Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Naphthalimide derivatives as multifunctional molecules for detecting 5-formylpyrimidine by both PAGE analysis and dot-blot assays

Yafen Wang[a, ‡], Chaoxing Liu[a, ‡], Wei Yang[a, ‡], Guangrong Zou[a], Xiong Zhang[a], Fan Wu[a], Shuyi Yu[a], Xiaomeng Luo[a], and Xiang Zhou[a, *]

[a] College of Chemistry and Molecular Sciences, Key Laboratory of Biomedical Polymers of Ministry of Education, The Institute for Advanced Studies, Hubei Province Key Laboratory of Allergy and Immunology, Wuhan University, Wuhan, Hubei, 430072, P. R. China.

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1. General methods and materials

All chemical reagents were purchased from Adamas-beta® (Shanghai, China) and Shanghai Shaoyuan Co. Ltd. (Shanghai, China) unless mentioned otherwise. Dibenzocyclooctyne-PEG4-biotin conjugate (cas: 1255942-07-4) was bought from Sigma-Aldrich. All the canonical oligonucleotides were synthesized and purified by GeneCreate Co., Ltd. (Wuhan, China). ODNs bearing 5fU was synthesized and purified according to our precious report.[1] ODNs containing 5fC moieties was bought from Takara Biotechnology (Dalian, China). DNA MALDI-TOF Mass Spectra were collected on MALDI-TOF-MS (Shimadzu, Japan) or Autoflex III MALDI-TOF L200 (Bruker Daltonics, Germany). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance 400 NMR or 300 NMR spectrometer, respectively. HRMS was acquired with Thermo Scientific™ Dionex Ultimate 3000 hybrid LTQ Orbitrap Elite Velos Pro (Thermo Scientific, USA). HPLC data was recorded on LC-6AD (Shimadzu, Japan) which equipped with an Inertsil ODS-SP column (5 μm, 250×4.6 mm) (GL Science Inc. Japan) with mobile phase A (100 mM TEAA buffer, pH=7.0) and B (CH_3CN) with a flow rate of 1 mL/min at 35°C (B conc.: 5-5-30% / 0-5-30 min) as described before.[2] Degradase Plus and enzyme reaction buffer were purchased from Zymo Research (Zymo Research, USA). LC-MS data were collected with the Agilent™ 1220 Infinity LC combined with the 6120 Single Quadrupole mass spectrometer (Agilent Technologies). Fluorescent emission spectra were acquired with PerkinElmer LS 55 (PerkinElmer, USA). Gel Imaging was monitored with Pharos FX Molecular imager (Bio-Rad, USA). UV absorption spectra were recorded on UV-2550 (Shimadzu, Japan). The nucleic acid stains YeaRed Nucleic Acid Gel Stain (NO.: 10202ES76) was purchased from YEASEN Biotechnology Co. Ltd., (Shanghai, China). And the Super GelRed (NO.: S-2001) was bought from US Everbright Inc. (Suzhou, China).

ODN reaction protocols. Protocol 1 (selective labelling 5fU): ODNs (100 μM, 1 μL), NAAH (10 mM in DMSO, 1 μL), PS buffer (1 M, pH=7.0, 10 μL) and 88 μL ddH_2O were added together into 1.5 mL microcentrifuge tube at 37°C for 90 minutes in a thermomixer (Eppendorf, 1500 r.p.m.), respectively. Protocol 2 (selective labelling 5fC): ODNs (100 μM, 1 μL), 4-Nitro-o-phenylenediamine (50 mM in DMSO, 1 μL), NaOAc buffer (1 M, pH=5.0, 10 μL) and 88 μL ddH_2O were added together into 1.5 mL microcentrifuge tube at 37°C for 8 hours, after that, NAAH (50 mM in DMSO, 1 μL) was added for another 4 hours' incubation in a thermomixer (Eppendorf, 1500 r.p.m.).

Enzymatic digest of ODNs protocol. DNAs, Degradase Plus (1 μL) and 10×Degradase Plus reaction buffer (2.5 μL) (Zymo Research) were mixed in a final volume of 25 μL at 37°C for 2 hours. The digested mixture was filtered by an ultrafiltration tube (3 kDa cutoff, Amicon, Millipore) to remove the enzymes to yield the corresponding nucleosides for further LC-MS detection.

Denaturing PAGE analysis. ODNs were in 10 μL 80% deionized formamide. A 20% denaturing PAGE was prepared by using 1xTBE buffer (89 mM Boric acid, 89 mM Tris base, 2 mM EDTA) containing 7.0 M urea. The denaturing PAGE was carried out in 1xTBE buffer at a constant voltage of 150 V for about 2-3 h at room temperature. We scanned the final PAGE products with Pharos FX Molecular imager operated in the fluorescence mode (λ_{ex}=488 nm). Then the gel was stained with nucleic acid stains to get all DNA bands (λ_{ex}=532 nm).

Dot-blot assay of avidin-HRP detection ODN-5fU and ODN-5fC. ODNs were treated using above protocol 2. Then after removing chemicals, they were spotted on an Amersham Hybond-N+ membrane (GE Healthcare). After dry, the DNAs were fixed to the membrance by photo-linking with UV light (254 nm, 5 min twice) and then wash with 1×TBST twice. The membrance was block with 5% BSA (Biosharp, China) at 37℃ for 1h followed by wash with 1×TBST five times. After that, the membrance was incubated with avidin-HRP (1:2000) (Thermo Scientific) at 37℃ for 1h then washed with 1×TBST four times. Finally, the results were visualized by enhanced chemiluminescence (SuperSignal™ West Pico Chemiluminescent Substrate, Cat: 34077, Thermo Scientific) using Molecular Imager® ChemiDocTM XRS+ Imaging System (Bio-Rad).

2. Synthesis

2-(3-azidopropyl)-6-bromo-1H-benzo[de]isoquinoline-1,3(2H)-dione (1)

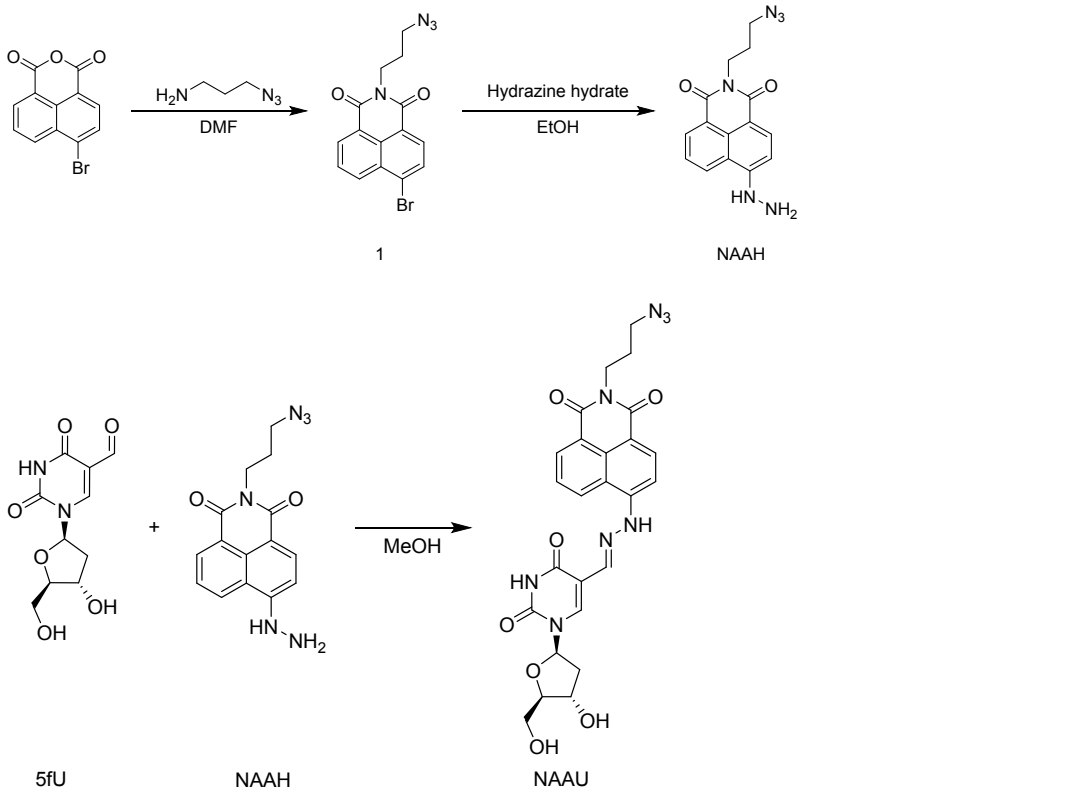
4-bromo-1,8-naphthalic anhydride (2.77 g, 10 mmol) was added into DMF (100 mL) and stirred at 40°C until completely dissolved. Then 3-azidopropan-1-amine (1 g, 10 mmol) was added dropwise while the reaction mixture was kept stirring and stayed at 60°C. After 1 h, the precipitate was filtered off and collected through prewashed with cold methanol and dried under vacuum to yield 3.22 g (90% yield) as a pale solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (dd, *J* = 10.7, 7.9 Hz, 2H), 8.33 (d, *J* = 7.9 Hz, 1H), 8.22 (d, *J* = 7.9 Hz, 1H), 8.03 – 7.96 (m, 1H), 4.11 (t, *J* = 6.9 Hz, 2H), 2.64 (t, *J* = 6.8 Hz, 2H), 1.90 (p, *J* = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.44, 163.39, 132.99, 131.95, 131.74, 131.33, 130.15, 129.53, 129.19, 128.72, 123.22, 122.44, 49.18, 37.97, 27.39. HRMS (ESI+) $C_{15}H_{12}BrN_4O_2^+$ $[M+H]^+$ calculated 359.01381, found 359.01463.

2-(3-azidopropyl)-6-hydrazinyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (NAAH)

1 (358 mg, 1 mmol) was dispersed int0 100 mL ethanol in a round bottom flask with three necks under Ar atmosphere. Then 1 mL hydrazine hydrate was added dropwise while the reaction mixture was kept stirring and stayed at reflux temperature. After 5 h, the precipitate was filtered off and collected through washed with cold trichloromethane and dried under vacuum to yield 248 mg (80% yield) as a deep red solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.08 (s, 1H), 8.56 (d, *J*=8.0, 1H), 8.37 (d, *J*=6.9, 1H), 8.25 (d, *J*=8.4, 1H), 7.59 (t, *J*=7.5, 1H), 7.21 (d, *J*=8.5, 1H), 4.66 (s, 2H), 4.07 (s, 2H), 3.44 – 3.40 (m, 2H), 1.95 – 1.77 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.33, 163.42, 153.66, 134.67, 131.01, 129.77, 128.69, 124.50, 122.12, 118.83, 107.72, 104.39, 49.32, 37.40, 27.68. HRMS (ESI+) $C_{15}H_{15}N_6O_2^+$ $[M+H]^+$ calculated 311.12510, found 311.12504.

