Supporting Information for:

Systematic Study of Protein Response Mechanism of Self-assembling ^{19}F-NMR/MRI Nanoprobes toward Rational Design and Improved Sensitivity

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Supplementary Figures

Supplementary Methods

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Supplementary Figures

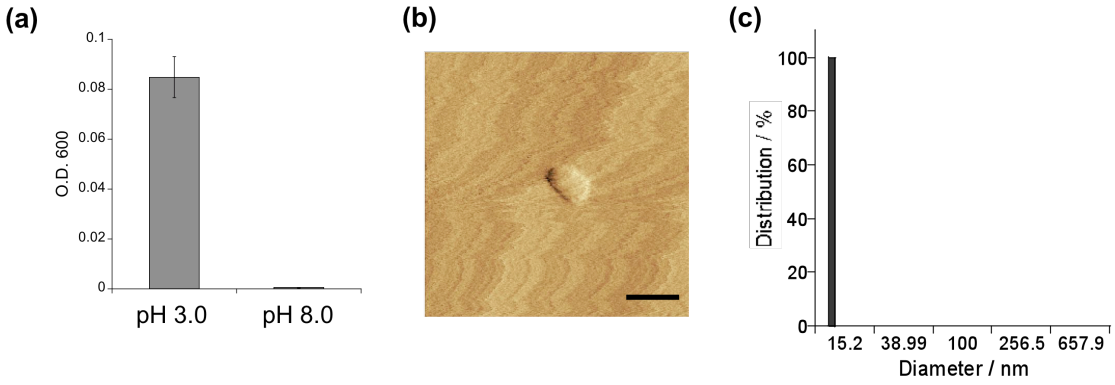


Figure S1. Evaluation of self-assembling properties of probe **2**. (**a**) Optical densities of probe **2** (100 μM) were determined at pH 3.0 or at pH 8.0 using a citrate/phosphate buffer system of constant ionic strength[S1] (0.5 ion strength, 0.2 mM TFA, 10% D_2O (v/v)). (**b**) Atomic force microscopy (AFM) image of probe **2** (5 μM) was determined at pH 3.0 citrate/phosphate buffer (0.5 ion strength, 0.2 mM TFA, 10% D_2O (v/v)) (scale bar 100 nm). (**c**) DLS analysis of particle-size distribution of the probe **2** (100 μM) in pH 3.0 citrate/phosphate buffer (0.5 ion strength).

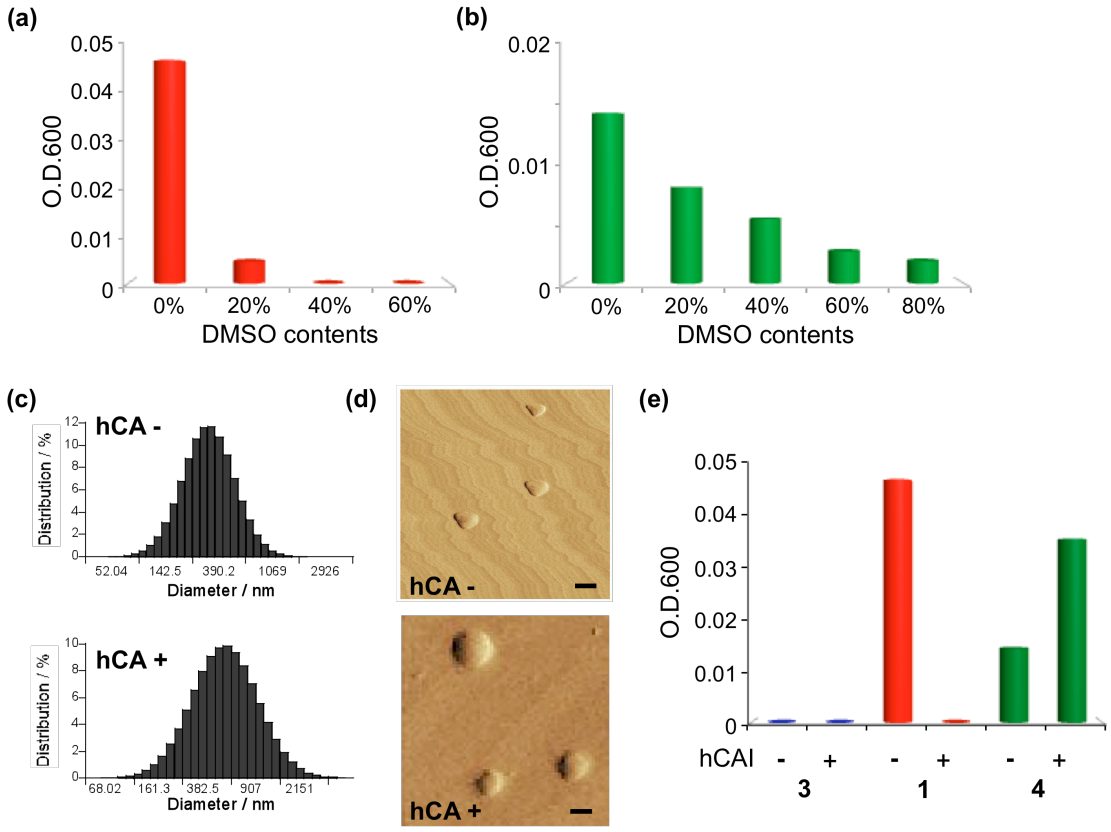


Figure S2. Evaluation of self-assembling properties of hCAI-type probes **1**, **3** and **4**. (**a**, **b**) Optical densities of probe **1** (**a**, 25 μM) or **4** (**b**, 25 μM) in HEPES buffer (pH 7.2) containing DMSO. (**c**) DLS analysis of particle-size distribution of the self-assembled probe **4** (25 μM) without (top) or with (bottom) hCAI (25 μM) in 50 mM HEPES buffer (pH 7.2, 0.2 mM TFA). (**d**) Atomic force microscopy (AFM) images of probe **4** (5 μM) without (top) or with (bottom) hCAI (5 μM) in 50 mM HEPES buffer (pH 7.2) (scale bar 100 nm). (**e**) Optical densities of probe **3**, **1**, and **4** (25 μM) without or with hCAI (25 μM) in 50 mM HEPES buffer (pH 7.2, 0.2 mM TFA).

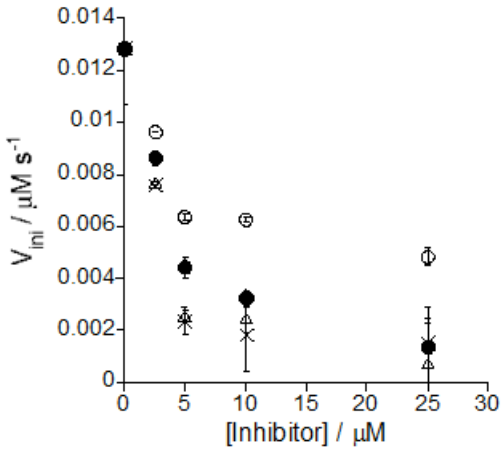


Figure S3. Inhibitory experiments of hCAI with ^{19}F NMR probes. Initial rates of hydrolysis reaction of *p*-nitrophenylacetate (250 μM) and hCAI (1 μM) with various concentrations of ^{19}F NMR probes **1** (×), **3** (Δ), **4** (○) and benzenesulfonamide (●). Experiments were performed in triplicate to obtain mean and standard deviation values (shown as error bars).

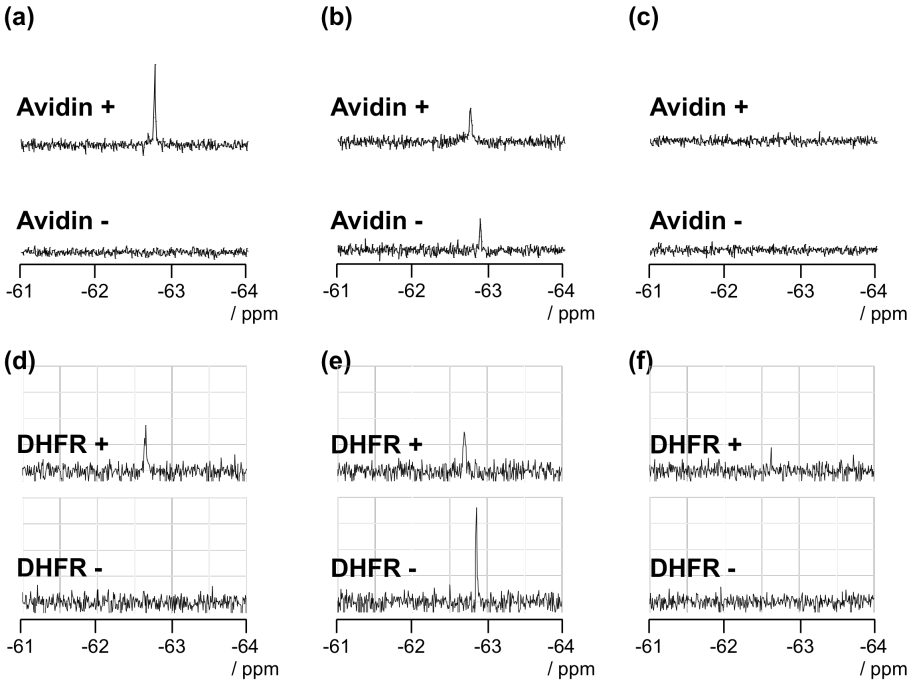


Figure S4. (**a** - **c**) ^{19}F NMR spectra of probes **5**, **6** and **7** (a, b and c, 25 μM) with or without avidin (top or bottom, 25 μM) in 50 mM HEPES buffer (pH 7.2, 0.2 mM TFA as an internal standard for signal intensity and chemical shift, 10% D_2O (v/v)). (**d** - **f**) ^{19}F NMR spectra of probes **8**, **9** and **10** (d, e and f, 25 μM) with or without DHFR (top or bottom, 25 μM) in 50 mM HEPES buffer (pH 7.2, 0.2 mM TFA, 25 μM NADPH, 10% D_2O (v/v)). All the experiments were performed at 25 ºC.

