Synthesis of G-quadruplex-targeting flexible macrocyclic molecules via click reactions

Qiang Zhang, Wei Tan, and Gu Yuan*

Beijing National Laboratory for Molecular Sciences, Key laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, Department of Chemical Biology, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China
E-mail: guyuan@pku.edu.cn

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Abstract

Four flexible macrocycles, such as **cTz** (**1**), **cTN** (**2**), **cPT** (**3**), and **cPTN** (**4**), were efficiently synthesized through cyclodimerizing triazo-alkynyl-containing monomers via Cu(I)-catalyzed click reactions with optimized conditions. The starting materials were pyrrole and triazole derivatives containing amine and carboxyl groups, followed by amide coupling to introduce the triazo and alkynyl groups to prepare the cyclization precursors. The electrospray ionization mass spectrometry (ESI-MS) results indicated that **cTN** (**2**) and **cPT** (**3**) showed the ability to bind with *c-myb* G-quadruplex. Therefore, **cTN** (**2**) and **cPT** (**3**) molecules might be potential leading compounds for anti-cancer drug discovery.

Keywords: Flexible macrocyclic molecules, click reaction, efficiently cyclization, G-quadruplex recognition

Introduction

DNA G-rich sequences widely exist in human genome and are potential to form G-quadruplex structures.[1,2] The G-quadruplex involved in the significant regions such as promoters and telomeres have been proven to play important roles in life process, including gene transcription and expression, cell division and apoptosis.[1,3-10] GGA repeats sequences were reported to consist in the promoter of *c-myb* and show the ability to form a G-quadruplex structure.[11-13] Further studies showed that Myc-associated zinc finger protein can bind to the G-quadruplex and repress *c-myb* promoter activity.[14] Thus, the G-rich sequences in *c-myb* promoter region can act as a critical element to regulate the expression of the *c-myb*. Furthermore, the small molecules that selectively bind with *c-myb* G-quadruplex and stabilize this structure are considered as potential anti-cancer drugs.

The classic G-quadruplex macrocyclic ligands, such as telomestatin and porphyrins, stack on the terminal planar of G-quadruplex by π-π interaction with high affinity.[15-24] Recently, a novel flexible cyclic polyamide was synthesized and proven to selectively recognize the *c-myb* G-quadruplex in our laboratory.[25] This flexible cyclic polyamide was efficiently cyclized by dimerizing two half-length precursors via amide-bonds formation. Herein, instead of amide coupling, we applied more efficient Cu(I)-catalyzed click reaction for cyclization to obtain four flexible macrocyclic G-quadruplex ligands (Figure 1).

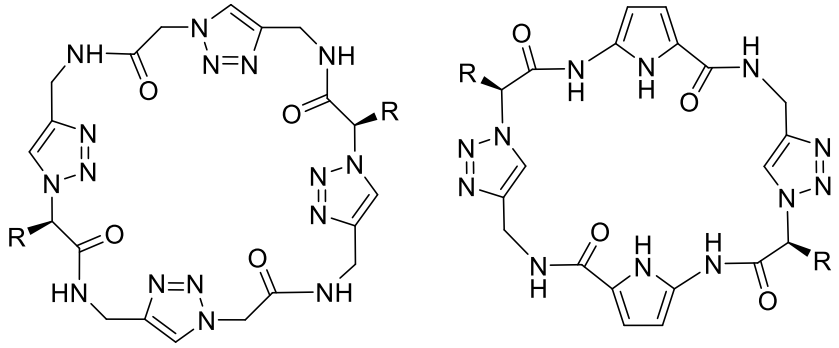
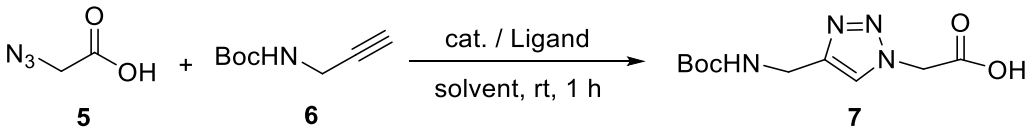


Figure 1. Structures of the macrocyclic molecules. **cTz** (**1**), **cTN** (**2**), **cPT** (**3**) and **cPTN** (**4**).

