Use of Diethanolamine as a Viscous Solvent for Mixture Analysis by Multidimensional Heteronuclear ViscY NMR Experiments

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Supporting Information Placeholder

ABSTRACT: The use of diethanolamine/DMSO-d_6 as viscous binary solvent is reported for the individualization of low-polarity mixture components by heteronuclear *ViscY* NMR experiments under spin diffusion conditions. Solvent viscosity induces the slowing down of molecular tumbling, hence promoting magnetization transfer by dipolar longitudinal cross-relaxation. As a result, all ^{1}H nuclei resonances within the same molecule may correlate in a 2D NOESY spectrum, giving access to mixture analysis. We state the individualization of four low-polarity chemical compounds dissolved in diethanolamine/DMSO-d_6 solvent blend using homonuclear selective 1D and 2D ^{1}H and ^{19}F NOESY and HOESY experiments and heteronuclear 2D ^{1}H-^{19}F, ^{1}H-^{31}P HSQC-NOESY and 3D ^{1}H-^{19}F-^{1}H, ^{1}H-^{31}P-^{1}H HSQC-NOESY experiments by taking profit from spin diffusion.

Identification of organic molecules within complex mixtures is still a crucial issue in the cosmetics, fine chemical, and pharmaceutical industries. Inventive and affordable solutions in conformity with regulations remain to be discovered for the structural elucidation of mixed small compounds, coming from either by chemical synthesis or by extraction from natural or biotechnological resources. Usually, concomitant chromatography and NMR spectroscopy is performed. [1-4] However, physical separation of mixture components is not every time functional and may turn out to be too long and thus too expensive to implement. In this context, to consider NMR without prior physical separation reveals to be a relevant alternative. Only a few approaches have been explored to date, such as diffusion-ordered spectroscopy (DOSY), assisted or not by matrix effect, [5-8] multiquantum spectroscopy combined (or not) with broadband homonuclear decoupling, sparse sampling, pure shift, ultrafast data acquisition, multiplet selective excitation, or tensor decomposition methods. [9-15] Original signal processing algorithms have also been proposed to resolve individual components of mixture spectra.[8, 16, 17]

The use of viscous solvents has initiated the way to alternative NMR approaches for mixture analysis that depend on the tailoring of molecular dynamics of the dissolved molecules. Indeed, the tumbling rate of small and medium-sized molecules can be lowered in aqueous and organic viscous solvents [18-32] since the value of the overall correlation time τ_c is governed by the medium viscosity according to the microviscosity theory of Gierer and Wirtz. [33] As a result, the analytes unveil a negative NOE regime, and their resonances can be clustered according to their ability to exchange magnetization through intramolecular spin diffusion. The 2D NOESY spectrum of a mixture reveals correlations between all the proton resonances of each mixed molecule when recorded in spin diffusion conditions, thus permitting to tag each resonance in the 1D mixture spectrum according to the compound it comes from. The 2D NOESY spectrum is not acquired to reach the 3D structure of a neat compound but to access the individual spectra of compounds within a mixture. In 2008, Simpson and coworkers *et al.* first reported the use of a highly viscous solvent such as CTFEP for organic mixture separation using 1D and 2D NOESY spectroscopy. [23] The use of supercooled water (in narrow capillaries) was published in 2012 by the same team for mixture individualization of small metabolites. [25] Our team described innovative outcomes in mixture analysis by NMR under spin diffusion conditions (*ViscY* experiments) [30] using glycerol and glycerol carbonate (in 2011), [24] DMSO/glycerol (in 2016), [26] DMSO/water (in 2017), [27] sucrose solution, agarose gel (in 2019), [28] sulfolane-based solvents (in 2020) [30] and phosphoric acid solutions (2021). [31]

So far, low-polarity molecules within mixtures have been only explored in a small number of viscous solvents. In this context, diethanolamine was prepared by dilution with DMSO-d_6, thus providing a solvent blend. For method assessment, one mixture made of four low-polarity heteronuclei-containing compounds were studied by *ViscY* experiments, including homonuclear selective 1D and 2D ^{1}H and ^{19}F experiments and heteronuclear 2D ^{1}H-^{13}C, ^{1}H-^{19}F, ^{1}H-^{31}P HSQC-NOESY and 3D ^{1}H-^{19}F-^{1}H, ^{1}H-^{31}P-^{1}H HSQC-NOESY experiments.

Among amino alcohols group, ethanolamines have been synthesized industrially since the 1930s then large-scale produced after 1945, when alkoxylation with ethylene oxide substituted the previous chlorohydrin way. In industry, ethanolamines are employed extensively in the production of emulsifiers, detergent raw materials, textile medical; in gas purification; in cement production, as milling additives; and as building blocks for agrochemicals. [34] Amid ethanolamines, diethanolamine (DEA) is a white crystalline solid at room temperature but tends to absorb water, carbon dioxide from the air and to supercool. As a result, it is often met as a colorless, viscous liquid. It presents a good dielectric constant (ε = 24.69 at 303.15 K) [35] and dipole moment (μ = 2.81 D). [36] Hence, it is completely miscible with water and other polar organic solvents. Although DEA unveils a higher melting point (300.9 K) [36] than DMSO (292 K), [37] its melting point can be lowered by the addition of DMSO. For instance, the melting point of the DEA/DMSO-d_6 solvent blend reaches 258 K after adding 40% of DMSO-d_6 by volume. This outcome opens a route to work at or below room temperature, which is particularly appropriate for thermosensitive compounds. Spin diffusion may therefore take place on a wide range of temperatures, from room to sub-zero temperatures due to the viscosity increase of the binary solvent upon temperature. [38] Nevertheless, the viscosity of the DEA-based solvent at room temperature remains sufficiently low, even if pure DEA reveals a higher viscosity (η = 566,3 cP at 298 K) [39] than

one of DMSO-d_6 (η = 2.007 cP at 298 K), [40] so that samples are prepared and moved into the NMR tube without any trouble in opposition to highly viscous solvents such as glycerol (η = 934 cP at 298 K). [41] However, the main experimental pitfall of considering non-deuterated DEA is mandatory suppression of strong residual ^{1}H resonances. Nonetheless, this is managed *via* selective pulses involved in a double pulsed field gradient spin echo (DPFGSE) sequence. [42] Furthermore, the high volume of added DMSO-d_6 allows the employ of automatic spectrometer tools such as field-locking and shimming.

