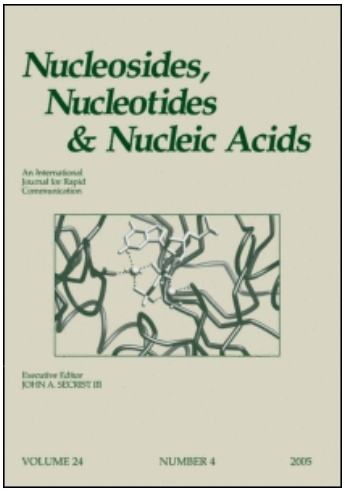


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Evaluation of Oligodeoxynucleotides Containing 3'-*C*-Aminomethyl-and 3'-*C*-Amethylthymidine

Guangyi Wang[a]; Patrick J. Middleton[a]; Liyan He[a]; Vesna Stoisavljevic[a]; Wiliiied E. Seifert[a]
[a] Research Department, ICN Pharmaceuticals, Inc., Costa Mesa, CA, USA

To cite this Article Wang, Guangyi , Middleton, Patrick J. , He, Liyan , Stoisavljevic, Vesna and Seifert, Wiliiied E.(1997) 'Synthesis and Evaluation of Oligodeoxynucleotides Containing 3'-*C*-Aminomethyl-and 3'-*C*-Amethylthymidine', Nucleosides, Nucleotides and Nucleic Acids, 16: 4, 445 — 454
To link to this Article: DOI: 10.1080/07328319708001361
URL: http://dx.doi.org/10.1080/07328319708001361

NUCLEOSIDES & NUCLEOTIDES, 16(4), 445–454 (1997)

SYNTHESIS AND EVALUATION OF OLIGODEOXYNUCLEOTIDES CONTAINING 3'-*C*-AMINOMETHYL- AND 3'-*C*-METHYLTHYMIDINE

Guangyi Wang,* Patrick J. Middleton, Liyan He, Vesna Stoisavljevic, Wilfried E. Seifert
Research Department, ICN Pharmaceuticals, Inc., 3300 Hyland Avenue
Costa Mesa, CA 92626, USA

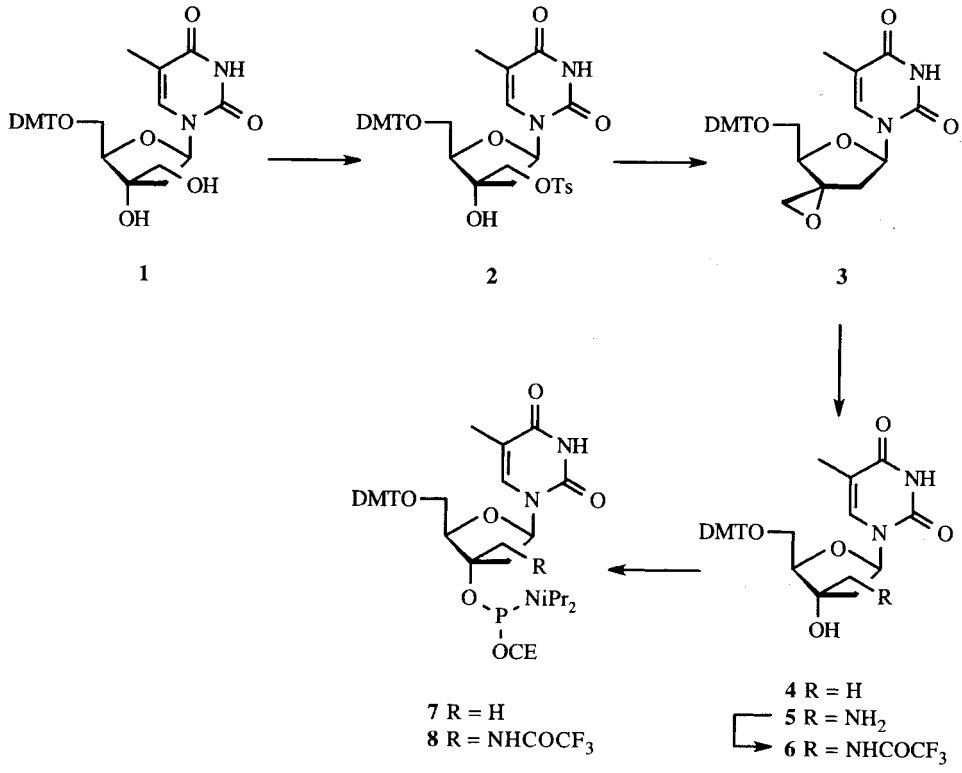
Abstract: 3'-*C*-Aminomethyl- and 3'-*C*-methylthymidine were synthesized and incorporated into oligodeoxynucleotides. Hybridization and enzyme stability of the modified oligonucleotides containing the 3'-*C*-branched thymidines are discussed.

