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Replacement of the Phosphodiester Linkage in Oligonucleotides by a C=C Double Bond: Comparison of the *cis* and *trans* Isomers

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Abstract: The two olefinic backbone replacements **III** and **IV** were synthesized and incorporated into oligonucleotides. The hybridization properties of the modified oligonucleotides to DNA and RNA complements and conformational analyses of **III** and **IV** are compared to the corresponding amide modifications **I** and **II**.

Recently we reported replacement of the phosphodiester linkage in oligonucleotides with a number of amide isomers, the two most promising **I** and **II** being recalled in **Figure 1**.[1] Detailed molecular mechanics studies of those two modifications predicted the *trans* conformation of the amide bond to be favored slightly over the *cis* conformation. We assumed this preference to be responsible for a favorable preorganisation of the amide-modified backbone explaining the observed duplex stabilization compared to natural phosphodiester backbones (from various different RNA sequences: average ΔT_m/mod = 0.4°C for **I**, 0.0°C for **II**).

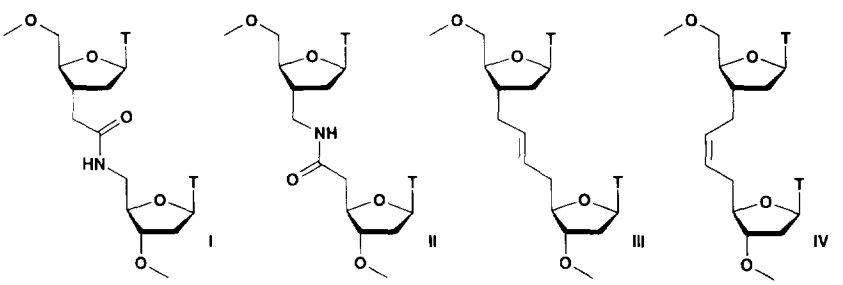


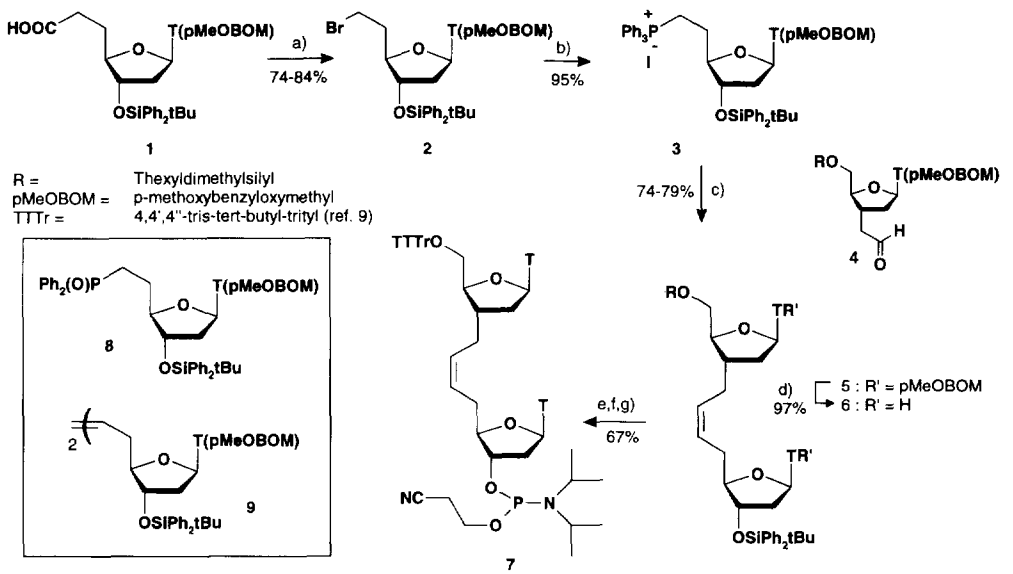
Figure 1.

In this context, the two olefinic backbone replacements **III** and **IV** could be considered as analogs of amides **I** and **II** with either a fixed *trans* or *cis* geometry. Based on the apparent geometrical preference of the amides **I** and **II** and the observed duplex stabilization of these modifications, we expected the *trans* olefin **III** to result in favorable hybridization properties. In this paper we report the synthesis, molecular mechanics and dynamics analysis, and hybridization properties of backbone modifications **III** and **IV**.[2]

