Supporting information

Structural study of Lindqvist polyoxovanadate-peptide conjugates

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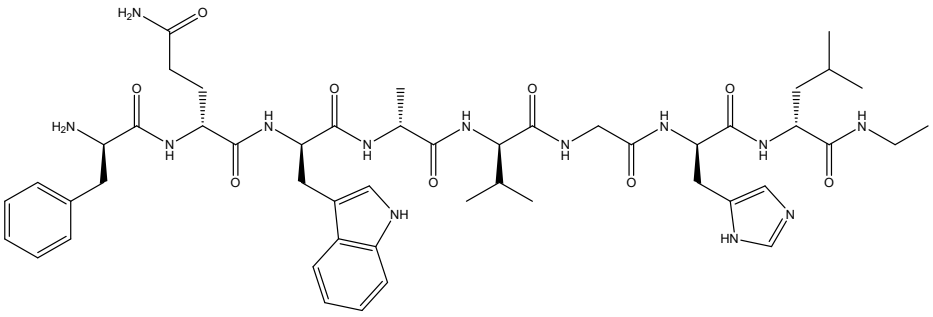
1) Synthesis of the peptides:

Pep-1: H-fQWAVGHLNHEt peptide (DB)



D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NHEt

The Fmoc-based synthetic strategy was utilized for manual solid-phase synthesis of the peptide in a 0.06 mmolar scale. Loading was achieved on Fmoc-deprotected ethyl-indole AM resin using equimolar HATU (hexafluorophosphate azabenzotriazole tetramethyl uronium) as the coupling reagent in the presence of a two-fold molar excess of DIPEA (N,N-Diisopropylethylamine), with Fmoc-Leu-OH (0.18 mmol). For each coupling step, a three-fold molar excess (0.24 mmol) of Fmoc-amino acids was used, with HBTU (hexafluorophosphate benzotriazole tetramethyl uronium)/HOBt (hydroxybenzotriazole) as the coupling reagents, and the reaction time was 40 min. The Fmoc group was removed using 20% piperidine in DMF. The peptide was then detached from the resin and all the side-chain protecting groups of the amino acid residues were removed using trifluoroacetic acid/anisole/triisopropylsilane/H_2O (95:2.5:2.0:0.5 v/v) for 45 min, and the desired peptide was precipitated by adding diethyl ether to the solid residue and centrifuging the mixture at 5000 rpm for 10 minutes. The supernatant was removed, and the peptide was dissolved in water, filtered to remove insoluble resin, and freeze-dried. The crude product was then purified by preparative HPLC system (Shimadzu, Tokyo, Japan) equipped with LC-8A pumps, SCL-8A controller and SPD-6A spectrophotometric detector, using a linear gradient (eluent A: 0.05% TFA in H_2O; eluent B: 0.05% TFA in 9:1 v/v CH_3CN-H_2O; 20-45%B in 35 minutes; flow 12 mL/min), and a Vydac C18 column

(300Å, 10µ, 250 x 22 mm). The yield was 35%.

ESI MS (+) in MeOH. m/z =984.5 is the peak of peptide ($[M+H]^+$ calculated = 984.5).

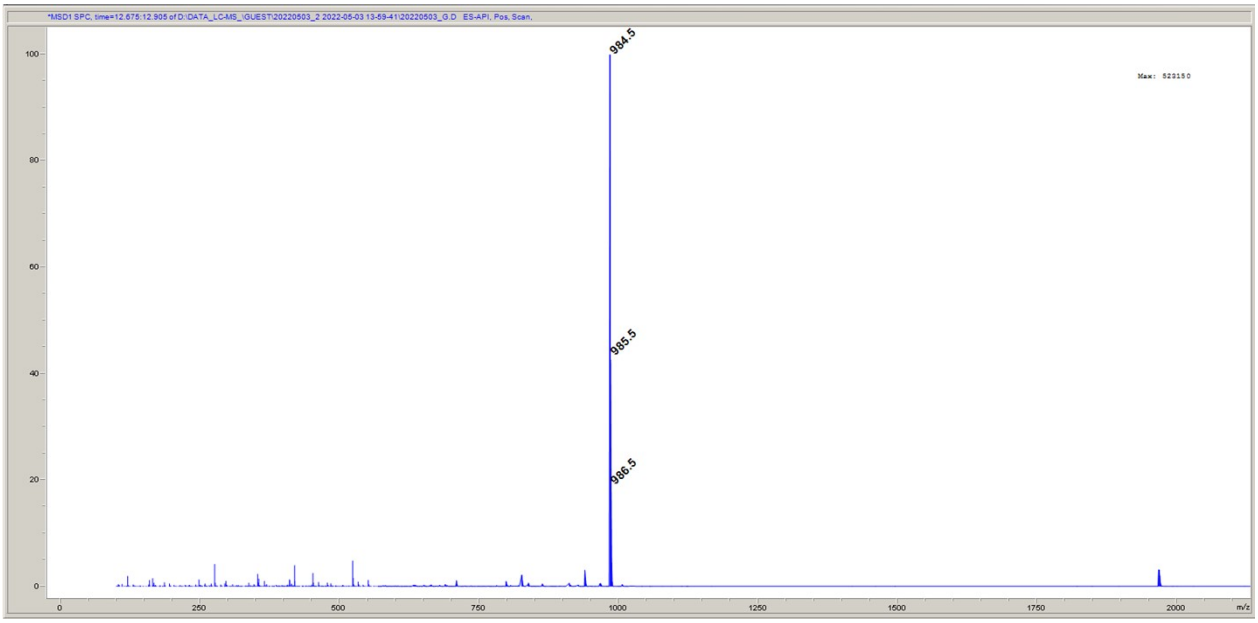


Fig. S.1 ESI-MS (+) spectrum of Pep-1 in MeOH.

Table 1. Proton resonances of Pep-1 (0.6 µM d_6-DMSO solution).

AA	NH (ppm)	CH_α (ppm)	Rest of the chain(ppm)
Phe	/	4.07	$-CH_{2\beta}$ 2.97 Aromatics 7.27
Gln	8.55	4.33	$-CH_{2\beta}$ (diastereotopic) 1.78 1.62 $-CH_{2\gamma}$ 1.95 $-NH_2$ not detected
Trp	8.30	4.56	$-CH_{2\beta}$ (diastereotopic) 3.11, 2.91 Unassigned aromatics
Ala	8.25	4.41	$-CH_3$ 1.20
Val	7.77	4.17	$-CH_\beta$ 1.97 $(-CH_{3\gamma})_2$ 0.85
Gly	8.23	$-CH_{2\alpha}$ 3.72, 3.79	/
His	8.13	4.61	$-CH_{2\beta}$ 3.03 -NH aromatics not detected -CH Unassigned aromatics
Leu	8.09	4.20	$-CH_{2\beta}$ 1.48 $-CH_\gamma$ 0.86 $(-CH_{3\delta})_2$ not detected
NHEt	8.04	$-CH_{2\alpha}$ 3.08	$-CH_{3\beta}$ 1.01

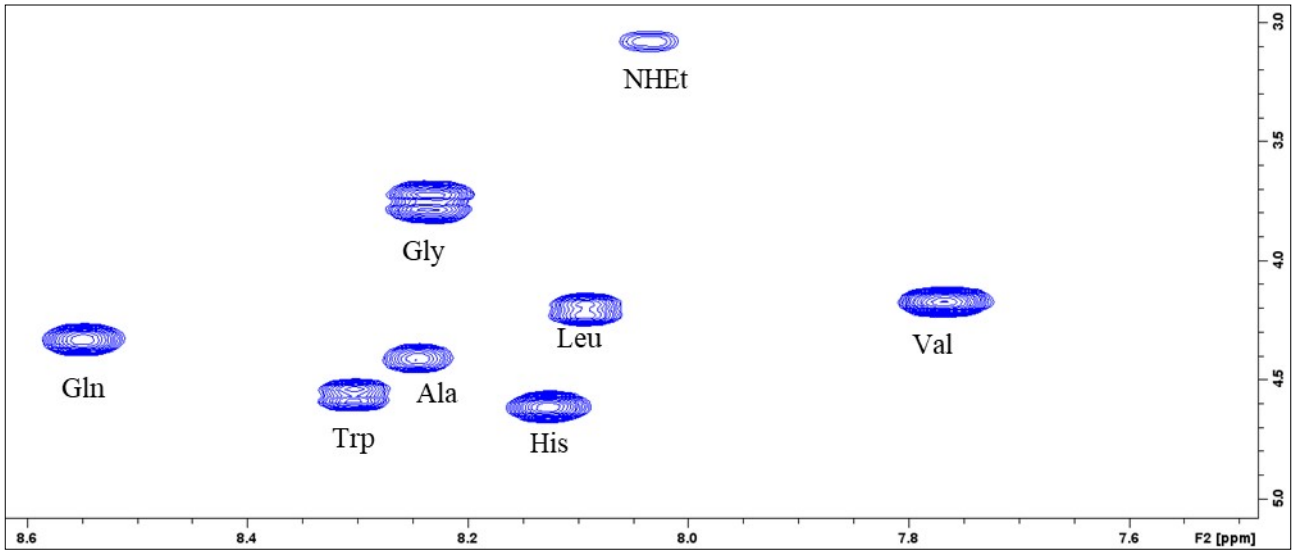


Fig. S.2 2D-^{1}H-NMR COSY spectrum of Pep-1 (150 µM) in d_6-DMSO.

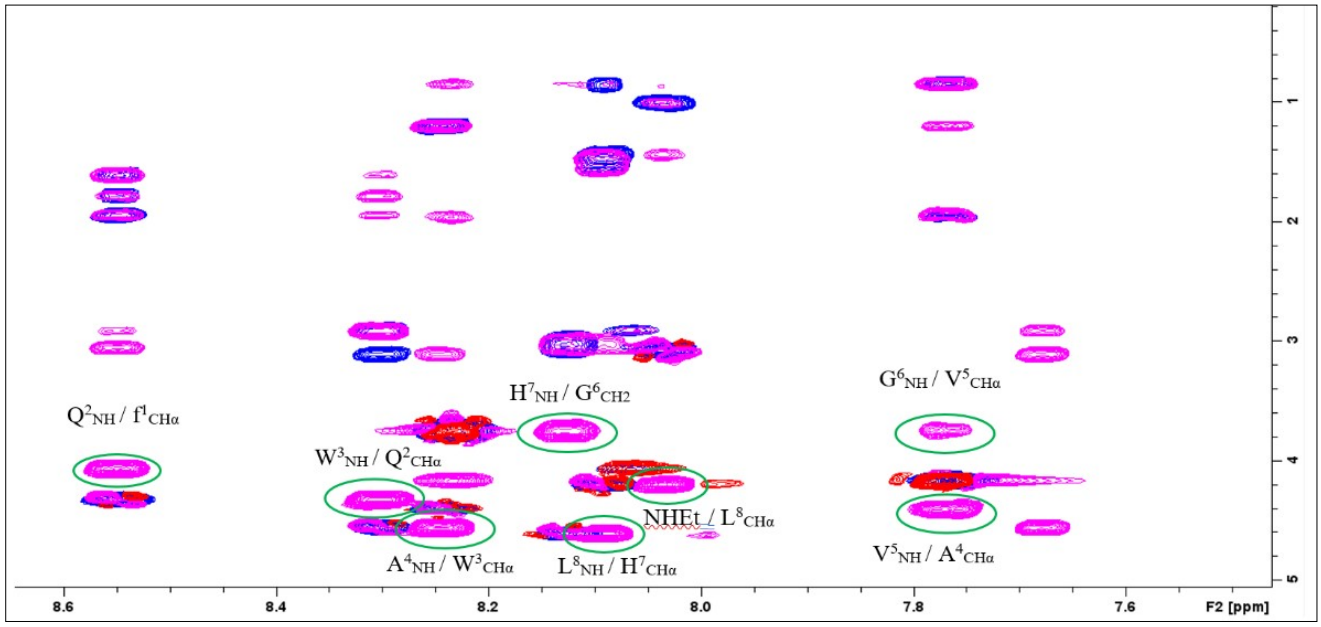
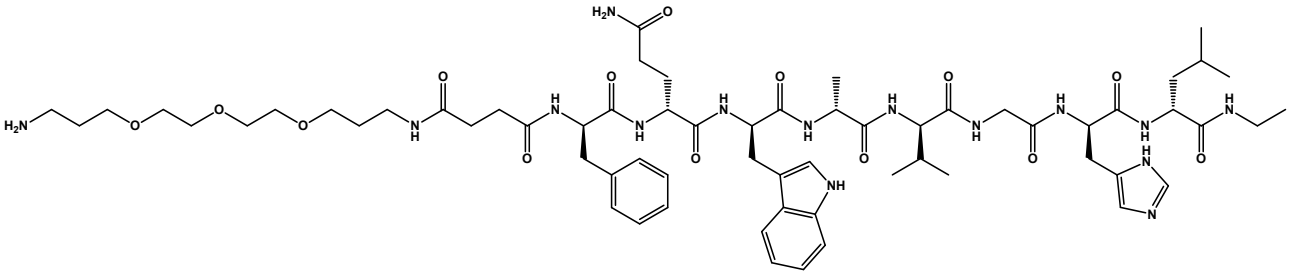


Fig. S.3 2D-^{1}H-NMR spectrum of Pep-1 (150 µM) in d_6-DMSO. TOCSY spectra (blue) and ROESY (pink), cross peak region.

Pep-2: Ttds-fQWAVGHL-NHEt (Ttds-DB)



H-Ttds-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NH-Et

The addition of the customized polyethylene glycol (PEG) -like linker, trioxatridecan-succinamic acid (Ttds), resulted in the synthesis of Pep-2. Ttds is a biocompatible and non-toxic substance that enhances water solubility, increases stability, and elicits minimal immune response. Pep-2 was synthesized according to the procedure previously described. The crude product was then purified by preparative HPLC system. ESI MS (+) in MeOH: at m/z =1286.7 and 644.3, the peaks of peptide are present ($[M+H]^+$: calculated m/z =1286.7 and $[M+2H]^{2+}$: calculated m/z= 643.8).

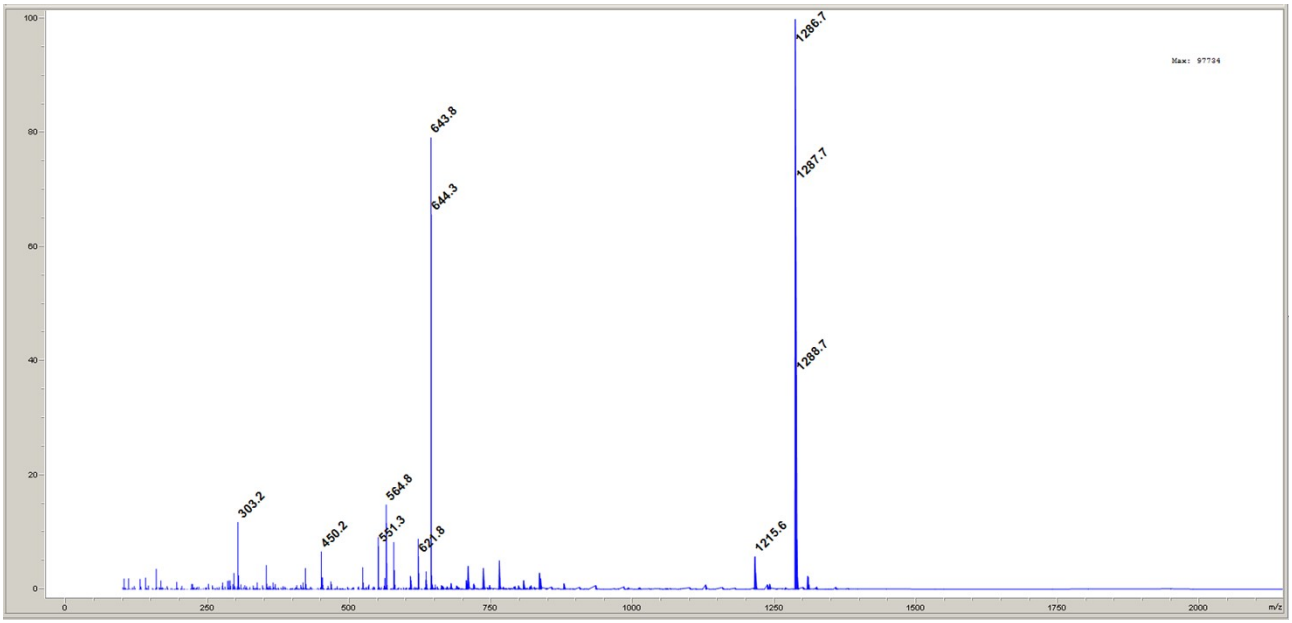


Fig. S.4 ESI-MS (+) spectrum of Pep-2 in MeOH.

Table 2. Proton resonances of Pep-2 (0.6 µM d_6-DMSO solution).

AA	NH (ppm)	CH_α (ppm)	Rest of the chain(ppm)
D-Phe	8.25	4.41	$-CH_{2\beta}$ (diastereotopic) 2.78, 2.95 Aromatics not observed
Gln	8.34	4.07	$-CH_{2\beta}$ (diastereotopic) 1.90, 1.77 $-CH_{2\gamma}$ 1.59
Trp	7.98	4.46	$-CH_{2\beta}$ (diastereotopic) 3.00, 3.16 Unassigned aromatics
Ala	7.90	4.35	$-CH_3$ 1.21
Val	7.61	4.13	$-CH_\beta$ 1.98 $(-CH_{3\gamma})_2$ 0.85
Gly	8.22	$-CH_{2\alpha}$ 3.70, 3.76	/
His	8.08	4.60	$-CH_{2\beta}$ 3.02 -NH aromatic not detected -CH Unassigned aromatics
Leu	8.06	4.20	$-CH_{2\beta}$ 1.47 $-CH_\gamma$ 0.85 $(-CH_{3\delta})_2$ not detected
NH-Ttds	7.79	$-CH_{2\alpha}$ 3.03	$-CH_{2\beta}$ 1.58 $-CH_{2\gamma}$ 3.35
NHEt	8.02	$-CH_{2\alpha}$ 3.06	$-CH_{3\beta}$ 1.01
NH_3^+ term	7.64	$-CH_{2\alpha}$ 2.84	$-CH_{2\beta}$ 1.76 $-CH_{2\gamma}$ 3.46

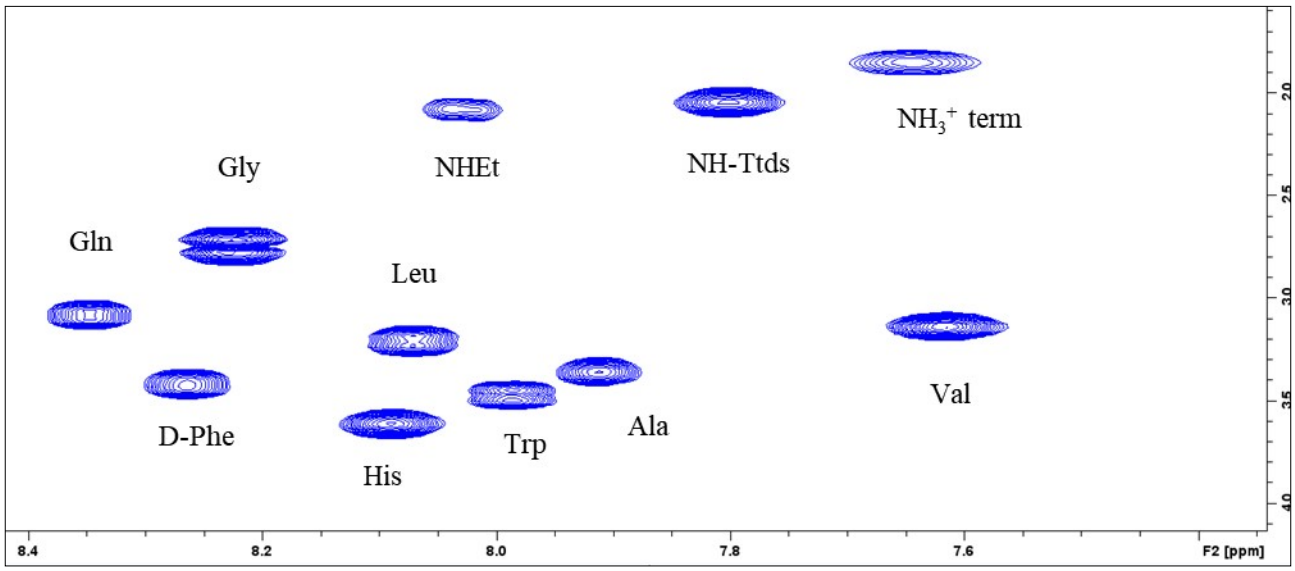


Fig. S.5 2D-^{1}H-NMR COSY spectrum of Pep-2 (150 µM) in d_6-DMSO.

