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# Speed versus stability – structure-activity effects on the assembly of two-component gels

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## SUPPORTING INFORMATION

### Contents

## 1. Synthesis and Characterisation

L-Lysine methyl ester was synthesised according to literature methods.[1] Boc-protected amino acids and all other reagents were sourced from commercial suppliers without further purification. Thin layer chromatography was performed on Merck aluminium backed plates, coated with 0.25 nm silica gel 60. Column chromatography was performed on silica gel 60 (35 – 75 µm) sup-plied by Fluka Ltd. NMR spectra were recorded on a JEOL ECX400 spectrometer ($^1$H 400 MHz, $^{13}$C 100 MHz). HR-ESI mass spectra were recorded on a Bruker Daltonics Micro-TOF mass spectrometer. Infrared spectra were recorded on a Perkin Elmer ATR-FTIR spectrometer Two with Perkin Elmer Spectrum in version 10.03. $[\alpha]_D$ values were recorded on a Jasco DIP-370 Digital Polarimeter. CD spectra were recorded on a Jasco J-810 Spectropolarimeter with Spectra Manager in version 1.53. Melting points were recorded on a Stuart melting point SMP3 machine.

**General Method for Peptide Coupling**

To an ice cooled solution of *N*-Boc protected amino acid (4.32 mmol) in $CH_2Cl_2$ (25 mL), TBTU (1.39 g, 4.32 mmol) and $Et_3N$ (0.6 mL, 4.32 mmol) were added under an $N_2$ atmosphere. The reaction was stirred for 30 minutes at the same temperature. L-lysine methyl ester dihydrochloride (0.47 g, 2.00 mmol) and $Et_3N$ (0.2 mL, 2.00 mmol) were then added to the mixture and stirred for 1 hour at room temperature. Water was added (ca. 25 mL), the organic phase was separated and the aqueous phase was extracted three times with $CH_2Cl_2$ (3x50 mL). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$, filtered and then the solvent was removed by rotary evaporation to obtain a highly viscous liquid. The crude product was purified by silica gel column chromatography ($CH_2Cl_2$/MeOH/$Et_3N$ 90:10:0.1) to furnish the desired peptide. This method was adapted from reference 2, where it was used for different peptides.

**Compound (Phe)$_2$Lys-CO$_2$Me.** Yield 58%, light yellow solid, m.p. 108-110°C; $R_f$ = 0.68 ($CH_2Cl_2$/MeOH/$Et_3N$ 90:10:0.1); $[\alpha]_D^{20}$ = +4.3 (c=1.0, MeOH); $^1$H NMR (400 MHz, MeOH-$d_4$) δ: 7.28-7.19 (m, Ar-*H,* 10H), 4.39-4.35 (m, Ar-$CH_2$-C*H*-NH, 2H), 4.27 (2 x d, both NHC*H*COOMe, *J* = 3.6 Hz, 1H), 3.69 (s, COOC$H_3$, 3H), 3.20-2.98 (m, Ph-C$H_2$- CHNH, 4H), 2.92-2.78 (m, CO-HN-