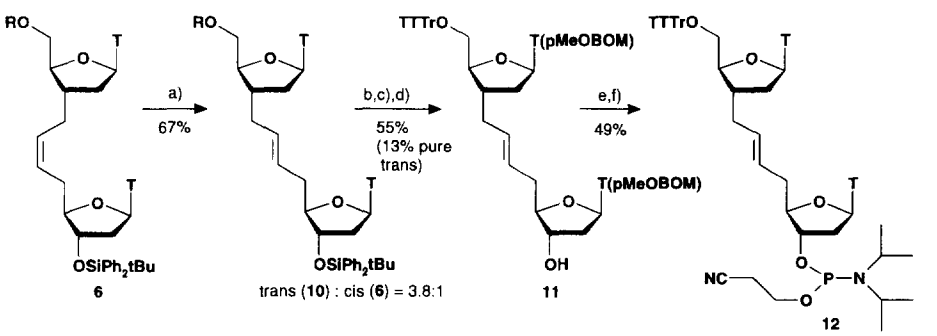
The synthesis of the *cis* olefin **7** is summarized in **Scheme 1**. Light induced decarboxylation of **1**[3] proceeded smoothly to give bromide **2** which was converted to the phosphonium salt **3** in almost quantitative yield.[4] Initial attempts to perform the *Wittig* condensation of **3** with aldehyde **4**[5] failed and only the corresponding phosphine-oxides **8** could be isolated. This unwanted reaction was particularly efficient when **3** was treated with $NaN(TMS)_2$ in THF/HMPA (4:1) at -78°C. Such hydrolyses of phosphonium salts to the corresponding phosphine-oxides are known but require usually much more vigorous conditions.[6] We postulate that **3** formed a tight hydrate, with a water molecule bound in a way favoring phosphine-oxide formation upon base treatment.

A rigorously dried (0.1 mbar, 130°C, 3 days) sample of **3** treated with $NaN(TMS)_2$ for 0.5h followed by the addition of 1 eq. of **4** resulted in no formation of phosphine oxide **8**, but, to our surprise, dimeric

structure **9** (**Figure 2**) was obtained.[7] Only when the ylide derived from **3** was treated within two minutes with aldehyde **4** at once the *Wittig* product could be obtained in good yield and formation of previously observed side products could be minimized.[8] Only the *cis* olefinic compound was detected under these conditions. Deprotection of the pMeOBOM protection group with DDQ (without interference of the double bond), followed by established chemistry resulted in **7**.[1,9]



Scheme 1. a) 1.1 eq. $Me_2C{=}CCl(NMe_2)$, $(ClCH_2)_2$, 0°C, 1.0h, then 1.1 eq. 2-mercaptopyridine 1-oxide Na^+ salt, 0°C, 0.5h, then 10 eq. CCl_3Br, *hν*, 5 min; b) 2 eq. Ph_3P, 2 eq. NaI, CH_3CN, 65°C, 36h; c) 1 eq. **3**, 2.1 eq. $NaN(TMS)_2$, THF, -78°C, 1-2 min, then 1 eq. **4**, -78°C, 1h, r.t., 0.3h; d) 2.7 eq. DDQ, CH_2Cl_2/H_2O (10:1), r.t., 3h; e) **6**, 2.3 eq. TBAF, THF, 50°C, 2h. f) 2.5 eq. TTTrCl, 3.5 eq. Et_3N, pyridine, 50°C, 16h; g) 3.0 eq. $((iPr_2)N)_2POCH_2CH_2CN$, 5 eq. $(iPr)_2NH_2^+$tetrazole$^-$, CH_2Cl_2, r.t., 2h.



Scheme 2. a) 6 x 0.5 eq. PhSH, 6 x 0.15 eq. AIBN, PhH, 6 x 3h, 80 °C; b) 2.3 eq. TBAF, THF, r.t., 5h; c) 2.5 eq. TTTrCl, 3 eq. Et_3N, pyridine, 60°C, 5h; d) 5.0 eq. DBU, 3 eq. pMeOBOMCl, DMF; separation of *cis* and *trans* isomers by $AgNO_3$-SiO_2 chromatography; e) 6 eq. DDQ, CH_2Cl_2, H_2O (10:1); f) 3 eq. $((iPr_2)N)_2POCH_2CH_2CN$, 5 eq. $(iPr)_2NH_2^+$tetrazole$^-$, CH_2Cl_2, r.t., 2h.

In order to obtain the trans-olefin **12**, isomerization of *cis* alkene **6** was accomplished using thiophenol and AIBN as a radical initiator resulting in a 3.8:1 mixture of the *trans* and *cis* alkene **10** and **6** (exposing **5** to those conditions resulted in partial cleavage of the pMeBOM groups).[10,11] Separation of the isomers at this stage was not possible. Thus, the mixture was deprotected and tritylated (steps b,c **Scheme 2**). Only after

protection of the thymine with pMeOBOM the two isomers could be separated by flash chromatography on silver nitrate impregnated silica-gel. Treatment of **11** with DDQ resulted in selective removal of the pMeBOM-protecting groups without substantial cleavage of the TTTr-group. With prolonged reaction time, cleavage of the TTTr-group was, indeed, observed. Conversion to the phosphoramidite **12** was accomplished as previously for the amides **I** and **II** .[1]

Both phosphoramidites **7** and **12** were incorporated into oligonucleotides[12] and the melting temperatures of the duplexes with their RNA and DNA 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]

