Topography of Nucleic Acid Helices in Solutions. XII. The Origin of the Oppositely Induced Circular Dichroism of Reporter Molecules Bound to Ribo- and Deoxyribonucleic Acid[1a]

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Abstract: A number of reporter molecules I, $R(CH_2)_nN^+(CH_3)_2(CH_2)_3N^+(CH_3)_3 \cdot 2Br^-$, where R is a chromophore absorbing in the 300–500-mμ region, have been synthesized. The effect of DNA and RNA on the absorption spectra of I is reported. A red shift and a hypochromic effect on the absorption spectra of the bound chromophore are observed. Moreover, it is found that DNA and RNA induce an opposite CD in the absorption band of the "unsymmetrical" reporter molecules, I. The origin of this effect appears to be the orientation of the chromophore with respect to the helix axis. The results are interpreted in terms of coupling of the transition moments of the reporter molecules, I, with the transition moments of the bases of the nucleic acids. Moreover, the evidence seems to indicate that the chromophore ring of I in the nucleic acid complex lies in the minor groove of the helix. The substituent of the "unsymmetrical" 4-nitroaniline reporter molecules appears to be pointing *in* and *out* for DNA and RNA complexes, respectively. Molecular models of the DNA- and RNA-reporter systems are formulated and discussed.

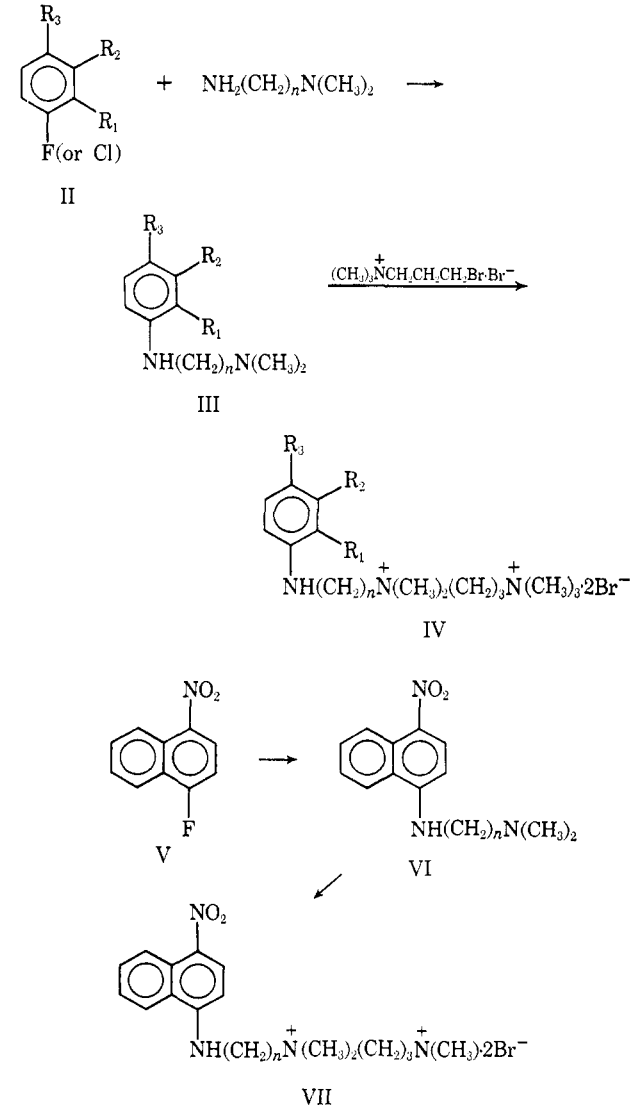
A number of methods are available for studying the structure of biological macromolecules in solutions.[2] Recently, a novel approach utilizing a "reporter group" covalently linked to a protein molecule has been described by Koshland's group. For example, it was shown that the absorption spectra of the bound "reporter group," *i.e.*, *p*-nitrophenol, changes depending on the environment which it is in, which in turn depends on the *local* primary, secondary, and tertiary structure of the macromolecule to which it is bound.[3]

In line with the above work we have recently reported on the use of reporter molecules specifically designed to interact strongly with polyanions, *e.g.*, nucleic acids.[4] In the present paper the synthesis and the interactions of a variety of reporter molecules, I, $R(CH_2)_nN^+(CH_3)_2(CH_2)_3N^+(CH_3)_3 \cdot 2Br^-$ (where R is a chromophore absorbing in the 300–500-mμ region), with nucleic acid helices are reported. It is shown that it is possible to distinguish between various nucleic acids by examining their effect on the absorption spectra and the induced CD of the bound reporter molecules. The method promises to be a useful tool of probing the structure of polynucleotides as well as other polyanions.

Results and Discussion

The synthetic scheme for the preparation of the reporter molecules is straightforward and is outlined in Scheme I and Chart I. An activated haloaromatic (II) is treated with N,N-dimethylethylenediamine or N,N-dimethylpropylenediamine to afford the corresponding tertiary amines III. The latter upon treatment with N,N,N-trimethyl-N-3-bromopropylammonium bromide yields the corresponding bis(quaternary ammonium)

Scheme I[a]



[a] See also Chart I.

(1) (a) For part XI in this series see E. J. Gabbay, *J. Am. Chem. Soc.*, **90**, 6574 (1968); (b) Department of Chemistry, University of Florida, Gainesville, Florida.

(2) H. R. Mahler and E. H. Cordes, "Biological Chemistry," Harper & Row Publishers, New York, N. Y., 1966.

(3) M. Burr and D. E. Koshland, Jr., *Proc. Natl. Acad. Sci. U. S.*, **52**, 1017 (1964); *J. Am. Chem. Soc.*, **89**, 5945 (1967).

(4) E. J. Gabbay, *ibid.*, **90**, 6574 (1968); E. J. Gabbay and J. Mitschele, *Biochem. Biophys. Res. Commun.*, **34**, 53 (1969).

Chart I

Compd	Deriv	R_1	R_2	R_3	n
1	III	NO_2	H	NO_2	2
2	III	NO_2	H	NO_2	3
3	III	H	H	NO_2	2
4	III	H	H	NO_2	3
5	III	NO_2	H	H	2
6	III	NO_2	H	H	3
7	IV	NO_2	H	NO_2	2
8	IV	NO_2	H	NO_2	3
9	IV	H	H	NO_2	2
10	IV	H	H	NO_2	3
11	IV	NO_2	H	H	2
12	IV	NO_2	H	H	3
15	III	CH_3	H	NO_2	2
16	III	CH_3	H	NO_2	3
17	III	H	CH_3	NO_2	2
18	III	H	CH_3	NO_2	3
19	VI				2
20	VI				3
21	IV	CH_3	H	NO_2	2
22	IV	CH_3	H	NO_2	3
23	IV	H	CH_3	NO_2	2
24	IV	H	CH_3	NO_2	3
25	VII				2
26	VII				3

salts, IV. These particular compounds have been synthesized since it is well known that polyammonium salts interact strongly with nucleic acids.[5] Moreover, we have recently shown that considerable information about the structure of nucleic acids *in solutions* may be obtained by studying the effect of substituted polyamines on the T_m of the helix–coil transition and the rate of RNase-catalyzed hydrolysis of polynucleotides.[6] For example, the following polyammonium salts have been synthesized: VIII, $R_1R_2R_3N^+(CH_2)_nN^+R_1R_2R_3 \cdot 2Br^-$; IX, spermine derivatives; X, spermidine derivatives; XI, optically active diammonium salts including amino acid derivatives, diamino acids, and lysyl dipeptides. The interaction of these compounds with nucleic acids has proved to be useful in probing the surface of polynucleotides. The results of such studies indicate that bisammonium salts bind to adjacent phosphate anions on the same chain. This conclusion has also been corroborated by our more recent studies using the reporter molecules **7** and **8**. For example, the single-stranded homopolymers, *i.e.*, polyriboadenylic (rA), polyriboinosinic (rI), polyribocytidylic (rC), and polyribouridylic (rU) acids, interact strongly with **7** and **8** as indicated by the hypochromic effect on the absorption band of the bound reporter molecule.[4,7] Moreover, the removal of the positive charges from the side chain of the reporter molecule, *e.g.*, compound **13**, $4\text{-}NO_2C_6H_4NHCH_2CH_2OH$, abolishes the affinity to the polynucleotides as indicated by the fact that the absorption spectra are unchanged in the presence and absence of the homopolymers.

(5) H. Tabor, *Biochemistry*, **1**, 496 (1962); H. R. Mahler and B. D. Mehrotra, *Biochim. Biophys. Acta.*, **68**, 211 (1963); B. D. Mehrotra and H. R. Mahler, *ibid.*, **91**, 78 (1964); W. Szer, *J. Mol. Biol.*, **16**, 585 (1966); W. Szer, *Biochem. Biophys. Res. Commun.*, **22**, 559 (1966); K. Matsuo and M. Tsuboi, *Bull. Chem. Soc. Japan*, **39**, 347 (1966); S. Higuchi and M. Tsuboi, *ibid.*, **39**, 1886 (1966).

(6) E. J. Gabbay, *Biochemistry*, **5**, 3036 (1966); *Biopolymers*, **5**, 727 (1967); R. Glaser and E. J. Gabbay, *ibid.*, **6**, 241 (1968); E. J. Gabbay and R. R. Shimshak, *ibid.*, **6**, 253 (1968); E. J. Gabbay and R. Kleinman, *J. Am. Chem. Soc.*, **89**, 7123 (1967); E. J. Gabbay, R. Kleinman, and R. R. Shimshak, *Biopolymers*, **6**, 993 (1968); E. J. Gabbay, R. Kleinman, and R. R. Shimshak, *J. Am. Chem. Soc.*, **90**, 1927 (1968); E. J. Gabbay, *ibid.*, **90**, 5257 (1968).

(7) The single-strand helix–reporter molecule complex is easily dissociable by the diammonium salts, $NH_3^+(CH_2)_nNH_3^+2Br^{2-}$, whereas increasing ionic strength, *e.g.*, increasing NaCl concentrations, exhibits a relatively small effect on the binding (E. Gabbay, M. Malin, and F. Passero, work in progress).

Absorption Properties of Reporter Molecules

In order to interpret the results of the interactions of the reporter molecules with nucleic acid systems a thorough analysis of the absorption properties of the chromophores is necessary. The near-uv spectra of reporters **7**, **9**, and **11** are shown in Figure 1. The mononitroanilines exhibit a single absorption maximum in the 300–500-mμ region, *e.g.*, 2-nitroaniline derivative, **11**, ϵ^{422} 5170; 4-nitroaniline derivative, **9**, ϵ^{383} 16,260. The 2,4-dinitroaniline derivative, **7**, shows two transitions in the 300–500-mμ region, *i.e.*, a maximum with ϵ^{350} 16,600, and a shoulder of lower intensity at approximately 400 mμ. These are assigned to the 4-nitroaniline and the 2-nitroaniline chromophores, respectively.[8] The effect of various solvents on the intensity and λ_{max} of the 4-nitroaniline transition of reporter **8** is shown in Table I. The transition is probably a charge-transfer $\pi^* \leftarrow \pi$ type since it has an extinction coefficient, ϵ_{max}, of 16,000 characteristic of an allowed transition, and a red shift in the maximum is observed as the polarity of the solvent is increased.[9] The result is consistent with previous studies on the 4-nitroaniline chromophore, *e.g.*, it has been shown that the excited state has a larger dipole moment than the ground state (μ_g = 6 D, and μ_e = 14 D).[10] Theoretically, the polarizability and the polarity of the solvent determine the energy of the ground and excited states.[11] The stabilization energy due to the polarizability effect is related to the refractive index n of the solvent.[9] It is clear from inspection of Table I that the energy of the transition depends mainly on the dielectric constant rather than the refractive index of the medium. This effect is important in interpreting the spectra of the bound reporter molecule (see below) and is in line with the Franck–Condon principle.[12]

Table I. The Effect of Various Solvents on 4-Nitroaniline Transition of Reporter **8**, $2,4\text{-}(NO_2)_2C_6H_3NH(CH_2)_3N^+(CH_3)_2(CH_3)_3N^+(CH_3)_3 \cdot 2Br^-$ [a]

Solvent	λ_{max}, mμ	E, kcal	$\epsilon \times 10^{-4}$	Dielec const	n
Ethanol	343.0	83.38	1.605	24	1.362
Acetonitrile	347.5	82.30	1.600	37	1.346
Formic acid	350.0	81.71	1.640	58	1.371
Water	356.0	80.34	1.616	80	1.333
Formamide	357.5	80.00	1.740	109	1.445

[a] All spectra were taken in 10-mm cells using a Cary 14 spectrophotometer at 25.0 + 0.2°.

(8) The blue shift observed for the two transitions (*i.e.*, 4-nitro- and 2-nitroanilines) upon introduction of the second nitro group is reasonable since the charge-transfer excited state of both transitions is expected to be destabilized by an electron-withdrawing substituent: J. N. Murrell, "The Theory of Electronic Spectra of Organic Molecules," John Wiley & Sons, Inc., New York, N. Y., 1963.

