Supporting Information

Are Two Better than One? Comparing Intermolecular and Intramolecular Indicator Displacement Assays in Pyrophosphate Sensors

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General Procedures for SPPS

General procedures for SPPS on 2-chlorotrityl chloride resin

Coupling of Fmoc-protected oxazole-based amino acids was carried out using O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyl-uronium hexafluorophosphate (HATU) as the coupling reagent in the presence of *N*-methylmorpholine (NMM). Progress of the couplings was monitored by LCMS.

Loading of Fmoc-protected oxazole-based amino acid onto 2-chlorotrityl chloride resin and capping

2-Chlorotrityl chloride resin (resin capacity 1.3 mmol g^{-1}) was swollen in anhydrous CH_2Cl_2 for 1 h in a sinter-fitted syringe. After filtering off the resin, a solution of the amino acid (1.7 equiv. relative to resin capacity) and DIPEA (3 equiv. relative to resin capacity) in anhydrous CH_2Cl_2-DMF (9:1 v/v, 0.2 M) was added. Agitated at room temperature for 72 h, the resin was drained and treated with a solution of methanol-diisopropylethylamine-CH_2Cl_2 (2:1:17 v/v/v, 3 x 5 mL x 10 min). The resin was then washed with DMF (3 x 6 mL), CH_2Cl_2 (3 x 6 mL) and diethyl ether (3 x 6 mL) before being dried under reduced pressure overnight. The resin loading was calculated to be 1.15 mmol g^{-1} according to gravimetric measurements.

Loading of amino acid onto Rink amide AM Resin

Rink Amide AM resin at a loading level of 0.41 mmol g^{-1} was placed in a sinter-fitted syringe and allowed to swell in anhydrous DCM for 1 h. After removal of DCM, the resin was treated with a solution of 20% v/v piperidine in DMF (3 x 5 mL x 10 min) and the resin was then drained and rinsed with DMF (3 x 5 mL), DCM (3 x 5 mL) and DMF (3 x 5 mL). Afterwards, a solution of Fmoc-protected amino acid (1.5 equiv.), DIPEA (3 equiv.) and HATU (2.5 equiv.) was added to the reaction syringe. After agitation at room temperature for 2 h, the resin was filtered off and washed with DMF (3 x 5 mL) and DCM (3 x 5 mL).

Fmoc deprotection

The resin-bound peptide was treated with a solution of 10% v/v piperidine in DMF (3 x 5 mL x 10 min) and subsequently filtered off and washed with DMF (3 x 5 mL), CH_2Cl_2 (3 x 5 mL) and DMF (3 x 5 mL) to give the free amine.

Solid Phase Peptide coupling

A solution of Fmoc-protected oxazole based amino acid (1.2 equiv. relative to loading), NMM (2.0 equiv. relative to loading), HATU (1.2 equiv. relative to loading) was added to the reaction vessel. The resulting suspension was shaken at room temperature for 50 min and then the resin was drained and washed with DMF (3 x 5 mL), CH_2Cl_2 (3 x 5 mL) and DMF (3 x 5 mL).

Capping of Rink amide AM Resin

Capping was accomplished by treatment of the resin with 20% v/v acetic anhydride in pyridine (3 x 5 min) and subsequent washing with DMF (3 x 6 mL), DCM (3 x 6 mL) and DMF (3 x 6 mL).

Peptide cleavage from 2-chlorotrityl resin

After the coupling, the resin was washed with DMF (5 x 5 mL) and CH_2Cl_2 (10 x 5 mL). Then the resin was treated with a solution of hexafluoroisopropanol-CH_2Cl_2 (1:4 v/v, 4 x 6 mL x 10 min). Afterwards, the resin was washed with CH_2Cl_2 (5 x 5 mL). All solutions were combined and evaporated to give the crude linear peptide.

Peptide cleavage from rink resin

After acetylation, the resin was washed with DMF (3 x 5 mL) and DCM (3 x 5 mL).The resin was then treated with a solution of trifluoroacetic acid/H_2O/triisopropylsilane (95:2.5:2.5 v/v, 2 x 1 h) and washed with DCM (2 x 2 mL). All solution were combined and evaporated to give the desired product.

Macrolactamisation

A solution of the crude linear peptide in DMF (0.05 M) was treated with DMTMM.BF_4 (1.3 equiv.) and DIPEA (3 equiv.) and the resulting mixture was stirred at room temperature for 16 h. The solution was concentrated and the resultant residue was partitioned between water (15 mL) and chloroform-isopropanol (3:1 v/v, 50 mL). The aqueous phase was further extracted with chloroform-isopropanol (3:1 v/v, 7 x 50 mL). The combined organic fractions were dried over $MgSO_4$ and evaporated to give the crude cyclic peptide which was then purified by

preparative RP-HPLC [gradient of 10% to 60% acetonitrile (0.1% TFA) in water (0.1% TFA) over 60 min] to afford the desired product as a colourless solid. Yields: 46%-53%.

General procedures for functionalization of peptide side chains and synthesis of metal complexes

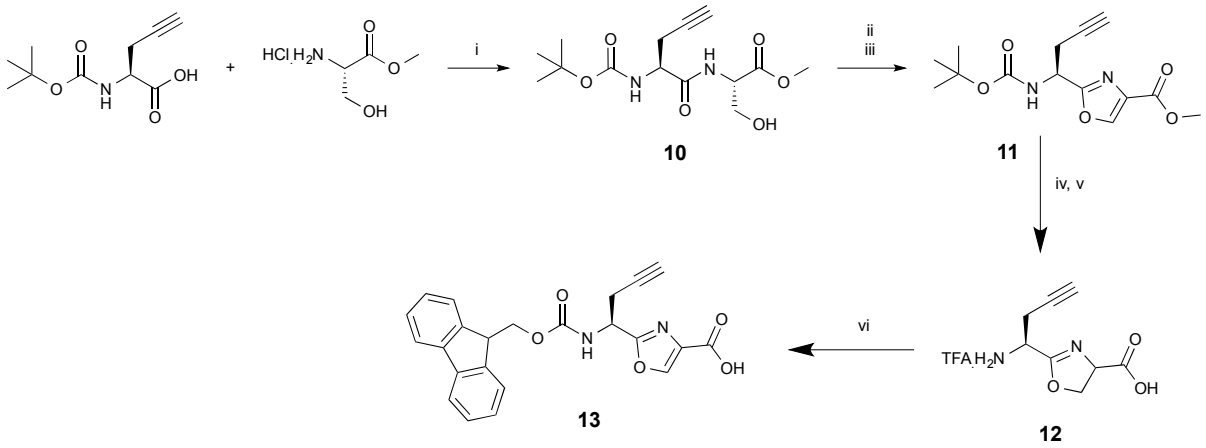
Step 1. Cbz deprotection: A solution of hydrogen bromide in acetic acid (33% v/v, 3.0 mL) was added to the Cbz-protected cyclic peptide (0.07 mmol) and stirred at room temperature for 2h. The reaction mixture was treated with diethyl ether (80 mL) to give a precipitate which was collected by centrifugation. The obtained dihydrobromide salt was then partitioned between chloroform-isopropanol (3:1 v/v, 20 mL) and aqueous NaOH (0.2 M). The aqueous phase was further extracted with chloroform-isopropanol (3:1 v/v, 9 x 20 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure to give the free diamine.

Step 2. Reductive amination: To a stirred solution of the free diamine in anhydrous DMF (5 mL) was added 2-pyridinecarboxaldehyde (20 equiv.) and sodium triacetoxyborohydride (20 equiv.). The resulting mixture was saturated with nitrogen and stirred at room temperature for 16 h. The mixture was then concentrated and treated with aq. NaOH (0.1 M, pH 8). The aqueous phase was further extracted with chloroform-isopropanol (3:1 v/v, 10 x 20 mL). The combined organic fractions were dried over $MgSO_4$ and the solvent was evaporated to give the crude functionalized peptide which was then purified by preparative RP-HPLC [gradient of 0 to 40% acetonitrile (0.05% ammonia) in water (0.05% ammonia) over 50 min] to afford the desired product.

Step 3. Metal complex preparation: To a solution of the peptide in methanol (3 mL) was added an aqueous solution of zinc nitrate (2.0 equiv.). The mixture was stirred at room temperature for 5 min and subsequent evaporation of the solvent afforded the Zn(II) complex in quantitative yield.

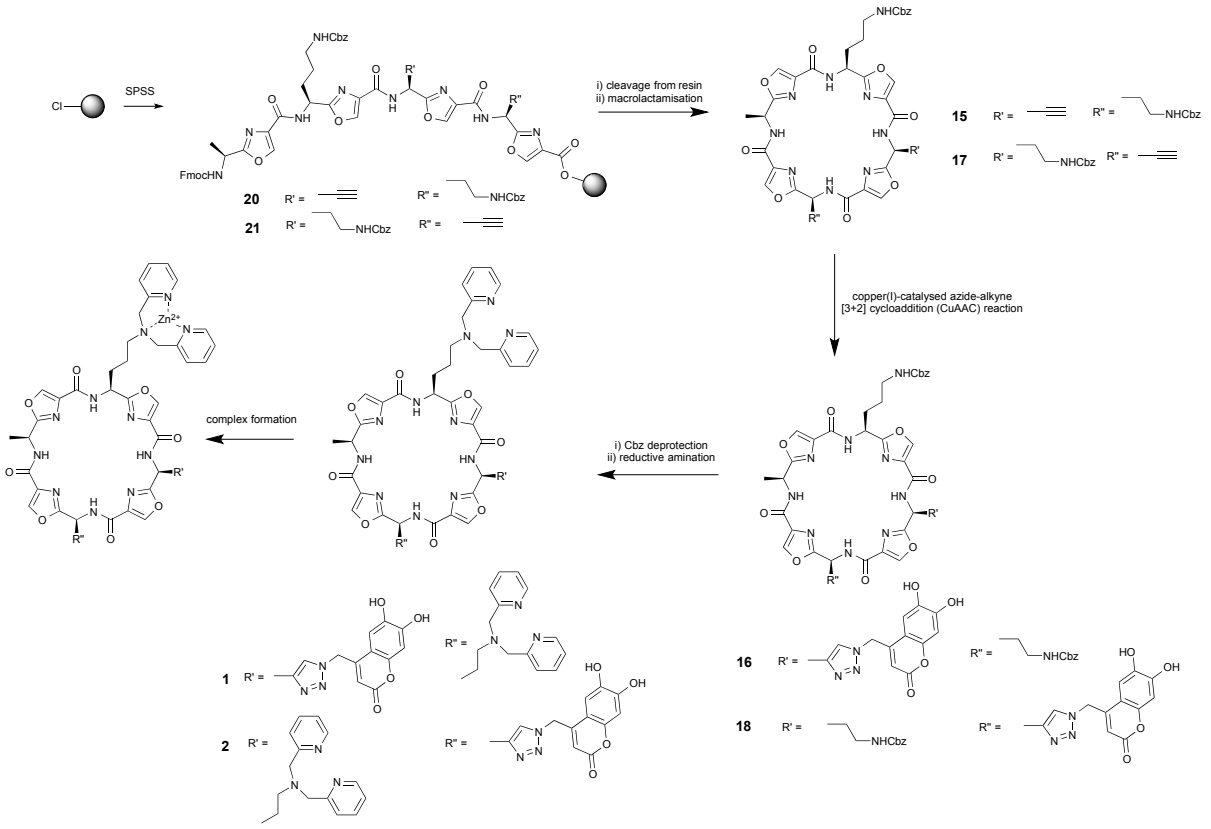
Reaction schemes

Scheme S1

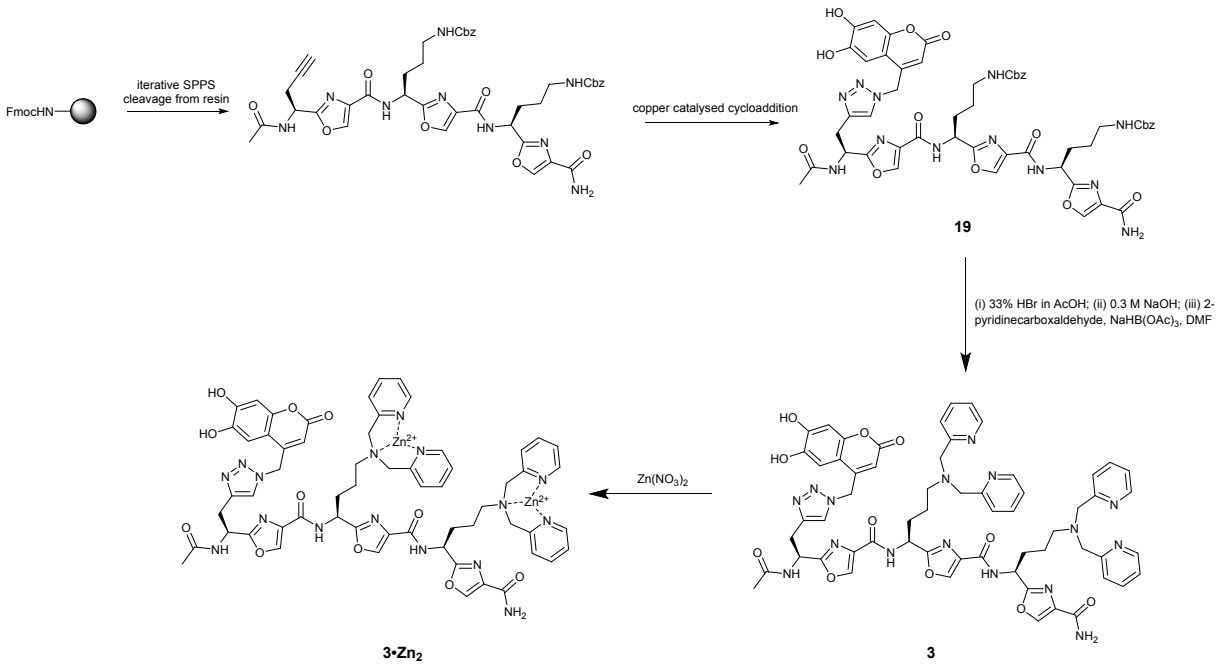


(i) HBTU, HOBt, DIPEA, CH_3CN, 0 °C, (ii) CH_2Cl_2, XtalFluor-E, -78 °C, (iii) $BrCCl_3$, DBU, CH_2Cl_2, 0 °C, (iv) NaOH, MeOH, H_2O, (v) TFA, CH_2Cl_2, (vi) Fmoc-succinimide, 1,4-dioxane.

