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# Rapid and Specific Post-Synthesis Modification of DNA through a Biocompatible Condensation of 1,2-Aminothiols with 2-Cyanobenzothiazole

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**Abstract:** Post-synthesis modification of DNA is an important way of functionalizing DNA molecules. Herein, we describe a method that first enzymatically incorporates a cyanobenzothiazole (CBT)-modified thymidine. The side-chain handle CBT can undergo a rapid and site-specific cyclization reaction with 1,2-aminothiols to afford DNA functionalization in aqueous solution. Another key advantage of this method is the formation of a single stereo/regioisomer in the process, which allows for precise control of DNA modification to yield a single component for aptamer selection work and other applications.

**Keywords:** bioorthogonal reactions · click chemistry · cyanobenzothiazole · DNA · post-synthesis modification

## Introduction

The unique physical and chemical characteristics of DNA as genomic materials also make them very useful for various other applications. For example, DNA has been explored for applications in nanoarchitectures,[1]nanorobots,[2] nanocomputing,[3] reaction encoding,[4] and nanosensing.[5] In addition, DNA can be used as therapeutic agents[6] and for the in vitro selection of aptamers for various applications.[7] For many applications, DNA must be equipped with specific functionalities, such as fluorescent markers, biotin, thiols, sugars, proteins, positively charged peptides (octaarginines), and boronic acid for endowing different properties.[8] Therefore, there is a critical need to develop chemistry that allows for the ready functionalization of DNA molecules. Nucleic acid functionalization can be conducted through various conjugation methods, such as Staudinger ligation, *N*-hydroxysuccinimide (NHS) ester chemistry, and the formation of thiourea, oxime, or disulfide linkages.[9] Recent advances in cycloaddition reactions have enabled faster and more efficient conjugation.[8a] Specifically, the labs of Seela[10] and Carell[11] first reported the functionalization of DNA nucleobases through the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction, which was developed by the groups of Sharpless[12] and Meldal.[13] This protocol is highly efficient and specific, and results in almost quantitative incorporation of different labels into the DNA. Although these seminal reports have allowed easy DNA modification, the CuAAC method has the issue of copper-catalyzed cleavage of DNA.[8a] In addition, $Cu^{I}$ poses stability problems for some other functional groups. For example, boronic acid was reported to be degraded during post-synthetic functionalization of DNA when using the CuAAC reaction.[14] Copper-free chemistry has thus been used recently by different researchers, including strain-promoted azide–alkyne cycloadditions,[15] Staudinger ligation,[16] Diels–Alder reaction,[17] hydrazone[18] and oxime[19] formation, and native peptide ligation.[20] Although these methods provide alternative ways for copper-free DNA labeling, they still possess some limitations, for example, low reaction rate, the need for other reagents/organic solvents for functionalization, and the possibility of generating stereo/regioisomers.

In our work of preparing modified DNA, we are especially interested in building boronic acid functionalized DNA libraries for aptamer selection.[8b] We have successfully demonstrated the synthesis and enzymatic incorporation of boronic acid modified TTPs (B-TTPs) into DNA[21] (route A, Scheme 1), and the chemical phosphoramidite synthesis of DNA with a strained alkyne handle, which allowed introduction of the boronic acid group through copper-free click chemistry (route B, Scheme 1).[14] Although these paved the way for enzyme-catalyzed functionalization with a boronic acid and the large scale synthesis of single-stranded boronic acid modified DNA for further applications, several limitations still need to be addressed, including the complex synthesis of different B-TTP moieties, $Cu^{I}$-mediated degradation of specific boronic acid functional groups, the potential problems during enzymatic recognition, and multiple stereoisomers generated due to lack of symmetry. In an attempt to

