS UMR 8576, 59655 Villeneuve d'Ascq Cedex, France

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ABSTRACT

NMR of weakly polar analytes in an apolar ultraviscous solvent has recently been proposed for mixture analysis as a pertinent alternative to the DOSY experiment. The present article reports the first use of glycerol and glycerol carbonate as polar solvents for the NMR analysis of a model mixture of dipeptides. This work demonstrates the high potentiality of these solvents for the analysis of mixtures made of polar and potentially bioactive compounds. Medium-sized molecules slowly reorient in glycerol and glycerol carbonate under particular temperature conditions, so that solute resonances may show spin diffusion in NOESY spectra, thus opening the way to mixture analysis. Glycerol and glycerol carbonate have turned out to be ultraviscous solvents of choice for the individualization of four structurally close mixed dipeptides: Leu–Val, Leu–Tyr, Gly–Tyr and Ala–Tyr by means of 1D and 2D NOESY experiments. Selective sample excitation and signal detection were implemented to eliminate the intense proton signals of the non-deuterated solvents. Moreover, the recording of a multiplet selective 2D NOESY–TOCSY has shown that the analytical power of NMR in highly viscous solvents is not limited to the extraction of mixture component 1D subspectra but may also yield some supplementary information about atom connectivity within components.

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1. Introduction

NMR spectroscopy plays a leading role among the spectroscopic techniques that are devoted to structure elucidation of unknown low-molecular weight organic molecules. Chemical purity is generally considered as a necessary prerequisite in this context. Purification and NMR spectroscopy can be carried out at the same time if a special LC–NMR probe is available. Sensitivity problems are then solved by the use of very high magnetic fields, of ultra-cooled probe coils and by sample concentration on solid adsorbents [1,2]. In some instances, however, separation is not possible, and NMR must be performed on mixtures. Mixture analysis by NMR is most often restricted to known compound identification and quantification. Finding the structure of unknown compounds within mixtures is a demanding exercise because the NMR response set has to be split into subsets, each one being related to a single mixture component. The task becomes all the more challenging when accidental peak superimposition occurs. Being able to assign each peak to a specific compound would reduce the necessity of chromatographic separation and would therefore greatly enhance the efficiency of synthetic and natural product chemists.

The DOSY experiment is presently considered the best way to make this dream possible. NMR signals are sorted according to the value of the translational diffusion coefficient of each molecule constituting the mixture [3–7]. However, 2D DOSY spectra have a limited resolution in the diffusion domain and therefore NMR signals from structurally close molecules may be difficult to group according to their compound of origin. The efficiency of the DOSY experiment can be enhanced under particular conditions by addition of auxiliary ingredients to the sample, such as solid chromatographic phases [8–13] or aggregate forming molecules [14,15]. Molecular mobility is then modulated by specific interactions between the analytes and the sample additive, thus resulting in significantly different measured diffusion coefficients.

A recent publication by Simpson and coworkers proposed an alternative approach that is based on spin diffusion [16]. By dissolving the sample in a highly viscous medium, the chlorotrifluoroethylene (PCTFE) polymer, relaxation falls into the very slow molecular tumbling regime where spin diffusion becomes very efficient: a 2D ^{1}H–^{1}H NOESY spectrum with a long mixing time can then correlate together the chemical shifts of the ^{1}H nuclei that belong to the same molecule. All the rows (or columns) in the spec-

* Corresponding author. Fax: +33 3 26 91 31 66.
E-mail address: jm.nuzillard@univ-reims.fr (J.-M. Nuzillard).

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trum that are identical thus contain the expected 1D spectrum of a single mixture component. This method was able to separate the spectra of strychnine and brucine, two structurally close alkaloids.

The PCTFE polymer, when mixed with perdeuterated organic solvents, has been previously used to modify the NOE responses of small or medium molecules [17–20]. This polymer does not impair the sample analysis as it is nearly chemically inert. As an aprotic solvent it can be used when dealing with lipophilic molecules, however, its low dielectric constant ($\varepsilon = 2.6$ at 25 °C) [21] does not favor the dissolution of polar compounds. This issue has been addressed in our study, and we wish to report the use of two small molecules, glycerol (GL) or glycerol carbonate (GC), used for the first time in the NMR analysis of a mixture of polar compounds.

Both GL and GC are viscous solvents with a high dielectric constant: $\varepsilon = 42.5$ [21] for GL and $\varepsilon = 109.7$ [22] for GC at 25 °C. Glycerol is a co-product of the industry scale manufacturing of biofuels, soaps and surfactants from triglycerides. Glycerol production currently exceeds demand. For this reason, new developments and new markets for glycerol are being actively explored. Glycerol has many applications such as being a hydrophilic component in neutral surfactants and an emulsifier in food, cosmetics and pharmaceuticals. In the domain of organic chemistry, one of the most attractive transformations of glycerol is the synthesis of glycerol carbonate [23,24]. This latter compound may play a central role in the near future for the industrial-scale production of solvents, lubricants and surfactants from renewable carbon sources. The existence of such ton-scale markets is not incompatible with the search for highly specific niche applications.

Medium-sized molecules slowly reorient in GL and GC under particular temperature conditions and therefore present a negative NOE regime. Spin diffusion can be observed in these conditions, so that all resonances of the protons within the same molecule correlate together in a 2D NOESY spectrum, thus opening the way to mixture analysis. In this context, this work focused on the assessment of GL and GC for the individual NMR characterization of four structurally close dipeptides: Leu–Val, Leu–Tyr, Gly–Tyr and Ala–Tyr within a single mixture. The major experimental pitfall of this approach was the mandatory elimination of the strong ^{1}H signals of GL and GC. Failing to correctly achieve this would have resulted in spectra in which solvent signals obscured solute signals. The first experiments that involved GL were carried out in perdeuterated GL. It is worth noting that this commercially available solvent is at most 99% D-enriched and is very expensive, about 100 Euros per gram. Therefore, 1D and 2D selective NOESY experiments that incorporate solvent suppression were implemented.

