Electronic supplementary information

Acceleration of hydrolytic ring opening of N7-alkylguanine by the terminal carbamoyl group of glycidamide

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Materials and Methods

General methods. HPLC analysis and purification were performed using a Gilson gradient system equipped with a Shimadzu CTO-20A column oven and a Waters 2998 photodiode array detector. For purification, elution was monitored by using a Gilson 151 UV/VIS detector. Analysis of oligonucleotides by mass spectrometry was performed at Hokkaido System Science Co., Ltd., using electrospray ionization (ESI) in the negative ion mode.

Analysis of the reaction of dG_F with GA. A mixture of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine (dG_F, purchased from Combi-Blocks, 100 nmol) and GA (50 μmol) in 0.1 M sodium phosphate (pH 7.0, 100 μL) was incubated at 37°C for 24 h, and the product was analyzed by HPLC using an Inertsil ODS-3 5 μm column (4.6 × 250 mm, GL Sciences) with a linear gradient of acetonitrile (7–13% for 20 min) in 0.1 M triethylammonium acetate (TEAA, pH 7.0), at a flow rate of 1.0 mL min^{-1}. The product and N7-GA-Gua prepared in the same manner using dG were purified by HPLC. LC-MS analysis of the dG_F adduct was performed on an X500R quadruple time-of-flight mass spectrometer coupled with a UPLC system (ACQUITY UPLC H-class plus, Waters), using an InertSustain AQ-C18 UHPLC-PEEK column (2.1 × 100 mm, 1.9 μm, GL Sciences) with a linear gradient of acetonitrile (0–38% for 7 min), at a flow rate of 0.2 mL min^{-1}. The mass spectrometer was operated in the positive electrospray-ionization mode with a capillary voltage of 5,500 V. The source temperature was 350°C, and the declustering potential was 40 V.

Spectroscopic analysis of the N7-GA-adduct and its hydrolyzed product. A 9-mer oligonucleotide containing dG_F in sequential abasic site analogues (dS), d(SSSSG_FSSSS), was synthesized using 2′-F-G-ANA-CE Phosphoramidite, dSpacer CE Phosphoramidite, and Universal Support III PS (Glen Research), and purified by reversed-phase HPLC using an

Inertsil ODS-3 5 μm column (4.6 × 250 mm) with a linear gradient of acetonitrile (7–13% for 20 min) in 0.1 M TEAA, at a flow rate of 1.0 mL min^{-1} in a column oven set at 50°C. An aliquot of this oligonucleotide (5 nmol) was treated with GA (5 μmol) in 50 mM sodium phosphate (pH 7.0, 25 μL) at 60°C for 4 h, and the product was purified by HPLC under the same conditions as described above. The eluate containing the GA-adducted oligonucleotide was concentrated on a rotary evaporator, and TEAA was removed by co-evaporation with water. This oligonucleotide (2.0 nmol) was dried on a Savant SpeedVac concentrator and then dissolved in 0.1 M sodium phosphate (pH 8.0, 20 μL). After an incubation at 37°C for 20 h, the reaction mixture (15 μL) was injected into a TSKgel DEAE-2SW column (4.6 × 250 mm, Tosoh Corporation). The product was separated with a linear gradient of ammonium formate (0.2–0.8 M for 20 min) in 20% aqueous acetonitrile, at a flow rate of 1.0 mL min^{-1} at 50°C.

Preparation of N7-adducted oligonucleotides. For GA 9-mer, 5'-phosphorylated d(TTTTG_FTTTT) (0.25 μmol) was mixed with GA (0.25 mmol) in 50 mM sodium phosphate (pH 7.0, 1.25 mL). After 12 h at 60°C, the product was isolated by reversed-phase HPLC using an Inertsil ODS-3 5 μm column (7.6 × 250 mm) with a linear gradient of acetonitrile (9–13% for 20 min) in 0.1 M TEAA, at a flow rate of 2.0 mL min^{-1} at 50°C, and the product peak was collected. The obtained product was further purified by anion-exchange HPLC using a TSKgel DEAE-2SW column (4.6 × 250 mm) with a linear gradient of ammonium formate (0–1.0 M for 20 min) in 20% aqueous acetonitrile, at a flow rate of 1.0 mL min^{-1} at 50°C, and then by reversed-phase HPLC using an Inertsil Ph-3 column (4.6 × 250 mm, GL Sciences) with isocratic separation at 10% acetonitrile in 0.1 M TEAA, at a flow rate of 1.0 mL min^{-1} at 50°C. The eluate was desalted by solid-phase extraction using a Sep-Pak cartridge (Waters Corporation). GO 9-mer and EB 9-mer were prepared in the same manner, except that purification of EB 9-mer on the Inertsil Ph-3 column was carried out with 11% acetonitrile in 0.1 M TEAA. The purity and the molecular weight of each adduct-containing oligonucleotide were confirmed by mass spectrometry (Fig. S6).