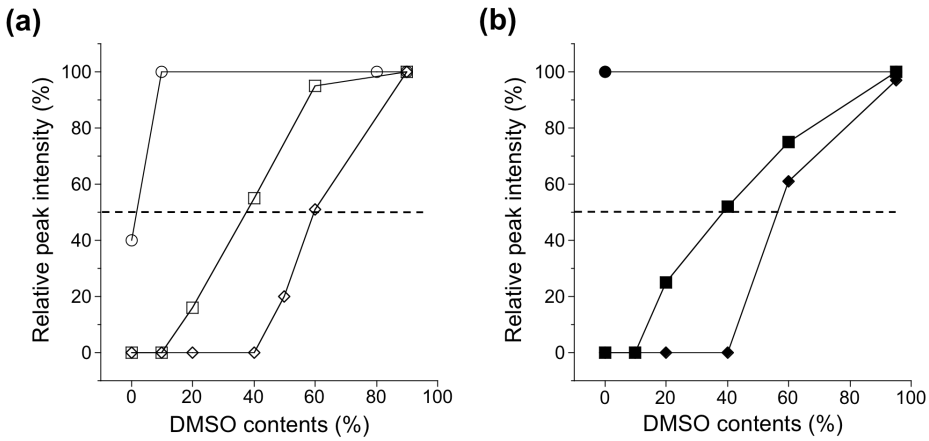


Figure S5. The stabilities of the self-assembling aggregates of avidin probes **5** – **7** (**a**, **5**; □, **6**; ○, **7**; ◇) or DHFR probes **8** – **10** (**b**, **8**; ■, **9**; ●, **10**; ◆) in aqueous solution (50 mM HEPES buffer, pH 7.2) with various DMSO contents.

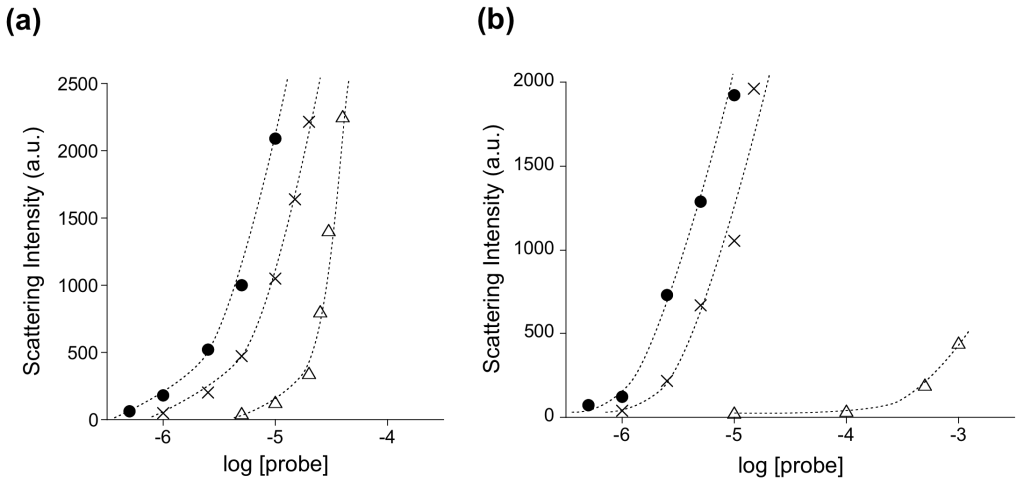


Figure S6. Concentration dependent change of scattering intensities for avidin probes **5** – **7** (**a**, **5**; ×, **6**; Δ, **7**; ●) and DHFR probes **8** – **10** (**b**, **8**; ×, **9**; Δ, **10**; ●) in aqueous solution (pH 7.2, 50 mM HEPES buffer). These scattering intensities were collected from DLS analyses with same laser strengths and sensitivities.

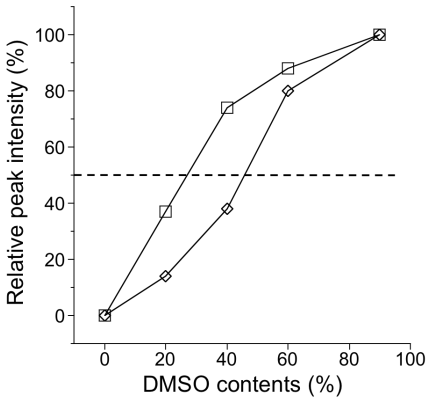


Figure S7. The stabilities of the self-assembling aggregates of probes **11** (◇) and **12** (□) in aqueous solution (50 mM HEPES buffer, pH 7.2, 0.2 mM TFA as an internal standard for signal intensity and chemical shift) with various DMSO-d_6 contents.

Table S1. Relaxation times of always on probe **3** and off/on probe **1** with hCAI.

	probe **3** only	probe **3** + hCAI	probe **1** + hCAI
T_1 / msec	430	840	950
T_2 / msec	350	82	85

Table S2. Relaxation times of probes **13**, **14**, **1** and **12** with hCAI.

	Probe **13** (F=1)	Probe **14** (F=3)	Probe **1** (F=6)	Probe **12** (F=12)
T_1 / msec	–[a]	894	950	949
T_2 / msec	–[a]	74	85	109
T_2*/ msec	–[a]	4.0	6.3	4.6

[a]Not determined due to the low signal intensity.

Supplementary Methods

pH profile of probe 2 in aqueous buffer. To examine the pH profile of the signal intensity change of ^{19}F-probe **2**, a citrate/phosphate buffer system of constant ionic strength[S1] was used (0.5 ion strength). The relative integral values of the signal intensity at various pH buffer solutions were calculated with the integral value of TFA (-75.6 ppm) on ^{19}F NMR spectroscopy. All the experiments were acquired at 25 ºC.

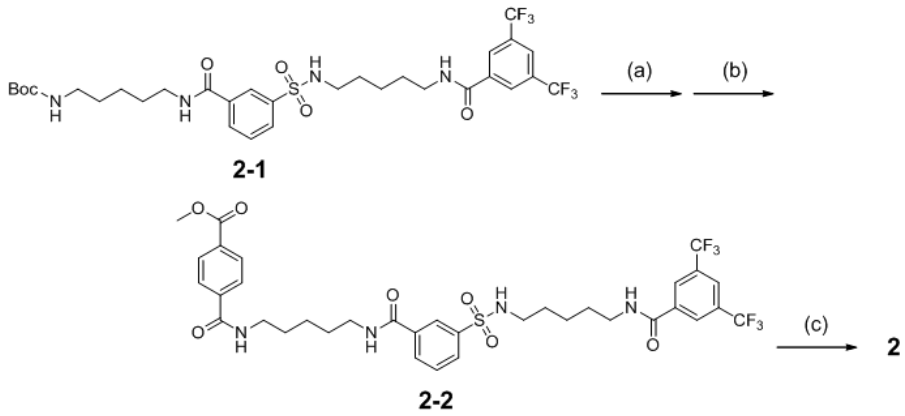
Measurements of light scattering and atomic force microscopy of ^{19}F-probes. The optical density was measured at 25 ºC in 50 mM HEPES buffer (pH 7.2, 0.2 mM TFA) using a quartz cell (1 cm). A DMSO stock solution of each compound was slowly added to the buffer solution to give a final concentration of 25 μM (0.25 % DMSO (v/v)). The DLS measurements were performed under the same conditions (laser strength; 668, channel width; 43.0 μsec, sensitivity; 50) on NICOMP-380zls with a tubular-type cell. All measurements were carried out in triplicate. In AFM imaging, a solution of each compound was spin coated onto a freshly cleaved mica surface and dried *in vacuo*. Images of the sample were obtained with a tapping-mode AFM.

DHFR expression and purification for sample preparation. The expression plasmid for DHFR from *Escherichia coli*, pBAD-DHFR[S2], was transformed into *E. coli* TOP10 (Invitrogen). The cells were grown in LB medium to an O.D. (600 nm) of 0.6, at which time expression of the protein was induced by the addition of L-arabinose to a final concentration of 0.1 % (wt/vol). After growth for an additional 16 h at 27 ºC, the cells were harvested by centrifugation. The cell pellets were resuspended in 50 mM Tris-HCl, 50 mM NaCl, pH 7.0 and lysed by sonication. The protein was purified from the soluble fraction of the lysate by TALON Metal Affinity Resins (Clontech) according to the manufacture's protocol and dialyzed against 1 × PBS buffer. The purified protein was subjected to site-specific cleavage with thrombin (GE Healthcare) to eliminate the fragment containing thioredoxin and His-tag in the N-terminus of the fusion protein. The eliminated fragment and thrombin were removed by TALON Metal Affinity Resins and Benzamidine Sepharose 6B (GE Healthcare) according to the manufacture's protocol. The concentration of DHFR was determined by the absorbance at 280 nm using the molar extinction coefficient (31,100 $M^{-1}cm^{-1}$)[S3].

Measurements of relaxation times. In the experiments of probe **3** or probe **1**, probe (150 μM) and hCAI (0 or 150 μM) were dissolved in 50 mM HEPES buffer (pH 7.2, 0.2 mM TFA, 10% D_2O (v/v), 1.2% DMSO (v/v), 500 μL). The 90-degree pulse width was 17 μsec. In the experiments of probe **14**, probe (200 μM) and hCAI (200 μM) were dissolved in 50 mM HEPES buffer (pH 7.2, 0.2 mM TFA, 10% D_2O (v/v), 0.8% DMSO (v/v), 500 μL). The 90-degree pulse width was 12 μsec. In the experiments of probe **12**, probe (100 μM) and hCAI (100 μM) were dissolved in 50 mM HEPES buffer (pH 7.2, 0.2 mM TFA, 10% D_2O (v/v), 0.4% DMSO (v/v), 500 μL). The 90-degree pulse width was 17 μsec. T_1 relaxation times were measured by the inversion recovery method, and T_2 relaxation times were measured by the Carr-Purcell-Meiboom-Gill (CPMG) method.

Synthesis

Synthesis of probe 2.



Scheme S1. Synthetic scheme of probe **2**. Reaction conditions: (a) TFA in dichloromethane (DCM), (b) Monomethyl terephthalic acid, ethyl-diisopropyl carbodiimide hydrochloric acid (EDCI), *N,N'*-diisopropylethylamine (DIPEA) in dry *N,N'*-dimethylformamide (DMF), (c) 1N NaOH aq. in MeOH.

Probe 2:

To a stirred solution of compound **2-1**[S4] (70 mg, 0.098 mmol) in DCM (3 mL) was added TFA (1 mL) at r.t.. The reaction mixture was stirred at r.t. for 2h. The solvent was removed under vacuum, and the residue was suspended with toluene (2 mL), the solvent was evaporated and dried under vacuum to yield deprotected compound **2-1** (72 mg, quantitative) as clear oil without further purification.

