SUPPORTING INFORMATION

Specific inhibitors explore a terminal electron transfer step in the Na^+-pumping NADH-ubiquinone oxidoreductase from *Vibrio cholerae*

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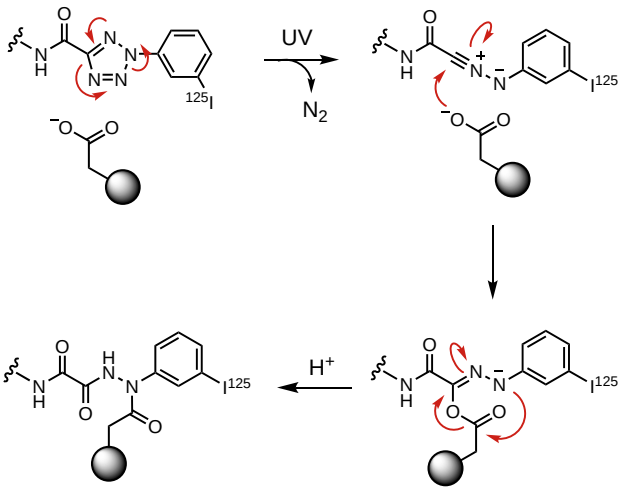


Figure S1

Proposed mechanism of the nucleophilic addition of carboxylic group to the carboxy-nitrile imine followed by the O → N acyl shift to generate the specific photo-adduct (ref. *33*).

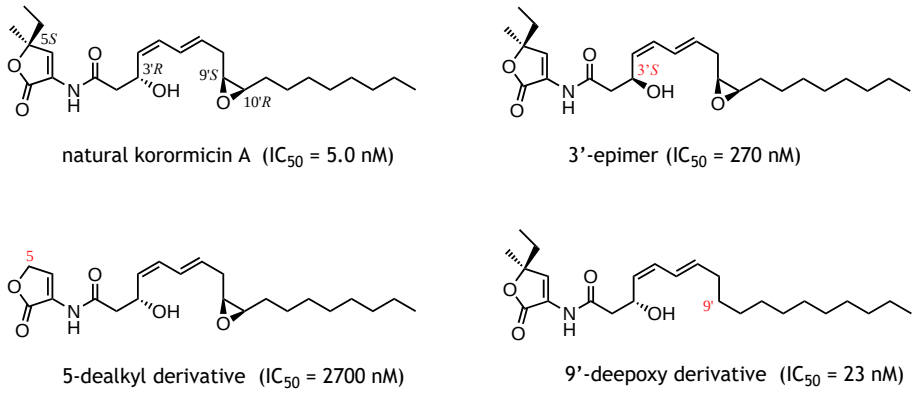


Figure S2

The structure-activity relationship of korormicin analogs that were synthesized in our laboratory.

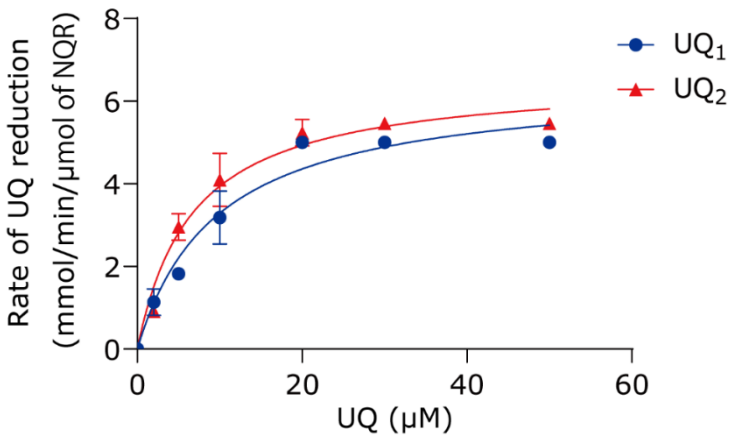


Figure S3

The NADH-UQ oxidoreductase activity with isolated *V. cholerae* Na^+-NQR (0.90 nM). UQ_1 or UQ_2 was used as a substrate quinone.

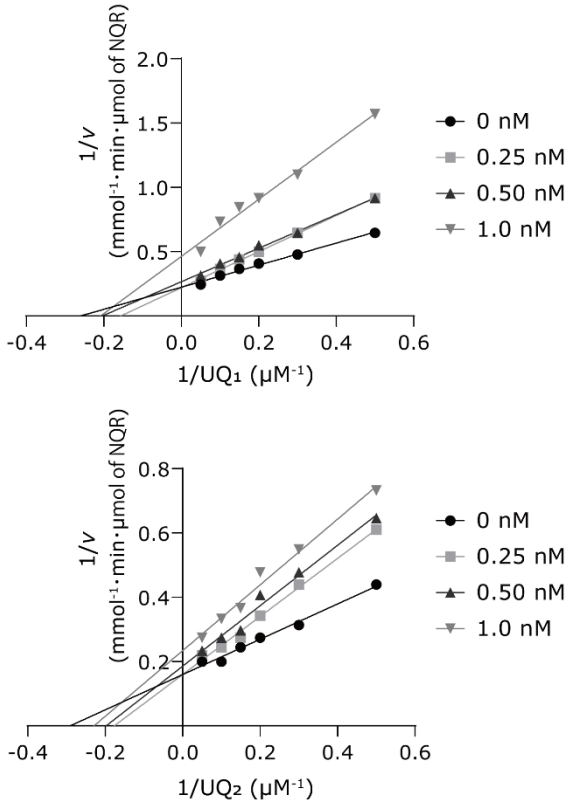


Figure S4

Double-reciprocal plots of initial velocity *vs*. UQ concentration at fixed concentrations of korormicin A (0.25, 0.50, and 1.0 nM). The concentration of isolated *V. cholerae* Na^+-NQR was 0.90 nM. Data are representative of two independent experiments.

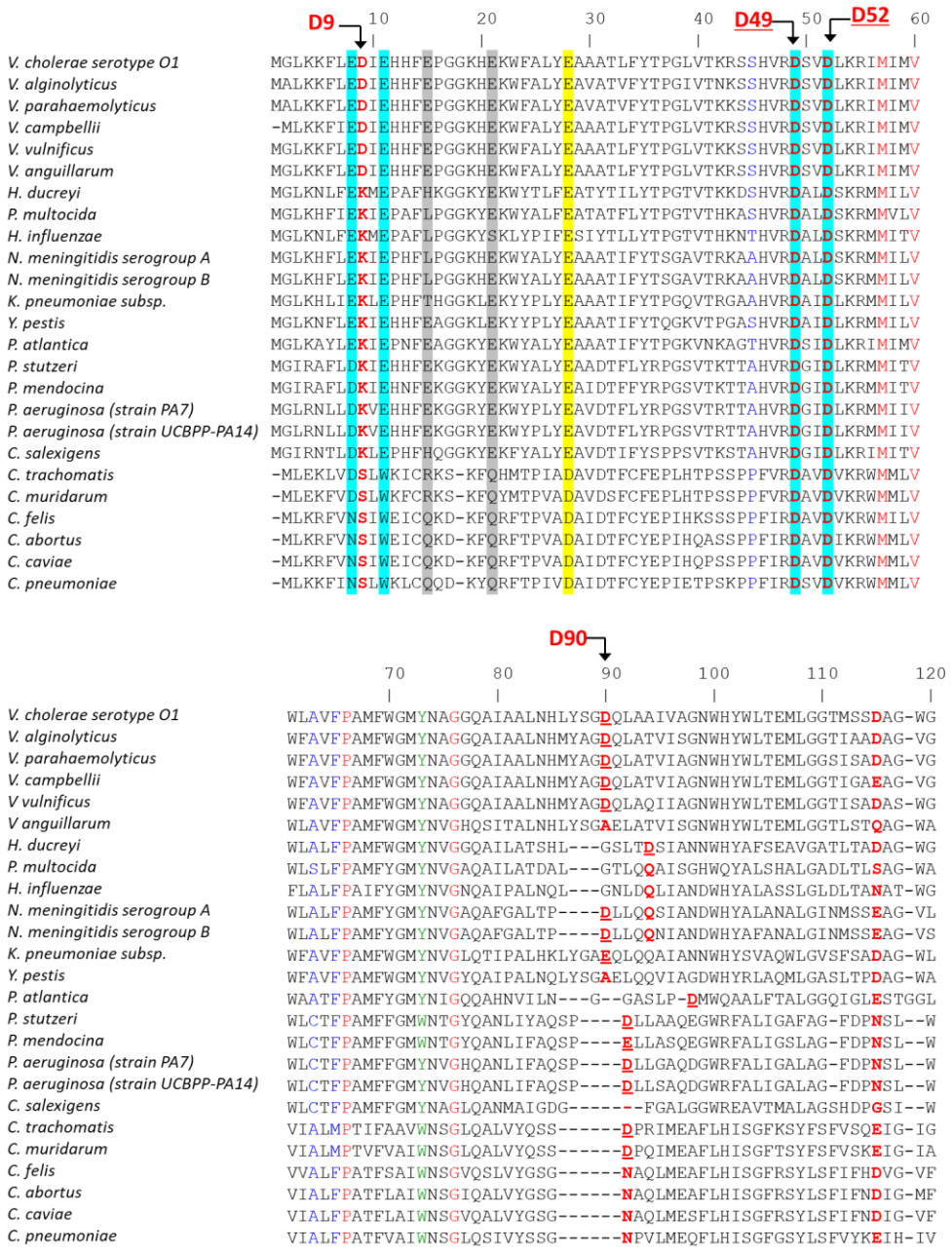


Figure S5

Alignment of the N-terminal region of the NqrB subunit in many bacterial species.

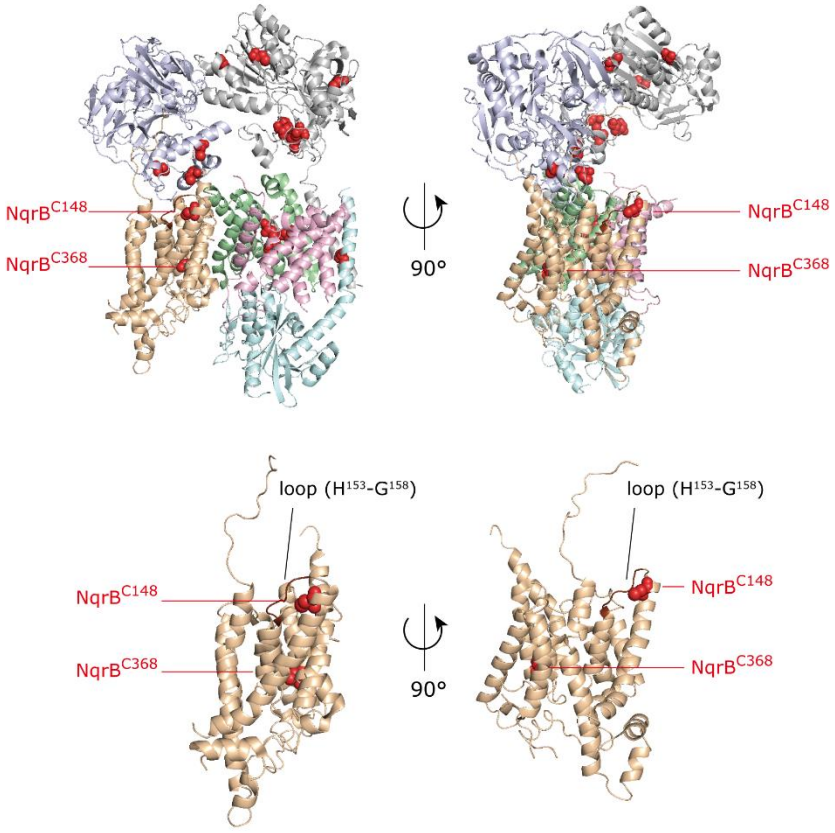


Figure S6

Upper panel: All cysteines in *V. cholerae* Na^+-NQR are indicated by *red spheres*.

Lower panel: Close up of the NqrB subunit.

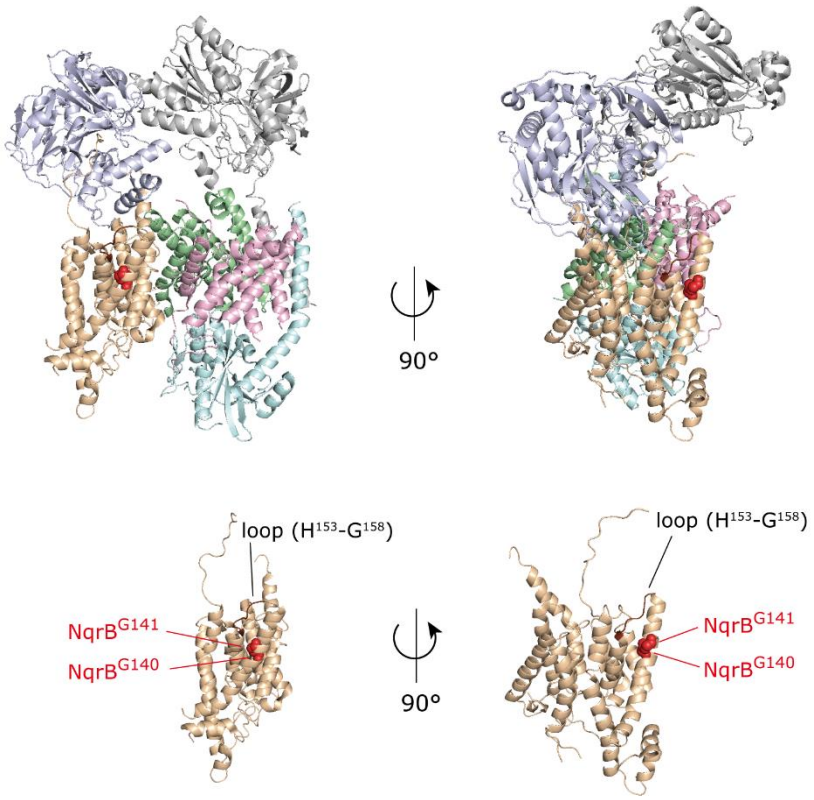


Figure S7

Upper panel: NqrB-G141 and G140 in *V. cholerae* Na^+-NQR are indicated by *red spheres*.

