Supplementary Information

Fluorescence quenching in oligonucleotides containing 7-substituted 7-deazaguanine bases prepared by the Nicking Enzyme Amplification Reaction

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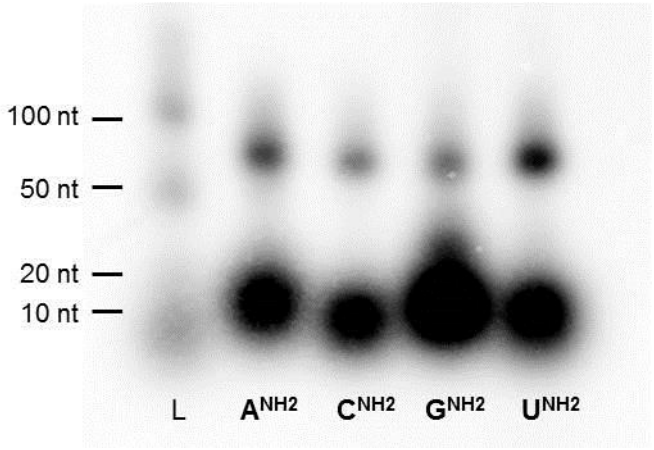


Figure S1. Agarose gel analysis of radiolabelled NEAR products (post-NEAR labelling). Lane 1 ($\mathbf{A}^{\mathbf{NH2}}$): $\mathbf{dA}^{\mathbf{NH2}}\mathbf{TP}$, dCTP, dGTP, dTTP; lane 2 ($\mathbf{C}^{\mathbf{NH2}}$): dATP, $\mathbf{dC}^{\mathbf{NH2}}\mathbf{TP}$, dGTP, dTTP; lane 3 ($\mathbf{G}^{\mathbf{NH2}}$): dATP, dCTP, $\mathbf{dG}^{\mathbf{NH2}}\mathbf{TP}$, dTTP; lane 4 ($\mathbf{U}^{\mathbf{NH2}}$): dATP, dCTP, dGTP, $\mathbf{dU}^{\mathbf{NH2}}\mathbf{TP}$. **L** = DNA ladder. Agarose gel is not well-suited for the analysis of radioactive samples, therefore, polyacrylamide gel was chosen as a better alternative (Figure 1).

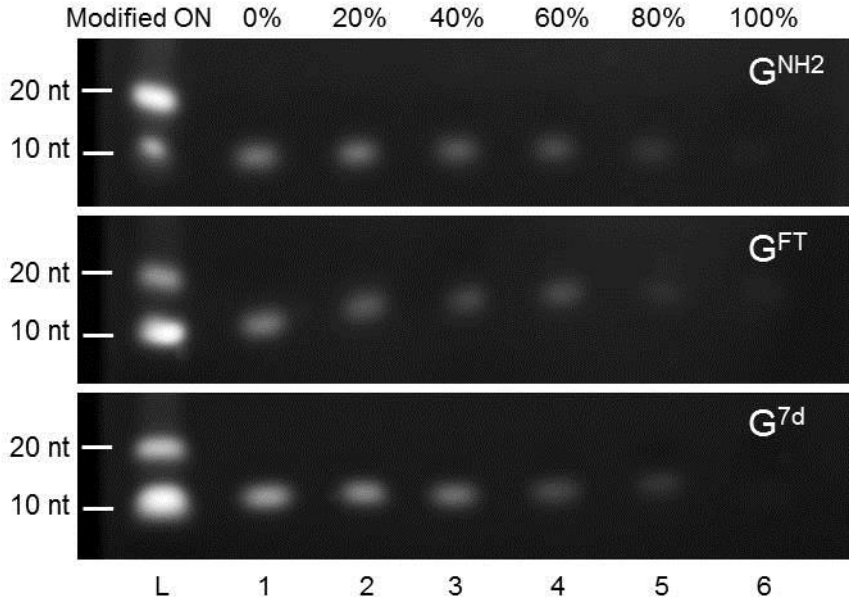


Figure S2. Analysis of GelRed fluorescence quenching of NEAR products on agarose gels. Each line contains the same amount of DNA, but the ratio of non-modified (containing natural **dG**) and modified (containing $\mathbf{dG}^{\mathbf{NH2}}$, $\mathbf{dG}^{\mathbf{FT}}$, or $\mathbf{dG}^{\mathbf{7d}}$) varies. **L** = DNA ladder. Lane 1: 100% natural; lane 2: 80% natural; lane 3: 60% natural; lane 4: 40% natural; lane 5: 20% natural; lane 6: 0% natural.

