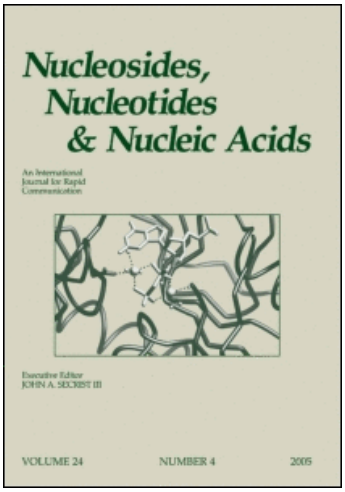

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SYNTHESIS AND BIOPHYSICAL STUDIES OF OLIGONUCLEOTIDES CONTAINING HYDROXAMATE LINKAGES

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ABSTRACT

Novel thymidine dimers containing hydroxamate linkages were synthesized, incorporated into oligonucleotide sequences and studied their hybridization properties against complementary DNA and RNA targets.

Inhibition of gene expression with antisense oligonucleotides by binding to mRNA in a sequence specific manner has become an attractive method for the treatment of viral diseases, cancer, and for the study of genetic disorders (1–3). As drug candidates, they should inhibit the translation process via duplex formation and/or able to activate RNase H to cleave the target message after hybridization. In addition, antisense oligonucleotides should possess *in vivo* nuclease stability, adequate binding affinity to the target mRNA, and the ability to reach target cells and tissues. So far, chemical modifications of oligonucleotides have resulted in increased solubility, nuclease stability, cellular uptake, binding properties and RNase H activation (4). However, the quest for new and novel modified oligonucleotides with improved properties is growing.

In this communication we report the synthesis of a novel class of thymidine dimers **15** and **16**, incorporation into oligonucleotide sequences and their hybridization studies against natural DNA/RNA. The hydroxamate dimers **15** and **16** were synthesized as shown in Scheme 1. The known aldehyde **4** (5) was coupled with *O*-methylhydroxylamine hydrochloride in the presence of pyridine

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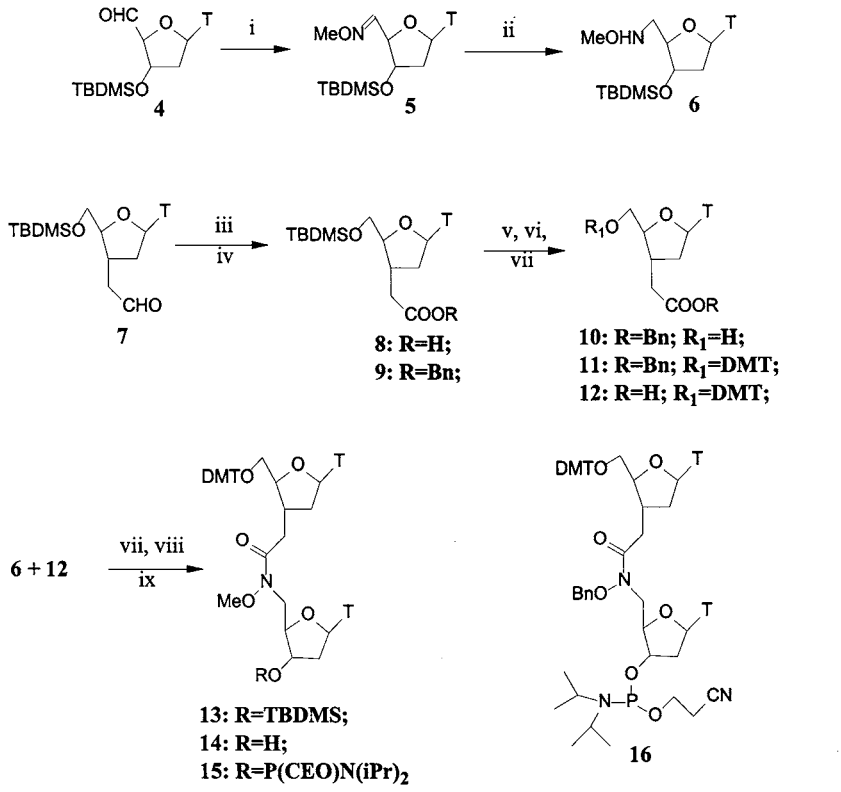

(i) MeOHN.HCl/Py/100°C; (ii) $NaCNBH_3$/ACOH/MeOH; (iii) $NaClO_2$/2-methyl-2-butene/t-BuOH/H_2O;
(iv) BnOH/EDC/N,N-Dimethylaminopyridine; (v) TBAF/THF/H_2O/Py; (vi) DMTCl/TEA/CH_2Cl_2;
(vii) 1N NaOH/THF/H_2O/H^+; (viii) HBTU/H-hydroxybenzotriazole/MeCN; (ix) (-Pr)$_2$NP(Cl)OCH_2CH_2CN -Pr_2NEt/CH_2Cl_2
T=Thymine.

