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Poly(2-amino-8-methyldeoxyadenylic acid): Contrasting Effects in Deoxy- and Ribopolynucleotides of 2-Amino and 8-Methyl Substituents

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ABSTRACT: Poly(2-amino-8-methyldeoxyadenylic acid) interacts readily with pyrimidine polynucleotides to form double helices only slightly less stable than those in which the purine polymer lacks the 8-Me group. In the ribo series, by contrast, complexes formed with poly(2-amino-8-methyladenylic acid) are very strongly destabilized by the 8-Me group, despite a larger stabilizing effect of the 2-NH_2 group in the ribo series. These results are interpreted in terms of a smaller steric interference of the 8-Me group with 2′-CH_2 than with 2′-CHOH, leading to a smaller population of syn structures in the deoxy chain and a consequent lower interference with homopolymer duplex formation. UV, circular dichroism (CD), and IR spectra of the new polymer and its complexes are reported and related to structural and energetic characteristics of the molecules. Since direct synthesis of 2-amino-8-methyldeoxyadenosine was not feasible, the corresponding riboside was prepared, the 3′- and 5′-positions were protected with a disilyloxy group, and a 2′-[(imidazol-1-yl)thiocarbonyl] group was introduced. Reduction with tributyltin hydride followed by deprotection gave the nucleoside, which was then converted to the triphosphate by standard methods. The homopolymer was prepared with terminal deoxynucleotidyl transferase.

Secondary and tertiary structures of nucleic acids are determined by a number of energetic and conformational factors. These are frequently not well resolved, and predictions are often difficult and unreliable. In order to help separate and analyze some of these factors, we have employed chemical modifications of nucleotides and polynucleotides to perturb their properties in defined and controllable ways. Thus, substitution of 2-NH_2 in poly(A) permits three interbase hydrogen bonds to be formed to U and T polymers. In the ribo

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series this change elevates the transition temperature of the complexes by approximately 30 deg, but in the deoxy series the elevation is only about 12 deg (Howard et al., 1976; Howard & Miles, 1984). A methyl group in the 8-position of poly(A) favors the syn conformation by steric interference with the sugar and prevents interaction with pyrimidine polynucleotides. When both 2-NH_2 and 8-CH_3 are present in poly(A), however, the combined effect of these groups results in a polymer that reacts with poly(U) to form a complex of relatively low stability (Limn et al., 1983; Howard et al., 1985). It is important to explore these chemical perturbations in the deoxy series since large differences between the two series have been found for effects of both the 2-NH_2 and the bulky 8-bromo substituent (Kanaya et al., 1984; Howard et al., 1985). We report here our investigation of the analogous deoxy polymer $(d2NH_28MeA)_n$, showing here also a wide difference from the corresponding ribo polymer.

Before pursuing these studies, it was first necessary to find a synthetic method for the constituent deoxynucleoside, for which satisfactory procedures are much more limited than with ribosides. Despite the rather large number of steps, the method of choice appears to be the recently developed conversion of ribo- to deoxynucleosides by reduction with tributyltin hydride [cf. Pankiewicz et al. (1982), Robins and Wilson (1981), and Barton and McCombie (1975)]. Details of chemical synthesis and enzymatic polymerization of the deoxynucleoside triphosphate are presented.

Materials and Methods

2-Amino-8-methyladenosine (V). The starting ribonucleoside (Silverton et al., 1982) was prepared by the following sequence: guanosine (I) → 8-methylguanosine (II) (50% yield) → tri-*O*-acetyl-8-methylguanosine (III) (75% yield) → 2-amino-6-chloro-8-methyltri-*O*-acetyl-β-D-ribofuranosylpurine (IV) (65% yield) → 2-amino-8-methyladenosine (V) (92% yield).

2-Amino-8-methyl-3′,5′-O-(tetraisopropyldisiloxanyl)adenosine (VI). To V (2.8 mmol, 0.83 g) in 14 mL of pyridine was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (3.1 mmol, 0.99 g) (Pankiewicz et al., 1982) with stirring. After 5 h, 5 mL of water was added and then evaporated in vacuo. The residue was partitioned between water and chloroform. The organic layer was evaporated in vacuo and then coevaporated 5 times with pyridine and twice with toluene. After chromatography over 15 g of silica gel with $CHCl_3$–MeOH (100:3), the product was crystallized from EtOAc to give 1.05 g of VI (82% yield): mp 175–180 °C; single spot on silica gel, R_f 0.6 [$CHCl_3$–MeOH (20:1)]; λ_{max} (95% EtOH) 257, 281 nm. Anal. Calcd for $C_{23}H_{42}N_6O_5Si_2 \cdot {}^3/_2H_2O$: C, 48.82; H, 8.02; N, 14.86. Found: C, 49.1; H, 8.56; N, 14.72.

2-Amino-8-methyl-3′,5′-O-(tetraisopropyldisiloxanyl)-2′-O-[(imidazol-1-yl)thiocarbonyl]adenosine (VII). A mixture of VI (0.98 mmol, 0.49 g) and thiocarbonyldiimidazole (2.4 mmol, 0.43 g) in 10 mL of dimethylformamide was stirred overnight at 25 °C and then partitioned between Et_2O and water at 0 °C. The organic layer was washed with water, dried with Na_2SO_4, and evaporated. The product was chromatographed over 10 g of silica gel with $CHCl_3$–MeOH (100:1 or 100:2). R_f on silica gel TLC [$CHCl_3$–MeOH (20:1)] was 0.56 (VI, 0.49); λ_{max} in 95% EtOH was 262 and 297 nm; yield was 11 000 AU_{279}.