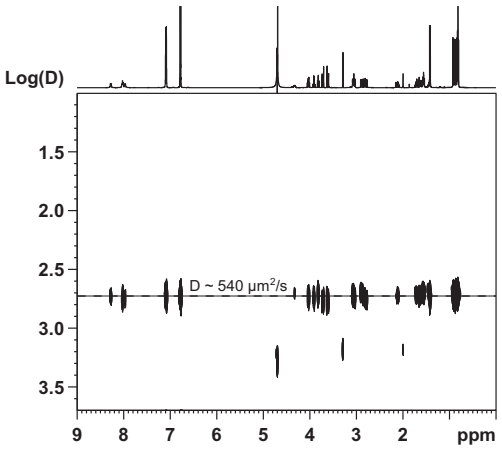

Fig. 1. ^{1}H DOSY spectrum of the dipeptide test mixture in water at 298 K. Data was acquired with the stebpgp1s19 pulse sequence. The diffusion time (Δ) was 50 ms and the gradient pulse length (δ) was 2 ms. The size of the raw data set was 32×8192. The gradient intensity values were equally spaced from 2% to 95%. Water suppression was achieved by a 3-9-19 pulse sequence with 1 ms gradient pulses of −20% intensity (WATERGATE). The DOSY spectrum was calculated using the Bruker TOPSPIN Software. Inverse Laplace transformation in the indirectly detected dimension was carried out by means of the MaxEnt algorithm. Log (D) was calculated with D expressed in $(\mu m)^2/s$.

2. Results and discussion

The self-diffusion coefficients of the dipeptides in the Leu–Val, Leu–Tyr, Gly–Tyr, and Ala–Tyr mixture were first measured in order to evaluate their ability to differentiate the mixture components [25]. The ^{1}H DOSY experiment was performed in water using WATERGATE solvent suppression (Fig. 1) [26]. The diffusion coefficients of the dipeptides are approximately identical ($D \sim 540\ \mu m^2/s$). The individual components of the mixture could not be clearly discriminated according to their translational

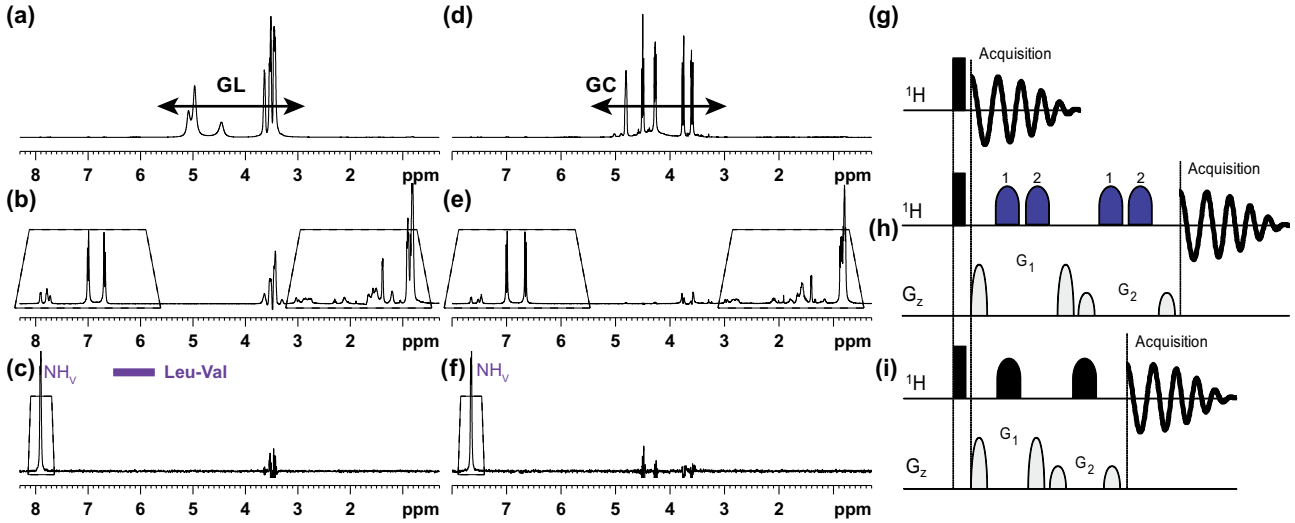

Fig. 2. 1D ^{1}H spectra (8 scans) and corresponding NMR pulse sequence of the dipeptide test mixture in GL (a, b, c, 318 K) and in GC (d, e, f, 288 K). G_1:G_2 = 70:30. The FIDs (32 k points, spectral width = 4500 Hz) were processed with LB = 0.3 Hz and zero-filled to 32 k points. (a, d, g) Non-selective excitation and detection. (b, e, h) Selective detection of two resonance bands. The 4 ms I-BURP-2 pulses cover 1250 Hz (dotted trapezium). The "1" and "2" labels respectively indicate their application to the high and low chemical shift regions. (c, f, i) Selective excitation of the valine amide proton doublet of Leu–Val (dotted trapezium) using a 20 ms, 1% truncated, 180° Gaussian pulse.