C*H*$_2$-CH$_2$, 2H), 1.85-1.21 (m, MeCOO-CH-C*H*$_2$-C*H*$_2$-C*H*$_2$- CH$_2$-NH, 6H), 1,37 (s, Boc-C*H*$_3$, 18H). $^{13}$C NMR (100 MHz, MeOH-d$_4$) δ: 174.6, 174.3, 173.9 (2x NH-CH-*C*O-NH and CH*C*OOCH$_3$), 157.6 (2x HN*C*OOC(CH$_3$)$_3$), 138.6, 138.4 (2x Ar-*C*q-CH$_2$), 130.5, 130.3, 129.5, 129.4, 127.7 (all Ar-*C*), 80.6 (2x *C*q(Boc)), 57.6, 57.2 (both Ar-CH$_2$-*C*H-NH), 56.6 (NH*C*HCOOMe), 52.7 (COO*C*H$_3$), 39.3 (Ar-CH$_2$-*C*H-NH), 38.9 (Ph-*C*H$_2$-CHNH), 32.2, 29.6 (MeCOO-CH-*C*H$_2$-CH2- *C*H2-CH2-NH), 28.7 (6x Boc-*C*H3), 23.8 (MeCOO-CH-CH2-*C*H2-CH2-CH2-NH). IR ν̃: 3329*m*, 2935*m*, 1740*m*, 1712*w*, 1687*s*, 1649*s*, 1518*s*, 1454*w*, 1366*m*, 1332*w*, 1292*m*, 1244*s*, 1212*w*, 1165*s*, 1045*m*, 1022*m*, 865*w*, 755*m*, 700*m*, 651*m*, 598*w*, 516*w*, 476*w*. HRMS: [M+Na]$^+$ ($C_{35}H_{50}N_4O_8Na$) Calc. *m/z* = 677.3521; Obs. *m/z* = 677.3540 Mean Err -2.5 ppm; [M+H]$^+$ ($C_{35}H_{51}N_4O_8$) Calc. *m/z* = 655.3701; Obs. *m/z* = 655.3717 Mean Err -2.6 ppm.

**Compound (Val)$_2$Lys-CO$_2$Me.** Yield 36%, colourless solid, m.p. 116-117°C; $R_f$ = 0.39 ($CH_2Cl_2$/MeOH/$Et_3N$ 90:10:0.1); $[\alpha]_D^{20}$ = -26.9 (c=1.0, MeOH); $^1$H NMR (400 MHz, MeOH-d$_4$) δ: 4.37 (q, HN-C*H*-COOCH$_3$, *J* = 4.7 Hz, 1H), 3.94, 3.80 (2x d, both -C*H*-NHBoc, *J* = 3.6 Hz, 2H), 3.70 (s, COOC*H*$_3$, 3H), 3.19 (t, CO-HN-C*H*$_2$-CH$_2$, *J* = 5.8 Hz, 2H), 2.06-1.95 (m, 2x (CH$_3$)$_2$-C*H*-CH-NHBoc, 2H), 1.94-1.48 (m, MeCOO-CH-C*H*$_2$-C*H*$_2$-C*H*$_2$- CH$_2$-NH, 6H), 1,44 (s, Boc-C*H*$_3$, 18H), 0.98, 0.93 (2x d, 4x Val-C*H*$_3$, J = 3.4 Hz, 12H). $^{13}$C NMR (100 MHz, MeOH-d$_4$) δ: 174.7, 173.9 (both NH-CH-*C*O-NH), 167.5 (CH*C*OOCH$_3$), 158.0 (2x HN*C*OOC(CH$_3$)$_3$), 80.5 (2x *C*q(Boc)), 61.9, 61.3 (both (CH$_3$)$_2$-CH-*C*H-NHBoc), 53.6 (NH*C*HCOOMe), 52.6 (COO*C*H$_3$), 39.8 (CO-NH-*C*H$_2$-), 32.2 (2x (CH$_3$)$_2$-*C*H-CH-NHBoc) 32.0, 29.7 (MeCOO-CH-*C*H$_2$-CH$_2$-*C*H$_2$-CH$_2$-NH), 28.8 (6x Boc-*C*H$_3$), 23.9 (MeCOO-CH-CH$_2$-*C*H$_2$- CH$_2$-CH$_2$-NH), 19.8, 19.7, 18.7, 18.6 (4x Val-*C*H$_3$). IR ν̃: 3326*m*, 2959*m*, 2871*w*, 1738*m*, 1686*m*, 1646*s*, 1520*s*, 1463*w*, 1366*m*, 1300*m*, 1245*s*, 1213*w*, 1168*s*, 1044*m*, 1019*m*, 928*w*, 877*w*, 849*w*, 798*m*, 745*w*, 653*m*. HRMS: [M+Na]$^+$ ($C_{27}H_{50}N_4O_8Na$) Calc. *m/z* = 581.3521; Obs. *m/z* = 581.3521 Mean Err 0.9 ppm; [M+H]$^+$ ($C_{27}H_{51}N_4O_8$) Calc. *m/z* = 559.3701; Obs. *m/z* = 559.3688 Mean Err 2.5 ppm.