Scheme 1.

at 100°C for 12 h to give oxime **5** in 99% yield. Reduction of **5** with sodium cyanoborohydride in dry methanol containing acetic acid for 6 h at room temperature provided **6**. Aldehyde **7** (6) was oxidized to the corresponding carboxylic acid **8** with sodium chlorite using 2-methyl-2-butene as HOCl scavenger (7). Esterification of **8** with benzyl alcohol using water soluble carbodiimide (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, EDC) (8) afforded benzyl ester **9** in 87% yield. Desilylation of **9** followed by dimethoxytritylation (9) and base hydrolysis provided second building block **12**. Carboxylic acid **12** was activated with *O*-(1*H*-benzotriaol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluoroborate (HBTU) and *N*-hydroxybenzotriazole (10) and then coupled with hydroxylamine **6** at room temperature to give a dimer **13** in 63% yield. Removal of the silyl protective group from **13** with tetra-*n*-butylammonium fluoride and subsequent phosphitylation (11) gave the target phosphoramidite dimer **15** (12). The second

Table 1. Tm values of Oligonucleotides Containing *O*-Methylhydroxamate Linkage[a]

	Tm (°C)		ΔTm/mod (°C)	
Sequence	DNA	RNA	DNA	RNA
5′ GAA GCC ATC AAG CAG GAA 3′				
5′ TTC CTG CTT GAT GGC TTC 3′	61.07	63.88		
5′ TTC CTG CTT GAT GGC ttC 3′	61.42	64.13	0.2	0.2
5′ ttC CTG CTT GAT GGC TTC 3′	60.75	64.03	−0.2	0.1
5′ ttC CTG CTT GAT GGC ttC 3′	61.33	64.18	0.1	0.1
5′ TTC CTG Ctt GAT GGC TTC 3′	60.25	63.03	−0.4	−0.4
5′ ttC CTG Ctt GAT GGC ttC 3′	60.31	63.07	−0.1	−0.1
5′ TTC CTG Ctt GAT GGC ttC 3′	60.26	62.97	−0.2	−0.2

[a]Tm is the temperature at the midpoint of the melting curve; The concentrations are as follows: Oligomer strands, 2 μM each. Melting temperatures (Tm) were determined (13) by measuring change in absorbance at 260 nm (cuvette, 1-mm path length) as a function of temperature in sodium phosphate buffer (10 mM, pH 7.0) containing 100 mM NaCl and 0.1 mM EDTA. All the values are averaged from at least three experiments. The letters "tt" denote thymidine dimer containing hydroxamate linkages.

dimer **16** was also prepared by using the same methodology depicted in scheme 1 (60% overall yield) and substituting *O*-benzylhydroxylamine hydrochloride for *O*-methylhydroxylamine hydrochloride during the formation of **5**.

Incorporation of the dimers **15** and **16** (12) into oligonucleotide sequences was accomplished using ABI 394 DNA synthesizer and protocol (14), and the coupling efficiency was found to be higher than 95%. The binding behavior of the modified oligonucleotides was assayed by examining their ultraviolet (UV) absorbance verses temperature profiles. An 18-mer oligonucleotide (5′-TTCCTGCTTGATGGCTTC-3′) was modified with **15** and **16** at different locations and hybridized to complementary DNA or RNA. Melting temperatures of the duplexes formed between oligonucleotides containing the hydroxamate linkages and their DNA and RNA complementary strands are summarized in Table 1. This study suggests that a smaller substitution (CH_3 group) on the hydroxamate dimer **15** did not affect duplex stability. On the other hand, dimer **16** with a bulky substitution (benzyl) in oligonucleotide sequence decreases (data not shown) its ability to form stable duplexes with complementary DNA/RNA. Interestingly, compared to unmodified oligonucleotides, incorporation of hydroxamate modified dimer at the 3′-end of the oligonucleotides, exhibited ten folds increase in resistance to exonucleases (15).

In summary, oligonucleotides containing hydroxamate dimers have been synthesized for the first time and studied for their ability to form stable duplexes. Interestingly, oligonucleotides containing hydroxamate dimer **15** not only displayed a similar or even slightly higher affinity for RNA target than the natural analogues, but also showed substantial resistance towards 3′-exonucleases.

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