2′-Deoxy-2-amino-8-methyl-3′,5′-(tetraisopropyldisiloxanyl)adenosine (VIII). A mixture of 2,2′-azobis(2-methyl propionitrile) (99 mg) and *n*-Bu_3SnH (3.6 mmol, 630 mg) [cf. Pankiewicz et al. (1982)] in 11 mL of dry toluene was added dropwise over 1 h to a refluxing solution of VII (9900 AU_{279}) in 9 mL of dry toluene. The solvent was evaporated in vacuo and the residue chromatographed on silica gel ($CHCl_3$–MeOH, 100:1 or 100:1.5). Fractions having a single spot at R_f 0.5 ($CHCl_3$–MeOH, 20:1) were combined and evaporated to give 5100 AU_{259} (95% EtOH), or about 48% yield. λ_{max} in the same solvent was 259 and 282 nm. Because the mobility on thin-layer chromatography (TLC) is dominated by the large tetraisopropyldisiloxanyl group, the R_f values of the compounds having it are only slightly different. That the desired 2′-OH reduction to 2′-H has occurred is further confirmed by specific chemical reactions and characterization described below.

2-Amino-8-methyl-2′-deoxyadenosine (IX). To a solution of VIII (5100 AU_{259}) in 1.4 mL of tetrahydrofuran was added dropwise a 1 M solution of *n*-Bu_4NF in 0.5 mL of the same solvent. After 1 h the reaction mixture was dissolved in pyridine–methanol–water (3:1:1) and passed through Dowex AG 1X2 (OH form) and Dowex 50X4 (pyridine form). Qualitative analyses for the presence of ribose and deoxyribose were carried out on the present reaction product IX, the precursor riboside V, adenosine (A), and deoxyadenosine (dA) after each had been developed on cellulose TLC in water-saturated butanol with the following results: With cysteine hydrochloride/3 N H_2SO_4 (deoxyribose; Buchanan, 1951), IX and dA were positive, and V and A were negative. With KIO_4/benzidine (ribose and other *cis*-glycols; Buchanan et al., 1950), IX and dA were negative, and V and A were positive. Quantitative analysis of IX by the Dische diphenylamine reaction (Dische, 1930) with a linear deoxyadenosine calibration curve indicated 0.33 mmol of product, or about 65% yield.

2-Amino-8-methyl-2′-deoxyadenosine 5′-(Cyanoethyl phosphate) (X). This nucleotide was prepared by the method of Tener (1961) but with the following modification. Instead of being added to the nucleoside in aqueous solution before the mixture was dried, cyanoethyl phosphate was first dried by evaporation 10 times from anhydrous pyridine. Failure to observe this precaution leads to depurination [cf. Howard and Miles (1984)]. To 0.32 mmol of dry IX in 15 mL of anhydrous pyridine was added 0.81 mmol of cyanoethyl phosphate pyridinium salt in anhydrous pyridine. Dicyclohexylcarbodiimide (DCC) (3.9 mmol, 0.81 g) was added and the mixture allowed to stand at room temperature overnight. With ice cooling, 15 mL of pyridine–water was added and the mixture again allowed to stand overnight. Dicyclohexylurea was removed, 200 mL of 50% pyridine–water was added, and the solution was applied to a DEAE Sephadex A-25 column (2.6 × 24 cm). A linear gradient of triethylammonium bicarbonate (0–0.1 M) was employed, and 23-mL fractions were collected. Fractions 85–100 were pooled and evaporated to give 2340 AU_{260}. On cellulose TLC (PrOH–concentrated NH_3–H_2O, 6:3:1), R_f values were as follows: X, 0.55; IX, 0.67; 2′dAMP, 0.21; dA, 0.68.

2-Amino-8-methyl-2′-deoxyadenosine 5′-Phosphate (XI). To the previous intermediate X (2340 AU_{260}) was added concentated NH_4OH (20 mL) and the solution allowed to stand overnight in a sealed flask at 45 °C. The solution was evaporated to dryness. The product moved as a single spot in the previous TLC system with R_f 0.22. UV maxima were at 257 and 281 nm in water at pH 7. A sample was completely hydrolyzed to the nucleoside IX by snake venom 5′-nucleotidase. 5′dAMP was hydrolyzed to dA, whereas 3′dAMP was unaffected by the same preparation of enzyme.

2-Amino-8-methyl-2′-deoxyadenosine 5′-Phosphomorpholidate (XII). To a solution of the nucleotide XI (0.19 mmol, 1852 AU_{260}) in water–butanol (14.3 mL of each) was

added 0.78 mmol of morpholine. The solution was heated to reflux and a solution of DCC (196 mg) in 24 mL of *tert*-BuOH was added dropwise during 7 h [cf. Moffatt and Khorana (1961)]. After 8 h, the reaction was stopped, and the mixture evaporated to dryness in vacuo. Water (50 mL) was added, dicyclohexylurea was filtered off, and the solution was extracted with ether (3 × 10 mL). Water was evaporated and the residue rendered anhydrous by several coevaporations from dry pyridine.

2-Amino-8-methyl-2'-deoxyadenosine 5'-Triphosphate (XIII). To the residue of XII, a solution of bis(tri-*n*-butylammonium) pyrophosphate (0.95 mmol) in 9.5 mL of dimethylformamide was added. After 2 days at room temperature, the reaction was quenched by the addition of water. The solvent was removed by evaporation and the nucleotide applied to a column of DEAE-Sephadex A-25 (2.6 × 22 cm), bicarbonate form. Elution was carried out with a linear gradient (0.005–0.5 M) of triethylammonium bicarbonate. The yield was 1020 AU_{258} or about 54%. On cellulose thin-layer electrophoresis in 0.05 M citrate buffer, pH 5, migration distances were (dA) −1.0, (dAMP) 2.1, and (XIII) 3.3 cm. UV maxima were 258 and 282 nm in water, pH 7.

