Interstrand cross-link of DNA by covalently linking a pair of abasic sites

Kohei Ichikawa,[a] Naoshi Kojima,[b] Yu Hirano,[b] Toshie Takebayashi,[b] Keiko Kowata[b] and Yasuo Komatsu[*b]

[†]*Nippon Steel Kankyo Engineering Co., Ltd., 2-1-38 Shohama, Kisarazu 292-0838, Japan,*

[‡]*Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), 2-17-2-1 Tsukisamu-Higashi, Toyohira-ku, Sapporo 062-8517, Japan*

Supplementary Information

Contents

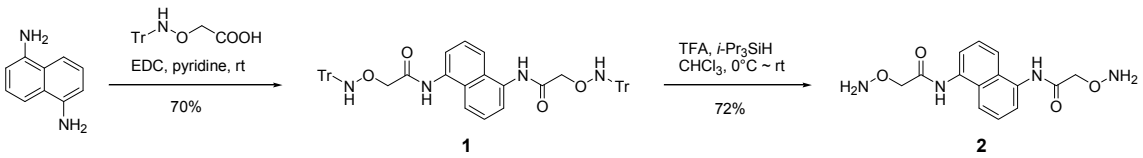
1) Synthesis of cross-linking molecules

1)-1. General information

Physical data were measured as follows: ^{1}H (270 MHz) and ^{13}C (67.8 MHz) NMR spectra were recorded on a JEOL FT-NMR spectrometer in DMSO-d_6 as the solvent with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), m (multiplet), or br (broad). All exchangeable protons were detected by disappearance on the addition of D_2O. Mass spectra were recorded on JEOL JMS-T100GC (EI-MS), JEOL JMS-700TZ (FAB) and on JEOL JMS-T100LP (ESI) spectrometers at the Instrumental Analysis Division, Equipment Management Center, Creative Research Institution at Hokkaido University. TLC was done on Merck silica gel 60F_{254} precoated plates (Merck, Germany). Silica gels used for column chromatography were Wakogel C-200 (particle size 75-150 μm, Wako Pure Chemical Industries, Japan), Silica Gel 60 (particle size 105-210 μm, Nacalai Tasque Inc. Japan), or Wakogel 100 C18 (reverse-phase, Wako Pure Chemical Industries, Japan).

1)-2. Synthesis of bisaminooxy molecules (aoNao, aoPao, ao^{P}Bao, aoOBao)

Scheme S1



N^{1},*N*^{5}-Bis(tritylaminooxyacetyl)-1,5-diaminonaphthalene (1)

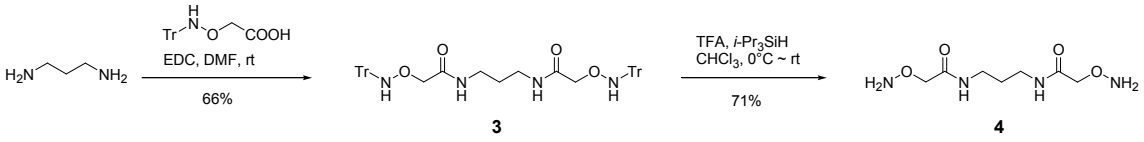
To a solution of 1,5-naphthalenediamine (158 mg, 1.00 mmol) and (*N*-tritylaminooxy)acetic acid[1] (333 mg, 1.00 mmol) in pyridine (30 mL) was added EDC (230 mg, 1.20 mmol) at RT, and the whole was stirred at the same temperature. Further amounts of (*N*-tritylaminooxy)acetic acid (333 mg, 1.00 mmol) and EDC (230 mg, 1.20 mmol) were added to the mixture after 3 and 6 h, respectively, and the stirring was continued for another 16 h. The mixture was concentrated in vacuo. The resultant white precipitate was suspended in H_2O (20 mL), which was washed with AcOEt (50 mL) and $CHCl_3$ (20 mL × 3). The precipitate was collected, washed with EtOH and Et_2O to give **1** (550 mg, 70%) as a white powder: FAB-LRMS *m/z* (+NaI) 789.3 (MH^+), 811.3 ($[M+Na]^+$); FAB-HRMS Calcd. for $C_{52}H_{44}N_4O_4Na$ ($[M+Na]^+$) 811.3260, found 811.3274; ^{1}H NMR (270 MHz, DMSO-d_6) δ: 9.62 (br s, 2 H, NH), 8.36 (br s, 2 H, NH), 7.77-7.71 (m, 4 H, naph), 7.52 (t, 2 H, naph, J = 7.9 Hz), 7.41-7.25 (m, 30 H, Tr), 4.23 (s, 4 H, CH_2); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ: 168.89 (C), 144.07 (C), 132.90 (C), 128.78

(CH), 127.63 (CH), 126.76 (CH), 125.43 (CH), 123.80 (C), 122.15 (CH), 120.11 (CH), 73.61 (C), 73.27 (CH_2).

N^1,N^5-Bis(aminooxyacetyl)-1,5-diaminonaphthalene (aoNao, 2)

To a suspension of **1** (394 mg, 0.50 mmol) in $CHCl_3$ (20 mL) was added triisopropylsilane (1.02 mL, 5.0 mmol) and trifluoroacetic acid (0.74 mL, 10.0 mmol) at 0°C, and the whole was stirred at RT for 3.5 h. The resultant white suspension was diluted with H_2O (30 mL), and the aqueous layer was washed with $CHCl_3$ (30 mL × 2) and AcOEt (30 mL). The aqueous layer was then diluted with CH_3CN (60 mL), and a solution was neutralized by DOWEX® 1×4-100 (OH^- form). The mixture was filtered, and the resin was washed with 80% aqueous CH_3CN. The filtrate was combined and concentrated in vacuo. The residue was purified by C-18 reverse phase column chromatography (1.0 × 15 cm, 0~40% CH_3CN in H_2O, 200 mL), and freeze-dried to give **2** (109 mg, 72%) as a white powder: FAB-LRMS *m/z* 305.2 (MH^+); FAB-HRMS calcd. for $C_{14}H_{17}N_4O_4$ (MH^+) 305.1250, found 305.1259; ^{1}H NMR (270 MHz, DMSO-d_6) δ: 9.88 (s, 2 H, NH), 7.93 (d, 2 H, naph, J = 8.6 Hz), 7.66 (d, 2 H, naph, J = 7.2 Hz), 7.54 (dd, 2 H, naph, J = 7.2, 8.6 Hz), 6.57 (s, 4 H, ONH_2), 4.28 (s, 4 H, CH_2); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ: 169.75 (C), 133.35 (C), 129.11 (C), 125.41 (CH), 122.66 (CH), 120.75 (CH), 74.42 (CH_2); Anal. Calcd. for $C_{14}H_{16}N_4O_4$: C, 55.26; H, 5.30; N, 18.41. Found: C, 55.30; H, 5.31; N, 18.32.

