Electronic Supplementary Information for

Sequence Selective Dual-Emission Detection of (*i*, *i*+1) Bis-Phosphorylated Peptide Using Diazastilbene-Type Zn(II)-Dpa Chemosensor

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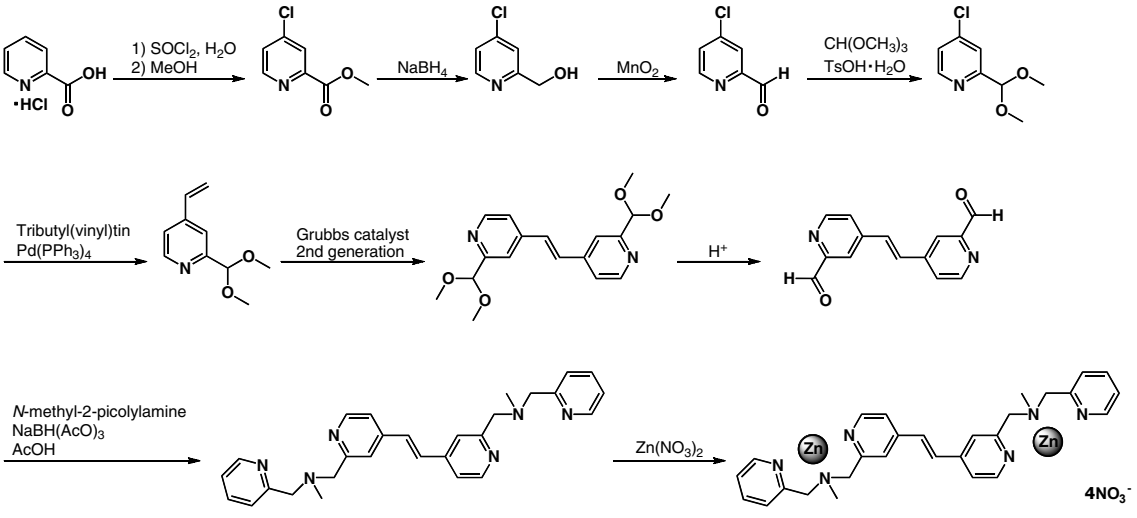
Materials and Methods

All chemical reagents were purchased from commercial suppliers (Aldrich, TCI, or Wako) and used without further purification. Fluorescence spectra were measured by a Perkin-Elmer LS55 fluorescence spectrometer. The slit width for excitation and emission were set at 15 and 10 nm, respectively. Microcalorimetric measurements were carried out using a Microcal Isothermal Titration Calorimeter VP-ITC. The titration curves obtained were analyzed using the ORIGIN software program. CD spectra were recorded with a Jasco J-720 spectropolarimeter.

Synthesis Probe 1-2Zn(II)

Scheme S1 Synthesis of **1**-2Zn(II).



4-Chloro-2-methoxycarbonylpyridine (7). To a solution of 2-pyridinecarboxylic acid hydrochloride (5.0 g, 31.7 mmol) in 15 mL of thionyl chloride was added dropwise water (0.57 mL, 31.7 mmol) at 0 ^{o}C, and the reaction mixture was refluxed and stirred for 3 days. After the reaction, thionyl chloride was removed under vacuum. The resulting residue was dissolved in 30 mL of toluene, and 1 mL methanol was added dropwise to the solution at 0 ^{o}C. The precitipate was collected by filtration and then dissolved in chloroform. The solution was washed with saturated aqueous $NaHCO_3$ and dried over Na_2SO_4. The purification by silica gel column chromatography (hexane : EtOAc = 3 : 1) gave **7** (2.52 g, 46 %) as a coloress solid. ^{1}H NMR (δ/ppm, $CDCl_3$, 400 MHz, TMS): 4.02 (s, 1H, OCH_3), 7.49 (d, 1H, 4.8 Hz, pyridine), 8.14 (s, 1H, pyridine), 8.65 (d, 1H, J = 4.8 Hz, pyridine). FAB-LRMS (m /z) : 172 $[M + H]^+$.

