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Fluorescence Quenching in Oligonucleotides Containing 7-Substituted 7-Deazaguanine Bases Prepared by the Nicking Enzyme Amplification Reaction

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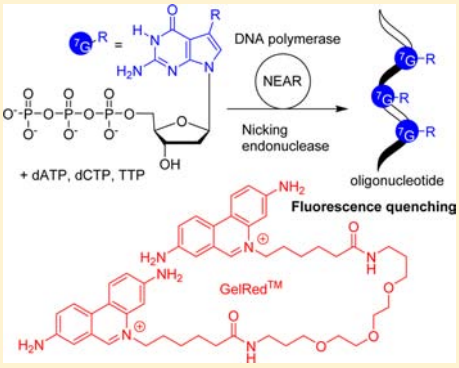
Supporting Information

ABSTRACT: Recently, we reported the use of the Nicking Enzyme Amplification Reaction (NEAR) for the enzymatic synthesis of short oligonucleotides (ONs) containing 5-substituted pyrimidine or 7-substituted 7-deazaadenine nucleotides. Since no oligonucleotide products were visible on agarose gels stained by an intercalating dye (GelRed), we assumed that the method did not work for 7-substituted 7-deazaguanine deoxyribonucleoside triphosphates. We revisited the work and found that the NEAR method works for 7-deazaguanine nucleotides as well but that the resulting modified ONs quench the fluorescence of DNA intercalators, rendering them invisible on gel electrophoresis stained by them. Here, we report on the modified methodology for the NEAR synthesis and analysis of G-modified ONs and on quantification of the fluorescence quenching.



INTRODUCTION

Enzymatic synthesis of base-modified oligonucleotides and nucleic acids becomes increasingly popular and useful for many applications in bioanalysis or chemical biology (for recent reviews, see refs 1−3). Recently, we have reported the use of the Nicking Enzyme Amplification Reaction (NEAR)[4] for the synthesis of short (10−22 nt) single-stranded oligonucleotides (ssONs) containing modified bases[5,6] and their applications as labeled primers in PCR.[6] The method consists of a primer extension (PEX) catalyzed by a DNA polymerase followed by nicking cleavage of the extended primer strand by nicking endonuclease (Nt.BstNBI), which releases the modified ssON. This ssON is too short to stay hybridized at the reaction temperature and recovers the primer and template for another cycle of the NEAR (Scheme 1). This method worked very well for a set of 5-substituted pyrimidine and for 7-substituted 7-deazaadenine 2′-deoxyribonucleoside triphosphates (dNTPs), and the products were analyzed by agarose gel electrophoresis using staining by intercalating dye GelRed.[5,6] However, when using 7-substituted 7-deazaguanine dNTPs, the gels stained by this dye did not show any spots corresponding to the modified ON products, and thus, we assumed that the method does not work for 7-deazaguanine nucleotides (although we proved that both PEX and nicking proceeds).[6] After the publication of our paper,[6] we were notified of several previous studies on the

Scheme 1. NEAR Synthesis of Modified ONs

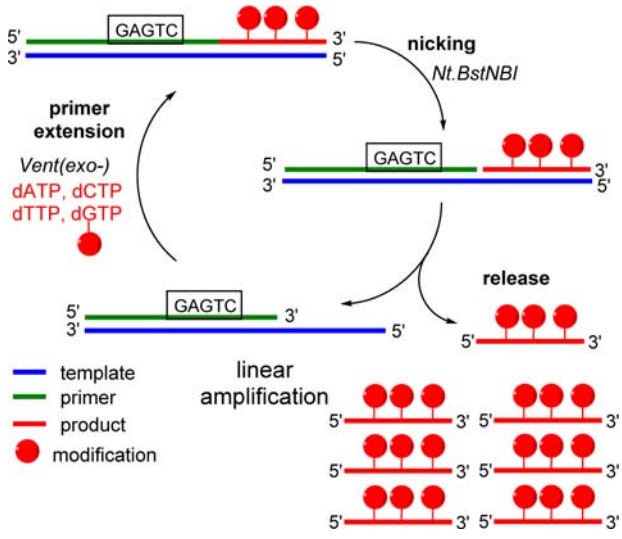


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Table 1. Sequences of Commercial Oligonucleotides Used in the Study[a]

name	sequence
TempNick	5′-TCGACTGGACTAcgagGACTCACTAGATCGG-3′
PrimNick	5′-CCGATCTAGTGAGTCctcg-3′
TempPEX-bio	5′-bio-CTAGCATGAGCTCAGTCCCATGCCGCCCATG-3′
PrimPEX	5′-CATGGGCGGCATGGG-3′

[a]Lower case letters, spacer bases; underlined letters, nicking enzyme recognition site; bio, biotin.

