Bioconjugate Chemistry

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General Method for Post-Synthetic Modification of Oligonucleotides Based on Oxidative Amination of 4-Thio-2′-deoxyuridine

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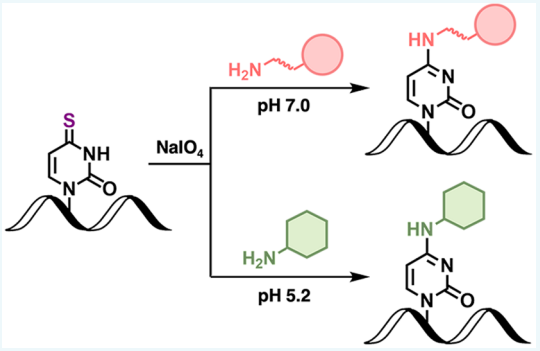
ABSTRACT: Functionalized oligonucleotides (ONs) are widely applied as target binding molecules for biosensing and regulators for gene expression. Numerous efforts have been focused on developing facile methods for preparing these useful ONs carrying diverse modifications. Herein, we present a general method for postsynthetic modification of ONs via oxidative amination of 4-thio-2′-deoxyuridine (4SdU). 4SdU-containing ON can be derived by both alkyl and aromatic amines. Using this approach, ONs are successfully attached with alkyne/azide, biotin and dansylamide moieties, and these as-prepared ONs possess the expected biorthogonal reactivity, streptavidin affinity and fluorescent property, respectively. Furthermore, we also directly install fluorophores to the ON nucleobase based on oxidative amination of 4SdU, and these fluorophores exhibit distinct luminescence behaviors before and after conjugation. We believe our method will be a versatile strategy for constructing various functionalized ONs used in a wide range of nucleic acid applications.



INTRODUCTION

Oligonucleotides (ONs) are chemically synthesized short nucleic acids, which can specifically recognize DNA or RNA sequences via complementary Watson−Crick base pairing.[1] ONs can serve as recognition units for identifying the nucleic acid analytes with certain sequences through hybridization, and usually modified with reporter units to construct the biosensors for nucleic acid quantification and imaging analysis, including real-time PCR,[2,3] complementary DNA microarray,[4,5] fluorescence in situ hybridization,[6,7] and live-cell RNA imaging.[8−10] On the other hand, ONs can also be rationally designed as drugs according to the sequences of target genes and used for treatment of cancer, neuropathy, musculoskeletal disorder, ophthalmopathy, and other diseases by specifically regulating the gene expression.[11] ON-based pharmaceuticals, such as antisense ONs[12,13] and small interfering RNAs,[14,15] are extensively modified to improve nuclease resistance, delivery efficiency, and cell targeting ability.[16,17] Consequently, bioconjugation of ONs for desired functions is essential to develop analytical tools for nucleic acids and improve the therapeutic efficacy of ONs-based medicines.

A variety of functional groups can be introduced into ONs through either solid-phase synthesis or postsynthetic modification. Solid-phase synthesis functionalizes ONs by using noncanonical phosphoramidite monomers.[16,18] Nevertheless, the procedures of solid-phase synthesis usually need case-by-case optimization for incorporating the modified building blocks, as well as the harsh conditions like strong acids, alkalis, and oxidants, make it challenging to modify ONs with labile groups through this approach.[19,20] Postsynthetic modifications utilize the easy-to-obtained ONs bearing reactive handles for conjugation of desired labels under mild conditions, providing a simpler, more cost-effective and universal method for functionalization of ONs compared to solid-phase synthesis.[19,21] Several reactions have been applied for postsynthetic modification of ONs, for instance, condensation of amine with activated esters,[22,23] thiol−ene addition,[24,25] azide−alkyne cycloaddition,[26,27] and inverse electron demand Diels−Alder reaction.[28,29] Although able to produce ONs with the required functions, these strategies hardly yield the ONs with extended pi-system, for example, fluorescent nucleobase-containing ONs, which usually exhibit unique photophysical characteristics.[30,31] In recent years, the Suzuki-Miyaura reaction has emerged for postsynthetic arylation of ONs, reacting aryl boronic acids/esters with halogenated nucleobases mediated by palladium.[32,33] Using this strategy, fluorogenic ON conjugates are prepared and found sensitive to the environment of neighboring bases, serving as probes for studying nucleic acid interactions and conformations.[34,35] However, the transition metal catalysts used in Suzuki-Miyaura coupling make it tough for removal of the potentially toxic

