Electronic Supplementary Material (ESI) for ChemComm.

Insight into the esterase like activity demonstrated by an imidazole appended self-assembling hydrogelator

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Electronic Supplementary Information

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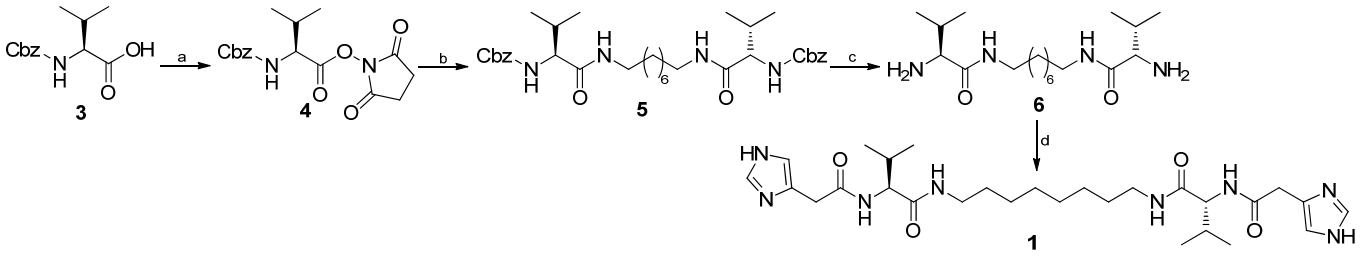
1. SYNTHESIS.



Scheme SI_1. Reagents and conditions: (a) DCC, NHS, THF, 5 ºC, 3 h, 91 %; (b) 1,8-diamonooctane, DME, 55 ºC, 6 h, 95 %; (c) Pd/C (5% wt), H_2, MeOH, 3 h, 99 %, (d) Imidazole 4-acetic acid, BOP, DIPEA, DCM, 0°C-R.T, 16 h, 70 %.

Synthesis of compounds 1, 4, 5 and 6.

(S)-2,5-dioxopyrrolidin-1-yl 2-(((benzyloxy)carbonyl)amino)-3-methylbutanoate (4): A solution of commercial available Carbobenzyloxy-*L*-valine**3** (5.0 g, 19.90 mmol) and *N*-hydroxysuccinimide (2.52 g, 21.89 mmol, 1.1 eq.) in dry THF (80 mL) was added dropwise under N_2 at 0 ºC with a dropping funnel to a solution of *N*,*N*′-Dicyclohexylcarbodiimide (4.52 g, 21.89 mmol, 1.1 eq.) in dry THF (25 mL). The mixture was further stirred for 1 h at 0 ºC. The solution was then allowed to stand into refrigerator for 2 h, which caused precipitation of *N*,*N*′-Dicyclohexylurea. After this time, the mixture was filtered under vacuum, and the filtrate was removed under reduced pressure and the crude residue was purified by crystallization in isopropanol to yield **4** (6.31 g, 18.11 mmol, 91%) as a white solid. The NMR spectra were consistent with those described in the literature.[1]

Dibenzyl ((2S,2'R)-(octane-1,8-diylbis(azanediyl))bis(3-methyl-1-oxobutane-2,1-diyl))dicarbamate (5): A solution of compound **4** (6.31 g, 18.11 mmol) in DME (80 mL) was added dropwise under N_2 at 25 ºC with a dropping funnel to a solution of commercial available 1,8-diamino octano (2.88 g, 19.92 mmol, 1.1 eq.) in DME (50 mL). The mixture was further stirred for 5 h at 50 ºC. The white solid obtained was filtered under vacuum, and

[1] Becerril, J.; Bolte, M.; Burguete, M. I.; Galindo, F.; García-España, E.; Luis, S. V.; Miravet, J. F., *J. Am. Chem. Soc.***2003**, *125*, 6677-6686.

the residue was washed with HCl 0.1 M (100 mL) and water (200 mL). The compound was dried under reduced pressure at 50 ºC to yield **5** (10.51 g, 17.21 mmol, 95%) as a white solid. The NMR spectra were consistent with those described in the literature.[1]

(S)-2-amino-N-(8-((R)-2-amino-3-methylbutanamido)octyl)-3-methylbutanamide (6): Palladium catalyst (5% Pd/C, 526 mg) was suspended in MeOH (250 mL) and stirred under H_2 at room temperature for 10 min. Subsequently, a solution of compound **5**, (10.51 g, 17.21 mmol) in MeOH (100 mL) was added via syringe, followed by stirring under H_2 at room temperature for 3 h. The reaction mixture was then filtered through Celite, and the solvent was removed under reduced pressure. The white solid obtained was filtered under vacuum, and the residue was washed with NaOH 0.1 M (100 mL) and water (200 mL). The compound was dried under reduced pressure at 50 ºC to yield **6**(5.84 g, 17.04 mmol, 99%) as a white solid.The NMR spectra are consistent with those described in the literature.[1]

(2S,2'S)-N,N'-(octane-1,8-diyl)bis(2-(2-(1H-imidazol-4-yl)acetamido)-3-methylbutanamide) (1):

Imidazole-4 acetic acid hydrochloride (95 mg, 0.6mmol.) was dissolved in dry DCM followed by addition of N,N-diisopropylethylamine (0.2mL.) and benzotriazol-1-yloxy-tris(dimethylamino)-phosphoniumhexafluorophosphate (258mg, 0.6mmol) at 0°C. To this solution was added **6** (100mg, 0.3mmol), dissolved in DCM in a dropwise manner. The reaction mixture was monitored by TLC until completion. This mixture was then sonicated and washed with 3 batches of 10 mL of 0.1M NaOH followed by washing with water and DCM over sintered funnel. The obtained solid was dried under vacuum to yield the final product in 70% yield.

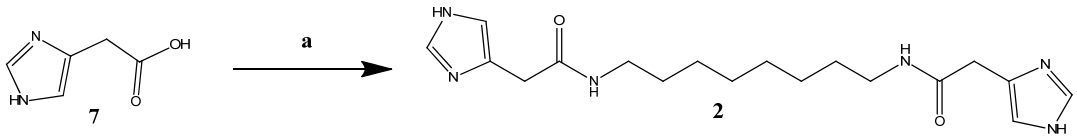
Compound 1:

^{1}H NMR (500 MHz, DMSO-d6): δ 8.06 (H14, t, *J* = 5.4 Hz, 2H), 7.90 (H8, d, *J* = 8.9 Hz, 2H), 7.60 (H2, s, 2H), 6.88 (H4, s, 2H), 4.10 (H9, dd, *J* = 8.9, 6.5 Hz, 2H), 3.44 (H6, d,2H,

J=5.8 Hz), 3.40 (H6′, d, 2H, *J*=5.8 Hz), 3.02 (H15, m, 2H), 2.98 (H15′, m, 2H), 1.95 (H10, oct., 2H, *J*=6.5 Hz),1.37 (H16, t, 4H, *J*=6.5 Hz), 1.22 (H17, H18, broad singlet, 8H), 0.80 (H11, d, *J* = 6.5 Hz, 6H), 0.78(H12, d, *J*=6.5 Hz, 6H)

^{13}C NMR (126 MHz, DMSO-d6):δ 171.0, 170.3, 135.4, 135.1, 113.8, 58.1, 38.8, 35.8, 30.8, 29.4, 29.0, 26.7, 19.6, 18.2.

