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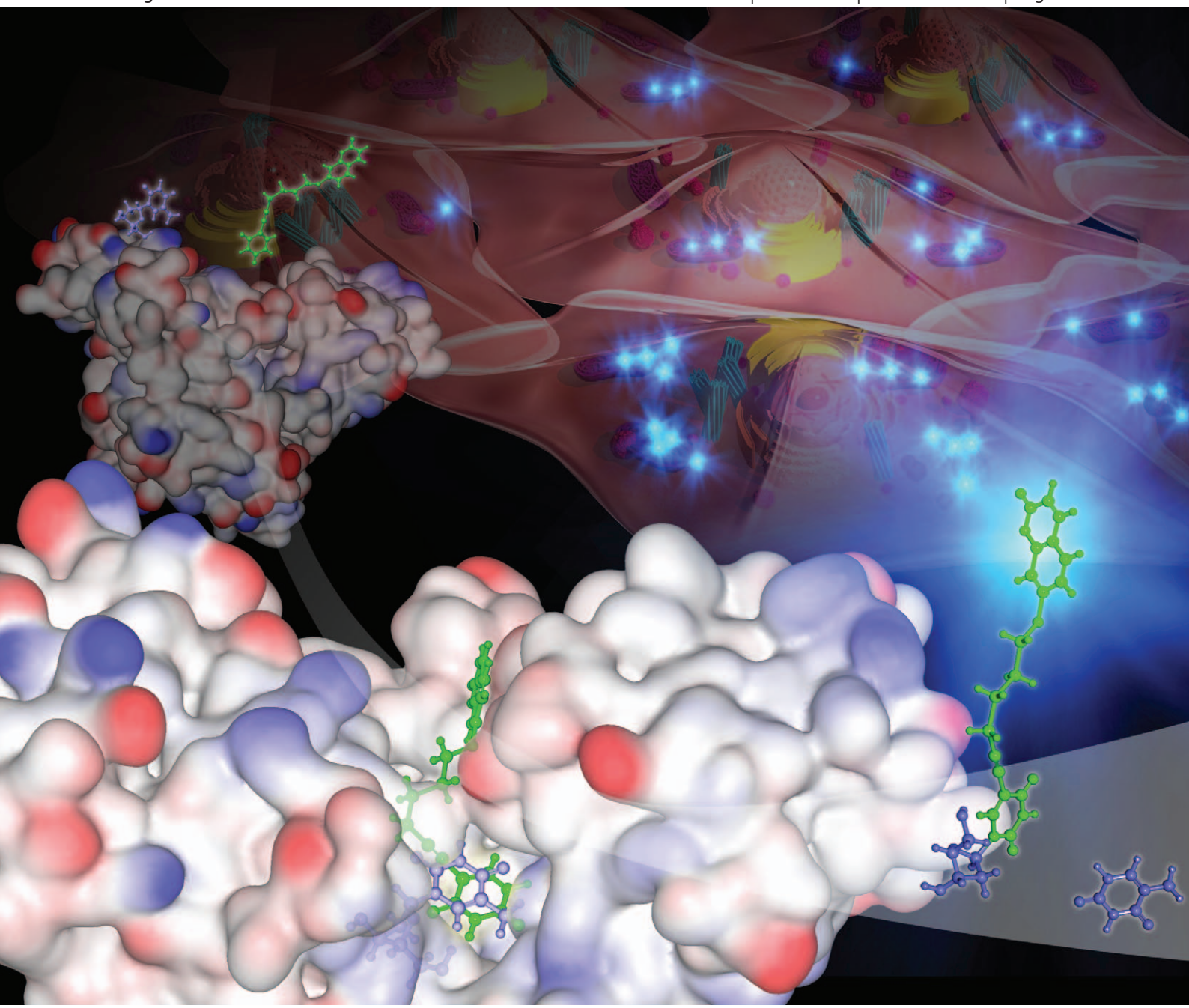

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One-pot approach to functional nucleosides possessing a fluorescent group using nucleobase-exchange reaction by thymidine phosphorylase†

Akihiko Hatano,* Masayuki Kurosu, Susumu Yonaha, Munehiro Okada and Sanae Uehara

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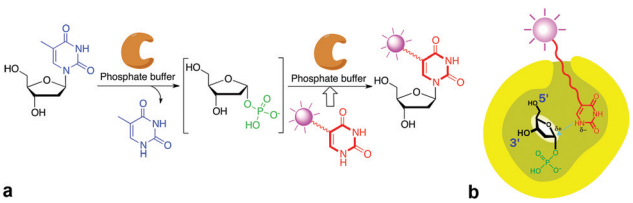
Herein, we describe β-selective coupling between a modified uracil and a deoxyribose to produce functionalized nucleosides catalyzed by thymidine phosphorylase derived from *Escherichia coli*. This enzyme mediates nucleobase-exchange reactions to convert unnatural nucleosides possessing a large functional group such as a fluorescent molecule, coumarin or pyrene, linked *via* an alkyl chain at the C5 position of uracil. 5-(Coumarin-7-oxyhex-5-yn)uracil (C4U) displayed 57.2% conversion at 40% DMSO concentration in 1.0 mM phosphate buffer pH 6.8 to transfer thymidine to an unnatural nucleoside with C4U as the base. In the case of using 5-(pyren-1-methyloxyhex-5-yn)uracil (P4U) as the substrate, TP also could catalyse the reaction to generate a product with a very large functional group at 50% DMSO concentration (21.6% conversion). We carried out docking simulations using MF myPrest for the modified uracil bound to the active site of TP. The uracil moiety of the substrate binds to the active site of TP, with the fluorescent moiety linked to the C5 position of the nucleobase located outside the surface of the enzyme. As a consequence, the bulky fluorescent moiety binding to uracil has little influence on the coupling reaction.

