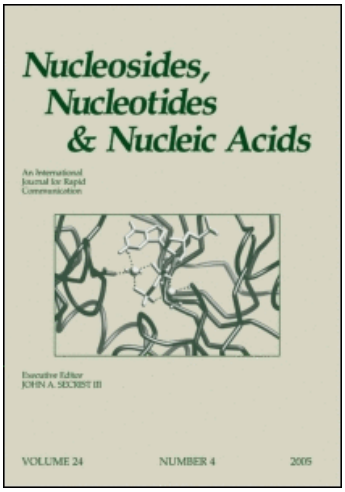

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Christopher J. Wilds[a]; George Minasov[b]; François Natt[c]; Peter von Matt[c]; Karl-Heinz Altmann[c]; Martin Egli[a]
[a] Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee, U.S.A. [b] Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL, U.S.A. [c] Novartis Pharma Inc., Basel, Switzerland

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STUDIES OF A CHEMICALLY MODIFIED OLIGODEOXYNUCLEOTIDE CONTAINING A 5-ATOM AMIDE BACKBONE WHICH EXHIBITS IMPROVED BINDING TO RNA

Christopher J. Wilds,[1] George Minasov,[2] François Natt,[3] Peter von Matt,[4] Karl-Heinz Altmann,[5] and Martin Egli[1,*]

[1]Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee
[2]Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL
[3]Therapeutic Area Functional Genomics, Novartis Pharma Inc., Basel, Switzerland
[4]Therapeutic Area Transplantation, Novartis Pharma Inc., Basel, Switzerland
[5]Therapeutic Area Oncology, Novartis Pharma Inc., Basel, Switzerland

ABSTRACT

Chimeric oligodeoxyribonucleotides where the phosphodiester linkage -C3′-O-PO_2–-O-CH_2-C4′- of DNA is substituted by the amide linkage -C3′-CH_2-CH*(CH_3)-CO-NH-CH_2-C4′ (*either R or S stereochemistry) have been prepared and their binding to RNA targets have been investigated. Incorporation of a single amide unit increases the T_m by approximately 1.4–1.9°C. Circular dichroic spectra of these modified duplexes are similar to the wildtype DNA/RNA.

Investigations of chemically modified oligonucleotides offer a wealth of insight about structure and thermodynamic stability which is of interest to those

*Corresponding author.

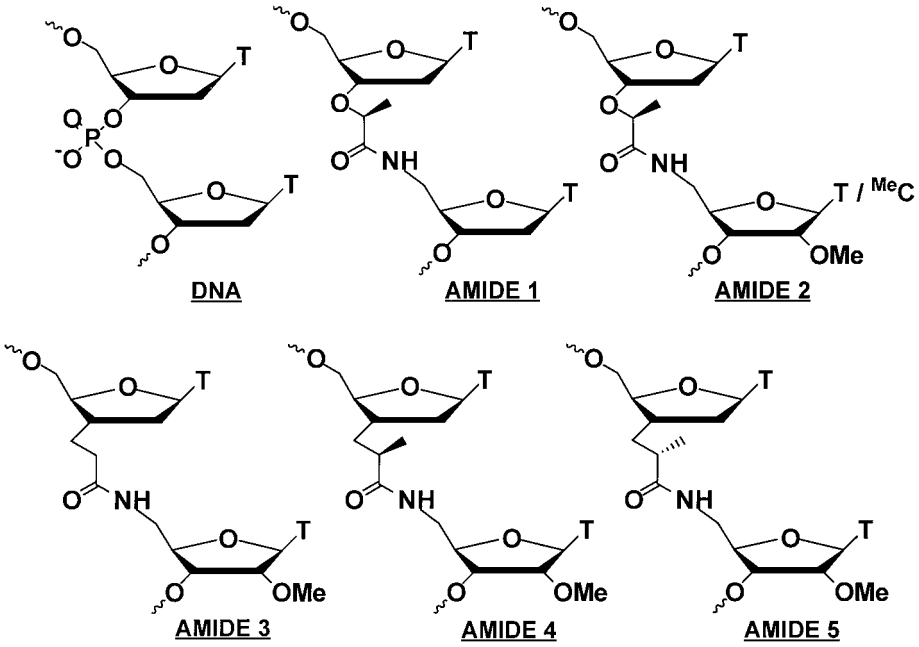

Figure 1.

pursuing studies of this family of molecules as therapeutic agents or trying to understand how DNA and RNA evolved as the molecules of life (1,2). We have found that the incorporation of some amide linked units into oligodeoxyribonucleotides produces chimeric molecules that bind to RNA targets with greater thermal stability compared to their wildtype DNA counterpart. Figure 1 shows the structure of the amide linkages we have investigated.