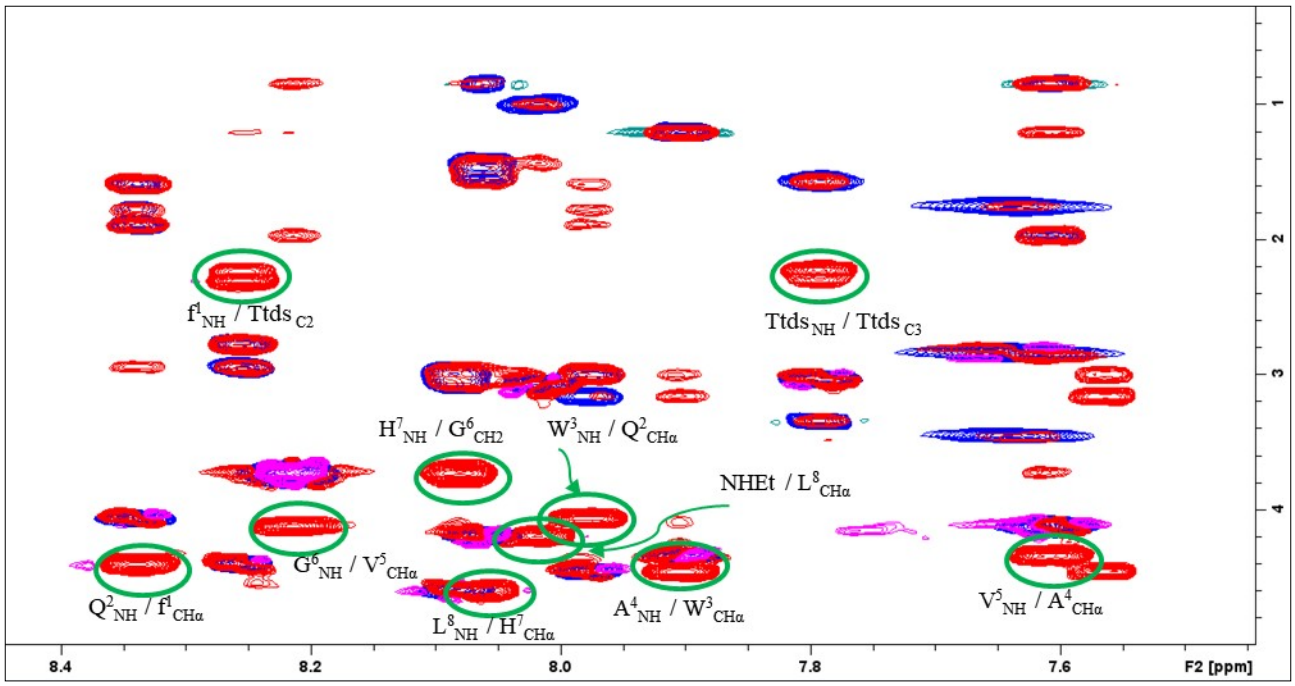
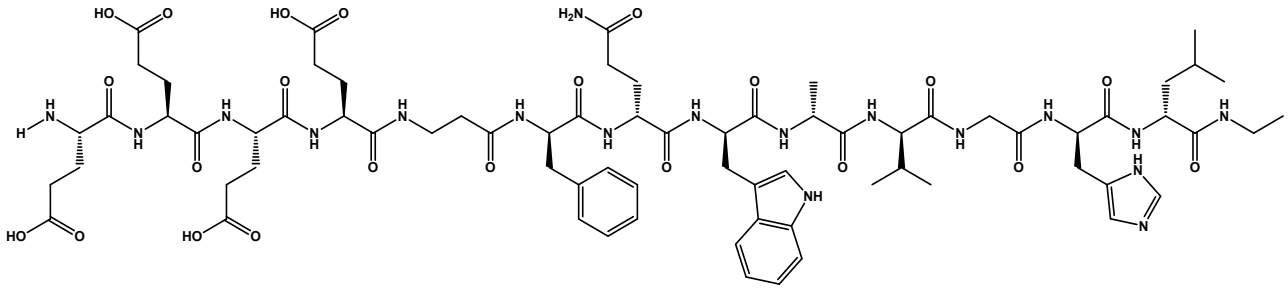


Fig. S.6 2D-^{1}H-NMR TOCSY and ROESY spectra of Pep-2 in d_6-DMSO.

Pep-3: EEEEβA-fQWAVGHL-NHEt (EEEA-DB)



H-Glu-Glu-Glu-Glu-βAla-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NH-Et

To mitigate peptide-POV interaction, a polyanionic spacer composed of four glutamic acid residues and a β-Ala was added to obtain Pep-3. This linker creates charge repulsion with the negatively charged POV. The extra β-alanine residue was introduced to enhance flexibility. Pep-3 was synthesized according to the procedure previously described for Pep-1.

ESI MS (+) in MeOH: $[M+H]^+$: calculated m/z = 1571.7, found = 1571.7 m/z; $[M+3H]^{3+}$: calculated m/z= 524.6, found = 523.3 m/z

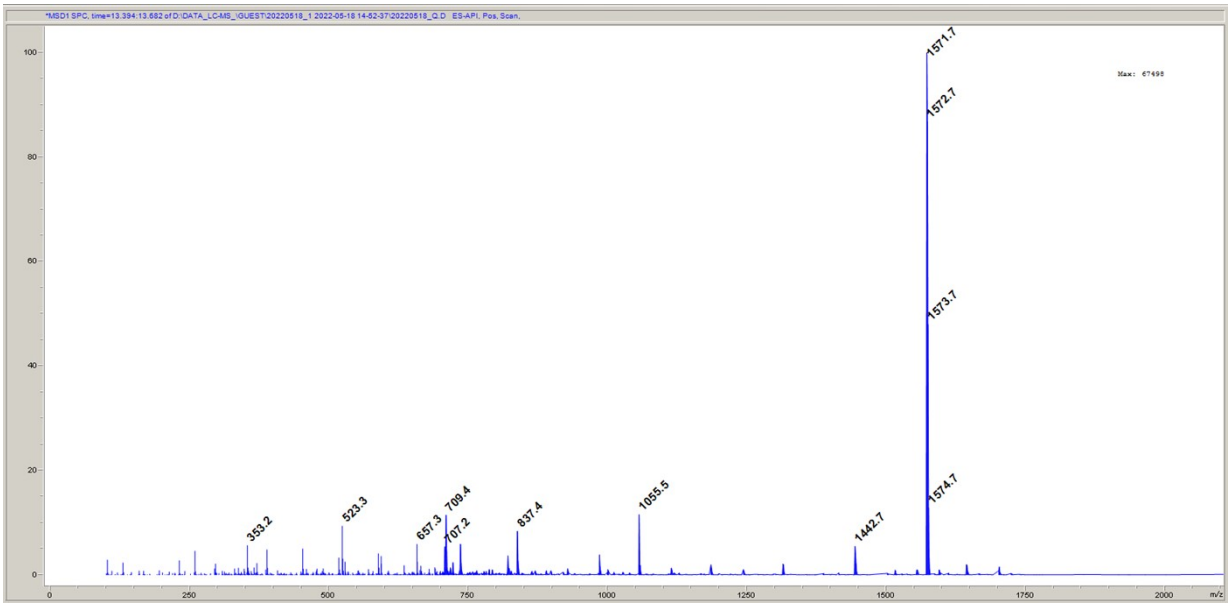


Fig. S.7 ESI-MS (+) spectrum of Pep-3 in MeOH.

Table 3. Proton resonances of Pep-3 (0.6 μM d_6-DMSO solution).

AA	NH (ppm)	CH_α (ppm)	Rest of the chain(ppm)
Glu[2]	8.62	4.35	$-CH_{2\beta}$ 2.30 $-CH_{2\gamma}$ 1.77
Glu[3]	8.21	4.25	$-CH_{2\beta}$ 2.25 $-CH_{2\gamma}$ 1.74
Glu[4]	8.01	4.15	$-CH_{2\beta}$ 2.19 $-CH_{2\gamma}$ 1.72
βAla	7.93	$-CH_{2\alpha}$ 3.11, 3.21	$-CH_{2\beta}$ 2.26
D-Phe	8.27	4.45	$-CH_{2\beta}$ (diastereotopic) 2.93, 2.78 Aromatics not observed
Gln	8.36	4.10	$-CH_{2\beta}$ 1.89 $-CH_{2\gamma}$ 1.58
Trp	8.01	4.48	$-CH_{2\beta}$ (diastereotopic) 3.07 Unassigned aromatics
Ala	7.97	4.37	$-CH_3$ 1.21
Val	7.61	4.14	$-CH_\beta$ 1.97 $(-CH_{3\gamma})_2$ 0.85
Gly	8.24	$-CH_{2\alpha}$ 3.70, 3.77	/
His	8.08	4.61	$-CH_{2\beta}$ 3.00 -NH aromatic not detected -CH Unassigned aromatics
Leu	8.07	4.20	$-CH_{2\beta}$ 1.47 $-CH_\gamma$ 0.85 $(-CH_{3\delta})_2$ not detected
NHEt	8.04	$-CH_{2\alpha}$ 3.08	$-CH_{3\beta}$ 1.00

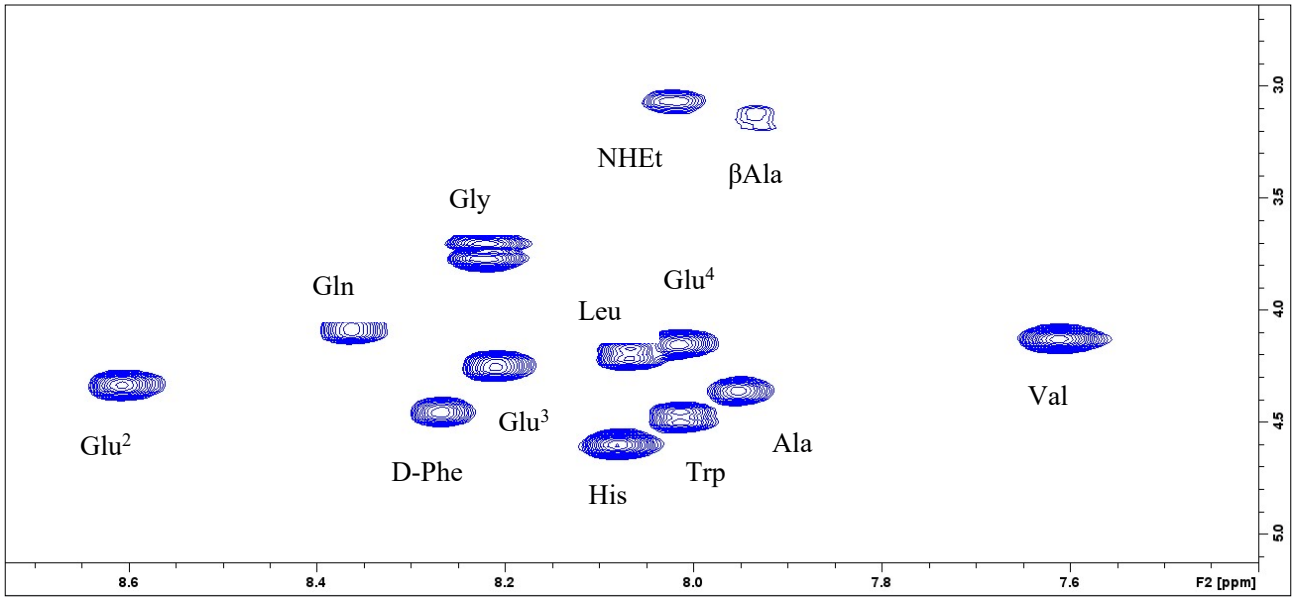


Fig. S.8 2D-^{1}H-NMR COSY spectrum of Pep-3 (150 µM) in d_6-DMSO.

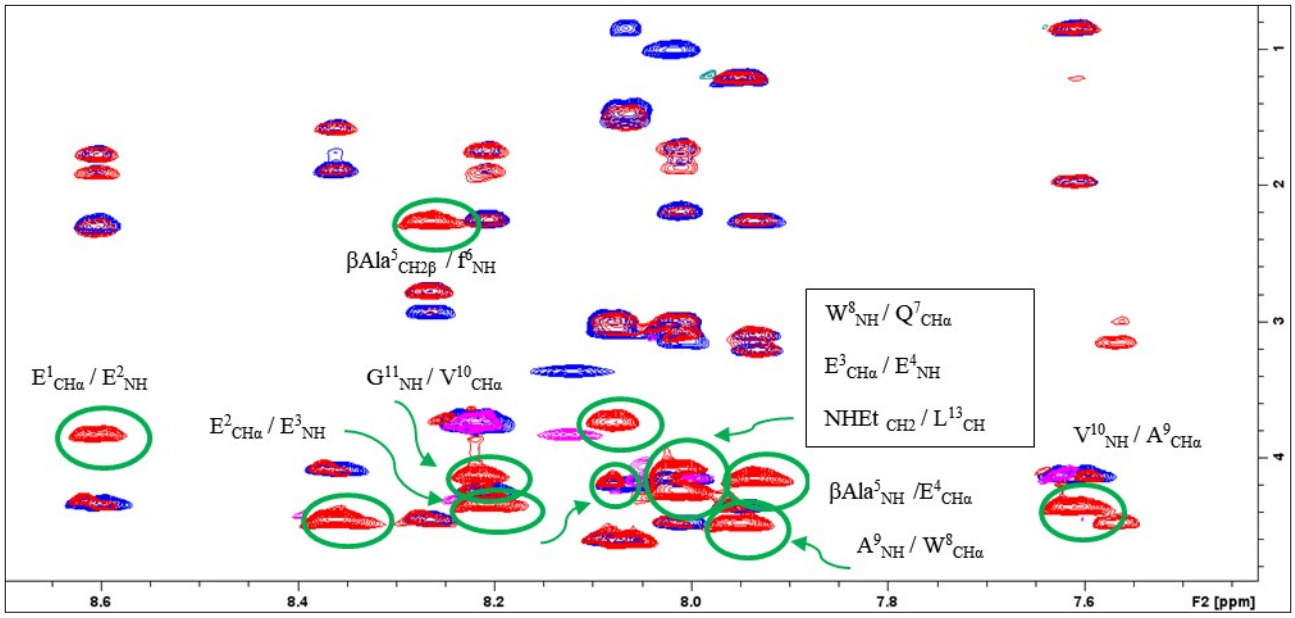
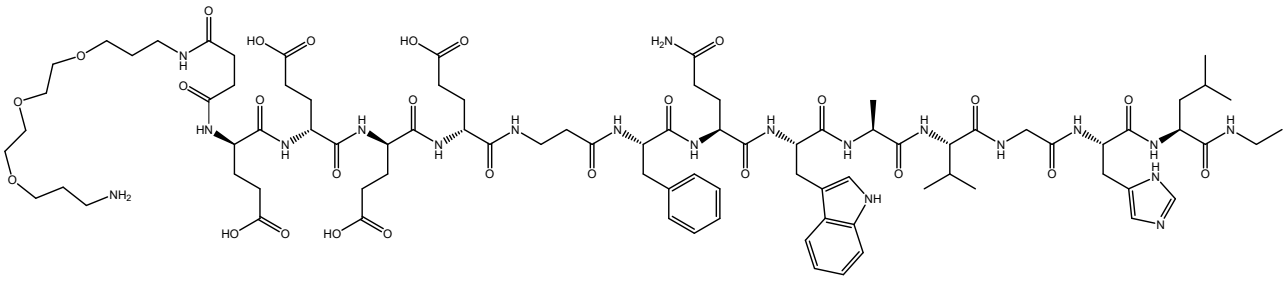


Fig. S.9 2D-^{1}H-NMR spectra of Pep-3 (150 µM) in d_6-DMSO. ROESY in red overlapped with TOCSY in blue, cross peak region.

Pep-4: Ttds- EEEEA-fQWAVGHL-NHEt (Ttds-EEEA-DB)



H-Ttds- Glu-Glu-Glu-Glu-βAla-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NH-Et

To mitigate peptide-POV interaction and improve water solubility, Pep-4 was synthesized by adding both the Glu-Glu-Glu-Glu-β-Ala and the 4,7,10-trioxa-1,13-tridecanediamine (Ttds). ESI-MS: the peaks of the charged peptide are present at m/z =1873.2 ($[M+H]^+$: calculated m/z =1874.1) and at m/z= 937.3 ($[M+2H]^{2+}$: calculated m/z =938.0.

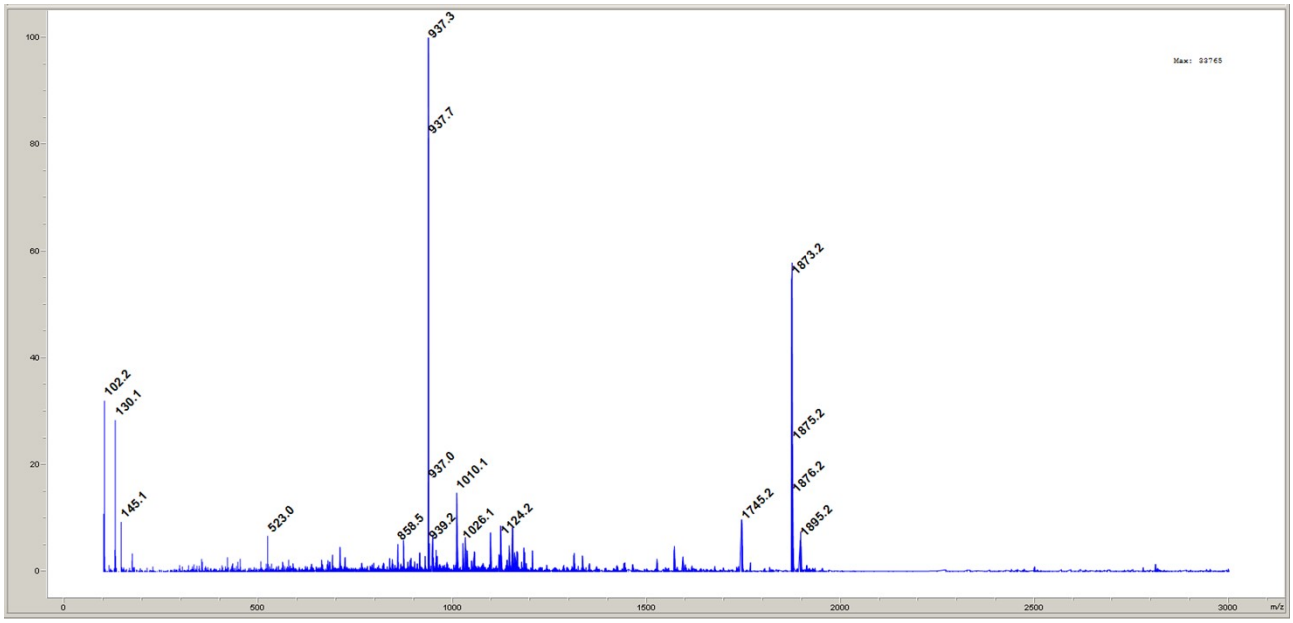


Fig. S.10 ESI-MS (+) spectrum of Pep-4 in MeOH.

Table 4. Proton resonances of Pep-4.

AA	NH (ppm)	CH_α (ppm)	Rest of the chain(ppm)
Glu[1]	8.18	4.17	$-CH_{2\beta}$ 2.26 $-CH_{2\gamma}$ 1.82
Glu[2]	8.09	4.19	$-CH_{2\beta}$ 2.27 $-CH_{2\gamma}$ 1.87
Glu[3]	7.83	4.20	$-CH_{2\beta}$ 2.25 $-CH_{2\gamma}$ 1.83
Glu[4]	7.79	4.13	$-CH_{2\beta}$ 2.20 $-CH_{2\gamma}$ 1.79
βAla	7.83	$-CH_{2\alpha}$ 3.11, 3.21	$-CH_{2\beta}$ 2.26
D-Phe	8.28	4.44	$-CH_{2\beta}$ (diastereotopic) 2.93, 2.78 Aromatics not observed
Gln	8.37	4.08	$-CH_{2\beta}$ 1.82 $-CH_{2\gamma}$ 1.57
Trp	8.01	4.48	$-CH_{2\beta}$ (diastereotopic) 3.01, 3.16 Unassigned aromatics
Ala	7.95	4.36	$-CH_3$ 1.21
Val	7.61	4.13	$-CH_\beta$ 1.98 $(-CH_{3\gamma})_2$ 0.86
Gly	8.23	$-CH_{2\alpha}$ 3.71, 3.75	/
His	8.07	4.61	$-CH_{2\beta}$ 3.03 -NH aromatic not detected -CH Unassigned aromatics
Leu	8.06	4.19	$-CH_{2\beta}$ 1.49 $-CH_\gamma$ 0.86 $(-CH_{3\delta})_2$ not detected
NH-Ttds	7.90	3.06	$-CH_{2\beta}$ 1.60 $-CH_{2\gamma}$ 3.37
NH_3^+ term	7.61	$-CH_{2\alpha}$ 2.85	$-CH_{2\beta}$ 1.77 $-CH_{2\gamma}$ 3.47
NHEt	8.03	$-CH_{2\alpha}$ 3.08	$-CH_{3\beta}$ 1.00

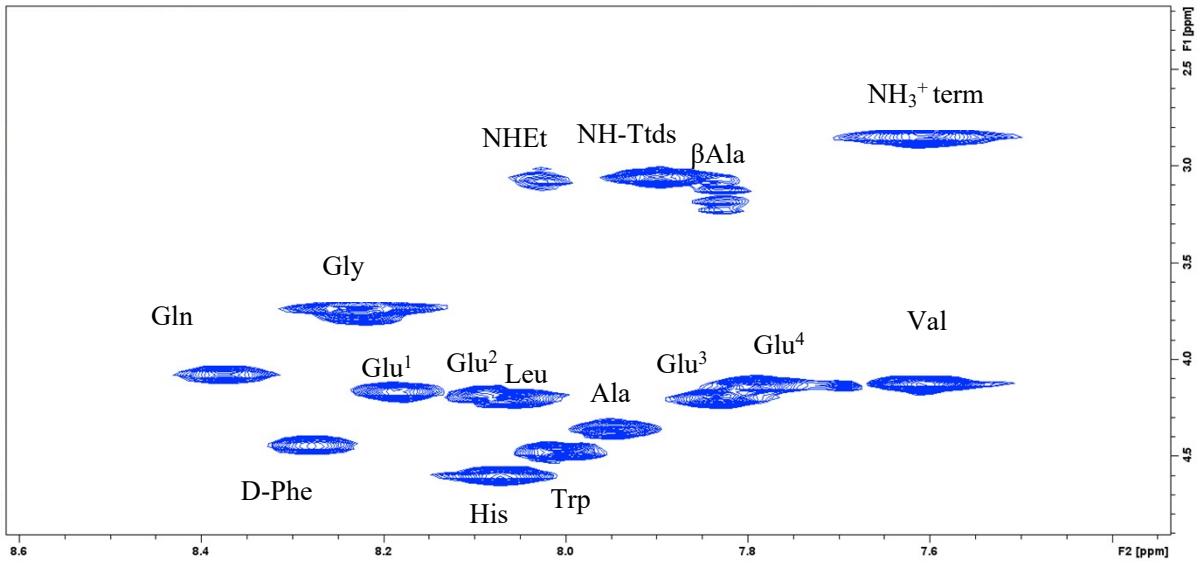


Fig. S.11 2D-^{1}H-NMR COSY spectrum of Pep-4 in d_6-DMSO.

