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Design and Synthesis of a New Type of Modified Nucleoside for Triple Helix-Mediated Adenine-Thymine Base Pair Recognition : Formation of Hydrogen Bonds to the Far-Side Adenine

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Abstract: We have synthesized a new class of modified nucleoside, X, that could recognize adenine-thymine base pairs. They were designed to form the triple helix of A-T•X motif, where X forms hydrogen bonds to the N7 and NH_2 of the far-side adenine in the major groove. Compounds **1** and **2** are a new type of triplex-forming modified nucleoside which are expected to act as DNA cleaving agents through the *p*-nitrophenyl group.

Triple-stranded DNA was discovered1 by Felsenfeld et al. in 1957, however, it is only relatively recently that it has attracted much attention. This is because of the potential therapeutic use in targeting and controling particular regions in the genome2-3. Numerous attempts have been reported concerning the stabilization of the triple helix and base triplet, base-pair preference and even *in vivo* application. Triple-helix formation is an efficient method for sequence-specific recognition of double-helical DNA, however, it is limited mostly to homopurine•homopyrimidine tracts as the third strand can bind only the near-side purine bases by forming two hydrogen bonds. It has been a challenging problem to overcome the sequence restriction and synthesize modified nucleosides that can recognize base-pairs selectively and form a triple helix.4

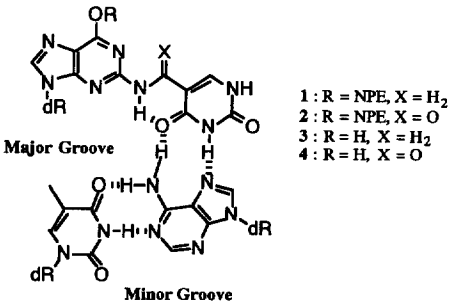

Figure 1. Two-dimensional model of an Adenosine-Thymidine . Modified nucleoside triplet.

Toward this end, we have been developing oligonucleotides which form hydrogen bonds to the purine base of a base pair in whichever strand the base is present. In this paper, we report the synthesis and some characterization of several novel modified nucleosides which could hydrogen bond to the far-side adenine of an AT base-pair to form a base triplet. We have previously employed the *p*-nitrobenzoyl group as a DNA cleaving moiety which cleaves

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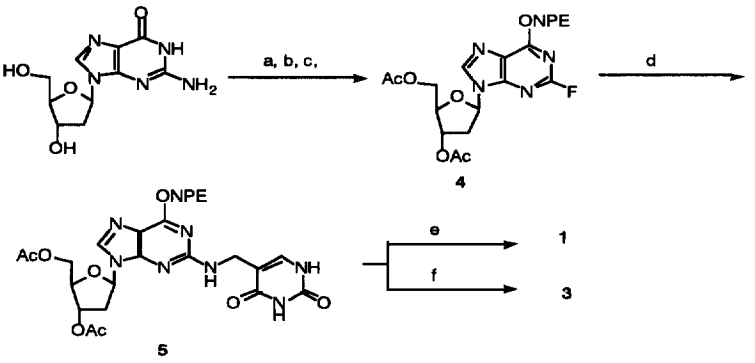
DNA via an excited triplet state on UV irradiation[5-7]. Compounds retaining the protecting *p*-nitrophenyl group are expected to act in a similar manner.

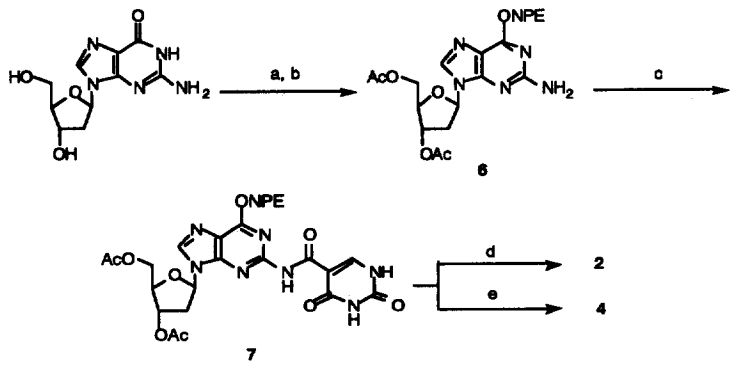
Structures of the compounds **1** ~ **4** we have synthesized and their expected base triplet are shown in Figure 1. In contrast to compounds **2** and **4** which are more rigid and planar as a result of the amide group, compounds **1** and **3** are flexible having the methylene linker ($X=H_2$) between the guanine and the uracil residues.

