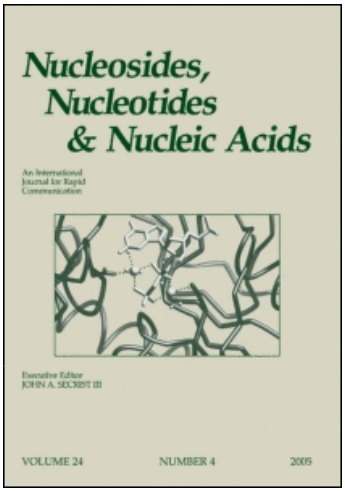

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of an Octameric Phosphodiester of 2, 3-dideoxy-ß-D-erythro-hex-2-eno-pyranosyl-thymine and Its Interactions with Poly-rA and Poly-dA

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To cite this Article Felder, Eduard , Gattlen, Raphael , Ossola, Flavio and Baschang, Gerhard(1992) 'Synthesis of an Octameric Phosphodiester of 2, 3-dideoxy-ß-D-erythro-hex-2-eno-pyranosyl-thymine and Its Interactions with Poly-rA and Poly-dA', Nucleosides, Nucleotides and Nucleic Acids, 11: 9, 1667 — 1671
To link to this Article: DOI: 10.1080/07328319208021358
URL: http://dx.doi.org/10.1080/07328319208021358

NUCLEOSIDES & NUCLEOTIDES, 11(9), 1667-1671 (1992)

SYNTHESIS OF AN OCTAMERIC PHOSPHODIESTER OF 2,3-DIDEOXY-ß-D-ERYTHRO-HEX--2-ENO-PYRANOSYL-THYMINE AND ITS INTERACTIONS WITH POLY-rA AND POLY-dA

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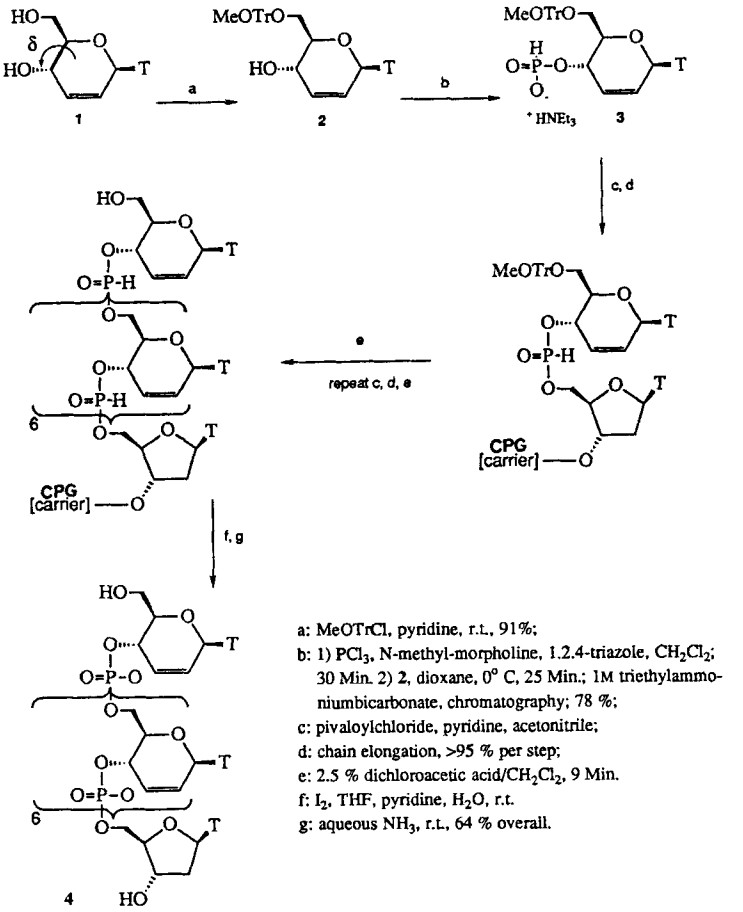
Abstract: 2,3-Dideoxy-ß-D-erythro-hex-2-eno-pyranosyl-thymine 1 was converted to an octanucleotide by the H-phosphonate method. This octamer did not hybridize with poly-rA in 1 M NaCl (pH 7) but did so, to a small extent, with poly-dA. One unsaturated nucleotide was incorporated in the middle of a hepta-thymidylate to probe the disorder caused by this foreign building block.

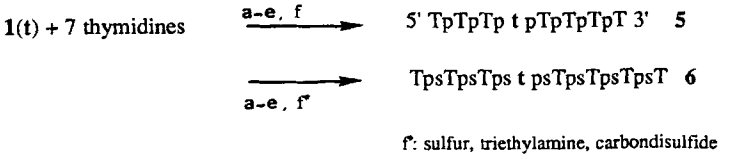
Introduction: The concept of antisense nucleotides, i.e. oligo- or poly-nucleotides which hybridize with specific mRNAs and thus intercept messages for certain proteins, has raised great interest throughout the scientific community[1]. In a search for potential substitutes of natural nucleotides which may interact with ribo- or deoxyribo-nucleic acids we selected an octanucleotide containing 2,3-dideoxy-β-D-erythro-hex-2-eno-pyranose instead of 2-deoxy-ribose. The geometry of this unsaturated sugar reveals a dihedral angle δ of approximately 85° which is in the range of δ= 77°-97° for ribose in A-RNA. This angle is crucial to the geometry of A-type helices and inspection of Dreiding models suggested the likelihood for such oligomers to adopt an A-type conformation. Moreover, a computer assisted study on a corresponding dinucleoside phosphate with the molecular mechanics program QUANTA (version 2.1, Polygen Inc.) indicated a δ= 93.3° after relaxation of the molecule under conditions enforcing the A-form specific distances and spatial orientations of the thymines (starting value δ= 85.5°). The corresponding values for a ribose dinucleoside phosphate were 88° before and 82° after relaxation.

