Supplementary Information

A tool for the selective sequestration of ATP and PP_i to aid in-solution phosphopeptide detection assays

Supplementary Information

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Figure S 1 UV-Vis spectra of 20 μM PV titrated with receptor 1 (0-200 μM) in 50 mM HEPES buffer, 5% DMSO pH 7.2

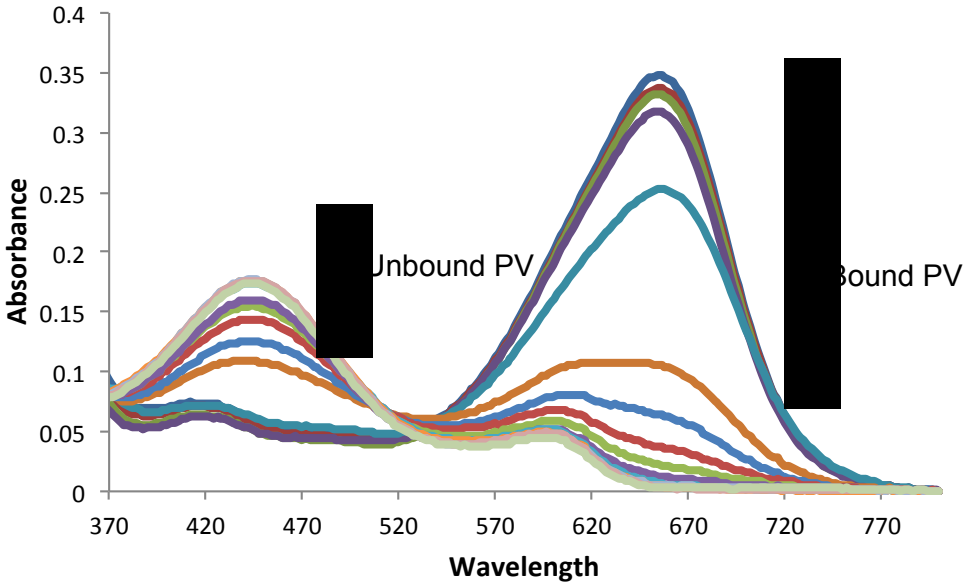


Figure S 2 UV –Vis spectra of [receptor 1:PV] complex (20 µM each) with various concentrations of analytes in 50 mM HEPES buffer, 5% DMSO pH 7.2

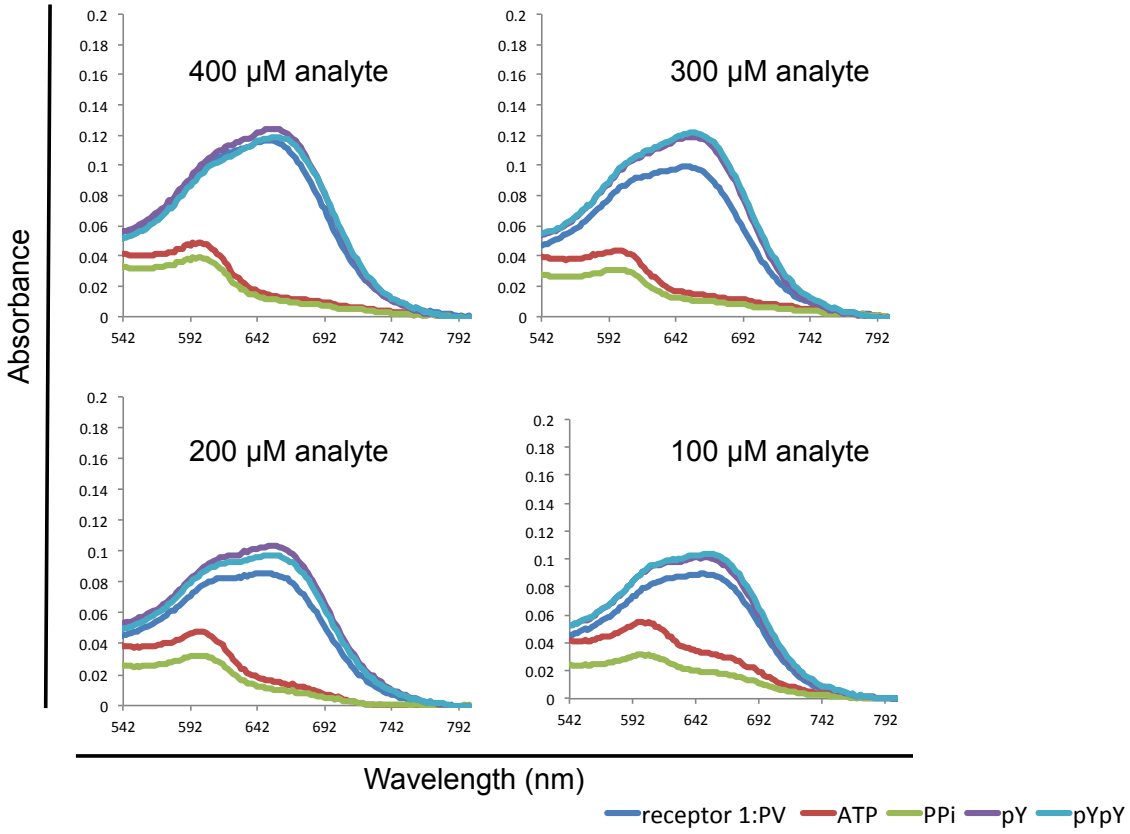
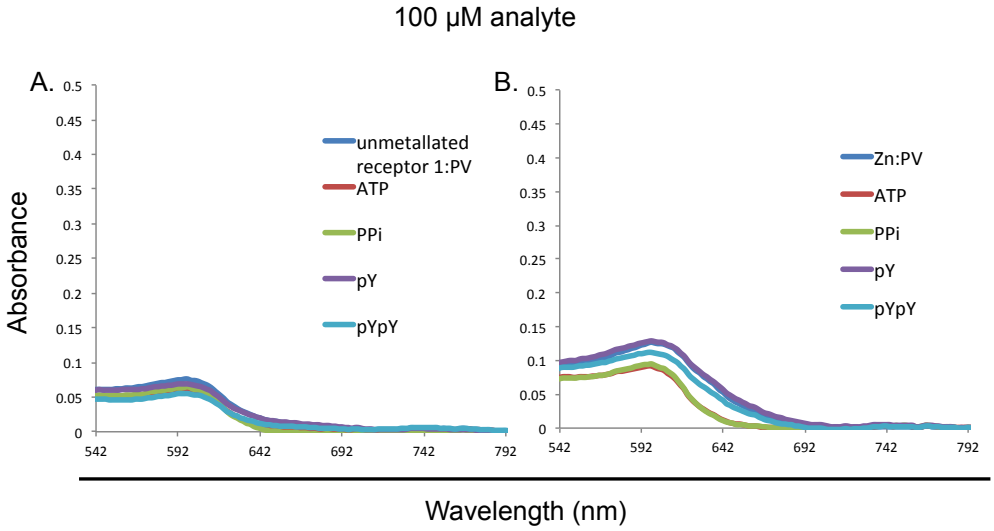
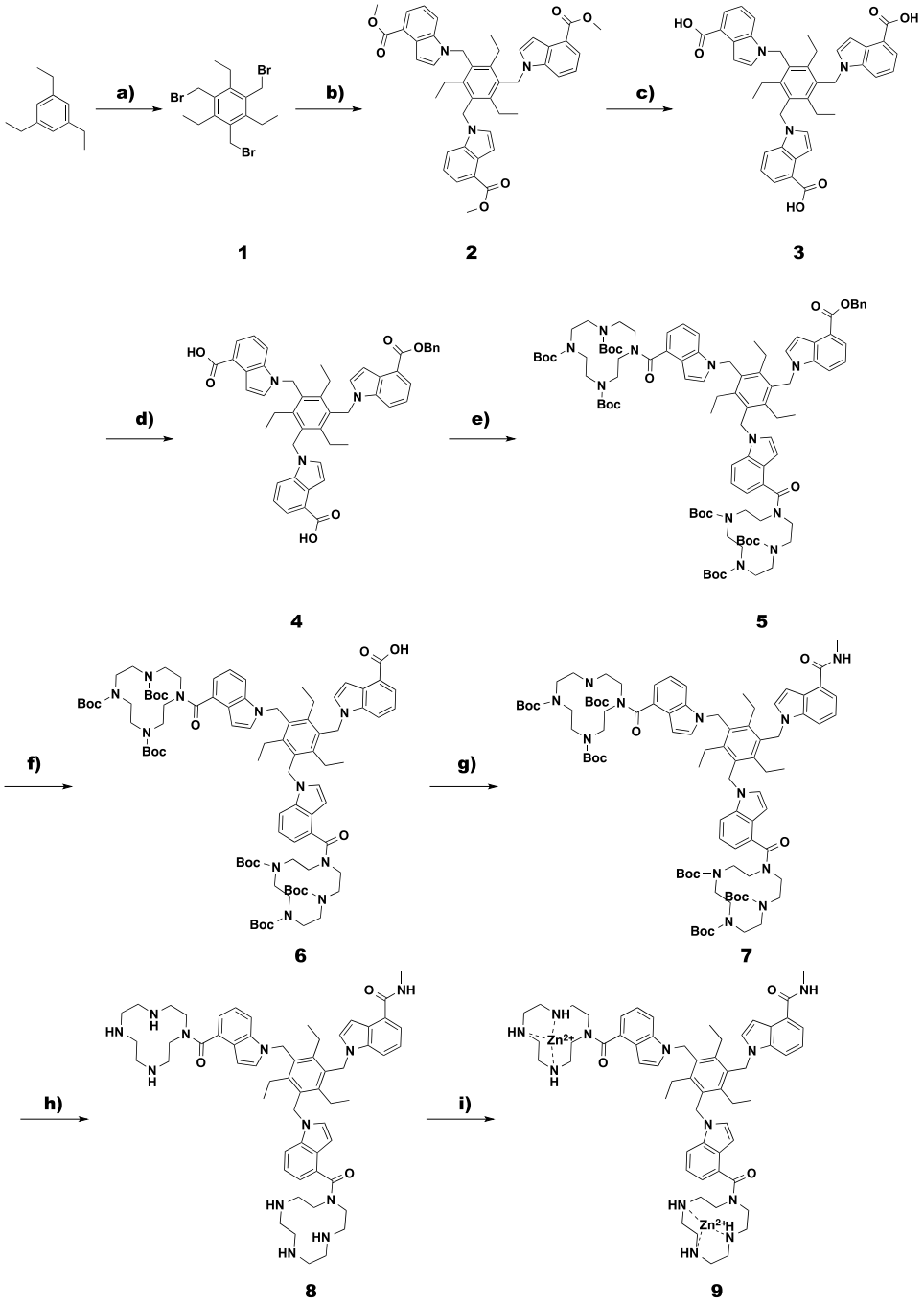


