***ELECTRONIC SUPPLEMENTARY MATERIAL* for**

ViscY Nuclear Magnetic Resonance experiments for in-situ Chemical Reaction Monitoring under Spin Diffusion Conditions

Francois Pedinielli, Ritchy Leroy, Salah-Eddine Akrial, Anthony Robert, Jean-Marc Nuzillard and Pedro Lameiras*

[a] Université de Reims Champagne-Ardenne, CNRS, ICMR UMR 7312, 51097 Reims, France

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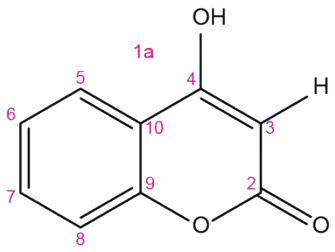
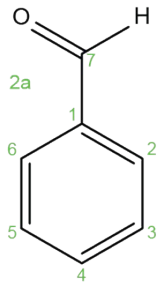
Chemical structures and NMR chemical shifts of reagents 1a, 2a and 3a, the final product 4a and the by-product 4b

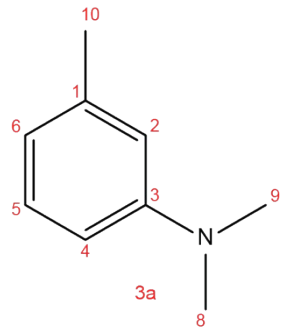
1a: 4-Hydroxycoumarin

	^{1}H chemical shifts in DMSO-d_6 /H_2O (7:3, v/v), at 298 K, at 500 MHz (^{1}H)		
	**1a: N°**	**δ ^{13}C (ppm)**	**δ ^{1}H (ppm)**
	C_3/H_3	91.76	5.60
	C_8/H_8	117.79	7.33
	C_5/H_5	124.53	7.81
	C_6/H_6	125.69	7.34
	C_7/H_7	**δ ^{13}C (ppm)**	7.62

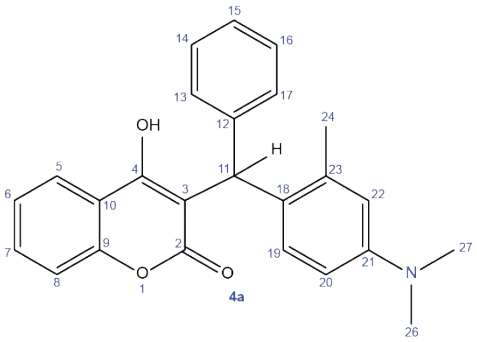
2a: Benzaldehyde

	^{1}H chemical shifts in DMSO-d_6 /H_2O (7:3, v/v), at 298 K, at 500 MHz (^{1}H)		
	**2a: N°**	**δ ^{13}C (ppm)**	**δ ^{1}H (ppm)**
	$C_{3,5}$/$H_{3,5}$	130.54	7,55
	$C_{2,6}$/$H_{2,6}$	130.89	7.85
	C_4/H_4	136.23	7,67
	C_1	136.94	-
	C_7/H_7	195.52	9.91

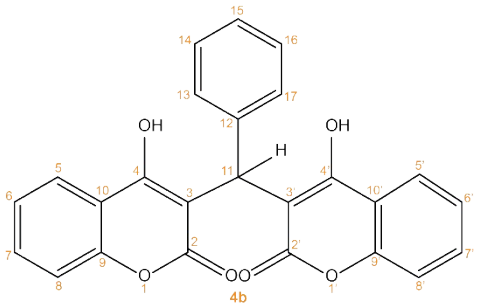
3a: *N, N*-Dimethyl-*m*-toluidine

	^{1}H chemical shifts in DMSO-d_6 /H_2O (7:3, v/v), at 298 K, at 500 MHz (^{1}H)		
	**3a: N°**	**δ ^{13}C (ppm)**	**δ ^{1}H (ppm)**
	C_{10}/H_{10}	22.28	2.18
	$C_{8,9}/H_{8,9}$	40.40	2.93
	C_4/H_4	115.07	6.91
	C_2/H_2	118.43	6.94
	C_6/H_6	125.46	6.88
	C_5/H_5	127.61	7.14
	C_1	138.64	-
	C_3	149.6	-