Synthesis: The known 2,3-dideoxy-β-D-erythro-hex-2-eno-pyranosyl-thymine **1** was prepared according to the literature[2] along with its α-anomer. Augustyns et al.[3] have used this compound as an intermediate for the synthesis of the saturated hexapyranosyl-thymine analog, which was incorporated into homooligomers with increased nuclease resistance. In order to separate the α- and β-isomers chromatography of the 4',6'-di-acetates on a silica gel column was carried out with dichloromethane/t-butyl-methylether (7:3) as eluent. The β-isomer was protected at the primary hydroxyl group with methoxytrityl chloride and converted to the 4'- hydrogen phosphonate **3** by reaction with PCl_3 and triazole[4] in 78% yield. Oligonucleotide chain assembly was performed on an Applied Biosystems DNA synthesizer (model 380 B), starting from commercially available long chain alkylamine controlled pore glass support (CPG) derivatized with 5'-dimethoxytrityl-thymidine. Derivative **3** was utilized for the coupling reactions following essentially the procedure described in lit. [4] (scheme 1). The synthesis cycle was modified by taking into account the slower acid deprotection kinetics of the monomethoxytrityl compared to the dimethoxytrityl group (9 min wash, 2.5% dichloroacetic acid). The latter protecting group would be more recommendable for the assembly of longer chains in order to minimize cycle time and exposure to acid, although proper control experiments have shown that even prolonged treatment of the β-nucleoside diacetate (18 h, 5% dichloroacetic acid, r.t.) does not lead to anomerization. Oxidation tests with iodine solutions and β-nucleoside **1** ruled out any undesirable incompatibility. Post synthesis work-up, including the manual oxidation step with iodine (I_2, THF, pyridine, water), was carried out as described[4] and followed by an ion exchange chromatography step on a Mono Q column (Pharmacia; NaCl gradient elution) which yielded 443 OD units of purified material (yield 64% calculated from 9 μmoles of thymidine loaded glass support). Electrospray ionization mass spectrometry showed the main fraction to have M= 2455.2 ± 0.3, (calculated M= 2455.1) for the octamer **4**. A minor fraction consisted of the heptamer (M= 2138.9). Moreover, we compared the ORD-curves of the β-nucleoside and the α-nucleoside with that of the octamer, to detect any partial anomerization in the oligomer. By this comparison we can assume the bases to have the all-β-configuration.



a: MeOTrCl, pyridine, r.t., 91%;
b: 1) PCl_3, N-methyl-morpholine, 1.2.4-triazole, CH_2Cl_2; 30 Min. 2) 2, dioxane, 0° C, 25 Min.; 1M triethylammoniumbicarbonate, chromatography; 78 %;
c: pivaloylchloride, pyridine, acetonitrile;
d: chain elongation, >95 % per step;
e: 2.5 % dichloroacetic acid/CH_2Cl_2, 9 Min.
f: I_2, THF, pyridine, H_2O, r.t.
g: aqueous NH_3, r.t., 64 % overall.

Scheme 1: solid phase synthesis of octanucleotide 4



Scheme 2: synthesis of mixed octamers

TABLE 1: Melting temperatures and hyperchromicity (%) of homo and hetero octanucleotides (4 and 5,6 resp.)

	poly-rA	poly-dA
4	[c]	17° (5%)
5	8° (24%)	12° (23%)
6	[c]	
$(Tps)_7T$	8° (24%)	18° (21%)

Note: temperatures Tm are at the inflexion point of UV absorption as f(temp)

* all experiments are in phosphate buffer pH 7 , 1M NaCl

• [c] = Tm not measurable

In order to assess the level of disorder caused by the foreign building block, the synthesis of two mixed octamers (**5**,**6**) was carried out in analogy to the preparation of **4** by using 5'-methoxytrityl-thymidine-3'-hydrogen phosphonate instead of **3**, where necessary (scheme 2). From a common hydrogen phosphonate precursor both **5** and the thiophosphate analog **6** can be obtained with an oxidation step, using either the iodine treatment (f) or a 0.1 M sulfur solution in triethylamine/carbon disulfide (1:9)[5] (f^*)

Hybridization experiments (Table 1): Incubation of octamer **4** with poly-rA in phosphate buffer pH 7 containing 1 M NaCl did not show hybridization. However an interaction with poly-dA could be observed with T_m= 17° and 5% hyperchromicity. Noticeably, the melting curve of **5** with poly-rA was virtually identical to that obtained with a reference phosphorothioate octanucleotide $(Tps)_7T$: T_m= 8°, 24% hyperchromicity. Derivative **6** showed only negligible interactions. In the poly-dA system **5** behaved slightly differently from $(Tps)_7T$ (T_m= 12°, 23% hyperchromicity and T_m= 18°, 21% hyperchromicity respectively).

Discussion: The preparation of short oligonucleotides (< 10 bases) being composed of either all unnatural monomers or containing single mutated residues is a stringent test for verifying the structural

compatibility of new synthetic entities with double helical structures. In the example we described, the modified nucleoside introduces a subtle perturbation which couldn't be predicted by rapid molecular modeling estimations, although the experiments confirmed the potential for unsaturated pyranosyl oligonucleotides to interact with natural nucleic acids.

Acknowledgement: We thank Dr. H. Fuhrer for NMR measurements, Mr. K. Jaekel and Dr. P. Moser for hybridization and ORD measurements, Dr. D. Mueller and Mr. U. Rindisbacher for mass spectrometry.

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Received 4/24/92
Accepted 7/16/92