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Supplementary Information

Direct ligand screening against membrane proteins on live cells enabled by DNA-Programmed Affinity Labelling

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Content

1. Abbreviations.

ACN: acetonitrile

CBS: carboxybenzene sulfonamide

DCC: *N*, *N*'-dicyclohexylcarbodiimide

DCM: dichloromethane

DIPEA: *N*, *N*'-diisopropylethylamine

DMF: *N*, *N*'-dimethylformamide

DMSO: dimethyl sulfoxide

EDC: 1-ethyl-3- (3-dimethylaminopropyl) carbodiimide hydrochloride

ESI-TOF-MS: electron-spray ionization time-of-flight mass spectrometry

FA: folic acid

FAM: carboxyfluorescein

FBS: fetal bovine serum

Fmoc: 9−fluorenylmethyloxycarbonyl

GLCBS: Gly-Leu-carboxybenzene sulfonamide

HBTU: *O*-benzotriazol-1-yl-*N*, *N*, *N*', *N*'-tetramethyluronium hexafluorophosphate

HOBt: *N*-hydroxylbenzotriazole

NHS: *N*-hydroxysuccinimide

PAGE: polyacrylamide gel electrophoresis

PBS: phosphate-buffered saline

SDS: sodium dodecyl sulfate

SE: succinimidyl ester

TAPS: tris(hydroxymethyl)methylamino]propanesulfonic acid

TBE: tris-borate-EDTA

TBTA: tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine

TEAA: triethylammonium acetate

TEA: trimethylamine

TFA: trifluoroacetic acid

TFE: 2, 2, 2-trifluoroethanol

TIPS: triisopropylsilane

TNBS: trinitrobenzenesulfonic acid

UV: ultraviolet

2. Materials and General Methods.

a) General.

Unless otherwise noted, all reagents and solvents were purchased from commercial sources and used as received. Common chemical reagents were purchased from Meryer (Shanghai) Chemical Technology Co., Ltd., J&K Scientific Ltd., and Sigma-Aldrich. Oligonucleotides were purchased from Sangon Biotech (Shanghai, China). Cell lines were purchased from ATCC. Cell culture reagents were purchased from Thermo Fisher Scientific. Proteins and antibodies were purchased from Sino Biologicals and Abcam. Water was purified with a Thermo Scientific Barnstead Nanopure system. Modified DNA oligonucleotides were purified using reverse-phase HPLC (Agilent 1200) using a gradient of acetonitrile (5-80%) in 100 mM TEAA (pH 7.0). Concentrations of oligonucleotides were determined based on the absorbance at 260 nm. Photo-crosslinking experiments were conducted with a UVata UV LED point light system at 365 nm wavelengths with an intensity of approximately 100 μJ /cm^2. Confocal images were acquired with a confocal laser microscope (Carl Zeiss LSM 710 NLO). Flow cytometer analysis was carried out with a Flow Cytometer and Cell Sorter Workstation (BD FACS AriaIII), and data analysis was performed using the FlowJo software. Error bars in data plots were standard deviation (SD) with the experiments individually repeated in triplicates. Synthesized small molecules were characterized by ^{1}H and ^{13}C NMR (500 MHz, Bruker AVAMCE III) and HRMS (Bruker APEX IV FTMS).

b) Proteins and antibodies information.

protein/antibody	vendor	catalogue #	information
folate receptor (FR)	Sino Biologics	11241-H08H	recombinant, human folate receptor 1 (Met1-Met233)
anti-actin antibody	Abcam	ab194952	immunogen: synthetic peptide within gamma actin aa 188-215 conjugated to Keyhole Limpet Haemocyanin (KLH); dilution: 1:5000
anti-CA-12 antibody	Sino Biologics	10617-RP02	immunogen: recombinant human carbonic anhydrase XII Protein (Met 1-Gln 291)

2. Supplementary figures and tables referenced in the main text.

a) Figure S1.

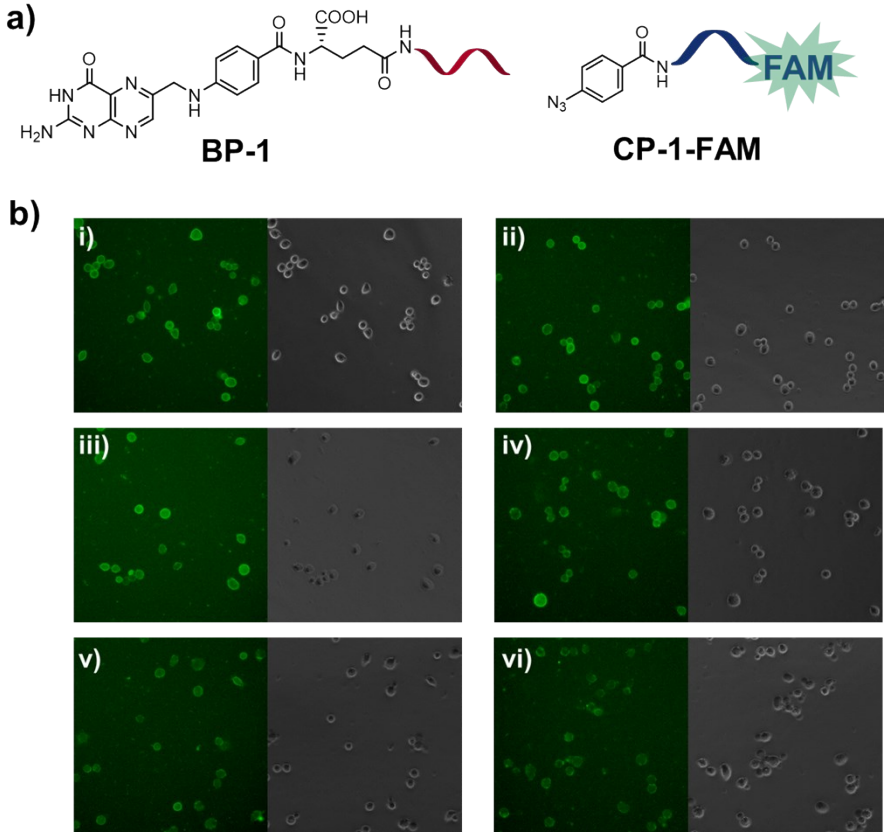


Figure S1: a) HeLa cells were labelled with **BP-1** and a fluorescently labelled **CP-1-FAM** as shown in Figure 1 of the main text. b) After labeling and washing away the excess probes, cell fluorescence was monitored with confocal imaging at different time points (0-6 hrs) to observe the internalization of DNA-tagged FR. i)-vi): 0 hr, 1 hr, 2 hrs, 3hrs, 4.5 hrs, and 6 hrs, respectively. Left: FAM channel; right: bright field. **CP-1-FAM**: same as **CP-1** in Figure 2 but with an additional fluorescein (FAM) group at the other end of DNA. Labelling conditions are the same as in Figure 2.

b) Figure S2.