Figure S 3 Control Experiments UV –Vis spectra of A. [unmetallated receptor 1:PV] complex (20 μM each) with 100 μM of analytes B. [$Zn(OTf)_2$:PV] complex (40μM $Zn(OTf)_2$:20 μM PV) with 100 μM of analytes in 50 mM HEPES buffer, 5% DMSO pH 7.2



Scheme S 1 Synthetic scheme of receptor 1 (9)



Supplementary Information

Supplementary Note 1 General Methods and Synthesis and Characterization

General methods: All reagents and solvents were purchased from Sigma–Aldrich. Silica gel chromatography was performed with Silica Gel 60 (particle size 40–63 μm) obtained from EMD. Thin layer chromatography (TLC) plates were obtained from EMD.

Synthesis and characterization: NMR spectra were recorded on a Bruker Advance III spectrometer at 23 °C, operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR spectroscopy in either $CDCl_3$ or CD_3OD. Chemical shifts (δ) are reported in parts per million (ppm) referenced to residual isotopic solvent. Coupling constants (J) are reported in Hertz (Hz). Low Resolution Mass Spectrometry (LRMS) was performed on a Waters Micromass ZQ model MM1. Purifications by prep-HPLC were performed using Atlantis Prep T3 10 μm C18 (2) 250 x 19 mm column run at 20 mL/min (preparative) using gradient mixtures of water with 0.1% TFA and 10:1 acetonitrile/water with 0.1% TFA. The crude mixture was injected as a solution 4:1 0.1% TFA in water / acetonitrile.

Synthesis and characterization of compounds 1 to 9 (Scheme S1):

a) 1,3,5-Tris(bromomethyl)-2,4,6-triethylbenzene (**1**): Triethylbenzene (8.01 g, 9.68 mL, 49.3 mmol), paraformaldehyde (15.5 g, 517.8 mmol) were dissolved in hydrobromic acid solution 33 wt. % in acetic acid (98 mL). Zinc bromide (17.7 g, 78.9 mmol) was added to the solution slowly at room temperature. The solution was heated to 90 ^{o}C for 10 hours. The reaction was then cooled to room temperature and the reaction mixture was filtered using vacuum filtration. The resulting white precipitate was washed several times with water and left to dry on the vacuum for 10 hours (20.79 g, 95%). δ_H (400 MHz, $CDCl_3$) 1.34 (t, J = 8.3 Hz, 9H), 2.94 (q, J = 7.6 Hz, 15.2 Hz, 6H), 4.58 (s, 6H); δ_C (100 MHz, $CDCl_3$) 15.5, 22.6, 28.4, 132.5, 144.9; LRMS (ESI+) m/z calc'd for $C_{15}H_{21}Br_3$[M + H]$^{+}$438.92, found 438.87

