Supplementary

Breaking the conservation of the guanine residues in the catalytic loop of 10-23 DNAzyme by position-specific nucleobase modifications for rate enhancement

Yang Liu, Zhiwen Li, Gaofeng Liu, Qi Wang, Wei Chen, Di Zhang, Maosheng Cheng, Zhibing Zheng, Keliang Liu, Junlin He

Table of contents

Experimental section 2

Oligodeoxyribonucleotide synthesis 7

T_m measurement 9

Table S1 Characterization of 10-23 DNAzyme and its analogs by MALDI-TOF MS 9

CD measurement 9

Fig. S1 CD spectrum of DNAzyme-substrate complexes 9

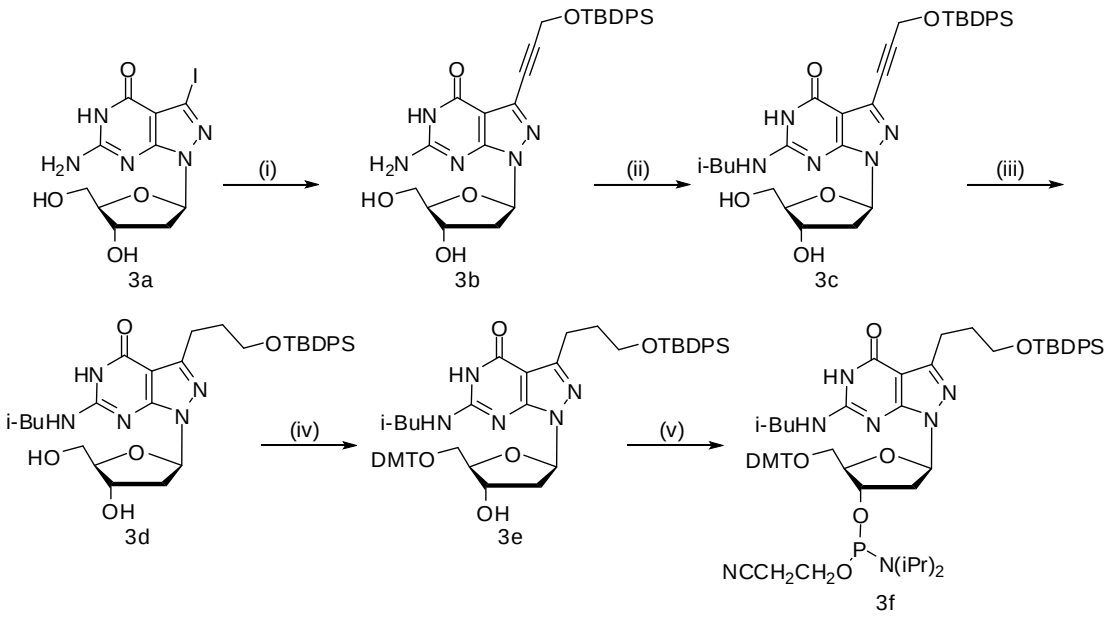
Radio-labeling of the chimeric substrates 10

The cleavage reaction under single-turnover and multiple-turnover conditions 11

Experimental section

MATERIALS AND METHODS

General Magnetic resonance spectroscopy (^{1}H, ^{13}C and ^{31}P NMR) were recorded on a JNM-ECA-400 spectrometer (JEOL, Japan), internal trimethylsilane (TMS) (^{1}H, ^{13}C) or external 85% H_3PO_4 (^{31}P) was used as the standard. Coupling constants (*J*) are given in Hertz (Hz). The reactions were monitored with thin layer chromatography (TLC) run on HS GF_{254} (Yantai Institute of Chemical Industry, China), and products were separated with flash column chromatography on silica gel (200-300 mesh, Qingdaohaiyang Chemicals Co., China). Elemental analyses on a Fisons-1108 (Fisons, Italy) and HR-MS on Q-FT-MS (Apex Qe, Brucker) were performed by the National Center of Biomedical Analysis (Beijing, China). Most of the commercially available chemicals were used as purchased without further purification. Pyridine was dried by refluxing with CaH_2.



(i) 3-*tert*-Butyldiphenylsiloxypropyne, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, in DMF, at r.t.; (ii) isobutyric anhydride, in pyridine, at r.t.; (iii) H_2, Pd/C, 5 atm, at 30 °C; (iv) DMTCl, in pyridine, at r.t.; (v) $(NCCH_2CH_2O)[(iPr)_2N]_2P$, $(iPr)_2EtN$ tetrazolium, in CH_2Cl_2, at r.t.