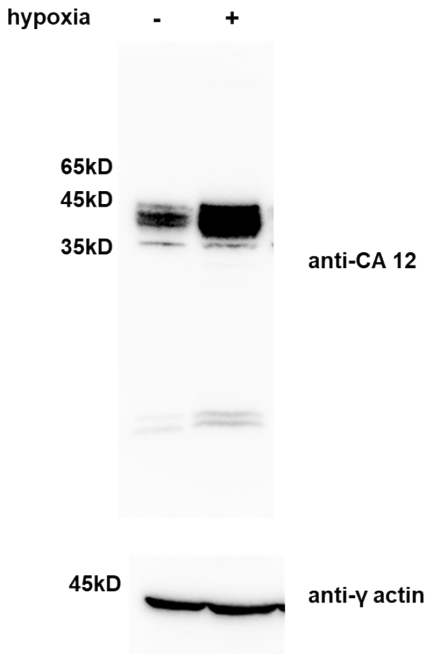


Figure S2: A549 cells were cultivated in DMEM (10% FBS, 100 unit/mL penicillin and 100 g/mL streptomycin) (+) with or (-) without hypoxia condition for 36 hours. After cell lysis, the cell lysates were analyzed with Western-blot. IB: immunoblotting.

c) Figure S3.

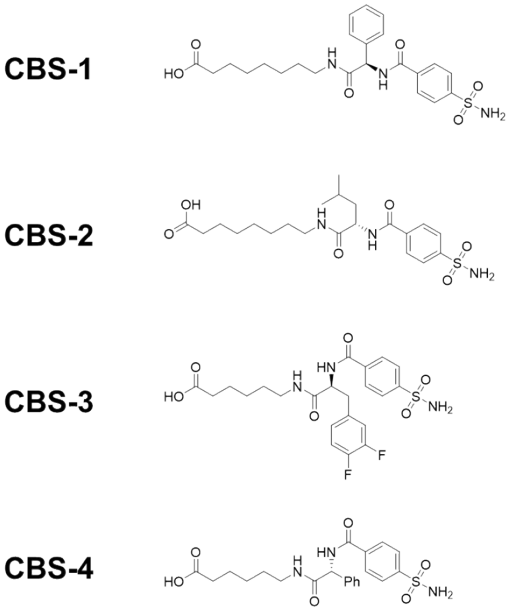


Figure S3: Structure of **CBS-1** to **CBS-4**, which were used in the on-cell screening against DNA-tagged CA-12 on A549 cells (Figure 4 of the main text). These compounds were prepared using the same method reported previously.[1]

d) Figure S4.

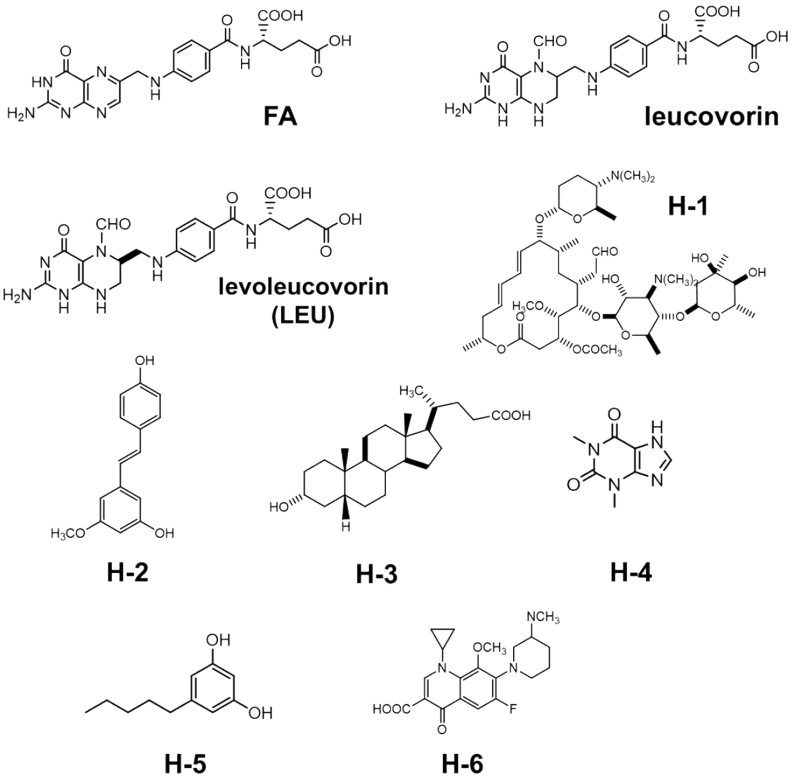


Figure S4: Structure of the nine hit compounds identified in the screening against DNA-tagged FR on HeLa cells using the 200-member compound library. **H-3**, **H-4**, **H-5**, and **H-6** showed limited solubility and a titration curve was not obtained; the titration curves of **FA** and leucovorin were not acquired since they are known FR ligands similar to the ones in Figure 3 of the main text; the titration curves of **LEU**, **H-1**, and **H-2** are shown in Figure 5 of the main text.

4. DNA structures, sequences, conjugate synthesis, purification and characterization.

a) Structures of small-molecule-modified DNAs.

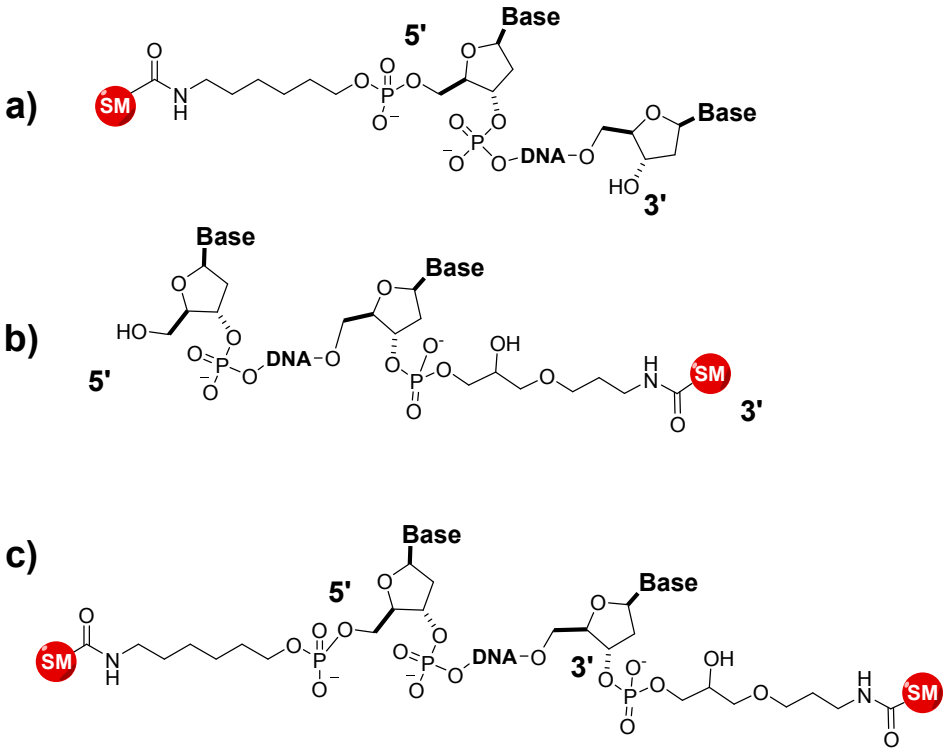


Figure S5: Representative structures of small-molecule-modified DNAs. a) 5'-small-molecule-conjugated DNA; b) 3'-small-molecule-conjugated DNA; c) DNA with modifications at both the 5'- and 3'-termini.

b) DNA sequences.

i) Sequences in Figure 2, 3, and 5:

BP-1: 5'-FA-TCTTAAGGTTTGCGGAGAGACGTAG-FAM-3'

CP-1: 5'-TCCGCAAACCTTAAGT-phenylazide-3'

DP: 5'-CTACGTCTCTCCGCAAACCTTAAG-3'

FP-1: 5'-FA-TCTTAA-FAM-3'

ii) Sequences in Figure 4:

BP-2: 5'-CBS-TCTTAAGGTTTGCGGAGAGACGTAG-FAM-3'

CP-1: 5'-TCCGCAAACCTTAAGT-phenylazide-3'

DP: 5'-CTACGTCTCTCCGCAAACCTTAAG-3'

FP-2: 5'-CBS-TCTTAA-FAM-3'

c) DNA sequence design.