Scheme S2



N^1,N^3-Bis(tritylaminooxyacetyl)-1,3-propanediamine (3)

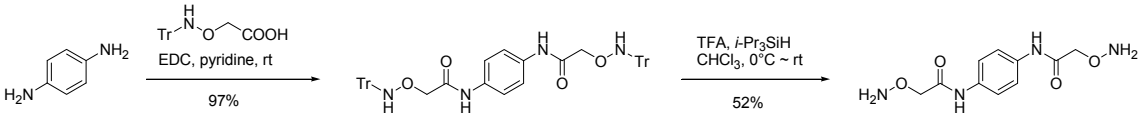
To a solution of 1,3-propanediamine (209 μL, 2.5 mmol) and (*N*-tritylaminooxy)acetic acid (2.00 g, 6.00 mmol) in DMF (30 mL) was added EDC (1.15 g, 6.0 mmol) at RT, and the whole was stirred at the same temperature for 15.5 h. AcOEt (200 mL) was added to the mixture, which was washed with H_2O (80 mL × 3) and brine (80 mL), then dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (4.0 × 11 cm, 75% AcOEt in Hexane) to give **3** (1.17 g, 66%) as a white powder: FAB-LRMS *m/z* (+NaI) 727.4 ($[M+Na]^+$); FAB-HRMS Calcd. for $C_{45}H_{44}N_4O_4Na$ ($[M+Na]^+$) 727.3260, found 727.3279; ^{1}H NMR (270 MHz, DMSO-d_6) δ: 8.23 (s, 2 H, NH), 7.57 (br t, 2 H, NH), 7.33-7.23 (m, 30 H, Tr), 3.79 (s, 4 H, CH_2), 3.09 (dt, 4 H, CH_2, J = 5.4, 6.6 Hz), 1.54 (t, 2 H, CH_2, J = 6.6 Hz); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ: 169.39 (C), 144.03 (C), 128.79 (CH), 127.56 (CH), 126.69

(CH), 73.55 (C), 73.11 (CH_2), 35.77 (CH_2), 29.10 (CH_2).

N^1,N^3-Bis(aminooxyacetyl)-1,3-propanediamine (aoPao, 4)

Compound **3** (529 mg, 0.75 mmol) was deprotected as described in the preparation of **2** to give **4** (118 mg, 71%) as a white powder: EI-LRMS *m/z* 220 (M^+); EI-HRMS calcd. for $C_7H_{16}N_4O_4$ (M^+) 220.1172, found 220.1175; ^{1}H NMR (270 MHz, DMSO-d_6) δ: 7.81 (br t, 2 H, NH, J = 5.3 Hz), 6.36 (s, 4 H, ONH_2), 3.93 (s, 4 H, CH_2), 3.13 (dt, 4 H, CH_2, J = 5.3, 6.9 Hz), 1.58 (t, 2 H, CH_2, J = 6.9 Hz); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ: 169.78 (C), 74.48 (CH_2), 35.53 (CH_2), 29.22 (CH_2); Anal. Calcd. for $C_{14}H_{17}N_4O_4(H_2O)_{0.2}$: C, 37.56; H, 7.39; N, 25.03. Found: C, 37.61; H, 7.21; N, 25.04.

Scheme S3



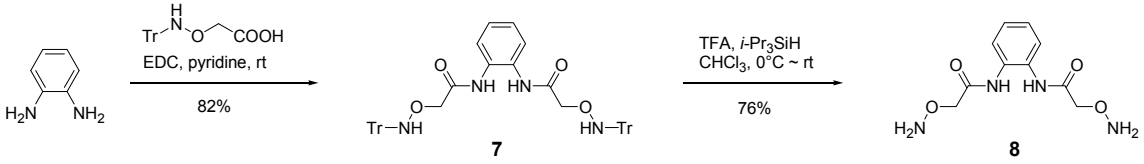
N^1,N^4-Bis(tritylaminooxyacetyl)-1,4-diaminobenzene (5)

To a solution of *p*-phenylenediamine (108 mg, 1.0 mmol) and (*N*-tritylaminooxy)acetic acid (833 mg, 2.50 mmol) in pyridine (25 mL) was added EDC (516 mg, 2.7 mmol) at RT, and the whole was stirred at the same temperature for 17 h. The mixture was concentrated in vacuo, and the residue was dissolved in AcOEt (70 mL). The solution was washed with H_2O (30 mL × 2) and brine (30 mL), and concentrated in vacuo. The resultant yellow solid was suspended in EtOH (50 mL) and the precipitate was collected, washed with EtOH and Et_2O to give **5** (717 mg, 97%) as a yellow powder: ESI-MS calcd. for $C_{48}H_{42}N_4O_4Na$ ($[M+Na]^+$) 761.3098, found 761.3102; ^{1}H NMR (270 MHz, DMSO-d_6) δ: 9.45 (s, 2 H, NH), 8.19 (s, 2 H, NH), 7.50 (s, 4 H, Ph), 7.36-7.25 (m, 30 H, Tr), 4.08 (s, 4 H, CH_2); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ: 168.06 (C), 144.15 (C), 133.92 (C), 128.85 (CH), 127.67 (CH), 126.80 (CH), 120.00 (CH), 73.60 (C), 73.14 (CH_2).

N^1,N^4-Bis(aminooxyacetyl)-1,4-diaminobenzene (aoPBao, 6)

Compound **5** (370 mg, 0.50 mmol) was deprotected as described in the preparation of **2** to give **6** (66.4 mg, 52%) as a white powder: ESI-MS calcd. for $C_{10}H_{14}N_4O_4Na$ ($[M+Na]^+$) 277.0907, found 277.0905; ^{1}H NMR (270 MHz, DMSO-d_6) δ: 9.61 (s, 2 H, NH), 7.59 (s, 4 H, Ph), 6.43 (s, 4 H, ONH_2), 4.11 (s, 4 H, CH_2); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ: 168.53 (C), 134.12 (C), 119.90 (CH), 74.51 (CH_2); Anal. Calcd. for $C_{10}H_{14}N_4O_4(H_2O)_{0.1}$: C, 46.91; H, 5.59; N, 21.88. Found: C, 47.03; H, 5.68; N, 21.51.

Scheme S4



N^1,N^2-Bis(tritylaminooxyacetyl)-1,2-diaminobenzene (7)

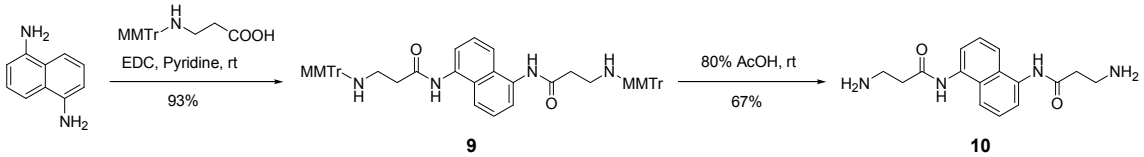
o-Phenylenediamine (108 mg, 1.0 mmol) was condensed with (*N*-tritylaminooxy)-acetic acid as described in the preparation of **5** to give **7** (604 mg, 82%) as a white powder: ESI-MS calcd. for $C_{48}H_{42}N_4O_4Na$ ($[M+Na]^+$) 761.3098, found 761.3100; ^{1}H NMR (270 MHz, DMSO-d_6) δ: 9.17 (s, 2 H, NH), 8.28 (s, 2 H, NH), 7.58 (dd, 2 H, Ph, J = 3.3, 6.3 Hz), 7.31-7.19 (m, 32 H, Tr, Ph), 3.97 (s, 4 H, CH_2); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ: 168.86 (C), 144.03 (C), 129.95 (C), 128.85 (CH), 127.64 (CH), 126.78 (CH), 125.35 (CH), 124.96 (CH), 73.77 (C), 73.63 (CH_2).