The choice of an optimal operating temperature is based on a compromise between overall spectral resolution and intensity of NOE cross-peaks between protons that are not nearby enough to present a NOE signal in a low-viscosity solvent. A temperature decrease promotes spin diffusion but modifies peak height through line widening due to a more active T_2 transverse relaxation. Sample cooling is thus necessary if the NOESY spectrum reveals positive NOE correlations (diagonal and off-diagonal cross-peaks of opposite signs). As regards the complexity of the mixtures, the analysis of ^{1}H NMR spectra may become intractable due to massive ^{1}H resonance overlapping. A traditional alternative solution consists of the spreading of the spectroscopic information along with a second and sometimes a third dimension that encodes chemical shifts of nuclei other than ^{1}H. [23, 26-28, 30-32, 43] This method to mixture analysis is exemplified in the viscous binary DEA/DMSO-d_6 solvent using the 2D ^{1}H-^{19}F and ^{1}H-^{31}P HSQC-NOESY and 3D ^{1}H-^{19}F-^{1}H, ^{1}H-^{31}P-^{1}H HSQC-NOESY experiments providing ^{1}H/^{19}F/^{31}P chemical shift lists for the mixture of four low polarity fluorine- and phosphorus-based compounds (see Figure 1).

EXPERIMENTAL SECTION

Chemical reagents. DMSO-d_6 was bought from Eurisotop (Gif-surYvette, France). Diethanolamine (DEA) was purchased from Acros Organics (Geel, Belgium). *N*-[5-(diphenylphosphinoylmethyl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl]-*N*-methylmethanesulfonamide, diethyl 4-fluorobenzylphosphonate, diethyl 2-fluorobenzylphosphonate and diethyl ((5-(3-fluorophenyl)pyridin-2-yl)methyl)phosphonate were purchased from ABCR (Karlsruhe, Germany). All compounds had 95% or higher purity and were dissolved at a concentration of 20 mM in DEA/DMSO-d_6 (6:4, v/v).

NMR Spectroscopy. All NMR experiments on the fluorine- and phosphorus-based compound test mixture were performed on a Bruker AVIII500 NMR spectrometer equipped with a 5 mm triple resonance TBO probe using the TOPSPIN software (Rheinstetten, Germany). Gradient pulses (maximum 0.535 Tm^{-1}) were generated by a 10 A amplifier. Temperature was controlled by a Bruker variable temperature (BVT) unit supplied with chilled air produced by a Bruker cooling unit.

All spectra of fluorine- and phosphorus-containing compounds were referenced so that the residual proton signal of DMSO-d_6 was observed at 2.50 ppm. Additional NMR data acquisition and processing parameters for Fig. 2-9 are reported in the Supporting Information (SI) file.

RESULTS AND DISCUSSION

Homonuclear *ViscY* NMR experiments

The fluorine- and phosphorus-containing compound test mixture (Figure 1) does not reveal an obvious differentiation in the DOSY spectrum (Figure S1) when dissolved in pure DMSO-d_6, especially, for 1b and 1c due to their analogous molecular weight and shape. The use of viscous DEA-based solvent such as DEA/DMSO-d_6 has unlocked the way to alternative strategies than DOSY, called *ViscY*, based on homo- and heteronuclear NOESY-based spin diffusion experiments for mixture analysis. The major experimental drawback of our analytical solution is the compulsory suppression of the strong residual ^{1}H signals of DEA (Figure 2a) for preventing obscure analyte signals since deuterated DEA would be too expensive to produce. The elimination of solvent signals was obtained using multiple presaturation selective pulses when involved in an excitation sculpting sequence (Figures 2b and 2e) [42] or using inversion selective pulses in a DPFGSE sequence (Figures 2c and 2f). [44, 45]

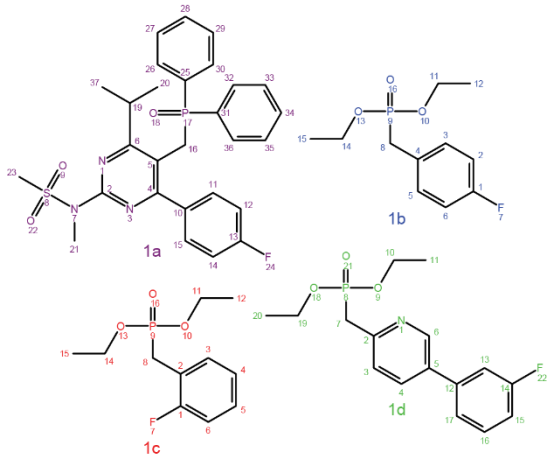

Figure 1. Chemical structures of the four fluorine- and phosphorus-containing compounds within mixture, 1a: *N*-[5-(diphenylphosphinoylmethyl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl]-*N*-methylmethanesulfonamide, 1b: Diethyl 4-fluorobenzylphosphonate, 1c: Diethyl 2-fluorobenzylphosphonate and 1d: Diethyl ((5-(3-fluorophenyl)pyridin-2-yl)methyl)phosphonate.

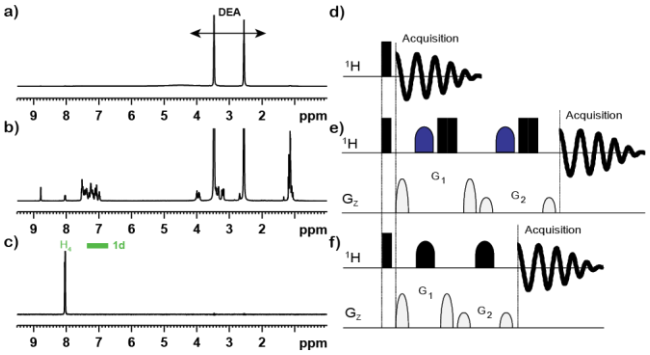

Figure 2. 1D proton spectra and corresponding NMR pulse sequence of the fluorine- and phosphorus-based compound test mixture (1a, 1b, 1c and 1d, 20 mM) dissolved in DEA/DMSO-d_6 (6:4, v/v), at 278 K, at 500 MHz (^{1}H). a, d) Non-selective excitation and detection. b, e) DEA suppression by excitation sculpting sequence using a pair of multiple presaturation selective pulses applied on both residual DEA signal resonances. c, f) Selective excitation of the aromatic proton H_4 of 1d using a 30 ms, 1% truncated, 180° Gaussian pulse.