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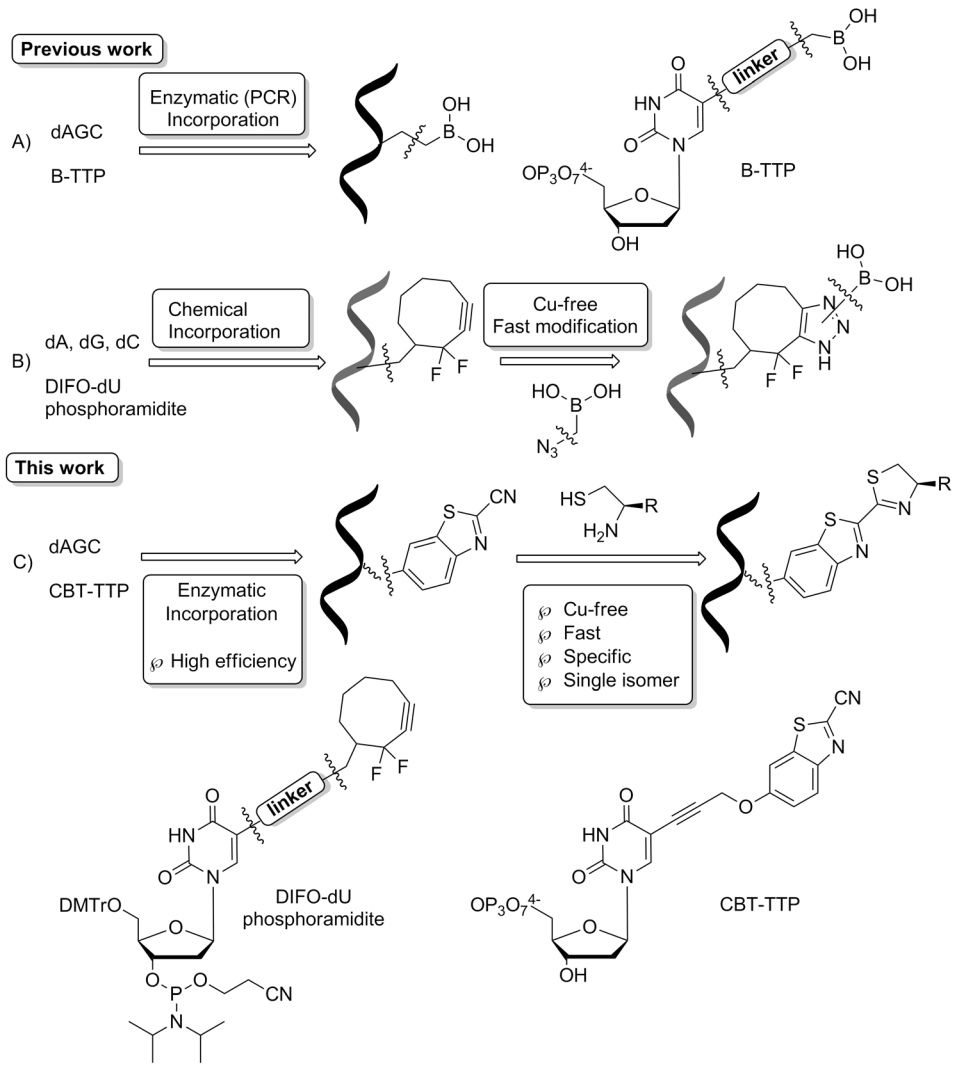


Scheme 1. Schematic representations of different boronic acid functionalized DNA modification strategies: A) Enzymatic incorporation of boronic acid modified nucleotides; B) chemical incorporation of a nucleotide modified with a reactive group, followed by Cu-free modification with their reaction partners; and C) enzymatic incorporation of a "general handle" modified nucleotide, followed by Cu-free post-synthesis modification with its partner. DIFO = difluorinated cyclooctyne, DMTr = 4,4-dimethoxytrityl.

circumvent these restrictions, and most importantly, to develop a novel platform for the rapid and specific post-synthesis modification of DNA that can address some limitations of current methods, we are further interested in the strategy of enzyme-catalyzed (such as the polymerase chain reaction (PCR)) synthesis of DNA with a "general handle" that can undergo a rapid and specific post-synthesis functionalization. Specifically, we need an approach that allows 1) high enzymatic incorporation efficiency of the "general handle" modified nucleotide, 2) fast, site-specific, and chemoselective post-synthesis modification with its reactive partner in aqueous solution, and 3) high yield of a single stereochemically pure product. The last point is especially important and remains a major obstacle for further application in DNA modification, such as aptamer selection. Because even if the yield of the desired isomer is 90% at a single base, the synthesis of a 90-mer DNA would have the possibility of generating multiple isomers with a random distribution of regio/stereoisomers at different positions. Herein, we report our recent endeavor in developing such a method that meets the aforementioned criteria for post-synthesis modification of DNA.