Polymerization. The nucleoside triphosphate was polymerized with terminal deoxynucleotidyl transferase (TdT) (Bollum, 1966) in an incubation mixture containing the following components: 1 mM XIII, 0.38 unit/mL TdT (PL), 0.625 A_{260} unit/mL $d(pA)_7$, 50 mM $MgCl_2$, 50 mM $ZnSO_4$, 200 mM potassium cacodylate, pH 7.5, and 0.29 mL of E730 diluent (Willis et al., 1980) per milliliter of incubation mixture. The reaction was incubated at 37 °C for 48 h before being stopped by the addition of concentrated NH_3. A precipitate was removed by centrifugation and then washed with water. The combined water solution was extracted with $CHCl_3$–isoamyl alcohol to remove protein. Some remaining bovine serum albumin (BSA) from E730 diluent was removed by differential elution from DEAE-cellulose. In one run, a sample of polymer (20 AU_{260}) was applied to 1.3 mL of DE-23 Cl^- (Whatman) in 12 mL of water. Elution with 12 mL of 0.5 mM NaCl removed BSA and unreacted triphosphate. The polynucleotide was eluted with 1 M NaCl in 7 M urea in the first 6 mL of effluent. The polymer was then passed over a Sephadex G-50 column. The yield was 15%. The molar absorbance, determined as described previously (Muraoka et al., 1980), was 7300 ± 20 at 257 nm. Gel electrophoresis of the polymer end labeled with ^{32}P (Maxam & Gilbert, 1980) indicated an average length of about 100 residues, though small amounts of oligomers of chain length less than 80 were present. A sample of the polymer was degraded with snake venom to the nucleoside IX. In BuOH saturated with water, the R_f of the degradation product on cellulose TLC was 0.44, the same as that of authentic IX. The riboside V had an R_f of 0.29. In PrOH–concentrated NH_3–H_2O (6:3:1), the product also cochromatographed with IX.

A Cary Model 118 spectrophotometer was used for UV spectral measurements. The spectrophotometer was interfaced to an LDACS computer system (Powell et al., 1980). Data were analyzed with a Digital Equipment Corp. Model 11/70 computer by the method described previously (Howard et al., 1976).

Melting curves were measured automatically with a Cary Model 118 spectrophotometer, the spectrophotometer and accessory equipment operating in a closed-loop mode with the LDACS computer system (Howard et al., 1977).

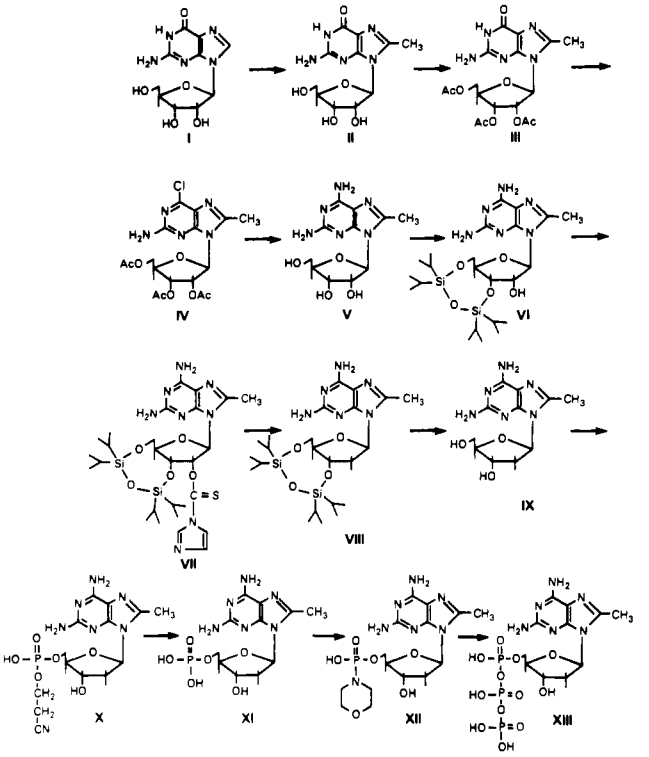

FIGURE 1: Synthesis of 2-amino-8-methyl-2'-deoxyadenosine 5'-triphosphate.

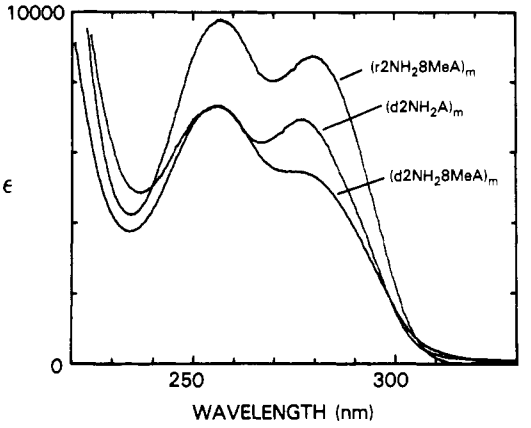

FIGURE 2: UV spectra of $(d2NH_28MeA)_n$, $(d2NH_2A)_n$, and $(r2NH_28MeA)_n$. Conditions of Table I.

Circular dichroism (CD) spectra were measured with a Jasco J-500A spectropolarimeter, also interfaced to the LDACS system.

Infrared spectra were measured with a Perkin-Elmer 580B spectrometer interfaced to an LDACS system and were processed by computer. Measurements were made in D_2O solution with CaF_2 windows, as described previously [cf. Miles (1971)].

