use at 110 °C (2 mm). Acetic anhydride and trichloroacetic acid (Aldrich) were used without further purification. Acetonitrile was dried by distillation from P_2O_5 followed by distillation from CaH_2. THF (Fisher Reagent Grade) was used without further purification. D_2O (100 atom %) for NMR experiments was purchased from Aldrich.

Preparation of Tomaymycin–d(ATGCAT)$_2$ Adduct. Tomaymycin–d(ATGCAT)$_2$ adducts for NMR experiments were prepared by adding excess solid TME to a 0.5-mL solution of d(ATGCAT)$_2$ in buffer (10 mM $NaHPO_4$, pH 7.0, 100 mM NaCl, 0.1 mM EDTA) at 5 °C. After vigorous stirring of the mixture for 4 days, excess tomaymycin was removed by centrifugation followed by extraction with ethyl acetate. Samples were lyophylized against D_2O three times and dissolved in 0.5 mL of D_2O. Samples used for NOE difference spectra were lyophilized to remove D_2O then dissolved in 0.5 mL of 90% H_2O/10% D_2O.

The adduct for fluorescence experiments was prepared by equilibrating a solution of d(ATGCAT)$_2$ in buffer with $A_{260} = 16$ and 5×10^{-4} M tomaymycin for 12 days at 5 °C. The hexamer concentration was about 3×10^{-4} M duplex, assuming an extinction coefficient of 4.8×10^3 M^{-1} cm^{-1} estimated from the base sequence[13] with 20% hyperchromicity as in similar oligomeric duplexes.[14] Unbound tomaymycin was removed by extracting four times with ethyl acetate and once with ether. Ether was removed with a stream of N_2. The sample was placed in a 4 × 10 mm stoppered cuvette.

^{1}H NMR Studies. A. Two-Dimensional NMR Spectra. ^{1}H NMR spectra were run on GN-500 instrument. All samples were run at 23 °C unless otherwise indicated and are referenced to HOD at 23 °C relative to external TSP. COSY spectra of the tomaymycin–d(ATGCAT)$_2$ adduct were obtained on a 2 mg/0.5 mL solution of the duplex adduct with a 3-s presaturation pulse before the standard 90°–t_1–90° pulse sequence to minimize the residual HOD signal. The COSY spectra contain 512 × 2K data points zero filled to give a 1K × 1K matrix. All other two-dimensional spectra contain 256 × 1K data points zero filled to give 512 × 512 matrices. Two-dimensional NOE spectra were run on a 10 mg/0.5 mL solution of duplex adduct, with a 90°–t_1–90°–$0.5t_m$–90°–180°–90°–$0.5t_m$–90° acquire sequence. The composite 180° pulse in the mixture time removes J coupling contributions from the cross-peaks.

B. NOE Difference Spectra. NOE difference spectra were performed at 5 °C in 90% H_2O/10% D_2O. A 1–3–3–1 pulse sequence was used to suppress the H_2O signal. Signals were irradiated for 1 s with a total recycle delay of 2.4 s.

C. Nonselective T_1 Inversion Recovery Experiments. Inversion recovery experiments utilized the standard 180°–t_1–90° pulse sequence. Samples were degassed by passing argon through the sample. A total of 32 t values ranging from 0.1 to 10 s were used with a total recycle delay of 12.9 s.

Time-Resolved Fluorescence Studies. Fluorescence decay measurements were made by the time-correlated single-photon counting technique as described before.[9] Decay curves were acquired at 5 °C to about 15×10^3 counts in the peak. The data were fitted by reference deconvolution[15] to a sum of exponentials

$$i(\lambda_{ex},\lambda_{em};t) = \sum \alpha_i(\lambda_{ex},\lambda_{em}) \exp(-t/\tau_i)$$

with amplitudes α_i and lifetimes τ_i. The amplitude α_i of a component i depends on a number of factors, including its molar extinction spectrum, fluorescence emission spectrum, radiative lifetime, and concentration. Decay curves measured at various excitation and emission wavelengths were analyzed by a global program,[16] assuming that the lifetimes but not the amplitudes are independent of wavelength. Errors in amplitudes and lifetimes were <10% and <5%, respectively, based on repeated experiments.