Synthetic oligonucleotides as antisense therapeutics have been explored for years.[1-3] A variety of oligonucleotide analogs have been prepared and evaluated in order to find favorable chemical modifications that can improve the enzyme stability, binding affinity, and other pharmacokinetic properties of antisense oligonucleotides.[4-6] Recently, oligonucleotides containing *C*-branched nucleosides have been reported to have certain promising properties. Oligonucleotide analogs containing 5'-*C*-branched nucleosides have shown improved stability to cellular nucleases while they retain good hybridization to both complementary DNA and RNA.[7-8] Oligonucleotides containing 4'-*C*-substituted thymidines exhibited better hybridization to DNA and equally good hybridization to RNA as compared to the unmodified while their enzyme stability was significantly improved.[9-11] Oligonucleotides containing 3'-*C*-branched nucleosides[12-13] were also explored and exhibited good hybridization and improved enzyme stability. In addition to their improved enzyme stability, the *C*-branches of the oligonucleotides containing *C*-branched nucleosides can be used as conjugation sites for a variety of functional moieties such as intercalators, alkylating agents, nucleic acid cleaving agents, etc. A linker at C3' of the 3'-*C*-branched nucleosides can be used to locate the functional moieties in the major groove of the DNA/DNA or DNA/RNA duplexes, which is potentially useful in oligonucleotide therapeutics and diagnostics. Recently, we have explored 3'-*C*-branched nucleosides along with a variety of other sugar modifications in search of better oligonucleotide analogs as antisense therapeutics. In this article, we will report synthesis and evaluation of oligonucleotides containing 3'-*C*-aminomethylthymidine and 3'-*C*-methylthymidine.

3'-*C*-Hydroxymethylthymidine **1** was prepared according to a published procedure.[12] 5'-*O*-(4,4'-dimethoxytrityl)thymidine was oxidized by PDC to a 3'-keto derivative, which was converted to a 3'-vinyl derivative by using dibromomethane, titanium tetrachloride, and zinc in THF, and in turn oxidized with osmium tetroxide to give **1**.[12] Tosylation of **1** with tosyl chloride in pyridine afforded **2** in good yield and treatment of **2** with sodium hydride in THF afforded **3** quantitatively. **3** was also prepared from epoxidation of 5'-*O*-DMT-3'-deoxy-3'-vinylthymidine with MCPBA (not shown). However, the yield was low because of formation of undesired, isomeric epoxides and epoxidation of the thymine base. Reduction of **3** with lithium aluminum hydride afforded the methyl derivative **4**. 3'-*C*-Methylthymidine derivatives have been previously synthesized in other laboratories by diferent strategies.[13-14] Reaction of **3** with saturated ammonia in methanol afforded the amino derivative **5**, which was protected with trifluoroacetyl to give **6**. Compounds **4** and **6** were converted to the phosphoramidites **7** and **8**, respectively, from reactions with 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite.

Oligodeoxynucleotides were synthesized by using phosphoramidite methodology.[15] The procedures used are identical to the standard except the coupling time for the modified thymidine phosphoramidites, for which a coupling time of 15-30 min was used and the coupling yields were in the range of 50-60%. Apparently, substituents at C3' of thymidine significantly lowered the coupling efficiency even though they are relatively small. The synthesized oligonucleotides were deprotected and purified according to a standard protocol.[16] The purified oligonucleotides containing one modified thymidine were obtained in an amount between 30-47 A_{260} units on 1.0 μmol scale. After desalting the purified oligonucleotides were characterized by electrospray mass spectrometry. The observed molecular weights are consistent with the calculated.

Hybridization of the oligonucleotides containing 3'-*C*-branched thymidines were studied by thermodynamic melting (Tm) experiments,[17] and the results are summarized in Table 1. As can be seen, most of the modified sequences exhibit comparable hybridization as the unmodified to both the complementary DNA and RNA, as indicated by about one degree drop per modification in Tm values. However, Sequence 5 containing a 3'-*C*-aminomethylthymidine in its center region exhibits a significant drop in Tm (ΔTm = 2.5 °C/modification) for hybridization to RNA. It is not certain whether the protonation of the amino group at pH 7.0 exerts adverse effects on the hybridization. The melting curves of all the modified oligonucleotides were similar to those of the unmodified, which indicates a cooperative melting. Recently, Schmit et al.[13] reported that the oligonucleotides containing 3'-*C*-methylthymidine hybridized well with complementary RNA with a minor decrease in Tm (ΔTm/modification = 0.1-1.5 °C), as compared with the unmodified oligonucleotide.