Results and Discussion

First, we optimized the Cu(I)-catalyzed click reaction conditions using azidoacetic acid **5** and Boc-protected propynylamine **6** as model substrates, as shown in Table 1. In presence of TEMED (*N*,*N*,*N'*,*N'*-tetramethylethylenediamine) as the ligand (100 mol%), CuCl (20 mol%) showed the higher catalytic activity than CuI and CuBr (entry 1-3). The reaction was promoted more effectively in THF than acetonitrile, and as the addition of water, the product yield increased to 71% when the THF / H_2O ratio reached 5 / 1 (entry 3-5). However, the further increasing amount of water suppressed the reaction due to the decreasing solubility of **6** (entry 6, 7). Varieties of nitrogen-containing compounds (TEMED, DIMED (*N*,*N*'-Dimethylethanediamine), ethylenediamine, L-proline, 2, 6-lutidine, DIEA (ethyldiisopropylamine)) were evaluated to study the influence of ligands on the reaction (entry 3, 8-12). The results revealed bidentate ligand DIMED exhibited the best activity (yield 85%, entry 8). The product yield was 23% even in the absence of ligand because formed trace triazole product promoted the reaction slightly (entry 13). In addition, more CuCl catalyst (50 mol%) could raise the product yield obviously (95%, entry 14).

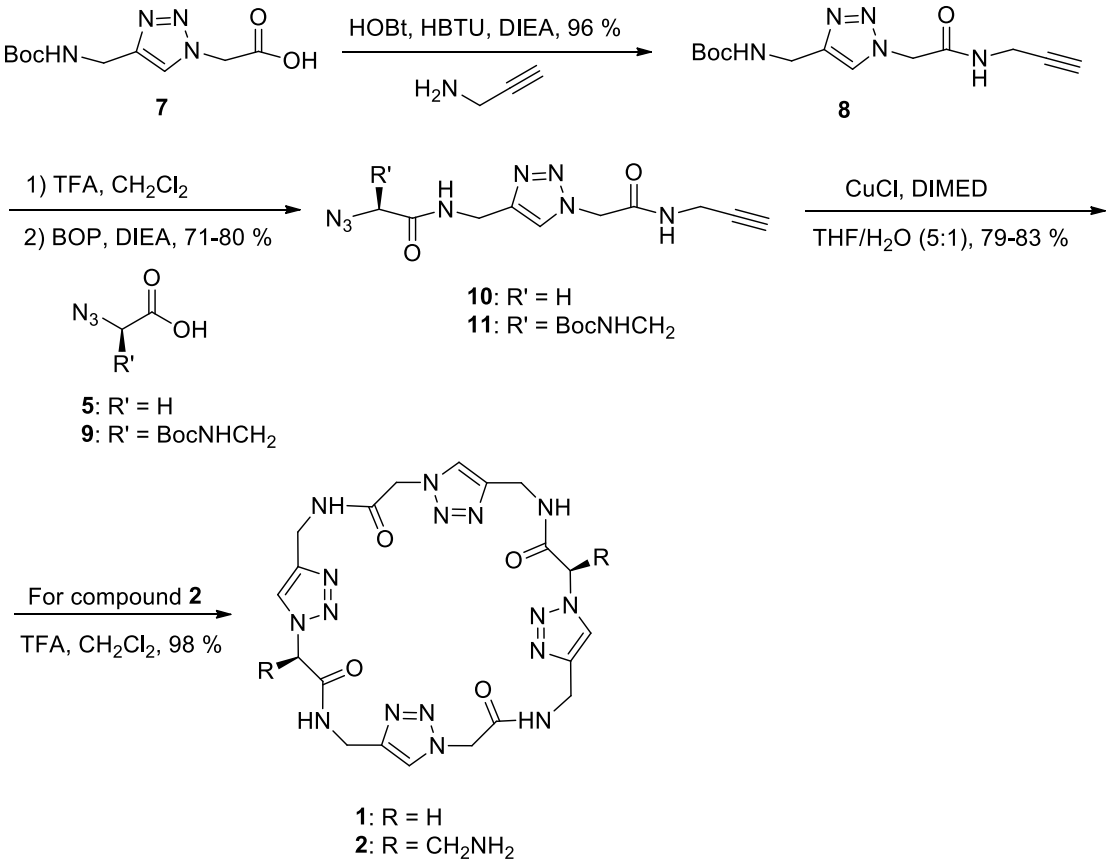
Table 1. Optimization of click reaction conditions [a]