Scheme S2

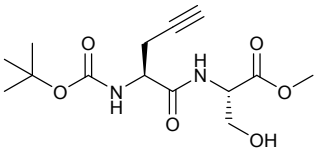


Scheme S3



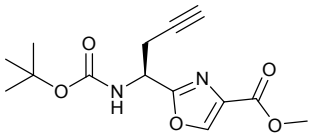
Synthesis

Boc-Pra-Ser-OMe, (10)



To a solution containing Boc-Pra-OH (10.0 g, 46.9 mmol) and the hydrochloride salt of H-Ser-OMe (7.30 g, 46.9 mmol) in CH_3CN (300 mL) was added HBTU (21.4 g, 56.4 mmol) and HOBt (7.60 g, 56.2 mmol). The resulting mixture was cooled to 0 °C and DIPEA (24.7 mL, 140 mmol) was added slowly. The reaction mixture was stirred at rt for 16 h. The solvent was removed under reduced pressure and the residue dissolved in chloroform (800 mL), washed with aqueous sodium hydrogen carbonate solution (3 x 60 mL), dried ($MgSO_4$) and concentrated to give a yellow oil, which was purified by flash column chromatography (silica gel; EtOAc/hexane, from 1:2 to 1:1, v/v) to afford the desired dipeptide **10** as a colourless oil (14.5 g, 82%). $[\alpha]_D^{20}$ = -4.3 (c 0.65 MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 4.58 – 4.47 (m, 1H), 4.36 – 4.22 (m, 1H), 3.91 (dd, *J* = 11.5, 4.0, 1H), 3.79 (dd, *J* = 11.5, 4.0, 1H), 3.74 (s, 3H), 2.75 – 2.64 (m, 2H), 2.63 – 2.51 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.7, 170.6, 155.7, 80.6, 79.4, 71.7, 62.7, 55.0, 53.1, 52.7, 28.3, 22.6; HRMS (ESI) calcd. for $C_{14}H_{22}N_2O_6Na$ $[M + Na]^+$ 337.1376, found 337.1370.

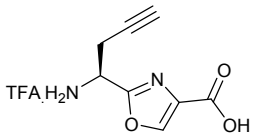
Boc-Pra-Oxz(Ser)-OMe, (11)



Step 1: A solution of dipeptide **10** (8.50 g, 27.0 mmol) in anhydrous CH_2Cl_2 (300 mL) was cooled to -78 °C, then XtalFluor-E (9.29 g, 40.6 mmol) was added portionwise. The reaction mixture was stirred at -78 °C for 3 h and then warmed to 0 °C. Aqueous saturated sodium hydrogen carbonate (300 mL) was added to quench the reaction and the organic phase was separated. The aqueous layer was subsequently warmed to room temperature and further extracted with chloroform (3 x 300 mL). The combined organic fractions were dried over $MgSO_4$ and concentrated under reduced pressure to yield the crude oxazoline as a yellow oil.

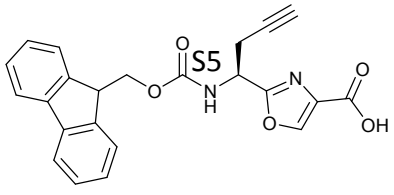
Step 2: $BrCCl_3$ (3.2 mL, 32.5 mmol) and DBU (4.8 mL, 32.1 mmol) was added to a solution containing oxazoline in anhydrous CH_2Cl_2 (300 mL) at 0 °C. The resulting mixture was stirred at room temperature for 3 h and then quenched by the addition of saturated ammonium chloride solution (400 mL). The aqueous layer was further extracted with chloroform (4 x 300 mL). The combined organic fractions were dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow oil which was purified by flash chromatography (silica gel, EtOAc/hexane, 1:4 v/v) to afford **11** as a colourless solid (4.80 g, 60%). mp 83-84 °C; $[\alpha]_D^{20}$ = -41.6 (c 0.68 MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.19 (s, 1H), 5.54 (d, *J* = 7.6, 1H), 5.10 (m, 1H), 3.87 (s, 3H), 2.87 - 2.80 (m, 2H), 1.96 (s, 1H), 1.41 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.6, 161.5, 154.9, 144.5, 133.4, 80.6, 78.2, 72.1, 52.3, 47.9, 28.3, 24.5; HRMS (ESI) calcd. for $C_{14}H_{18}N_2O_5Na$ $[M + Na]^+$ 317.1113, found 317.1108.

TFA.H-Pra-Oxz(Ser)-OH, (12)



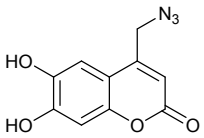
Step 1: A solution of **11** (2.94 g, 10.0 mmol) in methanol (50 mL) was treated with a solution of NaOH (4.00 g, 100 mmol) in water (15 mL), and the mixture was stirred at room temperature for 16 h. The reaction mixture was then acidified with hydrochloric acid (1.0 M) until pH 6 and evaporated to give a solid which was suspended in chloroform/isopropanol (3:1 v/v, 800 mL). The suspension was subsequently filtered and the filtrate was concentrated to give a colourless foam (2.80 g).
Step 2: The colourless foam obtained in *step 1* (2.80 g) was dissolved in CH_2Cl_2 (40 mL) and treated with trifluoroacetic acid (40 mL) at 0 °C. This was stirred at room temperature for 1.5 h, the reaction mixture was then concentrated to afford the product **12** as a yellow oil (2.79 g, quant. over two steps).

Fmoc-Pra-Oxz(Ser)-OH, (13)



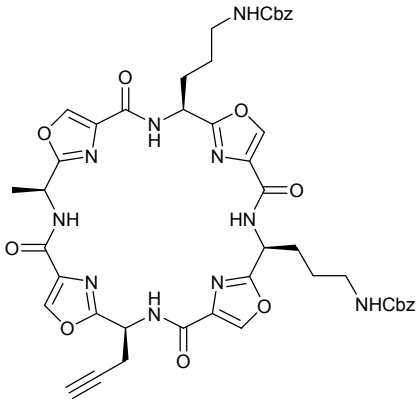
Compound **12** (2.79 g, 10.0 mmol) was treated with aqueous saturated sodium carbonate solution until pH 9 followed by addition of a solution of Fmoc-succinimide (3.20 g, 9.5 mmol) in 1,4-dioxane (20 mL). The mixture was diluted with 1,4-dioxane/water (1:1 v/v, 50 mL) and stirred at room temperature for 16 h. After removal of the solvents under reduced pressure, the residue was diluted with water (200 mL) and adjusted to pH 5 with hydrochloric acid (1.0 M, 125 mL). The solution was filtered to give the crude product which was triturated with hexane/Et_2O (5:1 v/v, 2 x 60 mL) to give **13** as a colourless solid (3.33 g, 83%). m.p. 136-138 °C; $[\alpha]_D^{20}$ = -39.1 (*c* 0.45 MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 8.46 – 8.41 (s, 1H), 7.79 (d, *J* = 7.5, 2H), 7.67 (d, *J* = 7.5, 2H), 7.39 (t, *J* = 7.5, 2H), 7.30 (t, *J* = 7.5, 2H), 5.04 (t, *J* = 6.8, 1H), 4.39 (t, *J* = 6.4, 2H), 4.23 (t, *J* = 6.8, 1H), 2.98 – 2.78 (m, 2H), 2.37 – 2.34 (s, 1H). ; ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.2, 162.6, 156.1, 144.3, 143.5, 141.1, 133.6, 127.5, 126.9, 124.9, 119.7, 78.0, 77.4, 71.5, 67.0, 46.9, 23.5; HRMS (ESI) calcd. for $C_{23}H_{18}N_2O_5Na$ $[M + Na]^+$ 425.1113, found 425.1104.

6,7-Dihydroxy-4-azidomethyl coumarin (9)



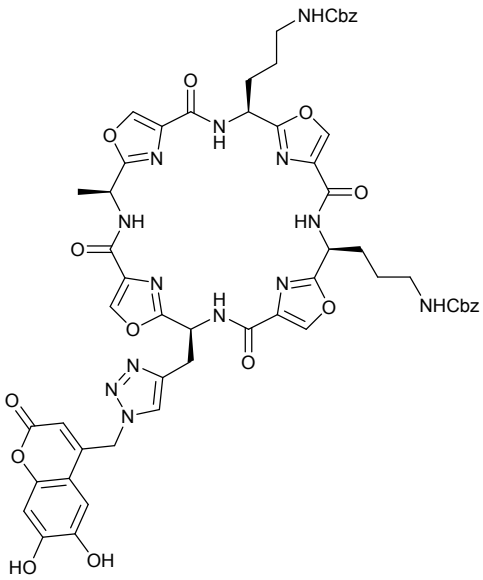
To a solution of 4-chloromethyl-6,7-dihydroxy coumarin (0.45 g, 2.0 mmol) in acetone (5 mL) was added a solution of sodium azide (0.25 g, 4.0 mmol) in water (0.5 mL). The mixture was then stirred at room temperature for 16 h and ice water (10 mL) was added, the resulting precipitate was isolated and dried to give the desired product **9** as a brown solid (0.33 g, 71%). IR ν_{max}/cm^{-1} 3485, 3427, 3124, 3088, 3045, 2190, 1728, 1624, 1468, 1412, 1362, 1283, 1238, 1215, 1156, 1047, 874; ^{1}H NMR (300 MHz, DMSO): δ 6.98 (s, 1H), 6.76 (s, 1H), 6.24 (s, 1H), 4.71 (s, 2H); HRMS (ESI) calcd. For $C_{10}H_7N_3O_4Na$ $[M + Na]^+$ 256.0334, found 256.0331.

Cyclo[Ala-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)-Pra-Oxz(Ser)], (15)



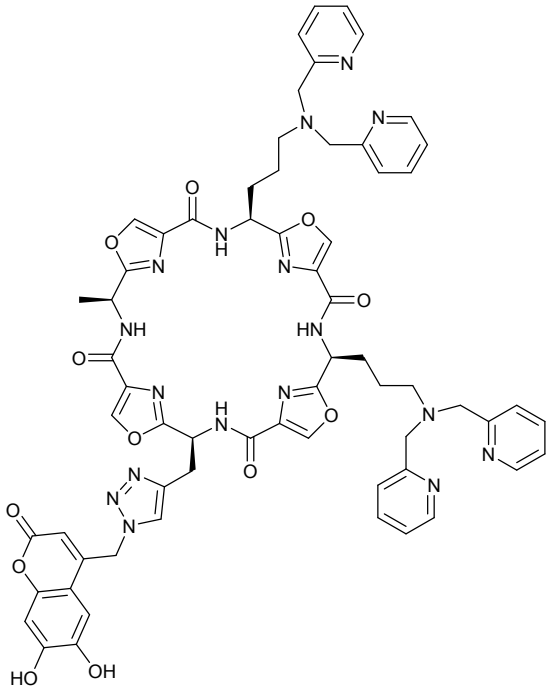
Cyclic peptide **15** was synthesized according to general procedures for SPPS on 2-chlorotrityl chloride resin followed by macrolactamisation). The crude product was used directly in the next step without purification.

Cyclo[Ala-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)-Ala(1-DHCMT)-Oxz(Ser)], (16)



Compound **16** was synthesized in 41% yield following the procedure described above; ^{1}H NMR (500 MHz, CD_3OD): δ 8.40 – 8.32 (m, 3H), 8.29 (s, 1H), 8.07 (s, 1H), 7.36 – 7.21 (m, 10H), 6.99 (s, 1H), 6.75 (s, 1H), 5.77 (s, 2H), 5.54 (s, 1H), 5.50 – 5.34 (m, 2H), 5.03 (d, *J* = 7.6, 4H), 3.24 – 3.05 (m, 4H), 2.20 – 1.90 (m, 4H), 1.74 – 1.45 (m, 4H), 1.65 (d, *J* = 6.6, 3H).

