Supporting Information

Site-Specific DNA Functionalization through the Tetrazene-Forming Reaction in Ionic Liquids

Seiya Ishizawa, Munkhtuya Tumurkhuu, Elizabeth J. Gross, Jun Ohata*
Department of Chemistry, North Carolina State University, Raleigh, NC 27695

Table of Contents

Supporting figures

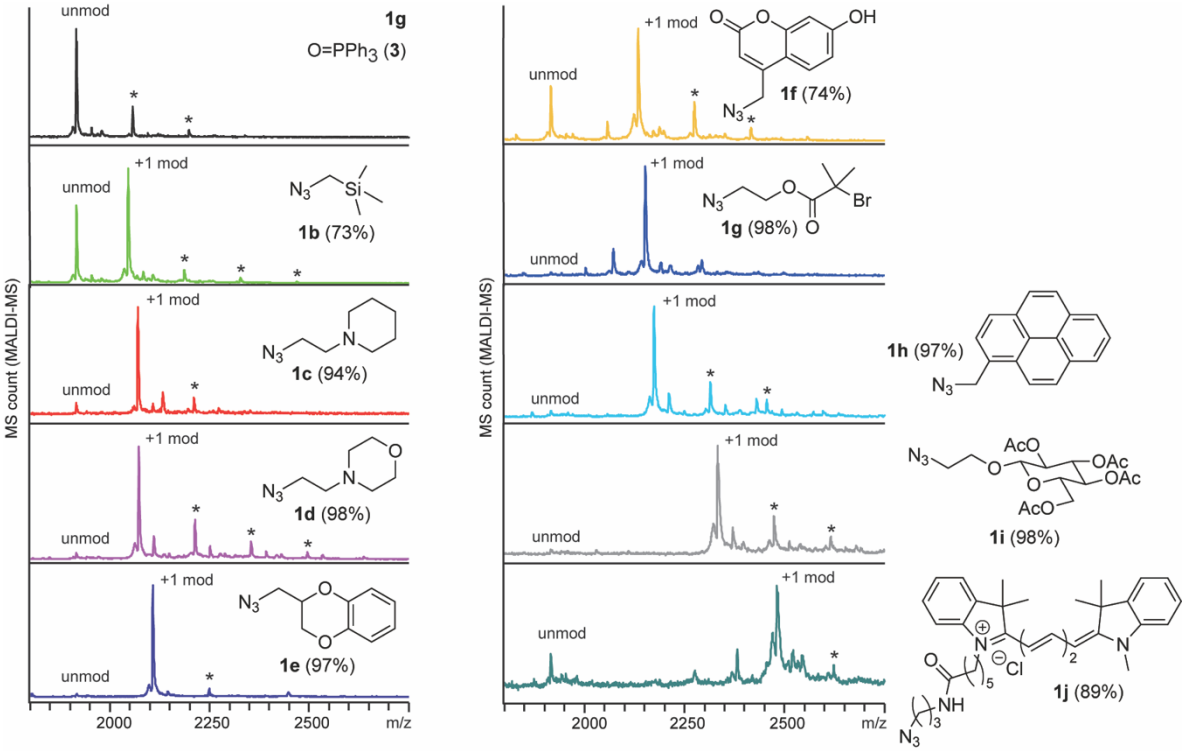
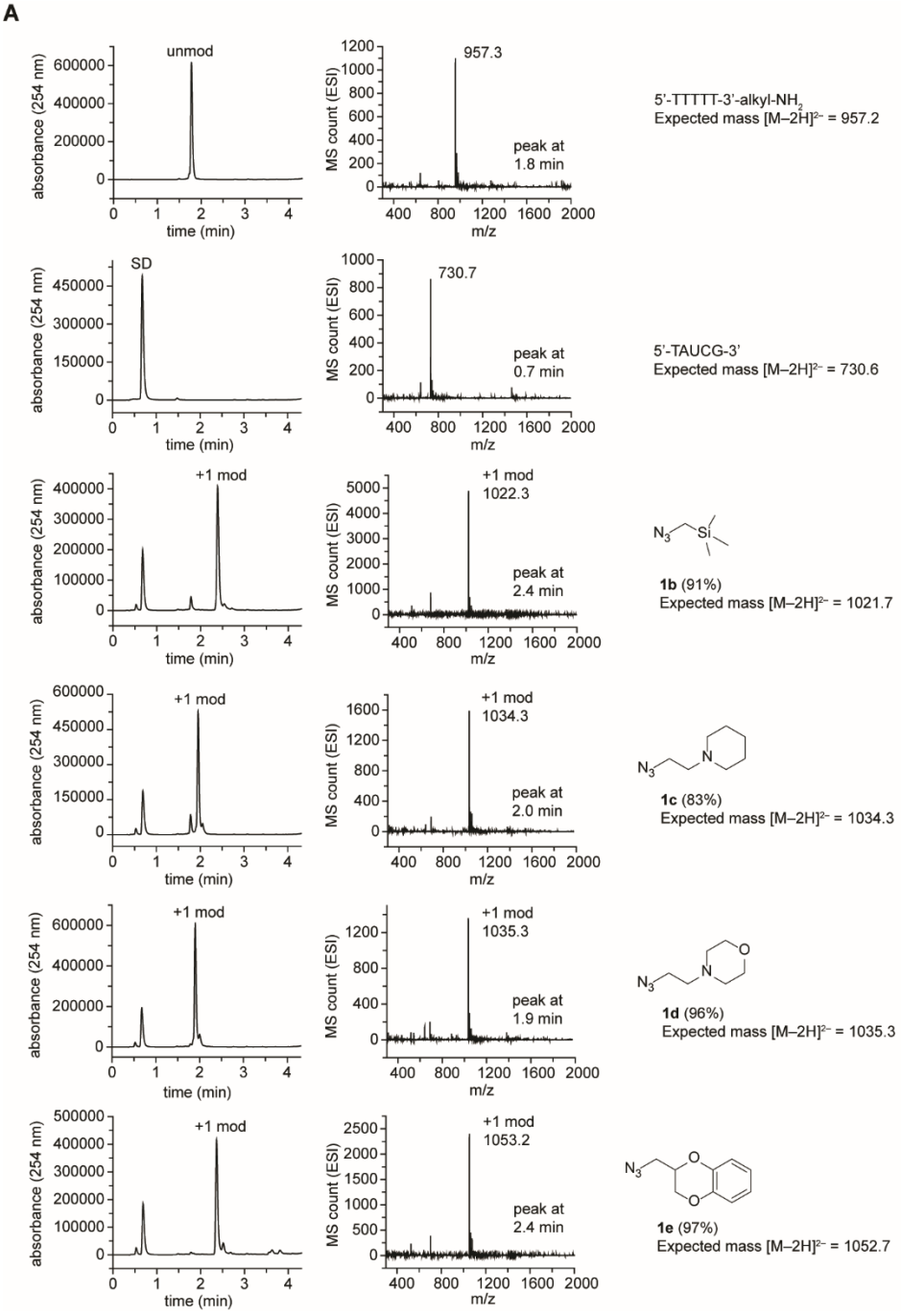


Figure S1. Data for Fig. 3. Matrix-assisted laser desorption/ionization (MALDI-MS) analysis of modification of 5'-TTTTT-3'-alkyl-NH_2 with various alkyl azide reagents. 3,4-diaminobenzophenone (DABP) was used as a matrix for all conditions. "*" sign denotes 1-butyl-1-methylpyrrolidinium (BMPy) adduct. BMPy = 142.3 Da. Modification reaction conditions: $KHCO_3$ (20 mM final concentration from 2 M stock solution), 5'-TTTTT-3'-alkyl-NH_2 (0.2 mM final concentration from 5 mM stock solution in H_2O), azide derivatives **1b-1j** (7.5 mM final concentration from 250 mM stock solution in DMSO), and PPh_3 (20 mM final concentration from 500 mM stock solution in DMSO) in BMPy OTf at 50 °C for 2 h. For the reaction with azide **1b**, the reaction solution was incubated overnight at 50 ºC. Conversion in the parentheses was calculated based on matrix-assisted laser desorption/ionization (MALDI-MS) analysis.

A


unmod
957.3
peak at 1.8 min
5'-TTTTT-3'-alkyl-NH2
Expected mass [M–2H]2– = 957.2
SD
730.7
peak at 0.7 min
5'-TAUCG-3'
Expected mass [M–2H]2– = 730.6
+1 mod
1022.3
peak at 2.4 min
1b (91%)
Expected mass [M–2H]2– = 1021.7
+1 mod
1034.3
peak at 2.0 min
1c (83%)
Expected mass [M–2H]2– = 1034.3
+1 mod
1035.3
peak at 1.9 min
1d (96%)
Expected mass [M–2H]2– = 1035.3
+1 mod
1053.2
peak at 2.4 min
1e (97%)
Expected mass [M–2H]2– = 1052.7
absorbance (254 nm)
time (min)
MS count (ESI)
m/z

