DOI: 10.1002/ejoc.200500395

Consequences of *P*-Chirality in Chimeric 2′-*O*-Methyloligoribonucleotides with Stereoregular Methylphosphonothioate Linkages

Lucyna A. Wozniak,*[a] Magdalena Janicka,[a] and Małgorzata Bukowiecka-Matusiak[a]

Dedicated to Professor Wojciech J. Stec on the occasion of his 65th birthday

Keywords: Chimeric oligonucleotides / Chirality / Conformational analysis / Circular dichroism / Nucleic acids / Thermodynamic stability

Stereoregular chimeric oligonucleotides modified with diastereomerically pure methylphosphonothioate linkages interact with complementary DNA or RNA templates in a stereodependent manner. There is an unprecedented large difference in the stability of duplexes of oligonucleotides modified with alternating methylphosphonothioate linkages (ΔT_m = 34 °C) that is dependent only on the stereochemistry of the methylphosphonothioate phosphorus centres. The nature of these interactions was studied by circular dichroism and NMR spectroscopy, and a thermodynamic analysis was performed. The collected data indicate that the absolute configuration at the *P*-chiral methylphosphonothioate linkages in chimeric 2′-*O*-methylribonucleotides could, in some cases, determine the global conformation of single–strand oligonucleotides and constrain the conformation of hybrid duplexes formed with RNA.

Introduction

There is an increasing body of data that demonstrates the broad applicability of synthetically modified oligonucleotides that have altered internucleotide linkages.[1–3] Such oligonucleotides have proven to be suitable tools to study the consequences of sequence-specific inhibition of gene expression,[4,5] including potential therapeutic (antisense or antigene strategies) applications,[6] or the mechanisms of the action of rybozymes.[7] It was recently demonstrated that modified oligonucleotides can also deliver precise information on protein functions and interactions with DNA provided that detailed information on their intrinsic structure is available.[8–11] The physicochemical and biological properties of modified oligonucleotides can be widely tuned since numerous procedures have been elaborated that enable various modifications to internucleotide phosphodiester bonds, sugar or base moieties.[12]

Modification of the internucleotide phosphorus centre allows the synthesis of oligonucleotides with enhanced affinity towards complementary templates, increased enzymatic stability, and improved membrane permeability.[13] The chimeric constructs consisting of various mixed internucleotide backbones, including those with diastereomerically pure *P*-chiral non-ionic modifications, are of special interest.[14,15] It has previously been established that *P*-chiral oligonucleotide methylphosphonates (*Oligo-MePO*) (**1**) interact with complementary DNA targets in a highly stereodependent manner. The profound effect of the *P*-chirality of methylphosphonates incorporated into *Oligo-MePO* on their physicochemical properties was first described by Miller et al.[16] and further confirmed by Lesnikowski and co-workers[17–19] in their studies following the first stereocontrolled synthesis of *Oligo-MePO*. Recently chimeric methylphosphonate oligonucleotides were successfully used to study the structures of the active sites and the mechanisms of the action of enzymes in detail.[20] Hecht and co-workers not only identified key determinants of the stability of enzyme–substrate complexes but demonstrated that the introduction of (S_P)-methylphosphonate linkages at the cleavage site could transform topoisomerase IB into a potent endonuclease.[21] Kurpiewski et al. used chimeric oligonucleotides modified both with diastereomerically pure (R_P)- and (S_P)-phosphorothioates and methylphosphonates to revise the mechanisms for the coupling between DNA recognition specificity and the catalysis in EcoRI endonuclease.[22] These studies allowed the roles of individual phosphoryl oxygen atoms in catalysis to be delineated and led to the conclusion that a "crosstalk ring" in the complex couples recognition to catalysis and connects the two catalytic sites to each other.

[a] Centre of Molecular and Macromolecular Studies,
Department of Bioorganic Chemistry,
Polish Academy of Sciences,
Sienkiewicza 112, 90-363 Lodz, Poland
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

Recently, we reported the large-scale synthesis of dinucleoside (3′,5′)-methylphosphonothioates and their separation into diastereomerically pure isomers,[23,24] which could be considered as substrates for *P*-chiral dimeric building blocks in the synthesis of chimeric oligonucleotides (*Oligo-MePS*) of predetermined *P*-chirality.[25] We demonstrated that *Oligo-MePS*,[26] considered as isosteric analogues of *Oligo-MePO*,[27] exhibit stereodependent binding properties towards complementary DNA or RNA targets. During our studies on the stereocontrolled synthesis of *Oligo-MePO*[28,29] we also evaluated stereospecific methods for *PS* → *PO* oxidation reactions[30] allowing conversion of *Oligo-MePS* to *Oligo-MePO*.

Results and Discussion

We now report the synthesis of chimeric 2′-*O*-methyloligoribonucleotides (*Oligo-MePS*, **2**) with diastereomerically pure dinucleoside (3′,5′)-methylphosphonothioates (**3**) of a predefined absolute configuration incorporated at the phosphonothioate centre. and the properties of hybrid duplexes formed with complementary DNA and RNA targets. It is known that the incorporation of particular electron-withdrawing (e.g. *O*-alkyl, *O*-alkoxyalkyl) 2′-substituents can augment the RNA-like C3′-*endo* sugar conformation of oligoribonucleotides, the modified oligomers pre-organising into the A conformation adopted by RNA duplexes.[31–34] Such modifications generally lead to a considerable increase in the stability of heteroduplexes with RNA, which is specially required for efficient antisense activity (steric blocking).[35] The highly enhanced plasma stability and the increased in vitro potency of entirely 2′-*O*-methyl-modified siRNA[36] and their potential as valuable tools to study siRNA mechanisms has also been demonstrated.[37]

For the model structural studies we designed several homopyrimidine chimeric 2′-*O*-methyloligoribonucleotides (**2**) modified with increasing numbers of methylphosphonothioate internucleotide bonds. The unusual differences in the affinity of stereoregular chimeric 2′-*O*-methyloligoribonucleotides towards DNA and RNA templates is discussed in the context of the predominant conformations of these hybrid duplexes and possible different solvation patterns.[38,39]

