***ELECTRONIC SUPPLEMENTARY INFORMATION* for**

ViscY NMR experiments in phosphoric acid as viscous solvent for the individualization of small molecules within mixtures by spin diffusion

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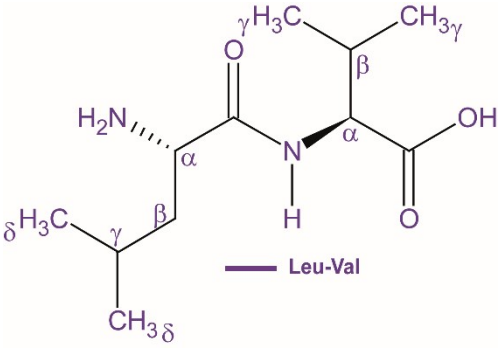
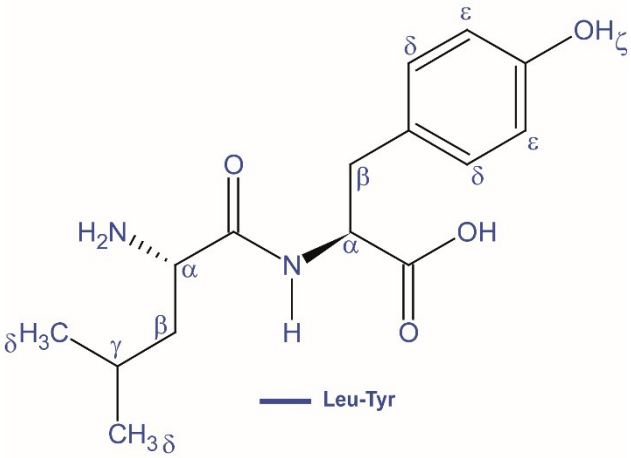
Dipeptide chemical structures:

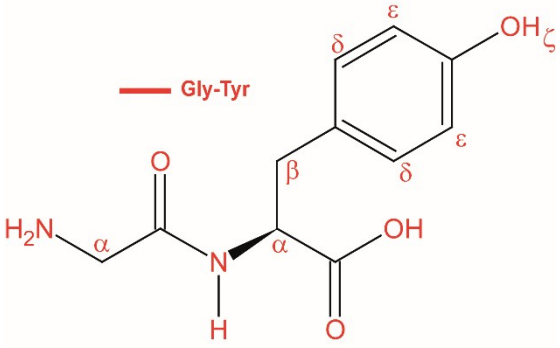
Leucine-Valine = Leu-Val = LV



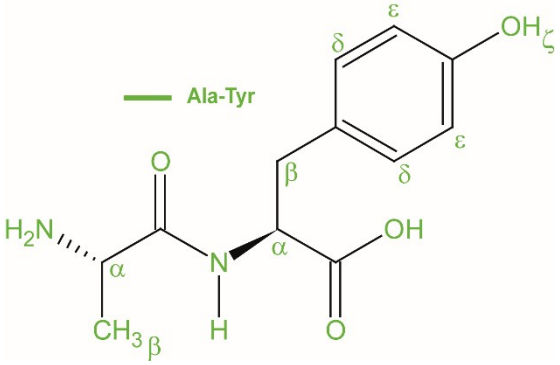
Leucine-Tyrosine = Leu-Tyr = LY



Glycine-Tyrosine = Gly-Tyr = GY



Alanine-Tyrosine = Ala-Tyr = AY



Additional NMR data acquisition and processing parameters for Fig. 1 -> 4 and Fig. 6 -> 9:

Fig. 1 a) Amide proton region of 2D NOESY spectrum of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr), 20 mM, dissolved in phosphoric acid (85%)/D_2O (8:2, v/v) solution, at 288 K, at 500 MHz (^1H) using the noesyesgpph pulse sequence. Data matrix was recorded in States-TPPI mode; its size was 512 × 4k with 8 scans per FID, a 1.5 s relaxation delay and a 1 s mixing time (t_m), resulting in a 183.32 min recording time (expt). The spectral width was 5000 Hz in both dimensions. G1: G2 = 70: 30. Data matrix was multiplied in both dimensions by a shifted squared sine bell function (SSB = 2) before zero filling to a 1k × 4k size. 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. b) Amide proton region of 2D NOESY spectrum of the same dipeptide test mixture (20 mM) dissolved in H_2O/D_2O (9:1 v/v), at pH = 5.0, t_m = 1 s, at 298 K, at 500 MHz (^1H). Spectrum b) was recorded and processed with the same parameters as those in 1a using the same noesyesgpph pulse sequence, resulting in a 171.02 min recording time (expt). 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.

Fig. 2 Multiplet selective excitation 1D ^1H NOESY spectra of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr), 20 mM, dissolved in phosphoric acid (85%)/D_2O (8:2, v/v) solution (a, b, c, and d), t_m = 1 s, at 288 K, at 500 MHz (^1H). 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 5000 Hz) were multiplied by an exponential function (LB = 0.3) before zero-filled to 64k points. The initial selective inversion pulses excite: a) the NH_V(LV) proton resonance (δ_1 = 40 ms, 8k scans, experiment time (expt) = 425.07 min); b) the $H\delta_L$(LY)/$H\delta_L$(LV)/$H\gamma_V$(LV) proton resonances (δ_1 = 8 ms, 1k scans, expt = 51.85 min); c) the $H\delta_Y$(LY)/$H\delta_Y$(GY)/$H\delta_Y$(AY) proton resonances (δ_1 = 20 ms, 1k scans, expt = 52.27 min); d) the $H\alpha_G$(GY) proton resonance (δ_1 = 40 ms, 4k scans, expt = 211.20 min); e) Pulse sequence: φ_1 = *x, y, -x, -y,* ψ = *x, -x*.

Fig. 3 a) F_1 band selective F_1 decoupled 2D NOESY spectrum of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr), 20 mM, dissolved in phosphoric acid (85%)/D_2O (8:2, v/v) solution, at 288 K, at 500 MHz (^1H), (256 scans per t_1 value, expt = 832.60 min, t_m = 1 s). b) Pulse sequence: φ_1 = *x, y, -x, -y,* ψ = *x, -x*. The initial selective 180° pulses (δ_1 = 12 ms) had a Gaussian shape and were applied to the four NH amide proton resonances. G1:G2 = 70:30. The following refocusing pulse was also applied to the NH protons and was a RE-BURP (δ_2 = 30 ms). G3:G4

= 80:23. WURST wideband adiabatic inversion pulses, δ_3 = 1.5 ms, start within t_m at 0.33 and 0.83 t_m with G5:G6:G7 = 40:-60:50. Gradient pulse length was 1 ms. d = 200 µs. Relaxation delay was 1.5 s. Data matrix was acquired in the States-TPPI mode, its size was 64 × 4k. Spectral widths were 5000 Hz in F_2 and 240 Hz in F_1. Data matrix was multiplied in both dimensions by a shifted squared sine bell function (SSB = 2) before zero filling to a 128 × 2k size.

Fig. 4 a) 2D ^{1}H-^{15}N HSQC-NOESY spectrum of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr), 20 mM, dissolved in phosphoric acid (85%)/D_2O (8:2, v/v) solution, at 288 K, at 600 MHz (^{1}H), using the hsqcetgpno pulse sequence. Data matrix was recorded in Echo-Antiecho mode; its size was 256 × 2k with 200 scans per FID, a 1.5 s relaxation delay and a 1 s mixing time (t_m), resulting in a 2158.07 min recording time. The spectral widths were respectively 6010 Hz in ^{1}H dimension and 1825 Hz in ^{15}N dimension. Data matrix was multiplied in both dimensions by a shifted squared sine bell function (SSB = 2) before zero filling to a 1k × 2k size.

