IIa used in these reactions was evident.

When 1 mmol of acetophenone was introduced into the mixture of sodium hydride and polymer IIa, the reaction was slower than with polymeric trityllithium Ia. After 1.5 h, excess hydride was carefully destroyed with water, and after workup 212 mg (yield 95%) of dibenzoylmethane was obtained.

When γ-butyrolactone was introduced into the mixture of the hydride and polymer IIa, TLC analysis of a sample of the reaction mixture revealed several spots. No attempt was made to isolate them.

Acknowledgment. We wish to thank the Camille and Henry Dreyfuss Foundation for partial support for this research.

The Structure of CC-1065, a Potent Antitumor Agent, and Its Binding to DNA

C. G. Chidester,* W. C. Krueger, S. A. Mizsak, D. J. Duchamp, and D. G. Martin

Contribution from The Upjohn Company, Kalamazoo, Michigan 49001. *Received May 11, 1981. Revised Manuscript Received July 20, 1981*

Abstract: The crystal and molecular structure of a potent antitumor agent, CC-1065 ($C_{37}H_{33}N_7O_8$), has been determined, and circular dichroism studies have demonstrated a strong interaction between CC-1065 and DNA. The triclinic unit cell, space group $P\bar{1}$, of dimensions a = 11.063 (3) Å, b = 13.311 (2) Å, c = 13.405 (2) Å, α = 85.18 (1)°, β = 99.52 (1)°, and γ = 103.36 (2)°, contains two CC-1065 molecules and disordered solvent molecules (two methanols and three waters). Intensity data for 6284 reflections were collected at −155 (2) °C; the final R value was 0.08 for 2411 significant high-angle reflections. NMR spectroscopy was very important in assigning kinds of atoms. The CC-1065 molecule consists of three substituted benzodipyrrole moieties linked by amide bonds. One terminal moiety has a cyclopropyl ring; the only asymmetric carbons in the molecule are at the cyclopropyl ring junctures. The two symmetry-independent molecules have the same configuration, but the absolute configuration is not known. The CC-1065 molecules are curved and somewhat twisted with the outer periphery hydrophilic and the inner surface lipophilic. The interaction between CC-1065 and a number of DNA polymers was studied by circular dichroism. The CD effect requires double-stranded DNA and is strongest with poly(dA-dT):poly(dA-dT).

A number of antitumor agents are known to bind to deoxyribonucleic acid by nonintercalative means.[1-5] Netropsin is an oligopeptide that binds preferentially to poly(dA):poly(dT) and is thought to interact through hydrogen bonds in the minor groove of DNA.[1,2] Anthramycin is a pyrrolo[1,4]benzodiazepine that binds covalently to guanine in the minor groove.[3,4] Braithwaite and Baguley have investigated netropsin, distamycin, and several bisamidine and bisquaternary ammonium heterocyclic drugs by viscometric, spectrophotometric, and fluorometric methods, and propose that they all bind in the minor groove of the DNA double helix.[5]

A new antitumor agent, CC-1065, also binds to DNA without intercalation.[6] CC-1065 is produced by a soil culture, streptomyces zelensis,[7] and was isolated in pure form[8] and found to be significantly more cytotoxic in vitro than actinomycin, vinblastine, or maytansine.[9] In vivo, CC-1065 is highly active against a variety of mouse tumors,[8] and, as a result, has been selected by the National Cancer Institute (as NSC298223) for preclinical toxicology studies.

Table I. ^{13}C NMR Shifts

chemical shift[a]	multiplicity[b]	tentative assignment[15]	chemical shift	multiplicity	tentative assignment
9.5	q	C3M	121.3	s	C23
20.9	d	C11	123.5	d	C2
21.6	t	C10	127.2	s	C16
26.6	t	C24	127.5	s	C29
27.6	t	C37	128.9	s	C22
31.5	s	C9	129.1	s	C35
49.4	t	C12	129.5	s	C4
53.3	t	C25	130.4	s	C19
54.8	t	C38	130.7	s	C32
60.0	q	C20M	132.4	s	C21
60.3	q	C33M	133.1	s	C34
105.9	d	C17	138.0	s	C20
106.3	d	C30	138.4	s	C33
110.6	d	C7	157.5	s	C40
113.0	s	C3	157.5	s	C5
117.3	s	C18	160.2	s	C27
117.7	s	C31	160.7	s	C8
118.2	s	C36	161.2	s	C14
			176.4	s	C6

[a] Chemical shifts in ppm relative to internal Me_4Si. [b] s = singlet, d = doublet, t = triplet, q = quartet.

The structure of a fragment of CC-1065, the base degradation product, was reported previously.[10] A preliminary report of the structure of CC-1065 has also been published.[11] We report here

(1) (1) Berman, H. M.; Neidle, S.; Zimmer, C.; Thrum, H. *Biochim. Biophys. Acta* **1979**, *561*, 124–131.
(2) Wartell, R. M.; Larson, J. E.; and Wells, R. D. *J. Biol. Chem.* **1974**, *249*, 6719–6731.
(3) Hurley, L. H.; Gairola, C.; Zmijewski, M. *Biochim. Biophys. Acta* **1977**, *475*, 521–535.
(4) Kohn, K. W.; Glaubiger, D.; Spears, C. L. *Biochim. Biophys. Acta* **1974**, *361*, 288–302.
(5) Braithwaite, A. W.; Baguley, B. C. *Biochemistry* **1980**, *19*, 1101–1106.
(6) Swenson, D. H.; Krueger, W. C.; Lin, A. H.; Schpok, S. L.; Li, L. H. *Proc. Am. Assoc. Cancer Res.* **1981**, *22*, 857.
(7) Hanka, L. J.; Dietz, A.; Gerpheide, S. A.; Kuentzel, S. L.; Martin, D. G. *J. Antibiot.* **1978**, *31*, 1211–1217.
(8) Martin, D. G.; Biles, C.; Gerpheide, S. A.; Hanka, L. J.; Krueger, W. C.; McGovern, J. P.; Mizsak, S. A.; Neil, G. L.; Stewart, J. C.; Visser, J. *J. Antibiot.* **1981**, *34*, 1119–1125.
(9) Martin, D. G.; Hanka, L. J.; Neil, G. L. *Proc. Am. Assoc. Cancer Res.* **1978**, *19*, 99.
(10) Chidester, C. G.; Duchamp, D. J.; Martin, D. G. *Abstr. Am. Cryst. Assoc., Winter Meeting* **1979**, *6* (Series 2, No. 2), 67.
(11) Martin, D. G.; Chidester, C. G.; Duchamp, D. J.; and Mizsak, S. A. *J. Antibiot.* **1980**, *33*, 902–903.