Table 2. Sequences of ON and DNA Products Containing 7-Deazaguanosine Derivatives[a]

name		sequence
ON_NEAR		5′-P-TAGTCCAGTCGA-3′
ON_PEX	1st strand	5′-CATGGGCGGCATGGGACT**G**AGCTCAT**G**CTAG-3′
	2nd strand	5′-bio-CTAGCATGAGCTCAGTCCCATGCCGCCCATG-3′

[a]Letters in bold font, position of 7-deazaguanosine derivative; P, phosphate group; bio, biotin.

quenching of fluorescence of DNA intercalators by 7-deazaguanine.[7−13] Therefore, we revisited the study and report here on the NEAR synthesis of ONs containing 7-substituted 7-deazaguanines, the analysis of the products by gel electrophoresis, and on the quantification of fluorescence quenching.

RESULTS AND DISCUSSION

On the basis of the literature,[7−13] we assumed that the NEAR with modified **dG^{X}TPs** may have proceeded but that the products could have been invisible on agarose gels due to fluorescence quenching. Therefore, we performed the NEAR and directly analyzed the reaction mixture by MALDI-TOF analysis. The NEAR with modified **dG^{X}TPs** was performed according to our previously published procedure using a primer−template pair (**PrimNick** and **TempNick**; Table 1) leading to the formation of 12-mer products **ON_NEAR** with three G bases (Table 2).[6] In all of the cases (using **dG^{NH2}TP**,[14] **dG^{FT}TP**,[6] **dG^{7d}TP**, or **dG^{I}TP**; Chart 1), the formation of the desired product was observed in MALDI-TOF (Table 3). In

Chart 1. List of Modified dN^{X}TPs Used in This Study

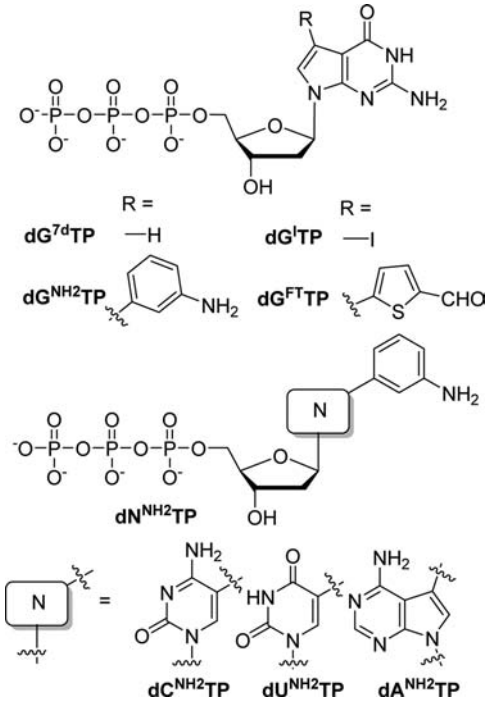

Table 3. Preparative Yields and MALDI-TOF Analyses of NEAR Products

product	preparative yield	M(calcd) $[M + H]^+$	M(found) $[M + H]^+$
ON_NEAR_G^{NH2}	9.3 nmol	3994.8 Da	3994.4 Da
ON_NEAR_G^{FT}	5.3 nmol	4051.6 Da	4051.6 Da
ON_NEAR_G^{7d}	n.d.	3721.6 Da	3721.6 Da
ON_NEAR_G^{I}	n.d.	4101.1 Da	4101.9 Da

the case of **dG^{NH2}TP** and **dG^{FT}TP**, the NEAR reactions on semipreparative scale were also performed, yielding 9.3 and 5.3 nmol, respectively, of a 12-mer product bearing three modifications (for yields, see Table 3). This means that the efficiency of semipreparative NEAR with **dG^{X}TP** is comparable with that of other modified dNTPs.[6]

