Supporting Information

Deactivation of dimeric DNA-binding peptides through a palladium-mediated self-immolative cleavage

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Contents

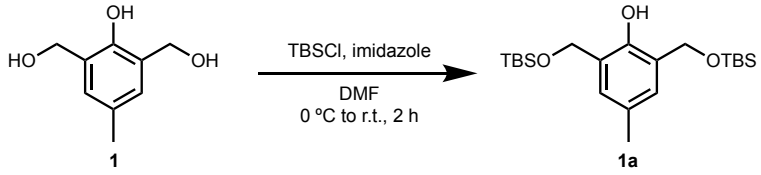
1 General methods and materials

All reagents were purchased from commercial sources: DMF and TFA were purchased from Scharlau, CH_2Cl_2 from Panreac, CH_3CN from Merck, $[Pd(allyl)Cl]_2$ from Sigma Aldrich. All peptide synthesis reagents were purchased from *GL Biochem (Shanghai) Ltd*. and *NovaBiochem*. Amino acids were purchased as protected Fmoc amino acids with the standard side chain protecting groups.

2 Synthetic procedures

2.1 Synthesis of the self-immolative linker

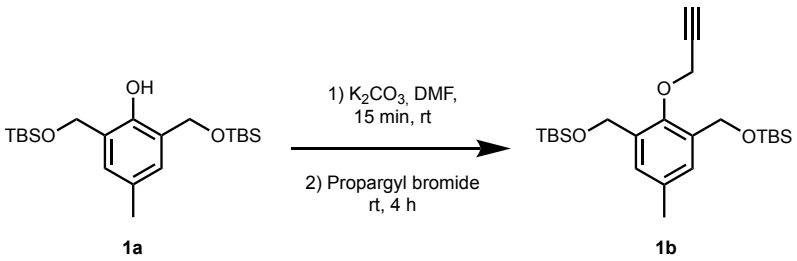
2.1.1 Synthesis of 1a



Compound **1a** was synthesized as previously described.[1]

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H), 6.94 (s, 2H), 4.86 (s, 4H), 2.29 (s, 3H), 0.98 (d, *J* = 1.4 Hz, 18H), 0.16 (d, *J* = 1.2 Hz, 12H).

2.1.2 Synthesis of 1b



Compound **1a** (1 g, 2.53 mmol) and K_2CO_3 (1.4 g, 10.12 mmol, 4 equiv) were dissolved in DMF (5 mL). After 15 min stirring at rt, propargyl bromide (80 wt. % in toluene, 340 µL, 3.03 mmol, 1.2 equiv) was added dropwise and the reaction was stirred at rt for 4 hours. The mixture was diluted with ether and washed with brine (x3), to completely remove DMF. The combined organic layers were dried with Na_2SO_4, filtered and the solvent was evaporated. The residue was purified by flash chromatography (5% ether/hexanes) to give the desired product (0.860 g, 78% yield) as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (s, 2H), 4.78 (s, 4H), 4.58 (d, *J* = 2.6 Hz, 2H), 2.55 – 2.49 (m, 1H), 2.33 (s, 3H), 0.95 (s, 18H), 0.12 (s, 12H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 150.9, 134.1, 133.8, 128.1, 79.4, 75.2, 62.0, 60.5, 26.0, 21.1, 18.4, -5.3.

[1] C.-C. Song, R. Ji, F.-S. Du and Z.-C. Li, *Macromolecules* 2013, **46**, 21, 8416–8425.

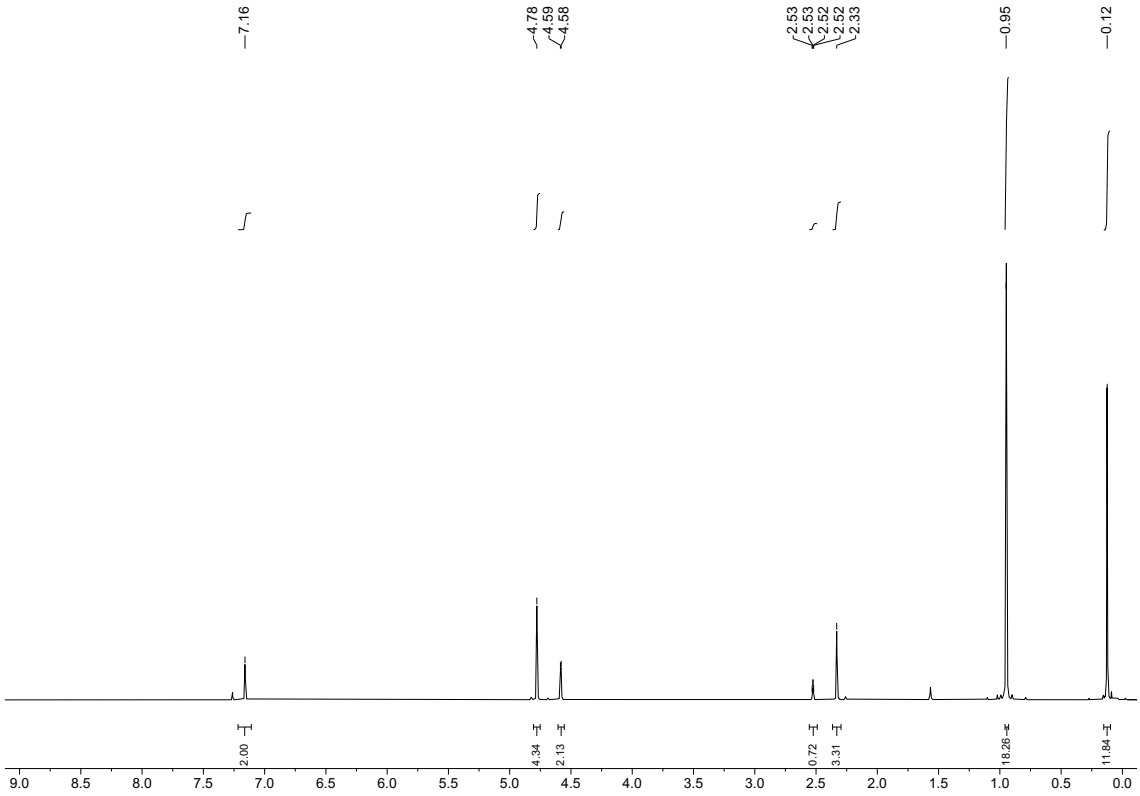


Figure S1. ^{1}H NMR spectrum of **1b** in $CDCl_3$.