0002-7863/81/1503-7629$01.25/0

Table II. Crystal Data for 7-[[1,6-Dihydro-4-hydroxy-5-methoxy-7-[(4,5,8,8a-tetrahydro-7-methyl-4-oxocyclopropa[c]benzo[1,2-b:4,3-b']dipyrrol-2(1H)-yl)carbonyl]benzo[1,2-b:4,3-b']dipyrrol-3(2H)-yl]carbonyl]-1,6-dihydro-4-hydroxy-5-methoxybenzo[1,2-b:4,3-b']dipyrrole-3(2H)-carboxamide

$C_{37}H_{33}N_7O_8 \cdot CH_3OH \cdot 1.5(H_2O)$	
molecular wt, 703.7·59.1	
triclinic, $P\bar{1}$	
a = 11.063 (3) A	α = 85.18 (1)°
b = 13.311 (2) A	β = 99.52 (1)°
c = 13.405 (2) A	γ = 103.36 (2)°
Z = 2	V = 1892.0 (5) A
d_{meas} = 1.38 g cm^{-3}	μ(Cu K) = 7.4 cm^{-1}
d_{calcd} = 1.37 g cm^{-3}	

the details of the molecular and crystal structure and results of circular dichroism (CD) studies which characterize its interaction with DNA. The CD studies suggest that CC-1065 is bound, like the antitumor agents previously mentioned, in the minor groove of DNA. However, CC-1065 differs from the other antitumor agents in the geometry of potential hydrogen-bond donors, and models suggest that this feature could cause CC-1065 to bind in the major groove of DNA.

CC-1065 has another structural feature of general interest; in one of the two molecules found in the crystal, there is an amide group which is extremely distorted from planarity. Previously the most distorted amide groups reported have been in highly strained ring compounds, tricyclic and tetracyclic spirodilactams,[12,13] and a cyclic tetrapeptide.[14] CC-1065 offers an opportunity to study an amide distortion which is not caused by covalent bonding constraints.

Experimental Section

NMR Study. The ^{13}C NMR spectrum of CC-1065 (in Me_2SO-d_6), obtained on a Varian CFT-20 spectrometer, indicated a pure compound containing 11 aliphatic and 26 unsaturated carbons (Table I). The aliphatic carbons were consistent by off-resonance decoupling with a methyl, three methylenes, a methine, a quaternary carbon, three methylenes on nitrogen, and two *O*-methyl carbons.

The ^1H NMR spectrum, obtained on a Varian XL-100, displayed seven exchangeable protons [δ 12.88, 11.44, 11.41, 11.20, 11.06, and 6.84 (2 H)] and confirmed the presence of four protons on unsaturated carbons [all singlets, δ 7.06 (2 H), 6.86, and 6.45]. The chemical shifts of the two *O*-methyl groups (δ 3.89 and 3.85) and the methyl (δ 2.00) indicated they were on unsaturated carbons. Two of the methylenes on nitrogen appeared as triplets at δ 4.66 and 4.04. Irradiation of a four-proton multiplet at δ 3.26 collapsed them to singlets. The third methylene on nitrogen appeared as the AB of an ABX pattern centered at δ 4.38. The methine, X of the ABX pattern, was found by spin decoupling to be buried under the Me_2SO signals. This methine was also coupled to the remaining cyclopropylmethylene which appeared as broad singlets at δ 1.98 and 1.48.

Circular Dichroism (CD) Measurements. CD measurements were obtained on a Cary 60 spectropolarimeter equipped with a Model 6003 CD attachment which was calibrated with D-10-camphorsulfonic acid.[16] Difference CD (ΔCD) spectra were calculated by subtracting the CD of CC-1065 from the CDs of mixtures of CC-1065 and DNA. For all experiments the concentration of CC-1065 in aqueous pH 7.2, 0.01 M phosphate buffer was 0.37 × 10^{-5} M. Aqueous solutions of CC-1065 were formed by adding small amounts of a concentrated solution of CC-1065 in DMF to the buffer.

X-ray Data Collection. Crystal data are collected in Table II. CC-1065 crystallized from a water and methanol solution as small prisms. A crystal with dimensions 0.3 × 0.5 × 0.2 mm was used for the data collection. Intensities of the 6289 unique reflections with $2\theta < 138°$ were measured at low temperature (−155 (2) °C), using graphite monochromatized Cu Kα radiation (λ = 1.5418 Å) on a Syntex $P\bar{1}$ diffractometer controlled by a Harris computer. The step-scan method was used with scan range ≥3° in 2θ and a scan rate of 1°/min. Crystal orientation was determined automatically, using seven orienting reflections. Ten reflections were monitored periodically during the data collection; there was no trend toward deterioration in intensities. Standard deviations of observed intensities were approximated by $\sigma^2(I) = \sigma^2_{\text{counting statistics}} + (0.026I)^2$, where the coefficient of I in the last term was calculated from those deviations in intensities of monitored reflections that were not explained by counting statistics.[14] Intensities were much weaker at high angles. Only 10% of the reflections in the range $130° < 2\theta < 138°$ had intensities greater than three standard deviations. Lorentz and polarization corrections were applied to the data, and Wilson statistics were used to place the data on an approximate absolute scale (Wilson B = 4.6 Å^2). Unit cell parameters were determined automatically, using a least-squares calculation based on accurately determined Kα_1 2θ values for 21 selected high 2θ reflections.[17]

Structure Determination and Initial Refinement. Since the unit cell is triclinic and CC-1065 was known to have high optical activity, it was apparent at the outset that the space group must be $P\bar{1}$. However, there were two molecules in the unit cell and the distribution of intensities of the reflections was centrosymmetric, so an approximate centrosymmetric relationship seemed likely. A partial trial solution was obtained in space group $P\bar{1}$ by direct methods, using the program DIREC written by one of the authors (D.J.D.). All except one atom in each CC-1065 molecule were found in subsequent structure factor and Fourier calculations; two other atoms in each molecule appeared to be disordered. A tedious series of least-squares refinements, followed by difference Fourier maps, was required before the final structure was known. Most of the O and N form factors were assigned at an early stage because the structure of the base degradation product (same as moieties II and III) was known from a previous structure determination.[10] There are only three atoms in the CC-1065 molecules not related by an approximate center of symmetry, and one of these, the cyclopropyl CH_2, was not found until relatively late in the refinement. When the refinement was changed to the correct space group, $P\bar{1}$, it was necessary to break the centrosymmetric relationship. Six solvent atoms were added to the calculation at positions not related by the center. Then cycles of refining only solvent atoms were interspersed with cycles of refining only CC-1065 atoms. Coordinates for "disordered" atoms were refined as though the atoms were half-populated, each at two locations. All but these atoms and the solvent atoms were allowed to be anisotropic with temperature factors in block matrices.

Solvent Model and Final Refinement. The model for solvent evolved gradually. In solvent refinements, isotropic temperature factors were refined and population factors were not. Population factors were adjusted after each refinement to try to have temperature factors in the same range. Additional solvent atoms found in difference maps were added until there were 19 positions for solvent atoms and about half did not have a pseudosymmetric relationship with another atom. In order to have the solvent model make sense physically, population factors were constrained so that different atoms would not occupy the same space at the same time, and packing distances, especially hydrogen-bonding distances, were considered in assigning either O or C form factors. The compound was crystallized from water and methanol, and the model included some of each.

After a suitable solvent model developed, an attempt was made to sort out the atoms in the disordered region. A difference map without the "disordered" atoms was used to decide which positions for the two atoms should be assigned to molecule A and which to B, so that in the final refinement no atoms would be disordered. After further refinement and additional maps, a three-membered ring was observed in molecule A which persisted, but it was not clear from the difference map that this additional atom must be carbon. NMR evidence was considered in order to make this assignment; the addition of a cyclopropyl carbon made the structure agree completely with the NMR data. That there was also a cyclopropyl ring in molecule B was not evident until much later. Even when the refinement with all the data was considered converged, this atom, C10′, was only 70% populated.