Entry	Catalyst	Ligand	Solvent	Yield (%)
1	20 mol% CuI	100 mol% TEMED	THF / H_2O 5 / 1	5
2	20 mol% CuBr	100 mol% TEMED	THF / H_2O 5 / 1	62
3	20 mol% CuCl	100 mol% TEMED	THF / H_2O 5 / 1	71
4	20 mol% CuCl	100 mol% TEMED	CH_3CN	4
5	20 mol% CuCl	100 mol% TEMED	THF	66
6	20 mol% CuCl	100 mol% TEMED	THF / H_2O 1 / 2	57
7	20 mol% CuCl	100 mol% TEMED	H_2O	62
8	20 mol% CuCl	100 mol% DIMED	THF / H_2O 5 / 1	85
9	20 mol% CuCl	100 mol% ethylenediamine	THF / H_2O 5 / 1	76
10	20 mol% CuCl	100 mol% L-proline	THF / H_2O 5 / 1	0
11	20 mol% CuCl	100 mol% 2, 6-lutidine	THF / H_2O 5 / 1	78
12	20 mol% CuCl	100 mol% DIEA	THF / H_2O 5 / 1	35
13	20 mol% CuCl	no ligand	THF / H_2O 5 / 1	23
14	50 mol% CuCl	100 mol% DIMED	THF / H_2O 5 / 1	95

[a] Reaction conditions: azidoacetic acid (**5**, 13 mg, 0.13 mmol) and Boc-protected propynylamine (**6**, 20 mg, 0.13 mmol) in solvent (1.0 mL) at rt for 1 h.

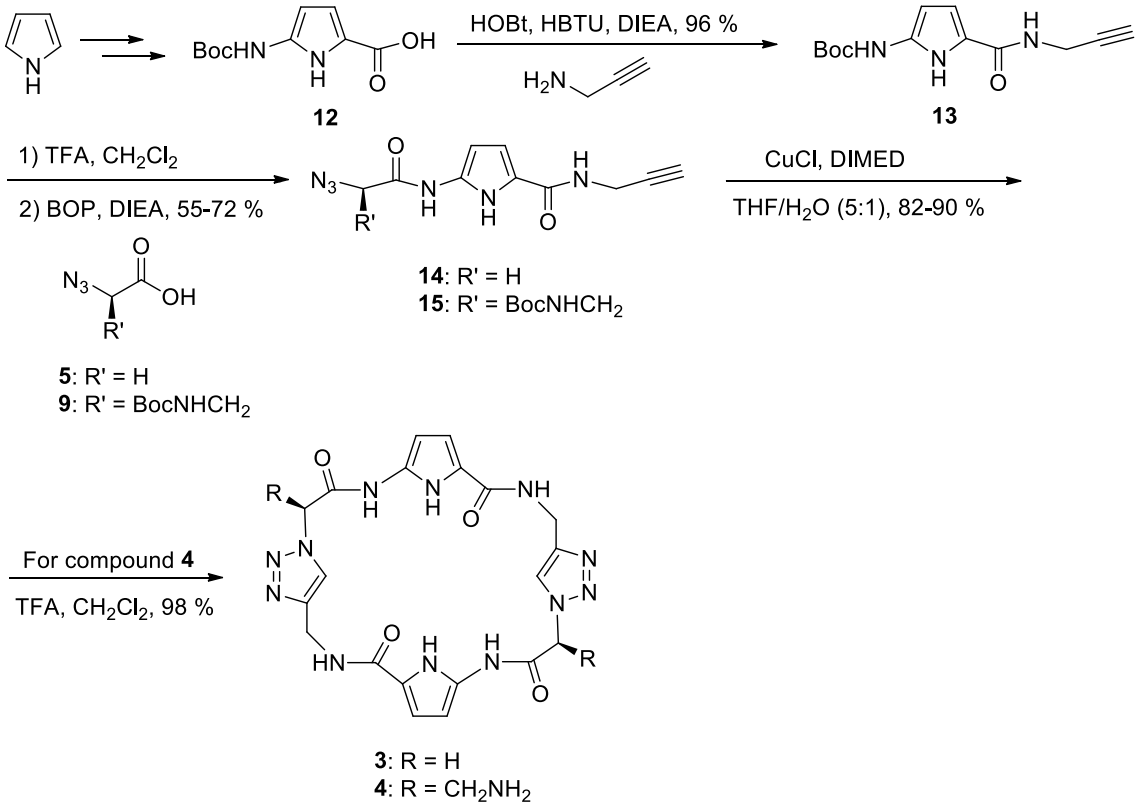
As shown in Scheme 1, propynylamine, and azidoacetic acid **5** or its derivative **9** were efficiently linked to the C- and N-terminal of **7** by amide bonds to afford cyclization precursors **10** and **11**, respectively. In our case, uronium-based coupling reagent HBTU (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) and phosphonium-based coupling reagent BOP (benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate) were utilized to activate the carboxylic acid of **7**, and **5** or **9** in order to facilitate the amide-bond coupling reaction. The triazo-alkynyl-containing precursors were readily cyclized by the formation of two triazoles with high yields at the concentration of 2 mM in the optimized click reaction condition. In addition, our starting material **12** for **cPT** (**3**) and **cPTN** (**4**) was obtained from pyrrole via five steps according to the procedures reported[25,26]. **cPT** (**3**) and **cPTN** (**4**) were also synthesized from triazole derivative **7** by the similar method (Scheme 2).



