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Efficient chemical synthesis of a pyrimidine (6–4) pyrimidone photoproduct analog and its properties

Kousuke Satou,[a] Yasuo Komatsu,[a,*] Takuya Torizawa,[b] Koichi Kato,[b] Ichio Shimada,[b] Osamu Nikaido[c] and Eiko Ohtsuka[d]

[a]*Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan*
[b]*Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan*
[c]*Kanazawa University, Kanazawa 920-0934, Japan*
[d]*Health Sciences University of Hokkaido, Toubetsu 061-0293, Japan*

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Abstract

We synthesized an analog of a pyrimidine (6–4) pyrimidone photoproduct with a formacetal linkage instead of the phosphodiester in the (6–4) photoproduct, and investigated the interaction between a monoclonal antibody specific to the natural (6–4) photoproduct and a DNA containing the photoproduct analog.

Keywords: nucleic acids; nucleic acid analogs; photochemistry; antigens; antibodies.

Ultraviolet-light (UV) causes damage in DNA and generates photoproducts, predominantly in tandem pyrimidine sites such as cyclobutane pyrimidine dimers, pyrimidine (6–4) pyrimidone, and Dewar photoproducts. The (6–4) photoproducts are formed by UV-light 3–5-fold less frequently than the *cis–syn* cyclobutane type of pyrimidine dimer; however, they cause mutations more frequently than the cyclobutane types.[1,2] Enzymes that repair the (6–4) photoproduct have been reported.[3] To investigate the catalytic mechanism of the enzymes, DNA that contains the (6–4) photoproduct at a specific site is required as a probe. Although the chemical synthesis of the photoproduct dimer unit has been reported,[4] the yield of the photoreaction is not satisfactory. A large amount of the photoproduct is required for physicochemical analyses. Recently, a cyclobutane-type thymine dimer in which the phosphodiester was modified was reported;[5] however, the efficient synthesis of the (6–4) photoproduct has yet to be developed. We now have synthesized an analog of the (6–4) photoproduct and obtained it in a satisfactory yield.

* Corresponding author. E-mail: komatsu@pharm.hokudai.ac.jp (Y. Komatsu)

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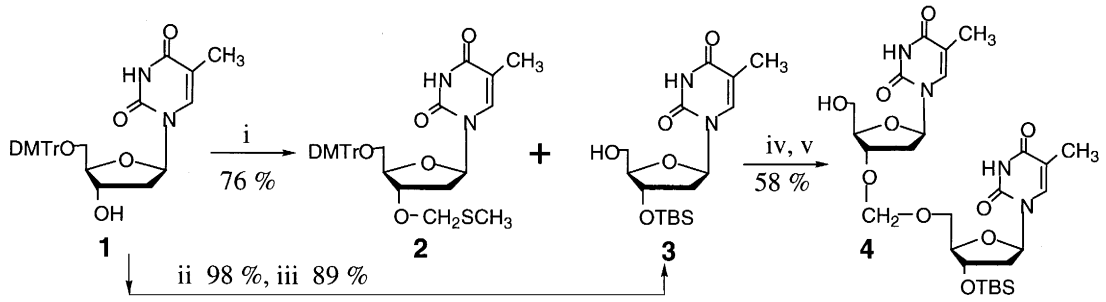
1. Results and discussion

We synthesized a thymine dimer (compound **4**) with a formacetal linkage using a standard procedure[6,7] except a *tert*-butyldimethylsilyl group was used for the 3′-hydroxyl group protection (Scheme 1). First, we compared the reactivity of compound **4** with that of **6** by HPLC[8] (Fig. 1). Compound **4** was converted to the (6–4) photoproduct **5** faster than the normal thymine dimer **6** as shown in Fig. 1b. The generation of T[6c4]T **5** became maximal by irradiation with 40 kJ/m^2, whereas T[6p4]T **7** with a phosphodiester required 100 kJ/m^2 irradiation for the maximum yield. Compound **4** with a formacetal linkage was found to be converted to T[6c4]T efficiently. The (6–4) photoproducts decomposed to unknown products by further UV irradiation.



Scheme 1. (i) CH_3SCH_2Cl, NaH, THF; (ii) TBDMSCl, imidazole, THF; (iii) 80% CH_3COOH/H_2O; (iv) NBS, 2,6-lutidine, molecular sieves 4 Å, CH_2Cl_2; (v) 80% CH_3COOH/H_2O

We scaled up the reaction,[9] and the yield of the photoreaction of T[6c4]T **5**[10] was 30% and about four-fold higher than that of T[6p4]T **7**. T[6p4]T is a diastereomeric mixture at the phosphotriester configuration; however, **5** was obtained as a unique product due to its lack of chirality.

