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SITE-SPECIFIC MODIFICATION OF THE PYRIMIDINE RESIDUE DURING THE DEPROTECTION OF THE FULLY-PROTECTED DIURIDYLIC ACID.

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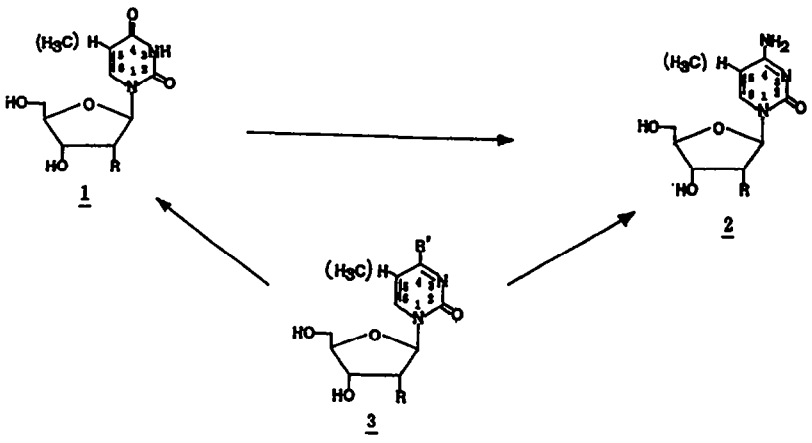
Abstract: A study of four different O-4 and N-3 protected uridine derivatives, 4 to 7, for their stabilities under different conditions versus their abilities to undergo nucleophilic substitution reaction at C-4 by an appropriate oxygen or a nitrogen nucleophile has established a general strategy for the site-specific modification of a particular pyrimidine residue in a model fully protected diuridylic acid to give either UpC, CpC or UpU, depending upon the deprotection condition.

Despite a tremendous surge of developments[1] in the synthesis of specific sequences of DNA and RNA molecules, still adequate chemical methodologies are not available for site-specific modification of aglycones of DNA and RNA. The only methodology that is so far reported[2-5] for the selective modification of uracil or thymine (1) to cytidine or 5-methylcytidine (2) employs either a triazolyl or a tetrazolyl group (R' in 3) at the C-4 position of the pyrimidine[6-14]. A suitable oxygen or a nitrogen nucleophile can then convert compound 3 to either a uracil (thymine) or a cytosine (5-methylcytosine) moiety. However, the scope of this procedure is limited by the fact that while it employs a C-4 substituted uracil (thymine) building block for the specific modification at the C-4 center, it leaves other uracil (thymine) moieties in the nucleic acid unprotected, thus promoting the formation of by-products due to side reactions[6-14]. This may be illustrated with a dinucleotide, (1) UpU* (* = triazolyl or a tetrazolyl group at C-4) can give either UpC or UpU and (2) U*pU* can give either CpC or UpU, depending upon the deprotection condition. Although in the first case, a site-specific modification to UpC has been achieved, but in the second case, clearly there is no specificity in modifications.

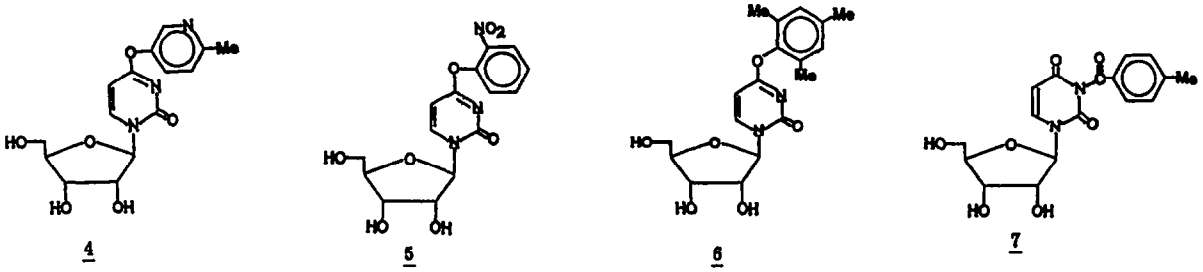
We herein report two different procedures of site-specific modification of uracil to cytosine in which all uracil residues have been appropriately protected in such a way that it was possible to induce a specific modification to one of the protected uracil block to cytosine. For this purpose, we considered four different types[15-17] of O-4 and N-3 protected uracil building blocks, as shown in 4 to 7, for the synthesis of fully protected diuridylic acids 17 and 18, which could be converted to either UpC, CpC or UpU depending upon deprotection condition. Table 1 shows the stabilities of Compounds 4 to 6 and their abilities to undergo nucleophilic substitution by an oxygen or nitrogen nucleophile; while Compound 7[17], under all conditions shown, is converted to the uracil moiety.