The operating temperature is a critical factor in *ViscY* experiments owing to its immediate influence on solvent viscosity and thus on overall rotational correlation times τ_C. [23, 24, 32, 33] As a result, we have determined the optimized temperature at which NOE cross-peaks were positive (negative NOE enhancements, slow-motion regime, see Figure 3a), well-resolved, and as intense as possible between protons not supposed to be near enough to present a NOE in low-viscous medium. The optimal temperature of 278 K has been chosen *via* 2D NOESY experiments (see Fig-

ure 3, full NOESY spectrum in Figure S3 at 278 K and Figures S2a-e: NOESY spectra at 298, 288, 278, 268 and 258 K in SI). The use of viscous DEA/DMSO-d_6 solvent mixture allows complete intramolecular magnetization exchange by spin diffusion, detected over distances of > 15 Å within all fluorine- and phosphorus-based compounds. In contrast, the NOESY spectrum acquired in neat DMSO-d_6 at 298 K presents fewer NOE cross-peaks, all of the reverse sign (positive NOE enhancements, fast motion regime, see full NOESY spectrum in Figure S4 in SI). Consequently, the grouping of proton resonances is achievable under *ViscY* conditions, making the individualization of the mixture compounds possible. To reach the same result in DMSO-d_6 would have required additional experiments.

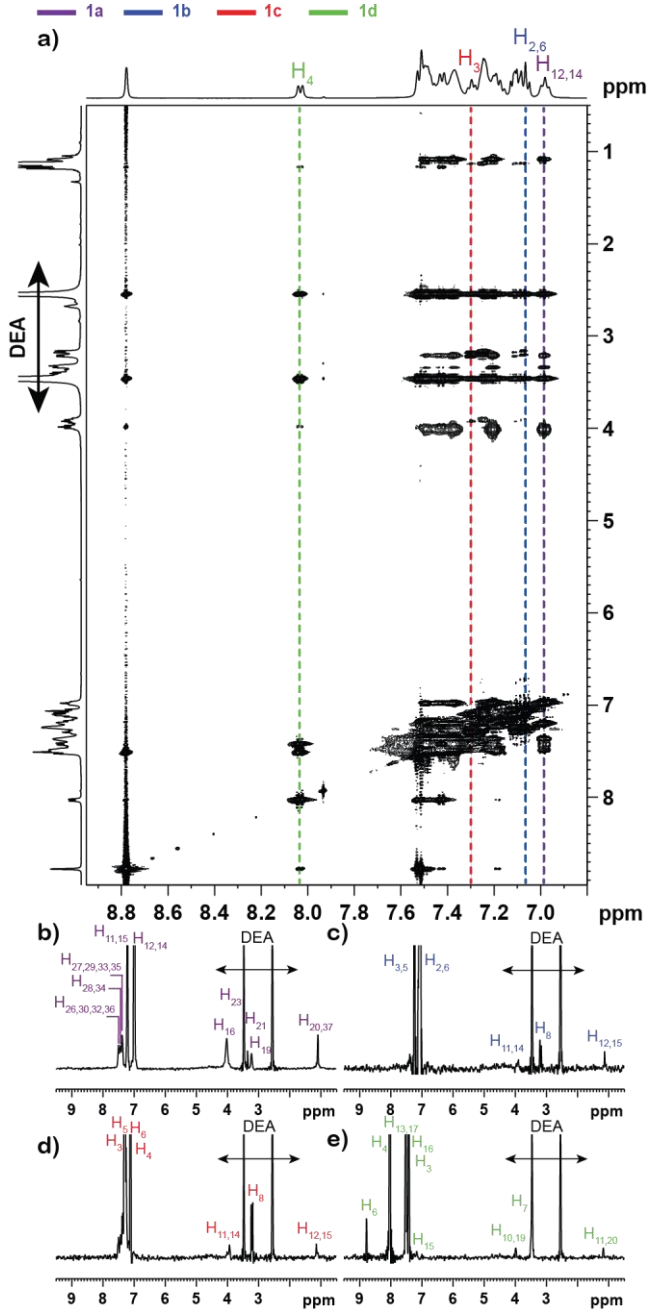

Figure 3. a) Low-field proton region of the 2D NOESY spectrum of the fluorine- and phosphorus-based compound test mixture (1a, 1b, 1c and 1d, 20 mM) dissolved in DEA/DMSO-d_6 (6:4, v/v), t_m = 0.5 s, at 278 K, at 500 MHz (^{1}H). ^{1}H vertical slices extracted from the 2D ^{1}H NOESY at 6.98 ppm (b, $H_{12,14}$(1a), purple dotted line), at 7.07 ppm (c, $H_{2,6}$(1b), blue dotted line), at 7.30 ppm (d, H_3(1c), red dotted line), and at 8.04 ppm (e, H_4(1d), green dotted line).

Owing to resonance peaks overlapping in the 1D and 2D spectra of the four fluorine- and phosphorus-containing molecules within mixture, it has been somewhat difficult to unambiguously assign each proton resonance to a specific compound. In this framework, detecting only the resonances of interest during signal acquisition has addressed this issue by selectively exciting a suitable set of proton resonances in 1D selective NOESY experiments.

During the 1D selective NOESY experiment, the magnetization of the single spin selectively excited is tipped to the *z*-axis where it can spread along with the molecular proton network by spin diffusion. The 1D selective NOESY pulse sequence begins with a multiplet selective excitation block (Figure 4e). [44, 45] Two wide-band inversion pulses have been incorporated during the mixing time to thwart the recovery of the intense DEA signal during the acquisition time that originates from longitudinal relaxation.[45]

To select an appropriate set of selectively excited proton resonances allows obtaining the individual 1D ^{1}H spectrum of each mixed molecule in the viscous DEA/DMSO-d_6 (6:4, v/v) binary solvent by taking benefit from spin diffusion. Figure 4 shows the selective excitation 1D NOESY spectrum of each mixed fluorine- and phosphorus-based compound. In particular, the individualization of 1a is achieved by the selective excitation of the aromatic protons $H_{12,14}$ at 6.98 ppm. Interestingly, spin diffusion can connect signals from the four aromatic moieties of 1a (Figure 4a). All proton resonances of 1b are observed using the selective excitation of the aromatic protons $H_{2,6}$ at 7.07 ppm (Figure 4b). Selective excitations of the aromatic protons H_3 at 7.30 ppm and H_4 at 8.04 ppm make it possible respectively to gather all proton resonances of 1c and 1d (Figures 4c and d).