HR ESMS: m/z: $[M+H]^+$ Calcd for $C_{28}H_{46}N_8O_4$: 559.3676; found: 559.3721; Δ=0.2 ppm.



Scheme SI_2. Reagents and conditions: (a) BOP, 1,8-diaminooctane, DIPEA, DCM, 0°C-R.T., 12 hr, 65%.

N-(8-(2-(1H-imidazol-4-yl)acetamido)octyl)-2-(1H-imidazol-5-yl)acetamide (2):

7(221 mg, 1.4 mmol.) was dissolved in dry DCM followed by addition of N,N-diisopropylethylamine (0.5 mL.) and Benzotriazol-1-yloxy-tris(dimethylamino)-phosphoniumhexafluorophosphate (616 mg, 1.4 mmol) at 0°C. To this solution was added a suspension of 1,8-diaminooctane(90mg, 0.6 mmol) in DCM in a dropwise manner. The reaction mixture was monitored by TLC until completion. This mixture was then sonicated and washed with 3 batches of 10 mL of 0.1M NaOH followed by washing with water and DCM over cintered funnel. The obtained solid was dried under vacuum to yield the final product in 65% yield.

Compound 2:

^{1}H NMR (500 MHz, DMSO-d6):δ 11.81 (H1, broad s, 2H), 7.79 (H8, broad s, 2H), 7.49 (H2, s, 2H), 6.85 (H5, s, 2H), 3.42 (H6, 2H(with water of DMSO)), 3.02 (H9, m, 4H), 1.44 (H10, m, 4H), 1.21 (H11, H12, m, 8H).

^{13}C NMR (126 MHz, DMSO-d6): δ 170.9, 135.4, 135.2, 117.5, 39.4, 39.2, 34.7, 29.0, 26.4.

HR ESMS: m/z: $[M+H]^+$Calcd for $C_{18}H_{28}N_6O_2$: 361.2307; found: 361.2350; Δ=0.6 ppm.

2. Gelation Experimental procedure:

The desired amount of compound**1**was dissolved in 0.1M TRIS-HCl buffer and sonicated to get a suspension. This suspension was then heated to dissolve the compound to get a clear solution and then was allowed to stand for 1 hour to come to room temperature forming hydrogels. The minimum gelation concentration was obtained using tube inversion method and did not vary significantly for different pH values.

3. Potentiometric Titration Experiments:

1 was dissolved in an excess of aqueous HCl(0.1M) and then titrated with aqueous NaOH (0.1M). The pH was recorded withaglass pH electrode and the data analyzed with HYPERQUAD2008 and HYSS2009 to afford acidity constants. The pKa was determined for two concentrations as reported below.

Conc.	**pKa** (calculated error less than 5%)
17.2 mM	6.6, 5.9
1.8 mM	6.3, 6.2

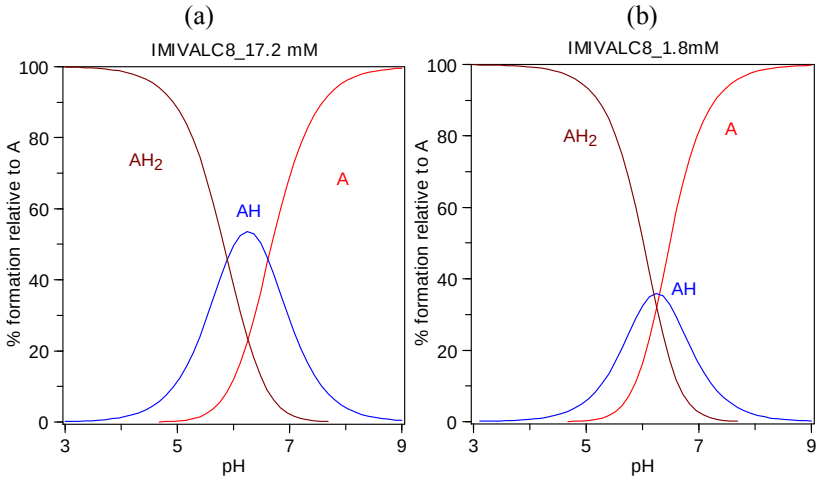
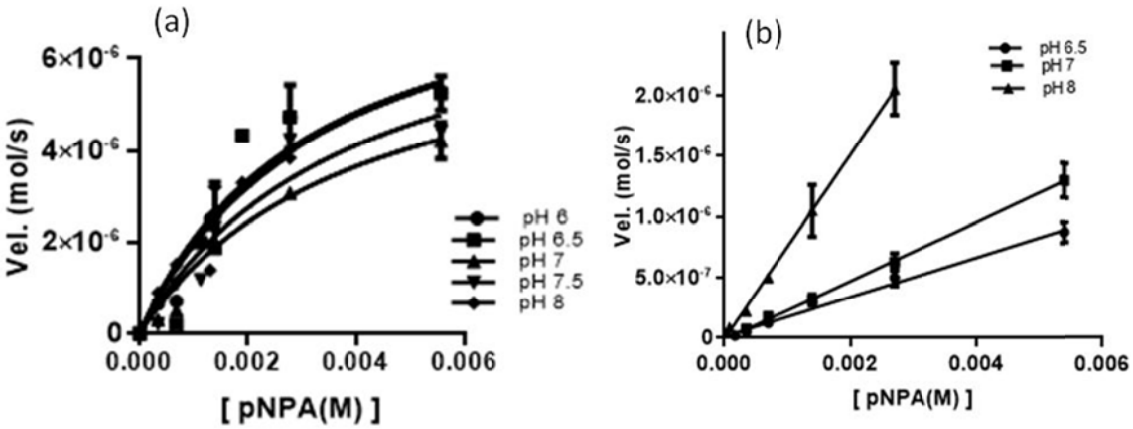


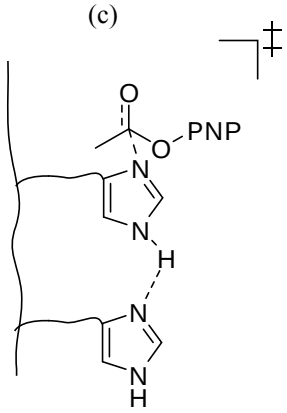
Figure S1: Relative abundance of different protonated and deprotonated species of **1**with respect to pH at (a) 17.2 mM (b) 1.8 mM.

4. UV kinetics experiment:

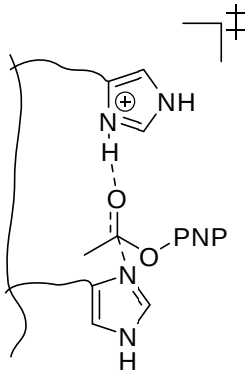
Kinetics of hydrolysis was measured using a JASCO FP-8300 spectrometer by recording the absorbance at 400nm. The 800 µL of a known concentration of catalyst was vortexed with 1mL of 0.1M TRIS-HCl buffer and 100µl of it was added to 1cm quartz cell containing 1.8mL of buffer. To this 100µl of substrate dissolved in acetonitrile was added from a freshly prepared stock solution. The final concentration of **1** under self-assembled state or **2** used for hydrolysis was 17×10^{-5} M. The catalysis experiments were generally done in triplicate. Similar procedure was followed for hydrolysis by non-aggregating concentrations of **1** with final concentrations of 0.09mM, 0.045mM, 0.025mM at pH 6, pH 7 and pH 8 respectively. Graph pad prism version 6·05 software was used for data fitting.