1 2 3

R = OH or H

R'= Leaving group



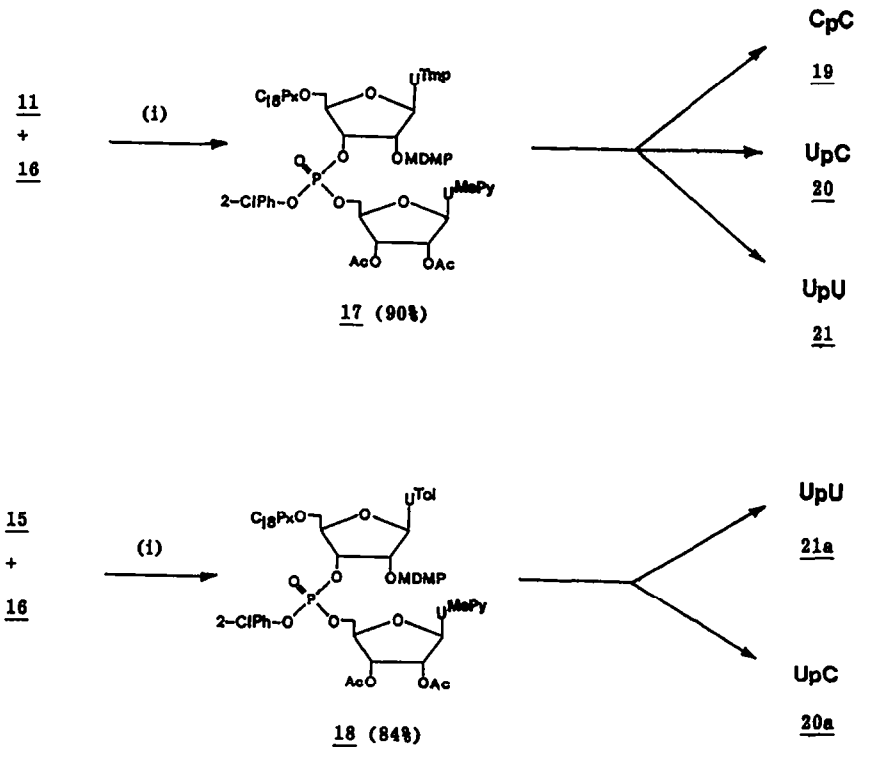
4 5 6 7

Studies in Table 1 showed that the O-4-(6-methyl-3-pyridyl (MePy)[16] protected block 4 was smoothly converted to cytidine quantitatively with 3 M ammonia in dry methanol, while the O-4-(2-nitrophenyl)[15] protected block 5, gave a 7:3 mixture of cytidine and the 4-methoxypyrimidone derivative (R' = OMe in 3), respectively in the latter reaction condition. It may be noted that the O-4-(2,4,6- trimethylphenyl)[15] (TMP) derivative 6 was completely stable under the above condition. On the other hand, the attack of a nitrogen or an oxygen nucleophile, under conditions B and C, respectively, in Table 1, converted blocks 4 to 6 to the corresponding cytidine and uridine derivatives quantitatively. A consideration of reactivity of 4 vs. the stability of 6 clearly suggested that a fully protected diuridylic acid U*pU$^+$ (* = TMP, + = MePy), depending upon the deprotection condition, should give either UpU, UpC or CpC. We, thus, prepared[19-21] U*pU$^+$ (17) in 90% yield using the phosphotriester approach[18] and carried out three sets of deprotection: (1) treatment with 4-nitrobenzaldoximate[22] ion in water:dioxane (2:10, v/v), followed by a usual work up and purification step gave UpU (21) in 88% yield; (2) treatment with fluoride ions in pyridine-tetrahydrofuran-water (1:8:1, v/v/v)[23], followed by the treatment of 3 M ammonia in dry methanol and then the nucleophilic displacement with the oximate ion[22] gave UpC (20) in 70% yield, and (3) treatment with fluoride ions in pyridine-tetrahydrofuran-water (1:8:1, v/v/v)[23] followed by aqueous ammonia (d = 0.9) gave CpC (19) in 81% yield (cf. Table 2 for details of deprotection conditions).

