Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Synthesis of oligonucleotides possessing versatile probes for PET labelling and their rapid ligand-free click reaction

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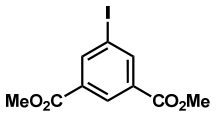
Experimental Section

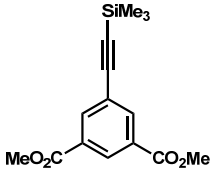
General Methods. All reactions were carried out under an argon atmosphere, unless otherwise noted. All reagents and solvents were purchased from commercial vendors and used without further purification, unless indicated otherwise. Pyridine was distilled over CaH_2 and stored over activated molecular sieves 4Å. ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM AL-400 spectrometer (400 MHz for ^1H NMR, 100 MHz for ^{13}C NMR, and 162 MHz for ^{31}P NMR). Chemical shifts (δ) were expressed in parts per million and internally referenced (0.00 ppm for tetramethylsilane (TMS)/$CDCl_3$, 2.49 ppm for DMSO-d_6, or 3.31 ppm for CD_3OD for ^1H NMR, 77.0 ppm for $CDCl_3$, 29.8 ppm, 39.5 ppm for DMSO-d_6, or 49.0 ppm for CD_3OD for ^{13}C NMR and 0.00 ppm for H_3PO_4/$CDCl_3$ for ^{31}P NMR). IR spectra were measured with a Shimadzu FTIR-8400S instrument. Mass spectra were taken on a JEOL JMS-SX102A instrument. Elemental analyses were performed by YANACO MT-5 instrument. Flash column chromatography was performed using silica gel C-300 from Wako Pure Chemical Industries, Ltd. and 60N (neutral) from Kanto Chemical Co., Inc. Absorbance was measured by GE Health care NanoVue spectrophotometer.

Dimethyl 5-iodoisophthalate (5)



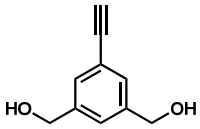
Dimethyl 5-aminoisophthalate (**4**) (6.276 g, 30.0 mmol) was stirred with 2N HCl (40 mL). When most of the ester has dissolved, the reaction mixture was cooled to 0 ºC and a solution of sodium nitrite (2.52 g, 36.5 mmol) in water (23 mL) was added dropwise the mixture was stirred for an additional 2 h at 0 ºC. An ice-cold solution of potassium iodide (7.48 g, 45.0 mmol) in water (70 mL) was added dropwise. The resulting mixture was warm to room temperature and was then stirred for 12 h. The aqueous mixture was extracted with EtOAc, and the combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (*n*-hexane) and recrystallized from MeOH to give dimethyl 5-iodoisophthalate as a pale yellow solid (5.29 g, 55%); mp 103–105 ºC, ^1H NMR (400 MHz, $CDCl_3$) δ 8.26 (t, J = 1.4 Hz, 1H), 8.53 (d, J = 1.4 Hz, 2H), 3.94 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2 (2C), 142.9 (2C), 132.6 (2C), 130.5, 93.8, 53.1 (2C); MS (EI) m/z 320 (M^+), HRMS (EI) Calcd for $C_{10}H_9O_4I$ (M^+): 319.9546. Found: 319.9553. Anal. Calcd for $C_{10}H_9O_4I$: C, 37.52; H, 2.83. Found: C, 37.44; H, 2.86.

Dimethyl 5-trimethylsilylisophthalate (6)



A solution of trimethylsilylacetylene (83.9 μL, 0.6 mmol) and piperidine (500 μL 5.0 mmol) in THF (5mL) was added dropwise to a mixture of dimethyl 5-iodoisophthalate (96.0 mg 0.3 mmol), bis(triphenylphosphine)palladium dichloride (16.8 mg, 4 mol%), cuprous iodide (4.6 mg, 8 mol%), and triphenylphosphine (11.8 mg, 15 mol%). The mixture was stirred for 12 h at room temperature, then the solvent was removed in vacuo. The organic residue was washed with saturated NH_4Cl solution and brine (each three times), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (*n*-hexane/EtOAc, 100:1) to give dimethyl 5-trimethylsilylisophthalate as a colorless solid (82.7 mg, 95 %); mp 103–105 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (t, J = 1.7 Hz, 1H), 8.29 (d, J = 1.7 Hz, 2H), 3.95 (s, 6H). 0.26 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8 (2C), 137.1 (2C), 131.0 (2C), 130.5, 124.4, 102.9, 96.9, 52.7 (2C), 0.0 (3C); MS (EI) m/z 290 (M^+), HRMS (EI) Calcd for $C_{15}H_{18}O_4Si$ (M^+): 290.0974. Found: 290.0979. Anal. Calcd for $C_{15}H_{18}O_4Si$: C, 62.04; H, 6.25. Found: C, 61.86; H, 6.23.