Preparation of FAPy-containing oligonucleotides. GA 9-mer, GO 9-mer and EB 9-mer (70 nmol) were each dissolved in 0.1 M sodium phosphate (pH 8.0, 1.0 mL) and incubated at 37°C (GA 9-mer) or 60°C (GO 9-mer and EB 9-mer). After 30 h (GA 9-mer), 10 h (GO 9-mer) or 18 h (EB 9-mer), the products were purified by anion-exchange HPLC using a TSKgel DEAE-2SW column (4.6 × 250 mm) with a linear gradient of ammonium formate (0.2–1.0 M for 20 min) in 20% aqueous acetonitrile, at a flow rate of 1.0 mL min^{-1} at 50°C, followed by desalting by solid-phase extraction using a Sep-Pak cartridge. The obtained 9-mers containing each FAPy derivative were analyzed by mass spectrometry (Fig. S7).

Comparison of the hydrolytic ring-opening reactions. Solutions (200 μL) containing GA 9-mer, GO 9-mer or EB 9-mer (2.0 nmol) in 0.1 M sodium phosphate (pH 8.0) were divided into five tubes and incubated at 37°C. After 6, 12, 24, 36 and 48 h, 0.2 M sodium phosphate (pH 5.0, 20 μL) was added on ice. The samples were analyzed by anion-exchange HPLC using a TSKgel DEAE-2SW column (4.6 × 250 mm) with a linear gradient of

ammonium formate (0.2–1.0 M for 20 min) in 20% aqueous acetonitrile, at a flow rate of 1.0 mL min^{-1} at 50°C. The data acquired by using a Waters 2998 photodiode array detector were processed with the Empower 3 software to obtain the peak area values. The same experiments were repeated three times. In the case of EB 9-mer, the solutions were also incubated at 60°C and analyzed by HPLC in the same manner.

Computational details. For the theoretical analyses of the reaction coordinates, a spin-restricted density functional theory (RDFT) method was performed, using the Becke 3-parameter Lee-Yang Parr (B3LYP) functional set. As the basis set, 6-31G* was used. The solvent effect of water was approximated by the polarizable continuum model (PCM), using the integral equation formalism variant (IEFPCM). The optimized initial, IM1, IM2 and product structures were confirmed to have no imaginary frequencies. The optimized TS2 structures were also confirmed to have single negative frequencies. The reactions from IM2 to the product through TS2 were confirmed by the intrinsic reaction coordinate (IRC). All the above calculations were performed by Gaussian 09 rev. D01.[S1] However, an appropriate transition state between IM1 and IM2 (i.e., TS1) was not obtained; therefore, potential energy curves between IM1 and IM2 were examined by the nudged elastic band (NEB) method, performed using the B3LYP/6-31G* level of theory under water solvent conditions approximated by the conductor-like polarizable continuum (CPCM) in Orca 4.1.2.[S2] In addition, we confirmed that there was no energy barrier between the initial and MI1 structures by NEB. All the initial model structures of the FAPy derivatives with a pentose moiety (see Fig. S10a) were constructed by GaussView 6.

Reaction of 2'-deoxyguanosine with GA, GO and EB. The mixtures of 2'-deoxyguanosine (100 nmol) and GA, GO or EB (50 μmol) in 0.1 M sodium phosphate (pH 7.0, 100 μL) were incubated at 37°C, and after 48 h, the products were separated by reversed-phase HPLC using an Inertsil ODS-3 5 μm column (4.6 × 250 mm) with a linear gradient of acetonitrile (0–15% for 20 min) in 0.1 M TEAA, at a flow rate of 1.0 mL min^{-1}.

S1 M. J. Frisch, et al., Gaussian 09, Revision D.01, Gaussian, Inc., Wallingford CT, 2013.

S2 F. Neese, ORCA – An Ab Initio, Density Functional and Semi-empirical Program Package, Version 4.1.2.

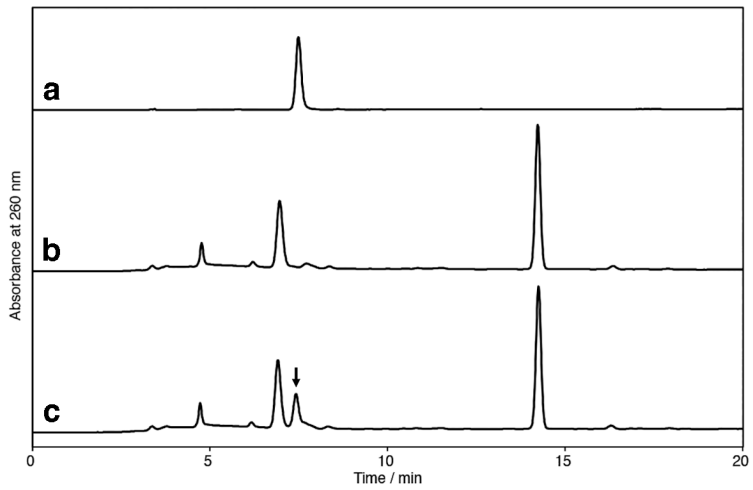

Fig. S1 Prevention of the glycosidic bond cleavage by the 2'-F modification. (**a**) HPLC analysis of N7-GA-Gua prepared by the reaction of dG with GA (peak i in Fig. 4a). An Inertsil ODS-3 column (4.6 × 250 mm) was used at a flow rate of 1.0 mL min^{-1} with a linear gradient of 7–13% acetonitrile. (**b**) HPLC analysis of the reaction mixture containing dG_F and GA in 0.1 M sodium phosphate (pH 7.0) after incubation at 37°C for 24 h. LC-MS analysis showed that the *m*/*z* value of the product at 7.0 min was 373.1257. The calculated *m*/*z* value of N7-GA-dG_F is 373.1266. (**c**) Co-injection of the reaction mixture (**b**) with N7-GA-Gua (**a**). The peak of N7-GA-Gua is indicated by an arrow.

