Supporting Information for

Development of Thiol-Responsive Amide Bond Cleavage Device and Its Application for Peptide Nucleic Acid-Based DNA Releasing System

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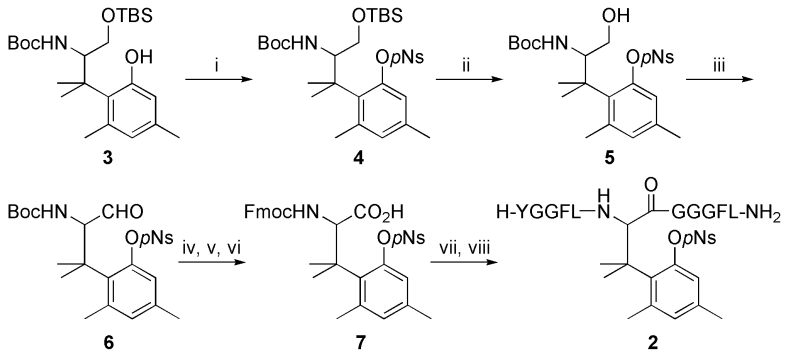
General Methods*:* All reactions were carried out under argon. For column chromatography, silica gel (KANTO KAGAKU N-60) was employed. Exact mass spectra were recorded on Waters MICROMASS® LCT PREMIER™ or Bruker Esquire200T. NMR spectra were recorded using JEOL GSX400 spectrometer at 400 MHz frequency for ^{1}H and 100 MHz frequency for ^{13}C in $CDCl_3$. Chemical shifts are calibrated to the solvent signal. For HPLC separations, a Cosmosil $5C_{18}$-AR-II analytical column (Nacalai Tesque, 4.6×250 mm, flow rate 1 mL/min) or a $5C_{18}$-AR-II semi-preparative column (Nacalai Tesque, 10×250 mm, flow rate 2.5 mL/min) was employed, and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% TFA in H_2O (*v*/*v*, soluvent A) and 0.1% TFA in MeCN (*v*/*v*, solvent B) was used for HPLC elution. Fluorescent spectra were recorded on Hitachi F-4500. Oligodeoxynucleotides were purchased form Nippon EGT Co., Ltd.

Preparation of Thiol-Responsive Amino Acid



Reagents and conditions. (i) *p*-nitrobenzenesulfonyl chloride (*p*NsCl), K_2CO_3, acetone, reflux, quant.; (ii) AcOH, H_2O, THF, quant.; (iii) PCC, CH_2Cl_2, 91%; (iv) $NaClO_2$, NaH_2PO_4, *tert*-BuOH, H_2O, acetone, 2-methyl-2-butene; (v) HCl, AcOEt; (vi) FmocOSu, Na_2CO_3, H_2O, MeCN, 70% (3 steps); (vii) Fmoc SPPS; (viii) TFA/Triethylsilane/H_2O=95/2.5/2.5.

2-*tert*-Butoxycarbonylamino-3,3-dimethyl-3-[2,4-dimethyl-6-(nitrobenzene-4-sulfonyloxy)-phenyl] propanol *tert*-butyldimethylsilyl ether (4).

To a stirred solution of phenol **3** (25.0 mg, 57.1 μmol) in acetone were added K_2CO_3 (81.0 mg, 818 μmol) and *p*-nitrobenzenesulfonyl chloride (33.8 mg, 90% purity, 137 μmol), and the resulting suspension was refluxed for 5.5 h. The resulting mixture was quenched by the addition of water and was extracted with AcOEt. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by column chromatography (SiO_2, hexane/AcOEt=10/1) and 35.6 mg of sulfonate **2** (57.1 μmol, quant) was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ=-0.07 (6H, s), 0.83 (9H, s), 1.31 (3H, s), 1.37 (9H, s), 1.44 (3H, s), 2.22 (3H, s), 2.62 (3H, s), 3.38 (1H, dd, *J*=11.3 and 4.7 Hz), 3.43 (1H, dd, *J*=11.3 and 4.7 Hz), 4.27 (1H, dt, *J*=9.8 and 4.7 Hz), 4.73 (1H, d, *J*=9.8 Hz), 6.89 (1H, s), 6.94 (1H, s), 8.24 (2H, d, *J*=8.7 Hz), 8.42 (2H, d, *J*=8.7 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ=-5.5 (CH_3), -5.4 (CH_3), 18.2 (C), 20.3 (CH_3), 25.7 (CH_3), 25.8 (CH_3×3), 26.7 (CH_3), 28.3 (CH_3×3), 28.5 (CH_3), 44.3 (CH_3), 56.5 (CH), 63.3 (CH_2), 78.8 (C), 120.6 (CH), 124.3 (CH×2), 129.8 (CH×2), 133.4 (CH), 133.9 (C), 136.7 (C), 140.0 (C), 142. (C)4, 149.2 (C), 150.8 (C), 155.9 (C); HRMS (ESI-TOF) calc. for $C_{30}H_{47}N_2O_8SSi$ ([*M*+H]$^+$): 623.2822, found; 623.2827.