DNA sequences were designed by using several online DNA property calculation tools including: OligoCalc (http://biotools.nubic.northwestern.edu/OligoCalc.html)[2] and OligoAnalyzer (https://www.idtdna.com). These tools were mostly used to calculate the thermal stability (Tm values), the sequence complementarity, and the potential mismatch issues of the DNA sequences. Thermodynamic parameter calculations (entropy, enthalpy, and free energy) were conducted with the CompareTm calculator developed by Weber;[3] specific experimental conditions, such as the ionic strength (salt concentration, bivalent ion concentration, etc.) and the probe concentration, were included in the calculation to obtain more accurate results.

d) DNA modification and purification.

i) General methods for DNA modification with small molecules.

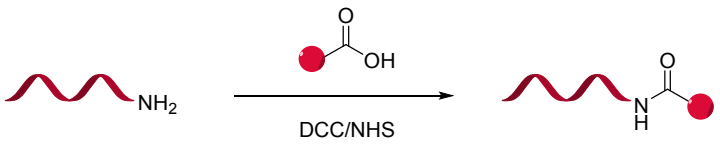
Modified oligonucleotides with amine, FAM, and biotin modifications were purchased from Shanghai Sangon Biotech and used after HPLC purification. Most small molecules were coupled with amine-modified oligonucleotides through amidation reactions and then purified by reverse-phase HPLC using a gradient of acetonitrile (5-80%) in 100 mM TEAA (pH 7.0), followed by lyophilization. Oligonucleotides were quantitated by a BioTek Epoch UV-Vis spectrometer based on the calculated extinction coefficient at 260 nm. Oligonucleotides were characterized by a Kratos PC Axima CFR plus V2.4.1 [for MALDI-MS, matrix: 8:1 (50 mg/mL 3-HPA or THAP in 1:1 water: acetonitrile) : (50 mg/mL ammonium citrate in water)] mass spectrometer or an Agilent 1290 Infinity II UHPLC /6230 TOF for ESI-MS (API-AJS ion source).

ii) General procedure of ethanol precipitation (for oligonucleotide purification).

To an aqueous DNA solution (ideally the sample volume is less than 400 μL), 0.1 volume of 3 M NaOAc (pH 5.0) was added to adjust the sample pH to ~5.0, followed by the addition of 1/40 volume of 20 mg/mL glycogen (Aldrich, final glycogen concentration: 133.33 μg/mL) and 2.5 volume of absolute ethanol. The solution was maintained at -80 °C for at least 1 hour and then centrifuged at 14,000 g for 15 minutes at 4°C.

The supernatant was discarded and the pellet was rinsed once carefully with cold 70% ethanol. After centrifuge at 14, 000 g for another 5 minutes at 4 °C, the supernatant was discarded and the pellet was dried by a speedvac. Recovered sample was dissolved in appropriate buffer for subsequent analysis or experiments.

iii) 5'-FA-modified DNA.



FA (4.0 mg, 9 μmol), DCC (3.7 mg, 18 μmol) and NHS (2.1 mg, 18 μmol) were dissolved in 100 μL anhydrous DMSO and then the solution was vortexed for 24 hours at room temperature. The formed urea precipitate was removed by centrifugation for 5 min. The supernatant was diluted by anhydrous DMSO with a ratio of 1:5.

5'-amine modified DNA (Shanghai Sangon Biotech) was dissolved in 40 μL phosphate buffer (pH = 7.2). 15 μL diluted supernatant (containing the active ester FA-SE) from the previous step was added to the DNA solution and the reaction was stirred at room temperature for 48 hours. After brief centrifugation, the reaction mixture was desalted by a NAP-5 column (GE Pharmacia) before the product was purified by HPLC. Purified 5'-FA-DNA was characterized by MALDI-MS or ESI-TOF-MS.

iv) 3'-Phenylazide-modified DNA.

4-azidobenzoic acid (2.0 mg, 12 μmol), DCC (2.5 mg, 12 μmol) and NHS (1.4 mg, 12 μmol) were dissolved in 120 μL anhydrous DMSO and then the solution was vortexed for 1 hour at room temperature. The formed urea precipitate was removed by filtration. 40 μL of the filtrate was added to a solution containing 40 μL 3'-amine-modified DNA and 40 μL phosphate buffer (0.25 M, pH = 7.2). The mixture was maintained with sonication at 37 ºC for 1 hour. The reaction mixture was desalted by a NAP-5 column (GE Pharmacia) or ethanol precipitation before the product was purified by HPLC. Purified 5'-phenylazide-DNA was

characterized by MALDI-MS or ESI-TOF-MS.

v) CBS-modified DNA.

CBS (carboxybenzene sulfonamide; 2.4 mg, 12 μmol), DCC (2.5 mg, 12 μmol) and NHS (1.4 mg, 12 μmol) were dissolved in 120 μL anhydrous DMSO and then the solution was vortexed for 1 hour at room temperature. The formed urea precipitate was removed by filtration. 40 μL of the filtrate was added to a solution containing 40 μL 5'-amine-modified DNA and 40 μL phosphate buffer (0.25 M, pH = 7.2). The mixture was maintained with sonication at 37 ºC for 1 hour. The reaction mixture was desalted by a NAP-5 column (GE Pharmacia) or ethanol precipitation before the product was purified by HPLC. The purified 5'-CBS-DNA conjugate was characterized by MALDI-MS or ESI-TOF-MS.

e) Small-molecule-DNA conjugate characterization.

DNA	expected mass	observed mass
BP-1	8944.9	8950.0
CP-1	5179.0	5181.1
FP-1	3265.9	3265.2
DP	7229.2	7232.7
BP-2	8704.5	8708.8
FP-2	2696.7	2695.5

5. Cell culture.

5.1 HeLa cell culture and FR overexpression.

To obtain cells with high-level FR expression, HeLa cells were cultured in folic-acid-depleted RPMI 1640 medium supplemented with 10% FBS, 100 unit/mL penicillin and 100 μg/mL streptomycin to induce the upregulation of folate receptor expression on cell membrane. After at least 6 passages, the cells were ready for the labeling experiments.

5.2 A549 cell culture and validation of CA-12 overexpression.

A549 cells with elevated CA-12 expression were obtained with hypoxia cultivation (AnaeroPack; Mitsubishi Gas Chemical) at 37 °C for 36 h in DMEM (10 % FBS, 100 unit/mL penicillin, and 100 g/mL streptomycin). The cells were then lysed with modified RIPA buffer (25 mM HEPES, pH = 7.5, 150 mM NaCl, 1 % NP-40, 1 % triton, 1 % sodium deoxycholate, 1 mM EDTA, supplemented with Pierce® Universal nuclease, Roche EDTA-free Protease Inhibitor Cocktail) at 37 °C for 15 min. Cell lysates were obtained by collecting the supernatant after centrifugation at 13,300 rpm for 10 min to remove insoluble cell debris. The lysate protein concentration was determined with a BCA Protein Assay Kit. The cell lysates were then resolved with SDS-PAGE and electro-transferred onto an immune-blot PVDF membranes. The membrane was blocked with 5 % non-fat milk in TBST buffer, incubated with anti-CA-12 antibody, and anti-γ-actin antibody, followed by incubation with HRP conjugated goat anti-rabbit IgG antibody or goat anti-mouse IgG antibody. The membranes were developed with Clarity™ Western ECL Substrate.

