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Synthesis, complete ¹H assignments and conformations of the self-complementary hexadeoxyribonucleotide [d(CpGpApTpCpG)]₂ and its fragments by high field NMR

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ABSTRACT

The two deoxyribonucleotides $[d(CpGpApTpCpG)]_{and} [d(CpGpCpG)]_{avere}$ synthesized by the phosphotriester method. Their duplex form under the conditions of the ¹H-nmr experiments was proven by end ³²P labeling with T4 polynucleotide kinase followed by butt end joining employing the absolute specificity of T4 ligase for double stranded DNA and analysis using gel electrophoresis and autoradiography. Complete nmr assignment of the ¹H chemical shifts and coupling constants was achieved. The assignments were secured using sequential decoupling, NOE difference measurements, and two-dimensional COSY and SECSY experiments. Spectrum simulation confirmed the experimental values of chemical shifts and coupling constants. The techniques for the assignment outlined together with ³¹P and 2-D heteronuclear shift correlation permit an approach to a systematic analysis of more complex single-strand and duplex oligodeoxyribonucleotides.

INTRODUCTION

Biochemical evidence indicates that nucleic acids are among the principal macromolecular cellular targets for many clinically effective anticancer agents including doxorubicin, actinomycin, bleomycin, 3-alkyl-1-(2-chloroethyl)-l-nitrosoureas, cis-platinum, mitomycin C and others (2-4). Physical methods including electron microscopy (5), X-ray diffraction (6) and ethidium binding assays in conjunction with certain cellular repair enzymes (7,8) have proven useful in identifying some of the several lesions induced in nucleic acids by such agents. 1 H and 13 C-NMR are potentially among the more informative techniques for characterizing drug-DNA or drug-oligodeoxyribonucleotide complexes (9). However progress has been slow owing to the inherent difficulties in making unambiguous assignments of the complex spectra resulting from all but the shortest fragments of oligomers (1). And yet the awareness of unique conformational changes that may occur in longer oligomers (e.g. the B to Z conformational transition for [d(CpGpCpGpCpG)], at high medium ionic strength (11)) of specific and extended molecular recognition requirements (e.g. an XTTTX sequence for the minor groove binding antiviral agent distamycin (12)) and of topological effects (13) demand extended analyses of longer oligonucleotide sequences. The advent of high-field NMR cryospectrometers and the attendant newer analytical techniques of SECSY, NOESY, and COSY twodimensional spectroscopy may permit the resolution of some of these problems and provide unique information on the structure and conformation in solution of certain anticancer drug-oligodeoxyribonucleotide complexes.

We recently required to examine the properties of certain oligodeoxyribonucleotides to investigate molecular recognition characteristics of agents designed to recognize and bind to specific sequences (8). Accordingly we report the synthesis and high-field NMR characterization, assignment and solution conformation of the self-complementary hexadeoxyribonucleotide $[d(CpGpApTpCpG)]_2$ as well as those of appropriate fragments necessary to confirm the assignments.

MATERIALS AND METHODS

The two oligodeoxyribonucleotides d(CpGpApTpCpG) and d(CpGpCpG) were synthesized via a modified phosphotriester approach (14,15). By means of its higher lipophilic properties, the ter-butylbenzoyl group (tbuBz) was found more suitable than the commonly used iso-butyryl group for the protection of the exocyclic amino function of the guanine residues (16). Two partially protected dinucleoside monophosphates d-[(MeO)₂Tr]bzCptbubzG and d-[(MeO)2Tr]bzApT and the fully protected dinucleoside monophosphate $d-[(Me0)_{2}Tr]bzCpTbubzGbz$ (p = 2-ch]oropheny] phosphate) were prepared and then assembled through selective detritylation (15), 3'-O-phosphorylation (14) and condensation (14) steps. After deprotection (17) and purification on a DEAE-Sephadex A-25 column, the two oligodeoxyribonucleotides d(CpGpApTpCpG) and d(CpGpCpG) were treated with Dowex 50 W (Na⁺ form) to yield the sodium salts. Their homogeneities were ascertained by HPLC analysis using a C-18 reverse phase column. After complete digestion of aliquots by crotalus durrissus terrificus snake venom phosphodiesterase, the two samples showed the expected nucleoside and nucleotide ratios.