In our previous work,[6] we used agarose gel electrophoresis on 4% agarose prestained with GelRed, an ethidium bromide homodimer, for the analysis of the outcome of the NEAR. Figure 1a shows that this assay failed to detect the presence of any NEAR products incorporating modified 7-deazaguanine nucleotides. To visualize any NEAR products, including those containing 7-deazaguanosine derivatives, which presumably quench the fluorescence of the intercalating dyes,[7−13] two radioactivity-based assays were developed. The NEAR was also performed in the same way as that reported in ref 6 using aminophenyl-modified **dN^{NH2}TPs**,[14] Vent(exo-) polymerase, Nt.BstNBI nickase, **TempNick** template, and **PrimNick** primer (Table 1) to produce 12-mer products **ON_NEAR** with three modified nucleobases. The first assay was based on radiolabeling of purified NEAR products by T4 polynucleotide kinase (PNK) according to standard protocols followed by polyacrylamide gel electrophoresis (PAGE).[15] Although the labeling by PNK seemed to be problematic because in this particular case, there could be an exchange reaction between the cold phosphate group at the 5′ end of NEAR products and hot phosphate groups at the γ-position of ATP, no special conditions or reactants were needed, and all NEAR products, including those with **G^{NH2}**, were successfully visualized (Figure 1b). The second assay made use of in situ radiolabeling during the NEAR mediated by the addition of [α-^{32}P]-dATP, followed by PAGE. This method was also suitable for analyzing NEAR products with modification on any nucleobase, including 7-deazaguanosine (Figure 1c). Both detection methods also independently proved that the NEAR method works for **dG^{NH2}TP** with efficiency similar to that for other modified nucleotides.

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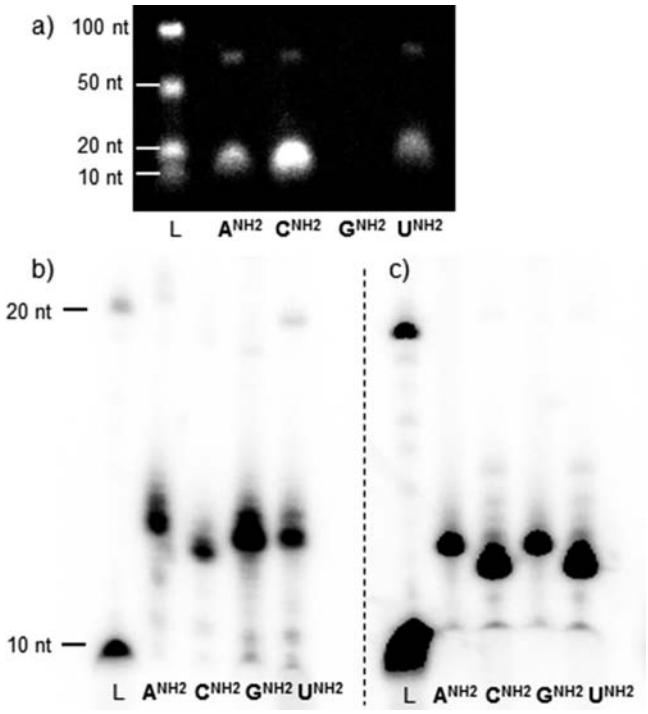

Figure 1. Gel analysis of NEAR products. (a) GelRed stained agarose-gel analysis of NEAR products; (b) PAGE analysis of radiolabeled NEAR by post-NEAR labeling by PNK; (c) PAGE analysis of radiolabeled NEAR in situ labeling. **L** = DNA ladder. Lane 1 ($\mathbf{A}^{\mathbf{NH2}}$), **dA^{NH2}TP**, dCTP, dGTP, dTTP; lane 2 ($\mathbf{C}^{\mathbf{NH2}}$), dATP, **dC^{NH2}TP**, dGTP, dTTP; lane 3 ($\mathbf{G}^{\mathbf{NH2}}$), dATP, dCTP, **dG^{NH2}TP**, dTTP; lane 4 ($\mathbf{U}^{\mathbf{NH2}}$), dATP, dCTP, dGTP, **dU^{NH2}TP**.