As shown in Scheme 1, compounds **1** and **3** were constructed as follows. The 6-*O*-*p*-nitrophenyl-ethyl-2-fluoroinosine **4** was prepared by Behr's method[8], starting from the 2'-deoxyguanosine. The coupling reaction with the aminomethyluracil hydrochloride[10] proceeded only in the presence of *N*, *N*-diisopropylethylamine at 80 °C in DMF to give compound **5** only. Deprotection of **5** with NH_3 / MeOH gave **1**. When reaction was performd with DBU in CH_3CN, compound **3**, in which both of the protecting groups were removed, was obtained.



Scheme 1 a) Ac_2O, Et_3N, DMAP, CH_3CN, rt., quant., b) *p*-nitrophenylethanol, DEAD, PPh_3, dioxan, rt. quant., c) PVPHF, tert-butyl nitrite, toluene, 85%, d) 6-aminomethyluracil hydrochlorate, *N*, *N*-diisopropylethyl-amine, DMF, 80 °C, 35%, e) NH_3, MeOH, rt. quant., f) DBU, CH_3CN, rt. 75%

As shown in Scheme 2, compounds **2** and **4** were constructed from the same starting material as that used for compound **1**, 2'-deoxyguanosine. The two hydroxy groups of 2'-deoxyguanosine were protected with acetate. As in Scheme 1, protection of the carbonyl group with the *p*-nitrophenylether afforded compound **6**. Coupling reaction with **6** using DCC, 2,4-hydroxypyrimidine-5-carboxylic acid and 1.2 eq. LiCl in THF afforded the amide **7**. This was the only successful coupling reaction condition. Cyanophosphonic acid diethyl ester, 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide and phenyl *N*-phenylphosphoramidochloridate did not produce any of the desired compound. Deprotection of **7** in a similar manner as in scheme 1 gave compounds **2** and **4**. We have newly found that the NPE group of **7** and not of **5** can be removed simply by the reaction with TBAF.



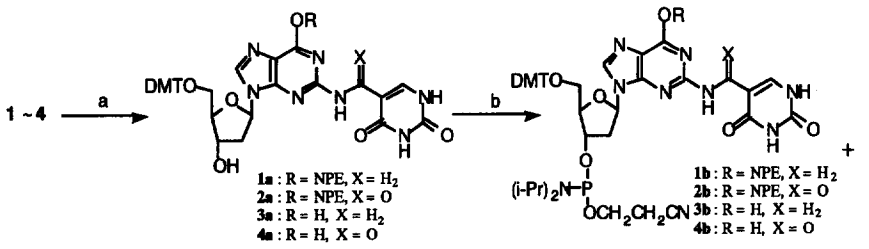
Scheme 2 a) Ac_2O, Et_3N, DMAP, CH_3CN, rt., quant., b) *p*-nitrophenylethanol, DEAD, PPh_3, dioxan, rt. quant., c) 2, 4-dihydroxypyrimidine-5-carboxylic acid, DCC, LiCl, THF, rt., 42%, d) NH_3, MeOH, rt. quant. e) DBU, CH_3CN, 80%.

To establish the triplex-forming ability of monomers **1** and **2**, ^{1}H-NMR titration experiments with 2'-deoxyadenosine were carried out. Compounds **5** and **7'** (TBDMS ether of **2**), which have the identical hydrogen-bond forming functional groups as **1** and **2** respectively, were employed to increase the solubility of compounds in $CDCl_3$. Both compounds exhibited complexation-induced chemical shifts (CIS) similar to thymidine. Similar titration with *N*6-acetylamino-2'-deoxyadenosine further increased the CIS of AH-8 in the case of **7'** (Table 1). The results clearly indicate that the newly synthesized compounds are strong candidates for the triplex-forming nucleoside units which overcome the homopurine /homopyrimidine sequence restriction.