A sample of the hexanucleotide (10 nmoles) was labeled with ^{32}P at the 5'hydroxyl using T4 polynucleotide kinase in a 20 µl mix containing: 70 mM Tris.Cl pH 7.6, 10 mM MgCl₂, 5 mM DTT, 40 nmoles γ ^{32}P -ATP (NEN, 0.5 Ci/mmole). The reaction was initiated by the addition of 20 units of T4 polynucleotide kinase (P. L. Biochemicals) and incubated at room temperature for 1 h. The reaction was monitored by incorporation of radioactivity into TCA precipitable material. The kinased hexanucleotide was then ligated in a reaction mixture containing 100 mM Tris.Cl pH 7.6; 10 mM $MgCl_2$, 7.5 mM DTT and 3 mM ATP. T4 DNA ligase (5 units) was added and the reaction incubated for 20 h at either room temperature or 4°C. Samples were analyzed on 10% polyacrylamide gels run in 50 mM Tris.borate, 50 mM boric acid, 2 mM EDTA at 150 v, 8 mA for 5 h. Bands were visualized by autoradiography (Kodak X-OMAT AR) at -70°C.

All NMR samples were made up in 99.6% D_2O (Merck, Sharp & Dohme) containing 40 mM potassium phosphate and 20 mM sodium or magnesium chloride buffered at pH 7.0. These solutions were lyophilized three times from 99.96% D_2O (M, S and D) and finally made up to 0.5 ml with 99.996% D_2O (Aldrich) and TSP added as internal standard. The ¹H-NMR spectra were recorded at 25°C on the degassed annealed samples (5°C for 36 hr) on either Bruker WH400 or WH200 spectrometers and using the Aspect 2000 data system.

Inversion recovery T_1 measurements (18) used a variable delay time between 0.01 - 10 secs while for NOE measurements a delay time of 10-12 secs was used. The two-dimensional correlated spectra (COSY and SECSY) (19-22) used two 90° pulses and detected N-type signals with the frequency window in F_1 of 900 Hz (WH200) and 1800 Hz (WH400). The COSY technique (21) was more generally applicable since the spectrum contains coupled signals separated by relatively large chemical shifts. For J-resolved spectra (23) 90° and 180° pulses were used with a window of 25 Hz (WH400) (i.e. the window required was at least half of the width of the largest multiplet). The ¹H simulations were calculated on the ASPECT 2000 data system using the PANIC program (24) which has a capacity for a maximum of an 8-spin system. Since 9 spins are present in the four central sugars of the hexamer (seven protons plus two flanking phosphorus atoms), two groups of resonances were calculated for each sugar, i.e. 1', 2'1, 2'2, 3', P, 4' (Group 1), P, 3', 4', 5'1, 5'2, P (Group 2). All six simulations of the Group 1 type were then superimposed using the FTP400 program giving simulations of the 1', 2'1, 2'2 and 3' resonances, and similarly with the group 2 type giving 4', 5'l and 5'2 resonances.

RESULTS AND DISCUSSION

In order to demonstrate that the synthetic hexanucleotide (Fig. 1) was a duplex during the NMR experiments we made use of the substrate specificity of T4 DNA ligase. This enzyme can catalyze the end to end joining of base paired duplexes when the nucleoside at the 5' end carries a phosphate group and the complementary nucleoside opposite it carries a free 3' hydroxyl mojety (25). Prior to the ligation T4 polynucleotide kinase was used to



<u>Figure 1. One strand of the duplex [d(CpGpApTpCpG)]</u> showing numbering and torsional angles.

transfer a radioactive phosphate group from γ^{32} P-ATP to the 5' position of the synthetic hexanucleotide (26). Both the kinase and ligase reactions were carried out at room temperature to duplicate the NMR scan conditions, even



Figure 2. The synthetic hexanucleotide is a substrate for T4 DNA ligase. Lane 1: $[d(CpGpApTpCpG)]_{2}$ labeled with ^{32}P by T4 polynucleotide kinase. Lane 2: labeled hexanucleotide after incubation with T4 DNA ligase at 4°C for 20 h. Lane 3: labeled hexanucleotide after incubation with T4 DNA ligase at room temperature for 20 h.