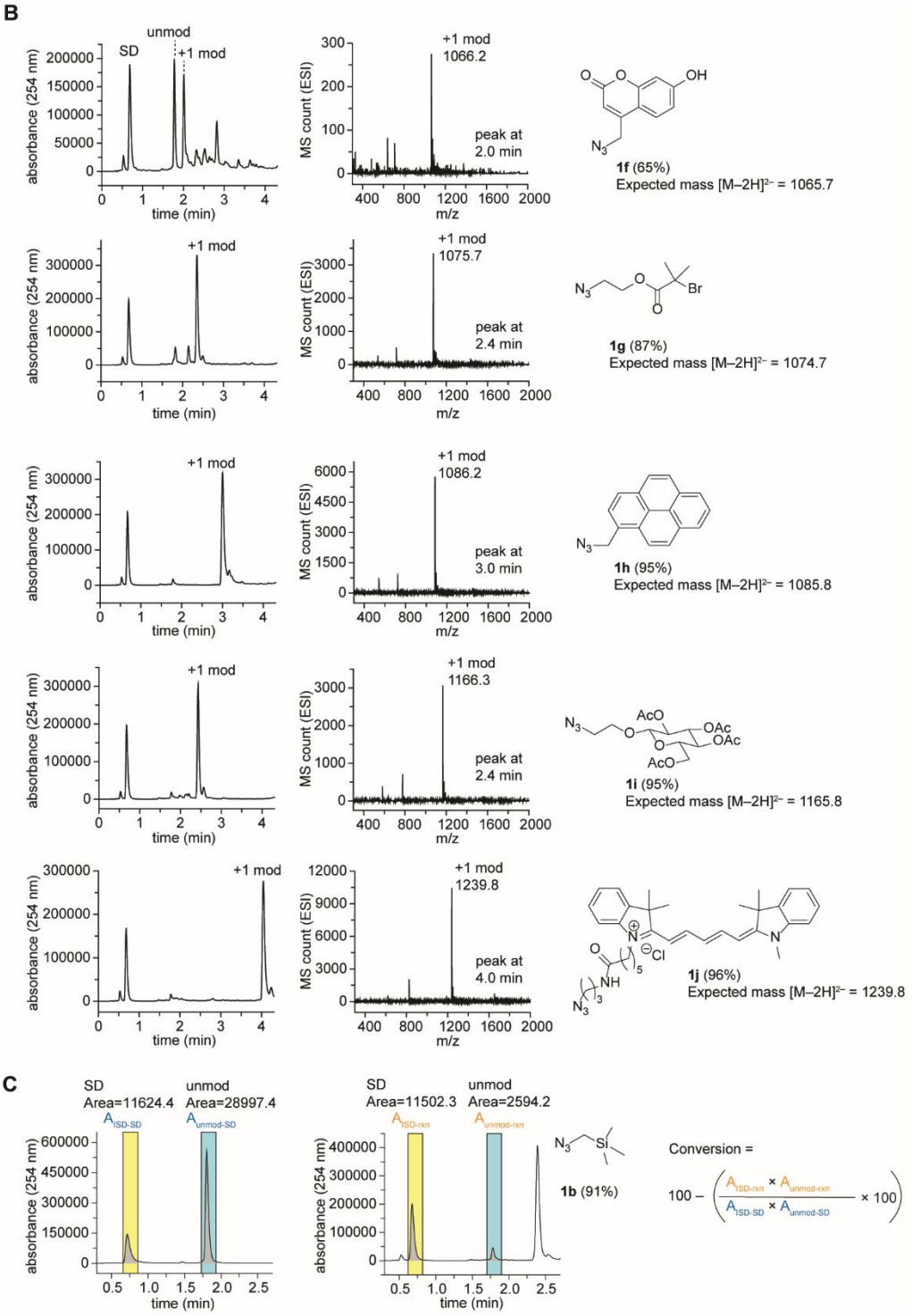


Figure S2. LC-MS analysis of a crude reaction of 5'-TTTTT-3'-alkyl-NH_2 with various alkyl azide reagents. (A,B) UV chromatogram (254 nm) of the analysis of the reaction mixture and mass spectrum corresponds to unmodified (unmod), standard (SD), and modified (mod) nucleotide in liquid chromatogram. SD: 5'-TAUCG-3'. Conversion in the parentheses was calculated based on UV chromatogram analysis (details described in Fig.S2C). (C) Representative images for area integration of UV chromatogram. Left: Unmodified 5'-TTTTT-3'-alkyl-NH_2 (LC analysis triplicated to give average of area for the conversion calculation) Middle: Modification of 5'-TTTTT-3'-alkyl-NH_2 with azide **1b**. Peak integration was performed on Origin software, and the conversion was calculated based on the equation shown in the figure (right).

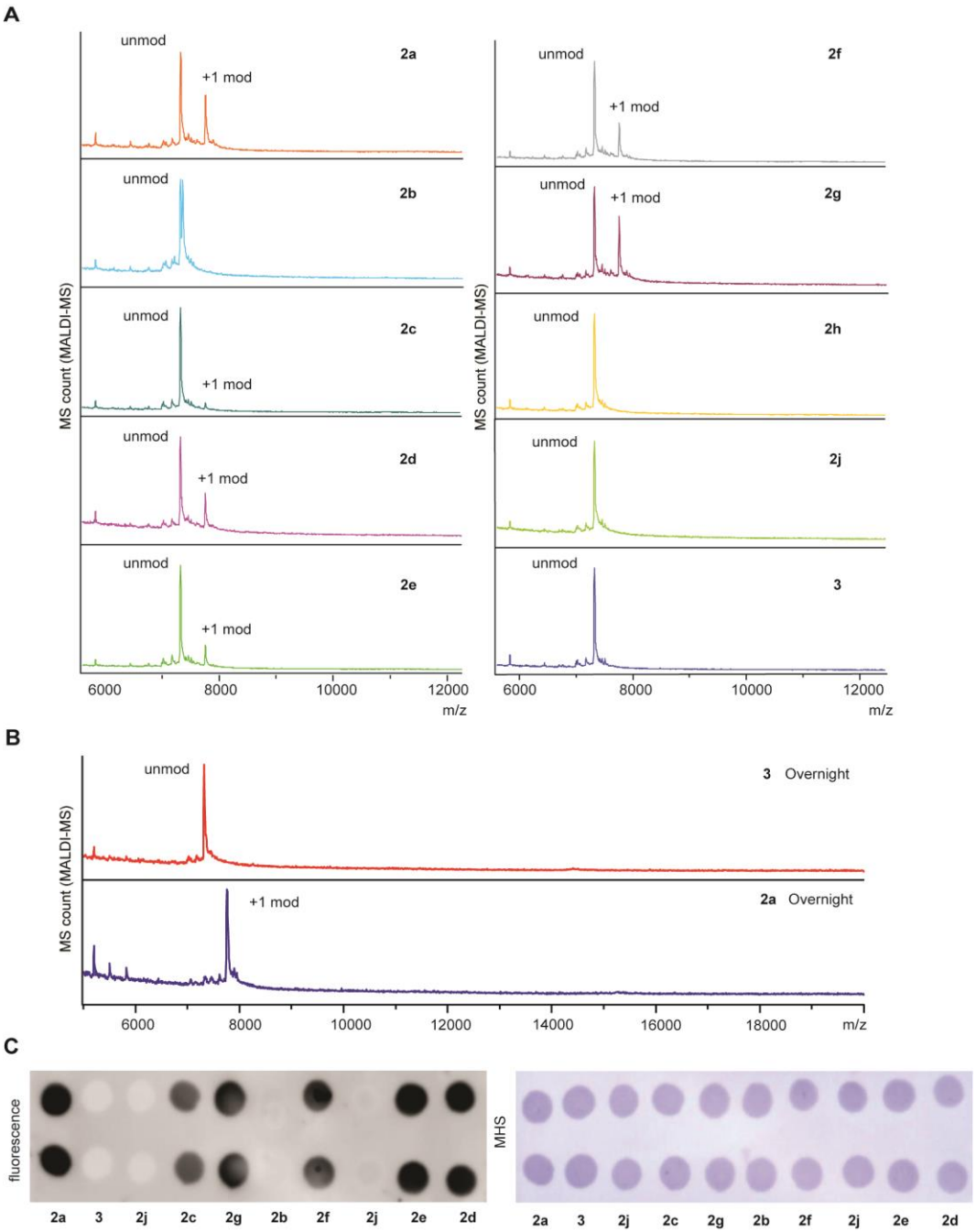


Figure S3. Analysis of modification of DNA aptamers with various phosphine reagents. (A) MALDI-MS analysis. 3-HPA was used as a matrix for all the conditions. Reaction conditions: HSA DNA aptamer (0.1 mM final concentration from 5 mM stock solution in H_2O), $KHCO_3$ (20 mM final concentration from 2 M stock solution in H_2O), biotin azide **S1** (7.5 mM final concentration from 100 mM stock solution in DMSO), and phosphines (3.0 mM final concentration from 150 mM stock solution in DMSO/toluene (1:1 ratio)) in EMIM OAc/DMF (1:1 ratio) at 50 °C for 2 h. (B) MALDI-MS analysis of overnight modification of HSA aptamer. 3-HPA was used as a matrix. Reaction conditions are the same as (A) except for the concentration of phosphines (20 mM final concentration from 500 mM stock solution in DMSO) and the reaction time (overnight). (C) Representative blot membrane images for the anti-biotin southern blot (Cy5) and total stain with Mayer's hemalum solution (MHS) of the HSA DNA aptamer.

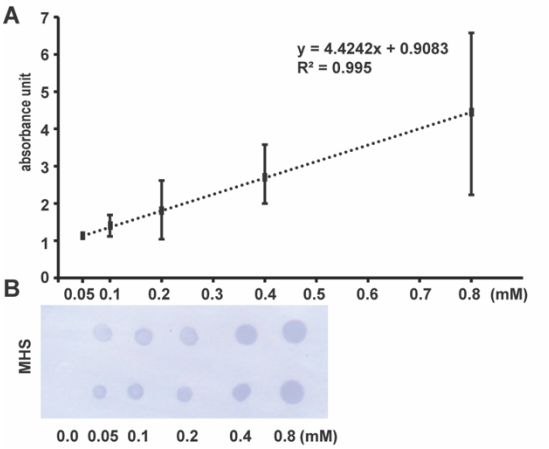


Figure S4. Mayer's hemalum solution screening of pentanucleotide (5'-TAUCG-3'). (A) Each lane contains two technical duplicates of each condition. Different concentrations of 5'-TAUCG-3' were used (0, 0.05, 0.1, 0.2, 0.4, 0.8 mM). The colorimetric signals were quantified by ImageJ and normalized by that of the average of 0.05 mM (5'-TAUCG-3'). The experiment was repeated three times to obtain the standard deviation (error bars, n = 3) and coefficient of determination (R-squared test) for the images. (B) Representative images of MHS dot blotting of 5'-TAUCG-3' in different concentrations.

