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Guanidine modification improves functions of natural RNA-targeting alkaloid

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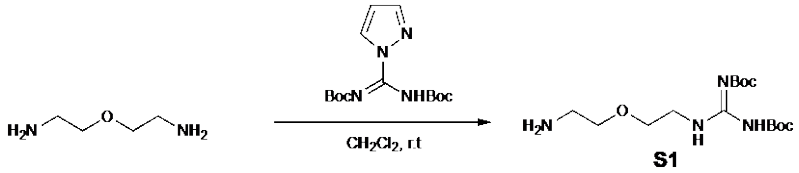
Materials

RNA oligonucleotides for constructing C-bulged RNA, 5′-GCAGCUCGUCCUG-3′ and 5′-CAGGAUAGCUGC-3′, and G-quadruplex-forming RNA oligonucleotide, 5′-GCCGGGCUGGGGCUGGGCGGGGAAA-3′, were purchased from Fasmac Co., Ltd. (Kanagawa, Japan) or GeneDesign, Inc. (Osaka, Japan) as a grade of HPLC purification. RNA oligonucleotides were dialyzed into H_2O before analysis by NMR. DNA oligonucleotides used for the construction of plasmid vectors were purchased from Fasmac Co., Ltd. Berberine was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Other chemical reagents used in this study were those in grade for organic synthesis and biochemical experiments.

Synthesis of guanidine-modified berberine

Berberine derivatives modified by guanidine were synthesized according to Scheme S1. Briefly, carboxy modified berberine, synthesized from berberrubine chloride (Nagara Science Co., Japan), in which one methoxy group of berberine is replaced to a hydroxy group, was coupled with guanidine derivatives having an amino linker with a different length according to the reaction steps below.

- **Synthesis of *N,N′*-[[2-(2-aminoethoxy)ethyl]carbonimidoyl]bis-,C,C′-bis(1,1-dimethylethyl) ester (S1)**



S1

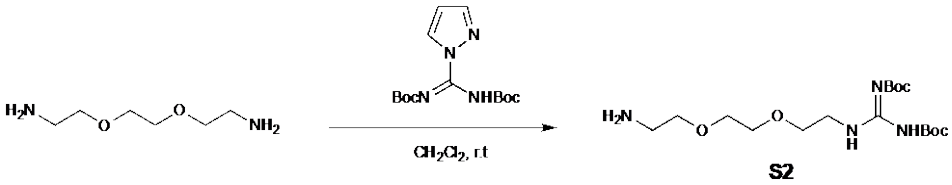
To a solution of 2,2′-oxybis(ethylamine) (34.2 μL, 322 μmol) in CH_2Cl_2 (3 mL) was added *N,N*′-bis(tert-butoxycarbonyl)-1H-pyrazole-1-carboxamidine (10 mg, 32 μmol). The reaction mixture was stirred at room temperature for 2 h and then concentrated. The crude was extracted with $CHCl_3$ (10 mL) and washed with H_2O (10 mL × 2) and brine (10 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated to afford colorless viscous oil (12 mg, crude).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 11.48 (1H, s), 8.75 (1H, s), 3.67-3.63 (2H, m), 3.60-3.57 (2H, m), 3.51 (2H, t, *J* = 5.2 Hz), 2.88 (2H, t, *J* = 4.4 Hz), 1.51 (9H, s), 1.49 (9H, s).

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 163.6, 156.2, 153.2, 83.1, 79.3, 73.3, 68.9, 41.9, 40.6, 28.3, 28.1.

HRMS (ESI-TOF) calcd. for $C_{15}H_{31}N_4O_5^+$ $[M+H]^+$: 347.2289, found: 347.2298.

- **Synthesis of 12-amino-3-[[(1,1-dimethylethoxy)carbonyl]amino]-,1,1-dimethylethyl ester (S2)**



S2

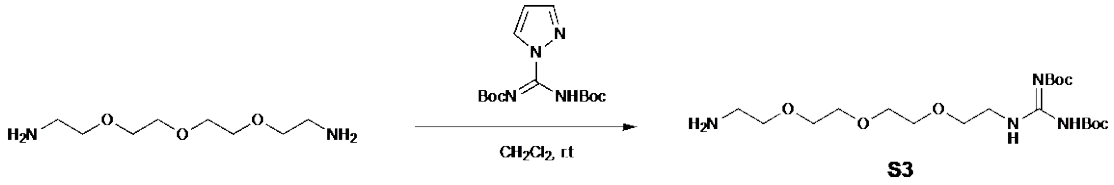
To a solution of 1,2-bis(2-aminoethoxy)ethane (472 µL, 3.22 mmol) in CH_2Cl_2 (30 mL) was added *N,N*'-bis(tert-butoxycarbonyl)-1H-pyrazole-1-carboxamidine (100 mg, 322 µmol). The reaction mixture was stirred at room temperature for 2h and then concentrated. The crude was extracted with DCM (10 mL) and washed with H_2O (15 mL + 8 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated to afford colorless viscous oil (103 mg, crude).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 11.48 (1H, s), 8.63 (1H, s), 3.65-3.60 (8H, m), 3.53 (2H, t, *J* = 5.2 Hz), 2.87 (2H, t, *J* = 5.2 Hz), 1.50 (9H, s), 1.49 (9H, s).

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 163.7, 156.4, 153.2, 83.1, 79.4, 73.7, 70.6, 70.4, 69.5, 42.0, 40.8, 28.4, 28.2.

HRMS (ESI-TOF) calcd. for $C_{17}H_{35}N_4O_6^+$ $[M+H]^+$: 391.2551, found: 391.2563.

- **Synthesis of 12-amino-3-[[(1,1-dimethylethoxy)carbonyl]amino]-, 1,1-dimethylethyl ester (S3)**



S3

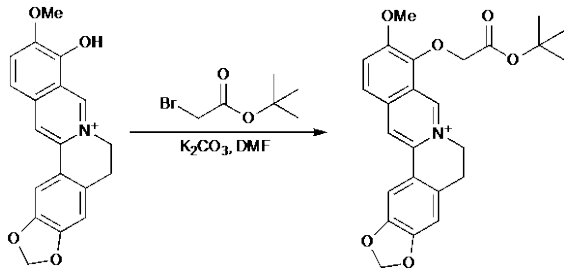
To a solution of 1,11-diamino-3,6,9-trioxaundecane (313 mg, 1.63 mmol) in CH_2Cl_2 (5 mL) was added *N,N*'-bis(tert-butoxycarbonyl)-1H-pyrazole-1-carboxamidine (49.7 mg, 160 µmol) on ice bath. The reaction mixture was raised to room temperature and stirred for 3 h and then concentrated. The crude was extracted with $CHCl_3$ (25 mL) and washed with H_2O (25 mL × 2 + 15 mL + 10 mL) and brine (20 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated to afford colorless viscous oil (64.1 mg, 92%).

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 11.48 (1H, s), 8.61 (1H, s), 3.69-3.67 (4H, m), 3.66-3.61 (8H, m), 3.51 (2H, t, *J* = 4.8 Hz), 2.86 (2H, t, *J* = 4.8 Hz), 1.50 (9H, s), 1.49 (9H, s).

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 163.7, 156.4, 153.1, 83.1, 79.4, 73.6, 70.8, 70.7, 70.7, 70.5, 69.5, 41.9, 40.8, 28.4, 28.2.

HRMS (ESI-TOF) calcd. for $C_{19}H_{39}N_4O_7^+$ $[M+H]^+$: 435.2813, found: 435.2837.

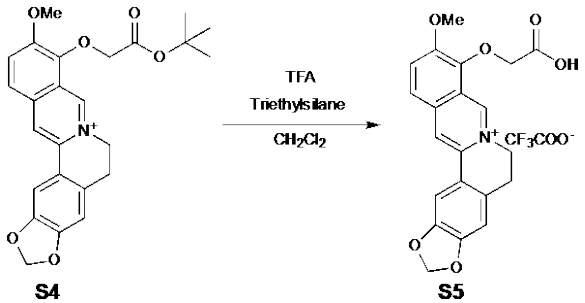
- **Synthesis of 9-(2-(tert-butoxy)-2-oxoethoxy)-10-methoxy-5,6-dihydro-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-7-ium (S4)**



S4

To a solution of berberrubine chloride (15 mg, 42 μmol) in DMF (1.5 mL) were added K_2CO_3 (11.3 mg, 81.8 μmol) and *t*-butyl-2-bromoacetate (12.5 μL, 85.2 μmol), the reaction mixture changed to brown from yellow. After stirring at room temperature for 21 h reaction mixture was filtrated and yellow solid precipitated on cotton. Precipitation was dissolved in MeOH and then evaporated to afford yellow solid (5.5 mg, 13 μmol). The residue filtration liquor was recrystallized using ethyl acetate: MeOH: hexane = 1.7 mL: 1 mL: 6 mL to afford yellow fine powder (7.0 mg, 16 μmol, total yield is 68%).
^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.93 (1H, s), 8.95 (1H, s), 8.19 (1H, d, J = 6.0 Hz), 7.98 (1H, d, J = 6.0 Hz), 7.81(1H, s), 7.09 (1H, s), 6.18 (1H, s), 4.97 (2H, s), 4.94 (2H, t, J = 4.0 Hz), 4.03 (3H, s), 3.21 (2H, t, J = 4.0 Hz), 1.41 (9H, s).
^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 167.9, 149.9, 148.9, 147.7, 145.7, 141.7, 137.6, 133.0, 130.7, 126.9, 123.1, 121.0, 120.4, 120.1, 108.5, 105.5, 102.1, 81.7, 69.6, 57.3, 55.4, 27.7, 26.4.
HRMS (ESI-TOF) calcd. for $C_{25}H_{26}NO_6^+$ $[M]^+$: 436.1755, found: 436.1766.

