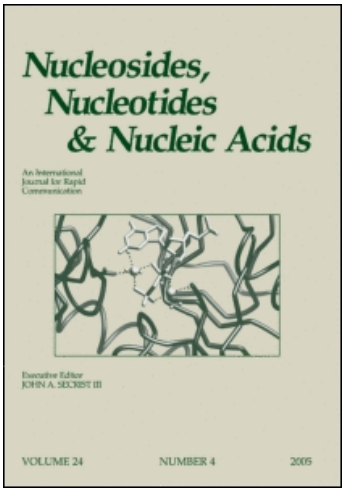

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Dodecanucleotides Containing (E)-5-(2-Bromovinyl)-2'-Deoxyuridine: Influence of a Bulky Major Groove Substituent on Duplex Stability and Endodeoxyribonuclease Eco Ri Recognition

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To cite this Article Seela, Frank , Driller, Hansjürgen , Herdering, Wilhelm and De Clercq, Erik(1988) 'Dodecanucleotides Containing (E)-5-(2-Bromovinyl)-2'-Deoxyuridine: Influence of a Bulky Major Groove Substituent on Duplex Stability and Endodeoxyribonuclease Eco Ri Recognition', Nucleosides, Nucleotides and Nucleic Acids, 7: 3, 347 — 363
To link to this Article: DOI: 10.1080/07328318808068715
URL: http://dx.doi.org/10.1080/07328318808068715

NUCLEOSIDES & NUCLEOTIDES, 7(3), 347-363 (1988)

DODECANUCLEOTIDES CONTAINING (E)-5-(2-BROMOVINYL)-2'-DEOXYURIDINE: INFLUENCE OF A BULKY MAJOR GROOVE SUBSTITUENT ON DUPLEX STABILITY AND ENDODEOXYRIBONUCLEASE ECO RI RECOGNITION.

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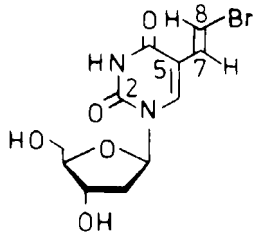
ABSTRACT Self-complementary dodecanucleotides containing BVDU (1) have been prepared by solid-phase synthesis employing the phosphoramidite technique. The phosphoramidite of BVDU was obtained after 4,4'-dimethoxytritylation and subsequent phosphitylation of the nucleoside 1. Melting experiments of the dodecamers containing one BVDU residue showed that the stability of such duplexes is only slightly affected by the bulky bromovinyl residue. The dodecamers d(GTAGAAbv^{5}UTCTAC) (9) and d(GTAGAATbv^{5}UCTAC) (10) were subject to hydrolysis experiments with the endodeoxyribonuclease Eco RI under star activity conditions. Whereas regioselective hydrolysis was decreased in case of the oligomer 10, compared to the non-modified oligomer 8, the oligomer 9 was not hydrolyzed at all under these conditions. This can be explained by a steric interference of the bulky bromovinyl substituent with the nitrogen-7 of the adjacent adenine moiety being a proton acceptor site during endonuclease binding. A complete assignment of ^{1}H and ^{13}C NMR spectra of BVDU (1) is also reported.

INTRODUCTION

(E)-5-(2-Bromovinyl)-2'-deoxyuridine (BVDU) [1] is one of the most potent inhibitors of herpes simplex virus type 1 (HSV-1) and varicella-zoster virus (VZV) [2,3]. It inhibits replication of these viruses at a concentration as low as 1-10 ng/mL. In vivo BVDU is degraded to bromovinyluracil due to the action of pyrimidine nucleoside phosphorylases.

The mode of action of BVDU is based on (i) a preferential phosphorylation by the virus-induced dThd kinase [3], and (ii) the interaction of BVDU 5′-triphosphate with viral DNA polymerase which may lead to incorporation of BVDU into DNA [4,5]. As this would obviously affect the functioning of DNA, synthetic oligomers containing BVDU are of interest. They allow the study of structural parameters induced by the antiviral agent and the interaction of BVDU-modified oligomers with enzymes.