## Results and Discussion

To start this project, we first explored the well-developed repertoire of biocompatible reactions to find the appropriate reaction partners for high reaction selectivity, absence of stereoisomers, and compatibility with biological systems. The condensation of 1,2-aminothiol with 2-cyanobenzothiazole (CBT) was chosen.[22] This reaction, known as the last step of the biosynthesis of luciferin,[22] has drawn much attention recently, largely due to the rejuvenation work of the groups of Rao[23] and Chin.[24] In employing this reaction for post-synthesis DNA labeling, we have the option of either incorporating a 1,2-aminothiol or CBT into the nucleotide. We selected the pathway of making the CBT-modified nucleotide, since it does not involve protection/deprotection during the synthesis. In addition, the 1,2-aminothiol provides good solubility in aqueous solution to the click-labeling agent. Based on the known fact that 5-position modification of deoxyuridine can be tolerated by polymerases and reverse transcriptases[25] and our own successful experience in developing a series of 5-position boronic acid functionalized deoxyuridines,[21c] we prepared a CBT-modified thymidine triphosphate in eight steps. Specifically, by starting from commercially available 1,4-benzoquinone (**1**), ethyl 6-hydroxybenzo[d]thiazole-2-carboxylate (**5**) was synthesized by following literature procedures.[26] Propargylation, followed by ammonolysis and dehydration provided a modified CBT with a terminal alkyne moiety (**8**). Subsequent Sonogashira reaction yielded CBT-T **9**. Triphosphorylation was accomplished by the classical one-pot three-step method[27] (Scheme 2) to afford final compound CBT-TTP **10**.

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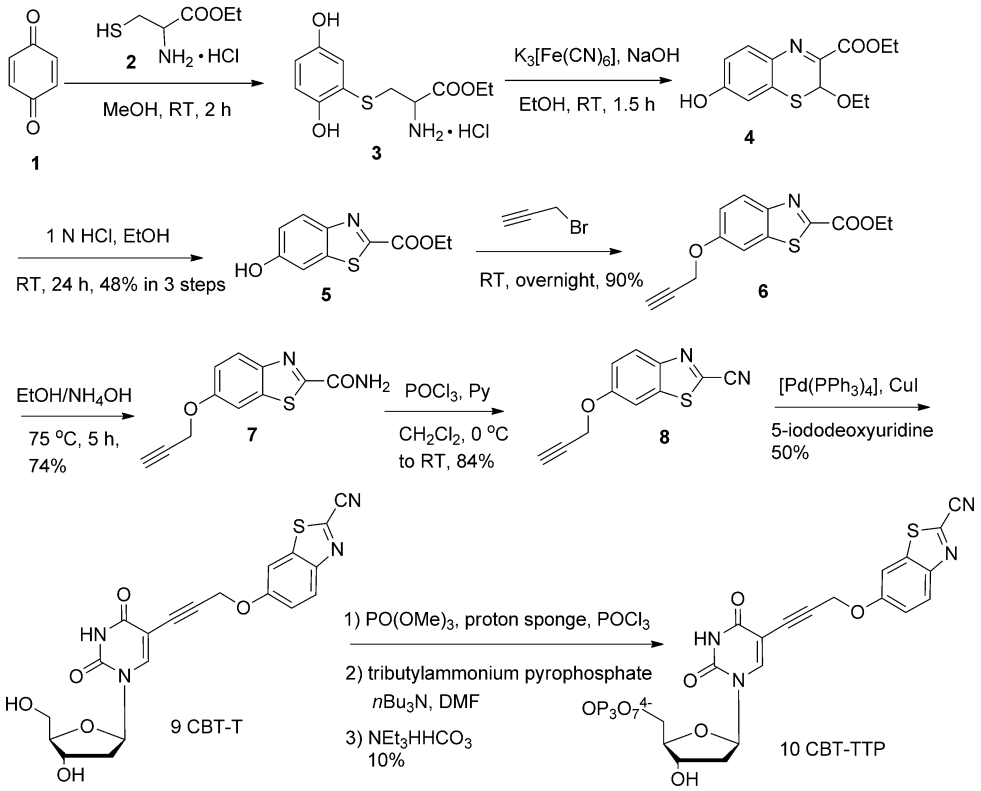


Scheme 2. Synthesis of **10** (CBT-TTP).

Before the incorporation study, we first studied the reaction profile. CBT has been reported to react rapidly and specifically with 1,2-aminothiol with a second-order rate constant of $9\,M^{-1}s^{-1}$.[23a] We reasoned that nucleotide-modified CBT would proceed in the same fashion. To confirm this, a kinetic study was performed for the reaction between the precursor CBT-T (**9**, Scheme 2) and 1,2-aminothiol. The second-order rate constant was determined to be $22\,M^{-1}s^{-1}$ (see Figure S1 in the Supporting Information) under near-physiological conditions (phosphate buffer, pH 7.4).