Table 1. Half-lives (min) of nucleophilic substitution reactions of compounds 4, 5 and 6

Compound	3 M ammonia in dry methanol (condition: A)		Aq. ammonia (d=0.9) (condition: B)		4-nitrobenzaldoximate (condition: C)	
	$t_{1/2}$	t_∞	$t_{1/2}$	t_∞	$t_{1/2}$	t_∞
4	240	1440[a]	30	210[d]	1	3[e]
5	180	960[b]	20	210[d]		1[e]
6	c		420	2880[d]	150	1560[e]
7	e		e		e	

[a] Cytidine was the only product isolated in 93% yield.
[b] A mixture of cytidine and the 4-methoxypyrimidone derivative was isolated in 7:3 ratio respectively.
[c] Stable for 72 h at 20 °C
[d] Cytidine was the only product formed in quantitative yield.
[e] Only uridine was formed in 85-93% yield.



(i) 2-mesitylenesulfonyl-3-nitro-(1,2,4-triazolide) (4 eq.) in dry pyridine.

Scheme 1

We reasoned that a fully protected oligouridylic acid prepared from the blocks 4 and 7 should also be able to give site-specific modifications depending upon the deprotection condition. A fully protected diuridylic acid U$pU+ ($ = 4-toluoyl group at N-3[17], + = 6-methyl-3-pyridyl at O-4[16]) was, therefore, synthesized using the phosphotriester approach[18-21] and it was deprotected in two different ways: (1) 4- nitrobenzaldoximate ions[22] in aqueous dioxane followed by an usual work-up and purification step gave UpU (21a) in 73% yield, while (2) a treatment with fluoride ions in pyridine-tetrahydrofuran-water (1:8:1, v/v/v)[23], followed by the treatment with 3 M ammonia in dry methanol gave UpC (20a) in 81% yield (Table 2).

The fully protected diuridylic acids have been prepared from building blocks 11, 15 and 16. General routes of preparation of 11 and 15 are shown in Scheme 2. Compound 16 has been prepared using our literature procedure[19-21]. Appropriate condensation reactions[18-21] of the building blocks, 11, 15 and 16, are shown in Scheme 1. Fully protected dinucleotide 17 and 18 have been then deprotected in different ways as detailed in Table 2. All deprotected dinucleotides 19 to 21, thus synthesized, have been rigorously characterized[19-21] by alkaline and enzymatic digestions followed by quantitation of monomeric components by Hplc[24] as shown in Table 3.

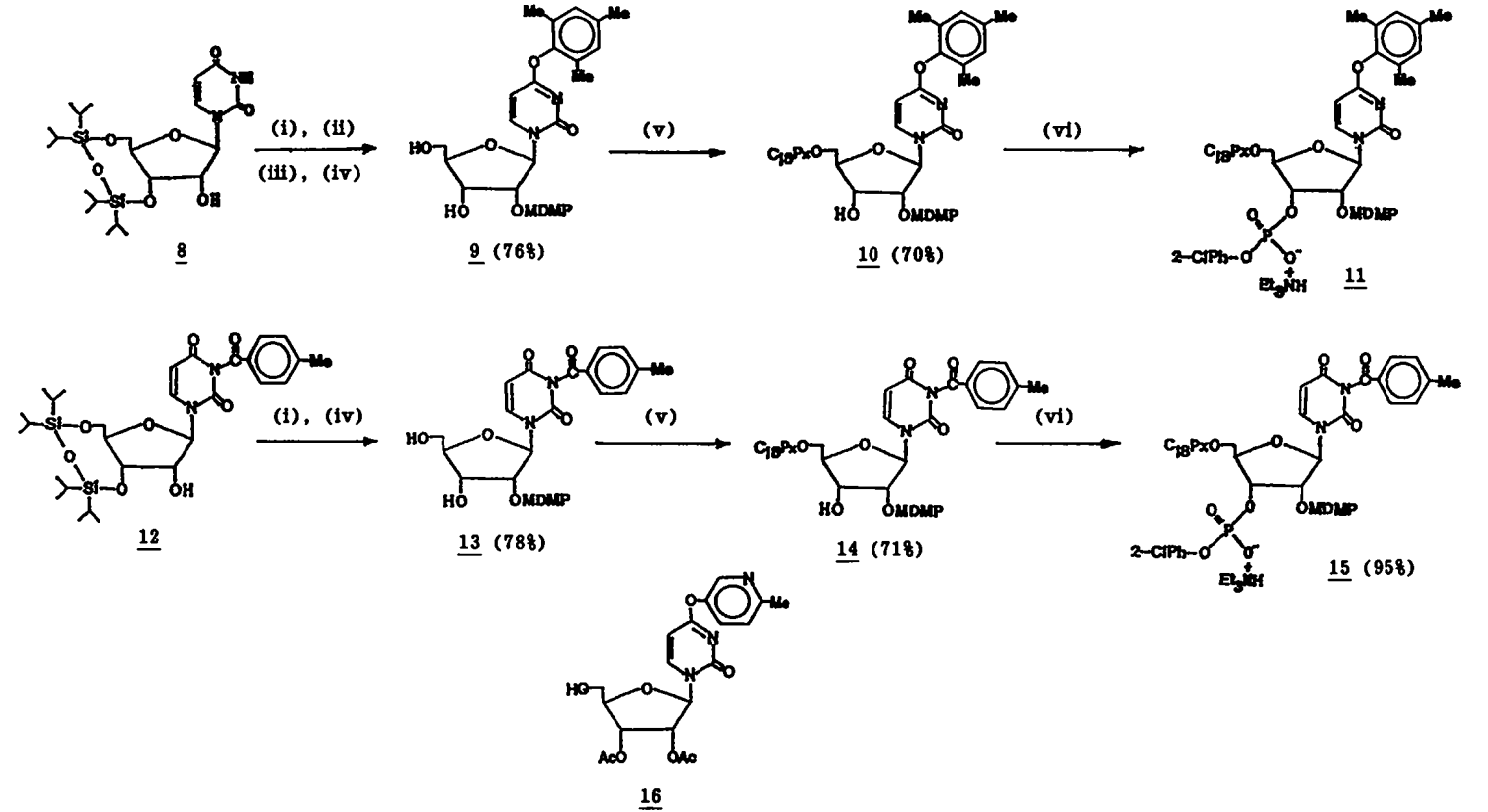
The present work clearly demonstrated that a judicious choice of a O-4 or N-3 protecting group for uracil moiety can serve as a good leaving group for the site-specific modification as well as to protect its urethane function from electrophilic attack.

