Supporting Information

Ultrafast and Selective Labeling of Endogenous Proteins Using Affinity-based Benzotriazole Chemistry

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Part I: Biological Experiments

1. FKBP12 Labeling using Compound 1 or LDNASA

FKBP12 Protein Preparation

A pET19_His-tag_FKBP12 plasmid containing His tag was used in this study. FKBP12 was expressed in Escherichia coli strain BL21 (DE3). Cells were cultured at 37 °C in LB media supplied with 100 μg/mL ampicillin. When the OD600 reached 0.6, 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce protein expression at 20 °C on a shaker (170 rpm) overnight (10 h). Cells were collected in the centrifuge Avanti J20-XP (Beckman Coulter) at 6000 rpm, 4 °C for 20 min, and lysed by a microfluidizer (CONSTANT SYSTEMS. Ltd) in lysis buffer [50 mM NaH₂PO₄, 300 mM NaCl pH 8.0, 10% (vol/vol) glycerol, 2 mM βME, 1 mM PMSF, and 0.1% (vol/vol) Antifoam 204 (Sigma-Aldrich, cat. No.: A6426)]. The cleared lysate was purified by affinity chromatography on a 5 mL
HisTrap™ HP column (GE Healthcare) followed by size-exclusion chromatography on a HiLoad 26/60 Superdex 200 pg column (GE Healthcare) in buffer containing 50 mM HEPES, 50 mM NaCl, pH 7.3 using the NGC system (Bio-Rad).

The purified recombinant FKBP12 protein (5 μM) was incubated with compound 1 (10 μM) or LDNAS (38) (10 μM) in the absence or presence of SLF (28) (100 μM) in buffer (50 mM HEPES, 50 mM NaCl, pH 7.3) at 37 °C. Aliquots at different time points were taken and then quenched by dispensing into the same volume of 2× SDS-PAGE Sample Buffer (62.5 mM Tris–HCl, pH 6.8, 20% glycerol, 2% SDS, 0.002% bromophenol blue, 5% 2-mercaptoethanol) supplemented with 200 μM SLF. The resulting mixture was incubated at 70 °C for 5 min. The samples were subjected to SDS-PAGE and electro-transferred onto an nitrocellulose blotting membrane (Bio-Rad, cat. No.: 1704158) using Trans-Blot® Turbo™ transfer system (Bio-Rad) according to the manufacturer’s instructions. The protein transferred to the membrane was stained with Ponceau S [0.1 % (w/v) in 5% acetic acid, Sigma-Aldrich, cat. No.: P7170-1L], then washed with tris-buffered saline with Tween 20 (TBST). The membranes were blocked in 5% skim milk/TBST at room temperature for 1 hour. After washing with TBST (5 min × 3), the membranes were incubated with Streptavidin-HRP (Sigma-Aldrich, cat. No.: RABHRP3-600UL, 1:1,000) in 5% BSA/TBST at room temperature for 1 hour. After washing with TBST (5 min × 3), the signals were visualized using ECL substrate (Bio-Rad, cat. No.: 1705061) by ChemDoc™ MP Imaging System (Bio-Rad) according to the manufacturer’s instructions. The images were analyzed using Image Lab software (Bio-Rad) and quantified using ImageJ software.

The assay was carried out in three independent biological replicates. The representative images are shown in Figures 2a and S1.

2. Determination of Second-order Rate Constant

\[
A + B \rightarrow AB \\
[A] = [FKBP12], [A]₀ = 5 \mu M; [B]=[probe], [B]₀ = 10 \mu M; I = \text{band intensity which is proportional to the product: } [A]₀[A]. \text{Given the integrated second-order rate law equation:}
\]

\[
\ln \left[\frac{[B]}{[A]}\right] = k([B]₀ - [A]₀)t
\]

and the relationship of the band intensity with the concentration of product:

\[
I = I_{\text{max}}(1 - \frac{[A]}{[A]₀})
\]

It can be derived:

\[
I = I_{\text{max}}(1 - \frac{1 - [A]₀}{e([B]₀-[A]₀)kt - [A]₀[B]₀})
\]

where \(I_{\text{max}}\) = maximal intensity. The band intensity (I) at each time point is plotted against time (t) and fitted to the equation to give the second-order reaction rate constant using GraFit (Erithacus Software Limited).

3. First-order rate constant

The reaction of affinity labeling for covalent protein labeling is:

\[
P + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P\cdot R \overset{k_{\text{chem}}}{\rightarrow} P\cdot R
\]

\[
k_{\text{obs}} = \frac{k_{\text{chem}}}{1 + \frac{K_d}{[R]}}
\]

where \(k_{\text{obs}}\) is the observed reaction rate at a specific concentration of reagent [R]; \(K_d\) is the dissociation constant of protein-ligand binding, defined as \(k_{-1}/k_{1}\); \(k_{\text{chem}}\) is the rate constant for the irreversible
chemical modification. When $[R] >> K_d$, $k_{obs} \approx k_{chem}$, which is determined by the intrinsic reactivity of the reactive group (BTA). In the three examples shown in the paper, the affinities range from low nanomolar to submicromolar, which represent typical binding affinities of protein-ligand interactions. Therefore, under the conditions used, the observed ultrafast reaction rate indeed reflects the reactivity of the BTA chemistry driven by proximity effect.

4. Peptide Mapping of the Labeled FKBP12 by compound 1

4.1. Preparation of Labeled FKBP12 Sample

FKBP12 (10 μM) was incubated with 1 (20 μM) in buffer (50 mM HEPES, 50 mM NaCl, pH 7.3) at 37 °C for 20 min. Then the reaction mixture was purified using PD Spintrap™ G-25 column (GE, cat. No.: 28-9180-04) according to the manufacturer’s instructions.

4.2. Digestion

10 μg (72.4 μL) of the sample was digested without reduction/alkylation with trypsin (1:20 ratio) at 37°C overnight. The digestion reaction was stopped by addition of 5 μL of 20% trifluoroacetic acid (TFA). Finally, the digested peptides were diluted to 150 μL and thereafter desalted using a SPE Pierce C18 Spin Column (Thermo Scientific). These columns were activated by 2 × 200 μL of 50% acetonitrile (ACN) and equilibrated with 2 × 200 μL of 0.5% TFA. The digested peptides were adsorbed to the column using two repeated cycles of 40 μL sample loading and the column was washed using 3 × 200 μL of 0.5% TFA. Finally, the peptides were eluted with 3 × 50 μL of 70% ACN. The solvent was evaporated. Peptides were resolved in 50 μL of 0.1% formic acid and further diluted 4 times prior to nano-LC-MS/MS.

4.3. LC-MS/MS

The nanoLC-MS/MS experiments were performed using a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a nano-electrospray ion source. The peptides were separated by C18 reversed phase liquid chromatography using an EASY-nLC 1000 system (Thermo Fisher Scientific). A set-up of pre-column and analytical column was used. The pre-column was a 2 cm EASYcolumn (ID 100 μm, 5 μm particles, Thermo Fisher Scientific) while the analytical column was a 15 cm EASY-column (ID 75 μm, 3 μm particles, Thermo Fisher Scientific). Peptides were eluted with a 35 min optimized gradient: 0-2 min 2% B (B – buffer B, ACN with 0.1% Formic acid), 2-17 min – 2-50% B, 17-22 min – 50-80% B, 22-26 min – 80% B, 26-27 min – 80-100% B, 27-35 min – 100% B at a rate of 300 nL/min. The mass spectrometer was operated in positive ion mode acquiring a survey mass spectrum with resolving power 70,000 (full width half maximum), m/z 400-1750 using an automatic gain control (AGC) target of 3×10^6. The 10 most intense ions were selected for higher-energy collisional dissociation (HCD) fragmentation (30% normalized collision energy) and MS/MS spectra were generated with an AGC target of 5×10^5 at a resolution of 17,500. The mass spectrometer worked in data-dependent mode.

4.4. Data Analysis

The acquired data was processed by pFind Software† (version 3.1.5) using a database containing targeted protein sequence FKBP12. The following parameters were used for data processing: maximum 20 ppm error tolerances for both the survey scan and MS/MS analysis. The search parameters were set to Taxonomy: targeted protein, Enzyme: Trypsin, open search mode, maximum of three miss cleavages sites. An FDR of maximum 1% for peptide identification was accepted.

5. FKBP12 Labeling in HeLa Cell Lysate using Compound 1

A mixture of purified recombinant FKBP12 (final 2 μM), HeLa cell lysate (0.5 mg/mL), and compound 1 (final 2 μM) in the absence or presence of SLF (20 μM) in buffer (50 mM HEPES, 50 mM NaCl, pH 7.3) was incubated at 37 °C. Aliquots at different time points were taken and then quenched by
dispensing into a same volum of 2× SDS-PAGE Sample Buffer (62.5 mM Tris–HCl, pH 6.8, 20% glycerol, 2% SDS, 0.002% bromophenol blue, 5% 2-mercaptoethanol) supplemented with 20 µM SLF. The resulting mixture was incubated at 70 °C for 3 min. The samples were subjected to SDS-PAGE and western blot as shown above.

For detection of FKBP12: the membrane was incubated with the primary antibody Rabbit anti-FKBP12 antibody (Abcam, cat. No.: ab2918, 1:1,000) in 5% BSA/TBST at 4 °C overnight. After washing with TBST (5 min × 3), the membrane was incubated with the secondary antibody Goat Anti-Rabbit IgG, H & L Chain Specific Peroxidase Conjugate (Merck, Calbiochem, cat. No.: 401315-2ML, 1:10,000) in 5% skim milk/TBST at room temperature for 1 hour. After washing with TBST (5 min × 3), the signals were visualized using ECL substrate (Bio-Rad, cat. No.: 1705061) on ChemDoc™ MP Imaging System (Bio-Rad) according to the manufacturer’s instructions.

The assay was carried out in three independent biological replicates. The representative images are shown in Figure 3a.

6. GRAMD1A_StART Domain Labeling using Compound 2

6.1. Protein Preparation

A pMAL_MBP-GRAMD1A 359-547 plasmid containing pMAL vector and MBP tag was used in this study. The StART domain (GRAMD1A 359-547) was expressed in Escherichia coli strain BL21 (DE3). Cells were cultured at 37 °C in LB media supplied with 100 µg/mL ampicillin. When the OD600 was up to 0.6, 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce protein expression at 20 °C on a shaker (170 rpm) overnight (≈ 12 h). Cells were collected in the centrifuge Avanti J20-XP (Beckman Coulter) at 6000 rpm, 4 °C for 20 min, and lysed by pressure treatment (CONSTANT SYSTEMS, Ltd) in lysis buffer [50 mM Tris, pH 7.5, 300 mM NaCl, 10% (vol/ vol) glycerol, 2 mM βME, 1 mM PMSF, 0.1% (vol/vol) Triton X-100, and 0.01%-0.1% (vol/vol) Antifoam 204 (Sigma-Aldrich, cat. No.: A6426)]. The cleared lysate was purified by affinity chromatography on a 5 mL HisTrap™ HP column (GE Healthcare) using NGC system (Bio-Rad). The MBP tag was cleaved by 0.1 equivalent of His-MBP-TEV Protease and dialysis against 100 volume of buffer [(50 mM Tris pH 7.5, 300 mM NaCl, 10% (vol/ vol) glycerol)] overnight at rt. Proteins were further purified by affinity chromatography on a 5 mL MBPTrap™ HP column 5 mL (GE Healthcare) in buffer containing 50 mM Tris pH 7.5, 300 mM NaCl, 10% (vol/ vol) glycerol, followed by size-exclusion chromatography on a HiLoad 26/60 Superdex 200 pg column (GE Healthcare) in buffer containing 25 mM Tris pH 7.5, 150 mM NaCl.

6.2. Compounds

![Chemical structure of Compound 2 and DBCO-TAMRA](image)

6.3. Labeling Assay
GRAMD1A_StART (5 μM) was incubated with compound 2 (10 μM) in the assay buffer (50 mM HEPES, 50 mM NaCl, pH 7.3) at 37 °C. Aliquots at different time points were taken and then quenched by 1% SDS. DBCO-TMR (5 eq, Jena Bioscience, cat. Nr. CLK-A131) was added at room temperature for 12 h for copper-free click reaction. Then 5 × SDS-PAGE Sample Buffer was added, and the samples were denatured. The reactions were analyzed by SDS–PAGE and imaged by in-gel fluorescence scanning and subsequent Coomassie blue staining using the ChemDoc™ MP Imaging System (Bio-Rad) according to the manufacturer’s instructions. The images were analyzed using Image Lab software (Bio-Rad) and quantified using ImageJ software.