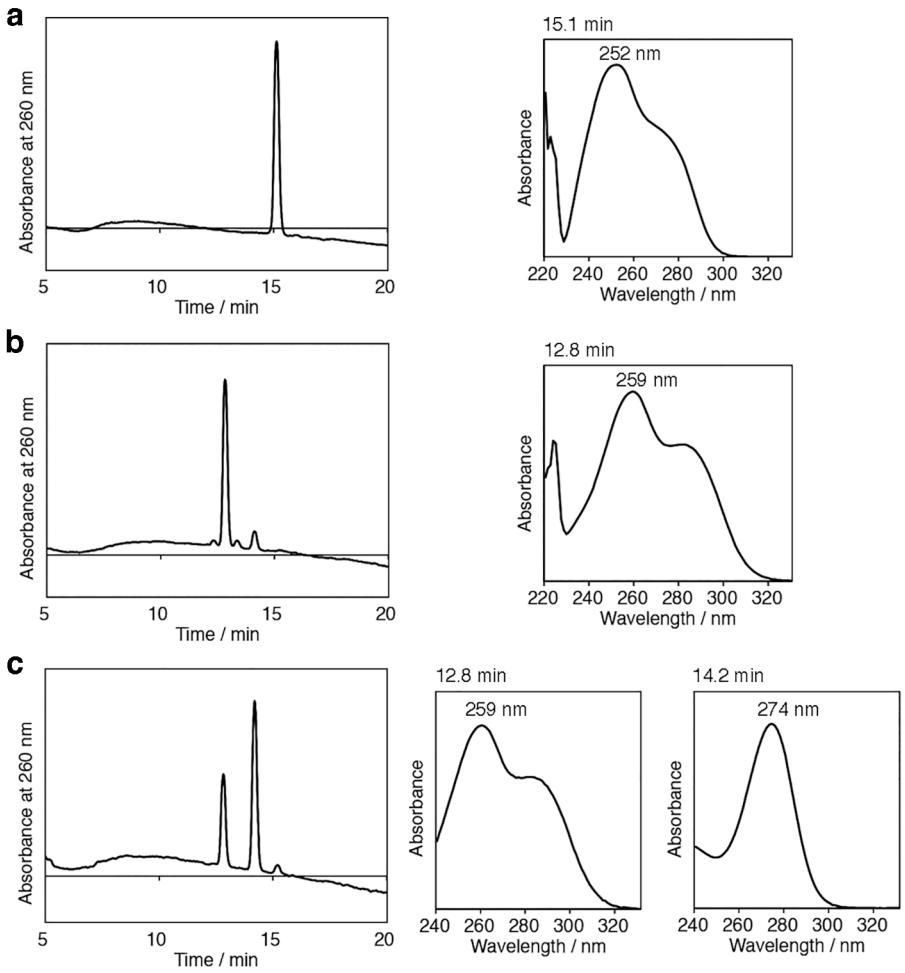

Fig. S2 Spectroscopic analyses of N7-GA-adduct formation and hydrolysis in oligonucleotides. (**a**) Anion-exchange HPLC analysis of d(SSSSG$_F$SSSS), (**b**) GA-adducted d(SSSSG$_F$SSSS) after purification by reversed-phase HPLC and (**c**) GA-adducted d(SSSSG$_F$SSSS) after an incubation in 0.1 M sodium phosphate (pH 8.0) at 37°C for 20 h. The HPLC conditions are described in the Materials and Methods. UV-absorption spectra of the peaks are shown with the retention time and the absorption maximum. These spectra were extracted with the data processing software for the photodiode array detector.