Lower panel: Close up of the NqrB subunit.

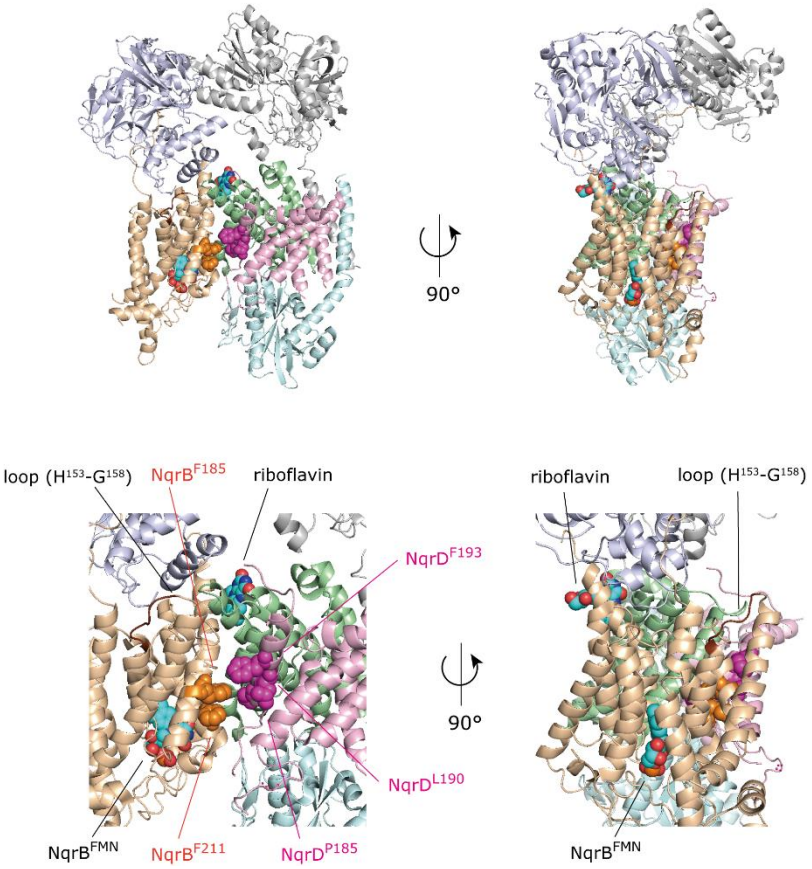


Figure S8

Upper panel: Phe^{185} and Phe^{211} in TMH 4 and 5 of NqrB, respectively, and Pro^{185}, Leu^{190}, and Phe^{193} in TMH 6 of NqrD in *V. cholerae* Na^+-NQR are indicated.

Lower panel: Close up of the membrane domain.

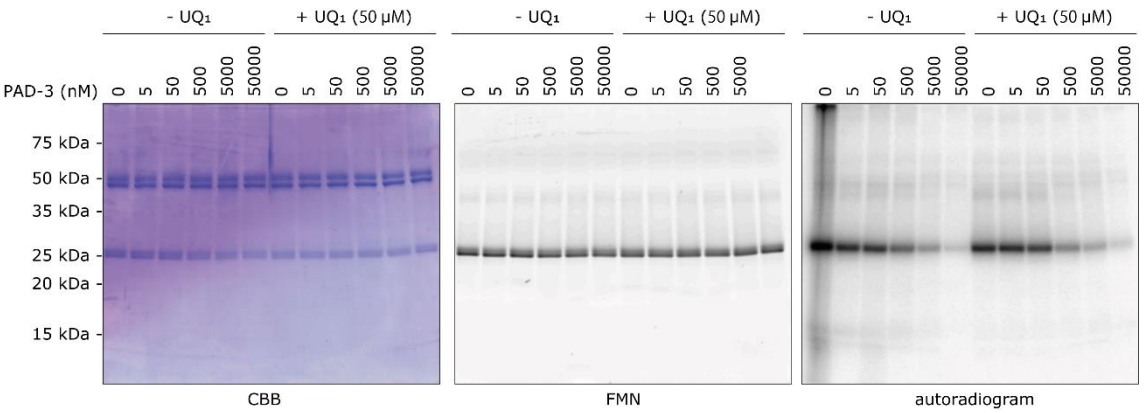


Figure S9

The competition tests between [^{125}I]PAD-3 and PAD-3 in the presence of UQ_1 (50 μM), which partially suppresses the labeling by [^{125}I]PAD-3 (see Figure 4). Data are representative of two independent experiments.

General procedures

All moisture- and air-sensitive reactions were performed in oven-dried glassware under N_2 (or Ar) atmosphere with dry solvents using standard syringe septum techniques. ^{1}H-NMR spectra were recorded at 400 MHz or 500 MHz with Bruker AVANCE III 400 or AVANCE III 500 spectrometers, respectively, using tetramethylsilane (TMS) as an internal standard. ^{13}C-NMR spectra were recorded at 100 MHz or 125 MHz. Chemical shifts (δ) are given in ppm relative to TMS with coupling constants (*J*) in Hz. Thin-layer chromatography (TLC) was performed on Merk TLC plate silica-gel $60F^{254}$, and the spot was detected by iodine, anis, phosphomolybdic acid or UV absorbance. Dry solvents were used either as purchased or freshly distilled using common practices where appropriate. HPLC purification was carried out with a Shimadzu LC-20 AD system, and elution profiles were detected with a Shimadzu SPD-10A.

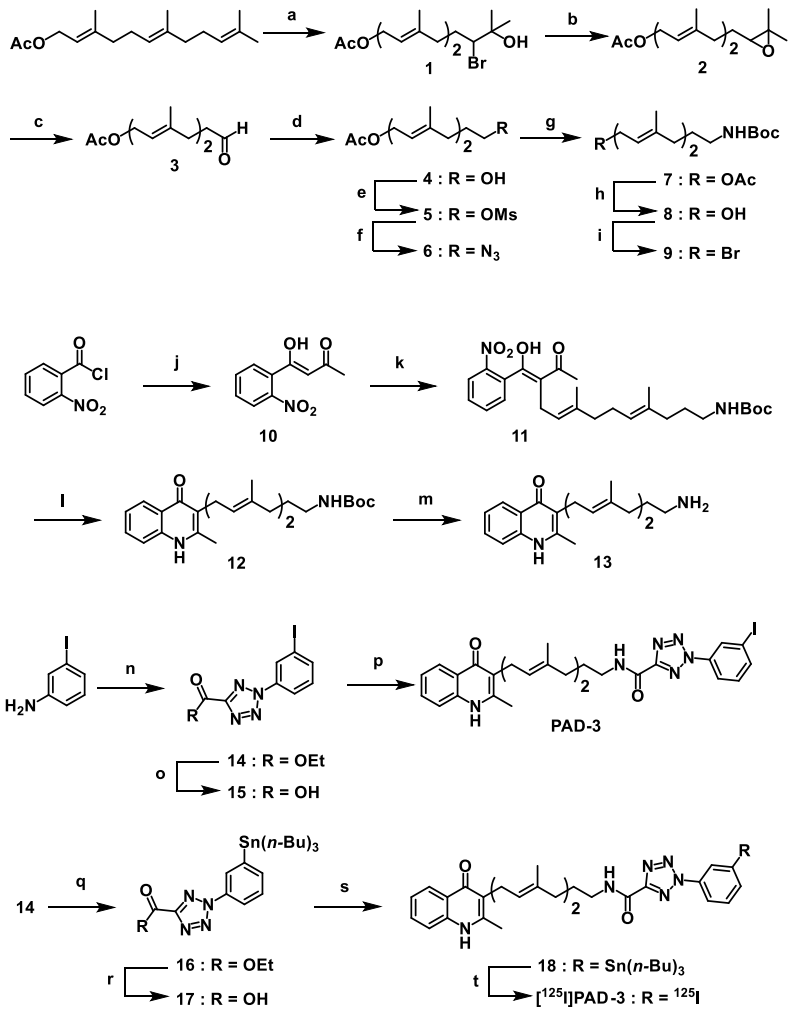
Abbreviations

Ac_2O, acetic anhydride; DCC, *N,N'*-dicyclohexylcarbodiimide; DIBAL-H, diisobutylaluminium hydride; DMAP, 4-dimethylaminopyridine; DME, 1,2-dimethoxyethane; DMF, dimethylformamide; HATU, hexafluorophosphate azabenzotriazole tetramethyl uronium; HMPA, hexamethylphosphoric triamide; HOAt, 1-hydroxy-7-azabenzotriazole; NaHMDS, sodium hexamethylenedisilane; NBS, *N*-bromosuccinimide; PCC, pyridinium chlorochromate; PPTS, pyridinium *p*-toluenesulfonate; rt, rt; TBAF, tetrabutylammonium fluoride; TBS, *tert*-butyldimethylsilyl; THF, tetrahydrofuran; TMS, trimethylsilyl; Tr, trityl.

Outline of the syntheses of PAD-3 and [^{125}I]PAD-3

PAD-3 and **[^{125}I]PAD-3** were synthesized according to the previous methods [*14*, *34*, *48*], as outlined in Scheme S1. The reaction of **10** with prenyl bromide **9**, followed by the reductive cyclization with Fe dust gave the key intermediate **12**. The amidation of **13** with appropriate carboxylic acids provided **PAD-3** and **18**. **[^{125}I]PAD-3** was prepared from a stanyl precursor **18** using chloramine T and [^{125}I]NaI as an oxidant and a radioisotope donor, respectively.

Scheme S1.



Reagents and conditions: (a) NBS, THF/H_2O, rt, 1.5 h, 64%; (b) NaH, THF, rt, 21 h, 80%; (c) $NaIO_4$, H_5IO_6, THF/H_2O, rt, 3 h, 83%; (d) $NaBH_4$, MeOH, -10 °C, 2 h, 85%; (e) MsCl, Et_3N, THF, 0 °C, 3 h, 95%; (f) NaN_3, DMF, 40 °C, 5 h, 87%; (g) H_2, Boc_2O, Pb-poisoned 5% Pd on $CaCO_3$, MeOH, rt, 23 h, 62%; (h) K_2CO_3, MeOH,

rt, 1 h, quant.; (i) Et_3N, MsCl, LiBr, THF, 0 ℃, 1.5 h, 79%; (j) *i*-Pr_2NH, *n*-BuLi, acetone, THF, -78 ℃ to rt, 21 h, 35%; (k) **9**, NaH, THF, 0 ℃ to 60 ℃, 5 h, crude; (l) 6.0 M HCl aq., Fe dust, EtOH, reflux, 24 h, 31% (2 steps); (m) conc. HCl, MeOH, rt, 24 h, quant.; (n) 1) $NaNO_2$, conc. HCl, EtOH, 0 ℃, 10 min, 2) ethyl glyoxylate, benzenesulfonyl hydrazide, pyridine, -10 ℃, 3.5 h, 71%; (o) 2.0 M NaOH aq., EtOH, rt, 1 h, quant.; (p) **13**, Et_3N, HATU, HOAt, DMF, 80 ℃, 3 h, 19%; (q) bis(tributyltin), $Pd(PPh_3)_4$, dioxane, 100 ℃, 6 h, 23%; (r) 2.0 M NaOH aq., EtOH, rt, 10 min, quant.; (s) **13**, Et_3N, HATU, HOAt, DMF, 50 ℃, 3 h, 35%; (t) [^{125}I]NaI, chloramine T, water, rt, 10 min.

*Synthesis of **1***

To an ice-cooled solution of *trans*, *trans*-farnesyl acetate (5.0 g, 18 mmol) in a mixture of THF/water (75 mL/37 mL), NBS (4.2 g, 23 mmol) was added, and the mixture was stirred for 1.5 h at rt. The reaction mixture was extracted with Et_2O and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 15% EtOAc/*n*-hexane) afforded **1** as a colorless oil (4.2 g, 12 mmol, 64%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.32 (t, J = 7.0 Hz, 1H), 5.16 (t, J = 3.8 Hz, 1H), 4.57 (d, J = 7.0 Hz, 2H), 3.94 (dd, J = 11.4, 1.9 Hz, 1H), 2.30 (m, 2H), 2.11-2.02 (m, 6H), 2.03 (s, 3H), 1.68 (s, 3H), 1.57 (s, 3H), 1.31 (d, J = 4.4 Hz, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.39, 142.18, 133.78, 125.60, 118.70, 72.67, 70.99, 61.63, 39.59, 38.35, 32.27, 26.81, 26.36, 26.08, 21.29, 16.69, 16.07; ESI-MS (*m/z*) 161.1 $[M+H]^+$.

*Synthesis of **2***

To an ice-cooled suspension of NaH (50% in mineral oil, 1.1 g, 23 mmol) in THF (77 mL), **1** (4.2 g, 12 mmol) was added under N_2 atmosphere, and the mixture was stirred for 21 h at rt. The reaction was quenched with saturated aqueous NH_4Cl, extracted with Et_2O, washed with brine, and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 15% EtOAc/*n*-hexane) afforded **2** as a colorless oil (2.6 g, 9.2 mmol, 80%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.34 (t, J = 7.1 Hz, 1H), 5.15 (t, J = 3.9 Hz, 1H), 4.58 (d, J = 7.1 Hz, 2H), 2.70 (t, J = 6.2 Hz, 1H), 2.14-2.05 (m, 6H), 2.05 (s, 3H), 1.70 (s, 3H), 1.63-1.62 (m, 2H), 1.28 (d, J = 15.9 Hz, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.34, 142.31, 134.82, 124.47, 118.58, 64.37, 61.59, 58.52, 39.65, 36.52, 27.67, 26.39, 25.11, 21.28, 18.97, 16.68, 16.23; ESI-MS (*m/z*) 303.2 $[M+Na]^+$.