**Compound (Ala)$_2$Lys-CO$_2$Me.** Yield 50%, colourless highly viscous liquid, $R_f$ = 0.65 ($CH_2Cl_2$/MeOH/$Et_3N$ 90:10:0.1); $[\alpha]_D^{20}$ = -13.3 (c=1.0, MeOH); $^1$H NMR (400 MHz, MeOH-d$_4$) δ: 4.39 (q, HN-C*H*-COOCH$_3$, *J* = 4.7 Hz, 1H), 4.13-4.02 (m, 2x -C*H*-NHBoc, 2H), 3.70 (s, COOC*H*$_3$, 3H), 3.25-3.14 (m, CO-HN-C*H*$_2$-CH$_2$, 2H), 1.90-1.46 (m, MeCOO-CH-C*H*$_2$-C*H*$_2$-C*H*$_2$-CH$_2$-NH, 6H), 1,44 (s, Boc-C*H*$_3$, 18H), 1.31, 1.28 (2x d, both Ala-C*H*$_3$, *J* = 7.2 Hz, 6H). $^{13}$C NMR (100 MHz, MeOH-d$_4$) δ: 176.0, 174.0 (both NH-CH-*C*O-NH), 167.5 (CH*C*OOCH$_3$), 157.6 (2x

HN*C*OOC($CH_3$)$_3$), 80.6 (2x *C*q(Boc)), 53.5 (NH*C*HCOOMe), 52.7 (COO*C*$H_3$), 51.7, 51.4 (both $CH_3$-*C*H-NH), 39.3 (CO-NH-*C*$H_2$-), 32.2, 29.7 (MeCOO-CH-*C*$H_2$-$CH_2$-*C*$H_2$-$CH_2$-NH), 28.8 (6x Boc-*C*$H_3$), 23.8 (MeCOO-CH-$CH_2$-*C*$H_2$-$CH_2$-$CH_2$-NH), 18.6, 18.4 (both Ala-*C*$H_3$). IR ṽ: 3298*w*, 2934*w*, 1745*w*, 1713*m*, 1627s, 1505*s*, 1453*m*, 1409*w*, 1366*s*, *1247m*, 1165*s*, 1143*s*, 1062*m*, 1022*m*, 914*m*, 857*w*, 782*m*, 740*w*, 578*m*, 557*m*. HRMS: $[M+Na]^+$ ($C_{23}H_{42}N_4O_8Na$) Calc. *m/z* = 525.2895; Obs. *m/z* = 525.2905 Mean Err -1.1 ppm; $[M+H]^+$ ($C_{23}H_{43}N_4O_8$) Calc. *m/z* = 503.3075; Obs. *m/z* = 503.3073 Mean Err 0.9 ppm.

**General Method for Saponification of the Methyl Ester to Yield Target Peptide Acids**

The dipeptide methyl ester (0.50 g, 1 equivalent) (Table 10) was dissolved in methanol (25 mL). The solution was cooled to 0°C and then aqueous sodium hydroxide solution (1M, 3 equivalents) was added. The reaction was stirred at room temperature under nitrogen for 24 hours. The solvent was removed by rotary evaporation, water was added (35 mL) and then the mixture was acidified to pH 3 with aqueous sodium hydrogen sulfate solution (1M). The product was extracted three times with ethyl acetate (3x50 mL). The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and then the solvent was removed by rotary evaporation. Diethyl ether (20 mL) was added and the solvent was removed from the mixture by rotary evaporation to give the product. This method was adapted from reference 3.