- **Synthesis of 9-(carboxymethoxy)-10-methoxy-5,6-dihydro-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-7-ium (S5)**



S4 **S5**

To a solution of **S4** (42.1 mg, 96.4 μmol) in CH_2Cl_2 (650 μL) were added triethyl silane (23.1 μL, 145 μmol) and TFA (2.54 mL). Under room temperature the reaction mixture was stirred for 1 h and then after evaporation and co-evaporation with MeCN three times the crude compound was purified by

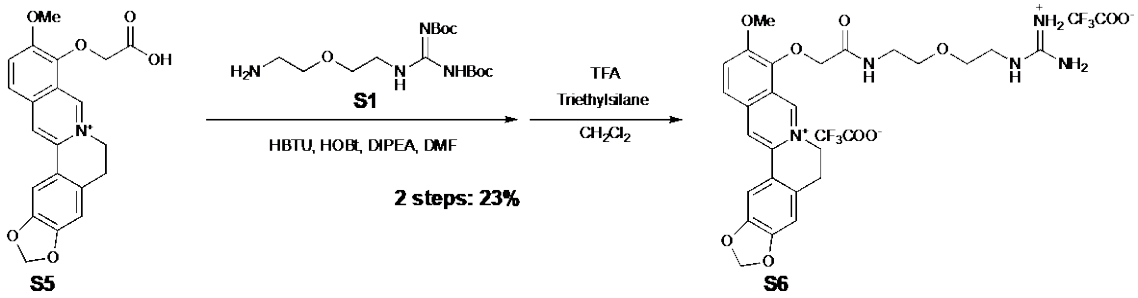
silica gel column chromatography (ethyl acetate: MeOH = 50:1→30:1→20:1→15:1→10:1, 0.5% acetic acid) to afford yellow solid (30.7 mg, 83.7%).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 10.61 (1H, s), 8.82 (1H, s), 8.10 (1H, d, *J* = 9.2 Hz), 7.88 (1H, d, *J* = 8.8 Hz), 7.78 (1H, s), 7.09 (1H, s), 6.16 (2H, s), 4.87 (2H, t, *J* = 6.0 Hz), 4.43 (2H, s), 4.01 (3H, s), 3.20 (2H, t, *J* = 6.0 Hz).

^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 170.4, 149.9, 149.4, 147.7, 146.3, 142.2, 137.4, 132.9, 130.6, 126.8, 123.1, 121.5, 120.5, 120.0, 108.5, 105.5, 102.1, 69.7, 57.2, 55.4, 26.4.

HRMS (ESI-TOF) calcd. for $C_{21}H_{18}NO_6^+$ $[M]^+$: 380.1129, found: 380.1139.

- **Synthesis of Ber-EG1-Gua (S6)**



To a solution of compound **S5** (7.0 mg, 18 μmol) in DMF (500 μL) were added DIPEA (9.62 μL, 55.2 μmol), HBTU (20.9 mg, 14.8 μmol) and HOBt (3.9 mg, 29 μmol), compound **S1** (6.8 mg, 20 μmol) in DMF (500 μL) was added subsequently at r.t 30 min later. After 2.5 h the reaction mixture was concentrated. The crude was roughly purified through column chromatography ($CHCl_3$:MeOH = 100:1→50:1→20:1→15:1→10:1→5:1).

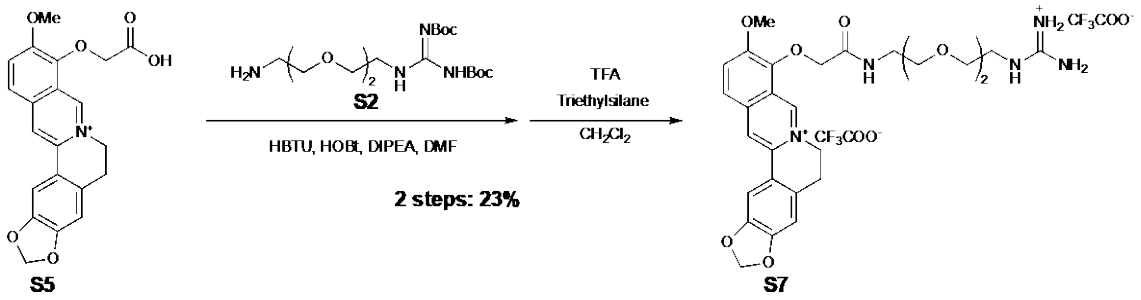
This crude was dissolved in TFA (480 μL) and CH_2Cl_2 (120 μL) mixture solution, and then triethyl silane (4.6 μL, 29 μmol) was added. After 1.5 h the reaction mixture was concentrated and dissolved in DMSO then filtrated through membrane (DISMIC®-13HP, ADVANTEC, 0.45 μm). The fitrate was purified by HPLC to afford yellow solution (4.26 μmol, 23%).

^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.96 (1H, s), 8.93 (1H, s), 8.28 (1 H, t, *J* = 5.4 Hz), 8.20 (1H, d, *J* = 9.0 Hz), 8.02 (1H, *J* = 9.0 Hz), 7.80 (1H, s), 7.53 (1H, t, *J* = 5.4 Hz), 7.10 (1H, s), 6.17 (2H, s), 4.91 (2H, t, *J* = 6.0 Hz), 4.80 (2H, s), 4.05 (3H, s), 3.52- 3.50 (4H, m), 3.28 (4H, q, *J* = 5.4 Hz), 3.21 (2H, t, *J* = 6.0 Hz).

^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 168.1, 157.0, 150.0, 149.9, 147.8, 145.8, 141.9, 137.6, 133.0, 130.7, 126.7, 123.9, 121.4, 120.5, 120.2, 108.5, 105.5, 102.2, 71.8, 68.9, 68.2, 57.2, 55.5, 40.9, 38.1, 26.4.

HRMS (ESI-TOF) calcd. for $C_{26}H_{30}N_5O_6^{2+}$ $[M+H]^{2+:}$ 254.6132, found: 254. 6146.

- **Synthesis of Ber-EG2-Gua (S7)**



To a solution of compound **S5** (5.0 mg, 13 μmol) in DMF (500 μL) were added DIPEA (6.9 μL, 39 μmol), HBTU (14.7 mg, 38.9 μmol) and HOBt (2.9 mg, 29 μmol). Compound **S2** (5.4 mg, 14 μmol) in DMF (500 μL) was added subsequently at r.t 1 h later. After 2 h additional HBTU (7.1 mg, 19 μmol) was added. After another 30 min the reaction mixture was concentrated and roughly purified through column chromatography ($CHCl_3$:MeOH = 80:1→60:1→50:1).

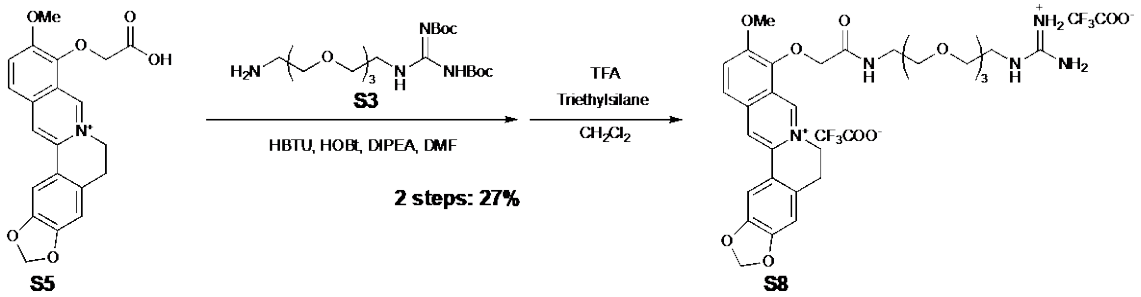
This crude was dissolved in TFA (700 μL) and CH_2Cl_2 (230 μL) mixture solution, and then triethyl silane (6.3 μL, 39 μmol) was added. After 1.5 h the reaction mixture was concentrated and dissolved in DMSO then filtrated through membrane (DISMIC®-13HP, ADVANTEC, 0.45 μm). The filtrate was purified by HPLC to afford yellow solution (3 μmol, 23%).

^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.97 (1H, s), 8.94 (1H, s), 8.27 (1 H, t, *J* = 5.4 Hz), 8.20 (1H, d, *J* = 9.0 Hz), 8.01 (1H, d, *J* = 9.6 Hz), 7.80 (1H, s), 7.60 (1H, t, *J* = 5.4 Hz), 7.10 (1H, s), 6.18 (2H, s), 4.92 (2H, t, *J* = 6.0 Hz), 4.80 (2H, s), 4.05 (3H, s), 3.55 (4H, s), 3.50-3.3.48 (4H, m), 3.33 (2H, q, *J* = 6.0 Hz), 3.27 (2H, q, *J* = 5.4 Hz), 3.21 (2H, t, *J* = 6 .0Hz).

^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 168.4, 157.5, 150.4, 150.3, 148.2, 146.3, 142.4, 138.0, 133.4, 131.1, 127.1, 124.3, 121.8, 120.9, 120.6, 108.9, 105.9, 102.6, 72.1, 70.1, 69.9, 69.4, 68.9, 57.6, 55.9, 41.3, 38.8, 26.8.

HRMS (ESI-TOF) calcd. for $C_{28}H_{35}N_5O_7^{2+}$ $[M+H]^{2+}$: 276.6263, found: 276.6279.

- **Synthesis of Ber-EG3-Gua (S8)**



To a solution of compound **S5** (5.0 mg, 13 μmol) in DMF (500 μL) were added DIPEA (6.9 μL, 39 μmol), HBTU (15.4 mg, 40.7 μmol) and HOBt (3.3 mg, 24 μmol). Compound **S3** (5.7 mg, 13 μmol) in DMF (500 μL) was added subsequently at r.t 1.5 h later. After 1.5 h additional HBTU (14.1 mg, 37.3 μmol) was added. After another 20 min the reaction mixture was concentrated and roughly purified through column chromatography ($CHCl_3$:MeOH = 80:1→60:1→50:1→20:1→5:1).

This crude was dissolved in TFA (680 μL) and CH_2Cl_2 (230 μL) mixture solution, then triethyl silane (6.3 μL, 39 μmol) was added. After 1 h the reaction mixture was concentrated and dissolved in DMSO then filtrated through membrane (DISMIC®-13HP, ADVANTEC, 0.45 μm). The filtration liquor was purified by HPLC to afford yellow solution (3.5 μmol, 27%).

