Bioorganic & Medicinal Chemistry Letters, Vol.3, No.4, pp. 615-618, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00
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NUCLEOSIDES & NUCLEOTIDES. 118. SYNTHESIS OF OLIGONUCLEOTIDES CONTAINING A NOVEL 2'-DEOXYURIDINE ANALOGUE THAT CARRIES AN AMINOALKYL TETHER AT 1'-POSITION; STABILIZATION OF DUPLEX FORMATION BY AN INTERCALATING GROUP ACCOMMODATED IN THE MINOR GROOVE[1]

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Key Words: oligonucleotide, oligonucleotide synthesis, linker, minor groove, intercalator, thermal denaturation

(*Received in USA* 30 *November* 1992)

Abstract: A novel 2'-deoxyuridine analogue carrying an aminoalkyl tether at 1'-position of the sugar moiety was synthesized and incorporated into oligonucleotides, then an intercalating group was attached to the amino group. Duplexes, consisting of the oligonucleotides and a complementary strand, were more stable than a unmodified parent duplex.

Oligonucleotide analogues carrying various functional groups such as intercalating, alkylating, DNA degrading, and fluorescent groups have been synthesized and used for biological and biophysical studies.[2] Many methods to attach the functional groups at several sites on the oligonucleotides such as 5' and 3' ends, 5-position of uracil residues, *N*-4 of cytosine residues, or phosphate residues have been reported.[2,3,4] When the above oligonucleotides form duplexes with complementary strands, the functional groups are located at the ends of duplexes, in the major grooves, or along with sugar-phosphate backbones, but not in the minor grooves. However, few reports[5,6,7] have been suggested that the minor groove should be the suitable site to accommodate functional groups; Yamana *et al.*[6] reported that an oligonucleotide with anthraquinone at 2'-position formed a more stable duplex than a unmodified parent oligonucleotide, or Kido *et al.*[7] reported that an alkylating group attached at 2'-position of an oligonucleotide reacted with purine residues of a complementary strand. In this communication, we report a synthesis of oligonucleotides containing **1**, **2**, and **3** (Fig. 1) which carry functional groups at the 1'-position of the deoxyribose moiety. The functional groups should be accommodated in the minor groove when the oligonucleotide analogues form duplexes with complementary strands, since the 1'-position of the sugar moiety in a duplex is located in the minor groove.

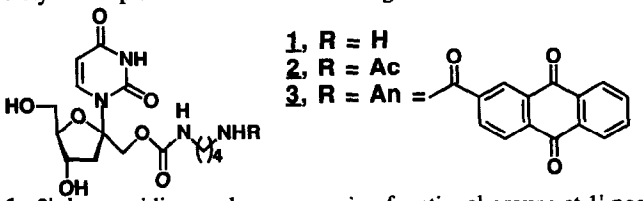

Figure 1. 2'-deoxyuridine analogues carrying functional groups at 1'-position.

A scheme for a synthesis of 1'-substituted-2'-deoxyuridine analogues[8] and an amidite unit **11** is shown in Fig. 2. The O^2,2'-anhydro linkage of **4**[9] was hydrolyzed under acidic condition, then radical deoxygenation of the newly generated hydroxyl group gave a deoxy derivative **5**, which was converted into TIPDS-protected derivative **6**. 1,4-Diaminobutane was conjugated with the unprotected hydroxyl group of **6** to give a deoxyuridine analogue **7** carrying an aminoalkyl tether at the 1'-position.[10] Then, **7** was converted into the nucleoside-3'-phosphoramidite **11** by the reported methods.[10]