Molecular Modeling Studies. The crystal structure of tomaymycin methyl ether[17,18] was used as the initial structure in this investigation. After removal of the methoxy group, partial atomic charges were obtained from ab initio calculations using GAUSSIAN-80 UCSF and a STO-3G basis set.[19] The resulting 11-demethoxytomaymycin methyl ether structure was minimized by using the program AMBER and all-atom force field parameters presented by Weiner et al.[21] A distance-dependence dielectric constant was used, and the structure was refined until the root mean square gradient was less than 0.1 kcal/mol Å. The minimized structure was docked in the appropriate location and orientations on the hexanucleotide duplex with the aid of the interactive graphics program MIDAS,[22] and then the binding energies were minimized by using AMBER and the parameters described above. The helix distortion energy was determined by subtracting the energy of the helix in the tomaymycin adduct from that of the separately minimized isolated helix. Distortion energy induced in the tomaymycin molecule was determined in the same way.

Structural effects of water and counterions on complexing were neglected in the energy calculations. Although these effects influence the absolute values of binding energies, they should be negligible in comparing relative binding energies wherein the same molecule is used at the same binding site on the duplex.

Acknowledgment. This work was supported by grants from the U.S. Public Health Service (CA35318, GM22873, CA37798) and the Welch Foundation. We thank Dr. Karol Maskos for assistance with fluorescence decay measurements and Professor Peter A. Kollman for copies of the programs AMBER and GAUSSIAN-80.

Stereoelectronic Factors Influencing the Biological Activity and DNA Interaction of Synthetic Antitumor Agents Modeled on CC-1065

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The synthesis, physicochemical properties, and biological activities of a series of novel spiro cyclopropyl compounds, modeled on the potent antitumor antibiotic CC-1065 (1), are described. Many of these synthetic analogues are significantly more effective than 1 against murine tumors. In particular, compound **27** exhibits high activity and potency. Structure–activity analysis supports a molecular mechanism for biological action involving hydrophobic interaction of the drug with DNA and acid-catalyzed alkylation of DNA.

The structurally novel antibiotic CC-1065 (1) is an extremely potent cytotoxin whose biological action has been attributed to its sequence selective binding and covalent bonding to the minor groove of DNA.[1-7] Because of its

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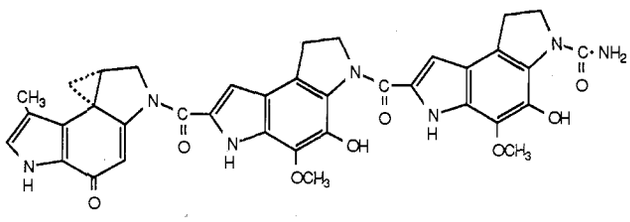
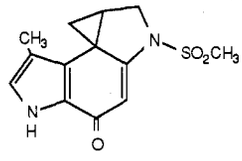


1

unique structure and mode of action, 1 was initially selected for development as an anticancer agent by the NCI.[8] Development ceased, however, when it was discovered that 1 caused delayed deaths in mice at therapeutic doses.[9] Attempts to circumvent this toxicity by pharmacological interventions[9,10] or to obtain, by chemical modifications of 1, an analogue that did not cause delayed deaths[11,12] were unsuccessful.