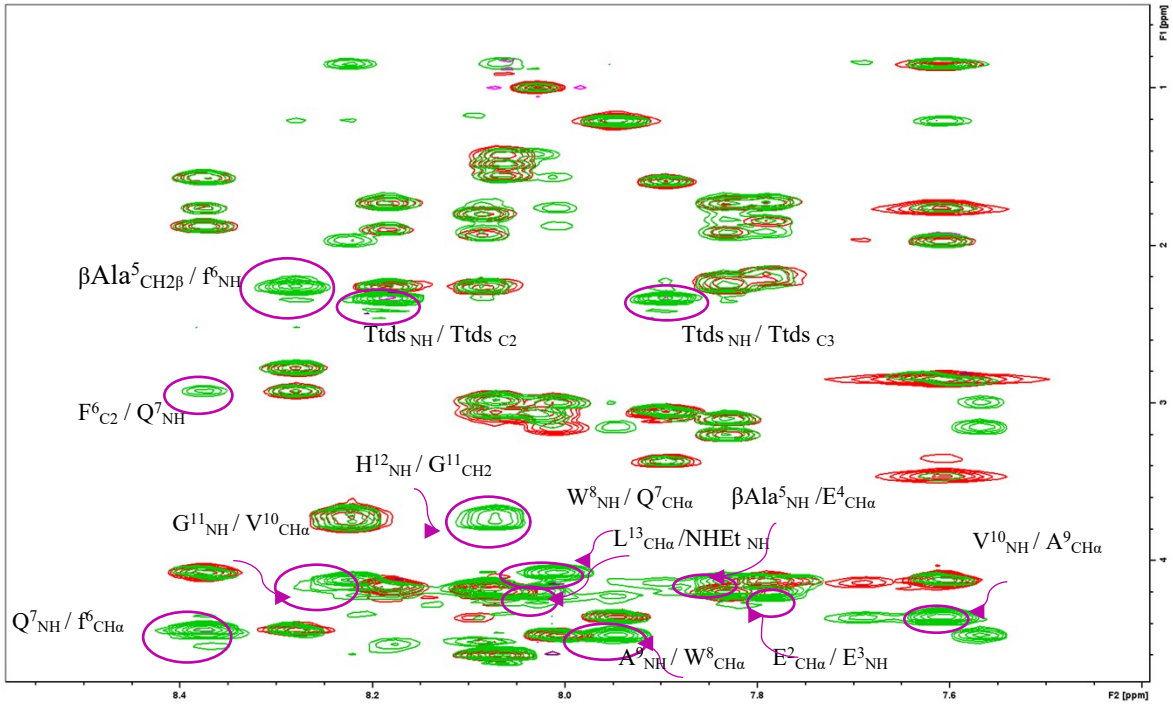
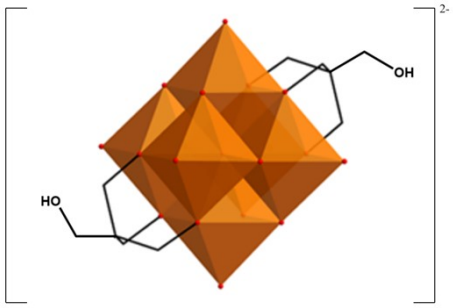


Fig. S.12 2D-^{1}H-NMR spectra TOCSY and ROESY spectra of Pep-4 in d_6-DMSO.

2) Synthesis of POMs precursors

Synthesis of $[(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OH\}_2]$ (V_6-OH)



Reaction:

$$6NaVO_3 + 4HCl + 2(C_4H_9)_4BrN + 2(HOCH_2)_3CCH_2OH \xrightarrow{H_2O,\ 80°C,\ 48\ hours} [(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OH\}_2] + 5H_2O + 4NaCl + 2NaBr$$

Procedure:

In a round-bottom flask $NaVO_3 \cdot H_2O$ (5.00 g, 0.036 mol) was dissolved in 120 mL of H_2O under mild heating (≈50°C) and vigorous stirring until a clear almost colorless solution was obtained. The solution was cooled to room temperature and a 3.84 M solution of HCl (8.65 mL, 0.033 mol) was added dropwise with a burette, monitoring the pH level with a universal indicator until it reached pH=3. During the titration experiment the solution passed from being colorless to yellow, then orange, then red, till when it stabilized on a bright orange coloration. $(HOCH_2)_3CCH_2OH$ (3.75 g, 0.028 mol) was added to the solution and the mixture was heated at 80°C for 48 hours using a reflux condenser. The final brown/dark orange mixture was cooled to room temperature and added dropwise to a previously prepared TBABr solution (10 g, 0.031 mol in 20 mL H_2O) under powerful stirring until a red-orange solid was formed. The crude product was filtered on a fitted funnel and washed with 50 mL of H_2O, 80 mL of diethyl ether, monitoring its purity by ESI-MS and FT-IR, then it was dried under vacuum. A light orange powder was obtained (5.07 g, 67% yield).

Characterization:

FTIR (KBr, cm^{-1}): 3376 (m, br), 2962 (s), 2943 (s), 2873 (s), 1640 (m), 1481 (s), 1383 (m), 1263 (w), 1123 (s), 1074 (m), 1034 (s), 951 (s), 884 (w), 810 (s), 794 (s), 713 (s), 650 (m), 580 (s), 513 (w), 490 (w), 420 (s).

ESI-MS (-) CH_3CN: 1022 ($[M\text{-}TBA]^-$), 780.6 ($[M\text{-}2TBA+H]^-$).

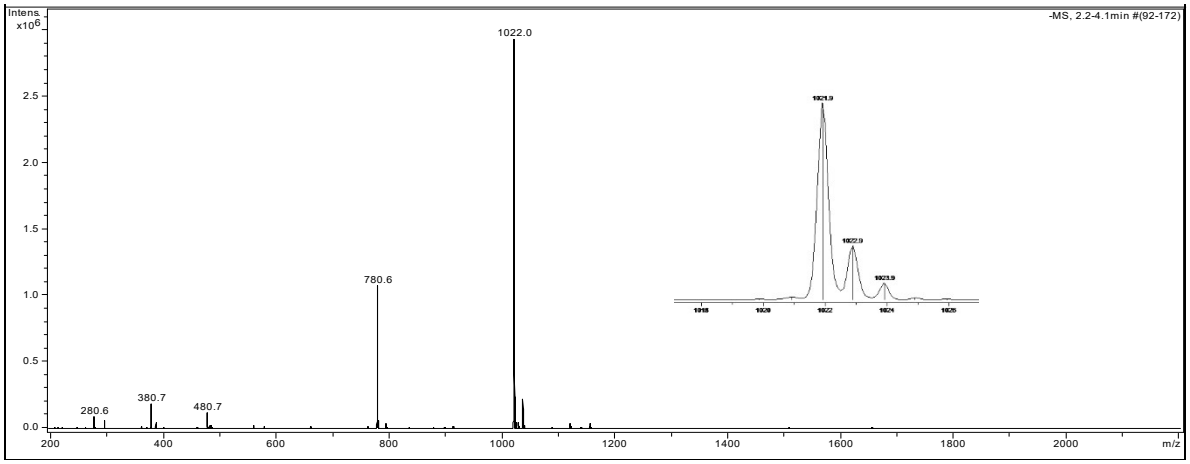


Fig. S.13. ESI-MS (-) spectrum of compound V_6-OH in CH3CN. Inset: magnification of the main signal at m/z=1022.

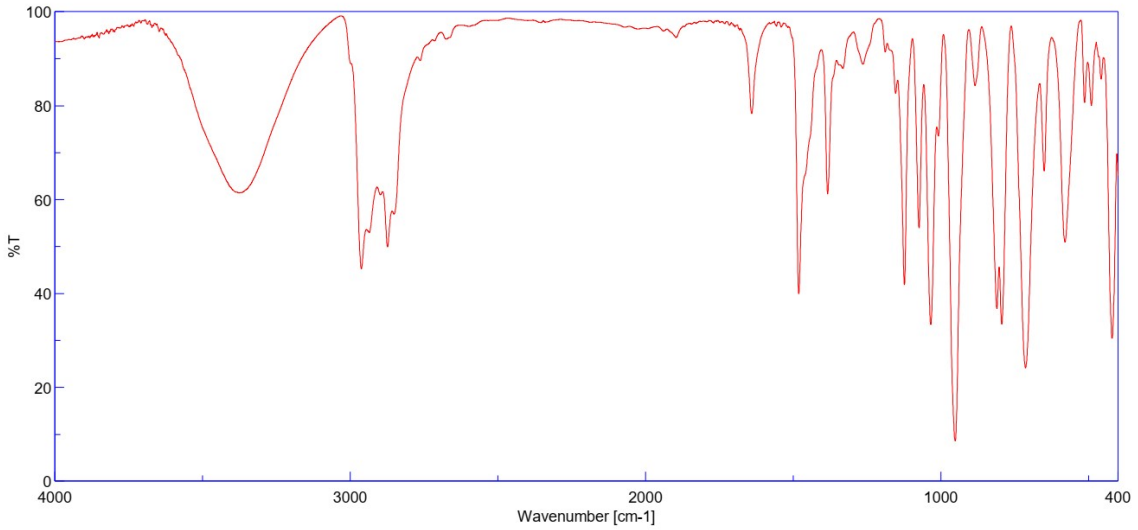
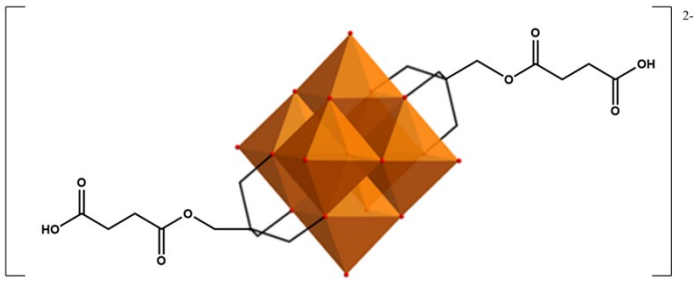


Fig. S.14 FT-IR spectrum of V_6-OH.

Synthesis of $[(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2COOH\}_2]$ (V_6-Succ)



Reaction:

$$[(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OH\}_2] + 2C_4H_4O_3 \xrightarrow[ACN,\ 50°C,\ 48\ hours]{DMAP,\ TEA} [(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2COOH\}_2]$$

Procedure:

$[(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OH\}_2]$ (1.11962 g, $8.85x10^{-4}$ mol) was dissolved in 40 mL of acetonitrile in a round-bottom flask to obtain a clear orange solution. TEA (triethylamine, 0.34 mL, 2.44×10^{-3} mol) and DMAP (4-Dimethylaminopyridine, 0.06 g, $4.91x10^{-4}$ mol) were added to the solution, causing a color change from orange to red-orange. The mixture was then refluxed for 48 hours at 50°C, and the resulting turbid orange solution was filtered to remove an orange precipitate. The solution was subsequently subjected to rotary evaporation under vacuum to evaporate the solvent, yielding a white precipitate that was filtered out. The resulting dark red solution was transferred to a beaker of cold water, and the red powder that formed was collected using a fritted funnel, washed with cold water, and finally dried under vacuum to yield 0.68 g of product (52% yield).

Characterization:

FTIR (KBr, cm^{-1}): 3444 (m, br), 2960 (s), 2932 (m), 2872 (m), 1738 (s), 1649 (w), 1469 (s), 1396 (w), 1380 (w), 1336 (w), 1265 (m), 1247 (m), 1224 (m), 1191 (m), 1161 (m), 1132 (s), 1061 (s), 1036 (m), 1000 (m), 950 (s), 933 (s), 879 (w), 810 (s), 723 (s), 582 (m), 417 (s).

ESI-MS (-) CH_3CN: 1221.9 ($[M\text{-}TBA]^-$), 980.8 ($[M\text{-}2TBA+H]^-$).

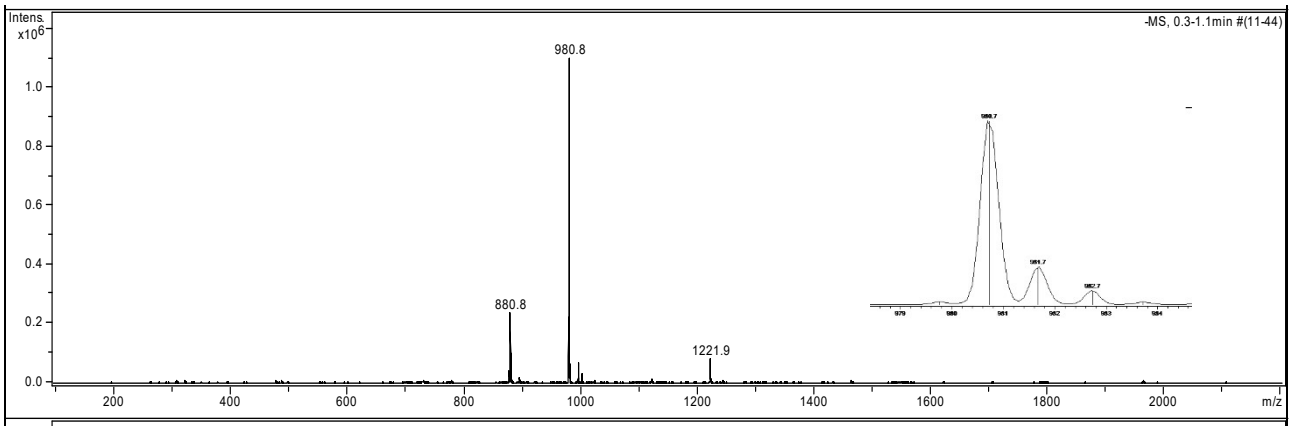


Fig. S.15 ESI-MS (-) spectrum of V_6-Succ in CH_3CN. Inset: magnification of the main signal at m/z=981.

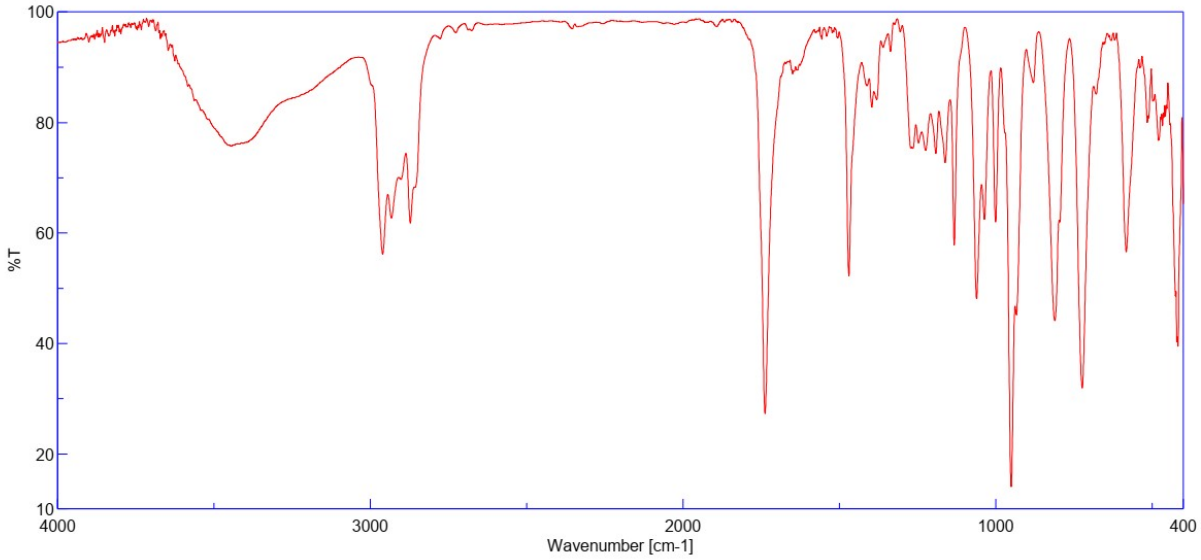
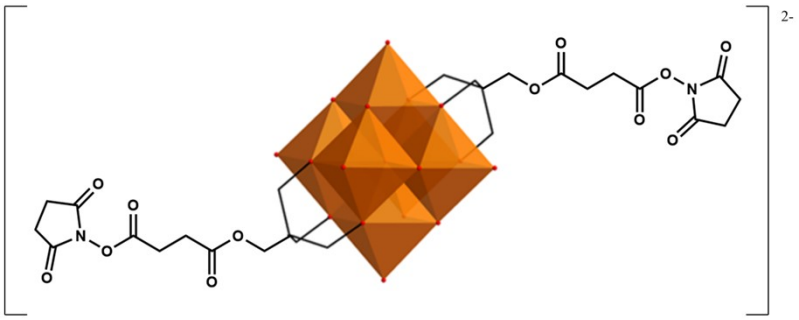


Fig. S.16 FT-IR spectrum of V_6-Succ.

Synthesis of $[(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2CO(C_4H_4NO_3)\}_2]$ (V_6-NHS)



Reaction:

$$[(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2COOH\}_2] + 2C_4H_5NO_3 \xrightarrow[DMF\ R.T.]{DCC} [(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2CO(C_4H_4NO_3)\}_2]$$

Procedure:

V_6-Succ (95.28mg, 6.50×10^{-2} mmol) was dissolved in a vial containing 2 mL of DMF, producing a clear red-orange solution. NHS (N-Hydroxysuccinimide, 34.26 mg, 0.26 mmol) was then added to the solution, and no noticeable color change was observed. Next, DCC (N,N'-Dicyclohexylcarbodiimide, 85.01mg, 0.39 mmol) was added to the mixture, causing a color change to a darker red shade. The solution was stirred for 24 hours at room temperature. The resulting brown mixture was subjected to diethyl ether atmosphere for 5 days, leading to the formation of red needle-shaped crystals. These crystals were collected, washed multiple times with diethyl ether, and finally dried under vacuum to yield 53.89 mg of product (yield 50%).

Characterization:

FTIR (KBr, cm^{-1}): 3444 (m, br), 2962 (s), 2937 (m), 2875 (m), 1814 (w), 1783 (w), 1739 (s), 1667 (w), 1482 (w), 1383 (w), 1252 (w), 1205 (w), 1132 (w), 1085 (m), 954 (s), 883 (w), 809 (s), 719 (s), 648 (w), 582 (m), 422 (m).

ESI-MS (-) CH_3CN: 1174.7 ($[M-2TBA+H]^-$).

^1H NMR (300MHz, CD_3CN, δ, ppm): 0.96 (t, $^+N(CH_2CH_2CH_2CH_3)_4$ TBA, 24H), 1.35 (s, $^+N(CH_2CH_2CH_2CH_3)_4$ TBA, 16H), 1.60 (m, $^+N(CH_2CH_2CH_2CH_3)_4$ TBA, 16H), 2.66 (t, -$OCCH_2CH_2CO$-, 4H), 2.77 (s, DMF solvent impurities, overlapped with -CH_2- from NHS, (3+4)H), 2.85 (m, -$OCCH_2CH_2CO$-, 4H), 3.10 (m, $^+N(CH_2CH_2CH_2CH_3)_4$ TBA, 16H), 3.95 (s, -CCH_2O- TRIS, 4H), 5.01 (s, -$C(CH_2O\text{-})_3$, 12H).

^{51}V NMR (78.9MHz, CD_3CN, δ, ppm): -497.4 (s, br)

UV-Vis (CH_3CN): Maximum at 195 nm (ε_{195} = 38400 $cm^{-1}M^{-1}$), and shoulder at 221 nm (ε_{221}=44800 $cm^{-1}M^{-1}$).

Elemental Analysis: Calculated for $C_{50}H_{96}V_6O_{25}N_4$: C 41.16%, H 6.63%, N 3.84%. Experimental: C 42.18%, H 6.55%, N 4.10%.

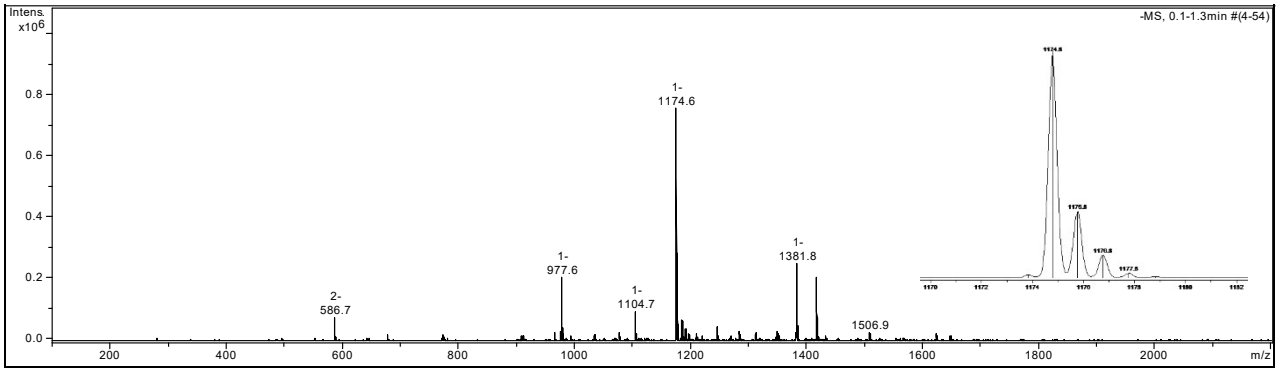


Fig. S.17 ESI-MS (-) spectrum of V_6-NHS in CH_3CN. Inset: magnification of main signal at m/z=1174.