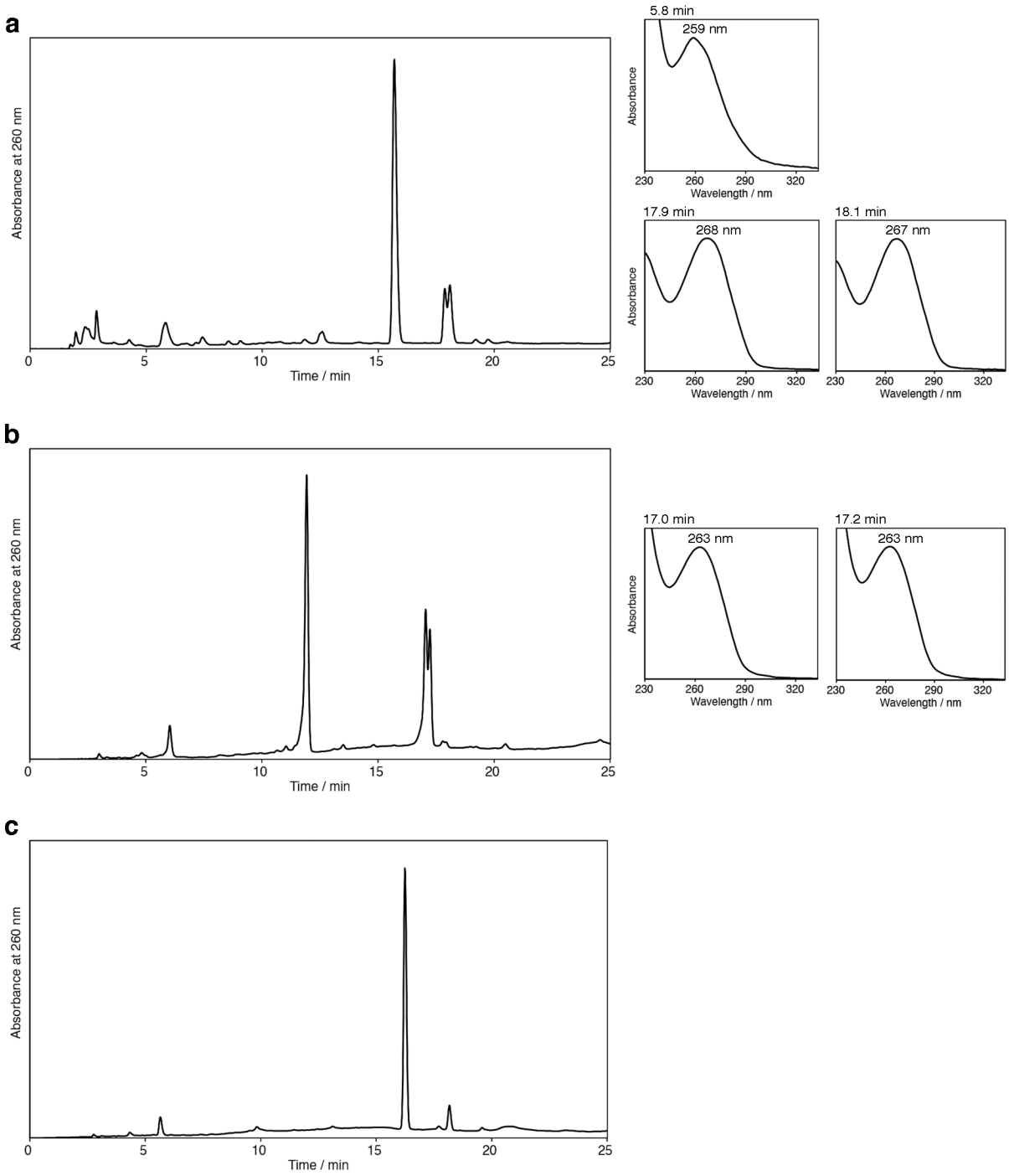

Fig. S3 Reversed-phase HPLC analyses of the reaction mixtures after incubating 2'-deoxyadenosine (**a**), 2'-deoxycytidine (**b**), and thymidine (**c**) with GA at 60°C for 20 h in 50 mM sodium phosphate (pH 7.0). (**a**) An XBridge C18 5 μm column (4.6 × 150 mm, Waters Corporation) was used at a flow rate of 1.0 mL min^{-1} with a linear gradient of acetonitrile (0–7.5% for 20 min) in 0.1 M TEAA. (**b** and **c**) An Inertsil ODS-3 column (4.6 × 250 mm) was used at a flow rate of 1.0 mL min^{-1} with a linear gradient of acetonitrile (0–10% (**b**) or 0–15% (**c**) for 20 min) in 0.1 M TEAA. The largest peak in each chromatogram is the starting material, and the UV-absorption spectra of the product peaks are shown.

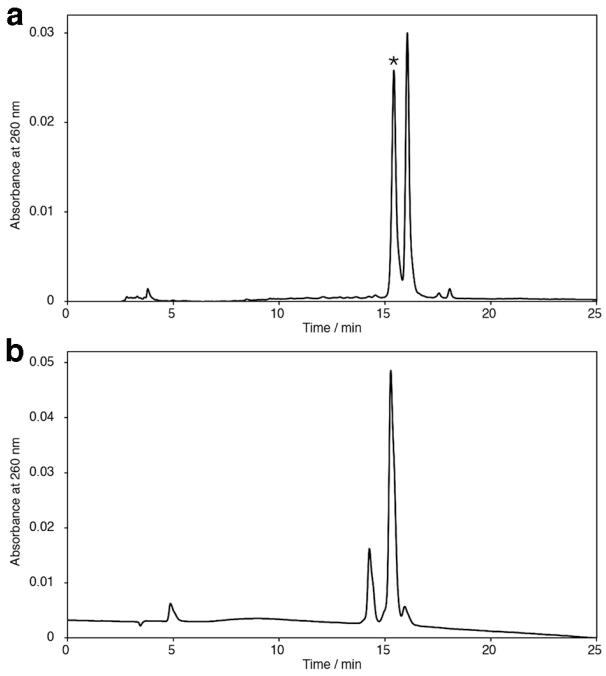

Fig. S4 Preparation of GA 9-mer and GA-FAPy 9-mer. (**a**) Reversed-phase HPLC analysis of the reaction mixture containing the 9-mer, p-d(TTTTG$_F$TTTT), and GA in 50 mM sodium phosphate (pH 7.0), after an incubation at 60°C for 12 h. An Inertsil ODS-3 5 μm column (4.6 × 250 mm) was used with a linear gradient of acetonitrile (7–17% for 20 min) in 0.1 M TEAA, at a flow rate of 1.0 mL min^{-1} at 50°C. The peak with a longer retention time is the starting 9-mer, and the product indicated with an asterisk (GA 9-mer) was isolated and purified further. (**b**) Anion-exchange HPLC analysis of GA 9-mer after an incubation in 0.1 M sodium phosphate (pH 8.0) at 37°C for 30 h. A TSKgel DEAE-2SW column (4.6 × 250 mm) was used with a linear gradient of ammonium formate (0.2–1.0 M for 20 min) in 20% aqueous acetonitrile, at a flow rate of 1.0 mL min^{-1} at 50°C. The peak with a retention time of 14.3 min is remaining GA 9-mer.