Cyclo[Ala-Oxz(Ser)-Orn(DPA)-Oxz(Ser)-Orn(DPA)-Oxz(Ser)-Ala(1-DHCMT)-Oxz(Ser)] (1)



Step 1: Cyclic peptide **16** (0.05 g, 0.043 mmol) was treated with a solution of hydrogen bromide in acetic acid (33% v/v, 2.5 mL) and the mixture was stirred at room temperature for 30 min. The resulting solution was then added to diethyl ether (60 mL) dropwise. The precipitate was collected by centrifuge and dissolved in MeOH-water (4:1 v/v), the solution was then treated with basic ion exchange resin (Ambersep® 900 hydroxide) to give the free diamine in quantitative yield.

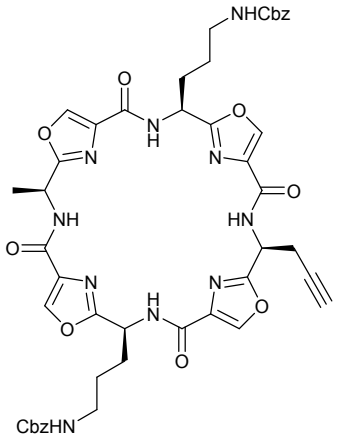
Step 2: Synthesis of **1** was achieved according to the general procedure for reductive amination. Subsequent preparative RP-HPLC purification [gradient of 0 to 30% acetonitrile (0.05% ammonia) in water (0.05% ammonia) over 50 min] afforded the desired product as a yellow solid (32 mg, 55%). $[\alpha]_D^{20}$ = -107.8 (*c* 0.18 MeOH); IR

ν_{max}/cm^{-1} 3352 (br), 3112, 3036, 1743, 1718, 1663, 1592, 1524, 1346, 1212; ^{1}H NMR (500 MHz, MeOD) δ = 8.39 (m, 4H), 8.32 (s, 1H), 8.31 (s, 1H), 8.30 (s, 1H), 8.28 (s, 1H), 7.91 (s, 1H), 7.73 (m4H), 7.57 (t, *J* = 8.6, 4H), 7.25 – 7.20 (m, 4H), 6.98 (s, 1H), 6.73 (s, 1H), 5.76 (m, 3H), 5.49 (s, 1H), 5.46 (q, *J* = 7.0, 1H), 5.29 (m, 2H), 3.76 (m, 8H), 3.52 (m, 2H), 2.58 (m, 4H), 2.14 – 2.02 (m, 2H), 2.02 – 1.95 (m, 2H), 1.92 – 1.83 (m, 2H), 1.65 (d, *J* = 7.1, 3H), 1.67 - 1.55 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 165.0, 164.6, 163.6, 161.0, 160.0, 159.7, 151.1, 151.0, 149.1, 143.9, 143.3, 142.8, 136.9, 135.9, 135.8, 135.6, 124.8, 123.1, 122.5, 108.1, 102.9, 60.1, 53.3, 49.7, 46.7, 46.4, 42.6, 30.5, 29.2, 23.4, 19.0; HRMS (ESI) calcd. for $C_{64}H_{62}N_{17}O_{12}$ $[M + H]^+$ 1260.4758, found 1260.4760.

1·Zn$_2$

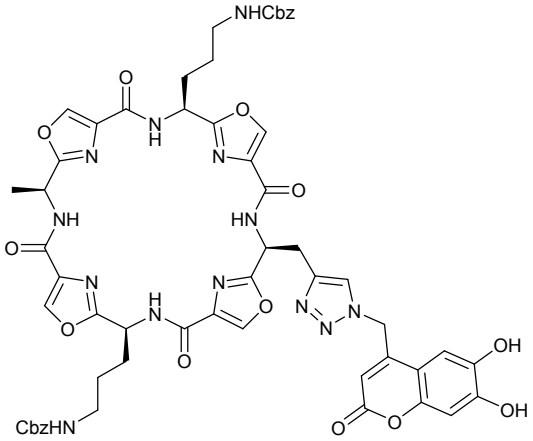
The general procedure for metal complex formation was followed to give **1·Zn$_2$** from **1**. HRMS (ESI) calcd. for $C_{64}H_{59}N_{17}O_{12}Zn_2$ $[M - 2H]^{2+}$ 692.65505, found 692.65510.

Cyclo[Ala-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)-Pra-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)], (17)



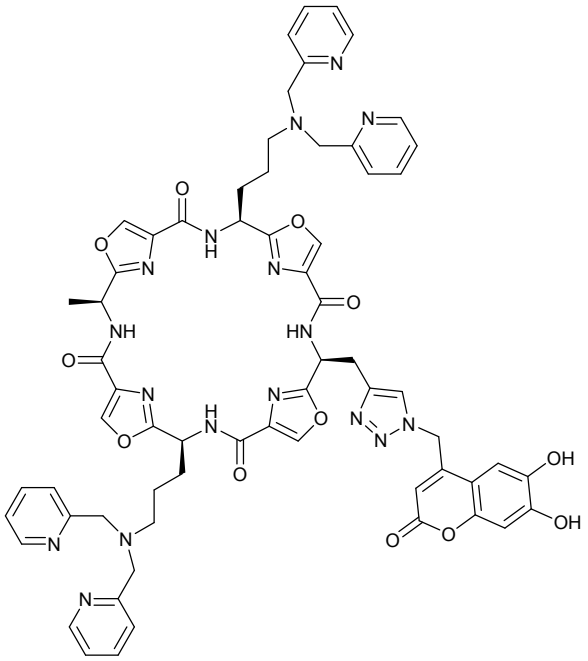
Cyclic peptide **17** was synthesized according to the general procedures for SPPS on 2-chlorotrityl chloride resin followed by macrolactamisation. The crude product was used directly in the next step without purification.

Cyclo[Ala-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)-Ala(1-DHCMT)-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)], (18)



To a solution of the crude cyclic peptide **18** (0.17 g, 0.2 mmol) in DMF (5 mL) was added azide **10** (0.04 g, 0.2 mmol), $CuSO_4.5H_2O$ (0.05 g, 0.2 mmol), and sodium ascorbate (0.04 g, 0.2 mmol). The suspension was then allowed to stir at rt for 16 h. The suspension was concentrated and the residue partitioned between aqueous EDTA solution (20 mL) and chloroform/isopropanol (3:1 v/v, 200 mL). The aqueous phase was further extracted with chloroform/isopropanol (3:1 v/v, 7 x 30 mL) and the combined organic fractions were evaporated to give the crude product which was then purified by preparative RP-HPLC [gradient of 10% to 60% acetonitrile (0.1% TFA) in water (0.1% TFA) over 60 min] to afford the desired product as a brown solid (91 mg, 39%). ^{1}H NMR (300 MHz, CD_3OD): δ 8.40 - 8.33 (m, 3H), 8.31 (s, 1H), 7.92 (s, 1H), 7.40 - 7.21 (m, 10H), 6.99 (s, 1H), 6.76 (s, 1H), 5.82 – 5.71 (m, 3H), 5.51 (s, 1H), 5.44 – 5.35 (m, 3H), 5.10 – 4.98 (m, 4H), 3.65 – 3.39 (m, 2H), 3.25 – 3.07 (m, 4H), 2.23 – 1.86 (m, 4H), 1.74 – 1.44 (m, 4H), 1.66 (s, 3H).

Cyclo[Ala-Oxz(Ser)-Orn(DPA)-Oxz(Ser)-Ala(1-DHCMT)-Oxz(Ser)-Orn(DPA)-Oxz(Ser)], (2)

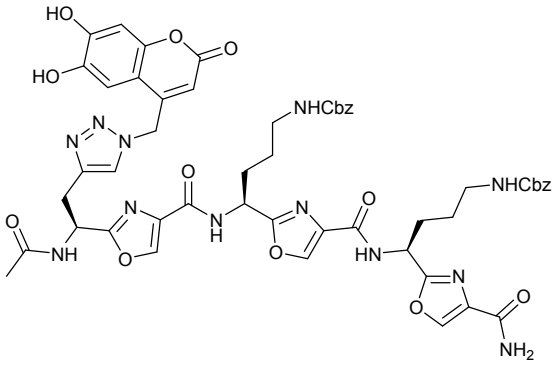


Cyclic peptide **2** was prepared as a yellow solid (32 mg, 55%) following the procedure described above for synthesis of **1**. $[\alpha]_D^{20}$ = -100.3 (*c* 0.22 MeOH); IR ν_{max}/cm^{-1} 3365 (br), 3142, 3025, 1752, 1745, 1678, 1601, 1516, 1455, 1362, 1184, 1118; ^{1}H NMR (400 MHz, CD_3OD) δ 8.43 - 8.36 (m, 4H), 8.36 - 8.33 (m, 3H), 8.31 (s, 1H), 7.93 (s, 1H), 7.75 (t, *J* = 7.9, 4H), 7.58 (d, *J* = 8.0, 4H), 7.27 - 7.18 (m, 4H), 6.98 (s, 1H), 6.70 (s, 1H), 5.80 - 5.72 (m, 3H), 5.53 – 5.44 (m, 1H), 5.39 (s, 1H), 5.30 - 5.23 (m, 2H), 3.76 (s, 8H), 3.58 – 3.43 (m, 2H), 2.61 - 2.54 (m, 4H), 2.13 – 2.00 (m, 2H), 2.00 – 1.83 (m, 2H), 1.66 (d, *J* = 6.5, 3H), 1.65 – 1.52 (m, 2H), 1.29 (s, 2H), ^{13}C NMR: (125 MHz, DMSO-d6): 164.5, 164.3, 164.3, 163.2, 160.4, 159.6, 159.5, 159.5, 159.3, 159.3, 150.7, 148.7, 148.4, 143.5, 142.8, 142.3, 142.3, 142.18, 136.4, 135.5, 135.3, 135.2, 124.3, 122.6, 122.6, 122.0, 108.0, 107.5, 102.5, 59.7, 52.9, 52.8, 49.2, 46.4, 42.2, 31.3, 30.0, 29.4, 29.0, 29.0, 29.0, 28.7, 28.7, 26.2, 22.9, 22.1, 18.6, 13.9, 13.6; HRMS (ESI) calcd. for $C_{64}H_{62}N_{17}O_{12}$ $[M + H]^+$ 1260.4764, found 1260.4774.

2·Zn$_2$

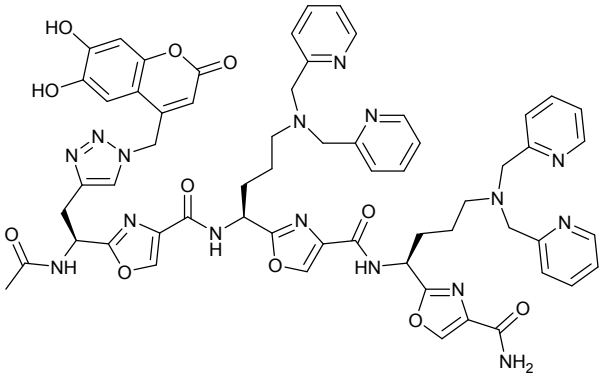
The general procedure for metal complex formation was followed to give **2·Zn$_2$** from **2**. HRMS (ESI) calcd. for $C_{64}H_{59}N_{17}O_{12}Zn_2$ $[M - 2H]^{2+}$ 692.65505, found 692.65528.

Ac-Ala(1-DHCMT)-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)-Orn(Cbz)-Oxz(Ser)-NH$_2$ (19)



Preparation of **19** was achieved according to the general procedures for SPPS on Rink Amide AM resin. After acetylation, the click reaction was carried out using azide **9** (1.5 equiv.), $CuSO_4.5H_2O$ (0.5 equiv.) and sodium ascorbate (0.5 equiv.).

