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Comparison of two Amides as Backbone Replacement of the Phosphodiester Linkage in Oligodeoxynucleotides

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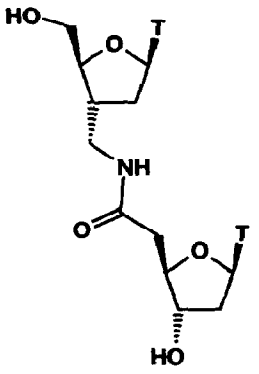
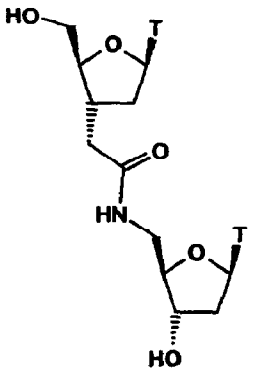
Abstract: *The two isomeric amide modifications 1 and 2 show similar effects on the melting temperature of RNA/DNA duplexes, when they replace the natural phosphodiester linkage in the DNA strand. The synthesis of the amide dimer 1 is presented.*

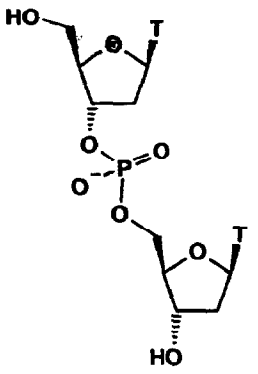
In recent years modified oligodeoxyribonucleotides (ODNs) have become an area of increased activity.[1] The ability of the antisense ODNs to interfere specifically with a mRNA target provides a powerful tool for the control of cellular and viral gene expression. However, this strategy is restricted by the poor resistance towards cellular nucleases and by the low cellular uptake of the natural phosphodiester-linked antisense ODNs (wild type). Therefore, chemical modifications of ODNs are required for their effective use as potential antisense drugs.[2]



1 ("amide-4")



2 ("amide-3")