Heteronuclear *ViscY* NMR experiments

The multiplet selective excitation 1D ^{1}H NOESY experiment has demonstrated that grouping proton resonances belonging to the same compound within mixture thus allowing its individualization is possible by taking profit from spin diffusion. However, in the study of other more complex mixtures, ^{1}H spectral overlap may happen. As a result, compounds of interest may not present resolved proton resonances, thus preventing the use of 1D selective ^{1}H NOESY experiments. In this context, the simplification of mixture analysis by means of heteronuclear chemical shift resonance labelling has been extended to ^{19}F NMR spectroscopy since fluorine is a 100% natural abundant and mono-valent nucleus offering a wider chemical shift range than ^{1}H nucleus that may facilitate the individualization of fluorinated molecules within mixtures.[46, 47] This nucleus can be integrated into the spin network in which magnetization is transferred by longitudinal cross relaxation, under spin diffusion conditions. This opens the route to the observation of ^{1}H-^{19}F spin diffusion through the HOESY experiment.

The first step has also been to set the temperature that provides the best compromise between spin diffusion and spectral resolution. Figure S3 shows the ^{1}H-^{19}F HOESY spectra from 298 K to 258 K with DEA suppression using excitation sculpting.[42] At 278 K, the magnetization exchange from each ^{19}F nucleus to the proton network is optimal for every molecule, however, it remains partial. Spin diffusion is logically active in aromatic moieties wearing ^{19}F nuclei, less active in distant molecule parts such as ethoxy, isopropyl or CH_2-P moieties (Figure 5a). By extracting an appropriate set of rows from each ^{19}F frequency, the individual ^{1}H spectrum of every compound is almost obtained (Figures 5b, c, d and e). In the study of complex mixtures of fluorinated compounds, structure elucidation may turn out challenging due to too much ^{1}H resonance overlapping. Selectively exciting a single

appropriate ^{19}F resonance may be a remedy to this issue. Figure 6 shows how each selectively excited ^{19}F nucleus at -112.65, -116.31, -117.26 and -112.42 ppm is able to transfer its magnetization over nearly all protons of each molecule, except those from faraway and flexible moieties (ethoxy, isopropyl or CH_2-P).

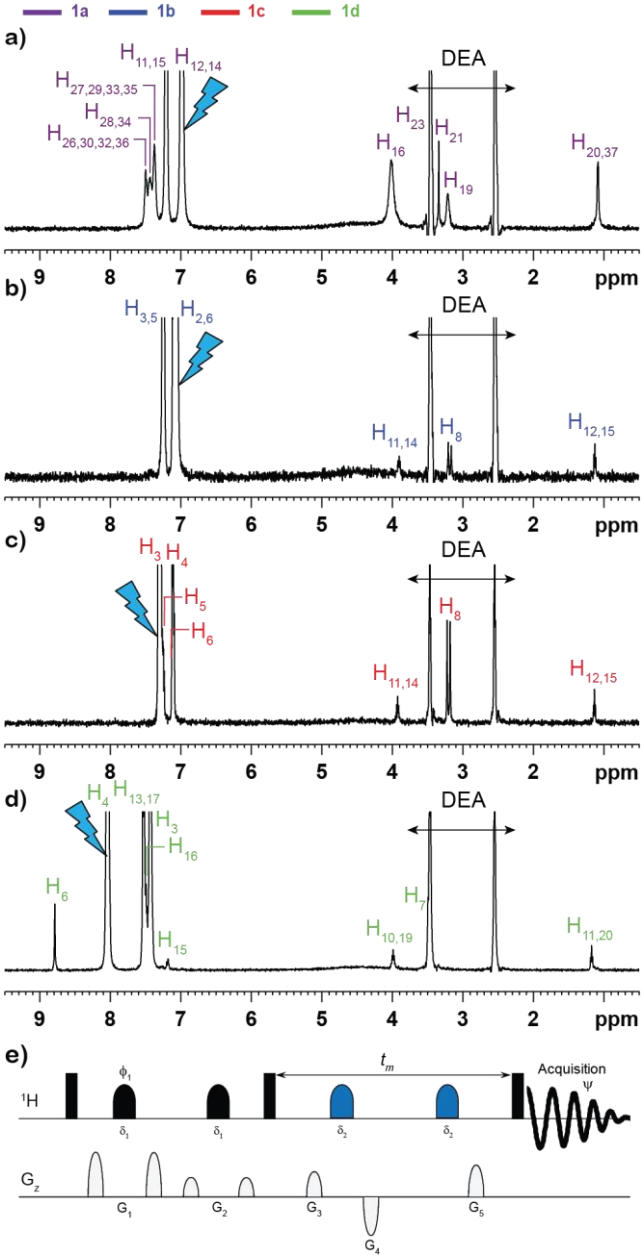

Figure 4. Multiplet selective excitation 1D ^{1}H NOESY spectra of the fluorine- and phosphorus-based compound test mixture (1a, 1b, 1c and 1d, 20 mM) dissolved in DEA/DMSO-d_6 (6:4, v/v), t_m = 0.5 s, at 278 K, at 500 MHz (^{1}H). The initial selective inversion pulses excite: a) $H_{12,14}$(1a), b) $H_{2,6}$(1b), c) H_3(1c) and d) H_4(1d) proton resonances. e) Pulse sequence: φ_1 = *x, y, -x, -y,* ψ = *x, -x.*

It appeared that spin diffusion involving only protons was more efficient than that involving fluorine and proton nuclei for every mixed molecule in DEA/DMSO-d_6 because of the distant location of each fluorine from the rest of the proton network and the internal flexibility of ethoxy, isopropyl and CH_2-P moieties. In this framework, to take advantage of the broader chemical shift dispersion of ^{19}F and the spin diffusion efficiency of ^1H, we have considered the HSQC-NOESY experiment since the existence of at least one $^3J^{19}F$-^1H (or $^4J^{19}F$-^1H) coupling constant for each mixed molecule. A complete proton spectrum should be obtained for each component within mixture starting only from one single fluorine resonance in the indirect dimension. [23, 26-28]