When the NEAR products after postsynthetic radiolabeling by PNK were analyzed on agarose gels (analogous to the fluorescent staining), the spots were very broad (Figure S1, Supporting Information), and thus, this method was less suitable than PAGE. Also, a semiquantitative analysis of GelRed-stained agarose gels of NEAR products prepared from 0 to 100% mixtures of unmodified and modified **ON_NEAR** containing **dG^{NH2}**, **dG^{FT}**, or **dG^{7d}** was performed. The images (Figure S2, Supporting Information) show that the higher the modified **dG^{X}** content, the weaker are the spots corresponding to the NEAR products. The NEAR products are visible up to 80% of **G^{NH2}**. The NEAR products containing 100% of **G^{X}** are barely visible but still can be detected. For instance, the intensity of **ON_NEAR_G^{FT}** and **ON_NEAR_G^{NH2}** on agarose gels comprised only 9% of the intensity of **ON_NEAR** of the same concentration. This was the reason why these products were overlooked in our previous work.[6] A similar decrease in the intensity of the **dG^{X}**-containing spots on gels, proportional to the density of labeling, was also observed in PEX products with different ratios of **dG^{X}** and dG prepared using Vent(exo-) polymerase, the **TempPEX-bio** template, and the **PrimPEX** primer. However, the fluorescence of PEX products made from 100% **dG^{X}TP**s was higher than in that the case of NEAR products since only a part of the PEX products sequence is modified (Figure S3, Supporting Information).

As mentioned above, a number of papers previously reported the decrease of ethidium bromide fluorescence upon intercalation in DNA containing 7-deazaguanine and 7-halogeneted 7-deazaguanine compared to that in natural DNA.[7−13] From our results with GelRed-stained gels and **dG^{X}** DNA, it may be concluded that quenching of fluorescence takes place also in the case of 7-aryl-7-deazaguanine DNA intercalated by ethidium derivatives. To prove that the quenching of fluorescence really occurs, we studied the fluorescence of GelRed and its complexes with DNA in solutions using time-resolved fluorescence spectroscopy.

Fluorescence decay curves of GelRed and its complexes with either unmodified ssDNA (**ON_NEAR**) or the **dG^{NH2}**-containing analogue (**ON_NEAR_G^{NH2}**) are shown in Figure 2a. Fluorescence lifetimes are much longer for the GelRed-**ON_NEAR** complex compared to that of GelRed in water (the mean lifetimes calculated from the 3-component reconvolution fitting are 12.1 and 1.3 ns, respectively). These results are in line with the measurements of lifetimes of monomeric ethidium bromide intercalated in dsDNA.[9] Comparison of the decay curves of GelRed with oligonucleotides **ON_NEAR** and **ON_NEAR_G^{NH2}** differing only by the presence of modified G reveals a strong quenching effect of 7-aryl-7-deazaguanine. The shortening of the mean lifetime from 12.1 to 4.3 ns indicates that the quenching is a dynamic process occurring with the singlet excited state of the fluorophore. To obtain more information about the nature of the quenching, we performed the Stern−Volmer titration experiment in time-resolved mode. We studied fluorescence decays of the GelRed-**ON_NEAR** complex in the presence of 2′-deoxy-7-(5-formylthiophene-2-yl)-7-deazaguanosine 5′-*O*-monophosphate (**dG^{FT}MP**) as an external model quencher (Figure 2b). Brief analysis of the decays confirms that **dG^{FT}** is an extremely efficient quencher of fluorescence. The Stern−Volmer plots (Figures 2c and S5, Supporting Information) show that both the fluorescence lifetime and intensity decrease in the presence of the quencher. The fluorescence intensity decreases more strongly than the lifetime in 0−1 mM quencher concentration range. This indicates that **dG^{FT}MP** can act both as static and dynamic quencher. However, the saturation of the F/F_0 curve at higher concentrations (Figures 2 and S5, Supporting Information) indicates the existence of a fraction of fluorophores inaccessible to the quencher. Altogether, the fluorescence lifetime measurements show that 7-aryl-7-deazaguanines are efficient quenchers of fluorescence of GelRed acting both as static and dynamic quenchers.