The assay was carried out in three independent biological replicates. The representative images are shown in Figure 3c.

7. Peptide Mapping of the Labeled GRAMD1A StART Domain by Compound 2

7.1. Preparation of Labeled GRAMD1A StART Domain Sample

Purified recombinant protein GRAMD1A StART domain (10 μM) was incubated with the labeling compound 2 (20 μM) in buffer (50 mM HEPES, 50 mM NaCl, pH 7.3) at 37 °C for 15 min or with just buffer for negative controls. Then the reaction mixture was purified using PD Spintrap™ G-25 column (GE, cat. No.: 28-9180-04) according to the manufacturer’s instructions. Each sample was repeated for a total of triplicates for compound-2 labelled and controls.

7.2. Applied Sample Processing and Methods

10 μg of protein per sample were digested without reduction/alkylation with sequencing grade trypsin (Promega) at 1:20 substrate:enzyme ratio and 37°C, overnight. The digestion reaction was stopped by sample acidification down to pH <3. The digested peptides were thereafter desalted using a Sep-Pak C-18 desalting columns or filters (Waters). Eluted peptides were dried by sped-vacuum concentration and dissolved in 20 μL of 0.1% formic acid 2% acetonitrile for subsequent LC-MS/MS analysis.

7.3. LC-MS/MS

NanoLC-MS/MS of labelled and unlabelled control protein digests were performed using a system made by a nano-Ultimate 3000 LC coupled to an EASY Spray source as ElectroSpray Ionization (ESI) source and a Q Exactive HF mass spectrometer (Thermo Scientific). Each sample replicate was injected twice, first with the equivalent of 0.5 μg and then with 3 μg according to estimated protein amount pre-digestion. After being injected, samples were preconcentrated and further desalted in-line using a PepMap C18 nano trap column. Peptide separation was performed using an EASY-Spray C18 reversed-phase nano LC column (Acclaim PepMap RSLC; length, 15 cm; inner diameter, 2 μm; particle size, 2 μm; pore size, 100 Å; Thermo Scientific) at 55 °C and a flow rate of 300 nL/min. Peptides were separated using a binary solvent system consisting of 0.1% (v/v) formic acid (FA), 2% (v/v) acetonitrile (ACN) (solvent A) and 98% ACN (v/v), 0.1% (v/v) FA (solvent B) and eluted with a gradient of 3–26% B in 40 min, 26–95% B in 10 min. Subsequently, the analytical column was washed with 95% B for 5 min before re-equilibration with 3% B for the last 5 minutes. Mass spectra were acquired in a mass-to-charge (m/z) range of 375–1500 with a resolution of 60,000 at m/z 200. Automatic gain control target was set to 1 × 10^6 with a maximum injection time of 100 ms. The 17 most abundant peptide ions were selected for higher-energy collision dissociation (HCD) with normalized collision energy value set at 28%. The ion abundance threshold was set at 0.1% with charge exclusion of z = 1–6 ions. The MS/MS spectra were acquired at a resolution of 17,500, with a target value of 4 × 10^5 ions and a maximum injection time of 120 ms. The fixed first m/z was set to 100, and the isolation window to 2 m/z. The instrument was operated in the positive ion mode for data-dependent acquisition of MS/MS spectra with a dynamic exclusion time of previously selected precursor ions of 45 s. To avoid carry over blanks were run in between each sample.
7.4. Data Analysis

The acquired data was processed by PEAKS Software (Bioinformatics Solutions Inc.) using a reduced human proteome database including the protein sequence of the GRAMD1A StART domain. The search parameters, for both labeled and unlabeled sample included: 10 ppm of mass error tolerance for parent ions; 0.05 Da of fragment mass error tolerance; trypsin and partial trypsin enzyme specificity with a maximum of 3 missed cleavages; N and Q deamidation, M oxidation and the labelling modification of 201.07 Da with no specified amino acid specificity as variable modifications. An FDR of maximum 1% for peptide identification was accepted.

The results are shown in Figure S6.

8. CA12 Labeling in Cells using Compound 3

8.1. Cell Culture

MCF7 (ATCC® HTB-22™) cells were cultured in Dulbecco’s modified Eagle medium (DMEM) (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and non-essential amino acids at 37°C with 5% CO2. Cells were routinely tested for mycoplasma contamination using the LookOut mycoplasma PCR detection kit (Sigma-Aldrich). Cell lines were authenticated by ATCC with no further authentication carried out after purchase.

8.2. Compounds

![Compound 3](image)

8.3. Cell Labeling

MCF7 cells were washed 3 times with PBS and incubated with 1 µM 3 (or 1 µM 3 + 100 µM EZA) in serum free DMEM for the indicated length of time. Cells were then washed 3 times with PBS prior to fluorescent microscopy or western blot analysis.

8.4. Fluorescent Microscopy

For live-cell imaging, MCF7 cells were seeded on µ-Slide 8-well glass-bottom chamber slides (Ibidi) and labeled as described above for 30 min. Imaging was performed on a Leica SP8 FALCON inverted confocal system (Leica Microsystems) equipped with a HC PL APO 63x/1.40 oil immersion lens and a temperature-controlled hood maintained at 37°C and 5% CO2. Hoechst 33342 and fluorescein were excited using a 405 nm Diode laser and a tuned white light laser, respectively. Scanning was performed in line-by-line sequential mode. The representative images are shown in Figure 4a.

8.5. Immunoblotting

Cells were washed 3 times with PBS, lysed in ice-cold lysis buffer (20 mM Tris-HCl pH 8, 300 mM KCl, 10% Glycerol, 0.25% Nonidet P-40, 0.5 mM EGTA, 1 mM PMSF, 1× complete protease inhibitor (Roche)), passed 6× through a 21G needle, and cleared by centrifugation (20 min/12,700 rpm/4°C, Eppendorf centrifuge 5427R). Protein concentrations were determined via Bradford assay (Bio-Rad...
Protein Reagent) and lysates normalized. Lysates were then mixed with 4x sample buffer and boiled for 10 min prior to separation by SDS-PAGE and transferred to a nitrocellulose membrane (Bio-Rad) using a Trans-Blot Turbo transfer system (Bio-Rad). After a 1 hour block in TBST with 5% skim milk (at room temperature), membranes were incubated with primary antibody overnight at 4°C [antibodies: anti-CA12 ((Cell Signaling Technology, cat. No.: 5864, 1:1000), anti-fluorescein (Sigma-Aldrich, cat. No.: SAB4600050, 1:1000) and anti beta-actin (Sigma-Aldrich, cat. No.: A2228, 1:10,000)]. Membranes were then washed with TBST and incubated for 1 hour at room temperature with the appropriate HRP-conjugated secondary antibody in blocking buffer. Protein detection was carried out using chemiluminescence (Bio-Rad) and imaged using a ChemiDoc imaging system (Bio-Rad). The representative blots are shown in Figure 4c.

8.6. Immunoprecipitation

MCF7 cells were labeled with 3 for 30 minutes and lysates harvested, as described above. FITC antibody (Thermo Fisher; 71-1900) was pre-bound to protein A Dynabeads (Thermo Fisher; 10001D) and incubated with lysates for 30 minutes at room temperature. Beads were washed 3 times with PBS-T and the target antigen eluted by re-suspending in Laemmli sample buffer and heating at 70°C for 10 minutes. The representative blots are shown in Figure 4d.

9. Supplementary Figures

![Figure S1. In vitro labeling of FKBP12 protein by LDNASA. a) Structure of LDNASA. b) SDS-PAGE and western blotting analysis of labeling reaction of 5 µM FKBP12 with 10 µM LDNASA.](image-url)
Figure S2. Hydrolysis kinetics of compound **1**. a) Compound **1** (20 µM) was incubated in labeling buffer (50 mM HEPES, 50 mM NaCl, pH 7.3) at 37 ℃. b) Compound **1** (200 µM) was incubated in DMSO at 37 ℃. Unhydrolyzed reagents at different time points were quantified by RP-HPLC and fitted by a single exponential function to obtain half-life.
Figure S3. Mass spectra of FKBP12 before and after labeling with 1. a) ESI-MS of FKBP12. b) MALDI-TOF analysis of the labeling of 10 μM FKBP12 with 20 μM 1 at 37 °C for 20 min. Expected increase of mass with mono-labeling: 314; observed: 318.
a) GHHHHHAENLYPOGRM GVQETISPQ DGRTFPKRGQ TCVVHYTGL
   EDGKFDSSR DRNKFKKEML G5OEVIRGWE EGVQMSVGQ RAKLTISPDY
   Peptide 2

b) AYGATGHPGI IPPHATIVFD VELLKLE
Figure S4. LC-MS/MS analysis of the labeling site of FKBP12 after reaction with 1. a) The primary sequence of the FKBP12 protein used in this study. Peptide 1 contains the major labeled Lys52 (colored in red) and Peptide 2 contains another (minor) labeled Lys35 (colored in blue) after tryptic digestion. b) The crystal structure of FKBP12-SLF complex (PDB ID: 1FKG). c) Ion chromatogram of the total tryptic digestion products. d,e) Mass spectrum of Peptide 1 (d) and Peptide 2 (e). f,g) MS/MS spectra of the labeled Peptide 1 (f) and Peptide 2 (g). The labeled K52 was colored in red and K35 in blue.

Figure S5. ESI-MS analysis of the labeling of 5 μM GRAMD1A (StART domain) with 10 μM 2. The deconvoluted mass signals of GRAMD1A (StART domain) are 21922.0 and 22176.4. After labeling by 2 at 37 °C for 8 min, the observed mass are 22122.9 and 22377.5.
a) 
1 GHMLAALLPD LSGRLINSV FHVGAERLQQ MLFSDSPLQ GFLQQCKFTD
51 VTLPWSGDS KCHQRRVLY TIPISPNLGP KSASVVEQT LFRRGPQAGG
101 CVVDSEVLTO GIPYQDFYFT AHRYCIIGLA RNKARLRVSS EIRYRKQPSWS
151 LVKSLIE185KNS WSGIEDYFHHLERELAKAEK LSLLEGGKDA RG

b) 

Ligand in this complex.

(c) 
5.0E9 TIC (m/z 350-1500)

(d) 
3.2E8 RIC (m/z 666.32)

(e) 

Relative Abundance

100
50
0

200 400 600 800 1000 1200 1400 m/z
**Figure S6.** LC-MS/MS analysis of the labeling site of GRAMD1A StART domain after reaction with compound 2. a) The primary sequence of the recombinant GRAMD1A_StART domain protein used in this study. The underling arrow shows the trypsin-digested peptide fragment containing modified Lys158. b) The homology model of the GRAMD1A StART domain with docked autogramin-2 based on the Hydrogen–Deuterium Exchange Mass Spectrometry (HDX-MS) results (ref. 20). c) LC-MS chromatogram of all peptides contained in the recombinant GRAMD1A_StART domain protein digest, showing the total ion current (TIC) and total ion intensity. d,e) Reconstructed ion chromatogram (RIC) with relative intensity (d) and MS/MS spectrum with fragmentation pattern (e) of the identified peptide fragment labeled on Lys158.
Part II: Synthesis of Compounds

10. General Information

All commercially available compounds were used as provided without further purifications unless otherwise noted. The solvents were treated to anhydrous prior to use according to the standard methods or purchased from commercial sources in anhydrous grade. Flash column chromatography was performed using silica gel (VWR, particle size 40 - 63 µm).

$^1$H, $^13$C were recorded on a Bruker 400 MHz Avance III, Bruker 500 MHz Avance III, or Bruker 600 MHz Avance III HD using CDCl$_3$, CD$_3$OD, CD$_2$Cl$_2$ or (CD$_3$)$_2$SO as solvent at room temperature. $^1$H and $^13$C-NMR spectra were calibrated to the solvent signals of CDCl$_3$ (7.26 and 77.16 ppm), CD$_3$OD (3.31 and 49.00 ppm), CD$_2$Cl$_2$ (5.32 and 53.84 ppm) or (CD$_3$)$_2$SO (2.50 and 39.52 ppm). The abbreviations $s$, $d$, $t$, $q$ and $m$ stand for singlet, doublet, triplet, quartet and multiplet in that order.