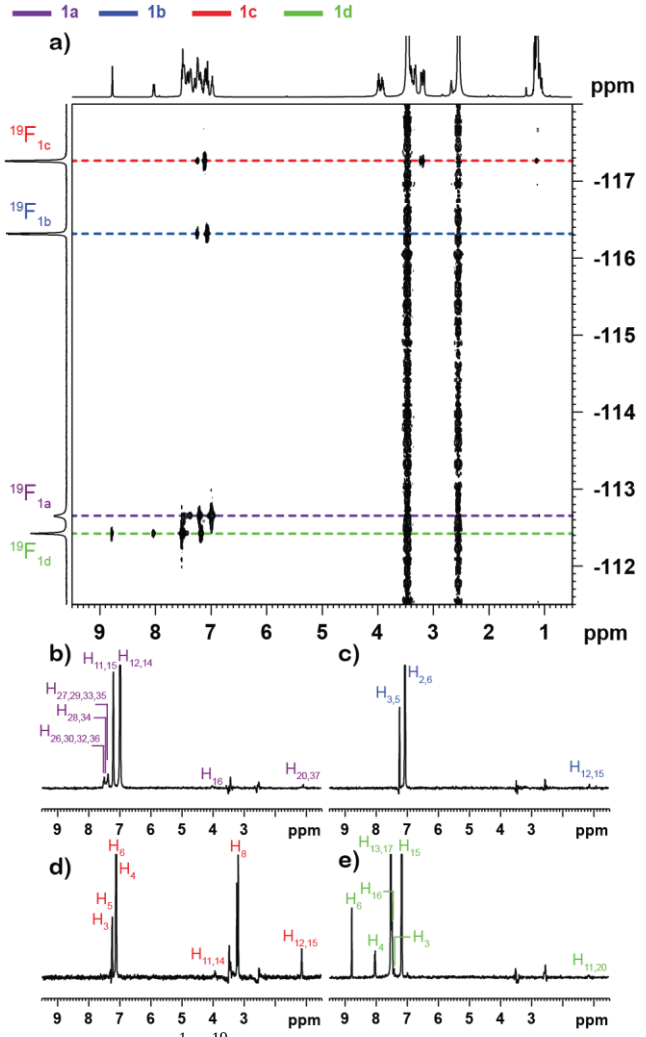

Figure 5. a) 2D ^{1}H-^{19}F HOESY spectrum of the fluorine- and phosphorus-based compound test mixture (1a, 1b, 1c and 1d, 20 mM) dissolved in DEA/DMSO-d_6 (6:4, v/v), t_m = 0.5 s, at 278 K, at 500 MHz (^{1}H). ^{1}H horizontal slices extracted from the 2D ^{1}H-^{19}F HOESY at -112.65 ppm (b, ^{19}F(1a), purple dotted line), at -116.31 ppm (c, ^{19}F(1b), blue dotted line), at -117.26 ppm (d, ^{19}F(1c), red dotted line), and at -112.42 ppm (e, ^{19}F(1d), green dotted line).

The 2D ^{1}H-^{19}F HSQC-NOESY spectrum of the fluorine- and phosphorus-based compound test mixture dissolved in DEA/DMSO-d_6 (6:4, v/v) solvent blend recorded at 278 K is drawn in Figure 7. Under these *ViscY* operating conditions, all the protons of each molecule can correlate with all other protons by spin diffusion after having marked the fluorine chemical shift in F_1, except for the flexible ethoxy moieties of 1d. The selection of four slices through ^{19}F resonances at -112.65 ppm (Figure 7b, purple dotted line), -116.31 ppm (Figure 7c, blue dotted line), at -117.26 ppm (Figure 7d, red dotted line), and at -112.42 ppm (Figure 7e, green dotted line) permits to produce, respectively, complete proton spectra corresponding to 1a, 1b, 1c and nearly-one corresponding to 1d. We have demonstrated the capability to individualize fluorine- and phosphorous-containing compounds within mixture by means of the 2D ^{1}H-^{19}F HSQC-NOESY experiment under *ViscY* conditions.

Nonetheless, to consider another heteronucleus than fluorine presenting a broad chemical shift range such as phosphorus may turn out to be a complementary and pertinent alternative in *ViscY* experiments to reach the individual spectrum of fluorine- and phosphorus-based molecules in a mixture, since phosphorus reveals also a 100% natural abundance and presents sharp NMR peaks. However, on the contrary of ^{19}F nucleus which has a magnetogyric ratio close to the one of ^{1}H, the ^{31}P nucleus reveals positive HOE (heteronuclear NOE) that tends towards zero in the spin diffusion limit. [48] Consequently, phosphorus-based compounds will never produce negative HOE when long molecular overall correlation times are observed. A workaround to this experimental issue is to consider ^{31}P nuclei as chemical shift markers and to induce magnetization transfer along the intramolecular proton network using the 2D ^{1}H-^{31}P HSQC-NOESY experiment.

We report in Figure 8 the individualization of the four fluorine- and phosphorus-containing compounds within mixture dissolved in the DEA/DMSO-d_6 (6:4, v/v) binary solvent by the 2D ^{1}H–^{31}P HSQC-NOESY spectrum recorded at 278 K, since the presence of at least one $^{2}J^{31}$P-^{1}H (or $^{3}J^{31}$P-^{1}H) coupling constant for each mixed molecule. All the protons of each compound can correlate with all other protons by spin diffusion after having marked the phosphorus chemical shift in F_1. An appropriate selection of horizontal slices through phosphorus resonances at 27.32, 26.91 ppm, 25.63 ppm and at 25.48 ppm enables the extraction of the four complete proton spectra respectively corresponding to 1a, 1b, 1c and 1d. We have established one more time the ability to extract all the proton chemical shifts for individual fluorine- and phosphorus-based components in a mixture under *ViscY* conditions. That may prove to be a useful tool in the structure assignment of phosphorus-based molecules within more complex mixtures.

Depending on the complexity of the mixtures, the analysis of ^{1}H NMR spectra may become problematic due to the overlapping of ^{1}H resonances. A common remedy to this difficulty consists in the spreading of the spectroscopic information along a second axis that encodes chemical shifts of nuclei other than ^{1}H. We have reported in this work the use of 2D ^{1}H-^{19}F and ^{1}H-^{31}P HSQC-NOESY experiments under spin diffusion conditions for providing ^{1}H, ^{19}F and ^{31}P chemical shift lists for all mixture components.[26] However, resorting to a second dimension may not be sufficient for the individualization of complex mixture constituents and considering a third dimension may turn out to be a pertinent alternative in a reasonable acquisition time using a sparse sampling method. In this framework, the 3D ^{1}H-^{19}F-^{1}H and ^{1}H-^{31}P-^{1}H HSQC-NOESY spectra of the fluorine- and phosphorus-based compound test mixture dissolved in DEA/DMSO-d_6 (6:4, v/v) solvent blend has been recorded at 278 K.