Introduction

Functionalization of deoxyuridine at the C5 position is of interest for the purpose of labeling[1] and the preparation of bioactive nucleoside derivatives.[2] The C5 position of the pyrimidine is located in the major groove of DNA and does not interfere with the stability of the DNA duplex and recognition by DNA polymerases. Thus, the C5 position of deoxyuridine was selected for chemical modification.[3] Moreover, functionalized appendages will increase the versatility of nucleic acids for imaging,[4] novel materials[5] and catalysts.[6]

Thymidine phosphorylase (TP, EC2.4.2.4) is a unique metabolic control enzyme, which catalyses the conversion of thymidine to deoxyribose-1α-phosphate and a free thymine base in phosphate buffer (Scheme 1). Additionally, TP can be used to change the nucleobase moiety of thymidine to a modified uracil.[7,8] It was found that TP can catalyze the coupling reaction of a deoxyribose moiety and many different uracil derivatives substituted at the C5 position. In another case, the preparation of nucleosides of heterocycle pyrimidine and purine bases using immobilized TP[9] and bacterial cells[10] was also reported. TP was immobilized on a solid support in order to generate a stable and recyclable biocatalyst for nucleoside synthesis. The immobilized bacterial cells produced nucleosides of several modified purine compounds. The range of available modified pyrimidines is very limited, and only five uracil derivatives modified at the C5 position were converted to unnatural nucleosides. The modeled structure of *E. coli* TP was obtained from Protein Data Bank (PDB code: closed form 1TPT, 2TPT, open form 1AZY).[11,12] The thymine base is bound



Scheme 1 (a) Catalytic reaction of thymidine phosphorylase and the synthesis of unnatural nucleosides containing a fluorescent group at the C5 position of uracil. (b) Illustration of the catalytic intermediate structure between the modified uracil at the C5 position and the deoxyribose-1α-phosphate in the active site of thymidine phosphorylase. The functional group linked to the C5 position of uracil projects outwards from the edge of the active site. By contrast, the uracil moiety is located within the active site itself.

Department of Chemistry, Shibaura Institute of Technology, 307 Fukasaku, Minuma-ku, Saitama, 337-8570, Japan. E-mail: a-hatano@sic.shibaura-it.ac.jp; Fax: +81-48-687-5013; Tel: +81-48-687-5035

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to the thymine binding site and its 4 position O atom, 3 position N atom, and 2 position O atom are engaged in direct interactions with Arg-171, Ser-186 and Lys-190, respectively. The methyl group at the C5 position of thymine is turned towards the exit of the active site pocket of TP. We hypothesized that large functional groups attached *via* an alkyl chain at the C5 position of uracil would not obstruct the formation of the ES complex between TP and uracil derivatives.

C5-alkynylation and alkenylation of 5-iodo-deoxyuridine have in the past typically involved transition metal-catalyzed cross-coupling reactions such as Sonogashira coupling[13] and Heck reaction.[14] These cross-coupling reactions are a convenient tool for introducing a functional group at the C5 position of uracil. We reasoned that TP and Pd catalyzed cross-coupling reactions could be used to easily obtain many different functional nucleosides containing a fluorescent group at low cost.

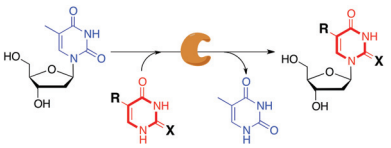
Herein, we report an enzymatic base-exchange reaction to convert thymidine to unnatural nucleosides possessing a functional group at the C5 position by thymidine phosphorylase. We also describe the influence of the solvent and alkyl chain length between the fluorescent group and the uracil on the enzyme catalyzed reaction.