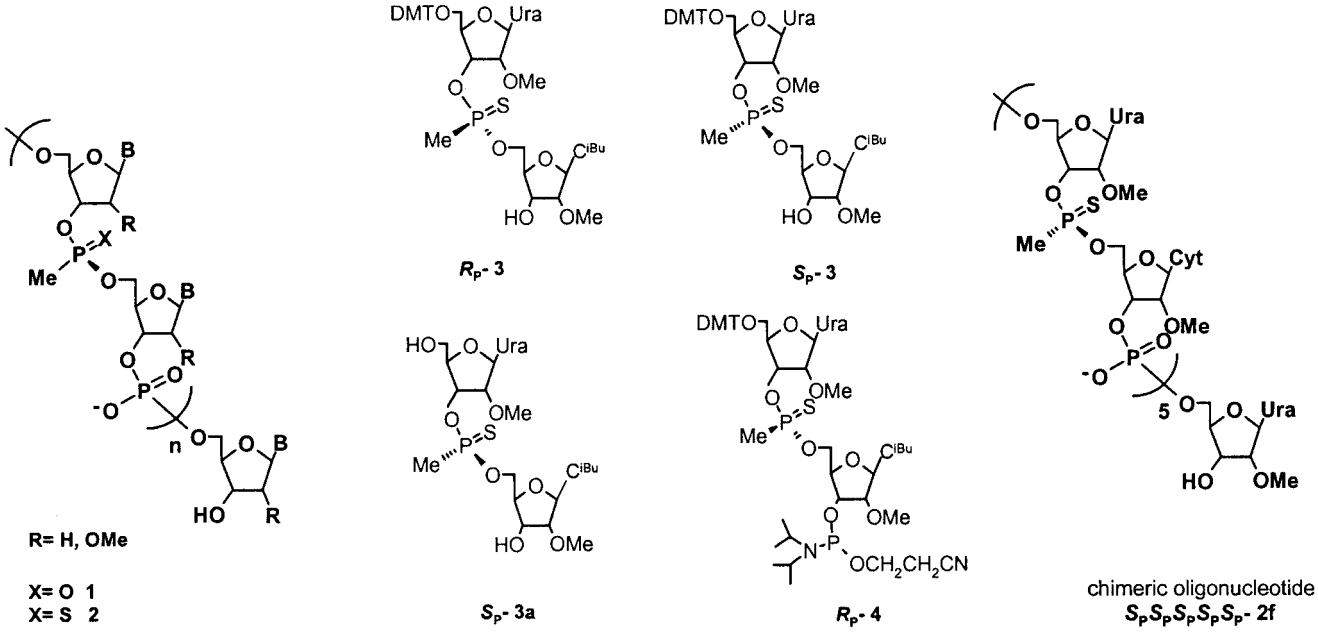
Synthesis

The dinucleoside (3′,5′)-methylphosphonothioate dimeric building blocks (**4**) were prepared from the corresponding dinucleotides ($U_{MePS}C)_{2'\text{-OMe}}$ (**3**) in good yield (61%) using the triazolidite one-pot method described previously (Scheme 1).[25,40]

The assignment of the (S_P) and (R_P) absolute configurations to FAST- and SLOW-eluting isomers **3**, respectively, by means of X-ray analysis[23] and ^{1}H ROESY NMR spectroscopy has previously been reported.[24]

The diastereomerically pure isomers **3** were 3′-*O*-phosphitylated with $(CNCH_2CH_2O)(iPr_2N)_2P$/1*H*-tetrazole in MeCN to give (R_P)- or (S_P)-5′-*O*-DMT-[2′-OMe-uridin-3′-yl (3′,5′)-5′-*O*-2′-OMe-cytidine methylphosphonothioate]-3′-*O*-(β-cyanoethyl)-*N*,*N*-diisopropylphosphoramidites (**4**) and used as dimeric building blocks for condensation by the phosphoramidite method on a solid support (Table 1).

Modified chimeric oligonucleotides **2**, the phosphodiesters d$(AG)_5$A (**9**) and r$(AG)_5$A (**10**) were prepared as complementary templates and the isosequential phosphodiesters d$(TC)_5$T (**11**) and (2′-OMe)$(UC)_5$U (**12**) used as reference oligomers (Table 1) were prepared on a 1 μmol scale. Chimeric oligonucleotides with a single dinucleoside (3′,5′)-



Scheme 1.

Table 1. MALDI-TOF MS analysis of chimeric 2′-*O*-methyloligoribonucleotides **2** and **13** and reference oligomeric phosphodiesters **9**–**12**.

Oligonucleotide		*m/z*[a]
5′-r(U_{MePS}C UC UC UC U_{MePS}C U)$_{2'\text{-OMe}}$-3′	(R_P,R_P)-**2a**	3479 (3478)
5′-r(U_{MePS}C UC UC UC U_{MePS}C U)$_{2'\text{-OMe}}$-3′	(S_P,S_P-**2b**	3481 (3478)
5′-r(UC UC U_{MePS}C UC UC U)$_{2'\text{-OMe}}$-3′	(R_P)-**2c**	3467 (3463)
5′-r(UC UC U_{MePS}C UC UC U)$_{2'\text{-OMe}}$-3′9	(S_P)-**2d**	3466 (3463)
5′-r(U_{MePS}C U_{MePS}C U_{MePS}C U_{MePS}C U)$_{2'\text{-OMe}}$-3′	(R_P,R_P,R_P,R_P)-**2e**	3521 (3523)
5′-r(U_{MePS}C U_{MePS}C U_{MePS}C U_{MePS}C U)$_{2'\text{-OMe}}$-3′	(S_P,S_P,S_P,S_P,S_P)-**2f**	3523 (3523)
5′-d(AG·AG·AG·AG·AG·A)-3′	**9**	3459 (3460)
5′-r(AG·AG·AG·AG·AG·A)-3′	**10**	3616 (3616)
5′-d(TC TC TC TC TC T)-3′	**11**	3305 (3306)
5′-r(UC UC UC UC UC U)$_{2'\text{-OMe}}$-3′	**12**	3450 (3453)
5′-r(UC UC U_{MePO}C UC UC U)$_{2'\text{-OMe}}$-3′	Oxo-(R_P)-**13a**	3452 (3447)
5′-r(UC UC U_{MePO}C UC UC U)$_{2'\text{-OMe}}$-3′	Oxo-(S_P)-**13b**	3452 (3447)

[a] Calculated molecular weights are given in brackets.

methylphosphonate linkage [(R_P)-**13a** and (S_P)-**13b**] were prepared by stereospecific oxidation of the internucleotide methylphosphonothioate centres of the corresponding methylphosphonothioate oligonucleotides (R_P)-**2c** and (S_P)-**2d**, respectively, with Oxone®.[29]

Formation of Hybrid Duplexes with Complementary DNA and RNA Templates

Extensive studies and theoretical analyses have been focused on the factors contributing to the thermal stability of the duplexes. Hydrogen-bonding architecture, π–π base-stacking interactions and hydration networks are among the major stabilizing interactions in double-helical structures. Since duplex formation is an enthaply-driven process, it was concluded that entropy-driven hydrophobic effects are hidden by the unfavourable entropy of bond rotations. However, electrostatic or van der Waals interactions, which are enthaply-driven effects, may be more important in nucleic acids than solvent-induced interactions. Moreover, there is no unified picture as to the relative importance of solvophobic, electrostatic or van der Waals effects on the stability of hybrid duplexes of modified oligonucleotides. Modifications of the internucleotide phosphorus centre, in particular those leading to changes in lipophilic/hydrophobic properties (reduction of total negative charge), will influence the relative energies of solvation of the bases when stacked or unstacked and the surface area desolvated on stacking.[39,41]

Here we analyze the factors that contribute to the stability of hybrid duplexes formed between the chimeric oligonucleotides **2** and **13** and complementary DNA and RNA templates by CD spectroscopy and thermodynamic analysis and assume that additional information can be gained from the conformational analysis of diastereomerically pure dinucleoside (3′,5′)-methylphosphonothioate (**3a**) by ^{1}H NMR spectroscopy.

NMR Analysis of Dinucleoside (3′,5′)-Methylphosphonothioate (3a) in Water

NMR analysis of the partially protected, diastereomerically pure dinucleoside (3′,5′)-methylphosphonothioates (**3b**) has proved diagnostic for the assignment of the absolute configurations and conformations of the corresponding dinucleotides.[24] Analogously, fully deprotected (S_P)-OH-2′-OMe-uridin-3′-yl (3′,5′)-5′-*O*-2′-OMe-cytidine methylphosphonothioate (**3a**) was analyzed by ^{1}H NMR spectroscopy in order to assign the conformation of both 2′-*O*-methylribose rings in water (Figure 1).

