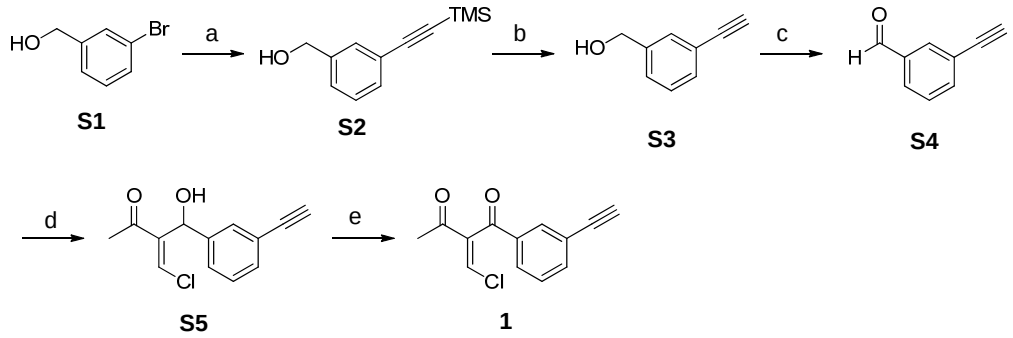
Supporting Information

Pin-Point Chemical Modification of RNA with Diverse Molecules through the Functionality Transfer Reaction and the Copper-Catalyzed Azide-Alkyne Cycloaddition Reaction

Kazumitsu Onizuka, [a,b] Atsushi Shibata, [a,b] Yosuke Taniguchi, [a,b] Shigeki Sasaki*[a,b]

[a]Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan, and CREST, Japan Science and Technology Agency, 4-1-8 Motomachi, Kawaguchi, Saitama 332-0012, Japan.,
*Corresponding author: 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan, TEL: +81-92-642-6615, FAX: +81-92-642-6786, e-mail: sasaki@phar.kyushu-u.ac.jp

Scheme S1 Synthesis of the diketone transfer group (**1**)



(a) TMS-acetylene, $PdCl_2(PPh_3)_2$, CuI, Et_3N, 70 °C, 66%; (b) TBAF, THF, rt, 91%; (c) DMP, CH_2Cl_2 ,rt, 96%; (d) 3-butyn-2-one, $TiCl_4$, dimethyl sulfide, CH_2Cl_2, 0°C, 55% (*E* isomer), 21% (*Z* isomer); (e) DMP, CH_2Cl_2 ,rt, 95%.

Synthesis of S3.[1] $PdCl_2(PPh_3)_2$ (35 mg, 0.050 mmol) and CuI (9.5 mg, 0.050 mmol) were added into a solution of 3-bromobenzylalcohol (**S1**)(935 mg, 5.00 mmol) in dry triethylamine (12.5 mL) under argon at room temperature, followed by the slow addition of TMS-acetylene (750 μL, 5.31 mmol). The mixture was refluxed under argon for 24 hrs. After cooling to room temperature, the mixture was diluted with diethylether and the whole was filtered through a celite pad. The filtrate was evaporated to give a crude oil, which was chromatographed on a silica gel column (hexane:AcOEt=4:1) to give **S2** as a brown oil (673 mg, 66%). TBAF•3(H_2O) was added into a solution of **S2** (300 mg, 1.47 mmol) in THF (3 mL) and the mixture was stirred for 1.5 h at room temperature. The solvents were evaporated to give a crude oil, which was chromatographed on a silica gel column (hexane:AcOEt=4:1) to give **S3** as a pale yellow oil (177 mg, 91%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm:7.48 (1H, s), 7.40 (1H, d, J = 7.6 Hz), 7.33 (1H, d, J = 7.6 Hz), 7.29 (1H, t, J = 7.6 Hz), 4.65 (2H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): 141.06, 131.23, 130.46, 128.49, 127.28, 122.28, 83.46, 77.24, 64.62. IR (cm^{-1}): 3330(br), 3293, 2103, 1482, 1017. ESI-HRMS (m/z): calcd. for C_9H_8O 133.0648 ($[M+H]^+$), found 133.0640.

Synthesis of S5.[2] Dess-Martin periodinane (DMP)(61 mg, 0.462 mmol) was added into a solution of **S3** in CH_2Cl_2 (1 mL), and the mixture was stirred for 1 h under argon at room temperature. The mixture was quenched by the addition of 20% aqueous $Na_2S_2O_3$ solution (15 mL) and saturated aqueous $NaHCO_3$ (15 mL), and the whole was extracted with AcOEt (20 ml×3). The organic solvents were washed with brine, dried over Na_2SO_4, then evaporated to give a crude oil, which was chromatographed on a silica gel column (hexane:AcOEt=5:1) to give **S4** as pale yellow solids (58 mg, 96%). 3-Butyn-2-one (74 μL, 0.946 mmol) and dimethyl sulfide ((2.3 μL, 0.031 mmol) were added into a solution of **S4** in dry CH_2Cl_2 (230 μl) at 0 °C under argon, followed by the addition of dry CH_2Cl_2 solution of $TiCl_4$

(1M, 315 μL, 0.315 mmol). The whole was stirred at 0 °C for 2.5 h. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$, and the whole was filtered through a celite pad. The filtrate was dried over Na_2SO_4, and evaporated to give a crude oil, which was chromatographed on a silica gel column (hexane:AcOEt=8:1) to give **S5** as a pale yellow oil (*E*-isomer: 40.7 mg, 55%; *Z*-isomer: 15.8 mg, 21%). *E*-isomer: ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 7.45 (1H, s), 7.44 (1H, s), 7.36 (2H, d, *J* = 7.0 Hz), 7.28 (1H, t, *J* = 7.0 Hz), 5.89 (1H, s), 4.35 (1H, brs), 3.04 (1H, s), 2.30 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): 198.06, 142.92, 141.80, 136.02, 131.20, 128.82, 128.47, 125.70, 122.25, 83.55, 77.25, 70.38, 27.04. IR (cm^{-1}): 3472(br), 3291, 2105, 1667, 1592, 1368, 1234. *Z*-isomer: ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 7.95 (1H, t, *J* = 1.2 Hz), 7.86 (1H, dt, *J* = 7.6, 1.2 Hz), 7.71 (1H, dt, *J* = 7.6, 1.2 Hz), 7.56 (1H, s), 7.46 (1H, t, *J* = 7.6 Hz), 3.13 (1H, s), 2.32 (3H, s). IR (cm^{-1}): 3472(br), 3291, 2105, 1667, 1592, 1368, 1234. ESI-HRMS (m/z): calcd. for $C_{13}H_{11}ClO_2$ 257.0340, 259.0314 ($[M+Na]^+$), found 257.0335, 259.0338.