The function minimized in the refinement was $\sum w(|F_o|^2 - |F_c|^2)^2$ where weights w were taken as the reciprocals of the variances $\sigma^2(F_o^2)$. Positions of hydrogen atoms, especially methyl and hydroxy hydrogens, were verified by locating them in difference maps. In the final cycles, parameters for 24 of the 66 CC-1065 hydrogens were included in the calculation but were not refined. The program arrays were not large enough to include the remaining hydrogen parameters. The final R ($R = \sum||F_o| - |F_c||/\sum|F_o|$) for this refinement was 0.145 for all 6284 reflections and 0.102 for the 3207 reflections for which F_o^2 was greater than three standard deviations. The standard deviation of fit was 2.9.

Finally, several refinement cycles were done leaving out about 650 low-angle ($2\theta < 50°$) reflections. This resulted in improvement in dis-

(12) Ealick, S. E.; van der Helm, D. *Acta Crystallogr., Sect. B* **1977**, *B33*, 76–80.
(13) Ealick, S. E.; Washecheck, D. M.; van der Helm, D. *Acta Crystallogr., Sect. B* **1976**, *B32*, 895–900.
(14) Flippen, J. L.; Karle, I. L. *Biopolymers* **1976**, *15*, 1081–1092.
(15) Enomoto, Y.; Furutani, Y.; Naganawa, H.; Hamada, M.; Takeuchi, T.; Umezawa, H. *Agric. Biol. Chem.* **1978**, *42*, 1331–1336. PDE I and II served as models in the ^{13}C shift assignments.
(16) Krueger, W. C.; Pschigoda, L. M. *Anal. Chem.* **1971**, *43*, 675–677.
(17) Duchamp, D. J. *ACS Symp. Ser.* **1977**, *No. 46*, 98–121.

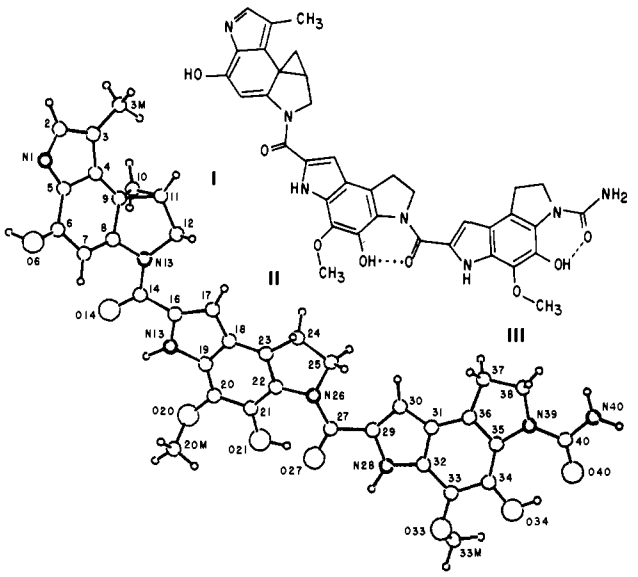


Figure 1. Structure and numbering.

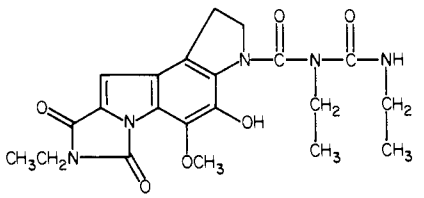
tances and angles and standard deviations, and gradually the partially populated carbon in molecule B became fully populated. The final R was 0.137 for all 5637 reflections with $2\theta > 50°$, and 0.080 for the 2411 reflections with $F_o^2 > 3\sigma(F_o^2)$. The standard deviation of fit was 1.7.

The choice of space group ($P1$ instead of $P\bar{1}$) was made because CC-1065 has high optical activity. However, $P\bar{1}$ could be considered as a possible space group for this structure, (in $P\bar{1}$ there would be one symmetry-independent molecule with three atoms, C10, C11, and C12, disordered). In order to test this possibility, a complete refinement to convergence was also carried out in space group $P\bar{1}$. In other cases, structures which have been reported in space group $P1$ have been shown to have an improved fit in $P\bar{1}$;[18] but in this case the agreement was significantly poorer in $P\bar{1}$ (the numbers corresponding to 0.137, 0.080, and 1.7 in $P1$ were 0.171, 0.100, and 1.9), and in addition the C9–C10 distance grew to be unreasonably long, 1.68 Å. Therefore space group $P1$ was the correct choice.

Atomic form factors were from "International Tables for X-Ray Crystallography"[19] except for hydrogen form factors, which were taken from Stewart, Davidson, and Simpson.[20] All calculations were done on an IBM 370 computer, using the CRYM crystallographic programs, which were written by one of the authors (D.J.D.). The final (high-angle refinement) coordinates for CC-1065 atoms are listed in Table III. Final solvent parameters and a description of the solvent model are in Table IV.[21]

Discussion

Bond Distances and Pseudosymmetry. CC-1065 is almost a trimer, with moieties II and III alike, and moiety I similar. Bond distances (Table V) have been grouped to facilitate comparison between the two symmetry-independent molecules and among similar moieties in the same molecule. Bond distances in the base degradation product of CC-1065 (**1**), a monomer like II or III



1

which was derivatized with three ethyl isocyanates ($C_{21}H_{25}N_5O_6$),[10] have been included in the table because they are more accurate than CC-1065 distances. Figure 1 shows chemical structure and numbering. Whereas standard deviations of bond distances are about 0.01 Å (0.009–0.014 Å), individual bond lengths are not as close to expected values as they should be from consideration only of standard deviations. Moreover, the mean difference between lengths of the same bond in the two molecules is 0.06 Å, larger than would be expected considering that for the most part the conformation of the two molecules is similar. Probably the pseudosymmetric relationship between the molecules is affecting the atom coordinates in the refinement. In fact, for all but the three atoms not related by pseudosymmetry, the mean distance between the observed position of an atom and the coordinates obtained by averaging with the $1-x$, $1-y$, $1-z$ coordinates of the related atom in the other molecule is only 0.05 Å. The pseudosymmetric agreement is closer than the agreement in bond lengths.

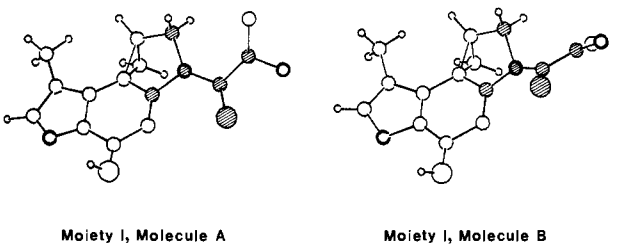


Figure 2. Moiety I of CC-1065. Amide group atoms are shaded.

