Supporting Information

Development of a series of near-infrared dark quenchers based on Si-rhodamines and their application to fluorescent probes

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Abbreviations:

AcOEt: ethyl acetate

AcOH: acetic acid

Ac_2O: acetic anhydride

DCM: dichloromethane

DIEA: *N*,*N*-diisopropylethylamine

DMEM: Dulbecco's modified Eagle's medium

DMF: *N*,*N*-dimethylformamide

DMSO: dimethyl sulfoxide

EMEM: Eagle's minimal essential medium

ESI: electrospray ionization

EtOH: ethanol

Φ_{fl}: fluorescence quantum yield

FBS: fetal bovine serum

HATU: 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate methanaminium

HBSS: Hanks' balanced salt solution

HBTU: *O*-benzotriazole-*N,N,N',N'*-tetramethyluronium hexafluorophosphate

HOBt: 1-hydroxybenzotriazole

HPLC: high-performance liquid chromatography

HRMS: high-resolution mass spectrometry

MeOH: methanol

MMP: matrix metalloproteinase

MS: mass spectrometry

NHS: *N*-hydroxysuccinimide

NMR: nuclear magnetic resonance

ODS: octadecylsilane

PBS: phosphate-buffered saline

r.t.: room temperature

TEA: triethylamine

TFA: trifluoroacetic acid

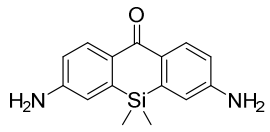
THF: tetrahydrofuran

WSCD: water-soluble carbodiimide

Experimental Section

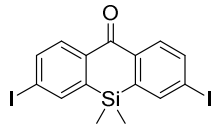
Synthesis.

3,6-Diamino-Si-xanthone (2)



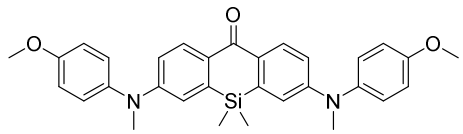
Prepared according to the literature.[SR1]

3,6-Diiodo-Si-xanthone (3)



Compound **2** (228 mg, 0.85 mmol) was dissolved in a mixture of 5 mL CH_3CN and 10 mL 2 N HCl aq., and the solution was cooled to 0°C. A solution of $NaNO_2$ (140 mg, 1.70 mmol) in 2 mL H_2O was added dropwise, and the mixture was stirred for 15 min. KI (2.82 g, 8.50 mmol) in 2 mL H_2O was added, and stirring was continued for 1 hr. Then 20 mL H_2O was added, and the whole was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Purification of the residue by column chromatography (silica gel, CH_2Cl_2) provided **3** (72 mg, 17% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.51 (s, 6H), 7.92 (dd, J = 8.10 Hz, J = 1.5 Hz, 2H), 7.98 (d, J = 1.5 Hz, 2H), 8.09 (d, J = 8.1 Hz, 2H); ^{13}C NMR (75 MHz, DMSO): δ –1.7, 101.8, 131.8, 131.4, 139.5, 139.5, 140.9, 141.8, 187; HRMS (ESI^+): Calcd for $[M+H]^+$, 490.8825; found, 490.8835 (+1.0 mmu).

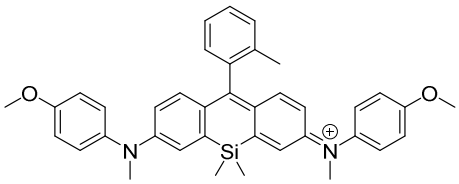
3,6-Bis(*N*-methyl-*p*-anisidine)-Si-xanthone (4)



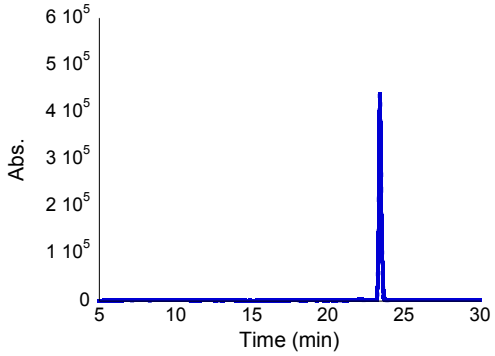
Compound **3** (60 mg, 0.12 mmol), Cs_2CO_3 (557 mg, 1.70 mmol) and *N*-methylanisidine (131 mg, 0.96 mmol) were dissolved in toluene in a 50 mL Schlenk flask, and the mixture was deaerated under argon. $Pd(OAc)_2$ (5.2 mg, 0.02 mmol) and BINAP (14.8 mg, 0.02 mmol) were added under argon. The mixture was heated to 100°C and stirred overnight. Then, it was cooled to r.t. and H_2O was added. The whole was

extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Purification of the residue by column chromatography (silica gel, CH_2Cl_2) provided **4** (12 mg, 20% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.36 (s, 6H), 3.36 (s, 6H), 3.84 (s, 6H), 6.77-6.83 (m, 4H), 6.95 (d, J = 8.7 Hz, 4H), 7.17 (d, J = 8.7 Hz, 4H), 8.30 (d, J = 9.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ –1.3, 40.3, 55.5, 115.0, 115.1, 115.9, 127.8, 130.6, 131.2, 140.3, 151.0, 157.4, 185.1; HRMS (ESI$^+$): Calcd for $[M+H]^+$, 509.2260; found, 509.2216 (–4.4 mmu).

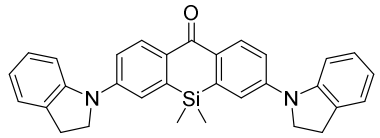
SiNQ660 (5)



To a flame-dried flask flushed with argon, **4** (12 mg, 0.02 mmol) and anhydrous THF (5 mL) were added. The solution was heated to 80°C, and 1 M *o*-tolyl magnesium bromide (120 μL, 0.12 mmol) was added to it. The mixture was stirred for 2 hrs and cooled to r.t. The reaction was quenched by addition of 2 N HCl and stirring was continued at r.t. for 15 min. The whole was extracted with CH_2Cl_2, and the organic layer was dried over Na_2SO_4 and evaporated to dryness. Purification of the residue by HPLC (eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 640 nm; eluent A (H_2O containing 0.1% TFA (v/v)) and eluent B (CH_3CN with 20% H_2O containing 0.1% TFA (v/v))) provided SiNQ650 (**5**) trifluoroacetic salt (13 mg, 93% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.49 (s, 3H), 0.51 (s, 3H), 2.03, (s, 3H), 3.61 (s, 6H), 3.86 (s, 6H), 6.50 (dd, J = 2.1, 9.0 Hz, 2H), 6.99-7.06 (m, 7H), 7.06-7.18 (m, 6H), 7.27-7.37(m, 3H); HRMS (ESI$^+$): Calcd for $[M]^+$, 583.2781; found, 583.2740 (–4.1 mmu). The HPLC chromatogram after purification is shown below. Elution was done with a 20-min linear gradient from 20% to 80% solvent B. 1.0 ml/min flow rate. Detection at 640 nm.

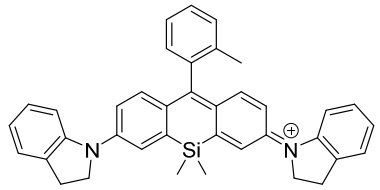


3,6-Diindoline-Si-xanthone (6)



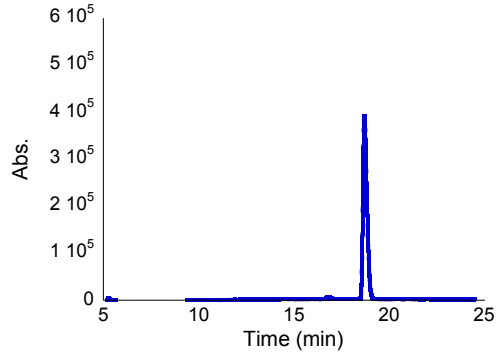
Compound **3** (60 mg, 0.12 mmol), Cs_2CO_3 (78 mg, 0.24 mmol) and indoline (28 mg, 0.24 mmol) were dissolved in toluene in a 50 mL Schlenk flask, and the mixture was deaerated under argon. $Pd(OAc)_2$ (2.6 mg, 0.01 mmol) and BINAP (7.4 mg, 0.01 mmol) were added to the solution under argon. The mixture was heated to 100°C and stirred overnight. The solution was cooled to r.t. and H_2O was added to it. The whole was extracted with CH_2Cl_2, and the organic layer was dried over Na_2SO_4 and evaporated to dryness. Purification of the residue by column chromatography (silica gel, CH_2Cl_2/ hexane = 8/2) provided **6** (31 mg, 54% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.52 (s, 6H), 3.20 (t, *J* = 8.1 Hz, 4H), 4.10 (t, *J* = 8.1 Hz, 4H), 6.86 (t, *J* = 7.5 Hz, 2H), 7.16 (t, *J* = 7.5 Hz, 2H), 7.23 (d, *J* = 7.2 Hz, 2H), 7.31-7.37 (m, 4H), 7.42 (d, *J* = 3.0 Hz, 2H), 8.47 (d, *J* = 9.0 HZ, 2H); ^{13}C NMR (75 MHz, DMSO): δ –1.20, 28.1, 51.8, 109.6, 117.6, 119.1, 120.3, 125.4, 127.2, 131.5, 132.1, 133.0, 140.4, 145.4, 146.3; HRMS (ESI^+): Calcd for $[M+H]^+$, 473.2049; found, 473.2007 (–4.2 mmu).

SiNQ780 (7)

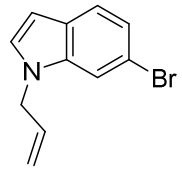


To a flame-dried flask flushed with argon, **6** (44 mg, 0.09 mmol) and anhydrous THF (5 mL) were added. The solution was heated to 80°C, and 1 M *o*-tolyl magnesium bromide (1 mL) was added to it. The mixture was stirred for 2 hrs and cooled to r.t. The reaction was quenched by addition of 2 N HCl and stirring was continued at r.t. for 15 min. The whole was extracted with CH_2Cl_2, and the organic layer was dried over Na_2SO_4 and evaporated to dryness. Purification of the residue by HPLC (eluent, a 20-min linear gradient, from 60% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 720 nm; eluent A (H_2O containing 0.1% TFA (v/v)) and eluent B (CH_3CN with 20% H_2O containing 0.1% TFA (v/v))) provided SiNQ780 (**7**) trifluoroacetic salt (15 mg, 25% yield). ^{1}H NMR (300 MHz, CD_3CN): δ 0.65 (s, 6H), 2.16 (s, 3H), 3.26 (t, *J* = 8.1 Hz, 4H), 4.32 (t, *J* = 8.1 Hz, 4H), 7.12-7.49 (m, 16H), 7.57(d, *J* = 8.1 Hz, 2H), 7.72 (d, *J* = 2.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ –1.5, –1.1, 19.5, 28.2. 53.1, 114.5, 117.2, 124.0, 125.6, 126.4, 127.8, 128.9, 129.2, 130.4, 130.5, 135.5, 135.7, 138.1, 141.7, 142.0, 148.8, 149.5; HRMS (ESI^+): Calcd for $[M]^+$, 547.2570; found, 547.2536 (–3.4 mmu). The HPLC chromatogram after purification is shown below.

Elution was done with a 20-min linear gradient from 80% to 100% solvent B.1.0 ml/min flow rate. Detection at 720 nm.

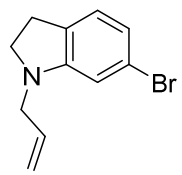


1-Allyl-6-bromoindole (8)



6-Bromoindoline (3.25 g, 16.8 mmol) was dissolved in DMF (20 mL), and the solution was cooled to 0°C. NaH (737 mg, 20.2 mmol, 60%) was added to the solution, and the mixture was stirred at 0°C for 1 hr. Allyl bromide (2 mL) was added to it, and stirring was continued at r.t. for 2 hrs. Then, H_2O was added, and the whole was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Purification of the residue by column chromatography (silica gel, CH_2Cl_2/hexane = 2/8) provided **8** (3.31 g, 85% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.56 (d, *J* = 6.0 Hz, 2H), 4.98 (d, *J* = 16.8 Hz, 1H), 5.14 (d, *J* = 10.2 Hz, 1H), 5.82-5.93 (m, 1H), 6.44 (d, *J* = 3.0 Hz, 1H), 6.99 (d, *J* = 3.0 Hz, 1H), 7.17 (dd, *J* = 1.5, 8.1 Hz, 1H), 7.43-7.45 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 48.7, 101.6, 112.5, 115.1, 117.4, 122.1, 122.6, 127.4, 128.4, 132.9, 136.8.