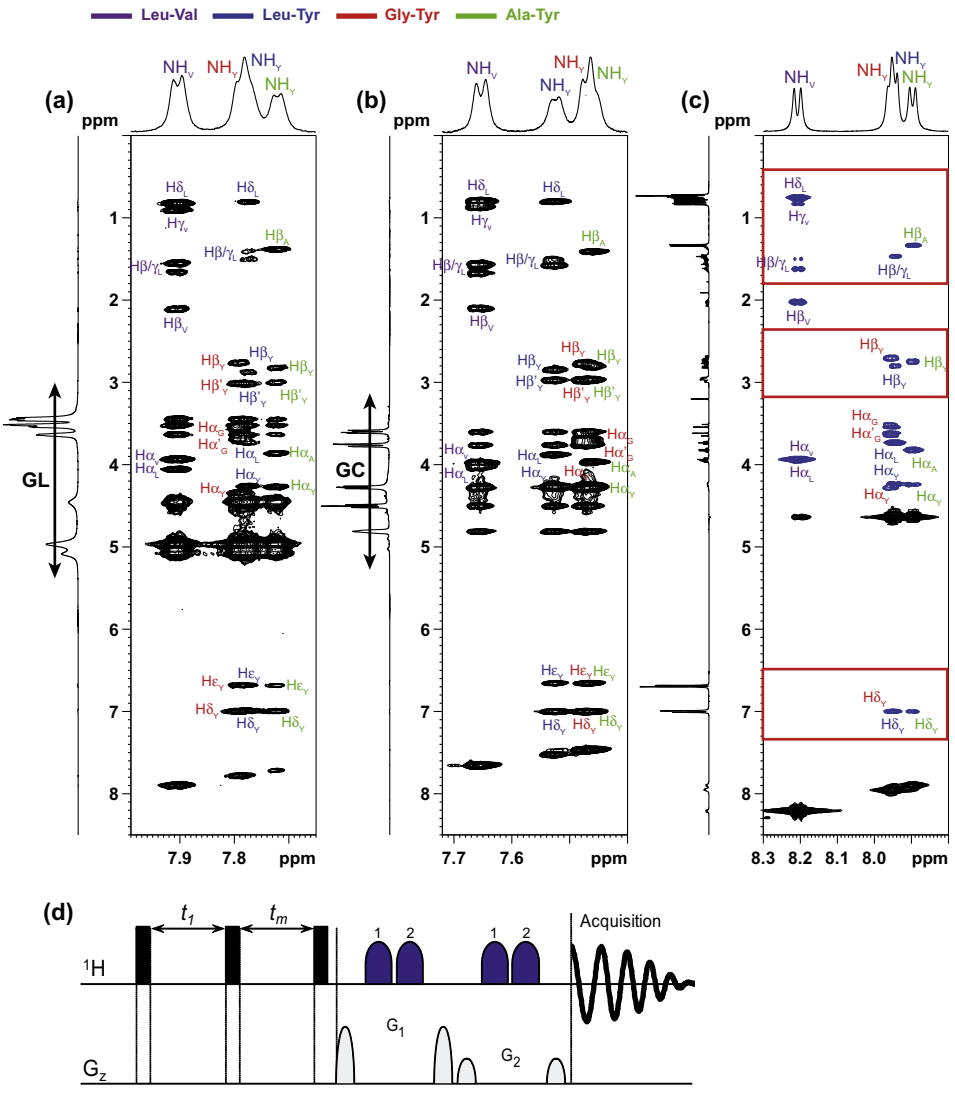

Fig. 3. Amide proton region of band-selective detection 2D NOESY spectra of dipeptide test mixture (a) dissolved in GL at 318 K, (b) dissolved in GC at 288 K using the pulse sequence in (d). Shaped pulses and gradient pulses were identical to those in Fig. 2b,e and h. The NOESY part of the sequence was adapted from the noesyph pulse program. All data matrixes were recorded in TPPI mode; their size was 400 × 2 k with 16 scans per FID, a 1.3 s relaxation delay and a 1 s mixing time (t_m), resulting in a 278.62 min recording time. The spectral width was 4500 Hz in both dimensions. G_1:G_2 = 70:30. All data matrixes were multiplied in both dimensions by a shifted sine bell function (SSB = 2) before zero filling to a 1 k × 2 k size. (c) Amide proton region of 2D NOESY spectrum of dipeptide test mixture dissolved in water at 298 K. Spectrum (c) was recorded and processed with the same parameters as those in (a and b) using the noesyesgpph pulse sequence. A pair of 2 ms rectangular shaped inversion pulses was applied on the water signal resonance. The length of the water suppression gradient pulses was 1 ms and their intensity 31% for the first pair and 11% for the second pair. The red frames correspond to spectral regions of interest in which water as solvent has a major effect on the number and sign of observable NOESY cross peaks.

diffusion behavior, due to their similar molecular weight and shape. This observation prompted us to investigate the resolving power of spin diffusion based experiments, using either GL or GC as solvent.

A NOESY spectrum of a sample recorded in non-deuterated GL or GC would only show the solvent signals. This is illustrated in Fig. 2a, 2d and 2g by simple excitation–detection spectra. GL and GC signals cover a spectral region of about 2 and 1.5 ppm, respectively. The elimination of these signals can be achieved by means of selective pulses when included in an excitation sculpting sequence [27]. The selective pulses must be applied so that they are able to invert the equilibrium magnetization of the nuclei of interest and leave untouched the one of the solvent nuclei. This approach has been named "band selective acquisition". For this purpose, two possibilities were considered. The first one involved resonance inversion in the two frequency bands on either side of the solvent signal by means of two consecutive band selective pulses. The second alternative is similar to water suppression: a solvent band selective soft inversion pulse is followed by a hard inversion pulse, so that solvent magnetization is not modified if the hard and soft pulse actions exactly compensate each other. Taking into account that the viscous medium induces a quick transverse relaxation of the sample magnetization, the first option was preferred because two wideband inversion pulses may be shorter than a single narrow band one. The spectra in Fig. 2b, 2e and 2h illustrate the quality of solvent suppression that was achieved by band selective detection.