(9) The large red shift observed on changing solvents (ethanol to formamide) which correspond to 3.4 kcal is indicative of an intramolecular charge-transfer transition: J. N. Murrell, ref 8, p 305.

(10) L. Czekalla, *Z. Elektrochem.*, **64**, 1221 (1960).

(11) Y. Ooshika, *J. Phys. Soc. Japan*, **10a**, 541 (1955); E. G. McRae, *J. Phys. Chem.*, **61**, 562 (1957); E. Lippert, *Z. Elektrochem.*, **61**, 962 (1957).

(12) N. S. Bayliss and E. G. McRae, *J. Phys. Chem.*, **58**, 1002 (1954).

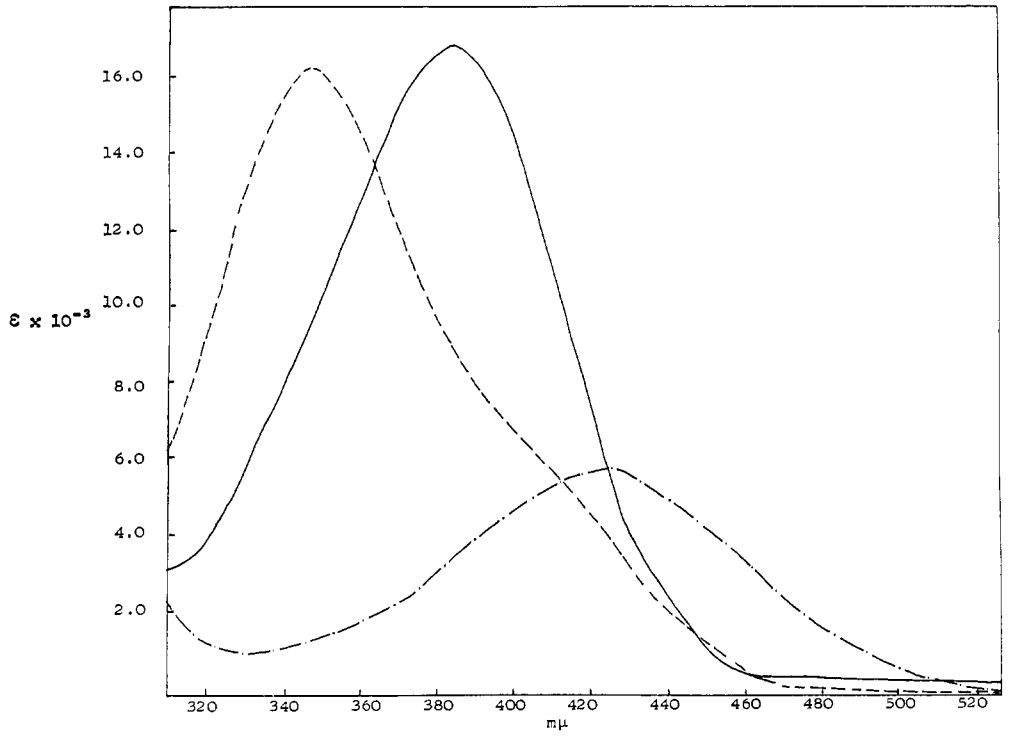


Figure 1. Near-uv spectra of reporter molecules **7** (– –), **9** (—), and **11** (–·–) in water–buffer (see Table II).

Table II contains a summary of the absorption spectra of reporter molecules **7–12** and **21–26** in two solvent systems, *i.e.*, 95% ethanol and 0.01 *M* sodium phosphate buffer, pH 6.50. Again, it is clear by inspection of Table II that a red shift is observed for the 4-nitroaniline transitions, *i.e.*, reporters **7–10**, **21–24**, and for the 2-nitroaniline transition, *i.e.*, reporters **11** and **12**, as the polarity of the solvent is increased. The results are consistent and in agreement with the previous interpretations. It is also noteworthy that a blue shift is observed in 4-nitro- and 2-nitroaniline transitions as the positive charge on the quaternary nitrogen is brought closer to the ring chromophore, *e.g.*, reporters **7** and **8**, **9** and **10**, **11** and **12**, **21** and **22**, and **23** and **24**. Moreover, methyl group substitution at either position 2 or 3 of the 4-nitroaniline ring lowers the extinction coefficient, ϵ_{max}, of the transition.[13]

Table II. Summary of the Absorption Spectra of Reporter Molecules **7–12** and **21–26** in 95% Ethanol and in Water[a,b]

Reporter	95% ethanol				H_2O–buffer[b]			
	λ_{max}, mμ	ϵ_{max}	λ_{max}, mμ	ϵ_{max}	λ_{max}, mμ	ϵ_{max}	λ_{max}, mμ	ϵ_{max}
7	338	16,600			350	16,600		
8	343	16,050			356	16,160		
9	366	16,500			383	16,260		
10	379	18,240			397	17,870		
11	415	5,500			422	5,170		
12	427	5,850			437	5,530		
21	371	14,840			387	14,440		
22	381	16,450			400	15,300		
23	366	13,600			381	13,600		
24	373	15,370			393	14,700		
25	414	15,360	333(s)	2800	428	14,400	322(w)	2500
26	428	16,300	333(s)	2290	443	15,500	322(w)	2400

[a] All spectra were taken in 10-mm cells using a Cary 14 spectrophotometer at 25.0 ± 0.2°. [b] In 0.01 *M* sodium phosphate buffer (0.01 *M* in Na^+), pH 6.50.

Reporters **25** and **26** were synthesized in order to obtain two separate transitions in the same molecule in the region above 300 mμ. The absorption spectra of **25** in two solvent systems is shown in Figure 2. It is clear that there are two transitions—one at 414 mμ (ε 15,360) and a shoulder peak at 333 mμ (ε 2800) in ethanol. The lower energy transitions shift to the red in going to more polar solvent, *e.g.*, 414 to 428 mμ and 428 to 443 mμ for reporters **25** and **26**, respectively. This behavior is consistent with the charge-transfer 4-nitroaniline transitions. The higher energy transition at 310–350 mμ of reporters **25** and **26** is characteristic of the naphthylamine chromophore and is therefore assigned to it.[14]

Absorption Properties of Bound Reporter Molecules

In all cases the nucleic acid bound reporter molecules exhibit two characteristic effects on the absorption spectra: (1) a red shift and (2) hypochromism. A summary of the results is shown in Tables III, IV, and V for reporters **7**, **8**; **9–12**, **21–24**; and **25–26**, respectively. The origin of the red shift may be attributed to two factors, namely, (1) higher polarity and higher polarizability of the nucleic acid surface as compared with that of water, and (2) the effect of the electrostatic field. A close proximity of the nitroaniline ring to the negatively charged phosphodiester group in the reporter–nucleic acid complex is expected. This polar environment would then lead to stabilization of the charge-transfer excited state causing the red shift. Polarizability fac-

(13) The lower extinction coefficients for 2- and 3-methyl-substituted 4-nitroanilines as compared with unsubstituted 4-nitroaniline derivative is probably due to steric hindrance to coplanarity in the former: J. N. Murrell, ref 8, Chapter 11.

(14) L. Lang, "Absorption Spectra in the Ultraviolet and Visible Region," Vol. 5, Academic Press, New York, N. Y., 1965, p 219.

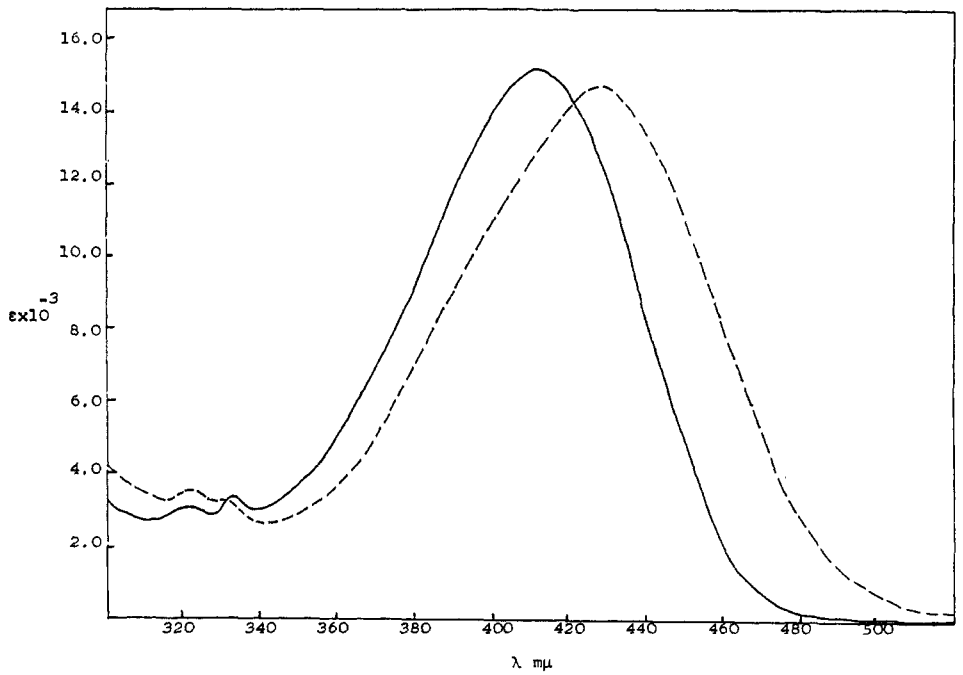


Figure 2. Near-uv spectra of reporter molecule **25** in ethanol (—) and in water (- -).

tors may also play a role but their effect on the transition is likely to be small due the the Franck–Condon principle.[12] These arguments are in line with the effects of solvents on the nitroaniline transitions (Table I) where

Table III. The Effect of Ribo- and Deoxyribonucleic Acids on the Absorption Spectra of Reporter Molecules **7** and **8** $[2,4\text{-}(NO_2)_2C_6H_3NH(CH_2)_nN^+(CH_3)_2(CH_2)_3N^+(CH_3)_3 \cdot 2Br^-]$[a]

	Reporter molecule							
	7 (n = 2)				8 (n = 3)			
Conditions	λ_{max}, mμ	ϵ_{max}	% H[b]	P/R[c]	λ_{max}, mμ	ϵ_{max}	% H[b]	P/R[c]
1. 95% EtOH	338	16,600			343	16,050		
2. H_2O–buffer[d]	350	16,600			356	16,160		
3. Salmon testes								
DNA(N)[e]	355	10,060	65	80	362	10,340	56	80
DNA(D)[f]	355	10,780	53	80	361	10,300	57	80
4. Calf thymus								
DNA(N)[e]	355	10,000	66	74	362	9,680	67	74
DNA(D)[f]	355	10,860	52	74	361	10,660	52	74
5. Yeast								
RNA(N)[e]	354	12,300	35	80	361	11,000	46	80
6. Torula								
RNA(N)[e]	355	11,800	41	80	361	12,330	31	80

[a] At 25.0 + 0.02° in 0.01 M sodium phosphate buffer pH 6.40–6.50 (0.01 M in Na^+). Spectra were taken in 10-mm cells using Cary 14 spectrometer. Values of λ_{max} and ϵ_{max} in the presence of nucleic acid reported in this table are limiting values, *i.e.*, additional change in spectra is not observed at further excess of nucleic acid. [b] Per cent hypochromicity (% H) = $[\epsilon_{H_2O}^{max}/(\epsilon_p^{max} - 1)]100$, where $\epsilon_{H_2O}^{max}$ and ϵ_p^{max} are the extinction coefficients in the absence and presence of the polynucleotides. [c] P/R indicates the ratio of polynucleotide phosphate/reporter molecule. In all cases reported 5×10^{-5} M of reporter molecules was used. [d] In 0.01 M sodium phosphate buffer (0.01 M in Na^+), pH 6.50. [e] Native nucleic acids. [f] Denatured nucleic acid (see Experimental Section).

Table IV. The Effect of Ribo- and Deoxyribonucleic Acids on the Absorption Spectra of Reporter Molecules **9–12** and **21–24**[a]

	Calf thymus DNA			Salmon testes DNA			Yeast RNA			Torula RNA		
Reporter	λ_{max}, mμ	ϵ_{max}	% H[b]	λ_{max}, mμ	ϵ_{max}	% H[b]	λ_{max}, mμ	λ_{max}	% H[b]	λ_{max}, mμ	ϵ_{max}	% H[b]
9	393	12,200	33	393	12,240	33	393	13,080	24	392	13,950	16
10	405	13,840	26	405	13,240	32	404	15,600	12	404	15,200	15
11	428	4,250	22	428	4,110	25	429	4,390	18	428	4,610	12
12	442	4,470	24	443	4,355	26	442	4,701	17	441	4,990	11
21	399	10,260	41	399	10,440	38	397	11,430	26	397	11,600	24
22	412	11,470	33	412	11,470	33	410	13,290	15	410	13,100	17
23	392	11,200	22	392	10,700	27	391	12,400	10	391	12,600	8
24	403	11,860	24	403	11,580	27	402	13,100	12	402	12,900	14

[a,b] Same as Table III. [c] P/R ratio (polynucleotide phosphorus/reporter molecule) used in these studies is approximately 74–80 for all reporters with the exception of **11** and **12** where P/R ratio of 12 was used; 5×10^{-5} M **9**, **10**, and **21–24**, and 3.3×10^{-4} M **11** and **12** was employed.