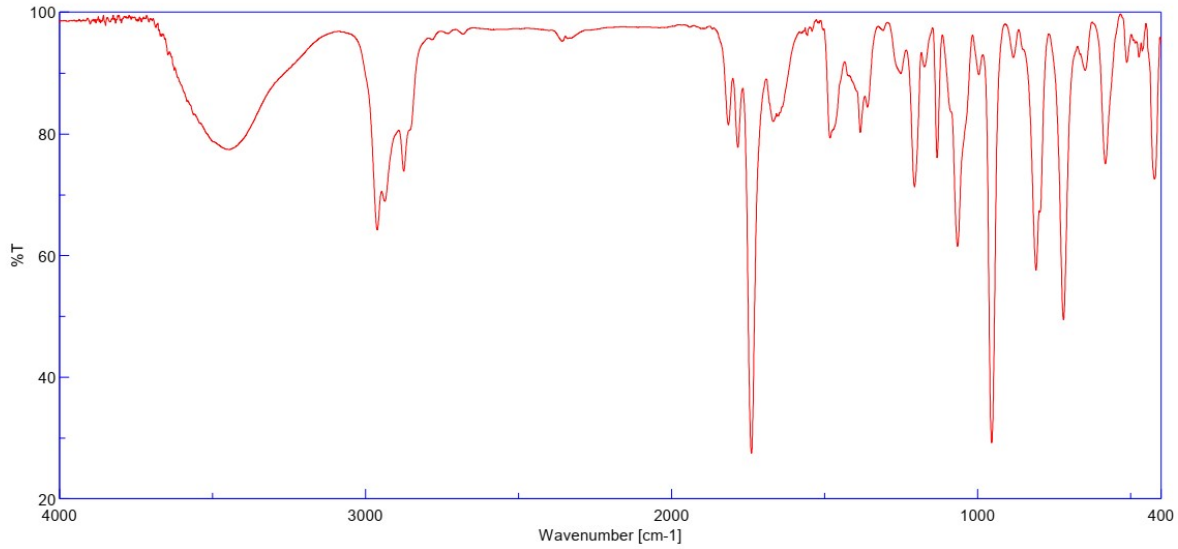


Fig. S.18 FT-IR spectrum of V_6-NHS.

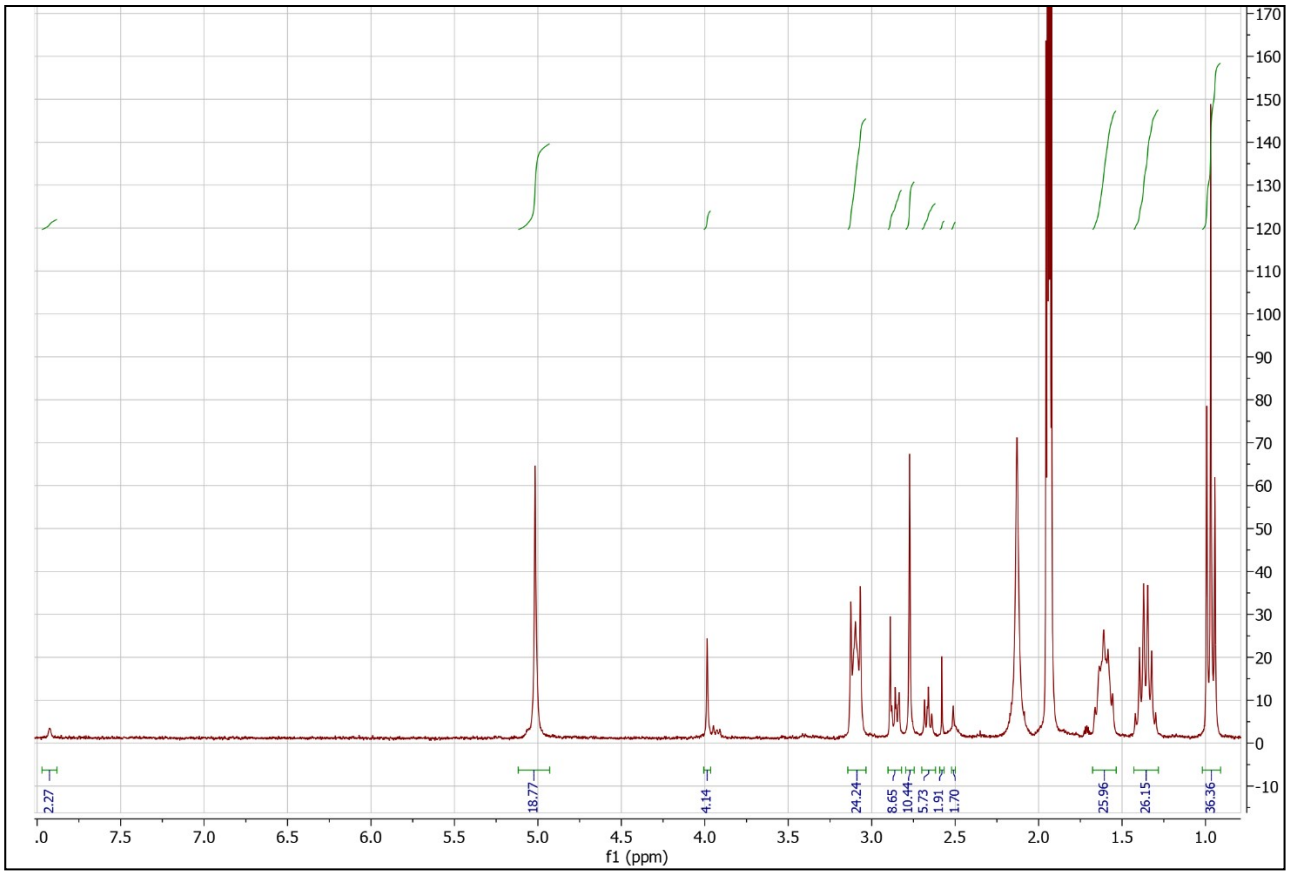
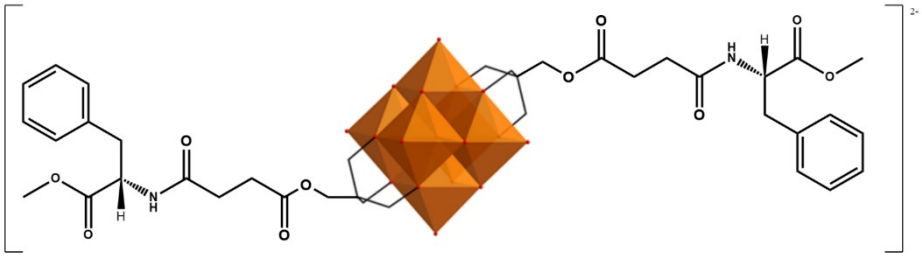


Fig. S.19 ^{1}H NMR 300MHz (CD_3CN) of V_6-NHS.

Synthesis of $[(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2COPheOMe\}_2]$ (V_6-Phe)



Reaction:

$[(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2CO(C_4H_4NO_3)\}_2]$ + 2L-PheOMe·HCl

$\xrightarrow[DMF\ R.T.]{DIPEA}$ $[(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2COPheOMe\}_2]$

Procedure:

V_6-NHS (40.50 mg; 0.0244 mmol) was dissolved in 2.0 mL of DMF in a vial. L-PheOMe·HCl (14.70 mg, 0.068 mmol) and DIPEA (13 mg, 18 µL, 0.1 mmol) were added to the reaction mixture, and the reaction system was stirred for 24 hours at room temperature. Then the desired product was crystallized under diethyl ether atmosphere. After one day, the dark orange greasy product was collected, washed four times with diethyl ether and dried under vacuum (0.03810 g, 86% yield).

Characterization:

FTIR (KBr, cm^{-1}): 3435 (m, br), 2961 (s), 2936 (m), 2874 (m), 1740 (s), 1668 (s), 1539 (m), 1465 (m), 1386 (m), 1212 (w), 1161 (m), 1131 (s), 1058 (s), 953 (s), 809 (s), 719 (s), 583 (s), 420 (s).

ESI-MS (-) CH_3CN: 1544.2 ($[M\text{-}TBA]^-$), 1302.9 ($[M\text{-}2TBA+H]^-$), 650.9 ($[M\text{-}2TBA]^{2-}$)

^1H NMR (300MHz, CD_3CN; δ, ppm): 0.97 (t, $^+N(CH_2CH_2CH_2CH_3)_4$ TBA, 24H), 1.37 (m, $^+N(CH_2CH_2CH_2CH_3)_4$ TBA, 18H), 1.62 (m, $^+N(CH_2CH_2CH_2CH_3)_4$ TBA, 16H), 3.11 (t, $^+N(CH_2CH_2CH_2CH_3)_4$ TBA, 18H), 3.63 (s, $-OCH_3$, 6H), 5.03 (s, $-C(CH_2O-)_3$, 12H), 3.91 (s, $-CCH_2O-$, 4H), 6.66 (d, -C=ONH-, 2H), 7.16-7.36 (m, aromatic ring Phe, 10H).

^{51}V NMR (78.9MHz, CD_3CN; δ, ppm): -499.75 (s, br)

UV (CH_3CN): Maximum at 191 nm (ε_{191}=150400 $cm^{-1}M^{-1}$), shoulder at 209 nm (ε_{209}=69600 $cm^{-1}M^{-1}$).

CD (CH_3CN): Maxima at 195 nm ([θ]=122438 deg*cm^2*$dmol^{-1}$) and approximately at 216 nm ([θ]=35933 deg*cm^2*$dmol^{-1}$).

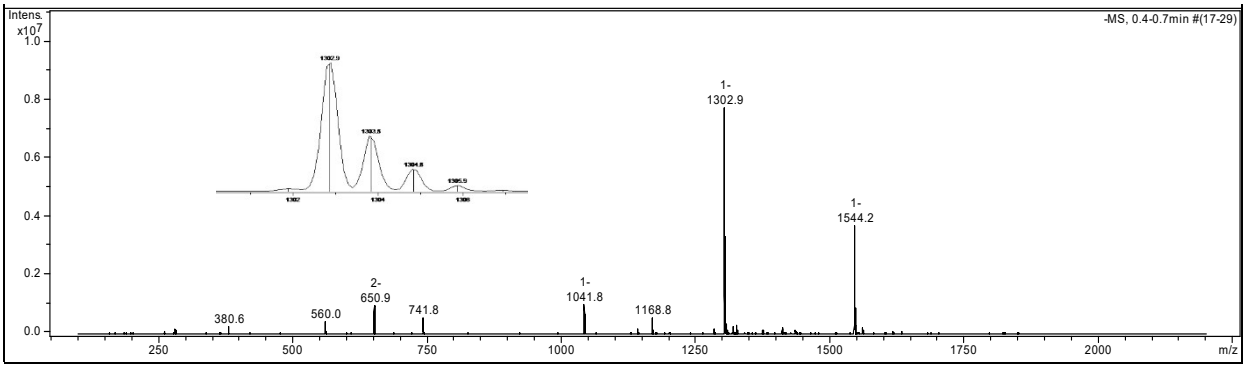


Fig. S.20 ESI-MS (-) spectrum of V_6-Phe in CH_3CN. Inset: magnification of main signal at m/z=1303.

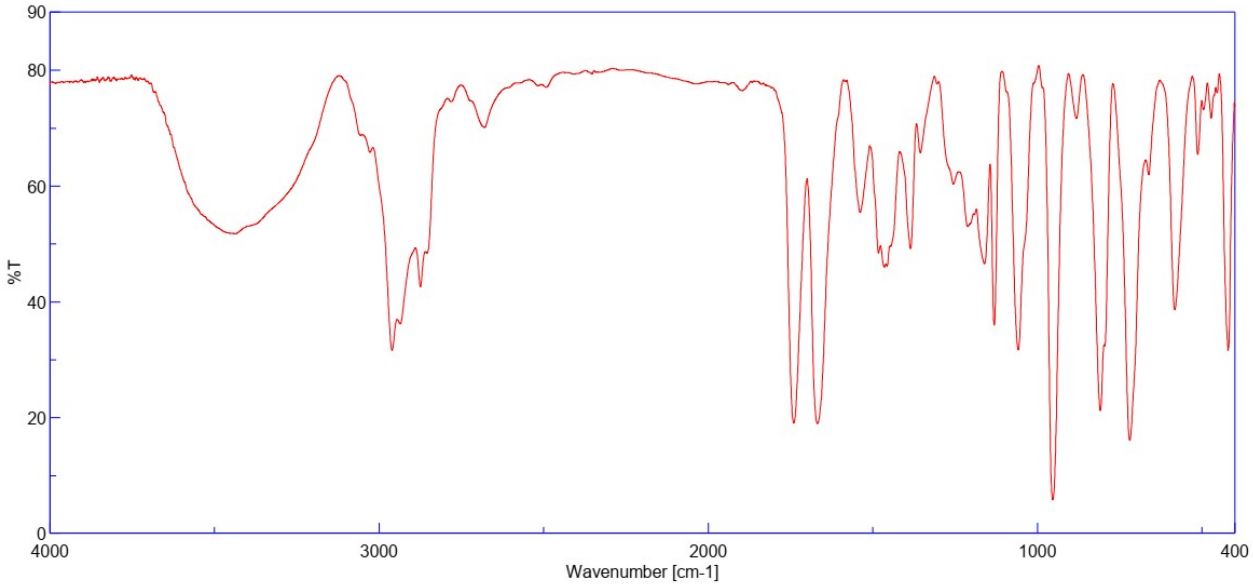


Fig. S.21 The FT-IR spectrum of V_6-Phe.

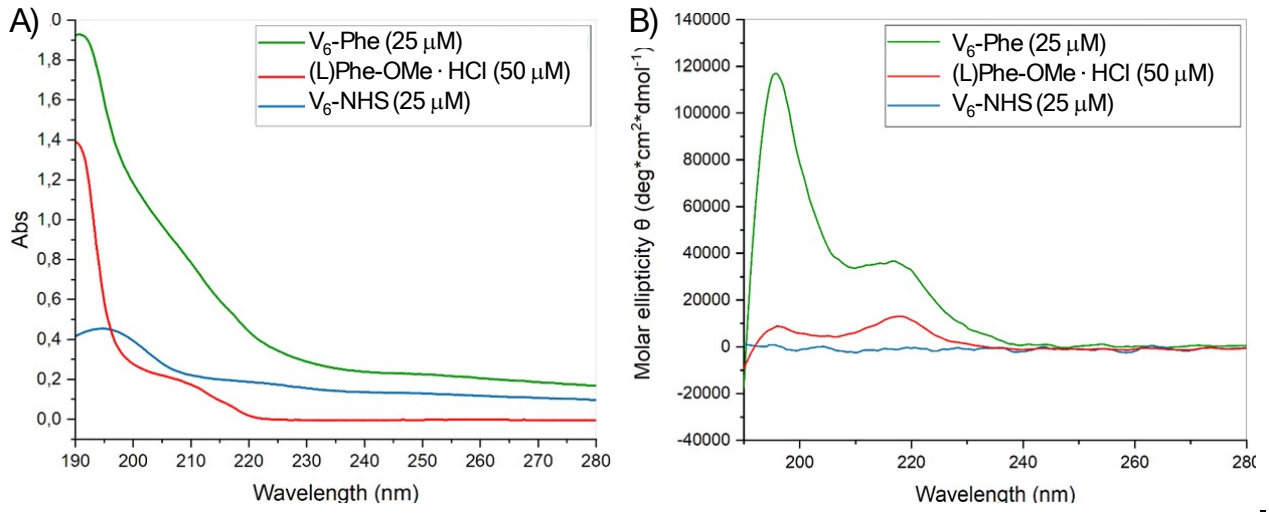


Fig. S.22 A) UV spectra of V_6-Phe, and of the precursors at similar overall concentrations (double for the amino acid). B) Molar ellipticity of V_6-Phe, V_6-NHS and phenylalanine ((L)-Phe-OMe·HCl).

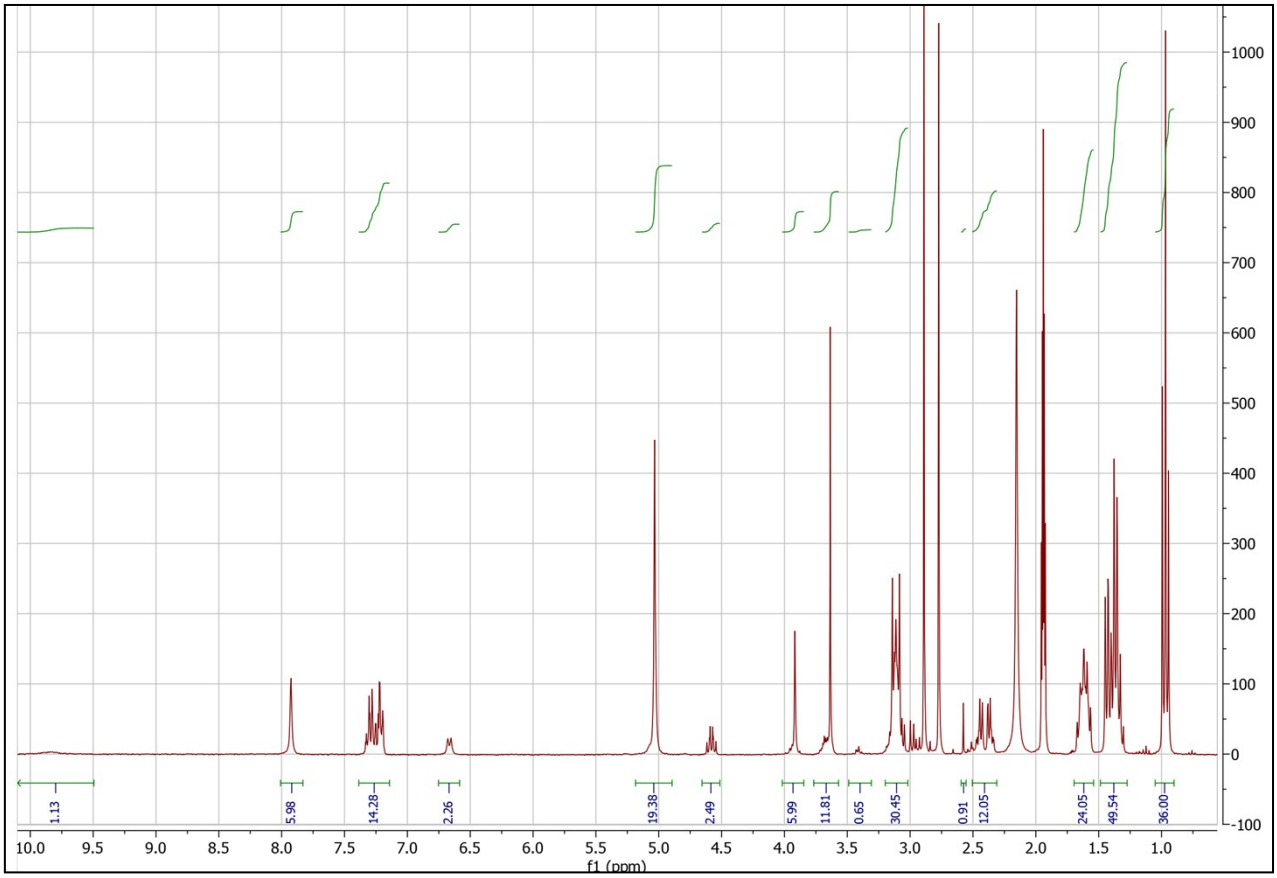
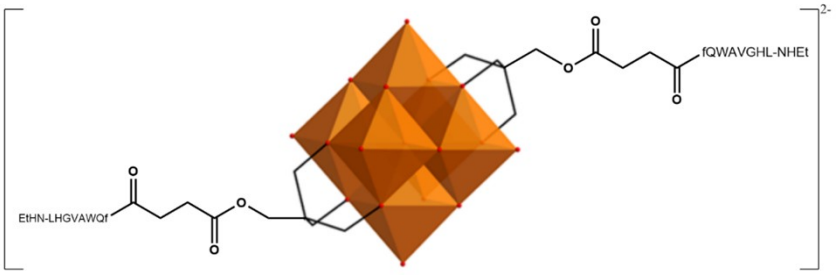


Fig. S.23 The ^{1}H NMR 300 (CD_3CN) of V_6-Phe.

3) Synthesis of the hybrid POM-peptide conjugates

Synthesis of POV-1: $[(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2CO(C_{49}H_{68}N_{13}O_9)\}_2]$ (V_6-DB)



Reaction:

$$[(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2CO(C_4H_4NO_3)\}_2] + 2\ C_{49}H_{68}N_{13}O_9 \xrightarrow[DMF\ R.T.]{DIPEA} [(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2CO(C_{49}H_{68}N_{13}O_9)\}_2]$$

Procedure:

V_6-NHS (8.17 mg; 0.0049 mmol) was dissolved in 0.8 mL of DMF in a vial. First Pep-1 (10.05 mg, 0.010 mmol) and then DIPEA (9.6 mg, 13 µL, 0.075 mmol) were added to the reaction mixture, and the orange solution was stirred for 24 hours at room temperature. Then, the desired product was crystallized under diethyl ether atmosphere. After a few hours, a white powder was recovered and washed four times with diethyl ether and dried under vacuum (0.01217 g, 73% yield).

