of various versions of the manuscript.

Registry No. DNA polymerase, 9012-90-2.

REFERENCES

Baril, E., Bonin, P., Burstein, D., Mara, K., & Zamecnik, P. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 4931-4935.
Battula, N., & Loeb, L. A. (1974) *J. Biol. Chem. 249*, 4086-4093.
Eichler, D. C., Fisher, P. A., & Korn, D. (1977) *J. Biol. Chem. 252*, 4011-4014.
Fisher, P. A., & Korn, D. (1979) *J. Biol. Chem. 254*, 11040-11046.
Fisher, P. A., Chen, J. T., & Korn, D. (1981) *J. Biol. Chem. 256*, 133-141.
Fry, M. (1983) in *Enzymes of Nucleic Acid Synthesis and Modification* (Jacob, S. T., Ed.) pp 39-92, CRC Press, Boca Raton, FL.
Fry, M., Silber, J., Loeb, L. A., & Martin, G. M. (1984) *J. Cell. Physiol. 118*, 225-232.
Hübscher, U. (1983a) *Experientia 39*, 1-25.
Hübscher, U. (1983b) *EMBO J. 2*, 133-136.
Hübscher, U., Gerschwiler, P., & McMaster, G. K. (1982) *EMBO J. 1*, 1513-1519.
John, B., & Miklos, G. L. G. (1979) *Int. Rev. Cytol. 58*, 1-114.
Kaftory, A., & Fry, M. (1978) *Nucleic Acids Res. 5*, 2679-2693.
Karawaya, E., Swack, J., Albert, W., Fedorko, J., Minna, J. D., & Wilson, S. M. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 7777-7781.
Knopf, K. W., & Weissbach, A. (1977) *Biochemistry 16*, 3190-3194.
König, H., Riedel, D. H. & Knippers, R. (1983) *Eur. J. Biochem. 135*, 435-442.
Lamothe, P., Baril, B., Chi, A., Lee, L., & Baril, E. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 4723-4727.
Lynch, W. E., Surrey, S., & Lieberman, I. (1975) *J. Biol. Chem. 250*, 8179-8183.
Noguchi, H., Reddy, G. P. V., & Pardee, A. B. (1983) *Cell (Cambridge, Mass.) 32*, 443-451.
Novak, B., & Baril, E. F. (1978) *Nucleic Acids Res. 5*, 221-239.
Ottiger, H. P., & Hübscher, U. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 3993-3998.
Otto, B., Baynes, M., & Knippers, R. (1977) *Eur. J. Biochem. 73*, 17-24.
Pritchard, C. G., & DePamphilis, M. L. (1983) *J. Biol. Chem. 258*, 9801-9809.
Pritchard, C. G., Weaver, D. T., Baril, E. F., & DePamphilis, M. L. (1983) *J. Biol. Chem. 258*, 9810-9819.
Rapaport, E., & Feldman, L. (1984) *Eur. J. Biochem. 138*, 111-115.
Rapaport, E., Zamecnik, P. C., & Baril, E. F. (1981) *J. Biol. Chem. 256*, 12148-12151.
Reddy, G. P. V., & Pardee, A. B. (1983) *Nature (London) 303*, 86-88.
Richter, A., Knippers, R., & Otto, B. (1978) *FEBS Lett. 91*, 293-296.
Sapp, M., König, H., Riedel, H. D., Richter, A., & Knippers, R. J. (1985) *J. Biol. Chem. 260*, 1550-1556.
Silber, J., Fry, M., Martin, G. M., & Loeb, L. A. (1985) *J. Biol. Chem. 260*, 1304-1310.
Tseng, B. Y., & Ahlem, C. N. (1983) in *Mechanisms of DNA Replication and Recombination* (Cozzareli, N., Ed.) pp 511-516, Liss, New York.
Wang, E.-C., Henner, D., & Furth, J. J. (1976) *Nucleic Acids Res. 3*, 129-147.
Wang, T. S.-F., Hu, S.-Z., & Korn, D. (1984) *J. Biol. Chem. 259*, 1854-1865.
Weisman-Shomer, P., Kaftory, A., & Fry, M. (1979) *J. Cell. Physiol. 101*, 219-228.
Yagura, T., Kozu, T., Seno, T., Saneyoshi, M., Hiraga, S., & Nagano, H. (1983) *J. Biol. Chem. 258*, 13070-13075.

Oligomers with Alternating Thymidine and 2′-Deoxytubercidin: Duplex Stabilization by a 7-Deazapurine Base†

Frank Seela* and Andreas Kehne
Laboratory of Bioorganic Chemistry, Department of Chemistry, University of Paderborn, D-4790 Paderborn, Federal Republic of Germany

Received May 23, 1985

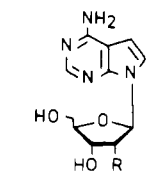
ABSTRACT: Self-complementary oligonucleotides with an alternating sequence of 2′-deoxytubercidin and thymidine and a chain length of 6 and 12 monomeric units have been synthesized by employing the phosphoramidite technique on solid support. The modified nucleoside **1a** used in these experiments has been prepared from 4-chloro-2-(methylthio)pyrrolo[2,3-*d*]pyrimidine and the halogenose **6** by applying phase-transfer conditions. The oligomers containing 7-deaza-2′-deoxyadenosine (**1a**) are compared to those having a parent d(A-T) sequence. Replacement of the adenine by the 7-deazaadenine base results in duplex stabilization of the oligomers $d(A\text{-}T)_3$ and $d(A\text{-}T)_6$. This is demonstrated by an increased T_m and a decreased susceptibility of $d(c^7A\text{-}T)_3$ and $d(c^7A\text{-}T)_6$ toward nuclease S1.