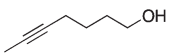
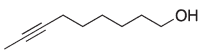
Results and discussion

We carried out the base-exchange reaction to synthesize unnatural nucleosides possessing various uracil derivatives modified at the C2 and C5 positions (Table 1). 2-Thiouracil and 2-thiothymine were reacted with each corresponding nucleoside using TP in 53% and 43% yield, respectively. However, 2,4-dithiouracil could not be converted to the corresponding nucleoside using this enzyme. 5-Fluorouracil and 5-trifluoromethyluracil were also prepared. ^{19}F labeled derivatives are very attractive tools for *in vivo* molecular imaging such as magnetic resonance studies.[15] In addition, deoxy-5-fluorouridine, which was converted from 5-fluorouracil, displays potent antitumor activity. Each 5-fluorine compound showed a high conversion for base-exchange reactions (–F: 86%, $-CF_3$: 78%). Based on X ray diffraction data of TP[11,12] we reasoned that the C5 position of uracil is not directly involved in the molecular interaction with the enzyme. Moreover, the C5 position of uracil is turned to the exit of the active site pocket of the enzyme. To test this prediction, we investigated the effect of altering the length of the alkyl chain of the modified uracil at the C5 position. 5-Ethyluracil was converted to the nucleoside possessing its base by TP in 93% yield after 39 h. In the case of 5-propyluracil the corresponding nucleoside was generated by the enzyme in 47% yield after 50 h. These findings suggest that elongation of the alkyl chain at the C5 position of uracil did not drastically interfere with the conversion of the enzymatic base-exchange reaction, although the incubation time of the reaction had to be extended. Substrates possessing a reactive group, such as an alkene (vinyl) or alkyne, displayed good conversion. The presence of these reactive groups makes possible the facile introduction of a functional molecule into DNA or RNA.[16] Next, we attempted to introduce the tag moiety into nucleosides using TP. We also endeavored to couple the deoxyribose moiety derived from thymidine and the modified uracil linked hydroxyl alkyne groups (n = 2, 4, and 6) at the C5 position. 5-(1-Hydroxybut-5-yn)uracil (n = 2) was converted to the corresponding nucleoside in good yield (85%). Elongation of the alkyl linker (n = 4 and 6) between the uracil and the hydroxyl group afforded conversion but only in moderate yield, possibly due to steric repulsion of the extended alkyl chain at the C5 position of uracil hindering entry of the substrate into the enzymatic pocket.

Next, we undertook the base-exchange reaction mediated by TP using the modified uracil linked to a fluorescent group (Fig. 1). We synthesized three modified uracils linked to a fluorescent group by different lengths of alkyl chains (n = 2, 4, and 6). No reaction occurred in phosphate buffer, presumably due to the decreased nucleophilicity of the uracil derivatives, although poor substrate solubility may also have played a role. We then attempted to perform the reaction in buffer containing a polar organic solvent such as DMSO. The influence of the mixing ratio of DMSO to buffer is shown in Fig. 1. 5-(Coumarin-7-oxyhex-5-yn)uracil (C4U) displayed the highest conversion at a DMSO concentration of <40% to transfer thymidine to unnatural nucleoside with C4U as a base (57.2% yield). The conversion was drastically enhanced as the concentration of DMSO increased up to 40%. This result indicates that the contact frequency between a substrate and an enzyme

Table 1 Nucleobase-exchange reaction from thymidine to unnatural nucleosides using thymidine phosphorylase. Substituted uracils possessing various groups at the C2 and C5 positions. Thymidine (50 mM, 50 μmol), substituted uracil (5 mM, 5.0 μmol), phosphate buffer (1.0 mM, pH 6.8, 1.0 mL), thymidine phosphorylase (1 unit mL^{-1}, 1.0 unit) at 37 °C



R	X	Time/h	Conversion/%
$-CH_3$	S	0.2	53
–H	S	0.2	43
–F	O	0.2	86
$-CF_3$	O	1	78
$-CH_2OH$	O	2	89
$-CH_2CH_3$	O	39	93
$-CH_2CH_2CH_3$	O	50	47
$-HC{=}CH_2$	O	0.3	86
$-C{\equiv}C-H$	O	2	89
	O	4	56
	O	20	85
	O	88	42
	O	5	57

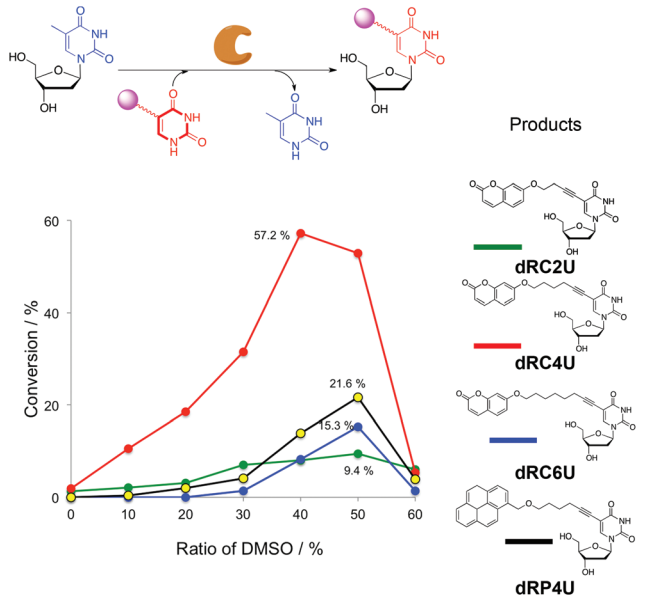


Fig. 1 Effect of the ratio of DMSO concentration on conversion of the base-exchange reaction between the thymidine and the modified uracil catalyzed by thymidine phosphorylase. The modified uracil is connected to a fluorescent molecule *via* an alkyl linker of different chain length (n = 2, 4, and 6). The reaction mixture contained 50 mM thymidine (50 μmol), 5 mM modified uracil (5.0 μmol) in 1.0 mM phosphate buffer (pH 6.8, 1 mL) and DMSO, 1 unit mL^{-1} (1.0 unit) of thymidine phosphorylase at 37 °C.