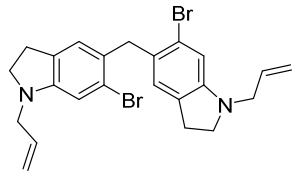
1-Allyl-6-bromoindoline (9)



Compound **8** (3.00 g, 12.8 mmol) was dissolved in AcOH (10 mL), and the solution was cooled to 0°C. $NaBH_3CN$ (995 mg, 15.3 mmol) was added to it, and the solution was stirred at r.t. for 2 hrs. Then, the mixture was neutralized with 2 N NaOH aq. The whole was extracted with CH_2Cl_2, and the organic layer

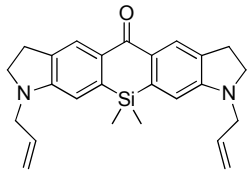
was dried over Na_2SO_4 and evaporated to dryness. Purification of the residue by column chromatography (silica gel, CH_2Cl_2/hexane = 2/8) provided **9** (2.12g, 70% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.88 (t, *J* = 9.0 Hz, 2H), 3.36 (t, *J* = 9.0 Hz, 2H), 3.65 (d, *J* = 6.0 Hz, 2H), 5.17-5.29 (m, 2H), 5.80-5.92 (m, 1H), 6.55 (d, *J* = 1.8 Hz, 1H), 6.71 (dd, *J* = 1.8, 7.2 Hz, 1H), 6.87 (d, *J* = 7.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.0, 51.4, 53.2, 110.0, 117.5, 119.9, 120.9, 125.3, 129.1, 133.3, 153.5; HRMS (ESI^+): Calcd for $[M+H]^+$, 238.0231; found, 238.0257 (+2.6mmu).

Compound 10



Compound **9** (2.12 g, 8.9 mmol) and 36% formaldehyde in water containing 10% MeOH (222 mg, 26.7 mmol) were dissolved in AcOH (30 mL). The mixture was heated to 60°C, stirred for 15 min, cooled to r.t., and neutralized with 2 N NaOH aq. The whole was extracted with CH_2Cl_2, and the organic layer was dried over Na_2SO_4 and evaporated to dryness. Purification of the residue by column chromatography (silica gel, CH_2Cl_2/hexane = 1/9 to 2/8 to 3/7) provided **10** (672 mg, 16% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.84 (t, *J* = 8.10 Hz, 4H), 3.33 (t, *J* = 8.1 Hz, 4H), 3.66 (d, *J* = 6.0 Hz, 4H), 3.96 (s, 2H), 5.18-5.30 (m, 4H), 5.81-5.94 (m, 2H), 6.67 (s, 2H), 6.71 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.2, 40.5, 51.9, 53.4, 110.9, 117.5, 123.0, 126.2, 128.2, 130.1, 133.7, 151.8; HRMS (ESI^+): Calcd for $[M+H]^+$, 489.0364; found, 489.0384 (+2.0 mmu).

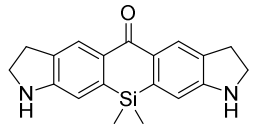
Compound 11



To a flame-dried flask flushed with argon, **10** (660 mg, 1.36 mmol) and anhydrous THF (10 mL) were added. The solution was cooled to –78°C, and 1 M *sec*-BuLi (3.26 mL, 3.26 mmmol) was added to it. The mixture was stirred at –78°C for 30 min. Dichlorodimethylsilane (438 mg, 413 μL, 3.40 mmol) was added, and the mixture was warmed to r.t., and then stirred for 2 hrs. The reaction was quenched by addition of 2 N HCl and stirring was continued at r.t. for 15 min. The whole was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in acetone (30 mL), and the

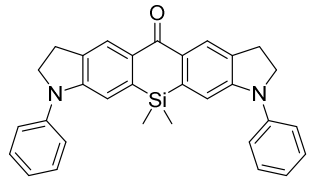
solution was cooled to r.t. To this solution, $KMnO_4$ (537 mg, 3.40 mmol) was added in small portions over a period of 2 hrs with vigorous stirring. The mixture was stirred for another 1 hr at r.t., then diluted with CH_2Cl_2, filtered through a Celite filter and evaporated to dryness. The residue was purified by column chromatography (silica gel, CH_2Cl_2/hexane = 1/1) to give **11** (92 mg, 17% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.41 (s, 6H), 3.05 (t, *J* = 8.1 Hz, 4H), 3.49 (t, *J* = 8.1 Hz, 4H), 3.85 (d, *J* = 6.0 Hz, 4H), 5.20-5.32 (m, 4H), 5.81-5.94 (m, 2H), 6.52 (s, 2H), 8.21 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ –1.19, 27.8, 50.4, 52.3, 108.1, 117.6, 126.2, 131.5, 132.1, 133.1, 139.9, 153.7, 185.0; HRMS (ESI^+): Calcd for $[M+H]^+$, 401.2049; found, 401.2092 (+4.3 mmu).

Compound 12



Compound **11** (78 mg, 0.20 mmol) and 3,6-dimethylbarbituric acid (120 mg, 0.80 mmol) were dissolved in CH_2Cl_2 (5 mL), and the solution was degassed with argon. $Pd(PPh_3)_4$ (22 mg, 0.02 mmol) was added to it under argon, and the mixture was stirred for 12 hrs at 40^{o}C and then evaporated to dryness. The residue was purified by column chromatography (silica gel, CH_2Cl_2) to give **12** (32 mg, 50% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.38 (s, 6H), 3.12 (t, *J* = 8.1 Hz, 4H), 3.67 (t, *J* = 8.1 Hz, 4H), 4.13 (br, 2H), 6.73 (s, 2H), 8.26 (s, 2H); ^{13}C NMR (300 MHz, $CDCl_3$): δ –1.3, 29.1, 46.9, 110.8, 126.6, 131.2, 132.5, 139.7, 154.0, 185.4; HRMS (ESI^+): Calcd for $[M+H]^+$, 321.1423; found, 321.1419 (–0.4 mmu).

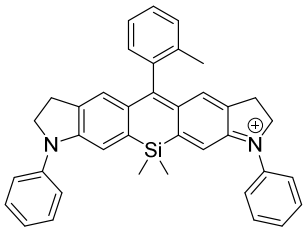
Compound 13



Compound **12** (32 mg, 0.10 mmol), Cs_2CO_3 (150 mg, 0.46 mmol) and iodobenzene (60 mg, 0.30 mmol) were dissolved in toluene in a 50 mL Schlenk flask, and the solution was deaerated under argon. $Pd(OAc)_2$ (2.0 mg, 0.01 mmol) and BINAP (6.0 mg, 0.01 mmol) were added under argon, and the mixture was heated at 100^{o}C and stirred overnight. It was then cooled to r.t., H_2O was added, and the whole was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Purification of the residue by column chromatography (silica gel, CH_2Cl_2) provided **13** (20 mg, 42% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.40 (s, 6H), 3.22 (t, *J* = 6.0 Hz, 4H), 4.06 (t, *J* = 6.0 Hz, 4H), 7.08 (t, *J* = 5.7 Hz, 1H), 7.21 (s, 2H), 7.31 (d, *J* = 5.7 Hz, 2H), 7.42 (t, *J* = 5.7Hz, 2H), 8.32 (s, 2H); ^{13}C NMR (300 MHz, $CDCl_3$): δ –1.2, 27.7, 52.4,

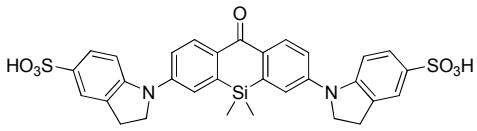
110.1, 118.8, 122.4, 126.9, 129.4, 132.9, 133.6, 139.8, 143.1, 149.5, 184.8; HRMS (ESI^+): Calcd for $[M+H]^+$, 473.2049; found, 473.2077 (+2.8 mmu).

Compound 14



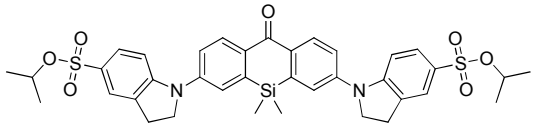
To a flame-dried flask flushed with argon, **13** (20 mg, 0.04 mmol) and anhydrous THF (5.0 mL) were added. The solution was heated to 80°C, and then 1 M *o*-tolyl magnesium bromide (2.0 mL, 2.0 mmol) was added to it. The mixture was stirred for 2 hrs and cooled to r.t. The reaction was quenched by addition of 2 N HCl and stirring was continued at r.t. for 15 min. The whole was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness. Purification of the residue by HPLC (eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 640 nm) provided **14** trifluoroacetic salt (5 mg, 19% yield). ^1H NMR (300 MHz, CD_2Cl_2): δ 0.37 (s, 3H), 0.40 (s, 3H), 2.02 (s, 3H), 3.02 (t, *J* = 6.0 Hz, 4H), 4.19 (t, *J* = 5.7 Hz, 4H), 6.87 (s, 2H), 7.06 (d, *J* = 5.7 Hz, 1H), 7.22 (s, 2H), 7.27-7.35 (m, 8H), 7.41 (d, *J* = 5.4 Hz, 1H), 7.46 (t, *J* = 6.0 Hz, 4H); ^{13}C NMR (300 MHz, $CDCl_3$): δ –1.6, –1.3, 19.6, 26.9, 55.0, 116.8, 122.4, 126.3, 127.6, 129.2, 129.3, 130.5, 130.9, 131.3, 135.2, 135.2, 136.1, 139.5, 139.8, 151.6, 154.8, 169.3; HRMS (ESI^+): Calcd for $[M]^+$, 547.2570; found, 547.2532 (–3.8 mmu).

3,6-DiSO_3H indoline-Si-xanthone (15)



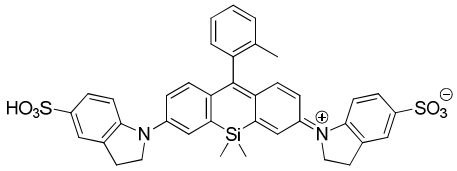
Compound **6** (400 mg, 0.93 mmol) was dissolved in CH_2Cl_2 (10 mL), and the solution was cooled to 0°C and stirred for 10 min. Chlorosulfuric acid (259 mg, 204 μL, 2.23 mmol) was added dropwise, and the mixture was stirred for 2 hrs at 0°C. The reaction was quenched by addition of H_2O, and the mixture was evaporated to remove CH_2Cl_2. Purification of the solution by HPLC (eluent, a 20-min linear gradient, from 0% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 350 nm) provided **15** (465 mg, 74% yield). ^1H NMR (300 MHz, DMSO):δ 0.54 (s, 6H), 3.22 (t, *J* = 9.0 Hz, 4H), 4.15 (t, *J* = 9.0 Hz, 4H), 7.29 (d, *J* = 8.7 Hz, 2H), 7.45 (dd, *J* = 2.4, 8.7 Hz, 2H), 7.49 (s, 2H), 7.63-7.66 (m, 4H), 8.37 (d, *J* = 8.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ. –1.44, 27.1, 51.7, 108.1, 117.5, 119.5, 122.9, 125.1, 130.8, 131.8, 132.1, 140.1, 140.5, 144.9, 145.7, 183.6; HRMS (ESI^-): Calcd for $[M-H]^-$, 630.0951; found, 630.0991 (+4.0 mmu).

3,6-Di-*i*-PrSO$_3$ indoline-Si-xanthone (16)

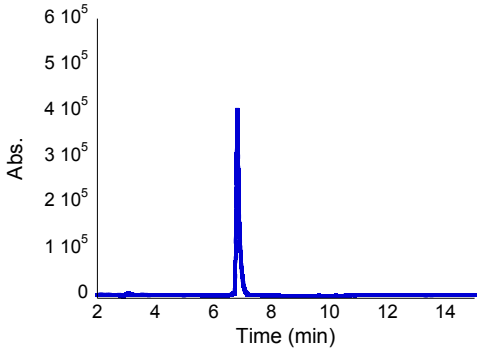


Compound **15** (100 mg, 0.16 mmol) was dissolved in *i*-PrOH (5 mL) and triisopropyl orthoformate (2.0 mL), and the mixture was heated to 55^{o}C and stirred for 4 hrs. The deposited product was filtered off and washed with hexane. Compound **16** (80 mg, 71% yield) was obtained without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.54 (s, 6H), 1.31 (d, *J* = 6.0 Hz, 12H), 3.27 (t, *J* = 8.7 Hz, 4H), 4.22 (t, *J* = 8.7 Hz, 4H), 4.76 (sep, *J* = 6.0 Hz, 1H), 7.25 (dd, *J* = 1.5, 8.1 Hz, 2H), 7.41-7.46 (m, 4H), 7.69 (s, 2H), 7.70 (dd, *J* = 2.4, 7.8 Hz, 2H), 8.50 (d, *J* = 8.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ –1.30, 22.9, 27.4, 52.4, 108.0, 119.2, 130.8, 124.6, 127.4, 128.8, 131.7, 132.7, 134.8, 140.5, 145.1, 150.1, 184.9; HRMS (ESI$^+$): Calcd for $[M+H]^+$, 717.2124; found, 717.2169 (+4.5 mmu).

