Discovery of Linear Receptors for Multiple $H_2PO_4^-$ Ions Using Dynamic Combinatorial Chemistry

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Supporting Information

S1 Experimental

S1.1 General Experimental Procedures

All chemicals, unless otherwise stated, were purchased from Aldrich, Alfa Aesar, Lancaster or NovaBioChem and used as received. All solvents were distilled prior to use with the exception of deuterated solvents and HPLC or LC-MS grade solvents. CD_3OD-d_4, DMSO-d_6 and $CDCl_3$ were purchased from Euriso-top. The $CDCl_3$ was filtered through Alumina (Merck) immediately prior to use for analysis of hydrazones. Where reactions were carried out under a N_2 (g) MeOH weas freshly distilled under N_2 (g) atmosphere CaH_2. The HPLC and LC-MS grade MeOH were purchased from Fisher. The water used in the eluent was purified by a Millipore system. HPLC-grade formic acid was purchased from Romil and LC-MS grade formic acid was purchased from Fluka. Column chromatography was carried out using silica gel 60 F (Merck).

^{1}H and ^{13}C-NMR spectra were recorded on a Bruker DPX-400 spectrometer, operating at 400 MHz (^{1}H), 100 MHz (^{13}C), a Bruker DMX-500 spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C) and a Bruker Cryoprobe TCI-500 operating at 500 MHz (^{1}H) and 125 MHz (^{13}C). Unless specified otherwise, all spectra were obtained at 298 K and are referenced to the internal solvent residue. Chemical shifts (δ) are quoted in ppm and have uncertainties of ± 0.01 ppm for ^{1}H, and ± 0.05 ppm for ^{13}C. Coupling constants (*J*) are listed in Hz. The following abbreviations are used for convenience in reporting the multiplicity for NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; sep, septet; m, multiplet and br, broad. Amino acid protons are labelled according to the traditional scheme: α, β, γ, and δ. The NMR data was processed using Bruker Topspin 2.0.

Assignment of all ^{1}H and ^{13}C resonances was achieved using standard 2D NMR techniques; COSY, NOESY, HMQC and HMBC.

UV/Vis spectroscopy was carried out on a Varian Cary 400 instrument, using quartz cuvettes and operating a room temperature. Absorption maxima, λ_{max} and molar absorptivities, ε are given in nm and M^{-1} cm^{-1}, respectively. Circular Dichroism (CD) spectroscopy was performed on a Chirascan at operating at 25 °C, using 0.5 cm and 1 cm quartz cuvettes. Ellipticity maxima (θ_{max}) are given in nm. Molar ellipticity coefficients M_θ were calculated as $M = 100 \times \theta / c \times l$, where ellipticity (θ) is in degrees, concentration in mM and pathlength (l) in cm, thus giving deg mM^{-1} cm^{-1}. Melting points were measured using a Gallenkamp apparatus and are uncorrected. Exact masses were recorded using a Waters Micromass LCT Premier mass spectrometer.

HPLC analysis was performed on an Agilent Technologies 1200 Series system coupled to a diode array UV/Vis detector. LC-MS was carried out on an Agilent 1100 LC/MSD trap XCT system operating in alternating ultrascan mode with nebuliser, 25 psi; dry gas, 8 $lmin^{-1}$; dry temperature, 340 °C; capillary 3500 V; skimmer 40 V; capillary exit, 241 V; Oct1 DC, 12V; Oct 1 DC, 4.45 V; Trap drive 167.9 V; Oct RF 210.4 Vpp; lens 1, -5; lens 2, -60; scan range, 100-2200 m/z; max accumulation time, 200 ms and smart target 50000.

Separations were achieved using a Waters Symmetry C_{18} 3.5 μm 4.6 × 150 mm column maintained at 45 °C. The mobile phase solutions prepared were 0.1% formic acid in H_2O (A) and 0.1% formic acid in MeOH (B). Different eluent gradients and injection volumes were used the separation of different mixtures. The specific methods are outlined below.

Method A Injection Volume: 10 μl

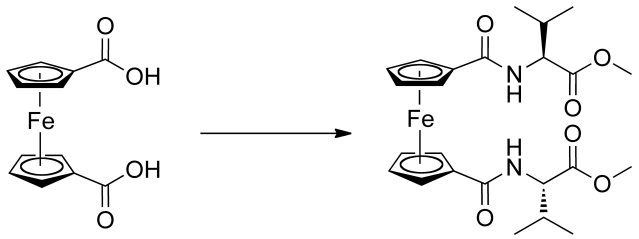
Time / min	% Solution A	% Solution B
0	50	50
3	30	80
10	27	83
17	0	100
20	0	100

Method B Injection Volume: 5 μl

Time / min	% Solution A	% Solution B
0	50	50
13	30	80
27	10	90
30	0	100

S1.2 Synthesis of Building Block Fc-[CO-Val-NHNH$_2$]$_2$ (V)

*Fc-[CO-Val-OMe]$_2$ (**1**)*[1]



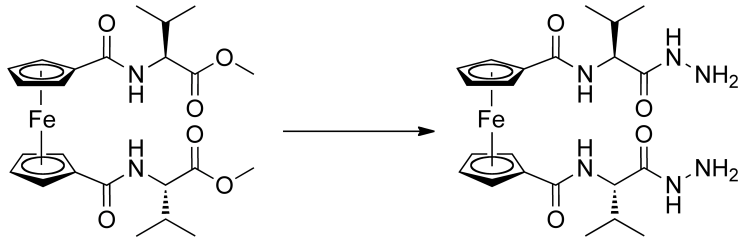
Fc-[COOH]$_2$ (2.00 g, 7.37 mmol), HOBt hydrate (2.64 g, 15.6 mmol (~20% H_2O)) and EDC (3.74 g, 19.5 mmol) were dissolved in DMF (120 ml) under an atmosphere of N_2 (g) to form a red solution. Et_3N (6.6 ml, 47 mmol) and then H-Val-OMe.HCl (3.27 g, 19.5 mmol) were added. The suspension formed was stirred overnight under an atmosphere of N_2 (g). Brine (150 ml) and H_2O (50 ml) were then added and the mixture was extracted with ethyl acetate (3 × 100 ml). The combined organic extracts were washed with $NaHCO_3$ (aq.) (2 × 150 ml), dried over Na_2SO_4, filtered and evaporated *in vacuo* to leave an orange solid. The crude material was purified by column chromatography (silica, crude material adsorbed on silica, hexane: ethyl acetate (1:1) to ethyl acetate) to yield the product as an orange solid. Yield: 2.64 g, 72 %. M.p.: 189-190 °C.

^{1}H-NMR (400 MHz; $CDCl_3$): δ 7.51 (d, ^3J = 8.4 Hz, 2H, N**H**), 4.85 (s, 2H, Cp-C**H**), 4.76 (s, 2H, Cp-C**H**), 4.66 (dd, ^3J = 7.9 Hz, 2H, α**H** of Val), 4.52 (s, 2H, Cp-C**H**), 4.36 (s, 2H, Cp-C**H**), 3.80 (s, 6H, OC**H**$_3$), 2.23-2.14 (m, 2H, β**H** of Val), 1.00 (d, ^3J = 3.1 Hz, 6H, γ**H** of Val), 0.99 (d, ^3J = 3.1 Hz, 6H, γ**H** of Val).

HR-MS (ESI): *m/z* = 501.1697 $[M + H]^+$ (calc. for $C_{24}H_{33}FeN_2O_6$: 501.1688).

CD (2 mM, $CHCl_3$:MeOH (96:4)): λ_{max} = 479 nm (M_θ = +6.9 deg mM^{-1} cm^{-1}), 358 nm (M_θ = –7.7 deg mM^{-1} cm^{-1}), 314 nm (M_θ = +12.2 deg mM^{-1} cm^{-1}).

*Fc-[CO-Val-NHNH$_2$]$_2$ (**V**)*



To a solution of Fc-[CO-Val-OMe]$_2$ (**1**) (2.00 g, 4.00 mmol) dissolved in MeOH (50 ml) under N_2 (g) atmosphere was added $N_2H_4.H_2O$ (20 ml) and the solution was stirred for two days. The yellow precipitate that formed was collected by vacuum filtration, washed with cold MeOH and dried *in vacuo*. Yield: 1.54 g, 77 %. M.p.: 220 °C (decomp.).

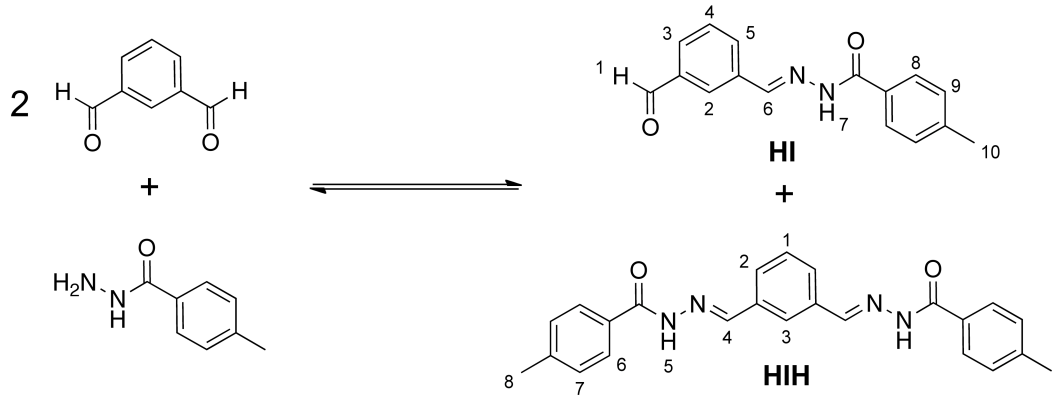
^{1}H-NMR (400 MHz; $CDCl_3$:CD_3OD (9:1): δ 8.72 (s, 2H, hydrazide N**H**), 7.98 (d, *J* = 8.3 Hz, 2H, amide N**H**), 4.75 (s, 2H, Cp-C**H**), 4.65 (s, 2H, Cp-C**H**), 4.45 (s, 2H, Cp-C**H**), 4.33 (s, 2H, Cp-C**H**), 4.08 (m, 2H, α**H** of Val), 2.20 (m, 2H, β**H** of Val), 1.02 (d, *J* = 6.6 Hz, 6H, γ**H** of Val), 0.94 (d, *J* = 6.6 Hz, 6H, γ**H** of Val). ^{13}C-NMR (100 MHz; $CDCl_3$:CD_3OD (9:1): δ 173.0 (**C**=O), 171.4 (**C**=O), 77.4 (Cp-**C**H), 76.0 (Cp-**C**), 72.1 (Cp-**C**H), 71.8 (Cp-**C**H), 70.2 (Cp-**C**H), 59.2 (α**C** of Val), 29.4 (β**C** of Val), 19.7 (γ**C** of Val), 19.4 (γ**C** of Val).

HR-MS (ESI): *m/z* = 523.1713 $[M + Na]^+$ (calc. for $C_{22}H_{32}FeN_6NaO_4$: 523.1732).

CD (2 mM, $CHCl_3$:MeOH (96:4)): λ_{max} = 483 nm (M_θ = +5.8 deg mM^{-1} cm^{-1}), 359 nm (M_θ = –5.5 deg mM^{-1} cm^{-1}), 312 nm (M_θ = +11.0 deg mM^{-1} cm^{-1}).

S1.3 Synthesis of Linear Oligomers: HIVIH, HIVIVIH and HIVIVIVIH

HI and HIH



Isophthalaldehyde (**I**) (1.79 g, 13.3 mmol), and 4-methylbenzohydrazide (**H**) (1.00 g, 6.66 mmol) were dissolved in $CHCl_3$ (100 ml). Acetic acid (2 ml) and 3Å molecular sieves (~1 g) were added and the reaction mixture was stirred overnight. MeOH (20 ml) was added to dissolve the thick white precipitate that had formed. The molecular sieves were removed by filtration and then the solution was washed with $NaHCO_3$ (aq.) (3 × 100 ml). MeOH was added to the organic fraction, which was then dried over Mg_2SO_4, filtered and evaporated *in vacuo* and adsorbed onto silica. Purification by column chromatography (silica, hexane: ethyl acetate (6:4) to ethyl acetate to ethyl acetate: MeOH (98:2) gave **HI** and **HIH** as white solids.

HI

Yield: 659 mg, 37%. M.p.: 165-168 °C.