In a previous publication (Seela & Kehne, 1985) we have reported the synthesis of an appropriately protected phosphoramidite of 2′-deoxytubercidin (**1a**) that was employed in oligonucleotide synthesis in solution. By this means 2′-deoxytubercidylyl-(3′,5′)-2′-deoxytubercidin has been prepared; it exhibits unusual properties toward nucleoside processing enzymes like nucleases. To extend our studies from di-

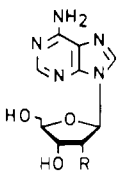
† This research was supported by the Deutsche Forschungsgemeinschaft and the Stiftung Volkswagenwerk.

0006-2960/85/0424-7556$01.50/0

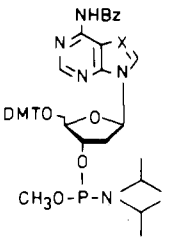
Chart I



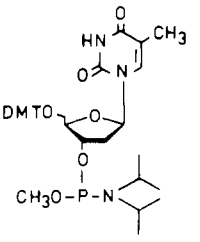
1a: R = H
b: R = OH



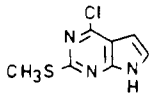
2a: R = H
b: R = OH



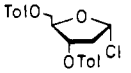
3a: X = CH
b: X = N



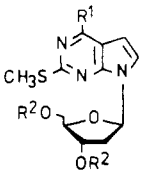
4



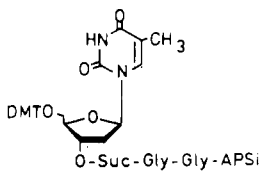
5



6



7a - c



8

Bz = Benzoyl; Tol = Toluoyl
DMT = 4,4'-Dimethoxytrityl

a: R^1 = Cl; R^2 = Tol
b: R^1 = Cl; R^2 = H
c: R^1 = NH_2; R^2 = H

Suc = Succinyl; Gly = Glycine
APSi = Aminopropyl silica gel

nucleoside monophosphates containing rare pyrrolo[2,3-*d*]-pyrimidine bases to oligonucleotides of biological interest, our work is now focused toward alternating d(A-T) sequences containing 2′-deoxytubercidin (**1a**) instead of 2′-deoxyadenosine (**2a**).

Studies on DNA containing long stretches of alternating A-T and T-A base pairs have provided evidence that these sequences form unique secondary structures that are biologically important (Sassone-Corsi et al., 1981; Klug et al., 1979). In particular, proteins like the *lac* repressor interact with poly[d(A-T)] 1000 times more strongly than with regular DNA (Klug et al., 1979). An X-ray structure of the tetramer $d(A\text{-}T)_2$ (Viswamitra et al., 1982) has shown that it forms a complementary duplex where a break of the helix form dT to dA is observed because of a weaker T-A interaction compared to that in the A-T configuration.

The unique properties of d(A-T) oligomers prompted us to substitute the purine by the corresponding pyrrolo[2,3-*d*]pyrimidine base. 7-Deazapurine nucleosides have been isolated as monomers from certain microorganisms (Suhadolnik, 1979) but are also constituents of nucleic acids (McCloskey & Nishimura, 1977). As monomers, adenosine analogues like tubercidin (Anzai et al., 1957) as well as guanosine derivatives such as cadeguomycin (Wu et al., 1982) are antimetabolites of natural origin. Several tRNAs mainly from *Escherichia coli* contain the 7-deazaguanosine derivative queuosine (Kasai et al., 1975). 7-Deazaguanosine itself has recently been incorporated into homopolynucleotides (Seela et al., 1982a,b). Due to the lack of N-7 in 2′-deoxytubercidin (**1a**) it can be expected that its dipole moment and as a consequence the π-electron interactions are different in oligomers containing the deoxy analogue **1a** of the naturally occurring antibiotic tubercidin (**1b**) (see Chart I).

In the following we report on the synthesis of the self-complementary hexamer $d(c^7A\text{-}T)_3$ (**15a**) and of the dodecamer $d(c^7A\text{-}T)_6$ (**15b**) employing solid-phase techniques (Caruthers, 1982) and using the phosphoramidites **3a** (Seela & Kehne, 1985) and **4** (McBride & Caruthers, 1983). For comparison of structural data of the modified oligomers **15a** and **15b** with the regular oligomers, the oligonucleotides **14a** and **14b** have been synthesized by the same technique (see Chart II).

