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Synthesis of 5-[3,6-di(pyridin-2-yl)pyridazine-4-yl]-2′-deoxyuridine-5′-*O*-triphosphate—a potential probe for fluorescence detection and imaging DNA

Anilkumar R. Kore *, Bo Yang, Balasubramanian Srinivasan

Life Sciences Solutions Group, Thermo Fisher Scientific, 2130 Woodward Street, Austin, TX 78744-1832, USA

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ABSTRACT

Efficient synthesis of 5-[3,6-di(pyridin-2-yl)pyridazine-4-yl]-2′-deoxyuridine-5′-*O*-triphosphate, a potential substrate for fluorescence detection and imaging of DNA is reported. Inverse electron demand Diels–Alder (invDA) reaction between the electron-rich 5-vinyl-2′-deoxyuridine-5′-*O*-triphosphate and electron-deficient tetrazine dienophile 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine resulted in the formation of 5-[3,6-di(pyridin-2-yl)pyridazine-4-yl]-2′-deoxyuridine-5′-*O*-triphosphate in 80% yield. This class of molecules will find applications in polymerase chain reactions for fluorescence detection and imaging applications of cellular DNA.

Fluorescent detection and labeling of DNA are important tools in synthetic biology.[1] Bioorthogonal chemical reactions are the highly chemoselective chemical reactions used to ligate an exogenous probe to a metabolically labeled biomolecule[2] and they are attractive alternatives to immunohistochemical staining.[3] Almost all bioorthogonal chemical reporters of DNA and RNA synthesis have mainly utilized azide-alkyne click reactions. Developing alternative bioorthogonal chemical reactions still remains as an opportunity in this area for DNA imaging in the labeling of cellular DNA synthesis. Inverse electron demand Diels–Alder (invDA) reactions were proposed as a potential alternative to azide-alkyne click reactions.[4] Particularly invDA reactions between electron-deficient tetrazines and electron-rich dienophiles are attractive for bioorthogonal chemical reactions mainly because these invDA reactions are reversible and also compatible with biological buffers and cell media.[5] Strained dienophiles such as norbornene,[5b–d,6b] *trans*-cyclooctene,[6a,7] and cyclopropene[8] were used in invDA reactions for labeling synthetic oligonucleotides (in vitro) as well as cellular and cell surface proteins. Polymerase chain reaction (PCR) and in vitro transcription reactions are the robust tools to synthesize modified RNAs and DNAs and they require triphosphate version of nucleosides as substrates. Since vinyl aromatic compounds are known to react with tetrazines,[9] 5-vinyl-2′-deoxynucleoside-5′-*O*-triphosphate can serve as a potential labeling substrate during DNA synthesis using PCR. Also vinyl functionality is the smallest possible dienophile for labeling via invDA reactions as larger substituents on nucleosides are known to inhibit their cellular metabolism.[10] Inverse electron demand Diels–Alder (invDA) reaction between the electron-rich 5-vinyl-2′-deoxynucleoside residues of modified DNAs obtained via PCR and the electron deficient tetrazine dienophiles such as Tamra-Tz[4] can be efficiently used for fluorescence detection and cellular DNA imaging (Fig. 1). This type of orthogonal chemical reactions aids cellular visualization of metabolically labeled biomolecules.[4] In this Letter, to investigate the chemical reactivity of vinyl functionality when present in the nucleoside triphosphate substrates, a model inverse electron demand Diels–Alder (invDA) reaction between the electron-rich 5-vinyl-2′-deoxyuridine-5′-*O*-triphosphate **4** (5-VdUTP) and electron-deficient tetrazine dienophile 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine **5** (Py_2-TZ) is reported leading to the facile formation of 5-[3,6-di(pyridin-2-yl)pyridazine-4-yl]-2′-deoxyuridine-5′-*O*-triphosphate **7** (5-VdUTP-Tz-Ox).