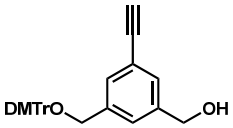
5-Ethynyl-1,3-benzenedimethanol (1)



Lithium aluminum hydride (2.60 g, 114 mmol) was added to a solution of dimethyl 5-trimethylsilylisophthalate (6.64 g, 22.9 mmol) in THF (100 mL) at room temperature. The mixture was heated at 60 ºC for 12 h. The solution was then cooled in an ice bath and saturated $NaHCO_3$ solution is carefully added dropwise. The mixture was diluted with EtOAc. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (*n*-hexane/EtOAc, 2:1) to give 5-ethynyl-1,3-benzenedimethanol as a yellow solid (2.26 g, 69%); mp 79–81 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (s, 2H,), 7.38 (s, 1H), 4.70 (d, J = 6.0 Hz, 4H), 3.08 (s, 1H), 1.68 (t, J = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.4 (2C), 129.6 (2C), 125.7 (2C), 122.5, 83.3, 77.2, 64.6 (2C); MS (EI) m/z 162 (M^+), HRMS (EI) Calcd for $C_{10}H_{10}O_2$ (M^+): 162.0681. Found: 162.0685. Anal. Calcd for $C_{10}H_{10}O_2$: C, 74.06; H,

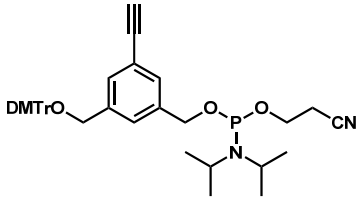
6.21. Found: C, 73.83; H, 6.19.

1-[3-(4,4'-Dimethoxytrityloxymethyl)-5-(hydroxymethyl)ethynyl]benzene (7)



A mixture of 5-ethynyl-1,3-benzenedimethanol (649 mg, 2.45 mmol) and DMTrCl (1.6 g, 4.72 mmol) in pyridine (20 mL) was stirred for 12 h at room temperature. The mixture was then poured into saturated $NaHCO_3$ solution. The layer were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography on neutral silica gel (*n*-hexane/EtOAc, 2:1) to give 1-[3-(4,4'-dimethoxytrityloxymethyl)-5-(hydroxymethyl)ethynyl]benzene as a yellow solid (941 mg, 50%); mp 56–58 ºC, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.41 (s, J = 7.6 Hz, 2H), 7.35–7.23 (m, 10H), 6.91 (d, J = 3.2 Hz, 4H), 5.26 (t, J = 5.6 Hz, 1H), 4.47 (d, J = 5.6 Hz, 2H), 4.16 (s, 1H), 4.06 (s, 2H), 3.73 (s, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 160.1 (2C), 146.5, 143.3, 141.1, 137.3 (2C), 131.2 (4C), 130.3, 130.1, 129.2 (2C), 128.8 (2C), 127.8, 127.0, 123.7, 114.1 (4C), 87.9, 84.5, 78.5, 66.3, 64.6, 55.7 (2C); IR (KBr) 3271, 3055, 3033, 2999, 2931, 2896, 2860, 2833, 1607, 1506, 1445, 1300, 1250, 1175, 1034, 829 cm^{-1}; MS (EI) m/z 464 (M^+), HRMS (EI) Calcd for $C_{31}H_{28}O_4$ (M^+): 464.1987. Found: 464.1993. Anal. Calcd for $C_{31}H_{28}O_4 \cdot 1/2H_2O$: C, 78.62; H, 6.17. Found: C, 78.67; H, 6.12.