**Compound (Phe)$_2$Lys-CO$_2$H.** Yield 68%, colourless solid, m.p. 100-102°C; $[\alpha]_D^{20}$ = +15.4 (c=1.0, MeOH); $^1$H NMR (400 MHz, MeOH-$d_4$) δ: 7.27-7.22 (m, Ar-*H,* 10H), 4.43-4.27 (m, Ar-$CH_2$-C*H*-NH, NHC*H*COOH, 3H), 3.18-2.97 (m, Ph-C*H*$_2$-CHNH, 4H), 2.93-2.74 (m, CO-HN-C*H*$_2$-$CH_2$, 2H), 1.91-1.27 (m, HOOC-CH-C*H*$_2$-C*H*$_2$-C*H*$_2$-$CH_2$-NH, 6H), 1,38 (s, Boc-C*H*$_3$, 18H). $^{13}$C NMR (100 MHz, MeOH-$d_4$) δ: 175.5, 175.1, 174.5 (2x NH-CH-*C*O-NH and CH*C*OOH), 157.8, 157.6 (both HN*C*OOC($CH_3$)$_3$), 138.7, 138.6 (both Ar-*C*q-$CH_2$), 130.4, 130.3, 129.5, 129.4, 127.7 (all Ar-*C*), 80.6 (2x *C*q(Boc)), 57.6, 57.2 (both Ar-$CH_2$-*C*H-NH), 56.4 (NH*C*HCOOH), 39.6 (Ar-$CH_2$-*C*H-NH), 38.9 (Ph-*C*$H_2$-CHNH), 32.3, 29.7 (HOOC-CH-*C*$H_2$-$CH_2$- *C*$H_2$-$CH_2$-NH), 28.7 (6x Boc-*C*$H_3$), 23.9 (MeCOO-CH-$CH_2$-*C*$H_2$-$CH_2$-$CH_2$-NH). IR ṽ: 3287*m*, 2930*m*, 1663*s*, 1628*s*, 1538*s*, 1455*w*, 1392*m*, 1366*m*, 1288*m*, 1247*m*, 1165*s*, 1087*w*, 1053*m*, 1020*m*, 857*w*, 742*m*, 698*s*, 565*w*, 496*m*. HRMS: $[M+2Na]^+$ ($C_{34}H_{47}N_4O_8Na_2$) Calc. *m/z* = 685.3184; Obs. *m/z* = 685.3175

Mean Err 1.6 ppm; $[M+Na]^+$ ($C_{34}H_{48}N_4O_8Na$) Calc. *m/z* = 663.3364; Obs. *m/z* = 663.3383 Mean Err -2.0 ppm.

**Compound $(Val)_2Lys\text{-}CO_2H$.** Yield 71%, colourless solid, m.p. 92-93°C; $[\alpha]_D^{20}$ = -19.4 (c=1.0, MeOH); $^1H$ NMR (400 MHz, MeOH-$d_4$) δ: 4.37 (dd, HN-C*H*-$COOCH_3$, *J* = 4.5 Hz, 1H), 3.94, 3.81 (2x d, both -C*H*-NHBoc, *J* = 3.4 Hz, 2H), 3.19 (t, CO-HN-C$H_2$-$CH_2$, *J* = 6.6 Hz, 2H), 2.06-1.95 (m, 2x $(CH_3)_2$-C*H*-CH-NHBoc, 2H), 1.94-1.50 (m, MeCOO-CH-C$H_2$-C$H_2$-C$H_2$-$CH_2$-NH, 6H), 1,44 (s, Boc-C$H_3$, 18H), 0.98, 0.93 (2x d, 4x Val-C$H_3$, *J* = 3.4 Hz, 12H). $^{13}C$ NMR (100 MHz, MeOH-$d_4$) δ: 175.8, 175.2 (both NH-CH-*C*O-NH), 158.5 (2x HN*C*OOC$(CH_3)_3$), 80.7 (2x *C*q(Boc)), 61.9, 61.4 (both $(CH_3)_2$-CH-*C*H-NHBoc), 53.5 (NH*C*HCOOMe), 39.9 (CO-NH-*C*$H_2$-), 32.1 (2x $(CH_3)_2$-*C*H-CH-NHBoc) 31.9, 29.6 (MeCOO-CH-*C*$H_2$-$CH_2$-*C*$H_2$-$CH_2$-NH), 28.7 (6x Boc-*C*$H_3$), 23.9 (MeCOO-CH-$CH_2$-*C*$H_2$-$CH_2$-$CH_2$-NH), 19.7, 19.6, 18,5, 18.4 (4x Val-*C*H3). IR ṽ: 3298*m*, 2965*m*, 1650*s*, 1526*s*, 1455*w*, 1390*m*, 1365*s*, 1295*m*, 1246*m*, 1161*s*, 1044*m*, 1018*m*, 915*w*, 872*m*, 781*w*, 647*m*. HRMS: $[M+Na]^+$ ($C_{26}H_{48}N_4O_8Na$) Calc. *m/z* = 567.3364; Obs. *m/z* = 567.3376 Mean Err -2.0 ppm; [M + H]+ ($C_{26}H_{49}N_4O_8$) Calc. *m/z* = 545.3545; Obs. *m/z* = 545.3561 Mean Err -2.9 ppm.