<u>Figure 3.</u> Resolution enhanced 400 MHz ¹H-nmr spectrum of the non-exchangeable protons for the region $\delta 1.8 - \delta 6.4$ of [d(CpGpApTpCpG)]₂ with computer simulation underneath.

though this is not optimal for either enzyme. Samples from both the kinase and ligase reactions were run on a 10% polyacrylamide slab gel (27). The ladder pattern of concatenated hexanucleotide duplex units can be clearly seen in the autoradiogram (Fig. 2) for the sample incubated with DNA ligase. Since the enzyme is specific for duplex DNA we conclude that the synthetic hexanucleotide is indeed a duplex.

Several recent studies have permitted strategies to be developed for the ¹H nmr analysis of short oligonucleotides using combinations of onedimensional [(sequential decoupling (28-30) and NOE difference measurements (31-38)] and two-dimensional [J-Resolved (33,39) COSY (40,41), SECSY (49), NOESY (42,43) and heteronuclear shift correlation (40,44)] nmr techniques. The HH and PH coupling constants obtained from the spectra have enabled the sugar-phosphate backbone and sugar ring conformations to be deduced, whilst the dependency of base and sugar chemical shifts on the shielding characteristics of the adjacent bases have been observed (45). In addition, NOE difference measurements have provided information on the orientation of bases with respect to the sugar residues in both single and duplex strands (31-38).



Figure 4. Full 2D-COSY spectrum of [d(CpGpApTpCpG)]2.

The non-exchangeable 1 H resonances of the hexamer (shown in Fig. 3 with simulation) and its fragments were assigned using sequential decoupling, NOE difference measurements and modified J-resolved and COSY techniques. The modification to the 2D experiments was to suppress the HOD signal such that adjacent correlations (COSY) and cross-sections (J-resolved) could be more clearly observed. This was done by applying an initial 180° pulse followed by a delay equivalent to the HOD relaxation time prior to the first pulse in the normal 2D experiment (46). This efficiently relaxes out the HOD resonance.

The connectivities of resonance of each sugar residue were readily identified from the COSY experiment (Figs. 4,5) and could be assigned to having a pyrimidine substituent (C,T) from the characteristically higher field 2'1, 2'2 resonances. In addition the terminal cytosine in both tetramer and hexamer could be identified by the higher field shifts of the 5'], 5'2 resonances. Confirmation of these connectivities was observed in NOE difference experiments a saturation of the 3' resonances. In these cases NOE differences were observed with all other protons of that residue (in addition to proximate base protons) in order of size 2'1 > 4' > 2'2 > 1'-



Figure 5. 2D-COSY expansion of the region $\sim\delta$ 1.7 - δ 3.2 for [d(CpGpApTpCpG)]₂

5'1 > 5'2 (1', 5'1, 5'2 are weak and <2%).

Each group of sugar resonances was then assigned to its appropriate base. The moderate NOE's observed on saturation of H8 (purines) and H6 (pyrimidines) with the 2'1, 2'2 resonances of the attached sugar and, where appropriate, with the 2'1, 2'2 resonances of the 5'-neighbor were particularly useful in this regard.

The H5 and H6 protons of the cytosines were readily assigned, with their connectivities indicated in the COSY experiment (Fig. 4) and also by the strong NOE differences observed on saturation of either resonance. In both tetramer and hexamer the terminal cytosine resonances were found to be between 0.11 and 0.32 ppm to lower field respectively than the corresponding in-chain cytosine resonances. This assignment was confirmed by the moderate NOE differences observed with the 2'1, 2'2 resonance of the terminal cytosine sugar residue. In contrast the in-chain cytosine show NOE's with both attached sugar and with 2'1, 2'2 of the 5'-neighbor. In addition in the hexamer saturation of the thymine base methyl group shows moderate NOE's with both cytosine base resonances (δ 7.50 and δ 5.71) confirming the assignment.

A comparison of the chemical shifts of the base protons in the hexamer



Figure 6. 2D-COSY expansion of the region $\sim \delta 3.3 - \delta 5.4$ for [d(CpGpApTpCpG)]₂.

and $[d(CpGpCpG)]_2$ provided information on the ring current shielding effects of neighboring bases. It was then evident that the major contributions were from the purine bases and that the contribution of A>G. In the oligomers examined there is a G unit at the 3' end so that any differences in chemical shifts of the H₅ and H₆ of the cytosine must arise from the 5'-substituent. In $[d(CpGpCpG)]_2$ the in-chain cytosine has purines at both the 3' and 5' ends whereas the corresponding cytosine in $[d(CpGpApTpCpG)]_2$ has a pyrimidine at the 5'-end. The chemical shifts for H₅ and H₆ in the tetramer are to higher field than in the hexamer by 0.17 and 0.12 ppm respectively confirming that there is a contribution to the shielding from the 5'-substituent. It was also observed that the terminal cytosines have H₅ in the tetramer $[d(CpGpCpG)]_2$ to lower field than in the hexamer but in this case by only 0.03 ppm and with H₆ to higher field by 0.02 ppm. Both resonances compare closely with the values observed for the dCpG dinucleotide (i.e. all cases 5' substituent -OH).

