# Supporting Information

## Monitoring inorganic pyrophosphatase activity with a fluorescent dizinc(II) complex of a macrocycle bearing one dansylamidoethyl antenna

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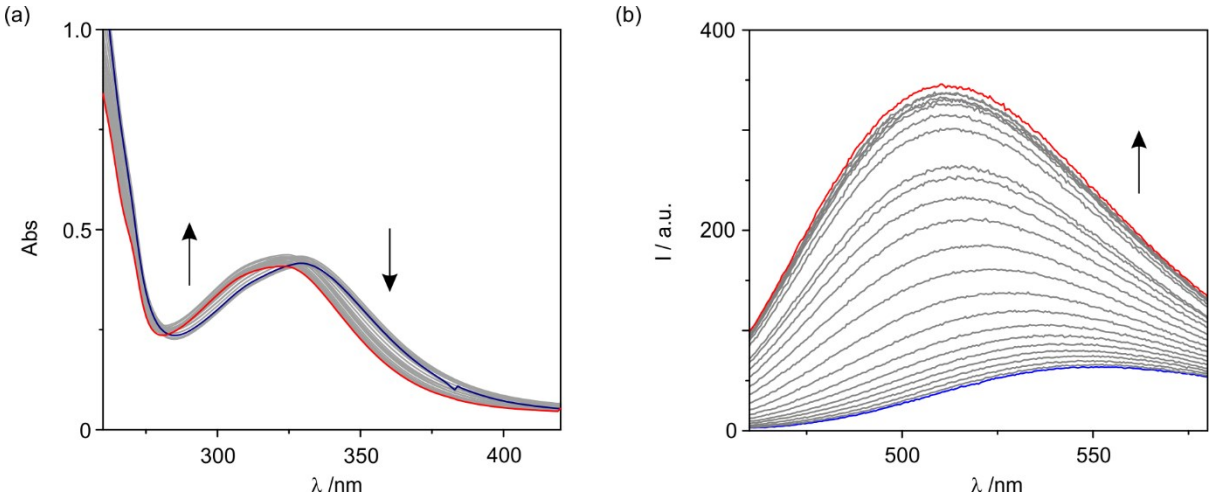


**Fig. S1** Titrations of **L** with $Zn(NO_3)_2$ aqueous solutions, followed by absorption (**a**) and fluorescence emission (**b**) spectra. Blue and red spectra correspond to 0 and 5.0 equiv. of $Zn^{2+}$, respectively); $C_L$ = 10 μM (absorption) and 75 μM (fluorescence), at pH 7.5, in 2 mM PIPPS aqueous solution ($\lambda_{exc}$ = 330 nm; exc. slit = 5 nm; em. slit 5 nm).

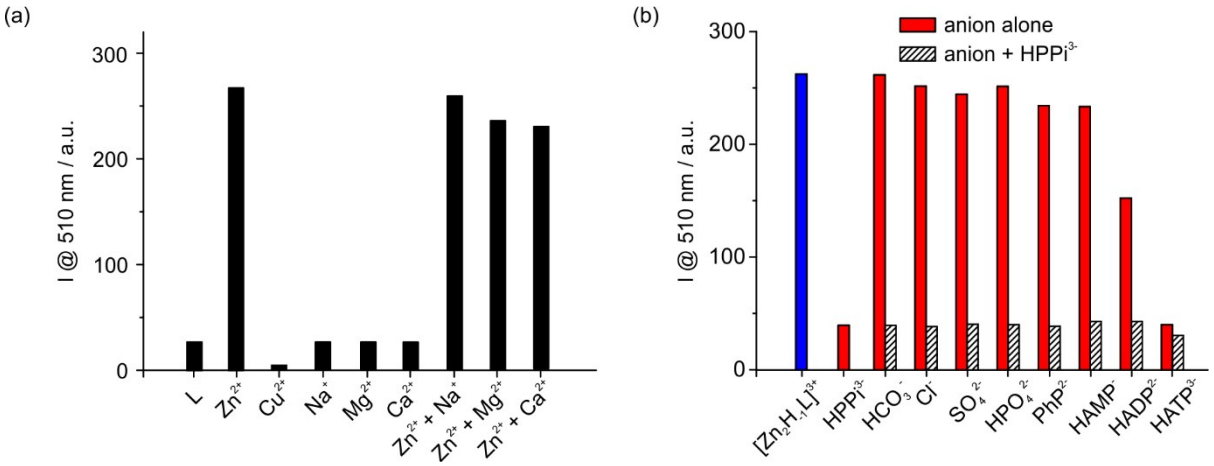


**Fig. S2** Fluorescence intensity change (**a**) of **L** alone, and in presence of 2 equiv. of metal ions, $Zn^{2+}$, $Cu^{2+}$, $Na^{+}$, $Mg^{2+}$ and $Ca^{2+}$, respectively; then to the $[Zn_2H_{-1}\mathbf{L}]^{3+}$ complex was added 1000 equiv. of $Na^{+}$, $Mg^{2+}$ and $Ca^{2+}$ cations, respectively. Fluorescence intensity change (**b**) of $[Zn_2H_{-1}\mathbf{L}]^{3+}$ (blue) upon addition of 5 equiv. of each anion (red bars), and then to these solutions were added 5 equiv. of $HPPi^{3-}$ (hatched bars). At 510 nm, $C_L$= 10 μM aqueous solution at pH 7.5 (2 mM PiPPs); $T$ = 298.2 K; $\lambda_{exc}$ = 330 nm; exc. slit = 5 nm; em. slit 5 nm.