Characterization:

FTIR (KBr, cm^{-1}): 3291 (s, br), 3061 (w), 2962 (m), 2933 (m), 2872 (m), 1738 (m), 1647 (s, br), 1533 (s, br), 1452 (w), 1389 (w), 1233 (w), 1160 (w), 1130 (w), 1055 (m), 955 (s), 880 (w), 809 (m), 716 (m), 586 (m), 420 (m).

ESI-MS (-) CH_3CN: 1455.9 ($[M\text{-}2TBA]^{2-}$).

UV (TFE/H_2O) 1:10: Maximum at 191.5 nm (ε_{191}=169600 $cm^{-1}M^{-1}$) and shoulder at 218 nm (ε_{212}=112800 $cm^{-1}M^{-1}$).

CD (TFE/H_2O) 1:10: Minimum at 207 nm ([θ]=-102122 deg*cm^2*$dmol^{-1}$), and maximum at 192 nm ([θ]=114616 deg*cm^2*$dmol^{-1}$).

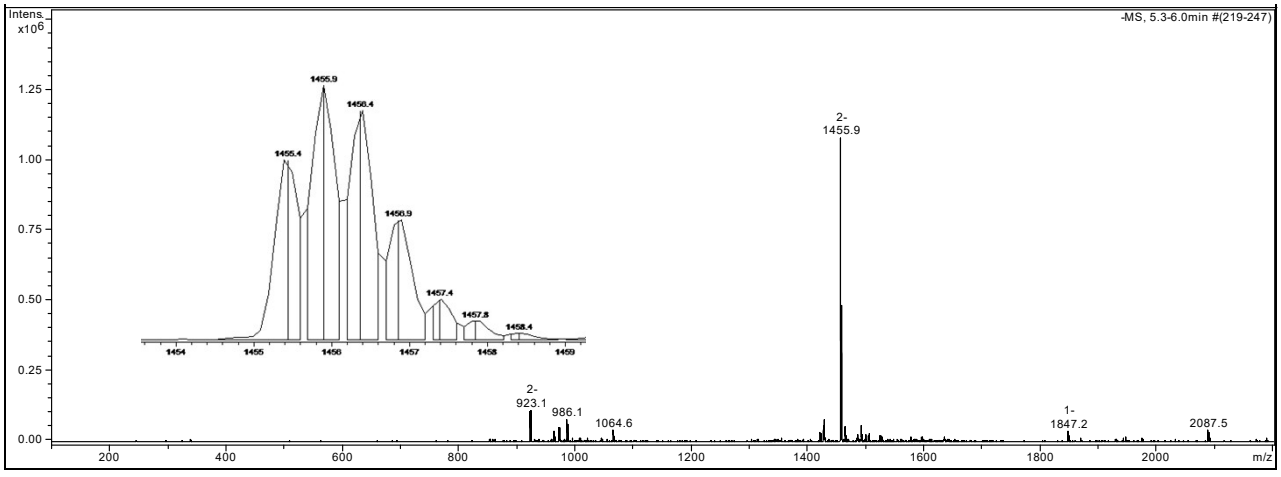


Fig. S.24 ESI-MS (-) spectrum of POV-1 in CH_3CN. Inset: magnification of the main signal at m/z=

1456.

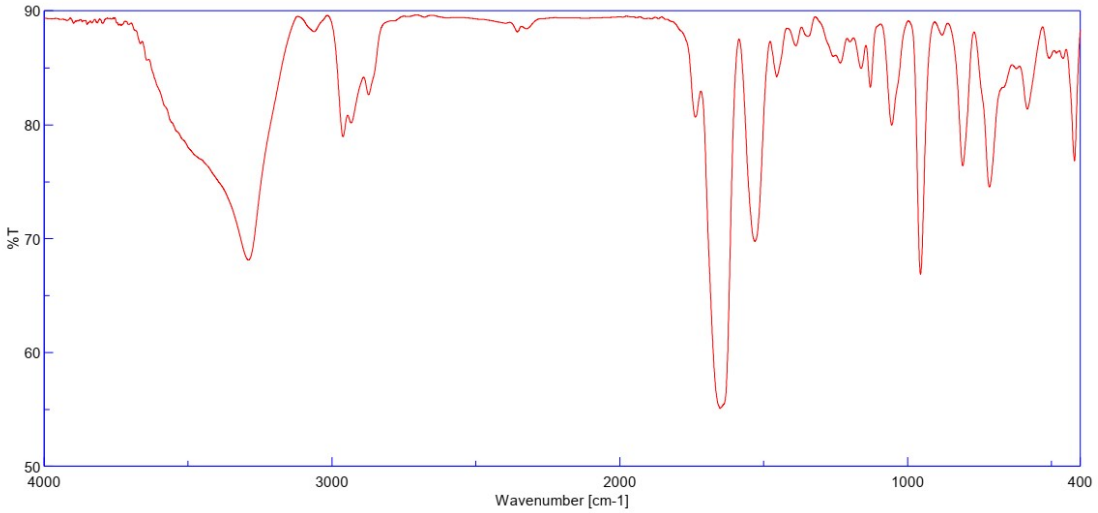


Fig. S.25 FT-IR spectrum of POV-1.

Table 5. Proton resonances of POV-1.

AA	NH (ppm)	CH_α (ppm)	Rest of the chain(ppm)
Gln	8.32	4.05	$-CH_{2\beta}$ (diastereotopic) 1.75, 1.85 $-CH_{2\gamma}$ 1.55 $-NH_2$ not detected
D-Phe	8.28	4.43	$-CH_{2\beta}$ (diastereotopic) 2.79, 2.90 Unassigned aromatics
Trp	7.94	4.49	$-CH_{2\beta}$ (diastereotopico) 2.98, 3.15 Unassigned aromatics
Ala	7.93	4.35	$-CH_3$ 1.20
Val	7.63	4.15	$-CH_\beta$ 2.00 $(-CH_{3\gamma})_2$ 0.85
Gly	8.22	$-CH_{2\alpha}$ 3.73	/
His	8.02	4.48	$-CH_{2\beta}$ 2.93 -NH aromatic not detected -CH Unassigned aromatics
Leu	7.94	4.15	$-CH_{2\beta}$ 1.47 $-CH_\gamma$ 0.81 $(-CH_{3\delta})_2$ not detected
NHEt	8.08	$-CH_{2\alpha}$ 3.07	$-CH_{3\beta}$ 0.99

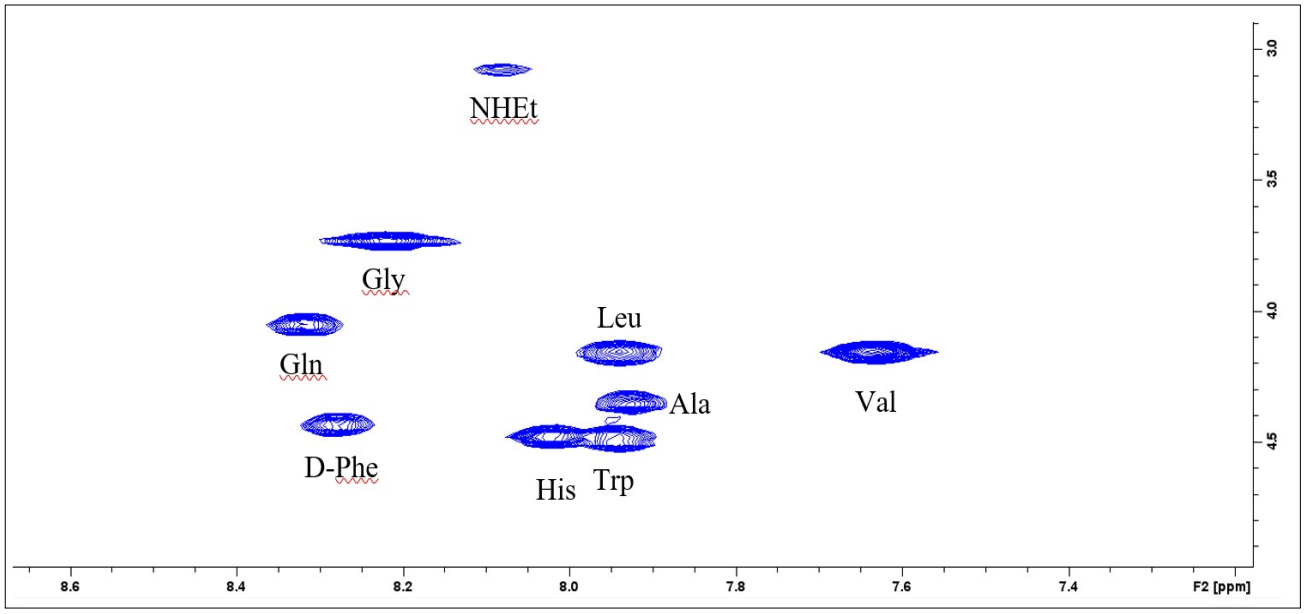


Fig. S.26 COSY spectrum of POV-1 (150 µM) in d_6-DMSO.

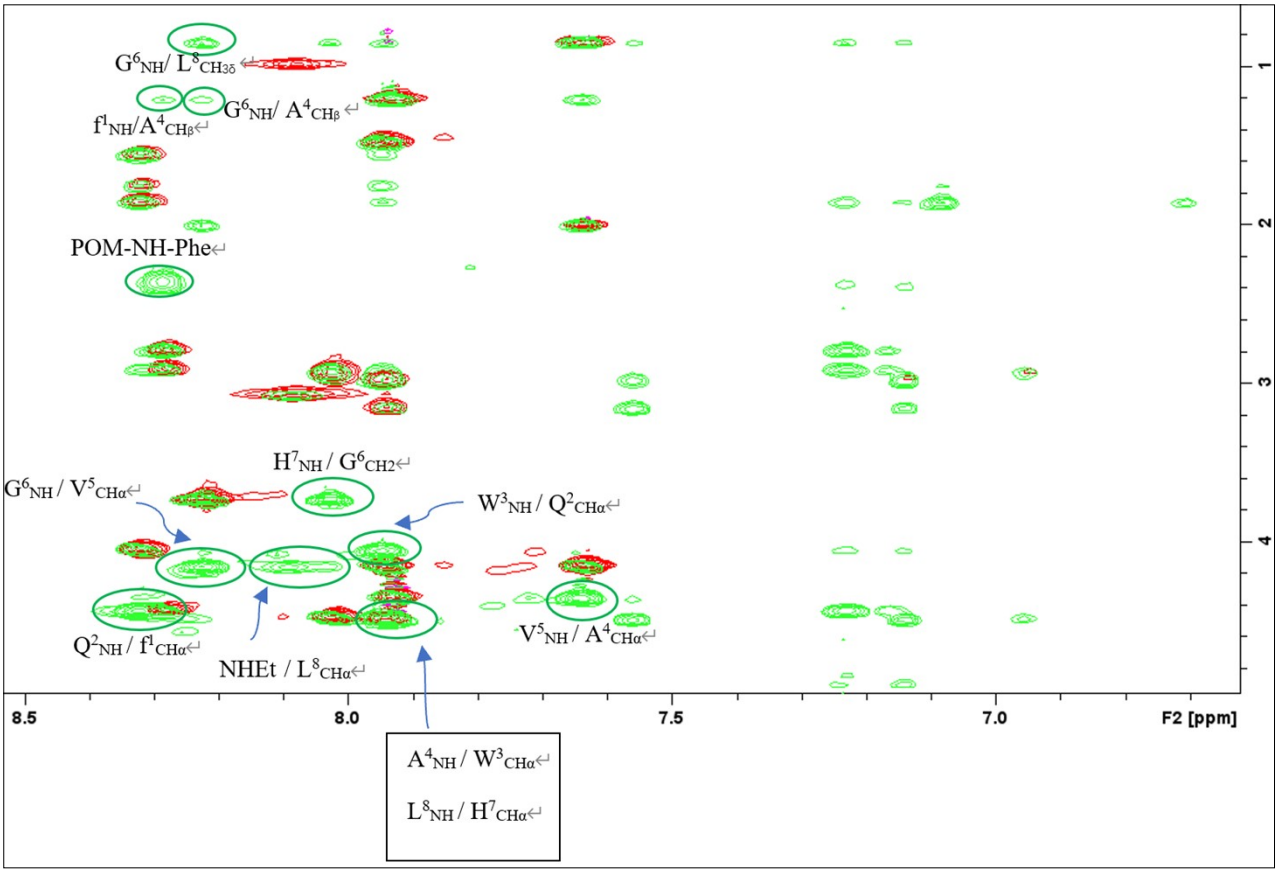


Fig. S.27 TOCSY and ROESY of POV-1 (150 µM) in d_6-DMSO

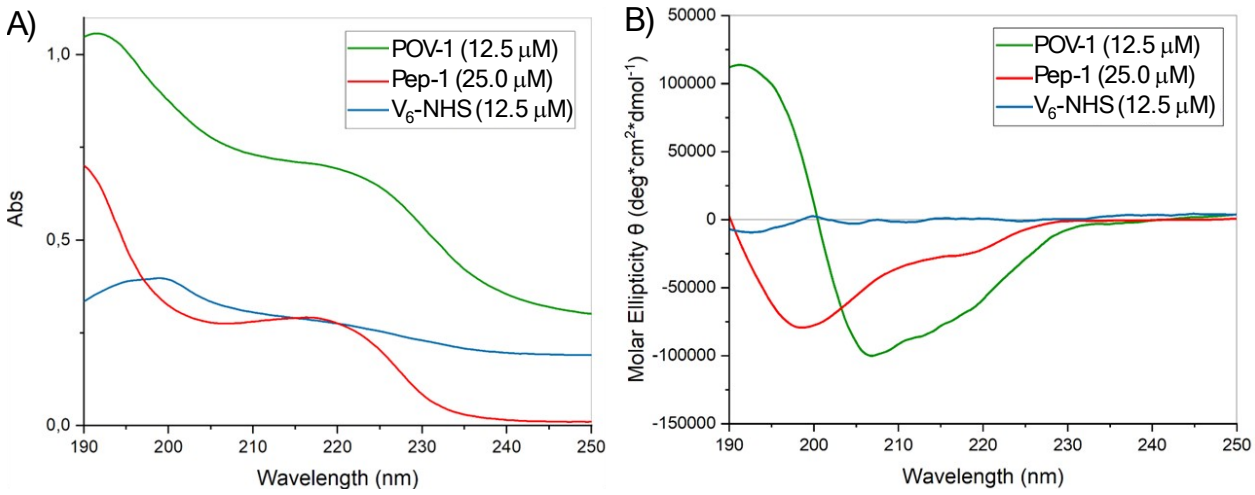
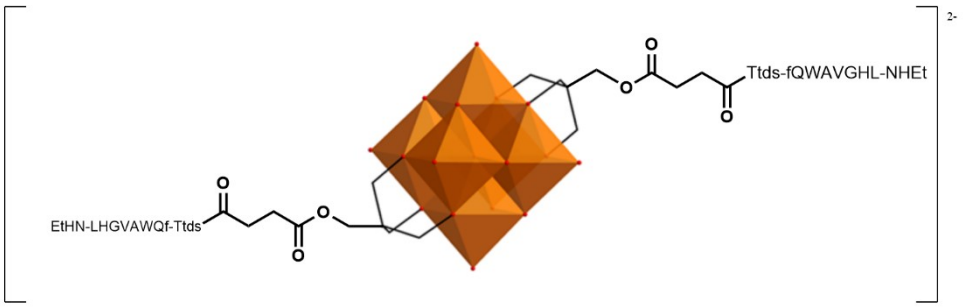


Fig. S.28 A) UV spectrum of POV-1, V_6-NHS and Pep-1 in TFE/H_2O 1:10. B) Molar ellipticity graph of POV-1, V_6-NHS and Pep-1 in TFE/H_2O 1:10.

Synthesis of POV-2: $[(C_4H_9)_4N]_3[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2CO(C_{63}H_{94}N_{15}O_{14})\}_2]$ (V_6-TtdsDB)



Reaction:

$[(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2CO(C_4H_4NO_3)\}_2] + 2\ C_{63}H_{94}N_{15}O_{14} \xrightarrow[DMF\ R.T.]{DIPEA} [(C_4H_9)_4N]_3[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2CO(C_{63}H_{94}N_{15}O_{14})\}_2]$

Procedure:

V_6-NHS (8.47 mg, 0.0051mmol) was dissolved in 0.8 mL of DMF in a vial. Ttds-fQWAVGHL-NHEt (15.15 mg, 0.012 mmol) and DIPEA (9.6 mg, 13 µL, 0.073 mmol) were added to the reaction mixture one by one, and the yellow-orange solution was stirred for 24 hours at room temperature. The desired product was obtained by crystallization under diethyl ether atmosphere. After 96 h, the dark red product was recovered, washed four times with diethyl ether and then dried under vacuum (0.095 g of product, 46% yield).

Characterization:

FTIR (KBr, cm^{-1}): 3429 (m, br), 3292 (s), 3071 (m), 2930 (m), 2874 (m), 1743 (m), 1664 (s), 1639 (s), 1541 (m), 1432 (w), 1386 (w), 1192 (w), 1139 (m), 1954 (m), 957 (s), 808 (m), 718 (m), 582 (m), 507 (w), 422 (m).

ESI-MS (-) CH_3CN: 1758.5 ($[M-2TBA]^{2-}$)

UV (TFE/H_2O 1:10): maximum at <190 nm (ε_{190}=289600 $cm^{-1}M^{-1}$) and shoulder at 218 nm (ε_{214}=120160 $cm^{-1}M^{-1}$).

CD (Pure TFE): The minimum, in TFE, is located at 206 nm ([θ]=-342728 $deg*cm^2*dmol^{-1}$), while the maximum is at 192 nm ([θ]=759366 $deg*cm^2*dmol^{-1}$)

^{51}V NMR (78.9MHz, CD_3CN; δ, ppm): -494.7 (s, br).

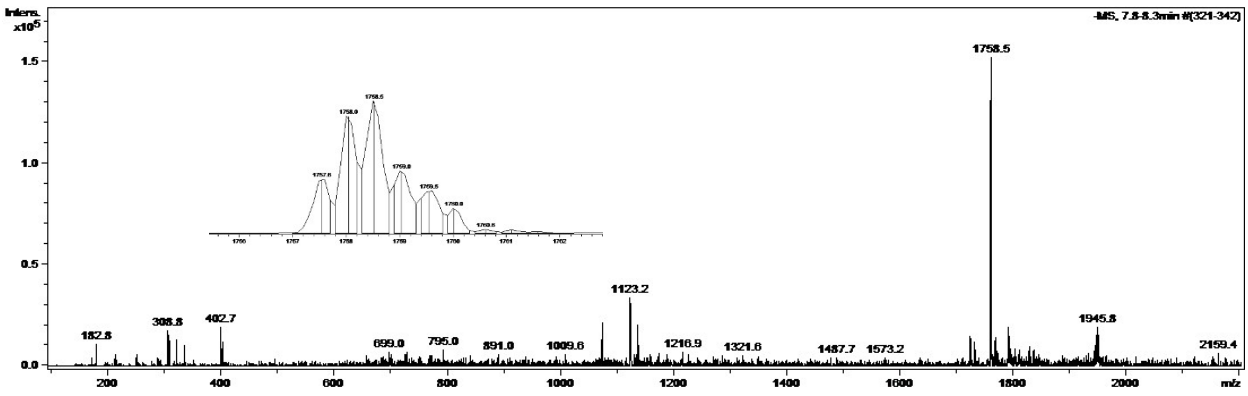


Fig. S.29 ESI-MS (-) spectrum of POV-2 in CH_3CN. Inset: magnification of the main signal at m/z= 1758.

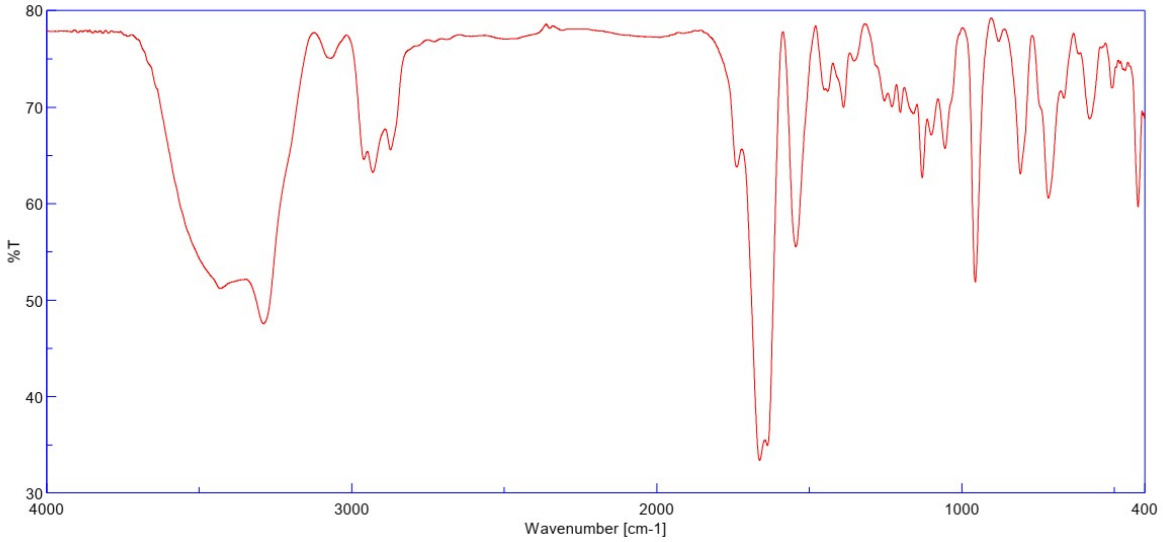


Fig. S.30 FT-IR spectrum of POV-2.

Table 6. Proton resonances of POV-2.