Synthesis of 1. Dess-Martin periodinane (DMP)(101 mg, 0.238 mmol) was added into a solution of **S5** in CH_2Cl_2 (1.5 mL), and the mixture was stirred for 4 h under argon at room temperature. The mixture was quenched by the addition of 20% aqueous $Na_2S_2O_3$ solution (10 mL) and saturated aqueous $NaHCO_3$ (10 mL), and the whole was extracted with AcOEt (10 ml×3). The organic solvents were washed with brine, dried over Na_2SO_4, then evaporated to give a crude oil, which was chromatographed on a silica gel column (hexane:$CHCl_3$ =4:1) to give **1** as white solids (26.4 mg, 95%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 7.95 (1H, t, *J* = 1.2 Hz), 7.86 (1H, dt, *J* = 7.6, 1.2 Hz), 7.71 (1H, dt, *J* = 7.6, 1.2 Hz), 7.56 (1H, s), 7.46 (1H, t, *J* = 7.6 Hz), 3.13 (1H, s), 2.32 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): 192.36, 191.88, 143.37, 137.59, 135.43, 135.01, 132.81, 129.18, 129.15, 123.40, 82.16, 78.77, 27.44. IR (cm^{-1}): 3288, 2111, 1688, 1668, 1594, 1368, 1325, 1248. ESI-HRMS (m/z): calcd. for $C_{13}H_9ClO_2$ 233.0364 ($[M+H]^+$), 235.0338 ($[M+2+H]^+$), found 233.0348, 235.0338.

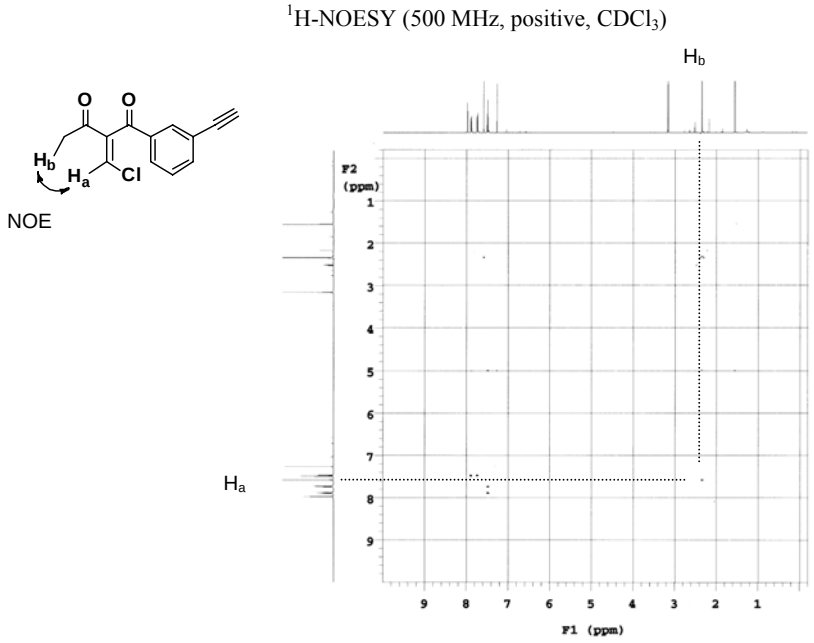
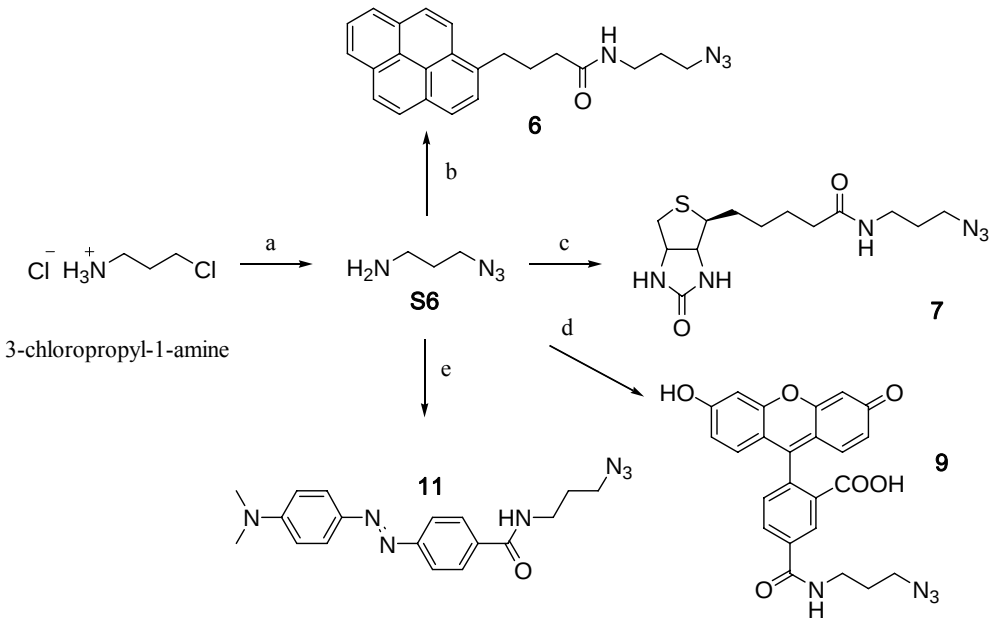


Fig. S1 Determination of the Z-configuration of **1** by ^{1}H-NOESY.