5-formyl-2'-deoxyuridine-NAAH adduct (NAAU)

5-formyl-2'-deoxyuridine[3] (256 mg, 1 mmol, 1 eq) and NAAH (310mg, 1 mmol, 1 eq) were dissolved into 50 mL methanol at a 100 mL round bottom flask. The reaction mixture was kept stirring at 50°C for 1 h, the precipitate was filtered off and collected through prewashed with cold methanol and dried under vacuum to yield 526 mg (96% yield) as a red solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.67 (s, 1H), 11.41 (s, 1H), 8.76 (d, *J*=8.5, 1H), 8.71 (s, 1H), 8.47 (d, *J*=7.1, 1H), 8.39 (d, *J*=7.0, 2H), 7.74 (dd, *J*=19.8, 8.2, 2H), 6.25 (t, *J*=6.3, 1H), 5.32 (d, *J*=3.5, 1H), 5.26 (s, 1H), 4.35 (s, 1H), 4.11 (t, *J*=6.1, 2H), 3.91 (s, 1H), 3.73 (s, 2H), 3.44 (t, *J*=6.3, 2H), 2.24 (s, 2H), 1.94 – 1.83 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.13, 163.43, 162.36, 150.11, 146.73, 137.23, 137.07, 134.07, 131.02, 129.50, 128.52, 125.13, 122.27, 118.77, 110.89, 108.96, 107.19, 88.35, 85.55, 70.83, 61.63, 49.25, 40.93, 37.45, 27.55. HRMS (ESI+) $C_{25}H_{25}N_8O_7^+$ $[M+H]^+$ calculated 549.18407, found 549.18524.



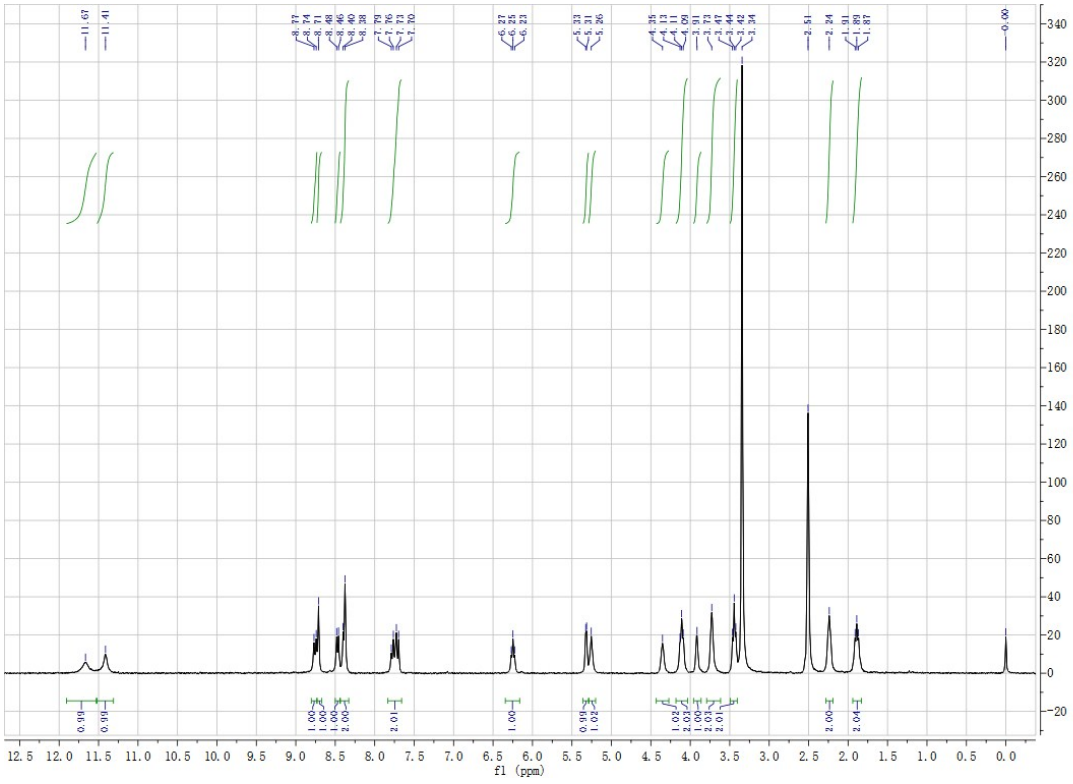
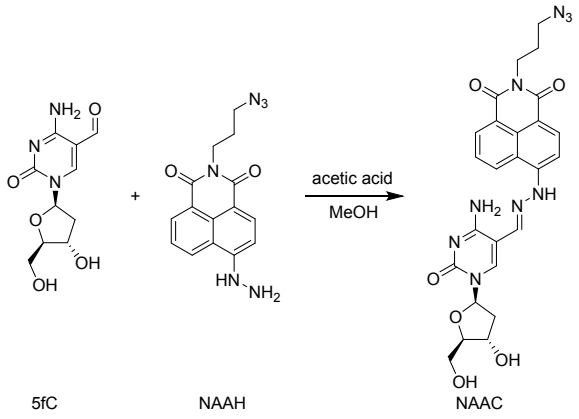


Figure S1: Synthesis of 5-formyl-2'-deoxyuridine-NAAH adduct (NAAU) and ^{1}H NMR spectrum of NAAU.

5-formyl-2'-deoxycytidine-NAAH adduct (NAAC)

5-formyl-2'-deoxycytidine[4] (51 mg, 0.2 mmol, 1 eq), NAAH (62 mg, 0.2 mmol, 1 eq) were dissolved into 3 mL methanol at a 10 mL round bottom flask. Then 150 uL acetic acid was added while the reaction mixture was kept stirring at 50°C. After 3 h, the precipitate was filtered off and collected through prewashed with cold methanol and dried under vacuum to yield 43 mg (40% yield) as a red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (s, 1H), 8.77 (d, *J* = 8.6 Hz, 1H), 8.50 (d, *J* = 7.2 Hz, 1H), 8.39 – 8.36 (m, 3H), 8.22 (s, 1H), 8.01 (s, 1H), 7.80 (t, *J* = 7.9 Hz, 1H), 7.39 (d, *J* = 8.5 Hz, 1H), 6.18 (t, *J* = 6.3 Hz, 1H), 5.28 (d, *J* = 4.2 Hz, 1H), 5.19 (t, *J* = 5.4 Hz, 1H), 4.36 – 4.19 (m, 1H), 4.11 (t, *J* = 6.8 Hz, 2H), 3.87 (dd, *J* = 6.7, 3.3 Hz, 1H), 3.76 – 3.56 (m, 2H), 3.44 (t, *J* = 6.7 Hz, 2H), 2.33 – 2.01 (m, 2H), 1.89 (p, *J* = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.18, 163.38, 162.37, 153.55, 146.26, 145.91, 143.83, 134.05, 131.27, 129.58, 128.61, 125.38, 122.39, 118.93, 111.13, 106.21, 101.18, 88.21, 86.32, 70.36, 61.42, 49.29, 41.44, 37.57, 27.60. HRMS (ESI+) $C_{25}H_{26}N_9O_6^+$ [M+H]$^+$ calculated 548.20006, found 548.20120.



5fC NAAH NAAC

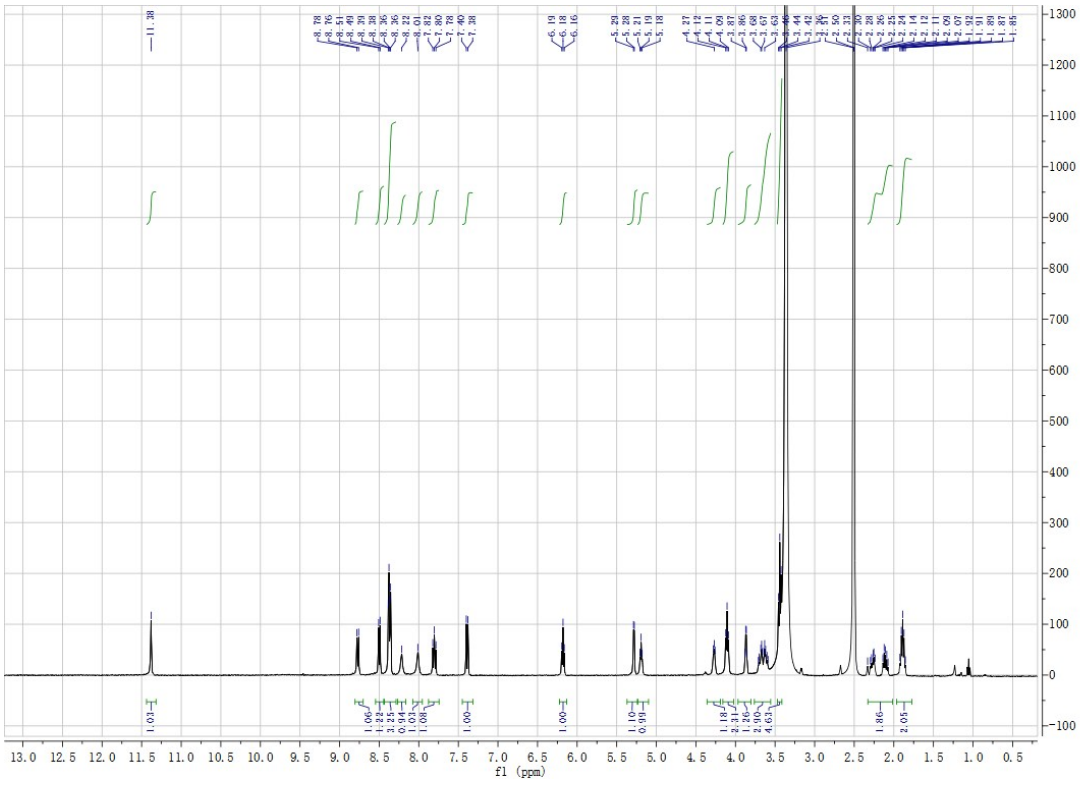


Figure S2: Synthesis of 5-formyl-2'-deoxycytidine-NAAH adduct (NAAC) and ^{1}H NMR spectrum of NAAC.