^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.97 (1H, s), 8.94 (1H, s), 8.27 (1 H, t, *J* = 5.4 Hz), 8.20 (1H, d, *J* = 9 Hz), 8.01 (1H, d, *J* = 9.6 Hz), 7.80 (1H, s), 7.60 (1H, t, *J* = 5.4 Hz), 7.10 (1H, s), 6.18 (2H, s), 4.93 (2H, t, *J* = 6.0 Hz), 4.80 (2H, s), 4.05 (3H, s), 3.52-3.49 (4H, m), 3.49-3.47 (8H, m), 3.33 (2H, q, *J* = 6.0 Hz), 3.26 (2H, q, *J* = 5.4 Hz), 3.22 (2H, t, *J* = 6.0 Hz).

^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 167.9, 157.1, 149.9, 149.9, 147.8, 145.9, 142.0, 137.6, 133.0, 130.7, 126.7, 123.8, 121.4, 120.5, 120.2, 108.5, 105.5, 102.1, 71.7, 69.7, 69.7, 69.6, 68.9, 68.5, 57.2, 55.5, 40.9, 38.4, 26.4.

HRMS (ESI-TOF) calcd. for $C_{30}H_{39}N_5O_8^{2+}$ $[M+H]^{2+:}$ 298.6394, found: 298.6405.

Synthesized compounds, Compoud-**S1** to Compound-**S8**, were confirmed by ^{1}H NMR and ^{13}C NMR (Figures S1 to S8).

Fluorescence analysis

C-bulged RNA or RNA G-quadruplex was prepared by annealing the sense and antisense strands or G-quadruplex-forming oligonucleotide, respectively. Varying concentrations (0 to 4 μM) of the RNAs were mixed with berberine derivatives (parental berberine and guanidine-modified derivatives) (50 nM) in a buffer containing 50 mM MES-LiOH (pH 7), 0.5 mM $MgCl_2$, 0.1% DMSO, and 0.01% Tween-20 in the presence of the indicated concentrations of KCl, and subsequently incubated at 25°C at least 60 min. The fluorescence intensity of berberine derivatives in the mixture was measured using a fluorescence microplate reader InfiniteM200 PRO (Tecan, Mannedorf, Switzerland) at 365 nm excitation and 530 nm emission at 25°C. The values of the association constant (K_A) between RNA and berberine derivatives were calculated from changes in the fluorescence signals depending on the RNA concentration according to equation **1** below, assuming 1:1 binding stoichiometry.

$$F = F_{initial} + \left(\frac{F_{final} - F_{initial}}{2 \times [BRB]}\right) \times \left\{\left([BRB] + [RNA] + \frac{1}{K_A}\right) - \sqrt{\left([BRB] + [RNA] + \frac{1}{K_A}\right)^2 - 4 \times [BRB] \times [RNA]}\right\} \quad \mathbf{1}$$

where F is the fluorescence intensity of berberine derivatives at each concentration of RNA, $F_{initial}$ is the fluorescence intensity of berberine derivatives in the absence of RNA, F_{Final} is the fluorescence intensity of berberine derivatives complexed with RNA, and $[RNA]$ and $[BRB]$ are the concentrations of RNA and berberine derivatives, respectively.

Molecular dynamics simulation

Structures of berberine derivatives were constructed by Discovery Studio Visualizer.[1] These derivatives were optimized at HF/6-31G(d) level using Gaussian 09.[2] Structures of complexes between RNA and the berberine derivatives were prepared by fitting the berberine backbone to the parental complex, which was previously analysed by NMR (PDBID: 7A3Y). The box size was set to be a cube with a minimum distance of 20 Å from the complex structure to the box wall. The box was filled with TIP3P water molecules[3] and 0.1 M sodium and chloride ions using AmberTools[4] and ACPYPE[5]. The force fields were applied to ff14SB[6] for RNA and general Amber force field (GAFF)[7] for berberine and its derivatives. The atom charges of berberine derivatives on GAFF were determined using AmberTools based on those calculated by Gaussian 09 at HF/6-31G(d) level. Optimizations of solvent molecules and the entire system were performed for 10,000 steps using GROMACS[8] before starting the molecular the dynamics simulation. The temperature of the system was gradually increased to 310.15 K for 100 ps and subsequently maintained for 1 ns under constant volume. The equilibration simulations of these systems were performed for 1 ns under 1 atm. The sampling simulations were performed for 100 ns under 1 atm. The time step was set to 2 fs with constraints of H-bonds by the LINCS algorithm.[10] Electrostatic interactions were treated with the particle mesh Ewald method[9] using a cut-off of 10 Å. The structurers from 40 to 100 ns were analysed. Analysis of hydrogen bonds was performed using AmberTools.

NMR spectroscopy

NMR data of RNA and its complex with Ber-EG1-Gua were collected on a Bruker AVANCE NEO 800 MHz NMR spectrometer equipped with a cryogenic probe. C-bulged RNA was dissolved in 10 mM Na_2HPO_4 buffer (pH 7) with the addition of 100 mM NaCl, 1 mM EDTA, and 10% ^2H_2O. The final RNA

concentration in 300 µL buffer was 500 µM. Ber-EG1-Gua was added to the RNA sample at a 1:1 molar ratio to record spectra of the RNA/Ber-EG1-Gua complex. 1D ^{1}H and 2D NOESY and TOCSY spectra were acquired at 25°C utilizing excitation sculpting solvent suppression. The processing and assignment of spectra were performed using NMRPipe and CcpNmr software.

Melting analyses of target RNA

Melting profiles of the C-bulged RNA (2 µM) were measured by absorption at 260 nm in a buffer containing 50 mM MES-LiOH (pH 7), 0.5 mM $MgCl_2$, 100 mM KCl, and 0.1% DMSO in the absence or presence of 10 µM berberine derivatives (berberine or Ber-EG1-Gua) with a heating rate of 0.5°C min^{-1} using a Shimadzu-1800 UV/Vis spectrophotometer equipped with a temperature controller. The thermodynamic parameters were calculated from the melting curves following the two-state melting transition model of oligonucleotides.[11]

Preparation of reporter mRNA

Plasmid vector consisting of a reporter gene, *Renilla* luciferase, in which 5′ untranslated region (UTR) contains a secondary structure module of C-bulged RNA, was constructed from previously described pCMV-TnT-T7-RL reporter vector.[12] DNA fragments prepared by annealing sense (5′-CTAGCATTTAGGTGACAGAAGCAGCTCGTCCTGGGCGAACAGGATAGCTGCAGAAAAAA-3′) and antisense (5′-TCGAGTTTTTTCTGCAGCTATCCTGTTCGCCCAGGACGAGCTGCTTCTGTCACCTAAATG-3′), were cloned into *Nhe*I and *Xho*I sites of pCMV-TnT-T7-RL. The sequence of the inserted DNA fragment was analysed using a contract service (Fasmac Co., Ltd.). A control vector, in which 5′ UTR contains canonical hairpin structure consisting of 9 base pairs, and a vector containing G-quadruplex-forming sequence at 5′ UTR were previously constructed.[13, 14] DNA fragments for *in vitro* transcription of reporter mRNA were prepared by PCR amplification using sense and antisense primers listed in Table S1. Reporter mRNAs were transcribed from the DNA fragments using ScriptMAX Thermo T7 Transcription Kit (Toyobo Co., Ltd., Osaka, Japan), and purified using the RNeasy Mini Kit (Qiagen, Venlo, Netherlands)

***In vitro* translation**

Reporter mRNA (final concentration of 100 nM in translation solution) was translated *in vitro* using Rabbit Reticulocyte Lysate System (Promega, Madison, WI, USA) in the absence and presence of indicated concentrations of berberine or Ber-EG1-Gua. Reaction mixtures were incubated at 37 °C for 60 min. The translation reaction was terminated by 10-fold dilution using PBS containing RNase A (20 µg/mL) and puromycin (20 µM), followed by incubation at 37 °C for 10 min. Levels of *Renilla* luciferase expression were determined by measuring the luminescence signal using Varioskan Flash (Thermo Fisher Scientific, Waltham, MA, USA) after adding coelenterazine (at a final concentration of 5 µM).

Reference

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Table S1 K_A values between the C-bulged RNA and berberine derivatives at 25℃.

	berberine	Ber-EG1-Gua	Ber-EG2-Gua	Ber-EG3-Gua
K_A ($\times 10^6$ M^{-1}) [a]	2.70 ± 0.10	28.5 ± 6.30	17.3 ± 1.80	7.90 ± 1.50

a) Values are average ± S.D. calculated from triplicate experiments.

Table S2 K_A values between the C-bulged RNA and berberine or Ber-EG1-Gua at 25℃ in the presence of different potassium concentration.

	K_A ($\times 10^6$ M^{-1}) [a]			
KCl	30 mM	100 mM	300 mM	1 M
berberine	2.10 ± 0.17	2.70 ± 0.10	0.920 ± 0.091	0.294 ± 0.127
Ber-EG1-Gua	79.5 ± 42.5	28.5 ± 6.30	1.90 ± 0.37	0.366 ± 0.267

a) Values are average ± S.D. calculated from triplicate experiments.

Table S3 Total number of simulated hydrogen bonds formed between RNA and berberine derivatives.[a]

	Ber-EG1-Gua	Ber-EG2-Gua	Ber-EG3-Gua
Ligand→RNA[b]	344,345	295,807	295,633
RNA→Ligand[c]	2,429	34,495	24,581
Total	346,774	330,302	320,214

a) Three sets of trajectory images from 40 to 100 ns (total of 180,000 frames) were analyzed.

