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AN URIDINE DERIVATIVE CONTAINING A HYDROPHOBIC FLUORESCENT PROBE AT THE 2'-POSITION: SYNTHESIS AND ITS INCORPORATION INTO OLIGONUCLEOTIDES

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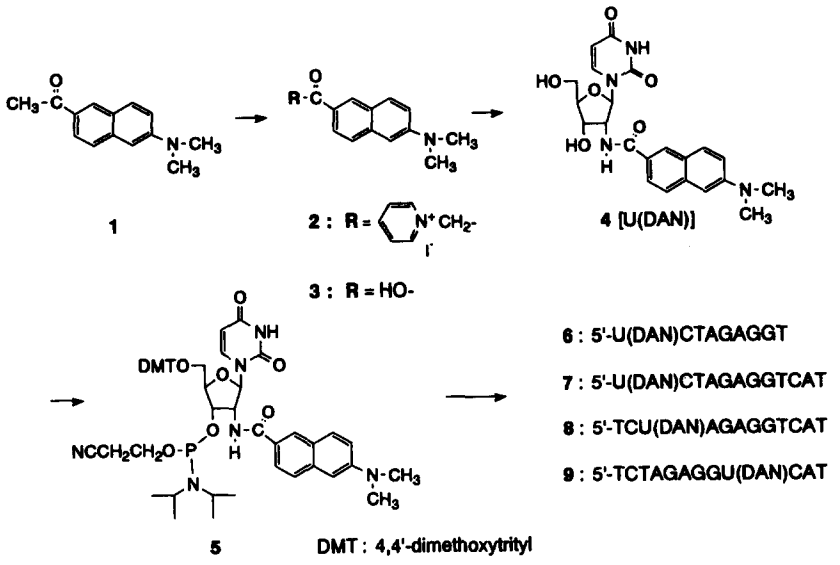
Abstract : **The synthesis of 2'-(6-dimethylamino-2-naphthamide)uridine [U(DAN)] has been described. The nucleoside was converted to the 5'-dimethoxytrityl nucleoside phosphoramidite which could be used for incorporation of U(DAN) into the desired positions of the oligonucleotide sequence.**

Environmentally sensitive fluorescent probe provides a useful mean to estimate the polarity of biological molecules.[1,3] A dansyl fluorophore (DNS), involving an electron donor and an electron acceptor attached to an aromatic ring system, is a typical molecule having such spectral properties. Placement of DNS derivatives in a hydrophobic less-polar environment causes significant increase in quantum yield and blue-shift of the emission.[1] In the previous report,[2] we have demonstrated that an oligonucleotide possessing a DNS group at the sugar-2' via a linker of an appropriate length exhibits enhanced emission together with blue-shift of the emission upon binding to the complementary DNA segment. It is thus clear that the DNS group incorporated into the sugar position of an oligonucleotide was buried in more hydrophobic interior of the duplex.

We have explored a new molecule as a hydrophobic fluorescent probe that could readily be incorporated into the 2'-position of oligonucleotide derivatives. It has been reported that the fluorescence of 6-propionyl-2-(dimethylamino)naphthalene and its related compounds show extremely high sensitivity to the environment.[3] In this polarity-sensitive fluorescent molecule, the alkylamino group as the electron donor and the C=O group as the electron acceptor are attached to the 2 and 6 positions of a naphthalene derivative where the distance between the donor and acceptor groups is a maximum. We reasoned that this molecule with some modification would be used for incorporating into the 2'-amino-2'-deoxyribonucleoside and the resulting nucleoside can be employed for the synthesis of oligonucleotide derivatives having the new hydrophobic probe at the 2'-position. In this report, we describe a general method for the preparation of the uridine derivative containing 6-dimethylamino-2-naphthamide group at the 2'-position [U(DAN)]. The protected uridine phosphoramidite **5** was found to be useful for incorporation of U(DAN) into any desired positions of oligonucleotide sequences.



The synthesis of uridine derivative containing the new hydrophobic fluorescent probe was initiated by the synthesis of 6-dimethylamino-2-naphthoic acid **3**. The key step in this synthesis was the transformation of the acetyl group at the naphthalene ring into carboxylic acid, since 6-acetyl-2-(dimethylamino)naphthalene **1** can be easily synthesized according to the literature procedure.[4] The compound **1** (1.5 g, 7.0 mmol) was allowed to react with iodine (1.8 g, 7.0 mmol) in 30 mL of dry pyridine under the refluxed conditions for 1 h and then the solution was allowed to stand overnight,[5] affording the pyridinium iodide **2**[6] in a yield of 66%. The compound **2** (1.9 g, 4.5 mmol) was then treated with 0.5 M NaOH at 100°C for 1 h. The resulting solution was acidified with HCl to pH 3 affording the acid **3**[7] as a powder (0.7 g, 73 %).