(a)
Vel. (mol/s)
6×10⁻⁶
4×10⁻⁶
2×10⁻⁶
0
0.000
0.002
0.004
0.006
[pNPA(M)]
pH 6
pH 6.5
pH 7
pH 7.5
pH 8
(b)
Vel. (mol/s)
2.0×10⁻⁶
1.5×10⁻⁶
1.0×10⁻⁶
5.0×10⁻⁷
0
0.000
0.002
0.004
0.006
[pNPA(M)]
pH 6.5
pH 7
pH 8

(c)

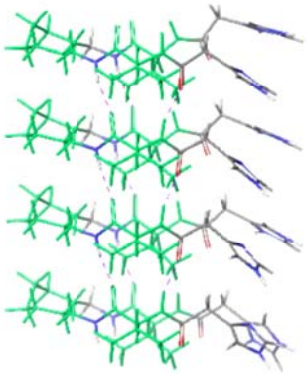

O
O
PNP
N
N
H
N
N
H

(d)

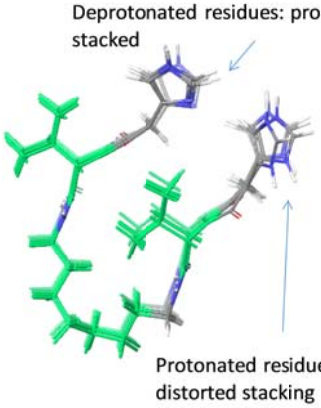

NH
N
H
O
O
PNP
N
N
H

(e)

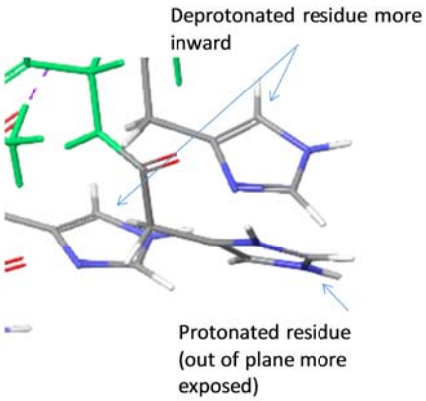
(i)



(ii)

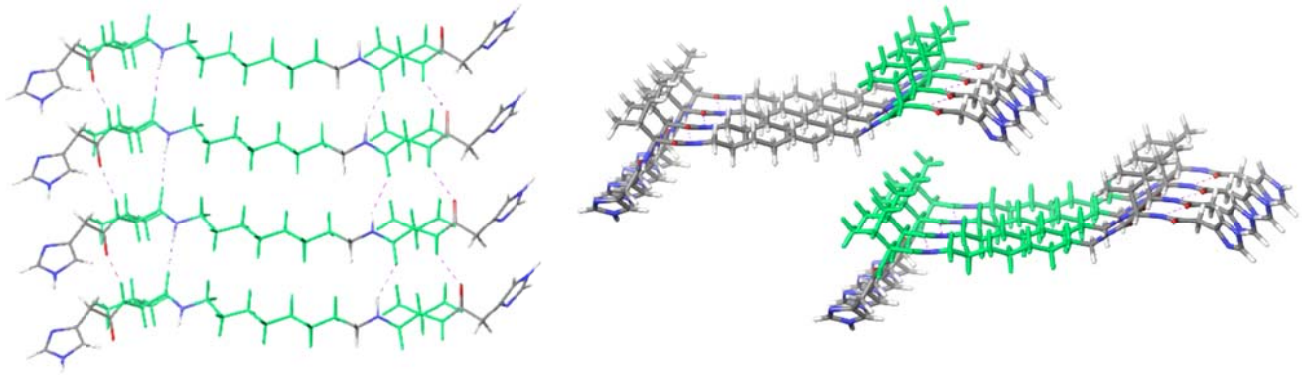

Deprotonated residues: properly stacked
Protonated residues: distorted stacking

(iii)


Deprotonated residue more inward
Protonated residue (out of plane more exposed)

(f)

(i) (ii)



Stacking of molecules for the deprotonated case for higher pH.
The green parts depict the hydrophobic regions.

(g)

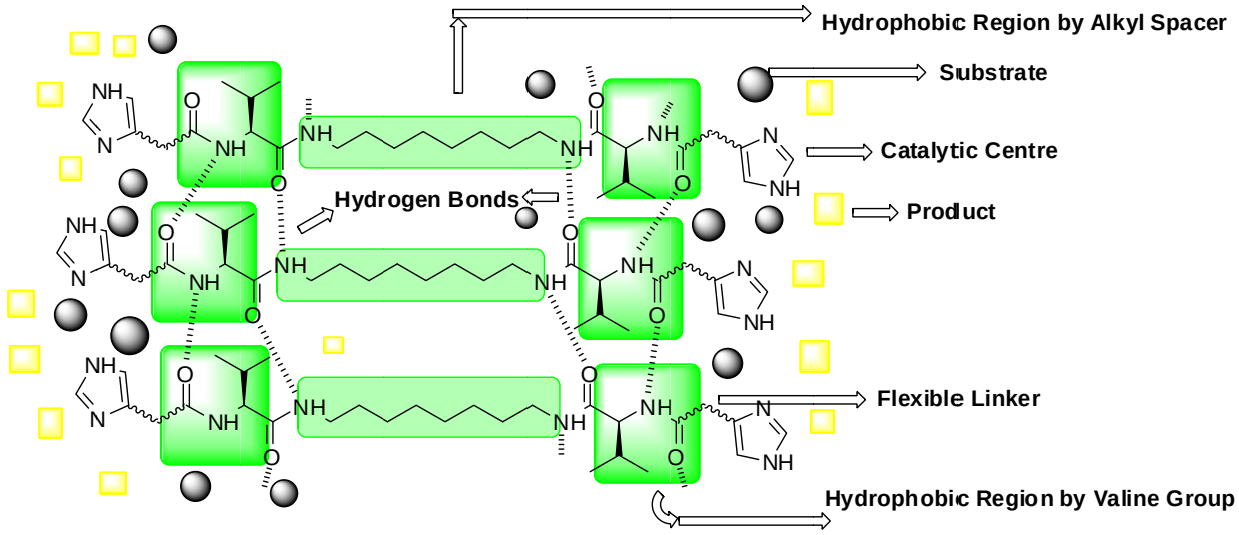


Fig.S2: (a) Michaelis-Menten graph for the hydrolysis of pNPA by **1** for different pH values (b) Hydrolysis of pNPA by **2** for different pH values. Schematic representation of proposed transition states for the hydrolysis of pNPA by (c) neutral imidazole residues and (d) cooperation between protonated and neutral imidazole residues. (e) **(i)** probable stacking of molecules in the case of lower pH values: shown here is one of the many probabilities, in this case stacking with folded molecules. Green part depicts the hydrophobic regions due to alkyl chain and valine group. For the sake of simplicity half of the imidazoles on one side of the molecules are taken as protonated. They can be present in random proportions as well **(ii)**the distorted stacking between the protonated residues that gives rise to the twist in fibers as seen in TEM and confirmed by CD **(iii)** protonated residue depicted being outward and stacked with higher slip angle making them more exposed to the solvent and substrates.
(f) **(i)** Proper stacking of molecules at higher pH values **(ii)** two such stacked fibers together forming hydrophobic regions (g) General schematic of molecular stacking and avalability of hydrophobic sites near imidazole residues for catalysis.