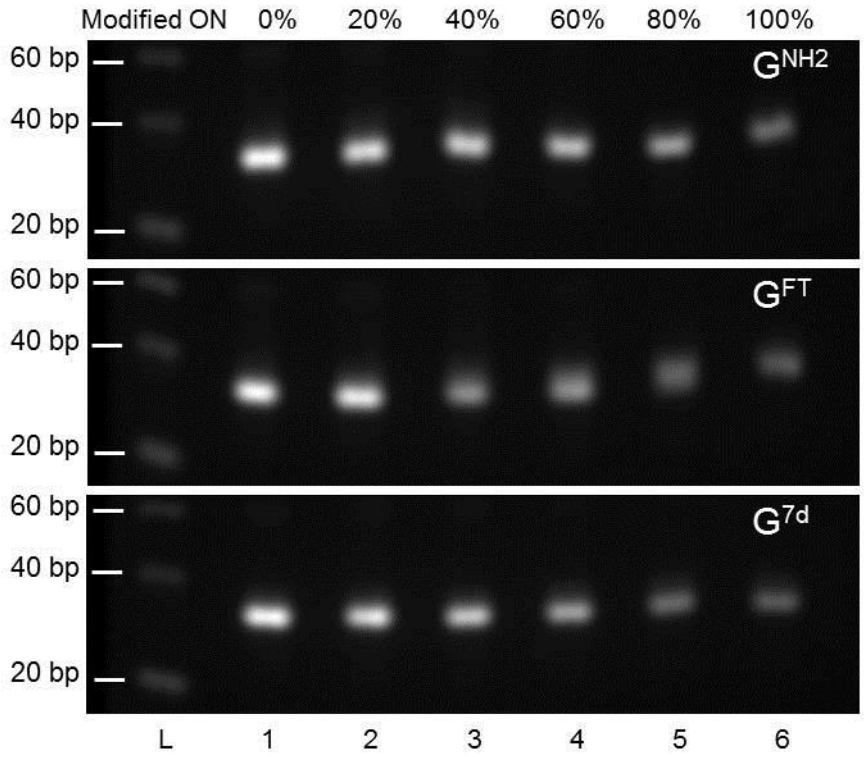


Figure S3. Analysis of GelRed fluorescence quenching of PEX products from agarose gels. Only in the newly synthesized stretch natural guanosines are substituted for 7-deazaderivatives (content of modified **dG**^{X} 21%). Each line contains the same amount of DNA, but the ratio of non-modified (containing natural **dG**) and modified (containing **dG**^{X}) varies. **L** = DNA ladder. Lane 1: 100% natural; lane 2: 80% natural; lane 3: 60% natural; lane 4: 40% natural; lane 5: 20% natural; lane 6: 0% natural.