Scheme S2. Synthesis of the azide derivative with the short linker[3]



(a) NaN_3, H_2O, 80°C, 75%, (b)1-pyrenebutyric acid *N*-hydroxysuccinimide, DMF, rt, 99%, (c) Biotin *N*-hydroxysuccinimide, DMF, rt, 81%, (d) 5-carboxyfluorescein, TBTU, DIPEA, DMF, rt, 50%, (e) Dabcyl-COOH, TBTU, DIPEA, DMF, rt, 83%.

Synthesis of 3-azidopropan-1-amine (S6).[4] A solution of NaN_3 (1.5 g, 23.1 mmol) and 3-chloropropyl-1-amie（1 g, 7.69 mmol）in H_2O (7.7 mL) was stirred at 80°C for 18 h. The solvents were evaporated to the half volume, and the mixture was alkalinized with KOH (400 mg) at 0 °C, then extracted with Et_2O (15 mLx3). The organic layers were dried over Na_2SO_4, and evaporated to give a pale yellow oil (580 mg, 75%).

Synthesis of 6 (Pyrene-N_3). 1-Pyrenebutyric acid *N*-hydroxysuccinimide (100 mg, 0.259 mmol) and 3-azidopropylamine(0.58 M in DMF, 670 μL, 0.390 mmol) in DMF solution (0.67 mL) was stirred for 24 h at room temperature. Et_2O (15 ml) and 10% aqueous NaOH (15 mL) were added into the reaction mixture and the whole was extracted with Et_2O (15 ml×2). The organic solvents were washed with brine, dried over Na_2SO_4, then evaporated to give a crude oil, which was chromatographed on a silica gel column to give **6** as pale yellow solids (95.7 mg, 99%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 8.27 (1H, d, *J* = 9.2 Hz), 8.15 (2H, dd, *J* = 7.6, 2.1 Hz), 8.09 (1H, d, *J* = 9.2 Hz), 8.08 (1H, d, *J* = 7.6 Hz), 8.01 (2H, s), 7.97 (1H, t, *J* = 7.6 Hz), 7.83 (1H, d, *J* = 7.6 Hz), 5.49 (1H, brs), 3.37 (2H, t, *J* = 7.0 Hz), 3.30 (2H, t, *J* = 6.7 Hz), 3.28 (2H, t, *J* = 6.7 Hz), 2.26-2.18 (4H, m), 1.72 (2H, quint, *J* = 6.7 Hz).IR (cm^{-1}): 3300, 2096, 1643, 1548, 1260, 842. ESI-MS (m/z): 371.2 ($[M+H]^+$).

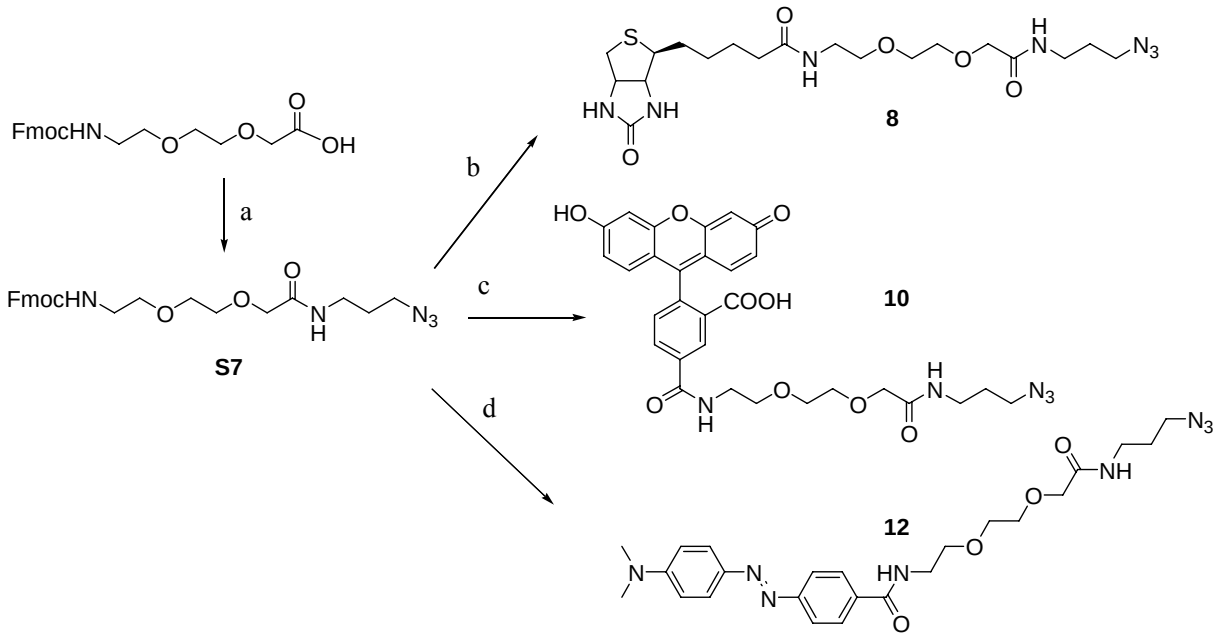
Synthesis of 7 (Biotin-N_3, short). 7 was obtained in 83 % yield from biotin by the same procedure described for the synthesis of **6** as white solids. ^{1}H-NMR (400 MHz, CD_3OD) δ ppm 4.48 (1H, dd, *J* = 7.8, 4.9 Hz), 4.29 (1H, dd, *J* = 7.8, 4.6 Hz), 3.34 (2H, t, *J* = 6.7 Hz), 3.24 (2H, t, *J* = 6.7 Hz), 3.21-3.18 (1H, m), 2.92 (1H, dd, *J* = 12.8, 4.9 Hz), 2.70 (1H, d, *J* = 12.8 Hz), 2.20 (2H, t, *J* = 7.3 Hz), 1.75 (2H, quint, *J* = 6.7 Hz), 1.69-1.55 (4H, m), 1.43 (2H, quint, *J* = 7.3 Hz). IR (cm^{-1}): 3252(br), 2422, 2102, 1691, 1635. ESI-MS (m/z) 327.5 ($[M+H]^+$).