Scheme 1.

TABLE 1. Sequences synthesized, hybridization data, and enzyme stability

	Sequence	Tm °C DNA	ΔTm °C/Mod.	Tm °C RNA	ΔTm °C/Mod.	$t_{1/2}$ min.
1.	5'-ATCTCTCCGCTTCCTTTC-3'	58.3		64.4		5
2.	5'-ATCTCTCCGCTTCCTTXC-3'	57.1	-1.2	63.7	-0.7	
3.	5'-ATCTCTCCGCTTCCTXXC-3'	56.2	-1.1	63.3	-0.6	29
4.	5'-ATCTCTCCGCTTCCXTXC-3'	55.6	-1.4	62.8	-0.8	28
5.	5'-ATCTCTCCGCXTCCTTTC-3'	57.2	-1.1	61.9	-2.5	
6.	5'-AXCTCTCCGCTTCCTTTC-3'	59.2	+0.9	64.3	-0.1	
7.	5'-ATCTCTCCGCTTCCTTYC-3'	56.7	-1.6	63.9	-0.5	
8.	5'-ATCTCTCCGCTTCCTYYC-3'	56.0	-1.2	62.8	-0.8	15
9.	5'-ATCTCTCCGCTTCCYTYC-3'	56.9	-0.7	63.0	-0.7	9
10.	5'-ATCTCTCCGCTYCCTTTC-3'	58.1	-0.2	63.5	-0.9	
11.	5'-AYCTCTCCGCTTCCTTTC-3'	58.4	+0.1	64.2	-0.2	

X = 3'-*C*-aminomethylthymidine, Y = 4'-*C*-methylthymidine.

Jorgensen et al.[12] reported a minor drop in Tm (ΔTm/modification = 0-2 °C) for hybridization of oligonucleotides containing 3'-*C*-hydroxymethylthymidine with complementary DNA. These results are similar to what we have obtained. It appears that a small substituent at C3' of thymidine does not destabilize hybridization significantly although it is too early to draw any conclusion.

Since 3'-exonucleases play a predominant role in *in vivo* DNA degradation, we employed a similar procedure to that used by Wengel and collaborators[18] to investigate the stability of oligonucleotides to snake venom phosphodiesterase. The increase in absorbance (hyperchromicity) at 260 nm was monitored during the digestion and the absorbance versus time was recorded. The half-lives ($t_{1/2}$) in Table 1 are defined as the time when 50% hyperchromicity is reached. The results are shown in Figure 1-2 and Table 1. At these conditions, the unmodified oligonucleotide was almost completely digested within 20 minutes and had a half-life of five minutes. The 3'-*C*-branched oligonucleotides behaved quite differently to degradation by the phosphodiesterase. Sequences 3 and 4 have half-lives of 28-29 minutes while the half-life of sequence 9 is only 9 minutes. It seems that the larger substituents at C3' would make the modified oligonucleotide more stable to the nuclease. Since each sequence contains only two modified nucleosides, incorporation of more modified nucleosides in the 3'-region of the oligonucleotides should increase enzyme stability significantly.

In summary, 3'-*C*-aminomethyl- and 3'-*C*-methylthymidine have been synthesized and incorporated into oligodeoxynucleotides. Hybridization studies have shown that the modified oligonucleotides containing the 3'-*C*-branched thymidines have comparable binding affinity as the unmodified sequence to both complementary DNA and RNA. The modified oligonucleotides have exhibited improved stability to snake venom phosphodiesterase.

EXPERIMENTAL

NMR spectra were recorded on a GE 500 MHz spectrometer. ^{1}H chemical shifts are reported in ppm relative to tetramethylsilane as internal standard and ^{31}P chemical shifts in ppm to phosphoric acid as an external standard. Fab Mass spectra were obtained on a Fisons Autospec spectrometer. Anhydrous solvents (water <0.005%) such as THF, DMF, dichoromethane, and pyridine were purchased from Fluka or Aldrich and used directly without further treatment. Thin layer chromatography plates and silica gel for flash chromatography were supplied by ICN.