[1-[3-[[(2-Cyanoethoxy)(*N*,*N*-diisopropylamino)phosphanyl]oxymethyl]-5-(4,4'-dimethoxytrityloxymethyl)ethnyl]benzene (2)

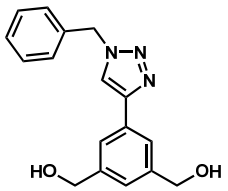


Chloro(2-cyanoethoxy)(*N,N*-diisopropylamino)phosphane (440 μL, 2.0 mmol) was added dropwise to a solution of 1-[3-(4,4'-dimethoxytrityloxymethyl)-5-(hydroxylmethyl)ethynyl]benzene (646 mg, 1.0 mmol) and *N,N*-diisopropylethylamine (860 μL, 5.0 mmol) in THF (5.0 mL). The mixture was stirred for 1 h at room temperature. The mixture was then poured into saturated $NaHCO_3$ solution and $CHCl_3$. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, and

concentrated in vacuo. The residue was purified by flash column chromatography on neutral silica gel (*n*-hexane/EtOAc, 1:1) to give [1-[3-[[(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphanyl]oxymethyl]-5-(4,4'-dimethoxytrityloxymethyl)ethnyl]benzene as a yellow solid (253 mg, 37%); ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.3. MS (EI) m/z 664 (M^+), HRMS (EI) Calcd for $C_{40}H_{45}N_2O_5P$ (M^+): 664.3066. Found: 664.3056.

Solid Support Synthesis. A mixture of 1-[3-(4,4'-dimethoxytrityloxymethyl)-5-(hydroxymethyl)ethynyl]benzene **7** (674.9 mg, 1.45 mmol), succinic anhydride (580.8 mg, 5.80 mmol), and DMAP (1.91 mg, 0.017 mmol) in pyridine (3.9 mL) was stirred for 72 h at room temperature. The mixture was then poured into saturated $NaHCO_3$ solution and $CHCl_3$. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give the corresponding succinate. Aminopropyl controlled pore glass (CPG) (838 mg, 77 μmol) was added to a solution of the succinate and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (720 mg, 3.76 mmol) in DMF (15 mL), and the mixture was kept for 72 h at room temperature. After the resin was washed with pyridine, a capping solution (8 mL, 0.1 M DMAP in pyridine/Ac_2O, 9:1, v/v) was added and the whole mixture was kept for 16 h at room temperature. The resin was washed with pyridine, EtOH, MeCN, and dried in vacuo. The amount of loaded compound **7** to the solid support (**3)** was 48.9 μmol/g from calculation of released dimethoxytrityl cation by a solution of 70% $HClO_4$/EtOH, 3:2 (v/v).

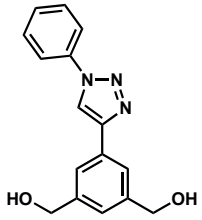
1-Benzyl-4-[3,5-bis(hydroxymethyl)phenyl]-1H-1,2,3-triazole (22)



Sodium ascorbate (59.4 mg, 0.3 mmol) and copper (II) sulfate pentahydrate (74.9 mg, 0.3 mmol) were added to a stirred suspension of **1** (48.7 mg, 0.3 mmol) and benzylazide (39.9 mg 0.3 mmol) in 50% EtOH (4 mL), and stirring is continued for 8 h. The mixture was diluted with EtOAc. The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH, 10:1~5:1) to give 1-benzyl-4-[3,5-bis(hydroxymethyl)phenyl]-1H-1,2,3-triazole as a colorless solid (71.7 mg, 85%); mp 150–152 ºC, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (s, 1H), 7.65 (s, 2H),

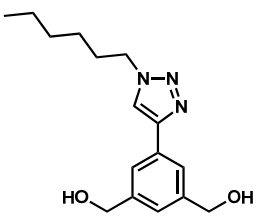
7.38–7.33 (m, 5H), 7.26 (s, 1H), 5.62 (s, 2H), 5.23 (t, J = 5.6 Hz, 2H), 4.51 (d, J = 5.6 Hz, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ 149.1, 143.8 (2C), 136.8, 131.7, 130.0 (2C), 129.6, 129.1 (2C), 126.4 124.1 (2C), 122.3, 64.9, 55.0; IR (KBr) 3408, 3367, 3178, 2878, 2372, 1607, 1456, 1229, 1159, 1070, 1057, 1024, 716 cm^{-1}; MS (EI) m/z 295 (M^+), HRMS (EI) Calcd for $C_{17}H_{17}N_3O_2$ (M^+): 295.1321. Found: 295.1327. Anal. Calcd for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.8; N, 14.23. Found: C, 68.90; H, 5.68; N, 14.27.