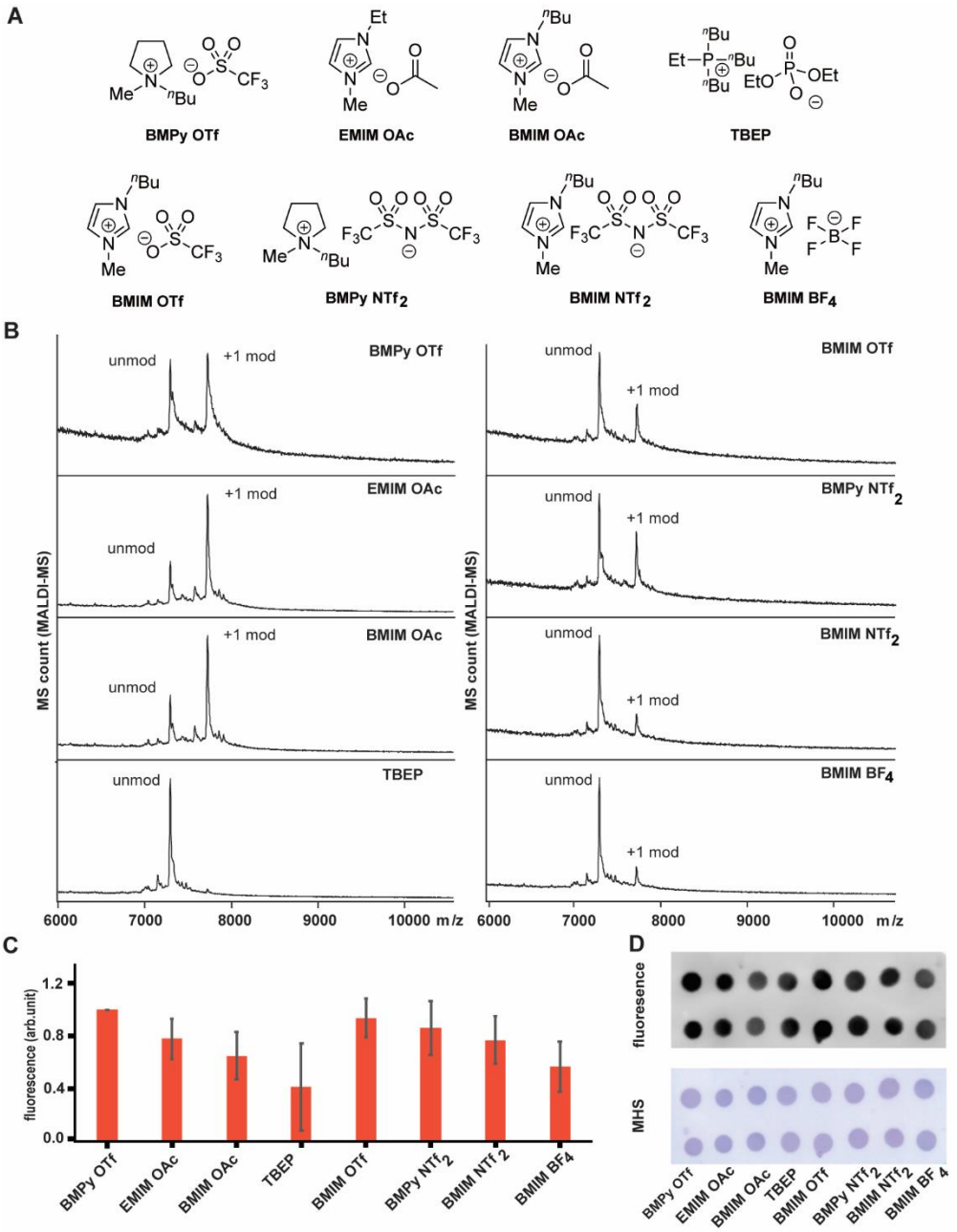


Figure S5. Ionic liquid screening on urea formation reaction on HSA DNA aptamers. Reaction conditions: HSA DNA aptamer (0.1 mM final concentration from 5 mM of stock solution in H_2O), $KHCO_3$ (20 mM final concentration from 2 M of stock solution in H_2O), biotin azide **S1** (7.5 mM final concentration from 250 mM of stock solution in DMSO), and PPh_3 or $O{=}PPh_3$ (20 mM final concentration from 250 mM of stock solution in DMSO) in different ionic liquids with DMF (1:1 ratio) at 50 °C for 2 h. (A) Chemical structure of ionic liquids. (B) MALDI-MS analysis. 3-HPA was used as a matrix for all the conditions. (C) Bar graph showing the anti-biotin southern blot for modification of the HSA DNA aptamer with biotin azide **S1** with different ionic liquids. The fluorescence was quantified by Image J and normalized by that of the average of BMPy OTf. (D) Representative blot membrane images for the anti-biotin southern blot (Cy5) and total stain with Mayer's hemalum solution (MHS) of the ionic liquid screening in HSA DNA aptamer modification. The experiment was repeated three times to obtain the standard deviation (error bars, *n* = 3) for the images.

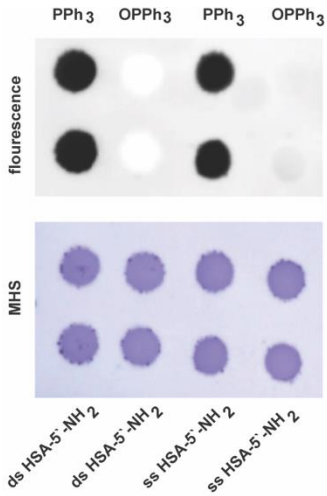


Figure S6. Blot membrane images for the anti-biotin southern blot (Cy5) and total stain with Mayer's hemalum solution (MHS) of the double- and single-stranded HSA DNA aptamer modification. Reaction conditions: HSA DNA aptamer (0.1 mM final concentration from 1 mM stock solution in H_2O), $KHCO_3$ (20 mM final concentration from 2 M stock solution in H_2O), biotin azide **S1** (7.5 mM final concentration from 100 mM stock solution in DMSO), and phosphines (20 mM final concentration from 500 mM stock solution in DMSO) in EMIM OAc/DMF/DMSO (2:1:1 ratio) at 50 ºC overnight. Before the reaction, the dsDNA was assembled with complementary ssDNA, according to the procedure described in *Represenitive procedure for the formation of DNA duplexes* (page S22). ds:double-stranded. ss: single-stranded.

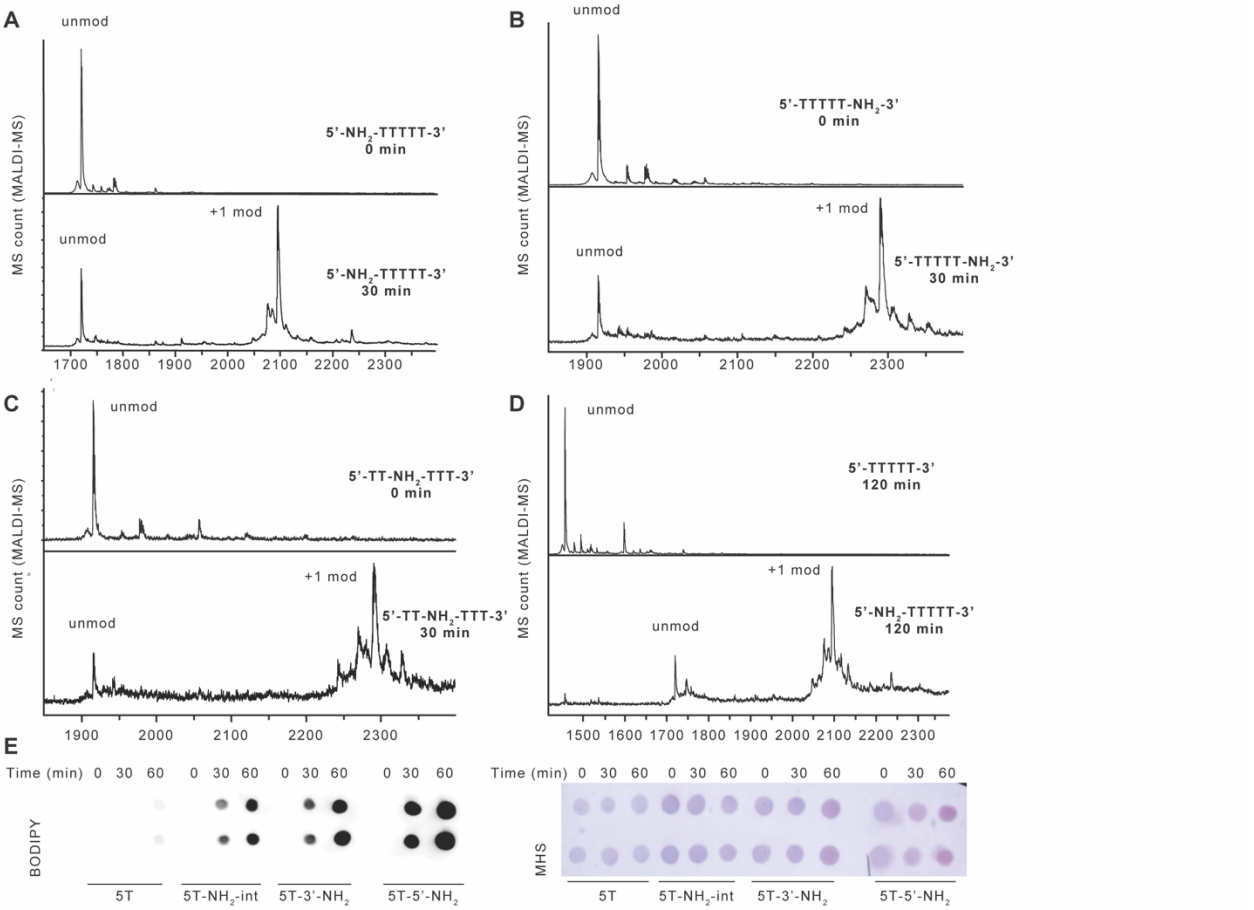


Figure S7. Data for Fig. 5. Kinetics analysis of the TTTTT DNAs with and without the amine modification. (A-C) MALDI-MS kinetics of the unmodified (top) and modified (bottom) DNA. DABP was used as a matrix. A) 5'-NH_2-TTTTT-3', 0 and 30 min B) 5'-TTTTT-NH_2-3', 0 and 30 min C) 5'-TT-NH_2-TTT-3' D) 5'-TTTTT-3', 120 min (top) and 5'-NH_2-TTTTT-3', 120 min (bottom). E) Raw membrane images of the dot blot analysis on a nylon membrane (Roche 11209299001) for the kinetics experiment. Each lane contains a technical duplicate of the same sample. Left: BODIPY fluorescence. Right: Total staining by Mayer's hemalum solution (MHS). Reaction conditions: Pentanucleotides (0.2 mM final concentration from 5 mM stock solution in H_2O), $KHCO_3$ (20 mM final concentration from 2 M stock solution in H_2O), BODIPY azide **1k** (3 mM final concentration from 100 mM stock solution in DMSO), and PPh_3 (3 mM final concentration from 150 mM stock solution in DMSO) in BMPy OTf/DMF (1:1 ratio) at 50 °C.