An attempt was also made to identify another fluorogenic DNA stain which could substitute GelRed in detection assays. A number of commercially available gel dyes, commonly used for DNA detection on agarose gels, were tested for the visualization of ssONs (NEAR products) containg **dG^{X}**. Unfortunately, none of the tested dyes (GelRed, GelGreen, SYBR Gold, SYBR Green I and II, and SYBR Safe) exhibited any fluorescence in the presence of ssONs bearing 7-deazaguanosine derivatives (Supporting Information, Figure S6).

CONCLUSIONS

We show here that the scope of the NEAR is wider and more general than reported previously[6] and that the method works well also for the synthesis of ONs containing 7-substituted 7-deazaguanines (as well as 5-substituted pyrimidines and 7-substituted 7-deazaadenines reported previously[5,6]). However, radioactivity-based assays must be used for the detection of these 7-deazaG-modified ONs on gels because these bases efficiently quench the fluorescence of fluorogenic DNA stains.

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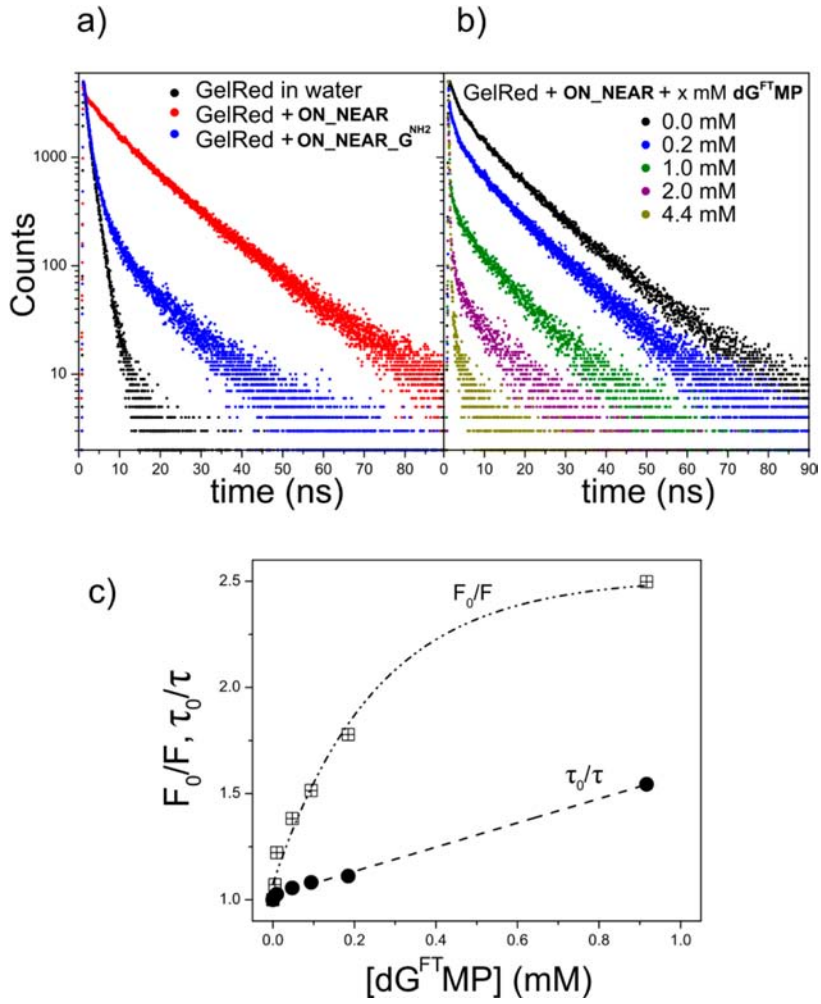

Figure 2. (a) Fluorescence decay curves for GelRed in water (black), GelRed in the presence of **ON_NEAR** (red), and GelRed in the presence of **ON_NEAR_G^{NH2}** (blue); (b) fluorescence decay curves of the GelRed-**ON_NEAR** complex in the presence of various concentrations of **dG^{FT}MP**; (c) Stern−Volmer plot for the quenching of ssDNA-intercalated GelRed by **dG^{FT}MP**.