Synthesis of 9 (FAM-N_3, short). 9 was obtained in 50% yield from 5-carboxyfluorescein by the same procedure described for the synthesis of **6**. ^{1}H-NMR (400 MHz, CD_3OD) δ ppm 8.41 (1H, d, *J* = 1.5

Hz), 8.18 (1H, dd, *J* = 7.9, 1.5 Hz), 7.29 (1H, t, *J* = 7.9 Hz), 6.68 (2H, d, *J* = 2.2 Hz), 6.59 (2H, d, *J* = 8.6 Hz), 6.53 (2H, dd, *J* = 8.6, 2.2 Hz), 3.51 (2H, t, *J* = 6.7 Hz), 3.44 (2H, d, *J* = 6.7 Hz), 1.91 (2H, quint, *J* = 6.7 Hz). IR (cm^{-1}) 3400-2600, 2099, 1741, 1607, 1453, 1248, 1112. ESI-MS (m/z) 459.3 ($[M+H]^+$).

Synthesis of 11 (Dabcyl-N_3, short). 11 was obtained in 83 % yield from Dabcy-COOH by the same procedure described for the synthesis of **6**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 7.88 (2H, d, *J* = 9.2 Hz), 7.85 (4H, s), 6.74 (2H, d, *J* = 9.2 Hz), 6.39 (1H, brs), 3.56 (2H, q, *J* = 6.4 Hz), 3.45 (2H, t, *J* = 6.4 Hz), 3.09 (6H, s), 1.92 (2H, quint, *J* = 6.4 Hz). IR (cm^{-1}): 3292, 2096, 1627, 1611, 1548, 822. ESI-MS (m/z): 352.3 ($[M+H]^+$).

Scheme S3. Synthesis of the azide derivative with the long linker



(a) **S6**, DIPEA, TBTU, DMF, 58%, (b) **S7**, octanethiol, TBAF·$3H_2O$, bis(1-methyl-1*H*-tetrazole-5-yl)disulfide, DIPEA, biotin, TBTU, DMF, rt, 60%, (c) **S7**, octanethiol, TBAF·$3H_2O$, bis(1-methyl-1*H*-tetrazole-5-yl)disulfide, DIPEA, 5-carboxyfluorescein, TBTU, DMF, rt, 15%, (e) **S7**, octanethiol, TBAF·$3H_2O$, bis(1-methyl-1*H*-tetrazole-5-yl)disulfide, DIPEA, Dabcyl-COOH, TBTU, DMF, rt, 61%.

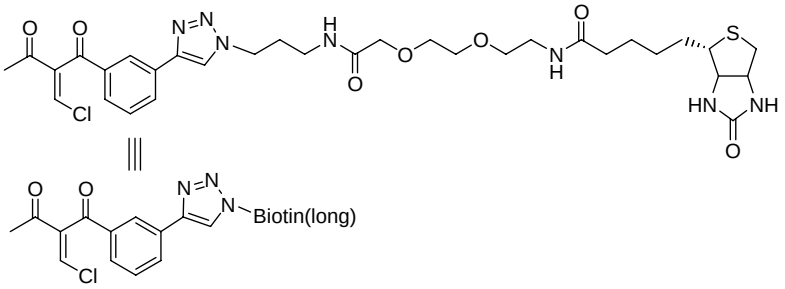
Synthesis of the linker-N_3 (S7) 3-Azidopropylamine (0.58 M in DMF, 3.4 mL), DIPEA (690 μL , 2.47 mmol) and TBTU (509 mg, 1.59 mmol) were added into a solution of *N*-Fmoc-[2-(2-aminoethoxy)ethoxy]acetic acid (510 mg, 1.32 mmol) in DMF (5 mL), and the mixture was stirred for 30 min at room temperature. The mixture was quenched by the addition of 5% aqueous $NaHCO_3$ (50 mL) and extracted with Et_2O (30 mL x 3). The organic layers were dried over Na_2SO_4, and evaporated to give a crude oil, which was chromatographed on a silica gel column to give 22 as a colorless oil (359 mg, 58%). ^{1}H-NMR (400 MHz, $CDCl_3$), δ ppm 7.74 (2H, d, *J* = 7.3 Hz), 7.57 (2H, d, *J* = 7.3 Hz), 7.38 (2H, t, *J* = 7.3 Hz), 7.29 (2H, t, *J* = 7.3 Hz), 6.95 (1H, s), 5.22 (1H, s), 4.41 (2H, d, *J* = 6.7 Hz), 4.20 (1H, t, *J* = 6.7 Hz), 3.97 (2H, s), 3.64-3.56 (6H, m), 3.40-3.29 (6H, m), 1.77 (2H, quint, *J* = 6.7 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): 169.90, 156.43, 143.87, 141.29, 127.65, 126.98, 124.91, 119.94, 70.89, 70.48, 70.05, 66.61, 49.17, 47.25, 40.81, 36.34, 28.73. IR (cm^{-1}) 3334, 2097, 1717, 1668, 1534, 1255, 1107. ESI-HRMS (m/z): calcd. for $C_{24}H_{29}N_5O_5$ 468.2241 ($[M+H]^+$), found 468.2236.