Table V. The Effect of Ribo- and Deoxyribonucleic Acids on the Absorption Spectra of Reporter Molecules **25** and **26** [4-$NO_2C_{10}H_6NH(CH_2)_nN^+(CH_3)_2(CH_2)_3N^+(CH_3)_3 \cdot 2Br$][a]

Conditions	Reporter molecule: 25 (n = 2) λ_{max}, mμ	ϵ_{max}	% H[b]	P/R[c]	26 (n = 3) λ_{max}, mμ	ϵ_{max}	% H[b]	P/R[c]
1. 95% EtOH	414	15,360			428	16,300		
2. H_2O–buffer[d]	428	14,400			443	15,500		
3. Calf thymus DNA	447	12,400	16	74	456	12,400	25	74
4. Salmon testes DNA	447	12,300	17	80	456	12,190	27	80
5. Yeast RNA	445	12,700	13	80	456	13,220	17	80
6. Torula RNA	445	13,000	11	80	456	13,330	16	80

[a–c] Same as Table III.

it is observed that the energy of the transition depends mainly on the dielectric constant rather than the refractive index of the solvent.

The second factor, the effect of the electrostatic field on the energy of the nitroaniline transition, is also indicated. For example, the introduction of a positive charge in the vicinity of the 4-nitroaniline ring results in a blue shift. This effect is clearly observed for 4-$NO_2C_6H_4NH(CH_2)_nR$ where R = OH, n = 2; R = $-N^+H(CH_3)_2$ and n = 2 and 3 (Table VI). The extent of the

(a) side-by-side arrangement (card stack)

A ↑ B ↑ where $\bar{\nu}_A < \bar{\nu}_B$ would lead to hypochromism (lower intensity) for the lower energy transition and hyperchromism (higher intensity) for the higher energy transition

direction of transition moments: A is hypochromic, B is hyperchromic

(b) head-to-tail arrangement

A → B → where $\bar{\nu}_A < \bar{\nu}_B$: A is hyperchromic, B is hypochromic

(c) herringbone arrangement

A ↑ → B no intensity interchange

Figure 3. The effect of two interacting transition moments for two different transitions, A and B, in adjacent chromophores on the intensity of the absorption spectra for transitions with frequencies ν_A and ν_B, respectively (see ref 15).

blue shift can be seen to depend on the distance between the positively charged nitrogen and the ring chromophore, *e.g.*, decreasing the distance by a methylene group causes a 15 and 4 mμ blue shift in water and ethanol, respectively. The possibility exists that the red shift observed upon binding the reporter molecule diammonium salts, compounds **7–12** and **21–26**, to polynucleotides may be due to the neutralization of the vicinal positive charge as a result of binding to adjacent phosphate anions.

Table VI. The Effect of the Internal Electrostatic Field on the 4-Nitroaniline Transition in 4-$NO_2C_6H_4NH(CH)_{2n}R$[a]

Solvent	n = 2, R = OH λ, mμ	ε	n = 3, R = $-N^+H(CH_3)_2$ λ, mμ	ε	n = 2, R = $-N^+H(CH_3)_2$ λ, mμ	ε
H_2O–buffer[b]	405	18,480	400	17,000	385	14,800
Ethanol	387	20,060	380	18,200	376	15,160

[a,b] Same as Table II.

The hypochromic effect exhibited by the bound reporter molecule may be explained in terms of coupling of the transition moments of the chromophore with the transition moments of the bases in the nucleic acid helix.[15] Figure 3 depicts the intensity interchange in absorption spectra of ordered systems. The diagram represents dispersion-force interaction between two different transitions of approximately equal intensity, A and B, in adjacent chromophores, arranged (a) side-by-side, (b) head-to-tail, (c) herringbone fashion; $\bar{\nu}_A$ and $\bar{\nu}_B$ are the frequencies corresponding to the two transitions. Although the theoretical treatment of hypochromism is not rigorous the following important generalization may still be made.[15] (1) When the chromophores are aligned side-by-side (card stack) the lowest and highest energy transitions will be hypochromic and hyperchromic, respectively. (2) When the chromophores are aligned head-to-tail, the lowest energy transition will be hyperchromic, intensity then being gained at the expense of the higher energy transitions. (3) For a herringbone arrangement, with the transition moments for the lower and higher energy transitions perpendicular to one another, there will be no intensity interchange.

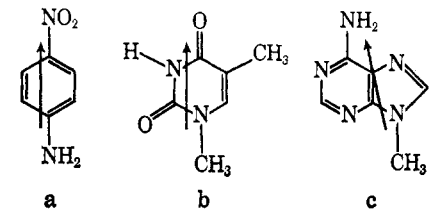


Figure 4. Direction of transition moments for (a) *p*-nitroaniline [E. Lippert, *Z. Elektrochem.*, **61**, 982 (1957); M. B. Ledger and P. Sappan, *Spectrochim. Acta*, **23A**, (1967)]; (b and c) 1-methylthymine and 9-methyladenine [R. Stewart and N. Davidson, *J. Chem. Phys.*, **39**, 255 (1963)].

In all the cases so far examined the low-energy transition, *i.e.*, nitroaniline chromophore, exhibits a hypochromic effect upon binding to nucleic acid systems. Coupling of the transition moments of the reporter molecule and the bases of the polynucleotides is indicated. Moreover, the geometry of the complex is such that the transition moments of the two chromophores has a nonvanishing side-by-side arrangement (Figure 3a). Figure 4 depicts the direction of the transition

(15) Hypochromism in ordered molecules may be qualitatively accounted for in terms of interaction of transition moments [I. Tinoco, Jr., *J. Am. Chem. Soc.*, **82**, 4745 (1960); *J. Chem. Phys.*, **34**, 1067 (1961); H. Devoe and I. Tinoco, Jr., *J. Mol. Biol.*, **4**, 518 (1962); W. Rhodes, *J. Am. Chem. Soc.*, **83**, 3609 (1961)].

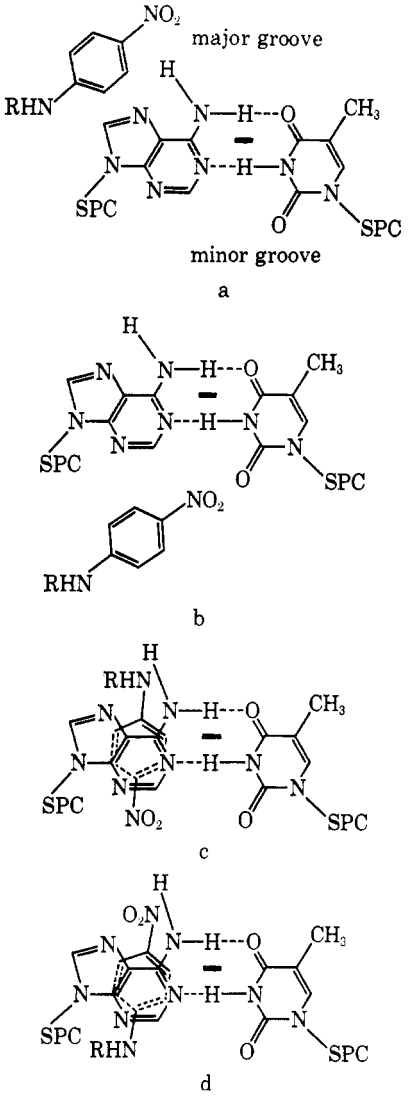


Figure 5. Schematic representation of the four possible complexes formed between base pairs, *e.g.*, A–T, in a nucleic acid and the 4-nitroaniline chromophore. In arrangements a and b the ring chromophore lies in the major and minor groove, respectively. In arrangements c and d the chromophore is inserted between base pairs from the major and minor groove, respectively. The major and minor grooves may easily be discerned from the position of the sugar phosphate chain (SPC).

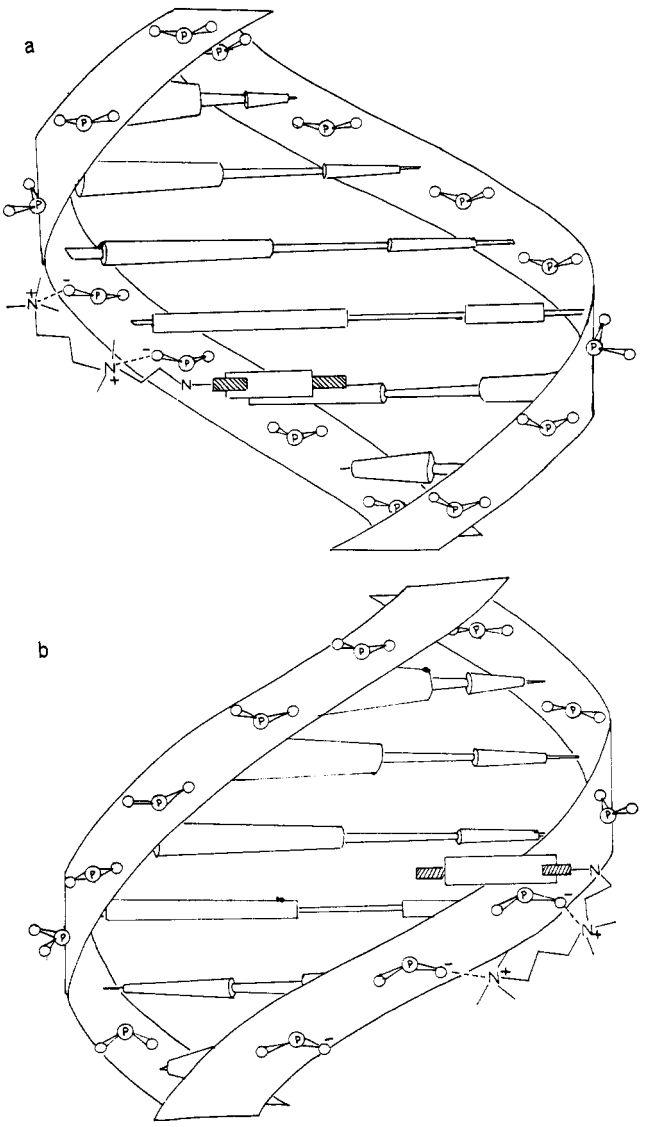


Figure 6. Schematic representation of the major (a) and minor (b) grooves of a nucleic acid helix showing the postulated attachment of the reporter molecules (see text).

moments of 4-nitroaniline, a purine, and a pyrimidine. In the reporter–nucleic acid complex it is clear that a card-stack arrangement of transition moments may be obtained in four possible ways as shown in Figure 5. In arrangements a and b the nitroaniline ring may be situated in the major and minor groove of the helix, respectively, whereas the intercalation model, arrangements c and d, the chromophore is inserted between the base pairs from the major and minor groove, respectively. All of these models (or slight modifications thereof) would predict the hypochromism observed in the bound reporter molecule. It is possible, however, to choose between these complexes on the basis of molecular models and experimental observations. If it is assumed that the reporter molecule binds to nucleic acids principally by the electrostatic binding of the side-chain diammonium groups to adjacent phosphate anions of a polynucleotide chains[16] then it is possible to rule out the intercalation models c and d of Figure 5 for steric reasons. The electrostatic binding assumption is reasonable since the reporter–nucleic acid complex is easily dissociable in high ionic strength solutions as well as in the presence of simple diammonium salts of the type $NH_3^+(CH_2)_nNH_3^+ \cdot 2Br^-$.[17] Moreover, the removal of the positive charges from the side chain of the reporter molecule, *e.g.*, compound **13**, $NO_2C_6H_4NHCH_2CH_2OH$, abolishes the affinity to polynucleotides as indicated by the fact that the absorption spectra of the latter is unchanged in the presence and absence of nucleic acids. The last two experimental observations argue against the intercalation model, Figure 5c and d. Moreover, inspection of molecular models of the reporter–nucleic acid complex where the diammonium groups are bound to adjacent anions on the helical chain rules out the intercalation models, *i.e.*, the methylene chain is not long enough to allow the nitroaniline ring to insert between base pairs. Figure 6 depicts schematically the two most plausible complexes that not

(16) It has been shown using a variety of techniques that diammonium salts bind to adjacent phosphate anions of the same strand of nucleic acid system (see ref 6).

(17) E. Gabbay, F. Passero, and M. Malin, work in progress, 1968.