Fig. 6 a) 2D ^{1}H NOESY spectrum of the phosphorus-containing compound test mixture (20 mM), at 298 K, at 500 MHz (^{1}H) using the noesyesgpph pulse sequence, dissolved in phosphoric acid (85%)/DMSO-d_6 (7:3, v/v) solution. Data matrix was recorded in States-TPPI mode; 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 170.92 min recording time (expt). The spectral width was 4500 Hz in both dimensions. G1: G2 = 70: 30. Data matrix was multiplied in both dimensions by a shifted squared sine bell function (SSB = 2) before zero filling to a 1k × 4k size. 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. b) 2D ^{1}H NOESY spectrum of the same phosphorus-containing compound test mixture (20 mM), mixing time (t_m) = 0.5 s, at 500 MHz (^{1}H) using the noesygpphpp pulse sequence, dissolved in neat DMSO-d_6. Spectrum b) was recorded and processed with the same parameters as those in 6a, resulting in a 145.74 min recording time (expt).

Fig. 7 Multiplet selective excitation 1D ^{1}H NOESY spectra of the phosphorus-containing compound test mixture (1a, 1b, 1c and 1d, 20 mM) dissolved in phosphoric acid (85%)/DMSO-d_6 (7:3 v/v) solution (a, b, c and d), at 298 K, t_m = 0.5 s, at 500 MHz (^{1}H). Pulse sequence: *φ1 = x, y, -x, -y, ψ = x, -x* (Fig. 2e). 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 s. The FIDs (4k points, spectral width 5000 Hz, zero filling 64 k points). The initial selective inversion pulses excite: a) the $H_{15,19}$ (1a) proton resonances (δ_1 = 40 ms, 4k scans, experiment time (expt)

= 234.67 min); b) the H_3 (1b) proton resonance (δ_1 = 10 ms, 2k scans, expt = 115.27 min); c) the H_9 (1c) proton resonance (δ_1 = 20 ms, 2k scans, expt = 115.93 min); d) the H_{10} (1d) proton resonance (δ_1 = 40 ms, 2k scans, expt = 117.33 min).

Fig. 8 a) 2D ^{1}H-^{31}P HSQC-NOESY spectrum of the phosphorus-containing compound test mixture (20 mM) dissolved in phosphoric acid (85%)/DMSO-d_6 (7:3, v/v) solution, using the hsqcetgpno pulse sequence, at 298 K, at 500 MHz (^{1}H). Data matrix was recorded in Echo-Antiecho mode; its size was 256 × 2k with 64 scans per FID, a 1.5 s relaxation delay and a 0.5 s mixing time (t_m), resulting in a 692.97 min recording time. The spectral widths were respectively 4500 Hz in ^{1}H dimension and 12148 Hz in ^{31}P dimension. Data matrix was multiplied in both dimensions by a shifted squared sine bell function (SSB = 2) before zero filling to a 1k × 4k size. * Impurity.

Fig. 9 a) 2D ^{1}H-^{31}P HMBC, b) ^{1}H-^{31}P HSQC, c) ^{1}H-^{1}H COSY and d) ^{1}H-^{1}H NOESY (t_m = 0.5 s) spectra using the NOAH-4 BSCN sequence of phosphorus-containing compound test mixture (50 mM) dissolved in phosphoric acid (85%)/DMSO-d_6 (7:3, v/v) solution, at 298 K, at 500 MHz (^{1}H). Data matrix was recorded in Echo-Antiecho mode; its size was 2k × 2k with 120 scans per FID and 512 t_1-increments per module, a 1.5 s relaxation delay, resulting in a 3833.97 min recording time. The spectral widths were respectively 4500 Hz in ^{1}H dimension and 12148 Hz in ^{31}P dimension. After splitting, matrix was multiplied in both dimensions by a shifted squared sine bell function (SSB = 0 or 2) before zero filling to a 1k × 4k size. * Impurity.

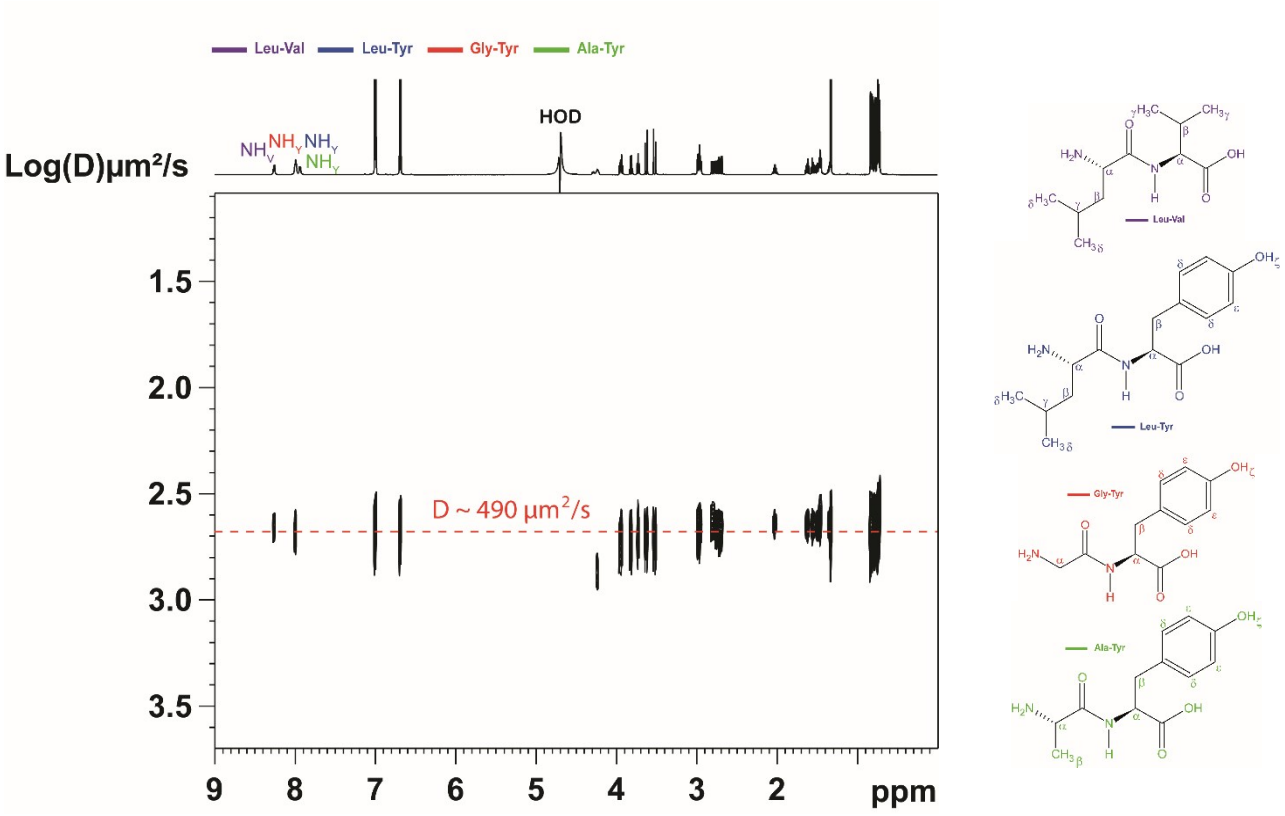


Fig. S1 2D ^{1}H DOSY spectrum of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr), 20 mM, dissolved in H_2O/D_2O (9:1 v/v), at pH = 5.0, at 298 K, at 500 MHz (^{1}H). Data were acquired by means of the stebpgp1s19 pulse sequence. The diffusion time (*Δ*) was 50 ms and the gradient pulse length (*δ*) was 2 ms. The size of the raw data set was 32 x 8 192, with 16 scans per FID, and a 1.5 s relaxation delay, resulting in a 20.52 min recording time. 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 $(\mu m)^2/s$.

