Solution structure of a nucleic acid photoproduct of deoxyfluorouridylyl-(3'-5')-thymidine monophosphate (d-FpT) determined by NMR and restrained molecular dynamics: structural comparison of two sequence isomer photoadducts (d-U5p5T and d-T5p5U)

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ABSTRACT

Acetone-sensitized irradiation using UV-B (sun lamp, $\lambda_{\text{max}} = 313 \text{ nm}$) of deoxyfluorouridylyl-(3'-5')-thymidine monophosphate (d-FpT, F = fluorouracil), produces two major photoproducts, the *cis-syn* cyclobutane-type photodimer and a defluorinated (5-5) photoadduct, d-U5p5T. Product distribution is dependent on the pH of the irradiation solution, as was the case of irradiated d-TpF. At high pH (8-10) the (5-5) photoadduct is the major photoproduct. Irradiation of d-FpT shows a much faster photodegradation rate than the sequence isomer d-TpF. Multinuclear NMR experiments establish the formation of (5-5) covalent bonding between the C5 (d-U5p-, where the fluorine had been) and the C5 (-p5T) and the C6 (-p5T) acquires an OH group. NOE interproton distances and dihedral angles derived from J coupling analysis are constrained to refine model structures of d-U5p5T in restrained molecular dynamics calculations. The resultant structures obtained show 5S-6S as the most probable chiralities of the C5 and C6 atoms of the thymine, which is the opposite chirality to the corresponding atoms in the sequence isomer d-T5p5U. The orientation of the C5 substituents (-p5T fragment), the CH_3 and the uracil are pseudo-axial and pseudo-equatorial respectively. Glycosidic angles are in the *anti* regions for both the d-U5p- and -p5T residues. Averaged backbone conformations of the two photoadducts, d-U5p5T and d-T5p5U, are similar, although the overall structure of d-U5p5T appears much more flexible than that of d-T5p5U. In particular, the sugar conformations of the 5'-end residues show a remarkable difference in flexibility.

INTRODUCTION

Ultraviolet light causes a variety of damage to DNA and model compounds, producing various photoproducts (e.g. cyclobutane-type dimers, adducts and hydrates) depending on experimental conditions. Sensitivity of cells to UV light and X-rays may be notably increased by substituting 5-halogenated uracil (chloro-, bromo- or iodo-) for thymine in DNA (1). The earliest aspects of the related photosensitization studies have been surveyed in a comprehensive review (2). Additional photochemical reactions can occur which may produce additional photoproducts (3,4). It was shown in a previous report that a fluorinated model nucleic acid exhibited enhanced sensitivity to sensitized UV-B irradiation compared with the normal analog and an unusual photoproduct, the so called (5-5) photoadduct, was produced (5). The biological significance of this class of (5-5) photoadducts is currently not known, since their biological presence has not yet been determined. However, these lesions appear to be generally produced by other halogen-containing nucleic acid bases (Khalili and Alderfer, unpublished results).

Sensitized irradiation (20% acetone and UV-B light) of deoxyfluorouridylyl-(3'-5')-thymidine monophosphate (d-FpT) produced the *cis-syn* cyclobutane-type photodimer and a defluorinated photoadduct (d-U5p5T). The [photodimer]:[photoadduct] distribution was shown to be pH dependent. Interestingly, d-FpT has shown a much faster photodegradation rate than d-TpF. This photoadduct was structurally characterized through use of multinuclear magnetic resonance experiments. The detailed mechanism leading to formation of the defluorinated photoadduct is not currently known. In this photoadduct the intact C5-C6 double bond of thymine is modified such that the C5 of thymine is covalently bonded with the C5 of uracil (where fluorine had been). In this paper we report NMR and molecular modeling studies on the three-dimensional structure of d-U5p5T, which are contrasted with the sequence isomer d-T5p5U. When the thymine

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is replaced with a fluorouracil at the 5'-end the parent compound (d-FpT) exhibits unusual photosensitivity compared with d-TpF, as well as an opposite direction of solvent approach in leading to the addition of the C6 OH group in the photoadduct, d-U5p5T. In the structure of d-T5p5U, the chiralities of both C5 and C6 of the thymidine are the *R* configuration (13), while in d-U5p5T they are both the *S* configuration. Compared with d-T5p5U, the sugar of the uridine in d-U5p5T was not conformationally pure and exhibited a large degree of conformational freedom in the torsion angle γ_2 (O5'-C5'-C4'-C3'). From a methodological point of view it is generally impossible to find a unique conformer in such a flexible molecule from NOE experiments alone, due to the rapid time-averaging behavior of the molecule. Nevertheless, the observed NOEs were very reasonably interpreted as an ensemble of structures (conformers) based on the results of restrained molecular dynamics calculations and experimental J couplings.

