***ELECTRONIC SUPPLEMENTARY INFORMATION* for**

Polar Mixture Analysis by NMR under Spin Diffusion Conditions in Viscous Sucrose Solution and Agarose Gel

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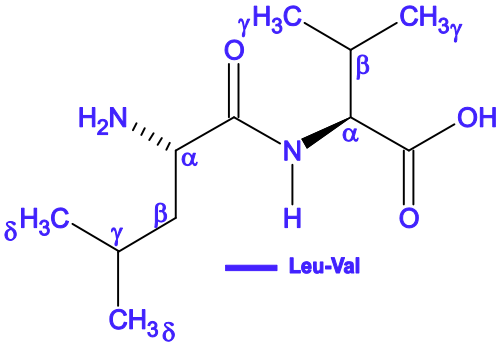
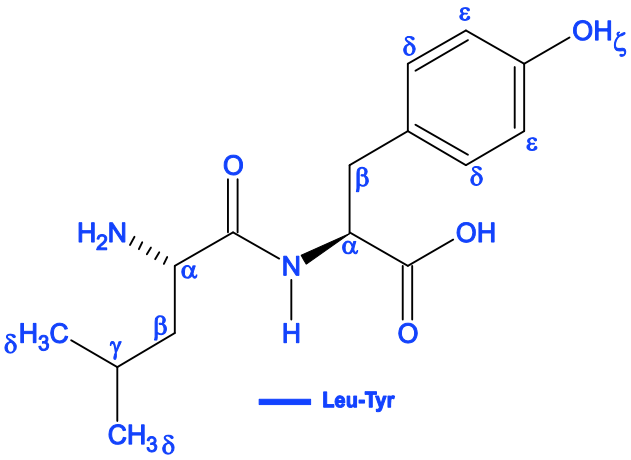
Peptide structures:

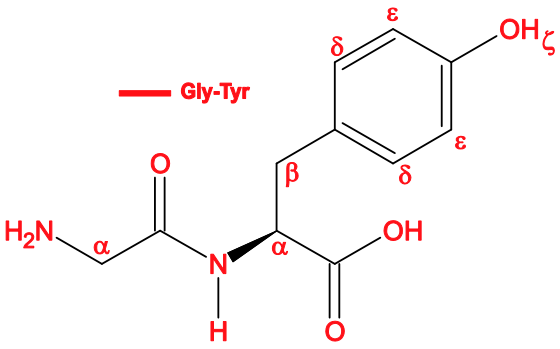
Leucine-Valine = Leu-Val = LV



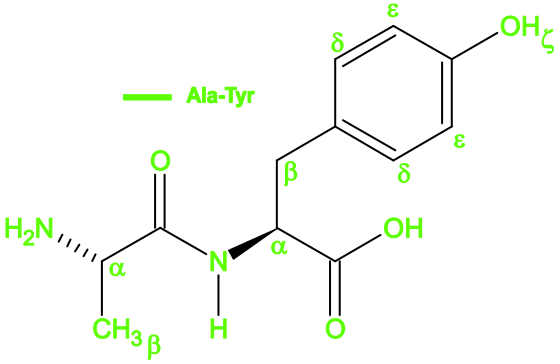
Leucine-Tyrosine = Leu-Tyr = LY



Glycine-Tyrosine = Gly-Tyr = GY



Alanine-Tyrosine = Ala-Tyr = AY



Additional NMR data acquisition and processing parameters for Figure 1 up to Figure 5:

Fig. 1. 1D proton spectra (8 scans), at 600 MHz (^{1}H), and corresponding NMR pulse sequence of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr) dissolved in sucrose/D_2O (5:5, w/w + 10% D_2O, v/v) (a, b, c, 283 K). G1: G2 = 70: 30. The FIDs (32k points, spectral width = 6 010 Hz) were processed with LB = 0.3 Hz and zero-filled to 32k points. a, d) Non-selective excitation and detection. b, e) Selective detection of two resonance bands. The 3 ms I-BURP-2 pulses cover 1 560 Hz (dotted trapezium). The "1" and "2" labels respectively indicate their application to the high and low chemical shift regions. c, f) Selective excitation of the valine amide proton doublet of Leu-Val (dotted trapezium) using a 10 ms, 1% truncated, 180° Gaussian pulse.