2-*tert*-Butoxycarbonylamino-3,3-dimethyl-3-[2,4-dimethyl-6-(nitrobenzene-4-sulfonyloxy)-phenyl]propanol (5).

Glacial acetic acid (450 μL) and water (153 μL) were added to a solution of silyl ether **4** (37.0 mg, 59.4 μmol) in THF (153 μL). The reaction mixture was stirred overnight. After extraction with AcOEt, the organic phase was washed with saturated aqueous solution of $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography (SiO_2, hexane/AcOEt=4/1 then 1/1) and 30.2 mg of alcohol **5** (59.4 μmol, quant.) was obtained as a

pale yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ=1.25 (3H, s), 1.39 (9H, s), 1.44 (3H, s), 2.19 (3H, s), 2.62 (3H, s), 3.44 (1H, dd, *J*=11.6 and 8.1 Hz), 3.52 (1H, dd, *J*=11.6 and 2.6 Hz), 4.42 (1H, m), 4.78 (1H, d, *J*=9.3 Hz), 6.81 (1H, s), 6.91 (1H, s), 8.23 (2H, d, *J*=8.2 Hz), 8.44 (2H, d, *J*=8.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ=20.3 (CH_3), 25.7 (CH_3), 26.6 (CH_3), 28.1 (CH_3), 28.3 (CH_3×3), 43.7 (C), 58.0 (CH), 63.5 (CH_2), 79.5 (C), 120.9 (CH), 124.4 (CH×2), 129.7 (CH×2), 133.5 (C), 133.6 (CH), 137.0 (C), 139.8 (C), 142.3 (C), 149.0 (C), 150.9 (C), 156.6 (C); HRMS (ESI-TOF) calc. for $C_{24}H_{33}N_2O_8S$ ([*M*+H]$^+$): 509.1958, found; 509.1950.

2-*tert*-Butoxycarbonylamino-3,3-dimethyl-3-[2,4-dimethyl-6-(nitrobenzene-4-sulfonyloxy)-phenyl] propanal (6).

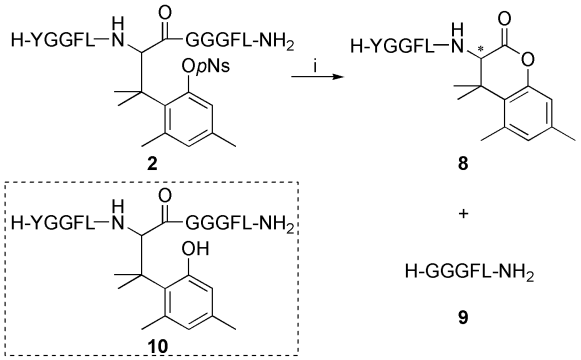
To a stirred solution of alcohol **5** (30.2 mg, 59.4 μmol) in dichloromethane (887 μL) was added PCC (131 mg, 475 μmol), and the resulting suspension was stirred overnight. To the reaction mixture was added the Celite and the obtained mixture was filtrated through the Celite pad. The resulting solution was concentrated in vacuo. The crude product was purified by column chromatography (SiO_2, hexane/AcOEt=1/0 then 4/1) and 27.3 mg of aldehyde **6** (53.9 μmol, 91%) was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ=1.31 (3H, s), 1.39 (9H, s), 1.42 (3H, s), 2.24 (3H, s), 2.69 (3H, s), 5.09 (1H, d, *J*=8.3 Hz), 5.22 (1H, d, *J*=8.3 Hz), 6.95 (1H, s), 6.98 (1H, s), 8.21 (2H, d, *J*=8.7 Hz), 8.43 (2H, d, *J*=8.7 Hz), 9.27 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ=20.4 (CH_3), 25.4 (CH_3), 26.5 (CH_3), 27.7 (CH_3), 28.2 (CH_3×3), 43.2 (C), 65.2 (CH), 79.9 (C), 121.2 (CH), 124.4 (CH×2), 129.8 (CH×2), 131.5 (C), 133.8 (CH), 137.8 (C), 139.7 (C), 142.1 (C), 148.8 (C), 150.9 (C), 155.9 (C), 200.1 (C); HRMS (ESI-TOF) calc. for $C_{24}H_{31}N_2O_8S$ ([*M*+H]$^+$): 507.1801, found; 507.1801.