6. Fluorescence-based on-cell ligand competition experiments.

6.1 DPAL-mediated cell labeling and ligand competition with adherent cells (Figure 2).

Cells were cultured on coverslips in 24-well plates at 37 °C. Before labeling, the medium was removed and the cells were washed three times with 1x PBS buffer (pH = 7.4). To prepare the labeling probes, small-molecule-conjugated BP and CP (1 μM each) were mixed in 1x PBS buffer, heated to 90 °C for 3 min and then cooled down to room temperature slowly. To the cells were added the prepared DNA probes and then incubated at 4 °C for 1.5 h, followed by UV irradiation over ice at 365 nm for 10 seconds by a Uvata UV LED point light source. After labeling, the cells were washed three times with 1x PBS buffer to remove unlabeled probes. After irradiation and the removal of unlabeled probes, 1 μM DP dissolved in 1x PBS buffer was added for toehold displacement at room temperature for 20 min. The cells were then washed three times with 1x PBS buffer to remove the BP/DP duplexes. Afterwards, the cells were added with 1 μM **FP-1** in a solution either containing the respective small molecule or without small molecule competitor and incubated at room temperature for 20 min, followed by washing with 1x PBS buffer for three times to remove unbound probes. The cells were then fixed with 4 % PFA at room temperature for 15 minutes and washed for two more times with 1x PBS buffer. Cell images were acquired with Carl Zeiss LSM 710 NLO system.

6.2 DPAL-mediated cell labeling and ligand competition with suspension cells (Figure 3-5).

The experimental procedure and conditions are the same as in **6.1** above, except that, prior to labeling, the cells were harvested and washed three times with 1x PBS buffer by gentle resuspension and centrifugation at 500× g for 5 min. After the labeling and ligand screening, the cells were fixed by re-suspension in 4% PFA and incubation at room temperature for 15 min, followed by washing two more times with 1x PBS buffer. Flow cytometry analysis was carried out with a BD FACS AriaIII system.

6.3 On-cell ligand titration experiments with DNA-labeled cells.

The experimental procedure and conditions are the same as in **6.2** above, except solutions containing different concentration of compounds were used.

6.4 On-cell ligand titration experiments with unlabeled cells.

The experimental procedure and conditions are the same as in **6.2** above, except cells were directly incubated with FP and compound solutions without the DPAL-mediated labeling step.

7. Fluorescence Polarization (FLP).

Fluorescence polarization experiments were performed on a PerkinElmer VICTOR X5 multi-label plate reader following previous reports.[4-6] In brief, the compounds were dissolved in DMSO and then diluted with 1× PBS serially. The solution containing the sample was combined with the protein target and the fluorescent probe **FA-FAM** at specified concentrations, incubated for 90 min at 23 ºC in a 384-well microplate, and then subjected to FLP measurement immediately. Conditions: **FA-FAM**, 200 nM; FR, 10 μM; solution volume, 60 μL. All measurements were conducted for three times. The obtained anisotropy data were fitted and plotted following previously reported methods.[4,6,7]

8. Calculation method to determine disassociation constant (K_d) values.

For on-cell screening experiments, first, apparent K_d values were obtained by fitting the respective titration curves using the DoseResp function in the OriginPro software (ver. 8.6). Next, disassociation constants were calculated using the Prusoff-Cheng equation.[8] In the equation, the concentration of **FP-1** was used as the ligand concentration [L], and the enhanced binding affinity of **FP-1** when hybridized on the DNA tag was used as the K_d.[9] To calculate the enhanced binding affinity of **FP-1**, we used the CompareTm DNA property calculator developed by Weber[3] and the calculation method reported previously.[9] In brief, the free energy gain from **FP-1**/CP hybridization was ~4.85 kcal/mol using the calculator, corresponding to a ~3,135 orders of magnitude affinity increase. Previously, we have determined the K_d of the FA-DNA conjugate **FP-1** to be 710 nM.[9] Assuming a simple additive effect without either a cooperativity or penalty factor on the apparent binding affinity,[10] the enhanced binding affinity **FP-1** on the DNA-tagged FR would be ~0.226 nM.

For the fluorescence polarization (FLP) experiments, first, apparent K_d values were also obtained by fitting the respective titration curves using the DoseResp function in the OriginPro software (ver. 8.6). Next, disassociation constants were calculated using the Prusoff-Cheng equation.[8] In the equation, the concentration and the reported binding affinity of **FP-FAM**[11] was used as the ligand concentration [L] and the K_d, respectively. [FA-FAM] = 200 nM, K_d = 0.1 nM.

9. Compound synthesis and characterization.

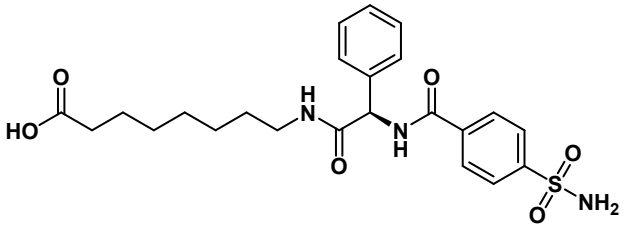
a) General methods for compound synthesis.

In general, all solvents used in compound synthesis were used as supplied by Fisher Chemicals, Merck or Sigma-Aldrich in HPLC or analytical grade. All other reagents were purchased from ACROS Organics, J&K Scientific Ltd., Merck, Millipore, Sigma-Aldrich, Alfa Aesar or TCI and used as supplied. Proton (^{1}H) nuclear magnetic resonance (NMR) spectrum were recorded at 298 K on a Bruker AV400 (400 MHz) or a Bruker AVIII500 (500 MHz) spectrometer. Carbon (^{13}C) NMR spectrum were recorded at 298 K on a Bruker AV400 (100 MHz) spectrometer or on a Bruker AVIII500 (125 MHz) spectrometer. High-resolution mass spectrometry (HRMS) spectrum were recorded on a Bruker Apex IV FTMS mass spectrometer using ESI (electrospray ionization) or Agilent 6230 API-AJS ESI-TOF mass spectrometer. Calculated and measured m/z values are reported as dimensionless quantities.

b) General synthesis routes.

Assembly of all peptides was carried out using the Fmoc-based SPPS (solid-phase peptide synthesis) method manually in a glass reaction vessel fitted with a sintered glass frit. The first amino acid was assembled on 2-chlorotrityl resin (0.1 mmol, loading of 1.0 mmol/g, GL Biochem, Lot No: GLS190613-48101) by adding 2.0 equiv. of *N*-Fmoc-protected amino acid (relative to resin loading), 4.0 equiv. of DIPEA in DCM for 4 hours. Coupling reactions were performed manually by using 2.0 equiv. of *N*-Fmoc-protected amino acid (relative to resin loading) activated *in situ* with 2.0 equiv. of HTAU, 2.0 equiv. of HOBt and 4.0 equiv. of DIPEA in DMF for 4 hours. The coupling efficiency in manual synthesis was assessed by TNBS tests. Fmoc-protecting groups were removed by treatment with a piperidine/DMF solution (1:4) for 10 min. The process was repeated three times and the efficiency of deprotection was verified by UV absorption of the piperidine washing solutions at 299 nm. Synthetic linear peptides were recovered directly upon acid cleavage. Before cleavage, the resin was washed thoroughly with DCM. The peptide was released from the resin using a cleavage solution of TFE/AcOH/DCM (2:1:7, 2 × 30 min).