		[d(CpGpC	;pG)] ₂ in	D ₂ 0 a	at 25°C				
	H5	H6	нв н	יו	H2'1 2'2	H3'	H4 '	H5'] I	15'2
C(1)	7.62	5.84 -	- 5	.77	2.42, 1.9	1 4.71	4.12	3.3	74
G(2)		8.	.01 5	.95	2.75	5.01	4.39	4.13,	4.02
C(3)	7.42	5.54 -	- 5	.87	2.39, 1.9	6 4.87	4.22	4.	11
G(4)		7.	95 6	.18	2.69, 2.4	3 4.72	4.21	4.	11
н-н	and H-	P Coupli	ng Const	ants f	for [d(CpG	pCpG)] ₂			
	5,6	1',2'1	1',2'2	2'1,2	2'2 2'1,3	' 2'2,3'	3',4'	4',5'1	4',5'2
C(1)	7.7	7.2	6.0	14.5	5 8.5	0.5	2.8	5.0	3.0
G(2)	-	9.4	5.5	(a)	4.5	1.0	0.5	3.5	~0.5
C(3)	7.5	9.1	5.7	14.5	5 8.8	0.5	3.1	4.5	2.4
G(4)	-	8.0	6.0	13.5	5 8.2	4.8	2.7	3.4	~0.5
	5'1,5	'2 3'	,P 5'	1,P	5'2,P	<u> </u>			
C(1)	12.5	4.	.7	-	-				
G(2)	11.0	5.	.0 2	2.0	(b)				
C(3)	(a)	6.	.1	(a)	(a)				
G(4)	(a)	-		(a)	(a)				

Table 1. ¹H Chemical Shifts (ppm) from TSP for Bases and Sugars of $[d(CpGpCpG)]_2$ in D_2O at 25°C

a. Since resonances overlap and have second order components it was not possible to obtain geminal coupling constants (5'1,P'; 5'2,P).

b 5'2 resonance is under 5'1,5'2 for C(3) and G(4).

The H6 proton of thymine in the hexamer is readily assigned by the strong NOE difference observed on saturation of the adjacent methyl group and also by the long-range connectivity observed in the 2D-COSY experiment (Fig. 4). The moderate NOE's observed between H6 and 2'1, 2'2 resonance (δ 2.04 and δ 2.47 respectively) of its attached sugar confirmed the assignment. Both H6 and the methyl resonances appear upfield by approximately 0.2 ppm when compared with the dinucleotides d(ApT) and d(pApT). This is plausibly due to the 5'-adenine exerting a greater shielding effect due to a closer crientation with the thymine ring. This proximity is also indicated by the moderate NOE difference effects that are observed between the methyl

	H2	Н5	H6	H8	H1'	H2'	H2'2	H3'	H4 '	H5'1	H5'2
C(1)		5.89	7.61		5.76	1.88	2.41	4.71	4.07	3.75	3.71
G(2)				7.99	5.60	2.76	2.86	5.03	4.36	4.00	4.11
A(3)	7.95			8.32	6.34	2.73	3.00	5.05	4.52	4.24	4.29
T(4)			7.23		5.99	2.04	2.47	4.89	4.21	4.34	4.17
C(5)		5.71	7.50		5.80	2.03	2.39	4.86	4.16	4.	.10 ^b
G(6)				7.96	6.18	2.64	2.39	4.70	4.21	4.	.10 ^b

Table 2. ¹H Chemical Shift (ppm) from TSP for Bases and Sugars of [d(CpGpApTpCpG)]₂ in D₂O at 25°C

^a The chemical shift assignment of the 5'l and 5'2 protons is arbitrary.
 ^b Both groups of 5 protons are overlapping and coincident and show second order effects, quoted chemical shift is the centre of the two signals.