Obviously, all sample signals that are present in the solvent frequency band are also eliminated. This might not be a problem in selectively detected 2D NOESY spectra if at least one signal per analyte is preserved. This signal could correlate with all the others along the F_1 axis, including those that fall in the solvent spectral region.

The authors of the recent publication using PCTFE as an NMR solvent have pointed out that temperature is an important parameter in spin diffusion experiments because it is directly related to

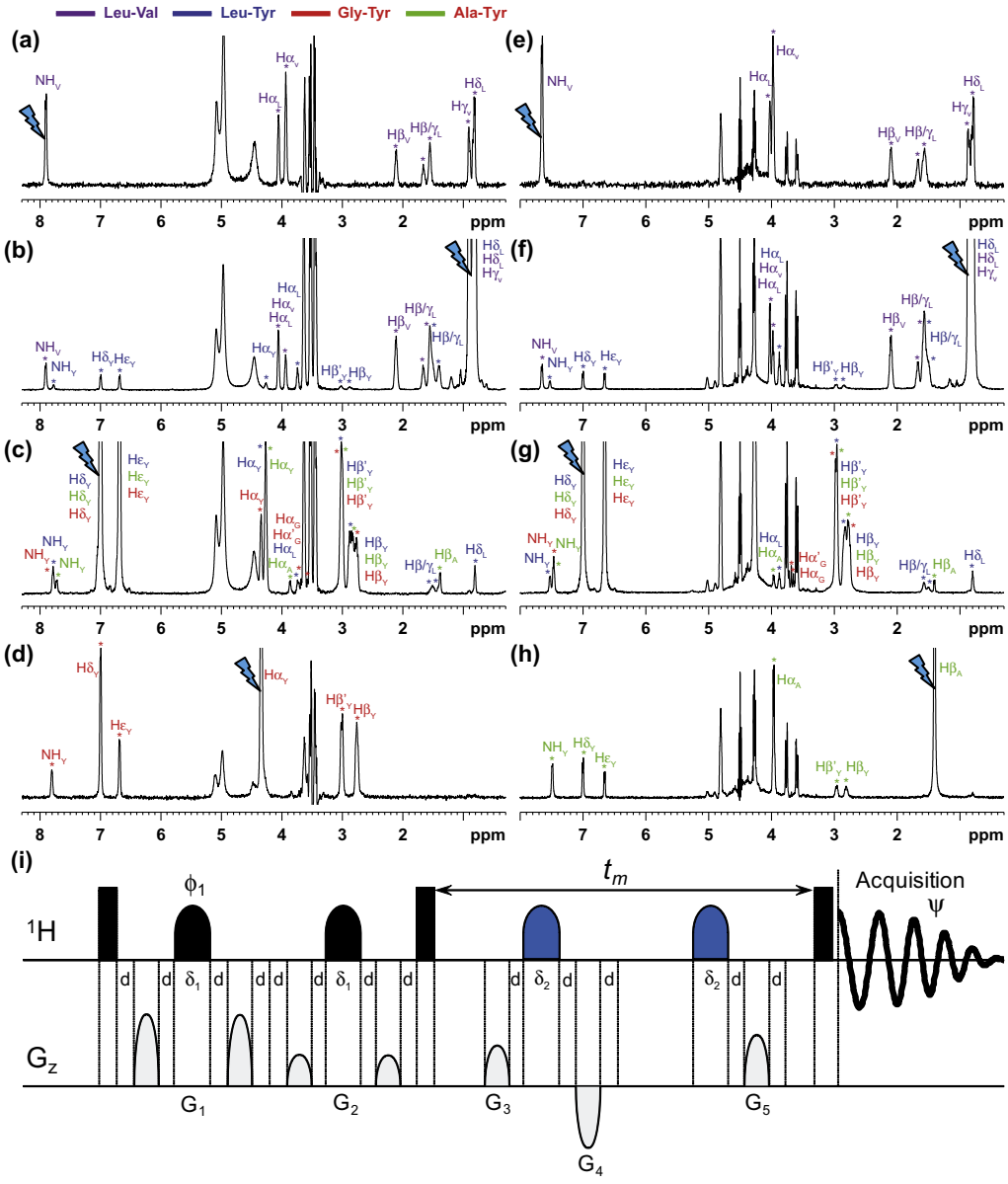

Fig. 4. Multiplet selective excitation ^{1}H 1D NOESY spectra of the dipeptide test mixture in GL (a, b, c, d, 318 K) and in GC (e, f, g, h, 288 K). (i) Pulse sequence: $\varphi_1 = x, y, -x, -y$, $\psi = x, -x$. The initial selective excitation was achieved by a 1% truncated 180° Gaussian pulse. G_1:G_2 = 70:30. WURST wideband adiabatic inversion pulses, δ_2 = 1.5 ms, starting within t_m (1 s) at 0.33 and 0.83 t_m with G_3:G_4:G_5 = 40:−60:50. Gradient pulse length was 3 ms. d = 200 μs. Relaxation delay was 2 s. The FID (16 k points, spectral width 4500 Hz) was multiplied by an exponential function (LB = 0.3) before zero filling to 16 k points. The initial selective inversion pulses excite: (a, e) the NH_V(LV) proton resonance (δ_1 = 20 ms, 8192 scans, 531.32 min); (b, f) the $H\delta_L$(LY)/$H\delta_L$(LV)/$H\gamma_V$(LV) proton resonances (δ_1 = 5 ms, 800 scans, 51.63 min); c, g) $H\delta_Y$(LY)/$H\delta_Y$(GY)/$H\delta_Y$(AY) proton resonances (δ_1 = 8 ms, 800 scans, 51.72 min); (d, h) respectively the $H\alpha_Y$(GY) and $H\beta_A$(AY) proton resonances (δ_1 = 50 and 40 ms, 2048 scans, 134.88 min and 134.2 min).

solvent viscosity and therefore to rotational correlation times [16]. The criterion for optimal temperature selection is the intensity of the NOESY cross peaks between nuclei that are not supposed to be close enough to show a NOE in a low viscosity medium. A temperature reduction causes a spin diffusion increase accompanied by a signal intensity decrease through transverse relaxation related line broadening. Sample cooling is mandatory if the NOESY spectrum shows positive NOE responses (diagonal and off-diagonal peaks of opposite signs).

