Supporting Information

"Click Handle"-Modified 2'-Deoxy-2'-Fluoroarabino Nucleic Acid as A Synthetic Genetic Polymer Capable of Post-Polymerization Functionalization

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Table of Contents

I. Supplementary Results

Figure S1. Quantitation of synthesized compound C8-alkyne-FANA UTP 11. **S4**

Figure S2. ESI-MS analysis of Man-cmFANA1. **S5**

Figure S3. Click Conjugation of Man-N3 to different cmFANA polymer sequences. **S6**

Figure S4. ESI-MS analysis of Lac-cmFANA1. **S7**

Figure S5. ESI-MS analysis of 6'-SA-Lac-cmFANA1. **S8**

Figure S6. Ethidium Bromide channel for Tgo polymerase activity assay **S9**

Figure S7. Denaturing PAGE analysis of the reverse transcription of cmFANA and mannose-cmFANA conjugate by several DNA polymerases. **S10**

Figure S8. Next-generation sequencing analysis of the libraries before and after DNA-display. **S11**

II. Supplementary Method

General information **S12**

Part 1: Chemical Syntheses **S12**

Experimental details for the synthesis of C8-alkyne-FANA UTP **S12**

Experimental details for the synthesis of azido-sugars **S23**

Part 2: Enzymatic Reactions **S25**

Polymerase expression and purification **S25**

Analyze polymerase activity on FANA synthesis **S26**

Generation of single-stranded cmFANA conjugates with 5'-biotinylation **S26**

cmFANA nuclease stability assay **S29**

Reverse transcription **S30**

DNA-display cycle as PCR strategy for carbohydrate-conjugated cmFANA **S31**

Mock selection experiment using the DNA-display cycle **S36**

Next-Generation Sequencing **S38**

Table S1. DNA sequences used in this study **S40**

Reference **S42**

NMR spectrum **S43**

I. Supplementary Results

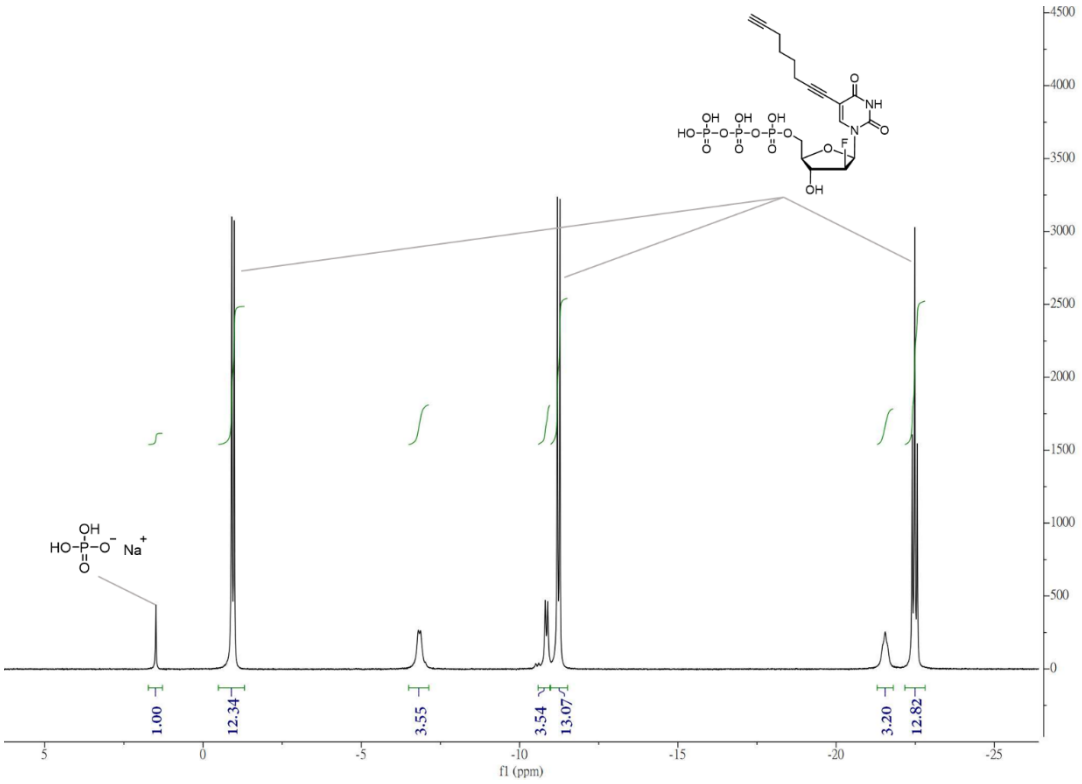


Figure S1. Quantitation of C8-alkyne-FANA UTP (11). To avoid the overestimation of the amount of **11** due to the presence of other salts (e.g., NaCl), we quantified the amount of **11** using an internal standard in ^{31}P-NMR. 2 μmole of Sodium phosphate monobasic salt was added to the NMR tube as internal standard to accurately quantify the amount of **11** through integration comparison in ^{31}P-NMR. The amount of **11** was calculated to be 0.026 mmol using this method.

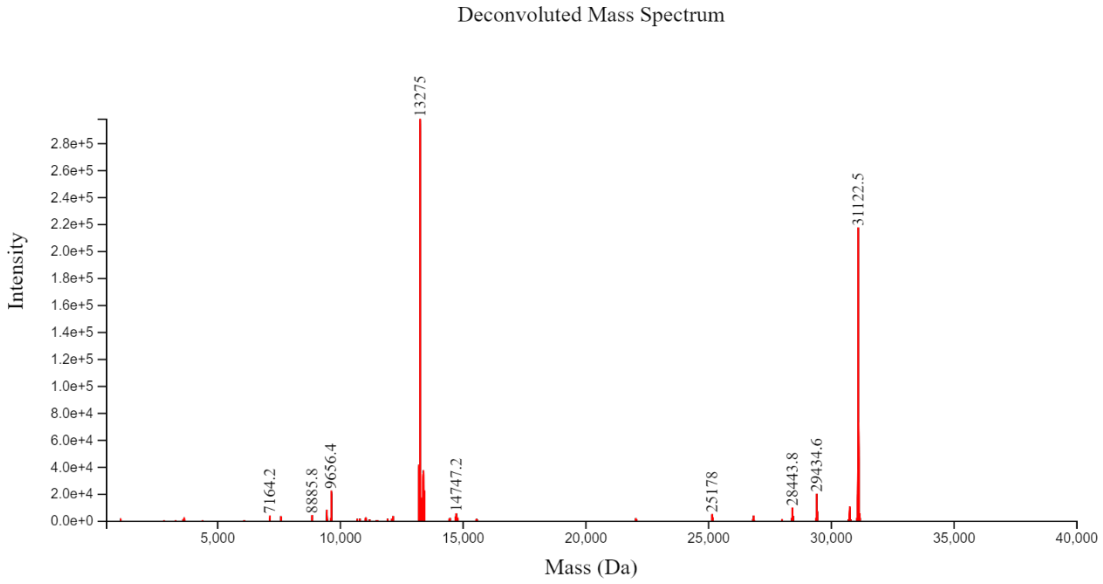


Figure S2. ESI-MS analysis of Man-cmFANA1. The deconvoluted mass spectrum showed the correct product of the conjugation of azidoethyl-penta-*O*-acetyl-mannose (Man-N3) to cmFANA1 (80 nt). The discovered mass (31122.5 Da) agreed with the calculated mass of desired product Man-cmFANA1. A subunit of streptavidin (13275 Da) is observed in the sample after the streptavidin magnetic particle was treated with concentrated ammonia at high temperature to elute the biotinylated Man-FANA strand.

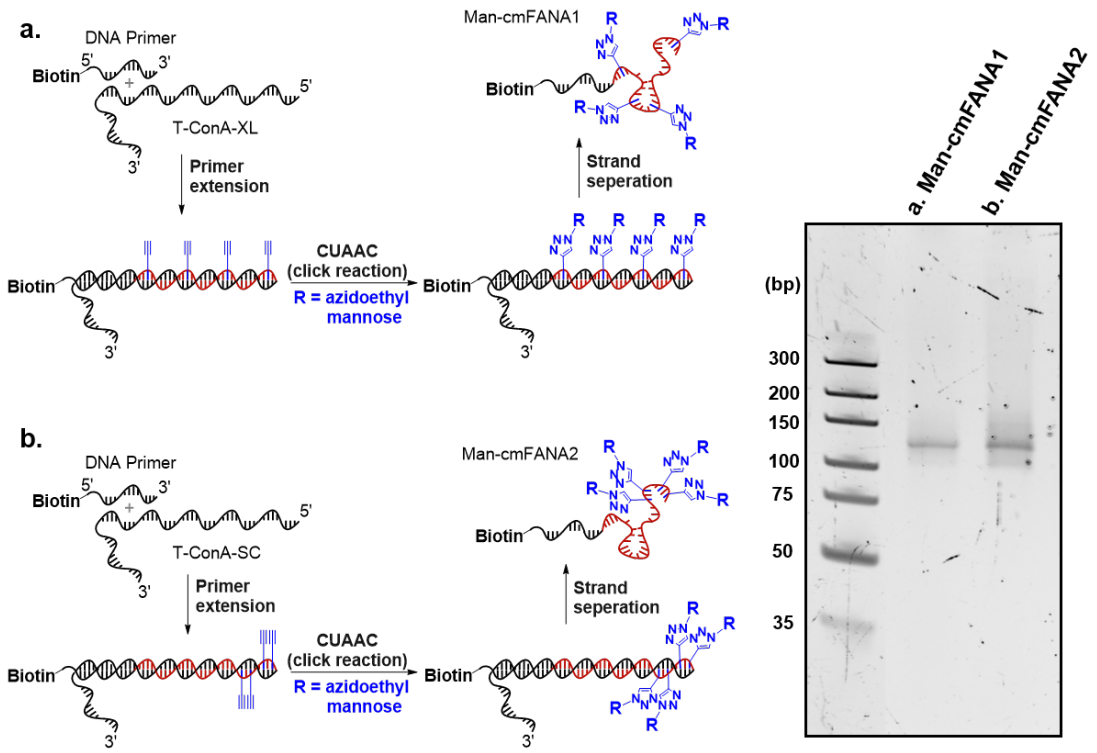


Figure S3. Click Conjugation of Man-N3 to different cmFANA polymer sequences. cmFANA1 **(a)** and cmFANA2 **(b)** contains same composition of nucleotides but different arrangement. Both went through the same process to obtain single-stranded cmFANA polymers and were both subjected to Man-N3 click conjugation. 10%TBE-Urea denaturing gel analysis showed same mobility of the two polymers, indicating quantitative conjugation reaction despite the proximity of the alkyne side chains in cmFANA2.

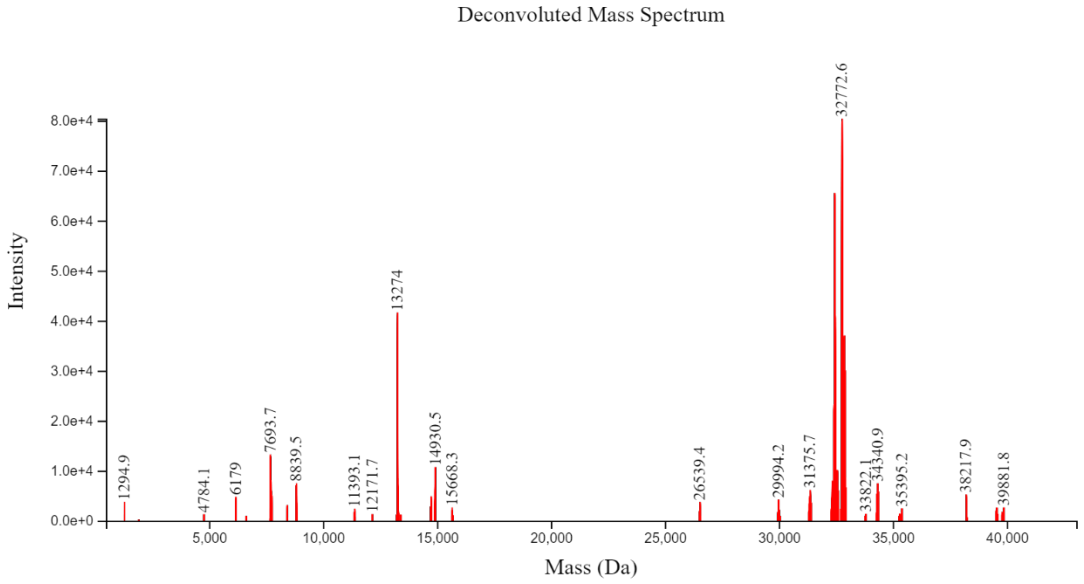


Figure S4. ESI-MS analysis of Lac-cmFANA1. The deconvoluted mass spectrum showed the correct product of the conjugation of azido lactose (Lac-N3) to cmFANA (80 nt). The discovered mass (32772.6 Da) agreed with the calculated mass of desired product Lac-cmFANA1. A subunit of streptavidin (13275 Da) is observed in the sample after the streptavidin magnetic particle was treated with concentrated ammonia at high temperature to elute the biotinylated Lac-FANA strand.

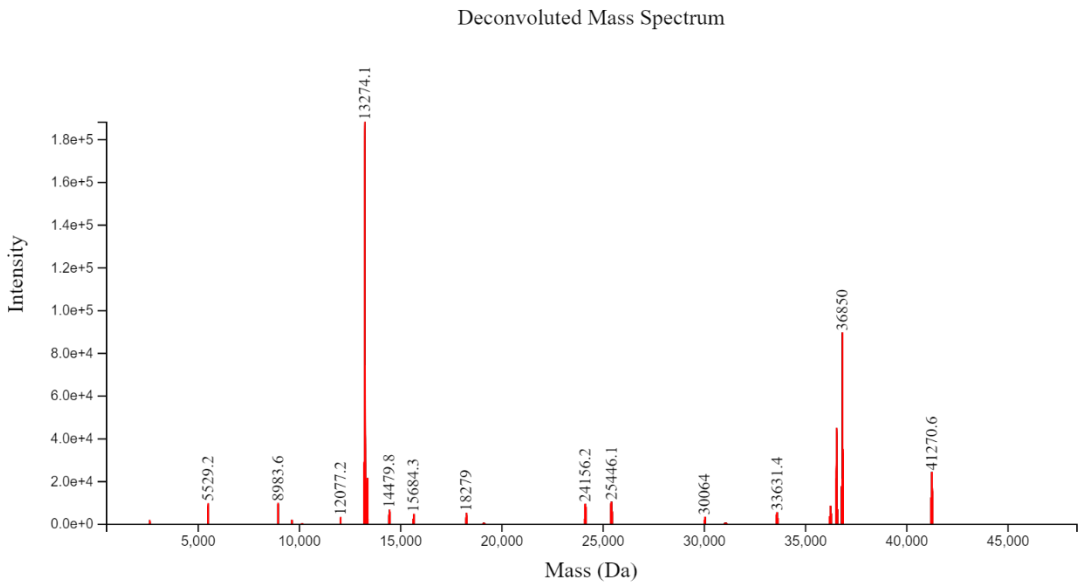


Figure S5. ESI-MS analysis of 6'-SA-Lac-cmFANA1. The deconvoluted mass spectrum showed the correct product of the conjugation of azido-6'-sialyllactose (6'-SA-Lac-N3) to cmFANA1 (80 nt). The discovered mass (36850 Da) agreed with the calculated mass of desired product SA-Lac-cmFANA1. A subunit of streptavidin (13275 Da) is observed in the sample after the streptavidin magnetic particle was treated with concentrated ammonia at high temperature to elute the biotinylated SA-Lac-FANA strand.

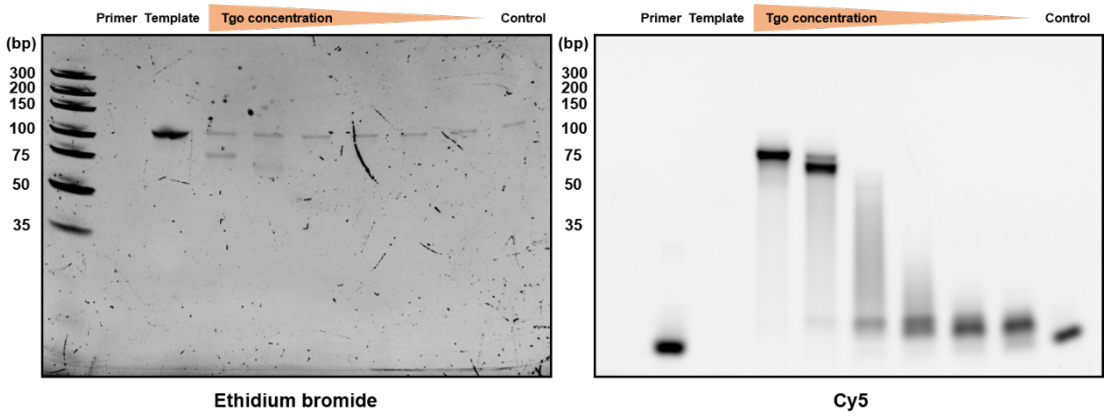


Figure S6. Ethidium Bromide channel for Tgo polymerase activity assay. Tgo polymerase activity assay suggested that C8-alkyne-FANA UTP can successfully serve as a substrate. Various amount of the polymerase was added to the reaction. The extended product was detected via Cy5 fluorescent tag at the 5'-end of the primer. Original PAGE form Ethidium bromide channel aligned with image of Cy5 channel (comparable to **Figure 3**). Trace amount of DNA template (**T-lib-XL**,100 nt) denatured from the extension product can be observed.