Table 1. CIS on titration with 3',5'-diacetyl-2'-deoxyadenosine and its *N*6-acetyl derivative[a] in $CDCl_3$.[b]

	Hydrogen(s)	CIS (ppm) 3', 5'-diacetyl-2'-deoxyadenosine		CIS (ppm) 3', 5', *N*6-triacetyl-2'-deoxyadenosine
		0.5 eq.	1.0 eq.	0.5 eq.
Thymidine[a]	A-H2	+0.058	+0.107	+0.032
	A-H8	+0.018	+0.035	+0.016
	A-NH_2	+0.28	+0.544	+0.199
5	A-H2	+0.032	+0.051	–
	A-H8	–0.003	+0.007	–
	A-NH_2	+0.255	+0.431	–
7'	A-H2	+0.084	+0.121	+0.065
	A-H8	+0.028	+0.042	+0.037
	A-NH_2	+0.494	+0.762	+0.240

a)Two hydroxy group were protected with acetate ester. b) A 0.025 M $CDCl_3$ solution of adenosine derivatives was titrated with 0.1 M $CDCl_3$ solution of tymidine, **5** or **7'**.



Scheme 3 a) DMTCl, Et_3N, DMAP, $CHCl_2$, b) 2-cyanoethyl tetraisopropylphosphorodiamidite, 1H-tetrazole, CH_3CN, molecular sieves 3Å

Compounds **1~4**, after protection of the 5'-hydroxy group with DMT ether, were treated with 2-cyanoethyl tetraisopropylphosphorodiamidite in the usual manner to produce 3'-phosphoroamidite **1b~4b**. These are the building blocks for the incoorporation of the compounds into oligonucleotides. The work is currently underway, which will be reported elsewhere.

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[10] **5**: IR(film) ν_{max} cm^{-1}: 3400, 2930, 2815, 0730, 1720, 1670, 1615, 1540 1520, 1345, 1240, 1049, 940, 750, 400, 535. ^{1}H-NMR($CDCl_3$, 500MHz) δ : 9.88 (1H, br), 9.11 (1H, br), 8.14 (2H, d, J = 8.6), 7.77 (1H, s), 7.46 (2H, d, J = 8.6 Hz), 6.24 (1H, t, J = 6.7 Hz), 5.72 (1H, br), 5.64 (1H, s), 5.46 (1H, dd, J = 3.1, 3.4 Hz), 4.71 (2H, t, J = 6.7 Hz), 4.42-4.32 (5H, m), 3.26 (2H, t, J = 6.7 Hz), 3.05 (1H, m), 2.55 (1H, m), 2.12 (3H, s), 2.05 (3H, s). HRFABMS(M$^+$ + H) Found, 625.2021. Calcd. For $C_{27}H_{29}O_{10}N_8$, 625.2007.

[11] **7'**: IR(film) ν_{max} cm^{-1}: 3820, 3800, 3735, 3649, 2925, 2855, 1735, 1715, 1697, 1560, 1540, 1520, 1480, 1460, 1340, 1240, 1075. ^{1}H-NMR(DMSO-d_6, 500MHz) δ : 11.88 (2H, br), 11,43 (1H, S), 8.37 (1H, s), 8.27 (1H, s), 8.16 (2H, d, J = 8.6 Hz), 7.65 (2H, d. J = 8.6 Hz), 6.30 (1H, t, J = 6.7 Hz), 4.76 (2H, d, J = 6.7 Hz), 4.68 (1H, m), 3.82 (2H, m), 3.65 (1H, dd, J = 7.3 13.1 Hz), 3.32 (2H, d, J =6.7 Hz), 3.15 (1H, m), 2.27 (1H, m). HRFABMS(M$^+$ + H) Found, 783.3305. Calcd. For $C_{35}H_{51}O_9N_8Si_2$, 783.3318.