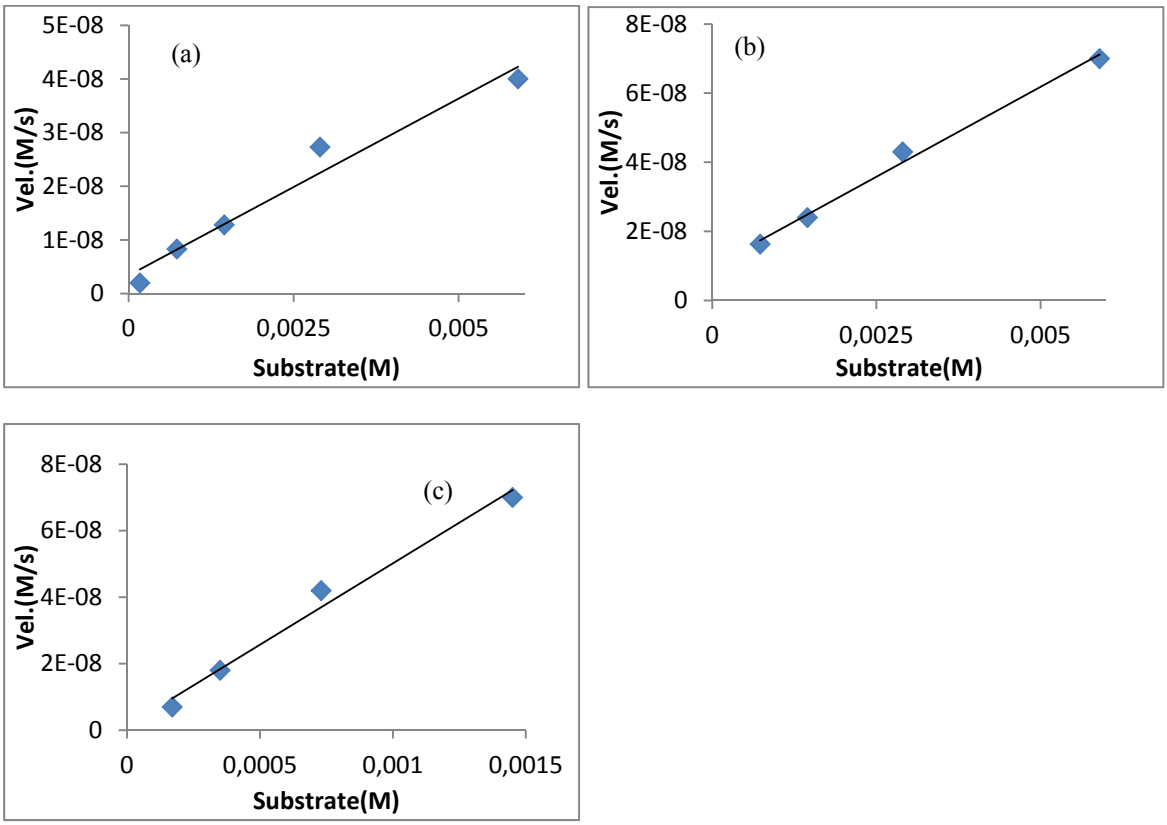


Figure S3: Observed hydrolysis rate by **1** under non aggregating conditions for (a) pH 6, (b) pH 7 and (c) pH 8.K_{sec} values for pH 6, pH 7 and pH 8 are 0.07$M^{-1}s^{-1}$, 0.23 $M^{-1}s^{-1}$, 1.8 $M^{-1}s^{-1}$ respectively.

5. Circular Dichroism:

CD experiments were performed using JASCO J-810 spectrometer for gels of **1** prepared directly in 1mm quartz cells. Measurements were performed at room temperature and spectra were recorded from 180 nm to 450 nm with 1.0 nm step and 1.0 nm bandwidth.

NMR Solubility Experiment:

All the NMR experiments were performed on Varian 500MHz spectrometer. For each data point freshly prepared samples were used by pouring hot solution of compound dissolved in buffered water into NMR tubes which were then allowed to stand for 24 hours. In this tube, another concentric NMR tube containing 50mM hydroquinone dissolved in D_2O was

introduced as an internal reference. NMR spectra in water were recorded at different concentrations of **1** ranging from completely soluble to gel state. The peak of water was presaturated to obtain the spectra. The solubility constants at different pH values were obtained by plotting the relative intergral of a chosen peak in the spectra against the concentration of **1** for different concentration.

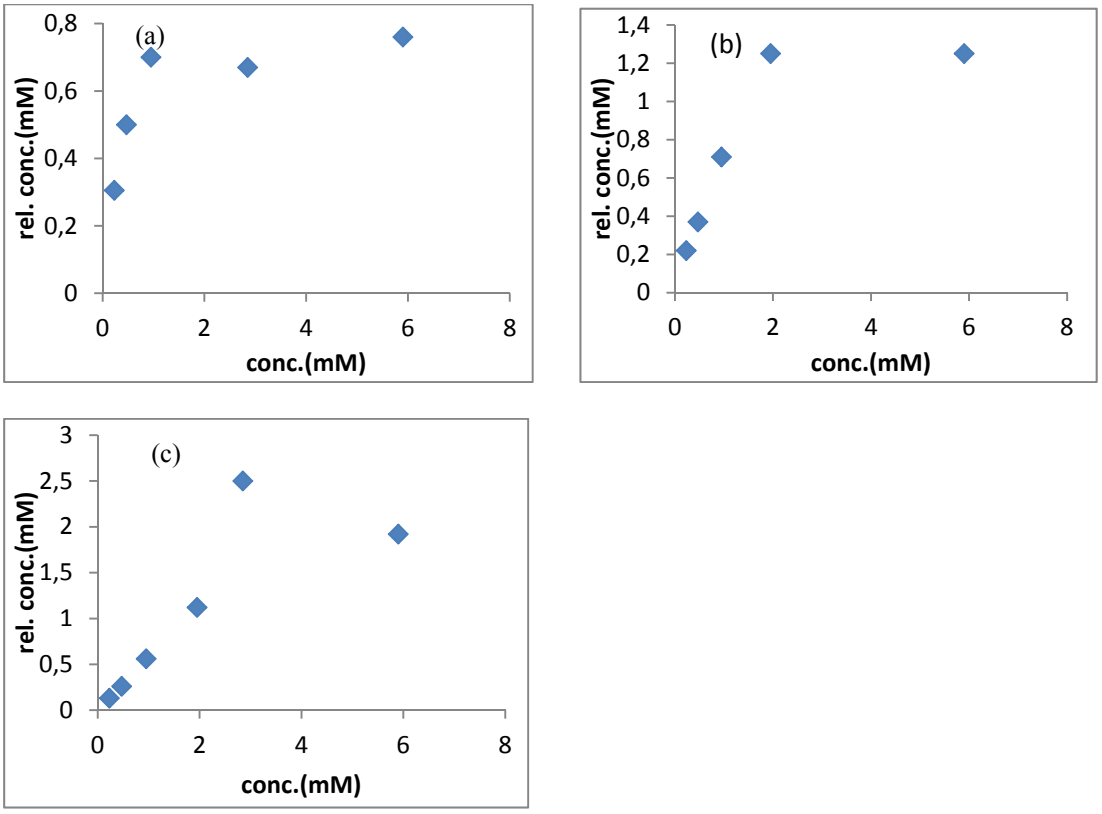


Figure S4: NMR solubility experiment graph for **1** at (a)pH 8, (b) pH 7 and (c) pH 6. Relative integral are w.r.t to 50mM hydroquinone in D_2O which was present in a concentric tube inside the NMR tube with the sample. The integral for hydroquinone peak was always taken as 100.