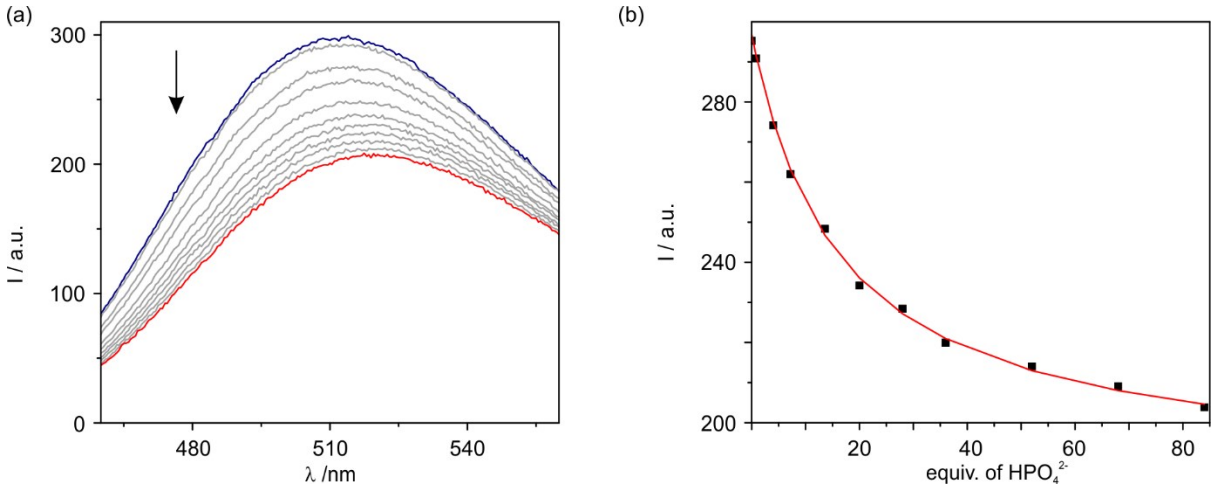


**Fig. S3** Fluorescence spectra (**a**) recorded in the course of the titration of $[Zn_2H_{-1}\mathbf{L}]^{3+}$ ($2.0\times10^{-5}$ M) with $HPO_4^{2-}$ anion; and experimental (■) and calculated values (red solid line) for the binding study of $[Zn_2H_{-1}\mathbf{L}]^{3+}$ receptor in function o $HPO_4^{2-}$ (**b**). The titration was performed in aqueous solution buffered with PIPPS (2 mM) at pH 7.5 and $T$ = 298.2 K; λ = 512 nm, log $K_{app}$ = 3.81; exc. slit = 5 nm; em. slit 5 nm.

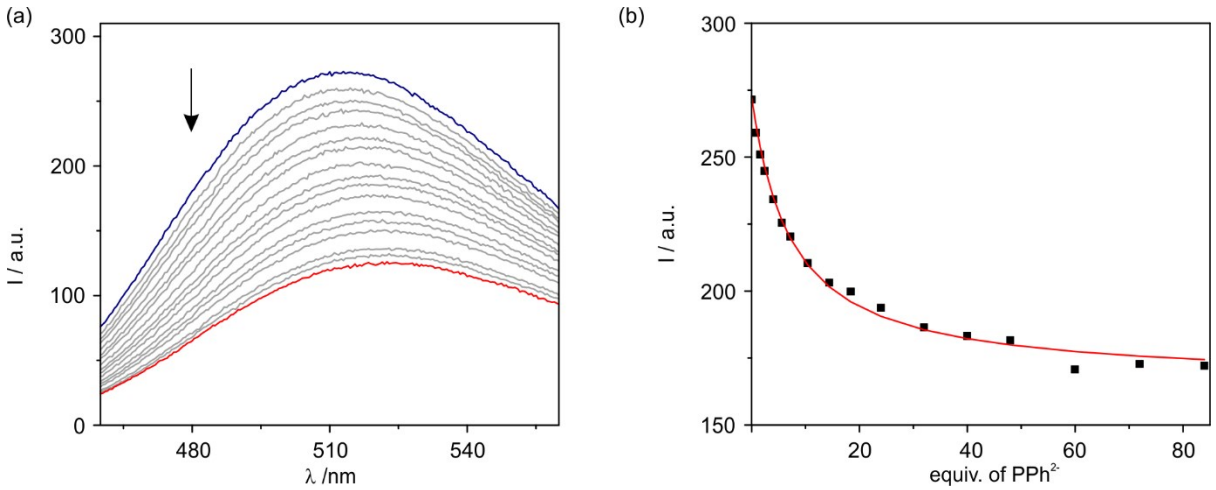


**Fig. S4** Fluorescence spectra (**a**) recorded along the titration of $[Zn_2H_{-1}\mathbf{L}]^{3+}$ ($2.0\times10^{-5}$ M) with $PPh^{2-}$ anion, and experimental (■) and calculated values (red solid line) for the binding study of $[Zn_2H_{-1}\mathbf{L}]^{3+}$ receptor in function of $PPh^{2-}$ anion (**b**). The titration was performed in aqueous solution buffered with PIPPS (2 mM) at pH 7.5 and $T$ = 298.2 K; λ = 512 nm; log $K_{app}$ = 4.20; exc. slit = 5 nm; em. slit 5 nm.