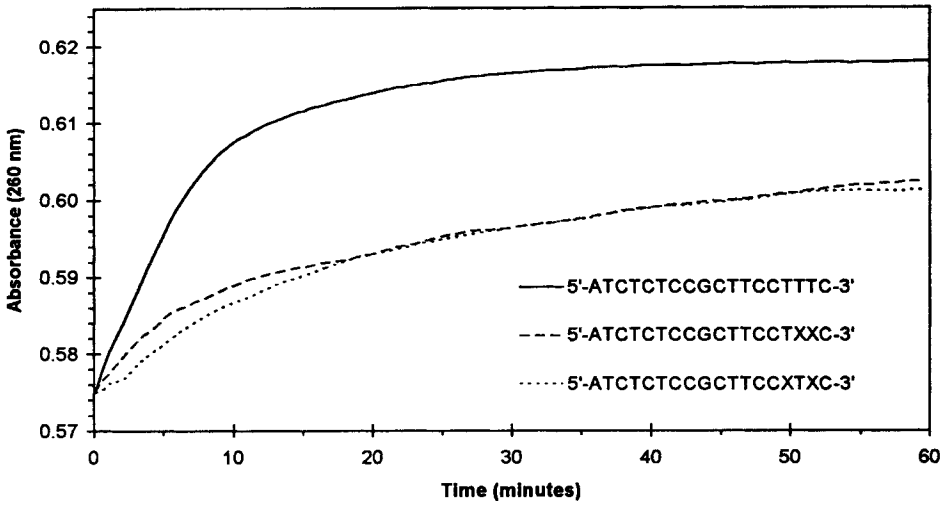


Fig. 1. Enzyme digestion time course of ODNs containing 3'-*C*-aminomethylthymidine [X]

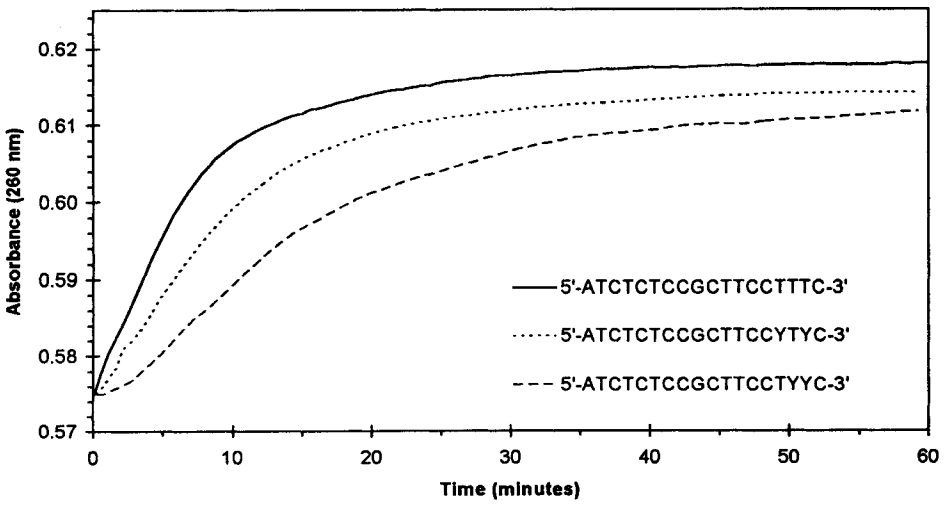


Fig. 2. Enzyme digestion time course of ODNs containing 3'-*C*-methylthymidine [Y]

5'-*O*-(4,4'-Dimethoxytrityl)-3'-*C*-p-tosyloxymethylthymidine 2. A solution of **1** (2.12 g, 3.69 mmol, prepared according to a known procedure[12]), p-tosyl chloride (1.76 g, 9.23 mmol), and DMAP (0.18 g, 1.48 mmol) in anhydrous pyridine (13 mL) was stirred at room temperature overnight, cooled to 0 °C, diluted with EtOAc (500 mL), washed with 10 % $NaHCO_3$, dried over Na_2SO_4, and concentrated. The crude was purified by chromatography on silica (5 % CH_3OH in CH_2Cl_2) to give 2.39 g (89 %) of **2** as a colorless foam; ^1H NMR ($CDCl_3$) δ 1.28 (s, 3H, 5-CH_3), 2.23 (dd, 1H, J = 12.7 Hz, 9.9 Hz, H2'a,), 2.44 (s, 3H, ArCH_3), 2.49 (m, 1H, H2'b), 3.00 (d, 1H, J = 10.9 Hz, H5'a), 3.33 (s, 1H, 3'-OH), 3.62 (dd, 1H, J = 11.1 Hz, 3.5 Hz, H5'b), 3.81 (m, 7H, 1H of CH_2OTs, 2OCH_3), 4.06 (s, 1H, H4'), 4.28 (d, 1H, J = 9.9 Hz, H of CH_2OTs), 6.53 (dd, 1H, J = 9.4 Hz, 5.0 Hz, H1'), 6.81-6.84 (m, 4H, DMT), 7.16-7.62 (m, 9H, DMT), 7.74 (s, 1H, H6), 8.70 (s, 1H, NH); FABMS m/z 729 (MH^+), 728 (M^+), 303 (DMT).