MATERIALS AND METHODS

Materials

d-FpT was chemically synthesized using the solution phosphotriester method (5). Irradiation of a solution containing 20% aqueous acetone, 0.1 M sodium phosphate and 1 mg/ml d-FpT, pH 10.0, using Westinghouse FS20T12 sun lamps produced the defluorinated photoadduct d-U5p5T as a major photoproduct.

NMR sample preparation

Purified d-U5p5T was repeatedly lyophilized from D₂O and finally dissolved in 400 μ l D₂O (99.9%) containing 0.05 M sodium phosphate buffer (pH 7.0). Samples were prepared at several concentrations, with a maximum of 180 OD/ml (measured at 260 nm).

NMR experiments

NMR spectra were obtained on a Bruker AM-400, operating at 400 MHz for ¹H, 100.6 MHz for ¹³C, 162 MHz for ³¹P and 376.3 MHz for ¹⁹F. A two-dimensional NOE spectrum (mixing time of 550 ms) was acquired using the TPPI (6) phase-sensitive mode at 27°C. Residual water was presaturated (only during relaxation delay) with low decoupling power. The observation frequency was placed at the low field end of the ¹H spectrum to avoid anti-diagonal artifacts (7). Acquisition parameters included a spectral width of 7000 Hz, with 2048 data points in the *t*₂ domain and 512 *t*₁ increments, each containing 64 transients. The data was processed on a Sun SPARC 4/370 with NMR2/NMRZ software from New Methods Research Inc. (East Syracuse, NY) and Tripos Inc. (St. Louis, MO). After zero-filling, the data matrix was 2K $\times$ 2K. Shifted sine bell functions of 90° and 45° were applied to data points in the *t*₂ and *t*₁ dimensions respectively, before Fourier transformation.

RESULTS AND DISCUSSION

Characterization of d-U5p5T

The relative photosensitivity of the dinucleoside monophosphates to UV radiation was determined to assess the photochemical effect of the chemical change resulting from replacing thymine with fluorouracil at the 5'-end. The sequence isomer

Figure 1. First-order rate analysis of photodegradation of dinucleoside monophosphates.

d-FpT showed a much higher degradation rate than d-TpF (Fig. 1). For identification of the photoadduct, d-U5p5T, the same procedures as described previously were employed (5). The fluorine resonance identified as fluoride ion (F⁻) was accompanied directly by formation of the photoadduct d-U5p5T. Absence of the fluorine atom from the base moiety of the photoadduct was obvious from the lack of fluorine coupling to any carbon (i.e. all carbons were singlets with broadband proton decoupling). Direct evidence for a C5-C5' linkage between two bases was provided by the proton-coupled C5 resonances of each base. The C5 (-p5T) resonance appeared as a doublet of quartets. Upon hydrogen decoupling, the methyl resonance became a doublet of doublets, resulting from the remaining proton coupling of both H6s to the C5 (-p5T) resonance. The coupling between C5 (-p5T) and H6 (d-U5p-) was consistent with a bond between two C5s. Saturation of the thymine C5-C6 double bond was confirmed from the upfield shift of the H6 base proton (Table 1; $\delta_{H6(T)} = 5.3$ p.p.m.) to a chemical shift position which is typical for C6 hydroxylated pyrimidines (8,9).

Analysis of the coupling constants

The coupling constants of the sugar protons (Table 1) were interpreted in terms of pseudo-rotational parameters (ϕ , *P*) and populations of the *N*- and *S*-type. Both the uridine and thymidine sugars showed a preference for the *S*-type conformer, but they were not conformationally pure (as shown in Table 2). The results of rotamer distribution analyses are summarized in Table 3. The torsion angle γ (O5'-C5'-C4'-C3') of the uridine was predominantly *g*+, while a large conformational freedom for rotation of the analogous bond (γ) in the thymidine was observed between *trans* and *gauche*. Both torsion angles β (P-O5'-C5'-C4') and ϵ (C4'-C3'-O3'-P) showed *trans* conformational preferences.