We converted **4** to the thymine dimer amidite unit using the same preparation route as the T[6c4]T amidite **10** shown in Scheme 2, and incorporated TcT in the tetra-oligonucleotide, d(ATcTA). The photoreactivity of TcT in the oligonucleotide was also compared with that in a normal tetra-oligonucleotide, d(ATpTA). The analog d(AT[6c4]TA) was obtained more efficiently than the natural oligonucleotide (data not shown). This result is consistent with that obtained in the dimer reaction (T[6c4]T), and indicates that the thymine dimer with a formacetal linkage is also useful in post-synthetic photoreactions. It is thought that the high reactivity of TcT is derived from the two proximal thymine bases connected by the formacetal linkage.

After T[6c4]T **5** was converted to the amidite unit **10** (Scheme 2), **10** was inserted in the center of a tetra (AT[6c4]TA-biotin) or an octa (CAAT[6c4]TAAG-biotin) oligonucleotide,[11] using a 3′-biotin support for immobilization on a streptavidin surface.

Analysis of monoclonal antibody binding: The monoclonal antibody, 64M5, specifically binds to oligonucleotides with the (6–4) photoproduct,[12–14] and thus we investigated whether 64M5 would recognize the (6–4) photoproduct analog with a formacetal linkage using surface plasmon resonance.

64M5 bound with oligonucleotides containing a T[6c4]T photoproduct, although the binding affinities of 64M5 to those antigens were about 10-fold lower than those to the cognate T[6p4]T antigens (Table 1). These low affinities may be due to the formacetal linkage of the T[6c4]T photoproduct. Since there might be some electrostatic contacts between the phosphodiester of the natural T[6p4]T and the basic amino acid residues in the antibody, the binding affinity of 64M5 for the T[6c4]T antigens might be decreased. However, the binding magnitude of 64M5 to the T[6c4]T antigens was significant, because the dissociation constant is in the order of 10^{-8} M. This result indicates that the phosphodiester of T[6p4]T is