1-Phenyl-4-[3,5-bis(hydroxymethyl)phenyl]-1H-1,2,3-triazole (23)



1-Phenyl-4-[3,5-bis(hydroxymethyl)phenyl]-1H-1,2,3-triazole was prepared from **1** (48.7 mg, 0.3 mmol), phenylazide (35.7 mg 0.3 mmol), sodium ascorbate (59.4 mg, 0.3 mmol), and copper (II) sulfate pentahydrate (74.9 mg, 0.3 mmol) in 50% EtOH (4 mL) by the method described for 1-benzyl-4-[3,5-bis-(hydroxymethyl)phenyl]-1H-1,2,3-triazole. Purification by flash column chromatography on silica gel ($CHCl_3$/MeOH, 10:1~5:1) to give 1-phenyl-4-[3,5-bis(hydroxymethyl)phenyl]-1H-1,2,3-triazole as a colorless solid (71.7 mg, 85%); mp 146–148 ºC, ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.1 (s, 1H,), 7.98 (d, 2H, J = 7.2 Hz), 7.79 (s, 2H), 7.62 (t, 2H, J = 7.2 Hz), 7.50 (t, 1H, J = 7.2 Hz), 7.28 (s, 1H), 5.29 (t, 2H, J = 5.2 Hz), 4.57 (d, 4H, J = 5.2 Hz). ^{13}C-NMR (100 MHz, CD_3OD) δ 149.5, 143.9 (2C), 138.4, 131.5, 130.9 (2C), 130.0, 126.6, 124.2 (2C), 121.4 (2C), 120.3, 65.0. IR (KBr) 3400, 3329, 3265, 3134, 3096, 2872, 2841, 1599, 1506, 1418, 1238, 1076, 1065, 987, 835, 754, 687 cm^{-1}. MS (EI) m/z 281 (M^+), HRMS Calcd for $C_{16}H_{15}N_3O_2$: 281 (M^+), Found: 281.1171. Anal. Calcd for $C_{16}H_{15}N_3O_2$: C, 68.31; H, 5.37; N, 14.94, Found: C, 68.13; H, 5.44; N, 14.92.

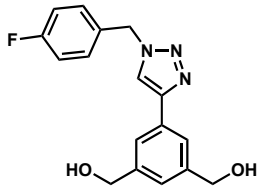
1-Hexyl-4-[3,5-bis(hydroxymethyl)phenyl]-1H-1,2,3-triazole (24)



1-Hexyl-4-[3,5-bis(hydroxymethyl)phenyl]-1H-1,2,3-triazole was prepared from **1** (48.7