9. Compound Synthesis and Characterization

Scheme S1. Synthetic scheme of building blocks 9, 13, 16, and 19

Synthesis of compound 6 following published procedures.$^{3,4}$ $N,N$-Disopropylethylamine (DIPEA) (12.03 mL, 69.5 mmol, 2.5 equiv.) was added dropwise to a solution of methyl (2S)-piperidinecarboxylate hydrochloride 4 (CAS No.: 16850-39-0) (4.992 g, 27.8 mmol, 1 equiv.) in dry CH$_2$Cl$_2$ (10 mL) at 0 °C (ice-water bath). Then methyl chlorooxoacetate 5 (CAS No.: 5781-53-3) (3.20 mL, 34.8 mmol, 1.25 equiv) was added dropwise using a syringe. After the addition, the resulting mixture was stirred at room temperature for 2 hours. The mixture was diluted with CH$_2$Cl$_2$ (70 mL), washed with water (80 mL x 2), and followed by brine (80 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (eluted with petroleum ether : EtOAc = 2:1) to afford compound 6 (6.033 g, 26.3 mmol, 95% yield) as a colorless oil. This product exists as an approximate 2.5:1 mixture of the trans and cis conformations. The analytical data matched literature reports.$^{3,4}$

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.22 (d, $J = 5.7$ Hz, 0.75H), 4.52 (d, $J = 5.2$ Hz, 0.25H), 4.47 – 4.40 (m, 0.26H), 3.87 (s, 2.21H), 3.83 (s, 0.80H), 3.76 (s, 0.85H), 3.75 (s, 2.15H), 3.62 – 3.50 (m, 0.83H), 3.32 (td, $J = 13.1$, 3.2 Hz, 0.82H), 2.89 (td, $J = 13.3$, 3.4 Hz, 0.28H), 2.30 – 2.24 (m, 1.14H), 1.78 – 1.66 (m,
3.43H), 1.57 – 1.46 (m, 1.01H), 1.43 – 1.32 (m, 1.22H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 170.7, 170.6, 163.1, 162.9, 161.5, 161.0, 56.8, 52.9, 52.8, 52.7, 52.6, 51.7, 44.4, 39.7, 27.4, 26.5, 25.1, 24.2, 21.1, 20.9.

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{OMe} \\
\text{6} \\
+ \\
\text{ClMg} \\
\text{7} \\
\text{THF} \\
-78 \degree C, 3 h \\
\text{N} \\
\text{O} \\
\text{OMe} \\
\text{8} \\
\end{array}
\]

Synthesis of compound 8 following published procedures\(^3\): To a solution of 6 (6.028 g, 26.3 mmol, 1 equiv.) in THF (26.3 mL) at -78 \degree C was added dropwise 1,1-dimethylpropylmagnesium chloride 7 (1.0 M in diethyl ether, 34.2 mL, 34.2 mmol, 1.3 equiv.) under argon atmosphere. The resulting mixture was stirred at -78 \degree C for 3 hours, and then was poured into saturated aqueous NH\(_4\)Cl (50 mL). The aqueous layer was extracted with EtOAc (50 mL \(\times\) 3). The combined organic layers were washed with water (50 mL \(\times\) 2), and followed by brine (50 mL). The organic layer was dried over anhydrous Na\(_2\)SO\(_4\), filtered, and concentrated. The residue was purified by silica gel column chromatography (eluted with petroleum ether : EtOAc = 10:1) to afford compound 8 (6.326 g, 23.5 mmol, 89\% yield) as a colorless oil. This product exists as an approximate 5:1 mixture of the trans and cis conformations. The analytical data matched literature reports\(^3\).

\[^1\]H NMR (400 MHz, CDCl\(_3\)) \(\delta 5.26\) (d, \(J = 5.7\) Hz, 1.00H), 4.48 – 4.43 (m, 0.20H), 4.23 (d, \(J = 5.2\) Hz, 0.21H), 3.76 (s, 0.61H), 3.75 (s, 3.00H), 3.41 – 3.37 (m, 1.03H), 3.21 (td, \(J = 12.9, 3.2\) Hz, 1.02H), 2.91 (td, \(J = 13.1, 3.5\) Hz, 0.21H), 2.33 – 2.27 (m, 1.03H), 2.24 – 2.19 (m, 0.21H), 1.79 – 1.62 (m, 6.38H), 1.56 – 1.34 (m, 2.79H), 1.23 (s, 3.08H), 1.19 (s, 3.08H), 1.18 (s, 0.63H), 1.16 (s, 0.60H), 0.88 (t, \(J = 7.5\) Hz, 3.12H), 0.83 (d, \(J = 7.5\) Hz, 0.63H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 208.0, 207.8, 171.0, 170.9, 167.7, 166.6, 56.6, 52.7, 52.5, 51.3, 46.9, 44.1, 39.0, 32.6, 27.7, 26.5, 25.0, 24.6, 23.8, 23.6, 23.2, 23.1, 21.3, 9.0, 8.9.

\[
\begin{array}{c}
\text{N} \\
\text{O} \\
\text{OMe} \\
\text{8} \\
\text{H}_2\text{O/MeOH} \\
\text{rt. overnight} \\
\text{N} \\
\text{O} \\
\text{OH} \\
\text{9} \\
\end{array}
\]

Synthesis of compound 9 following published procedures\(^3\): To a solution of compound 8 (5.925 g, 22.0 mmol, 1 equiv.) in MeOH (88 mL) at 0 \degree C was added 33 mL of 1 N LiOH aqueous dropwise over a period of 15 min. The resulting mixture was stirred at 0 \degree C for 1 hour, and then warmed to room temperature and stirred at room temperature overnight. The mixture was acidified with 1 N HCl and extracted with CH\(_2\)Cl\(_2\) (100 mL \(\times\) 3). The combined organic layers were washed with water (50 mL), and followed by brine (50 mL), dried over anhydrous Na\(_2\)SO\(_4\), filtered. The solvent was removed under reduced pressure to afford compound 9 (4.961 g, 19.4 mmol, 88\% yield) as a white solid. This product exists as an approximate 5:1 mixture of the trans and cis conformations. The analytical data matched literature reports\(^3\).

\[^1\]H NMR (400 MHz, CDCl\(_3\)) \(\delta 11.00\) (broad, s, 1.20H), 5.32 (d, \(J = 5.5\) Hz, 1.00H), 4.48 (d, \(J = 13.6\) Hz, 0.19H), 4.27 (d, \(J = 5.5\) Hz, 0.19H), 3.43 – 3.38 (m, 1.14H), 3.24 (td, \(J = 12.8, 3.1\) Hz, 1.01H), 2.93 (td, \(J = 13.2, 3.3\) Hz, 0.19H), 2.34 (d, \(J = 13.8\) Hz, 1.02H), 2.26 (d, \(J = 14.1\) Hz, 0.19H), 1.83 – 1.61 (m, 6.35H), 1.58 – 1.38 (m, 2.62H), 1.23 (s, 3.21H), 1.19 (s, 4.07H), 0.88 (t, \(J = 7.5\) Hz, 3.58H).

\[
\begin{array}{c}
\text{HO} \\
\text{NH}_2 \\
\text{10} \\
\text{Boc}_2\text{O, DIPEA} \\
\text{DMF, rt, 24 h} \\
\text{HO} \\
\text{NH}_2 \\
\text{Boc} \\
\text{11} \\
\end{array}
\]

Synthesis of compound 11 following published procedures\(^5\): To a solution of di-tert-butyl dicarbonate (CAS No.: 24424-99-5) (3.275 g, 15.0 mmol, 3.0 equiv.) in DMF (5 mL) was added 3,4-diaminobenzoic acid 10 (CAS No.: 619-05-6) (761.0 mg, 5.0 mmol, 1.0 equiv.) followed by disopropylethylamine (DIPEA) (1.039 mL, 6.0 mmol, 1.2 equiv.). The reaction mixture was stirred at room temperature under nitrogen for 24 hours and then poured into 50 mL of
water. The solution pH was adjusted to 6 using 1 M HCl and extracted with EtOAc (20 mL × 4). The combined organic layers were washed with water (20 mL × 4) and brine (20 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was dried under vacuum to give 11 (1.390 g, 3.94 mmol, 79% yield) as a brown solid. The analytical data matched literature reports.5

1H NMR (400 MHz, (CD₃)₂SO) δ 12.78 (br s, 1H), 8.75 (s, 1H), 8.70 (s, 1H), 8.09 (s, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.64 (d, J = 8.3 Hz, 1H), 1.48 (s, 1H); 13C NMR (100 MHz, (CD₃)₂SO) δ 166.8, 153.2, 152.7, 134.4, 128.9, 125.6, 125.3, 125.1, 122.1, 80.0, 79.7, 28.1, 28.0.

Synthesis of compound 13 following published procedures6:
To a solution of 1,4-diaminopentane 12 (CAS No.: 462-94-2) (511.0 mg, 5.0 mmol, 5.0 equiv) in CH₂Cl₂ (15 mL), was added dropwise a solution of compound 11 (352.4 mg, 1.0 mmol, 1.0 equiv), PyBOP® (CAS No.: 128625-52-5) (572.4 mg, 1.1 mmol, 1.1 equiv), and DIPEA (225 µL, 1.3 mmol, 1.3 equiv) in CH₂Cl₂ (10 mL) at room temperature. The resulting mixture was stirred at 40 °C for 1 hour. The solvent was removed under reduced pressure, the residue was dissolved in EtOAc (100 mL), washed with saturated aqueous solution of NaHCO₃ (50 mL × 2) and brine (50 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified by silica gel column chromatography (eluted with CH₂Cl₂: MeOH = 5:1) to afford compound 13 (371.7 mg, 0.8514 mmol, 85% yield) as a white solid. 1H NMR (400 MHz, CDCl₃) δ 7.70 (s, 1H), 7.48 (d, J = 8.3 Hz, 1H), 7.36 (d, J = 8.3 Hz, 1H), 7.01 (s, 1H), 3.33 – 3.28 (m, 3H), 3.19 – 3.15 (m, 1H), 2.69 (t, J = 6.9 Hz, 2H), 1.90 – 1.87 (m, 1H), 1.50 (s, 9H), 1.48 (s, 9H), 1.34 – 1.29 (m, 3H); 13C NMR (100 MHz, CDCl₃) δ 167.2, 154.5, 153.8, 134.2, 130.5, 129.3, 124.3, 123.9, 123.0, 81.3, 81.2, 41.1, 39.9, 31.0, 29.1, 28.39, 28.36, 24.0; HRMS (ESI): calcd for C₂₂H₃₇N₄O₅ [M + H]+ 437.2758, found: 437.3084.

Synthesis of compound 16 following published procedures7:
To a solution of 2-(tert-butoxycarbonylamino)-1-ethanol 14 (CAS No.: 26690-80-2) (1.612 g, 10 mmol) in dry acetonitrile (50 mL), N,N′-disuccimidyl carbonate 15 (CAS No.: 74124-79-1) (5.252 g, 20.5 mmol, 2.05 equiv.) and NEt₃ (2.85 mL, 20.5 mmol, 2.05 equiv.) were added and the resulting mixture was stirred at 40 °C for 1 hour. The solvent was removed under reduced pressure, the residue was dissolved in EtOAc (100 mL), washed with saturated aqueous solution of NaHCO₃ (50 mL × 2) and brine (50 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified by silica gel column chromatography (eluted with CH₂Cl₂: MeOH = 30:1) to afford compound 16 (1.786 g, 5.91 mmol, 59% yield) as a colorless oil. The analytical data matched literature reports.7

1H NMR (400 MHz, CDCl₃) δ 4.98 (br s, 1H), 4.35 (t, J = 5.2 Hz, 2H), 3.46 (q, J = 5.9 Hz, 2H), 2.82 (s, 4H), 1.43 (s, 9H); 13C NMR (100 MHz, CDCl₃) δ 168.7, 155.8, 151.5, 80.0, 70.6, 39.3, 28.4, 25.6.