The synthetic pathway leading to the formation of 5-[3,6-di(pyridin-2-yl)pyridazine-4-yl]-2′-deoxyuridine-5′-*O*-triphosphate, a potential substrate for fluorescence detection and imaging of DNA is depicted in Scheme 1 starting from 5-iodo-2′-deoxyuridine **1** (5-IdU). 5-IdU is subjected to palladium catalyzed vinylation using tri-*n*-butyl(vinyl)stannane to afford 5-vinyl-2′-deoxyuridine **2** (5-VdU) in 92% yield.[11] Having 5-VdU in hand, the next step would be the triphosphate formation to get the 5-vinyl-2′-deoxyuridine-5′-

* Corresponding author. Tel.: +1 512 721 3589; fax: +1 512 651 0201.
E-mail address: anil.kore@thermofisher.com (A.R. Kore).

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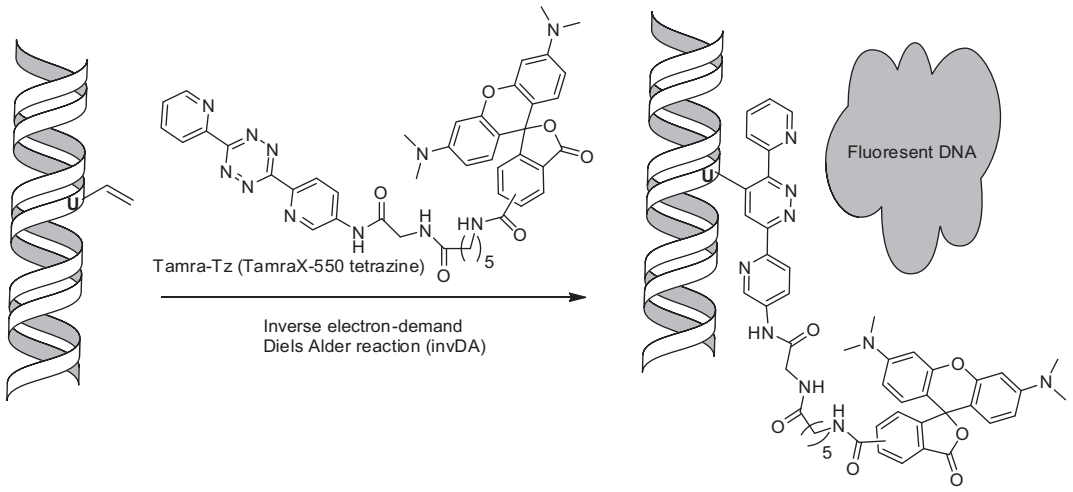
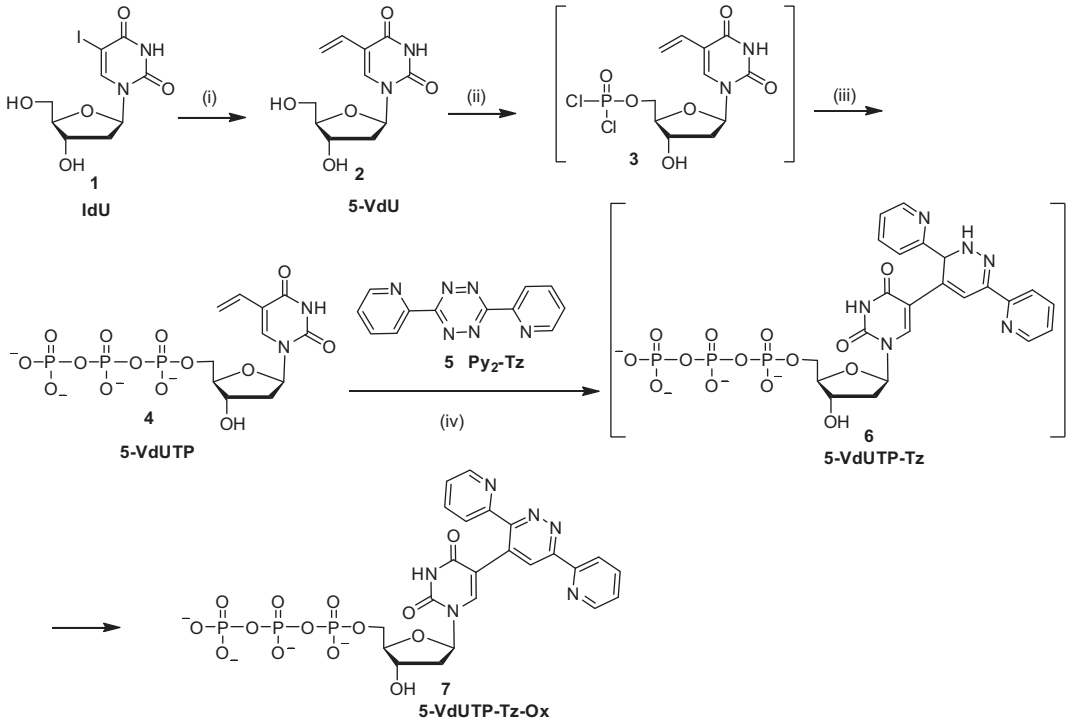