N^1,N^2-Bis(aminooxyacetyl)-1,2-diaminobenzene (aoOBao, 8)

Compound **7** (370 mg, 0.50 mmol) was deprotected as described in the preparation of **2** to give **8** (97.1 mg, 76%) as a white foam: ESI-MS calcd. for $C_{10}H_{14}N_4O_4Na$ ($[M+Na]^+$) 277.0907, found 277.0905; ^{1}H NMR (270 MHz, DMSO-d_6) δ: 9.44 (s, 2 H, NH), 7.59 (dd, 2 H, Ph, J = 3.6, 5.9 Hz), 7.21 (dd, 2 H, Ph, J = 3.6, 5.9 Hz), 6.47 (s, 4 H, ONH_2), 4.16 (s, 4 H, CH_2); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ: 169.31 (C), 130.11 (C), 125.28 (CH), 124.86 (CH), 74.48 (CH_2); Anal. Calcd. for $C_{10}H_{14}N_4O_4(H_2O)_{0.1}$: C, 46.91; H, 5.59; N, 21.88. Found: C, 46.79; H, 5.48; N, 21.64.

1)-3. Synthesis of bisamino molecule (aNa)

Scheme S5



N^1,N^5-Bis[3-(*N*-monomethoxytrityl)aminopropionyl]-1,5-diaminonaphthalene (9)

To a solution of 1,5-naphthalenediamine (160 mg, 1.00 mmol) and *N*-monomethoxytrityl-β-alanine (1.08 g, 3.00 mmol) in pyridine (30 mL) was added EDC (630 mg, 3.30 mmol) at RT, and the whole was stirred at the same temperature for 20 h. The mixture was concentrated in vacuo, and the residue was precipitated from EtOH (50 mL). The

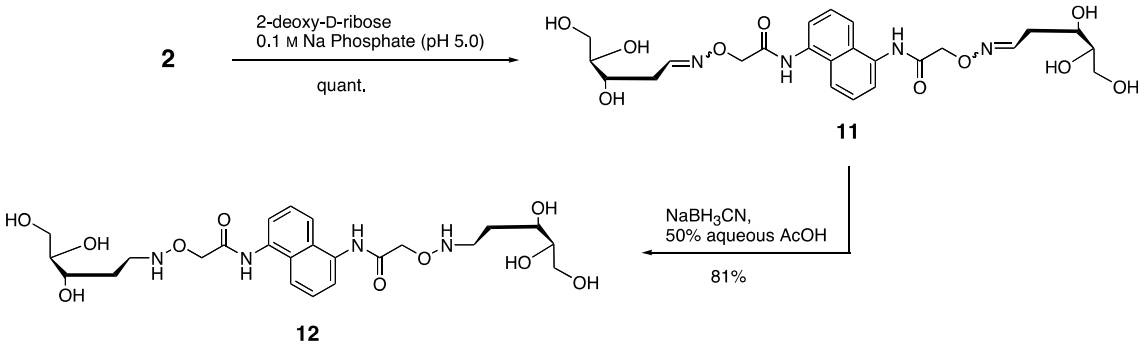
precipitate was collected to give **9** (787 mg, 93%) as a white powder: ESI-MS *m/z* 845.4 (MH^+); ^{1}H NMR (270 MHz, DMSO-d_6) δ: 10.00 (br s, 2 H, NH), 7.97 (d, 2 H, naph, *J* = 8.3 Hz), 7.70 (d, 2 H, naph, *J* = 7.3 Hz), 7.48 (dd, 2 H, naph, *J* = 7.3, 8.3 Hz), 7.45-7.42 (m, 8 H, MMTr), 7.33-7.25 (m, 12 H, MMTr), 7.20-7.15 (m, 4 H, MMTr), 6.86-6.83 (m, 4 H, MMTr), 3.71 (s, 6 H, OMe), 2.81 (br t, 2 H, NH, *J* = 7.8 Hz), 2.67 (m, 4 H, CH_2), 2.37 (m, 4 H, CH_2); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ: 171.23 (C), 157.27 (C), 146.27 (C), 137.78 (C), 133.80 (C), 129.48 (CH), 128.51 (C), 128.16 (CH), 127.61 (CH), 125.92 (CH), 125.17 (CH), 121.79 (CH), 119.99 (CH), 112.94 (CH), 69.76 (C), 54.85 (CH_3), 40.05 (CH_2), 36.69 (CH_2).

N^1,N^5-Bis(3-aminopropionyl)-1,5-diaminonaphthalene (aNa, 10)

A solution of **9** (340 mg, 0.40 mmol) in 80% aqueous acetic acid (20 mL) was stirred at RT for 1 h. The mixture was concentrated in vacuo, and the residue was co-evaporated with EtOH (5 mL × 3). The residue was dissolved in H_2O (40 mL), which was washed with $CHCl_3$ (40 mL × 2) and ethyl acetate (40 mL). The aqueous layer was then diluted with CH_3CN (60 mL), and a solution was neutralized by DOWEX® 1×4-50 (OH^- form). The mixture was filtered, and the resin was washed with 80% aqueous CH_3CN. The filtrate was combined and concentrated in vacuo. The residue was purified by C-18 reverse phase column chromatography (2.5 × 12 cm, 0~10% CH_3CN in H_2O), and freeze-dried to give **10** (80 mg, 67%) as a white foam: FAB-LRMS *m/z* 301.0 (MH^+); FAB-HRMS calcd. for $C_{16}H_{21}N_4O_2$ (MH^+) 301.1665, found 301.1667; ^{1}H NMR (270 MHz, DMSO-d_6) δ: 7.93 (d, 2 H, naph, *J* = 8.6 Hz), 7.88 (d, 2 H, naph, *J* = 7.3 Hz), 7.51 (dd, 2 H, naph, *J* = 7.3, 8.6 Hz), 2.94 (t, 4 H, CH_2, *J* = 6.3 Hz), 2.52 (t, 4 H, CH_2, *J* = 6.3 Hz); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ: 171.36 (C), 134.15 (C), 127.81 (C), 125.41 (CH), 120.44 (CH), 119.01 (CH), 39.22 (CH_2), 38.14 (CH_2); Anal. Calcd. for $C_{16}H_{20}N_4O_2(H_2O)_{0.25}$: C, 63.04; H, 6.78; N, 18.38. Found: C, 62.95; H, 6.61; N, 18.20.