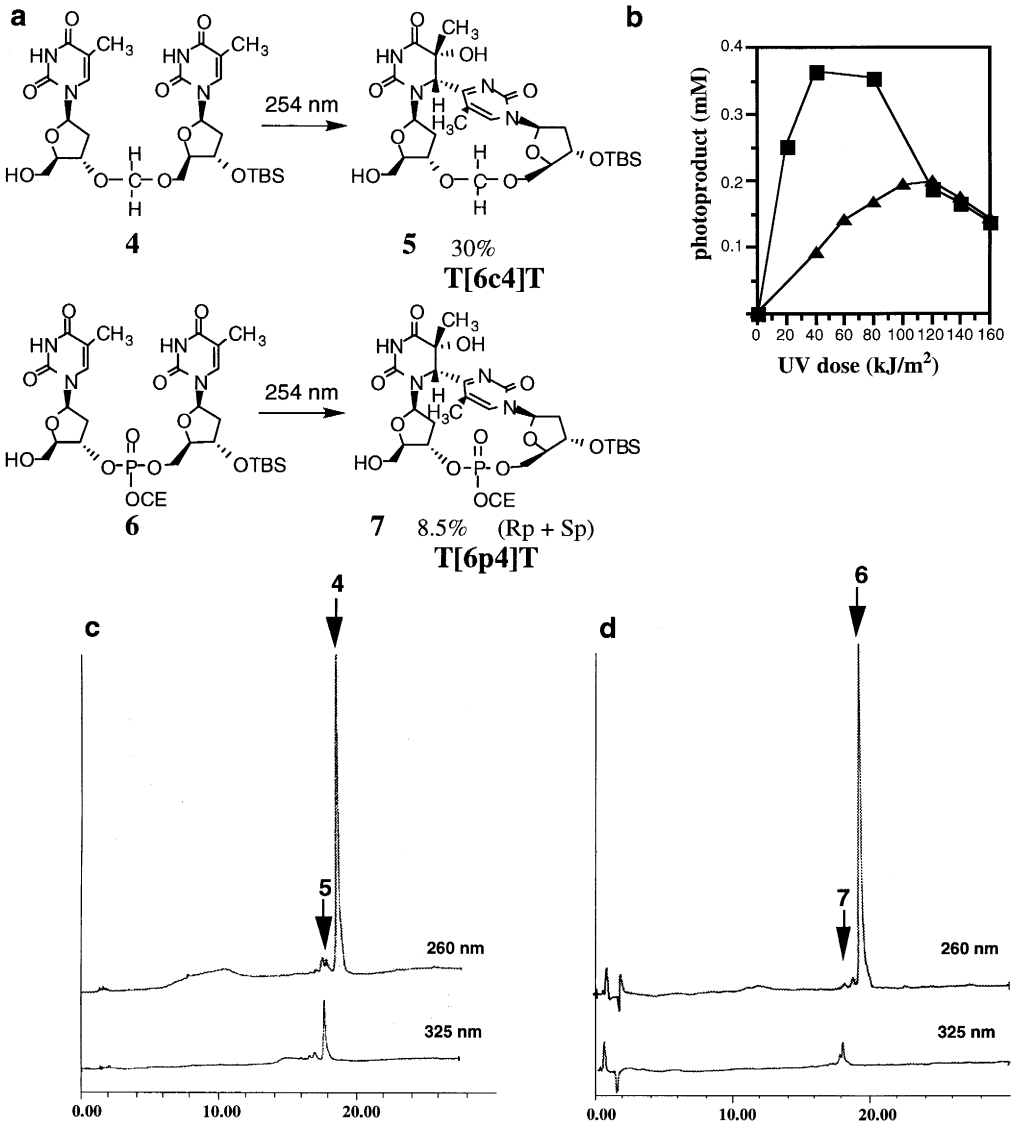
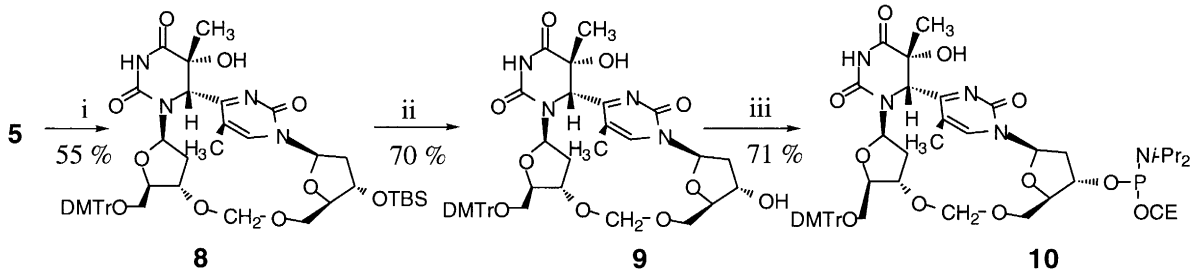


Fig. 1. (a) Photoreactions to give both T[6p4]T (compound **7**) and T[6c4]T (compound **5**) photoproducts. (b) Plots of the concentration of the (6–4) photoproduct versus UV dose. T[6p4]T (▲) and T[6c4]T (■). Analysis of photoreactions from compound **4** to **5** (c) and from compound **6** to **7** (d) by HPLC using a reverse phase column after the irradiation of UV-light (80 kJ/m^2)

important for the binding, but is not absolutely required for the recognition by the monoclonal antibody. The dissociation rate constants (k_{diss}), rather than the association rate constants (k_{ass}), of T[6c4]T were decreased as compared with those of T[6p4]T. If there are some conformational changes in the 64M5-T[6p4]T complex, such as an induced fit, then the structural change might be difficult to accommodate in the 64M5–T[6c4]T complex. Then, the antibody would easily dissociate from the complex.

Various organisms have repair enzymes that recognize UV-damaged DNA, such as the (6–4) photoproduct. For physicochemical analyses of enzyme-photoproduct interactions, large amounts of the photoproducts are required. The thymine dimer connected by a formacetal linkage increased the yield



Scheme 2. (i) DMTrCl, pyridine; (ii) TBAF, THF; (iii) *i*-Pr_2NP(OCE)Cl, *i*-Pr_2NEt, CH_2Cl_2

Table 1
Kinetic and thermodynamic parameters for antigen–antibody complexes

	k_{ass} ($M^{-1}s^{-1}$) x 10^{-5}	k_{diss} (s^{-1}) x 10^3	*Kd* (M) x 10^9	Δ H° (kcal/mol)	Δ S° (cal/mol/K)	Δ G° (kcal/mol)
T[6p4]T-4	2.6±0.9 (**1.0**)	1.1±0.02 (**1.0**)	3.3±1.3 (**1.0**)	-20.5	-29.5	-11.7
T[6c4]T-4	1.0±0.3 (**0.4**)	4.4±0.04 (**4.0**)	40±15 (**12**)	-18.5	-27.2	-10.4
T[6p4]T-8	2.9±0.7 (**1.1**)	0.1±0.005 (**0.1**)	0.38±0.09 (**0.1**)	-20.8	-26.5	-12.9
T[6c4]T-8	2.8±0.5 (**1.1**)	0.67±0.01 (**0.6**)	2.9±1.0 (**0.9**)	-20.1	-28.3	-11.7

