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SUPPLEMENTARY INFORMATION

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Ribosomal synthesis and folding of peptide-helical aromatic foldamer hybrids

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Supplementary Information for:
Ribosomal synthesis and folding of peptide-helical aromatic foldamer hybrids

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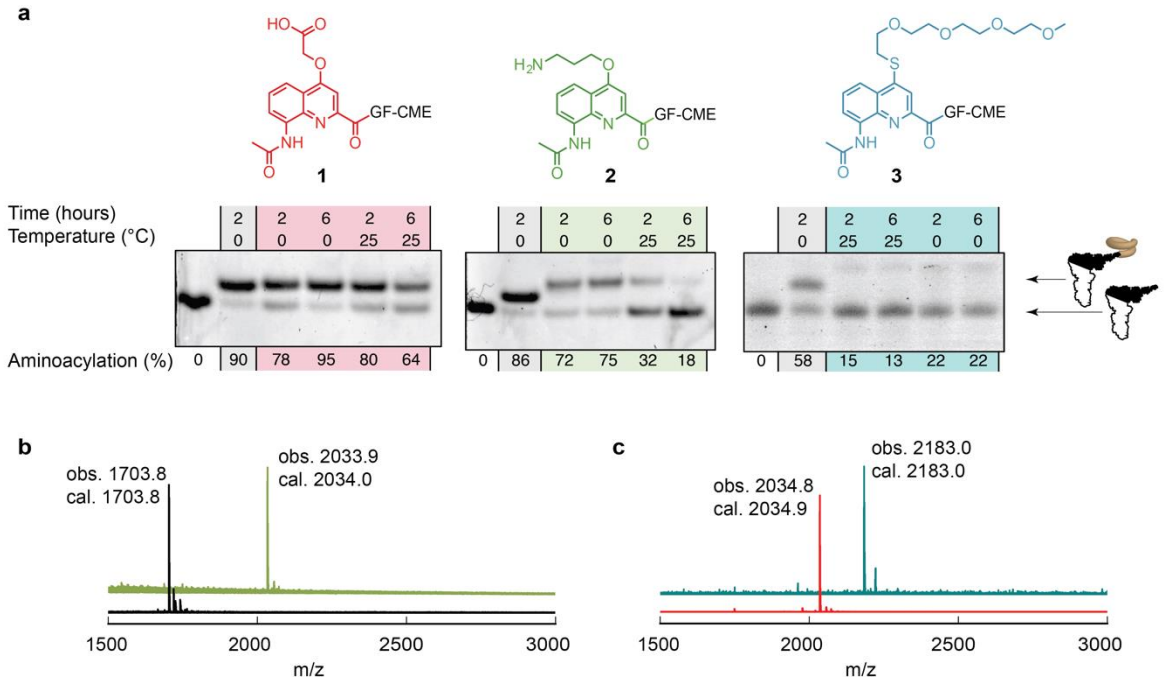
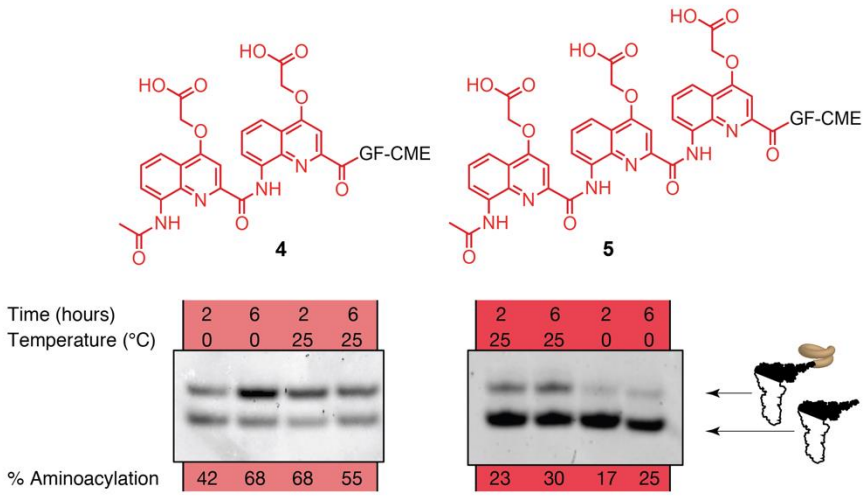
1. Supplementary Figures

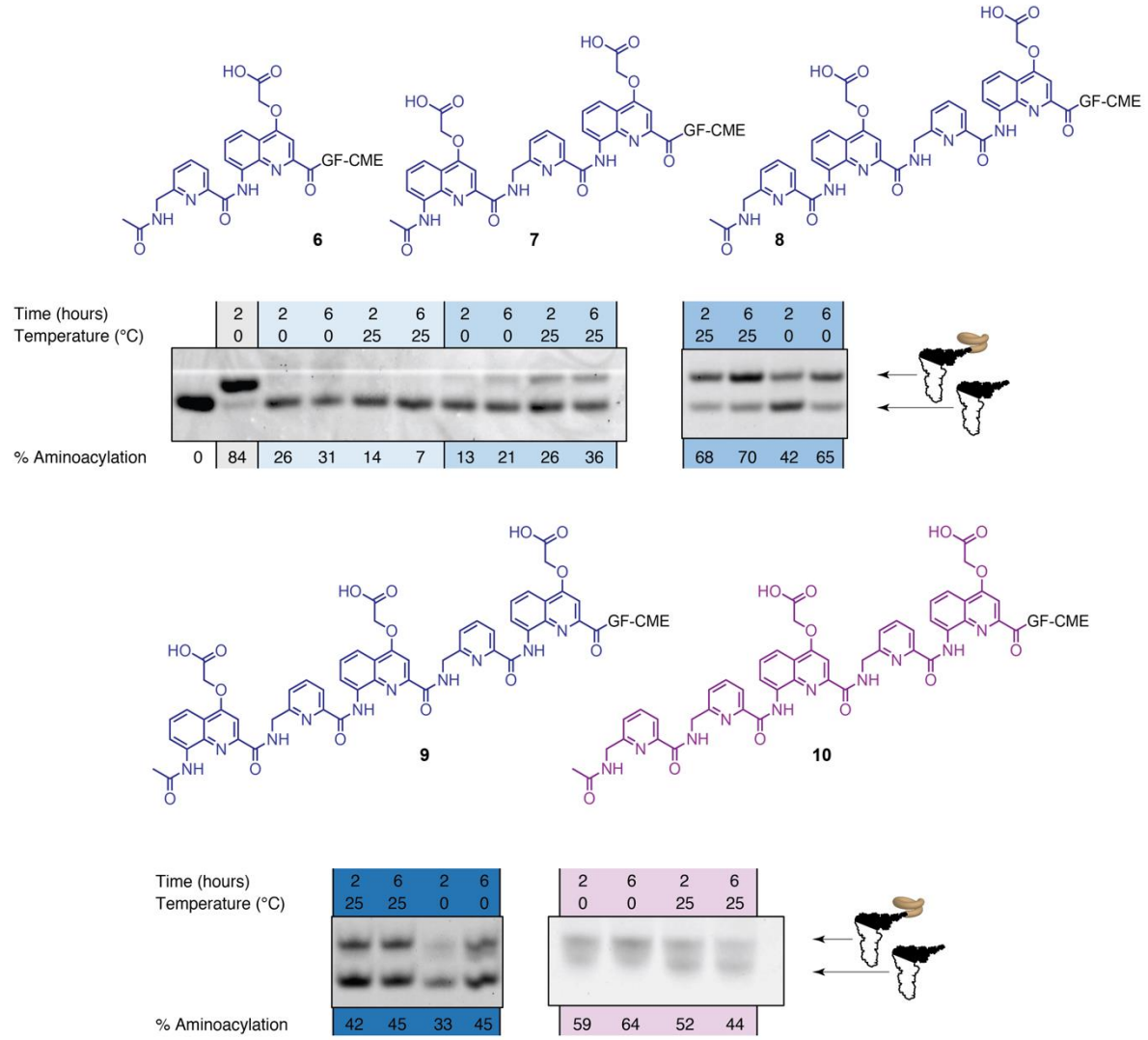
1.1. *In vitro* translation



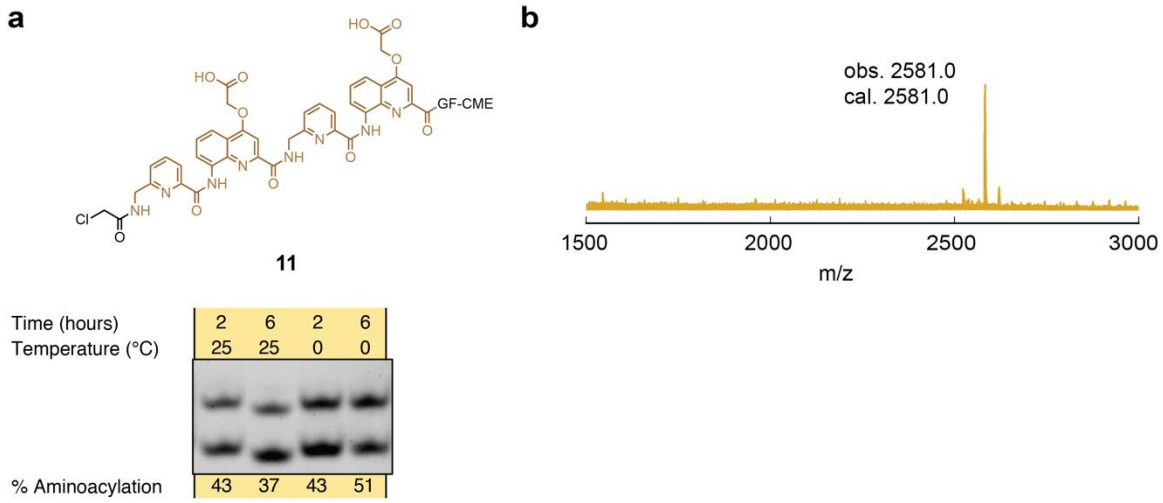
Supplementary Figure 1. Aminoacylation of tRNA mimic is possible for substrates containing a Q monomer with different side-chains, and these substrates are accepted by the ribosome during *in vitro* translation. (a) Aminoacylation of microhelix (RNA mimic of tRNA acceptor step) using substrates **1**-**3**. Substrates are activated as cyanomethyl esters (CME) through the glycine phenylalanine linker that allows for attachment of the foldamer moiety to the RNA by flexizyme. Products were separated using a denaturing acrylamide gel: microhelix without foldamer attached migrates faster (lower band) than microhelix with foldamer attached (upper band). (b-c) Products detected by MALDI-TOF-MS after coupled *in vitro* transcription and translation using a DNA template that encodes for KKKFDYKDDDDK after the initiator (as shown in Fig. 1f). *In vitro* translation product with 'natural' formyl methionine is shown in black. Substrates **1**-**3** were attached to initiator tRNA using flexizyme, these complexes were added to *in vitro* translation mixtures lacking the amino acids methionine. The expected products of foldamer-peptide hybrids could be detected for all substrates: **1** (red), **2** (blue) and **3** (green). (obs. = observed mass, cal. = calculated for $M+H^+$).



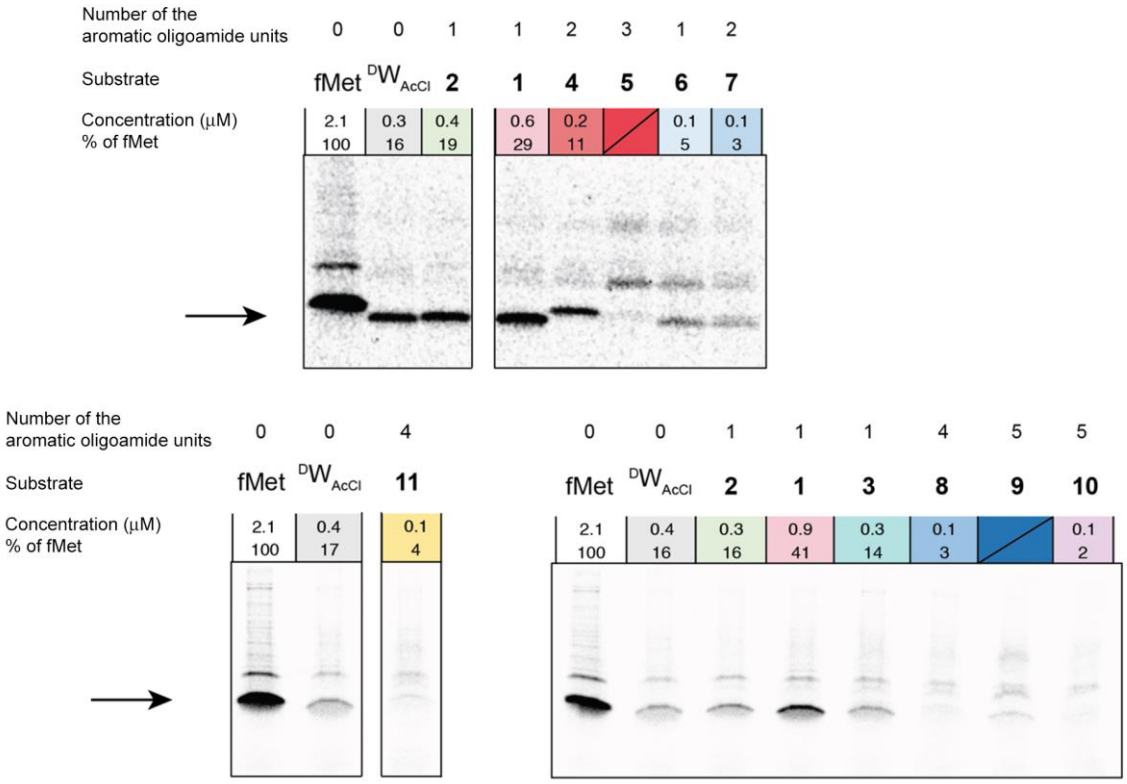
Supplementary Figure 2. Aromatic oligoamide foldamers are suitable substrates for flexizyme-mediated aminoacylation. Substrates containing two or three monomers of **Q** (with aspartate-like side-chains), **4** and **5** respectively, could be attached to a tRNA mimic using flexizyme.



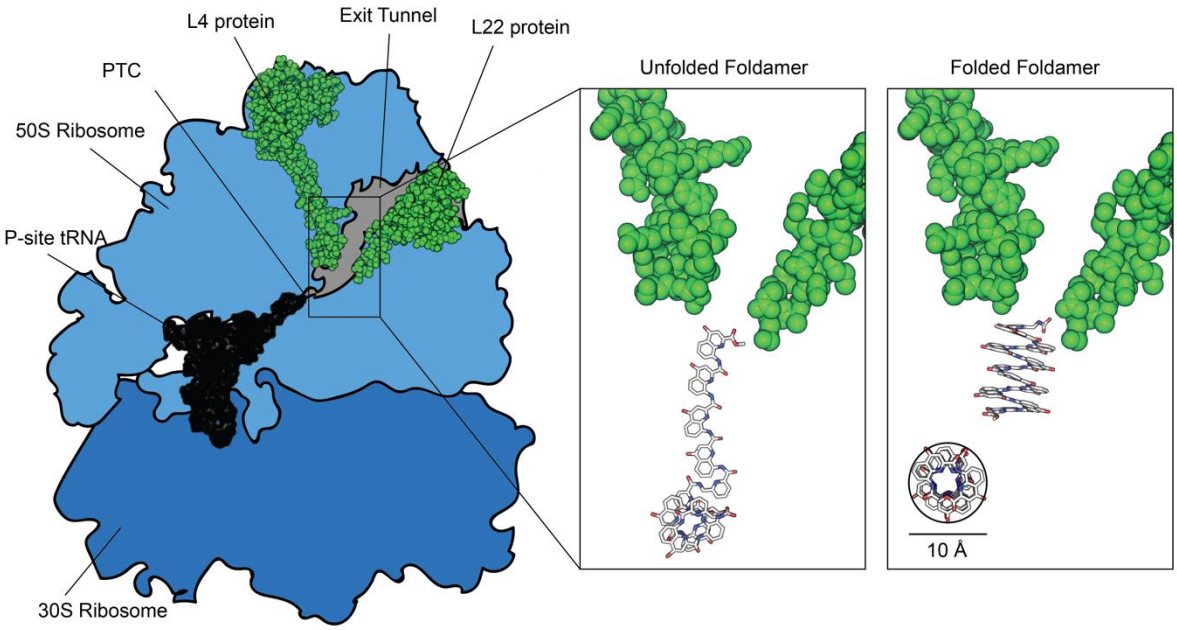
Supplementary Figure 3. Aromatic oligoamide foldamers are suitable substrates for flexizyme-mediated aminoacylation. Substrates containing foldamer oligomers made up of **Q** (with aspartate-like side-chains) and **P** monomers, **6**-**10**, could be attached to a tRNA mimic using flexizyme.



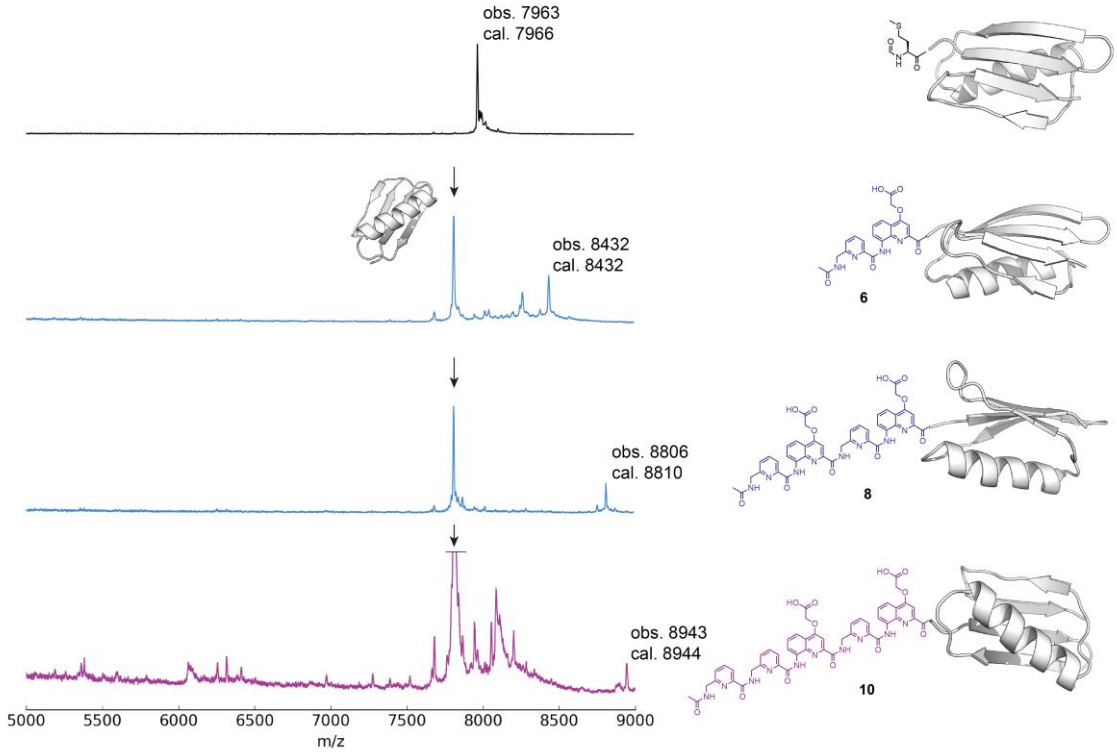
Supplementary Figure 4. Substrate containing an aromatic oligoamide foldamer with an N-terminal chloroacetamide can be used in the aminoacylation of a tRNA mimic and be using to initiate *in vitro* translation. (a) Aromatic foldamer substrate **11** with an N-terminal chloroacetoamide can be attached to a tRNA mimic using flexizyme. (b) Substrate **11** could be attached to an initiator tRNA using flexizyme and initiate *in vitro* translation, giving the expected foldamer-peptide hybrid detected using MALDI-TOF-MS.



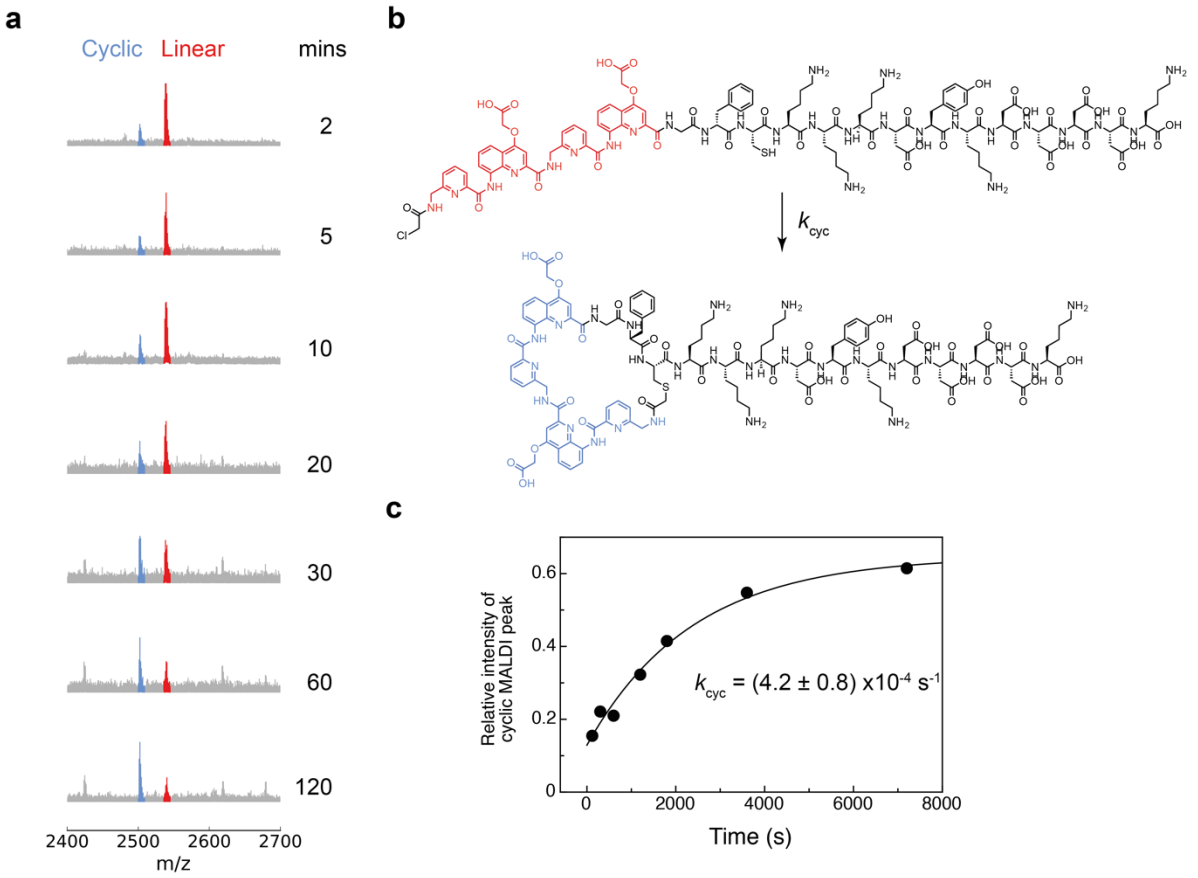
Supplementary Figure 5. Substrates containing fewer aromatic oligoamide units initiate peptide synthesis more efficiently. Translation reactions were performed using radioisotope C^{14} enriched aspartate, allowing detection of the translation product after separation using denaturing gel electrophoresis. The entire translation reaction was loaded, without flag-tag purification. Arrow indicates the major product (corresponding to the expected product), the intensity of this band was compared to that for free aspartate (not shown) to calculate the concentration of product. Lane labelled fMet corresponds to initiation using formyl methionine (i.e. no genetic code reprogramming). Lane labelled $^{D}W_{ClAc}$ corresponds to N-terminal ClAc tryptophan with D- stereochemistry, a commonly used substrate for genetic code reprogramming. No calculated concentrations are given for substrates where the expected product was not detected by MALDI-TOF-MS. Lower yields were observed for the substrates containing longer foldamer units, often with no clear dominant band, even when the correct product could be detected using MALDI-TOF-MS. Note: migration of small peptides on these Tricine-SDS gels is sequence dependent, slower migration (further up the gel) does not necessarily mean a larger product.



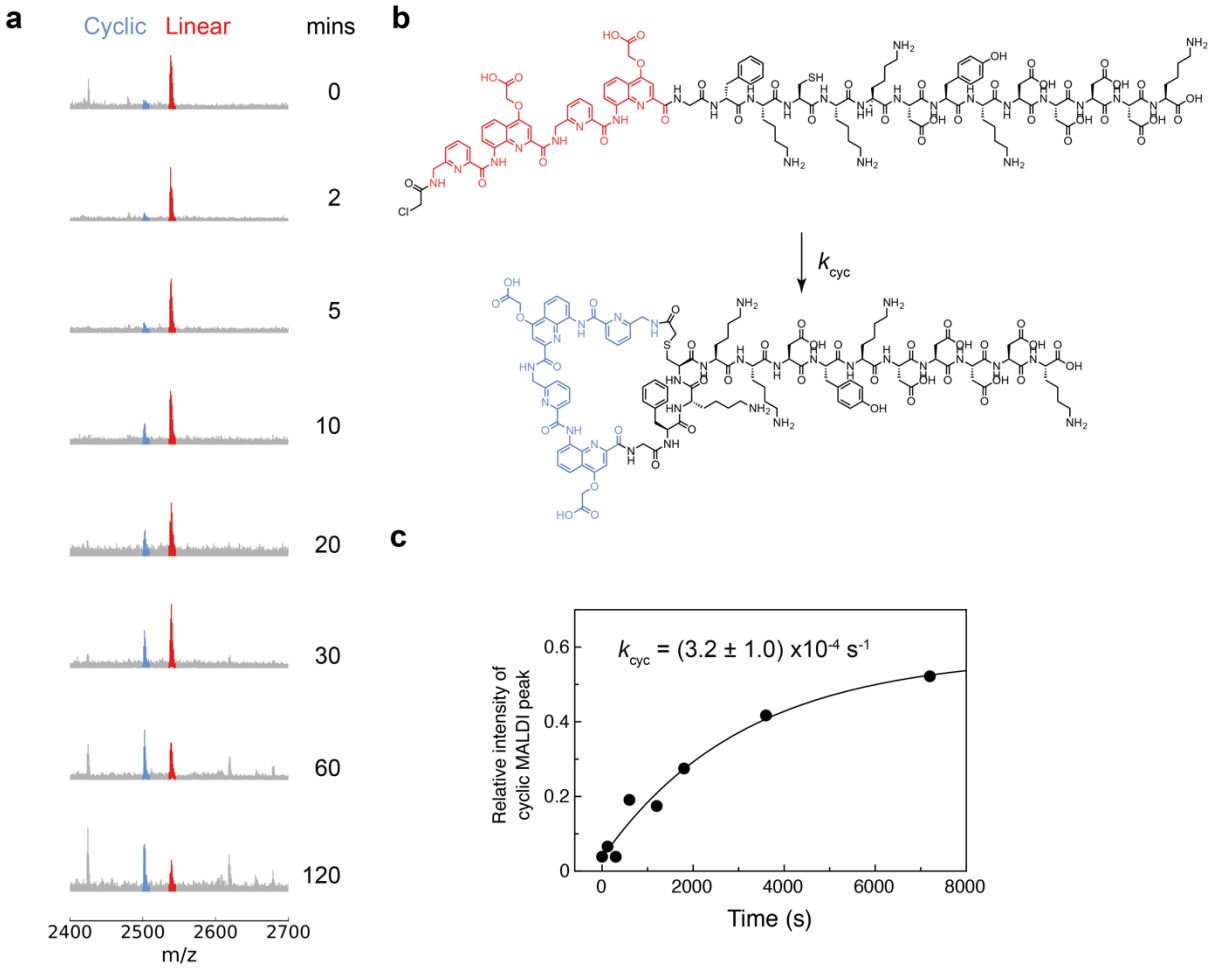
Supplementary Figure 6. The geometry of the exit tunnel may prevent folded, helical aromatic oligoamides from passing, but allow the unfolded conformation. During peptide synthesis by the ribosome (blue), the growing peptide chain must pass from the peptidyl transfer center (PTC), through the exit tunnel and into the free solution. The narrowest point in the tunnel is ~1 nm in diameter where the two ribosomal proteins L4 and L22 (green) are nearest. The size of this constriction is such that an unfolded foldamer (sticks) may be able to pass this narrowest point, whereas the well-folded, helical foldamer may not. Helix shown is the crystal structure of PQQQQPQQQQ with the side-chain removed, as in Figure 1b[1]. Unfolded structure created using manual manipulation (UCSF Chimera). Ribosome and protein structures taken from pdb 5AFI[2].



Supplementary Figure 7. Initiation of protein synthesis using aromatic oligoamide foldamer substrates. MALDI-TOF-MS spectra, from top to bottom: Translation of Protein G (plus C-terminal flag tag) with no genetic code reprogramming, i.e. initiation with formyl methionine (black); Initiation using substrate **6** (blue), the major product (indicated with an arrow) corresponds to Protein G starting at the second position, a feature of *in vitro* translation initiation using difficult substrates. However, the desired product can be observed; Initiation using substrate **8** (blue); Initiation using the largest foldamer in this study, substrate **10** (purple). The low yield of this reaction means the desired product is one of the minor products, the peak corresponding to second position initiation is cropped.

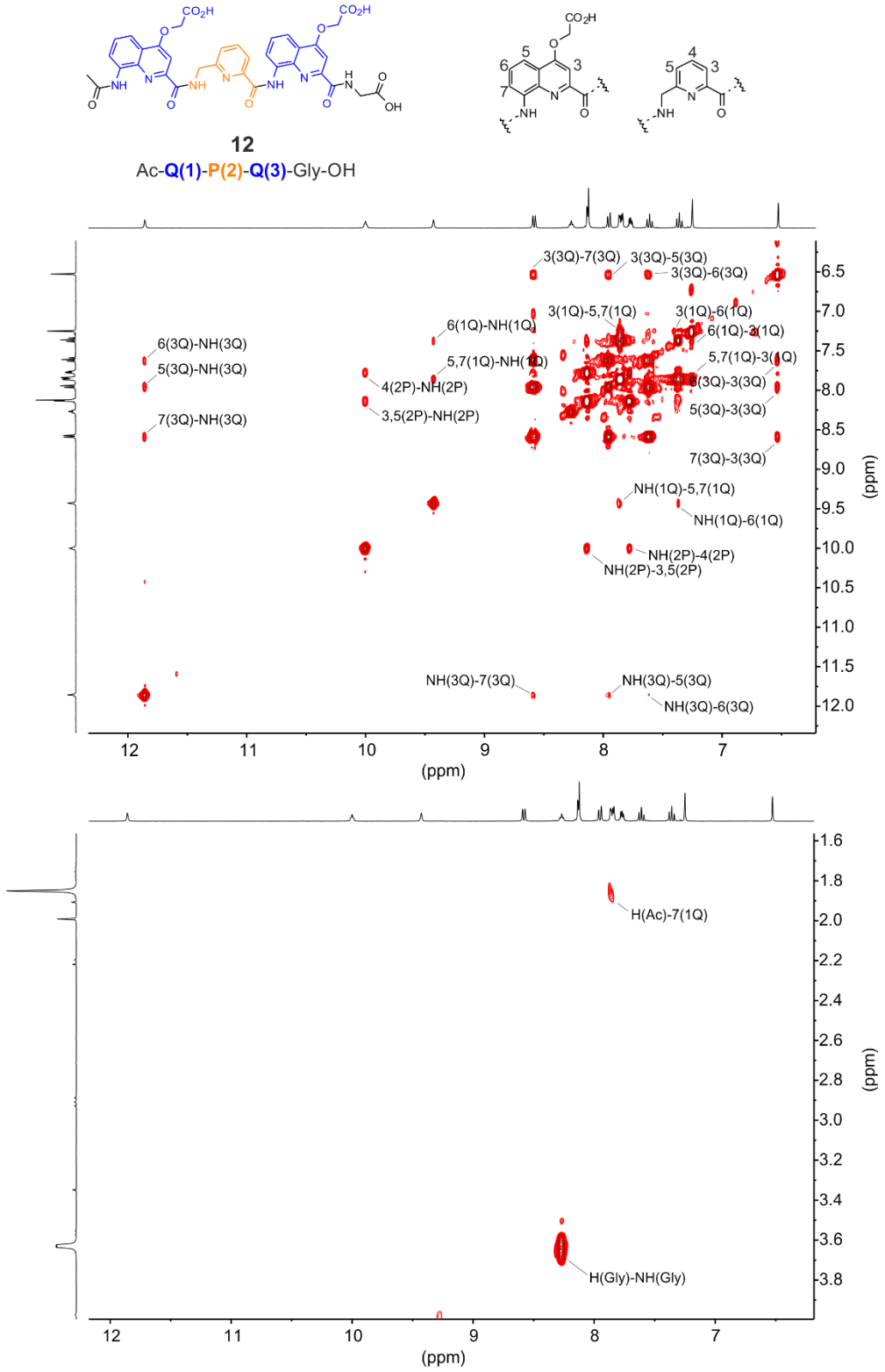


Supplementary Figure 8. Macrocyclization kinetics of a foldamer-peptide hybrid with cysteine in position 2. (a) MALDI-TOF-MS spectra of translation products using substrate **11** and a DNA template coding for cysteine immediately after the foldamer initiator. The peak corresponding to the linear product decreases in intensity over time, whereas the peak corresponding to the cyclic product increases. (b) Macrocyclization reaction between chloroacetoamide (ClAc) group and thiol of cysteine side chain. (c) Relative MALDI peak intensity of cyclic product (compared to the total for linear and cyclic products) over time can be fit to a single exponential process with the rate constant k_{cyc}. It is assumed that MALDI ionization efficiency is the same for both linear and cyclic forms.

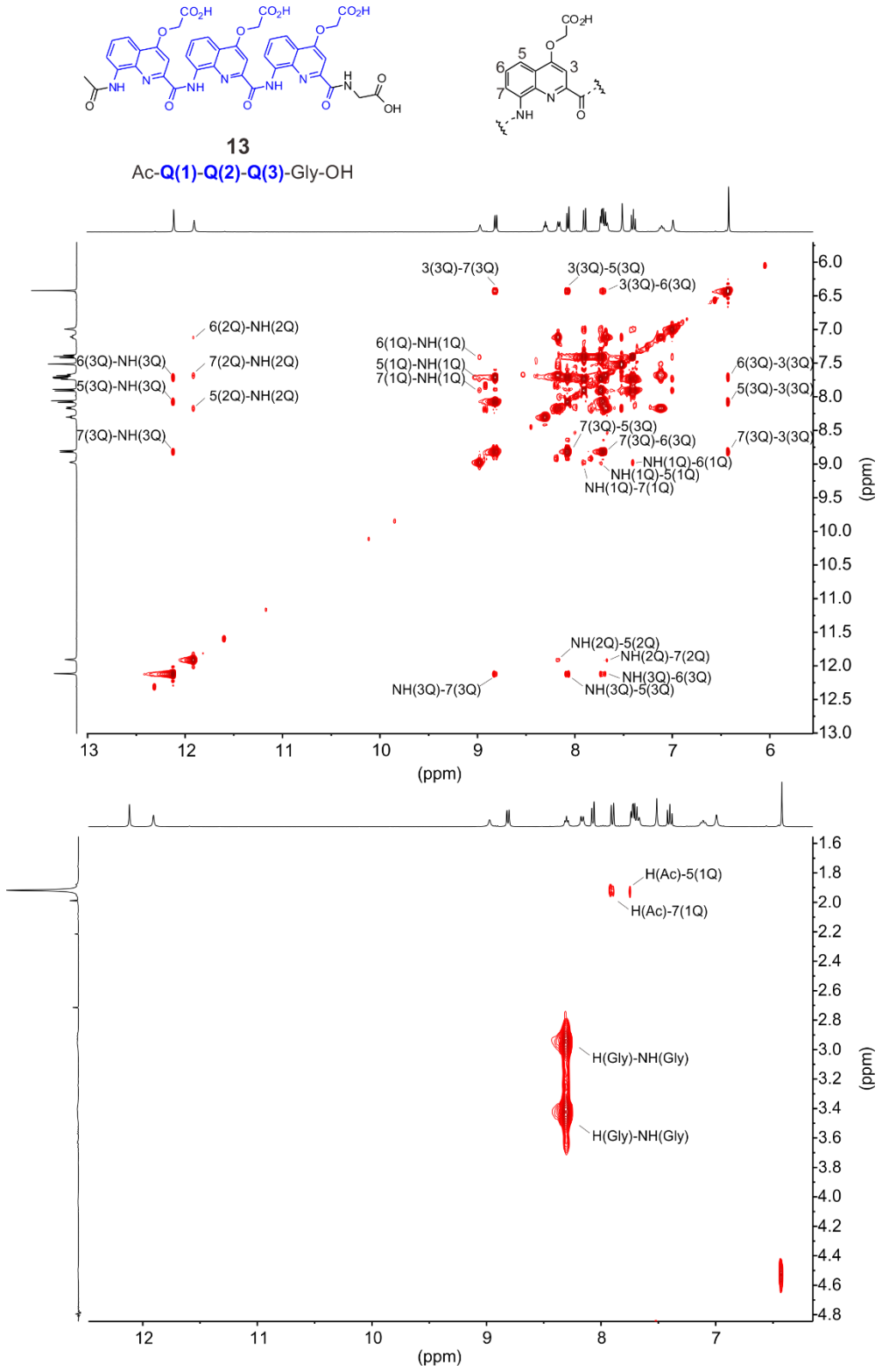


Supplementary Figure 9. Macrocyclization kinetics of a foldamer-peptide hybrid with cysteine in position 3. (a) MALDI-TOF-MS spectra of translation products using substrate **11** and a DNA template coding for cysteine at the third position. The peak corresponding to the linear product decreases in intensity over time, whereas the peak corresponding to the cyclic product increases. (b) Macrocyclization reaction between chloroacetoamide (ClAc) group and thiol of cysteine side chain. (c) Relative MALDI peak intensity of cyclic product (compared to the total for linear and cyclic products) over time can be fit to a single exponential process with the rate constant k_{cyc}. It is assumed that MALDI ionization efficiency is the same for both linear and cyclic forms.

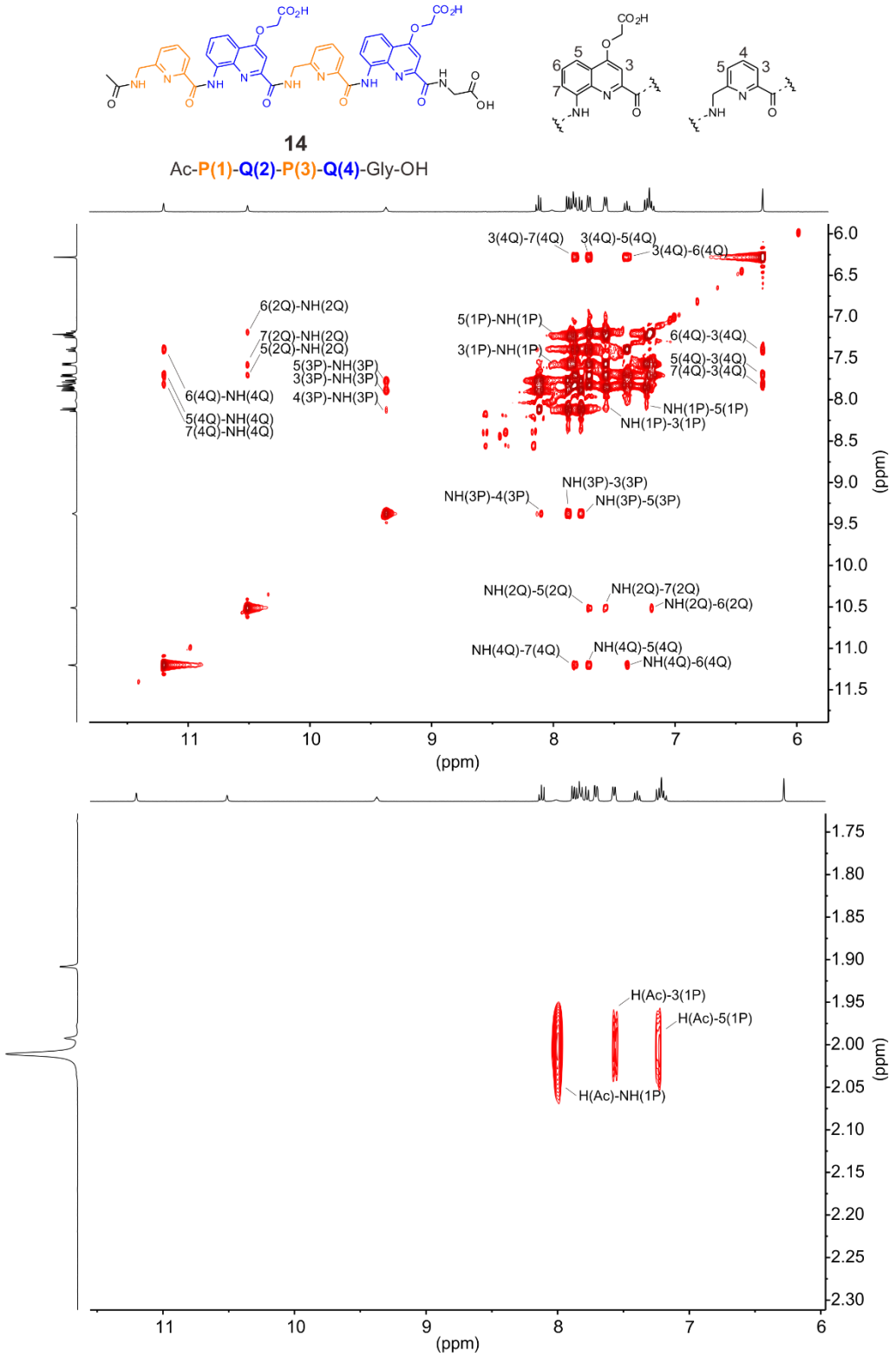
1.2. Solution-state structural studies



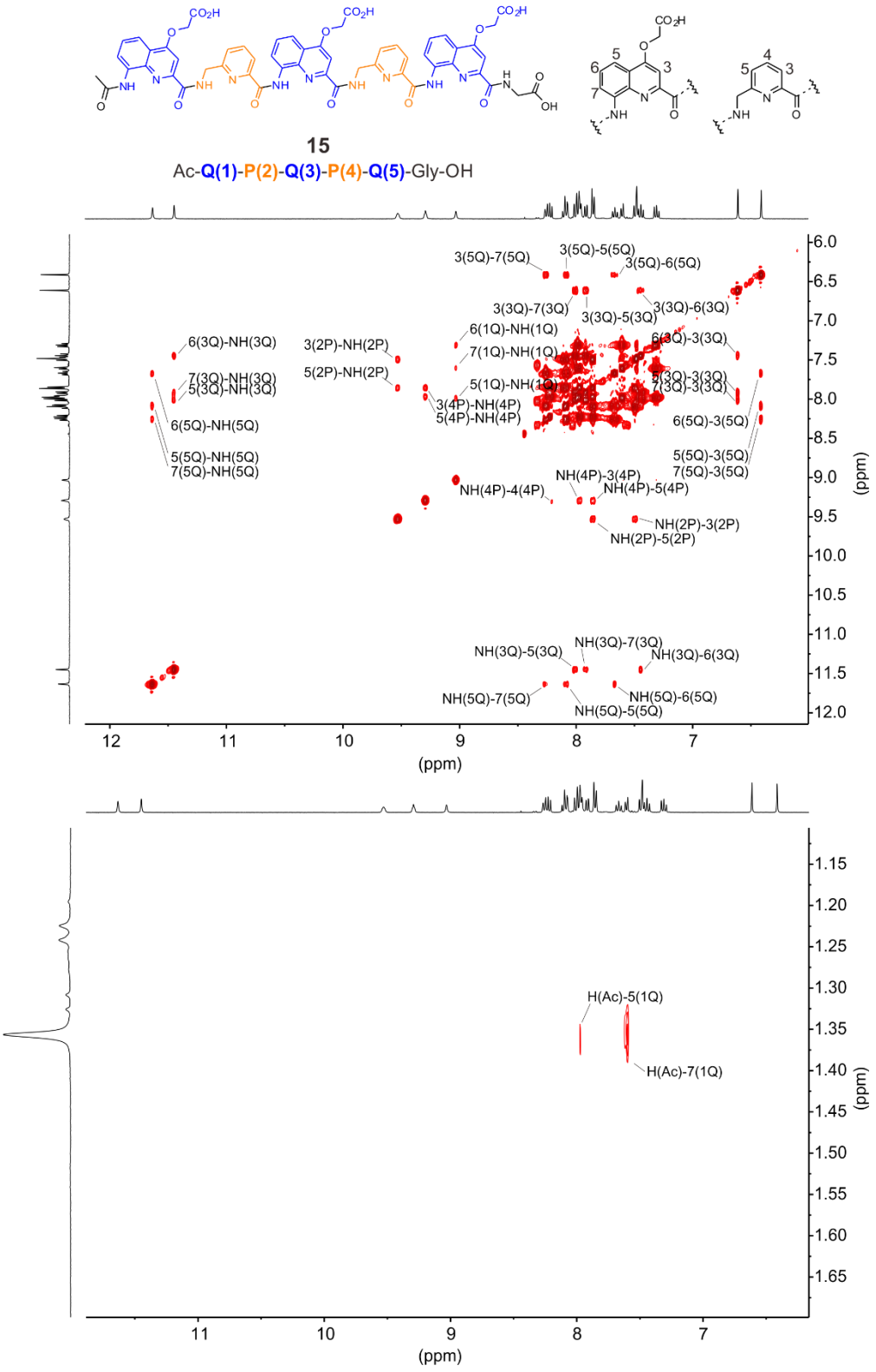
Supplementary Figure 10. Excerpts from TOCSY spectrum of **12** in H_2O/D_2O (9:1 vol/vol, with 50 mM $NaHCO_3$) at room temperature.