Scheme 1. Synthesis of macrocyclic molecules **cTz** (**1**) and **cTN** (**2**).

The binding affinity of compound **1-4** towards the *c-myb* G-quadruplex (Q1) was evaluated by ESI-MS. The mass spectra showed that as the molar ratio of ligand/Q1 was 4:1, the complex ion of Q1 with one **cTN** ([Q1+**cTN**]$^{5-}$) at *m/z* 1672.4 appeared in the spectrum with an intensity of nearly 20% (Figure 2a). For **cPT**, the complex ion ([Q1+**cPT**]$^{5-}$) appeared at *m/z* 1649.5 with an intensity of 50%, and that of Q1 with two **cPT** ([Q1+2**cPT**]$^{5-}$) at *m/z* 1748.0 with an intensity of 10% (Figure 2b). The results indicated **cTN** (**2**) and **cPT** (**3**) exhibited the ability to bind with Q1 (Figure 2). To evaluate the binding affinity of **cTN** (**2**) and **cPT** (**3**) to Q1, the parameter IR_a[27-30] was defined as the relative abundance ratio of bound ions ($\sum Ir[Q1+n\mathbf{Ligand}]^{5-}$, n = 1, 2) to that of both unbound and bound species ($\sum Ir[Q1]^{5-} + \sum Ir[Q1+n\mathbf{Ligand}]^{5-}$, Equation 1). The IR_a values were 0.15 and 0.29 for **cTN** (**2**) and **cPT** (**3**), respectively.

$$IR_a = \frac{Ir[Q1+n\mathbf{Ligand}]^{5-}}{Ir[Q1]^{5-} + \sum Ir[Q1+n\mathbf{Ligand}]^{5-}} \qquad (\mathbf{1})$$



Scheme 2. Synthesis of macrocyclic molecules **cPT** (**3**) and **cPTN** (**4**).

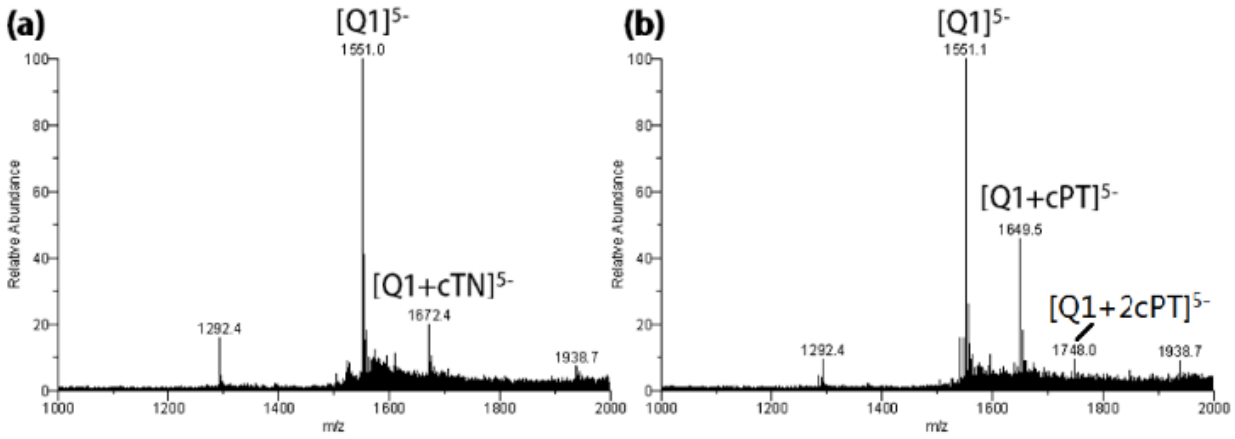


Figure 2. ESI mass spectra of the *c-myb* G-quadruplex (Q1) with **cTN** (**2**, a) and **cPT** (**3**, b) in 100 mM NH_4OAc, 25% CH_3OH.