EXPERIMENTAL SECTION

General Remarks. Synthesis and characterization data for 7-iodo-2′-deoxy-7-deazaguanosine 5′-*O*-triphosphate (**dG^{I}TP**),[16] 7-(3-aminophenyl)-2′-deoxy-7-deazaguanosine 5′-*O*-triphosphate (**dG^{NH2}TP**),[17] 7-(5-formylthiophene-2-yl)-2′-deoxy-7-deazaguanosine 5′-*O*-triphosphate (**dG^{FT}TP**),[6] 7-(3-aminophenyl)-2′-deoxy-7-deazaadenosine 5′-*O*-triphosphate (**dA^{NH2}TP**),[14] 5-(3-aminophenyl)-2′-deoxycytidine 5′-*O*-triphosphate (**dC^{NH2}TP**),[14] and 5-(3-aminophenyl)-2′-deoxyuridine 5′-*O*-triphosphate (**dU^{NH2}TP**)[14] were reported previously. For the synthesis of 7-(5-formylthiophene-2-yl)-2′-deoxy-7-deazaguanosine 5′-*O*-monophosphate (**dG^{FT}MP**), see the Supporting Information.

Synthetic oligonucleotides (NEAR template **TempNick**, NEAR primer **PrimNick**, biotinylated PEX template **Temp-PEX-bio**, PEX primer **PrimPEX**; ss ladder components **L10-L100**; for sequences, see Table 1) were purchased from Generi Biotech. The single-stranded ladder was prepared according to a published procedure. The double-stranded 20 bp ladder was obtained from Takara. SYBR Green, SYBR Gold, SYBR Safe, and Nucleic Acid Stains Dimer Sampler Kit were purchased from Life Technologies, and GelGreen and GelRed from Lab Mark. Enzymes Vent(exo-) DNA polymerase and Nt.BstNBI, as well as natural nucleoside triphosphates (dATP, dCTP, dGTP, and dTTP), were purchased from New England Biolabs. KOD XL DNA polymerase was purchased from Merck. 2′-Deoxy-7-deazaguanosine 5′-*O*-triphosphate (**dG^{7d}TP**) was obtained from Jena Bioscience. Streptavidin Magnetic Particles were obtained from Roche. Acetonitrile for HPLC and TEAA buffer were purchased from Sigma-Aldrich. All solutions were prepared in Milli-Q water. Oligonucleotide samples were concentrated on CentriVap Vacuum Concentrator System (Labconco). Mass spectra of the prepared ONs were measured by MALDI-TOF, on UltrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Germany), with 1 kHz smartbeam II laser. UV spectra were measured on NanoDrop1000 (ThermoScientific).

NEAR General Procedure. The reaction mixture contained the template (0.125 μM), primer (0.125 μM), modified **dN^{X}TP** (156 μM), natural dNTPs (125 μM), 1× ThermoPol buffer (10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl/pH 8.8/, 0.1% Triton-X-100, and 2 mM $MgSO_4$), and 0.5× NEBuffer 3 (50 mM NaCl, 25 mM Tris-HCl/pH 7.9/, 5 mM $MgCl_2$, and 0.5 mM DTT). The amounts of enzymes depend on whether only natural dNTPs or modified **dN^{X}TP** together with the three remaining natural dNTPs were incorporated, being 0.075 U/μL Vent(exo-), 0.90 U/μL Nt.BstNBI and 0.10 U/μL Vent(exo-), and 0.60 U/μL Nt.BstNBI, respectively. The reaction mixture

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was incubated at 55 °C for 3 h. The reaction was stopped by cooling to 4 °C.