Table 1. ¹H and ¹³C NMR parameters of d-U5p5T and d-T5p5U

	d-U5p5T		d-T5p5U	
	d-U5p-	-p5T	d-T5p-	-p5U
¹ H chemical shift (p.p.m.) ^a				
H1'	6.271	6.072	6.184	6.424
H2'	2.701	2.363	2.304	2.489
H2''	2.659	2.162	2.526	2.236
H3'	4.720	4.363	4.865	4.678
H4'	4.050	3.984	3.881	4.058
H5'	3.986	4.118	3.843	4.105
H5''	3.919	4.000	3.813	4.030
CH ₃	—	1.550	1.531	—
H6	8.050	5.250	5.290	7.902
¹ H- ¹ H coupling constants (Hz)				
J1'2''	2.39	5.60	9.50	5.33
J2'2''	-12.87	-13.63	-14.45	-13.87
J1'2'	5.49	8.21	1.79	10.06
J2'3'	5.37	8.94	7.54	7.26
J2''3'	2.20	3.22	10.33	1.87
J3'4'	8.79	5.28	8.12	3.48
J4'5'	2.80	1.67	2.51	2.91
J4'5''	1.94	6.61	2.79	1.73
J5'5''	-13.19	-11.16	-12.26	-11.59
¹ H- ³¹ P coupling constants (Hz)				
J3',P	7.33	—	7.93	—
J4',P	—	1.34	—	2.68
J5',P	—	1.34	—	2.66
J5'',P	—	2.34	—	2.07
¹³ C chemical shifts (p.p.m.) ^b				
C1'	17.696	16.691	16.305	16.056
C2'	-29.128	-29.016	-31.445	-29.256
C3'	2.641	2.800	4.072	2.744
C4'	17.032	16.798	15.294	17.610
C5'	-8.632	-1.547	-8.422	-2.206
C2	86.456	84.067	87.094	84.211
C4	96.454	107.873	107.598	96.071
C6	12.811	73.515	14.385	72.821
CH ₃	—	-46.008	—	-45.808
¹³ C- ³¹ P coupling constants (Hz)				
JC2',P	1.30	—	<0.4	—
JC3',P	4.76	—	5.09	—
JC4',P	7.69	10.48	10.49	9.24
JC5',P	—	4.36	—	4.96

^aChemical shifts reported from TSP.^bChemical shifts reported from dioxane at 30°C.**Table 2.** Furanose distribution of d-U5p5T and d-T5p5U

	% ² E ^a	% ³ E ^b	Equilibrium ^c		Form II		Φ
			f _I	P _I	f _{II}	P _{II}	
d-U5p5T							
U5p-	38	62	0.36	-4.6	0.64	122.4	37.5
-p5T	58	37	0.15	-19.6	0.85	122.7	31.0
d-T5p5U							
T5p-	18	82	0.06	294.4	0.94	24.9	40.0
-p5U	74	26	0.05	-10.7	0.95	30.5	37.0

^aEquation used: $f_{C2'-endo} = J_{1'2'}/(J_{1'2'} + J_{3'4'})$, from Alderfer and Ts'o (1977).^bEquation used: $f_{C3'-endo} = J_{3'4'}/(J_{1'2'} + J_{3'4'})$, from Alderfer and Ts'o (1977).^cCalculations are performed by using the program PSEUROT: f_I and f_{II} are mole fractions, P_I and P_{II} are phase angles, Φ is puckering amplitude.**Table 3.** Rotamer distribution of d-U5p5T and d-T5p5U

	γ (C5'-C4') ^a		β (O5'-C5') ^b		ε (C3'-O3') ^c			
	g+	t	g-	t	g+	g-	g	t
d-U5p5T								
U5p-	1.00	0.00	0.00	—	—	—	0.03	0.84
-p5T	0.57	0.43	0.00	1.00	0.00	0.00	—	—
d-T5p5U								
T5p-	1.00	0.00	0.00	—	—	—	0.03	0.84
-p5U	0.53	0.47	0.00	1.00	0.00	0.00	—	—