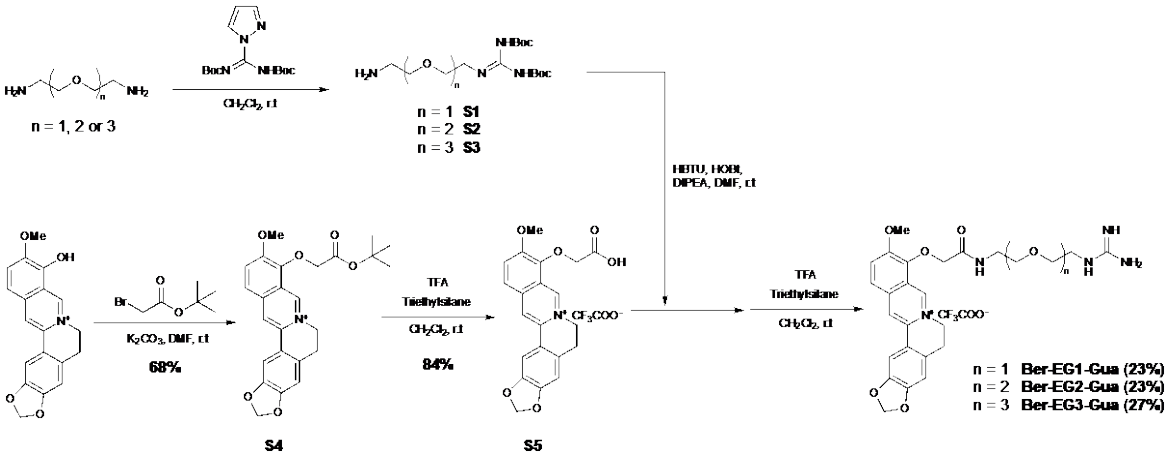
b) "Ligand→RNA" indicates number of hydrogen bonds from berberine derivative (donor) to RNA (acceptor)

c) "RNA→Ligand" indicates number of hydrogen bonds from RNA (donor) to berberine derivative (acceptor)

Table S4 Distribution of hydrogen bonds from berberine derivatives to phosphates in RNA backbone.

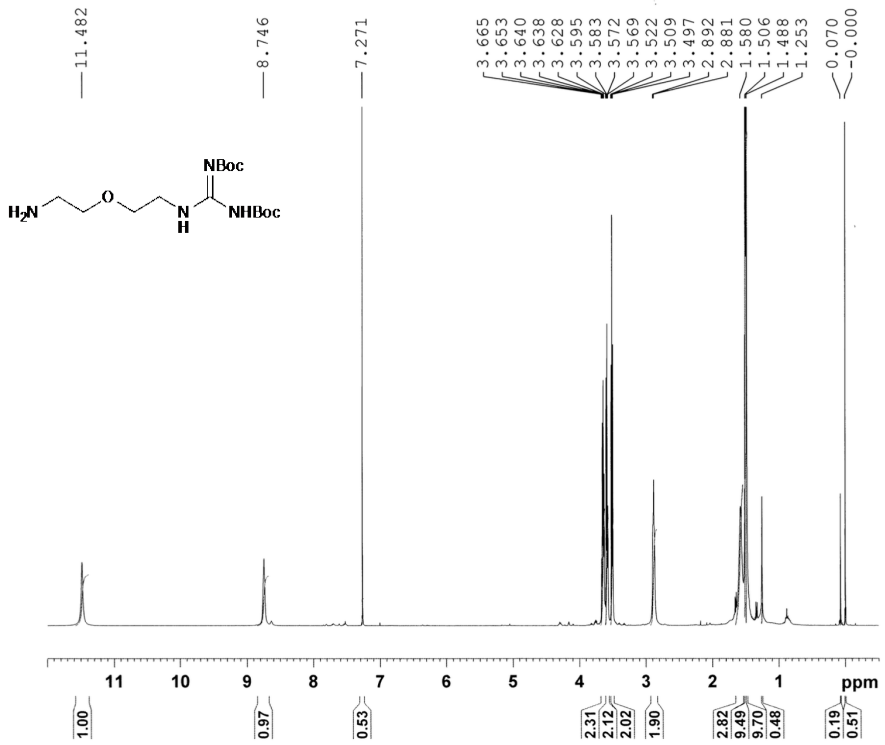
Nucleotide position[a]	Ber-EG1-Gua	Ber-EG2-Gua	Ber-EG3-Gua
C2	132	11,437	9,059
A3	153	16,687	30,863
G4	15	9,208	26,975
C5	0	9,499	3,372
U6	0	99	0
C7	0	0	19
G8	0	29,869	6,736
U9	0	2	411
A15	22	41	290
G16	24,449	8,181	2,438
G17	102,697	36,477	26,226
A18	108,053	46,087	42,171
U19	15,939	16,975	29,059
A20	3,979	1,594	12,679
G21	0	0	845
C22	0	242	0
Total	255,439	186,398	191,143

a) Nucleotide positions not shown in the column did not form hydrogen bonds with berberine derivatives.



Scheme S1. Synthesis of Ber-EGn-Gua.

^{1}H NMR ($CDCl_3$, 400 MHz)



^{13}C NMR ($CDCl_3$, 150 MHz)

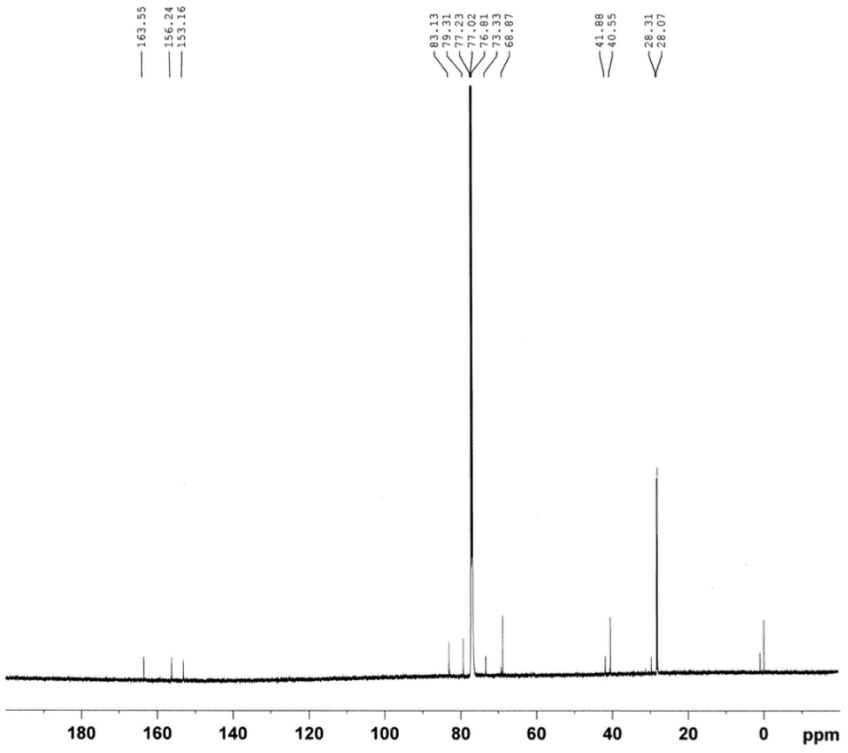
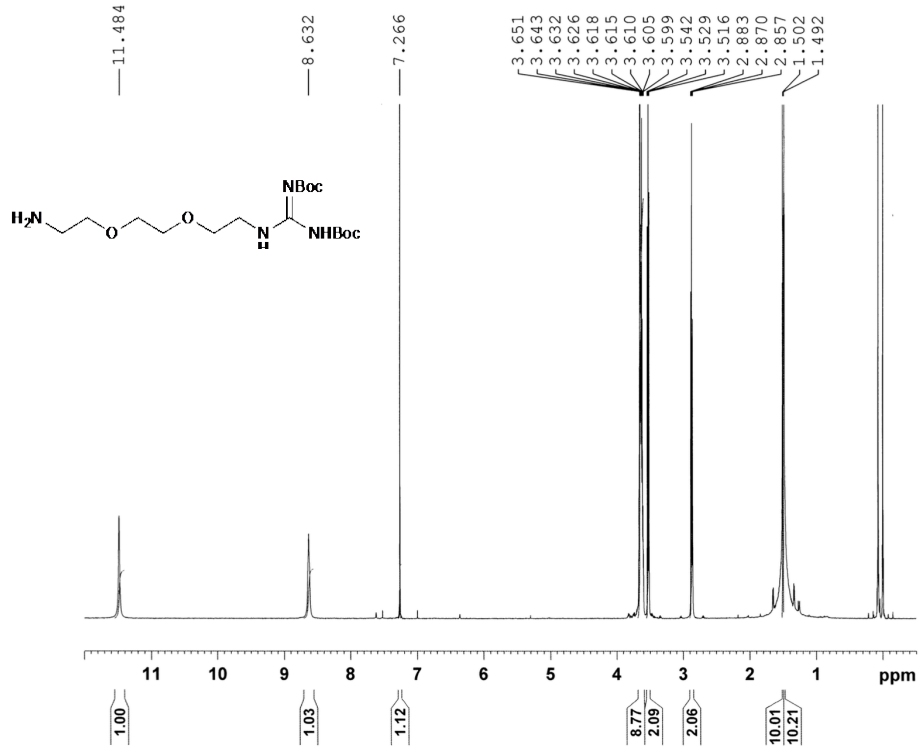


Figure S1 ^{1}H NMR and ^{13}C NMR of Compound-**S1**

^{1}H NMR ($CDCl_3$, 400 MHz)



^{13}C NMR ($CDCl_3$, 150 MHz)

