ViscY nuclear magnetic resonance experiments for in situ chemical reaction monitoring under spin diffusion conditions

François Pedinielli, Ritchy Leroy, Salah-Eddine Akrial, Anthony Robert, Jean-Marc Nuzillard, Pedro Lameiras

COMMUNICATION

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François Pedinielli,[a] Ritchy Leroy,[a] Salah-Eddine Akrial, [a] Anthony Robert,[a] Jean-Marc Nuzillard[a] and Pedro Lameiras*[a]

***Visco*sity-enhanced spectroscop*Y* (*ViscY*) offers a new way to analyze complex mixtures of time-varying composition. This communication reports the use of the viscous binary solvent DMSO-d_6/water to induce NMR spin diffusion for *in-situ* chemical reaction monitoring and real-time characterization of a 3-substituted 4-hydroxycoumarin derivative and its side-product.**

Synthetic organic chemists are still looking for innovative and powerful real-time analytical methods to elucidate the structures of products and by-products yielded by chemical reactions. Such challenging investigations provide insights into reaction mechanisms when one or more intermediates are formed. NMR spectroscopy has become an essential tool to address this challenge due to its high analytical throughput. The reduction of NMR data acquisition times was achieved through the availability of increasingly intense magnetic fields, the improvement of probe technology, the continuous development of new pulse sequences and the improvement of the existing ones. Over the last twenty years, high-field NMR spectroscopy has delivered highly advanced technologies and methods for chemical reaction monitoring. From an instrumental point of view, two operation modes are possible, hereafter referred to as the static [1] and the mobile modes. [2]

The static mode is implemented in three ways: *in situ,* [1] *off-line* [3] and rapid-injection, [4] each resorting to standard NMR tubes of 5 or 10 mm outer diameter in which chemical reaction monitoring takes place without any modification of the spectrometer setup. In the *in-situ* approach, the organic reagents are quickly introduced into the NMR tube to let the chemical reaction start. In contrast, in the *off-line* method, the reaction is started, and aliquots are periodically taken from the reaction medium and then transferred into a usual NMR tube for analysis. Finally, the rapid-injection approach requires additional tools, such as an insert inside NMR tubes and a syringe pump outside the magnet. A needle delivers the reagent(s) required to start the reaction. Reagent mixing inside NMR tubes is performed within a few tens of milliseconds, thus allowing the investigation of fast chemical reactions that would not be appropriately monitored by *in-situ* and *off-line* approaches.

The mobile mode eliminates operator intervention once the chemical reaction has started but requires dedicated devices (syringe, pneumatic driver, pump and flowprobe) [5] and will not be discussed further.

The *in-situ* method remains the most popular way to monitor chemical reactions in high-field NMR spectroscopy for its practicality and minimal cost, as it does not require any particular device adjunction to the NMR spectrometer. However, the so-called dead time that separates sample preparation and data acquisition constitutes a drawback of the *in-situ* approach as it precludes the study of quick reactions.

A relevant way to monitor chemical reactions using the *in-situ* approach is to resort to *ViscY* NMR (*Visco*sity-enhanced spectroscop*Y*) experiments to elucidate the chemical structure of all mixed compound more rapidly in the reaction vessel than could be achieved by usual NMR experiments. *ViscY* is a collective name for NMR experiments that take benefit from spin diffusion in viscous solvents for the individualization of NMR spectra of the components of small molecule mixtures. [6-8] According to the microviscosity theory of Gierer and Wirts, the value of the overall rotational correlation time τ_c of a compound in solution depends on the medium's viscosity (see equation in ESI). [9] Therefore, when the medium viscosity is high, the tumbling rate of small and mid-sized molecules is slow so that the longitudinal cross-relaxation becomes very efficient and thus promotes spin diffusion over entire molecular spin networks (quantitative aspects described in ESI [10]). [11] As a result, molecules exhibit a negative Nuclear Overhauser Effect (NOE), and their resonances can be grouped according to their ability to share magnetization by intramolecular spin diffusion. Each ^{1}H