6-bromo-2-(prop-2-yn-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (2)

4-bromo-1,8-naphthalic anhydride (2.77 g, 10 mmol) and propargyl amine (0.64 mL, 10 mmol) were dissolved in DMF (60 mL) and stirred at 60°C for 3 h. Then, the solution was poured into 500 mL ice cubes. The resulting precipitate was filtered off and collected through prewashed with cold water and dried under vacuum to yield 2.89 g (92% yield) as a brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (d, *J*=6.9, 1H), 8.57 (d, *J*=8.2, 1H), 8.43 (d, *J*=7.5, 1H), 8.03 (d, *J*=7.5, 1H), 7.85 (t, *J*=7.7, 1H), 4.94 (s, 2H), 2.20 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.66, 162.61, 133.57, 132.43, 131.97, 131.78, 130.34, 130.13, 129.41, 128.73, 122.87, 122.09, 79.58, 73.73, 29.69. HRMS(ESI+) $C_{15}H_9BrNO_2^+$ $[M+H]^+$ calculated 313.98112, found 313.98173.

6-hydrazinyl-2-(prop-2-yn-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (NAPH)

2 (314 mg, 1 mmol) was dispersed in 100 mL ethanol in round bottom flask with three necks under Ar atmosphere. Then 1 mL hydrazine hydrate was added dropwise while the reaction mixture was kept stirring and stayed at reflux temperature. After 5 h, the precipitate was filtered off and collected through washed with cold trichloromethane and dried under vacuum to yield 225 mg (85% yield) as a deep red solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.23 (s, 1H), 8.64 (d, *J*=8.3, 1H), 8.43 (d, *J*=7.2, 1H), 8.30 (d, *J*=8.5, 1H), 7.64 (t, *J*=7.8, 1H), 7.26 (d, *J*=8.6, 1H), 4.74 (s, 2H), 4.71 (s, 2H), 3.08 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.45, 162.38, 153.86, 134.88, 131.21, 129.76, 129.06, 124.51, 121.72, 118.86, 107.15, 104.54, 80.37, 72.73, 28.99. HRMS(ESI+) $C_{15}H_{12}N_3O_2^+$ $[M+H]^+$ calculated 266.09240, found 266.09297.

5-formyl-2'-deoxyuridine-NAPH adduct (NAPU)

5-formyl-2'-deoxyuridine[3] (25.6 mg, 0.1 mmol, 1eq) and NAPH (26.5 mg, 0.1 mmol, 1 eq) were dissolved into 10 mL methanol at a 25 mL round bottom flask. The reaction mixture was kept stirring at 50°C for 1 h, the precipitate was filtered off and collected through prewashed with cold methanol and dried under vacuum to yield 46.3 mg (92% yield) as a red solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.69 (s, 1H), 11.48 (s, 1H), 8.82 – 8.73 (m, 2H), 8.53 – 8.40 (m, 3H), 7.82 – 7.72 (m, 2H), 6.26 (d, *J* = 6.0 Hz, 1H), 5.32 – 5.26 (m, 2H), 4.77 (s, 2H), 4.35 (s, 1H), 3.91 (s, 1H), 3.73 (s, 1H), 3.11 (s, 2H), 2.24 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.66, 162.52, 162.38, 150.12, 147.12, 137.66, 137.26, 134.43, 131.40, 129.55, 129.03, 125.29, 121.93, 118.86, 110.38, 108.90, 107.35, 88.36, 85.56, 80.19, 72.92, 70.80, 61.61, 40.96, 36.14. HRMS(ESI+) $C_{25}H_{22}N_5O_7^+$ $[M+H]^+$ calculated 504.15137, found 504.15317.

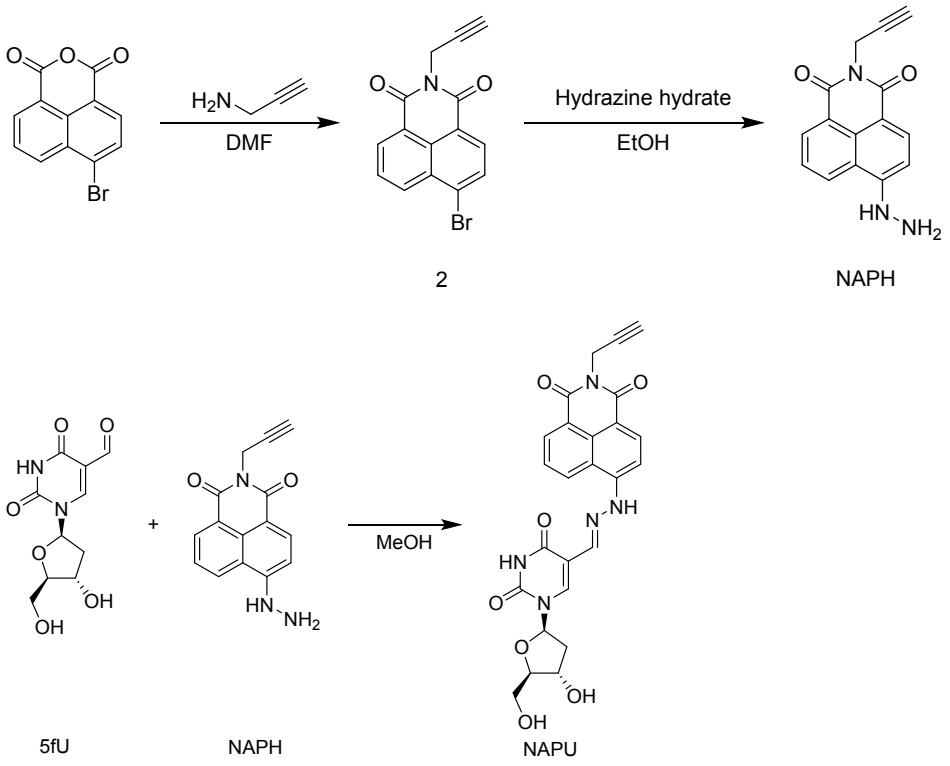


Figure S3: Synthesis of 5-formyl-2'-deoxyuridine-NAPH adduct (NAPU).

3. Table of oligonucleotides sequences

Oligomer	**Sequence (from 5'to 3')**
ODN-T	GACTCAA**T**AGCCGTA
ODN-5fU	GACTCAA**5fU**AGCCGTA
ODN-5fC	GACTCAA**5fC**AGCCGTA
ODN-5hmU	GACTCAA**5hmU**AGCCGTA
ODN-5hmC	GACTCAA**5hmC**AGCCGTA
ODN-U	GACTCAA**U**AGCCGTA
ODN-C	GACTCAA**C**AGCCGTA
ODN2-T	CATAG**T**GCTCAAGAGAAATCTCGATGG
ODN2-5fU	CATAG**5fU**GCTCAAGAGAAATCTCGATGG
ODN2- Primer	HEX-CCATCGAGATTTCTC
ODN2-5fC	AAATCA**5fC**CCTATCCTCCTTCAGGACCAACGTAC

4. DNA MALDI-TOF Mass Spectra

5’-GACTCAA**5fU**AGCCGTA-3’ ⟶ 5’- GACTCAA**(NAAU)**AGCCGTA-3’

calculated 4865.9, found 4864.2.

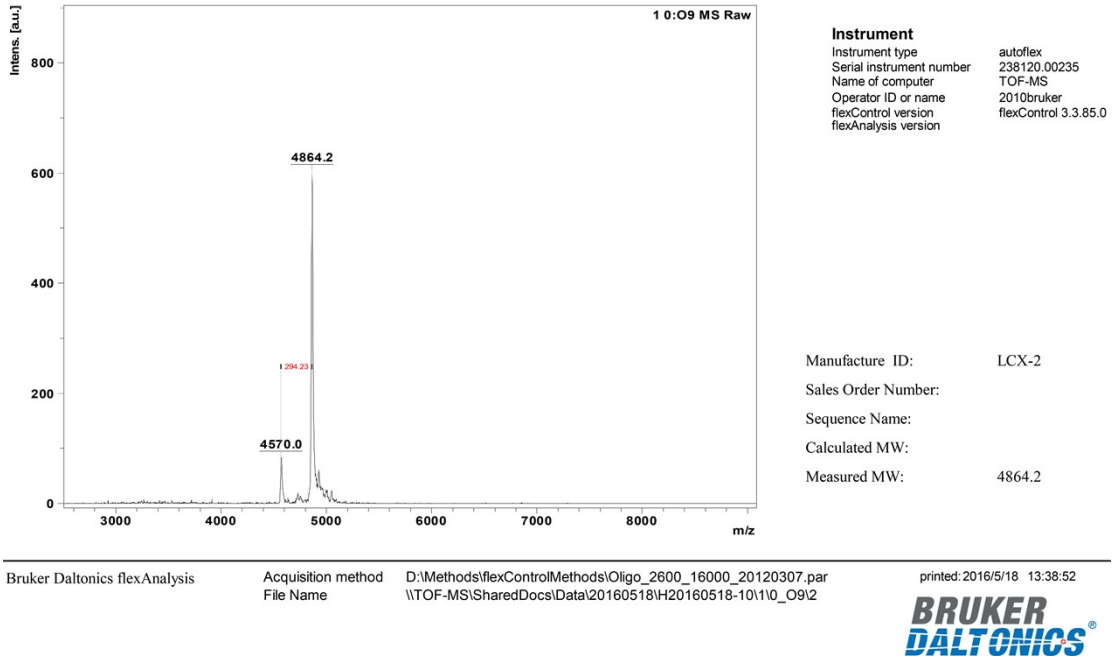


Figure S4: MALDI-TOF-spectrum of ODN-NAAU.

5’-GACTCAA**5fU**AGCCGTA-3’ ⟶ 5’-GACTCAA**Biotin-(NAAU)**AGCCGTA-3’

calculated 5615.2, found 5623.1.