**Compound $(Val)_2Lys\text{-}CO_2H$.** Yield 97%, colourless viscous liquid, $[\alpha]_D^{20}$ = -17.1 (c=1.0, MeOH); $^1H$ NMR (400 MHz, MeOH-$d_4$) δ: 4.37 (q, HN-C*H*-COOH, *J* = 4.5 Hz, 1H), 4.13-4.02 (m, 2x - C*H*-NHBoc, 2H), 3.19 (br s, CO-HN-C$H_2$-$CH_2$, 2H), 1.94-1.46 (m, HOOC-CH-C$H_2$-C$H_2$-C$H_2$-$CH_2$-NH, 6H), 1,44 (s, Boc-C$H_3$, 18H), 1.32, 1.28 (2x d, both Ala-C$H_3$, J = 7.2 Hz, 6H). $^{13}C$ NMR (100 MHz, MeOH-$d_4$) δ: 176.0, 175.2 (both NH-CH-*C*O-NH), 167.5 (CH*C*OOH), 157.6 (2x HN*C*OOC$(CH_3)_3$), 80.6 (2x *C*q(Boc)), 53.4 (NH*C*HCOOH), 51.7, 51.4 (both $CH_3$-*C*H-NH), 40.0 (CO-NH-*C*$H_2$-), 32.3, 29.8 (HOOC-CH-*C*$H_2$-$CH_2$-*C*$H_2$-$CH_2$-NH), 28.7 (6x Boc-*C*$H_3$), 23.8 (HOOC-CH-$CH_2$-*C*$H_2$-$CH_2$-$CH_2$-NH), 18.7, 18.4 (both Ala-*C*$H_3$). IR ṽ: 3300*w*, 2977*w*, 2934*w*, 1712*m*, 1627*s*, 1510*s*, 1453*m*, 1409*w*, 1366*s*, 1246*s*, 1163*s*, 1064*m*, 1021*m*, 914*w*, 857*w*, 781*m*, 742*w*, 578*m*, 559*m*, 462*w*. HRMS: $[M+Na]^+$ ($C_{22}H_{40}N_4O_8Na$) Calc. *m/z* = 511.2738; Obs. *m/z* = 511.2747 Mean Err -1.4 ppm; $[M+H]^+$ ($C_{22}H_{41}N_4O_8$) Calc. *m/z* = 489.2919; Obs. *m/z* = 489.2919 Mean Err -2.6 ppm

## 2 NMR Stoichiometry Studies

The stoichiometry of the complex responsible for gelation was studied with the help of $^1$H NMR. The samples all contained $(AA)_2$Lys-$CO_2$H (20 mM) and diphenylmethane (DPM) as an internal standard (20 mM) in toluene-$d_8$. To 0.5 mL of this solution, different amounts of phenylethylamine were added. The concentration of both compounds which was mobile within the sample was then determined by integration of relevant peaks and comparison to the internal standard. Data are provided in Figures S1-S3.

As is apparent, the initial amine added is not visible within the NMR spectrum as it becomes immobilised within solid-like gel fibres. This also decreases the mobility of the peptides. The fact that on addition of one equivalent of amine, equal amounts of amine and peptide are mobile within the sample is consistent with the complexes within the solid-like fibres having 1:1 stoichiometry.

