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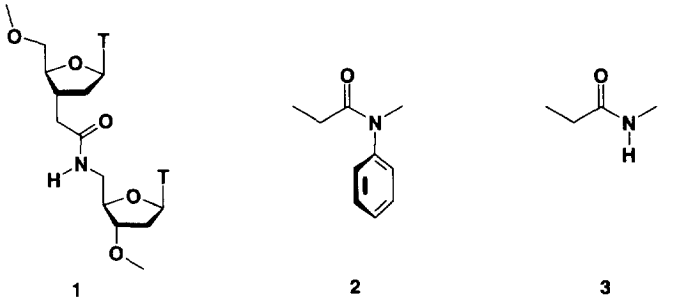
# Hydrophobic Effects in Duplexes with Modified Oligonucleotide Backbones and RNA

**Adrian Waldner*, Alain De Mesmaeker, Sebastian Wendeborn**

Central Research Laboratories, Ciba-Geigy Ltd.
CH-4002 Basel, Switzerland
E-Mail: adrian.waldner@chbs.MHS.CIBA.COM; FAX: +41 61 697 82 52

***Abstract:*** The incorporation of hydrophobic substituents on the amide functionality of modified oligonucleotides drastically influences the thermal stability of the corresponding duplexes with the RNA complement. Copyright © 1996 Elsevier Science Ltd

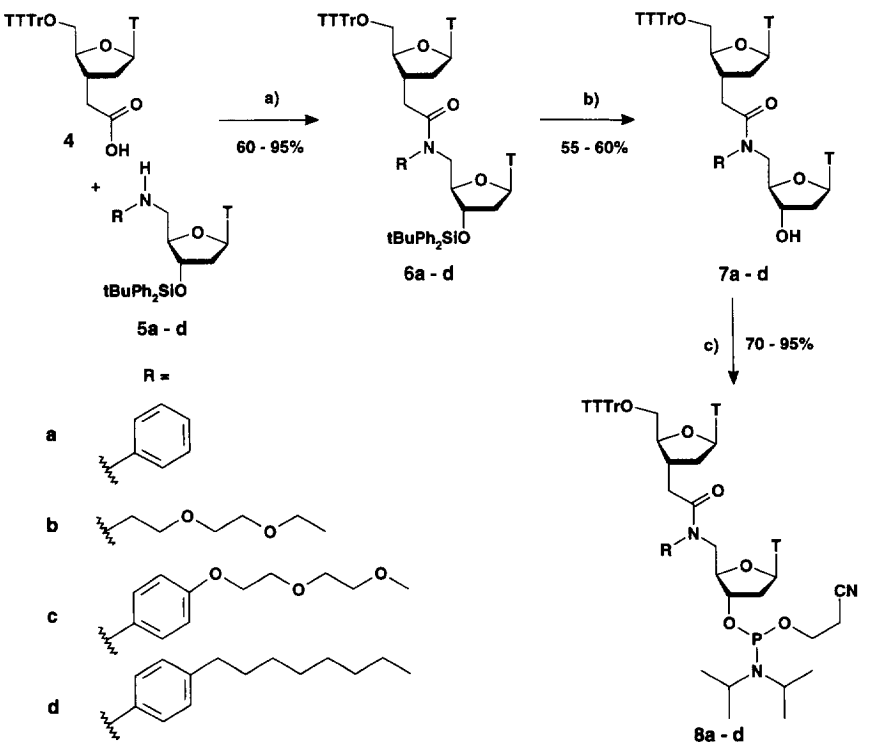
In the course of our investigations on modifications of the internucleosidic linkage in oligonucleotides we showed that two out of five amide replacements for the natural phosphodiester moiety exhibit promising properties with respect to the thermal stability of the duplex formed with an RNA complement as well as to the stability towards enzymatic degradation by nucleases [1]. These two modifications are preferred due to conformational factors, such as the preorganization of the backbone and the puckering of the sugars, influencing the stability of duplexes formed between modified oligonucleotides and their RNA complement [2]. In addition to these very important factors, duplexes are, in general, further stabilized by water molecules present in their major and minor groove, thus forming hydrogen bonds towards the bases and the phosphate groups, respectively [3]. Therefore, the incorporation of hydrophobic substituents in the backbone should influence the hydration and, as a consequence, the stability of duplexes and their biophysical properties.




1 2 3

**Figure:** Amide-3 backbone modification **1** and preferred rotamers in tertiary, N-phenyl and secondary acetamides, **2** and **3**, respectively.

In order to test this effect, it was important to exclude the changes of the duplex stability which are caused by other factors than the desired one. For example, the replacement of the hydrogen atom in the amide-3 modified backbone **1** (**Fig.**)[4] by other substituents gives rise to the formation of rotamers. N-alkyl-N-phenyl acetamides **2** are an exception in that they all occur preferentially in only one rotameric form [5], in which the phenyl ring occupies the position of the hydrogen atom of simple N-alkyl-acetamides **3**. This behaviour is explained by a rotation of the phenyl ring around the N-C(phenyl) bond thus interrupting partially the conjugation of the lone pair of the N-atom and the phenyl ring and, as a consequence, avoiding steric congestion. The introduction of different substituents in the para position of the phenyl ring would therefore allow to determine the influence of the respective substituents on the duplex stability in dependence of their hydrophobic or hydrophilic properties, since the amide conformation would not be affected.