Under *ViscY* operating conditions, from the 3D ^{1}H-^{19}F-^{1}H HSQC-NOESY spectrum (Figure S6 in SI), the selection of four 2D slices (^{1}H F_2 and F_3 dimensions) through ^{19}F resonances (F_1 dimension) at -112.65 ppm (Figure S6b), -116.31 ppm (Figure S6c), at -117.26 ppm (Figure S6j), and at -112.42 ppm (Figure S6k) permits to generate partially, respectively, 2D NOESY spectra corresponding to 1a, 1b, 1c and 1d. The extraction of four 1D slices from these 2D NOESY spectra through ^{1}H resonances at 6.98 ppm (Figure S6d, purple dotted line), 7.25 ppm (Figure S6e, blue dotted line), at 7.25 ppm (Figure S6l, red dotted line), and at 7.53 ppm (Figure S6m, green dotted line) reveals incomplete proton spectrum for every mixture test component. However, the positive sum of all 1D slices along F_2 produces a complete ^{1}H spectrum allowing the individualization of each mixing compound after taking benefit from the third ^{19}F dimension.

In a similar way, from the 3D ^{1}H-^{31}P-^{1}H HSQC-NOESY spectrum (Figure 9), the extraction of four 2D slices (^{1}H F_2 and F_3 dimensions) through ^{31}P resonances (F_1 dimension) at -112.65 ppm (Figure 9b), -116.31 ppm (Figure 9c), at -117.26 ppm (Figure 9j), and at -112.42 ppm (Figure 9k) permits to create partly, respectively, 2D NOESY spectra corresponding to 1a, 1b, 1c and 1d. The selection of four 1D slices from these 2D NOESY spectra through ^{1}H resonances at 7.37 ppm (Figure 9d, purple dotted line), 7.25 ppm (Figure 9e, blue dotted line), at 3.20 ppm (Figure 9l, red dotted line), and at 3.46 ppm (Figure 9m, green dotted line) reveals the individual proton spectrum for every mixture test component (except for 1a). Nonetheless, the positive-sum of every slice along F_2 gives four entire ^{1}H spectra (Figures 9f,g,n,p) also permitting the individualization of each mixing molecule after taking profit from the third ^{31}P dimension.

We have also demonstrated the capability to individualize fluorine- and phosphorous-containing compounds within mixture by means of the 3D ^{1}H-^{19}F-^{1}H and ^{1}H-^{31}P-^{1}H HSQC-NOESY experiments. That may prove to be a useful 3D NMR tool in the structure assignment of molecules within more complex mixtures under *ViscY* conditions.

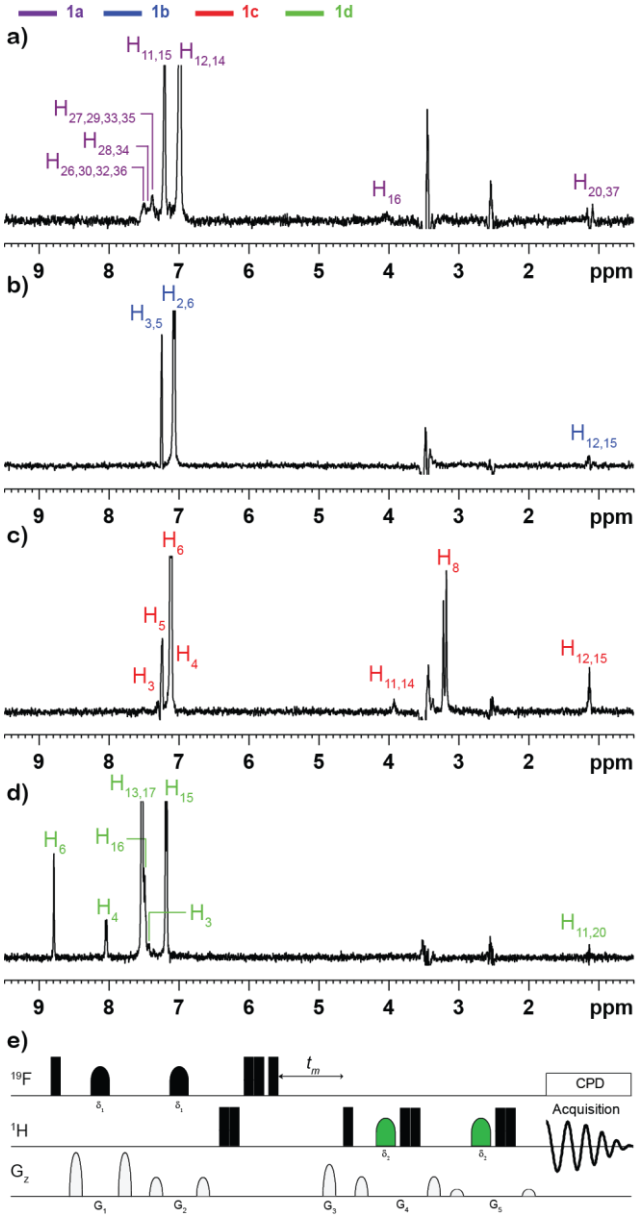

Figure 6. Multiplet selective excitation 1D ^{1}H-^{19}F HOESY spectra of the fluorine- and phosphorus-based compound test mixture (1a,

1b, 1c and 1d, 20 mM) dissolved in DEA/DMSO-d_6 (6:4, v/v), t_m = 0.5 s, at 278 K, at 500 MHz (^{1}H). The initial selective inversion pulses excite the fluorine resonance, at a) -112.65, b) -116.31, c) -117.26 and d) -112.42 ppm. e) Pulse sequence.