5'-*O*-(4,4'-Dimethoxytrityl)-3'-*C*,*O*-methylenethymidine 3. To a stirred suspension of NaH (60 % in mineral oil, 180 mg, 4.5 mmol) in anhydrous THF (10 mL) at 0 °C under argon was added dropwise a solution of **2** (1.5 g; 2.06 mmol) in THF (10 mL). The resulting reaction mixture was stirred at room temperature for 2 h, cooled to 0 °C, and quenched by addition of water. The mixture was diluted with ethyl acetate, washed with water twice, then with 10% $NaHCO_3$ twice, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica (5% CH_3OH in $CHCl_3$) to give 0.97 g (85%) of **3** as a colorless foam, ^1H NMR ($CDCl_3$) δ 1.45 (s, 3H, 5-CH_3), 2.34 (dd, 1H, J = 14.0 Hz, 5.8 Hz, H2'a,), 2.65-2.70 (m, 2H, H2'b, 1H of epoxy), 3.05 (d, 1H, J = 4.0 Hz, H of epoxy), 3.08 (dd, 1H, J = 10.7 Hz, 1.7 Hz, H5'a), 3.57 (dd, 1H, J = 10.7 Hz, 2.1 Hz, H5'b), 3.80 (s, 6H, 2OCH_3), 4.00 (s, 1H, H4'), 6.53 (dd, 1H, J = 8.9 Hz, 5.9 Hz, H1'), 6.83-6.86 (m, 4H, DMT), 7.24-7.41 (m, 9H, DMT), 7.77 (s, 1H, H6), 8.86 (br, 1H, NH); FABMS m/z 557 (MH^+), 556 (M^+), 303 (DMT).

5'-*O*-(4,4'-Dimethoxytrityl)-3'-*C*-methylthymidine 4. To a stirred suspension of lithium aluminum hydride (58 mg, 1.53 mmol) in anhydrous THF (10 mL) at 0 °C under argon was added dropwise a solution of **3** (385 mg; 0.692 mmol) in THF (10 mL). The reaction mixture was stirred at 0 °C for 1 h and the reaction quenched by slow addition of 10 % $NaHCO_3$. The resulting mixture was diluted with ethyl acetate, washed with 10% $NaHCO_3$ twice, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica (5% CH_3OH in $CHCl_3$) to yield 306 mg (79%) of **4** as a foam; ^1H NMR ($CDCl_3$) δ 1.23 (s, 3H, 5-CH_3, or 3'-CH_3), 1.28 (s, 3H, 3'-CH_3, or 5-CH3), 2.18 (dd, 1H, J = 12.7 Hz, 9.4 Hz, H2'a,), 2.36 (dd, 1H, J = 12.7 Hz, 5.1 Hz, H2'b), 3.14 (dd, 1H, J = 10.7 Hz, 2.5 Hz, H5'a), 3.63 (dd, 1H, J = 10.7 Hz, 3.8 Hz, H5'b),

3.79, 3.796 (2s, 6H, $2OCH_3$), 4.01 (t, 1H, J = 2.9 Hz, H4'), 6.48 (dd, 1H, J = 9.4 Hz, 5.1 Hz, H1'), 6.82-6.85 (m, 4H, DMT), 7.25-7.40 (m, 9H, DMT), 7.82 (s, 1H, H6), 8.32 (br, 1H, NH); FABMS m/z 559 (MH^+), 558 (M^+), 303 (DMT).

5'-*O*-(4,4'-Dimethoxytrityl)-3'-*C*-trifluoroacetamidomethylthymidine 6. To a stirred solution of **3** (200 mg, 0.36 mmol) in methanol (5 mL) was added a saturated solution of ammonia in methanol (20 mL). The resulting solution in a capped flask stood at room temperature for 24 h. Excess ammonia and methanol were evaporated and the residue chromatographed on silica (15% methanol in CH_2Cl_2) to give 151 mg (73.2 %) of **5** as a colorless foam; ^1H NMR (DMSO) δ 1.14 (s, 3H, 5-CH_3), 2.01-2.11 (m, 2H, H2'a, H2'b), 2.48 (d, 1H, J = 12.8 Hz, H of CH_2N), 2.63 (d, 1H, J = 12.8 Hz, H of CH_2N), 3.13 (dd, 1H, J = 10.6 Hz, 3.6 Hz, H5'a), 3.72 (s, 6H, $2OCH_3$), 3.89 (t, 1H, J = 3.5 Hz, H4'), 6.25 (dd, 1H, J = 9.5 Hz, 5.6 Hz, H1'), 6.87-6.90 (m, 4H, DMT), 7.20-7.36 (m, 9H, DMT), 7.65 (s, 1H, H6).

