Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 521–524

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

2'-DEOXY-2'(*S*)-ETHINYL OLIGONUCLEOTIDES: A MODIFICATION WHICH SELECTIVELY STABILIZES OLIGOADENYLATE PAIRING TO DNA COMPLEMENTS

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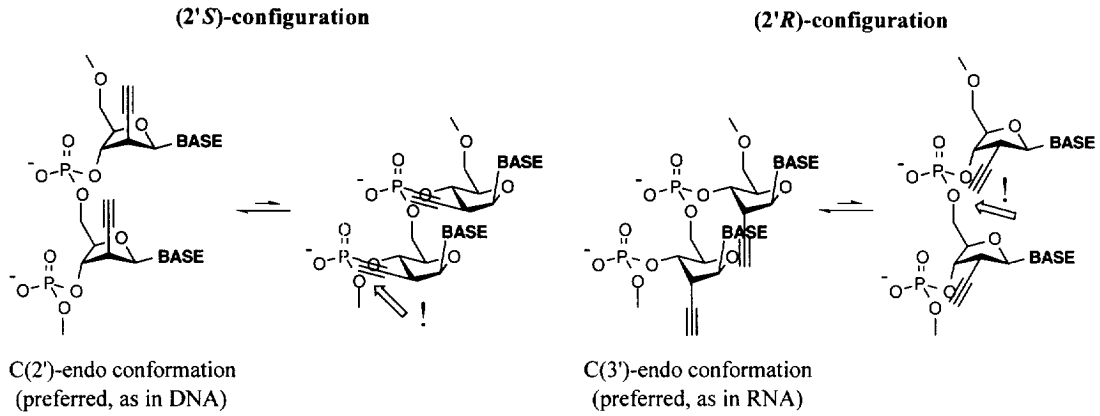
Received 19 December 1997; accepted 26 January 1998

Abstract: Oligonucleotides consisting of 2'-deoxy-2'(*S*)-ethinyl-thymidine, -uridine, and -adenosine have been prepared. Whereas the modified pyrimidine oligonucleotides uniformly lead to weaker pairing affinity with DNA and RNA complements, the corresponding adenine oligonucleotides show enhanced thermal stability in duplexes with complementary DNA and decreased stability with RNA.

The design and synthesis of structurally modified oligonucleotides has now been persued for nearly three decades.[1,2] This effort was mainly targeted at enhancing the thermal stability of duplexes and triplexes consisting of such oligonucleotide analogs and complementary DNA and RNA single or double strands. In general, modified oligonucleotides, like regular oligodeoxynucleotides, form slightly stronger hybrids with complementary RNA than with DNA.[3] Notable exceptions are anhydrohexitol oligonucleotides (HNA), 2',5'-linked DNA, and 2',3'-bridged DNA which, in a certain sequence context, bind much stronger to RNA.[4-6] However, no attempt has been made to design oligonucleotides which exclusively bind a DNA complement but not an RNA one. Such specificity would be highly desirable in the application of oligonucleotide-based sensors of relevant DNA sequences in complex biological probes where both nucleic acids might be present.

The design of such oligonucleotide analogs relies on the premise that DNA should be an easier target than RNA, since DNA double helices are capable of adopting a multitude of conformations whereas RNA is confined to A-form duplexes.[7] Selectivity for a DNA complement should thus be possible if an A-form conformation of the resulting double helix is not accessible. To restrict the conformational freedom of individual nucleosides in an ideal B-form double helix we sought to introduce an (*S*)-configured ethinyl substituent at the C(2')-position of

Figure 1



C(2')-endo conformation (preferred, as in DNA)

C(3')-endo conformation (preferred, as in RNA)

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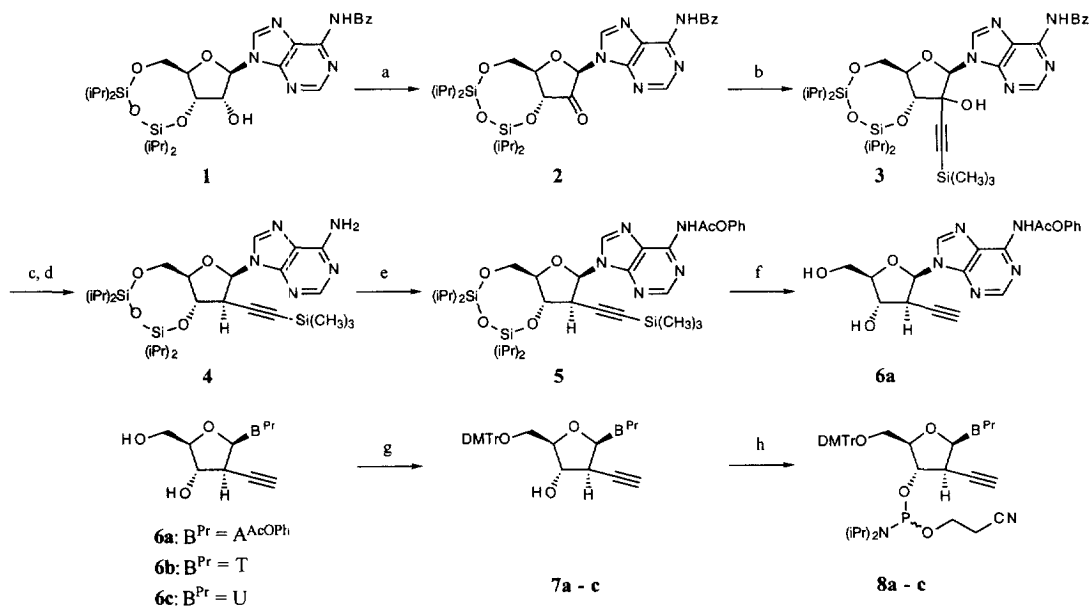
0960-894X/98/$19.00
PII: S0960-894X(98)00060-2