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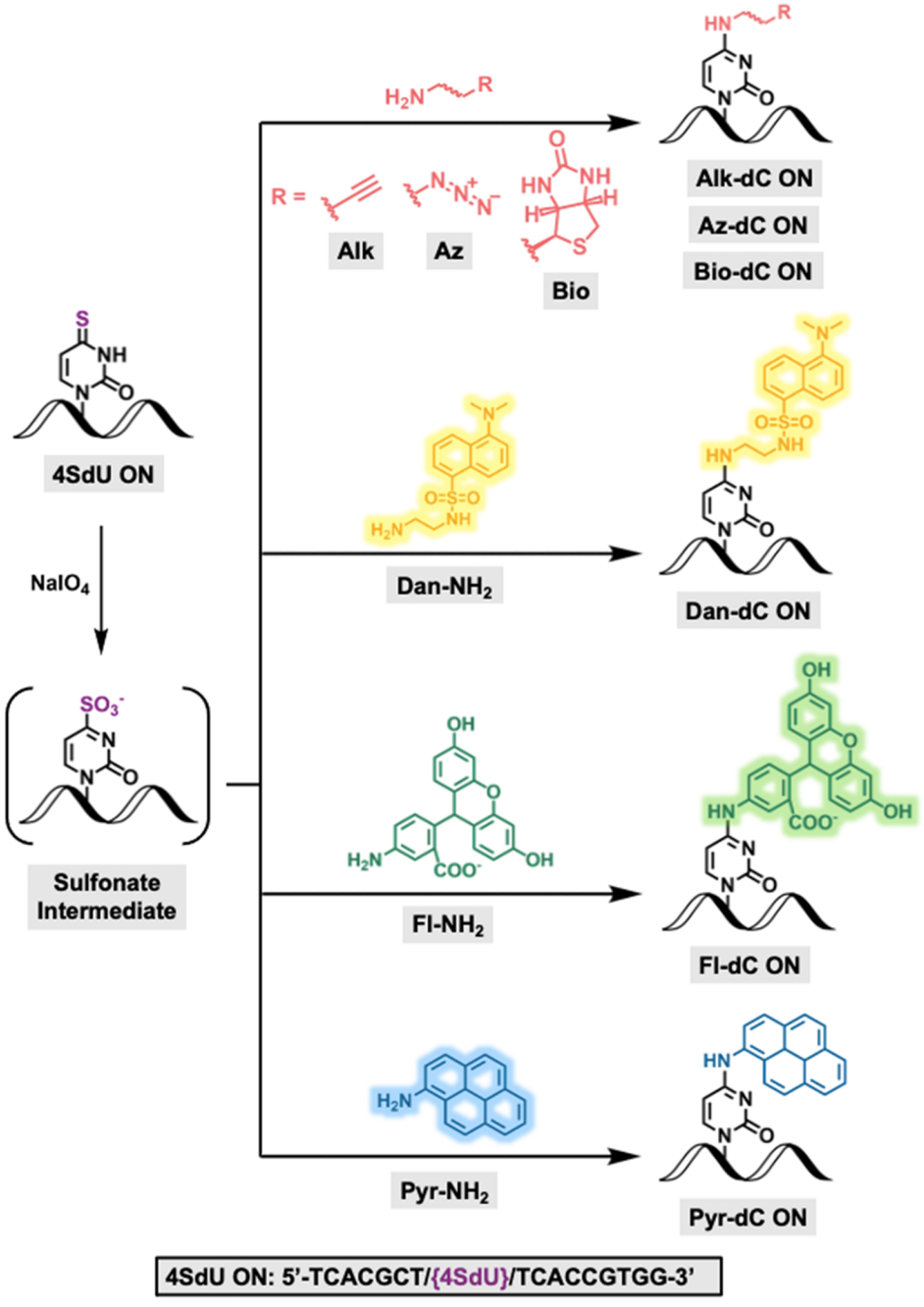
Scheme 1. General Method for Post-Synthetic Modification of 4-Thio-2′-deoxyuridine-Containing Oligonucleotide (4SdU ON) with $NaIO_4$ and Functional Amines via Oxidative Amination Reaction



metals from the reaction solution.[36] Therefore, a general reaction still remain in need for postsynthetic modification of ONs, especially for postsynthetic construction of arylated nucleobases on ONs in transition metal-free manner.