Fig. 2. a) Amide proton region of band-selective detection 2D NOESY spectrum of dipeptide test mixture (10 mM), at 600 MHz (^{1}H) a) dissolved in sucrose/H_2O (5:5, w/w + 10% D_2O, v/v), at 283 K. 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 165.97 min recording time (expt). Shaped pulses and gradient pulses were identical to those in Figures 1b and 1e in ESI. The NOESY part of the sequence was adapted from the noesyph pulse sequence. The spectral width was 6 009.615 Hz in both dimensions. G1: G2 = 70: 30. Data matrix was multiplied in both dimensions by a shifted sine bell function (SSB = 2) before zero filling to a 1k × 4k size. b) Amide proton region of 2D NOESY spectrum of dipeptide test mixture dissolved in H_2O/D_2O (9:1, v/v), at pH = 5,0, at 298 K. Spectrum b) was recorded and processed with the same parameters as those in 2a using the noesyesgpph pulse sequence. A pair of 2 ms rectangular shaped inversion pulses was applied on the water signal resonance. The length of the water suppression gradient pulses was 1 ms and their intensity 31% for the first pair and 11% for the second pair. The red frames correspond to spectral regions of interest in which water as solvent has a major effect on the number and sign of observable NOESY cross peaks.

Fig. 3. Multiplet selective excitation 1D ^{1}H NOESY spectra of the dipeptide test mixture (10 mM) dissolved in sucrose/H_2O (5:5, w/w + 10% D_2O, v/v) (a, b, c, d, 283 K), t_m = 0.5 s, at 600 MHz (^{1}H). e) Pulse sequence: $\varphi_1 = x, y, -x, -y, \psi = x, -x$. 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 (16k points, spectral width 6 009.615 Hz) were multiplied by an

exponential function (LB = 0.3) before zero filling to 64k points. The initial selective inversion pulses excite: a) the NH_V(LV) proton resonance (δ_1 = 10 ms, 2 048 scans, experiment time (expt) = 134.65 min); b) the $H\delta_L$(LY)/$H\delta_L$(LV)/$H\gamma_V$(LV) proton resonances (δ_1 = 5 ms, 512 scans, expt = 33.67 min); c) the $H\delta_Y$(LY)/$H\delta_Y$(GY)/$H\delta_Y$(AY) proton resonances (δ_1 = 10 ms, 800 scans, expt = 25.32 min); d) the $H\alpha_Y$(GY) proton resonance (δ_1 = 60 ms, 4 096 scans, expt = 275.07 min).

Fig. 4. a) F_1 band selective F_1 decoupled 2D NOESY spectrum of the dipeptide test mixture (10 mM) dissolved in sucrose/H_2O (5:5, w/w + 10% D_2O, v/v), at 283 K, at 600 MHz (^{1}H), (64 scans per t_1 value, expt = 222.54 min, t_m = 0.5 s). b) Pulse sequence: *φ1 = x, y, -x, -y, ψ = x, -x*. The initial selective 180° pulses (δ_1 = 3 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 = 24 ms). G3: G4 = 80: 23. WURST wideband adiabatic inversion pulses, δ_3 = 1 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 2 s. Data matrix was acquired in the States-TPPI mode, its size was 64 × 4k. Spectral widths were 5 699.088 Hz in F2 and 312 Hz in F1. Data matrix was multiplied in both dimensions by a shifted sine bell function (SSB = 2) before zero filling to a 128 × 4k size.

Fig. 5. a) 2D ^{1}H-^{15}N HSQC-NOESY spectrum of the dipeptide test mixture (20 mM) dissolved in sucrose/H_2O (5:5, w/w + 10% D_2O, v/v), at 283 K, using the hsqcetgpnosp pulse sequence with solvent multiple presaturation, at 600 MHz (^{1}H). Data matrix was recorded in Echo-Antiecho mode; its size was 128 × 2k with 512 scans per FID, a 1.5 s relaxation delay and a 0.5 s mixing time (t_m), resulting in a 2451,8 min recording time. The spectral widths were respectively 6009.615 Hz in ^{1}H dimension and 1824.63 Hz in ^{15}N dimension. Data matrix was multiplied in both dimensions by a shifted sine bell function (SSB = 2) before zero filling to a 1k × 2k size. Comparison of four ^{1}H horizontal slices extracted from the 2D ^{1}H-^{15}N HSQC-NOESY at 123.93 (b, b', Ala-Tyr, green row), 124.49 (c, c', Gly-Tyr, red row), 126.01 (d, d', Leu-Tyr, blue row), and 126.91 ppm (e, e', Leu-Val, purple row) with the conventional 1D proton spectra of each pure dipeptide dissolved (20 mM) in sucrose/H_2O (5:5, w/w + 10% D_2O, v/v), at 283 K, at 600 MHz (^{1}H).