6-amino-1-[2-Deoxy-β-D-erythro-pentofuranosyl]-1,5-dihydro-3-(3-tert-butyldiphenylsilyloxy prop-1-ynyl)-4H-pyrazolo[3,4-d]-pyrimidin-4-one (**3b**)

To the solution of 7-iodo-8-aza-7-deaza-deoxyguanosine (**3a**) (1.5 g, 4 mmol) in DMF (20 ml) were added $Pd(PPh_3)_2Cl_2$ (0.48 g, 0.7 mmol), CuI (0.24 g, 1.3 mmol). The mixture was stirred at r.t. Tert-butyldiphenylsiloxypropyne (1.6 g, 5.4 mmol) and triethylamine (2 ml) were added in this order. After stirring at 40 ºC for 3 h, the mixture was evaporated in vacuum, and the residue was subjected to flash chromatography to obtain the product as colorless product (1.7 g, 81%). R_f (CH_2Cl_2/CH_3OH 9:1) 0.5. ^{1}H NMR (DMSO-d_6): δ 1.02 (s, 9 H, tBu), 2.19 (m, 1 H, C2'-H_α), 2.68 (m, 1 H, C2'-H_β), 3.39, 3.51 (2 m, 2 H, C5'-H), 3.79 (m, 1 H, C4'-H), 4.39 (m, 1 H, C3'-H), 4.62 (s, 2 H, CH_2), 4.77 (t, 1 H, J = 5.8, C5'-OH), 5.28 (d, 1 H, J = 4.2, C3'-OH), 6.32 (t, 1 H, J = 6.4, C1'-H), 6.80 (br, 2 H, 6-NH_2), 7.48, 7.73 (2 m, 10 H, arom. H), 10.82 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ 18.8, 26.5, 37.7, 52.8, 62.4, 70.9, 77.3, 83.2, 87.6, 90.1, 100.2, 128.0, 129.4, 130.0, 132.3, 135.1, 155.2, 155.5, 156.9. Anal. Calcd for $C_{29}H_{33}N_5O_5Si$ (M 559.69): C, 62.23; H, 5.94; N, 12.51. Found: C, 62.15; H, 5.86; N, 12.49.

6-amino-1-[2-Deoxy-β-D-erythro-pentofuranosyl]-1,5-dihydro-3-(3-tert-butyldiphenylsilyloxy prop-1-yl)-4H-pyrazolo[3,4-d]-pyrimidin-4-one (**3c**)

To the solution of compound **3b** (1.6 g, 2.86 mmol) in dried pyridine (30 ml) was added trimethylchlorosilane (7.5 ml, 80 mmol). After stirring for 15 min, isobutyric anhydride (2.1 ml, 12.3 mmol) was added. The reaction mixture was stirred for 3 h. It was then cooled in an ice bath and water (7.5 ml) was added. After 5 min, conc.aq. ammonia (1.5 ml) was added. The solution was concentrated for flash chromatography to give the product as colorless solid (1.3 g, 72%). R_f (CH_2Cl_2/CH_3OH 15:1) 0.5. ^{1}H NMR (DMSO-d_6): δ 1.02 (s, 9 H, tBu), 1.12, 1.14 [2 d, J = 1.1, $CH(CH_3)_2$], 2.25 (m, 1 H, C2'-H_α), 2.76 (m, 2 H, C2'-H_β, $CH(CH_3)_2$], 3.37, 3.49 (2 m, 2 H, C5'-H), 3.80 (m, 1 H, C4'-H), 4.42 (m, 1 H, C3'-H), 4.64 (s, 2 H, CH_2), 4.72 (m, 1 H, C5'-OH), 5.28 (d, 1 H, J = 3.9, 3'OH), 6.40 (t, 1 H, J = 6.3, C1'-H), 7.47,7.72 (2 m, 10 H, arom. H), 11.80 (s, 1 H, NH), 11.97 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ 18.7, 18.8, 18.9, 26.5, 33.1, 34.9, 37.8, 52.7, 62.2, 70.8, 76.6, 83.7, 87.8, 91.1, 103.2, 128.0, 129.6, 130.0, 132.3, 135.1, 150.5, 152.9, 155.0, 180.6. Anal. Calcd for $C_{33}H_{39}N_5O_6Si$ H_2O (M 647.79): C, 61.19; H, 6.38; N, 10.81. Found: C, 60.93; H, 6.14; N, 10.95.

1-[2-Deoxy-β-D-erythro-pentofuranosyl]-1,5-dihydro-6-[(2-methylpropanoyl)amino]-3-(3-ter

t-butyldiphenylsilyloxyprop-1-yl)-4H-pyrazolo[3,4-d]-pyrimidin-4-one (**3d**)