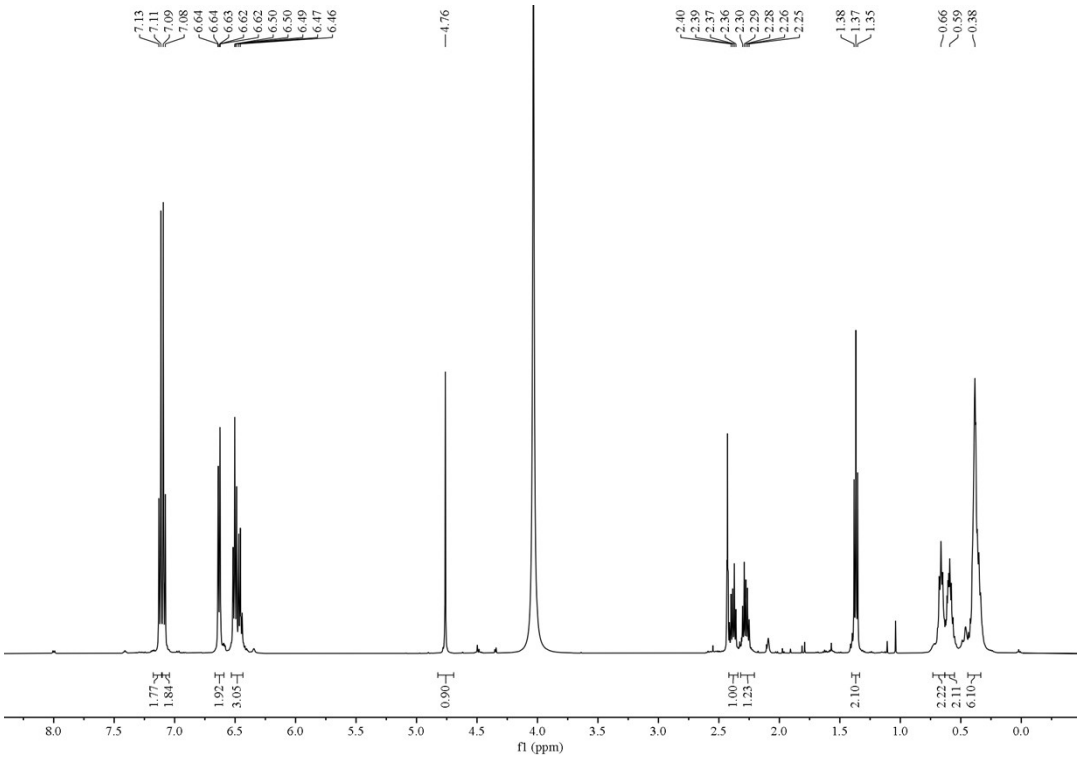
c) Experimental procedure and characterization data.



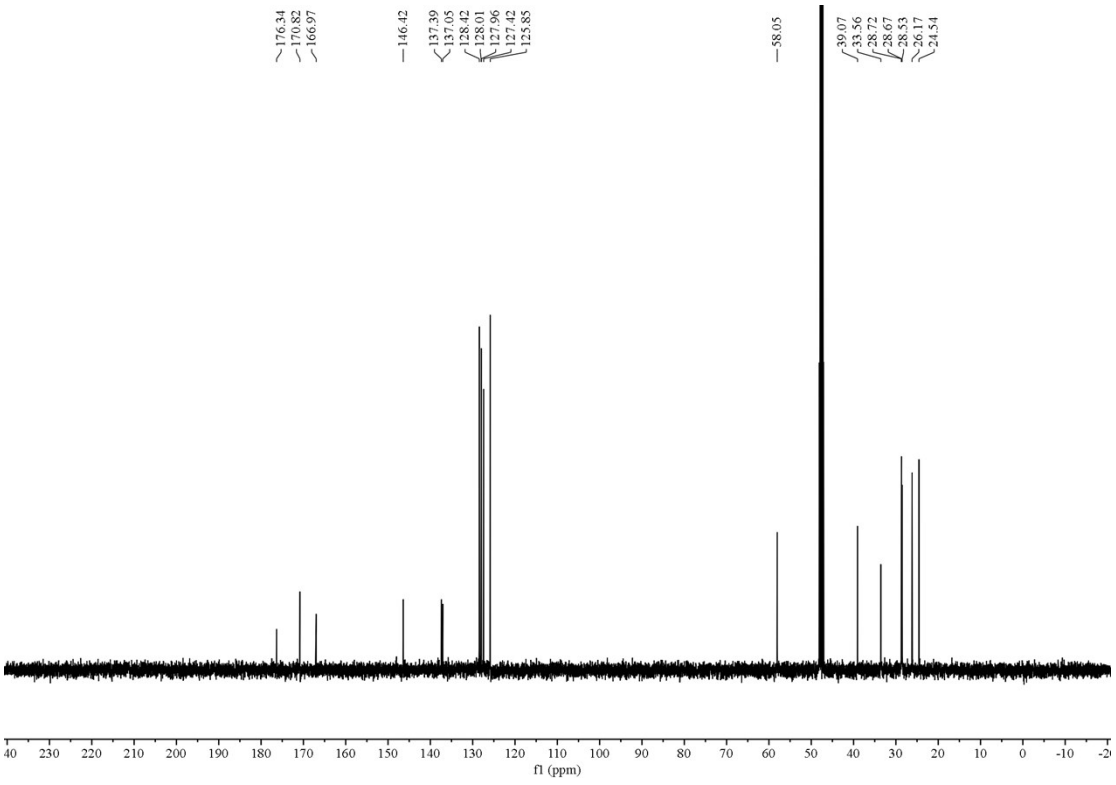
- **CBS-1:**

The linear tripeptide was assembled on 2-chlorotrityl resin (0.1 mmol, loading of 1.0 mmol/g) using the general procedure in the following sequence: *N*-Fmoc-8-aminooctanoic acid, Fmoc-*L*-phenylglycine, 4-sulfamoylbenzoic acid. The peptide was released from the resin using a cleavage solution of TFE/AcOH/DCM (2:1:7). The linear protected peptide was obtained as a white powder after precipitation and washing with diethyl ether; the precipitate was dissolved in CH_3CN/H_2O (1:1) finally purified with reverse-phase HPLC. The desired tripeptide **CBS-1** was recovered as a white powder after lyophilization (41 % yield). HRMS (*m/z*, $C_{23}H_{29}N_3O_6S$, ESI): calculated $[M+H]^+$: 476.1850; found: 476.1854. ^1H NMR (500 MHz, CD_3OD) δ 7.12 (d, *J* = 8.6 Hz, 2H), 7.09 (d, *J* = 8.6 Hz, 2H), 6.67 – 6.59 (m, 2H), 6.53 – 6.44 (m, 3H), 4.76 (s, 1H), 2.42 – 2.34 (m, 1H), 2.32 – 2.21 (m, 1H), 1.37 (t, *J* = 7.4 Hz, 2H), 0.73 – 0.63 (m, 2H), 0.63 – 0.55 (m, 2H), 0.44 – 0.33 (m, 6H). ^{13}C NMR (125 MHz, Methanol-d_4) δ 176.34, 170.82, 166.97, 146.42, 137.39, 137.05, 128.42, 128.01, 127.96, 127.42, 125.85, 58.05, 39.07, 33.56, 28.72, 28.67, 28.53, 26.17, 24.54.