4a: The final product

	^{1}H chemical shifts in DMSO-d_6 /H_2O (7:3, v/v), at 298 K, at 500 MHz (^{1}H)		
	**4a: N°**	**δ ^{13}C (ppm)**	**δ ^{1}H (ppm)**
	C_{24}/H_{24}	20.23	2.20
	C_{26}/H_{26}, C_{27}/H_{27}	41.12	2.98
	C_{11}/H_{11}	43.18	5.83
	C_3	107.63	-
	C_{22}/H_{22}	115.15	7.20
	C_{10}	116.42	-
	C_{20}/H_{20}	116.49	7.20
	C_8/H_8	116.79	7.39
	C_5/H_5	123.85	8.03
	C_6/H_6	124.33	7.37
	C_{15}/H_{15}	126.24	7.19
	C_{14}/H_{14}, C_{16}/H_{16}	128.40	7.27
	C_{13}/H_{13}, C_{17}/H_{17}	129.05	7.13
	C_{19}/H_{19}	129.45	7.15
	C_7/H_7	132.55	7.63
	C_{18}	132.87	-
	C_{23}	137.88	-
	C_{12}	142.70	-
	C_{21}	151.41	-
	C_9	152.83	-
	C_4	161.65	-
	C_2	162.60	-

4b: The by-product

	^{1}H chemical shifts in DMSO-d_6 /H_2O (7:3, v/v), at 298 K, at 500 MHz (^{1}H)		
 4b	**4b: N°**	δ **^{13}C (ppm)**	δ **^{1}H (ppm)**
	C_{11}/H_{11}	37.2	6.16
	C_3, $C_{3'}$	104.63	-
	C_8/H_8, $C_{8'}/H_{8'}$	116.99	7.25
	$C_{10}/C_{10'}$	120.54	-
	C_6/H_6, $C_{6'}/H_{6'}$	124.93	7.24
	C_5/H_5, $C_{5'}/H_{5'}$	125.38	7.78
	C_{15}/H_{15}	126.65	7.06
	C_{13}/H_{13}, C_{17}/H_{17}	127.50	7.01
	C_{14}/H_{14}, C_{16}/H_{16}	129.27	7.14
	C_7/H_7, $C_{7'}/H_{7'}$	132.93	7.50
	C_{12}	142.70	-
	C_9, $C_{9'}$	153.49	-
	C_2, $C_{2'}$	166.95	-
	C_4, $C_{4'}$	169.70	-

Additional NMR data acquisition and processing parameters for Figures 1 -> 5:

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). Data matrix was recorded in States-TPPI mode using the noesyesgpph pulse sequence; its size was 512 × 4k with 8 scans per FID, a 1.5 s relaxation delay, and a 0.5 s mixing time (t_m), resulting in a 164.90 min recording time (expt). The spectral width was 5 498.53 Hz in both dimensions. 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. Data matrix was multiplied in both dimensions by a shifted squared sine bell function (SSB = 2) before zero filling to a 1k × 4k size. ^{1}H vertical slices extracted from the 2D ^{1}H NOESY spectrum at 5.50 ppm [(b) H3(1a), pink dotted line], at 6.58 ppm [(c) H2, H4, H6(3a), red dotted line] and at 7.67 ppm [(d) H4(2a), green dotted line].

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 278 K, at 500 MHz (^{1}H). The initial selective excitation was achieved by a 1% truncated 180° Gaussian pulse. G1:G2 = 70:30. WURST wideband adiabatic inversion pulses, δ_2 = 1.5 ms, starting within t_m at 0.33 and 0.83 t_m with G3:G4:G5 = 40:-60:50. Gradient pulse length was 1 ms. d = 200 µs. Relaxation delay was 2.0 s. The FIDs (8k points, spectral width 5498.53 Hz) were multiplied by an exponential function (LB = 0.3) before zero-filled to 64k points. The initial selective inversion pulses excite: a) the H_5(1a) proton resonance (δ_1 = 100 ms, 4k scans, experiment time (expt) = 237.87 min); b) the H_{10}(3a) proton resonances (δ_1 = 40 ms, 4k scans, expt = 229.60 min); c) the H_7(2a) proton resonance (δ_1 = 20 ms, 4k scans, expt = 226.80 min); d) Pulse sequence: φ_1 = *x, y, -x, -y,* ψ = *x, -x*.