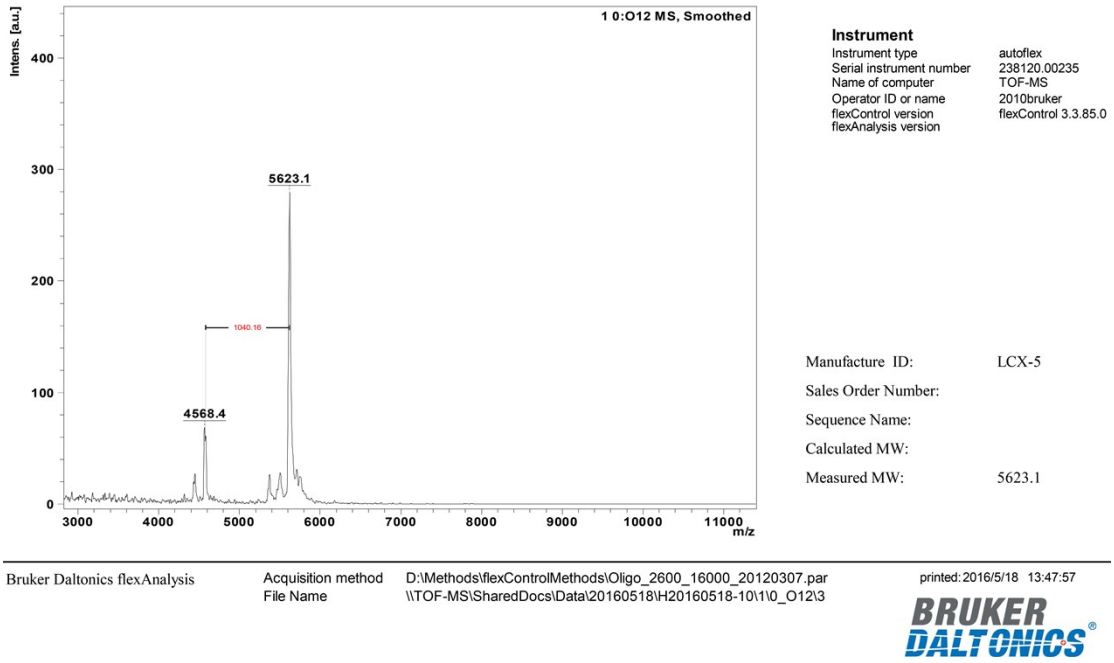


Figure S5: MALDI-TOF-spectrum of ODN-biotin-NAAU.

5’-CATAG**5fU**GCTCAAGAGAAATCTCGATGG-3’ ⟶ 5’-CATAG**(NAAU)**GCTCAAGAGAAATCTCGATGG-3’

calculated 8635.5, found 8630.6.

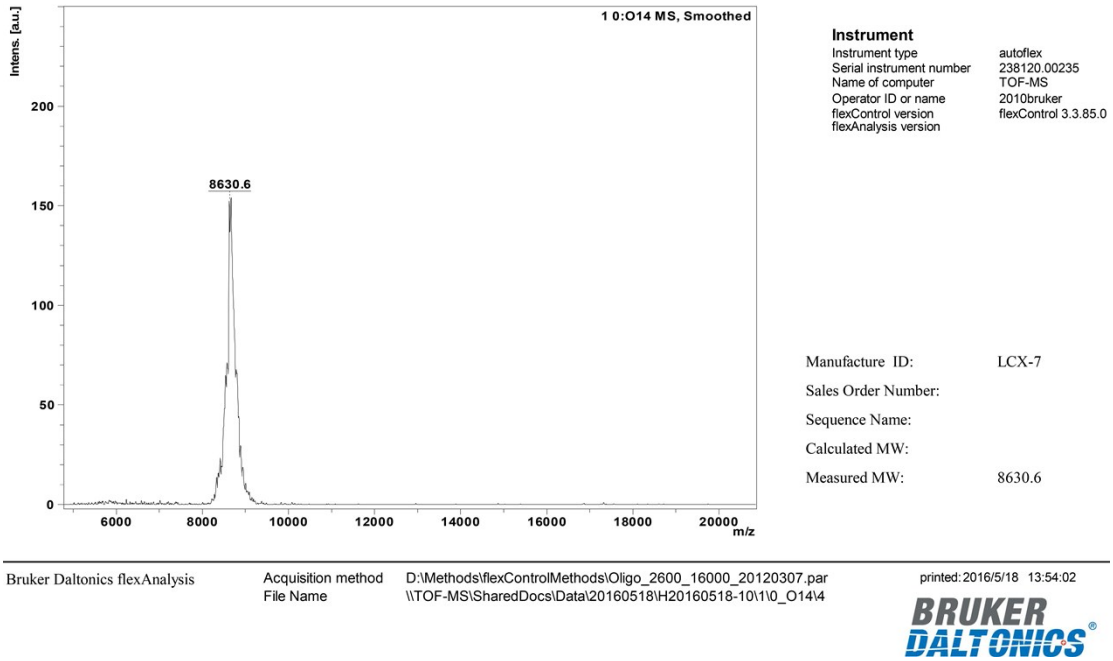


Figure S6: MALDI-TOF-spectrum of ODN2-NAAU.

5’-GACTCAA**5fC**AGCCGTA-3’ ⟶ 5’- GACTCAA**(NAAC)**AGCCGTA-3’

calculated 4864.9, found 4865.1.

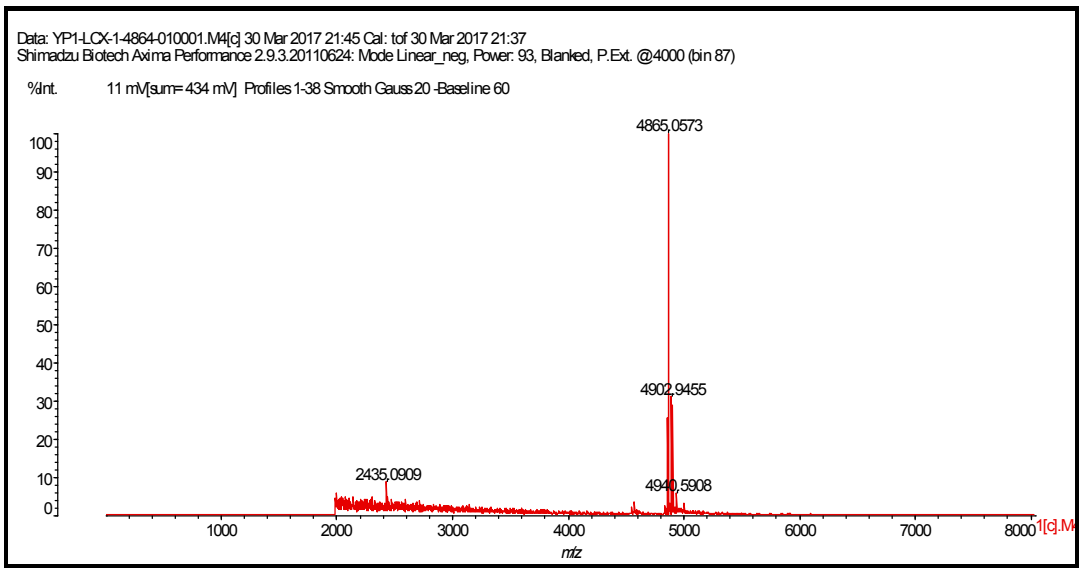


Figure S7: MALDI-TOF-spectrum of ODN-NAAC.

5’-GACTCAA**5fC**AGCCGTA-3’ ⟶ 5’-GACTCAA**Biotin-(NAAC)**AGCCGTA-3’

calculated 5614.3, found 5613.8.

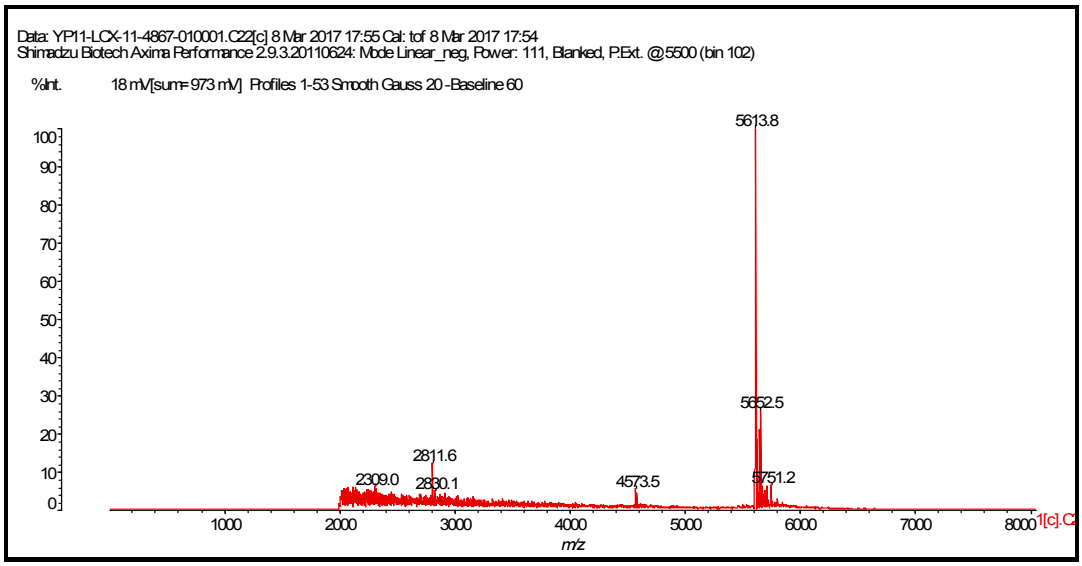


Figure S8: MALDI-TOF-spectrum of ODN-biotin-NAAC.

5’-AAATCA**5fC**CCTATCCTCCTTCAGGACCAACGTAC-3’ ⟶ 5’-AAATCA**(NAAC)**CCTATCCTCCTTCAGGACCAACGTAC-3’

Calculated 10262.8, found 10227.3, 10268.4, 10303.3.

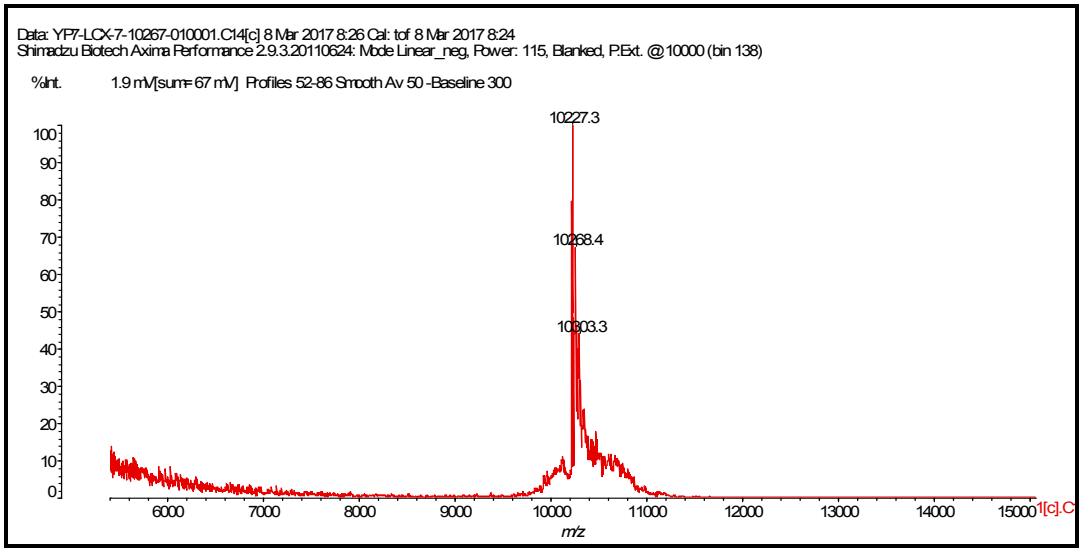


Figure S9: MALDI-TOF-spectrum of ODN2-NAAC.