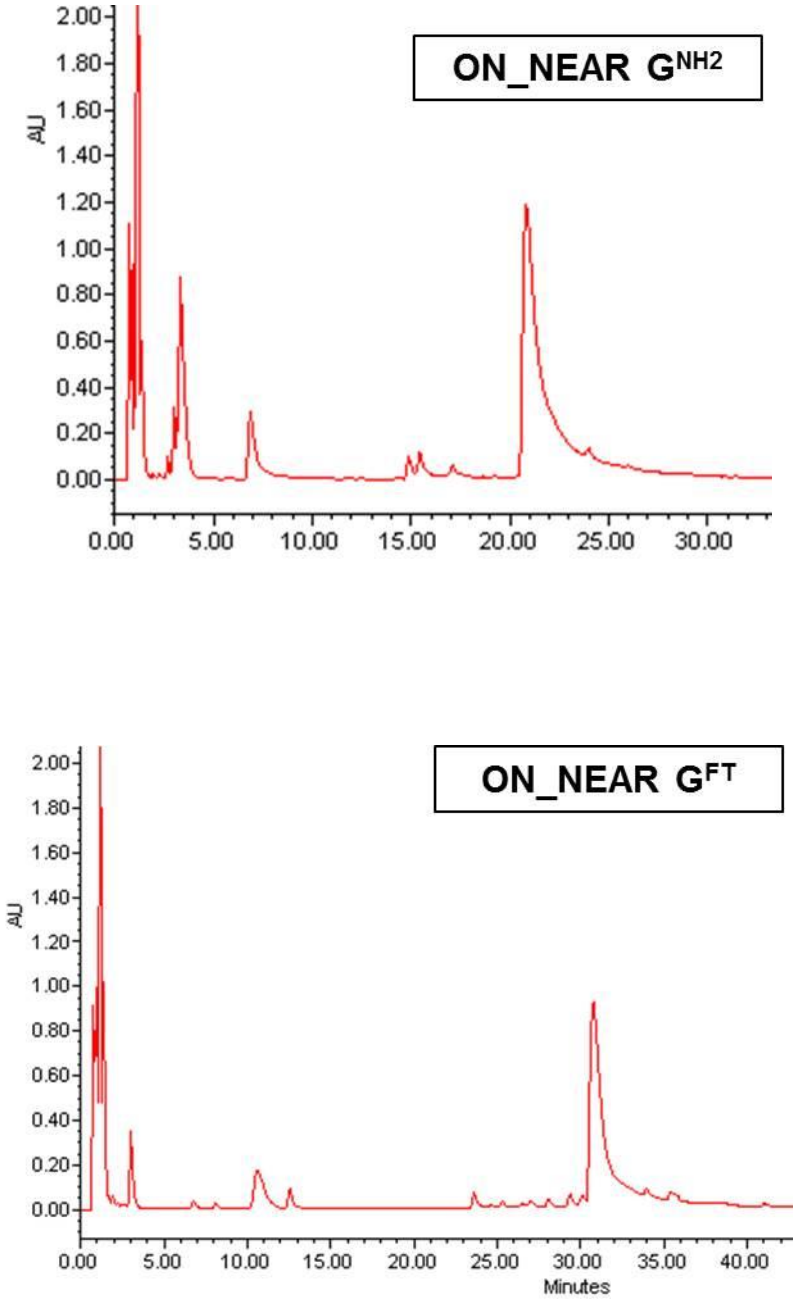


Figure S4. HPLC chromatograms of crude NEAR mixtures (preparative reactions). Column XBridge OST C18 2.5 μM (4.6 × 50 mm). Mobile phase A: 0.1 M TEAA, B: acetonitrile/0.1M TEAA, 20/80 (v/v). Flow rate: 1 mL/min. Gradient 40 to 80 % B in 53 min.

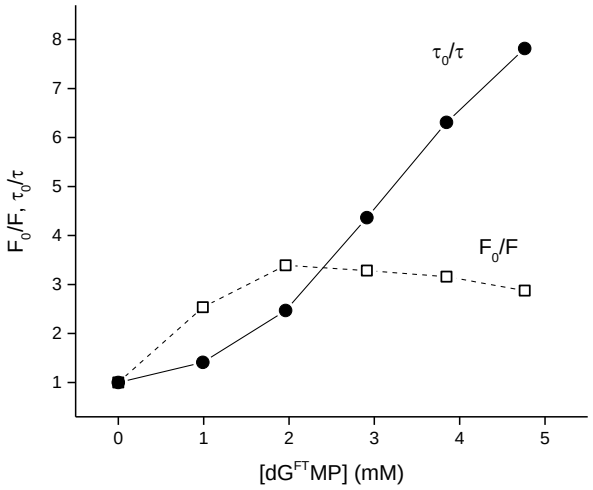


Figure S5. The Stern-Volmer plot for the quenching of GelRed–**ON_NEAR** by **$dG^{FT}MP$** in 0 – 5 mM range