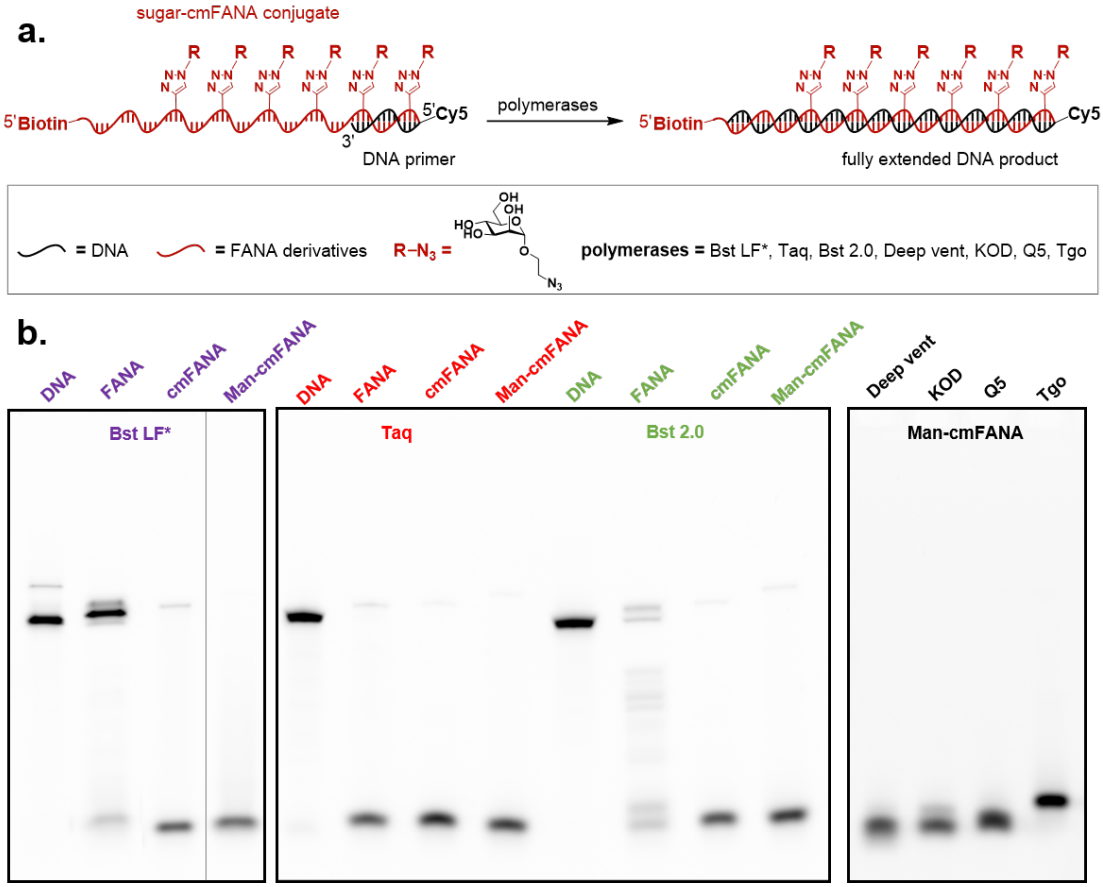


Figure S7. Denaturing PAGE analysis of the reverse transcription of cmFANA1 and mannose-cmFANA1 conjugate by several DNA polymerases. (a) Reaction scheme of DNA template synthesis through primer extension with Bst LF* as well as several other polymerases using FANA, cmFANA1, and sugar-cmFANA1 conjugate as template. **(b)** The following templates were used in the reverse-transcription experiments: DNA, regular FANA (no base-modifications), cmFANA1 (without sugar-clicked), and Mannose-cmFANA1 conjugate. Lab prepared Bst LF* polymerization assays in **purple**; commercial Bst2.0 assays in **green**; Taq assays in **red**. For the rest: Mannose-cmFANA conjugate teated with Deep Vent, KOD, Q5, and Tgo showed in **black**. PAGE condition: 10%TBE-urea gel, image on Cy5 channel.

Figure S8. Next-generation sequencing analysis of libraries before and after DNA-display.

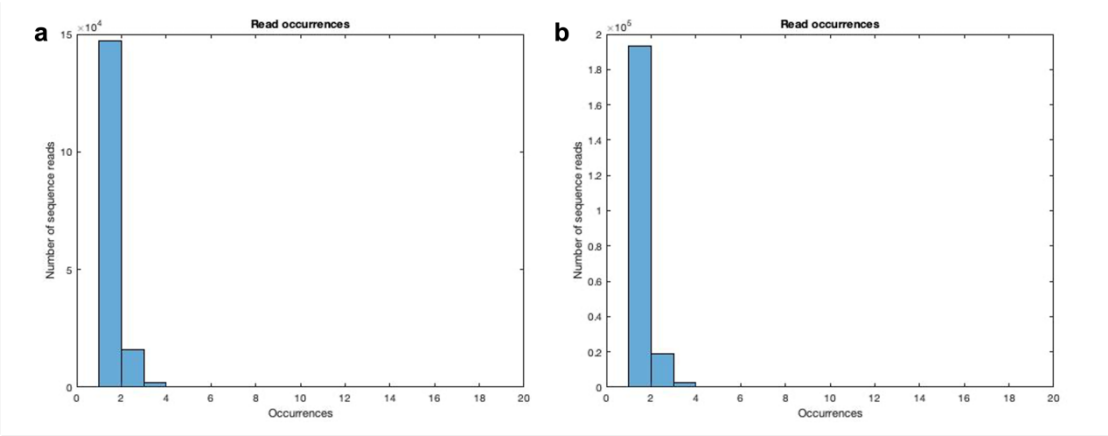


Figure S8. Diversity of the library before and after a DNA-display cycle. The plots show the uniqueness of the sequences in the library measured by the occurrences of the sequences in the library (a) before and (b) after the DNA-display cycle. The ratio of unique sequences in the two libraries remained unchanged at 86%, indicating the diversity of the library was not affected by the DNA-display cycle.

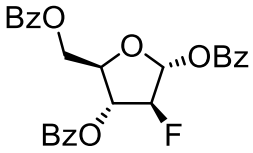
II. Supplementary Methods

General information

All solvents and reagents for chemical synthesis were purchased from commercial sources and used as supplied. All DNA oligonucleotides were purchased from Integrated DNA Technologies. FANA NTPs (FANA ATP, FANA CTP, FANA GTP, FANA UTP) were obtained from Metkinen Chemistry. Next-generation sequencing was performed using the Amplicon-EZ service provided by Genewiz.

Part 1: Chemical Syntheses

2-Deoxy-2-fluoro- 1,3,5-tri- *O*-benzoyl-α-D-arabinofuranose (1)

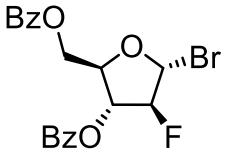


1,3,5-tri- *O*-benzoyl-α-(D)-ribofuranose (20 g, 43.25 mmol) in CH_2Cl_2 (200 mL) was added DAST (11.42 mL, 86.5 mmol) by syringe under nitrogen. The reaction mixture was then heated in oil bath and stirred for 16 hours under reflux. After TLC (EA/Hex = 1/3, Rf = 0.35) indicated generation of product and full consumption of starting material, the reaction was moved to ice-bath to cool and quenched by slow addition of $NaHCO_3$ (300 mL). Aqueous layer was further extracted with CH_2Cl_2 (2x 50 mL). The organic layer was collected and dried over Na_2SO_4 and

evaporated to dryness. Silica gel column chromatography (Hex/EtOAc 7:1 to 5:1 to 3:1 to 2:1) yielded **1** (17.1 g, 36.82 mmol, 85%) as white crystals.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14 – 7.38 (m, 15H), 6.76 (d, *J* = 9.1 Hz, 1H), 5.63 (dd, *J* = 19.5, 3.3 Hz, 1H), 5.39 (d, *J* = 48.3 Hz, 1H), 4.84 – 4.66 (m, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.38, 165.54, 164.66, 134.06, 133.93, 133.36, 130.09, 130.04, 130.02, 129.74, 129.28, 128.86, 128.78, 128.69, 128.55, 99.51 (d, J = 36.4 Hz) , 97.10 (d, J = 184.8 Hz) , 84.17, 76.89 (d, J = 28.3 Hz) , 63.71. **^{19}F NMR** (564 MHz, $CDCl_3$) δ -190.86 (ddd, *J* = 48.3, 19.6, 9.3 Hz, 1F). **HRMS (ESI)**: Calcd. for $C_{26}H_{25}FNO_7$ $[M+NH_4]^+$: 482.16096; found: 482.16097.

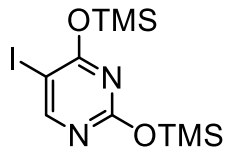
2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranosyl bromide (2)



A mixture of **1** (17 g, 36.6 mmol) in CH_2Cl_2 (170 mL) was stirred in ice-bath while adding 33% HBr in AcOH (19 mL, 110 mmol) by syringe. The mixture was allowed to stir in room temperature overnight. After TLC (EA/Hex = 1/3) indicated full consumption of starting material, the reaction was then quenched by $NaHCO_3$ (300 mL) and the aqueous layer was further extracted with CH_2Cl_2 (2x 50 mL). The organic layer was collected and dried over Na_2SO_4, evaporated to dryness, and purified by flash column chromatography to yield **2** (12.4 g, 29.3 mmol, 80%) as yellowish syrup.

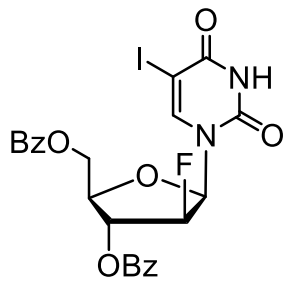
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.16 – 8.03 (m, 4H), 7.68 – 7.52 (m, 2H), 7.52 – 7.38 (m, 4H), 6.64 (d, *J* = 12.2, 1.0 Hz, 1H), 5.67 – 5.52 (m, 2H), 4.87 – 4.69 (m, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.13, 165.65, 134.03, 133.38, 130.13, 129.93, 129.55, 128.79, 128.68, 128.53, 100.73 (d, J = 191.5 Hz) , 87.73 (d, J = 31.7 Hz) , 84.85, 76.33 (d, J = 31.7 Hz) , 62.62. **HRMS (ESI)**: Calcd. for $C_{19}H_{17}BrFO_5$ $[M+H]^+$: 423.02379; found: 423.02328.

2,4-bis-*O*-(trimethylsilyl)-5-iodouracil (3)



To a mixture of 5-iodouracil (7.5 g, 31.5 mmol), 80 mL of dry CH3CN, and ammonium sulfate (0.416 g, 3.15 mmol) was added hexamethyldisilazane (5 g, 6.7 mL, 31.5 mmol) at 40 °C. The resulting mixture was then stirred overnight under reflux, then cooled, and concentrated to a yellow syrup and was directly used for the subsequent step.

(2'S)-2'-deoxy-2'-fluoro-3',5'-di-O-benzoyl-5-iodouridine (4)

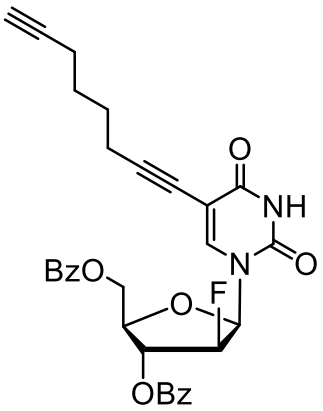


A mixture of **2** (10.58 g, 25 mmol) in dry CH_2Cl_2 (150 mL) was added **3** in dry CH3CN (30 mL)

and sodium iodide (3 g, 20 mmol) and was allowed to stir under room temperature for 7 days. The reaction was tracked by TLC (EA/Hex =2/3, Rf = 0.23). Then, the reaction mixture was diluted with CH_2Cl_2 and washed with water. The organic layer was dried over Na_2SO_4 an then concentrated to syrup. Column chromatography (Hex/EtOAc 4:1 to 3:1 to 2:1 to 1:1) yielded **4** (6.97 g ,12 mmol, 48%) as white solid.

^{1}H NMR (600 MHz, DMSO-*d*) δ 11.94 (s, 1H), 8.13 –7.55 (m, 10H), 6.30 (dd, *J* = 19.4, 3.8 Hz, 1H), 5.69 (dd, *J* = 20.2, 4.7, 1.7 Hz, 1H), 5.53 (dd, *J* = 50.7, 3.8, 1.7 Hz, 1H), 4.80 – 4.70 (m, 2H), 4.62 (q, *J* = 4.3 Hz, 1H). **^{13}C NMR** (151 MHz, DMSO-*d*) δ 165.63, 164.91, 160.33, 149.78, 144.98, 134.00, 133.66, 129.72, 129.32, 129.19, 128.89, 128.82, 128.64, 93.12 (d, J = 191.1 Hz) , 83.66 (d, J = 16.3 Hz) , 79.03, 76.41 (d, J = 29.9 Hz) , 69.60, 63.30. **HRMS (ESI)**: Calcd. for $C_{23}H_{19}FIN_2O_7$ $[M+H]^+$: 581.02155; found: 581.02124.

(2'S)-2'-deoxy-2'-fluoro-3',5'-di-O-benzoyl-5-(Octa-1,7-diynyl)- uridine (5)



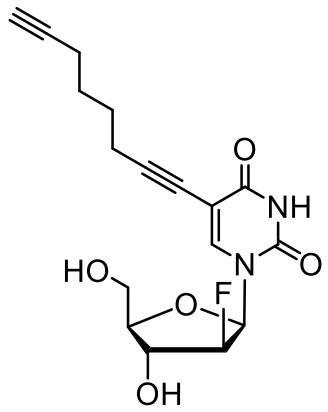
A mixture of **4** (6.8 g, 11.72 mmol), $Pd(PPh_3)_2Cl_2$ (0.842 g, 1.2 mmol), and CuI (0.229 g, 1.2 mmol) in anhydrous DMF (100 mL) were added triethylamine (20 mL). The mixture was then

stirred under room temperature for 5 minutes before adding 12 equiv. of Octa-1,7-diyne (19 mL, 145 mmol). The reaction mixture then was allowed to stir overnight under nitrogen gas at room temperature. After full evaporation of DMF, the residue was dissolved in CH_2Cl_2 and washed with $NaHCO_3$. The aqueous layer was further extracted with CH_2Cl_2 and the organic layer was collected and concentrated to syrup and purified by column chromatography to give **5** (4.97 g, 8.9 mmol, 76%) as white crystal.

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.49 (s, 1H), 8.06 (ddd, *J* = 28.2, 8.3, 1.4 Hz, 4H), 7.76 (d, *J* = 1.9 Hz, 1H), 7.67 – 7.54 (m, 2H), 7.46 (dt, *J* = 14.4, 7.8 Hz, 4H), 6.33 (dd, *J* = 21.1, 2.9 Hz, 1H), 5.62 (dd, *J* = 17.6, 2.9 Hz, 1H), 5.34 (dd, *J* = 50.0, 2.9 Hz, 1H), 4.85 – 4.72 (m, 2H), 4.53 (q, *J* = 3.9 Hz, 1H), 2.30 (td, *J* = 6.7, 2.6 Hz, 2H), 2.16 (dt, *J* = 6.3, 3.1 Hz, 2H), 1.94 (t, *J* = 2.6 Hz, 1H), 1.58 (dtd, *J* = 7.3, 3.7, 2.1 Hz, 4H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 166.32, 165.30, 161.79, 149.39, 142.43, 134.27, 133.52, 130.10, 129.88, 129.40, 128.86, 128.69, 128.25, 100.98, 94.77, 92.66 (d, J = 192.5 Hz), 85.16 (d, J = 16.6 Hz), 84.20, 81.73, 77.41, 77.20, 76.88 (d, J = 31.3 Hz), 71.15, 68.74, 63.42, 27.61, 27.41, 19.18, 18.01.

HRMS (ESI): Calcd. for $C_{31}H_{28}FN_2O_7$ $[M+H]^+$: 559.18751; found: 559.18737.

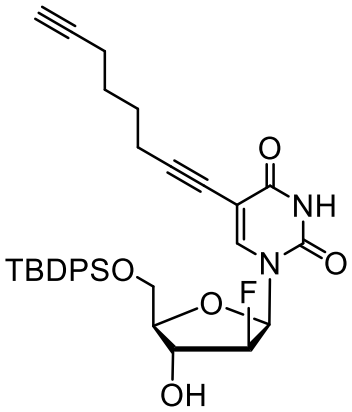
(2'S)-2'-deoxy-2'-fluoro-5-(Octa-1,7-diynyl)- uridine (C8-alkyne-FANA uridine, 6)



A mixture of **5** (4.97 g, 8.9 mmol) in methanol (50 mL) was added 25% NaOMe in methanol (9 mL). The mixture was then stirred for 2 hours at room temperature. After TLC (methanol/CH_2Cl_2 = 1/15) indicated full consumption of starting material, the reaction was added with ion exchange resin (Dowex 50WX8 (H^+)) and stirred for 15 minutes. The resin was filtered and the mixture solution was concentrated to syrup and purified by column chromatography to give **6** (2.62 g, 7.48 mmol, 84%) as white crystal.