Synthesis of compound 19 following published procedures8:
To a solution of biotin 17 (CAS No.: 58-85-5) (488.6 mg, 2.0 mmol) and N-hydroxysuccinimide 18 (CAS No.: 6066-82-6) (230.2 mg, 2.0 mmol, 1.0 equiv) in DMF (15 mL), N,N′-dicyclohexylcarbodiimide (DCC, CAS No.: 538-75-0) (495.1 mg, 2.4 mmol, 1.2 equiv.) was added. The mixture was stirred overnight at room temperature during which time a white precipitate was formed. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue solid was washed with petroleum ether (30 mL × 3) and dried under vacuum to give compound 19 (670.2 mg, 1.963 mmol, 98% yield) as a white solid. The analytical data matched literature reports.8

1H NMR (400 MHz, (CD₃)₂SO) δ 6.42 (s, 1H), 6.36 (s, 1H), 4.32 – 4.29 (m, 1H), 4.15 (t, J = 5.9 Hz, 1H), 3.13 – 3.08 (m, 1H), 2.86 – 2.81 (m, 5H), 2.67 (t, J = 7.3 Hz, 2H), 2.58 (d, J = 12.4 Hz, 1H), 1.73
- 1.60 (m, 4H), 1.55 – 1.44 (m, 2H); $^{13}$C NMR (100 MHz, (CD$_3$)$_2$SO) δ 170.3, 168.9, 162.7, 61.0, 59.2, 55.2, 39.8, 30.0, 27.8, 27.6, 25.5, 24.3.

Scheme S2. Synthetic scheme of compound 1

Synthesis of compound 22 following published procedures$^{3,4}$.
To a solution of compounds 20 (16.620 g, 0.1 mol) and 21 (13.620 g, 0.1 mol, equiv.) in EtOH (200 mL) at 0 °C, was added an ice-water cooled 2 N KOH aqueous (200 mL) dropwise. The mixture was stirred at 0 °C for 30 min, and then at room temperature overnight. The mixture was diluted with water (200 mL), and cooled down to 0 °C, then acidified to pH 6 using concentrated HCl. A yellow solid precipitated. Two workup procedures for aqueous layer and the solid: 1) The aqueous layer was extracted with EtOAc ((150 mL × 3), dried over anhydrous Na$_2$SO$_4$, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (CH$_2$Cl$_2$ : MeOH = 10 : 1) to give 4.766 g of 22 as yellow solid. 2) The solid was dissolved in EtOAc (200 mL), washed with water (150 mL × 2), brine (150 mL), dried over anhydrous Na$_2$SO$_4$, filtered and concentrated. The residue was
washed with CH₂Cl₂ (50 mL) and petroleum ether (50 mL × 2) to give 17.34 g of 22 as yellow solid. Total 22: 22.106 g (77.8 mmol, 78% yield). The analytical data matched literature reports. ¹,²

¹H NMR (400 MHz, CDCl₃) δ 7.77 (d, J = 15.6 Hz, 1H), 7.69–7.65 (m, 1H), 7.56 (d, J = 7.7 Hz, 1H), 7.40–7.33 (m, 2H), 7.21 (dd, J = 8.3, 1.8 Hz, 1H), 7.12 (dd, J = 7.8, 2.1 Hz, 2H), 6.88 (d, J = 8.3 Hz, 1H), 3.93 (s, 3H), 3.92 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 191.1, 156.7, 151.7, 149.3, 145.9, 139.8, 130.0, 127.8, 123.5, 120.9, 120.5, 119.9, 115.4, 111.3, 111.0, 56.14, 56.11.

Synthesis of compound 23 following published procedures ⁹,¹⁰:
To a suspension of palladium (10 wt%) on carbon (1.706 g) and ammonium formate (5.679 g, 90 mmol, 1.5 equiv.) in MeOH (80 mL) was added compound 22 (17.058 g, 60 mmol). The resulting black suspension was stirred under reflux for 3.5 h. After being cooled to room temperature, the reaction mixture was filtered through a pad of Celite® 545, and the Celite® 545 pad was washed with MeOH (40 mL). The combined filtrate was heated to 50 ℃, and water (120 mL) was added. The resulting precipitate suspension was stirred at room temperature overnight. The solid was collected via filtration, and was washed with water (50 mL × 2), and petroleum ether (100 mL × 2), and dried under vacuum to give compound 23 (12.728 g, 44.5 mmol, 74% yield) as a white solid. The analytical data matched literature reports. ⁹,¹⁰

³H NMR (400 MHz, CDCl₃) δ 7.48–7.46 (m, 2H), 7.29 (t, J = 8.1 Hz, 1H), 7.05 (dd, J = 8.0, 1.8 Hz, 1H), 6.78–6.75 (m, 3H), 4.74 (broad s, 1H), 3.84 (s, 3H), 3.84 (s, 3H), 3.25 (t, J = 7.6 Hz, 2H), 2.99 (t, J = 7.6 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 200.3, 156.7, 149.0, 147.5, 138.3, 133.9, 130.0, 120.8, 120.5, 120.3, 114.6, 112.0, 111.5, 56.1, 56.0, 40.9, 30.0.

Synthesis of compound 25 following published procedures ⁶:
To a suspension of 23 (7.158 g, 25 mmol, 1 equiv.) and K₂CO₃ (6.910 g, 50 mmol, 2 equiv.) in acetone (50 mL), was added tert-butyl bromoacetate 24 (7.335 mL, 50 mmol, 2 equiv.) dropwise over a period of 20 min. The resulting mixture was stirred at room temperature for 24 hours. The resulting white solid was filtered off and the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (petroleum ether : EtOAc = 5 : 1) to give 25 (7.806 g, 19.5 mmol, 78% yield) as a colorless oil. The analytical data matched literature reports. ⁹

³H NMR (400 MHz, CDCl₃) δ 7.56 (d, J = 7.6 Hz, 1H), 7.46 (t, J = 1.9 Hz, 1H), 7.36 (t, J = 7.9 Hz, 1H), 7.12 (dd, J = 8.3, 2.7 Hz, 1H), 6.81–6.76 (m, 3H), 4.55 (s, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.25 (t, J = 7.6 Hz, 2H), 3.00 (t, J = 7.6 Hz, 2H), 1.48 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 198.9, 167.7, 158.3, 149.0, 147.5, 138.4, 133.9, 129.8, 121.6, 120.3, 120.2, 113.2, 111.9, 111.5, 82.7, 65.8, 56.1, 56.0, 40.9, 29.9, 28.2.

Synthesis of compound 26 following published procedures ⁹,¹¹:
(+)-DIP-Chloride™ (CAS No.: 112246-73-8) (9.624 g, 30 mmol, 2 equiv.) in THF (15 mL) was added dropwise to a solution of 25 (6.008 g, 15 mmol, 1 equiv.) in THF (30 mL) at -20 ℃. The solution was stirred at -10 ℃ for 20 hours. The solvent was removed under reduced pressure. The residue was diluted with diethyl ether (50 mL) followed by addition of diethanolamine (CAS No.: 111-42-2) (15.765 g, 150 mmol) and stirred at room temperature overnight. The mixture was diluted with EtOAc (100 mL), filtered through Celite® 545, and the filtrate was concentrated. The residue was purified by flash column
chromatography on silica gel (petroleum ether : EtOAc = 5 : 1) to give 26 (3.028 g, 7.5 mmol, 50% yield) as a colorless oil. The analytical data matched literature reports.  

1H NMR (400 MHz, CDCl₃) δ 7.25 (t, J = 7.9, 1H), 6.96 – 6.91 (m, 2H), 6.80 – 6.77 (m, 2H), 6.73 – 6.70 (m, 2H), 4.65 (dd, J = 7.8, 5.2 Hz, 1H), 4.50 (s, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 2.72 – 2.56 (m, 2H), 2.00 – 1.93 (m, 2H), 1.47 (s, 9H); 13C NMR (100 MHz, CDCl₃) δ 168.1, 158.2, 148.9, 147.3, 146.6, 134.4, 129.6, 120.3, 119.2, 113.7, 112.3, 111.8, 111.3, 82.5, 73.8, 65.7, 56.0, 55.9, 40.7, 31.7, 28.1.

Synthesis of compound 27 following published procedures:

To a solution of compound 26 (2.576 g, 6.4 mmol) and compound 9 (1.797 g, 7.04 mmol, 1.1 equiv.) in CH₂Cl₂ (25.6 mL) at 0 °C, was added dicyclohexylcarbodiimide (DCC) (1.452 g, 7.04 mmol, 1.1 equiv.) and 4-dimethylamino)pyridine (DMAP) (86.0 mg, 0.704 mmol, 0.11 equiv.). The mixture was stirred at 0 °C for 2 hours, and then at room temperature overnight. The mixture was diluted with EtOAc (100 mL), filtered through Celite® 545, and the filtrate was concentrated. The residue was purified by flash column chromatography on silica gel (petroleum ether : EtOAc = 4 : 1) to give 27 (3.986 g, 6.23 mmol, 97% yield) as a colorless oil. The analytical data matched literature reports.

1H NMR (400 MHz, CDCl₃) δ 7.27 (t, J = 7.9 Hz, 1H), 6.97 – 6.94 (m, 1H), 6.92 – 6.90 (m, 1H), 6.86 – 6.81 (m, 1H), 6.79 – 6.76 (m, 1H), 6.69 – 6.66 (m, 2H), 5.79 – 5.75 (m, 1H), 5.31 (d, J = 5.1 Hz, 1H), 4.52 (s, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.35 (d, J = 13.3 Hz, 1H), 3.14 (td, J = 13.0, 3.0 Hz, 1H), 2.62 – 2.47 (m, 2H), 2.36 (d, J = 14.1 Hz, 1H), 2.28 – 2.19 (m, 1H), 2.09 – 2.05 (m, 1H), 1.77 – 1.60 (m, 6H), 1.48 (s, 9H), 1.23 (s, 3H), 1.20 (s, 3H), 1.11 (d, J = 5.8 Hz, 1H), 0.88 (t, J = 7.5 Hz, 3H); 13C NMR (100 MHz, CDCl₃) δ 207.9, 169.8, 168.0, 167.4, 158.2, 149.0, 147.4, 141.5, 133.6, 129.8, 120.3, 120.0, 114.4, 113.4, 111.8, 111.4, 82.5, 65.9, 56.0, 56.0, 51.4, 46.8, 44.3, 38.1, 32.6, 31.3, 28.2, 26.6, 25.1, 23.7, 23.2, 21.3, 8.9.

Synthesis of compound 28 (SLF) following published procedures:

To a solution of compound 27 (3.986 g, 6.23 mmol) in CH₂Cl₂ (62.3 mL) at 0 °C was added trifluoroacetic acid (TFA) (20 mL). The mixture was stirred at room temperature for 2 hours. The volatile materials was removed under reduced pressure, and the residue was co-evaporated with toluene (5 mL × 2). The residue was purified by flash column chromatography on silica gel (CH₂Cl₂ : MeOH = 30 : 1) to give 28 (3.574 g, 6.12 mmol, 98% yield) as a colorless foam. The analytical data matched literature reports.

1H NMR (400 MHz, CDCl₃) δ 9.92 (broad s, 1H), 7.23 (d, J = 8.2 Hz, 1H), 6.91 (d, J = 7.7 Hz, 1H), 6.86 – 6.82 (m, 2H), 6.74 (d, J = 8.1 Hz, 1H), 6.65 (d, J = 2.3 Hz, 1H), 6.63 (s, 1H), 5.76 – 5.70 (m, 1H), 5.28 – 5.25 (m, 1H), 4.66 (d, J = 2.7 Hz, 2H), 3.82 (s, 3H), 3.81 (s, 3H), 3.32 (d, J = 12.9 Hz, 1H), 3.15 (td, J = 13.0, 2.9 Hz, 1H), 2.62 – 2.47 (m, 2H), 2.35 (d, J = 13.6 Hz, 1H), 2.24 – 2.15 (m, 1H), 2.07 – 1.98 (m, 1H), 1.77 – 1.58 (m, 5H), 1.48 – 1.43 (m, 1H), 1.37 – 1.30 (m, 1H), 1.17 (s, 3H), 1.15 (s, 3H), 0.83 (t, J = 7.5 Hz, 3H); 13C NMR (100 MHz, CDCl₃) δ 207.7, 172.7, 169.5, 167.7, 157.8, 149.0, 147.4, 141.8, 133.5, 130.0, 120.4, 120.2, 115.0, 111.9, 111.8, 111.5, 65.1, 56.1, 56.0, 51.8, 46.9, 44.5, 38.2, 32.6, 31.4, 26.6, 25.0, 23.6, 23.1, 21.2, 8.8.
Synthesis of compound 29:
To a solution of compound 28 (233.5 mg, 0.4 mmol, 1.0 equiv.) in DMF (2 mL) was added compound 13 (192.1 mg, 0.44 mmol, 1.1 equiv.), EDC-HCl (115.0 mg, 0.46 mmol, 1.5 equiv.), HOBt·H₂O (91.9 mg, 0.6 mmol, 1.5 equiv.), and DIPEA (208 µL, 1.2 mmol, 3.0 equiv.). The mixture was stirred at room temperature overnight. The mixture was diluted with EtOAc (40 mL), washed with water (10 mL) and brine (10 mL × 3), dried over Na₂SO₄, filtered and concentrated. The residue was purified by flash column chromatography on silica gel (CH₂Cl₂ : MeOH = 10 : 1) to give 29 (310.3 mg, 0.31 mmol, 77% yield) as a colorless oil.