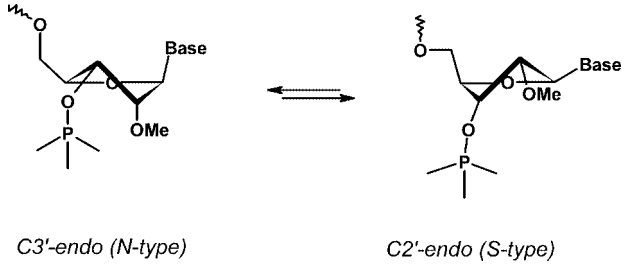


Figure 1. Representations of the *S*-type (DNA-like) and *N*-type (RNA-like) structures of the sugar conformations.

The conformation of the ribose rings was studied using a two-state model and described in terms of an equilibrium between *N* and *S* conformers in solution.[41,42] The 3′-*endo* pucker of the ribose ring is typical of A-RNA and the introduction of an electronegative 2′-*O*-methyl group leads to enhanced 3′-*endo* pucker stabilization.[34] From the ^{1}H NMR spectra (2D ROESY and NOESY, ^{31}P NMR/^{1}H NMR correlations) it became evident that unlike in crystalline (R_P)-**3b**,[23] both in organic solution and particularly in D_2O (10 mM Tris-HCl, 100 mM NaCl, and 10 mM $MgCl_2$ pH = 7.4), a considerable fraction of the uridine moiety is present as the *S* conformer with the more populated 2′-*endo* fraction observed for (S_P)-**3b** (*N*: 45% at 25 °C in $CDCl_3$). The small coupling constant ($J_{1',2'}$ = 2.9 Hz) for cytidine indicates that this sugar moiety prefers the *N* conformation, whereas a value of $J_{1',2'}$ = 5.85 Hz for the uridine moiety is indicative of the greater conformational flexibility of this moiety in solution and a significant content of the 2′-*endo* puckered conformer in dynamic equilibrium between the 2′-*endo* and 3′-*endo* conformations.[43]

The conformational analysis based on individual coupling constants $J_{1'2'}$, $J_{2'3'}$, and $J_{3'4'}$ was performed using the PSEUROT 6.2 program and confirmed that a relatively

high proportion of the uridine moiety adopts the *S* conformer (62%). These results indicate that the (S_P)-methylphosphonothioate modification of the internucleotide bond in 2′-*O*-methylribonucleotides has significantly increased the population of the *S* conformers relative to the unmodified internucleotide phosphodiester bonds in 2′-*O*-methylribonucleotides.

Conformation of Chimeric Oligonucleotides as Determined by Circular Dichroism

Although quantitative structural information cannot be gained from analysis of the CD spectra of oligonucleotides, these spectra can provide reliable characteristics of their global conformation in solution if compared with the CD spectra of reference samples.[44]

To compare the properties of the chimeric oligonucleotides with different modifications we recorded the CD spectra of single-stranded oligonucleotides and hybrid duplexes (Figure 2). The CD spectra of single-stranded chimeric oligonucleotides **2a**–**2d** and **12** are similar to the corresponding hybrid duplexes with RNA **10** in having a negative molar ellipticity of moderate magnitude at wavelengths above 220 nm, a crossover point between 245 and 249 nm, and a large positive broad band from 250 to 300 nm with a maximum at 266 nm characteristic of the C3′-*endo* pucker. In contrast, the spectrum of the reference oligodeoxynucleotide $d(TC)_5T$ **(11)** exhibits a relatively low and flat band with a maximum at 274 nm and as such is considered as a reference for the B-like (C2′-*endo*) DNA structure.[45]

From the spectra of the duplexes (Figure 2, b) we concluded that (R_P) modifications [vide (R_P,R_P)-**2a** and (R_P)-**2c**], a single (S_P) modification in (S_P)-**2d**, or the flanking modifications of (S_P,S_P)-**2b** do not cause significant changes in the total conformation of the hybrid duplexes with RNA templates. A major change is observed, however, for the hybrid duplex formed between (S_P,S_P,S_P,S_P,S_P)-**2f** and an RNA template. The positive band at 268 nm for this duplex is less intense with the crossover point shifted to 249 nm, implying an increasing distortion of the global conforma-

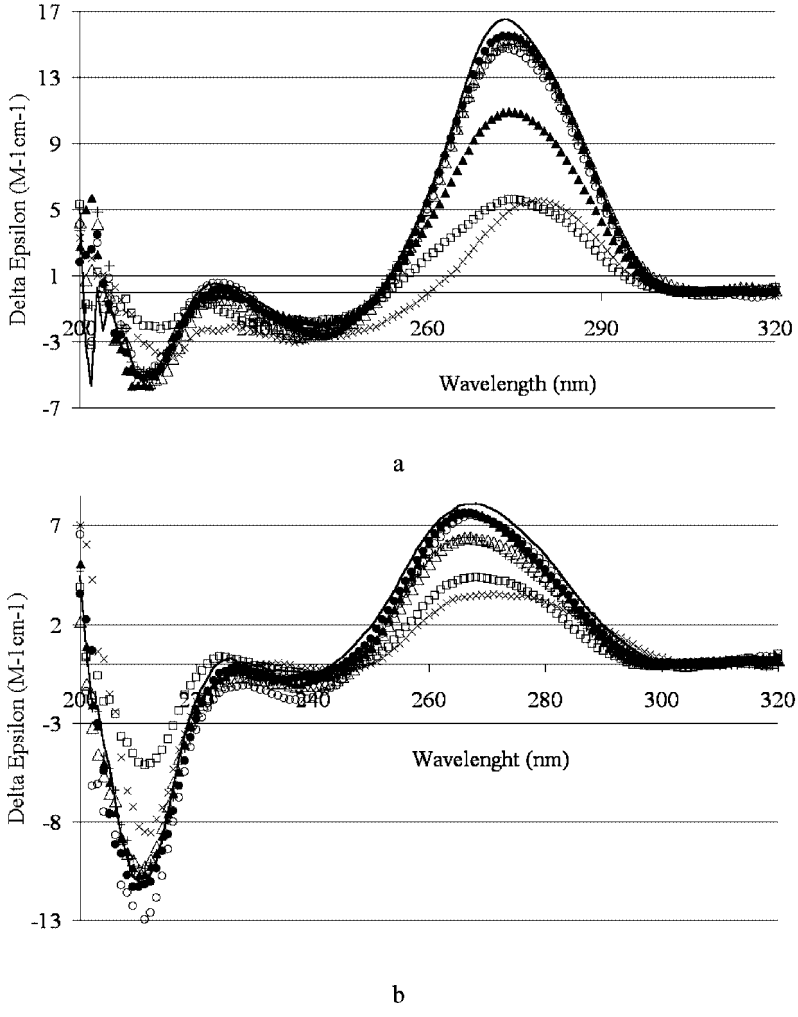


Figure 2. Circular dirchroism (CD) spectra of a) free single strands of oligonucleotides **2**: **2a** (●), **2b** (+), **2c** (○), **2d** (Δ), **2e** (▲), **2f** (□), **11** (×), and **12** (−); b) heteroduplexes of chimeric oligonucleotides **2** with RNA **10**: **2a**/RNA (●), **2b**/RNA (+), **2c**/RNA (○), **2d**/RNA (Δ), **2e**/RNA (▲), **2f**/RNA (□), **11**/RNA (×), and **12**/RNA (−). Reagents and conditions: 10 mM Tris-HCl, 100 mM NaCl, and 10 mM $MgCl_2$ (pH = 7.4), T = 20 °C, concentration = 4 μM. See Table 1 for base sequences of the corresponding oligonucleotides.