The solution of compound **3c** (1.26 g, 2 mmol) in THF (100 ml) containing Pd/C (0.126 g) was sealed under hydrogen atmosphere in a stainless steel oven (5 kg). The reaction was maintained at 30 ºC for 5 h. The catalyst was filtered off, and the filtrate was concentrated for flash chromatography to obtain the product as colorless solid (1.18 g, 93%). R_f (CH_2Cl_2/CH_3OH 15:1) 0.52. ^{1}H NMR (DMSO-d_6): δ 0.98 (s, 9 H, tBu), 1.12, 1.13 [m, 6 H, CH(CH_3)$_2$], 1.98 (m, 2 H, 7-$CH_2CH_2CH_2$), 2.16 (m, 1 H, C2'-H_α), 2.68 [m, 1 H, CH(CH_3)$_2$], 2.76 (m, 1 H, C2'-H_β), 2.88 (m, 2 H, 7-C$H_2$$CH_2CH_2$), 3.46 (2 m, 2 H, 7-$CH_2CH_2CH_2$), 3.69-3.80 (m, 3 H, C5'-H, C4'-H), 4.38 (m, 1 H, C3'-H), 4.73 (br, 1 H, C5'-OH), 5.24 (br,, 1 H, C3'-OH), 6.34 (t, 1 H, J = 6.4, C1'-H), 7.42, 7.60 (2 m, 10 H, arom. H), 11.73 (s, 1 H, NH), 11.84 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ 18.8, 24.3, 26.6, 30.5, 34.8, 37.8, 62.4, 62.9, 71.0, 83.2, 87.6, 100.6, 127.5, 127.8, 129.7, 133.3, 134.5, 135.0, 149.3, 150.0, 153.4, 156.2, 180.4. Anal. Calcd for $C_{33}H_{43}N_5O_6Si$ (M 633.81): C, 62.54; H, 6.84; N, 11.05. Found: C, 62.59; H, 6.84; N, 10.76.

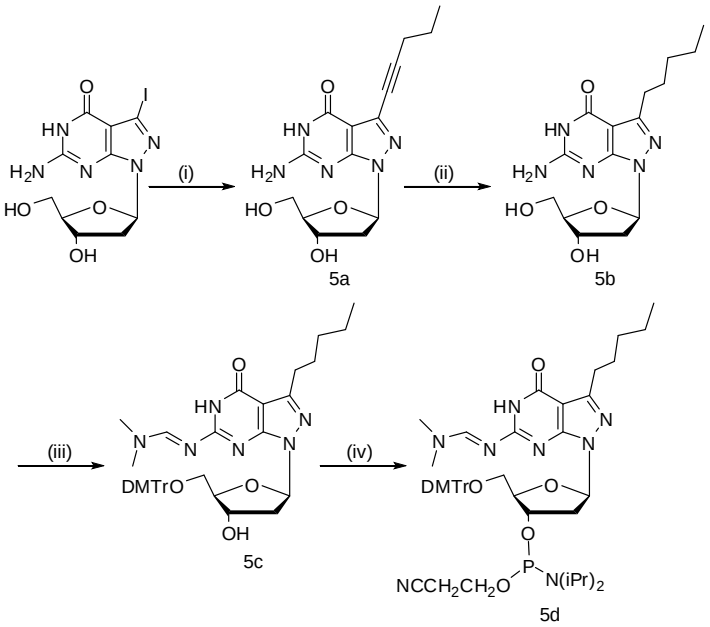
1-[2-Deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl]-1,5-dihydro-6-[(2-methylpropanoyl)amino]-3-(3-tert-butyldiphenylsilyloxyprop-1-yl)-4H-pyrazolo[3,4-d]-pyrimidin-4-one (**3e**)

Compound **3d** (1 g 1.58 mmol) was dissolved in dried pyridine (1.5 ml) after it was co-evaporated with dried pyridine three times. To the solution, DMT-Cl (0.76 g, 2.24 mmol) was added in portions. Methanol (10 ml) was added to stop the reaction, and the solution was concentrated for flash chromatography, the product was obtained as colorless solid (1 g, 67.6%). R_f (CH_2Cl_2/CH_3OH 30:1) 0.70. ^{1}H NMR (DMSO-d_6): δ 0.97 (s, 9 H, tBu), 1.12, 1.14 [d, 6 H, J = 6.7, (CH_3)$_2$CH], 1.78 (m, 7-$CH_2CH_2CH_2$), 2.22 (m, 1 H, C2'-H_α), 2.68-2.80 [m, 4 H, C2'-H_β, CH(CH_3)$_2$, 7-C$H_2$$CH_2CH_2$], 3.03 (m, 2 H, 7-$CH_2CH_2CH_2$), 3.62 (m, 2 H, C5'-H), 3.66, 3.67 (2 s, 6 H, 2 OCH_3), 3.95 (m, 1 H, C4'-H), 4.46 (m, 1 H, C3'-H), 5.29 (d, 1 H, J = 4.5, C3'-OH), 6.38 (m, 1 H, C1'-H), 6.71- 7.60 (m, 23 H, arom. H), 11.77 (s, 1H, NH), 11.87 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 18.8, 24.3, 26.6, 30.4, 34.8, 38.1, 54.8, 63.0, 64.7, 71.2, 83.5, 85.2, 85.6, 100.6, 112.9, 126.4, 127.5, 127.6, 127.7, 127.8, 129.6, 129.7, 133.2, 134.9, 135.0, 135.6, 145.0, 149.1, 149.9, 153.2, 156.2, 157.8, 157.9, 180.5. Anal. Calcd for

$C_{54}H_{61}N_5O_8Si$ (M 936.18): C, 69.28 H, 6.57; N, 7.48. Found: C, 69.17; H, 6.58; N, 7.36.