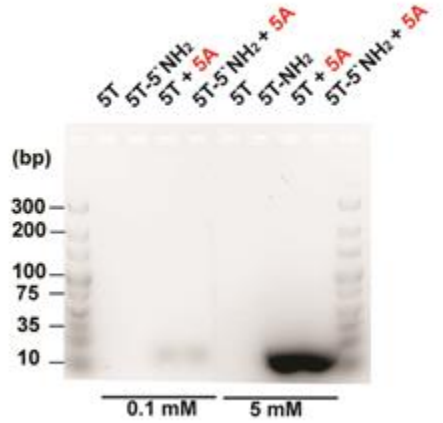


Figure S8. An agarose gel image for TTTTT with or without amine at 5' position. 5T detivative (5 µL of 0.1 mM or 5 mM) with or without AAAAA (5 µL of 0.1 mM or 5 mM, respectively) at 60 °C for 10 min and separated by electrophoresis in 4% of agarose gel containing 1.5% of SYBR Gold nucleic acid gel stain (Cy3 excitation and emission channel).

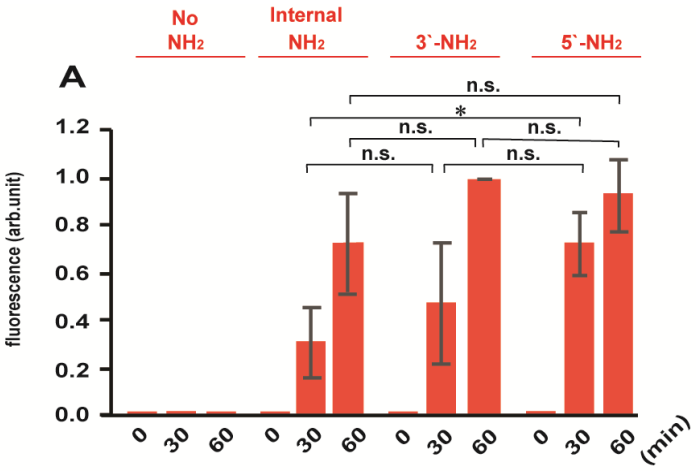


Figure S9. Statistical analysis of Fig. 5A. Statistical significance was determined by a two-tailed unpaired Student's *t*-test was used: "*" represents $p<0.05$; "ns" represents $p>0.05$.

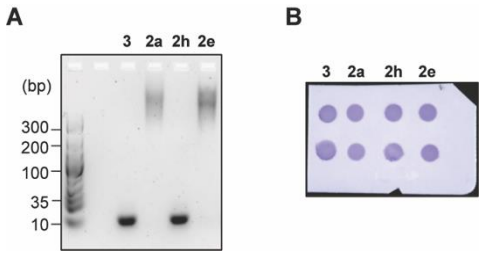


Figure S10. Data for Fig. 6. Attachment of cholesterol on a DNA aptamer toward the RNA hairpin of human immunovirus (HIV)-1 transactivation-responsive (TAR) element through the urea-forming reaction. Sequence of the HIV-1-TAR aptamer: 5'-CCCTAGTTAGCCATCTCCC-3'. (A) Full-width agarose gel image of HIV-1-TAR-5'-NH_2 aptamer modified with cholesterol azide (**1l**) in the presence of triphenylphosphine oxide (**3**), triphenylphosphine (**2a**) JohnPhos (**2h**), and sulfonate-substituted triphenylphosphine (**2e**) with DNA ladder (leftmost lane). Modification conditions: HIV-1-TAR-5'-NH_2 aptamer (0.1 mM final concentration from 5 mM stock solution in H_2O), $KHCO_3$ (20 mM final concentration from 2 M stock solution in H_2O), cholesterol azide **1l** (7.5 mM final concentration from 100 mM stock solution in DMSO/toluene (7:3)), and phosphine **3**, **2a**, or **2e** (20 mM final concentration from 500 mM stock solution in DMSO) or phosphine **2h** (20 mM final concentration from 500 mM stock solution in DMSO/toluene (1:1)) in EMIM OAc/DMF/DMSO (2:1:1 ratio) at 50 °C for 2 h. (B) Representative blot membrane images for the total stain with Mayer's hemalum solution (MHS) of the TAR DNA aptamer modification. Before used in the gel (and cell) experiment, the dsDNAs were prepared from the complementary ssDNA, according to the procedure described in *Representive procedure for the formation of DNA duplexes* (page S22). ds:double-stranded. ss: single-stranded.

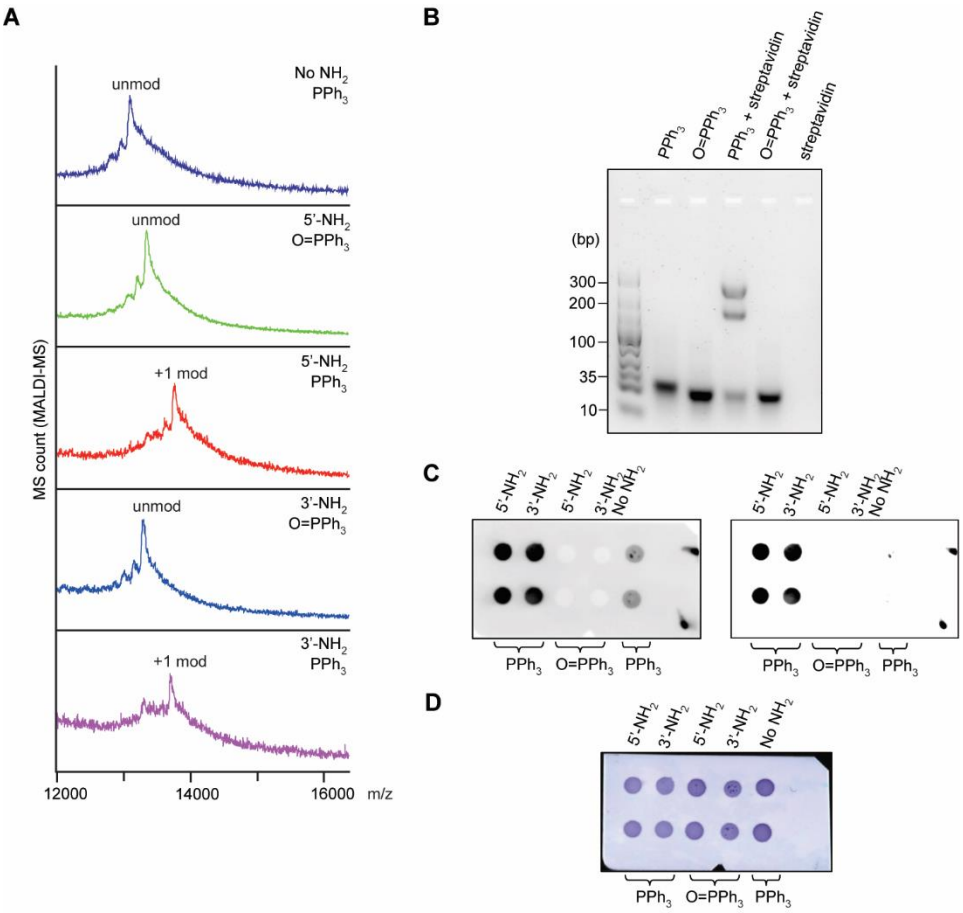


Figure S11. Data for Fig. 7. Urea-forming reaction of 5' or 3' alkylamine modified DNA aptamer toward Her2 receptor. Sequence of the Her2 aptamer: 5'-GCAGCGGTGTGGGGGCAGCGGTGTGGGGGCAGCGGTGTGGGG-3'. (A) Matrix-assisted laser desorption/ionization (MALDI-MS) analysis of the reaction of 5', 3' alkylamine modified or not modified DNA aptamer toward Her2 receptor with biotin azide **S1** and triphenylphosphine oxide (negative control) or triphenylphosphine. 3-HPA was used as a matrix. (B) Agarose gel analysis of Her2 aptamer modified with biotin azide **S1** and triphenylphosphine oxide (negative control) or triphenylphosphine. The gel was run in the presence or absence of streptavidin (5 equivalent to DNA aptamer). Appearance of multiple bands by the streptavidin-biotin interactions were also observed in previous reports.[1,2] (C) Representative blot membrane images for the anti-biotin southern blot (Cy5) and total stain with Mayer's hemalum solution (MHS). Two images corresponds to the same blot membrane, but different contrast of fluorescence. (D) of the Her2 DNA aptamer modification. Before used in the cell staining experiment, the dsDNA was assembled with complementary ssDNA, according to the procedure described in *Representive procedure for the formation of DNA duplexes* (page S22). ds:double-stranded. ss: single-stranded.