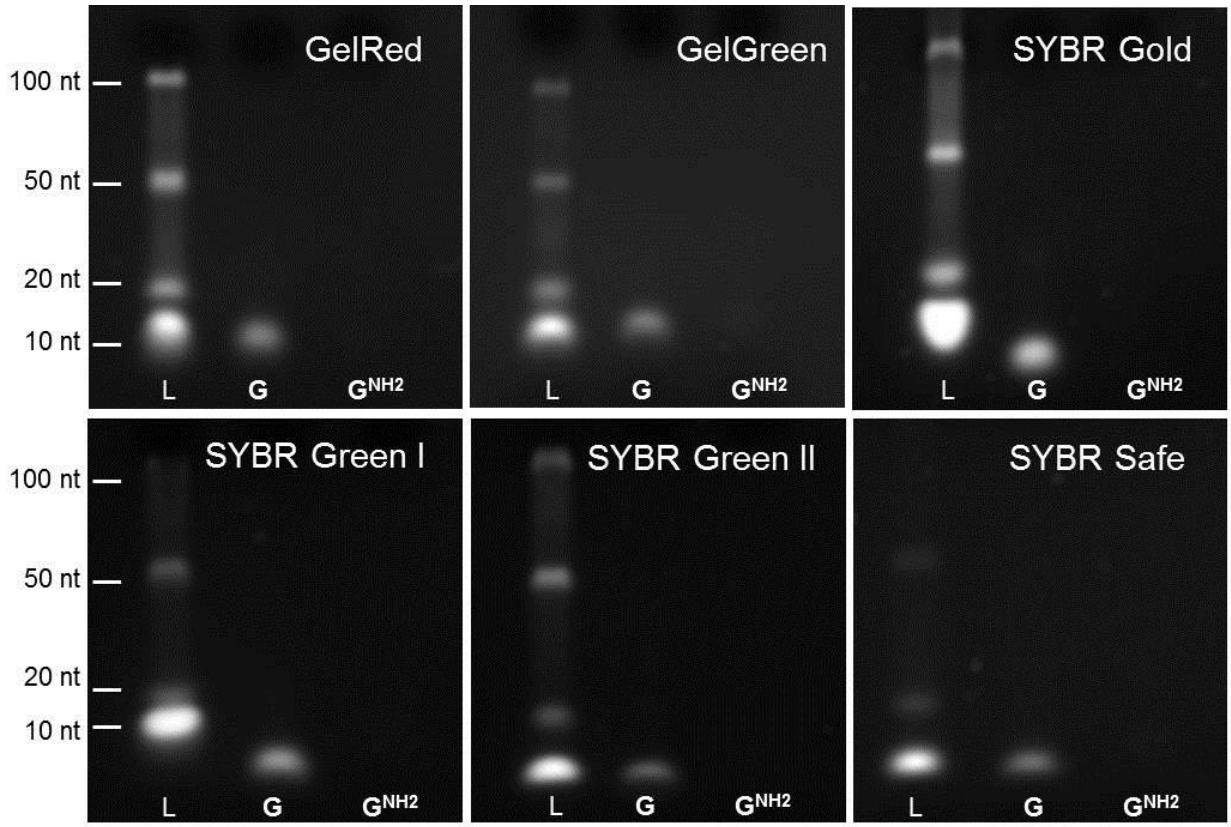


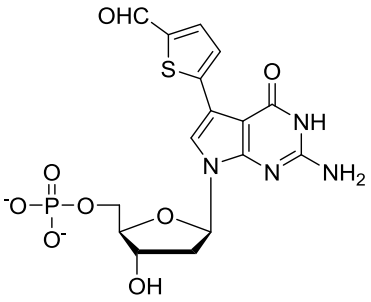
Figure S6. Testing of different gel dyes for the visualization of ssONs containg 7-deazaguanosine derivatives instead of natural guanosine. **L** = DNA ladder, **G** ssON containing natural guanosine, **G^{NH2}** ssON containing modified 7-deazaguanosine.

Synthesis of dG^{FT}MP

General remarks

5-Formylthiophene-2-boronic acid was purchase from Frontier Scientific. NMR spectra were measured on Bruker Avance 500 at 500 MHz for ^{1}H, 200 MHz for ^{31}P and 125.7 MHz for ^{13}C, in 50mM phosphate buffer (in D_2O) at pD 7.1 (reference to dioxane as internal standard, δ_H = 3.75 ppm, δ_C = 67.19 ppm). ^{31}P NMR spectra were referenced to the phosphate buffer signal (δ = 2.35 ppm). Chemical shifts are given in ppm (δ scale), coupling constants (*J*) in Hz. Mass spectra were measured by ESI. High resolution mass spectra were measured on a LTQ Orbitrap XL (Hermo Fischer Scientific) spectrometer using ESI ionization technique.

2'-Deoxy-7-(5-formylthiophene-2-yl)-7-deazaguanosine 5'-O-monophosphate (**dG^{FT}MP**)



A water–acetonitrile mixture 2:1 (2 mL) was added through a septum to an argon-purged vial containing 7-iodo-2'-deoxy-7-deazaguanosine monophosphate (**dG^{I}MP**) (61 mg, 0.116 mmol), 5-formylthiophene-2-boronic acid (118 mg, 0.753 mmol, 6.5 equiv.) and Cs_2CO_3 (330 mg, 1.013 mmol, 8.5 equiv.). In a separate flask, $Pd(OAc)_2$ (4.1 mg, 18 mol%) and TPPTS (50 mg, 0.087 mmol, 5 equiv. to Pd) were combined under argon atmosphere and a mixture of water–acetonitrile 2:1 (2 mL) was added. After dissolution, the catalyst solution was added to the reaction mixture through a septum and a mixture of water–acetonitrile 2:1 (2 mL) was used to collect remaining drops. The resulting mixture was stirred at 100 °C for 1 h. The reaction mixture was then concentrated on a rotatory evaporator and the product was disolved in H_2O (3 mL) and filtered throught 0.2 μm filter. The concentrate was injected on C18 reversed phase HPLC column (Phenomenex, Luna 10 μm C18, 100A HPLC Column 250 × 21.2 mm). Mobile phase A corresponds to 0.1 M TEAB in HPLC-grade water, mobile phase B to 0.1 M TEAB in aqueous MeOH (50%, v/v). The gradient started with 100 % mobile phase A going linearly to 15 % mobile phase B in 10 min, then to 40 % mobile phase B in 25 min, then to 70 % mobile phase B in 50 min and then to 100 % mobile phase B in 70 min. The flow rate was 10 mL/min. The fractions containing the product were evaporated on a vacuum concentrator. Several co-distillations with water and conversion to sodium salt form (Dowex 50WX8 in Na+ cycle), followed by freeze-drying from water gave 2 different pure compounds. The compound resulting of separation at 25

minutes was **dG^{7d}MP** as a white powder (10.4 mg, 23 %), the other compound resulting from the separation at 50 minutes was **dG^{FT}MP** as a vibrant yellow powder (25.9 mg, 45 %).