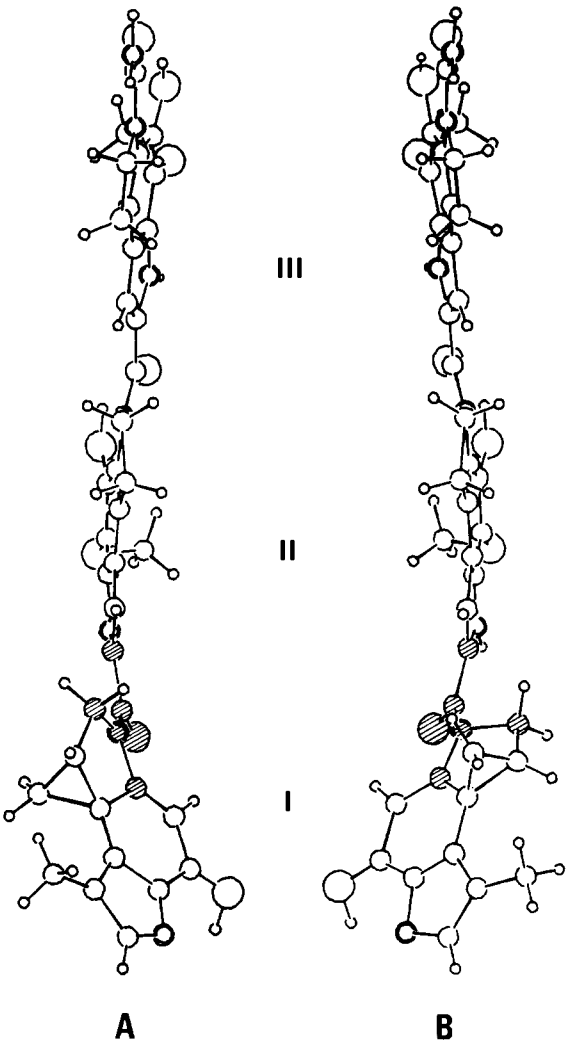


Figure 3. Edge view. The molecules were rotated independently. Nonplanar amide and corresponding planar amide atoms are shaded.

Conformation. Except for the cyclopropyl CH_2, the O-methyl carbons, and C12 in moiety I, each of the moieties I, II, and III

(18) Marsh, R. E.; Schomaker, V., *Inorg. Chem.* **1979**, *18*, 2331–2336.
(19) "International Tables for X-ray Crystallography"; Kynoch Press: Birmingham, England, **1962**; Vol III, pp 202–205.
(20) Stewart, R. F.; Davidson, E. R.; Simpson, W. T. *J. Chem. Phys.* **1965**, *42*, 3175–3187.
(21) Supplementary material available. See paragraph at end of paper.

Table III. Final Coordinates ($\times 10^4$) and Standard Deviations for Nonhydrogen Atoms in CC-1065

	molecule A				molecule B			
	X	Y	Z		X	Y	Z	
N1	8441 (7)	4006 (7)	5471 (6)	N1'	-8468 (8)	5985 (7)	-5474 (6)	
C2	7844 (9)	3280 (8)	5989 (7)	C2'	-7785 (12)	6873 (9)	-6028 (10)	
C3	6528 (9)	3012 (9)	5623 (8)	C3'	-6529 (10)	7022 (9)	-5627 (7)	
C3M	5509 (12)	2320 (11)	6074 (9)	C3M'	-5501 (11)	7888 (11)	-5967 (10)	
C4	6409 (9)	3661 (8)	4762 (7)	C4'	-6436 (9)	6240 (8)	-4793 (7)	
C5	7679 (8)	4283 (8)	4702 (7)	C5'	-7511 (9)	5655 (8)	-4728 (7)	
C6	7925 (10)	5069 (8)	3870 (7)	C6'	-7857 (9)	4828 (8)	-4005 (7)	
O6	8965 (6)	5625 (6)	3844 (5)	O6'	-8950 (6)	4246 (6)	-3988 (6)	
C7	6860 (8)	5207 (7)	3153 (7)	C7'	-6746 (9)	4642 (8)	-3231 (8)	
C8	5722 (8)	4674 (8)	3238 (7)	C8'	-5600 (9)	5283 (8)	-3264 (7)	
C9	5433 (10)	3800 (8)	4001 (7)	C9'	-5295 (8)	6031 (8)	-4113 (7)	
C10	4437 (11)	2830 (10)	3549 (9)	C10'	-4130 (11)	5950 (9)	-4580 (8)	
C11	3987 (10)	3633 (8)	3996 (8)	C11'	-3992 (8)	6664 (7)	-3807 (7)	
C12	3495 (10)	4379 (9)	3241 (8)	C12'	-3577 (9)	6289 (8)	-2755 (8)	
N13	4506 (7)	4746 (6)	2614 (5)	N13'	-4539 (6)	5263 (6)	-2597 (6)	
C14	4433 (9)	5099 (8)	1597 (6)	C14'	-4493 (8)	4827 (8)	-1653 (7)	
O14	5316 (6)	5231 (6)	1129 (5)	O14'	-5406 (6)	4525 (6)	-1241 (5)	
N15	3089 (6)	5535 (6)	107 (5)	N15'	-3085 (6)	4464 (6)	-56 (6)	
C16	3216 (9)	5346 (8)	1135 (7)	C16'	-3259 (9)	4637 (8)	-1138 (7)	
C17	2203 (8)	5596 (7)	1521 (7)	C17'	-2307 (8)	4453 (9)	-1512 (6)	
C18	1512 (7)	5970 (7)	692 (6)	C18'	-1431 (9)	4079 (8)	-665 (7)	
C19	1955 (7)	5900 (7)	-218 (7)	C19'	-2103 (9)	4089 (7)	158 (6)	
C20	1485 (9)	6220 (8)	-1118 (7)	C20'	-1449 (8)	3832 (7)	1172 (6)	
O20	1985 (5)	6062 (5)	-1989 (4)	O20'	-1985 (6)	3914 (5)	1992 (4)	
C20M	2676 (12)	6992 (9)	-2442 (10)	C20B	-2594 (12)	2967 (10)	2388 (8)	
C21	383 (8)	6583 (8)	-1218 (7)	C21'	-378 (9)	3415 (8)	1288 (7)	
O21	-168 (6)	6818 (5)	-2207 (4)	O21'	146 (6)	3219 (6)	2209 (5)	
C22	-68 (8)	6726 (8)	-344 (7)	C22'	131 (8)	3315 (7)	375 (6)	
C23	455 (9)	6389 (7)	621 (7)	C23'	-373 (8)	3662 (7)	-560 (7)	
C24	-273 (9)	6556 (8)	1380 (7)	C24'	300 (8)	3520 (7)	-1402 (6)	
C25	-1415 (8)	6942 (7)	822 (6)	C25'	1477 (9)	3138 (9)	-763 (7)	
N26	-1143 (6)	7150 (6)	-267 (5)	N26'	1217 (7)	2934 (6)	271 (6)	
C27	-1712 (8)	7734 (7)	-958 (6)	C27'	1797 (9)	2322 (8)	931 (7)	
O27	-1329 (5)	8038 (5)	-1776 (4)	O27'	1488 (6)	2065 (7)	1819 (5)	
N28	-3378 (6)	8636 (6)	-1518 (5)	N28'	3432 (6)	1378 (6)	1530 (5)	
C29	-2888 (8)	8026 (8)	-729 (7)	C29'	2904 (8)	1971 (8)	794 (7)	
C30	-3613 (9)	7862 (8)	6 (7)	C30'	3595 (9)	2187 (7)	-25 (6)	
C31	-4564 (8)	8367 (8)	-310 (7)	C31'	4653 (8)	1676 (7)	269 (7)	
C32	-4455 (9)	8836 (7)	-1258 (6)	C32'	4508 (9)	1196 (8)	1261 (7)	
C33	-5319 (9)	9388 (7)	-1812 (8)	C33'	5321 (8)	613 (8)	1724 (6)	
O33	-5130 (6)	9865 (5)	-2718 (5)	O33'	5155 (6)	148 (5)	2694 (5)	
C33M	-6001 (13)	9356 (10)	-3528 (7)	C33B	6050 (10)	507 (10)	3581 (8)	
C34	-6342 (8)	9513 (7)	-1313 (7)	C34'	6328 (9)	507 (8)	1290 (7)	
O34	-7077 (7)	10090 (6)	-1823 (5)	O34'	7115 (5)	-70 (5)	1788 (5)	
C35	-6466 (8)	9008 (7)	-343 (7)	C35'	6451 (8)	986 (8)	307 (7)	
C36	-5616 (8)	8452 (8)	150 (7)	C36'	5617 (9)	1523 (8)	-191 (8)	
C37	-5833 (8)	8108 (8)	1248 (8)	C37'	5892 (9)	1907 (7)	-1191 (7)	
C38	-6987 (8)	8508 (8)	1317 (6)	C38'	7025 (8)	1468 (8)	-1335 (7)	
N39	-7319 (7)	9034 (6)	364 (6)	N39'	7367 (7)	949 (6)	-330 (6)	
C40	-8429 (8)	9370 (8)	168 (7)	C40'	8409 (9)	602 (7)	-124 (7)	
O40	-8802 (6)	9758 (6)	-683 (5)	O40'	8791 (6)	261 (7)	725 (5)	
N40	-9088 (8)	9310 (7)	959 (8)	N40'	9124 (7)	719 (7)	-895 (6)	