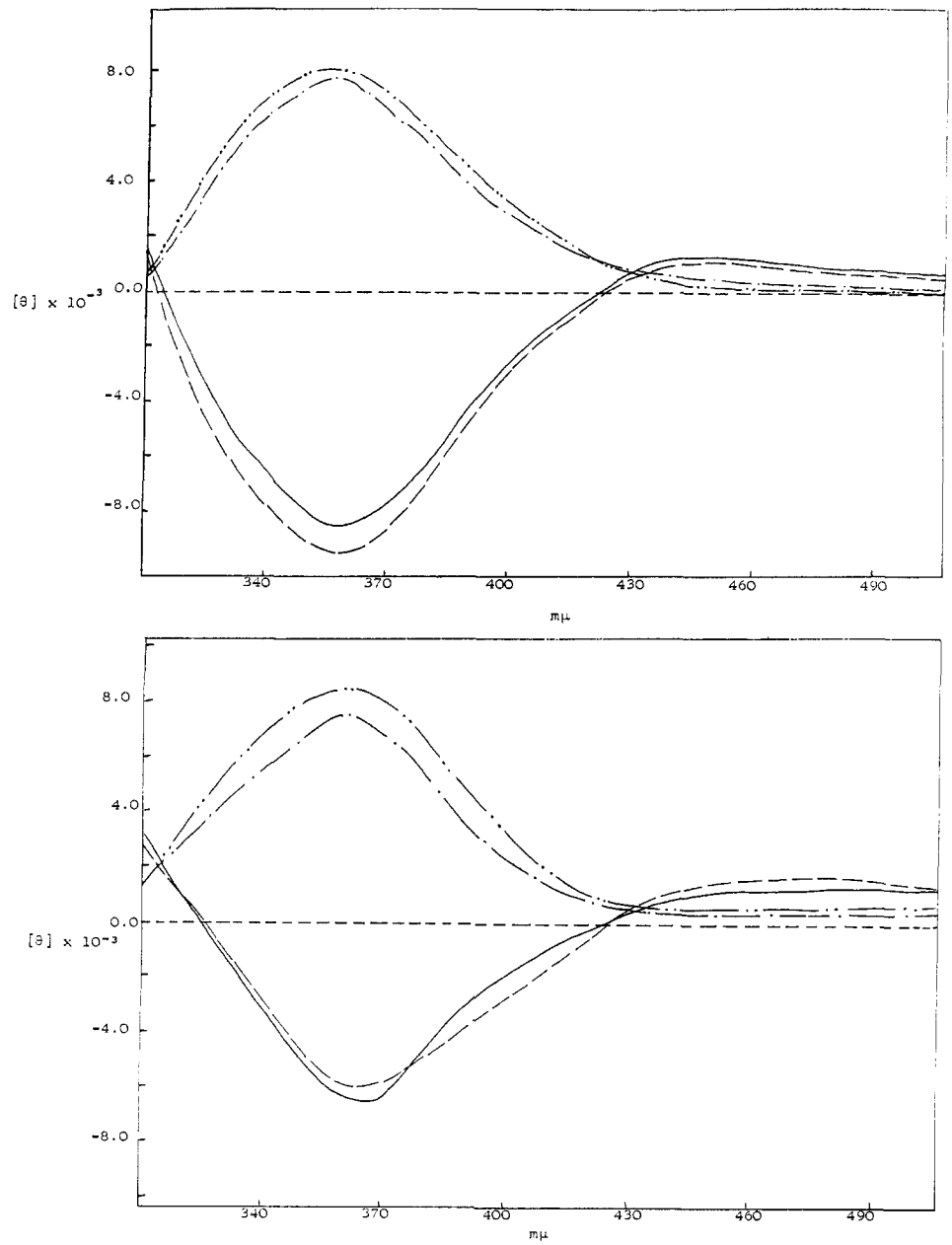


Figure 7. The induced CD spectra of reporter molecules **7** and **8** in the presence of nucleic acids. (a, top) Reporter **7** in the presence of yeast RNA (-··-), torula RNA (-·-), calf thymus DNA (—), and salmon testes DNA (- - -). (b, bottom) Same as a but using reporter **8** (see also Table VII).

only fit the molecular framework requirements but all the experimental data as well (see below). It is observed that the two arrangements shown in Figure 6a and b would bring the chromophore into close edgewise proximity to the bases in the major and minor groove, respectively. It is difficult to decide which of the complexes, *i.e.*, Figure 6a or 6b and/or both, is formed in solutions. In both arrangements, the direction of the transition moment of the 4-nitroaniline chromophore is expected to be more nearly parallel with that of the adjacent base as compared with the 2-nitroaniline chromophore. These models would predict a greater hypochromicity for the bound 4-nitroaniline than the bound 2-nitroaniline chromophore which in fact is observed (Tables III and IV). It should be pointed out that the absorption spectra and the molecular framework models do not give the exact geometry of the reporter–nucleic acid complex. For example, the angle that the plane of the nitroaniline ring makes with the helical axis in the major or minor groove can be anywhere from 0 to 90°. It is likely, however, that the plane of the chromophore is perpendicular with respect to the helix axis as indicated by the induced CD studies (see below). Flow dichroism studies are now in progress to resolve this question. It is important to note that in all cases so far examined the bound reporter molecule show a greater hypochromicity upon binding to DNA than to RNA. This finding is significant and consistent (as will be shown below) with the idea that the ring chromophore is bound more closely to DNA than to RNA.

Table VII. The Effect of Various Nucleic Acids on the Induced CD of Reporter Molecules **7** and **8**, $2,4\text{-}(NO_2)C_6H_4NH(CH_2)_nN^+(CH_3)_2(CH_2)_3N^+(CH_3)_3 \cdot 2Br^-$ [a]

Polynucleotide	7 (n = 2) λ^p, mμ	7 (n = 2) $[\theta]^p \times 10^{-3}$	7 (n = 2) λ^t, mμ	7 (n = 2) $[\theta]^t \times 10^{-3}$	7 (n = 2) P/R[b]	8 (n = 3) λ^p, mμ	8 (n = 3) $[\theta]^p \times 10^{-3}$	8 (n = 3) λ^t, mμ	8 (n = 3) $[\theta]^t \times 10^{-3}$	8 (n = 3) P/R[b]
1. Calf thymus										
DNA(N)			360	−9.40	22			365	−6.73	22
DNA(D)[c]			357	−1.45	22			365	−2.76	22
DNA(D)[d]			357	−3.12	22					
2. Salmon testes										
DNA(N)			360	−9.60	24			365	−6.24	24
DNA(D)[c]			360	−3.48	24			370	−2.41	24
DNA(D)[d]			360	−4.08	24					
3. Yeast										
RNA(N)	357	7.80			24	360	8.68			24
RNA(D)[c]	355	7.74			24	361	8.92			24
4. Torula										
RNA(N)	358	7.44			24	360	7.44			24
RNA(D)[c]	357	7.62			24	360	7.23			24

[a] CD curves were measured in a Cary 60 recording spectropolarimeter equipped with a Model 6001 CD accessory at 26.0 ± 0.4° in 10-mm cells. The solution contained 1.67×10^{-4} M of **7** or **8** in 0.01 M sodium phosphate buffer (0.01 M in sodium), pH 6.40–6.50. [b] The ratio of moles of polynucleotide phosphorus to moles of reporter molecules in solution. [c] Denatured nucleic acid (see Experimental Section). [d] Denatured DNA after standing at room temperature for 26 hr.

Induced Circular Dichroism of the Bound Reporter Molecule

Gabbay[1] has recently reported that DNA and RNA induce an opposite CD in the absorption band of the bound 2,4-dinitroaniline reporter molecules, **7** and **8**. The results are reproduced in Figure 7 and Table VII. Ribose-containing nucleic acids induce a positive Cotton effect similar to what has been found for the double-stranded homopolymers, *i.e.*, polyriboadenylic–polyribouridylic, and polyribocytidylic–polyriboinosinic acid helices.[4] Concentration studies was made and a dependence of the observed molar ellipticity on the P/R (moles of polynucleotide phosphorus/mole of reporter molecules) ratio was found. In all cases, as $P/R \rightarrow 0$ the molar ellipticity $[\theta] \rightarrow 0$; as $P/R \rightarrow \infty$ ($P/R = 24$ was the largest ratio studies) $[\theta]$ approaches a limiting value. Moreover, the molar ellipticity of the DNA–reporter complex depends on n, *i.e.*, it is larger for $n = 2$ than $n = 3$. On the other hand, the molar ellipticity of the RNA–reporter complex is relatively unchanged for native torula RNA and is smaller for $n = 2$ than for $n = 3$ for yeast RNA (Table VII). Denatured DNA induces a lower CD in the absorption band of **7** and **8** than native DNA. The results are consistent with the idea that the induced CD depends on the interaction with double-stranded helical rather than the single-stranded random coil regions of DNA. On allowing the denatured DNA to anneal (26 hr at room temperature) the molar ellipticity, $[\theta]$, increases slightly in line with the above interpretation. Native and denatured RNA complexes with **7** and **8** show approximately the same molar ellipticities. The results are not surprising since the commercial preparation of yeast RNA and torula RNA involves a similar denaturation step.

It remains at this point to explain the origin of the oppositely induced CD in the 4-nitroaniline transition of the bound 2,4-dinitroaniline chromophore. The effect of nucleic acid on the absorption spectra of reporter molecules together with molecular framework model studies has allowed certain conclusions concerning the structure of the complex to be made. For example, the arrangements shown in Figure 6a and 6b where the reporter molecule is bound electrostatically to adjacent phosphate anions and the chromophore ring lying alongside the bases in the major or minor groove of the helix best describe the available data (see above). It is clear by inspection that there are two possible conformations for the "unsymmetrical" nitroanilines for each arrangement as shown in Figure 8. The 2-nitro

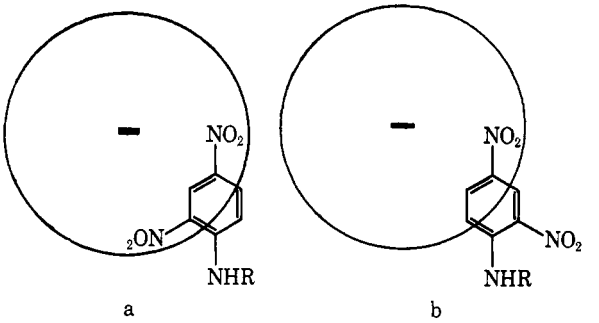


Figure 8. Schematic representation of a nucleic acid helix (top view) showing the *in* (a) and *out* (b) geometries (see text).

group in the 2,4-dinitroaniline ring may be pointing either *in* or *out* of the helix. The oppositely induced circular dichroism that is found for the RNA- and DNA–reporter complexes may be caused by either the *in* or *out* geometries or differences due to the binding to the major or minor grooves. In order to test these hypotheses the induced CD in the absorption band of the bound 2-nitro- and 4-nitroaniline reporter molecules were examined. It is predicted by the first model that the symmetrical chromophore, 4-nitroaniline, should give only one geometry, whereas the 2-nitroaniline chromophore should yield the two possible conformations, *i.e.*, *in* and/or *out*. The second model which depends on the mode of binding, *i.e.*, Figure 6a or 6b, would predict oppositely induced CD for all the chromophores. The results are shown in Tables VIII and IX. It is clear from inspection of the data that both RNA and DNA induce a positive single Cotton effect in the absorption band of the "symmetrical" reporter molecules

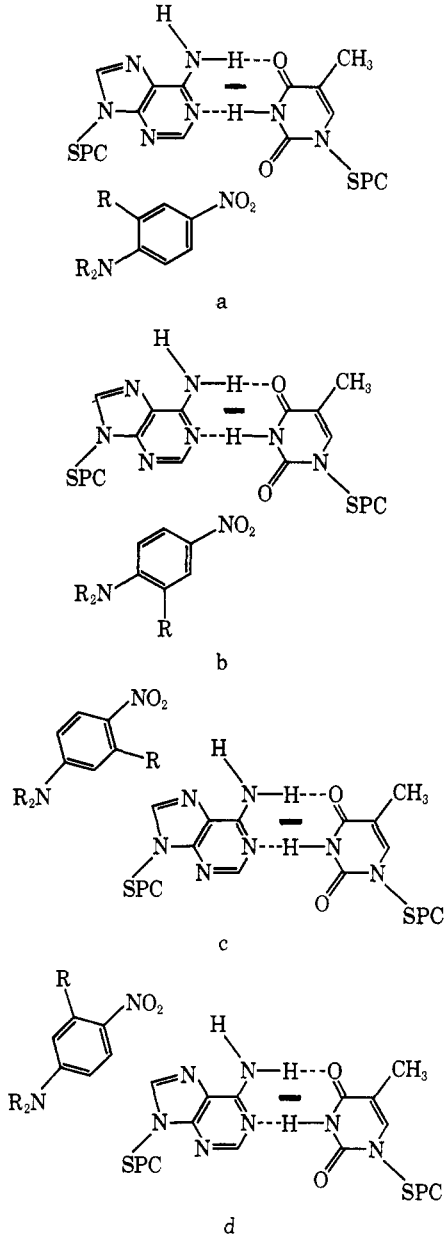


Figure 9. Schematic representation of the four possible complexes formed between base pairs, *e.g.*, A–T, in a nucleic acid and "unsymmetrical" substituted 4-nitroaniline chromophores. In arrangements a and b the ring chromophore lies in the minor groove with substituent group sticking *in* and *out* of the helix, respectively. In arrangements c and d the ring chromophore lies in the major groove with the substituent group sticking *in* and *out* of the helix, respectively. The major and minor grooves may easily be discerned from the position of the sugar phosphate chain (SPC).