1H NMR (400 MHz, CDCl₃) δ 7.87 (s, 1H), 7.77 – 7.64 (m, 2H), 7.51 (dd, J = 8.5, 2.0 Hz, 1H), 7.31 (t, J = 7.9 Hz, 1H), 7.10 – 6.93 (m, 4H), 6.88 – 6.84 (m, 1H), 6.82 – 6.79 (m, 1H), 5.79 (dd, J = 8.1, 5.4 Hz, 1H), 5.33 – 5.31 (m, 1H), 4.49 (s, 2H), 3.86 (s, 3H), 3.85 (s, 3H), 3.40 – 3.32 (m, 5H), 2.63 – 2.55 (m, 2H), 2.39 (d, J = 13.4 Hz, 1H), 2.30 – 2.21 (m, 1H), 2.12 – 2.08 (m, 1H), 1.81 – 1.57 (m, 10H), 1.52 (s, 9H), 1.51 (s, 9H), 1.39 – 1.35 (m, 3H), 1.24 (s, 3H), 1.23 (s, 3H), 1.14 (d, J = 2.4 Hz, 1H), 0.90 (t, J = 7.5 Hz, 3H); 13C NMR (100 MHz, CDCl₃) δ 207.7, 170.9, 169.4, 167.9, 167.1, 166.6, 166.3, 157.3, 155.9, 154.0, 153.2, 148.6, 147.1, 141.6, 133.2, 130.3, 129.8, 128.7, 123.9, 120.0, 119.8, 113.7, 113.3, 111.2, 80.5, 76.3, 67.1, 60.2, 55.7, 55.6, 51.1, 46.5, 44.0, 39.6, 38.7, 37.9, 32.3, 29.0, 28.8, 28.1, 24.7, 23.9, 23.2, 23.0, 21.0, 20.8, 14.0, 8.6; HRMS (ESI): calcd for C₅₄H₇₆N₅O₁₃ [M + H]+ 1002.5434, found: 1002.5530.

Synthesis of compound 30:
To a stirred solution of compound 29 (200.4 mg, 0.2 mmol, 1.0 equiv.) in CH₂Cl₂ (4 mL) was added trifluoroacetic acid (TFA, 2 mL) at room temperature. The reaction solution was stirred for 45 min at room temperature. CH₂Cl₂ and TFA was removed under reduced pressure, and further removed by co-evaporation with toluene (1 mL × 2). To a stirred solution of this residue in dry DMF (2 mL) was added compound 16 (66.5 mg, 0.22 mmol, 1.1 equiv.), and NEt₃ (83.4 µL, 0.6 mmol, 3 equiv.). The mixture was stirred at 40 ℃ for 12 hours. The mixture was diluted with EtOAc (20 mL), washed with water (10 mL) and brine (10 mL × 3), dried over anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified by flash chromatography on silica gel (eluted using EtOAc with 1% NEt₃) to give compound 30 (135.5 mg, 0.137 mmol, 68% yield) as white solid.

1H NMR (400 MHz, CDCl₃) δ 7.57 (s, 1H), 7.42 (d, J = 9.0 Hz, 1H), 7.20 (d, J = 6.9 Hz, 1H), 6.90 (d, J = 7.9 Hz, 1H), 6.85 (d, J = 2.4 Hz, 1H), 6.78 – 6.68 (m, 3H), 6.60 (dd, J = 7.5, 3.9 Hz, 3H), 5.69 (dd, J = 8.2, 5.5 Hz, 1H), 5.23 (d, J = 5.5 Hz, 1H), 4.38 (s, 2H), 4.09 (t, J = 5.4 Hz, 2H), 3.77 (s, 3H), 3.77 (s, 3H), 3.45 – 3.16 (m, 8H), 2.53 – 2.45 (m, 2H), 2.29 (d, J = 13.8 Hz, 1H), 2.24 – 2.06 (m, 2H), 1.97 (s, 2H), 1.65 – 1.45 (m, 10H), 1.36 (s, 9H), 1.30 – 1.22 (m, 3H), 1.14 (s, 3H), 1.13 (s, 3H); 13C NMR (100 MHz, CDCl₃) δ 208.0, 169.8, 169.7, 168.3, 168.2, 167.4, 167.2, 166.6, 157.5, 156.2, 155.0, 148.9,
Synthesis of compound 31:

To a stirred solution of compound 30 (69.2 mg, 70 µmol, 1.0 equiv.) in CH$_2$Cl$_2$ (2 mL) was added trifluoroacetic acid (TFA, 1 mL) at room temperature. The reaction solution was stirred at room temperature for 30 min. CH$_2$Cl$_2$ and TFA were removed under reduced pressure, and further removed by co-evaporation with toluene (1 mL × 2). To a stirred solution of this residue in dry DMF (2 mL) was added compound 19 (26.3 mg, 77 µmol, 1.1 equiv.), and NEt$_3$ (48.7 µL, 350 µmol, 5 equiv.). The mixture was stirred at room temperature for 10 hours. The volatile was removed under reduced pressure and the residue was purified by flash chromatography on silica gel (CH$_2$Cl$_2$: MeOH = 10:1 with 1% NEt$_3$) to give compound 31 (58.6 mg, 50.5 µmol, 72% yield) as white solid.

$^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.22 (br s, 1H), 7.69 (br s, 1H), 7.51 – 7.35 (m, 2H), 7.25 (t, $J$ = 7.9 Hz, 1H), 7.03 – 6.88 (m, 3H), 6.80 (d, $J$ = 8.4 Hz, 1H), 6.74 (d, $J$ = 8.4 Hz, 1H), 6.64 (d, $J$ = 5.6 Hz, 3H), 6.46 (br s, 1H), 6.02 (br s, 1H), 5.73 (t, $J$ = 7.0 Hz, 1H), 5.26 (d, $J$ = 5.1 Hz, 1H), 4.80 (br s, 1H), 4.43 (s, 2H), 4.33 (br s, 1H), 4.13 (s, 2H), 3.81 (s, 3H), 3.80 (s, 3H), 3.47 – 3.19 (m, 11H), 3.14 (t, $J$ = 12.9 Hz, 1H), 2.98 (s, 1H), 2.62 – 2.45 (m, 5H), 2.36 – 1.93 (m, 4H), 1.78 – 1.40 (m, 15H), 1.34 – 1.27 (m, 6H), 1.18 (s, 3H), 1.17 (s, 3H); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 207.9, 174.9, 174.2, 169.9, 169.6, 168.2, 167.5, 167.3, 166.5, 164.3, 157.4, 155.5, 148.8, 147.3, 144.8, 141.8, 133.3, 130.0, 123.6, 122.4, 120.2, 119.9, 115.5, 114.0, 113.4, 111.7, 111.3, 76.5, 67.2, 64.1, 61.7, 60.2, 55.9, 55.8, 51.3, 50.3, 46.7, 44.2, 40.5, 39.8, 38.9, 38.8, 38.1, 35.6, 32.4, 31.2, 29.3, 29.1, 28.0, 26.4, 25.4, 24.9, 24.1, 23.4, 23.2, 21.1, 8.7; HRMS (ESI): calcd for C$_{57}$H$_{79}$N$_8$O$_{13}$S$^+$ [M + H]$^+$ 1115.5482, found: 1115.5639.

Synthesis of compound 1:

Compound 31 (30.7 mg, 26.5 µmol) was suspended in AcOH (0.5 mL) and water (0.5 mL). To this solution was added NaNO$_2$ (9.1 mg, 132.5 µmol, 5 equiv). The mixture was stirred at room temperature for 3 hours. The mixture was diluted with EtOAc (20 mL), washed with water (10 mL) and brine (10 mL × 2), dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated. The residue was purified by flash
chromatography on silica gel (CH$_2$Cl$_2$ : MeOH = 20:1) to give crude compound 1 (19.9 mg) as white foam. The crude product was further purified by preparative-HPLC [(column: Macherey-Nagel, NUCLEODUR C18 HTec, 5 µm, 250×21 mm; mobile phase: CH$_3$CN (containing 0.1% formic acid) : H$_2$O (containing 0.1% formic acid) = 20 : 80 → 90 : 10 (linear gradient over 45 min); flow rate: 18 mL/min; detection UV wavelength: 220 nm)]. The combined collected fractions were lyophilized to give 1 (9.5 mg, 8.4 µmol, 32% yield) as a white powder.

*Note:* This product exists as an approximate 6.5:1 [based on the ratio of 2.6H (δ 0.85, t) and 0.4H (δ 0.80, t)] mixture of the trans and cis conformations due to the epimerization of the N-C bond in SLF.$^3$

$^1$H NMR (600 MHz, CDCl$_3$) δ 8.52 (s, 1H), 8.14 – 8.12 (m, 1H), 8.09 – 8.04 (m, 2H), 7.91 (s, 1H), 7.29 – 7.27 (m, 1H), 6.96 (d, J = 7.8 Hz, 1H), 6.89 (s, 1H), 6.86 (t, J = 6.0 Hz, 1H), 6.82 – 6.80 (m, 1H), 6.79 – 6.76 (m, 1H), 6.69 – 6.66 (m, 3H), 5.73 (dd, J = 8.1, 5.4 Hz, 1H), 5.30 – 5.29 (m, 1H), 4.70 (t, J = 6.4 Hz, 1H), 4.66 – 4.64 (m, 1H), 4.54 – 4.49 (m, 2H), 4.07 – 4.02 (m, 1H), 3.91 – 3.88 (m, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.54 – 3.47 (m, 2H), 3.40 – 3.34 (m, 2H), 3.20 (td, J = 13.2, 3.0 Hz, 1H), 2.96 – 2.90 (m, 1H), 2.88 – 2.83 (m, 1H), 2.77 – 2.73 (m, 1H), 2.62 – 2.58 (m, 1H), 2.55 – 2.50 (m, 1H), 2.40 (d, J = 13.6 Hz, 1H), 2.26 – 2.20 (m, 1H), 2.14 (q, J = 7.0 Hz, 1H), 2.07 – 2.02 (m, 1H), 1.81 – 1.59 (m, 14H), 1.55 – 1.45 (m, 5H), 1.42 – 1.23 (m, 4H), 1.20 (s, 3H), 1.19 (s, 3H), 1.12 (s, 1H), 0.85 (t, J = 7.4 Hz, 2.60H), 0.80 (t, J = 7.5 Hz, 0.40H).

$^{13}$C NMR (151 MHz, CDCl$_3$) δ 208.2, 174.2, 169.9, 168.7, 167.6, 166.5, 157.5, 154.9, 149.0, 148.8, 147.5, 147.1, 142.0, 137.2, 133.4, 131.2, 130.2, 126.4, 120.3, 120.0, 113.9, 113.6, 112.3, 111.90, 111.86, 111.5, 76.7, 68.8, 67.4, 59.9, 58.7, 56.1, 56.0, 54.5, 51.5, 46.9, 44.3, 40.4, 39.0, 38.4, 35.6, 35.3, 32.6, 31.4, 29.3, 28.8, 27.6, 27.4, 26.6, 25.3, 25.1, 24.2, 23.5, 23.4, 21.3, 8.9.

