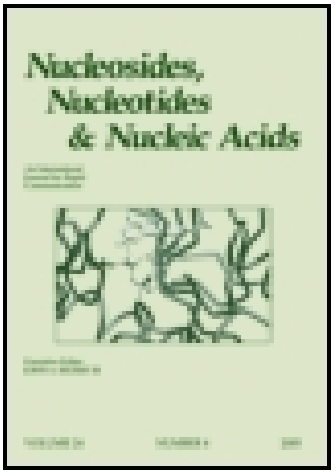

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Evaluation of Oligonucleotides with Novel Modifications

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EVALUATION OF OLIGONUCLEOTIDES WITH NOVEL MODIFICATIONS.

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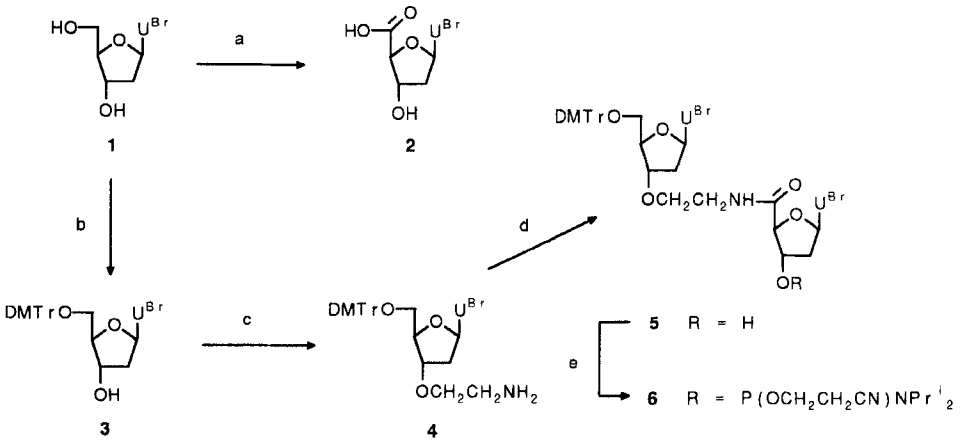
Abstract: Oligodeoxynucleotides modified with carboxamide linked dimeric nucleotides and an acyclic nucleoside were prepared and investigated for their hybridization properties toward DNA.

Modified oligonucleotides are promising new therapeutic agents and have stimulated much research in order to develop oligonucleotides which are nuclease resistant while retaining appropriate affinity and specificity.[1,2] We recently discovered that oligodeoxynucleotides modified with a dimeric nucleotide with a 5-atom carboxamide linker connecting two thymidine moieties showed good hybridization toward complementary DNA.[3] The hybridization properties expressed as the change of the melting point (T_m) relative to an unmodified sequence showed a lowering of T_m of 2-2.5 °C for incorporation of one modification and 1.5 °C / modification for incorporation of two and caused complete inhibition of exonuclease III cleavages.[3] These results promted us to further investigate this 5-atom carboxamide linker.

Using the same chemistry as for the T*T dimer[3] we prepared a U^{Br}*U^{Br} dimer (scheme I) and incorporated it 1-2 times in the middle of a 14-mer homo-pyrimidine oligodeoxynucleotide with thymidine and 2'-deoxycytidine replaced by 5-bromo-2'-deoxyuridine and 5-methyl-2'-deoxycytidine, respectively. Hybridization studies under physiological conditions gave similar results as for the T*T dimer but with increased T_m's (app. 11 °C) caused by the substitution of thymidine and 2'-deoxycytidine with 5-bromo-2'-deoxyuridine and 5-methyl-2'-deoxycytidine, respectively.[4] These preliminary results indicate



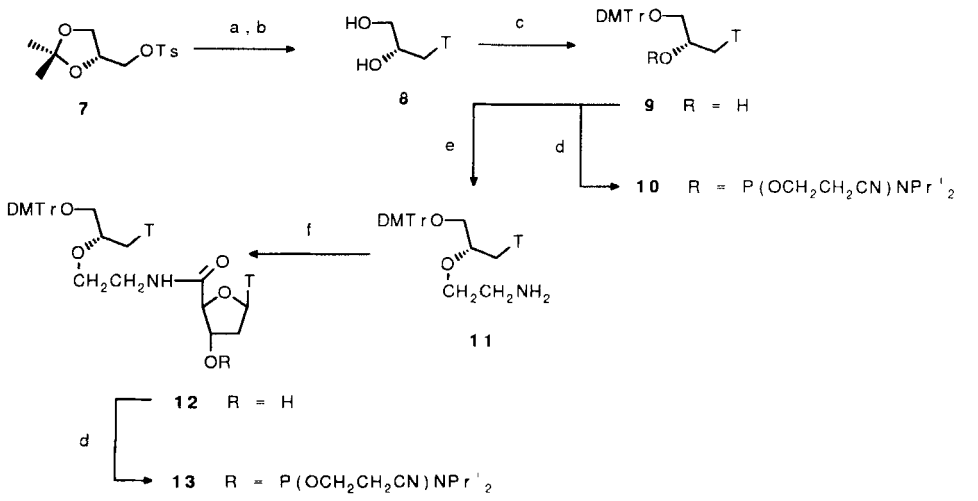
a) Pt/O_2, H_2O, b) DMTrCl, pyridine, c) $ClCH_2CH_2NH_2 \cdot HCl$, OH^- (excess), benzene/dioxane, 70 °C d) **2**, DPPA, Et_3N, DMF, e) $NCCH_2CH_2OP(Cl)NPr^i_2$, CH_2Cl_2, $EtNPr^i_2$. U^{Br} = 5-bromouracil-1-yl.