^{1}H NMR (600 MHz, MeOD) δ 7.99 (d, *J* = 1.5 Hz, 1H), 6.16 (dd, *J* = 16.7, 3.9 Hz, 1H), 5.02 (dt, *J* = 52.1, 2.9 Hz, 1H), 4.33 (ddd, *J* = 19.5, 4.9, 2.6 Hz, 1H), 3.92 (q, *J* = 4.6 Hz, 1H), 3.79 (ddd, *J* = 48.2, 12.3, 4.5 Hz, 2H), 2.41 (t, *J* = 6.8 Hz, 2H), 2.21 (ddd, *J* = 14.0, 6.0, 2.7 Hz, 2H), 2.01 (s, 1H), 1.67 (dddd, *J* = 22.0, 11.3, 5.8, 3.5 Hz, 4H). **^{13}C NMR** (151 MHz, MeOD) δ 164.46, 150.90, 144.58, 100.72, 97.38, 95.47 (d, J = 190.5 Hz), 85.68, 85.34 (d, J = 16.9 Hz), 84.70, 74.70 (d, J = 25.0 Hz) , 72.57, 69.71, 61.66, 28.75, 28.58, 19.65, 18.53. **HRMS (ESI)**: Calcd. for $C_{17}H_{20}FN_2O_5$ $[M+H]^+$: 351.13508; found: 351.13487.

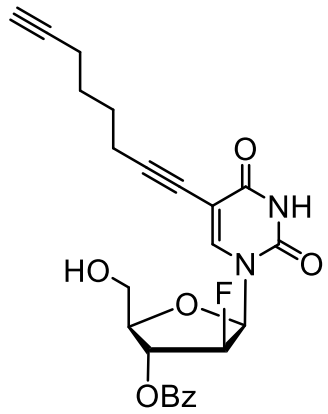
(2'S)-2'-deoxy-2'-fluoro-5'-O-(*tert*-butyldiphenylsilyl)-5-(Octa-1,7-diynyl)- uridine (7)



To a solution of **6** (2.5 g, 7.14 mmol) and imidazole (0.972 g, 14.28 mmol) in dry DMF (25 mL) was added TBDPS-Cl (1.85 mL, 7.14 mmol) dropwise in ice-bath. The reaction mixture was then allowed to stir overnight at room temperature. After TLC (EA/Hex = 1/1) indicated full consumption of starting material, the reaction was added with methanol (10 mL) and the solution was evaporated to dryness. The residue was dissolved with CH_2Cl_2 and washed with $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 an then concentrated to syrup and purify by column chromatography to give **7** (3.57 g, 6.07 mmol, 85%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.52 (d, *J* = 1.7 Hz, 1H), 7.77 (d, *J* = 1.7 Hz, 1H), 7.74 – 7.38 (m, 10H), 6.22 (dd, *J* = 19.6, 3.2 Hz, 1H), 5.11 (dd, *J* = 51.5, 3.3, 1.3 Hz, 1H), 4.51 (dt, *J* = 19.5, 4.5 Hz, 1H), 4.02 (q, *J* = 4.3 Hz, 1H), 3.98 – 3.83 (m, 2H), 2.31 (t, *J* = 6.7 Hz, 2H), 2.14 (td, *J* = 6.7, 2.6 Hz, 2H), 1.90 (t, *J* = 2.7, 0.7 Hz, 1H), 1.69 – 1.46 (m, 4H), 1.09 (s, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 162.20, 149.55, 142.93, 135.74, 135.68, 133.00, 132.96, 130.17, 130.12, 128.08, 100.72, 95.70, 94.95, 94.42, 84.87, 84.66 (d, J = 16.8 Hz), 84.23, 75.19 (d, J = 26.2 Hz), 71.21, 68.74, 62.72, 27.70, 27.46, 26.94, 19.36, 19.23, 18.06. **HRMS (ESI)**: Calcd. for $C_{33}H_{38}FN_2O_5Si$ $[M+H]^+$: 589.25285; found: 589.25194.

(2'S)-2'-deoxy-2'-fluoro-3' -O-benzoyl-5-(Octa-1,7-diynyl)- uridine (8)

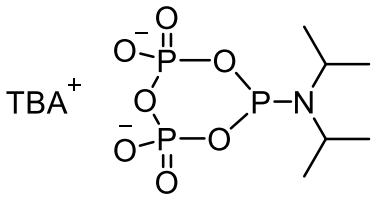


To a solution of **7** (3.5 g, 5.94 mmol) and pyridine (3 mL) in CH_2Cl_2 (350 mL) was slowly added benzoyl chloride (1.03 mL, 8.91 mmol) in ice-bath. The reaction mixture was then allowed to stir for 3 hours at room temperature. After TLC (EA/Hex = 1/1) indicated full consumption of starting material, the reaction was washed with 1M HCl (2x 50 mL), water (50 mL), and brine (50 mL). The organic layer was collected and dried over Na_2SO_4, and evaporated to dryness. This residue was then dissolved with THF (300 mL) and added with 1M TBAF in THF (7 mL, 7 mmol) in ice-bath. The reaction mixture was then allowed to stir at room temperature for 1 hour. After TLC (EA/Hex = 1/1) indicated full consumption of starting material, the reaction solution was evaporated to dryness and the residue was dissolved with CH_2Cl_2 and washed with water and brine. The organic layer was collected, dried over Na_2SO_4, and concentrated to syrup and purify by column chromatography to give **8** (2.43 g, 5.35 mmol, 90 %) as white crystal.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.47 (s, 1H), 8.04 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.82 (d, *J* = 1.8 Hz, 1H), 7.64

(ddt, *J* = 8.6, 7.2, 1.3 Hz, 1H), 7.53 – 7.47 (m, 2H), 6.28 (dd, *J* = 19.0, 3.4 Hz, 1H), 5.54 (dd, *J* = 19.2, 4.0, 1.5 Hz, 1H), 5.42 – 5.27 (m, 1H), 4.22 (q, *J* = 4.2 Hz, 1H), 4.12 – 3.98 (m, 2H), 2.45 (t, *J* = 6.8 Hz, 2H), 2.25 (td, *J* = 6.8, 2.6 Hz, 2H), 1.98 (t, *J* = 2.6 Hz, 1H), 1.76 – 1.65 (m, 4H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 165.70, 161.26, 149.15, 142.53, 134.37, 130.14, 128.93, 128.38, 101.02, 95.11, 92.99 (d, J = 193.7 Hz) , 84.45, 84.29 (d, J = 16.9 Hz), 83.14, 76.30 (d, J = 29.9 Hz), 71.41, 68.78, 62.04, 27.78, 27.54, 19.36, 18.17. **HRMS (ESI)**: Calcd. for $C_{24}H_{24}FN_2O_6$ $[M+H]^+$: 455.16129; found: 455.15885.

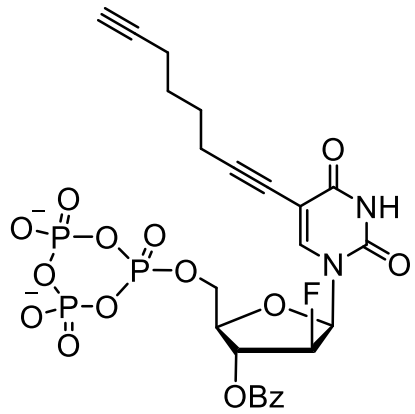
Cyclic pyrophosphoryl-P-amidite (c-PyPA, 9)



To a solution of sodium pyrophosphate (1.5 g, 5.64 mmol) in water was added Dowex 50WX8 (H^+) ion exchange resin and stirred for 30 minutes. After filtering out the resin, the solution was neutralized with tetrabutylammonium (TBA) hydroxide and lyophilization to convert into their tetrabutylammonium (TBA) salts. Then, the pyrophosphate was co-evaporated with acetonitrile (2 × 30 mL) and dissolved with dry acetonitrile (70 mL). 4 Å molecular sieves and triethylamine (2.8 mL, 20 mmol) were then added under nitrogen gas. The mixture was cooled to -10 ºC before slowly adding previously distilled $(iPr)_2N$-PCl_2 (1.14 g, 5.64 mmol) and the reaction mixture was stirred for 5 minutes at -10 ºC. The mixture was checked by 31P-NMR to confirmed full conversion and stored at -20 ºC and is ready to use for the subsequent step.

^{31}P NMR (243 MHz, CD_3CN) δ 126.13 (t, *J* = 24.6 Hz, 1P), -18.62 (d, *J* = 24.3 Hz, 2P).

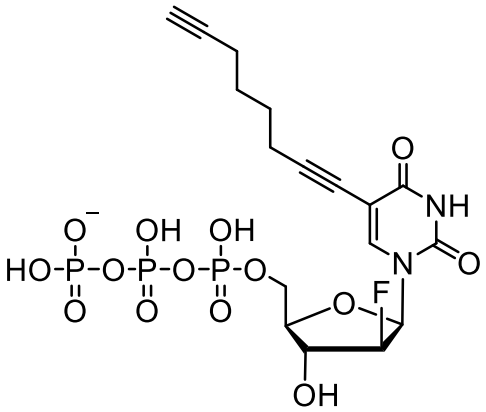
5-(Octa-1,7-diynyl)- 2'-deoxy-2'-fluoro-3'-O-benzoyluridine-5′-cyclotriphosphate (10)



A mixture of 1.2 equiv. **8** (545 mg, 1.2 mmol) and 4 equiv. of 5-(Ethylthio)-1H-tetrazole (ETT, 625 mg, 4.8 mmol) were co-evaporated with dry acetonitrile (2x 5 mL) and dried under high-vacuum before directly adding in 1 equiv. of the reaction mixture **9** (80 mM in acetonitrile, 12.5 mL, 1 mmol) under nitrogen gas. The resulting mixture was allowed to stir at room temperature for 15 minutes and checked with 31P-NMR to confirm completion of the coupling (a shift of triplet from +126 ppm to +103 ppm indicated the coupling of **8** and **9**). Then, the reaction was cooled in ice-bath before adding in 1.5 equiv. of ≤77% mCPBA (260 mg, 1.5 mmol) and stirred for 10 minutes in ice-bath. The reaction was tracked by 31P-NMR (the triplet at +103 ppm fully consumed and formation of a triplet at -24 ppm) to confirm full oxidation. This reaction mixture **10** was stored at -20 ºC and can be directly used for the ring-opening step.

^{31}P NMR (243 MHz, $CDCl_3$) δ -24.21 (t, *J* = 21.2 Hz, 1P), -25.59 (dd, *J* = 21.3, 5.5 Hz, 2P).

5-(Octa-1,7-diynyl)- 2'-deoxy-2'-fluoro-uridine-5′-triphosphate (11)

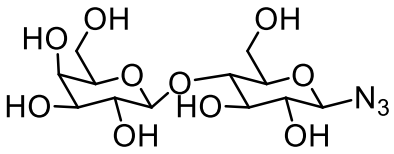


The reaction mixture **10** (500 μL, 48 μmol) was added 500 μL of D_2O and stirred at room temperature for 3 hours. Then, 15 mL of 28%-30% NH_4OH was added to the mixture and stirred for 18 hours. After concentration of the mixture solution by reducing pressure, precipitation with 0.5 M $NaClO_4$ in acetone (15 mL, stand at -4°C for 20 minutes, precipitate collected) followed by preparative HPLC (a gradient of 95% 0.1 M TEAA, PH 7/ 5% acetonitrile to 60% 0.1 M TEAA, PH 7/ 40% acetonitrile in 40 minutes). The fractions were collected and concentrated by reduce pressure, and precipitation with 0.5 M $NaClO_4$ in acetone was performed again to give **11** as colorless solid in sodium form. The accurate amounts of **11** was determined by 31P-NMR by using sodium phosphate monobasic salt as internal standard (**Figure S1**).

^{1}H NMR (600 MHz, D_2O) δ 8.01 (d, *J* = 1.9 Hz, 1H), 6.31 (dt, *J* = 17.6, 3.6 Hz, 1H), 5.33 – 5.07 (ddd, 1H), 4.58 (ddd, *J* = 19.1, 4.4, 2.4 Hz, 1H), 4.36 – 4.19 (m, 3H), 2.48 (t, *J* = 6.9, 3.3 Hz, 2H), 2.39 (t, *J* = 5.2, 2.7 Hz, 1H), 2.30 (td, *J* = 6.5, 3.2 Hz, 2H), 1.78 – 1.58 (m, 4H). **^{13}C NMR** (151 MHz, D_2O) δ 165.09, 150.47, 144.16, 99.79, 96.09, 94.40 (d, J = 191.8 Hz), 86.02, 83.87 (d, J = 16.4 Hz), 82.04, 73.52 (d, J =

26.3 Hz), 70.90, 69.23, 64.55, 26.96, 26.76, 18.20, 17.07. **^{31}P NMR** (243 MHz, D_2O) δ 0.96 (d, *J* = 18.3 Hz, 1P), -9.69 (d, *J* = 17.4 Hz, 1P), -20.12 (t, *J* = 17.8 Hz, 1P). **^{19}F NMR** (564 MHz, D_2O) δ -199.67 (dt, *J* = 51.7, 18.6 Hz, 1F). **HRMS (ESI)**: Calcd. for $C_{17}H_{21}FN_2O_{14}P_3$ $[M-H]^-$: 589.01950; found: 586.01862.

1-azido-lactose (Lac-N_3)

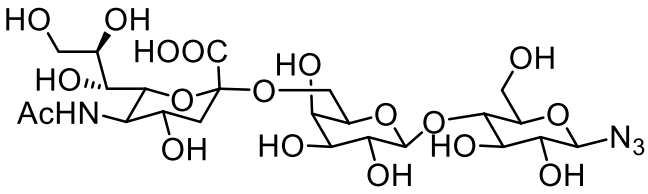


To a mixture of D-lactose (500 mg, 1.39 mmol), diisopropylethylamine (2.4 mL, 13.88 mmol), and NaN3 (910 mg, 13.88 mmol) in water (6 mL) was cooled in ice-bath before adding 3 equiv. of 2-chloro-1,3-dimethyl-imidazolinium chloride (710 mg, 4.20 mmol), and the reaction mixture was then stirred for 1 hour in ice-bath. After concentration of the reaction mixture by reduced pressure, ethanol was applied to the mixture and the solid was removed by filtration. The filtrate was again concentrated and the residue was dissolved in water and washed with CH_2Cl_2. The product was purified by ion-exchange column chromatography (Amberlite IR-120B Na^+ form) and lyophilized to give 1-azido-lactose (496 mg, 1.35 mmol, 97%) as white crystal.

^{1}H NMR (500 MHz, Deuterium Oxide) δ 4.91 (d, J = 8.8 Hz, 1H), 4.59 (d, J = 7.8 Hz, 1H), 4.18 – 4.01 (m, 2H), 4.01 – 3.76 (m, 8H), 3.66 (dd, J = 10.0, 7.8 Hz, 1H), 3.52 – 3.38 (m, 1H).

HRMS (ESI): Calcd. for C12H21N3NaO10 $[M+Na]^+$: 390.1119; found: 390.1123.

1-azido-2,6-sialyllactose (SA-Lac-N3)



To a mixture of 6'-sialyllactose (27 mg, 0.041 mmol), diisopropylethylamine (72 µL, 0.411 mmol), and NaN3 (27 mg, 0.411 mmol) in Deuterium Oxide (300 µL) was cooled in ice-bath before adding 3 equiv. of 2-chloro-1,3-dimethyl-imidazolinium chloride (21 mg, 0.123 mmol), and the reaction mixture was then stirred for 1 hour in ice-bath. The reaction mixture was then concentrated before adding N, N-Dimethylformamide to dissolve the product. The solid was removed by filtration and the filtrate was again concentrated and the residue was dissolved in water and washed with CH_2Cl_2. The product was purified by ion-exchange column chromatography (Amberlite IR-120B Na^+ form) and lyophilized to give 1-azido-2,6-sialyllactose (23.5 mg, 0.034, 84%) as white solid. The NMR data were in good agreement with those published in the literature by Tanaka et al.[1]

^{1}H NMR (500 MHz, Deuterium Oxide) δ 4.86 (d, J = 8.9, 1.2 Hz, 1H), 4.51 (d, J = 7.9 Hz, 1H), 4.09 – 3.57 (m, 18H), 3.47 – 3.39 (t, 1H), 2.84 – 2.75 (dd, 1H), 2.14 – 2.09 (s, 3H), 1.80 (t, J = 12.2 Hz, 1H). **HRMS (ESI)**: Calcd. for $C_{23}H_{37}N_4O_{18}$ $[M-H]^-$: 657.2108; found: 657.2116.