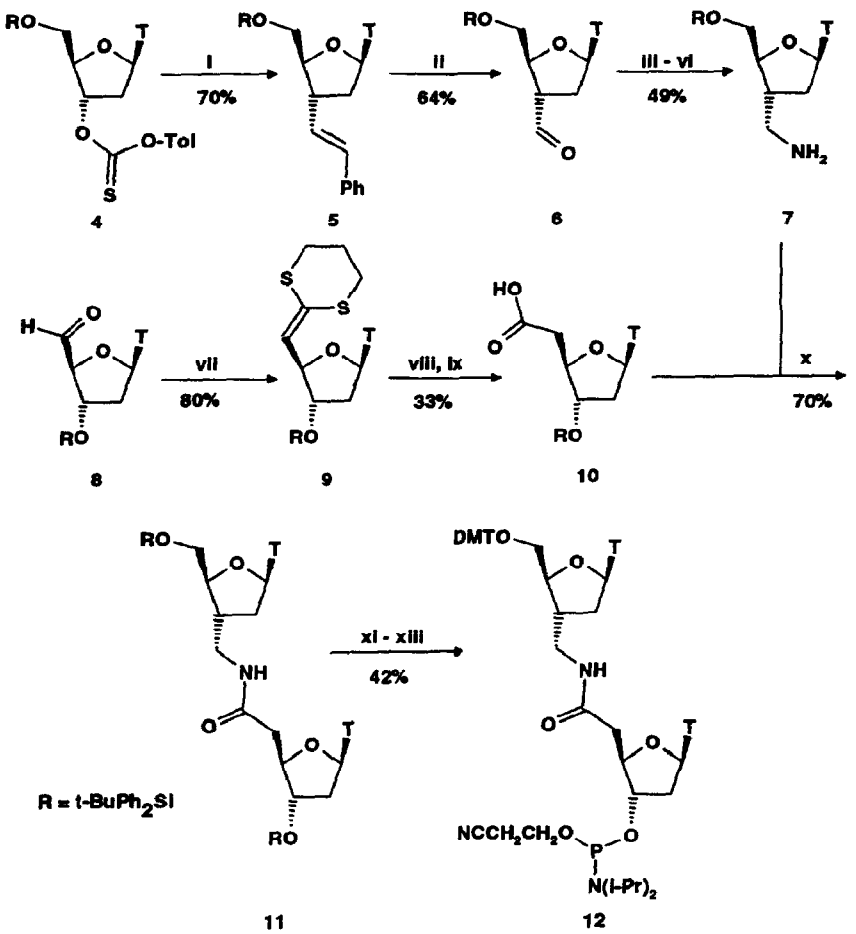


3 (wild type)

We now wish to report our results on the amide modification **1** ("amide-4"),[3] and compare them to the ones we obtained from its structural isomer, the amide modification **2** ("amide-3") (permutation of the C=O and NH groups), as well as to the natural phosphodiester linkage **3**.[4] The synthesis of the modified dimer **1** is outlined in the **Scheme**. Aldehyde **6** is the key intermediate for the synthesis of amide dimer **1**. The known procedures[2a,5] for the preparation of **6** are unsatisfactory. Therefore, we developed an efficient synthesis of this compound using a stereoselective radical addition of the 3'-centered radical to β-tri-n-butyltin-styrene.[6] A single isomer, **5**, was obtained. The cleavage of the C=C double bond was achieved by OsO_4 and $NaIO_4$. After reduction of **6**, the resulting alcohol was tosylated. Displacement with LiN_3 and reduction of the azide

with n-Bu_3SnH furnished the amine **7**. After several attempted syntheses of the acid **10**, we obtained this compound from the aldehyde **8**[7] by *Wittig* reaction with 1,3-dithia-2-cyclohexylidene triphenylphosphorane[8], followed by methanolysis of the resulting olefin **9** in the presence of aqueous $HgCl_2$. The desired methylester was obtained in 40% yield. The major side product (40%) is the 4'-α epimer. The hydrolysis of the methylester to the acid **10** had to occur with aqueous NaOH in THF under controlled conditions due to the sensitivity of the starting material towards bases. The coupling of the acid **10** with the amine **7** and the further elaboration of the resulting dimer **11** to **12** was performed as described for the amide modification 2.[4]

Scheme:



i) Ph-CH=CH-$SnBu_3$, AIBN, PhH, 80°C. ii) OsO_4, $NaIO_4$, dioxane/H_2O (3:1), RT. iii) $NaBH_4$, MeOH, RT. iv) TsCl, DMAP, pyridine, $CHCl_3$, RT. v) LiN_3, NaI, DMF, 100°C. vi) n-Bu_3SnH, AIBN, PhH, 80°C. vii) 1,3-dithia-2-cyclohexylidene triphenylphosphorane, THF, -78°C → RT. viii) $HgCl_2$, MeOH/H_2O (9:1), reflux. ix) NaOH (0.5 M), H_2O, THF, RT. x) N-methylmorpholine, O-(1H-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium-tetrafluoroborat, N-hydroxybenzotriazole, CH_3CN, RT, 7, N-methylmorpholine, RT. xi) TBAF, THF, AcOH, RT. xii) DMTCl, pyridine, RT. xiii) $(i\text{-}Pr_2N)_2POCH_2CH_2CN$, $(i\text{-}Pr)_2NH_2^+$ tetrazole$^-$, CH_2Cl_2, RT.

The solid phase synthesis of the ODNs containing the modified dimer **1** and their purification were performed using standard procedures.[9] The stability of the dimer **1** under the conditions required for the solid phase synthesis was verified. In particular, we observed no change of the compound **1** by ^{1}H-NMR spectroscopy after treatment with aqueous NH_3 (25%) at 60° for 12 hours.