Synthesis of 8 (Biotin-N_3, long). [5] Octanethiol (160 μl, 0.922 mmol) and TBAF·$3H_2O$ (59 mg, 0.187 mmol) were added into a solution of **S7** (44 mg, 0.0941 mmol) in DMF solution (1 mL) under argon, and the mixture was stirred for 5 minutes, followed by the sequential addition of bis(1-methyl-1*H*-tetrazole-5-yl)disulfide (130 mg, 0.565 mmol), DIPEA (60 μl, 0.344 mmol), biotin (28 mg, 0.115 mmol), TBTU (36 mg, 0.112 mmol). The mixture was stirred for 30 minutes, and quenched by the addition of 5 % aqueous $NaHCO_3$ (15 ml), then extracted with CH_2Cl_2 (10 ml×4). The organic layers were dried over Na_2SO_4, and evaporated to give a crude oil, which was chromatographed on a silica gel column to give 8 as white solids (26.4 mg, 60%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 7.06 (1H, brs), 6.50 (1H, t, *J* = 5.2 Hz), 6.24 (1H, s), 5.35 (1H, s), 4.48 (1H, dd, *J* = 7.6, 4.9 Hz), 4.30 (1H, dd, *J* = 7.6, 4.9 Hz), 3.99 (2H, s), 3.67-3.62 (4H, m), 3.56 (2H, t, *J* = 5.2 Hz), 3.45-3.35 (6H, m), 3.12 (1H, td, *J* = 7.3, 4.6 Hz), 2.89 (1H, dd, *J* = 12.8, 5.2 Hz), 2.72 (1H, d, *J* = 12.8 Hz), 2.22 (2H, t, *J* = 7.3 Hz), 1.81 (2H, quint, *J* = 6.7 Hz), 1.76-1.59 (4H, m), 1.46-1.41 (2H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): 173.38, 170.09, 163.74, 70.87, 70.61, 70.04, 70.00, 61.87, 60.10, 55.54, 49.25, 40.52, 39.04, 36.43, 35.83, 28.80, 28.10, 25.54. IR (cm^{-1}): 3279, 2098, 1699, 1662, 1545, 1262, 1112. ESI-HRMS (m/z): calcd. for $C_{19}H_{33}N_7O_5S$ 472.2337 ($[M+H]^+$), found 472.2359.

Synthesis of 10 (FAM-N_3, long). **10** was obtained in 15 % yield from 5-carboxyfluorescein by the same procedure described for the synthesis of **8**. ^{1}H-NMR (400 MHz, CD_3OD), δ ppm 8.44 (1H, s), 8.19 (1H, dd, *J* = 7.9, 1.5 Hz), 7.30 (1H, d, *J* = 7.9 Hz), 6.68 (2H, d, *J* = 2.4 Hz), 6.63 (2H, d, *J* = 8.9Hz), 6.54 (2H, dd, *J* = 8.9, 2.4 Hz), 3.99 (2H, s), 3.75-3.70 (6H, m), 3.65 (2H, t, *J* = 6.7Hz), 3.34-3.26 (4H, m), 1.74 (2H, quint, *J* = 6.7 Hz). IR (cm^{-1}) 3279, 2099, 1745, 1642, 1610, 1456, 1251, 1111. ESI-HRMS (m/z): calcd. for $C_{30}H_{29}N_5O_9$ 604.2038 ($[M+H]^+$), found 604.2037.

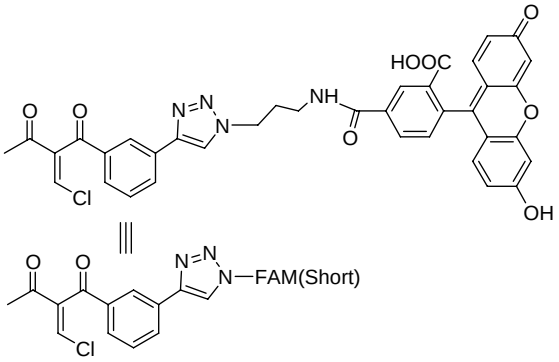
Synthesis of 12 (Dabcyl-N_3, long). **12** was obtained in 61 % yield from Dabcyl-COOH by the same procedure described for the synthesis of **8**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 7.89-7.84 (6H, m), 6.96 (1H, brs), 6.74 (2H, d, *J* = 9.2 Hz), 6.73 (1H, brs), 4.02 (2H, s), 3.70-3.67 (8H, m), 3.38-3.29 (4H, m), 3.09 (6H, s), 1.76 (2H, quint, *J* = 6.7 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): 170.28, 167.35, 155.00, 152.88, 143.59, 134.17, 127.86, 125.53, 122.18, 111.53, 70.92, 70.53, 70.09, 69.98, 49.21, 40.26, 39.82, 36.52, 28.70. IR (cm^{-1}): 3312, 2096, 1655, 1633, 1600, 1539, 1365, 1138. ESI-HRMS (m/z): calcd. for $C_{24}H_{32}N_8O_4$ 497.2619 ($[M+H]^+$), found 497.2620.

Synthesis of 2 (Biotin-long). **2(Biotin-long)** was obtained as a yellow oil by the same method as described for the synthesis of **3 (FAM-short)**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 8.31 (1H, s), 8.13 (1H, d, *J* = 7.9 Hz), 8.10 (1H, s), 7.81 (1H, d, 7.9 Hz), 7.59 (1H, s), 7.55 (1H, t, *J* = 7.9 Hz), 6.61 (1H, s), 5.85 (1H, s), 5.28 (1H, s), 5.03 (1H, s), 4.52-4.49 (1H, m), 4.30-4.29 (1H, m), 3.95 (2H, s), 3.62-3.56 (6H, m), 3.45-3.36 (6H, m), 3.11-3.10 (1H, m), 2.88 (1H, dd, *J* = 12.5, 4.9 Hz), 2.70 (1H, d, *J* = 12.5 Hz), 2.35 (3H, s), 2.21 (4H, m), 1.67-1.61 (4H, m), 1.47-1.44 (2H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): 173.38, 170.09, 163.74, 70.87, 70.61, 70.04, 70.00, 61.87, 60.10, 55.54, 49.25, 40.52, 39.04, 36.43, 35.83, 28.80, 28.10, 25.54. IR (cm^{-1}): 3291, 1666, 1547, 1458, 1236, 1102. ESI-HRMS (m/z): calcd. for $C_{32}H_{42}ClN_7O_7S$ 704.2628 ($[M+H]^+$), 706.2614 ($[M+2+H]^+$), found 704.2644, 706.2678.



