Supplementary Information

A tool for the selective sequestration of ATP and PPi 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
Supplementary Information

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

![UV-Vis spectra of [receptor 1:PV] complex](image)

- 400 μM analyte
- 300 μM analyte
- 200 μM analyte
- 100 μM analyte

Absorbance

Wavelength (nm)
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$_3$OD. 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 °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%). $\delta_H$ (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); $\delta_C$ (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 °C and sodium hydride 60% w/w (4.5 g, 113.0
Supplementary Information

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%): $\delta_H$ (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); $\delta_C$ (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$_3$O$_6$ [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$_2$O (3:1:1 88 mL) and sodium hydroxide was added (1.4 g, 35.5 mmol). The solution was then heated to 65 °C 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%). $\delta_H$ (400 MHz, (CD$_3$)$_2$SO) 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); $\delta_C$ (100 MHz, (CD$_3$)$_2$SO) 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$_3$O$_6$ [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
Supplementary Information

chromatography (50% dichloromethane, 50% (1% acetic acid, 7% methanol, 92% dichloromethane)) and collected as a dark orange oil. (1.10 g, 17%) δH (400 MHz, CDCl3) 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); δC (100 MHz, CDCl3) 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 C49H45N3O6 [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. Boc3Cyclen (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%) δH (400 MHz, CDCl3) 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); δC (100 MHz, CDCl3) δC (100 MHz, CDCl3) 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
Supplementary Information

(0.15 g, 10% wt.) dissolved in 1 mL methanol was added slowly to the solution. The solution was purged several times with H₂ and reacted at room temperature under H₂ 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% H₂O, 12 % methanol, 70% dichloromethane) and collected as an off-white solid. (0.789 g, 55%) δH (400 MHz, CDCl₃) 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); δC (100 MHz, CDCl₃) 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 C₈₉H₁₂₃N₁₁O₁₆ [M+Na]+ 1613.91, found 1613.16.

g) Hexa-tert-butyl 10,10’-((2,4,6-triethyl-5-(4-(methylcarbamoyl)-1H-indol-1-yl)methyl)-1,3-phenylene)bis(methylene))bis(1H-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%) δH (400 MHz, CDCl₃) 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); δC (100 MHz, CDCl₃) 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 C₈₉H₁₂₆N₁₂O₁₅ [M+Na]+ 1625.90, found 1625.95
h) 1-(3,5-Bis((4-(1,4,7,10-tetraazacyclododecane-1-carbonyl)-1H-indol-1-yl)methyl)-
2,4,6-triethylbenzyl)-N-methyl-1H-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\textsubscript{H} (400 MHz, CD\textsubscript{3}OD) 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\textsubscript{C} (100 MHz, CD\textsubscript{3}OD) 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\textsubscript{59}H\textsubscript{78}N\textsubscript{12}O\textsubscript{3} [M+Na]\textsuperscript{+} 1025.63, found 1025.67

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

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

**1D \textsuperscript{1}H**
1D \textsuperscript{1}H 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 \textsuperscript{1}H/\textsuperscript{13}C gc2hsqcse**
2D \textsuperscript{1}H/\textsuperscript{13}C HSQC spectra were acquired at 25°C 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 \textsuperscript{1}H/\textsuperscript{13}C gc2hmmbc**
2D \textsuperscript{1}H/\textsuperscript{13}C 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.
Supplementary Information

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, δ($^1$H)=3.31 ppm, δ($^{13}$C)=49.00 ppm).
Figure S 4 2D NMR spectra of 8

[Image of 2D NMR spectra]
Table S1 Full NMR characterization of compound 8.