Experimental Procedures

Melting points were determined on a Linström apparatus (Wagner & Munz, Munich, FRG) and are not corrected. Elemental analyses were performed by Microanalytisches Labor Beller (Göttingen, FRG). NMR[1] spectra were recorded on a Bruker WM 250 spectrometer; values are in parts per million relative to tetramethylsilane as internal standard. Chemical shifts are positive when downfield from the appropriate standard. UV spectra were recorded on a Uvicon 810 spectrometer (Kontron, Munich, FRG). Thin-layer chromatography was performed on silica gel SIL G-25 UV_{254} plates (Macherey-Nagel, Dueven, FRG). Dowex ion-exchange resin was purchased from Serva (Heidelberg, FRG). Solvent systems used for TLC were (A) CH_2Cl_2–ethyl acetate (95:5) and (B) $CHCl_3$–MeOH (9:1). Dichloromethane and acetonitrile used for oligonucleotide synthesis were predried with P_2O_5 and redistilled from CaH_2. Tetrazole, 4-(dimethylamino)pyridine, and trichloroacetic acid were sublimated under reduced pressure. Snake venom phosphodiesterase (EC 3.1.4.1, *E. coli*) and nuclease S1 (EC 3.1.30.1) were products of Boehringer

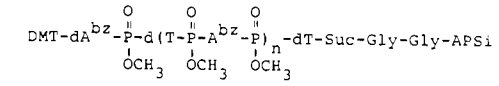
[1] Abbreviations: c^7A (tubercidin, 7-deazaadenosine), 4-amino-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine; 2′-deoxytubercidin, 4-amino-7-(β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine; T, thymidine; A, adenosine; cadeguomycin, 2-amino-4-oxo-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid; queuosine, 2-amino-5-[[(1*S*,4*R*,5*S*)-(4,5-dihydroxycyclopent-2-en-1-yl)amino]methyl]-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidin-4-one; $d(A\text{-}T)_3$, 2′-deoxyadenylyl-(3′,5′)-thymidylyl-(3′,5′)-2′-deoxyadenylyl-(3′,5′)-thymidylyl-(3′,5′)-2′-deoxyadenylyl-(3′,5′)-thymidine; $d(A\text{-}T)_6$, $d(A\text{-}T)_3$ with three additional A-T units; $d(c^7A\text{-}T)_3$ and $d(c^7A\text{-}T)_6$, $d(A\text{-}T)_3$ and $d(A\text{-}T)_6$ with each A replaced by c^7A; Gly, glycine; THF, tetrahydrofuran; Suc, succinate; Tris, tris(hydroxymethyl)aminomethane; Bz, benzoyl; Tol, toluoyl; DMT, 4,4′-dimethoxytrityl; APSi, 3-aminopropyl silica gel; $\tau_{1/2}$, half-rate time; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance.

Table I: ^{13}C NMR Chemical Shifts of 7-Deazapurine and Purine 2'-Deoxyribonucleosides in Me_2SO-d_6[a]

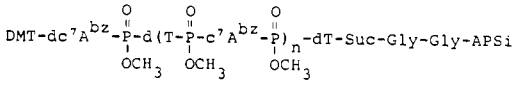
carbon	1a	1b	2a	2b	7a	7b	7c
C-2	151.3	151.3	152.1	152.1	163.5	163.2	163.1
C-4/C-6	157.3	157.4	148.8	149.0	151.5	151.4	157.1
C-4a/C-4	102.8	103.0	119.2	119.2	114.0	113.8	100.1
C-5/-	99.3	99.3			100.0	99.6	100.0
C-6/C-8	121.4	122.1	139.2	139.6	126.9	126.8	120.2
C-7a/C-5	149.6	149.9	155.9	155.9	150.7	150.4	150.8
C-1'	83.2	87.6	83.7	87.8	81.1	83.1	82.6
C-2'	39.8	73.5	39.8	73.3	35.9	40.4	39.9
C-3'	70.9	70.5	70.8	70.4	74.5	70.6	71.0
C-4'	87.1	84.9	87.8	85.6	83.8	87.4	87.1
C-5'	62.0	61.7	61.7	61.5	63.8	61.5	62.0
SCH_3					13.6	13.6	13.1
C=O					165.2		
CH_3					20.9		

[a] The NMR signals (δ) are relative to tetramethylsilane as internal standard.

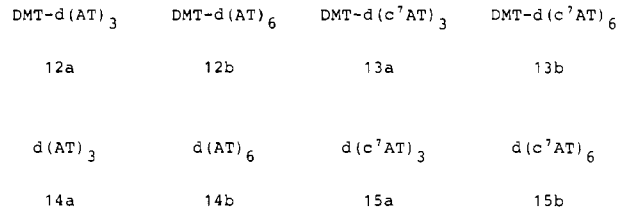
Chart II



10a n = 2
b n = 5



11a n = 2
b n = 5



(Mannheim, FRG). 2'-Deoxyadenosine and thymidine were purchased from Pharma-Waldhof (Heidelberg, FRG).

Melting Curves. The melting curves were measured in Teflon-stoppered cuvettes with 2-mm light path length in a thermostatically controlled cell holder with a Shimadzu 210-A recording spectrometer connected with a Kipp & Zonen BD 90 recorder. The increase of absorbance at the appropriate wavelength as a function of time was recorded while the temperature of the solution was increased linearly with time at a rate of 20 °C/h by using a Lauda PM-350 programmer and a Lauda RCS 6 bath equipped with an R 22 unit (MWG, Lauda, FRG). The actual temperature was measured in the reference cell with a Pt resistor.