To a stirred solution of the deprotected compound **2-1** (72 mg) in dry DMF (5 mL) and DIPEA (40 μL, 0.30 mmol) was added EDCI (29 mg, 0.15 mmol), HOBt·H_2O (23 mg, 0.15 mmol) and *p*-terephthalic acid monomethyl ester (23 mg, 0.13 mmol) at r.t.. The reaction mixture was stirred at r.t. for 12h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in 100 mL EtOAc, and washed with 5% citric acid aq. (50 mL, twice), saturated $NaHCO_3$ aq. (50 mL, twice) and brine (50 mL, twice). The organic solvent was dried over anhydrous $MgSO_4$, concentrated under vacuum and purified on column chromatography (SiO_2/$CHCl_3$: MeOH = 20 : 1) to yield **2-2** (65 mg, 2steps; 86%) as white solid without further purification.

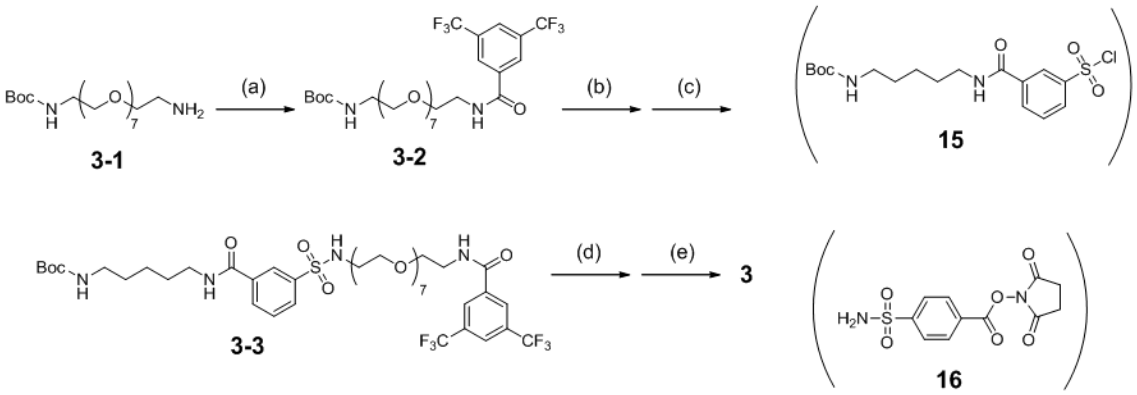
To a stirred solution of **2-2** (65 mg) in MeOH (3 mL) was added 1N NaOH aq. (3 mL) at r.t.. The reaction mixture was stirred at r.t. for 2h. The solvent was removed under vacuum, the residue

was dissolved in 5% citric acid aq. (50 mL) and extracted with EtOAc (50 mL, 3 times), and the organic layer was washed with brine (50 mL). The organic layer was dried over anhydrous MgSO4, concentrated under vacuum and purified on column chromatography (SiO_2/$CHCl_3$: MeOH : AcOH = 20 : 1 : 0.1 to 10 : 1 : 0.1) to yield 36 mg (56%) as white solid.

^{1}H NMR (400 MHz, CD_3OD, TMS) : δ/ppm, 8.72 (br, 1H), 8.63 (br, 1H), 8.42 (s, 2H), 8.29 (m, 1H), 8.15 (s, 1H), 8.06 (d, J_H = 8.4 Hz, 2H), 7.99 (m, 2H), 7.85 (d, J_H = 8.4 Hz, 2H), 7.64 (m, 1H), 3.42 (m, 4H), 3.36 (m, 2H), 2.89 (t, J_H = 7.2 Hz, 2H), 1.68 (m, 4H), 1.58 (m, 2H), 1.50 (m, 4H), 1.38 (m, 2H).

HR-FAB MS (NBA): calcd for $C_{34}H_{37}N_4F_6O_7S_1$ $[M+H]^+$ = 759.2282; obsd 259.2261.

Synthesis of probe 3.



Scheme S2. Synthetic scheme of probe **3**. Reaction conditions: (a) 3,5-bis(trifluoromethyl)benzoylchloride, *N,N'*-dimethyl-4-aminopyridine (DMAP) in dry DCM, (b) TFA in DCM, (c) **15**, DIPEA in dry DCM, (d) TFA in DMF, (e) **16**, DIPEA in dry DMF

Compound **3-2**:

To a stirred solution of 3,5-bis(trifluoromethyl)benzoylchloride (410 mg, 1.5 mmol) in dry DCM (8 mL) was added compound **3-1** (460 mg, 0.98 mmol), 4-dimethylaminopyridine (DMAP, 6.1 mg, 0.050 mmol) at r.t.. The reaction mixture was stirred for 3h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (300 mL), and washed with saturated $NaHCO_3$ aq. (100 mL, twice), water (100 mL, 3 times) and brine (100 mL, once). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum to yield 380 mg (54 %) as pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.37 (s, 2H), 7.99 (s, 1H), 3.30-3.75 (m, 32H), 1.43 (s, 9H).

Compound **3-3**:

To a stirred solution of compound **3-2** (190 mg, 0.27 mmol) in DCM (3 mL) was added TFA (1 mL) at r.t.. The reaction mixture was stirred at r.t. for 2h. The solvent was removed under vacuum, the residue was suspended with toluene (2 mL) and the solvent was evaporated and dried under vacuum to yield deprotected compound **3-2** (195 mg, quantitative) as clear oil without further purification.

To a stirred solution of the deprotected compound **3-2** (195 mg) in dry DCM (4 mL) and

DIPEA (130 μL, 0.98 mmol) was added compound **15**[S4] (73 mg, 0.18 mmol) and DMAP (4.4 mg, 0.036 mmol) at r.t.. The reaction mixture was stirred at r.t. for 5h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (200 mL), and washed with saturated $NaHCO_3$ aq. (100 mL, 3 times) and brine (100 mL, once).The organic solvent was dried over anhydrous $MgSO_4$, concentrated under vacuum and purified on column chromatography (SiO_2 / $CHCl_3$: MeOH = 20 : 1) to yield 75 mg (2 steps; 43 %) as pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.38 (s, 2H), 8.27 (s, 1H), 7.98-8.00 (m, 3H), 7,57 (t, J_H = 8.0 Hz, 1H), 3.40-3.72 (m, 32H), 3.11-3.13 (m, 4H), 1.45-1.68 (m, 6H), 1.43 (s, 9H).

Probe **3**:

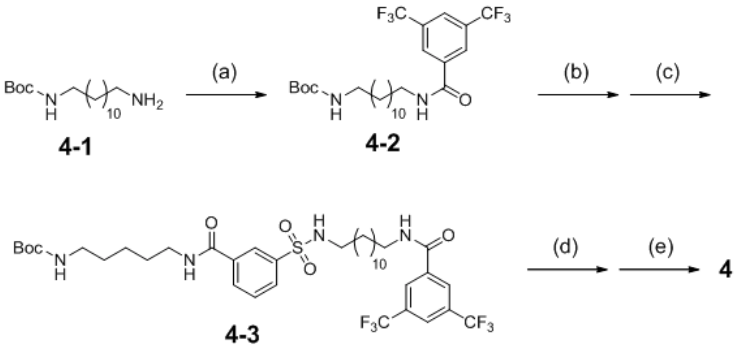
To a stirred solution of compound **3-3** (75 mg, 0.077 mmol) in DCM (4.5 mL) was added TFA (2 mL) at r.t.. The reaction mixture was stirred at r.t. for 2h. The solvent was removed under vacuum, the residue was suspended with toluene (4 mL) and the solvent was evaporated and dried under vacuum to yield deprotected compound **3-3** (80 mg, quantitative) as clear oil without further purification.

To a stirred solution of the deprotected compound **3-3** (80 mg) in dry DMF (3 mL) and DIPEA (47 μL, 0.35 mmol) was added compound **16**[S4] (16 mg, 0.053 mmol) at r.t.. The reaction mixture was stirred at r.t. for 5h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (100 mL), and washed with saturated $NaHCO_3$ aq. (50 mL, 3 times) and brine (50 mL, twice).The organic solvent was dried over anhydrous $MgSO_4$, concentrated under vacuum and purified on column chromatography (SiO_2 / $CHCl_3$: MeOH = 8 : 1) to yield 33 mg (2 steps; 59 %) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.40 (s, 2H), 8.25 (s, 1H), 7.96-8.02 (m, 3H), 7.81-7.84 (m, 4H), 7,57 (t, J_H = 8.0 Hz, 1H), 3.40-3.72 (m, 34H), 3.12 (t, J_H = 6.0 Hz, 2H), 1.63-1.80 (m, 6H).

HR-FAB MS (NBA): calcd for $C_{44}H_{60}N_5F_6O_{14}S_2$ $[M+H]^+$ = 1060.3438; obsd 1060.3457.

Synthesis of probe 4.



Scheme S3. Synthetic scheme of probe **4**. Reaction conditions: (a) 3,5-bis(trifluoromethyl)benzoylchloride, DMAP in dry DCM, (b) TFA in DCM, (c) **15**, DIPEA in dry DCM, (d) TFA in DMF, (e) **16**, DIPEA in dry DMF

Compound **4-2**:

To a stirred solution of 3,5-bis(trifluoromethyl)benzoylchloride (1.0 g, 3.8 mmol) in dry DCM (25 mL) was added compound **4-1** (760 mg, 2.5 mmol), DMAP (15 mg, 0.13 mmol) at r.t.. The reaction mixture was stirred for 3h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (500 mL), and washed with saturated $NaHCO_3$ aq. (200 mL, twice), water (200 mL, 3 times) and brine (200 mL, once). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum to yield 690 mg (63 %) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.23 (s, 2H), 7.99 (s, 1H), 3.49 (m, 2H), 3.10 (m, 2H), 1.26-1.43 (m, 29H).

Compound **4-3**:

To a stirred solution of compound **4-2** (250 mg, 0.56 mmol) in DCM (8 mL) was added TFA (3 mL) at r.t.. The reaction mixture was stirred at r.t. for 2h. The solvent was removed under vacuum, the residue was suspended with toluene (5 mL) and the solvent was evaporated and dried under vacuum to yield deprotected compound **4-2** (255 mg, quantitative) as clear oil without further purification.