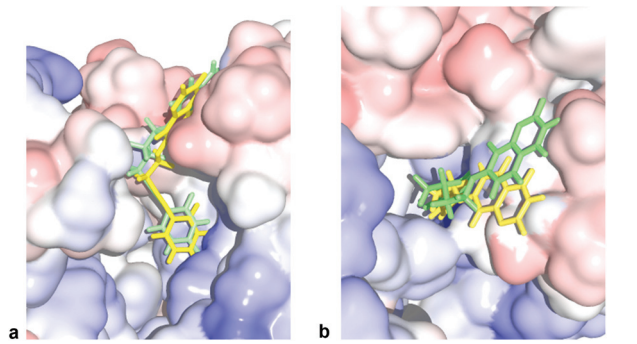


Fig. 2 Docking simulation of modified uracils such as C2U and C4U with thymidine phosphorylase as calculated by MF myPresto. (a) Side view of docking simulation of 5-(coumarin-7-oxyhex-5-yn)uracil (C4U, green) and 5-(coumarin-7-oxybut-3-yn)uracil (C2U, yellow). (b) Upper view of same complexes by docking simulation.

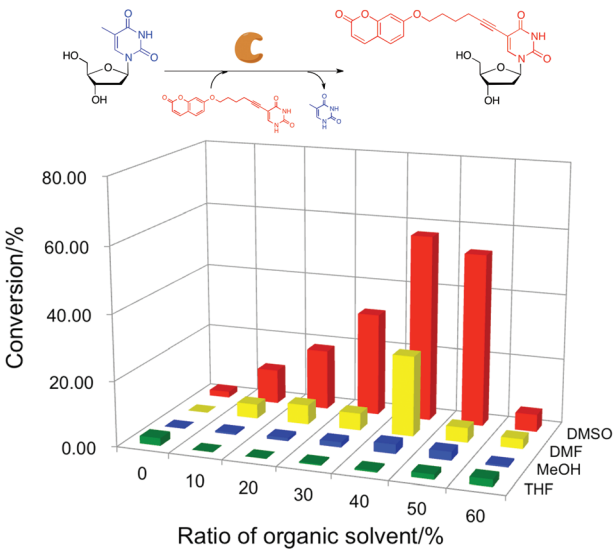


Fig. 3 Effect of variation and mixing ratio of the organic solvents on the conversion of base-exchange reaction of thymidine to 5-(coumarin-7-oxyhex-5-yn) uracil (C4U, n = 4) using thymidine phosphorylase. The reaction mixture contained 50 mM thymidine (50 μmol), 5 mM modified uracil (5.0 μmol) in 1.0 mM phosphate buffer (pH 6.8, 1 mL) and DMSO, 1 unit mL^{-1} (1.0 unit) of thymidine phosphorylase at 37 °C.

is very important for turnover of the catalytic reaction for C4U. However, TP appears to be inactivated in solution when the concentration of DMSO reaches 60% or above. A similar result was obtained using 5-(coumarin-7-oxyoct-7-yn)uracil (C6U) and 5-(pyren-1-methyloxyhex-5-yn)uracil (P4U) in terms of production of unnatural nucleosides upon changing the DMSO ratio in solution. These substrates showed maximum conversion at a 50% DMSO ratio. Interestingly, the yield of 5-(coumarin-7-oxybut-3-yn)uracil (C2U) possessing an alkyl chain n = 2 did not significantly vary across a wide range of DMSO concentrations (from 30 to 50%) in the reaction solution. These results seem to show that chain length between the C5 position of uracil and the fluorescent group influences the reactivity. Indeed, the distance between the uracil moiety and the fluorescent group was too close in the molecular structure of C2U to promote the reaction. We believe a coumarin group obstructs the binding of the uracil moiety to the active site of TP.

To further investigate the influence of the alkyl chain length of uracil substrates C2U and C4U on reactivity, we carried out docking simulations using MF myPrest[17] for the modified uracil bound to the active site of TP (Fig. 2).[11,12] TP derived from *Escherichia coli* is thought to belong to the induced fit type of enzyme (based on the X-ray diffraction structure of a protein-substrate complex, 2TPT and 1AZY).[12,18] Two substrates whose length of alkyl chain was different (n = 2 and 4) were located approximately in the same position in the enzyme pocket. The conformer C2U is similar to C4U in a side view (Fig. 2a). However, an upper view shows the coumarin group of C2U is situated closer to the entrance of the active site crevice of TP than that of C4U (Fig. 2b). This result was anticipated from the observed reactivity of the two substrates. In addition, the docking studies explained why the reactivity of C2U was relatively independent of the ratio of organic solvent (DMSO concentration from 30 to 50%) in the reaction solution as shown in Fig. 1.