6. ANS Experiment:

From a stock solution of concentration0.8mM ANS in H_2O, 100µL solution was introduced in the 0.8mL of the vortexed gels at different pH values. An aliquot of 15 µL from this was diluted to 215µL in the TRIS-HCl buffer of respective pH and the fluorescence spectrum was recorded.

7. TEM: TEM micrographs were obtained using a JEOL 2100 transmission electron microscope. The TEM samples were prepared by directly applying gels at m.g.c on carbon coated TEM grid. A 5µL droplet of purified water was used to remove the salts and the excess solution was wicked off using filter paper. The samples were immediately stained using 5µl droplet of 1% phosphotungstic acid and was allowed to stand for 5 min. The excess solution was removed using a filter paper. The grids were then left under covered petri dish to dry before obtaining images.

8. HPLC analysis for *L* and *D*-phenylalanine methyl ester catalysis: 400 µL of gels and solutions of **1**and **2**respectively, were prepared in TRIS-HCl buffer at pH 7 and were allowed to stand for 24 hours. The gels were then vortexed in which 50µL of *L*- or *D*-phenylalnine methyl ester hydrochloride of respective concentrations prepared in the same buffer were introduced. The reaction vessels were then analysed by HPLC for a specific time by using a gradient of 5% to 95% acetonitrile(containing 1% TFA) w.r.t to water in 45 minutes.

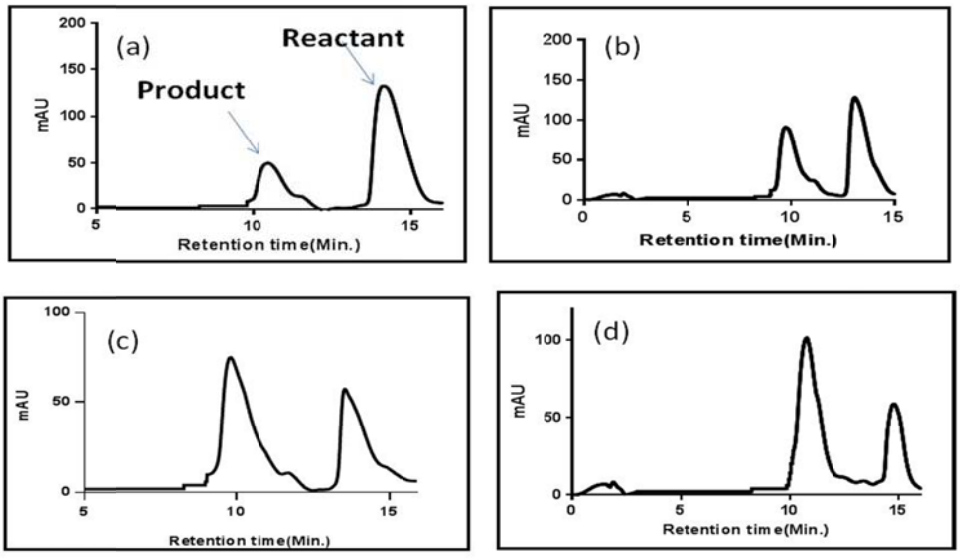


Fig. S6: HPLC chromatogram of the hydrolysis of phenylalanine methyl ester hydrochloride [12.3mM] using **1** [5.6mM] for (a) 8 hours (b) 30 hours (c) 60 hours (d) 72 hours.

The peak at 13.8 minutes corresponds to: phenylalanine methyl ester and the peak at 10 minutes corresponds to the product formed after hydrolysis.

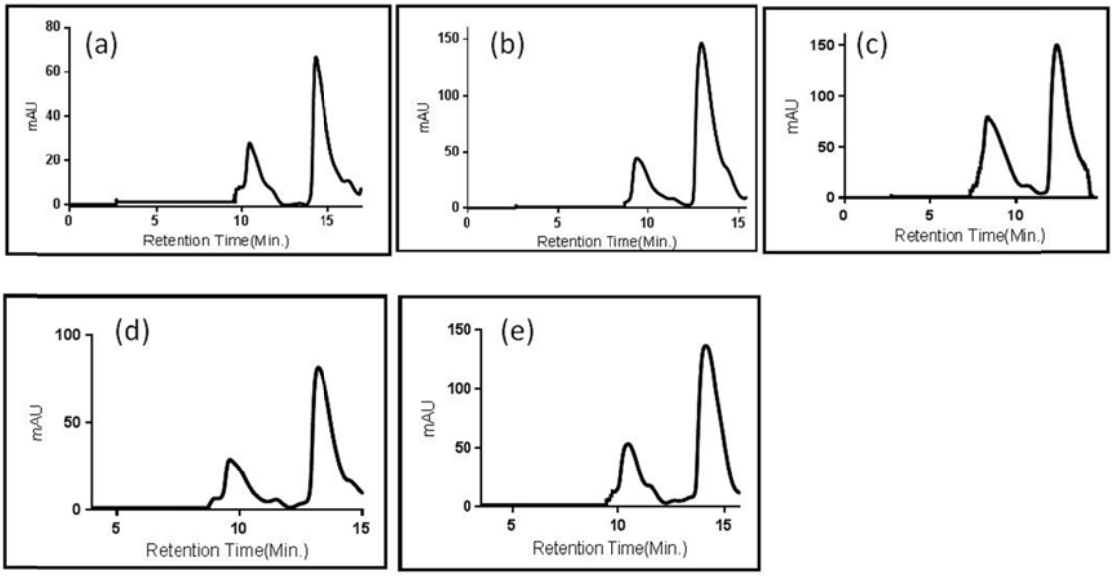


Fig. S7: HPLC chromatogram of the hydrolysis of different concentrations of *L*-phenylalanine methyl ester using **1** [5.7mM] for 12 hours (a) 4.2 mM (b) 8.2 mM (c) 12.3 mM (d)16.5 mM (e)20.7 mM.