When measured with complementary RNA strands, mono-modified oligonucleotides (entry 1, 2 **Table 1**) resulted in ΔT_m/mod of ca. -1°C for both, the *cis* and the *trans* olefin, the corresponding fully modified oligonucleotides (entry 3, 4 **Table 1**) resulted in a ΔT_m/mod = -0.6°C for the *trans* olefin and a ΔT_m/mod = -1.3°C for the *cis* olefin. Both, the *cis* and the *trans* olefin showed much lower T_m's with complementary DNA targets (ΔT_m/mod = -2.3°C (*cis*); -3.0°C (*trans*)). Even though this data suggests that the *trans* olefin-backbone modification **III** forms a slightly more stable duplexes with RNA, the difference to the corresponding *cis* olefinic substitution **IV** is surprisingly small. The amide **I** (**Figure 1**) (as amides in general) prefers the *trans*-conformation. However, other modifications mimicking the *cis*-olefin now have to be considered as potential backbone-replacements as well. Nevertheless, the double bond analogs decrease the stability of the duplex compared to the amides **I**, **II**, possibly due to their hydrophobic properties.

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

	sequence		I	II	III	IV
1	TTTt-t$(CT)_5$	RNA	+ 0.4	- 0.8	- 1.0	- 1.1
2		DNA	- 1.7			
3	GTG(t-t)$_5$GCG	RNA	- 0.1	+0.4	- 0.6	- 1.3
4		DNA	- 1.5	- 1.4	- 3.0	- 2.3

Conformational analysis and molecular dynamics simulations have been carried out with settings identical to those in the work on amide-modified oligodeoxynucleotides,[1b, 3, 13] using the AMBER force field[14a] as incorporated in the package InsightII(2.3.5) and DISCOVER(2.9) of BIOSYM Technologies, San Diego, with adjusted parameters added for the C=C double bond environment.[14b]

The conformational analysis was carried out on octamer hybrid duplexes with one C=C modification in the middle of the DNA strand, r(GA_6G)·d(CTTt-tTTC). The lowest-energy conformers for *trans* and *cis* are shown in **Figure 2**. They were found to be identical in energy (comparing the energies of the entire octamer duplex after complete minimization). Torsion angles are close to those found for the respective amide modifications **I** and **II**.[13] The major disparity with the amide modifications resides in the intrinsic energy difference between the *trans* and the *cis* peptide bond, which fades for the double bond.

Molecular dynamics (MD) were carried out on alternatingly modified 14*mer* hybrid duplexes r(GA_{12}G)·d(CT(t-t)$_5$TC). **Figure 2** depicts the average dynamics structures, starting with the lowest-energy geometries for the *trans* and the *cis* C=C modifications. Both modifications yield stable duplexes with standard Watson-Crick base pairing. All results globally parallel those of the amide modifications. Although no persisting conformational transitions have been observed on the 100 picoseconds time scale, in contrast to the amides (especially **II**), the influence of the backbone conformation on the sugar puckering was found to be similar. For the lowest-energy *trans* C=C modification, sugars attached to the C=C modification with C3' (residues *i*)[13] display an average phase angle of pseudorotation[15] *P* of 68° while the residues with *trans* C=C attached to C5' (residues ***i+1***)[13] adopt an average *P* value of 97°. The corresponding *P* values for the lowest-energy *cis* C=C modification are 91° and 99°, respectively. In all simulations the RNA strand remained unaffected by the modified DNA strand.[15] The MD analysis of a mixed alternating sequence with three *trans* and two *cis* C=C segments confirmed that both modifications can be incorporated into the same DNA strand.

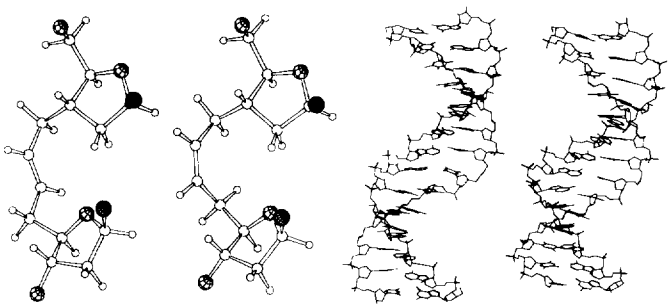


Figure 2. Lowest-energy conformations for *trans* and *cis* C=C modified backbone (only N1 of bases shown for clarity) and MD average structures (without hydrogens) of 14*mer* hybrid duplexes with alternatingly modified DNA strands (left with *trans*, right with *cis* C=C modifications, C5'-end of DNA strands at top left).

We demonstrated that both, *cis* and *trans* backbones **III, IV** are compatible with a DNA:RNA duplex structure. Consequently, both rotamers of amides **I, II** are suitable for the formation of a stable duplex. This is of special importance in the case of N-substituted amides were both rotamers are present.[1b] However, other factors such as solubility of the backbone might be also a critical issue.

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