Figure 3. 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}). The FIDs (8 scans, 32k points, spectral width = 5500.55 Hz) were zero-filled to 64k points. 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. * Impurity

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, water signal suppression using excitation sculpting, at 248 K, at 500 MHz (^{1}H). Data matrix was recorded in States-TPPI mode using the noesyesgpph pulse sequence; its size was 512 × 4k with 8 scans per FID, a 1.5 s relaxation delay, and a 0.5 s mixing time (t_m), resulting in a 164.90 min recording time (expt). The spectral width was 5 498.53 Hz in both dimensions. 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. Data matrix was multiplied in both dimensions by a shifted squared sine bell function (SSB = 2) before zero filling to a 1k × 4k size. ^{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 excitation was achieved by a 1% truncated 180° Gaussian pulse. G1:G2 = 70:30. WURST wideband adiabatic inversion pulses, δ_2 = 1.5 ms, starting within t_m at 0.33 and 0.83 t_m with G3:G4:G5 = 40:-60:50. Gradient pulse length was 1 ms. d = 200 µs. Relaxation delay was 2.0 s. The FIDs (8k points, spectral width 5498.53 Hz) were multiplied by an exponential function (LB = 0.3) before zero-filled to 64k points. The initial selective inversion pulses excite: a) the H_{24}(4a) proton resonance (δ_1 = 60 ms, 13k scans, experiment time (expt) = 754.87 min); b) the H_{11}(4b) proton resonance (δ_1 = 30 ms, 4k scans, expt = 114.07 min).

Spin diffusion and its quantitative aspects

Steady-state NOE

The magnitude of indirect NOE effects is favoured for slowly tumbling molecules, so that quantitative steady-state NOE measurements largely lose usefulness for two main reasons. The indirect effects are of the same sign as direct effects and therefore cannot be easily discriminated. Moreover, they grow rapidly and may reach and maintain very negative intensities. In the extreme case of extended saturation times, the NOE initially produced between two spins can spread throughout the entire molecule until all nuclei undergo the same NOE enhancement (see Figure S1). This dispersion of saturation throughout the molecule is often referred to as spin diffusion for fairly obvious reasons and may be compared to heat diffusing through a conductive solid. **The limit of slow tumbling is also referred to as the spin diffusion limit. For this reason, steady-state NOEs in the negative NOE regime cannot provide reliable distance or proximity information. Instead, it is mandatory to consider the rate at which NOEs grow between spins to grasp distance information, suggesting the use of kinetic measurements by means of transient NOE experiments.**

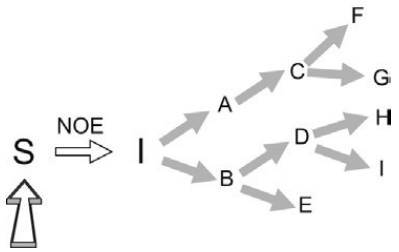


Figure S1. Schematic illustration of the spin diffusion process. In this the original *S*–*I* NOE is efficiently relayed onto neighbouring nuclei and propagated throughout the molecule. Reproduced from [10].

Transient NOE (considered in our study)

The steady-state NOE experiments are complicated to use for the exploitation of quantitative NOE intensities into internuclear distances due to the influence of all neighbouring spins (relaxation leakage, indirect effects, spin diffusion, etc.). Furthermore, molecules in a slow motion regime that exhibit negative NOEs may also disturb the possible extraction of reliable spatial proximity constraints. However, the rate of the NOE growth towards steady-state may be directly related to distances under appropriate conditions. Considering the kinetics of NOE turns out to be a pertinent alternative. One way is to consider the saturation of the target resonance for shorter periods than those needed to reach the steady-state for detecting and sampling NOE intensities. Repeating the experiment with progressively incremented saturation periods makes it possible to record the NOE evolution kinetics. Another more universal approach (considered in our study) is to obtain kinetic data by instantaneously inverting the target resonance(s) instead of saturating it and then allowing the NOE to grow in the absence of any external action. The new populations are then sampled by means of a 90-degree pulse (see Figure S2). In this

context, the NOE initially builds for a while but at the end fades away as longitudinal spin relaxation acts for restoring the equilibrium condition. The observed resonance intensity variations are hence called transient NOEs.

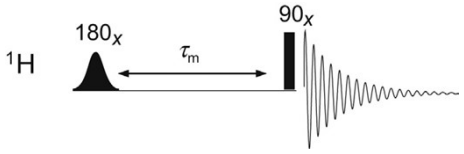


Figure S2. General scheme for observing transient NOEs. Following inversion of a target resonance, the NOE develops during the mixing time t_m, after which the system is sampled. Reproduced from [10].