Table 2. Melting temperatures of hybrid duplexes: chimeric oligomers **2** and **13** with complementary DNA **9** and RNA **10**.

Oligonucleotide		T_m [°C] of duplexes[a]	
		DNA **9**	RNA **10**
5′-r(U_{MePS}C UC UC UC U_{MePS}C U)$_{2′\text{-OMe}}$-3′	(R_P,R_P)-**2a**	40.4	75.5
5′-r(U_{MePS}C UC UC UC U_{MePS}C U)$_{2′\text{-OMe}}$-3′	(S_P,S_P)-**2b**	28.1	65.9
5′-r(UC UC U_{MePS}C UC UC U)$_{2′\text{-OMe}}$-3′	(R_P)-**2c**	41.4	76.3
5′-r(UC UC U_{MePS}C UC UC U)$_{2′\text{-OMe}}$-3′	(S_P)-**2d**	29.0	71.3
5′-r(U_{MePS}C U_{MePS}C U_{MePS}C U_{MePS}C U_{MePS}C U)$_{2′\text{-OMe}}$-3′	(R_P,R_P,R_P,R_P,R_P)-**2e**	41.0	71.2
5′-r(U_{MePS}C U_{MePS}C U_{MePS}C U_{MePS}C U_{MePS}C U)$_{2′\text{-OMe}}$-3′	(S_P,S_P,S_P,S_P,S_P)-**2f**	n.d.	37.0
5′-d(TC TC TC TC TC T)-3′	**11**	44.0	57.7
5′-r(UC UC UC UC UC U)$_{2′\text{-OMe}}$-3′	**12**	41.0	74.9
5′-r(UC UC U_{MePO}C UC UC U)$_{2′\text{-OMe}}$-3′	Oxo-(R_P)-**13a**	43.8	74.4
5′-r(UC UC U_{MePO}C UC UC U)$_{2′\text{-OMe}}$-3′	Oxo-(S_P)-**13b**	36.0	70.0

[a] T_m calculated from the first derivative;[46] the error in the temperature measurement did not exceed 0.1 °C.

tion from C3′-*endo* which can only be caused by multiple alternating (S_P)-methylphosphonothioate modifications. The smaller amplitude of the **2f**/RNA CD spectrum is probably due to a similar conformation deviation to that seen for single-strand **2f** with alternating (S_P) modifications, as presented in Figure 2 (a). Such an effect is not observed for the alternating (R_P) modifications of **2e** (Figure 2, a). This can be rationalized in terms of a different pre-organization caused by steric effects and/or the solvation of single-strand oligonucleotides with alternating (R_P) and (S_P) modifications.[31,33] The process of duplex formation is accompanied by changes in the solvation pattern, which are manifested in a more intense CD spectrum for **2c**/RNA than for **2d**/RNA and a less intense spectrum for duplex **13a**/RNA than for **13b**/RNA. This correlates with a significant decrease in the amplitude at 274 nm for (R_P)-**13a** compared with (S_P)-**13b** and has significant thermodynamic consequences (vide infra).

Thermodynamic Studies

The data collected in Table 2 reveal a strong relationship between the absolute configuration at the phosphorus atom of the internucleotide methylphosphonothioate linkage and the stability of the hybrid duplexes with complementary DNA and RNA templates.

In all cases, the chimeric oligonucleotides with (R_P)-methylphosphonothioates (**2a**, **2c**, and **2e**) elicit stronger affinity towards complementary DNA and RNA than those with the (S_P) modifications. Oligomers oxo-**13a** and oxo-**13b**, modified with a single methylphosphonate linkage, appear to form hybrid duplexes with a stability close to those determined for methylphosphonothioates. The differences in the binding stabilities caused by incorporation of a single modification into chimeric oxo-(R_P)-**13a** or oxo-(S_P)-**13b** (ΔT_m = 4.4 °C for RNA and ΔT_m = 7.8 °C for DNA) is in this case smaller than the corresponding difference between **2c** and **2d**. The T_m values of the duplexes formed with the target RNA **10** and the (R_P)-modified chimeric oligomers **2a** and **2c** are about 18 °C higher than those formed with RNA **10** and the reference phosphodiester **11**. Interestingly, the chimeric **2e** with alternating (R_P)-methylphosphonothioate modifications forms a complex with RNA **10** with T_m = 71.2 °C and the complex formed between phosphodiester 2′-*O*-methyloligonucleotide **12** and RNA **10** provides a complex of similar thermal stability. Analogous behaviour was observed for the hybrid duplexes (R_P,R_P)-**2a**/**10** and (R_P)-**2c**/**10**. In this context, a destabilizing effect of the (S_P)-methylphosphonothioate function should be pointed out. The modifications located at the flanking positions of *Oligo-MePS* (**2a** and **2b**) also contribute to the stability of the hybrid duplexes in a stereodependent manner. The stability of duplexes formed by (R_P,R_P)-**2a** and (S_P,S_P)-**2b** is similar to that formed by (R_P)-**2c** and (S_P)-**2d** and is in agreement with the results of studies of chimeric oligonucleotides modified with incorporated methylphosphonates reported by Reynolds et al.[14]

From the data collected in Table 2 and Table 3 it is evident that several combinations of $\Delta H°$ and $\Delta S°$ elicit the same binding affinity, indicating that different factors govern a particular duplex formation process. Regardless of the position of the methylphosphonothioate modification in chimeric oligonucleotides **2**, the hybrid duplexes consisting of oligonucleotides with incorporated (R_P)-*MePS* [(R_P,R_P)-**2a**, (R_P)-**2c**, and (R_P,R_P,R_P,R_P,R_P)-**2e**] and a DNA/RNA template are thermally more stable than their (S_P)-*Oligo-MePS*–DNA/RNA counterparts, that is, they have higher melting temperatures and lower $\Delta G_{37}°$ values. The thermodynamic properties of the duplexes formed by stereoregular chimeric oligonucleotides **2** strongly depend on the nature of the template. 2′-*O*-Methyloligoribonucleotides are considered as structural analogues of RNA with a C3′-*endo* pucker[32,38] and therefore possess an increased affinity towards RNA templates.[34,36] Note that in the case of a single *P*-chiral modification incorporated into the middle of *Oligo-MePS* [(R_P)-**2c** and (S_P)-**2d**], the difference in stability caused by the opposite absolute configuration [(R_P)-**2c**/DNA vs. (S_P)-**2d**/DNA] is ΔT_m = 12.4 °C for DNA template **9**, and ΔT_m = 5 °C for duplexes (R_P)-**2c**/RNA and (S_P)-**2d**/RNA. These differences in duplex stabilities are not reflected in the CD spectra of single-strand **2c** and **2d**, either in the shape or intensity of the spectra of the duplexes formed by both oligonucleotides. Oligonucleotide (R_P)-**13a**, with a single methylphosphonate modification, forms a hybrid duplex with a stability comparable to that determined for the corresponding methylphosphonothioate analogue,

Table 3. Standard thermodynamic parameters[46] for hybrid duplexes of chimeric oligonucleotides and DNA/RNA templates.