^aKarplus-type equation used: $J_{HH} = 10.5 \cos^2 \phi - 1.2 \cos \phi$ (Altona and Sundaralingam, 1973) and rotamer equations from Davies and Danyluk (1974).^bEquation used: $J_{HH} = 15.3 \cos^2 \phi - 6.1 \cos \phi + 1.6$ (Lankhorst *et al.*, 1984) and rotamer equations from Davies and Danyluk (1974).^cEquation used: $f_{g-} = (J_{PC2'} - 1.0)/9.2$; $f_t = J_{PC4'}/9.2$ (Alderfer and Ts'o, 1977).

NOE results

Observed NOEs are summarized in Table 4. One of the major structural components of this molecule is the configuration of the C5 and C6 atoms of the thymine. Here the original C5–C6 double bond was modified to a single bond with concomitant bonding of C5 (U5p-) and C5 (-p5T) and addition of OH to the C6 (-p5T). The pattern and intensity distribution of observed NOEs are expected to yield information on these configurations. A strong NOE between two H6 protons and two medium-sized NOEs between the C5 CH₃ group (-p5T) and two H6 protons were observed. An inter-residue NOE between H6 (U5p-) and CH₃ (-p5T) was significantly more intense than the intra-residue NOE between H6 (-p5T) and CH₃ (-p5T), even though both cross-peaks were classified as medium in the calculation. Another important structural aspect of this system is the conformation of the glycosidic bond. In the case of an *anti* conformation, the H6 base proton is close to the H2' or H3' of the sugar residue, whereas the H1' proton is pointing in the other direction and distant from the base protons. On the other hand, if the glycosidic bond is *syn*, the H6 base proton is close to the sugar H1' proton but more distant from the H2', H3' and H2'' protons (10). In the uridine fragment, a strong NOE between H6 and H3' and a weak NOE between H6 and H2' was observed, while in the thymidine fragment, only a medium-sized NOE between H6 and H2' was observed. Therefore, these observations indicate *anti* conformations of the glycosidic bonds of both residues. Initial attempts were made to assign specific configurations to the C5 and C6 atoms among four possible pairs (5R–6R, 5S–6R, 5R–6S, 5S–6S) using a combination of molecular modeling techniques and a qualitative application of the observed NOEs. Since this dinucleoside monophosphate photoadduct was in the fast molecular tumbling time-frame, only a few NOE cross-peaks were observed between proton pairs at distances up to 3.5 Å (11). The limited number of critical NOEs between certain proton pairs precluded an unequivocal assignment of the chirality of the C5 and C6 atoms based on a simple qualitative evaluation of the NOE data.

Molecular modeling and structure refinement

In general it is difficult to determine the structure of a unique conformer of a flexible molecule in solution solely by NOE experiments. In this small molecule, only three inter-residue NOEs (including one rather weak cross-peak) were observed. The flexible sugar of the 5'-end fragment showed a two-state conforma-

tion distribution, whose populations were calculated by J coupling analysis. In addition, modeling results suggested that two major inter-residue NOEs, H6 (d-U5p)-H6 (-p5T) and H6 (d-U5p)-CH₃ (-p5T), would be more affected by different chirality than by local flexibilities. In this regard, each conformational state of an equilibrium mixture was assumed to be a pseudo-conformer in the first approximation of the real conformational space of this molecule. In order to estimate which configurational and conformational features were most compatible with the observed NOEs, 16 structures were generated using CHARMM (12) by considering all possible chiralities (*R* and *S*) of the C5 and C6 atoms of the thymine, the flexible sugar puckering of the uridine (²E↔³E) and the torsional angle γ_2 (*trans*↔*gauche*), i.e. $4 \times 2 \times 2 = 16$ structures. Volume integrated NOE cross-peaks, grouped as strong ($d < 2.5$ Å), medium ($d < 3.0$ Å) and weak ($d < 3.5$ Å), were incorporated as a set of distance constraints. In addition, some torsional angles (ϵ , β , γ , τ_0 , τ_2), which were calculated based on the observed NMR data, were also constrained. Unusually short atomic contacts were removed by energy minimization with a 30-step Powell algorithm before performing restrained molecular dynamics calculations. Restrained molecular dynamics were applied to the 16 model structures for 5 ps at 300K using the XPLOR program (13). Further restrained energy minimization using the Powell algorithm was done until the energy gradient reached zero. The most probable solution structure was determined on the basis of the number of NOE violations, average energy and the RMSD of NOE, angle and bond constraints. The results of the restrained molecular dynamics of the 16 structures are summarized in Table 5. The lowest average