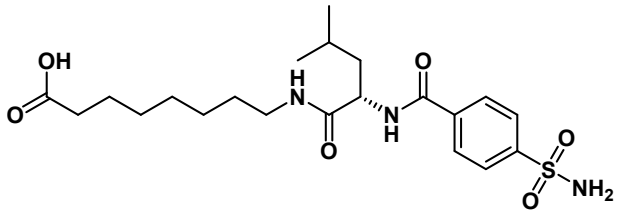
^{1}H-NMR spectrum of **CBS-1**



^{13}C-NMR spectrum of **CBS -1**

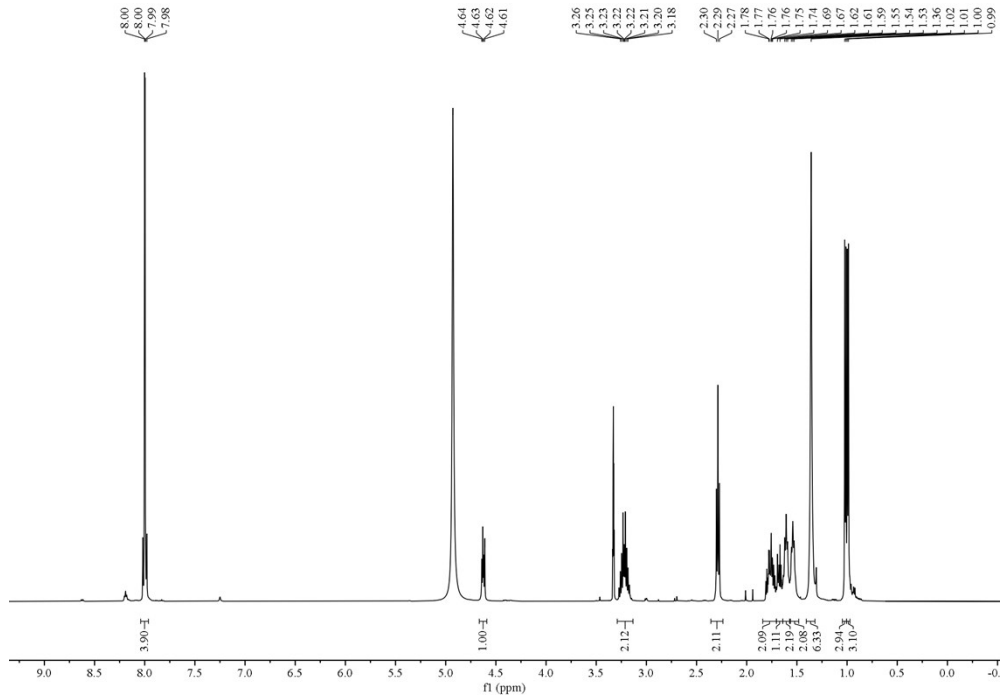


- **CBS-2:**

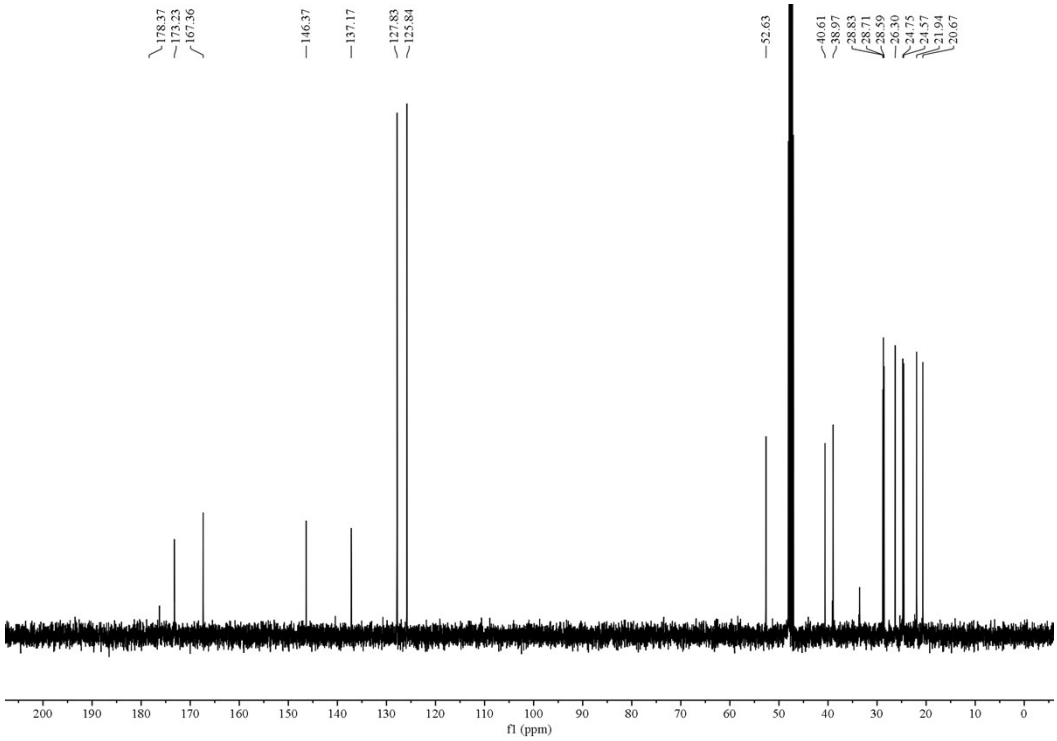


The linear tripeptide was assembled on 2-chlorotrityl resin (0.1 mmol, loading of 1.0 mmol/g) using the general procedure in the following sequence: *N*-Fmoc-8-aminooctanoic acid, Fmoc-*L*-leucine, 4-sulfamoylbenzoic acid. The peptide was released from the resin using a cleavage solution of TFE/AcOH/DCM (2:1:7). The linear protected peptide was obtained as a white powder after precipitation and washing with diethyl ether; the precipitate was dissolved in CH_3CN/H_2O (1:1) finally purified with reverse-phase HPLC. The desired tripeptide **CBS-2** was recovered as a white powder after lyophilization (45 % yield). HRMS (*m/z*, $C_{21}H_{33}N_3O_6S$, ESI): calculated $[M+H]^+$: 456.2163; found: 456.2168. ^{1}H NMR (500 MHz, CD_3OD) δ 8.04 – 7.96 (m, 4H), 4.63 (dd, *J* = 9.7, 5.3 Hz, 1H), 3.29 – 3.13 (m, 2H), 2.29 (t, *J* = 7.4 Hz, 2H), 1.84 – 1.70 (m, 2H), 1.70 – 1.64 (m, 1H), 1.64 – 1.57 (m, 2H), 1.56 – 1.48 (m, 2H), 1.36 (s, 6H), 1.02 (d, *J* = 6.3 Hz, 3H), 0.99 (d, *J* = 6.3 Hz, 3H). **^{13}C NMR** (125 MHz, Methanol-d_4) δ 178.37, 173.23, 167.36, 146.37, 137.17, 127.83, 125.84, 52.63, 40.61, 38.97, 28.83, 28.71, 28.59, 26.30, 24.75, 24.57, 21.94, 20.67.