4-Chloro-2-hydroxymethylpyridine (8). A mixture of **7** (1.5 g, 8.74 mmol) and $CaCl_2$ (3.8 g, 35.0 mmol) and $NaBH_4$ (0.66 g, 17.5 mmol) in 10 mL of methanol and 5 mL of dryTHF was stirred at 0 ^{o}C for 1h. The reaction mixture was quenched with water and extracted with EtOAc. The organic layer was dried over Na_2SO_4, and concentrated in vacuo to give **8** (1.23 g, 98 %) as a colorless oil. ^{1}H NMR (δ/ppm, $CDCl_3$, 400 MHz, TMS): 4.75 (s, 2H, CH_2), 7.22 (d, 1H, J = 5.2 Hz, pyridine), 7.31 (s, 1H, pyridine), 8.45 (d, 1H, J = 5.2 Hz, pyridine). FAB-LRMS (m /z) : 144 $[M + H]^+$.

4-Chloro-2-formylpyridine (9). A suspension of **8** (1.23 g, 8.56 mmol) and MnO_2 (9.2 g) in 15 mL of chloroform was refluxed for 2 h. After filtration with celite pad, the filtrate was concentrated under vacuum to give **9** (1.2 g, quantitative yield.) as a yellow oil. ^{1}H NMR (δ/ppm, $CDCl_3$, 400 MHz, TMS): 7.52 (dd, 1H, J = 2.0, 5.2, pyridine), 7.95 (ds, 1H, J = 2.0 Hz, pyridine), 8.68 (d, 1H, J = 5.2 Hz, pyridine), 10.2 (s, 1H, CHO). CI-MS (m /z) : 140 $[M + H]^+$.

4-Chloro-2-dimethoxymethylpyridine (3). A mixture of **9** (0.6 g, 4.24 mmol) and trimethyl orthoformate (0.7 mL, 8.9 mmol) and *p*-toluenesulfonic acid monohydrate (32 mg, 0.17 mmol) in 15 mL of drymethanol was refluxed for 5 h. After removal of the solvent in vacuo, the residue was dissovled in EtOAc and washed 2 N NaOHaq. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give **3** (0.57 g, 80 %) as a yellow oil. ^{1}H NMR (δ/ppm, $CDCl_3$, 400 MHz, TMS): 3.41 (s, 6H, OCH_3), 5.36 (s,

1H, CH), 7.27 (dd, 1H, *J* = 2.0, 5.2 Hz, pyridine), 7.58 (ds, 1H, *J* = 2.0 Hz, pyridine), 8.51 (d, 1H, *J* = 5.2 Hz, pyridine). FAB-LRMS (m /z) : 188 $[M + H]^+$.

2-Dimethoxymethyl-4-vinylpyridine (4). A mixture of **3** (0.57 g, 3.04 mmol) and tributyl(vinyl)tin (1.1 mL, 3.65 mmol) and $Pd(PPh_3)_4$ (0.35 g, 0.3 mmol) in 20 mL of drytoluene was refluxed for 24 h under Ar atmosphere. After removing the precitipate by filtration with celite pad, the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane : EtOAc = 3 : 1) to give **4** (0.45 g, 82 %) as a yellow oil. ^{1}H NMR (δ/ppm, $CDCl_3$, 400 MHz, TMS): 3.42 (s, 6H, OCH_3), 5.37 (s, 1H, CH), 5.50 (d, 1H, *J* = 10.8 Hz, vinyl-CH_2), 6.00 (d, 1H, *J* = 17.6 Hz,vinyl-CH_2), 6.68 (dd, 1H, *J* = 10.8, 17.6 Hz, vinyl-CH), 7.23 (d, 1H, *J* = 4.8 Hz, pyridine), 7.53 (s, 1H, pyridine) 8.56 (d, 1H, *J* = 4.8 Hz, pyridine). FAB-LRMS (m /z) : 188 $[M + H]^+$.