Ac-Ala(1-DHCMT)-Oxz(Ser)-Orn(DPA)-Oxz(Ser)-Orn(DPA)-Oxz(Ser)-NH$_2$, (3)



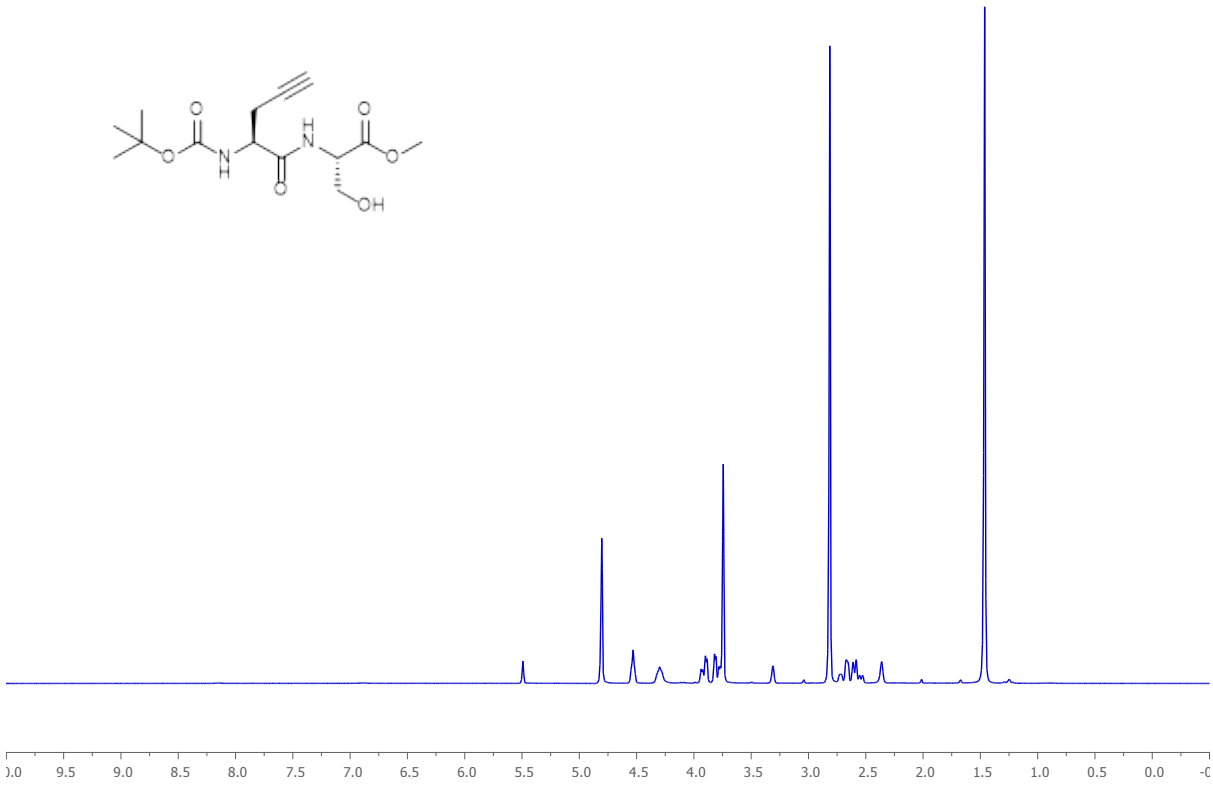
Step 1: A solution of hydrogen bromide in acetic acid (33% v/v, 6 mL) was added to the tripeptide 19 (0.13 g, 0.12 mmol) and the suspension was stirred at room temperature for 50 min. The resulting mixture was then added to diethyl ether (80 mL) dropwise. The yellow precipitate was collected by centrifuge and dissolved in MeOH/water (4:1 v/v), the solution was then treated with basic ion exchange resin (Ambersep® 900 hydroxide) to give the free diamine (0.10 g, quant.).

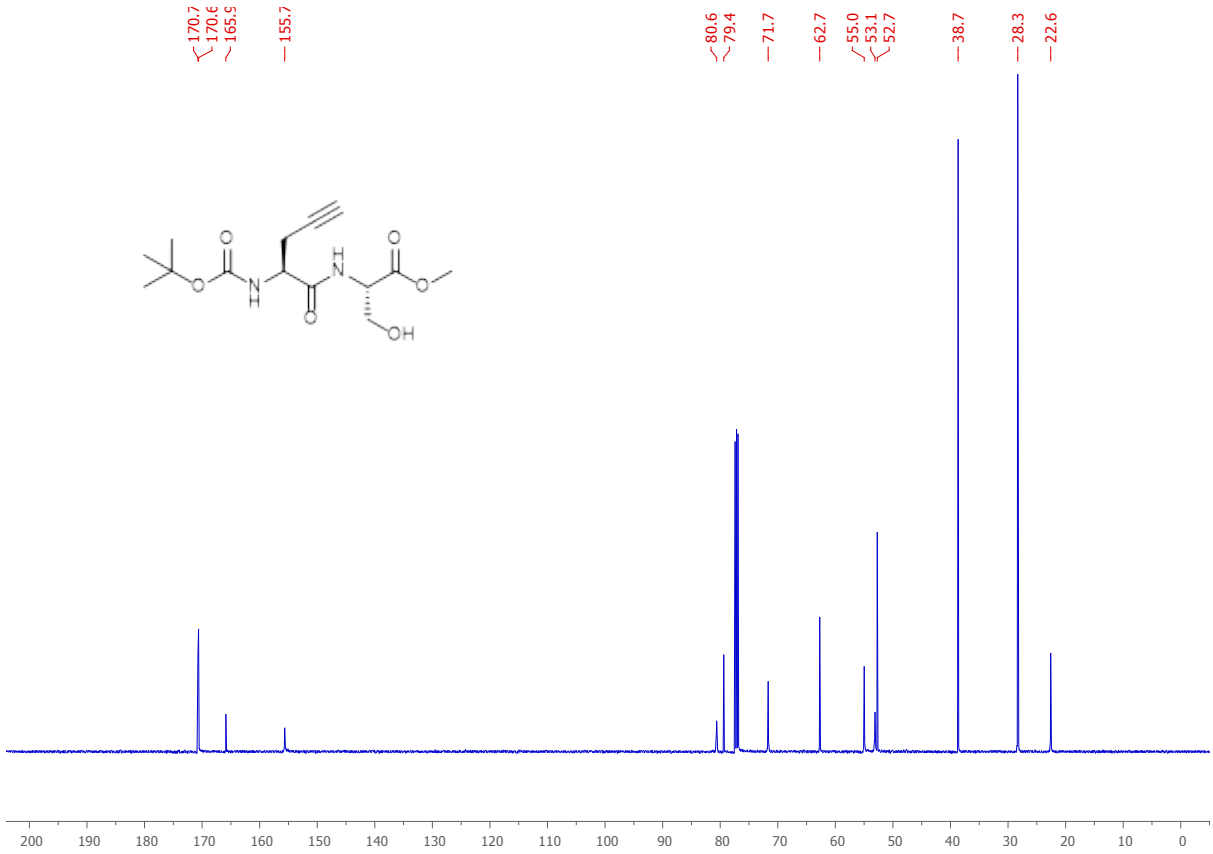
Step 2: Synthesis of **3** was achieved according to the general procedure for reductive amination. Preparative RP-HPLC purification [gradient of 0 to 22% acetonitrile (0.05% ammonia) in water (0.05% ammonia) over 50 min] gave the desired product **3** as a yellow solid (68 mg, 48%). $[\alpha]_D^{20}$ = -30.2 (*c* 0.21 MeOH); IR ν_{max}/cm^{-1} 3126, 3063, 1746, 1377, 1214; ^{1}H NMR (400 MHz, CD_3OD) δ 8.42 – 8.35 (m, 4H), 8.32 (s, 1H), 8.30 (s, 1H), 8.28 (s, 1H), 7.90 (s, 1H), 7.74 (t, *J* = 7.5, 4H), 7.58 (d, *J* = 7.5, 4H), 7.26 – 7.19 (m, 4H), 7.01 (s, 1H), 6.73 (s, 1H), 5.76 (s, 2H), 5.47 (s, 1H), 5.49 – 5.40 (m, 1H), 5.23 – 5.13 (m, 2H), 3.76 (s, 8H), 3.45 (dd, *J* = 15.2, 7.5, 1H), 3.37 – 3.32 (m, 1H), 2.58 (t, *J* = 6.1, 4H), 2.18 – 2.03 (m, 2H), 2.02 – 1.90 (m, 2H), 1.93 (s, 3H), 1.70 – 1.47 (m, 4H); ^{13}C NMR (75 MHz, DMSO) δ 169.4, 163.9, 163.7, 163.4, 161.9, 160.6, 160.0, 160.0, 159.4, 151.3, 150.7, 148.8, 148.4, 143.2, 143.1, 142.6, 142.4, 142.0, 136.6, 136.1, 135.4, 124.4, 122.8, 122.2, 108.7, 102.8, 59.8, 53.1, 46.9, 46.6, 29.6, 28.9, 23.1, 22.3. HRMS (ESI) calcd. for $C_{60}H_{61}N_{16}O_{11}$ $[M + H]^+$ 1181.4706, found 1181.4710.

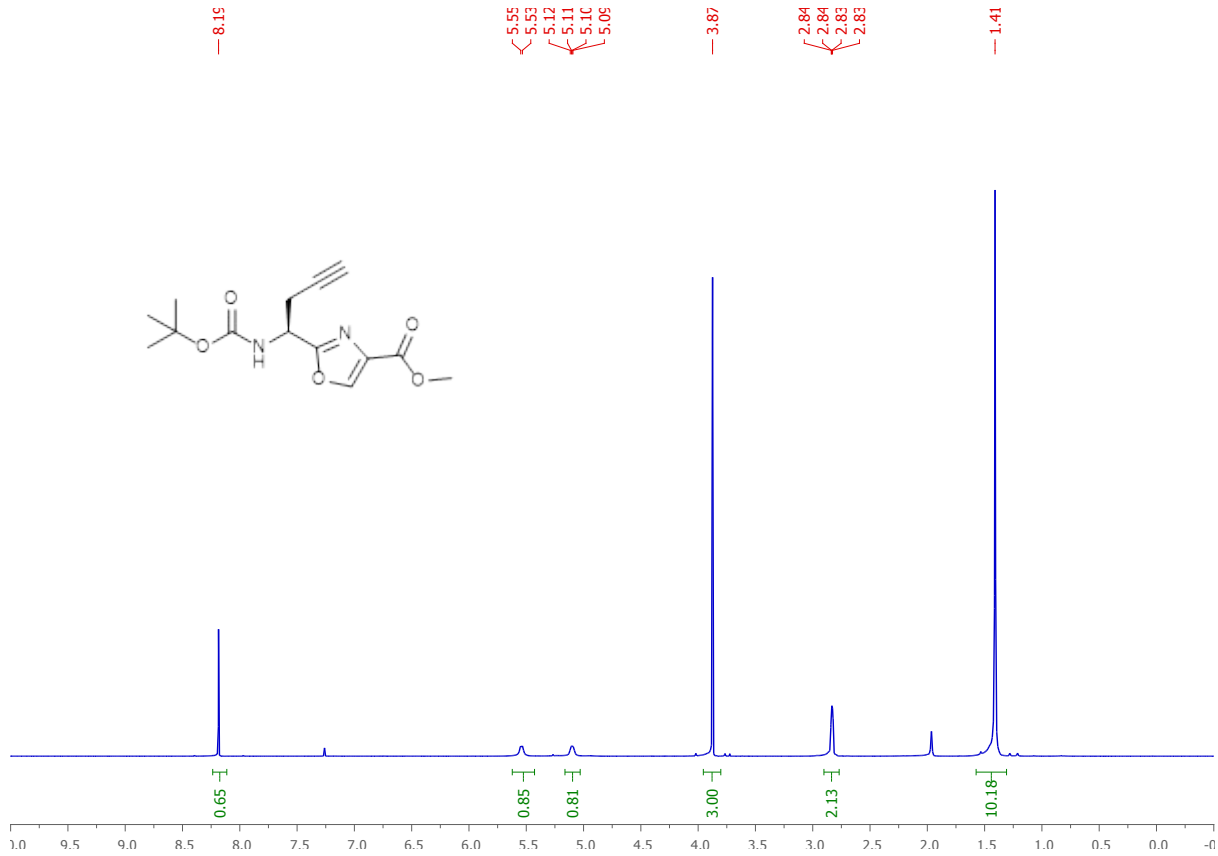
3·Zn_2

The general procedure for metal complex formation was followed to give **3·Zn_2** from **3**. HRMS (ESI) calcd. for $C_{60}H_{58}N_{17}O_{12}Zn_2$ $[M - 2H]^{2+}$ 653.15215, found 653.15217.