^{1}H-NMR (400 MHz; $CDCl_3$:CD_3OD (9:1)): δ 11.11 (s, 1H, **H**7), 9.92 (s, 1H, **H**1), 8.29 (s, 1H, **H**6), 8.10 (s, 1H, **H**2), 8.04 (d, ^3J = 7.6, 1H, **H**3), 7.80 (dt, ^3J = 7.6, ^4J = 1.4, 1H, **H**5), 7.74 (d, ^3J = 8.0, 2H, **H**8), 7.47 (dd, apparent t, ^3J = 7.6, 1H, **H**4), 7.18 (d, ^3J = 8.0, 2H, **H**9), 2.32 (s, 3H, **H**10). ^{13}C-NMR (100 MHz; $CDCl_3$:CD_3OD (9:1)): δ 192.4 (H**C**=O), 165.3

(**C**=O), 146.9 (H**C**=N), 142.8 (Ar-**C**), 136.6 (Ar-**C**), 135.1 (Ar-**C**), 132.9 (Ar-**C**H), 130.9 (Ar-**C**H), 129.8 (Ar-**C**), 129.5 (Ar-**C**H), 129.3 (Ar-**C**H), 129.3 (Ar-**C**H), 127.6 (Ar-**C**H), 21.5 (**C**H_3).

HR-MS (ESI): *m*/*z* = 289.0965 $[M + Na]^+$ (calc. for $C_{16}H_{14}N_2NaO_2$: 289.0953).

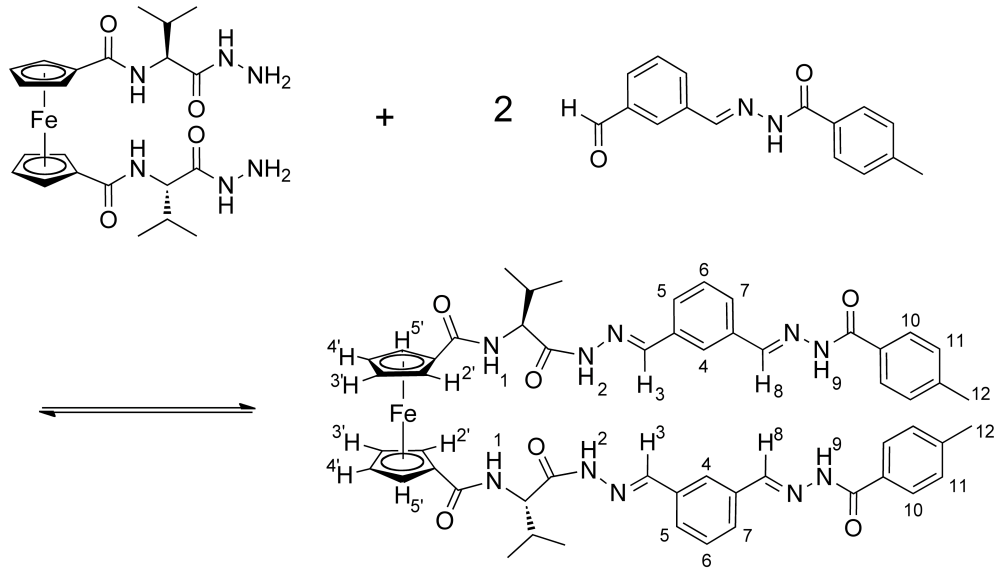
HIH

Yield: 321 mg, 24%. M.p.: 260 °C (decomp.).

^{1}H-NMR (400 MHz; $CDCl_3$:CD_3OD (9:1)): δ 11.34 (s, 2H, H5), 8.23 (s, 2H, H4), 8.00 (s, 1H, H3), 7.80 (d, ^3J = 8.1, 4H, H7), 7.69 (d, ^3J = 7.6, 2H, H2), 7.26 (m, 1H, H1), 7.20 (d, ^3J = 8.1, 4H, H6), 2.34 (s, 6H, H8). ^{13}C-NMR (100 MHz; $CDCl_3$:CD_3OD (9:1)): δ 165.5 (C=O), 148.2 (HC=N), 142.8 (Ar-C), 134.6 (Ar-C), 129.9 (Ar-C), 129.3 (Ar-CH), 129.3 (Ar-CH), 129.0 (Ar-CH), 127.7 (Ar-CH), 127.0 (Ar-CH), 21.5 (CH_3).

HR-MS (ESI): *m*/*z* = 399.1836 $[M + H]^+$ (calc. for $C_{24}H_{23}N_4O_2$: 399.1821).

HIVIH



Fc-[CO-Val-$NHNH_2$]$_2$ (**V**) (300 mg, 0.600 mmol) and **HI** (319 mg, 1.20 mmol) were dissolved in a mixture of $CHCl_3$ (65 ml), MeOH (20 ml) and acetic acid (1 ml) to form a yellow solution. 3Å molecular sieves (~1 g) were added to the solution, which was then

stirred overnight. MeOH (20 ml) and $CHCl_3$ (20 ml) were added to the mixture and it was filtered to remove the molecular sieves. The solution was then washed with $NaHCO_3$ (aq.) (2 × 100 ml). Additional methanol was added to dissolve the precipitate that formed and then the solution was then dried over Na_2SO_4, filtered, evaporated *in vacuo* and adsorbed onto silica. Purification by column chromatography (silica, CH_2Cl_2:MeOH (96:4) to CH_2Cl_2: MeOH (94:6)) gave the product as a pale yellow solid. Yield: 330 mg, 55%. M.p.: 200 °C (decomp.).

*The NMR spectra for **HIVIH** (4 mM) in the presence of $Bu_4NH_2PO_4$ (24 mM).*

^{1}H-NMR (500 MHz, $CDCl_3$:CD_3OD (96:4), 278 K): δ 13.39 (br s, 2H, **H**2 (NH)), 12.32 (s, 2H, **H**9 (NH)), 8.77 (s, 2H, **H**8), 8.68 (s, 2H, **H**3), 8.12 (d, ^3J = 7.4 Hz, 2H, **H**5/**H**7), 8.06 (s, 2H, **H**4), 8.05 (d, ^3J = 7.4 Hz, 2H, **H**7/**H**5), 7.94 (d, ^3J = 7.8 Hz**,** 4H, **H**10), 7.28 (dd apparent t, ^3J = 7.4 Hz, 2H, **H**6), 7.19 (d, ^3J = 7.8 Hz, 4H, **H**11), 5.49 (br s, 2H, Cp-**H**2´), 4.85 (s, 2H, Cp-**H**3´), 4.81 (br s, 2H, Cp-**H**5´), 4.52 (d, ^3J = 9.5 Hz, 2H, α**H** of Val) 4.29 (s, 2H, Cp-**H**4´), 2.40-2.30 (m, 2H, β**H** of Val), 2.35 (s, 6H, **H**12), 1.08 (d, ^3J = 6.5 Hz, 6H, γ**H** of Val), 1.03 (d, ^3J = 6.5 Hz, 6H, γ**H** of Val). ^{13}C-NMR (125 MHz, $CDCl_3$:CD_3OD (96:4), 278 K): δ 170.8 (**C**=O), 169.8 (**C**=O), 165.3 (**C**=O), 149.3 (**C**8), 148.8 (**C**3), 148.1 (Ar-**C** quaternary), 147.9 (Ar-**C** quaternary), 135.3 (Ar-**C** quaternary), 135.1 (Ar-**C** quaternary), 129.1 (**C**11), 128.6 (**C**6), 128.0 (**C**10), 126.8 (**C**7/**C**5), 126.7 (**C**4), 126.6 (**C**5/**C**7), 72.5 (Cp-**C**4'), 70.6 (Cp-**C**3'), 60.8 (α**C** of Val), 30.9 (β**C** of Val), 21.6 (**C**12), 19.9 (γ**C** of Val), 19.7 (γ**C** of Val).

HR-MS (ESI): m/z = 997.3820 $[M + H]^+$ (calc. for $C_{54}H_{57}FeN_{10}O_6$: 997.3812).

UV/Vis ($CHCl_3$:MeOH (96:4)): λ_{max} = 296 nm (ε = 75000 M^{-1} cm^{-1}), 446 nm (ε = 325 M^{-1} cm^{-1}).

CD (2 mM, $CHCl_3$:MeOH (96:4)): λ_{max} = 486 nm (M_θ = +3.8 deg mM^{-1} cm^{-1}), 420 nm (M_θ = −3.2 deg mM^{-1} cm^{-1}), 360 nm (M_θ = −2.7 deg mM^{-1} cm^{-1}).

HIVIVIH and HIVIVIVIH

HI (133 mg, 0.50 mmol), Fc-[CO-Val-$NHNH_2$]$_2$ (**V**) (250 mg, 0.50 mmol) and isophthalaldehyde (**I**) (33 mg, 0.25 mmol) were dissolved in a mixture of $CHCl_3$ (65 ml), MeOH (10 ml) and acetic acid (1 ml). Molecular sieves (3Å, ~1 g) were added and the solution was stirred overnight. Additional MeOH and $CHCl_3$ were then added to dissolve precipitated materials. The solution was filtered to remove the molecular sieves then washed with Na_2CO_3 (aq.) (2 × 100 ml) and water (100 ml). Additional MeOH was added to the collected organic layer to dissolve precipitated material; it was then dried over Na_2SO_4, filtered and evaporated *in vacuo* to give a pale yellow solid. Analysis by HPLC showed a mixture of several species consisting mainly of **HIVIH**, **HIVIVIH** and **HIVIVIVIH**. Column chromatography (silica: CH_2Cl_2 to MeOH:CH_2Cl_2 (9:1)) enabled the separation of **HIVIH**, **HIVIVIH** and **HIVIVIVIH**. **HIVIVIVIH** was isolated with adequate purity as a pale yellow solid. The separated **HIVIVIH** was further purified by column chromatography (silica: MeOH:Et_3N:CH_2Cl_2 (4:1:95)) to give the product as a pale yellow solid.

HIVIVIH

Yield: 14 mg, 4%. M.p.: 230 °C (decomp.)

MS (ESI) *m*/*z* = 1617.6 $[M + Na]^+$, 820.4 $[M + 2Na]^{2+}$, 1593.6 $[M – H]^-$, 1615.5 $[M – 2H + Na]^-$ (calc. for $C_{84}H_{90}Fe_2N_{16}O_{10}$: 1594.573).

UV/Vis ($CHCl_3$:MeOH (96:4)): λ_{max} = 293 nm (ε = 130000 M^{-1} cm^{-1})

CD (0.5 mM, $CHCl_3$:MeOH (96:4)): λ_{max} = 485 nm (M_θ = +7 deg mM^{-1} cm^{-1}), 420 nm (M_θ = –4.5 deg mM^{-1} cm^{-1}), 360 nm (M_θ = –5 deg mM^{-1} cm^{-1}).

HIVIVIVIH

Yield: 9 mg, 2%.

MS (ESI) *m/z* = 1098.5 $[M + 2H]^{2+}$, 1109.1 $[M + H + Na]^{2+}$, 1120.4 $[M + 2Na]^{2+}$, 2191.9 $[M – H]^-$, 1095.4 $[M – 2H]^{2-}$ (calc. for $C_{114}H_{124}Fe_3N_{22}O_{14}$: 2192.772).

UV/Vis ($CHCl_3$:MeOH (96:4)): λ_{max} = 290 nm (ε = 170000 M^{-1} cm^{-1})

CD (0.5 mM, $CHCl_3$:MeOH (96:4)): λ_{max} = 485 nm (M_θ = +16 deg mM^{-1} cm^{-1}), 420 nm (M_θ = –7 deg mM^{-1} cm^{-1}), 360 nm (M_θ = –11 deg mM^{-1} cm^{-1}).