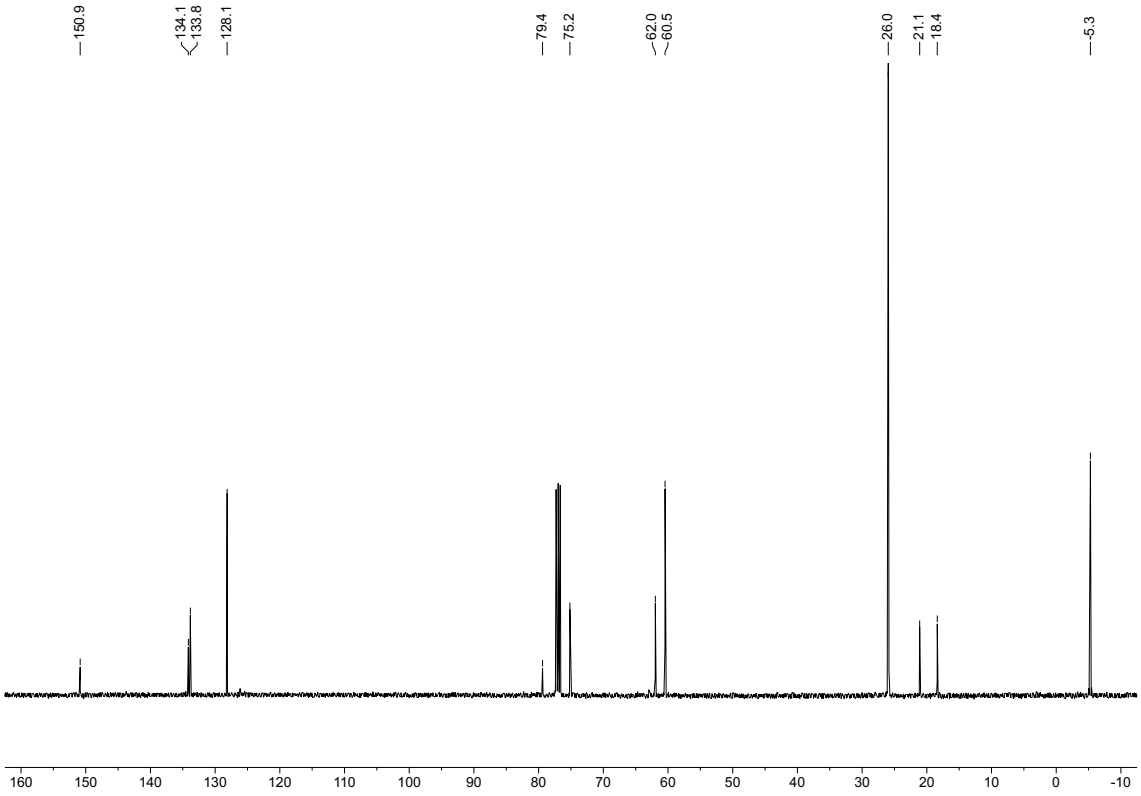


Figure S2. ^{13}C NMR spectrum of **1b** in $CDCl_3$.

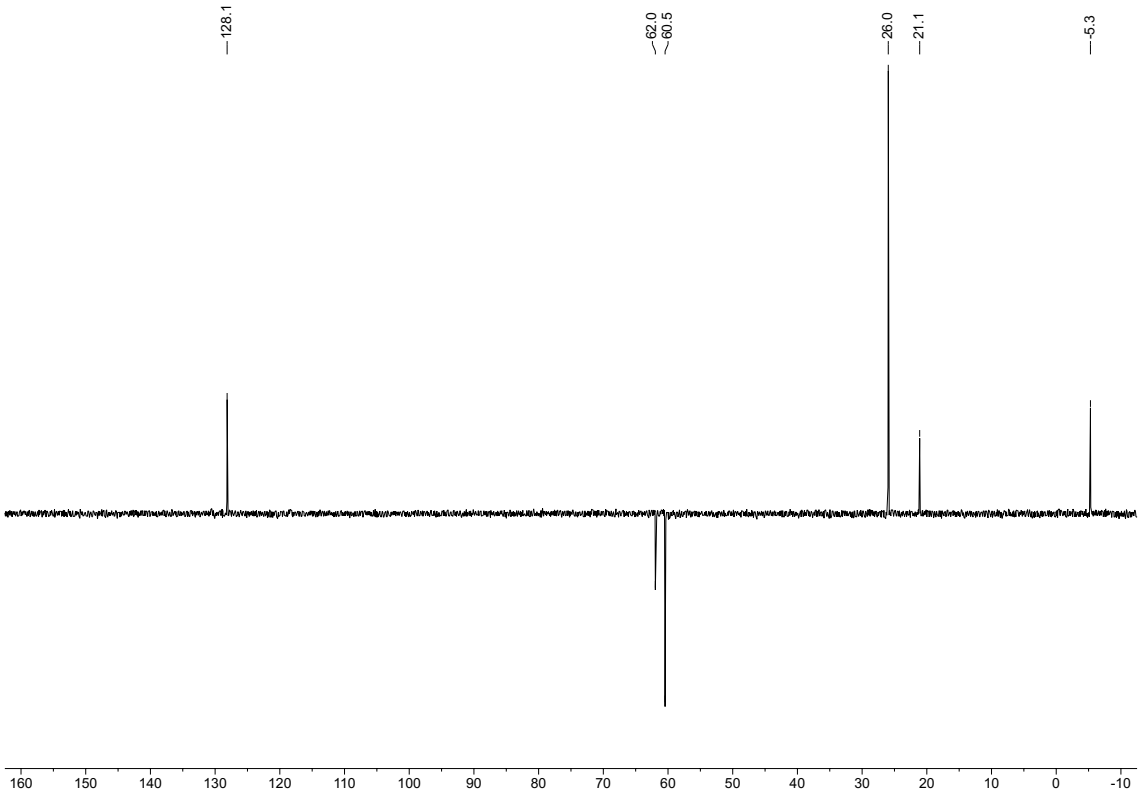
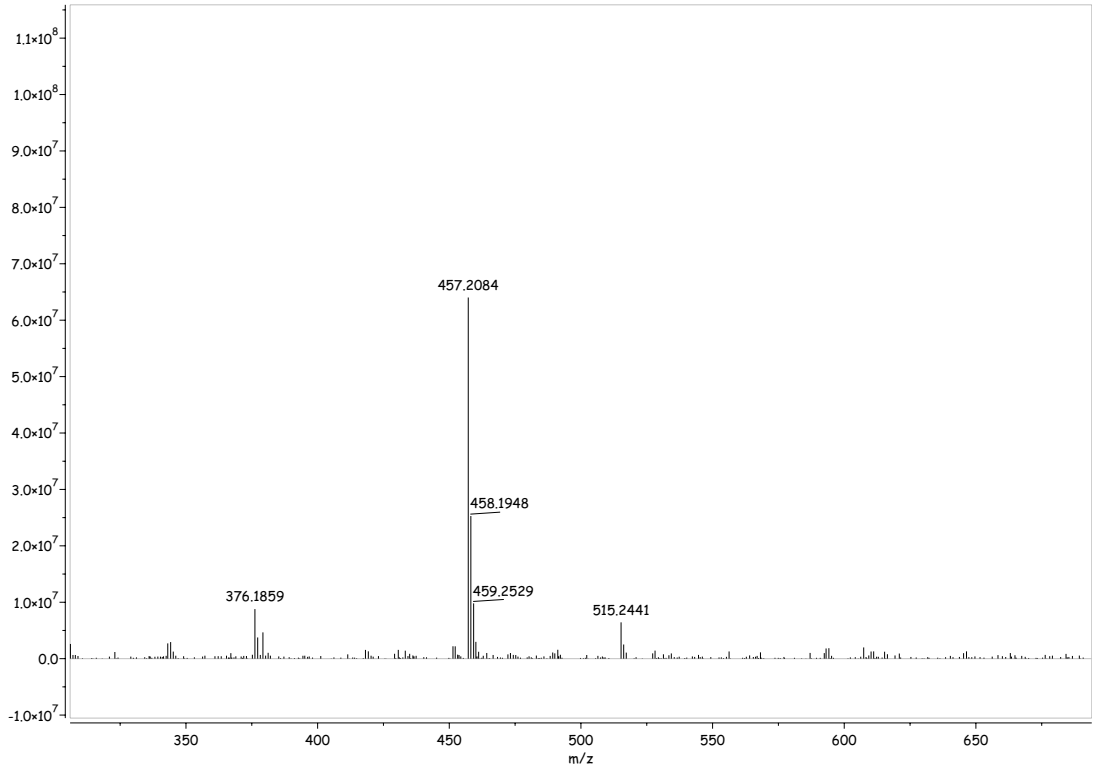
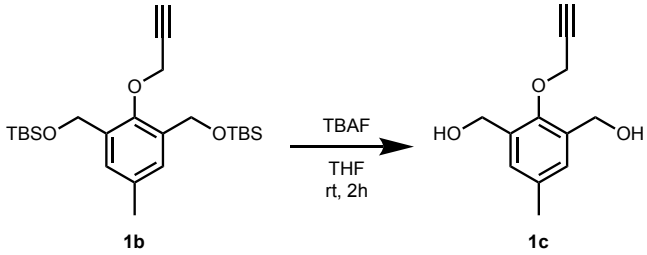


Figure S3. DEPT NMR spectrum of **1b** in $CDCl_3$.