The temperature conditions that are adapted to GL and GC were experimentally determined using the pulse sequence in Fig. 3d, in which the usual NOESY part is followed by a selective detection period. Figs. S1 and S2 provided as Supplementary Data report the evolution of the 2D NOESY correlations of the amide protons in the dipeptide test mixture upon sample temperature modification. The spectra revealed the expected intra-molecular correlations but also showed a non-negligible amount of magnetization transfer between the dipeptides and the solvent. The intermolecular Overhauser effect, usually not efficient in itself, clearly appeared due to the high concentration of the solvent molecules. Moreover, as the solvent and the analytes contained labile hydrogen atoms, the existence of a large network of hydrogen bonds could favor the observation of intermolecular Overhauser effects. Slightly higher temperatures (298 K, 308 K, 318 K and 328 K) were tested when the solvent was GL compared to 298 K, 288 K and 278 K with GC. In GL, 318 K was the temperature at which spin diffusion took place across all the protons of the dipeptides with the highest amide proton spectral resolution and with a good signal to noise ratio. The NH magnetization did not reach the tyrosine Hε protons at 328 K and only very weakly reached the tyrosine Hε

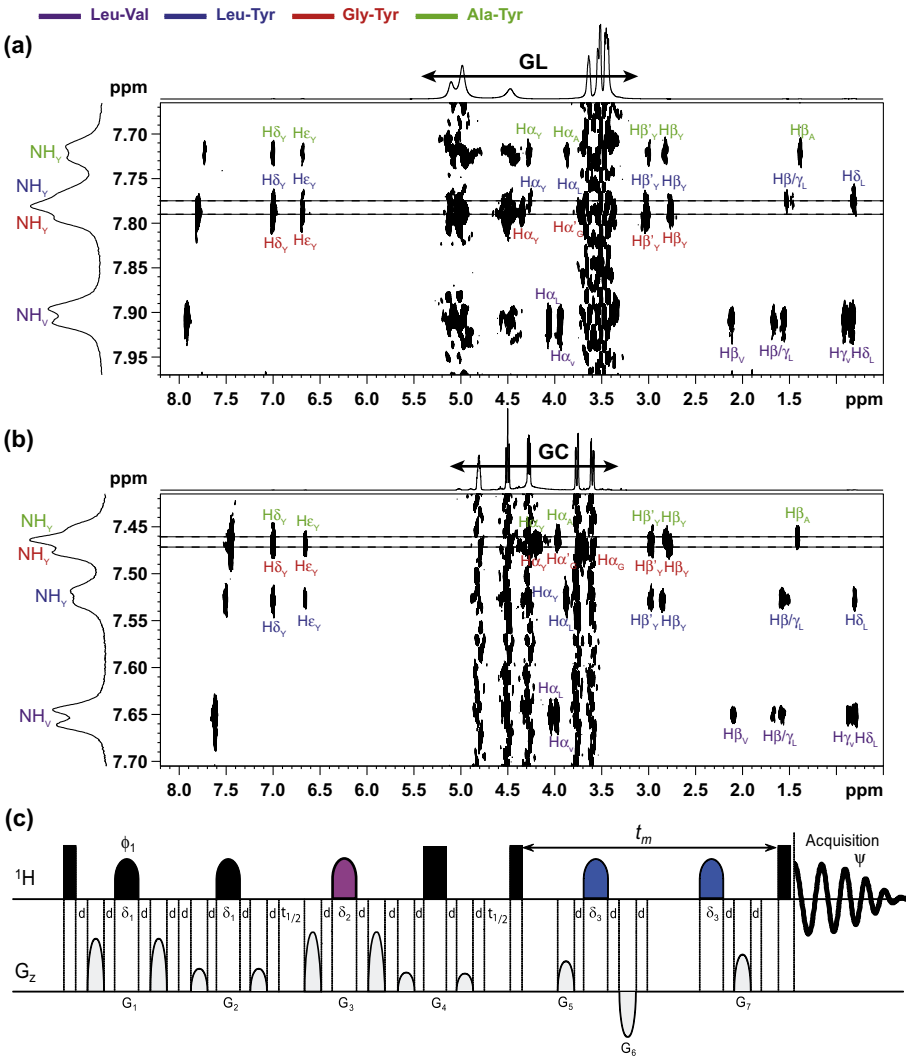

Fig. 5. F_1 band selective F_1 decoupled ^{1}H 2D NOESY spectrum of the dipeptide test mixture in GL (a, 256 scans per t_1 value, 318 K, 462.29 min) and in GC (b), 248 scans per t_1 value, 288 K, 447.84 min). (c) Pulse sequence. $\varphi_1 = x, y, -x, -y, \psi = x, -x$. The initial selective 180° pulses (δ_1 = 8 ms) had a Gaussian shape and were applied to the four NH amide proton resonances. G_1:G_2 = 70:30. The following refocusing pulse was also applied to the NH protons and was a RE-BURP (δ_2 = 30 ms). G_3:G_4 = 80:23. WURST wideband adiabatic inversion pulses, δ_3 = 1.5 ms, start within t_m (1 s) at 0.33 and 0.83 t_m with G_5:G_6:G_7 = 40:−60:50. Gradient pulse length was 3 ms, d = 200 μs. Relaxation delay was 2 s. Both data matrixes were acquired in the TPPI mode, their size was 32 × 2 k. Spectral widths were 4500 Hz in F_2 and 156 Hz in F_1. Both data matrixes were multiplied in both dimensions by a shifted sine bell function (SSB = 2) before zero filling to a 128 × 2 k size.