Determination of Hypochromicity. The hypochromicity was calculated by the formula $H = [(\epsilon_{monomer} - \epsilon_{oligomer})/\epsilon_{monomer}] \times 100$ by enzymatic cleavage. The oligomer was digested with snake venom phosphodiesterase in Tris-HCl buffer (0.1 M, pH 8.5). Hypochromicity was calculated from the absorbance before and after the cleavage.

HPLC Separation. High-performance liquid chromatography was carried out on a 4 × 250 mm (7-μm) RP-18 LiChrosorb column (Merck, FRG) by using an LKB HPLC with two pumps (Model 2150), a variable wavelength monitor (Model 2151), and a controller (Model 2152), connected with an integrator (Hewlett-Packard 3390A). The solvent systems and gradients consisting of 0.1 M triethylammonium acetate, pH 7.0 (A), and acetonitrile (B) were used in the following order: solvent system I, 12% B; solvent system II, 28% B; solvent system III, 30% B; solvent system IV, 10–25% B for 10 min. The flow rate was 1 mL/min.

Minireactor for Oligonucleotide Synthesis. Solid-phase synthesis was carried out in a HPLC LiChroCART cartridge (25-4). This cartridge was placed into a manu-fix 25-4 device (Merck, Darmstadt, FRG) equipped with a male and a female Luer adapter. The unit can then be connected with a syringe and a needle.

7-(2-Deoxy-β-D-erythro-pentofuranosyl)-4-chloro-2-(methylthio)-7H-pyrrolo[2,3-d]pyrimidine (**7b**). Compound **5** (500 mg, 3.0 mmol) was stirred with the deoxyhalogenose **6** (1030 mg, 2.65 mmol) and tetrabutylammonium hydrogen sulfate (10 mg, 30 μmol) in 20 mL of 50% sodium hydroxide solution/dichloromethane (1:3 v/v) for 10 min with a vibromixer. After separation of the layers the hydroxide solution was extracted with 10 mL of dichloromethane 2 times. The organic layers were dried over sodium sulfate, and after evaporation the residue was purified by chromatography on silica gel with chloroform as eluent. Separation of the main zone yielded the protected nucleoside **7a**, which crystallized from methanol as colorless needles (708 mg, 52%) with a melting point of 117 °C. Compound **7a** (600 mg, 1.2 mmol) was treated with sodium methoxide (0.5 M, 50 mL) at room temperature for 2 h. After evaporation of the solvent the product was purified by Dowex ion-exchange chromatography (20 × 2.5 cm, OH^- form) with water (500 mL) and water/methanol (2:3, 2 L) as eluents. After evaporation compound **7b** crystallized as colorless needles from methanol as solvent (274 mg, 73%): mp 196 °C; TLC (silica gel, solvent B) R_f 0.4; UV (methanol) λ_{max} 251, 275, 308 nm (ϵ 24 400, 7200, 5600, λ_{min} 233, 268, 291 nm (ϵ 8800, 7000, 5600); ^1H NMR (Me_2SO-d_6) δ 2.25 (1 H, m, H-2'b), 2.51 (1 H, m, H-2'a), 2.57 (3 H, s, SCH_3), 3.53 (2 H, m, H-5'a,b), 3.85 (1 H, m, H-4'), 4.38 (1 H, m, H-3'), 4.94 (1 H, t, HO-5', J = 5.4 Hz), 5.33 (1 H, d, HO-3', J = 4.2 Hz), 6.57 (1 H, dd, H-1', J = 7.3 Hz), 6.61 (1 H, d, H-5, J = 3.8 Hz), 7.78 (1 H, d, H-6, J = 3.8 Hz); ^{13}C NMR data, see Table I. Anal. Calcd for $C_{12}H_{14}N_3O_3ClS$: C, 45.65; H, 4.48; N, 13.31; Cl, 11.22; S, 10.14. Found: C, 46.04; H, 4.65; N, 13.16; Cl, 11.01; S, 10.24.

4-Amino-7-(2-deoxy-β-D-erythro-pentofuranosyl)-2-(methylthio)-7H-pyrrolo[2,3-d]pyrimidine (**7c**). Compound **7b** (150 mg, 480 μmol) was treated with 50 mL of methanol, saturated with ammonia at 0 °C, for 2 h at 120 °C and 3 × 10^7 Pa. After evaporation of the solvent the product was purified by Dowex ion-exchange chromatography (10 × 2.5 cm, OH^- form) with water (500 mL) and water/methanol (2:3, 1 L) as eluents. Crystallization from methanol yielded **7c** as colorless needles (113 mg, 80%), which were identical

with authentic material (Seela & Kehne, 1983) in all physical parameters.

