

Supporting Information

A Diazirine-Based Photoaffinity Probe for Facile and Efficient Aptamer-Protein Covalent Conjugation

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Experimental Section

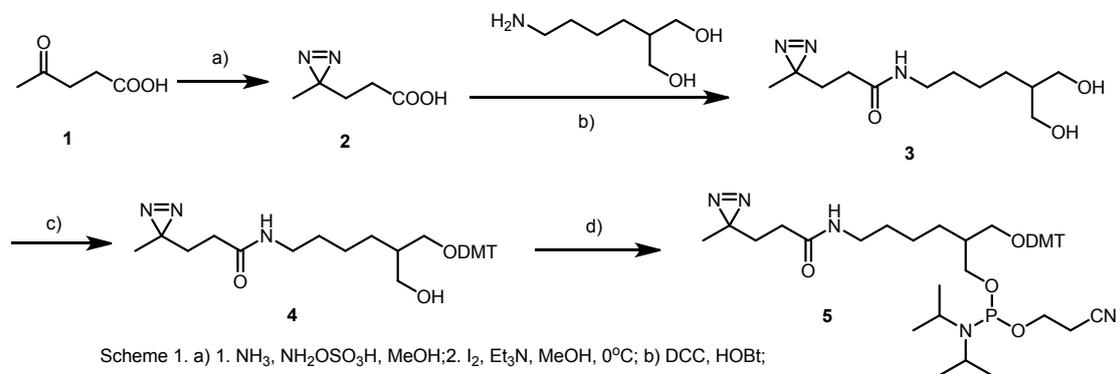
1. Materials.

All the DNA synthesis reagents, 4, 4'-dimethoxytrityl chloride, and N, N'-diisopropylethylamine were purchased from ChemGenes (Wilmington, MA, USA). 6-amino-2-hydroxymethylhexan-1-ol, dicyclohexylcarbodiimide, hydroxybenzotriazole, 4-dimethylaminopyridine and chemical solvents were purchased from J&K (Guangzhou, China).

¹H and ¹³C-NMR spectra were recorded on a Bruker AM 500 (¹H: 500 MHz, ³¹P: 202 MHz) at room temperature. Chemical shifts are expressed in parts per million (ppm) and the spectra calibrated to residual solvent signals of CDCl₃ (7.27 ppm (¹H)). Coupling constants are given in hertz (Hz) and the following notations indicate the multiplicity of the signals: s (singlet), d (doublet), brs (broad singlet), t (triplet), q (quartet), m (multiplet), ap. (apparent).

2. Synthesis of diazirine phosphoramidite

scheme 1



Compound 2:

Liquid NH₃ (500 mL) was added to 4-hydroxybutan-2-one (45 g, 511.0 mmol) at -78 °C, and the solution was stirred at reflux (-35 °C) 5h. A MeOH (400 mL) solution

of hydroxylamine-o-sulfonic acid (64 g, 566.0 mmol) was added portion-wise to the NH_3 solution and refluxed ($-35\text{ }^\circ\text{C}$) 1h. The NH_3 was allowed to slowly evaporate (overnight), and the slurry residue was filtered and washed with MeOH. The filtrate was evaporated and Et_3N (71.7 mL, 511.0 mmol) was added followed by I_2 (78.00 g, 307.0 mmol) at 0°C . When the red color of I_2 was persistent, the I_2 addition was stopped and the solution was concentrated in vacuum. Brine was added and the organic layer was extracted with ethyl acetate (EA). The EA layer was dried and concentrated in vacuum to obtain compound 2. ^1H NMR (500 MHz, CDCl_3): δ 2.24 (t, 3H), 1.73 (t, H), 1.04 (s, 1H).

