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Replacement of the Phosphodiester Linkage in Oligonucleotides by an Acetylenic Bond: Comparison between Carbon-, Sulfur-, and Oxygen-Containing Analogs

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Abstract: Four acetylene-containing dimers were synthesized and incorporated into oligonucleotides. The hybridization properties of the modified oligonucleotides to RNA complements and their conformational analysis are reported. Copyright © 1996 Elsevier Science Ltd

In our continuing effort to understand geometric factors of different backbone modifications in antisense oligonucleotides influencing the thermodynamic stability of the duplex with RNA, we recently reported replacement of the phosphodiester linkage with non-hydrolysable functionalities, among these were amides as well as *cis*- and *trans*-olefins.[1] These backbone modifications have in common a high degree of preorganization stabilizing the duplex in some cases. Here we present the synthesis and computer modeling of acetylene containing backbone modifications (**Figure 1**). Besides a very good fit into molecular models of DNA-RNA duplexes, these backbone replacements should also have a very high degree of preorganization since the acetylenic bond arranges four atoms in a straight line thereby reducing considerably the rotational freedom in the backbone.

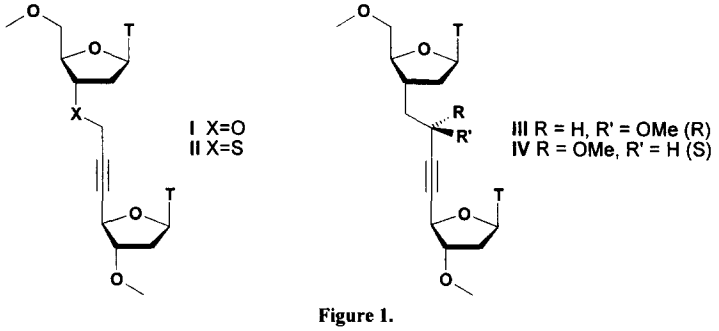
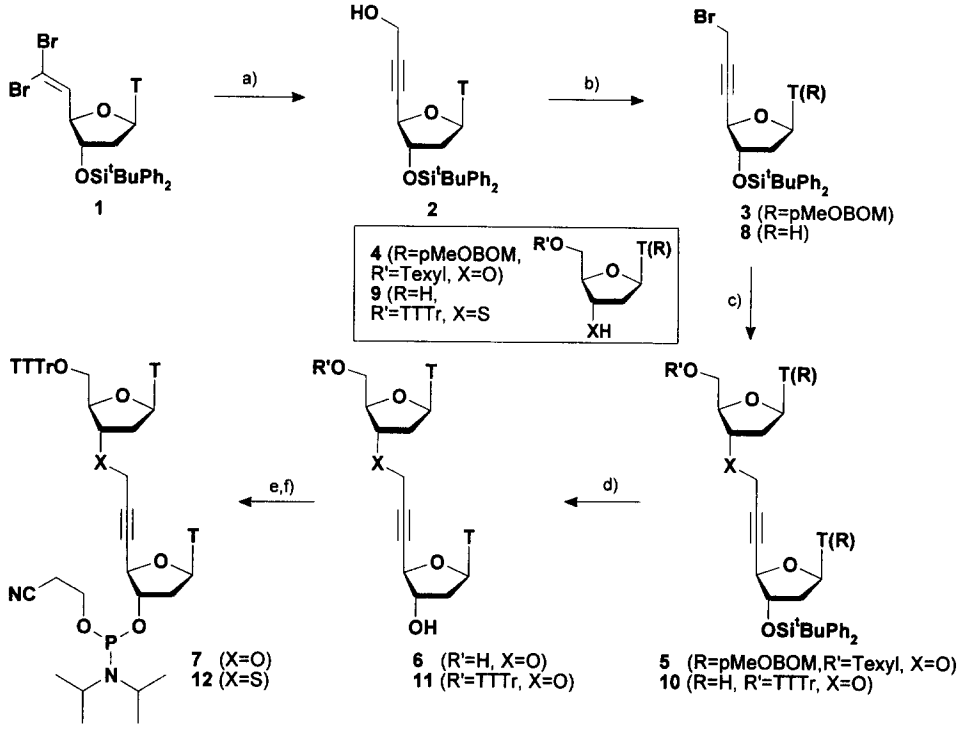
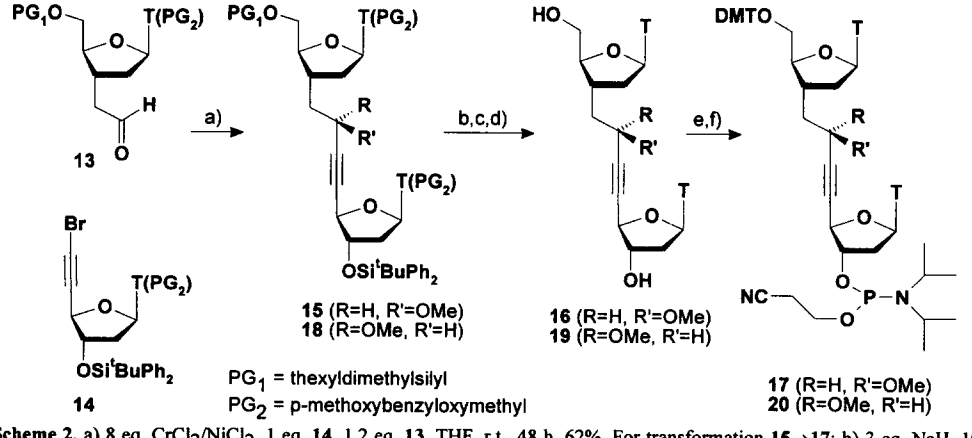


Figure 1.