To a stirred solution of the deprotected compound **4-2** (255 mg) in dry DCM (6 mL) and DIPEA (200 μL, 1.5 mmol) was added compound **15** (150 mg, 0.37mmol) and DMAP (8.8 mg, 0.074 mmol) at r.t.. The reaction mixture was stirred at r.t. for 5h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (500 mL), and washed with

saturated $NaHCO_3$ aq. (200 mL, 3 times) and brine (200 mL, once).The organic solvent was dried over anhydrous $MgSO_4$, concentrated under vacuum and purified on column chromatography (SiO_2 / $CHCl_3$: MeOH = 50 : 1) to yield 70 mg (2 steps; 23 %) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.29 (s, 1H), 8.26 (s, 2H), 7.94-7.98 (m, 2H), 7,58 (t, J_H = 8.0 Hz, 1H), 3.42-3.52 (m, 4H), 2.92-3.07 (m, 4H), 1.19-1.69 (m, 35H).

Probe **4**:

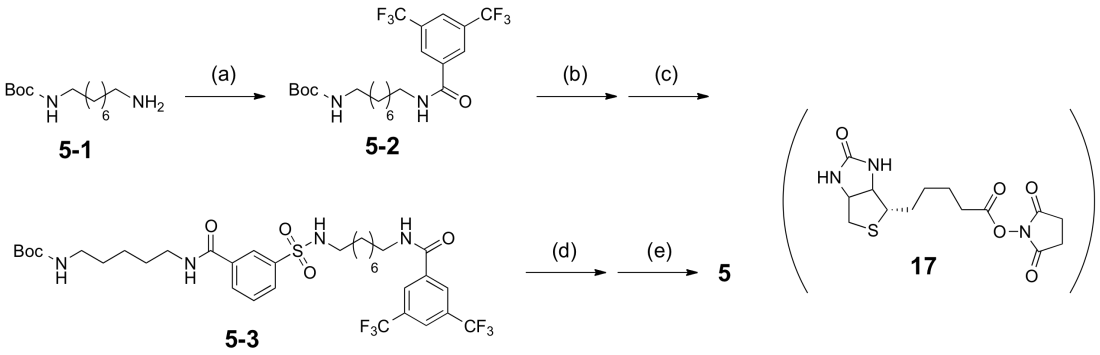
To a stirred solution of compound **4-3** (50 mg, 0.060 mmol) in DCM (2 mL) was added TFA (1 mL) at r.t.. The reaction mixture was stirred at r.t. for 2h. The solvent was removed under vacuum, the residue was suspended with toluene (2 mL) and the solvent was evaporated and dried under vacuum to yield deprotected compound **4-3** (56 mg, quantitative) as clear oil without further purification.

To a stirred solution of the deprotected compound **4-3** (56 mg) in dry DMF (3 mL) and DIPEA (54 μL, 0.41 mmol) was added compound **16** (14 mg, 0.050 mmol) at r.t.. The reaction mixture was stirred at r.t. for 5h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (100 mL), and washed with saturated $NaHCO_3$ aq. (50 mL, 3 times) and brine (50 mL, twice).The organic solvent was dried over anhydrous $MgSO_4$, concentrated under vacuum and purified on column chromatography (SiO_2 / $CHCl_3$: MeOH = 10 : 1) to yield 28 mg (2 steps; 65 %) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.42 (s, 2H), 8.15 (s, 1H), 7.91-8.03 (m, 6H), 7,64 (t, J_H = 8.0 Hz, 1H), 3.35-3.48 (m, 6H), 2.80 (t, J_H = 7.2 Hz, 2H), 1.12-1.72 (m, 26H).

HR-FAB MS (NBA): calcd for $C_{40}H_{51}N_5F_6O_7S_2$ $[M]^+$ = 891.3134; obsd 891.3126.

Synthesis of probe 5.



Scheme S4. Synthetic scheme of probe **5**. Reaction conditions: (a) 3,5-bis(trifluoromethyl)benzoylchloride, DMAP in dry DCM, (b) TFA in DCM, (c) **15**, DIPEA in dry DCM, (d) TFA in DCM, (e) **17**, DIPEA in dry DMF

Compound **5-2**:

To a stirred solution of 3,5-bis(trifluoromethyl)benzoylchloride (1.3 g, 4.9 mmol) in dry DCM (10 mL) was added compound **5-1** (1.0 g, 4.1 mmol), DMAP (25 mg, 0.26 mmol) at r.t.. The reaction mixture was stirred for 3h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (500 mL), and washed with saturated $NaHCO_3$ aq. (200 mL, twice), water (200 mL, 3 times) and brine (200 mL, once). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum to yield 1.2 g (62 %) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.24 (s, 2H), 7.99 (s, 1H), 3.49 (m, 2H), 3.10 (m, 2H), 1.31-1.66 (m, 21H).

Compound **5-3**:

To a stirred solution of compound **5-2** (1.2 g, 2.2 mmol) in DCM (8 mL) was added TFA (2 mL) at r.t.. The reaction mixture was stirred at r.t. for 2h. The solvent was removed under vacuum, the residue was suspended with toluene (5 mL) and the solvent was evaporated and dried under vacuum to yield deprotected compound **5-2** (1.3 g, quantitative) as clear oil without further purification.

To a stirred solution of the deprotected compound **5-2** (1.3 g) in dry DCM (8 mL) and DIPEA (1.0 mL, 7.5 mmol) was added compound **15** (600 mg, 1.5 mmol) and DMAP (36 mg, 0.30 mmol) at r.t.. The reaction mixture was stirred at r.t. for 5h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (200 mL), and washed with saturated $NaHCO_3$

aq. (100 mL, 3 times) and brine (100 mL, once).The organic solvent was dried over anhydrous $MgSO_4$, concentrated under vacuum and purified on column chromatography (SiO_2 / $CHCl_3$: MeOH = 50 : 1) to yield 780 mg (2 steps; 71 %) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.33 (s, 2H), 8.23 (s, 1H), 7.98-8.00 (m, 2H), 7,52 (t, J_H = 8.0 Hz, 1H), 3.40-3.47 (m, 4H), 2.93-3.09 (m, 4H), 1.19-1.69 (m, 35H).

Probe **5**:

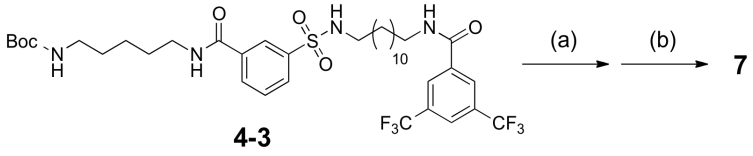
To a stirred solution of compound **5-3** (66 mg, 0.088 mmol) in DCM (4 mL) was added TFA (1 mL) at r.t.. The reaction mixture was stirred at r.t. for 2h. The solvent was removed under vacuum, the residue was suspended with toluene (2 mL) and the solvent was evaporated and dried under vacuum to yield deprotected compound **5-3** (70 mg, quantitative) as clear oil without further purification.

To a stirred solution of the deprotected compound **5-3** (70 mg) in dry DMF (4 mL) and DIPEA (42 μL, 0.32 mmol) was added compound **17**[S4] (20 mg, 0.059 mmol) at r.t.. The reaction mixture was stirred at r.t. for 5h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (100 mL), and washed with saturated $NaHCO_3$ aq. (50 mL, 3 times) and brine (50 mL, twice).The organic solvent was dried over anhydrous $MgSO_4$, concentrated under vacuum and purified on column chromatography (SiO_2 / $CHCl_3$: MeOH = 20 : 1) to yield 30 mg (2 steps; 58 %) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.42 (s, 1H), 8.38 (s, 2H), 8.14 (m, 1H), 7,97 (m, 2H), 7.60 (t, J_H = 8.0 Hz, 1H), 4.53 (m, 1H), 4.18 (m, 1H), 3.30-3.40 (m, 6H), 3.10 (m, 2H), 2.89-2.94 (m, 2H), 2.75 (m, 1H), 2.14 (t, J_H = 7.4 Hz, 2H), 1.10-1.77 (m, 24H).

HR-FAB MS (NBA): calcd for $C_{39}H_{52}N_6F_6O_6S_2$ $[M]^+$ = 878.3294; obsd 878.3283.

Synthesis of probe 7.



Scheme S5. Synthetic scheme of probe **7**. Reaction conditions: (a) TFA in DCM, (b) **17**, DIPEA in dry DMF

Probe 7:

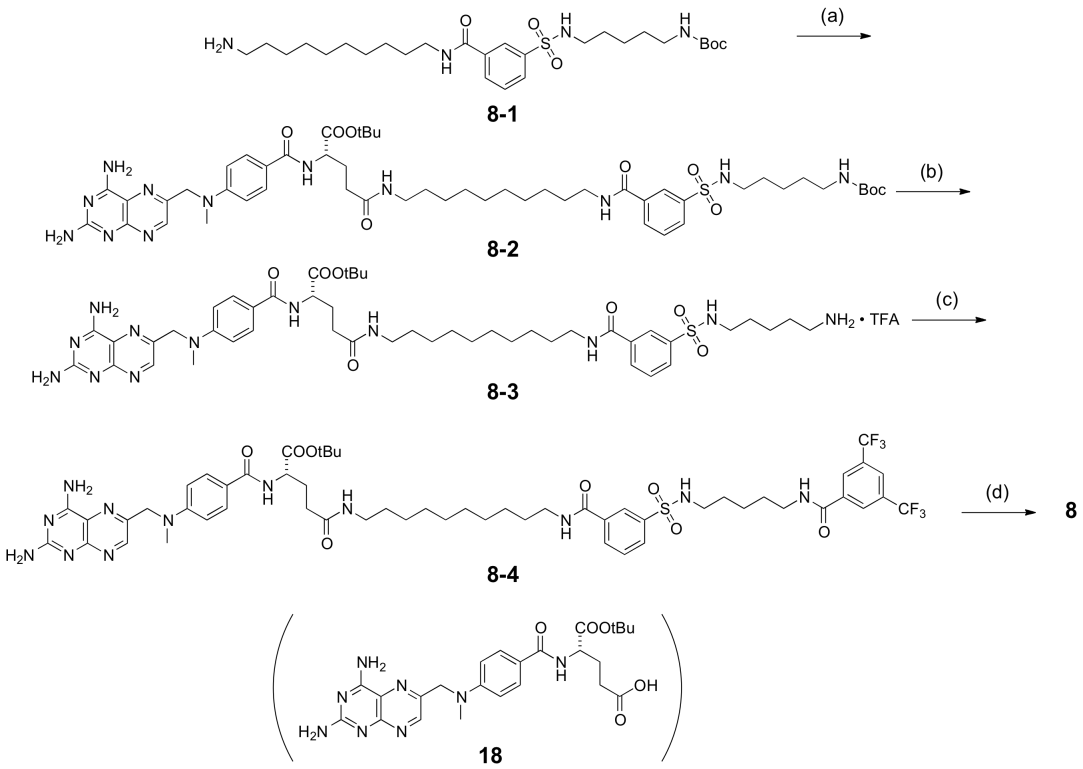
To a stirred solution of compound **4-3** (45 mg, 0.060 mmol) in DCM (2 mL) was added TFA (1 mL) at r.t.. The reaction mixture was stirred at r.t. for 2h. The solvent was removed under vacuum, the residue was suspended with toluene (2 mL) and the solvent was evaporated and dried under vacuum to yield deprotected compound **4-3** (50 mg, quantitative) as clear oil without further purification.