Solid-Phase Synthesis of the Oligomers **14a**, **14b**, **15a**, *and* **15b**. The minireactor was charged with 40 mg of the modified silica gel **8** containing 70 μmol of immobilized 2′-deoxythymidine/g of solid support. The following reaction cycle was used for oligomerization: (1) detritylation by addition of 5 mL of a 3% solution of trichloroacetic acid in dichloromethane for 5 min; (2) washing with dichloromethane (5 mL) and acetonitrile (5 mL); (3) drying with argon for 5 min; (4) coupling with a 20-fold excess of the nucleoside phosphoramidite in 0.5 mL of a 0.1 M solution of tetrazole in absolute acetonitrile for 15 min; (5) washing with acetonitrile (3 mL) and dichloromethane (3 mL); (6) capping of unreacted hydroxyl groups by addition of 1 mL of a mixture (1:1 v/v) of (a) (dimethylamino)pyridine (600 mg) in absolute tetrahydrofuran (10.7 mL) and (b) acetic anhydride/tetrahydrofuran/2,6-lutidine (8:1:1 v/v/v) for 5 min; (7) washing with dichloromethane (3 mL) and tetrahydrofuran/pyridine/water (40:20:1 v/v/v, 1 mL); (8) oxidizing by adding a 0.1 M solution of iodine dissolved in tetrahydrofuran/pyridine/water (40:20:1 v/v/v, 1 mL) for 1 min; (9) washing with tetrahydrofuran/pyridine/water (40:20:1 v/v/v, 3 mL), tetrahydrofuran (5 mL), and dichloromethane (10 mL). Step 9 completed the addition of one nucleoside. The oligomer was then elongated by beginning at step 1.

The last reaction cycle ended at washing step 9, omitting the capping procedure at step 6. After being dried with argon for 10 min the reactor was filled with thiophenol/triethylamine/dioxane (1:1:2 v/v/v, 1 mL) for demethylation of the phosphotriesters and rotated for 90 min. After being washed with methanol (20 mL) and diethyl ether (20 mL) the polymer support was poured into 10 mL of a 25% solution of ammonia and treated for 16 h at room temperature. The solution was then decanted from the solid support, and the base protecting groups were removed by storing at 60 °C for 24 h in the case of the alternating d(A-T) sequences **12a** and **12b** and for 72 h for the modified oligomers **13a** and **13b**. After filtration the solution was concentrated to 1 mL, and the dimethoxytritylated oligomers **12a**, **12b**, **13a**, and **13b** were purified by reverse-phase HPLC using the solvent system II or III (see Figure 1, Table II). The collected fractions containing the 5′-protected oligomers were evaporated, and the dimethoxytrityl residue was completely removed by treatment with 80% acetic acid (5 mL) for 20 min. The acid was then removed by evaporation under reduced pressure. The residue was dissolved in 5 mL of water, and the solution was extracted with diethyl ether (5 mL) 5 times. After purification on reverse-phase HPLC (solvent system IV, Figure 1b) and lyophilization, the purified products were dissolved in 1 mL of water and stored frozen at −20 °C. Usually between 0.4 and 0.6 μmol of pure oligonucleotides was obtained, which represents a yield between 15% and 20% on the basis of the polymer-linked thymidine.

Enzymatic Hydrolysis of the Oligomers. The nucleotide oligomer (about 0.3 A_{260} unit) was dissolved in 1 mL of 0.1 M Tris-HCl buffer, pH 8.5, and treated with snake venom phosphodiesterase (5 μg) at room temperature for 2 h. After further incubation with alkaline phosphatase (5 μg) at room temperature for 15 min the mixture was analyzed by HPLC with solvent system I as eluent. Quantification of the material was made on the basis of the peak areas, which were divided by the extinction coefficients of the containing nucleoside. (ϵ_{267}: dA, 12.4; dT, 9.7. ϵ_{270}: dTu, 12.3; dT, 9.4.)

Hydrolysis of the Nucleotide Oligomers by Nuclease S1. The cleavage of the oligonucleotides by nuclease S1 was determined spectrophotometrically. For this purpose 5 μmol of each hexamer, **14a** and **15a**, dissolved in 1 mL of 0.03 M sodium acetate buffer, pH 4.5, 1 M NaCl, and 1 mM $ZnSO_4$ was treated with 250 units of nuclease S1 in a quartz cuvette of 1-cm light path length. The increase of absorbance was recorded as a function of time at 25 °C. In the case of the dodecamers **14b** and **15b**, 1.2 μmol of each oligomer was dissolved in 1 mL of the buffer described above but containing 0.1 M $MgCl_2$. Digestion was performed at 20 °C. The percentage of cleavage was calculated at that temperature and related to the hypochromicity determined by phosphodiesterase cleavage.

Results and Discussion

Earlier we have shown that the phosphoramidite of the appropriately protected 7-deaza-2′-deoxyguanosine can be successfully used in solid-phase synthesis of oligonucleotides (Seela & Driller, 1985). In order to produce the oligomers **15a** and **15b**, sufficient amounts of 2′-deoxytubercidin had to be prepared. Besides semisynthetic procedures the nucleoside has been synthesized by applying phase-transfer glycosylation on 4-amino-2-(methylthio)-7*H*-pyrrolo[2,3-*d*]pyrimidine with the halogenose **6** (Seela & Kehne, 1983). Since it was shown in the series of arabinonucleosides that phase-transfer glycosylation of 4-chloro-2-(methylthio)-7*H*-pyrrolo[2,3-*d*]pyrimidine results in higher yields of glycosylated material (Winkeler & Seela, 1980), we employed the chromophore **5** in the preparation of the deoxynucleoside **1a**.