*Synthesis of **3***

To an ice-cooled solution of **2** (2.6 g, 9.2 mmol) in THF (60 mL), a solution of $NaIO_4$ (3.9 g, 18

mmol) in water (10 mL) and a solution of H_5IO_6 (1.1 g, 4.6 mmol) in THF (43 mL) were added carefully. Then, the mixture was allowed to warm to rt over 1.5 h, followed by further stirring for 1.5 h. The reaction mixture was quenched with 1.0 M aqueous HCl, extracted with EtOAc, washed with saturated aqueous $NaHCO_3$ and water, and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 15% EtOAc/*n*-hexane) afforded **3** as a colorless oil (1.8 g, 7.6 mmol, 83%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.72 (t, J = 1.9 Hz, 1H), 5.29 (t, J = 6.5 Hz, 1H), 5.10 (t, J = 6.0 Hz, 1H), 4.55 (d, J = 7.1 Hz, 2H), 2.48 (m, 2H), 2.29 (t, J = 7.4 Hz, 2H), 2.08 (m, 2H), 2.03 (m, 5H), 1.67 (s, 3H), 1.59 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 202.76, 171.32, 142.07, 133.68, 124.97, 118.74, 61.56, 42.32, 39.49, 32.02, 26.26, 21.26, 16.63, 16.32.

*Synthesis of **4***

To a cooled solution of **3** (1.8 g, 7.6 mmol) in MeOH (76 mL), $NaBH_4$ (340 mg, 9.1 mmol) was slowly added in small portions at -10 ˚C. After stirring for 2 h at -10 ˚C, the mixture was quenched with ice-cold water. After the removal of MeOH under reduced pressure, the residue was saturated with NaCl, extracted with Et_2O and dried over anhydrous Na_2SO_4. The purification by silica-gel column chromatography (Wako gel® C-200, 30% EtOAc/*n*-hexane) afforded **4** as a colorless oil (1.6 g, 6.5 mmol, 85%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.34 (t, J = 6.4 Hz, 1H), 5.13 (t, J = 6.6 Hz, 1H), 4.59 (d, J = 7.0 Hz, 2H), 3.62 (t, J = 6.5 Hz, 2H), 2.12 (m, 2H), 2.08-2.04 (m, 4H), 2.06 (s, 3H), 1.70 (s, 3H), 1.69-1.65 (m, 2H), 1.61 (s, 3H), 1.50 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.38, 142.22, 135.32, 124.34, 118.68, 62.87, 61.62, 39.62, 36.09, 30.86, 26.22, 21.26, 16.60, 16.09; ESI-MS (*m/z*) 263.2 $[M+Na]^+$.

*Synthesis of **5***

To an ice-cooled solution of **4** (400 mg, 1.7 mmol) in THF (17 mL), MsCl (380 mg, 3.3 mmol) and Et_3N (670 mg, 6.6 mmol) were added under N_2 atmosphere, and the mixture was stirred for 3 h at 0 ˚C. The reaction was quenched with brine, extracted with Et_2O and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 30% EtOAc/*n*-hexane) afforded **5** as a colorless oil (500 mg, 1.6 mmol, 95%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.33 (t, J = 3.2 Hz, 1H), 5.14 (t, J = 6.6 Hz, 1H), 4.59 (d, J = 7.1 Hz, 2H), 4.20 (t, J = 6.5 Hz, 2H), 3.00 (s, 3H), 2.16-2.04 (m, 6H), 2.05 (s, 3H), 1.87-1.83 (m, 2H), 1.70 (s, 3H), 1.61 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.21, 142.03, 133.58, 125.26, 118.63, 69.75, 61.46, 39.45, 37.47, 35.28, 27.37, 26.21, 21.17, 16.55, 15.95.

*Synthesis of **6***

To an ice-cooled solution of **5** (500 mg, 1.6 mmol) in DMF (16 mL), NaN_3 (200 mg, 3.1 mmol) was added under N_2 atmosphere. Then, the mixture was allowed to warm to 40 ˚C, followed by further stirring for 5 h. The reaction mixture was quenched with ice-cold water, extracted with Et_2O and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 10% EtOAc/*n*-hexane) afforded **6** as a colorless oil (360 mg, 1.4 mmol, 87%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.31 (t, J = 6.5 Hz, 1H), 5.10 (t, J = 6.6 Hz, 1H), 4.56 (d, J = 7.1 Hz, 2H), 3.20 (t, J = 6.9 Hz, 2H), 2.13-2.01 (m, 6H), 2.03 (s, 3H), 1.68 (s, 3H), 1.70-1.61 (m, 2H), 1.57 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.30, 142.17, 134.08, 125.09, 118.65, 61.56, 51.06, 39.57, 36.66, 27.15, 26.29, 21.23, 16.62, 16.00; ESI-MS (*m/z*) 268.2 $[M+Na]^+$.

*Synthesis of **7***

To a solution of **6** (360 mg, 1.4 mmol) in MeOH (7 mL), Boc_2O (360 mg, 1.6 mmol) and 5% $Pd/CaCO_3$ poisoned with Pb (36 mg) was added carefully. Then, and the suspension was stirred for 23 h at rt under H_2 atmosphere. The mixture was filtered through a celite pad and purified by silica-gel column chromatography (Wako gel® C-200, 10% EtOAc/*n*-hexane) to provide **7** as a colorless oil (284 mg, 0.84 mmol, 62%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.30 (t, J = 6.5 Hz, 1H), 5.06 (t, J = 3.9 Hz, 1H), 4.54 (d, J = 7.1 Hz, 2H), 3.04 (d, J = 6.4 Hz, 2H), 2.07 (t, J = 5.9 Hz, 2H), 2.03 (m, 2H), 2.01 (s, 3H), 1.96 (t, J = 7.6 Hz, 2H), 1.66 (s, 3H), 1.55 (s, 3H), 1.57-1.50 (m, 2H), 1.40 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.30, 156.13, 142.26, 134.91, 124.41, 118.59, 79.18, 61.56, 40.49, 39.60, 37.03, 28.61, 28.30, 26.26, 21.22, 16.60, 16.03; ESI-MS (*m/z*) 340.2 $[M+H]^+$, 362.2 $[M+Na]^+$.

*Synthesis of **8***

To a solution of **7** (390 mg, 1.2 mmol) in MeOH (7.7 mL), K_2CO_3 (80 mg, 0.58 mmol) was added, and the mixture was stirred for 1 h at rt. The reaction was quenched with brine, extracted with EtOAc, and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 35% EtOAc/*n*-hexane) afforded **8** as a colorless oil (370 mg, 1.2 mmol, quant.): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.37 (t, J = 7.4 Hz, 1H), 5.08 (t, J = 6.32 Hz, 1H), 4.52 (s, 1H), 4.12 (d, J = 6.8 Hz, 2H), 3.04 (d, J = 6.5 Hz, 2H), 2.13-2.07 (m, 2H), 2.03-1.95 (m, 4H), 1.64 (s, 3H), 1.56 (s, 3H), 1.58-1.51 (m, 2H), 1.41 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 139.10, 134.59, 124.82, 124.14, 79.32, 59.56, 40.40, 39.54, 36.96, 28.63, 28.00, 26.17, 16.37, 15.99, 14.40; ESI-MS (*m/z*) 298.2 $[M+H]^+$, 320.2 $[M+Na]^+$, 332.2 $[M+Cl]^-$.

Synthesis of ***9***

To a solution of **8** (370 mg, 1.2 mmol) and Et_3N (630 µL, 6.3 mmol) in THF (9 mL), MsCl (430 mg, 3.8 mmol) was added under N_2 atmosphere, and the mixture was stirred for 1 h at -10 ˚C. After the addition of LiBr (1.09 g, 12.5 mmol), the mixture was allowed to warm to 0 ˚C over 1.5 h. The reaction was quenched with saturated aqueous $NaHCO_3$, extracted with Et_2O, washed with brine, and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 15% EtOAc/*n*-hexane) afforded **9** as a colorless oil (360 mg, 1.0 mmol, 79%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.50 (t, J = 8.5 Hz, 1H), 5.08-5.05 (m, 1H), 4.50 (s, 1H), 4.00 (d, J = 8.4 Hz, 2H), 3.05 (d, J = 6.4 Hz, 2H), 2.10-2.05 (m, 4H), 1.97 (t, J = 7.4 Hz, 2H), 1.70 (d, J = 1.2 Hz, 3H), 1.58-1.53 (m, 2H), 1.57 (s, 3H), 1.42 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 156.14, 143.65, 135.09, 124.22, 120.90, 40.51, 39.63, 37.04, 29.84, 28.65, 28.31, 26.20, 16.14, 16.10.

Synthesis of ***10***

To an ice-cooled solution of *i*Pr_2NH (2.0 mL, 14 mmol) in THF (17.6 mL), *n*-BuLi (5.8 mL, 2.6 M in *n*-hexane, 15 mmol) was added under N_2 atmosphere, and the mixture was stirred for 30 min at 0 ˚C, followed by the addition of acetone (627 mg, 11 mmol), and the mixture was stirred for 1 h at -78 ˚C. To the reaction mixture, 2-nitrobenzoyl chloride (2.0 g, 11 mmol) in THF (4.0 mL) was added carefully, and the mixture was gradually warmed to rt and stirred for 21 h. The reaction mixture was quenched with saturated aqueous NH_4Cl, extracted with Et_2O and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 15-60% EtOAc/*n*-hexane and subsequent $CHCl_3$) afforded **10** as an orange oil (783 mg, 3.8 mmol, 35%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.90 (dd, J = 7.9, 1.2 Hz, 1H), 7.64 (td, J = 7.5, 1.3 Hz, 1H), 7.58 (dd, J = 8.0, 1.7 Hz, 1H), 7.54 (td, J = 5.2, 1.4 Hz, 1H), 5.78 (s, 1H), 2.14 (s, 3H); ESI-MS (*m/z*) 208.1 $[M+H]^+$, 230.1 $[M+Na]^+$, 206.0 $[M-H]^-$.

Synthesis of ***11***

To an ice-cooled suspension of NaH (50% in mineral oil, 44 mg, 0.91 mmol) and HMPA (327 mg, 1.8 mmol) in THF (2.0 mL) under Ar atmosphere, **10** (170 mg, 0.83 mmol) in THF (2.0 mL) was slowly added and the mixture was stirred for 15 min at 0 ˚C. Then, prenyl bromide **9** (360 mg, 0.99 mmol) in THF (2.0 mL) was added to the mixture, and the reaction was warmed to rt over 1 h, followed by heating at 60 ˚C for 4 h. The reaction mixture was poured onto saturated aqueous NH_4Cl, extracted with Et_2O, washed with brine, dried over anhydrous $MgSO_4$, and purified by silica-gel column chromatography (Wako gel® C-200, 20–30% EtOAc/*n*-hexane) to provide a keto-enol

tautomeric mixture of **11** as an orange oil (330 mg, crude): ESI-MS (*m/z*) 487.3 $[M+H]^+$, 509.3 $[M+Na]^+$, 485.3 $[M-H]^-$, 521.3 $[M+Cl]^-$.

*Synthesis of **12***

To a solution of **11** (330 mg, crude) in EtOH (33 mL), 6.0 M aqueous HCl (570 µL) and Fe dust (280 mg, 5.0 mmol) were added, and the mixture was stirred for 24 h at 90 ˚C. Additional 6.0 M aqueous HCl (570 µL) and Fe dust (5.0 mmol) were added to the mixture until TLC indicated the completion of the reaction. The mixture was diluted with EtOAc and water, filtered through celite, extracted with EtOAc, washed with brine, dried over anhydrous $MgSO_4$, and purified by silica-gel column chromatography (Wako gel® C-200, 60% EtOAc/*n*-hexane) to provide **12** as an yellow solid (110 mg, 0.26 mmol, 31%, 2 steps from **10**): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.37 (d, J = 7.9 Hz, 1H), 7.55-7.49 (m, 2H), 7.26 (t, J = 7.4 Hz, 1H), 5.10 (t, J = 6.9 Hz, 1H), 4.96-4.92 (m, 1H), 3.41 (d, J = 6.7 Hz, 2H), 2.99 (q, J = 6.7 Hz, 2H), 2.41 (s, 3H), 2.05-1.94 (m, 4H), 1.83 (t, J = 8.4 Hz, 2H), 1.71 (s, 3H), 1.50 (s, 3H), 1.46 (s, 9H), 1.44 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 177.13, 156.61, 147.33, 139.61, 134.36, 134.12, 131.15, 126.10, 124.49, 124.41, 123.50, 123.12, 119.82, 117.85, 60.61, 40.81, 39.48, 36.93, 28.68, 25.89, 24.17, 18.58, 16.21, 16.12, 14.39; ESI-MS (*m/z*) 439.3 $[M+H]^+$, 437.4 $[M-H]^-$, 473.3 $[M+Cl]^-$.

*Synthesis of **13***

To a solution of **12** (40 mg, 0.091 mmol) in EtOAc (0.9 mL), concentrated HCl (12 drops) was added, and the mixture was stirred for 24 h at rt. Then, the reaction mixture was basified with 6.0 M aqueous NaOH, extracted with EtOAc, dried over anhydrous $MgSO_4$. The organic layer was concentrated under reduced pressure to afford **13** as a red oil (31 mg, quant.): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.29 (d, J = 7.8 Hz, 1H), 7.57 (d, J = 8.1 Hz, 1H), 7.44 (t, J = 7.2 Hz, 1H), 7.21 (t, J = 7.6 Hz, 1H), 5.06-4.98 (m, 2H), 3.32 (d, J = 6.5 Hz, 2H), 2.67 (m, 2H), 2.39 (s, 3H), 2.05-2.00 (m, 2H), 1.95-1.92 (m, 4H), 1.68 (s, 3H), 1.53-1.48 (m, 2H), 1.51 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 176.98, 147.88, 139.61, 134.62, 134.46, 131.10, 125.77, 124.78, 124.24, 123.21, 123.16, 119.61, 118.06, 58.39, 39.62, 36.98, 26.18, 24.26, 18.64, 18.46, 16.17, 16.11; ESI-MS (*m/z*) 339.2 $[M+H]^+$, 361.3 $[M+Na]^+$, 337.3 $[M-H]^-$, 373.2 $[M+Cl]^-$.

*Synthesis of **14***

To a solution of ethyl 2-oxoacetate (47% in toluene, 700 mg, 3.2 mmol) in EtOH (13 mL), benzenesulfonyl hydrazide (430 mg, 2.5 mmol) was added, and the mixture was stirred for 1 h at rt.