5 (151 mg; 0.26 mmol) was dissolved in a solution of S-ethyl thiotrifluoroacetate (0.17 mL, 1.3 mmol) in THF (3 mL) and triethylamine (0.1 mL) added. The resulting solution was stirred at room temperature for 1.5 h. Solvent was evaporated and the residue chromatographed on silica (7% CH_3OH in CH_2Cl_2) to yield 160 mg (91%) of **6** as a colorless foam; ^1H NMR (DMSO) δ 1.14 (s, 3H, 5-CH_3), 2.11-2.25 (m, 2H, H2'a, H2'b), 3.01-3.09 (m, 2H, 1H of CH_2N, H5'a), 3.41-3.45 (m, 1H, H of CH_2N), 3.53-3.58 (m, 1H, H5'b), 3.72 (s, 6H, $2OCH_3$), 3.99 (t, 1H, J = 2.8 Hz, H4'), 6.25 (dd, 1H, J = 9.6 Hz, 5.4 Hz, H1'), 6.87-6.90 (m, 4H, DMT), 7.21-7.39 (m, 9H, DMT), 7.68 (s, 1H, H6), 9.23 (t, br, 1H, CF_3CONH), 11.4 (s, 1H, NH); FABMS m/z 670 (MH^+), 669 (M^+), 303 (DMT).

5'-*O*-(4,4'-Dimethoxytrityl)-3'-*C*-methylthymidine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) 7. To a stirred solution of **4** (123 mg, 0.22 mmol) and diisopropylethylamine (0.23 mL, 1.32 mmol) in anhydrous dichloromethane (3 mL) at 0 °C under nitrogen was added dropwise 2'-cyanoethyl-N,N-diisopropylchlorophosphoramidite (156 mg, 0.66 mmol). The resulting solution was stirred at room temperature under argon for 2 h, cooled to 0 °C, diluted with ethyl acetate (20 mL), and washed with cold 5% $NaHCO_3$/10% NaCl three times, dried (Na_2SO_4), and concentrated. The crude was purified by chromatography on silica (Et_3N-EtOAc-hexanes, 5:45:50) to give **7** (86%, 51 mg of the higher R_f diastereomer and 93 mg of the lower R_f diastereomer) as colorless foam; the higher R_f diastereomer: ^1H NMR (acetone-d_6) δ 1.18 (s, 6H, CMe_2), 1.19 (s, 6H, CMe_2), 1.23 (s, 3H, 3'-CH_3), 1.48 (s, 3H, 5-CH_3), 2.25-2.30 (m, 1H, H2'a), 2.62-2.66 (m, 1H, H2'b), 2.72 (t, 2H, J = 6.0 Hz, CH_2CN), 3.24 (dd, 1H, J

= 10.9 Hz, 2.9 Hz, H5'a), 3.59 (dd, 1H, J = 10.9 Hz, 3.8 Hz, H5'b), 3.63-3.76 (m, 3H, $NCHMe_2$, $POCH_2$), 3.77-3.83 (m, 7H, $2CH_3O$, $NCHMe_2$), 4.31 (m, 1H, H4'), 6.49 (dd, 1H, J = 9.6 Hz, 5.0 Hz, H1'), 6.90-6.93 (m, 4H, DMT), 7.26-7.47 (m, 9H, DMT), 7.81 (s, 1H, H6), 9.98 (s, 1H NH); ^{31}P ($CDCl_3$) δ 140.36; FABMS m/z 781 (MNa^+), 759 (MH^+), 303 (DMT); the lower R_f diastereomer: ^1H NMR (acetone-d_6) δ 1.16-1.19 (m, 12H, $2CMe_2$), 1.22 (d, 3H, J = 1.0 Hz, 3'-CH_3), 1.48 (s, 3H, 5-CH_3), 2.27 (dd, 1H, J = 13.1 Hz, 9.8 Hz, H2'a), 2.65-2.69 (m, 1H, H2'b), 2.80 (t, 2H, J = 6.0 Hz, CH_2CN), 3.23 (dd, 1H, J = 10.9 Hz, 2.9 Hz, H5'a), 3.60 (dd, 1H, J = 10.8 Hz, 3.8 Hz, H5'b), 3.62-3.77 (m, 3H, $POCH_2$, $NCHMe_2$), 3.79 (s, 6H, $2OCH_3$), 3.82-3.89 (m, 1H, $NCHMe_2$), 4.24 (s, 1H, H4'), 6.54 (dd, 1H, J = 9.7 Hz, 4.9 Hz, H1'), 6.90-6.93 (m, 4H, DMT), 7.26-7.48 (m, 9H, DMT), 7.82 (s, 1H, H6), 10.0 (s, 1H NH); ^{31}P ($CDCl_3$) δ 140.05.