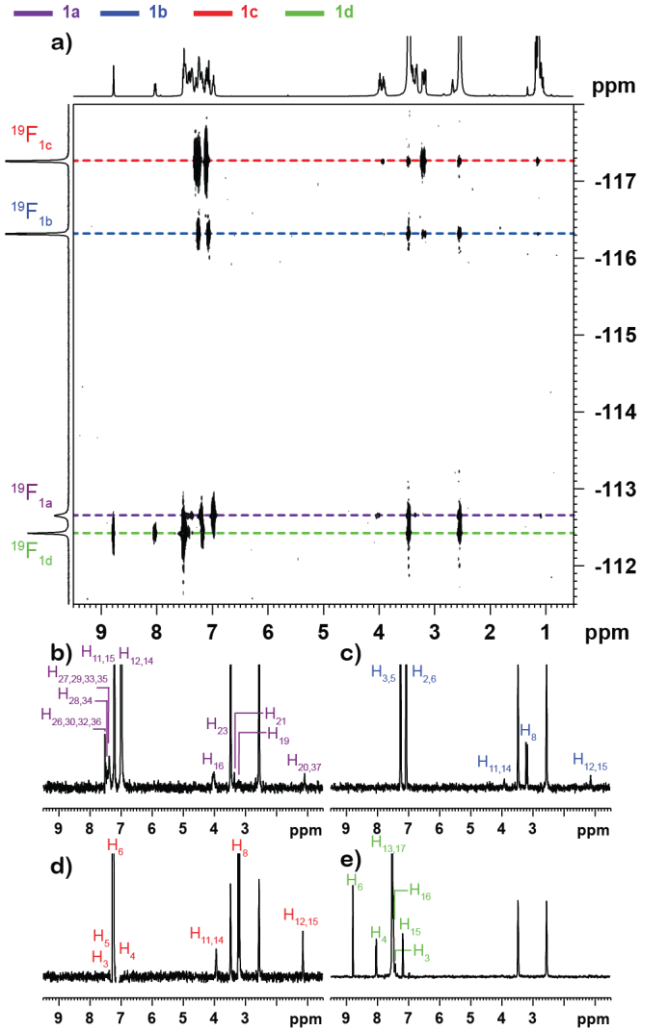

Figure 7. a) 2D ^{1}H-^{19}F HSQC-NOESY spectrum of the fluorine- and phosphorus-based compound test mixture (1a, 1b, 1c and 1d, 20 mM) dissolved in DEA/DMSO-d_6 (6:4, v/v), at 278 K, at 500 MHz (^{1}H). ^{1}H horizontal slices extracted from the 2D ^{1}H-^{19}F HSQC-NOESY at -112.65 ppm (b, ^{19}F(1a), purple dotted line), at -116.31 ppm (c, ^{19}F(1b), blue dotted line), at -117.26 ppm (d, ^{19}F(1c), red dotted line), and at -112.42 ppm (e, ^{19}F(1d), green dotted line).

CONCLUSIONS

Till now, small, and low-polarity compounds within complex mixtures have been only studied in a tiny number of viscous solvents. In this framework, we have proved that the use of DEA/DMSO-d_6 as a viscous binary solvent allows the individualization of four mixed low-polarity constituents, by taking advantage of NMR spin diffusion using heteronuclear *ViscY* experiments.

The component individualization within the *N*-[5-(diphenylphosphinoylmethyl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl]-*N*-methylmethanesulfonamide, Diethyl 4-fluorobenzylphosphonate, diethyl 2-fluorobenzylphosphonate and diethyl ((5-(3-fluorophenyl)pyridin-2-yl)methyl)phosphonate mixture in DEA/DMSO-d_6 (6:4, v/v) solvent has been accomplished at 278 K by means of homonuclear selective 1D and 2D ^{1}H and ^{19}F NOESY and HOESY experiments and heteronuclear 2D ^{1}H-^{19}F, ^{1}H-^{31}P HSQC-NOESY and 3D ^{1}H-^{19}F-^{1}H, ^{1}H-^{31}P-^{1}H HSQC-NOESY experiments.

We have highlighted that the viscous solvent blend DEA/DMSO-d_6 present valuable advantages compared to other viscous solvents previously described. [24] DEA/DMSO-d_6 blend reveals a low viscosity at room temperature so that NMR samples are prepared and transferred into NMR sample tube without any trouble, contrarily to other solvent blends based on glycerol or glycerol carbonate. [24] Adding DMSO-d_6 also opens the way of working at or below room temperature, which is especially appropriate for thermosensitive compounds since the freezing point of the solvent blend drops with the amount of added DMSO-d_6. Thus, spin diffusion is effective in a wide temperature range. The large amount of DMSO-d_6 makes also possible to run easily automatic field locking and shimming as for usual solvents. Finally, the residual proton resonances of DEA are readily suppressed with the usual excitation sculpting method.[42]

Future examinations in the field of mixture analysis considering heteronuclear *ViscY* experiments will deal with the study of other low-polarity mixtures composed of small and mid-sized molecules for assessing spin diffusion power of viscous DEA-based binary solvents.

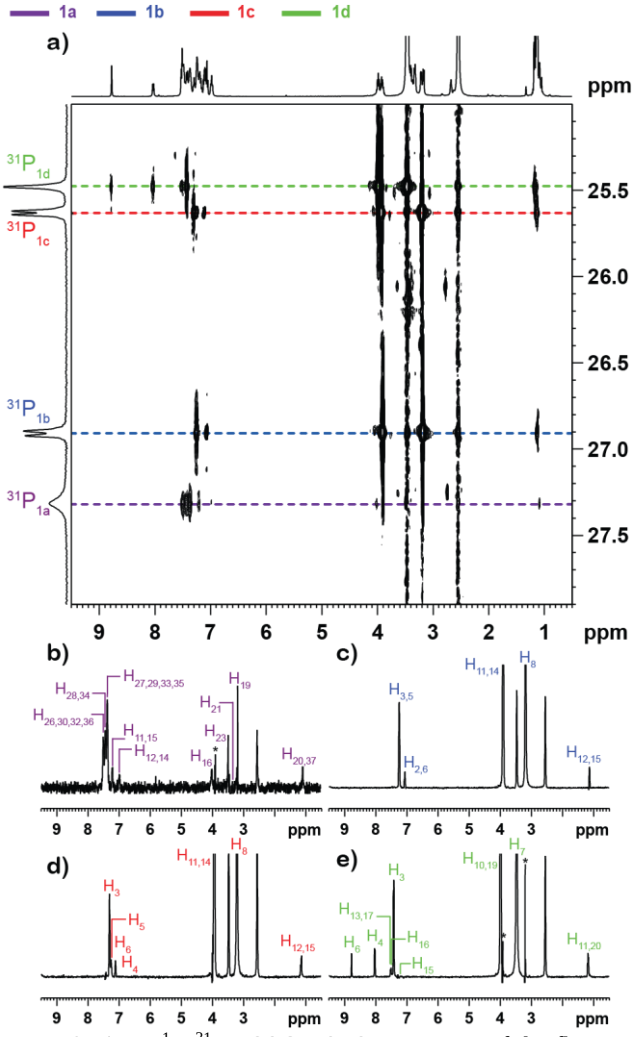

Figure 8. a) 2D ^{1}H-^{31}P HSQC-NOESY spectrum of the fluorine- and phosphorus-based compound test mixture (1a, 1b, 1c and 1d, 20 mM) dissolved in DEA/DMSO-d_6 (6:4, v/v)), t_m = 0.5 s, at 278 K, at 500 MHz (^{1}H). ^{1}H horizontal slices extracted from the 2D ^{1}H-^{31}P HSQC-NOESY at 27.32 ppm (b, ^{31}P(1a), purple dotted

line), at 26.91 ppm (c, ^{31}P(1b), blue dotted line), at 25.63 ppm (d, ^{31}P(1c), red dotted line), and at 25.48 ppm (e, ^{31}P(1d), green dotted line). * Artefacts.