NMR spectra



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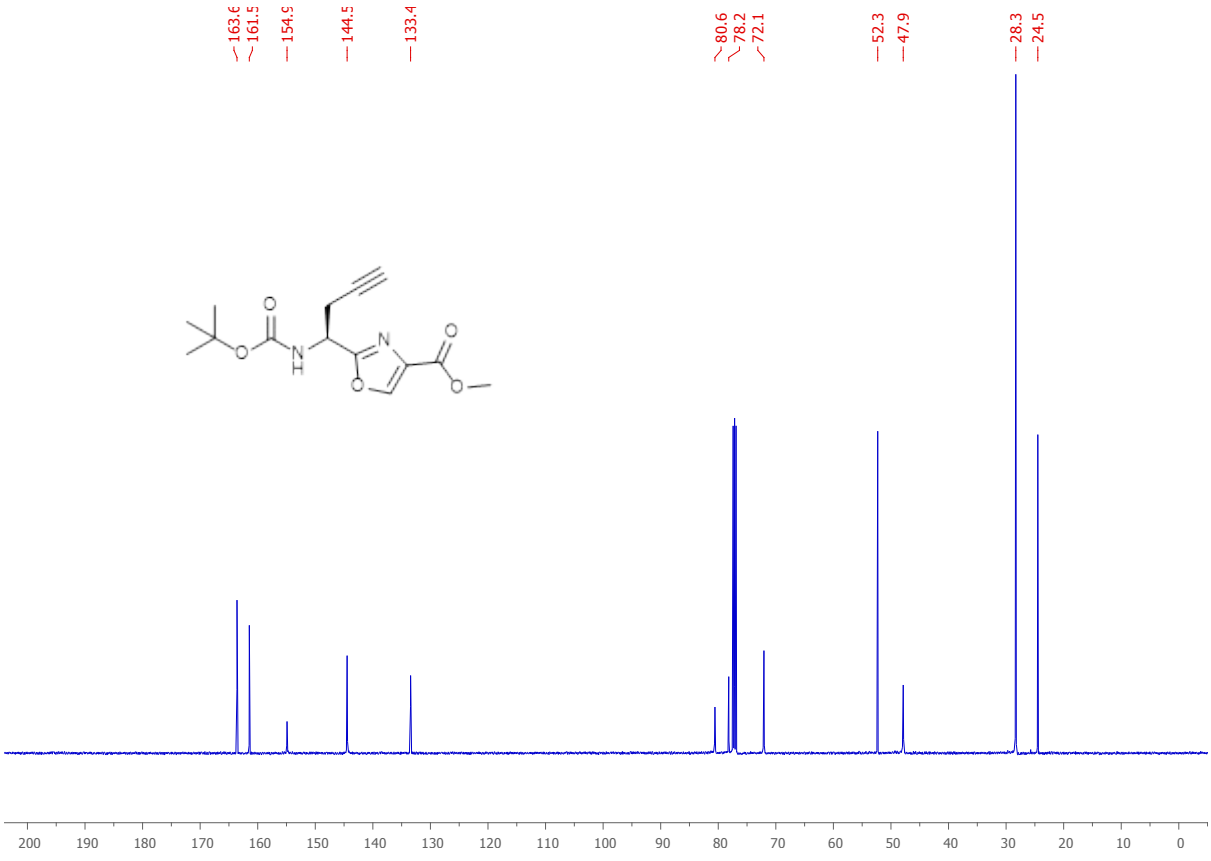
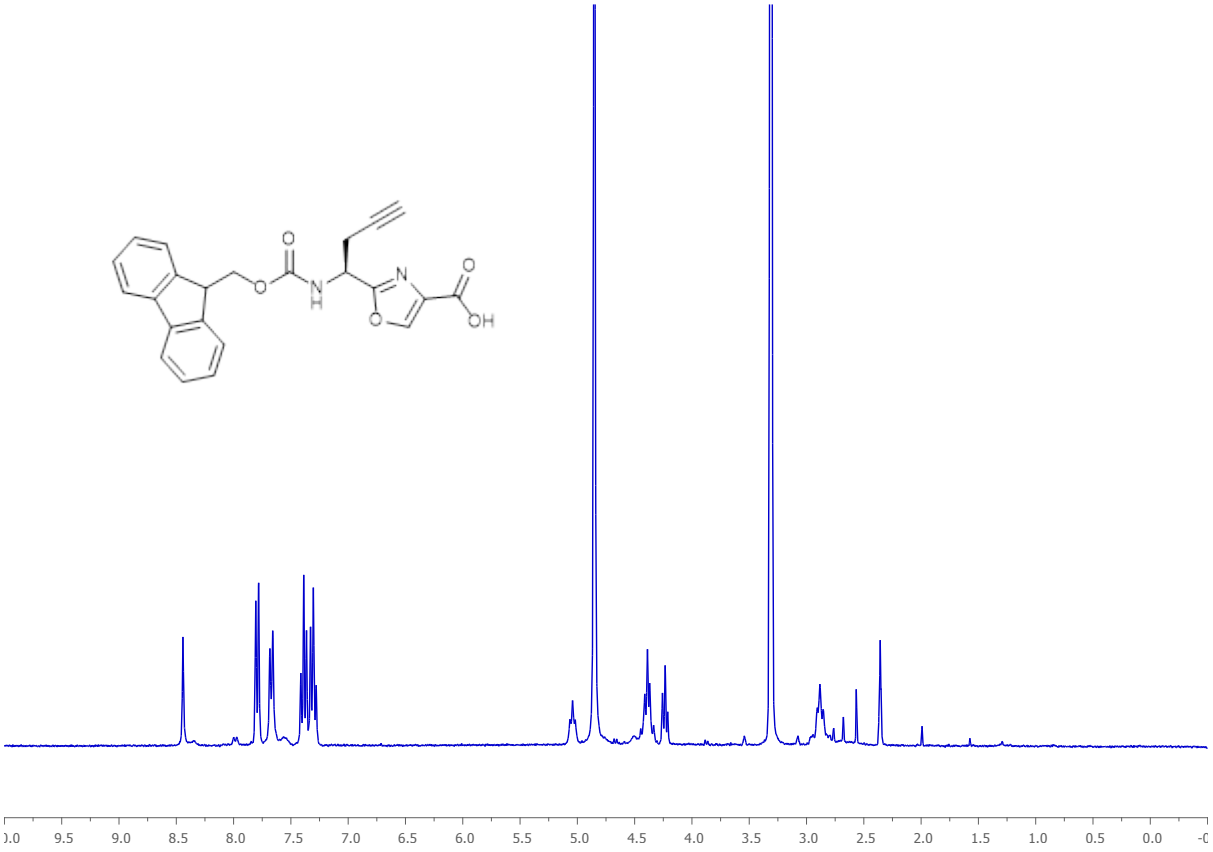
5.53
5.12
5.11
5.10
5.09
3.87
2.84
2.84
2.83
2.83
1.41
0.65
0.85
0.81
3.00
2.13
10.18


163.6
161.5
154.9
144.5
133.4
80.6
78.2
72.1
52.3
47.9
28.3
24.5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7.0
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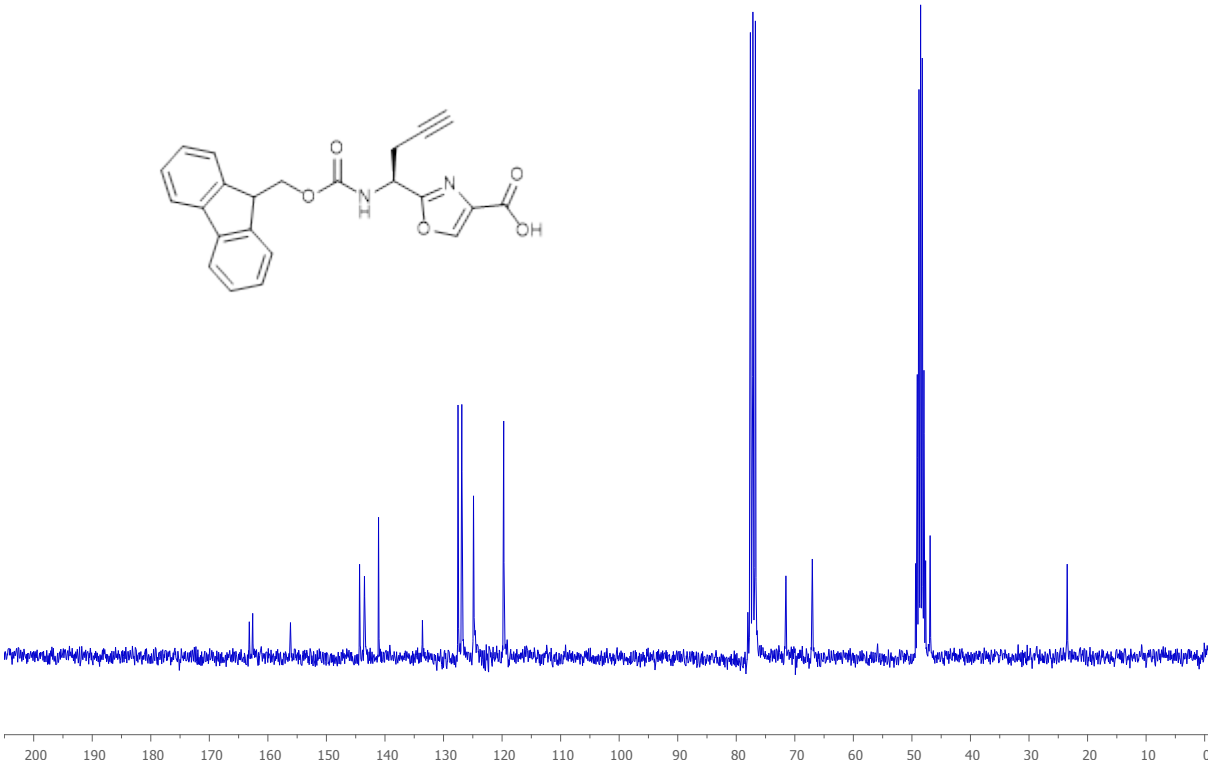
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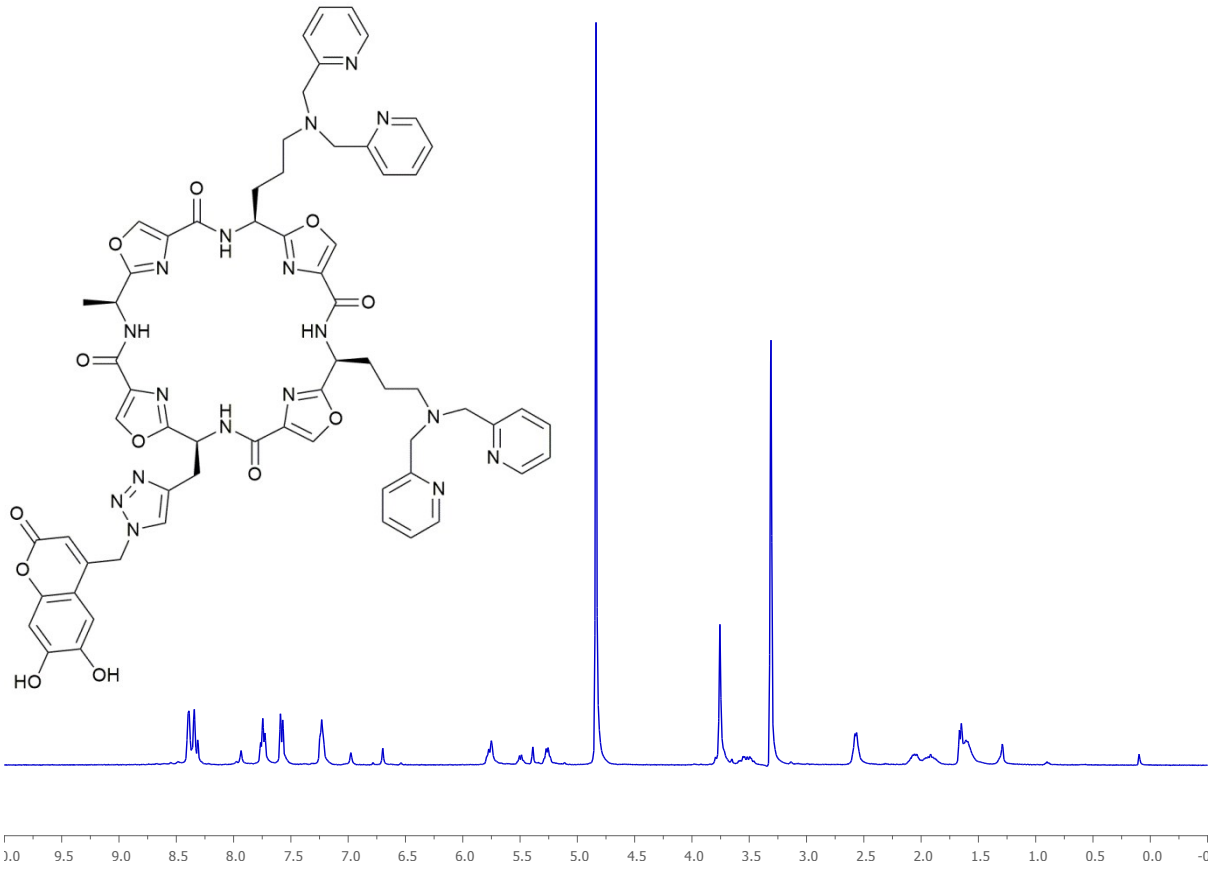
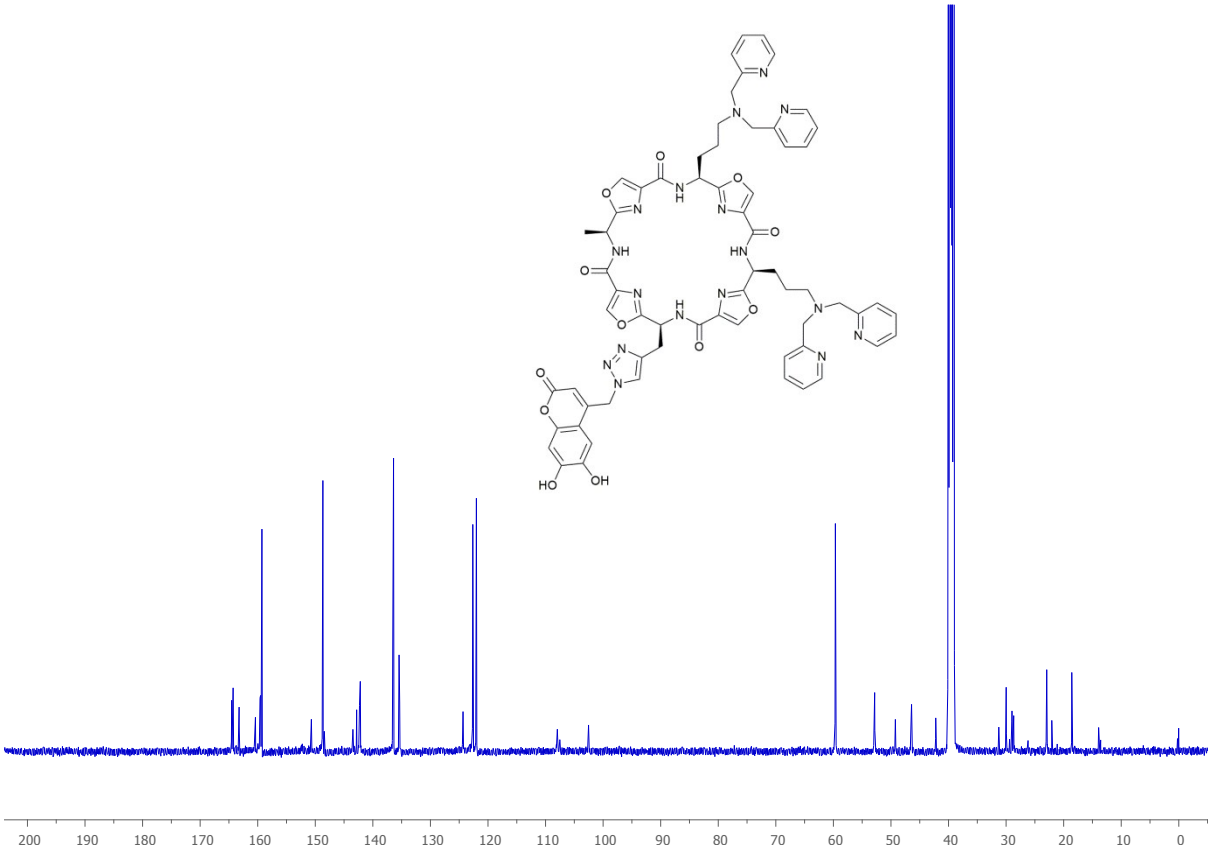