deoxynucleosides. We assumed that in such an oligodeoxynucleotide the individual nucleosides are adopting a C(2')-*endo* conformation, typical of B-form DNA double helices, because the alternative C(3')-*endo* (RNA) conformation would lead to sterically unfavorable interactions with the phosphodiester residue or the nucleobase of the 3'-neighbouring nucleotide (*figure 1*). The same reasoning would imply that the inverse configuration of the ethinyl residue should favor an RNA-like conformation. While several modifications are known[1,2,8] which preorganize an oligonucleotide on a mononucleotide level this modification is expected to maintain a certain duplex conformation through interactions in a dinucleotide step within the duplex.

The synthesis of 2'-deoxy-2'(*S*)-ethinyl pyrimidine nucleosides **6b** and **6c** has previously been described in the context of antiviral agents.[9] Preparation of the corresponding deoxyadenosine derivative has been claimed as well.[10] However, we were not successful in reproducing this procedure and therefore followed a strategy similar to the synthesis of **6b** and **6c**. Oxidation of **1**[11], followed by lithium acetylide addition and radical deoxygenation of the intermediate tertiary alcohol **3** led to **4** in a stereoselective[12] manner (*scheme 1*). Phenoxyacetyl protection of the exocyclic amino group[13] and removal of the silyl protecting groups gave 2'-deoxy-2'(*S*)-ethinyl adenosine derivative **6a**. Nucleosides **6** were then converted to the phosphoramidite-building blocks **8** according to standard procedures.[14]

Scheme 1



*a) Dess-Martin periodinane, CH_2Cl_2,, r.t., 20h, 96%; b) BuLi, TMSC≡CH, THF, -78°C, 3h, 95%; c) $H_3COOCCOCl$, DMAP, CH_2Cl_2, r.t., 12h; d) Bu_3SnH, AIBN, toluene, 90°C, 4h, 67% (2 steps); e) PhOAc-imidazole, $Et_3O^+BF_4^-$, CH_2Cl_2, r.t., 3h, then **3** in CH_2Cl_2, r.t., 19h, 74%; f) TBAF, AcOH, THF, r.t., 1h, 95%; g) DMTrCl, pyridine, r.t., 3h, **6a**: 61%, **6b**: 79%, **6c**: 71%; h) $(iPr_2N)(OCH_2CH_2CN)PCl$, iPr_2NEt, THF, r.t., 1h, **6a**: 80%, **6b**: 87%, **6c**: 92%*

With the suitable building blocks at hand, oligonucleotides **9** - **18** (*table 1*) were prepared on a 1.3 µmol scale by automated solid-phase synthesis.[15] Average coupling yields for ethinyl-modified nucleosides (94-97%) were slightly lower than for unmodified phosphoramidites. The oligonucleotides were purified by ion-exchange

HPLC and checked for homogeneity by reverse-phase HPLC. The integrity of the isolated oligonucleotides **9** - **18** was subsequently confirmed by MALDI-ToF mass spectrometry.[16]

Replacing one thymidine residue with 2'(*S*)-ethinyl-thymidine at a central position in the reference duplex $d(T_{10})$•$d(A_{10})$ (**9•14**) leads to a dramatic decrease in thermal stability (**11•14**, ΔT_m = -13°C) (*table 1*). Contrary to our expectations, a somewhat smaller decrease is observed in the corresponding DNA-RNA heteroduplex **11**•poly(A) (ΔT_m = -8°C). A rationale for this destabilization might be that the ethinyl substituent unfavorably interacts with the thymine methyl group in the same nucleoside thus forcing the base to adopt a conformation not suitable for pairing. This could be tested by replacing uracil for thymine. Besides the expected overall decrease in stability due to this modification — $d(U_{10})$•$d(A_{10})$ melts 17°C lower than $d(T_{10})$•$d(A_{10})$ — the ethinyl substitution still negatively affects duplex stability (**12•14**, ΔT_m = -4°C; **12**•poly(A), ΔT_m = -4°C). This effect is somewhat diminished in a completely modified sequence **13** but again, relative to the reference systems, the RNA complement forms a more stable duplex than the DNA one.