Next, we studied the incorporation of CBT-TTP into DNA by an enzyme-catalyzed reaction. Specifically, primer extension, by using CBT-TTP and the Klenow fragment, was conducted by using a short sequence of 21-mer oligonucleotide (nt) Template-1 and a 14-mer FAM-labeled primer (Figure 1; FAM = 6-carboxyfluorescein), which have been successfully used in our previous incorporation studies of different functionalized DNA.[21] Klenow fragment (3′–5′ exo$^-$) was used to avoid cleavage of the template. The primer was designed in such a way that the first incorporated base would be a T, so there are two possible scenarios in the extension: either a fully extended product or no extension at all. The obtained DNA products were studied by using polyacrylamide gel electrophoresis (PAGE). As shown in Figure 1 A, negative controls without dTTP (Lane 1, Figure 1 A), without the Klenow fragment (Lane 2, Figure 1 A), and primer plus template only (Lane 3, Figure 1 A) showed no full length DNA sequence. Instead, annealing products

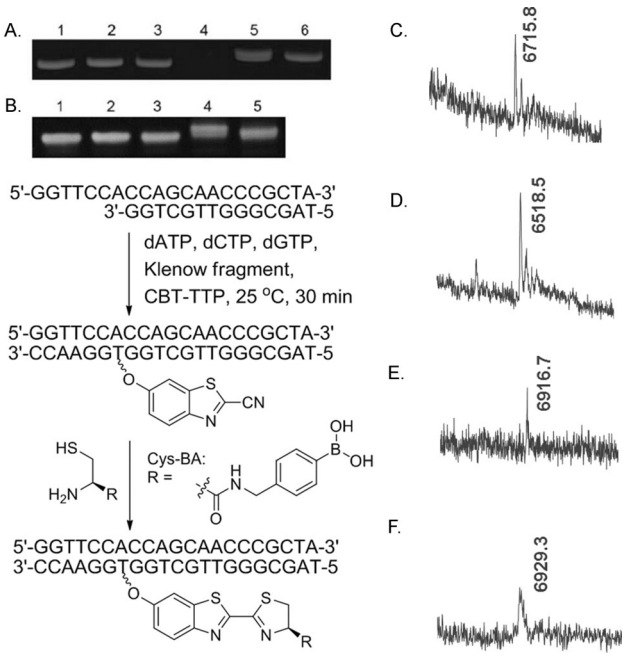


Figure 1. A) Primer extension with CBT-TTP catalyzed by the Klenow fragment (3′–5′ exo$^-$), 20% PAGE analysis: 1) no dTTP, 2) no enzyme, 3) Template-1 + primer, 4) primer only, 5) dNTPs, 6) the same as Lane 5 except with CBT-TTP instead of dTTP; B) Post-synthesis labeling by using "click" reagent Cys-BA, 20% PAGE analysis: 1) dNTPs-$DNA_{21}$, 2) CBT-$DNA_{21}$, 3) dNTPs-$DNA_{21}$ + Cys-BA, 4) CBT-$DNA_{21}$ + Cys-BA, 5) Cys-BA "click"-labeled CBT-$DNA_{21}$ treated with $H_2O_2$; C,D,E,F) MALDI spectra of DNA products corresponding to gel lanes B2, B3, B4, and B5, respectively.

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were observed. Negative control with primer only (Lane 4, Figure 1A) did not show annealing product due to the absence of template. On the other hand, primer extension product with CBT-TTP (Lane 6, Figure 1A) in place of dTTP gave a similar full-length DNA band to that of the positive control with Klenow fragment and natural dNTPs only (Lane 5, Figure 1A). Such results indicated that synthesized CBT-TTP could be recognized as similar to dTTP by the Klenow fragment, and incorporated into DNA. After demonstrating the successful incorporation of CBT-TTP, we further explored the feasibility of post-synthesis modification of DNA. Since it is a long-term interest in our group to develop boronic acid functionalized DNA for further applications, a 1,2-aminothiol conjugated boronic acid (Cys-BA) probe was synthesized as an example for post-synthesis modification (Figure 1, see Scheme S1 in the Supporting Information for the synthetic route). The same 21-nt template and 14-nt primer without the FAM label were used. As shown from Figure 1B, fully extended product was observed by using both dNTPs (Lane 1, Figure 1B) and CBT-TTP (Lane 2, Figure 1B). However, after being treated with Cys-BA (1 mM, final concentration (50 equiv) for 30 min, supplemented with tris(2-carboxyethyl)phosphine (TCEP, 1 mM final concentration)) with the primer extension product, only CBT-$DNA_{21}$ (CBT incorporated DNA product, 21-nt) showed the post-synthesis product as expected (Lane 4, Figure 1B). The reduced mobility of Cys-BA "click"-labeled CBT-$DNA_{21}$ indicated the reaction, which is presumably due to the interaction between boronic acid and polyacrylamide matrix.[14,21b] The result was further supported by the band with different mobility after treating the Cys-BA "click"-labeled CBT-$DNA_{21}$ with $H_2O_2$ (1 mM final concentration, 1 h; Lane 5, Figure 1B), which is due to the well-known oxidation reaction of converting the phenyl boronic acid functional group into a phenol group.[28] As a control, the mobility for the dNTPs-$DNA_{21}$ (extended DNA product by using dNTPs, 21-nt) band did not change after treatment with Cys-BA (Lane 3, Figure 1B), which indicated no reactions, as expected.