b) Trimethyl,1,1',1''-((2,4,6-triethylbenzene-1,3,5-triyl)tris(methylene))tris(1H-indole-4-carboxylate) (**2**):1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene (**1**) (10.0 g, 22.6 mmol) and methyl indole-4-carboxylate (12.5 g, 68.0 mmol) were dissolved in anhydrous THF (226 mL). The reaction was cooled to 0 ^{o}C and sodium hydride 60% w/w (4.5 g, 113.0

mmol) was slowly added. The solution was heated to room temperature for 48 hours, at which TLC confirmed that the reaction had gone to completion. The solution was quenched with methanol and concentrated *in vacuo.* The residue was extracted several times into EtOAc from neutral water after which the organic layers were combined and washed once with water. Following drying and concentration *in vacuo,* the product was purified by flash chromatography (2:1 hexanes:EtOAc) to afford (**2**) (13.1 g, 80%): δ_H (400 MHz, $CDCl_3$) 0.964 (t, *J* = 7.52 Hz, 9H), 2.61 (q, *J* = 7.4 Hz, 14.9 Hz, 6H), 3.98 (s, 9H), 5.38 (s, 6H), 6.74 (d, *J* = 3.8 Hz, 3H), 7.09 (d, *J* = 4.0 Hz, 3H), 7.32 (t, *J* = 7.7 Hz, 3H), 7.70 (d, *J* = 8.3 Hz, 3H), 7.96 (d, *J* = 7.6 Hz, 3H); δ_C (100 MHz, $CDCl_3$)15.3, 23.3, 43.5, 51.7, 102.8, 113.6, 121.0, 121.9, 123.5, 127.4, 128.4, 130.7, 137.1, 146.1, 167.8; LRMS (ESI+) m/z calc'd for $C_{45}H_{45}N_3O_6$ $[M + Na]^+$ 746.33, found 746.56

c) 1,1',1''-((2,4,6-Triethylbenzene-1,3,5-triyl)tris(methylene))tris(1H-indole-4-carboxylic acid) (**3**): **2** (6.4 g, 8.85 mmol) was dissolved in THF: MeOH: H_2O (3:1:1 88 mL) and sodium hydroxide was added (1.4 g, 35.5 mmol). The solution was then heated to 65 ^oC for 5 hours at which TLC confirmed that the reaction had gone to completion. The solution was then extracted several times into EtOAc from 0.1 M HCl solution after which the organic layers were combined and washed once with water. Following drying and concentration *in vacuo,* the product was collected as a white solid (**3**) (5.6 g, 90%). δ_H (400 MHz, $(CD_3)_2SO$) 0.76 (t, *J* = 6.8 Hz, 9H), 2.58 (q, *J* = 6.8 Hz, 14.6 Hz, 6H), 5.45 (s, 6H), 6.87 (d, *J* = 3.2 Hz, 3H), 6.95 (d, *J* = 3.2 Hz, 3H), 7.27 (t, *J* = 7.9 Hz, 15.4 Hz, 3H), 7.76 (d, *J* = 7.5 Hz, 3H), 7.94 (d, *J* = 8.3 Hz, 3H), 12.62 (s, 3H); δ_C (100 MHz, $(CD_3)_2SO$) 15.2, 23.3, 43.7, 102.6, 114.9, 120.9, 122.3, 123.2, 128.1, 128.3, 131.1, 137.2, 145.8, 168.6; LRMS (ESI-) m/z calc'd for $C_{42}H_{39}N_3O_6$ $[M + Na]^-$ 704.28, found 704.27

d) 1,1'-((5-((4-((Benzyloxy)carbonyl)-1H-indol-1-yl)methyl)-2,4,6-triethyl-1,3-phenylene)bis(methylene))bis(1H-indole-4-carboxylic acid) (**4**): **3** (5.6 g, 8.05 mmol), potassium tert-butoxide (0.99 g, 8.86 mmol) and benzyl bromide (1.05 mL, 8.86 mmol), were dissolved in DMF (161 mL) at room temperature. The reaction was left at room temperature for 10 hours. The solution was then extracted several times into EtOAc from 0.1 M HCl solution after which the organic layers were combined and washed once with water. Following drying and concentration *in vacuo,* the product was purified by flash

chromatography (50% dichloromethane, 50% (1% acetic acid, 7% methanol, 92% dichloromethane)) and collected as a dark orange oil. (1.10 g, 17%) δ_H (400 MHz, $CDCl_3$) 1.01 (t, *J* = 7.5 Hz, 6H), 1.15 (t, *J* = 7.0 Hz, 3H), 2.45 (q, *J* = 7.6 Hz, 16.0 Hz, 2H), 2.67 (q, *J* = 7.6 Hz, 15.3 Hz, 4H), 5.40 (s, 4H), 5.41 (s, 2H), 5.44 (s, 2H), 6.67 (d, *J* = 3.4 Hz, 2H), 6.77 (d, *J* = 3.6 Hz, 1H), 7.10 (d, *J* = 3.2 Hz, 1H), 7.24 (d, *J* = 33.2 Hz, 2H), 7.31-7.42 (m, 6H), 7.49 (d, *J* = 7.2 Hz, 2H), 7.74 (d, *J* = 8.1 Hz, 1H), 7.79 (d, *J* = 8.13 Hz, 2H), 8.00 (s, 1H), 8.06 (d, *J* = 7.68 Hz, 2H); δ_C (100 MHz, $CDCl_3$) 15.2, 15.4, 23.3, 43.4, 50.4, 66.3, 103.1, 113.7, 120.9, 121.7, 122.7, 123.6, 123.8, 127.2, 127.6, 128.1, 128.4, 128.7, 130.5, 136.2, 137.0, 145.9, 146.2, 167.0, 172.8 LRMS (ESI-) m/z calc'd for $C_{49}H_{45}N_3O_6[M + H]^-$ 794.33, found 794.35