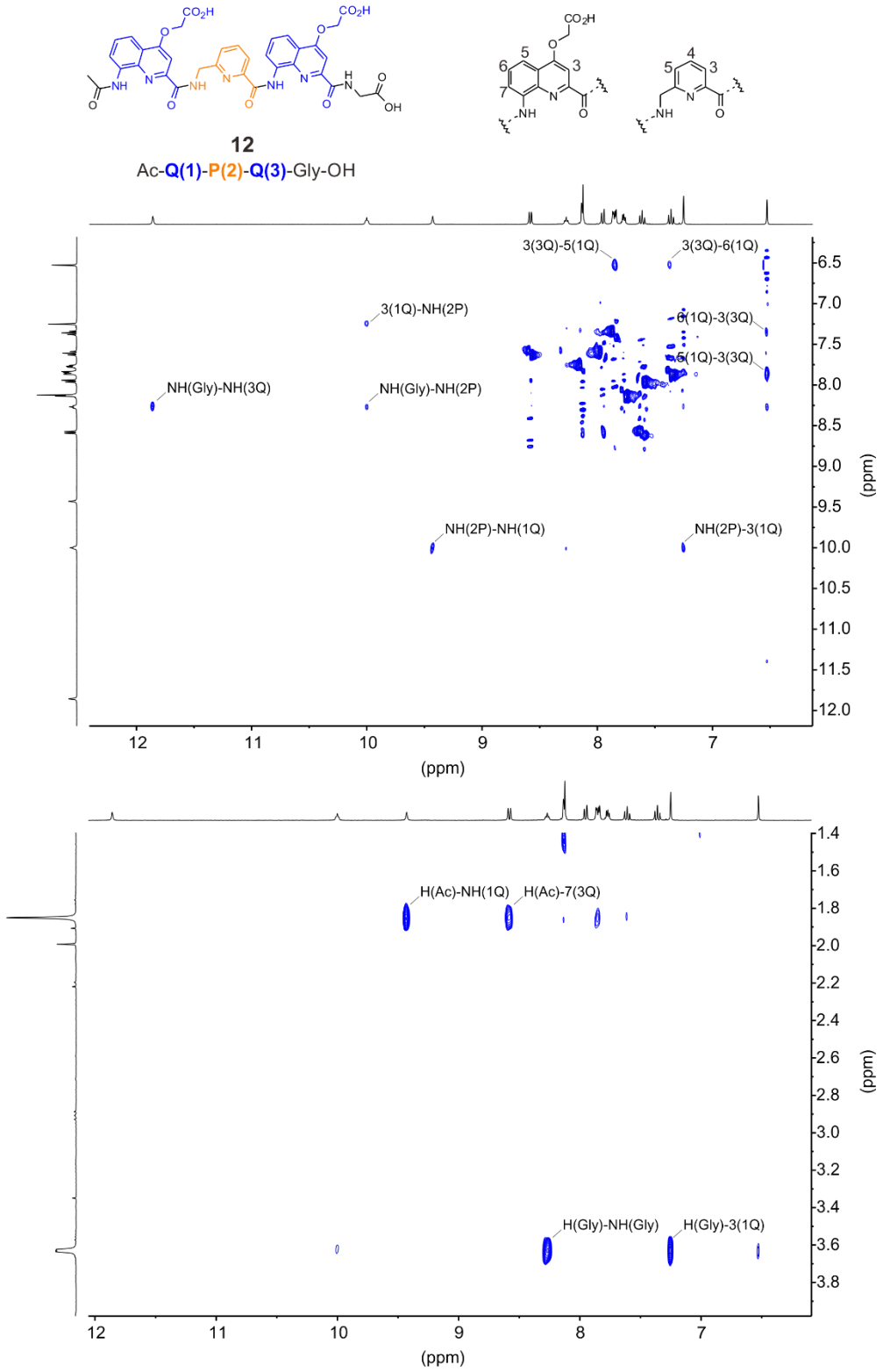
Supplementary Figure 11. Excerpts from TOCSY spectrum of **13** in H_2O/D_2O (9:1 vol/vol, with 50 mM $NaHCO_3$) at room temperature.



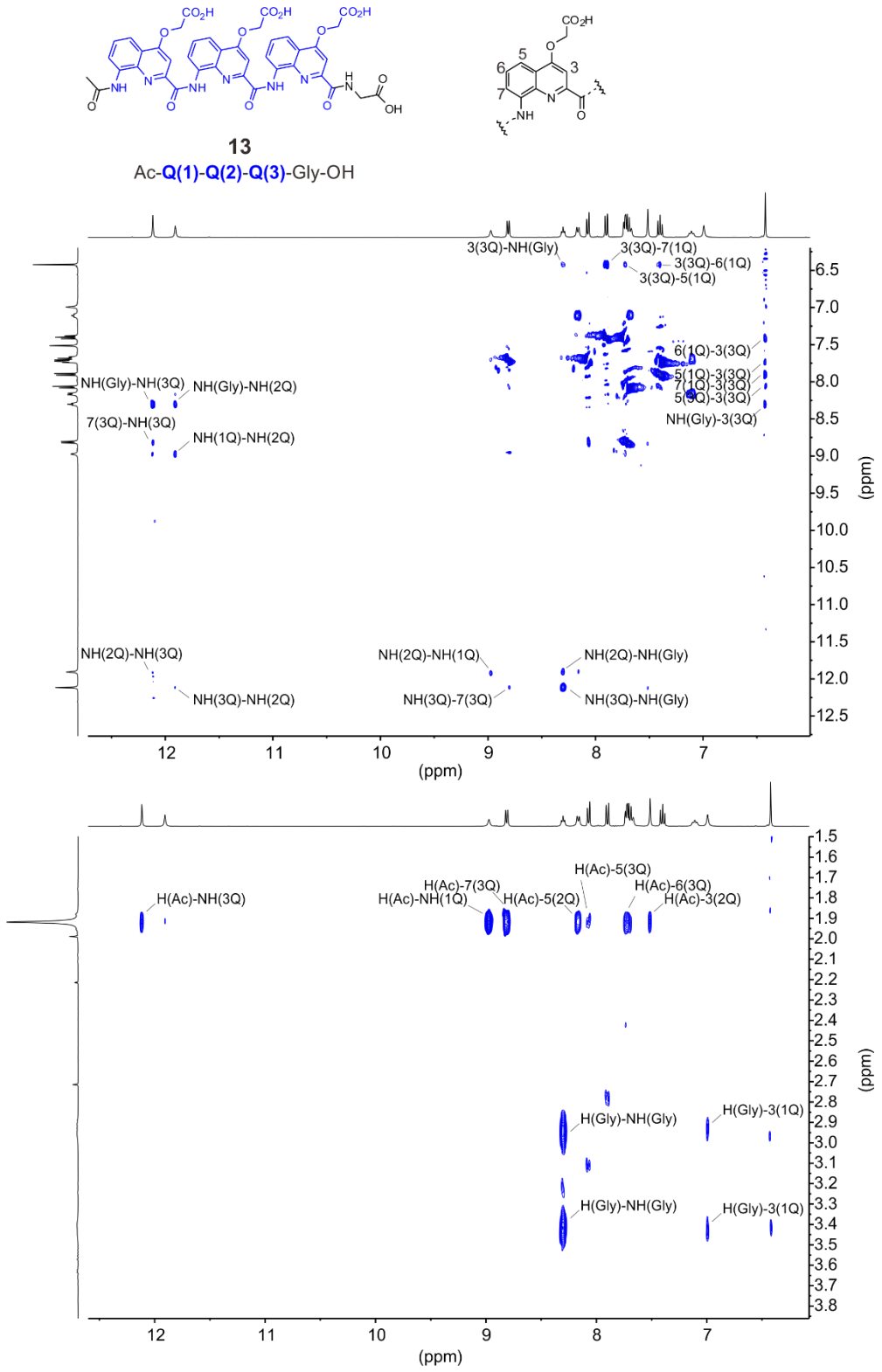
Supplementary Figure 12. Excerpts from TOCSY spectrum of **14** in H_2O/D_2O (9:1 vol/vol, with 50 mM $NaHCO_3$) at room temperature.



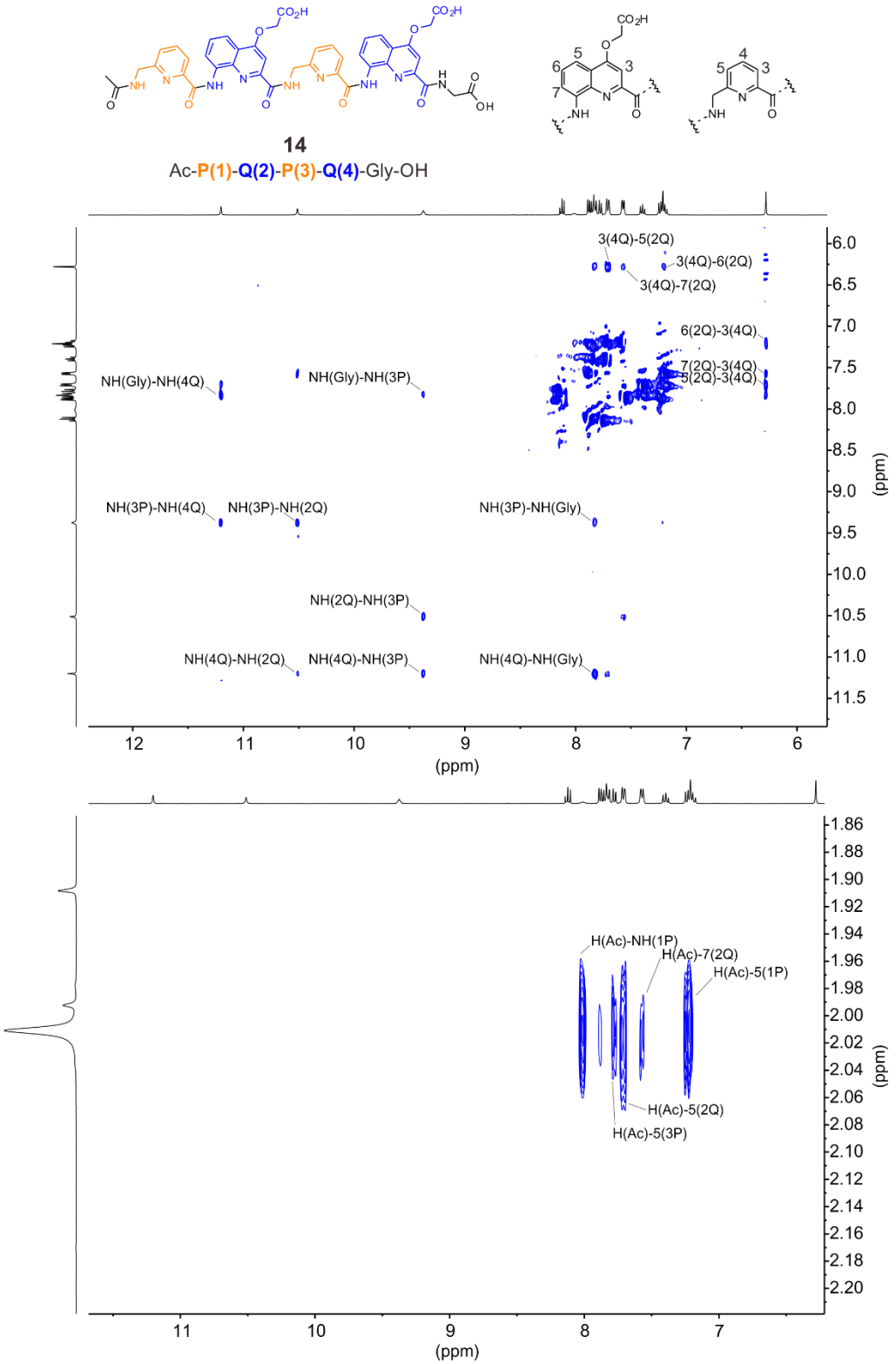
Supplementary Figure 13. Excerpts from TOCSY spectrum of **15** in H_2O/D_2O (9:1 vol/vol, with 50 mM $NaHCO_3$) at room temperature.



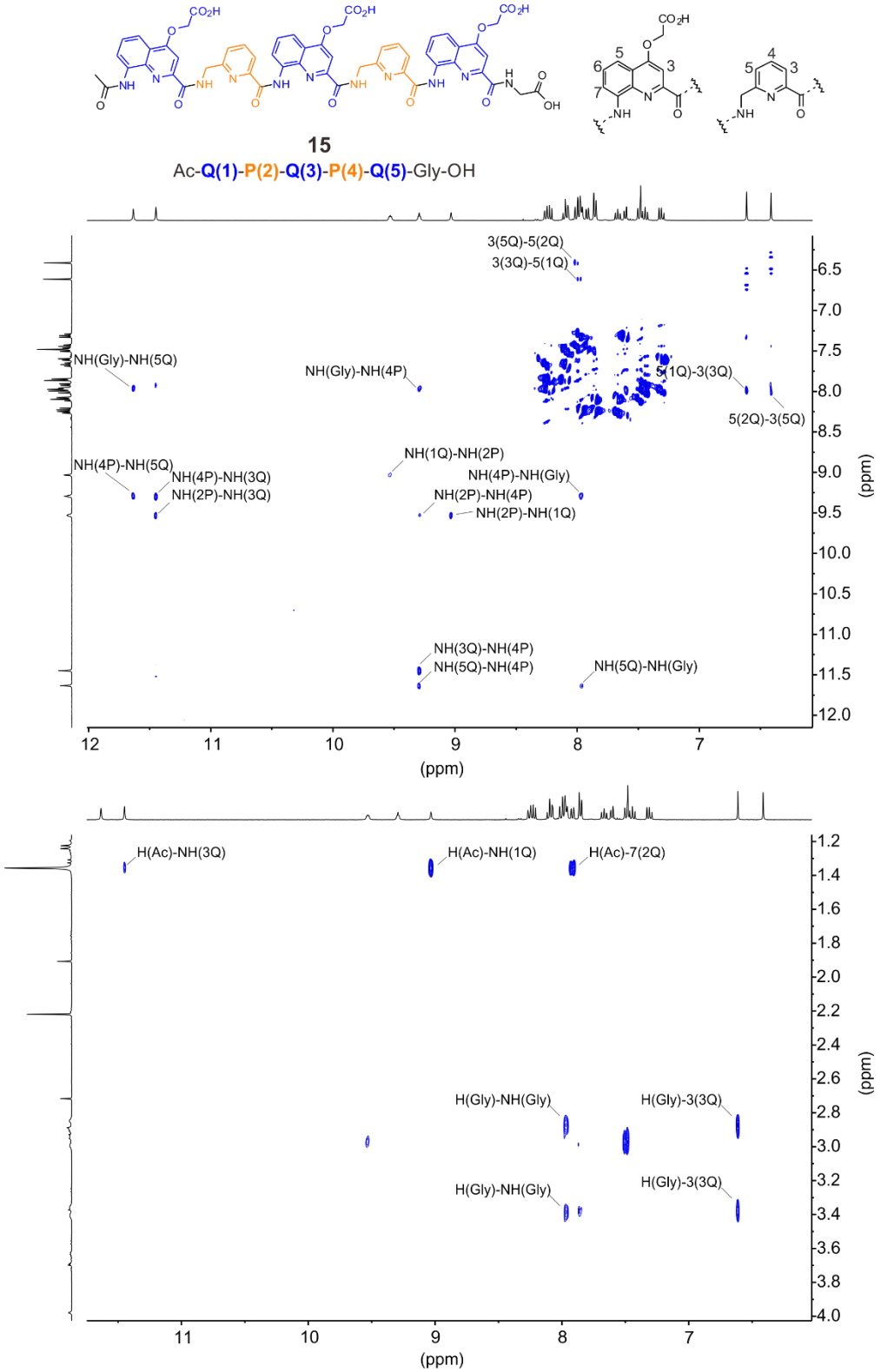
Supplementary Figure 14. Excerpts from ROESY spectrum of **12** in H_2O/D_2O (9:1 vol/vol, with 50 mM $NaHCO_3$) at room temperature.



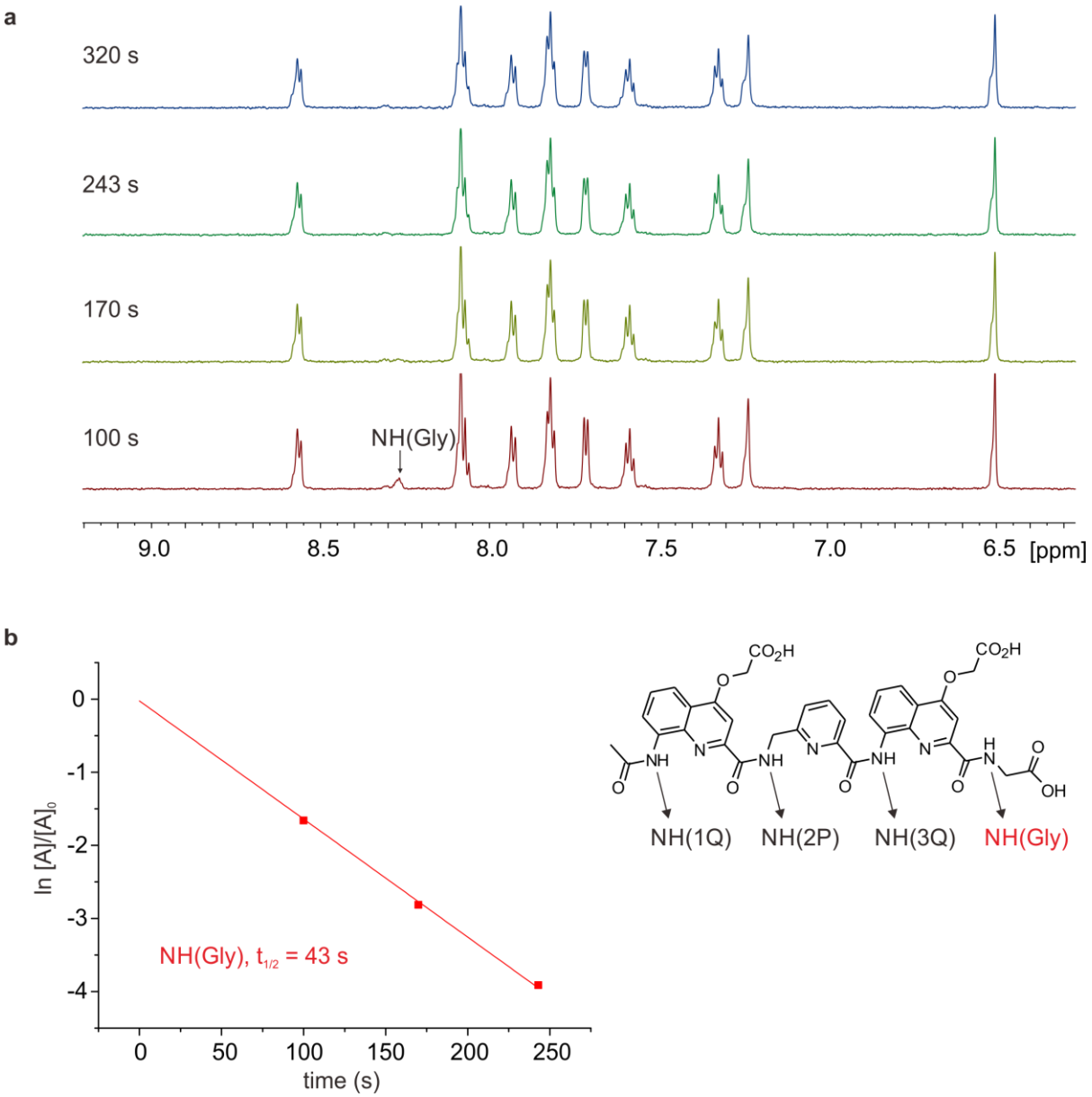
Supplementary Figure 15. Excerpts from ROESY spectrum of **13** in H_2O/D_2O (9:1 vol/vol, with 50 mM $NaHCO_3$) at room temperature.



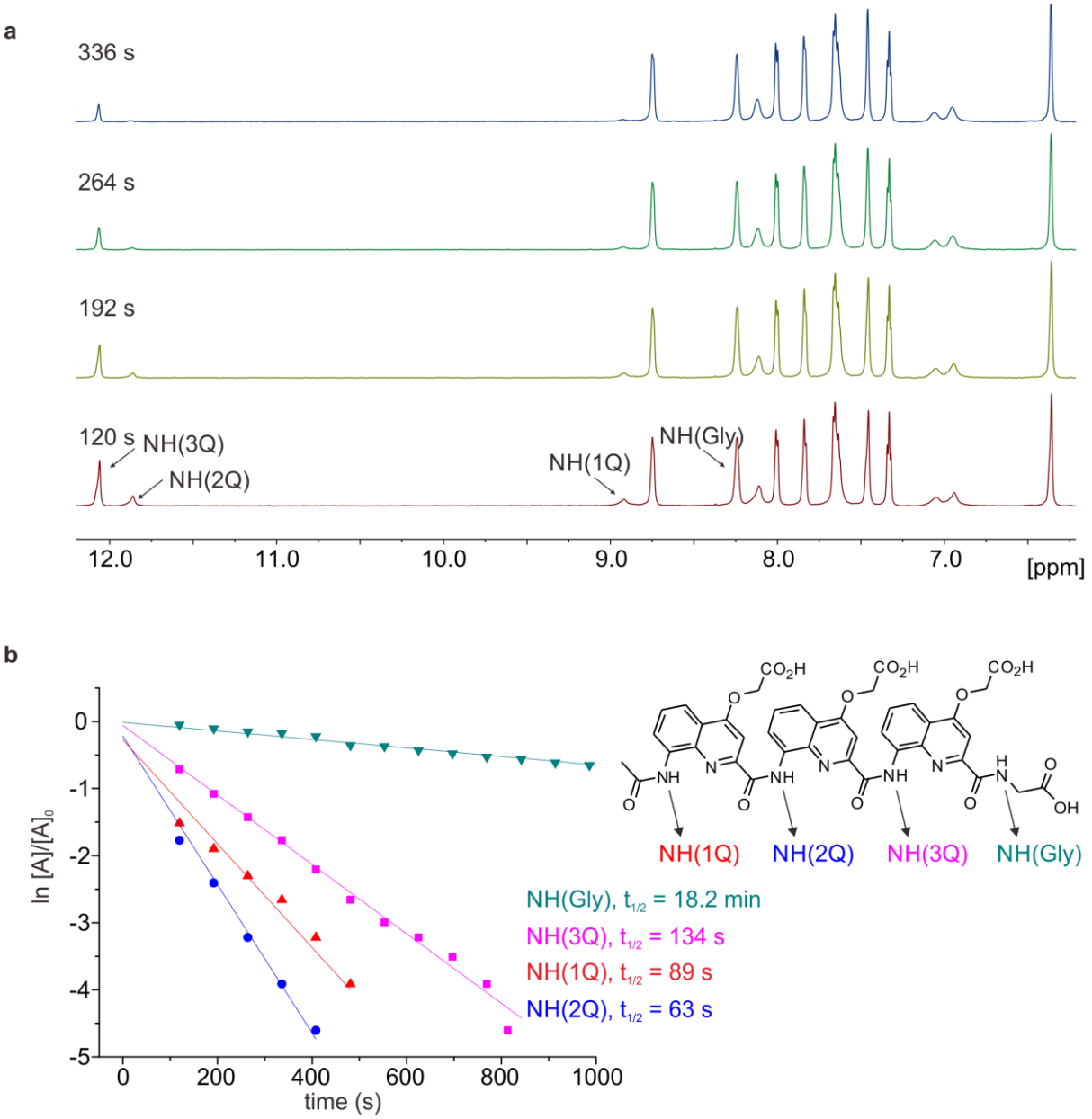
Supplementary Figure 16. Excerpts from ROESY spectrum of **14** in H_2O/D_2O (9:1 vol/vol, with 50 mM $NaHCO_3$) at room temperature.



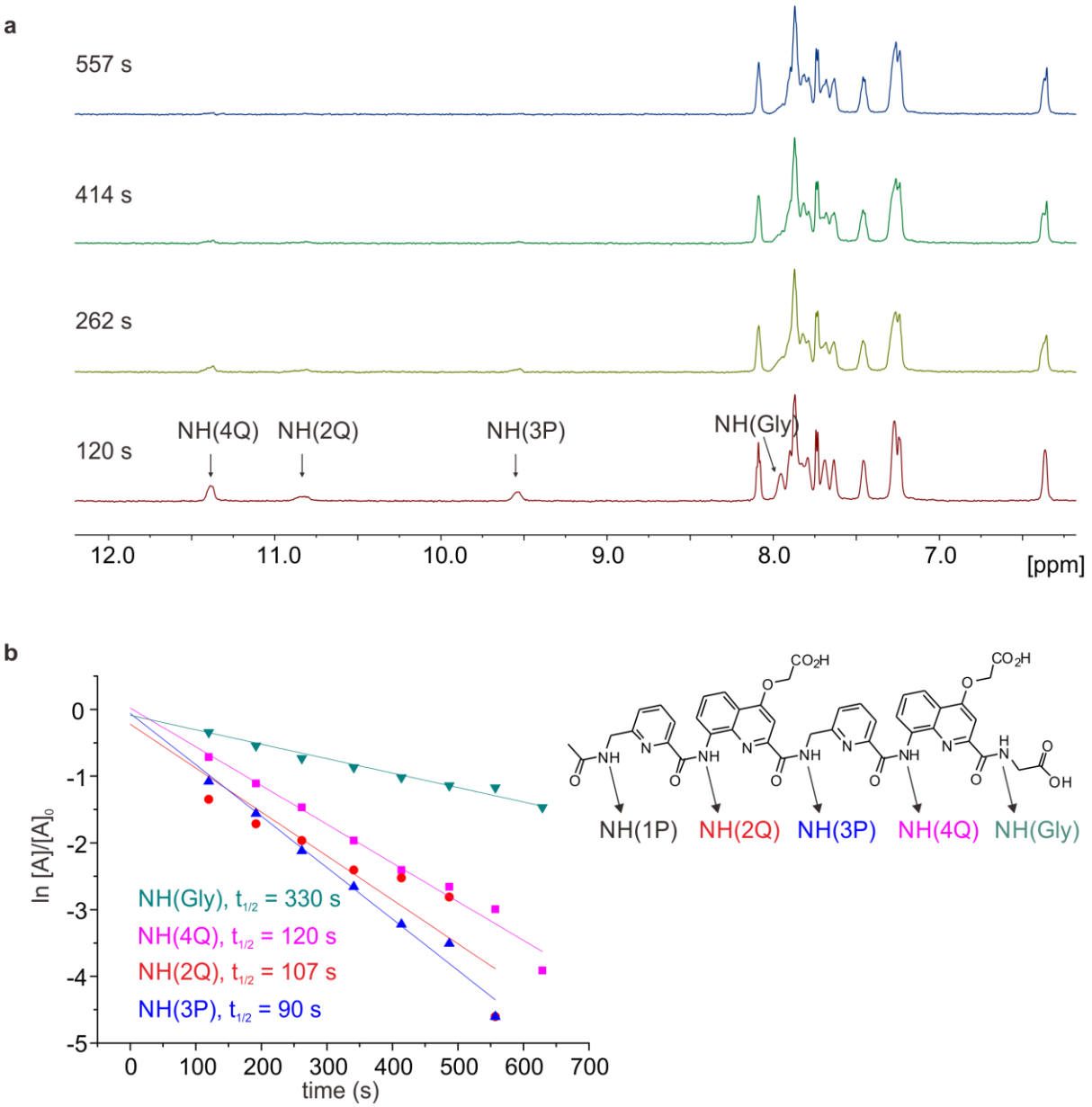
Supplementary Figure 17. Excerpts from ROESY spectrum of **15** in H_2O/D_2O (9:1 vol/vol, with 50 mM $NaHCO_3$) at room temperature.



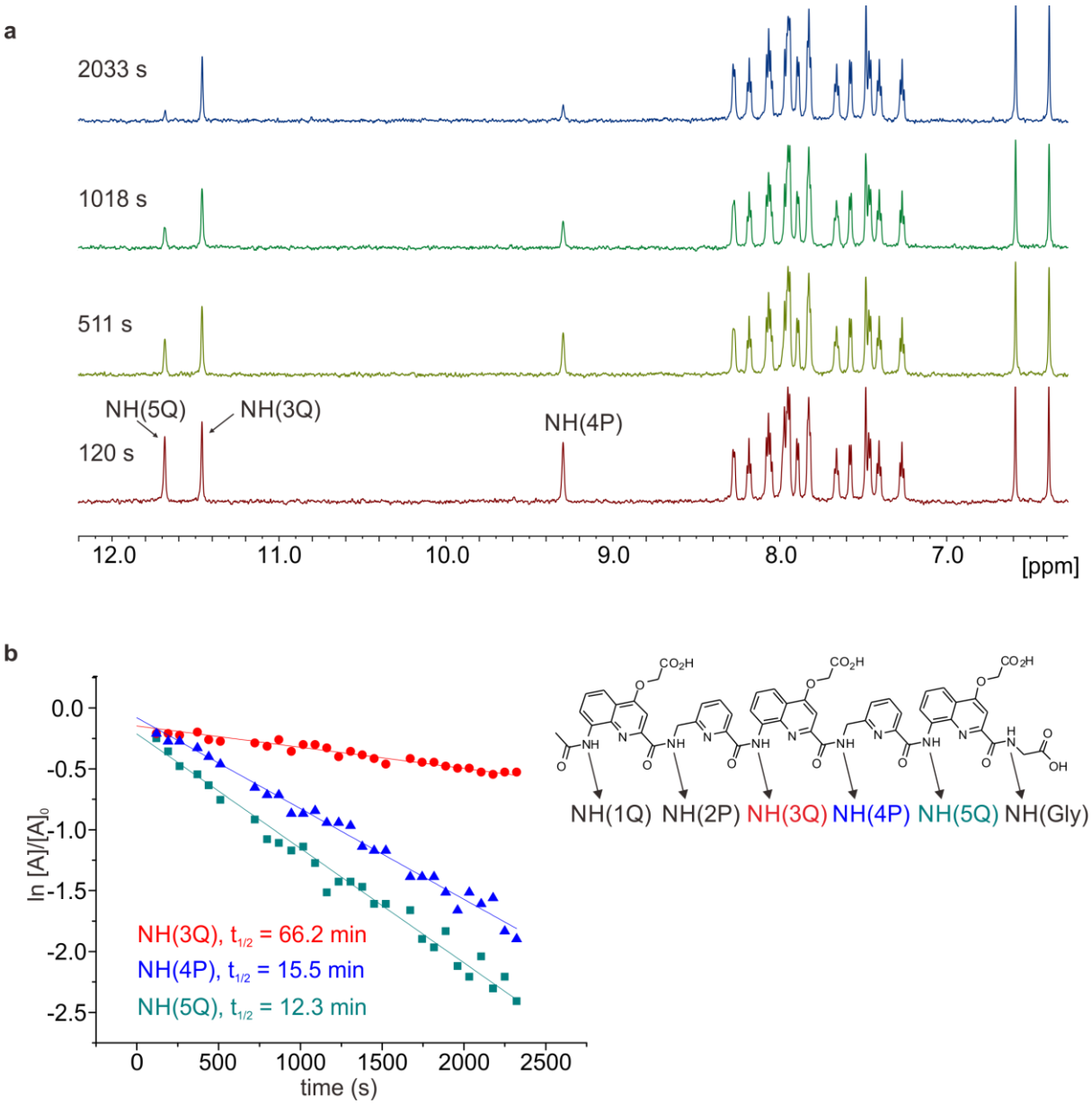
Supplementary Figure 18. (a) Part of ^{1}H spectra of **12** recorded over time at 298 K after dissolution in D_2O (with 50 mM $NaHCO_3$). (b) Pseudo first-order rate plot and half-life ($t_{1/2}$) of amide protons of **12**.



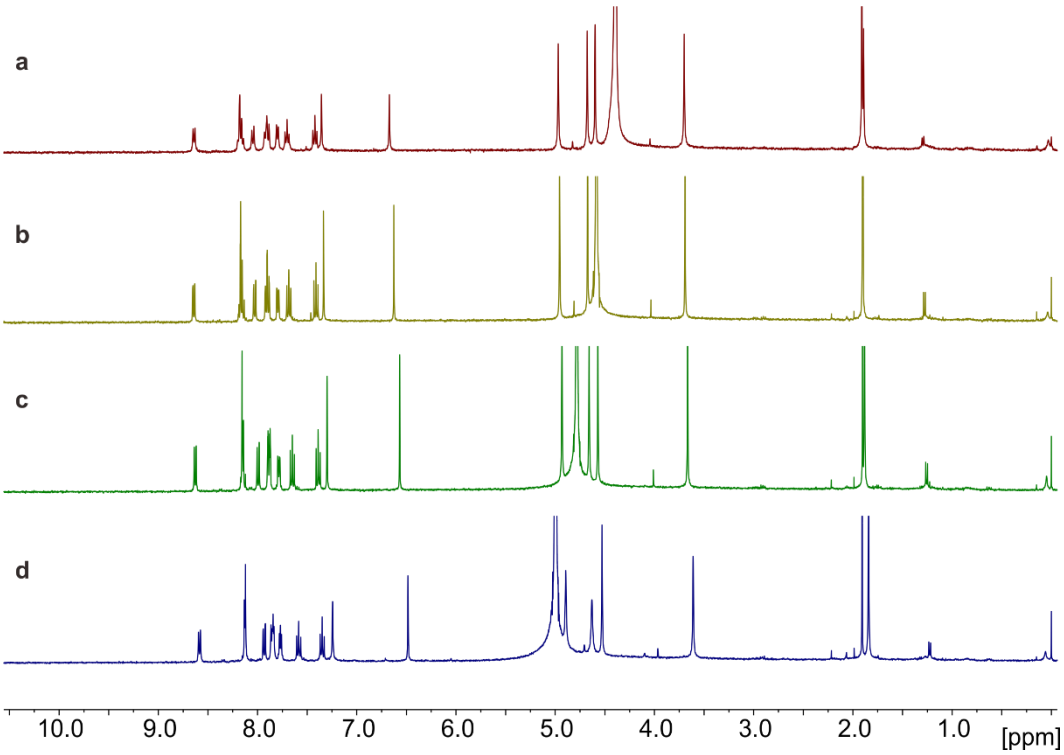
Supplementary Figure 19. (a) Part of ^{1}H spectra of **13** recorded over time at 298 K after dissolution in D_2O (with 50 mM $NaHCO_3$). (b) Pseudo first-order rate plot and half-life ($t_{1/2}$) of amide protons of **13**.



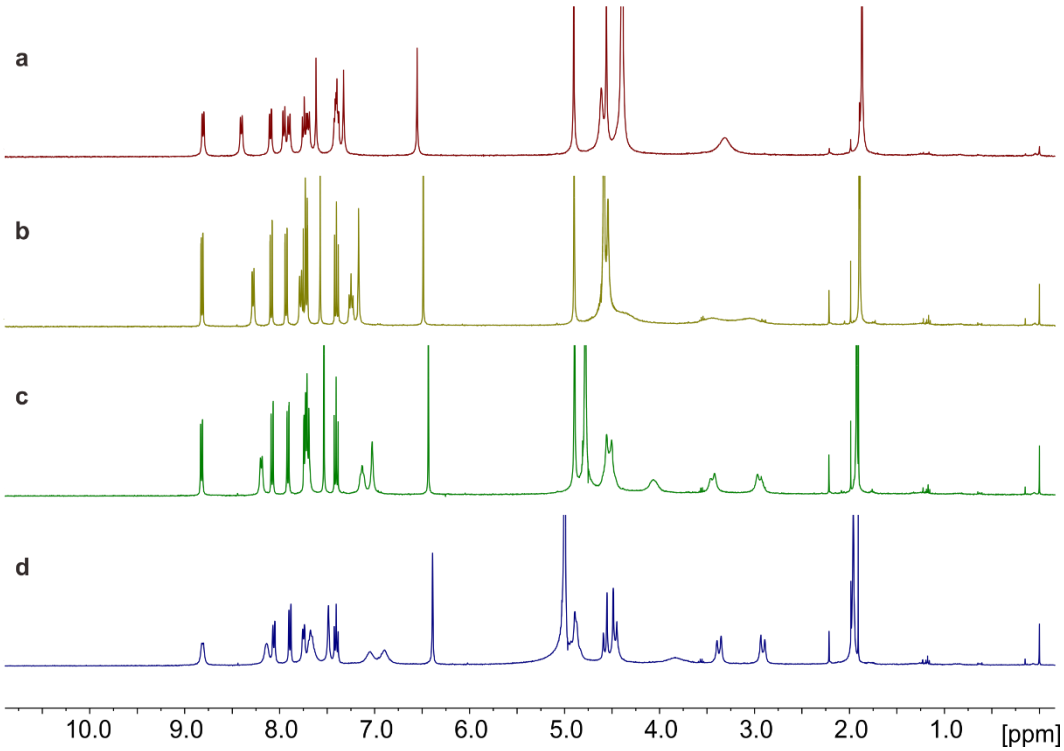
Supplementary Figure 20. (a) Part of ^{1}H spectra of **14** recorded over time at 298 K after dissolution in D_2O (with 50 mM $NaHCO_3$). (b) Pseudo first-order rate plot and half-life ($t_{1/2}$) of amide protons of **14**.



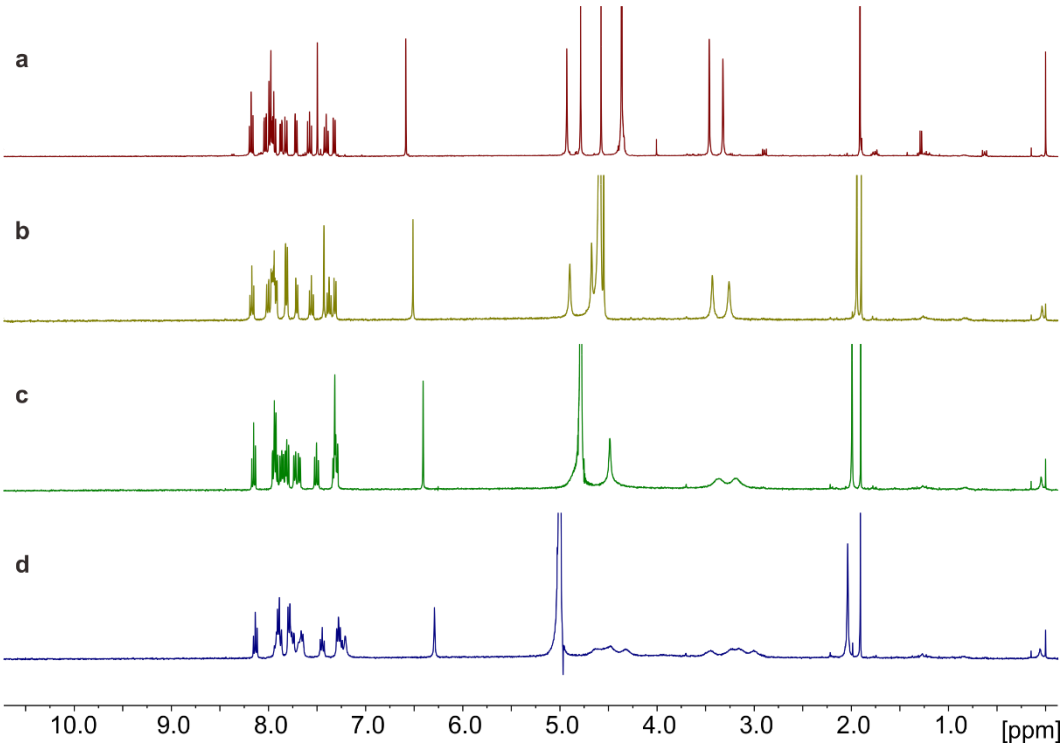
Supplementary Figure 21. (a) Part of ^{1}H spectra of **15** recorded over time at 298 K after dissolution in D_2O (with 50 mM $NaHCO_3$). (b) Pseudo first-order rate plot and half-life ($t_{1/2}$) of amide protons of **15**.



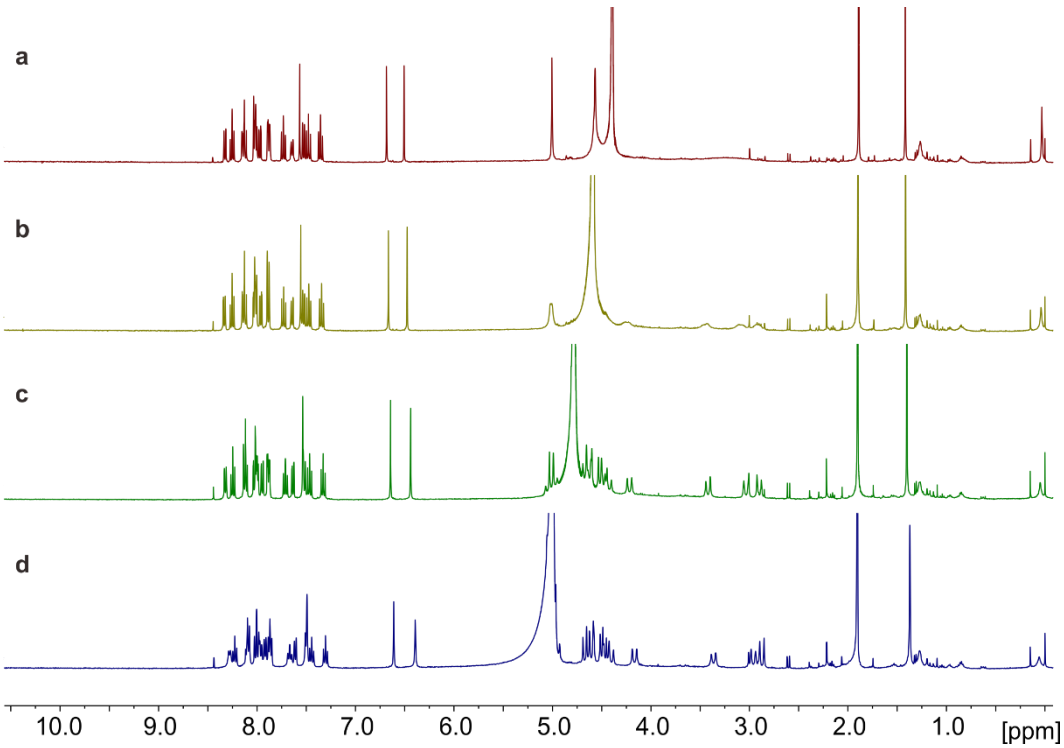
Supplementary Figure 22. ^{1}H spectra of **12** in D_2O (with 50 mM $NaHCO_3$) recorded at (a) 338 K; (b) 318 K; (c) 298 K; and (d) 278 K.



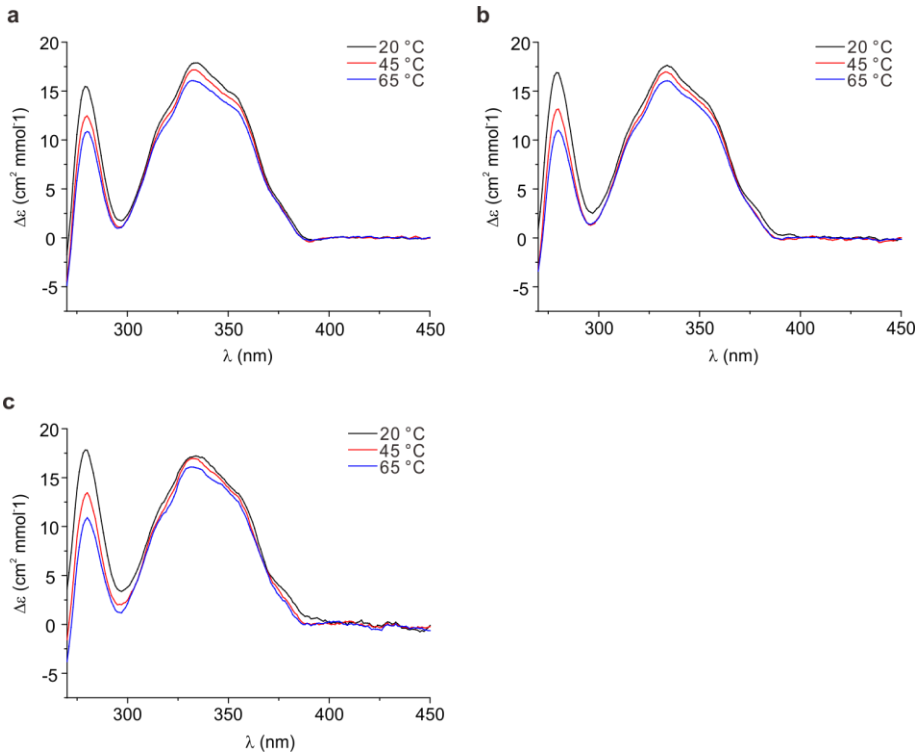
Supplementary Figure 23. ^{1}H spectra of **13** in D_2O (with 50 mM $NaHCO_3$) recorded at (a) 338 K; (b) 318 K; (c) 298 K; and (d) 278 K.



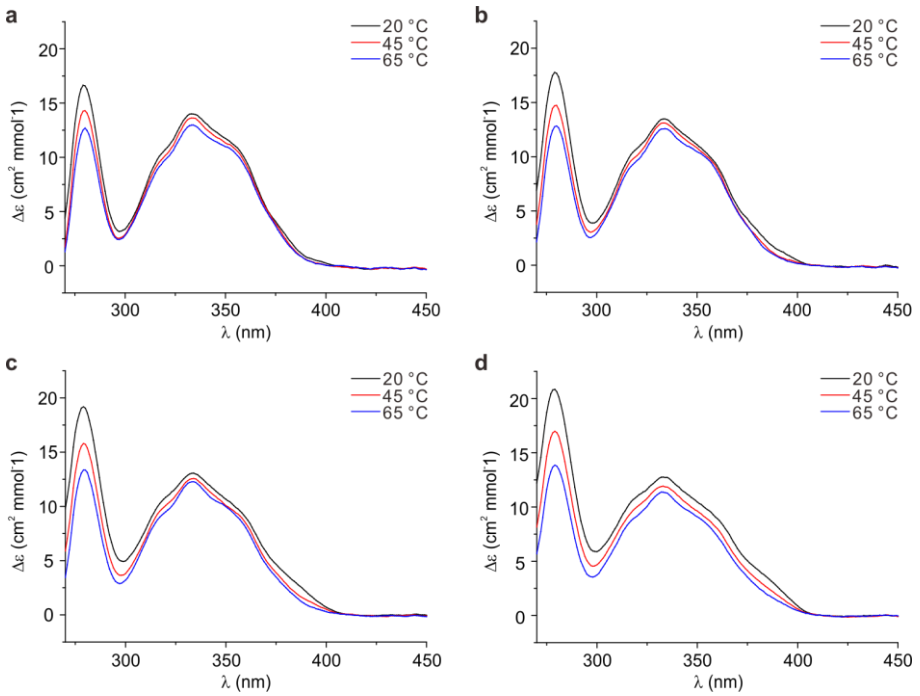
Supplementary Figure 24. ^{1}H spectra of **14** in D_2O (with 50 mM $NaHCO_3$) recorded at (a) 342 K; (b) 318 K; (c) 298 K; and (d) 278 K.