The synthesis of these amide dimer building blocks was accomplished via a combination of standard nucleoside and peptide coupling protocols to produce the requisite phosphoramidites for solid phase oligonucleotide synthesis (3,4). These dimeric units were incorporated into an oligodeoxyribonucleotide and purified trityl-ON via reverse phase HPLC.

The results of thermal denaturation experiments for sequences containing single and multiple amide inserts are shown in Table 1. Modified oligomers containing **AMIDE 1**, studied previously by De Napoli et al., were found to be destabilizing relative to wildtype DNA. The introduction of a 2′-OMe ribo group into the sugar at the 3′-end of the dimer (**AMIDE 2**) was found to be stabilizing by about 2.9°C.

In the case of **AMIDES 3–5**, the 3′-position of the linkage is replaced by a methylene group. Modification **3** is stabilizing as a single unit whereas multiple inserts results in destabilization of the duplex. Introduction of a methyl group in the backbone with either the S or R stereochemistry (**AMIDES 4** and **5**) results in further thermal stability. This is suprising in terms of introducing "more" atoms into the backbone of DNA. Interestingly, a sequence containing alternating

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Table 1.

	SEQUENCE A		SEQUENCE B	
TTunit	T_m (°C)[a]	ΔT_m/mod[b] (°C)	T_m (°C)[a]	ΔT_m/mod[b] (°C)
DNA (dT - dT)	53.4		51.0	
AMIDE 1	51.5	−1.9	42.1	−1.6
AMIDE 2	54.4	+1.0	n.m.	
AMIDE 2*	61.8	+1.2	n.m.	
AMIDE 3	54.0	+0.6	47.3	−0.8
AMIDE 4	54.8	+1.4	54.0	+0.6
AMIDE 5	55.3	+1.9	52.0	+0.2
dT −2′OMe ribo T	54.4	+1.0	48.2	−0.6

SEQUENCE A:
DNA: 5′-TTT **TT**C TCT CTC TCT-3′ (*5′−**TT TT TC TC TC TC TC** T −3′)
RNA target: 5′− AGA GAG AGA GAA AAA −3′
SEQUENCE B:
DNA: 5′-GCG **TT TT TT TT TT** GCG-3′
RNA target: 5′- CGC AA AA AA AA AA CGC-3′
[a]at a total strand concentration of 8 μM.
[b]compared to all DNA.
Buffer: 10 mM phosphate buffer with a total sodium concentration of 100 mM (supplemented as NaCl), 10 mM EDTA, pH = 7.

DNA / 2′OMe-RNA units is destabilizing relative to the all DNA but a single insert is stabilizing (5).

Circular dichroism spectra of these modified duplexes reveal little difference relative to the wildtype DNA/RNA duplex, indicating that there is little change in the global conformation of the modified duplex relative to "natural" DNA / RNA (data not shown). All duplexes show signatures that are consistent with a A-type structure with a large positive CD band around 260 nm and a large negative band centered around 210 nm (6).

In general, A-like duplexes are more stable than B-like ones (7). It is well known that 2′-OMe ribo units in oligomers that are singly or uniformly modified adopt a C3′-endo structure which helps to account for their greater stability (5). Similarly, oligonucleotides with an all-carbon backbone are believed to be in a C3′-endo conformation as well due to the absence of a gauche effect between O3′ and O4′, driving the sugar into the C2′-endo conformation (8). All of these amide modifications have CD signatures similar to those of wildtype DNA/RNA hybrids and little increase in the overall A-like character of the duplex is observed. Thermodynamic results for the duplexes containing multiple inserts of **AMIDE 4** and **5** modification show a less negative entropic component relative to wildtype DNA / RNA (data not shown). This suggests that the origin of stability may arise from preorganization, presumably due to reduced conformational flexibility of the amide relative to the phosphodiester backbone.

These modified amide units were incorporated into the DNA strand of a DNA / RNA duplex, yielding crystals with the space group $P4_3$ (a = b = 47.23 Å, c = 24.46 Å) that diffract to 2.5 Å resolution. To our knowledge this is the first time a crystal structure has been obtained for a backbone modified oligodeoxyribonucleotide hybridized to RNA. The X-ray crystal structure confirms that the amide backbone has a *trans* conformation and that the distance between neighboring base pairs is not affected by incorporation of this longer backbone (data not shown).

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