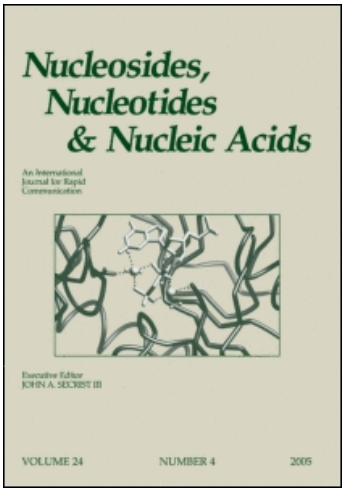

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Synthesis and Physicochemical Properties of 2′-Deoxy-2′,2″-difluoro-β-D-ribofuranosyl and 2′-Deoxy-2′,2″-difluoro-α-D-ribofuranosyl Oligonucleotides

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ABSTRACT

We present procedures for nucleoside and oligonucleotide synthesis, binding affinity (T_m) and structural analysis (CD spectra) of 2′-deoxy-2′,2″-difluoro-α-D-ribofuranosyl and 2′-deoxy-2′,2″-difluoro-β-D-ribofuranosyl oligothymidylates. Possible reasons for the thermal instability of duplexes formed between these compounds and RNA or DNA targets are discussed.

Key Words: 2′-Deoxy-2′-2″-difluorothymidine; α/β-Anomers; Oligonucleotide; Thermal stability; Circular dichroism.

Gemcitobine (2′-deoxy-2′,2″-difluorocytidine) is a potent anticancer agent that exerts cytotoxic activity, in part, through incorporation of its nucleoside triphosphate into DNA and perturbation of DNA-mediated processes. As has been shown previously, single insertion of gemcitobine to a DNA/DNA duplex reduces the

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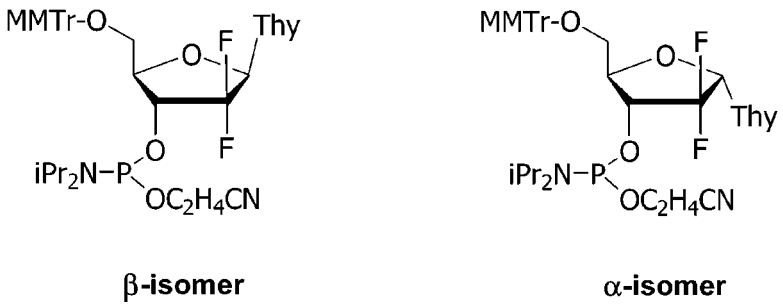

Figure 1. Structure of monomer building blocks, prepared for oligonucleotide synthesis.

thermal stability of the complex by 2–4°C, depending on the base sequence and location.[1] Unfortunately, no information regarding the behavior of antisense strands consisting exclusively of 2′-deoxy-2′,2″-difluoro-nucleosides toward RNA or DNA complements is available. We have attempted to define these properties through the synthesis of 2′-deoxy-2′,2″-difluorothymidine (diF-T) oligonucleotides and exami nation of their physicochemical properties.

The synthesis of gemcitobine was originally accomplished by Hertel and coworkers[2] and later expanded to include other 2′,2″-difluoro-pyrimidine[3] and purine nucleosides.[4] Accordingly, we employed the method of Chou et al.[3] for the synthesis of diF-T. Briefly, the 3′,5′-benzoyl-protected difluorinated nucleoside was obtained as a mixture of α and β anomers upon coupling of the sugar precursor to the thymine base, and separated by selective crystallization from ethanol. The anomeric identity of α-diF-T and β-diF-T was established by 2D NOESY ^{1}H NMR. After appropriate deprotection, both anomeric nucleosides were converted to their 5′-monomethoxytrityl-protected 3′-phosphoramidite building blocks (Fig. 1) for oligonucleotide assembly on solid support.

Oligonucleotides were assembled on the Expedite 8909 using 1 μmol RNA protocols to obtain both α-diF-T_{18} and β-diF-T_{18} oligonucleotides in *ca.* 30–40% isolated yields. After ammonium hydroxide cleavage from the support and deprotection, the pure oligonucleotides were isolated by denaturing PAGE and/or anion-exchange HPLC.