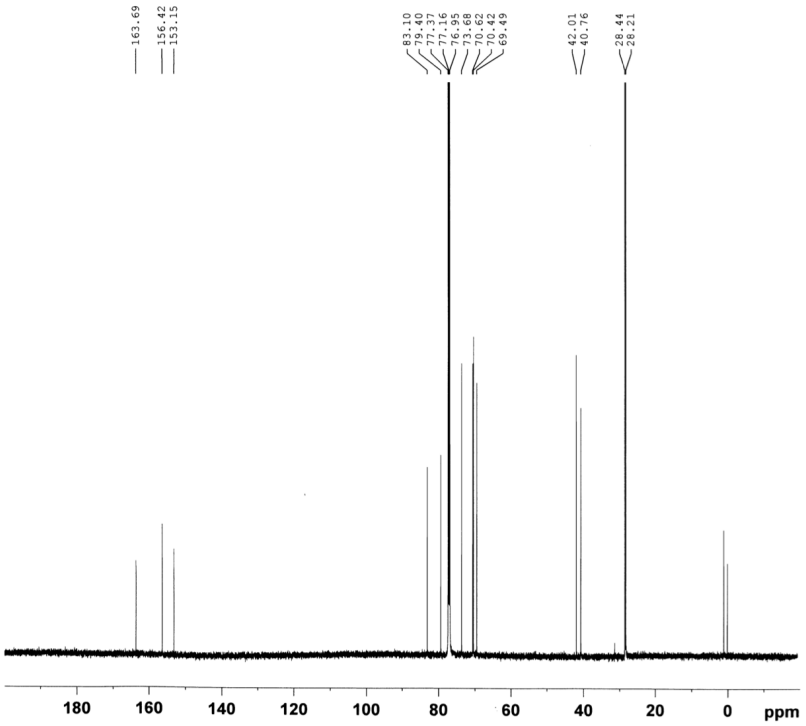
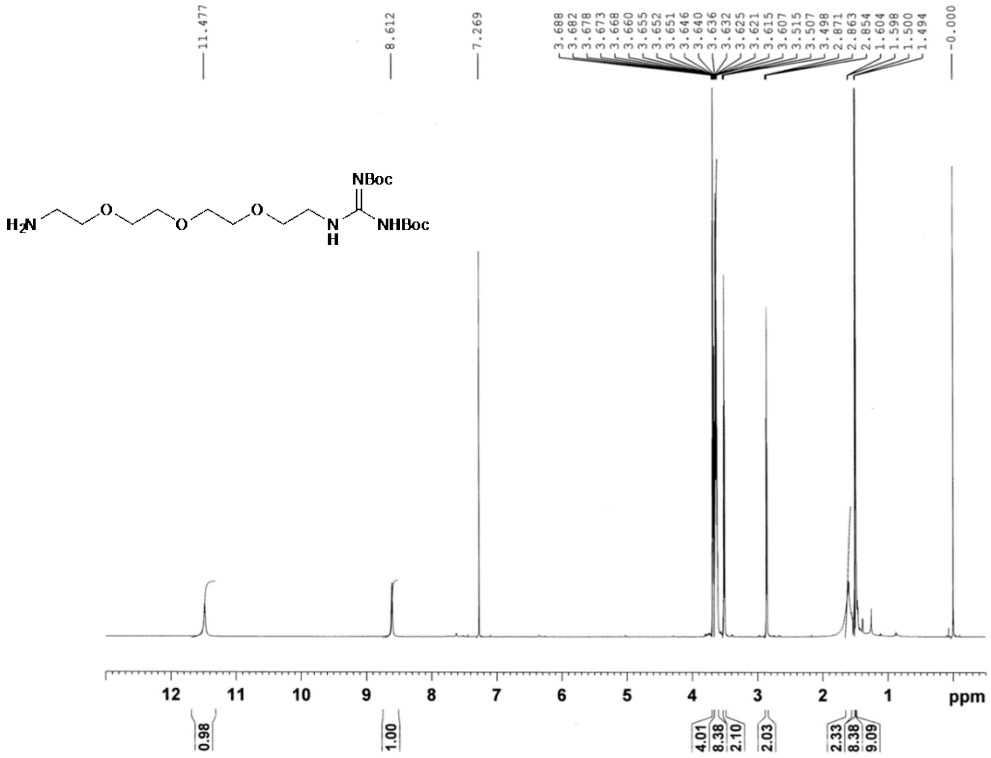


Figure S2 ^{1}H NMR and ^{13}C NMR of Compound-**S2**

^{1}H NMR ($CDCl_3$, 600 MHz)



^{13}C NMR ($CDCl_3$, 150 MHz)

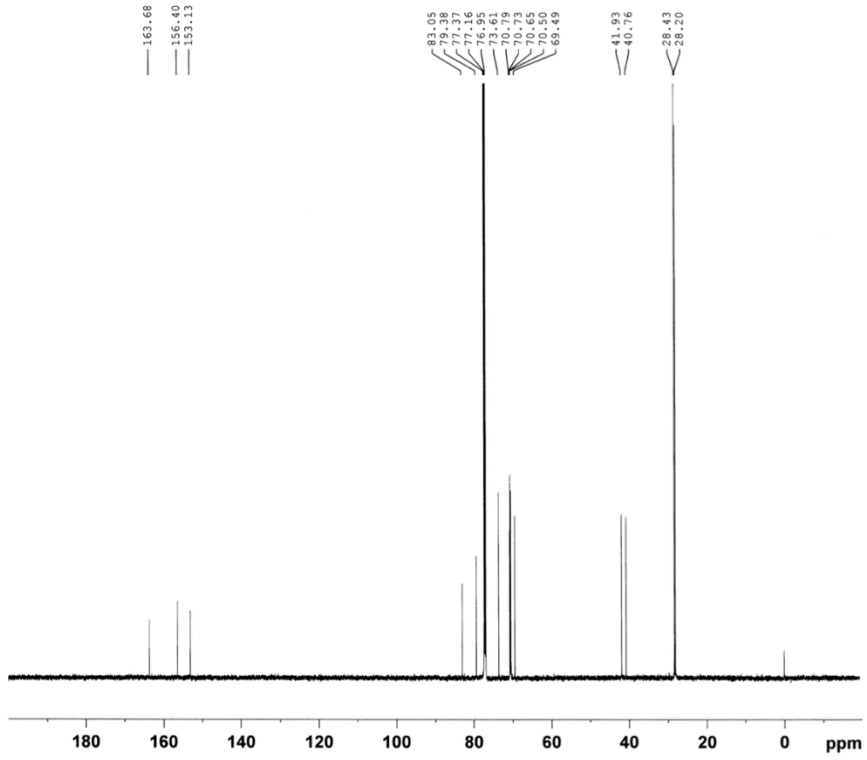
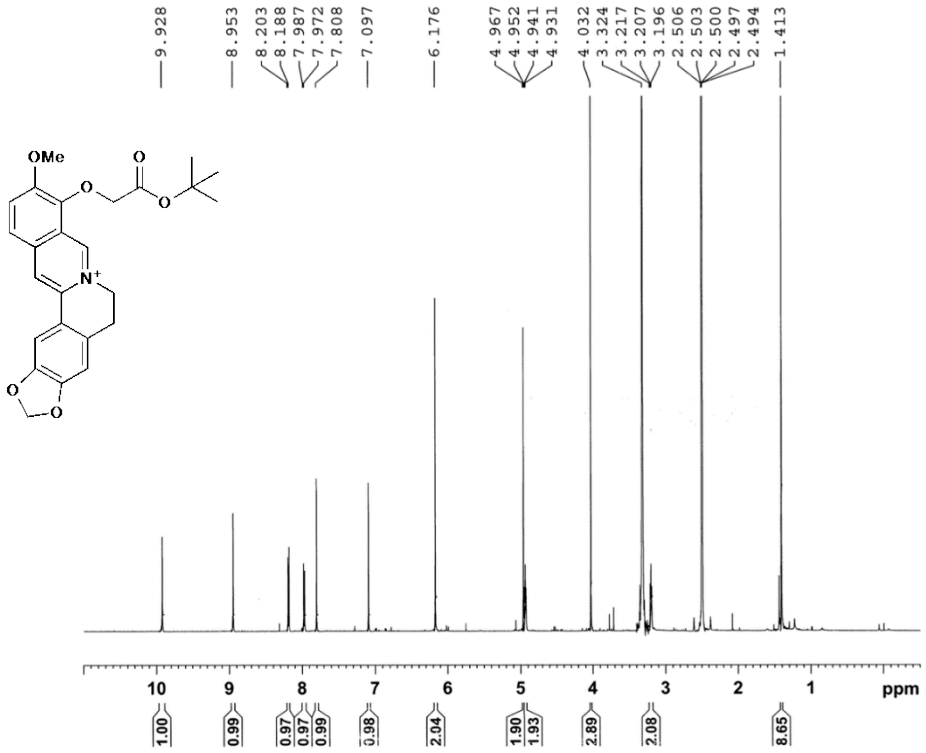


Figure S3 ^{1}H NMR and ^{13}C NMR of Compound-**S3**

^{1}H NMR (DMSO-d_6, 600 MHz)



^{13}C NMR ($CDCl_3$, 150 MHz)

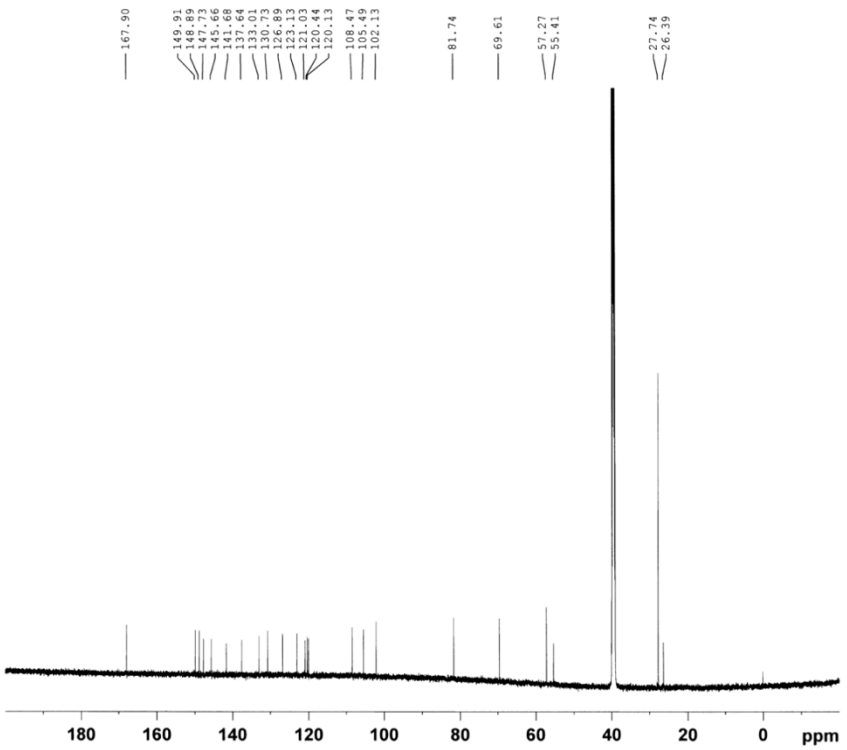
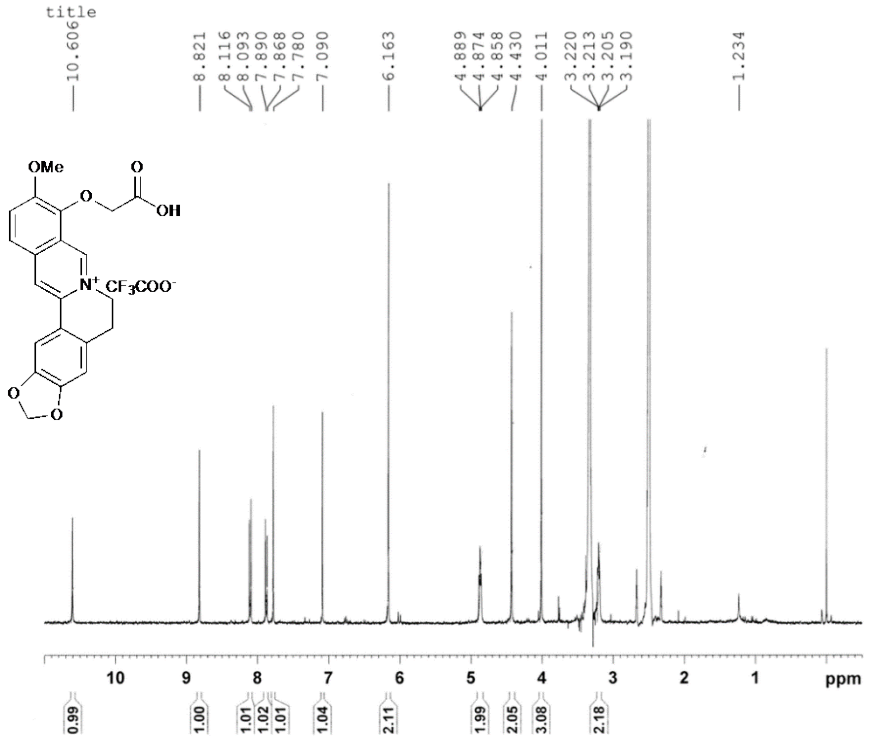