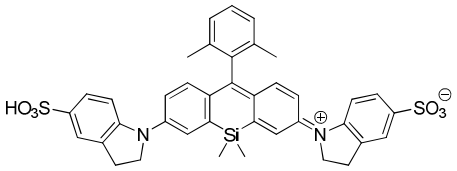
wsSiNQ780 derivative 1 (17)



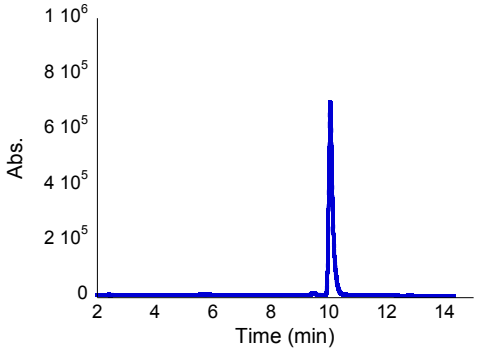
To a flame-dried flask flushed with argon, **16** (12.8 mg, 0.02 mmol) and anhydrous THF (5 mL) were added. The solution was heated to 80^{o}C, and 1 M *o*-tolyl magnesium bromide (300 μL, 0.30 mmol) was added to it. The mixture was stirred for 2 hrs, then cooled to r.t., and the reaction was quenched by addition of 2 N HCl. Stirring was continued at r.t. for 15 min, and then the whole was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in 2 N HCl (less than 5.0 ml) and CH_3CN (2.0 mL) and the solution was heated to reflux under argon. It was then cooled to r.t., and purified by HPLC (eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 740 nm) to provide **17** (4.0 mg, 32% yield). ^{1}H NMR (300 MHz, DMSO): δ 0.67 (s, 3H), 0.70 (s, 3H), 2.05 (s, 3H), 3.25 (t, *J* = 7.50 Hz, 4H), 4.41 (t, *J* = 7.50 Hz, 4H), 7.15 (d, *J* = 9.10 Hz, 2H), 7.27 (d, *J* = 7.50 Hz, 1H), 7.45-7.59 (m, 11H), 7.82 (d, *J* = 2.10 Hz, 2H); HRMS (ESI$^-$): Calcd for $[M–2H]^-$, 705.1549; found, 705.1586 (+3.7 mmu). The HPLC chromatogram after purification is shown below. Elution was done with a 20-min linear gradient from 80% to 100% solvent B. 1.0 ml/min flow rate. Detection at 740 nm.



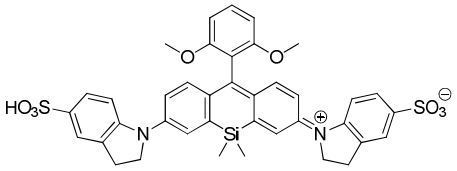
wsSiNQ780 derivative 2 (18)



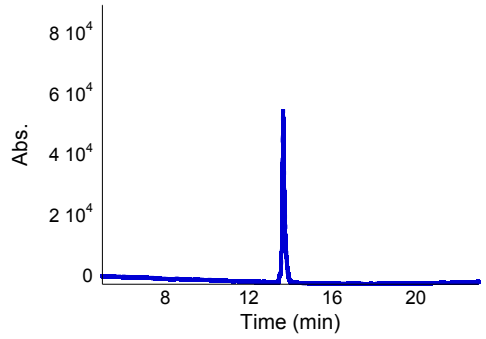
To a flame-dried flask flushed with argon, 2-bromo-*m*-xylene (28 mg, 0.15 mmol) and anhydrous THF (3.0 mL) were added. The solution was cooled to –78°C, and 1 M *sec*-BuLi (150 μL, 0.15 mmol) was added to it. The mixture was stirred for 30 min, and then a solution of **16** (11 mg, 0.02 mmol) in anhydrous THF (2.0 mL) was added to it at –78°C, and the whole was allowed to warm to r.t. over 2 hrs. The reaction was quenched by addition of 2 N HCl and the solution was stirred at r.t. for 15 min. The whole was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in 2 N HCl (less than 5.0 ml) and CH_3CN (2.0 mL), and the solution was heated to reflux under argon. It was then cooled to r.t., and purified by HPLC (eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 740 nm) to provide **18** (5 mg, 46% yield). ^{1}H NMR (300 MHz, DMSO): δ 0.70 (s, 6H), 2.05 (s, 6H), 3.33 (br, 4H), 4.43 (t, J = 6.6 Hz, 4H), 7.27-7.43 (m, 7H), 7.62 (d, J = 8.7 Hz, 2H), 7.75-7.84 (m, 6H); HRMS (ESI$^-$): Calcd for $[M-2H]^-$, 719.1706; found, 709.1702 (–0.4 mmu). The HPLC chromatogram after purification is shown below. Elution was done with a 20-min linear gradient from 20% to 100% solvent B. 1.0 ml/min flow rate. Detection at 740 nm.



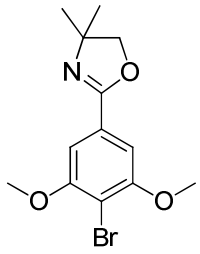
wsSiNQ780 derivative 3 (19)



To a flame-dried flask flushed with argon, 2-bromo-1,3-dimethoxybenzene (33 mg, 0.15 mmol) and anhydrous THF (4.0 mL) were added. The solution was cooled to –78°C, and 1 M *sec*-BuLi (150 μL, 0.15 mmol) was added to it. The mixture was stirred for 30 min, then a solution of **16** (14 mg, 0.02 mmol) in anhydrous THF (2.0 mL) was added at –78°C, and the whole was warmed to r.t. for 2 hrs. The reaction was quenched by addition of 2 N HCl and stirring was continued at r.t. for 15 min. The whole was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in 2 N HCl (less than 5.0 ml) and CH_3CN (2 mL), and the solution was heated to reflux under argon. It was then cooled to r.t., and purified by HPLC (eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 740 nm) to provide **19** (6 mg, 41% yield). ^{1}H NMR (300 MHz, DMSO): δ 0.66 (s, 6H), 3.24 (t, J = 7.5 Hz, 4H), 3.66 (s, 6H), 4.39 (t, J = 7.5 Hz, 4H), 6.93 (d, J = 8.7 Hz, 2H), 7.30 (d, J = 9.6 Hz, 2H), 7.43 (dd, J = 2.1, 9.6 Hz, 2H), 7.49-7.61 (m, 7H), 7.78 (d, J = 2.1 Hz, 2H); HRMS (ESI$^-$): Calcd for $[M–2H]^-$, 751.1604; found, 751.1567 (–3.7 mmu). The HPLC chromatogram after purification is shown below. Elution was done with a 20-min linear gradient from 20% to 80% solvent B. 1.0 ml/min flow rate. Detection at 740 nm.

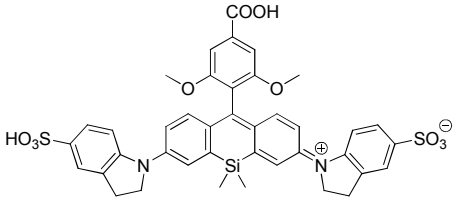


2-(4-Bromo-3,5-dimethoxyphenyl)-4,4-dimethyl-4,5-dihydrooxazole (20)

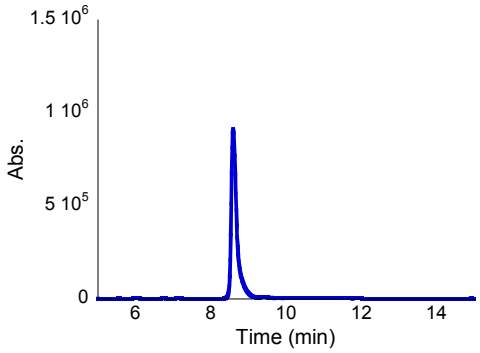


4-Bromo-3,5-dimethoxybenzoic acid (2.09 g, 8.00 mmol) and 2-amino-2-methylpropanol (1.80 g, 20.19 mmol) were added to pyridine (20 mL) and CH_3CN (20 mL). Then DIEA (6.97 mL) and CCl_4 (3.87 mL) were added, and the solution was cooled to 0°C and stirred for 1 hr. PPh_3 (10.5g, 40.1 mmol) in pyridine (2.0 mL) and CH_3CN (2.0 mL) were added dropwise, and the mixture was stirred for 12 hrs, then evaporated to dryness. Purification of the residue by column chromatography (silica, CH_2Cl_2/hexane = 1/5 to 1/3 to 1/1) provided **20** (500 mg, 20% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (s, 6H), 3.95 (s, 6H), 4.10 (s, 2H), 7.15 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3, 56.7, 67.9 79.2, 104.4, 128.3, 128.4, 132.0, 156.7, 161.5; HRMS (ESI$^+$): Calcd for $[M+H]^+$, 314.0392; found, 314.0347 (–4.5 mmu).

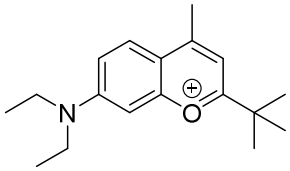
2',6'-Dimethoxy wsSiNQ780 COOH (21)



To a flame-dried flask flushed with argon, **20** (65 mg, 0.21 mmol) and anhydrous THF (5.0 mL) were added. The mixture was cooled to –78°C, and 1 M *sec*-BuLi (210 μL) was added to it. The resulting solution was stirred for 15 min, and a solution of **16** (30 mg, 0.04 mmol) in anhydrous THF (5 mL) was added. The mixture was warmed to r.t., stirred for 1 hr, and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in 6 N HCl (5.0 mL) and CH_3CN (1.0 mL) and the mixture was heated to 60°C and stirred for 4 hrs. It was then cooled to r.t., and purified by HPLC (eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 740 nm) to provide **21** (20 mg, 60% yield). ^{1}H NMR (300 MHz, DMSO): δ 0.66 (s, 6H), 3.25 (t, J = 7.5 Hz, 4H), 3.73 (s, 6H), 4.40 (t, J = 7.5 Hz, 4H), 7.26 (d, J = 9.3 Hz, 2H), 7.40-7.58 (m, 10H), 7.78 (d, J = 2.1 Hz, 2H, h); HRMS (ESI$^-$): Calcd for $[M-2H]^-$, 791.1502; found, 791.1528 (+2.6 mmu). The HPLC chromatogram after purification is shown below. Elution was done with a 20-min linear gradient from 1% to 100% solvent B. 1.0 ml/min flow rate. Detection at 650 nm.

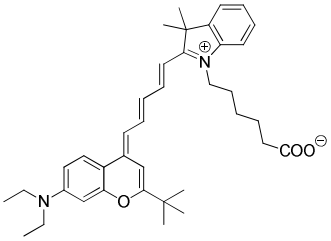


Compound 22



This compound was prepared according to the literature.[SR2] A mixture of *N*,*N*-diethyl-3-aminophenol (2.60 g, 16.0 mmol) and methyl pivaloyl acetate (4.40g, 28.0 mmol) was heated to 180°C under argon, stirred for 18 hrs, and then cooled to r.t. The resulting solid was roughly purified by column chromatography (silica, AcOEt/hexane = 1:1). The crude compound was dissolved in anhydrous THF (20 mL), and the solution was cooled to 0°C under argon. 3 M MeMgBr (5.5 mL) was added to it, and the mixture was stirred for 2 hrs. The solution was poured into the ice water and tetrafluoroboronic acid (3.0 mL) was added to it. Stirring was continued for 1 hr, and then the whole was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was added to AcOEt and the precipitate was filtered off and dried under vacuum. Compound **22** tetrafluoroboronic salt (1.23 g, 12% yield) was obtained without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.36 (t, *J* = 7.2 Hz, 6H), 1.49 (s, 9H), 2.86 (s, 3H), 3.69 (q, *J* = 7.2 Hz, 4H), 6.84 (d, *J* = 2.1 Hz, 1H), 7.15 (s, 1H), 7.40 (dd, *J* = 2.1, 8.7 Hz, 1H), 8.12 (d, *J* = 8.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.4, 19.9, 28.2, 37.9, 46.2, 95.3, 111.3, 117.5, 118.5, 129.4, 156.5, 159.7, 164.2, 179.8; HRMS (ESI$^+$): Calcd for $[M]^+$, 272.2014; found, 272.2015 (+0.1 mmu).