Part 2: Enzymatic Reactions

Polymerase expression and purification

The Tgo polymerase were expressed and purified following known protocol.[2] The pGDR11 plasmids encoding the Tgo and Bst-LF* polymerases are kind gifts from the Chaput lab. Briefly, after transformation E. coli cell XL1-Blue with polymerase expression plasmids, the cells were inoculated in 1 L of LB-ampicillin (100 μg mL^{-1}) liquid medium on shaker at 37 °C. Protein expression was induced by adding IPTG to the medium for a final concentration of 0.5 mM at OD600 = 0.6 and incubated overnight on shaker at 15 °C. Cells were transferred into 1L jar and centrifuged 10 min at 9500 × g at 4 °C and lysed in 40 mL buffer (10 mM Tris pH 8.0, 500 mM NaCl, 10% glycerol) by sonication on ice. The cell lysate was heated for 60 minutes at 80 °C then cooled on ice for 30 minutes to denature endogenous E. coli proteins before centrifuged again for 30 minutes at 40,000 × g at 4 °C. The supernatant was recovered. Nucleic acids were precipitated by adding 10% (v/v) polyethyleneimine to a final concentration of 0.5% and incubating for 15 min on ice before centrifuging for 20 min at 40,000 × g at 4 °C. Recombinant polymerase was recovered from excess polyethyleneimine by adding 60% (w/v) ammonium sulfate to the supernatant, incubating for 30 min on ice, and then centrifuging for 30 min at 40,000 × g at 4 °C. Protein pellets were suspended in 4 °C buffer (10 mM Tris pH 8.0, 50 mM NaCl, 10% glycerol) and purified by 5 mL heparin high-performance (HP) affinity column (step elutions of 100, 250, 500, and 1000 mM NaCl). Finally, Fractions corresponding to protein of

the correct size were verified by sodium dodecyl sulfate–PAGE, combined, quantified by UV absorbance at 280 nm, and added with glycerol up to 50% final concentration to store long-term at -20 °C.

Analyze polymerase activity on FANA synthesis

Polymerase activity assays were performed in eight separate PCR reaction tubes with each containing 1 μM of DNA primer **Tcy5-RP**, 1 μM of DNA template **T-lib-XL**, 100 μM of FANA NTPs (A, C, and G) as well as C8-alkyne-FANA UTP (**11**), and different amount of Tgo polymerase (2 μM, 1 μM, 0.5 μM, 0.25 μM, 0.125 μM, 62.5 nM, 31.25 nM, 15.62 nM, respectively) in 1× ThermoPol buffer of 10 μL reaction volumes. The primer-template complex in the reaction mixtures was annealed at 90 °C for 5 minutes and cooling at 4 °C for 10 minutes. Primer-extension reactions were performed for 1 hour at 55 °C before quenching with 100 μL (10 equivalents, v/v) of formamide stop buffer (99% deionized formamide, 25 mM EDTA). Samples were denatured for 15 min at 95 °C before analyzing by 10% TBE-Urea denaturing PAGE due to high stability of the chimeric DNA/FANA heteroduplex. Gels were visualized through Cy5 imaging channel.

Generation of single-stranded cmFANA conjugates with 5'-biotinylation

Single-stranded cmFANA were generated first in a total volume of 1 mL in 20 PCR tubes

containing 1 μM of 5'-biotinylated DNA primer **T-ConA-RP-Bio**, 1 μM of DNA template (**T-ConA-XL**, or **T-ConA-SC**), 100 μM of FANA NTPs (A, C, and G) as well as C8-alkyne-FANA UTP (**11**), and 1 μM of lab expressed Tgo polymerase in 1× ThermoPol buffer. The primer-template complex in the reaction mixtures was annealed at 90 °C for 5 minutes and cooling at 4 °C for 10 minutes. Primer-extension reactions were performed for 3 hours at 55 °C and then hold at 4 °C.

The primer extension reactions in 20 PCR tubes were transferred into a 15 mL centrifuge tube and added with 0.25 mL of 3 M sodium acetate (pH 5.2) solution and 6.875 mL of 100% ethanol, followed by freezing at -80 °C for 30 minutes or liquid nitrogen for 5 minutes. The frozen stock was then centrifuged for 30 min at 4000 x g at 4 °C to precipitate the DNA-cmFANA hybrid. The pellet was dissolved with 300 μL water, followed by purification using MinElute spin columns where the primer extension product was eluted with 90 μL of TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8). The collected primer extension product was again applied with ethanol precipitation, in which 20 μL of 3 M sodium acetate (pH 5.2) and 600 μL of 100% ethanol were added to the solution, followed by freezing and centrifugation of the frozen stock 30 min at 21,000 x g at 4 °C. The precipitated DNA-cmFANA hybrid was resuspended in 10 μL 1X PBS buffer.

To perform click conjugation, the 10 μL DNA-cmFANA hybrid solution was added with 10 μL of 100 mM azido-sugar (2-azidoethyl 2,3,4,6-tetra-O-acetyl-α-(D)-mannopyranoside, 1-azido-

lactose, or 1-azido-2,6-sialyllactose) in DMSO and 20 μL 20 mM sodium phosphate buffer (pH 8) in a 1.5 mL Eppendorf tube. The cap of the tube was cut off and sealed with a rubber septum, and the system was purged with nitrogen gas for 15 minutes. The click reaction was initiated by adding 10 μL of a 10 mM, premixed solution of Cu:TBTA = 1:1 (prepared with 1 mg CuBr + 0.7 mL of 10 mM TBTA in 4:3:1 water:DMSO:tBuOH), followed by nitrogen gas purge for 5 minutes and incubated for 3 hours. The click reaction mixture was then applied with ethanol precipitation, in which 5 μL of 3 M sodium acetate (pH 5.2) and 165 μL of 100% ethanol were added to the solution, followed by freezing and centrifugation of the frozen stock 30 min at 21,000 x g at 4 °C. The sugar-conjugated DNA-cmFANA hybrid pellet was resuspended with 180 μL 1X B&W buffer (5 mM Tris-HCl pH 7.5, 0.5 mM EDTA, 1 M NaCl) and ready for the next step.

To perform bead separation, 175 μL MyOne C1 streptavidin beads was added to a new 1.5 mL Eppendorf tube. The beads were captured on the side of the tube with a magnet and the supernatant was removed. The beads were washed three times with 175 μL 1X B&W. The click product solution was added to the beads and mixed on a rotator for 30 minutes at room temperature. Then, the beads were captured and the supernatant was removed. The beads with sugar-conjugated DNA-cmFANA hybrid were washed three times with 175 μL 1X B&W, and then treated twice with 50 μL of freshly-prepared 0.25 M NaOH solution to generate single-stranded sugar-cmFANA on the beads. The beads were captured and the supernatant was

removed. To separate the biotinylated single-stranded sugar-cmFANA from the streptavidin beads, 150 µL of concentrated ammonium hydroxide (18 M) was added and the tube was sealed tightly and then heated on a thermal block at 70 °C for 10 min (an extra 3 hours of incubation with the 18 M ammonium hydroxide at room temperature was required before heating to deprotect the acetyl group on the mannose). The sample was cooled on ice before opening the cap. The beads were captured by magnet and the supernatant was transferred to a separate tube. Another 50 µL of 18 M ammonium hydroxide was applied to the beads and the heating procedure was again performed.

The supernatants from the two ammonium hydroxide treatment steps were combined and added with 2.25 mL *n*-butanol. This mixture was then vortexed and centrifuged for 10 min at 16,000 ×g at 4 °C. The supernatant was removed and the pellet was dried over vacuum centrifugation, and then resuspended in 100 µL water. 25 µL of 5 M NH_4OAc and 207.5 µL of cold 100% ethanol were added, and the solution mixture was frozen and centrifuged for 30 min at 21,000 ×g at 4 °C to precipitate the base-modified cmFANA. Finally, the pellet was washed once with 70% v/v cold ethanol in water, then dissolved in 50 µL water. The amount of the single-stranded cmFANA product was quantified by NanoDrop Spectrophotometer and stored at -20 °C.

cmFANA nuclease stability assay

Nuclease stability assays were performed in 50 µL reaction volumes containing 1 µM of single-

stranded DNA (**ConA-aptamer-Cy5**) or single-stranded fluorescein-cmFANA conjugate obtained from previous step, and 50% Human Serum (HS). The reactions were incubated at 37 °C for 2 days, and in each time point (0 min, 5 min, 15 min, 30 min, 1 hr, 2 hr, 6 hr, 24 hr, 48 hr), 5 μM of the reaction mixture was collected, freeze and lyophilized, added with 10 μL of formamide stop buffer (99% deionized formamide, 25 mM EDTA), and store at -20 °C. Samples were denatured for 15 min at 95 °C before analyzing by 10% TBE-Urea denaturing PAGE. Gels were visualized through Cy5 or fluorescein imaging channel.

Reverse transcription

Reverse transcriptions were performed in 10 μL reaction volumes containing 1 μM of DNA primer **TConA-FANA-cy5RP**, 1 μM of single-stranded FANA, cmFANA1, or Man-cmFANA1, 1× ThermoPol buffer supplemented with 2 mM MgCl2, 500 μM of each DNA dNTP, and DNA polymerase (0.8 U/μL of commercial Bst2.0, Bst3.0, Taq, Deep vent, KOD, Q5, 1 μM lab expressed Tgo, or 1 μM Bst LF*). The primer-template complex in the reaction mixtures was annealed by heating at 90 °C for 5 minutes followed by cooling at 4 °C for 10 minutes. Then, the reactions were incubated for 3 hours at 50 °C (55°C for Tgo) before quenching with 100 μL (10 equivalents, v/v) of formamide stop buffer (99% deionized formamide, 25 mM EDTA). Samples were denatured for 15 min at 95 °C before analyzing by 10% TBE-Urea denaturing PAGE. Gels were visualized through Cy5 imaging channel.

DNA-display cycle for carbohydrate-conjugated cmFANA

The DNA-display cycle was adapted from a selection with modified aptamers (SELMA) protocol by the Krauss lab.[3] To begin, 10 µL of 10 µM ssDNA **Library 1 (N40)**, 20 µL of 10× NEBuffer 2, 12 µL of 10 µM **Hairpin Regenerating Primer**, and 150 µL of water was added to a PCR tube to make a master mix of a total volume of 192 µL. The master mix was separated into two PCR tubes with 96 µL master mix in each PCR tube. The PCR tubes were placed in a thermal cycler and heated with an annealing ramp of 95°C to 45°C at a rate of 0.1 °C/sec to anneal the ssDNA library template-primer. Then, 2 µL of 10 mM DNA dNTPs mix and 2 µL of 5 U/µL DNA polymerase I (Klenow) was added into each PCR tube and the reaction mixtures was incubated for 25 min at 25°C before directly adding 1.5 µL of 20 U/µL Exo I to each tube and further incubated for 30 minutes at 37°C and then 20 minutes at 80°C to denature the enzyme. To perform ethanol precipitation, the reaction mixture of the two PCR tubes were combined in a 1.5 mL Eppendorf tube and added nuclease-free water to a total volume of 300 µL, before added with 40 µL of 3 M sodium acetate pH 5.2 and 1mL of 100% ethanol, followed by freezing at -80 °C for 30 minutes and centrifugation of the frozen stock 30 min at 21,000 x g at 4 °C. The supernatant was removed and the pellet of dsDNA regeneration product was resuspended with 95 µL water (reserve 5 µL for PAGE analysis) before combining with 90 µL 2X B&W buffer.

To remove unwanted biotinylated strand, the dsDNA regeneration product solution (180 μL) was first added to 150 μL of MyOne C1 streptavidin beads (supernatant removed and prewashed three times) and mixed on a rotator for 30 minutes at room temperature. The beads were then capture on magnet and the supernatant was removed. The beads were washed three times with 150 μL 1X B&W before adding 40 μL of 100 mM NaOH and sit for 4 minutes to elute the non-biotinylated ssDNA. The supernatant containing non-biotinylated ssDNA was then transferred to another tube containing 4 μL of 1 M HCl and 1 μL of 1 M Tris-Cl, pH 8.0 for neutralization. The ssDNA was then quantified by NanoDrop Spectrophotometer and stored at -20 °C.

cmFANA synthesis by ssDNA hairpin self-extension reaction was performed in 43 μL reaction volumes containing 35 μL of ssDNA library hairpin previously made, 5 μL of 10× Thermopol buffer, and water to a total volume of 43 μL. The ssDNA was self-annealed to its hairpin formation by heating for 5 minutes at 95 °C and slowly cooled down to 4 °C for 10 minutes. The mixture was then added with 1 μL of 10 mM premixed FANA NTPs (FANA ATP, FANA CTP, and FANA GTP only), 1 μL of 10 mM C8-alkyne-FANA UTP (**11**), and 5 μL of 8 μM lab expressed Tgo polymerase, followed by incubation for 3 hours at 55 °C to perform cmFANA synthesis. The reaction was then added with water to a total volume of 100 μL and treated with ethanol precipitation, in which 10 μL of 3 M sodium acetate (pH 5.2) and 330 μL of 100% ethanol were added to the solution, followed by freezing at -80 °C for 30 minutes and

centrifugation of the frozen stock 30 min at 21,000 x g at 4 °C. The supernatant was removed and the pellet was resuspended with 22 µL PBS buffer 1X, where 2 µL was reserved for PAGE analysis (10% TBE gel).

Click conjugation of hairpin DNA-cmFANA hybrid with Man-N3 was performed by combining the 20 µL hairpin DNA-cmFANA hybrid solution with 30 µL of 100 mM 2-azidoethyl α-(D)-mannopyranoside in DMSO and 40 µL of 20 mM sodium phosphate buffer (pH 8), followed by addition of 20 µL of a 10 mM, premixed solution of Cu:TBTA = 1:1 (prepared with 1 mg CuBr + 0.7 mL of 10 mM TBTA in 4:3:1 water:DMSO:tBuOH). For preparing positive control for mock selection experiment, exchange 2-azidoethyl α-(D)-mannopyranoside with Azide-PEG3-biotin. The reaction was incubated for 3 hours at room temperature under nitrogen gas bubbling to displace oxygen in the system. The click reaction mixture was again applied with ethanol precipitation, in which 12 µL of 3 M sodium acetate (pH 5.2) and 400 µL of 100% ethanol were added to the solution, followed by freezing and centrifugation of the frozen stock 30 min at 21,000 x g at 4 °C. The pellet was resuspended with 22 µL of water and 2 µL was reserved for PAGE analysis (10% TBE gel).

Strand displacement of the self-annealed hairpin was performed by combining 20 µL of the hairpin with 6 µL of 10 µM DNA primer **H-FP**, 5 µL of 10× Thermopol buffer, and 16 µL of water. After heating for 15 seconds at 65 °C in the thermal cycler, the mixture was quickly added with 1 µL of 10 mM DNA dNTP mix and 2 µL of 8 U/µl Bst2.0 WarmStart DNA

Polymerase to a total reaction volume of 50 μL, and the reaction was continued to incubate at 65 °C for another 5 minutes, followed by 60 °C for 5 minutes and then hold at 4 °C. Ethanol precipitation (add water to 100 μL, add 10 μL of 3 M sodium acetate pH 5.2 and 330 μL of 100% ethanol, freeze, and centrifuge for 30 min at 21,000 x g at 4 °C.) was performed after the reaction. The pellet was resuspended with 80 μL of water and the mixture was stored at -80 °C. To confirm strand displacement result, 8 μL of the strand displacement solution was added with 1 μL exonuclease I (Exo I) buffer, and 1 μL of 20 U/μl Exo I, and then Incubate for 30 min at 37°C and reserve on ice for PAGE analysis. Both strand displacement samples before and after exonuclease digestion should be analyzed by PAGE (10% TBE gel) to confirm the success of strand displacement.

PCR amplification of the dsDNA was performed by combining the 1 μL strand displacement product with 2.5 μL of 10 μM **H-FP-Bio**, 2.5 μL of 10 μM **H-RP**, 10 μL of 5× Q5 reaction buffer, 1 μL of 10 mM DNA dNTPs mix, 1 μL of 2 U/μL Q5 High-Fidelity DNA Polymerase, and 32 μL of water. PCR conditions were as follow: 98 °C for 30 second; 12 cycles of 98 °C for 10 second, 60 °C for 25 second, 72 °C for 26 second; 72 °C for 2 minutes, and hold at 4 °C. To remove excess primers and denature the enzymes, 1 μL of 20 U/μl exonuclease I (Exo I) was directly added to the reactions and incubate in a thermal cycler for 30 minutes at 37°C and then 20 minutes at 80°C. The four reaction mixtures were combined. Ethanol precipitation (add water to 100 μL, add 10 μL of 3 M sodium acetate pH 5.2 and 330 μL of 100% ethanol, freeze,

and centrifuge for 30 min at 21,000 x g at 4 °C.) was again performed. The pellet of PCR product was resuspended into 180 µL 1X B&W buffer and ready for the next step.

To remove unwanted biotinylated strand, the PCR product solution was first added to 150 µL of MyOne C1 streptavidin beads (supernatant removed and prewashed three times) and mixed on a rotator for 30 minutes at room temperature. The beads were then capture on magnet and the supernatant was removed. The beads were washed three times with 150 µL 1X B&W before adding 40 µL of 100 mM NaOH and sit for 4 minutes to elute the non-biotinylated ssDNA. The supernatant containing non-biotinylated ssDNA was then transferred to another tube containing 4 µL of 1 M HCl and 1 µL of 1 M Tris-Cl, pH 8.0 for neutralization. The ssDNA was then quantified by NanoDrop Spectrophotometer and stored at -20 °C.