1)-4. Synthesis of dR_2-aoNao and dR_2-aoNao (red)

Scheme S6



dR_2-aoNao (11)

A mixture of **2** (40 mg, 0.13 mmol) and 2-deoxy-D-ribose (70 mg, 0.52 mmol) in 0.1 M Na Phosphate buffer (pH 5.0, 10 mL) was stirred at RT for 1.5 h. The reaction mixture was purified by C-18 reverse phase column chromatography (2.0 × 12 cm, 20% CH_3CN in H_2O), and freeze-dried to give **11** (71 mg, quantitative) as a white form. Since compound **11** contained two symmetrical oxime linkages, it was obtained as a mixture of *E-E*, *Z-Z homo- and E-Z hetero*-isomers, in a ratio of 3:1 (*E*/*Z*). The *E*/*Z* ratio was assigned based on the chemical shift of the oxime proton (C*H*=N–O) in the ^{1}H NMR spectrum[2,3]: ESI-MS (+NaI) Calcd. for $C_{24}H_{32}N_4O_{10}Na$ ($[M+Na]^+$) 559.2016, found 559.2011; ^{1}H NMR (270 MHz, DMSO-d_6) δ: 9.82 (br s, 1.5 H, NH), 9.77 (br s, 0.5 H, NH), 7.87 (m, 2 H, naph), 7.71 (t, 1.5 H, C*H*=N–O, *J* = 6.3 Hz, *E*-isomer), 7.62 (m, 2 H, naph), 7.53 (m, 2 H, naph), 7.04 (t, 0.5 H, C*H*=N–O, *J* = 5.1 Hz, *Z*-isomer), 4.87 (d, 0.5 H, OH, *J* = 5.9 Hz), 4.79 (d, 1.5 H, OH, *J* = 5.9 Hz), 4.75 (s, 1 H, CH_2), 4.68 (m, 5 H, CH_2, OH), 4.43 (m, 2 H, OH), 3.60-3.52 (m, 4 H), 3.42-3.27 (m, 4 H), 2.74-2.23 (m, 4 H).

dR$_2$-aoNao (red) (12)

To a solution of **11** (54 mg, 0.10 mmol) in 50% aqueous acetic acid (10 mL) was added $NaBH_3CN$ (25 mg, 0.40 mmol) at RT. After stirring the mixture for 2 h, further amount of $NaBH_3CN$ (25 mg, 0.40 mmol) was added, and stirring was continued for another 15 h. The reaction mixture was concentrated in vacuo, and the residue was co-evaporated with EtOH (3 mL × 3). The residue was purified by C-18 reverse phase column chromatography (2.0 × 7.0 cm, 20% CH_3CN in H_2O), and freeze-dried to give **12** (44 mg, 81%) as a white solid: ESI-MS (+NaI) Calcd. for $C_{24}H_{36}N_4O_{10}Na$ ($[M+Na]^+$) 563.2324, found 563.2311; ^{1}H NMR (270 MHz, DMSO-d_6) δ: 9.87 (s, 2 H, NH), 7.91 (d, 2 H, naph, *J* = 8.6 Hz), 7.67 (d, 2 H, naph, *J* = 7.3 Hz), 7.54 (dd, 2 H, naph, *J* = 7.3, 8.6 Hz), 7.05 (br t, 2 H, NH), 4.54 (d, 2 H, OH, *J* = 5.0 Hz), 4.52 (d, 2 H, OH, *J* = 5.6 Hz), 4.39 (t, 2 H, OH, *J* = 5.6 Hz), 4.33 (s, 4 H, CH_2), 3.54 (ddd, 2 H, *J* = 3.8, 5.8, 10.1 Hz), 3.45-3.31 (m, 4 H), 3.27 (m, 2 H), 3.13-2.94 (m, 4 H), 1.84 (m, 2 H), 1.49 (m, 2 H); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ: 169.75 (C), 133.37 (C), 128.98 (C), 125.43 (CH), 122.46 (CH), 120.63 (CH), 74.89 (CH), 72.88 (CH_2), 69.96 (CH), 63.44 (CH_2), 48.56 (CH_2), 30.23 (CH_2).

2) Preparation of oligonucleotides

All oligodeoxynucleotides (ODNs) were chemically synthesized by using standard phosphoramidite chemistry at 0.2 μmol scale or 1.0 μmol scale. The amidite units were purchased from Glen Research, USA. The synthesis was carried out with An Applied Biosystems DNA/RNA synthesizer (model 3400). The synthesized DNAs were purified as previously reported[1].

3) ICL reactions

3)-1. Standard ICL reactions

5'-End fluorescein labeled ODN-I (12 pmol) was dissolved in the UDG reaction solution (29. 5 μL) in the presence or absence of the complementary ODN-II (13.2 pmol). The reaction solution contained 20.4 mM Tris-HCl (pH 8), 1.02 mM EDTA-Na_2, 1.02 mM dithiothreitol, and heated at 90 °C for 1 min, then immediately transferred into ice bath. After the solution was incubated at 37 °C for 5 min, 2 units/μL UDG (0.5 μL; BioLabs) was added to the solution to start the UDG reaction. After incubation at 37 °C for 60 min, the reaction solution was heated at 90 °C for 1 min, and then cooled in the ice bath for 10 min to reconstitute the duplex. After the reaction solution was preincubated at room temperature for 5 min, 2 mM bis(aminooxy) compound (aoNao, aoPao, aoOBao, aoPBao; 6 μL) was added to the reaction solution, followed by incubation at 27 °C. An aliquot (3 μL) was taken from the reaction solution (36 μL) at time intervals and added to the stop solution (5 μL) containing 40 mM EDTA-Na_2, 8 M urea, 0.08 % bromophenol blue and 40 mM glutaraldehyde. The reaction products were analyzed by the electrophoresis using 20 % polyacrylamide gel containing 8 M urea. Percentages of the labeled products were determined form the amounts of the ICL-duplex, the aminooxy-conjugate with a single strand and the starting material. ICL reactions of other combinations (I/II-A, I/II-f, I/II-MM, I/II-MM2) were also carried out according to this method.