5’-AAATCA5fCCCTATCCTCCTTCAGGACCAACGTAC-3’ ⟶ 5’-AAATCA**Biotin-(NAAC)**CCTATCCTCCTTCAGGACCAACGTAC-3’

Calculated 11012.2, found 10974.9, 11017.2, 11058.6.

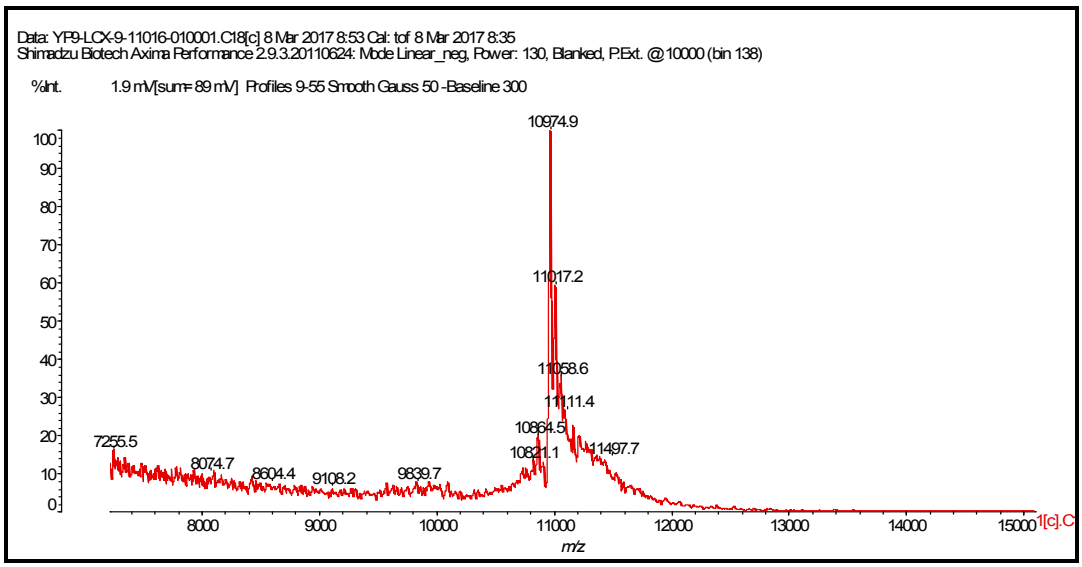


Figure S10: MALDI-TOF-spectrum of ODN2-biotin-NAAC.

5’-GACTCAA**5fU**AGCCGTA-3’ ⟶ 5’- GACTCAA**(NAPU)** AGCCGTA-3’

calculated 4820.8, found 4819.5.

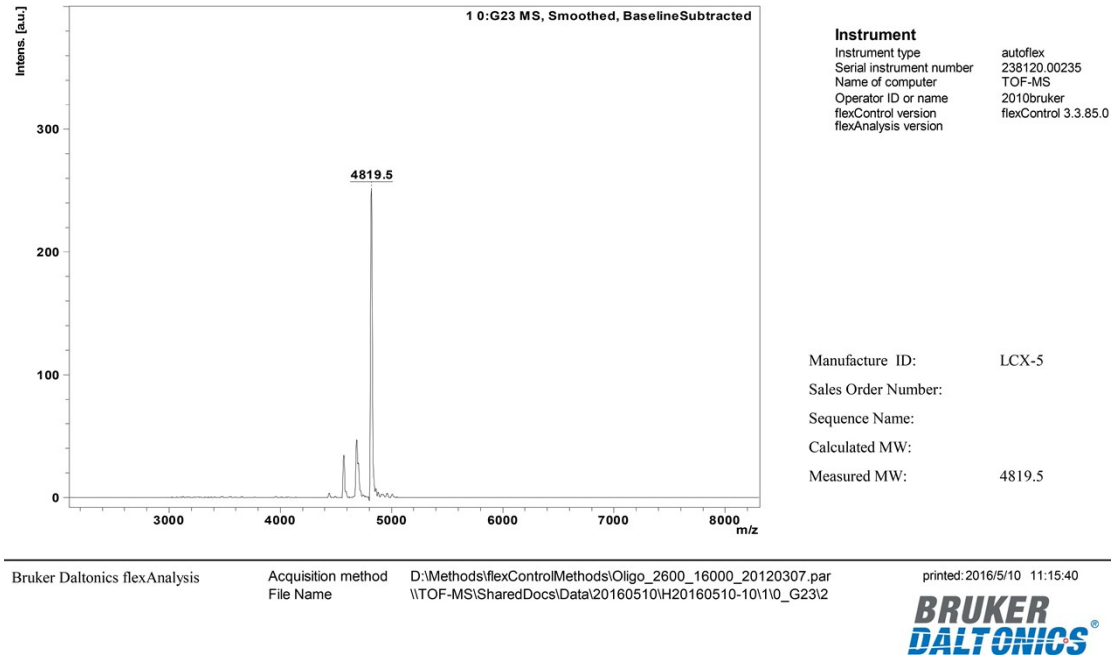


Figure S11: MALDI-TOF-spectrum of ODN-NAPU.

5’-CATAG**5fU**GCTCAAGAGAAATCTCGATGG-3’ ⟶ 5’-CATAG**(NAPU)**GCTCAAGAGAAATCTCGATGG-3’

calculated 8590.5, found 8591.6.

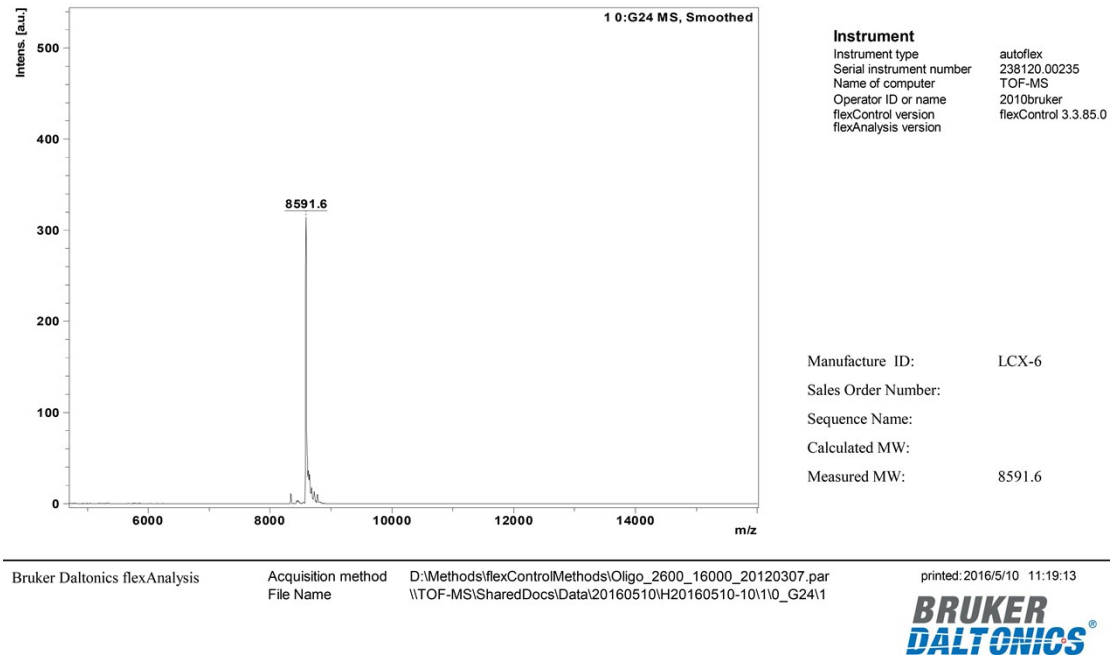


Figure S12: MALDI-TOF-spectrum of ODN2-NAPU.

5’-GACTCAA**5fU**AGCCGTA-3’ ⟶ 5’- GACTCAA**(bzmU)**AGCCGTA-3’

calculated 4706.8, found 4706.6; calculated M-NH_2 4690.8, found 4690.6; calculated M+Na^+ 4728.8, found 4729.0.

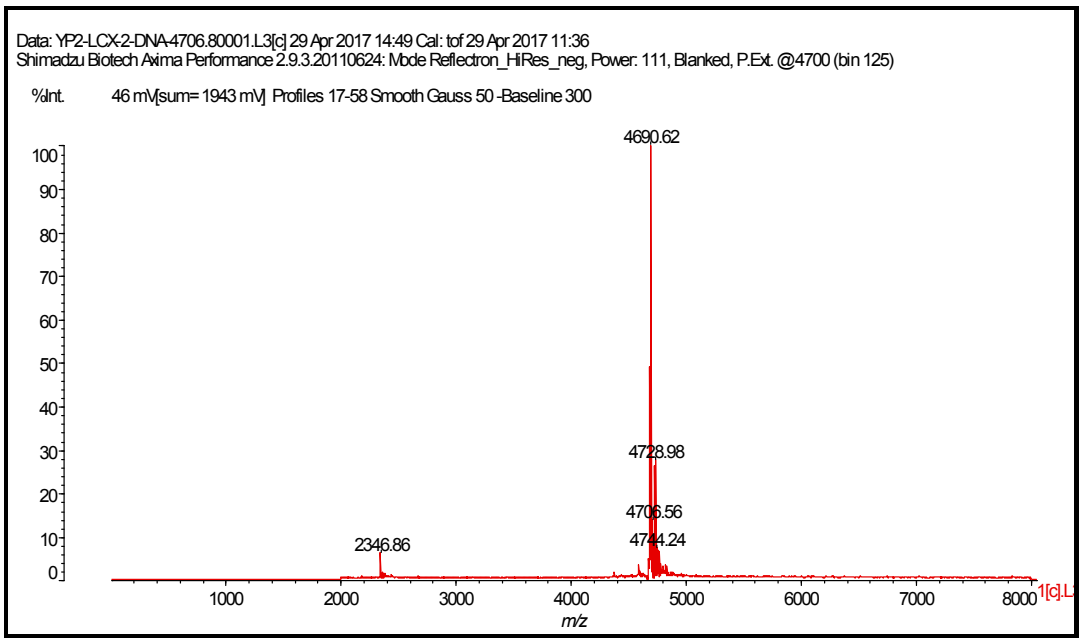


Figure S13: MALDI-TOF-spectrum of ODN-bzmU.