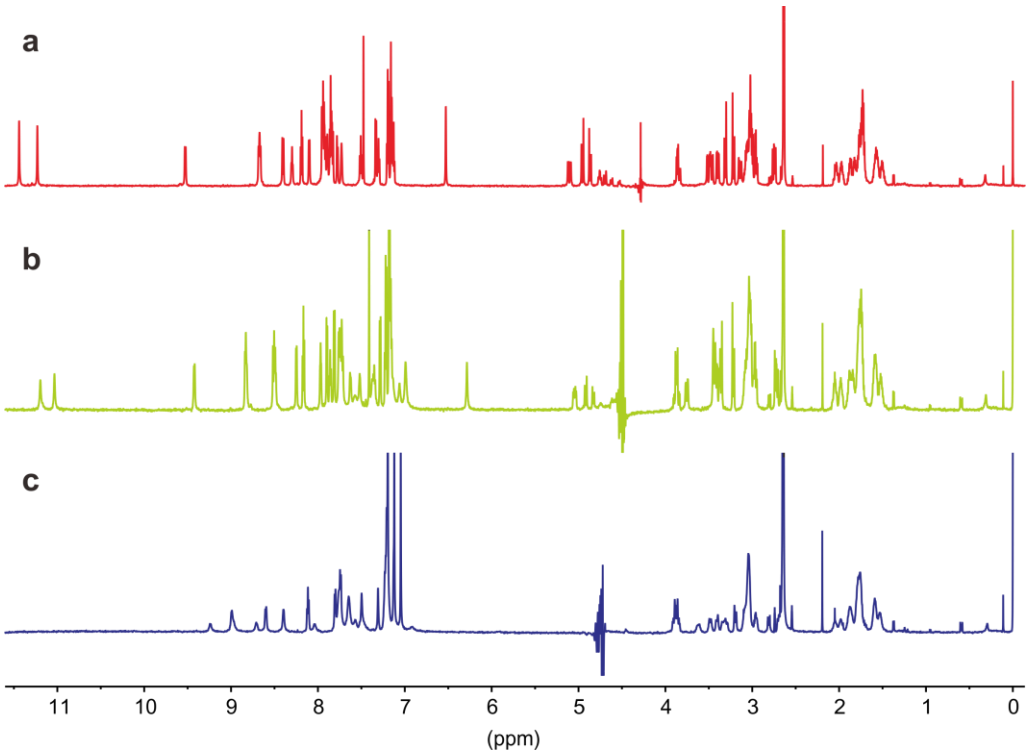
Supplementary Figure 25. ^{1}H spectra of **15** in D_2O (with 50 mM $NaHCO_3$) recorded at (a) 338 K; (b) 318 K; (c) 298 K; and (d) 278 K.



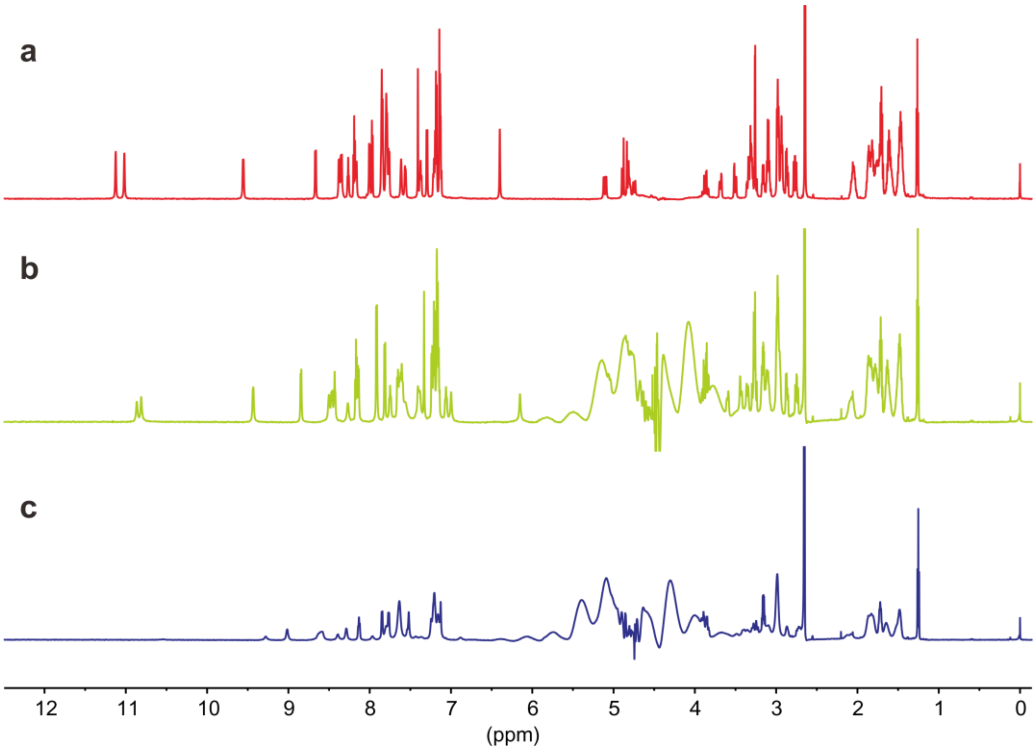
Supplementary Figure 26. Circular dichroism spectra of **16** in (a) 7:3; (b) 8:2; and (c) 9:1 of H_2O:DMSO-d_6 (vol/vol) recorded at various temperatures (293 K, 318 K, and 338 K).



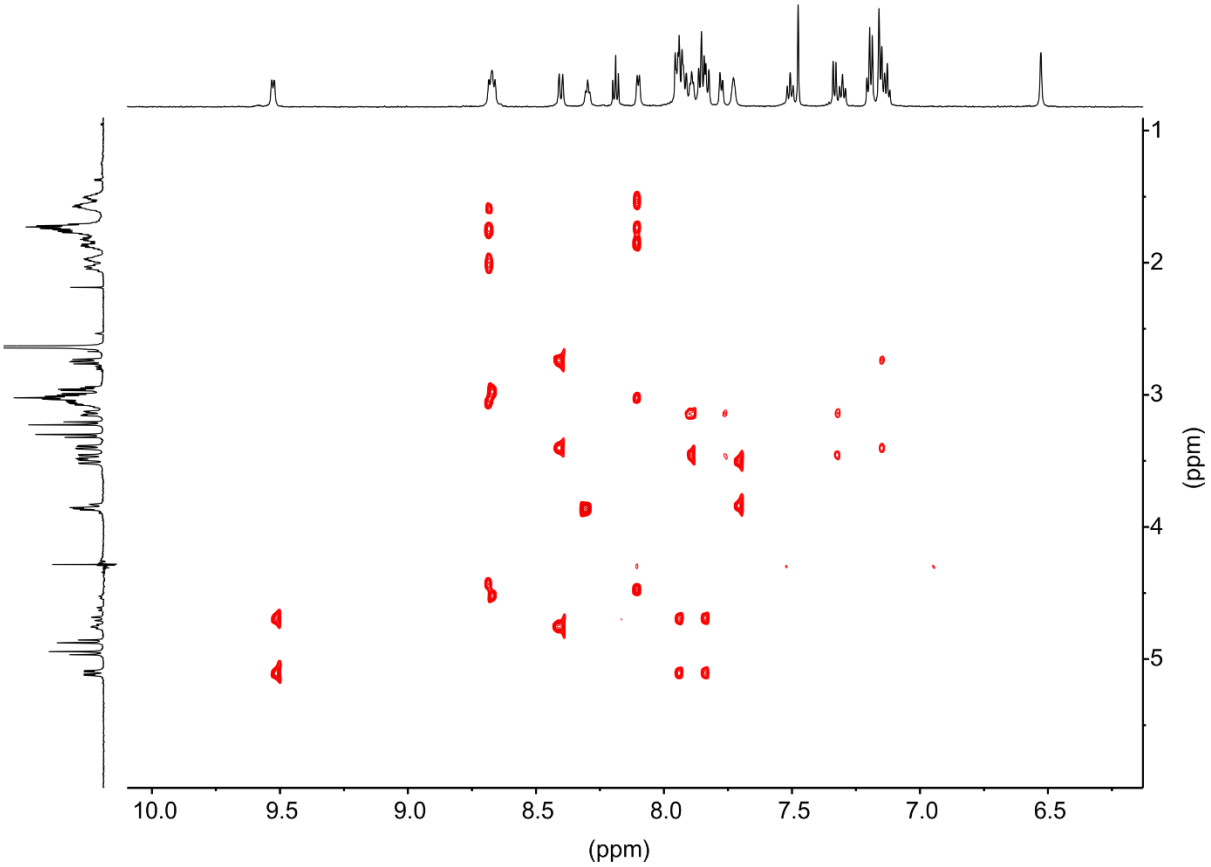
Supplementary Figure 27. Circular dichroism spectra of **17** in (a) 7:3; (b) 8:2; (c) 9:1; and (d) 10:0 of H_2O:DMSO-d_6 (vol/vol) recorded at various temperatures (293 K, 318 K, and 338 K).



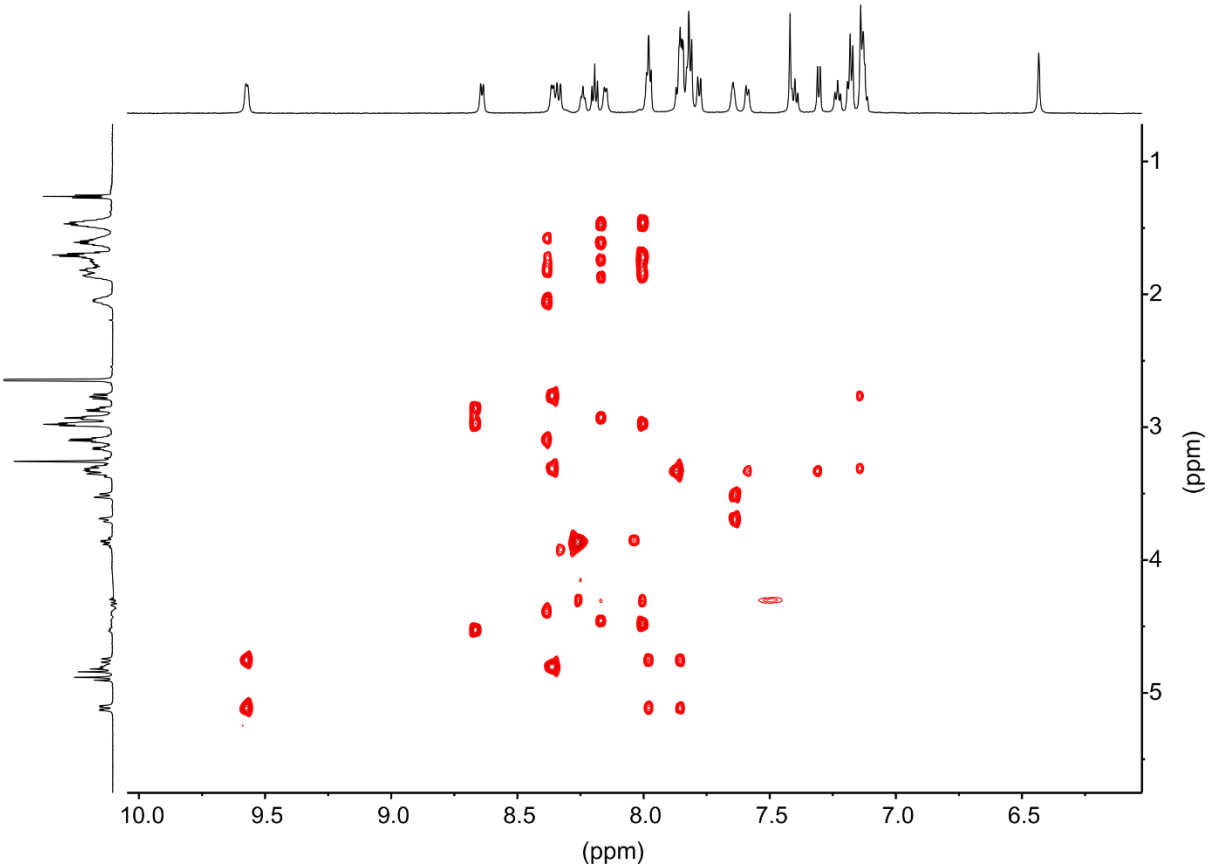
Supplementary Figure 28. ^{1}H spectra of **16** in H_2O:DMSO-d_6 (7:3, vol/vol) recorded at (a) 338 K; (b) 318 K; and (c) 298 K with water suppression using excitation sculpting.



Supplementary Figure 29. ^{1}H spectra of **17** in H_2O:DMSO-d_6 (7:3, vol/vol) recorded at (a) 338 K; (b) 318 K; and (c) 298 K with water suppression using excitation sculpting.

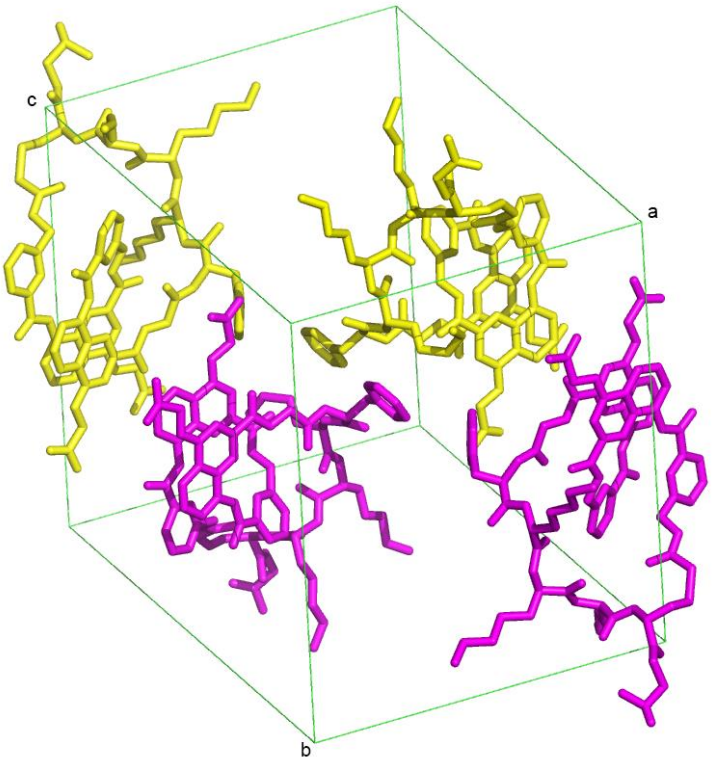


Supplementary Figure 30. Excerpts from TOCSY spectrum of **16** in H_2O:DMSO-d_6 (7:3, vol/vol) at 338 K.

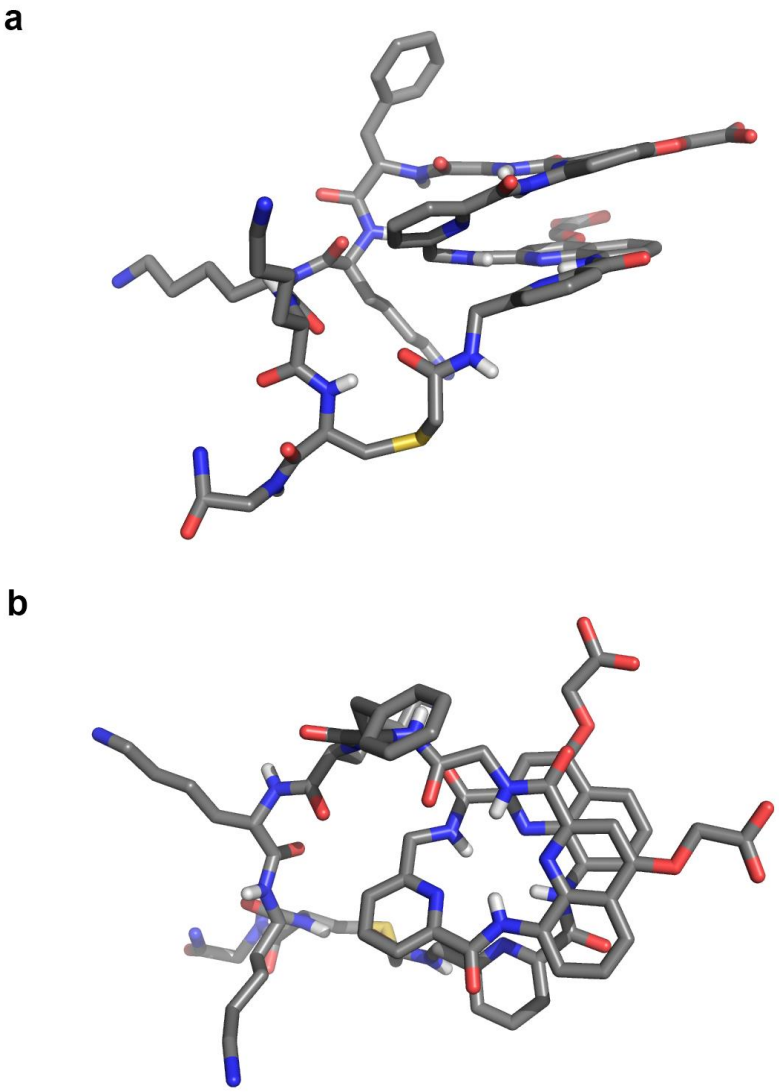


Supplementary Figure 31. Excerpts from TOCSY spectrum of **17** in H_2O:DMSO-d_6 (7:3, vol/vol) at 338 K.

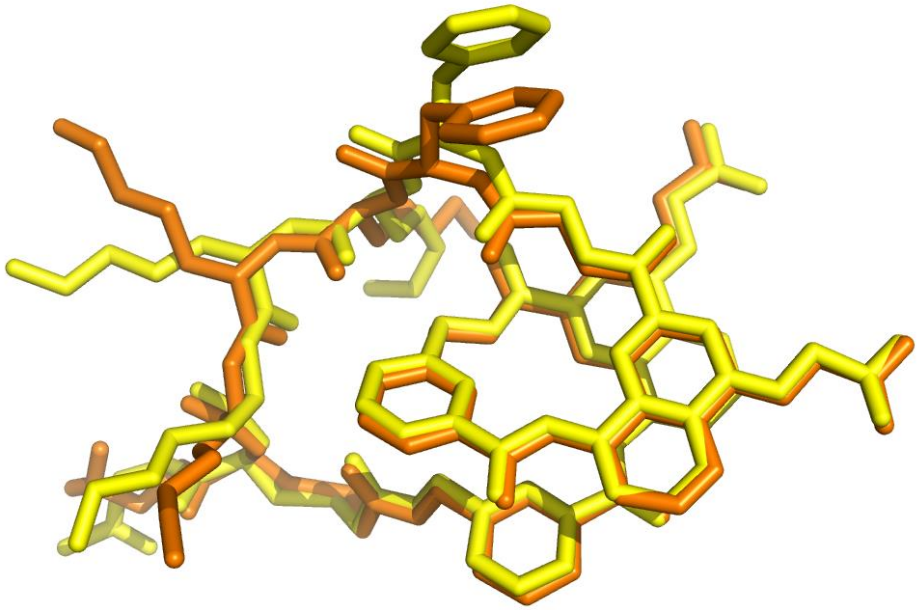
1.3. Solid-state structures



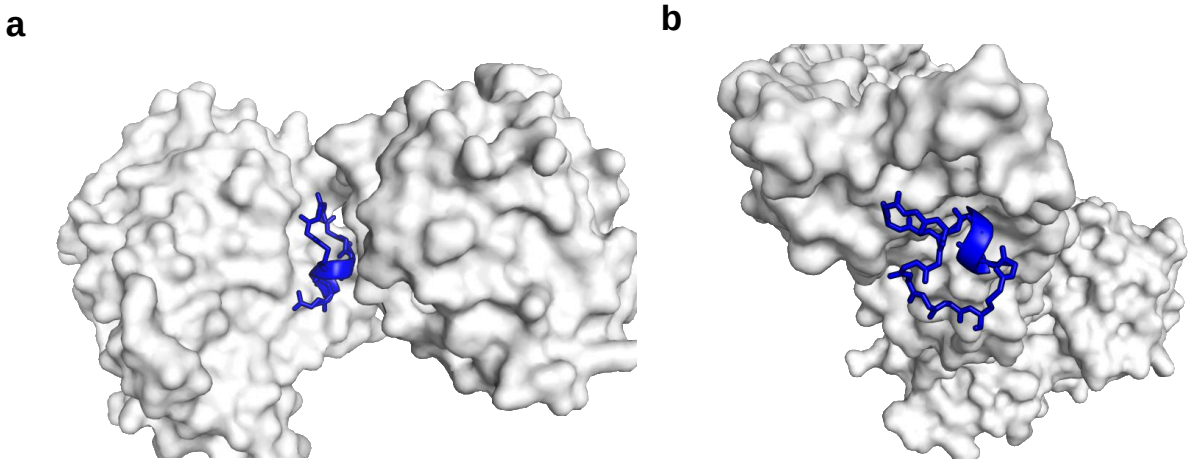
Supplementary Figure 32. Solid-state structure elucidated by X-ray crystallography of **17** showing four individual molecules in the unit cell. Two L-**17** molecules are shown in yellow, while the other two D-**17** are shown in magenta.



Supplementary Figure 33. Side view (a) and top view (b) of L-**17** in the crystal structure.



Supplementary Figure 34. Superposition of two L-**17** in the asymmetric unit showing almost identical backbone structure.



Supplementary Figure 35. Helix-loop structural motifs in macrocycle peptides. a) Macrocyclic peptide Ce-2d bound to iPGM, pdb 5kgn. b) Peptide aCAP bound to CmABCB1, pdb 3wmg.

2. Supplementary Methods

2.1. Methods for *in vitro* translation

2.1.1. Preparation of eFx

All oligonucleotides were purchased from Operon (Japan). The DNA template for the flexizyme eFx (DNA_eFx, below) was assembled by primer extension and PCR as described previously[3]. The DNA template was transcribed by T7 RNA polymerase and purified using 12% denaturing PAGE. The RNA product (RNA_eFx) was dissolved in water, adjusted to 250 µM and stored at -20 °C.

2.1.2. Preparation of $tRNA^{fMet}_{CAU}$

The DNA template for $tRNA^{fMet}_{CAU}$ (DNA_tRNA, below) was assembled by primer extension and PCR as described previously[4]. The DNA template was transcribed by T7 RNA polymerase and purified using 8% denaturing PAGE, the RNA product (RNA_tRNA) was dissolved in water and the concentration adjusted to 250 µM and stored at -20 °C.

2.1.3. Preparation of translation DNA template

DNA template for coupled transcription-translation (DNA_mRNA) was prepared using the primers:

F1 = TAATACGACTCACTATAGGGTTAACTTTAACAAGGAGAAAAACATG

R1 = GTCGTCGTCCTTGTAGTCAAACTTCTTCTTCATGTTTTTCTC

R2 = CGAAGCTTACTTGTCGTCGTCGTCCTTGTAGTC

DNA_mRNA contains the T7 promoter for transcription to mRNA, and the ribosome binding site for translation, producing the peptide ‘MKKKFDYKDDDDK’. Where ‘M’ is either formyl methionine or the foldamer species described in the main text, and ‘DYKDDDDK’ is a ‘FLAG-tag’ for purification.

2.1.4. Microhelix

Microhelix RNA was purchased from GeneDesign (Japan). Microhelix is a short RNA designed to mimic the acceptor stem of tRNA, the site of aminoacylation. It is based on the acceptor stem of *E.coli* Asn tRNA.

2.1.5. Aminoacylation of microhelix

3 µL H_2O, 1 µL microhelix (250 µM), 1 µL eFx (250 µM) and HEPES-KOH pH 8.4 (500 mM) were mixed, heated to 95 °C for 2 min, cooled at room temperature for 5 min. 2 µL $MgCl_2$ (3 M) added and left for a further 5 min. Aminoacylation was started by adding 2 µL cyanomethyl substrate in DMSO. Reaction was incubated on ice or at room temperature for 2-6 hours. (Final concentrations: 25 µM microhelix, 25 µM eFx and 5 mM cyanomethyl ester in 50 mM HEPES-KOH pH 8.4, 600 mM $MgCl_2$, 20%

DMSO)
The reaction was stopped by pelleting any insoluble substrate, collecting the substrate, adding 4 reaction volumes (40 µL) of 0.3 M NaOAc pH 5.2, and the product precipitated using 10 reaction volumes of EtOH (100 µL). The pellet was washed with 0.1 M NaOAc pH 5.2, 70% EtOH and analyzed by 20% denaturing acid PAGE (50 mM sodium acetate, 6 M urea). The RNA was stained with ethidium bromide and analyzed by FLA-5100 (Fuji, Japan)[3].

2.1.6. Aminoacylation of $tRNA^{fMet}_{CAU}$ with foldamer substrates

25 µM $tRNA^{fMet}_{CAU}$, 25 µM eFx and 5 mM cyanomethyl ester substrate were incubated in 50 mM HEPES-KOH pH 8.4, 600 mM $MgCl_2$ in 20% DMSO using the time and temperature optimized for each substrate (see Table below).

Aminoacylation times for cyanomethyl ester substrates

Substrate	Time (hours)	Temperature (°C)
1	6	0
2	6	0
3	2	0
4	2	25
5	2	25
6	6	0
7	6	25
8	6	25
9	6	25
10	6	0
11	6	0

The reaction was stopped by pelleting any insoluble substrate, collecting the supernatant, adding 4 reaction volumes (40 µL) of 0.3 M NaOAc pH 5.2, and the product precipitated using 10 reaction volumes of EtOH (100 µL). The pellet was washed twice with 0.1 M NaOAc pH 5.2 70% EtOH, once with 70% EtOH, dried and stored at -80 °C.

2.1.7. *in vitro* translation and MALDI-TOF-MS

A custom *in vitro* translation mixture was assembled, with each protein/RNA component purified separately from *E.coli*.
Final concentrations: 1.2 µM ribosome, 0.1 µM T7 RNA polymerase, 4 µg/mL creatine kinase, 3 µg/mL myokinase, 0.1 µM pyrophosphatase, 0.1 µM nucleotide-diphosphatase kinase, 2.7 µM IF1, 0.4 µM IF2, 1.5 µM IF3, 30 µM EF-Tu, 30 µM EF-Ts, 0.26 µM EF-G, 0.25 µM RF2, 0.17 µM RF3, 0.5 µM RRF, 0.6 µM MTF, 0.73 µM AlaRS, 0.03 µM ArgRS, 0.38 µM AsnRS, 0.02 µM CysRS, 0.06 µM GlnRS, 0.23 µM GluRS, 0.09 µM GlyRS, 0.02 µM HisRS, 0.4 µM IleRS, 0.04 µM LeuRS, 0.03 µM

MetRS, 0.68 µM PheRS, 0.16 µM ProRS, 0.04 µM SerRS, 0.09 µM ThrRS, 0.03 µM TrpRS, 0.02 µM ValRS, 0.13 µM AspRS, 0.11 µM LysRS, 0.02 µM TyrRS.
Additionally, 50 mM Hepes-KOH (pH 7.6), 100 mM potassium acetate, 2 mM GTP, 2 mM ATP, 1 mM CTP, 1 mM UTP, 20 mM creatine phosphate, 12 mM $Mg(OAc)_2$, 2 mM spermidine, 2 mM DTT, and 1.5 mg/mL *E. coli* total tRNA (Roche). 19 of the 20 canonical amino acids were included at 500 µM: methionine was not added nor the formyl donor, usually 10-formyl-5,6,7,8-tetrahydrofolic acid, required for initiation using formyl methionine.
Solutions containing the above plus 0.04 µM DNA template and 50 µM foldamer-$tRNA^{fMet}_{CAU}$ (prepared using eFx, above) were incubated for 30 min at 37 °C. The foldamer-peptide hybrid was isolated using M2-anti-FLAG magnetic agarose (Sigma), and eluted using 0.2% TFA. Solution was mixed with α-cyano-4-hydrocinnamic acid before spotting on a MALDI plate. Foldamer-peptide detected on positive mode (UltraFlex, Bruker).

2.1.8. *In vitro* translation and quantification

In vitro translation was carried out as above, expect that the reaction was supplemented with 50 mM [C^{14}]-Asp. The reaction was carried out in 2.5 µL at 37 °C for 30 min and the products were analyzed by Tricine-SDS-PAGE (without FLAG-tag purification).

2.1.9. Sequences (all shown 5' to 3')

***eFx*:**

DNA_eFx =
GGCGTAATACGACTCACTATAGGATCGAAAGATTTCCGCGGCCCCGAAAGGGGATTAGCGTTAGGT
RNA_eFx =
GGAUCGAAAGAUUUCCGCGGCCCCGAAAGGGGAUUAGCGUUAGGU

***$tRNA^{fMet}_{CAU}$*:**

DNA_tRNA =
GGCGTAATACGACTCACTATAGGCGGGGTGGAGCAGCCTGGTAGCTCGTCGGGCTCATAACCCGAAGATCGTCGGTTCAAATCCGGCCCCCGCAACCA
RNA_tRNA =
GGCGGGGUGGAGCAGCCUGGUAGCUCGUCGGGCUCAUAACCCGAAGAUCGUCGGUUCAAAUCCGGCCCCCGCAACCA

***Translation template (for peptide MKKKFflag)*:**

DNA_mRNA =
TAATACGACTCACTATAGGGTTAACTTTAACAAGGAGAAAAACATGAAGAAGAAGTTTGACTACAAGGACGACGACGACAAGTAAGCTTCG
mRNA =

GGGUUAACUUUAACAAGGAGAAAAACAUGAAGAAGAAGUUUGACUACAAGGA
CGACGACGACAAGUAAGCUUCG

***Microhelix*:**
Microhelix_RNA = GGCUCUGUUCGCAGAGCCGCCA

2.2. Methods for NMR spectroscopy

NMR spectra were recorded on three different spectrometers: Avance II NMR spectrometer (Bruker Biospin) with a vertical 7.05 T narrow-bore/ultrashield magnet operating at 300 MHz for ^{1}H observation by means of a 5-mm direct BBFO H/X probe with single axis Z gradient capabilities; Avance III HD NMR spectrometer (Bruker Biospin) with a vertical 9.4 T narrow-bore/ultrashield magnet operating at 400 MHz for total correlation spectroscopy (TOCSY) and rotating-frame overhauser effect spectroscopy (ROESY) by means of a 5-mm Smartprobe with single axis Z-gradient capabilities; Avance III NMR spectrometer (Bruker Biospin) with a vertical 16.45 T narrowbore/ultrashield magnet operating at 700 MHz for ^{1}H observation by means of a 5-mm TXI $^{1}H/^{13}C/^{15}N$ probe with single axis Z-gradient capabilities. ^{1}H NMR spectra were recorded at 300, 400, or 700 MHz. 2D NMR and H-D exchange spectra were recorded at 400 MHz and 700 MHz, respectively. Chemical shifts are reported in ppm relative to residual solvent signals of $CDCl_3$ (δ 7.26) and DMSO-d_6 (δ 2.50). TSP (trimethylsilylpropanoic acid) was used as an internal reference for NMR in D_2O and H_2O:D_2O mixed solvent. Data processing was performed with Bruker TOPSPIN 2.1 software. All bidimensional NMR experiments (TOCSY and ROESY) were recorded at 298K, with 2048 (F2) x 256 (F1) data points in States-TPPI mode. Processing was done after a sine-bell multiplication in both dimensions and Fourier transformation in 4K x 4K real points (linear prediction was use in F1). For the TOCSY experiment of compound **12**, acquisition was performed with following parameters: recycling delay of 1.5 s; 16 scans per increment; sweep width of 8012.8 Hz in both dimensions; mixing time of 300 ms; water suppression using excitation sculpting. For the ROESY experiment of compound **12**, acquisition was performed with following parameters: recycling delay of 1.5 s; 48 scans per increment; sweep width of 8012.8 Hz in both dimensions; mixing time of 300 ms; water suppression using excitation sculpting. For the TOCSY experiment of compound **13**, acquisition was performed with following parameters: recycling delay of 1.5 s; 32 scans per increment; sweep width of 6578.9 Hz in both dimensions; mixing time of 300 ms; water suppression using excitation sculpting. For the ROESY experiment of compound **13**, acquisition was performed with following parameters: recycling delay of 1.5 s; 32 scans per increment; sweep width of 6578.9 Hz in both dimensions; mixing time of 300 ms; water suppression using excitation sculpting. For the TOCSY experiment of compound **14**, acquisition was

performed with following parameters: recycling delay of 1.5 s; 64 scans per increment; sweep width of 6849.3 Hz in both dimensions; mixing time of 300 ms; water suppression using excitation sculpting. For the ROESY experiment of compound **14**, acquisition was performed with following parameters: recycling delay of 2 s; 64 scans per increment; sweep width of 6849.3 Hz in both dimensions; mixing time of 300 ms; water suppression using excitation sculpting. For the TOCSY experiment of compound **15**, acquisition was performed with following parameters: recycling delay of 1.5 s; 32 scans per increment; sweep width of 6024 Hz in both dimensions; mixing time of 300 ms; water suppression using excitation sculpting. For the ROESY experiment of compound **15**, acquisition was performed with following parameters: recycling delay of 1.5 s; 96 scans per increment; sweep width of 6024Hz in both dimensions; mixing time of 300 ms; water suppression using excitation sculpting. For the TOCSY experiment of compound **16**, acquisition was performed with following parameters: recycling delay of 2 s; 88 scans per increment; sweep width of 11111 Hz in both dimensions; mixing time of 300 ms; water suppression using excitation sculpting. For the TOCSY experiment of compound **17**, acquisition was performed with following parameters: recycling delay of 2 s; 96 scans per increment; sweep width of 11111 Hz in both dimensions; mixing time of 300 ms; water suppression using excitation sculpting.

2.3. Crystallization and X-ray diffraction measurements

For crystallization, lyophilized powder of L-**17** was dissolved using ultra-pure water such that the final concentration was 2.5 mM. Commercial sparse matrix screens were used for crystallization experiments at 293 K using standard hanging drop vapor diffusion method in 24-well Linbro-style plates. Colorless, bipyramid prisms (size 0.10 x 0.06 x 0.06 mm) were obtained from drops prepared by mixing 1.25 µL of L-**17** and an equal volume of a crystallization reagent containing 1.2 M ammonium sulfate, 100 mM HEPES buffer (pH 7.5), 2% v/v polyethylene glycol (PEG) 400 equilibrated against 500 µL of 2.0 M ammonium sulfate in the reservoir. Crystallographic data was collected using X-ray facility (UMS 3033) in IECB using a micro-focus rotating anode (2.9 kW) Rigaku FRX diffractometer, with Cu Kα radiation and a PILATUS 200K hybrid pixel detector. However, the crystal diffracted only to a maximum resolution of 2.5 Å in the tetragonal space group $P4_32_12$ with unit cell parameters $a = b = 53.38$ Å, $c = 60.79$ Å.

Subsequently, D- enantiomer of **17** was synthesized and the lyophilized powder was dissolved in ultra-pure water. Racemic mixture of L/D-**17** was prepared by mixing the two enantiopure solutions to final concentration of 2.5 mM. Colorless prisms of L/D-**17** were obtained from drops prepared by mixing 0.8 µL of L/D-**17** and 1.7 µL of a crystallization reagent containing 50 mM sodium cacodylate buffer (pH 6.5), 300 mM potassium bromide, 20% v/v PEG 400 equilibrated against 500 µL of 40% v/v PEG 400 in the reservoir. The crystals were mounted on cryo-loops after quick soaking in

paratone-N oil and flash frozen. Diffraction measurements at atomic resolution were obtained in the X-ray facility at IECB (UMS 3033) using a micro-focus rotating anode (2.9 kW) Rigaku FRX diffractometer, with Cu Kα radiation and a PILATUS 200K hybrid pixel detector. Diffraction data were processed and scaled using the *CrysAlisPro* package[5].

Structure determination and refinement

The structure was solved with *Shelxt*[6] and refined by full-matrix least-squares method on F[8] with *Shelxl-2014*[7] within the *Olex2* suite[8]. The structure was resolved in centrosymmetrical space group $P\bar{1}$ with two independent molecules in the asymmetric unit. Non-hydrogen atoms for main-chain of the macrocycle and all side chains (except 3 out of 6 lysines) were refined with anisotropic displacement parameters. In case of lysine side chains and solvent molecules, the non-hydrogen atoms were refined with anisotropic or isotropic displacement parameters. Hydrogen atoms were included for the macrocycles in idealized positions using HFIX and refined with a riding model. A bromine ion was identified and refined anisotropically with 0.8 occupancy. For solvent molecules, positions of hydrogen atom were not determined. After several attempts to model the disordered water molecules, the PLATON/SQUEEZE[9] procedure was implemented. DFIX, DELU, SIMU and ISOR instructions were used to model displacement parameters and the geometry of molecules.

Crystallographic data for L/D-**17**

Formula	$C_{144.25}H_{160}Br_{0.80}O_{30}S_2$
M	3641.62
T/K	100
λ/Å	1.5418
Crystal system	Triclinic
Space group	$P\bar{1}$
a/Å	19.8457 (4)
b/Å	23.3538 (6)
c/Å	27.1601 (5)
α/°	106.247 (2)
β/°	106.538 (2)
γ/°	101.356 (2)
V/Å³	11049.9
Z	2
ρ/g mm⁻³	1.094
Color and shape	Colorless, prisms
Size (mm)	0.10 x 0.04 x 0.02
μ/mm⁻¹	1.08
Total reflections	126672

Unique data [Fo > 2σFo]	33165
R_{int}	0.0396
Parameters/restraints	2401/313
R1, wR2	0.1355, 0.4202
Goodness-of-fit	1.689
Total potential solvent accessible void volume from SQEEZE/ Å³	2105.3
Electron count/cell	646
CCDC number	1554263

The final cif files were examined using IUCr's *checkcif* algorithm. The L/D-**17** crystal was observed to have large fraction of disordered solvent molecules, side chains (of lysine) and glycine. Two A-level and a number of B-level alerts remain in the checkcif reports and are listed in the following:

A-level alerts

PLAT430_ALERT_2_A Short Inter D...A Contact O28W .. O1BA .. 2.50 Ang.
PLAT430_ALERT_2_A Short Inter D...A Contact O28W .. O36W .. 2.53 Ang.

These alerts concern disordered solvent molecules.

B-level alerts

THETM01_ALERT_3_B The value of sine(theta_max)/wavelength is less than 0.575
Calculated sin(theta_max)/wavelength = 0.5710
PLAT084_ALERT_3_B High wR2 Value (i.e. > 0.25) ..... 0.42 Report

These alerts are inherent to the quality of the data.

PLAT220_ALERT_2_B Non-Solvent Resd 1 N Ueq(max)/Ueq(min) Range 6.6 Ratio
PLAT220_ALERT_2_B Non-Solvent Resd 1 O Ueq(max)/Ueq(min) Range 6.5 Ratio
PLAT230_ALERT_2_B Hirshfeld Test Diff for N2 -- C0BA .. 11.3 s.u.
PLAT234_ALERT_4_B Large Hirshfeld Difference N1BA -- C2BA .. 0.26 Ang.
PLAT241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of N2 Check
PLAT241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C060 Check
PLAT241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C42 Check
PLAT241_ALERT_2_B High 'MainMol' Ueq as Compared to Neighbors of C50 Check
PLAT242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C2BA Check
PLAT242_ALERT_2_B Low 'MainMol' Ueq as Compared to Neighbors of C06O Check

These alerts concerns main-chain and side-chain atoms of the macrocycle; however they do not indicate incorrect atom-type assignment.

PLAT306_ALERT_2_B Isolated Oxygen Atom (H-atoms Missing ?) ..... O2 Check

This alert corresponded to hydration water molecules. The thermal nature of water molecules and the level of apparent electron densities assured their modeling.

Positions of hydrogen atoms were not determined.

PLAT430_ALERT_2_B Short Inter D...A Contact O00D .. O37W .. 2.71 Ang.
And 36 other PLAT430 Alerts

These alerts corresponded to hydration water molecules. The thermal nature of water molecules and the level of apparent electron densities assured their modeling. Positions of hydrogen atoms were not determined.

Crystallographic data and refinement statistics are illustrated in the Table above. Atomic coordinates and structure factors for have been deposited in the Cambridge Crystallographic Data Centre (CCDC) with accession code 1554263. The data is available free of charge upon request (www.ccdc.cam.ac.uk/).

2.4. Materials and methods for chemical synthesis and characterizations

Fmoc-Q^{AsptBu}-OH, Fmoc-Q^{OrnBoc}–OH, and Fmoc-Q^{TEG}–OH monomers were prepared by following the reported synthetic protocols[10,11]. Synthetic procedure for Fmoc-P-OH will be described elsewhere.

Chemical reagents were purchased from commercial suppliers (Sigma-Aldrich, Alfa-aesar, or TCI) and used without further purification. 2-Chlorotrityl resin was purchased from Iris biotech. Wang and rink amide MBHA resin were purchased from Novabiochem. Tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2) were dried over alumina columns. *N,N*-diisopropylethylamine (DIPEA) was distilled over CaH_2 prior to use. Column chromatography purifications were performed on silica gel (230-400 mesh, 40-63 µm, Merck). Reactions were monitored by thin layer chromatography on silica gel 60-F254 plates (Merck). RP-HPLC analyses and purifications were performed with JASCO HPLC systems (PU-2089 Plus, UV-2077 Plus, HV-2080-01, and AS-2055 Plus for analytical HPLC; DG-2080-53, PU-2086 Plus, and UV-2075 Plus for semi-preparative HPLC). 0.1% (v/v) TFA/Milli-Q water (solvent A) and 0.1% TFA/CH_3CN (solvent B) compose the mobile phase for System A. 12.5 mM aqueous NH_4OAc-NH_4OH adjusted to pH 8.5 (solvent C) and CH_3CN (solvent D) compose the mobile phase for System B. RP-HPLC analyses were carried out on a Macherey-Nagel Nucleodur C18 HTec column (4.6×100 mm, 5 µm) for System A and a Macherey-Nagel Nucleodur C18 HTec column (4×100 mm, 5 µm) for System B at a flow rate of 1 mL min^{-1}. Semi-preparative RP-HPLC purifications were carried out on a Macherey-Nagel Nucleodur C18 HTEC column (10×100 mm, 5 µm) for System A and a Macherey-Nagel Nucleodur C18 HTec column (10×250 mm, 5 µm) for System B at a flow rate of 3 mL min^{-1}. Eluate from column was monitored by UV detection at 254 nm and 300 nm using a diode array detector. High-resolution electrospray mass spectra were recorded on a Thermo Exactive orbitrap instrument. Circular dichroism spectra were recorded on a Jasco J-815 spectrometer with 2 mm path length quartz cells. Scans were measured over a wavelength range of 270-500 nm, with a response time of 0.5 s and a scanning speed of 50 nm/min.

2.5. Methods for solid phase synthesis

The resin was swollen in DMF for 1 h with gentle agitation prior to use. For removal of Fmoc protection group, 20% piperidine in DMF was added to the resin and stirred for 10 min at room temperature. The resin was then washed briefly with DMF and the process was repeated two more times. After deprotection of Fmoc group, the resin was thoroughly washed with DMF prior to coupling or capping.

2.5.1. General method (1) for acid chloride activation

Acid chloride activation of Fmoc-Q$^{(Xxx)}$-OH exemplified by Fmoc-Q^{AsptBu}-OH

The Fmoc-Q^{AsptBu}-OH monomer (96 mg, 0.177 mmol) was dissolved in anhydrous DCM (2 mL), and Ghosez reagent (47 µL, 0.354 mmol) was added via syringe. The resulting mixture was allowed to stir for 2 h at room temperature. Solvent was then removed directly under vacuum line to afford Fmoc-Q^{AsptBu}-Cl in quantitative yield. This acid chloride was directly used in the following coupling reaction without purification.

Acid chloride activation of Fmoc-P-OH

To a solution of Fmoc-P-OH monomer (66.3 mg, 0.177 mmol) in MeOH (2mL) was added concentrated HCl (17.4 µL, 0.177 mmol). The resulting mixture was stirred at room temperature for 30 min, and the solvent was then evaporated under reduced pressure. The resulting oil was co-evaporated three times with toluene, three times with *n*-hexane, and further dried for 1 h under vacuum to give protonated Fmoc-P-OH monomer as a white solid. The protonated Fmoc-P-OH monomer was dissolved in anhydrous DCM (2 mL), and Ghosez reagent (47 µL, 0.354 mmol) was added via syringe. The resulting mixture was allowed to stir for 2 h at room temperature. Solvent was then removed directly under vacuum line to afford Fmoc-P-Cl in quantitative yield. This acid chloride was directly used in the following coupling reaction without purification.

2.5.2. General method (2) for coupling of acid chloride to aromatic amine exemplified by preparation of Fmoc-P-Q^{AsptBu}-resin

NH_2-Q^{AsptBu}-resin was thoroughly washed with anhydrous THF to ensure all reagents have been removed. The resin was then suspended in anhydrous THF (0.5 mL), followed by addition of DIEA (47 µL, 0.266 mmol, 9 equiv). Fmoc-P-Cl (0.177 mmol, 6 equiv) was dissolved in THF (1 mL) under N_2, and half of the solution was transferred to the resin via syringe. The resin was then allowed to stir for 90 min at room temperature, and washed briefly with anhydrous THF. And the coupling process was repeated overnight with remaining half of the Fmoc-P-Cl solution. The resin was then thoroughly washed with anhydrous THF and DMF. In the cases of solid phase synthesis on Wang resin, the reaction mixture was treated with microwaves (50 W, 50 ^oC, 5 min) instead of stirring at room temperature.

2.5.3. General method (3) for coupling of acid to aliphatic amine exemplified by preparation of Fmoc-Q^{AsptBu}-P-resin

NH_2-P-resin was thoroughly washed with anhydrous DMF to ensure all reagents have been removed. Fmoc-Q^{AsptBu}-OH (48 mg, 0.089 mmol, 3 equiv), HBTU (34 mg, 0.089 mmol, 3 equiv), and HOBT (14 mg, 0.0.089 mmol, 3 equiv) were dissolved in anhydrous DMF (2 mL), followed by addition of DIEA (31 µL, 0.177 mmol, 6 equiv). The resulting mixture was directly added to the resin, and suspension was allowed to stir 1 h at room temperature. The resin was then briefly washed with anhydrous DMF,

and the coupling process was repeated once. The resin was then thoroughly washed with anhydrous DMF. In the cases of solid phase synthesis on Wang resin, the reaction mixture was treated with microwaves (25 W, 70 ^{o}C, 10 min) instead of stirring at room temperature.

2.5.4. General method (4) for N-terminal acetylation/chloroacetylation

If N-terminal is aliphatic amine of P

After removal of Fmoc group, the resin was thoroughly washed with anhydrous DMF. The resin was then suspended in 1 mL of anhydrous DMF, followed by addition of DIEA (10 equiv) and acetic anhydride (5 equiv). The mixture was stirred at room temperature for 30 min. The resin was then briefly washed with anhydrous DMF, and the procedure was repeated once. The resulting resin was then thoroughly washed with DMF, DCM, and DCM/MeOH (1:1, v/v) and dried under vacuum. Chloroacetic anhydride was used in cases of chloroacetylation.

If N-terminal is aromatic amine of Q

After removal of Fmoc protecting group, the resin was thoroughly washed with anhydrous THF. The resin was then suspended in 1 mL of anhydrous THF, followed by addition of DIEA (6 equiv) and acetyl chloride (3 equiv). The mixture was stirred at room temperature for 1 h. The resin was then briefly washed with anhydrous THF, and the procedure was repeated once. The resulting resin was then thoroughly washed with DMF, DCM, and DCM/MeOH (1:1, v/v) and dried under vacuum. Chloroacetyl chloride was used in cases of chloroacetylation.

2.5.5. General method (5) for 2CT resin cleavage

Resin was suspended in 1,1,1,3,3,3-hexafluoro-2-propanol/DCM (3:2, v/v) and stirred at room temperature for 2 h. The resin beads were then removed by filtration and washed with DCM. The collected filtrate was then concentrated under reduced pressure to give the desired product.