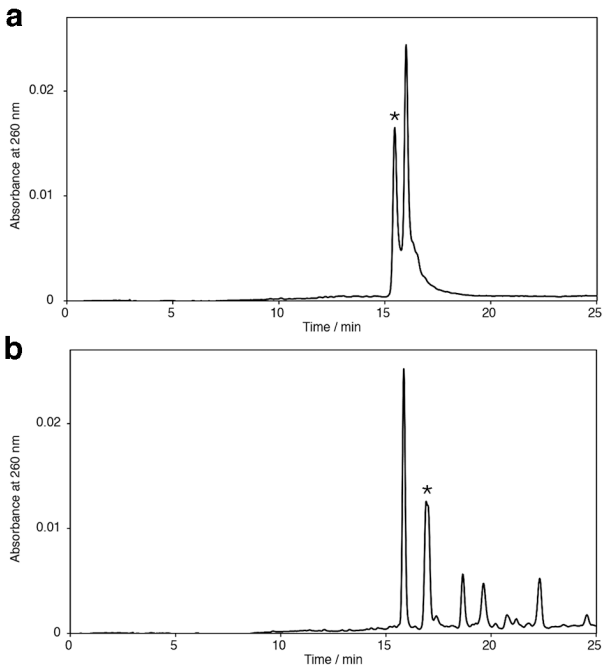

Fig. S5 Reversed-phase HPLC analyses of the reaction mixtures containing the 9-mer, p-d(TTTTG$_F$TTTT), and GO (**a**) or EB (**b**) in 50 mM sodium phosphate (pH 7.0), after an incubation at 60°C for 12 h. The largest peak is the starting material in each case, and the products indicated with an asterisk were isolated and purified further. The HPLC conditions are the same as described in the legend to Fig. S4a.