This impasse was surmounted with the first total synthesis, in racemic form, of the sulfonamide **2**.[13] It shares with 1 the unusual and potentially alkylating cyclopropa[*c*]pyrrolo[3,2-*e*]indol-4(5*H*)-one (CPI) system. This



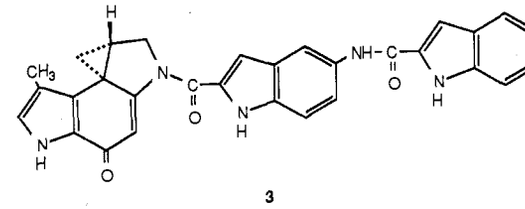
2

truncated "left-hand segment" is as effective as 1 against P388 leukemia in mice, though at an almost 1000-fold higher dose. It does not show the remarkable DNA-binding properties of 1, and it does not produce delayed deaths in mice.[12]

These results have some significant implications. First, they suggest that the capability to covalently *bond* to DNA through the CPI system is important for the antitumor activity of both 1 and **2**. The strong DNA *binding* of 1 appears not to be necessary for antitumor action. (We emphasize the distinction between DNA *bonding* and DNA *binding*. Bonding refers to a covalent attachment, which is, in this case, alkylation of DNA by the drug. Binding results from noncovalent interactions between DNA and drug species. In principle, a drug may bind, becoming fixed in a mutual orientation with DNA, but without bonding, or it may bind and bond, or it may form a covalent bond, but still rotate fairly freely if noncovalent binding forces are weak or absent.) The second, and crucial implication is that the alkylation of DNA by the CPI moiety, and hence the antitumor activity, is not inextricably related to the delayed-death phenomenon of 1.

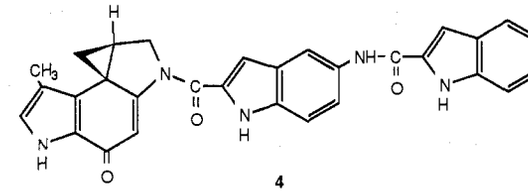
Encouraged by this observation, and with a viable synthetic route for the critical CPI segment in hand, we prepared a series of N^2-substituted CPI analogues which partly bridged the structural gap between 1 and **2**. We sought to separate and identify structural features affecting reactivity toward nucleophiles and those influencing noncovalent interactions with DNA and to relate these features to biological activity and potency.

An exciting therapeutic lead has emerged from these studies. The recently described DNA-binding agent **3** (U-71184), an enantiomer of the racemate **27** (Table I), not only retains the high potency of 1 but greatly surpasses



3

the natural product in antitumor efficacy, without causing delayed lethality.[14] Both **3** and 1 possess the 7b*R*,8a*S* configuration in the CPI segment. The mirror image of **3**, namely **4** (U-71185), is biologically inactive at comparable dose levels.



4

In this paper, we describe the synthesis and properties of the series of racemic analogues that informed us of the desirable steric and electronic features for the CPI appendage and that led us to select **27** for resolution.[15]

Chemistry

The compounds in Table I were synthesized by the routes shown in Schemes I and II.[13,14] Commercially available 4-chloro-3-nitroanisole was first converted to the benzyl ether **32**. Malonate displacement on **32** afforded **33**, which was reduced to diol **34a** with Dibal and then to aniline **34b** by catalytic hydrogenation. Methanesulfonylation led to in situ cyclization to indoline **35a**, which then underwent acetate displacement to **35b**. Regioselective nitration gave **36a**, which was reduced with hydrogen over platinum to the aniline **36b**. Gassman's

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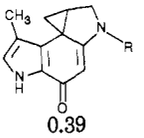
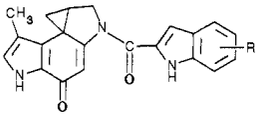
Table I. Physicochemical and Biological Properties of Racemic CC-1065 and CPI Analogues

no.	R	formula	R_f[a]	$k \times 10^5$[b]	ICD $\times 10^{-3}$[c]	ID_{50}[d]	P388 in vivo[e] OD	P388 in vivo[e] % ILS
								