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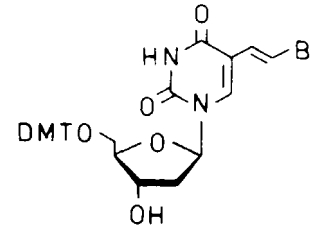

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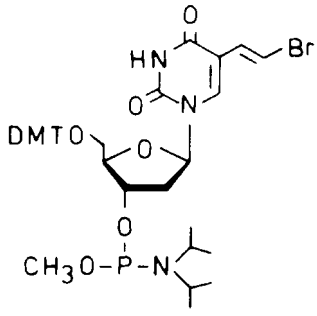

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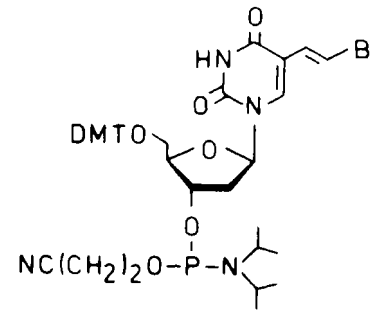

4

Recently, BVDU 5′-triphosphate was polymerized in the presence of dATP by DNA polymerase to yield high-molecular weight alternating polymers, which were then subjected to physicochemical studies [6]. One drawback of this approach is the non-homogeneity of DNA-fragments with respect to chain length and limitation of sequence changes due to enzymatic synthesis.

We now report on the synthesis of the BVDU-phosphoramidites 3 and 4 which can be employed in solid-phase oligonucleotide synthesis of any particular sequence. As the phosphoramidites 3 and 4 bear protecting groups compatible to those of regular monomers employed in automatized DNA synthesizers any kind of a defined oligomer containing BVDU can now be prepared. In this study self complementary dodecamers containing one BVDU residue were synthesized and their melting profiles as well as their regiospecific phosphodiester hydrolysis by the Eco RI endodeoxyribonuclease have been studied.

RESULTS AND DISCUSSION

Recent progress of oligonucleotide synthesis which makes use of solid support [7] and phosphoramidite chemistry allows access to oligomers containing more than 100 nucleotides. As polymeric supports and phosphoramidites of regular DNA-constituents are commercially available, incorporation of BVDU into oligomers requires the synthesis of the 5′-protected phosphoramidites 3 or 4. BVDU (1) itself was synthesized according to a procedure of Walker et al. [1].

From earlier work it was apparent that ^{13}C as well as ^{1}H NMR chemical shifts of BVDU were not unambiguously assigned. Therefore we made a complete assignment by two-dimensional homo- and heteronuclear NMR spectroscopy. First, the gated-decoupled NMR spectrum of 1 was measured in [D_6]-DMSO solution. Chemical shifts and coupling constants are shown in Tables 1 and 2. According to the coupling pattern (TABLE 2) all carbon signals of BVDU can be unambiguously assigned. A differentiation between carbon-7 and 8 was possible based on the more complex coupling pattern of C-7. Furthermore, carbon-8 showed the larger $^{1}J_{C,H}$ coupling constant induced by the electronegative bromine substituent. Carbon-2 was identified by the $^{3}J_{C,H}$ couplings with the anomeric proton and carbon-6. Next, the ^{13}C NMR data of BVDU

TABLE 1. ^{13}C NMR chemical shifts (δ) of compound 1 and related nucleosides in [D_6]DMSO

	dT	1	2
C-2	150.5	149.2	149.3
C-4	163.8	161.6	161.8
C-5	109.4	109.7	110.1
C-6	136.2	139.4	139.4
C-1′	83.8	84.5	84.5
C-2′	39.8	39.8	DMSO
C-3′	70.5	69.9	70.2
C-4′	87.3	87.5	86.0
C-5′	61.4	60.9	63.8
CH_3	12.3	-	-
C-7	-	129.8	129.8
C-8	-	106.5	107.1

TABLE 2. $J_{C,H}$ Values (Hz) of BVDU 1 and dT

$J_{C,H}$		BVDU	dT
C(2),	H-C(6)	8.0	8.0
	H-C(1′)	1.6	2.1
C(4),	H-C(6)	7.9	10.0
	H-C(7)	7.4	-
	H-C(CH_3)	-	3.8
C(5),	H-C(6)	-	-
	H-C(7)	4.0	-
	H-C(8)	4.0	-
	H-C(CH_3)	-	6.0
C(6),	H-C(6)	182.0	181.0
	H-C(1′)	4.6	4.0
	H-C(CH_3)	-	6.0
	H-C(7)	4.6	-
C(7),	H-C(6)	5.8	-
	H-C(7)	185.5	-
	H-C(8)	5.8	-
C(8),	H-C(7)	13.0	-
	H-C(8)	206.8	-
C(1′),	H-C(1′)	169.8	168.0
C(3′),	H-C(3′)	148.5	150.4
C(4′),	H-C(4′)	148.4	148.0
C(5′),	H-C(5′)	140.4	140.3

were correlated with those of dT. Similar chemical shifts were observed for almost all parts of the molecule being identical (TABLE 1). A 3.2 ppm downfield shift of carbon-6 of BVDU as compared to dT was found. Correlation of BVDU with dT spectra allowed the assignment of carbon-5. Assignment of carbon-4 was made according to its $^{3}J_{C,H}$ coupling pattern with 6-H and 7-H. A still open question was the assignment of the vinylic protons (7-H and 8-H) of compound 1. From the two-dimensional [^{1}H,^{13}C] correlation spectrum of 1 (FIG. 1) it is apparent that the olefinic signal located at high field was 7-H and that at lower field 8-H. Other proton assignments can also be taken from this correlation and are in agreement with those reported earlier [1].