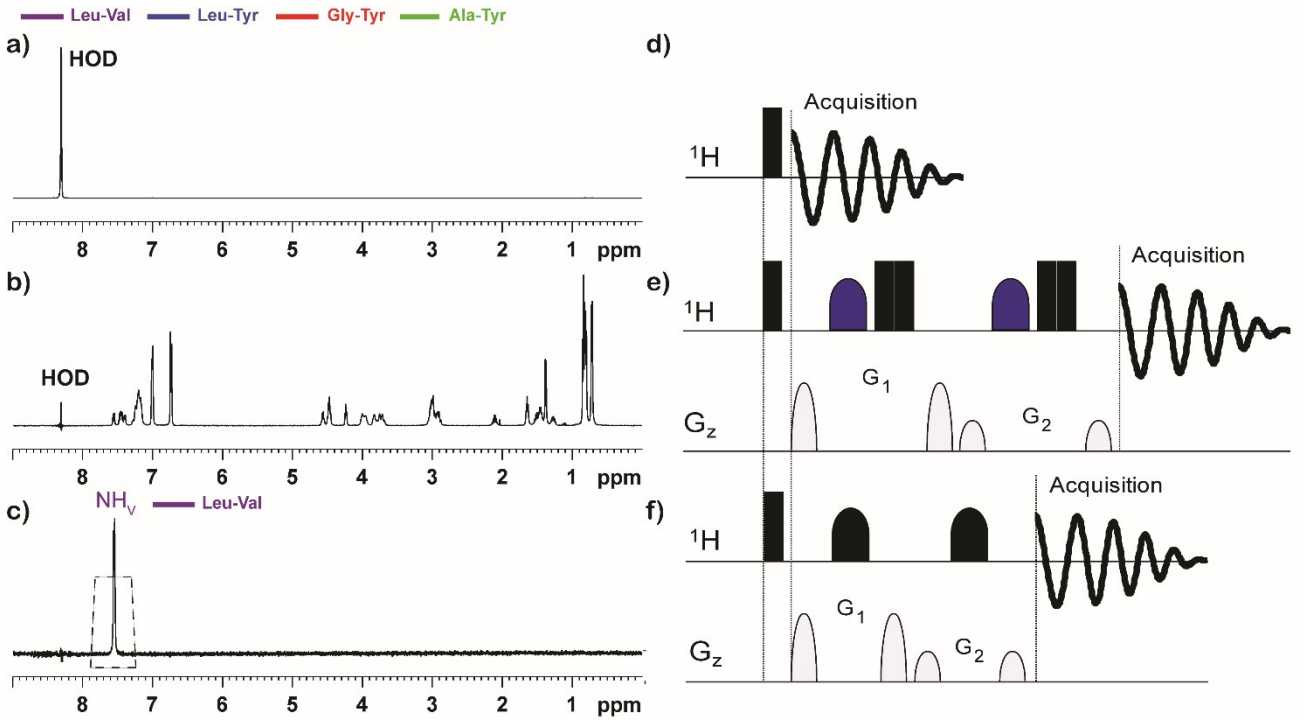


Fig. S2 1D ^{1}H spectra (8 scans) and corresponding NMR pulse sequence of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr), 20 mM, dissolved in phosphoric acid (85%)/D_2O (8:2, v/v) solution, at 288 K, at 500 MHz (^{1}H). G1:G2 = 70:30. The FIDs (32k points, spectral width = 5000 Hz) were zero-filled to 64k points. a, d) Non-selective excitation and detection. b, e) Water suppression by excitation sculpting sequence using a pair of 2 ms rectangular shaped inversion pulses applied on the water signal resonance. The length of the gradient pulses was 1 ms and their intensity 31% for the first pair and 11% for the second pair. c, f) Selective excitation of the valine amide proton doublet of Leu-Val (dotted trapezium) using a 40 ms, 1% truncated, 180° Gaussian pulse.

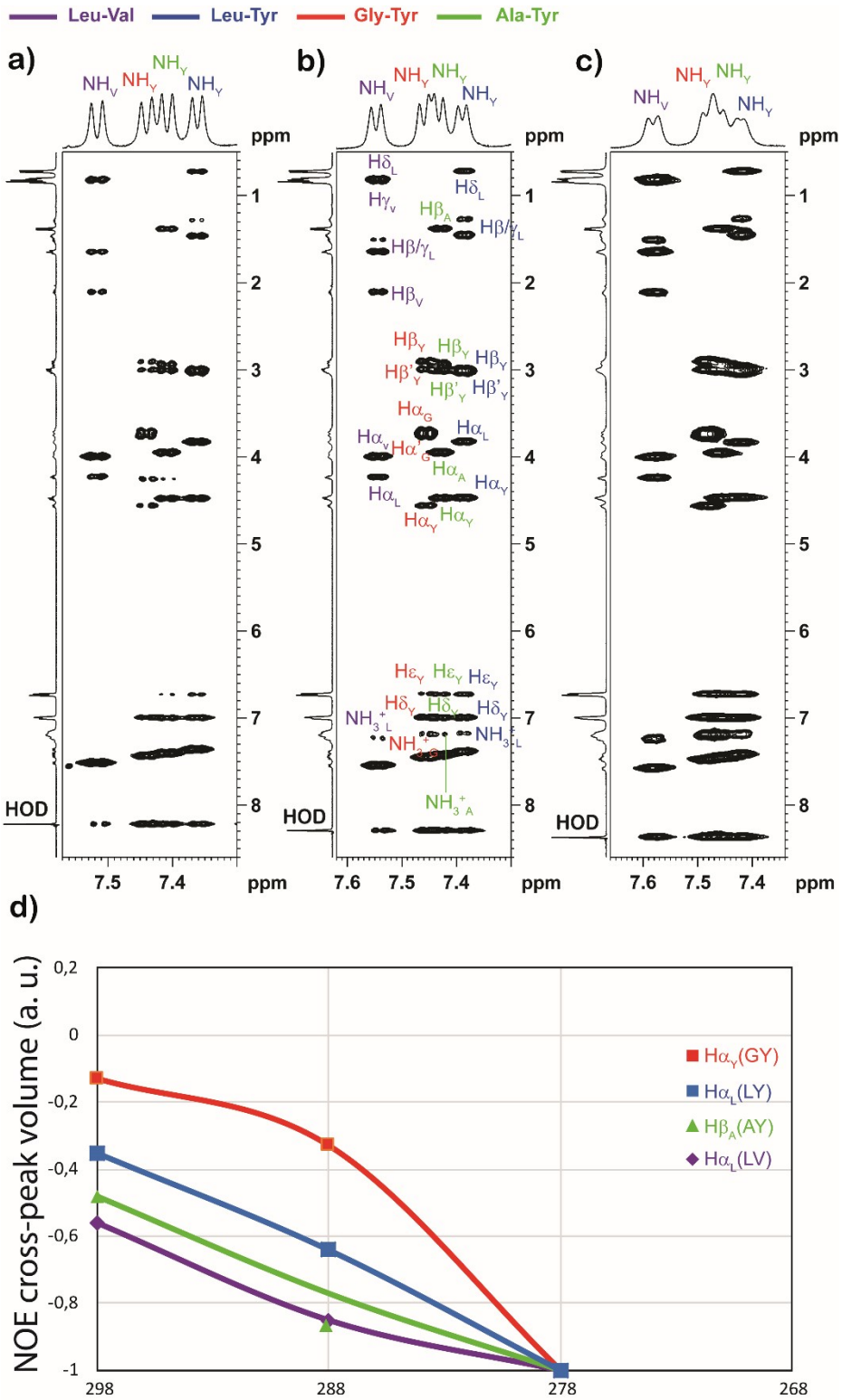


Fig. S3 a-c) Amide proton region of 2D NOESY spectra of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr), 20 mM, dissolved in phosphoric acid (85%)/D_2O (8:2, v/v) solution, mixing time (t_m) = 1 s, at 500 MHz (^1H) using the noesyesgpph pulse sequence, at a) 298 K, b) 288 K, and c) 278 K. See caption of Fig. 1a in ESI for the acquisition and processing parameters. d) Plots of the NOE cross-peak volume observed with temperature of $H\alpha_Y$(GY), $H\alpha_L$(LY), $H\beta_A$(AY) and $H\alpha_L$(LV) proton resonances of the dipeptide test mixture (polynomial regression of order 2).

Fig. S3a-c reports the evolution of amide proton NOESY cross-peaks upon sample temperature modification. Ambient and lower temperatures (298 K, 288 K, and 278 K) have been chosen in phosphoric acid (85%)/water binary solvent. The NH magnetization reaches the tyrosine H_ϵ protons at 278 K and 288 K and hardly at 298 K, nonetheless line broadening is too active at 278 K due to transverse relaxation. Consequently, all following NMR spectra in phosphoric acid (85%)/water solution have been carried out at 288 K, being the optimal temperature at which the NOESY spectrum of the dipeptide test mixture presents correlations from the amide proton resonance of each dipeptide to all proton resonances of the same dipeptide without significant signal broadening.