1-[2-Deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl]-1,5-dihydro-6-[(2-methylpropanoyl)amino]-3-(3-tert-butyldiphenylsilyloxyprop-1-yl)-4H-pyrazolo[3,4-d]-pyrimidin-4-one 3'-(2-cyanoethyl-diisopropylphosphoramidite) (**3f**)

To a solution of compound **3e** (0.5 g, 0.53 mmol) in dried dichloromethane (15 ml) were added bis-N,N'-diisopropylamino(2-cyanoethyl)phosphate (0.3 g, 1 mmol), and diisopropylethylamine tetrazolium (0.05 g). This clear solution was stirred for 90 min. The solution was diluted with dichloromethane (15 ml), and subsequently washed with 5%$NaHCO_3$ and brine, respectively. The organic layer was dried with anhydr. Na_2SO_4, and concentrated for flash chromatography to obtain the product as colorless foam (0.44 g, 73%). R_f (CH_2Cl_2/CH_3COCH_3 50:1) 0.68. ^{1}H NMR ($CDCl_3$): δ 1.03-1.18 {m, 27 H, tBu, N[CH(C*H*$_3$)$_2$]$_2$, CH(C*H*$_3$)$_2$}, 1.97 (m, 2 H, 7-CH_2C*H*$_2$$CH_2$), 2.28 (m, 1 H, C2'-$H_\alpha$), 2.44 [m, 1 H, C*H*(CH$_3$)$_2$], 2.62 (m, 1 H, C2'-$H_\beta$), 2.92 (m, 2 H, 7-C*H*$_2$$CH_2CH_2$), 3.08-3.34 (m, 2 H, OCH_2C*H*$_2$CN), 3.36-3.85 {m, 15 H, C5'-H, 2 OCH_3, C4'-H, N*H*(CH$_3$)$_2$]$_2$,OC*H*$_2$$CH_2CN$, 7-$CH_2CH_2$C*H*$_2$}, 4.24 (m, 1 H, C3'-H), 4.79 (m, 1 H, NH), 6.39 (m, 1 H, C1'-H), 6.37, 7.13-7.67 (m, 23 H, arom. H). ^{13}C NMR ($CDCl_3$): δ 18.8, 19.2, 20.2, 24.4, 24.5, 24.6, 24.9, 26.8, 30.9, 36.3, 37.3, 37.5, 43.0, 43.1, 55.1, 58.0, 58.1, 58.3, 63.6, 64.2, 74.1, 74.3, 74.4, 85.0, 85.4, 86.0, 101.9, 112.9, 117.6, 126.6, 127.5, 127.6, 128.1, 128.2, 129.4, 130.0, 133.9, 135.5, 135.9, 136.0, 136.1, 144.8, 148.8, 150.5, 150.7, 153.0, 153.2, `156.8, 158.3, 178.7. ^{31}P NMR ($CDCl_3$): 148.29, 148.35. HRMS for $C_{63}H_{78}N_7O_9PSi$-Na^+ (M 1158.5260): 1158.5285.



(i) 1-pentyne, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, in DMF, at r.t.; (ii) H_2, Pd/C (10%), 5 atm, at 30 °C;
(iii) (a) dimethylaminoformamide, in methanol, at r.t.; (b) DMTrCl, in pyridine, at r.t.;
(iv) $(NCCH_2CH_2O)[(iPr)_2N]_2P$, $(iPr)_2EtN$ tetrazolium, in CH_2Cl_2, at r.t.

6-amino-1-[2-Deoxy-β-D-erythro-pentofuranosyl]-1,5-dihydro-3-(pent-1-ynyl)-4H-pyrazolo[3,4-d]-pyrimidin-4-one (**5a**)

As described for the synthesis of compound **3b**, compound **5a** was prepared from the cross-coupling reaction between 7-iodo-8-aza-7-deaza-deoxyguanosine (**3a**) (1.5 g, 3.81 mmol) and 1-pentyne (0.39 g, 5.75 mmol) in DMF (20 ml) in the presence of $Pd(PPh_3)_2Cl_2$ (0.27 g, 0.38 mmol), CuI (0.16 g, 0.76 mmol), and triethylamine (0.3 ml). The product was purified as colorless solid (1.1 g, 86%) by flash chromatography and applied to the next step. R_f (CH_2Cl_2/CH_3COCH_3 9:1) 0.30.