protons at 298 K and 328 K. The effect of spin diffusion was also less visible at 298 K towards the side chains protons (Hβ/γ/δ) and the Hα protons, due to transverse relaxation induced peak broadening. The temperature of 308 K could have been chosen as providing good resonance peak sharpness and efficient spin diffusion. However, at this temperature the two amides protons of Leu–Tyr and Gly–Tyr accidentally overlap. Consequently, all following NMR spectra in GL were recorded at 318 K.

In GC, the optimal temperature was found to be 288 K because the NH magnetization was weakly transferred to the tyrosine Hε protons at 298 K and only very weakly to the side chains protons (Hβ/γ/δ) at 278 K. Furthermore, the intra and intermolecular NOE cross peaks were sharper at 288 K than at 298 K, especially in the Hα proton region of band-selective detection 2D NOESY spectra.

The full band selective detection NOESY spectra of the dipeptide test mixture, when recorded in GL and GC at optimal temperature, are drawn in Figs. S3 and S4 (Supplementary Data). They show correlations from the Hβ of alanine and Hδ of leucine to the aromatic Hδ/ε of the neighboring tyrosine residue, and vice versa. These clearly originate from spin diffusion and are not visible when the spectra are recorded in water, using excitation sculpting for solvent signal suppression [28], as shown in Fig. S5 (Supplementary Data). The NH signals in Leu–Tyr, Gly–Tyr and Ala–Tyr only correlate with one of the two tyrosine Hβ protons and with none of the aromatic Hε protons. In addition, the NH signal in Leu–Tyr does not correlate with the Hδ terminal side chain protons of leucine (Fig. 3). It is also worth noting that at 298 K the four dipeptides rapidly reorient in water and therefore present a positive NOE regime (blue[1] correlation peaks in the usual NOESY spectrum), thus spin diffusion cannot be observed. The individualization of the four dipeptides in water would have required the simultaneous use of NOESY and COSY or TOCSY, according to the usual resonance assignment strategy [29].

The band selective detection 2D NOESY experiment showed only the Hα proton resonances in F_1 because they were eliminated in F_2 due to their proximity to the solvent signals. For analytical purposes, it appeared that detecting all the resonances of interest during signal acquisition would furnish supplementary structural information about particular mixture components. The 1D selective NOESY pulse sequence, when followed by an incremented

[1] For interpretation of color in Figs. 3–6, the reader is referred to the web version of this article