^{1}H NMR (500.0 MHz, D_2O, ref$_{dioxane}$ = 3.75 ppm): 2.40 (ddd, 1H, J_{gem} = 14.0, $J_{2'b,1'}$ = 6.2, $J_{2'b,3'}$ = 3.2, H-2'b); 2.65 (ddd, 1H, J_{gem} = 14.0, $J_{2'a,1'}$ = 8.1, $J_{2'a,3'}$ = 6.3, H-2'a); 4.89 (t, 2H, $J_{5',4'}$ = $J_{H,P}$ = 5.5, H-5'); 4.13 (td, 1H, $J_{4',5'}$ = 5.5, $J_{4',3'}$ = 3.2, H-4'); 4.63 (dt, 1H, $J_{3',2'}$ = 6.3, 3.2, $J_{3',4'}$ = 3.2, H-3'); 6.23 (dd, 1H, $J_{1',2'}$ = 8.1, 6.2, H-1'); 7.41 (s, 1H, H-6); 7.61 (d, 1H, $J_{3,4}$ = 4.1, H-3-thienyl); 7.75 (dd, 1H, $J_{4,3}$ = 4.1, $J_{4,CHO}$ = 0.5, H-4-thienyl); 9.65 (d, 1H, $J_{CHO,4}$ = 0.5, CHO). ^{13}C NMR (125.7 MHz, D_2O, ref$_{dioxane}$ = 69.3 ppm): 40.56 (CH_2-2'); 66.84 (d, $J_{C,P}$ = 4.5, CH_2-5'); 74.28 (CH-3'); 85.53 (CH-1'); 88.16 (d, $J_{C,P}$ = 8.1, CH-4'); 100.26 (C-4a); 115.95 (C-5); 120.98 (CH-6); 129.53 (CH-3-thienyl); 141.67 (C-5-thienyl); 143.41 (CH-4-thienyl); 151.28 (C-2-thienyl); 154.73 (C-7a); 155.59 (C-2); 162.79 (C-4); 188.79 (CHO). ^{31}P (^{1}H dec.) NMR (202.3 MHz, D_2O,): 4.29. MS (ESI$^-$): *m/z (%)*: 477.0 (5), 455.0 (100). HRMS (ESI$^-$): *m/z* calculated for $C_{16}H_{16}O_8N_4PS$: 455.04319; found 455.04297.