An X-ray structure of BVDU [8] published recently showed the orientation of the olefinic side chain as depicted in formula 1. The conformation derived from the solid state seems to be the same as formed in solution. From the difference of the NOESY (FIG. 2b) and COSY spectra (FIG. 2a) of compound 1 measured between 5.8 and 8.4 ppm it can be seen, that 7-H shows a stronger NOE than 8-H. As NOESY cross peaks point to close spatial proximities of 7-H and 6-H, the preferred conformation of the side chain in solution is as in formula 1.

To employ compound 1 in oligonucleotide synthesis the nucleoside was protected at the 5′-hydroxyl group with 4,4′-dimethoxytrityl chloride. Compound 2 was isolated crystalline. Phosphitylation of 2 with bis(diisopropylamino)methoxyphosphane [9] gave 3 which was purified by flash-chromatography. ^{31}P NMR spectroscopy showed that a mixture of R_P-and S_P-diastereoisomers was formed. Similar to the methylphosphoramidite the cyanoethyl compound 4 was prepared from 2 and β-cyano-ethoxychloro-N,N-diisopropylphosphane [10]. To ensure that the phosporamidites 3 and 4 were suitable intermediates for oligomerization, they were employed upon synthesis of the oligomer 5 in an automated DNA-synthesizer. Synthesis and purification of the oligomer

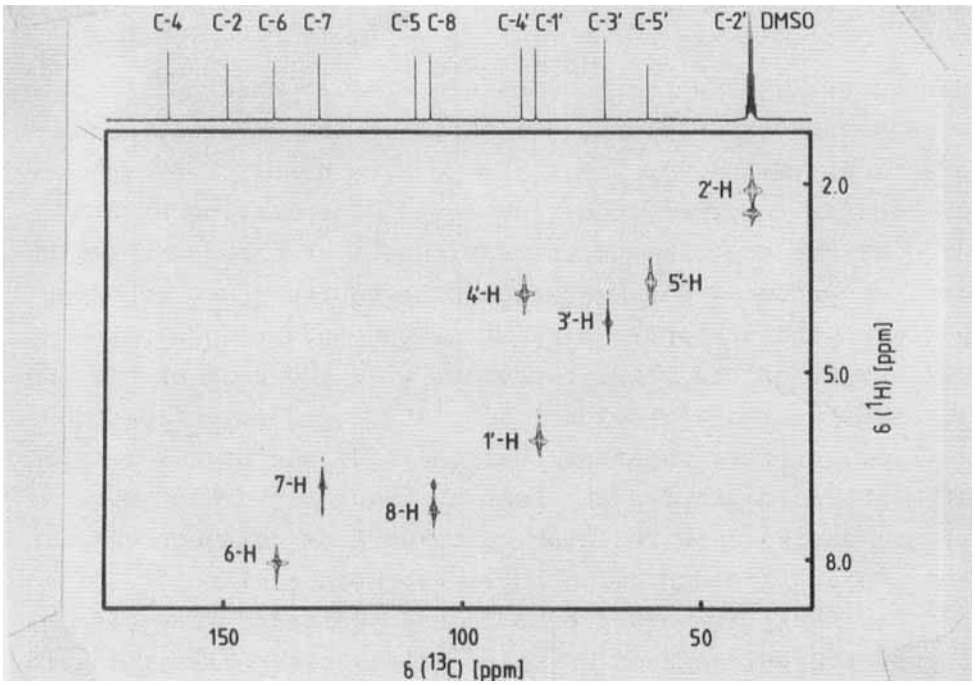

FIG. 1. Two-dimensional [^{1}H, ^{13}C]-correlation spectrum (XHCORR) of BVDU (1) in [D_6]DMSO. For details see Experimental Section.