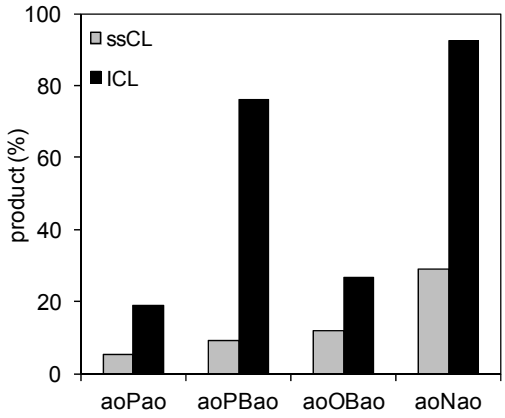
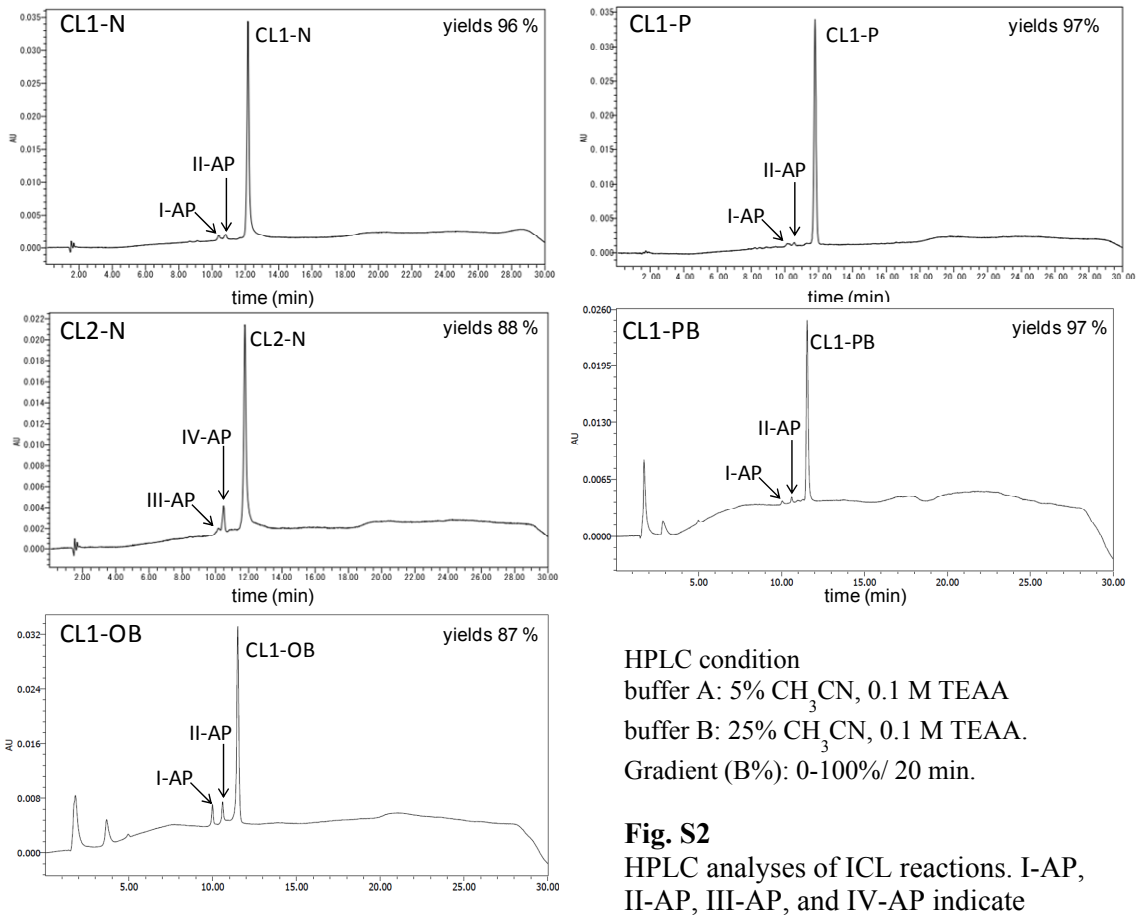


Fig. S1
ODN-I (gray bar) or ODN-I/II (black bar) was treated with cross-linkers and percentages of their conjugates after 60 min reaction were plotted.

3-2) Preparation of ICL-duplexes (CL1-N, CL2-N, CL1-P, CL1-OB, CL1-PB)

Complementary ODNs (I/II or IV/V; 2.52 nmol) without fluorescein residue were treated with UDG (2 units) in the reaction solution (100 μL) as described in the section of the standard ICL reaction. After the UDG treatment, aoNao (25.2 nmol for CL1-N or 50 nmol for CL2-N), aoPao (252 nmol), aoPBao (25.2 nmol), or aoOBao (50.4 nmol) was added to the double stranded ODNs. The reaction solution of aoPBao contained 3.4 % dimethylsulfoxide. The reaction solution was incubated at 27 °C (125 min for CL1-N, CL2-N; 180 min for CL1-P,

CL1-PB, CL1-OB). The reaction products were subjected to ethanol precipitation and purified with reverse phase HPLC (Fig. S2). The molecular weights of the ICL-duplexes were analyzed with MALDI-TOF/MS, and all ICL-duplexes were found to be correctly constructed (CL1-N calcd. 12272.15, found 12266.63; CL2-N calcd. 12314.17, found 12314.81; CL1-P calcd. 12188.15, found 12187.48; CL1-PB calcd. 12222.13, found 12224.66; CL1-OB, calcd. 12222.13, found 12223.73).



HPLC condition
buffer A: 5% CH_3CN, 0.1 M TEAA
buffer B: 25% CH_3CN, 0.1 M TEAA.
Gradient (B%): 0-100%/ 20 min.

Fig. S2
HPLC analyses of ICL reactions. I-AP, II-AP, III-AP, and IV-AP indicate ODNs having an AP site, respectively because they were treated with UDG.

3)-3. Reductive treatment of ICL-duplexes (CL1-N, CL1-P, CL1-OB, CL1-PB)

The ICL-duplexes (CL1-N, CL1-P, CL1-OB and CL1-PB, 10 pmol) were incubated in 200 mM phosphate buffer (pH1) containing 200 mM sodium cyanoborohydride. After 30 min, the products (1 pmol) were analyzed by the 20 % denaturing polyacrylamide gel electrophoresis, and the gel was stained with SYBER Gold (Life Technologies). As shown in Fig. S3, the multiple bands seen under the non reductive condition were changed to a single band by the reduction in the ICL-duplexes. These results indicated that the multiple bands were caused by the *cis-trans* structures of the oxime linkage at the two conjugation sites.