9 and **10**, *i.e.*, $4\text{-}NO_2C_6H_4NH(CH_2)_nN^+(CH_3)_2(CH_2)_3N^+(CH_3)_3\cdot 2Br^-$ where $n = 2$ and 3, respectively. However, the induced CD of the bound "nonsymmetrical" chromophore, reporters **11** and **12**, *i.e.*, $2\text{-}NO_2C_6H_4NH(CH_2)_nN^+(CH_3)_2(CH_2)_3N^+(CH_3)_3\cdot 2Br^-$, shows a dependence on n as well as on the nucleic acid. For example, the complex formed between reporter **11** where $n = 2$ and RNA or DNA shows a weak positively induced Cotton effect.[18] On the other hand, RNA and DNA induce opposite CD in the absorption band of reporter molecule **12** where $n = 3$. These results are in line with the first hypothesis, namely that the oppositely induced CD arises from "unsymmetrical" nitroaniline nucleic acid complex having two geometries, *in* and/or *out* as shown in Figure 8. Moreover, it is possible that the *in* and *out* geometries are in equilibrium and the more predominant one determines the sign of the induced Cotton effect. This situation is probably obtained in the case of the DNA–reporter **11** complex where the molar ellipticity $[\theta]$ is almost negligible (Table IX).

Table VIII. The Effect of Various Nucleic Acids on the Induced CD of $4\text{-}NO_2C_6H_4NH(CH_2)_nN^+(CH_3)_2(CH_2)_3N^+(CH_3)_3\cdot 2Br^-$ [a]

	9 (n = 2)			10 (n = 3)		
	λ_P, mμ	$[\theta]_P \times 10^{-3}$	P/R [b]	λ_P, mμ	$[\theta]_P \times 10^{-3}$	P/R [b]
1. Calf thymus DNA	388	2.51	20	395	6.43	20
2. Salmon testes DNA	385	2.51	22	395	5.77	22
3. Yeast RNA	395	4.06	22	398	6.21	22
4. Torula RNA	395	4.77	22	398	5.55	22

[a,b] Same as Table VII.

In summary, it is concluded on the basis of molecular framework models and experimental observations (both of which rules out intercalation of the reporter) that the nucleic acid–reporter complex may best be described by two arrangements whereby the chromophore ring of the reporter lies in the major or minor groove of the nucleic acid helix. Moreover, for each of these conformations the substituted 4-nitroaniline chromophore may have two orientations, *in* and/or *out* as shown in Figures 8 and 9. In arrangements shown in Figure 9a and b the substituted 4-nitroaniline ring lies in the minor groove with the substituent group at position 2 or 3 pointing either *in* or *out* of the helix, respectively. Similarly, arrangements shown in Figure 9c and d depict the substituted 4-nitroaniline chromophore lying in the major groove with the substituent group pointing *in* and *out* of the helix, respectively. It is possible to distinguish between these complexes. The hypochromism and the induced CD in the absorption band of the bound substituted 4-nitroaniline chromophore can best be explained in terms of the preference of RNA to bind the reporter molecule with the substituent group pointing *out*. The DNA, however, may bind the "unsymmetrical" reporter molecule either with substituent group *in* or *out* depending on the nature of the latter. The evidence for this conclusion has up till now been inconclusive and may be summarized as follows. (1) The DNA–reporter complex always shows a greater hypochromism than the corresponding RNA–reporter complex. It suggests that the chromophore ring of the reporter molecule lies closer to the bases in DNA than in RNA. (2) *Both* RNA and DNA induce a *positive* circular dichroism in the "symmetrical" unsubstituted 4-nitroaniline chromophore, *i.e.*, reporters **9** and **10**. The introduction of a substituent in the 4-nitroaniline ring, *i.e.*, 2,4-dinitroaniline reporter molecules, **7** and **8**, leads to an oppositely induced CD in the absorption band of the 4-nitroaniline transition of the RNA and DNA complexes. The RNA complex with 2,4-dini-

(18) It should be noted that the induced molar ellipticity $[\theta]$ for the 2-nitroaniline–nucleic acid complex is an order of magnitude lower than that of the 4-nitroaniline–nucleic acid complex. This difference may reflect not only the distance between the transition moment associated with two chromophores and that of the bases in nucleic acids but also the angle between the magnetic dipoles associated with each. Other explanations are also possible (see text).

Table IX. The Effect of Various Nucleic Acids on the Induced CD of 2-$NO_2C_6H_4NH(CH_2)_nN^+(CH_3)_2(CH_2)_3N^+(CH_3)_3 \cdot 2Br^-$ [a-c]

	11 (n = 2)				12 (n = 3)	
	λ^p, mμ	$[\theta]^p \times 10^{-2}$	λ^p, mμ	$[\theta]^p \times 10^{-2}$	λ^t, mμ	$[\theta]^t \times 10^{-2}$
1. Calf thymus DNA	430	0.74			440	−5.38
2. Salmon testes DNA	430	1.48			440	−5.38
3. Yeast RNA	430	6.67	440	5.30		
4. Torula RNA	430	5.19	440	4.61		

[a,b] Same as Table VII. [c] No sharp maximum but a broad peak.

Table X. The Effect of Various Nucleic Acids on the Induced CD of Reporter Molecules **21–24**[a]

	Calf thymus DNA		Salmon testes DNA		Yeast RNA		Torula RNA	
Reporter	$\lambda^{p \text{ or } t}$, mμ	$[\theta] \times 10^{-3}$	$\lambda^{p \text{ or } t}$, mμ	$[\theta] \times 10^{-3}$	λ^p, mμ	$[\theta]^p \times 10^{-3}$	λ^p, mμ	$[\theta]^p \times 10^{-3}$
21	412	−4.08	410	−4.56	393	5.04	394	4.80
22	400	0.92	400	1.37	405	3.44	405	3.44
	440	−0.92	438	−0.69				
23	402	−3.00	400	−3.23	391	6.35	390	6.23
24	400	0.80	400	0.70	405	4.59	405	5.04

[a] Same as Table VII.

Table XI. The Effect of Various Nucleic Acids on the Induced CD of Reporter Molecules **25** and **26**, [4-$NO_2C_{10}H_5NH(CH_2)_nN^+(CH_3)_2(CH_2)_3N^+(CH_3)_2 \cdot 2Br^-$][a]

	Reporter molecule											
	25 (n = 2)						26 (n = 3)					
Conditions	λ^p, mμ	$[\theta]^p \times 10^{-3}$	λ^t, mμ	$[\theta]^t \times 10^{-3}$	λ^p, mμ	$[\theta]^p \times 10^{-3}$	λ^p, mμ	$[\theta]^p \times 10^{-3}$	λ^t, mμ	$[\theta]^t \times 10^{-3}$	λ^p, mμ	$[\theta]^p \times 10^{-3}$
1. Calf thymus DNA			450	−7.20	323	6.00			465	−1.12	330	1.98
2. Salmon testes DNA			450	−6.12	322	5.52			465	−1.73	330	2.73
3. Yeast RNA	440	6.48			No	CD	465	7.43			No	CD
4. Torula RNA	440	5.76			No	CD	463	7.31			No	CD

[a] Same as Table VII.

troaniline reporter molecules exhibits a *positive* induced CD similar to that observed with unsubstituted symmetrical 4-nitroaniline chromophore whereas the DNA complex gives rise to a *negative* induced CD. This result suggests that in the RNA complex there is no interaction with the nitro group at the 2 position whereas in the DNA complex there is. The conclusion is consistent with the interpretation that the substituent at the 2 position of the chromophore is *out* in RNA and *in* in DNA complexes. The above reasoning is far from being rigorous and more definitive experiments were sought. The induced CD spectra of the bound unsymmetrically substituted 4-nitroaniline reporter molecules, **21–26**, were examined. The results are summarized in Tables X and XI for reporters **21–24** and **25** and **26**, respectively. It is very instructive to examine first the induced CD results obtained with 4-nitronaphthylamine chromophore, *i.e.*, reporter **25**, which is shown in Figure 10 and summarized in Table XI. This particular chromophore was synthesized since it contains two separate transitions above 300 mμ, *i.e.*, a transition at approximately 440 mμ which is assigned to the charge-transfer "4-nitroaniline" type transition and a lower intensity band at approximately 330 mμ associated with naphthylamine chromophore (see above). Figure 10 shows the effect of DNA and RNA on the induced CD in the absorption bands of **25**. An oppositely induced CD is observed in the lower energy "4-nitroaniline" type transition, *i.e.*, RNA–reporter complex shows a *positive* and DNA–reporter complex shows a *negative* induced CD. However, the most significant result is obtained with the higher energy transition associated with naphthylamine ring. For example, it is observed that an induced CD in this transition is obtained *only* in the DNA–reporter complex. The RNA–reporter **25** complex does not exhibit an induced CD in the 300–360-mμ region. These results which indicate that the "naphthylamine" transition is not perturbed by the local environment in the RNA complex but strongly perturbed in the DNA complex can be satisfactorily explained in terms of the *in* and *out* geometry discussed above. It is concluded that the fused phenyl ring of the 4-nitronaphthylamine system of **25** is pointing *in* in DNA and *out* in RNA complexes. The conclusion is entirely consistent with the previous observations and lends firm support to our earlier hypothesis.

Now that the idea that the "unsymmetrical" substituted 4-nitroaniline reporter molecule assumes only the *out* geometry in RNA complex and *in* and/or *out* geometry in DNA[19] is established it is instructive to examine the contents of Tables X and XI in greater details. Several interesting phenomena are observed. (1) In the RNA–reporter complex a positive induced CD is observed in the absorption band of the bound unsubstituted "symmetrical" 4-nitroaniline as well as the substituted "unsymmetrical" 4-nitroaniline chromophores. For example, the introduction of a nitro group at the 2 position of the 4-nitroaniline chromophore does not change the sign of the induced CD in the 4-nitroaniline transition of the RNA-bound chromophore (Table VII). This behavior is also observed with the introduction of

(19) In the DNA–reporter complex the *in* geometry is allowed but it should be noted that the *out* geometry may also exist as will be shown (see text).

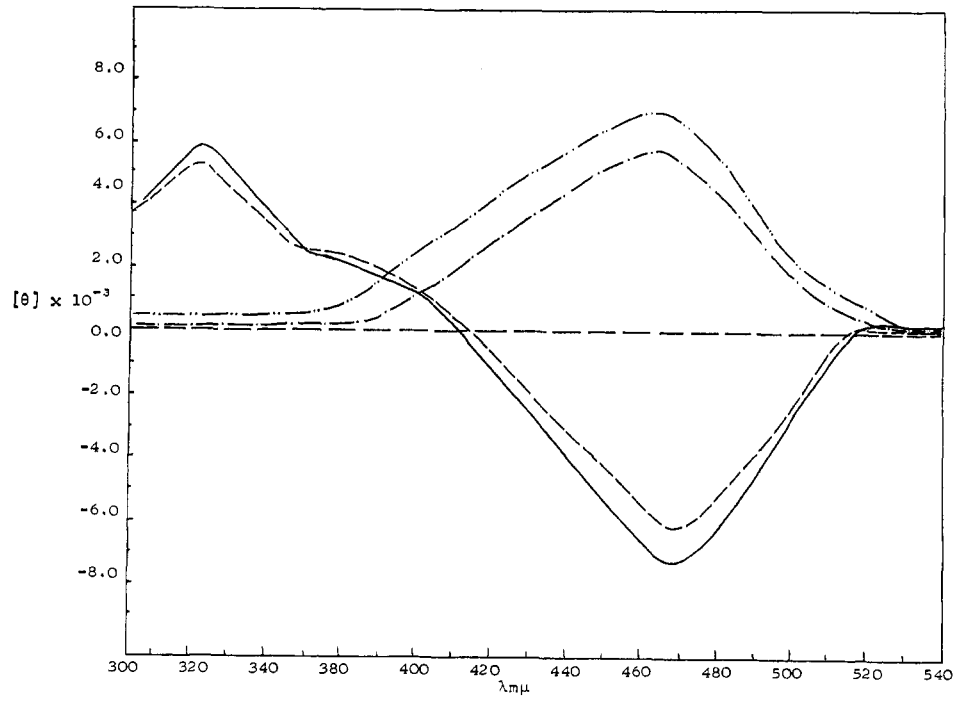


Figure 10. The induced CD spectra of reporter molecule **25** in the presence of nucleic acids, *i.e.*, yeast RNA (—··—), torula RNA (—·—), calf thymus DNA (—), and salmon testes DNA (— —).