e) Hexa-*tert*-butyl 10,10'-(1,1'-((5-((4-((benzyloxy)carbonyl)-1H-indol-1-yl)methyl)-2,4,6-triethyl-1,3-phenylene)bis(methylene))bis(1H-indole-4,4'-carbonyl))bis(1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate) (**5**): **4** (1.03 g, 1.33 mmol) and TBTU (1.4 g, 4.25 mmol) were dissolved in DMF (13.3 mL) at room temperature and left for 10 minutes. Boc_3Cyclen (2.0 g, 4.25 mmol) and DIPEA (1.15 mL, 6.65 mmol) were added to the reaction mixture and the reaction was left at room temperature for 48 hours. The solution was then extracted several times into EtOAc from a saturated sodium bicarbonate solution after which the organic layers were combined and washed once with water. Following drying and concentration *in vacuo,* the product was purified by flash chromatography (4:1, EtOAc: hexanes) and collected as a yellow solid. (1.51 g, 67%) δ_H (400 MHz, $CDCl_3$) 0.94 (t, *J* = 3.0 Hz, 9H), 1.43 (s, 5H), 1.45 (s, 31H), 1.46 (s, 18H), 2.59 (q, *J* = 8.5 Hz, 7.9 Hz, 6H), 3.32 – 3.50 (m, 32H), 5.30 (s, 4H), 5.36 (s, 2H), 5.41 (s, 2H), 6.39 (d, *J* = 3.2 Hz, 2H), 6.63 (d, *J* = 3.3 Hz, 2H), 6.70 (d, *J* = 3.7 Hz, 1H), 7.10 (d, *J* = 7.5 Hz, 3H), 7.22 – 7.40 (m, 6H), 7.47 (d, *J* = 8.4 Hz, 4H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.99 (d, *J* = 8.4 Hz, 1H); δ_C (100 MHz, $CDCl_3$) δ_C (100 MHz, $CDCl_3$) 13.7, 15.1, 15.2, 19.1, 23.2, 28.0, 28.3, 28.4, 31.5, 43.5, 53.3, 61.7, 71.0, 71.6, 79.6, 80.2, 100.4, 109.9, 117.9, 121.5, 125.7, 126.1, 127.1, 127.6, 128.7, 128.9, 130.5, 136.4, 137.0, 146.0, 156.7

f) 1-(2,4,6-Triethyl-3,5-bis((4-(4,7,10-tris(tert-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane-1-carbonyl)-1H-indol-1-yl)methyl)benzyl)-1H-indole-4-carboxylic acid (**6**): **5** (1.51 g, 0.90 mmol) was dissolved in methanol (7.9 mL) and Pd/C

(0.15 g, 10% wt.) dissolved in 1 mL methanol was added slowly to the solution. The solution was purged several times with H_2 and reacted at room temperature under H_2 for 16 hr. The resulting solution was filtered through celite and the product was dried and concentrated *in vacuo*. The product was purified by column chromatography (70% dichloromethane. 30% (1% H_2O, 12 % methanol, 70% dichloromethane)) and collected as an off-white solid. (0.789 g, 55%) δ_H (400 MHz, $CDCl_3$) 0.96 (t, J = 6.9 Hz, 9H), 1.16 (s, 17H), 1.46 (s, 20H), 1.51 (s, 17H), 2.62 (q, J = 6.9 Hz, 7.5 Hz, 6H), 3.20 – 3.75 (m, 32H), 5.33 (s, 4H), 5.38 (s, 2H), 6.42 (d, J = 2.6 Hz, 2H), 6.65 (d, J = 3.5 Hz, 2H), 6.73 (d, J = 3.1 Hz, 1H), 7.13 (s, 2H), 7.24 – 7.36 (m, 4H), 7.49 (d, J = 8.3 Hz, 2H), 7.73 (d, J = 8.3 Hz, 1H), 8.02 (d, J = 7.9 Hz, 1H); δ_C (100 MHz, $CDCl_3$) 13.8, 15.1, 15.3, 19.1, 23.2, 28.1, 28.3, 28.4, 31.5, 43.4, 53.3, 61.7, 65.7, 71.0, 71.6, 79.6, 80.2, 100.4, 109.9, 117.9, 121.5, 125.7, 126.1, 128.7, 128.9, 130.5, 136.4, 137.0, 146.0, 156.7; LRMS (ESI+) m/z calc'd for $C_{88}H_{123}N_{11}O_{16}$ $[M+Na]^+$ 1613.91, found 1613.16.

g) Hexa-*tert*-butyl 10,10'-(1,1'-((2,4,6-triethyl-5-((4-(methylcarbamoyl)-1*H*-indol-1-yl)methyl)-1,3-phenylene)bis(methylene))bis(1*H*-indole-1,4-diyl-4-carbonyl))bis(1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylate) (**7**): **6** (80.0 mg, 0.05 mmol) and TBTU (32 mg, 0.1 mmol) were dissolved in DMF (0.5 mL) at room temperature and left for 10 minutes. Methylamine hydrochloride (3.09, 0.1 mmol) and DIPEA (43.3 μL, 0.25 mmol) were added to the reaction mixture and the reaction was left to react at room temperature for 16 h. The product was then extracted several times into EtOAc from a saturated sodium bicarbonate solution after which the organic layers were combined and washed once with water. Following drying and concentration *in vacuo,* the product was purified by flash chromatography (10% MeOH in DCM) and collected as a yellow oil **7** (72.6 mg, 56%) δ_H (400 MHz, $CDCl_3$) 0.96 (t, J = 7.6 Hz, 9H), 1.15 (s, 17H), 1.45 (s, 20H), 1.49 (s, 17H), 2.62 (q, J = 6.9 Hz, 7.5 Hz, 6H), 3.04 (s, 3H), 3.15- 3.83 (m, 32 H), 5.31 (s, 4H), 5.34 (s, 2H), 6.40 (d, J = 2.6 Hz, 2H), 6.65 (d, J = 3.5 Hz, 2H), 6.75 (d, J = 3.1 Hz, 1H), 7.13 (s, 2H), 7.24 – 7.36 (m, 4H), 7.49 (d, J = 8.3 Hz, 2H), 7.73 (d, J = 8.3 Hz, 1H), 8.02 (d, J = 7.9 Hz, 1H); δ_C (100 MHz, $CDCl_3$) 13.7, 15.1, 15.2, 19.1, 23.2, 28.0, 28.3, 28.4, 31.5, 43.5, 53.3, 61.7, 71.0, 71.6, 79.6, 80.2, 100.4, 109.9, 117.9, 121.5, 125.7, 126.1, 128.7, 128.9, 130.5, 136.4, 137.0, 146.0, 156.7; LRMS (ESI+) m/z calc'd for $C_{89}H_{126}N_{12}O_{15}$ $[M+Na]^+$ 1625.90, found 1625.95

h) 1-(3,5-Bis((4-(1,4,7,10-tetraazacyclododecane-1-carbonyl)-1*H*-indol-1-yl)methyl)-2,4,6-triethylbenzyl)-*N*-methyl-1*H*-indole-4-carboxamide (**8**): **7** (72.6 mg, 0.05 mmol) was dissolved in 50% (v/v) TFA/DCM and allowed to react for 0.5 h. The solvents were then removed *in vacuo* and the crude product purified by rp –HPLC to produce the product **8** as a clear oil (62.7 mg, 62%) δ_H (400 MHz, CD_3OD) 0.93 (t, *J* = 7.9 Hz, 6H), 0.93 (t, *J* = 7.9 Hz, 6H), 2.33-2.85 (bm, 24H), 2.91-3.42 (bm, 12H), 3.46-3.98 (bm, 5H), 5.47 (s, 4H), 5.51(s, 2H), 6.66-6.86 (m, 2H), 6.38-6.45 (m, 3H), 7.06-7.81 (m 9H), 7.97 (d, *J* = 7.9 Hz, 1H) δ_C (100 MHz, CD_3OD) 14.0, 14.1, 22.7, 25.2, 43.3, 44.6, 46.8, 99.6, 110.2, 111.6, 111.6, 117.5, 120.7, 121.0, 121.2, 125.5, 126.5, 126.6, 128.4, 130.7, 130.8, 131.5, 136.5, 137.0, 145.8, 145.9, 174.0; LRMS (ESI+) m/z calc'd for $C_{59}H_{78}N_{12}O_3$ $[M+Na]^+$ 1025.63, found 1025.67