	$\Delta H°$ [kcal/mol]		$\Delta S°$ [cal/mol K]		$\Delta G_{37}°$ [kcal/mol]	
	DNA **9**	RNA **10**	DNA **9**	RNA **10**	DNA **9**	RNA **10**
(R_P,R_P)-**2a**	–46.0 ± 0.2	–74.2 ± 1.4	–121 ± 1	–188 ± 4	–8.5 ± 0.3	–16.01 ± 1.8
(S_P,S_P)-**2b**	–30.6 ± 0.4	–66.6 ± 0.9	–77 ± 1	–170 ± 3	–6.7 ± 0.5	–13. 9 ± 1.2
(R_P)-**2c**	–45.9 ± 0.2	–84.6 ± 1.7	–120 ± 1	–218 ± 5	–8.6 ± 0.3	–17.1 ± 2.3
(S_P)-**2d**	–38.5 ± 0.3	–78.2 ± 1.1	–102 ± 1	–202 ± 3	–7.0 ± 0.5	–15.5 ± 1.4
(R_P,R_P,R_P,R_P,R_P)-**2e**	–44.5 ± 0.6	–63.5 ± 0.8	–116 ± 2	–158 ± 2	–8.7 ± 0.9	–14.6 ± 1.0
(S_P,S_P,S_P,S_P,S_P)-**2f**	n.c.	–42.9 ± 1.1	n.c.	–112 ± 4	n.c.	–8.2 ±1.6
Ref. 2′-OMe **12**	–43.4 ± 0.4	–75.6 ± 0.9	–113 ± 1	–192 ± 3	–8.4 ± 0.6	–16.2 ± 1.3
Ref. deoxy **11**	–62.2 ± 0.6	–64.0 ± 1.0	–173 ± 1	–168 ± 3	–8.4 ± 0.4	–11.9. ±1.4
Oxo-(R_P)-**13a**	–54.2 ± 0.3	–89.2 ± 0.8	–146 ± 1	–231 ± 2	–9.0 ± 0.3	–17.5 ± 1.1
Oxo-(S_P)-**13b**	–46.8 ± 0.3	–70.9 ± 0.3	–126 ± 1	–179 ± 1	–7.6 ± 0.3	–15.4 ± 0.4

albeit the value of T_m for **2c**/RNA (76.3 °C) is 2.8 °C higher than that for the corresponding (R_P)-**13a**/RNA (74.4 °C). A comparison of the CD spectra of oligonucleotides with a single modification, (R_P)-**13a** and (S_P)-**13b** [$\Delta H°$ = –89 kcal/mol for (R_P)-**13a** vs. $\Delta H°$ = –71 kcal/mol for (S_P)-**13b**], with the corresponding (R_P)-**2c** and (S_P)-**2d** oligonucleotides [$\Delta H°$ = –85 kcal/mol for (R_P)-**2c** and $\Delta H°$ = –78 kcal/mol for (S_P)-**2d**] (methylphosphonate vs. methylphosphonothioate) reveals a stronger influence of the (R_P) configuration in **13a** on the shape of the CD spectrum, manifested as a significant decrease in amplitude at 266 nm in comparison to that for **2d**. It is possible that the difference in the hybridization stability of the (R_P) and (S_P) chimeric oligonucleotides **2** is a result of unfavourable steric interactions between the methyl group of the (S_P) isomer and the flanking residues in the major groove (Figure 1, b), as was postulated for methylphosphonate analogues.[31] For the (R_P) diastereomer the methyl groups are directed away from the neighbouring residues in the major groove (Figure 1, a). Hydration of the axial projecting methyl group and less effective exposure to the solvent of the equatorial methyl group in the (S_P) diastereomer add to the relative destabilization of the hybrid duplexes formed with the target DNA/RNA strand. Previously, the favourable contribution of solvent interactions to the stability of the (R_P) isomers over the (S_P) diastereomers in *Oligo-MePO* was supported by ab initio and free-energy perturbation calculations for oligonucleoside (3′,5′)-methylphosphonates.[31]

For oligonucleotides **2a**, **2c**, and **2e** containing (R_P)-*MePS* moieties, more stable duplexes are formed with smaller changes in both enthalpy and entropy than is the case for the hybrid duplexes formed between unmodified oligonucleotides **12** and RNA or DNA templates. For (S_P)-*MePS* modified oligonucleotides (S_P,S_P)-2**b**, (S_P)-**2d**, and (S_P,S_P,S_P,S_P,S_P)-**2f**, smaller changes in the thermal stability per modification and the formation of less stable duplexes are observed.

An increase in the number of modifications results in a decrease in the enthalpy of the duplexes with (R_P) [from $\Delta H°$ = –84.6 kcal/mol for (R_P)-**2c** to $\Delta H°$ = –63.5 kcal/mol for **2e**] and (S_P) modifications ($\Delta H°$ = –78.2 kcal/mol for **2d** and $\Delta H°$ = –42.9 kcal/mol for **2f**). The same effect is observed for the entropy contribution [$-T\Delta S$ = –218 cal/mol K for (R_P)-**2c**, increasing to $-T\Delta S$ = –158 cal/mol K for **2e**]. This difference is even more profound for the (S_P)-modified hybrid duplexes with values of $-T\Delta S$ = –202 cal/mol K for (R_P)-**2d**, and $-T\Delta S$ = –112 cal/mol K for (S_P,S_P,S_P,S_P,S_P)-**2f**, respectively. Generally, in all cases but **2f**, the increase in entropy can be interpreted to result from a change in the solvation pattern of the hydrophobic groups.[47]

Since the CD spectrum of the single-stranded oligonucleotide (S_P,S_P,S_P,S_P,S_P)-**2f** is significantly distinct from those of the other chimeric oligonucleotides **2**, it can be concluded that this modification causes a major conformational change. Hybrid duplex (S_P,S_P,S_P,S_P,S_P)-**2f**/RNA is the least stable (since hybrid duplex **2f**/DNA was not detected under the experimental conditions), with a relatively low negative entropy ($\Delta S°$ = –112 cal/mol K) and enthalpy ($\Delta H°$ = –43 kcal/mol). These data together with NMR studies of the (S_P) dinucleotide **3a** imply that the unique behaviour of the oligonucleotide **2f** with alternating (S_P) modifications is a result of a major difference in the conformation of this oligonucleotide, which is shifted toward a B-like structure with a main C2′-*endo* pucker. According to the thermodynamic criterion, the hybrid duplex **2f**/RNA is very similar to the one formed between reference $r(UC)_5$-$U_{2'\text{-OMe}}$ (**11**) and DNA template **9**, that is, a duplex of DNA/RNA type (Figure 3).

Regardless of the number of (R_P) or (S_P) modifications in both types of hybrid duplexes (with RNA and DNA templates), the enthalpy factor is higher for the (S_P)-modified oligonucleotides than for the corresponding (R_P) diastereoisomers and this can be rationalized in terms of the dominant enthalpy of solvation responsible for the global conformation of the duplexes.[47] On the other hand, the lower stability of hybrid duplexes with (S_P) modifications is to a greater extent controlled by an entropy factor. The presence of non-ionic hydrophobic internucleotide methylphosphonothioate centres with sulfur fixed between neighbouring nucleosides makes the solvation entropy larger and positive. In contrast, the entropy of the (R_P)-modified oligonucleotides (**2a** and **2c**) is lower than the entropy of the (S_P) counterparts (**2b** and **2d**). Note that the number of (R_P) modifications is not a crucial factor determining the stability of duplexes with DNA and RNA, but an increasing number of (S_P) modifications rapidly reduces the stability of duplexes with both DNA and RNA templates. Interestingly, modifications located at the flanking positions of *Oligo-*