9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0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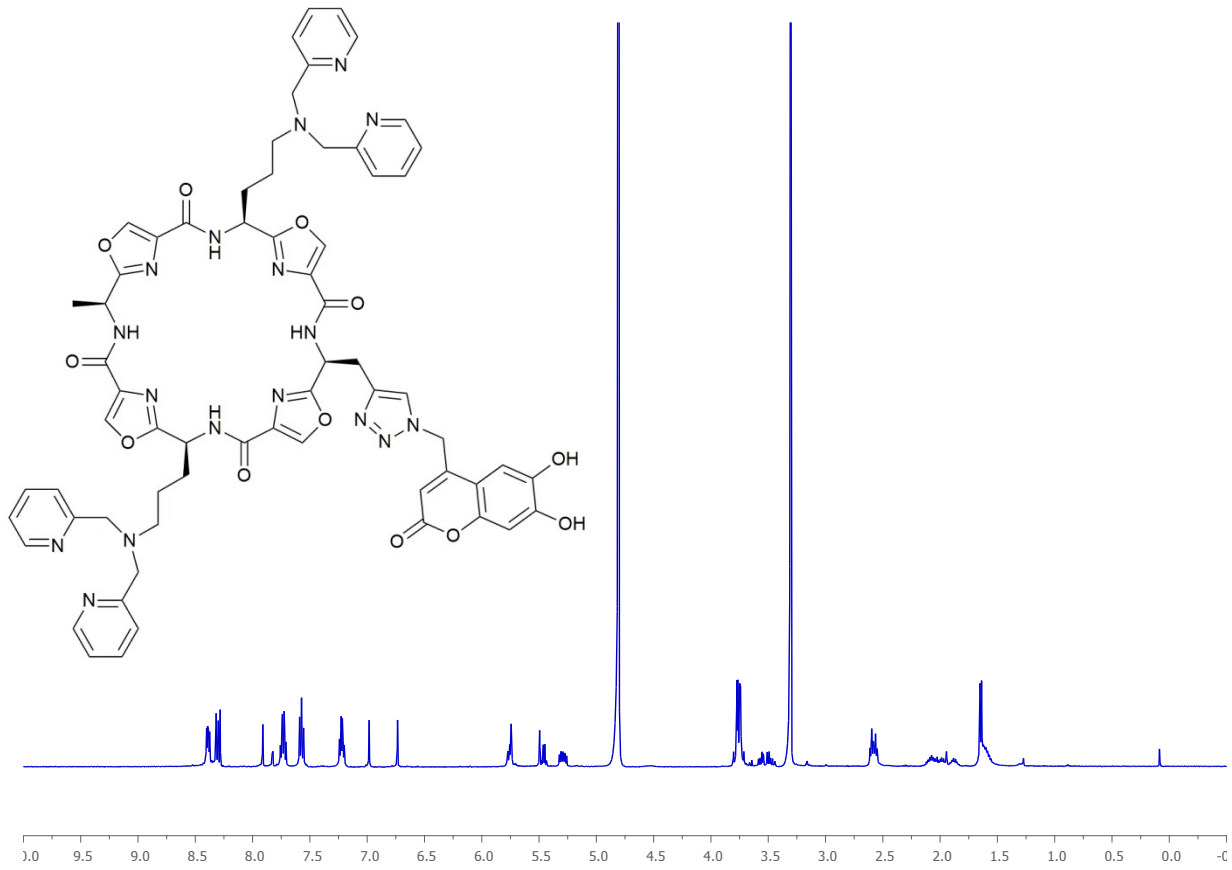
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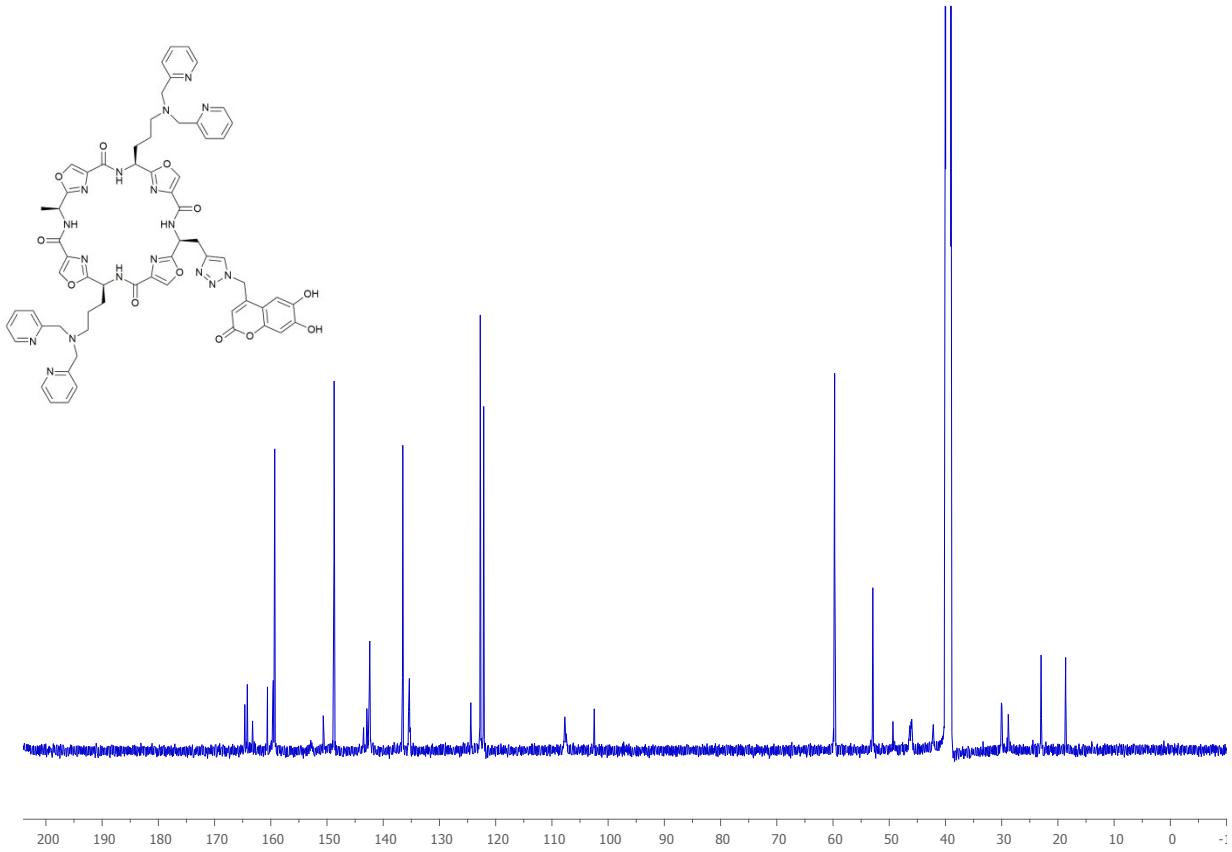
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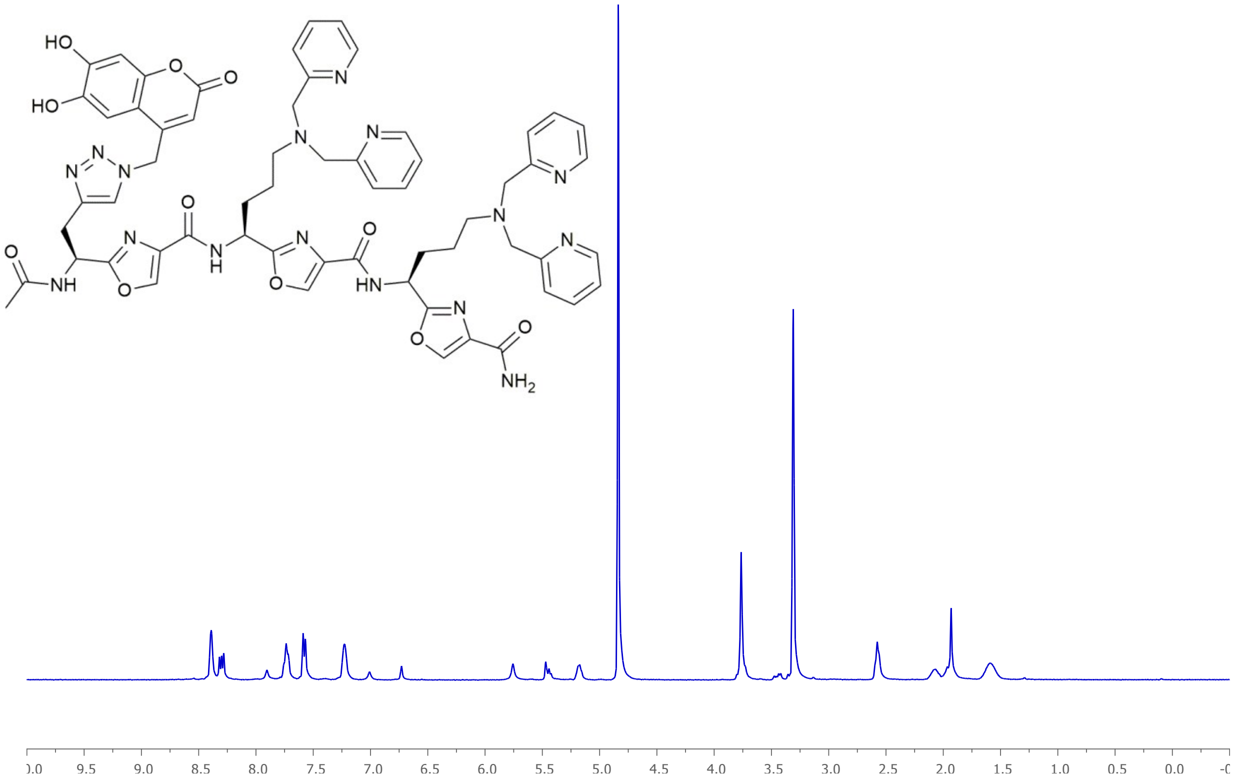
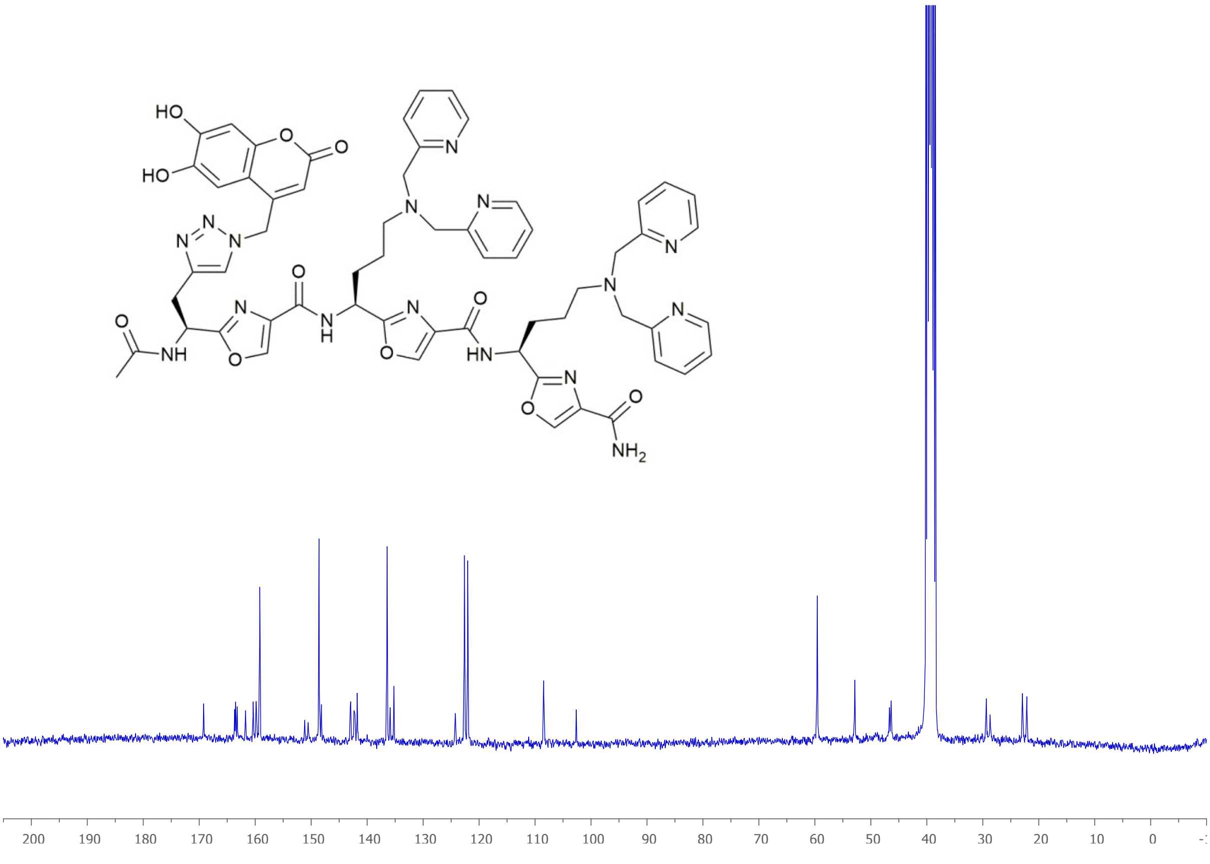