Supplementary Note 2 Methods for characterization of **8**

Acquisition:
All ^{13}C NMR spectra of final compounds were acquired at 25°C using an Agilent DD2 spectrometer ($\nu(^1H)$ = 699.758 MHz, $\nu(^{13}C)$ = 175.973 MHz; Agilent, Walnut Creek, CA) equipped with a 5mm variable temperature ^1H-$^{19}F\{^{13}C/^{15}N\}$ Triple Resonance Cold Probe and VnmrJ4.0 acquisition software.

1D ^1H
1D ^1H spectra were acquired using the standard VnmrJ4.0 s2pul pulse sequence. The spectrum was acquired over a 7142.9 Hz spectral window with 71154 points, a 5s recycle delay, and 32 transients.

2D $^1H/^{13}C$ gc2hsqcse
2D $^1H/^{13}C$ HSQC spectra were acquired at 25ºC using the default VnmrJ4.0 gc2hsqcse pulse sequence. The spectra were acquired with a 7142.9 Hz spectral window and 2048 points in the direct dimension and a 35195.8 Hz spectral window and 256 increments in the indirect dimension. Spectra were collected with a 0.64s recycle delay, and 4 transients.

2D $^1H/^{13}C$ gc2hmbc
2D 1H/13C HMBC spectra were acquired using the default VnmrJ4.0 gHMBCAD pulse sequence. The spectra were acquired over a 5733.9 Hz spectral window and 2048 points in the direct dimension and a 42238.6 Hz spectral window and 256 increments in the indirect dimension. Spectra were collected with a 0.64s recycle delay, and 32 transients.

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2D ^{1}H/^{1}H gCOSY

2D COSY spectra were acquired using the default VnmrJ4.0 gCOSY pulse sequence. The spectra were acquired over a 7142.9 Hz spectral window and 2048 points in the direct dimension and a 7142.9 Hz spectral window and 512 increments in the indirect dimension. Spectra were collected with a 0.64s recycle delay, and 2 transients.

