Supporting Information

Material and Apparatus

DNA Sequences

Experimental Section

NMR Spectrum
Material and Apparatus

Puromycin-2HCl was purchased from sigma. Polynucleotide kinase, T4 DNA ligase, T7 RNA polymerase, ATP and NTP were purchased from Thermo. [γ-32P]ATP was purchased from Perkin Elmer. DNA polymerase, M-MLV reverse transcriptase and dNTP were purchased from Beijing TransGen Biotech Co., Ltd.. All reactions that required anhydrous or oxygen-free conditions were carried out by standard procedures under nitrogen atmosphere. All reagents for the synthesis were commercially available. All modify reaction of puromycin were monitored by thin-layer chromatography(TLC). The oligonucleotides were synthesized by Sangon Biotech(Shanghai) Co., Ltd.. 1H NMR spectra were recorded on commercial instruments(300 MHz). All the molecular weight of the intermediate or end products were measured by the high resolution mass spectrometry(HRMS). The solvents were dried by distillation over the appropriate drying reagents. Puromycin-tethered oligonucleotides was purified by reverse phase HPLC. All the solutions were prepared with deionized and sterilized water. The PCR reaction was manipulated in a C1000 thermal cycler(Bio-Rad). Radioactive isotope γ-32P was detected by Cyclone Plus Phosphor Imager (Perkin Elmer).

DNA Sequences

Table 1S Oligonucleotides (presented 5’-3’)

<table>
<thead>
<tr>
<th>Name/Sequence</th>
</tr>
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<tbody>
<tr>
<td>16aa-Template-Flag: GAATTCTAATACGACTCACTATAGGAGGACGAAATGAGGACGATGACGATAAGCAGCTGCGTAA</td>
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<tr>
<td>CTCTAAAAAA</td>
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<tr>
<td>7aa-Template-His: GAATTCTAATACGACTCACTATAGGAGGACGAAATGAGGACGATGACGATAAGCAGCTGCGTAA</td>
</tr>
<tr>
<td>PCR-P: GAATTCTAATACGACTCACTATA</td>
</tr>
<tr>
<td>PCR-RP-Flag: CTTATCGTCATCGTCC</td>
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<tr>
<td>PCR-RP-Flag-16aa: TTTTTTTTAGAGTTACGCAGC</td>
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<td>PCR-RP-His: ATGATGATGGTGGTG</td>
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<td>PCR-RP-Full-length RNA: AAAAAAAAAAAAGAGTTACGCAGCTG</td>
</tr>
<tr>
<td>Tail: CAGCTGCGTTAACCTCTTTTTTTTTTTTTTTTTT</td>
</tr>
<tr>
<td>L1: ACGCCGCCAAMMMMMMMMMMMMMMMMMMMMMMMMMMMMMGAGTTACGCAGCTG</td>
</tr>
<tr>
<td>L2: AAAAAAAAAAAAGAGTTACGCAGCTG</td>
</tr>
<tr>
<td>Pu16: Puro-rCrC-2PEG-AAAAAAAAAAAAAAAAAAAAAAA</td>
</tr>
<tr>
<td>30P: AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACC-puro</td>
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<td>Flag16aa-30P Splint: TTTTTTTTTTTTTTTTTTAGAGT</td>
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<tr>
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<td>Flag-Tail Splint: ACGCAGCTGTTATCGTC</td>
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<tr>
<td>Flag16aa-Tail Splint: ACGCAGCTGTTTTTTTTAGA</td>
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<tr>
<td>Pu16-L1 Splint: TGCCGGCCGTTTTTTTTTTTT</td>
</tr>
</tbody>
</table>

Puromycin; RP: Reverse Primer; 30P: puromycin couple to the 3’-end of oligonucleotides was used for mRNA display.
Experimental Section

Synthesis

Synthesis of compound P1

Puromycin hydrochloride (100 mg, 0.184 mmol) was suspended in 2 mL acetonitrile and stirred at 0 °C. After triethylamine (0.06 mL) was added, the solution became clarify. N-(9-Fluorenylmethoxycarbonyloxy) succinimide (68.2 mg, 0.202 mmol) was dissolved in 1 mL acetonitrile then gradually added to the reaction. After stirred at 0 °C for another 30 min, white turbidity appeared. The reaction mixture was stirred at 0 °C for 2 h, then stirred at room temperature for 1 h. The white powder product P1 was obtained by the filtration. Yield: 93%.