Compound 3:

Compound 2 (105.3 mg, 0.82 mmol), 6-amino-2-hydroxymethylhexan-1-ol (132 mg, 0.902 mmol), DCC (203 mg, 0.984 mmol) and HOBt (133 mg, 0.984 mmol) were suspended in DMF and stirred at room temperature overnight. The solution was purified using column chromatography (Si_2O) using (EA/MeOH= 15:1) as eluent to yield compound 3 as a white solid (126 mg, 60%). ^1H NMR (500 MHz, CDCl_3): δ 3.73(m, 2H), 3.61(m, 2H), 3.19(m, 2H), 1.98(t, 2H), 1.72(t, 2H), 1.66(m, 1H), 1.45(m, 2H), 1.30-1.20(m, 4H), 0.99(s, 3H). ESI-MS calculated for $\text{C}_{12}\text{H}_{23}\text{N}_3\text{O}_3\text{Na}$: 280.33 ($[\text{M}+\text{Na}]^+$), found: 280.2.

Compound 4:

Compound 3 (424.4 mg, 1.65 mmol) and 4-dimethylaminopyridine (20 mg, 0.165 mmol) were dissolved in 10 mL dry pyridine in a 100 mL round-bottom flask and the solution was kept under dry nitrogen (using a balloon full of dry nitrogen gas) on an ice bath (approximately $0\text{--}5^\circ\text{C}$). DMT-Cl (560 mg, 1.65 mmol) was dissolved in 4 mL dry CH_2Cl_2 in a 50 mL round-bottom flask under nitrogen and added to the above pyridine solution slowly under dry nitrogen in an ice bath with stirring. The reaction flask was removed from the ice bath and stirred at room temperature for 24 h. The solvent (pyridine and CH_2Cl_2) was removed using a rotary evaporator to obtain a crude oily product. Water was added and the organic layer was extracted with EA. The EA layer was dried with Na_2SO_4 and concentrated in vacuo. The solution was purified using column chromatography (Si_2O) using (EA) as eluent to give compound 4 as a colorless oil (512.4 mg, 55.6%). ^1H NMR (500 MHz, CDCl_3): δ 7.41-6.82(m, 13H), 3.79(s, 6H), 3.67-3.61(m, 2H), 3.27-3.08(m, 4H), 2.40(s, 1H), 1.93(m, 2H), 1.75(m, 2H), 1.59(s, 4H), 1.44(m, 2H), 1.02(s, 3H). ESI-MS calculated for $\text{C}_{33}\text{H}_{41}\text{N}_3\text{O}_5\text{Na}$: 582.7 ($[\text{M}+\text{Na}]^+$), found: 582.3.

Compound 5:

To a solution containing compound 4 (181.2 mg, 0.324 mmol) in anhydrous CH_2Cl_2 (5 mL) at $0\text{ }^\circ\text{C}$, N, N'-Diisopropylethylamine (DIPEA) (0.155 mL, 0.907 mmol) was added in 3 minutes (slowly) under N_2 atmosphere. Then, 2-cyanoethyl diisopropyl chlorophosphoramidite (0.072 mL, 0.324 mmol) was added drop-wise under N_2 atmosphere, and the reaction mixture was stirred at $0\text{ }^\circ\text{C}$ for 2 h. The solvent was evaporated, and the residue was purified by column chromatography (Si_2O)

(EA/PE/triethylamine 1:1:0.01) and dried to afford the title compound as a colorless oil (159.8 mg, 65%). ¹H NMR (500 MHz, CDCl₃): δ 7.41-6.82(m, 13H), 3.79(s, 6H), 3.76-3.5(m, 6H), 3.27-3.08(m, 4H), 2.6-2.5(m, 4H), 1.93(m, 2H), 1.85(m, 1H), 1.72(m, 2H), 1.4(s, 4H), 1.2(m, 2H), 1.15(d, 6H), 1.12(d, 6H), 1.02(s, 3H). ³¹P NMR (202MHz, CDCl₃): δ 147ppm. ESI-MS calculated for C₄₂H₅₈N₅O₆PNa: 782.9 ([M+Na]⁺), found: 782.5.

3. DNA Synthesis

All the oligonucleotides used in this work were synthesized in house. The DNA product was synthesized on a 12-Column DNA Synthesizer (PolyGen GmbH). The products were cleaved from the solid support, deprotected with ammonia treatment, and purified by HPLC. The purified probe was quantified by determining the UV absorption at 260 nm, after which the probe was dissolved in DI water and stored at -20°C for future experiments.