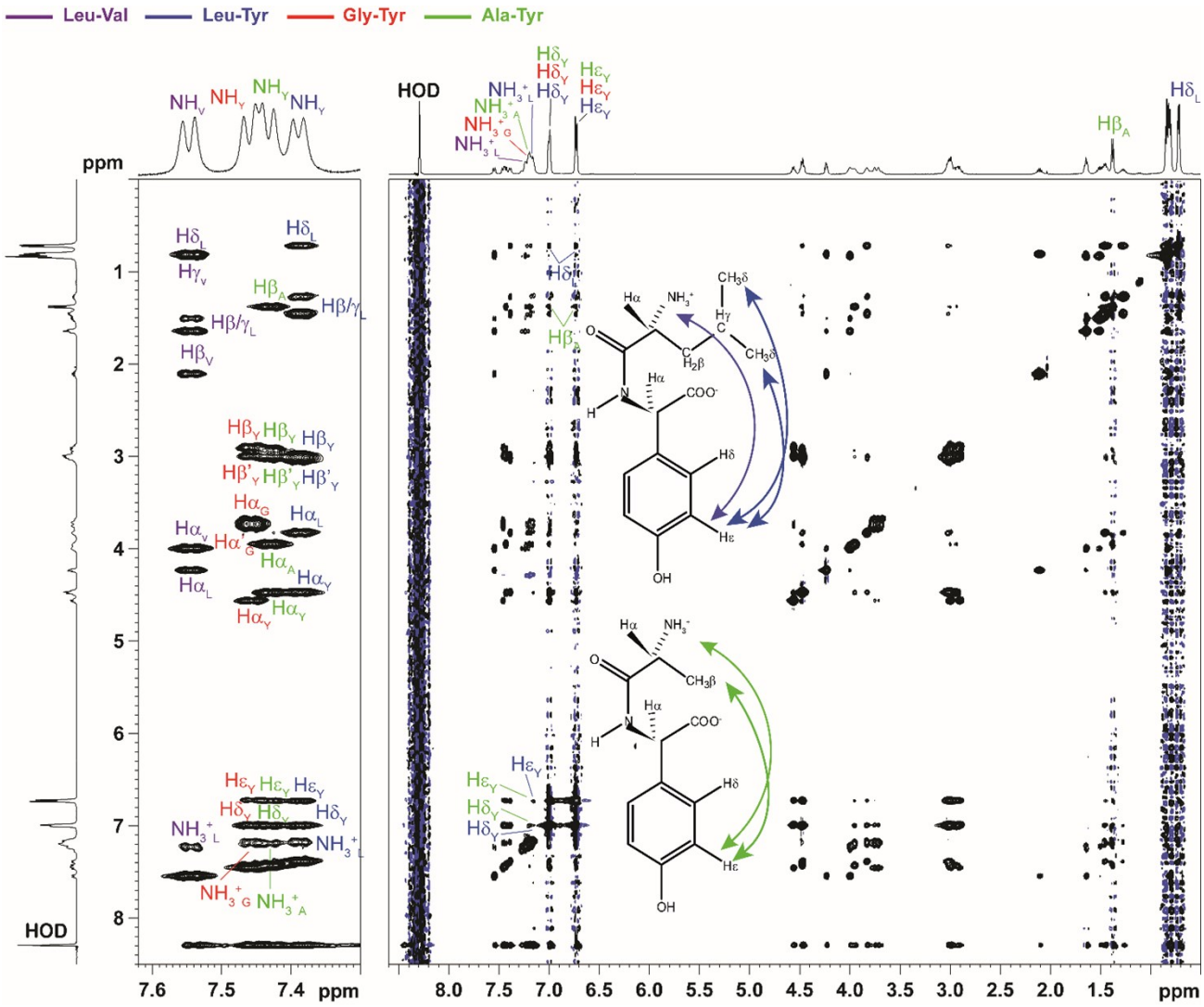


Fig. S4 2D ^{1}H NOESY spectrum of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr), 20 mM, dissolved in phosphoric acid (85%)/D_2O (8:2, v/v) solution, mixing time (t_m) = 1 s, at 288 K, at 500 MHz (^{1}H). See caption of Fig. 1a in ESI for the NMR acquisition and processing parameters.

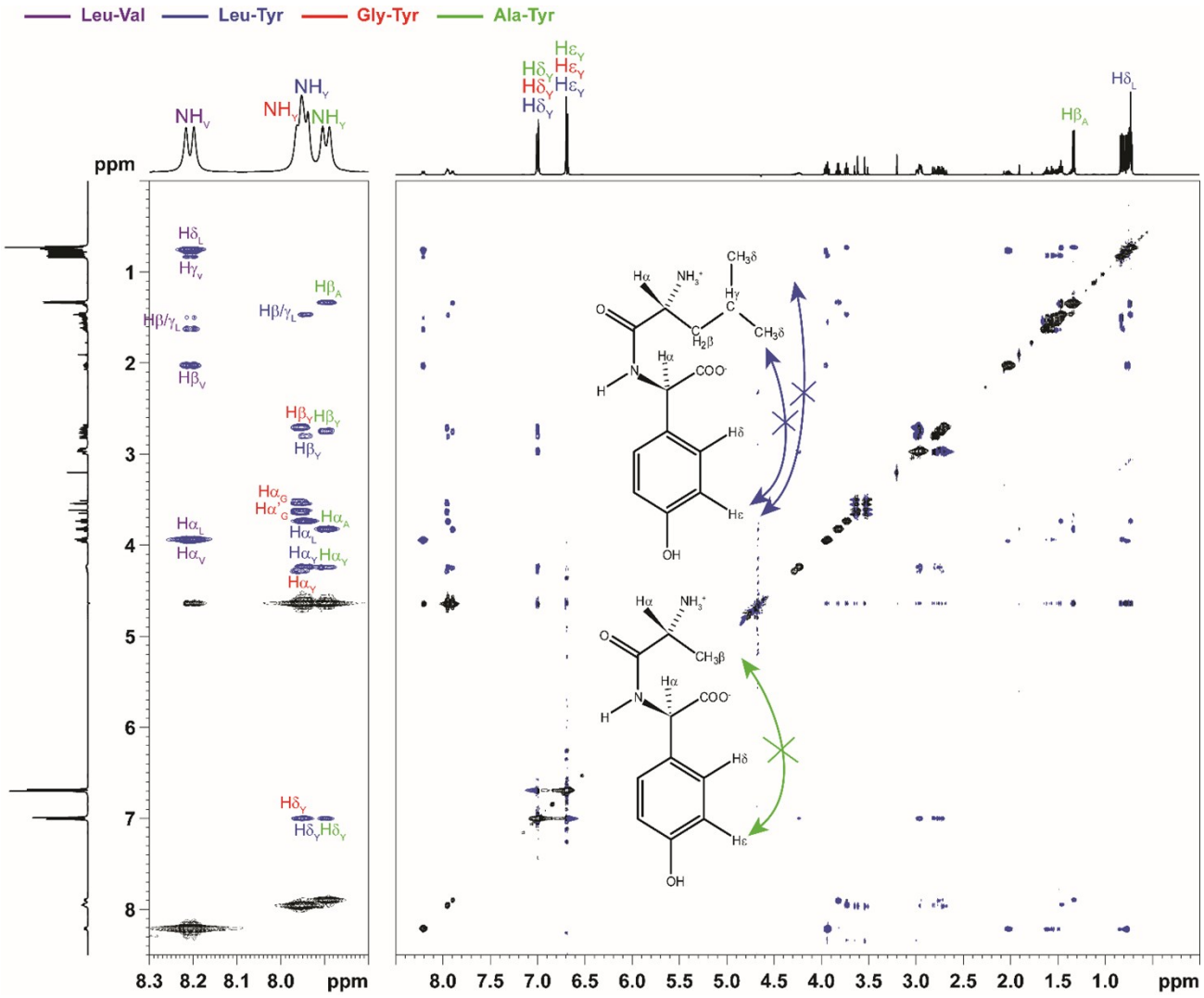


Fig. S5 2D ^{1}H NOESY spectrum of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr), 20 mM, dissolved in H_2O/D_2O (9:1 v/v), at pH = 5.0, mixing time (t_m) = 1 s, at 298 K, at 500 MHz (^{1}H). See caption of Fig. 1b in ESI for the NMR acquisition and processing parameters.