1	$(PDE-I)_2$	$C_{37}H_{33}N_7O_8$	0.39		280	0.07	0.1	62
2	SO_2Me	$C_{13}H_{14}N_2O_3S$	0.82	0.3	0	100	25	60
5	SO_2Ph	$C_{18}H_{16}N_2O_3S$	0.58			900		
6	H	$C_{12}H_{12}N_2O$	0.87	stable	0	900	10 (HDT)	NA
7	$CO_2C(CH_3)_3$	$C_{17}H_{20}N_2O_3$	0.35	0.5	0	300		
8	$COCH_3$	$C_{14}H_{14}N_2O_2$	0.90		0	20	3	45
9	$CO(CH_2)_4CH_3$	$C_{18}H_{22}N_2O_2$	0.35	0.8	0	3	2	100
10	$CO(CH_2)_8CH_3$	$C_{22}H_{30}N_2O_2$	0.07		0	100	40 (HDT)	NA
11	COPh	$C_{19}H_{16}N_2O_2$	0.63	0.9	<1	3	12	71
12	CO-4-$(NHCOPh)C_6H_4$	$C_{26}H_{21}N_3O_3$	0.42		11	0.5	0.5	83
13	CO-2-pyridyl	$C_{18}H_{15}N_3O_2$	0.76	1.2	0	10	15	69
14	CO-2-naphthyl	$C_{23}H_{18}N_2O_2$	0.46		0	7	6	73
15	CO-2-quinolyl	$C_{22}H_{17}N_3O_2$	0.50	1.2	1	10	6	142
16	CO-6-$(NHCO(CH_2)_4CH_3)$-2-quinolyl	$C_{28}H_{28}N_4O_3$	0.15		*f*	100	21	127
17	CO-2-pyrrolyl	$C_{17}H_{15}N_3O_2$	0.70		10	2	1	71
18	CO-2-indolyl	$C_{21}H_{17}N_3O_2$	0.45	1.0	36	0.2	0.4	90
19	CO-1-Me-2-indolyl	$C_{22}H_{19}N_3O_2$	0.43	1.0	3	10	8	44
20	CO-2-benzofuranyl	$C_{21}H_{16}N_2O_3$	0.47	1.1	6	0.04	0.25	110
21	CO-2-benzothiopheneyl	$C_{21}H_{16}N_2O_2S$	0.41	1.1	7	0.3	0.80	100
22	CO-3-Me-2-indenyl	$C_{23}H_{20}N_2O_2$	0.39		4	30	17 (HDT)	NA
								
23	5-OMe	$C_{22}H_{19}N_3O_3$	0.47	1.0	22	0.01	0.05	95
24	6-OH, 7-OMe	$C_{22}H_{19}N_3O_4$	0.63	1.0	37	0.1	0.05	55
25	$5-NHCONH_2$	$C_{22}H_{19}N_5O_3$	0.89	1.0	47	0.6	0.06	65
26	5-NHCOPh	$C_{28}H_{22}N_4O_3$	0.43		36	0.07	0.05	82
27	5-NHCO-2-indolyl	$C_{30}H_{23}N_5O_3$	0.30	*g*	70	0.01	0.05	159 (4)
28	5-NHCO-2-benzofuranyl	$C_{30}H_{22}N_4O_4$	0.30	1.0	50	0.02	0.05	170 (1)
29	5-NHCO-5-$(NHCONH_2)$-2-indolyl	$C_{31}H_{25}N_7O_4$			75	0.3	0.1	121 (2)
30	5-NHCO-5-(NHCOPh)-2-indolyl	$C_{37}H_{28}N_6O_4$	0.30		33	0.01	0.1	77

[a] R_f represents chromatographic measure of lipophilicity determined by liquid–liquid chromatography on C-18 bonded silica gel. See Experimental Section. [b] Pseudo-first-order rate constant, in s^{-1}, for solvolytic ring opening at pH 3, followed spectrophotometrically. See Experimental Section. [c] ICD = induced circular dichroism, expressed as molar ellipticity, in the presence of calf-thymus DNA. See Experimental Section. [d] ID_{50} = the nanomolar concentration of drug required to inhibit, by 50%, the growth of murine L1210 leukemia cells in a 3-day assay. [e] Drug given by intraperitoneal injection to mice implanted intraperitoneally with 10^6 P388 leukemia cells. See Experimental Section. OD = optimum dose in mg/kg per day, given on days 1, 5, and 9. % ILS = the percentage increase in life span of treated animals over that of control mice bearing tumor, when treated at the optimal dose. Numbers in parentheses are the numbers of mice (out of a group of six) that survived for 30 days. (HDT) = highest dose tested; toxicity not reached. NA = no activity (<20% ILS). [f] Extreme insolubility of the drug gave anomalous and irreproducible results. [g] The drug was not soluble in this medium in the concentration range used.