Synthesis of 3 (FAM-short). Sodium ascorbate (0.4 mg, 0.0020 mmol), **9** (2.4 mg, 0.010 mmol), TBTA (1.1 mg, 0.0021 mmol), and $CuSO_4$•$5H_2O$ (0.3 mmol, 0.0012 mmol) were added into a solution of **1** in DMSO/H_2O (180 / 20 μL) at rt, and the mixture was stirred for 45 min. The reaction mixture

was diluted with AcOEt/Et_2O (1:1, 10 ml), quenched with 5% aqueous HCl (10 ml). The aqueous phase was extracted with AcOEt (10 ml×2). The combined organic phases were washed with brine (10 ml), dried over Na_2SO_4, and evaporated to give the residue, which was purified by silica gel chromatography (AcOEt:acetone = 8:1) to give **2**(FAM-short) (6.4 mg, 90%) as yellow solids. ^{1}H-NMR (400 MHz, acetone) δ ppm 9.11 (1H, brs), 8.58 (1H, s), 8.44 (1H, d, *J* = 0.9 Hz), 8.40 (1H, s), 8.30 (1H, dd, *J* = 7.9, 1.5 Hz), 8.27 (1H, brs), 8.18 (1H, dd, *J* = 7.6, 0.9 Hz), 8.04 (1H, s), 7.85 (1H, dd, *J* = 7.9, 0.6 Hz), 7.61(1H, t, *J* = 7.9 Hz), 7.35 (1H, d, *J* = 7.6 Hz), 6.75 (2H, d, *J* = 2.1 Hz), 6.65 (2H, d, *J* = 8.6 Hz), 6.61 (2H, dd, *J* = 8.6, 2.1 Hz), 4.64 (2H, t, *J* = 6.7 Hz), 3.59 (2H, q, *J* = 6.7 Hz), 2.48 (3H, s), 2.36 (2H, quint, *J* = 6.7 Hz). IR (cm^{-1}) 3400-2600, 1751, 1610, 1453, 1233, 1114. ESI-HRMS (m/z): calcd. for $C_{37}H_{27}ClN_4O_8$ 691.1590 ($[M+H]^+$), 693.1583 ($[M+2+H]^+$), found 691.1598, 693.1629.



The synthesis of the functionality transfer ODN1(alkyne). A solution of **1** in CH_3CN (800 pmol, 380 μM final concentration) was added into a solution of **ODN1** (315 pmol, 150 μM, final concentration) in carbonate buffer (25 mM, final concentration) at rt. The reaction progress was followed by HPLC by monitoring UV at 254 nm (Fig. S2).

The functionality transfer reaction with ODN1(alkyne) and RNA2. A portion of the above solution (1.5 μM **ODN1**(alkyne), final concentration) was added into a solution of **RNA2** (1 μM, final concentration) in the carbonate buffer (50 mM, final concentration) containing NaCl (100 mM, final concentration). The pH was adjusted to 9.6. The HPLC charts are shown in Fig. S2, and the time course of the reaction is shown in Fig. 4 in the text. HPLC conditions: column: SHISEIDO C18, 4.6×250 mm, solvents: A: 0.1M TEAA Buffer, B: CH_3CN, B: 10% to 30% /20 min, 30% to 100% /25 min, flow rate: 1.0 ml/min, UV: 254 nm.

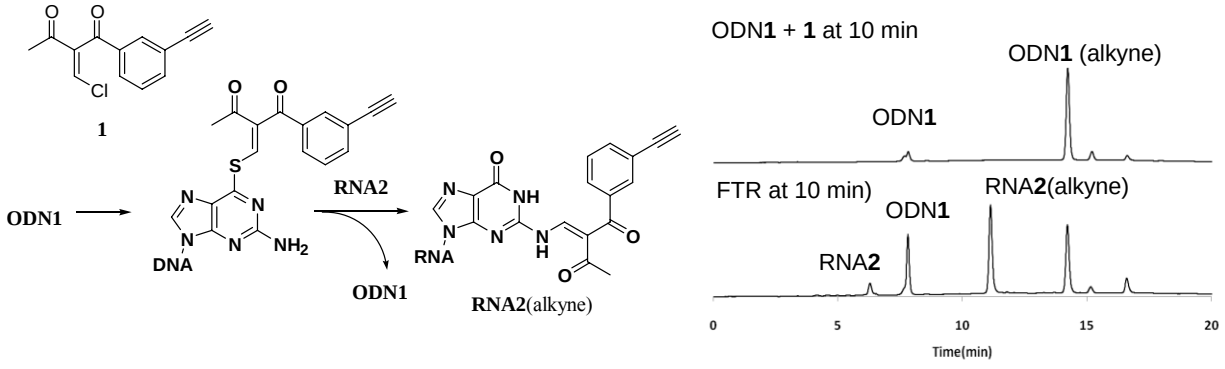


Fig. S2. The HPLC charts for the formation of *S*-functionalized ODN**1**(alkyne) and the following functionality transfer reaction analyzed at 10 min. The transfer yield was obtained by quantification of the peak of **RNA2** and **RNA3**.

The synthesis of ODN1F(Biotin long) and ODN1F(FAM short) and the following functionality transfer reaction to RNA2. The reactions were performed as described for the reaction with **ODN1**(Alkyne).