Figure S4 ^{1}H NMR and ^{13}C NMR of Compound-**S4**

^{1}H NMR (DMSO-d_6, 400 MHz)



^{13}C NMR (DMSO-d_6, 150 MHz)

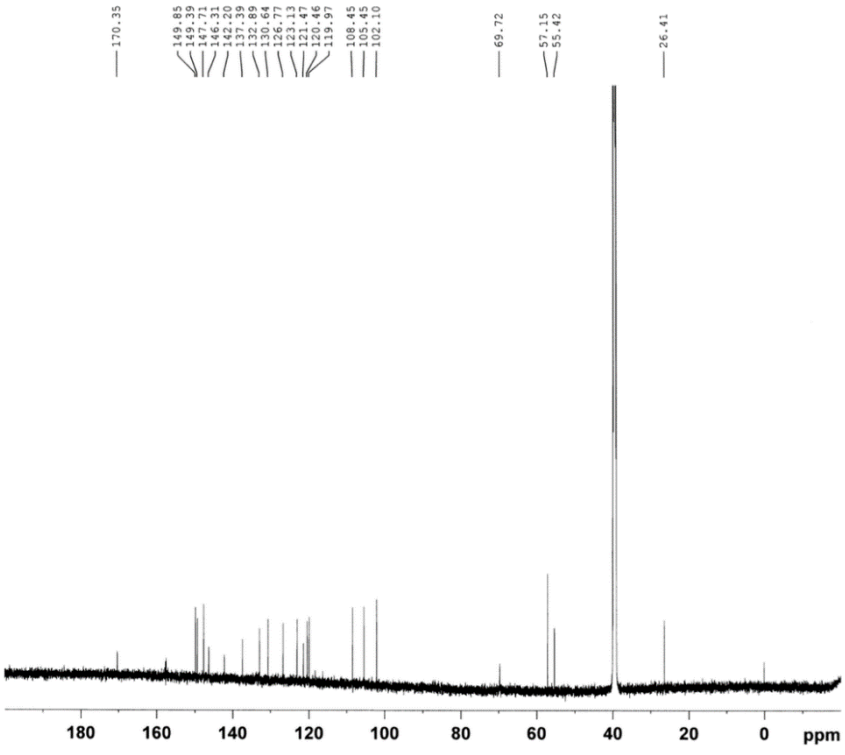
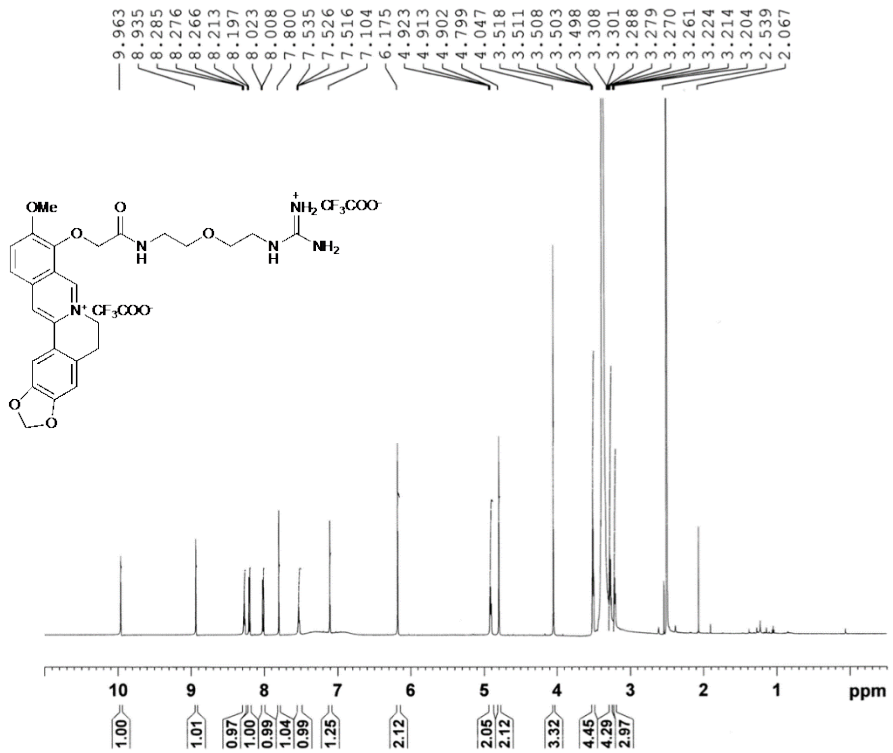


Figure S5 ^{1}H NMR and ^{13}C NMR of Compound-**S5**

^{1}H NMR (DMSO-d_6, 600 MHz)



^{13}C NMR (DMSO-d_6, 150 MHz)

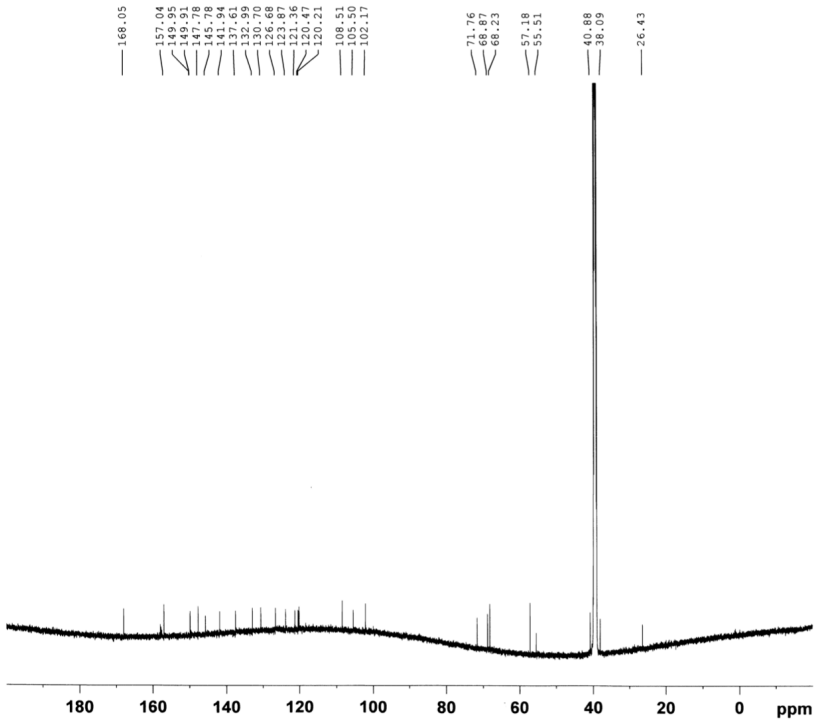
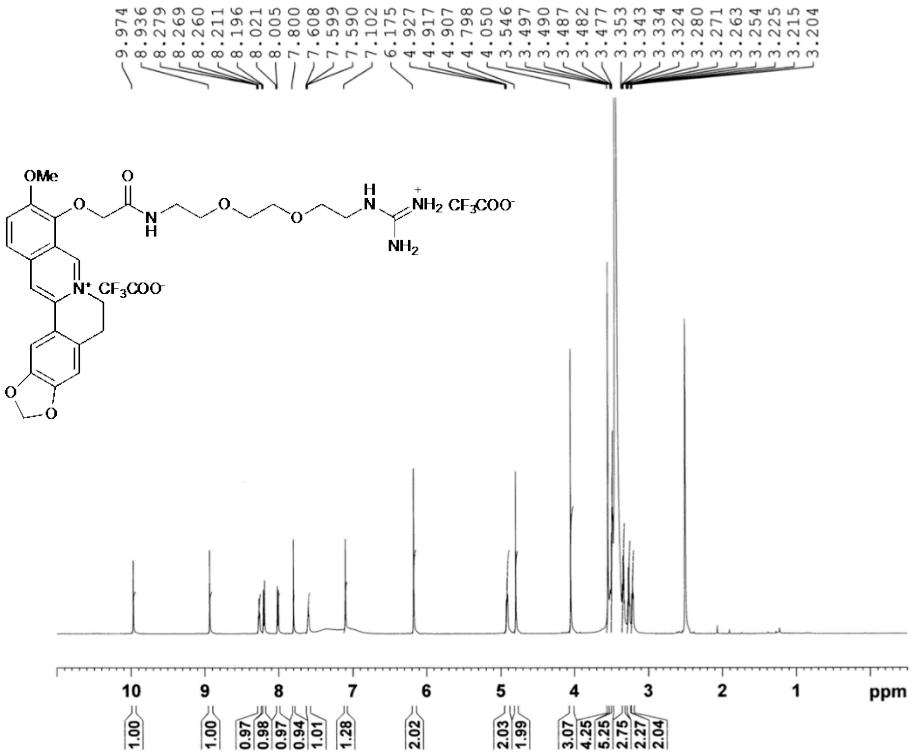