mg, 0.3 mmol), hexylazide (38.1 mg 0.3 mmol), sodium ascorbate (59.4 mg, 0.3 mmol), and copper (II) sulfate pentahydrate (74.9 mg, 0.3 mmol) in 50% EtOH (4 mL) by the method described for 1-benzyl-4-[3,5-bis-(hydroxymethyl)phenyl]-1H-1,2,3-triazole. Purification by flash column chromatography on silica gel ($CHCl_3$/MeOH, 10:1~5:1) to give 1-hexyl-4-[3,5-bis(hydroxymethyl)phenyl]-1H-1,2,3-triazole as a colorless solid (27.2 mg, 29%); mp 60–62 ºC, ^{1}H NMR (400 MHz, DMSO-d_6) δ ; 8.56 (s, 1H), 7.65 (s, 2H), 7.21 (s, 1H), 7.25-7.19 (m, 3H), 5.23 (t, 2H, J = 5.6 Hz), 4.53 (d, 4H, J = 5.6 Hz), 4.37 (t, 2H, J = 6.8 Hz), 1.98-1.85 (m, 2H), 1.26-1.15 (m, 6H), 0.84 (s, 3H),^{13}C-NMR (100 MHz, CD_3OD) δ 148.8, 143.9 (2C), 131.9, 126.4, 124.1 (2C), 122.3, 65.0 (2C), 51.5, 32.6, 31.3, 27.2, 23.6, 14.3. IR (KBr) 3310, 3292, 3275, 3254, 3150, 3132, 3096, 2953, 2907, 2856, 2727, 2615, 2376, 1717, 1699, 1541, 1429, 1155 cm^{-1}. MS (EI) m/z 289 (M^+), HRMS Calcd for $C_{16}H_{23}N_3O_2$: 289.1790 Found: 289.1800. Anal. Calcd for $C_{16}H_{23}N_3O_2$: C, 66.41; H, 8.01; N, 14.52, Found: 66.59; H, 8.26; N, 13.88.

1-(4-Fluoro)benzyl-4-[3,5-bis(hydroxymethyl)phenyl]-1H-1,2,3-triazole (25)



1-(4-Fluoro)benzyl-4-[3,5-bis(hydroxymethyl)phenyl]-1H-1,2,3-triazole was prepared from **1** (48.7 mg, 0.3 mmol), 4-fluorobenzylazide (45.3 mg 0.3 mmol), sodium ascorbate (59.4 mg, 0.3 mmol), and copper (II) sulfate pentahydrate (74.9 mg, 0.3 mmol) in 50% EtOH (4 mL) by the method described for 1-benzyl-4-[3,5-bis-(hydroxymethyl)phenyl]-1H-1,2,3-triazole. Purification by flash column chromatography on silica gel ($CHCl_3$/MeOH, 10:1~5:1) to give 1-(4-fluoro)-benzyl-4-[3,5-bis(hydroxymethyl)phenyl]-1H-1,2,3-triazole as a colorless solid (71.7 mg, 85%); mp 145–147 ºC, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (s, 1H), 7.65 (s, 2H), 7.43 (dt, J = 6.0, 2.4 Hz, 2H), 7.25–7.19 (m, 3H), 5.62 (s, 2H), 5.22 (t, J = 5.6 Hz, 2H), 4.52 (d, J = 5.6 Hz, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ 164.3 (d, J = 242.0 Hz), 149.3, 143.9 (2C), 133.0, 131.8, 131.4 (d, J = 9.0 Hz, 2C), 126.5, 124.1 (2C), 122.3, 116.8 (d, 2C, J = 22.2 Hz), 65.0 (2C), 55.0. IR (KBr) 3364, 3352, 3111, 3080, 2916, 2966, 2735, 2623, 1603, 1510, 1443, 1219, 1022, 883 cm^{-1}; MS (EI) m/z 313 (M^+), HRMS (EI) Calcd for $C_{17}H_{16}FN_3O_2$ (M^+): 313.1227. Found: 313.1217. Anal. Calcd for $C_{17}H_{16}FN_3O_2$: C, 65.17; H, 5.15; N, 13.41. Found: C, 64.97; H, 5.00; N,

13.34.

RNA Synthesis. Synthesis was carried out with a DNA/RNA synthesizer (ABI Expedite 3400 or NTS H-6) by phosphoramidite method. Deprotection of the bases and phosphates was performed in concentrated NH_4OH/EtOH, 3:1 (v/v) at room temperature for 12 h. TBDMS groups were removed by 1.0 M tetrabutylammonium fluoride (TBAF) in THF at room temperature for 12 h. The reaction was quenched with 0.1 M TEAA buffer (pH 7.0) and desalted on a Sep-Pak C18 cartridge. Deprotected 2-5As were purified by reverse phase HPLC. Deprotected short single strand RNAs were purified by 20 % PAGE containing 7 M urea.