AA	NH (ppm)	CH_{α} (ppm)	Rest of the chain(ppm)
D-Phe	8.26	4.40	$-CH_{2\beta}$ (diastereotopico) 2.78, 2.94 Aromatics not observed
Gln	8.33	4.07	$-CH_{2\beta}$ (diastereotopico) 1.77, 1.89 $-CH_{2\gamma}$ 1.59
Trp	7.97	4.48	$-CH_{2\beta}$ (diastereotopico) 3.00, 3.17 Unassigned aromatics
Ala	7.92	4.36	$-CH_3$ 1.21
Val	7.65	4.17	$-CH_{\beta}$ 2.00 $(-CH_{3\gamma})_2$
Gly	8.22	$-CH_{2\alpha}$ 3.73	/
His	8.02	4.47	$-CH_{2\beta}$ 2.92 -NH aromatic not detected -CH Unassigned aromatics
Leu	7.95	4.13	$-CH_{2\beta}$ 1.47 $-CH_{\gamma}$ 0.81 $(-CH_{3\delta})_2$ not detected
NH-Ttds	7.79	$-CH_{2\alpha}$ 3.04	$-CH_{2\beta}$ 1.58 $-CH_{2\gamma}$ 3.35
NHEt	8.11	$-CH_{2\alpha}$ 3.08	$-CH_{3\beta}$ 1.00

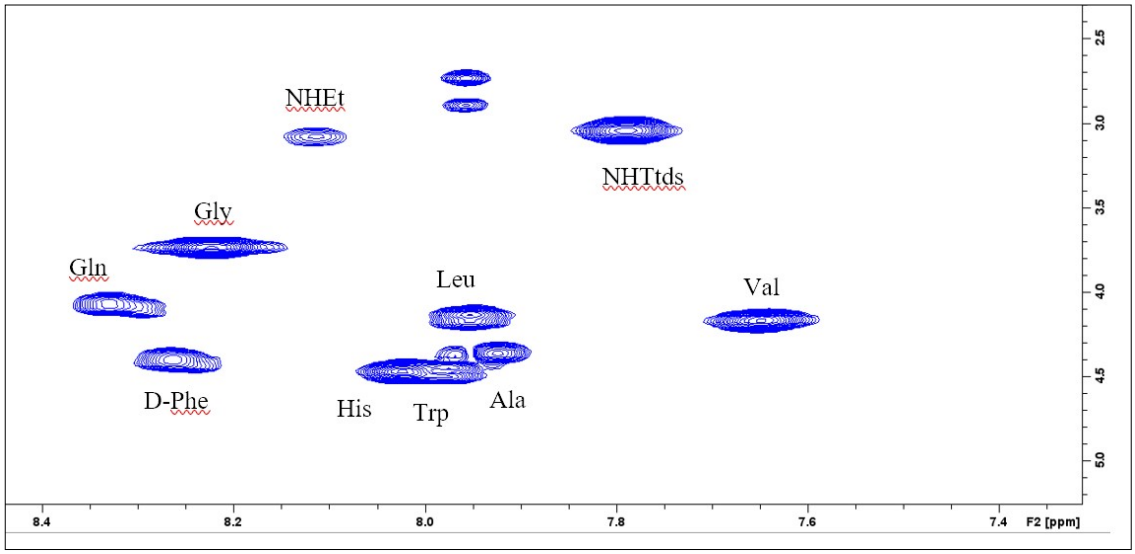


Fig. S.31 COSY spectrum of POV-2 in d_6-DMSO.

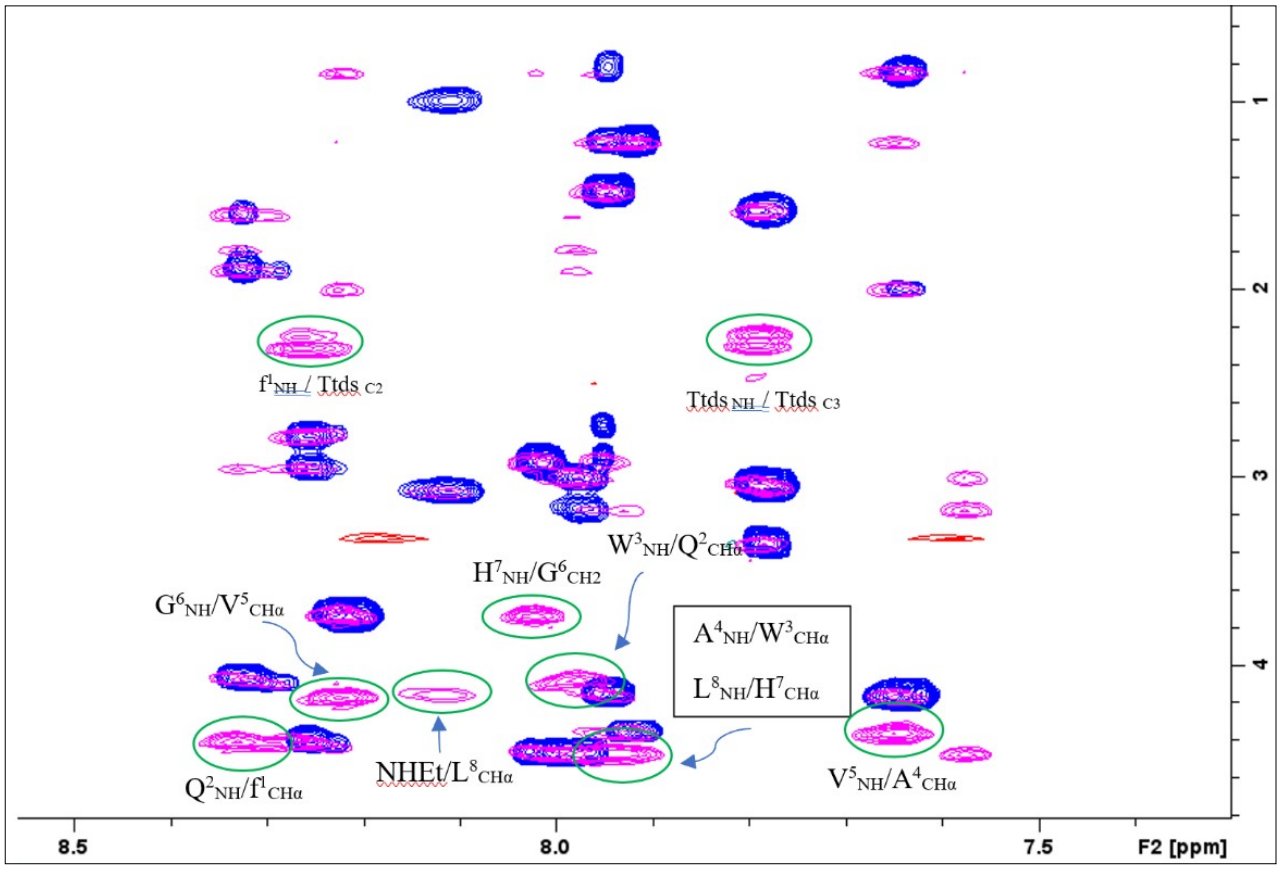


Fig. S.32 TOCSY and ROESY spectra of POV-2.

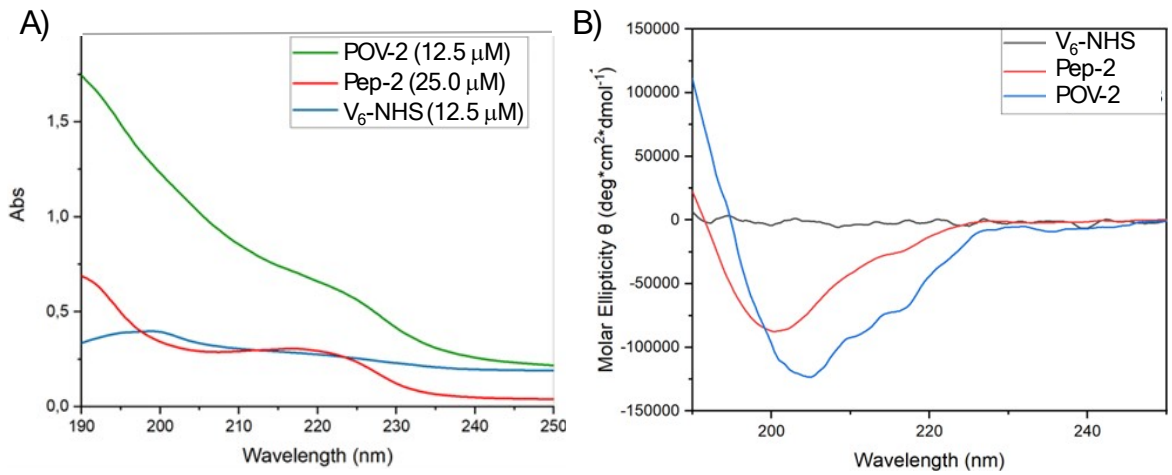
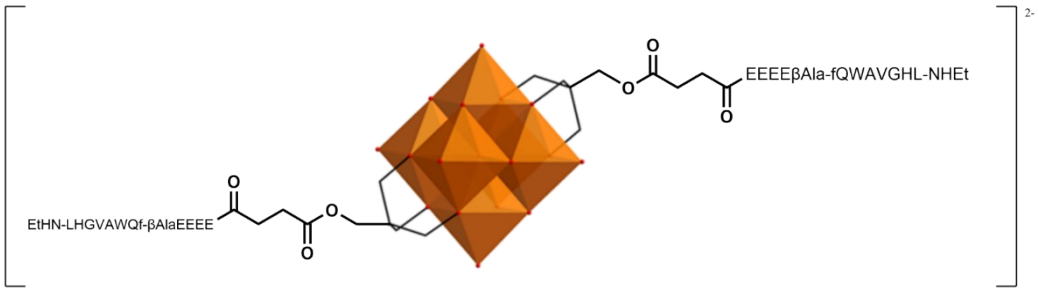


Fig. S.33 A) UV spectrum of POV-2, V_6-NHS and Pep-2 in TFE/H_2O 1:10. B) Molar ellipticity graph of POV-2, V_6-NHS and Pep-2 in TFE/H_2O 1:10.

Synthesis of POV-3: $[(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2CO(C_{72}H_{101}N_{18}O_{22})\}_2]$ (V_6-EEEEA-DB)



Reaction:

$[(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2CO(C_4H_4NO_3)\}_2] + 2\ C_{72}H_{101}N_{18}O_{22} \xrightarrow[DMF\ R.T.]{DIPEA} [(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2CO(C_{72}H_{101}N_{18}O_{22})\}_2]$

Procedure:

V_6-NHS (13.64 mg; 0.00823 mmol) was dissolved in 1 mL of DMF in a vial. First EEEEβA-fQWAVGHL-NHEt (Pep-3, 29.93 mg, 0.019 mmol) and then DIPEA (14.8 mg, 20 µL, 0.11 mmol) were added to the reaction mixture, and the orange solution was stirred for 24 hours at room temperature. The desired product was crystallized under diethyl ether. After 72 hours the light orange product was recovered, washed three times with diethyl ether and then dried under vacuum (0.04277 g, 92% yield).

Characterization:

FTIR (KBr, cm^{-1}): 3440 (m, br), 3289 (s), 3056 (w), 2955 (m), 2934 (m), 2879 (w), 1659 (s), 1539 (m), 1387 (m), 1255 (m), 1130 (w), 1052 (w), 958 (s), 808 (m), 718 (w), 578 (w), 473 (w), 426 (w).

ESI-MS (-) CH_3CN: 2163.7 ($[M\text{-}TBA\text{-}H]^{2-}$), 2043.1 ($[M\text{-}2TBA]^{2-}$), 1362.0 ($[M\text{-}2TBA\text{-}H]^{3-}$)

UV (TFE/H_2O 1:10): Maximum <190 nm (ε_{190}=289600 $cm^{-1}M^{-1}$) and a shoulder at 214 nm (ε_{214}=137600 $cm^{-1}M^{-1}$).

CD (TFE): Minimum located at 206 nm ([θ]=-338766 $deg*cm^2*dmol^{-1}$), while the maximum is at 192 nm ([θ]=756593 $deg*cm^2*dmol^{-1}$).

^{51}V NMR (78.9MHz, CD_3CN; δ, ppm): -493.9 (s, br).

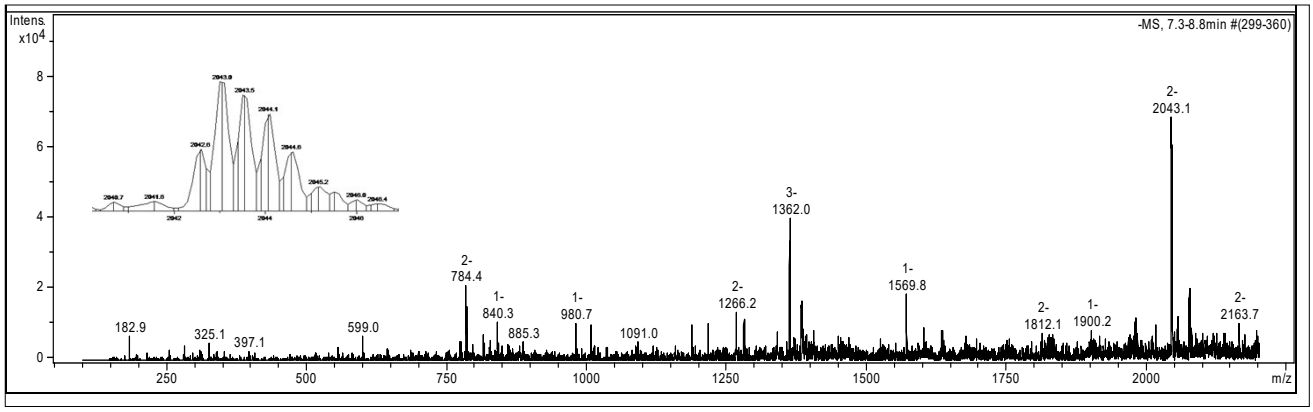


Fig. S.34 ESI-MS (-) spectrum of POV-3. Inset: magnification of the main signal at m/z= 2043.

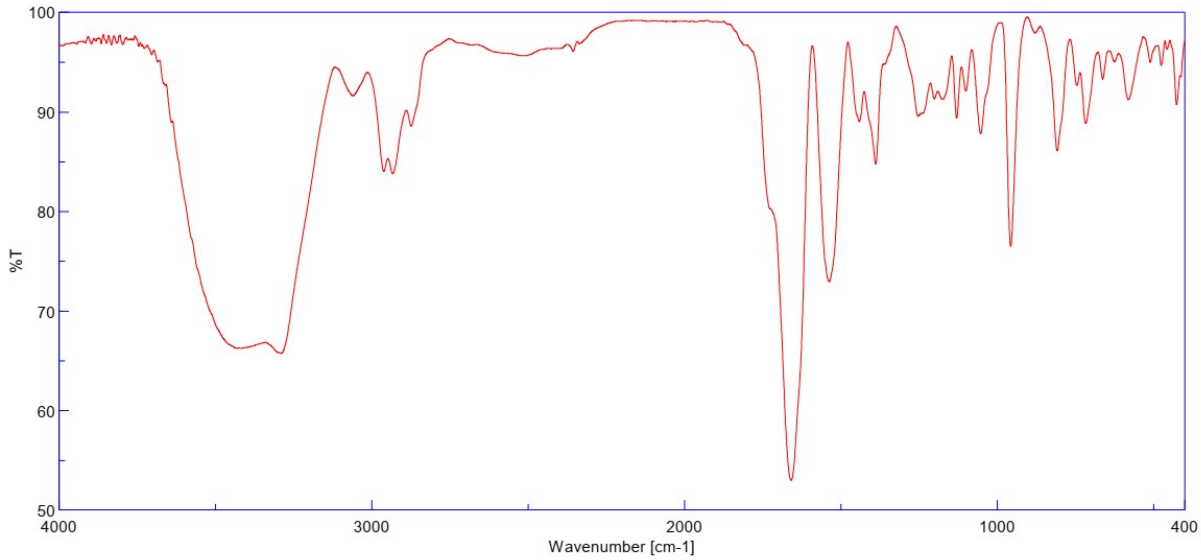


Fig. S.35 FT-IR spectrum of POV-3.

Table 7. Proton resonances of POV-3.

AA	NH (ppm)	CH_{α} (ppm)	Rest of the chain(ppm)
Glu[1]	8.14	4.23	$-CH_{2\beta}$ 2.24 $-CH_{2\gamma}$ (diastereotopico) 1.72, 1.87
Glu[2]	8.06	4.19	$-CH_{2\beta}$ 2.25 $-CH_{2\gamma}$ (diastereotopico) 1.77, 1.90
Glu[3]	7.97	4.21	$-CH_{2\beta}$ 2.24 $-CH_{2\gamma}$ (diastereotopico) 1.75, 1.90
Glu[4]	7.90	4.14	$-CH_{2\beta}$ 2.20 $-CH_{2\gamma}$ (diastereotopico) 1.73, 1.86
βAla	7.84	$-CH_{2\alpha}$ 3.16	$-CH_{2\beta}$ 2.27
D-Phe	8.29	4.45	$-CH_{2\beta}$ (diastereotopico) 2.94, 2.78 Aromatics not observed
Gln	8.34	4.10	$-CH_{2\beta}$ (diastereotopico) 1.78, 1.89 $-CH_{2\gamma}$ 1.59
Trp	8.03	4.48	$-CH_{2\beta}$ (diastereotopico) 3.00, 3.17 Unassigned aromatics
Ala	7.99	4.38	$-CH_3$ 1.21
Val	7.68	4.18	$-CH_{\beta}$ 2.01 $(-CH_{3\gamma})_2$ 0.86
Gly	8.24	$-CH_{2\alpha}$ 3.74	/
His	8.02	4.46	$-CH_{2\beta}$ 2.90 -NH aromatic not detected -CH Unassigned aromatics
Leu	7.93	4.14	$-CH_{2\beta}$ 1.48 $-CH_{\gamma}$ 0.81 $(-CH_{3\delta})_2$ not detected
NHEt	8.13	$-CH_{2\alpha}$ 3.08	$-CH_{3\beta}$ 1.00

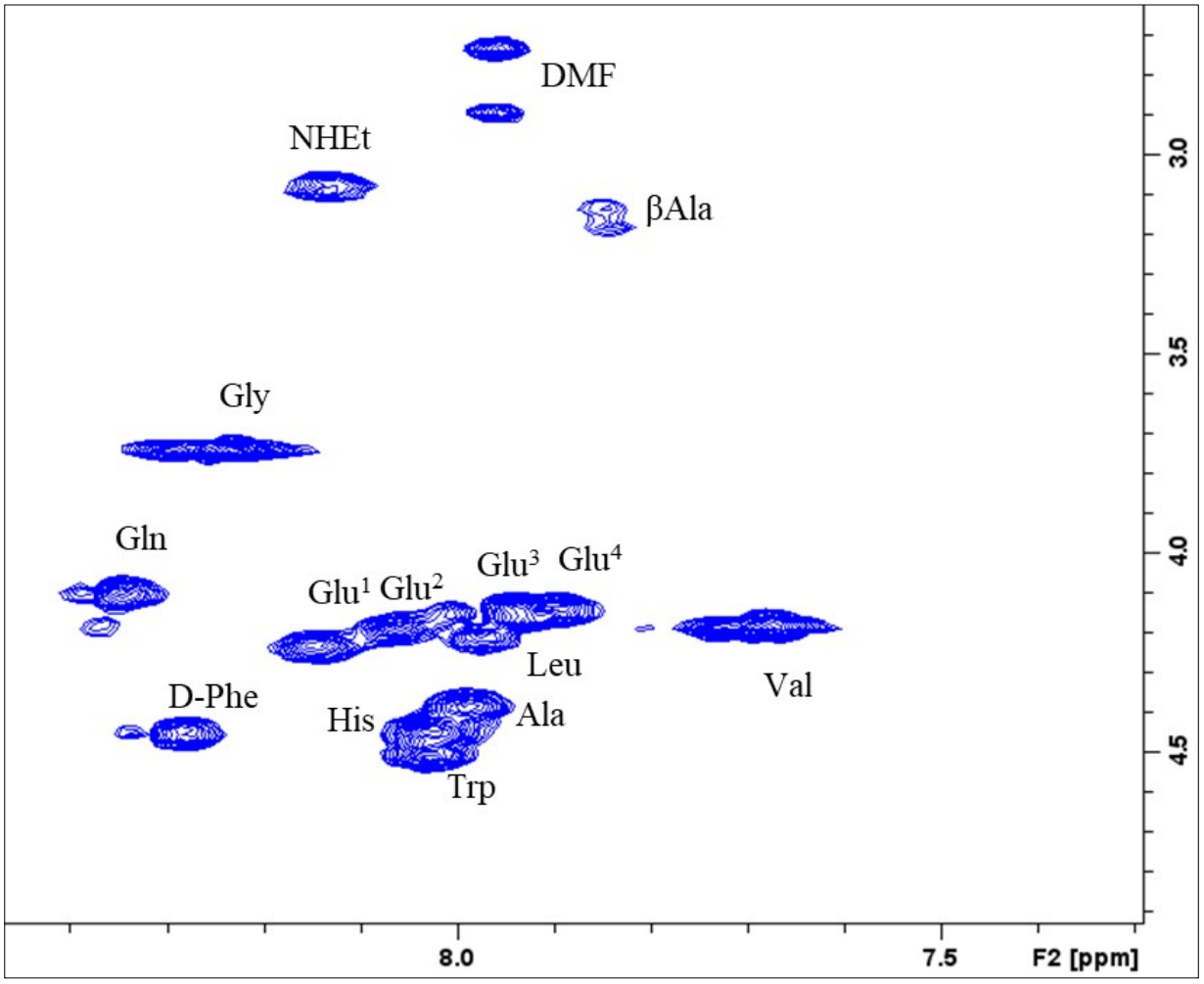


Fig. S.36 COSY spectrum of POV-3 (150 µM) in d_6-DMSO.