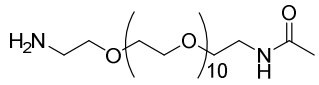
Modified DY 730 COOH (23)



Compound **22** tetrafluoroboronic salt (128 mg, 0.36 mmol) and malonaldehyde dianilide hydrochloride

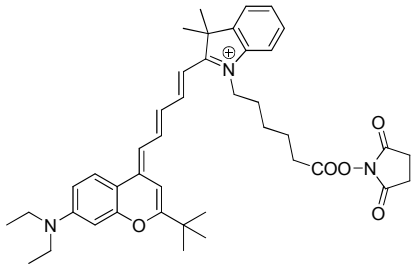
(121 mg, 0.47 mmmol) were dissolved in AcOH (2 mL) and Ac_2O (2 mL). The mixture was heated to 150°C, refluxed under argon for 30 min, and then evaporated to dryness. 1-(5-Carboxypentyl)-2,3,3-trimethyl-3*H*-indol-1-ium bromide (140 mg, 0.47 mmol), EtOH (20 mL) and TEA (5.0 mL) were added to the residue, and the mixture was stirred for 2 hrs, then evaporated to dryness. Purification of the residue by HPLC (eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 680 nm) provided **23** (40 mg, 19% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21 (t, *J* = 7.2 Hz, 6H), 1.40-1.50 (m, 14H), 1.58-1.67 (m, 11H), 17.4-1.79 (m, 2H), 2.28 (t, *J* = 7.2 Hz, 2H), 3.51 (q, *J* = 7.5 Hz, 4H), 3.97 (t, *J* = 7.5 Hz, 2H), 6.22 (d, *J* = 13.8 Hz, 1H), 6.59 (d, *J* = 12.6 Hz, 1H), 6.66 (d, *J* = 3.0 Hz, 1H), 6.84 (d, *J* = 14.1 Hz, 1H), 6.94-6.97 (m, 2H), 7.19-7.23 (m, 2H), 7.37 (t, *J* = 6.6 Hz, 1H), 7.45 (d, *J* = 7.2 Hz, 1H), 7.90-8.14 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.7, 25.2, 26.8, 27.6, 27.7, 28.2, 34.2, 37.4, 44.7, 45.6, 49.9, 97.5, 100.3, 104.2, 111.4, 11.7, 113.4, 114.0, 123.1, 125.7, 126.9, 129.4, 142.2, 143.3, 149.3, 153.7, 158.0, 171.6, 173.2; HRMS (ESI$^+$): Calcd for $[M]^+$, 581.3743; found, 581.3697 (–4.6 mmu).

_N_-(35-Amino-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacontyl)acetamide (24)



t-Butyl(35-amino-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacontyl)carbamate (1.0 g, 1.55 mmol) was dissolved in Ac_2O (2.0 mL) and pyridine (2.0 mL). The mixture was stirred for 2 hrs at r.t. and then evaporated to dryness. The residue was dissolved in TFA (2.0 mL). This solution was stirred for 4 hrs and evaporated to dryness to afford crude **24** (1.2 g). HRMS (ESI$^+$): Calcd for $[M+H]^+$, 587.3755; found, 687.3708 (–4.7 mmu).

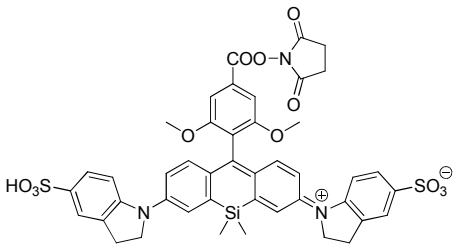
Modified DY730 COO-SE (25)



Compound **23** (29 mg, 0.05 mmol) was dissolved in CH_2Cl_2 (3 mL). WSCD/HCl (95 mg, 0.50 mmol), NHS (58 mg, 0.50 mmol) and DIEA (4 drops) were added to it, and the mixture was stirred for 4 hrs and then evaporated to dryness. The residue was roughly purified by HPLC (eluent, a 20-min linear gradient,

from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 740 nm) to obtain crude **25** trifluoroacetic salt (30 mg).

2',6'-Dimethoxy wsSiNQ780 COOH-SE (26)



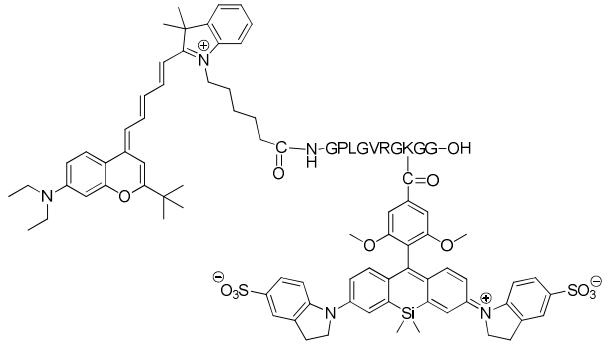
Compound **21** (40 mg, 0.05 mmol) was dissolved in DMF (3.0 mL). WSCD/HCl (95 mg, 0.50 mmol), NHS (58 mg, 0.50 mmol) and DIEA (4 drops) were added to the solution, and the mixture was stirred for 4 hrs and then evaporated to dryness. The residue was roughly purified by HPLC (eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 740 nm) to obtain crude **26** (40 mg).

Compound 27

H_2N-GPLGVR(Pbf)GK(Boc)GG-OH

Peptide **27** was synthesized on an automatic peptide synthesizer using standard protocols for fluorenylmethoxycarbonyl (Fmoc) solid-phase synthesis with 2-chlorotrityl resin (0.06 mmol). The peptide was cleaved with CH_2Cl_2 containing 1% TFA (2.0 mL) and precipitated by adding water. Crude **27** was filtered off and dried under vacuum. LRMS (ESI^+): 1250 $[M+H]^+$. The peptide was used for the next step without further purification.

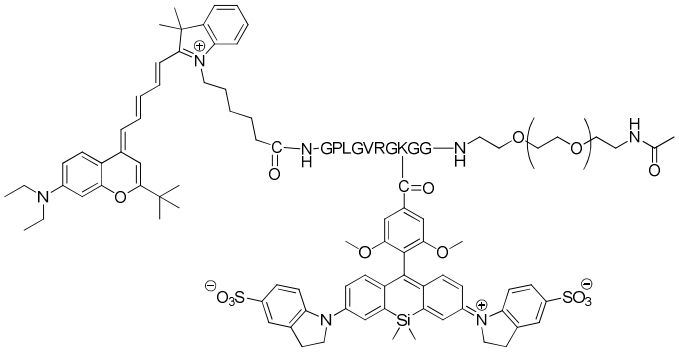
Compound 28



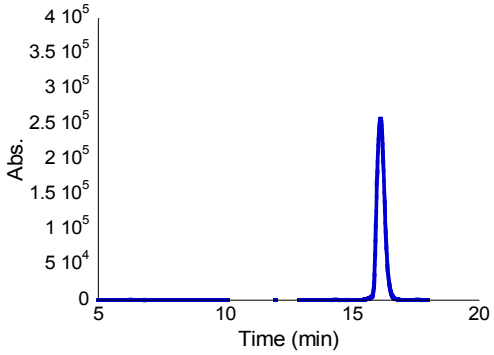
Compound **25** trifluoroacetic salt (20 mg, crude) and peptide **27** (20 mg, crude) were dissolved in DMF (2.0 mL), and then DIEA (4 drops) was added. The mixture was stirred for 4 hrs and evaporated to dryness.

The residue was dissolved in TFA (2.0 mL), triethylsilane (10 μL) and H_2O (10 μL), and the mixture was stirred for 2 hrs and then evaporated to dryness. The residue was roughly purified by HPLC. The solution was lyophilized, and the residue was dissolved in DMF (2.0 mL). Compound **26** (5.2 mg, crude) and DIEA (4 drops) were added to the solution, and the mixture was stirred for 4 hrs and then evaporated to dryness. Purification of the residue by HPLC (eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 740 nm) provided **28** (5.0 mg, 7.0% yield). LRMS (ESI^+): 1120 $[M+2H]^{2+}$.

MMP probe 1 (29)



Compound **28** (5.0 mg, 0.03 mmol) was dissolved in DMF (2.0 mL). Compound **24** (15 mg, crude), HATU (38 mg, 0.01 mmol) and DIEA (4 drops) were added, and the mixture was stirred for 6 hrs and then evaporated to dryness. Purification of the residue by HPLC (eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 740 nm) provided **29** (3.2 mg, 51% yield). LRMS (ESI^+): 1404 $[M+2H]^{2+}$. The HPLC chromatogram after purification is shown below. Elution was done with a 20-min linear gradient from 20% to 100% solvent B. 1.0 ml/min flow rate. Detection at 740 nm.

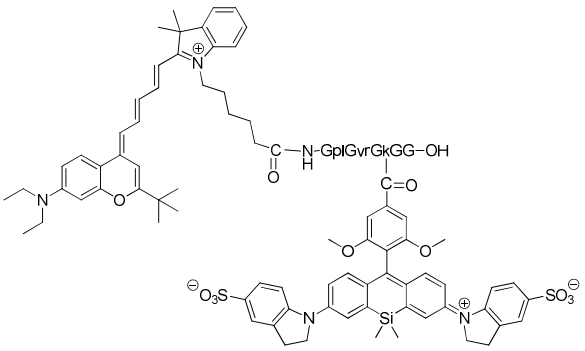


Compound 30

H_2N-GpIGvr(Pbf)Gk(Boc)GG−OH

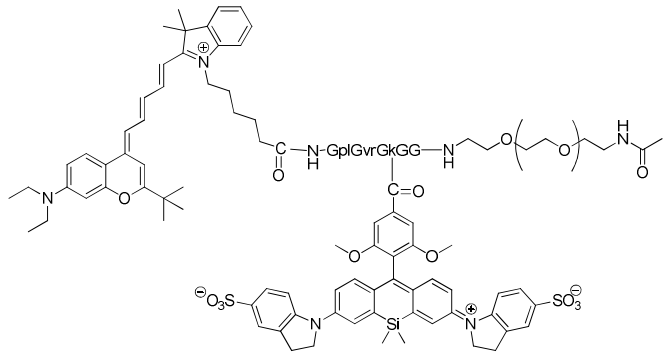
Peptide **30**, which contains D-amino acids, was synthesized on an automatic peptide synthesizer using standard protocols for fluorenylmethoxycarbonyl (Fmoc) solid-phase synthesis with 2-chlorotrityl resin (0.06 mmol reaction site). It was cleaved with 2.0 mL CH_2Cl_2 containing 1% TFA. The peptide was precipitated by addition of water. Compound **30** was filtered off and dried under vacuum. LRMS (ESI^+): 1250 $[M+H]^+$. The peptide was used for the next step without further purification. Lower-case letters stand for D-amino acids in the peptide.

Compound 31

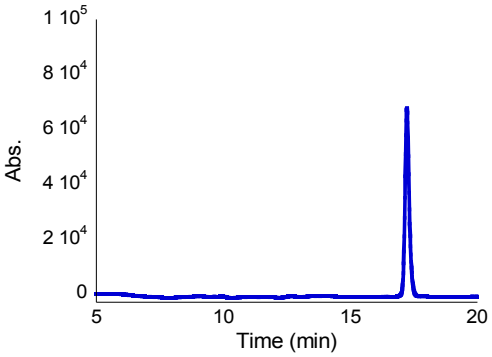


Compound **25** trifluoroacetic salt (20 mg, crude) and peptide **30** (20 mg, crude) were dissolved in DMF (2.0 mL), and DIEA (4 drops) was added to the solution. The mixture was stirred for 4 hrs and evaporated to dryness. The residue was dissolved in TFA (2.0 mL), triethylsilane (10 μL) and H_2O (10 μL), and the solution was stirred for 2 hrs and evaporated to dryness. The residue was roughly purified by HPLC. The eluate was lyophilized, and the residue was dissolved in DMF (2.0 mL). Compound **26** (5.2 mg, crude) and DIEA (4 drops) were added, and the mixture was stirred for 4 hrs, and then evaporated to dryness. Purification of the residue by HPLC (eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 740 nm) provided **31** (5.2 mg, 7.0% yield). LRMS (ESI^+): 1120 $[M+H+Na]^{2+}$. Lower-case letters represent D-amino acids in the peptide.