NMR spectra of dG^{FT}MP

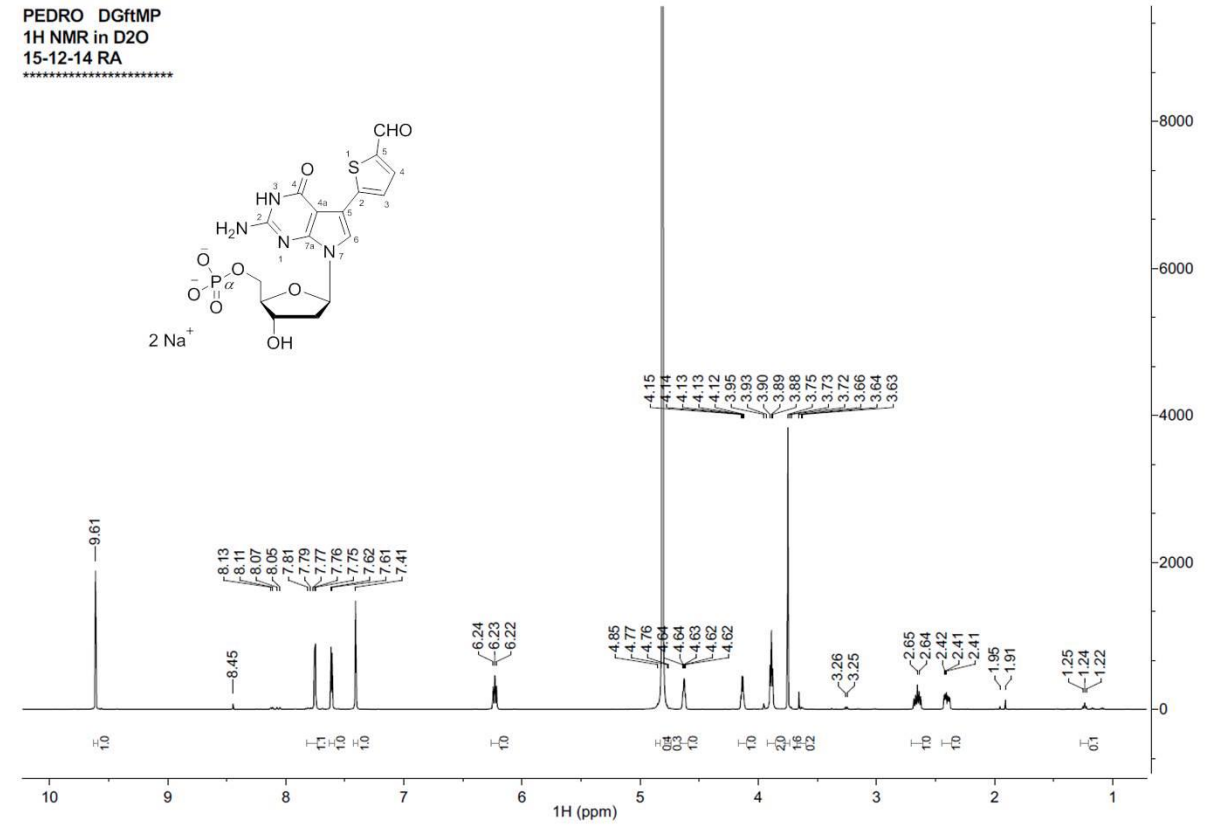


Figure S7. ^{1}H NMR of **dG^{FT}MP**

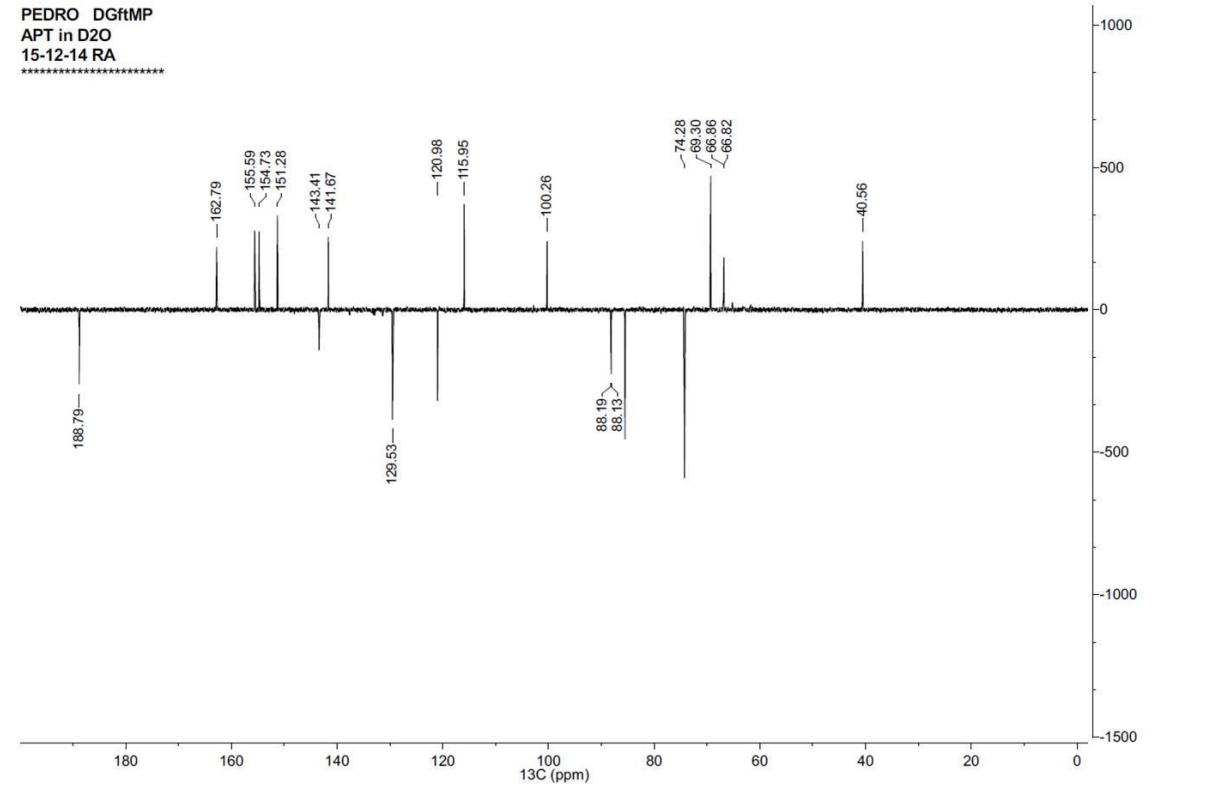


Figure S8. ^{13}C NMR of **dG^{FT}MP**

MALDI-TOF Spectra of NEAR products

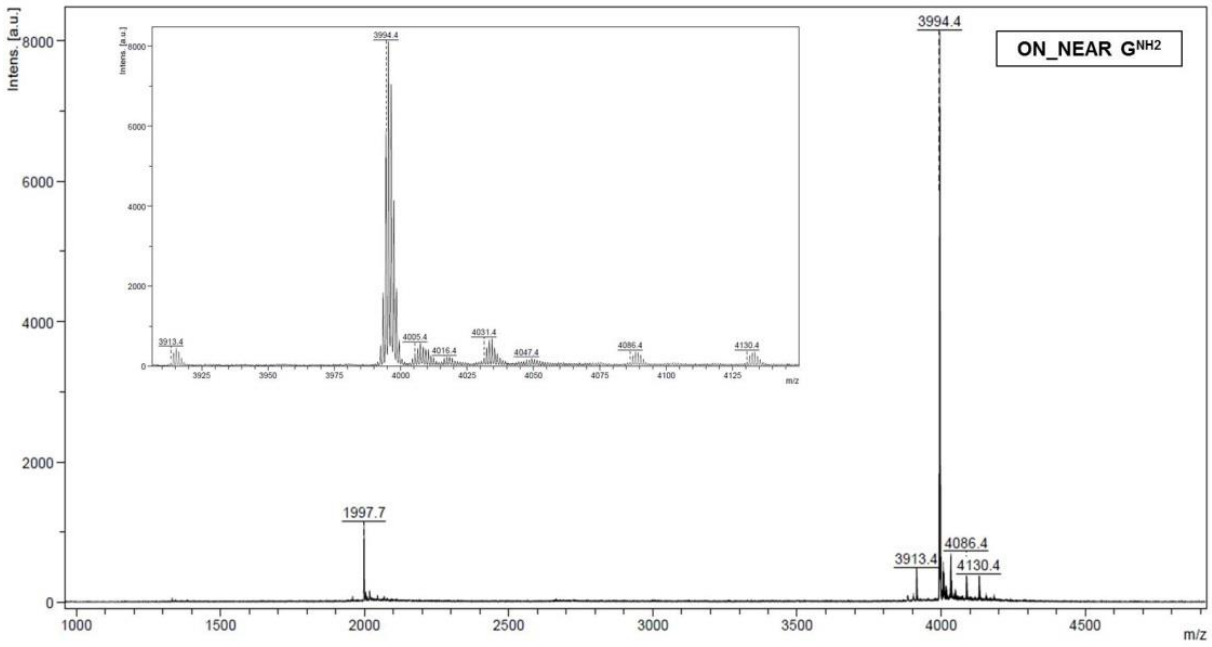