Fig. 6. 1D ^{1}H spectra of the dipeptide test mixture (10 mM) dissolved a) in H_2O/D_2O (9:1, v/v), at 298 K, at 600 MHz (^{1}H) and b) in agarose gel 1%, at 273 K, at 600 MHz (^{1}H) with water suppression using excitation sculpting.

Fig. 7. Multiplet selective excitation 1D ^{1}H NOESY spectra of the dipeptide test mixture (10 mM) dissolved in agarose gel 1% (a, b, c, 273 K), t_m = 0.5 s, at 600 MHz (^{1}H). The initial selective inversion pulses excite: a) the $H\beta_Y$(AY) proton resonance (δ_1 = 40 ms, 2 800 scans, expt = 186,25 min; b) the $H\delta_Y$(LY)/$H\delta_Y$(GY)/$H\delta_Y$(AY) proton resonances (δ_1 = 10 ms, 1 024 scans, expt = 67,25 min); c) the $H\delta_L$(LY)/$H\delta_L$(LV)/$H\gamma_V$(LV) proton resonances (δ_1 = 5 ms, 1 024 scans, expt = 67,08 min); Pulse sequence: *φ1 = x, y, -x, -y, ψ = x, -x* (see Figure 3e).

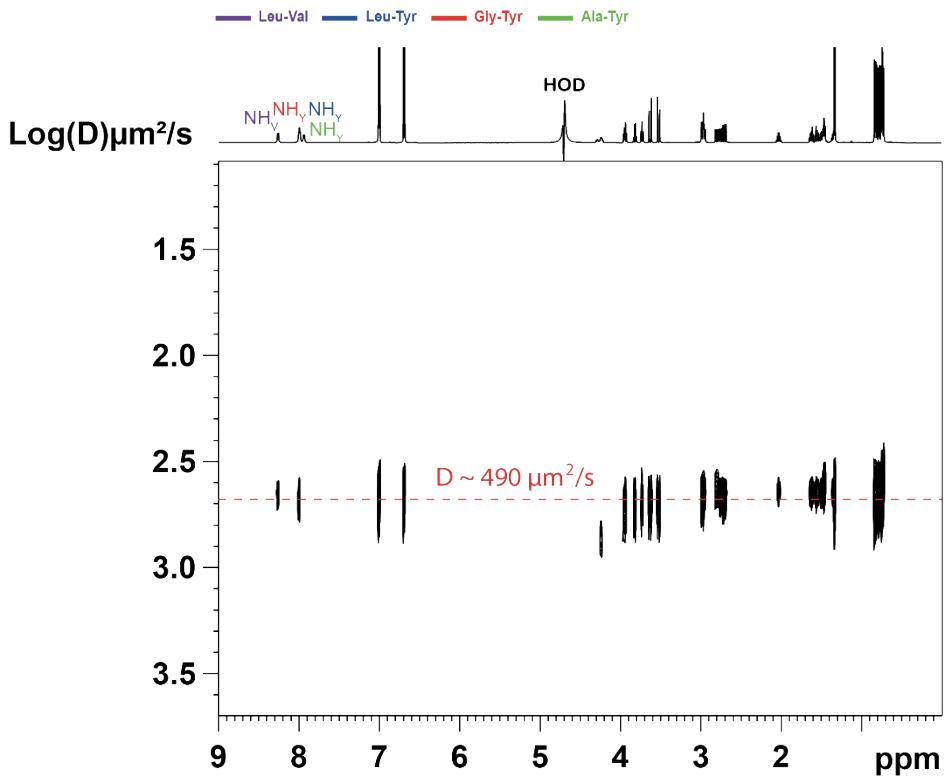


Fig. S-1. 2D ^{1}H DOSY spectrum of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr), 10 mM, dissolved in H_2O/D_2O (9:1, v/v), at pH = 5.0, at 298 K, at 600 MHz (^{1}H). Data were acquired by means of the stebpgp1s19 pulse sequence. The diffusion time (Δ) was 50 ms and the gradient pulse length (δ) was 1.2 ms. The size of the raw data set was 32 x 16 384, with 8 scans per FID, and a 10 s relaxation delay, resulting in a 49.78 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 $(\mu m)^2/s$.