Experimental

^1H and ^{31}P NMR spectra were recorded using a Jeol FX 90Q spectrometer at 89.5 and 23.5 MHz respectively using TMS and phosphoric acid as internal standards respectively. UV spectra were measured using a Cary/ Varian 2200 spectrometer. TLC was carried out using pre-coated silica gel F_{254} plates in the following solvent system: (A) ethanol-dichloromethane (9:1, v/v); (B) ethanol-dichloromethane (9.5:0.5, v/v); (C) ethanol-dichloromethane (8:2, v/v). The short column chromatographic separation were carried out using Merck G 60 silica gel with dichloromethane and Methanol as eluents.

Preparation of 4-O-(2,4,6-trimethylphenyl)uridine (6): 2',3',5'-O-tri- acetyl-uridine (368 mg, 1 mmol) was treated with 2-mesitylenesulfonyl chloride (547 mg, 2.5 mmol), triethylamine (0.35 ml, 2.5 mmol) and 4-N,N-dimethylaminopyridine (DMAP) (24 mg, 0.2 mmol) followed by 2,4,6-trimethylphenol (952 mg, 7 mmol) and trimethylamine (1 ml, 10 mmol). After 2 h, the reaction was worked up in the usual way and treated with methanolic ammonia. After 2.5 h, the mixture was evaporated and purified by short column chromatography. Yield: 347 mg (96%). ^1H NMR (DMSO + D_2O): δ 8.45 (d, 7.8 Hz, 1H) H-6; 6.94 (s, 2H) TMP; 6.32 (d, 7.7 Hz, 1H) H-5; 5.76 (d, 3.1 Hz, 1H) H-1'; 3.97 (m, 3H) H-2', H-3', H-4'; 3.69 (m, 2H) H-5'; 2.25 (s, 3H) TMP; 2.01 (s, 6H) TMP. UV (methanol): λ max 281 nm (pH 2), 280 nm (pH 7), 281 nm (pH 13),

Preparation of 4-O-(2-nitrophenyl)uridine (5): 3',5'-O-(1,1,3,3-tetra- isopropyl-1,3-disiloxyl)--4-O-(2-nitrophenyl) uridine (200 mg, 0.33 mmol), was treated with 0.1 M tetrabutylammonium fluoride in dry tetrahydrofuran (3 ml) for 3 min. It was then evaporated and triturated with petroleum ether. The residue was purified subsequently by short column chromatography. Yield: 102 mg (85%). ^1H NMR (DMSO-d_6): δ 8.59 (d, 7.4 Hz, 1H) H-6; 8.21-7.52 (m, 4H) arom.; 6.45 (d, 7.5 Hz, 1H) H-5; 5.55 (m, 1H) H-1'; 3.98 (m, 5H) H-2',H-3', H-4', H-5'. UV (methanol): λ max 282 nm (broad) (pH 2), 283 nm (pH 7), 281 nm (pH 13).