(a) a methyl group at the 2 position, *i.e.*, reporters **21** and **22** (Table X); (b) a methyl group at the 3 position, *i.e.*, reporters **23** and **24** (Table X); and (c) a fused phenyl ring at the 2 and 3 positions, *i.e.*, reporters **25** and **26** (Table XI). The results strongly suggest that the substituents in the RNA–reporter complex do not interact with the RNA "surface" and therefore are pointing *out* as shown in Figure 9b and d. (2) In the DNA–reporter complex a *positive* induced CD is observed in the absorption band of the bound unsubstituted "symmetrical" 4-nitroaniline and in general a *negative* induced CD is obtained upon the introduction of a substituent in the ring chromophore. The introduction of a nitro group at the 2 position of the 4-nitroaniline chromophore, *i.e.*, reporters **7** and **8**, yields a negative induced CD in the 4-nitroaniline transition of the DNA-bound chromophore. Similar behavior is also observed with the introduction of a methyl group at positions 2 and 3; however, the situation is a little more complicated but nonetheless very enlightening and fits in nicely with our earlier hypothesis. For example, the reporter molecule **21**, *i.e.*, $2\text{-}CH_3\text{-}4\text{-}NO_2C_6H_3NH(CH_2)_nN^+(CH_3)_2(CH_2)_3N^+(CH_3)_3\cdot 2Br^-$ where $n = 2$, shows a negative induced CD for the calf thymus and salmon testes DNA complexes; however, increasing the distance between the ring nitrogen and the adjacent positively charged nitrogen by a CH_2 group, *i.e.*, reporter **22** where $n = 3$, yields what appears to be a double Cotton effect with a peak and a trough at 400 and 440 mμ, respectively (Table X). Obviously, it is not due to a double Cotton effect[20] but more likely is due to the presence of two complexes in which the methyl group at the 2 position is either *in* or *out* of the helix.

(20) Double Cotton effects are characteristic of exciton type interactions which are favored by chromophore–chromophore stacking. See M. Kasha, *Radiation Res.*, **20**, 55 (1963); M. Kasha, *Rev. Mod. Phys.*, **31**, 162 (1959); C. A. Bush and I. Tinoco, Jr., *J. Mol. Biol.*, **23**, 601 (1967).

It is necessary to assume that the *in* geometry results in a negative induced CD with a λ^t slightly shifted to the red of λ_{max} and the *out* geometry results in a positive induced CD with a λ^p slightly shifted to the blue of λ^t. This is a reasonable assumption and is totally consistent with and moreover support all the data at hand. It is tempting to be able to determine the equilibrium concentrations of the *in* and *out* geometries in the DNA complex but this is not possible since the molar ellipicities, $[\theta]^{in}$ and $[\theta]^{out}$, for the *in* and *out* geometries are not known. If it is assumed that the absolute value of $[\theta]$ for the *in* and *out* geometries is the same then it is possible to conclude that the DNA–reporter **22** complex contains approximately equal concentrations of the *in* and *out* geometries. However, this assumption are not valid since the induced molar ellipticity, $[\theta]$, according to present theories, depends not only on the distance between the transition moment of the bases in the nucleic acid and the transition moment of the reporter molecule but also on the cross product of the magnetic moment vectors associated with each transition.[20] Since the *in* and *out* geometries are vastly different it is clear that the assumption is unjustifiable. The dependence of the induced CD in the DNA–reporter complex on n appears to be general. For example, reporter molecules **23** and **24**, $3\text{-}CH_3\text{-}4\text{-}NO_2C_6H_3NH(CH_2)_nN^+(CH_3)_2(CH_2)_3N^+(CH_3)_3$ where $n = 2$ and 3, respectively, show a negatively induced CD for DNA–reporter **23** complex and a very weak positively induced CD for DNA–reporter **24** complex (Table X). Similarly, reporter molecules **25** and **26**, $4\text{-}NO_2C_{10}H_6NH(CH_2)_nN^+(CH_3)_2(CH_2)_3N^+(CH_3)_3$ where $n = 2$ and 3, respectively, shows a strong negatively induced CD for the DNA–reporter **25** complex and a weaker negatively induced CD for the DNA–reporter **26** complex in the "4-nitroaniline" transition (Table XI). It is very instructive to note that the lower the absolute value of the negatively

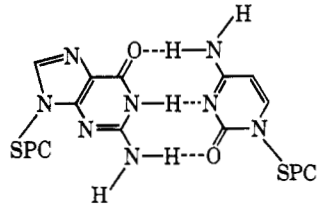


Figure 11. The Watson–Crick–Wilkins G–C base pairs. The additional hydrogen bond is seen to occupy the minor groove.

induced CD in the 450-mμ transition is always accompanied by a lower value of the positive induced CD in the higher energy transition at 330 mμ. For example, calf thymus DNA–reporter **25** complex shows a greater absolute value of $[\theta]^t$ at 450 mμ and $[\theta]^p$ at 323 than the complex between salmon testes DNA and reporter **25**. Similar behavior is also observed with reporter **26**. These results are entirely consistent with the interpretation that there is an equilibrium between the *in* and *out* geometries whereby the *in* geometry leads to a negatively induced CD in the "4-nitroaniline" transition as well as a net positively induced CD in the "naphthylamine" transition. The higher the concentration of the *in* geometry the higher is the observed induced $[\theta]^t$ for the "4-nitroaniline" and $[\theta]^p$ for the naphthylamine transition.

Is the Reporter in the Major or Minor Groove of the Helix?

The ring chromophore of the reporter molecule probably lies in the minor groove of the nucleic acid helix. In order to understand the evidence leading up to this conclusion molecular framework models of a nucleic acid helix was carefully examined. Several features of pertinent interest may be cited. (1) The major and minor grooves of the Watson–Crick type double helix arise as a result of the position of substitution of the sugars, *i.e.*, D-ribofuranoside and D-deoxyribofuranoside in RNA and DNA, respectively. It is clear by inspection of Figure 11 that the sugar–phosphate backbone connected to the individual bases is closer on one side of the plane of the base pairs than the other. As a consequence two important features emerge: (a) substitution at the 5 position of pyrimidines will place the group in the major groove, and (b) the additional hydrogen bond in the G–C base pair lies in the minor groove (Figure 11). (2) Due to the antiparallel nature of the two sugar–phosphate backbone chains in native nucleic acids[21] as well as the stereochemistry of D-ribofuranoside ring (*i.e.*, the *trans* substitution of the 2′-hydroxyl group with respect to the base at the 1′ position) the 2′-hydroxyl groups in RNA lie in the minor groove. This geometry is illustrated schematically in Figure 12 for the single-stranded stacked polyriboadenylic acid helix. Formation of a double-stranded RNA-type helix, *e.g.*, poly A·poly U, would place the 2′-hydroxyl group of both chains in the minor groove. This argument is not only self-evident from symmetry consideration but it may also be demonstrated by molecular framework models of two antiparallel right-handed double-stranded polyribonucleic acid helix.

(21) V. M. Ingram, "The Biosynthesis of Macromolecules," W. A. Benjamin, Inc., New York, N. Y., 1965.

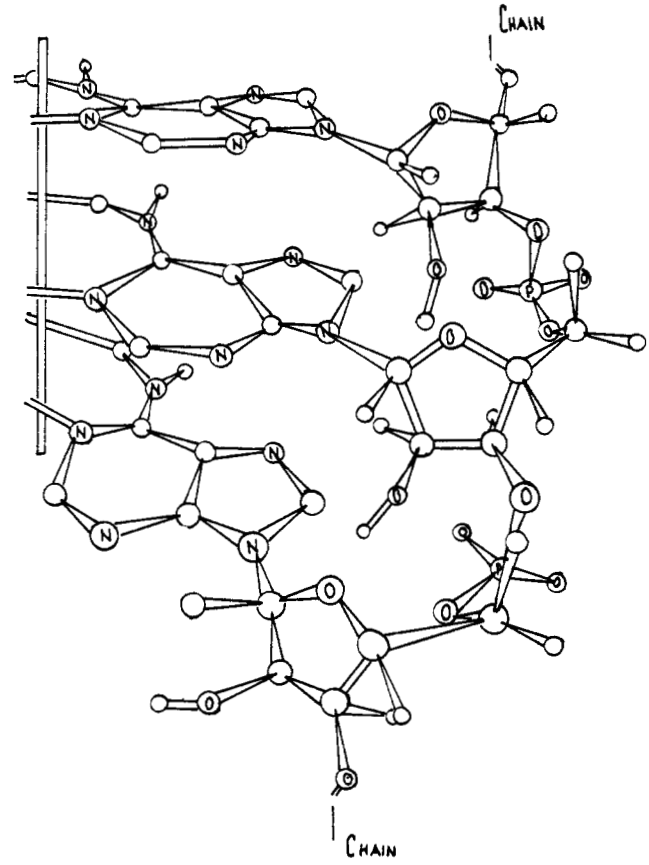


Figure 12. Model of a section of polyriboadenylic acid in the stacked configuration. Note (1) *trans* substitution of the 2′-hydroxyl group with respect to the base at the 1′ position, and (2) the 2′-hydroxyl group lies in the minor groove.

It is well known from the X-ray data of fibers of DNA and RNA that the former may exist either in the A or B form depending on the per cent of water content of the fiber.[22] Ribose-containing nucleic acids, on the other hand, show diffuse X-ray patterns which resemble the A form of DNA. The A and B forms differ in terms of the orientation of the plane of the base pairs with respect to the helix axis, *i.e.*, in the A and B form the bases are approximately 70 and 90° with respect to the helix axis. It should be noted that in either forms the characteristics of the major and minor grooves discussed above are not significantly altered. The major groove of DNA differs from that of RNA due to the presence of the 5-methyl substituent in one of the pyrimidine bases, *i.e.*, thymine. It is reasoned therefore that the complex between the "unsymmetrical" substituted reporter molecule and nucleic acids whereby the ring chromophore lies in the major groove of the helix with the substituent group pointing *in* will be sterically unfavorable in DNA as compared with RNA. Moreover, since experimental observation indicates that the substituent group always prefers the *out* arrangement in RNA it is plausible to interpret the data in terms of steric hindrance of forming the *in* arrangement in the RNA complex. It is therefore concluded that the substituted reporter molecule lies in the minor groove of the RNA helix with its substituent group pointing *out*. The basis of this conclusion is also due to the fact that the minor groove of RNA is relatively unaccessible as compared with DNA due to the presence of the 2′-hydroxyl

group (see above). Molecular model studies of the reporter–nucleic acid complex are also in line with this interpretation, *i.e.*, steric hindrance of binding the substituted reporter molecule to the minor groove of RNA with the substituent group pointing *in*. However, this mode of binding to DNA presents less steric interference due to the absence of the 2′-hydroxyl group. The fact that the steric interference to the *in* geometry in the DNA complex increases with *n* (the number of methylene groups between the ring nitrogen and the adjacent positively charged nitrogen of I) is consistent with this hypothesis and suggests that the hydrophobic cavity in the minor groove of DNA is of finite size. Rotation of the ring chromophore to yield the *out* complex is expected at higher values of *n* due to the enhanced interaction possibilities between the base pairs and the substituent group. This effect is clearly demonstrated in DNA–reporter complexes of **21** and **22**; **23** and **24**; and **25** and **26** where in each set as *n* increases from 2 to 3 more of the *out* complex appears to be formed.

In summary, the experimental data suggest that the reporter molecule lies in the minor groove of RNA as well as DNA. This conclusion is based primarily on a careful analysis of the structure of a nucleic acid helix and the induced circular dichroism of bound reporter molecules. A scheme of interpreting the data in a consistent manner is presented. The validity of the conclusions reached in this paper remains a problem which is being actively pursued.

Experimental Section

Melting points were determined on Meltemp apparatus and are uncorrected. Unless otherwise stated proton magnetic resonance spectra of the neutral compounds were taken in CCl_4 with TMS acting as internal standard, and in D_2O with TMS as external standard for the polar diammonium salts. The spectra were recorded on a Varian A-60 spectrometer. Analyses were performed by George Robertson, Florham Park, N. J.

N-2,4-Dinitrophenyl-N¹,N¹-dimethylethylenediamine (1). To 5 g (0.0204 mol) of 2,4-dinitrochlorobenzene in 5 ml of ethanol was added 2.4 g (0.024 mol) of N,N-dimethylethylenediamine at room temperature over a period of 10 min. The mixture was then heated in a sealed tube at 80–100° for 1 hr. The solvent was then evaporated, and the residue dissolved in 20 ml of water and washed with two 50-ml portions of ether. The water solution was made basic with sodium hydroxide pellets and the oil was extracted with ethyl acetate. The latter was dried over $MgSO_4$ and evaporated, and the product was recrystallized from ether–petroleum ether (bp 30–60°) to yield 5.5 g (88% yield) of N-2,4-dinitrophenyl-N¹,N¹-dimethylethylenediamine (**1**), mp 75°. The pmr spectrum in CCl_4 showed a one-proton broad singlet at τ 1.10, one-proton doublet at 1.16 (J_{AB} = 2.6 cps), one-proton doublet of doublets at 1.89 (J_{AB} = 2.6, J_{BC} = 9.6 cps), one-proton doublet at 3.12 (J_{BC} = 9.6 cps), three-proton multiplet at 6.58, three-proton triplet at 7.30 (J = 6 cps), and six-proton singlet at 7.65.

Anal. Calcd for $C_{10}H_{14}N_4O_4$: C, 47.25; H, 5.52. Found: C, 47.26; H, 5.57.

In a similar manner, the following compounds were synthesized.