are approximately planar. The mean deviations from the best planes calculated for ring atoms in each moiety are about 0.05 Å. In both molecules, the angle between the planes of II and III is about 16°, and the angle between the planes of I and II is about 55°. There is an internal hydrogen bond between II and III which constrains the twist between them.

Within moiety I, which has the only asymmetric carbon centers, the ring conformation is the same for both molecules (Figure 2). They both have the same absolute configuration. However, the molecules are as close to mirror images as their asymmetry allows. Figure 3 is an edge-on view of the molecules. They have been rotated independently in order to show their approximate mirror relationship rather than the almost centric relationship they have in the crystal. The atoms which are not mirror related have been marked with cross-hatching. In order to make molecule B have the same conformation as molecule A instead of approximate mirror-image conformation, large torsion angle rotations would be necessary about the bonds linking moieties I and II: the C14–C16 bond, and the amide C14–N13 bond.

Nonplanar Amide. It is apparent from Figure 3 that the amide group in molecule B is extremely distorted from planarity. C12′ is 1.2 Å out of the plane of the other five atoms. Table VI lists torsion angles for the two amide bonds, the urea bonds, and the C–C bonds between moieties. The torsion angles involving C12′ are −53.4° and 131.6°, very far from the ideal values for a planar group of 0° and 180°.

Dunitz and Winkler, in their studies of amide group deformations,[22,23] defined two out-of-plane bending parameters, χ_N and χ_C, and a twisting parameter, τ'. These parameters, for several compounds with deformed amide groups, are listed in Table VII. χ_C, the parameter for out-of-plane bending at the carbonyl end of the amide bond, is expected to be small, and this is true for all of the compounds listed, including CC-1065. The strained ring compounds, two polycyclic spirodilactams,[12,13] and a cyclic tetrapeptide[14] have high χ_N, describing bending at the nitrogen end of the bond, but a much lower twist angle τ'. Two other compounds, amicetin[24] and tryptophane,[25] were included in the table for comparison because their amide bonds are not in rings. Certainly molecule B in CC-1065 has an exceptionally large twist.

(22) Dunitz, J. D.; Winkler, F. K. *Acta Crystallogr., Sect. B* **1975**, *B31*, 251–263.
(23) Winkler, F. K.; Dunitz, J. D. *J. Mol. Biol.* **1971**, *59*, 169–182.
(24) Smith, J. L.; Sundaralingam, M. *Acta Crystallogr., Sect. B* **1981**, *B37*, 1095–1101.
(25) Stenkamp, R. E.; Jensen, L. H. *Acta Crystallogr., Sect. B* **1973**, *B29*, 2872–2878.

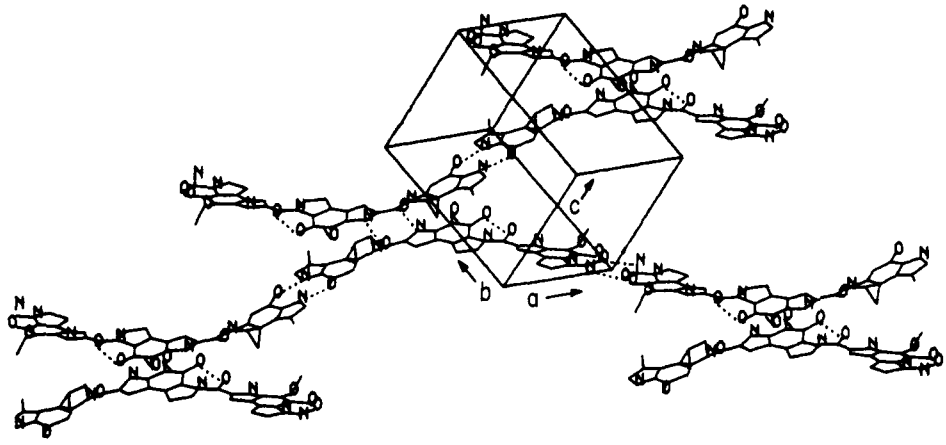


Figure 4. Packing. Solvent atoms were omitted for clarity.