The use of dedicated viscous solvents allows the tailoring of molecular dynamics of the dissolved molecules. Indeed, the tumbling rate of small and medium-sized molecules can be slowed down in aqueous and organic viscous solvents. As a result, the solutes present a **negative NOE regime**, and their resonances can be grouped according to their ability to **exchange magnetization through intramolecular spin diffusion**. However, **quantitative measurements are still accessible by considering the growth of the initial rate of negative transient NOE as linear**. The magnitude of an enhancement between two spins I and S after a period τ will be proportional to the cross-relaxation rate σ_{IS}, which in turn depends on r_{IS}^{-6}:

$$\eta_I\{S\} = k\sigma_{IS}\tau = k' r_{IS}^{-6}\tau$$

The proportionality constants k and k' contain the molecular correlation time τ_c in addition to known physical constants, and {S} is now considered to indicate inversion rather than saturation of spin S. Taking a known internal distance r_{XY} as a reference allows one to determine r_{IS}. If the NOE between reference nuclei X and Y is measured, then:

$$\frac{\eta_I\{S\}}{\eta_X\{Y\}} = \frac{r_{IS}^{-6}}{r_{XY}^{-6}}$$

The knowledge of the two NOE intensities thus determines the unknown internuclear distance. From a single experiment, distances can be estimated assuming the initial rate approximation is valid for all interactions. This relies on all internuclear vectors with identical correlation times, which may not be the case if internal motion is present.

Overall rotational correlation τ_c

$$\tau_c = \frac{4\pi\eta a^3}{3kT}$$

Equation ES1: overall rotational correlation τ_c versus the medium's viscosity η, with *T* the medium temperature, a molecular radium, *k* constant.

The value of the overall rotational correlation time τ_c of a molecule in solution lies on the medium's viscosity. As a result, when the medium viscosity is high, the tumbling rate of small and mid-sized molecules is reduced so that the longitudinal cross-relaxation becomes very efficient and thus promotes spin diffusion over entire molecular spin networks.