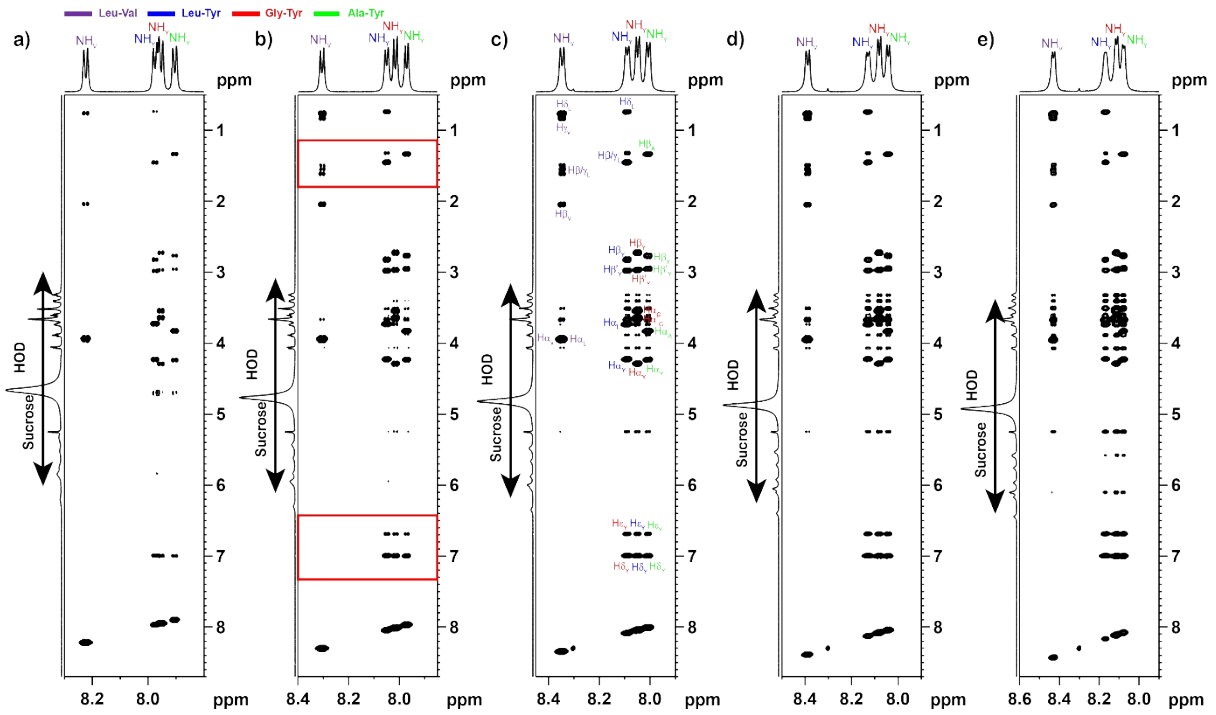


Fig. S-2. Amide proton region of band-selective detection 2D NOESY spectra of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr), 10 mM, dissolved in sucrose/H_2O (5:5, w/w + 10% D_2O, v/v), at a) 298 K, b) 288 K, c) 283 K, d) 278 K, and e) 273 K, at 600 MHz (^1H). The red frames correspond to spectral regions of interest in which temperature variations show a major effect on signal intensity. See caption of Figure 2a in ESI for the acquisition and processing parameters.

Figure S-2 reports the evolution of the 2D NOESY correlations of the amide protons in the dipeptide test mixture upon sample temperature modification. The spectra reveal the expected intra-molecular correlations but also present a certain amount of magnetization transfer between the labile dipeptide amide protons and sucrose/water blend protons whose intensity is related to temperature. Ambient and lower temperatures (298 K, 288 K, 283 K, 278 K and 273 K) have been tested in sucrose solution. The NH magnetization weakly reaches the tyrosine Hε protons at 288 K and not at all at 298 K. At 278 K, the effect of spin diffusion is still active but the NH proton resolution is damaged and even more at 273 K due to transverse relaxation inducing peak broadening. Consequently, all following NMR spectra in sucrose solution have been recorded at 283 K, being the optimal temperature at which the NOESY spectrum of the dipeptide test mixture shows correlations from the amide proton resonance of each dipeptide to all the proton resonances of the same dipeptide without significant signal broadening.