Figure 1. Inverse electron demand Diels–Alder reaction (invDA) between vinyluridine incorporated DNA and Tamra-Tz for fluorescence detection and imaging.



Scheme 1. Synthesis of 5-[3,6-di(pyridin-2-yl)pyridazine-4-yl]-2′-deoxyuridine-5′-*O*-triphosphate. Reagents and conditions: (i) $(n\mathrm{Bu})_3\mathrm{SnCH{=}CH_2}$, $\mathrm{Pd(PPh_3)_4}$, $\mathrm{Cl_2}$, anhydrous THF, reflux, 12 h, 92%; (ii) $\mathrm{POCl_3}$, $(\mathrm{CH_3O})_3\mathrm{PO}$, 1,8-bis(dimethylamino)naphthalene, −5 °C, 0.5 h, 95% (by hplc); (iii) $(\mathrm{NHBu_3})_2\mathrm{H_2P_2O_7}$, $\mathrm{Bu_3N}$, $\mathrm{CH_3CN}$, −5 °C, 0.5 h, 71%; (iv) 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine **5**, dioxane/$\mathrm{H_2O}$, rt, ambient atmospheric oxygen, 16 h, 80%.

O-triphosphate **4** (5-VdUTP). There are many methods available in literature to synthesize triphosphates and were reviewed by us recently.[12] Earlier, we reported an improved protection free, gram-scale, one-pot methodology for the chemical synthesis of 2′-deoxynucleoside-5′-*O*-triphosphates (dNTPs).[13] The chemistry involves the formation of nucleoside dichlorophosphoridate using $\mathrm{POCl_3}$ as the reagent at the monophosphorylation step in the presence or absence of a base followed by reaction with tributylammonium pyrophosphate and hydrolysis of the resulting cyclic intermediate leading to dNTPs. The use of substoichiometric amounts of pyrophosphate (0.95 equiv or less) for the triphosphorylation step not only helped to achieve excellent triphosphate conversion but also simplified the purification task. The utility of this improved methodology was successfully demonstrated by synthesizing various natural and non-natural nucleoside triphosphates and tetraphosphates at either 3′-*O* or 5′-*O* positions in high yields and purity.[14] The modified one-pot methodology was evaluated to convert 5-VdU into 5-VdUTP which involves the formation of 5-vinyl-2′-deoxyuridine-5′-*O*-dichlorophosphoridate **3** using $\mathrm{POCl_3}$ as the reagent at the monophosphorylation step followed by reaction with tributylammonium pyrophosphate and hydrolysis of the resulting cyclic intermediate. It is interesting to observe that 5-VdU can be converted into 5-VdUTP by the one-pot procedure (Scheme 1) in 71% yield. Initial optimization experiments reveal that monophosphorylation of 5-VdU proceeds smoothly (up to 95% conversion into 5-vinyl-2′-deoxyuridine-5′-*O*-dichlorophosph-