Table IV. Final Coordinates ($\times 10^3$), Temperature Factors, and Standard Deviations for Solvent Atoms[a]

	X	Y	Z	B	occupancy
O(W1A)	172 (3)	12 (2)	296 (2)	5.2 (0.5)	0.33
O(W2A)	−281 (2)	955 (2)	−358 (1)	4.3 (0.4)	0.33
O(M3A)	183 (3)	89 (2)	492 (2)	6.6 (0.6)	0.33
C(M3A)	164 (5)	166 (4)	433 (4)	7.8 (1.0)	0.33
O(M4A)	1028 (5)	908 (4)	654 (4)	9.7 (1.1)	0.25
C(M4A)	970 (5)	836 (4)	585 (4)	7.4 (1.0)	0.33
O(W5A)	147 (3)	183 (3)	667 (3)	8.2 (0.8)	0.33
O(W1B)	−179 (2)	967 (2)	−309 (2)	7.6 (0.5)	0.50
O(W2B)	250 (5)	5 (4)	328 (3)	7.9 (0.9)	0.25
O(M3B)	−9 (4)	245 (4)	444 (3)	7.8 (0.9)	0.25
C(M3B)	−38 (5)	190 (4)	508 (4)	6.1 (1.0)	0.25
O(W4B)	895 (4)	841 (3)	362 (3)	7.2 (0.9)	0.25
O(W5B)	927 (4)	749 (3)	541 (3)	7.0 (0.8)	0.25
O(W1C)	103 (3)	−125 (3)	311 (3)	5.5 (0.7)	0.25
O(M2C)	−64 (3)	131 (2)	−306 (2)	8.6 (0.6)	0.50
C(M2C)	35 (3)	110 (3)	681 (2)	7.9 (0.7)	0.50
O(M3C)	877 (3)	853 (2)	461 (2)	7.5 (0.6)	0.33
C(M3C)	802 (4)	883 (3)	492 (3)	4.7 (0.7)	0.33
O(W4C)	21 (4)	183 (4)	459 (4)	7.7 (0.9)	0.25

[a] In general, a unit cell is occupied by either A group atoms, or B, or C group atoms; the groups are mutually exclusive with the exception that the half-populated B group water and C group methanol can coexist with atoms in the A group.

The barrier for cis–trans isomerism of the amide bond for various $(CH_3)_2NCOR$ amides has been found to be anywhere from 16 to 21 kcal/mol.[26,27] Ab initio quantum mechanics calculations of the potential curve for internal rotation in formamide predict a barrier of about 20 kcal/mol and an energy requirement of about 14–16 kcal/mol for a rotation of 54°.[28,29] Dunitz and Winkler have proposed a form for the energy surface for amides in condensed phases.[22] For (τ', χ_N) of (−64°, 38°), their map predicts an energy of about 8 kcal/mol. This is a better estimate for the energy required by the nonplanar amide in CC-1065, because the most likely explanation for the observed twist is that it allows the formation of three hydrogen bonds.

Packing, Hydrogen Bonds. The CC-1065 molecules have an overall curved shape with hydrogen-bond donors and acceptors on the outer periphery of the curve and with a lipophilic inner surface. Figure 4 is a packing drawing (with solvent atoms left out) showing hydrogen bonds, which are also listed in Table VIII. In every case, the intermolecular hydrogen bonds are between symmetry-independent molecules; there are no hydrogen bonds between symmetry-related molecules. Type A and type B molecules stack with the central aromatic moieties parallel and about 3.4 Å apart. The stacked molecules curve in opposite ways, and each have double hydrogen bonds to three molecules of the opposite type, a pair at either end and a pair in the middle. These six intermolecular hydrogen bonds and two intramolecular hydrogen bonds are the same for A and B molecules and involve all but one of the ten potential hydrogen-bond donors and acceptors, N28 in moiety III. This nitrogen forms a hydrogen bond with a molecule of solvent. There are few interactions between CC-1065 and solvent; the other solvent interactions involve the two oxygens which are near N28: O21 and O27, which are also internally hydrogen bonded to each other, and one of the NH_2 hydrogens at the end of moiety III.

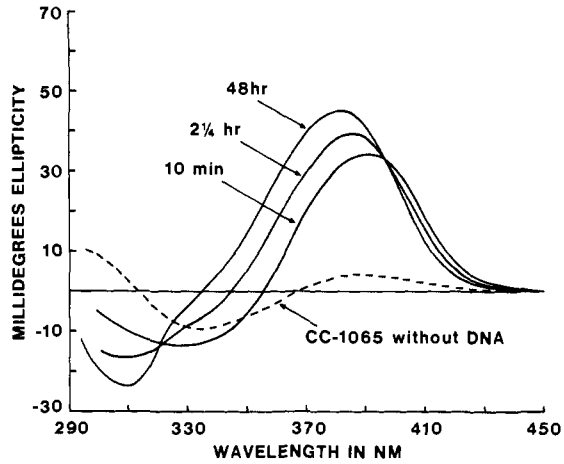


Figure 5. Circular dichroism of CC-1065 + calf-thymus (CT)-DNA. The concentrations in 40% DMF:60% pH 7.2, 0.01 M phosphate buffer were: CC-1065 = 0.37×10^{-5} M; CT-DNA = $30(0.37 \times 10^{-5})$ M.

Interaction with DNA: CD Studies. CC-1065 dissolved in DMF forms a colloidal suspension when added to aqueous phosphate buffer solution. Large particles settle out of solution after 24 h. The same results are obtained when the aqueous buffer contains substances which do not bind CC-1065, for instance netropsin, poly(dA) or poly(dT). In other experiments, DNA polymers in aqueous buffer solubilize CC-1065, so that after 24 h CC-1065 neither settles out of solution nor remains in colloidal suspension. These polymers bind CC-1065 and can be divided into two classes—those that induce a large CD in the CC-1065 electronic transition near 380 nm (mechanism 1 binding), and those that do not (mechanism 2 binding). A third mechanism of binding may also occur. When CC-1065 in DMF is added to a solution of CT-DNA in 40% DMF:60% aqueous buffer, the CD curve changes gradually over a period of days (Figure 5). Isoelliptic points occur near 320 and 395 nm. Since CC-1065 appears to be completely dissolved in this mixture, and since the CD curve of unbound CC-1065 does not pass through the isoelliptic points, these results suggest that CC-1065 binds instantly to CT-DNA

(26) Siddall, T. H. III; Stewart, W. E.; Knight, F. D. *J. Phys. Chem.* **1970**, *74*, 3580–3583.
(27) Neuman, R. C., Jr.; Jonas, V. *J. Org. Chem.* **1974**, *39*, 929–931.
(28) Shipman, L. L.; Christoffersen, R. E. *J. Am. Chem. Soc.* **1973**, *95*, 1408–1416.
(29) Christensen, D. H.; Kortzeborn, R. N.; Bak, B.; Led, J. J. *J. Chem. Phys.* **1970**, *53*, 3912–3922.

Table V. Bond Distances in Å for Molecule A (unprimed) and Molecule B (primed) and for a Previously Determined Monomer[10,a]