Finally, to regenerate the hairpin sequence to give $(N+1)^{th}$-generation, the 45 µL of ssDNA product was added with 10 µL of 10× NEBuffer 2, 6 µL of 10 µM **Hairpin Regenerating Primer**, and 35 µL of water. The ssDNA-primer was annealed in thermal cycler with an annealing ramp of 95°C to 45°C at a rate of 0.1 °C/sec. Then, 2 µL of 10 mM DNA dNTPs mix and 2 µL of 5 U/µl DNA polymerase I (Klenow) was added and the reaction mixture was incubated for 15 min at 25°C before directly adding 1.5 µL of 20 U/µl Exo I and further incubated for 30 minutes at 37°C and then 20 minutes at 80°C. Ethanol precipitation (30 µL of 3 M sodium acetate pH 5.2 and 900 µL of 100% ethanol, freeze, and centrifuge for 30 min at 21,000 x g at 4 °C.) was again performed. The pellet of dsDNA regeneration product was

resuspended into 180 µL 1X B&W buffer. Bead separation was again practiced with exact same procedure described in the previous step to remove unwanted biotinylated strand. The ssDNA hairpin of $(N+1)^{th}$-generation was quantified by NanoDrop Spectrophotometer and stored at -20 °C.

A second-generation ssDNA hairpin self-extension reaction was performed as described above and an aliquot of the extended hairpin DNA-cmFANA hybrid product was analyzed by PAGE (10% TBE gel) to confirm the success of the DNA-display cycle.

Mock selection experiment using the DNA-display cycle

The mock selection experiment started with the preparation of a positive control using single sequence ssDNA template **TConA-Hairpin** for cmFANA synthesis by ssDNA hairpin self-extension reaction following the procedure mentioned previously. After click conjugation of hairpin DNA-cmFANA hybrid with Azide-PEG3-biotin conjugate followed by ethanol precipitation and resuspension with water (procedure from DNA-display cycle). The concentration of the positive control was determined by Nanodrop and adjust by water to 1/50 of the concentration of Man-N3 conjugated hairpin library DNA-cmFANA hybrid prepared following procedure from DNA-display cycle. Then, 1 µL of the positive control was added to 20 µL of Man-cmFANA-DNA hybrid library to make the ratio of library:control = 1000:1. Strand displacement of the mixture was performed following the procedure from DNA-display

cycle. After ethanol precipitation and resuspension with 80 µL of water, the mixture was added with 80 µL 2X B&W buffer. Selection to recover positive control was performed by incubating the strand displacement product (160 µL 1X B&W buffer.) with 60 µL of MyOne C1 streptavidin beads (supernatant removed and prewashed three times) and mixed on a rotator for 30 minutes at room temperature. The beads were then capture on magnet and the supernatant was removed. The beads were washed six times with 160 µL 1X B&W before transferred to a PCR tube and added with 10 µL of Exo I buffer, 88 µL water, and 2 µL of 20 U/µl Exo I and incubated for 30 minutes at 37°C. The beads were then capture on magnet and the supernatant was collected. To amplify the selected sequences, 1 µL selection product was added with 2.5 µL of 10 µM **H-FP-Bio**, 2.5 µL of 10 µM **H-RP**, 10 µL of 5× Q5 reaction buffer, 1 µL of 10 mM DNA dNTPs mix, 1 µL of 2 U/µL Q5 High-Fidelity DNA Polymerase, and 32 µL of water. A total of three 50 µL PCR mixtures were prepared. PCR amplification was performed following the procedure from DNA-display cycle, following ethanol precipitation and resuspension with 25 µL water. Digestion by restriction enzymes were performed as follow:

1. 5 µL of PCR product, 1 µL of 10X NEBuffer, 3 µL water, and 1 µL BsrI, incubate at 65°C for 20 min;
2. 5 µL of PCR product, 1 µL of 10X rCutSmart Buffer, 3 µL water, and 1 µL SmLI, incubate at 55°C for 50 min.

Finally, the digest results were analyzed by PAGE (10% TBE gel) to confirm successful

enrichment of positive control.

Next-Generation Sequencing

The next-generation sequencing of the library was performed according to the Amplicon-EZ workflow provided by Genewiz. Briefly, dsDNA hairpin regeneration products (134 bp, **Figure 6**) of from the first or second cycle of DNA-display were further amplified by PCR with extended forward (**Adapter-FP**) and reverse primers (**Adapter-RP**) to add extra 16bp to the dsDNA amplicon sample to install the adapter and a spacer such that the length of the PCR product is 150 bp (**dsDNA amplicon sample**) required for Genewiz Amplicon-EZ sequencing. The sequencing results were analyzed by a MATLAB code (available upon request to the authors) that provided the total number of reads, read length, number of unique sequences, the most repeated sequence, and read number of the most repeated sequence. The results are as follows.

For the library before DNA display, there are 165333 unique reads among 191753 total reads, unique sequence ratio = 86%.

For the library before DNA display, there are 215202 unique reads among 249597 total reads, unique sequence ratio = 86%.

For the library after mock selection, there are 36074 unique reads among 176991 total reads, unique sequence ratio = 20%. The most repeated sequence has 97927 reads to itself, with the

sequence being:

GATAGCATCCTATAGCCGTTTGCACAAGTTCTTCTGCCACTCAAGCTCACCAGTCG

TGCAGATGCAACCCCGTACCCGTTAAGATTACTTCGGACTGGGATCATTTTATATTT

TATATTTTTATTTTATATTCGGGTACGGGCAGGAGTC

This sequence is identical to the positive control template.

Table S1. DNA sequences used in this study

Name	**DNA sequence**
T1 (81 nt)	5' – ATC CAG AGT GAC GCA GCA CGG AAC GTC TTT GTA ACT TGA AAT ACC GTG GTA GGT TGG CTA GGT TGG ACA CGG TGG CTT AGT – 3'
T-lib-XL (100 nt, product 76 nt)	5' – ATC CAG AGT GAC GCA GCA (N1:25 25 25 25)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1) TGG ACA CGG TGG CTT AGT AAC AAC AAC AAC AAC AAC AAC AAC – 3'
T-FP (18 nt)	5' – ATC CAG AGT GAC GCA GCA – 3'
T-RP (18 nt)	5' – ACT AAG CCA CCG TGT CCA – 3'
Tcy5-RP (18 nt)	5' – /5Cy5/ACT AAG CCA CCG TGT CCA – 3'
Generation of single-stranded cmFANA conjugates	
T-ConA (80 nt)	5' – CC TAT AGC CGT TTG CAC AAG TTC TTC TGC CAC TCA AGC TCA CCA GTC GTG CAG ATG CAA CGA TTA CTT CGG ACT GGG ATC – 3'
T-ConA-XL (98 nt, product 80 nt)	5' – CC TAT AGC CGT TTG CAC AAG TTC TTC TGC CAC TCA AGC TCA CCA GTC GTG CAG ATG CAA CGA TTA CTT CGG ACT GGG ATC AAC AAC AAC AAC AAC AAC – 3'
T-ConA-SC (98 nt, product 80 nt)	5' – CC TAA AAC TAA AAC ACA ATC AAA GTG CCG TTT GCG TTC TTC TGC CGC TCC CGC GTG

	CTG CGA TTA CTT CGG ACT GGG ATC AAC AAC AAC AAC AAC AAC – 3’
T-ConA-RP-Bio (20 nt)	5’ – /5BiotinTEG/ GAT CCC AGT CCG AAG TAA TC – 3’
cmFANA1 80 nt, 14 **U*** sites)	5’ – /5BioTinTEG/ GAT CCC AGT CCG AAG TAA TCG **U*****U***G CA**U*** C**U***G CAC GAC **U***GG **U***GA GC**U*** **U***GA G**U***G GCA GAA GAA C**U*****U*** G**U***G CAA ACG GC**U*** A**U***A GG – 3’
cmFANA2 80 nt, 14 **U*** sites)	5’ – /5BioTinTEG/ GAT CCC AGT CCG AAG TAA TCG CAG CAC GCG GGA GCG GCA GAA GAA CGC AAA CGG CAC **U*** **U*** **U*** GA **U*** **U***G **U*** G **U*** **U*** **U*** **U***A G **U*** **U*** **U*** **U***A GG – 3’
Reverse transcription	
single-stranded FANA derivatives (cmFANA1) (80 nt, 14 **U*** sites)	5’ – /5BioTinTEG/ GAT CCC AGT CCG AAG TAA TCG **U*****U***G CA**U*** C**U***G CAC GAC **U***GG **U***GA GC**U*** **U***GA G**U***G GCA GAA GAA C**U*****U*** G**U***G CAA ACG GC**U*** A**U***A GG – 3’
TConA-FANA-RP (20 nt)	5’ – CC TAT AGC CGT TTG CAC AAG – 3’
TConA-FANA-cy5RP (20 nt)	5’ – /5Cy5/CC TAT AGC CGT TTG CAC AAG – 3’
DNA displacement assay and mock selection experiment	
TConA-Hairpin (134nt)	5’ – CC TAT AGC CGT TTG CAC AAG TTC TTC TGC CAC TCA AGC TCA CCA GTC GTG CAG ATG CAA C CC CGT ACC CG T TAA GA TTA CTT CGG ACT GGG ATC A TTT TAT ATT TTA TAT TTT TAT TTT ATA TT C GGG TAC GGG – 3’
Library 1 (N40) (94nt)	5’ –CC TAT AGC CGT TTG CAC AAG (N1:25 25 25 25)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1) CCC GTA CCC GTT AAG ATT ACT TCG GAC TGG GAT C – 3’
H-RP (20 nt)	5’ – GAT CCC AGT CCG AAG TAA TC – 3’
H-FP (20 nt)	5’ – CCT ATA GCC GTT TGC ACA AG – 3’
H-FP-Bio (20 nt)	5’ – /5BioTinTEG/ CCT ATA GCC GTT TGC ACA AG – 3’
Hairpin Regenerating Primer (60 nt)	5’ – /BioTEG/ CCC GTA CCC GAA TAT AAA ATA AAA ATA TAA AAT ATA AAA T GA TCC CAG TCC GAA

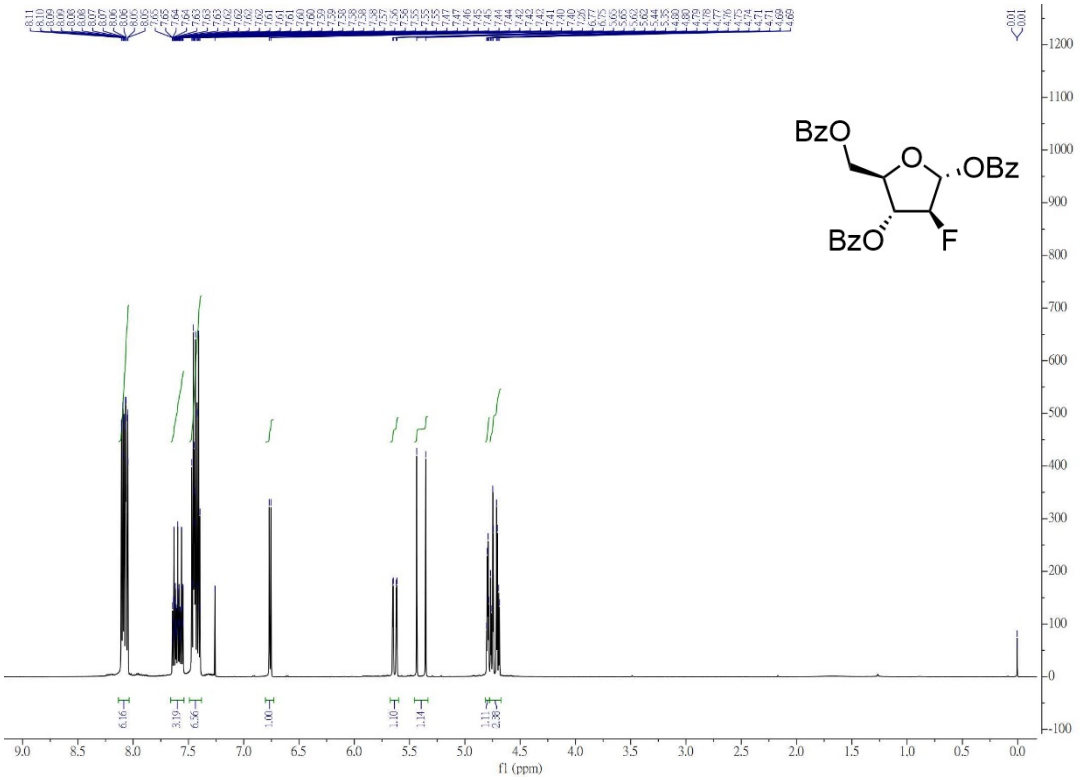
	GTA ATC – 3’
cmFANA nuclease stability assay	
ConA-aptamer-Cy5 (80 nt)	5’ – /5Cy5/ GAT CCC AGT CCG AAG TAA TCG UUG CAU CUG CAC GAC UGG UGA GCU UGA GUG GCA GAA GAA CUU GUG CAA ACG GCU AUA GG – 3’
Next-generation sequencing	
Atapter-FP (60 nt)	5’– ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT GAT AGC ATC CTA TAG CCG TTT GCA CAA – 3’
Atapter-RP (60 nt)	5’– GAC TGG AGT TCA GAC GTG TGC TCT TCC GAT CTG ACT CCT GCC CGT ACC CGA ATA TAA AAT– 3’
Hairpin Regeneration Product (134 bp)	5’ –CC TAT AGC CGT TTG CAC AAG (N1:25 25 25 25)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1)(N1)(N1) (N1) CC CGT ACC CG T TAA GA TTA CTT CGG ACT GGG ATC A TTT TAT ATT TTA TAT TTT T AT TTT ATA TT C GGG TAC GGG – 3’
dsDNA amplicon sample (150 bp)	5’ –GAT AGC ATC CTA TAG CCG TTT GCA CAA GNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNC CCG TAC CCG TTA AGA TTA CTT CGG ACT GGG ATC ATT TTA TAT TTT ATA TTT TTA TTT TAT ATT CGG GTA CGG GCA GGA GTC – 3’

U*: C8-alkyne-FANA dU.

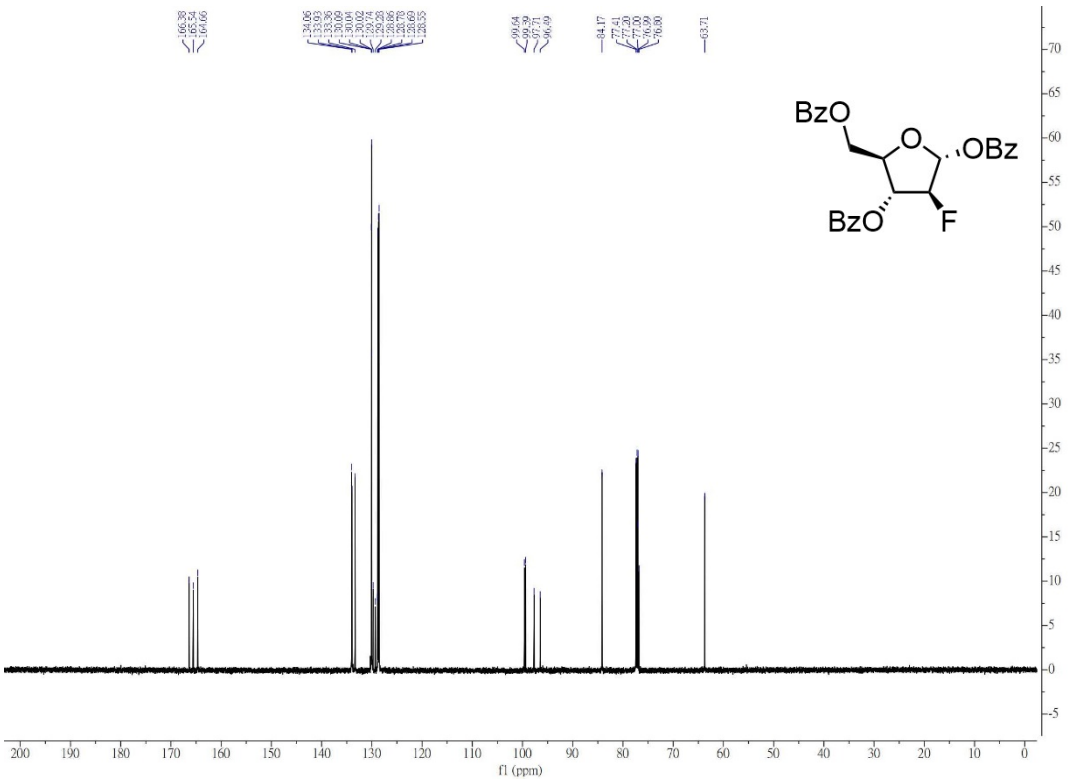
Reference

1. T. Tanaka, H. Ishitani, Y. Miura, K. Oishi, T. Takahashi, T. Suzuki, S.-i. Shoda and Y. Kimura, *ACS Macro Lett.*, 2014, **3**, 1074-1078.
2. A. Nikoomanzar, M. R. Dunn and J. C. Chaput, *Curr. Protoc. Nucleic Acid Chem.*, 2017, **69**, 4.75.1–4.75.20.
3. J. S. Temme and I. J. Krauss, *Curr. Protoc. Chem. Biol.*, 2015, **7**, 73-92.