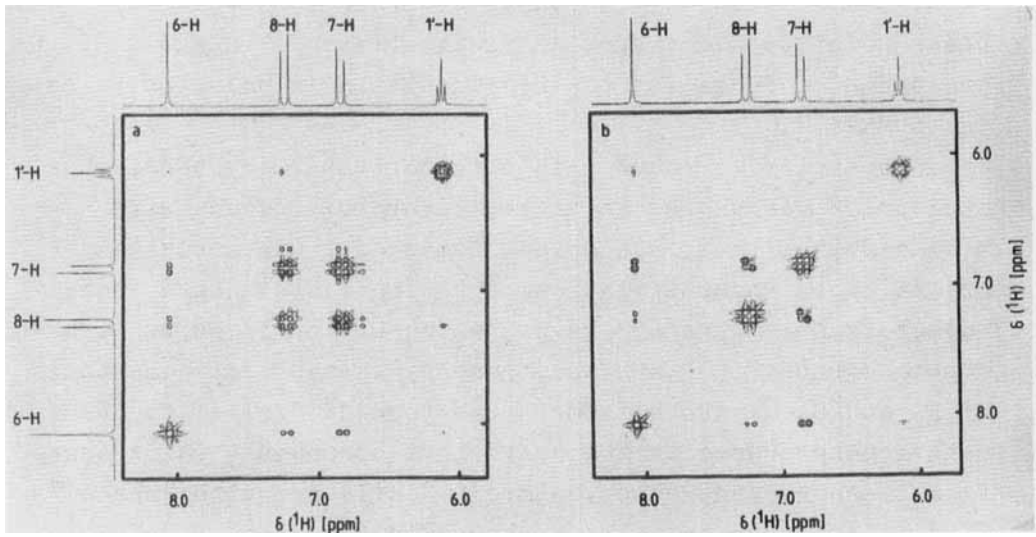

FIG. 2. a) Symmetrized two-dimensional [^{1}H, ^{1}H]-correlation spectrum (COSY) and b) symmetrized [^{1}H, ^{1}H]-correlation spectrum (NOESY) of BVDU (1) in [D_6]DMSO. For details see Experimental Section.

was carried out according to the methodology described in the Experimental Section. The composition of the oligomer was determined by hydrolysis with snake venom phosphodiesterase followed by alkaline phosphatase. Reverse-phase HPLC separated the nucleosides which then allowed the calculation of the dT/BVDU ratio. According to $\varepsilon(dT)=8800$ and $\varepsilon(BVDU)=11800$ a ratio of 11:1 was obtained. Both phosphoramidites (3 and 4) were then used in solid-phase synthesis of the alternating oligomer 7 and of palindromic oligonucleotides 9 and 10. The latter contained the recognition site of the endodeoxyribonuclease Eco RI.

5	d(TTTTTbv^{5}UTTTTTT)	8	d(GTAGAATTCTAC)
6	d(ATATATATATAT)	9	d(GTAGAAbv^{5}UTCTAC)
7	d(ATATAbv^{5}UATATAT)	10	d(GTAGAATbv^{5}UCTAC)

In order to show that the oligomers 6-10 form duplexes under conditions employed later for enzymatic hydrolysis experiments melting profiles were determined in 10 mM TRIS-HCl buffer (pH 7.5), containing 20 mM $MgCl_2$, and 80 mM NaCl. As Figure 3 shows, sigmoidal melting curves were observed indicating cooperative helix-coil transition in all cases. Table 3 summarizes the T_m-values and shows that the modified oligomers (7, 9, and 10) exhibited very similar T_m-values as the parent compounds (6 and 8).

These findings indicate that short DNA-fragments can accommodate one bulky bromovinyl residue without changing its secondary structure. As this residue is located in the major groove of the DNA helix steric interference with other bases is not expected. However, in alternating oligomers containing only BVDU and dA it is apparent that the accumulation of bromovinyl residues influences DNA secondary structure either by steric repulsion and/or hydrophobization of the major groove.

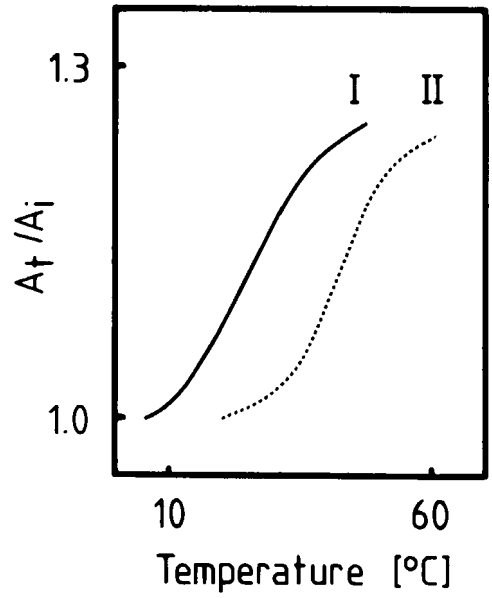

FIG. 3. Melting profiles of $[d(ATATAbv^5UATATAT)]_2$ (I) and $[d(GTAGAATbv^5UCTAC)]_2$ (II) in water, containing 2.5 µM oligomer, 10 mM TRIS-HCl (pH 7.5), 20 mM $MgCl_2$ and 80 mM NaCl. A_t/A_i is the ratio of absorbance at 260 nm at a given temperature (t) to the initial temperature (i).