Understandably, mobility studies alone would not be enough to prove the post-synthesis modifications. MALDI-MS was used to further examine the DNA products. Specifically, dNTP-$DNA_{21}$ treated with Cys-BA (Lane 3, Figure 1B) had the same peak with a $m/z$ of 6518 (Figure 1D, calcd: 6519 $[M+H]^+$) as the original DNA product. Such results indicate that Cys-BA does not interfere/react with dNTP-$DNA_{21}$, as expected. In contrast, full extension of the primer by using CBT-TTP instead of dTTP yielded a CBT-$DNA_{21}$ (Lane 2, Figure 1B) with a $m/z$ of 6716 (Figure 1C, calcd: 6717 $[M+H]^+$) in MALDI-MS. When treated with Cys-BA, CBT-$DNA_{21}$ was converted to a "click"-labeled product (Lane 4, Figure 1B) with a $m/z$ of 6917 (Figure 1E, calcd: 6917 $[M-2H_2O+H]^+$), corresponding to the Cys-BA "click"-labeled CBT-$DNA_{21}$ product. After treating the "click" product (Lane 4, Figure 1B) with $H_2O_2$, a product (Lane 5, Figure 1B) with a $m/z$ of 6929 (Figure 1F, calcd: 6927 $[M+H]^+$), corresponding to the boronic acid oxidation product, was observed, as expected.[14,21b] Such results confirmed the intended click modifications. In addition, the effect of a CBT and boronic acid moiety on the thermostability of a DNA duplex was investigated through thermodenaturation. The results (see Figure S8 in the Supporting Information) suggest that the incorporation of one CBT or boronic acid moiety in a 21 bp DNA duplex only slightly decreased its stability ($T_m$ decreased from 73.00°C to 70.96 and 70.24°C, respectively).

After successful incorporation of one CBT moiety into DNA by using the Klenow fragment catalyzed primer extension reaction, we further explored the feasibility of incorporating multiple CBT moieties. Thus, 21-nt Template-2 and Template-3 were designed to incorporate two and three CBT-TTP, respectively. To our surprise, the primer extension reaction catalyzed by the Klenow fragment was unsuccessful, as indicated by the presence of multiple incomplete bands, even at elevated temperature or with a longer reaction time (results not shown). Knowing family B polymerases are relatively more tolerant to modified TTP,[29] a family B polymerase (KOD XL) from *Thermococcus kodakaraensis*, which is a mixture of the natural form and an exo$^-$ mutant, was thus chosen for the reaction. As shown in Figure 2A, the electrophoretic mobility of modified $DNA_{21}$

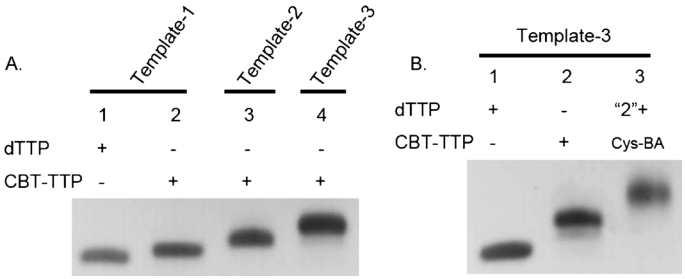


Figure 2. A) Primer extension with CBT-TTP catalyzed by KOD XL DNA polymerase, 20% PAGE analysis: 1) Template-1 with dTTP, 2) Template-1 with CBT-TTP, 3) Template-2 with CBT-TTP, 4) Template-3 with CBT-TTP; B) Post-synthesis labeling by using extension product of Template-3 and "click" reagent Cys-BA, 20% PAGE analysis: 1) dNTPs-$DNA_{21}$, 2) CBT-$DNA_{21}$, 3) CBT-$DNA_{21}$ + Cys-BA.