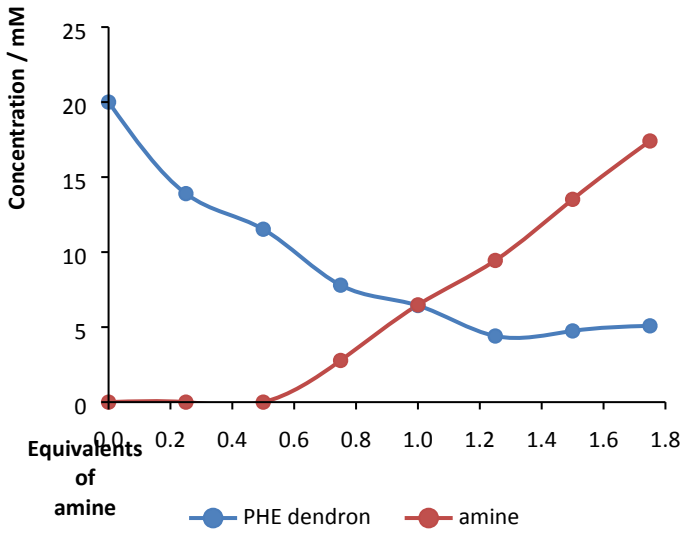


Figure S1. Titration of phenylethylamine into $(Phe)_2$Lys-$CO_2$H (20 mM) with the amount of mobile peptide dendron and amine being determined by NMR spectroscopy at 25°C.

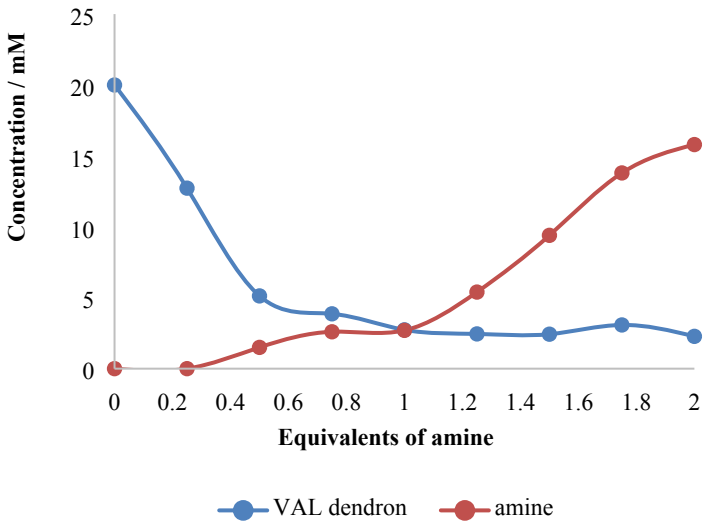


Figure S2. Titration of phenylethylamine into $(Val)_2Lys\text{-}CO_2H$ (20 mM) with the amount of mobile peptide dendron and amine being determined by NMR spectroscopy at 25°C.

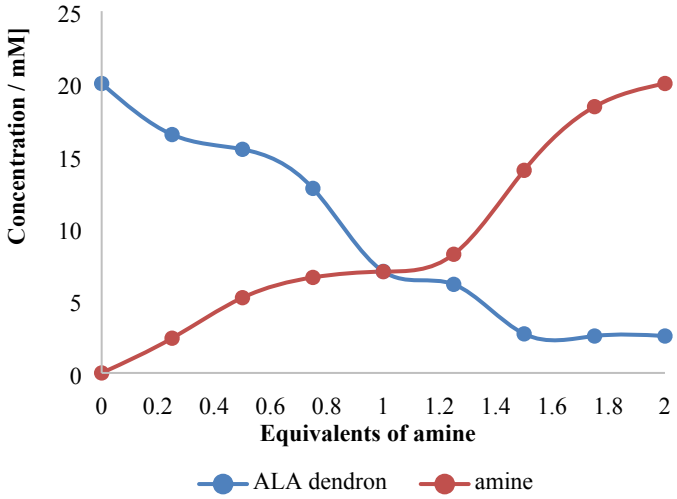


Figure S3. Titration of phenylethylamine into $(Ala)_2Lys\text{-}CO_2H$ (20 mM) with the amount of mobile peptide dendron and amine being determined by NMR spectroscopy at 25°C (N.B. This study was performed at higher concentration than the VT NMR studies on this compound).