In order to evaluate the most effective organic solvent, we attempted the base-exchange reaction with TP in a mixture of a buffer and a polar organic solvent. These results are shown in Fig. 3. We reasoned that the presence of a polar solvent, such as DMSO, DMF, THF or MeOH, would increase the solubility of the modified uracil and facilitate homogeneous mixing in the phosphate buffer. The production of unnatural

nucleosides having C4U as the base was most effective when the reaction solvent contained DMSO. In particular, a DMSO content of 40% gave the highest yield of unnatural nucleoside (57.2%). Addition of DMSO drastically enhanced the conversion of unnatural nucleosides possessing a bulky fluorescent moiety. Introduction of more polar solvents (methanol, tetrahydrofuran or DMF) increased the solubility of the substrate, but the enzymatic reaction appeared to be inhibited. We believe elevated levels of methanol or THF might denature TP. The coupling reaction of the modified uracil and deoxyribose-1α-phosphate was effectively performed with a ratio of DMSO up to 50%, presumably caused by an increase in the collision frequency between the modified uracil and the TP. However, when the ratio of organic solvent was increased to more than 60% the enzyme was denatured. When the ratio of DMSO was 40 to 50%, the productivity of unnatural nucleosides was the highest due to a good balance between solubility of the substrate and enzymatic activity in the reaction solution.

Conclusions

In summary, we have developed an efficient method for generating unnatural nucleosides possessing a functionalized molecule using TP. Recognition of the C5 position of uracil by TP was flexible. Thus, the production of unnatural nucleosides possessing a fluorescent group such as a coumarin or pyrene was possible using this enzyme in a one-pot reaction. We believe the uracil moiety of the substrate binds to the active site of TP, with the fluorescent moiety linked to the C5 position of the nucleobase located outside the surface of the enzyme. As a consequence, the bulky fluorescent moiety binding to uracil has little influence on the coupling reaction. Our research describes a novel method for the synthesis of functionalized nucleosides that can be used as fluorescent probes. Further chemical modifications at the C5 position of uracil will provide new synthetic approaches to generate many more functionalized unnatural nucleosides.

Experimental section

General

All solvents and reagents were of reagent-grade quality, and used without further purification. The TLC analysis was carried out using silica gel 60 F254 1.05554 (Merck). Column chromatography was performed using Silica gel 60 N (Kanto Chemical Co.). The ^{1}H, ^{13}C and COSY NMR spectra were recorded using a JEOL ECS 400 spectrometer (400.0 MHz for ^{1}H; 100.4 MHz for ^{13}C). The spectra were referenced to TMS in chloroform-d_3, CD_3OD-d_4-d_4, and DMSO-d_6. The chemical shifts (δ) are reported in ppm; multiplicity is indicated by: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet), and br (broad). The coupling constants, J are reported in Hz. FABMS and MALDI-TOFMS were recorded using a JEOL JMS-700 V, and Applied Biosystems Voyager DE-STR, respectively. ESI-MS were recorded using the Accu TOF TLC JMS-T100TD (JEOL). The UV spectra were measured using a JASCO UV 630 BIO spectrometer in a 1 cm quartz cell.

Enzymatic reactions

Incubations generally contained 50 mM thymidine (10 μmol, 0.12 g), 5 mM of various uracil derivatives (1 μmol), 1 unit mL^{-1} of thymidine phosphorylase in 10 mL of 1 mM phosphate buffer (pH 6.8) and 0–60% of DMSO. Mixtures were stirred at 37 °C until the reactions reached equilibrium. Product formation was monitored by UV absorption as shown in ESI.† After removal of H_2O *in vacuo*, the residue was purified using HPLC by eluting with a H_2O–MeCN system to afford a pure unnatural nucleoside possessing a large functional group.

Thymidine phosphorylase (from *Escherichia coli*, EC 2.4.2.4) was purchased from Sigma-Aldrich Chemical Co. The units of enzymatic activity indicate the transition of native substrates as 1.0 μmol each of thymidine and phosphate to thymine and deoxyribose-1α-phosphate per 1 minute. In this research, we used the "unit" such as the amount of enzyme activity although we were using unnatural substrates.