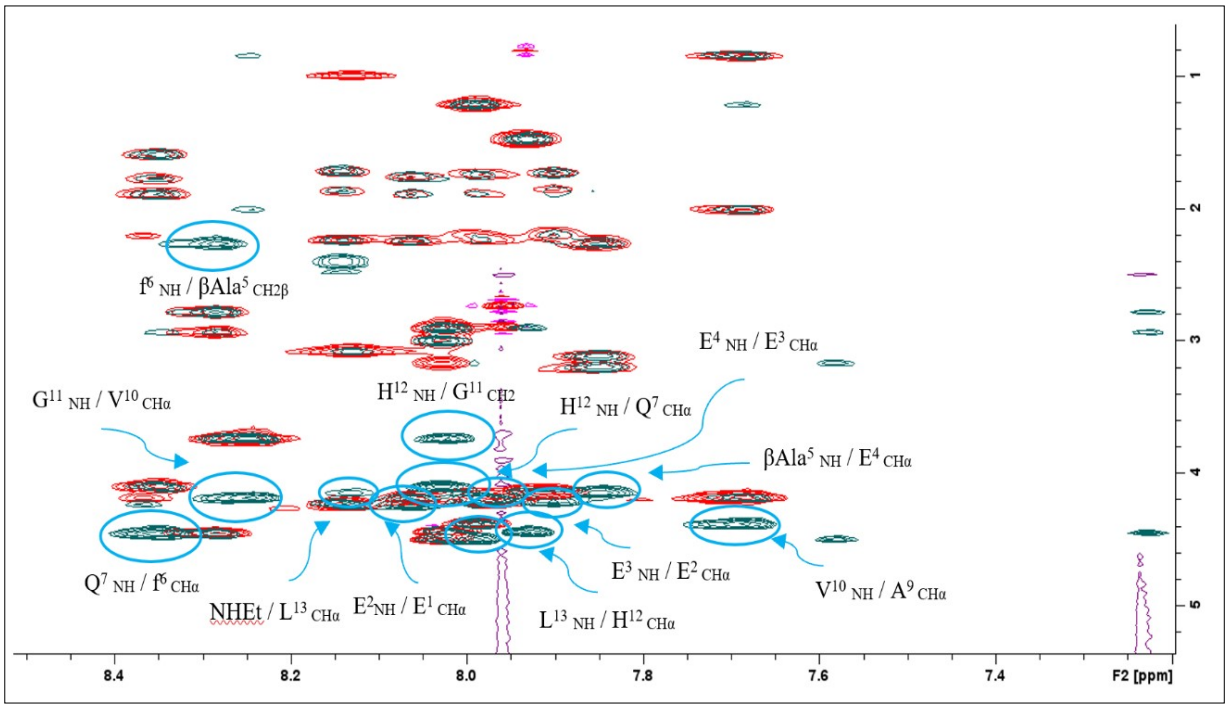


Fig. S.37 ^{1}H ROESY of POV-3 (in green) overlapped with TOCSY (in red). It is possible to notice the correlation between the amino acids His 12 and Gln 7.

In POV-3, the presence of an interaction between His and Gln (i, i+5) has been detected, suggesting the presence of a π-turn.

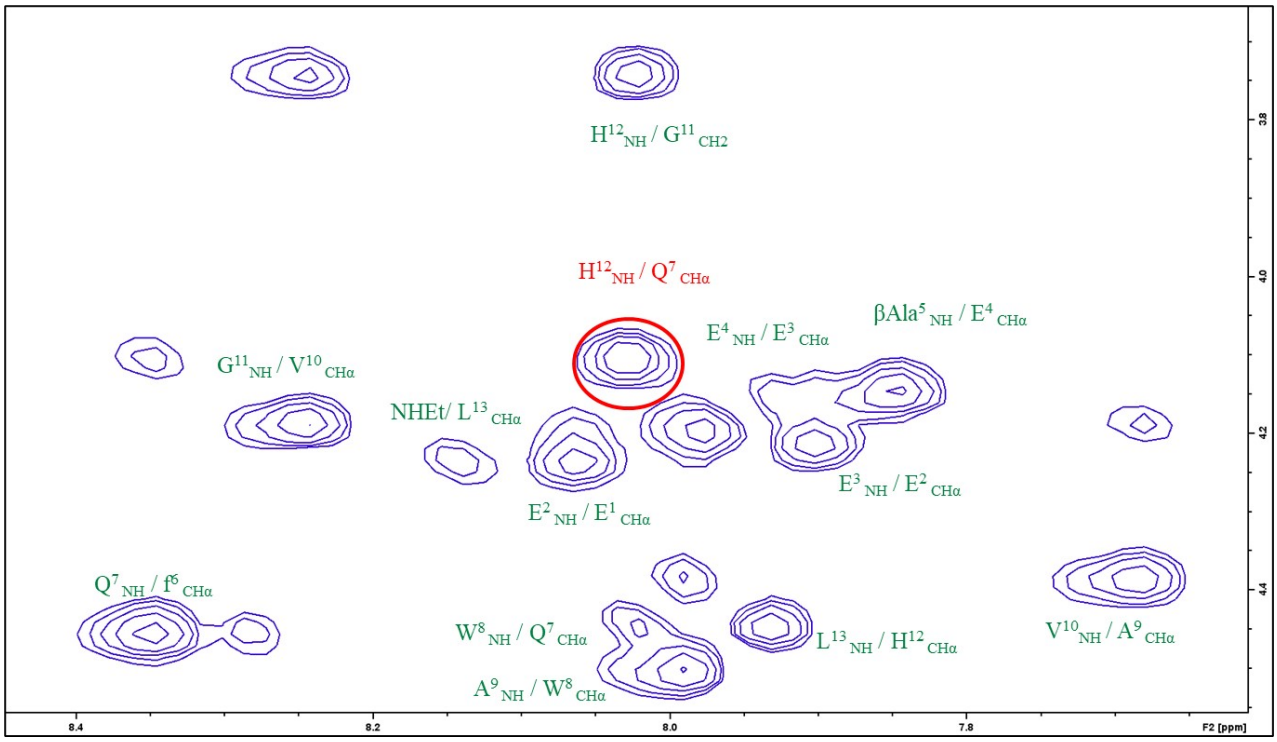


Fig. S.38 ^{1}H ROESY of POV-3, the correlation between the amino acids His 12 and Gln 7 is circled in red.

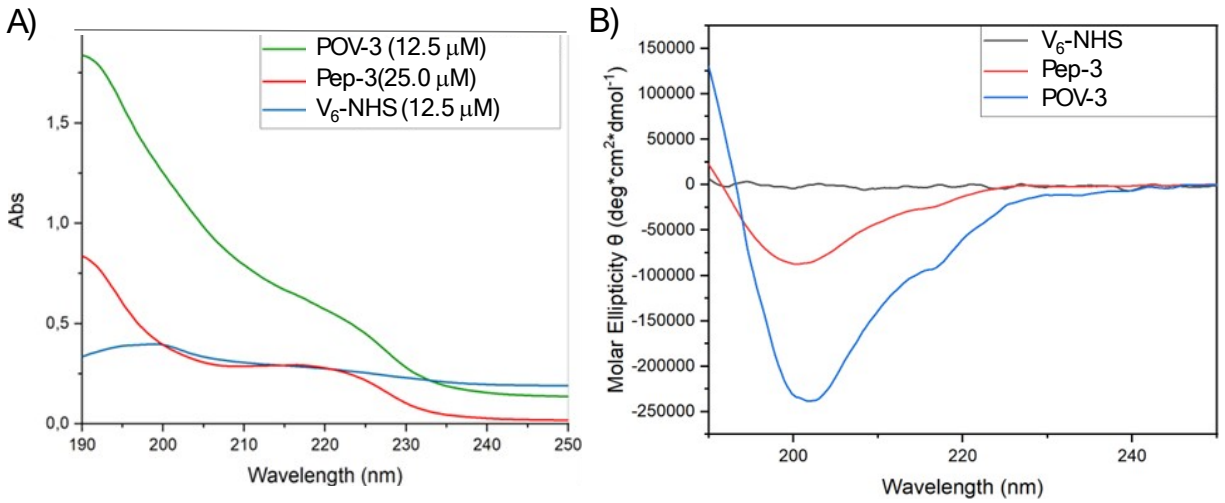
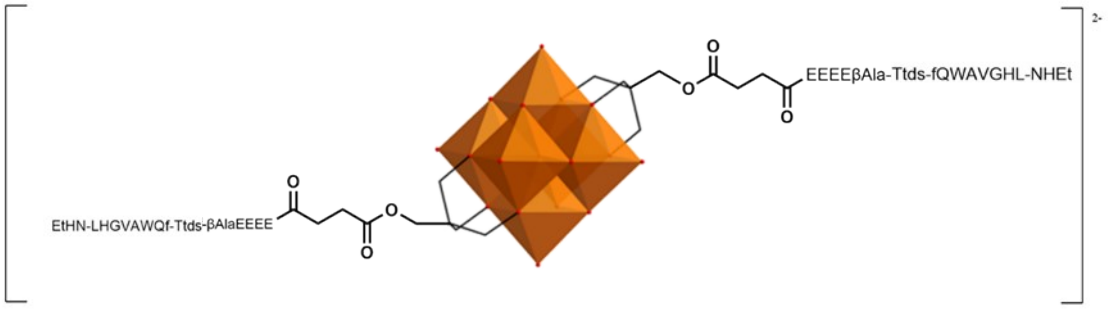


Fig. S.39 A) UV spectrum of POV-3, V_6-NHS and Pep-3 in TFE/H_2O 1:10; B) Molar ellipticity graph of POV-3, V_6-NHS and Pep-3 in TFE/H_2O 1:10.

Synthesis of POV-4: $[(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2CO(C_{86}H_{127}N_{20}O_{27})\}_2]$ (V_6-EEEEβAlaTtdsDB)



Reaction:

$[(C_4H_9)_4N]_2[V6\text{-}O\text{-}O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2CO(C_4H_4NO_3)\}_2] + 2\ C_{86}H_{127}N_{20}O_{27} \xrightarrow[DMF\ R.T.]{DIPEA} [(C_4H_9)_4N]_2[V_6O_{13}\{(OCH_2)_3CCH_2OOCCH_2CH_2CO(C_{86}H_{127}N_{20}O_{27})\}_2]$

Procedure:

V_6-NHS (7.58mg, 0.00457 mmol) was dissolved in 0.8 mL of DMF in a vial. Ttds-EEEEβAla-fQWAVGHL-NHEt (Pep-4, 6.72mg, 0.00358 mmol) and DIPEA (4.45 mg, 6 µL, 0.06 mmol) were added to the system. The mixture was stirred for 24 hours at room temperature. The desired product was obtained by crystallization under diethyl ether atmosphere. After 24 h, a dark red solid formed. It was washed with diethyl ether and then dried under vacuum (6.75 g of product, 84% yield).

Characterization:

FTIR (KBr, cm^{-1}): 3412 (m, br), 3287 (s), 3061(m), 2924 (m), 2873 (m), 1637 (s), 1536 (s), 1449 (w), 1390 (w), 1257 (w), 1057 (m), 956 (s), 879 808 (m), 713 (m), 626 (w) 588 (w), 507 (w), 423 (m).

ESI-MS (-) CH_3CN: ($[M\text{-}2TBA\text{-}2H_2O\text{-}7H+Na^+]^{7-}$ = 665.9)

UV (TFE/H_2O 1:10): maximum at <190 nm (ε190=688700 $cm^{-1}M^{-1}$) and shoulder at 218 nm (ε218=350635 $cm^{-1}M^{-1}$).

CD (Pure TFE): The minimum in TFE is located at 205 nm ([θ]= -699199 $deg*cm^2*dmol^{-1}$), while the maximum is at 193 nm ([θ]= 1540910 $deg*cm^2*dmol^{-1}$)

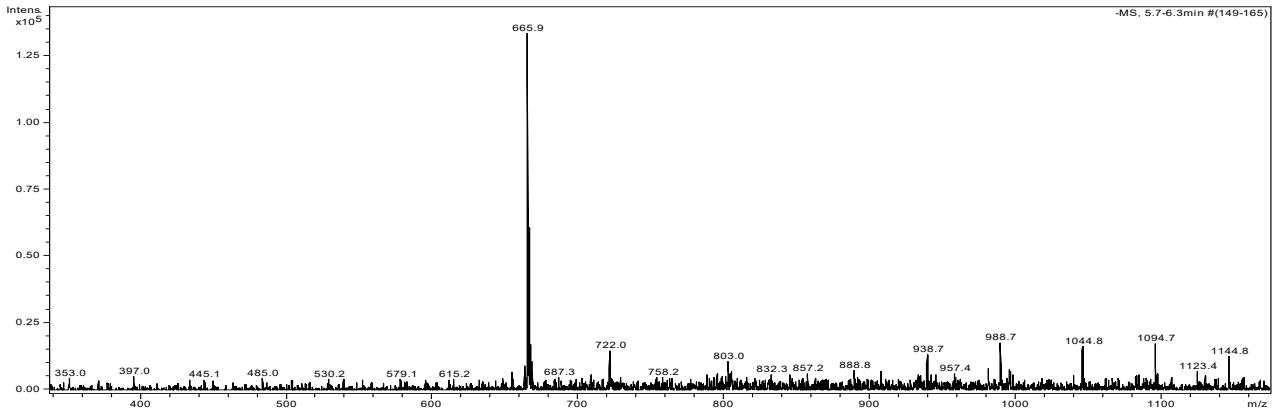


Fig. S.40 ESI-MS (-) spectrum of POV-4 in CH_3CN.

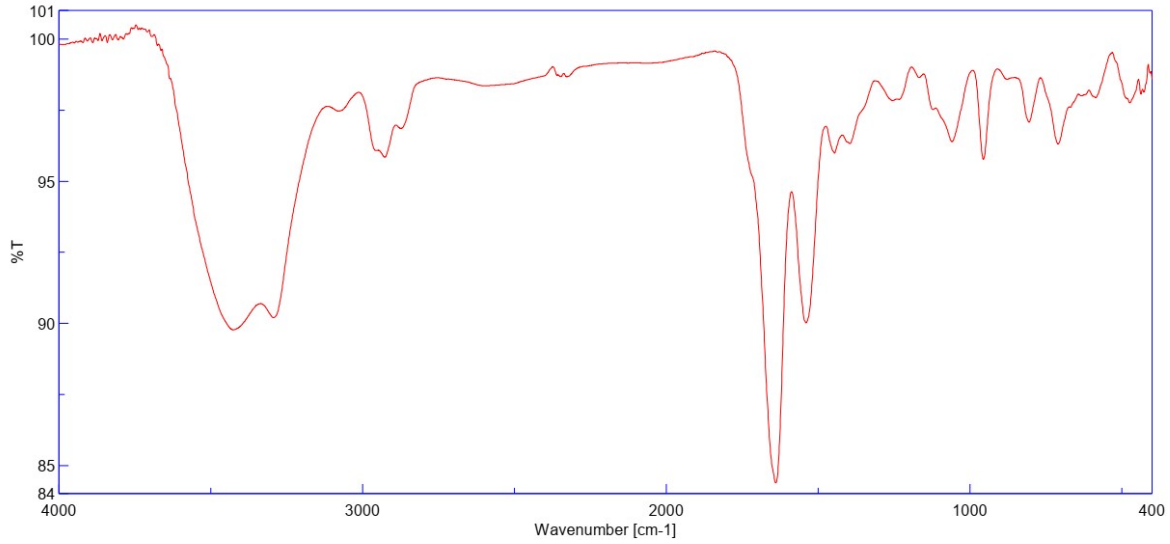


Fig. S.41 FT-IR spectrum of POV-4.

Table 8. Proton resonances of POV-4.

AA	NH (ppm)	CH_{α} (ppm)	Rest of the chain(ppm)
Glu[1]	8.27	4.14	$-CH_{2\beta}$ 2.25 $-CH_{2\gamma}$ 1.82
Glu[2]	8.20	4.16	$-CH_{2\beta}$ 2.25 $-CH_{2\gamma}$ 1.82
Glu[3]	7.87	4.17	$-CH_{2\beta}$ 2.21 $-CH_{2\gamma}$ 1.83
Glu[4]	7.78	4.12	$-CH_{2\beta}$ 2.20 $-CH_{2\gamma}$ 1.79
βAla	7.76	$-CH_{2\alpha}$ 3.15	$-CH_{2\beta}$ 2.25
D-Phe	8.32	4.44	$-CH_{2\beta}$ (diastereotopic) 2.92, 2.77 Aromatics not observed
Gln	8.37	4.09	$-CH_{2\beta}$ (diastereotopic) 1.76, 1.88 $-CH_{2\gamma}$ 1.56
Trp	8.02	4.51	$-CH_{2\beta}$ (diastereotopico) 2.99, 3.16 Unassigned aromatics
Ala	7.97	4.38	$-CH_3$ 1.20
Val	7.71	4.18	$-CH_{\beta}$ 2.00 $(-CH_{3\gamma})_2$ 0.85
Gly	8.27	$-CH_{2\alpha}$ 3.73	/
His	8.03	4.43	$-CH_{2\beta}$ 2.89 -NH aromatic not detected -CH Unassigned aromatics
Leu	7.93	4.14	$-CH_{2\beta}$ 1.47 $-CH_{\gamma}$ 0.80 $(-CH_{3\delta})_2$ not detected
NH-Ttds	7.93	3.05	$-CH_{2\beta}$ 1.59 $-CH_{2\gamma}$ 3.37
NH-V6-O-	7.80	$-CH_{2\alpha}$ 3.05	$-CH_{2\beta}$ 1.59 $-CH_{2\gamma}$ 3.37
NHEt	8.12	$-CH_{2\alpha}$ 3.07	$-CH_{3\beta}$ 0.99

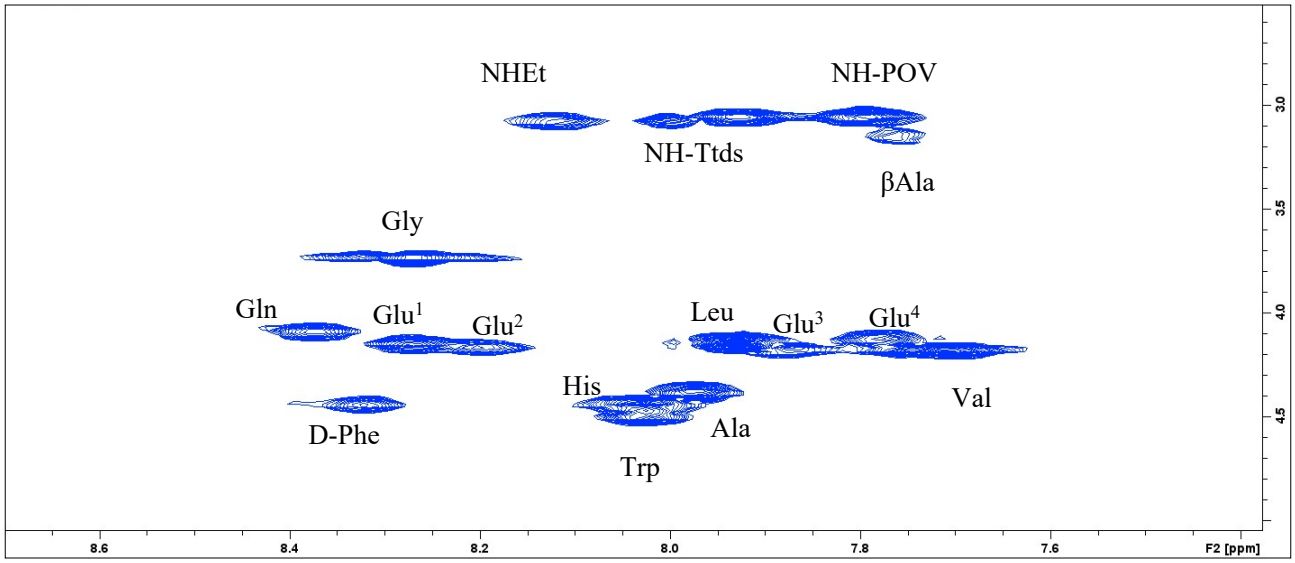


Fig. S.42 COSY spectrum of POV-4 in d_6-DMSO.