The solvent was removed under reduced pressure, and the residue was dissolved in pyridine (13 mL). Separately, to an ice-cooled suspension of 3-iodoaniline (500 mg, 2.3 mmol) and concentrated HCl (0.9 mL) in H_2O (1.7 mL) and EtOH (2.1 mL), $NaNO_2$ (160 mg, 2.3 mmol) was added. After 10-minute stirring, this mixture was added dropwise to the previously prepared pyridine solution at -10 ˚C. The mixture was warmed up to rt over 3.5 h with stirring. The reaction mixture was quenched with ice-cooled water, extracted with CH_2Cl_2, washed with 2.0 M aqueous HCl and brine, and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 10% EtOAc/*n*-hexane) afforded **14** as an orange solid (550 mg, 1.6 mmol, 71%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.58 (t, J = 1.8 Hz, 1H), 8.20 (m, 1H), 7.89 (m, 1H), 7.32 (t, J = 8.1 Hz, 1H), 4.58 (q, J = 7.2 Hz, 2H), 1.48 (t, J = 20.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 157.79, 139.88, 131.44, 130.67, 129.48, 129.20, 119.68, 94.50, 63.17, 14.41; ESI-MS (*m/z*) 345.0 $[M+H]^+$, 380.8 $[M+Cl]^-$.

*Synthesis of **15***

To a solution of **14** (60 mg, 0.17 mmol) in EtOH (1.5 mL), 2.0 M aqueous NaOH (3 mL) was added, and the mixture was stirred for 1 h at rt. Then, the reaction mixture was acidified with 1.0 M aqueous HCl, extracted with EtOAc, and dried over anhydrous $MgSO_4$. The organic layer was concentrated under reduced pressure to afford **15** as a red oil (60 mg, crude): ^{1}H-NMR (400 MHz, MeOD) δ 8.52 (t, J = 1.8 Hz, 1H), 8.19 (ddd, J = 0.96, 1.16, 0.96 Hz, 1H), 7.96 (ddd, J = 0.92, 0.64, 0.96 Hz, 1H), 7.40 (t, J = 8.1 Hz, 1H); ^{13}C-NMR (100 MHz, MeOD) δ 141.04, 138.64, 136.54, 132.83, 130.24, 120.85, 95.16; ESI-MS (*m/z*) 317.0 $[M+H]^+$, 315.0 $[M-H]^-$.

*Synthesis of **PAD-3***

To a solution of **15** (21 mg, 0.066 mmol) in DMF (0.45 mL), HATU (20 mg, 0.053 mmol), HOAt (7 mg, 0.053 mmol), Et_3N (13 mg, 0.13 mmol), and a solution of **13** (15 mg, 0.044 mmol) in DMF (0.45 mL) were added under N_2 atmosphere, and the mixture was stirred for 3 h at 80 ˚C. The reaction was quenched with brine, extracted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$, and purified by silica-gel column chromatography (Wako gel® C-200, 60% EtOAc/*n*-hexane) to provide **PAD-3** as a white solid (5.2 mg, 0.0082 mmol, 19%): ^{1}H-NMR (400 MHz, MeOD) δ 8.52 (t, J = 1.8 Hz, 1H), 8.31 (m, 1H), 8.21 (dd, J = 8.2, 1.0 Hz, 1H), 8.14 (dt, J = 8.2, 0.9 Hz, 1H), 7.78 (dt, J = 7.9, 1.0 Hz, 1H), 7.50 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.41 (m, 1H), 7.31 (t, J = 8.1 Hz, 1H), 7.24 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H), 5.10-5.05 (m, 2H), 3.39-3.34 (m, 4H), 2.40 (s, 3H), 2.09-2.05 (m, 2H), 2.00 (m, 4H), 1.75 (s, 3H), 1.68 (quin, J = 7.6 Hz, 2H), 1.56 (s, 3H); ^{13}C-NMR (100 MHz, MeOD) δ 177.52, 160.33, 157.33, 151.05, 148.30, 139.96, 139.44, 137.32,

135.03, 134.28, 131.69, 129.23, 125.81, 125.38, 124.18, 123.66, 123.01, 120.00, 119.69, 117.79, 94.55, 39.80, 38.81, 37.25, 27.67, 26.50, 24.22, 18.14, 16.26, 16.08; ESI-MS (*m/z*) 637.1 $[M+H]^+$, 635.1 $[M-H]^-$, 671.1 $[M+Cl]^-$.

*Synthesis of **16***

A solution of **14** (85 mg, 0.25 mmol), $Pd(PPh_3)_4$ (29 mg, 0.025 mmol), and bis(tributyltin) (440 mg, 0.75 mmol) in dioxane (2.5 mL) was stirred for 6 h at 100 ˚C under Ar atmosphere. The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica-gel column chromatography (Wako gel® C-200, 5% EtOAc/*n*-hexane) to provide **16** as an yellow oil (29 mg, 0.057 mmol, 23%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.23 (d, J = 2.3 Hz, 1H), 8.08 (m, 1H), 7.63 (d, J = 7.2 Hz, 1H), 7.51 (t, J = 7.9 Hz, 1H), 4.58 (q, J = 7.2 Hz, 2H), 1.58 (m, 12H), 1.50 (t, J = 7.1 Hz, 3H), 1.35 (m, 6H), 0.92 (t, J = 7.3 Hz, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 158.18, 145.61, 138.90, 137.52, 136.24, 129.13, 127.68, 120.26, 63.00, 29.22, 27.52, 16.64, 14.43, 13.86; ESI-MS (*m/z*) 509.2 $[M+H]^+$.

*Synthesis of **17***

To a solution of **16** (29 mg, 0.057 mmol) in EtOH (0.60 mL), 2.0 M aqueous NaOH (1.2 mL) was added, and the mixture was stirred for 10 min at rt. Then, the reaction mixture was neutralized with 1.0 M aqueous HCl, and extracted with CH_2Cl_2. The organic layer was concentrated under reduced pressure to afford **17** as a white solid (30 mg, quant.): ^{1}H-NMR (400 MHz, MeOD) δ 8.24 (d, J = 2.3 Hz, 1H), 8.09 (ddd, J = 1.24, 1.04, 1.28 Hz, 1H), 7.65 (m, 1H), 7.58 (m, 1H), 1.60 (m, 6H), 1.39-1.34 (m, 6H), 1.17 (m, 6H), 0.90 (t, J = 7.3 Hz, 9H); ^{13}C-NMR (100 MHz, MeOD) δ 146.17, 139.41, 138.00, 130.39, 128.32, 121.08, 30.34, 28.49, 14.14, 10.76.

*Synthesis of **18***

To a solution of **17** (40 mg, 0.084 mmol) in DMF (0.3 mL), HATU (27 mg, 0.071 mmol), HOAt (10 mg, 0.071 mmol), Et_3N (18 mg, 0.18 mmol), and a solution of **13** (20 mg, 0.059 mmol) in DMF (0.3 mL) were added under N_2 atmosphere, and the mixture was stirred for 3 h at 50 ˚C. The reaction was quenched with brine, extracted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$, and purified by silica-gel column chromatography (Wako gel® C-200, 50% EtOAc/*n*-hexane) to provide **18** as an yellow oil (17 mg, 0.021 mmol, 35%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.43 (s, 1H), 8.34 (d, J = 7.9 Hz, 1H), 8.22 (d, J = 2.4 Hz, 1H), 8.06 (dq, J = 8.1, 1.2 Hz, 1H), 7.61 (d, J = 7.1 Hz, 1H), 7.52-7.42 (m, 4H), 7.22 (m, 1H), 5.08 (t, J = 6.5 Hz, 1H), 4.99 (t, J =

6.0 Hz, 1H), 3.38 (m, 2H), 2.36 (s, 3H), 2.10-2.02 (m, 4H), 1.91 (t, J = 7.3 Hz, 2H), 1.72 (m, 5H), 1.63-1.51 (m, 9H), 1.35-1.24 (m, 8H), 1.13-1.09 (m, 6H), 0.87 (t, J = 7.3 Hz, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 177.33, 157.29, 159.95, 145.71, 139.32, 138.90, 136.45, 133.86, 131.22, 129.17, 127.55, 126.44, 124.92, 124.44, 123.74, 123.18, 120.15, 120.07, 117.48, 39.34, 38.84, 37.00, 29.84, 29.21, 28.06, 27.51, 25.58, 24.07, 18.86, 16.05, 13.86, 10.06; ESI-MS (*m/z*) 801.4 $[M+H]^+$.

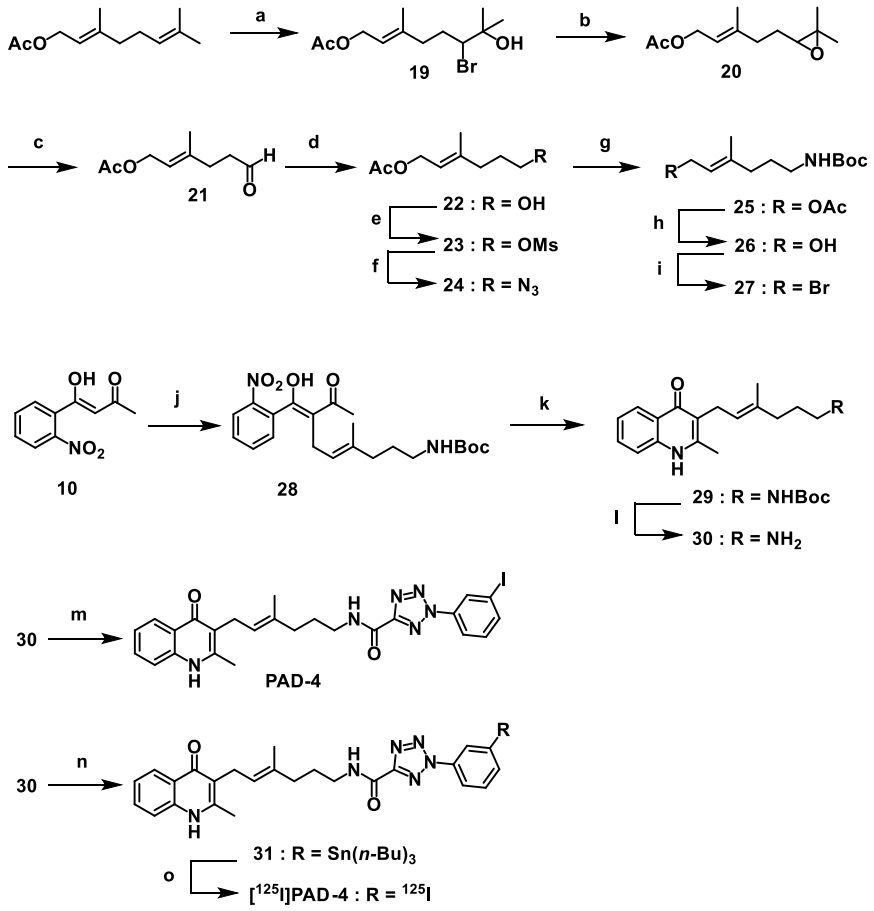
Synthesis of ***[^{125}I]PAD-3***

To a mixture of a stanyl-precursor **18** (1.0 mM in EtOH, 20 μL) and chloramine T (2.0 mM in 1.0 M KPi buffer (pH 7.4), 5 μL) in screw-capped 1.5 mL plastic-tube was added [^{125}I]NaI (PerkinElmer, NEZ 033A, 1 mCi, 2,000 Ci/mmol, 10 μL). The mixture was incubated at rt for 10 min. The reaction was terminated with 5% (w/v) aqueous $NaHSO_3$ (50 μL), and the resulting mixture was carefully extracted with $CHCl_3$ (3 × 100 μL). The combined organic layer was concentrated using vacuum-centrifugal evaporator. The crude product was dissolved in MeOH (30 μL), and subjected to HPLC purification (Shimadzu LC-10AS, Kyoto, Japan) using an ODS column (COSMOSIL 5C_{18}-MS-II, 4.6 x 150 mm, Nacalai Tesque, Kyoto, Japan) at a flow rate of 0.8 mL/min with isocratic 80% MeOH/water system as an eluent. The fraction was collected every 30 s (400 μL). To check the elution pattern of the radioactivities and their radiochemical purity, each fraction was measured by γ-counting system and radio-TLC analysis. The strong radioactive fractions, corresponding to the retention time of **PAD-3**, were combined and the solvent was evaporated by a vacuum-centrifugal evaporator. **[^{125}I]PAD-3** was stored as an ethanolic solution (0.20 μM) at 4 ˚C. The radiochemical yield of **[^{125}I]PAD-3** from the initial [^{125}I]NaI was 6.7%.

Outline of the syntheses of PAD-4 and [^{125}I]PAD-4

The syntheses of **PAD-4** and **[^{125}I]PAD-4** were outlined in Scheme S2. **PAD-4** and **[^{125}I]PAD-4** were synthesized according to the same procedures as those used for the preparation of **PAD-3** and **[^{125}I]PAD-3**, respectively, using geranyl acetate as a starting material.

Scheme S2.



Reagents and conditions: (a) NBS, THF/H_2O, rt, 40 min, 81%; (b) NaH, THF, rt, 22 h, 98%; (c) $NaIO_4$, H_5IO_6, THF/H_2O, rt, 3 h, 47%; (d) $NaBH_4$, MeOH, -10 ℃, 3 h, 63%; (e) MsCl, Et_3N, THF, 0 ℃, 1 h, quant.; (f) NaN_3, DMF, 40 ℃, 22 h, 80%; (g) H_2, Boc_2O, Pb-poisoned 5% Pd on $CaCO_3$, MeOH, rt, 5 h, 23%; (h) K_2CO_3, MeOH, rt, 1 h, 80%; (i) Et_3N, MsCl, LiBr, THF, 0 ℃, 30 min, 63%; (j) **27**, NaH, THF, 0 ℃ to 60 ℃, 5.5 h, crude; (k) 6.0 M HCl aq., Fe dust, EtOH, reflux, 22 h, 22% (2 steps); (l) conc. HCl, MeOH, rt, 26 h, quant.; (m) **15**, Et_3N, HATU, HOAt, DMF, 70 ℃, 3 h, 63%; (n) **17**, Et_3N, HATU, HOAt, DMF, 40 ℃, 4 h, 32%; (o) [^{125}I]NaI, chloramine T, water, rt, 10 min.