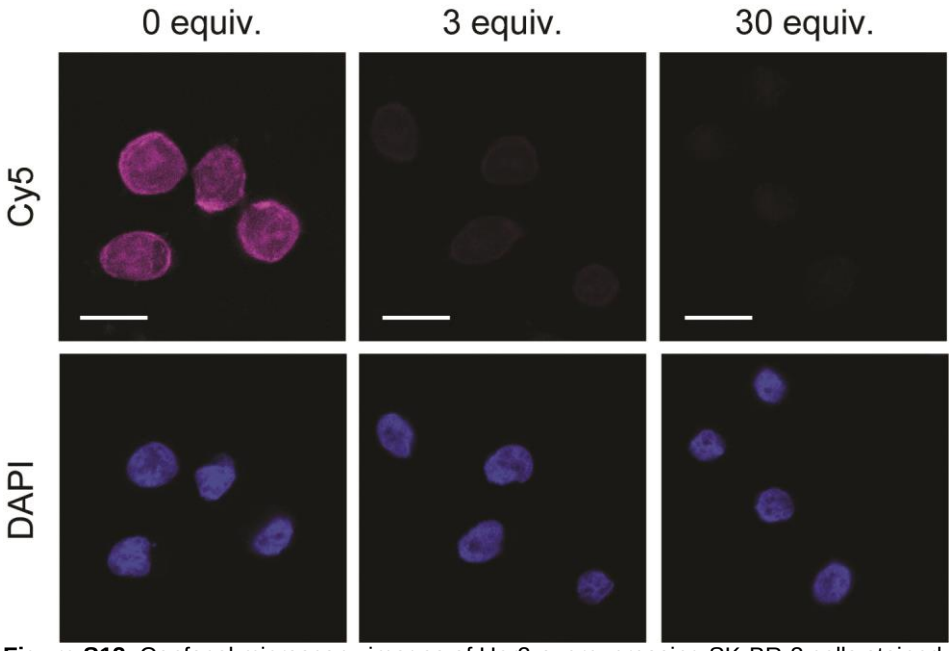


Figure S12. Confocal microscopy images of Her2-overexpressing SK-BR-3 cells stained with biotin-modified Her2 aptamer pretreated without (left), with 3 equivalent or with 30 equivalent (right) of unmodified Her2 aptamer. The cells were visualized by streptavidin fluorophore (Cy5) conjugate (1:50 dilution) shown in magenta. Blue: Nuclear stain with DAPI.

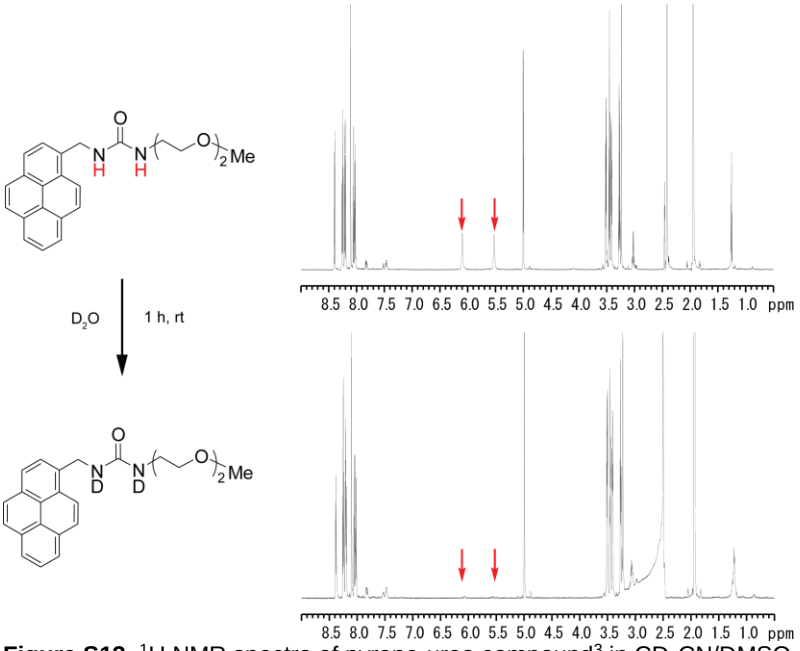


Figure S13. ^{1}H NMR spectra of pyrene-urea compound[3] in CD_3CN/DMSO-d_6 (95:5) without (top) and with (bottom) treatment with D_2O (1 h, rt).

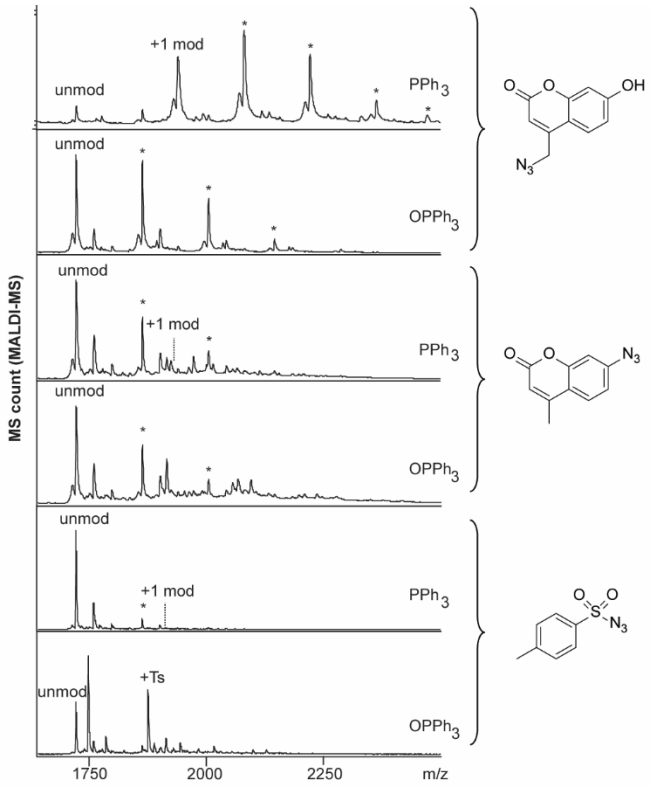


Figure S14. Matrix-assisted laser desorption/ionization (MALDI-MS) analysis of urea-forming reaction with 5'-NH_2-TTTTT-3' and alkyl (top two), aryl (middle two), and sulfonyl (bottom two) azides in ionic liquids. "*" sign denotes 1-butyl-1-methylpyrrolidinium (BMPy) adduct. BMPy = 142.3 Da. Reaction conditions: $KHCO_3$ (20 mM), 5'-NH_2-TTTTT-3' (0.2 mM), azides (7.5 mM), and PPh_3 (20 mM) in BMPy OTf at 50 °C for 2 h.

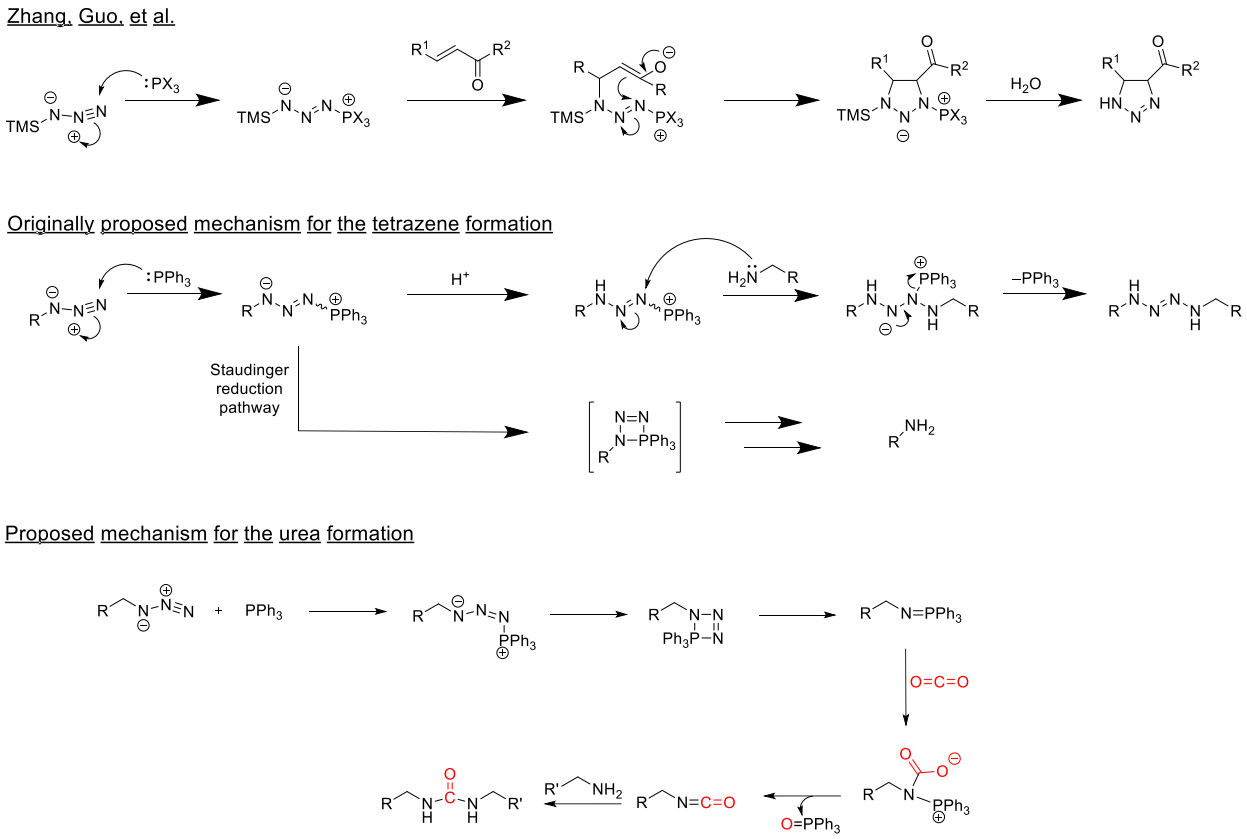


Figure S15. Reaction mechanisms for phosphine-involved azide conversion proposed by Zhang and Guo (top)[4], putative mechanism of tetrazene formation (middle), and that of the urea formation (bottom).