HRMS (ESI): calcd for C$_{57}$H$_{76}$N$_{9}$O$_{13}$S$^+$ [M + H]$^+$ 1126.5278, found: 1126.5284.

![Scheme S3. Synthetic scheme of LDNasa (38)](image)
LDNAsA (38) was synthesized according reference 12.

Synthesis of compound 34 following published procedures12:
To a stirred solution of 4-sulfamoylbenzoic acid 32 (140.8 mg, 0.7 mmol) in dry DMF (2.8 mL), was added compound 33 (141.6 mg, 0.7 mmol, 1 equiv.), EDC-HCl (201.3 mg, 1.05 mmol, 1.5 equiv.), HOBt·xH2O (160.8 mg, 1.05 mmol, 1.5 equiv.) and DIPEA (242 µL, 1.4 mmol, 2 equiv.). The mixture was stirred at room temperature overnight. The solution was dissolved with EtOAc (25 mL), and washed with brine (25 mL × 3). The organic layer was dried over Na2SO4, filtrated and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (CH2Cl2: MeOH = 20:1) to give compound 34 (184.1 mg, 0.478 mmol, 68% yield) as white solid. The analytical data matched literature reports.12

1H NMR (600 MHz, CD3OD) δ 7.97 (d, J = 8.6 Hz, 2H), 7.94 (d, J = 8.4 Hz, 2H), 3.42 – 3.37 (m, 2H), 3.05 (t, J = 7.0 Hz, 2H), 1.65 (p, J = 7.3 Hz, 2H), 1.52 (p, J = 7.2 Hz, 2H), 1.43 – 1.39 (m, 11H); 13C NMR (151 MHz, CD3OD) δ 168.9, 158.6, 147.6, 139.2, 128.9, 127.3, 79.8, 41.2, 41.0, 30.6, 30.1, 28.8, 25.2.

Synthesis of compound 35 following published procedures12:
To a stirred solution of compound 34 (38.6 mg, 0.1 mmol) in dry DMF (2 mL) was added biotin 17 (40.3 mg, 0.165 mmol, 1.65 equiv.), EDC-HCl (57.5 mg, 0.3 mmol, 3 equiv.), 4-dimethylaminopyridine (DMAP) (4.0 mg, 0.033 mmol, 0.33 equiv.) and DIPEA (52 µL, 0.3 mmol, 3 equiv.). The mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (CH2Cl2 : MeOH = 10:1) to yield compound 35 (51.2 mg, 83.7 µmol, 84%) as a white solid. The analytical data matched literature reports.12

1H NMR (600 MHz, CDCl3) δ 8.08 – 7.74 (m, 2H), 4.42 (m, 1H), 4.24 – 4.13 (m, 1H), 3.77 – 3.52 (m, 2H), 3.50 – 2.53 (m, 8H), 2.42 – 2.13 (s, 2H), 1.60 – 1.11 (m, 18H).

Synthesis of compound 36 following published procedures12:
To a stirred solution of compound 35 (50.8 mg, 83 µmol) in CH2Cl2 (4 mL) was added trifluoroacetic acid (TFA) (2 mL) at room temperature. The reaction solution was stirred at room temperature for 30 min. TFA and CH2Cl2 was removed under reduced pressure and further removed by co-evaporation with toluene (1 mL × 2). The residue was dissolved in dry DMF (0.83 mL), add compound 28 (48.4 mg, 83 µmol, 1 equiv.), EDC-HCl (23.9 mg, 124.5 µmol, 1.5 equiv.), HOBt·xH2O (19.1 mg, 124.5 µmol, 1.5 equiv.), DIPEA (242 µL, 1.4 mmol, 2 equiv.). The mixture was stirred at room temperature overnight. The solution was dissolved with EtOAc (25 mL), and washed with brine (25 mL × 3). The organic layer was dried over Na2SO4, filtrated and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (CH2Cl2: MeOH = 20:1) to give compound 36 (184.1 mg, 0.478 mmol, 68% yield) as white solid. The analytical data matched literature reports.12
equiv.) and DIPEA (72 µL, 415 µmol, 5 equiv.). The mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (CH₂Cl₂ : MeOH = 10:1) to yield compound 36 (83.5 mg, 77.5 µmol, 93%) as colorless oil. The analytical data matched literature reports.¹²

⁵¹H NMR (600 MHz, CDCl₃) δ 10.25 (br s, 3H), 7.93 (s, 4H), 7.81 (s, 1H), 7.20 (t, J = 7.9 Hz, 1H), 6.99 – 6.79 (m, 3H), 6.76 (d, J = 8.3 Hz, 1H), 6.72 – 6.66 (m, 1H), 6.61 – 6.58 (m, 2H), 5.67 (t, J = 7.0 Hz, 1H), 5.19 (d, J = 5.8 Hz, 1H), 4.39 (s, 2H), 3.76 (s, 3H), 3.74 (s, 3H), 3.60 – 3.56 (m, 8H), 3.31 – 3.18 (m, 4H), 2.79 – 2.60 (m, 2H), 2.55 – 2.42 (m, 2H), 2.30 – 2.14 (m, 4H), 1.98 – 1.94 (m, 2H), 1.71 – 1.45 (m, 15H), 1.12 (d, J = 4.9 Hz, 6H), 0.87 – 0.67 (m, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 207.9, 169.5, 168.2, 167.2, 166.2, 157.3, 148.8, 147.2, 141.7, 139.1, 133.3, 129.9, 128.0, 127.8, 120.1, 119.9, 117.7, 115.8, 113.9, 113.3, 111.7, 111.3, 110.1, 76.4, 67.1, 57.9, 55.8, 55.8, 51.2, 50.1, 46.6, 44.1, 39.9, 38.8, 38.0, 32.3, 31.1, 29.1, 28.7, 26.3, 24.8, 24.0, 23.9, 23.7, 23.1, 21.0, 18.4, 18.2, 18.2, 17.2, 8.6.

Synthesis of compound 38 (LDNASA) following published procedures¹²:
To a stirred solution of compound 36 (83.5 mg, 77.5 µmol) in dry DMF (1 mL) was added iodoacetonitrile 37 (56.1 µL, 775 µmol, 10 equiv.) and DIPEA (67 µL, 387.5 µmol, 5 equiv.). The mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (CH₂Cl₂ : MeOH = 20:1) to yield compound 38 (38.4 mg, 34.4 µmol, 44% yield) as colorless oil. The analytical data matched literature reports.¹²

¹⁵¹H NMR (600 MHz, CDCl₃) δ 9.12 (br s, 1H), 8.13 (d, J = 8.5 Hz, 2H), 8.00 (d, J = 8.5 Hz, 2H), 7.79 (t, J = 5.6 Hz, 1H), 7.29 – 7.26 (m, 1H), 6.96 (d, J = 7.7 Hz, 1H), 6.93 – 6.92 (m, 1H), 6.89 (t, J = 6.3 Hz, 1H), 6.82 – 6.80 (m, 1H), 6.77 – 6.75 (m, 1H), 6.68 – 6.65 (m, 2H), 5.76 – 5.73 (m, 1H), 5.67 – 5.61 (m, 2H), 5.28 – 5.27 (m, 1H), 4.79 (d, J = 2.6 Hz, 2H), 4.47 (t, J = 6.6 Hz, 1H), 4.44 (s, 2H), 4.25 – 4.18 (m, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 3.78 – 3.72 (m, 1H), 3.68 (q, J = 7.0 Hz, 2H), 3.43 (s, 1H), 3.42 – 3.29 (m, 5H), 3.18 – 3.13 (m, 2H), 3.05 – 3.02 (m, 1H), 2.88 – 2.84 (m, 1H), 2.72 – 2.49 (m, 5H), 2.35 (d, J = 13.7 Hz, 1H), 2.26 – 2.18 (m, 2H), 2.07 – 2.01 (m, 1H), 1.68 – 1.43 (m, 8H), 1.40 – 1.34 (m, 3H), 1.19 – 1.16 (m, 6H), 0.85 (t, J = 7.5 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 208.0, 171.9, 169.7, 168.4, 167.4, 165.4, 163.7, 157.4, 148.9, 147.4, 141.9, 140.7, 140.1, 133.4, 130.1, 129.0, 127.8, 120.2, 120.1, 115.1, 113.9, 113.5, 111.8, 111.4, 76.5, 67.3, 61.9, 60.3, 56.0, 55.9, 54.7, 51.3, 46.7, 44.2, 40.5, 40.1, 38.7, 38.2, 35.4, 33.4, 32.5, 31.3, 29.2, 28.6, 28.0, 27.8, 26.5, 25.0, 23.9, 23.5, 23.3, 21.2, 8.8; HRMS (ESI): calcd for C₅₆H₇₃N₇O₁₃S₂Na⁺ [M + Na⁺] 1138.4600, found: 1138.4640.
Scheme S4. Synthetic scheme of compounds 2 and Autogramin-2 (57)

Synthesis of compound 40 following published procedures:\(^\text{13}\):
Powdered potassium hydroxide (44.88 g, 0.8 mol) was added portionwise to a solution of diethylene glycol 39 (10.610 g, 0.1 mol, 1 equiv.) and p-toluenesulfonyl chloride (38.140 g, 0.2 mol, 2 equiv.) in CH\(_2\)Cl\(_2\) (100 mL) at 0 °C. The reaction mixture was stirred at from 0 °C to room temperature overnight. Water (200 mL) was then added and the water phase was extracted with CH\(_2\)Cl\(_2\) (50 mL × 3). The combined organic layers were dried over anhydrous Na\(_2\)SO\(_4\), filtrated and concentrated under reduced pressure to afford 40 (39.415 g, 95.1 mmol, 95% yield) as a white solid. The analytical data matched literature reports.\(^\text{13}\)

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.77 (d, \(J = 8.4\) Hz, 4H), 7.34 (d, \(J = 8.1\) Hz, 4H), 4.11 – 4.05 (m, 4H), 3.63 – 3.57 (m, 4H), 2.44 (s, 6H); \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 145.1, 133.0, 130.0, 128.1, 69.1, 68.9, 21.8.
Synthesis of compound 41 following published procedures:

Sodium azide (650.0 mg, 10 mmol) was added portionwise to a solution of 40 (4.145 g, 10 mmol) in acetonitrile (25 mL) at room temperature. The reaction mixture was stirred under reflux for 48 hours. After cooling down to room temperature, the white solid was filtered off. The filtrate was concentrated under reduced pressure and purified by flash chromatography on silica gel (petroleum ether : EtOAc = 5:1) to give compound 41 (1.411 g, 4.946 mmol, 49% yield) as colorless liquid. The analytical data matched literature reports.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.80 (d, $J = 7.5$ Hz, 2H), 7.34 (d, $J = 7.9$ Hz, 2H), 4.21 – 4.12 (m, 2H), 3.70 (t, $J = 4.0$ Hz, 2H), 3.60 (t, $J = 4.3$ Hz, 2H), 3.32 (t, $J = 4.5$ Hz, 2H), 2.44 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 145.0, 133.0, 130.0, 128.1, 70.3, 69.3, 68.8, 50.7, 21.8.

Synthesis of compound 43 following published procedures:

To a solution of NaN$_3$ (2.600 g, 40 mmol) in water (80 mL) was added compound 42 (3.372 g, 20 mmol) at room temperature. The reaction mixture was stirred under reflux for 24 hours. The crude mixture was extracted with CH$_2$Cl$_2$ (40 mL × 3). The combined organic layers were washed with brine (20 mL) and dried over Na$_2$SO$_4$, filtered, and evaporated to dryness to obtain compound 43 (3.172 g, 18.1 mmol, 91% yield) as colorless oil with was pure enough for further usage without purification. The analytical data matched literature reports.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.76 – 3.71 (m, 2H), 3.70 – 3.65 (m, 6H), 3.64 – 3.59 (m, 2H), 3.40 (t, $J = 5.0$ Hz, 2H), 2.16 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 72.6, 70.8, 70.5, 70.2, 61.9, 50.8.