In this work, we developed a general method for postsynthetic modification of ONs through oxidative amination of 4-thio-2′-deoxyuridine (4SdU), converting the 4SdU on ONs into deoxycytidine (dC) derivates by sodium periodate ($NaIO_4$) and multiple amines (Scheme 1). We found both alkyl and aromatic amines could be used to derive 4SdU on ONs, thereby allowing a series of functional groups introduced into ONs. Using this versatile method, we successfully synthesized ONs carrying alkyne/azide for biorthogonal chemistry, biotin for streptavidin pull-down, and dansylamide for fluorescent signal generation. We also created arylated cytosine in ONs attached with two fluorophores, fluorescein and pyrene, respectively at the nitrogen of cytosine 4-position, showing different fluorescence intensity changes before and after the derivatization.

RESULTS AND DISCUSSION

Design of General Method for Post-Synthetic Modification of 4SdU ON through Oxidative Amination. The 4-thiouracil is a noncanonical nucleobase replacing the oxygen at the uracil 4-position with a sulfur, which acts as both a naturally occurring base in prokaryotic tRNAs[37,38] and

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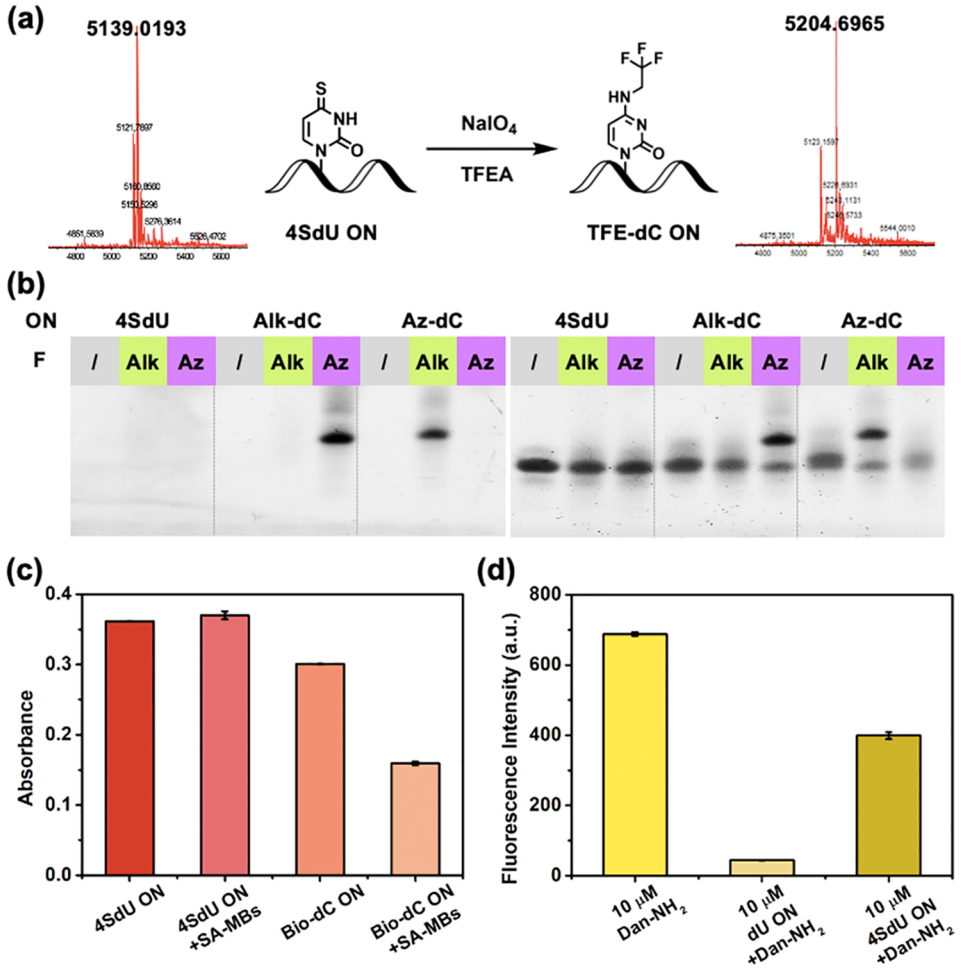