1-[2-Deoxy-β-D-erythro-pentofuranosyl]-1,5-dihydro-3-(pent-1-yl)-4H-pyrazolo[3,4-d]-pyrimidin-4-one (**5b**)

With the hydrogenation reaction described for compound **3c**, compound **5b** was prepared from **5a** (1.0 g, 3.0 mmol) as a colorless solid (0.8 g, 90%). R_f (CH_2Cl_2/CH_3OH 20:1) 0.41. ^1H NMR (DMSO-d_6): δ 0.88 (t, J = 6.9, 3 H, Me), 1.30 (m, 4 H, CH_2CH_2), 1.67 (m, 2 H, CH_2), 2.15 (m, 1 H, C2'-H_α), 2.67 (m, 3 H, CH_2, C2'-H_β), 3.40, 3.52 (2 m, 2 H, C5'-H), 3.78 (m, 1 H, C4'-H), 4.40 (m, 1 H, C3'-H), 4.82 (t, 1 H, J = 5.7, C5'-OH), 5.23 (d, 1 H, J = 4.5, C3'-OH), 6.27 (t, 1 H, J = 6.5, C1'-H), 6.66 (br, 2 H, 6-NH_2), 10.55 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ 13.9, 21.8, 27.5, 27.6, 30.8, 37.9, 62.6, 71.2, 82.8, 82.9, 87.4, 97.5, 149.4,

154.9, 156.1, 158.1. Anal. Calcd for $C_{15}H_{23}N_5O_4$ (M 337.37): C, 53.40; H, 6.87; N, 20.76. Found: C, 53.42; H, 6.69; N, 20.61.

1-[2-Deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl]-1,5-dihydro-6-(dimethylformamido)-3-(pent-1-yl)-4H-pyrazolo[3,4-d]-pyrimidin-4-one (**5c**)

A solution of compound **5b** (0.76 g, 2.25 mmol) and N, N-dimethylformamide dimethyl acetal (0.8 g, 6.75 mmol) in methanol (10 ml) was stirred overnight at r.t. until a complete conversion was finished. The solution was evaporated, and the oily residue was coevaporated with dried pyridine for three times. The residue was dissolved in dried pyridine, and DMTrCl (1.24 g, 3.65 mmol) was added in portions and the mixture was stirred at r.t. for 3 hours. Methanol (10ml) was added and the solution was evaporated. The residue was purified by flash chromatography to afford a colorless solid (0.9 g, 64.3% for two steps). R_f (CH_2Cl_2/CH_3OH 30:1) 0.65. ^{1}H NMR (DMSO-d_6): δ 0.78 (t, *J* = 6.2, 3 H, Me), 1.16 (m, 4 H, CH_2CH_2), 1.42 (m, 2 H, CH_2), 2.20 (m, 1 H, C2'-H_α), 2.58 (m, 3 H, C5'-CH_2, C2'-H_β), 3.04 (m, 5 H, CH_2, Me), 3.18 (s, 3 H, Me), 3.70 (2 s, 6 H, 2 OMe), 3.90 (m, 1 H, C4'-H), 4.46 (m, 1 H, C3'-H), 5.26 (d, 1 H, *J* = 4.8, C3'-OH), 6.44 (m, 1 H, C1'-H), 6.71-7.34 (m, 13 H, arom. H), 8.70 (s, 1 H, CH), 11.19 (s, 1 H, NH). ^{13}C NMR (DMSO-d_6): δ 13.8, 21.8, 27.4, 27.6, 30.9, 34.8, 38.3, 40.7, 54.9, 65.0, 71.3, 82.7, 85.2, 90.9, 112.9, 126.4, 127.6, 127.7, 129.5, 129.7, 135.7, 145.1, 149.2, 155.1, 157.9, 158.4, 159.0, 159.1. Anal. Calcd for $C_{39}H_{46}N_6O_6$ (M 694.82) : C, 67.42; H, 6.67; N, 12.10. Found: C, 67.19; H, 6.37; N, 11.89.

1-[2-Deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-D-erythro-pentofuranosyl]-1,5-dihydro-6-(dimethylformamido)-3-(pent-1-yl)-4H-pyrazolo[3,4-d]-pyrimidin-4-one
3'-(2-cyanoethyl-diisopropylphosphoramidite) (**5d**)

As described for compound **3e**, compound **5d**was synthesized from compound **5c** (0.5 g, 0.75 mmol) and bis-N,N'-diisopropylamino(2-cyanoethyl)phosphate (0.73 g, 1.44 mmol), in the presence of diisopropylethylamine tetrazolium (0.22 g, 1.44 mmol). It was purified with flash chromatography as colorless solid (0.5 g, 87%). R_f (CH_2Cl_2/CH_3OH 50:1) 0.82. ^{1}H NMR ($CDCl_3$): δ 0.83 (t, *J* = 6.7, 3 H, Me), 1.18 (m, 16 H, 4 Me, CH_2CH_2), 1.58 (m, 2 H, CH_2), 2.30-2.85 (m, 5 H, C2'-H, C5'-H, CH_2), 3.00-3.32 (m, 9 H, C5'-H, 2 Me, CH_2), 3.53-3.89 (m,