Figure S9. MALDI-TOF spectrum of **ON_NEAR G^{NH2}**.

M (calc.) = 3 994.8 Da, M (found) = 3 994.4 Da ([M+H]$^+$).

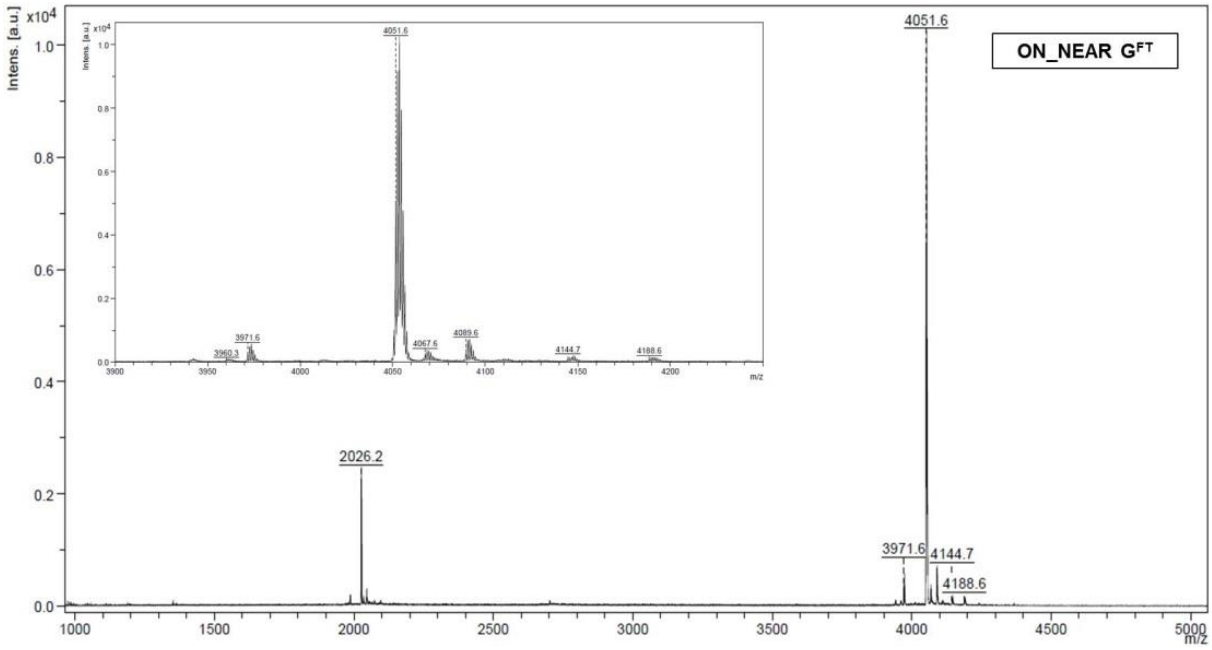


Figure S10. MALDI-TOF spectrum of **ON_NEAR G^{FT}**.

M (calc.) = 4 051.6 Da, M (found) = 4 051.6 Da ([M+H]$^+$).