[a] *Université de Reims Champagne-Ardenne, CNRS, ICMR UMR 7312, 51097 Reims, France.*
[b] *Address here.*
[c] *Address here.*
† Footnotes relating to the title and/or authors should appear here.
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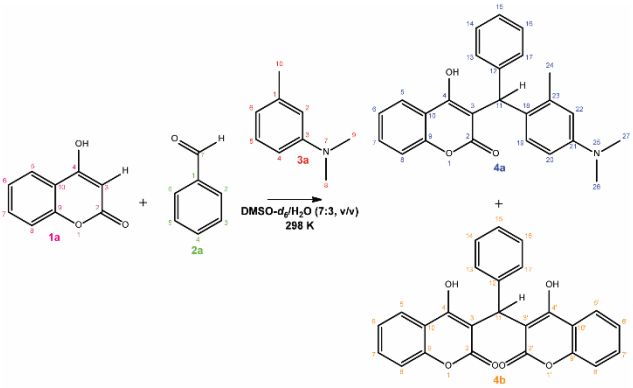
resonance from a molecule can correlate with the other ones from the same molecule in a 2D NOESY spectrum (diagonal and off-diagonal peaks of same signs), thus giving access to the individual ^{1}H NMR spectra of all mixture components. Our research team reported original results in mixture analysis by *ViscY* NMR using glycerol and glycerol carbonate (in 2011), [12] DMSO/glycerol (in 2016), [13] DMSO/water (in 2017), [14] sucrose solution, agarose gel (in 2019), [15] sulfolane-based solvents (in 2020), [6] phosphoric acid solutions (in 2021), [7] and diethanolamine (in 2022). [8]

The present work focuses on the monitoring of a model reaction, the synthesis of a 3-substituted 4-hydroxycoumarin derivative (4a) through *C*-alkylation of 4-hydroxycoumarin (1a, 20 mM) with benzaldehyde (2a, 20 mM) and *N,N-m*-dimethyltoluidine (3a, 20 mM) (see Scheme 1) in the viscous binary solvent DMSO-d_6/H_2O (7:3, v/v) by 1D and 2D NMR *ViscY* experiments such as selective 1D NOESY and conventional 2D NOESY experiments. This chemical reaction was chosen due to its compatibility with DMSO and water as solvents, as reported by Kumar *et al.* in 2012. [16]



Scheme 1. Chemical reaction of 4-hydroxycoumarin (1a, 20 mM) with benzaldehyde (2a, 20 mM) and *N,N*-m-dimethyltoluidine (3a, 20 mM) in DMSO-d_6/H_2O (7:3, v/v) at 298 K. The expected final product was 4a, but the reaction also yielded side-product 4b.

The preparation of the reaction mixture was achieved below the zero Celsius temperature using a bath of crushed water ice cooled by the addition of acetone to prevent the reaction from starting. H_2O was preferred to D_2O to avoid the chemical exchange of the ethylenic proton H_3 of 4-hydroxycoumarin with the deuterium nucleus of D_2O, a reaction that was observed in a preliminary study. The proposed NMR analytical procedure consisted of successive periods that differed by sample temperature. A first period at 238 or 248 K prevented the chemical reaction advancement to let time-consuming 1D selective and 2D NOESY spectra be recorded. The chemical reaction was then let to take place at room temperature. Successive ^{1}H spectra with short acquisitions monitored reaction. Even though the evolution of the chemical reaction was stopped from the beginning, we determined the value of the dead time t_0 at 7 min 13 s, which is the necessary time from the sample preparation to the first NMR acquisition at 238 K, including field locking and probehead tuning, matching, and shimming.

The chemical shifts of the reagents were determined by conventional 1D and 2D NMR at 238 K (page S2 in ESI), at which the chemical reaction was initially blocked. At 238 K, the NMR spin diffusion was sufficiently active for the individualization of two among three reagents by means of 2D ^{1}H-^{1}H NOESY (Figure 1) and selective 1D ^{1}H NOESY experiments (Figure 2).

The NOESY spectrum recorded at 238 K in DMSO-d_6/H_2O revealed a sufficient number of negative NOE cross peaks to collect the individual ^{1}H spectrum of 1a and 3a by extracting suitable columns, whereas compound 2a did not show complete spin diffusion (Figure 1). However, the identification of its chemical shift pattern remained possible.