The condensation of **3** (0.5 g, 2.3 mmol) with 2'-deoxy-2'-aminouridine (0.56 g, 2.3 mmol) was carried out by using DCC (0.53 g, 2.5 mmol) and 1-hydroxybenzotriazole (0.35 g, 2.5 mmol) in DMF (12 mL) at r.t. overnight. After removal of urea formed, the material was applied to a silica gel column eluted with CH_2Cl_2-MeOH (9:1, v/v). The desired compound **4** [U(DAN)][8] was obtained in a yield of 37 %.

The fluorescence properties of U(DAN) depending upon the solvent polarity was examined. Table 1 showed the fluorescence spectra of U(DAN) and 2'-(dansylamino)uridine [U(DNS)][2] in the aqueous solution

Table 1. UV and fluorescence spectral data for U(DAN) and U(DNS).

	absorption maxima	emission maximum (intensity)
U(DAN)	259.0 nm 331.0 nm	456.0 nm (1926) in i-PrOH-H_2O = 2:8 (v/v)
		443.0 nm (8981) in i-PrOH-H_2O = 8:2 (v/v)[a]
U(DNS)	255.0 nm 345.0 nm	547.5 nm (305) in i-PrOH-H_2O = 2:8 (v/v)
		536.0 nm (1746) in i-PrOH-H_2O = 8:2 (v/v)

UV spectra were obtained in i-PrOH-H_2O (2:8). Fluorescence spectra were measured for solutions containing U(DAN) at a concentration of 1.9×10^{-5} M and U(DNS) of 9.1×10^{-5} M in which excitation was done at 331 nm and 345 nm, respectively. [a]Fluorescence quantum yield for U(DAN) was estimated to be 0.22 based on quinine sulfate in 1.0 N sulfuric acid as a standard.

containing different amount of i-propanol (i-PrOH). The emission maximum of U(DAN) appeared at the shorter wavelength when compared with that of U(DNS). For both compounds, the large fluorescence enhancement and blue-shift of the emission were observed with increase in the propanol content. The degree of these changes were almost identical for both compounds, thus clearly indicating that the newly synthesized uridine derivative has the same potential in use for a hydrophobic fluorescent probe as U(DNS). The important feature of the compound U(DAN) is the observed spectral overlap between the fluorescence of U(DAN) and the absorption spectrum of the fluorescein (λmax at 450 nm and 480 nm). It is therefore expected that the present fluorescent uridine derivative may be used as a donor for fluorescence energy transfer to an acceptor with fluorescein label.

We next examined the utility of U(DAN) in the synthesis of oligonucleotide derivatives. This nucleoside was converted by the usual procedure to the phosphoramidite **5** which could be used for incorporation of U(DAN) into the oligonucleotide sequences **6-9**. The oligonucleotides containing U(DAN) at the different position were synthesized by the fully automated solid phase phosphoramidite methods. Efficient coupling (98%) of **5** was achieved when a large excess of the amidite was used.[9] The protected oligonucleotides bound to the supports were treated with concentrated ammonium hydroxide at 55°C for 10 h to afford deprotected oligomers. Final purification of oligomers **6-9** was done with denaturing polyacrylamide gel (20 %) electrophoresis.[10]

The binding of the oligonucleotides containing U(DAN) to their complementary DNA was investigated by UV melting behaviors in a pH 7 buffer solution. All the UV melting profiles for the U(DAN)-modified duplexes exhibited sigmoidal curves whose shapes were similar to those for the unmodified duplexes. Table 2 summarized the Tm values obtained from the UV melting curves. The oligonucleotides containing U(DAN) at the terminal fraying end retain their normal binding affinity for DNA, whereas the incorporation of U(DAN) into the other sites caused destabilization of the modified duplexes. A similar duplex stability depending on the site of incorporation of U(DNS) into oligonucleotides has been observed.[2] The fluorescence changes of oligomers **6-9** upon binding to DNA were shown in Table 2. Upon hybridization to DNA, all the fluorescence

Table 2. Binding and fluorescence properties of oligonucleotides containing U(DAN).

oligonucleotide	Tm (°C)	relative fluorescence intensity (emission blue-shift)
5'-dTCAGAGGT	25[a]	
6	28[a]	0.88 (2.5 nm)
5'-dTCATAGAGGTCAT	40[b]	
7	43[b]	0.76 (1.5 nm)
8	35[b]	0.70 (3.0 nm)
9	33[b]	0.88 (1.5 nm)