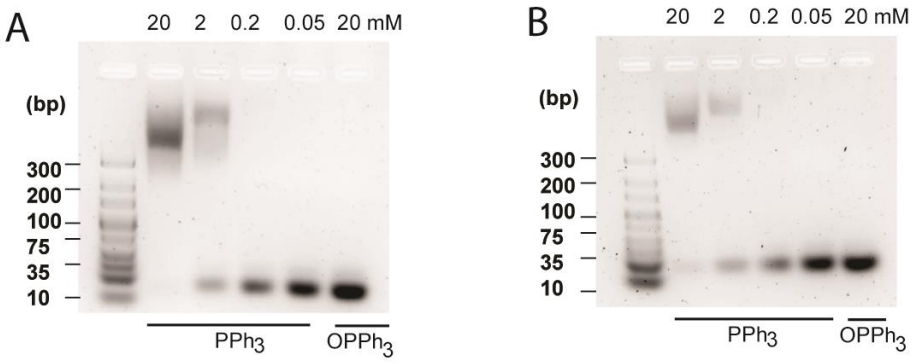


Figure S16. Agarose gel analysis of HIV-1-TAR-5'-NH_2 aptamer modified with cholesterol azide **1l** and different concentrations of triphenylphosphine (or triphenylphosphine oxide negative control at 20 mM). DNA samples were visualized by SYBR Gold nucleic acid gel stain (Cy3 excitation and emission) in 4% of agarose gel. Reaction conditions: HIV-1-TAR-5'-NH_2 (0.1 mM), $KHCO_3$ (20 mM), cholesterol azide **1l** (7.5 mM), and PPh_3 (20, 2, 0,2 0,05 mM) or $OPPh_3$ (20 mM) in EMIM OAc/DMF/DMSO (2:1:1) at 50 °C for (A) overnight or (B) 2h. Observation of different size of aggregates between 20-mM and 2-mM PPh_3 conditions is presumably due to the different degree of modification efficiency of those conditions.[5]

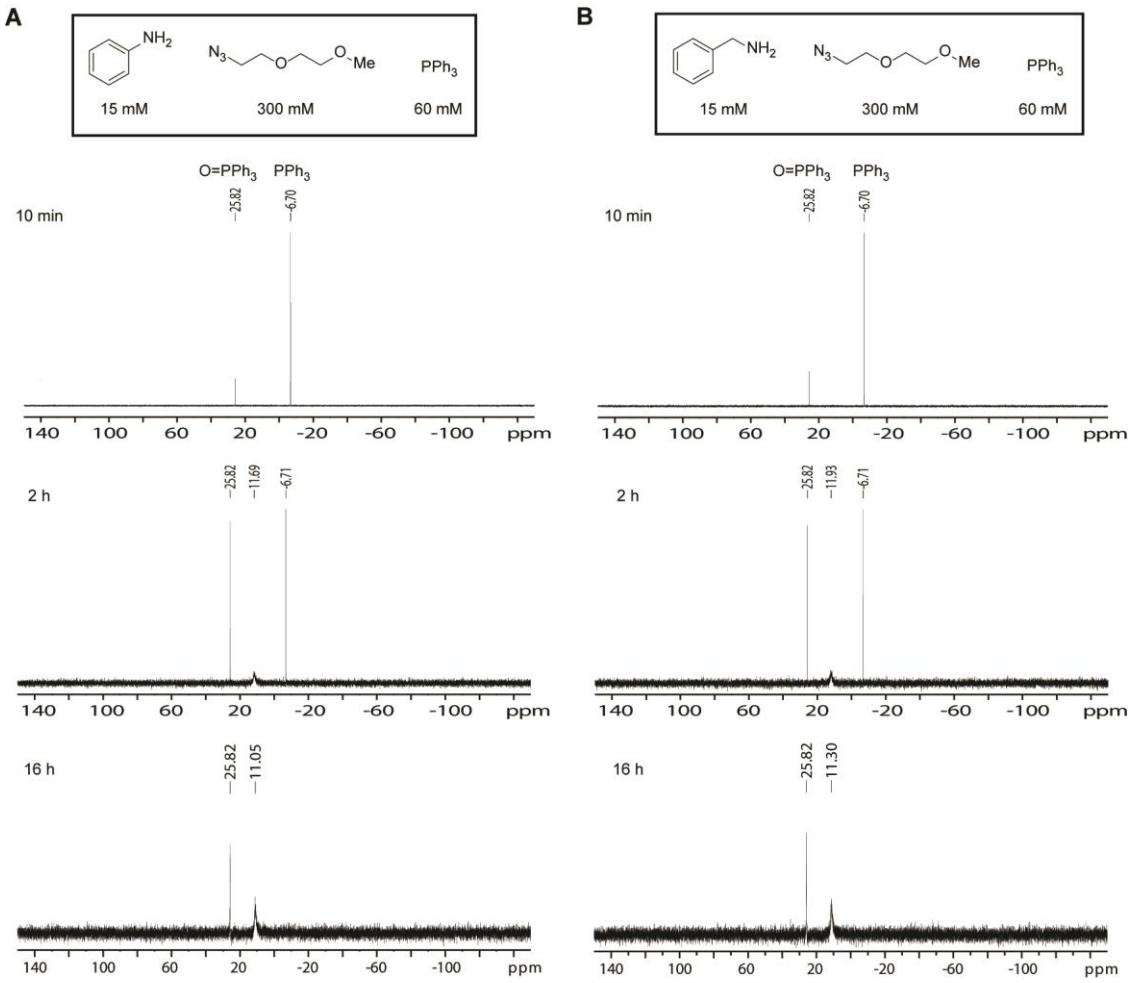


Figure S17. ^{31}P NMR spectra of the reaction of PPh_3 (60 mM) with Azido-PEG1-OMe (300 mM) in BMPy OTf/DMSO-d_6 (1:1) at different time points (10 min, 2 h, and 16 h) in the presence of (A) aniline (15 mM) or (B) benzylamine (15 mM).

General information

Materials and reagents

All the chemicals including DNAs were purchased from commercial vendor unless otherwise noted. A list of azides and DNAs used in this study is available in Table S1 and Table S2, respectively. DNA ladder (Ultra low range, 10-300 bp) was purchased from Invitrogen (10597012). Benzodioxane-azide (**1e**) was synthesized according to the reported literature.[6]

Instrumentation

MALDI-MS was performed on a Bruker Daltonics Autoflex-TOF. Matrices used were 20 mg/mL 3,4-diaminobenzophenone (DABP) in ammonium citrate (20 mg/mL solution in H_2O)/acetonitrile (1:1) or saturated solution of 3-hydroxypicolinic acid (3-HPA) in ammonium citrate (20 mg/mL solution in H_2O)/acetonitrile (1:1). For DABP, a 1:1 ratio of sample (0.5 or 1 µL) and matrix solution (0.5 or 1 µL) were mixed on a ground-steel MALDI plate (Bruker 8280784). For 3-HPA, a 2:1 ratio of matrix (1 µL) and sample (1 µL) were used; first, 3-HPA (1 µL) was added and dried, then sample (1 µL) was added and dried, and another 3-HPA (1 µL) was added and dried on the same well. The DABP

matrix solution was freshly prepared every time, and the 3-HPA matrix solution was prepared every 1 month and stored at –80 ºC.

LC-MS was performed on Thermo Vanquish LC system and LTQ-XL linear ion trap MS system with a C18 column (Hypersil Gold 25003-032130, particle size: 3 μm, diameter: 2.1 mm, length: 30 mm). The flow rate was 0.4 mL/min. Triethylammonium acetate buffer (5 mM, pH 7.2) was used as eluent with the gradient of acetonitrile (5-40% for 3.5 min, and then 90% for 1.5 min). The analysis of the reactions were performed by the UV detection of the unmodified DNA at 254 nm, compared with the internal standard (single stranded DNA, TAUCG 0.05 mM). The conversion of the reaction was calculated by the decrease of the peak area of starting material (unmodified DNA) using the internal standard (see Fig.S2 caption).

Agarose gel electrophoresis was performed as described below. 4.0 g of Agarose (Research Products International A20090) was suspended in 100 mL of TAE Buffer (10X EMD Millipore 574797, 4% w/v final concentration) and 10 times diluted with water. The agarose was dissolved by heating in a microwave (1050 W) for 2 min. For the total staining purpose, 1.5 mL of SYBR™ Gold nucleic acid gel stain (Invitrogen S11494) was added while stirring. The hot solution was poured into the container of the Electrophoresis Unit (Walter EL-100) and cooled at rt for 20 min. In a 1.7-mL Eppendorf tube, 1 μL of the samples were mixed with 5 μL of TrackIt™ Cyan/Yellow Loading Buffer (Invitrogen 10482035), and the mixed samples were loaded to the gel. 1 μL of Ultra Low Range DNA Ladder (Invitrogen 10597012) was mixed with 1 μL of TrackIt™ Cyan/Yellow Loading Buffer (Invitrogen 10482035), and the mixed solution was loaded to the gel. The electrophoresis was run by using the power supply (BIO-RAD Power Pac™ Basic Supply 1645050) for 40–50 min at constant 150 V. The resulting gel was analyzed by Amersham ImageQuant 800 (Cytiva).