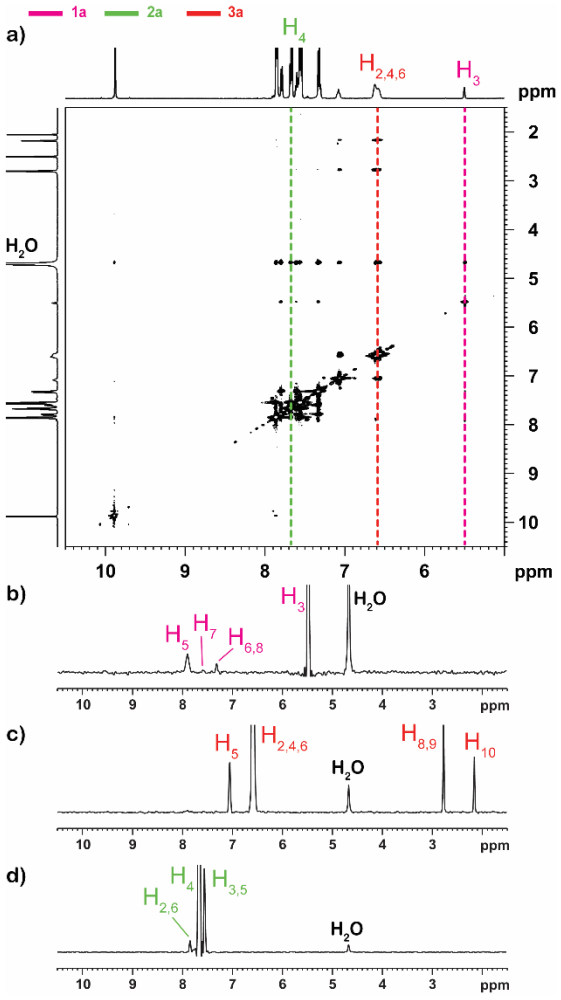


Figure 1. (a) Low-field proton region of the 2D NOESY spectrum of 1a (20 mM), 1b (20 mM) and 1c (20 mM) mixed in DMSO-d_6/H_2O (7:3, v/v), t_m = 0.5 s, with water suppression using excitation sculpting, at 238 K, at 500 MHz (^{1}H). ^{1}H vertical slices extracted from the 2D ^{1}H NOESY spectrum at 5.50 ppm [(b) H_3(1a), pink dotted line], at 6.58 ppm [(c) H_2, H_4, H_6(3a), red dotted line] and at 7.67 ppm [(d) H_4(2a), green dotted line].

Another way to individualize the spectrum of each reagent was to detect the resonances of interest during signal acquisition using selective 1D ^{1}H NOESY experiments to prevent proton resonance overlapping (Figure 2). This approach allowed the clustering of resonances belonging to 1a, 3a and 2a even though 2a showed positive and negative NOE peaks due to its

incompletely slowed molecular tumbling. Besides, a few intermolecular negative NOEs with very low intensity were detected (from 2a to 1a and 3a, from 3a to 2a).