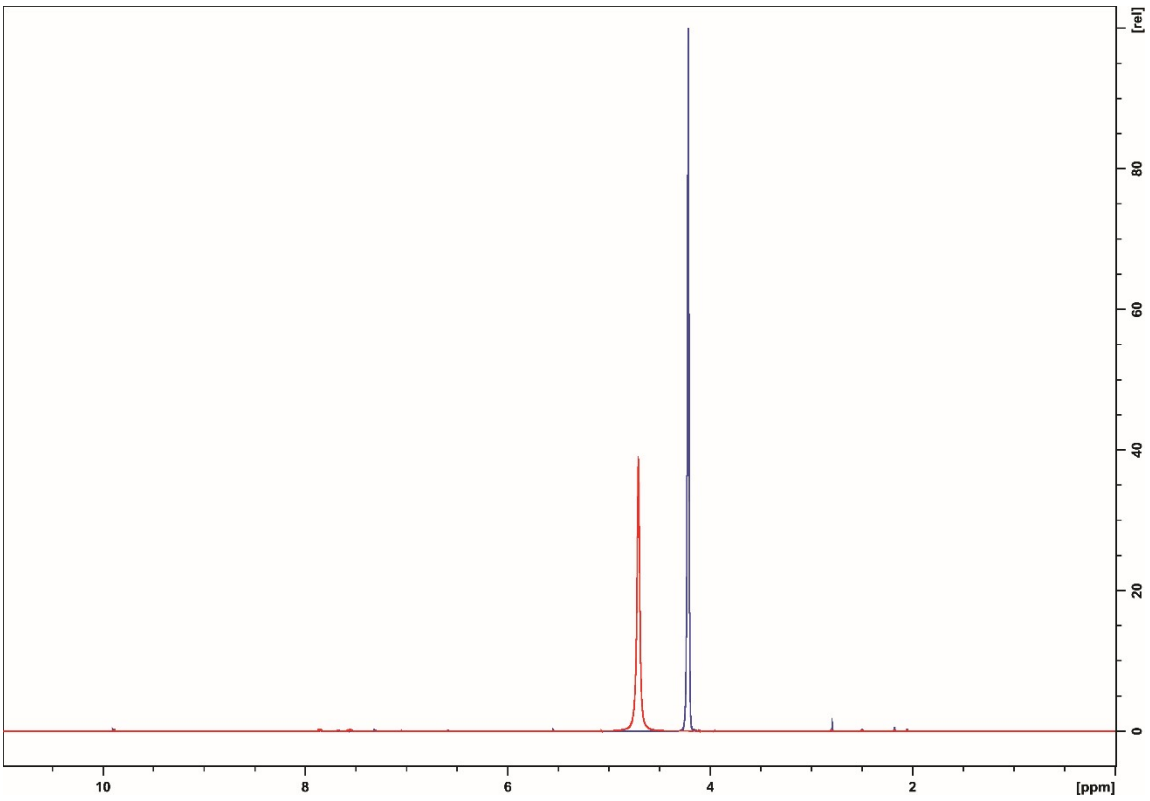


Figure S3. 1D ^{1}H NOESY spectra of 1a (20 mM), 2a (20 mM) and 3a (20 mM) mixed in DMSO-d_6/H_2O (7:3, v/v), in red at 238 K (Peak Width at Half Height = 14.36 Hz), and in blue at 298 K (Peak Width at Half Height = 8.06 Hz), at 500 MHz (^{1}H). The FIDs (32k points, spectral width 5498.53 Hz, NS =1) were acquired before zero-filled to 64k points.