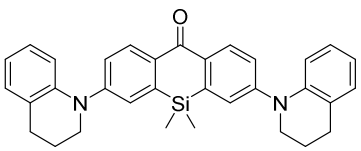
MMP probe 2 (32)



Compound **31** (5.0 mg, 0.03 mmol) was dissolved in DMF (2.0 mL). Compound **24** (15 mg, crude), HATU (38 mg, 0.01 mmol) and DIEA (4 drops) were added to the solution, and the mixture was stirred for 6 hrs, and then evaporated to dryness. Purification of the residue by HPLC (eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 740 nm) provided **32** (5.4 mg, 87% yield). LRMS (ESI^+): 1404 $[M+2H]^{2+}$. The HPLC chromatogram after purification is shown below. HPLC analysis; eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 1.0 ml/min; detection wavelength, 740 nm. Lower-case letters represent D-amino acids in the peptide.



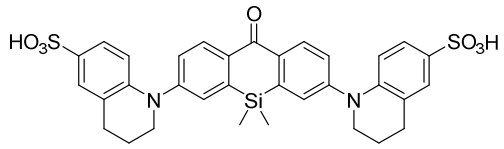
Compound 33



Compound **3** (245 mg, 0.50 mmol), Cs_2CO_3 (3.25 g, 2.00 mmol) and tetrahydroquinoline (1.33 g, 2.00 mmol) were dissolved in toluene (10-20 ml) in a 50 mL Schlenk flask, and the mixture were deaerated with argon. $Pd(OAc)_2$ (22 mg, 0.05 mmol) and BINAP (62 mg, 0.05 mmol) were added to it under argon. The

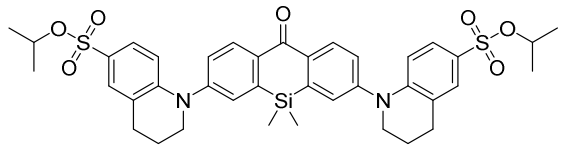
mixture was heated to 100°C and stirred overnight. It was then cooled to r.t. and H_2O was added. The whole was extracted with CH_2Cl_2, and the organic layer was dried over Na_2SO_4 and evaporated to dryness. Purification of the residue by column chromatography (silica gel, CH_2Cl_2/hexane = 1/1) provided **33** (150 mg, 60% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.43 (s, 6H), 2.06 (qt, *J* = 6.0 Hz, 4H), 2.82 (t, *J* = 6.0 Hz, 4H), 3.74 (t, *J* = 6.0 Hz, 4H), 6.87 (t, *J* = 7.5 Hz, 2H), 7.04 (dt, *J* = 1.5, 8.7 Hz, 2H), 7.12 (d, *J* = 7.5 Hz, 2H), 7.28 (d, *J* = 8.1 Hz, 2H), 7.34 (dd, *J* = 3.0, 9.0 Hz, 2H), 7.41 (d, *J* = 2.4 Hz, 2H), 8.39 (d, *J* = 8.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ –1.43, 23.6, 27.4, 49.1, 118.7, 120.9, 121.8, 123.6, 126.3, 128.4, 129.3, 131.3, 134.1, 140.2, 142.1, 150.4, 185.3; HRMS (ESI$^+$): Calcd for $[M+H]^+$, 501.2362; found, 501.2412 (+5.0 mmu).

Compound 34



Compound **33** (50 mg, 0.10 mmol) was dissolved in CH_2Cl_2 (10 mL), and the solution was cooled to 0 °C and stirred for 10 min. Chlorosulfuric acid (182 μL) was added dropwise, and the mixture was stirred for 2 hrs at 0°C. The reaction was quenched by addition of H_2O, and the whole was evaporated to remove CH_2Cl_2. Purification of the residual solution by HPLC (eluent, a 20-min linear gradient, from 0% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 350 nm) provided **34** (78 mg, quant). ^{1}H NMR (300 MHz, DMSO): δ 0.46 (s, 6H), 1.97 (q, *J* = 6.0 Hz, 4H), 2.76 (t, *J* = 6.0 Hz, 4H), 3.74 (t, *J* = 6.0 Hz, 4H), 7.03 (d, *J* = 8.1 Hz, 2H), 7.27 (d, *J* = 8.1 Hz, 2H), 7.38-7.42 (m, 4H), 7.55 (d, *J* = 2.1 Hz, 2H), 8.21 (d, *J* = 8.7 Hz, 2H); ^{13}C NMR (75 MHz, DMSO): δ –1.6, 23.1, 26.9, 38.7, 38.9, 39.2, 39.5, 39.8, 40.1, 40.3, 48.8, 117.2, 121.5, 123.7, 123.8, 126.7, 127.4, 130.5, 133.2, 140.3, 141.7, 149.9, 183.8; HRMS (ESI$^-$): Calcd for $[M–H]^-$, 658.1264; found, 658.1243 (–2.1 mmu).

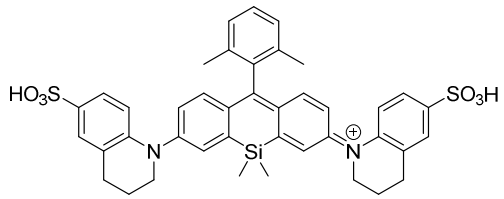
3,6-Di-*i*-PrSO$_3$ tetrahydroquinoline-Si-xanthone (35)



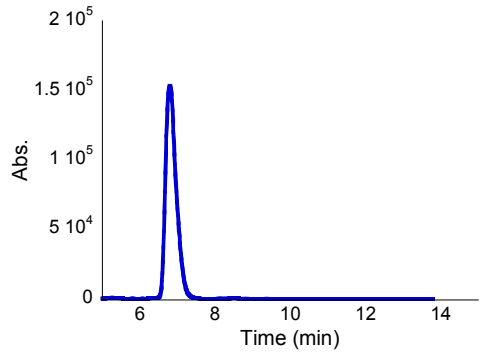
Compound **34** (66 mg, 0.10 mmol) was dissolved in CH_2Cl_2 (3.0 mL) and DMF (1.0 mL), and the mixture was cooled to 0°C under argon. Oxalyl chloride (85 μL, 0.50 mmol) was added to it, and the mixture was stirred for 2 hrs, and then evaporated to dryness. The residue was dissolved in CH_2Cl_2 under argon. Pyridine (15 mL) and *i*-PrOH (5.0 mL) were added to it, and the mixture was stirred at r.t. for 2 hrs, then

evaporated to dryness. Purification of the residue by column chromatography (silica gel, CH_2Cl_2/AcOEt = 1/1) provided **35** (26 mg, 35% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.48 (s, 6H), 1.31 (d, *J* = 6.6 Hz, 12H), 2.14 (tt, *J* = 6.0 Hz, 4H), 2.93 (t, *J* = 6.00 Hz, 4H), 3.80 (t, *J* = 6.0 Hz, 4H), 4.75 (sep, *J* = 6.0 Hz, 1H), 6.96 (d, *J* = 8.7 Hz, 2H), 7.42-7.50 (m, 6H), 7.62 (d, *J* = 1.5 Hz, 2H), 8.47 (d, *J* = 7.8 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ –1.47, 22.2, 22.9, 27.5, 50.4, 115.4, 125.3, 125.4, 126.3, 126.5, 127.5, 129.3, 131.8, 136.8, 140.8, 147.5, 149.4, 185.5; HRMS (ESI$^+$): Calcd for $[M+Na]^+$, 767.2257; found, 767.2210 (–4.7 mmu).

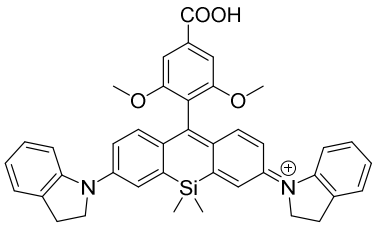
Compound 36



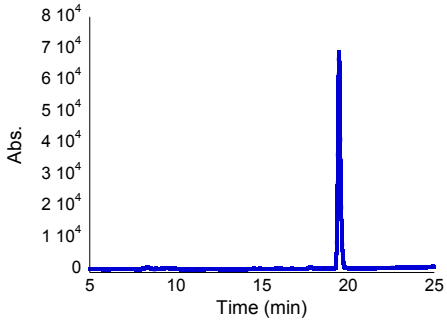
To a flame-dried flask flushed with argon, 2-bromo-*m*-xylene (28 mg, 0.15 mmol) and anhydrous THF (5.0 mL) were added. The solution was cooled to –78°C, then 1 M *sec*-BuLi (150 μL, 0.15 mmol) was added to it, and the mixture was stirred for 30 min. Compound **35** (11 mg, 0.02 mmol) in anhydrous THF (2.0 mL) was added at –78°C, and the whole was stirred at r.t. for 2 hrs. The reaction was quenched by addition of 2 N HCl and stirring was continued at r.t. for 15 min. The whole was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in 2 N HCl (less than 5.0 ml) and CH_3CN (2.0 mL), and the solution was heated to reflux under argon, and then cooled to r.t. Purification of the solution by HPLC (eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 740 nm) provided **36** (6 mg, 54% yield). ^{1}H NMR (300 MHz, DMSO): δ 0.58 (s, 6H), 1.98 (br, 10H), 2.76 (t, *J* = 6.0 Hz, 4H), 4.01 (t, *J* = 6.6 Hz, 4H), 7.04 (d, *J* = 9.6 Hz, 2H), 7.24 (dd, *J* = 2.4, 9.3 Hz, 2H), 7.28 (d, *J* = 7.8 Hz, 2H), 7.36-7.47 (m, 5H), 7.53 (s, 2H), 7.81 (d, *J* = 2.1 Hz, 2H); HRMS (ESI$^-$): Calcd for $[M-2H]^-$, 747.2019; found, 747.2051 (+3.2 mmu). The HPLC chromatogram after purification is shown below. HPCL analysis; eluent, a 20-min linear gradient, from 1% to 100% solvent B; flow rate, 1.0 ml/min; detection wavelength, 650 nm.



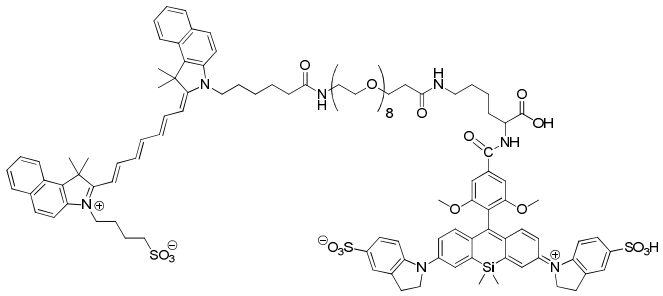
Compound 37



To a flame-dried flask flushed with argon, **20** (100 mg, 0.32 mmol) and anhydrous THF (10 mL) were added. The mixture was cooled to –78°C, and 1 M *sec*-BuLi (310 μL) was added to it. The mixture was stirred for 15 min, and then a solution of **6** (30 mg, 0.06 mmol) in anhydrous THF (3.0 m) was added. The whole was warmed to r.t., stirred for 1 hr, and then extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in 6 N HCl (5.0 mL) and CH_3CN (1.0 mL) and the mixture was heated to 60°C and then stirred for 4 hrs. Purification of the solution by HPLC (eluent, a 20-min linear gradient, from 40% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 740 nm) provided **37** (25 mg, 65% yield). ^{1}H NMR (300 MHz, acetone-d6): δ 0.70 (s, 6H), 3.35 (t, *J* = 7.2 Hz, 4H), 3.81 (s, 6H), 4.47 (t, *J* = 7.2 Hz, 4H), 7.16 (t, *J* = 7.2 Hz, 2H), 7.32 (t, *J* = 7.2 Hz, 2H), 7.40-7.48 (m, 6H), 7.52 (s, 2H), 7.68 (d, *J* = 8.1 Hz, 2H), 7.93 (s, 2H); HRMS (ESI$^+$): Calcd for $[M+H]^+$, 637.2523; found, 637.2529 (+0.6 mmu). The HPLC chromatogram after purification is shown below. HPCL analysis; eluent, a 20-min linear gradient, from 40% to 100% solvent B; flow rate, 1.0 ml/min; detection wavelength, 740 nm.