^{1}H NMR of 1 (600 MHz, $CDCl_3$)



^{13}C NMR of 1 (600 MHz, $CDCl_3$)



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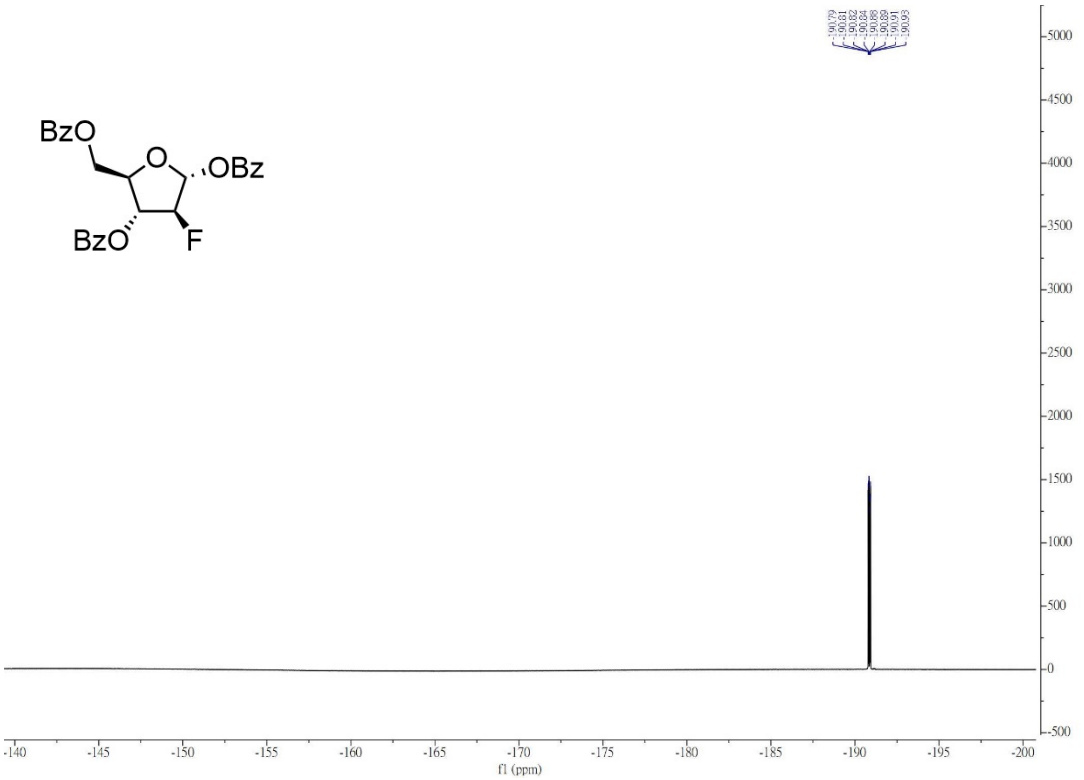

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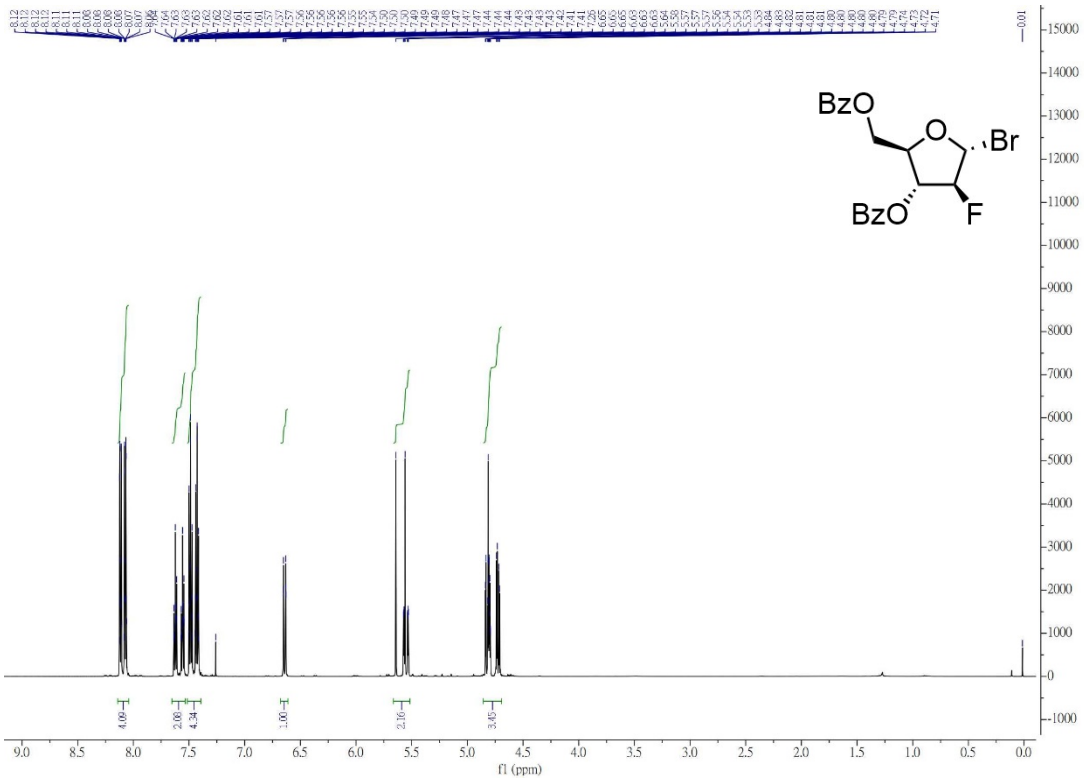
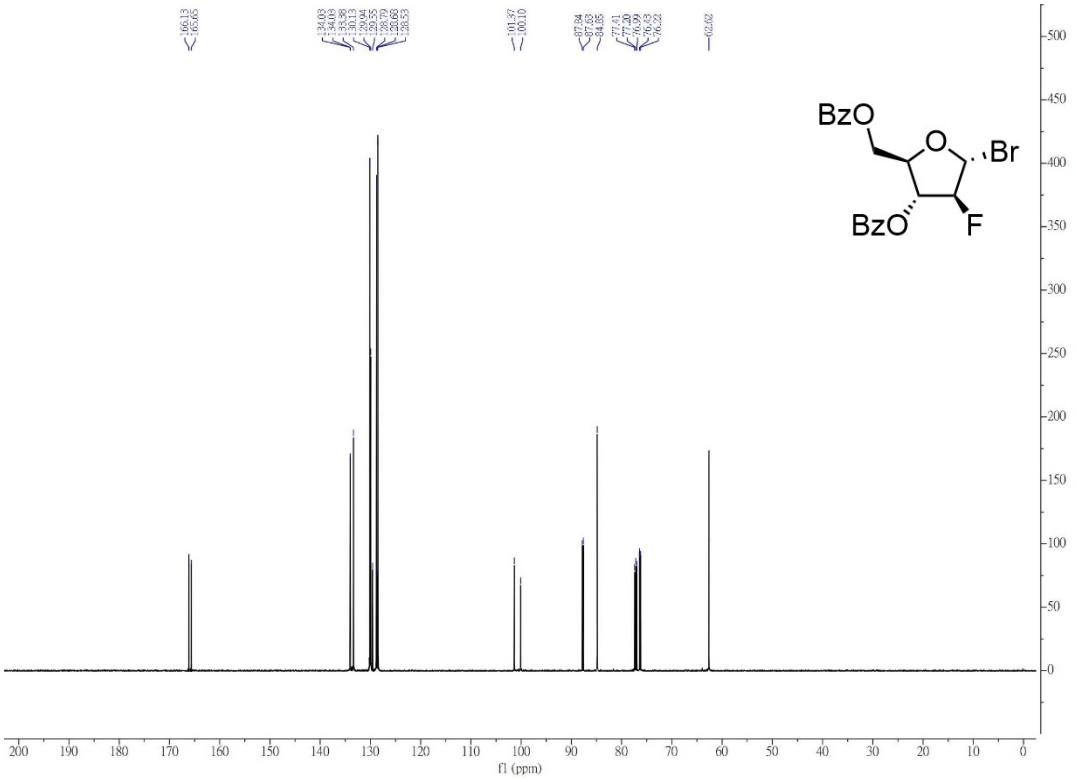


^{13}C NMR of 2 (600 MHz, DMSO-d)



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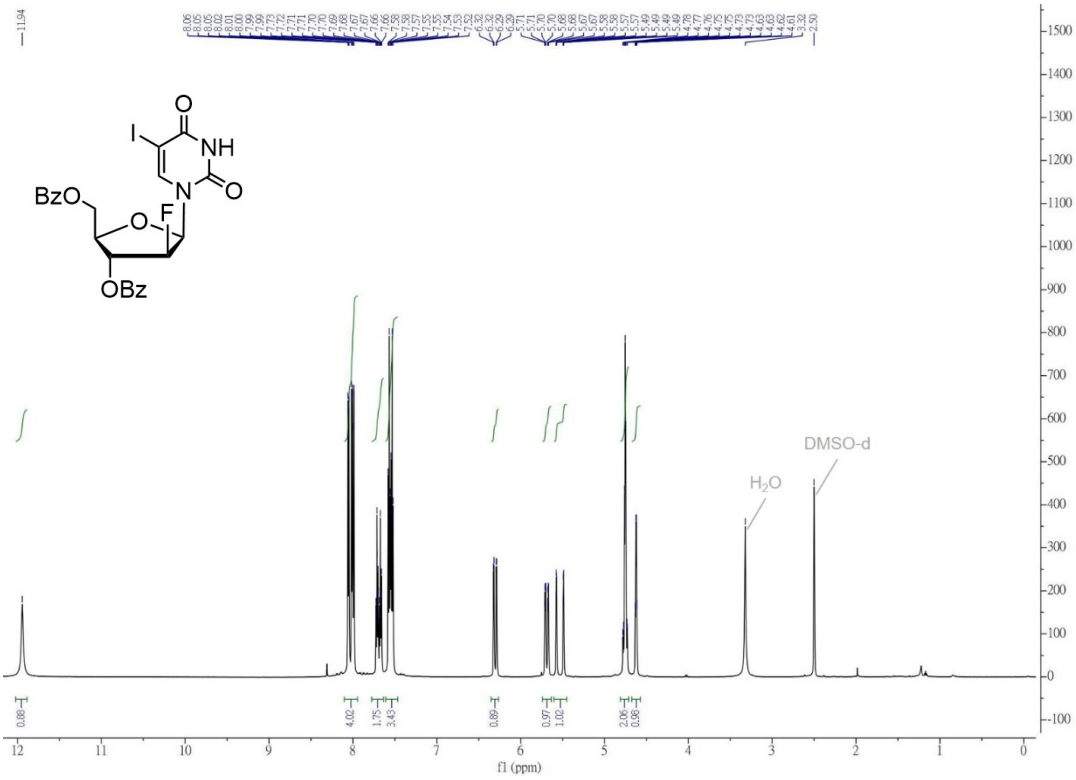

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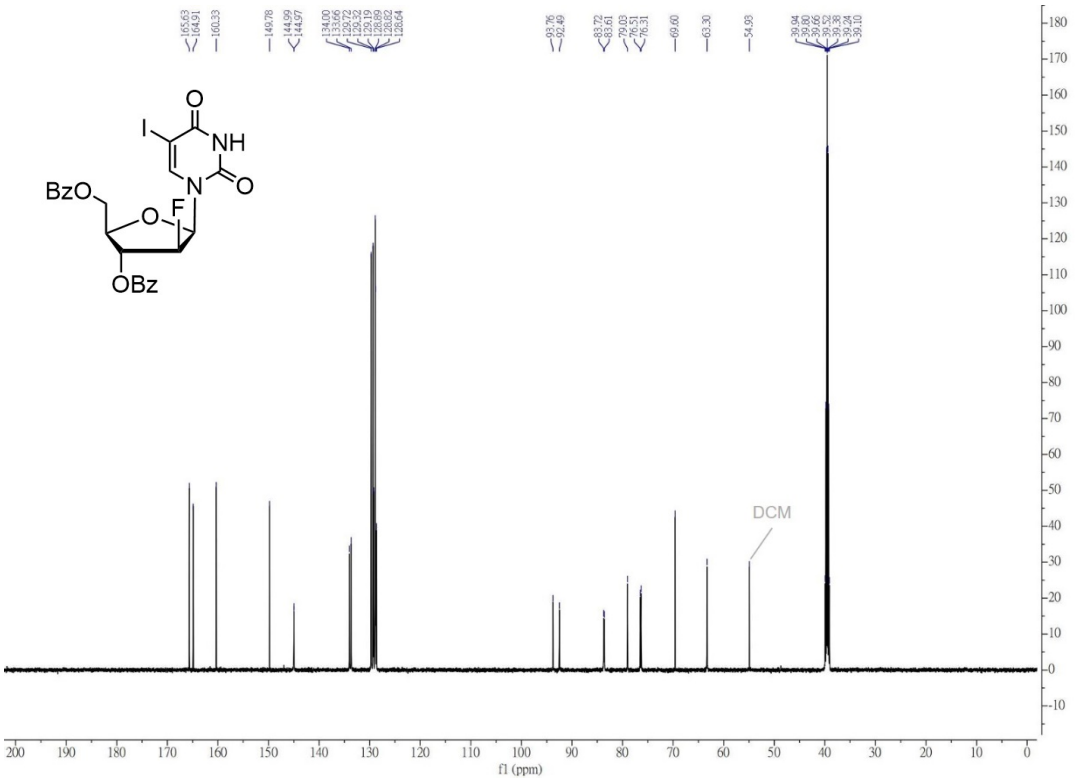

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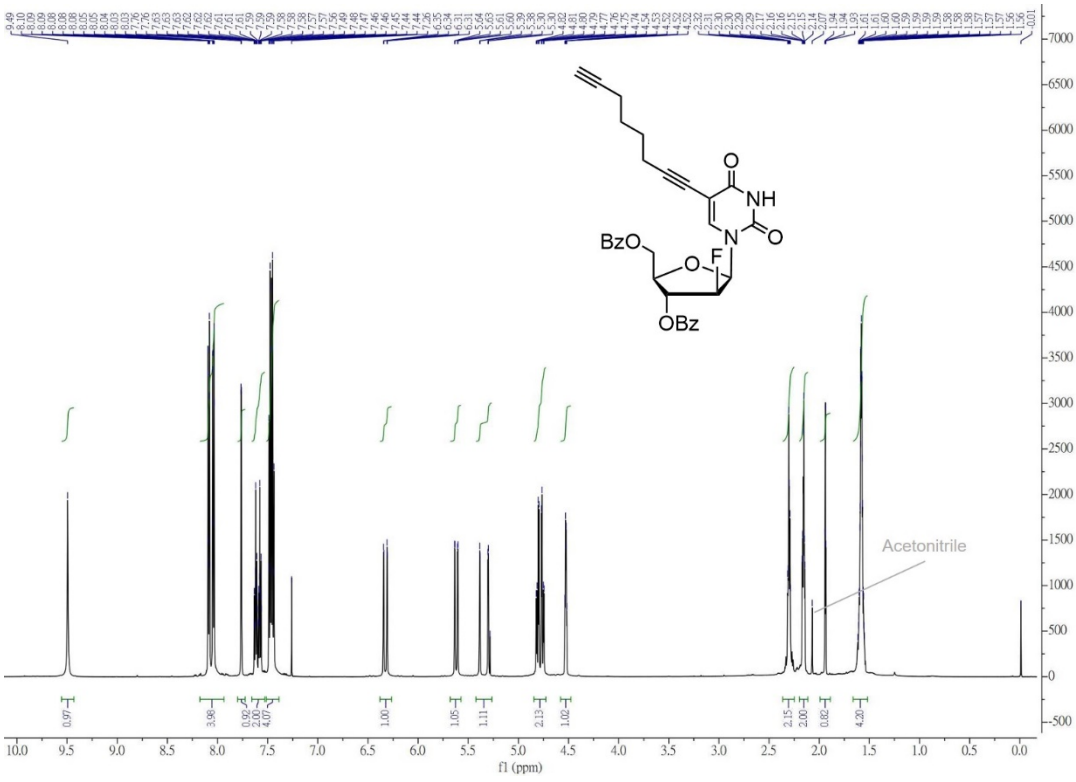