Figure 1. Modification of 4SdU ON with alkyl amines through oxidative amination. (a) MALDI-TOF MS analysis of 4SdU ON before (left, 4SdU ON, calc. 5139.4, found 5139.0) and after (right, TFE-dC ON, calc. 5204.4, found 5204.7) the treatment with $NaIO_4$ and TFEA. (b) PAGE analysis of ONs treated with fluorescent alkyne or azide through CuAAC reaction. The gel was imaged without staining (left) and subsequently stained by SYBR Gold (right). (c) Absorbance at 260 nm of 4SdU ON and Bio-dC ON before and after SA-MB adsorption. (d) Fluorescence intensity at 515 nm of 10 μM Dan-NH_2, 10 μM dU ON, and 10 μM 4SdU ON treated with $NaIO_4$ and Dan-NH_2. Condition: 90% DMF + 10% 10 mM sodium phosphate buffer (pH 7.0), λ_{ex} = 343 nm.

metabolic label for studying RNA dynamics.[39,40] The aryl thiol, a resonance form of aromatic thioketone in 4-thiouracil, can be oxidized to sulfonate intermediates and further substituted by various nucleophiles such as alcohols, amines, and mercaptans.[41−43] This chemical reactivity of 4-thiouridine (s^4U), the ribonucleosides of 4-thiouracil, has been utilized for the researches of RNA metabolism through s^4U labeling and recording.[44,45] We speculate this reaction can also be adopted for modification of ONs with diverse chemical groups using the corresponding amines. The deoxyribonucleoside version of 4-thiouracil, 4-thio-2′-deoxyuridine (4SdU) can be incorporated into ONs through a well-developed solid-phase synthesis method, and 4SdU-containing ONs are commercially available from vendors.[46,47] Thus, we consider 4SdU may serve as a reactive handle for postsynthetic modification of ONs, and a series of functional amines can be derived to ONs via oxidative amination of 4SdU (Scheme 1).

Modification of 4SdU ON with Alkyl Amines through Oxidative Amination. We initially studied the performance of oxidative amination of a 4SdU-containing ON (4SdU ON, Scheme 1) with 10 mM $NaIO_4$ and 600 mM trifluoroethylamine (TFEA) in 100 mM sodium acetate buffer (pH 5.2), which condition has been utilized to derive s^4U in RNAs.[45] Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) and high performance liquid chromatography coupled with ultraviolet detector (HPLC-UV) analysis revealed the effective amination of 4SdU ON to give the product, TFE-dC ON with small amount of hydrolysis byproduct (Figures 1a, S1, and S2 and Table 1). Meanwhile, to understand the necessary role of 4SdU for the oxidative amination, we also carried out the experiment under the same condition using dU ON, and ON with the same sequence as 4SdU ON except for altering the 4SdU with dU. As expected, no detectable oxidative amination product was observed for dU ON as characterized by MS (Figure S3), suggesting the TFEA derivatization indeed occur selectively on 4SdU. To further illustrate the reaction mechanism, we conducted oxidative amination on the small molecule 4-thiouracil with $NaIO_4$ and TFEA and found the generation of TFE-derived cytosine according to nuclear magnetic resonance (NMR) analysis (Figure S4).

TFEA is a relatively weak base and its protonated form has a low pK_a value (pK_a = 5.7), which ensures sufficient TFEA molecules presented as the nonprotonated form in the acetate

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Table 1. Yields of Isolated Products Obtained by the Oxidative Amination Reaction of 4SdU ON with $NaIO_4$ and the Amines

sample name	yields of isolated products
TFE-dC ON	93%
Et-dC ON	84%
Bz-dC ON	92%
Alk-dC ON	91%
Az-dC ON	82%
Bio-dC ON	76%
Dan-dC ON	74%
Ph-dC ON	95%
Fl-dC ON	66%
Pyr-dC ON	63%

buffer (pH 5.2). Considering the majority of alkyl amines tend to be protonated in acidic solution, we adjusted the pH condition to neutral for keeping the alkyl amines in our work reactive to oxidative amination. First, 100 mM ethylamine (Et-NH_2) and benzylamine (Bz-NH_2) were tested to react with 4SdU ON and 10 mM $NaIO_4$ in 50 mM sodium phosphate buffer (pH 7.0) containing 25% volume of dimethylformamide (DMF). The amination products, Et-dC ON and Bz-dC ON, appeared predominantly in MALDI-TOF MS and HPLC-UV analysis (Figures S5 and S6 and Table 1), indicating oxidative amination of 4SdU ON could still proceed with common alkyl amines. Then, we further evaluated oxidative amination of 4SdU with alkyl amines carrying useful functional groups, including alkyne (Alk) and azide (Az) for biorthogonal chemistry, biotin (Bio) for streptavidin adsorption, and dansylamide (Dan) for fluorescent signal generation. A series of alkyl amine-modified ONs were obtained as Alk-dC ON, Az-dC ON, Bio-dC ON, and Dan-dC ON respectively, and their production were evidenced via MALDI-TOF MS and HPLC-UV analysis (Figures S7 and S8 and Table 1).