RESULTS

Synthesis and Characterization of $(d2NH_28MeA)_n$. The deoxynucleoside $d2NH_28MeA$ was prepared by the reactions shown in Figure 1, as detailed under Materials and Methods and discussed below.

At neutral pH, the UV spectrum of the homopolymer has λ_{max} 256.4 (7300) and ~278 (sh) nm and λ_{min} 234 (3760) nm, compared to monomer values of 258, 283, and 236 nm (Figure 2; Table I). The corresponding ribo polymer (Howard et al., 1985) has values of 257 (9660), 280 (8770), and 235 nm (4960). The ribo polymer has a cooperative melting curve at very low temperature (T_m = 5 °C) but changes relatively little

Table I: Spectroscopic Data

	Ultraviolet[a]			
	λ_{max} (nm)	ϵ_{max}	λ_{min} (nm)	ϵ_{min}
$(d2NH_28MeA)_n$	256.4	7300	234	3760
	274 (sh)			
$(d2NH_28MeA)_n{\cdot}(rU)_n$	258.4	7010	234.4	3010
$(d2NH_28MeA)_n{\cdot}(dT)_n$	261.2	7030	236.4	3080
	Circular Dichroism[a]			
	λ_{max} (nm)	$\epsilon_L - \epsilon_R$	λ_{min} (nm)	$\epsilon_L - \epsilon_R$
$(d2NH_28MeA)_n$	292.0	+1.3	275.6	−0.8
	258.6	+3.5	241.4	+0.1
	225.0	+1.2		
$(d2NH_28MeA)_n{\cdot}(rU)_n$	263.8	+9.7	287	−0.9
	219.6	+4.1	243.4	−1.5
$(d2NH_28MeA)_n{\cdot}(dT)_n$	266.2	+6.6	292.4	−0.8
	221	+2.5	245.6	−2.6
	Infrared[b]			
			ν_{max} (cm^{-1})	ϵ_{max}
$(d2NH_28MeA)_n{\cdot}(rU)_n$ (1:1, pD 7.0, 7 °C)			1687	336
			1669	432
			1625	273
			~1598 (sh)	90
$(d2NH_28MeA)_n{\cdot}(dT)_n$ (1:1, pD 7.0, 6 °C)			1673	570
			1638	276
			1620	324
			~1600 (sh)	100
$d2NH_28MeATP$			1618	s
			~1605 (sh)	m

[a] Conditions: 0.002 M sodium cacodylate, pH 7.0; 0.1 M NaCl; 20.0 °C. [b] D_2O solution, 0.0125 M sodium cacodylate, pD 7.4, and 0.125 M sodium chloride. For the nucleoside triphosphate, s indicates strong and m indicates medium intensity.

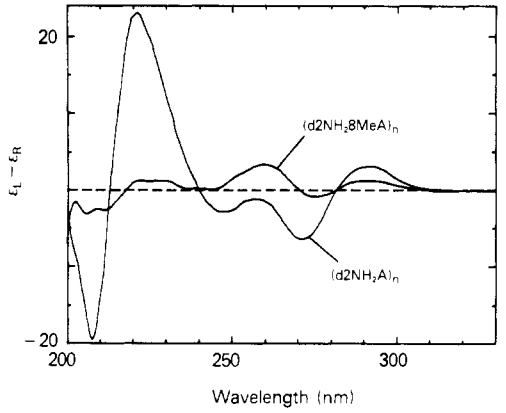

FIGURE 3: CD spectra of the homopolymers $(d2NH_28MeA)_n$ and $(d2NH_2A)_n$. Conditions of Table I.

above 20 °C. The unusually high molar absorbance may be due to an unstacked conformation. The deoxy polymer $(d2NH_28MeA)_n$ has a noncooperative temperature profile of absorbance and gives no evidence of a regular ordered form, though the polymer is presumably stacked. The CD spectrum of the deoxy polymer has maxima at 289.2 ($\epsilon_L - \epsilon_R$, 1.3), 258.6 (3.5), and 225 (1.2) nm and minima at 275.6 (−0.8) and 241.4 nm (0.1) (Figure 3).

Interaction with $(rU)_n$ and $(dT)_n$: Optical Spectroscopy and Thermal Stability. CD spectra of the double helices formed by each of these polymers with $(d2NH_28MeA)_n$ are shown in Figure 4. These are quite similar to complexes formed by the corresponding ribo polymer (Howard et al., 1985) and differ widely from computer summations of spectra of the uninteracted polymers. Ultraviolet melting curves are sigmoidal and have T_m of 59 °C in 0.1 M NaCl for the complex with $(rU)_n$ and 44.5 °C for that with $(dT)_n$ duplex, indicating quite stable complexes. Salt dependence curves in Figure 5 give $dT_m/d \log [Na^+]$ values of 17.5 °C for the former complex and 21 °C for the latter. Infrared spectra of the complexes in Figures 6 and 7 are consistent with these results and

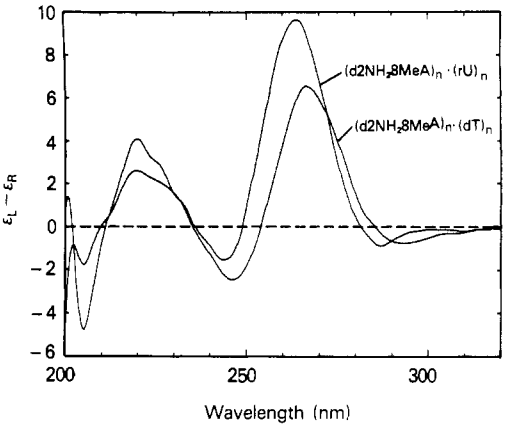

FIGURE 4: CD spectra of the double helices $(d2NH_28MeA)_n{\cdot}(rU)_n$ and $(d2NH_28MeA)_n{\cdot}(dT)_n$. Conditions of Table I.