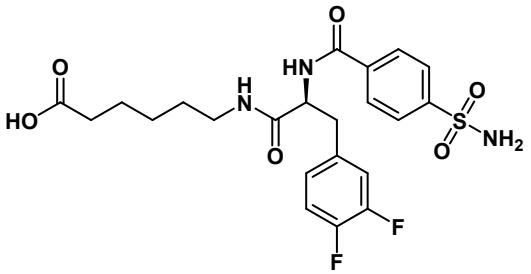
^{1}H-NMR spectrum of **CBS-2**



^{13}C-NMR spectrum of **CBS-2**

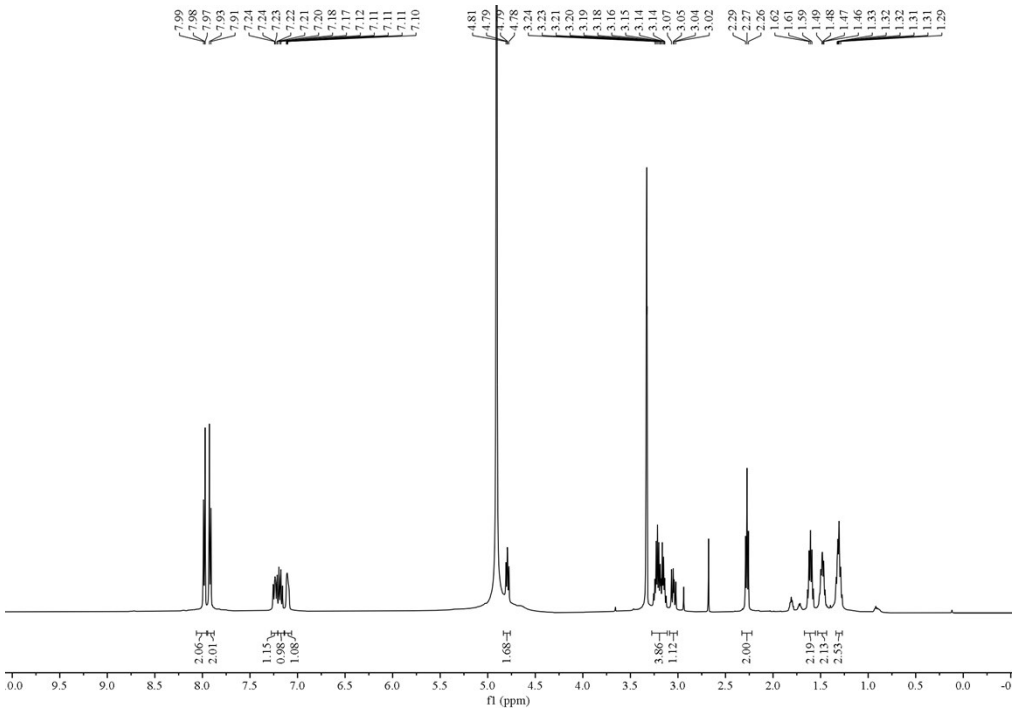


- **CBS-3:**

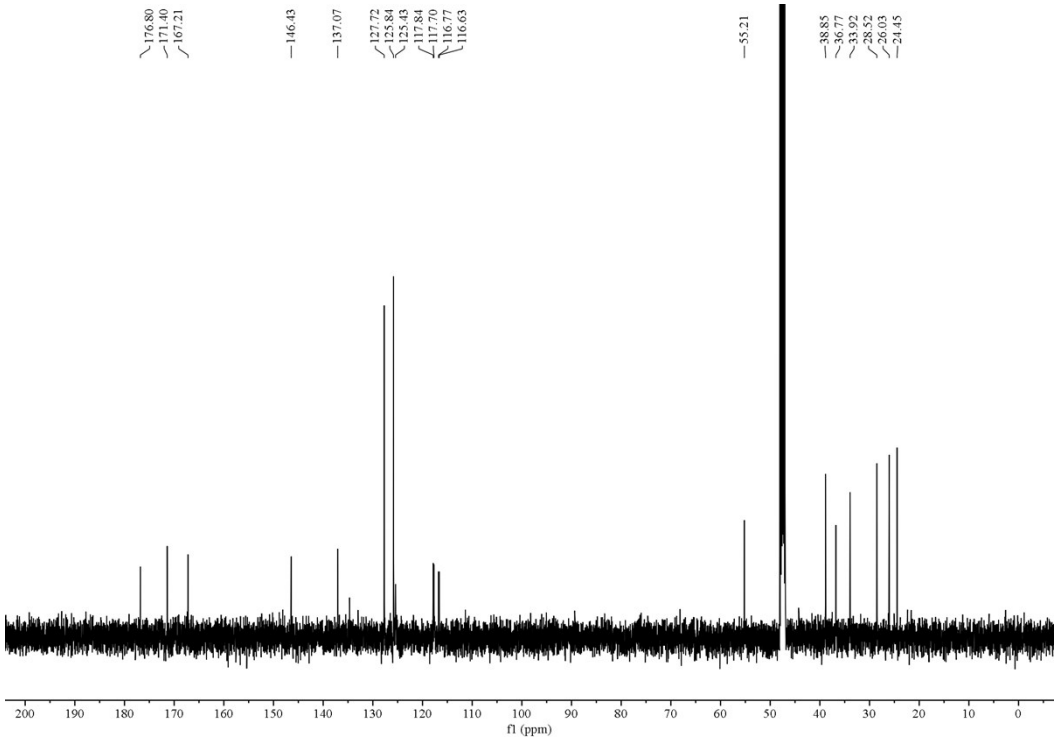


The linear tripeptide was assembled on 2-chlorotrityl resin (0.1 mmol, loading of 1.0 mmol/g) using the general procedure in the following sequence: Fmoc-6-aminohexanoic acid, Fmoc-3,4-difluoro-*L*-phenylalanine, 4-sulfamoylbenzoic acid. The peptide was released from the resin using a cleavage solution of TFE/AcOH/DCM (2:1:7). The linear protected peptide was obtained as a white powder after precipitation and washing with diethyl ether; the precipitate was dissolved in CH_3CN/H_2O (1:1) finally purified with reverse-phase HPLC. The desired tripeptide **CBS-3** was recovered as a white powder after lyophilization (50 % yield). HRMS (*m/z*, $C_{22}H_{25}F_2N_3O_6S$, ESI): calculated $[M+H]^+$: 498.1505; found: 498.1507. ^{1}H NMR (500 MHz, CD_3OD) δ 7.98 (d, *J* = 8.5 Hz, 2H), 7.92 (d, *J* = 8.5 Hz, 2H), 7.28 – 7.21 (m, 1H), 7.21 – 7.14 (m, 1H), 7.14 – 7.06 (m, 1H), 4.79 (dd, *J* = 8.7, 6.7 Hz, 2H), 3.28 – 3.11 (m, 4H), 3.04 (dd, *J* = 13.8, 8.7 Hz, 1H), 2.27 (t, *J* = 7.4 Hz, 2H), 1.67 – 1.55 (m, 2H), 1.53 – 1.44 (m, 2H), 1.34 – 1.27 (m, 3H). ^{13}C NMR (125 MHz, Methanol-d_4) δ 176.80, 171.40, 167.21, 146.43, 137.07, 127.72, 125.84, 125.43, 117.84, 117.70, 116.77, 116.63, 55.21, 38.85, 36.77, 33.92, 28.52, 26.03, 24.45.