N-2,4-Dinitrophenyl-N¹,N¹-dimethylpropylenediamine (2) was obtained from the reaction of 2,4-dinitrochlorobenzene and N,N-dimethylpropylenediamine. The product was recrystallized from ether–petroleum ether to obtain 71% yields of **2**, mp 95°. The pmr spectrum in CCl_4 showed a one-proton broad singlet at τ 1.01, a one-proton doublet at 1.16 (J_{AB} = 2.6 cps), a one-proton doublet of doublets at 1.93 (J_{AB} = 2.6, J_{BC} = 9.6 cps), a one-proton doublet at 3.19 (J_{BC} = 9.6 cps), a two-proton multiplet at 6.63, a two-proton triplet at 7.55 (J = 6 cps), a six-proton singlet at 7.63, and a three-proton multiplet at 8.17.

Anal. Calcd for $C_{11}H_{16}N_4O_4$: C, 49.26; H, 5.97. Found: C, 49.50; H, 6.18.

N-4-Nitrophenyl-N¹,N¹-dimethylethylenediamine (3) was obtained from the reaction of 4-nitrofluorobenzene and N,N-dimethylethylenediamine. The product was recrystallized from ethanol–water to yield 3.95 g (38% yield) of **3**, mp 37–40°. The pmr spectrum in $CDCl_3$ showed a two-proton doublet at τ 1.93 (J_{AB} = 9.2 cps), a two-proton doublet at 3.45 (J_{AB} = 9.2 cps), a one-proton broad singlet at 4.53, a three-proton complex multiplet at 6.78, a three-proton triplet at 7.43 (J = 6.0 cps), and a six-proton singlet at 7.75.

Anal. Calcd for $C_{10}H_{15}N_3O_2$: C, 57.42; H, 7.18. Found: C, 56.80; H, 7.38.

N-4-Nitrophenyl-N¹,N¹-dimethylpropylenediamine (4) was prepared from 4-nitrofluorobenzene and N,N-dimethylpropylenediamine. The product was recrystallized from ethanol–water to yield 5.7 g (49% yield) of **4**, mp 57°. The pmr spectrum in CCl_4 showed a two-proton doublet at τ 2.04 (J_{AB} = 9.2 cps), a two-proton doublet at 3.56 (J_{AB} = 9.2 cps), a one-proton broad singlet at 3.84, a two-proton multiplet at 6.75, a two-proton triplet at 7.58 (J = 6.2 cps), a six-proton singlet at 7.78, and a two-proton multiplet at 8.22.

Anal. Calcd for $C_{11}H_{17}N_3O_2$: C, 59.20; H, 7.64. Found: C, 58.55; H, 7.87.

N-2-Nitrophenyl-N¹,N¹-dimethylethylenediamine (5) was prepared from the reaction of 2-nitrofluorobenzene and N,N-dimethylethylenediamine. The product was not amenable to crystallization in several solvent systems and was therefore purified as the crystalline hydrobromide salt (mp 182–184°) and converted to the oily tertiary amine **5**. The pmr spectrum of the hydrobromide salt of **5** in D_2O showed a four-proton complex multiplet at τ 3.05 ± 0.75, a four-proton A_2B_2 complex multiplet at 6.55, and a six-proton singlet at 7.13.

Anal. Calcd for $C_{10}H_{15}N_3O_2 \cdot HBr$: C, 41.38; H, 5.52. Found: C, 41.80; H, 5.62.

N-2-Nitrophenyl-N¹,N¹-dimethylpropylenediamine (6) was prepared from the reaction of 2-nitrofluorobenzene and N,N-dimethylpropylenediamine. The product was purified as the hydrobromide salt (mp 165°) and converted back to the oil **6**. The pmr spectrum of the hydrobromide salt of **6** in D_2O showed a four-proton multiplet at τ 3.08 ± 0.78, a four-proton A_2B_2 multiplet at 6.85, a six-proton singlet at 7.22, and a two-proton multiplet at 8.05.

Anal. Calcd for $C_{11}H_{17}N_3O_2 \cdot HBr$: C, 43.42; H, 5.92. Found: C, 43.86; H, 6.06.

N,N,N-Trimethyl-N¹,N¹-dimethyl-N¹-(β-2,4-dinitroanilinoethyl)-1,3-diammoniumpropane Dibromide (7). To 1.27 g (0.005 mol) of N-2,4-dinitrophenyl-N¹,N¹-dimethylethylenediamine in 5 ml of ethanol is added 2.60 g (0.01 mol) of N,N,N-trimethyl-N-3-bromopropylammonium bromide (**14**). The reaction mixture is heated in a sealed tube at 100–120° for 24 hr. After cooling and addition of excess acetone, trituration of the oil resulted in a solid product which upon recrystallization from ethanol–acetone afforded 1.67 g (65% yield) of **7**, mp 239°. The pmr spectrum in D_2O showed a one-proton doublet at τ 1.52 (J_{AB} = 2.5 cps), a one-proton doublet of doublets at 1.79 (J_{AB} = 2.5 cps, J_{BC} = 8 cps), a one-proton doublet at 2.80 (J_{BC} = 8 cps), an eight-proton complex multiplet at 6.15 ± 0.60, a six-proton singlet at 6.50, a nine-proton singlet at 6.63, and a two-proton multiplet at 7.33 ± 0.35.

Anal. Calcd for $C_{16}H_{29}N_5O_4Br_2$: C, 37.30; H, 5.63. Found: C, 37.20; H, 5.65.

In a similar manner, the following compounds were synthesized.

N,N,N-Trimethyl-N¹,N¹-dimethyl-N¹-(γ-2,4-dinitroanilinopropyl)-1,3-diammoniumpropane dibromide (8) was prepared from the reaction of N-2,4-dinitrophenyl-N¹,N¹-dimethylpropylenediamine and N,N,N-trimethyl-N-3-bromopropylammonium bromide (**14**). The product was recrystallized from ethanol–acetone in 74% yield, mp 249°. The pmr spectrum in D_2O showed a one-proton doublet at τ 1.56 (J_{AB} = 2.7 cps), a one-proton doublet of doublets at 1.96 (J_{AB} = 2.7, J_{BC} = 9.6 cps), a one-proton doublet at 3.08 (J_{BC} = 9.6 cps), an eight-proton broad multiplet at 6.35, a fifteen-proton singlet at 6.68, and a four-proton multiplet at 7.60.

Anal. Calcd for $C_{17}H_{31}N_5O_4Br_2 \cdot H_2O$: C, 36.74; H, 6.10. Found: C, 37.26; H, 6.05.

N,N,N-Trimethyl-N¹,N¹-dimethyl-N¹-(β-4-nitroanilonoethyl)-1,3-diammoniumpropane dibromide (9) was prepared from the reaction of N-4-nitrophenyl-N¹,N¹-dimethylethylenediamine (**3**) and **14**. The product is obtained by recrystallization from ethanol–acetone in 74% yield, mp 205–210°. The pmr spectrum in D_2O showed a two-proton doublet at τ 2.20 (J_{AB} = 9.5 cps), a two-proton doublet at 3.38 (J_{AB} = 9.5 cps), eight-proton multiplets at 6.35 ± 0.25, a six-proton singlet at 6.64, a nine-proton singlet at 6.77, and a two-proton multiplet at 7.53.

Anal. Calcd for $C_{16}H_{30}N_4O_2Br_2 \cdot 0.5H_2O$: C, 40.08; H, 6.47. Found: C, 40.41; H, 6.94.

N,N,N-Trimethyl-N¹,N¹-dimethyl-N¹-(γ-4-nitroanilinopropyl)-1,3-diammoniumpropane dibromide (10) was prepared from the reaction of N-4-nitrophenyl-N¹,N¹-dimethylpropylenediamine (**4**) and **14**. The product is very hygroscopic; however, it was obtained as a crystalline solid by lyophilization, mp 75-110° with gas evolution. The pmr spectrum in D_2O showed a two-proton doublet at τ 2.49 (J_{AB} = 9 cps), a two-proton doublet at 3.70 (J_{AB} = 9 cps), an eight-proton complex multiplet at τ 6.67, a fifteen-proton singlet at 6.84, and a four-proton multiplet at 7.84.

Anal. Calcd for $C_{17}H_{32}N_4O_2Br_2 \cdot H_2O$: C, 40.64; H, 6.77. Found: C, 40.59; H, 7.10.

N,N,N-Trimethyl-N¹,N¹-dimethyl-N¹-(β-2-nitroanilinoethyl)-1,3-diammoniumpropane dibromide (11) was prepared from the reaction of N-2-nitrophenyl-N¹,N¹-dimethylethylenediamine (**5**) and **14**. The product is obtained by recrystallization from ethanol-acetone in 22% yield, mp 165°. The pmr spectrum in D_2O showed a four-proton complex multiplet at τ 2.15 ± 0.60, an eight-proton complex multiplet at 6.00 ± 0.25, a six-proton singlet at 6.35, a nine-proton singlet at 6.45, and a two-proton multiplet at 7.75 ± 0.25.

Anal. Calcd for $C_{16}H_{30}N_4O_2Br_2 \cdot 1.5H_2O$: C, 38.64; H, 6.68. Found: C, 38.39; H, 6.98.

N,N,N-Trimethyl-N¹,N¹-dimethyl-N¹-(β-2-nitroanilinopropyl)-1,3-diammoniumpropane dibromide (12) was prepared from the reaction of N-2-nitrophenyl-N¹,N¹-dimethylpropylenediamine (**6**) and **14**. The product was obtained by recrystallization from ethanol-acetone in 49% yield, mp 119–120°. The pmr spectrum in D_2O showed a four-proton multiplet at τ 2.15 ± 0.60, an eight-proton multiplet at 6.17, a fifteen-proton singlet at 6.45, and a four-proton multiplet at 7.40.

Anal. Calcd for $C_{17}H_{32}N_4O_2Br_2 \cdot 1.5H_2O$: C, 39.93; H, 6.89. Found: C, 40.67; H, 7.37.

N-4-Nitrophenylethanolamine (13) was obtained from the reaction of 1 equiv each of ethanolamine and of 4-nitrofluorobenzene in ethanol. The product was obtained as a solid which was recrystallized from ethanol–water, mp 110°.

Anal. Calcd for $C_8H_{10}N_2O_3$: C, 52.74; H, 5.49. Found: C, 52.20; H, 5.46.

N,N,N-Trimethyl-N-3-bromopropylammonium bromide (14) was prepared as follows. To 40 g (0.20 mol) of 1,3-dibromopropane was added 28 ml of 25% trimethylamine (0.12 mol) in methanol. The reaction mixture was heated in a sealed tube at 90° for 24 hr. The solvent was evaporated, and the solid mass was collected and washed with acetone. The crude product was dissolved in 30 ml of ethanol and filtered, and 30 ml of acetone was added. A solid (2–3 g) separated from the solution which was filtered and 100 ml of 30:70 ether–acetone mixture was added to the mother liquor. The product separated as crystalline platelets (13.2 g, 45% yield), mp 201–203°. The pmr spectrum in D_2O showed a four-proton multiplet at τ 6.27, a nine-proton singlet at 6.63, and a two-proton multiplet at 7.42.

Anal. Calcd for $C_6H_{15}NBr_2 \cdot 0.5H_2O$: C, 26.67; H, 5.92. Found: C, 26.83; H, 6.26.

N-2-Methyl-4-nitrophenyl-N¹,N¹-dimethylethylenediamine (15). To 4.64 g (0.030 mol) of 2-fluoro-5-nitrotoluene (Aldrich Chemical Co.) in 5 ml of ethanol is added 4.03 (0.046 mol) of N,N-dimethylethylenediamine (Aldrich). The reaction was carried out in a sealed tube at 110° for 24 hr. After cooling, 10 ml of 50% aqueous HBr and 50 ml of acetone was added to the reaction mixture. The hydrobromide salt of **15** was separated, collected, and converted to the free amine by the addition of excess NaOH solution. The product was recrystallized from ether–petroleum ether and collected under N_2 to give 4.51 g (62% yield) of **15**, mp 67–68°. The pmr spectrum in $CDCl_3$ showed a one-proton doublet of doublets at τ 2.10 (J_{AB} = 2.5, J_{AC} = 8.5 cps), a one-proton doublet at 2.20 (overlaps with A proton), a one-proton doublet at 3.61 (J_{AC} = 8.5 cps), a one-proton broad singlet at 4.87, a two-proton multiplet at 6.83,a two-proton triplet at 7.42 (J = 6.3 cps), a six-proton singlet at 7.73, and a three-proton singlet at 7.88.

Anal. Calcd for $C_{11}H_{17}N_3O_2 \cdot 0.5H_2O$: C, 56.90; H, 7.26. Found: C, 56.71; H, 7.43.

In the same manner the following compounds were also prepared.

N-2-Methyl-4-nitrophenyl-N¹,N¹-dimethylpropylenediamine (16) was prepared from the reaction of 2-fluoro-5-nitrotoluene and N,N-dimethylpropylenediamine (Aldrich Chemical Co.) in 55% yield. The product was recrystallized from ether–petroleum ether and collected under N_2, mp 89–90°. The pmr spectrum in $CDCl_3$ showed a one-proton doublet of doublets at τ 2.16 (J_{AB} = 2.5, J_{AD} = 8.6 cps), a two-proton complex multiplet at 6.73, a two-proton triplet at 7.56 (J = 6.5 cps), a six-proton singlet at 7.78, and a two-proton complex multiplet at 8.20.