TABLE 3. T_m Values of the oligomers 6-10 at 260 and 280 nm

oligomer		Tm (^{0}C)[a]	
		260 nm	280 nm
$d(ATATATATATAT)_2$ [15]	(6)	26	-[b]
$d(ATATAbv^5UATATAT)_2$	(7)	25	-[b]
$d(GTAGAATTCTAC)_2$ [11]	(8)	44	44
$d(GTAGAAbv^5UTCTAC)_2$	(9)	43	43
$d(GTAGAATbv^5UCTAC)_2$	(10)	43	44

[a] T_m Values were determined in 10 mM TRIS-HCl buffer, pH 7.5, containing 20 mM $MgCl_2$, 80 mM NaCl and an oligomer concentration of 2.5 µM; [b] no transition.

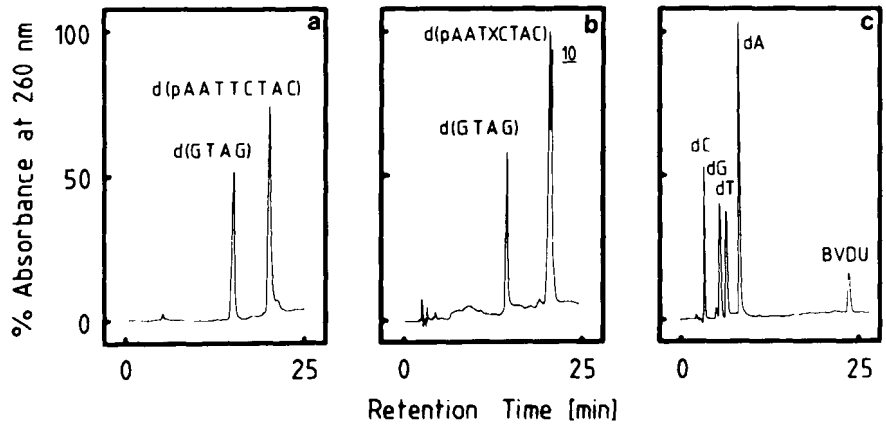

FIG. 4. Reverse-phase HPLC profile of d(GTAGAATTCTAC) (a) and d(GTAGAATbv^{5}UCTAC) (b) after hydrolysis with the endodeoxyribonuclease Eco RI. The right hand pattern (c) shows the profile of a complete enzymatic hydrolysis of the oligomer 10 by snake venom phosphodiesterase followed by alkaline phosphatase.

It has been reported earlier that enzymatically synthesized oligomers containing dA and BVDU are hydrolyzed by DNA-processing enzymes non-specific for nucleobase residues at slower rates than the parent d(A-T) [6]. We have studied the regiospecific phosphodiester hydrolysis occuring between dG and dA residues within the d(GAATTC) recognition sequence of the endodeoxyribonuclease Eco RI.

We have compared hydrolysis rates of compounds 9 and 10 with those of the parent oligomer 8. Under regular assay conditions the non-modified oligomer 8 was hydrolyzed completely within 2 h (FIG. 4a). In contrast, neither 9 nor 10 were cleaved even after prolonged treatment with the enzyme (24 h). These results indicate that the endonuclease Eco RI can accept methyl groups as in dT or other small substituents at C-5 of the pyrimidine moiety but cannot interact with the DNA-fragment if bulkier substituents as the (E)-5-(2-bromovinyl) group are located in the major groove. We have also measured hydrolysis by Eco RI under star activity