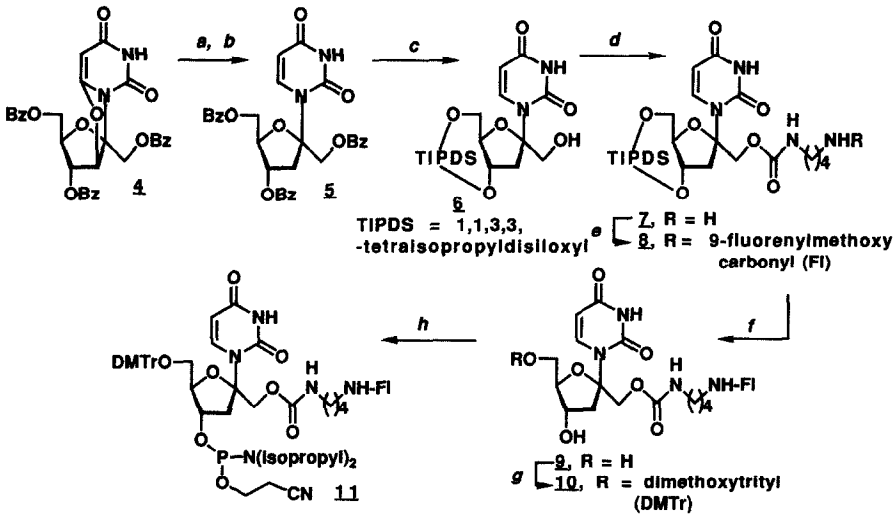

Figure 2. A scheme for a synthesis of the amidite **11**. *a*; a mixture of 2 N HCl and DMF (2 : 5, v/v), r.t. 95%. b; 1) 1,1'-thiocarbonyldiimidazole, DMF. 2) Bu_3SnH, AIBN, toluene. 92%. c; 1) NaOMe, MeOH. 2) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane. 57%. *d*; 1,1'-carbonyldiimidazole, 1,4-diaminobutane, dioxane. 95%. *e*; 9-fluorenylmethyl chloroformate, pyridine. 86%. *f*; Bu_4NF, THF. 98%. *g*; dimethoxytrityl chloride, pyridine. 83%. *h*; 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, *N*,*N*-diisopropylethylamine, CH_2Cl_2. 80%.

Hexadecanucleotides, 5'-**1**$(T)_{15}$-3' (**I**) and 5'-$(T)_7$**1**$(T)_8$-3' (**II**), were synthesized on a DNA synthesizer (391 DNA Synthesizer, Applied Biosystems, USA) using the amidite **11**. The coupling yield of **11** was 74%[12] using a 0.12 M solution of **11** in acetonitrile and 2 minutes for coupling time. Fully protected oligonucleotides linked to the solid support were deprotected and purified by the same procedure as for the purification of natural oligonucleotides. Starting from 1 μmol of thymidine residues linked to controlled pore glass, 21 OD units (at 254 nm) of **I** and 53 OD units of **II** were obtained after purification.

After de-tritylation, each oligonucleotide in this preparation showed a single peak by HPLC analysis with a C-18 column (Inertsil ODS-2, GL Science Inc., Japan) without further purification. An example is shown in Fig. 3a. After complete hydrolysis of the oligonucleotides to nucleosides by snake venom phosphodiestrase (Boehringer Mannheim, Germany) and calf intestine alkaline phosphatase (Takara Shuzo Co., Ltd., Japan), the nucleoside composition of each oligonucleotide was analyzed by HPLC, but no sharp peak corresponding to **1** was detected (data not shown). Therefore, to certify the existence of the amino group, the oligonucleotides were treated with acetic anhydride[12] to give oligonucleotides 5'-**2**$(T)_{15}$-3' (**I**-Ac) and 5'-$(T)_7$**2**$(T)_8$-3' (**II**-Ac) (Fig. 3b). The acetylated oligonucleotides were completely hydrolyzed and the nucleoside composition (T : **1** = 15 : 1) was confirmed by HPLC detected at 254 nm (Fig. 3d). Nucleoside **2** was confirmed by co-elution with an authentic compound. Furthermore, an intercalating group was attached to the amino group of **I** and **II**. The oligonucleotides **I** and **II** were incubated with *N*-(anthraquinone-2-carboxy)succinimide overnight to give 5'-**3**$(T)_{15}$-3' (**I-An**) and 5'-$(T)_7$**3**$(T)_8$-3' (**II-An**) (**An** = anthraquinone) (Fig. 3c).[14] The nucleoside composition of **I-An** and **II-An** was also confirmed (Fig. 3e).

Stability of duplexes formed by the oligonucleotides with poly rA were studied by thermal denaturation (Table 1)[15]. T_{16} was used as a control. The stability of the duplexes depended upon the kinds of the functional groups and their positions. Depending upon the kinds of the functional groups, the orders of Tm's, **I-An** > **I** ≥

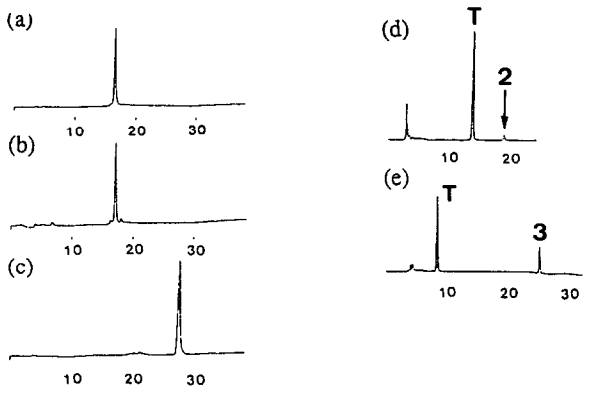

Figure 3. HPLC profiles. (a); **II**, a linear gradient of CH_3CN from 10 to 20% (20 minutes) in 0.1 M triethyl-ammonium acetate buffer pH 6.8. (b); **II**-Ac, 10 to 20%. (c); **II-An**, 20 to 33%. (d); the nucleoside mixture obtained by complete hydrolysis of **II**-Ac, a linear gradient of methanol from 8 to 20% (20 minutes) in water. (e); the nucleoside mixture obtained by complete hydrolysis of **II-An**, 20 to 45% (10 minutes) to 80% (5 minutes) to 80% (15 minutes). The peaks were observed at 254 nm.