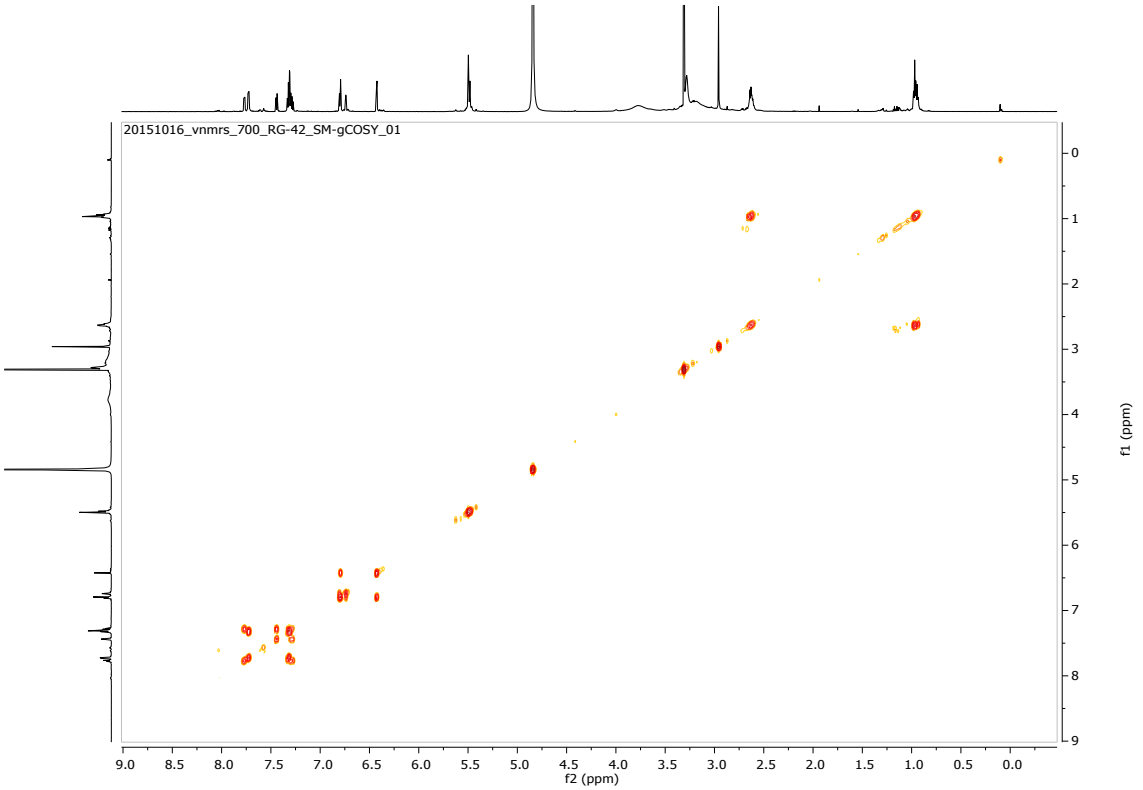
2D ^{1}H/^{1}H ROESY

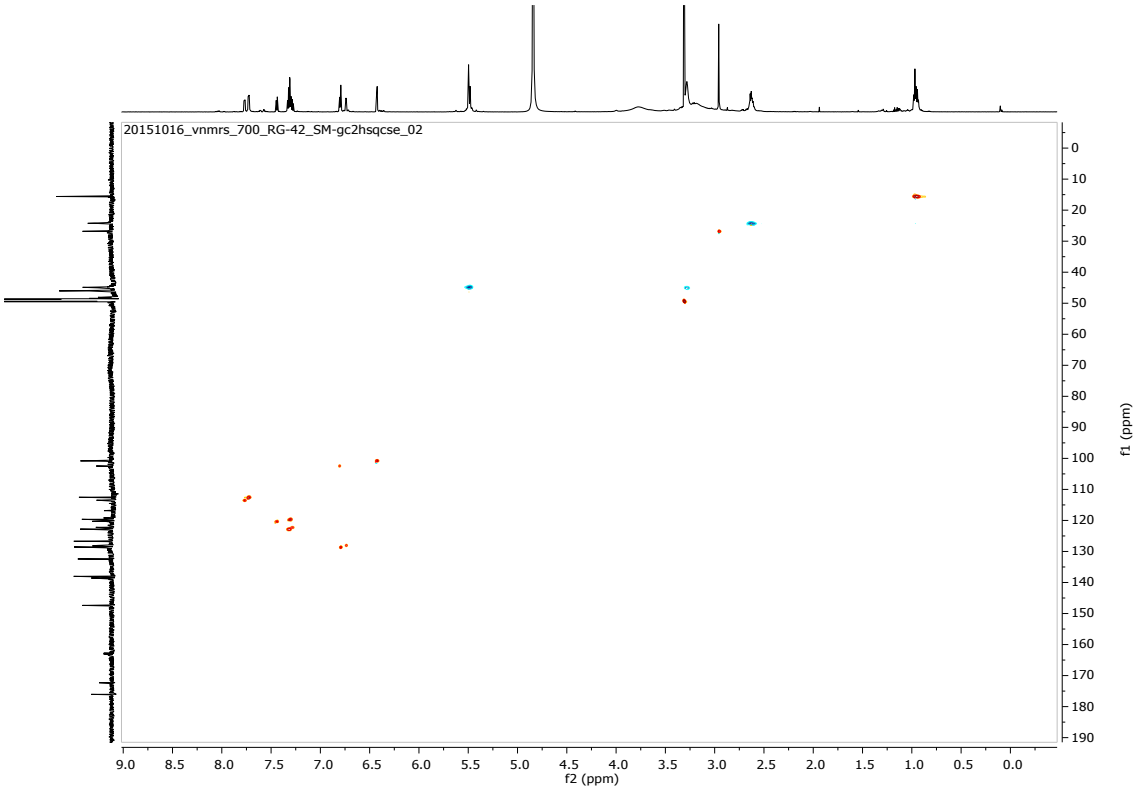
2D ROESY spectra were acquired using the default VnmrJ4.0 ROESY pulse sequence. The spectra were acquired over a 7142.9 Hz spectral window and 2048 points in the direct dimension and a 7142.9 Hz spectral window and 256 increments in the indirect dimension. Spectra were collected with a 1.5s recycle delay, and 16 transients.

NMR Processing:

NMR spectra were processed using MestReNova NMR processing software (v9.0.1, Santiago de Compostela, Spain). All spectra were Fourier transformed, phase corrected, and baseline corrected. Window functions were applied as necessary prior to Fourier transformation and all 2D spectra were two-fold linear predicted in the F1 dimension prior to Fourier transformation. Chemical shifts were referenced relative to the residual solvent peaks (d_4-MeOD, $\delta(^{1}H)$=3.31 ppm, $\delta(^{13}C)$=49.00 ppm).

Figure S 4 2D NMR spectra of 8





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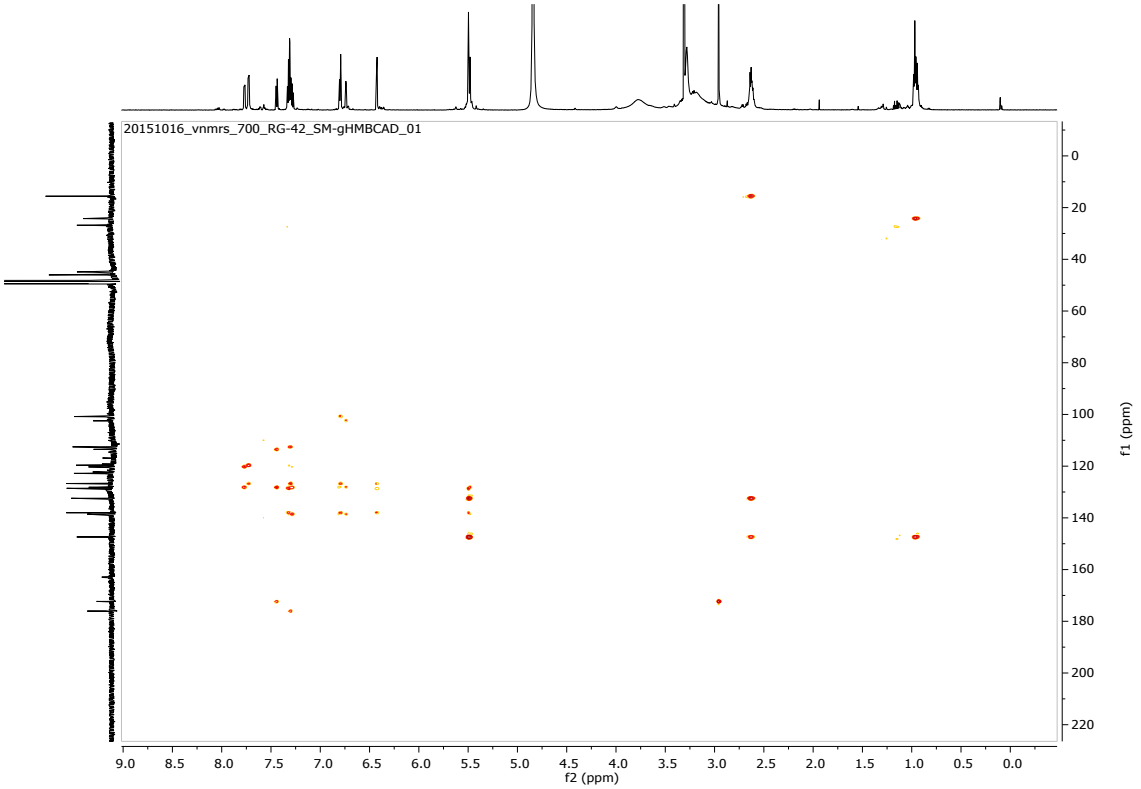
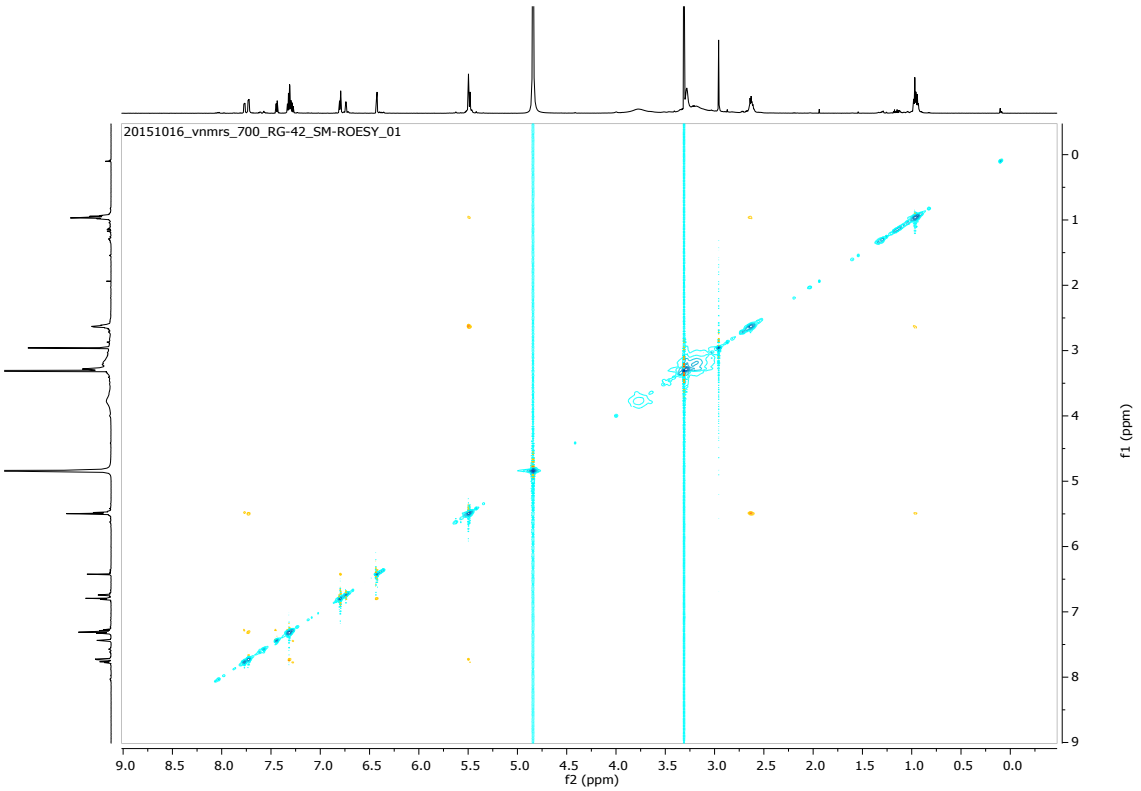