MALDI-TOF/MS Analysis of 2-5As and single strand RNAs. Spectra were obtained with a time-of-flight mass spectrometer equipped with a nitrogen laser (337 nm, 3-ns pulse) (SHIMAZU KRATOS). A solution of 3-hydroxypicolinic acid (3-HPA) and diammonium hydrogen citrate in H_2O was used as the matrix. 2-5A tetramer: Calcd for $C_{40}H_{48}N_{20}O_{25}P_4$ $[M–H]^-$: 1332.2. Found: 1332.5. **8**: Calcd for $C_{40}H_{46}N_{15}O_{23}P_4$ $[M–H]^-$: 1228.2. Found: 1229.8. **9**: Calcd for $C_{40}H_{46}N_{15}O_{23}P_4$ $[M–H]^-$: 1228.2. Found: 1231.2. **18**: Calcd for $C_{231}H_{281}N_{89}O_{161}P_{23}$ $[M–H]^-$: 7589.0. Found: 7589.9. **19**: Calcd for $C_{221}H_{268}N_{87}O_{154}P_{22}$ $[M–H]^-$: 7285.0. Found: 7285.3. **10**: $C_{47}H_{53}N_{18}O_{23}P_4$ $[M–H]^-$: 1361.3. Found: 1364.2. **11**: $C_{46}H_{51}N_{18}O_{23}P_4$ $[M–H]^-$: 1347.2. Found: 1349.3. **12**: $C_{46}H_{59}N_{18}O_{23}P_4$ $[M–H]^-$: 1355.3. Found: 1359.9. **14**: Calcd for $C_{47}H_{52}FN_{18}O_{23}P_4$ $[M–H]^-$: 1379.2. Found: 1381.9. **15**: Calcd for $C_{47}H_{52}FN_{18}O_{23}P_4$ $[M–H]^-$: 1379.2. Found: 1381.5. **16**: Calcd for $C_{46}H_{59}N_{15}O_{27}P_5$ $[M–H]^-$: 1408.2. Found: 1410.3. **17**: Calcd for $C_{53}H_{65}N_{18}O_{27}P_5$ $[M–H]^-$: 1559.3. Found: 1558.5. **20**: Calcd for $C_{238}H_{287}FN_{92}O_{161}P_{23}$ $[M–H]^-$: 7740.1. Found: 7733.2. **21**: Calcd for $C_{228}H_{274}FN_{90}O_{154}P_{22}$ $[M–H]^-$: 7436.1. Found: 7435.7.

General procedure for rapid CuCAAC reaction. To an eppendorf tube was added RNA contained 5-ethynyl-1,3-benzenedimethanol **1 (**100 μM solution in miliQ water, 20 μL, 2 nmol), azide (1 mM solution in DMSO, 3 μL, 3 nmol), $CuSO_4{\cdot}5H_2O$ (100 mM solution in miliQ water, 2 μL, 200 nmol), Sodium Ascorbate (100 mM solution in miliQ water, 2 μL, 200 nmol), DMSO (17 μL), MeCN (22 μL), and sterile 0.1 M phosphate buffer (pH 7.0) (156 μL). The mixture was vortexed for approximately 1 sec**,** and then left for 15 min. The mixture was passed through a membrane filter (ADVANTEC, DISMIC$^{@}$-13CP, 0.45 μm) and washed with miliQ water (300 μL). The filtrate was purified by reverse-phase HPLC to give the corresponding RNA possessing 1,4-triazole unit. HPLC analysis was performed using a Shimadzu LC-10ADvp system consisting of

an SCL-10A*vp* controller, two LC-10ADvp pumps, a DGU-12A degasser, an SPD-10A VP UV detector (set at 260 nm). Inertsil®ODS-3 column (5 μm, 4.6 × 150 mm) was used for HPLC separation and analysis.