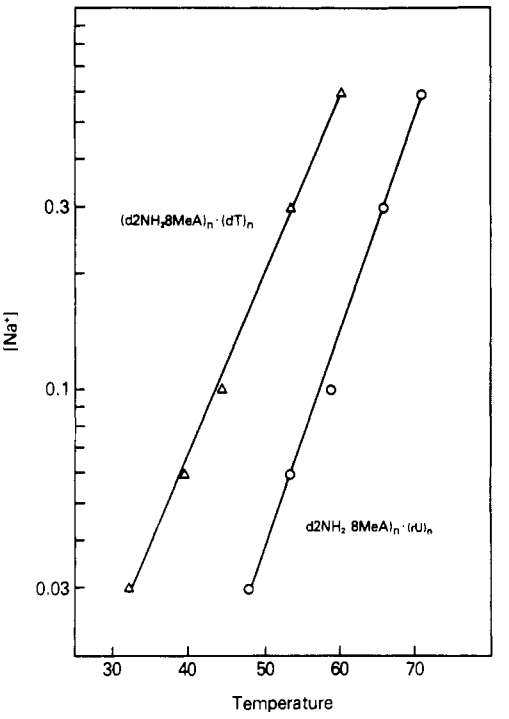

FIGURE 5: Phase diagrams for the helices $(d2NH_28MeA)_n{\cdot}(dT)_n$ (left) and $(d2NH_28MeA)_n{\cdot}(rU)_n$ (right). Equations of the lines are $T_m = 20.952 \log [Na^+] + 64.832$ (left) and $T_m = 17.537 \log [Na^+] + 75.246$.

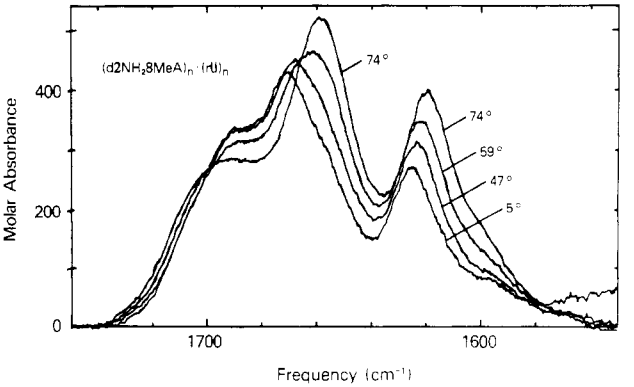

FIGURE 6: Infrared spectra of $(d2NH_28MeA)_n{\cdot}(rU)_n$ as a function of temperature. In the intact helix at 4 °C, U carbonyl bands are observed at 1687 and 1669 cm^{-1}, and a strong A ring vibration is observed at 1625 cm^{-1}. Concentration of each polymer, 6.25 mM in D_2O solution; pD is 7.4; $[Na^+]$ is 0.15 M.

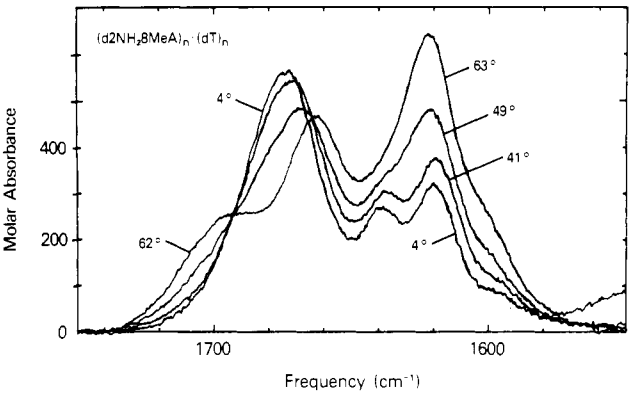

FIGURE 7: Infrared spectra of $(d2NH_28MeA)_n \cdot (dT)_n$ as a function of temperature. In the intact helix at 4 °C, the single strong band at 1673 cm^{-1} is formed of two overlapping carbonyl bands of T. These bands are resolved in the random-coil form of $(dT)_n$ at 63 °C: 1692 and 1663 cm^{-1}. The 1639- and 1620-cm^{-1} bands at 4 °C are ring vibrations of T and A, respectively. Concentration of each polymer, 5.1 mM in D_2O solution; pD is 7.4; $[Na^+]$ is 0.15 M.

show that helices are double and not triple stranded (see below). The infrared melting curves (Figure 8) similarly demonstrate specific base-pairing interactions of the polymers and confirm the transition temperatures observed in the UV for much more dilute solutions.

DISCUSSION

Chemical Synthesis of d2NH₂8MeATP. In the past, many deoxynucleotides have been relatively inaccessible because suitable synthetic approaches were not available. The object of our present study presented special problems in that acid causes rapid depurination of $2NH_2dA$ and its derivatives. Direct methylation of $2NH_2dA$, carried out in strong acid, was thus ruled out. Glycosylation with deoxyribose gives a mixture of α and β anomers, which are difficult to separate, and may also risk depurination. A recent synthetic approach to deoxynucleosides by free radical reduction of ribonucleoside thiono esters under neutral conditions [cf. Pankiewicz et al. (1982), Robins and Wilson (1981), and Barton and McCombie (1975)] was evidently the only feasible approach in the present case and is probably the method of choice in general. The desired ribonucleoside V was prepared as shown in Figure 1 [cf. Silverton et al. (1982)] and protected with a bifunctional silylating reagent to obtain VI. Thiocarbonyldiimidazole reacted slowly but in high yield to give the thiono ester VII. The deoxygenation was carried out in good yield with tributyltin hydride to give VIII. Deprotection gave the product IX, which was shown to have a deoxy structure and to be free of ribo contaminants. Phosphorylation was carried out in 70% yield by the method of Tener (1961) as modified by drying the pyridinium cyanoethyl phosphate by repeated evaporation from dry pyridine before addition of the nucleoside. Omission of this step leads to depurination. Application of the Moffatt and Khorana (1961) procedure led to the desired triphosphate XIII.