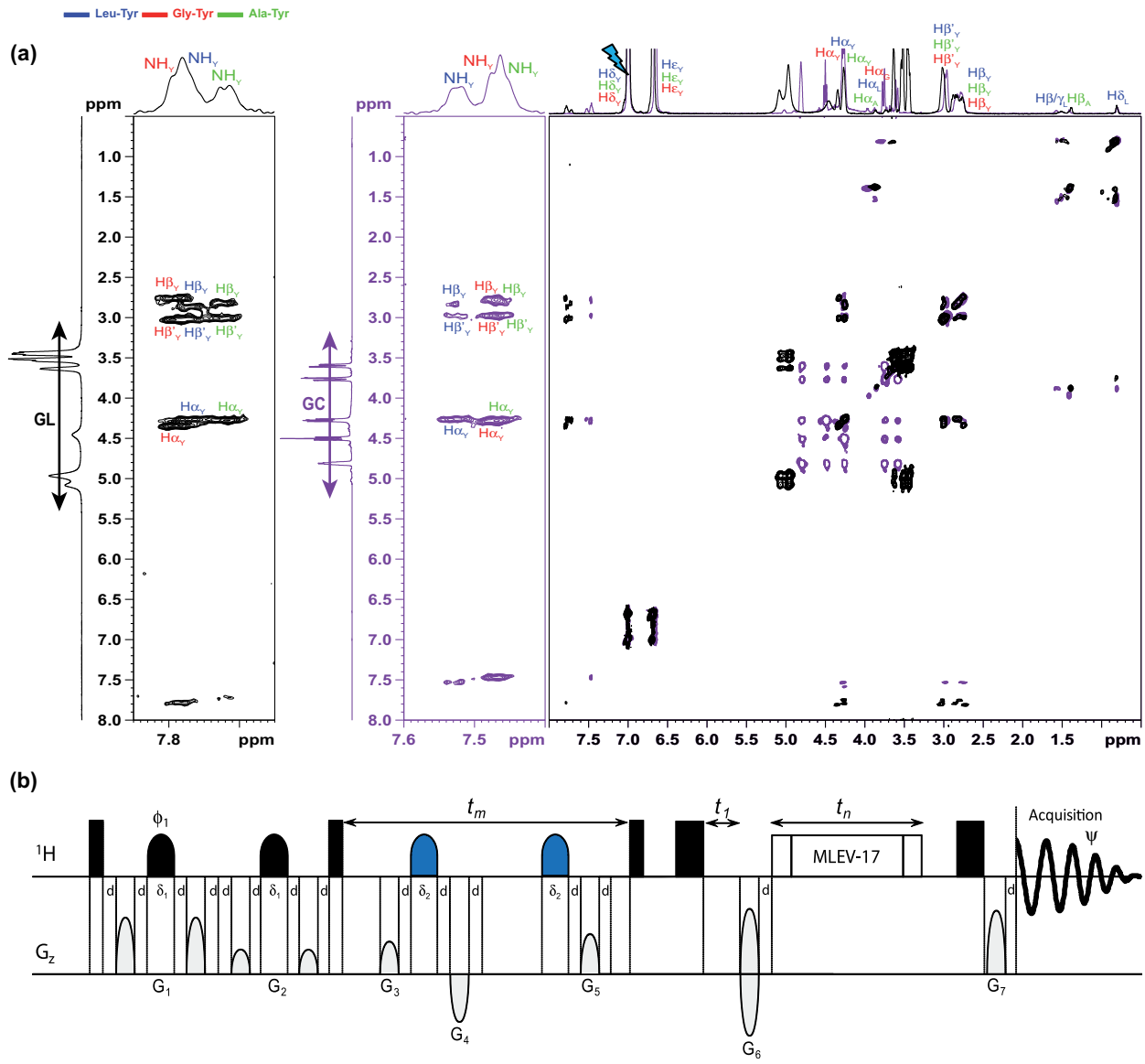

Fig. 6. Multiplet selective ^{1}H NOESY–TOCSY spectra of the dipeptide test mixture (a) in GL (in black, 318 K) and in GC (in purple, 288 K). (b) Pulse sequence: $\varphi_1 = x, y, -x, -y$, $\psi = x, -x$. The initial selective 180° pulses (δ_1 = 8 ms) have a Gaussian shape and were applied to the tyrosine superimposed Hδ$_Y$ resonances of Leu–Tyr, Gly–Tyr and Ala–Tyr. G_1:G_2 = 70:30. WURST wideband adiabatic inversion pulses, δ_2 = 1.5 ms, starting within t_m (1 s) at 0.33 and 0.83 t_m with G_3:G_4:G_5 = 40:−60:50. Gradient pulse length was 3 ms. d = 200 μs. Relaxation delay was 2 s. Isotropic mixing (MLEV-17, t_n = 100 ms) and trim pulses (2.5 ms) were emitted at a 8.3 kHz. Both data matrixes were acquired in echo/antiecho mode (G_6:G_7 = ±80:80), their size was 256 × 2 k with 64 scans per t_1 value. Their acquisition time was 927.48 min. Spectral widths were 4500 Hz in F_1 and F_2. Both data matrixes were multiplied in the two dimensions by a shifted sine bell function (SSB = 2) before zero filling to a 1 k × 2 k size. For clarity reasons, the solvent signal ridges were manually removed by means of an image processing tool; they were initially as intense as those in Fig. 5.

delay and an isotropic mixing period should deliver a 2D TOCSY spectrum for each mixture component. With the goal of directly observing the Hα proton resonances in peptides and of being able to go beyond the recording of simple 1D spectra of mixture components, four 1D NOESY spectra were first separately recorded on the dipeptide test mixture. Then, a F_1 band selective 2D NOESY spectrum was recorded in order benefit from the multiplex advantage of 2D NMR.

In the 1D selective NOESY experiment, a single spin is excited, its magnetization is further flipped to bring it to the $-z$ axis where it can spread by spin diffusion along the molecular proton network. The main difficulty here, that can be easily overcome, is to avoid the reintroduction of solvent signals at detection time [27]. The spectra in Fig. 2c,f and i shows that the selective excitation of an amide proton signal can be achieved with almost complete destruction of the solvent signals. The recording of 1D selective NOESY spectra brings a clear resolution improvement over the extraction of 1D slices through a 2D NOESY data set. The latter should be considered as a "pilot" spectrum for the identification of specific resonances for different compounds.

The 1D selective NOESY spectra in Fig. 4 show that all dipeptides were differentiated by spin diffusion in GL and GC, using an appropriate set of selectively excited resonances. Indeed, the selective excitation of the NH amide proton, at δ 7.90 in GL and at δ 7.65 in GC, caused a magnetization exchange exclusively with the protons of the Leu–Val dipeptide because the tyrosine Hδ/Hε proton resonances do not appear in the 1D NOESY spectra (Fig. 4a and e).

The selective excitation of the side chain Hδ and Hγ protons (between δ 0.7 and 1) made a magnetization exchange possible with all protons of the two Leu–Val and Leu–Tyr dipeptides (Fig. 4b and f). By comparison of the 1D NOESY spectra in Fig. 4a,e,b and f an almost complete proton assignment of

Leu–Tyr was possible. Only the $H\alpha_Y$ proton resonance of Leu–Tyr was masked by the GC proton signal.

The selective excitation of the aromatic Hδ/Hε protons of Leu–Tyr, Gly–Tyr and Ala–Tyr revealed all of the proton resonances of Leu–Tyr, Gly–Tyr and Ala–Tyr, with the exception of the tyrosine Hα proton in GC because it was hidden by the proton signals of GC (Fig. 4c and g).

In order to differentiate all resonances from Gly–Tyr and Ala–Tyr, one of the three tyrosine Hα protons at δ 4.34 in GL was selectively excited (Fig. 4d) along with the side chain Hβ proton of Ala–Tyr at δ 1.41 in GC (Fig. 4h). Fig. 4d shows the transfer of the tyrosine Hα magnetization over all protons of Gly–Tyr. Those of Leu–Tyr and Ala–Tyr are absent because the side chain proton ($H\beta_A/\beta_L/\gamma_L/\delta_L$) resonances are not present in the 1D NOESY spectrum. Fig. 4h shows the assignment of all proton resonances of Ala–Tyr, with the exception of the proton $H\alpha_Y$ resonance which was hidden by the GC proton signal.