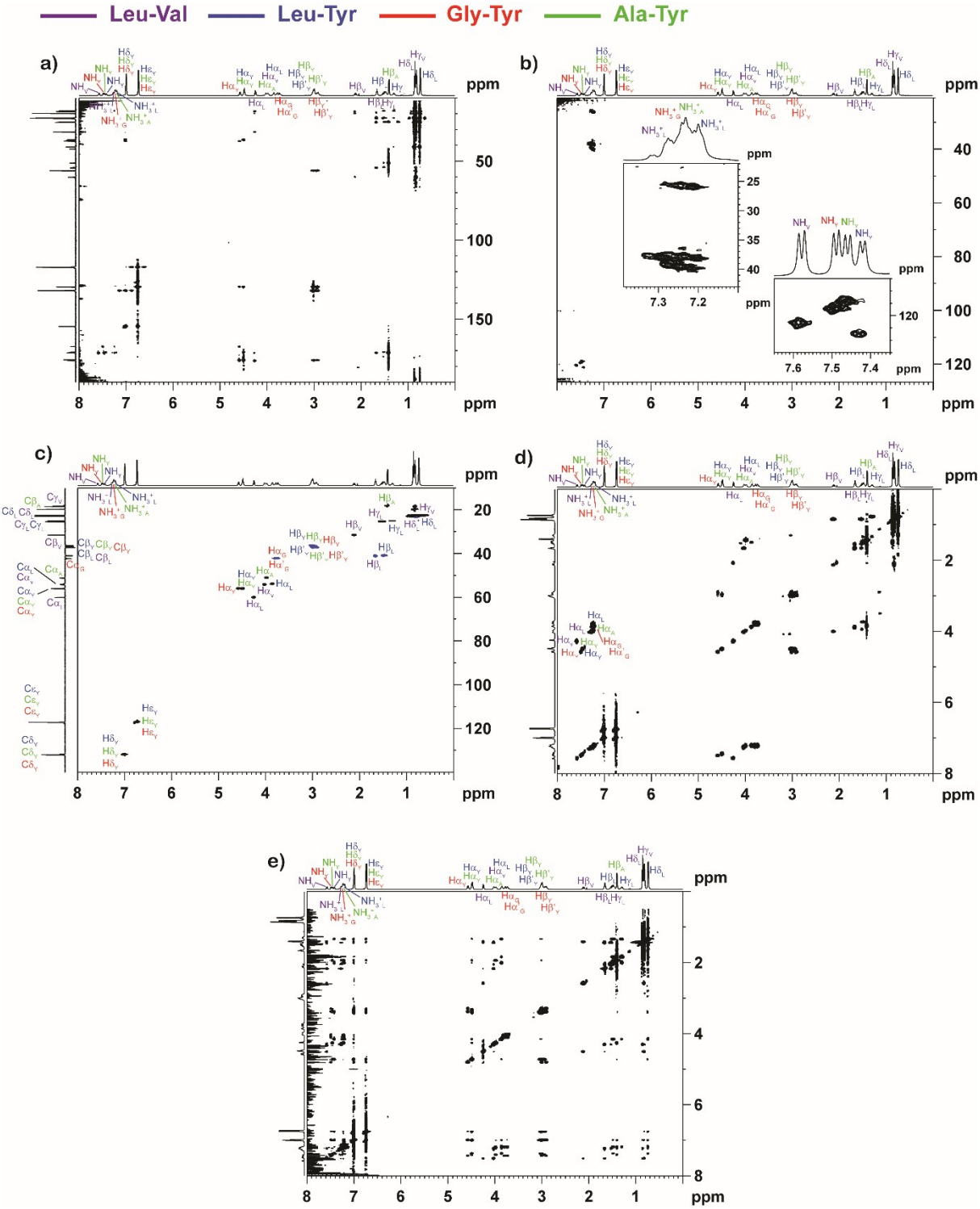
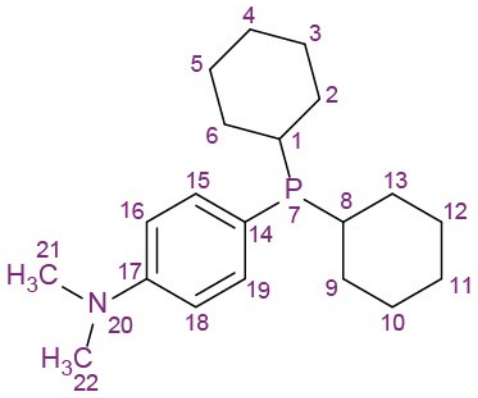


Fig. S6 a) 2D ^{1}H-^{13}C HMBC, b) ^{1}H-^{15}N HMQC, c) ^{1}H-^{13}C HSQC, d) ^{1}H-^{1}H COSY and e) ^{1}H-^{1}H NOESY (t_m = 1 s) spectra using the NOAH-5 BMSCN sequence of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr), 50 mM, dissolved in phosphoric acid (85%)/D_2O (8:2, v/v) solution, at 288 K, at 600 MHz (^{1}H). Data matrix was recorded in Echo-Antiecho mode; its size was 2.5k × 2k with 80 scans per FID and 512 t_1-increments per module, a 1.5 s relaxation delay, resulting in a 2030.73 min recording time. The spectral widths were respectively 6010 Hz in ^{1}H dimension and 27166 Hz/6264 Hz in ^{13}C/^{15}N dimension. After splitting, matrix was multiplied in both dimensions by a shifted squared sine bell function (SSB = 0 or 2) before zero filling to a 1k × 4k size.

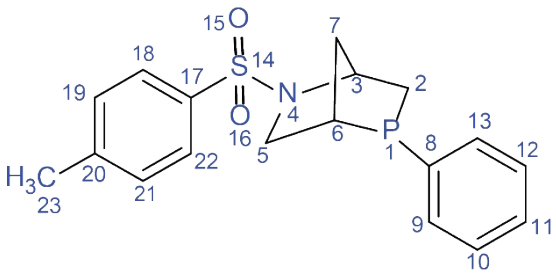
Phosphorus-containing compound structures:

1a: Dicyclohexyl(4-*N*,*N*-dimethylamino)phenyl) phosphine



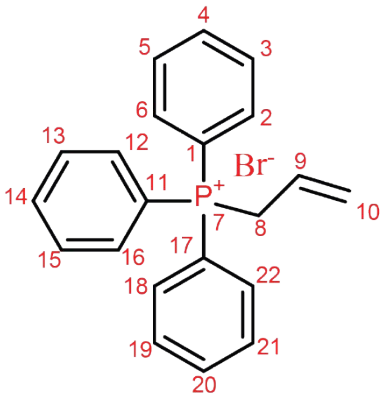
	^{1}H and ^{31}P chemical shifts in Phosphoric Acid (85%)/DMSO-d_6 (7:3, v/v), at 298 K, at 500 MHz (^{1}H)		
	1a: N°	**δ ^{1}H (ppm)**	**δ ^{31}P (ppm)**
	$H_{2a,13a}$	0.87	-
	$H_{3a,12a}$	1.02	-
	$H_{6a,9a}$	1.04	-
	$H_{4a,11a}$	1.05	-
	$H_{5a,10a}$	1.11	-
	$H_{2b,13b}$	1.34	-
	$H_{4b,11b}$	1.46	-
	$H_{3b,12b}$	1.49	-
	$H_{5b,10b}$	1.50	-
	$H_{6b,9b}$	1.73	-
	$H_{1,8}$	2.46	-
	$H_{21,22}$	3.02	-
	$H_{16,18}$	7.61	
	$H_{15,19}$	7.67	-
	P_7	-	28.36