Phase-transfer glycosylation (Seela et al., 1986b) was carried out in a biphasic mixture of dichloromethane and 50% aqueous sodium hydroxide. The organic layer contained the chromophore **5**, the halogenose **6**, and tetrabutylammonium hydrogen sulfate as the phase-transfer catalyst. Glycosylation took place by thorough mixing with a vibromixer and was complete within 10 min at room temperature. From the crude glycosylation product **7a**, which can be obtained crystalline from methanol, the protecting groups were removed by treatment with sodium methoxide to yield the chloronucleoside **7b** in 73% yield. Nucleophilic displacement of the halogen with methanolic ammonia resulted in the formation of 2-(methylthio)-2′-deoxytubercidin (**7c**), which was then desulfurized with Raney nickel catalyst to give compound **1a** in 65% yield. The preparation of the protected nucleoside **7a** by a more laborious and time-consuming procedure (Kazimierczuk et al., 1984) (absolute acetonitrile, nitrogen atmosphere) resulted in similar yields of the reaction product.

Since sufficient amounts of 2′-deoxytubercidin (**1a**) have been available by the phase-transfer glycosylation technique, the pyrrolo[2,3-*d*]pyrimidine nucleoside **1a** was appropriately protected at the heterocyclic amino and 5′-hydroxyl group and converted into the phosphoramidite **3a** (Seela & Kehne, 1985). The incorporation into the self-complementary hexamer **15a** and the corresponding dodecamer **15b** followed a protocol that has been already used in the synthesis of the hexamer $d(c^7G\text{-}C)_3$ (Seela & Driller, 1985) following a procedure developed by Caruthers (1982). As solid support the modified silica gel **8**, carrying 5′-dimethoxytritylated thymidine via an alkaline labile succinate–glycine linker, was used. Two glycine residues were inserted between the (3-aminopropyl)silyl functionalized macroporous silica and the 3′-succinyl residue in order to ensure a high coupling yield during the first oligomerization step (Gait et al., 1982).

Purification of the DMT-protected oligonucleotides was accomplished by reverse-phase HPLC. Figure 1a shows a typical HPLC pattern of the crude condensation product of the reaction cycles after debenzoylation. Figure 1b exhibits

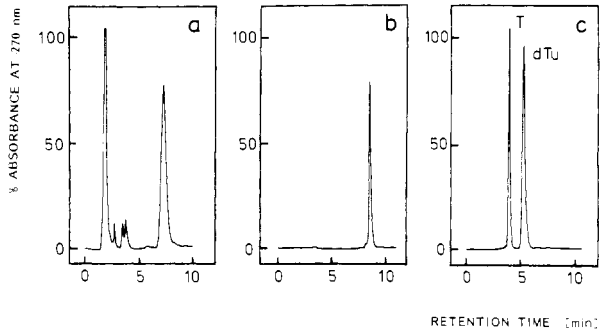


FIGURE 1: HPLC elution profile of (a) the crude reaction mixture of the deacylated DMT-oligomer **13b** (solvent system II), (b) the purified oligomer **15b** (solvent system IV), and (c) the enzymatically hydrolyzed oligomer **15b**. Digestion was performed with snake venom phosphodiesterase followed by alkaline phosphatase (see also Experimental Procedures).

Table II: HPLC Retention Times of Oligomers on a Reverse-Phase Column[a]

compd	oligomer	retention time (min)	solvent system	molar content of purine vs. pyrimidine nucleosides
12a	DMT-d(A-T)$_3$	8.0	III	
12b	DMT-d(A-T)$_6$	7.3	II	
13a	DMT-d(c^{7}A-T)$_3$	6.8	II	
13b	DMT-d(c^{7}A-T)$_6$	7.3	II	
14a	d(A-T)$_3$	6.4	IV	1:1
14b	d(A-T)$_6$	8.9	IV	1:1
15a	d(c^{7}A-T)$_3$	8.2	IV	1:1
15b	d(c^{7}A-T)$_6$	8.6	IV	1:1

[a] The nucleotide content was calculated after digestion of the oligomers with snake venom phosphodiesterase followed by alkaline phosphatase. The column used in the procedure was an RP-18 250 × 4 mm (7 μm).

the HPLC pattern of the purified detritylated dodecamer **15b**.

The structure of the oligomers was confirmed by hydrolysis with snake venom phosphodiesterase to give the 5′-monophosphates of thymidine and **1a** and the free nucleoside **1a**. Quantification of the nucleoside content was accomplished after subsequent digestion with alkaline phosphatase. From the HPLC pattern (Figure 1c) it is apparent that hydrolytic cleavage of **15b** yielded molar equivalents of the incorporated nucleosides, which confirms the structure of this oligomer. Corresponding results were obtained from the syntheses of compounds **14a**, **14b**, and **15a**. The data are summarized in Table II. From digestion with snake venom phosphodiesterase the hypochromicities of the oligomers were calculated. Values of 10% (**14a**), 16% (**15a**), 20% (**14b**), and 22% (**15b**) were determined (260 nm for **14a** and **14b**; 270 nm for **15a** and **15b**).