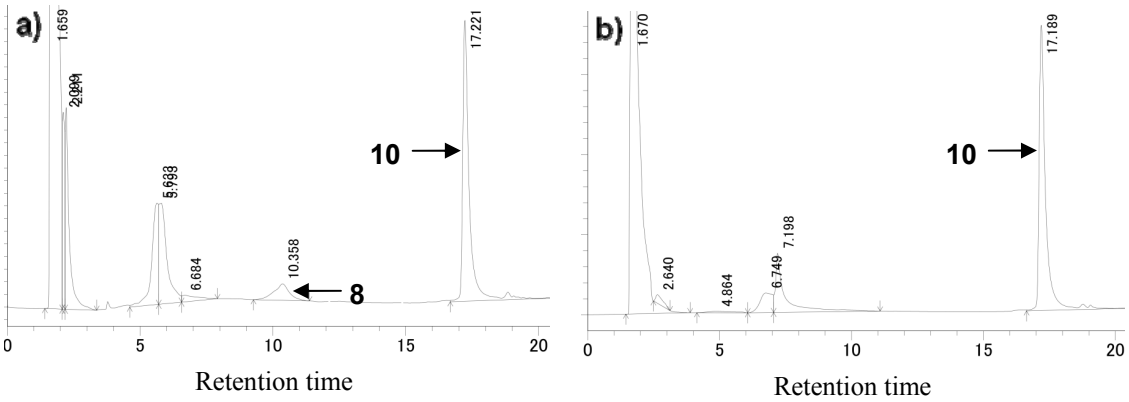


Fig. S1. RP-HPLC analysis of rapid CuCAAC reaction between **8** and benzylazide.
a) Reaction performed with a molar ration benzylazide/RNA = 1.
b) Reaction performed with a molar ration benzylazide/RNA = 1.5.

Construction of an expression plasmid. Recombinant human RNase L was expressed and purified according to the reported procedure with a slight modification as below[1]. cDNA encoding human RNase L was amplified by reverse transcription-polymerase chain reaction (RT-PCR) from total RNA of HepG2 cells. Reverse transcription was performed using SuperScriptII® reverse transcriptase (Gibco) according to the manufacture's instructions. PCR was carried out using *Pfu* turbo DNA polymerase (Stratagene) and oligonucleotide primers (sense, 5′-CCGAATTCATGGAGAGCAGGGAT-3′ containg an *Eco*RI site; antisense, 5′-GGGTAAAGCTTATGGACTAGTGTAGTCTGGG-3′ containg a *Hin*dIII site) The PCR product was ligated into the *Eco*RI and *Hin*dIII. The nucleotide sequence of the cDNA in the resulting vector (pKKRNL) was subsequently verified by dideoxy sequencing.

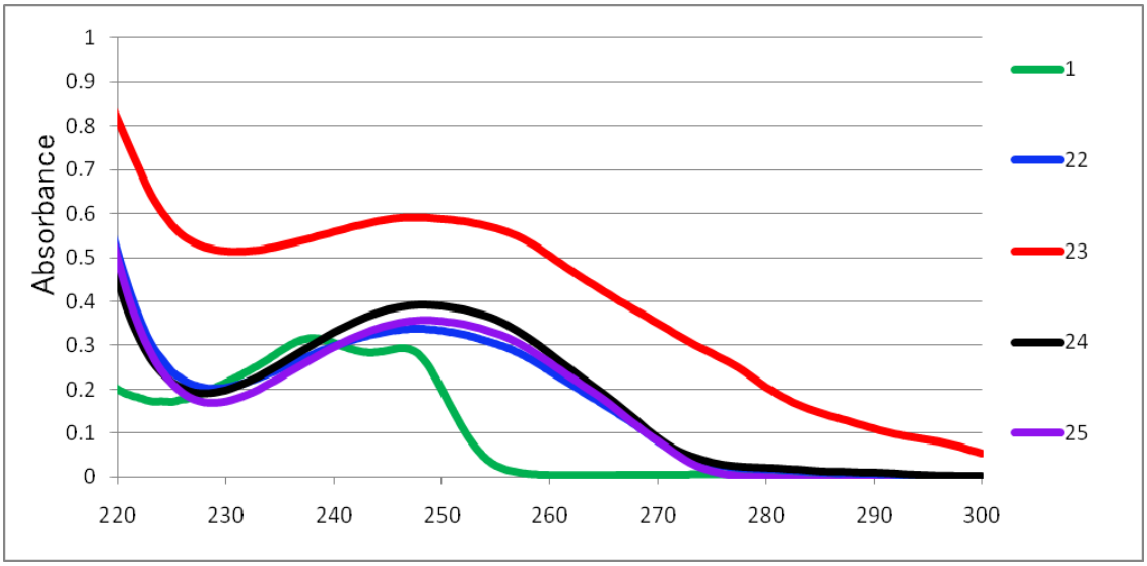
Expression of RNase L. *E.coli* JM105 harboring pKKRNL was grown at 30 ºC overnight in 40 mL of LB medium containing 50 μg/mL ampicillin. The culture was transferred to 4 liters of the same medium and then incubated on a rotary shaker at 30 ºC. When OD_{600} reached 0.6, expression was induced with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside. The bacterial cells were collected after an additional 5 cultivation by centrifugation at 5000 ×g for 10 min and stred at –20 ºC.