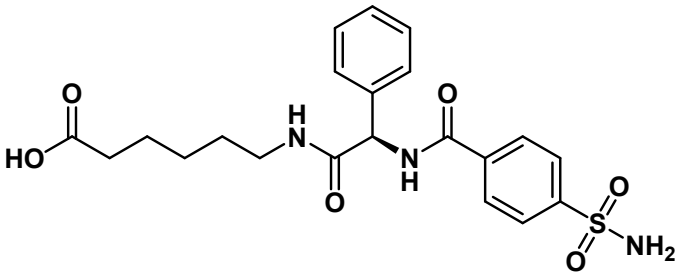
^{1}H-NMR spectrum of **CBS-3**



^{13}C-NMR spectrum of **CBS-3.**

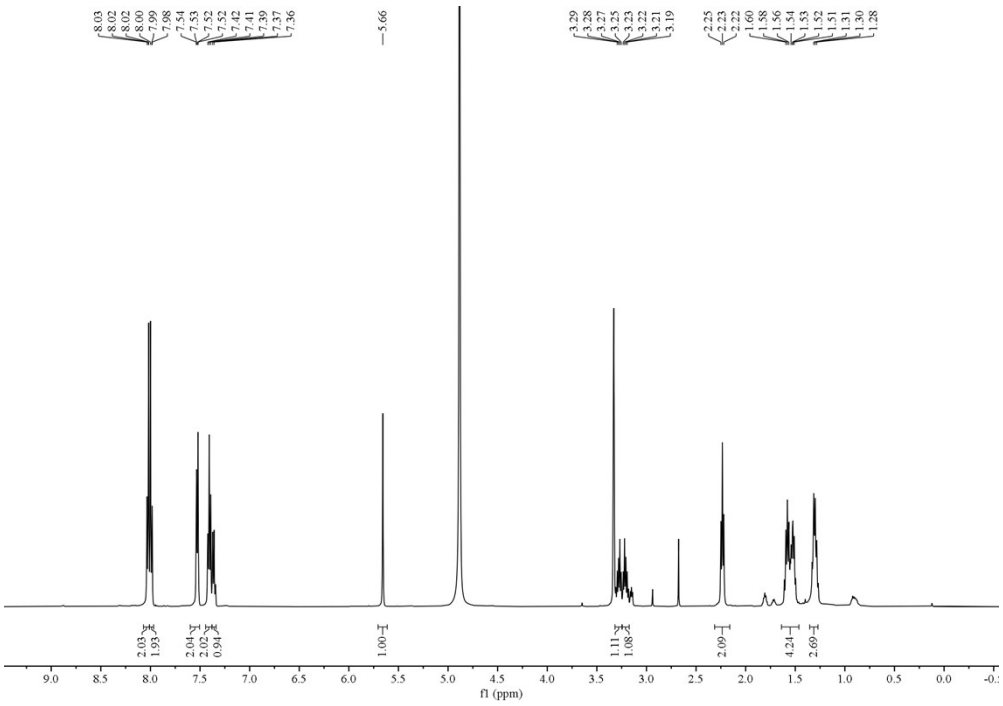


- **CBS-4:**

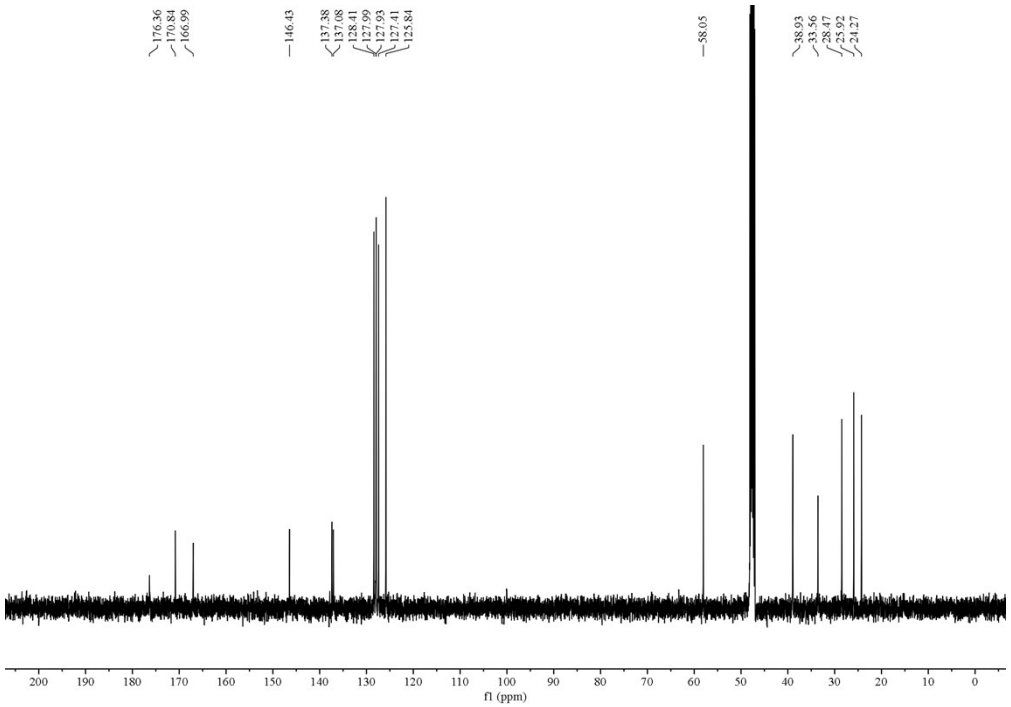


The linear tripeptide was assembled on 2-chlorotrityl resin (0.1 mmol, loading of 1.0 mmol/g) using the general procedure in the following sequence: Fmoc-6-aminohexanoic acid, Fmoc-*L*-phenylglycine, 4-sulfamoylbenzoic acid. The peptide was released from the resin using a cleavage solution of TFE/AcOH/DCM (2:1:7). The linear protected peptide was obtained as a white powder after precipitation and washing with diethyl ether; the precipitate was dissolved in CH_3CN/H_2O (1:1) finally purified with reverse-phase HPLC. The desired tripeptide **CBS-4** was recovered as a white powder after lyophilization (44 % yield). HRMS (*m/z*, $C_{21}H_{25}N_3O_6S$, ESI): calculated $[M+H]^+$: 448.1537; found: 448.1534. ^1H NMR (500 MHz, CD_3OD) δ 8.03 (d, *J* = 8.5 Hz, 2H), 7.99 (d, *J* = 8.5 Hz, 2H), 7.57 – 7.51 (m, 2H), 7.44 – 7.38 (m, 2H), 7.38 – 7.34 (m, 1H), 5.66 (s, 1H), 3.32 – 3.24 (m, 1H), 3.25 – 3.17 (m, 1H), 2.23 (t, *J* = 7.4 Hz, 2H), 1.64 – 1.46 (m, 4H), 1.35 – 1.27 (m, 3H). ^{13}C NMR (125 MHz, Methanol-d_4) δ 176.36, 170.84, 166.99, 146.43, 137.38, 137.08, 128.41, 127.99, 127.93, 127.41, 125.84, 58.05, 38.93, 33.56, 28.47, 25.92, 24.27.

^{1}H-NMR spectrum of **CBS-4**



^{13}C-NMR spectrum of **CBS-4**



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