Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Fluorescent probe for detection of histone deacetylase activity based on aggregation-induced emission

Koushik Dhara,[a] Yuichiro Hori,[a] Reisuke Baba[a] and Kazuya Kikuchi[*ab]

[a] Division of Advanced Science and Biotechnology
Graduate School of Engineering
Osaka University, Osaka 565-0871 (Japan)
[b] Immunology Frontier Research Center, Osaka University
Osaka 565-0871 (Japan)
Fax: (+81) 6-6879-7875

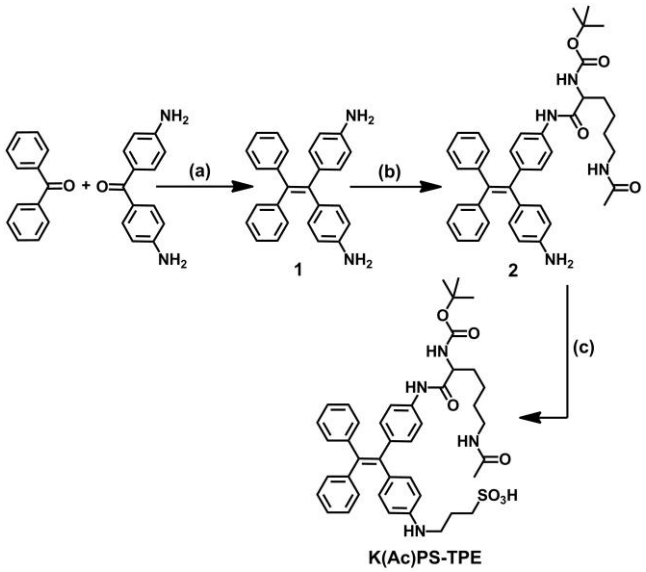
[*] To whom correspondence should be addressed: kkikuchi@mls.eng.osaka-u.ac.jp
Homepage: http://www-molpro.mls.eng.osaka-u.ac.jp/

1. Materials and Instruments.

General chemicals for organic synthesis were of the highest grade available, supplied by Tokyo Chemical Industries, Wako Pure Chemicals, or Aldrich Chemical Co. They were used without further purification. Reversed-phase HPLC analysis was performed with an Inertsil ODS4 column (4.6 mm × 250 mm, GL Sciences Inc.) using an HPLC system that comprised a pump (PU-2080, JASCO) and a detector (MD-2010 and FP-2020, JASCO). High-resolution mass spectra (HRMS) were obtained on a JEOL JMS-700. ESI-TOF MS was performed on a Waters LCT-Premier XE. Fluorescence spectra were measured using a Hitachi F4500 spectrometer. The slit width was 10.0 nm for both excitation and emission. The photomultiplier voltage was set as 700 V. K(Ac)PS-TPE was dissolved in dimethyl sulfoxide (DMSO) to obtain a 1 mM stock solution, and the solution was diluted to the desired final concentration with the appropriate aqueous buffer solution. NMR spectra were recorded on a JEOL JNM-AL400 instrument at 400 MHz for ^{1}H and at 100.4 MHz for ^{13}C NMR using tetramethylsilane as internal standard. We have expressed Sirt1 in our laboratory. The plasmid encoding Sirt1 was kindly provided by Prof. Eric M. Verdin.

2. Protein Expression and Purification.

E. coli, BL21 (DE3) (Novagen), was transformed with pGEX-4T-1-Sirt1 and grown to an OD_{600} of 0.6–0.8 in Luria-Bertani medium containing 100 μg/mL ampicillin at 37 °C. At this point, the temperature was lowered to 25 °C and the protein was expressed overnight by adding isopropyl-β- D-thiogalactopyranoside (IPTG) to the medium (final concentration: 100 μM). The cells were harvested by centrifugation at 5,000 rpm for 10 min at 4 °C; resuspended in sonication buffer (5.8 mM sodium phosphate, 137 mM NaCl, 2.7 mM KCl, 10 mM DTT, pH 7.3); and lysed by sonication. The supernatant of the cell lysate was obtained by centrifugation at 15000 rpm for 15 min at 4 °C and passed through a column packed with GST-Bind resin. Then, the resin was washed with 5.8 mM sodium phosphate buffer (pH 7.3) containing 137 mM NaCl and 2.7 mM KCl, and eluted with 50 mM Tris-HCl buffer (pH 8.0) containing 10 mM glutathione in accordance with the manufacturer's protocol. Finally, buffer exchange was conducted by ultracentrifugation (VIVASPIN 6, MWCO 30,000 PES, Sartorius Stedim) to keep the purified protein in assay buffer (25 mM Tris-HCl [pH 8.0], 100 mM NaCl, 0.05% Tween-20, 20% glycerol). The purity and size of the protein were assessed by SDS-PAGE.