Purification of recombinant RNase L. All steps were carried out at 4 ºC. Frozen cells were thawed and then homogenized in 200 mL of buffer R1 comprising 25 mM Tris-HCl (pH 7.5), 50 mM KCl, 10% glycerol, 1 mM EDTA, 5 mM $MgCl_2$, 0.1 mM ATP and 14 mM 2-mercaptoethanol by sonication disruption. Cell debris was removed from the homogenate by centrifugation at 12000 ×g for 15 min at 4 ºC. The cell extract was loaded onto a SP-Sepharose column (2.4 × 13 cm) that had been preequilibrated with buffer R1 at the flow rate of 50 mL/h. The column was washed with buffer R1 for 5 h. RNase L was eluted with 500 mL of buffer R1 containing a linear gradient of NaCl, from 0 to 1 M. Each fraction was subjected to SDS-PAGE and the fractions containing RNase L were pooled. The pooled fractions were diluted 3-fold with buffer R1 and then loaded onto a Red-Sepharose column (2.4 × 6 cm) preewuilibrated with buffer R1 at the flow rate of 30 mL/h. After extensive washing of the column with buffer R1, the enzyme was eluted with 300 mL of buffer R1 containing a linear gradient of NaCl, from 0 to 1.5 M. The fractions containg RNase L were pooled and concentrated to 5 mL with a YM-10 ultrafiltration membrane. A sample was then subjected to gel filtration on a Sephacryl S200-column (1.6 × 90 cm) that had been preequilibrated with buffer R1 at the flow rate of 30 mL/h. The fractions containg RNase L were pooled and concentrated to 5 mL with a YM-10 ultrafiltration membrane. A sample was measured by Bradford protein assay.

RNA cleavage by recombinant human RNase L activated with 2-5A analogues. 2-5As (2-5A tetramer, **14**, **15,** and **17**) (final concentrations, 0.5 to 500 nM), each mixed with recombinant human RNase L (final concentration, 100 mM) in a buffer comprising 20 mM Tris-HCl (pH 7.5), 10 mM magnesium acetate, 8 mM 2-mercaptoethanol, 90 mM KCl, and 0.1 mM ATP (total, 18 μL), and then the mixtures were incubated on ice. After 30 min, 2 μL of a solution containing 2 μM r($C_{11}U_2C_7$) with fluorescein at the 5′-end was added to each mixture (total, 20 μL), and then the solution was incubated at 30 ºC. After 30 min, the reaction was terminated by the addition of 20 μL of a loading solution containing 9 M urea, 1 mM EDTA, 0.02% xylene cyanol and 0.02% bromophenol blue. An aliquot (18 μL) of the mixture was electrophoresed on a 20% polyacrylamide gel containing 7 M urea. The fluorescence intensities on the gel were determined with a LAS-4000 fluorescence gel scanner and Multi Gauge 3.0 software (Fujifilm, Tokyo, Japan).

Determination of molar extinction coefficient. The molar extinction coefficient (ε_{260}) of **1**, **22**, **23**, **24** and **25** (30 μM) in MeOH was measured with a SHIMADZU UV-2450

UV-VIS spectrophotometer. **1**: 167 cm^{-1}. **22**: 8030 cm^{-1}. **23**: 16800 cm^{-1}. **24**: 9367 cm^{-1} **25**: 6700 cm^{-1}.



Reference

1. A. Yoshimura, M. Nakanishi, C. Yatome and Y. Kitade, *J. Biochem.*, 2002, **132**, 643–648.