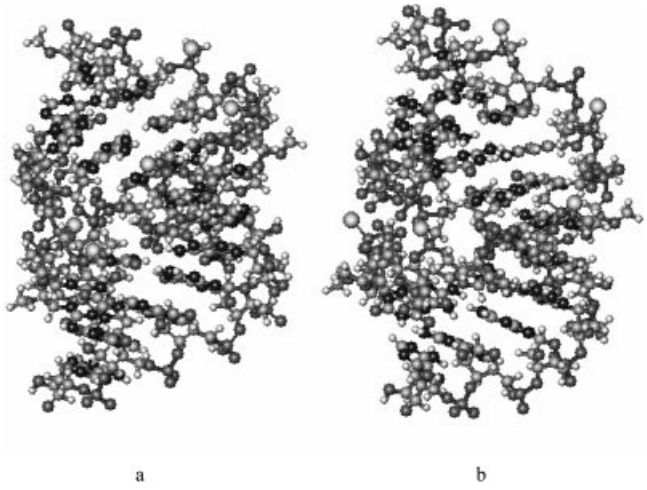


Figure 3. Energy-minimized structures of the hybrid duplexes a) (R_P,R_P,R_P,R_P,R_P)-**2e**/RNA and b) (S_P,S_P,S_P,S_P,S_P)-**2f**/RNA. The duplex structures were generated with the HyperChem. 7.5 molecular modelling program[49] and optimized using the AMBER96 force field method with default parameters.[50]

MePS [**2a** and (S_P,S_P)-**2b**] also contribute to the stability of the hybrid duplexes in a stereodependent manner. The effect of stabilization of duplexes formed by chimeric oligonucleotides **2a** and **2b** modified at the flanking positions (two modifications) is similar to that of oligonucleotides **2c** and **2d** with a single modification in the middle of the oligonucleotides. Moreover, *Oligo-MePS* **2e** with alternate (R_P) modifications (five methylphosphonothiates) forms with the RNA template **10** a relatively stable duplex with T_m = 71.2 °C, which is of comparable stability to the duplexes formed between the target RNA **10** and (R_P)-modified chimeric oligomers **2a** and **2c**, which are about 18 °C ($\Delta\Delta G_{37}$° = 7.6 kcal/mol) more stable than those formed between RNA **10** and the reference deoxyribonucleoside phosphodiester **11**.

The domination of the enthalpy factor is directly observed in the CD spectra of the single-stranded oligoncleotides or duplexes, even in the significant difference in amplitude of the positive band at 268 nm for single-strand (R_P,R_P,R_P,R_P,R_P)-**2e** compared to its hybrid duplex with RNA. The incorporation of the alternating (S_P)-methylphosphonothioate modifications into (S_P,S_P,S_P,S_P,S_P)-**2f** results in a chimeric oligonucleotide structure that forms a **2f**/RNA hybrid duplex with the lowest entropy ΔS° = –112 cal/mol K. Moreover, analogous to the *Oligo-MePS*/DNA duplexes, the unmodified DNA/RNA duplexes are less stable than the corresponding *Oligo-MePS*/RNA heteroduplexes and their association results in a greater change in both enthalpy and entropy.

Direct comparison of the thermodynamic parameters (Table 3) additionally suggests duplexes (S_P,S_P,S_P,S_P,S_P)-**2f**/RNA **10** and the reference 2′-*O*-methyloribonucleotide phosphodiester **12**/DNA **9** have similar structures.

Further studies, including NMR experiments and molecular dynamics simulations, which are in progress, on specific solute–solvent hydrogen-bond acceptor–donor interactions[39] responsible for the decrease in the stability of (S_P)-modified oligonucleotides should clarify to what extent conformational effects induced by the (S_P)-methylphosphonothioate linkages are significant factors affecting the stability of hybrid duplexes.

The reported differences in the stability of the hybrid duplexes described herein illustrate the potential for possible tuning of the properties of chimeric oligonucleotides modified with diastereomerically pure dimeric methylphosphonothioate building blocks. These results may provide interesting data in respect of the potential application of this type of oligonucleotide as therapeutic agents,[25] for studying and controlling oligonucleotide expression (antisense approach, RNase H independent or by steric blocking of translation machinery, siRNA), and as precise stereochemical tools to study the interactions of oligonucleotides with other biomolecules.

Experimental Section

Reactions were carried out under dry argon. Solvents and reagents were purified according to standard laboratory techniques and distilled directly into reaction vessels. Column chromatography and TLC analyses were performed on silica gel (Kieselgel 60, 240–400 mesh, E.Merck Inc.) and silica gel HP TLC precoated F_{254} plates (purchased from E. Merck Inc.), respectively. NMR spectra were recorded with a Bruker Advance DRX 500 spectrometer operating at 500.13 MHz (^{1}H) and 202.46 MHz (^{31}P). Chemical shifts (δ) are reported relative to TMS (^{1}H) and 80% H_3PO_4 (^{31}P) as external standards. Mass spectra were recorded with Finnigan Mat 95 (NBA, Cs^+ gun operating at 13 keV) and Voyager Elite (MALDI-TOF) spectrometers.

(R_P)- and (S_P)-5′-*O*-DMT-2′-OMe-uridin-3′-yl (3′,5′)-3′-*O*-(N^4-Isobutyryl 2′-OMe-cytidine) 3′-Methylphosphonothioates (3): Dinucleotides **3** were obtained according to previously described procedures.[23]

FAST (S_P)-3: R_f = 0.5, $CHCl_3$/EtOH, 9:1; ^{31}P NMR (202.46 MHz, $CDCl_3$): δ = 100.1 ppm. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.59 [t, $^3J_{HH}$ = 7.59 Hz, 6 H, CH(CH_3)$_2$], 1.94 (d, $^2J_{PH}$ = 15.61 Hz, 3 H, P-CH_3), 2.1 [dq, 1 H, CH(CH$_3$)$_2$], 2.56 (m, 1 H), 3.44 (dd, 1 H), 3.6 [s, 3 H, (C)CH_3O-C2′], 3.74 [s, 3 H, (Ura)CH_3O-C2′], 3.8 [s, 6 H, (Ura)CH_3O-DMT], 3.92 (m, 1 H), 4.18 (dd, 1 H,), 4.26 [d, $^3J_{HH}$ = 6.56 Hz, 1 H, (Ura)H2′], 5.36 [m, 1 H, (C)H2′], 5.87 [s, 1 H, (C)H1′], 6.05 [d, $^3J_{HH}$ = 3.41 Hz, 1 H, (Ura)H1′] ppm. FAB MS: m/z = 962.6 [M – H]$^+$ ($C_{46}H_{55}N_5O_{14}PS$: calcd. 963.99).