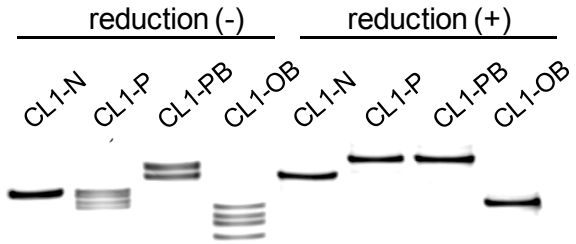


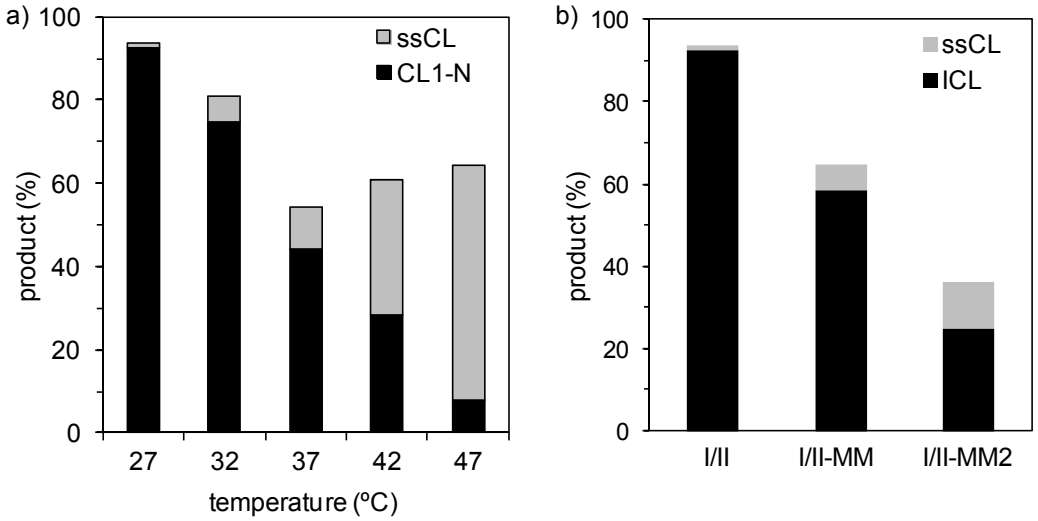
Fig. S3 Denaturing polyacrylamide gel analysis of all ICL-duplexes which were reduced with or without sodium sodium cyanoborohydride.

3)-4. Observed rate constants for ICL reactions

Observed rate constants of aoNao to the single (I) and double stranded forms (I/II-A and I/II-f) were determined as a previous report[1]. Since both II-A and II-f did not present an AP site, this reaction was analyzed as a single reaction site. Reactions under excessive amounts of aoNao were regarded as pseudo-first-order reactions and the observed rate constants (k_{obs}) were calculated from a least squares fit of the data points on a plot of the percentages of the products versus time, using the following equation P/P + S = A[1 – exp(–*k*obs × t)], where P is the concentration of the labeled products, S is the concentration of the remaining target-ONT, and A is a factor showing reactable ONTs ([reactable ONT] = A[total ONT]).

3)-5. Effects of temperature and mismatched base pairs on ICL yields

We examined effects of reaction temperature and mismatched bases pairs adjacent to an AP pair on ICL efficiency. Percentages of ICL-duplex and conjugates with a single strand (ssCL) after 60 min were shown in two graphs (Fig. S4, a, b).



c)

```
5' GAATTGCTXGGAAGAGGTTT 3' I
3' CTTAACGAXCCTTCTCCAAA 5' II
3' -------TX---------- 5' II-MM
3' -------TXG--------- 5' II-MM2
```

Fig. S4
a) Percentages of ICL-duplex (CL1-N) and ssCL at different reaction temperatures. b) Percentages of ICL duplexes and ssCL in the reactions using single (II-MM) or double (II-MM2) mismatched duplexes (c).

3)-6. ICL reaction of aNa in the presence of $NaBH_3CN$ under acidic condition

We first carried out UDG reaction as described in the standard ICL reaction except for the use of acidic buffer (50 mM phosphate buffer (pH 6)). After the UDG reaction, 4 mM aNa (3 μL) and 1.8 M $NaBH_3CN$ (3 μL) were added to the reaction solution. The reaction products were analyzed by the method described in the standard ICL reaction (Fig. S5).

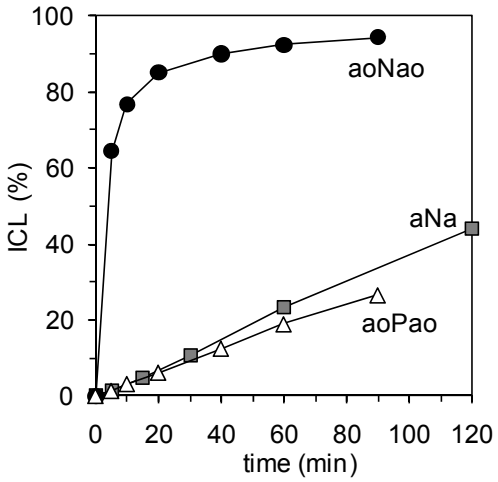


Fig. S5
Percentages of ICL-duplexes when I/II was treated with aoNao (black circles), aoPao (open triangles) or aNa (gray squares).

4) Thermal denaturation experiments

Thermal denaturation experiments of ICL-duplexes (CL1-N, CL1-P, CL2-N) and double strands without ICL (I/II, I/II-A, III/IV) were carried out (Fig. S6). Each ODN was dissolved in 10 mM sodium cacodylate buffer (pH 7) containing 10 mM NaCl to the concentration of 1.5 μM. The solutions were heated at 90°C for 3 min, gradually cooled to anneal each duplex. UV absorbance of these duplexes was measured by the use of UV-2500PC (Shimadzu) and Tm values were calculated from their plots.

a)

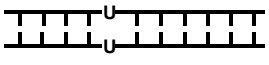
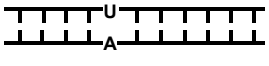
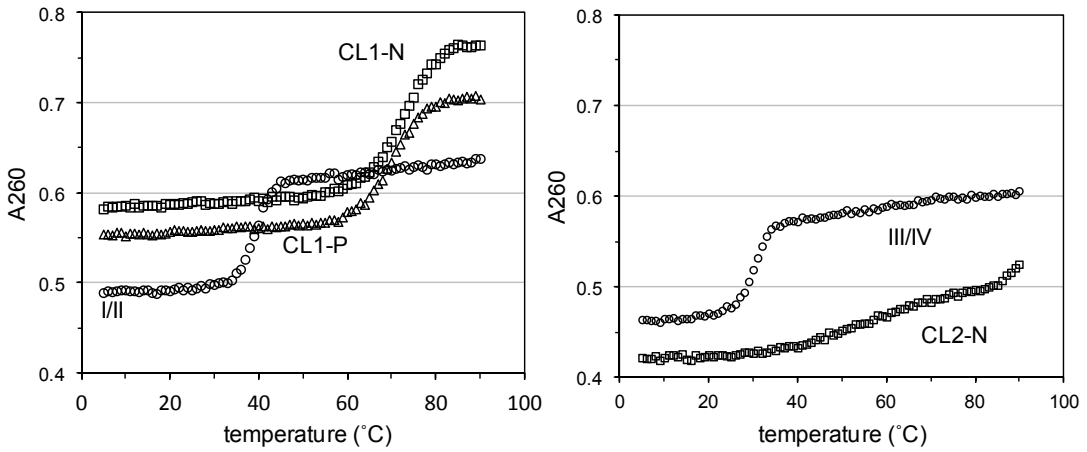
duplex		base pair or ICL	Tm
	I/II	U:U	40.5
	I/II-A	U:A	47.1
	CL1-N	aoNao	75.7
	CL1-P	aoPao	70.5
	III/IV	U:U	30.7
	CL2-N	aoNao	N.D.