HRMS (m/z): Calcd. for $C_{24}H_{42}NaO_3Si_2$ $[M+Na]^+$: 457.2570. Found: 457.2084 $[M+Na]^+$.

2.1.3 Synthesis of 1c



Compound **1b** (360 mg, 0.829 mmol) was dissolved in THF (4.1 mL). TBAF (2.5 mL 1M solution in THF) was added to the solution. The reaction mixture was stirred at rt for 2 h. The solvent was evaporated, and the resulting residue was redissolved in CH_2Cl_2 and washed with water (3x). The combined aqueous layers were extracted with CH_2Cl_2. The combined organic layers were dried with Na_2SO_4, filtered and the solvent was evaporated to give the desired product **1c** (147 mg, 86% yield) as transparent-white needles.

^{1}H NMR (400 MHz, CD_3OD) δ 7.16 (s, 2H), 4.64 (s, 4H), 4.58 (d, *J* = 2.5 Hz, 2H), 2.92 (t, *J* = 2.4 Hz, 1H), 2.29 (s, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ 151.5, 134.0, 134.0, 128.6, 78.8, 75.4, 61.7, 58.9, 19.6.

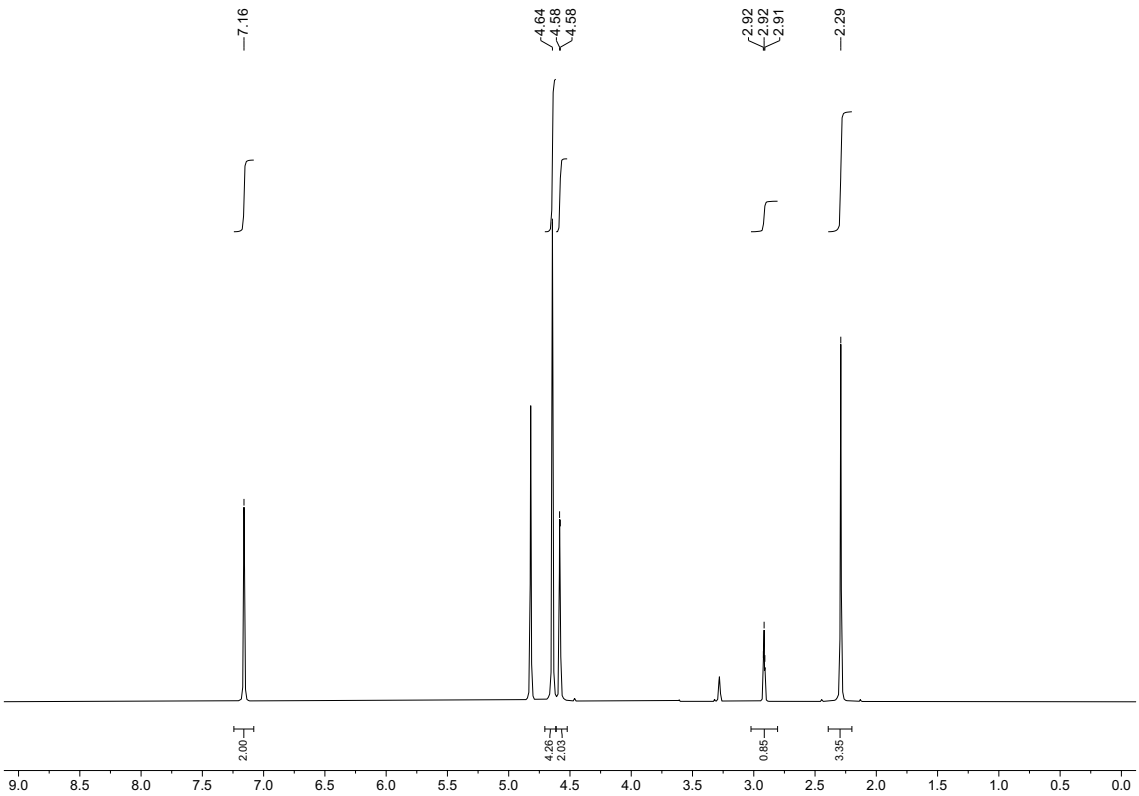


Figure S4. ^{1}H NMR spectrum of **1c** in CD_3OD.

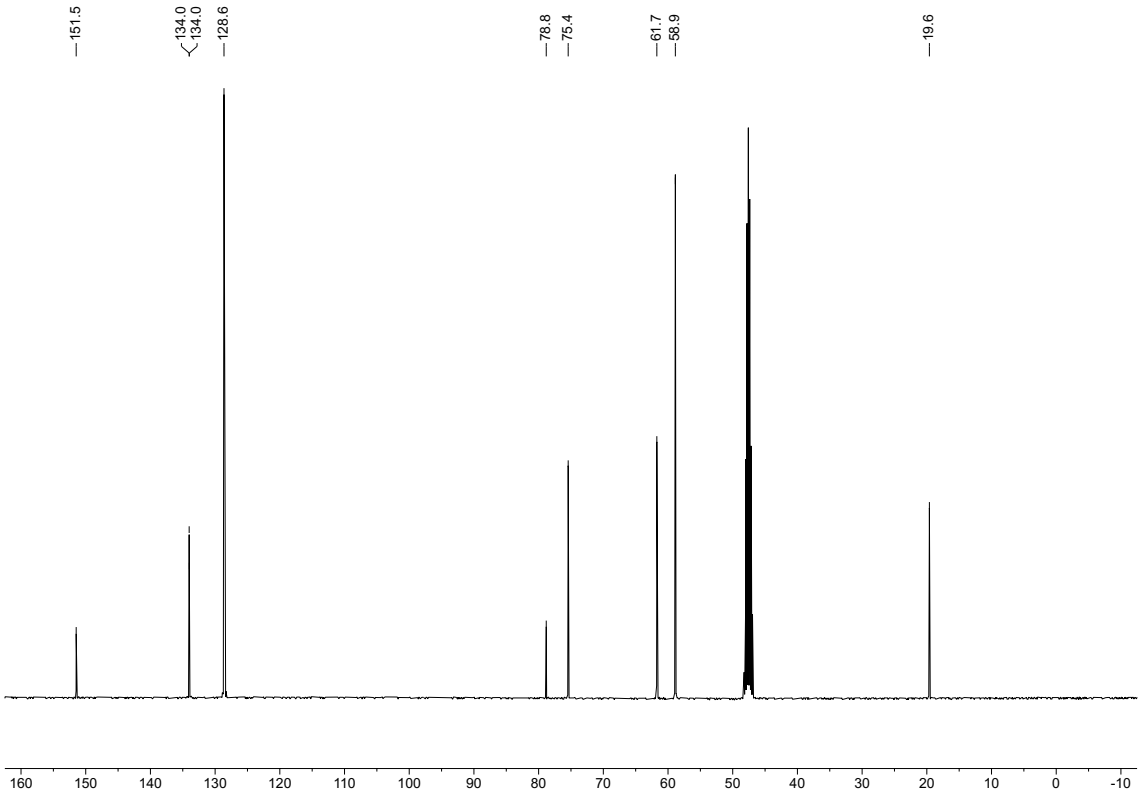


Figure S5. ^{13}C NMR spectrum of **1c** in CD_3OD.