NEAR on an Analytical Scale. The analytical reactions were performed according to the general procedure in a volume of 30 μL. The products were analyzed by agarose gel electrophoresis using 4% agarose gels stained with GelRed (Lab Mark). Samples were prepared by mixing 1.6 μL of 6× DNA Loading Dye (Thermo Scientific) and 8 μL of the reaction mixture or ss DNA ladder. The gel was run for 70 min at 120 V and imaged using an electronic dual wave transilluminator equipped with GBox iChemi XRQ Bio imaging system (Syngene). For MALDI-TOF mass spectrometry analysis, the excess nucleotides and buffer salts were removed by filtration on Illustra MicroSpin G-25 columns (GE Healthcare).

NEAR on Preparative Scale. The preparative reactions were performed according to the general procedure in a volume of 750 μL. After the reaction was stopped, the solution was concentrated on a vacuum concentrator to approximately 150 μL. The viscous concentrate was injected on an HPLC XBridge OST C_{18} Column (Waters; 2.5 μm particle size, 4.6 mm × 50 mm) and separated using a gradient of triethylammonium acetate (TEAA) and acetonitrile at a flow rate of 1 mL/min. Mobile phase A corresponds to 0.1 M TEAA in HPLC-grade water, mobile phase B to acetonitrile/0.1 M TEAA in HPLC-grade water 20/80 (v/v). The gradient started with 60% mobile phase A and 40% mobile phase B, going linearly to 80% mobile phase B in 53 min. The fractions containing the product were evaporated on a vacuum concentrator. The residue was diluted with a known volume of water, and a UV spectrum was measured. The concentration of the product was calculated from the absorbance at 260 nm and the extinction coefficient obtained from an online calculator by IDT Biophysics.[18] The products were analyzed by MALDI-TOF mass spectrometry.

NEAR with in Situ Radiolabeling. In-situ radiolabeling NEAR reactions (30 μL) were performed according to the general procedure, adding modified **dN^{X}TP** (156 μM), natural dNTPs (125 μM), and [α-^{32}P]-dATP (222 TBq/mmol, 370 MBq/mL, 0.4 μL). The reaction mixture was incubated at 55 °C for 3 h. The reaction was stopped by cooling to 4 °C. The NEAR products were purified on Illustra MicroSpin G-25 columns (GE Healthcare). Samples (2 μL) were mixed with PAGE stop solution (2 μL, 80% [v/v] formamide, 20 mM EDTA, 0.025% [w/v] bromophenol blue, and 0.025% [w/v] xylene cyanol) and subjected to vertical electrophoresis in 15% denaturing polyacrylamide gel containing 1× TBE buffer (pH 8) and 7 M urea at 42 mA for 50 min. The gels were dried (85 °C, 50 min), audioradiographed, and visualized by a phosphorimager (Typhoon FLA 9500, GE Healthcare).

Post-NEAR Radiolabeling. The NEAR was performed according to the general procedure in a volume of 30 μL. The excess nucleotides and buffer salts were removed by filtration on an Illustra MicroSpin G-25 column (GE Healthcare). To the filtrate, T4 DNA polynucleotide buffer (5 μL), T4 polynucleotide kinase (2 μL), and [γ-^{32}P]-ATP (2 μL) were added. The total volume was adjusted to 50 μL by adding Milli-Q water. The mixture was incubated at 37 °C for 1 h. After the incubation, the mixture was once more purified by filtration on an Illustra MicroSpin G-25 column (GE Healthcare). The aliquot (2 μL) was mixed with PAGE stop solution (2 μL, 80% [v/v] formamide, 20 mM EDTA, 0.025% [w/v] bromophenol blue, and 0.025% [w/v] xylene cyanol) and subjected to vertical electrophoresis in 15% denaturing polyacrylamide gel containing 1× TBE buffer (pH 8) and 7 M urea at 42 mA for 50 min. The gel was dried (85 °C, 50 min), audioradiographed, and visualized by a phosphorimager (Typhoon FLA 9500, GE Healthcare).

Primer Extension on Preparative Scale. The reaction mixture (150 μL) contained Vent(exo-) DNA polymerase (2 U/μL, 6 μL), natural dNTPs (4 mM, 7.5 μL), modified **dG^{X}TP** (4 mM, 10 μL), primer **PrimPEX** (100 μM, 3 μL), and 5′-biotinylated template **TempPEX-bio** (100 μM, 3 μL) in ThermoPol reaction buffer (15 μL) supplied by the manufacturer. The reaction mixture was incubated for 20 min at 60 °C in a thermal cycler. The reaction was stopped by cooling to 4 °C. The products were purified by QIAquick Nucleotide Removal Kit (Qiagene).