*Synthesis of **19***

To an ice-cooled solution of geranyl acetate (5.0 g, 25 mmol) in a mixture of THF/water (100 mL/50 mL), NBS (5.7 g, 33 mmol) was added, and the mixture was stirred for 40 min at rt. The reaction mixture was extracted with Et_2O and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 20-40% EtOAc/*n*-hexane) afforded **19** as a colorless oil (5.9 g, 20 mmol, 81%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.38 (t, J = 7.0 Hz, 1H), 4.56 (d, J = 7.0 Hz, 2H), 3.93 (dd, J = 11.4, 1.9 Hz, 1H), 2.39 (m, 1H), 2.15-2.10 (m, 2H), 2.04-1.97 (m, 5H), 1.68 (s, 3H), 1.32 (d, J = 5.1 Hz, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.29, 140.57, 119.90, 72.68, 70.48, 61.42, 38.32, 31.99, 26.82, 26.19, 16.63.

*Synthesis of **20***

To an ice-cooled suspension of NaH (50% in mineral oil, 1.9 g, 40 mmol) in THF (130 mL), **19** (5.9 g, 20 mmol) was added under N_2 atmosphere, and the mixture was stirred for 22 h at rt. The reaction was quenched with saturated aqueous NH_4Cl, extracted with Et_2O, washed with brine, and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 20% EtOAc/*n*-hexane) afforded **20** as a colorless oil (4.1 g, 19.5 mmol, 98%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.36 (tq, J = 7.1, 1.3 Hz, 1H), 4.57 (d, J = 7.2 Hz, 2H), 2.68 (t, J = 6.2 Hz, 1H), 2.21-2.11 (m, 2H), 2.03 (s, 3H), 1.70 (s, 3H), 1.67-1.63 (m, 2H), 1.28 (s, 3H), 1.24 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.29, 141.48, 119.13, 64.13, 61.47, 58.59, 36.40, 27.29, 25.04, 21.24, 18.96, 16.68; ESI-MS (*m/z*) 235.2 $[M+Na]^+$.

*Synthesis of **21***

To an ice-cooled solution of **20** (4.10 g, 19.5 mmol) in THF (120 mL), a solution of $NaIO_4$ (8.3 g, 39 mmol) in water (20 mL) and a solution of H_5IO_6 (2.2 g, 9.8 mmol) in THF (22 mL) were added carefully. Then, the mixture was allowed to warm to rt over 1.5 h, followed by further stirring for 1.5 h. The reaction mixture was quenched with 1.0 M aqueous HCl, extracted with EtOAc, washed with saturated aqueous $NaHCO_3$ and water, and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 20% EtOAc/*n*-hexane) afforded **21** as an yellow oil (1.6 g, 9.2 mmol, 47%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.75 (s, 1H), 5.33 (tq, J = 7.0, 1.4 Hz, 1H), 4.55 (d, J = 7.0 Hz, 2H), 2.55 (m, 2H), 2.35 (t, J = 7.5 Hz, 2H), 2.02 (s, 3H), 1.70 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 201.86, 171.23, 140.19, 119.55, 61.29, 41.93, 31.64, 21.20, 16.79.

*Synthesis of **22***

To a cooled solution of **21** (1.6 g, 9.2 mmol) in MeOH (92 mL), $NaBH_4$ (420 mg, 11.0 mmol) was slowly added in small portions at -10 ˚C. The mixture was stirred for 3 h at -10 ˚C, and quenched with ice-cold water. After the removal of MeOH under reduced pressure, the residue was saturated with NaCl, extracted with Et_2O and dried over anhydrous Na_2SO_4. The purification by silica-gel column chromatography (Wako gel® C-200, 30-50% EtOAc/*n*-hexane) afforded **22** as a colorless oil (1.0 g, 5.8 mmol, 63%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.35 (t, J = 7.2 Hz, 1H), 4.56 (d, J = 7.0 Hz, 2H), 3.62 (t, J = 6.3 Hz, 2H), 2.11 (t, J = 7.2 Hz, 2H), 2.03 (s, 3H), 1.70-1.66 (m, 5H), 1.33 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 142.06, 118.91, 62.76, 61.52, 35.98, 30.69, 21.24, 16.58, 14.41.

*Synthesis of **23***

To an ice-cooled solution of **22** (1.0 g, 5.8 mmol) in THF (58 mL), MsCl (1.3 g, 12 mmol) and Et_3N (2.3 g, 23 mmol) were added under N_2 atmosphere, and the mixture was stirred for 1 h at 0˚C. The reaction was quenched with brine, extracted with Et_2O and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 40% EtOAc/*n*-hexane) afforded **23** as a colorless oil (1.5 g, 5.8 mmol, quant.): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.34 (m, 1H), 4.55 (d, J = 7.0 Hz, 2H), 4.18 (t, J = 6.4 Hz, 2H), 2.98 (s, 3H), 2.13 (t, J = 7.2 Hz, 2H), 2.02 (s, 3H), 1.90-1.83 (m, 2H), 1.68 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.22, 140.19, 119.95, 69.45, 61.31, 37.55, 35.25, 27.11, 21.18, 16.48, 14.38.

*Synthesis of **24***

To an ice-cooled solution of **23** (1.5 g, 5.8 mmol) in DMF (58 mL), NaN_3 (750 mg, 12 mmol) was added under N_2 atmosphere. Then, the mixture was allowed to warm to 40 ˚C, followed by further stirring for 22 h. The reaction mixture was quenched with ice-cold water, extracted with Et_2O and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 15% EtOAc/*n*-hexane) afforded **24** as a colorless oil (910 mg, 4.6 mmol, 80%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.35 (t, J = 6.5 Hz, 1H), 4.56 (dd, J = 7.1, 0.4 Hz, 2H), 3.24 (t, J = 6.8 Hz, 2H), 2.10 (t, J = 7.3 Hz, 2H), 2.03 (s, 3H), 1.74-1.67 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.27, 140.79, 119.64, 61.40, 51.07, 30.56, 26.93, 21.22, 16.51.

*Synthesis of **25***

To a solution of **24** (910 mg, 4.6 mmol) in MeOH (23 mL), Boc_2O (1.5 g, 6.9 mmol) and 5% Pd/$CaCO_3$ poisoned with Pb (91 mg) was added carefully. Then, the suspension was stirred for 5 h at

rt under H_2 atmosphere. The mixture was filtered through celite and purified by silica-gel column chromatography (Wako gel® C-200, 15-30% EtOAc/*n*-hexane) to provide **25** as a colorless oil (290 mg, 1.1 mmol, 23%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.32 (t, J = 7.1 Hz, 1H), 4.55 (d, J = 7.1 Hz, 2H), 3.07 (d, J = 6.5 Hz, 2H), 2.02 (m, 5H), 1.67 (s, 3H), 1.59 (q, J = 8.1 Hz, 2H), 1.42 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.29, 156.14, 141.64, 119.02, 61.46, 40.46, 36.89, 28.63, 28.16, 21.24, 16.54, 14.40; ESI-MS (*m/z*) 272.2 $[M+H]^+$, 294.2 $[M+Na]^+$.

*Synthesis of **26***

To a solution of **25** (740 mg, 2.7 mmol) in MeOH (18 mL), K_2CO_3 (190 mg, 1.4 mmol) was added, and the mixture was stirred for 1 h at rt. The reaction was quenched with brine, extracted with EtOAc, and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 40% EtOAc/*n*-hexane) afforded **26** as a colorless oil (500 mg, 2.2 mmol, 80%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.40 (t, J = 6.9 Hz, 1H), 4.52 (s, 1H), 4.12 (m, 2H), 3.08 (d, J = 6.5 Hz, 2H), 2.02 (m, 2H), 1.65 (s, 3H), 1.59 (quin, J = 7.4 Hz, 2H), 1.42 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 156.15, 138.96, 124.32, 59.47, 40.27, 36.81, 28.64, 28.03, 21.25, 16.27, 14.40; ESI-MS (*m/z*) 252.2 $[M+Na]^+$.

*Synthesis of **27***

To a solution of **26** (500 mg, 2.2 mmol) and Et_3N (1.10 g, 11 mmol) in THF (15 mL), MsCl (750 mg, 6.5 mmol) was added under N_2 atmosphere, and the mixture was stirred for 1 h at -10 ˚C. After the addition of LiBr (1.9 g, 22 mmol), the mixture was allowed to warm to 0 ˚C over 30 min. The reaction was quenched with saturated aqueous $NaHCO_3$, extracted with Et_2O, washed with brine, and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 15% EtOAc/*n*-hexane) afforded **27** as a colorless oil (400 mg, 1.4 mmol, 63%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.52 (tq, J = 8.4, 1.2 Hz, 1H), 3.98 (d, J = 8.4 Hz, 2H), 3.07 (t, J = 7.0 Hz, 2H), 2.08-2.02 (m, 2H), 1.70 (s, 3H), 1.60 (quin, J = 7.3 Hz, 2H), 1.42 (m, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 142.97, 121.23, 111.37, 48.36, 36.93, 29.51, 28.75, 28.65, 28.10, 16.05.

*Synthesis of **28***

To an ice-cooled suspension of NaH (50% in mineral oil, 60 mg, 1.3 mmol) and HMPA (450 mg, 2.5 mmol) in THF (2.9 mL) under Ar atmosphere, **10** (240 mg, 1.1 mmol) in THF (2.9 mL) was slowly added and the mixture was stirred for 15 min at 0 ˚C. Then, prenyl bromide **27** (400 mg, 1.4 mmol) in THF (1.8 mL) was added to the mixture, and the reaction was warmed to rt over 1 h, followed by heating at 60 ˚C for 4.5 h. The reaction mixture was poured onto saturated aqueous

NH_4Cl, extracted with Et_2O, washed with brine, dried over anhydrous $MgSO_4$, and purified by silica-gel column chromatography (Wako gel® C-200, 30–50% EtOAc/*n*-hexane) to provide a keto-enol tautomeric mixture of **28** as a red oil (420 mg, crude): ESI-MS (*m/z*) 441.2 $[M+Na]^+$.

*Synthesis of **29***

To a solution of **28** (420 mg) in EtOH (50 mL), 6.0 M aqueous HCl (850 μL) and Fe dust (420 mg, 7.5 mmol) were added, and the mixture was stirred for 22 h at 90 ˚C. Additional 6.0 M aqueous HCl (850 μL) and Fe dust (7.5 mmol) were added to the mixture until TLC indicated the completion of the reaction. The mixture was diluted with EtOAc and water, filtered through a celite pad, extracted with EtOAc, washed with brine, dried over anhydrous $MgSO_4$, and purified by silica-gel column chromatography (Wako gel® C-200, 70% EtOAc/*n*-hexane) to provide **29** as a white powder (93 mg, 0.25 mmol, 22%, 2 steps from **10**): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 11.21 (s, 1H), 8.36 (d, J = 8.0 Hz, 1H), 7.53-7.48 (m, 2H), 7.28-7.24 (m, 1H), 5.07 (s, 1H), 4.68 (s, 1H), 3.40 (d, J = 6.4 Hz, 2H), 2.99 (d, J = 6.5 Hz, 2H), 2.44 (s, 3H), 1.86 (t, J = 7.0 Hz, 2H), 1.68 (s, 3H), 1.41 (m, 11H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 177.29, 156.28, 147.42, 139.60, 134.59, 131.30, 125.97, 124.37, 123.29, 123.17, 119.73, 117.96, 60.62, 40.55, 36.92, 28.63, 24.42, 18.68, 16.34, 14.40; ESI-MS (*m/z*) 371.2 $[M+H]^+$, 393.2 $[M+Na]^+$, 369.2 $[M-H]^-$, 405.2 $[M+Cl]^-$.

*Synthesis of **30***

To a solution of **29** (30 mg, 0.081 mmol) in MeOH (0.90 mL), concentrated HCl (3 drops) was added, and the mixture was stirred for 26 h at rt. Then, the reaction mixture was basified with 6.0 M aqueous NaOH, extracted with EtOAc, and dried over anhydrous $MgSO_4$. The organic layer was concentrated under reduced pressure to afford **30** as a red oil (27 mg, quant.): ^{1}H-NMR (400 MHz, MeOD) δ 8.22 (m, 1H), 7.61 (ddd, J = 8.4, 6.9, 1.4 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.33 (ddd, J = 8.2, 7.0, 1.1 Hz, 1H), 5.13 (ddd, J = 8.0, 5.6, 1.2 Hz, 1H), 3.39 (d, J = 6.6 Hz, 2H), 2.58 (t, J = 7.9 Hz, 2H), 2.46 (s, 3H), 2.03 (m, 2H), 1.81 (s, 3H), 1.57 (quin, J = 7.6 Hz, 2H); ^{13}C-NMR (100 MHz, MeOD) δ 178.62, 149.90, 140.71, 135.97, 132.76, 126.44, 125.16, 124.65, 124.12, 120.80, 118.83, 61.67, 42.23, 38.11, 24.96, 18.38, 16.39; ESI-MS (*m/z*) 271.2 $[M+H]^+$, 293.2 $[M+Na]^+$, 269.2 $[M-H]^-$.

*Synthesis of **PAD-4***

To a solution of **15** (32 mg, 0.10 mmol) in DMF (0.8 mL), HATU (36 mg, 0.094 mmol), HOAt (13 mg, 0.094 mmol), Et_3N (24 mg, 0.23 mmol), and a solution of **30** (21 mg, 0.078 mmol) in DMF (0.8 mL) were added under N_2 atmosphere, and the mixture was stirred for 3 h at 70 ˚C. The reaction

was quenched with brine, extracted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$, and purified by silica-gel column chromatography (Wako gel® C-200, 5% MeOH/$CHCl_3$) to provide **PAD-4** as an orange powder (28 mg, 0.049 mmol, 63%): ^{1}H-NMR (400 MHz, MeOD) δ 8.53 (t, J = 1.8 Hz, 1H), 8.25 (dd, J = 8.2, 0.9 Hz, 1H), 8.15 (dt, J = 8.2, 1.2 Hz, 1H), 7.90 (dd, J = 7.8, 0.9 Hz, 1H), 7.56 (td, J = 7.0, 1.4 Hz, 1H), 7.45 (m, 1H), 7.36-7.32 (m, 2H), 5.18 (t, J = 6.8 Hz, 1H), 3.48-3.40 (m, 2H), 2.47 (s, 3H), 2.11 (t, J = 7.4 Hz, 2H), 1.83 (s, 3H), 1.82-1.76 (m, 2H), 1.27 (s, 2H), 0.89 (s, 1H); ^{13}C-NMR (100 MHz, MeOD) δ 203.56, 193.48, 178.49, 160.92, 157.89, 148.88, 140.30, 139.66, 137.79, 134.79, 132.07, 129.60, 126.02, 124.67, 124.01, 120.06, 118.16, 100.28, 94.77, 40.13, 37.48, 27.80, 24.67, 18.40, 16.42; ESI-MS (*m/z*) 569.1 $[M+H]^+$, 591.1 $[M+Na]^+$, 567.1 $[M-H]^-$, 603.0 $[M+Cl]^-$.