Compound P1: White powder $^\text{1H-NMR(DMSO)} \delta$: 8.44(s, 1H), 8.23(s, 1H), 8.16(d, $J$=6.0 Hz, 1H), 7.88(d, $J$=9.0 Hz, 2H), 7.65-7.56(m, 2H), 7.42(m, 2H), 7.33-7.23(m, 4H), 6.82(d, $J$=8.2 Hz, 2H), 6.09(s, 1H), 5.99(s, 1H), 5.17(s, 1H), 4.49(s, 2H), 4.33(s, 1H), 4.17-4.11(m, 3H), 3.95(s, 1H), 3.68(m, 4H), 3.48-3.46(m, 5H), 3.32(s, 3H), 2.96(m, 1H), 2.92(m, 1H); $^\text{13C-NMR(DMSO)} \delta$: 172.42, 158.24, 156.19, 149.53, 144.26, 141.12, 138.93, 130.78, 130.31, 128.07, 127.50, 125.82, 120.53, 120.03, 113.91, 89.99, 83.92, 73.67, 66.15, 61.24, 56.79, 55.37, 50.69, 47.02; HRMS(ESI) $m/z$ calcld for C$_{37}$H$_{40}$N$_7$O$_7$[M+H]$^+$: 694.2984, found: 694.2986.

Synthesis of compound P2

Compound P1 (25 mg) was dissolved in 1 mL pyridine, and triethylamine (25uL) was added into the reaction solution, then stirred at 0 °C. 4, 4-Dimethoxytriphenylmethyl chloride (36.5 mg) was dissolved in 0.7 mL pyridine and injected into the reaction mixture. After stirring on ice for 30 min and room temperature for 2h, the product P2 was directly purified by flash column chromatography eluting with petroleum ether and ethyl acetate(1:5). Yield: 90%. Compound P2: pale yellow powder, $^\text{1H-NMR(DCM)} \delta$: 8.19(s, 1H), 8.01(s, 1H), 7.73-7.71(m, 2H), 7.70-7.48(m, 2H), 7.38-7.36(m, 4H), 7.32-7.17(m, 11H), 6.97(m, 2H), 6.79-6.72(m, 6H), 5.72(s, 1H), 5.49(s, 1H), 4.60(s, 1H), 4.52(m, 1H), 4.30(m, 3H), 4.15(m, 2H), 3.75(s, 6H), 3.70(s, 3H), 3.52-3.50(m, 4H), 3.46(m, 2H), 3.42(m, 1H), 2.95(m, 1H), 2.83(m, 1H), 2.36(m, 2H); $^\text{13C-NMR(DCM)} \delta$: 171.31, 158.68, 158.54, 154.87, 151.34, 148.68, 144.40, 143.77, 143.67, 141.26, 139.49, 137.37, 136.06, 135.70, 135.63, 130.27, 130.19, 130.10, 129.15, 128.23, 127.83, 127.69, 125.68, 125.01, 120.62, 119.95, 114.26, 114.18, 113.17, 91.24, 86.51, 83.70, 77.20, 74.54, 67.11, 63.36, 56.51, 55.23, 55.19, 55.16, 51.99, 47.10, 38.28;
HRMS(ESI) m/z calcd for C_{51}H_{61}N_{7}NaO_{14}[^{[M+Na]}^+] : 1018.4158, found: 1018.4160.

Synthesis of compound P3

Compound P2 (420 mg) was suspended in 6 mL pyridine, then 4-dimethylamiopryidine (10 mg) was added. After dissolved, the reaction solution was cooled on ice and follow by gradually adding acetic anhydride (0.6 mL). The reaction mixture was stirred at 0°C for 3 h. The product P3 was purified by flash column chromatography eluting with petroleum ether and ethyl acetate (1:1). Yield: 94%. Compound P3: White powder, \([\alpha]_{D}-17.7\) (c= 0.045, CHCl₃); ¹H-NMR (300 MHz, CDCl₃): δ 8.73(s, 1H), 8.64(s, 1H), 8.05(s, 1H), 7.75(d, J=9.0 Hz, 2H), 7.51(d, J=9.0 Hz, 2H), 7.44-7.26(m, 15H), 7.06(d, J=6.0 Hz, 2H), 6.83-6.75(m, 7H), 6.24-6.20(m, 1H), 5.8-5.77(m, 1H), 5.77-5.72(m, 1H), 5.11-5.02(m, 1H), 4.37-4.11(m, 5H), 3.80-3.66(m, 12H), 3.44-3.02(m, 2H), 2.96-3.12(m, 2H), 1.99(s, 3H); ¹³C NMR (75 MHz, CDCl₃): δ: 170.70, 169.21, 158.69, 158.51, 154.91, 152.59, 150.11, 144.38, 143.60, 141.26, 136.36, 135.60, 130.14, 128.25, 127.81, 127.76, 127.06, 126.83, 124.93, 124.87, 120.42, 119.99, 114.22, 113.14, 87.05, 86.53, 82.19, 74.95, 67.08, 63.10, 60.34, 56.33, 55.21, 55.15, 50.11, 47.04, 45.63, 38.50, 37.34, 29.65, 28.15, 14.15, 8.59; HRMS (ESI) m/z calcd for C_{60}H_{59}N_{7}NaO_{10}[^{[M+Na]}^+] : 1060.4240, found: 1060.4242.