Anal. Calcd for $C_{12}H_{19}N_3O_2 \cdot 0.5H_2O$: C, 58.54; H, 8.13. Found: C, 58.11; H, 8.03.

N-3-Methyl-4-nitrophenyl-N¹,N¹-dimethylethylenediamine (17) was prepared from the reaction of 5-fluoro-2-nitrotoluene (Aldrich Chemical Co.) and N,N-dimethylethylenediamine in 37% yield. The product was recrystallized from ether–petroleum ether and collected under N_2, mp 60–61°. The pmr spectrum in $CDCl_3$ showed a one-proton doublet at τ 2.06 (J_{AB} = 9.6 cps), a two-proton complex multiplet at 3.66 ± 0.10, a one-proton broad singlet at 4.88, a two-proton complex multiplet at 6.83, a two-proton triplet at 7.46 (J = 6.5 cps), a three-proton singlet at 7.43, and a six-proton singlet at 7.78.

Anal. Calcd for $C_{11}H_{17}N_3O_2$: C, 59.19; H, 7.62. Found: C, 59.01; H, 7.70.

N-3-Methyl-4-nitrophenyl-N¹,N¹-dimethylpropylenediamine (18) was prepared from the reaction of 5-fluoro-2-nitrotoluene and N,N-dimethylpropylenediamine in 30% yield. The product was recrystallized from ether–petroleum ether and collected under N_2, mp 47–48°. The pmr spectrum in $CDCl_3$ showed a one-proton doublet at τ 2.05 (J_{AB} = 9 cps), a two-proton complex multiplet at 3.37, a one-proton broad singlet at 4.16, a two-proton complex multiplet at 6.73, a three-proton singlet at 7.42, a two-proton triplet at 7.58 (J = 6.5 cps), a six-proton singlet at 7.75, and a two-proton multiplet at 8.17.

Anal. Calcd for $C_{12}H_{19}N_3O_2$: C, 60.76; H, 8.02. Found: C, 61.54; H, 8.14.

N-4-Nitronaphthyl-N¹,N¹-dimethylethylenediamine (19) was prepared from the reaction of 1-fluoro-4-nitronaphthalene (Aldrich Chemical Co.) and N,N-dimethylethylenediamine in 76% yield. The product was recrystallized from ethanol–water, mp 82°. The pmr spectrum in $CDCl_3$ showed a one-proton complex multiplet at τ 1.12, a one-proton doublet at 1.71 (J_{BJ} = 9 cps), a three-proton complex multiplet at 2.5 ± 0.33, a one-proton doublet at 3.77 (J_{BJ} = 9 cps), a one-proton broad singlet at 3.77, a two-proton complex multiplet at 6.76, a two-proton triplet at 7.36, and a six-proton singlet at 7.74.

Anal. Calcd for $C_{14}H_{17}N_3O_2$: C, 64.87; H, 6.56. Found: C, 65.35; H, 6.79.

N-4-Nitronaphthyl-N¹,N¹-dimethylpropylenediamine (20) was prepared from the reaction of 1-fluoro-4-nitronaphthalene and N,N-dimethylpropylenediamine in 96% yield. The product was recrystallized from ethanol–water, mp 61–65°. The pmr spectrum in $CDCl_3$ showed a one-proton complex multiplet at τ 1.12, a one-proton doublet at 1.75 (J_{BJ} = 9.0 cps), a four-proton complex multiplet at 2.40 ± 0.84, a one-proton doublet at 3.88 (J_{BJ} = 9 cps), a two-proton multiplet at 6.67, a two-proton multiplet at 7.54, a six-proton singlet at 7.68, and a two-proton complex multiplet at 8.08.

Anal. Calcd for $C_{15}H_{19}N_3O_2$: C, 65.93; H, 6.96. Found: C, 65.31; H, 6.10.

N,N,N-Trimethyl-N¹,N¹-dimethyl-N¹-(β-2-methyl-4-nitroanilinoethyl)-1,3-diammoniumpropane Dibromide (21). To 1.00 g (0.0045 mol) of **15** in 5 ml of ethanol is added 1.5 g (0.0058 mol) of N,N,N-trimethyl-N-3-bromopropylammonium bromide. The reaction was carried out in a sealed tube at 110° for 24 hr. After evaporation of the solvent, an oil separated which was triturated in a Dry Ice–acetone bath. The resulting hygroscopic solid was recrystallized from ethanol–acetone under N_2 to give 1.34 g (62% yield) of **21**, mp 226–227°. The pmr spectrum in D_2O showed a two-proton complex multiplet at τ 2.05 ± 0.15, a one-proton multiplet at 3.20, an eight-proton multiplet at 6.05 ± 0.20, a six-proton singlet at 6.20, a nine-proton singlet at 6.40, a two-proton multiplet at 7.20 ± 0.20, and a three-proton singlet at 7.60.

Anal. Calcd for $C_{17}H_{32}N_4Br_2O_2 \cdot H_2O$: C, 40.64; H, 6.82. Found: C, 40.69; H, 7.11.

In the same manner the following compounds were also prepared.

N,N,N-Trimethyl-N¹,N¹-dimethyl-N¹-(γ-2-methyl-4-nitroanilinopropyl)-1,3-diammoniumpropane dibromide (22) was prepared from the reaction of the free amine **16** and N,N,N-trimethyl-N-3-bromopropylammonium bromide in 64% yield. The hygroscopic product was recrystallized from ethanol–acetone, mp 142–143°. The pmr spectrum in D_2O showed a two-proton complex multiplet at τ 2.10, a one-proton multiplet at 3.25, an eight-proton multiplet at 6.15 ± 0.15, a fifteen-proton singlet at 6.35, a four-proton multiplet at 7.45, and a three-proton singlet at 7.70.

Anal. Calcd for $C_{18}H_{34}N_4Br_2O_2 \cdot 2H_2O$: C, 40.44; H, 7.12. Found: C, 39.73; H, 7.41.

N,N,N-Trimethyl-N¹,N¹-dimethyl-N¹-(β-3-methyl-4-nitroanilinoethyl)-1,3-diammoniumpropane dibromide (23) was prepared from the free amine **17** and N,N,N-trimethyl-N-3-bromopropylammo-

nium bromide in 60% yield. The product was recrystallized from ethanol–acetone and collected under N_2, mp 167–168°. The pmr spectrum in D_2O showed one proton at τ 1.90, a two-proton multiplet at 3.20 ± 0.20, an eight-proton multiplet at 6.05 ± 0.20, a six-proton singlet at 6.40, a nine-proton singlet at 6.50, a three-proton singlet at 7.25, and a two-proton multiplet at 7.25 ± 0.25.

Anal. Calcd for $C_{17}H_{32}N_4Br_2O_2 \cdot H_2O$: C, 40.64; H, 6.82. Found: C, 40.89; H, 7.04.

N,N,N-Trimethyl-N¹,N¹-dimethyl-N¹-(γ-3-methyl-4-nitroanilinopropyl-1,3-diammoniumpropane dibromide (24) was prepared from the reaction of the amine **18** and N,N,N-trimethyl-N-3-bromopropylammonium bromide in 20% yield, mp 88–89°. The pmr spectrum in D_2O showed one proton at τ 1.90, a two-proton multiplet at 3.15, an eight-proton multiplet at 6.00 ± 0.25, a fifteen-proton singlet at 6.30, a four-proton multiplet at 7.25, and a three-proton singlet at 7.40.

Anal. Calcd for $C_{18}H_{34}N_4O_2Br_2 \cdot 2H_2O$: C, 40.45; H, 7.16. Found: C, 40.11; H, 7.25.

N,N,N-Trimethyl-N¹,N¹-dimethyl-N¹-(β-4-nitronaphthylaminoethyl)-1,3-diammoniumpropane dibromide (25) was prepared from the corresponding amine **19** and N,N,N-trimethyl-N-3-bromopropylammonium bromide in 63% yield, mp 75°. The pmr spectrum in D_2O showed a six-proton multiplet at τ 2.76 ± 1.20, an eight-proton multiplet at 5.95, a six-proton singlet at 6.33, a nine-proton singlet at 6.57, and a two-proton multiplet at 7.10.

Anal. Calcd for $C_{20}H_{32}N_4O_2Br_2 \cdot H_2O$: C, 44.61; H, 6.32. Found: C, 44.23; H, 6.90.

N,N,N-Trimethyl-N¹,N¹-dimethyl-N¹-(γ-4-nitronaphthylaminopropyl)-1,3-diammoniumpropane dibromide (26) was prepared from the corresponding amine **20** and N,N,N-trimethyl-N-3-bromopropylammonium bromide in 65% yield, mp 202°. The pmr spectrum in D_2O showed a six-proton complex multiplet at τ 7.00 ± 0.33, an eight-proton multiplet at 6.67, a nine-proton singlet at 6.80, a six-proton singlet at 6.86, and a four-proton multiplet at 7.75.

Anal. Calcd for $C_{21}H_{34}O_2Br_2 \cdot 2H_2O$: C, 44.21; H, 6.67. Found: C, 43.69; H, 6.92.

Native (N) calf thymus and salmon testes DNA are Worthington products lot no. 642, and 6CFA, respectively. Yeast RNA (lot no. 6234) and torula RNA (lot no. 55711) were obtained from Worthington and Calbiochem, respectively. These preparations of RNA are largely of ribosomal origin and are partially degraded. Denatured (D) nucleic acids were obtained by heating in a boiling water bath for 15 min and then immediate quenching in ice water.

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Optically Active Solvents in Nuclear Magnetic Resonance Spectroscopy. IX. Direct Determinations of Optical Purities and Correlations of Absolute Configurations of α-Amino Acids

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Abstract: The nuclear magnetic resonance spectra of the enantiomers of a given α-amino acid methyl ester are found to differ appreciably in optically active 2,2,2-trifluorophenylethanol (**2**) solvent. It is demonstrated that this spectral nonequivalence is widely applicable to the absolute determination of the optical purities and the correlation of the absolute configurations of monosubstituted glycines. The nmr method is also shown to be applicable to optical purity determinations of disubstituted glycines and β-amino acids. A conformational model capable of explaining the origin and senses of the nmr spectral nonequivalence of the enantiomeric amino acid methyl esters is suggested.

We have recently demonstrated that enantiomers, although exhibiting identical properties in achiral media, may be readily distinguished by nuclear magnetic resonance spectroscopy in appropriate optically active solvents.[1–7] As a result of this phenomenon, optical purities and absolute configurations of a variety of alcohols,[1–4] α-hydroxy acids,[5] amines,[6] and sulfoxides[7] may now be directly determined from the relative peak areas and senses of nonequivalence[8] of the resonances of enantiotopic nuclei in chiral solvents.[9]

Other workers have also observed the nmr spectral nonequivalence of enantiomers in optically active solvents. Their studies have included racemic alkylarylcarbinols in asymmetric sulfoxides,[10] diastereomeric interactions between dissymmetric nickel(II) complexes and (+)-α-pinene,[11] spectral dissimilarities of enantio-

(1) W. H. Pirkle, *J. Amer. Chem. Soc.*, **88**, 1837 (1966).
(2) T. G. Burlingame and W. H. Pirkle, *ibid.*, **88**, 4294 (1966).
(3) W. H. Pirkle and T. G. Burlingame, *Tetrahedron Lett.*, 4039 (1967).
(4) W. H. Pirkle and S. D. Beare, *J. Amer. Chem. Soc.*, **89**, 5485 (1967).
(5) W. H. Pirkle and S. D. Beare, *Tetrahedron Lett.*, 2579 (1968).
(6) W. H. Pirkle, T. G. Burlingame, and S. D. Beare, *ibid.*, 5849 (1968).
(7) W. H. Pirkle and S. D. Beare, *J. Amer. Chem. Soc.*, **90**, 6250 (1968).
(8) The term "sense of nonequivalence," used for the sake of brevity, refers to the relative field position of a resonance of one isomer relative to the corresponding resonance of its enantiomer (in a given chiral solvent). If the solute is partially resolved, the relative field position of the larger of the two sets of unequally intense resonances will denote the sense. Should the solute be optically pure, the sense of nonequivalence can be determined through comparison of the spectrum of the solute in the *S* solvent enantiomer with its spectrum in the *R* solvent enantiomer.
(9) We shall use the terminology of Mislow (see M. Raban and K. Mislow in "Topics in Stereochemistry," Vol. 1, N. L. Allinger and E. L. Eliel, Ed., Interscience Publishers, New York, N. Y., 1967, pp 1–38) to describe nuclei according to their spatial relationships. Accordingly, enantiotopic nuclei in chiral environments are diastereotopic and may exhibit different nmr spectra.
(10) F. A. L. Anet, L. M. Sweeting, T. A. Whitney, and D. J. Cram, *Tetrahedron Lett.*, 2617 (1968).
(11) R. E. Ernst, M. J. O'Conner, and R. H. Holm, *J. Amer. Chem. Soc.*, **90**, 5305 (1968).