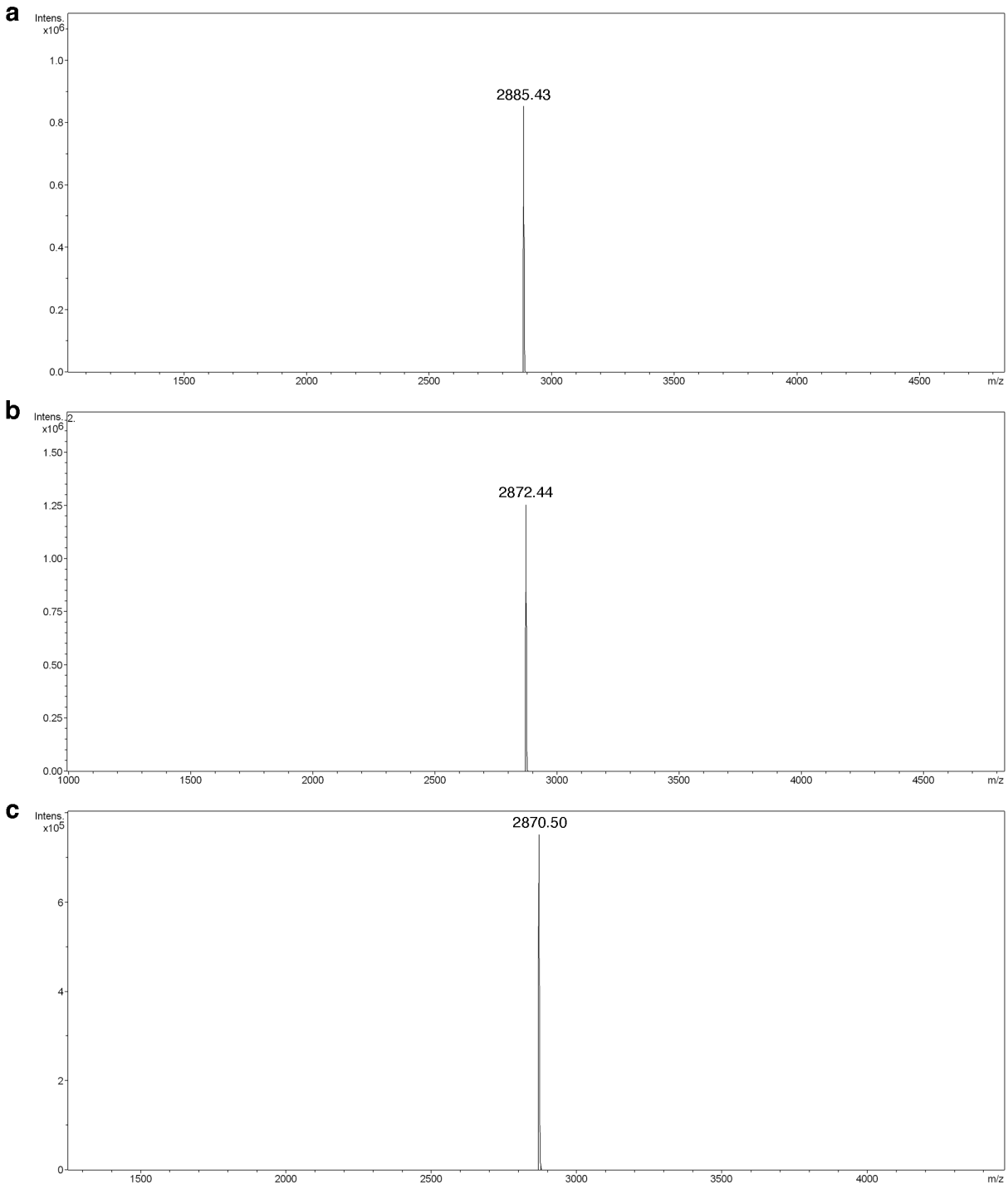

Fig. S6 Analyses of GA 9-mer (**a**), GO 9-mer (**b**) and EB 9-mer (**c**) by ESI mass spectrometry in the negative ion mode. The calculated *m*/*z* values for GA 9-mer, GO 9-mer and EB 9-mer are 2884.84, 2871.84 and 2869.87, respectively.

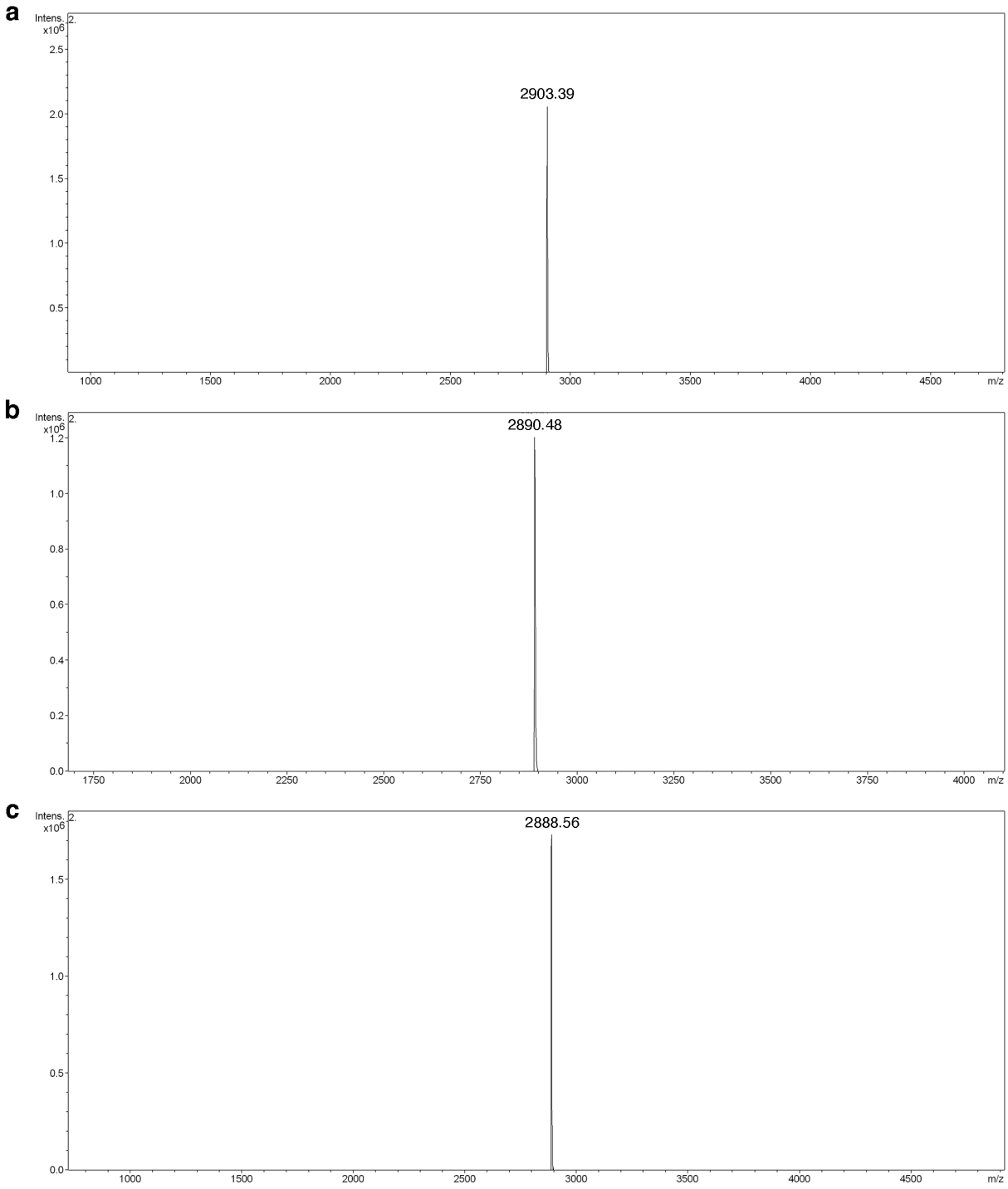

Fig. S7 Analyses of GA-FAPy 9-mer (**a**), GO-FAPy 9-mer (**b**) and EB-FAPy 9-mer (**c**) by ESI mass spectrometry in the negative ion mode. The calculated *m*/*z* values for GA-FAPy 9-mer, GO-FAPy 9-mer and EB-FAPy 9-mer are 2902.86, 2889.86 and 2887.88, respectively.