Scheme S1. Synthetic route to obtain K(Ac)PS-TPE. a) $TiCl_4$, Zn, THF, pyridine, -5 °C; b) *N*-α-*t*-butoxycarbonyl-*N*-ε-acetyl-L-lysine, WSCD·HCl, HOBt·H_2O, DMF; c) 1,3-propanesultone, n-butanol. THF: tetrahydrofuran, DMF: *N*,*N*-dimethylformamide.

3. Synthesis of Compounds (Scheme S1).

Synthesis of 1. Under an Ar atmosphere, a three-necked flask equipped with a magnetic stirrer was charged with zinc powder (3.2 g, 48 mmol) and 60 mL THF. The mixture was cooled to -5 °C, and $TiCl_4$ (2.6 mL, 24 mmol) was slowly added by using a syringe, while the temperature was kept under -5 °C. The suspended mixture was warmed to room temperature and stirred for 0.5 h, then heated at reflux for 3 h. The mixture was again cooled to -5 °C, charged with pyridine (1 mL, 12 mmol), and stirred for 15 min. The solution of two carbonyl compounds of benzophenone (0.875 g, 0.4.8 mmol) and 4,4′-diaminobenzophenone (0.849 g, 4 mmol) at a 1.2:1.0 mole ratio (in 30 mL THF) was added slowly. After addition, the reaction mixture was heated at reflux until the carbonyl compounds were consumed (monitored by TLC). After 2.5 h, the reaction was quenched with 10% K_2CO_3 aqueous solution and taken up with ethyl acetate. The organic layer was collected and concentrated. The crude material was purified by chromatography using 40% ethyl acetate in hexane to give the desired products (0.269 g, y. 18%) as a solid (mp 224-227 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.97 (s, 4H), 6.25–6.27 (d, 4H), 6.58–6.60 (d, 4H), 6.91–6.93 (d, 4H), 7.02–7.11 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.9, 144.9, 141.7, 135.2, 131.7, 131.0, 130.8, 127.5, 125.4; HRMS (FAB$^+$) m/z: 362.1777 (calculated for $[M]^+$: 362.1783).

Synthesis of 2. *N*-α-*t*-butoxycarbonyl-*N*-ε-acetyl-L-lysine (119 mg, 0.412 mmol, 1.0 eq.) and compound **1** (150 mg, 0.412 mmol, 1 eq.) were dissolved in dry DMF (15 mL), then WSCD·HCl (119 mg, 0.621 mmol, 1.5 eq.) and HOBt·H_2O (96 mg, 0.621 mmol, 1.5 eq.) were added at 0 °C. The mixture was stirred at 0 °C for 2 h under an Ar atmosphere, then warmed to room temperature, and stirred for 3 h. The solvent was removed under vacuum, and the

crude product was diluted with ethyl acetate and washed with 4% $NaHCO_3$ aq., 10% citric acid, and brine. The organic layer was dried over $MgSO_4$ and removed under vacuum to give the crude compound **2**. The crude material was purified by chromatography using 3% MeOH in DCM as eluent to give the desired products (32 mg, y. 12%) as a solid (mp 138-142 °C), although a side product, which contains 2 equivalents of lysine, was also obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.14–4.16 (t, 1H), 1.62–1.93 (m, 2H), 1.50–1.57 (m, 2H), 1.39–1.43 (m, 11H), 3.22–3.27 (q, 2H), 1.95 (s, 3H), 6.39–6.41 (d, 2H), 6.76–6.78 (d, 2H), 6.69–7.10 (m, 12H), 7.26–7.28 (d, 2H), 8.40 (s, 1H),5.74 (s, 1H) and 5.23–5.25 (d, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.4, 23.3, 28.3, 28.8, 31.2, 38.6, 100.5, 114.3, 118.8, 126.0, 126.1, 127.6, 127.8, 131.3, 131.4, 132.1, 132.5, 133.9, 138.1, 139.1, 140.2, 154.1, 164.3, 172.7; HRMS (FAB$^+$) m/z: 632.3356 (calculated for $[M]^+$: 632.3363).