2-Deoxy-[5-(coumarin-7-oxybut-3-yn)]uridine (dRC2U). ^{1}H NMR (CD_3OD-d_4): δ 2.23 (1H, J = 6.8, 6.8, 13.6 Hz, ddd), 2.32 (1H, J = 6.0, 6.4, 13.2 Hz, ddd), 2.93 (1H, J = 6.8 Hz, t), 3.79 (2H, J = 3.4, 11.8, 31.0 Hz, ddd), 3.94 (2H, J = 3.4 Hz, q), 4.27 (2H, J = 6.6 Hz, t), 4.41 (1H, J = 3.4, 6.8 Hz, dt), 6.26 (1H, J = 6.4 Hz, t), 6.27 (1H, J = 9.2 Hz, d), 6.99 (2H, *m*), 7.56 (1H, J = 8.4 Hz, d), 7.91 (1H, J = 9.6 Hz, d), 8.28 (1H, s); ^{13}C NMR (CD_3OD-d_4): δ 19.8, 40.3, 61.3, 66.5, 70.7, 72.8, 85.6, 87.8, 89.6, 99.3, 101.2, 112.2, 112.9, 129.2, 143.5, 144.4, 149.9, 155.7, 162.0, 162.2, 163.4; ESI-MS m/z 441 $[M + H]^+$; Anal. Calcd for $C_{22}H_{20}N_2O_8$: C, 60.00; H, 4.58; N, 6.36. Found: C, 60.03; H, 4.53; N, 6.15.

2-Deoxy-[5-(coumarin-7-oxyhex-5-yn)]uridine (dRC4U). ^{1}H NMR (DMSO-d_6): δ 1.61 (1H, J = 7.3 Hz, quin), 1.82 (1H, J = 6.9 Hz, quin), 2.06 (2H, J = 5.4, 6.4 Hz, dd), 2.40 (2H, J = 7.2 Hz, t), 3.74 (1H, J = 3.6 Hz, q), 4.07 (2H, J = 6.4 Hz, t), 4.38 (1H, J = 4.2 Hz, quin), 5.05 (1H, J = 5.0 Hz, t), 5.25 (1H, J = 4.4 Hz, d), 6.05 (1H, J = 6.4 Hz, t), 6.21 (1H, J = 9.2 Hz, d), 6.90 (2H, m), 7.55 (1H, J = 8.4 Hz, d), 7.91 (1H, J = 9.6 Hz, d), 8.06 (1H, s), 11.5 (1H, br); ^{13}C NMR (DMSO-d_6): δ 19.0, 25.2, 28.1, 61.5, 68.4, 70.7, 73.6, 79.6, 85.2, 88.1, 93.6, 99.5, 101.7, 112.8, 112.9, 113.3, 130.0, 143.2, 144.9, 150.0, 155.9, 160.9, 162.3, 162.4; ESI-MS m/z 491 $[M + Na]^+$; HRMS (ESI): calcd for $C_{24}H_{24}N_2O_8Na$ $[M + Na]^+$: 491.1418, found: 491.1430.

2-Deoxy-[5-(coumarin-7-oxyoct-7-yn)]uridine (dRC6U). ^{1}H NMR (CD_3OD-d_4): δ 1.55 (6H, m), 1.82 (6H, J = 6.4 Hz, quin), 2.23 (2H, m), 2.39 (2H, J = 6.8 Hz, t), 3.31 (2H, br), 3.75 (2H, J = 3.2, 6.8, 6.8 Hz, ddd), 3.92 (1H, J = 3.0, 6.0 Hz, dd), 4.06 (2H, J = 6.4 Hz, t), 4.36 (1H, J = 3.1, 6.0 Hz, tt), 6.22 (2H, m), 6.84 (1H, J = 2.4 Hz, d), 6.88 (1H, J = 2.2, 8.8 Hz, dd), 7.48 (1H, J = 8.8 Hz, d), 7.84 (1H, J = 9.6 Hz, d), 8.17 (1H, s); ^{13}C NMR (CD_3OD-d_4: δ 18.7, 25.1, 28.1, 28.6, 40.2, 61.3, 68.3, 70.7, 78.2, 85.5, 87.7, 93.7, 99.9, 100.8, 111.9, 112.5, 112.9, 129.1, 129.3, 142.8, 144.5, 149.9, 142.8, 144.5, 149.9, 155.8, 162.1, 162.8, 163.3; ESI-MS m/z 519 $[M + Na]^+$; HRMS (ESI): calcd for $C_{26}H_{28}N_2O_8Na$ $[M + Na]^+$: 519.1732, found: 519.1743.

2-Deoxy-[5-(pyrene-1-methyloxyhex-5-yn)]uridine (dRP4U). ^{1}H NMR (CD_3OD-d_4): δ 1.67 (2H, J = 7.2 Hz, quin), 1.81 (2H, m), 2.19 (2H, m), 2.37 (2H, J = 6.8 Hz, t), 3.69 (1H, m), 3.88 (1H, J = 3.2, 6.0 Hz, tt), 4.33 (1H, J = 3.2, 6.4 Hz, tt), 5.22 (2H, s), 6.20 (2H, J = 6.6 Hz, t), 8.11 (9H, m), 8.38 (1H, J = 9.2 Hz, d); ^{13}C NMR (CD_3OD-d_4): δ 19.9, 26.4, 30.0, 30.7, 41.6, 62.6, 71.1, 72.1, 72.4, 72.9, 86.9, 89.1, 94.9, 101.3, 124.7, 125.6, 125.9, 126.1, 126.4, 127.2, 128.5, 128.5, 128.8, 130.7, 132.3, 132.7, 132.8, 133.0, 144.3, 151.4, 164.8; ESI-MS m/z 561 $[M + Na]^+$; HRMS (ESI) calcd for $C_{32}H_{30}N_2O_6Na$: 561.2002, found: 561.2124.