To a stirred solution of the deprotected compound **4-3** (50 mg) in dry DMF (2 mL) and DIPEA (47 μL, 0.36 mmol) was added compound **17** (14 mg, 0.040 mmol) at r.t.. The reaction mixture was stirred at r.t. for 5h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (100 mL), and washed with saturated $NaHCO_3$ aq. (50 mL, 3 times) and brine (50 mL, twice).The organic solvent was dried over anhydrous $MgSO_4$, concentrated under vacuum and purified on column chromatography (SiO_2 / $CHCl_3$: MeOH = 10 : 1) to yield 29 mg (2 steps; 76 %) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.45 (s, 1H), 8.27 (s, 2H), 8.14 (m, 1H), 7,97 (m, 2H), 7.60 (t, J_H = 8.0 Hz, 1H), 4.53 (m, 1H), 4.34 (m, 1H), 3.41-3.51 (m, 6H), 3.14 (m, 2H), 2.89-2.94 (m, 2H), 2.75 (m, 1H), 2.14 (t, J_H = 7.4 Hz, 2H), 1.10-1.77 (m, 32H).

HR-FAB MS (NBA): calcd for $C_{43}H_{60}N_6F_6O_6S_2$ $[M]^+$ = 934.3920; obsd 934.3950.

Synthesis of probe 8.



Scheme S6. Synthetic scheme of probe **8**. Reaction conditions: (a) **18**, EDCI, HOBt·H_2O, DIPEA in dry DMF, (b) TFA in DCM, (c) 3,5-bis(trifluoromethyl)benzoic acid, EDCI, HOBt·H_2O, DIPEA in dry DMF, (d) TFA

Compound **8-2**:

To a stirred solution of compound **8-1**[S5] (404 mg, 0.75 mmol) in dry DMF (15 mL) was added compound **18**[S6] (400 mg, 0.78 mmol), EDCI (260 mg, 1.36 mmol), HOBt·H_2O (183 mg, 1.19 mmol) and DIPEA (400 μl, 2.30 mmol) at r.t.. The reaction mixture was stirred overnight under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in $CHCl_3$ (300 mL), and washed with saturated $NaHCO_3$ aq. (300 mL, once) and brine (300 mL, once). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum. The crude residue was purified by column chromatography (SiO_2 / $CHCl_3$: MeOH = 10 : 1) to yield 412 mg (53%) as yellow solid. ^{1}H NMR (400 MHz, CD_3OD) : δ/ppm, 8.55 (s, 1H), 8.29 (m, 1H), 8.03-7.96 (m, 2H), 7.74 (d, J_H = 9.2 Hz, 2H), 7.64 (t, J_H = 8.0 Hz, 1H), 6.84 (d, J_H = 9.2 Hz, 2H), 4.82 (s, 2H), 4.44-4.40 (m, 1H), 3.37 (t, J_H = 7.2 Hz, 2H), 3.23 (s, 3H), 3.10 (t, J_H = 7.2 Hz, 2H), 2.95 (t, J_H = 7.2 Hz, 2H), 2.85 (t, J_H = 7.2 Hz, 2H), 2.36-2.31 (m, 2H), 2.24-2.16 (m, 1H), 2.12-2.02 (m, 1H), 1.60 (m, 2H), 1.46 (s, 9H),

1.41-1.21 (m, 29H)

Compound **8-3**:

To a stirred solution of compound **8-2** (387 mg, 0.38 mmol) in DCM (20 mL) was added TFA (1.5 ml) on ice. The reaction mixture was stirred for 6h at 0 ºC. The solution was neutralized with 25% NH_3 aq, and the solvent was removed under vacuum. The residue was added to isopropanol/DCM (1:10). After the solution was filtered, the filtrate was concentrated under vacuum. The crude residue was purified by column chromatography (SiO_2/ $CHCl_3$: MeOH : 25% NH_3aq = 3:1:0.04) to yield 500 mg (quantitative) as yellow solid.

^{1}H NMR (400 MHz, CD_3OD) : δ/ppm, 8.60 (s, 1H), 8.29 (m, 1H), 8.05-7.96 (m, 2H), 7.74 (d, J_H = 8.8 Hz, 2H), 7.65 (t, J_H = 8.0 Hz, 1H), 6.84 (d, J_H = 8.8 Hz, 2H), 4.89 (s, 2H), 4.43-4.40 (m, 1H), 3.37 (t, J_H = 7.2 Hz, 2H), 3.25 (s, 3H), 3.11 (t, J_H = 7.2 Hz, 2H), 2.89-2.86 (m, 4H), 2.35 (m, 2H), 2.24-2.15 (m, 1H), 2.11-2.02 (m, 1H), 1.61 (m, 2H), 1.51-1.23 (m, 29H)

Compound **8-4**:

To a stirred solution of compound **8-3** (70 mg, 0.075 mmol) in dry DMF (15 mL) was added 3,5-bis(trifluoromethyl)benzoic acid (26 mg, 0.10 mmol), EDCI (18 mg, 0.094 mmol), HOBt·H_2O (13 mg, 0.085 mmol) and DIPEA (117 μl, 0.672 mmol) at r.t.. The reaction mixture was stirred overnight under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (50 mL), and washed with saturated $NaHCO_3$ aq. (50 mL, twice) and brine (50 mL, once). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum. The crude residue was purified by column chromatography (SiO_2 / $CHCl_3$: MeOH = 15 : 1) to yield 23 mg (27%) as yellow solid.

^{1}H NMR (400 MHz, CD_3OD) : δ/ppm, 8.56 (s, 1H), 8.42 (s, 2H), 8.29 (m, 1H), 8.15 (s, 1H), 8.03-7.96 (m, 2H), 7.74 (d, J_H = 9.2 Hz, 2H), 7.64 (t, J_H = 8.0 Hz, 1H), 6.86 (d, J_H = 9.2 Hz, 2H), 4.84 (s, 2H), 4.43-4.39 (m, 1H), 3.38-3.33 (m, 4H), 3.25 (s, 3H), 3.10 (t, J_H = 7.2 Hz, 2H), 2.89 (t, J_H = 6.8 Hz, 2H), 2.35-2.31 (m, 2H), 2.24-2.16 (m, 1H), 2.10-2.01 (m, 1H), 1.61-1.54 (m, 4H), 1.53-1.22 (m, 27H)

Probe **8**:

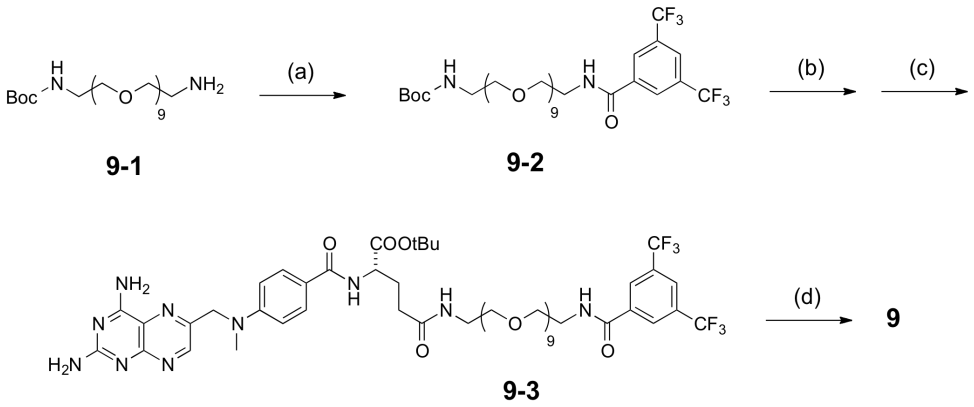
Compound **8-4** (20 mg, 0.017 mmol) was added to TFA (2 ml) and the solution was stirred for 6h. After evaporation, the residue was washed with 1N HCl in EtOAc (4 ml, once) and diethylether

(10 ml, twice). The residue was purified by column chromatography (SiO_2 / $CHCl_3$: MeOH : AcOH = 5 : 1:0.06) to yield 16 mg (85%) as yellow solid.

^{1}H NMR (400 MHz, CD_3OD) : δ/ppm, 8.65 (s, 1H), 8.42 (s, 2H), 8.28 (m, 1H), 8.15 (s, 1H), 8.03-7.97 (m, 2H), 7.77 (d, J_H = 9.2 Hz, 2H), 7.65 (t, J_H = 8.0 Hz, 1H), 6.88 (d, J_H = 9.2 Hz, 2H), 4.93 (s, 2H), 4.57-4.53 (m, 1H), 3.37-3.33 (m, 4H), 3.12 (t, J_H = 7.2 Hz, 2H), 2.89 (t, J_H = 7.2 Hz, 2H), 2.39-2.34 (m, 2H), 2.31-2.23 (m, 1H), 2.16-2.09 (m, 1H), 1.60-1.56 (m, 4H), 1.51-1.24 (m, 18H)

HR-ESI MS: calcd for $C_{51}H_{63}N_{12}F_6O_8S_1$ $[M+H]^+$ = 1117.4517; obsd 1117.4495.

Synthesis of probe 9.