3,3-Dimethyl-3-[2,4-dimethyl-6-(nitrobenzene-4-sulfonyloxy)phenyl]-2-(9-fluorenylmethyl-carbonylamino)propionic acid (7).

To a solution of aldehyde **6** (27.3 mg, 53.9 μmol) in acetone/*tert*-BuOH/H_2O (55/35/10 *v*/*v*/*v*, 1.17 mL) were added 2-methyl-2-butene (42.6 μL, 377 μmol), NaH_2PO_4 (10.3 mg, 85.8 μmol) and $NaClO_2$ (29.3 mg, 324 μmol), and the resulting mixture was stirred overnight. The reaction mixture was poured into saturated aqueous solution of NH_4Cl and it was extracted with diethyl ether. The organic phase was dried over Na_2SO_4 and concentrated in vacuo. Hydrogen chloride in AcOEt (4 M, 432 μL) was added to the crude product, and the resulting mixture was stirred for 1 h. After concentration in vacuo, the obtained residue was dissolved in acetonitrile/10% *w*/*v* aqueous solution of Na_2CO_3 (1/1, 486 μL). To the resulting solution was added FmocOSu (19.1 mg, 56.6 μmol), and the reaction mixture was stirred overnight. After acidification with 5% *w*/*v* aqueous solution of $KHSO_4$, the obtained mixture was extracted with diethyl ether. The organic phase was washed with brine and concentrated in vacuo. The obtained crude product was purified by a preparative TLC

(SiO_2, chloroform/MeOH=10/1) and 24.2 mg of Fmoc derivative **7** (37.5 μmol, 70% (3 steps)) was obtained as a pale yellow amorphousness: ^{1}H NMR ($CDCl_3$, 400 MHz) δ=1.52 (3H, s), 1.55 (3H, s), 2.15 (3H, s), 2.63 (3H, s), 4.15 (1H, t, *J*=7.2 Hz), 4.27 (1H, dd, *J*=10.4 and 7.2 Hz), 4.35 (1H, dd, *J*=10.4 and 7.2 Hz), 4.44 (1H, br s), 5.19 (1H, d, *J*=9.0 Hz), 5.70 (1H, d, *J*=9.0 Hz), 6.70 (1H, s), 6.90 (1H, s), 7.22-7.30 (2H, m), 7.38 (2H, t, *J*=7.4 Hz), 7.49 (1H, d, *J*=7.4 Hz), 7.55 (1H, d, *J*=7.4 Hz), 7.75 (2H, d, *J*=7.4 Hz), 8.19 (2H, d, *J*=8.5 Hz), 8.39 (2H, d, *J*=8.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ=20.3 (CH_3), 25.0 (CH_3), 27.5 (CH_3), 27.9 (CH_3), 43.4 (C), 47.1 (CH), 59.6 (CH), 67.1 (CH_2), 119.9 (CH), 121.0 (CH), 121.0 (CH), 124.4 (CH×2), 125.0 (CH×2), 126.9 (CH×2), 127.6 (CH×2), 129.6 (CH×2), 132.1 (C), 133.6 (CH), 137.3 (C), 139.9 (C), 141.2 (C), 142.2 (C×2), 143.6 (C), 143.6 (C), 148.7 (C), 150.9 (C), 156.1 (C), 175.4 (C); HRMS (ESI-TOF) calc. for $C_{34}H_{33}N_2O_9S$ ([*M*+H]$^+$): 645.1907, found; 645.1919.

Preparation and Processing Reaction of Thiol Responsive Model Peptide



Preparation of thiol-responsive model peptide 2

Peptide **2** was synthesized by Fmoc SPPS according to a previous report.[S1] Analytical HPLC condition: linear gradient of solvent B in solvent A, 10 to 80% over 30 min. Retention time=22.6 and 23.2 min, respectively for each diastereomer of peptide **2**. Semi-preparative HPLC condition: linear gradient of solvent B in solvent A, 30 to 70% over 30 min. MS (ESI-IT) calc. for $C_{68}H_{88}N_{13}O_{17}S$ ([*M*+H]$^+$): 1390.6, found; 1390.7 and 1390.7.