4. General procedure for the labelling reaction

All the labelling reactions were performed on ice in labelling buffer(1x PBS buffer, pH=7.4). The samples were incubated in the dark for 10 min at 37°C and then irradiated for 10 min with UV light at 365 nm (5UV Lamp, bulb power 8 W, UVP crosslinker).

Table S1 Aptamers with diazirine modification.

SA aptamer	5'-FITC-ATT GAC CGC TGT GTG ACG CAA CAC TCA AT-3'
SA-1-drz	5'- X ATT GAC CGC TGT GTG ACG CAA CAC TCA AT TTT- FITC -TTT-3'
SA-15-drz	5'- FITC -ATT GAC CGC TGT GT X G ACG CAA CAC TCA AT-3'
SA-6-drz	5'- FITC -ATT G X C CGC TGT GTG ACG CAA CAC TCA AT-3'
TBA	5'- FITC -AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-3'
TBA-1-drz	5'- X AGT CCG TGG TAG GGC AGG TTG GGG TGA CTT FITC -TTTTT-3'
TBA-11-drz	5'- FITC -AGT CCG TGG T X AG GGC AGG TTG GGG TGA CT-3'
TBA-19-drz	5'- FITC -AGT CCG TGG TAG GGC AGG X TTG GGG TGA CT-3'
TBA-25-drz	5'- FITC -AGT CCG TGG TAG GGC AGG TTG GGG x-TGA CT-3'

X: diazidite

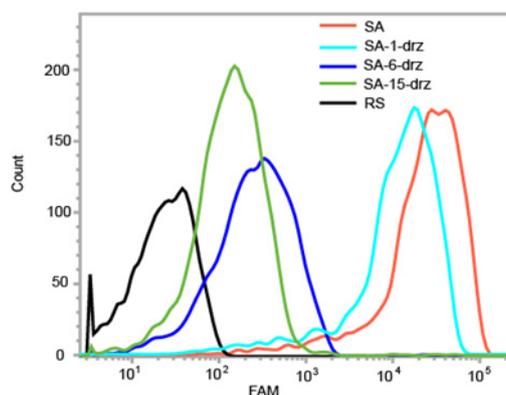


Figure S1. Binding affinity of diazirine-modified SA aptamers against target protein SA beads.

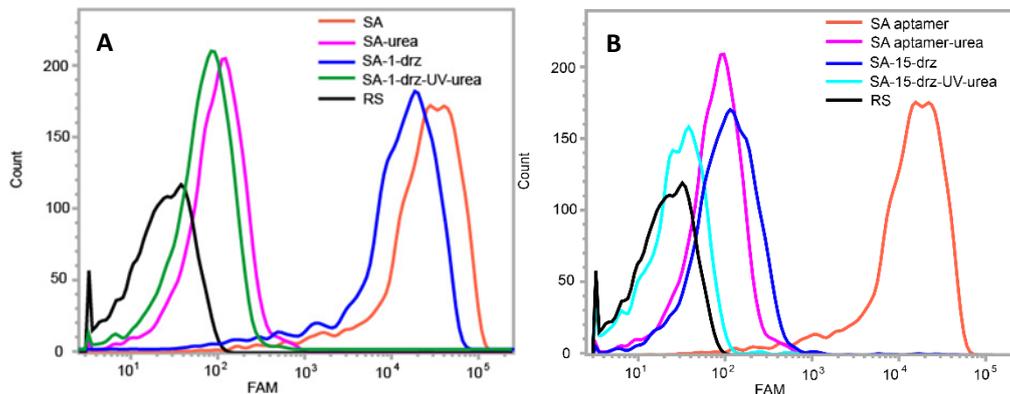


Figure S2. The covalent binding of modified aptamer SA-1-drz (A) and SA-15-drz (B) with SA-beads was monitored by flow cytometry.