moiety I: A, B		moiety II: A, B		moiety III: A, B		monomer
N1-C2	1.26, 1.47	N15-C16	1.37, 1.44	N28-C29	1.39, 1.35	1.39
N1-C5	1.30, 1.45	N15-C19	1.44, 1.28	N28-C32	1.38, 1.38	1.42
C2-C3	1.43, 1.38	C16-C17	1.42, 1.32	C29-C30	1.34, 1.41	1.37
C3-C3M	1.46, 1.52					
C3-C4	1.39, 1.47	C17-C18	1.37, 1.50	C30-C31	1.37, 1.47	1.42
C4-C5	1.47, 1.27	C18-C19	1.40, 1.43	C31-C32	1.38, 1.44	1.42
C4-C9	1.39, 1.50	C18-C23	1.39, 1.39	C31-C36	1.43, 1.38	1.41
C5-C6	1.48, 1.43	C19-C20	1.31, 1.48	C32-C33	1.41, 1.36	1.37
C6-O6	1.22, 1.28	C20-O20	1.42, 1.36	C33-O33	1.35, 1.41	1.38
C6-C7	1.43, 1.52	C20-C21	1.39, 1.40	C33-C34	1.45, 1.38	1.42
		O20-C20M	1.44, 1.39	O33-C33M	1.43, 1.45	1.44
		C21-O21	1.40, 1.30	C34-O34	1.32, 1.35	1.33
C7-C8	1.31, 1.36	C21-C22	1.39, 1.46	C34-C35	1.43, 1.43	1.45
C8-C9	1.50, 1.48	C22-C23	1.41, 1.37	C35-C36	1.38, 1.35	1.38
C8-C13	1.48, 1.36	C22-N26	1.45, 1.44	C35-N39	1.45, 1.44	1.44
C9-C10	1.58, 1.55					
C9-C11	1.56, 1.50	C23-C24	1.46, 1.50	C36-C37	1.54, 1.45	1.49
C10-C11	1.48, 1.43					
C11-C12	1.48, 1.49	C24-C25	1.53, 1.60	C37-C38	1.51, 1.54	1.53
C12-N13	1.48, 1.55	C25-N26	1.53, 1.45	C38-N39	1.44, 1.49	1.49
N13-C14	1.40, 1.34	N26-C27	1.33, 1.34	N39-C40	1.38, 1.32	1.37
C14-O14	1.22, 1.20	C27-O27	1.24, 1.29	C40-O40	1.26, 1.23	1.24
C14-C16	1.48, 1.49	C27-C29	1.52, 1.45	C40-N40	1.37, 1.38	1.42

[a] Standard deviations range from 0.010 to 0.014 Å for A and B molecules of CC-1065, and 0.01 Å for the monomer.

Table VI. Torsion Angles (deg) for Amide and Urea Bonds and Carbon-Carbon Bonds between Moieties (Standard Deviations Are in Parentheses)

	A	B
C8-N13-C14-O14	10.2 (1.3)	−10.6 (1.4)
C8-N13-C14-C16	−167.2 (0.7)	164.2 (0.8)
C12-N13-C14-O14	−168.4 (0.8)	131.6 (0.8)
C12-N13-C14-C16	14.0 (1.3)	−53.4 (1.0)
N13-C14-C16-N15	−170.3 (0.7)	162.0 (0.7)
N13-C14-C16-C17	21.6 (1.4)	−28.7 (1.4)
O14-C14-C16-N15	12.1 (1.2)	−22.8 (1.2)
O14-C14-C16-C17	−155.9 (0.9)	146.3 (1.0)
C22-N26-C27-O27	−3.5 (1.2)	−6.3 (1.3)
C22-N26-C27-C29	175.8 (0.7)	−179.0 (0.7)
C25-N26-C27-O27	167.1 (0.7)	−173.6 (0.8)
C25-N26-C27-C29	−13.4 (1.0)	13.6 (1.3)
N26-C27-C29-N28	180.0 (0.8)	177.9 (0.8)
N26-C27-C29-C30	−1.1 (1.5)	0.3 (1.5)
O27-C27-C29-N28	−0.5 (1.0)	4.6 (1.1)
O27-C27-C29-C30	178.3 (1.0)	−173.0 (0.8)
C35-N39-C40-O40	−3.8 (1.3)	−2.5 (1.4)
C35-N39-C40-N40	179.2 (1.0)	−177.1 (0.7)
C38-N39-C40-O40	−174.1 (1.0)	172.6 (0.8)
C38-N39-C40-N40	8.9 (1.0)	−1.9 (1.1)

Table VII. Nonplanar Amide Parameters[b]

ω_1, deg	τ', deg	χ_N, deg	χ_C, deg	ref
14.0, −167.2	24.3	∓1.4	2.5	molecule A[a]
−53.4, 164.2	−64.1	±37.8	−5.1	molecule B[a]
−148.0	20.5	−43.7	−0.2	13
149.0	−20.8	41.2	0.0	12
156.5	−22.9	21.3	−2.4	14
−159.2	23.6	−15.8	2.2	24
162.3	−17.8	10.5	−6.9	25

[a] This paper, N13-C14. [b] $\tau' = \omega_1 + \omega_2$, $|\omega_1 - \omega_2| < \pi$; $\chi_N = (\omega_2 - \omega_3 + \pi) \bmod 2\pi$; $\chi_C = (\omega_1 - \omega_3 + \pi) \bmod 2\pi$; where torsion angles are: ω_1 = C-C-N-C; ω_2 = O-C-N-(C' or H); ω_3 = O-C-N-C O-C-N-C.

and that one bound species slowly converts to another. This "third mechanism" might be covalent binding, for instance a cyclopropyl alkylation reaction, or it might be a rearrangement of CC-1065 on the DNA lattice, or both.

The CD results for CC-1065 bound to T-4 phage DNA, which has glycosylated cytosine residues in the major groove, and competitive binding experiments with netropsin, suggest that CC-1065

Table VIII. Hydrogen Bonds

donor atom	acceptor atom	distance, Å	symmetry of acceptor
O6	N1′	2.78 (1)	$x+2,y,z+1$
O6′	N1	2.81 (1)	$x-2,y,z-1$
N15	O14′	3.21 (1)	$x+1,y,z$
N15′	O14	2.95 (1)	$x-1,y,z$
O21	O27	2.45 (1)	intramolecular
O21′	O27′	2.51 (1)	intramolecular
O34	O40	2.58 (1)	intramolecular
O34′	O40′	2.46 (1)	intramolecular
N40	O40′	2.87 (1)	$x-2,y+1,z$
N40′	O40	2.84 (1)	$x+2,y-1,z$
OM3B	O6′	3.22 (4)	$x+1,y,z+1$
OM3B	O21′	3.11 (4)	x,y,z
OW5B	O21	3.24 (4)	$x+1,y,z+1$
OM4A	O27	3.14 (5)	$x+1,y,z+1$
OW1B	O27	2.77 (2)	x,y,z
OW1A	O27′	2.94 (2)	x,y,z
N28	OW2A	3.03 (2)	x,y,z
N28	OW1B	3.01 (2)	x,y,z
N28′	OW1A	3.04 (3)	x,y,z
N28′	OW2B	2.99 (4)	x,y,z
N40	OW1A	2.89 (3)	$x-1,y+1,z$
N40	OW1C	2.91 (3)	$x-1,y+1,z$
N40′	OW1B	3.27 (2)	$x+1,y-1,z$
N40′	OM2C	2.98 (3)	$x+1,y,z$

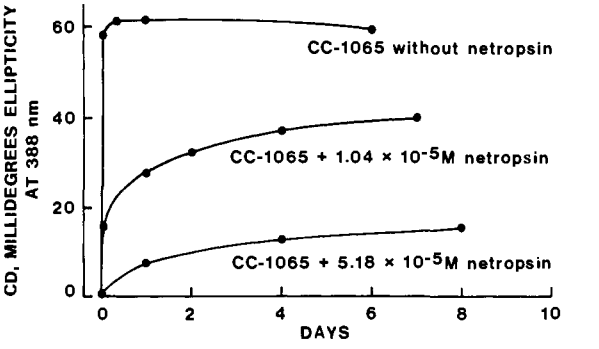


Figure 6. Competitive binding curve of netropsin and CC-1065 with CT-DNA. Concentrations in pH 7.2, 0.01 M phosphate buffer were: CC-1065 = 0.37×10^{-5} M; CT-DNA = 5.18×10^{-5} M.