Next, we examined the functions of these alkyl amine-derived ONs. We first performed the copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction on 4SdU ON, Alk-dC ON, and Az-dC ON, each with fluorescent alkyne and azide, ALKYNE-FLUOR 488 (Alk488) and AZIDE-FLUOR 545 (Az545). Polyacrylamide gel electrophoresis (PAGE) analysis demonstrated click reactions selectively occurred between Alk-dC ON and Az545 as well as Az-dC ON and Alk488 (Figure 1b). MALDI-TOF MS analysis of the CuAAC products, Az545-Alk-dC ON and Alk488-Az-dC ON, showed no unreacted Alk-dC ON and Az-dC ON left after reaction with Az545 and Alk488, suggesting Alk-dC ON and Az-dC ON were highly active for biorthogonal chemistry (Figure S9). We next investigated the interaction between Bio-dC ON and streptavidin magnetic beads (SA-MBs) by UV spectral and PAGE analysis. Following the user guide, 4SdU ON and Bio-dC ON were treated with SA-MBs adsorption. Based on the quantification of original and leftover ONs by UV absorbance at 260 nm, we found approximately 47% of molecules were adsorbed by SA-MBs from the samples of Bio-dC ON, while 4SdU ON stayed almost untouched (Figures 1c and S10). The specific binding of Bio-dC ON to SA-MBs was further verified by disappearance of upper biotinylated ON band in the PAGE analysis (Figure S11). Collectively, Bio-dC ON prepared by our method preserved the capacity of biotin for streptavidin-binding. Then we measured the fluorescence of Dan-dC ON and compared it with that of Dan-NH_2. The fluorescence intensity of 10 μM Dan-dC ON was at the same order of