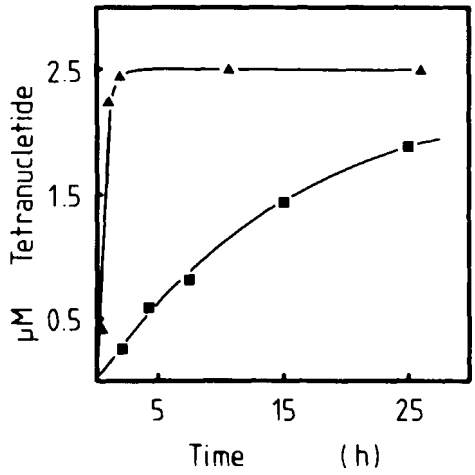

FIG. 5. Hydrolysis rate of the oligomers 8 and 10 by the endodeoxyribonuclease Eco RI. The concentration of each of the oligomers was 2.5 μM. Samples (1 mL water) contained 100 mM TRIS/HCl, pH 7.5, 20 mM $MgCl_2$, and 80 mM NaCl in the case of 8 and 25 mM TRIS/HCl, pH 8.5, 2 mM $MgCl_2$, in case of 10. The amount of the endodeoxyribonuclease Eco RI was 100 units. The reaction mixture was incubated at 30 ^{0}C. Aliqouts were analysed by reverse-phase HPLC (solvent system III). (▲) d(GTAGAATTCTAC) (8), (■) d(GTAGAATbv^{5}UCTAC) (10)

conditions [11] which reduce recognition of the parent sequence d(GAATTC). In this case the oligomer 10 was subject to slow hydrolysis whereas 9 was still resistant towards the enzyme (FIG. 4b).

From the crystal structure of the complex between Eco RI endonuclease and the cognate oligonucleotide d(TCGCGAATTCGCG) it is known that the 7-nitrogens of the dA adjacent to dT are binding sites for the enzyme [12]. Investigations on oligonucleotides where the dA residues are replaced by 2'-deoxytubercidin are in agreement [13] with these findings. An explanation for the different results obtained with the BVDU-modified oligomers 9 and 10 can be given based on the fact that in 9 the bromovinyl residue is in proximity of the 7-nitrogens of the adenine bases whereas in 10 these positions are free and therefore accessible for

the enzyme. Further studies should give more information on the stereochemical influence of *BVDU* (1) on its nearest nucleobase within the DNA-duplex.

EXPERIMENTAL SECTION

Melting points were determined on a Linström apparatus (Wagner & Munz, FRG) and are not corrected. Elemental analysis were performed by Mikroanalytisches Labor Beller (Göttingen, FRG). 1D- and 2D-FT NMR spectra were measured on an AC 250 spectrometer equipped with an Aspect 3000 data system and an array processor (Bruker, FRG). Operational frequencies: ^{1}H: 250.133 MHz; ^{13}C: 62.898 MHz. δ Values are in ppm relative to tetramethylsilane as internal standard (^{1}H and ^{13}C) or to external 85% phosphoric acid (^{31}P). Chemical shifts are positive when downfield from the appropriate standard. Digital resolutions: ^{1}H: 0.275 Hz/pt; ^{13}C: 0.526 Hz/pt; Homonuclear correlation spectroscopy ([^{1}H,^{1}H]-COSY) was performed using the pulse sequence D_1-90^0-D_0-90^0-FID with a relaxation period D_1 of 1 sec and an initial delay D_0 of 3 µsec. The experiments were carried out with 2048 data points and 512 data points in the t_2 and t_1 directions. 2D-Nuclear Overhauser enhancement spectra (NOESY, magnitude mode) were carried out with 2048 data points and 1024 data points in the t_2 and t_1 dimensions. The pulse sequence was as follows: D_1-90^0-D_0-90^0-D_9-90^0-FID. The initial delay was set to 3 µsec, the relaxation delay D_1 to 5 sec, and the mixing time D_9 to 500 msec. 2D [^{1}H, ^{13}C] correlation spectrum was carried out with 2048 data points and 1024 data points in the t_2 and t_1 dimensions. The pulse sequence was as follows:

^{1}H : D_0-90^0-D_0- -D_0-D_3-90^0 BB
^{13}C: D_1 -180^0- 90^0-D_4-FID

The delays were set to: D_0=3 µsec, D_1=2.5 sec, D_3=0.00345 sec, and D_4=0.5 D_3. For all 2D-NMR experiments a typical 90^0 pulse width of 10.8 sec was used. After zero-filling,

sine-bell multiplication of the domain data, and fourier transformation, the contour plots with a digital resolution of 2.9 Hz/pt were obtained. UV spectra were recorded on a U 3200 spectrophotometer (Hitachi, Japan). Thin-layer chromatography (TLC) was performed on silica gel SIL G-25 UV_{254} plates (Macherey-Nagel, FRG). Flash chromatography was performed with silica gel 60 H (Merck, FRG) at 0.5 bar (N_2). Solvent systems for TLC: (A) CH_2Cl_2-MeOH (9:1), (B) CH_2Cl_2-acetone (8:2), (C) CH_2Cl_2-ethyl acetate-triethyl amine (45:45:10), (D) CH_2Cl_2-ethyl acetate (9:1). 2′-Deoxyadenosine, 2′-deoxycytidine, 2′-deoxyguanosine, and thymidine were purchased from Pharma-Waldhof (FRG). The protected phosphoramidites were synthesized according to the procedures of Baron and Caruthers [9] and Sinha et al. [10] in the case of the cyanoethylphosphoramidites.