***trans*-4,4'-diaza-3,3'-Dimethoxymethylstilbene (5)**. A mixture of **4** (0.31 g, 1.72 mmol) and Grubbs catalyst (2nd generation, 57 mg, 86 μmol) in 15 mL of dry-toluene was refluxed for 24 h. After removing the precitipate by filtration with celite pad, the filtrated was concentrated in vacuo. The residue was dissolved with diisopropyl ether and insoluble was removed by filtration. The obtained solid was futher washed with hexane / diisopropyl ether (1 : 1) to give **5** (0.1 g, 38 %) as a brown solid. ^{1}H NMR (δ/ppm, $CDCl_3$, 400 MHz, TMS): 3.44 (s, 12H, OCH_3), 5.41 (s, 2H, CH), 7.28 (s, 2H, alkenyl-CH), 7.34 (d, 2H, *J* = 5.2 Hz, pyridine), 7.68 (s, 2H, pyridine), 8.62 (d, 2H, *J* = 5.2 Hz, pyridine). FAB-LRMS (m /z) : 331 $[M + H]^+$.

***trans*-4,4'-Diaza-3,3'-diformylstilbene (6)**. A solution of **5** (50.0 mg, 0.15 mmol) in 7 mL of THF and 1 mL of conc.HClaq was stirred at 50 ^{o}C for 4 h. The mixture was neutralized with saturated $NaHCO_3$aq, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give **6** (35.7 mg, quantitative yield) as a brown oil. ^{1}H NMR (δ/ppm, $CDCl_3$, 400 Hz, TMS): 7.39 (s, 2H, alkenyl-CH), 7.61 (d, 2H, *J* = 5.2 Hz, pyridine), 8.11 (s, 2H, pyridine), 8.83 (d, 2H, *J* = 5.2 Hz, pyridine), 10.1(s, 2H, CHO). FAB-LRMS (m /z) : 239 $[M + H]^+$.

***trans*-3,3'-Di(*N*-methyl-2-picolylaminomethyl)-4,4'-diazastilbene (1)**. A mixture of **6** (35.7 mg, 0.15 mmol) and *N*-methyl-2-picolylamine hydrobromide (67.0 mg, 0.33 mmol) and $NaBH(OAc)_3$ (0.15 g, 0.6 mmol) in 15 mL of dryCH_2Cl_2 and acetic acid (1 drop) was stirred at r.t. for 7 h. The mixture was neutralized with saturated $NaHCO_3$aq, and extracted with chloroform. The organic layer was washed with brine and dried over Na_2SO_4. After removal of the solvent in vacuo, the residue was purified by silica gel column chromatography (chloroform : methanol = 20 : 1, containing NH_3aq) to give compound **1** (0.45 g, 82 %) as a yellow oil. ^{1}H NMR (δ/ppm, CD_3OD, 400 MHz, TMS): 2.30 (s, 6H, CH_3), 3.78 (s, 8H, CH_2), 7.30 (t, 2H, J = 4.8 Hz, pyridine), 7.51 (s, 2H, alkenyl-CH), 7.54 (dd, 2H, J = 2.0, 5.2 Hz, pyridine), 7.65 (d, 2H, J = 8.0 Hz, pyridine), 7.80-7.84 (m, 4H, pyridine), 8.46-8.49 (m, 4H, pyridine). FAB-LRMS (m /z) : 451 $[M + H]^+$.