(i) 3-methoxy-1,5-dicarbomethoxy-2-pentene (14 eq.), benzenesulfonic acid (0.14 eq.) in dry dioxane; (ii) 2-mesitylenesulfonyl chloride (3 eq.); triethylamine (3 eq.) and 4-dimethylaminopyridine (o.2 eq.) in dichloromethane. (iii) Trimethylphenol (5 eq.), trimethylamine (10 eq.) and Dabco (0.5 eq.) in dichloromethane. (iv) 0.1 M tetrabutylammonium fluoride in tetrahydrofuran. (v) 9-chloro-9-(4-octadecyloxyphenyl)xanthen (2 eq.) in dry pyridine. (vi) 2-chlorophenylphosphoro-bis-(1,2,4-triazolide) (2 eq.) in dry pyridine.

Table 2. Deprotection and purification of dinucleotides 17 and 18

Fully protected compound	Yield (%)	Product	Deprotection condition	Purification
17	81	CpC (19)	0.05 M TBAF[a] in THF: H_2O: pyridine (8:1:1, v/v/v), 4 h, aq. ammonia 50 °C. 3 days, 80% acetic acid, 4h at 20 °C.	DEAE Sephadex A25 0-0.3 M NH_4HCO_3
17	70	UpC (20)	0.5 M TBAF[a] in THF: H_2O: pyridine (8:1:1, v/v/v), 2.5 h, 3 M ammonia in dry methanol, 2 days, 20 °C, TMG/NBO[b] in dioxane-H_2O (10:2 v/v) 2 days, 80% AcOH 4 h at 20 °C.	DEAE Sephadex A25 0-0.3 M NH_4HCO_3
17	88	UpU (21)	TMG/NBO[b] in dioxane-H_2O (10:2 v/v) 2 days, ammonia 24 h at 20 °C, 80% AcOH, 4 h at 20 °C.	DEAE Sephadex A25 aq. 0-0.3 M NH_4HCO_3
18	73	UpU (21a)	TMG/NBO[b] in dioxane-H_2O (10:2, v/v) 24 h, ammonia 72 h at 20 °C, 80% AcOH 6 h at 20 °C.	DEAE-Sephadex A25 aq. 0-0.3 M triethylammonium hydrogen carbonate
18	81	UpC (20a)	0.05 M TBAF[a] in THF: H_2O: pyridine (8:1:1, v/v/v), 2.5 h, 3 M ammonia in dry methanol, 5 days, 80% acetic acid, 6 h at 20 °C	DEAE-Sephadex A25 0-0.3 M triethylammonium hydrogen carbonate

[a] tetrabutylammonium fluoride; [b] syn-4-nitrobenzaldoxime and tetramethylguanidine.

Table 3. HPLC Quantitation after hydrolysis of the dinucleotides 19 to 21

Compounds	Fragments obtained after hydrolysis at 37 °C			R_t[a] (min)	Ratio	
	0.2 M NaOH	Spleen phosphodiesterase	Snake venom phosphodiesterase		obs.	calc.
CpC	C			1.96	1	1
(19)	2'(3')-CMP			7.35, 7.88	0.91	1
CpC		C		2.03	1	1
(19)		3'-CMP		6.00	0.89	1
CpC			C	1.92	1	1
(19)			5'-CMP	4.67	1.02	1
UpC	C			2.05	1	1
(20)	2'(3')-UMP			9.78	0.96	1
UpC		C		1.95	1.19	1
(20)		3'-UMP		8.74	1	1
UpC			U	2.25	1	1
(20)			5'-CMP	3.78	1.04	1
UpU	U			2.16	1	1
(21)	2'(3')-UMP			7.39	1.12	1
UpU		U		2.10	1.13	1
(21)		3'-UMP		8.07	1	1
UpU			U	2.13	1	1
(21)			5'-UMP	4.75	1.04	1
UpC	U			2.0	0.87	1
(20a)	2'(3')-UMP			7.18	1	1
UpC		C		1.99	0.94	1
(20a)		3'-UMP		8.09	1	1
UpC			U	2.24	1	1
(20a)			5'-UMP	4.33	1.08	1
UpU	C			2.04	1	1
(21a)	2'(3')-UMP			6.64	1.06	1
UpU		C		2.0	1	1
(21a)		3'-UMP		6.35	1	1
UpU			U	2.80	1	1
(21a)			5'-CMP	5.92	0.98	1

[a] Hplc elution of all monomeric components have been characterized by co-injection with authentic samples.

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