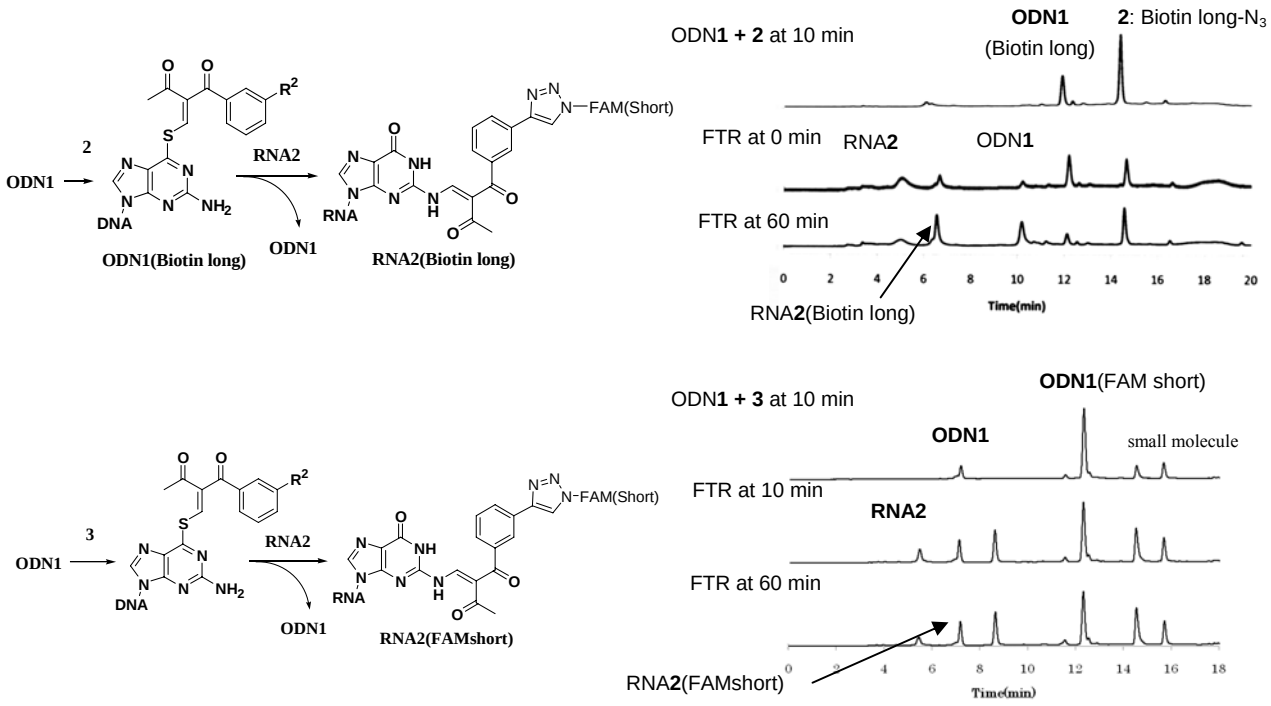


Fig. S3. The HPLC charts for the formation of *S*-functionalized ODN**1**(alkyne) and the following functionality transfer reaction analyzed at 10 min. The transfer yield was obtained by quantification of the peak of **RNA2** and **RNA2**(modified).

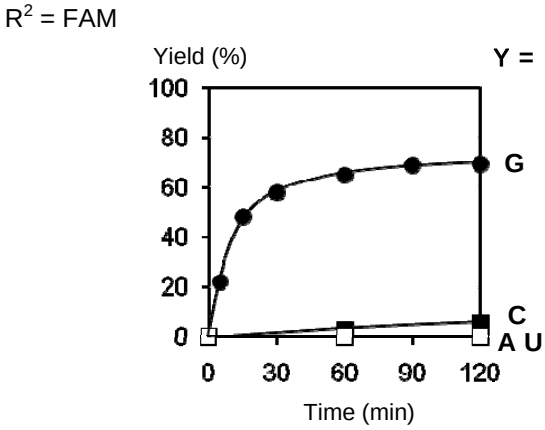
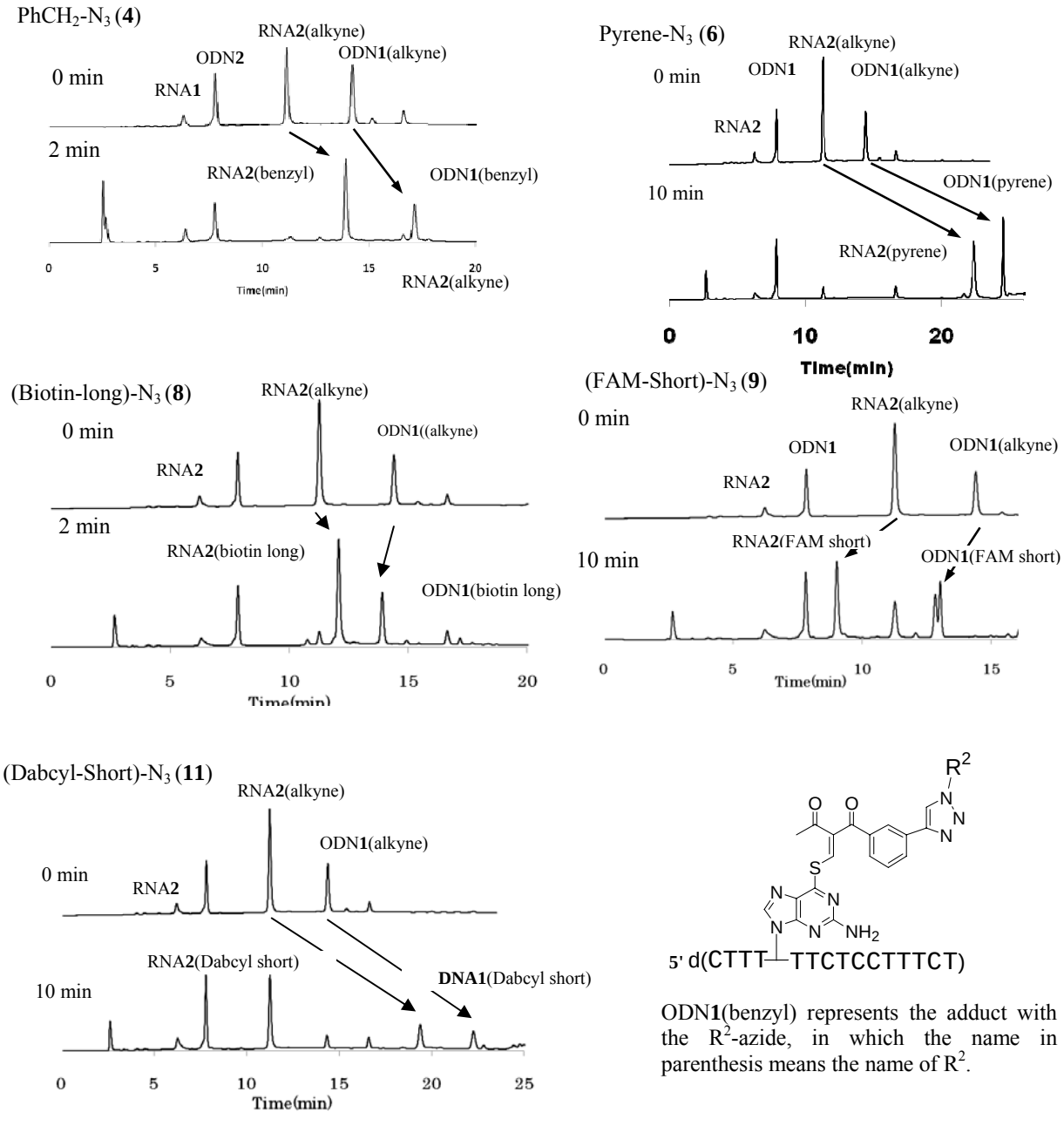