Scheme S7. Synthetic scheme of probe **9**. Reaction conditions: (a) 3,5-bis(trifluoromethyl)benzoylchloride, DMAP in dry DCM, (b) TFA in DCM, (c) **18**, EDCI, HOBt·H_2O, DIPEA in dry DMF, (d) TFA in DCM

Compound **9-2**:

To a stirred solution of 3,5-bis(trifluoromethyl)benzoylchloride (48 mg, 0.30 mmol) in dry DCM (5 mL) was added compound **9-1** (110 mg, 0.20 mmol), DMAP (0.72 mg, 0.060 mmol) at r.t.. The reaction mixture was stirred for 3h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (200 mL), and washed with saturated $NaHCO_3$ aq. (50 mL, twice), water (50 mL, 3 times) and brine (50 mL, once). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum to yield 170 mg (quantitative) as white solid.
^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.39 (s, 2H), 7.97 (s, 1H), 3.49-3.72 (m, 38H), 3.30 (m, 2H), 1.44 (s, 9H).

Compound **9-3**:

To a stirred solution of compound **9-2** (50 mg, 0.063 mmol) in DCM (1 mL) was added TFA (1 mL) at r.t.. The reaction mixture was stirred overnight at r.t.. The solvent was removed under vacuum, the residue was suspended with toluene (2 mL) and the solvent was evaporated and dried under vacuum to yield deprotected compound **9-2** (50 mg, quantitative) as clear oil without further purification.

To a stirred solution of the deprotected compound **9-2** (50 mg) in dry DMF (5 mL) was added compound **18** (35 mg, 0.069 mmol), EDCI (20 mg, 0.11 mmol), HOBt·H_2O (20 mg, 0.13 mmol) and DIPEA (88 μL, 0.51 mmol) at r.t.. The reaction mixture was stirred overnight at r.t. under Ar

atmosphere. The solvent was removed under vacuum, the residue was purified on column chromatography (SiO_2 / $CHCl_3$: MeOH = 10 : 1) to yield 16 mg (21 %) as yellow solid.

^{1}H NMR (400 MHz, CD_3OD) : δ/ppm, 8.57 (s, 1H), 8.45 (s, 2H), 8.15 (s, 1H), 7.75 (d, J_H = 9.2 Hz, 2H), 6.86 (d, J_H = 9.2 Hz, 2H), 4.85 (s, 2H), 4.43-4.40 (m, 1H), 3.68 (t, J_H = 4.8 Hz, 2H), 3.64-3.51 (m, 36H), 3.47 (t, J_H = 5.2 Hz, 2H), 3.25 (s, 3H), 2.36 (m, 2H), 2.25-2.16 (m, 1H), 2.09-2.00 (m, 1H), 1.47 (s, 9H).

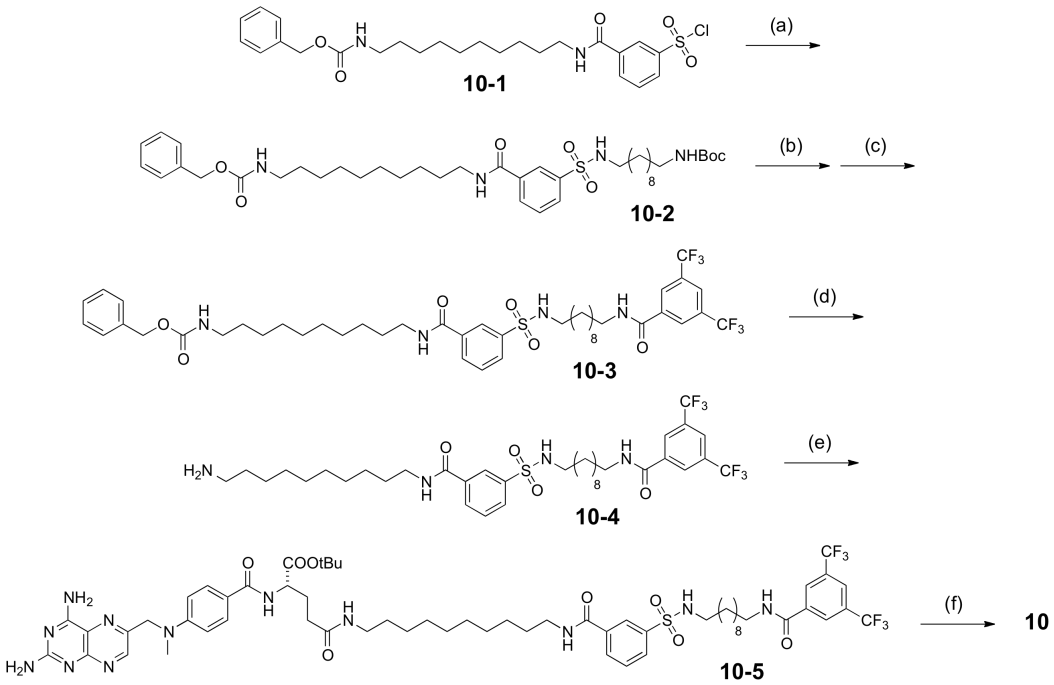
Probe **9**:

To a stirred solution of compound **9-3** (16 mg, 0.014 mmol) in DCM (1 mL) was added TFA (1 mL) at r.t.. The reaction mixture was stirred overnight at r.t.. After evaporation, the residue was suspended with 1N HCl in EtOAc (1 mL) and the solvent was evaporated. The residue was purified by reprecipitation with MeOH and diethylether. The precipitate was collected by filtration and dried *in vacuo* to 6.9 mg (45%) as yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) : δ/ppm, 12.46 (br, 1H), 9.25 (br, 2H), 9.07 (t, J_H = 5.2 Hz, 1H), 9.04 (br, 2H), 8.70 (s, 1H), 8.50 (s, 2H), 8.31 (s, 1H), 8.28 (d, J_H = 7.2 Hz, 1H), 7.89 (t, J_H = 5.2 Hz, 1H), 7.73 (d, J_H = 9.2 Hz, 2H), 6.81 (d, J_H = 9.2 Hz, 2H), 4.86 (s, 2H), 4.30-4.25 (m, 1H), 3.57-3.46 (m, 38H), 3.23 (s, 3H), 3.18-3.14 (m, 2H), 2.19 (t, J_H = 7.2 Hz, 2H), 2.02 (m, 1H), 1.91 (m, 1H)

HR-ESI MS: calcd for $C_{49}H_{67}N_{10}F_6O_{14}$ $[M+H]^+$ = 1133.4742; obsd 1133.4713.

Synthesis of probe 10.



Scheme S8. Synthetic scheme of probe **10**. Reaction conditions: (a) *N*-tert-butoxycarbonyl-1,10-diaminodecane, DIPEA in dry DCM (b) TFA in DCM, (c) 3,5-bis(trifluoromethyl)benzoic acid, EDCI, HOBt·H_2O, DIPEA in dry DMF, (d) Pd-C / H_2 in dry MeOH / DCM, (e) **18**, EDCI, HOBt·H_2O, DIPEA in dry DMF, (f) TFA in DCM

Compound **10-2**:

To a stirred solution of compound **10-1**[S5] (340 mg, 0.67 mmol) in dry DCM (20 ml) was added a solution of *N*-(*tert*-butoxycarbonyl)-1,10-diaminodecane hydrochloride (227 mg, 0.74 mmol) and DIPEA (0.55 ml, 3.2 mmol) in dry DCM (80 ml). The reaction mixture was stirred for 4h at r.t.. After evaporation, the residue was dissolved in $CHCl_3$ (300 ml) and washed with 5% aqueous citric acid (150 ml) and brine (150 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated to yield 502 mg (quantitative) as white solid.

^{1}H NMR (400 MHz, DMSO-d_6) : δ/ppm, 8.69 (m, 1H), 8.24 (s, 1H), 8.07 (m, 1H), 7.90 (m, 1H), 7.68 (t, J_H = 8.0 Hz, 1H), 7.63 (t, J_H = 5.6 Hz, 1H), 7.37-7.30 (m, 5H), 7.20 (br, 1H), 6.73 (br, 1H), 4.99 (s, 2H), 3.28 (m, 2H), 2.96 (dd, J_H = 12.8, 7.8 Hz, 2H), 2.87 (dd, J_H = 13.2, 7.8 Hz, 2H), 2.73 (dd, J_H = 13.2, 7.8 Hz, 2H), 2.01 (m, 2H), 1.36-1.14 (m, 39H)

Compound **10-3**:

To a stirred solution of compound **10-2** (200 mg, 0.27 mmol) in DCM (2 mL) was added TFA (1.5 mL) at r.t.. The reaction mixture was stirred for 6h at r.t.. The solvent was removed under vacuum, the residue was suspended with toluene (2 mL) and the solvent was evaporated and dried under vacuum to yield deprotected compound **10-2** as clear oil without further purification.

To a stirred solution of the deprotected compound **10-2** in dry DMF (10 mL) was added 3,5-bis(trifluoromethyl)benzoic acid (80 mg, 0.31 mmol), EDCI (80 mg, 0.42 mmol), HOBt·H_2O (70 mg, 0.46 mmol) and DIPEA (500 μL, 2.9 mmol) at r.t.. The reaction mixture was stirred overnight at r.t. under Ar atmosphere. The solvent was removed under vacuum, the residue was purified on column chromatography (SiO_2 / $CHCl_3$: MeOH = 30 : 1) to yield 240 mg (quantitative) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.27 (s, 2H), 8.20 (s, 1H), 8.02-7.96 (m, 3H), 7.59 (t, J_H = 7.6 Hz, 1H), 7.34-7.30 (m, 5H), 6.71 (br, 1H), 6.32 (br, 1H), 5.08 (s, 2H), 4.76 (br, 1H), 4.54 (br, 1H), 3.50-3.41 (m, 4H), 3.17 (m, 2H), 2.98 (m, 2H), 1.63-1.58 (m, 2H), 1.48-1.43 (m, 2H), 1.34-1.20 (m, 28H)

Compound **10-4**:

To a solution of compound **10-3** (240 mg, 0.27 mmol) in dry MeOH (5.0 ml) and dry DCM (3.0 ml) was added 10 wt.% Pd/C (120 mg). The reaction mixture was stirred at room temperature overnight under H_2 atmosphere. After the solution was filtered, the filtrate was evaporated. The resulting residue was purified on column chromatography (SiO_2 / $CHCl_3$: MeOH = 10 : 1) to yield 119 mg (59%) as colorless oil.