(Lanes 2, 3, and 4) was further decreased with the incorporation of the CBT moiety at one or more positions. This clearly indicated the successful incorporation of multiple CBT-TTP units into DNA through enzyme-catalyzed reactions. This was further confirmed by MALDI analysis of the incorporated product by using Templates-2 and -3 (calcd for Template-2: 6929 $[M+H]^+$; found: 6930; calcd for Template-3: 7135 $[M+H]^+$; found: 7136, see Figures S6 and S7 in the Supporting Information). The extension product formed by using Template-3 was further used for post-synthesis modification by Cys-BA. As is shown in Figure 2B, the incorporated product (Lane 2) could be successfully labeled by Cys-BA, showing a slower-moving band (Lane 3), which is consistent with the phenomena observed when using the template with incorporation of one CBT moiety.

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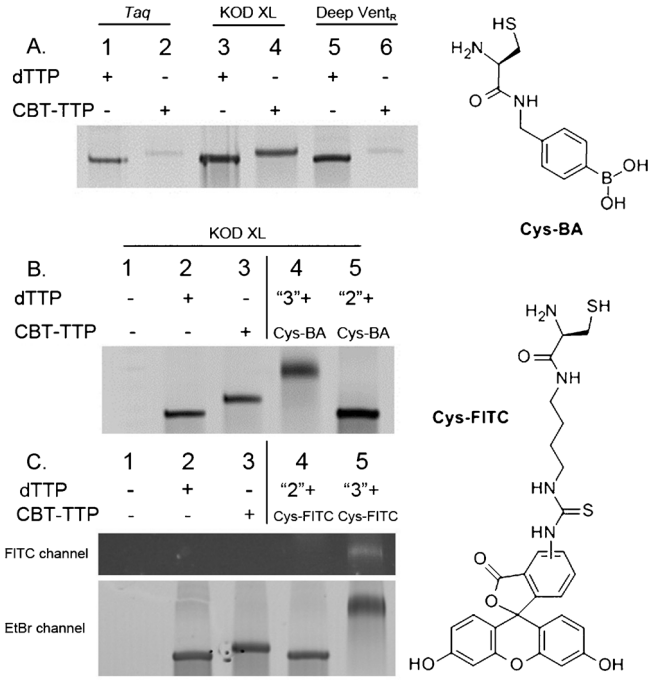
Encouraged by the successful incorporation of CBT-TTP into short DNA sequences, we further investigated the PCR amplification of a longer 90-mer DNA strand.[8b] We first studied the incorporation by using three commercially available polymerases, including a family A polymerase from *Thermus aquaticus* (*Taq*) and family B polymerases from *Thermococcus litoralis* (Deep $Vent_R$exo$^-$) and *Thermococcus kodakaraensis* (KOD XL), which was used for primer extension experiments. As can be seen from the results summarized in Figure 3A, incorporation of CBT-TTP was inefficient







**Cys-BA**




**Cys-FITC**

Figure 3. A) Enzyme screening for PCR incorporation, Lanes 1, 3, and 5 are using dNTPs, and lanes 2, 4, and 6 are using CBT-TTP instead of dTTP. Lanes 1, 2: *Taq* polymerase; 3, 4: KOD XL polymerase; 5, 6: Deep Vent (exo$^-$) polymerase. B) "Click" labeling of dNTPs/CBT-$DNA_{90}$ with Cys-BA: 1) no dTTP, 2) dNTPs, 3) CBT-TTP, 4) DNA product after post-synthesis modification of CBT-$DNA_{90}$ (Lane 3) with Cys-BA for 30 min, 5) DNA yielded after post-synthesis modification of dNTPs-$DNA_{90}$ (Lane 2) with Cys-BA for 30 min. C) "Click" labeling of dNTPs/CBT-$DNA_{90}$ with Cys-FITC: 1) no dTTP, 2) dNTP, 3) CBT-TTP, 4) DNA product after post-synthesis modification of CBT-$DNA_{90}$ (Lane 3) with Cys-FITC for 30 min, 5) DNA yielded after post-synthesis modification of dNTPs-$DNA_{90}$ (Lane 2) with Cys-FITC for 30 min. The FITC channel only detects DNA products with green fluorescence, whereas the EtBr channel detects all DNA products. (15% PAGE; see the Supporting Information for the detailed protocols).