Synthesis of K(Ac)PS-TPE. To a solution of compound **2** (59 mg, 0.093 mmol) in 5 mL of *n*-butanol was added 1,3-propanesultone (8 μL, 0.093 mmol), and one drop of triethylamine. The reaction mixture was refluxed for 1 h. Then the reaction mixture was stirred at room temperature overnight. The solvent was removed under vacuum and the crude product was purified by reversed-phase HPLC (Figure S1), using H_2O/acetonitrile containing 0.1% formic acid. Although it was found out that compound 2 was degraded to form the compound 1 under the reaction condition, the objective K(Ac)PS-TPE was obtained as a solid (mp 226-229 °C) of 7% yield (5 mg) after the purification. ^{1}H NMR (400 MHz, CD_3OD) δ 3.97–4.01 (t, 1H), 3.32–3.36 (t, 2H), 3.13–3.17 (t, 2H), 2.92–2.96 (t, 2H), 2.12–2.16 (m, 2H), 1.89 (s, 3H), 1.63–1.75 (m, 2H), 1.50–1.53 (m, 2H), 1.28–1.43 (m, 11H), 6.93–7.24 (m, 16H), 7.33–7.35 (d, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 22.5, 22.9, 24.3, 28.7, 30.0, 30.9, 31.8, 33.1, 40.1, 52.4, 80.6, 120.6, 122.9, 127.9, 128.9, 129.0, 132.2, 132.4, 132.7, 134.3, 138.4, 140.0, 140.2, 144.5, 158.7, 166.1, 173.2; HRMS (FAB$^-$) m/z: 753.3300 (calculated for $[M-H]^-$: 753.3322).

4. HPLC analyses.

HPLC separation was carried out with an increasing ratio of buffer B (0.1% HCOOH in acetonitrile) to buffer A (0.1% HCOOH in H_2O). All samples were evaluated by increasing the amount of buffer B from 20 to 90% over 20 min. Before injecting the samples, 4,4′-diaminobenzophenone was mixed with the samples as an internal standard to calculate the integrated peak areas of KPS-TPE and K(Ac)PS-TPE.

5. Fluorescence spectroscopy.

The fluorescence spectra of K(Ac)PS-TPE were recorded at an excitation wavelength of 345 nm. The measurement was conducted every 30 min using a sample containing K(Ac)PS-TPE (10 μM) and NAD^+ (500 μM) with or without Sirt1 (500 nM) in the reaction buffer (20 mM HEPES, 0.2 mM Tris, pH 8.0, 150 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, 0.17 % glycerol, 0.0004% Tween-20, 1% DMSO) (pH 8.0) at 37 °C. For the inhibition assay, tenovin-6 was added to the reaction mixture at a final concentration of 1 mM.

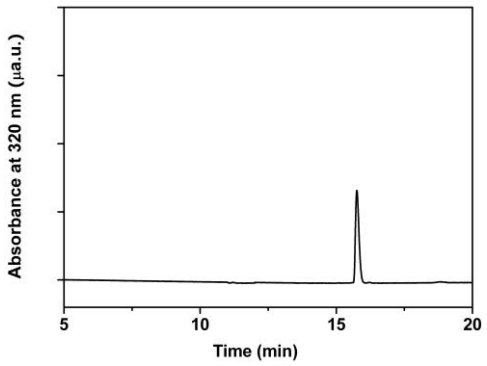


Fig. S1 Analytical reversed-phase HPLC diagram of K(Ac)PS-TPE.

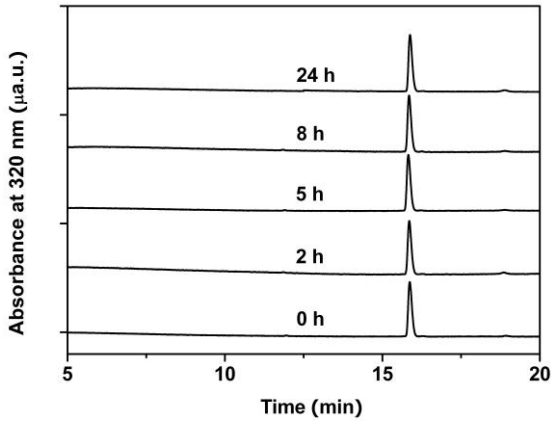


Fig. S2 Reversed-phase HPLC analyses of the stability of the probe (10 μM). K(Ac)PS-TPE was incubated for the designated period in reaction buffer (20 mM HEPES, 0.2 mM Tris, pH 8.0, 150 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, 0.17% glycerol, 0.0004% Tween-20, 1% DMSO) at 37 °C.