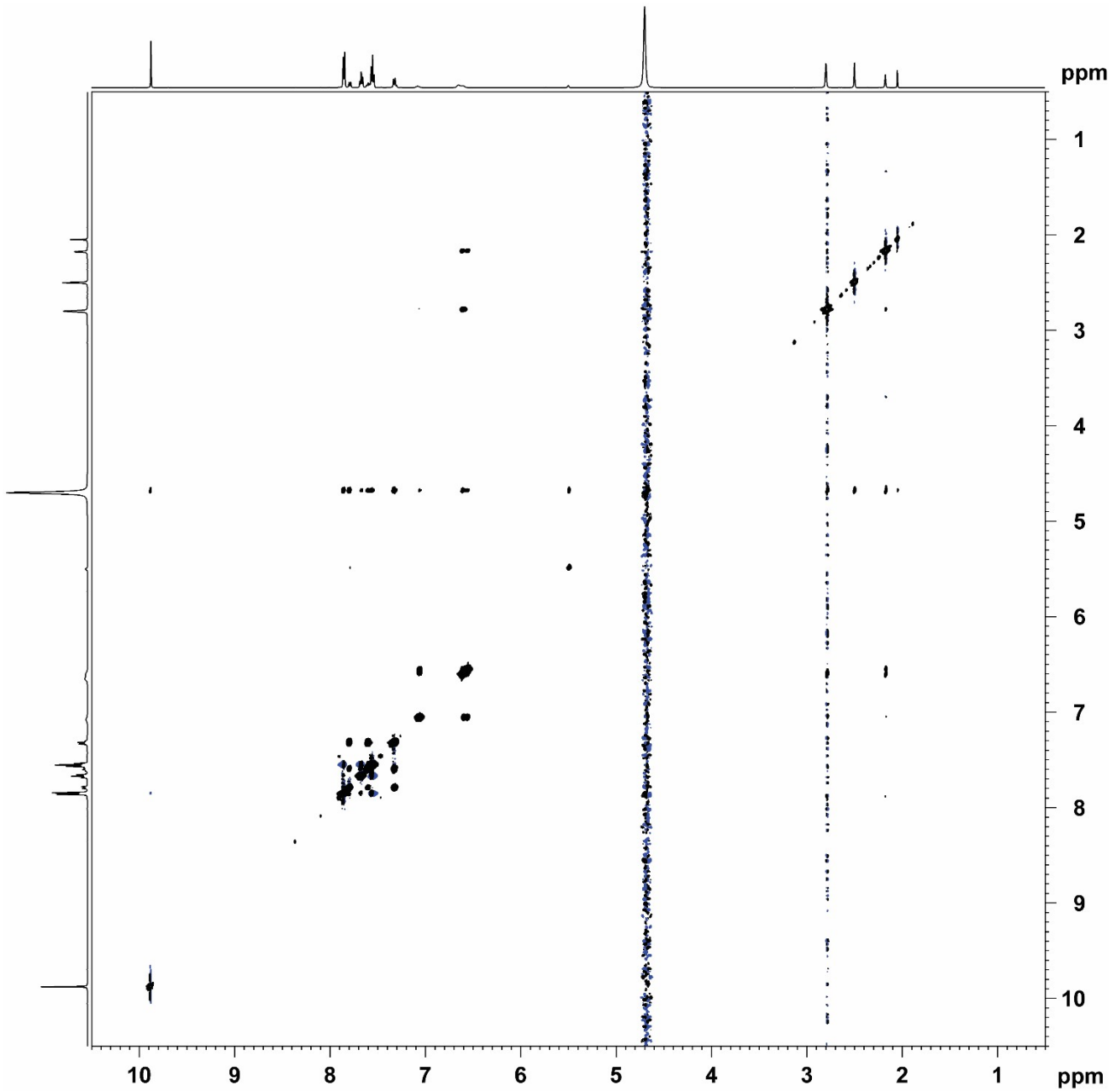


Figure S4. Full 2D ^{1}H-^{1}H NOESY spectrum of 1a (20 mM), 1b (20 mM) and 1c (20 mM) mixed in DMSO-d_6/H_2O (7:3, v/v), t_m = 0.5 s, with water suppression using excitation sculpting, at 238 K, at 500 MHz (^{1}H). Data matrix was recorded in States-TPPI mode using the noesyesgpph pulse sequence; its size was 512 × 4k with 8 scans per FID, a 1.5 s relaxation delay, and a 0.5 s mixing time (t_m), resulting in a 164.90 min recording time (expt). The spectral width was 5 498.53 Hz in both dimensions. 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. Data matrix was multiplied in both dimensions by a shifted squared sine bell function (SSB = 2) before zero filling to a 1k × 4k size.