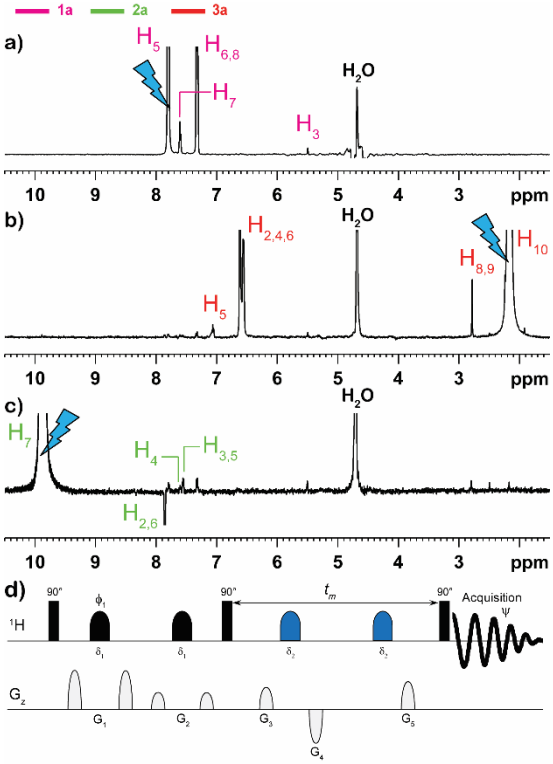


Figure 2. Multiplet selective excitation 1D ^{1}H NOESY spectra of 1a (20 mM), 2a (20 mM) and 3a (20 mM) mixed in DMSO-d_6/H_2O (7:3, v/v), t_m = 0.5 s, at 238 K, at 500 MHz (^{1}H). The initial selective inversion pulses excite (a) H_5(1a), (b) H_{10}(3a), and (c) H_7(2a) proton resonances. (d) Pulse sequence: φ_1 = x, y, −x, −y, ψ = x, −x.

The chemical reaction temperature was set at 298 K after the initial data acquisition, and the reaction mixture was left to evolve for several days. Spectral monitoring was achieved by the acquisition of a series of ^{1}H spectra. The resulting kinetic profile (Figure 3) revealed the appearance of the final product 4a directly after reaching the temperature of 298 K (after 11 days (286.8 h) from the beginning), deduced from the detection of proton H_{11} at 5.83 ppm. The benzylated coumarin dimer (side-product) (4b) also appeared after 11 days, clearly attested by the observation of proton H_{11} at 6.16 ppm. The reaction was considered as completed after 43 days (1034.6 h), resulting in an 8.6% yield of 4a and a 22.7% yield of 4b. The chemical structure of 4a and 4b were confirmed by taking profit from spin diffusion at 248 K, using *ViscY* NOESY experiments.

The individual ^{1}H spectrum of 4a and 4b were extracted from the columns of their H_{11} resonances in the 2D NOESY spectrum (Figure 4). The identical individual spectra were also recorded after selectively exciting H_{24} and H_{11} resonances, respectively, from 4a and 4b, by means of the selective 1D ^{1}H NOESY experiment (Figure 5). This latter experiment facilitated the structural identification of the final product 4a and its by-product 4b by acquiring only ^{1}H resonances of interest.

To conclude, we reported the in-situ monitoring of an organic reaction involving *ViscY* NMR experiments for the first time. It was applied to the synthesis of a 3-substituted 4-hydroxycoumarin derivative (4a) through C-alkylation of 4-hydroxycoumarin with benzaldehyde (2a) and *N,N*-m-dimethyltoluidine (3a) in the viscous binary solvent DMSO-d_6/H_2O (7:3, v/v) for the individualization of every reagent (1a, 2a and 3a), of the side-product (4b) and of the final product (4a). For this purpose, an experimental approach was developed by considering sub-zero Celsius temperatures to block the chemical reaction and room temperature to let it take place.