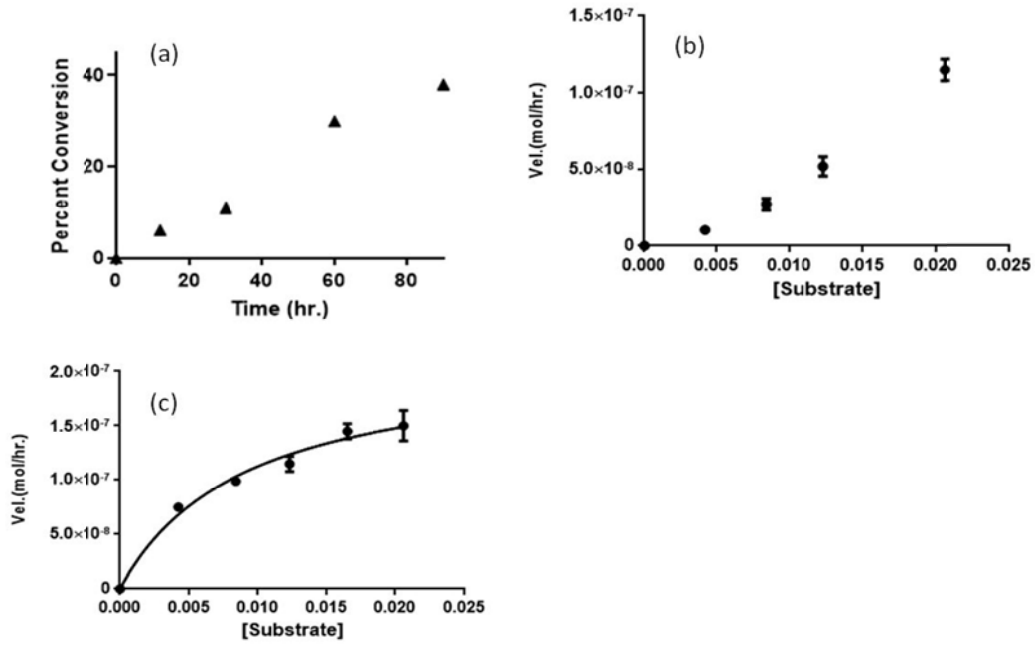


Fig. S8: (a) Percentage conversion by hydrolysis of 12.3Mm L-phenylalanine methyl ester by **2** over time. (b) Rate of hydrolysis of different concentrations of L-phenylalanine methyl esterusing **2** [5.7mM] for 24 hours (c) Michaelis-Menten fit for the hydrolysis of D-phenylalanine methyl ester by **1**.

Compound **1**	k_{cat}	K_M
L-phenylalanine methyl ester hydrochloride	$(3.7\pm0.3)\times10^{-5}hr^{-1}$	(29±1.6)mM
D-phenylalanine methyl ester hydrochloride	$(1.5\pm0.1)\times10^{-5}hr^{-1}$	(9±1.5)mM

9. Fourier Transform Infrared Spectroscopy:

The gels at m.g.c were washed with water to remove salts and non-aggregating parts and then lyophilized. The resultant dry solid was diluted with 200-300 mg of KBr in an agate mortar and then was compressed at 10 ton c.a. 2-3 minutes to make pellets. JASCO FTIR-6200 spectrometer was used to obtain the IR spectra.

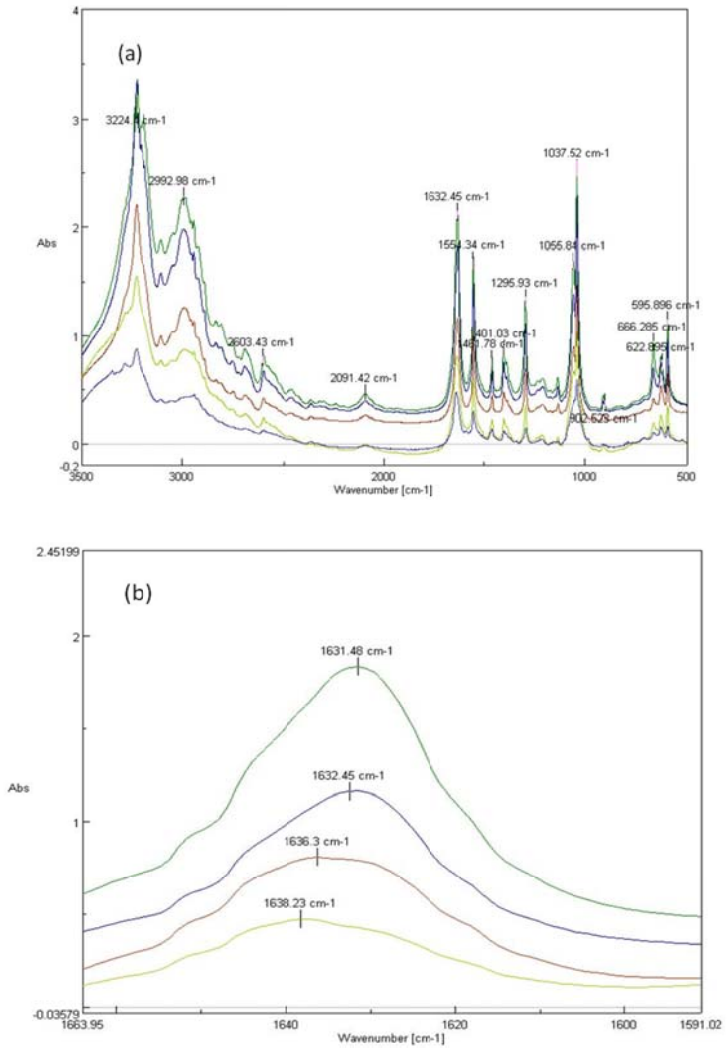


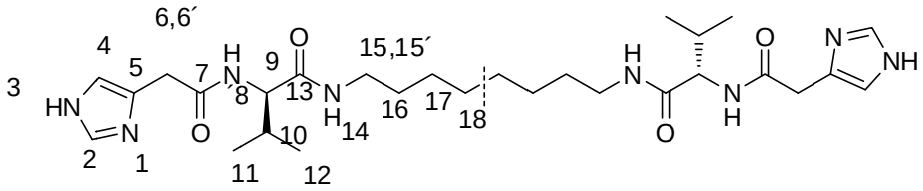
Figure S9: (a) IR spectra for gels of **1** at different pH values.