1b: Exo-phenyl Kwon [2.2.1] bicyclic phosphine



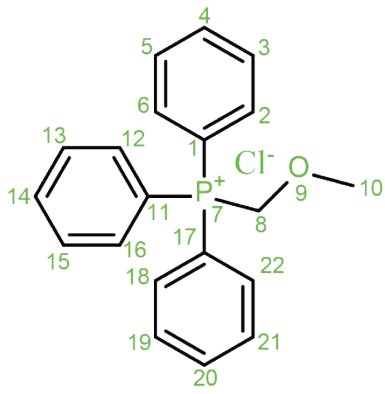
	^{1}H and ^{31}P chemical shifts in Phosphoric Acid (85%)/DMSO-d_6 (7:3, v/v), at 298 K, at 500 MHz (^{1}H)		
	1b: N°	**δ ^{1}H (ppm)**	**δ ^{31}P (ppm)**
	H_{7a}	1.18	-
	H_{7b}	1.35	-
	H_{2a}	1.90	-
	H_{23}	2.10	-
	H_{2b}	2.25	-
	H_6	2.85	-
	H_{5a}	3.14	-
	H_{5b}	3.59	-
	H_3	4.29	-
	$H_{19,21}$	7.16	-
	H_{11}	7.32	-
	$H_{18,22}$	7.42	-
	$H_{9,13}$	7.43	-
	$H_{10,12}$	7.45	
	P_1	-	63.64

1c: Allyltriphenylphosphonium bromide



^{1}H and ^{31}P chemical shifts in Phosphoric Acid (85%)/DMSO-d_6 (7:3, v/v), at 298 K, at 500 MHz (^{1}H)		
1c: N°	**δ ^{1}H (ppm)**	**δ ^{31}P (ppm)**
H_8	3.66	-
H_{10}	5.03	-
H_9	5.36	-
$H_{2,6}$	7.32	-
$H_{12,16}$	7.32	-
$H_{18,22}$	7.32	-
$H_{3,5}$	7.41	-
$H_{13,15}$	7.41	-
$H_{19,21}$	7.41	-
H_4	7.55	-
H_{14}	7.55	-
H_{20}	7.55	-
P_7	-	20.41

1d: (Methoxymethyl)triphenylphosphonium chloride



^{1}H and ^{31}P chemical shifts in Phosphoric Acid (85%)/DMSO-d_6 (7:3, v/v), at 298 K, at 500 MHz (^{1}H)		
1d: N°	**δ ^{1}H (ppm)**	**δ ^{31}P (ppm)**
H_{10}	3.21	-
H_8	4.79	-
$H_{2,6}$	7.32	-
$H_{12,16}$	7.32	-
$H_{18,22}$	7.32	-
$H_{3,5}$	7.42	-
$H_{13,15}$	7.42	-
$H_{19,21}$	7.42	-
H_4	7.58	-
H_{14}	7.58	-
H_{20}	7.58	-
P_7	-	16.71

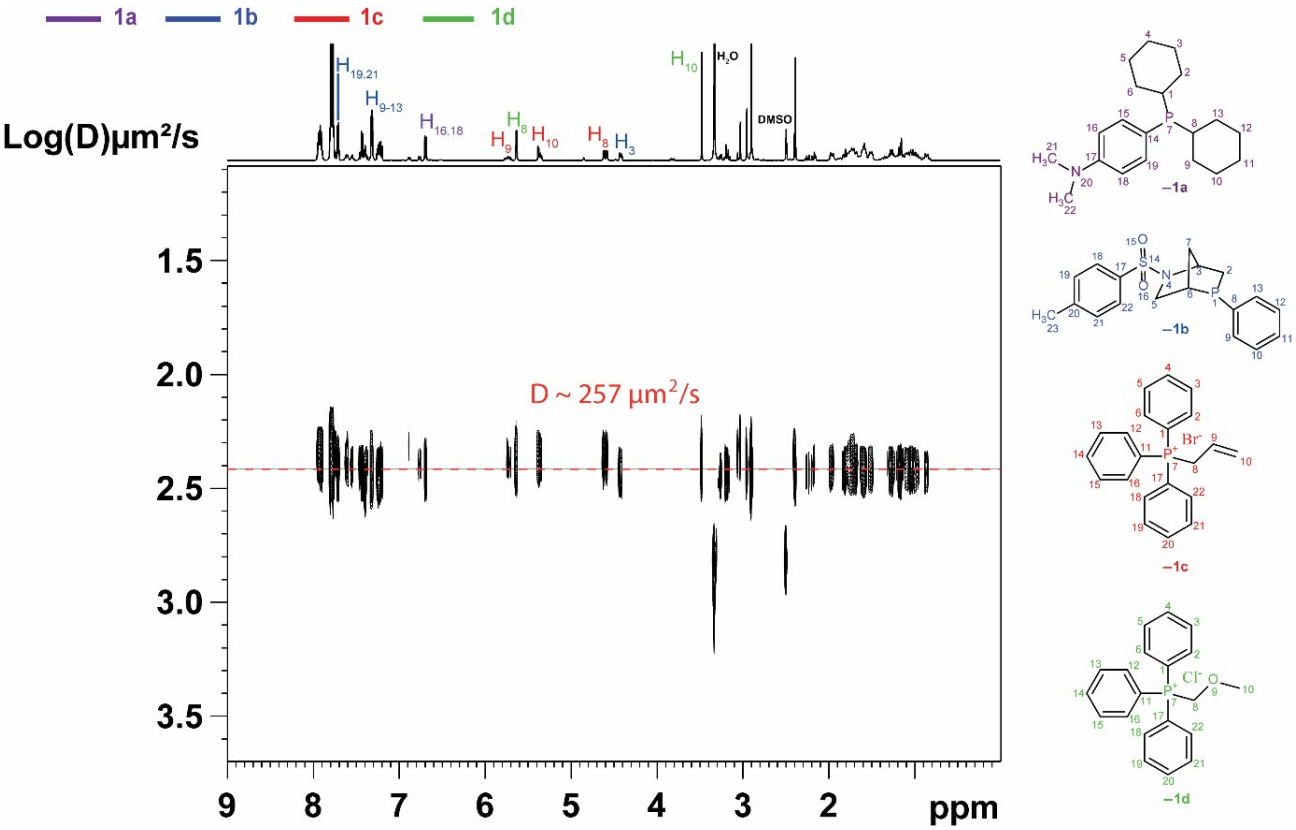


Fig. S7 2D ^{1}H DOSY spectrum of the phosphorus-containing compound test mixture (1a, 1b, 1c and 1d), 20 mM, dissolved in neat DMSO-d_6, at 298 K, at 500 MHz (^{1}H). Data were acquired by means of the ledbpgp2s pulse sequence. The diffusion time (Δ) was 70 ms and the gradient pulse length (δ) was 1.9 ms. The size of the raw data set was 32 x 16k, with 8 scans per FID, and a 15 s relaxation delay, resulting in a 72.95 min recording time. The gradient intensity values were equally spaced from 2% to 95%. 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 (µm)^2/s.