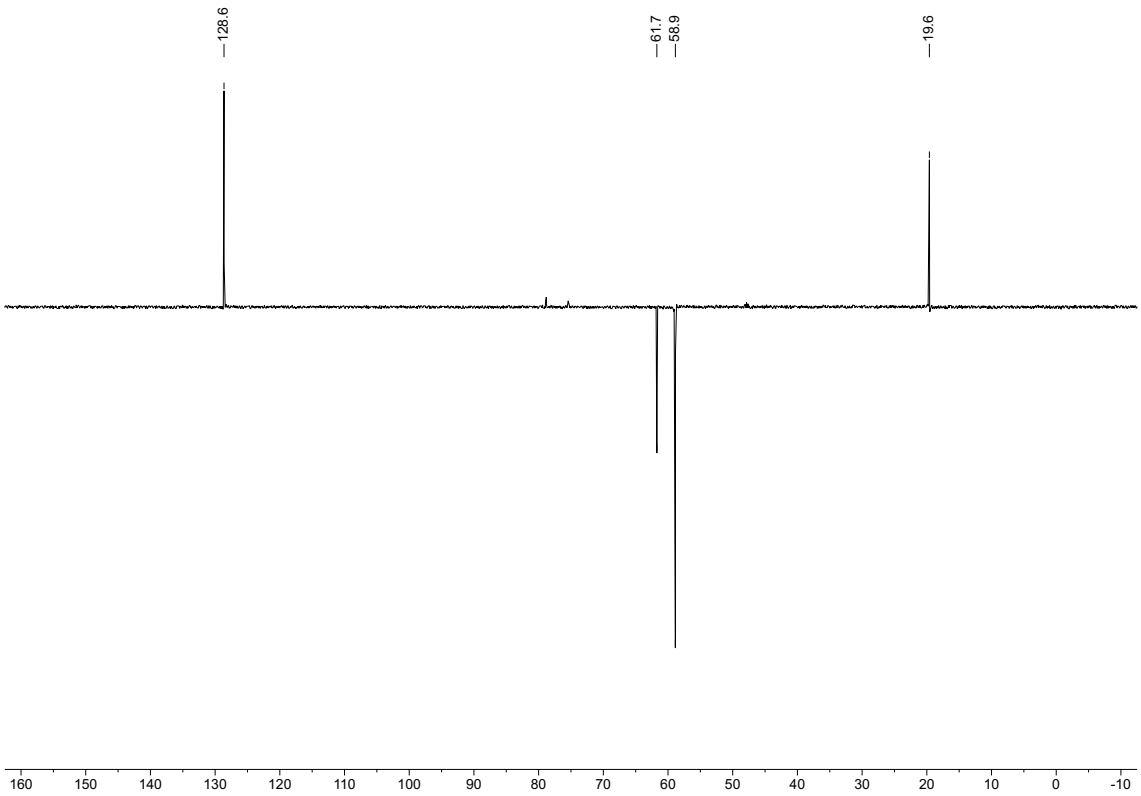
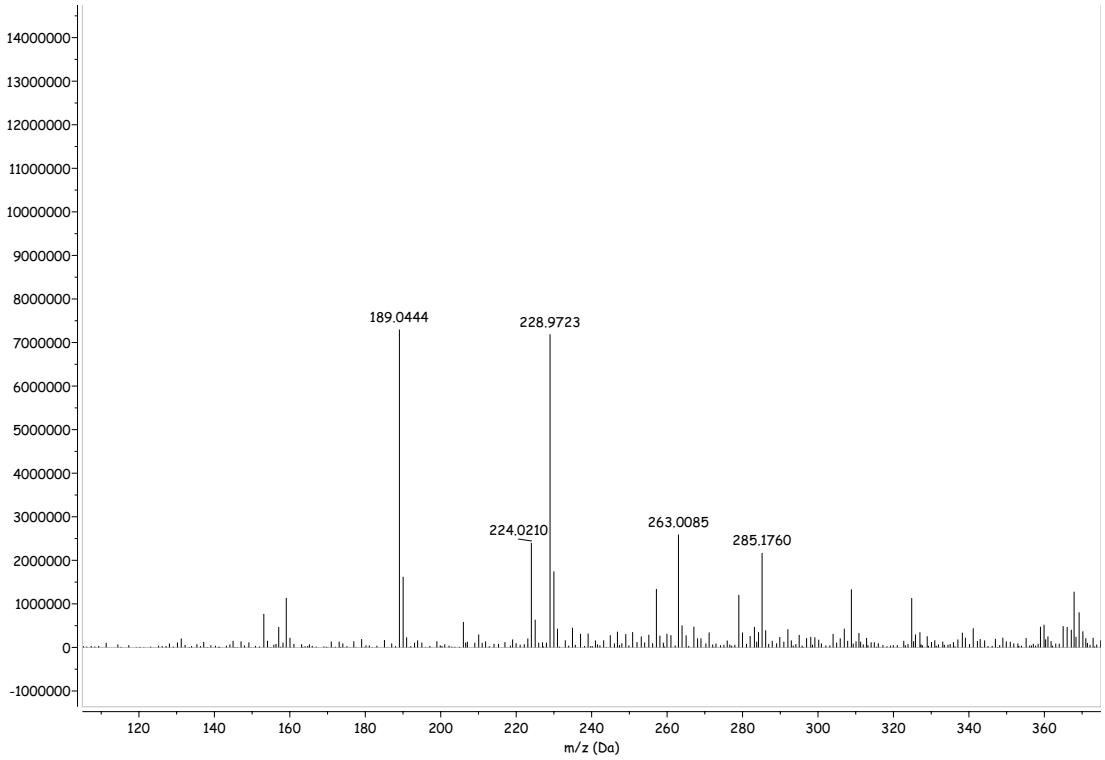
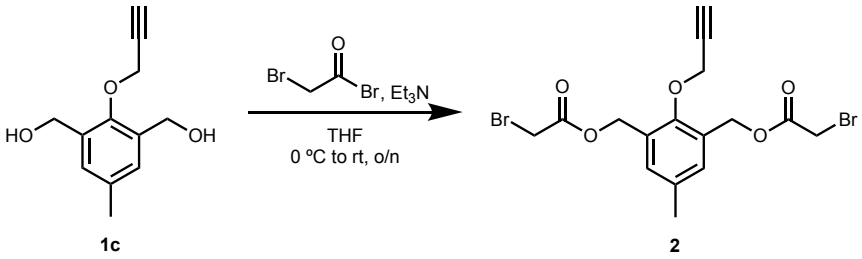


Figure S6. DEPT NMR spectrum of **1c** in CD_3OD.



HRMS (m/z)**:** Calcd. for $C_{12}H_{14}NaO_3$ $[M+Na]^+$: 229.0841. Found: 228.9723 $[M+Na]^+$.

2.1.4 Synthesis of 2



1c 2

Compound **1c** (50 mg, 0.243 mmol) and Et_3N (76 µL, 0.55 mmol) were dissolved in THF (1.2 mL) and cooled down to 0 °C. Bromoacetyl bromide (47 µL, 0.53 mmol) was added dropwise over the solution. The reaction mixture was stirred at rt overnight. The white precipitate was filtered off and the solution was diluted with CH_2Cl_2 and washed with Milli-Q water. The combined organic layers were dried with Na_2SO_4, filtered and the solvent was evaporated. The residue was purified by flash chromatography (10% AcOEt/hexanes) to give the desired product **2** (62 mg, 57% yield) as an orange oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (s, 2H), 5.31 (s, 4H), 4.68 (d, *J* = 2.4 Hz, 2H), 3.91 (s, 4H), 2.60 (t, *J* = 2.4 Hz, 1H), 2.36 (t, *J* = 0.7 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 167.0, 153.5, 135.1, 132.2, 128.8, 78.4, 76.4, 63.3, 63.1, 25.8, 20.8.