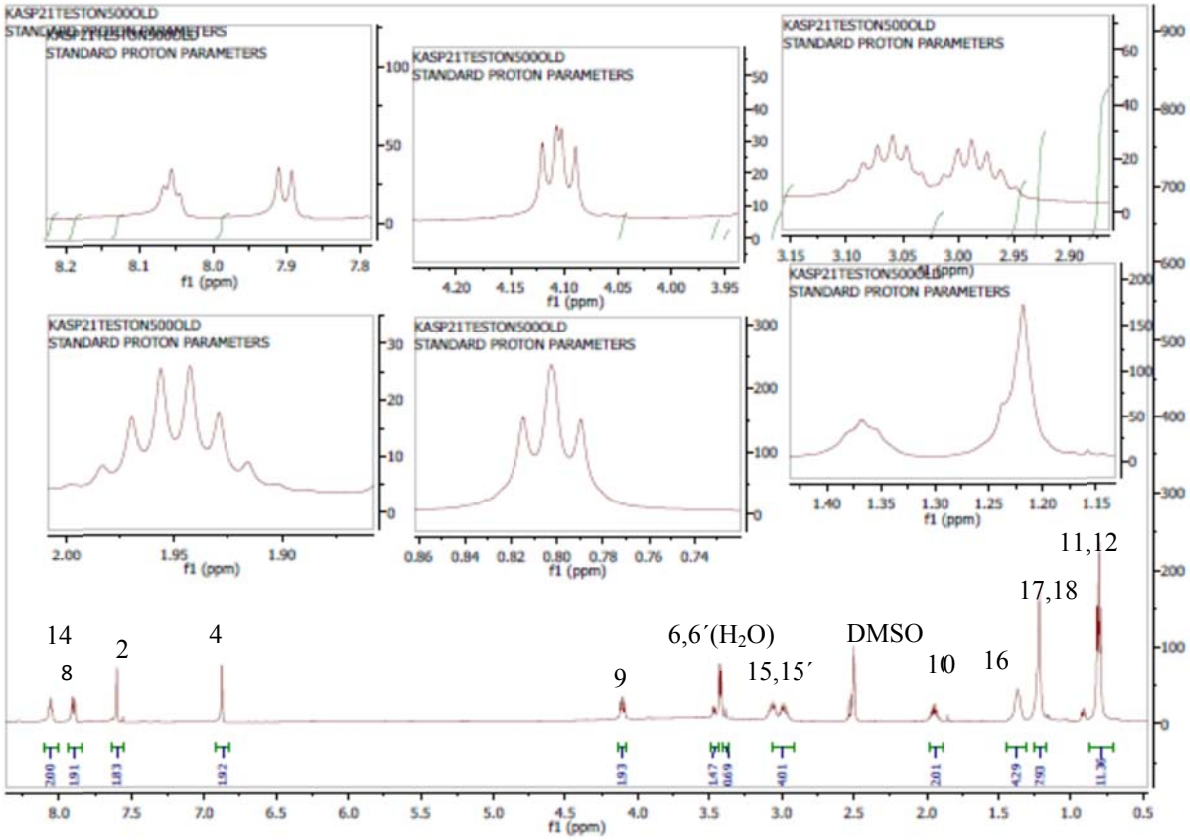
(b) Expanded amide I band region for β sheet: pH 8 (light green), pH 7.5(red), pH 7(blue), pH 6 (dark green).

10. NMR:

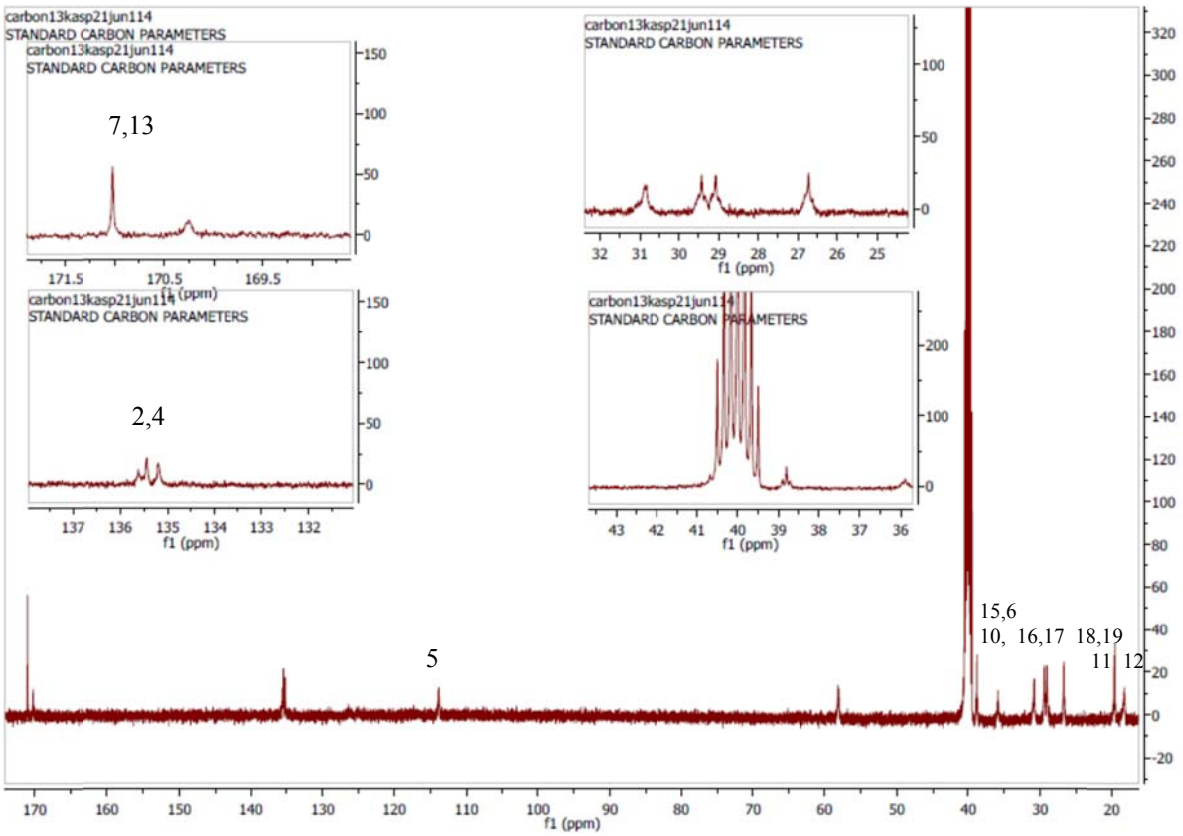
Compound 1:

^{1}H NMR:



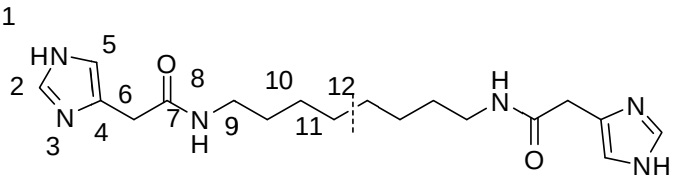


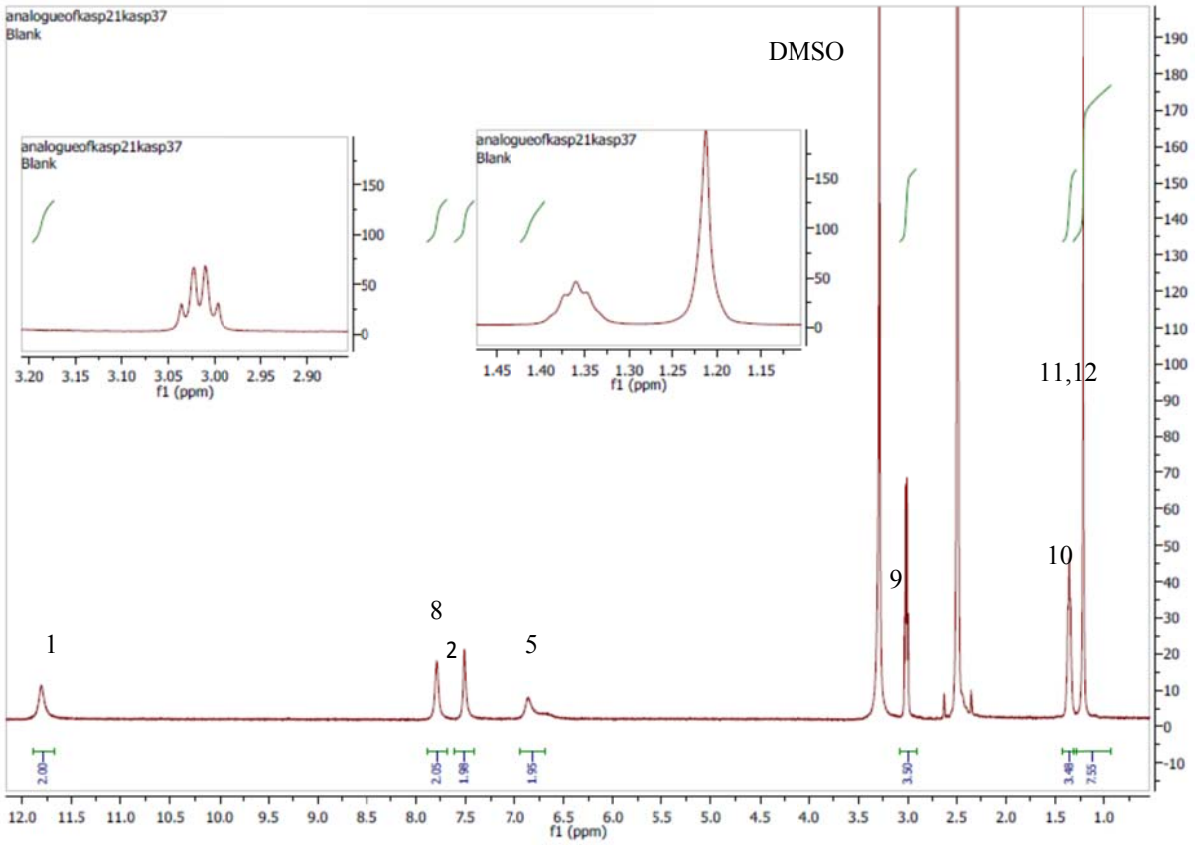
^{13}C NMR:


carbon13kasp21jun114
STANDARD CARBON PARAMETERS
7,13
2,4
5
15,6
10, 16,17 18,19
11 12
f1 (ppm)

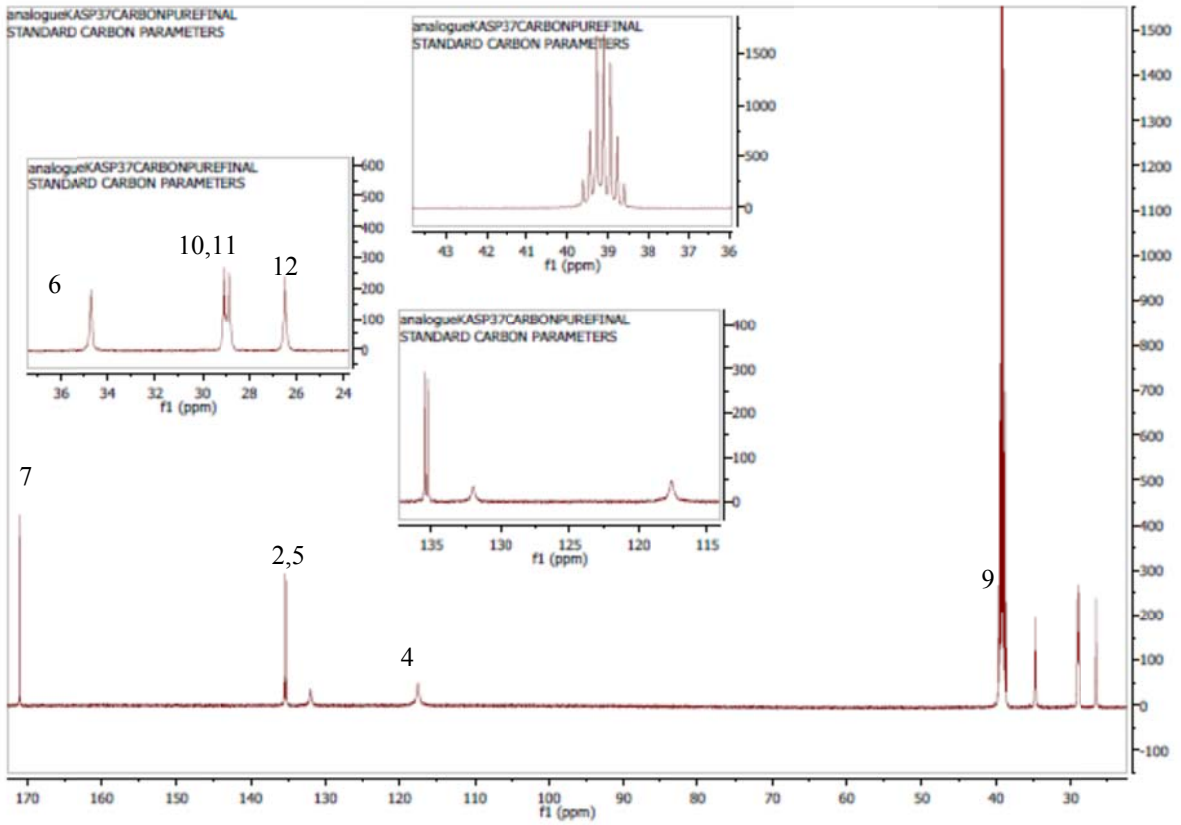
Compound 2:

^{1}H NMR:





^{13}C NMR:



11.

a. Temperature dependent NMR experiment:

To establish the retainment of molecules in aggregated state upon dilution, as used for catalysis, a temperature dependent NMR experiment was performed. An aliquot from the gel of compound **1** was diluted to 0.17mM following the same method as used in catalysis. This sample was transferred to an NMR tube containing a concentric tube with an internal standard, hydroquinone(50mM). The tube was subjected to rise in temperature and respective NMR integrals were recorded. With subsequent rise in temperature the relative integral of compound **1** rose w.r.t the fixed integral of hydroquinone (set as 100). Thus it can be deduced

that the molecules which were in aggregated state (NMR invisible), on application of heat disintegrated and started getting solubilised into the solution (NMR visible).

b.Comparison of amount of molecules in solution for gel and diluted aliquot of gel by NMR:

A gel of **1** was prepared in an NMR tube with an internal standard of hydroquinone(50mM). The concentration of molecules in solution was found to be 2.3 mM. Subsequently an aliquot of gel was diluted to 0.17mM and the concentration of **1** in solution was found to be 0.08mM. The ratio of molecules in solution for gel and aliquot is 1.17 which confirms that **1** still retains most of its aggregate form upon dilution.

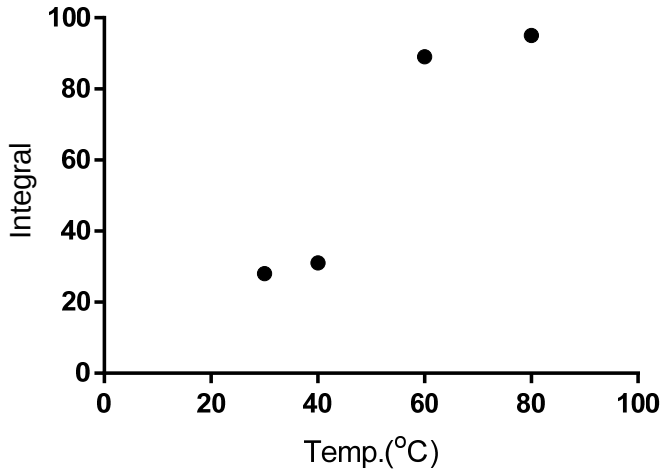


Fig. S10: Integral of a selected peak of compound **1** w.r.t to the fixed integral of hydroquinone versus temperature.