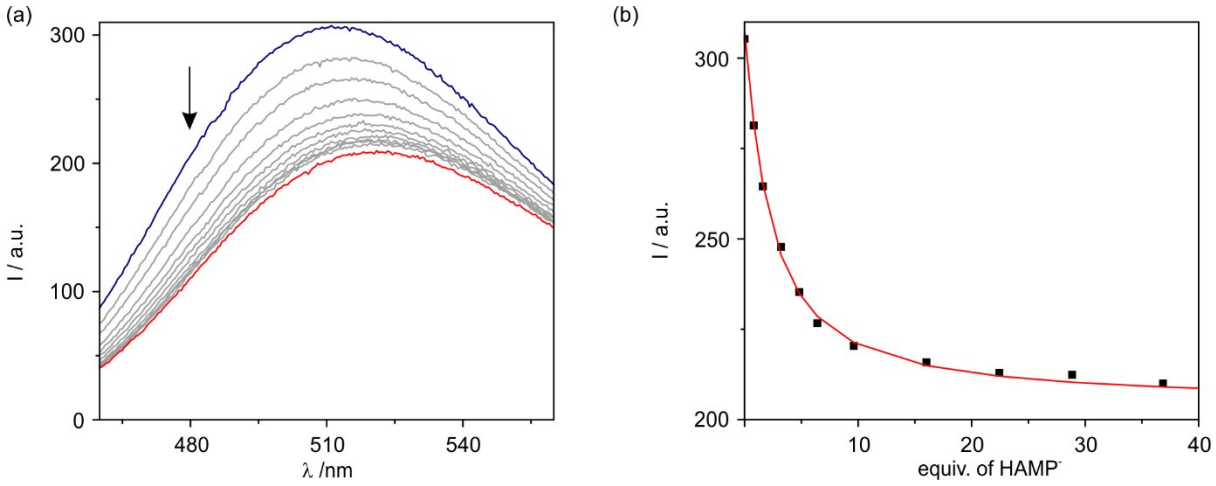


**Fig. S5** Fluorescence spectra (**a**) recorded in the course of the titration of $[Zn_2H_{-1}\mathbf{L}]^{3+}$ ($2.0\times10^{-5}$ M) with $HAMP^-$ anion, and experimental (■) and calculated values (red solid line) for the binding study of receptor $[Zn_2H_{-1}\mathbf{L}]^{3+}$ in function of AMP anion (**b**). The titration was performed in aqueous solution buffered with PIPPS (2 mM) at pH 7.5 and $T$ = 298.2 K, λ = 512 nm, log $K_{app}$ = 4.78, exc. slit = 5 nm; em. slit 5 nm.

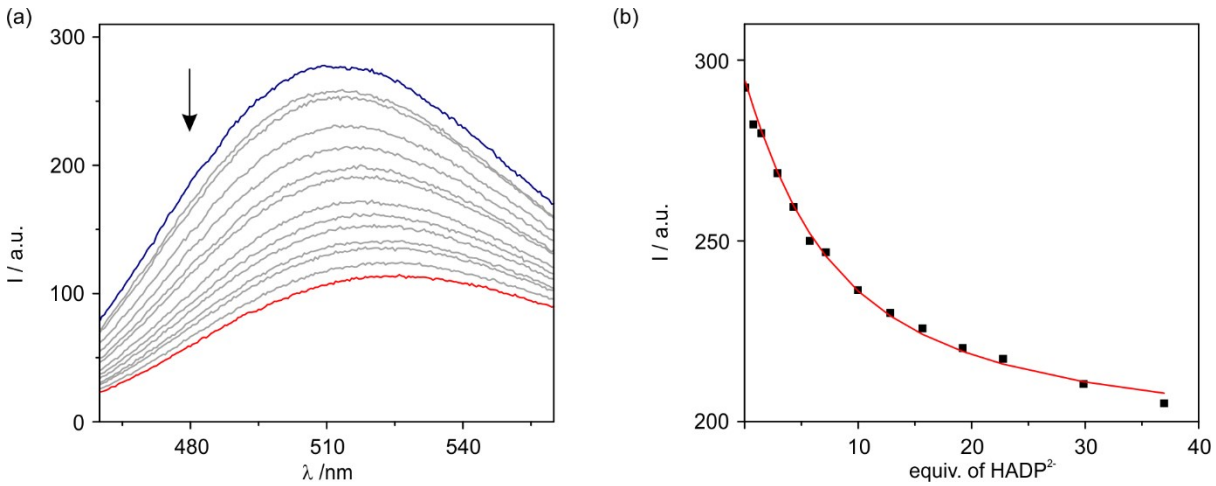


**Fig. S6** Fluorescence spectra (**a**) recorded in the course of the titration of $[Zn_2H_{-1}\mathbf{L}]^{3+}$ ($2.0\times10^{-5}$ M) with $HADP^{2-}$ anion; and experimental (■) and calculated values (red solid line) for the binding study of receptor $[Zn_2H_{-1}\mathbf{L}]^{3+}$ in function of. $HADP^{2-}$ (**b**) anion. The titration was performed in aqueous solution buffered with PIPPS (2 mM) at pH 7.5 and $T$ = 298.2 K, λ = 512 nm, log $K_{app}$ = 4.95, exc. slit = 5 nm; em. slit 5 nm.