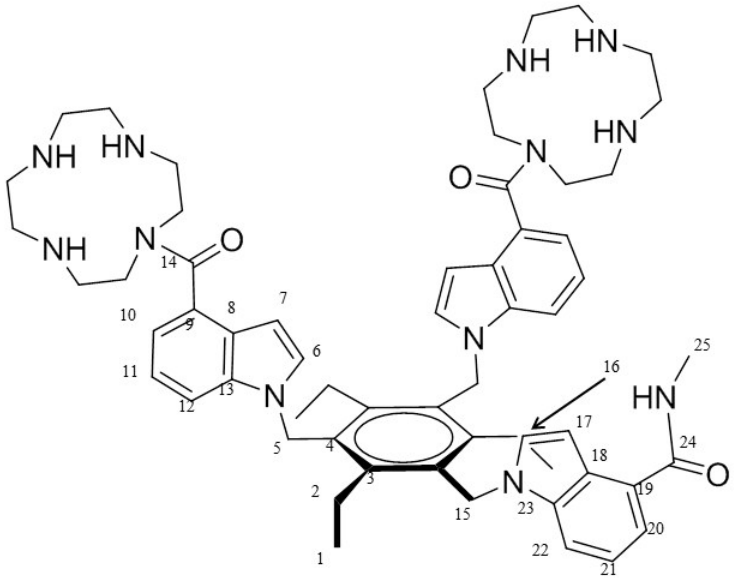


Table S 1 Full NMR characterization of compound 8.

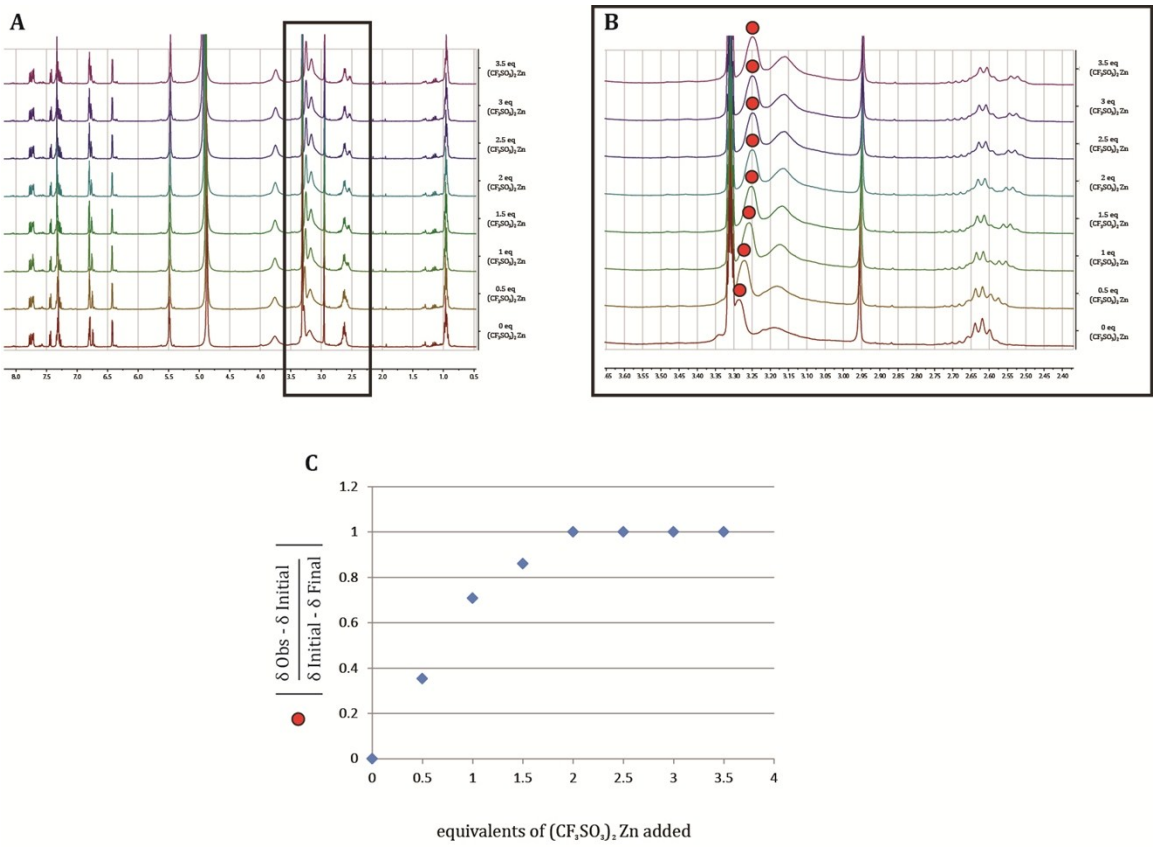
C-numbering	δ (C13)	δ (H1)	m 1H	INT	J H-H	gCOSY	HSQC (H --> C)	HSQC (m) / RING ID	HMBC (H--> C)	ROE (H--> H)
1	15.59	0.96	m	9.00		2.63	15.59	CH/CH3	24.22 (vs), 147.42 (vs)	2.63, 5.50 (w)
2	24.19	2.63	m	6.00		0.96	24.19	CH2	15.60 (vs), 132.42 (vs), 147.37 (vs)	0.96
3	147.41									
4	132.42									
5	44.73	5.50	s	6(for 5.5 and 5.48), 4			44.73	CH2	128.70* (s), 132.49 (vs), 138.02 (m), 147.41 (vs)	2.63, 7.73, 0.96 (w)
6	128.7*	6.80	d	2.00	3.3 Hz	6.43	128.70	CH/CH3	100.78 (s) 126.70 (s), 138.02 (m)	6.43
7	100.81	6.43	d	2.00	3.3 Hz	6.80	100.81	CH/CH3	126.7 (w), 128.7* (w), 138.02 (m)	6.8
8	126.70									
9	128.7*									
10	119.58	7.30	m	2.00		7.73	119.58	CH/CH3	112.5 (s), 126.70 (s), 176.04 (s)	7.73
11	122.80	7.32	m	2.00		7.73	122.80	CH/CH3	112.5(s), 128.7* (s), 138.02 (s)	
12	112.53	7.73	d	2.00	7.5 Hz	7.32 (broad peak)	112.53	CH/CH3	119.65 (s), 126.71 (s)	7.3, 5.50
13	138.02									
14	176.04									
15	44.73	5.48	s	6(for 5.5 and 5.48), 2			44.73	CH2	128.20** (m), 132.49 (vs) , 147.41 (vs)	2.63, 7.77 (w)
16	102.45	6.81	d	1.00	3.1 Hz	6.74	102.45	CH/CH3	100.8 (s), 128.20** (s), 138.02 (s)	
17	128.07***	6.74	d	1.00	3.1 Hz	6.81	128.07***	CH/CH3	102.44 (s), 128.20** (s), 138. 58 (s)	
18	128.20**									
19	138.56									
20	120.29	7.44	d	1.00	7.9 Hz	7.29	120.29	CH/CH3	113.5 (s), 128.20** (s), 172.31 (s)	7.29 (w),
21	122.32	7.29	dd	1.00	7.9 Hz	7.77, 7.44	122.32	CH/CH3	120,3 (w), 128.20** (s), 138.56 (s)	7.77 (w), 7.44 (w)
22	113.50	7.77	d	1.00	7.9 Hz	7.29	113.50	CH/CH3	120.3 (s), 128.20** (s)	5.48 (w), 7.29 (w)
23	128.20**									
24	172.31									
25	26.82	2.96	s	3.00			26.82	CH/CH3	172.31	
Cyclen		3.78	bs	10.00						
Cyclen	45.03	3.28	bs	8.00			45.03	CH2		
Cyclen		318	bs	12.00						