	Tm (°C)
(I)	34
(I-Ac)	32
(I-An)	38
(II)	30
(II-Ac)	30
(II-An)	35
T_{16}	31

Table 1[13]

I-Ac (in the duplexes modified at 5'-end) and **II-An** > **II** ≥ **II**-Ac (in the duplexes modified in the center), were observed. The result was characterized as, **An**-conjugated duplex > free aminobutyl duplex ≥ acetylated duplex, and the phenomenon can be interpreted as follows. A positive charge on the amino group was expected to stabilize the duplex formation electrostatically. However, the acetylation (*i.e.* the erasure of the positive charge) did not destabilize the duplex formation much, probably because the effect of the positive charge was not crucial in the conditions used for the thermal denaturation. In contrast to the acetylation, the **An**-derivatization stabilized the duplex formation efficiently even though the **An**-derivatization, like the acetylation, erased the positive charge on the free amino group. Intercalation of **An** or hydrophobic interaction between **An** and the surface of the minor groove could stabilize the duplex formation effectively. In addition to the kinds of the functional groups, the stability of the duplexes depended upon the position of the functional groups. The duplexes carrying the free amino group and the acetamide group in the center (**II** and **II**-Ac) were less stable than the control duplex, while the duplexes carrying the functional groups at 5'-ends (**I**, **I**-Ac, **I-An**) were slightly more stable than the control. Attachment of the bulky group at the 1'-position of the sugar in the center of the duplexes may disturb the duplex conformation, but attachment at 5'-end may not. Nevertheless, the **An**-derivatization, even in the center of the duplex, stabilized the duplex formation efficiently. A study for conjugation of various functional groups to the amino function is in progress.

Acknowledgment. This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, and Culture, Japan.

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8. According to Holý,[9] the nucleosides can be named as psicofuranosyluracil derivatives. In this report, for convenience, we named them as 2'-deoxyuridine derivatives.
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10. Nucleoside **6** (0.6 mmol) and 1,1'-carbonyldiimidazole (2.4 mmol) were dissolved in dioxane (5 mL) and the solution was stirred at room temperature under an Ar atmosphere. After 30 min, water was added to the solution and the mixture was extracted with ethyl acetate. The organic layer was washed with NaH_2PO_4 solution and NaCl solution, and dried over Na_2SO_4. The organic layer (100 mL) was added to a solution of 1,4-diaminobutane (12 mmol) in ethyl acetate (50 mL) with stirring, then the solution was washed with water. The organic layers were dried over Na_2SO_4, and concentrated to give **7** (0.57 mmol, 95%). Compound **7** (0.42 mmol) and 9-fluorenylmethyl chloroformate (0.63 mmol) were dissolved in pyridine and the mixture was kept at room temperature for 1 h. The solvent was evaporated and the residue was chromatographed over a silica gel column (Ø2.7 x 7 cm) with 3% ethanol in $CHCl_3$ as a eluent to give **8** (0.36 mmol, 86%). FAB-MS *m/z* 837 (M^++1).
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12. The coupling yield of **11** was lower than that of the thymidine-3'-phosphoramidite probably due to steric effect of the linker at the 1'-position. The purity of **11** determined by ^{31}P-NMR was more than 99%. ^{31}P NMR ($CDCl_3$) δ 149.32 (0.5 P), 149.12 (0.5 P). Phosphoric acid was used as an internal standard.
13. Each solution of the oligonucleotide (1 OD unit) and acetic anhydride (2 μL) in 0.2 M HEPES buffer (500 μL, pH 7.8) was kept at room temperature for 50 minutes. The oligonucleotide was passed through a short C-18 column (PSPEC18101, YMC, Japan) and was used for next studies without further purification.
14. *N*-(Anthraquinone-2-carboxy)succinimide was synthesized by the reported method with minor modificatior (Telser *et al.*, 1989, *J. Am. Chem. Soc.*, **111**, 6966). MS *m/z* 349 (M^+). *Anal.* Calcd for $C_{19}H_{11}NO_6$: C; 65.33, H; 3.18, N; 4.01. Found: C; 65.04, H; 3.05, N; 3.97. A solution of *N*-(anthraquinone-2-carboxy)succinimide (0.5 mg) in DMF (70 μL) was added to a solution of **II** (1.5 OD units at 254 nm) in 50 mM $NaHCO_3$-Na_2CO_3 (70 μl, pH 10.3) and the mixture was kept at room temperature overnight. After the pH of the mixture was adjusted to 3 by adding 0.1 N HCl, the solution was washed with ethyl acetate (4 times), then sat. $NaHCO_3$ was added to the water layer to neutralize it. The reaction was purifiec by HPLC in order to remove the reagents to give 1.3 OD units (at 254 nm) of **II-An.**
15. Each solution contains each oligonucleotide (69 μM/base) and poly rA (69 μM/base) in a buffer of 0.01 M sodium phosphate and 0.05 M NaCl (pH 7.0). Thermally induced transition of each mixture was monitored at 260 nm on a Gilford Response II.