ortho alkylation procedure for anilines with subsequent in situ cyclization to the oxindole[16] was carried out in one of two ways. In the original synthesis, which utilized **37b**, the steric hindrance of the α methyl group resulted in low yields for this step.[13] To circumvent this problem, we carried out the reaction with sulfide **37a**. This gave, initially, the desoxy oxindole **38a**. The α-oxindole methyl group of **38b** was introduced by subsequent base-catalyzed alkylation. Alternatively, use of cyclic sulfide **37c** allowed efficient formation of **38c** without the additional alkylation step. Borane–methyl sulfide reduction[17] then afforded CPI precursor **39** in an overall yield of 4% (nine steps) from **32**. Red-Al (Aldrich) removed the methanesulfonyl group to give the air-sensitive dihydrobenzodipyrrole **40**, which was generally not purified, but condensed directly with a carboxylic acid in the presence of a carbodiimide dehydrating agent or with an active acylating (sulfonylating, carbamoylating) agent. The product **41**, typically obtained in 50–80% yield, was then reacted with methanesulfonyl chloride in pyridine to afford **42**. The methanesulfonate **42** (80–100% crude yield) was subjected to debenzylation either with catalytic hydrogenolysis, with in situ generated trimethylsilyl iodide, or with boron tribromide to give **43**. Usually without isolation, **43** (which was generally accompanied by some of the CPI product) was treated with triethylamine. This afforded, in yields in the range of 10–40%, most of the CPI analogues listed in Table I. An alternate route explored in some cases was debenzylation of **41** to produce **44**, followed by an intramolecular Mitsunobo dehydration to afford the CPI analogue.[18] Solubility and purification problems limited the utility of this route. It was readily apparent that sulfonyl substitution on the pyrrolidine nitrogen of CPI markedly affects its chemical reactivity relative to acyl substitution. Under aqueous alkaline conditions, both **1**[11] and its acyl analogues