SLOW (R_p)-3: R_f = 0.45, $CHCl_3$/EtOH, 9:1; ^{31}P NMR ($CDCl_3$): δ = 98.26 ppm. ^{1}H NMR ($CDCl_3$): δ = 1.59 [t, $^3J_{HH}$ = 7.59 Hz, 6 H, CH(CH_3)$_2$], 1.76 (d, $^2J_{PH}$ = 15.32 Hz, 3 H, P-CH_3), 2.1 [dq, 1 H, CH(CH$_3$)$_2$], 2.56 (m, 1 H), 3.44 (dd, 1 H), 3.55 [s, 3 H, (C)CH_3O-C2′], 3.74 [s, 3 H, (Ura)CH_3O-C2′], 3.81 [s, 6 H, (Ura)CH_3O-DMT], 3.92 (m, 1 H), 4.18 (dd, 1 H,), 4.26 [d, $^3J_{HH}$ = 6.56 Hz, 1 H, (Ura)H2′], 5.36 [m, 1 H, (C)H2′], 5.95 [s, 1 H, (C)H1′], 6.02 [d, $^3J_{HH}$ = 3.43 Hz, 1 H, (Ura)H1′], 7.42 [d, $^3J_{HH}$ = 7.4 Hz, 1 H, (C)H5], 7.92 [d, $^3J_{HH}$ = 8.2 Hz, 1 H, (Ura)H6], 8.16 [d, $^3J_{HH}$ = 7.5 Hz, 1 H, (C)H6] ppm. FAB$^-$MS: m/z = 962.4 [M – H]$^+$ (calcd. 963.99).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-methyluridyl (3′,5′)-3′-*O*-[β-Cyanoethyl-*N*,*N*-diisopropylaminophosphoramidityl]-2′-(N^4-isobutyryl-2′-*O*-methylcytidine) Methylphosphonothioate (4): The diastereomerically pure dinucleosides **3** (600 mg) were coevaporated

twice with dry benzene (5 mL) and CH_2Cl_2 (5 mL) and kept overnight in a desiccator under high vacuum (r.t., 0.01 Torr), dissolved in CH_2Cl_2 (dried with CaH_2, and stored under argon with 4 Å molecular sieves) (3 mL), and treated with diisopropylethylamine (4.4 equiv.) and β-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (2.2 equiv.) with vigorous stirring. The reaction progress was monitored by TLC (HP TLC plate analysis; chloroform/MeOH, 9:1 v/v). When the reaction was complete, the reaction mixture was loaded directly into a silica gel column equilibrated with an ethyl acetate/CH_2Cl_2/MeCN (3:1:1 v/v) mixture containing 1% of Et_3N. The column was run under a positive pressure of argon using the same eluent. Fractions containing product **4** were concentrated to afford pure products **4** as colorless foams. These were dissolved in dichloromethane and precipitated in cold hexane. White powders were isolated after centrifugation, dried under vacuum, and stored at –20 °C under argon. Yields: 80–82%. ^{31}P NMR: (R_P)-**4** ($CDCl_3$): δ = 99.3, 99.1 [(R_P)-methylphosphonothioate signals] and 149.5, 148.8 ppm (phosphoramidite signals). FAB MS: *m*/*z* = 1162.6 [M – H]$^-$, 1164.7 [M + H]$^+$ ($C_{55}H_{71}N_7O_{15}PS$: calcd. 1163.42). ^{31}P NMR: (S_P)-**4** ($CDCl_3$): δ = 97.4, 97.5 ppm (S_P)-methylphosphonothioate signals) and 151.67, 151.34 ppm (phosphoramidite signals). FAB MS: *m*/*z* = 1164.7 [M – H]$^+$ ($C_{55}H_{71}N_7O_{15}PS$: calcd. 1163.42).

Solid-phase Synthesis of Chimeric Oligonucleotides 2 Using Dimeric Building Block 4: Oligomers **2** and **8**–**13** were prepared on a 1 μmol scale using an ABI 392 Synthesizer (Applied Biosystems Inc., Foster City, USA). Incorporation of the dimeric building blocks **4** required an increase in the coupling time to 180 s without any further modifications of the protocol. Removal of the base-protecting groups and cleavage from the solid support was achieved by treatment of the CPG-bound oligomers with NH_4OH/MeOH solution (2:1 v/v) at room temperature for 8 h followed by HPLC purification of oligomers (DMT-ON; ODS Hypersil column, Shandon, UK, 210×4.6 mm, gradient 2–40% MeCN, 0.1 M TEAB buffer, pH 7.5). Standard removal of the 5′-DMT group was followed by RP HPLC purification (ODS Hypersil C18, gradient 2–20% MeCN, 0.1 M TEAB buffer, pH 7.5). The purity of all oligomers was confirmed by gel electrophoresis. Their structures were confirmed by MALDI-TOF spectrometry (see Table 1).

Melting Profiles and Thermodynamic Calculations: All absorption measurements were carried out in a cell of 1 cm pathlength with a UV/Vis 916 spectrophotometer equipped with a Peltier Thermocell (GBC UV/Vis 916, GBC, Dandenong, Australia). The concentration of oligomers was determined spectrophotometrically by UV absorbance at λ_{max} in water using the extinction coefficients calculated by the published method.[48] The unmodified phosphodiester oligonucleotides d(TC)$_5$T (**11**) and r(UC)$_5$U$_{2'-OMe}$ (**12**) as well as chimeric oligonucleotides **2** were lyophilized and redissolved in 10 mM Tris-HCl, 100 mM NaCl, 10 mM $MgCl_2$ buffer (pH = 7.4) and complementary templates were dissolved in the same buffer at a total concentration of duplexes of 4 μM. *Oligo-MePS* **2**, and the appropriate templates were mixed together, heated at 90 °C for 5 min, and kept overnight at 4 °C. Melting profiles were recorded between 3 and 95 °C in both directions with a temperature gradient of 0.3 °C/min. The melting temperatures were calculated using the first-order derivative method. Thermodynamic parameters were obtained numerically by fitting an analytical curve resulting from a two-state model to experimental melting profiles. The procedure applied for fitting was a nonlinear least-squares method included in the Sigma Plot software (version 5.01).

CD Spectra: CD spectra were recorded in the same buffer at a concentration of 4 μM on a CD6 dichrograph (Jobin–Yvon, Longjumeau, France) using cells with a 5 mm pathlength, 2 nm band width, and 1–2 s integration time. Each spectrum was smoothed with a 9- or 15-point algorithm (included in the manufacturer's software, version 2.2.1) after averaging of at least three scans.

Supporting Information: Synthesis, NMR and MS (MALDI-TOF) data are available along with colour figures illustrating molecular modelling results for hybrid duplexes (**2f**/RNA).

Acknowledgments

This work was financially assisted by the State Committee for Scientific Research (KBN-Grant 4 TO9A 073 25 awarded to L. A. W.). The authors are indebted to Prof. W. J. Stec for his interest in this work and helpful discussions, Dr. S. Kazmierski for NMR analysis, and T. Kębłowska for skillful technical assistance.