10 H, CH_2, 2CH, 2 OMe), 4.23 (m, 1 H, C4'-H), 4.79 (m, 1 H, C3'-H), 6.58 (m, 1 H, C1'-H), 6.70-7.43 (m, 13 H, arom. H), 8.76 (s, 1 H, CH), 8.81 (s, 1 H, NH). ^{13}C NMR ($CDCl_3$): δ 14.0, 20.1, 20.2, 20.3, 22.3, 24.4, 24.5, 24.6, 27.8, 28.2, 31.5, 35.0, 37.3, 37.5, 41.3, 42.9, 43.0, 55.0, 58.3, 58.5, 64.4, 64.5, 74.0, 74.5, 74.6, 83.4, 83.5, 85.0, 85.9, 86.0, 100.8, 112.8, 117.5, 126.4, 127.5, 128.1, 128.2, 130.0, 136.0, 136.1, 144.8, 150.6, 155.6, 158.1, 158.5, 159.5. ^{31}P NMR ($CDCl_3$): 148.43, 148.64. HRMS for $C_{48}H_{63}N_8O_7P$-Na^+ (M 917.4450): 917.4465.

Oligodeoxynucleotide synthesis

The chimera substrate was purchased from Takara (Dalin, China). 33-mer DNAzyme sequences were synthesized on an ABI 392 DNA/RNA synthesizer (Applied Biosystems, USA) on a 1 μmol scale with the DMT-off mode according to the User Protocol. The DMT-off oligodeoxyribonucleotides were deprotected with conc. aq. ammonia and purified with electrophoresis on a 20% polyacrylamide gel containing 7 M urea. Desaltation was conducted with Sep-Pak column (C18, Waters, USA) and washed with sterilized and deionized water. The product was lyophilized and stored at -30˚C. MALDI-TOF was performed on a Kratos Axima-CFR^{TM}-plus instrument (Shimatzu, Japan) with 2', 4', 6'-trihydroxyacetophenone (THAP) as the matrix (Table S1).

Table S1 Characterization of 10-23 DNAzyme and its analogs by MALDI-TOF MS

Name		MW (calc)	MW (found)
10-23DZ	5’-d(tgc tct cca GGC TAG CTA CAA CGA cct gca cct)-3’	9994.4	9994.0
DZ-G1-1	5’-d(tgc tct cca **1**GC TAG CTA CAA CGA cct gca cct)-3’	9992.5	9997.8
DZ-G2-1	5’-d(tgc tct cca G**1**C TAG CTA CAA CGA cct gca cct)-3’	9992.5	9992.3
DZ-G6-1	5’-d(tgc tct cca GGC TA**1** CTA CAA CGA cct gca cct)-3’	9992.5	9996.2
DZ-G14-1	5’-d(tgc tct cca GGC TAG CTA CAA C**1**A cct gca cct)-3’	9992.5	9995.5
DZ-G1-2	5’-d(tgc tct cca **2**GC TAG CTA CAA CGA cct gca cct)-3’	9994.4	9996.5
DZ-G2-2	5’-d(tgc tct cca G**2**C TAG CTA CAA CGA cct gca cct)-3’	9994.4	9994.5
DZ-G6-2	5’-d(tgc tct cca GGC TA**2** CTA CAA CGA cct gca cct)-3’	9994.4	9994.8
DZ-G14-2	5’-d(tgc tct cca GGC TAG CTA CAA C**2**A cct gca cct)-3’	9994.4	9995.7
DZ-G1-3	5’-d(tgc tct cca **3**GC TAG CTA CAA CGA cct gca cct)-3’	10052.5	10057.5
DZ-G2-3	5’-d(tgc tct cca G**3**C TAG CTA CAA CGA cct gca cct)-3’	10052.5	10057.6
DZ-G6-3	5’-d(tgc tct cca GGC TA**3** CTA CAA CGA cct gca cct)-3’	10052.5	10056.3
DZ-G14-3	5’-d(tgc tct cca GGC TAG CTA CAA C**3**A cct gca cct)-3’	10052.5	10057.2
DZ-G1-4	5’-d(tgc tct cca **4**GC TAG CTA CAA CGA cct gca cct)-3’	10051.5	10057.4
DZ-G2-4	5’-d(tgc tct cca G**4**C TAG CTA CAA CGA cct gca cct)-3’	10051.5	10048.4
DZ-G6-4	5’-d(tgc tct cca GGC TA**4** CTA CAA CGA cct gca cct)-3’	10051.5	10048.9
DZ-G14-4	5’-d(tgc tct cca GGC TAG CTA CAA C**4**A cct gca cct)-3’	10051.5	10055.5
DZ-G1-5	5’-d(tgc tct cca **5**GC TAG CTA CAA CGA cct gca cct)-3’	10064.6	10064.7
DZ-G2-5	5’-d(tgc tct cca G**5**C TAG CTA CAA CGA cct gca cct)-3’	10064.6	10062.7
DZ-G6-5	5’-d(tgc tct cca GGC TA**5** CTA CAA CGA cct gca cct)-3’	10064.6	10065.4
DZ-G14-5	5’-d(tgc tct cca GGC TAG CTA CAA C**5**A cct gca cct)-3’	10064.6	10064.1
DZ-G1-G14-4	5’-d(tgc tct cca **4**GC TAG CTA CAA C**4**A cct gca cct)-3’	10108.6	10110.1
DZ-G2-G14-4	5’-d(tgc tct cca G**4**C TAG CTA CAA C**4**A cct gca cct)-3’	10108.6	10106.3
10-23-DZt	5’-d(agg atc ta GGC TAG CTA CAA CGA tgg ctc ca)-3’	9529.2	9530.3
DZt-G1-4	5’-d(agg atc ta **4**GC TAG CTA CAA CGA tgg ctc ca)-3’	9586.3	9588.9
DZt-G2-4	5’-d(agg atc ta G**4**C TAG CTA CAA CGA tgg ctc ca)-3’	9586.3	9585.9
DZt-G6-4	5’-d(agg atc ta GGC TA**4** CTA CAA CGA tgg ctc ca)-3’	9586.3	9587.1
DZt-G14-4	5’-d(agg atc ta GGC TAG CTA CAA C**4**A tgg ctc ca)-3’	9586.3	9586.6