Figure S6 ^{1}H NMR and ^{13}C NMR of Compound-**S6**

^{1}H NMR (DMSO-d_6, 600 MHz)



^{13}C NMR (DMSO-d_6, 150 MHz)

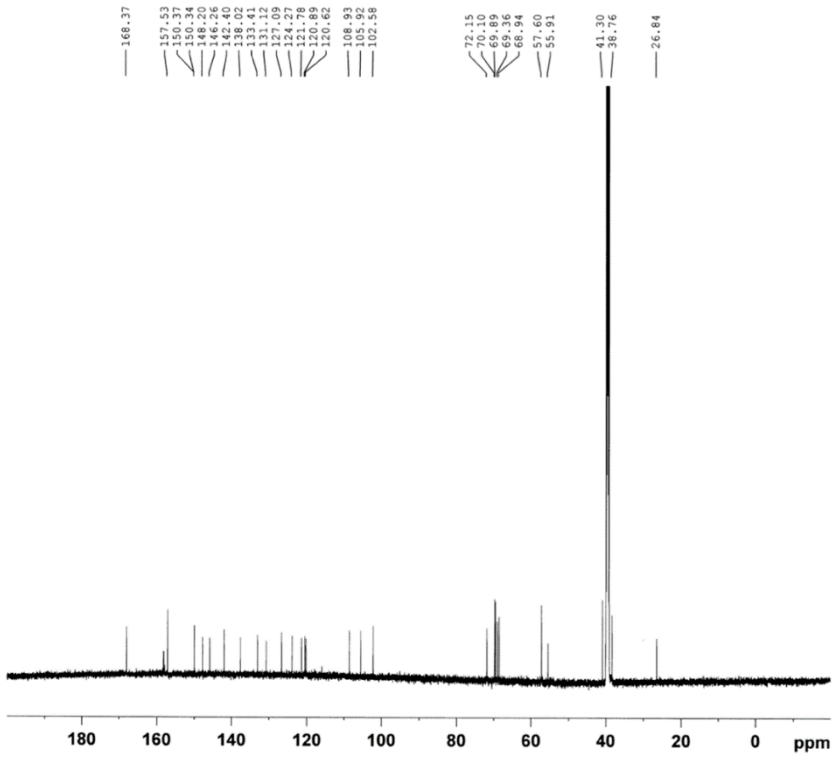
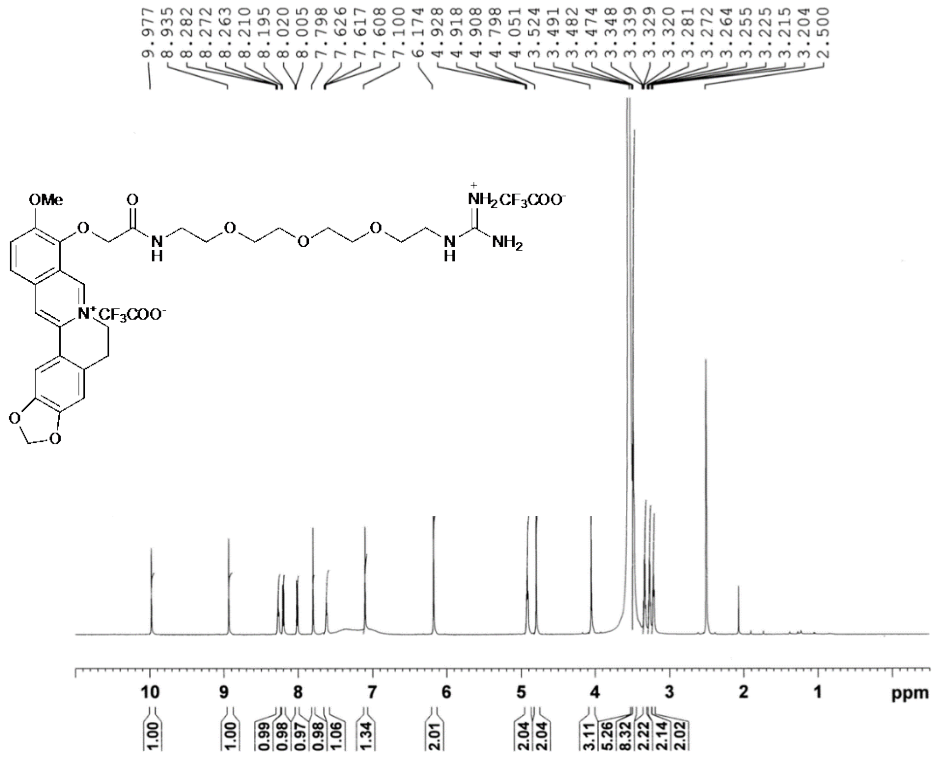


Figure S7 ^{1}H NMR and ^{13}C NMR of Compound-**S7**

^{1}H NMR (DMSO-d_6, 600 MHz)



^{13}C NMR (DMSO-d_6, 150 MHz)

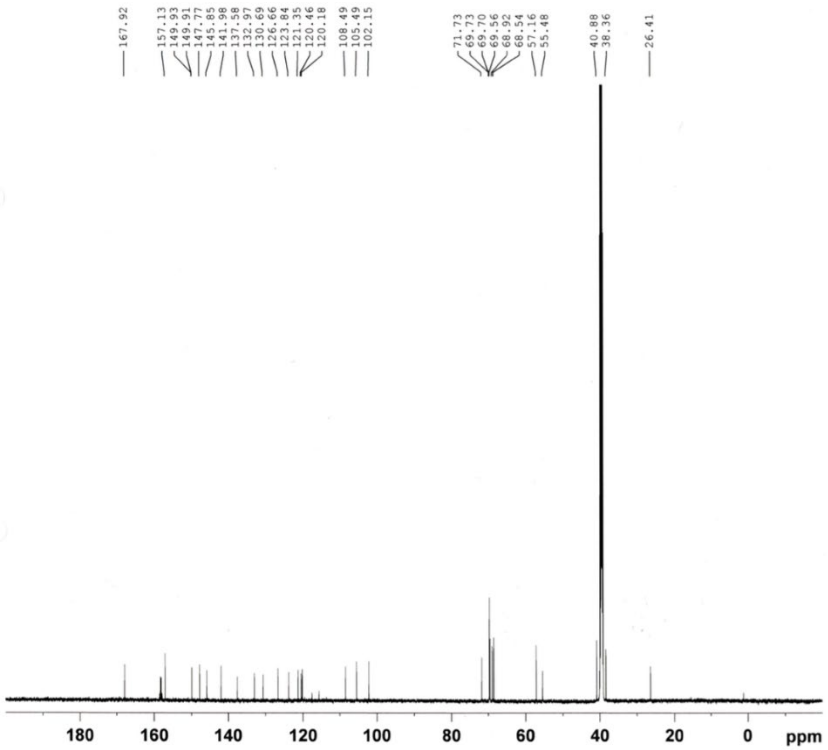


Figure S8 ^{1}H NMR and ^{13}C NMR of Compound-**S8**

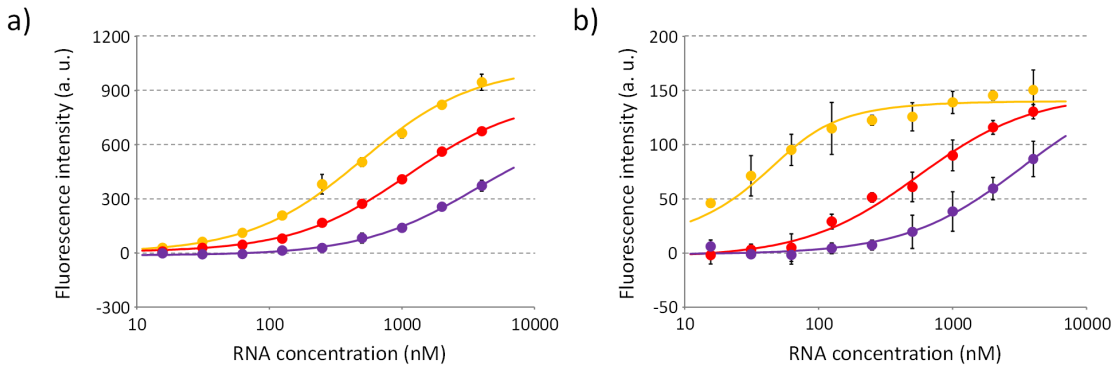


Figure S9 Effect of potassium concentration on interaction between the C-bulged RNA and berberine or Ber-EG1-Gua. (a) Berberine or (b) Ber-EG1-Gua at 50 nM was mixed with various concentrations of the C-bulged RNA in a buffer containing 50 mM MES-LiOH (pH 7), 0.5 mM $MgCl_2$, 0.1% DMSO, and 0.01% Tween-20 in the presence of 30 mM (yellow), 300 mM (red), or 1 M (purple) KCl. The fluorescence signals were measured at 25℃ after 60-min incubation using 365 nm excitation and 530 nm emission. Values and errors represent the average ± S.D. of quadruplicate experiments.