5'-*O*-(4,4'-Dimethoxytrityl)-3'-*C*-trifluoroacetamidomethylthymidine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) 8. To a stirred solution of **6** (160 mg, 0.24 mmol) and diisopropylethylamine (0.25 mL, 1.44 mmol) in anhydrous dichloromethane (3 mL) at 0 °C under nitrogen was added dropwise 2'-cyanoethyl-N,N-diisopropylchlorophosphoramidite (170 mg, 0.72 mmol). The resulting solution was stirred at room temperature for 3 h, cooled to 0 °C, diluted with cold ethyl acetate (15 mL), washed with cold 5% $NaHCO_3$/10% NaCl three times, dried over Na_2SO_4, and concentrated. The crude was purified by chromatography on silica (Et_3N- EtOAc-hexanes, 1:10:10) to give **8** (60%, 71 mg of the higher R_f diastereomer and 54 mg of the lower R_f diastereomer) as colorless foam; the higher R_f diastereomer: ^1H NMR ($CDCl_3$) δ 1.15 (s, 3H, 5-CH_3), 1.20 (d, 6H, J = 6.8 Hz, CMe_2), 1.25 (d, 6H, J = 6.8 Hz, CMe_2), 2.40 (s, 1H, H2'a), 2.41 (s, 1H, H2'b), 2.65 (t, 2H, J = 5.7 Hz, CH_2CN), 3.12 (dd, 1H, J = 14.4 Hz, 1.9 Hz, H of CH_2N), 3.19 (d, 1H, J = 11.1 Hz, H5'a), 3.62-3.70 (m, 3H, $NCHMe_2$, $POCH_2$), 3.78-3.87 (m, 8H, $2CH_3O$, H5'b, $NCHMe_2$), 4.24 (dd, 1H, J = 14.3 Hz, 9.0 Hz, H of CH_2N), 4.59 (m, 1H, H4'), 6.52 (t, 1H, J = 7.3 Hz, H1'), 6.86-6.89 (m, 4H, DMT), 7.26-7.46 (m, 9H, DMT), 7.77 (s, 1H, H6), 8.23 (br, 1H NH); ^{31}P ($CDCl_3$) δ 142.20; FABMS m/z 781 (MNa^+), 759 (MH^+), 303 (DMT); the lower R_f diastereomer: ^1H NMR ($CDCl_3$) δ 1.21(d, 6H, J = 6.7 Hz, CMe_2), 1.22 (s, 3H, 5-CH_3), 1.26 (d, 6H, J = 6.7 Hz, CMe_2), 2.33 (dd, 1H, J = 13.3 Hz, 9.8 Hz, H2'a), 2.64 (t, 2H, J = 6.1 Hz, CH_2CN), 2.79-2.85 (m, 1H, H2'b), 3.19 (dd, 1H, J = 11.1 Hz, 2.3 Hz, H5'a), 3.29 (dd, 1H, J = 14.1 Hz, 4.3 Hz, H of CH_2N), 3.62-3.73 (m, 4H, H5'b, $POCH_2$, $NCHMe_2$), 3.78-3.87 (m, 7H, $NCHMe_2$, $2OCH_3$), 4.06 (dd, 1H, J =14.1 Hz, 7.3 Hz, H of CH_2N), 4.33 (s, 1H, H4'), 6.54 (dd, 1H, J = 9.7 Hz, 5.1 Hz, H1'), 6.85-6.88 (m, 4H, DMT), 7.09 (t, 1H, J = 5.0 Hz, $NHCOCF_3$), 7.26-7.45 (m, 9H, DMT), 7.77 (s, 1H, H6), 8.19 (br, 1H NH); ^{31}P ($CDCl_3$) δ 141.13.

Oligonucleotide synthesis

Oligodeoxynucleotides were synthesized on an ABI 394 DNA Synthesizer equipped with automatic dimethoxytrityl cation detection, which monitors the coupling yields. The modified phosphoramidites were dissolved in a mixture of anhydrous THF (Aldrich, 25%) and anhydrous acetonitrile (Perkin Elmer-ABI, 75%) for obtaining a clear solution (0.12-0.13 M). The synthesis followed a standard protocol for ABI 394 DNA Synthesizer except the coupling time for the modified phosphoramidites that was raised up to 30 min. The crude DMT-on oligonucleotides were deprotected in 30% ammonia (55 °C, 8h) and purified on C8 reverse phase HPLC column (0.1 M triethylammonium acetate buffer pH 7.5/acetonitrile).