*Synthesis of **31***

To a solution of **17** (26 mg, 0.054 mmol) in DMF (0.4 mL), HATU (37 mg, 0.097 mmol), HOAt (13 mg, 0.097 mmol), Et_3N (25 mg, 0.24 mmol), and a solution of **30** (27 mg, 0.081 mmol) in DMF (0.4 mL) were added under N_2 atmosphere, and the mixture was stirred for 4 h at 40 ˚C. The reaction was quenched with brine, extracted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$, and purified by silica-gel column chromatography (Wako gel® C-200, 60% EtOAc/*n*-hexane) to provide **31** as an orange oil (19 mg, 0.026 mmol, 32%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 10.6 (s, 1H), 8.35 (d, J = 8.0 Hz, 1H), 8.21 (m, 1H), 8.01 (dq, J = 8.0, 0.9 Hz, 1H), 7.59 (d, J = 7.1 Hz, 1H), 7.49-7.43 (m, 4H), 7.24 (m, 1H), 5.14 (t, J = 6.1 Hz, 1H), 3.44 (m, 2H), 2.46 (s, 3H), 1.99 (t, J = 7.3 Hz, 2H), 1.73 (s, 3H), 1.67 (t, J = 7.2 Hz, 2H), 1.58-1.50 (m, 6H), 1.37-1.28 (m, 8H), 1.13-1.09 (m, 6H), 0.88 (t, J = 7.3 Hz, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 177.38, 160.02, 156.95, 146.91, 145.50, 139.48, 138.70, 136.27, 134.13, 131.30, 129.09, 127.50, 126.13, 124.40, 123.80, 123.24, 120.10, 119.72, 117.74, 39.71, 37.01, 29.19, 27.48, 24.45, 18.79, 16.34, 13.84, 10.02, 8.31; ESI-MS (*m/z*) 733.3 $[M+H]^+$, 755.3 $[M+Na]^+$, 731.1 $[M-H]^-$, 767.2 $[M+Cl]^-$.

*Synthesis of **[^{125}I]PAD-4***

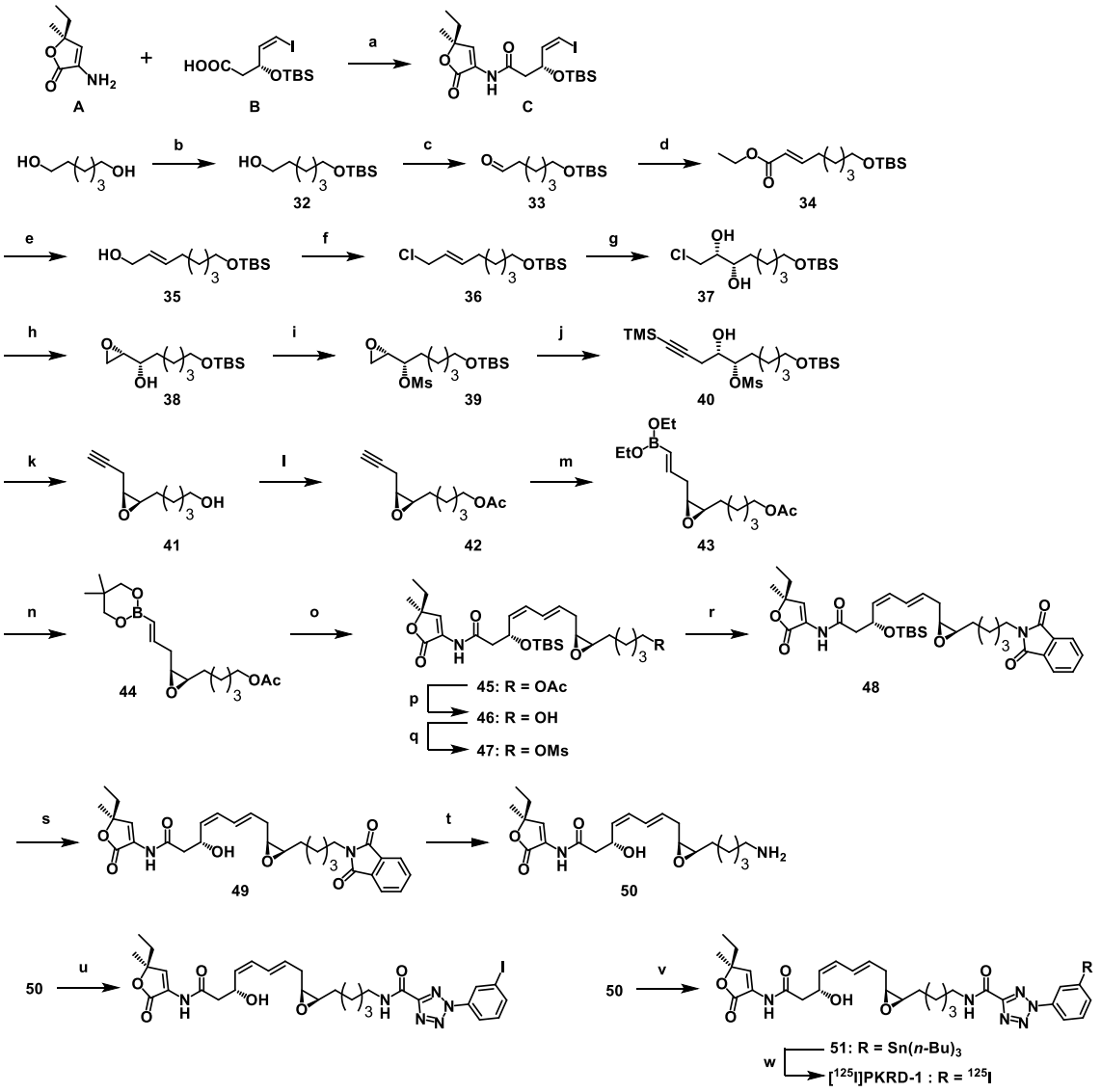
To a mixture of a stanyl-precursor **31** (1.0 mM in EtOH, 20 μL) and chloramine T (2.0 mM in 1.0 M KPi buffer (pH 7.4), 5 μL) in screw-capped 1.5 mL plastic-tube, [^{125}I]NaI (PerkinElmer, NEZ 033A, 1 mCi, 2,000 Ci/mmol, 10 μL) was added. The mixture was incubated at rt for 10 min. The reaction was terminated with 5% (w/v) aqueous $NaHSO_3$ (50 μL), and the resulting mixture was carefully extracted with $CHCl_3$ (3 × 100 μL). The combined organic layer was concentrated using vacuum-centrifugal evaporator. The crude product was dissolved in MeOH (30 μL), and subjected to

HPLC purification (Shimadzu LC-10AS, Kyoto, Japan) using an ODS column (COSMOSIL $5C_{18}$-MS-II, 4.6 x 150 mm, Nacalai Tesque, Kyoto, Japan) at a flow rate of 0.8 mL/min with isocratic 70% MeOH/water system as an eluent. The fraction was collected every 30 s (400 μL). To check the elution pattern of the radioactivities and their radiochemical purity, each fraction was measured by γ-counting system and radio-TLC analysis. The strong radioactive fractions, corresponding to the retention time of cold-type **PAD-4**, were combined and the solvent was evaporated by a vacuum-centrifugal evaporator. **[^{125}I]PAD-4** was stored as an ethanolic solution (0.10 μM) at 4 ˚C. The radiochemical yield of **[^{125}I]PAD-4** from the initial [^{125}I]NaI was 1.6%.

Outline of the syntheses of PKRD-1 and [^{125}I]PKRD-1

The synthetic procedure of **[^{125}I]PKRD-1** was outlined in Scheme S3. The key intermediates **A** and **B** were synthesized according to the method of Kobayashi *et al* and Uehara *et al* [*49*, *50*]. Mono-TBS ether protection of 1,6-hexanediol gave **32**. After oxidation of **32**, Horner-Wadsworth-Emmons reaction provided **34**, which was treated with DIBAL-H, followed by halogenation with Appel reaction. Sharpless asymmetric dihydroxylation with AD-mix-α of **36** gave **37**. After epoxidation of **37**, mesylation of hydroxy group gave **39**. The epoxide of **39** was opened by lithium trimethylsilylacetylide to afford **40**. After deprotection of TMS group and subsequent ring closure reaction, acetyl protection of hydroxy group gave **42**. Hydroboration and subsequent transesterification provided **44**. Suzuki-Miyaura cross coupling of **C** and **44** gave **45**. After deprotection of acetyl group and mesylation of hydroxy group, S_N2 reaction with potassium salt of phthalimide gave **48**. Deprotection of TBS group and phthalimide group provided **50**. The amidation of **50** with appropriate carboxylic acids provided **PKRD-1** and **51**. **[^{125}I]PKRD-1** was prepared from a stanyl precursor **51** using chloramine T and [^{125}I]NaI as an oxidant and a radioisotope donor, respectively.

Scheme S3.



Reagents and conditions: (a) DCC, DMAP, PPTS, CH_2Cl_2, rt, 12 h, 54%; (b) TBSCl, NaH, THF, rt, 20 °C, 61%; (c) PCC, NaOAc, celite, CH_2Cl_2, rt, 1 h, 73%; (d) triethyl phosphonoacetate, NaH, THF, rt, 30 min, 50%; (e) DIBAL-H, CH_2Cl_2, -78 °C, 1 h, 80%; (f) MsCl, LiCl, 2,4,6-trimethylpyridine, DMF, 0 °C, 3 h, 81%; (g) AD-mix-α, $MeSO_2NH_2$, *t*-BuOH/H_2O, 4 °C, 24 h; (h) NaOH, THF, rt, 1 h, 78% (2 steps); (i) MsCl, Et_3N, CH_2Cl_2, 0 °C, 40 min; (j) TMS acetylene, *n*-BuLi, $BF_3{\cdot}Et_2O$, THF, -78 °C, 1 h; (k) K_2CO_3, MeOH, rt, 10 h, 38% (3 steps); (l) Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt, 4 h, 88%; (m) 1) α-(-)-pinene, $BH_3{\cdot}SMe_2$, THF, 0 °C, 2 h, 2) acetaldehyde, THF, reflux, 19 h; (n) 2,2-dimethyl-1,3-propanediol, THF, rt, 5 h, 42% (2 steps); (o) **C**, $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O, 90 °C, 1 h, 42%; (p) K_2CO_3, MeOH/H_2O, rt, 3 h, 67%; (q) MsCl, Et_3N, CH_2Cl_2, 0 °C, 1 h; (r) potassium phthalimide, DMF, rt, 3 days, 69% (2 steps); (s) TBAF, THF, rt, 30 min, quant.; (t) $MeNH_2$, THF/MeOH, 0 °C, 4 h, 49%; (u) **15**, HATU, HOAt, Et_3N, DMF, rt, 24 h, 59%; (v) **17**, HATU, HOAt, Et_3N, DMF, rt, 24 h, 21%; (w) [^{125}I]NaI, chloramine T, water, rt, 10 min.

*Synthesis of **A***

This compound was synthesized according to the procedure described in ref *4*: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.83 (s, 1H), 3.72 (br, 2H), 1.66-1.83 (m, 2H), 1.42 (s, 3H), 0.86 (t, J = 7.4 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.80, 132.67, 119.81, 86.60, 32.75, 25.14, 8.42.

*Synthesis of **B***

This compound was synthesized according to the procedure described in ref *5*: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.28-6.33 (m, 2H), 4.81-4.86 (m, 1H), 2.54-2.56 (m, 2H), 0.86 (s, 9H), 0.11 and 0.07 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 176.23, 142.89, 81.57, 72.95, 41.71, 25.88 (3C), 18.15, -4.17, -4.79.

*Synthesis of **C***

To a solution of **A** (29 mg, 0.20 mmol) and **B** (70 mg, 0.20 mmol) in CH_2Cl_2 (1.5 mL), DMAP (6 mg, 0.04 mmol), PPTS (16 mg, 0.06 mmol), and DCC (46 mg, 0.22 mmol) were added under Ar atmosphere at rt. After stirring for 12 h at rt, the reaction mixture was quenched with water, extracted with CH_2Cl_2 and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 10-20% EtOAc/*n*-hexane) afforded **C** as a yellow oil (52 mg, 0.11 mmol, 54%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.35 (s, 1H), 6.36-6.29 (m, 2H), 4.80 (td, J = 7.2, 3.8 Hz, 1H), 2.61 (dd, J = 14.4, 3.8 Hz, 1H), 2.52 (dd, J = 14.4, 7.0 Hz, 1H), 1.90-1.70 (m, 2H), 1.47 (s, 3H), 0.87 (s, 9H), 0.86 (t, J = 7.4 Hz, 3H), 0.09 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.14, 168.95, 142.05, 133.84, 124.67, 88.28, 81.98, 73.28, 43.78, 32.06, 25.71 (3C), 24.32, 18.00, 8.15, -4.45, -4.99; ESI-MS (*m/z*) 480.2 $[M+H]^+$, 518.2 $[M+K]^+$.

*Synthesis of **32***

To an ice-cooled suspension of NaH (60% in mineral oil, 3.38 g, 85 mmol) under N_2 atmosphere, 1,6-hexanediol (10.0 g, 85 mmol) in THF (340 mL) was slowly added and the mixture was stirred for 30 min at 0 ˚C. After stirring for 2 h at rt, TBSCl (12.8 g, 84.6 mmol) was added to the mixture, and the reaction mixture was stirred at rt for 20 h. The reaction mixture was quenched with H_2O, extracted with Et_2O and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 20% EtOAc/*n*-hexane) afforded **32** as a colorless oil (12.0 g, 52 mmol, 61%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.64 (t, J = 6.8 Hz, 2H), 3.60 (t, J = 6.4 Hz, 2H), 1.58-1.35 (m, 8H), 0.89 (s, 9H), 0.05 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 68.19, 63.38, 33.00 (2C), 26.19 (3C), 25.84, 25.75, 18.59, -5.05 (2C).