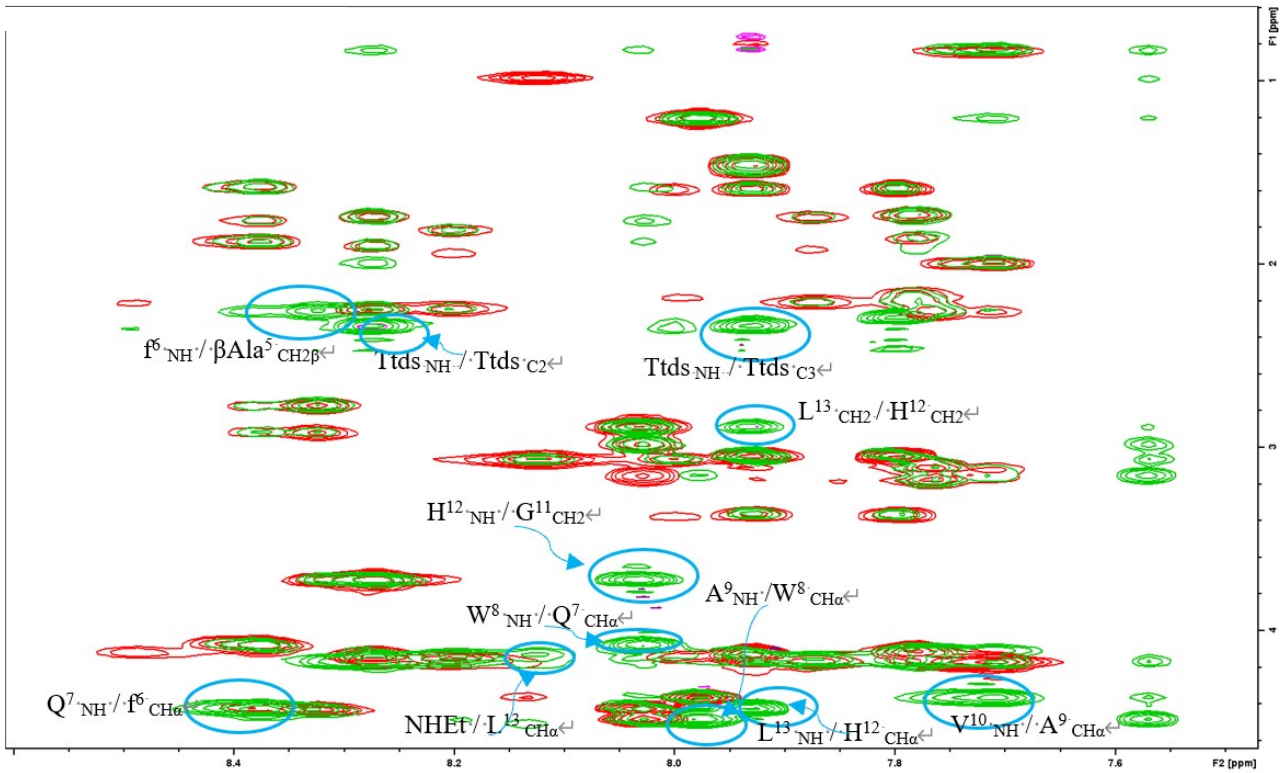


Fig. S.43 TOCSY and ROESY spectra of POV-4 in d_6-DMSO.

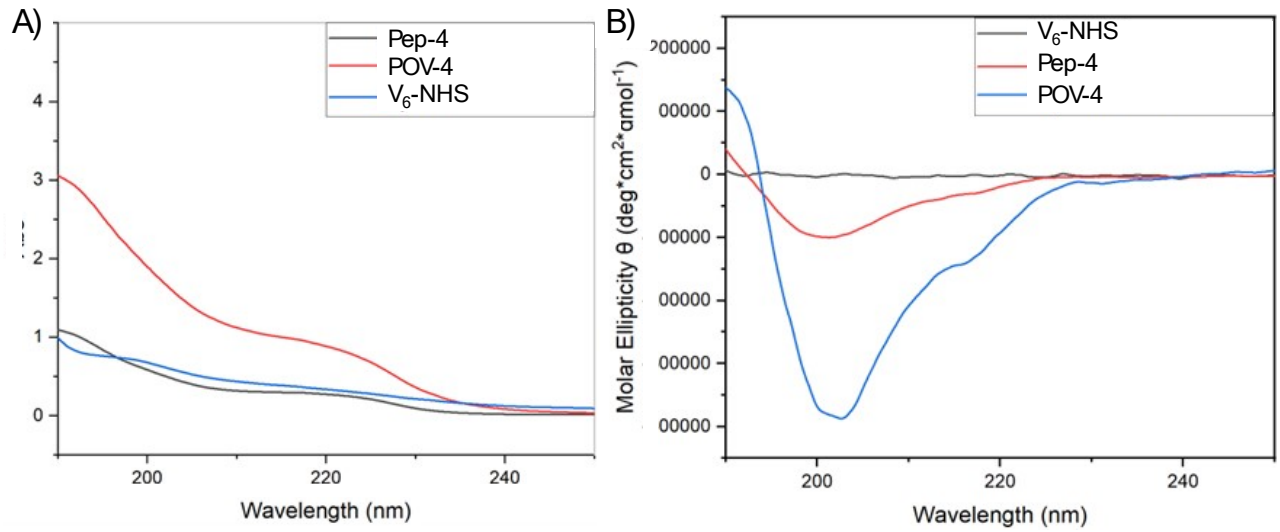


Fig. S.44 A) UV spectrum of POV-4, V_6-NHS and Pep-4 in TFE/H_2O 1:10. B) Molar ellipticity graph of POV-4, V_6-NHS and Pep-4 in TFE/H_2O 1:10.

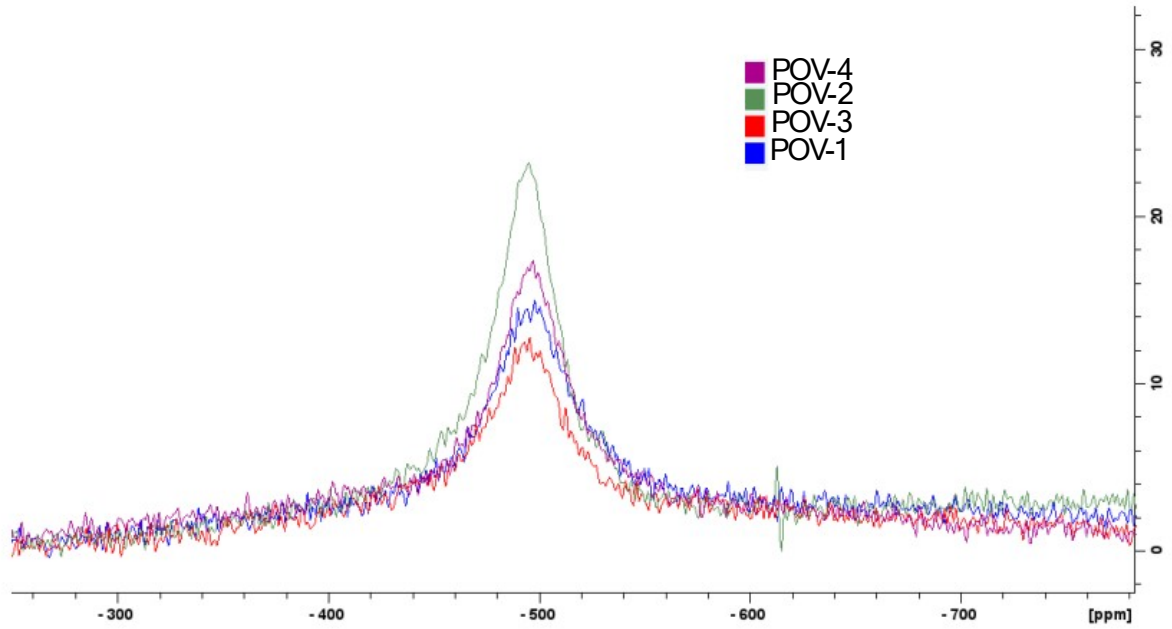


Fig. S.45 Overlaid ^{51}VNMR spectra (in d_6-DMSO) for POV-1, POV-2, POV-3, POV-4. (Vanadium peroxopicolinate 5x10^{-3}M, pH=1 in water, was taken as external reference, with δ = -600 ppm).

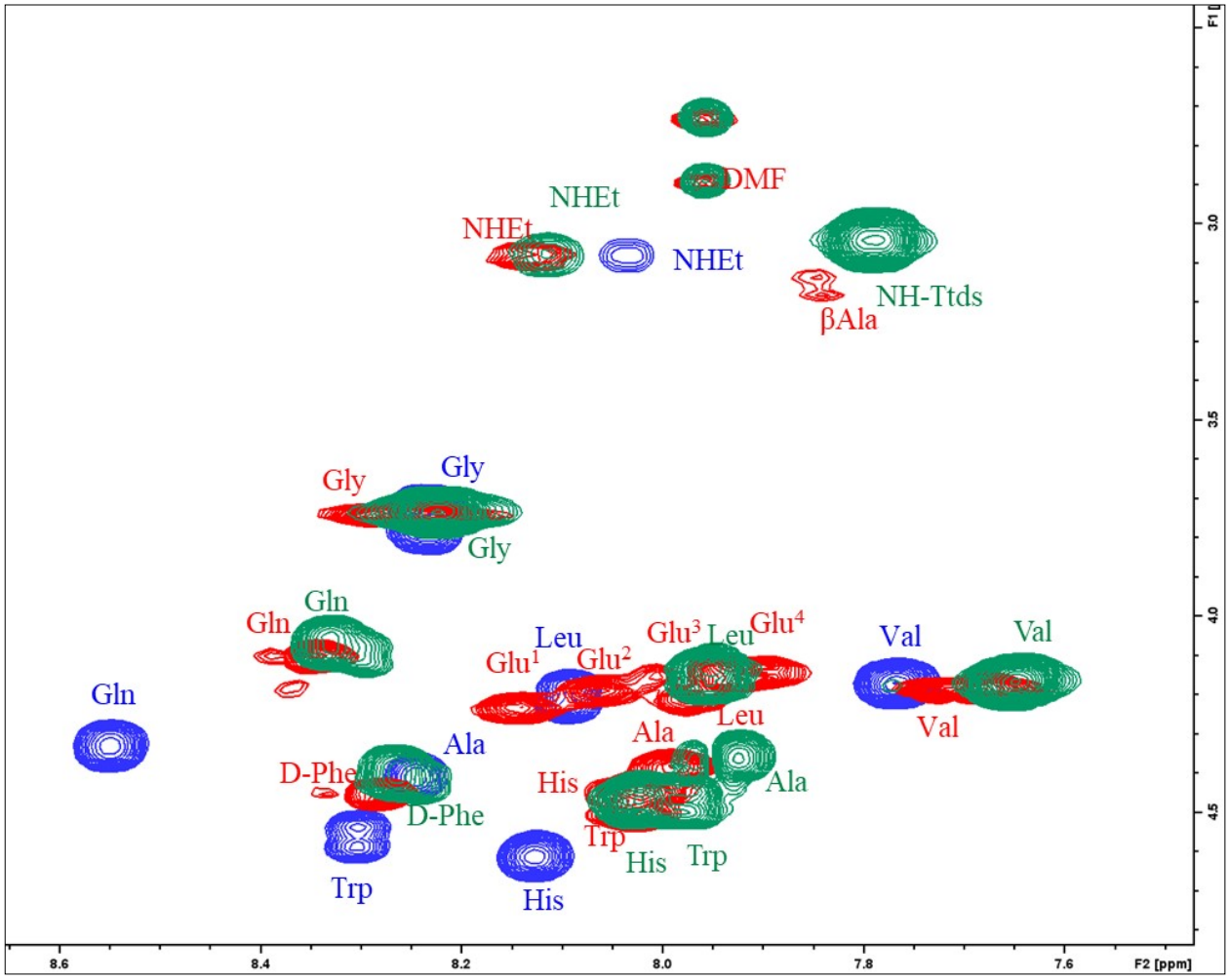


Fig. S.46 Overlaid COSY spectra (in d_6-DMSO) for NH–CH_α cross peaks region belonging to free and V_6-grafted DB. Pep-1 (blue), POV-2 (green), POV-3 (red).

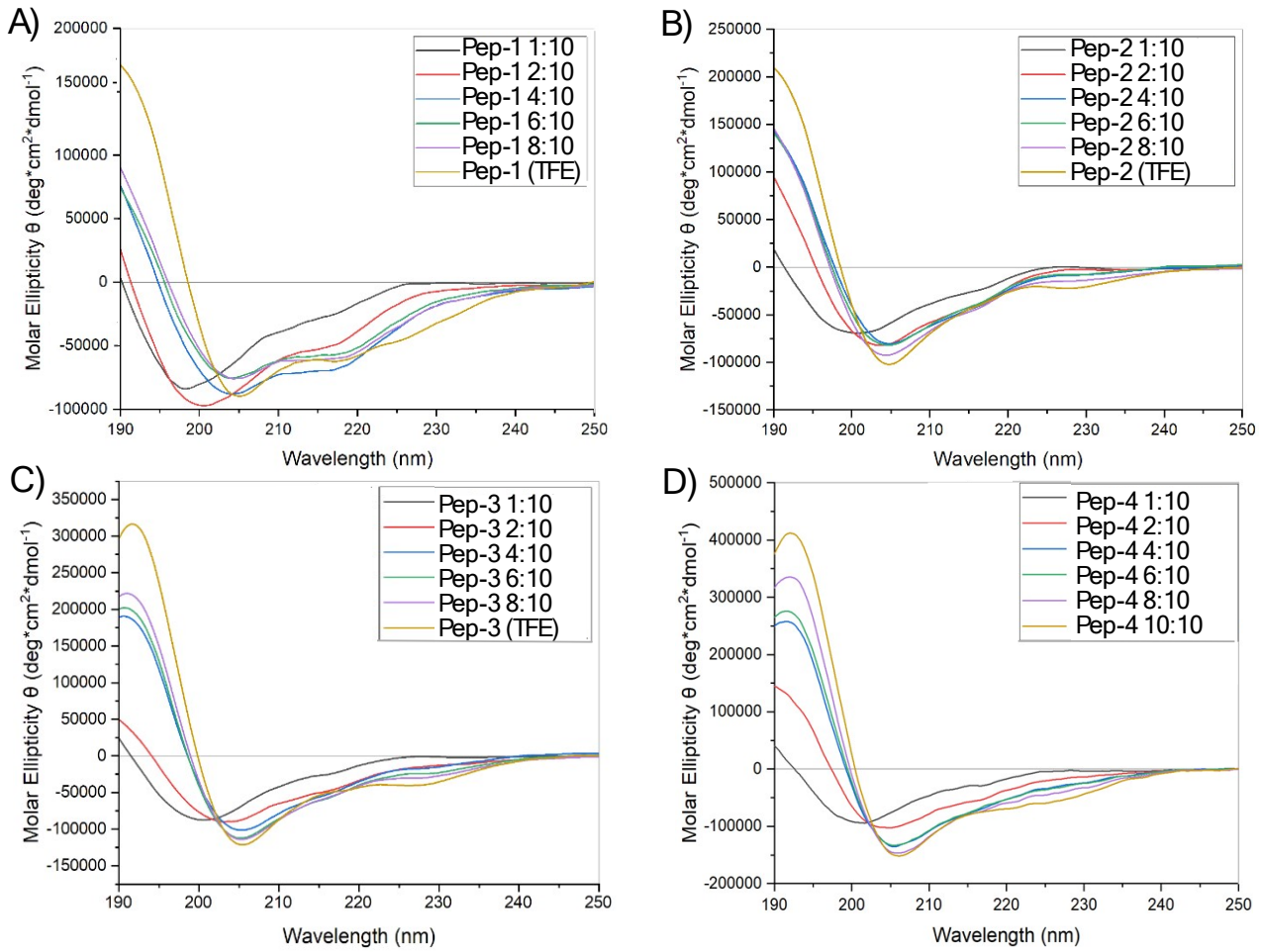


Fig. S.47 Far-UV CD spectra of: A) Pep-1, B) Pep-2, C) Pep-3, D) Pep-4 (25 μM) at variable % of trifluoroethanol (TFE) in water.

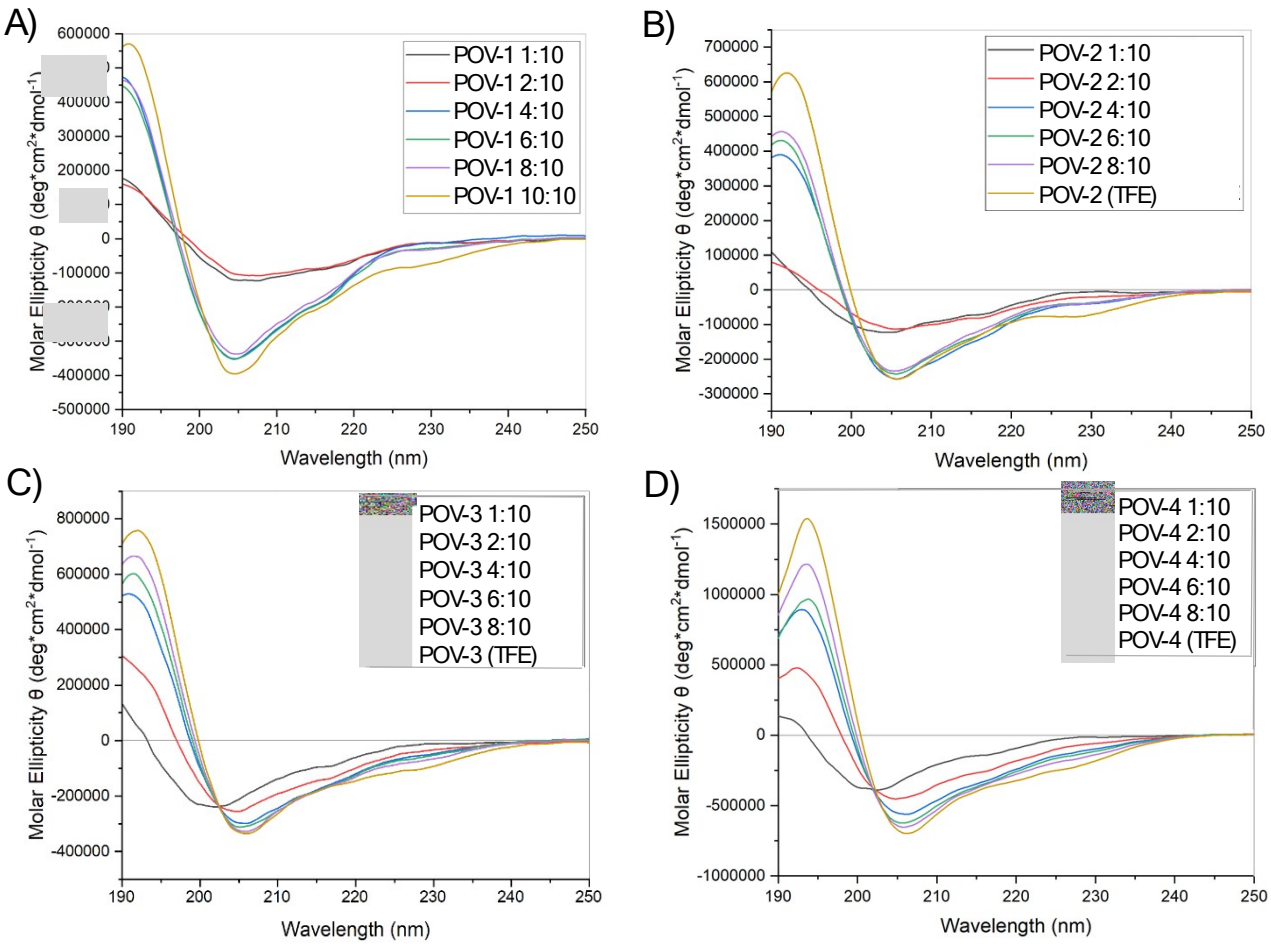


Fig. S.48 Far-UV CD spectra of: A) POV-1, B) POV-2, C) POV-3, D) POV-4 (12.5 µM) at variable % of trifluoroethanol (TFE) in water.

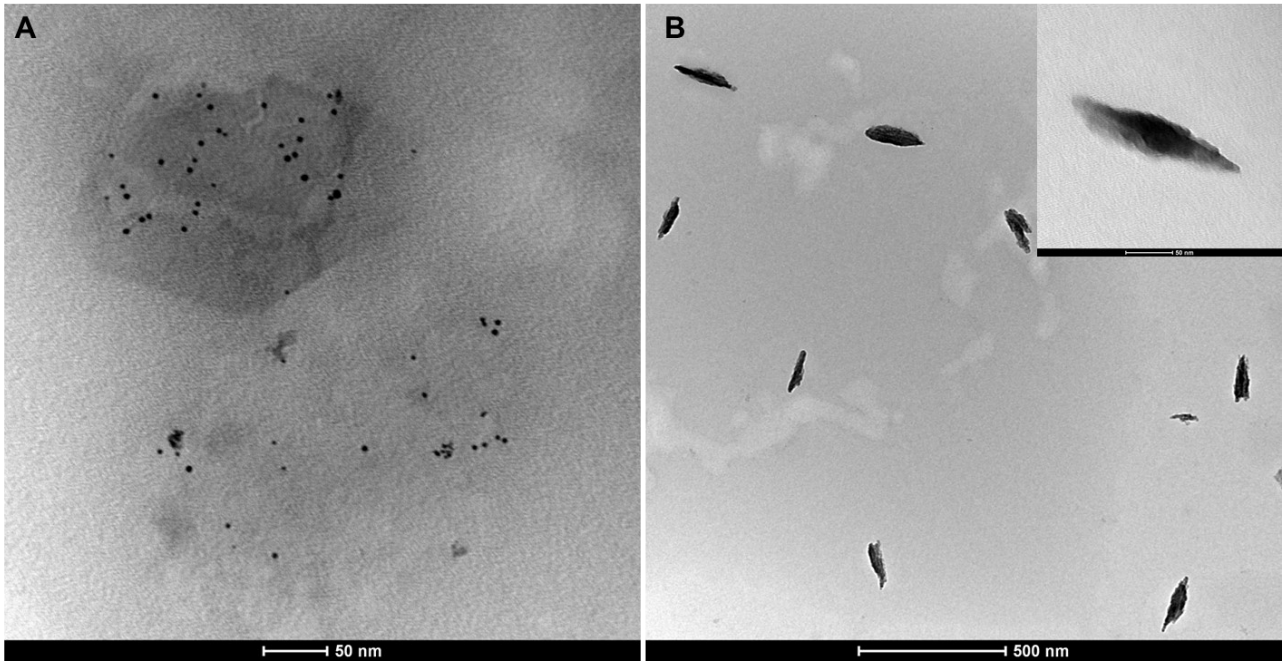


Fig. S.49 TEM measurements, 10^{-4} M solutions of (A) V_6-OH, (B) V_6-Phe in DMSO/water.