| C-numbering | δ (H) | δ (13C) | J (Hz) | MT | IH (p.p.m.) | J (H-H) | δC097 | IH-C | δSEC (1H→C) | δSEC (13C) | δSEC (1H→C) | H H H | δSEC (1H→C) |
|-------------|-------|---------|--------|----|---------|--------|-------|------|-----------|------------|------------|--------|---------|----------------|
| 1           | 15.69 | 0.96    | 0.00   | 0.2 | 2.63    | 15.50  | CHCH3 | 24.22 (s), 147.41 (s) | 2.63, 5.40 (w) |
| 2           | 24.19 | 2.65    | 0.00   | 0.36 | 24.19   | CH2    | 15.60 (s), 152.42 (s), 147.37 (s) | 0.96 |
| 3           | 167.41 | | | | |
| 4           | 132.42 | | | | |
| 5           | 44.73 | 5.50    | s      | 5.50 | 44.73   | CH2    | 128.70 (s), 132.49 (s), 136.02 (m), 147.41 (s) | 2.63, 7.73, 9.96 (w) |
| 6           | 128.7* | 6.80    | d     | 2.00 | 3.34    | 0.43   | 128.70 | CHCH3 | 100.76 (s), 126.70 (s), 136.02 (m) | 6.43 |
| 7           | 100.7 | 0.43    | d     | 2.00 | 3.34    | 0.43   | 128.70 | CHCH3 | 100.76 (s), 126.70 (s), 136.02 (m) | 6.43 |
| 8           | 136.02 | | | | |
| 9           | 128.7* | | | | |
| 10          | 119.58 | 7.70    | m     | 2.00 | 7.70    | 119.58 | CHCH3 | 113.5 (s), 128.70 (s), 136.02 (m) | 7.70 |
| 11          | 122.80 | 7.70    | m     | 2.00 | 7.70    | 122.80 | CHCH3 | 113.5 (s), 128.70 (s), 136.02 (m) | 7.70 |
| 12          | 112.53 | 7.75    | d     | 2.00 | 7.57    | 112.53 | CHCH3 | 113.5 (s), 126.71 (s) | 7.3, 8.50 |
| 13          | 126.02 | | | | |
| 14          | 136.03 | | | | |
| 15          | 44.73 | 5.48    | s      | 5.48 | 44.73   | CH2    | 128.70 (s), 132.49 (s), 147.41 (s) | 2.63, 7.77 (w) |
| 16          | 122.45 | 6.81    | d     | 2.00 | 3.14    | 6.74   | 122.45 | CHCH3 | 100.8 (s), 126.70 (s), 136.02 (m) | 2.63, 7.77 (w) |
| 17          | 100.7* | 6.81    | d     | 2.00 | 3.14    | 6.74   | 122.45 | CHCH3 | 100.8 (s), 126.70 (s), 136.02 (m) | 2.63, 7.77 (w) |
| 18          | 126.02* | | | | |
| 19          | 136.03 | | | | |
| 20          | 120.29 | 7.74    | d     | 2.00 | 7.94    | 7.29   | 120.29 | CHCH3 | 113.5 (s), 128.70 (s), 136.02 (m) | 7.29 (w) |
| 21          | 122.32 | 7.74    | d     | 2.00 | 7.94    | 7.29   | 122.32 | CHCH3 | 113.5 (s), 128.70 (s), 136.02 (m) | 7.29 (w) |
| 22          | 113.50 | 7.77    | d     | 2.00 | 7.94    | 7.29   | 113.50 | CHCH3 | 120.3 (s), 128.70 (s), 136.02 (m) | 7.29 (w) |
| 23          | 128.7* | | | | |
| 24          | 122.32 | | | | |
| 25          | 26.82 | 2.96    | s     | 3.00 | 26.82   | CHCH3 | 126.31 | | |
| Cyclen      | 3.76  | bs      | 10.00  | | | | | | | |
| Cyclen      | 49.00 | bs      | 8.00   | | | | | | | |
| 26          | 318.76 | bs      | 12.00  | | | | | | | |

* ** ** * cluster of 13C peaks too close to assign separately
Supplementary Information

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). \(\delta\)\textsubscript{H} (400 MHz, CD\textsubscript{3}OD) 1H NMR (400 MHz, Methanol-d4)

\(\delta\) 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). \(\delta\)\textsubscript{C} (100 MHz, CD\textsubscript{3}OD) 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.
A) Titration of 8 with Zn(CF$_3$SO$_3$)$_2$. Stacked $^1$H NMR spectra (in a 400MHz spectrometer) of 8 (18 mg, 0.018 mmol) in CD$_3$OD, 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$_3$SO$_3$)$_2$ (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$_3$SO$_3$)$_2$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.
Supplementary Information

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, NaSO₄ 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:

\[ pY = \text{Ac-AYpYAA-NH}_2 \]

\[ pYpY = \text{Ac-ApYpYAA-NH}_2 \]

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 \( \mu \text{L} \) 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.