*Synthesis of **33***

To a suspension of silica gel (5.0 g), NaOAc (1.88 g, 23 mmol) and PCC (4.92 g, 23 mmol) in CH_2Cl_2 (75 mL), **32** (3.54 g,15 mmol) was added under N_2 atmosphere, and the mixture was stirred for 1 h at rt. The reaction mixture was diluted with Et_2O, filtered, and purified by silica-gel column chromatography (Wako gel® C-200, 15% EtOAc/*n*-hexane) to provide **33** as a colorless oil (2.54 g, 11 mmol, 73%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.76 (s, 1H), 3.61 (t, J = 6.4 Hz, 2H), 2.43 (dt, J = 7.4, 1.8 Hz, 2 H), 1.51-1.65 (m, 6 H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 202.96, 63.09, 44.13, 32.75, 26.17 (3C), 25.69, 22.12, 18.57, -5.08 (2C).

*Synthesis of **34***

To an ice-cooled suspension of NaH (60% in mineral oil, 521 mg, 13 mmol) in THF (12 mL) under N_2 atmosphere, triethyl phosphonoacetate (4.0 g, 18 mmol) was added and the mixture was stirred for 5 min at 0 ˚C. After stirring for 30 min at rt, **2** (2.50 g, 11 mmol) in a small amount of THF (2 mL) was slowly added to the mixture, and then the reaction mixture was stirred at rt for 30 min. The reaction mixture was quenched with ice-cooled H_2O, extracted with *n*-hexane and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 10% EtOAc/*n*-hexane) afforded **33** as a colorless oil (1.65 g, 5.5 mmol, 50%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.95 (dt, J = 15.6, 7.0 Hz, 1 H), 5.80 (dt, J = 15.6, 5.8 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2 H), 3.60 (t, J = 6.5 Hz, 3H), 2.20 (q, J = 8.7 Hz, 2H), 1.39-1.59 (m, 6H), 0.89 (s, 9H), 0.04 (s, 6 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 167.01, 149.46, 121.56, 63.23, 60.34, 32.78, 32.39, 30.06, 28.03, 26.19 (3C), 18.58, 14.50, -5.06 (2C).

*Synthesis of **35***

To a solution of **34** (1.50 g, 5.0 mmol) in CH_2Cl_2 (10 mL), DIBAL-H (1.0 M in toluene, 10.0 mL, 10 mmol) was slowly added under N_2 atmosphere at -78 ˚C. After stirring for 1 h at -78 ˚C, the reaction mixture was quenched with saturated aqueous potassium sodium tartrate, and stirred for 2 h at 0 ˚C. The mixture was filtered through celite, and extracted with Et_2O and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 15% EtOAc/*n*-hexane) afforded **35** as a colorless oil (1.04 g, 4.0 mmol, 80%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.66 (m, 2H), 4.11 (m, 1H), 3.60 (t, J = 6.6 Hz, 2H), 2.05 (m, 2H), 1.24-1.61 (m, 6H), 0.89 (s, 9H), 0.05 (s, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 133.60, 129.02, 64.07, 63.40, 32.91, 32.40, 29.13, 26.20 (3C), 25.58, 18.59, -5.04 (2C).

*Synthesis of **36***

To a solution of **35** (658 mg, 2.6 mmol) in DMF (42 mL) under N_2 atmosphere, LiCl (1.08 g, 26 mmol) and 2,4,6-trimethyl pyridine (3.15 g, 26 mmol) were added. After stirring for 5 min at 0 ˚C, MsCl (871 mg, 7.6 mmol) was added to the mixture, and then the reaction mixture was stirred for 3 h at 0 ˚C. The reaction mixture was quenched with ice-cooled H_2O, extracted with *n*-hexane, washed with 1.0 M aqueous HCl (3 times) and saturated aqueous $NaHCO_3$ (3 times), and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 5% EtOAc/*n*-hexane) afforded **36** as a colorless oil (575 mg, 2.1 mmol, 81%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.76 (m, 1H), 5.60 (m, 1H), 4.04 (dd, J = 7.0, 0.8 Hz, 2H), 3.60 (t, J = 6.6 Hz, 2H), 2.06 (dt, J = 7.0, 6.8 Hz, 2H), 1.31-1.55 (m, 6H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 136.32, 126.18, 63.35, 45.70, 32.86, 32.25, 28.85, 26.20 (3C), 25.55, 18.58, -5.05 (2C).

*Synthesis of **37***

To a solution of AD-mix-α (14.3 g), $MeSO_2NH_2$ (948 mg, 10 mmol) in *t*-BuOH (50 mL) and H_2O (50 mL), **36** (2.67 g, 10 mmol) was slowly added under N_2 atmosphere at 0 ˚C. After stirring for 24 h at 0 ˚C, the reaction mixture was quenched with saturated aqueous $NaHCO_3$, extracted with Et_2O, washed with 1.0 M aqueous KOH, and dried over anhydrous $MgSO_4$. The resulting mixture was filtered and concentrated *in vacuo* to afford crude **37** as a light-yellow oil (2.11 g, <10 mmol), which was used for the next step without further purification.

*Synthesis of **38***

To a solution of **37** (2.11 g, <10 mmol, crude) in THF (22 mL) under N_2 atmosphere, NaOH (4.0 g, 100 mmol) was added. After stirring for 1 h at rt, the reaction mixture was diluted with Et_2O, quenched with brine, extracted with Et_2O, and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 30% EtOAc/*n*-hexane) afforded **38** as a colorless oil (2.14 g, 7.8 mmol, 78%, 2 steps): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.61 (t, J = 6.5 Hz, 2H), 3.44 (m, 1H), 2.98 (m, 1H), 2.82 (m, 1H), 2.71 (m, 1H), 1.39-1.65(m, 8H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 71.81, 63.34, 60.60, 55.53, 45.37, 34.69, 32.95, 26.20 (3C), 26.07, 18.59, -5.05 (2C).

*Synthesis of **39***

To a solution of **38** (2.41 g, 7.8 mmol) in CH_2Cl_2 (20 mL) and Et_3N (2.5 g, 25 mmol) under N_2

atmosphere, MsCl (0.9 mL, 12 mmol) was added at 0 ˚C. After stirring for 1 h at 0 ˚C, the reaction mixture was diluted with Et_2O, quenched with saturated aqueous $NaHCO_3$, extracted with Et_2O, and dried over anhydrous $MgSO_4$. The resulting mixture was filtered and concentrated *in vacuo* to afford crude **39** as a light yellow oil (2.69 mg, <7.8 mmol), which was used for the next step without further purification.

*Synthesis of **40***

To a solution of TMS acetylene (314 mg, 3.2 mmol) in THF (5 mL), *n*-BuLi (2.7 M in *n*-hexane, 1.0 mL, 2.65 mmol) was added at -78 ˚C under N_2 atmosphere. After stirring for 30 min at -78 ˚C, $BF_3 \cdot Et_2O$ (383 mg, 2.7 mmol) was added. After stirring for 10 min at -78 ˚C, **39** (560 mg, <1.6 mmol, crude) in THF (2.5 mL) was added. After stirring for 1 h at -78 ˚C, the mixture was allowed to warm to 0˚C. The reaction mixture was quenched with saturated aqueous NH_4Cl, extracted with Et_2O and dried over anhydrous $MgSO_4$. The resulting mixture was filtered and concentrated *in vacuo* to afford crude **40** as a light yellow oil (638 mg, < 1.6 mmol, crude), which was used for the next step without further purification.

*Synthesis of **41***

To a solution of **40** (1.50 g, <3.33 mmol) in MeOH (14 mL), K_2CO_3 (1.80 g, 13 mmol) was added at rt. After stirring for 10 h at rt, the reaction mixture was diluted with Et_2O, quenched with saturated aqueous NH_4Cl, extracted with Et_2O, and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 10% EtOAc/*n*-hexane) afforded **41** as a colorless oil (282 mg, 1.7 mmol, 38%, 3 steps): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.66 (t, J = 6.4 Hz, 2H), 3.16 (m, 1H), 2.97 (m, 1H), 2.60 (ddd, J = 17.2, 5.5, 2.5 Hz, 1H), 2.28 (ddd, J = 17.2, 7.3, 2.8 Hz, 1H), 2.05 (s, 1H), 1.39-1.64 (m, 8H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 79.61, 70.59, 63.02, 57.07, 54.96, 32.82, 27.69, 26.51, 25.82, 18.73.

*Synthesis of **42***

To an ice-cooled solution of **41** (300 mg, 1.8 mmol) in CH_2Cl_2 (4 mL), DMAP (11.0 mg, 0.09 mmol), Ac_2O (449 mg, 4.4 mmol), and Et_3N (233 mg, 2.3 mmol) were added under N_2 atmosphere at 0 ˚C. After stirring for 90 min at 0 ˚C and further 4 h at rt, the reaction mixture was quenched with saturated aqueous NH_4Cl, extracted with Et_2O, washed with saturated aqueous $NaHCO_3$, and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 50% EtOAc/*n*-hexane) afforded **42** as a colorless oil (329 mg, 1.6 mmol, 88%): ^{1}H-NMR (400 MHz,

$CDCl_3$) δ 4.07 (t, J = 6.7 Hz, 2H), 3.16 (m, 1H), 2.97 (m, 1H), 2.61 (ddd, J = 17.2, 5.5, 2.5 Hz, 1H), 2.28 (ddd, J = 17.2, 7.3, 2.1Hz, 1H), 2.06 (s, 1H), 2.05 (s, 3H), 1.43-1.70 (m, 8H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.40, 70.64, 64.56, 57.00, 54.93, 28.73, 27.64, 26.38, 26.02, 21.22, 18.73.

*Synthesis of **43***

To a solution of $BH_3 \cdot SMe_2$ (152 mg, 2.0 mmol) in THF (0.8 mL), (-)-α-pinene (682 mg, 5.0 mmol) was added under N_2 atmosphere at 0 ˚C. After stirring for 1 h at 0 ˚C and 2 h at rt, the reaction mixture was cooled to -30 ˚C. After addition of **42** (100 mg, 0.48 mmol), the reaction mixture was stirred for 10 min at -30 ˚C and 2 h at 0 ˚C. After addition of acetaldehyde (1.15 g, 26 mmol), the reaction mixture was stirred for 19 h at 40 ˚C. The resulting mixture was concentrated *in vacuo* to afford crude **43** as a light yellow oil (<1.6 mmol, crude), which was used for the next step without further purification.

*Synthesis of **44***

To a solution of **43** (<1.6 mmol, crude) in THF (0.8 mL), 2,2-dimethyl-1,3-propanediol (197 mg, 1.9 mmol) was added under N_2 atmosphere at rt. After stirring for 5 h at rt, the resulting mixture was concentrated *in vacuo*. The crude product was purified by silica-gel column chromatography (Wako gel® C-200, 10% EtOAc/*n*-hexane) to afford **44** as a colorless oil (61 mg, 0.20 mmol, 42%, 2 steps): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.53 (dt, J = 17.9, 5.3 Hz, 1H), 5.50 (dt, J = 17.9, 1.6 Hz, 1H), 4.05 (t, J = 4.0 Hz, 2H), 3.64 (s, 4H), 3.03 (m, 1H), 2.94 (m, 1H), 2.48 (m, 1H), 2.26 (m, 1H), 2.04 (s, 3H), 1.37-1.66 (m, 8H), 0.93 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.18, 145.57, 125.91 (br), 72.08, 64.41, 56.89, 55.54, 33.96, 31.78, 30.37, 28.54, 27.63, 25.87, 21.85, 21.00.

*Synthesis of **45***

To a solution of **C** (51 mg, 0.11 mmol) and $Pd(PPh_3)_4$ (6.2 mg, 0.0055 mmol) in DME (1.5 mL), **44** (42 mg, 0.13 mmol) and aqueous Na_2CO_3 (12 mg, 0.11 mmol in 0.8 mL H_2O) were added. After stirring for 1 h at 90 ˚C, the reaction mixture was cooled to rt, and quenched with brine, extracted with EtOAc and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 20-30% EtOAc/*n*-hexane) afforded **45** as a yellow oil (26 mg, 0.046 mmol, 42%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.34 (s, 1H), 6.40 (ddd, J = 15.0, 11.5, 0.8 Hz, 1H), 5.98 (t, J = 11.2 Hz, 1H), 5.78 (dt, J = 15.0, 6.9 Hz, 1H), 5.36 (dd, J = 10.7, 9.0 Hz, 1H), 5.01 (td, J = 8.0, 3.4 Hz, 1H), 4.07 (t, J = 6.6 Hz, 1H), 3.02-2.90 (m, 2H), 2.57 (dd, J = 14.2, 3.8 Hz, 1H), 2.49 (dd, J = 14.3, 7.7 Hz, 1H), 2.41-2.22 (m, 2H), 2.05 (s, 3H), 1.90-1.72 (m, 2H), 1.69-1.40 (m, 11H), 0.89-

0.80 (m, 12H), 0.07 (s, 3H), 0.06 (s, 3H).

*Synthesis of **46***

To a solution of **45** (26 mg, 0.046 mmol) in MeOH (1.8 mL), aqueous K_2CO_3 (19 mg, 0.14 mmol in 0.6 mL H_2O) was added. After stirring for 3 h at rt, the reaction mixture was quenched with saturated aqueous NH_4Cl, extracted with EtOAc and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 40-50% EtOAc/*n*-hexane) afforded **46** as a yellow oil (16 mg, 0.031 mmol, 67%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.34 (s, 1H), 6.39 (ddd, *J* = 14.9, 11.2, 1.0 Hz, 1H), 5.98 (t, *J* = 11.1 Hz, 1H), 5.78 (dt, *J* = 15.0, 7.0 Hz, 1H), 5.36 (dd, *J* = 10.9, 8.8 Hz, 1H), 5.01 (td, *J* = 7.8, 3.8 Hz, 1H), 3.66 (t, *J* = 6.5 Hz, 1H), 3.02-2.94 (m, 2H), 2.56 (dd, *J* = 14.1, 3.8 Hz, 1H), 2.52-2.41 (m, 2H), 2.30 (dt, *J* = 15.3, 6.9 Hz, 1H), 1.91-1.72 (m, 2H), 1.68-1.42 (m, 11H), 0.89-0.78 (m, 12H), 0.06 (s, 3H), 0.05 (s, 3H).