Synthesis of compound P4

Compound P3 (380 mg) was dissolved in 5 mL anhydrous dichloromethane, and stirred at 0°C. Dichloroacetic acid (0.2 mL) was added and the reaction mixture was stirred at 0°C for 1 h, then stirred at room temperature for 30 min. The product was purified by flash column chromatography eluting with petroleum ether and ethyl acetate (1:1). Yield: 92%. Compound P4: White powder, [\alpha]_{D}-65.1 (c= 0.086, CHCl₃); ¹H-NMR (300 MHz, CDCl₃): δ 8.33(s, 1H), 7.84(s, 1H), 7.77-7.75(d, J=7.2 Hz, 2H), 7.53(d, J=7.2 Hz, 2H), 7.43-7.26(m, 4H), 7.14(s, 2H), 6.89(d, J=7.8 Hz, 2H), 6.18(m, 1H), 5.88(m, 1H), 5.65(m, 1H), 5.44(m, 1H), 4.82(m, 1H), 4.43-4.35(m, 3H), 4.21-4.12(m, 2H), 3.98(d, J=13.0 Hz, 1H), 3.78(s, 3H), 3.74-3.60(d, J=3.0 Hz, 1H), 3.54(m, 4H), 3.09-2.62(m, 4H), 2.00(s, 3H); ¹³C NMR (151 MHz, Py): δ: 174.04, 170.81, 159.88, 158.10, 156.02, 153.49, 151.10, 145.65, 145.39, 142.53, 138.85, 131.78, 128.96, 128.92, 128.38, 126.55, 124.70, 121.29, 115.20, 89.53, 85.71, 76.73, 69.10, 67.72, 65.33, 62.83, 58.50, 56.05, 51.46, 48.60, 39.99, 39.09, 31.59, 30.06, 24.98, 24.10, 21.43, 15.08, 12.02; HRMS (ESI) m/z calcd for C_{41}H_{40}N_{7}NaO_{8}[^{[M+Na]}^+] : 758.2935, found: 758.2937.

Synthesis of compound P5
Compound P4 (40 mg) was dissolved in 300 uL pyridine under nitrogen atmosphere, then stirred at 35 °C for 15 min and cooled to 0 °C. N,N'-diisopropylethylamine (DIPEA, 35 uL) and 2-cyanoethoxy N,N'-diisopropyl chlorophosphoramidate (25 uL) were added and stirred at 0 °C for 1 h, then stirred at 15 °C for another 1 h. The product was purified by base-deactivated column eluting with dichloromethane and ethyl acetate (1:3). Yield: 91%. Compound P5: White powder, \[\alpha\] D\textsubscript{20} -52.3 (c= 0.065, CHCl\textsubscript{3}); \textsuperscript{1}H-NMR(300MHz, CDCl\textsubscript{3}) δ: 8.31 (s, 1H), 8.06 (s, 1H), 7.98 (s, 1H), 7.77 (d, J=6 Hz, 2H), 7.54 (d, J=9 Hz, 2H), 7.42-7.37 (m, 2H), 7.32-7.30 (d, J=6 Hz, 2H), 7.15-7.14 (m, 2H), 6.89-6.86 (d, J=9 Hz, 2H), 6.17-6.13 (m, 1H), 6.09-6.08 (d, J=3 Hz, 1H), 5.68-5.63 (m, 1H), 5.30 (s, 1H), 5.12-5.01 (m, 1H), 4.44-4.34 (m, 3H), 4.21-4.02 (m, 2H), 3.91-3.78 (m, 5H), 3.62-3.52 (m, 6H), 3.03 (s, 2H), 2.68-2.64 (m, 2H), 2.04-2.01 (m, 1H), 1.64 (s, 3H), 1.26-1.13 (m, 12H); \textsuperscript{13}C NMR(75 MHz, CDCl\textsubscript{3}) δ: 171.09, 152.49, 143.62, 141.25, 136.45, 130.25, 128.24, 127.74, 127.05, 124.91, 119.98, 116.83, 114.21, 94.75, 82.84, 74.91, 67.11, 58.17, 58.10, 55.21, 49.99, 47.03, 45.33, 45.25, 43.21, 43.05, 31.87, 26.35, 24.66, 24.58, 24.49, 23.37, 22.94, 22.91, 22.86, 22.83, 20.47, 20.10, 20.00; \textsuperscript{31}P NMR(121 MHz, CDCl\textsubscript{3}) δ: 149.83; HRMS(ESI) m/z calcd for C\textsubscript{49}H\textsubscript{60}N\textsubscript{9}O\textsubscript{9}P\textsubscript{+}[M+H]+: 936.4151, found: 936.4153.