2.5.6. General method (6) for cyanomethyl ester installation on C-terminal carboxylic acid

Resin-cleaved crude product obtained from general method (5) was dissolved in anhydrous DMF, followed by addition of potassium carbonate (1.5 equiv) and bromoacetonitrile (1.3 equiv). The reaction mixture was stirred under N_2 at room temperature for 1 h. The mixture was then diluted with EtOAc and washed three times with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The resulting residue was purified by column chromatography to afford the desired product.

2.5.7. General method (7) for Boc/*tert*-Bu deprotection

For deprotection of Boc group, cyanomethyl ester compound obtained from general method (6) was dissolved in 2 mL of TFA/DCM (1:1, v/v) and stirred at room temperature for 2 h. The solvent was then removed under reduced pressure, and the

resulting oil was triturated and washed with diethyl ether to afford the desired product. TFA/DCM/TIS (50:48:2, v/v/v) mixture was used for *tert*-Bu deprotection.

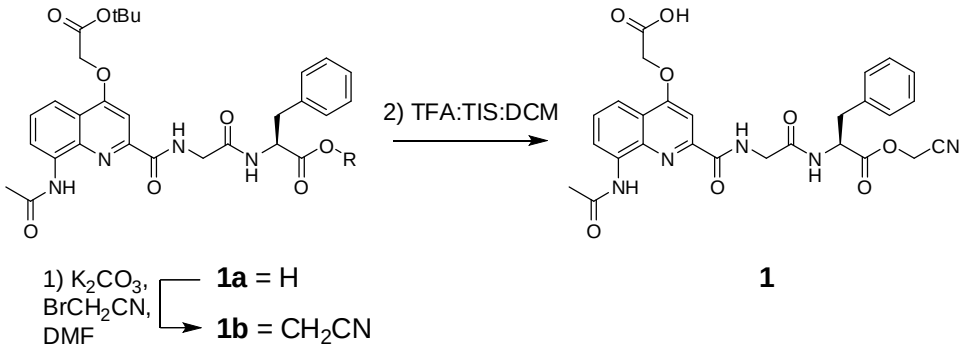
2.5.8. General method (8) for Wang resin cleavage

Resin was suspended in TFA/TIS(triisopropylsilane)/H_2O (95:2.5:2.5, v/v/v) and stirred at room temperature for 2 h. The resin beads were then removed by filtration and washed with TFA. The collected filtrate was then concentrated under reduced pressure. After solvent evaporation, the resulting oil was precipitated in diethyl ether to give the desired product.

2.5.9. General method (9) for Rink amide resin cleavage

Resin was suspended in TFA/EDT(1,2-ethanedithiol)/TIS/H_2O (94:1:2.5:2.5, v/v/v/v) and stirred at room temperature for 2 h. The resin beads were then removed by filtration and washed with TFA. The collected filtrate was then concentrated under reduced pressure. After solvent evaporation, the resulting oil was precipitated at 5 ^oC in diethyl ether to give the desired product.

2.6. Experimental procedures

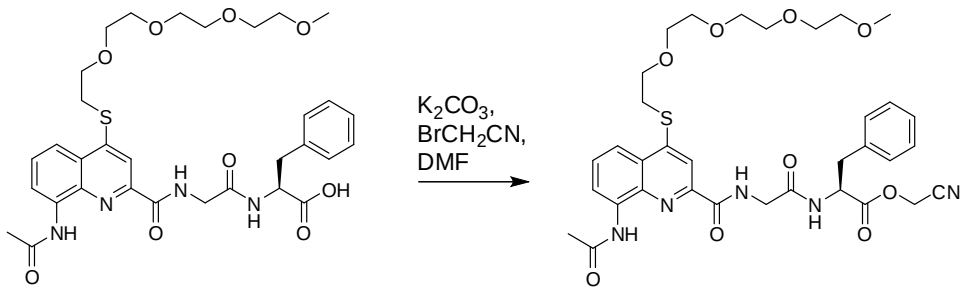


Compound 1a: Compound **1a** was prepared from H-L-Phe-2CT-resin on a 29.5 µmol scale (54.6 mg, manufacturer's loading: 0.54 mmol g^{-1}) using general methods (3), (4), and (5). The crude product obtained from resin cleavage was used directly in subsequent reaction without further purification (12.5 mg, 75%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.27 (s, 1H), 9.77 (t, *J* = 6.3 Hz, 1H), 8.75 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.25 (d, *J* = 7.8 Hz, 1H), 7.87 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.63 (t, *J* = 8.1 Hz, 1H), 7.54 (s, 1H), 7.15-7.27 (m, 5H), 5.11 (s, 2H), 4.39-4.49 (m, 1H), 3.91-4.10 (m, 2H), 2.87-3.11 (m, 2H), 2.32 (s, 3H), 1.45 (s, 9H). HRMS (ESI^+): *m/z* calcd for $C_{29}H_{31}N_4O_8$ $[M-H]^-$ 563.2147 found 563.2152.

Compound 1b. Compound **1b** was synthesized from carboxylic acid **1a** (10 mg, 17.7 µmol) using general method (6). The crude residue was purified by silica gel column chromatography eluting with EtOAc:cyclohexane (7:3, v/v) to give **1b** as a white solid

(8.7 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.34 (s, 1H), 8.81 (d, *J* = 7.8 Hz, 1H), 8.62 (t, *J* = 5.6 Hz, 1H), 7.94 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.56 (t, *J* = 8.1 Hz, 1H), 7.50 (s, 1H), 7.08-7.24 (m, 5H), 6.53 (d, *J* = 7.8 Hz, 1H), 4.92-5.01 (m, 1H), 4.78 (AB quartet, *J* = 15.6 Hz, 1H), 4.77 (s, 2H), 4.49 (AB quartet, *J* = 15.6 Hz, 1H), 4.05-4.28 (m, 2H), 3.08-3.23 (m, 2H), 2.34 (s, 3H), 1.53 (s, 9H). HRMS (ESI$^+$): *m/z* calcd for $C_{31}H_{34}N_5O_8$ $[M+H]^+$ 604.2402 found 604.2392.

Compound 1. Compound **1** was synthesized from cyanomethyl ester **1b** (6.0 mg, 9.94 μmol) using general method (7). The crude residue was purified by semi-preparative RP-HPLC with a gradient from 5% to 100% solvent B over 30 min (System A) to give **1** as a white solid (1.05 mg, 19%). ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 10.27 (s, 1H), 9.79 (t, *J* = 6.2 Hz, 1H), 8.75 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.66 (d, *J* = 7.2 Hz, 1H), 7.88 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.62 (t, *J* = 8.1 Hz, 1H), 7.54 (s, 1H), 7.18-7.3 (m, 5H), 5.09 (br, 2H), 4.98 (s, 2H), 4.53-4.63 (m, 1H), 3.93-4.10 (m, 2H), 2.95-3.13 (m, 2H), 2.33 (s, 3H). HRMS (ESI$^+$): *m/z* calcd for $C_{27}H_{26}N_5O_8$ $[M+H]^+$ 548.1776 found 548.1761.

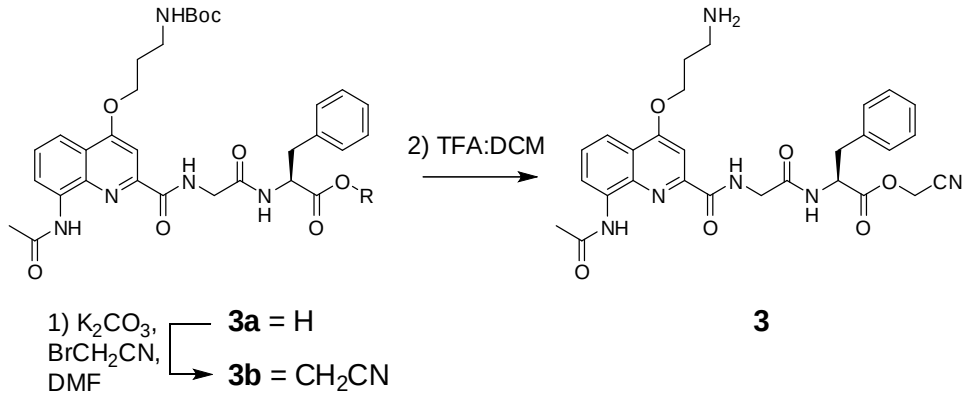


2a **2**

Compound 2a: Compound **2a** was prepared from H-L-Phe-2CT-resin on a 10.8 μmol scale (20 mg, manufacturer's loading: 0.54 mmol g^{-1}) using general methods (3), (4), and (5). The crude product obtained from resin cleavage was used directly in subsequent reaction without further purification (5 mg, 70%). ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 10.31 (s, 1H), 9.79 (t, *J* = 6.2 Hz, 1H), 8.75 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.27 (d, *J* = 7.8 Hz, 1H), 8.07 (s, 1H), 7.77 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.15-7.32 (m, 5H), 4.38-4.50 (m, 1H), 3.91-4.11 (m, 2H), 3.81 (t, *J* = 6.2 Hz, 2H), 3.51-3.63 (m, 4H), 3.44-3.50 (m, 6H), 3.36-3.41 (m, 2H), 3.21 (s, 3H), 3.03-3.12 (m, 2H), 2.85-2.97 (m, 2H), 2.32 (s, 3H). HRMS (ESI$^+$): *m/z* calcd for $C_{32}H_{41}N_4O_9S$ $[M+H]^+$ 657.2589 found 657.2579.

Compound 2. Compound **2** was synthesized from carboxylic acid **2a** (5 mg, 7.61 μmol) using general method (6). The crude residue was purified by silica gel column chromatography eluting with EtOAc:cyclohexane (9:1, v/v) and MeOH:DCM (5:95, v/v) to give **2** as a white solid (4 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.39 (s, 1H), 8.78 (d, *J* = 7.8 Hz, 1H), 8.68 (t, *J* = 5.7 Hz, 1H), 7.99 (s, 1H), 7.71 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.54 (t, *J* = 8.3 Hz, 1H), 7.10-7.25 (m, 5H), 6.61 (d, *J* = 8.1 Hz, 1H), 4.92-5.01 (m, 1H), 4.79 (AB quartet, *J* = 15.6 Hz, 1H), 4.71 (AB quartet, *J* = 15.6 Hz, 1H), 4.05-4.30 (m, 2H), 3.88 (t, *J* = 6.3 Hz, 2H), 3.60-3.7 (m, 8H), 3.51 (m, 2H), 3.34-3.41 (m, 5H), 3.09-

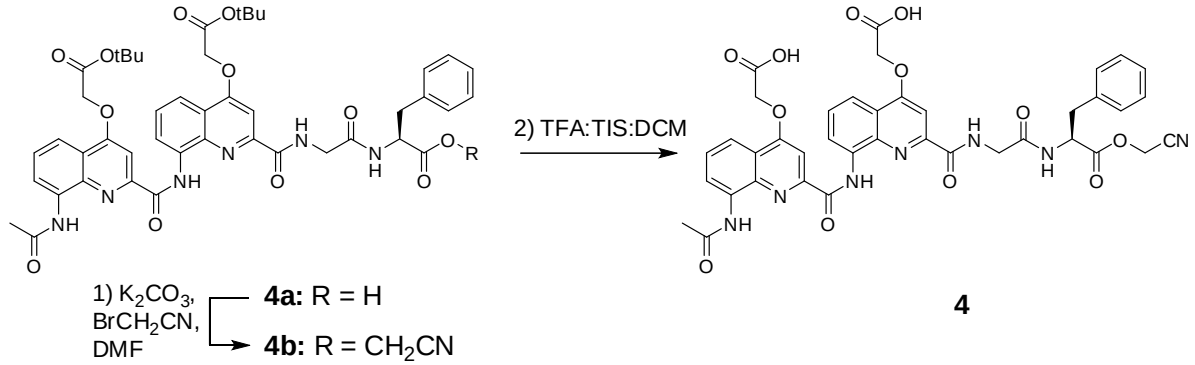
3.24 (m, 2H), 2.30 (s, 3H). HRMS (ESI$^+$): *m/z* calcd for $C_{34}H_{42}N_5O_9S$ $[M+H]^+$ 696.2698 found 696.2690.



Compound 3a: Compound **3a** was prepared from H-L-Phe-2CT-resin on a 27 μmol scale (50 mg, manufacturer's loading: 0.54 mmol g^{-1}) using general methods (3), (4), and (5). The crude product obtained from resin cleavage was used directly in subsequent reaction without further purification (10.9 mg, 67%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.25 (s, 1H), 9.76 (t, *J* = 6.3 Hz, 1H), 8.73 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.27 (d, *J* = 7.8 Hz, 1H), 7.85 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.62 (s, 1H), 7.60 (t, *J* = 8.1 Hz, 1H), 7.15-7.32 (m, 5H), 6.97 (t, *J* = 5.4 Hz, 1H), 4.40-4.50 (m, 1H), 4.35 (t, *J* = 6.0 Hz, 2H), 3.91-4.10 (m, 2H), 2.84-3.24 (m, 6H), 2.32 (s, 3H), 1.95-2.06 (m, 2H), 1.35 (s, 9H). HRMS (ESI$^+$): *m/z* calcd for $C_{31}H_{38}N_5O_8$ $[M-H]^-$ 606.2569 found 606.2573.

Compound 3b. Compound **3b** was synthesized from carboxylic acid **3a** (9.7 mg, 16 μmol) using general method (6). The crude residue was purified by silica gel column chromatography eluting with EtOAc:cyclohexane (7:3, v/v) to give **3b** as a white solid (9.9 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.29 (s, 1H), 8.82 (t, *J* = 5.6 Hz, 1H), 8.72 (d, *J* = 7.5 Hz, 1H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.44 (t, *J* = 7.8 Hz, 2H), 7.12-7.29 (m, 6H), 6.66 (d, *J* = 7.8 Hz, 1H), 4.93-5.02 (m, 1H), 4.79 (AB quartet, *J* = 15.6 Hz, 1H), 4.71 (AB quartet, *J* = 15.6 Hz, 1H), 3.98-4.31 (m, 4H), 3.34-3.46 (m, 2H), 3.08-3.24 (m, 2H), 2.25 (s, 3H), 2.07-2.17 (m, 2H), 1.45 (s, 9H). HRMS (ESI$^+$): *m/z* calcd for $C_{33}H_{39}N_6O_8$ $[M+H]^+$ 647.2824 found 647.2817.

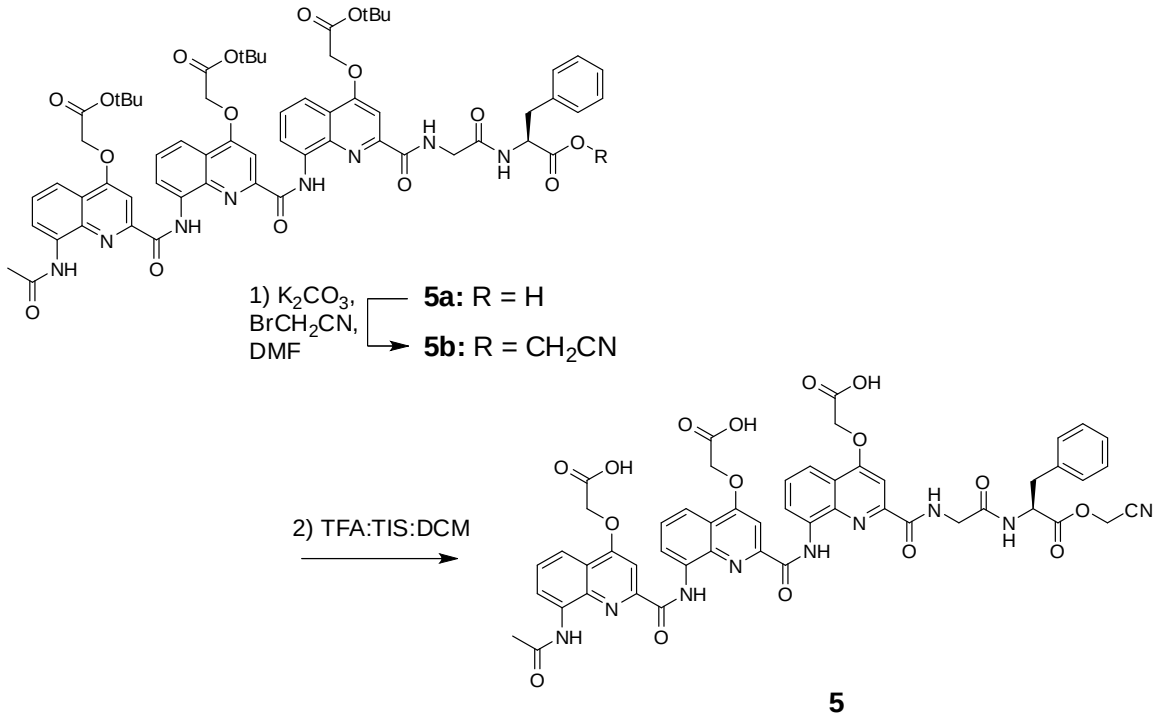
Compound 3. Compound **3** was synthesized from cyanomethyl ester **3b** (9.1 mg, 14.1 μmol) using general method (7). The crude residue was purified by semi-preparative RP-HPLC with a gradient from 5% to 40% solvent B over 15 min (System A) to give **3** as a white solid (7.3 mg, 79%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.26 (s, 1H), 9.79 (t, *J* = 6.0 Hz, 1H), 8.75 (dd, *J* = 7.8, 0.9 Hz, 1H), 8.68 (d, *J* = 7.5 Hz, 1H), 7.88 (dd, *J* = 8.4, 1.1 Hz, 1H), 7.75 (br, 1H), 7.65 (s, 1H), 7.62 (t, *J* = 8.1 Hz, 1H), 7.2-7.3 (m, 5H), 4.99 (s, 2H), 4.53-4.63 (m, 1H), 4.45 (t, *J* = 6.0 Hz, 2H), 4.03 (m, 2H), 3.51 (s, 1H), 2.95-3.15(m, 5H), 2.33 (s, 3H), 2.19 (quintet, *J* = 6.5 Hz, 2H). HRMS (ESI$^+$): *m/z* calcd for $C_{28}H_{31}N_6O_6$ $[M+H]^+$ 547.2300 found 547.2292.



Compound 4a: Compound **4a** was prepared from H-L-Phe-2CT-resin on a 29.5 µmol scale (54.6 mg, manufacturer's loading: 0.54 mmol g^{-1}) using general methods (1), (2), (3), (4), and (5). The crude product obtained from resin cleavage was used directly in subsequent reaction without further purification (16.5 mg, 73%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.88 (s, 1H), 10.00 (s, 1H), 9.26 (t, *J* = 6.0 Hz, 1H), 8.85 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.68 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.24 (d, *J* = 7.8 Hz, 1H), 8.02 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.94 (dd, *J* = 8.7, 1.2 Hz, 1H), 7.78 (t, *J* = 8.1 Hz, 1H), 7.68 (t, *J* = 8.0 Hz, 2H), 7.55 (s, 1H), 7.10-7.30 (m, 5H), 5.20 (s, 2H), 5.15 (s, 2H), 4.22-4.31 (m, 1H), 3.78-3.97 (m, 2H), 2.73-2.98 (m, 2H), 2.09 (s, 3H), 1.48 (s, 9H), 1.47 (s, 9H). HRMS (ESI$^+$): *m/z* calcd for $C_{45}H_{49}N_6O_{12}$ $[M+H]^+$ 865.3403 found 865.3388.

Compound 4b. Compound **4b** was synthesized from carboxylic acid **4a** (12.5 mg, 14.45 µmol) using general method (6). The crude residue was purified by silica gel column chromatography eluting with EtOAc:cyclohexane (1:1, v/v) to give **4b** as a white solid (9.5 mg, 77%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.76 (s, 1H), 9.55 (s, 1H), 8.91 (dd, *J* = 7.5, 0.9 Hz, 1H), 8.75 (d, *J* = 7.5 Hz, 1H), 8.57 (t, *J* = 4.5 Hz, 1H), 8.09 (td, *J* = 8.4, 1.2 Hz, 2H), 7.64 (dt, *J* = 31.5, 8.1 Hz, 2H), 7.64 (d, *J* = 21.0 Hz, 2H), 7.02-7.25 (m, 5H), 6.18 (d, *J* = 6.9 Hz, 1H), 4.91 (s, 2H), 4.88 (s, 2H), 4.70 (AB quartet, *J* = 15.6 Hz, 1H), 4.59 (AB quartet, *J* = 15.6 Hz, 1H), 4.52-4.60 (m, 1H), 4.03-4.11 (m, 2H), 2.91-3.07 (m, 2H), 2.17 (s, 3H), 1.55 (s, 9H), 1.54 (s, 9H). HRMS (ESI$^+$): *m/z* calcd for $C_{47}H_{50}N_7O_{12}$ $[M+H]^+$ 904.3512 found 904.3493.

Compound 4. Compound **4** was synthesized from cyanomethyl ester **4b** (8.3 mg, 9.18 µmol) using general method (7). The crude residue was purified by semi-preparative RP-HPLC with a gradient from 5% to 100% solvent B over 30 min (System A) to give **4** as a white solid (1.6 mg, 22%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.89 (s, 1H), 10.00 (s, 1H), 9.28 (t, *J* = 6.0 Hz, 1H), 8.84 (d, *J* = 7.5 Hz, 1H), 8.67 (dd, *J* = 7.5, 0.9 Hz, 1H), 8.60 (d, *J* = 7.5 Hz, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.95 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.77 (t, *J* = 8.1 Hz, 1H), 7.68 (t, *J* = 8.1 Hz, 2H), 7.54 (s, 1H), 7.13-7.25 (m, 5H), 5.17 (br, 2H), 5.13 (br, 2H), 4.88 (s, 2H), 4.34-4.44 (m, 1H), 3.80-3.96 (m, 2H), 2.84-3.00 (m, 2H), 2.08 (s, 3H). HRMS (ESI$^+$): *m/z* calcd for $C_{39}H_{32}N_7O_{12}$ $[M-H]^-$ 790.2114 found 790.2135.



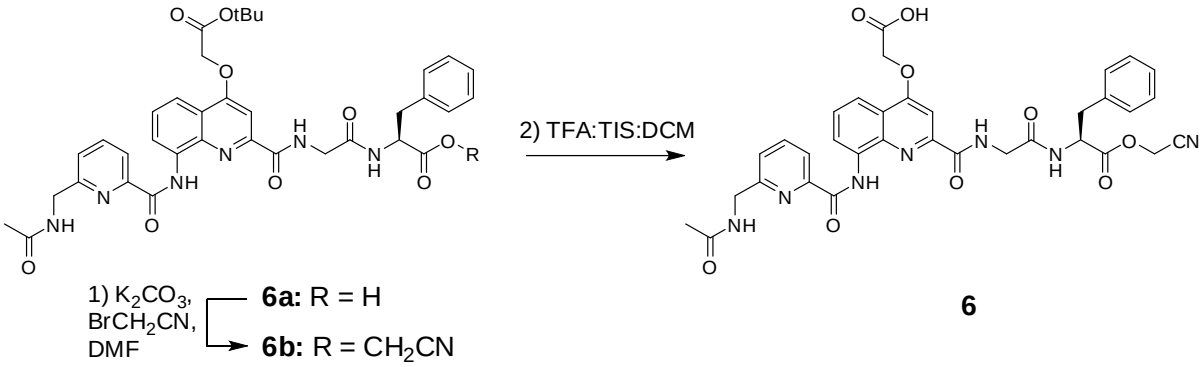
5

Compound 5a: Compound **5a** was prepared from H-L-Phe-2CT-resin on a 29.5 μmol scale (54.6 mg, manufacturer's loading: 0.54 mmol g^{-1}) using general methods (1), (2), (3), (4), and (5). The crude product obtained from resin cleavage was used directly in subsequent reaction without further purification (26.4 mg, 77%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.21 (s, 1H), 11.95 (s, 1H), 9.29 (s, 1H), 8.94 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.84 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.36 (t, *J* = 5.7 Hz, 1H), 7.98-8.06 (m, 2H), 7.88 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.65-7.82 (m, 5H), 7.61 (s, 1H), 7.32 (t, *J* = 8.4 Hz, 1H), 7.03-7.27 (m, 5H), 6.56 (s, 1H), 5.26 (s, 2H), 5.17 (s, 2H), 4.83 (s, 2H), 4.12-4.22 (m, 1H), 2.72-2.90 (m, 4H), 1.73 (s, 3H), 1.54 (s, 9H), 1.50 (s, 9H), 1.47 (s, 9H). HRMS (ESI$^+$): *m/z* calcd for $C_{61}H_{65}N_8O_{16}$ $[M+H]^+$ 1165.4513 found 1165.4500.

Compound 5b. Compound **5b** was synthesized from carboxylic acid **5a** (13.3 mg, 11.41 μmol) using general method (6). The crude residue was purified by silica gel column chromatography eluting with EtOAc:cyclohexane (1:1, v/v) to give **5b** as a white solid (5.8 mg, 42%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.16 (s, 1H), 11.86 (s, 1H), 9.00 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.97 (s, 1H), 8.89 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.16 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.09 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.85 (m, 2H), 7.67-7.75 (m, 4H), 7.58 (s, 1H), 7.27 (t, *J* = 8.4 Hz, 1H), 7.07-7.15 (m, 3H), 6.92-7.00 (m, 2H), 6.76 (s, 1H), 6.23 (d, *J* = 6.9 Hz, 1H), 4.96 (s, 2H), 4.92-4.96 (m, 2H), 4.57-4.80 (m, 5H), 3.52-3.73 (m, 2H), 2.83-2.99 (m, 2H), 1.86 (s, 3H), 1.58 (s, 9H), 1.56 (s, 9H), 1.54 (s, 9H). HRMS (ESI$^+$): *m/z* calcd for $C_{63}H_{66}N_9O_{16}$ $[M+H]^+$ 1204.4622 found 1204.4602.

Compound 5. Compound **5** was synthesized from cyanomethyl ester **5b** (5.8 mg, 4.82 μmol) using general method (7). The crude residue was purified by semi-preparative RP-HPLC with a gradient from 5% to 100% solvent B over 30 min (System A) to give **5** as a yellow solid (0.74 mg, 15%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.43 (br, 3H), 12.23 (s, 1H), 11.96 (s, 1H), 9.28 (s, 1H), 8.92 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.84 (dd, *J* =

7.8, 1.2 Hz, 1H), 8.46 (d, *J* = 7.2 Hz, 1H), 8.36 (t, *J* = 5.7 Hz, 1H), 8.04 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.88 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.66-7.85 (m, 4H), 7.61 (s, 1H), 7.33 (t, *J* = 8.4 Hz, 1H), 7.11-7.24 (m, 5H), 6.54 (s, 1H), 5.29 (s, 2H), 5.21 (s, 2H), 4.82-4.87(m, 4H), 4.25-4.35 (m, 1H), 3.51 (s, 2H), 2.83-2.95 (m, 2H), 1.72 (s, 3H). HRMS (ESI$^+$): *m/z* calcd for $C_{51}H_{40}N_9O_{16}$ [M-H]$^-$ 1034.2598 found 1034.2633.

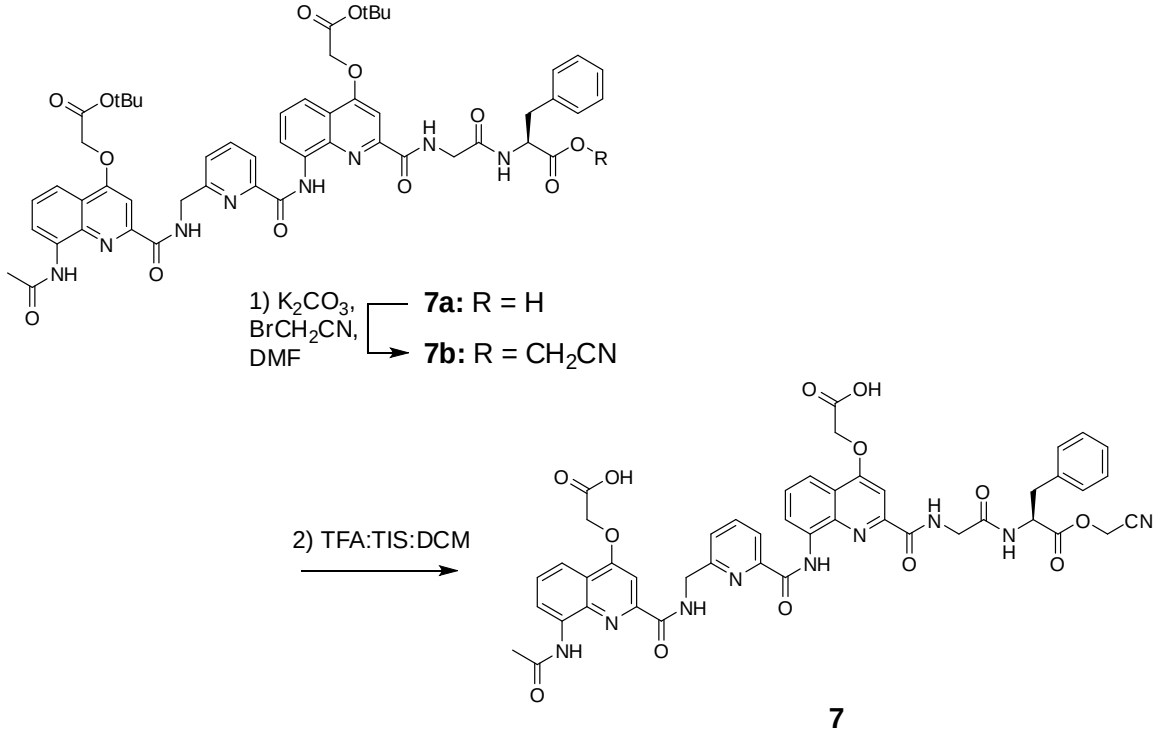


Compound 6a: Compound **6a** was prepared from H-L-Phe-2CT-resin on a 29.5 µmol scale (54.6 mg, manufacturer's loading: 0.54 mmol g^{-1}) using general methods (1), (2), (3), (4), and (5). The crude product obtained from resin cleavage was used directly in subsequent reaction without further purification (12 mg, 58%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.07 (s, 1H), 8.97 (dd, *J* = 7.5, 1.2 Hz, 1H), 8.86 (t, *J* = 6.0 Hz, 1H), 8.65 (t, *J* = 6.0 Hz, 1H), 8.31 (d, *J* = 7.8 Hz, 1H), 8.05-8.15 (m, 2H), 7.98 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.76 (t, *J* = 8.1 Hz, 1H), 7.60 (s, 1H), 7.58 (d, *J* = 1.5 Hz, 1H), 7.12-7.30 (m, 5H), 4.37-4.53 (m, 3H), 4.02-4.20 (m, 2H), 2.98-3.09 (m, 2H), 2.83-2.96 (m, 2H), 1.88 (s, 3H), 1.46 (s, 9H). HRMS (ESI$^+$): *m/z* calcd for $C_{36}H_{39}N_6O_9$ [M-H]$^-$ 697.2628 found 697.2629.

Compound 6b. Compound **6b** was synthesized from carboxylic acid **6a** (10.2 mg, 14.6 µmol) using general method (6). The crude residue was purified by silica gel column chromatography eluting with EtOAc:cyclohexane (4:1, v/v) to give **6b** as a white solid (10.1 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.93 (s, 1H), 9.03 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.74 (t, *J* = 6.0 Hz, 1H), 8.25 (d, *J* = 7.5 Hz, 1H), 8.08 (dd, *J* = 8.4, 0.9 Hz, 1H), 7.92 (t, *J* = 7.8 Hz, 1H), 7.68 (t, *J* = 8.1 Hz, 1H), 7.63 (s, 1H), 7.58 (d, *J* = 7.5 Hz, 1H), 7.02-7.16 (m, 5H), 6.85 (d, *J* = 7.5 Hz, 1H), 6.78 (t, *J* = 6.0 Hz, 1H), 4.81-4.91 (m, 3H), 4.75 (AB quartet, *J* = 15.6 Hz, 1H), 4.67 (AB quartet, *J* = 15.6 Hz, 1H), 4.49-4.64 (m, 2H), 4.15-4.35 (m, 2H), 3.03-3.19 (m, 2H), 2.03 (s, 3H), 1.54 (s, 9H). HRMS (ESI$^+$): *m/z* calcd for $C_{38}H_{40}N_7O_9$ [M+H]$^+$ 738.2882 found 738.2860.

Compound 6. Compound **6** was synthesized from cyanomethyl ester **6b** (9.5 mg, 12.9 µmol) using general method (7). The crude residue was purified by semi-preparative RP-HPLC with a gradient from 5% to 100% solvent B over 30 min (System A) to give **6** as a white solid (2.7 mg, 31%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.08 (s, 1H), 8.97 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.87 (t, *J* = 6.0 Hz, 1H), 8.71 (d, *J* = 7.2 Hz, 1H), 8.49 (t, *J* =

6.0 Hz, 1H), 8.06-8.17 (m, 2H), 7.98 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.76 (t, *J* = 8.1 Hz, 1H), 7.58-7.62 (m, 2H), 7.18-7.29 (m, 5H), 5.19 (s, 2H), 4.95 (s, 2H), 4.54-4.64 (m, 1H), 4.50 (d, *J* = 6.0 Hz, 2H), 4.04-4.22 (m, 2H), 2.95-3.09 (m, 2H), 1.89 (s, 3H). HRMS (ESI$^+$): *m/z* calcd for $C_{34}H_{30}N_7O_9$ [M-H]$^-$ 680.2110 found 680.2084.



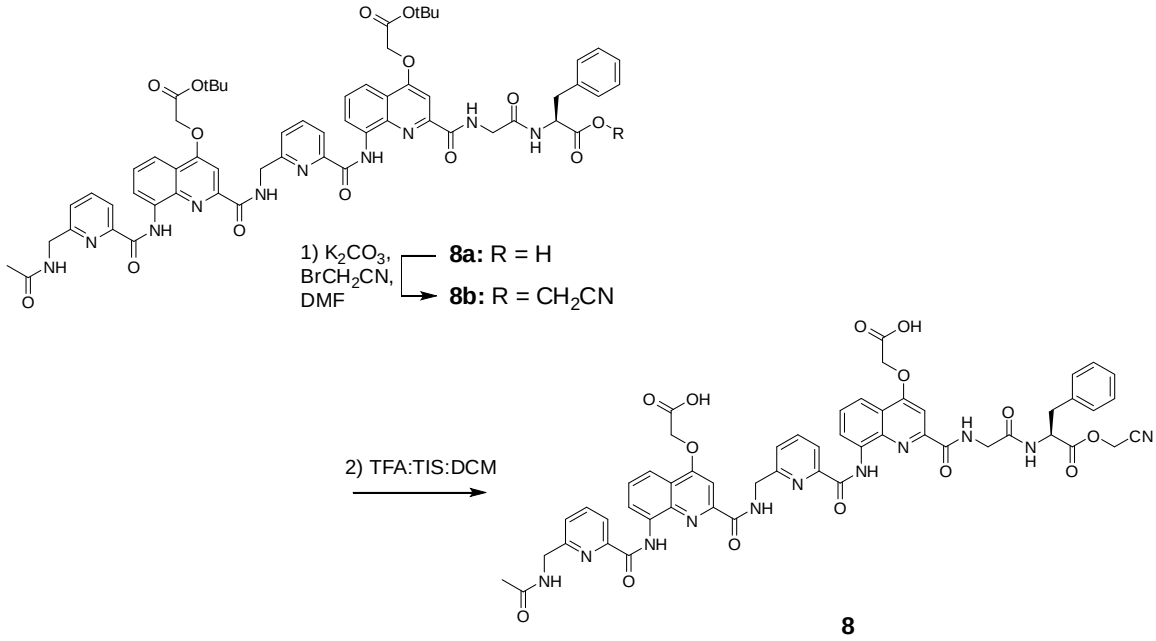
7

Compound 7a: Compound **7a** was prepared from H-L-Phe-2CT-resin on a 29.5 μmol scale (54.6 mg, manufacturer's loading: 0.54 mmol g^{-1}) using general methods (1), (2), (3), (4), and (5). The crude product obtained from resin cleavage was used directly in subsequent reaction without further purification (15 mg, 51%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.11 (s, 1H), 10.17 (s, 1H), 10.10 (t, *J* = 6.0 Hz, 1H), 8.98 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.90 (t, *J* = 6.0 Hz, 1H), 8.71 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.54 (d, *J* = 8.1 Hz, 1H), 8.17 (dd, *J* = 7.5, 1.2 Hz, 1H), 8.11 (t, *J* = 7.5 Hz, 1H), 8.04 (d, *J* = 7.5 Hz, 1H), 7.97 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.85 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.72-7.79 (m, 2H), 7.60 (t, *J* = 8.1 Hz, 1H), 7.56 (s, 2H), 7.00-7.31 (m, 5H), 5.10-5.20 (m, 4H), 5.05 (s, 2H), 4.72-4.83 (m, 2H), 4.44-4.56 (m, 1H), 4.06-4.33 (m, 2H), 2.72-2.96 (m, 2H), 2.09 (s, 3H), 1.46 (s, 9H), 1.42 (s, 9H). HRMS (ESI$^+$): *m/z* calcd for $C_{52}H_{55}N_8O_{13}$ [M-H]$^-$ 997.3738 found 997.3739.

Compound 7b. Compound **7b** was synthesized from carboxylic acid **7a** (13.8 mg, 13.81 μmol) using general method (6). The crude residue was purified by silica gel column chromatography eluting with EtOAc:cyclohexane (7:3, v/v) to give **7b** as a yellow solid (9.6 mg, 67%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.93 (s, 1H), 9.67 (s, 1H), 9.40 (t, *J* = 6.0 Hz, 1H), 9.08 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.75 (d, *J* = 7.5 Hz, 1H), 8.60 (t, *J* = 5.7 Hz, 1H), 8.30 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.07 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.90-

7.98 (m, 2H), 7.82 (dd, *J* = 7.5, 0.9 Hz, 1H), 7.67 (t, *J* = 8.1 Hz, 1H), 7.61 (s, 1H), 7.47-7.55 (m, 2H), 7.17-7.22 (m, 3H), 6.87-6.92 (m, 2H), 6.41 (br, 1H), 4.70-4.95 (m, 6H), 4.62-4.70 (m, 1H), 4.31-4.57 (m, 3H), 4.17-4.28 (m, 1H), 2.75-2.98 (m, 2H), 1.88 (s, 3H), 1.55 (s, 9H), 1.54 (s, 9H). HRMS (ESI$^+$): *m/z* calcd for $C_{54}H_{56}N_9O_{13}$ $[M+H]^+$ 1038.3992 found 1038.3984.

Compound 7. Compound **7** was synthesized from cyanomethyl ester **7b** (9.6 mg, 9.15 µmol) using general method (7) as a white solid (6.3 mg, 74%). ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 12.14 (s, 1H), 10.15 (s, 1H), 10.12 (t, *J* = 6.3 Hz, 1H), 8.98 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.90 (t, *J* = 6.0 Hz, 1H), 8.83 (d, *J* = 7.5 Hz, 1H), 8.71 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.18 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.12 (t, *J* = 7.8 Hz, 1H), 7.97 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.87 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.72-7.79 (m, 2H), 7.61 (t, *J* = 8.1 Hz, 1H), 7.6 (s, 1H), 7.54 (s, 1H), 7.01-7.13 (m, 5H), 5.18 (s, 2H), 5.11 (s, 2H), 4.78-4.86 (m, 4H), 4.53-4.63 (m, 1H), 4.09-4.29 (m, 2H), 2.82-2.98 (m, 2H), 2.10 (s, 3H). HRMS (ESI$^+$): *m/z* calcd for $C_{46}H_{38}N_9O_{13}$ $[M-H]^-$ 924.2595 found 924.2567.



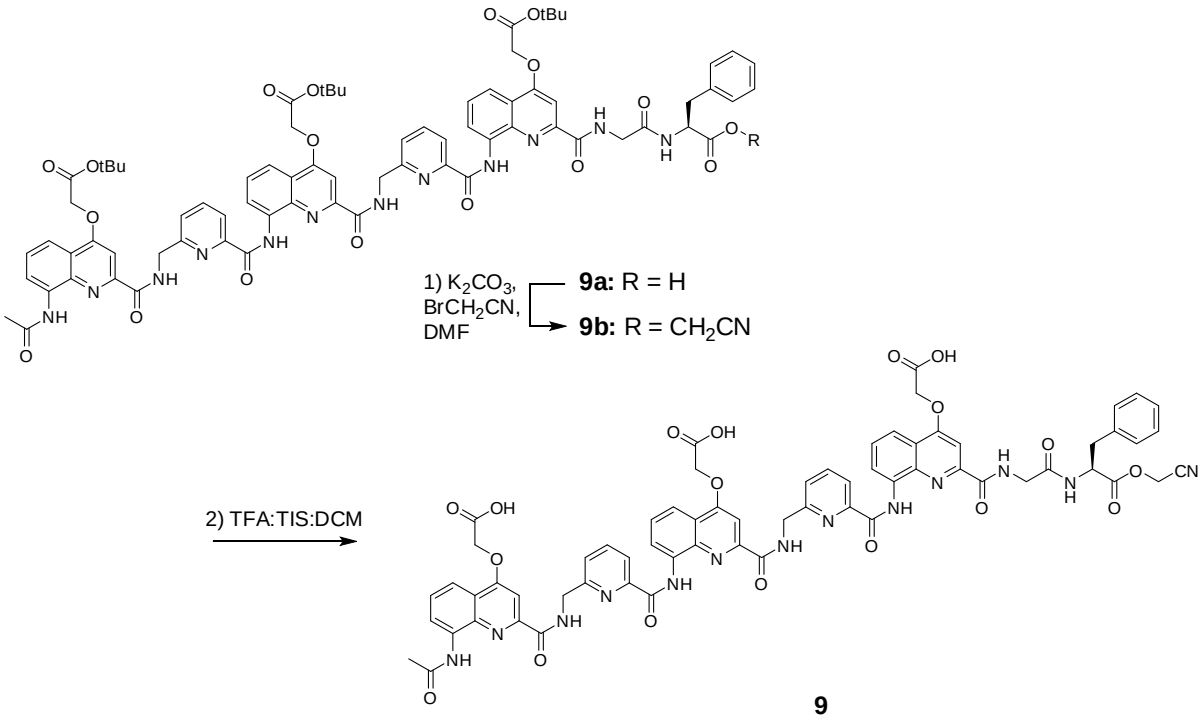
8

Compound 8a: Compound **8a** was prepared from H-L-Phe-2CT-resin on a 33.3 µmol scale (61.7 mg, manufacturer's loading: 0.54 mmol g^{-1}) using general methods (1), (2), (3), (4), and (5). The crude product obtained from resin cleavage was used directly in subsequent reaction without further purification (23 mg, 51%). ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 11.88 (s, 1H), 11.87 (s, 1H), 9.52 (t, *J* = 5.4 Hz, 1H), 8.60-8.68 (m, 2H), 8.57 (t, *J* = 5.4 Hz, 1H), 8.30 (t, *J* = 6.0 Hz, 1H), 8.03-8.15 (m, 2H), 7.99 (t, *J* = 7.5 Hz, 1H), 7.86-7.93 (m, 3H), 7.75 (dd, *J* = 6.9, 1.8 Hz, 1H), 7.68 (t, *J* = 8.1 Hz, 1H), 7.62 (t, *J* = 8.1 Hz, 1H), 7.59 (s, 1H), 7.38 (dd, *J* = 7.5, 1.2 Hz, 1H), 6.95-7.29 (m, 7H), 5.10 (s, 2H), 5.05 (s, 2H), 4.90-5.05 (m, 2H), 4.22-4.34 (m, 1H), 3.65-4.01 (m, 4H), 2.75-2.97 (m, 2H), 1.65 (s, 3H), 1.45-1.49 (m, 18). HRMS (ESI$^+$): *m/z* calcd for $C_{59}H_{61}N_{10}O_{14}$

$[M+H]^+$ 1133.4363 found 1133.4354.

Compound 8b. Compound **8b** was synthesized from carboxylic acid **8a** (15 mg, 13.24 μmol) using general method (6). The crude residue was purified by silica gel column chromatography eluting with EtOAc:cyclohexane (7:3, v/v) and MeOH:DCM (5:95, v/v) to give **8b** as a white solid (7 mg, 45%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.67 (s, 2H), 9.39 (t, *J* = 5.1 Hz, 1H), 8.64 (td, *J* = 7.5, 1.2 Hz, 2H), 8.27 (t, *J* = 5.1 Hz, 1H), 8.20 (dd, *J* = 6.9, 0.6 Hz, 1H), 8.08 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.97-8.05 (m, 2H), 7.93 (dd, *J* = 7.5, 0.6 Hz, 1H), 7.80 (t, *J* = 7.5 Hz, 1H), 7.68 (dd, *J* = 7.8, 0.6 Hz, 1H), 7.64 (s, 1H), 7.59 (t, *J* = 8.1 Hz, 1H), 7.53 (t, *J* = 8.1 Hz, 1H), 7.22-7.26 (m, 1H), 7.21 (s, 1H), 7.04-7.12 (m, 3H), 6.90-6.95 (m, 2H), 6.55 (d, *J* = 7.8 Hz, 1H), 6.31 (t, *J* = 5.4 Hz, 1H), 4.96-5.10 (m, 2H), 4.89-4.95 (m, 2H), 4.75-4.83 (m, 3H), 4.66 (AB quartet, *J* = 15.6 Hz, 1H), 4.52 (AB quartet, *J* = 15.6 Hz, 1H), 3.67-3.97 (m, 4H), 2.87-2.99 (m, 2H), 1.74 (s, 3H), 1.55 (s, 9H), 1.54 (s, 9H). HRMS (ESI$^+$): *m/z* calcd for $C_{61}H_{62}N_{11}O_{14}$ $[M+H]^+$ 1172.4472 found 1172.4451.

Compound 8. Compound **8** was synthesized from cyanomethyl ester **8b** (7.0 mg, 5.97 μmol) using general method (7) as a yellow solid (2.3 mg, 36%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.82 (s, 1H), 11. 78 (s, 1H), 9.53 (t, *J* = 5.1 Hz, 1H), 8.63 (d, *J* = 7.2 Hz, 1H), 8.52-8.59 (m, 2H), 8.46 (t, *J* = 5.7 Hz, 1H), 8.05-8.17 (m, 3H), 7.98 (t, *J* = 7.5 Hz, 1H), 7.87-7.94 (m, 3H), 7.75-7.80 (m, 1H), 7.56-7.70 (m, 3H), 7.36 (dd, *J* = 7.5, 0.6 Hz, 1H), 7.04-7.18 (m, 6H), 5.15 (s, 2H), 5.05 (s, 2H), 4.88-5.02 (m, 2H), 4.85 (s, 2H), 4.46-4.56 (m, 1H), 3.77-3.96 (m, 2H), 3.56-3.69 (m, 2H), 2.84-2.99 (m, 2H), 1.64 (s, 3H). HRMS (ESI$^+$): *m/z* calcd for $C_{53}H_{44}N_{11}O_{14}$ $[M-H]^-$ 1058.3075 found 1058.3078.