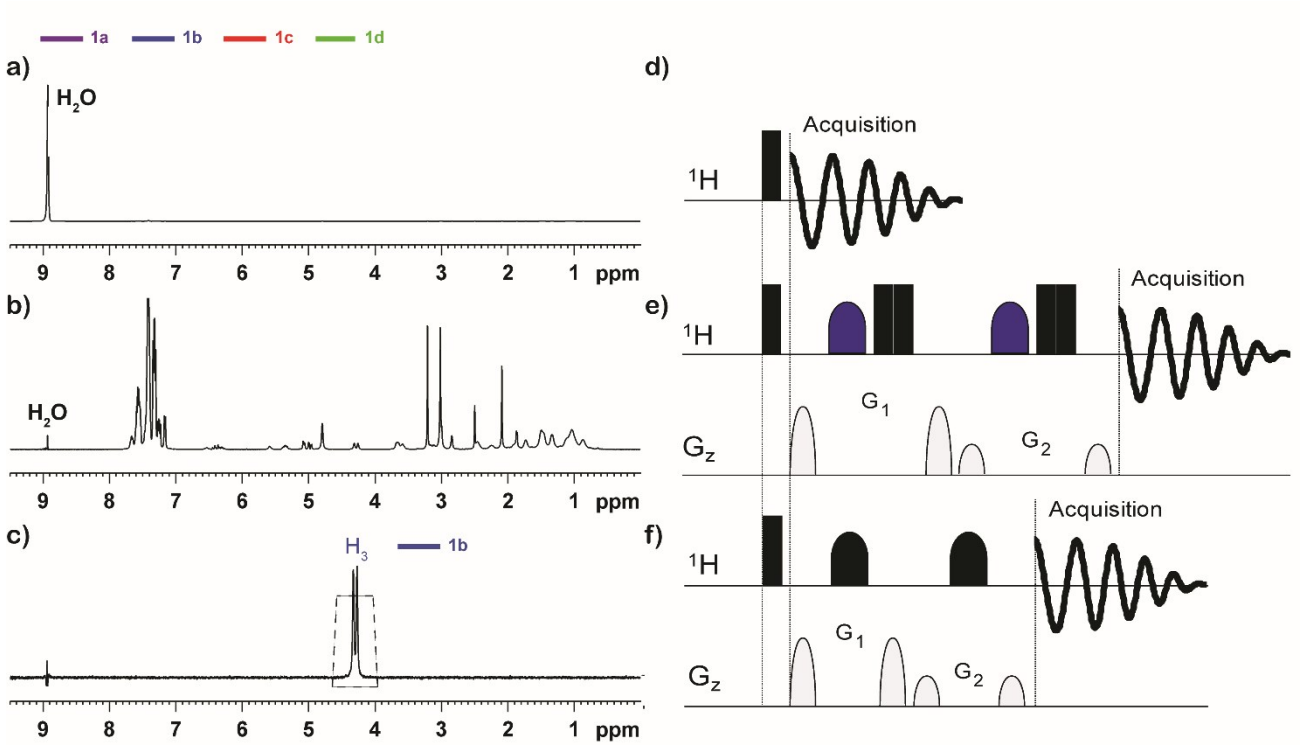


Fig. S8 1D ^{1}H spectra (8 scans) and corresponding NMR pulse sequence of the phosphorus-containing compound test mixture (1a, 1b, 1c and 1d), 20 mM, dissolved in phosphoric acid (85%)/DMSO-d_6 (7:3, v/v) solution, at 298 K, at 500 MHz (^{1}H). G1:G2 = 70:30. The FIDs (32k points, spectral width = 5000 Hz) were zero-filled to 64k points. a, d) Non-selective excitation and detection. b, e) Water suppression by excitation sculpting sequence using a pair of 2 ms rectangular shaped inversion pulses applied on the water signal resonance. The length of the gradient pulses was 1 ms and their intensity 31% for the first pair and 11% for the second pair. c, f) Selective excitation of the proton H3 of 1b (dotted trapezium) using a 10 ms, 1% truncated, 180° Gaussian pulse.

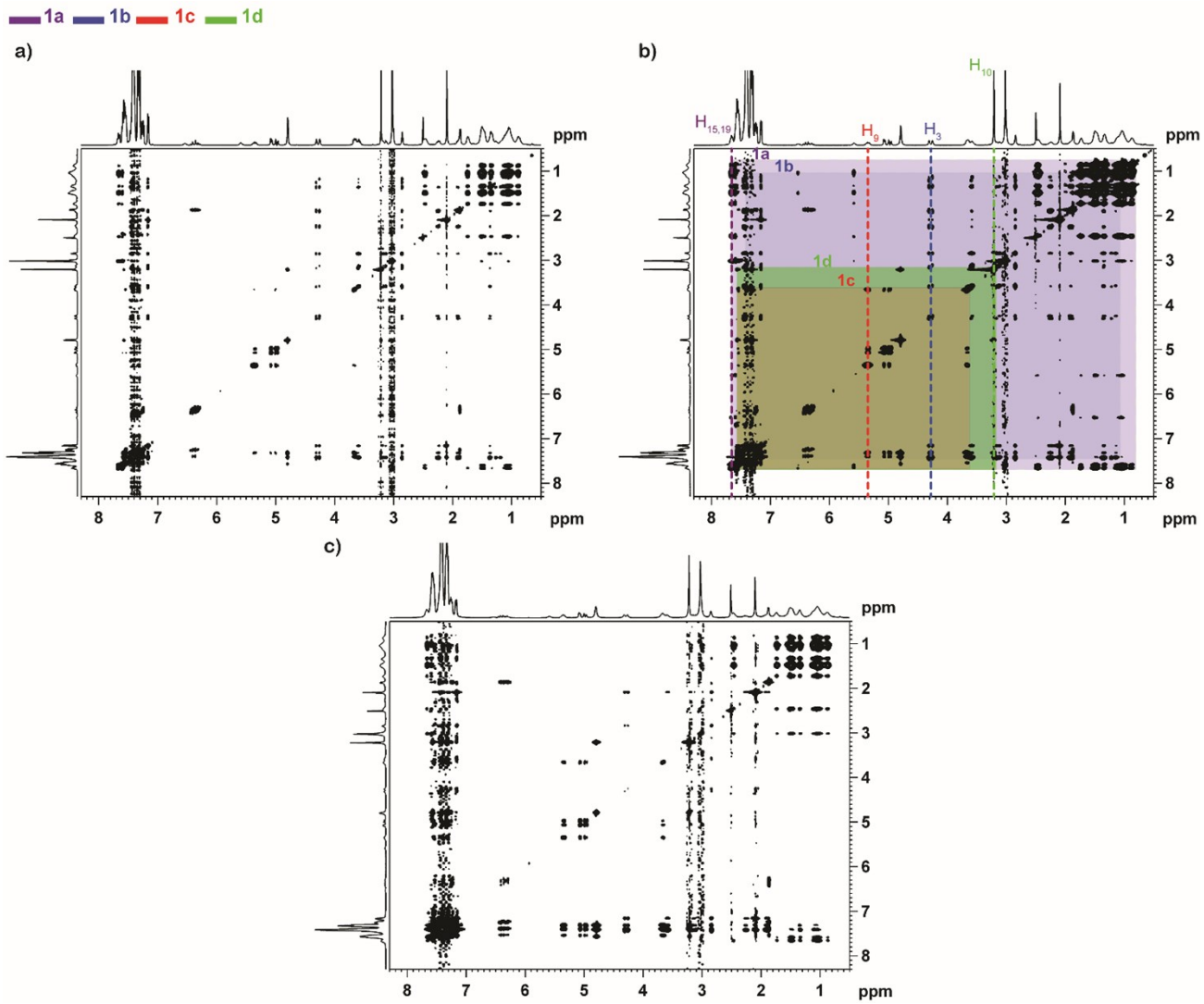


Fig. S9 2D ^{1}H NOESY spectra of the phosphorus-containing compound test mixture (1a, 1b, 1c and 1d), 20 mM, dissolved in phosphoric acid (85%)/DMSO-d_6 (7:3, v/v) solution, mixing time (t_m) = 0.5 s, at a) 308 K, b) 298 K and c) 288 K, at 500 MHz (^{1}H) using the noesyesgpph pulse sequence.

Fig. S9 reports the evolution of proton NOESY cross-peaks upon sample temperature change. Full spin diffusion over each mixture component is only observed at 298 K. Nonetheless, at 288 K, efficient spin diffusion is still observed but the spectral resolution is damaged due to active transverse relaxation inducing peak broadening. Consequently, all *ViscY* NMR experiments in phosphoric acid (85%)/DMSO-d_6 (7:3, v/v) solution have been carried out at 298 K, being the optimal temperature at which the NOESY spectrum of every phosphorus-containing compound within mixture present correlations between all proton resonances of each compound without signal broadening.