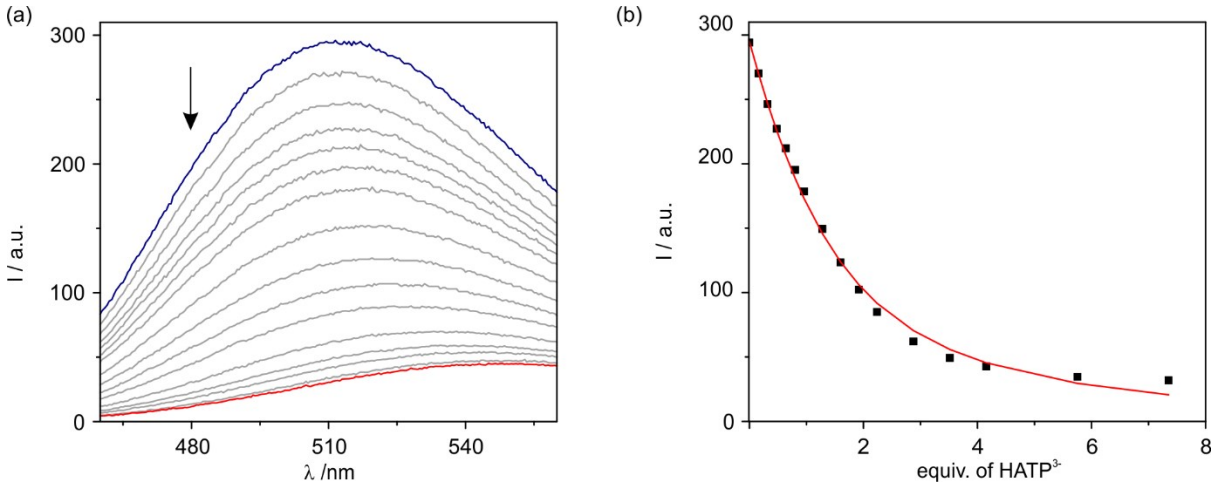


**Fig. S7** Fluorescence spectra (**a**) recorded in the course of the titration of $[Zn_2H_{-1}\mathbf{L}]^{3+}$ ($2.0\times10^{-5}$ M) with $HATP^{3-}$ anion; experimental (■) and calculated values (red solid line) for the binding study of receptor $[Zn_2H_{-1}\mathbf{L}]^{3+}$ in function of $HATP^{3-}$ anion (**b**). The titration was performed in aqueous solution buffered with PIPPS (2 mM) at pH 7.5 and $T$ = 298.2 K; λ = 512 nm, log $K_{app}$ = 5.24, exc. slit = 5 nm; emission slit 5 nm.

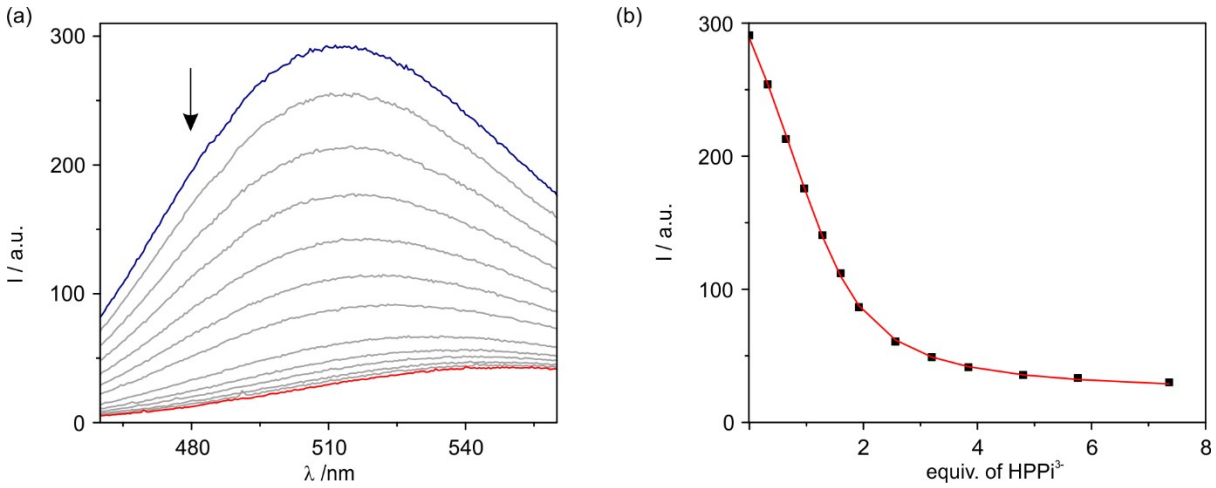


**Fig. S8** Fluorescence spectra (**a**) recorded in the course of the titration of $[Zn_2H_{-1}\mathbf{L}]^{3+}$ ($2.0\times10^{-5}$ M) with $HPPi^{3-}$ anion, and experimental (■) and calculated values (red solid line) for the binding study of receptor $[Zn_2H_{-1}\mathbf{L}]^{3+}$ in function of $HPPi^{3-}$ anion (**b**). The titration was performed in aqueous solution buffered with PIPPS (2 mM) at pH 7.5 and $T$ = 298.2 K; λ = 512 nm, log $K_{app}$ = 5.57, exc. slit = 5 nm; emission slit 5 nm.