when using *Taq* (Lane 2, Figure 3A) and Deep Vent (Lane 6, Figure 3A). On the other hand, efficient incorporation was obtained by using KOD XL polymerase, similar to the primer extension experiments for multiple CBT moiety incorporation. Further studies were then conducted by using KOD XL as the polymerase. As a negative control, no DNA product (Lane 1, Figure 3B) was observed without using dTTP or CBT-TTP. When the appropriate nucleotides are used, dNTPs-$DNA_{90}$ (DNA product using dNTPs, 90-nt, Lane 2, Figure 3B) and CBT-$DNA_{90}$ (CBT incorporated DNA product, 90-nt, Lane 3, Figure 3B) were obtained. This also indicated that CBT-moiety-containing DNA chains could be used as templates for amplification. dNTPs-$DNA_{90}$ (Lane 2) showed different mobility from that of CBT-$DNA_{90}$ (Lane 3), presumably because of the added molecular weight of the latter.

After post-synthesis modification with Cys-BA (1 mM final concentration for 30 min, supplemented with 1 mM TCEP), a new band with further reduced mobility was observed for the CBT-$DNA_{90}$ product (Lane 4, Figure 3B). As a control, the same band with the same mobility was observed after the subjection of dNTPs-$DNA_{90}$ to the same treatment with Cys-BA (Lane 5, Figure 3B). Such results demonstrated the feasibility of post-synthesis click-modification with a longer 90-mer DNA with multiple CBT moieties.

Finally, to further demonstrate the generality of this approach for post-synthesis modification of DNA. 1,2-Aminothiol conjugated with FITC (fluorescein isothiocyanate; Cys-FITC; Figure 3) was used instead of Cys-BA. The same phenomenon was observed as with Cys-BA. As can be seen from the EtBr channel in Figure 3C, which stained all DNA products, no DNA product (negative control, Lane 1, Figure 3C) was observed without using dTTP or CBT-TTP. On the other hand, dNTPs-$DNA_{90}$ (Lane 2, Figure 3C) and CBT-$DNA_{90}$ (Lane 3, Figure 3C) were observed with different mobilities. After post-synthesis modification with Cys-FITC (1 mM final concentration), supplemented with TCEP (1 mM final concentration) for 30 min, a new band with further reduced mobility was observed (Lane 5, Figure 3C). As a control, dNTPs-$DNA_{90}$ was also treated with Cys-FITC under the same conditions. No mobility changes were observed after treatment (Lane 4, Figure 3C), as expected. The successful post-synthesis modification of CBT-$DNA_{90}$ was further confirmed when imaging the same gel through the FITC channel, which only detects the green fluorescein signal. Only the band of Cys-FITC-labeled CBT-$DNA_{90}$ (Lane 5, Figure 3C) showed a FITC signal, with no observable green fluorescein signals for all the other bands, as expected.

## Conclusion

A novel method for post-synthesis modification of DNA is described thorough the design, synthesis, and successful enzymatic incorporation of a cyanobenzothiazole (CBT)-modified TTP. The CBT-TTP-incorporated DNA products can undergo rapid post-synthesis modification through a biocompatible condensation reaction with 1,2-aminothiol-conjugated boronic acid (Cys-BA) or the FITC analogue (Cys-FITC). This approach provides a novel method for the rapid and site-specific post-synthesis modification of DNA without the issue of regio- or stereoisomers and the formation of a single isomer makes this approach suitable for DNA-based aptamer selection work and other applications.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## Experimental Section

**General procedure for Klenow fragment catalyzed primer extension by using dTTP or CBT-TTP**: The reaction mixtures of a final volume of 50 μL contained 21-nt template (5′-GGTTCCACCAGCAACCCGCTA-3′ (20 μM)), 14-nt primer or 5′-FAM 14-nt primer (5′-(FAM)-TAGCGGGTTGCTGG-3′ (20 μM)), Tris-HCl (10 mM), NaCl (50 mM), $MgCl_2$ (10 mM), dithiothreitol (1 mM) at pH 7.9, Klenow fragment (0.5 U $\mu L^{-1}$), and dATP, dCTP, dGTP, dTTP, or CBT-TTP (200 μM). Reactions were performed by incubating the prepared solutions at 25 °C for 30 min. The primer-extension products were analyzed by 20% PAGE.