Electrospray mass spectrometry

Oligonucleotide samples for mass spectra were prepared as follow: a solution of 10-15 ODs of purified oligonucleotides in 1.0-1.5 mL of 0.1 M TEAA was loaded onto an ABI Oligonucleotide Purification Cartridge (ABI# 400771) filled with 2.0 M TEAA, washed with 0.1 M TEAA, and eluted with acetonitrile in water (1:1). About 80% of oligonucleotides can be recovered (based on UV absorbance reading). The desalted oligonucleotide solution was lyophilized, dissolved in HPLC grade water (1.0 mL), and lyophilized again. The electrospray mass spectra (ionization mode: negative) were obtained at Mass Consortium Corp., San Diego, CA.

Measurements of thermodynamic melting temperature (Tm)

The thermodynamic melting experiments were conducted on a Varian UV spectrometer equipped with an electronic temperature controller and Cary hybridization software. The complementary DNA (5'-GAAAGGAAGCGGAGA-GAT-3') was synthesized as described above and the complementary RNA (5'-GAAAGGAAG-CGGAGAGAU-3') purchased from Genset, San Diego. The samples for Tm measurements contained 2 μM of modified oligonucleotides and 2.0 μM of either the complementary DNA or RNA in a buffer (10 mM sodium phosphate, 0.1 mM EDTA, and 0.1 M sodium chloride, pH = 7.0). The Tm values in Table 1 were calculated with Cary software based on hyperchromicity.

Enzyme stability experiments

Oligonucleotides (0.75 OD) were incubated with snake venom phosphodiesterase (1.2 units) in 1.5 mL of buffer (0.1 M Tris·HCl, pH 7.5; 0.1 M NaCl; 14 mM $MgCl_2$) in a cuvette on a Varian UV spectrometer at 25 °C, and the increase of absorbance at 260 nm during the digestion versus time was monitored every 30 s. From these absorbance curves, the half-lives of oligonucleotides were calculated.

Acknowledgment. Authors wish to thank Drs. Jiejun Wu and John Greaves, Department of Chemistry, University of California at Irvine for obtaining NMR and MS spectra.

REFERENCES

1. Crooke, S. T.; Lebleu, B. *Antisense Research and Applications*; CRC Press: Boca Raton, 1993.
2. Lisziewicz, J.; Sun, D.; Metelev, V.; Zamecnik, P.; Gallo, R. C.; Agrawal, S. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3860.
3. Stein, C. A.; Cheng, Y. -C. *Science* **1993**, *261*, 1004.
4. Uhlmann, E.; Peyman, A. *Chemical Reviews* **1990**, *90*, 543-584.
5. Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 6123-94.
6. Sanhvi, Y. S.; Cook, P. D. *Carbohydrate Modifications in Antisense Research, ACS Symposium Series, No. 580*; American Chemical Society: Washington, D.C., 1994.
7. Saha, A. K.; Caulfield, T. J.; Hobbs, C.; Upson, D. A.; Waychunas, C.; Yawman, A. M. *J. Org. Chem.* **1995**, *60*, 788.
8. Wang, G.; Middleton, P.J. *Tetrahedron Lett.* **1996**, *37*, 2739.
9. Fensholdt, J.; Thrane, H.; Wengel, J. *Tetrahedron Lett.* **1995**, *36*, 2535;
10. Maag, H.; Schmidt, B.; Rose S. J. *Tetrahedron Lett.* **1994**, *35*, 6449.
11. Wang, G.; Seifert, W. E. *Tetrahedron Lett.* **1996**, *37*, 6515.
12. Jorgensen, P. N.; Stein, P. C.; Wengel, J. *J. Am. Chem. Soc.* **1994**, *116*, 2231;
13. Schmit, C.; Bevierre, M. -O.; Mesmaeker, A. D.; Altmann, K. -H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1969.
14. Fedorov, I.I.; Kazmina, E.M.; Novicov, N.A.; Gurskaya, G.V.; Bochkarev, A.V.; Jasco, M.V.; Victorova, L.S.; kukhanova, M.K.; Balzarini, J.; De Clercq, E.; Krayevski, A.A. *J. Med. Chem.* **1992**, *35*, 4567.
15. Eckstein, F. *Oligonucleotides and Analogues: A Practical Approach*; IRL Press: Oxford, 1991.
16. A protocol for ABI 394 Synthesizer from Perkin-Elmer (1994).
17. Puglisi, J. D.; Tinoco, I. Jr. *Methods Enzymol.* **1989**, *180*, 304.
18. Svendsen, M. L.; Wengel, J.; Dahl, O.; Kirpekar, F.; Roepstorff, P. *Tetrahedron* **1993**, *49*, 11341).

Received December 2, 1996
Accepted February 27, 1997