Fig. S4 Time course and the selectivity to the guanine base in the transfer reaction. The reaction was performed by using 15 μM of *S*-functionalized ODN**1F**(FAM(short)), and 10 μM of the target ORN**2**(**Y**) in 50 mM carbonate buffer containing 100 mM NaCl at pH 9.6 at 25°C, and followed by HPLC with monitoring UV at 254 nm.

The Click chemistry to the modified RNA2-M(alkyne) $CuSO_4$ (25 mM in H_2O, 0.15 mM at final concentration) was added to start the cycloaddition reaction into a solution of **RNA2**(Alkyne) (10 μM final concentration), sodium ascorbate (0.3 mM final concentration), TBTA (tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine) (0.6 mM final concentration) and the azide compound (0.75 mM final concentration) in DMSO-H_2O. The reaction was followed by HPLC, and the HPLC charts are compared in Fig. S5. HPLC conditions: column: SHISEIDO C18, 4.6×250 mm, solvent : A: 0.1M TEAA Buffer, B: CH_3CN, B: 10% to 30% /20 min, 30% to 100% /25 min, flow rate: 1.0 ml/min, UV: 254 nm.



ODN**1**(benzyl) represents the adduct with the R^2-azide, in which the name in parenthesis means the name of R^2.

Fig. S5 The HPLC chart changes of the reaction by using **4**, **6**, **8**, **9** and **11**.

Table S1 MALDI-TOF/MS data of the oligonucleotides obtained in this study.

DNA or RNA	5' Label	Base, R	calcd. ([M-H]$^-$)	found
ODN**1**	-	G^S	4767.8	4767.1
ODN**1**	-	G^S(alkyne)	4962.8	4963.0
ODN**1**(benzyl)	-	R^2 = Bn	5095.9	5097.4
ODN**1**(biotin-short)	-	R^2 = Biotin(short)	5289.0	5288.8
ODN**1**(biotin-long)	-	R^2 = Biotin(long)	5434.0	5433.7
ODN**1**(pyrene)	-	R^2 = Pyrene	5333.0	5332.3
ODN**1**(FAM-short)	-	R^2 = FAM(short)	5421.0	5420.7
ODN**1**(FAM-long)	-	R^2 = FAM(long)	5566.0	5566.8
ODN**1**(dabcyl-short)	-	R^2 = Dabcyl(short)	5314.0	5314.0
RNA**2(C)**	-	Y=C	5257.8	5258.1
RNA**2(G)**	-	Y=G	5297.8	5297.8
RNA**2(A)**	-	Y=A	5281.8	5281.7
RNA**2(U)**	-	Y=U	5258.8	5259.4
FAM-RNA**2(C)**	FAM	Y=C	5795.9	5795.1
FAM-RNA**2(G)**	FAM	Y=G	5835.9	5835.9
FAM-RNA**2(A)**	FAM	Y=A	5819.9	5819.5
FAM-RNA**2(U)**	FAM	Y=U	5796.9	5796.5
RNA**2**(alkyne)	-	R^1=alkyne	5492.8	5493.1
RNA**2(**benzyl)	-	R^2 = Bn	5625.9	5626.2
RNA**2**(biotin short)	-	R^2 = Biotin(short)	5819.0	5818.7
RNA**2** (biotin long)	-	R^2 = Biotin(long)	5964.0	5964.1
RNA**2**(pyrene)	-	R^2 = Pyrene	5863.0	5863.5
RNA**2**(FAM short)	-	R^2 = FAM(short)	5950.9	5950.2
RNA**2** (FAM long)	-	R^2 = FAM(long)	6096.0	6096.7
RNA**2**(Dabcyl short)	-	R^2 = Dabcyl(short)	5844.0	5843.8
RNA**2**(Dabcyl long)	-	R^2 = Dabcyl(long)	5989.1	5988.9

All oligonucleotides were separated by HPLC under the conditions described in the experimental part, and subjected to MALDI-TOF/MS measurements.

References

[1] Q. Zhang and J. M. Takacs, *Org. Lett.* **2003**, *5*, 3831-3834.
[2] a) S. Kinoshita, H. Kinoshita, T. Iwamura, S. Watanabe, T. Kataoka, *Chem. Eur. J.*, **2003**, *9*, 1496-1502. b) H-X. Wei, S. H. Kim, T. D. Caputo, D. W. Purkiss, G. Li, *Tetrahedron*, **2000**, *56*, 2397–2401.
[3] P. M. E. Gramlich, S. Warncke, J. Gierlich, T. Carell, *Angew. Chem., Int. Ed.*, **2008**, *47*, 3442-3444.
[4] B. Carboni, A. Benalil, M. Vaultier, *J. Org. Chem.* **1993**, *58*, 3736-3741.
[5] M. Ueki, N. Nishigaki, H. Aoki, T. Tsurusaki, T. Katoh, *Chem. Lett.*, **1993**, 721-724.