The 1D selective NOESY pulse sequence in Fig. 4i started with a multiplet selective excitation block [27,30]. Two wideband inversion pulses were inserted during the mixing time. Their position had to be adjusted in order to minimize the resurrected GC and GL magnetization that arose from longitudinal relaxation.

The sequential recording of 1D NOESY spectra could have been sensitivity-optimized by Hadamard encoding and decoding [31]. An easier experimental strategy was to focus on the close NH amide resonances as an initial source of magnetization and to resort to a F_1 band selective F_1 decoupled 2D NOESY experiment [32,33]. This approach is feasible only because the selected nuclei have their resonances in the same frequency band and because they are not coupled together. The initial band selective excitation step, in Fig. 5, only concerned the amide proton region. Decoupling in the F_1 direction involved a selective echo followed by a non selective echo in the middle of t_1, both gradient enhanced. The NH protons were thus submitted to a zero degree pulse without any signal evolution through scalar coupling during t_1. The doublet amide proton signal thus collapsed into a singlet of higher intensity. Carrying out this experiment in GL and in GC made the assignment of nearly all proton resonances of Leu–Val, Leu–Tyr, Gly–Tyr and Ala–Tyr possible. The individualization of the four mixed dipeptides was obtained with a shortened overall acquisition time, in comparison with the four 1D NOESY experiments that were initially performed in order to obtain the same result.

The selective 1D and 2D NOESY experiments are only able to group together the resonances that belong to the same compound. This information may be sufficient for the identification of known molecules. However, the structure elucidation of individual mixture components, and even their simple identification, generally requires the acquisition of homonuclear and heteronuclear connectivity data as well. To address this situation, the multiplet selective 2D NOESY–TOCSY experiment was implemented. It was intended to identify the various spin systems that belong to a single mixture component. The superimposed $H\delta_Y$ proton resonances in Leu–Tyr, Gly–Tyr and Ala–Tyr were chosen as the starting point of the spin diffusion process. The resulting spectra, recorded in GL and in GC, are shown in Fig. 6. The 1D NOESY spectrum that was recorded from the same starting point, in Fig. 4c and g, did not provide a clear assignment of the tyrosine Hα resonances of Leu–Tyr, Gly–Tyr and Ala–Tyr in GC because they were hidden by the solvent signals. The 2D NOESY–TOCSY experiment, in Fig. 6a, explicitly displays the tyrosine Hα resonances between δ 4.20 and 4.35. This 2D spectrum also reveals the coupling between the leucine Hδ, Hβ/γ and Hα protons of Leu–Tyr and between the alanine Hα and Hβ protons of Ala–Tyr. Interestingly, the side chain protons of Leu–Tyr and Ala–Tyr, located far away from the selectively excited aromatic protons of tyrosine, were clearly differentiated.

The assignment of the tyrosine Hα and Hβ protons of Leu–Tyr, Gly–Tyr and Ala–Tyr and the assignment of side chain protons of Leu–Tyr and Ala–Tyr (Fig. 6a) were confirmed in GL as well.

3. Conclusions

Differentiation between organic compounds within mixtures by NMR spectroscopy in ultraviscous solvents is a pertinent alternative to the DOSY experiment, each technique having its own scope and limitations. Structure elucidation of molecules within complex mixtures is still a very difficult task. Spin diffusion and DOSY experiments are two ways to reach this goal and might also improve identification reliability of known molecules. In this context, GL and GC obviously appeared to be ultraviscous solvents of choice in the individualization of four structurally close dipeptides: Leu–Val, Leu–Tyr, Gly–Tyr and Ala–Tyr by using spin diffusion in 1D and 2D NOESY experiments. Selective excitation and detection were implemented to eliminate the intense proton signals of the non-deuterated solvents.

This article does not contain any comparison between GL and GC because they can be complementary, depending on the nature of the mixture components. However, GC shows the sharpest resonance lines in the narrowest frequency band. The studied dipeptides exhibit an adequate NOE regime at a lower temperature in GC than in GL, which might be an advantage for the former if the analytes are thermally unstable. The high dissolution power of these two solvents, and particularly of GC, is due to a very high dielectric constant; this opens the way to the study of a wide range of polar, biologically active classes of organic molecules. Other glycerol derivatives and other viscous polar solvents may also contribute to widen the range of compounds that may benefit from spin diffusion based mixture analysis. Studies are currently underway.

4. Experimental

D_2O was purchased from Eurisotop (Gif-sur-Yvette, France). Leu–Val, Leu–Tyr, Gly–Tyr and Ala–Tyr were purchased from TCI Europe (Zwijndrecht, Belgium). All peptides had 95% or higher purity and were dissolved at a concentration of 10 mM in GL/D_2O, GC/D_2O and H_2O/D_2O solvent systems (9:1, v/v). GC was given by ENSIACET (Toulouse, France) and GL purchased from Sigma–Aldrich (Lyon, France).

All NMR experiments were performed on a Bruker Avance AVIII-500 NMR spectrometer equipped with a 5 mm BBFO+ probe using the Bruker TOPSPIN Software (Rheinstetten, Germany). Static field gradient pulses were generated by a 10 A amplifier, so that the sample is submitted to a nominal 0.535 $T \cdot m^{-1}$ gradient. Gradient intensity values are hereafter reported in percents of this value. Gradient pulses were followed by a 200 μs recovery delay. Temperature control was performed using a Bruker variable temperature (BVT-2000) unit in combination with a Bruker cooling unit (BCU-05) to provide chilled air. Spectra were calibrated so that the tyrosine Hδ proton resonances appeared at δ 7.0. See figure captions for specific experimental details.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmr.2011.06.021.

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