T_m measurements

A Cary-100 Bio UV-Visible spectrophotometer equipped with a Cary temperature controller (Varian, USA) was used for T_m measurement. Equal molar concentration of DNAzyme and

the full-DNA substrate was mixed in the reaction buffer (50 mM Tris-HCl, pH 7.5, 2 mM Mg^{2+}), and the absorbance at 260 nm was recorded during the temperature cooling process from 85 ˚C to 20 ˚C at a rate of 1 ˚C/min. Melting temperatures were obtained from the maxima of the first derivatives of the melting curves (Table S2).

Table S2 Thermal stability evaluation results of DNAzyme-substrate complexes [a]

Name	T_m	Name	T_m
10-23 DNAzyme	52.0±0.8		
DZ-G1-1+D19	51.0±0.5	**DZ-G1-5+D19**	50.3±0.5
DZ-G2-1+D19	51.0±0.5	**DZ-G2-5+D19**	51.7±0.4
DZ-G6-1+D19	51.0±0.5	**DZ-G6-5+D19**	50.0±0.5
DZ-G14-1+D19	51.0±0.5	**DZ-G14-5+D19**	50.1±0.5
DZ-G1-2+D19	51.0±0.5	**DZ-G1-G14-4+D19**	51.0±0.5
DZ-G2-2+D19	51.0±0.5	**DZ-G2-G14-4+D19**	50.8±0.4
DZ-G6-2+D19	51.0±0.5	**10-23Dzt+D17**	39.5±0.5
DZ-G14-2+D19	52.0±0.6	**DZt-G1-4+D17**	39.5±0.5
DZ-G1-3+D19	51.0±0.5	**DZt-G2-4+D17**	40.2±1.0
DZ-G2-3+D19	51.0±0.5	**DZt-G6-4+D17**	40.6±1.0
DZ-G6-3+D19	51.0±0.5	**DZt-G14-4+D17**	39.8±0.5
DZ-G14-3+D19	52.0±0.6		
DZ-G1-4+D19	51.0±0.5		
DZ-G2-4+D19	51.0±0.5		
DZ-G6-4+D19	51.7±0.8		
DZ-G14-4+D19	52.0±1.0		

CD Measurement

From the melting temperature around 51 ˚C of all the DNAzyme-substrate complexes, the CD data were collected at 20˚C. The samples from T_m measurement were used for CD spectrum in a quartz cuvette of 10 mm pathlength. Three scans were collected and averaged to improve the signal-to noise ratio in the range of wavelength from 350 to 200 nm.