Conclusions

Macrocyclic molecules **cTz** (**1**), **cTN** (**2**), **cPT** (**3**), and **cPTN** (**4**) were synthesized conveniently from simple starting materials and cyclized via efficient click reaction with high yields. ESI-MS array demonstrated the binding affinities of **cTN** (**2**) and **cPT** (**3**) with *c-myb* G-quadruplex. **cTN** (**2**) and **cPT** (**3**) molecules have potential to be excellent G-quadruplex ligands with further derivatization.

Experimental Section

General. Low resolution mass (LRMS) spectra and ESI-MS binding array spectra were recorded on Thermo Finnigan SURVEYOR-LCQDECA (ESI-Ion Trap) mass spectrometer. High resolution mass (HRMS) spectra were recorded on Bruker APEX IV (ESI-FTICR) mass spectrometer. NMR spectra were recorded on Bruker 400MHz spectrometer. 1*H*-Pyrrole, ethylenediamine, L-proline, sodium azide, and ethyl bromoacetate was purchased from Sinopharm Chemical Reagent Co., Ltd. Boc anhydride, 1-hydroxybenzotrizole (HOBt), HBTU, DIEA, BOP were purchased from Shanghai Medpep Co., Ltd. Propynylamine and DIMED were purchased from Energy Chemical Co., Ltd.. Trifluoroacetic acid (TFA), CuCl, CuBr, CuI, TEMED, and 2, 6-lutidine was purchased from J&K Chemical Co., Ltd. (S)-isoserine was purchased from Beijing HWRK Chem Co., Ltd.

Azidoacetic acid (5). To a solution of ethyl bromoacetate (20.0 g, 120 mmol) in DMF (20 mL) was added sodium azide (12.5 g, 192 mmol) with stirring at 0 °C. The mixture was allowed to warm to rt and stirred for 24 h. Saturated sodium carbonate solution was added and the aqueous layer was extracted three times with Et_2O. The organic layer was combined and washed by saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4. The solvent was evaporated to obtain colorless liquid. Without further purification, 1 M NaOH (60 mL) and methanol (60 mL) were added and stirred at 40 ^oC. Methanol was evaporated after 5 h. The pH of the remaining aqueous solution was adjusted about 2 by adding 2 M HCl. The aqueous layer was extracted three times with Et_2O. The organic layer was combined and washed with brine, dried over Na_2SO_4. The solvent was evaporated to give **5** as a colorless liquid (4.9 g，48.5 mmol，81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.97 (s, 1 H), 3.95 (s, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.3, 50.1. LRMS (ESI-Ion Trap): *m/z* calcd. for $C_2H_2N_3O_2$ $[M\text{-}H]^-$ 100.0 found 100.0.

***tert*-Butyl prop-2-yn-1-ylcarbamate (Boc-protected propynylamine, 6).** To a solution of propynylamine (5.5 g, 100 mmol) in EtOAc (50 mL) was added Boc_2O (32.0 g, 147 mmol) with stirring at 0 ^oC. The mixture was allowed to warm to rt and stirred overnight. The organic solution was washed by 1% HCl solution, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4. The solvent was evaporated to give **6** as a yellow solid (15.3 g, 99 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.75 (s, 1 H), 3.90 (d, $^3J_{(H,H)}$ 3.0 Hz, 2 H), 2.21 (t, $^3J_{(H,H)}$ 3.0 Hz, 1 H), 1.44

(s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.2, 80.2, 71.4, 30.2, 28.2, 27.3. LRMS (ESI-Ion Trap): *m/z* calcd. for $C_8H_{12}NO_2$ [*M*-H]$^-$ 154.1 found 154.0.

2-(4-(((tert-butoxycarbonyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)acetic acid (7). To a solution of **5** (1.3 g, 12.9 mmol) and **6** (2.0 g, 12.9 mmol) in THF (10 mL) was added water (2 mL), CuCl (0.64 g, 6.5 mmol), and DIMED (1.14 g, 12.9 mmol) sequentially with stirring at rt. 2 M HCl solution (2 mL) and EtOAc were added after 1 h. The organic layer was washed by 1% HCl solution and brine, dried over Na_2SO_4. The solvent was evaporated and the product was frozen to give **7** as a white solid (3.0 g, 11.7 mmol, 91%). ^{1}H NMR (400 MHz, acetone-*d*$_6$): δ 11.91 (br, 1 H), 7.96 (s, 1 H), 6.50 (s, 1 H), 5.33 (s, 2 H), 4.36 (s, 2 H), 1.44 (s, 9 H). ^{13}C NMR (100 MHz, acetone-*d*$_6$): δ 168.8, 156.8, 124.9, 82.0, 79.1, 51.2, 36.7, 28.2. LRMS (ESI-Ion Trap): *m/z* calcd. for $C_{10}H_{15}N_4O_4$ [*M*-H]$^-$ 255.1 found 255.0.