group of the thymine and H2 and H8 of adenine. The H8 resonance of adenine is to lower field than the other purine resonances ($\delta 8.32$). Saturation of this resonance showed the expected 2'1, 2'2 NOE's of the attached sugar ($\delta 2.73$ and $\delta 3.00$). The in-chain guanines of the tetramer and hexamer ($\delta 8.01$, and $\delta 7.99$ respectively) showed NOE's with their attached 2'1, 2'2 sugar resonances and also with the 2'1, 2'2 resonances of the terminal cytosine, already assigned. Similarly the terminal guanines for the tetramer and hexamer ($\delta 7.95$ and $\delta 7.96$ respectively) showed additional NOE's with the in-chain cytosine 2'1, 2'2 resonances.

The remaining base resonance for the hexamer was H2 of adenine (δ 7.95) which showed the expected absence of NOE's with any sugar resonances. The H2 and H8 adenine resonances have characteristic spin-lattice relaxation times (T₁). Since the nucleoside unit is expected to be in the <u>anti</u> conformation thus orienting H8 in close proximity, T₁ for H8 should be shorter than H2. This effect was observed in the inversion-recovery T₁ experiment.

Base-pair Stacking.

Inspection of CPK models indicates that the H_8 protons (G,A) and H_6 protons (C,T) are close to $H_{2'1,2'2}$ of the attached sugar and also $H_{2'1,2'2}$ of the 5'-neighbor. This proximity can be gauged by NOE difference measurements.

					(
	5,6	5,CH ₃	1',2'	1',2'2	2'1,2'2	2'1,3'	2'2,3'	
C(1)	7.6		7.0	6.5	14.5	6.0	0.0	
G(2)			10.2	5.0	13.3	4.5	0.5	
A(3)			8.9	5.8	13.9	5.5	1.0	
T(4)		0.5	9.1	6.0	13.7	7.7	1.0	
C(5)	7.5		7.2	6.5	14.6	7.0	3.1	
G(6)			8.0	6.7	14.3	6.3	0.5	
	3',4'	4',5'1	4',5'2	5'1,5'2	3',P	5'1,P	5'2,P	
C(1)	2.9	4.0	5.1	12.6	3.1	••		
G(2)	0.5	2.8	5.9	11.2	4.5	4.3	3.5	
A(3)	2.9	2.5	5.0	12.2	2.0	4.4	3.4	
T(4)	1.9	3.5	6.5	13.0	4.1	3.5	3.0	
C(5)	3.5	3.5	6.1	a	4.0	a	a	
G(6)	3.4	3.0	6.3	a		a	a	

Table 3. Coupling Constants [J(Hz)] for [d(CpGpApTpCpG)]

a These 5'1 and 5'2 signals overlap and exhibit second order effects

Proton saturated	NOE same sugar (taken as 100%)*	NOE 5-neighbor
с _{Н8}	only C _{2'1,2'2}	-
с _{Н8}	^G 2'1,2'2	^C 2'1 ~25%, C _{2'2} ~50%
А _{Н8}	A2'1,2'2	^G 2'1,2'2 ~100%
т _{н6}	^T 2'1,2'2	A _{2'1} ~60%, A _{2'2} ~75%
с _{Н6}	^C 2'1,2'2	^T 2'1,2'2 ~ ^{95-100%}
\dot{G}_{H}	G _{2'1,2'2} saturated signal.	C _{2'1,2'2} ~ 10-15%

*NOE \simeq 15% '8 compared to the saturated signal.

The CPK model shows that only with the bases packed as closely as possible does the base proton come close to both 2'-methylene groups. The above NOE difference results suggest that the GA, and TC pairs are closely stacked (possibly because they are like bases and stack more efficiently) while the

Table 4.	Population	Distribution of	Conformers	in	[d(CpGpApTpCpG)]	at	25°C
	in Aqueous	Buffer pH 7.0 ^a					-