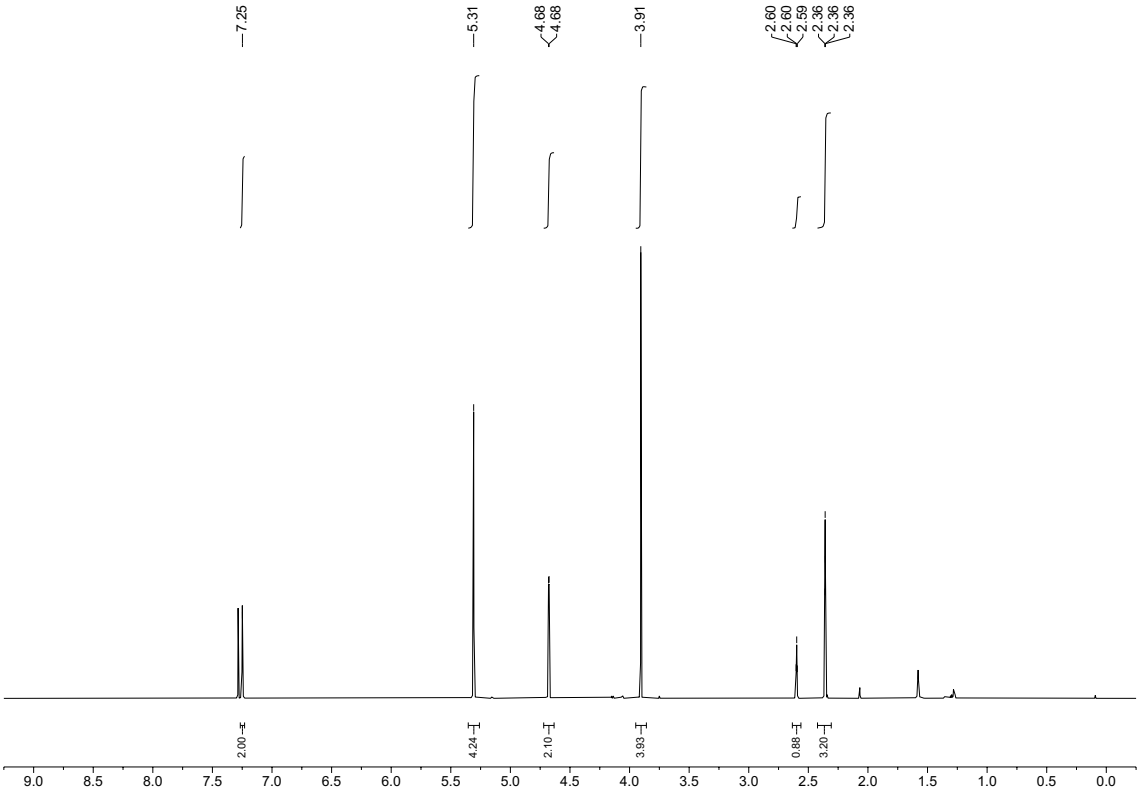


Figure S7. ^{1}H NMR spectrum of **2** in $CDCl_3$.

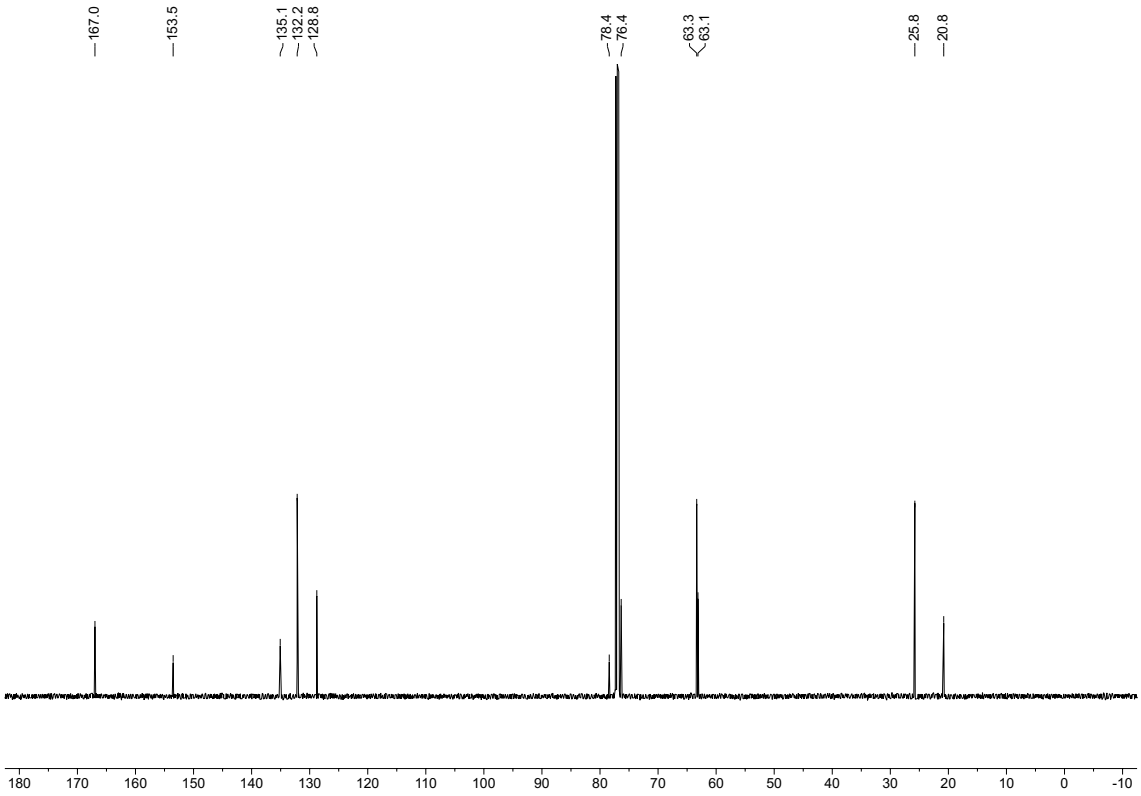


Figure S8. ^{13}C NMR spectrum of **2** in $CDCl_3$.