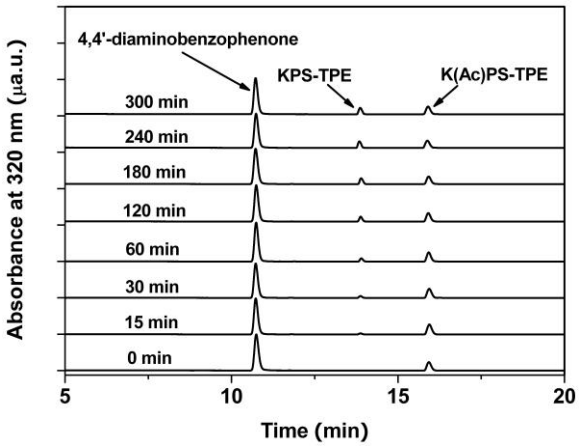


Fig. S3 Reversed-phase HPLC analyses of the reaction progress of K(Ac)PS-TPE (10 μM) with Sirt1 (500 nM) in the reaction buffer (pH 8.0) at 37 °C. 4,4′-Diaminobenzophenone was used as internal standard.

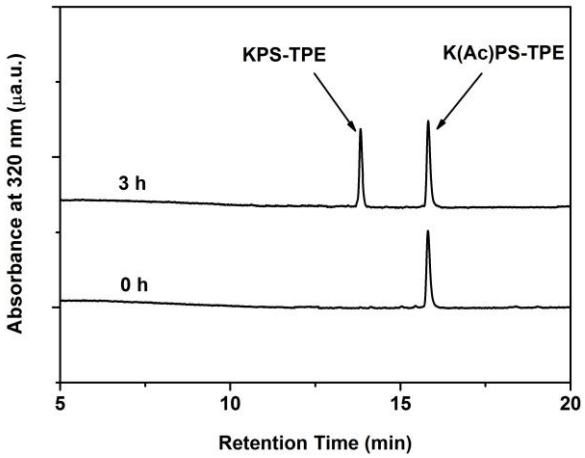


Fig. S4 Reversed-phase HPLC analyses of the reaction progress of K(Ac)PS-TPE (10 μM) with Sirt1 (500 nM) in the reaction buffer (pH 8.0) in the presence of 10 μM of sodium acetate at 37 °C.

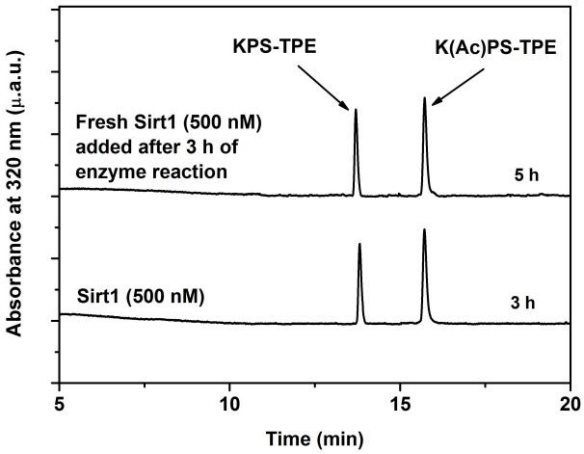


Fig. S5 Reversed-phase HPLC analyses of the reaction progress of K(Ac)PS-TPE (10 μM) with Sirt1 in the reaction buffer (pH 8.0) at 37 °C. Fresh Sirt1 was added to enzyme reaction mixture that had been already incubated for 3 h, and then the enzyme reaction was checked with reversed-phase HPLC.

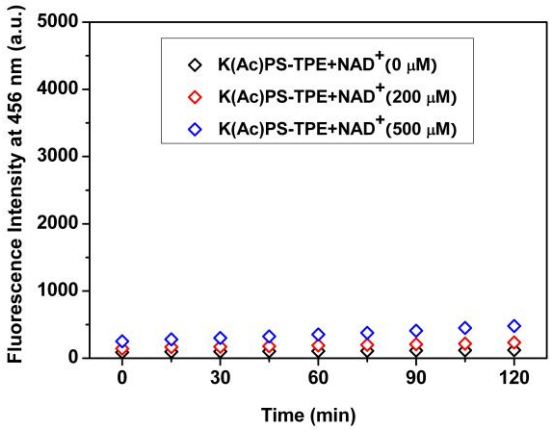


Fig. S6 Fluorescent intensity was plotted against incubation time of K(Ac)PS-TPE (10 μM) in the presence of various concentrations of NAD^+ in the reaction buffer (pH 8.0) at 37 °C. Excitation wavelength: 345 nm.