	%S(% ² E)	ε ^t	%β ^t	%y ^t
C(1)	71	183°	-	47
G(2)	95	189°	85	52
A(3)	75	178°	85	64
T(4)	83	187°	92	38
C(5)	67	187°	-	42
G(6)	70	-	-	45

a
$$\$S = [J_{1'2'}/J_{1'2'} + J_{3'4'}] \times 100; \ \varepsilon^{t} = 240^{\circ} -\theta , \ ^{3}J_{H3',P} = 17.6\cos^{2}\theta - 3.8\cos\theta; \ \$\beta^{t} = [(23.9 - \Sigma')/18.9] \times 100; \ \Sigma' = J_{H5'1,P} + J_{H5;2,P}; \ \$\gamma^{t} = [(13.3 - \Sigma)/9.7] \times 100; \ \Sigma = J_{4',4'1} + J_{4',5'2} (31).$$

others are further apart. The low values for ^{E}CG and CG^{E} may be due to "fraying" at the termini (9), and the value for the AT residues is probably more typical for adjacent purine-pyrimidine substituted sugars. Conformation of the Deoxyribose Rings

The deoxyribose rings can be represented in two idealized conformations, the S-type form $\binom{3}{2}T$) and the N-type $\binom{2}{3}T$) (31) (Fig. 7). Evidence, so far, suggests that the deoxyribose rings exist predominantly in the Stype form (72-100% S) for 5'-terminal and central residues, with the 3'-terminal residues showing more N-type character (56-70% S). It can be seen from the above structures (Fig. 7) that the conformation of the ring is directly related to the torsion angles between H₁, and H_{2'1}, H₃, and H_{4'}. Knowing the corresponding vicinal coupling constants an estimate of the population of the S-conformation can be calculated as shown in Table 4 (31). From the NOE





experiments described in the previous section, the conformations of the terminal residues in the hexamer are somewhat atypical which may be due to "fraying" of the duplex (9). However examination of the data for the other residues shows a similar trend to that observed previously. Backbone Conformations

Conformation around C5' - 05' (β). An estimate of the population around the torsion angle β (Fig. 1) can be obtained from the equation shown in Table 4. For the hexamer values in the range 85-92% β^{t} conformer are obtained indicating a large amount of right-handed stacking expected for the duplex. Conformation around C_{4'} - C_{5'} (γ) (See Fig. 1). The values of the vicinal $J_{4'}, 5'1, J_{4'}, 5'2$ coupling constants can be used to estimate the γ^{t} population according to the equation shown in Table 4. In previous studies on single-stranded molecules, the γ^{t} conformation has been shown to be most populated (76%) with γ^{t} (18%) and γ^{-} (6%) (30). The results for the hexamer show values in the range 38 - 64% γ^{t} , somewhat lower than those observed above and may result from different conformational requirements on duplex formation. Conformation Around C_{3'} - O_{3'} (ϵ) The torsion angle ϵ (Fig. 1) between C_{4'} and P can be determined by first estimating the angle θ between H₃, and P and then subtracting θ from 240°;

$${}^{3}J_{H3',P} = 17.6 \cos^{2}\theta - 3.8 \cos^{2}\theta$$

 $\epsilon^{t} = 240^{\circ} - \theta$

The average value observed for the hexamer is 184.8° which corresponds closely to the calculated value of 177° for duplex B-DNA and 186° calculated for $d(A)_{6}d(T)_{6}$ duplex (31).

<u>Conclusion</u> The application of NOE difference measurements and two-dimensional COSY and SECSY techniques together with sequential decoupling permitted the complete assignment of all the non-exchangeable protons of $[d(CpGpApTpCpG)]_2$ The application of these procedures together with heteronuclear shift correlation between ¹H and ¹³C or ³¹P resonances to such sequences of biological significance will be reported in due course.

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- 1. Abbreviations: NMR, nuclear magnetic resonance; SECSY, spin echo correlation spectroscopy; NOESY, nuclear Overhauser effect correlation spectroscopy; COSY, correlation spectroscopy; PANIC, parameter adjustment in nmr by iteration calculation; NOE, nuclear Overhauser effect; tbuBz - <u>p</u> tert-butylbenzoyl; DMTr - [(MeO)_Tr] - p,p'-dimethoxytrityl; Ar - <u>o</u>-chloro-phenyl; <u>p</u> represents the <u>o</u>-chlorophenylphosphate group, DTT, dithiothreitol, TCA, trichloroacetic acid, TSP, 3(trimethylsilyl)-1-propanesulfonic acid, sodium salt hydrate. The symbols used to represent oligodeoxyribonucleotides and their protected derivatives and the designation of the torsional angles in the structural diagram follow the recommendations of the IUPAC-IUC Commission on Biochemical Nomenclature (1983) Eur. J. Biochem. 131, 9-15.
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