Enzymic Synthesis of $(d2NH_28MeA)_n$. For the enzyme terminal deoxynucleotidyl transferase (Bollum, 1966), the triphosphate XIII is a reluctant substrate and indeed appears to inhibit reaction of other substrates. Under conditions giving 60% polymerization with dATP, for example, yield of $(dA)_n$ dropped to 10% when only 1% of XIII was added to dATP. When only XIII was present, the yield of $(d2NH_28MeA)_n$ was about 5%.

Studies on the effect of primers, univalent cations, divalent cations, and protective concentrations of BSA (Kato et al., 1967; Chirpich, 1978; Coleman, 1977; Willis et al., 1980) were

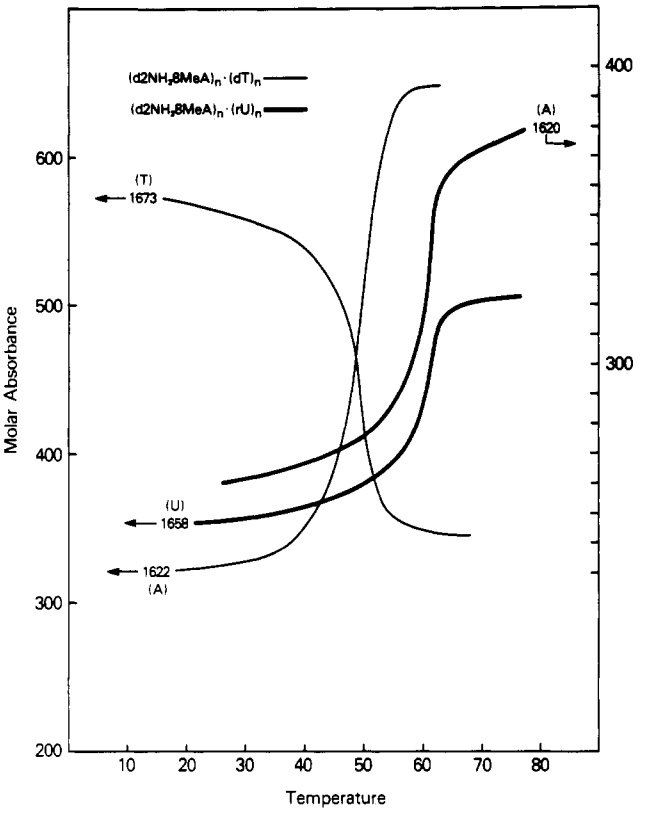

FIGURE 8: Infrared temperature profiles of resolved A and T vibrations (light lines) exhibit the same melting behavior, demonstrating that the temperature-dependent changes in Figure 7 result from specific interaction of the two polymers. Similarly, parallel temperature profiles of A and U bands (heavy lines) demonstrate specific interaction from the spectra of Figure 6.

applied with this substrate to obtain more favorable conditions for synthesis (Materials and Methods). The polymerization yield was increased to 15%, with $d(pA)_7$ as a primer. Polyacrylamide gel electrophoresis of 5′-^{32}P-labeled polymer showed that the bulk of the material had a chain length of approximately 100 ± 30.

Attempts to make the alternating copolymer $(d2NH_28MeA\text{-}dT)_n$ were unsuccessful but worth noting. Since an 8-methyl group favors the syn conformation, it appeared possible that a Z structure would be the favored or perhaps exclusive form of the polymer if it could be synthesized. Experiments with *Escherichia coli* DNA polymerase I under both unprimed and $(dA\text{-}dT)_n$ primed synthesis conditions, however, were uniformly negative under a variety of conditions. Though a negative result cannot demonstrate the point, it is consistent with an inability of the enzyme to synthesize directly a polymer with Z conformation.

Electronic Spectra. Like other $2NH_2A$ derivatives, $(d2NH_28MeA)_n$ shows in its UV spectrum resolved B_{2u} and B_{1u} transitions at 256 and 278 nm [Figure 2; cf. Howard et al. (1976) and Howard and Miles (1984)]. The CD spectrum of this polymer, again like three of the others in this series, exhibits exciton splitting of the B_{2u} transition at ~278 nm but not of the B_{1u} transition at 256 nm (Howard et al., 1976; Howard & Miles, 1984; Figure 3). The significant point for the present ribo–deoxy comparison is that the ribo analogue $(r2NH_28MeA)_n$ does not fit this pattern. The single-stranded form of $(r2NH_28MeA)_n$ differs in CD and in conformation from the analogue that lacks the 8-Me group, $(r2NH_2A)_n$. In the heteroduplexes formed with $(rT)_n$ and $(rBrU)_n$, however, the conformation changes to that possessed by $(r2NH_2A)_n$ in the analogous duplexes. The results were interpreted in terms

of a single-stranded form in which the syn conformation was favored, inhibiting pair formation with pyrimidine polymers, as it does in the case of $(r8MeA)_n$ (Limn et al., 1983). Formation of a third hydrogen bond by the 2-NH_2 group evidently supplies sufficient free energy to drive the conformational equilibrium to the anti form, permitting formation of complexes like those of the polymer that lacks the 8-methyl group. Consideration of thermal destabilization by the 8-Me group in the deoxy series will be given in a subsequent paragraph.