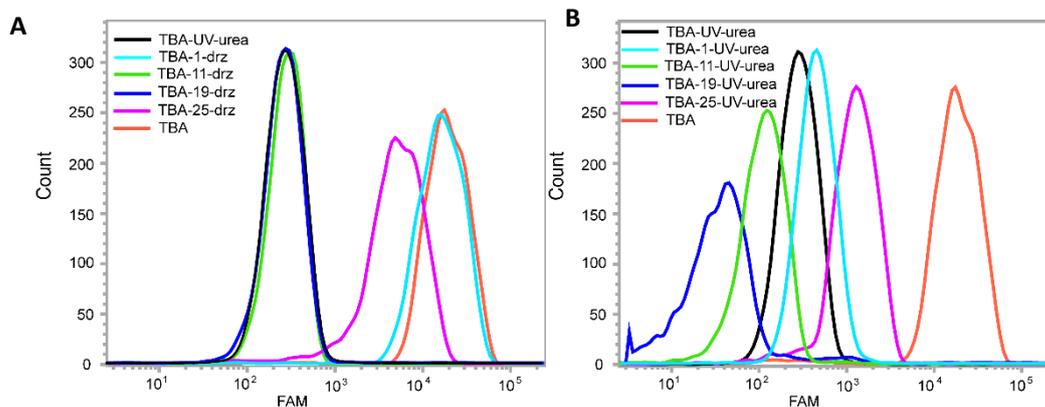
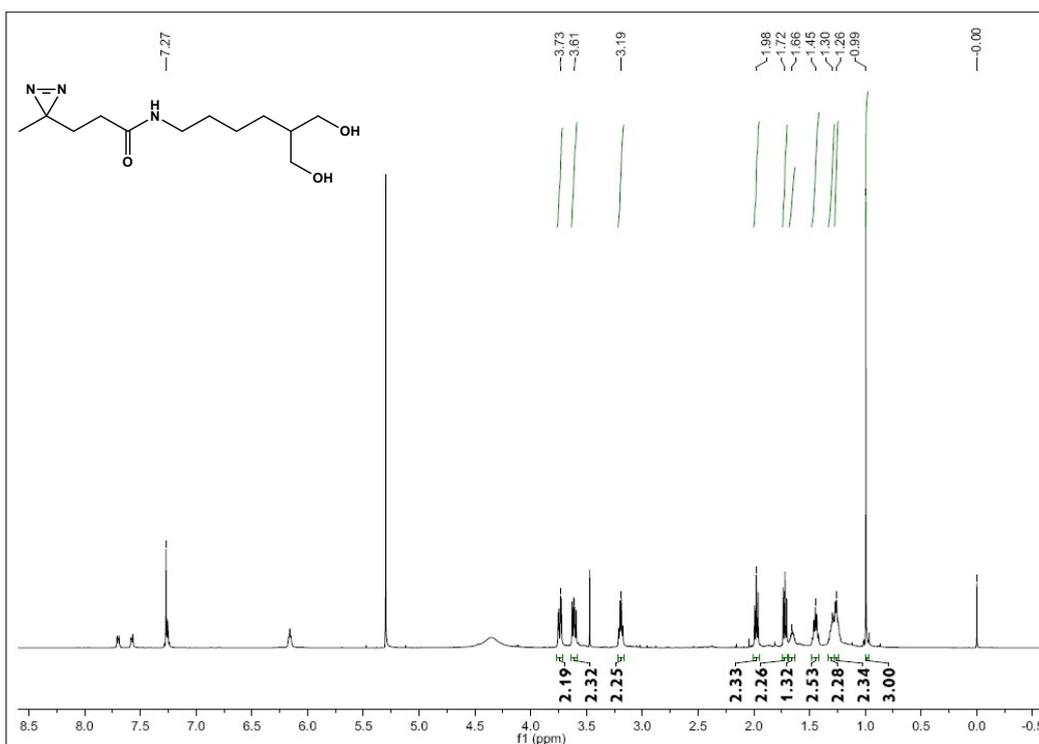
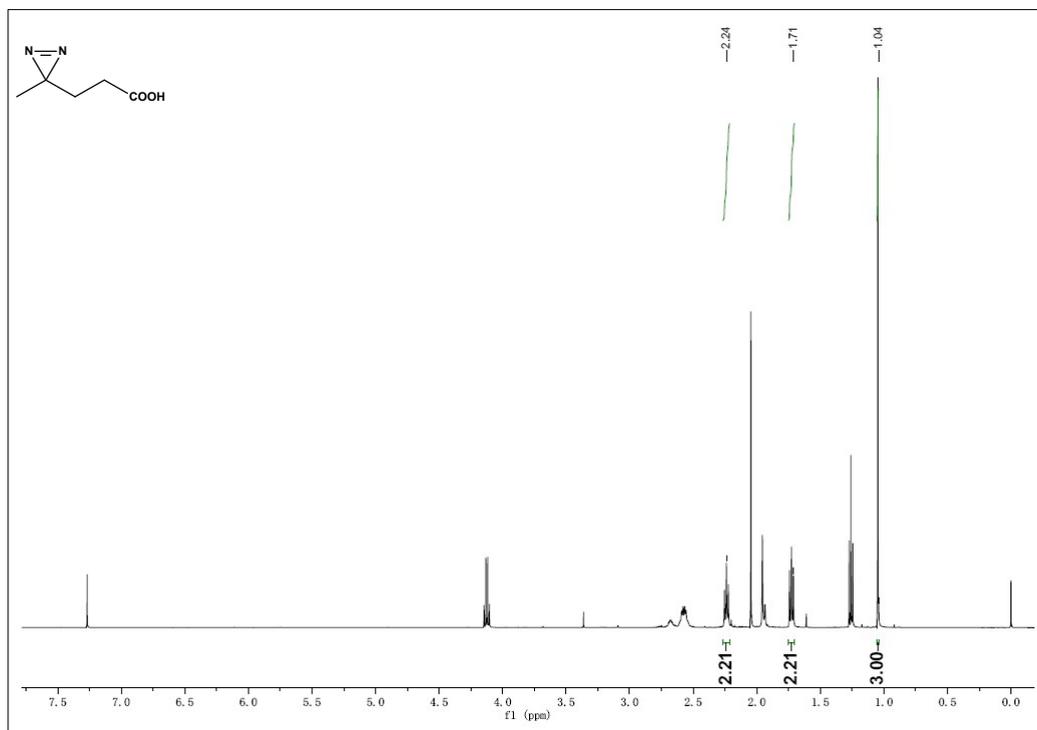
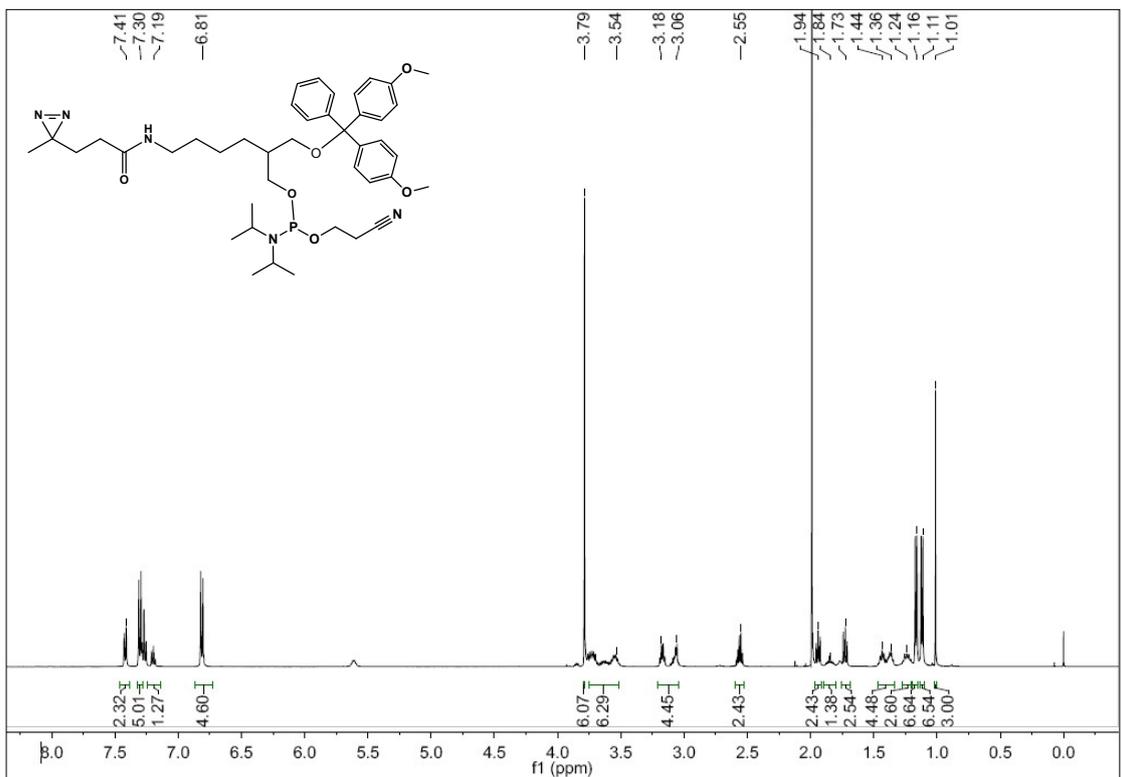