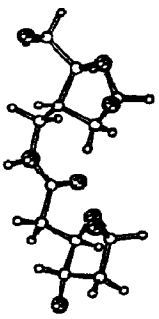
Table: Melting temperatures T_m[10] of duplexes of modified oligonucleotides with their RNA and DNA complements [a]

		T_m (°C)	ΔT_m(°C)/mod.[4a] [b]	
	Oligomer sequence	wild type	**1**	**2**
A	CTCGTACC**TaT**TCCGGTCC	63.3	-0.5	0.9
B	CTCGTAC**TaTTaT**CCGTCC	61.8	-0.3	0.5
C	GCG**TaTTaTTaTTaTTaT**GCG	50.2	0.4	-0.3
D	TTT**TaT**CTCTCTCTCT	51.6	-0.8	0.4
E	GCG**TaTTaTTaTTaTTaT**GCG	54.1	-1.4	-1.5

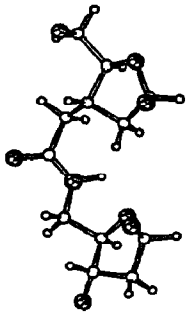
[a] **A** - **D** with RNA complement, **E** with DNA complement. [b] ΔT_m = [T_m (modified ODN) - T_m (wild type)] / number of modifications. 4μM of each strand, pH 7, 100 mM total [Na^+], 0.1 mM EDTA.

The melting temperatures of the duplexes formed between these ODNs and their RNA and DNA complements are summarized in the **Table**. Both modifications **1** and **2** show similar effects on the melting temperature of the duplexes with the corresponding RNA complements, when they replace the natural phosphodiester linkage. The similarity of the effects on the T_m values for the amides **1**, **2** is in agreement with their closely related geometries (see below). These amides are the two analogs of the same *trans* C=C bond. The amide **2** modification is superior to the amide **1** as a single replacement of the phosphodiester moiety (entries **A**, **B**, **D**). In contrast, the amide **1** increases the T_m value when introduced five times in alternating mode with phosphodiester bonds (entry **C**), whreas for the amide **2** a destabilization of the duplex was observed in this case. Interestingly, both amide modifications **1** and **2** destabilize, to the same extent (entry **E**), the duplexes formed with complementary DNA strands.

Figure:



1 ("amide-4")



2 ("amide-3")

In order to study the conformational features and the dynamical behaviour of such modified oligonucleotides, molecular mechanics and molecular dynamics (MD) investigations were carried out on the modified hybrid

octamer d(CTTTaTTTC)•r(GAAAAAAG) using the AMBER all atom forcefield[11]. The lowest-energy geometries of the **TaT** modified portion found for **1** and **2** by a conformational analysis procedure[12] are shown in the **Figure** (bases omitted for clarity). The two amide modifications display very close backbone geometries. However, differences in the dynamical behaviour were observed during MD simulations. A detailed account on the computer simulations will be published elsewhere.

In summary, we have identified two amide backbone modifications **1** and **2** displaying similar or slightly higher affinity for a complementary RNA strand than the wild type. Moreover, we have shown that the introduction of these modifications in an alternating mode with phosphodiester bonds increases very substantially the resistance of the latter moieties towards nucleases. Due to their very promising properties, we are currently incorporating these two new backbone modifications in biological relevant sequences.

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3. The names "amide-3" and "amide-4" refer to the order of their synthesis in our laboratories.
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9. For details see: Sinha, N.D., Biernat, J., McManus, J., Köster, H. *Nucl. Acids Res.* **1984**, *12*, 4539-57.
10. For exp. details see: Freier, S.M., Albergo, D.D., Turner, D.H. *Biopolymers* **1983**, *22*, 1107-31. All values are averages of at least three experiments. The absolute experimental error of the T_m values is ± 0.5°C.
11. AMBER force field (Weiner, S.J., Kollman, P.A., Nguyen, D.T., Case, D.A., *J. Comp. Chem.*, **1986**, *7*, 230-52), as incorporated in the Insight II (version 2.2.0)/DISCOVER (version 2.9) software from BIOSYM Technologies, San Diego, CA 92121 (USA).
12. The same procedure as used for the study of **2** (see ref. 4a.).

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