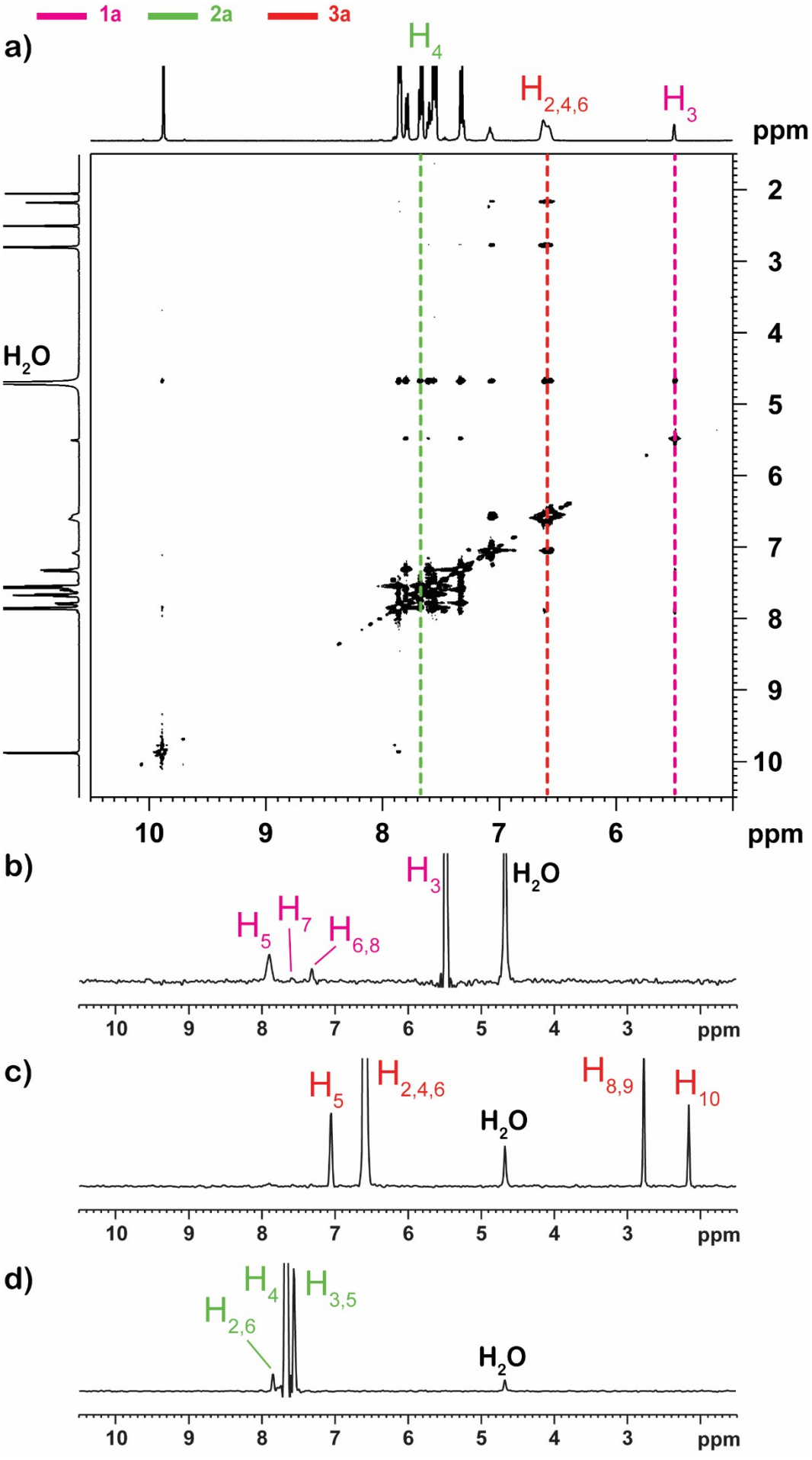

1a
2a
3a
a)
H4
H2,4,6
H3
ppm
H2O
2
3
4
5
6
7
8
9
10
10
9
8
7
6
ppm
b)
H3
H2O
H5
H7
H6,8
10
9
8
7
6
5
4
3
ppm
c)
H5
H2,4,6
H2O
H8,9
H10
10
9
8
7
6
5
4
3
ppm
d)
H4
H3,5
H2,6
H2O
10
9
8
7
6
5
4
3
ppm

Figure S5. (double column) 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). Data matrix was recorded in States-TPPI mode using the noesyesgpph pulse sequence; its size was 512 × 4k with 8 scans per FID, a 1.5 s relaxation delay, and a 0.5 s mixing time (t_m), resulting in a 164.90 min recording time (expt). The spectral width was 5 498.53 Hz in both dimensions. 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. Data matrix was multiplied in both dimensions by a shifted squared sine bell function (SSB = 2) before zero filling to a 1k × 4k size. ^{1}H vertical slices extracted from the 2D ^{1}H NOESY spectrum at 5.50 ppm [(b) H3(1a), pink dotted line], at 6.58 ppm [(c) H2, H4, H6(3a), red dotted line] and at 7.67 ppm [(d) H4(2a), green dotted line].