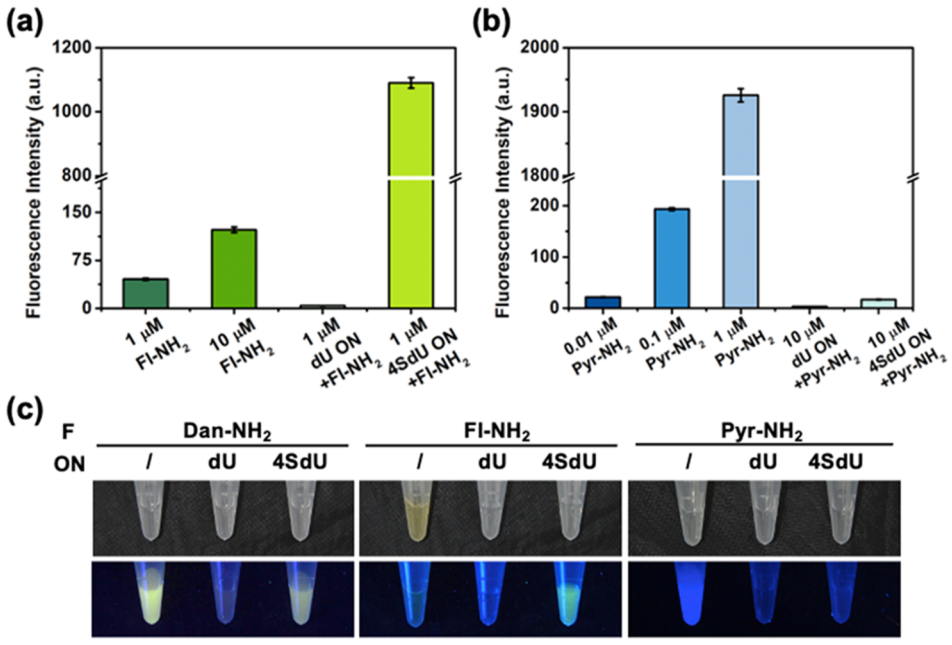

Figure 2. Modification of 4SdU ON with aromatic amines through oxidative amination. (a) Fluorescence intensity at 520 nm of 1 μM Fl-NH_2, 10 μM Fl-NH_2, 1 μM dU ON, and 1 μM 4SdU ON treated with $NaIO_4$ and Fl-NH_2. Condition: 100 mM sodium phosphate buffer (pH 8.0), λ_{ex} = 490 nm. (b) Fluorescence intensity at 436 nm of 0.01 μM Pyr-NH_2, 0.1 μM Pyr-NH_2, 1 μM Pyr-NH_2, 10 μM dU ON, and 10 μM 4SdU ON treated with $NaIO_4$ and Pyr-NH_2. Condition: 90% DMF + 10% 10 mM sodium phosphate buffer (pH 7.0), λ_{ex} = 372 nm. (c) The images of amino-containing fluorophores as well as ONs treated with $NaIO_4$ and the corresponding fluorophores under white light (top) and UV light (365 nm, bottom).

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magnitude with that of 10 μM Dan-NH_2, though there was about 40% reduction of fluorescence mainly due to uncomplete fluorescence labeling of ON (Figure 1d). The 10 μM dU ON treated with $NaIO_4$ and Dan-NH_2 displayed weak fluorescence, which was not arising from covalent bond formation between Dan-NH_2 and dU ON because there were no visible Dan-derived ON bands emerged in PAGE analysis (Figure S12). Accordingly, the chemically modified ONs prepared by our method reserved the functions of modifications, revealing that oxidative amination did not cause undesired chemical changes to functional groups.

Modification of 4SdU ON with Aromatic Amines through Oxidative Amination. After the achievement in modifying 4SdU ON with various alkyl amines as shown above, we set to explore whether the oxidative amination of 4SdU ON was also compatible with aromatic amines. In principle, when oxidative amination occurs between aromatic amine and 4-thiouracil, arylated cytosine may be formed. As an extended pi-system, arylated cytosine potentially exhibit unparalleled properties and valuable functions. First, to testify the tolerance of oxidative amination to aromatic amines, we applied 250 mM aniline (Ph-NH_2) to react with 4SdU ON and 10 mM $NaIO_4$ in 100 mM sodium acetate buffer (pH 5.2) containing 50% volume of DMF. The efficient production of Ph-dC ON was validated by MALDI-TOF MS and HPLC-UV analysis (Figures S13 and S14 and Table 1). Subsequently, we moved on to select two aromatic amine-containing fluorophores, aminofluorescein (Fl-NH_2) and aminopyrene (Pyr-NH_2) for 4SdU amination on the ON, and the obtained ON conjugates, Fl-dC ON and Pyr-dC ON were characterized by MALDI-TOF MS and HPLC-UV analysis (Figures S15 and S16 and Table 1). The selectivity of oxidative amination for Fl-NH_2 and Pyr-NH_2 conjugated to 4SdU ON was analyzed by PAGE, where 4SdU ON derived by Fl-NH_2 and Pyr-NH_2 migrated slightly slower and faster than 4SdU ON respectively, while dU ON could be derived by neither Fl-NH_2 nor Pyr-NH_2 (Figure S12).

Afterward, we investigated the fluorescence properties of Fl-dC ON and Pyr-dC ON. The solution of 1 μM Fl-dC ON exhibited 24-fold fluorescence enhancement than 1 μM Fl-NH_2 in the same buffer, while the fluorescence of dU ON after treatment with $NaIO_4$ and Fl-NH_2 was negligible (Figure 2a). On the contrary, 10 μM Pyr-dC ON reduced the fluorescence to less than 1% of even 1 μM Pyr-NH_2 in the same solvent, and dU ON treated with $NaIO_4$ and Pyr-NH_2 was almost nonfluorescent (Figure 2b). This phenomenon is attributed to the different mechanisms of derivation-caused fluorescence intensity change for Fl-NH_2 and Pyr-NH_2. Fl-NH_2 has a low quantum yield due to intramolecular excited-state electron transfer from the amino-substituted phenyl to xanthene ring. Fl-NH_2 recovers the strong luminous emission of fluorescein when its primary amine is made unavailable by covalent labeling or electrostatic interaction.[48] On the other hand, Pyr-NH_2 itself is a high quantum yield fluorophore in organic solvents. When the amine of Pyr-NH_2 is attached with an electron-drawing group, its fluorescence will be quenched resulting from photoinduced electron transfer (PET) effect.[49] In comparison to the ON modified with alkyl amine-containing fluorophore like Dan-NH_2 retaining its intrinsic emission characteristic, conjugation of aromatic amine-containing fluorophores directly to the heterocycle of ON nucleobase could significantly affect their luminescent properties (Figure 2c). Based on these derivatization-caused fluorescence intensity change, we could further confirm the formation of fluorophore-arylated cytosine in ONs. Therefore, our method using oxidative amination of 4SdU ON could be a promising approach for facile creation of arylated nucleobase in ONs.