5. HPLC data

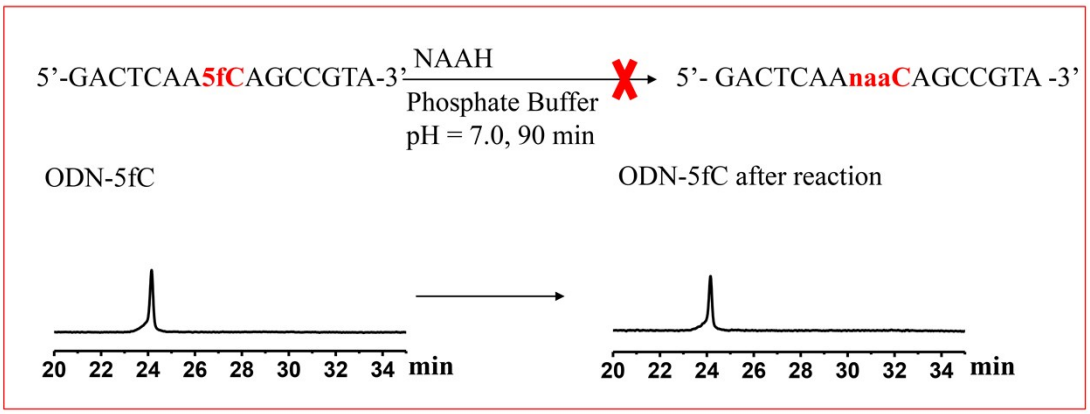


Figure S14. HPLC spectrum about ODN-5fC after incubation with NAAH under the ODN reaction protocol 1.

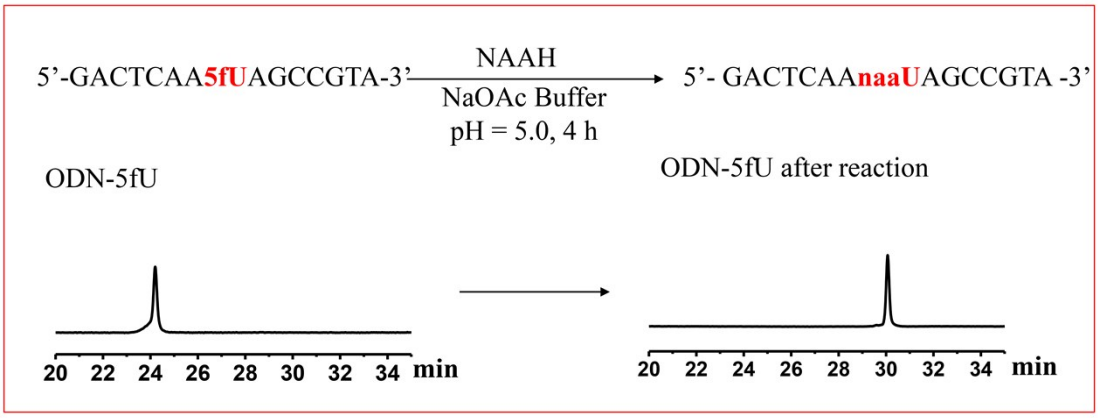


Figure S15. HPLC spectrum about ODN-5fU after incubation with NAAH in the NaOAc buffer (pH=5.0) for 4 hours.

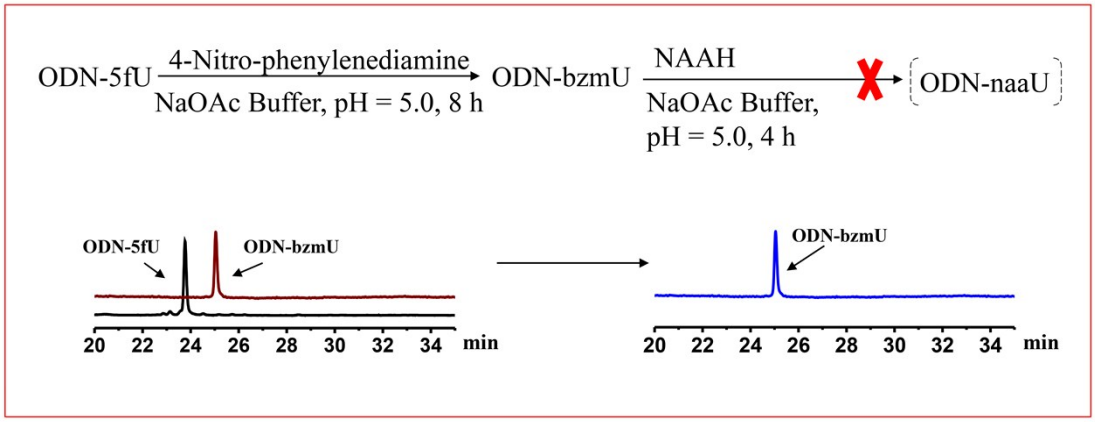


Figure S16. HPLC spectra recorded on the ODN-5fU under the ODN reaction protocol 2. Black line: ODN-5fU before reaction. Brown line: ODN-5fU after incubation with 4-Nitro-o-phenylenediamine in the NaOAc buffer (pH=5.0) for 8 hours. Blue line: after above steps, it was subjected to NAAH for another 4 hours' incubation.

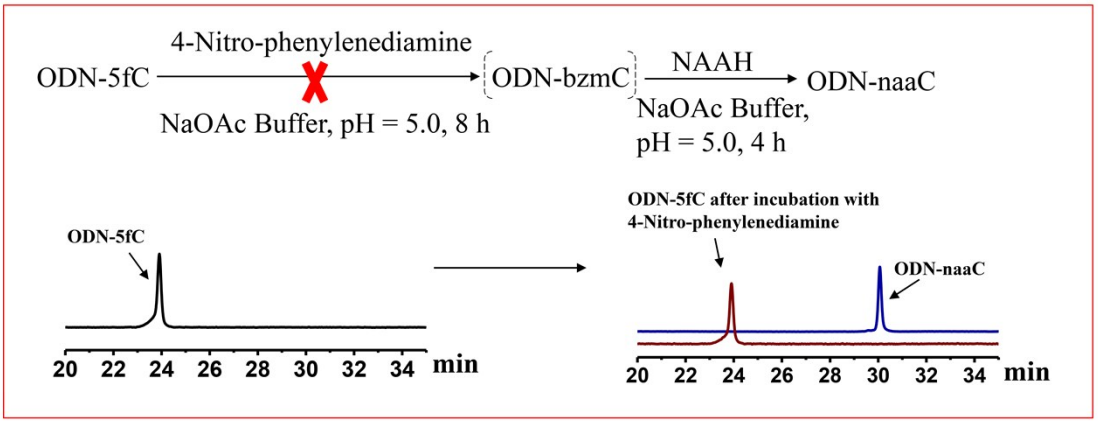
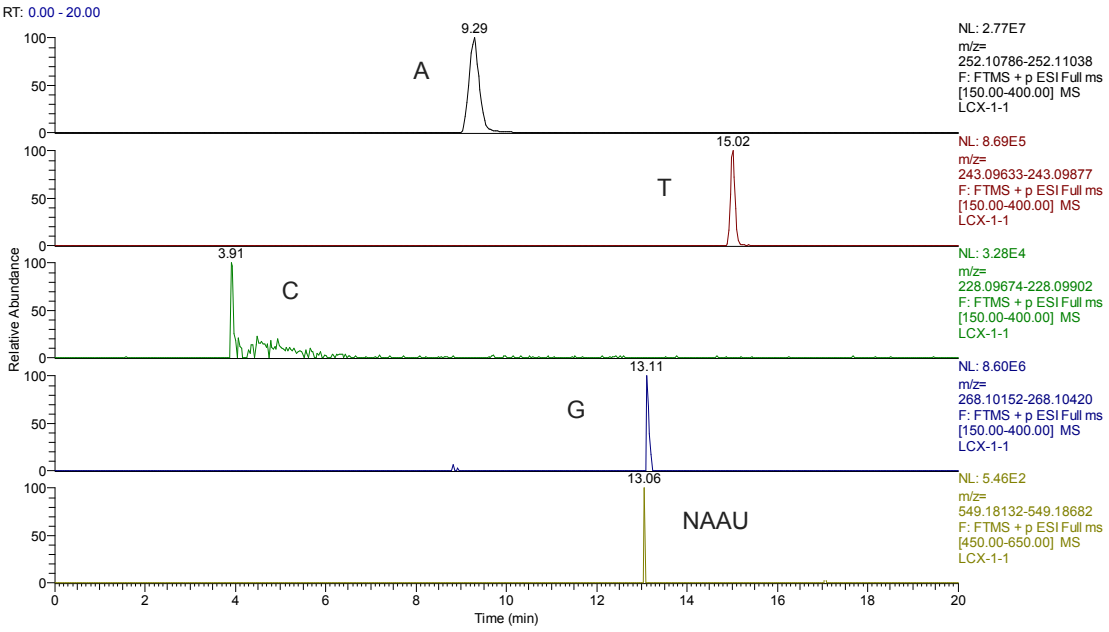


Figure S17. HPLC spectra recorded on the ODN-5fC under the ODN reaction protocol 2. Black line: ODN-5fC before reaction. Brown line: ODN-5fC after incubation with 4-Nitro-o-phenylenediamine in the NaOAc buffer (pH=5.0) for 8 hours. Blue line: after above steps, it was subjected to NAAH for another 4 hours' incubation.