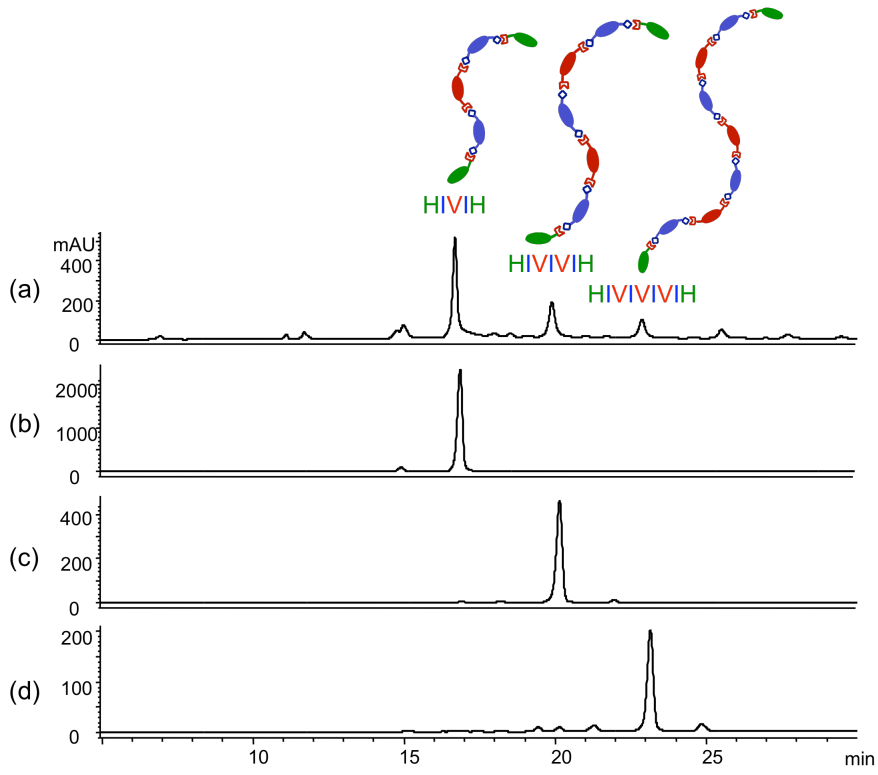


Figure S1. HPLC chromatograms (290 nm) of: (a) the reaction mixture in the synthesis of **HIVIVIH** and **HIVIVIVIH**; (b) isolated **HIVIH** (c) isolated **HIVIVIH** and (d) isolated **HIVIVIVIH**.

S1.4 Synthesis and Isolation of Macrocycles (VI) and $(VI)_2$

Fc-[CO-Val-$NHNH_2$]$_2$ (**V**) (200 mg, 0.40 mmol) and isophthalaldehyde (**I**) (54 mg, 0.40 mmol) were dissolved in a solution of $CHCl_3$ (400 ml) and acetic acid (4 ml) and stirred for 5 days. The solution was then washed with $NaHCO_3$ (aq.) (3 × 200 ml) and brine (1 × 200 ml), dried over Na_2SO_4, filtered and evaporated to yield a yellow solid. The material was purified by column chromatography (silica, crude material absorbed on Celite). The first column (CH_2Cl_2 to MeOH:CH_2Cl_2 (1:9)) was used to separate **(VI)** and **$(VI)_2$** from higher oligomers. A second column (isocratic, MeOH:Et_3N:CH_2Cl_2 (5:2.5:92.5)) was required to separate **(VI)** and **$(VI)_2$**. The isolated fractions were first washed with Na_2CO_3 (aq.) and then dried over Na_2SO_4 before evaporation *in vacuo* to yield as pale yellow solids: **(VI)** (17 mg, 7 %); **$(VI)_2$** (30 mg, 13 %). The mixture of macrocycles in solution, and the isolated samples were analysed using HPLC, employing HPLC Method A.

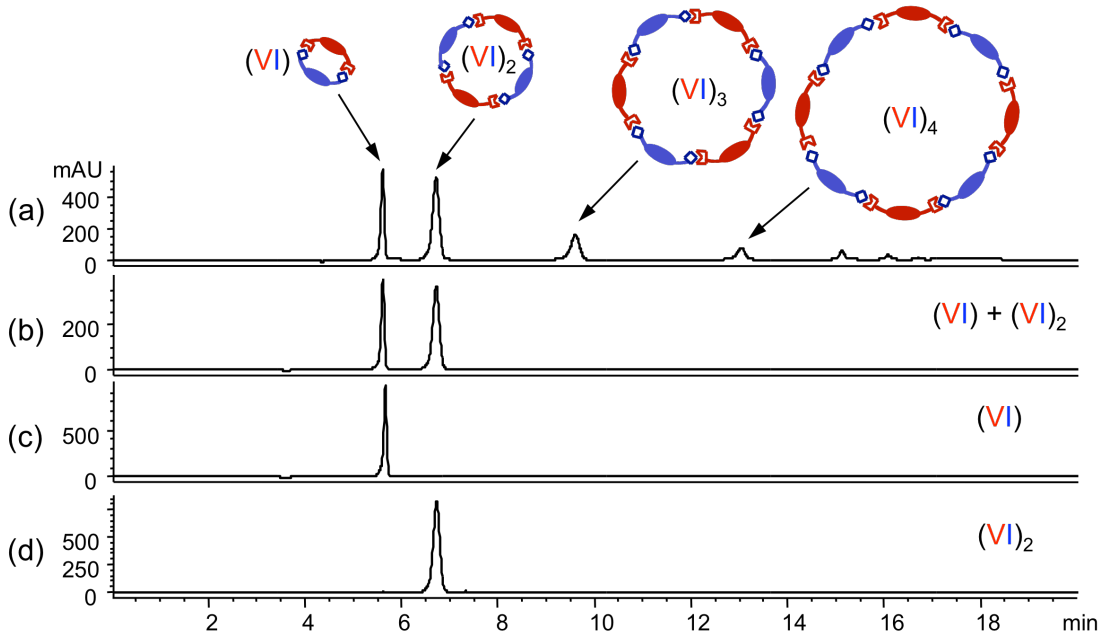
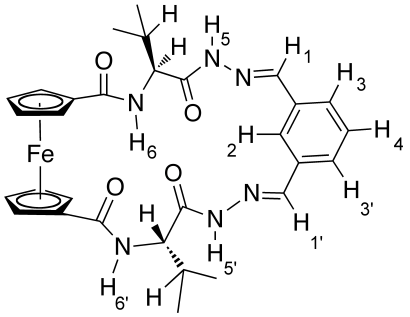


Figure S2. HPLC chromatograms (290 nm) showing (a) the mixture of macrocycles formed and three fractions isolated *via* column chromatography (CH_2Cl_2 – CH_2Cl_2:MeOH (9:1)): (b) **(VI)** and **$(VI)_2$** and two fractions separated by a second column (CH_2Cl_2:MeOH:Et_3N (92.5:5:2.5)): (c) **(VI)** and (d) **$(VI)_2$**.

(VI)



M.p.: 260 °C (decomp.).

^{1}H-NMR (500 MHz, CD_2Cl_2:CD_3OD (9:1), 278 K): δ 10.29 (s, 1H N**H**5/N**H**5′), 9.98 (br m, 2H, N**H**5/N**H**5′ and N**H**6), 8.79 (s, 1H, **H**2), 7.81 (s, 2H, **H**1 and **H**1′), 7.40 (dd apparent t, 1H, **H**4), 7.33 (br m, 3H, **H**3, **H**3′ and N**H**6′), 5.45 (br m, 1H α**H**′ of Val), 4.68 (br s, 1H, Cp-C**H**), 4.54 (br s, 1H Cp-C**H**), 4.39-4.20 (m, 6H, Cp-C**H**) , 4.00 (br m, 1H, α**H** of Val), 2.91 (br m, 1H, β**H** of Val), 2.25 (br m, 1H, β**H**′ of Val), 1.25 (br m, 3H, γ**H** of Val), 1.08 (br m, 3H, γ**H** of Val), 1.05 (d, ^3J = 6.7 Hz, 6H, γ**H**′ of Val) ppm.

HR-MS (ESI): *m/z* = 621.1890 $[M + Na]^+$ (calc. for $C_{30}H_{34}FeN_6NaO_4$: 621.1889).

UV/Vis: λ_{max} = 278 nm (ε = 40 000 M^{-1} cm^{-1}), 434 nm (ε = 100 M^{-1} cm^{-1}).

CD (1 mM $CHCl_3$:MeOH (9:1)): λ_{max} = 427 nm (M_θ = +0.4 deg mM^{-1} cm^{-1}), 340 nm (M_θ = +3.5 deg mM^{-1} cm^{-1})

(VI)$_2$

M.p.: 260 °C (decomp.).

HR-MS (ESI): *m/z* = 1219.3837 $[M + H]^+$ (calc. for $C_{60}H_{68}Fe_2N_{12}NaO_8$: 1219.3874).

UV/Vis: λ_{max} = 288 nm (ε = 80 000 M^{-1} cm^{-1}), 449 nm (ε = 290 M^{-1} cm^{-1}).

CD (0.5 mM $CHCl_3$:MeOH (9:1)): λ_{max} = 483 nm (M_θ = +3.0 deg mM^{-1} cm^{-1}), 418 nm (M_θ = –2.3 deg mM^{-1} cm^{-1}), 359 nm (M_θ = –1.7 deg mM^{-1} cm^{-1}).

S1.5 Dynamic Combinatorial Libraries

S1.5.1 Test for Reversibility

Three solutions were prepared in $CHCl_3$:MeOH (96:4) containing:

I. Fc-[CO-Val-$NHNH_2$]$_2$ (**V**) (5.55 mM), isophthalaldehyde (**I**) (5.55 mM) and 1-naphthoic acid (20 mM) in 6.5 ml;

II. **(VI)$_2$** (0.278 mM) and 1-naphthoic acid (20 mM) in 6.5 ml;

III. 4-methylbenzhydrazide (**H**) (25.0 mM, 5 ml).

Solutions I and II (900 μl) were each combined with solution III (40 μl) and solvent mixture $CHCl_3$:MeOH (96:4) (60 μl), and then stirred continuously for 10 days. The mixtures were analysed by LC-MS after 5 and 10 days using Method A. After 10 days, the chromatograms for the libraries showed the same distribution of species, indicating that thermodynamic equilibrium had been reached.

S1.5.2 $Bu_4NH_2PO_4$-Templated Library

A solution of 1-naphthoic acid (20 mM) in $CHCl_3$:MeOH (96:4) (50 ml) was prepared. This was then used to prepare a solution containing 1-naphthoic acid and the building blocks Fc-[CO-Val-$NHNH_2$]$_2$ (**V**) (0.50 mM), isophthalaldehyde (**I**) (0.50 mM) and 4-methylbenzhydrazide (**H**) (1.0 mM) (20 ml). This building block solution was used to prepare a solution containing acid, building blocks and $Bu_4NH_2PO_4$ (10 mM, 800 μl). Two DCLs were set-up: (a) a blank library formed from building blocks solution (1 ml) and (b) a templated library formed from building block solution (975 μl) and building block + anion solution (25 μl). The libraries were continuously stirred and analysed by HPLC-MS using Method B after 5 and 10 days. The experiment was repeated on a separate occasion to ensure reproducibility.

S1.6 UV/Vis Titration

A solution of **HIVIH** (15 μM) in $CHCl_3$:MeOH (96:4) (100 ml) was prepared. This solution was used to prepare a solution containing **HIVIH** (5 μM) and $Bu_4NH_2PO_4$ (1.0 mM, 4 ml). The UV/Vis spectrum of the **HIVIH** solution was recorded from 240 nm to 400 nm on a 2 ml solution in a 1 cm quartz cuvette. Small aliquots of **HIVIH** + $Bu_4NH_2PO_4$ solution were added (2.5 μl increasing to 20 μl up to a total of 300 μl). The UV/Vis spectrum was recorded after each addition. The changes in absorbance at 295 nm were plotted against the concentration of added $Bu_4NH_2PO_4$ to generate binding isotherms.