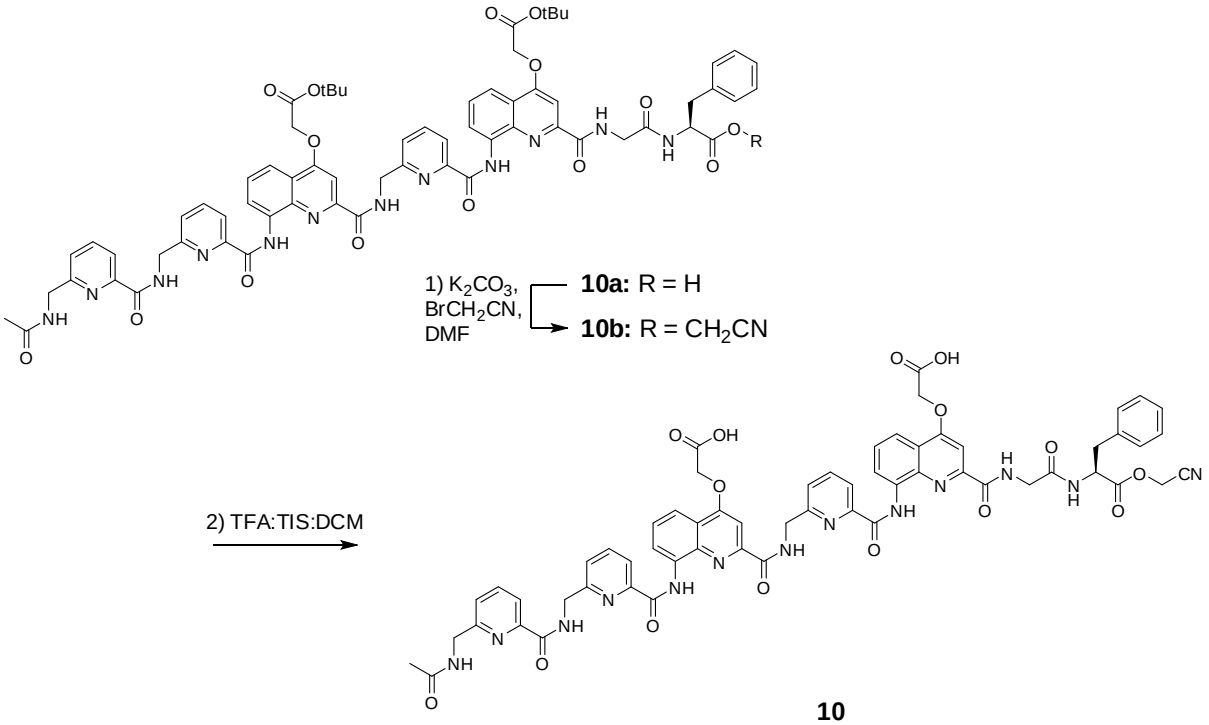


9

Compound 9a: Compound **9a** was prepared from H-L-Phe-2CT-resin on a 28.8 μmol scale (53.3 mg, manufacturer's loading: 0.54 mmol g^{-1}) using general methods (1), (2), (3), (4), and (5). The crude product obtained from resin cleavage was used directly in subsequent reaction without further purification (38 mg, 92%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.24 (s, 1H), 11.54 (s, 1H), 9.80 (t, *J* = 6.0 Hz, 1H), 9.63 (s, 1H), 9. 33 (t, *J* = 6.0 Hz, 1H), 8.81 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.46-8.53 (m, 2H), 8.13-8.19 (m, 2H), 7.92-8.04 (m, 2H), 7.68-7.76 (m, 2H), 7.65 (s, 1H), 7.53 (dd, *J* = 5.7, 1.5 Hz, 1H), 7.46 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.32-7.40 (m, 2H), 7.22-7.27 (m, 1H), 7.14 (s, 1H), 7.10 (s, 1H), 6.92-7.02 (m, 5H), 5.12 (s, 2H), 4.94 (s, 2H), 4.77 (s, 2H), 4.38-4.49 (m, 1H), 3.94-4.34 (m, 4H), 2.69-3.13 (m, 4H), 2.15 (s, 3H), 1.50 (s, 9H), 1.49 (s, 9H), 1.46 (s, 9H). HRMS (ESI$^+$): *m/z* calcd for $C_{75}H_{77}N_{12}O_{18}$ $[M+H]^+$ 1433.5473 found 1433.5475.

Compound 9b. Compound **9b** was synthesized from carboxylic acid **9a** (19.0 mg, 13.25 μmol) using general method (6). The crude residue was purified by silica gel column chromatography eluting with EtOAc:cyclohexane (7:3, v/v) to give **9b** as a white solid (7 mg, 36%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.04 (s, 1H), 11.58 (s, 1H), 9.13-9.20 (m, 2H), 9.09 (t, *J* = 6.0 Hz, 1H), 8.85 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.50-8.57 (m, 2H), 8.41 (t, *J* = 6.0 Hz, 1H), 8.32 (d, *J* = 7.2 Hz, 1H), 8.03-8.10 (m, 2H), 7.97 (t, *J* = 7.8 Hz, 1H), 7.81 (t, *J* = 7.8 Hz, 1H), 7.58-7.70 (m, 5H), 7.27-7.39 (m, 3H), 7.23 (s, 1H), 7.13 (s, 1H), 7.05-7.10 (m, 3H), 6.80-6.86 (m, 2H), 6.54 (2, *J* = 7.5 Hz, 1H), 4.97-5.18 (m, 2H), 4.89 (s, 2H), 4.74 (s, 2H), 4.70 (s, 2H), 4.54-4.63 (m, 1H), 4.44 (AB quartet, *J* = 15.6 Hz, 1H), 4.34 (AB quartet, *J* = 15.6 Hz, 1H), 4.09-4.28 (m, 4H), 2.75-2.90 (m, 2H), 2.10 (s, 3H), 1.57 (s, 18H), 1.56 (s, 9H). HRMS (ESI$^+$): *m/z* calcd for $C_{77}H_{78}N_{13}O_{18}$ $[M+H]^+$ 1472.5582 found 1472.5612.

Compound 9. Compound **9** was synthesized from cyanomethyl ester **9b** (7.0 mg, 4.75 μmol) using general method (7) as a white solid (5.18 mg, 84%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.70-14.05 (br, 2H), 12.20 (s, 1H), 11.55 (s, 1H), 9.72 (t, *J* = 6.6 Hz, 1H), 9.58 (s, 1H), 9.33 (t, *J* = 6.0 Hz, 1H), 8.76-8.84 (m, 2H), 8.43-8.52 (m, 2H), 8.37 (t, *J* = 4.8 Hz, 1H), 8.11-8.19 (m, 2H), 7.94-8.04 (m, 3H), 7.66-7.76 (m, 3H), 7.55 (dd, t, *J* = 8.4, 1.2 Hz, 1H), 7.46-7.52 (dd, t, *J* = 8.4, 1.2 Hz, 1H), 7.29-7.42 (m, 3H), 6.95-7.11 (m, 5H), 5.17 (s, 2H), 5.00-5.08 (m, 2H), 4.96 (s, 2H), 4.83 (s, 2H), 4.78 (s, 2H), 4.49-4.60 (m, 1H), 4.06-4.28 (m, 4H), 2.80-2.89 (m, 2H), 2.17 (s, 3H). HRMS (ESI$^+$): *m/z* calcd for $C_{65}H_{52}N_{13}O_{18}$ $[M-H]^-$ 1302.3559 found 1302.3554.



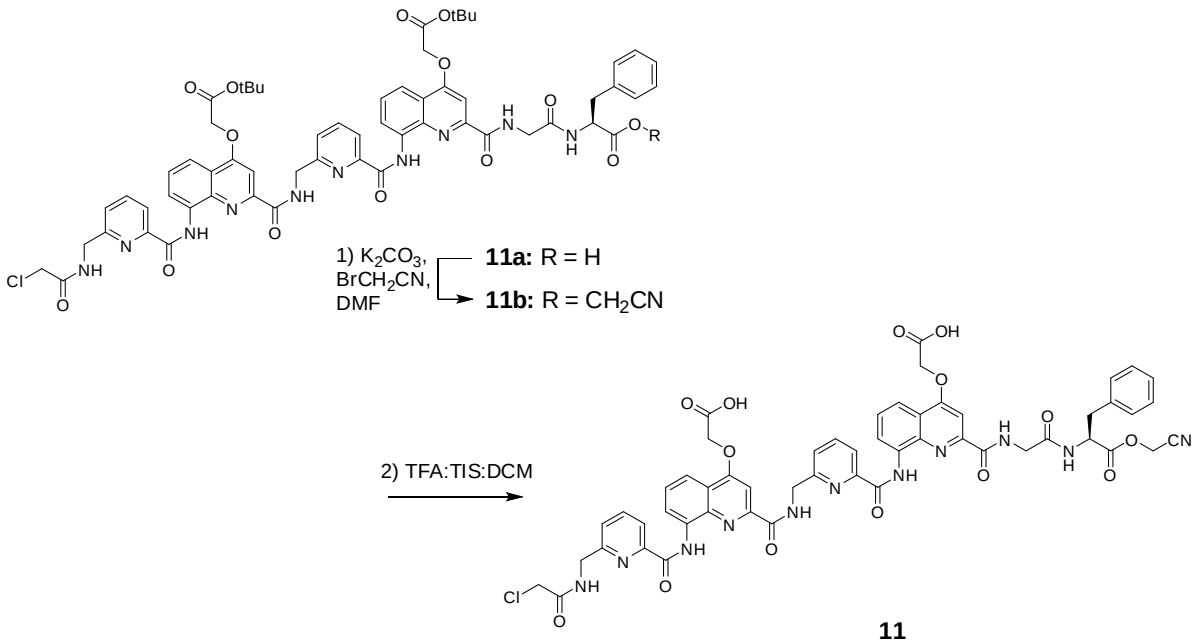
10

Compound 10a: Compound **10a** was prepared from H-L-Phe-2CT-resin on a 13.33 μmol scale (24.7 mg, manufacturer's loading: 0.54 mmol g^{-1}) using general methods (1), (2), (3), (4), and (5). The crude product obtained from resin cleavage was used directly in subsequent reaction without further purification (13 mg, 77%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.05 (s, 1H), 11.72 (s, 1H), 9.45 (t, *J* = 5.7 Hz, 1H), 9.23 (t, *J* = 6.0 Hz, 1H), 8.71 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.60 (dd, *J* = 7.5, 1.2 Hz, 1H), 8.49 (t, *J* = 5.7 Hz, 1H), 8.24 (t, *J* = 5.7 Hz, 1H), 8.02-8.15 (m, 2H), 7.95-8.00 (m, 2H), 7.85-7.93 (m, 2H), 7.73 (dd, *J* = 6.6, 1.8 Hz, 1H), 7.58-7.70 (m, 3H), 7.35-7.51 (m, 3H), 7.16-7.32 (m, 4H), 7.03-7.15 (m, 2H), 6.94-7.00 (m, 2H), 5.10 (s, 2H), 5.07 (s, 2H), 4.95-5.05 (m, 2H), 4.21-4.31 (m, 1H), 3.84-4.17 (m, 4H), 2.62-2.99 (m, 4H), 1.84 (s, 3H), 1.47-1.50 (m, 18H). HRMS (ESI$^+$): *m/z* calcd for $C_{66}H_{67}N_{12}O_{15}$ $[M+H]^+$ 1267.4843 found 1267.4859.

Compound 10b. Compound **10b** was synthesized from carboxylic acid **10a** (8.0 mg, 6.31 μmol) using general method (6). The crude residue was purified by silica gel column chromatography eluting with MeOH:DCM (4:96, v/v) to give **10b** as a white solid (5.0 mg, 61%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.15 (s, 1H), 11.71 (s, 1H), 9.36 (t, *J* = 6.0 Hz, 1H), 8.95 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.64 (t, *J* = 6.0 Hz, 1H), 8.54 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.37 (t, *J* = 6.6 Hz, 1H), 8.26 (d, *J* = 7.5 Hz, 1H), 8.06-8.12 (m, 2H), 7.95-8.03 (m, 2H), 7.62-7.80 (m, 4H), 7.42-7.52 (m, 2H), 7.11-7.23 (m, 4H), 7.01-7.10 (m, 4H), 6.78-6.88 (m, 3H), 5.01-5.25 (m, 2H), 4.94 (s, 2H), 4.88 (s, 2H), 4.62-4.72 (m, 1H), 4.46 (AB quartet, *J* = 15.6 Hz, 1H), 4.33 (AB quartet, *J* = 15.6 Hz, 1H), 3.88-4.29 (m, 6H), 2.72-2.84 (m, 2H), 2.19 (s, 3H). HRMS (ESI$^+$): *m/z* calcd for $C_{68}H_{68}N_{13}O_{15}$ $[M+H]^+$ 1306.4952 found 1306.4965.

Compound 10. Compound **10** was synthesized from cyanomethyl ester **10b** (5.0 mg, 3.83 μmol) using general method (7) as a white solid (2.87 mg, 63%). ^{1}H NMR (300

MHz, DMSO-d_6): δ 11.97 (s, 1H), 11.69 (s, 1H), 9.47 (t, *J* = 5.7 Hz, 1H), 9.06 (t, *J* = 6.0 Hz, 1H), 8.64-8.70 (m, 2H), 8.53 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.41 (t, *J* = 5.7 Hz, 1H), 8.05-8.18 (m, 3H), 7.91-8.01 (m, 3H), 7.87 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.75 (dd, *J* = 7.2 1.2 Hz, 1H), 7.57-7.70 (m, 3H), 7.44-7.48 (m, 2H), 7.35-7.40 (m, 1H), 7.19 (s, 1H), 7.01-7.13 (m, 6H), 5.17 (s, 2H), 5.08 (s, 2H), 4.89-5.05 (m, 2H), 4.81 (s, 2H), 4.47-4.56 (m, 1H), 3.88-4.11 (m, 6H), 2.82-2.95 (m, 2H), 1.86 (s, 3H). HRMS (ESI$^+$): *m/z* calcd for $C_{60}H_{52}N_{13}O_{15}$ [M-H]$^-$ 1192.3555 found 1192.3567.



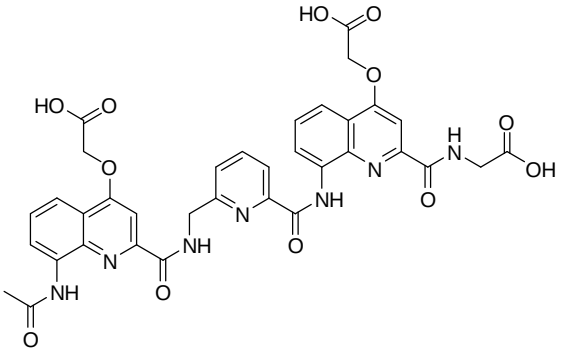
11

Compound 11a: Compound **11a** was prepared from H-L-Phe-2CT-resin on a 48.6 μmol scale (90 mg, manufacturer's loading: 0.54 mmol g^{-1}) using general methods (1), (2), (3), (4), and (5). The crude product obtained from resin cleavage was used directly in subsequent reaction without further purification (35 mg, 62%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.86 (s, 1H), 11.84 (s, 1H), 9.56 (t, *J* = 5.4 Hz, 1H), 8.79-8.87 (m, 1H), 8.63 (dd, *J* = 4.2, 0.9 Hz, 1H), 8.61 (dd, *J* = 3.9, 0.9 Hz, 1H), 8.52 (t, *J* = 5.7 Hz, 1H), 8.16-8.28 (m, 1H), 8.05-8.16 (m, 2H), 8.02 (t, *J* = 7.5 Hz, 1H), 7.94 (dd, *J* = 7.5, 0.9 Hz, 1H), 7.85-7.92 (m, 2H), 7.77 (dd, *J* = 7.2, 0.9 Hz, 1H), 7.68 (t, *J* = 8.1 Hz, 1H), 7.61 (t, *J* = 8.1 Hz, 1H), 7.57 (s, 1H), 7.37-7.43 (m, 1H), 7.15-7.33 (m, 4H), 7.02-7.13 (m, 2H), 5.10 (s, 2H), 5.04 (s, 2H), 4.90-5.01 (m, 2H), 4.25-4.35 (m, 1H), 3.95 (s, 2H), 3.70-3.93 (m, 2H), 2.73-2.99 (m, 2H), 1.44-1.48 (m, 18H). HRMS (ESI$^+$): *m/z* calcd for $C_{59}H_{60}N_{10}O_{14}Cl$ [M+H]$^+$ 1167.3974 found 1167.3944.

Compound 11b. Compound **11b** was synthesized from carboxylic acid **11a** (22 mg, 18.84 μmol) using general method (6). The crude residue was purified by silica gel column chromatography eluting with EtOAc:cyclohexane (9:1, v/v) to give **11b** as a white solid (10 mg, 44%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.67 (s, 2H), 9.36 (t, *J* = 5.4 Hz, 1H), 8.67 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.64 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.19-8.27 (m,

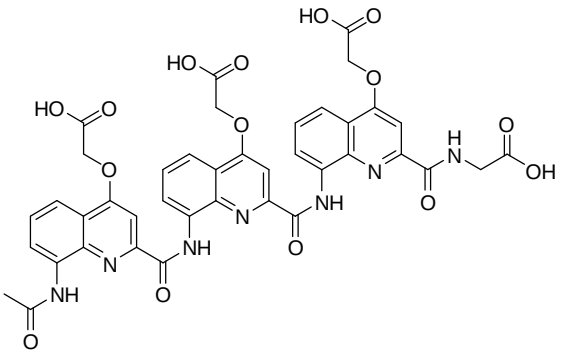
2H), 8.09 (dd, *J* = 8.7, 1.2 Hz, 1H), 8.02 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.94-8.04 (m, 2H), 7.82 (t, *J* = 7.5 Hz, 1H), 7.66-7.71 (d, *J* = 7.8 Hz, 1H), 7.65 (s, 1H), 7.60 (t, *J* = 8.1 Hz, 1H), 7.53 (t, *J* = 8.1 Hz, 1H), 7.21-7.25 (m, 3H), 7.06-7.12 (m, 3H), 6.92-6.97 (m, 2H), 6.51 (d, *J* = 7.8 Hz, 1H), 4.99-5.07 (m, 2H), 4.89-4.96 (m, 2H), 4.77-4.86 (m, 3H), 4.66 (AB quartet, *J* = 15.6 Hz, 1H), 4.51 (AB quartet, *J* = 15.6 Hz, 1H), 3.80-4.01 (m, 4H), 3.81 (s, 2H), 2.86-2.99 (m, 2H), 1.53-1.56 (m, 18H). HRMS (ESI$^+$): *m/z* calcd for $C_{61}H_{61}N_{11}O_{14}Cl$ $[M+H]^+$ 1206.4082 found 1206.4043.

Compound 11. Compound **11** was synthesized from cyanomethyl ester **11b** (7.0 mg, 5.8 μmol) using general method (7). The crude residue was purified by semi-preparative RP-HPLC with a gradient from 10% to 50% solvent D over 18 min (System B) to give **11** as a white solid (5.0 mg, 79%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.74 (s, 1H), 11.65 (s, 1H), 9.46 (t, *J* = 5.4 Hz, 1H), 8.90 (d, *J* = 6.6, 1H), 8.51-8.57 (m, 2H), 8.49 (dd, *J* = 7.5, 1.2Hz, 1H), 8.31 (t, *J* = 5.4 Hz, 1H), 8.05-8.16 (m, 2H), 7.86-7.99 (m, 3H), 7.76-7.84 (m, 2H), 7.50-7.62 (m, 3H), 7.37 (dd, *J* = 7.8, 0.9 Hz, 1H), 7.09-7.20 (m, 6H), 4.89-4.98 (m, 2H), 4.75-4.85 (m, 6H), 4.40-4.51 (m, 1H), 3.94 (s, 2H), 3.66-3.83 (m, 4H), 2.87-3.03 (m, 2H). HRMS (ESI$^+$): *m/z* calcd for $C_{53}H_{43}N_{11}O_{14}Cl$ $[M-H]^-$ 1092.2685 found 1092.2709.

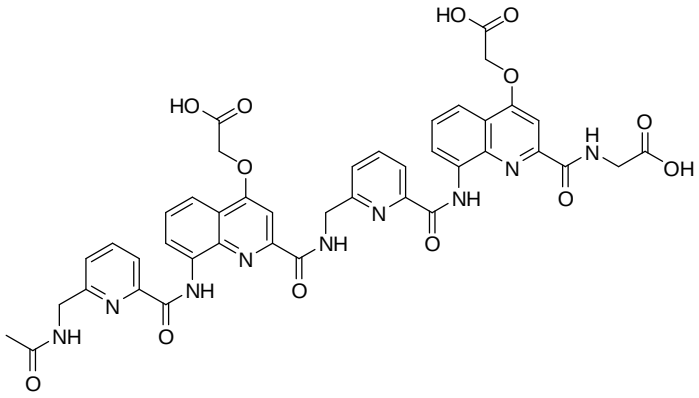


Compound 12: Compound **12** was prepared from Fmoc-Gly-Wang-resin on a 18.5 μmol scale (50 mg, manufacturer's loading: 0.37 mmol g^{-1}) using general methods (1), (2), (3), (4), and (8). The crude residue obtained from resin cleavage was purified by semi-preparative RP-HPLC with a gradient from 7% to 25% solvent D over 27 min (System B) to give **12** as a white solid (5.4 mg, 39%). ^{1}H NMR (400 MHz, 50 mM $NaHCO_3$ D_2O/H_2O (1:9, v/v)): δ 11.86 (s, 1H), 10.00 (t, *J* = 3.0 Hz, 1H), 9.43 (s, 1H), 8.58 (d, *J* = 6.0 Hz, 1H), 8.27 (t, *J* = 3.9 Hz, 1H), 8.11-8.15 (m, 2H), 7.95 (d, *J* = 6.6 Hz, 1H), 7.86 (d, *J* = 2.1 Hz, 1H), 7.84 (d, *J* = 3.0 Hz, 1H), 7.77 (t, *J* = 3.3 Hz, 1H), 7.61 (t, *J* = 6.0 Hz, 1H), 7.36 (t, *J* = 6.3 Hz, 1H), 7.25 (s, 1H), 6.53 (s, 1H), 3.63 (m, 2H), 1.85 (s, 3H). ^{1}H NMR (400 MHz, 50 mM $NaHCO_3$ in D_2O): δ 8.63 (dd, *J* = 7.6, 0.8 Hz, 1H), 8.16 (s, 1H), 8.11-8.18 (m, 1H), 7.99 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.89 (d, *J* = 1.6 Hz, 1H), 7.87 (d, *J* = 1.2 Hz, 1H), 7.79 (dd, *J* = 10.0, 2.4 Hz, 1H), 7.65 (t, *J* = 8.0 Hz, 1H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.30 (s, 1H), 6.57 (s, 1H), 4.93 (s, 2H), 4.66 (s, 2H), 4.57 (s, 2H), 3.67 (s, 2H), 1.88 (s, 3H). HRMS (ESI$^+$): *m/z* calcd for $C_{35}H_{30}N_7O_{12}$ $[M-H]^-$

738.1801 found 738.1778.

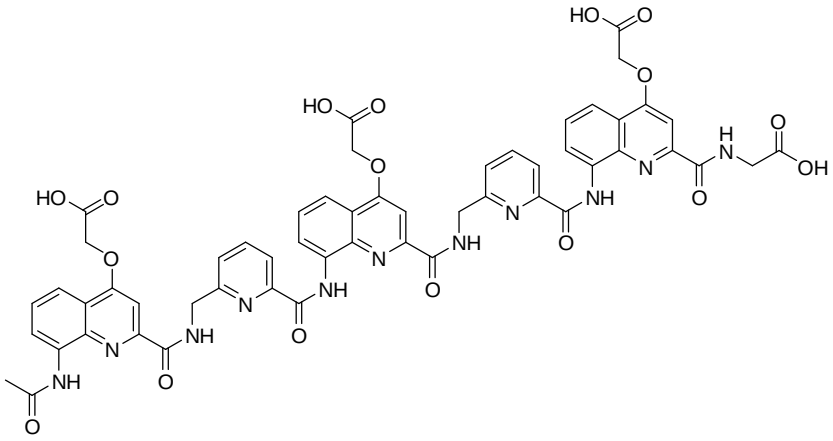


Compound 13: Compound **13** was prepared from Fmoc-Gly-Wang-resin on a 18.5 µmol scale (50 mg, manufacturer's loading: 0.37 mmol g^{-1}) using general methods (1), (2), (3), (4), and (8). The crude residue obtained from resin cleavage was purified by semi-preparative RP-HPLC with a gradient from 7% to 25% solvent D over 27 min (System B) to give **13** as a white solid (6.1 mg, 39%). ^{1}H NMR (400 MHz, 50 mM $NaHCO_3$ D_2O/H_2O (1:9, v/v)): δ 12.12 (s, 1H), 11.91 (s, 1H), 8.97 (s, 1H), 8.81 (d, *J* = 7.6 Hz, 1H), 8.30 (d, *J* = 5.6 Hz, 1H), 8.16 (d, *J* = 8.0 Hz, 1H), 8.07 (d, *J* = 8.0 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.64-7.75 (m, 3H), 7.51 (s, 1H), 7.40 (t, *J* = 8.0 Hz, 1H), 7.11 (t, *J* = 8.0 Hz, 1H), 6.99 (s, 1H), 6.42 (s, 1H), 1.92 (s, 3H). ^{1}H NMR (400 MHz, 50 mM $NaHCO_3$ in D_2O): δ 8.82 (d, *J* = 7.6 Hz, 1H), 8.19 (d, *J* = 7.2 Hz, 1H), 8.08 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.91 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.66-7.76 (m, 3H), 7.53 (s, 1H), 7.41 (t, *J* = 8.0 Hz, 1H), 7.13 (t, *J* = 8.0 Hz, 1H), 7.03 (s, 1H), 6.43 (s, 1H), 4.90 (s, 2H), 4.40-4.65 (m, 3H), 3.95-4.15 (br, 1H), 3.34-3.51 (m, 1H), 2.85-3.02 (m, 1H), 1.93 (s, 3H). HRMS (ESI^+): *m/z* calcd for $C_{40}H_{32}N_7O_{15}$ $[M-H]^-$ 848.1805 found 848.1779.

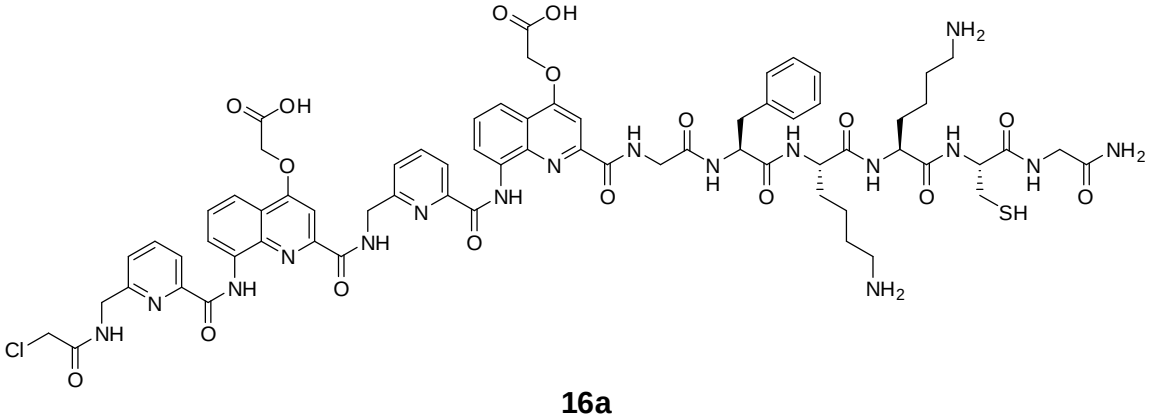


Compound 14: Compound **14** was prepared from Fmoc-Gly-Wang-resin on a 18.5 µmol scale (50 mg, manufacturer's loading: 0.37 mmol g^{-1}) using general methods (1), (2), (3), (4), and (8). The crude residue obtained from resin cleavage was purified by semi-preparative RP-HPLC with a gradient from 7% to 25% solvent D over 27 min

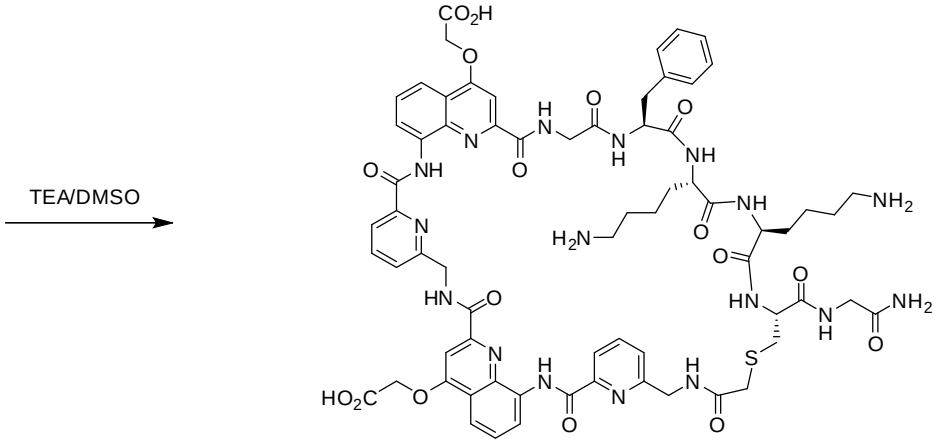
(System B) to give **14** as a white solid (4.6 mg, 29%). ^{1}H NMR (400 MHz, 50 mM $NaHCO_3$ D_2O/H_2O (1:9, v/v)): δ 11.20 (s, 1H), 10.51 (s, 1H), 9.35-9.40 (m, 1H), 8.12 (t, *J* = 7.6 Hz, 1H), 8.01 (br, 1H), 7.80-7.90 (m, 4H), 7.77 (d, *J* = 7.6 Hz, 1H), 7.68-7.74 (m, 2H), 7.54-7.60 (m, 2H), 7.40 (t, *J* = 8.0 Hz, 1H), 7.16-7.26 (m, 3H), 6.28 (s, 1H), 2.01 (s, 3H). ^{1}H NMR (400 MHz, 50 mM $NaHCO_3$ in D_2O): δ 8.15 (t, *J* = 8.0 Hz, 1H), 7.90-7.97 (m, 3H), 7.85-7.89 (m, 1H), 7.82-7.85 (m, 1H), 7.78-7.82 (m, 1H), 7.71-7.75 (m, 1H), 7.66-7.70 (m, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.27-7.35 (m, 3H), 6.41 (s, 1H), 4.49 (br, 4H), 3.27-3.5 (br, 2H), 3.04-3.27 (br, 2H), 1.99 (s, 3H). HRMS (ESI$^+$): *m/z* calcd for $C_{42}H_{36}N_9O_{13}$ $[M-H]^-$ 872.2282 found 872.2251.



Compound 15: Compound **15** was prepared from Fmoc-Gly-Wang-resin on a 18.5 µmol scale (50 mg, manufacturer's loading: 0.37 mmol g^{-1}) using general methods (1), (2), (3), (4), and (8). The crude residue obtained from resin cleavage was purified by semi-preparative RP-HPLC with a gradient from 7% to 25% solvent D over 27 min (System B) to give **15** as a white solid (5.2 mg, 25%). ^{1}H NMR (400 MHz, 50 mM $NaHCO_3$ D_2O/H_2O (1:9, v/v)): δ 11.63 (s, 1H), 11.45 (s, 1H), 9.50-9.56 (m, 1H), 9.26-9.32 (m, 1H), 9.03 (s, 1H), 8.19-8.28 (m, 2H), 8.06-8.13 (m, 2H), 7.94-8.03 (m, 4H), 7.92 (d, *J* = 7.6 Hz, 1H), 7.83-7.88 (m, 2H), 7.67 (t, *J* = 7.6 Hz, 1H), 7.60 (d, *J* = 7.2 Hz, 1H), 7.41-7.52 (m, 3H), 7.31 (t, *J* = 8.0 Hz, 1H), 6.61 (s, 1H), 6.41 (s, 1H), 1.36 (s, 3H). ^{1}H NMR (400 MHz, 50 mM $NaHCO_3$ in D_2O): δ 8.30-8.34 (m, 1H), 8.25 (t, *J* = 8.0 Hz, 1H), 8.09-8.15 (m, 2H), 7.97-8.05 (m, 3H), 7.95 (dd, *J* = 7.6, 0.8 Hz, 1H), 7.89 (d, *J* = 3.2 Hz, 1H), 7.87 (d, *J* = 3.2 Hz, 1H), 7.71 (t, *J* = 8.0 Hz, 1H), 7.63 (dd, *J* = 7.6, 0.8 Hz, 1H), 7.50-7.55 (m, 2H), 7.47 (t, *J* = 8.0 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 6.65 (s, 1H), 6.44 (s, 1H), 4.93-5.09 (m, 2H), 4.56-4.71 (m, 3H), 4.37-4.55 (m, 3H), 4.17-4.26 (d, *J* = 18.4 Hz, 1H), 3.38-3.46 (d, *J* = 17.6 Hz, 1H), 2.98-3.08 (m, 1H), 2.86-2.94 (m, 1H), 1.40 (s, 3H). HRMS (ESI$^+$): *m/z* calcd for $C_{54}H_{44}N_{11}O_{17}$ $[M-H]^-$ 1116.2766 found 1116.2730.



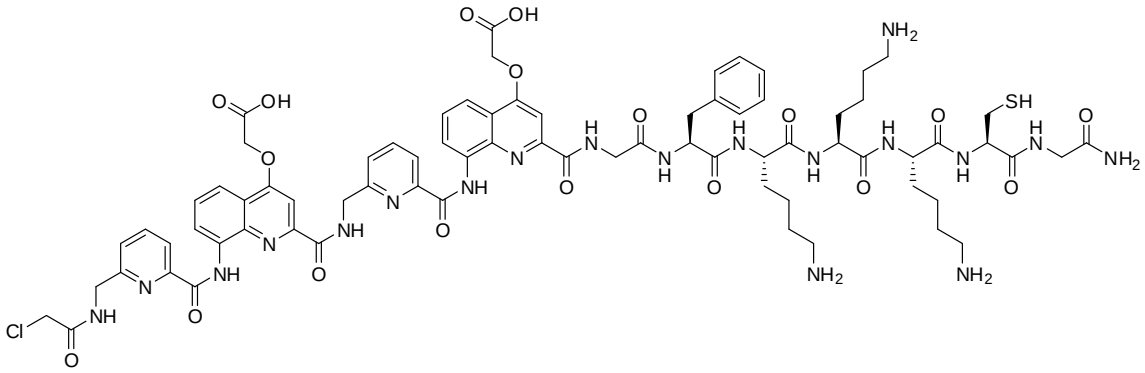
16a



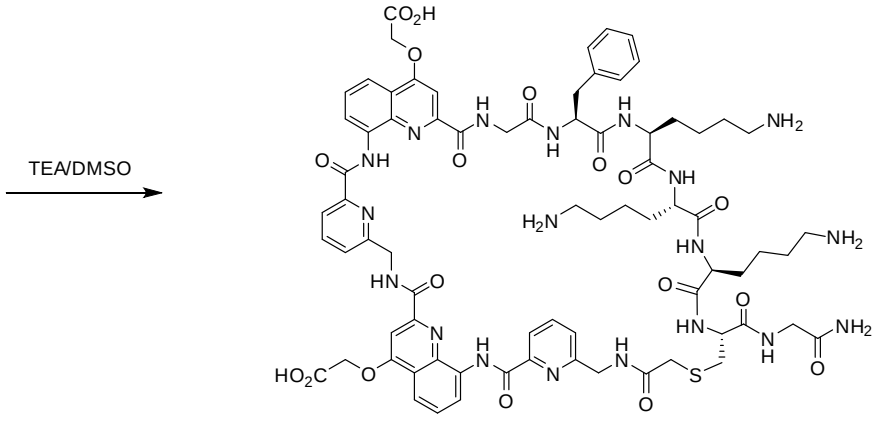
16

Compound 16a: Peptide Fmoc-Phe-Lys(Boc)-Lys(Boc)-Cys(Trt)-Gly was assembled on Rink amide MBHA resin on a 8.66 μmol scale (23.4 mg, manufacturer's loading: 0.37 mmol g^{-1}) using CEM Liberty Blue microwave automated peptide synthesizer. Fmoc-deprotection was carried out with 20% piperidine in DMF at room temperature. Each coupling step of Fmoc-amino acid was carried out with DIC (diisopropyl carbodiimide, 10 equiv) and oxyma (10 equiv) at 70 °C. Fmoc-P-Q^{AsptBu}-P-Q^{AsptBu}-Gly-OH block (compound **18**) was then coupled to the N-terminus of peptide using general method (3), followed by chloroacetylation and cleavage (general method (4) and (9), respectively) to give **16a** as a white solid. The crude product obtained from resin cleavage was used directly in subsequent reaction without further purification (10.3 mg, 80.8%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.75 (s, 1H), 11.63 (s, 1H), 9.56 (t, *J* = 5.1 Hz, 1H), 8.42-8.54 (m, 3H), 8.35 (t, *J* = 5.1 Hz, 1H), 8.12-8.25 (m, 4H), 7.97-8.12 (m, 4H), 7.85-7.93 (m, 3H), 7.80 (d, *J* = 7.5 Hz, 1H), 7.52-7.68 (m, 3H), 7.38 (d, *J* = 7.2 Hz, 1H), 7.28 (s, 1H), 7.00-7.20 (m, 6H), 6.96 (s, 1H), 5.11 (s, 2H), 4.85-5.03 (m, 4H), 4.45-4.58 (m, 1H), 4.32-4.43 (m, 1H), 4.16-4.31 (m, 2H), 3.94 (s, 2H), 3.78-3.90 (m, 1H), 3.50-3.71 (m, 3H), 3.41 (AB quartet, *J* = 7.2 Hz, 1H), 3.36 (AB quartet, *J* = 7.2 Hz, 1H), 2.92-3.04 (m, 1H), 2.63-2.85 (m, 7H), 1.59-1.78 (m, 2H), 1.40-1.58 (m, 6H), 1.18-1.40 (m, 4H).

Compound 16: Compound **16a** (10.3 mg, 7.0 μmol) was incubated in 0.5 M triethylamine/DMSO solution for 10 min at room temperature. The resulting residue was purified by semi-preparative RP-HPLC with a gradient from 26% to 29% solvent B over 14 min (System B) to give **16** as a white solid (5.2 mg, 52%). ^{1}H NMR (700 MHz, 8:2 H_2O/DMSO-d_6, 338 K): δ 11.30 (s, 1H), 11.02 (s, 1H), 9.38-9.42 (m, 1H), 8.65-8.73 (m, 2H), 8.38-8.43 (m, 1H), 8.36 (d, *J* = 9.8 Hz, 1H), 8.17 (t, *J* = 7.7 Hz, 1H), 8.09-8.14 (m, 1H), 7.91 (d, *J* = 7.0 Hz, 1H), 7.76-7.87 (m, 6H), 7.72 (d, *J* = 7.7 Hz, 1H), 7.64 (s, 1H), 7.55-7.60 (m, 1H) 7.36-7.41 (m, 2H), 7.27 (d, *J* = 7.7 Hz, 1H), 7.10-7.22 (m, 7H), 6.42 (s, 1H), 5.02-5.08 (m, 1H), 4.73-4.91 (m, 2H), 3.86-3.94 (m, 1H), 3.73-3.81 (m, 1H), 3.44-3.52 (m, 2H), 3.34-3.41 (m, 2H), 3.22-3.26 (m, 2H), 2.97-3.14 (m, 9H), 2.73-2.79 (m, 1H), 1.96-2.10 (m, 2H), 1.68-1.93 (m, 9H), 1.48-1.64 (m, 4H). HRMS (ESI$^+$): *m/z* calcd for $C_{68}H_{74}N_{17}O_{17}S$ $[M-H]^-$ 1432.5175 found 1432.5183.



17a

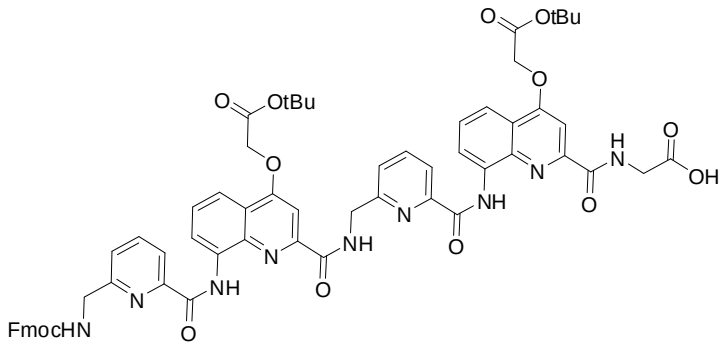


17

Compound 17a: Peptide Fmoc-Phe-Lys(Boc)-Lys(Boc)-Lys(Boc)-Cys(Trt)-Gly was assembled on Rink amide MBHA resin on a 14.2 μmol scale (38.4 mg, manufacturer's loading: 0.37 mmol g^{-1}) using CEM Liberty Blue microwave automated peptide synthesizer. Fmoc-deprotection was carried out with 20% piperidine in DMF at room temperature. Each coupling step of Fmoc-amino acid was carried out with DIC (diisopropyl carbodiimide, 10 equiv) and oxyma (10 equiv) at 70 °C. Fmoc-P-Q^{AsptBu}-

P-Q^{AsptBu}-Gly-OH block (compound **18**) was then coupled to the N-terminus of peptide using general method (3), followed by chloroacetylation and cleavage (general method (4) and (9), respectively) to give **17a** as a white solid. The crude product obtained from resin cleavage was used directly in subsequent reaction without further purification (16.8 mg, 74%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.74 (s, 1H), 11.62 (s, 1H), 9.56 (t, J = 4.6 Hz, 1H), 8.52 (t, J = 5.7 Hz, 1H), 8.44 (m, 2H), 8.35 (t, J = 4.5 Hz, 1H), 8.02-8.22 (m, 7H), 7.84-8.02 (m, 4H), 7.71-7.83 (m, 2H), 7.51-7.67 (m, 3H), 7.39 (d, J = 7.8 Hz, 1H), 7.31 (s, 1H), 7.00-7.18 (m, 6H), 6.95 (s, 1H), 5.15 (s, 2H), 4.84-5.03 (m, 4H), 4.45-4.58 (m, 1H), 4.32-4.42 (m, 1H), 4.14-4.30 (m, 3H), 3.95 (s, 2H), 3.80-3.90 (m, 1H), 3.64-3.68 (m, 2H), 3.54-3.60 (m, 2H), 2.91-3.03 (m, 1H), 2.65-2.85 (m, 10H), 2.41 (t, J = 8.1 Hz, 1H), 1.41-1.75 (m, 12H), 1.18-1.40 (m, 6H).

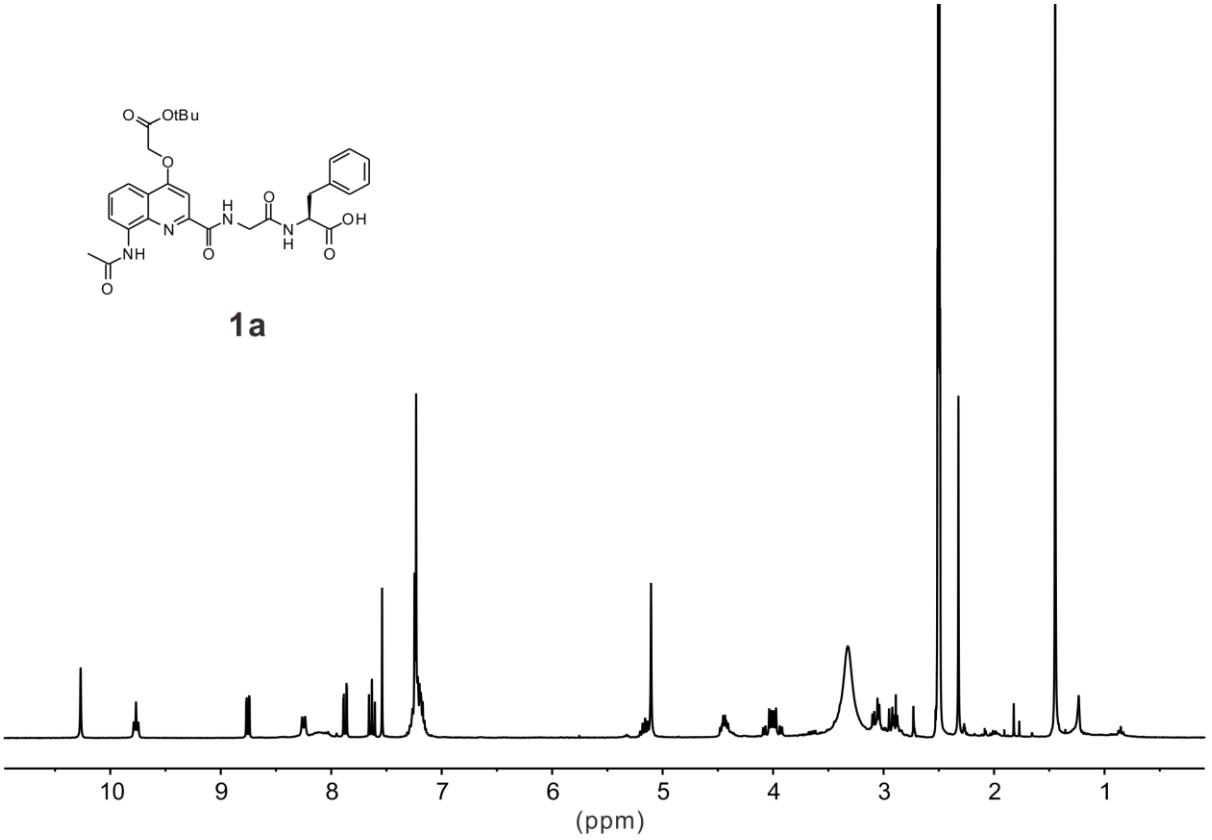
Compound 17: Compound **17a** (16.8 mg, 10.5 µmol) was incubated in 0.5 M triethylamine/DMSO solution for 10 min at room temperature. The resulting residue was purified by semi-preparative RP-HPLC with a gradient from 25% to 27.5% solvent B over 14 min (System B) to give **17** as a white solid (5.4 mg, 33%). ^{1}H NMR (700 MHz, 8:2 H_2O/DMSO-d_6, 338 K): δ 11.16 (s, 1H), 11.05 (s, 1H), 9.55-9.59 (m, 1H), 8.64 (d, J = 7.0 Hz, 1H), 8.31-8.39 (m, 2H), 8.24 (t, J = 5.6 Hz, 1H), 8.19 (t, J = 14 Hz, 1H), 8.15 (d, J = 6.3 Hz, 1H), 7.96-8.00 (m, 2H), 7.80-7.88 (m, 6H), 7.78 (d, J = 7.7 Hz, 1H), 7.63-7.66 (m, 1H), 7.59 (d, J = 7.0 Hz, 1H), 7.42 (s, 1H), 7.40 (t, J = 7.7 Hz, 1H), 7.31 (d, J = 7.7 Hz, 1H), 7.23 (t, J = 7.7 Hz, 1H), 7.10-7.20 (m, 5H), 6.43 (s, 1H), 5.08-5.15 (m, 1H), 4.73-4.92 (m, 3H), 3.82-3.92 (m, 1H), 3.67-3.72 (m, 1H), 3.49-3.54 (m, 1H), 3.23-3.38 (m, 5H), 3.14-3.19 (m, 1H), 3.06-3.13 (m, 2H), 2.89-3.01 (m, 5H), 2.84-2.89 (m, 1H), 2.74-2.80 (m, 1H), 2.00-2.11 (m, 2H), 1.68-1.91 (m, 9H), 1.54-1.66 (m, 3H), 1.41-1.52 (m, 4H). HRMS (ESI$^+$): *m/z* calcd for $C_{74}H_{86}N_{19}O_{18}S$ [M-H]$^-$ 1560.6124 found 1560.6125.