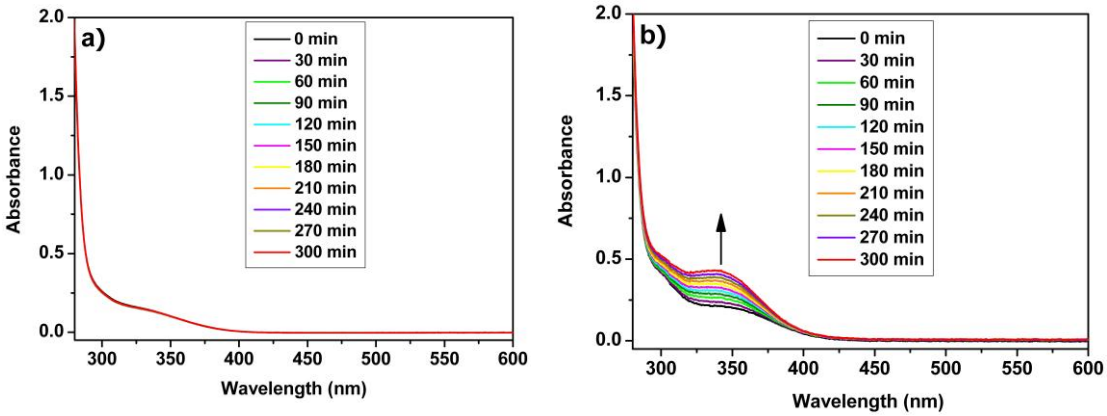


Fig. S7 Absorption spectra of K(Ac)PS-TPE (10 μM) a) in absence of Sirt1 and b) in the presence of Sirt1 (500 nM) in the reaction buffer (pH 8.0) at 37 °C. Both reactions were carried out in the presence of 500 μM NAD^+, and the spectra were measured every 30 min after addition of the enzyme.

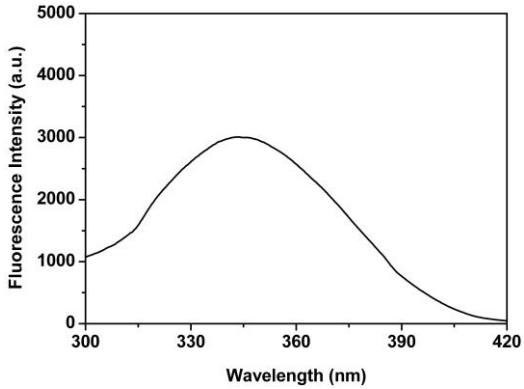


Fig. S8 Excitation spectra of K(Ac)PS-TPE (10 μM) in the reaction buffer (pH 8.0) at 37 °C.

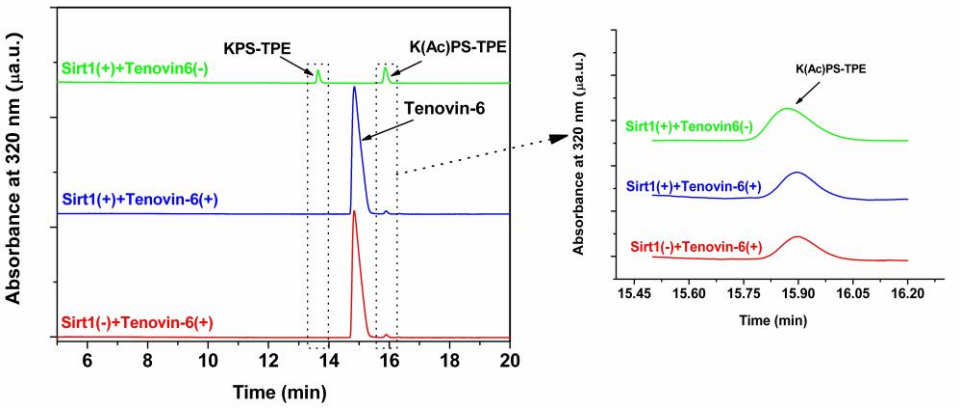


Fig. S9 Analytical reversed-phase HPLC diagram of K(Ac)PS-TPE (10 μM) in the absence and presence of various additives. Tenovin-6, NAD^+, and Sirt1 were used at a final concentration of 1 mM, 500 μM, and 500 nM respectively.