Gel fluorescence and southern blot imaging was conducted on Amersham ImageQuant 800 (Cytiva). Gel or blot fluorescence imaging were performed using 460-nm (Cy2), 535-nm (Cy3), and 635-nm (Cy5) light sources with corresponding emission bandpass filters at 525 nm (±20 nm), 605 nm (±40 nm), and 705 (±40 nm), respectively. Anti-biotin southern blot was performed with streptavidin-Cy5 conjugate (Jackson ImmunoResearch 016-170-084, 1:2,000 dilution for sourthen blot) after blocking with 5% BSA in TBST buffer. Quantification of the blot membrane images were performed by using ImageJ software, and signals were normalized to one of the strongest signals (set as 1.0) in the experiment. Weak, unanalyzable signals in the Image J software were set as 0.01, compared to the normalization sample (1.0).

Confocal microscope

Fluorescence microscope imaging was performed on Zeiss scanning microscope 710 with a 40x water immersion C-Apochromat objective lens (numerical aperture 1.1). Excitation at 405 nm (DAPI), 488 nm (Phalloidin-CF488), and 633 nm (Cy5) were used with filter settings 410-480 nm, 494-631 nm, and 638-759 nm, respectively. Image J software was used to generate images suitable for publication.

NMR was performed on Bruker AVANCE NEO 600 and 700.

Cell culture

SK-BR-3 and HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with Glutamax and 10% fetal bovine serum (FBS) using 24-well cell culture plate (Corning 3524) coated with poly-L-lysine under 5% CO_2 at 37 °C. Cells were fixed with 4% paraformaldehyde at 90% (SK-BR-3 cells) or 60% (HeLa cells) confluency, washed with PBS three times, and used for the cell stainning experiment (Fig. 6 and Fig. 7).

List of Materials

Table S1. A list of azide compounds presented in the manuscript

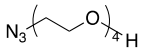
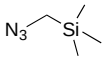
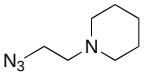
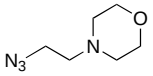
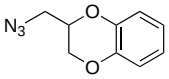
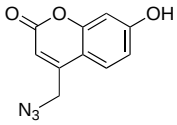
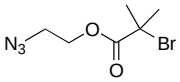
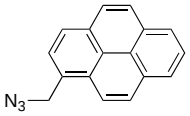
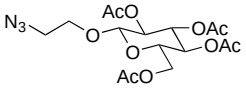
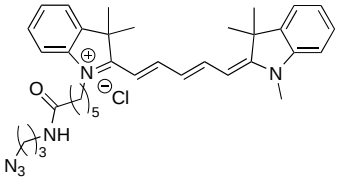
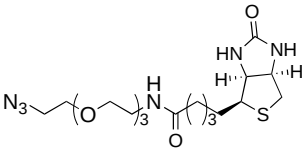
Structure	Compound #	Groups	Supplier (catalog number)
	**1a**	alcohol	ChemImpex (NC1851841)
	**1b**	trimethylsillyl (TMS)	Sigma-Aldrich (152854-5G)
	**1c**	piperidine	Sigma-Aldrich (CDS015394)
	**1d**	morpholine	Sigma-Aldrich (CDS005526)
	**1e**	benzodioxane	Home-made[6]
	**1f**	coumarin	Synthonix (A73367)
	**1g**	ester	Combi-Blocks (QH-5870)
	**1h**	pyrene	AK Scientific (AMTGC336)
	**1i**	acetylpyranose	Biosynth Carbosynth (MA34873)
	**1j**	Cy5	Lumiprobe (41430)
	**S1**	biotin	Sigma-Aldrich (762024)

Table S2. A list of DNA used in the study

Sequence	Name	Supplier
5'- /5AmMC12/TTT TT -3'	TTTTT-5'-NH_2	Integrated DNA Technologies
5'- TTT TT/3AmMC6T/ -3'	5'-TTTTT-3' -NH_2	Integrated DNA Technologies
5'-TTT /iAmMC6T/TT -3'	5'-TTT(NH_2)TT-3'	Integrated DNA Technologies
5'-TTT TT -3'	5'-TTTTT -3'	Integrated DNA Technologies
5'-ATT TT -3'	5'-ATTTT -3'	Integrated DNA Technologies
5'-GTT TT -3'	5'-GTTTT -3'	Integrated DNA Technologies
5'-CTT TT -3'	5'-CTTTT -3'	Integrated DNA Technologies
5'-UTT TT -3'	5'-UTTTT -3'	Integrated DNA Technologies
5'- /5AmMC12/TGC GGT TGT AGT ACT CGT GGC CG -3'	HSA aptamer-5'-NH_2	Integrated DNA Technologies
5'- /5AmMC12/CCC TAG TTA GCC ATC TCC C -3'	HIV-1-TAR aptamer-5'-NH_2	Integrated DNA Technologies
5'- GGG AGA TGG CTA ACT AGG G/3Cy5Sp/ -3'	Complementary HIV-1-TAR aptamer-3'-Cy5	Integrated DNA Technologies
5'-/5AmMC12/GCA GCG GTG TGG GGG CAG CGG TGT GGG GGC AGC GGT GTG GGG -3'	Her2 apramer-5'-NH_2	Integrated DNA Technologies
5'- GCA GCG GTG TGG GGG CAG CGG TGT GGG GGC AGC GGT GTG GGG/3AmMO/ -3'	Her2 apramer-3'-NH_2	Integrated DNA Technologies
5'- GCA GCG GTG TGG GGG CAG CGG TGT GGG GGC AGC GGT GTG GGG -3'	Her2 apramer	Integrated DNA Technologies
5'- CCC CAC ACC GCT GCC CCC ACA CCG CTG CCC CCA CAC CGC TGC -3'	Complementary Her2 apramer	Integrated DNA Technologies

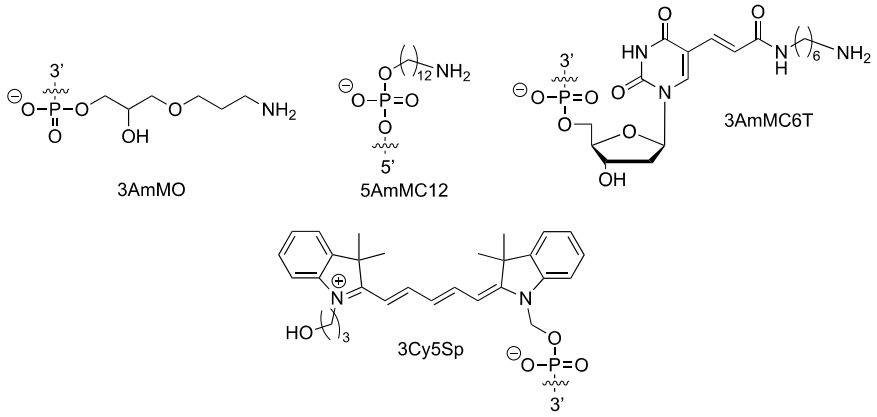


Figure S18. Structures of the DNA modifiers used in this study.

Experimental procedures

Typical DNA modification procedure in ionic liquids

To ionic liquids (typically 10–40 μL for analytical scale), potassium bicarbonate aqueous solution (20 mM final concentration from 2M stock solution in water), DNA aptamers or pentanucleotides (0.02–0.1 mM final concentration from 2–5 mM stock solution in water), alkyl azide (3–7.5 mM final concentration from 100–500-mM stock solution in DMSO), and PPh_3 or $O{=}PPh_3$ (3–20 mM final concentration from 150–500-mM stock solution in DMSO) were added. The final concentration of H_2O was kept lower than 6% v/v. The reaction mixture was incubated in a 50 °C incubator for 2 h and subjected to a *Post-reaction cleanup process for analytical scale reaction* before analysis.

Post-reaction cleanup process for analytical scale reaction

To the reaction mixture (typically 10–40 μL) in a 1.7-mL Eppendorf tube, cold acetone (600–900 μL, –20 °C) was added in one portion. For the samples in EMIM OAc, the precipitation was performed with cold acetone/methanol (5:1 ratio, 600–900 μL, –20 °C) instead of cold acetone. After the addition of acetone or acetone/methanol, the mixture was mixed by upside-down shaking and sit at –80 °C for 1 h or overnight. The precipitates were collected by centrifugation (15,000 rcf, 15 min, 4 °C), and acetone or acetone/methanol was removed. The pellet was further washed by acetone or acetone/methanol (5:1) addition and centrifugation processes before the pellet was air-dried on the bench at rt for 15 min. The dried pellet was reconstituted in 10–30 μL of ammonium bicarbonate (NH_4HCO_3) aqueous solution (5 mM) and analyzed by suitable analytical methods.

Southern blotting (dot blot)

The reconstituted samples (0.5 μL) were spotted onto a positively charged nylon membrane (Roche 11209299001). Mayer hemalum solution for the membrane stain was prepared by diluting the commercial solution (Sigma Aldrich 1.09249.0500) with water (100 times dilution) in a 50-mL falcon tube, and the diluted solution can be stored at rt. The membrane with the DNA was stained with the diluted Mayer hemalum solution for 5 min and rinsed with TBST buffer twice. The stained membrane was imaged by ImageQuant 800 to obtain the colorimetric image. Then the membrane was washed with TBST buffer for 5 min, blocked with 5% BSA in TBST buffer at rt for 20 min, incubated with streptavidin-Cy5 conjugate (1:2000) in the blocking buffer at rt for 40 min, washed with TBST buffer three times, and imaged by ImageQuant 800. For the quantification purpose, the experiment was triplicated on different days, and the fluorescence intensity was quantified by ImageJ software.

Pentanucleotide modification with azide 1a. Experiment for Fig. 2

The urea-forming reaction on pentanucleotides (5'-XTTTT-3' where X = adenosine, thymidine, cytosine, guanosine, deoxyuridine, and thymidine with alkylamine containing 12 carbon linker) were performed by *Typical DNA modification procedure in ionic liquids* (page S21) and *Post-reaction cleanup process for analytical scale reaction* (page S21) in 30 μL scale with following conditions. 5'-XTTTT-3' (0.2 mM final concentration from 5 mM stock solution in H_2O), $KHCO_3$ (20 mM final concentration from 2 M stock solution in H_2O), azide **1a** (7.5 mM final concentration from 250 mM stock solution in DMSO), and PPh_3 (20 mM final concentration from 500 mM stock solution in DMSO) in BMPy OTf at 50 °C for 2 h.