2-Deoxy-[5-(1-hydroxybut-3-yn)]uridine (dRU2OH). ^{1}H NMR (CD_3OD-d_4): δ 2.22 (1H, J = 6.8, 13.6 13.6 Hz, ddd), 2.30 (1H, J = 3.6, 6.0, 13.2 Hz, ddd), 2.59 (2H, J = 6.4 Hz, t), 3.31 (1H, br), 3.35(1H, br), 3.69 (2H, J = 6.6 Hz, t), 3.77 (2H, J = 3.2, 11.6, 31.2 Hz, ddd), 3.93 (1H, J = 3.2, 6.4 Hz, dd), 4.40 (1H, J = 3.2, 6.0 Hz, dd), 6.23 (1H, J = 6.8 Hz, t), 8.22 (1H, s); ^{13}C NMR (CD_3OD-d_4): δ 23.2, 40.5, 60.2, 61.3, 70.7, 72.3, 85.6, 87.7, 91.0, 99.5, 143.4, 149.9, 163.5; ESI-MS m/z 319 $[M + Na]^+$; HRMS (ESI) calcd for $C_{13}H_{16}N_2O_6Na$: 319.0906, found: 319.0815.

2-Deoxy-[5-(1-hydroxyhex-5-yn)]uridine (dRU4OH). ^{1}H NMR (CD_3OD-d_4): δ 1.63 (4H, m), 2.24 (2H, m), 2.39 (2H, J = 6.4 Hz, t), 2.56 (2H, J = 6.0 Hz, t), 3.74 (2H, J = 3.2, 11.8, 32.2 Hz, ddd), 3.90 (1H, J = 2.8, 5.6 Hz, dd), 4.39 (1H, J = 3.2, 6.0 Hz, tt), 6.22 (1H, J = 6.4 Hz, t), 8.20 (1H, s); ^{13}C NMR (CD_3OD-d_4): δ 18.6, 4.7, 31.4, 47.4, 61.1, 70.7, 71.6, 85.5, 87.7, 93.5, 99.9, 142.9, 150.2, 163.7; Anal. Calcd for $C_{15}H_{20}N_2O_6$: C, 55.55; H, 6.22; N, 8.64. Found: C, 55.58; H, 6.16; N, 8.46.

2-Deoxy-[5-(1-hydroxyoct-7-yn)]uridine (dRU6OH). ^{1}H NMR (DMSO-d_6): δ 1.27 (4H, br), 1.36 (2H, J = 6.4 Hz, quin), 1.56 (2H, J = 7.2 Hz, quin), 2.00 (1H, J = 6.2, 14.0 Hz, tt), 2.34 (1H, m), 2.59 (2H, J = 7.4 Hz, t), 3.33 (1H, J = 6.4, 11.6 Hz, dd), 3.87 (1H, J = 3.6, 7.2 Hz, dd), 4.18 (2H, J = 4.4, 9.6 Hz, tt), 4.36 (1H, J = 5.2 Hz, t), 5.10 (1H, J = 5.2 Hz, t), 5.29 (1H, J = 4.0 Hz, d), 6.12 (1H, J = 6.2 Hz, t), 6.37 (1H, s), 8.61 (1H, s); ^{13}C NMR (DMSO-d_6): δ 25.6, 26.9, 27.8, 28.7, 32.8, 41.7, 61.2, 70.2, 79.6, 87.9, 88.6, 100.2, 107.0, 137.3, 154.4, 159.0, 171.7; Anal. Calcd for $C_{17}H_{24}N_2O_6$: C, 57.94; H, 6.86; N, 7.95. Found: C, 57.73; H, 6.78; N, 7.79.

Docking studies

Docking studies were performed using the MF myPresto sievgene program (FiatLux) with an AMBER-type molecular force field. This simulation was performed using two basic criteria as follows. Firstly, a global minimum search was carried out that assumes contact between three atoms of the substrate and three atoms at the surface of the protein pocket. This simulation evaluates the distance and similarities between the protein pocket and substrate. Secondly, a local minimum search was performed that is reinitiated with different conformers. The receptor–substrate interactions accounted for van der Waals, coulomb, hydrogen bond, and hydrophobic interactions. The dimensionless raw docking score that was used for receptor–substrate docking is below. The "g" was a parameter and it was set to 0.01 mol kcal^{-1}. "E" value showed the potential functions for each interaction, van der Waals interaction (E_{vdW}), coulomb interaction (E_{elec}), hydrogen bond ($E_{H\text{-}bond}$) and hydrophobic interaction (E_{ASA}). In detail, see ref. 17.

$$\text{Sraw} = g(E_{vdW} + E_{elec} + E_{H\text{-}bond} + E_{ASA})$$

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Notes and references

1 Recent examples: J. Riedl, P. Ménová, R. Pohl, P. Orsá, M. Fojta and M. Hocek, *J. Org. Chem.*, 2012, **77**, 8287–8293; L. Wicke and J. W. Engels, *Bioconjugate Chem.*, 2012, **23**, 627–642; S. Ikeda and A. Okamoto, *Chem.–Asian J.*, 2008, **3**, 958–968; P. Kaden, E. M.-Enthart, A. Trifonov, T. Fiebig and H.-A. Wagenknecht, *Angew. Chem., Int. Ed.*, 2005, **44**, 1636–1639.