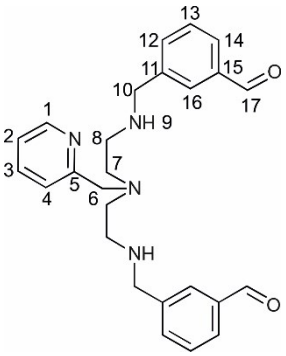


**Fig. S9** Atom labelling of the dialdehyde intermediate **3** for NMR assignment.

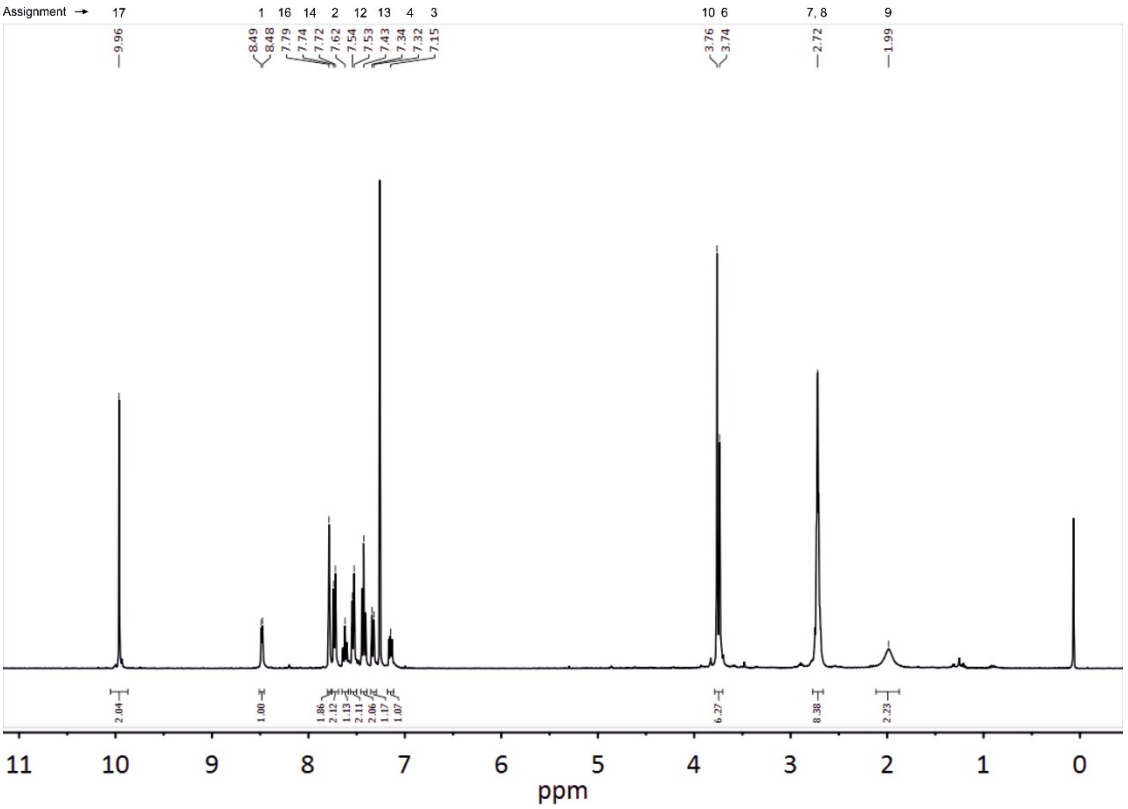


**Fig. S10** $^{1}$H NMR spectrum of the dialdehyde intermediate **3** in $CDCl_3$.

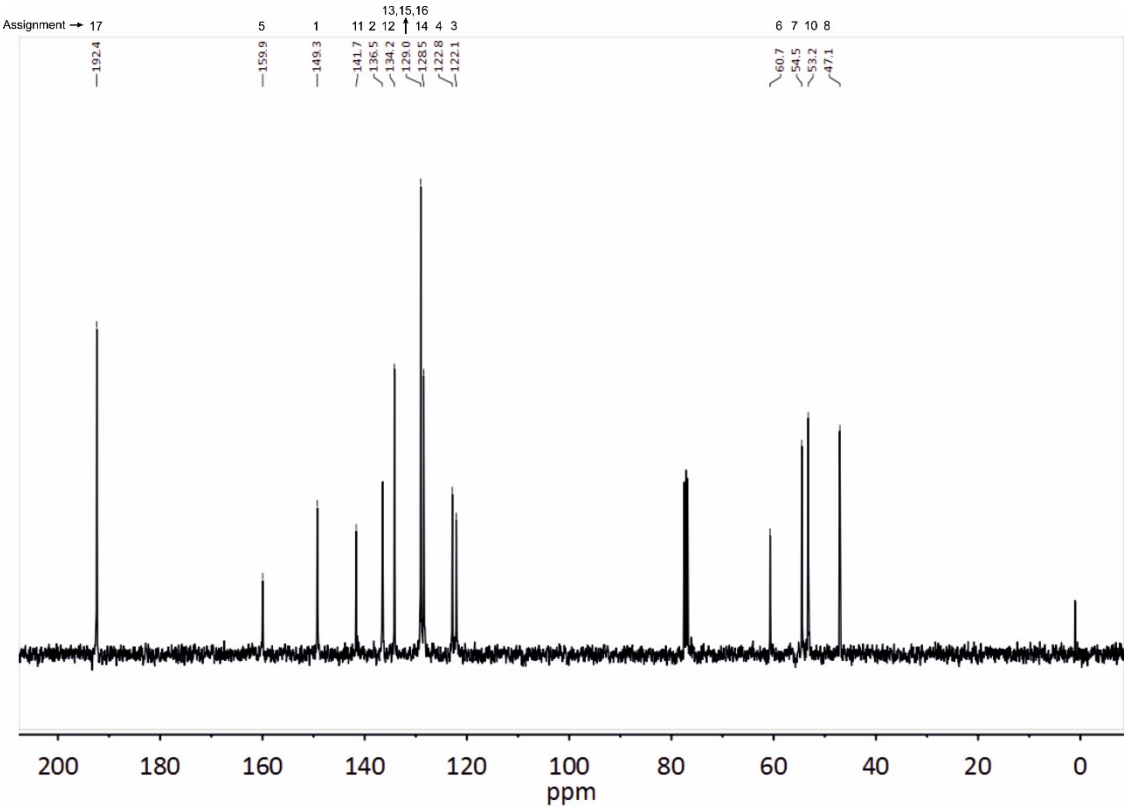


**Fig. S11** $^{13}C$ NMR spectrum of the dialdehyde intermediate **3** in $CDCl_3$.

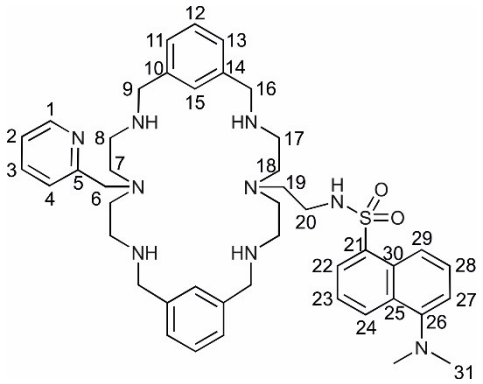