energy was observed in the structural group with the 5S-6S configuration, which also produced the lowest number of NOE violations. In the other structural groups a number of unacceptable NOE violations were observed, concomitant with relatively higher average energy. On the basis of these observations, the 5S-6S configuration is most probable for the photoadduct d-U5p5T. In the structure group with these configurations, the structure STR 5 was the most consistent with NOE observations, while others produced one or two NOE violations. In principle, when the population distributions of each conformer existing in a flexible molecule are known, it is possible to reconstruct an ensemble averaged structure from all the existing conformers in solution. By assuming four structures (STR 5, 6, 7 and 8) as a pseudo-ensemble of the real conformational space, their population distributions were estimated on the basis of the results of the rotamer distribution analysis (Table 3) and the furanose distribution analysis (using equations a and b in Table 2). For instance, STR 5 has a probability fraction of population of 0.35, a product of 0.57 (% g+) and 0.62 (% C3'-endo). Summation of all the products of this probability fraction and the interproton distance in each conformer gives the averaged distance. As shown in Table 4, only two deviations from experimental NOEs were observed. One is the distance between H6 (U5p-) and H2' (U5p-) and the other is between H6 and H3' in the same residue. These distances are quite sensitive to the sugar conformation. This discrepancy could arise from using inappropriate furanose populations or from an incorrect structure of the refined conformer. This result suggests that a slightly smaller population of the C2'-endo-type conformation may actually exist, rather than that indicated by the calculated value.

Table 4. Observed NOE and distance constraints used in energy minimization (A) and calculated distances in the refined structures (B)

(A) NOE and distance constraints				(B) Calculated distances of refined structures				
Proton (i)	Proton (j)	NOE ^a	distance	STR 5	STR 7	STR 6	STR 8	$\Sigma p_i d_i^b$
Uri H6	Thy H6	s	<2.5	2.559	2.394	2.352	2.228	2.414
Uri H6	Uri H3'	s	<2.5 ^c	1.952	3.898	2.269	4.152	2.817
Uri H6	Uri H1'	a	>3.5 ^a	3.739	3.737	3.718	3.736	3.732
Uri H6	Uri H2'	w	<3.5	3.304	2.547	3.506	2.580	3.076
Uri H6	Thy CH3	m	<3.5 ^d	2.537	2.460	2.524	2.520	2.514
Thy H6	Thy CH3	m	<3.5 ^d	3.368	3.039	3.006	3.004	3.141
Thy H6	Thy H2'	m	<3.0	3.090	2.815	3.035	2.707	2.953
Thy H6	Thy H1'	a	>3.5 ^a	3.729	3.656	3.449	3.716	3.639
Thy H6	Uri H2'	a	—	3.879	2.172	4.053	2.329	3.302
Thy H6	Uri H3'	w	<3.5	3.325	3.831	3.241	4.089	3.537
Uri H1'	Uri H2''	s	<2.5	2.284	2.385	2.252	2.401	2.316
Thy H1'	Thy H2''	s	<2.5	2.421	2.393	2.406	2.403	2.408
Uri H2'	Uri H2''	s	<2.5	1.795	1.798	1.797	1.799	1.797
Thy H2'	Thy H2''	s	<2.5	1.843	1.796	1.842	1.727	1.814
Uri H2'	Uri H3'	w	<3.5	2.505	2.105	2.517	2.164	2.366
Thy H2'	Thy H3'	m	<3.0	2.419	2.149	2.448	2.387	2.363
Thy H2'	Thy H5''	a		4.019	4.615	3.191	3.669	3.871
Thy H6	Thy H5''	a		5.261	5.448	2.554	2.929	4.199

^aa, non-NOE; s, strong; m, medium; w, weak.