2 M. M. Masud, T. Masuda, Y. Inoue, M. Kuwahara, H. Sawai and H. Ozaki, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 715–717; S. M. Park, H. Yang, S.-K. Park, H. M. Kim and B. H. Kim, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 5831–5834.

3 C. Höbartner, G. Sicoli, F. Wachowius, D. B. Gophane and S. T. Sigurdsson, *J. Org. Chem.*, 2012, **77**, 7749–7754; A. Roychowdhury, H. Illangkoon, C. L. Hendrickson and S. A. Benner, *Org. Lett.*, 2004, **6**, 489–492; A.-M. Chacko, W. Qu and H. F. Kung, *J. Org. Chem.*, 2008, **73**, 4874–4881.

4 H. Yamakoshi, K. Dodo, M. Okada, J. Ando, A. Palonpon, K. Fujita, S. Kawata and M. Sodeoka, *J. Am. Chem. Soc.*, 2011, **133**, 6102–6105; S. Ikeda, T. Kubota, D. O. Wang, H. Yanagisawa, T. Umemoto and A. Okamoto, *ChemBioChem*, 2011, **12**, 2871–2880.

5 D. J. Hurley and Y. Tor, *J. Am. Chem. Soc.*, 2002, **124**, 3749–3762; I. Bouamaied and E. Stulz, *Synlett*, 2004, **9**, 1579–1583.

6 D. M. Perrin, T. Garestier and C. Hélène, *J. Am. Chem. Soc.*, 2001, **123**, 1556–1563; C. H. Lam and D. M. Perrin, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 5119–5122.

7 A. Hatano, A. Harano and M. Kirihara, *Chem. Lett.*, 2006, 232–233.

8 A. Hatano, A. Harano, Y. Takigawa, Y. Naramoto, K. Toda, Y. Nakagomi and H. Yamada, *Bioorg. Med. Chem.*, 2008, **16**, 3866–3870.

9 S. Immacolata, S. C. Daniela, R. Silvia, U. Daniela and T. Marco, *Enzyme Microb. Technol.*, 2011, **49**, 52–58; I. Serraa, T. Bavaroa, D. A. Cecchinia, S. Dalyc, A. M. Albertinib, M. Terrenia and D. Ubiali, *J. Mol. Catal. B: Enzym.*, 2013, **95**, 16–22.

10 I. Votruba, A. Holý, H. Dvořáková, J. Günter, D. Hocková, H. Hřebabecký, T. Cihlar and M. Masojídková, *Collect. Czech. Chem. Commun.*, 1994, **59**, 2303–2330.

11 W. J. Cook, G. W. Koszalka, W. W. Hall, C. L. Burns and S. E. Ealick, *J. Biol. Chem.*, 1987, **262**, 3788–3789; M. R. Walter, W. J. Cook, L. B. Cole, S. A. Short, G. W. Koszalka, T. A. Krenitsky and S. E. Ealick, *J. Biol. Chem.*, 1990, **265**, 14016–14022.
12 M. J. Pugmire, W. J. Cook, A. Jasanoff, M. R. Walter and S. E. Ealick, *J. Mol. Biol.*, 1998, **281**, 285–299.
13 F. W. Hobbs Jr., *J. Org. Chem.*, 1989, **54**, 3420–3422.
14 H. Li, L. Czuchajowski and W. R. Trumble, *J. Heterocycl. Chem.*, 1997, **34**, 999–1003.
15 S. Mizukami, R. Takikawa, F. Sugihara, Y. Hori, H. Tochio, M. Wälchli, M. Shirakawa and K. Kikuchi, *J. Am. Chem. Soc.*, 2008, **130**, 794–795.
16 E. F. V. Scriven and K. Turnbull, *Chem. Rev.*, 2009, **109**, 4207–4220; A. H. El-Sagheerab and T. Brown, *Chem. Soc. Rev.*, 2010, **39**, 1388–1405; K. Fujimoto, S. Matsuda, N. Takahashi and I. Saito, *J. Am. Chem. Soc.*, 2000, **122**, 5646–5647; H. Xiong and F. Seela, *Bioconjugate Chem.*, 2012, **23**, 1230–1243.
17 Y. Fukunishi, Y. Mikami and H. Nakamura, *J. Mol. Graphics Modell.*, 2005, **24**, 34–45; Y. Fukunishi, Y. Mikami, K. Takedomi, M. Yamanouchi, H. Shima and H. Nakamura, *J. Med. Chem.*, 2006, **49**, 523–533.
18 A. Greube, K. Müller, E. T.-Petersen, A. Herrmann and P. Müller, *Biochemistry*, 2004, **43**, 464–472.