Synthesis of compound 43 following published procedures:

To a solution of compound 43 (1.402 g, 8 mmol) in dry acetonitrile (40 mL), N,N’-disuccimidyl carbonate 15 (4.099 g, 16 mmol, 2 equiv.) and NEt$_3$ (2.230 mL, 4 mmol, 2 equiv.) were added and the resulting mixture was stirred at 40 °C for 1 hour. The solvent was removed under reduced pressure, the residue was dissolved in EtOAc (50 mL), washed with saturated NaHCO$_3$ aqueous (50 mL), water (25 mL) and brine (25 mL), dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (eluted with CH$_2$Cl$_2$: MeOH = 30:1) to afford compound 44 (1.454 g, 4.6 mmol, 57% yield) as a colorless oil. The analytical data matched literature reports.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 4.49 – 4.43 (m, 2H), 3.81 – 3.76 (m, 2H), 3.68 (d, $J = 3.7$ Hz, 6H), 3.39 (t, $J = 5.1$ Hz, 2H), 2.83 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 168.7, 151.8, 71.1, 70.8, 70.5, 70.2, 61.9, 50.8.

Synthesis of compound 50 following published procedures:

To a solution of methyl 4-hydroxybenzoate 45 (CAS No.: 99-76-3) (228.3 mg, 1.5 mmol, 1.0 equiv.) in THF (7.5 mL) was added K$_2$CO$_3$ (629.1 m, 4.5 mmol, 3 equiv.) and compound 41 (427.9 mg, 1.5 mmol, 1.0 equiv.). The mixture was stirred under reflux overnight. After being cooled to room temperature, the mixture was diluted with EtOAc (20 mL), washed with water (20 mL). The aqueous layer was extracted
with EtOAc (10 ml). The combined organic layers were washed with brine (10 ml), dried over anhydrous Na$_2$SO$_4$, filtrated and concentrated under reduced pressure. To the residue was added 10 mL of 5 N NaOH aqueous, stirred at 100 °C for 2 hours. The mixture was cooled to room temperature, washed with CH$_2$Cl$_2$ (10 mL × 2), neutralized with concentrated HCl. The resulting precipitate was collected by filtration to give 46 (197.3 mg, 0.785 mmol, 52% yield) as white solid. The analytical data matched literature reports.$^{19}$

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.06 (d, $J = 8.8$ Hz, 2H), 6.97 (d, $J = 8.8$ Hz, 2H), 4.22 (t, $J = 4.7$ Hz, 2H), 3.99 – 3.84 (m, 2H), 3.76 (t, $J = 5.0$ Hz, 2H), 3.42 (t, $J = 5.0$ Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 172.0, 163.3, 132.5, 122.1, 114.5, 70.5, 69.7, 67.7, 50.8.

Synthesis of compound 56 following published procedures$^{20}$:

5,5-Dibromobarbituric acid 48 (CAS No.: 511-67-1) (1.001 g, 3.50 mmol, 0.6 equiv.) was added to a solution of N-Boc-4-piperidone 47 (CAS No.: 79099-07-3) (1.162 g, 5.83 mmol, 1.0 equiv.) in dry THF (15 mL). The mixture was stirred at room temperature overnight, during which time a white precipitate formed. This precipitate was filtered off, washed with THF (10 mL). The solvent in the filtrate was removed under reduced pressure to yield the bromopiperidinone 49, which was used without further purification. Crude 49 was dissolved in EtOH (15 mL), and thiourea 50 (487.8 mg, 6.41 mmol, 1.1 equiv.) was added. The mixture was stirred under reflux for 4 hours. The pale yellow precipitate was filtered off and the filtrate was concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL), washed with saturated NaHCO$_3$ aqueous (25 mL). The aqueous was extracted with EtOAc (10 mL × 2). The combined organic layers were washed with brine (50 mL), dried over Na$_2$SO$_4$, filtered, and the solvent removed under reduced pressure to yield a pale yellow solid. This was washed with petroleum ether/EtOAc (10 mL × 2). The combined organic layers were washed with brine (10 mL × 2), dried over anhydrous Na$_2$SO$_4$, filtrated and concentrated under reduced pressure. To the residue was added 10 mL of 5 N NaOH aqueous, stirred at 100 °C for 2 hours. The mixture was cooled to room temperature, washed with CH$_2$Cl$_2$ (10 mL × 2), neutralized with concentrated HCl. The resulting precipitate was collected by filtration to give 46 (197.3 mg, 0.785 mmol, 52% yield) as white solid. The analytical data matched literature reports.$^{19}$

$^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 4.37 (s, 2H), 3.68 (t, $J = 5.8$ Hz, 2H), 2.55 – 2.51 (m, 2H), 1.48 (s, 9H); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 170.1, 156.5, 144.2, 113.5, 81.7, 42.3, 42.0, 28.6, 27.4.

Synthesis of compound 52 following published procedures$^{20}$:

To a solution of compound 51 (199.1 mg, 0.78 mmol, 1.0 equiv.) and compound 46 (195.9 mg, 0.78 mmol, 1.0 equiv.) in DMF (7.8 mL) was added DIPEA (405 µL, 2.34 mmol, 3 equiv.) and PyBOP (CAS No.: 128625-52-5) (811.8 mg, 1.56 mmol, 2.0 equiv.). The mixture was stirred at 70 °C for 24 hours. After being cooled to room temperature, the mixture was diluted with EtOAc (30 mL), washed with water (15 mL × 3), brine (15 mL), dried over anhydrous Na$_2$SO$_4$, filtrated and concentrated under reduced pressure. To the residue was added 10 mL of 5 N NaOH aqueous, stirred at 100 °C for 2 hours. The mixture was cooled to room temperature, washed with CH$_2$Cl$_2$ (10 mL × 2), neutralized with concentrated HCl. The resulting precipitate was collected by filtration to give 46 (197.3 mg, 0.785 mmol, 52% yield) as white solid. The analytical data matched literature reports.$^{19}$

$^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.91 (d, $J = 8.9$ Hz, 2H), 6.98 (d, $J = 8.9$ Hz, 2H), 4.53 (s, 2H), 4.16 – 4.14 (m, 2H), 3.83 – 3.81 (m, 2H), 3.71 – 3.67 (m, 4H), 3.37 – 3.35 (m, 2H), 2.63 (t, $J = 5.5$ Hz, 2H), 1.47 (s, 9H); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 166.4, 163.8, 158.9, 156.2, 144.2, 113.5, 81.7, 42.3, 42.0, 28.6, 27.4.

Synthesis of compound 53:

1) PPh$_3$, THF, 50 °C, 10 h

2) H$_2$O, r, 6 h
A solution of compound 52 (242.8 mg, 0.497 mmol, 1.0 equiv.) and triphenylphosphine (156.4 mg, 0.596 mmol, 1.2 equiv.) in THF (2 mL) was stirred at 50 °C for 10 hours under argon atmosphere. After being cooled to room temperature, water (44.7 µL, 2.49 mmol, 5 equiv.) was added and stirred at room temperature for additional 6 hours. The mixture was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (petroleum ether : EtOAc = 1 : 1) to give compound 53 (153.2 mg, 0.331 mmol, 67% yield) as a white solid.

$^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.99 (d, $J = 8.5$ Hz, 2H), 7.07 (d, $J = 8.5$ Hz, 2H), 4.58 (s, 2H), 4.24 (t, $J = 4.5$ Hz, 2H), 3.86 (t, $J = 5.8$ Hz, 2H), 3.61 (t, $J = 5.3$ Hz, 2H), 2.84 – 2.71 (m, 2H), 1.49 (s, 9H);

$^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 185.5, 167.3, 163.8, 156.4, 147.7, 144.1, 131.1, 126.4, 115.5, 81.72, 81.69, 78.8, 73.2, 70.5, 68.9, 42.0, 28.7, 27.5; HRMS (ESI): calcd for C$_{22}$H$_{31}$N$_4$O$_5$S $\{M + H\}^+$ 463.2010, found: 463.2018.

Synthesis of compound 55:
To a stirred solution of compound 53 (83.2 mg, 0.18 mmol) in dry DMF (1.8 mL) was added benzotriazole-5-carboxylic acid 54 (CAS No.: 23814-12-2) (35.2 mg, 0.216 mmol, 1.2 equiv.), EDC-HCl (CAS No.: 25952-53-8) (51.8 mg, 0.27 mmol, 1.5 equiv.), 1-hydroxybenzotriazole hydrate (HOBt·xH$_2$O, CAS No.: 123333-53-9) (41.3 mg, 0.27 mmol, 1.5 equiv.), and DIPEA (155.8 µL, 0.9 mmol, 5 equiv.). The mixture was stirred at room temperature overnight. The mixture was diluted with EtOAc (20 mL), washed with water (20 mL) and brine (20 mL × 3), dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated. The residue was purified by flash chromatography on silica gel (CH$_2$Cl$_2$: MeOH = 20:1) to give compound 55 (30.1 mg, 49.5 µmol, 28% yield) as a white foam.

$^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.20 (s, 1H), 7.82 (s, 2H), 7.78 (d, $J = 8.5$ Hz, 1H), 7.70 – 7.64 (m, 1H), 7.33 (s, 1H), 6.79 (s, 2H), 4.51 (s, 2H), 3.82 – 3.64 (m, 10H), 2.54 (s, 2H), 1.46 (s, 9H); $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 167.6, 164.6, 162.8, 162.4, 158.0, 154.8, 142.7, 132.3, 132.2, 131.2, 129.9, 128.8, 128.7, 124.4, 119.5, 114.6, 80.6, 69.8, 69.4, 67.7, 40.1, 36.7, 31.6, 28.5, 14.3; HRMS (ESI): calcd for C$_{29}$H$_{34}$N$_7$O$_6$S $\{M + H\}^+$ 608.2286, found: 608.2282.

Synthesis of compound 2:
A solution of compound 55 (30.1 mg, 49.5 µmol), compound 44 (78.3 mg, 247.5 µmol, 5 equiv.), and pyridine (39.9 µL, 495 µmol, 10 equiv.) in dry DMF (1 mL) was stirred at 80 °C for 24 hours. The volatile material was removed under reduced pressure. The residue was purified by Preparative-HPLC [(column: Macherey-Nagel, NUCLEODUR C18 HTec, 5 µm, 250×21 mm; mobile phase: CH$_3$CN (containing 0.1% formic acid) : H$_2$O (containing 0.1% formic acid) = 20 : 80 → 90 : 10 (linear gradient over 40 min); flow rate: 18 mL/min; detection UV wavelength: 220 nm)]. The combined collected fractions were lyophilized to give 2 (3.0 mg, 7% yield) as a white powder.


$^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 8.44 (s, 1H), 8.11 (d, $J = 8.6$ Hz, 1H), 8.07 (dd, $J = 8.6, 1.4$ Hz, 1H), 7.88 (d, $J = 8.8$ Hz, 2H), 7.01 (d, $J = 8.6$ Hz, 2H), 4.79 – 4.78 (m, 2H), 4.59 (s, 2H), 4.25 – 4.23 (m, 2H), 3.99 – 3.98 (m, 2H), 3.80 – 3.73 (m, 10H), 3.70 – 3.68 (m, 2H), 3.67 – 3.65 (m, 2H), 3.34 (t, $J = 5.0$ Hz, 2H), 2.72 (m, 2H), 1.49 (s, 9H); $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 166.2, 165.4, 162.6, 157.1, 154.8, 148.7, 145.9, 133.5, 132.8, 129.7, 129.4, 124.5, 119.3, 118.6, 115.9, 114.0, 100.1, 80.5, 71.0, 70.9, 70.3, 70.0, 69.6, 68.8, 68.2, 67.7, 50.8, 40.1, 28.6; [Note: The carbon signals should be 32, but found 29. The sum of -CH$_2$- and CH$_3$- signals should be 15, but found 12 (see HSQC data). So three -CH$_2$- and/or CH$_3$- carbon signals were overlapping with others.]

HSQC (600, 150 MHz, CDCl$_3$) (CH$_2$ + CH$_3$ signals part, should be 14, but found 11): {4.78, 68.08}, {4.58, 41.87}, {4.24, 67.52}, {3.98, 68.78}, {3.92, 69.63}, {3.78, 70.10}, {3.74, 40.12}, {3.66, 70.41}, {3.34, 50.78}, {2.72, 26.68}, {1.49, 28.54}; HRMS (ESI): calcd for C$_{36}$H$_{44}$N$_{10}$O$_{10}$SNa$^+$ [M + Na]$^+$ 831.2855, found: 831.2861.