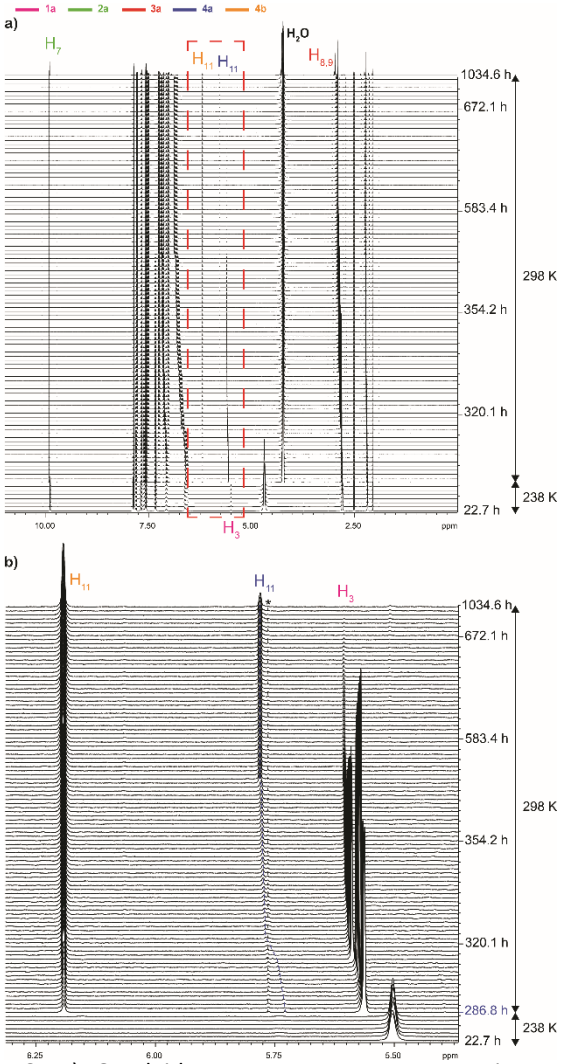


Figure 3. a) Overlaid 1D proton spectra over time of 4-hydroxycoumarin (1a, 20 mM), benzaldehyde (2a, 20 mM) and *N, N*-m-dimethyltoluidine (3a, 20 mM) mixed in DMSO-d_6/H_2O (7:3, v/v) at 298 K. b) Low-field proton region of overlaid proton spectra over time of 1a (H_3), 4a (H_{11}) and 4b (H_{11}). * Impurity

For several reasons, the binary solvent DMSO/water may be considered appropriate for *in-situ* chemical reaction monitoring using *ViscY* NMR experiments. This solvent blend presents a low viscosity at ambient temperature, a property that facilitates the preparation of the reaction mixture and its transfer into an NMR tube. Adding water to DMSO also permits working at or below room temperature, which is especially appropriate for thermally unstable organic species. The freezing point of the binary solvent blend decreases significantly with the amount of added H_2O. As a result, monitoring the chemical reaction of

interest will be facilitated by quenching it on purpose at a sub-zero Celsius temperature and by taking profit from spin diffusion for reagent/product individualization on a wide range of temperatures, from 238 K to room temperature. Furthermore, the large amount of DMSO-d_6 also enables spectrometer tools such as the automatic field-locking and shimming as for usual solvents, thus facilitating chemical reaction monitoring. Finally, the residual proton resonance of water is easily suppressed by presaturation or excitation sculpting. However, organic compounds of very low polarity may not be compatible with the use of DMSO/H_2O in chemical reaction monitoring for a low reagent solubility reason.

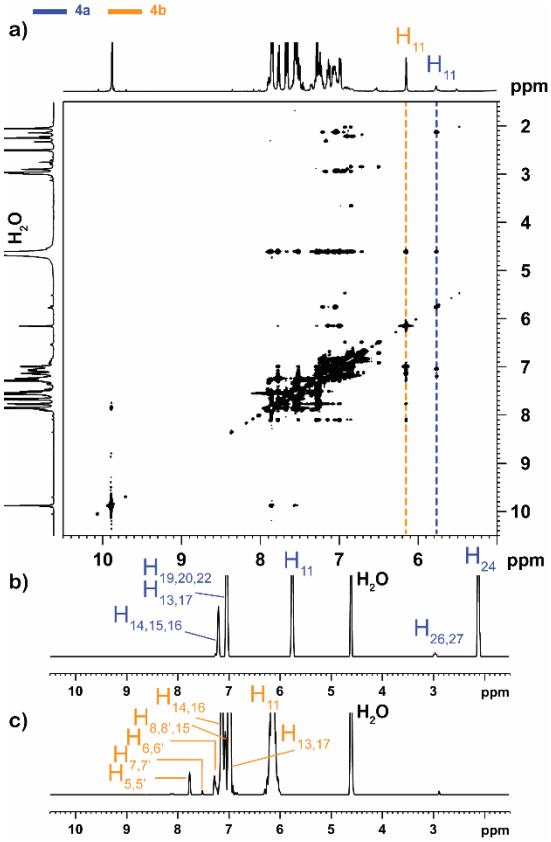


Figure 4. a) Low-field proton region of the 2D NOESY spectrum of 1a (20 mM), 2a (20 mM) and 3a (20 mM) mixed in DMSO-d_6/H_2O (7:3, v/v), after 43 days of chemical reaction, t_m = 0.5 s, water signal suppression using excitation sculpting, at 248 K, at 500 MHz (^{1}H). ^{1}H vertical slices extracted from the 2D ^{1}H NOESY spectrum at 5.76 ppm [(b) H_{11}(4a), blue dotted line], and at 6.15 ppm [(c) H_{11}(4b), orange dotted line].