^bAveraged distance over four conformers (see text): p_i is the population fraction, d_i is interproton distance in each conformer.

^cNot used for the refinement of structures with C2'-endo type sugar in the uridine.

^dAdditional 0.5 Å for methyl group.

Table 5. Structural assessment of refined model structures from restrained molecular dynamics

Starting structure	Chirality C5 C6		Furanose conformation	γ_2	RMSD NOE	Angle	Bond	Total energy ^a	NOE violation ^b	Average energy ^{b,c}
STR 1	<i>S</i>	<i>R</i>	³ E	g+	0.031	3.837	0.011	15.5	2	
STR 2	<i>S</i>	<i>R</i>	³ E	t	0.033	3.932	0.012	107.5	3	
STR 3	<i>S</i>	<i>R</i>	² E	g+	0.007	3.894	0.012	26.3	3	
STR 4	<i>S</i>	<i>R</i>	² E	t	0.007	4.019	0.012	119.4	4	67.4
STR 5	<i>S</i>	<i>S</i>	³ E	g+	0.058	3.818	0.011	21.3	0	
STR 6	<i>S</i>	<i>S</i>	³ E	t	0.045	3.971	0.013	21.0	2	
STR 7	<i>S</i>	<i>S</i>	² E	g+	0.014	3.780	0.010	27.0	1	
STR 8	<i>S</i>	<i>S</i>	² E	t	0.014	3.761	0.011	24.2	2	23.3
STR 9	<i>R</i>	<i>R</i>	³ E	g+	0.138	4.172	0.013	31.6	2	
STR 10	<i>R</i>	<i>R</i>	³ E	t	0.133	4.091	0.013	37.9	3	
STR 11	<i>R</i>	<i>R</i>	² E	g+	0.119	4.069	0.012	71.7	4	
STR 12	<i>R</i>	<i>R</i>	² E	t	0.153	3.955	0.013	71.7	4	53.2
STR 13	<i>R</i>	<i>S</i>	³ E	g+	0.022	3.976	0.012	23.7	2	
STR 14	<i>R</i>	<i>S</i>	³ E	t	0.048	4.128	0.012	25.3	3	
STR 15	<i>R</i>	<i>S</i>	² E	g+	0.053	4.045	0.013	42.1	3	
STR 16	<i>R</i>	<i>S</i>	² E	t	0.069	4.172	0.015	43.7	3	33.7

^akcal/mol.^bNumber of violations greater than 0.5 Å, including non-NOE interproton distance.^cAveraged over four structures with same chiralities. **Figure 2.** Stereo view of STR 5 of d-U5p5T (5S, 6S). **Figure 3.** Superimposed refined structures of d-U5p5T (left) and d-T5p5U (right).

Structural analysis and comparison with d-T5p5U

The calculated results showed that 5S–6S is the most probable chirality of the C5–C6 atoms in the thymine (Fig. 2). This is opposite to the chirality pair of the corresponding atoms in d-T5p5U. The opposite C5–C6 chiralities in the thymine portion of the d-T5p5U and d-U5p5T photoproducts and the experimental observation that only one of four possible configurational isomers is produced are useful in developing a mechanism of formation for these photoadducts. Thus it is proposed that both starting materials, d-TpF and d-FpT, exist in solution as stable base-stacked dinucleoside monophosphates with both bases in the *anti* conformation. In the excited base-stacked state, an initial event is an attack by the oxygen from a water molecule on the C6 of thymine from the outside of the base-stacked sandwich, leading to the observed chiralities of the respective C6s and, following concomitant C5–C5 bond formation from inside the stack, leads to the observed chiralities of the C5s. Thus the C6 OH

group has a relative orientation (originating from a similar directional approach of bulk solvent from outside the stack) which is the same in both of these sequence isomers. Saturation of the double bond between C5 and C6 of the thymine leads to the sp³ configuration on the corresponding carbon atoms, which was manifested as a twisted plane of the thymine. The average torsion angle (N1–C6–C5–C4) of the thymine is 27°, while the uridine is planar. A similar twisted, six-membered ring containing a sp³ carbon is observed in the refined structure of the (6–4) photoproduct of thymidylyl-(3′–5′)-thymidine (14) and in the structure of deoxy-m⁵HO⁶-uridylyl(5–5)-(5′–3′)-deoxyuridine monophosphate (d-T5p5U) (15). In d-U5p5T, the conformation of the C5 CH₃ and the uracil base were pseudo-equatorial and pseudo-axial respectively relative to the C5 (–p5T) atom. Similar conformational features are observed in d-T5p5U. The conformation of the glycosidic angles of both residues were *anti*, while *high anti* (T5p–) and *anti* (–p5U) are observed in d-T5p5U. This difference presumably relates to the differential flexibility of the