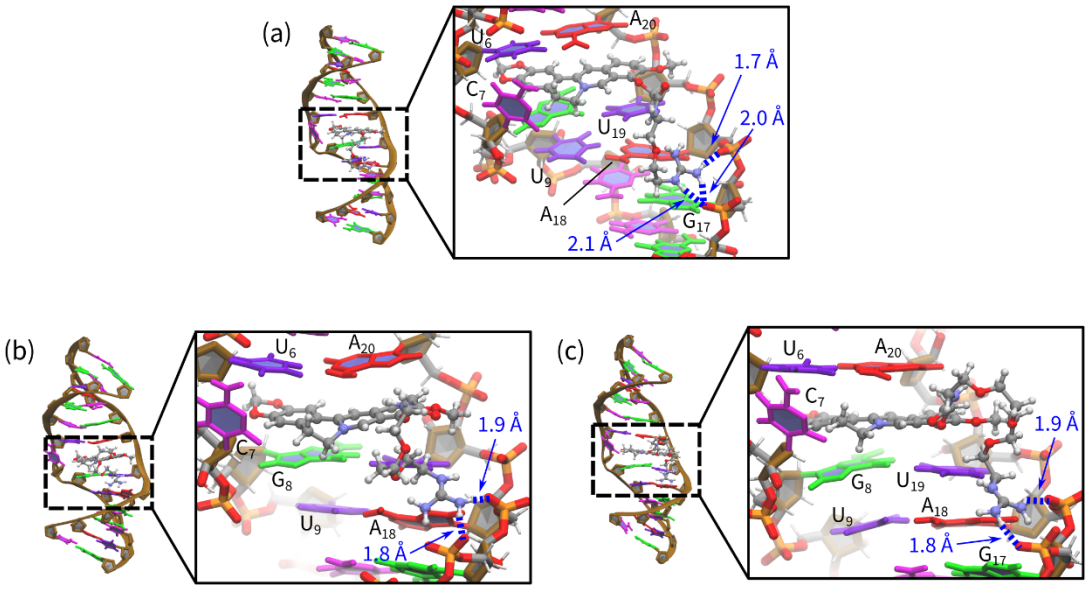


Figure S10 Typical hydrogen bonds formed between guanidine moiety of (a) Ber-EG1-Gua, (b) Ber-EG2-Gua, and (c) Ber-EG3-Gua) and phosphate backbone in RNA. Nucleotide positions are indicated. Distances of hydrogen-bonding atoms between guanidine moiety and phosphate backbone are shown in blue.

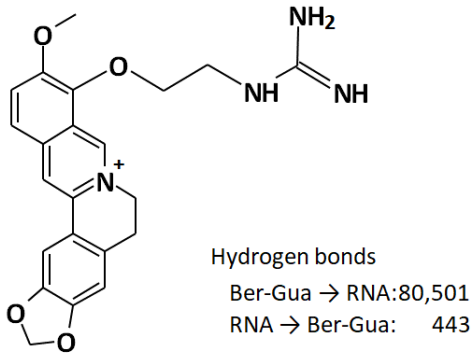


Figure S11 Chemical structure of Ber-Gua and numbers of hydrogen bonds formed between Ber-Gua and RNA in simulation study. Simulation study was performed with 3 sets of trajectory images from 40 to 100 ns (total of 180,000 frames). “Ber-Gua → RNA” indicates number of hydrogen bonds from berberine Ber-Gua (donor) to RNA (acceptor). “RNA → Ber-Gua” indicates number of hydrogen bonds from RNA (donor) to Ber-Gua (acceptor)

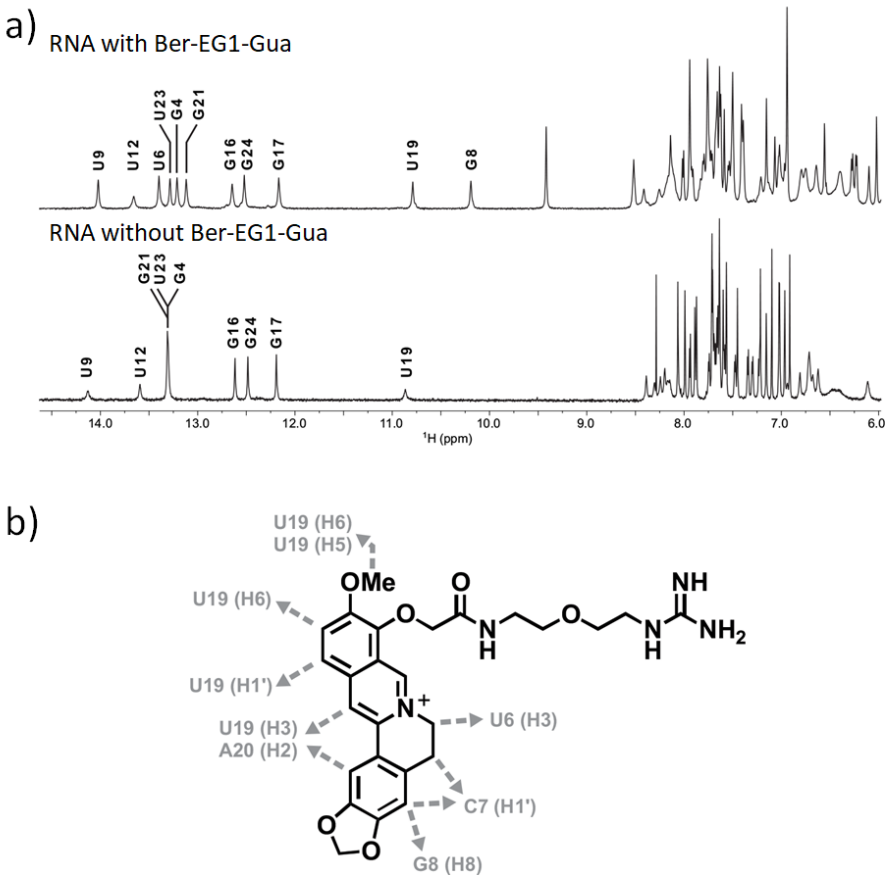
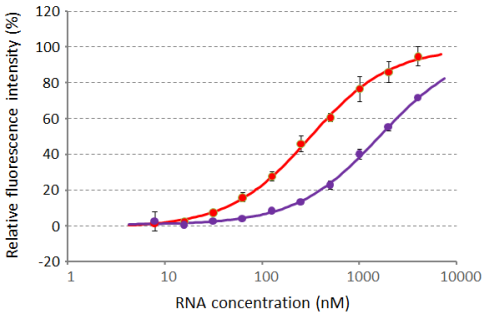


Figure S12 Structure of the complex between the C-bulged RNA and Ber-EG1-Gua analysed by NMR. (a) Comparison of aromatic and imino regions of 1D ^{1}H NMR spectra of RNA with and without Ber-EG1-Gua. Imino proton resonances are assigned. (b) NOE contacts between Ber-EG1-Gua and protons on RNA. Arrows indicate the point of contact with nucleotide protons.

a)

5′-GCCGGGCUGGGGCUGGGCGGGGAAA-3′

b)



	K_A (× 10^6 M^{-1})
berberine	0.63 ± 0.04
Ber-EG1-Gua	3.55 ± 0.10

Figure S13 Interaction between berberine and Ber-EG1-Gua with a model RNA G-quadruplex. (a) Sequence of G-quadruplex-forming RNA oligonucleotide. Guanine tracts involved in formation of G-quartets in the G-quadruplex structures are underlined. (b) Relative fluorescence intensities of berberine or Ber-EG1-Gua mixed with G-quadruplex-forming RNA oligonucleotide. Berberine (purple) or Ber-EG1-Gua (red) at 50 nM was mixed with various concentrations of RNA oligonucleotide in a buffer containing 50 mM MES-LiOH (pH 7), 0.5 mM $MgCl_2$, 100 mM KCl, 0.1% DMSO, and 0.01% Tween-20. The fluorescence signals were measured at 25℃ after 60-min incubation using 365 nm excitation and 530 nm emission. Values and errors represent the average ± S.D. of triplicate samples. Association constant (K_A) values between the model RNA G-quadruplex and berberine or Ber-EG1-Gua at 25℃ are shown right side of the panel.

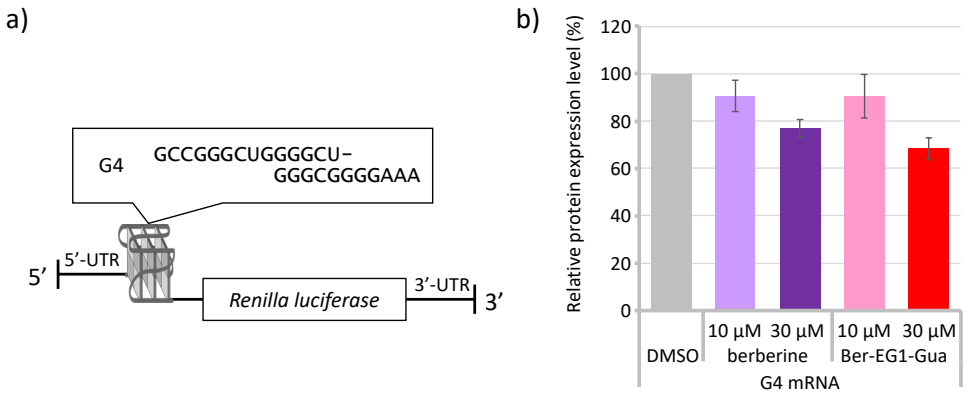


Figure S14 Suppression of protein expression from mRNA containing G-quadruplex module by berberine or Ber-EG1-Gua. (a) Reporter mRNA construct consisting of coding region of *Renilla Luciferase* and G-quadruplex-forming module at 5′ untranslated region (UTR). (b) Relative protein expression levels from the reporter mRNA. mRNA (100 nM) was mixed with rabbit reticulocyte lysate and translated at 37 ℃ for 60 min in the absence or presence of indicated concentrations of berberine or Ber-EG1-Gua. Expression levels of Renilla Luciferase were evaluated by luminescence signals after addition of coelenterazine. Luminescence signals were normalized by those obtained from the control sample in the absence of additional chemical. Values are average ± s.d. obtained from triplicated experiments.

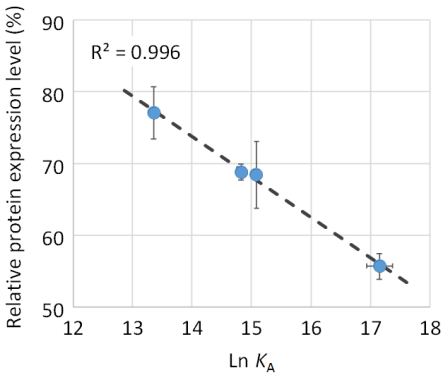


Figure S15 Correlation between suppression efficiency of protein expression and interaction affinity between berberine derivatives and RNAs. Relative protein expression levels in the presence of 30 μM berberine or Ber-EG1-Gua comparing to the absence of chemical were plotted on natural logarithm of K_A values calculated from fluorescence titration experiments in Figure 2 and Figure S11.