**Scheme:** a) 3.2 eq. 2-chloro-1-methyl-pyridinium iodide, 4.4 eq. $Et_3N$, $CH_2Cl_2$. b) 4.0 eq. TBAF, 4.0 eq. AcOH, THF. c) 3 eq. $((iPr_2)N)_2POCH_2CH_2CN$, 5 eq. $(iPr)_2NH_2^+$ tetrazole$^-$, $CH_2Cl_2$.

The synthesis of the N-substituted amide-3 dinucleotides **8a** - **d** is outlined in the **Scheme** and follows the route described for the corresponding N-methyl-amide-3 dimer [6] by substitution of the 5'-OTs with the corresponding amine. The substituted anilines were prepared by nitration of n-octyl-benzene and by etherification of p-nitro-phenol, respectively, followed by reduction of the nitro group. The ethylene glycol derived amine was prepared from diethylene glycol monoethyl ether by a

*Gabriel*-type synthesis. Due to the low reactivity of anilines, the procedure with N-hydroxybenzotriazole ester we used for the N-alkyl amide-3 derivatives was not successful. Therefore, we had to prepare a more reactive ester which in addition does not require a protection of thymine. The method of *Mukayama* [7] fits to these requirements. The dimers **8** were protected on the 5'-position by the tris-t-butyl-trityl group (TTTr) and activated on the 3'-position as phosphoramidites [8]. In contrast to the N-phenyl derivatives, the modification **b** (**6** - **8**) displays two rotamers, the major one being as depicted in the **Scheme** (3:1 in $CDCl_3$ at RT for **7b**). Both rotamers should be compatible with an overall duplex structure with an RNA complement [9]. We verified the stability of the N-phenyl amide-3 dimers **8a, c** and **d** under the conditions required for the oligomerization. In particular, no cleavage of the amide bond was observed by treatment with ammonia in methanol under reflux.

**Table:** Hybridization data [10] towards RNA

| Entry | # | Modification | Sequence[a] | $\Delta T_m$/modification (°C)[b] |
|---|---|---|---|---|
| 1 | **1** | Amide-3 | 1 | +0.4 |
| | | | 2 | -0.1 |
| 2 | **8a** | N-Phenyl-amide-3 | 1 | -0.7 |
| | | | 2 | n.d.[c] |
| 3 | **8b** | N-Ethoxyethoxyethyl-amide-3 | 1 | -0.7 |
| | | | 2 | -0.7 |
| 4 | **8c** | N-p-(Methoxy-diethoxy)-phenyl- | 1 | -2.0 |
| | | amide-3 | 2 | -1.3 |
| 5 | **8d** | N-p-Octylphenyl-amide-3 | 1 | -3.5 |
| | | | 2 | -3.8 |

a) Sequence 1: TTTT*TCTCTCTCTCT; Sequence 2: GCGT*TT*TT*TT*TT*TGCG
b) compared to the wild type phosphodiester; c) not determined

The **Table** summarizes the differences in melting temperature ($\Delta T_m$) of the duplexes formed between the modified oligonucleotides and their RNA complement. All amide-3 modifications, except the parent amide-3 compound **1** in one sequence (entry 1), show a negative influence of the additional substituent on the thermal stability of these duplexes. Nevertheless the degree of destabilization of these derivatives shows a good correlation with the size and with the hydrophobic properties of the internucleosidic moiety. Surprisingly, the N-phenyl substituent does not influence the duplex stability to a greater extent (entry 2). Its effect is comparable to the glycol substituent (entry 3), which was synthesized for comparison. The increase in size by "combining" the two substituents (entry 4) results in a severe destabilization of the duplexes. In this series, the N-(p-octyl)phenyl substituent (entry 5) displays the greatest hydrophobic effect which results in the highest destabilization of the duplexes measured. A steric contribution of the substituents pointing towards the minor groove might be present as reflected by the higher destabilization observed for **8c** (entry 4) compared to the combined effects of **8a** and **8b** (entries 2 and 3, respectively). A molecular modeling study [2] suggests that the N-H bond of amide-3 **1** points towards the minor groove of the duplex. The exclusion of water from the minor groove which stabilizes the duplex

correlates well with the size of the N-substituent and with its ability to form hydrogen bonds or to complex cations.

In conclusion, we have shown that the stability of duplexes formed between a modified oligonucleotide and its RNA complement is influenced by the nature of substituents which do not directly affect the conformation of the backbone and the sugar puckering of the modified strand in the duplex. The nature and the size of these substituents determine the degree of solvation by water and the complexation by counterions, most probably in the minor groove. Although the duplex stability is, so far, only negatively influenced, these substituents might have a positive effect on the cell permeation of modified oligonucleotides. Experiments in this direction are currently in progress.

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