6. LC-MS about digested DNA

a)



b)

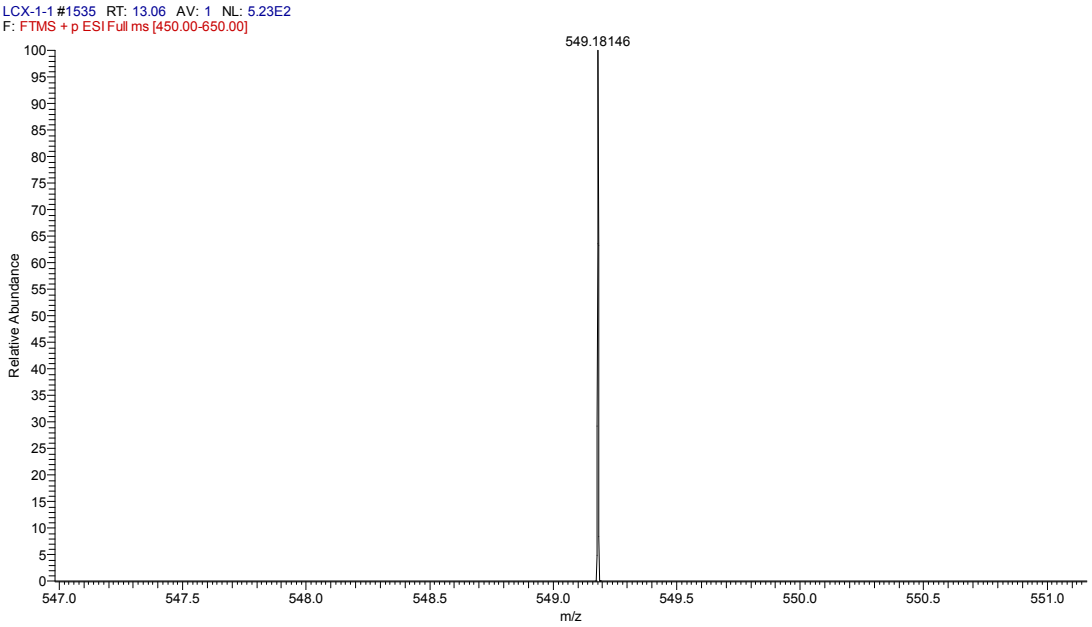
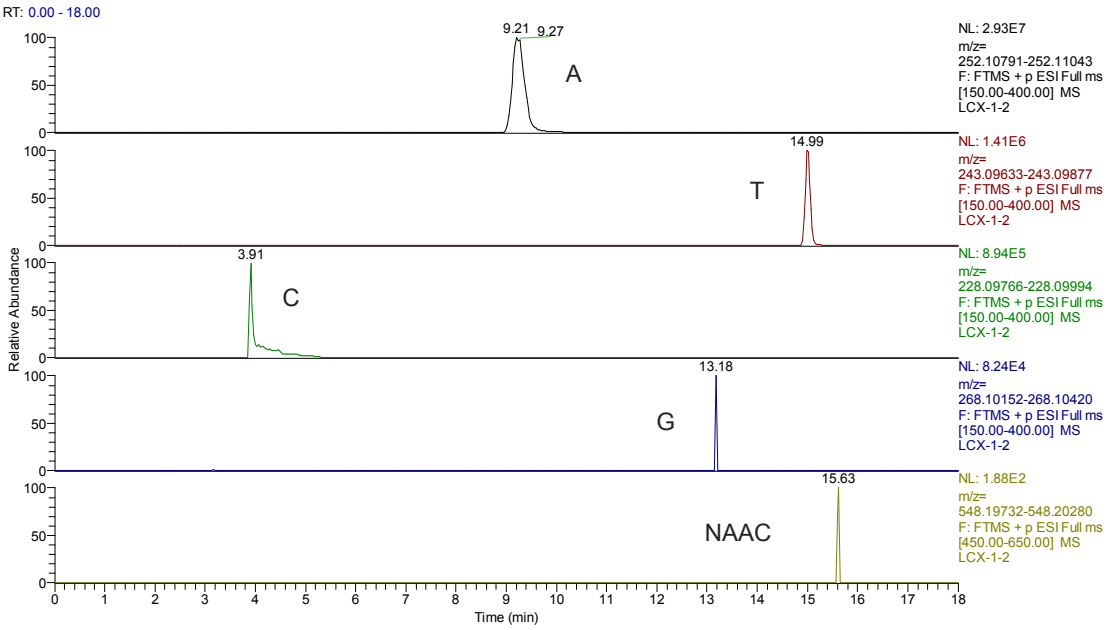


Figure S18. a) HPLC-MS extracted $[M+H]^+$ ion count for A, T, C, G, NAAU deoxynucleosides after digestion of ODN-5fU which was after labeled by NAAH. b) HRMS (ESI+) of NAAU in HPLC-MS after digestion, HRMS (ESI+) $C_{25}H_{25}N_8O_7^+$ $[M+H]^+$ calculated 549.18407, found 549.18146.

a)



b)

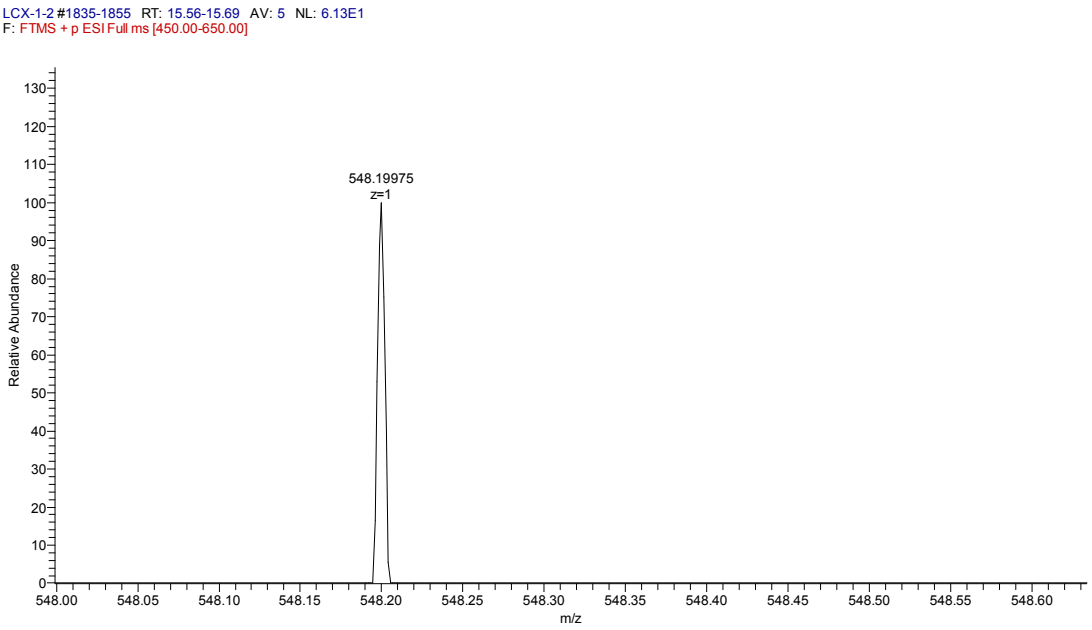


Figure S19. a) HPLC-MS extracted $[M+H]^+$ ion count for A, T, C, G, NAAC deoxynucleosides after digestion of ODN-5fC which was after labeled by NAAH. b) HRMS (ESI+) $C_{25}H_{26}N_9O_6^+$ $[M+H]^+$ calculated 548.20006, found 548.19975.

7. UV absorption spectra and fluorescent emission spectra

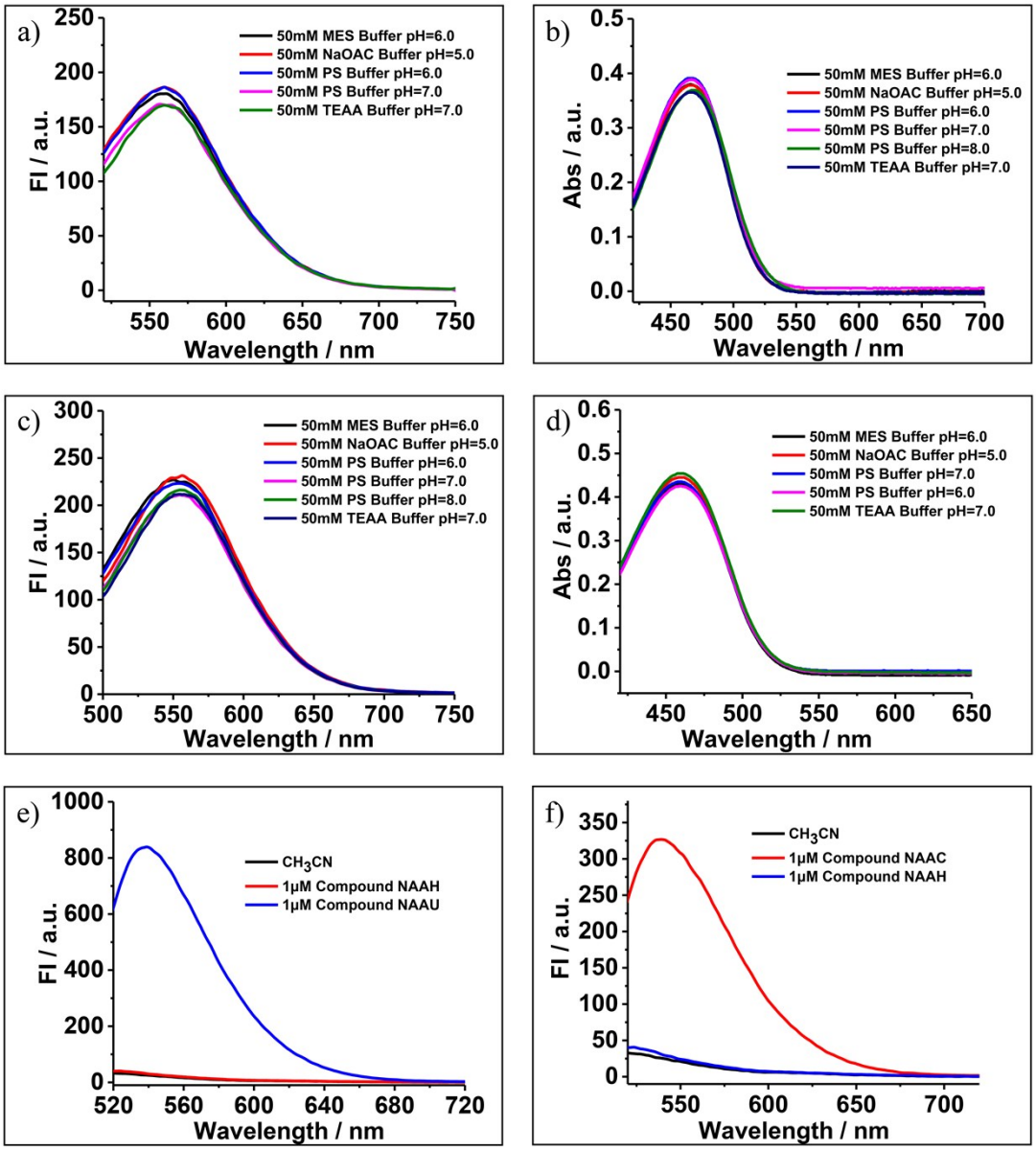


Figure S20. a) Fluorescence emission spectra of NAAU in different buffer solutions (λ_{ex}: 460 nm); b) UV absorption spectra of NAAU in different buffer solutions; c) Fluorescence emission spectra of NAAC in different buffer solutions (λ_{ex}: 460 nm); d) UV absorption spectra of NAAC in different buffer solutions; e) Fluorescence emission spectra of NAAU (1 μM) (blue line) and NAAH (1 μM) (red line) in acetonitrile solutions (λ_{ex}: 439 nm); f) Fluorescence emission spectra of NAAC (1 μM) (red line) and NAAH (1 μM) (blue line) in acetonitrile solutions (λ_{ex}: 439 nm)