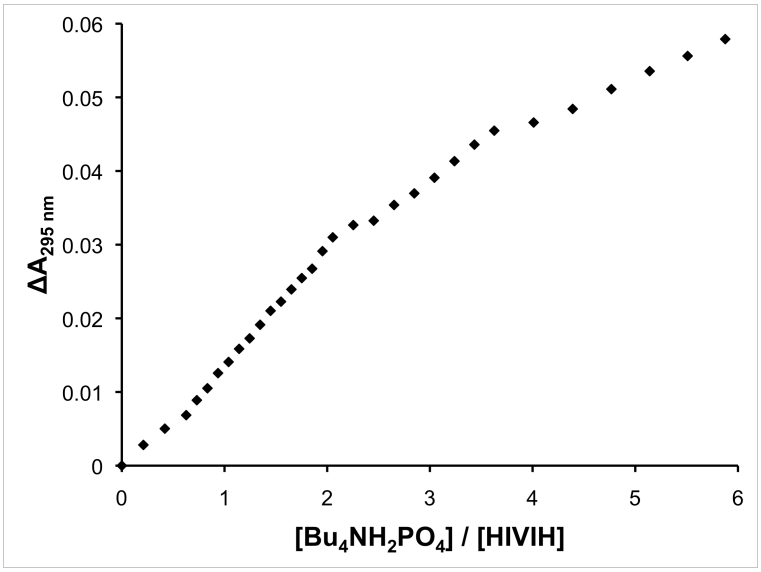


Figure S3. Binding isotherm (monitoring absorbance at 295 nm) for the interaction of $Bu_4NH_2PO_4$ with **HIVIH.**

S1.7 UV/Vis Job Plot

A solution of **HIVIH** (15 μM) was prepared in $CHCl_3$:MeOH (96:4) (100 ml), as was a solution of $Bu_4NH_2PO_4$ (15 μM, 5 ml). The UV/Vis spectrum of the **HIVIH** solution (2 ml) was recorded from 240 nm to 340 nm in a 1 cm quartz cuvette. Then aliquots of $Bu_4NH_2PO_4$ solution were added up to a total volume of 4 ml, recording the UV/Vis spectrum after each addition. The spectrum of the $Bu_4NH_2PO_4$ solution (2 ml) was then recorded. Aliquots of the **HIVIH** solution, up to a total volume of 4 ml, were added, again recording the spectrum after each addition. Plotting ΔA × [**HIVIH**] against [**HIVIH**] / ([**HIVIH**] + [$Bu_4NH_2PO_4$]) produced the Job plot.

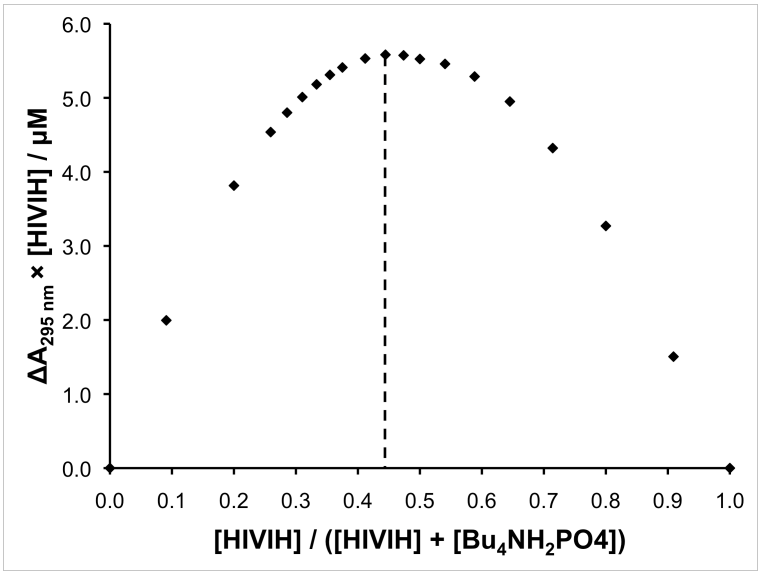


Figure S4. Job plot for the interaction of $Bu_4NH_2PO_4$ with **HIVIH.**

S1.8 NMR Titration

Two solutions were prepared in $CDCl_3$:CD_3OD (96:4) as follows:

HIVIH (4.0 mM, 1.5 ml);

solution I was then used to prepare solution II

$Bu_4NH_2PO_4$ (48 mM) and **HIVIH** (4.0 mM) in 750 µl.

Solution I (600 µl) was transferred to an NMR tube, and the ^{1}H-NMR spectrum acquired. Aliquots of solution II were added (beginning with 5 µl and gradually increasing to 60 µl until 5 or 6 equivalents of anion had been added). The NMR tube was shaken and the ^{1}H-NMR spectra were acquired after each addition.

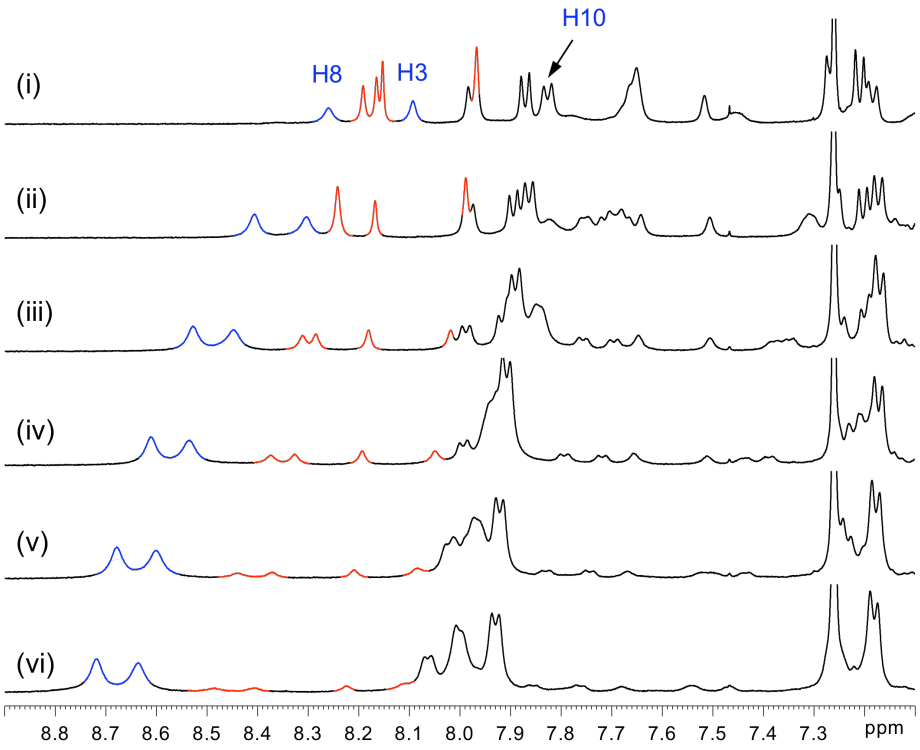


Figure S5. Partial ^{1}H-NMR spectra of **HIVIH** (4 mM) in the presence of $Bu_4NH_2PO_4$: (i) 0 mM, (ii) 4 mM, (iii) 8 mM, (iv) 12 mM, (v) 16 mM and (vi) 20 mM. Selected resonances corresponding to the isomer **HIVIH$_A$** and **HIVIH$_B$** are coloured in blue and red, respectively.

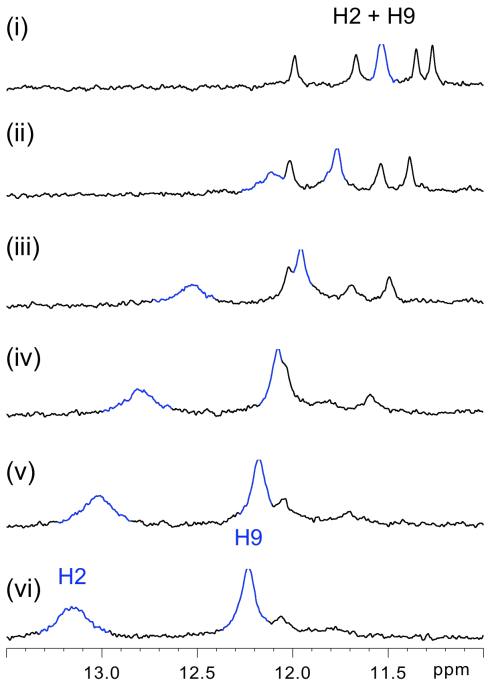


Figure S6. Partial ^{1}H-NMR spectra of **HIVIH** (4 mM) in the presence of $Bu_4NH_2PO_4$: (i) 0 mM, (ii) 4 mM, (iii) 8 mM, (iv) 12 mM, (v) 16 mM and (vi) 20 mM. Resonances corresponding to the isomer **HIVIH$_A$** are coloured in blue.

S2 Additional Experiments to Characterize HIVIVIH and HIVIVIVIH and Their Interactions with $Bu_4NH_2PO_4$

S2.1 Characterization of HIVIVIH and HIVIVIVIH

S2.1.1 UV/Vis Spectroscopy

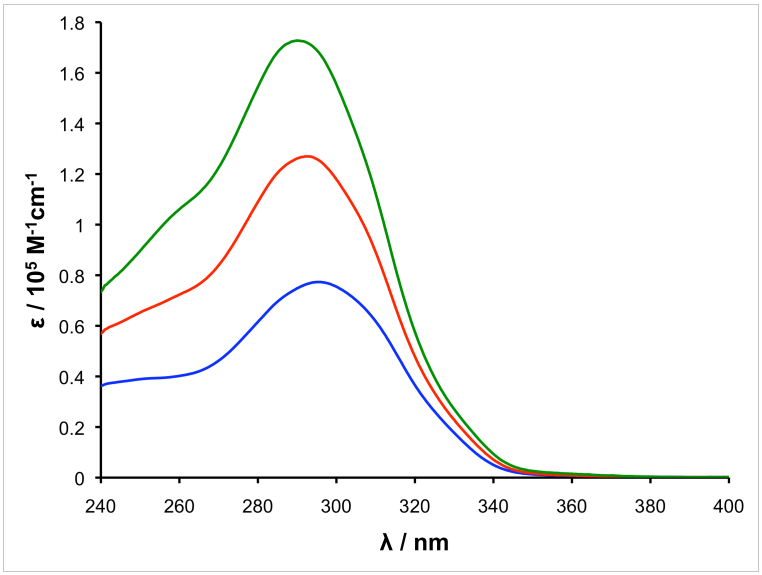


Figure S7. UV/Vis spectra: **HIVIH** (blue), **HIVIVIH** (red) and **HIVIVIVIH** (green) in $CHCl_3$:MeOH (96:4).

S2.1.2 CD Spectroscopy

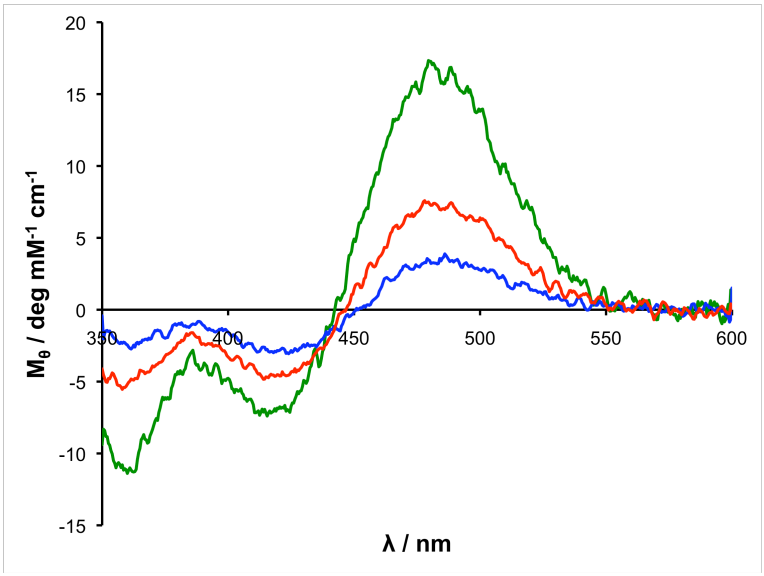


Figure S8. CD spectra of **HIVIH** (blue), **HIVIVIH** (red) and **HIVIVIVIH** (green) in $CHCl_3$:MeOH (96:4) (0.5 mM).

S2.1.3 NMR Spectroscopy

NMR spectra of **HIVIVIH** and **HIVIVIVIH** were recorded at varying temperatures in an unsuccessful attempt to simplify the spectra in order to assign resonances.