*Synthesis of **47***

To an ice-cooled solution of **46** (5.4 mg, 10.3 μmol) in CH_2Cl_2 (0.5 mL), Et_3N (6 μL, 43 μmol) and MsCl (3.3 μL, 43 μmol) were added under N_2 atmosphere at 0 ˚C. After stirring for 1 h at 0 ˚C, Et_3N (3 μL, 22 μmol), MsCl (1.6 μL, 21 μmol) were added to the reaction mixture. After stirring for 1 h at 0 ˚C, the reaction mixture was quenched with saturated aqueous NH_4Cl, extracted with EtOAc and dried over anhydrous $MgSO_4$. The resulting mixture was concentrated *in vacuo* to afford crude **47** (<10.3 μmol, crude, yellow oil), which was used for the next step without further purification.

*Synthesis of **48***

To a solution of **47** (<10.3 μmol, crude) in DMF (0.5 mL), potassium phthalimide (5.3 mg, 0.029 mmol) was added under N_2 atmosphere at 0 ˚C. After stirring for 3 days at rt, additional potassium phthalimide (2.6 mg, 0.015 mmol) was added to the reaction mixture. After stirring for 12 h at rt, the reaction mixture was quenched with saturated aqueous NH_4Cl, extracted with EtOAc and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 30% EtOAc/*n*-hexane) afforded **48** as a colorless oil (4.3 mg, 6.6 μmol, 64%, 2 steps): ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.84 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.71 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.33 (s, 1H), 6.39 (dd, *J* = 14.5, 11.8 Hz, 1H), 5.98 (t, *J* = 11.1 Hz, 1H), 5.77 (dt, *J* = 15.0, 6.9 Hz, 1H), 5.35 (dd, *J* = 10.6, 9.1 Hz, 1H), 5.01 (td, *J* = 8.0, 3.6 Hz, 1H), 3.70 (t, *J* = 7.3 Hz, 2H), 2.99-2.93 (m, 2H), 2.57 (dd, *J* = 14.2, 3.7 Hz, 1H), 2.49 (dd, *J* = 14.2, 7.6 Hz, 1H), 2.39 (dt, *J* = 15.6, 6.3 Hz, 2H), 2,31 (dt, *J* = 15.4, 6.1 Hz, 1H), 1.89-1.75 (m, 2H), 1.71 (quin, *J* = 7.4 Hz, 2H), 1.57-1.51 (m, 4H), 1.49-1.40 (m,

5H), 0.90-0.84 (m, 12H), 0.06 (s, 3H), 0.05 (s, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.91, 169.28, 168.63 (2C), 134.48, 134.07 (2C), 132.40 (2C), 132.29, 131.38, 129.02, 127.08, 124.95, 123.39 (2C), 88.36, 66.82, 57.05, 56.06, 46.14, 38.05, 32.28, 31.67, 28.70, 27.84, 26.91, 26.37, 25.94 (3C), 24.64, 18.24, 8.33, -4.15, -4.83; ESI-MS (*m/z*) 649.3 $[M-H]^-$.

*Synthesis of **49***

To a solution of **48** (9.4 mg, 0.014 mmol) in THF (0.8 mL), TBAF (1.0 M in THF, 0.030 mL, 0.028 mmol) was added under N_2 atmosphere. After stirring for 30 min at rt, the reaction mixture was quenched with saturated aqueous NH_4Cl, extracted with EtOAc and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 60% EtOAc/*n*-hexane) afforded **49** as a colorless oil (7.6 mg, 0.014 mmol, quant.): ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.85 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.16 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.36 (s, 1H), 6.46 (dd, *J* = 15.0, 11.8 Hz, 1H), 6.08 (t, *J* = 11.0 Hz, 1H), 5.82 (dt, *J* = 15.1, 6.7 Hz, 1H), 5.40 (dd, *J* = 10.6, 9.0 Hz, 1H), 5.06 (td, *J* = 8.0, 3.0 Hz, 1H), 3.68 (t, *J* = 7.3 Hz, 2H), 3.01 (td, *J* = 6.2, 4.2 Hz, 1H), 2.96 (td, *J* = 6.2, 4.3 Hz, 1H), 2.65 (dd, *J* = 15.8, 8.2 Hz, 1H), 2.60 (dd, *J* = 15.8, 3.7 Hz, 1H), 2.40 (dt, *J* = 15.8, 6.7 Hz, 1H), 2.31 (dt, *J* = 15.7, 6.5 Hz, 1H), 1.89-1.75 (m, 2H), 1.70 (quin, *J* = 7.4 Hz, 2H), 1.60-1.50 (m, 4H), 1.49 (s, 3H), 1.42 (quin, *J* = 7.2 Hz, 2H), 0.89 (t, *J* = 7.4 Hz, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.58, 169.39, 168.75 (2C), 134.33, 134.15 (2C), 132.80 (2C), 132.39, 131.00, 129.92, 126.97, 124.90, 123.46 (2C), 88.59, 64.98, 57.14, 55.88, 43.64, 38.06, 32.32, 31.58, 28.64, 27.77, 26.88, 26.26, 24.49, 8.43; ESI-MS (*m/z*) 559.2 $[M+Na]^+$, 535.2 $[M-H]^-$.

*Synthesis of **50***

To a solution of **49** (7.6 mg, 0.014 mmol) in THF/MeOH (2:10, 0.6 mL), 40% $MeNH_2$ (0.153 mL, 1.74 mmol) was added under N_2 atmosphere. After stirring for 4 h at 0 ˚C, the reaction mixture was concentrated *in vacuo* and purified by silica-gel column chromatography (Wako gel® C-200, MeOH) afforded **50** as a colorless oil (2.8 mg, 6.9 μmol, 49%): ^{1}H-NMR (500 MHz, MeOD) δ 7.40 (s, 1H), 6.56 (dd, *J* = 14.5, 11.7 Hz, 1H), 6.05 (t, *J* = 11.0 Hz, 1H), 5.79 (dt, *J* = 15.1, 6.9 Hz, 1H), 5.37 (t, *J* = 9.9 Hz, 1H), 5.02 (td, *J* = 8.2, 5.4 Hz, 1H), 3.04-2.97 (m, 2H), 2.72 (t, *J* = 7.2 Hz, 2H), 2.66 (dd, *J* = 14.5, 8.1 Hz, 1H), 2.50 (dd, *J* = 14.6, 5.0 Hz, 1H), 2.38-2.32 (m, 2H), 1.89-1.76 (m, 2H), 1.65-1.47 (m, 6H), 1.47-1.40 (m, 5H), 0.85 (t, *J* = 7.4 Hz, 3H); ^{13}C-NMR (125 MHz, MeOD) δ 172.54, 170.73, 136.36, 132.67, 132.35, 131.00, 128.89, 126.74, 89.30, 65.89, 58.55, 57.48, 45.24, 42.13, 33.03, 32.44, 28.75, 27.81, 27.57, 24.73, 8.51; ESI-MS (*m/z*) 405.2 $[M-H]^-$, 407.2 $[M+H]^+$, 429.2 $[M+Na]^+$.

*Synthesis of **PKRD-1***

To a solution of **50** (2.8 mg, 6.9 μmol) and **15** (3.3 mg, 0.010 mmol) in DMF (1 mL), Et_3N (3.0 μL, 0.021 mmol), HATU (3.2 mg, 8.3 μmol), and HOAt (1.1 mg, 8.3 μmol) were added under N_2 atmosphere. After stirring for 24 h at rt, the reaction mixture was diluted with CH_2Cl_2, quenched with brine, extracted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 40-60% EtOAc/*n*-hexane) afforded **PKRD-1** as a colorless oil (2.9 mg, 4.1 μmol, 59%): ^{1}H-NMR (500 MHz, MeOD) δ 8.56 (t, *J* = 1.8 Hz, 1H), 8.21 (ddd, *J* = 8.2, 2.0, 0.8 Hz, 1H), 7.97 (d, *J* = 7.9 Hz, 1H), 7.42 (t, *J* = 8.1 Hz, 1H), 7.39 (s, 1H), 6.56 (dd, *J* = 15.0, 12.0 Hz, 1H), 6.05 (t, *J* = 11.0 Hz, 1H), 5.79 (dt, *J* = 15.1, 6.9 Hz, 1H), 5.36 (t, *J* = 10.0 Hz, 1H), 5.03 (td, *J* = 8.2, 5.2 Hz, 1H), 3.48 (t, *J* = 7.1 Hz, 2H), 3.03-2.97 (m, 2H), 2.66 (dd, *J* = 14.6, 8.1 Hz, 1H), 2.50 (dd, *J* = 14.5, 5.1 Hz, 1H), 2.38-2.32 (m, 2H), 1.86-1.75 (m, 2H), 1.70 (quin, *J* = 7.2 Hz, 2H), 1.63-1.47 (m, 5H), 1.44 (s, 3H), 0.83 (t, *J* = 7.4 Hz, 3H); ^{13}C-NMR (125 MHz, MeOD) δ 177.58, 172.52, 170.71, 140.96, 138.71, 136.33, 132.81, 132.66, 132.35, 131.00, 130.28, 128.93, 126.73, 120.83, 95.11, 89.29, 65.88, 58.58, 57.49, 45.24, 40.75, 33.03, 32.47, 30.34, 28.75, 27.92, 27.40, 24.72, 8.52; ESI-MS (*m/z*) 703.0 $[M-H]^-$, 705.3 $[M+H]^+$, 727.1 $[M+Na]^+$.

*Synthesis of **51***

To a solution of **50** (3.7 mg, 9.1 μmol) and **17** (6.55 mg, 13.7 μmol) in DMF (1.5 mL), Et_3N (3.8 μL, 0.027 mmol), HATU (4.2 mg, 10.9 μmol), and HOAt (1.7 mg, 10.9 μmol) were added under N_2 atmosphere. After stirring for 24 h at rt, the reaction mixture was diluted with CH_2Cl_2, quenched with brine, extracted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and dried over anhydrous $MgSO_4$. The purification by silica-gel column chromatography (Wako gel® C-200, 10-20% EtOAc/*n*-hexane) afforded **51** as a yellow oil (1.7 mg, 1.9 μmol, 21%): ^{1}H-NMR (500 MHz, MeOD) δ 8.24 (t, *J* = 2.3 Hz, 1H), 8.10 (dt, *J* = 8.1, 1.1 Hz, 1H), 7.69 (d, *J* = 2.2 Hz, 1H), 7.60 (t, *J* = 7.5 Hz, 1H), 7.39 (s, 1H), 6.56 (dd, *J* = 15.1, 6.7 Hz, 1H), 6.04 (t, *J* = 11.0 Hz, 1H), 5.79 (dt, *J* = 15.1, 6.9 Hz, 1H), 5.36 (t, *J* = 9.9 Hz, 1H), 5.03 (td, *J* = 8.3, 5.2 Hz, 1H), 3.48 (t, *J* = 7.1 Hz, 2H), 3.03-2.97 (m, 2H), 2.66 (dd, *J* = 14.5, 8.1 Hz, 1H), 2.50 (dd, *J* = 14.6, 5.1 Hz, 1H), 2.38-2.33 (m, 2H), 1.86-1.75 (m, 2H), 1.71 (quin, *J* = 7.1 Hz, 2H), 1.64-1.45 (m, 12H), 1.44 (s, 3H), 1.37 (tt, *J* = 7.5, 7.5 Hz, 6H), 1.18 (t, *J* = 7.9 Hz, 6H), 0.90 (t, *J* = 7.3 Hz, 9H), 0.83 (t, *J* = 7.4 Hz, 3H); ^{13}C-NMR (125 MHz, MeOD) δ 172.52, 161.49, 159.18, 139.88, 137.78, 136.32, 132.66, 131.00, 130.49, 129.41, 128.93, 128.47, 126.72, 121.26, 89.28, 65.88, 58.59, 57.50, 45.24, 40.74, 33.03, 32.47, 30.37, 30.32 (3C), 28.76, 28.46 (3C), 27.95, 27.42, 24.73, 14.10, 10.78, 8.52; ESI-MS (*m/z*) 869.3 $[M+H]^+$, 891.3 $[M+Na]^+$.

Synthesis of ***[^{125}I]PKRD-1***

To a mixture of a stanyl-precursor **51** (1.0 mM in EtOH, 20 μL) and chloramine T (2.0 mM in 1.0 M KPi buffer (pH 7.4), 5 μL) in screw-capped 1.5 mL plastic-tube was added [^{125}I]NaI (PerkinElmer, NEZ 033A, 1 mCi, 2,000 Ci/mmol, 10 μL). The mixture was incubated at rt for 10 min. The reaction was terminated with 5% (w/v) aqueous $NaHSO_3$ (50 μL), and the resulting mixture was carefully extracted with $CHCl_3$ (3 × 100 μL). The combined organic layer was concentrated using vacuum-centrifugal evaporator. The crude product was dissolved in MeOH (30 μL), and subjected to HPLC purification (Shimadzu LC-10AS, Kyoto, Japan) using an ODS column (COSMOSIL 5C_{18}-MS-II, 4.6 x 150 mm, Nacalai Tesque, Kyoto, Japan) at a flow rate of 0.8 mL/min with isocratic 75% MeOH/water system as an eluent. The fraction was collected every 30 s (400 μL). To check the elution pattern of the radioactivities and their radiochemical purity, each fraction was measured by γ-counting system and radio-TLC analysis. The strong radioactive fractions, corresponding to the retention time of cold-type **PKRD-1**, were combined and the solvent was evaporated by a vacuum-centrifugal evaporator. **[^{125}I]PKRD-1** was stored as an ethanolic solution (0.20 μM) at 4 ˚C. The radiochemical yield of **[^{125}I]PKRD-1** from the initial [^{125}I]NaI was 4.6%.