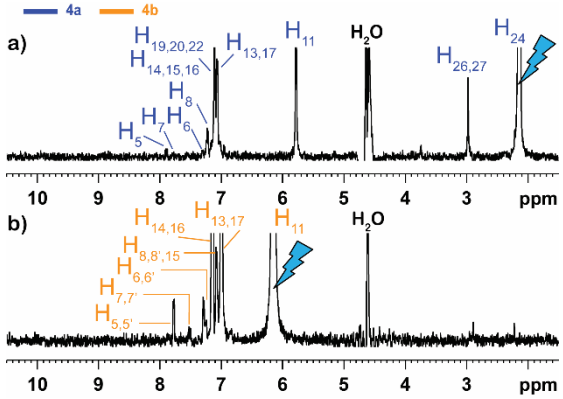


Figure 5. Multiplet selective excitation 1D ^{1}H NOESY spectra of 4a and 4b in DMSO-d_6/H_2O (7:3, v/v), after 43 days of chemical reaction, t_m = 0.5 s, at 248 K, at 500 MHz (^{1}H). The initial selective inversion pulses excite (a) H_{24}(4a), and (b) H_{11}(4b) proton resonances.

Acknowledgments

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Conflicts of interest

There are no conflicts to declare.

Notes and references

1. F. Susanne, D. S. Smith and A. Codina, *Org. Process Res. Dev*, 2012, **16**, 61-64.
2. P. A. Keifer, in *Annu. Rep. NMR Spectrosc.*, ed. G. A. Webb, Academic Press, 2007, vol. 62, pp. 1-47.
3. T. Bartik, B. Bartik, B. E. Hanson, T. Glass and W. Bebout, *Inorg. Chem.*, 1992, **31**, 2667-2670.
4. J. F. McGarrity, J. Prodolliet and T. Smyth, *Org. Magn. Reson.*, 1981, **17**, 59-65.
5. D. A. Foley, E. Bez, A. Codina, K. L. Colson, M. Fey, R. Krull, D. Piroli, M. T. Zell and B. L. Marquez, *Anal. Chem.*, 2014, **86**, 12008-12013.
6. F. Pedinielli, J.-M. Nuzillard and P. Lameiras, *Anal. Chem.*, 2020, **92**, 5191-5199.
7. F. Pedinielli, R. Leroy, A. Martinez, J.-M. Nuzillard and P. Lameiras, *Analyst*, 2021, **146**, 5316-5325.
8. R. Leroy, F. Pedinielli, G. Bourbon, J.-M. Nuzillard and P. Lameiras, *Anal. Chem.*, 2022, **94**, 9278-9286.
9. A. Gierer and K. Wirtz, *Zeitschrift Für Naturforschung Section A*, 1953, **8**, 532-538.
10. T. D. W. Claridge, in *High-Resolution NMR Techniques in Organic Chemistry. 3rd Ed.*, Elsevier Ltd., 2016, DOI: 10.1016/b978-0-08-099986-9.00009-9, pp. 315-380.
11. P. Lameiras and J.-M. Nuzillard, *Prog. Nucl. Magn. Reson. Spectrosc.*, 2021, **123**, 1-50.
12. P. Lameiras, L. Boudesocque, Z. Mouloungui, J. H. Renault, J. M. Wieruszeski, G. Lippens and J. M. Nuzillard, *J. Magn. Reson.*, 2011, **212**, 161-168.
13. P. Lameiras and J. M. Nuzillard, *Anal. Chem.*, 2016, **88**, 4508-4515.
14. P. Lameiras, S. Patis, J. Jakhlal, S. Castex, P. Clivio and J.-M. Nuzillard, *Chem. Eur. J.*, 2017, **23**, 4923-4928.
15. P. Lameiras, S. Mougeolle, F. Pedinielli and J.-M. Nuzillard, *Faraday Discuss.*, 2019, **218**, 233-246.
16. A. Kumar, M. Kumar, M. Gupta and L. Gupta, *RSC Adv.*, 2012, **2**, 8277-8280.