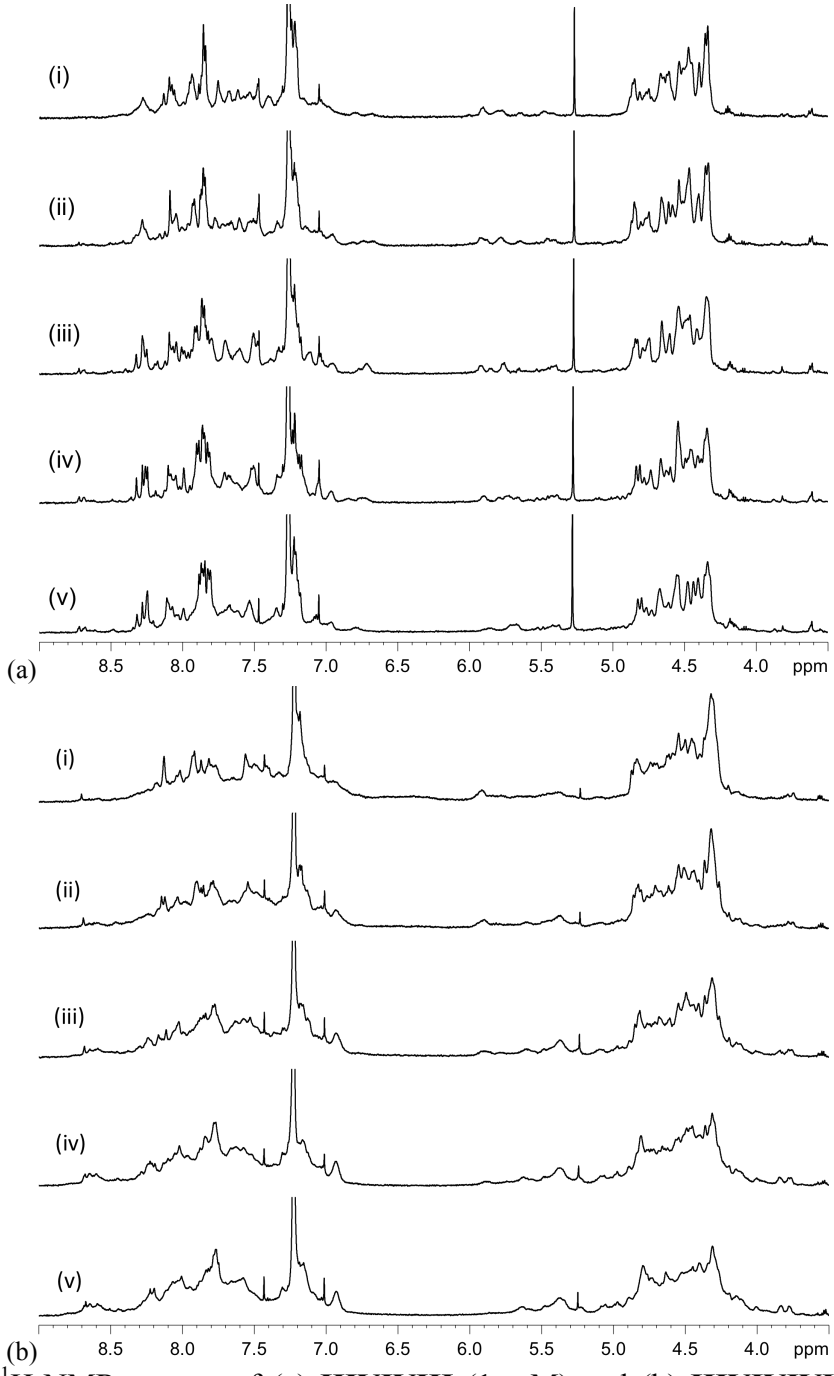


Figure S9. ^{1}H-NMR spectra of (a) **HIVIVIH** (1 mM) and (b) **HIVIVIVIH** (1 mM) in $CDCl_3$:CD_3OD (96:4) at (i) 328 K, (ii) 313 K, (iii) 298 K, (iv) 283 K and (v) 268 K.

S2.2 UV/Vis Titrations

Solutions of host (either **HIVIVIH**, 8.4 μM or **HIVIVIVIH**, 6.7 μM) in $CHCl_3$:MeOH (96:4) were prepared. From these solutions were prepared host + guest solutions (6 ml) ([$Bu_4NH_2PO_4$] = 0.75 mM for **HIVIVIH** and 0.62 mM for **HIVIVIVIH** titrations). The UV/Vis spectrum of the host solution was recorded in a 1 cm quartz cuvette from 240 nm to 340 nm. Aliquots of host + guest solution (5 μl) were added until ten equivalents of guest had been added. After each addition the cuvette was shaken and after a specified delay (3 minutes for titrations with **HIVIVIH** and 1 minute for titrations with **HIVIVIVIH**) the UV/Vis spectrum was recorded.

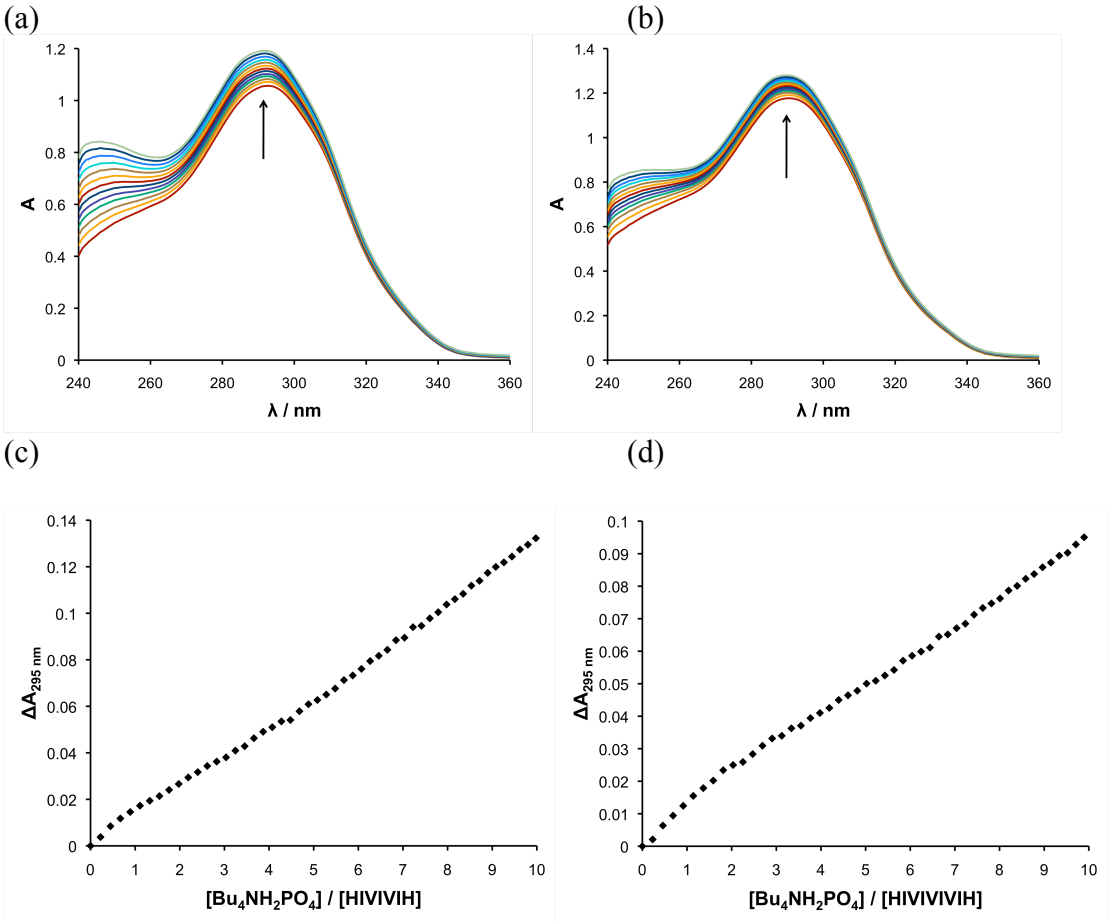


Figure S10. UV/Vis spectra showing the hyperchromic shifts in the absorbance maxima at 285-295 nm during titrations of $Bu_4NH_2PO_4$ with: (a) **HIVIVIH** and (b) **HIVIVIVIH** and binding isotherms (monitoring the change in absorbance at 295 nm for the titration of $Bu_4NH_2PO_4$ with (c) **HIVIVIH** and (d) **HIVIVIVIH**.

S2.3 UV/Vis Job Plots

UV/Vis Job plots for **HIVIVIH** and **HIVIVIVIH** were generated in the same manner as for **HIVIH** with the exception that the host and $Bu_4NH_2PO_4$ solutions were prepared with concentrations of 8.4 mM and 6.7 μM for **HIVIVIH** and **HIVIVIVIH**, respectively.

(a) (b)

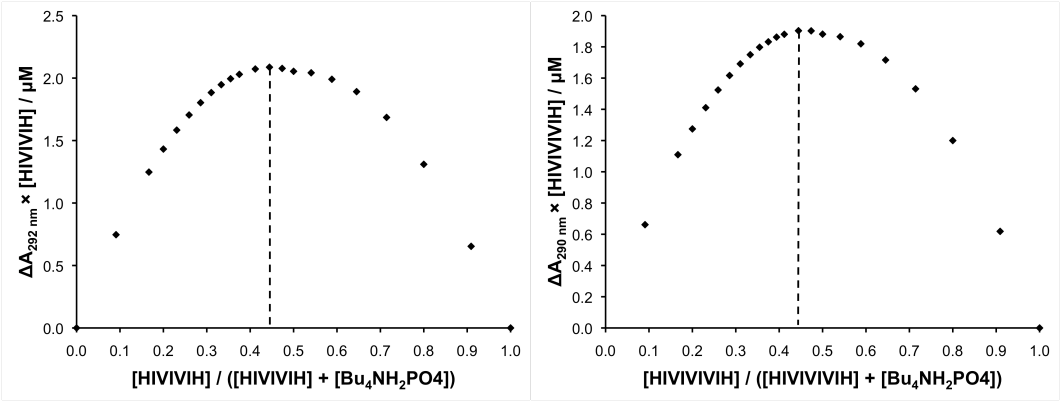


Figure S11. Job plots the interaction of $Bu_4NH_2PO_4$ with: (a) **HIVIVIH** and (b) **HIVIVIVIH**.

S2.4 NMR Titrations

S2.4.1 ***HIVIVIH*** + *$Bu_4NH_2PO_4$*

For the titration of **HIVIVIH** with $Bu_4NH_2PO_4$, two solutions were prepared as for the titration with **HIVIH**

HIVIVIH (1.0 mM, 900 μl);

$Bu_4NH_2PO_4$ (64 mM) and **HIVIVIH** (1.0 mM) in 200 μl.

Solution I (600 μl) was transferred to an NMR tube and the ^{1}H NMR spectrum acquired. Aliquots of solution II were added (5 μl initially increasing to 25 μl until 12 equivalent of $Bu_4NH_2PO_4$ had been added). After each addition, the NMR tube was shaken, and three minutes later the ^{1}H-NMR spectrum was acquired.

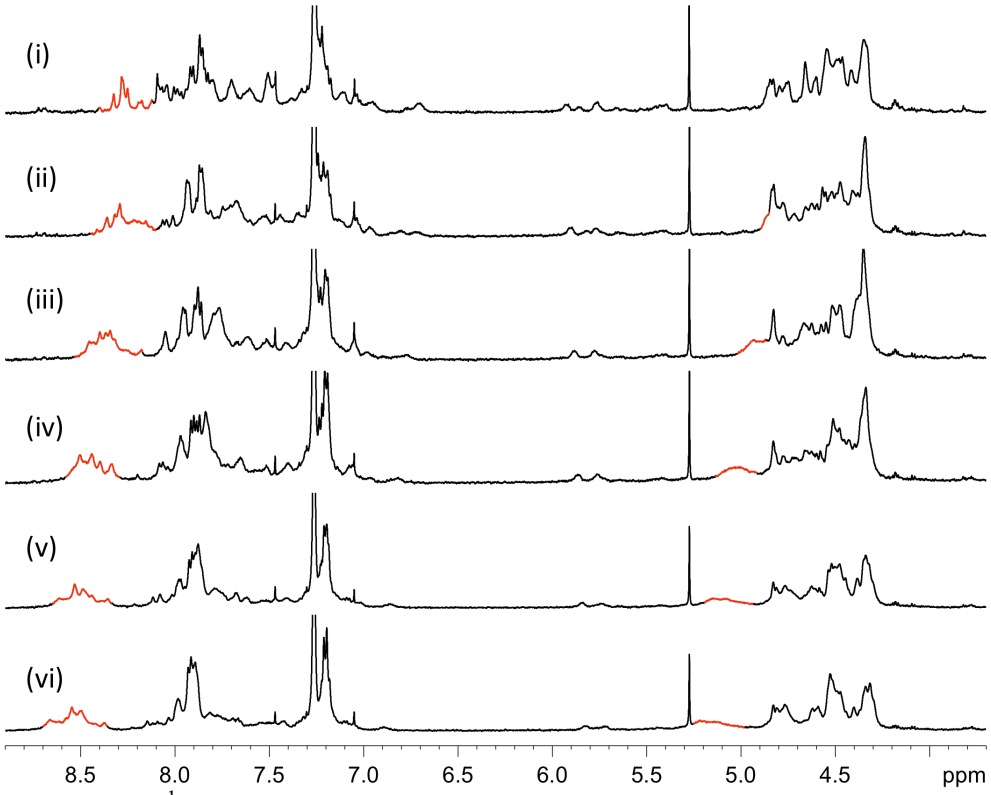


Figure S12. Partial ^{1}H-NMR spectra of **HIVIVIH** (1 mM) in the presence of $Bu_4NH_2PO_4$: (i) 0 mM, (ii) 1 mM, (iii) 2 mM, (iv) 3 mM, (v) 4 mM and (vi) 5 mM. Shifting imine proton resonances are coloured in red.