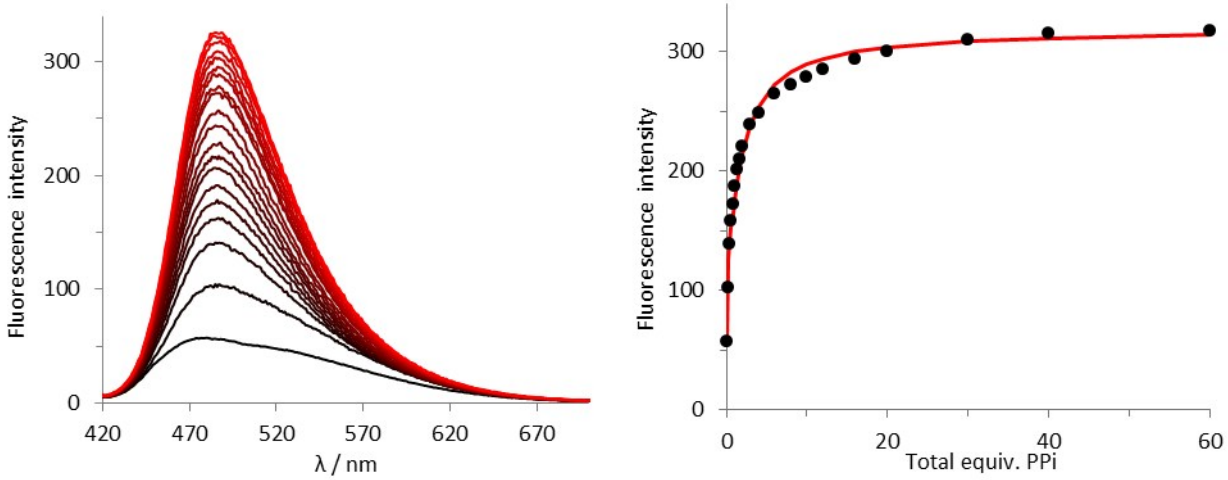


Fluorescence titrations of 1-3·Zn_2 with PPi, ADP and ATP

Stock solutions of receptors (5 μM of **1-3·Zn_2**, and of the anion (2 mM) were prepared respectively. An aliquot of receptor solution (2.5 mL) was placed in a 1 cm quartz glass cuvette and incubated at 25 °C for 5 min, the fluorescence emission spectrum from 400-700 nm or 360-600 nm was recorded (λ_{ex} = 390 nm for **1-3·Zn_2**). Aliquots of a solution containing the anion were then added to the sample cuvette, followed by stirring for 20 seconds, the fluorescence emission spectrum was collected.

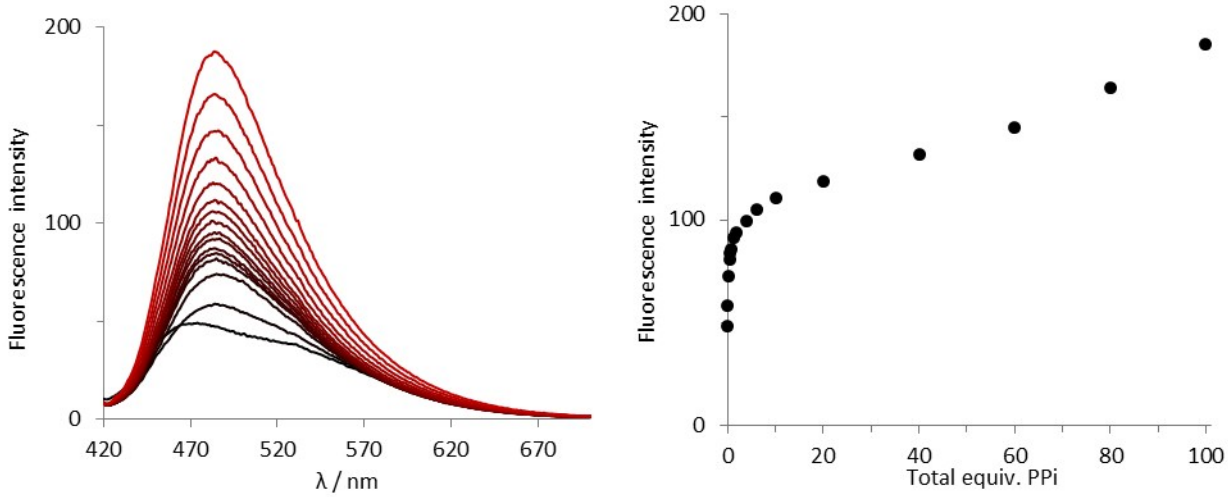
To assess the apparent association constant for the receptor-anion, the fluorescence data over the range 440-580 nm or 380-520 nm was fitted with nonlinear least squares curve fitting program HypSpec® (Hyperquad® package, global analysis)[119] based on 1:1 binding model.

1·Zn_2 with PPi



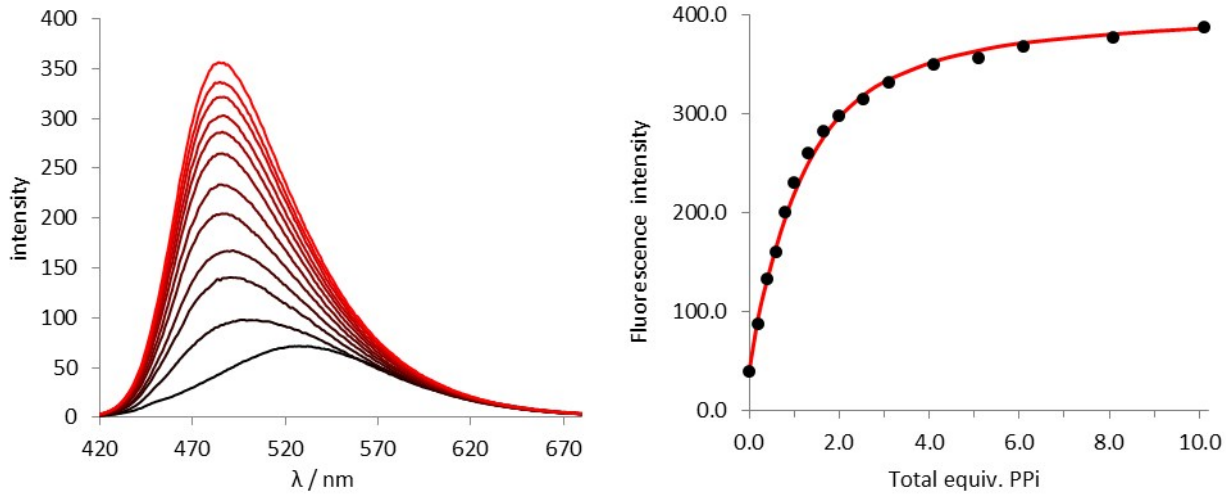
(left) Fluorescence emission spectra of **1·Zn_2** (5 μM) in response to PPi (0-300 μM) in HEPES (5 mM, 145 mM NaCl, pH 7.4) at 25 °C. λ_{ex} = 390 nm, slits = 5 nm/5 nm. (right) Binding isotherm (—) obtained by fitting the observed fluorescence intensities at 480 nm (●) to a 1:1 binding model using non-linear regression.

2·Zn_2 with PPi



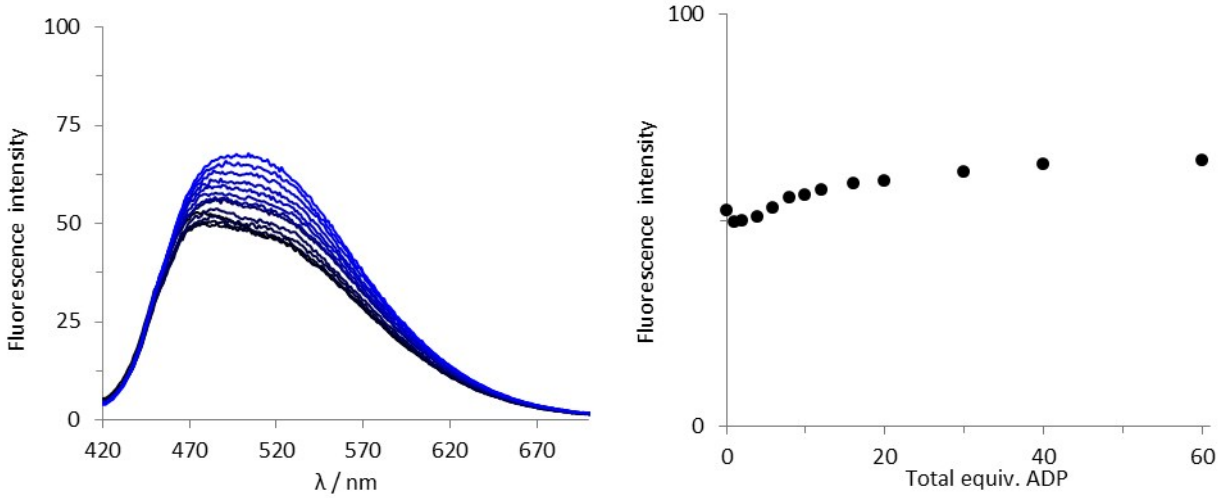
(left) Fluorescence emission spectra of **2·Zn_2** (5 μM) in response to PPi (0-500 μM) in HEPES (5 mM, 145 mM NaCl, pH 7.4) at 25 °C. λ_{ex} = 390 nm, slits = 5 nm/5 nm. (right) Observed fluorescence intensities at 480 nm (●).