Synthesis of compound 57 following published procedures$^{20}$:

A 10 mL flask was charged with compound 51 (25.5 mg, 0.10 mmol) and 4-isopropoxybenzoic acid 56 (21.6 mg, 0.10 mmol, 1.2 equiv. CAS No.: 13205-46-4) and DMF (1 mL). To this was added DIPEA (51.9 $\mu$L, 0.3 mmol, 3 equiv.) and PyBOP$^\circ$ (104.1 mg, 0.2 mmol, 2 equiv. CAS No.: 128625-52-5). The mixture was stirred at 70 °C for 24 hours. The mixture was diluted with EtOAc (10 mL), washed with saturated NaHCO$_3$ aqueous solution (10 mL $\times$ 3), saturated NH$_4$Cl solution (10 mL $\times$ 2), and brine (10 mL). The organic layer was dried over Na$_2$SO$_4$ and filtered, and the solvent removed under reduced pressure. The residue was purified by flash chromatography on silica gel (petroleum ether : EtOAc = 5 : 1) to give compound 57 (15.6 mg, 37.4 $\mu$mol, 37% yield) as a white solid. The analytical data matched literature reports.$^{20}$

$^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 11.29 (br s, 1H), 7.88 (d, $J = 8.3$ Hz, 2H), 6.91 (d, $J = 8.5$ Hz, 2H), 4.65 – 4.61 (m, 1H), 4.56 (s, 2H), 3.60 (s, 2H), 2.42 (s, 2H), 1.47 (s, 9H), 1.36 (d, $J = 6.2$ Hz, 6H); $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 164.6, 161.9, 158.1, 154.8, 130.0, 129.8, 123.9, 115.7, 115.6, 80.4, 70.3, 42.0, 41.1, 28.5, 26.3, 22.0.
Synthesis of compound 59 following published procedures:\(^{17}\):
To a solution of compound 58 (410.6 mg, 2 mmol) in dry acetonitrile (16 mL), compound 15 (1.025 g, 4 mmol, 2 equiv.) and NEt\(_3\) (556 µL, 4 mmol, 2 equiv.) were added and the resulting mixture was stirred at 40 °C for 1 hour. The solvent was removed under reduced pressure, the residue was dissolved in EtOAc (30 mL), washed with saturated aqueous solution of NaHCO\(_3\) (20 mL), water (20 mL) and brine (20 mL), dried over anhydrous Na\(_2\)SO\(_4\), filtered, and concentrated. The residue was purified by silica gel column chromatography (eluted with CH\(_2\)Cl\(_2\): MeOH = 50:1) to afford compound 59 (268.5 mg, 0.775 mmol, 39% yield) as a colorless oil. The analytical data matched literature reports:\(^{17}\).

\(^{1}\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 5.28 (s, 1H), 4.49 – 4.39 (m, 2H), 3.74 – 3.67 (m, 2H), 3.52 (t, \(J\) = 5.1 Hz, 2H), 3.29 (q, \(J\) = 5.5 Hz, 2H), 2.81 (s, 4H), 1.41 (s, 9H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 168.7, 156.1, 151.7, 79.3, 70.6, 70.0, 68.2, 40.4, 28.5, 25.5.
Synthesis of compound 60:
To a dry flask, was added compound 4-sulfamoylbenzoic acid 32 (402.4 mg, 2 mmol, 1.0 equiv. CAS No.: 138-41-0), DMF (3 mL), compound 12 (204.4 mg, 2 mmol, 1 equiv.), EDC-HCl (575.1 mg, 3 mmol, 1.5 equiv.), HOBt·xH₂O (459.3 mg, 3 mmol, 1.5 equiv.), and DIPEA (1.039 mL, 6 mmol, 3 equiv.). The mixture was stirred at room temperature overnight. Then compound 11 (704.8 mg, 2 mmol, 1 equiv.), EDC-HCl (460.1 mg, 2.4 mmol), HOBt·xH₂O (367.4 mg, 2.4 mmol), and DIPEA (692 µL, 4 mmol) was added. The mixture was further stirred at room temperature for 24 hours. The mixture was diluted with EtOAc (50 mL), washed with water (50 mL × 3) and brine (50 mL × 2), dried over anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified by flash chromatography on silica gel (CH₂Cl₂ : MeOH = 20:1) to give compound 60 (166.1 mg, 0.268 mmol, 13% yield) as a white solid.

¹H NMR (400 MHz, CD₃OD) δ 7.99 – 7.83 (m, 5H), 7.65 (d, J = 8.5 Hz, 1H), 7.56 (dd, J = 8.5, 2.1 Hz, 1H), 5.49 (s, 1H), 3.39 (dt, J = 10.3, 7.0 Hz, 4H), 1.68 (h, J = 6.8 Hz, 5H), 1.52 (s, 9H), 1.49 – 1.39 (m, 3H);
¹³C NMR (100 MHz, CD₃OD) δ 169.3, 168.8, 156.0, 155.3, 147.5, 139.2, 135.6, 135.5, 131.6, 130.7, 128.9, 127.3, 125.2, 123.8, 81.8, 81.7, 40.8, 30.1, 30.0, 28.61, 28.59, 25.3; HRMS (ESI): calcd for C₂₉H₄₂N₅O₈S⁺ [M + H]⁺ 620.2749, found: 620.2741.

Synthesis of compound 61:
To a stirred solution of compound 60 (166.1 mg, 0.268 mmol) in CH₂Cl₂ (2 mL) was added trifluoroacetic acid (TFA, 1 mL) at room temperature. The reaction solution was stirred for 30 min at room temperature. CH₂Cl₂ and TFA was removed under reduced pressure, and further removed by co-evaporation with toluene (1 mL × 2). To a stirred solution of this residue in dry DMF (2 mL) was added compound 59 (139.2 mg, 0.402 mmol, 1.5 equiv.), and NEt₃ (112 µL, 0.804 mmol, 3 equiv.). The mixture was stirred at room temperature overnight. The mixture was diluted with EtOAc (20 mL), washed with water (20 mL × 3) and brine (20 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified by flash chromatography on silica gel (CH₂Cl₂ : MeOH = 10:1) to give compound 61 (80.6 mg, 0.124 mmol, 46% yield) as white solid.

¹H NMR (400 MHz, CD₃OD) δ 8.01 – 7.91 (m, 4H), 7.73 (s, 1H), 7.49 (d, J = 8.5 Hz, 1H), 6.78 (d, J = 8.5 Hz, 1H), 4.28 (d, J = 4.7 Hz, 2H), 3.70 (s, 3H), 3.54 (s, 3H), 3.37 (dt, J = 14.0, 7.0 Hz, 6H), 3.24 (s, 3H), 1.65 (dd, J = 12.8, 6.0 Hz, 6H), 1.44 (s, 12H); ¹³C NMR (100 MHz, CD₃OD) δ 169.7, 168.6, 158.3, 157.2, 147.4, 147.1, 139.0, 128.9, 127.2, 123.9, 123.3, 117.7, 116.6, 116.2, 80.0, 70.9, 70.1, 65.5, 41.2, 40.9, 40.6, 30.2, 29.9, 28.7, 25.3; HRMS (ESI): calcd for C₂⁹H₄₃N₆O₉S⁺ [M + H]⁺ 651.2870, found: 651.2894.

Synthesis of compound 63:
To a stirred solution of compound 61 (80.6 mg, 0.124 mmol) in CH₂Cl₂ (2 mL) was added trifluoroacetic acid (TFA, 1 mL) at room temperature. The reaction solution was stirred for 30 min at room temperature. CH₂Cl₂ and TFA was removed under reduced pressure, and further removed by co-evaporation with toluene (1 mL × 2). To a stirred solution of this residue in dry DMF (1.24 mL) was added compound 62...
[NHS-Fluorescein (5/6-carboxyfluorescein succinimidyl ester), mixed isomer. Thermo Scientific, cat. No.: 46410] (58.7 mg, 0.124 mmol, 1 equiv.), and NEt$_3$ (52 µL, 0.372 mmol, 3 equiv.). The mixture was stirred at room temperature overnight. The mixture was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (CH$_2$Cl$_2$ : MeOH = 5:1) to give compound 63 (91.2 mg, 0.1003 mmol, 81% yield) as yellow solid.

$^1$H NMR (400 MHz, CD$_2$Cl$_2$/CD$_3$OD = 1:1) δ 8.39 (s, 1H), 8.14 – 7.99 (m, 1H), 7.94 – 7.78 (m, 4H), 7.63 – 7.55 (m, 1H), 7.36 (td, $J = 8.4$, 2.0 Hz, 1H), 7.20 – 7.10 (m, 1H), 6.71 – 6.59 (m, 3H), 6.59 – 6.41 (m, 4H), 4.66 (br s, 3H), 4.31 – 4.07 (m, 2H), 3.74 – 3.41 (m, 6H), 3.32 – 3.28 (m, 4H), 1.63 – 1.47 (m, 4H), 1.40 – 1.29 (m, 2H).

$^{13}$C NMR (100 MHz, CD$_3$OD) δ 170.4, 169.3, 168.2, 167.9, 167.8, 161.8, 156.7, 156.6, 154.0, 153.9, 146.5, 141.4, 138.7, 137.2, 134.7, 130.0, 129.9, 128.6, 126.9, 125.7, 125.0, 124.0, 123.0, 116.2, 113.9, 110.7, 110.7, 103.5, 70.02, 69.99, 65.2, 40.8, 40.6, 40.3, 29.8, 29.5, 24.8; HRMS (ESI): calcd for C$_{45}$H$_{45}$N$_6$O$_{13}$S$^+$ [M + H]$^+$ 909.2760, found: 909.2812.

Synthesis of compound 3: Compound 63 (41.1 mg, 45.2 µmol) was dissolved in DMF (1 mL). To this solution was added a solution of NaNO$_2$ (31.2 mg, 452 µmol, 10 equiv.) in water (0.5 mL), then AcOH (0.5 mL). The mixture was stirred at room temperature for 1 hour. The mixture was directly purified by preparative-HPLC [(column: Macherey-Nagel, NUCLEODUR C18 HTec, 5 µm, 250×21 mm; mobile phase: CH$_3$CN (containing 0.1% formic acid) : H$_2$O (containing 0.1% formic acid) = 20 : 80 → 100 : 0 (linear gradient over 40 min); flow rate: 18 mL/min; detection UV wavelength: 220 nm)]. The combined collected fractions were lyophilized to give 3 (16.4 mg, 17.8 µmol, 39% yield) as a yellow powder.

$^1$H NMR (400 MHz, (CD$_3$)$_2$SO) δ 10.18 (s, 2H), 8.96 – 8.67 (m, 2H), 8.63 (t, $J = 5.0$ Hz, 1H), 8.51 – 8.37 (m, 1H), 8.33 – 8.00 (m, 3H), 7.98 (dd, $J = 8.3$, 2.2 Hz, 2H), 7.92 (s, 1H), 7.90 – 7.82 (m, 2H), 7.46 (s, 2H), 7.40 – 7.27 (m, 1H), 7.05 – 6.80 (m, 2H), 6.77 – 6.62 (m, 2H), 6.62 – 6.50 (m, 3H), 4.78 – 4.63 (m, 1H), 4.42 – 4.21 (m, 1H), 3.96 – 3.82 (m, 2H), 3.76 – 3.49 (m, 4H), 3.41 (d, $J = 5.8$ Hz, 1H), 3.32 – 3.27 (m, 2H), 1.64 – 1.55 (m, 4H), 1.43 – 1.37 (m, 2H).

$^{13}$C NMR (100 MHz, (CD$_3$)$_2$SO) δ 165.8, 165.1, 164.9, 164.8, 159.8, 159.6, 151.8, 148.1, 146.2, 146.1, 145.1, 137.6, 132.6, 132.6, 131.3, 129.7, 129.3, 129.2, 127.8, 125.6, 118.9, 113.4, 112.71, 112.66, 109.15, 109.10, 109.08, 102.3, 68.8, 67.9, 67.6, 28.8, 28.8, 24.0. Note: three peaks overlap with (CD$_3$)$_2$SO (40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89) as shown in peaks of HSQC NMR: {3.34, 40.52}, {3.29, 38.70}, {3.51, 38.57}.

HRMS (ESI): calcd for C$_{35}$H$_{42}$N$_7$O$_{13}$S$^+$ [M + H]$^+$ 920.2556, found: 920.2669.
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