CONCLUSIONS

In summary, we developed a general method for postsynthetic modification of ONs with a wide range of functional groups based on oxidative amination of 4SdU. Both alkyl amines and aromatic amines could be used for modifying 4SdU ON through our method. The as-prepared ONs, including Alk-dC ON, Az-dC ON, Bio-dC ON, and Dan-dC ON, reserved the activities of their conjugated functional moieties for bioorthogonal chemistry, streptavidin adsorption and fluorescing respectively after the oxidative amination. Our postsynthetic modification approach, in particular, could produce the arylated cytosine in ONs. The obtained fluorophore-arylated ONs, Fl-dC ON, and Pyr-dC ON exhibited altered fluorescence properties, compared to the aromatic amine fluorophores before derivatization. Despite the requirement of high concentration amines for efficient oxidative amination, we still expect this method will be a promising strategy to construct various functionalized ONs for nucleic acid–based biosensing and therapy.

EXPERIMENTAL SECTION

Oxidative Amination Reaction of Nucleic Acids with TFEA. The oxidative amination reaction was conducted in an aqueous solution according to the literature:[45] $NaIO_4$ (100 mM, 4 μL) was added to a solution of oligonucleotides (200 μM, 10 μL), EDTA (10 mM, 4 μL), TFEA (1.88 μL, 24 μmol), and NaAc-HAc buffer (pH 5.2, 200 mM, 20 μL). The reaction was incubated at 45 °C for 1 h. Then, the oligonucleotides were purified using Amicon-3k ultrafilter with water to remove small molecules.

^{1}H NMR Analysis of 4-Thiouracil Treated with $NaIO_4$ and TFEA. 4-Thiouracil (3.2 mg, 25 μmol) and $NaIO_4$ (9.1 mg, 42.5 μmol) were dissolved in 250 μL of DMSO-d_6 respectively. TFEA (2.6 μL, 33 μmol) was added into 4-thiouracil. Subsequently $NaIO_4$ was added into 4-thiouracil and TFEA mixture. The reaction was incubated at 45 °C for 4 h, and ^{1}H NMR spectrum was measured immediately.

Synthesis of Dansyl Ethylenediamine. Dansyl ethylenediamine (Dan-NH_2) was prepared according to a published procedure:[50] Dansyl chloride (Dan-Cl, 487.39 mg, 1.8 mmol) was dissolved in 20 mL of DCM. The Dan-Cl was added dropwise to a 50 mL of DCM solution of ethylenediamine (6 mL, 90 mmol) in ice bath conditions. The mixture was stirred at room temperature for 1 h. The reaction was monitored by TLC with ethyl acetate vs acetone = 1:1 (V/V). The solution was acidified by 150 mL of 1 M HCl. The aqueous phase was collected and basified by 30 mL of 5 M NaOH. The product was extracted by 100 mL of DCM. The organic phase was dried over Na_2SO_4 and evaporated to dryness. Dan-NH_2 was obtained as a gray-white solid (461.3 mg, yield 87.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, 1H), 8.29 (d, 1H), 8.22 (d, 1H), 7.53 (dd, 1H), 7.49 (dd, 1H), 7.15 (d, 1H), 2.88 (dd, 2H), 2.86 (s, 6H), 2.67 (dd, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 151.89, 136.61, 129.88, 129.62, 129.59, 128.82, 128.34, 124.12, 119.61, 115.63, 46.65, 45.58, 42.01. ESI: calc.

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for $C_{14}H_{19}O_2N_3S$, $[M + H]^+$ 294.12762, found 294.12695; $[M-H]^-$ 292.11197, found 292.11230.