Kinetics Experiment for Fig. 5A

The urea-forming reaction on pentathymidine for the kinetics investigation were performed by *Typical DNA modification procedure in ionic liquids* (page S21) and *Post-reaction cleanup process for analytical scale reaction* (page S21) in 20 μL scale with following conditions. The resulting DNA

solution was spotted in a nylon membrane. Once the spots get dried, the membrane was rinsed with MeOH once and visualized by fluorescence (BODIPY). After that, the membrane was stained by the diluted MHS (*Southern blotting* section) for 5 min, rinsed with TBST buffer twice, and visualized by ImageQuant 800. Standard deviations (error bars, n = 3) and coefficient of determination (R-squared test) were calculated after three independent replicates. Reaction conditions: Pentathymidine (0.2 mM final concentration from 5 mM stock solution in H_2O), $KHCO_3$ (20 mM final concentration from 2 M stock solution in H_2O), BODIPY azide **1k** (3 mM final concentration from 100 mM stock solution in DMSO), and PPh_3 (3 mM final concentration from 150 mM stock solution in DMSO) in BMPy OTf at 50 °C for given time (0, 30, 60 min).

Modification of alkylamine-tagged DNA in the presence of DNA ladder, Experiment for Fig. 5C

To 10 µL of Ultra Low Range DNA Ladder (Invitrogen, 10597012), 1 µL of 3 M sodium acetate at pH 5.2 and 30 µL of ice-cold 100% ethanol were added. DNA samples were mixed and stored at –20 °C for 1 h to precipitate DNA. DNA pellet was collected by centrifugation (15,000 rcf, 15 min, 4 °C), and then supernatant was removed. And the pellet was washed with ice-cold 70% ethanol two times with the centrifugation processes. The pellet was air-dried on the bench at rt for 15 min after removing ethanol and reconstituted with 5 µL of distilled water. The concentrated DNA solution was analyzed by Nanodrop 2000 (Thermo-Scientific).

The pentanucleotides were modified with BODIPY azide **1k** in the presence of DNA Ladder by *Typical DNA modification procedure in ionic liquids* (page S21) and *Post-reaction cleanup process for analytical scale reaction* (page S21) in 20 µL scale with following conditions. TTTTT-5'-NH_2 (0.05 mM final concentration from 1 mM stock solution in H_2O), Ultra Low Range DNA Ladder (0.02 µg/µL final concentration from 0.9 µg/µL stock solution in H_2O), $KHCO_3$ (20 mM final concentration from 2 M stock solution in H_2O), BODIPY azide **1k** (3.75 mM final concentration from 100 mM of stock solution), and PPh_3 (3 mM final concentration from 150 mM stock solution in DMSO) were added into BMPy OTf: DMSO: DMF (2:1:1 ratio). After the acetone precipitation process, the reconstituted solution was added 6XDNA Loading dye (1 µL) and run by 4% agarose gel premade with SYBR™ Gold nucleic acid gel stain (1,5-2:10000) or plain gel for 40 min at constant 150 V. Total DNA samples was visualized by the fluorescence from SYBR™ Gold nucleic acid gel stain (Cy3), whereas modified DNA samples were visualized by the fluorescence from BODIPY (Cy2).

Representive procedure for the formation of DNA duplexes for Fig. 6, Fig. 7 and Fiure S5

The single-stranded DNA (5–9 µL, 0.02–0.1 mM final concentration from 0.04–0.2 mM stock solution in H_2O) and its complementary sequence (5–9 µL, 0.02–0.1 mM final concentration from 0.04–0.2 mM stock solution in H_2O) was hybridized by heating at 60 ºC for 10 min using Digital Dry Bath (Thermo Scientific, 88870002).

Cell staining with cholesterol modified DNA. Experiment for Fig. 6

The cholesterol modified DNA was prepared by *Typical DNA modification procedure in ionic liquids* (page S21) and *Post-reaction cleanup process for analytical scale reaction* (page S21) in 30 µL scale with following conditions. HIV-1-TAR-5'-NH_2 aptamer (0.1 mM final concentration from 5 mM stock solution in H_2O), $KHCO_3$ (20 mM final concentration from 2 M stock solution in H_2O), cholesterol azide **1l** (7.5 mM final concentration from 100 mM stock solution in DMSO/toluene (7:3)), and PPh_3 or O=PPh_3 (20 mM final concentration from 500 mM stock solution in DMSO) in EMIM OAc/DMF/DMSO (2:1:1 ratio) at 50 °C for 2 h. HeLa cells were cultured and fixed in a 24-well culture plate with coverslips, according to the procedure described in *Cell culture* (page S10). The cells were blocked in a 4:1 mixture of 1X PBS/Blocking One (Nacalai Tesque, 03953-95) solution at rt for 15 min. The blocking solution was removed, and the cells were incubated with dsDNA (final concentration ~2 µM) and phalloidin-CF488 conjugate (1:40 dilution, final concentration 5 U/mL,

Biotium 00042) in 19:1 mixture of 1X PBS/Blocking One at rt for 30 min. For the preparation of dsDNA solution, the cholesterol modified (PPh_3) or unmodified ($O=PPh_3$) ssDNA was hybridized with Cy5 conjugated complementary ssDNA, according to the procedure described in *Assembly of DNA duplexes* (page S22). After the incubation, cells were washed with PBS solution three times. The coverslip with stained cells were mounted onto a microscope slide with Prolong Gold Anti-fade Mountant (Thermo, P10144) and CoverGrip Coverslip Sealant (Biotium, 23005), and the cells were imaged using confocal microscope.

Cell staining with biotin modified Her2 Aptamer DNA. Experiment for Fig. 7

The biotin modified DNA was prepared by *Typical DNA modification procedure in ionic liquids* (page S21) and *Post-reaction cleanup process for analytical scale reaction* (page S21) in 30 µL scale with following conditions. Her2-5'-NH_2 aptamer (0.1 mM final concentration from 2 mM stock solution in H_2O), $KHCO_3$ (20 mM final concentration from 2 M stock solution in H_2O), biotin azide **S1** (7.5 mM final concentration from 250 mM stock solution in DMSO), and PPh_3 or $O=PPh_3$ (20 mM final concentration from 500 mM stock solution in DMSO) in EMIM OAc/DMF/DMSO (2:1:1 ratio) at 50 °C for 2 h. SK-BR-3 cells were cultured and fixed in a 24-well culture plate with coverslips, according to the procedure described in *Cell culture* (page S18). The cells were blocked in a 4:1 mixture of 1X PBS/Blocking One (Nacalai Tesque, 03953-95) solution at rt for 15 min. The blocking solution was removed, and the cells were incubated with ssDNA (biotin-modified or unmodified) or ds DNA (biotin-modified) at the final concentration of ~0.5 µM in 19:1 mixture of 1X PBS/Blocking One at rt for 1 h. For the preparation of dsDNA solution, the biotin modified ssDNA (PPh_3) was hybridized with complementary ssDNA, according to the procedure described in *Assembly of DNA duplexes* (page S22). For the sake of consistency across the samples, ssDNAs were also heated at 60 ºC for 10 min by the same method before used in the cell staining. After the incubation with the DNA solutions, the cells were washed with PBS solution three times and incubated with Streptavidin Cy5 (Jackson ImmunoResearch 016-170-084, 1:100 dilution) in 19:1 mixture of 1X PBS/Blocking One containing DAPI (0.1 µg/mL) at rt for 30 min. After the incubation, cells were washed with PBS solution three times. The coverslip with stained cells were mounted onto a microscope slide with Prolong Gold Antifade Mountant (Thermo, P10144) and CoverGrip Coverslip Sealant (Biotium, 23005), and the cells were imaged using confocal microscope.

Mayer staining procedure, Experiment for Figure S3

A pentanucleotides, 5'-TAUCG-3' sample was diluted as 0.05, 0.1, 0,2, 0,4, 0,8 mM, spotted in a nylon membrane, stained with the diluted MHS (*Southern blotting* section) for 5 min, dried overnight, and imaged by ImageQuant 800. The experiment was repeated three times to obtain the standard deviation (error bars, $n = 3$) for the images.

Gel shift assay for the Her2 aptamer modification, Experiment for Figure S11B

The biotin modified DNA for the gel shift assay was prepared by in 24 µL scale with following conditions. Her2 aptamer-5'-NH_2 (5 µM final concentration from 0.2 mM stock solution in 5 mM NH_4HCO_3 aq.) modified (PPh_3) or unmodified ($OPPh_3$) with biotin azide **S1** was incubated with or without streptavidin (25 µM final concentration from 1 mM stock solution in 50 mM MES buffer) at rt for 20 min. 5 µL of the resulting mixture was analyzed by the agarose gel electrophoresis and the DNA was visualized by SYBR™ Gold total staining.

References for supporting information

1 J. J. Ludtke, G. Zhang, M. G. Sebestyén and J. A. Wolff, *J Cell Sci*, 1999, **112 (Pt 12)**, 2033–2041.
2 Z. Liu, Y. Liu, Y. Sun, G. Chen and Y. Chen, *RSC Adv.*, 2017, **7**, 52581–52587.
3 H. M. El-Shaffey, E. J. Gross, Y. D. Hall and J. Ohata, *J. Am. Chem. Soc.*, 2021, **143**, 12974.
4 H. Wang, L. Zhang, Y. Tu, R. Xiang, Y.-L. Guo and J. Zhang, *Angew. Chem. Int. Ed.*, 2018, **57**, 15787–15791.
5 A. Ohmann, K. Göpfrich, H. Joshi, R. F. Thompson, D. Sobota, N. A. Ranson, A. Aksimentiev and U. F. Keyser, *Nucleic Acids Res.*, 2019, **47**, 11441–11451.
6 J. Li, M. Hu and S. Q. Yao, *Org. Lett.*, 2009, **11**, 3008–3011.