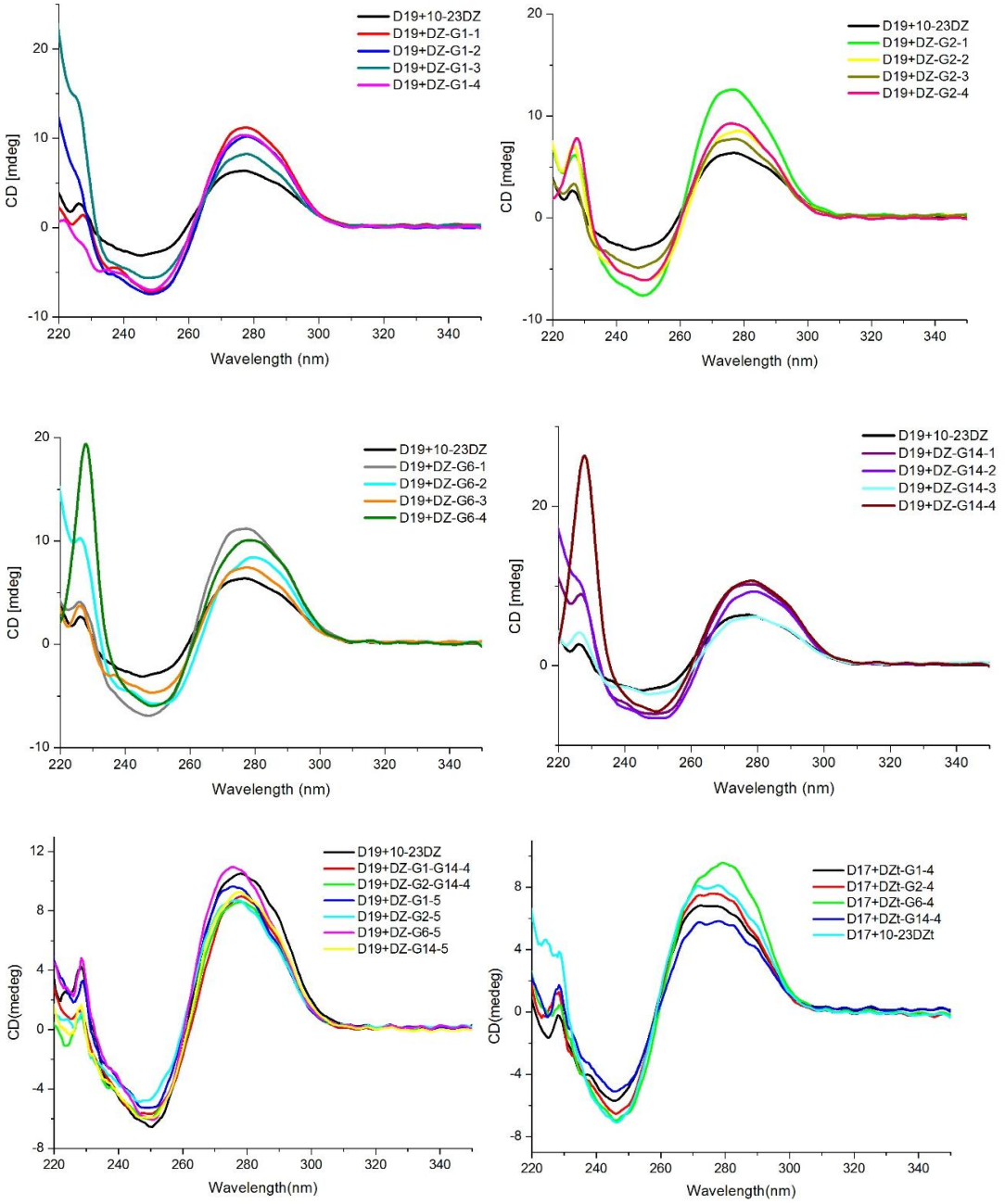


Fig. S1 CD spectrum of the complexes between DNAzymes modified with compounds **1-5** and the full-DNA substrate.

Radio-labeling of the chimeric substrate: the substrate 5'-d (AGG TGC AGG)-rA-rU-d(GGA GAG CA)-3' (11 nmol) was incubated together with 50 μCi of [γ-^{32}P] ATP and 10 U of T4 polynucleotide kinase (Takara, Dalian, China) in the phosphate buffer at 37 °C for 40 min. Then the solution was heated at 70 °C for 10 min to stop the reaction. The ^{32}P-labeled substrate was extracted using a SEP-PAK column and washed with sterilized water, methanol/water (70/30, v/v) was used to elute the product, which was then lyophilized and

stored at -30 °C.

Measurements of kinetic parameters: For the cleavage reaction of DNAzymes under single-turnover conditions, deoxyribozyme (2 μM) and the substrate (20 nM) were dissolved in the reaction buffer (50 mM Tris-HCl, pH 7.5), heated at 90 °C for 3 min and cooled to 37 °C for 10 min. An equal volume of the reaction buffer containing Mg^{2+} (4 mM) was added to initiate the reaction at 2 mM Mg^{2+}. Aliquots were taken from the reaction mixture at appropriate time points, and were immediately mixed with an equal volume of stopping solution (100 mM EDTA, 8 M Urea) to quench the reaction. The radio-labeled product and substrate were separated on a 20% polyacrylamide gel containing 7 M urea, and quantified by the densitometry of the gel images with a Molecular Dynamics Storm 840 Phosphoimager. The time dependent cleavage of the substrate was fitted with $P\% = P_{\infty}\%\ (1- \exp\ [-k_{obs}t])$, where $P\%$ is the cleavage percentage of the product at time t, $P_{\infty}\%$ is the final percentage of the product at $t = \infty$. All the reactions were performed as triplicates, and the averaged result was given for the calculation of the observed rate constant. Less than 20% variation was observed for identical experiments performed on different days.

For multiple-turnover assays in the reaction buffer (50 mM Tris-HCl, pH 7.5, 10 mM Mg^{2+}), each DNAyzme (1 nM) was reacted with substrate of different concentrations from 20 nM to 1000 nM. The initial rate of cleavage was calculated from the first five data points, corresponding to the initial 10% of the reaction. The maximum velocity V_{max} and K_M (Michaelis constant) were calculated from Eadie-Hofstee plots based on Michaelis-Menten mechanism. The DNAzyme kinetic parameters k_{cat} (cleavage rate constant) were calculated from V_{max} /[DNAzyme]. At least three independent experiments were conducted.