***tert*-Butyl ((1-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-1H-1,2,3-triazol-4-yl)methyl)carbamate (8).** To a solution of **7** (1.00 g, 3.91 mmol), HOBt (0.530 g, 3.93 mmol), and HBTU (2.20 g, 5.80 mmol) in DMF (7 mL) was added DIEA (0.7 mL, 4 mmol) with stirring at rt. Propynylamine (0.320 g, 5.82 mmol) was added after 15 min. The mixture was stirred under N_2 at rt overnight. The solution was added to EtOAc, followed by washing by 1% HCl solution, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography on silica gel ($CHCl_3$: CH_3OH 15: 1 – 10: 1) to give **8** as a light yellow solid (1.07 g, 3.65 mmol, 93%). ^{1}H NMR (400 MHz, MeOD-*d*$_4$): δ 7.88 (s, 1 H), 5.41 (s, 2 H), 4.32 (s, 2 H), 3.66 (d, $^3J_{(H,H)}$ 3.0, 2 H), 2.63 (t, $^3J_{(H,H)}$ 3.0, 2 H), 1.43 (s, 9 H). ^{13}C NMR (100 MHz, MeOD-*d*$_4$): δ 166.8, 147.5, 125.9, 82.8, 82.2, 72.3, 63.8, 42.9, 42.2, 31.3, 28.9. LRMS (ESI-Ion Trap): *m/z* calcd. for $C_{13}H_{18}N_5O_3$ [*M*-H]$^-$ 292.2 found 292.1.

(*R*)-2-Azido-3-((tert-butoxycarbonyl)amino)propanoic acid) azidoacetic acid derivative (9). To a solution of (*S*)-isoserine (5.00 g, 47.6 mmol) in ethanol (200 mL) was added hydrogen chloride gas with stirring at 0 ºC for 5 h. The mixture was allowed to warm to rt and stirred overnight. Ethanol was evaporated and EtOAc was added. Boc_2O (15.0 g, 68.8 mmol) and triethylamine (9.44 g, 93.5 mmol) were added and stirred at 0 ºC. The mixture was allowed to warm to rt and stirred overnight. The solvent was evaporated and the residue was washed with petroleum ether to obtain white solid intermediate. The intermediate compound was dissolved in CH_2Cl_2 (100 mL) and triethylamine (8.7 g, 86.3 mmol) was added. The mixture was treated dropwise with a solution of tosyl chloride (9.8 g, 51.3 mmol) in CH_2Cl_2 (80 mL). The mixture was allowed to warm to rt and stirred overnight. The organic solution was washed by 1% HCl solution, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4. The solvent was evaporated and the residue was dissolved in DMF (75 mL). Sodium azide (7.0 g, 108 mmol) was added with stirring at rt. The reaction mixture was warmed to 60 ºC and stirred overnight at this temperature. The mixture was then cooled to rt. saturated aqueous $NaHCO_3$ was added and the aqueous layer was extracted three times with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4. The solvent was evaporated and the residue was dissolved in methanol (60 mL). 1 M NaOH (60 mL) was added with stirring at 40 ºC. Methanol was evaporated after 5 h. The aqueous layer was washed twice with Et_2O. The pH of the remaining aqueous solution was

adjusted about 2 by adding 2 M HCl. The aqueous layer was extracted three times with Et_2O. The organic layer was combined and washed with brine, dried over Na_2SO_4. The solvent was evaporated to give **9** as a light yellow solid (7.1 g，30.9 mmol，65%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (d, $^3J_{(H,H)}$ 7.8 Hz, 1 H), 7.18 (s, 1 H), 4.17 (m, 1 H), 3.60 (m, 1 H), 3.42 (m, 1 H), 1.44 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.5, 171.9, 80.7, 61.5, 41.7, 28.3. LRMS (ESI-Ion Trap): *m/z* calcd. for $C_8H_{13}N_4O_4$ [*M*-H]$^-$ 229.1 found 229.0.