[1] C. A. Stein, A. M. Krieg (Eds.), *Applied Antisense Oligonucleotide Technology*, Wiley-Liss, New York, **1998**.
[2] M. Phillips (Ed.), "Antisense Technology" in *Methods in Enzymology*, Academic Press, **1999**, vol. 314, part B.
[3] *Protocols in Nucleic Acids Chemistry* (Eds.: S. L. Beaucage, D. E. Bergstrom, G. D. Glick, R. A. Jones), John Wiley & Sons, Inc., Hoboken, NJ, **2003**, 4.17.1–4.17.25.
[4] B. H. Loyd, R. V. Giles, D. G. Spiller, J. Grzybowski, D. M. Tidd, *Nucleic Acids Res.* **2001**, *29*, 3664–3673.
[5] S. T. Crooke, *Annu. Rev. Med.* **2004**, *55*, 61–95.
[6] E. Wickstrom (Ed.), *Clinical Trials of Genetic Therapy with Antisense DNA and DNA Vectors*, Marcel Dekker, Inc., New York, **1998**.
[7] M. Warashina, B. Nawrot, S. Obika, L. A. Wozniak, T. Kuwabara, T. Imanishi, W. J. Stec, K. Taira in *Synthetic Nucleic Acids as Inhibitors of Gene Expression: Mechanisms, Applications and Therapeutic Implications* (Ed.: L. M. Khachigian), CRC Press, Boca Raton, FL, **2005**, pp. 95–113.
[8] M. R. Kurpiewski, M. Koziołkiewicz, A. Wilk, W. J. Stec, L. Jen-Jacobson, *Biochemistry* **1996**, *35*, 8846–8854.
[9] S. A. Smith, L. W. McLaughlin, *Biochemistry* **1997**, *36*, 6046–6058.
[10] T. M. Okonogi, S. C. Alley, E. A. Harwood, P. B. Hopkins, B. H. Robinson, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4156–4160.
[11] P. R. Hardwidge, J. M. Zimmerman, L. J. Maher, *Nucleic Acids Res.* **2002**, *30*, 1879–1885.
[12] K. F. Pirollo, A. Rait, L. S. Sleer, E. H. Chang, *Pharmacol. Ther.* **2003**, *99*, 55–77.
[13] Y. S. Cho-Chung, C. A. Stein, A. M. Gewirtz (Eds.), "*Therapeutic Oligonucleotides: Antisense, Rnai, Triple-Helix, Gene Repair, Enhancer Decoys, Cpg, and DNA Chips*" in *Annals of the New York Academy of Sciences*, New York Academy of Sciences, New York, **2004**.
[14] M. A. Reynolds, R. I. Hogrefe, J. A. Jaeger, D. A. Schwartz, T. A. Riley, W. B. Marvin, W. J. Daily, M. M. Vaghefi, T. A. Beck, S. K. Knowles, R. E. Klem, L. J. Arnold Jr., *Nucleic Acids Res.* **1996**, *24*, 4584–4591.
[15] P. S. Miller, N. D. Annan, S. M. McParland, K. B. Pulford, *Biochemistry* **1982**, *21*, 2507–2512.
[16] P. S. Miller, N. S. Dreon, M. Pulford, K. B. McParland, *J. Biol. Chem.* **1980**, *255*, 9659–9665.
[17] M. Jaworska-Maslanka, W. Kacperczyk, D. Korczynski, Z. J. Lesnikowski, *Antisense Nucleic Acid Drug Dev.* **1997**, *7*, 23–30.
[18] Z. J. Lesnikowski, M. Jaworska, W. J. Stec, *Nucleic Acids Res.* **1990**, *18*, 2109–2115.
[19] W. J. Stec, Z. Leśnikowski in *Methods in Molecular Biology, Protocols for Oligonucleotides and Analogs* (Ed.: S. Agrawal), Humana Press, Inc., Totowa, NJ, **1993**.
[20] L. Tian, Ch. D. Claeboe, S. M. Hecht, S. Shuman, *Structure* **2004**, *12*, 31–40.

[21] L. Tian, Ch. D. Claeboe, S. M. Hecht, S. Shuman, *Mol. Cell* **2003**, *12*, 199–208.
[22] M. R. Kurpiewski, L. Engler, L. A. Wozniak, A. Kobylanska, M. Koziolkiewicz, W. J. Stec, L. Jen-Jacobson, *Structure* **2004**, *12*, 1775–1788.
[23] L. A. Wozniak, W. Majzner, W. J. Stec, *Arkivoc* **2004**, 101–110.
[24] S. Olejniczak, M. J. Potrzebowski, L. A. Wozniak, *Eur. J. Org. Chem.* **2004**, 1958–1966.
[25] L. A. Wozniak, M. Janicka, M. Bukowiecka-Matusiak, *J. Organomet. Chem.* **2005**, *690*, 2658–2663.
[26] D. Venkaterwarlu, K. E. Lind, V. Mohan, M. Manoharan, D. M. Ferguson, *Nucleic Acids Res.* **1999**, *27*, 2189–2195.
[27] L. A. Wozniak *Reviews on Heteroatom Chemistry*, vol. 19 (Ed.: Shigeru Oae), **1999**, pp. 173–202.
[28] L. A. Wozniak, J. Pyzowski, M. Wieczorek, W. J. Stec, *J. Org. Chem.* **1994**, *59*, 5843–5846.
[29] W. J. Stec, L. A. Wozniak, J. Pyzowski, W. Niewiarowski, *Antisense Res. Develop.* **1997**, *7*, 381–395.
[30] L. A. Wozniak, M. Koziolkiewicz, A. Kobylanska, W. J. Stec, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2641–2646.
[31] F. H. Hausheer, B. G. Rao, J. D. Saxe, U. C. Singh, *J. Am. Chem. Soc.* **1992**, *114*, 3201–3206;
[32] H. C. P. F. Roelen, E. de Vroom, A. G. van der Marel, J. H. van Boom, *Nucleic Acids Res.* **1988**, *16*, 7633–7645.
[33] D. Hamelberg, L. D. Williams, W. D. Wilson, *Nucleic Acids Res.* **2002**, *30*, 3615–3623.
[34] M. Egli, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1894–1909.
[35] T. Hamma, A. Saleh, I. Huq, T. M. Rana, P. S. Miller, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1845–1848.
[36] C. R. Allerson, N. Sioufi, R. Jarres, T. P. Prakhash, N. Naik, A. Berdeja, L. Wanders, R. H. Gaffney, E. E. Swayze, B. Bhat, *J. Med. Chem.* **2005**, *48*, 901–904.
[37] A. Grunweller, E. Wyszko, B. Bieber, R. Jahnel, V. A. Erdmann, J. Kurreck, *Nucleic Acids Res.* **2003**, *31*, 3185–3193.
[38] J. M. Kean, C. D. Cushman, H. M. Kang, T. E. Leonard, P. S. Miller, *Nucleic Acids Res.* **1994**, *22*, 4497–4503.
[39] P. Auffinger, E. Westhoff, *Angew. Chem. Int. Ed.* **2001**, *40*, 4648–4650.
[40] L. A. Wozniak, W. J. Stec in *Frontiers in Nucleosides and Nucleic Acids* (Ed.: R. F. Schinazi, D. C. Liotta), Informed Horizons, LLC, Tucker, USA.
[41] W. Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag, New York, **1984**.
[42] S. Neidle (Ed.), *Oxford Handbook of Nucleic Acid Structure*, Oxford University Press, Oxford, **1999**.
[43] T. Popenda, E. Biala, J. Milecki, R. W. Adamiak, *Nucleic Acids Res.* **1997**, *25*, 4589–4598.
[44] B. Norden, T. Kurucsev, *J. Mol. Recognit.* **1994**, *7*, 141–155.
[45] M. F. Summers, R. A. Byrd, K. A. Gallo, C. J. Samsom, G. Zon, W. Egan, *Nucleic Acids Res.* **1985**, *13*, 6375–6386.
[46] E. M. Evertsz, R. Karsten, T. M. Jovin, *Nucleic Acids Res.* **1994**, *22*, 3293–3303.
[47] A. Velazquez-Campoy, I. Luque, E. Freire, *Thermochim. Acta* **2001**, *380*, 217–227.
[48] P. Lubini, W. Zurcher, M. Egli, *Chem. Biol.* **1994**, *1*, 39–45.
[49] Hypercube, Inc.; www.hyper.com.
[50] W. D. Cornell, P. Cieplak, C. I. Bayly, I. R. Gould, K. M. Merz Jr., D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell, P. A. Kollman, *J. Am. Chem. Soc.* **1995**, *117*, 5179–5197.

Received: June 2, 2005
Published Online: October 12, 2005