CPG (70 µmol of immobilized protected 2′-deoxynucleoside/g of solid support) was purchased from Biosyntech (Hamburg, FRG). Snake venom phosphodiesterase (EC 3.1.4.1, from crotalus durissus), alkaline phosphatase (EC 3.1.3.1), and Eco RI (EC 3.1.23.13, from escherichia coli BS 5) were products of Boehringer Mannheim (FRG).

(E)-5-(2-Bromovinyl)-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyuridine (2).

Compound 1 (500 mg, 1.5 mmol) was dissolved in anhydrous pyridine. 4,4′-Dimethoxytrityl chloride (750 mg, 2.2 mmol) and N-ethyldiisopropylamine (520 µL, 3 mmol) were added and the solution was stirred for 1.5 h at room temperature. To this solution 5% aqueous $NaHCO_3$ (5 mL) was added and the solution was extracted twice with dichloromethane (20 mL). The combined organic layers were dried over sodium sulfate, filtered, and the solution was evaporated. The residue was purified by flash-chromatography (column 25x3 cm, solvent B). After evaporation of the solvent from the main zone colorless amorphous 2 (820 mg, 86%) was isolated and crystallized from n-hexane-diethyl ether (1:1) to yield color-

less plates with m.p. 132 °C. TLC (silica gel, solvent B) R_f 0.4. - UV(MeOH) λ_{max} 294 nm (ε 11200). - ^{1}H NMR ([D]$_6$DMSO) δ 2.25 (m, 2′-$H_{a,b}$), 3.19 (m, 5′-H), 3.73 (s, OCH_3), 3.89 (m, 4′-H), 4.29 (m, 3′-H), 5.32 (d, 3′-OH, J=4.6 hz), 6.18 (pt, 1′-H, J=6.5 hz), 6.44 (d, 7-H, J=13.5 hz), 7.39-7.24 (m, aromat. H and 8-H), 7.78 (s, 6-H), 11.64 (s, NH). - Anal. calcd. for $C_{32}H_{31}BrN_2O_7$ (635.5): C, 60.47; H, 4.92; N, 4.41. Found: C, 60.38; H, 5.16; N, 4.39.

(E)-5-(2-Bromovinyl)-3′-O-[(N,N-diisopropylamino)-methoxyphosphanyl])-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyuridine (3).

Compound 2 (500 mg, 0.79 mmol) dissolved in anhydrous dichloromethane (1 mL), was treated with N,N-diisopropylammonium tetrazolide (171 mg, 1.0 mmol) and bis(N,N-diisopropylamino)-methoxyphosphane (262 mg, 1.0 mmol) was added (syringe) under argon at room temperature. After 1 h the solution was diluted with ethyl acetate and extracted with saturated aqueous $NaHCO_3$. The organic layer was washed with aqueous sodium chloride and dried over sodium sulfate. After evaporation the foam was purified by flash chromatography on silica gel (column 25x3 cm, solvent C). Evaporation of the solvent of the main zone gave colorless amorphous 3 (600 mg, 95 %). TLC (silica gel, solvent C) R_f 0.9. -^{31}P NMR ($CDCl_3$) δ 150.67, 150.09 . Anal. calc. for $C_{39}H_{47}BrN_3O_8P$ (796.7): C, 58.75; H, 5.95; N, 5.27. Found: C, 58.62; H, 6.13; N, 5.27.

(E)-5-(2-Bromovinyl)-3′-O-[(β-cyanoethoxy)-(N,N-diisopropylamino)phosphanyl]-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyuridine (4).

Compound 2 (250 mg, 0.39 mmol), dissolved in anhydrous tetrahydrofurane was treated with N-ethyldiisopropylamine (77 mg, 0.6 mmoL) and (β-cyanoethoxy)-chloro-N,N-diisopropylphosphane (142 mg, 0.6 mmol) within 2 min at room temperature (syringe, argon). The resulting precipitate was filtered under argon and the filtrate was evaporated in

vacuo. The residue was dissolved in ethyl acetate (saturated with argon) and extracted with saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4 and applied to flash chromatography on silica gel (column 10x2 cm, solvent C). Coevaporation of the main zone with acetone afforded colorless amorphous 4 (268 mg, 82%).- TLC (silica gel, solvent D) R_f 0.3. - ^{31}P NMR ($CDCl_3$) δ 147.6, 147.2.