It has been reported that d(A-T) regions in DNA have a greater flexibility of the phosphate–sugar backbone than random sequences (Chen et al., 1985). This may be one reason for the special binding properties of this sequence to proteins (Riggs et al., 1972; Sassone-Corsi et al., 1981). In contrast, d(G-C) sequences are much stiffer than naturally occurring DNA (Thomas & Bloomfield, 1983). In the case of the tetranucleotide d(A-T)$_2$ Viswamitra and co-workers (Viswamitra et al., 1982) found a break in the geometry of the phosphate–sugar backbone between dT and dA. Subsequent model-building studies based on the crystal structure of d(A-T)$_2$ suggested that poly[d(A-T)] does not form a smooth B-DNA double helix. Overlaps of A/T and T/A are slightly different, and consequently the polynucleotide backbone shows

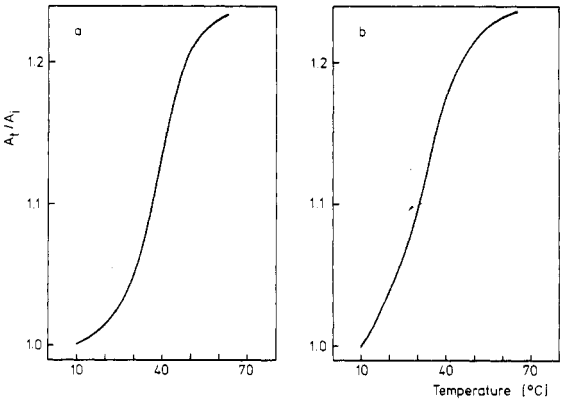


FIGURE 2: Melting profiles of (a) d(c^{7}A-T)$_6$ (**15b**) and (b) d(A-T)$_6$ (**14b**) in H_2O containing 30 μM oligonucleotide, 1 M NaCl, 0.1 M $MgCl_2$, and 60 mM sodium cacodylate, pH 7.0. A_t/A_i is the ratio of absorbance at 260 nm at a given temperature (t) to the initial temperature (i).

an alternating sequence-dependent structure. It was suggested that these differences in backbone conformation and in nucleobase overlap cause the high flexibility of these polynucleotides, which is much smaller in random DNA sequences.

Alteration of the π-electron system of one of the heterocycles in these alternating and self-complementary oligomers should influence base stacking and the overlap of the bases. It has been demonstrated (Seela et al., 1981) that polylysine synthesis in a ribosome-dependent protein synthesis system is enhanced if tubercidin (**1b**) is incorporated into poly(adenylic acid) instead of adenosine. Therefore, purine base modification might increase the flexibility of oligomers like **14a** and **14b**. Base pairing and base stacking in the short structures should be influenced. As a consequence the melting curves of **15a** and **15b** and the behavior toward the single-strand specific nuclease S1 were investigated and compared with those of **14a** and **14b**.

From melting experiments on d(A-T)$_3$ (**14a**) it is apparent that duplex formation occurs only below 5 °C. This is in contrast to d(c^{7}A-T)$_3$, where the upper part of a sigmoidal melting curve can be observed between 5 and 15 °C. A complete melting profile could not be obtained even at high-salt and -oligonucleotide concentrations. However, this experiment clearly indicates that the incorporation of 2′-deoxytubericidin (**1a**) into a d(A-T) sequence stabilizes the duplex structure. To obtain a complete melting profile the duplex formation of the dodecamers **14b** and **15b** was investigated. The melting behavior of these oligonucleotides (Figure 2) shows that duplex formation takes place in both cases under the described conditions. The lower T_m (34 °C) of the naturally occurring oligomer **14b** than that of the modified **15b** (38 °C) confirms that the pyrrolo[2,3-*d*]pyrimidine nucleoside **1a** stabilizes the secondary structure of oligomers containing alternating thymidines. The reasons may be 2-fold: (i) a shortened glycosyl bond (V. Zabel, W. Saenger, A. Kehne, and F. Seela, unpublished data) can influence nucleotide conformation, and (ii) the altered π-electron distribution in the nucleobase may account for stronger base stacking interactions. In contrast, replacement of adenosine (**2b**) by tubercidin (**1b**) in the ribopolynucleotide duplex poly(A)·poly(U) leads to a decrease of duplex stability (Seela et al., 1982).

The difference in helix properties of the two dodecamers **14b** and **15b** could explain their behavior toward nuclease S1. While the d(A-T) dodecamer is cleaved with a half-life of 40 min (for conditions, see Experimental Procedures), the modified dodecamer d(c^{7}A-T)$_6$ (**15b**) has a much slower rate of hydrolysis ($\tau_{1/2}$ = 60 min). Since nuclease S1 preferentially

cleaves single strands (Wiegand et al., 1974), it can be used as a probe for the DNA secondary structure. The different half-lives of the oligomers **14b** and **15b** can be interpreted on the basis that under identical conditions the population of single strands is greater in d(A-T)$_6$ than in d(c^{7}A-T)$_6$. On the other hand, if 2′-deoxytubercidin (**1a**) is incorporated into the *Eco*RI palindromic sequence d(GGAATTCC), a less stable duplex is formed (Seela et al., 1986a,b), which confirms that the nearest neighbor is important for a stabilization or destabilization of a duplex containing 2′-deoxytubercidin.