Fig. S6
a) Tm values of each duplex. U and A indicate deoxynucleotides. b) Plots of UV absorbance versus temperatures.

b)



5) Composition analyses of CL1-N and CL2-N

5)-1. Complete enzymatic digestion

ICL-duplexes could not be completely digested by either Nuclease P1 or snake venome phosphodiesterase, and we used both enzymes for the longer reaction time. CL1-N or CL2-N was first treated with Nuclease P1 (5 μg; 1 μL) in 50 mM sodium acetate buffer (pH 5.2: 10 μL) at 50 °C. After 4 hours, 0.5 M Tris-HCl (pH 8.5: 5 μL), distilled water (29.5 μL), snake venome phosphodiesterase (5 μg; 5 μL) and calf intestinal alkaline phosphatase (10 units; 0.5 μL) were added to the reaction solution, followed by a 37 °C incubation for 17 hours. Ethanol (125 μL) was added to the reaction solution for ethanol precipitation. After the supernatant was filtered with 0.45 μm filter (Advantec), the solvent was evaporated under reduced pressure, and the reaction products were dissolved in distilled water.

The digested products, which were derived from CL1-N and CL2-N, and the standard sample (dR_2-aoNao) for the cross-linked site were analyzed with HPLC using a reverse phase column (Fig. S7 b, d, f). The dR_2-aoNao could be detected at 290 nm based on the absorbance

of the naphthalene residue, and showed three peaks of each isomer derived from the double oxime linkages. All nucleosides and dR_2-aoNao were identified from the complete digestions of both ICL-duplexes, and each amount was confirmed to be the same as calculated. The nuclease digestion of CL2-N was also found to give about two folds of dR_2-aoNao in comparison with that of CL1-N.

5)-2. Reduction of the cleaved products

The standard sample of dR_2-aoNao was converted to a single product, dR_2-aoNao (red) by reducing the oxime linkages (Fig. S7c) in the presence of sodium cyanoborohydride, and the structure was identified as that described in the preparation of dR_2-aoNao (red) (**12**). To confirm the same reduction, the digested products of CL1-N were treated in the sodium phosphate buffer (pH 1) containing 200 mM sodium cyanoborohydride for 60 min at room temperature. The reaction provided dR_2-aoNao (red) as a single peak (Fig. S7e).

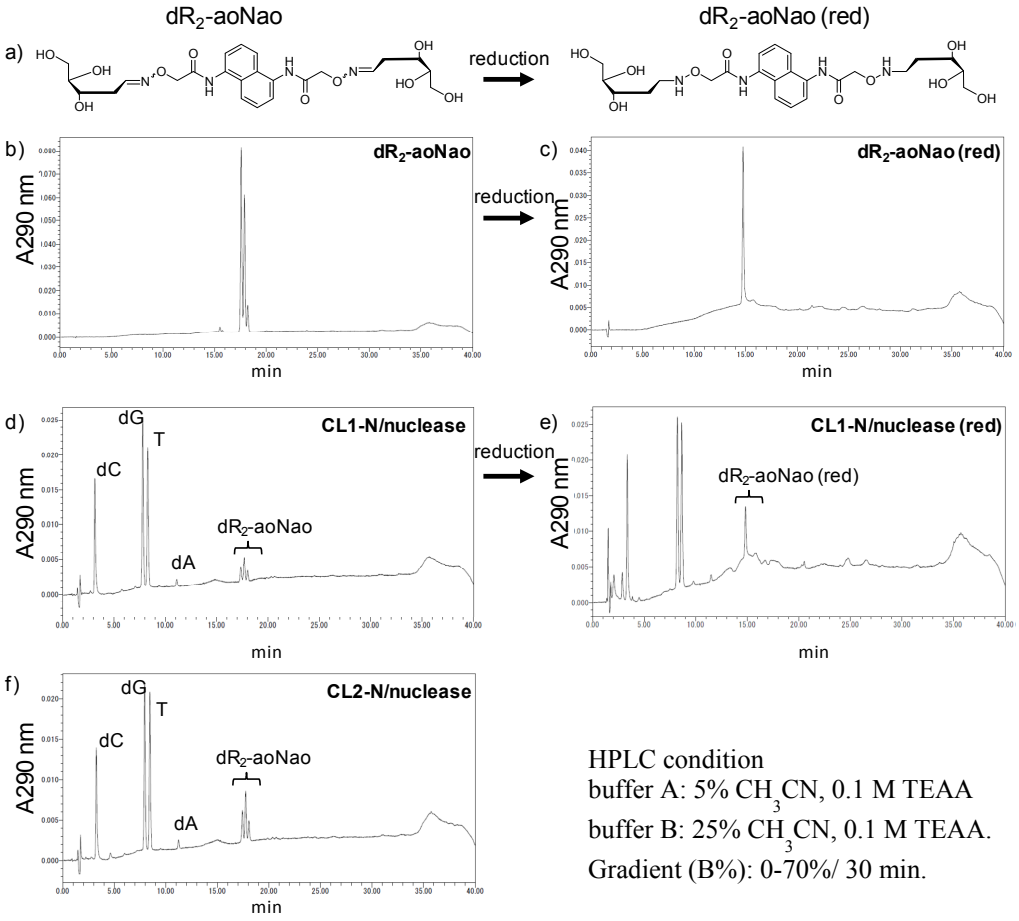


Fig. S7

HPLC analyses of complete digestions with nucleases and the reduction of them. a) Structures of ICL site (dR_2-aoNao) and its reduced form (dR_2-aoNao(red)). b) standard dR_2-aoNao (**11**), c) standard dR_2-aoNao (red) (**12**), d) complete digestion of CL1-N, e) the reduction of (d), f) complete digestion of CL2-N. CL1-N/nuclease and CL2-N/nuclease indicate nuclease digested ICL-duplexes.

6) Enzyme reactions on ICL-duplexes

6)-1. Preparation of ICL and PM-duplexes

5'-Fluorescein, biotin or thiol modified ODNs (I, bio-V, s-II, s-VI, s-VII; Fig. S8), were chemically synthesized by the standard amidite method. Fluorescein (F), biotin (bio) and thiol (s) modifications were performed by using specific amidite reagents (5'-fluorescein, 5'-biotin and dithiol phosphoramidite, Glen Research). All of these ODNs were synthesized to have deoxyuridine (U) at X.

ICL-duplexes (HRP-CL1-N, ALP-CL1-N, HRP/ALP-CL2-N) were obtained from the aoNao-promoting ICL reactions of bio-V/s-VI, I/s-II and bio-V/I/s-VII duplexes. All ODNs (2.52 nmol) dissolved in the UDG reaction solution (99 μL) containing 20.2 mM Tris-HCl (pH 8), 1.01 mM EDTA-Na_2, 1.01 mM dithiothreitol were heated at 90 °C for 1 min, and then annealed. After the solution was kept at room temperature for 5 min, 2 units/μL UDG (1 μL; BioLabs) was added to the solution to form AP sites. The reaction solution was incubated at 37 °C for 60 min, followed by the addition of aoNao (50.4 nmol for bio-V/I/s-VII and 25.2 nmol for bio-V/s-VI or I/s-II). Each ICL reaction was performed at 27 °C for 2 hours (bio-V/s-VI , I/s-II) or for 4 hours (bio-V/I/s-VII). After the reaction solutions were subjected to ethanol precipitation, each ICL-duplex was analyzed with HPLC using a reverse phase column (Fig. S9). All ICL-duplexes could be purified with the HPLC. Each standard PM-duplex (PM-bio, PM-F) was prepared from annealing double strands (I-A/s-II, bio-V-A/s-VI, Fig. S8).

