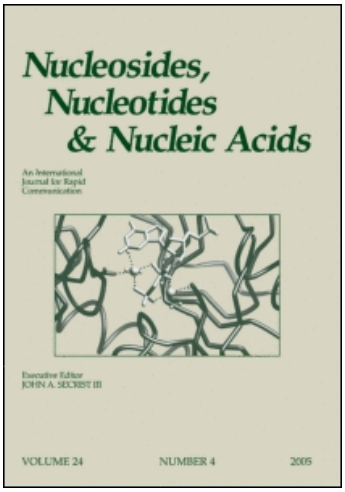

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SYNTHESIS AND CHARACTERIZATION OF SHORT OLIGONUCLEOTIDE SEGMENTS CONTAINING NONNATURAL INTERNUCLEOSIDE AMINE- AND AMIDE LINKAGES

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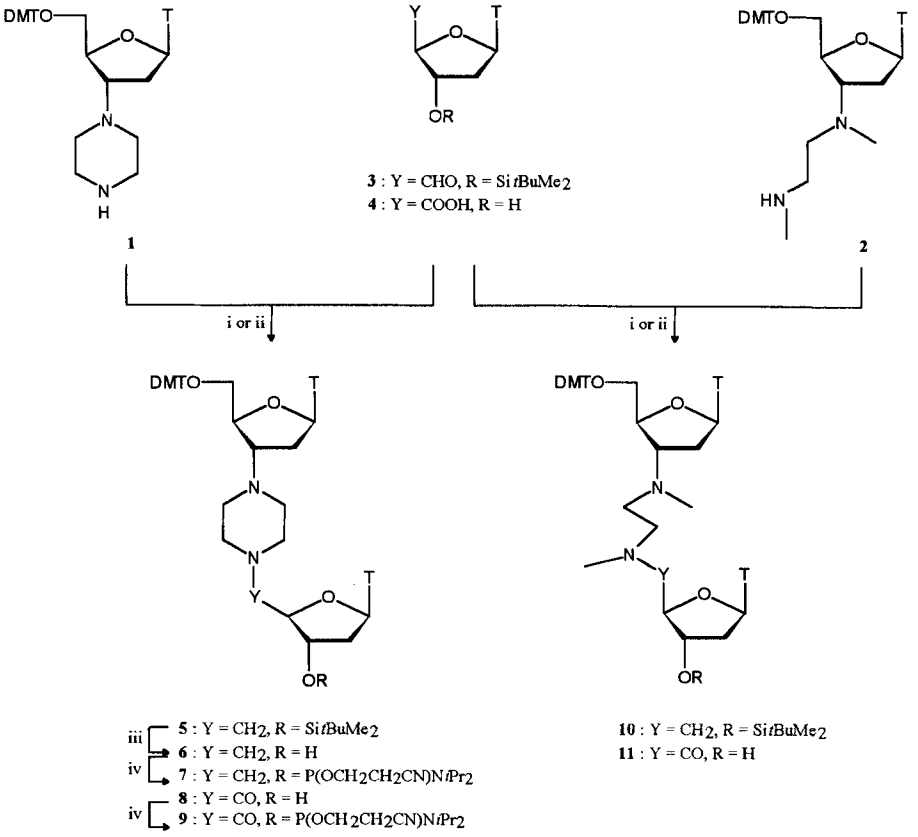
Abstract: The synthesis of thymidine dimers in which the phosphodiester linkage has been replaced by either piperazine or *N,N'*-dimethylethylenediamine are described. The dimers containing piperazine were incorporated into oligodeoxynucleotides on which thermal and enzymatic stability experiments were performed.

Modified oligonucleotides have achieved much attention because of their potential use as therapeutics in the treatment of AIDS and certain forms of cancer. The oligonucleotides can be modified in the nucleobase, the carbohydrate moiety or the internucleoside linkage. The oligonucleotides containing modified linkages can be divided into two classes, one in which the phosphor atom is retained and one in which the natural phosphodiester linkage is replaced by achiral, neutral moieties.[1] Here we wish to report the synthesis of the four novel diamine linked thymidine dimers **6**, **8**, **10** and **11** and the incorporation of **7** and **9** into oligonucleotide sequences. The thermal and enzymatic stability of these oligodeoxynucleotides were evaluated.