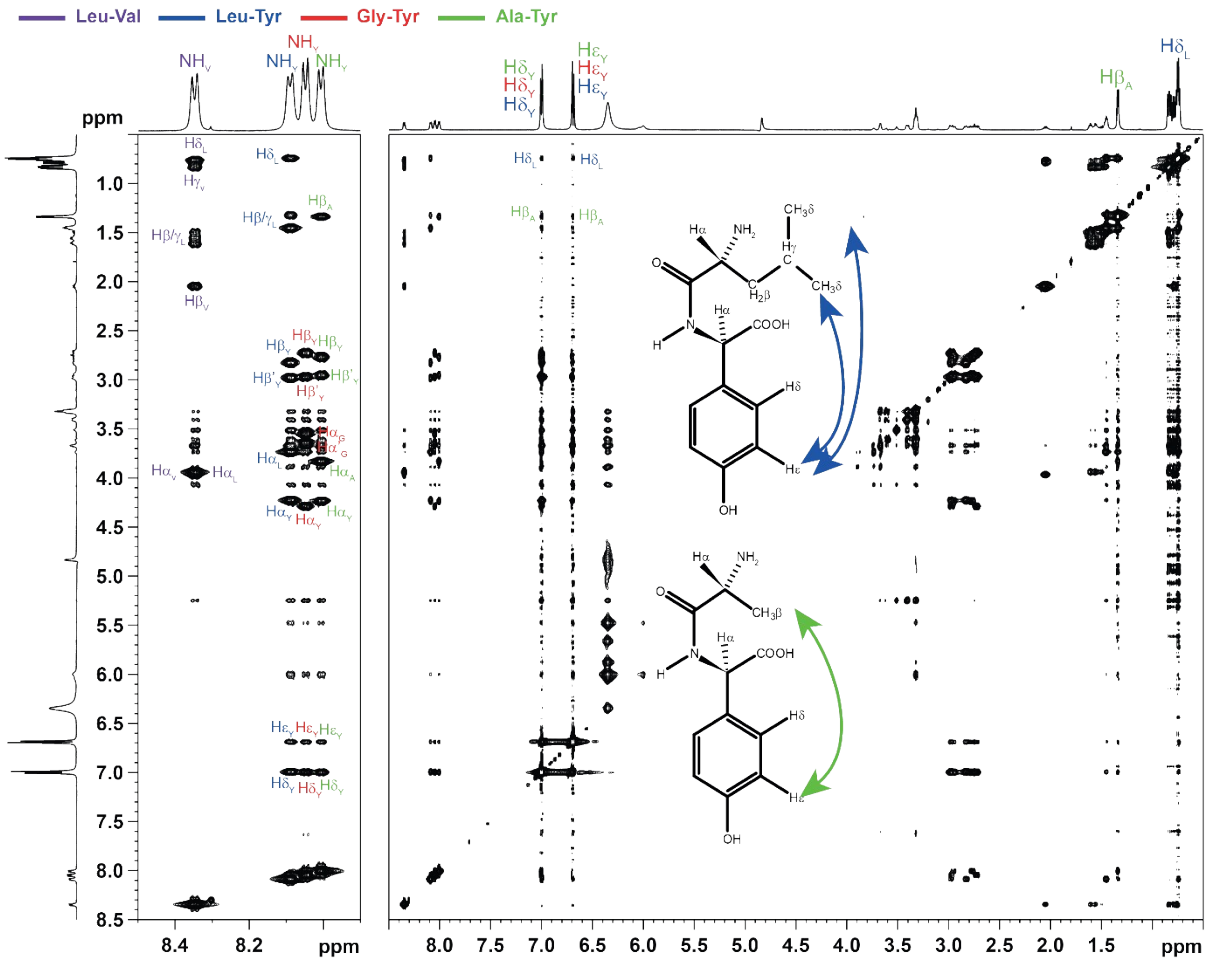


Fig. S-3. Band-selective detection 2D ^{1}H-^{1}H NOESY spectrum of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr), 10 mM, in sucrose/H_2O (5:5, w/w + 10% D_2O, v/v), at 283 K, at 600 MHz (^{1}H). See caption of Figure 2a in ESI for the NMR acquisition and processing parameters.