**Fig. S12** Atom labelling of **L**.6TFA for NMR assignment.

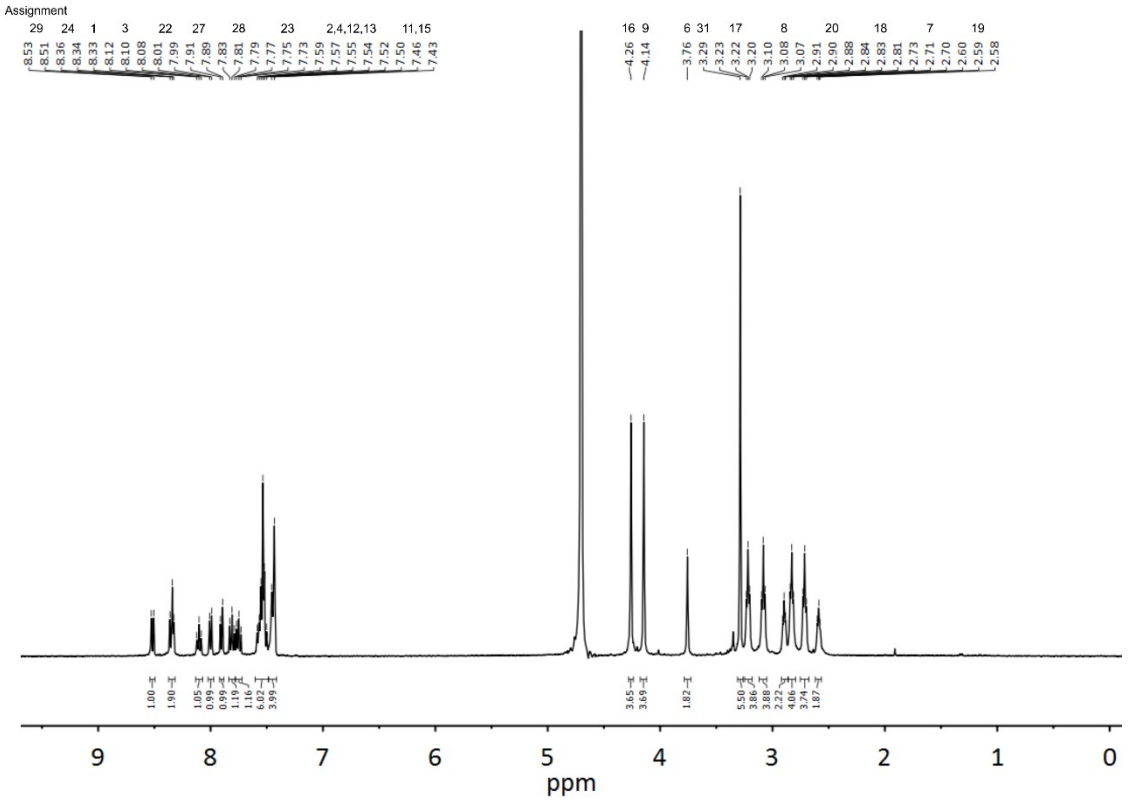


**Fig. S13** $^{1}$H NMR spectrum of **L**.6TFA in $D_2O$.

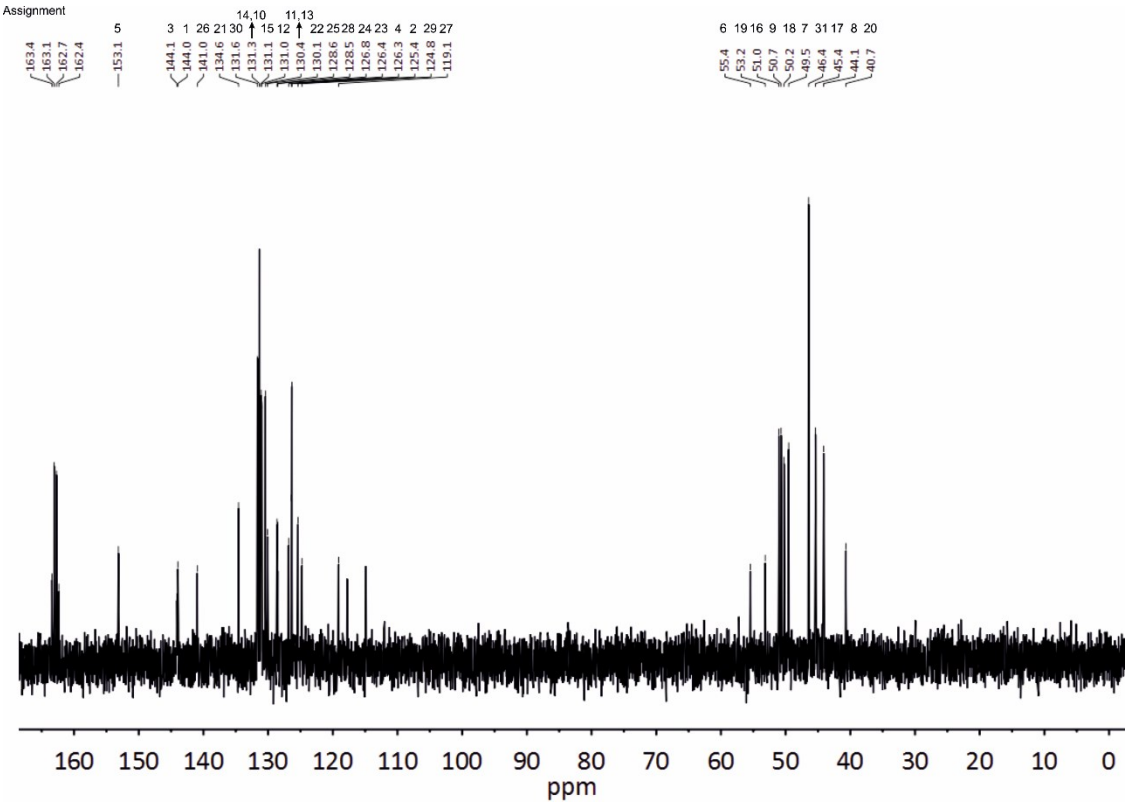


**Fig. S14** $^{13}$C NMR spectrum of L.6TFA in $D_2O$.

| *PCM/15xx Instrument Setup* | |
|---|---|
| Type | PCM/15xx |
| Instrument Status | On |
| Pump Mode | Gradient |
| Flow A | 1.00 |
| Flow B | 0.00 |
| Flow C | 0.00 |
| High Limit | 2500.0 |
| Low Limit | 0.0 |
| Total Flow | 1.00 |
| Use Events | Off |
| Solvent A | 0.1% TFA |
| Solvent B | 90% MeCN:10%H2O:0.1%TFA |
| Solvent C | |

**PCM/15xx Gradient Table**

| | Time | Flow | %A | %B | %C | Curve |
|---|---|---|---|---|---|---|
| 1 | | 1.00 | 100.0 | 0.0 | 0.0 | |
| 2 | 2.00 | 1.00 | 100.0 | 0.0 | 0.0 | 6 |
| 3 | 22.00 | 1.00 | 0.0 | 100.0 | 0.0 | 6 |
| 4 | 24.00 | 1.00 | 100.0 | 0.0 | 0.0 | 6 |
| 5 | 30.00 | 1.00 | 100.0 | 0.0 | 0.0 | 6 |