The CD spectra of Figure 4 provide suggestive evidence of the conformations of the heteroduplexes. The spectrum of $(d2NH_28MeA)_n{\cdot}(rU)_n$ has a weak negative band at 287 and a very strong positive band at 263 nm, quite similar to many RNA or A-DNA spectra and different from typical conservative B-DNA spectra [cf. Bush and Brahms (1973), Bush (1974), and Bloomfield et al. (1974)]. A negative first extremum, moreover, has been characteristic of all A-form structures containing the $2NH_2A$ residue that have been examined (Howard & Miles, 1984). Ribo–deoxy hybrids are usually considered to be A form [cf. Milman et al. (1967), Arnott (1970), O'Brien and MacEwan (1970), and Wang et al. (1982)], though $(rA)_n{\cdot}(dT)_n$ evidently has a B-like structure (Zimmerman & Pheiffer, 1981). On these grounds, we suggest an A conformation for this hybrid duplex. The CD spectrum of the deoxy–deoxy complex (Figure 4) is remarkably similar to that of the hybrid. On this basis, we would provisionally assign this also to the A form, while recognizing that CD is not an absolute method and that the correlation with structure is uncertain. There is no clearly established example of a deoxy–deoxy complex having an A conformation under these experimental conditions, but high alcohol concentration [cf. Brahms and Mommaerts (1964) and Gray and Ratliff (1975)] and high salt concentration (Borah et al., 1985) have been shown to cause B to A conversions in some cases. There is thus nothing inherently improbable about an A conformation in this case, and provisional evidence appears to support it.

Infrared Spectra. Infrared spectra of polynucleotide helices are highly characteristic, permiting similar structures to be recognized and distinguished from each other [see below; cf. Miles and Frazier (1964), Miles (1971), and Howard et al. (1966)]. Independently of the appearance of a particular spectrum, it is possible to establish that it arises from specific interaction of two components by monitoring simultaneously temperature profiles of resolved bands assignable to the two components [for discussion, cf. Miles and Frazier (1964), Miles (1971), and Howard et al. (1966)]. Thus, in Figure 8 the A ring vibration at 1620 cm^{-1} and the U carbonyl band at 1658 cm^{-1} melt congruently, showing that the spectrum at low temperature is due to specific interaction of the two bases. The T_m of 60 °C is the same as that observed in the ultraviolet. Similarly, parallel changes of the 1673-cm^{-1} T band and the 1622-cm^{-1} A band show specific interaction of these bases (Figure 8). Again, the T_m of 48 °C is the same as that obtained from the UV salt dependence curve at 0.15 M NaCl (Figure 5).

The spectrum of a 1:1 mixture of $(d2NH_28MeA)_n$ and $(rU)_n$ in Figure 6 has C2 and C4 carbonyl bands at 1689 and 1670 cm^{-1} and a moderately intense adenine ring vibration at 1625 cm^{-1}, typical of an A·U double helix and quite distinct from a triple helix (see below). Heating the solution causes the spectra to shift progressively to those of the single-stranded forms. Further confirmation of the combining ratio is obtained from spectra of a mixture of a ratio 1A:2U (Figure 1 of supplementary material). A triple helix would have a strong

Table II: Thermal Properties of Helices

	T_m for $[Na^+]$ of		dT_m/d log $[Na^+]$
	0.03 M	0.1 M	
(a) $(d2NH_28MeA)_n{\cdot}(dT)_n$	32.2	43.6	21.0 ± 0.6
(b) $(d2NH_2A)_n{\cdot}(dT)_n$	65.3	73.9	16.4 ± 0.8
(c) $(d2NH_28MeA)_n{\cdot}(rU)_n$	48.1	57.7	17.5 ± 0.8
(d) $(d2NH_2A)_n{\cdot}(rU)_n$	56.3	66.6 (3/1)	12.3 ± 0.8
(e) $(r2NH_28MeA)_n{\cdot}(rU)_n$	15.3	21.3	12.3 ± 0.7
(f) $(r2NH_2A)_n{\cdot}(rU)_n$	82.7	89.5	12.6 ± 0.4
(g) $(r2NH_2A)_n{\cdot}(rT)_n$	93	100	12.8 ± 0.7
(h) $(r2NH_28MeA)_n{\cdot}(rT)_n$	32.3	38.8	13.2 ± 0.5

maximum at 1657, a weaker band at ~1676, and a strong band at 1696 cm^{-1}. The adenine ring vibration at ~1630 cm^{-1} would be unresolved and very weak. We observe instead in the spectrum of the 2:1 mixture a strong maximum at 1670 cm^{-1} with a shoulder at 1658 cm^{-1}, representing the overlap of bands of the single-stranded U and the double-stranded A:U (Miles & Frazier, 1964). The highest frequency carbonyl band is at 1690 cm^{-1}. The well-resolved and moderately strong adenine ring vibration of a double helix is seen at 1625 cm^{-1}, confirming that a triple helix has not been formed [cf. Miles and Frazier (1964)].