Since 2′-deoxytubercidin (**1a**) lacks the 7-nitrogen of the purine moiety of 2′-deoxyadenosine (**2a**), it promises to be an ideal probe for studying DNA/protein interactions involving specifically this nitrogen binding site. When the pyrrolo-[2,3-*d*]pyrimidine nucleoside **1a** was incorporated into several palindromic sequences, recognized by restriction enzymes (Ono et al., 1984; Seela et al., 1986a), phosphodiester cleavage did not occur if 2′-deoxytubercidin was close to the cleavage site. For reasons discussed above, changes in the hydrogen bonding pattern and helix distortion always have to be considered when a regular base is replaced by a modified base at a highly specific binding site.

ACKNOWLEDGMENTS

We thank Dr. W. Saenger and Dr. H. Rosemeyer for critical reading of the manuscript.

REFERENCES

Anzai, K., Nakamura, G., & Suzuki, S. (1957) *J. Antibiot., Ser. A*, 201.

Caruthers, M. H. (1982) *Chemical and Enzymatic Synthesis of Gene Fragments* (Gassen, H. G., & Lang, A., Eds.) Verlag Chemie, Weinheim.

Chen, H. H., Rau, D. C., & Charney, E. (1985) *J. Biomol. Struct. Dyn. 2*, 709–719.

Gait, M., Matthes, W. D., Singh, M., Sproat, B. S., & Titmas, R. C. (1982) *Chemical and Enzymatic Synthesis of Gene Fragments* (Gassen, H. G., & Lang, A., Eds.) Verlag Chemie, Weinheim.

Kasai, H., Ohashi, Z., Harada, F., Nishimura, S., Oppenheimer, N. J., Crain, P. F., Liehr, J. G., von Minden, D. L., & McCloskey, J. A. (1975) *Biochemistry 14*, 4198–4208.

Kazimierczuk, Z., Cottam, H. G., Revankar, G. R., & Robins, R. K. (1984) *J. Am. Chem. Soc. 106*, 6379–6382.

Klug, A., Jack, A., Viswamitra, M. A., Kennard, O., Shakked, Z., & Steitz, T. A. (1979) *J Mol. Biol. 131*, 669–680.

McBride, L. J., & Caruthers, M. H. (1983) *Tetrahedron Lett. 24*, 245–248.

McCloskey, J. A., & Nishimura, S. (1977) *Acc. Chem. Res. 10*, 403.

Ono, A., Sato, M., Ohtani, Y., & Ueda, T. (1984) *Nucleic Acids Res. 12* (23), 8939–8949.

Riggs, A. D., Lin, S. Y., & Walls, R. D. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 761–764.

Sassone-Corsi, P., Cordon, J., Kędinger, C., & Chambon, P. (1981) *Nucleic Acids Res. 9*, 3941–3959.

Seela, F., & Kehne, A. (1983) *Liebigs Ann. Chem.*, 876–884.

Seela, F., & Driller, H. (1985) *Nucleic Acids Res. 13*, 911–926.

Seela, F., & Kehne, A. (1985) *Tetrahedron* (in press).

Seela, F., Tran-Thi, Q.-H., Mentzel, H., & Erdmann, V. (1981) *Biochemistry 20*, 2559–2564.

Seela, F., Ott, J., & Franzen, D. (1982a) *Nucleic Acids Res. 10*, 1389–1397.

Seela, F., Tran-Thi, Q.-H., & Franzen, D. (1982b) *Biochemistry 21*, 4338–4343.

Seela, F., Driller, H., & Kehne, A. (1986a) *Chem. Scr.* (in press).

Seela, F., Driller, H., Kehne, A., Menkhoff, S., Ott, J., & Winkeler, H.-D. (1986b) *Chemical Synthesis in Molecular Biology* (Blöcker H., Frank, R., & Fritz, H. J., Eds.) Verlag Chemie, Weinheim.

Suhadolnik, R. J. (1979) *Nucleosides as Biological Probes*, Wiley, New York.

Thomas, T. J., & Bloomfield, V. A. (1983) *Biophys. J. 41*, 420 a.

Viswamitra, M. A., Shakked, Z., Jones, P. G., Sheldrick, G. M., Salisbury, S. A., & Kennard, O. (1982) *Biopolymers 21*, 513–533.

Wiegand, R. G., Godson, G. N., & Radding, C. M. (1974) *J. Biol. Chem. 250*, 8848–8855.

Winkeler, H.-D., & Seela, F. (1980) *Chem. Ber. 113*, 2069–2080.

Wu, R. T., Okabe, T., Nashikomi, M., Okuda, S., Nishimura, T., & Tanaka, N. (1982) *Jpn. J. Antibiot. 35*, 279–284.