ICG-wsSiNQ780 (38)



Compound **26** (5.2 mg, crude) and N^{α}-Boc-L-lysine (24.6 mg, 0.10 mmol) were dissolved in DMF (1

mL), and DIEA (30 μL) was added to the solution. The mixture was stirred for 14 hrs, then evaporated to dryness and the residue was dissolved in CH_3CN (1 mL). The solution was cooled to 0°C, and TFA (1 mL) was added to it. The mixture was stirred for 30 min, then evaporated to dryness and the residue was roughly purified by HPLC (eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 740 nm). The crude product was dissolved in DMF (500 μL), and ICG-EG8-Sulfo-OSu (5 mg, 0.04 mmol)[SR3] and DIEA (30 μL) were added to the solution. The mixture was stirred for 18 hrs and then evaporated to dryness. Purification of the residue by HPLC (eluent, a 20-min linear gradient, from 20% to 100% solvent B; flow rate, 5.0 ml/min; detection wavelength, 740 nm) provided **38** (0.3 mg, 2.6% yield). HRMS (ESI⁻): Calcd for $[M–2H]^{2-}$, 2057.8177; found, 2057.8128 (–4.9 mmu). The HPLC chromatogram after purification is shown below. Elution was done with a 20-min linear gradient from 20% to 100% solvent B. 1.0 ml/min flow rate. Detection at 740 nm.

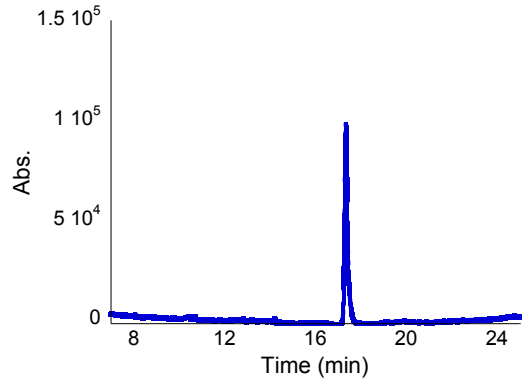


Table S1. Photophysical properties of SiNQ660 (**5**).

	Abs_{max} (nm)	Φ_{FL} [a]
MeOH	660	n.d.
Acetonitrile	660	n.d.
DMSO	672	n.d.
Chloroform	663	n.d.

[a] For determination of the fluorescence quantum yield (Φ_{FL}), Cy5.5 in PBS at pH 7.4 (Φ_{FL} = 0.23) was used as a fluorescence standard.[SR4]

Table S2. Photophysical properties of SiNQ780 (**7**).

	Abs_{max} (nm)	Φ_{FL} [a]
MeOH	779	n.d.
DMF	790	n.d.
Chloroform	790	n.d.

[a] For determination of the fluorescence quantum yield (Φ_{FL}), ICG in DMSO (Φ_{FL} = 0.13) was used as a fluorescence standard. [SR5]

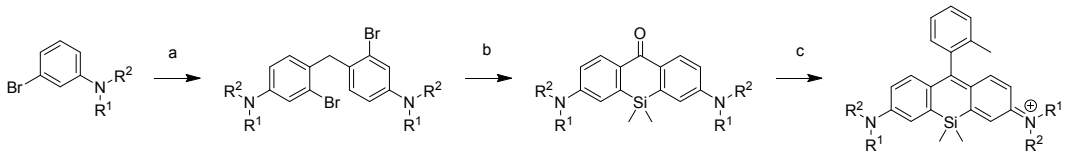
Table S3. Photophysical properties of compound **14**.

	Abs_{max} (nm)	Φ_{FL} [a]
MeOH	731	0.08

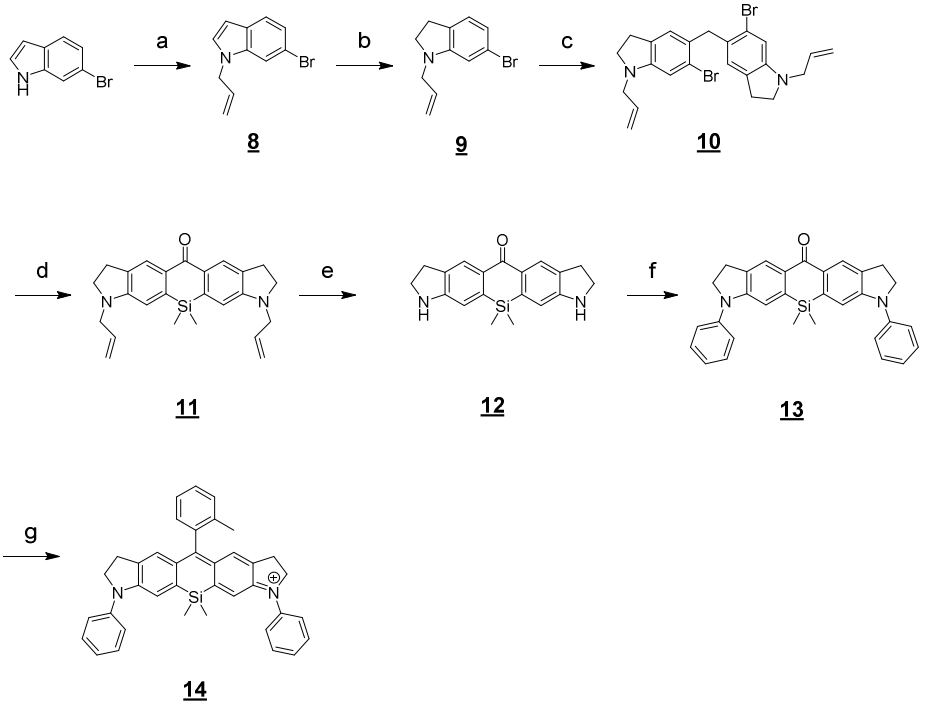
[a] For determination of the fluorescence quantum yield (Φ_{FL}), ICG in DMSO (Φ_{FL} = 0.13) was used as a fluorescence standard.[SR5]

Table S4. Photophysical property of wsSiNQ780 derivative **2** and **36** in PBS.

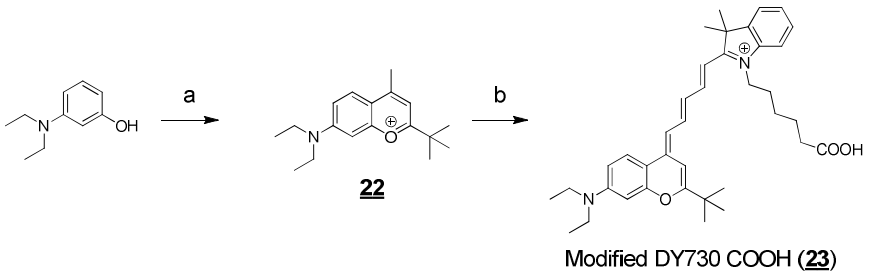
	Abs_{max} (nm)
wsSiNQ780 derivative **2**	763
36	732



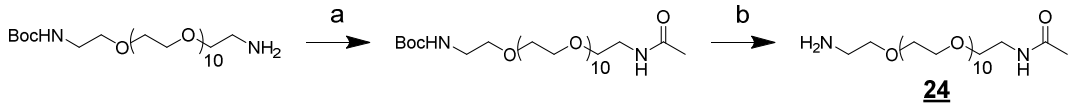
Scheme S1. a) HCHO, acetic acid; b) i) *sec*-BuLi, $(CH_3)_2SiCl_2$, THF, ii) $KMnO_4$, acetone; c) *o*-tolylmagnesium bromide, THF.



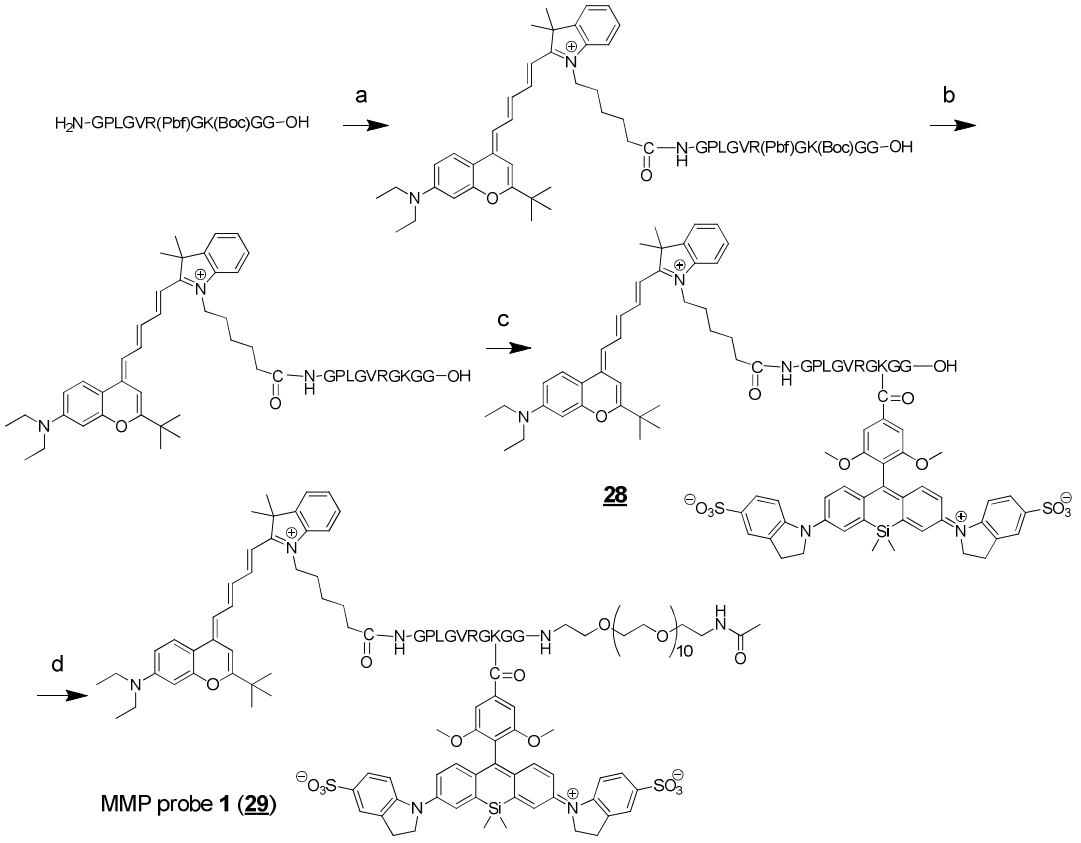
Scheme S2. (a) NaH, allyl bromide, DMF, 0 °C, 85%; (b) $NaBH_3CN$, CH_3COOH, 0°C, 70%; (c) formaldehyde, CH_3COOH, 80°C, 16%; (d) i) *sec*-BuLi, dichlorodimethylsilane, THF, −78°C, ii) $KMnO_4$, acetone, 17%; (e) $Pd(PPh_3)_4$, 1,3-dimethylbarbituric acid, CH_2Cl_2, 40°C, 50%; (f) iodobenzene, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, 100°C, 42%; (g) i) *o*-tolylmagnesium bromide, THF, 80°C, ii) 2N HCl aq., 18%.