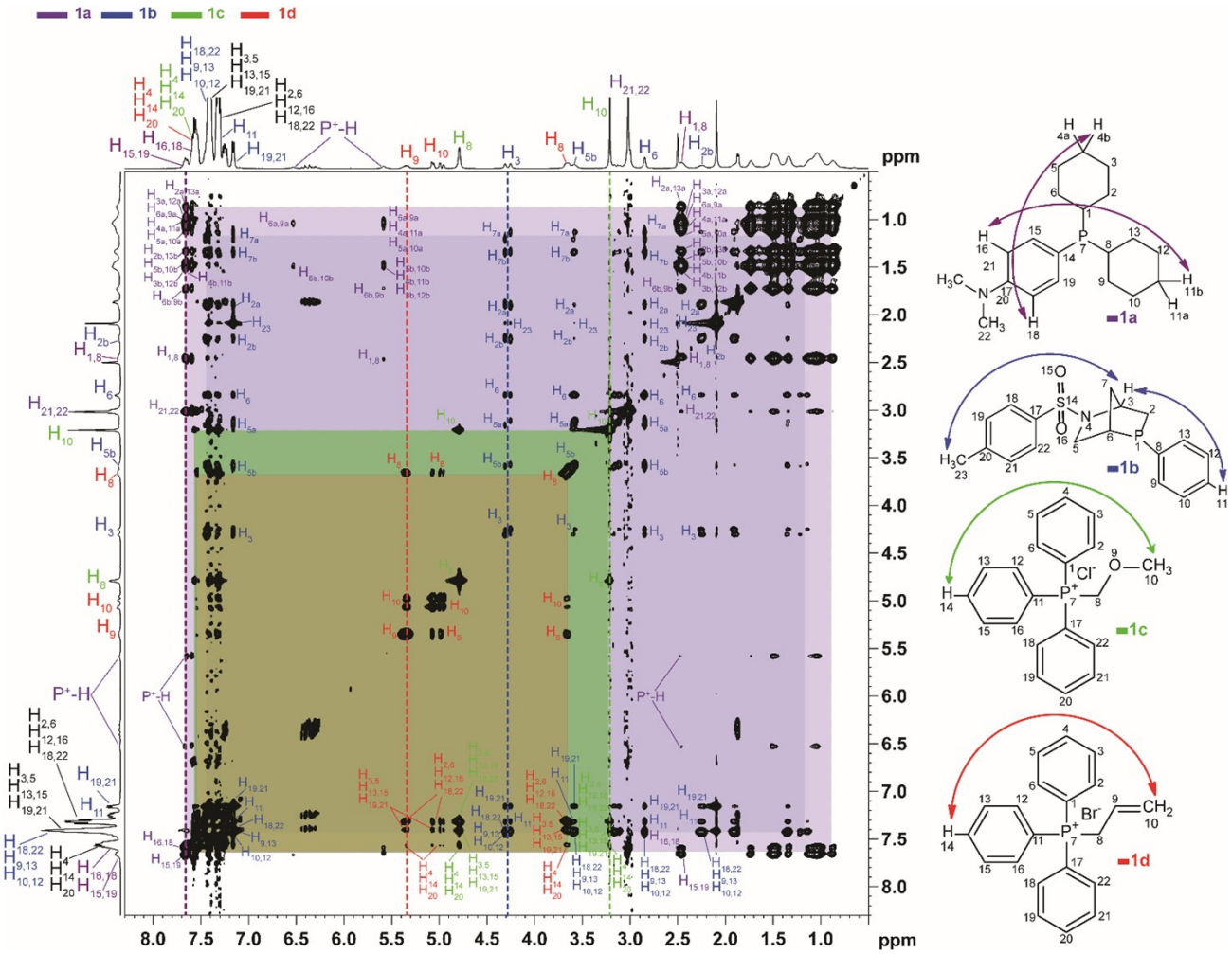


Fig. S10 2D ^{1}H NOESY spectrum of the phosphorus-containing compound test mixture (1a, 1b, 1c and 1d), 20 mM, dissolved in phosphoric acid (85%)/DMSO-d_6 (7:3, v/v) solution, mixing time (t_m) = 0.5 s, at 298 K, at 500 MHz (^{1}H) using the noesyesgpph pulse sequence. See caption of Fig. 6a in ESI for the NMR acquisition and processing parameters.

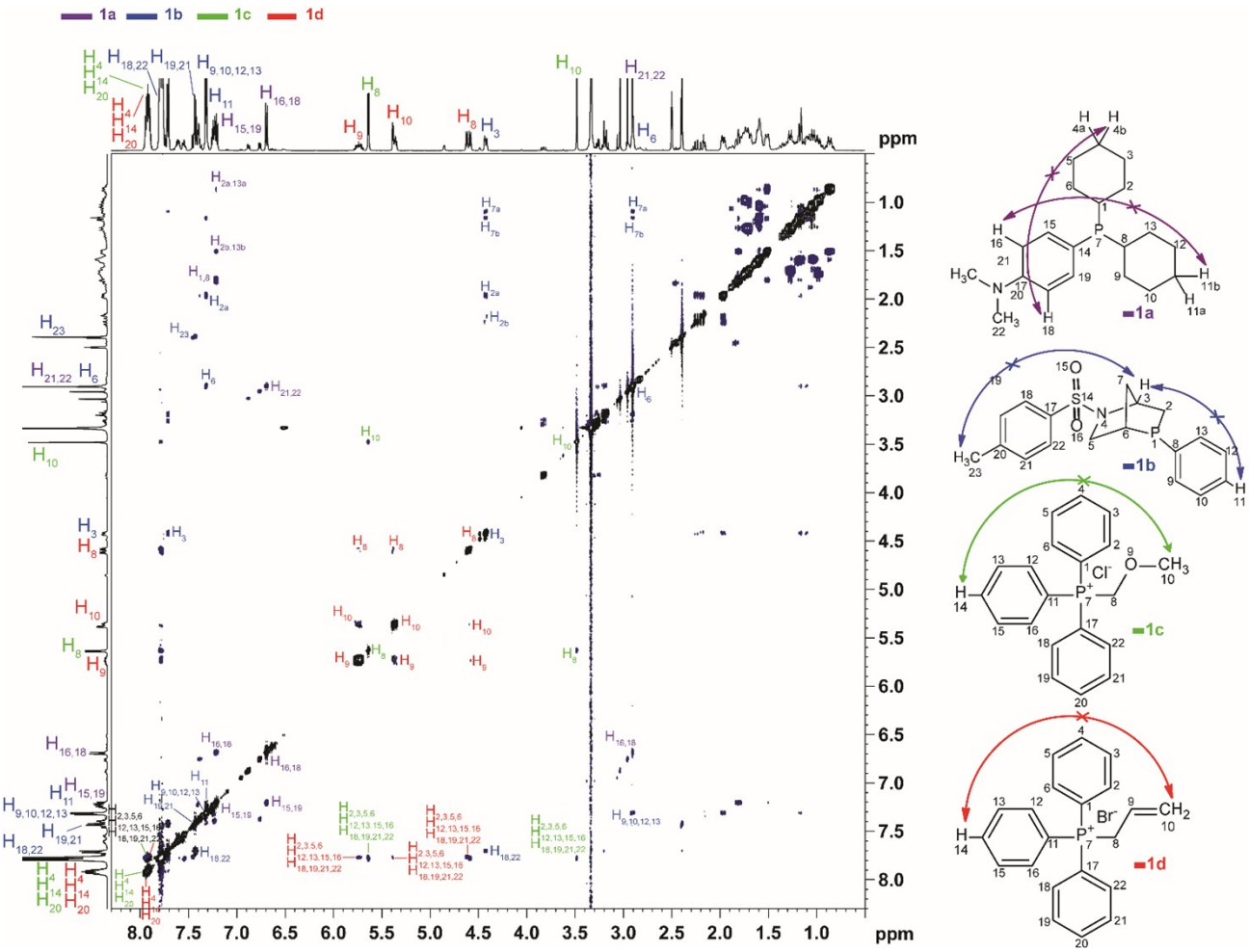


Fig. S11 2D ^{1}H NOESY spectrum of the phosphorus-containing compound test mixture (1a, 1b, 1c and 1d), 20 mM, dissolved in neat DMSO-d_6, mixing time (t_m) = 0.5 s, at 298 K, at 500 MHz (^{1}H) using the noesygpphpp pulse sequence. See caption of Fig. 6b in ESI for the NMR acquisition and processing parameters.