Scheme I

that good hybridization can be obtained by incorporating a $U^{Br}*U^{Br}$ dimer in both ends of an oligonucleotide while retaining stability toward exonuclease cleavages.

Oligonucleotides modified with acyclic nucleoside units have previously been shown to have good enzymatic stability but with unsatisfactory hybridization properties (a decrease in T_m of app. 6-13 °C / middle-modification).[5] In order to take advantage of the improved enzymatic stability, we prepared and incorporated an acyclic nucleoside and a mixed dimeric nucleotide consisting of both an acyclic and a cyclic part. The acyclic nucleoside and the dimeric nucleotide were choosen so they had less flexible structures because we believe that the somewhat poor hybridization properties of the acyclic oligonucleotide analogues obtained so far is due to their high flexibility.

Reaction of (R)-(-)-2,2-dimethyl-1,3-dioxolane-4-ylmethyl p-toluenesulfonate (**7**) with thymine followed by deprotection of the hydroxyl groups with acid and protection of the primary hydroxyl group with 4,4'-dimethoxytrityl chloride gave compound **9**. Compound **9** was alkylated with 2-chloroethylamine, connected through a carboxamide bond with 1,2-dideoxy-1-thyminyl-β-D-*erythro*-pentofuranoic acid using diphenyl phosphorazidate (DPPA) as the condensing reagent to give the mixed dimer **12**. Compound **9** and **12** were converted to their corresponding phosphoramidites **10** and **13**, respectively (scheme II).



a) Sodium salt of thymine, DMF, 100 °C, b) 80 % HOAc, reflux, c) DMTrCl, pyridine, d) $NCCH_2CH_2OP(Cl)NPr^i_2$, CH_2Cl_2, $EtNPr^i_2$, e) $ClCH_2CH_2NH_2 \cdot HCl$, OH^- (excess), benzene/dioxane, 70 °C, f) 1,2-dideoxy-1-thyminyl-β-D-*erythro*-pentofuranoic acid, DPPA, Et_3N, DMF. T = thymin-1-yl.

Scheme II

Incorporation of compound **10** in the middle of 17-mer oligodeoxynucleotides resulted in a decrease in T_m of app. 8 °C / modification but with only a decrease of app. 2-3 °C / modification for incorporation in one or both ends.[4] Stability toward SVPDE (3'-exonuclease) showed a large increase in half-life for 3'-end modified sequences while modifications in the middle apparently had no effect. However, the enzymatic hypochromicities calculated for the middle-modified analouges are smaller than the unmodified, indicating rapid 3'-exonucleolytic degradation until enzymatic stability is induced by the acyclic unit. Incorporation of the mixed dimer **13** in the middle of 14-mer oligodeoxynucleotides resulted in a larger decrease in T_m of app. 11-12 °C while one incorporation at the 3'-end gave a decrease of app. 5 °C.[4] To investigate if the hybridization properties could be improved, we incorporated both the acyclic monomer **10** and the mixed dimer **13** in the same sequence. In these cases there was a decrease in T_m of app. 19.5-23 °C for incorporation of one **10** and one **13**.[4] The best results were obtained by incorporation of **10** just after incorporation of **13**. Exchanging the position

of **10** and **13** gave a slightly decrease in T_m, while a greater decrease were observed when **10** and **13** were not incorporated just after each other. Conclusively, the hybridization properties obtained for the oligodeoxynucleotides modified with these novel acyclic thymidine-analogues are similar to previous results.[5]

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4) The melting experiments were carried out in a medium salt buffer, 1 mM EDTA, 20 mM Na_2HPO_4, 140 mM NaCl, pH 7.2. The increase in absorbance at 260 nm as a function of time was recorded while the temperature was raised lineary from 10-80 °C with a rate of 1 °C per minute.

5) Nielsen, P.; Kirpekar, F.; Wengel, J. *Nucleic Acids Res.* **1994**, *22*, 703 and references therein.