The kinetic parameters at 25 °C are listed. Values relative to T[6p4]T-4 are shown in parentheses. Kinetic parameters for the 64M5-antigen complexes were measured with a Pharmacia BIAcore instrument. Buffer conditions: 10 mM HEPES-NaOH (pH7.4), 150 mM NaCl, 3.4 mM $EDTANa_2$, 0.005 % Tween-20. Thermodynamic parameters were calculated from data obtained at various temperatures.

of the (6–4) photoreaction, and simplified the preparation of the photoproduct. Furthermore, T[6c4]T may be useful as a probe for analyzing the electrostatic contacts within repair enzymes.

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8. Compounds **4** and **6** were each dissolved in a 20% aqueous acetonitrile solution. The 1 mM solutions containing **4** or **6** were irradiated with UV-light (254 nm). After aliquots were taken from the solutions at each UV dose, the generation of the (6–4) photoproducts was analyzed by HPLC using a reverse phase column (μ-Bondasphere, C-18, 3.9 mm i.d.×150 mm, Waters), and the gradient was 10–100% B buffer content over 20 min (A buffer, 5% aqueous acetonitrile, 0.1 M triethylammonium acetate (TEAA); B buffer, 60% aqueous acetonitrile, 0.1 M TEAA). The elution was monitored by a photodiode array

detector (Waters TM 996). Since both **5** and **7** absorb UV-light at 325 nm efficiently, the concentrations of those products were calculated from the peak areas at 325 nm.

9. After compound **4** (274 mg, 0.45 mmol) was dissolved in 20% aqueous acetonitrile (450 ml), the 1 mM solution of **4** (50 ml×9) was irradiated with UV-light (254 nm, 50 kJ/m^2). Compound **5** (82 mg, 0.13 mmol) was isolated by silica gel column chromatography (Wakogel, C-300).
10. Chemical shifts and coupling constants of **5** were as follows; ^{1}H NMR ($CDCl_3$) δ (ppm); 7.19 (s, 1H, cT H6), 6.63 (d, 1H, cT H1′, J=6.6 Hz), 6.20 (d, 1H, Tc H1′, J=8.5 Hz), 5.03 (s, 1H, Tc H6), 4.82–4.79 (m, 1H, cT H3′), 4.68 (AB, 2H -OCH_2O-), 4.24 (m, 1H, cT H4′), 4.01 (m, 2H, cT H5′), 3.96 (m, 2H, Tc H5′), 3.83 (dd, 1H, Tc H3′, J=4.6, 12.9 Hz), 3.72 (m, 1H, Tc H4′), 2.83 (m, 1H, cT H2′), 2.56 (m, 1H, cT H2′′), 2.27 (s, 3H, cT -CH_3), 2.01 (m, 1H, Tc H2′), 1.62 (s, 3H, Tc -CH_3), 1.57 (m, 1H, Tc H2′′), 0.87 (s, 9H, TBS *t*-Bu), 0.05 (s, 6H, TBS -CH_3). Tc and cT indicate 5′- and 3′-side thymidines, respectively. The base structure was confirmed by ROESY ($CDCl_3$; Tc H3′ and cT -CH_3, Tc H6 and cT -CH_3, cT H6 and cT -CH_3, cT H6 and cT H2′, cT H6 and -CH_2-). FAB-HRMS calcd for $C_{27}H_{43}N_4O_{10}Si$ $[M+H]^+$ 611.2745, found 611.2741.
11. Oligonucleotides containing T[6c4]T were synthesized using a 3′ biotinon CPG support (CLONTECH) identical to the natural (6–4) photoproduct,[4] and were purified on a reverse phase column (μ-Bondasphere, 3.9 mm i.d.×150 mm, Waters). The gradient for T[6c4]T-4 and T[6c4]T-8, which were covalently connected with biotins, were 0–100% and 20–40% B buffer content, respectively, over 20 min (A buffer, 5% aqueous acetonitrile, 0.1 M TEAA; B buffer, 25% aqueous acetonitrile, 0.1 M TEAA). The overall yields of T[6c4]T-4 and T[6c4]T-8 were 31% and 14%, respectively.
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