A completely different picture evolves in the case of the ethinyl-modification of deoxyadenosine. Whereas monosubstitution in $d(T_{10})$•$d(A_{10})$ (**9•14**) does not affect thermal stability (**9•15**, ΔT_m = 0°C), the respective DNA-RNA complex **15**•poly(U) (ΔT_m = -2°C) is destabilized. The same substitution in $d(U_{10})$•$d(A_{10})$ (**10•14**) leads to strong stabilization (**10•15**, ΔT_m = +3°C). The fully modified oligonucleotide **16** clearly shows enhanced pairing to $d(T_{10})$ and $d(U_{10})$ as compared to the reference system but decreased affinity for the corresponding RNA complement.

Table1

	oligonucleotide sequence[a]	T_m [°C][b]			ΔT_m/modification		
		complementary oligonucleotide					
		$d(A_{10})$	poly(A)		$d(A_{10})$	poly(A)	
9	$d(T_{10})$	33	37		—	—	
10	$d(U_{10})$	16	28		—	—	
11	d(T-T-T-T-T^{Et}-T-T-T-T-T)	20	29		-13	-8	
12	d(U-U-U-U-U^{Et}-U-U-U-U-U)	12	24		-4	-4	
13	d(U^{Et}-U^{Et}-U^{Et}-U^{Et}-U^{Et}-U^{Et}-U^{Et}-U^{Et}-U^{Et}-U)	< 5	26		< -1.2	-0.2	
		$d(T_{10})$	$d(U_{10})$	poly(U)	$d(T_{10})$	$d(U_{10})$	poly(U)
14	$d(A_{10})$	33	16	56[c]	—	—	—
15	d(A-A-A-A-A-A^{Et}-A-A-A-A)	33	19	54[c]	0	+3	-2
16	d(A^{Et}-A^{Et}-A^{Et}-A^{Et}-A^{Et}-A^{Et}-A^{Et}-A^{Et}-A^{Et}-A)	38	25	35[c]	+0.6	+1.0	-2.3
		$d(U^{Et}{}_9U)$					
16	d(A^{Et}-A^{Et}-A^{Et}-A^{Et}-A^{Et}-A^{Et}-A^{Et}-A^{Et}-A^{Et}-A)	6					
		self-complementary sequences					
17	d(A-U-A-U-A-U-A-U-A-U)	17			—		
18	d(A^{Et}-U-A^{Et}-U-A^{Et}-U-A^{Et}-U-A^{Et}-U)	< 5			< -2.4		

a) *T^{Et}, U^{Et}, A^{Et} = 2'-deoxy-2'(S)-ethinyl-thymidine, -uridine, and -adenosine, respectively. b) Concentration of oligonucleotides (duplex) = 4μM in 10mM NaH_2PO_4, 1M NaCl, pH 7.0. Absorbance detected at 260nm. Heating rate: 0.5°C/min (heating-cooling-heating cycle). The accuracy of melting points is estimated to be ± 0.5°C. Melting points represent the mean value of three melting curves. c)* ***14***, ***15***, *and* ***16*** *are forming triplexes with poly(U) as shown by UV-titration experiments (data not shown).*[17]

This result is consistent with our initial assumptions on the conformation-driving effect of the 2'(*S*)-ethinyl group. The failure of this concept in an oligopyrimidine context might be due to the interference of the ethinyl group with the C(5)-methyl group of the 3'-neighbouring thymidine and, to a lesser extent, H-C(5) in the deoxyuridine case. This is further supported by the fact that the self-complementary sequence **18**, in which 2'-ethinyl deoxyadenosine precedes an unmodified deoxyuridine is destabilized by a similar amount (ΔT_m/mod. < -2.4°C) as in the case of the fully modified decadeoxyuridinylate **13**. The overall lower affinity of the modified oligopyrimidinenucleotides towards their complements, regardless of selectivity, could arise from an unfavorable entropic component in duplex formation since the single strand conformation likely differs from the conformation in the duplexes. In the modified oligoadenosinylates this preorganization towards a B-form pairing conformation might already be achieved in the single strand due to enhanced stacking of the nucleobases.

In conclusion, we have shown that 2'(*S*)-ethinyl-substituted oligodeoxyadenosine pairs stronger to a complementary DNA-strand than to the respective RNA counterpart. It remains to be seen whether this effect is limited to homo-purine strands and how such sequence limitations can be circumvented to allow the development of truly DNA-specific oligonucleotide analogs. The synthesis of 2'(*R*)-ethinyl-substituted oligonucleotides is also under way.

Acknowledgement: This work was supported by the Swiss National Science Foundation.

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16. m/z (monoanion, H^+-form): **11**: calc. 3003.0, found 3002.1; **12**: calc. 2862.8, found 2863.1; **13**: calc. 3055.0, found 3054.9; **15**: calc. 3093.2, found 3095.5; **16**: calc. 3285.4, found 3289.0; **18**: calc. 3074.1, found 3074.4. Matrix conditions as described in: Pieles, U.; Zürcher, W.; Schär, M.; Moser, H. E. *Nucleic Acids Res.* **1993**, *21*, 3191.
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