sugar puckering of the 5'-end residue. In d-U5p5T a more flexible sugar at the 5'-end was observed than the corresponding sugar in d-T5p5U, which is predominantly C3'-endo type. In both photoadducts the C2'-endo type was observed at the 3'-end. As shown in Table 6, the averaged backbone conformations of the two isomers are similar. However, the overall conformation of d-U5p5T appears much more flexible than that of d-T5p5U, as indicated in Figure 3, where superposition of the refined structures of the two isomers shows large differential fluctuations. A manifestation of differential flexibility of these two photoadducts in the sugar-phosphate regions is apparent from the temperature dependence of the ^{31}P chemical shifts ($\Delta\delta$ in Table 7) of d-U5p5T and d-T5p5U compared with normal d-FpT and d-TpF respectively. Over the temperature range 18–63°C, $\Delta\delta$ is reduced by 63% in d-T5p5U, while in contrast it is increased by 5% in d-U5p5T, suggesting that the backbone of d-U5p5T is significantly more flexible.

Table 6. Backbone torsional angles in the refined structures of d-T5p5U and d-U5p5T

	d-U5p5T					d-T5p5U			
	STR 5	STR 7	STR 6	STR 8	Average	STR I	STR II	Average	
γ_1	62	59	60	60	60	55	61	57	
δ_1	81	103	76	108	89	74	83	78	
ϵ_1	176	-159	-148	-169	-166	-132	-138	-135	
ζ_2	59	-41	-49	-43	-10	-70	-72	-71	
α_2	-177	-99	164	102	-178	-77	146	-136	
β_2	176	177	-170	178	-176	-179	174	178	
γ_2	59	55	-178	-172	112	57	181	110	
δ_2	134	92	148	135	129	142	156	148	
χ_1	-140	-148	-150	-146	-146	-91	-87	-89	
χ_2	-134	-133	-152	-129	-138	-99	-111	-104	

Table 7. ^{31}P chemical shift distribution of two photoadducts at two different temperatures

	18°C	63°C	$\Delta\delta^a$
d-FpT	-3.993	-3.714	0.279
d-U5p5T	-3.791	-3.498	0.293
d-TpF	-3.857	-3.601	0.256
d-T5p5U	-2.929	-2.833	0.096

$$^a\Delta\delta = \delta_{63^\circ} - \delta_{18^\circ}.$$

CONCLUSION

Multinuclear NMR experiments characterized the formation of a defluorinated photoadduct of d-FpT, deoxy-m⁵HO⁶-uridyl(5-5)-(3'-5')-deoxyuridine (d-U5p5T). The solution structure was determined by analysis of NMR data and restrained molecular dynamics calculations. In the refined structure, the deduced chiralities of the C5 and C6 atoms of the -p5T were 5*S* and 6*S*. These are opposite to those observed previously in d-T5p5U. A mechanism of formation for the (5-5) photoadduct was proposed to explain the observed chiralities of the C5 and C6 atoms in both sequence isomers. The presence of sp³ carbons in the six-membered ring of -p5T yielded a twisted plane, with an average torsion angle (N1-C6-C5-C4) of 27°. With respect to C5 (-p5T), the C5 CH₃ group and the uracil were pseudo-equatorial and pseudo-axial respectively. Both glycosidic angles were observed as the *anti* conformations. The averaged structures of the two photoadducts (d-U5p5T and d-T5p5U) showed similar backbone conformations, however, the overall conformation of d-U5p5T was much more flexible than that of d-T5p5U. In particular, the sugar conformation of the 5'-end residue in d-U5p5T showed an equilibrium mixture, while the C3'-endo type was predominant as the corresponding sugar in d-T5p5U.

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