Oxidative Amination Reaction of Nucleic Acids with Alkyl Amines. $NaIO_4$ (100 mM, 4 μL) was added to a solution of oligonucleotides (250 μM, 2 μL), EDTA (10 mM, 4 μL), alkyl amines dissolved in DMF (400 mM, 10 μL), sodium phosphate buffer (pH 7.0, 200 mM, 10 μL), and 10 μL of H_2O. For the reaction with Dan-NH_2, the proportion of DMF was adjusted to 50%. The reaction was incubated at room temperature for 3 h. Then, the oligonucleotides were purified using Amicon-3k ultrafilter with water.

Click Chemistry of Nucleic Acids Modified with Alkyne or Azide. The CuAAC reaction was carried out following the reported protocol:[51] Sodium ascorbate (150 mM, 10 μL) was added to a solution of oligonucleotides (25 μM, 20 μL), $CuSO_4$ (20 mM, 5 μL), THPTA (50 mM, 10 μL), Alk488 or Az545 (1 mM, 5 μL), and sodium phosphate buffer (pH 7.0, 200 mM, 50 μL). The reaction was incubated at room temperature for 2 h and stopped by adding 10 μL of 100 mM EDTA. Then, the oligonucleotides were purified using Amicon-3k ultrafilter with water.

PAGE Analysis of Nucleic Acids. The samples for denaturing PAGE analysis were prepared by mixing 10 pmol of nucleic acids, with equal volume of 8 M urea and 1/10 volume of DNA loading buffer. The samples were loaded on 20% denaturing PAGE and run for 2 h at 3 W. Green fluorescence of nucleic acids was observed by imaging using SYBR Green mode. Afterward, the gels were stained by SYBR Gold in 1 × TBE for 8 min and imaged using SYBR Gold mode.

Binding of Biotin-Derived Nucleic Acids by SA-MBs. SA-MBs (4 mg/mL, 300 μL) was added into a 1.5 mL of microcentrifuge tube. Apply magnet to side of tube for 30 s. The supernatant was removed and discarded. The beads were washed with 600 μL of binding buffer (containing 20 mM Tris-HCl at pH 7.5, 1 mM EDTA and 0.5 M NaCl) for three times. Then 100 μL of 2 μM biotin-derived nucleic acids was added into beads. The beads were vortexed and incubated at room temperature for 30 min. A magnet was applied and then the supernatant was transferred to a clean microcentrifuge tube.

Oxidative Amination Reaction of Nucleic Acids with Aromatic Amines. $NaIO_4$ (100 mM, 4 μL) was added to a solution of oligonucleotides (250 μM, 2 μL), EDTA (10 mM, 4 μL), aromatic amines dissolved in DMF (500 mM, 20 μL), and NaAc-HAc buffer (pH 5.2, 200 mM, 10 μL). The reaction was incubated at 45 °C for 3 h. Then, the oligonucleotides were purified by ethanol precipitation or *n*-butanol extraction and subsequently washed using Amicon-3k ultrafilter with water.

Fluorescence Measurement of Fluorophore-Derived Nucleic Acids. The concentration of nucleic acids was determined by its absorbance at 260 nm. The 200 μL of a solution of nucleic acids was used for measurement of fluorescence spectra. The bandwidth of excitation and emission was set at 5 nm.

HPLC-UV Analysis of Nucleic Acids for Quantification of Oxidative Amination Reaction. The reactant or product nucleic acids were analyzed on a HPLC system equipped with a UV detector. A C18 column (5 μM particle size, 120 Å pore size, 250 mm length, 4.6 mm inner diameter) was used at 35 °C with 50 mM triethylammonium acetate (TEAA) buffer (pH 7.0)-acetonitrile (93:7) as eluent A and 50 mM TEAA buffer (pH 7.0)-acetonitrile (50:50) as eluent B. The flow rate was 1.0 mL/min. The gradient elution procedure was as follows: 0 min 100% eluent A, 35 min 70% eluent A + 30% eluent B, 40 min 100% acetonitrile, 45 min 100% acetonitrile, 50 min 100% eluent A, and 60 min 100% eluent A. The yields of reactions were quantified according to the peak area ratio of the isolated product to the reactant, and these peak areas were normalized by that of inert impurity dC ON as an internal standard. The isolated products were collected and dried in vacuo and subsequently identified by MALDI-TOF MS analysis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.bioconjchem.1c00016.

Material and instrumentation, MALDI-TOF MS analysis, NMR analysis, absorption measurements, PAGE analysis (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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