The synthesis of the propargylic ether and thioether containing backbone modifications **I** and **II** is outlined in **Scheme 1**.[2] Both syntheses followed the same strategy and started with the vinylic dibromide **1**.[3] Treatment of **1** with excess *n*-butyl lithium resulted in the acetylenic anion, which, upon quenching with paraformaldehyde, gave propargyl alcohol **2**. For the synthesis of the propargyl ether we selectively protected the thymidine base and converted the alcohol to the corresponding bromide **3**. Ether formation between **3** and **4** proceeded in 37% yield to give **5**. Cleavage of the p-methoxybenzyloxymethyl group of the thymidines with DDQ, desilylation (TBAF), selective tritylation[4] of the primary alcohol and conversion of the secondary alcohol to the phosphoramidite resulted in **7**. For the synthesis of the propargylic thioether **12** we avoided



Scheme 1. a) 3.6 eq. BuLi, 3.0 eq. $(CH_2O)_n$, -78°→25°C, 60%. For transformation **2**→**7**: b) i: 2.0 eq DBU, 1.6 eq. pMeOBOMCl, DMF, 0°C, 3.5 h, 85%. ii: 1.5 eq. CBr_4, 2.0 eq. Ph_3P, CH_2Cl_2, 59-65%. c) 1.1 eq. NaH, 0.2 eq. nBu_4NI, **4**, THF, r.t., 3 h, 37%. d) i: 4.2 eq. DDQ, CH_2Cl_2/H_2O (20:1), r.t. 1.5 h, 86%. ii: 2.5 eq. TBAF, THF, r.t. 4 h, 89%. e) 2.5 eq. TTTrCl, 4.0 eq. Et_3N, pyridine, 60°C, 22 h, 84%. f) 3eq. $((iPr_2)N)_2POCH_2CH_2CN$, 5 eq. $(iPr)_2NH_2^+$tetrazole$^-$, CH_2Cl_2, r.t., 1.5 h, 88%. For transformation **2**→**12**: b) 1.5 eq. CBr_4, 2 eq. Ph_3P, CH_2Cl_2, 0°C, 1 h, 71%. b) 1 eq. **8**, 1 eq. **9**, 0.5 eq. 18-crown-6, Et_2O, $NaHCO_3$(aq), 12 h, 28-51%. c) 1.5 eq. TBAF, THF, r.t., 0.5 h, 77%. c) 3eq. $((iPr_2)N)_2POCH_2CH_2CN$, 5 eq. $(iPr)_2NH_2^+$tetrazole$^-$, CH_2Cl_2, 83-96%.



Scheme 2. a) 8 eq. $CrCl_2/NiCl_2$, 1 eq. **14**, 1.2 eq. **13**, THF, r.t., 48 h, 62%. For transformation **15**→**17**: b) 3 eq. NaH, 10 eq. MeI, THF, 0°C→r.t, 3 h, 95%. c) 3.5 eq. DDQ, CH_2Cl_2, H_2O (20:1), r.t., 17 h, 54%. d) 2.3 eq. TBAF, THF, r.t., 2.5 h, 100%. e) 1.7 eq. DMTCl, pyr. r.t., 18 h, 71%. f) 3.0 eq. $((iPr_2)N)_2POCH_2CH_2CN$, 5 eq. $(iPr)_2NH_2^+$tetrazole$^-$, CH_2Cl_2, r.t., 2h, 90%. For transformation **19**→**20**: b) 88%. c) 83%. d) 99%. e) 51%. f) 76%.

protection of the thymidine base, since selective thioether formation could be accomplished under much milder condition without danger of thymidine alkylation. Thioether formation between **8** and **9**[5] proceeded with up to 51%. The use of the TTTr-protected[4] monomer **9** in the coupling reaction with **8** shortened the synthesis since protection group manipulation could be avoided.

The synthesis of the carbon analogs **III** and **IV** is depicted in **Scheme 2**. The $CrCl_2/NiCl_2$ mediated coupling between acetylenic bromide **13** and aldehyde **14** resulted in clean formation of a 1:1 diastereomeric mixture of **15** and **18** in 62% yield.[3,6] The alcohols **15** and **18** were separated by flash chromatography. The stereochemistry of compounds **15** and **18** was tentatively assigned based on the chemical shifts of C(3')-CH_2 's of the corresponding methylmandelate esters as described by Trost.[7] Methylation of the separated propargylic alcohol **15** and **18**, protection group manipulation, and formation of the phosphoramidite resulted in compounds **17** and **20**, respectively.