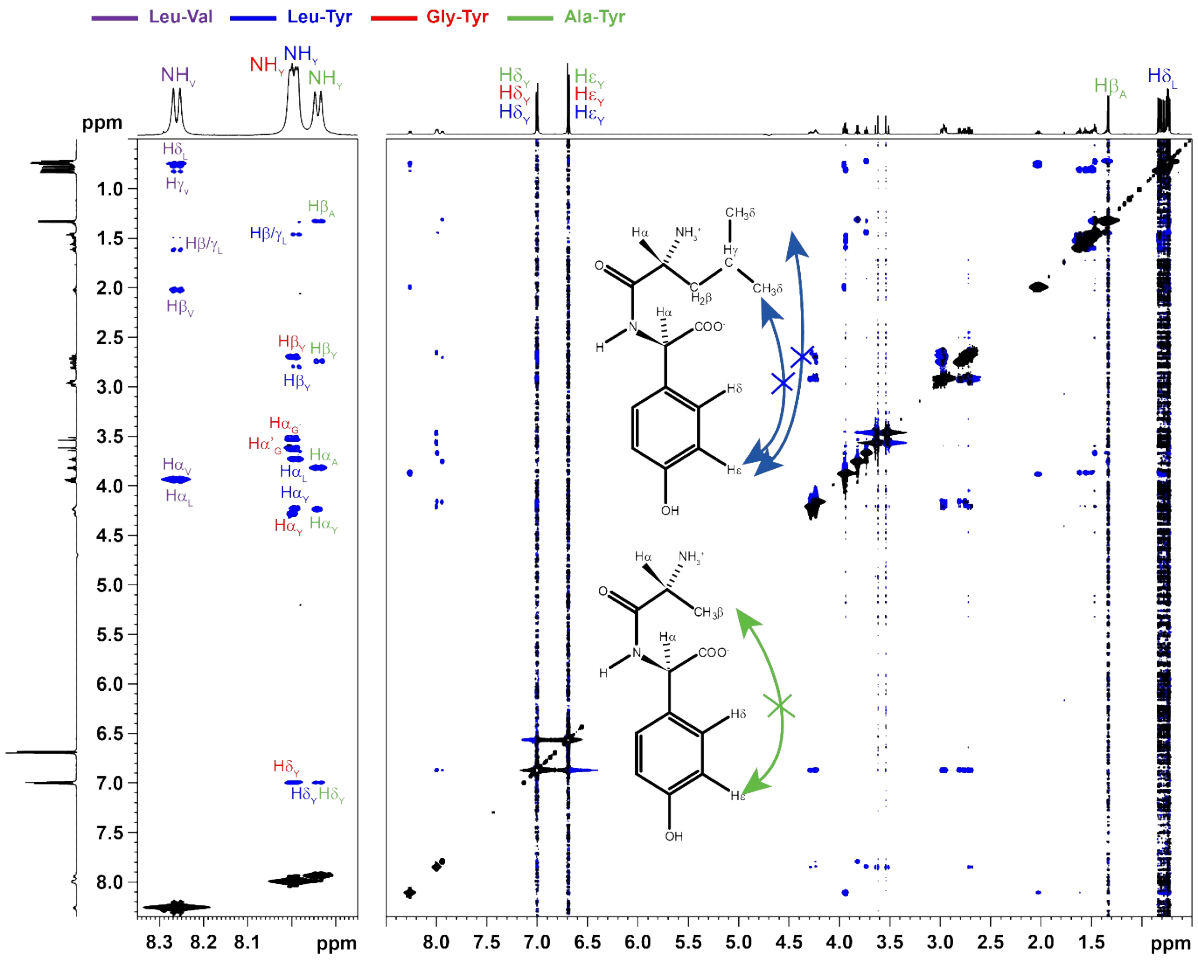


Fig. S-4. 2D ^{1}H-^{1}H NOESY spectrum of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr), 10 mM, dissolved in H_2O/D_2O (9:1, v/v), at pH = 5.0, at 298 K, at 600 MHz (^{1}H). See caption of Figure 2b in ESI for the NMR acquisition and processing parameters.