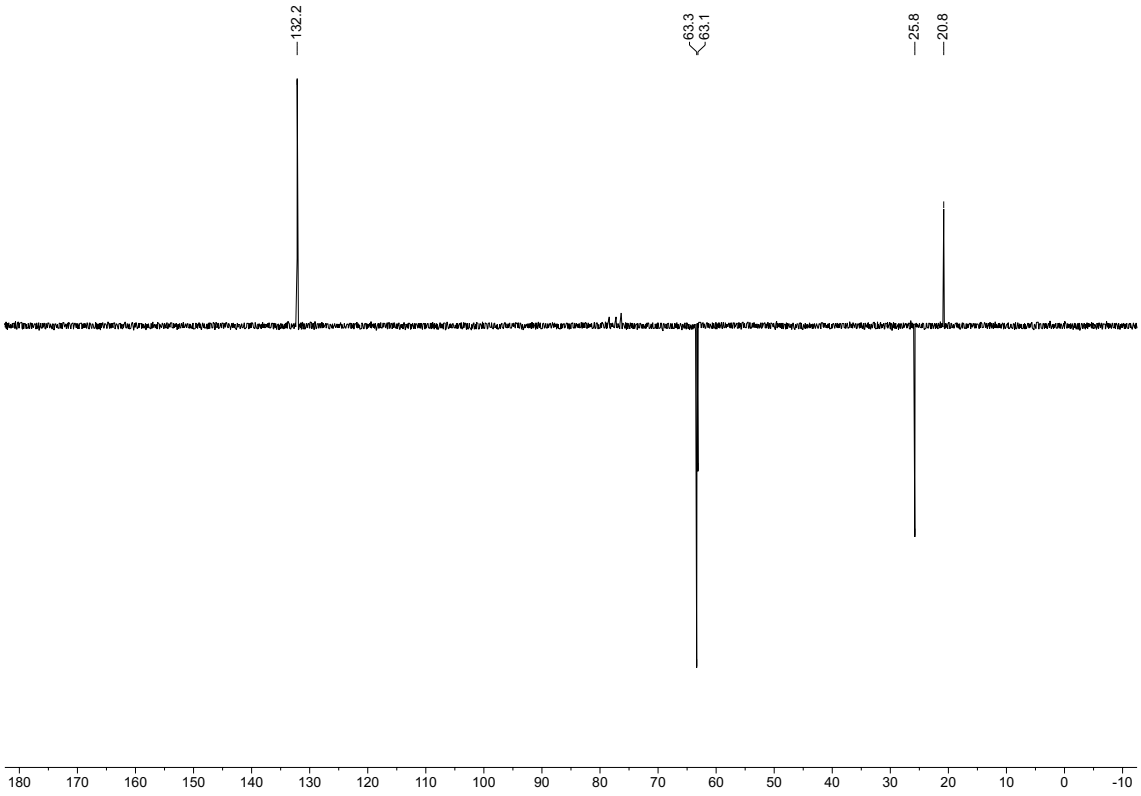
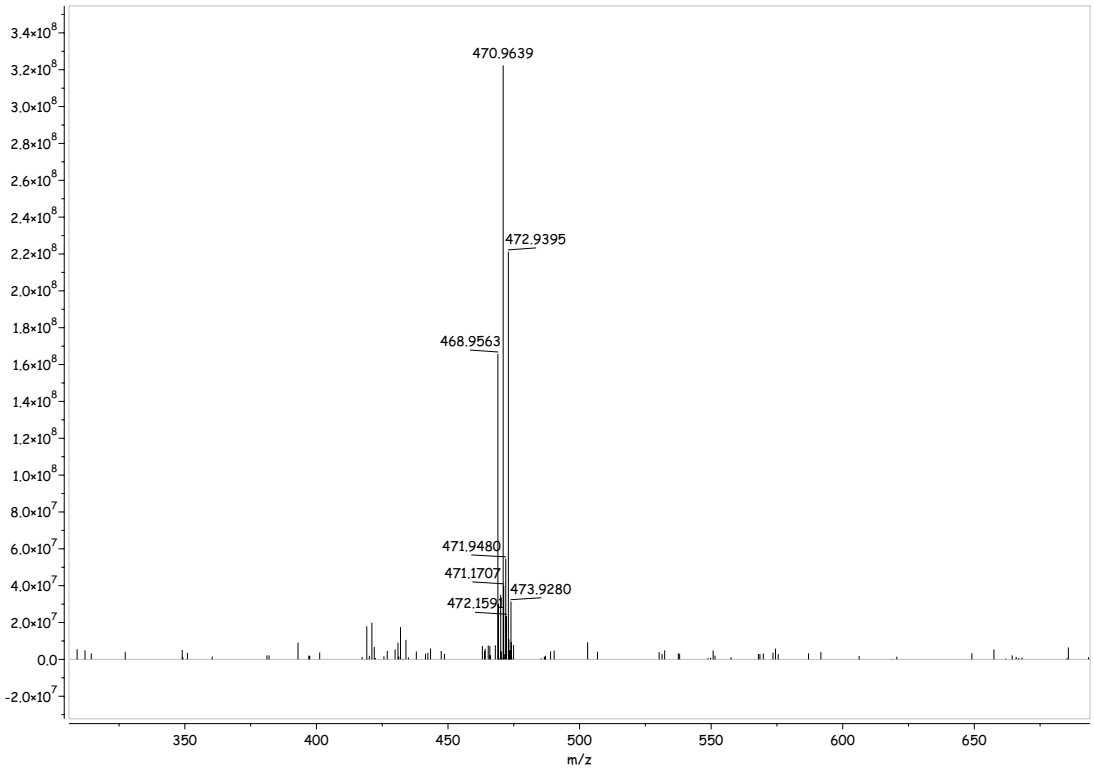


Figure S9. DEPT NMR spectrum of **2** in $CDCl_3$



HRMS (m/z)**:** Calcd. for $C_{16}H_{16}Br_2NaO_5$ $[M+Na]^+$: 468.9282. Found: 468.9563 $[M+Na]^+$.

2.2 Peptide Synthesis

General peptide synthesis procedures

All peptide synthesis reagents and amino acid derivatives were purchased from GL Biochem (Shanghai) and Novabiochem; amino acids were purchased as protected Fmoc amino acids with the standard side chain protecting scheme: Fmoc-Ala-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Glu(Ot-Bu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ile-OH, Fmoc-Thr(t-Bu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-His(Trt)-OH and Fmoc-Asp(Ot-Bu)-OH except for the orthogonally protected Fmoc-Lys(Alloc)-OH, which was purchased from Bachem. All other chemicals were purchased from Aldrich or Fluka. All solvents were dry and synthesis grade, unless specifically noted.

Peptides were synthesized using an automatic peptide synthesizer CEM Liberty Lite. Peptide synthesis was performed using standard Fmoc solid-phase method on a PAL–PEG–PS resin (0.19 mmol/g) with DIC as activator, Oxime as base and DMF as solvent. The deprotection of the temporal Fmoc protecting group was performed by treating the resin with 20% piperidine in DMF solution for 20 min. After the N-terminal aspartic acid, we coupled p-ABA chromophore. The cleavage/deprotection step was performed by treatment of the resin-bound peptide for 1.5–2h with the following cleavage cocktail: 940 µL TFA, 25 µL EDT, 25 µL H_2O and 10 µL TIS (1 mL of cocktail / 40 mg resin).

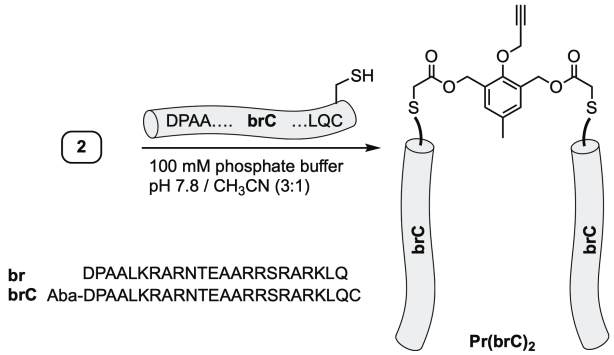
High-Performance Liquid Chromatography (HPLC) was performed using an Agilent 1100 series Liquid Chromatograph Mass Spectrometer system. Analytical HPLC was run using a Eclipse XDB-C18 analytical column (4.6 x 150 mm, 5 µm), 1 ml/min, gradient 5 to 75% B over 30 min. Purification of the peptides was performed on a semipreparative Phenomenex Luna–C18 (250 × 10 mm) reverse-phase column. The crude products were purified by RP−HPLC, 4 mL/min, gradient 10 to 50% B over 40 min. (A: H_2O 0.1% TFA, B: CH_3CN 0.1% TFA) and identified as the desired peptides.