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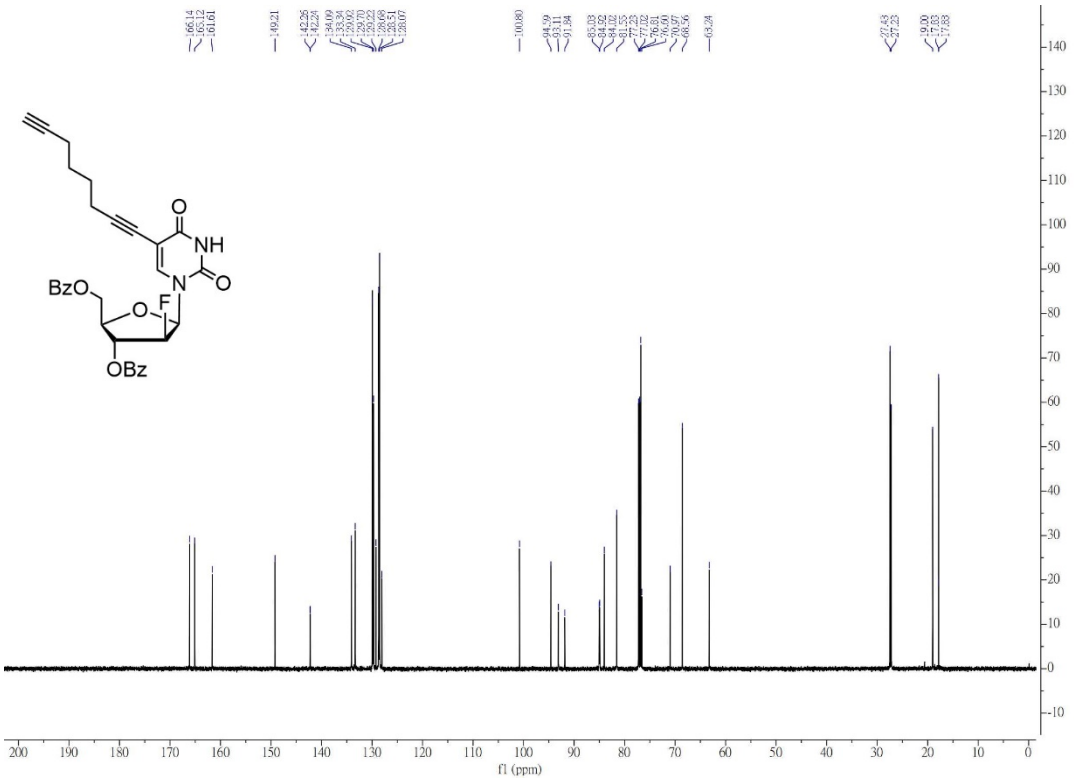

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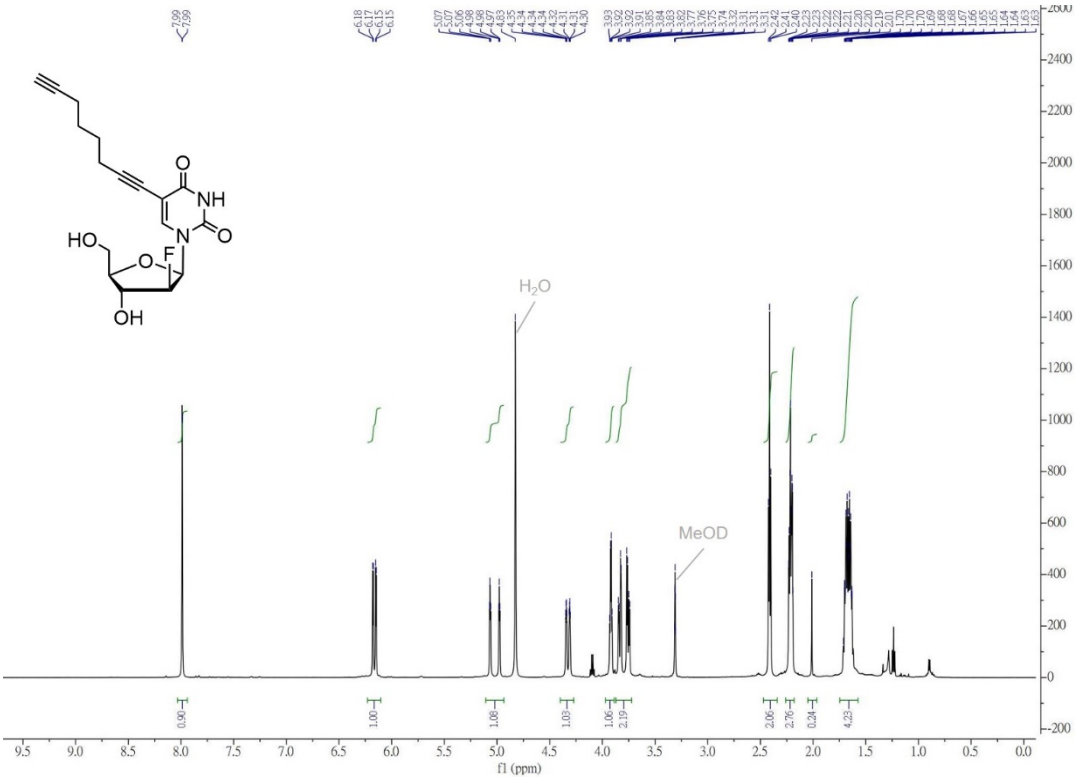

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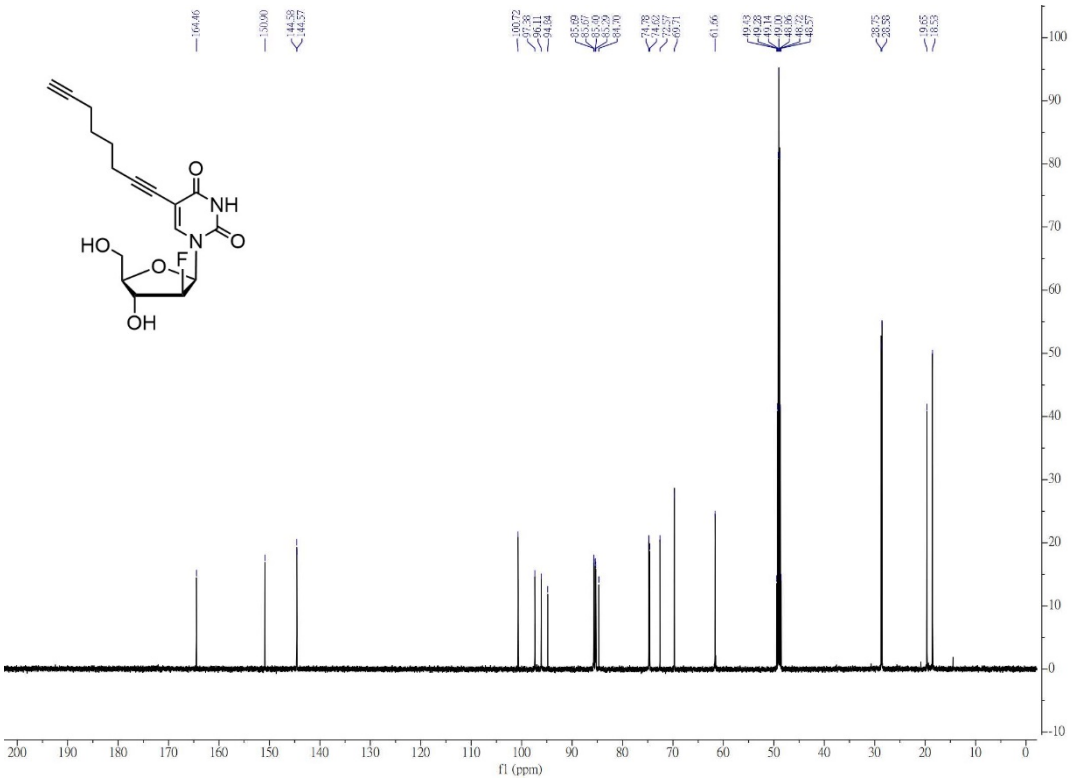
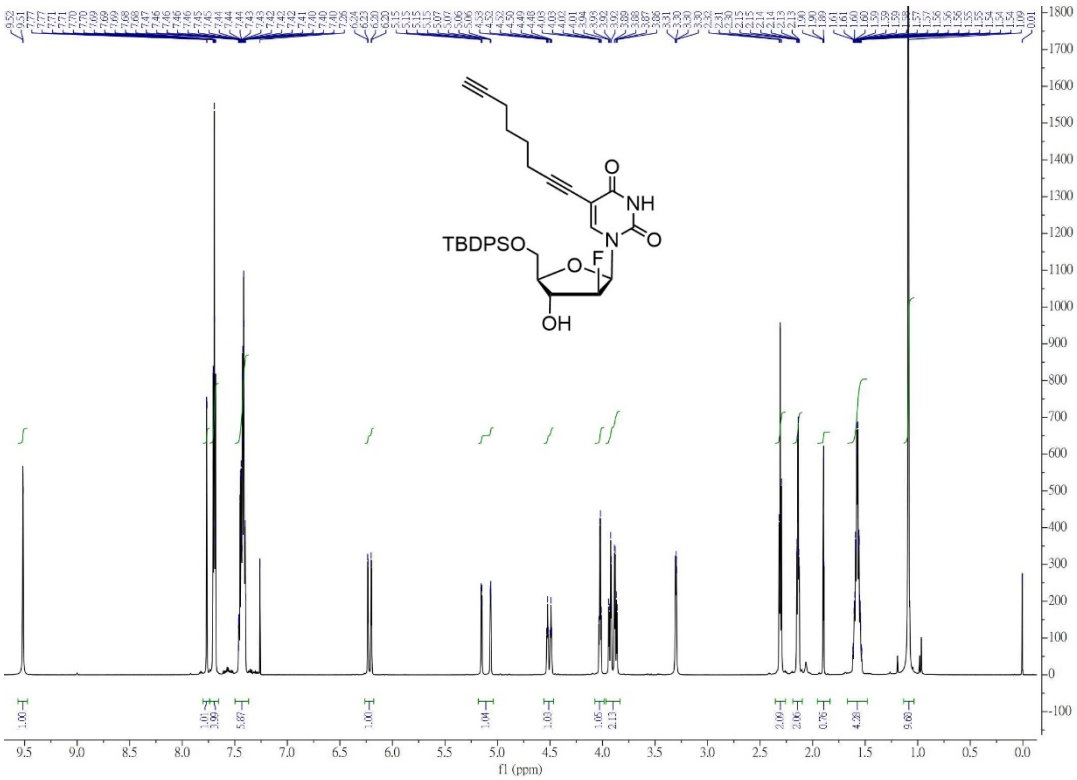
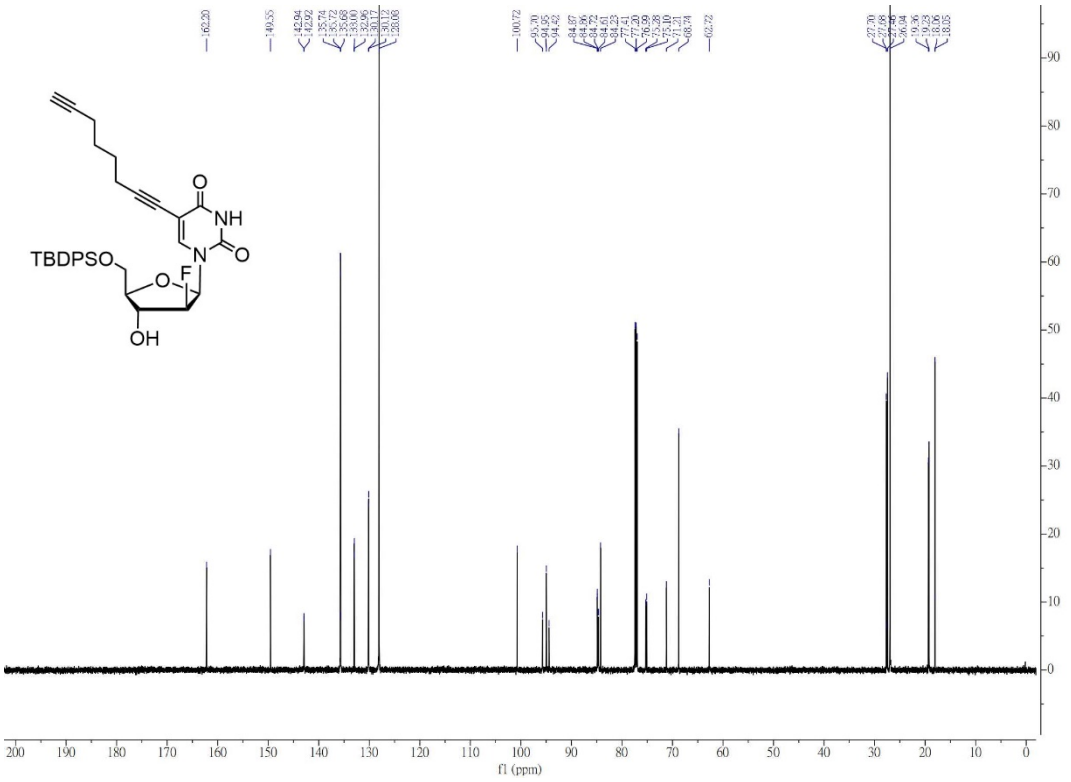


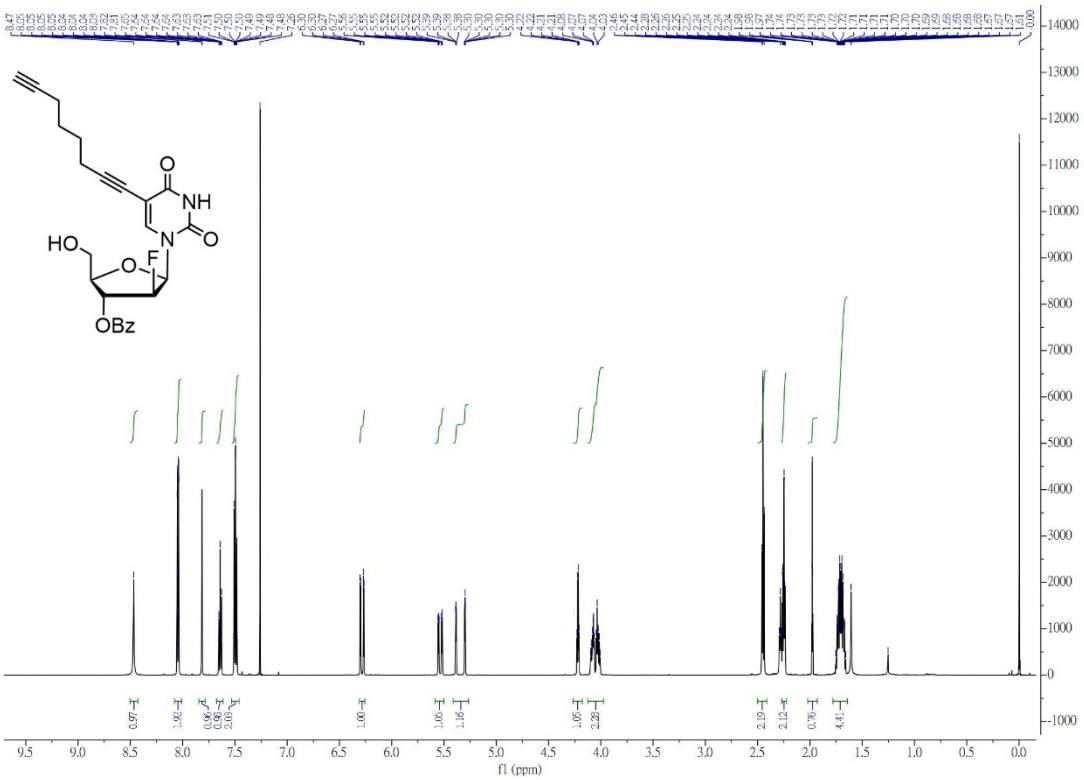
^{1}H NMR of 7 (600 MHz, $CDCl_3$)



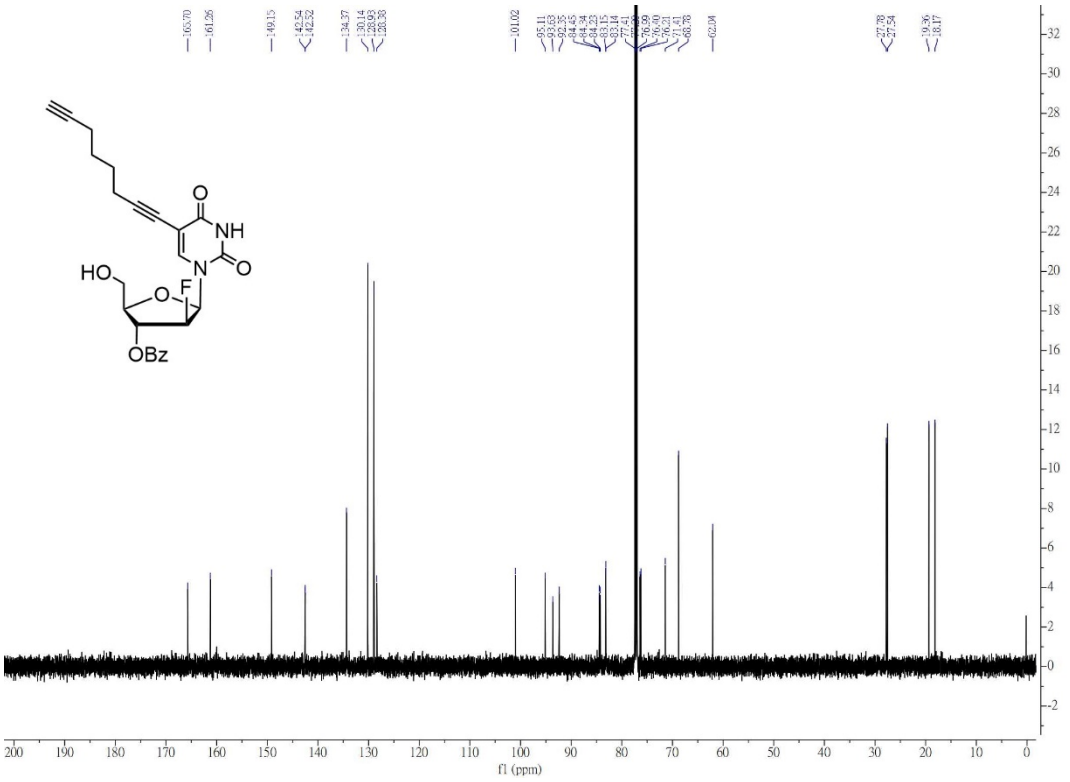
^{13}C NMR of 7 (600 MHz, $CDCl_3$)