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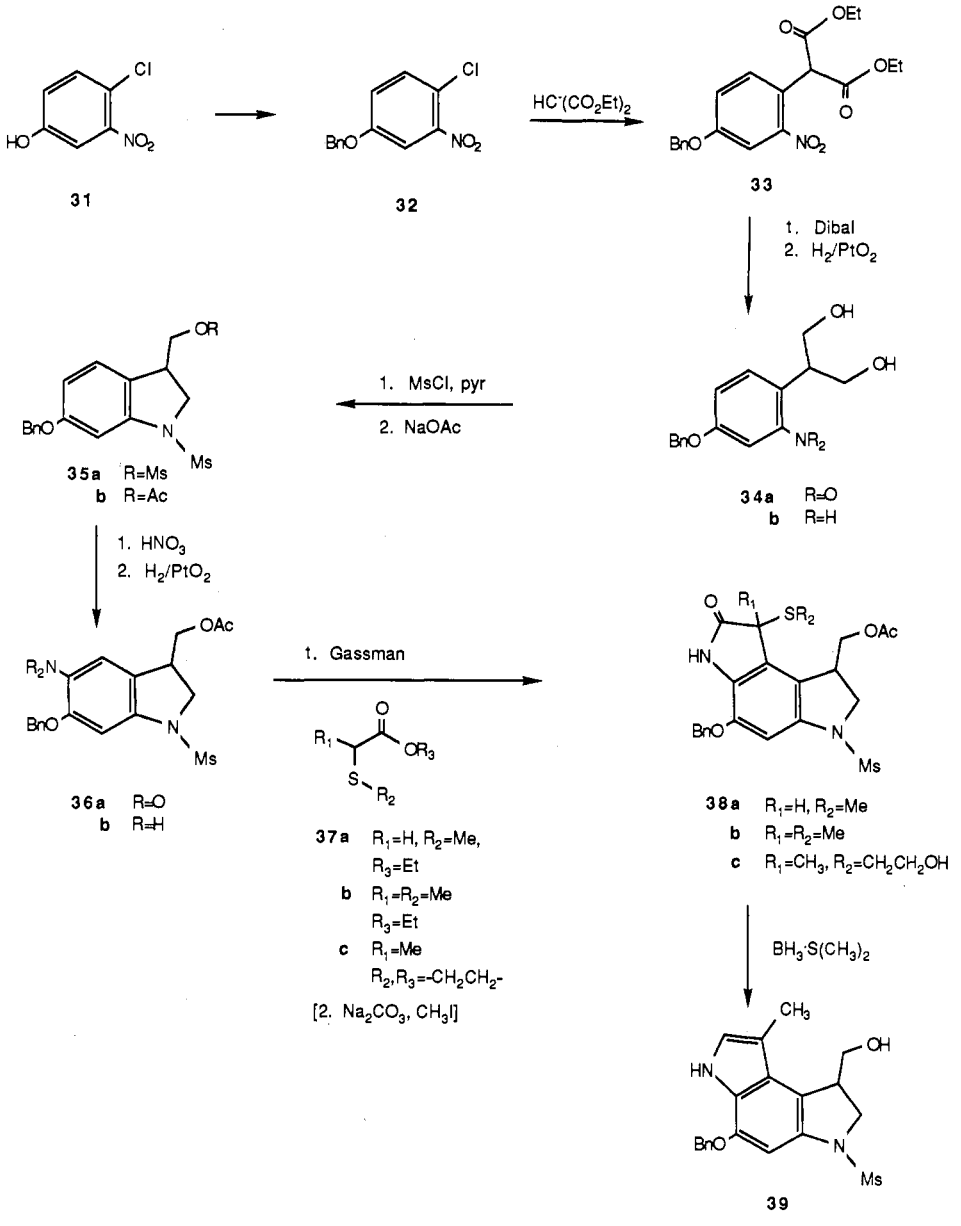
Scheme I



(Scheme II) are cleaved to give, after neutralization, the vinylogous amide **6**, with the CPI moiety still intact, and the free acid component. In contrast, **2** and **5** were stable to these conditions. Opening of the cyclopropyl ring of 1[11] and all of the analogues except **6** occurs under acidic conditions. The expectedly greater basicity of acyl- versus sulfonyl-substituted CPI analogues was reflected in the greater difficulty in their isolation. Excess base was necessary to drive the equilibrium to the ring-closed form (Scheme II), but timely removal of base was also necessary to avoid conversion to **6**.