Electrospray Ionization Mass Spectrometry (ESI/MS) was performed with an Agilent 1100 Series LC/MSD VL G1956A model in positive scan mode.

The synthesis of peptides **brC** (pABA-D-P-A-A-L-K-R-A-R-N-T-E-A-A-R-R-S-R-A-R-K-L-Q-C-$CONH_2$), **brGGC** (pABA-D-P-A-A-L-K-R-A-R-N-T-E-A-A-R-R-S-R-A-R-K-L-Q-C-$CONH_2$) and **$(brGGC)_2$** has been previously reported.[2]

[2] Mosquera, J.; Sanchez, M. I.; Vázquez, M. E.; Mascareñas, J. L. *Chem. Commun.* **2014**, *50*, 10975–10978.

2.2.1 Synthesis of dimeric peptide Pr(brC)$_2$



The monomeric peptide **brC** (1 mg, 2.7x10^{-4} mmol) was dissolved in desoxygenated phosphate buffer (75 µL, 100 mM, pH = 7.8). Dibromide **2** (1.3x10^{-4} mmol, 25 µL of a 5 mM solution in desoxygenated CH_3CN, 0.45 equiv) was added, and the solution was stirred at room temperature for 1.5 hours under argon atmosphere.

The resulting crude was purified by RP−HPLC, 4 mL/min, gradient 10 to 50% B over 40 min. (A: H_2O 0.1% TFA, B: CH_3CN 0.1% TFA, R_t = 18.1 min), and identified as product **Pr(brC)$_2$** by mass spectrometry. The covalent dimer was obtained as a white powder.

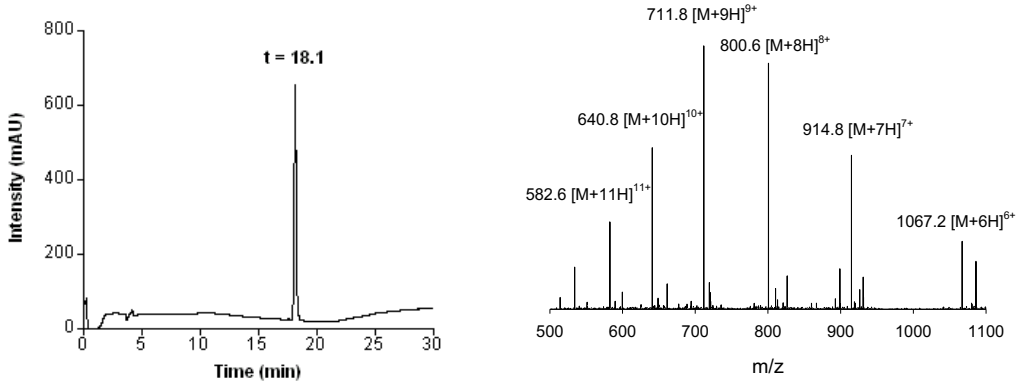
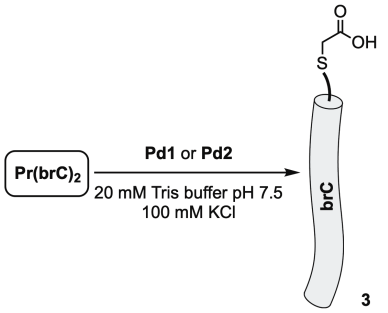


Figure S10. Left) HPLC chromatogram of the purified peptide **Pr(brC)$_2$**. Gradient 10 to 50% B over 40 min. Right) Mass spectrum of the peptide **Pr(brC)$_2$**. **EM–ESI$^+$ (m/z):** Calcd. for $C_{268}H_{454}N_{104}O_{75}S_2$ (**Pr(brC)$_2$**): 6397.35. Found: 1067.2 $[M+6H]^{6+}$; 914.8 $[M+7H]^{7+}$; 800.6 $[M+8H]^{8+}$; 711.8 $[M+9H]^{9+}$; 640.8 $[M+10H]^{10+}$; 582.6 $[M+11H]^{11+}$.

3 Palladium-triggered Pr(brC)$_2$ cleavage in the absence of DNA

Concentrations of the peptides were measured by UV at 20 °C in a Jasco V-630 spectrophotometer coupled to an ETC-717 Peltier, using a standard UV Hellma micro cuvette 108.002-QS (10 mm light path). Concentrations were obtained using the following molar extinction coefficient: 18,000 $M^{-1}cm^{-1}$ at 270 nm for the p-ABA (4-acetamidobenzoic acid) chromophore.



The dimeric peptide **Pr(brC)$_2$** (1x10^{-6} mmol) was dissolved in deoxygenated 20 mM Tris buffer 100 mM KCl (98.4 µL, pH 7.5). The corresponding palladium catalyst (1x10^{-5} mmol, 1.6 µL of a 6 mM solution in DMSO, 10 equiv) was added, and the solution was stirred at room temperature for 3 hours. The reaction was monitored by HPLC and the cleavage product identified by mass spectrometry.

3.1 HPLC monitoring

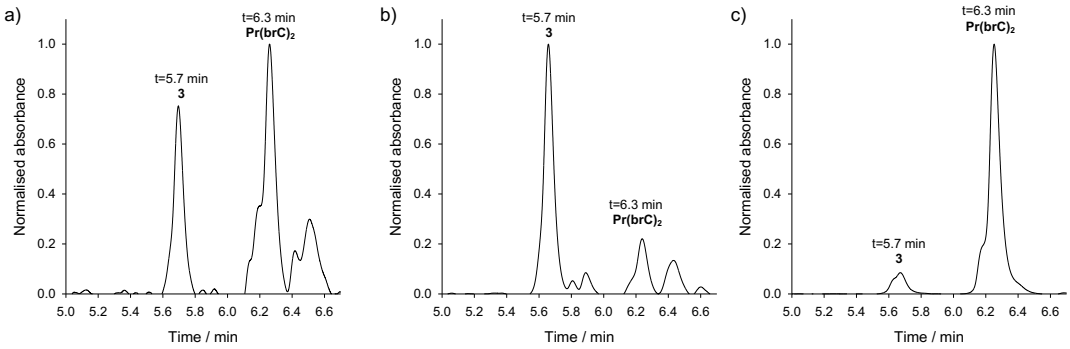


Figure S11. HPLC chromatograms of the Pd-mediated cleavage at different ratios of **Pr(brC)$_2$/Pd1**. Gradient 15 to 40% B over 12 min. a) 10 µM **Pr(brC)$_2$** and 10 µM **Pd1**; b) 10 µM **Pr(brC)$_2$** and 100 µM **Pd1**; c) 100 µM **Pr(brC)$_2$** and 10 µM **Pd1**. R_t = 6.3 min, starting **Pr(brC)$_2$**. R_t = 5.7 min, cleavage product **3**.

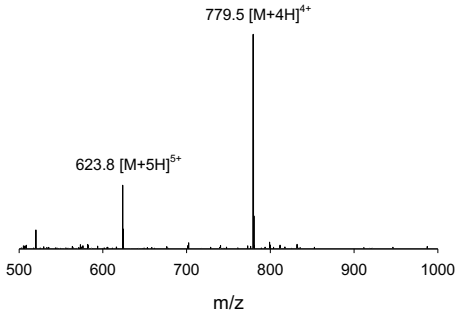


Figure S12. EM–ESI$^+$ (m/z): Calcd. for $C_{128}H_{222}N_{52}O_{37}S$ (**3**): 3111.68. Found: 779.5 $[M+4H]^{4+}$; 623.8 $[M+5H]^{5+}$.