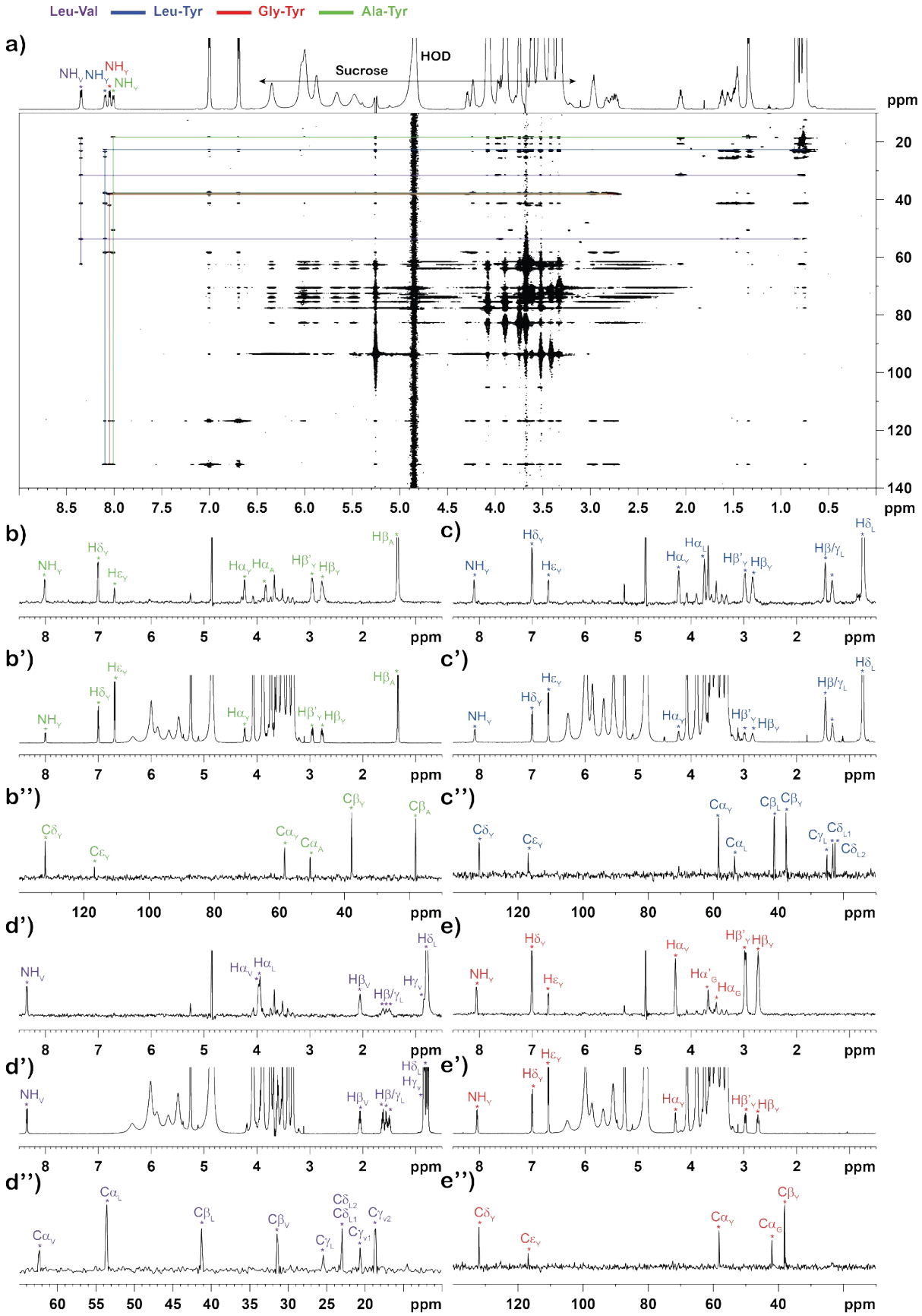


Fig. S-5. a) 2D ^{1}H-^{13}C HSQC-NOESY spectrum of the dipeptide test mixture (20 mM) in sucrose/H_2O (5:5, w/w + 10% D_2O, v/v), at 283 K, at 600 MHz (^{1}H), using the hsqcetgpnosp pulse sequence with solvent

multiple presaturation. Data matrix was recorded in Echo-Antiecho mode; its size was 1k × 2k with 48 scans per FID, a 1.5 s relaxation delay and a 0.5 s mixing time (t_m), resulting in a 1843.58 min recording time. The spectral widths were respectively 6 009.615 Hz in ^{1}H dimension and 21 129.352 Hz in ^{13}C dimension. Data matrix was multiplied in both dimensions by a shifted sine bell function (SSB = 2) before zero filling to a 1k × 2k size. Comparison of ^{1}H horizontal slices extracted from the 2D ^{1}H-^{13}C HSQC-NOESY at 18.14 and 38.08 ppm (b, b', Ala-Tyr, green rows (sum)), 22.64 and 37.66 ppm (c, c', Leu-Tyr, blue rows (sum)), 31.43 and 53.64 ppm (d, d', Leu-Val, purple rows (sum)) and 38.11 ppm (e, e', Gly-Tyr, red row) with the conventional 1D proton spectra of each pure dipeptide dissolved (20 mM) sucrose/H_2O (5:5, w/w + 10% D_2O, v/v), at 273 K, at 600 MHz (^{1}H). ^{13}C vertical slices extracted from the 2D ^{1}H-^{13}C HSQC-NOESY at 8.35 ppm (d'', Leu-Val, purple column), at 8.09 ppm (c'', Leu-Tyr, bleu column), at 8.05 ppm (e'', Gly-Tyr, red column), and at 8.01 ppm (a'', Ala-Tyr, green column).