S2.4.2 ***HIVIVIVIH*** *+ $Bu_4NH_2PO_4$*

The titration of **HIVIVIVIH** with $Bu_4NH_2PO_4$ was carried out in the same manner as the titration with **HIVIVIH** with the exception that there was no three minute delay before acquisition of the NMR spectra. The two solutions prepared were as follows:

(I) **HIVIVIVIH** (1.0 mM, 900 μl)

(II) $Bu_4NH_2PO_4$ (61 mM) and **HIVIVIVIH** (1.0 mM) in 200 μl.

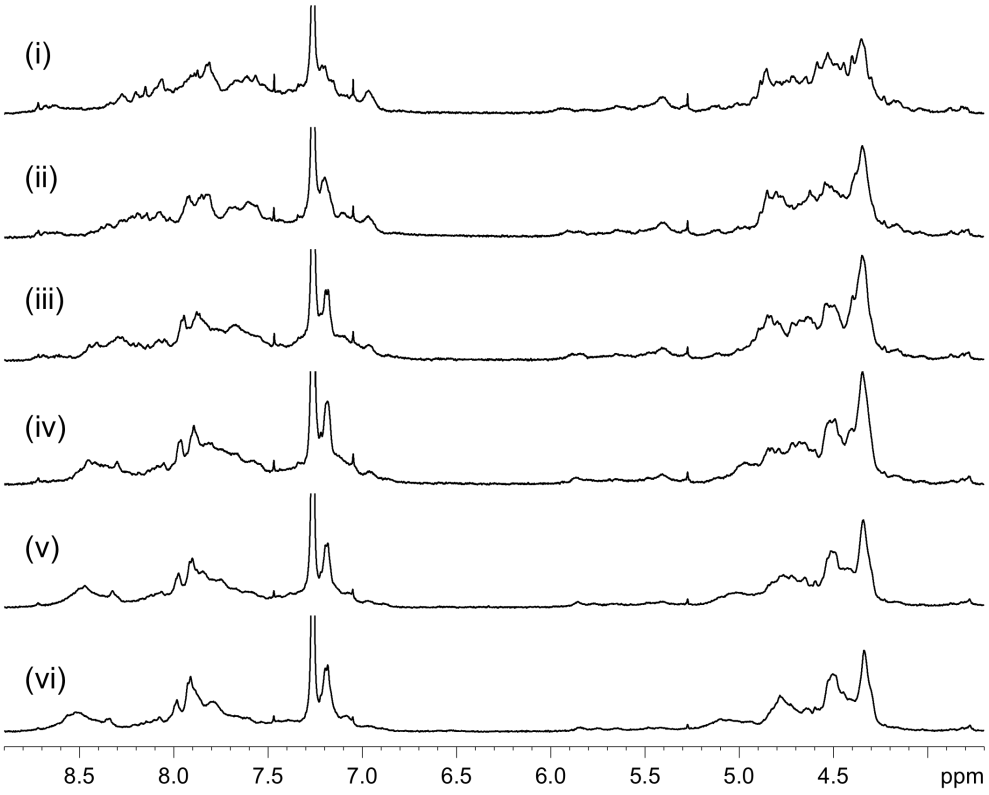


Figure S13. Partial ^{1}H-NMR spectra of **HIVIVIVIH** (1 mM) in the presence of $Bu_4NH_2PO_4$: (i) 0 mM, (ii) 1 mM, (iii) 2 mM, (iv) 3 mM, (v) 4 mM and (vi) 5 mM.

S3 NMR Spectra

S3.1 Fc-[CO-Val-NHNH$_2$]$_2$

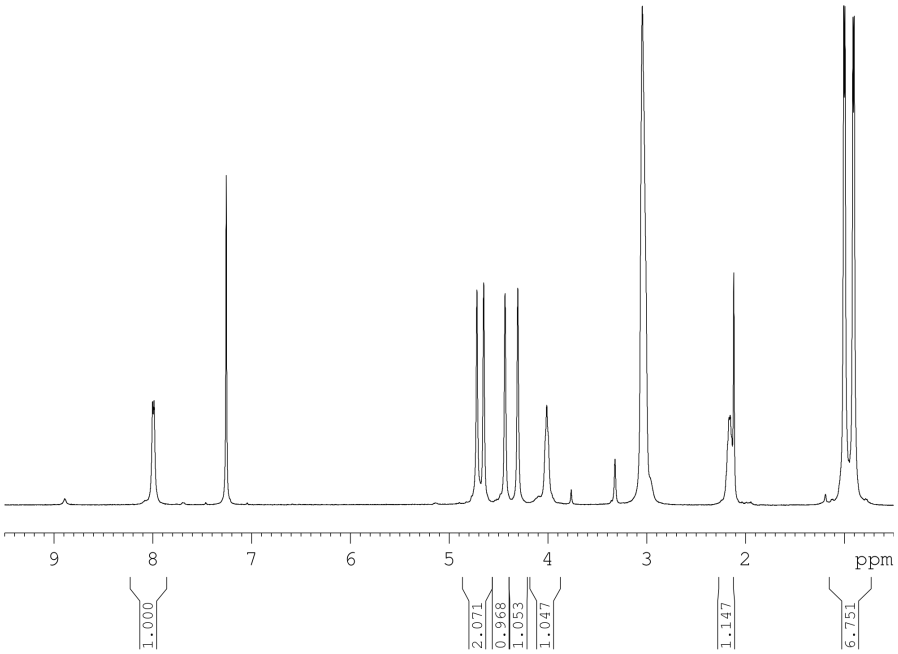


Figure S14. ^{1}H-NMR spectrum of Fc-[CO-Val-NHNH$_2$]$_2$ (**V**) in CDCl$_3$:CD$_3$OD (9:1).

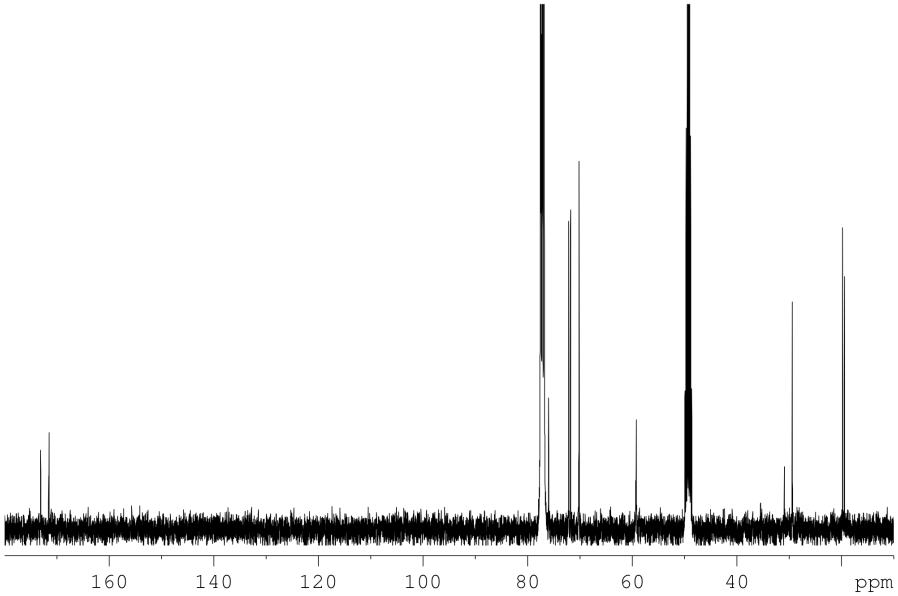


Figure S15. ^{13}C-NMR spectrum of Fc-[CO-Val-NHNH$_2$]$_2$ (**V**) in CDCl$_3$:CD$_3$OD (9:1).

S3.2 HI

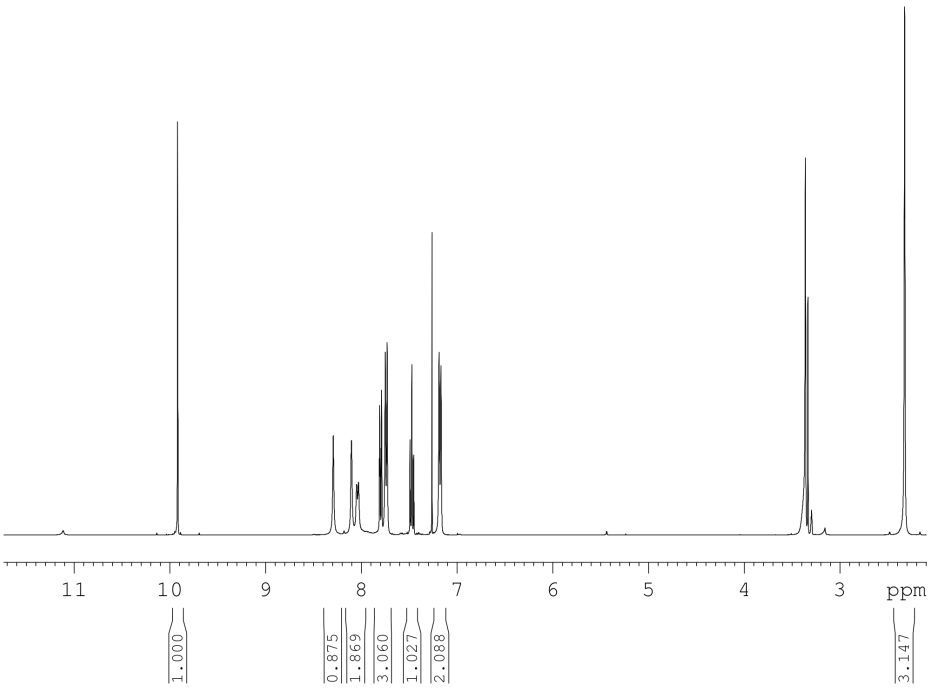


Figure S16. ^{1}H-NMR spectrum of **HI** in $CDCl_3$:CD_3OD (9:1).

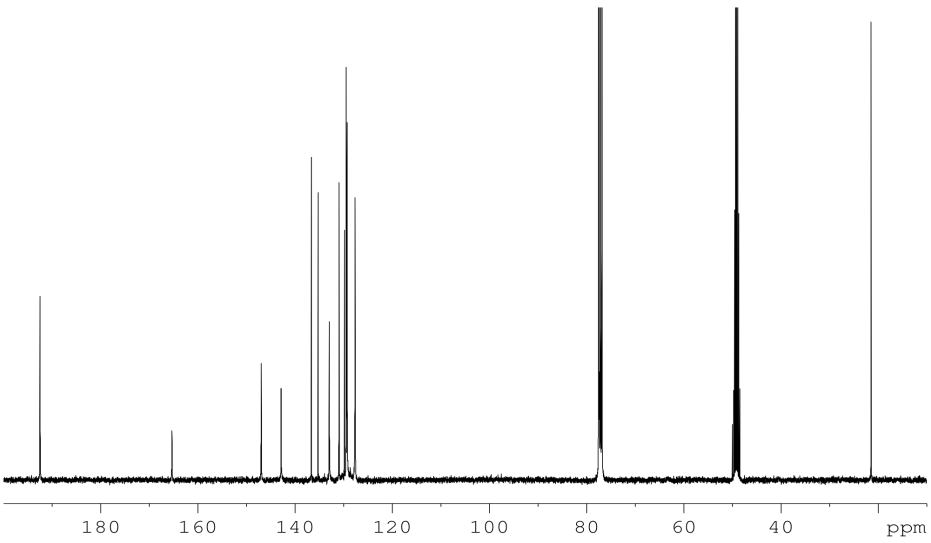


Figure S17. ^{13}C-NMR spectrum of **HI** $CDCl_3$:CD_3OD (9:1).