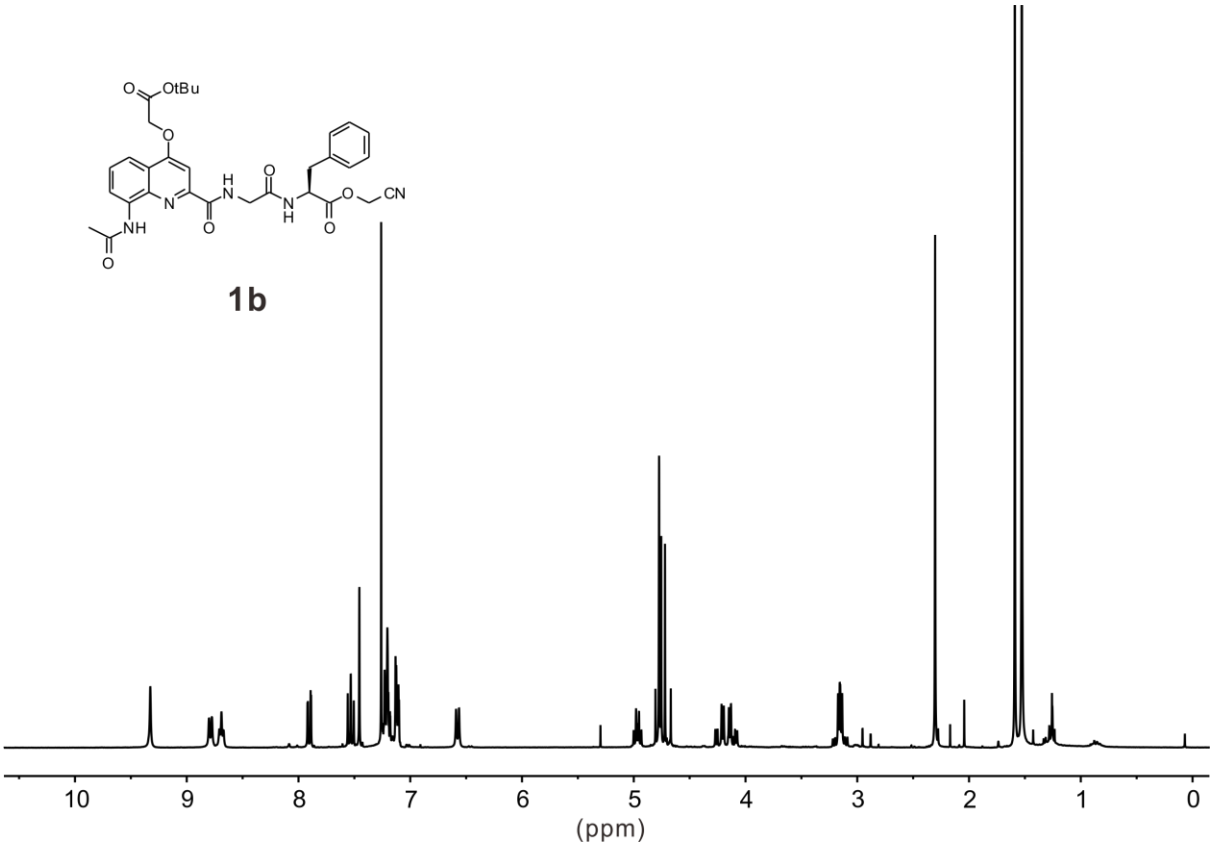


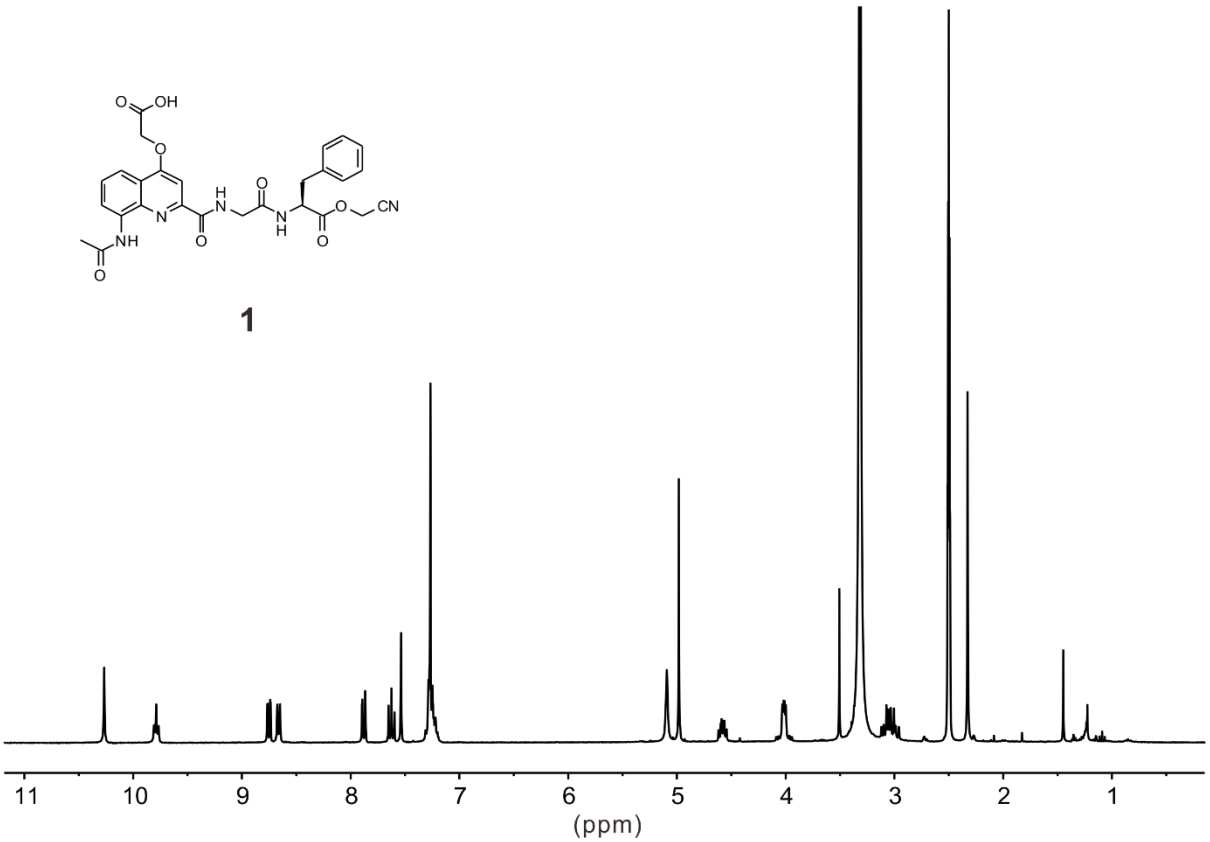
Compound 18: Compound **18** was prepared from H-L-Phe-2CT-resin on a 0.594 mmol scale (1.1 g, manufacturer's loading: 0.54 mmol g^{-1}) using general methods (1), (2), (3), and (5). The crude residue obtained from resin cleavage was purified by precipitation in MeOH:DCM (1:9, v/v) to afford compound **18** as a white solid (284 mg, 45%). ^{1}H NMR (300 MHz, $CDCl_3$/DMSO-d_6 (1:4, v/v)): δ 12.01 (s, 1H), 11.93 (s, 1H), 9.58 (br, 1H), 8.75-8.82 (d, J = 10.4 Hz, 1H), 8.69-8.74 (d, J = 10.4 Hz, 1H), 8.62 (t, J

= 7.8 Hz, 1H), 7.92-8.16 (m, 6H), 7.60-7.83 (m, 7H), 7.28-7.48 (m, 6H), 7.15-7.24 (m, 2H), 5.09 (s, 2H), 5.07 (s, 2H), 5.03 (s, 2H), 4.12-4.22 (m, 2H), 4.00-4.10 (m, 3H), 3.90-3.97 (m, 2H), 1.56 (s, 9H), 1.54 (s, 9H). HRMS (ESI^+): *m/z* calcd for $C_{63}H_{60}N_9O_{14}$ $[M+H]^+$ 1166.4254 found 1166.4249.

3. Supplementary Data (^{1}H NMR spectra and HPLC profiles)



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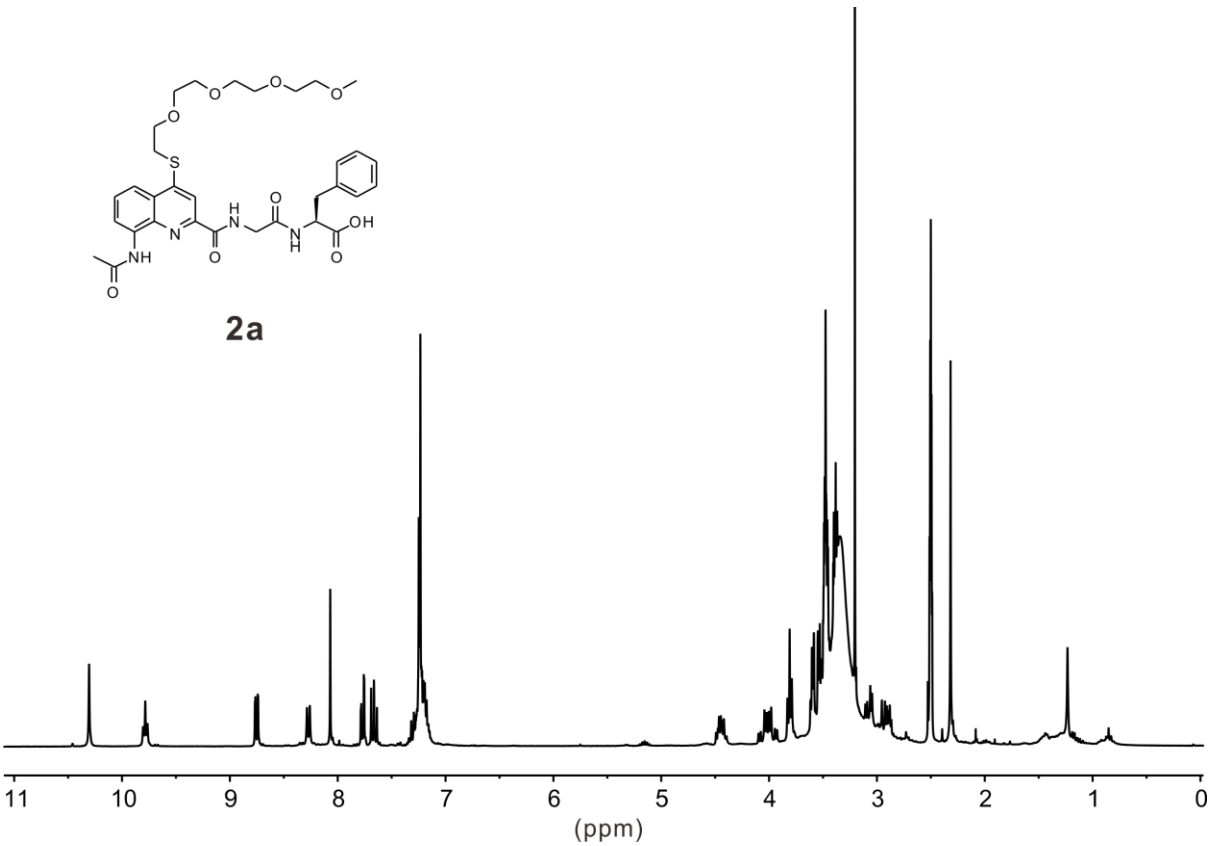
1
(ppm)


2a
11
10
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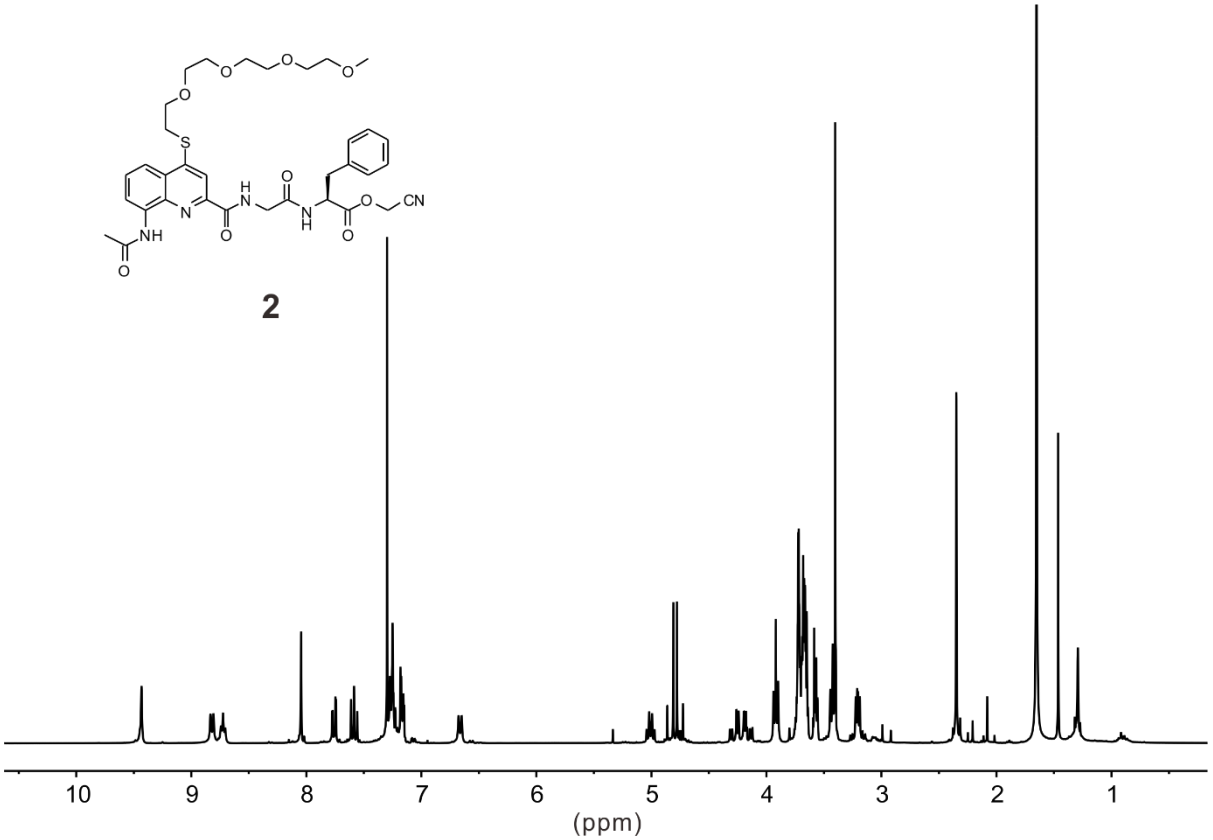
8
7
6
5
4
3
2
1
0
(ppm)


2
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9
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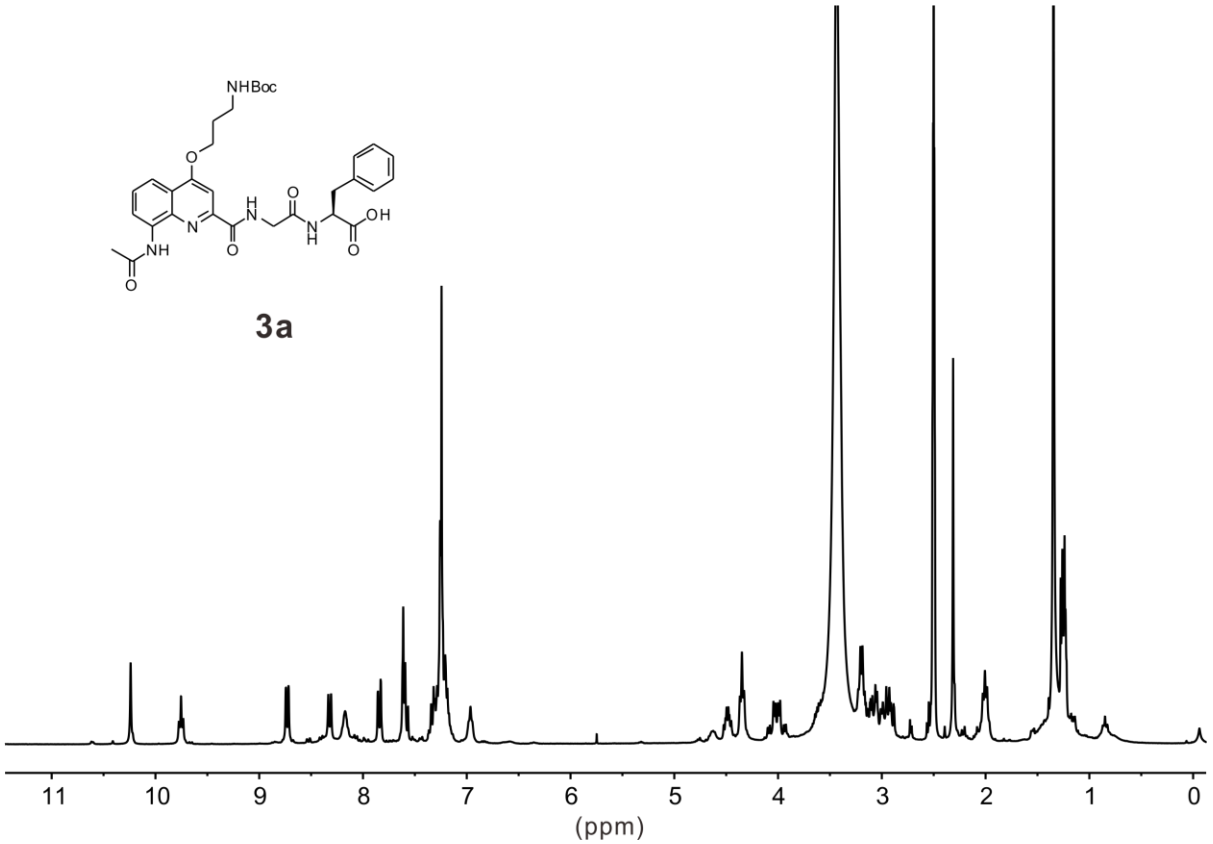
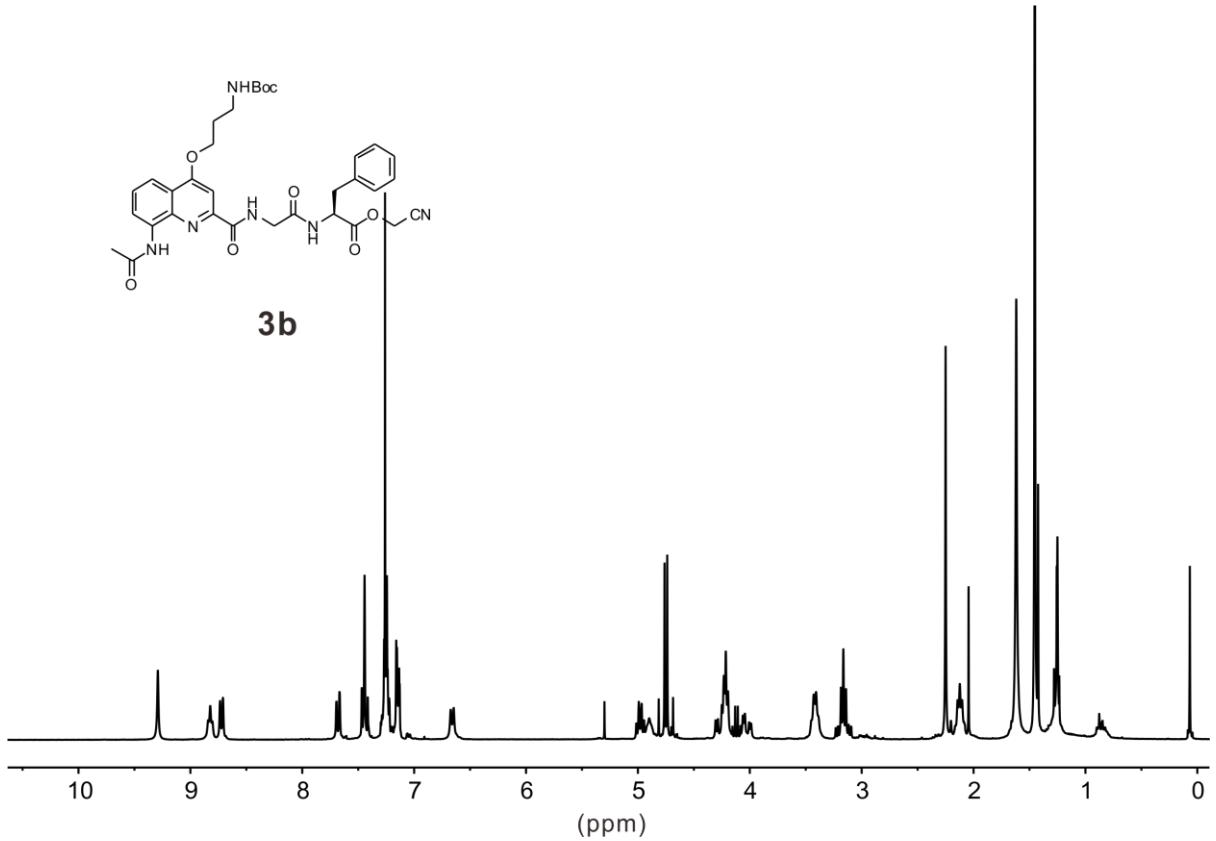
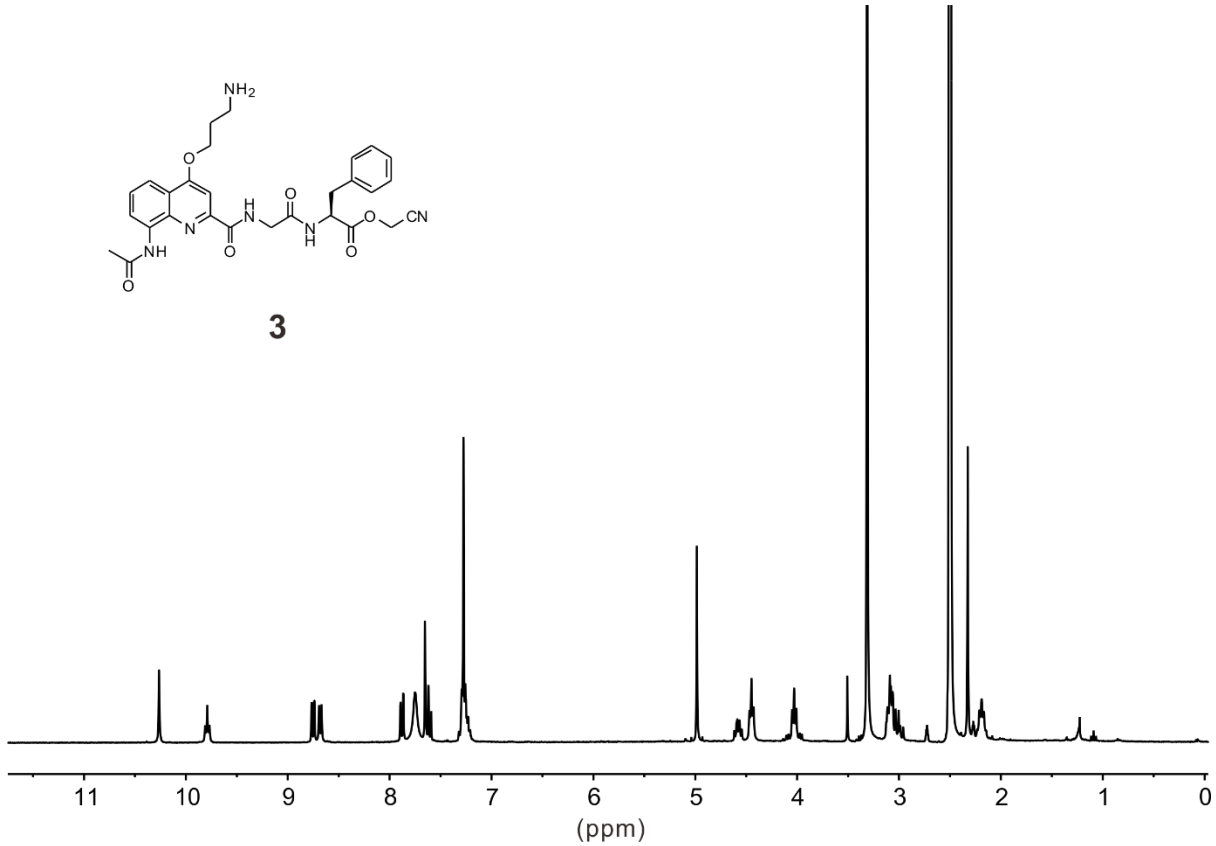
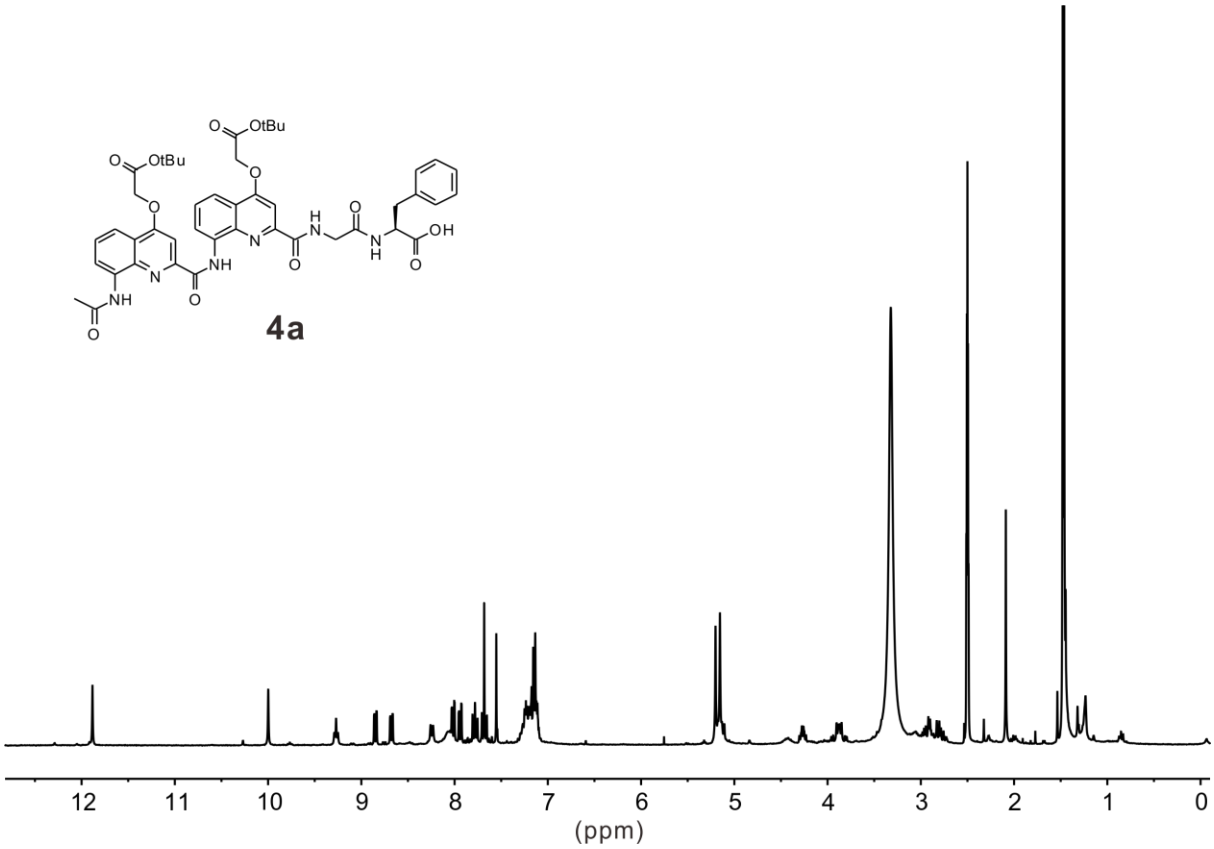
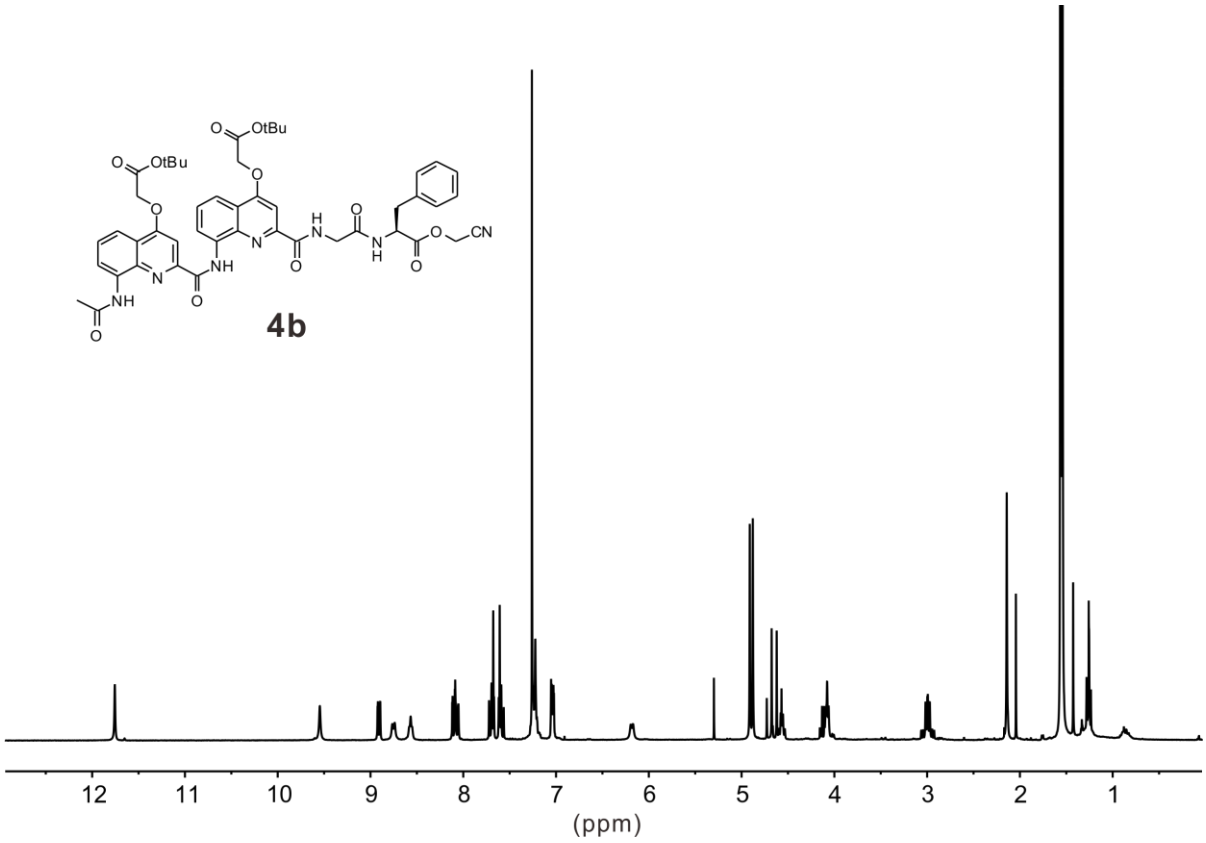
7
6
5
4
3
2
1
(ppm)

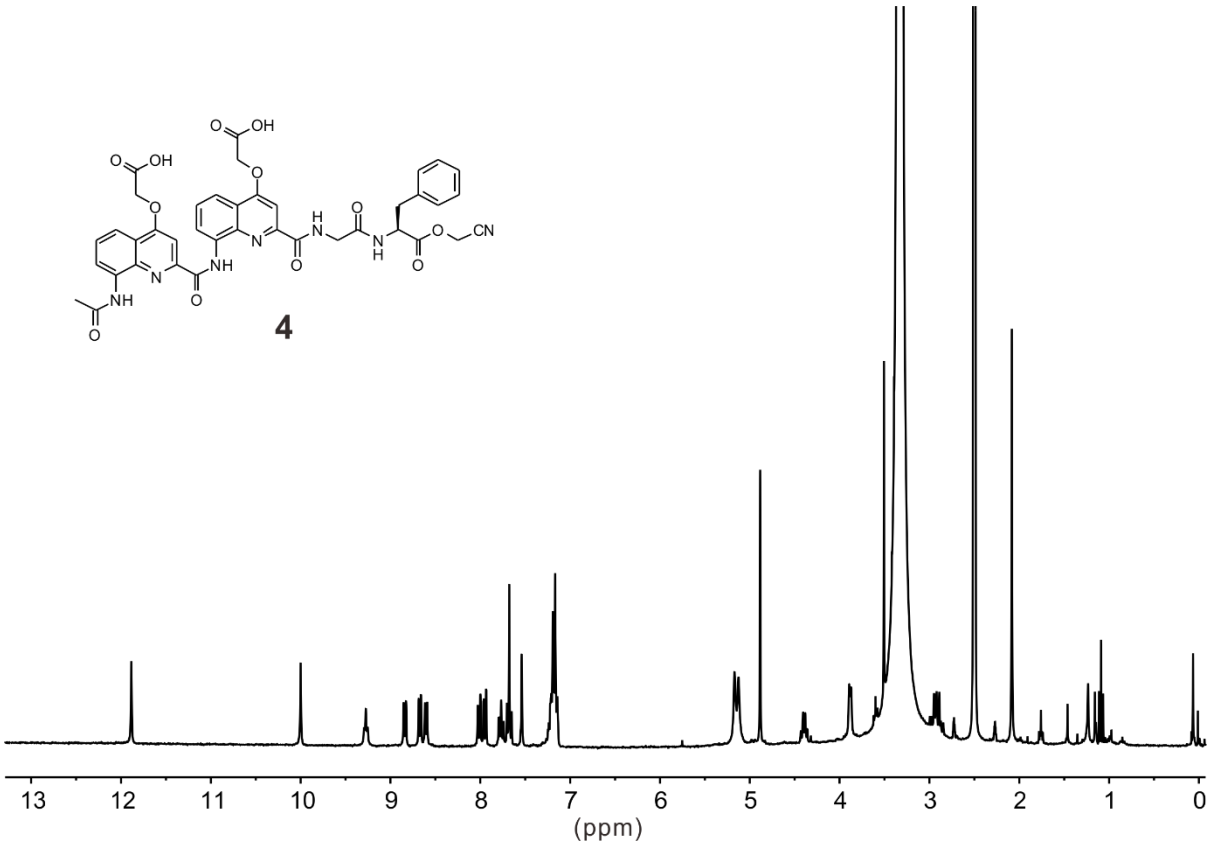

3a
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)


NHBoc
O
N
NH
O
H
N
O
H
N
O
O
O
CN
3b
10
9
8
7
6
5
4
3
2
1
0
(ppm)


NH2
O
N
NH
O
H
N
O
H
N
O
O
O
CN
3
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)


4a
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)


4b
12
11
10
9
8
7
6
5
4
3
2
1
(ppm)


4
13
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11
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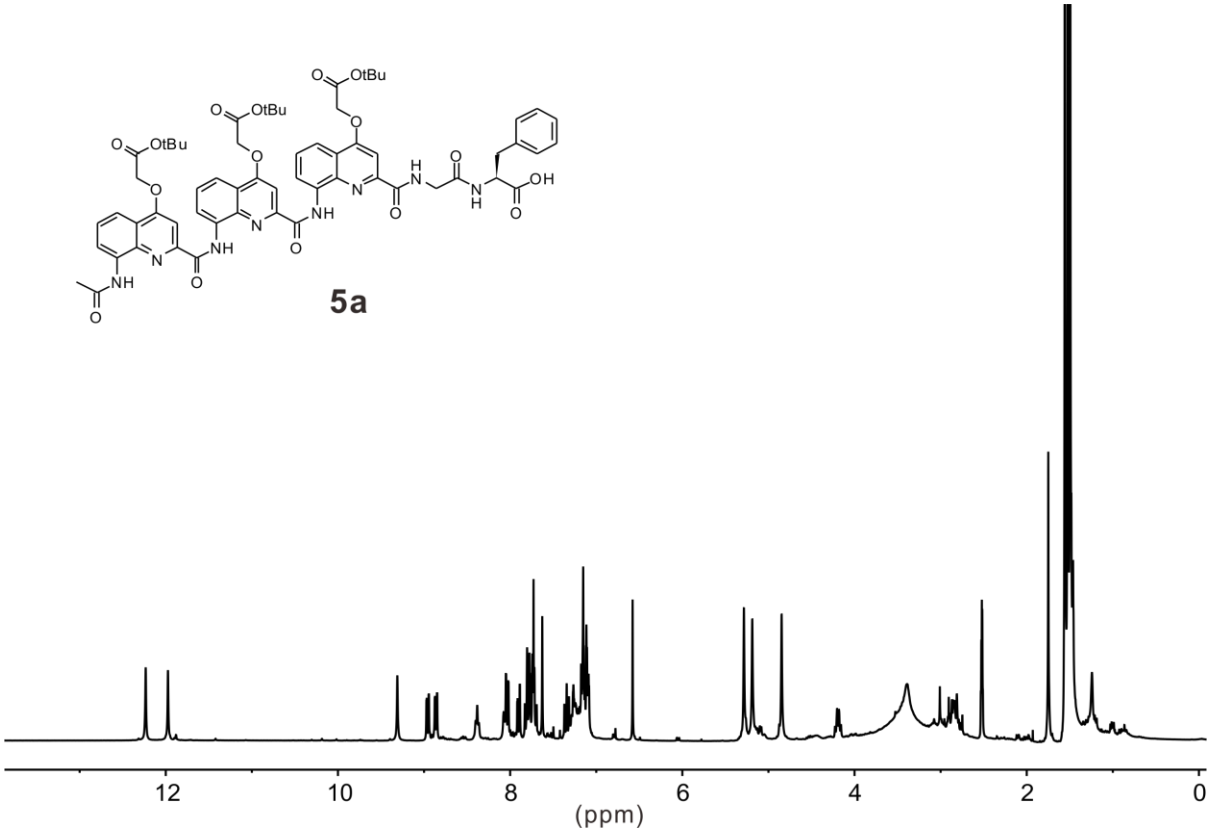
9
8
7
6
5
4
3
2
1
0
(ppm)


5a
12
10
8
6
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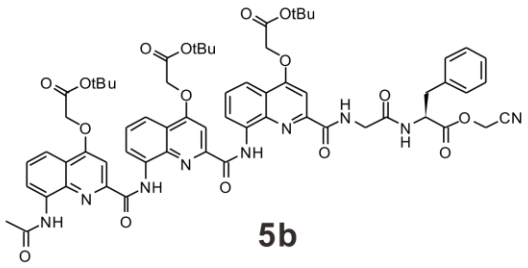
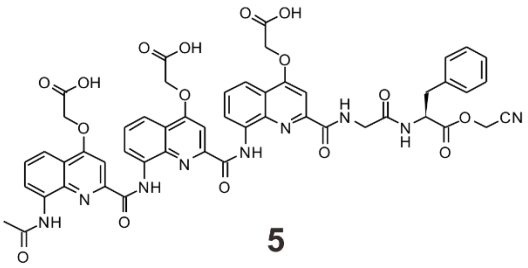
0
(ppm)


OtBu
OtBu
OtBu
NH
NH
NH
N
N
N
O
H
N
O
O
CN

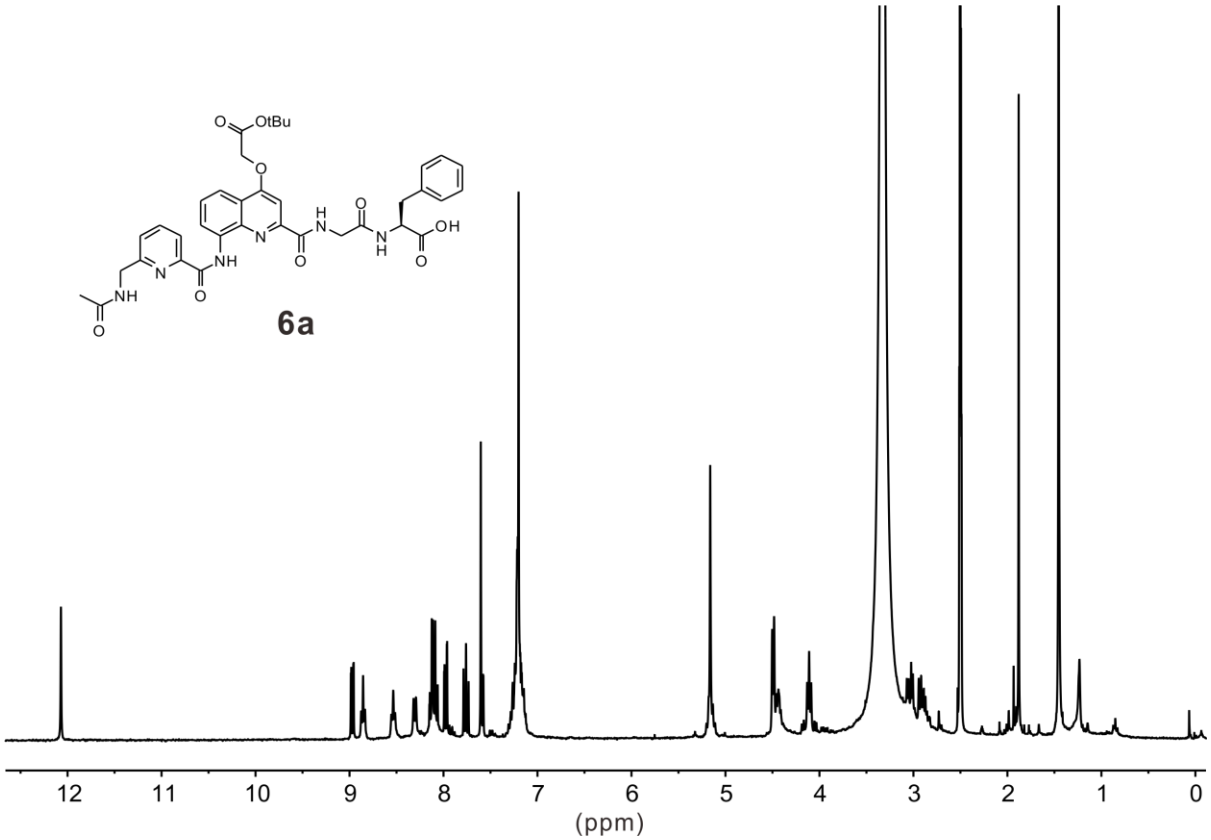
5b

12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)

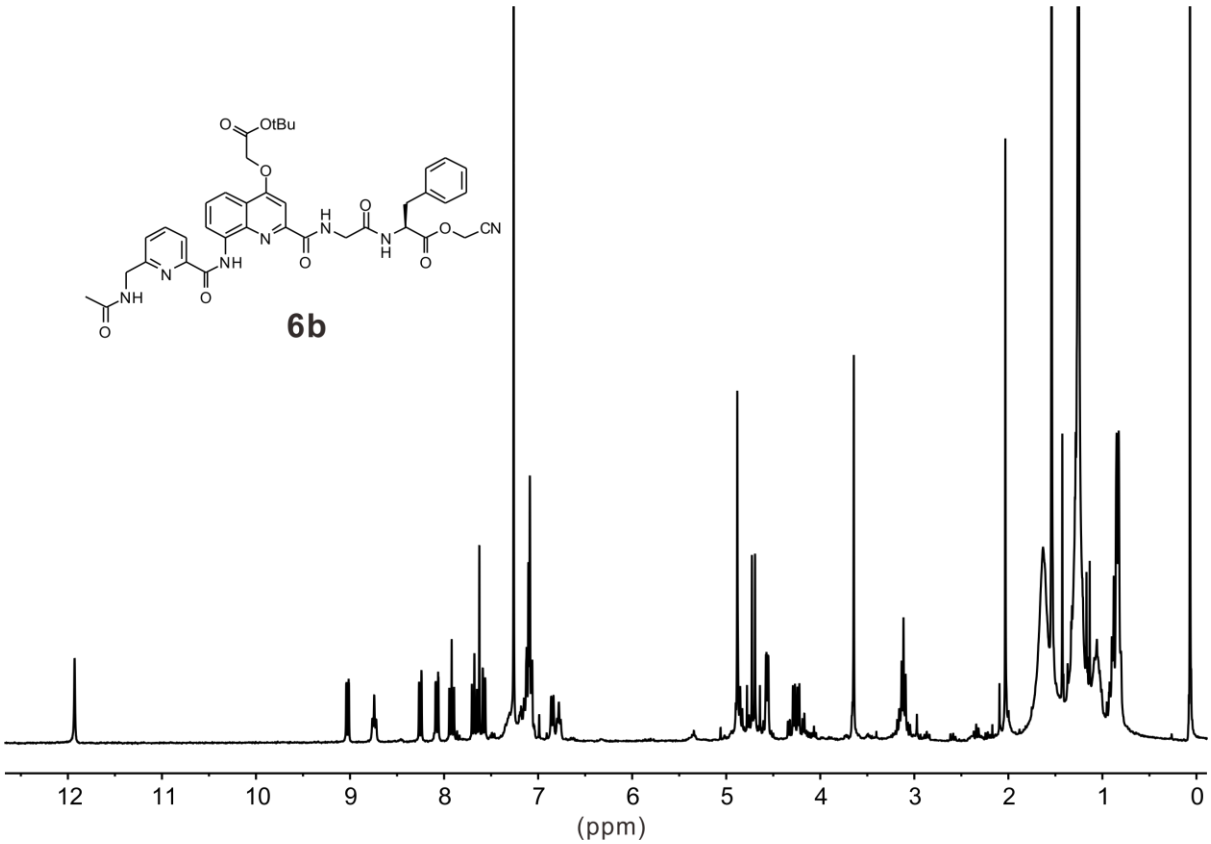

OH
OH
OH
NH
NH
NH
N
N
N
O
H
N
O
O
CN

5

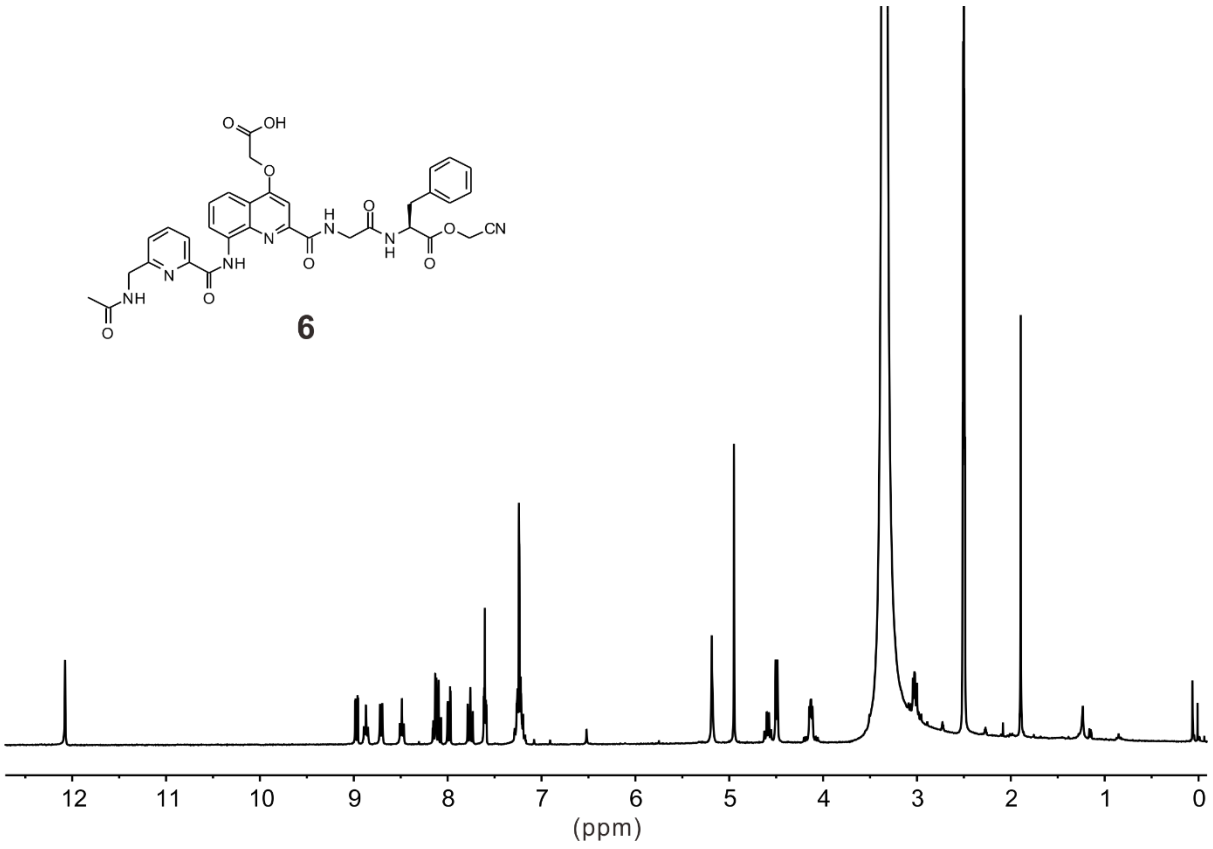
14
12
10
8
6
4
2
0
(ppm)