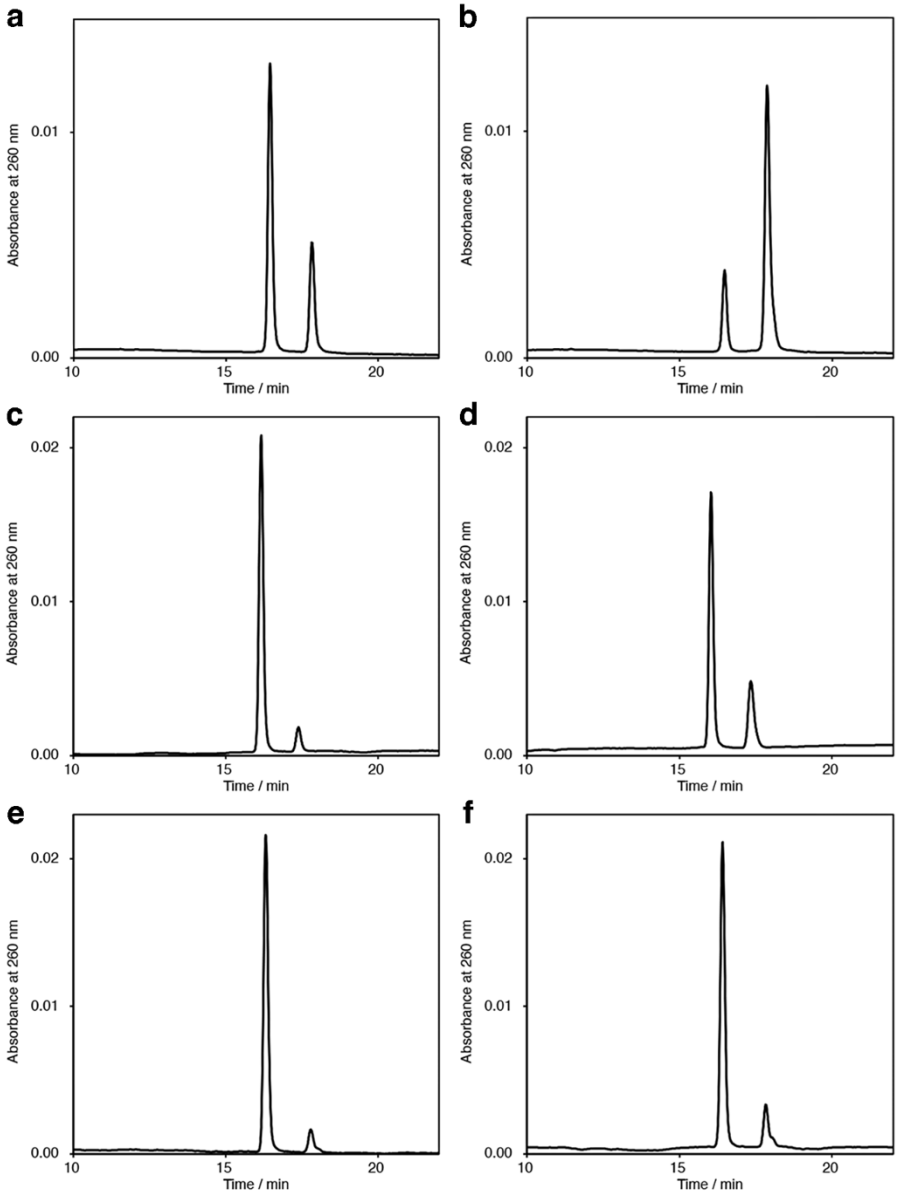

Fig. S8 Anion-exchange HPLC analyses of GA 9-mer (**a** and **b**), GO 9-mer (**c** and **d**), and EB 9-mer (**e** and **f**) after an incubation in 0.1 M sodium phosphate (pH 8.0) at 37°C for 6 h (**a**, **c** and **e**) or 24 h (**b**, **d** and **f**). The HPLC conditions are described in the Materials and Methods.

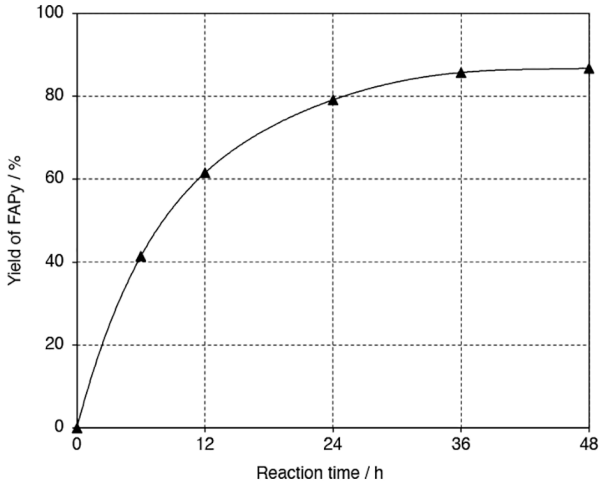

Fig. S9 Hydrolysis of EB 9-mer to EB-FAPy 9-mer at 60°C. The product was separated from the starting 9-mer by anion-exchange HPLC, and the yields were calculated from the peak areas.