UV melting curves were obtained in a pH 7 phosphate buffer containing 1.0 M[a] or 0.1 M[b] NaCl at a total strand concentration of 4.0 x 10^{-5} M. Fluorescence spectra were measured at 22°C before (Fs) and after hybrid formation (Fd) to yield relative fluorescence intensity (Fd/Fs). Excitation wavelength was 331 nm. All the single-stranded oligonucleotides with U(DAN) exhibited emission at 458 nm. After hybrid formation, the modified duplexes exhibited emission at the shorter wavelength. DNA fragments, 5'-dACCTCTGA for **6** and 5'-dATGACCTCTGA for **7-9**, were used as complementary sequences.

intensity of the modified oligonucleotides decreased and only a slight blue-shift of the emission maximum was observed. These results imply that the DAN group may not be transferred to more hydrophobic interior of the duplexes in the present system. These are probably because of the structural elements around the fluorophore that is differ from those of U(DNS).[2]

We have synthesized an uridine derivative containing a fluorescent probe at the 2'-position [U(DAN)] which can be used for incorporation into oligonucleotide sequences. Since the fluorescence of U(DAN) is sensitive to the local environment and potentially useful as a donor of energy transfer to a fluorescein label, it would be possible to find appropriate systems to demonstrate the utility of the present fluorescent ribonucleoside.

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6. Compound **2** : m.p.=212-215℃, Rf=0.52(n-PrOH:NH_4OH:H_2O=55:35:10), ^{1}H-NMR(270MHz, DMSO-d6) : δ 3.10(s, 6H, -N(CH_3)$_2$), 6.50(s, 2H, -CO-CH_2-$^+$N), 7.00(s, 1H, naphthyl), 7.34(dd, 1H, naphthyl), 7.78(d, 1H, naphthyl), 7.85(dd, 1H, naphthyl), 8.00(d, 1H, naphthyl), 8.27(t, 2H, pyridine), 8.57(s, 1H, naphthyl), 8.73(t, 1H, pyridine), 9.01(d, 2H, pyridine).
7. Compound **3** : m.p.=251-254℃, Rf=0.76(n-PrOH:NH_4OH:H_2O=55:35:10), ^{1}H-NMR(270MHz, DMSO-d6) : δ 3.04(s, 6H, -N(CH_3)$_2$), 6.94(s, 1H, naphthyl), 7.25(dd, 1H, naphthyl), 7.66(d, 1H, naphthyl), 7.79(dd, 1H, naphthyl), 7.88(d, 1H, naphthyl), 8.37(s, 1H, naphthyl), 12.61(s, 1H, -COOH).
8. Compound **4** [U(DAN)] : m.p.=187-189℃, Rf=0.23(CH_2Cl_2:MeOH=9:1), ^{1}H-NMR(270MHz, DMSO-d6) : δ 3.03(s, 6H, -N(CH_3)$_2$), 3.63(m, 2H, H5',5"), 3.99(m, 1H, H4'), 4.20(m, 1H, H3'), 4.75(m, 1H, H2'), 5.19(t, 1H, 5'-OH), 5.57(d, 1H, 3'-OH), 5.70(d, 1H, H5), 6.08(d, 1H, H1') 6.94(s, 1H, naphthyl), 7.26(dd, 1H, naphthyl), 7.66(d, 1H, naphthyl), 7.74(dd, 1H, naphthyl), 7.82(d, 1H, naphthyl), 7.97(d, 1H, H6), 8.02(d, 1H, amide), 8.26(s, 1H, naphthyl), 11.26(s, 1H, uracil N3H).
9. The synthesis of the modified oligonucleotides was accomplished by phosphoramidite chemistry, beginning with 5'-DMT nucleoside (0.2 μmol) bound to a CPG support. For the coupling of normal deoxyribonucleoside phosphormaidites, the standard protocol (50 μL of 0.1 M amidite and 50 μL of 0.1 M tetrazole in acetonitrile, 2 min) was used. For the coupling of the modified amidites **5**, 120 μL of the 0.1 M amidite and 120 μL of 0.1 M tetrazole in acetonitrile (10 min) were used.
10. The TOF Mass spectra data obtained on a Bruker ReflexII model using a linear negative mode; Oligomer **6** : 2646.7 (. 2646.9), **7** : 3857.6 (calcd. 3857.7), **8** : 3857.7 (calcd. 3857.7), **9** : 3857.9 (calcd. 3857.7).

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