^{1}H NMR (400 MHz, CD_3OD) : δ/ppm, 8.42 (s, 2H), 8.29 (m, 1H), 8.15 (s, 1H), 8.05-7.98 (m, 2H), 7.66 (t, J_H = 8.0 Hz, 1H), 3.42-3.32 (m, 4H), 2.90 (t, J_H = 7.6 Hz, 2H), 2.87 (t, J_H = 7.2 Hz, 2H), 1.64 (m, 6H), 1.41-1.23 (m, 26H)

Compound **10-5**:

To a solution of compound **10-4** (35 mg, 0.047 mmol) in dry DMF (2.0 ml) was added compound **18** (19 mg, 0.037 mmol), EDCI (14 mg, 0.073 mmol), HOBt·H_2O (12 mg, 0.078 mmol) and DIPEA (40 μL, 0.230 mmol) at r.t.. The reaction mixture was stirred overnight at r.t. under Ar atmosphere. After evaporation, the residue was dissolved in $CHCl_3$ (50 ml) and washed with saturated aqueous $NaHCO_3$ (50 ml, once) and brine (50 ml, 3 times). The organic layer was dried

over anhydrous Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (SiO_2 / $CHCl_3$: MeOH, 10:1) to yield 33 mg (71%) as yellow solid.

^{1}H NMR (400 MHz, CD_3OD) : δ/ppm, 8.55 (s, 1H), 8.42 (s, 2H), 8.30 (m, 1H), 8.14 (s, 1H), 8.04-7.96 (m, 2H), 7.74 (d, J_H = 9.2 Hz, 2H), 7.64 (t, J_H = 8.0 Hz, 1H), 6.84 (d, J_H = 9.2 Hz, 2H), 4.81 (s, 2H), 4.43-4.40 (m, 1H), 3.41-3.36 (m, 4H), 3.23 (s, 3H), 3.10 (t, J_H = 6.8 Hz, 2H), 2.85 (t, J_H = 6.8 Hz, 2H), 2.35-2.31 (m, 2H), 2.24-2.15 (m, 1H), 2.11-2.01 (m, 1H), 1.66-1.55 (m, 4H), 1.45 (s, 9H), 1.41-1.20 (m, 28H)

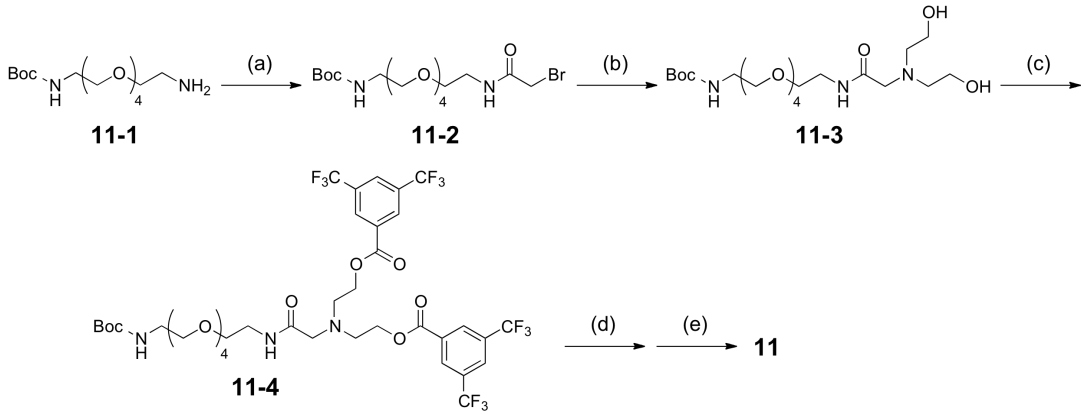
Probe **10**:

To a stirred solution of compound **10-5** (33 mg, 0.027 mmol) in DCM (3 mL) was added TFA (1.5 mL) at r.t.. The reaction mixture was stirred overnight at r.t.. After evaporation, the residue was suspended with 1N HCl in EtOAc (1 mL) and the solvent was evaporated. The residue was purified by column chromatography (SiO_2 / $CHCl_3$: MeOH : AcOH, 5:1:0.06) to yield 18 mg (57%) as yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) : δ/ppm, 8.96 (t, J_H = 5.6 Hz, 1H), 8.73 (t, J_H = 5.2 Hz, 1H), 8.53 (s, 1H), 8.48 (s, 2H), 8.26 (m, 2H), 8.06 (m, 1H), 7.88 (m, 1H), 7.78 (t, J_H = 4.8 Hz, 1H), 7.67-7.59 (m, 3H), 7.48 (d, J_H = 5.2 Hz, 1H), 7.38 (br, 2H), 6.74 (d, J_H = 8.8 Hz, 2H), 6.56 (br, 3H), 4.75 (s, 2H), 4.14 (m, 1H), 3.23 (m, 4H), 3.18 (s, 3H), 2.94 (dd, J_H = 12.8, 6.4 Hz, 2H), 2.70 (dd, J_H = 12.8, 6.4 Hz, 2H), 2.08 (m, 2H), 2.02 (m, 1H), 1.85 (m, 1H), 1.85 (m, 1H), 1.50 (m, 4H), 1.29-1.12 (m, 28H)

HR-ESI MS: calcd for $C_{56}H_{73}N_{12}F_6O_8S_1$ $[M+H]^+$ = 1187.5299; obsd 1187.5276.

Synthesis of probe 11.



Scheme S9. Synthetic scheme of probe **11**. Reaction conditions: (a) bromoacetylbromide, DIPEA in dry DCM, (b) diethanolamine in dry DMF, (c) 3,5-bis(trifluoromethyl)benzoylchloride, DMAP in dry DCM, (d) TFA in DCM, (e) **16**, DIPEA in dry DMF.

Compound **11-2**:

To a stirred solution of compound **11-1** (110 mg, 0.32 mmol) in dry DCM (6 mL) was added bromoacetylbromide (55 μL, 0.63 mmol) and DIPEA (110 μL, 0.53 mmol) at -10 ºC. The reaction mixture was stirred for 3h under Ar atmosphere. The solvent was quenched with saturated $NaHCO_3$ aq. (4mL) and washed with brine (5 mL, 3 times). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum to yield 120 mg (83 %) as brown oil.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 3.88 (s, 2H), 3.59-3.67 (m, 16H), 3.56 (t, J_H = 6.0 Hz, 2H), 3.52 (t, J_H = 6.0 Hz, 2H), 1.44 (s, 9H).

Compound **11-3**:

To a stirred solution of compound **11-2** (120 mg, 0.26 mmol) in dry DMF (4 mL) was added diethanolamine (110 mg, 1.1 mmol) at r.t.. The reaction mixture was stirred for 5h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (100 mL), and washed with brine (20 mL, once). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum to yield 53 mg (42 %) as brown oil.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 3.57-3.65 (m, 18H), 3.50 (t, J_H = 6.0 Hz, 2H), 3.42 (t, J_H = 6.0 Hz, 2H), 3.23 (t, J_H = 5.6 Hz, 2H), 2.70 (t, J_H = 5.6 Hz, 2H), 1.44 (s, 9H).

Compound **11-4**:

To a stirred solution of 3,5-bis(trifluoromethyl)benzoylchloride (92 mg, 0.33 mmol) in dry DCM (5mL) was added compound **11-3** (53 mg, 0.11 mmol) and DMAP (0.7 mg, 0.0056 mmol) at r.t.. The reaction mixture was stirred for 5h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (300 mL), and washed with saturated $NaHCO_3$ aq. (100 mL, twice), water (100 mL, 3 times) and brine (100 mL, once). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum to yield 80 mg (75 %) as pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.43 (s, 4H), 8.05 (s, 2H), 4.51 (t, J_H = 5.6 Hz, 4H), 3.50-3.59 (m, 16 H), 3.43 (m, 2H), 3.36 (s, 2H), 3.26 (m, 2H), 3.11 (t, J_H = 5.6 Hz, 4H),1.43 (s, 9H).

Probe **11**:

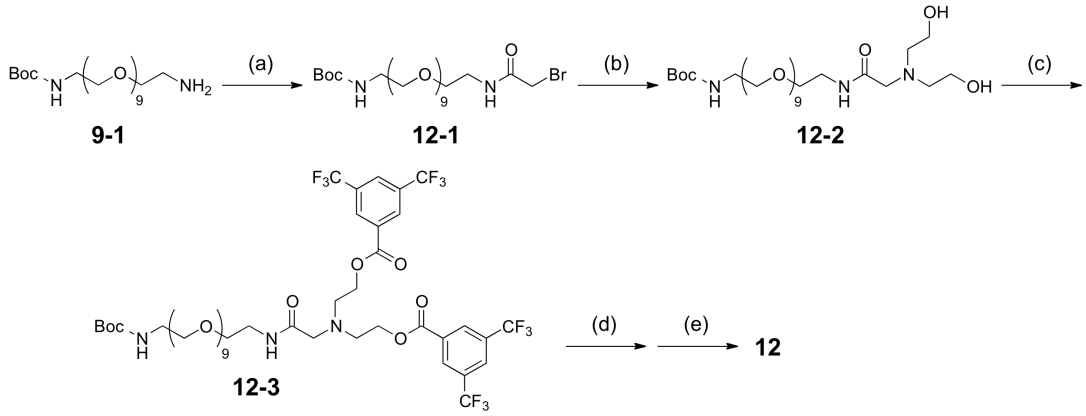
To a stirred solution of compound **11-4** (44 mg, 0.046 mmol) in DCM (4 mL) was added TFA (2 mL) at r.t.. The reaction mixture was stirred at r.t. for 2h. The solvent was removed under vacuum, the residue was suspended with toluene (5 mL) and the solvent was evaporated and dried under vacuum to yield deprotected compound **11-4** (50 mg, quantitative) as clear oil without further purification.

To a stirred solution of the deprotected compound **11-4** (50 mg) in dry DMF (4 mL) and DIPEA (33 μL, 0.25 mmol) was added compound **16** (18 mg, 0.059 mmol) at r.t.. The reaction mixture was stirred at r.t. for 5h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (100 mL), and washed with saturated $NaHCO_3$ aq. (50 mL, 3 times) and brine (50 mL, once).The organic solvent was dried over anhydrous $MgSO_4$, concentrated under vacuum and purified on column chromatography (SiO_2 / $CHCl_3$: MeOH = 20 : 1) to yield 13 mg (2 steps; 25 %) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.42 (s, 4H), 8.04 (m, 2H), 7.73 (m, 4H), 4.54 (t, J_H = 5.6 Hz, 4H), 3.40-3.73 (m, 16H), 3.35 (m, 2H), 3.29 (m, 2H), 3.15 (t, J_H = 5.6 Hz, 4H).