**General procedure for KOD-XL-catalyzed primer extension by using dTTP or CBT-TTP**: The reaction mixture of a final volume of 50 μL contained 21-nt template (Template-2: 5′-TCAGTCACCAGCAACCCGCTA-3′, Template-3: 5′-CACGACACCAGCAACCCGCTA-3′ (20 μM)), 14-nt primer (5′-TAGCGGGTTGCTGG-3′ (20 μM)), Tris-HCl (10 mM), NaCl (50 mM), $MgCl_2$ (10 mM), dithiothreitol (1 mM) at pH 7.9, KOD XL (0.5 U $\mu L^{-1}$), and dATP, dCTP, dGTP, dTTP, or CBT-TTP (200 μM). Reactions were performed by incubating the prepared solutions at 90 °C for 1 min, 20 °C for 1 min, and 66 °C for 20 min. The primer extension products were analyzed by 20% PAGE.

**Post-synthesis labeling of the primer extension products CBT-$DNA_{21}$**: The primer extension product CBT-$DNA_{21}$ was purified by using Millipore Amicon 3 kDa spin column. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) in PBS buffer (10 mM, 5 μL) as mixed with Cys-BA (2 mM, 25 μL) in 1×PBS buffer. The reaction was allowed to stand at RT for 10 min. Pre-purified CBT-$DNA_{21}$ (20 μL) was then added to the mixture, which was further incubated at 37 °C for 1 h. The negative control experiment was performed by following the same procedure except by using dNTPs-$DNA_{21}$, the primer extension product by using dNTPs, and other reagents. The resulting DNA products after post-synthesis modification were purified with Millipore Amicon 3 kDa spin column and analyzed by 20% PAGE. For MALDI analysis, the DNA product was further purified on a Sephadex G25 column. Fractions were collected and concentrated by using a Millipore Amicon 3 kDa spin column.

**General procedure for PCR incorporation by using dTTP or CBT-TTP**: The PCR mixture of a final volume of 50 μL contained DNA 90-nt template (5′-CCTTCGTTGTCTGCCTTCGTGAGCGGAGTCAGACG-CACGCTCGTACCTGTGCGCAAGCACTATGACGGACACCCTT-CAGAATTCGCACCA-3′ (10 nM)), primer 1 (5′-TGGTGCGAATTCT-GAAGGGT-3′ (1 μM)), primer 2 (5′-CCTTCGTTGTCTGCCTTCGT-3′ (1 μM)), and dATP, dCTP, dGTP, dTTP, or CBT-TTP (200 μM), DNA polymerase (0.5 U $\mu L^{-1}$), and 1×reaction buffer as provided by the vendor. Taq (New England Biolabs): Tris-HCl (10 mM), KCl (50 mM), $MgCl_2$ (1.5 mM), pH 8.3 at 25 °C. Deep Vent (New England Biolabs): Tris-HCl (20 mM), $(NH_4)_2SO_4$ (10 mM), KCl (10 mM), $MgSO_4$ (2 mM), Triton X-100 (0.1%), pH 8.8 at 25 °C, and KOD-XL (Novagen): Tris-HCl (20 mM), $MgCl_2$ (8 mM), DTT (7.5 mM), BSA (50 μg/ml), pH 7.5 at 25 °C. Ten thermal cycles were conducted with melting at 90 °C for 20 s, annealing at 48 °C for 20 s, and extending at 72 °C for 30 s with initial denaturing at 90 °C for 2 min and final extension at 72 °C for 5 min. The PCR products were then analyzed by 15% PAGE.

**Post-synthesis labeling of the PCR product CBT-$DNA_{90}$**: Post-synthesis labeling of the PCR products was performed by using similar procedures as those for primer extension. Specifically, CBT-$DNA_{90}$ prepared from PCR was purified by using a Millipore Amicon 10 kDa spin column. TCEP (5 μL of 10 mM) was mixed with Cys-BA or Cys-FITC (25 μL of 2 mM) and allowed to stand at RT for 10 min. Pre-purified CBT-$DNA_{90}$ (20 μL) was then added to the mixture, which was further incubated at 37 °C for 30 min. The negative control experiment was performed by following the same procedure except by using dNTPs-$DNA_{90}$, the PCR product using dNTPs, and other reagents. The resulting DNA product after post-synthesis modification was purified with a Millipore Amicon 10 kDa spin column and analyzed by 15% PAGE.

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