3·Zn_2 with PPi



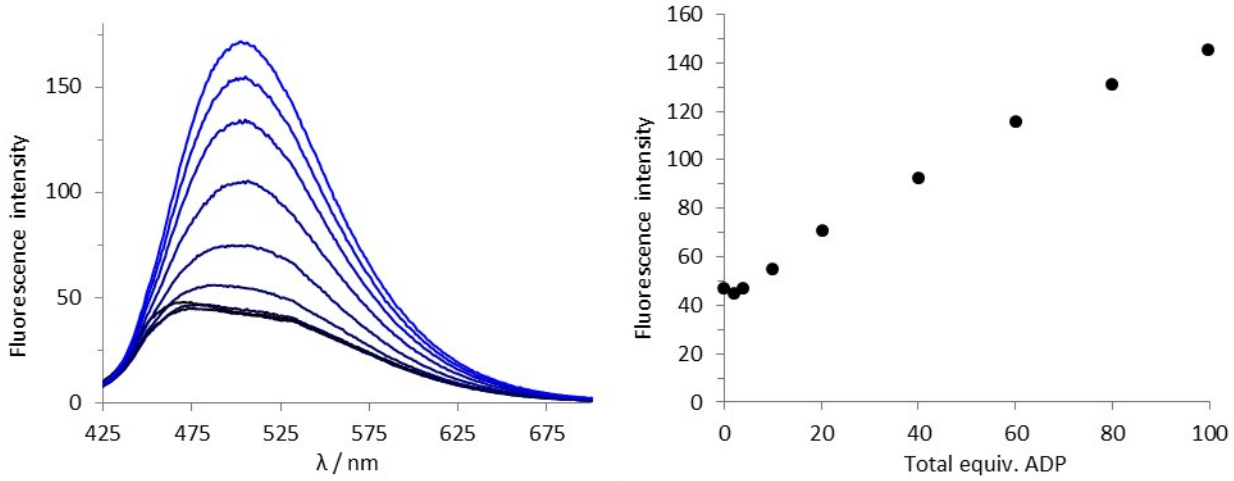
(left) Fluorescence emission spectra of **3·Zn$_2$** (5 µM) in response to PPi (0-10 µM) in HEPES (5 mM, 145 mM NaCl, pH 7.4) at 25 °C. λ_{ex} = 390 nm, slits = 5 nm/5 nm. (right) Binding isotherm (—) obtained by fitting the observed fluorescence intensities at 480 nm (●) to a 1:1 binding model using non-linear regression.

1·Zn$_2$ with ADP



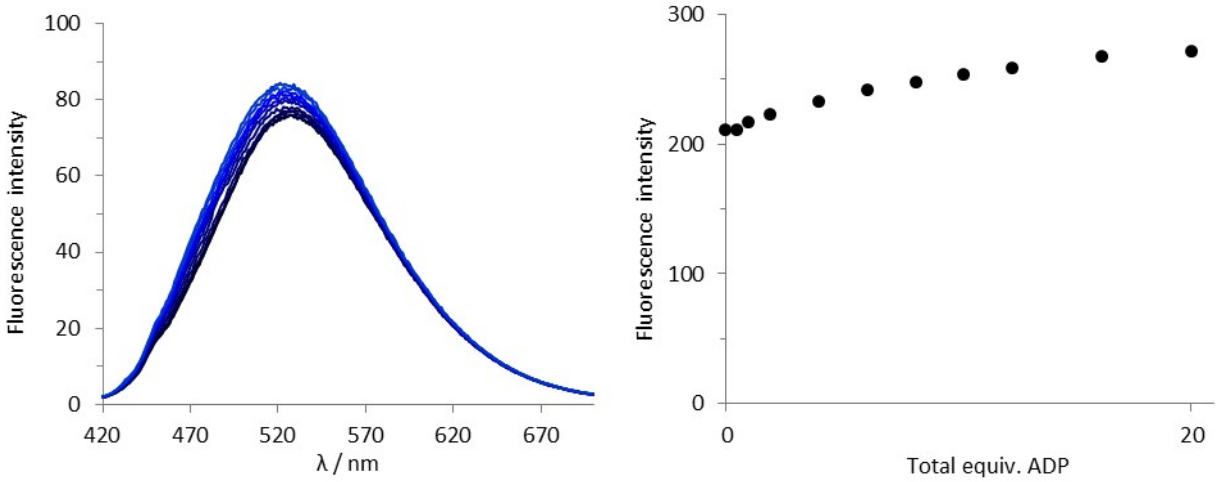
(left) Fluorescence emission spectra of **1·Zn$_2$** (5 µM) in response to ADP (0-300 µM) in HEPES (5 mM, 145 mM NaCl, pH 7.4) at 25 °C. λ_{ex} = 390 nm, slits = 5 nm/5 nm. (right) Observed fluorescence intensities at 480 nm (●).

2·Zn$_2$ with ADP



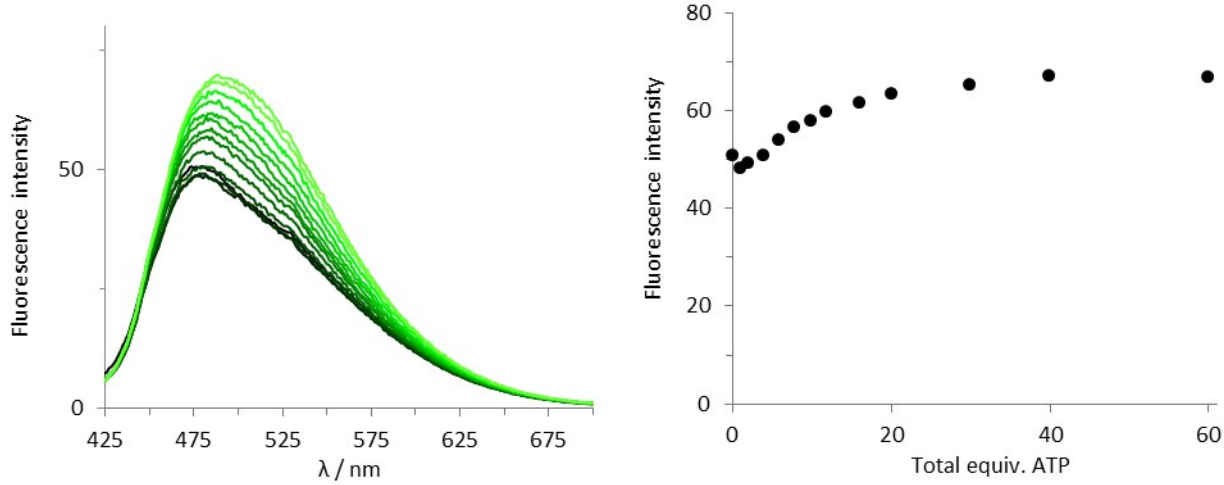
(left) Fluorescence emission spectra of **2·Zn$_2$** (5 µM) in response to ADP (0-500 µM) in HEPES (5 mM, 145 mM NaCl, pH 7.4) at 25 °C. λ_{ex} = 390 nm, slits = 5 nm/5 nm. (right) Observed fluorescence intensities at 480 nm (●).

3·Zn$_2$ with ADP



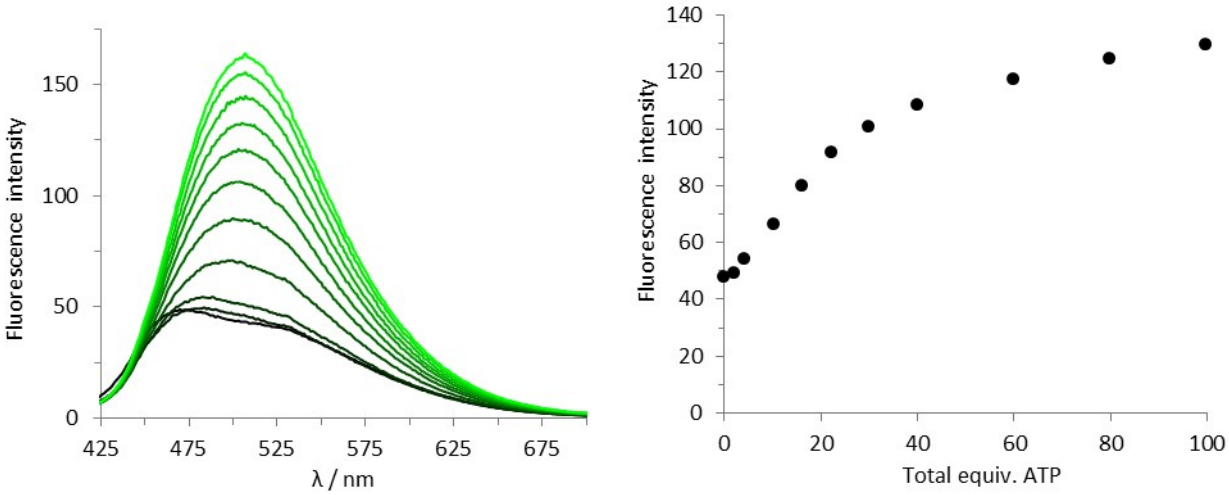
(left) Fluorescence emission spectra of **3·Zn$_2$** (5 µM) in response to ADP (0-100 µM) in HEPES (5 mM, 145 mM NaCl, pH 7.4) at 25 °C. λ_{ex} = 390 nm, slits = 5 nm/5 nm. (right) Observed fluorescence intensities at 480 nm (●).

1·Zn$_2$ with ATP



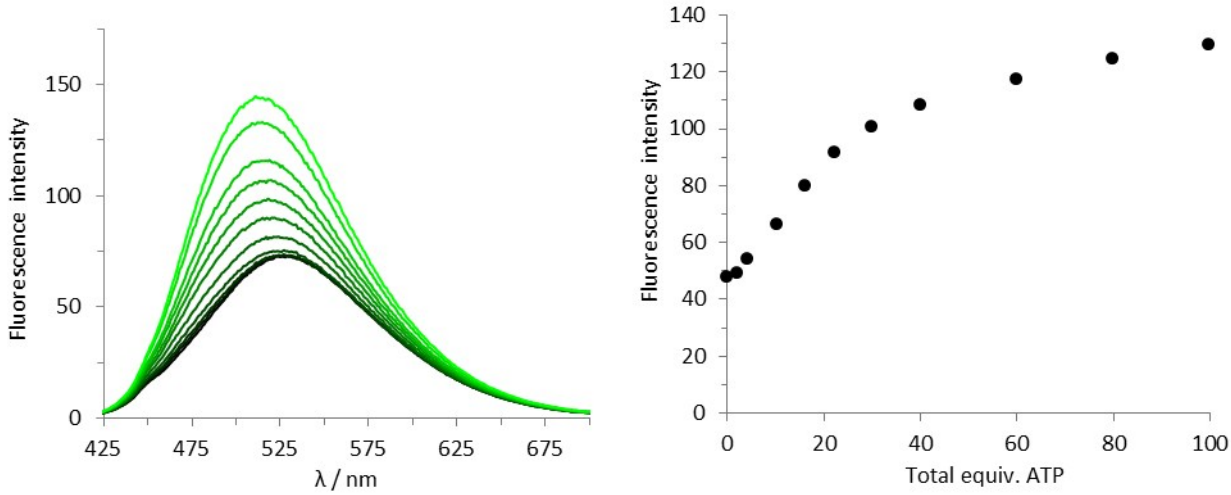
(left) Fluorescence emission spectra of **1·Zn$_2$** (5 µM) in response to ATP (0-300 µM) in HEPES (5 mM, 145 mM NaCl, pH 7.4) at 25 °C. λ_{ex} = 390 nm, slits = 5 nm/5 nm. (right) Observed fluorescence intensities at 480 nm (●).

2·Zn$_2$ with ATP



(left) Fluorescence emission spectra of **2·Zn$_2$** (5 μM) in response to ATP (0-500 μM) in HEPES (5 mM, 145 mM NaCl, pH 7.4) at 25 °C. λ_{ex} = 390 nm, slits = 5 nm/5 nm. (right) Observed fluorescence intensities at 480 nm (●).

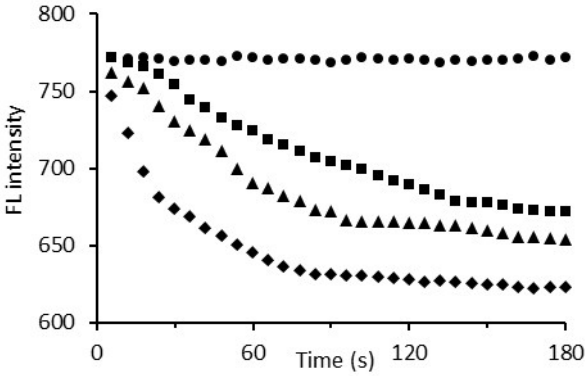
3·Zn$_2$ with ATP



(left) Fluorescence emission spectra of **3·Zn$_2$** (5 μM) in response to ATP (0-500 μM) in HEPES (5 mM, 145 mM NaCl, pH 7.4) at 25 °C. λ_{ex} = 390 nm, slits = 5 nm/5 nm. (right) Observed fluorescence intensities at 480 nm (●).

Procedure for fluorescence pyrophosphatase assay

A stock solution containing receptor and PPi (4-10 equiv. relative to the receptor concentration) in 10 mM Tris buffer (pH 7.5, 10 mM $MgCl_2$) was prepared. An aliquot of 3.0 mL of the solution was placed in a 1 cm quartz glass cuvette. After incubation at 30 °C for 8 min, inorganic pyrophosphatase (0.25, 0.5, 1.0, 2.0 units) was added to the sample cuvette. The resulting solution was stirred for 6 seconds and the fluorescence spectra were periodically collected.



Real-time assay of PPi (100 μM) hydrolysis catalysed by different amounts of pyrophosphatase (● = 0 units, ■ = 0.5 units, ▲= 1.0 units, ♦ = 2.0 units) in the presence of **1·Zn$_2$** (10 μM) in Tris buffer (10 mM, 10 mM $MgCl_2$, pH 7.5) at 30 °C, λ_{ex} = 390 nm, slits = 10 nm & 5 nm.