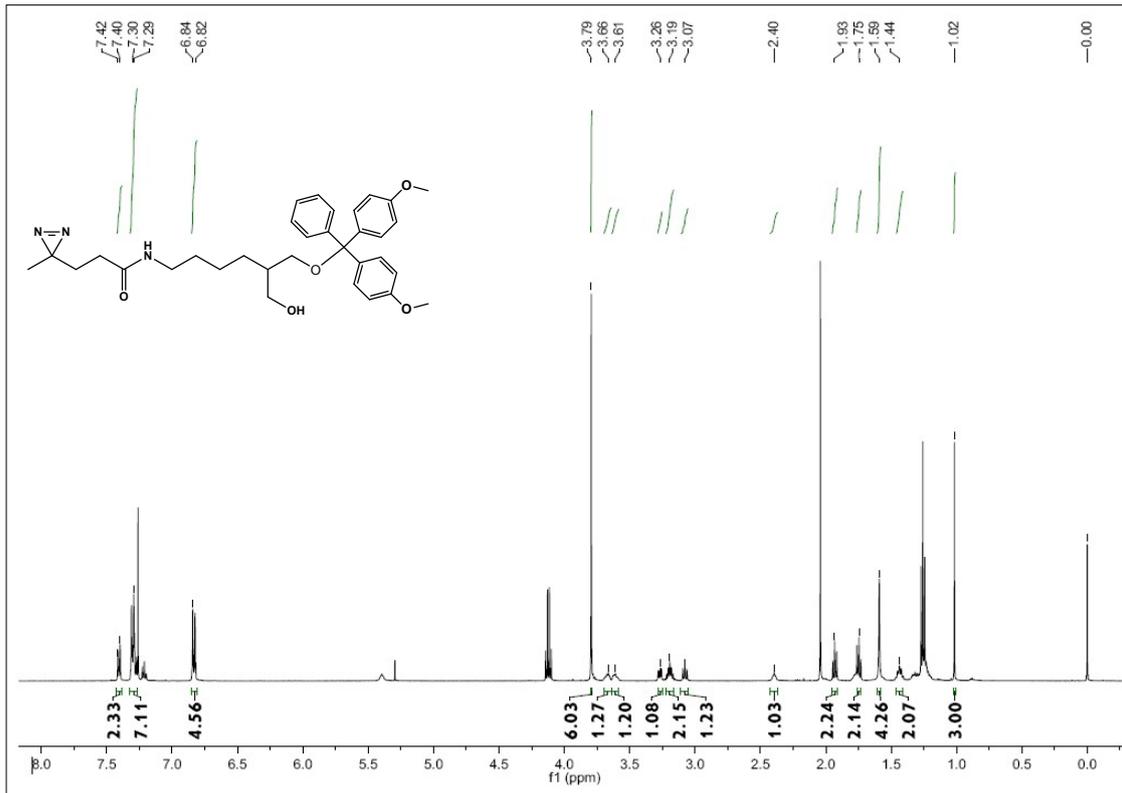


Figure S3. (A) The affinity of modified thrombin aptamers with target monitored by flow cytometry. (B) The covalent binding was monitored by flow cytometry using FITC-labeled aptamer and thrombin

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