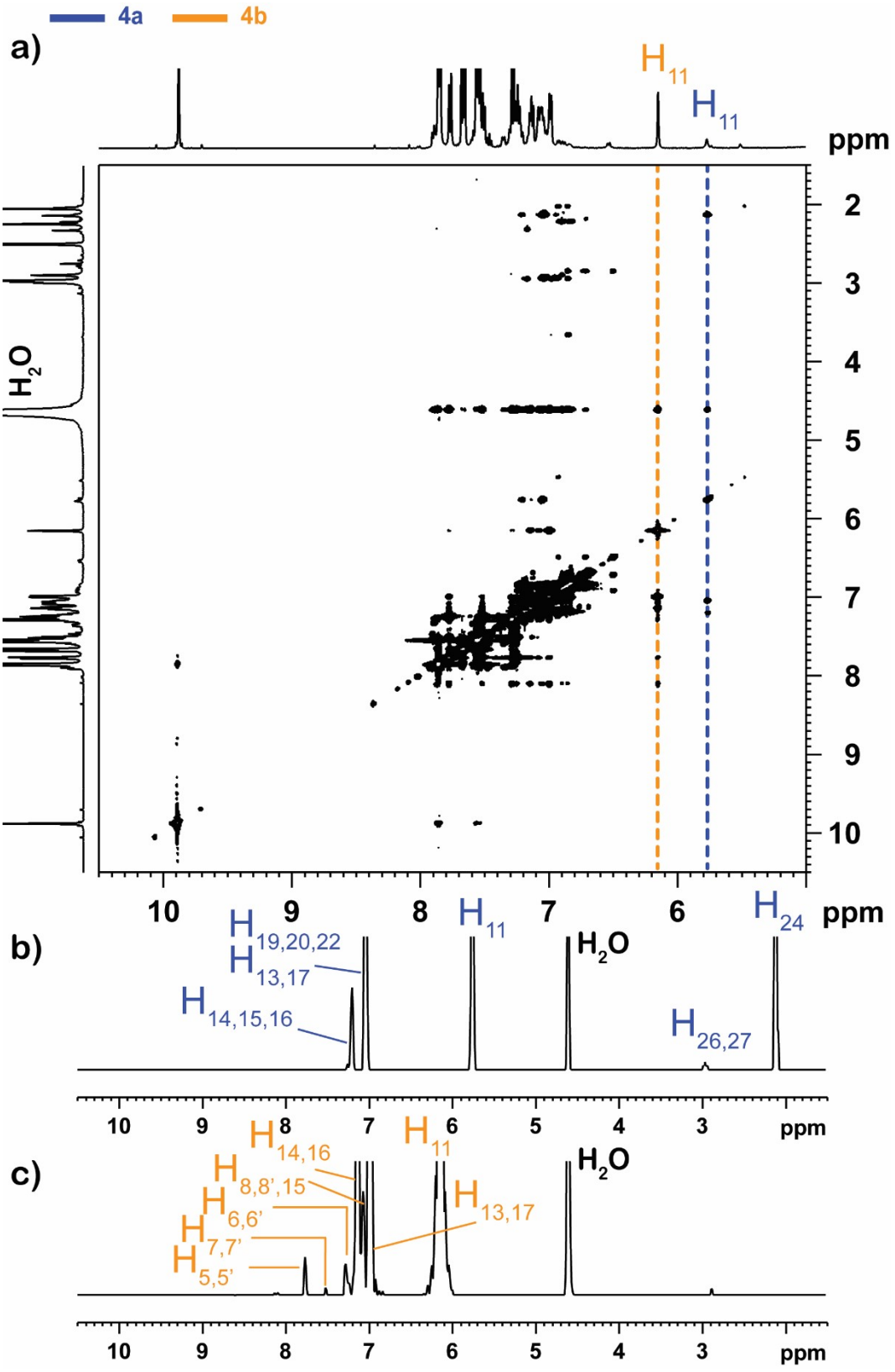

4a
4b
a)
H11
H11
ppm
H2O
2
3
4
5
6
7
8
9
10
10
9
8
H11
7
6
H24
ppm
b)
H19,20,22
H13,17
H14,15,16
H2O
H26,27
10
9
8
7
6
5
4
3
ppm
c)
H14,16
H8,8',15
H6,6'
H7,7'
H5,5'
H11
H13,17
H2O
10
9
8
7
6
5
4
3
ppm

Figure S6. (double column) 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, water signal suppression using excitation sculpting, at 248 K, at 500 MHz (^{1}H). Data matrix was recorded in States-TPPI mode using the noesyesgpph pulse sequence; its size was 512 × 4k with 8 scans per FID, a 1.5 s relaxation delay, and a 0.5 s mixing time (t_m), resulting in a 164.90 min recording time (expt). The spectral width was 5 498.53 Hz in both dimensions. 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. Data matrix was multiplied in both dimensions by a shifted squared sine bell function (SSB = 2) before zero filling to a 1k × 4k size. ^{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].

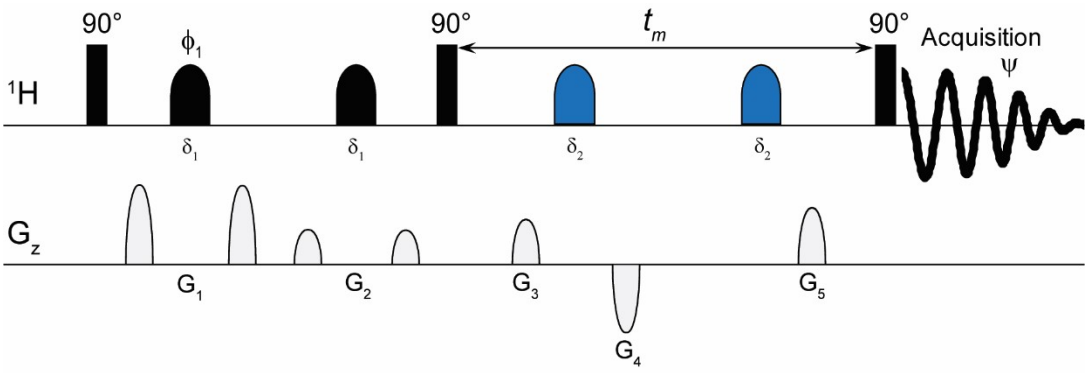


Figure S7. Multiplet selective excitation 1D ^{1}H NOESY pulse sequence: $\varphi_1 = x, y, -x, -y, \psi = x, -x$.

In the 1D selective-NOESY experiment, a single spin is excited, and its magnetization is further flipped to bring it to the -z-axis, in which it can spread by spin diffusion along the molecular proton network. The main difficulty here was avoiding reintroducing the water signal at detection time. The 1D selective-NOESY pulse sequence starts with a multiplet-selective excitation block. Two wideband inversion pulses were inserted during the mixing time. Their position was adjusted to minimize the amount of resurrected H_2O magnetization that arises by longitudinal relaxation.