## 3 Thermodynamic Analysis of Variable Temperature NMR Data

VT NMR spectra were measured under conditions in which probe temperature was increased at a rate of 1°C/min, and probe temperature was equilibrated for 5 minutes prior to measurement of the spectrum.

In terms of theory, for an ideal solution, the solubility (Sol) at a given temperature can be expressed by the van't Hoff equation (1).

$$\ln (Sol) = (- \Delta H_{diss} / RT_{eq}) + (\Delta S_{diss} / R) \qquad (1)$$

$\Delta H_{diss}$ and $\Delta S_{diss}$ denote the molar enthalpy and the molar entropy for the dissolution process (i.e., gel-sol transformation), $T_{eq}$ is the equilibrium temperature and R is the gas constant. The solubility is estimated as the concentration of mobile peptide gelator as determined by VT NMR referenced to the integration of mobile standard diphenylmethane.

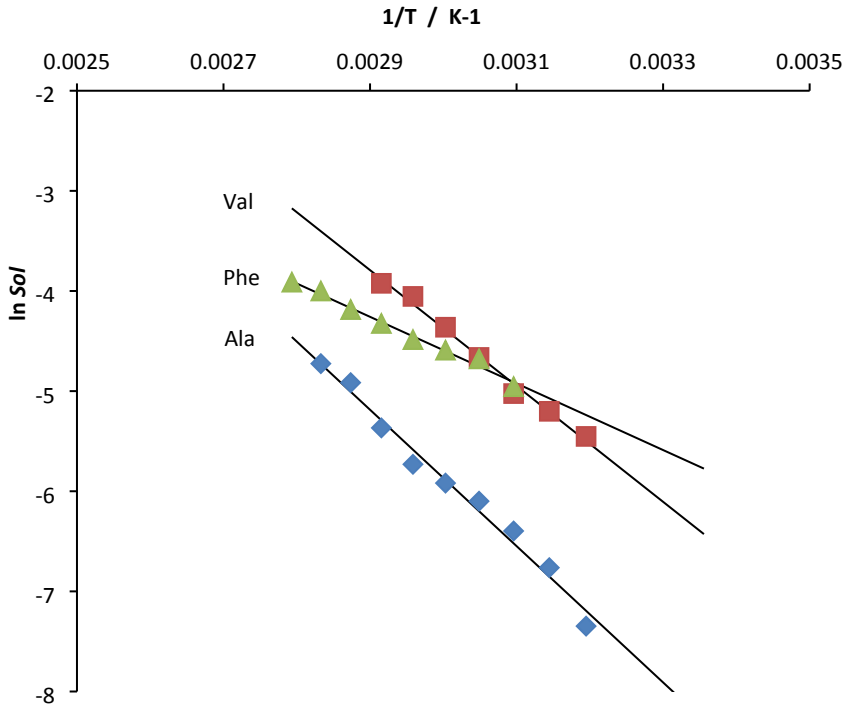


Figure S4. Plot of van't Hoff analysis of VT NMR data for $(Phe)_2Lys\text{-}CO_2H$ (20 mM) $(Val)_2Lys\text{-}CO_2H$ (20 mM) and $(Ala)_2Lys\text{-}CO_2H$ (10 mM) in the presence of equimolar phenylethylamine and a mobile internal standard (diphenylmethane).

The van't Hoff plots of ln*Sol* against 1/T for the systems under investigation are shown in Figure S4. These plots can be used to calculate $\Delta H_{diss}$ and $\Delta S_{diss}$ via their gradients and intercepts. The significant differences thermodynamics of the systems under investigation are clearly evident from this graph.

## 4 References


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3. G. M. Dykes, L. J. Brierley, D. K. Smith, P. T. McGrail and G. J. Seeley, *Chem. Eur. J.* **2001**, *7*, 4730–4739.