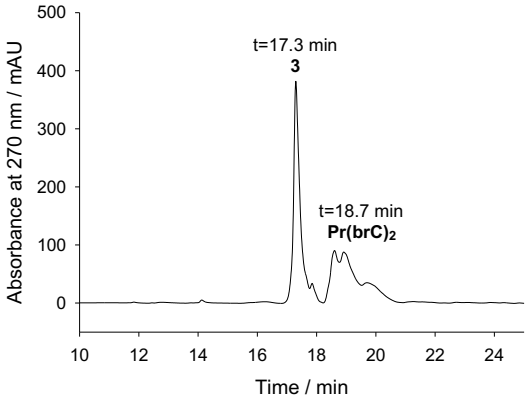


Figure S13. HPLC chromatograms of the Pd-mediated cleavage of **Pr(brC)$_2$** (10 μM) in the presence of 100 μM **Pd2**, after 3 h in 20 mM Tris buffer 100 mM KCl. Gradient 10 to 50% B over 40 min. R_t = 18.7 min, starting **Pr(brC)$_2$**. R_t = 17.3 min, cleavage product **3**.

4 DNA binding studies

Oligonucleotide sequences

Double stranded (only one strand is shown) oligonucleotides used for EMSA experiments were supplied by *Thermo Fischer* and the sequence was:

ATF/CREB 5'–CGG***ATGA***CG***TCAT***TTTTTTTC–3'
Random 5'-AGCGCACGTCCTGCACGTC-3'.

4.1 EMSA experiments

EMSAs were performed with a BioRad Mini Protean gel system, powered by an electrophoresis power supplies PowerPac Basic model, maximum power 150 V, frequency 50.60 Hz at 140 V (constant V). Binding reactions were performed over 30 min in 18 mM Tris-HCl (pH 7.5), 90 mM KCl, 1.8 mM $MgCl_2$, 1.8 mM EDTA, 9% glycerol, 0.11 mg/mL BSA, 2.2% NP-40. In the experiments we used 50 nM of the unlabeled ds oligonucleotides and a total incubation volume of 20 µL. After incubation for 30 min products were resolved by PAGE using a 10% non-denaturing polyacrylamide gel and 0.5x TBE buffer for 40 min at 20 °C, and analyzed by staining with SyBrGold (Molecular Probes: 5 µL in 50 mL of 1x TBE) for 10 min and visualized by fluorescence.

5x TBE buffer (0.445M Tris, 0.445 M Boric acid, 10 mM ETDA pH 8.0)

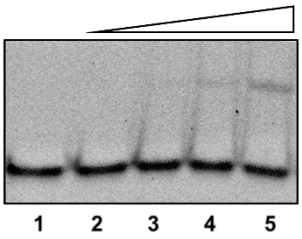


Figure S14. EMSA results for **Pr(brC)$_2$** with a non-specific DNA. Lanes 1–5: 50 nM random ds-DNA; lanes 2–5: 250, 500, 750 and 1000 nM **Pr(brC)$_2$**. Random ds-DNA (only one strand is shown): 5'-AGCGCACGTCCTGCACGTC-3'.

Clevage of Pr(brC)$_2$ in the presence of DNA: The target **ATF/CREB** DNA (500 nM) was was dissolved in desoxygenated 20 mM Tris buffer 100 mM KCl (98.4 µL, pH 7.5) and the peptide **Pr(brC)$_2$** ($5x10^{-7}$ mmol) was added. The mixture was incubated for 30 min at rt. Then, the corresponding palladium catalyst (1.6 µL of a 3 mM solution in DMSO) was added, and the solution was stirred at room temperature for 3 hours. Samples were diluted to a DNA concentration of 50 nM and analyzed by EMSA.

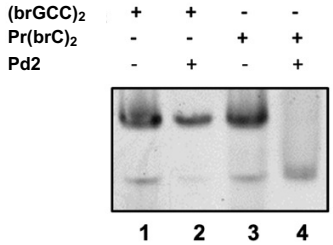


Figure S15. Palladium-promoted cleavage of **Pr(brC)$_2$** studied by EMSA. Lanes 1–4: 50 nM target **ATF/CREB**; lanes 1–2: 500 nM **(brGCC)$_2$**; lanes 3–4: 500 nM **Pr(brC)$_2$**. Concentration of **Pd2** is, when present, 5 µM.

4.2 Fluorescence Anisotropy

Measurements were made with a Jobin-Yvon Fluoromax-3, (DataMax 2.20) coupled to a Wavelength Electronics LFI−3751 temperature controller, using the following settings: integration time: 2.0 s; excitation slit width: 1.0 nm; emission slit width: 10.0 nm; excitation wavelength 490 nm; emission wavelength 520 nm.

FAM-ATF/CREB (FAM-5'–CGGATGACGTCATTTTTTTTC–3', one strand shown) (5 µL, 5 µM) was added to 995 µL of Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl, and the anisotropy was measured. Aliquots of a stock solution in water of the dimer **Pr(brC)$_2$** (12.5 µM) were successively added to this solution, and the anisotropic value was recorded after each addition.

Data were analysed by fitting a Hill equation:

$$F = F_0 + A\frac{[Pr(brC)_2]^n}{[Pr(brC)_2]^n + {K_d}^n}$$

Where F is the fluorescence anisotropy, F_0 the anisotropy of the free DNA, K_d the dissociation constant and n the Hill coefficient.

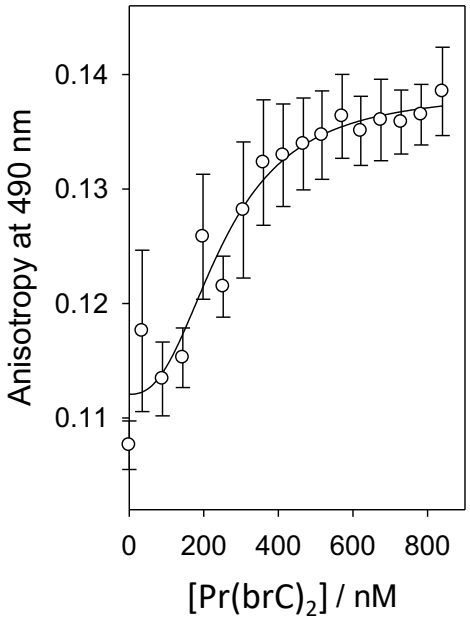


Figure S16. Fluorescence anisotropy data of **Pr(brC)$_2$** for the **FAM-ATF/CREB** dsDNA recorded at 490 nm against the total concentration of **Pr(brC)$_2$**. The solid line represents the fitting to the Hill equation, with an estimated K_D of 321 nM. Average of three replicates.

4.3 Circular Dichroism

Circular Dichroism experiments were performed in a *Jasco-715* coupled with a thermostat *Nestlab* RTE-111. The settings used were: Acquisition range: 320-195 nm; band width: 0.5 nm; resolution: 0.5 nm; accumulation: 5 scans; sensitivity 10 mdeg; response time: 0.5 s, speed: 100 nm/min. Measurements were made in a 2 mm cell at 20 °C. Samples contained 10 mM phosphate buffer pH 7.5 and 100 mM of NaCl, 5 µM peptide, 5 µM of corresponding dsDNA (when present) and 50 µM of corresponding palladium catalyst (when present).

The mixtures were incubated for 5 min before registering. The CD spectra of the peptides (when measured in the presence of DNA) were calculated as the difference between the spectrum of the peptide/DNA mixture and the measured spectrum of a sample of the DNA oligonucleotide. The spectra are the average of 5 scans and were processed using the "smooth" macro implemented in the program *Kaleidagraph* (v 3.5 by Synergy Software).