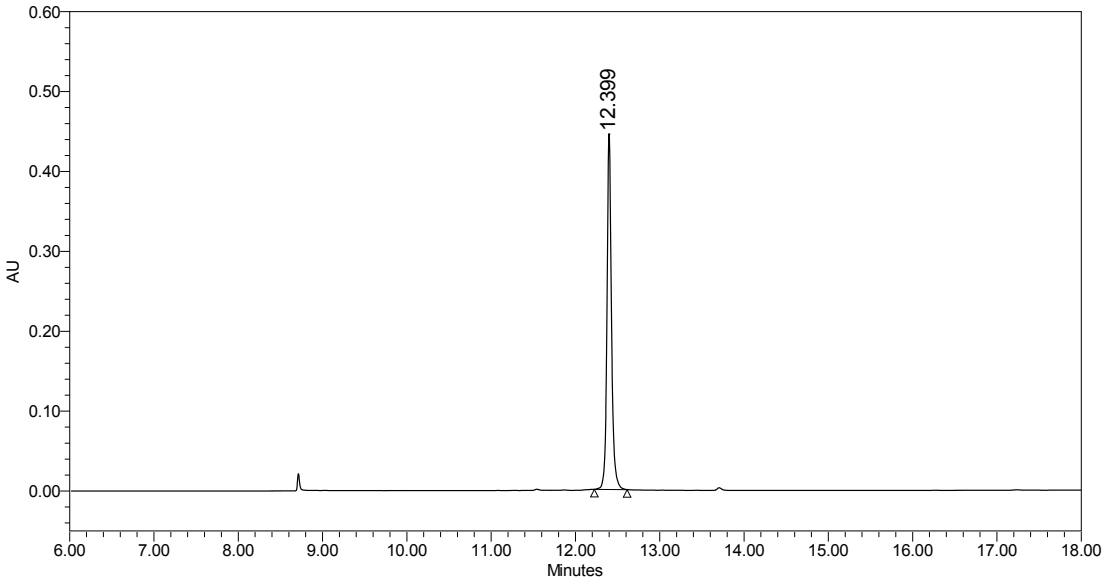


**Fig. S15** HPLC profile of **L**.6TFA.

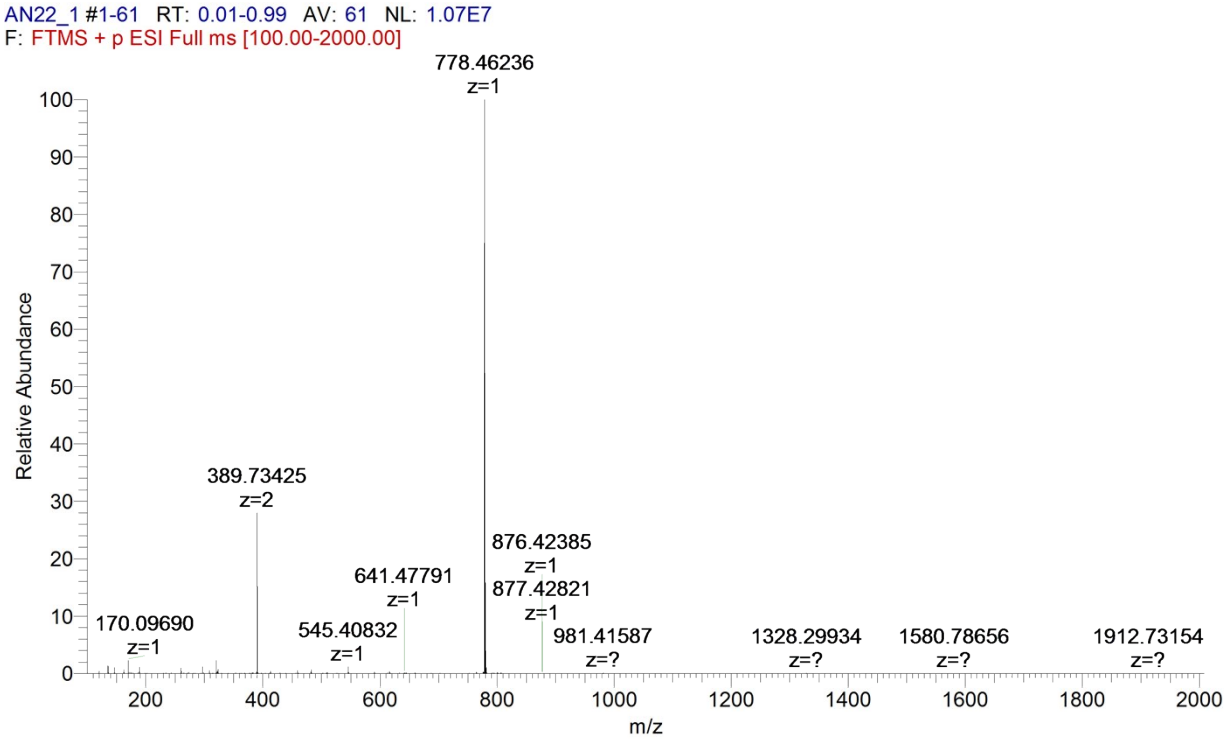


**Fig. S16** HRMS mass spectrum of **L**.6TFA in $H_2O$.