Processing reaction of model peptide 2

Typical procedure: A stock solution of peptide **2** (0.5% *w*/*v* in MeCN/H_2O=1/1, 4.2 μL; final concentration=0.02% *w*/*v*) was added to a mixture of phosphate buffer (pH 9.0, 100 mM, 21.0 μL; final concentration=20 mM), MeCN (31.5 μL; final concentration=30% *v*/*v*) and H_2O (38.9 μL). To reaction mixture was added an aqueous solution of sodium 2-mercaptoethanesulfonate (1% *w*/*v*, 10.5 μL; final concentration=0.1% *w*/*v*) and the obtained solution was incubated at 37 °C. Reaction progress was monitored by analytical HPLC and products were characterized by ESI-MS. Spectral data were identical with those reported previously.[S1]

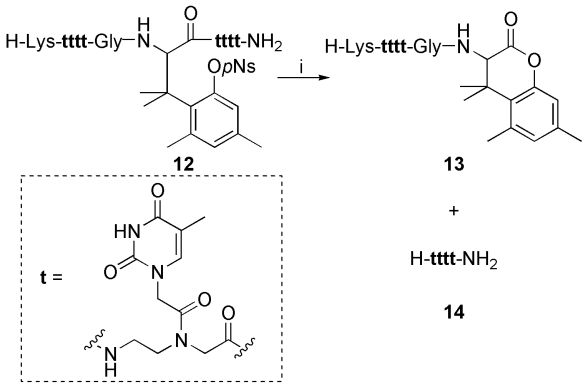
Analytical HPLC condition: linear gradient of solvent B in solvent A, 10 to 80% over 30 min. Retention time=10.9, 21.3 and 21.6 min, respectively for **9, 8** and **8'** (an epimer of **8** at the carbon designated by an asterisk). Yields were calculated by following equation.

$$\text{yield (\%)} = 100 \times [A(\mathbf{8}) + A(\mathbf{8'}) + A(\mathbf{9})] / [A(\mathbf{2}) + A(\mathbf{8}) + A(\mathbf{8'}) + A(\mathbf{9})]$$

$A(\mathbf{n})$: peak area of compound **n** in HPLC chart.

Preparation and Processing Reaction of Thiol-Responsive PNA

Preparation of thiol-responsive PNA 12



PNA **12** was synthesized by using Fmoc SPPS. Reagents and conditions are summarized in Table S1. Fmoc-**t**-OH was synthesized according to a literature.[S2] Analytical HPLC condition: linear gradient of solvent B in solvent A, 1 to 50% over 30 min. Retention time=22.0 min. MS (ESI-IT) calc. for $C_{115}H_{152}N_{38}O_{40}S$ ($[M+2H]^{2+}$): 1369.1, found; 1369.1.

Table S1

residue No.	reagents (eq.)[a]	reaction time
1	Fmoc-**t**-OH (5), PyBOP (5), NEM (10)	1 h
2	Fmoc-**t**-OH (5), PyBOP (5), NEM (10)	1 h
3	Fmoc-**t**-OH (5), PyBOP (5), NEM (10)	1 h
4	Fmoc-**t**-OH (5), PyBOP (5), NEM (10)	1 h
5	**7** (2), PyBOP (2), NEM (4)	overnight
6	Fmoc-Gly-OH (5), PyBOP (5), NEM (10)	1 h
7	Fmoc-**t**-OH (4), HCTU (3.8), DIEA (4)	2 h
8	Fmoc-**t**-OH (4), HCTU (3.8), DIEA (4)	2 h
9	Fmoc-**t**-OH (4), HATU (3.8), DIEA (4)	2 h
10	Fmoc-**t**-OH (4), HATU (3.8), DIEA (4)	12 h (twice)
11	Fmoc-Lys(Boc)-OH (4), HATU (3.8), DIEA (4)	12 h (twice)

(a) DMF/DMSO=3/1 was used as a solvent.