OtBu
O
O
N
NH
O
N
NH
O
O
N
H
O
N
H
OH
O
O
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)

6a


OtBu
O
O
N
NH
O
N
NH
O
O
N
H
O
N
H
O
O
CN
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)

6b


6
12
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9
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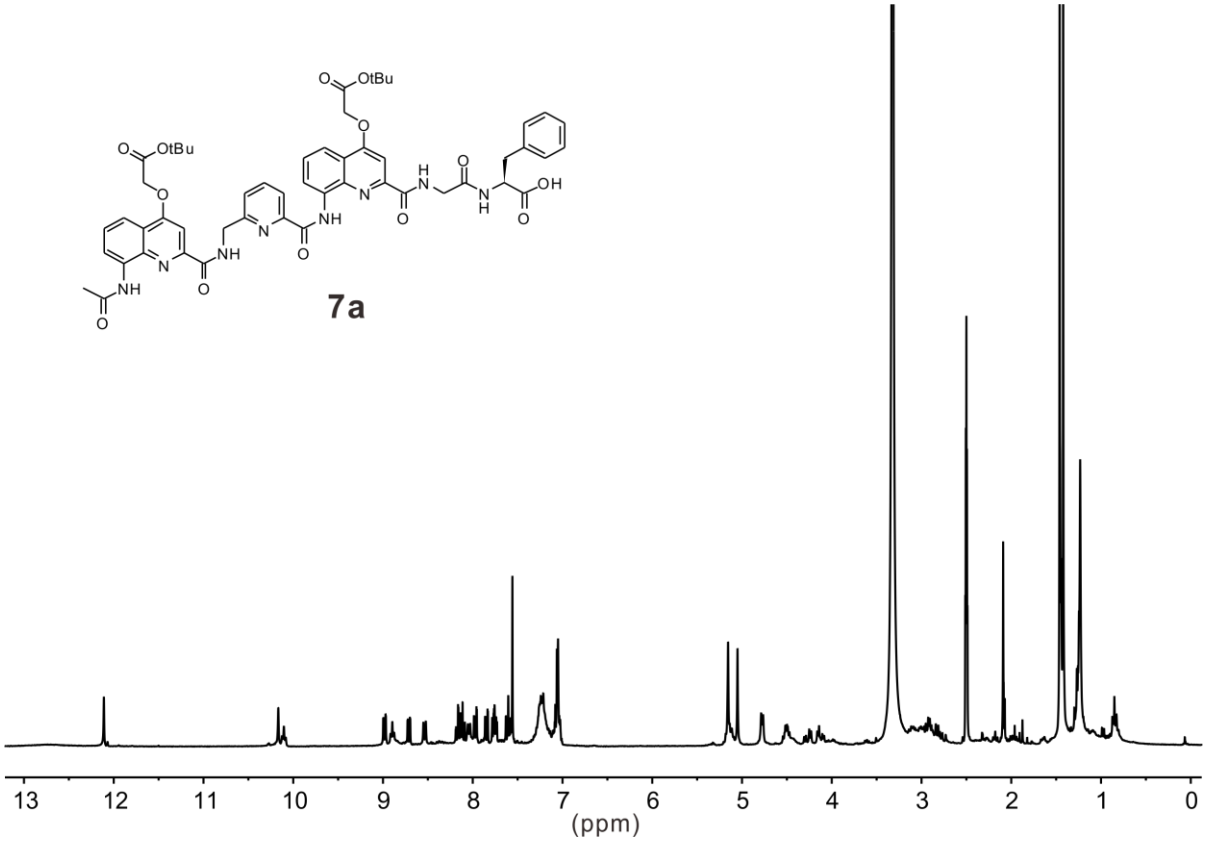
7
6
5
4
3
2
1
0
(ppm)


7a
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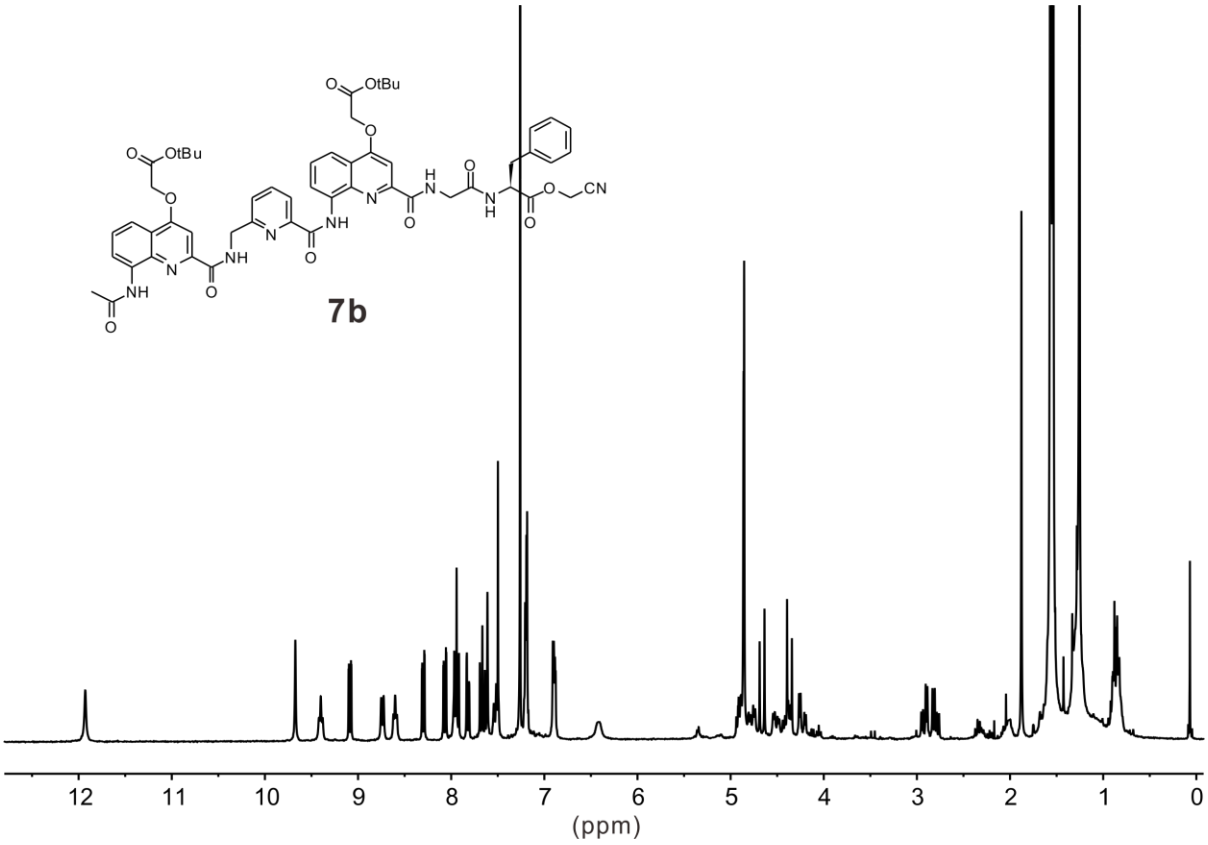
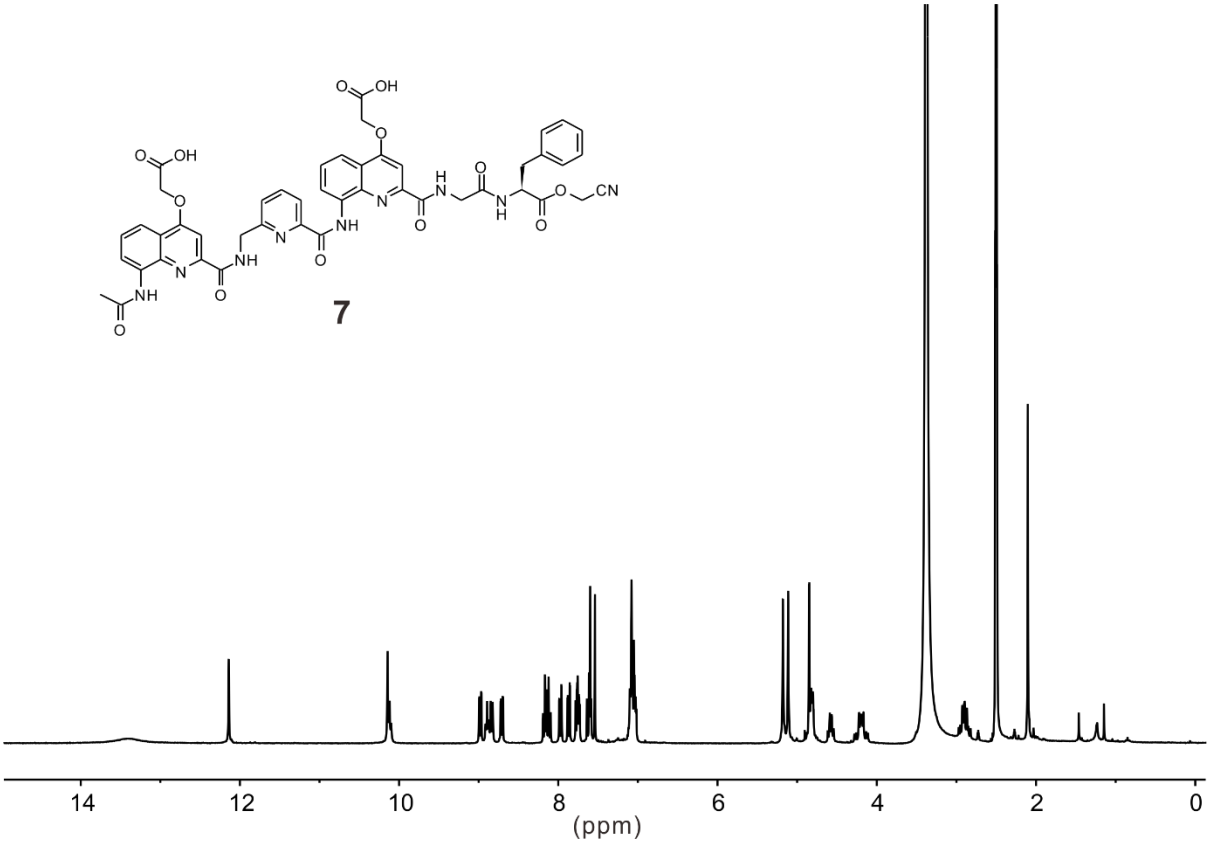
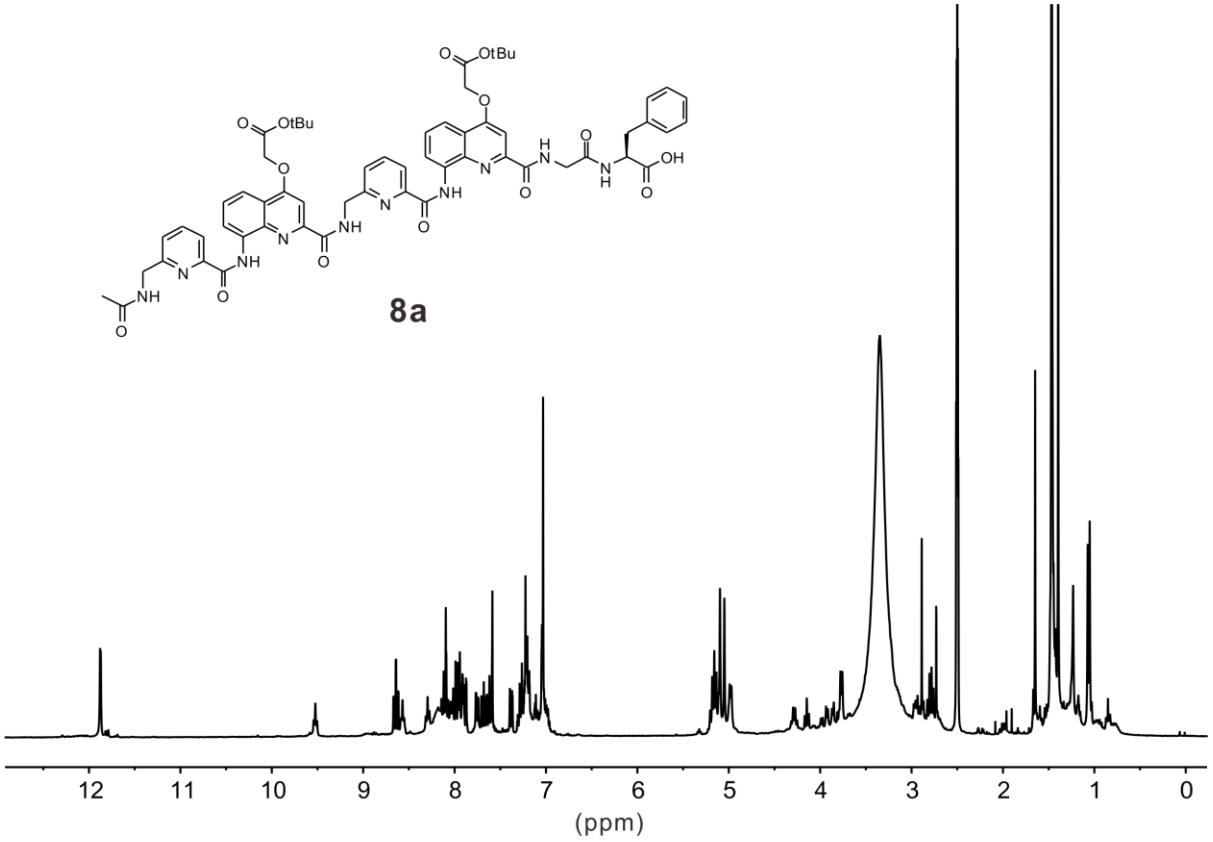
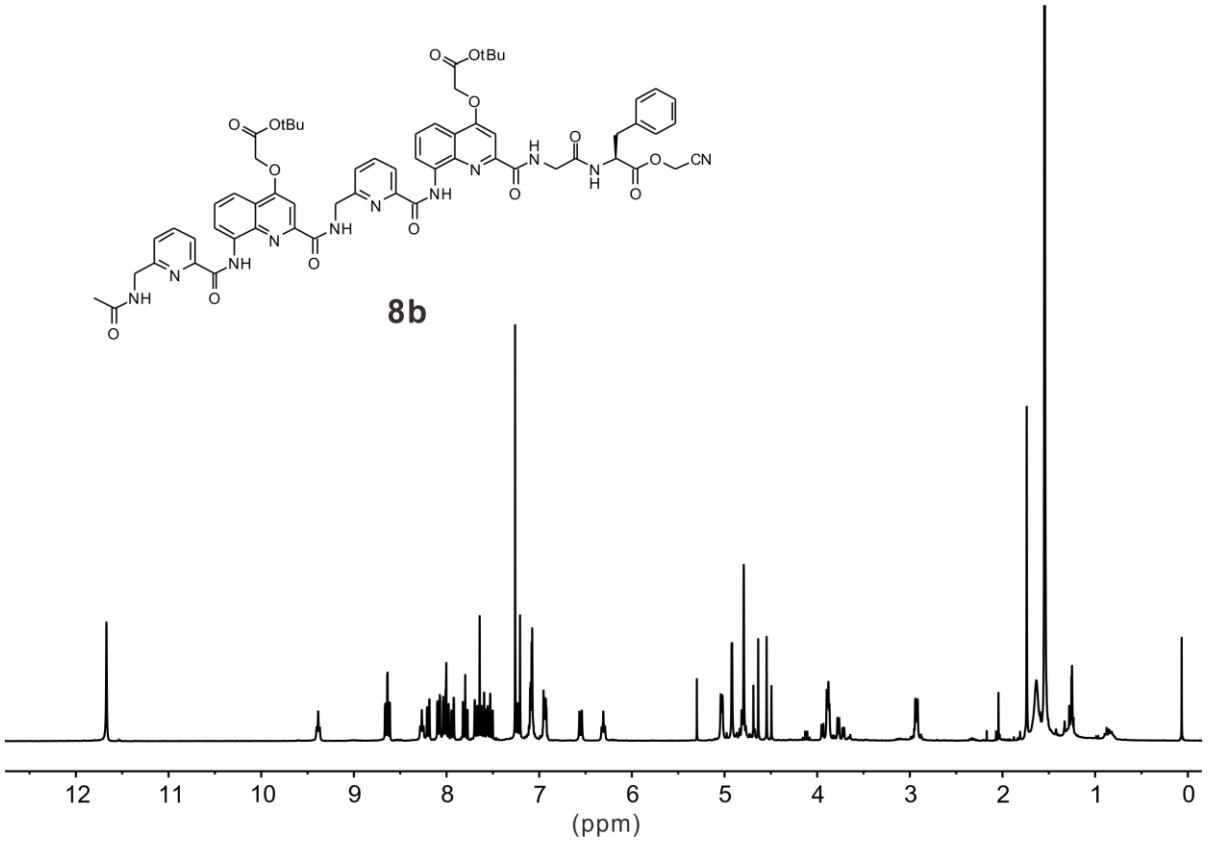
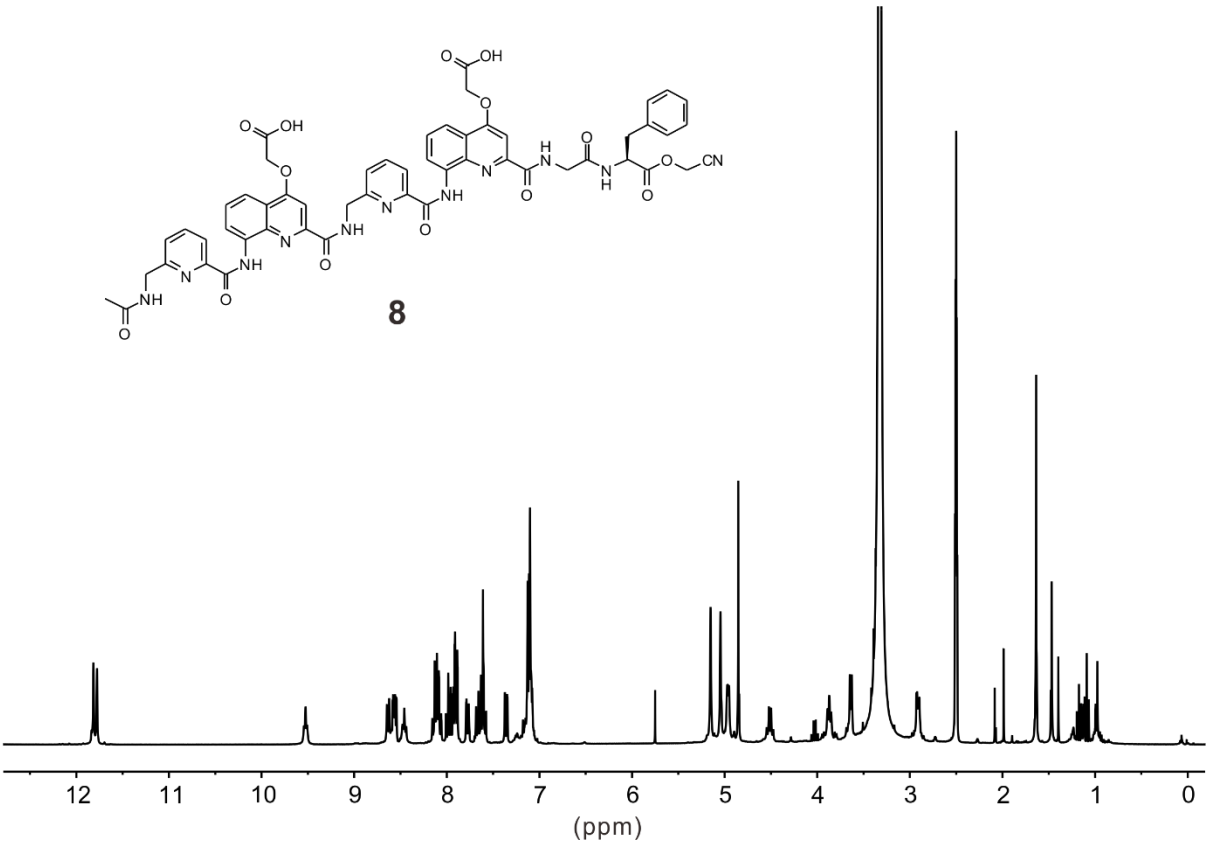
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)


7b
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)


7
14
12
10
8
6
4
2
0
(ppm)


8a
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)


8b
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)


8
12
11
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9
8
7
6
5
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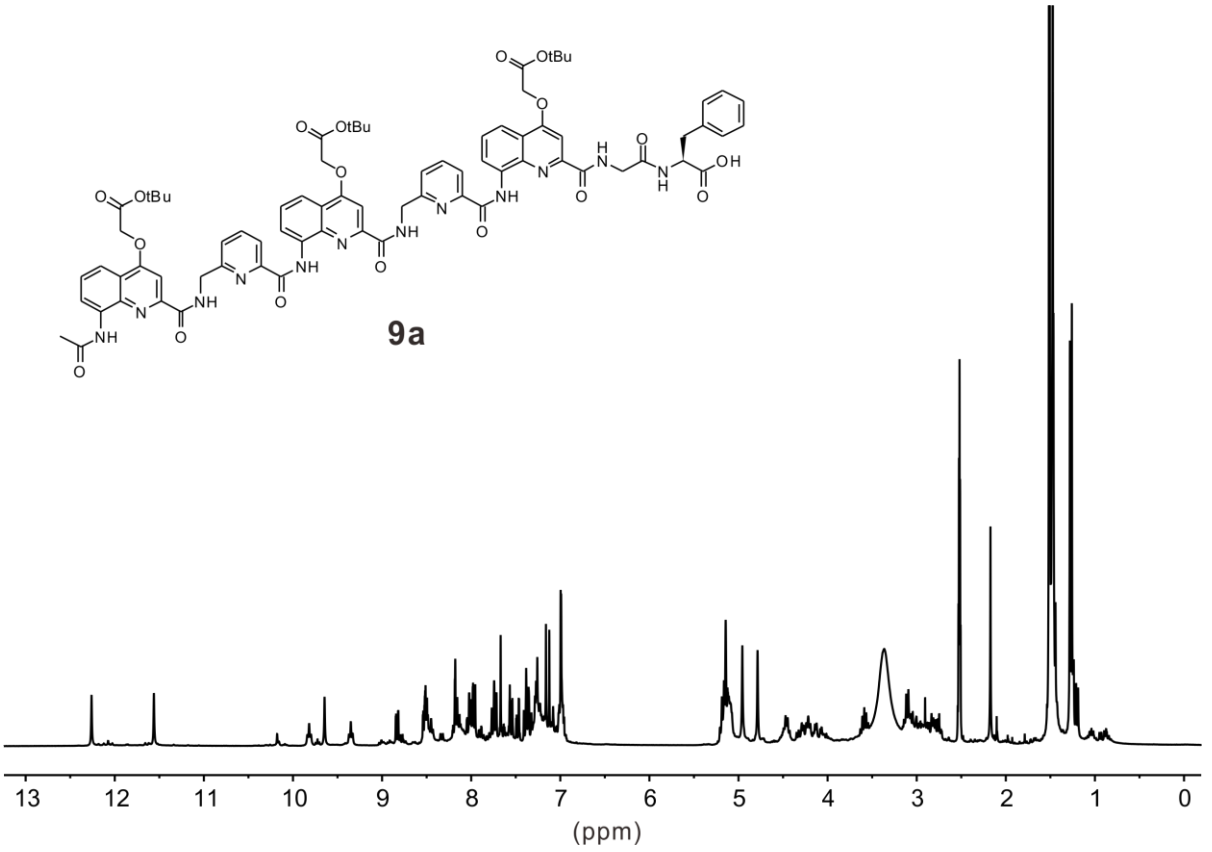
3
2
1
0
(ppm)


9a
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10
9
8
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6
5
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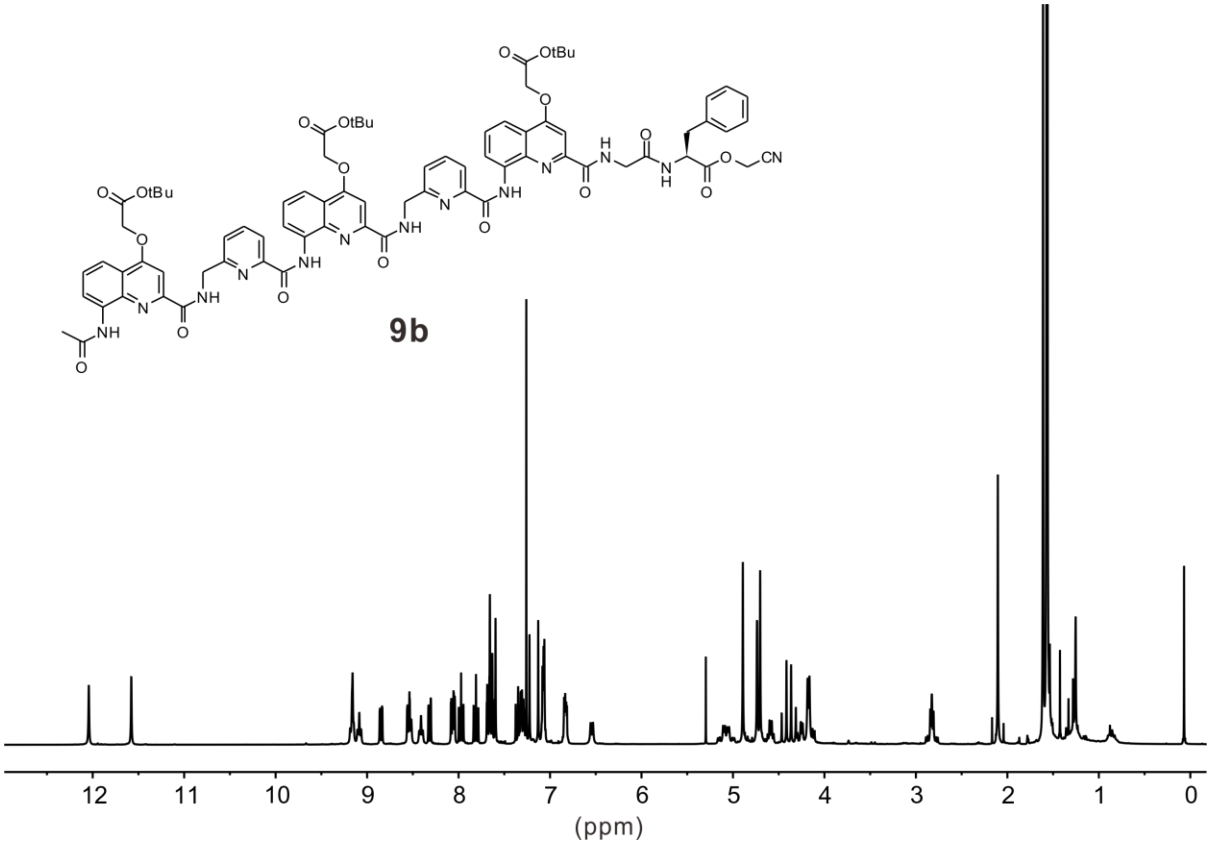
2
1
0
(ppm)


9b
12
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10
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6
5
4
3
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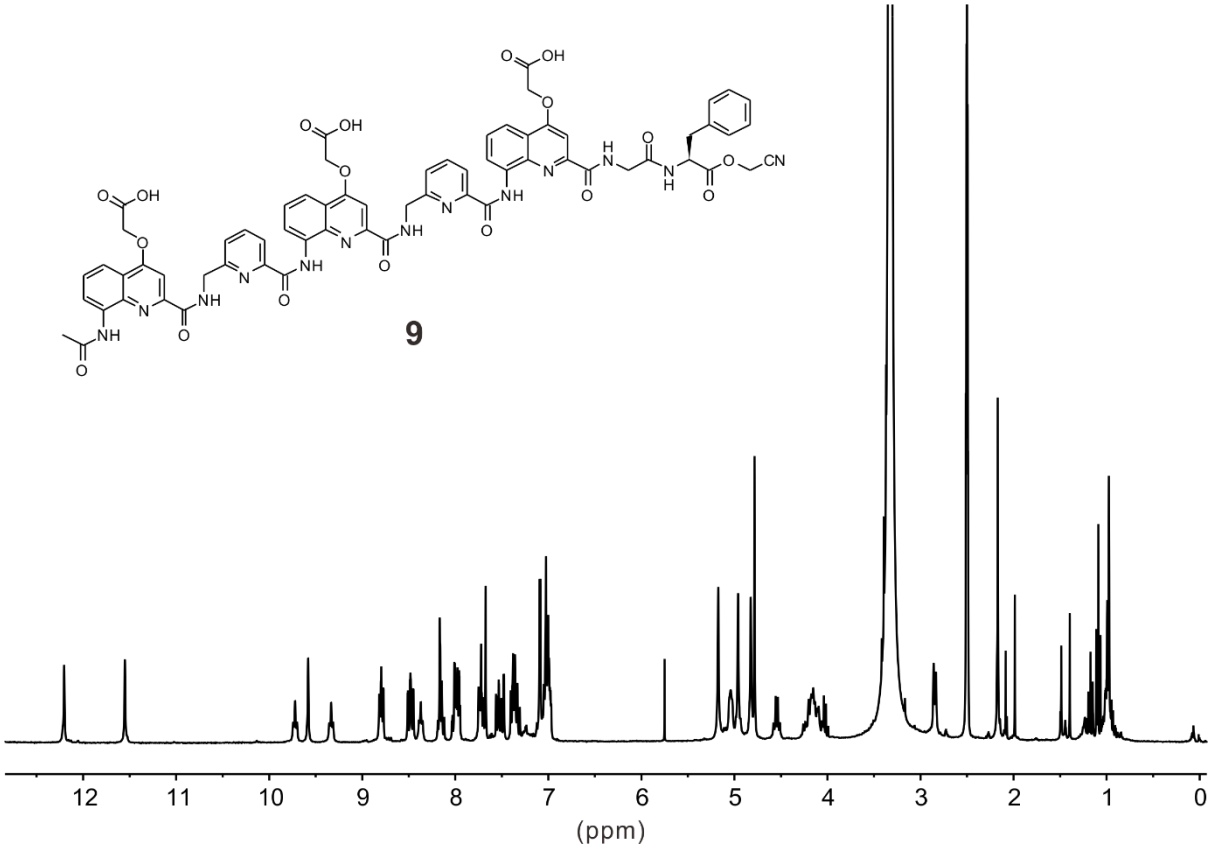
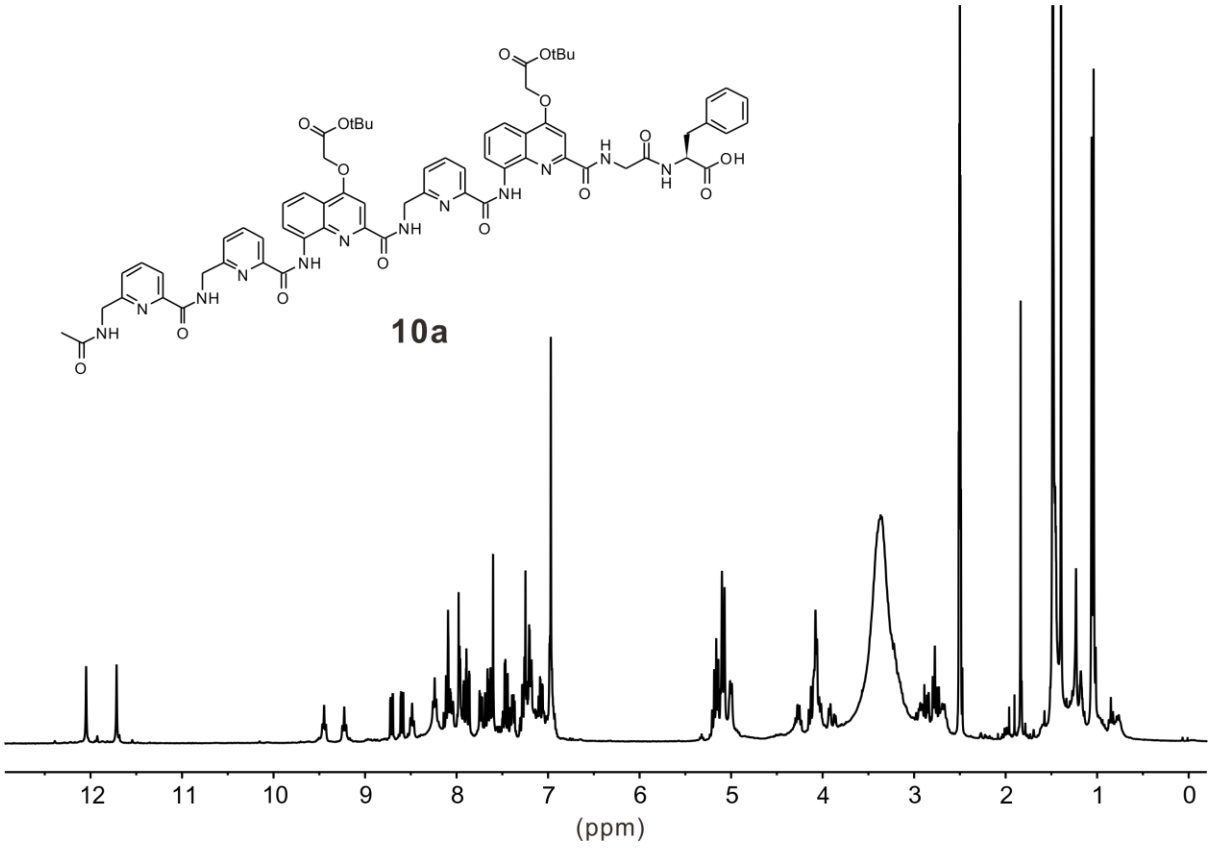
1
0
(ppm)


9
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)


10a
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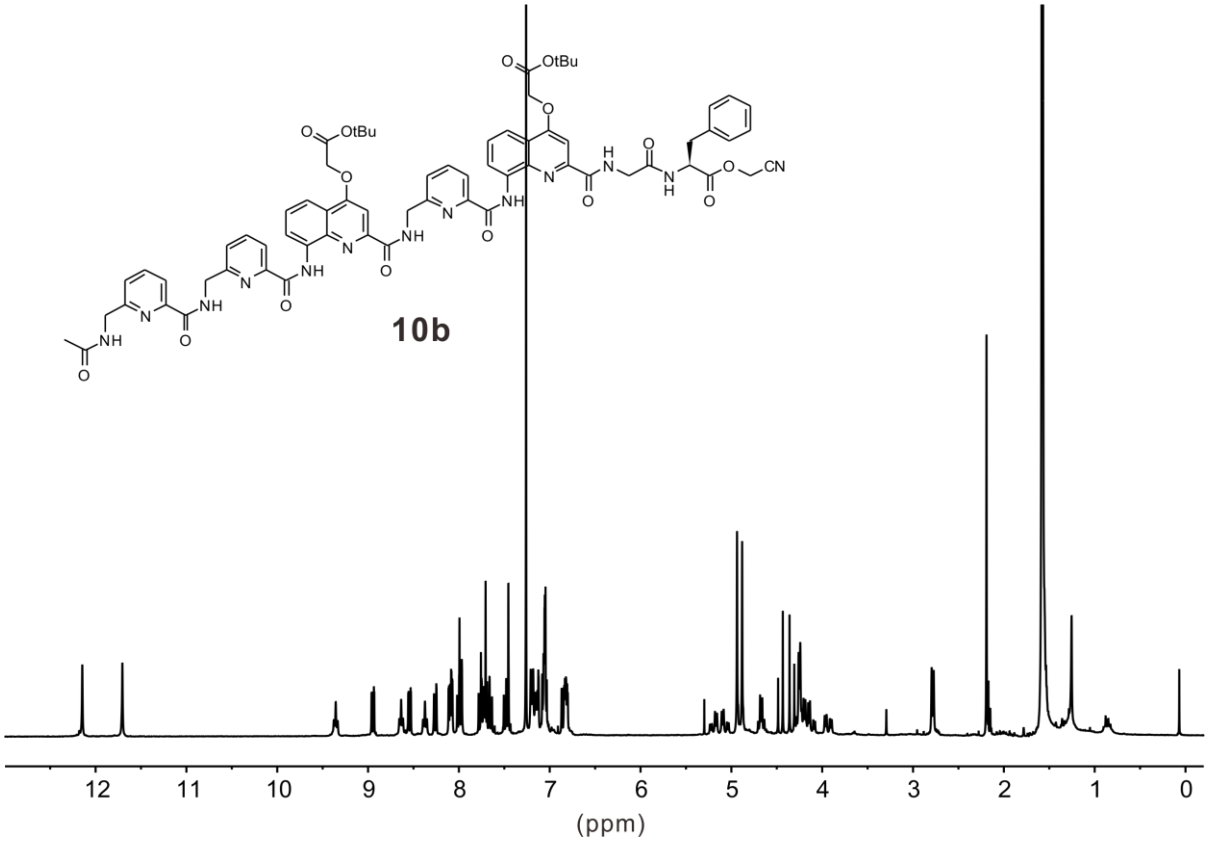
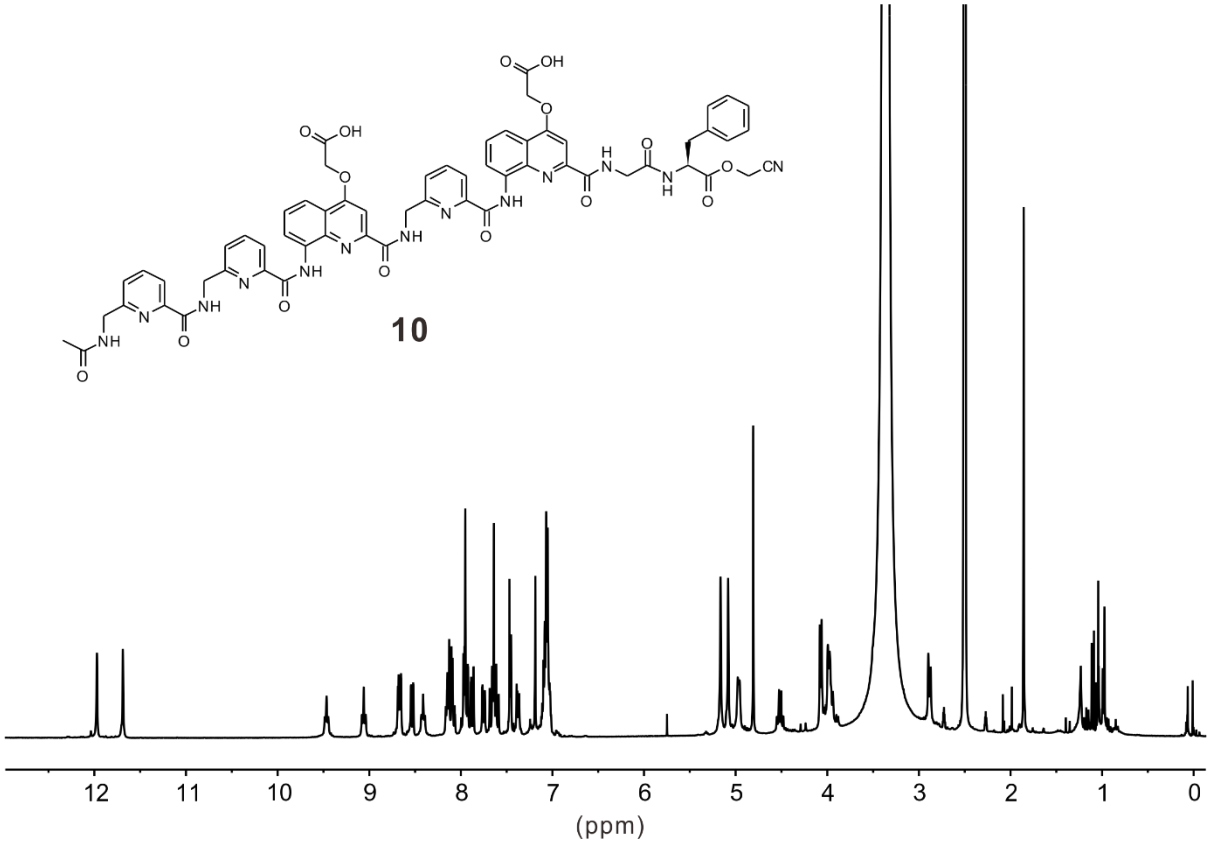
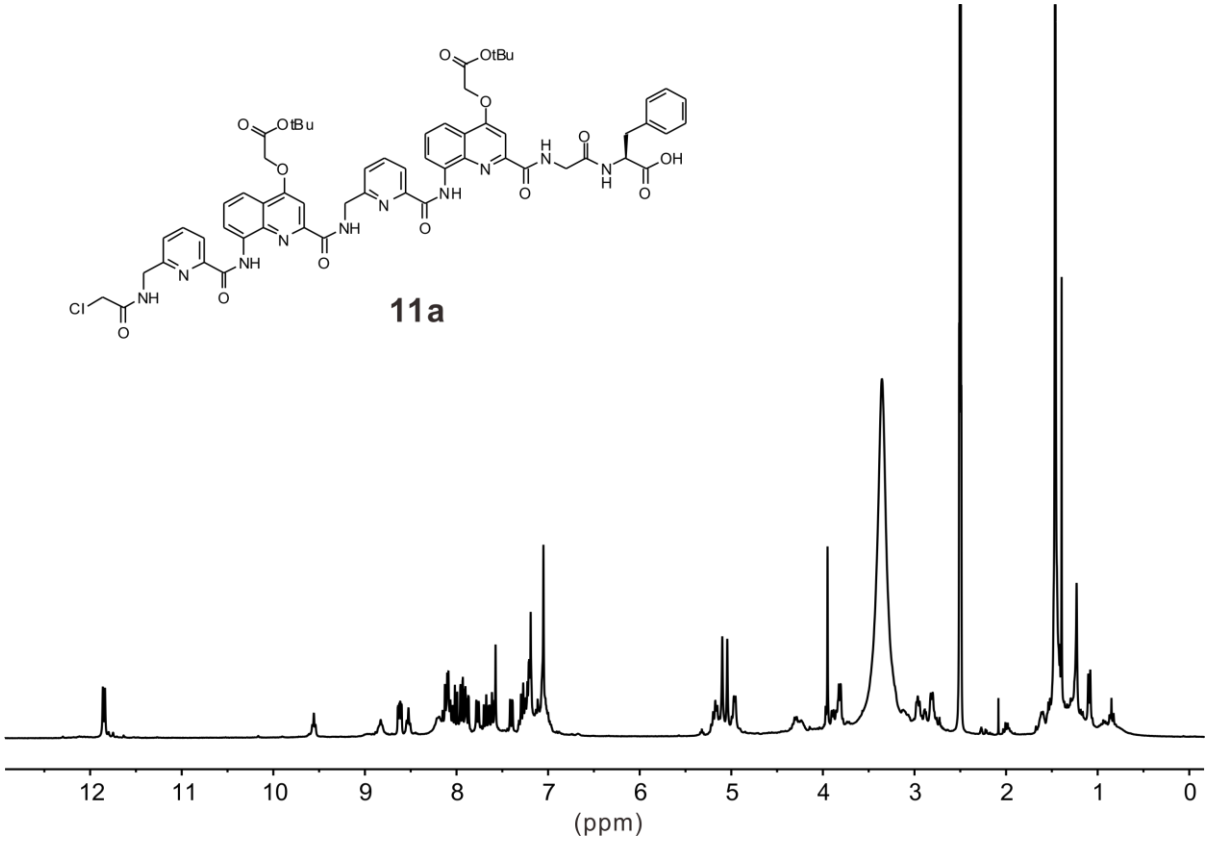
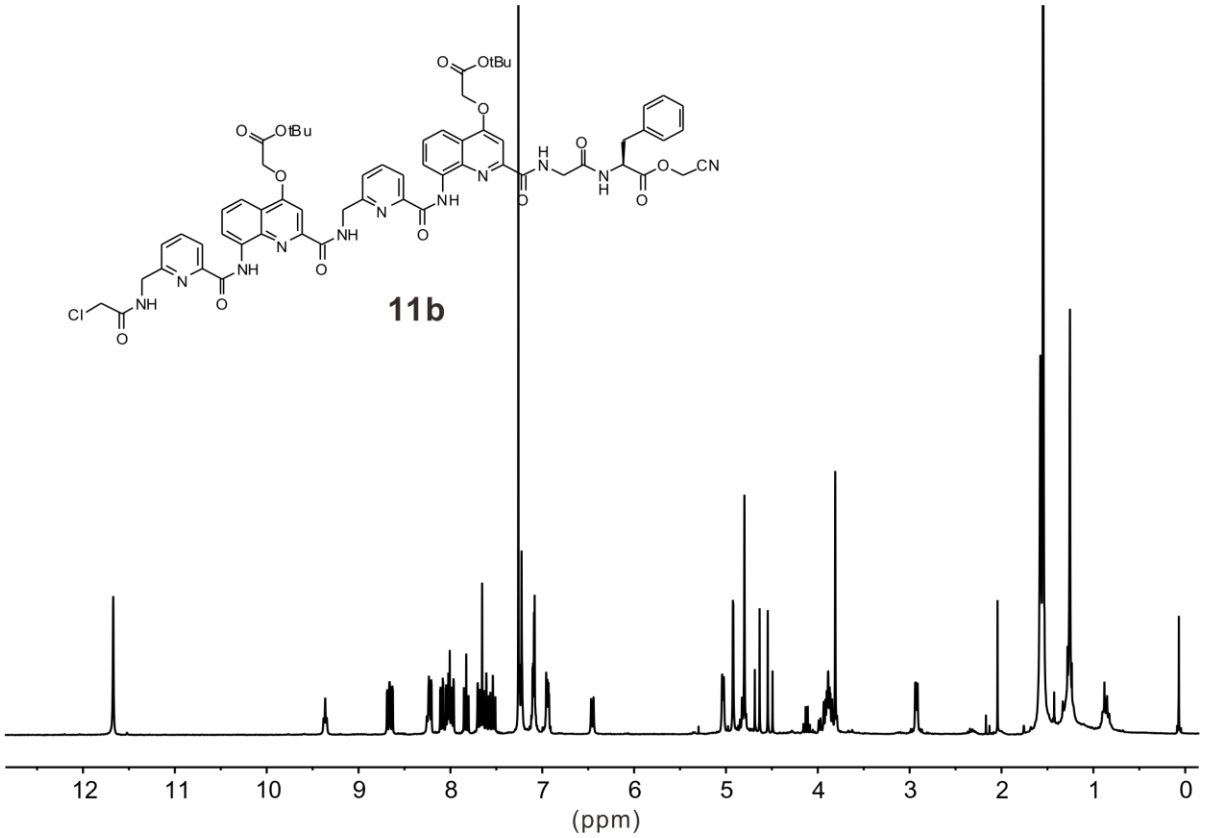
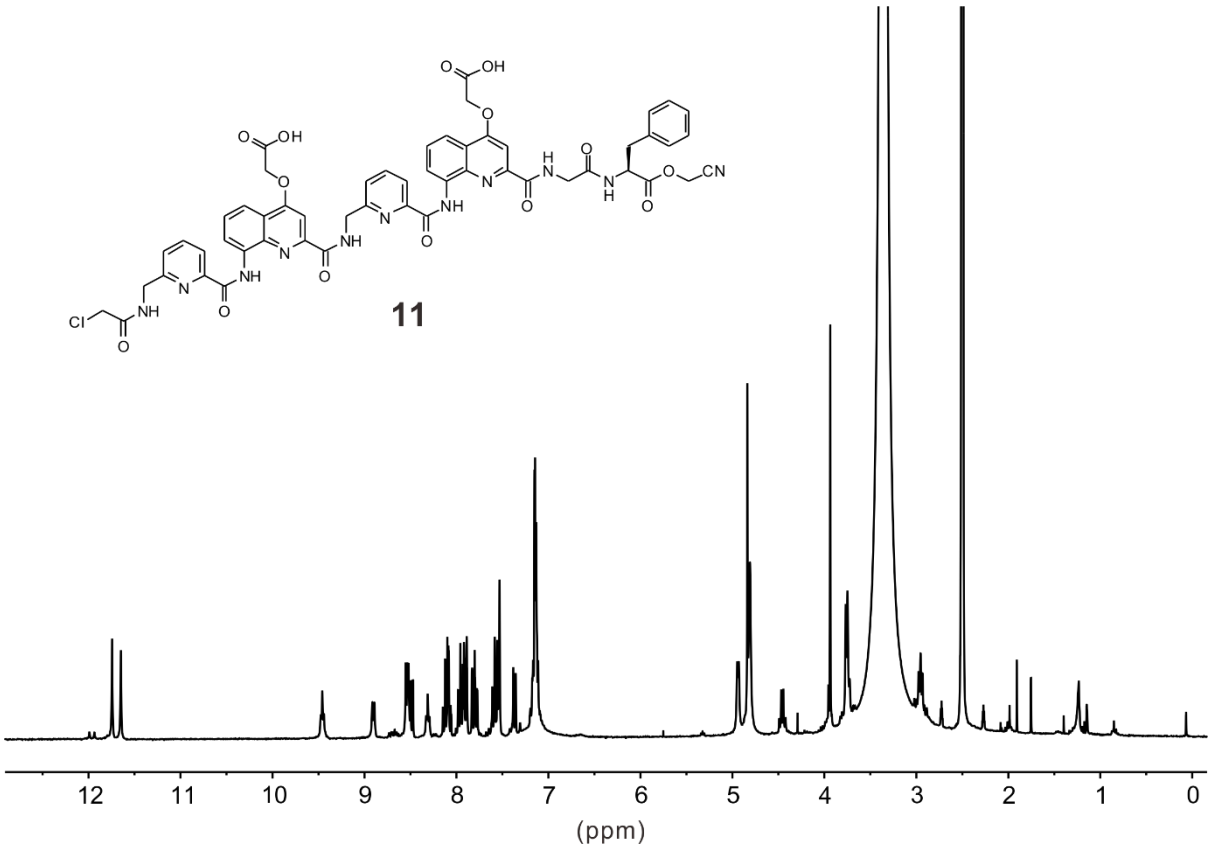
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)