**Coupling reaction between compound P5 and oligonucleotides**

The coupling reaction between P5 (0.1M, in acetonitrile) and oligonucleotides 16 (HO-rCrC-Spacer18-AAAAAAAAAAAAAA, rC: dC-5′-CE Phosphoramidite) was carried out in the ABI 394 DNA/RNA synthesizer by using the standard synthesize protocols (Figure S1), then the coupling product puromycin-tethered oligonucleotides at the 5′-end was purified by reverse phase HPLC (Waters, C18, acetonitrile-0.1 M triethyl ammonium acetate (TEAA) buffer(pH 7.0) gradient: 5% for 0.5 min, then 5-40% in 20 min, flow rate 1.0 mL/min, UV=260 nm): t\textsubscript{R}=8.74 min. HRMS (ESI) m/z calcd for Pu16 [M]+ = 5856.87, found = 5856.87) (Figure S2, S3). DNA Linker L1 (ACGCCGCCAAAAAAAAAAAAAAAAAAAAAGAGTTACGCAGCTG), which included a template-hybridized region, was labeled with [r-\textsuperscript{32}P]ATP by using polynucleotide kinase (PNK, thermo) and ligated to Pu16 by using T4 DNA ligase (thermo). Ligation products Pu16-L1 will be used in the in vitro translation reaction to capture the nascent peptide in the following research.

![Figure S1](image-url) **Figure S1** Coupling reaction. The coupling reaction between P5 and oligonucleotides was carried out in the DNA synthesizer with standard synthesize protocols, then the coupling product Pu16 was purified by HPLC. rC: dC-5′-CE Phosphoramidite.
**In vitro transcription**

mRNAs were prepared by using the double strand DNA genomes from PCR (Polymerase Chain Reaction) and T7 RNA polymerase (Thermo) to transcribe by incubation at 37 °C for 4 h. mRNA products then were purified by 10% denaturing PAGE and used for translation.

**In vitro translation**

The DNA tail which will hybridized to the puromycin-included primer Pu16-L1 then were labeled with ATP by using polynucleotide kinase and ligated to the *in vitro* transcription products mRNA. Ligation products (1 uM) then will act as the template for translation and hybridized to Pu16-L1 (2 uM) by heating to 84 °C for 30 s and cooling down on the bench for 5 min. Translations were performed in Rabbit Reticulocyte Lysate (RRL; Promega) at 20 °C for 30 min in standard condition and on ice for 40 min. After that, KCl and MgCl₂ were added into the reaction to the final concentration of 500 mM and 50 mM respectively, then the reaction tubes were kepted at room temperature for 1 h and followed with long incubations at -20 °C for more then 10 h.

Full-length RNA included RBS (Ribosome Binding Site) and ORF (Open Reading Frame) was used as the template for peptide’s expression. Fusion between peptides and Pu16-L1 was formed initially (**Figure S4**). But there is less or no peptide-DNA fusion products appeared again when we repeat the translation experiments with total RNA template.
**Reverse transcription**
Reverse transcriptions were performed with M-MLV reverse transcriptase (Transgene) at 42°C for 30 min and the results were analysed by 10% denaturing PAGE after ethanol precipitation.

**Preliminary validation of DNA-peptide fusions**
To perform the protein degradation experiments, proteinase K (Thermo) was added into the translation solution and incubated at 37°C for 30 min. Digestion results were analysed by 10% denaturing PAGE after extraction with phenol chloroform.
NMR Spectrum

$^1$H-NMR

P1

P2
$^{13}$C-NMR

P1
$^{31}$P NMR

P5

Current Data Parameters

Name: 130Pn-H	
ZMFS: 1.307 ± 0.004
Burn: 1

P2 - Acquisition Parameters

Frequency: 205 MHz
(np: 125 MHz)

P1 - Processing parameters

Spin: 130
Spin 125 MHz

P0 - Depth parameters

Probes: 60 cm

P0 - Magnetic field parameters

Name: 130Pn-H	
ZMFS: 1.307 ± 0.004
Burn: 1

P2 - Acquisition parameters

Frequency: 205 MHz
(np: 125 MHz)

P1 - Processing parameters

Spin: 130
Spin 125 MHz

P0 - Depth parameters

Probes: 60 cm

P0 - Magnetic field parameters

Name: 130Pn-H	
ZMFS: 1.307 ± 0.004
Burn: 1