The infrared spectrum of a 1:1 mixture of $(d2NH_28MeA)_n$ with $(dT)_n$ (Figure 7) clearly shows complex formation and may be compared with that of the two-stranded helix $(rA)_n{\cdot}(rT)_n$ (Howard et al., 1971). There is a single strong band at 1673 cm^{-1} resulting from overlap of the two carbonyl bands, which are observed at 1692 and 1662 cm^{-1} in uninteracted T and at 1684 and 1667 cm^{-1} in rA·rT. A resolved T ring vibration is observed at 1638 cm^{-1} and an A ring vibration at 1619 cm^{-1}. The triple helix rA·2rT, by contrast, has carbonyl bands at 1690, ~1670 (sh), and 1652 cm^{-1}, an unresolved T ring vibration at ~1637 cm^{-1}, and a very weak A band at 1623 cm^{-1} (Howard et al., 1971).

The infrared spectra thus confirm the stereochemical conclusion that a triple helix cannot be formed when A has a bulky substituent in the 8-position and agree with experimental evidence on analogous systems (Kanaya et al., 1984; Howard et al., 1985). To form the necessary hydrogen bonds for a third strand, the 2-oxygen of U would need to be less than 3 Å from the 8-methyl of A (Howard et al., 1985; Figure 6). The sum of the van der Waals radii of these groups is 3.4 Å, and the contact is not allowed.

Thermal Transitions of Double Helices: Stability of Complexes. Ultraviolet melting curves of the double helices formed with $(rU)_n$ and $(dT)_n$ are cooperative, with T_m values of 59 and 45 °C, respectively, in 0.1 M NaCl. Phase diagrams for these systems are given in Figure 5. We have shown in previous papers that a number of factors affecting helix stability may be separated by comparing pairs of complexes that differ only in a chemical feature of interest [cf. Ikeda et al. (1970), Howard et al. (1969), and Howard and Miles (1984)]. Thus in the ribo analogue of the present polymer, the T_m of the complex with poly(U) was depressed 68 deg below that of the corresponding helix lacking the 8-Me group (Table II, f–e, indicating a large destabilization by this group. A similar value of 61 °C was observed for the pair g–h. Results with the present deoxy polymer $(d2NH_28MeA)_n$, however, are entirely different. The T_m of the complex with $(rU)_n$ is depressed only 8 deg (d–c) (in 0.03 M Na^+ in which both undergo 2 → 1 transitions) and that with $(dT)_n$ 30 deg (b–a), or half the value observed for the same substituent in the ribo series. The relatively high stability of the complexes formed by $(d2NH_28MeA)_n$ is more striking in view of the lower contribution of $2NH_2A$ to elevation of T_m in the deoxy series

(Howard & Miles, 1984). It is evident that the smaller destabilizing effect of 8-Me in the deoxy series arises from less steric interference of this group with the 2′-CH_2 of deoxyribose than with the 2′-CHOH of ribose. We suggest that this difference results in a smaller population of the syn conformation and a lower energy barrier to duplex formation. The similarity of the CD spectra of $(d2NH_28MeA)_n$ and $(d2NH_2A)_n$ in sign, frequency, and band shape (Figure 3) suggests that the 8-Me group does not greatly alter the predominant single-strand conformation in the deoxy series, though significant differences in band intensities imply populations of other conformations. [As noted above, there is marked contrast with the ribo series: here CD spectra of $(r2NH_28MeA)_n$ and $(r2NH_2A)_n$ are radically different, indicating quite different conformations (Howard et al., 1985).] It is possible, however, that in the $(d2NH_28MeA)_n{\cdot}(dT)_n$ double helix the 8-Me group does significantly affect the conformation, for example, by destabilizing the C2′-endo conformation, causing the A form to be favored over the B, expected for a deoxy–deoxy pair. The only other example of a deoxy–deoxy complex likely to be in the A form ($[Na^+]$, 0.1 M) is $(d8BrA)_n{\cdot}(d5BrU)_n$, which also has a bulky 8-substituent (Kanaya et al., 1984). As we have noted above, however, the conformational assignments in both cases are based on CD spectra and are only provisional. In any event, it is clear that the constraints introduced by the free energy required to convert the purine strand to a suitable conformation (presumably anti) for pairing of the homopolymers can account for smaller destabilization by 8-Me, though there may also be a modulating effect on conformation in helical complexes after they are formed. It is, incidentally, a prediction of these considerations that $(d8MeA)_n$, not yet described, would form heteroduplexes of low to moderate stability, unlike the analogous ribo polymer $(8MeA)_n$ reported by Limn et al. (1983).

Acknowledgments

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Supplementary Material Available

Infrared spectrum of a 1:2 mixture of $(d2NH_28MeA)_n$ and $(rU)_n$ (1 page). Ordering information is given on any current masthead page.

Registry No. V, 80326-50-7; VI, 110488-50-1; VII, 110488-51-2; VIII, 110488-52-3; IX, 110488-53-4; X, 110488-54-5; XI, 110488-55-6; XI (homopolymer), 110488-58-9; XII, 110488-56-7; XIII, 110488-57-8; TdT, 9027-67-2; $(r2NH_28MeA)_n$, 97374-44-2; 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, 69304-37-6; thiocarbonyldiimidazole, 6160-65-2; morpholine, 110-91-8; bis(tri-*n*-butylammonium) pyrophosphate, 5975-18-8; $(d2NH_28MeA)_n{\cdot}(dT)_n$, 110613-09-7; $(d2NH_2A)_n{\cdot}(dT)_n$, 83546-40-1; $(d2NH_28MeA)_n{\cdot}(rU)_n$, 110613-10-0; $(d2NH_2A)_n{\cdot}(rU)_n$, 83546-41-2; $(r2NH_28MeA)_n{\cdot}(rU)_n$, 97374-46-4; $(r2NH_2A)_n{\cdot}(rU)_n$, 58382-91-5; $(r2NH_2A)_n{\cdot}(rT)_n$, 85588-09-6; $(r2NH_28MeA)_n{\cdot}(rT)_n$, 97374-45-3.

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