To assess the effect of α-diF-T_{18} and β-diF-T_{18} on possible duplex formation, various duplexes with complementary RNA or DNA as a second strand were prepared and compared with the duplexation behavior of α-dT_{18} and dT_{18} (Table 1). Surprisingly, β-diF-T_{18} does not form a duplex at all, even at high salt concentration (i.e., 1 M NaCl). On the other hand, α-diF-T_{18} is able to form duplexes with both complementary DNA and RNA, although its binding affinity is considerably weakened relative to α-dT_{18}. The CD spectra demonstrate that the conformational disposition of all fluorinated strands closely mimic their deoxy counterparts. In fact, both α-diF-T and α-dT oligonucleotides exhibit entirely analogous pairing behavior in that both bind in an antiparallel fashion with complementary rA_{18} while forming parallel duplexes with dA_{18} (Fig. 2).[5,6]

Modeling calculations conducted at the nucleoside level using AMBER 4.1 suggest that both difluorinated analogs possess considerable rigidity, which may prevent

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Table 1. Thermal stabilities of oligonucleotide duplexes at various ionic solution conditions.

Conditions[a]	RNA target T_m (°C)	DNA target T_m (°C)
β-dT_{18}		
Buffer A	40	47
Buffer B	53	59
β-diF-T_{18}[b]		
Buffer A	< 5	< 5
Buffer B	< 5	< 5
α-dT_{18}		
Buffer A	52.5	47
Buffer B	67	62
α-diF-T_{18}		
Buffer A	46	29
Buffer B	55	43

[a]Buffer A: 140 mM KCL, 5 mM Na_2HPO_4, 1 mM $MgCl_2$, pH 7.2. Buffer B; 1 M NaCl, 100 mM Na_2HPO^4, pH 7.
[b]No transition was etected for any of these duplexes.

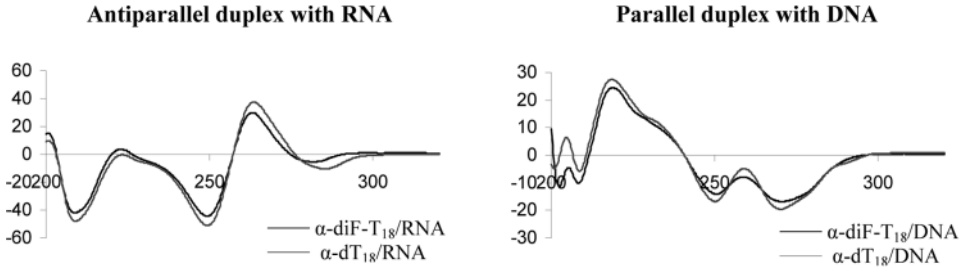

Figure 2. CD spectra for the parallel and antiparallel duplexes formed by α-dT_{18} and α-diF-T_{18} with complementary DNA and RNA strands. All spectra were obtained in buffer consisting of 1 M NaCl, 100 mM Na_2HPO_4, at pH 7 and 20 °C.

stable complexation to the target strands. Indeed, excessive rigidity in the β-diF-T oligomers rather than sugar conformation[7,8] likely abolishes stable binding to DNA or RNA, and implies that the greater flexibility of α-diF-T relative to β-diF-T enables greater tolerance toward heteroduplexation. In conclusion, the high structural similarity shared by α- and β-diF-oligonucleotides to the corresponding α- and β-deoxyoligonucleotides indicates that the difluoro-oligonucleotides behave as DNA mimics. However, α-diF-T heteroduplexes with RNA fail to elicit RNase H activity, suggesting that sugar pucker, minor groove dimensions or excessive conformational rigidity in the antisense strand prevents enzyme processing as compared to the natural DNA:RNA substrates. Unfortunately, an adequate assessment of this property with the β-configured difluoro-oligonucleotides is not currently possible given their inability to form hybrids with complementary RNA.

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