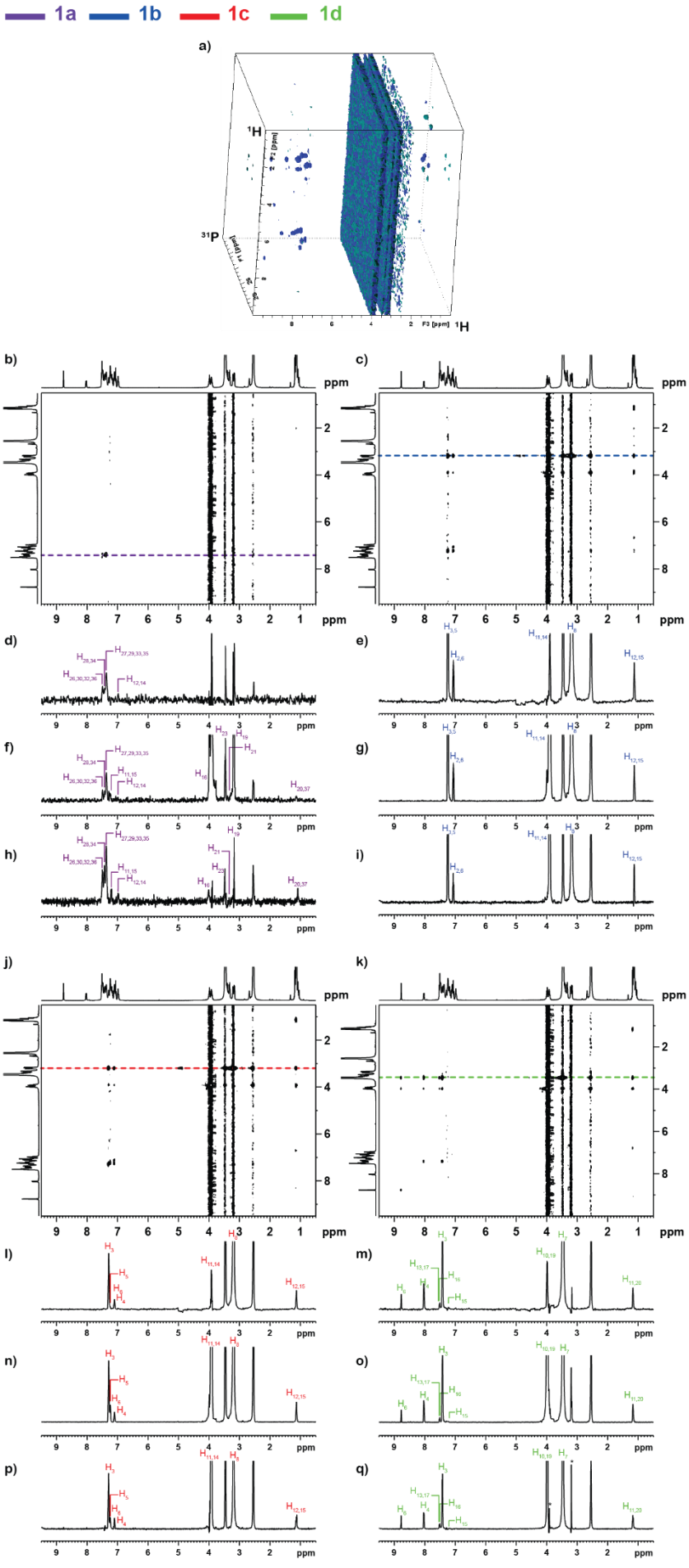
1a
1b
1c
1d
a)
^{1}H
^{31}P
F2 [ppm]
F3 [ppm]
^{1}H
b)
c)
d)
e)
f)
g)
h)
i)
j)
k)
l)
m)
n)
o)
p)
q)
ppm

Figure 9. a) 3D ^{1}H-^{31}P-^{1}H HSQC-NOESY spectrum of the fluorine- and phosphorus-based compound test mixture (1a, 1b, 1c and 1d, 20 mM) dissolved in DEA/DMSO-d_6 (6:4, v/v)), t_m = 0.5 s, at 278 K, at 500 MHz (^{1}H). 2D ^{1}H slices extracted from 3D ^{1}H-^{31}P-^{1}H HSQC-NOESY at -112.65 ppm (b, ^{31}P(1a)), at -116.31 ppm (c, ^{31}P(1b)), at -117.26 ppm (j, ^{31}P(1c)), and at -112.42 ppm (k, ^{31}P(1d)). Comparison of four 1D ^{1}H slices extracted from 2D ^{1}H NOESY (b,c,j,k) through ^{1}H resonances at 7.37 ppm (d, ^{1}H(1a), purple dotted line), 7.25 ppm (e, ^{1}H(1b), blue dotted line), at 3.20 ppm (l, ^{1}H(1c), red dotted line), and at 3.46 ppm (m, ^{1}H(1d), green dotted line) with positive sum of every slice along F_1 from 2D ^{1}H NOESY (f, ^{1}H(1a)), (g, ^{1}H(1b)), (n, ^{1}H(1c)), and (p, ^{1}H(1d)) and four ^{1}H horizontal slices extracted from 2D ^{1}H-^{31}P HSQC-NOESY at 27.32 ppm (h, ^{31}P(1a)), at 26.91 ppm (i, ^{31}P(1b)), at 25.63 ppm (p, ^{31}P(1c)), and at 25.48 ppm (q, ^{31}P(1d)). *Artefacts.

ASSOCIATED CONTENT

Supporting Information

Relevant NMR data acquisition and processing parameters used for the study and additional 2D ^{1}H NOESY and 1D ^{19}F and ^{31}P spectra (PDF file). The Supporting Information is available free of charge on the ACS Publications website.

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The authors declare no competing financial interests.

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