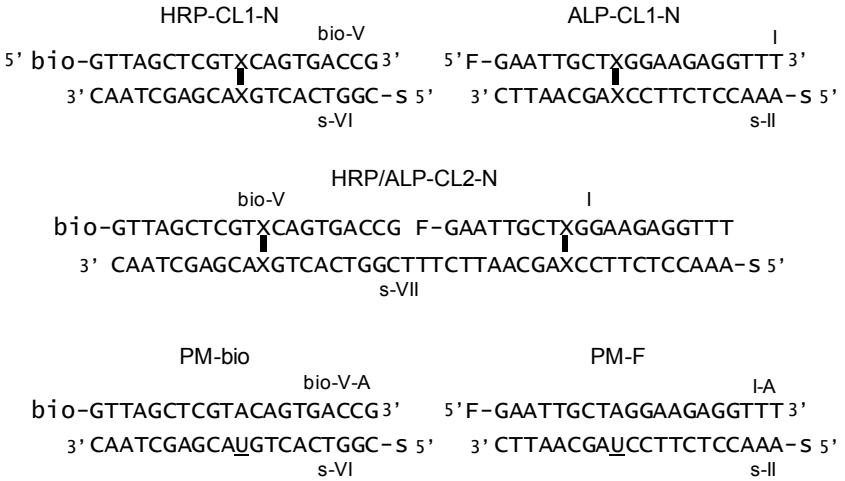


Fig. S8
Sequences of ICL- and PM-duplexes. F, bio, s and U indicate fluorescein, biotin, dithiol and deoxyuridine, respectively. X and bold lines indicate cross-linked AP sites.

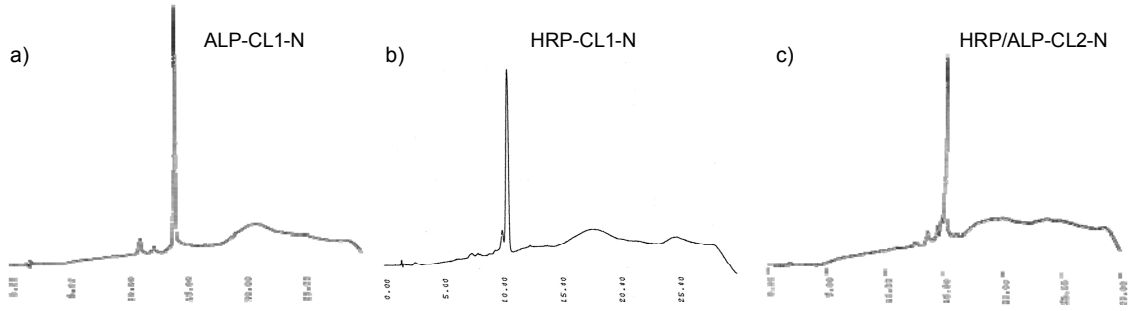


Fig. S9
HPLC analyses after ICL reactions. a) ALP-CL1-N, b) HRP-CL1-N, c) HRP/ALP-CL2-N.

6)-2. Preparation of the gold chip immobilizing ICL-duplexes

Gold chips were prepared as the previous report[4], and ICL-duplexes (HRP-CL1-N, ALP-CL1-N and HRP/ALP-CL2-N; 2 μM) and PM-duplexes (PM-bio and PM-F) dissolved in 0.5 M phosphate buffer (pH 7) were spotted on the chip in triplicate by using a glass capillary (internal diameter, 23.9±1.2 μm). After the gold chip was incubated in a sealed petri dish at room temperature for 16 hours, it was washed in water (100 mL) at 40 °C for 10 min, and then washed in 0.05 x PBS solution containing 0.1 % SDS at room temperature for 10 min. Masking the surface was carried out by immersing the chip in 1 mM mercaptohexanol aqueous solution (3 mL) for 60 min, and the chip was washed twice in PBS solution (100 mL). The chip surface was further masked with the BSA solution (1% BSA, 0.1 M Tris-HCl (pH 7.5), 0.15 M NaCl; 3 mL) for 2 hours to reduce nonspecific binding of proteins, and then washed with PBS solution (100 mL).

6)-3. Monitoring of enzymatic reactions on ICL-duplexes by using SECM

To bind alkaline phosphatase-anti-fluorescein antibody (ALP-Ab) with fluorescein -modified ODNs, the gold chip surface was covered with the ALP-Ab solution (3.8 units/mL; 200 μL) containing 1 % BSA, 0.15 M NaCl, 0.1 M Tris-HCl buffer (pH 7.5), and incubated for 20 min at room temperature in a humidified petri dish. The chip was washed twice in PBS solution (100 mL), and then used for SECM measurement in the presence of the ALP-substrate solution (3 mL) containing 2 mM *p*-aminophenol phosphate (PAPP), 0.1 M Tris-HCl (pH 8.5), and 1 mM $MgCl_2$. After the measurement of ALP activity, HRP-streptavidin conjugate (HRP-SA) was bound with biotin-modified ODNs in the HRP-SA solution (2 μg/mL; 200 μL) containing 1% BSA, 0.15 M NaCl, 0.1 M Tris-HCl (pH 7.5). The gold chip was incubated in a humidified petri dish at room temperature for 20 min, and then washed twice in PBS (100 mL). The gold chip was analyzed with SECM in the HRP-substrate solution (3 mL) containing 1 mM hydroquinone (H_2Q) and 1 mM H_2O_2 in PBS.

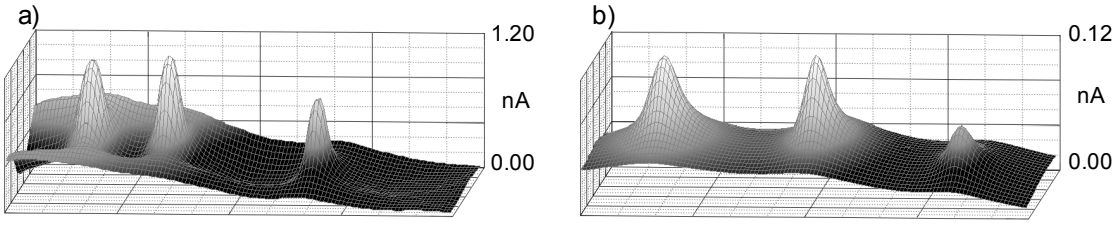


Fig. S10

SECM images of HRP (a) and ALP (b) reactions shown in Fig. 3b.

References

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(4) Palchetti, I.; Laschi, S.; Marrazza, G.; Mascini, M. *Anal. Chem.* **2007,** *79*, 7206.