***trans*-3,3'-Di(*N*-methyl-2-picolylaminomethyl)-4,4'-diazastilbene $Zn(NO_3)_2$ complex (1-2Zn(II))**. To a solution of **1** (42.9 mg, 95 μmol) in 10 mL of acetonitrile was added 306 mM of $Zn(NO_3)_2$aq (590 μL, 181 μmol). After stirring at r.t. for 2 h, the precitipate was collected and washed with acetonitrile to give the compound **1-2Zn(II)** (49.1 mg, 59 μmol, 62 %) as a colorless solid. ^{1}H NMR (δ/ppm, D_2O, 400 MHz, 25 ^oC): 2.27 (s, 6H, CH_3), 4.03-4.17 (m, 8H, CH_2), 7.45-7.52 (m, 6H, alkenyl-CH and pyridine), 7.63-7.67 (m, 4H, pyridine), 7.96 (t, 2H, J = 7.6 Hz, pyridin), 8.51-8.53 (m, 4H, pyridine). FAB-LRMS (m / z) : 768 $[M - NO_3^- + H]^+$. Anal. calcd for $C_{28}H_{30}N_6 \cdot 2Zn(NO_3)_2$: C, 40.55; H, 3.65; N, 16.89. Found : C, 40.71; H, 3.65; N, 17.02.

Synthesis of the tau fragment peptides

The peptides listed in Table 1 were synthesized using the standard Fmoc-based FastMoc coupling chemistry (0.1 mmol scale) on Fmoc-Amide Resin (Novabiochem). The peptide cleavage from resin and side-chain deprotection were conducted by the treatment with TFA–*m*-cresol–thioanisole (86:2:12) over 1 h at r.t. After removal of TFA in vacuo, the crude peptide was precipitated in tert-butyl-methyl ether and purified by reversed-phase HPLC (column; YMC-pack ODS-A, 20 × 250 mm) using CH_3CN (0.1%

TFA) / H_2O (0.1% TFA) solvent system with a linear gradient mode. Molecular weights of the peptides were confirmed by MALDI-TOF mass analysis as shown in Table S1.

Table S1 | MALDI-TOF mass analysis of synthetic tau fragment peptides

peptide	sequence	*m/z*, calcd. $[M-H]^-$	*m/z*, found
tau(400-409)-2P (i+1)	YSGD**pT**P**S**PRHLS	1376.51	1376.81
tau(204-217)-2P (i+2)	YGTPGSRSR**pT**P**pS**LPT	1733.75	1733.74
tau(231-238)-2P (i+2)	YTPPK**pS**P**pS**S	1121.42	1111.81
tau(210-220)-2P (i+3)	YSRTP**pS**LP**pT**PPT	1473.63	1473.41
tau(204-217)-2P (i+4)	YGTPG**pS**RSR**pT**PSLPT	1733.75	1733.37
tau(204-217)-2P (i+6)	YGTPG**pS**RSRTP**pS**LPT	1733.75	1733.90
tau(400-409)-1P	YSGDT**pS**PRHLS	1296.55	1297.73

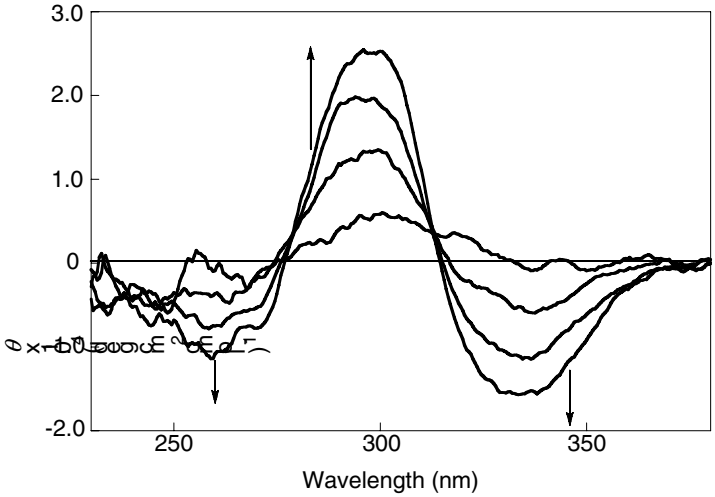


Fig. S1 CD spectral changes of **1**-2Zn(II) (20 μM) upon addition of Tau(400-409)-2P(*i*+1) in 50 mM HEPES buffer at pH 7.2 and 25 ^oC.