2-Azido-*N*-((1-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (10). To a suspension of **8** (0.250 g, 0.853 mmol) in CH_2Cl_2 (4 mL) was added the same volume of TFA (4 mL) under N_2. After being stirred at rt for 1 h, the solvent was evaporated to give a yellow solid and added to the mixture of **5** (0.130 g, 1.29 mmol), BOP (0.860 g, 1.94 mmol), and DIEA (0.5 mL, 3 mmol) in DMF (5 mL) was stirred under N_2 at rt overnight. The solution was added to EtOAc, followed by washing by 1% HCl solution, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography on silica gel ($CHCl_3$: CH_3OH 15: 1 – 10: 1) to give **10** as a light yellow solid (0.188 g, 0.681 mmol, 80%). ^{1}H NMR (400 MHz, acetone-d_6): δ 7.91 (s, 1 H), 5.32 (s, 2 H), 4.01 (s, 2 H), 3.94 (s, 2 H), 3.75 (s, 2 H), 2.62 (s, 1 H). ^{13}C NMR (100 MHz, acetone-d_6): δ 166.8, 148.0, 126.1, 82.8, 82.2, 63.8, 51.2, 42.9, 42.2, 31.3. LRMS (ESI-Ion Trap): *m/z* calcd. for $C_{10}H_{11}N_8O_2$ [*M*-H]$^-$ 275.1 found 275.0.

tert-Butyl (*S*)-(2-azido-3-oxo-3-(((1-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)propyl)carbamate (11). The synthesis of **11** was similar to that of **10**. **11** was a light yellow solid (0.245 g, 0.605 mmol, 71%). ^{1}H NMR (400 MHz, acetone-d_6): δ 7.90 (s, 1 H), 5.33 (s, 2 H), 4.16 (s, 2 H), 4.14 (s, 1 H), 4.36 (s, 2 H), 3.64 (m, 1 H), 3.44 (m, 1 H), 2.64 (s 1 H), 1.41 (s, 9 H). ^{13}C NMR (100 MHz, acetone-d_6): δ165.8, 161.2, 148.2, 126.2, 156.1, 81.7, 79.3, 71.8, 63.3, 51.2, 43.2, 31.3, 28.8, 28.6. LRMS (ESI-Ion Trap): *m/z* calcd. $C_{16}H_{22}N_9O_4$ [*M*-H]$^-$ 404.2 found 404.1.

cTz (1). To a solution of **10** (0.100 g, 0.362 mmol) in THF (150 mL) was added water (30 mL), CuCl (18 mg, 0.182 mmol), and DIMED (32 mg, 0.364 mmol) sequentially with stirring at rt. The concentration of substrate **10** was 2 mM. THF was evaporated after 1 d. The precipitate was collected by filtration and washed by proper amount of methanol to give **1** as a light yellow solid (0.083 g, 0.150 mmol, 83%) and showed very poor solubility in common organic solvents and water. LRMS (ESI-Ion Trap): *m/z* calcd. $C_{20}H_{23}N_{16}O_4$ [*M*-H]$^-$ 551.2 found 551.1.

cTN (2). The cyclization of **2** was similar to that of **1**. The precipitate was collected by filtration and washed by a small amount of methanol. The residue was added the solution of TFA / CH_2Cl_2 (1:1, v/v, 4 mL) with stirring at rt. The solvent was evaporated and added saturated solution of HCl in EtOAc, followed by adding Et_2O. The precipitate was collected by filtration to give the **2** hydrochloride salt as a light yellow solid (0.097 g, 0.142 mmol, 77%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.47 (s, 2 H), 9.32 (s, 2 H), 8.37 (s, 4 H), 7.62 (s, 2 H), 7.48 (s, 2 H), 5.53 (s, $^3J_{(H,H)}$ 5.5, 2 H), 4.87 (s, 4 H), 4.61 (m, 2 H), 4.32 (s, 4 H), 3.96 (m, 2 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.4, 164.9, 145.9, 121.7, 121.5, 60.0, 54.1, 38.0, 34.7, 34.4. HRMS (ESI-FTICR): *m/z* calcd. $C_{22}H_{31}N_{18}O_4$ [*M*+H]$^+$ 611.2776 found 611.2782.

***tert*-Butyl (5-(prop-2-yn-1-ylcarbamoyl)-1*H*-pyrrol-2-yl)carbamate (13).** The synthesis of **13** was similar to that of **8**. **13** was a light yellow solid (1.12 g, 4.26 mmol, 96%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.10 (s, 1 H), 9.01 (s, 1 H), 8.42 (t, $^{3}J_{(H,H)}$ 8.0 Hz, 1 H), 6.81 (s, 1 H), 6.70 (s, 1 H), 3.96 (s, 2 H), 3.09 (s, 1 H), 1.44 (s, 9 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.6, 163.7, 155.7, 125.7, 124.1, 113.2, 104.0, 80.4, 30.2, 28.2, 27.3. LRMS (ESI-Ion Trap): *m/z* calcd. $C_{13}H_{16}N_3O_3$ [*M*-H]$^-$ 262.1 found 261.9.