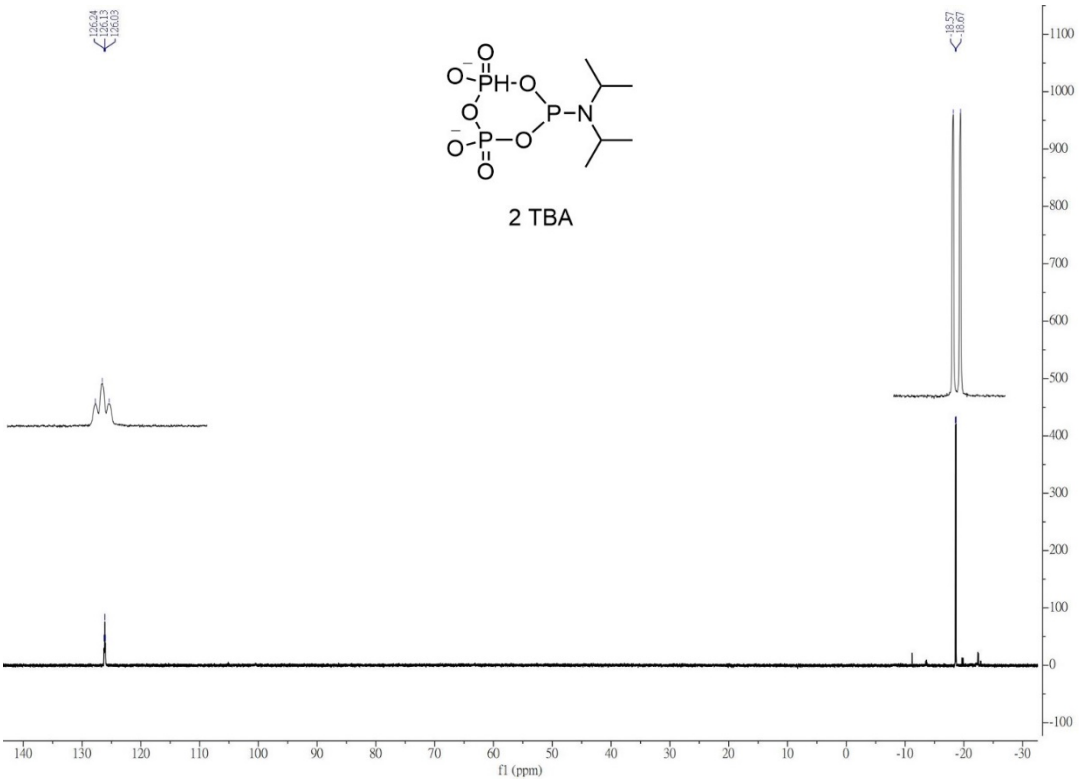
^{1}H NMR of 8 (600 MHz, $CDCl_3$)



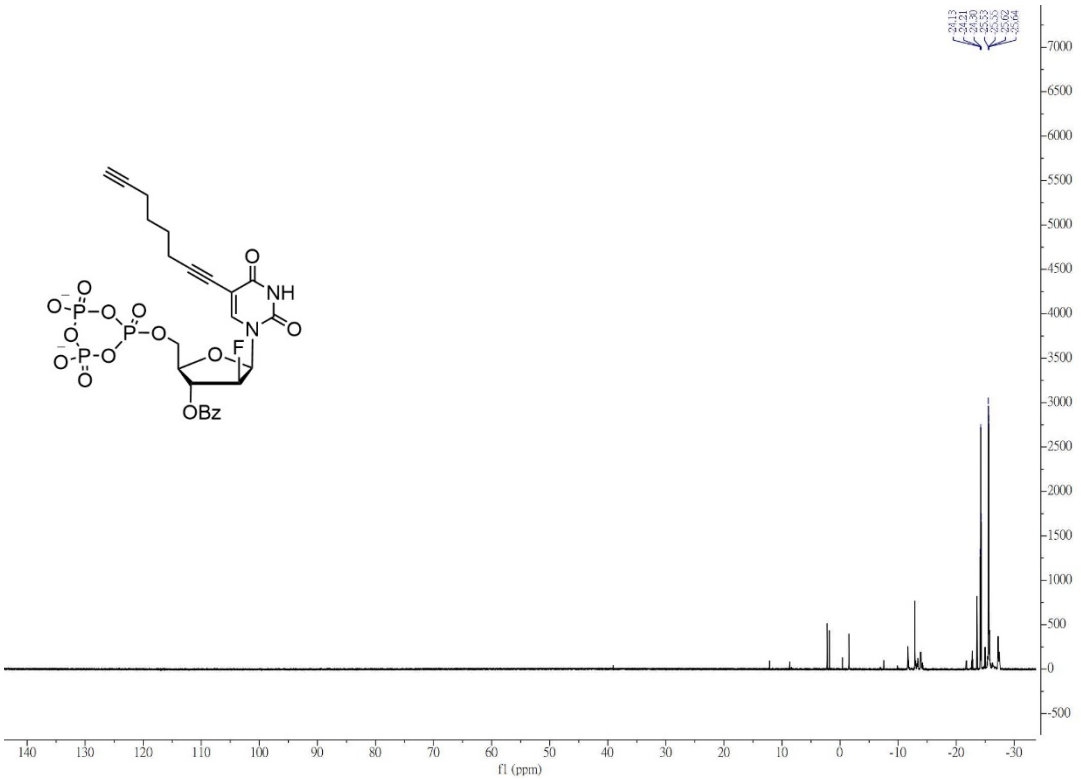
^{13}C NMR of 8 (600 MHz, $CDCl_3$)



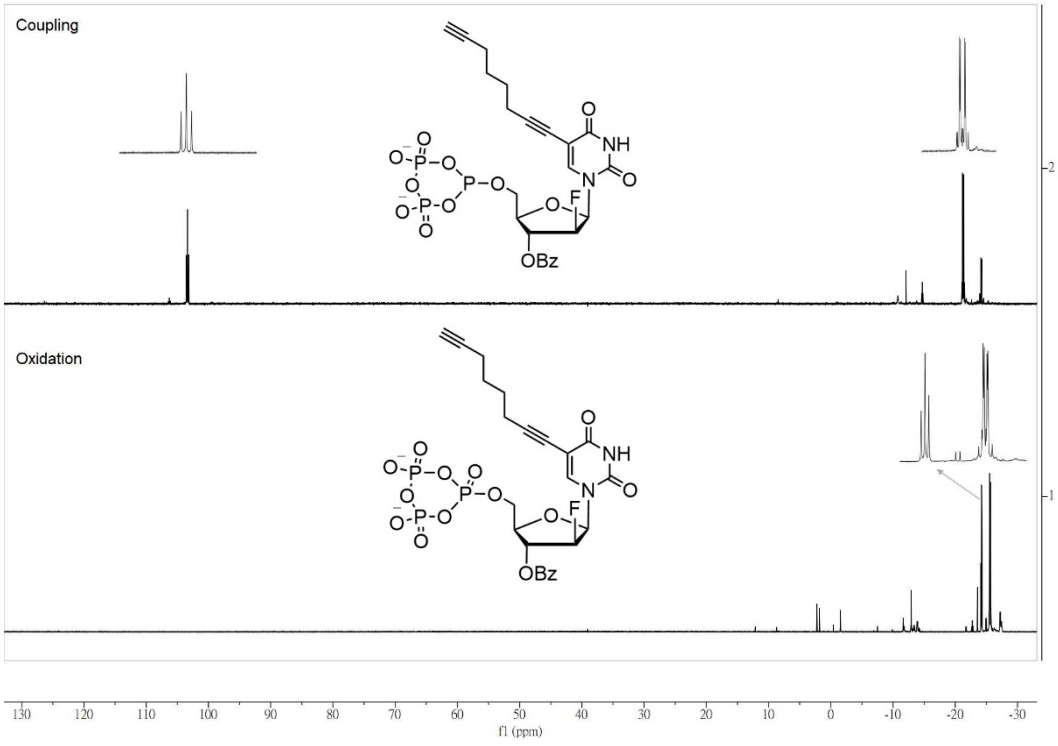
^{31}P NMR of 9 (600 MHz, CD_3CN)



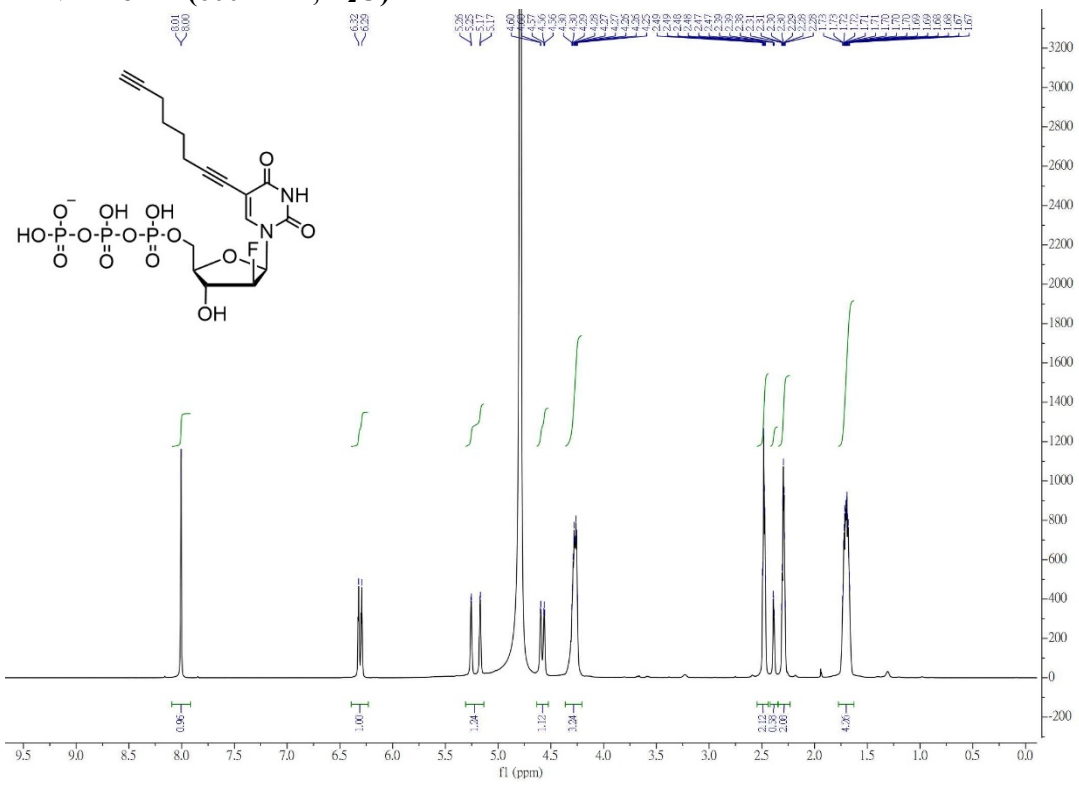
^{31}P NMR of 10 (600 MHz, $CDCl_3$)



^{31}P NMR of 10, coupling and oxidation (600 MHz, $CDCl_3$)



^{1}H NMR of 11 (600 MHz, D_2O)



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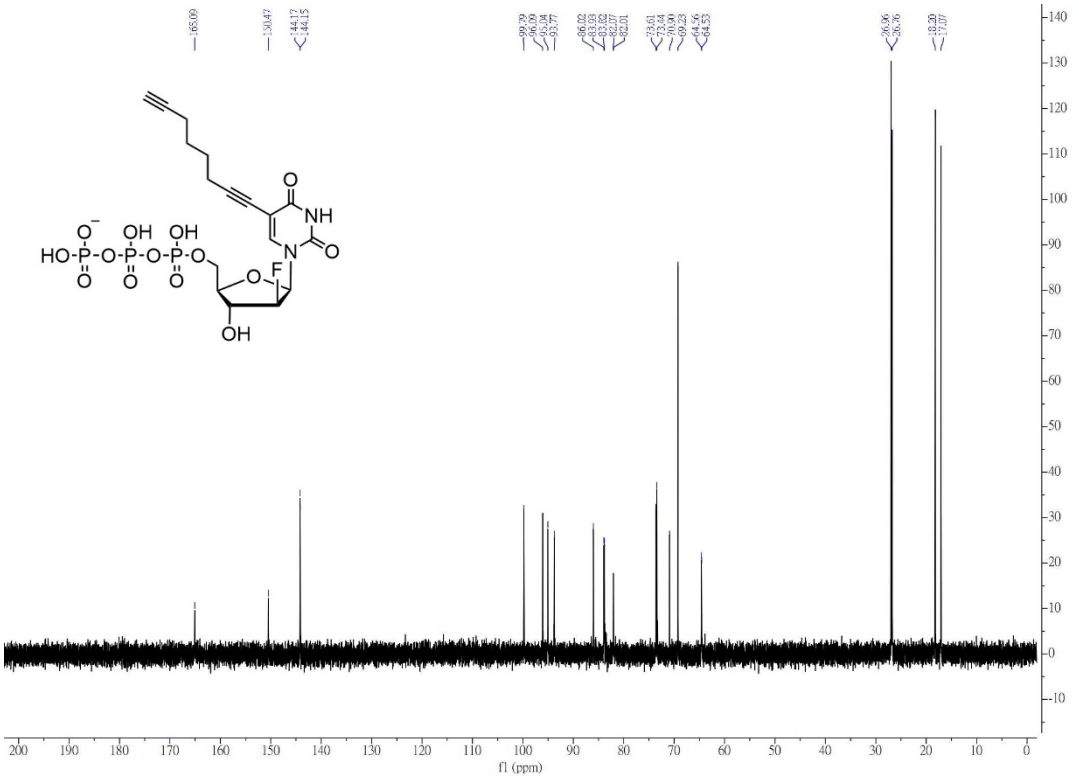

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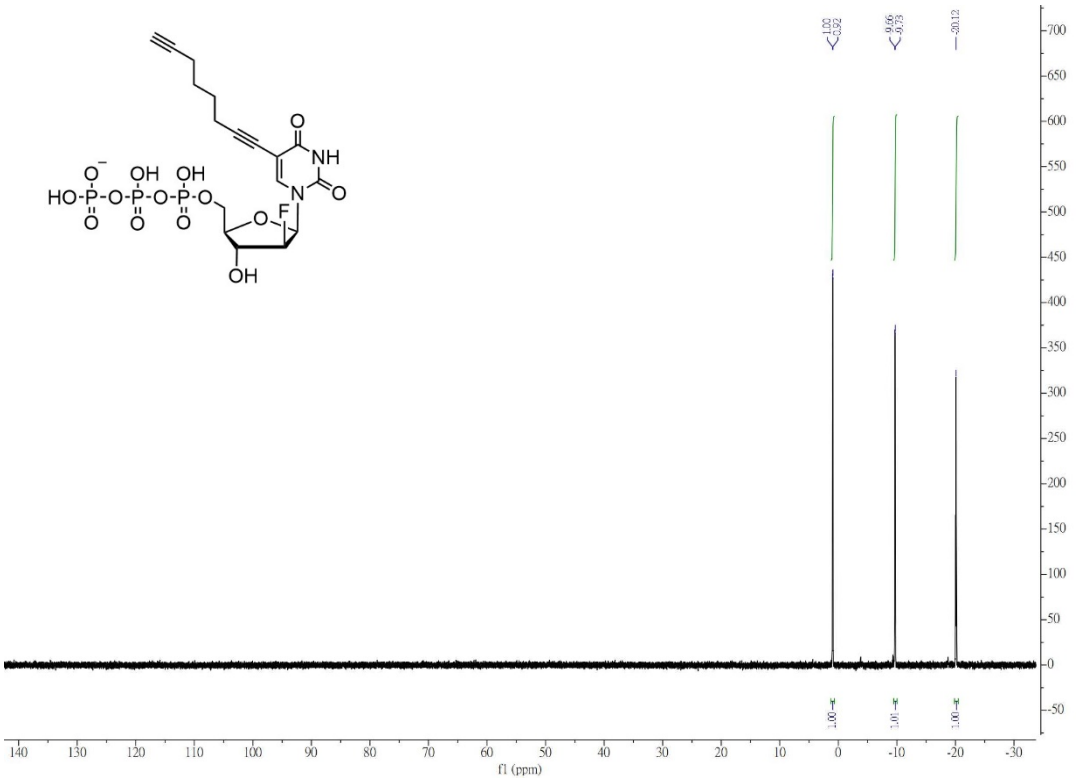

^{19}F NMR of 11 (600 MHz, D_2O)