S 3.3 HIH

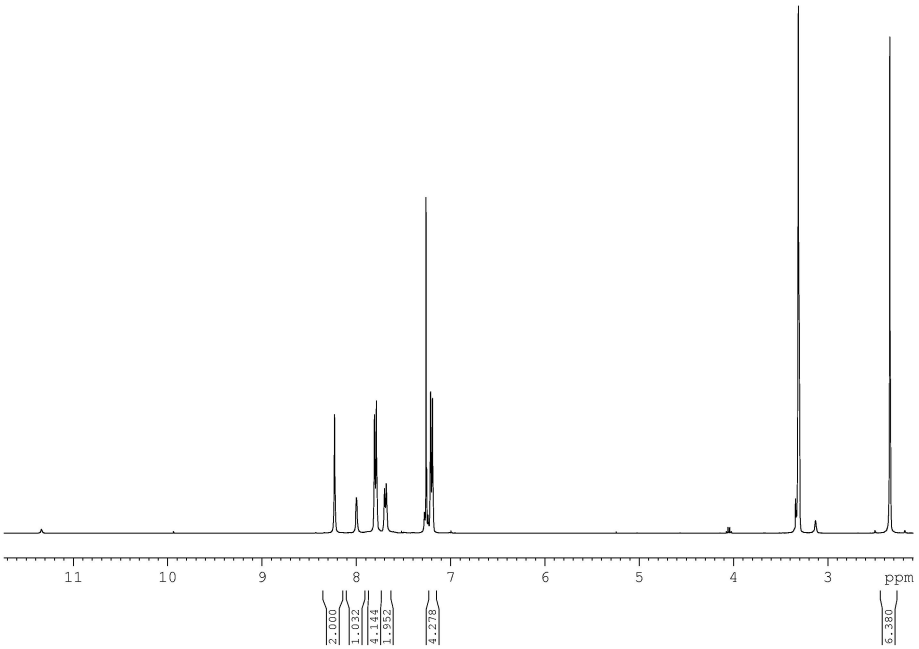


Figure S18. ^{1}H-NMR spectrum of **HIH** in $CDCl_3$:CD_3OD (9:1).

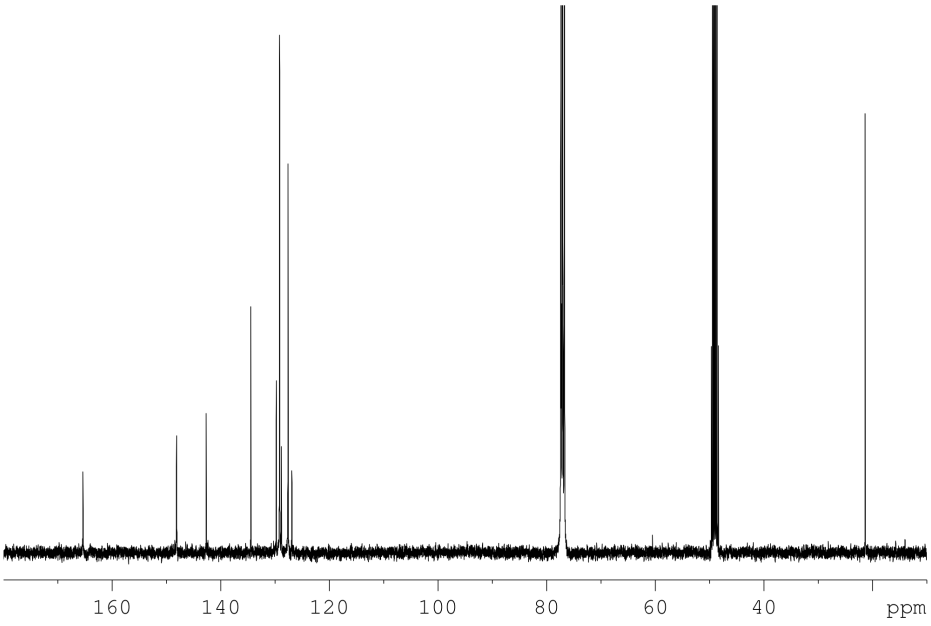


Figure S19. ^{13}C-NMR spectrum of **HIH** in $CDCl_3$:CD_3OD (9:1).

S3.4 HIVIH

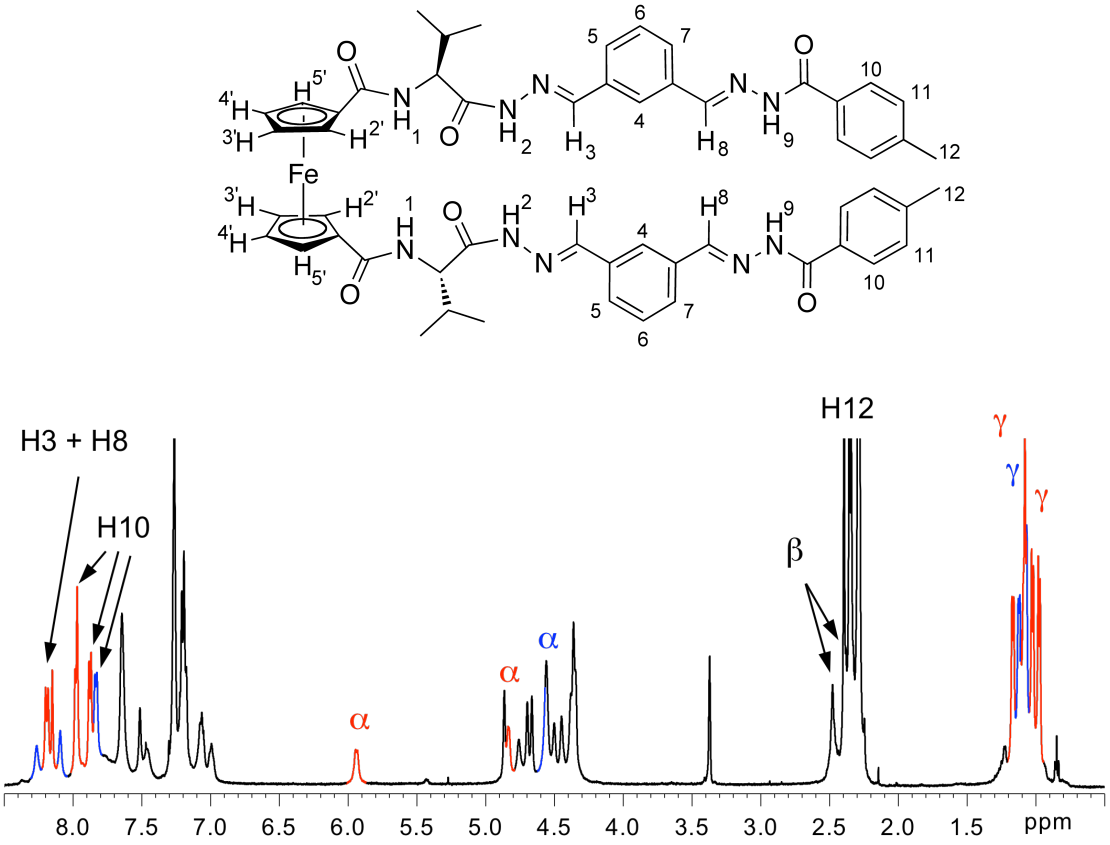


Figure S20. ^{1}H-NMR spectrum of **HIVIH** (4.0 mM) in $CDCl_3$:CD_3OD (96:4) at 298 K with selected peaks coloured and labelled from conformational isomer A (blue) and B (red).

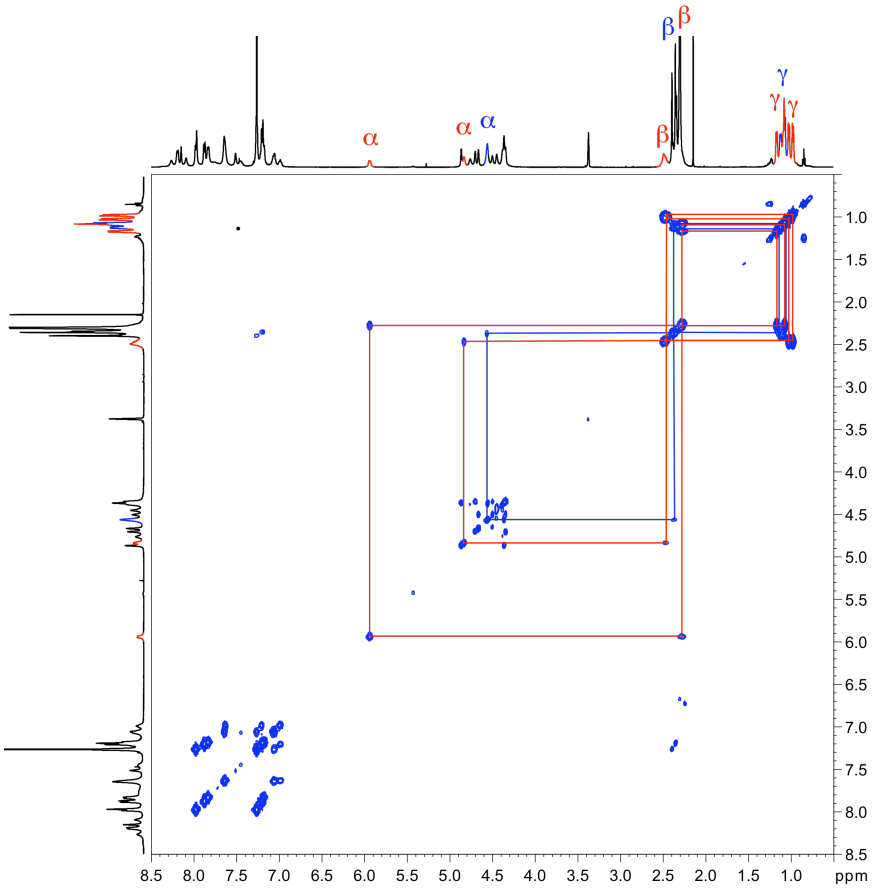


Figure S21. COSY spectrum of **HIVIH** (4.0 mM) showing the aliphatic and ferrocene regions. COSY cross-peaks between α, β and γ-Valine protons from conformational isomers A and B are highlighted.

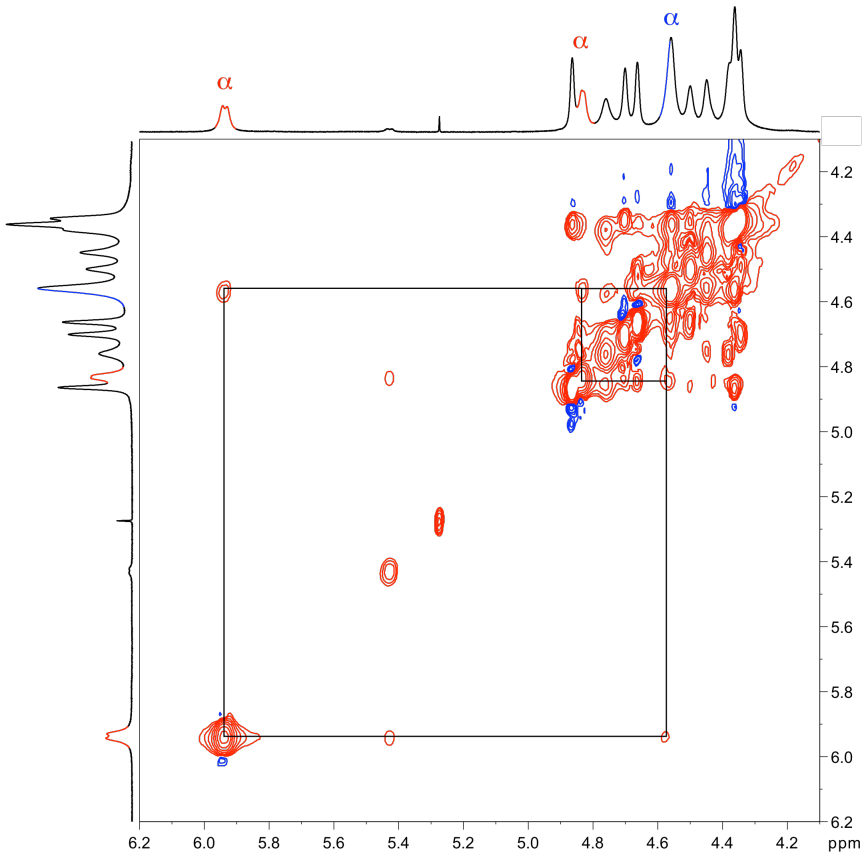


Figure S22. NOESY spectrum of **HIVIH** (4.0 mM) showing the α and ferrocene regions of the spectrum; exchange cross-peaks between the α-protons of different conformational isomers are highlighted.