Analysis of GelRed fluorescence quenching from agarose gels. Agarose gel was prestained with GelRed. The samples were mixed with loading dye (6× DNA Loading Dye, Thermo Scientific) and loaded on the gel in different ratios natural/modified DNA (from 100% natural to 100% modified, lowering the amount of natural DNA by 20% in each step). After running, the gel was imaged using electronic dual wave transilluminator equipped with GBox iChemi XRQ Bio imaging system (Syngene). Band intensities were calculated using ImageJ program.[19]

NEAR products (ss short ONs) were analyzed on 4% agarose gel. Each sample contained 4 μL of 10 μM solution (prepared by mixing natural and modified DNA in the given ratio), 1 μL of water, and 1 μL of loading dye. The gel was run at 120 V for 70 min.

PEX products (ds short ONs) were analyzed on 4% agarose gel. Each sample contained 4 μL of 2.4 μM solution (prepared by mixing natural and modified DNA in the given ratio), 1 μL of water, and 1 μL of loading dye. The gel was run at 120 V for 70 min.

Testing of the Fluorescent Quenching of Different Dyes. *GelRed, GelGree, SYBR Safe, SYBR Green I and II, and SYBR Gold.* Four percent agarose gel was prestained with the dye according to the manufacturer's instructions. After running, the gel was imaged using an electronic dual wave transilluminator equipped with a GBox iChemi XRQ Bio imaging system (Syngene).

Preparation of Samples for Time-Resolved Fluorescence Measurements. For the comparison of fluorescence decays of GelRed with natural and modified DNAs, solutions of GelRed (0.5× in water), **ON_NEAR**-GelRed complex (1 μM **ON_NEAR**, 0.5× GelRed in water), and **ON_NEAR_G^{NH2}**-GelRed complex (1 μM **ON_NEAR_G^{NH2}**, 0.5× GelRed in water) were prepared.

For the Stern−Volmer quenching studies, a solution of **ON_NEAR**-GelRed complex (1 μM ON_NEAR, 0.5× GelRed) in water was titrated by a solution of **dG^{FT}MP** in water.

Time-Resolved Fluorescence Measurements. All fluorescence lifetime measurements were performed at room temperature (23 °C). The time correlated single photon counting (TCSPC) setup (model 5000U, IBH, Glasgow, U.K.) includes a 470 nm diode laser (PicoQuant, Berlin, Germany) as the excitation source and a cooled Hamamatsu R3809U-50 microchannel plate photomultiplier. Excitation and emission monochromators were used to choose appropriate wavelengths, i.e., 470 ± 4 nm and 640 ± 16 nm. An emission cutoff filter of 500 nm was used to further limit the influence of light scattering. Full-width-at-half-maximum of the instrument

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response function (IRF) measured for the scattering solution was 110 ps, which results in a temporal resolution of ~22 ps.

Mean fluorescence lifetime was obtained either from the multiexponential reconvolution fitting performed using FluoFit v.4.5 by PicoQuant (Berlin, Germany) or from the calculation according to the following equation:

$$\tau = \frac{\int_0^\infty t \cdot F(t)\mathrm{d}t}{\int_0^\infty F(t)\mathrm{d}t} - \frac{\int_0^\infty t \cdot \mathrm{IRF}(t)\mathrm{d}t}{\int_0^\infty \mathrm{IRF}(t)\mathrm{d}t}$$

where t is the time after excitation and $F(t)$ the fluorescence decay.

■ ASSOCIATED CONTENT

Supporting Information

Agarose gel analysis of radiolabeled NEAR products; analysis of GelRed fluorescence quenching of NEAR products on agarose gels and PEX products from agarose gels; HPLC chromatograms of crude NEAR mixtures; Stern-Volmer plot for the quenching of GelRed−**ON_NEAR** by **dG^{FT}MP**; testing of different gel dyes; and copies of NMR and MALDI spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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