binds in the minor groove of DNA. The ΔCD values for T-4 DNA are the same as those for CT-DNA (Table IX). This would not

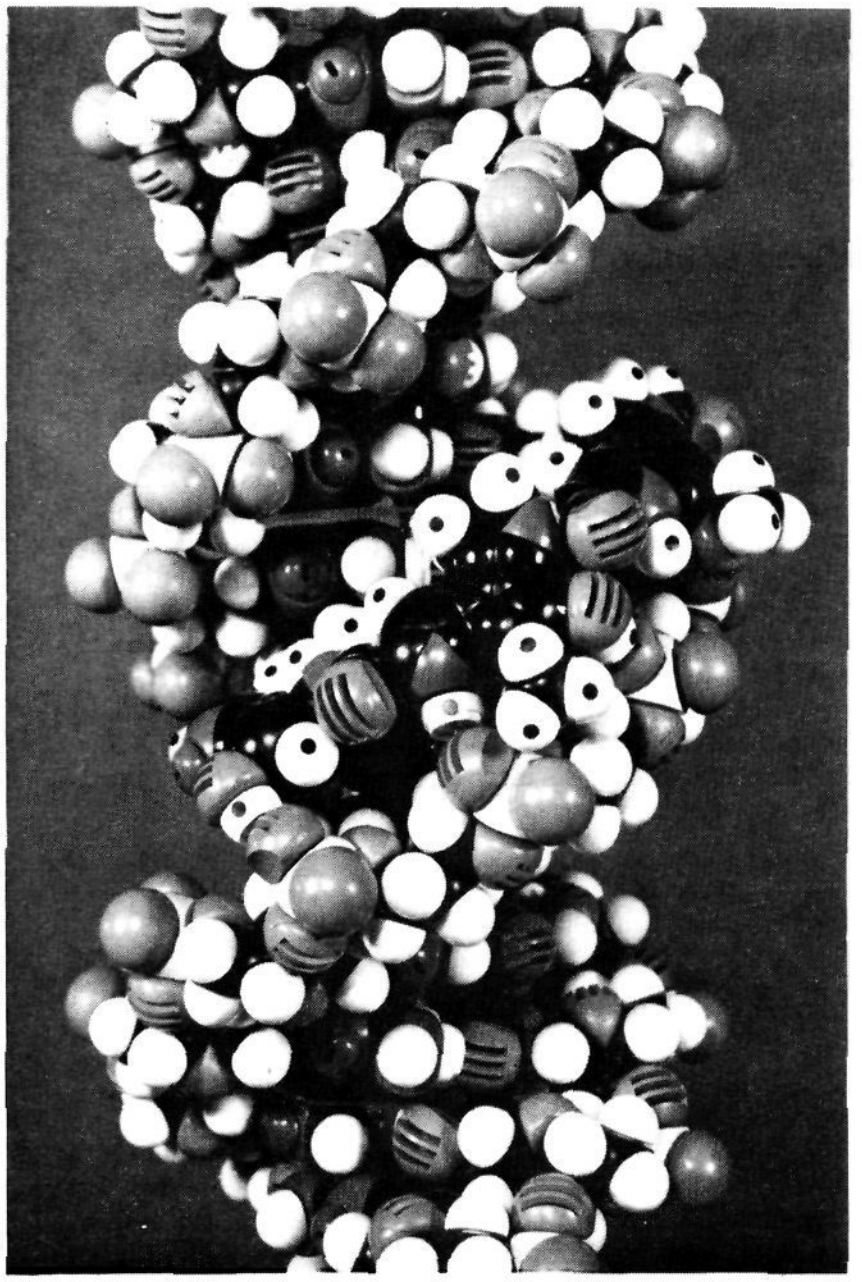


Figure 7.

be expected for major groove binding, unless the CC-1065 molecule can encompass the extra sugar moiety when it binds. In addition, netropsin, which binds to A-T base pairs in the minor groove,[1,2] inhibits the binding of CC-1065 when netropsin is pre-bound to CT-DNA (Figure 6). CC-1065 also prefers A-T base pairs, and at low concentrations of netropsin can displace netropsin from CT-DNA, a process which takes days. However, netropsin cannot displace CC-1065 from CT-DNA once CC-1065 is incubated with CT-DNA for 24 h, even at high netropsin concentrations. The latter observation supports the speculation that CC-1065 covalently binds to CT-DNA.

In summary, the CD binding results suggest that CC-1065: (1) binds to double stranded DNA by more than one mechanism, (2)

Table IX. Circular Dichroism Binding Results

polymer	ΔCD (mdeg ellipticity) at 388 nm
poly(dA-dT):poly(dA-dT)	62
poly(dA):poly(dT)	52
CT-DNA	*50*
T-4 phage DNA	49
poly(dG-dC):poly(dG-dC)	15
heat denatured CT-DNA	5
poly(dG):poly(dC)	−2
poly dA	−2
poly dT	−3

concentration of CC-1065 = 0.37×10^{-5} M
solvent: pH 7.2, 0.01 M phosphate buffer
concentration of DNA (phosphate) = $30(0.37 \times 10^{-5})$ M
1 day incubation
$\Delta CD = CD_{mixture} - CD_{CC\text{-}1065} = CD_{mixture} - 4.0$

prefers A-T base pairs to G-C pairs, and (3) requires the minor groove of DNA to show a large induced CD.

Model Study of CC-1065 and DNA. Model building with space-filling Corey–Pauling–Koltun (CPK) models of CC-1065 and of the DNA double helix shows that a molecule of CC-1065, in the conformation found in the crystal for A-type molecules, fits very well in either the major groove or the minor groove of DNA. The curve of the CC-1065 molecule mimics the turn of the DNA helix. Furthermore, the spacing of the hydrogen-bond donors along the periphery of CC-1065 is very close to the spacing between the phosphate groups in the DNA backbone. However, unlike the CD experiments, the model study favors major groove binding of CC-1065. When CC-1065 is placed in the minor groove, it seems that the hydrogen bonds would tend to be with the ester oxygens, whereas in the major groove, the phosphoryl and hydroxy oxygens are pointed in the right direction to form hydrogen bonds. These oxygens are much more likely candidates for hydrogen bonds than ester oxygens, and therefore it may be that, unlike other antitumor agents, CC-1065 binds in the major groove of DNA. Figure 7 is a CPK model of the DNA double helix with CC-1065 in the major groove, hydrogen bonded to five phosphate oxygens. It has been placed so that the cyclopropyl is in proximity to the NH_2 of an adenine, in a convenient position for an alkylation reaction.

Acknowledgment. The authors are grateful to Mrs. Charlotte Waber for her expert technical assistance and for her exceptional patience in finding a suitable crystal.

Supplementary Material Available: Tables of anisotropic thermal parameters and bond angles not involving hydrogen atoms, and hydrogen atom coordinates (5 pages). Ordering information is given on any current masthead page.