The phosphoramidites **7**, **12**, **17**, and **20** were incorporated into oligonucleotides[8] and the melting temperatures of the duplexes with their RNA complements were determined (4 μM each strand, 10 mM phosphate pH 7.0 (Na salts) 100 mM total Na^+, 0.1 mM EDTA) as summarized in **Table 1**.[1b]

Table 1. ΔT_m/mod (°C) for the backbone modifications I-IV.

Sequence		I	II	III	IV
1) TTTt-t$(CT)_5$	RNA	-1.8	-4.6	-3.6	-3.1
2) GCG(t-t)$_5$GCG	RNA	-3.1		-0.5	-2.3

In agreement with our preliminary conformational analysis, the four propargylic backbone modifications **I-IV** can be incorporated into duplexes with RNA, as single modifications (sequence 1) as well as alternating with phosphodiester bonds (sequence 2). Single replacement of a phosphodiester bond led to less severe destabilization of the duplex with modification **I**, where the upper sugar is linked to the propargylic residue through an oxygen atom. The largest T_m depression was measured for the sulfur containing backbone **II**. A different trend was observed with sequence 2, where five backbone replacements were introduced. Most interestingly, the modification **III**, tentatively assigned as the (R)-isomer, led to a small depression of the T_m of the duplex (ΔT_m/mod. = -0.5°C). In contrast, its (S)-epimer showed a pronounced destabilization (ΔT_m/mod. = -2.3°C).

Molecular mechanics (MM) and dynamics (MD) studies were carried out according to the scheme already used for all amide and double-bond modifications,[1,9] using AMBER force field.[10] The lowest-energy conformation for **I** is depicted in **Figure 2**. Another distinct conformation was found to be higher by more than 5 kcal·mol^{-1} (based on the energy of the entire duplex). This conformer was not considered for further investigation. A 14*mer* duplex with five alternating backbone modifications (all in the starting conformation shown in **Figure 2**) was used for molecular dynamics studies. The DNA-modified duplex was stable over the entire MD trajectory. The puckering behavior of the *i* and *i+1* residues (see **Figure 2**) was different, as also observed for other backbone modifications studied before.[1,9] The sugars of residues having the modified backbone portion attached to C3' (*i* residues) tend to prefer a C3'-endo puckering mode (average phase angle of pseudorotation *P* around 70°). A notable difference with respect to other "rigid" backbone modifications is the clearly preferred C2'-endo puckering of the sugar having the triple bond attached at C4', i.e., the *i+1* residue. In other modifications, these sugars generally adopted an average puckering mode close to that in unmodified RNA·DNA duplexes, i.e., $P \approx 110°$,[11] resulting from an oscillation between C3'-endo and C2'-endo. In this case, *P* averages around 150°. The triple bond, forcing four consecutive atoms in a row, clearly affects the sugar puckering to a larger extent than did the various amide or double-bond modifications. Globally however, the stable MD trajectory corroborates the experimental findings that this type of modification can be incorporated into the DNA strand of an RNA·DNA hybrid duplex without severe strain.

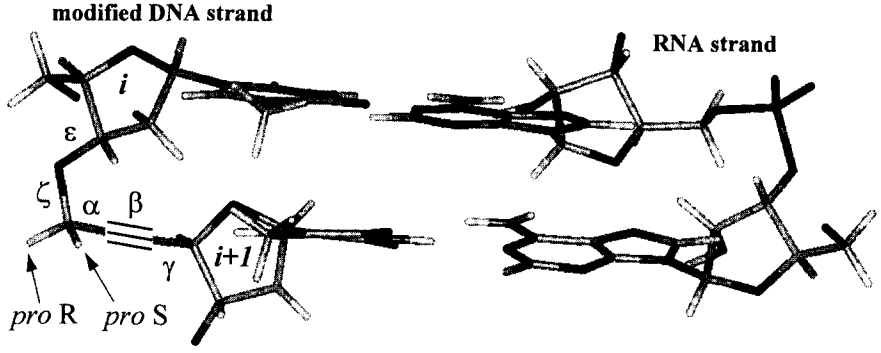


Figure 2. Lowest-energy conformation in the acetylene-modified portion (modification **I**, dimer cut out of an octamer hybrid duplex r(GA$_6$G)·d(CTTt-tTTC). The torsion angles ε and ζ assume values of -171° and -69°, respectively. Considering the sequence -CH$_2$-C≡C-C3'(*i+1*) as a pseudo bond, the torsion angle O3'(*i*)-CH$_2$-C4'(*i+1*)-C3'(*i+1*) is 157°.

Replacement of the oxygen atom by a CH_2 group resulted in a lowest energy conformation almost identical to the one shown in **Figure 2**. Substitution of the *pro-S* hydrogen by an OMe-group would result in 1,3-diaxial interactions with H-C(4') (***i*** residues), therefore destabilizing the duplex. However, no such interactions occur if *pro-R* hydrogen is replaced with a OMe-group. Provided the assignment of the stereochemistry of the two diastereomeric propargyl ethers was correct, this analysis is in agreement with the T_m data shown in **Table 1**.

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