*, **, *** cluster of 13C peaks too close to assign separately

i) Metallated receptor 1 **(9): 8** (28.9 mg, 0.03 mmol) was dissolved in anhydrous methanol (0.3 mL) and zinc(II) trifluoromethanesulfonate (20.9 mg, 0.06 mmol) was added and allowed to stir for 0.5 h at ambient temperature. The reaction was filtered and the methanol was then removed *in vacuo* to yield the final product as an off- white solid (35.9 mg). δ_H (400 MHz, CD_3OD) 1H NMR (400 MHz, Methanol-d4)

δ 0.96 (m, 9H), 2.58 (m, 6H), 2.95 (s, 3H), 3.29 – 3.08 (m, 24H), 3.75 (bs, 8H), 5.47 (s, 6H), 6.42 (d, J = 3.3 Hz, 2H), 6.76 (d, J = 3.3 Hz, 1H), 6.80 (m, 3H), 7.28 (dd, J = 7.91 Hz, 7.91 Hz, 1H), 7.32 (m, 4H), 7.43 (d, J = 7.3 Hz, 1H),7.72 (dd, J = 7.0, 2.2 Hz, 2H), 7.77 (d, J = 8.2 Hz, 1H). δ_C (100 MHz, CD_3OD) 15.49, 15.51, 15.57, 23.61, 24.07, 24.22, 26.83, 32.66, 44.85, 45.79, 48.49, 100.72, 110.00, 110.00, 112.72, 113.57, 114.34, 114.83, 116.65, 117.86, 118.96, 119.74, 120.29, 120.39, 121.27, 122.19, 122.82, 122.92, 125.46, 126.49, 126.56, 127.87, 128.05, 128.06, 128.09, 128.12, 128.86, 128.95, 132.29, 132.33, 137.91, 138.47, 147.23, 147.26, 172.31, 176.20.

Figure S 5 ^{1}HNMR titrations of 8 with zinc trifluoromethanesulfonate



A) Titration of **8** with $Zn(CF_3SO_3)_2$. Stacked ^{1}H NMR spectra (in a 400MHz spectrometer) of **8** (18 mg, 0.018 mmol) in CD_3OD, shown at the bottom; followed by ^{1}H NMR spectra of the initial solution upon the incremental additions of 0.5 eq. of $Zn(CF_3SO_3)_2$ (3.62mg, 0.009 mmol) as a powder, going upwards up to 3.5 eq. of Zn^{2+}. X-axis has values in ppm.

B) Expanded inset shown in **A**, red circle indicates cyclen shift upon binding to Zn^{2+}. X-axis has values in ppm.

C) Absolute change of cyclen chemical shifts plotted vs. equivalents of $(CF_3SO_3)_2$ Zn added in titration. It can be observed that after the addition of 2 Zn^{2+} no more shift in cyclen chemical shift is observed. This is indicative of a 2:1, Zn^{2+} to **8** complex formation, yielding **9**.

ProxyPhos was synthesized according to the previously published procedure. [1]

Supplementary Note 2 Biophysical experiments

Indicator Displacement Assay: HEPES free acid was purchased from BioShop Canada (cat. HEP005). ATP, AMP, PhoP, $NaSO_4$ and PPi were purchased from Sigma Aldrich. Pyrocatechol violet was purchased from Sigma Aldrich All peptides were purchased from CanPeptide at 95 % purity.

Full-length sequences for purchased peptides:

pY = Ac-AYpYAA-NH_2

pYpY = Ac-ApYpYAA-NH_2

All absorbance measurements were taken on a BioTek Cytation 3 instrument

ProxyPhos Assay: A Tecan Infinite M1000 microplate reader was used for all solution fluorescence intensity measurements at 400 Hz in black 384 well, flat bottom microplates. For 384 well plates a total sample volume of 60 μL was used throughout. Samples were excited at 350 nm (5 nm bandwidth) and fluorescence intensity was recorded an emission wavelength of 476 nm (20 nm bandwidth). All experiments were performed in triplicate.

1. Kraskouskaya, D., Bancerz, M., Soor, H. S., Gardiner, J. E. & Gunning, P. T. An excimer-based, turn-on fluorescent sensor for the selective detection of diphosphorylated proteins in aqueous solution and polyacrylamide gels. *J. Am. Chem. Soc.* **136,** 1234–1237 (2014).