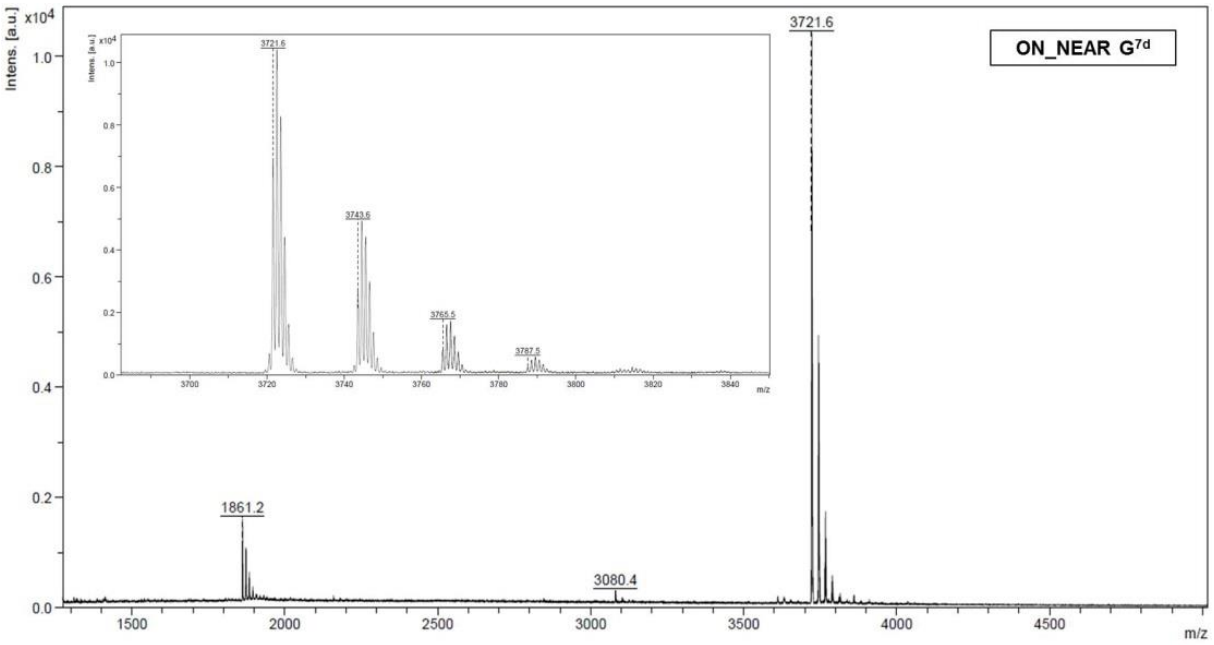


Figure S11. MALDI-TOF spectrum of **ON_NEAR G^{7d}**.

M (calc.) = 3 721.6 Da, M (found) = 3 721.6 Da ([M+H]$^+$).

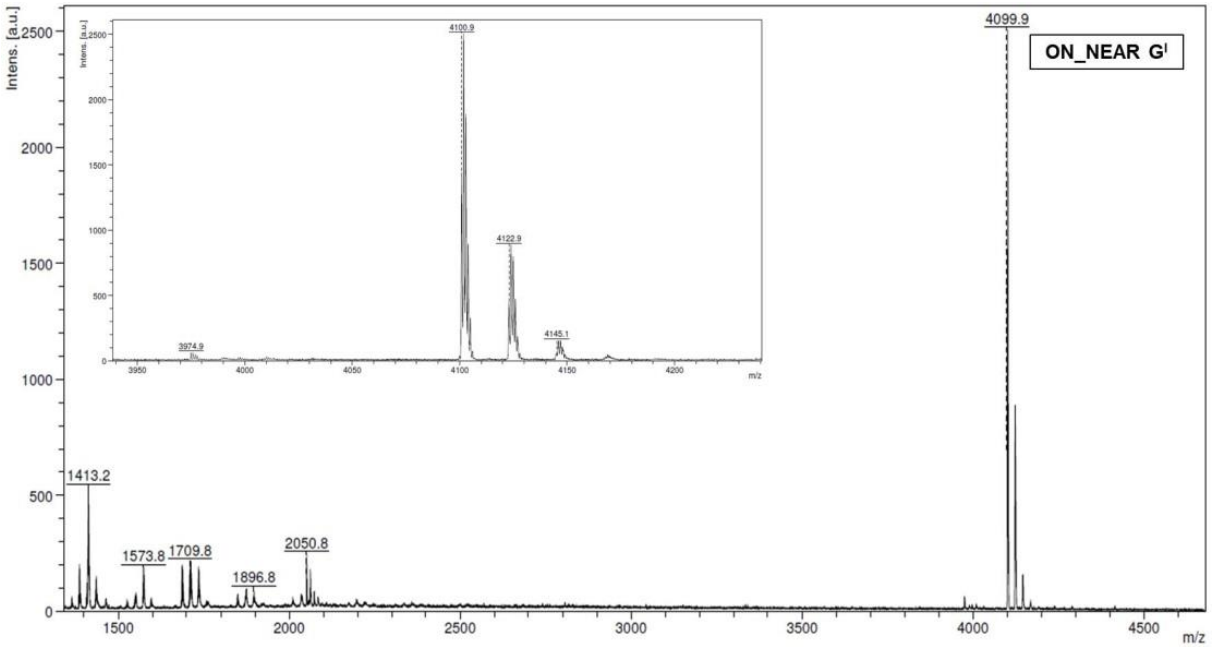


Figure S12. MALDI-TOF spectrum of **ON_NEAR G^{I}**.

M (calc.) = 4 101.1 Da, M (found) = 4 101.9 Da ([M+H]$^+$).