HR-FAB MS (NBA): calcd for $C_{41}H_{45}N_4F_{12}O_{12}S_1$ $[M+H]^+$ = 1044.2518; obsd 1044.2551.

Synthesis of probe 12.



Scheme S10. Synthetic scheme of probe **12**. Reaction conditions: (a) bromoacetylbromide, DIPEA in dry DCM, (b) diethanolamine in dry DMF, (c) 3,5-bis(trifluoromethyl)benzoylchloride, DMAP in dry DCM, (d) TFA in DCM, (e) **16**, DIPEA in dry DMF.

Compound **12-1**:

To a stirred solution of compound **9-1** (120 mg, 0.21 mmol) in dry DCM (8 mL) was added bromoacetylbromide (37 μL, 0.42 mmol) and DIPEA (70 μL, 0.53 mmol) at -10 ºC. The reaction mixture was stirred for 3h under Ar atmosphere. The solvent was quenched with saturated $NaHCO_3$ aq. (4mL) and washed with brine (5 mL, 3 times). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum to yield 120 mg (84 %) as brown oil. ^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 3.88 (s, 2H), 3.31-3.66 (m, 40H), 1.45 (s, 9H).

Compound **12-2**:

To a stirred solution of compound **12-1** (120 mg, 0.18 mmol) in dry DMF (4 mL) was added diethanolamine (75 mg, 0.71 mmol) at r.t.. The reaction mixture was stirred for 5h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (100 mL), and washed with brine (20 mL, once). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum to yield 120 mg (97 %) as brown oil.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 3.54-3.66 (m, 40H), 3.54 (s, 2H), 2.74 (t, J_H = 4.8 Hz, 4H), 1.44 (s, 9H).

Compound **12-3**:

To a stirred solution of 3,5-bis(trifluoromethyl)benzoylchloride (140 mg, 0.51 mmol) in dry DCM (5mL) was added compound **12-2** (120 mg, 0.17 mmol) and DMAP (1.1 mg, 0.0085 mmol) at r.t.. The reaction mixture was stirred for 5h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (300 mL), and washed with saturated $NaHCO_3$ aq. (100 mL, twice), water (100 mL, 3 times) and brine (100 mL, once). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum to yield 120 mg (57 %) as pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.43 (s, 4H), 8.05 (s, 2H), 4.51 (t, J_H = 6.0 Hz, 4H), 3.30-3.65 (m, 40 H), 3.11 (t, J_H = 6.0 Hz, 4H), 1.44 (s, 9H).

Probe **12**:

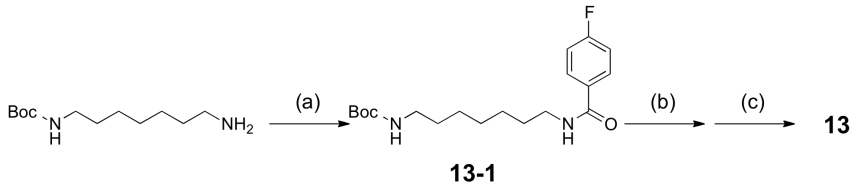
To a stirred solution of compound **12-3** (120 mg, 0.097 mmol) in DCM (4 mL) was added TFA (2 mL) at r.t.. The reaction mixture was stirred at r.t. for 2h. The solvent was removed under vacuum, the residue was suspended with toluene (5 mL) and the solvent was evaporated and dried under vacuum to yield deprotected compound **12-3** (130 mg, quantitative) as clear oil without further purification.

To a stirred solution of the deprotected compound **12-3** (130 mg) in dry DMF (5 mL) and DIPEA (48 μL, 0.36 mmol) was added compound **16** (15 mg, 0.057 mmol) at r.t.. The reaction mixture was stirred at r.t. for 5h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (100 mL), and washed with saturated $NaHCO_3$ aq. (50 mL, 3 times) and brine (50 mL, once).The organic solvent was dried over anhydrous $MgSO_4$, concentrated under vacuum and purified on column chromatography (SiO_2 / $CHCl_3$: MeOH = 15 : 1) to yield 47 mg (2 steps; 52 %) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 8.43 (s, 4H), 8.04 (m, 6H), 4.54 (t, J_H = 6.0 Hz, 4H), 3.14-3.68 (m, 42H), 3.14 (t, J_H = 6.0 Hz, 4H).

HR-FAB MS (NBA): calcd for $C_{51}H_{65}N_4F_{12}O_{17}S_1$ $[M+H]^+$ = 1265.3829; obsd 1265.3848

Synthesis of probe 13.



Scheme S11. Synthetic scheme of probe **13**. Reaction conditions: (a) 4-fluorobenzoylchloride, DIPEA in dry DCM, (b) TFA in DCM, (c) **16**, DIPEA in dry DMF

Compound **13-1**:

To a stirred solution of 4-fluorobenzoylchloride (50 mg, 0.31 mmol) in dry DCM (5 mL) was added *N*-(tert-butoxycarbonyl)-1,7-diaminoheptane (100 mg, 0.38 mmol) and DIPEA (160 μL, 1.2 mmol) at r.t.. The reaction mixture was stirred for 3h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (200 mL), and washed with saturated $NaHCO_3$ aq. (50 mL, twice), water (50 mL, 3 times) and brine (50 mL, once). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum to yield 100 mg (91 %) as white solid.
^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 7.77 (m, 2H), 7.11 (m, 1H), 3.45 (m, 2H), 3.11 (m, 2H), 1.26-1.63 (m, 10H).

Probe **13**:

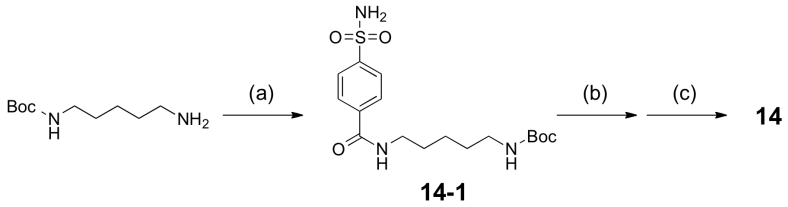
To a stirred solution of compound **13-1** (70 mg, 0.20 mmol) in DCM (3 mL) was added TFA (1 mL) at r.t.. The reaction mixture was stirred at r.t. for 2h. The solvent was removed under vacuum, the residue was suspended with toluene (2 mL) and the solvent was evaporated and dried under vacuum to yield deprotected compound **13-1** (76 mg, quantitative) as clear oil without further purification.

To a stirred solution of the deprotected compound **13-1** (76 mg) in dry DMF (5 mL) and DIPEA (85 μL, 0.64 mmol) was added compound **16** (50 mg, 0.17 mmol) at r.t.. The reaction mixture was stirred at r.t. for 5h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (200 mL), and washed with saturated $NaHCO_3$ aq. (50 mL, 3 times) and brine (50 mL, once).The organic solvent was dried over anhydrous $MgSO_4$, concentrated under vacuum and purified on column chromatography (SiO_2 / $CHCl_3$: MeOH = 5 : 1) to yield 24 mg (2 steps; 28 %) as white solid.

^{1}H NMR (400 MHz, CD_3OD) : δ/ppm, 7.82-7.98 (m, 6H), 7.18 (m, 2H), 3.44 (m, 2H), 3,39 (m, 2H), 1.24-1.45 (m, 10H).

HR-FAB MS (NBA): calcd for $C_{21}H_{27}N_3F_1O_4S_1$ $[M+H]^+$ = 436.1701; obsd 436.1692.

Synthesis of probe 14.



Scheme S12. Synthetic scheme of probe **14**. Reaction conditions: (a) **16** in dry DMF, (b) TFA in DCM, (c) 4-trifluoromethylbenzoylchloride, DIPEA, DMAP in dry DMF.

Compound **14-1**:

To a stirred solution of *N*-(tert-butoxycarbonyl)-1,5-diaminopentane (160 mg, 0.80 mmol) in dry DMF (4 mL) was added compound **16** (200 mg, 0.67 mmol) at r.t.. The reaction mixture was stirred for 3h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (100 mL), and washed with saturated $NaHCO_3$ aq. (50 mL, twice), water (50 mL, 3 times) and brine (50 mL, once). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum to yield 300 mg (97 %) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ/ppm, 7.97 (m, 4H), 3.40 (m, 2H), 3.05 (m, 2H), 1.65 (m, 2H), 1.53 (m, 2H), 1.31-1.49 (m,11 H).

Probe **14**:

To a stirred solution of compound **14-1** (60 mg, 0.16 mmol) in DCM (4 mL) was added TFA (2 mL) at r.t.. The reaction mixture was stirred at r.t. for 2h. The solvent was removed under vacuum, the residue was suspended with toluene (5 mL) and the solvent was evaporated and dried under vacuum to yield deprotected compound **14-1** (66 mg, quantitative) as clear oil without further purification.

To a stirred solution of the deprotected compound **14-1** (66 mg) in dry DMF (5 mL) and DIPEA (66 μL, 0.50 mmol) was added 4-trifluoromethylbenzoylchloride (27 mg, 0.13 mmol) and DMAP (0.8 mg, 0.0065 mmol) at r.t.. The reaction mixture was stirred at r.t. for 5h under Ar atmosphere. The solvent was removed under vacuum, the residue was dissolved in EtOAc (200 mL), and washed with saturated $NaHCO_3$ aq. (50 mL, 3 times) and brine (50 mL, once). The organic solvent was dried over anhydrous $MgSO_4$, concentrated under vacuum and purified on column chromatography (SiO_2 / $CHCl_3$: MeOH = 5 : 1) to yield 36 mg (2 steps; 49 %) as white solid.

^{1}H NMR (400 MHz, CD_3OD, TMS) : δ/ppm, 7.92-7.98 (m, 6H), 7.73 (m, 2H), 3.42 (m, 4H), 1.69 (m,

4H), 1.48 (m, 2H).

HR-FAB MS (NBA): calcd for $C_{20}H_{23}N_3F_3O_4S_1$ $[M+H]^+$ = 458.1317; obsd 458.1379.

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