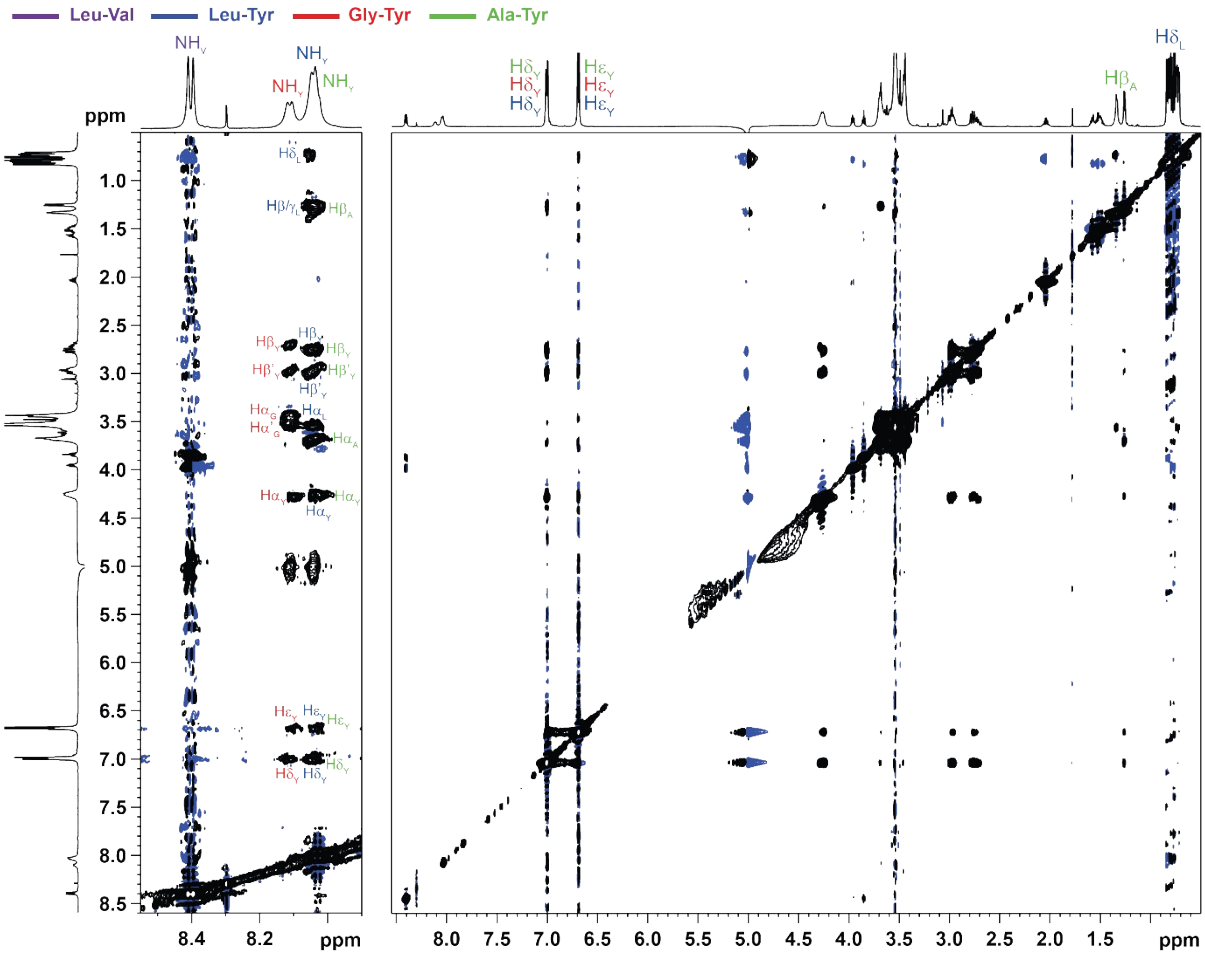


Fig. S-6. 2D ^{1}H-^{1}H NOESY spectrum of the dipeptide test mixture (Leu-Val, Leu-Tyr, Gly-Tyr and Ala-Tyr), 10 mM, dissolved in agarose gel 1%, at 273 K, at 600 MHz (^{1}H). See caption of Figure 2b in ESI for the NMR acquisition and processing parameters.