HATU: 2-(1*H*-7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HCTU: 5-chloro2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
NEM: *N*-ethylmorpholine
PyBOP: (1*H*-1,2,3-benzotriazolyloxy)tris(pyrrolidino)phosphonium hexafuluorophosphate

Processing reaction of PNA 12

A stock solution of PNA **12** (0.5% *w*/*v* in H_2O, 5.6 μL; final concentration=0.02% *w*/*v*) was added to a mixture of phosphate buffer (pH 9.0, 100 mM, 28.0 μL; final concentration=20 mM) and H_2O (105 μL). To reaction mixture was added an aqueous solution of sodium 2-mercaptoethanesulfonate **11** (10% *w*/*v*, 1.4 μL; final concentration=0.1% *w*/*v*) and the obtained solution was incubated at 37 °C for 24 h, which was followed by the second addition of thiol **11** (10% *w*/*v*, 1.4 μL; final concentration=0.1% *w*/*v*), and the reaction mixture was incubated at 37 °C for additional 24 h. Reaction progress was monitored by analytical HPLC and products were characterized by ESI-MS. Analytical HPLC condition: linear gradient of solvent B in solvent A, 1 to 50% over 30 min. Retention time=10.8 and 20.7 min, respectively for **14** and **13**. MS (ESI-IT) calc. for **14**: $C_{44}H_{60}N_{17}O_{16}$ ([*M*+H]$^+$): 1082.4, found; 1082.4. **13**: $C_{65}H_{89}N_{20}O_{20}$ ([*M*+H]$^+$): 1469.7, found; 1469.5.

Melting Temperature Experiments

The melting temperature experiments were performed according to the literature with slight modification.[S3] Melting curves were recorded by a fluorescence spectrometer (λ_{ex}=525 nm, λ_{em}=600 nm) after addition of an ethidium bromide (5.0 μM). Conditions: 4.0 μM concentration for each strand, 10 mM phosphate buffer (pH 9.0), 100 mM NaCl. thiol +: The sample was first treated

with thiol **11** (0.1% *w*/*v*) at 37 °C for 24 h, and then it was treated with additional thiol **11** (0.1% *w*/*v*) at 37 °C for 24 h.

Supplemental Figures

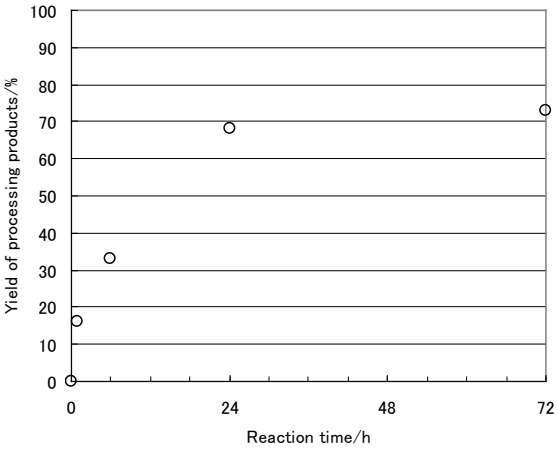


Figure S1. Yield of processing products relative to reaction time. Reaction conditions: Peptide **2** (0.02% *w*/*v* and thiol **11** (0.1% *w*/*v*) in 30% *v*/*v* MeCN/phosphate buffer (pH 9.0, 20 mM) were incubated at 37 °C.

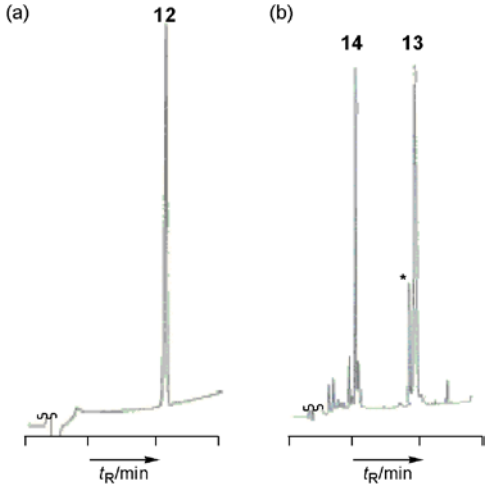


Figure S2. HPLC profiles (a) before treatment with thiol **11** and (b) after 24 h incubation with 0.1% *w*/*v* thiol **11** at 37 °C under Ar, followed by subsequent incubation with additional thiol 0.1% *w*/*v* thiol **11** at 37 °C for 24 h. Peptides were detected by UV absorbance at 220 nm. Asterisked peak is not a peptidic compound.

References

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S3 Rahman, S. M. A.; Seki, S.; Obika, S.; Yoshikawa, H.; Miyashita, K.; Imanishi, T. *J. Am. Chem. Soc*. **2008**, *130*, 4886-4896.