8. Polyacrylamide gel electrophoresis analysis

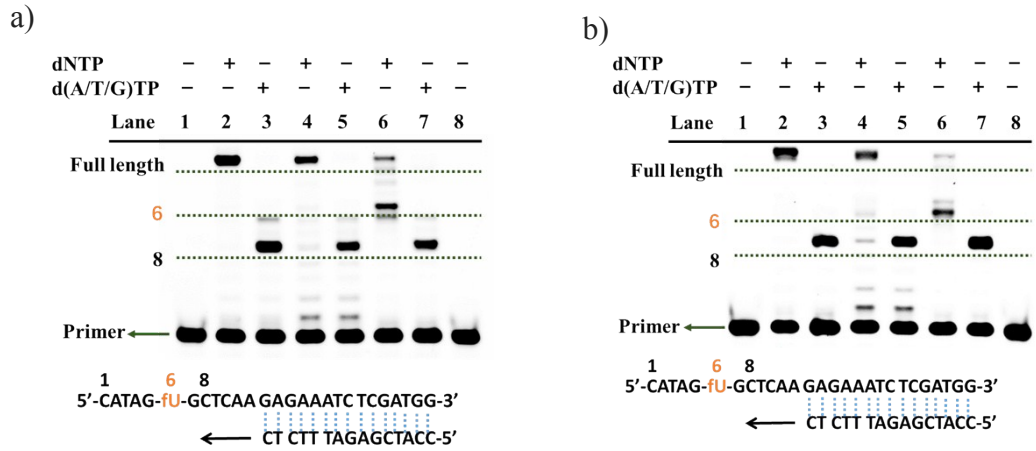


Figure S21. Comparing primer extension reactions with DNA polymerase on templates containing ODN-T, ODN-5fU and ODN-naaU in an identical sequence context. (a) and (b) represent *Bst* DNA polymerase large fragment (New England BioLabs) and *Bsu* DNA polymerase large fragment (New England BioLabs) respectively. Lane 1, 8: the HEX-labeled ODN2-primer marker; Lane 2, 3: unmodified DNA (ODN2-T); Lane 4, 5: ODN2-5fU; Lane 6, 7: ODN2-naaU (ODN2-5fU after incubation with NAAH). Lane 2, 4, 6: The primer extension system contains dNTPs (dA/T/C/GTP); Lane 3, 5, 7: The primer extension system contains dA/T/GTP. The reaction time is 1 min.

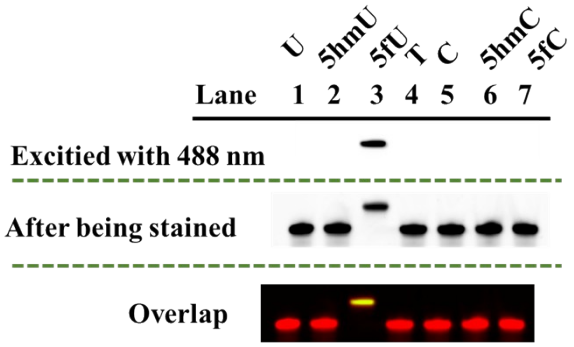


Figure S22. Polyacrylamide gel electrophoresis analysis of ODN-5fU after incubation with NAAH (lane 3) in the ODN reaction protocol 1 (selective labelling 5fU) before (above dash line, fluorescence mode, λ_{ex}: 488 nm) and after (below dash line, fluorescence mode, λ_{ex}: 532 nm) being stained with nucleic acid stains in comparison with those of other control DNAs such as ODN-U (lane 1), ODN-5hmU (lane 2), ODN-T (lane 4), ODN-C (lane 5), ODN-5hmC (lane 6),and ODN-5fC (line 7) under the same conditions.

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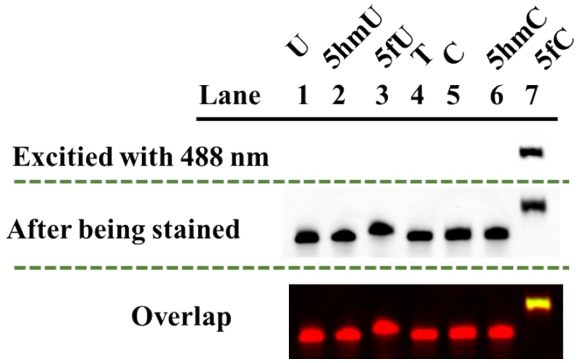


Figure S23. Polyacrylamide gel electrophoresis analysis of ODN-5fC after incubation with NAAH (lane 7) in the ODN reaction protocol 2 (selective labelling 5fC) before (above dash line, fluorescence mode, λ_{ex}: 488 nm) and after (below dash line, fluorescence mode, λ_{ex}: 532 nm) being stained with nucleic acid stains in comparison with those of other control DNAs such as ODN-U (lane 1), ODN-5hmU (lane 2), ODN-T (lane 4), ODN-C (lane 5), ODN-5hmC (lane 6),and ODN-5fU (line 3) under the same conditions.

a) b)

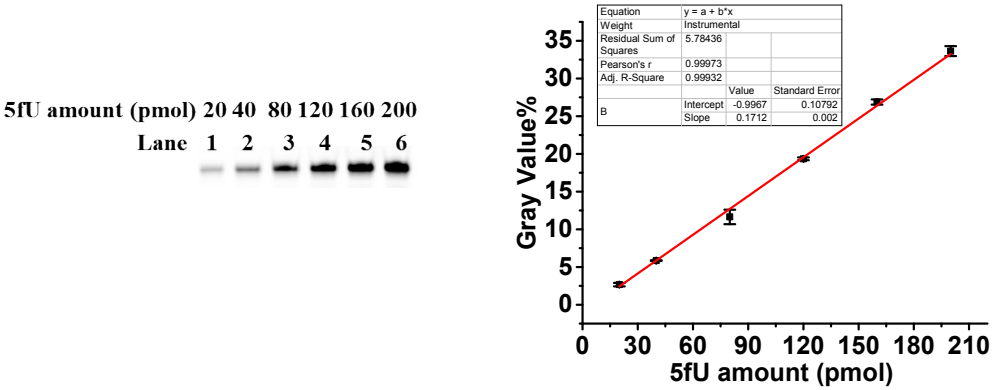


Figure S24. Correlation of the Gray Value (fluorescence mode, λ_{ex}: 488 nm) of ODN-5fU after incubation with NAAH with DNA concentration.

a) b)

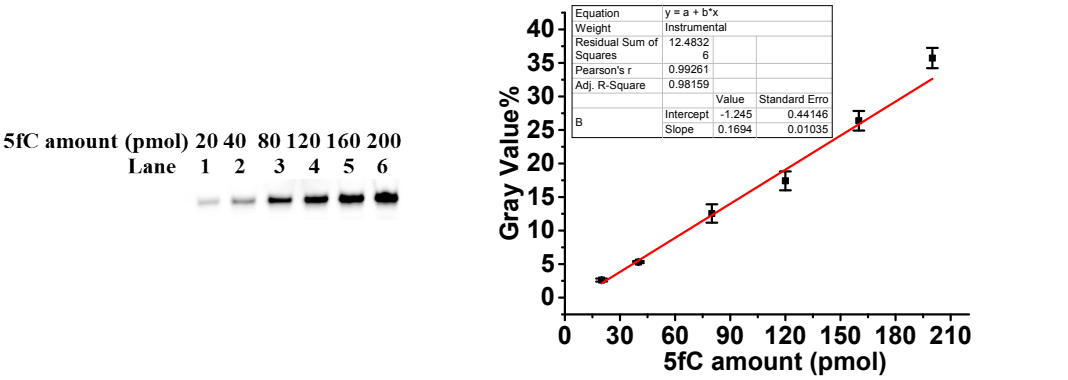
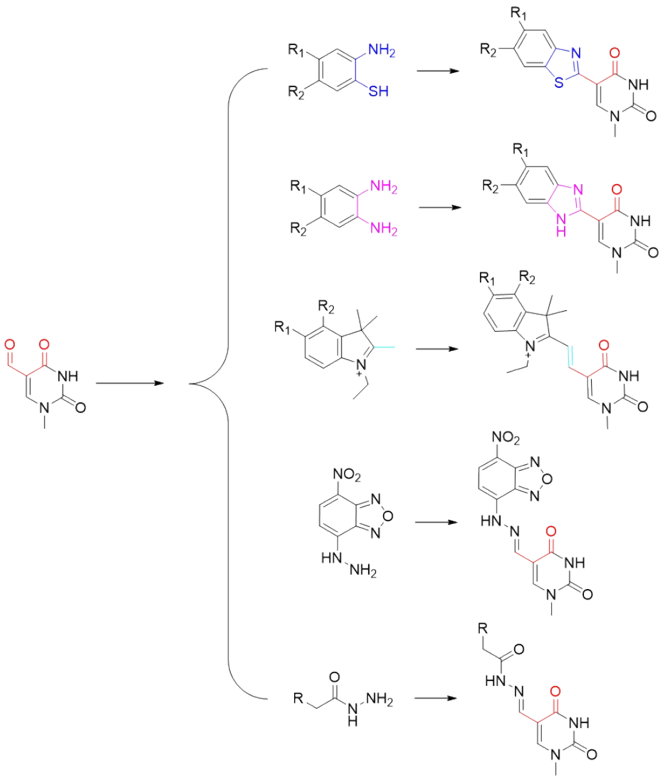


Figure S25. Correlation of the Gray Value (fluorescence mode, λ_{ex}: 488 nm) of ODN-5fC after incubation with NAAH with DNA concentration.

9. Scheme S1



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Scheme S1. Different groups reacted with 5fU reported before.

10. References

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