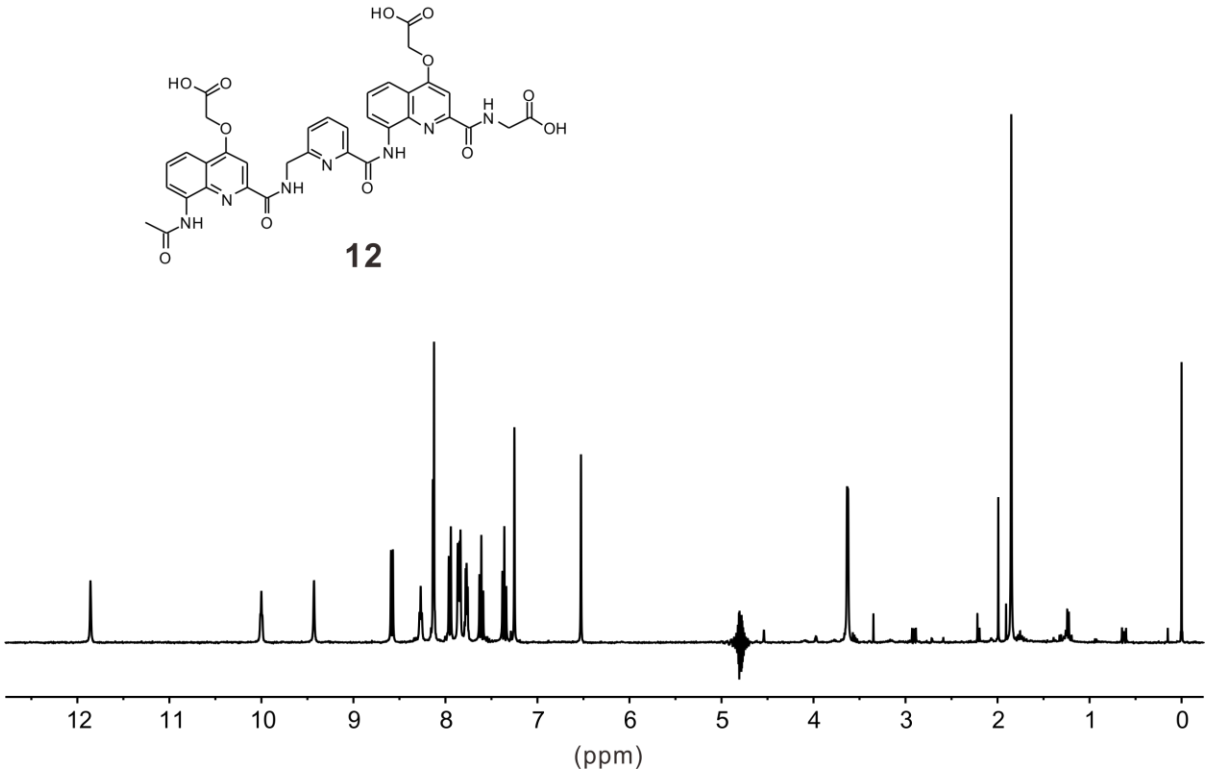

10b
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)

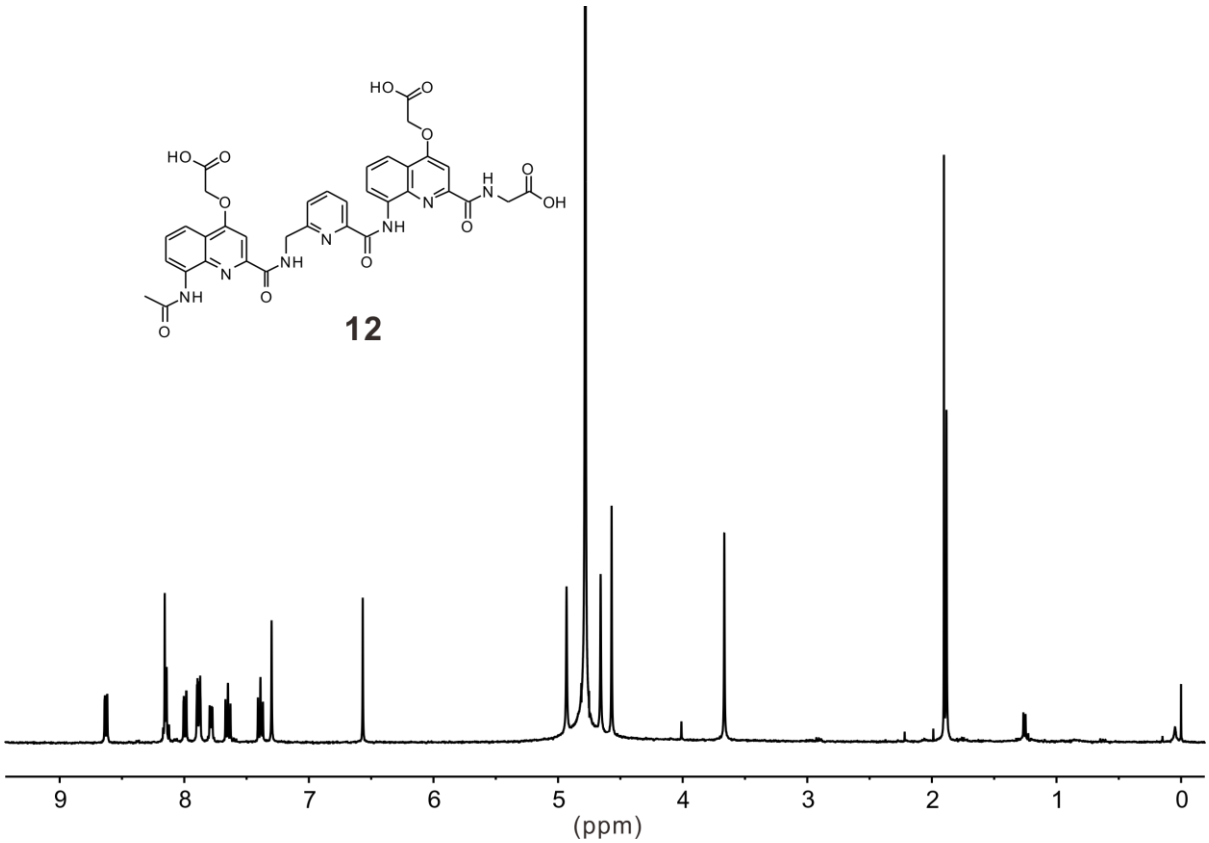

10
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)

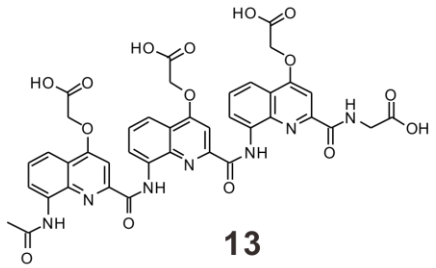

11a
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)


11b
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)

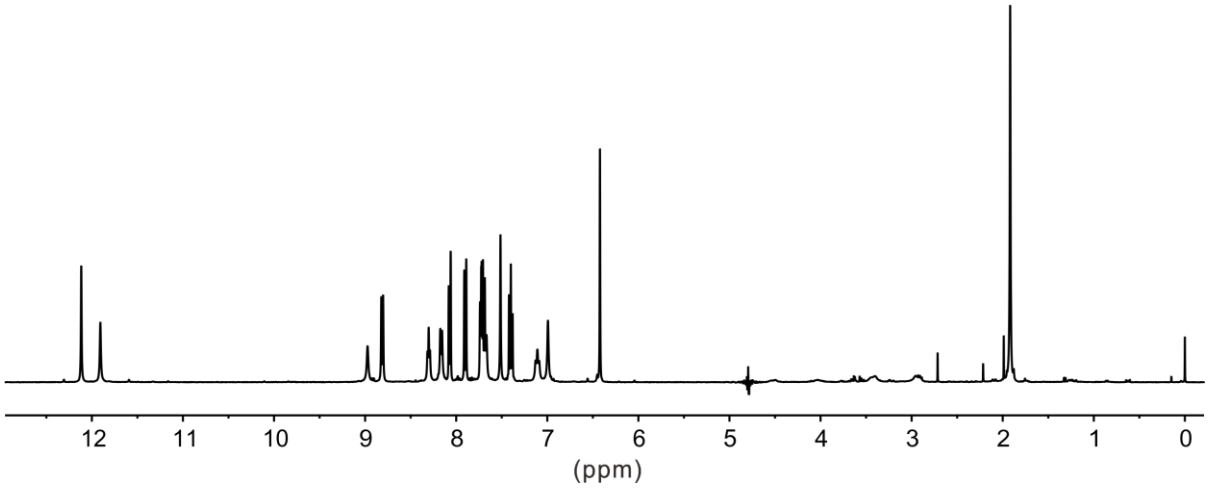

11
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)

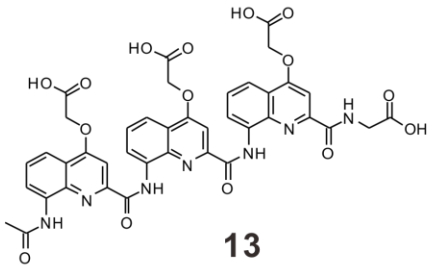

12
12 11 10 9 8 7 6 5 4 3 2 1 0
(ppm)


12
9 8 7 6 5 4 3 2 1 0
(ppm)

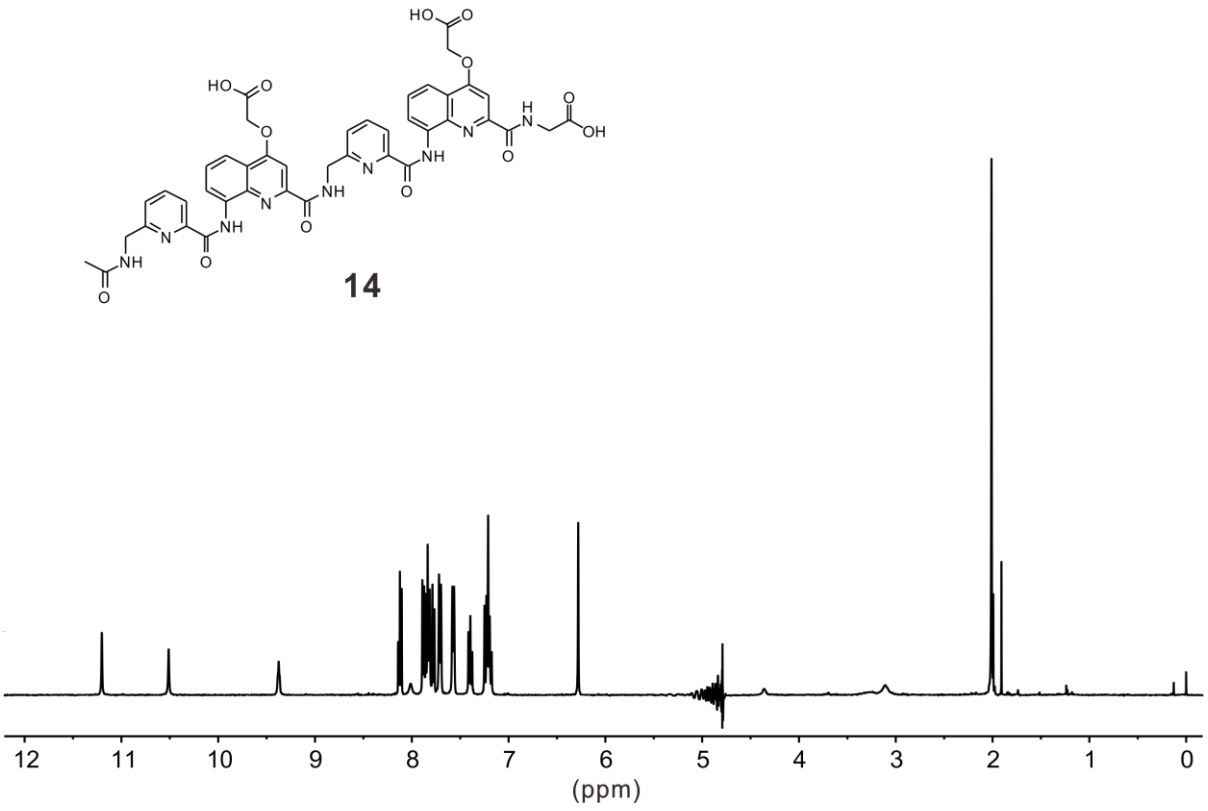


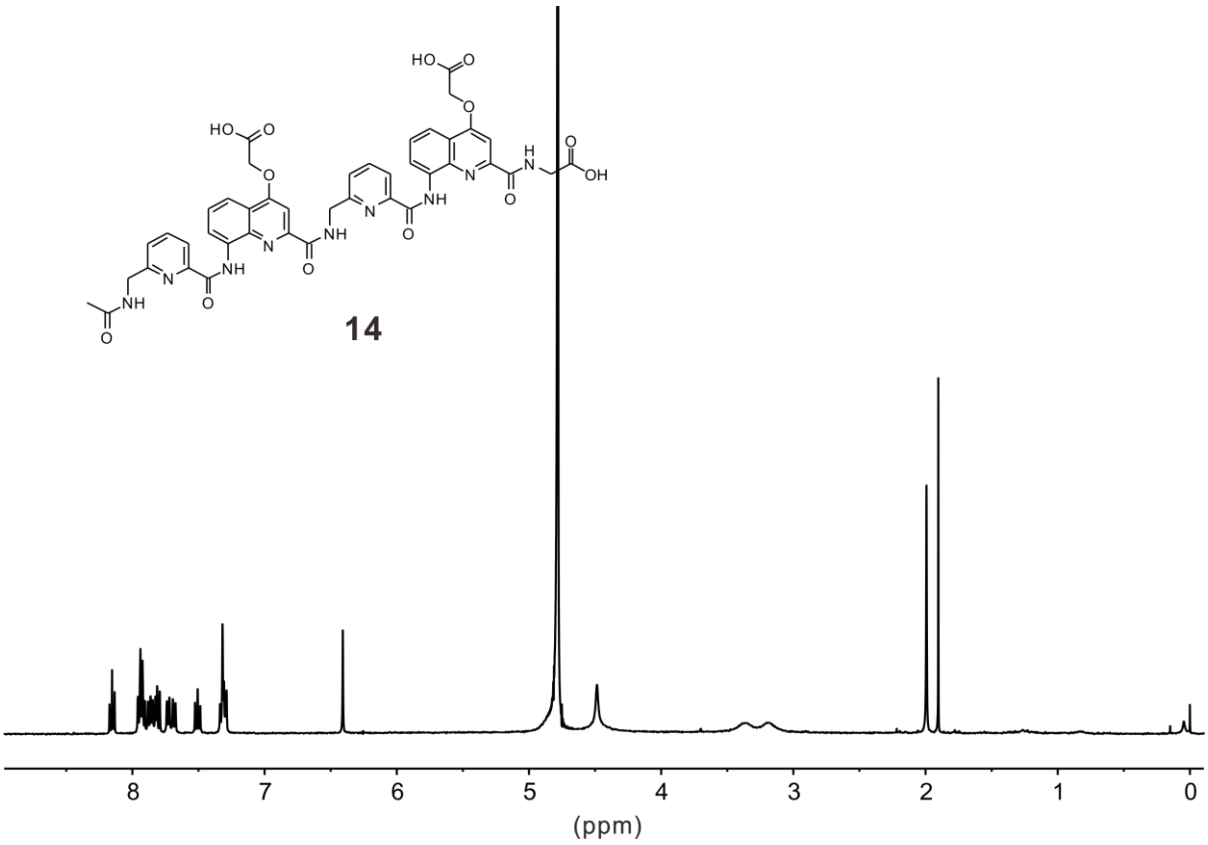
13

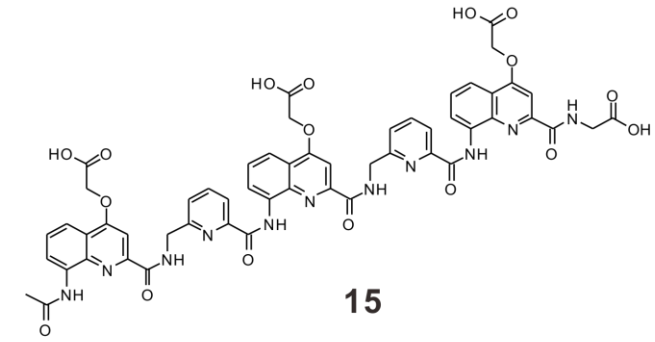




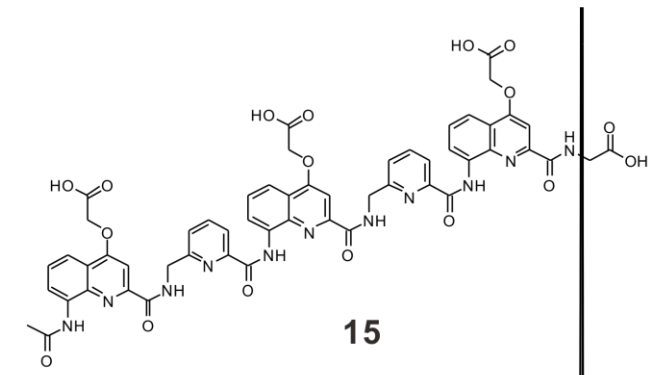
13

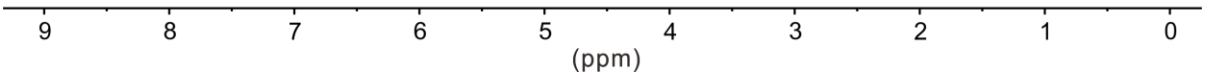

14
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)

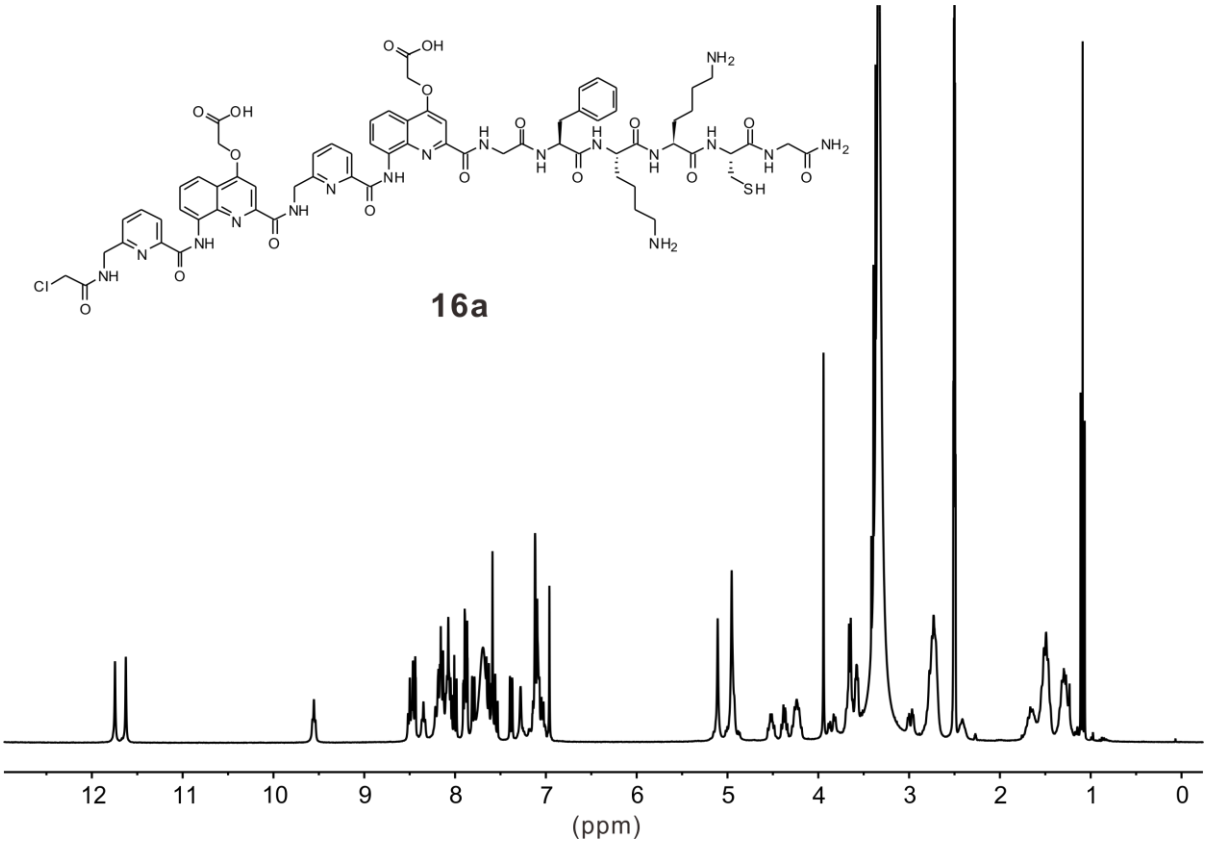

14
8
7
6
5
4
3
2
1
0
(ppm)


15

12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)


15


9
8
7
6
5
4
3
2
1
0
(ppm)


16a
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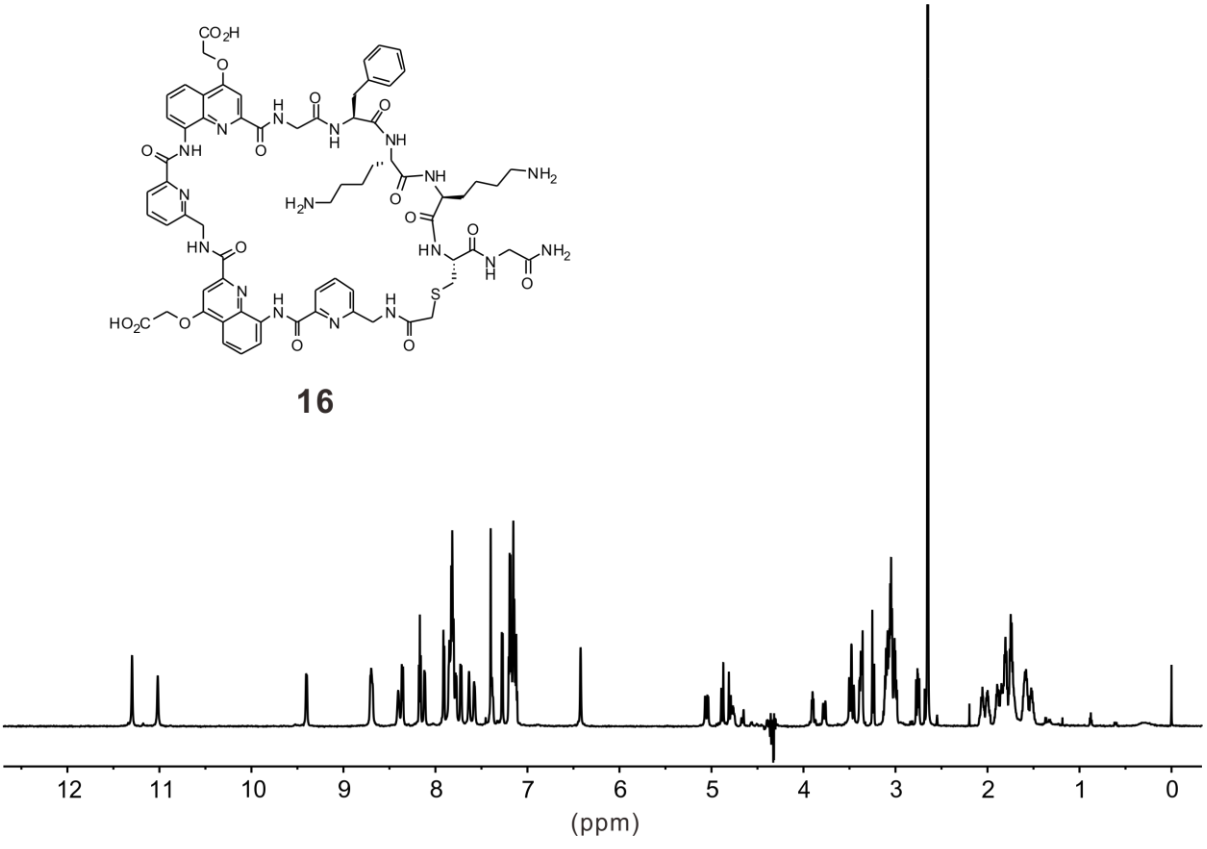
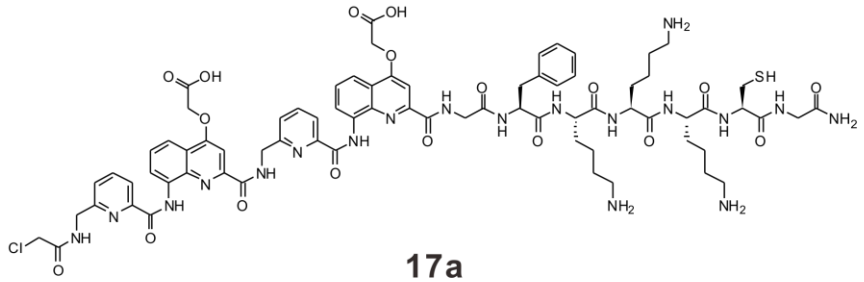
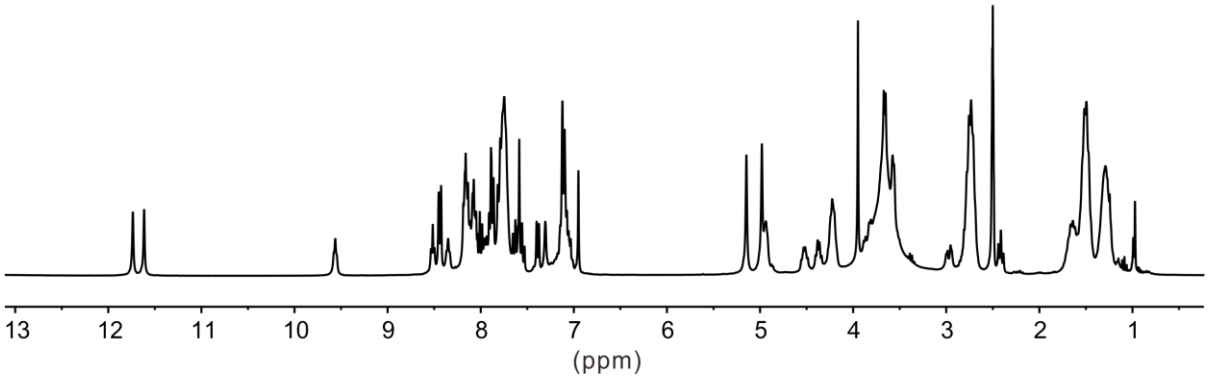
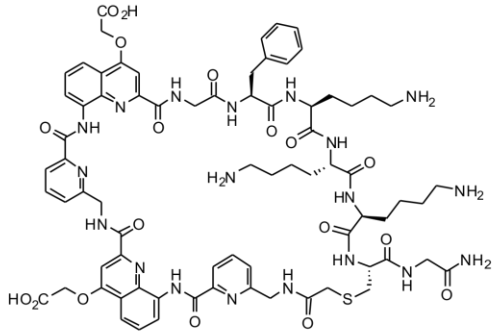
9
8
7
6
5
4
3
2
1
0
(ppm)


16
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)

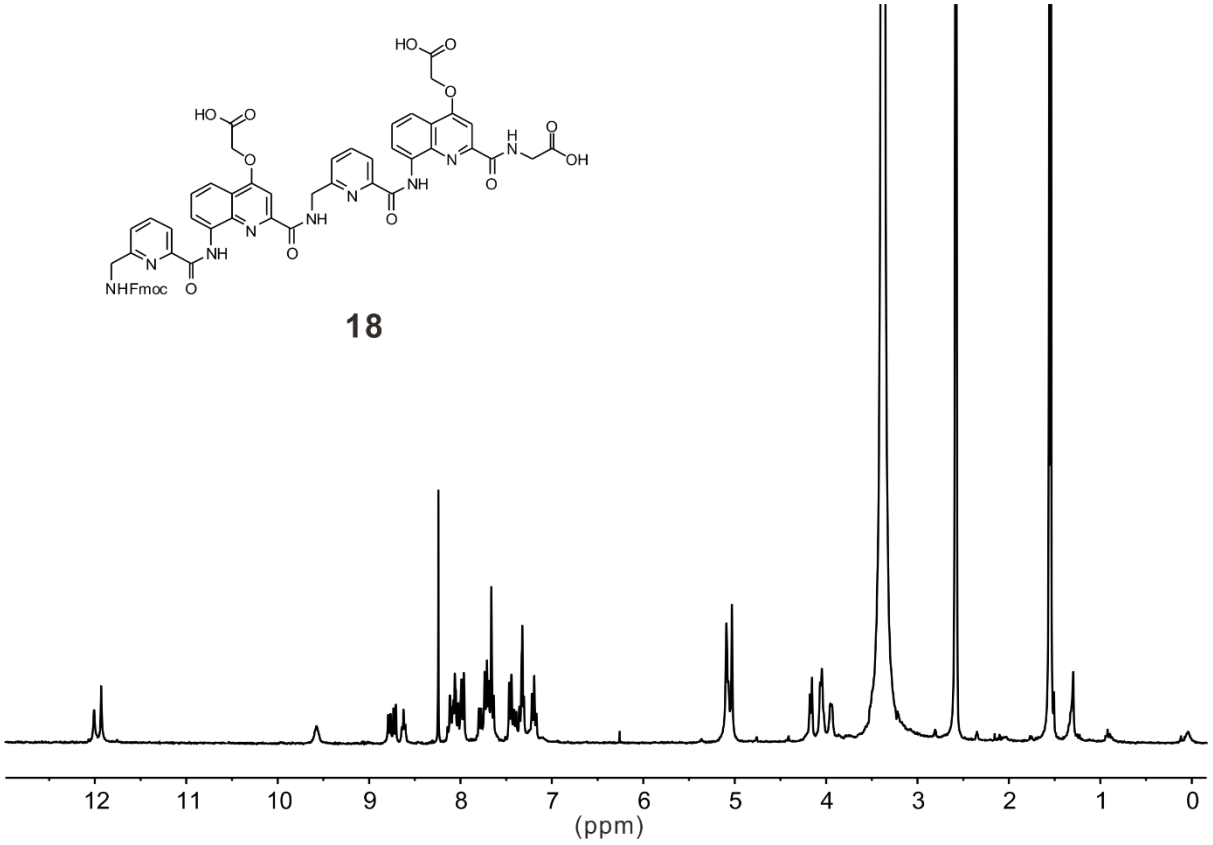


17a

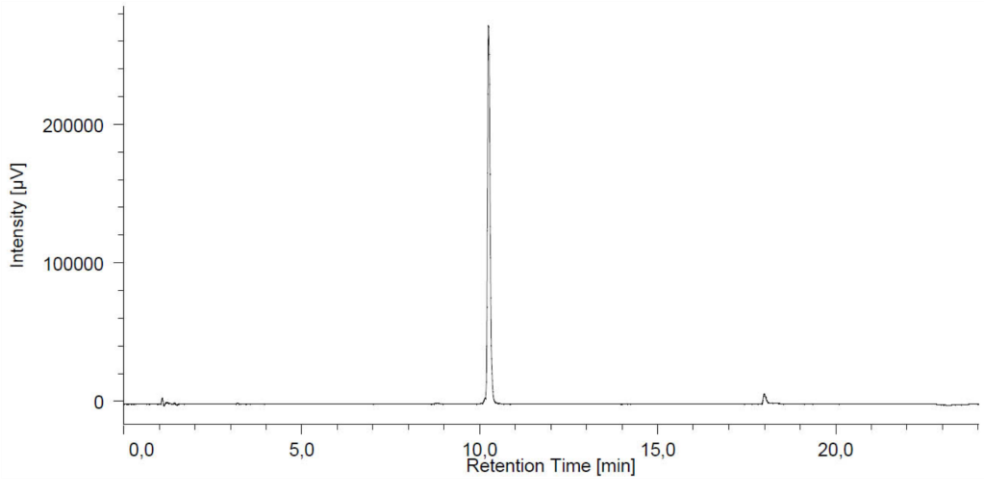




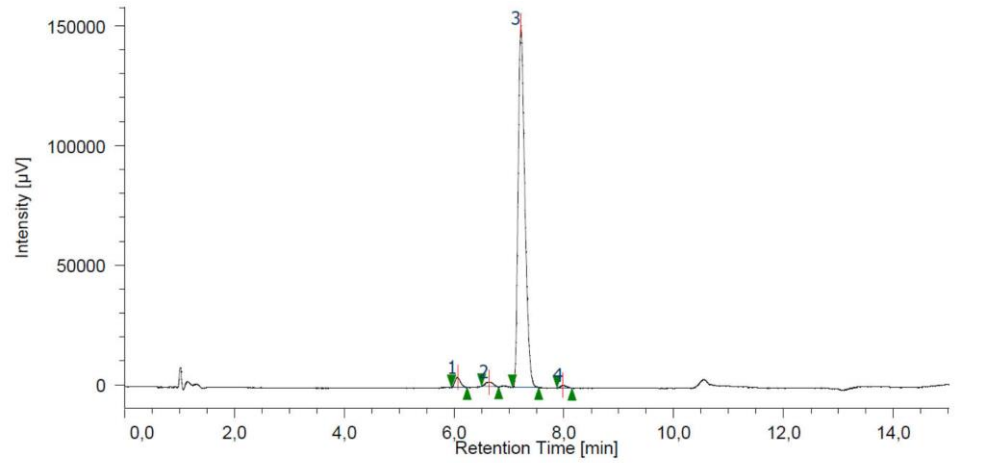
17


HO
O
O
O
N
H
N
O
OH
NH
O
N
N
NH
O
HO
O
O
N
NH
O
NHFmoc
O
12
11
10
9
8
7
6
5
4
3
2
1
0
(ppm)

18

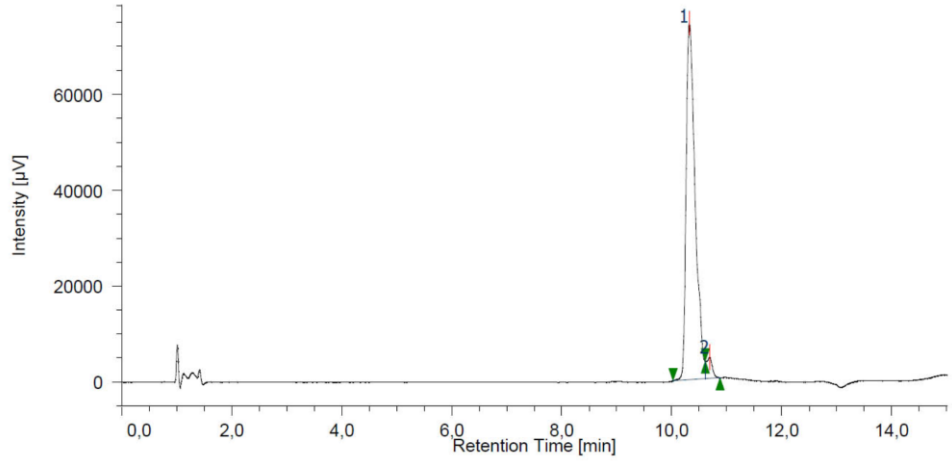


RP-HPLC chromatogram of **12**
gradient from 0% to 20% solvent D over 15 min (System B)



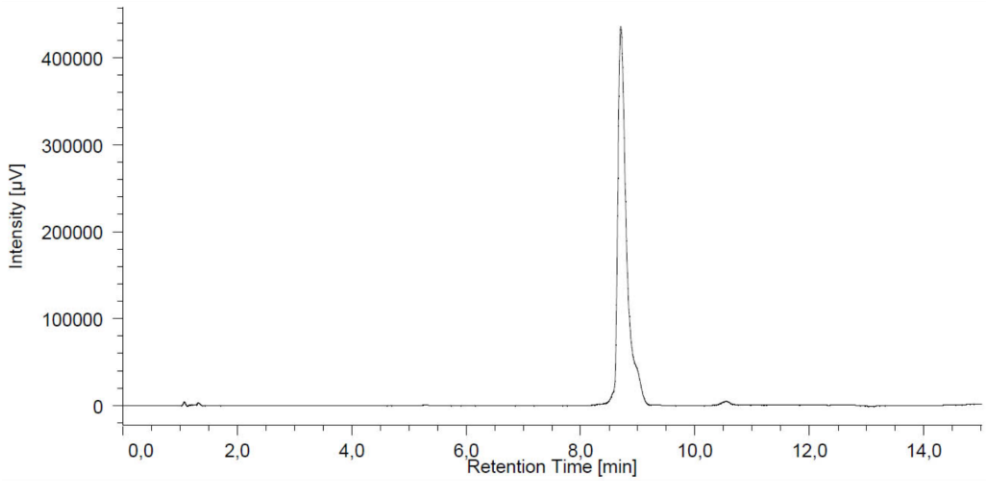
#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	6,058	29658	4177	2,209	2,644	N/A	15981	2,419	1,200	
2	Unknown	3	6,633	18434	1820	1,373	1,152	N/A	8674	2,298	1,104	
3	Unknown	3	7,217	1286302	150945	95,811	95,542	N/A	16583	3,514	1,352	
4	Unknown	3	7,983	8152	1046	0,607	0,662	N/A	22410	N/A	1,259	

RP-HPLC chromatogram of **13**
gradient from 5% to 15% solvent D over 8 min (System B)

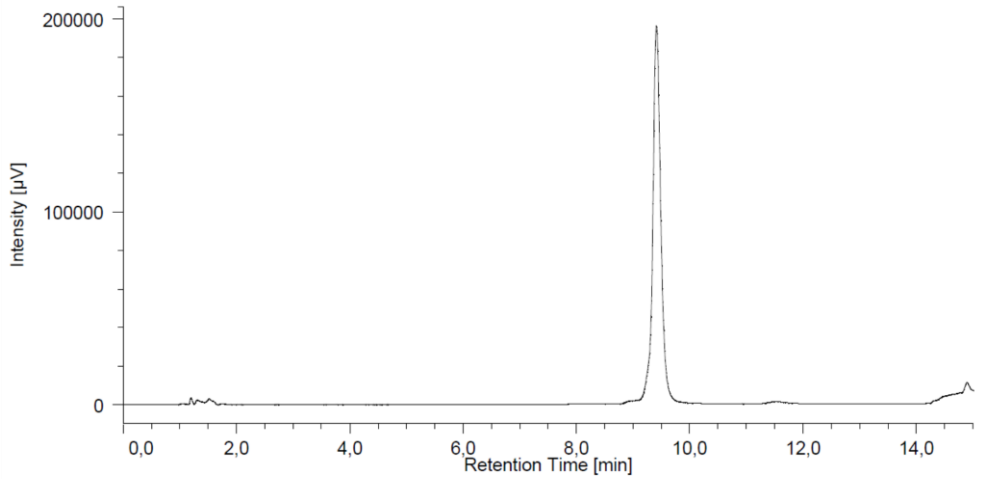


#	Peak Name	CH	tR [min]	Area [µV·sec]	Height [µV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	3	10,317	847529	74377	96,240	94,422	N/A	20188	N/A	1,737	
2	Unknown	3	10,683	33113	4393	3,760	5,578	N/A	N/A	N/A	N/A	

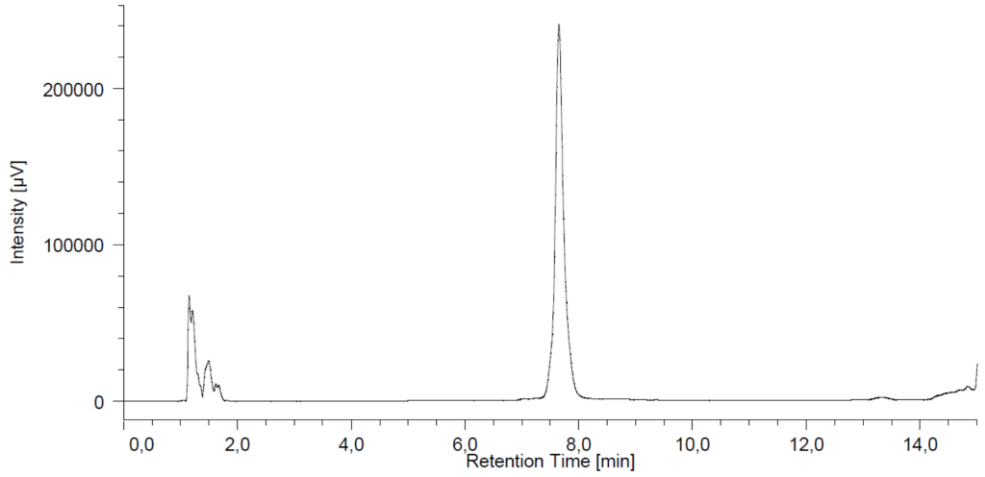
RP-HPLC chromatogram of **14**
gradient from 0% to 20% solvent D over 8 min (System B)



RP-HPLC chromatogram of **15**
gradient from 5% to 20% solvent D over 8 min (System B)



RP-HPLC chromatogram of **16**
gradient from 20% to 40% solvent B over 14 min (System A)



RP-HPLC chromatogram of **17**
gradient from 20% to 40% solvent B over 14 min (System A)

4. References

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