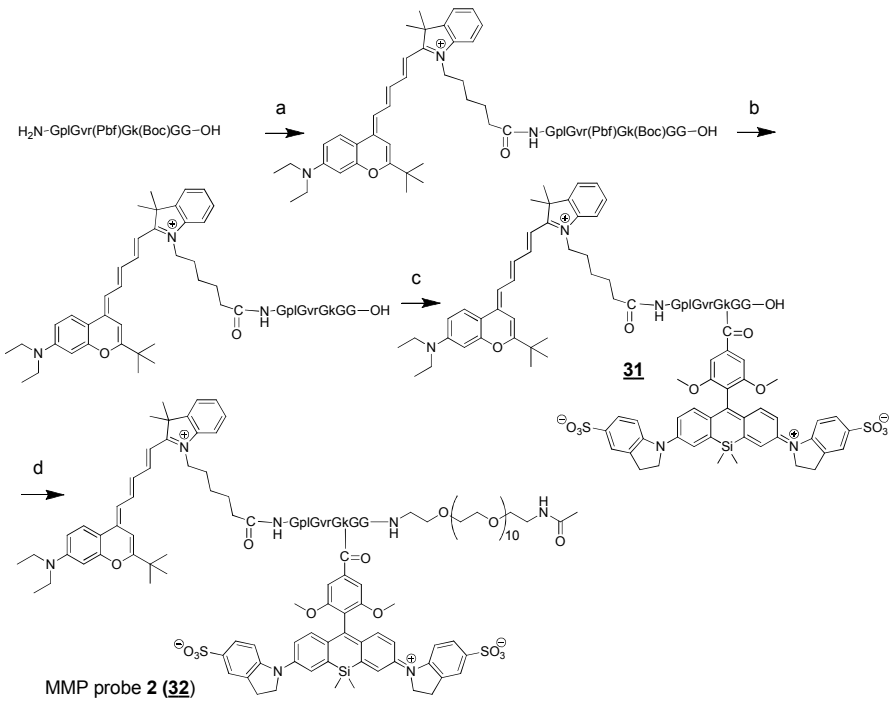
Scheme S3. a) i) Methyl 4,4-dimethyl-3-oxovalerate, neat, 180°C, ii) MeMgBr, THF, iii) tetrafluoroboric acid, H_2O, 22%; b) i) malonaldehyde dianilide hydrochloride, AcOH, Ac_2O, reflux, ii) 1-(5-carboxypentyl)-2,3,3-trimethyl-3*H*-indolium bromide, TEA, EtOH, 19%.



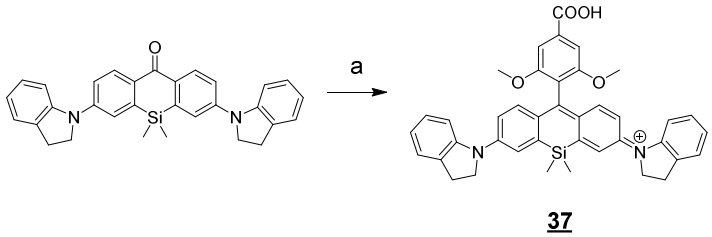
Scheme S4. a) Ac_2O, pyridine, crude; b) TFA, CH_2Cl_2, crude.



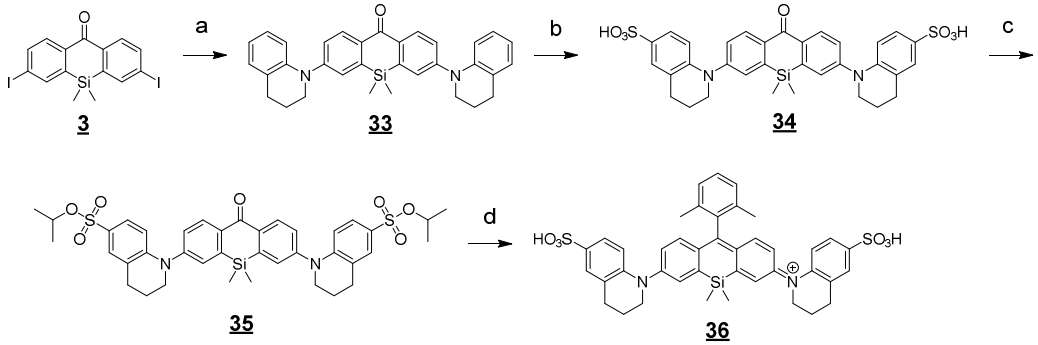
Scheme S5. a) **25**, DIEA, DMF, crude; b) TFA, triethylsilane, H_2O, crude; c) **26**, DIEA, DMF, 7.0% (in 3 steps); d) **24**, HATU, DIEA, DMF, 51%.



Scheme S6. a) **25**, DIEA, DMF, crude; b) TFA, triethylsilane, H_2O, crude; c) **26**, DIEA, DMF, 7.0% (in 3 steps); d) **24**, HATU, DIEA, DMF, 87%. Lower-case letters represent D-amino acids in the peptide.



Scheme S7. a) i) **20**, *sec*-BuLi, THF, 60°C, ii) 2 N HCl aq., acetone, reflux, 2 days, 65%.



Scheme S8. a) 1,2,3,4-tetrahydroqunoline, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, 80°C, 60%; b) $ClSO_3H$, CH_2Cl_2, quant; c) i) oxalyl chloride, DMF, CH_2Cl_2, ii) isopropanol, pyridine, 35%; d) i) 2-bromo-*m*-xylene, *sec*-BuLi, THF, −78°C, ii) 2 N HCl aq., reflux, 54%.

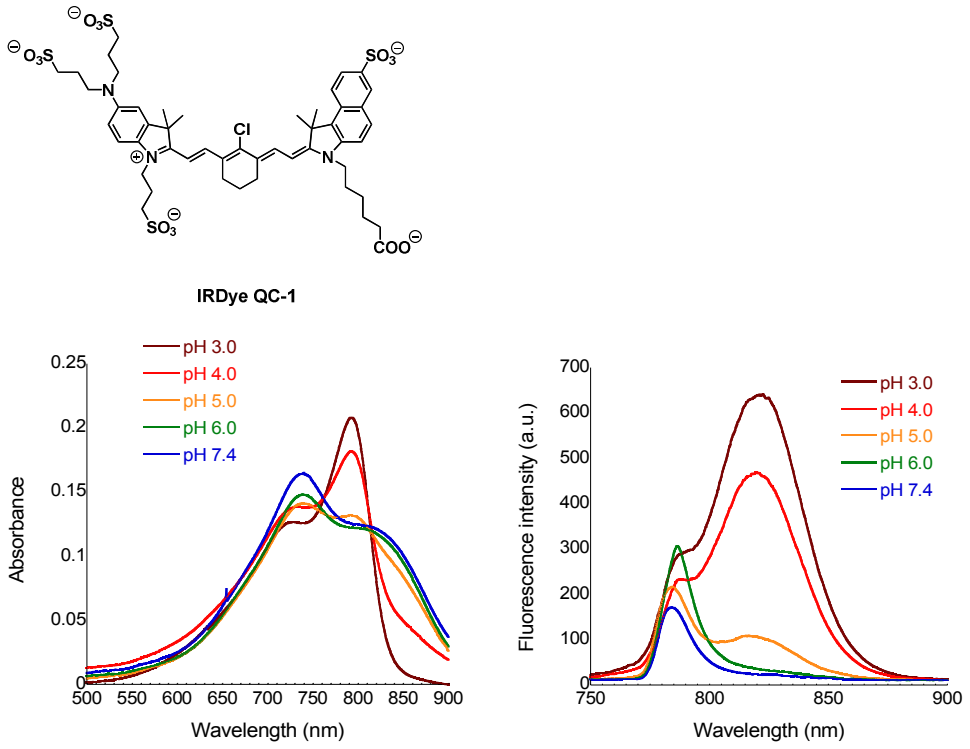


Figure S1. Chemical structure of IRDye QC-1 (top). Absorption (bottom, left) and fluorescence (bottom, right) spectra of 1 μM IRDye QC-1 in sodium phosphate buffer containing 0.1% DMSO as a cosolvent at various pH values. Excitation at 780 nm.

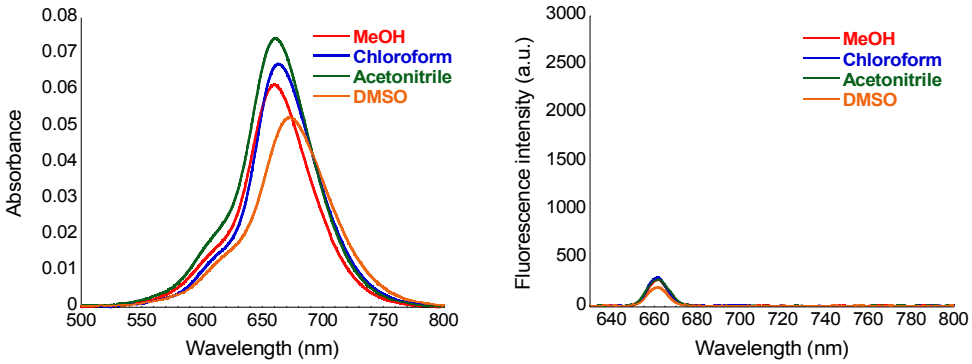


Figure S2. Absorption (left) and fluorescence (right) spectra of 1 μM SiNQ660 (**5**) in MeOH (red), acetonitrile (green), DMSO (orange) and chloroform (blue) containing 0.1% DMSO as a cosolvent. Excitation at 660 nm.

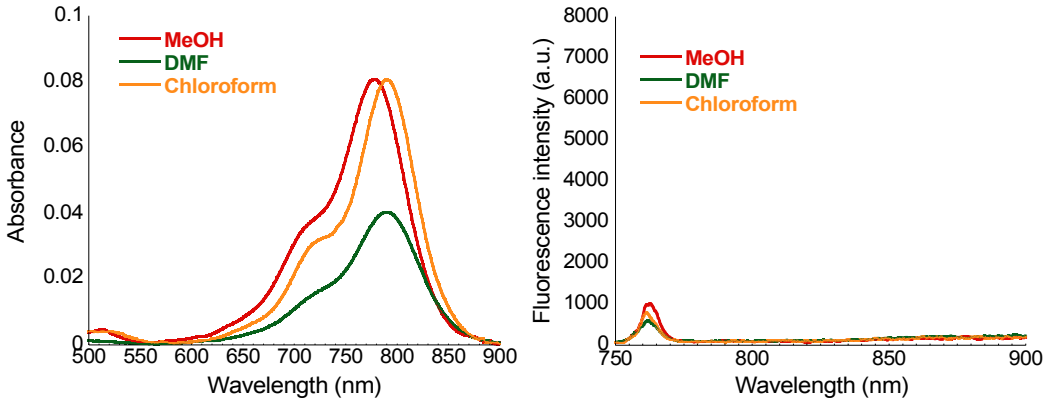


Figure S3. Absorption (left) and fluorescence (right) spectra of 1 μM SiNQ780 (**7**) in MeOH (red), DMF (green) and chloroform (orange) containing 0.1% DMSO as a cosolvent. Excitation at 760 nm.

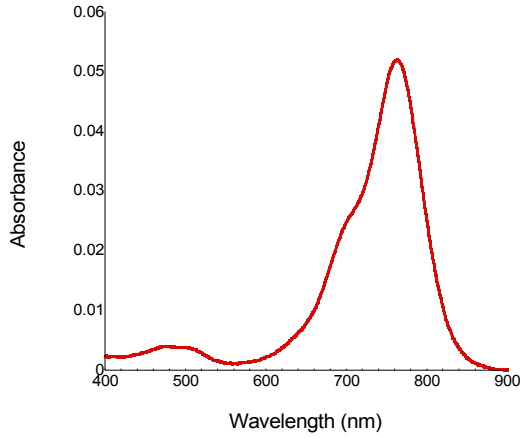


Figure S4. Absorption spectrum of wsSiNQ780 derivative **1** (1 μM) in PBS (pH 7.4) containing 0.1% DMSO as a cosolvent.

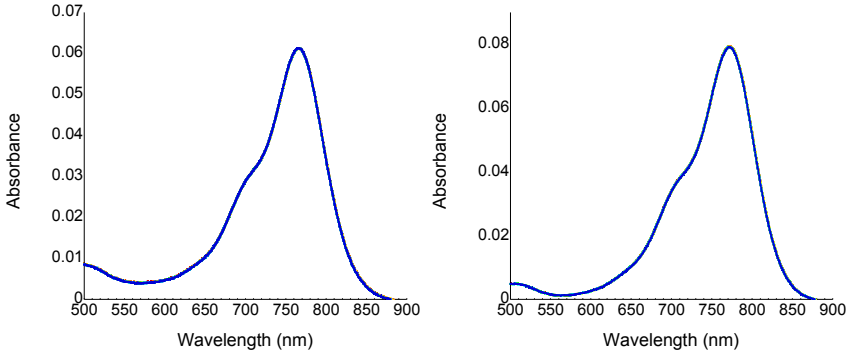


Figure S5. Absorption spectra of wsSiNQ780 derivative **2** (left) and **3** (right) in PBS containing 0.1% DMSO as a cosolvent for 1 hr. As shown in the figure, both compounds showed almost no absorption spectral change.