S3.5 HIVIH + 6 equiv. $Bu_4NH_2PO_4$

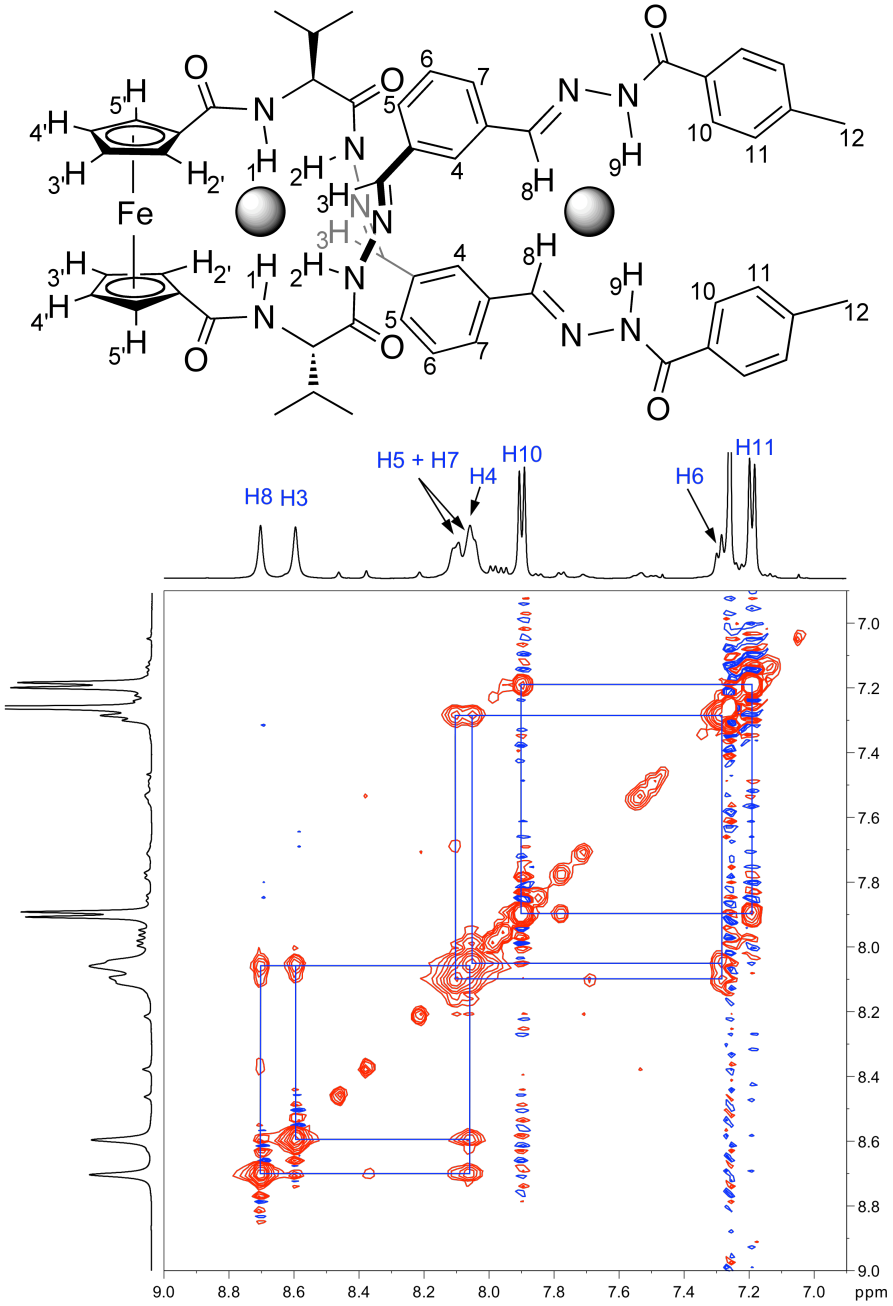


Figure S23. Partial NOESY spectrum of **HIVIH** (4 mM) in the presence of $Bu_4NH_2PO_4$ (24 mM) in $CDCl_3$:CD_3OD (96:4) at 278 K showing the aromatic and imine region. The nOe cross-peaks are marked in blue.

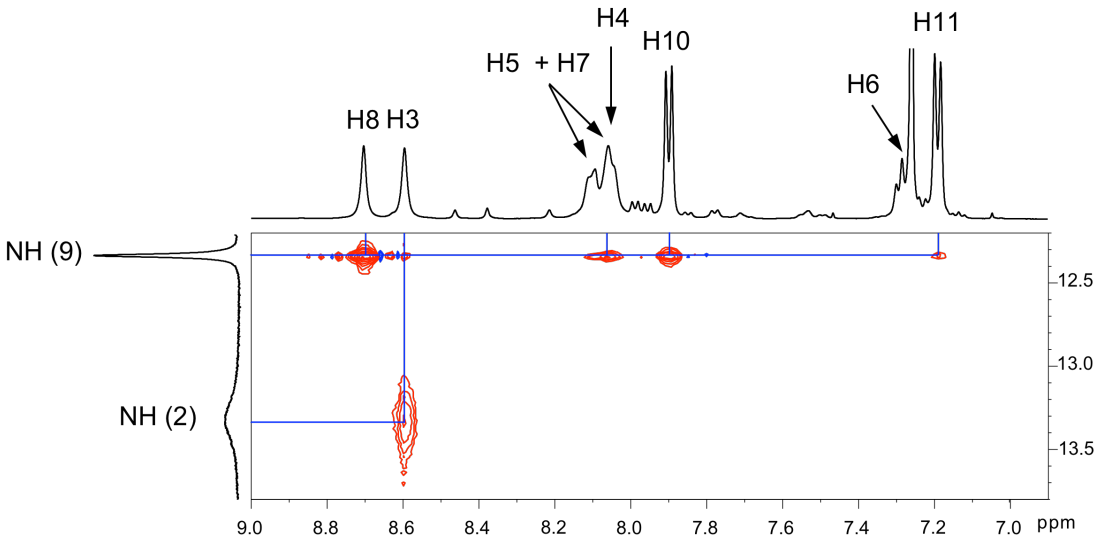


Figure S24. Partial NOESY spectrum of **HIVIH** (4 mM) in the presence of $Bu_4NH_2PO_4$ (24 mM) in $CDCl_3$:CD_3OD (96:4) at 278 K showing close contacts between imine protons (H3) and (H8) and hydrazone NH protons (H2) and (H9), respectively.

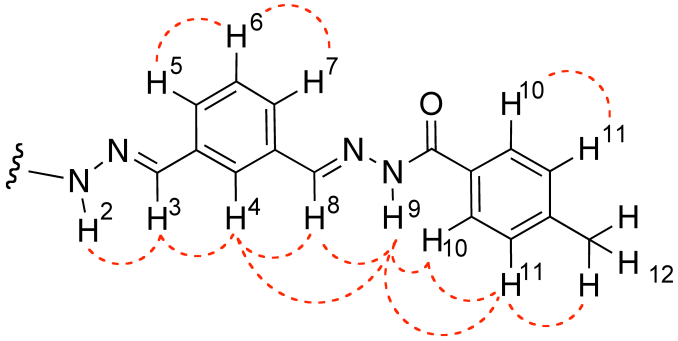


Figure S25. Partial structure of **HIVIH** showing the observed nOes with dashed red curves.

S3.6 $(VI)_2$

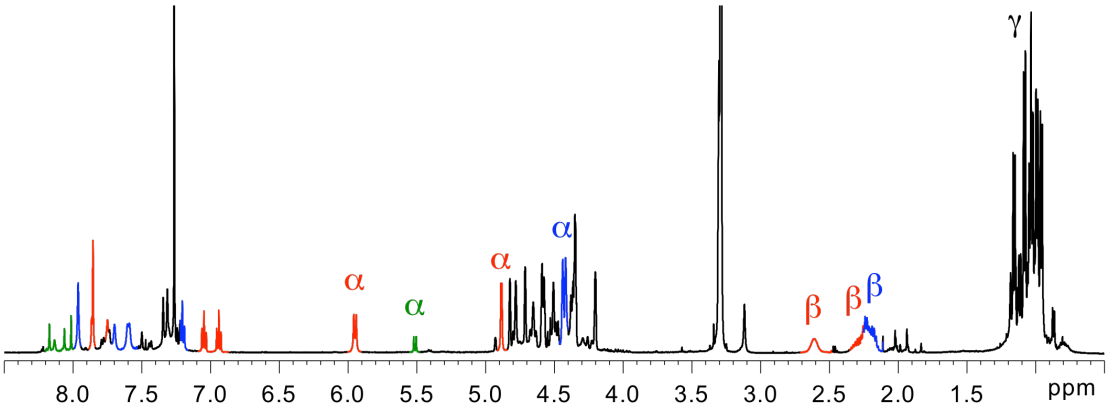


Figure S26. ^{1}H-NMR spectrum of **$(VI)_2$** in $CDCl_3$:CD_3OD (9:1) at 298 K. Macrocycle **$(VI)_2$** is present in solution as three different conformational isomers: one with D_2 symmetry (coloured in blue), one with C_2 symmetry (coloured in red) and one with C_1 symmetry (coloured in green).

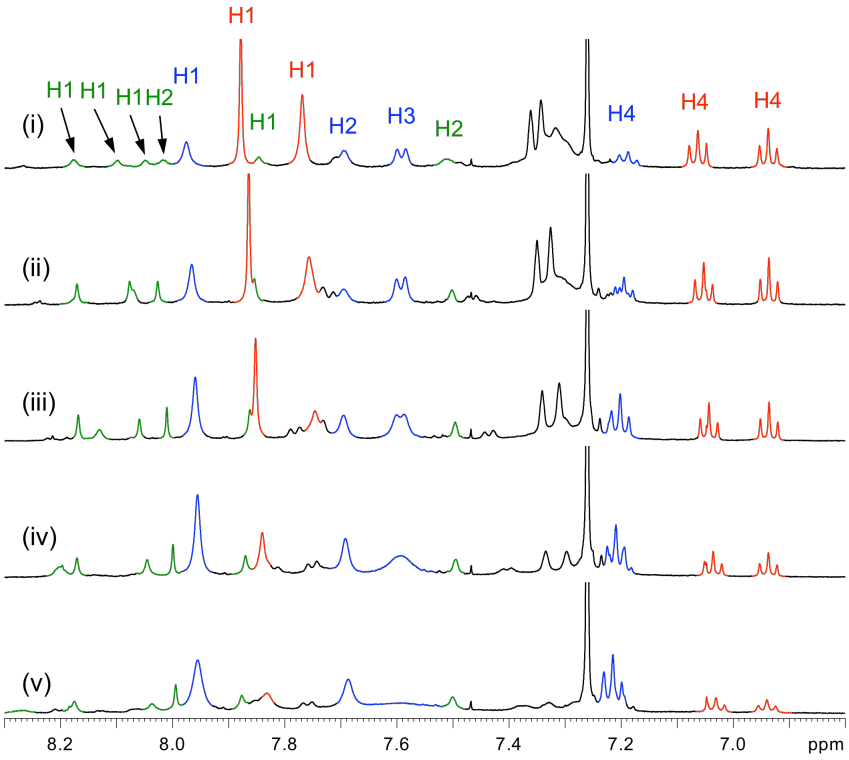


Figure S27. Partial ^{1}H-NMR spectra of **$(VI)_2$** in $CDCl_3$:CD_3OD (9:1) showing the aromatic region only at (i) 268 K, (ii) 283 K, (iii) 298 K, (iv) 313 K and (v) 328 K. Conformational isomers with D_2, C_2 and C_1 symmetry are coloured in blue, red and green, respectively.

[1] M. Oberhoff, L. Duda, J. Karl, R. Mohr, G. Erker, R. Frohlich and M. Grehl, *Organometallics*, **1996**, *15*, 4005-4011.