The key synthons **1** and **2** were synthesized by refluxing 1-[2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-3-*O*-methanesulfonyl-β-D-*threo*-pentofuranosyl]thymine prepared from 5'-*O*-(4,4'-dimethoxytrityl)thymidine using the protocol outlined by Fox and Miller[2] with either piperazine or *N,N'*-dimethylethylenediamine. Thus affording **1** in 30% and **2** in 19% yield after chromatographic purification. The 5'-aldehyde **3**[3] and 5'-carboxylic acid **4**[4] were prepared from thymidine as previously reported. The dimers **5** and **10** were synthesized by reductive amination of **3** with either **1** or **2** using titanium tetraisopropoxide in toluene and sodium cyanoborohydride giving **5** in 33% and **10** in 25% yield. **6** was obtained by treating **5** with tetrabutylammoniumfluoride (TBAF) in THF in 46% yield. The amide linkage in **8** and **11** was formed by dicyclohexylcarbodiimide (DCC)/*N*-hydroxysuccinimide (NHS) activation of **4** followed by addition of either **1** or **2** furnishing **8** and **11** in 60% and 73% yield, respectively. The two phosphoramidites 7 and **9**

were obtained by reacting **6** and **8** with 2-cyanoethyl-*N,N*-diisopropylphosphoramidochloridite in the presence of *N,N*-diisopropylethylamine hence giving **7** and **9** in yields of 84% and 82% after precipitation from hexanes.



Reagents : (i) 1) $Ti(OiPr)_4$/toluene/3A molecular sieves 2) $NaBH_3CN$, (ii) NHS/DCC/DMF, (iii) TBAF/THF (iv) $NCCH_2CH_2OP(Cl)NiPr_2$/$EtNiPr_2$/CH_2Cl_2. DMT=4,4'-dimethoxytrityl, T=thymin-1-yl.

The phosphoramidites **7** and **9** were incorporated into different oligonucleotide sequences by standard phosphoramidite methodology on an automated DNA-synthesizer[5] in an average yield of 50%.

The thermal stability of the duplexes formed by the modified oligodeoxynucleotides and their DNA complements were determined by melting experiments as previously described.[6] It can be seen that incorporation of **7** once or twice in the middle of an oligodeoxynucleotide results in a considerable lowering of the melting temperature T_m by approximately 11 °C per modification. Contrary to this, incorporation of the amide **9** once or twice in the middle only causes a slight decrease in T_m (approximately 2 °C per modification).

Table 1. Oligodeoxynucleotides Synthesized and Melting Experiments

	Sequence (5'→3')	T_m (°C) / h_T	ΔT_m (°C)		h_T	
		w.t.	**8**	**11**	**8**	**11**
A	CACCAACT*TCTTCCACA	64/1.34	-10.8	-1.6	1.25	1.28
B	CACCAACT*TCT*TCCACA	64/1.34	-11.1	-2.0	1.23	1.26
C	TTAACTTCTTCACAT*TC	55/1.34	-2.8	-2.4	1.33	1.34
D	T*TAACTTCTTCACAT*TC	55/1.34	-2.0	-1.6	1.31	1.30

T*T = modified thymidine dimer, w.t. = wild type DNA, ΔT_m = decrease in T_m per modification, h_T = thermal hyperchromicity

As expected, incorporation of the modified dimers **7** and **9** at the ends do not lead to a significant change in T_m values. The hyperchromicities of all the modified oligodeoxynucleotides deviate to some extent from the wild type oligodeoxynucleotides indicating a distortion of the secondary structure of the DNA duplexes formed compared to the wild type duplexes, the distortion being less for the end-modified oligomers as expected.

The fact that 3'-phoshpodiesterases play a predominant role in the *in vivo* degradation of natural oligodeoxynucleotides[7] prompted us to study the enzymatic stability of the modified oligodeoxynucleotides towards the 3'-exonuclease snake venom phosphodiesterase (SV PDE). Incorporation of **7** and **9** gave rise to an increase in stability towards 3'-exonucleases by a factor of 5 to 6, while incorporation in the middle lead to a rapid degradation of the 17-mers to a stabilized 9- or 12-mer (depending on the number of modifications in the oligodeoxynucleotide).

In conclusion, synthesis of novel diamine linked thymidine dimers have been accomplished. Two of these have been incorporated into different oligodeoxynucleotides. Oligomers containing the amide **9** are promising candidates as antisense oligonucleotides in that the duplex stability only slightly is affected and the stability towards nucleolytic degradation is increased considerably. On the contrary oligomers containing amine **7** seems to be of no use in antisense oligonucleotides due to the pronounced destabilization observed for duplexes formed with complementary DNA strands.

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