5-(2-Azidoacetamido)-*N*-(prop-2-yn-1-yl)-1*H*-pyrrole-2-carboxamide (14). The synthesis of **14** was similar to that of **10**. **14** was a light yellow solid (0.337 g, 1.37 mmol, 72%). ^{1}H NMR (400 MHz, MeOD-d_4): δ 7.26 (d, $^{3}J_{(H,H)}$ 7.2, 1 H), 6.83 (d, $^{3}J_{(H,H)}$ 7.2, 1 H), 4.09 (d, $^{3}J_{(H,H)}$ 4.1, 2 H), 3.99 (s, 2 H), 2.58 (t, $^{3}J_{(H,H)}$ 4.1, 1 H). ^{13}C NMR (100 MHz, MeOD-d_4): δ 167.2, 162.9, 124.4, 114.6, 104.3, 81.1, 72.0, 53.1, 29.4, 24.3. LRMS (ESI-Ion Trap): *m/z* calcd. $C_{10}H_9N_6O_2$ [*M*-H]$^-$ 245.1 found 245.0.

***tert*-Butyl (*R*)-(2-azido-3-oxo-3-((5-(prop-2-yn-1-ylcarbamoyl)-1*H*-pyrrol-2-yl)amino)-propyl)carbamate (15).** The synthesis of **15** was similar to that of **11**. **15** was a light yellow solid (0.390 g, 1.04 mmol, 55%). ^{1}H NMR (400 MHz, acetone-d_6): δ 10.62 (s, 1 H), 9.45 (s, 1 H), 7.75 (s, 1 H), 7.38 (s, 1 H), 6.92 (s, 1 H), 6.38 (s, 1 H), 4.14 (s, 2 H), 4.13 (s, 1 H), 3.64 (m, 1 H), 3.44 (m, 1 H), 2.64 (s 1 H), 1.41 (s, 9 H). ^{13}C NMR (100 MHz, acetone-d_6): δ 165.8, 161.2, 156.8, 124.6, 124.5, 113.4, 102.7, 81.7, 79.3, 71.8, 63.3, 43.2, 28.8, 28.6. LRMS (ESI-Ion Trap): *m/z* calcd. $C_{16}H_{20}N_7O_4$ [*M*-H]$^-$ 374.2 found 374.0.

cPT (3). The synthesis of **3** was similar to that of **1**. **3** was a tan solid (0.090 g, 0.183 mmol, 90%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.44 (s, 2 H), 9.98 (s, 2 H), 8.28 (s, 2 H), 7.92 (s, 2 H), 7.01 (s, 2 H), 6.91 (s, 2 H), 5.13 (s, 4 H), 4.49 (s, 4 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.3, 159.9, 156.5, 123.6, 123.4, 122.9, 111.6, 103.0, 53.1, 34.2. HRMS (ESI-FTICR): *m/z* calcd. $C_{20}H_{21}N_{12}O_4$ [*M*+H]$^+$ 493.1809 found 493.1791.

cPTN (4). The synthesis of **4** was similar to that of **2**. **4** hydrochloride salt was a yellow solid (0.100 g, 0.161 mmol, 81%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.37 (s, 2 H), 10.95 (s, 2 H), 8.74 (s, 2 H), 8.34 (s, 2 H), 8.18 (s, 2 H), 7.13 (s, 2 H), 6.81 (s, 2 H), 5.78 (s, 4 H), 4.47 (s, 4 H), 3.66 (m, 2 H), 3.39 (m, 2 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.5, 160.2, 123.5, 122.8, 122.6, 112.1, 102.5, 65.0, 60.0, 56.0, 34.1. HRMS (ESI-FTICR): *m/z* calcd. $C_{22}H_{27}N_{14}O_4$ [*M*+H]$^+$ 551.2340 found 551.2342.

ESI-MS binding array. The oligonucleotide 5'-$(GGA)_8$-3' representing the G-rich sequence in the *c-myb* promoter (Q1) was synthesized by Sangon Biotech Co., Ltd. (China) and further desalted using Microcon filters (Amicon, Beverly, MA, USA) for purification. ESI mass spectra were collected using Finnigan LCQ Deca XP Plus ion-trap mass spectrometer (Thermo Finnigan, San Jose, CA). The DNA sample was diluted in 100 mM NH_4OAc, 25% (volume fraction) methanol solution. Negative ion mode was used with a spray voltage of 2.7 kV and a capillary temperature of 120 °C.

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