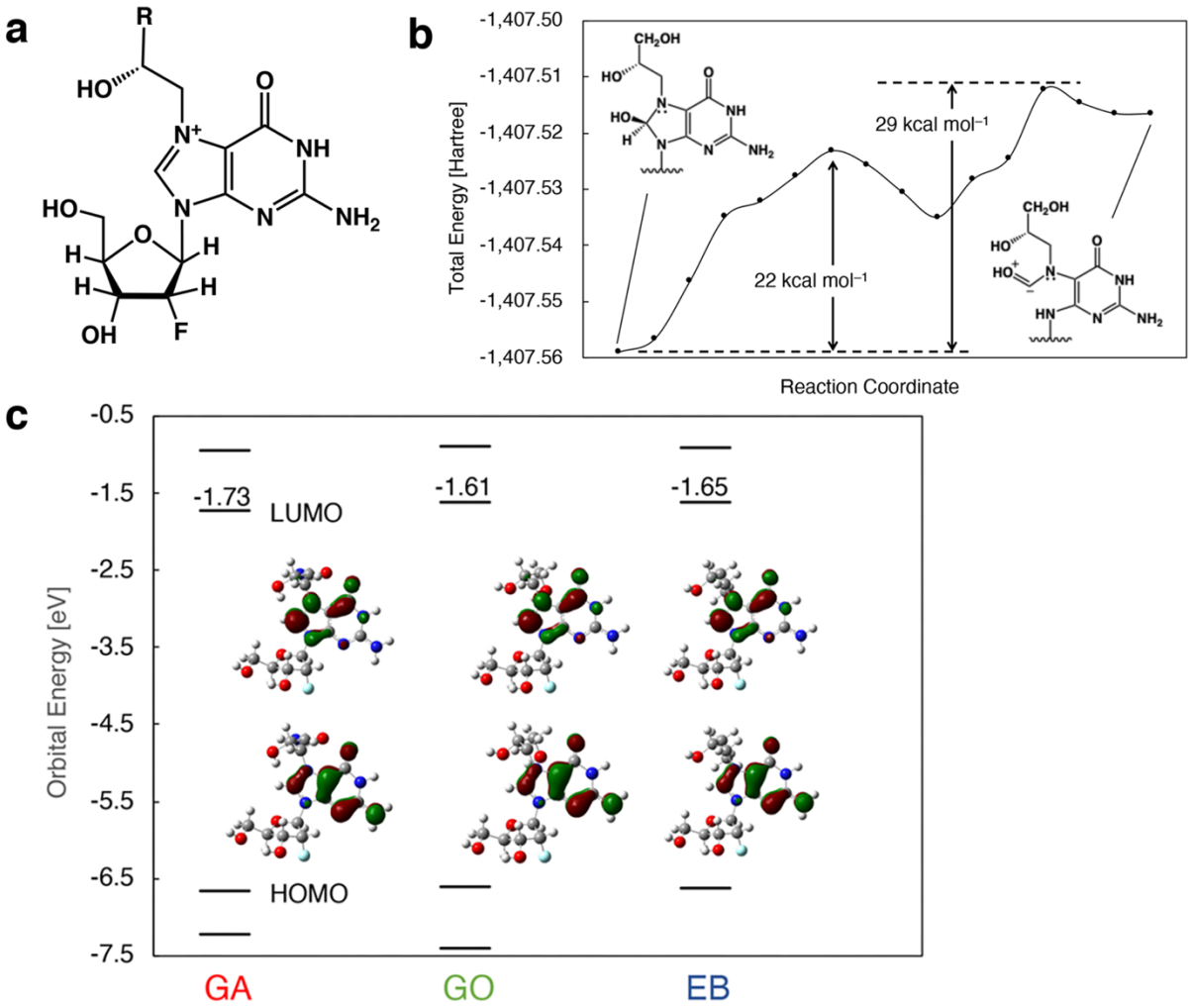

Fig. S10 (**a**) Illustration of the computational model structure of the FAPy derivatives (R = $CONH_2$, CH_2OH and CH_2CH_3 for GA, GO and EB, respectively). (**b**) Calculated potential energy curve of the GO adduct by the NEB method. (**c**) Frontier orbitals and their energies of the GA, GO and EB adducts.

Table S1. Selected Hirshfeld atomic charges in MI2 of the GA, GO and EB adducts. The atom labels are defined in the figure below the table. The reaction from IM2 to TS2 is a proton (H(4)) migration from O(3) to C(1). There is, however, no significant difference in the atomic charges between GA, GO and EB. On the other hand, the optimized distances between H(2) and O(3) in the GA, GO and EB adducts are 2.22, 2.09 and 2.10 Å, respectively. Therefore, H(2) is considered to make a weak intramolecular hydrogen bond with O(3). The amount of the positive charge on H(2) is in the order of GA > GO > EB. In other words, the intramolecular hydrogen bond formed in the GA adduct is stronger than those in GO and EB. From these results, it is suggested that the strength of the hydrogen bond between H(2) and O(3) contributes to the difference in the reaction barrier of TS2.

Atom	GA	GO	EB
C(1)	–0.103	–0.105	–0.107
O(1)	–0.227	–0.222	–0.234
N(1)	–0.076	–0.075	–0.075
N(2)	–0.129	–0.132	–0.129
H(1)	0.109	0.106	0.106
H(2)	0.167	0.161	0.154
O(3)	–0.178	–0.175	–0.177
H(4)	0.210	0.210	0.209