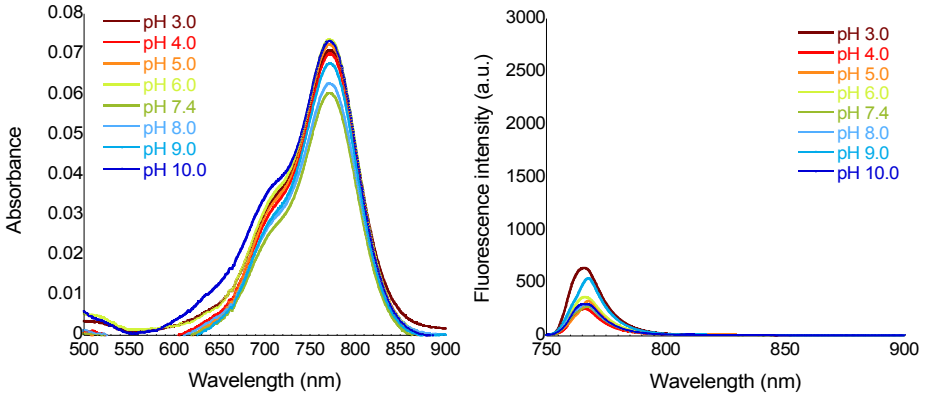


Figure S6. Absorption (left) and fluorescence (right) spectra of 1 μM wsSiNQ780 derivative **3** in sodium phosphate buffer at various pH values containing 0.1% DMSO as a cosolvent. Excitation at 760 nm.

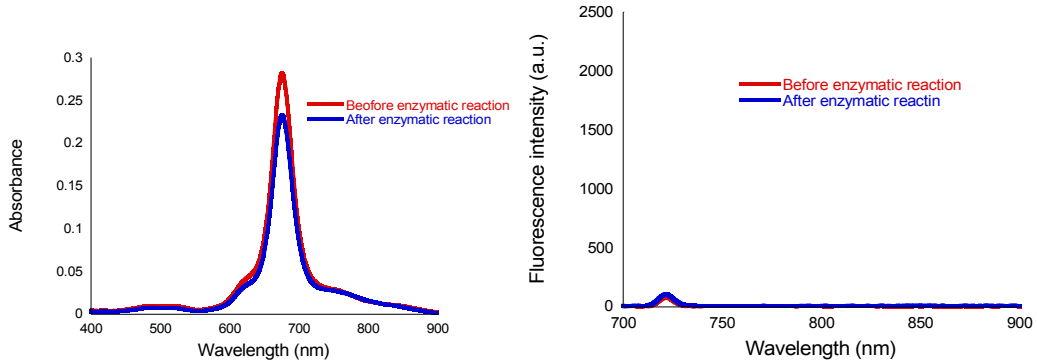


Figure S7. Absorption (left) and fluorescence (right) spectra of MMP probe **2** (final 1 μM) in TCN buffer containing 0.1% DMSO as a cosolvent. MT1-MMP catalytic domain (5 μg) was added and incubated for 2 hrs. Ex = 720 nm.

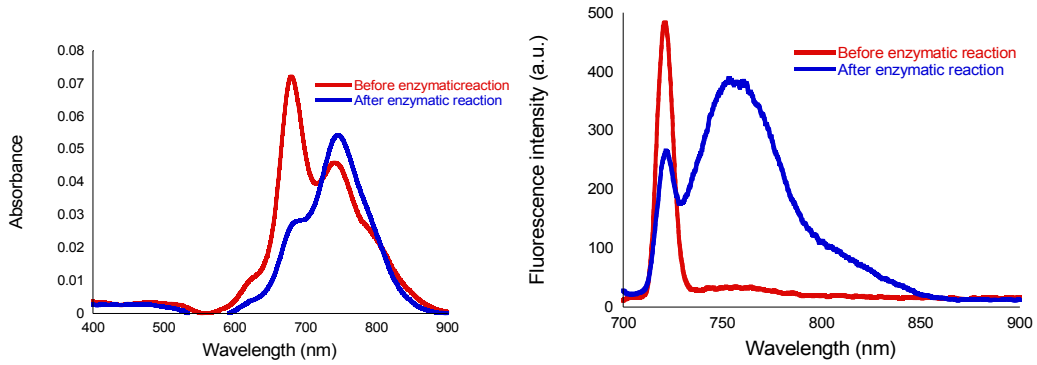


Figure S8. Absorption (left) and fluorescence (right) spectra of MMP probe **1** (final 1 μM) in TCNB buffer containing 0.1% DMSO as a cosolvent. MMP-9 catalytic domain (5 μg) was added to the solution and incubated for 2 hrs. Ex = 720 nm.

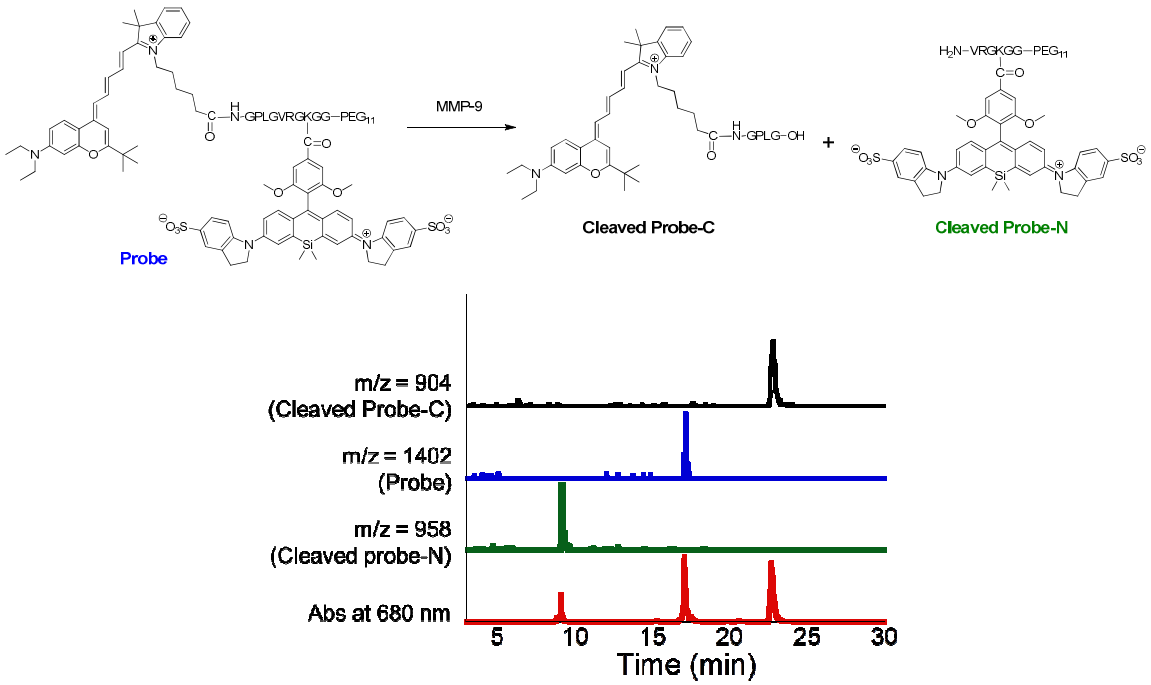


Figure S9. LC-MS chromatograms (lower) of the reaction mixture of MMP probe **1** incubated with MMP-9 for 18 hrs. The reaction scheme of MMP probe **1** with MMP-9 (upper) is also shown. The mass signals of Probe, Cleaved Probe-C and Cleaved Probe-N shown in the figure are m/z = 1402, 904 and 958, respectively.

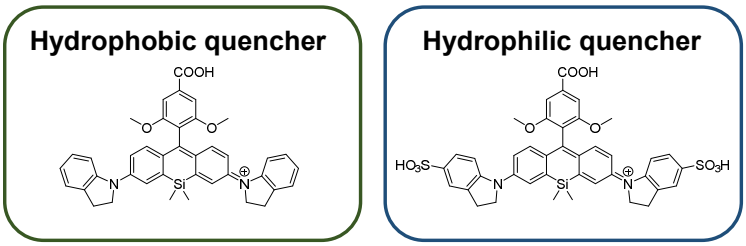


Figure S10. Chemical structures of hydrophobic (left) and hydrophilic (right) SiNQ780 derivatives, which can be selected for use as required.

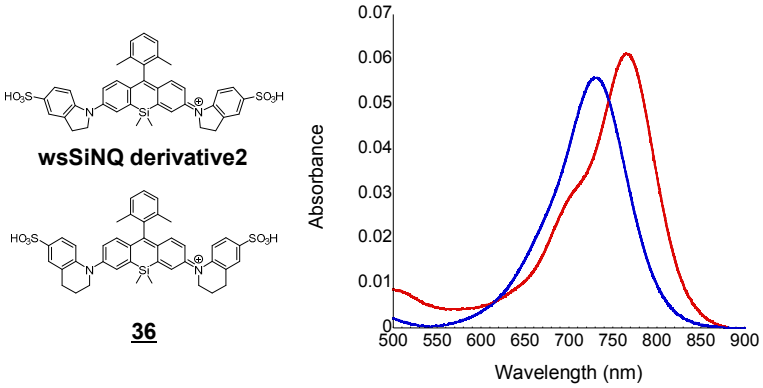


Figure S11. Absorption spectra of wsSiNQ780 derivative **2** (red) and **36** (blue) in PBS containing 0.1% DMSO as a cosolvent.

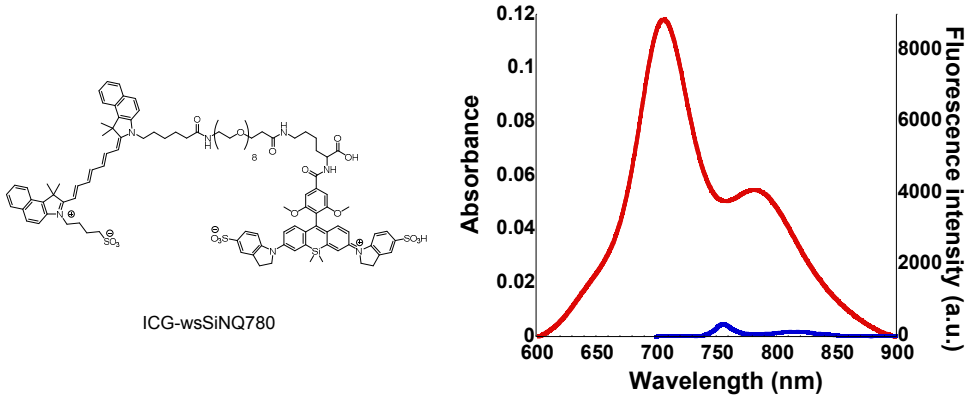


Figure S12. Chemical structure of ICG-wsSiNQ780 and its absorption (red) and fluorescence (blue) spectra in PBS containing 0.1% DMSO as a cosolvent. Excitation wavelength is 750 nm. ICG-wsSiNQ780 is almost non-fluorescent (Φ_{FL} = 0.001). The fluorescence of ICG (Φ_{FL} = 0.13 in DMSO) was highly quenched by wsSiNQ780.

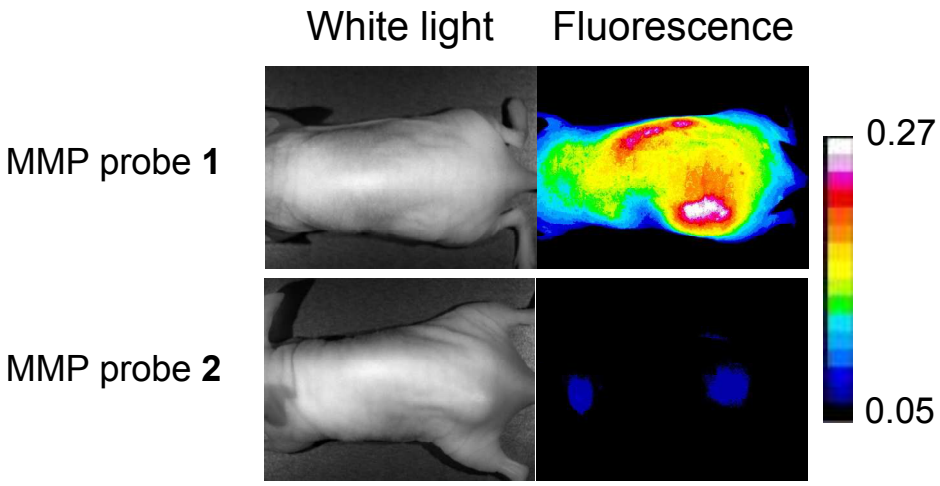


Figure S13. Fluorescence images of an HT-1080 tumor-bearing nude mouse injected with MMP probe **1** or **2** (100 μM in 100 μL PBS containing 1% DMSO as a cosolvent) via the tail vein.

(Supporting References)

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