Oligonucleotide synthesis.

The oligomer 5-10 were synthesized using an Applied Biosystem DNA-synthesizer (Model 680 B) employing methoxyphosphoramidite (compound 3) or cyanoethoxyphosphoramidite chemistry (compound 4). Synthesis was carried out on solid support (CPG) in a 1 µmol scale. The reaction cycle of detritylation, coupling, oxidation, and capping followed the user manual (Edition 1986). Cleavage of the CH_3-protecting group was accomplished by the action of thiophenol. Removal of the oligomer from the solid support was carried out with concentrated ammonia at room temperature at the stage of the 5′-tritylated oligomers. This procedure also removed the cyanoethyl protecting groups. Further incubation (60°C, 24h) cleaved the nucleobase protecting groups.

The 5′-protected oligomers were purified by HPLC (solvent system I), and detritylation was performed by the action of 80% acetic acid for 5 min. After removal of the acid the oligomer was dissolved in water (5 mL) and extracted with diethyl ether. The deprotected oligomers were then purified by reverse-phase HPLC with solvent system II. Samples of the main zone were lyophilized and desalted by reverse phase HPLC with solvent system IV. After lyophilization the oligomers (15-20 OD) were dissolved in water (100 µL) and stored frozen at -20 °C.

HPLC separation.

High performance liquid chromatography was carried out on 4x250 mm (10µm) RP-18 LiChrosorb column (Merck, FRG) which was connected with a 4x25 mm RP-18 LiChrosorb pre-

column (Merck, FRG) using a Hitachi HPLC with one pump (model 655A-12), a variable wavelength monitor (model 655A), and a controller (model L-5000), connected with an integrator (Hitachi, model D-2000). The following gradients containing triethylammonium acetate (pH 7.0)/5% acetonitril (A), acetonitril (B), water (C) and methanol/water (3:2) (D) were used: I: 15 min (15-60% B) in A; II: 15 min (0-25% B) in A; III: 30 min (0-20%B) in A; IV: 15 min C - 10 min D.

Enzymatic hydrolysis of the oligomers 6-10.

The oligomer (about 0.3 A_{260} units) was dissolved in 0.1 M TRIS/HCl buffer, pH 8.5, (500 µL) and digested with snake venom phosphodiesterase (2 µg) for 30 min at 37°C. Further incubation with alkaline phosphatase (1 µg, 25 °C, 15 min) yielded the mixture of nucleosides. After separation on HPLC (solvent system III) quantification was made at 260 nm on the basis of the peak areas and the extinction coefficients of the nucleosides (dC 7300, dG 11400, dT 8800, dA 15400, and BVDU 11800).

Melting curves.

The melting curves were measured in Teflon stoppered cuvettes with 1 cm light path length in a thermostatically controlled cell holder with a Shimadzu 210-A recording spectrophotometer connected with a Kipp & Zonen BD 90 recorder. The increase of absorbance at the appropriate wavelength as function of time was recorded while the temperature of the solution was increased linearly at a rate of 20°C/h using a Lauda PM 350 programmer and a Lauda RCS-6 bath equipped with a R 22 unit (MWG Lauda, FRG). The actual temperature was measured in the probe cell with a Pt-resistor.

Phosphodiester-hydrolysis of the oligomers 8-10 with the endodeoxyribonuclease Eco RI.

The oligonucleotide (2.5 µM single strand concentration) dissolved in water (1 mL), containing 10 mM TRIS/HCl,

pH 7.5, 20 mM $MgCl_2$, and 80 mM NaCl was digested with the endodeoxyribonuclease Eco RI (100 units). Star activity was induced by 25 mM TRIS/HCl, pH 8.5, and 2 mM $MgCl_2$. One unit is the enzyme activity that completely cleaves 1 µg λ DNA in 1 h at 37 °C in the incubation buffer [100 mM TRIS/HCl, 50 mM NaCl, and 10 mM $MgCl_2$, pH 7.5 at 37 °C] in a total volume of 50 µL. The reaction mixture was incubated at 30 °C. Aliqouts were analysed at different intervals of time by HPLC (solvent system III). The amount of the reaction products was calculated according to Dwyer-Hallquist et al. [15].

ACKNOWLEDGEMENTS

We thank Dr. H. Rosemeyer for valuable discussion. Financial support by the Stiftung Volkswagenwerk and the BMFT is gratefully acknowledged.

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Received August 28, 1987