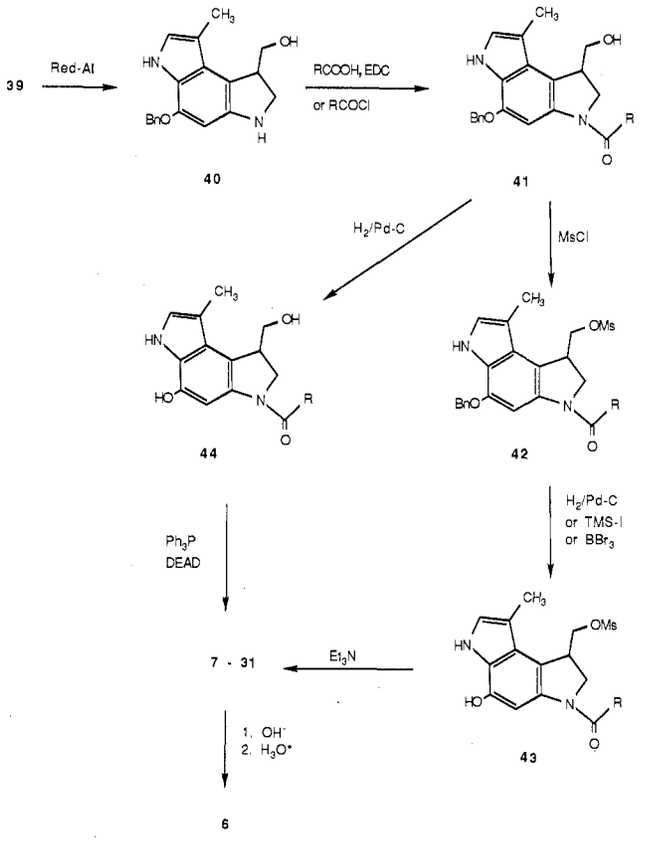
Results and Discussion

Table I lists the biological activities (in vitro L1210 and in vivo P388) of 1 and the racemic CPI analogues (**2**, **5–30**) as well as experimental parameters reflecting solvolytic ring opening reactivity, solvent partitioning, and in vitro DNA binding or interaction. Solvolytic reactivity is indicated by the pseudo-first-order rate constant for the acid-catalyzed opening of the cyclopropyl ring in methanolic buffer at pH 3. R_f values were obtained by DMF–saline elution on reversed-phase thin-layer chromatography. They are taken as a measure of relative hydrophilic/lipophilic partitioning, with higher R_f values corresponding to great hydrophilicity. "DNA binding" is operationally equated with the induced circular dichroism (ICD), expressed as molar ellipticity, observed in the presence of DNA under standardized conditions.

Solvolytic Reactivity. Both the association of biological activity with the CPI moiety[12] and the more recent isolation of an adenine adduct from the in vitro incubation of 1 with DNA[5,6] strongly suggest that at the molecular level biological action involves nucleophilic attack by DNA on the cyclopropyl methylene group of the CPI segment. The electronic nature of the group directly bonded to the pyrrolidine nitrogen of that system might therefore be expected to have a marked influence on bioactivity through its effect on chemical reactivity.

We had already noted, during the course of their preparation, the greater acid-catalyzed ring-opening reactivity of acyl- vs sulfonyl-substituted CPI analogues. To quantitate these differences, we measured the rates of acid-catalyzed solvolytic ring opening of a number of CPI analogues in methanolic buffer at pH 3. The reactions were followed by the disappearance of the long-wavelength (344–370 nm) absorption band of the CPI chromophore and showed pseudo-first-order kinetics for several half-lives. Product analysis confirmed the expected solvolytic

Scheme II



ring opened products resulting from water or methanol addition across the cyclopropylcyclohexadienone system,[19] in effect, an "alkylation" of solvent. At pH 5–7 in an otherwise comparable milieu, these analogues showed no significant decomposition over a 2-week period, confirming the pH dependence of the reaction.

Acyl analogues, whether bearing alkyl, aryl, or heteroaryl groups, show relatively little variation in the rates of solvolytic ring opening (Table I). Somewhat slower kinetics are observed for both the less basic sulfonamide **2** and the more basic carbamate **7**. In contrast, the vinylogous amide **6**, which in strongly acidic media forms a stable, spectroscopically distinct conjugate acid, is completely stable at pH 3.

Non DNA binding analogues show large differences in cytotoxic potency (Table I). Least cytotoxic are the sulfonamides **2** and **5**, the carbamate **7**, and the vinylogous amide **6**. Potencies of the non DNA binding acyl analogues fall in a fairly tight range, except for the extremely hydrophobic **10** and **16**. This correspondence between solvolytic reactivity and biological potency, in the absence of DNA interaction, suggests that acidic solvolysis is a reasonable model for the biologically important alkylation step. In effect, the DNA nucleophile attacks, in a concerted or stepwise manner, a protonated or otherwise electrophilically activated form of the CPI drug. The acid catalyst may be DNA itself.

DNA Binding. In the presence of double-helical DNA, the modest circular dichroism of the long-wavelength absorption of **1** is enormously enhanced, reaching a magnitude characteristic of inherently chiral chromophores.[20,21] This induced circular dichroism (ICD) is attributed to the tight binding (with restriction of rotational freedom of the drug molecule) between the helical minor groove of DNA and **1**. With the alternating deoxyinosine-deoxycytosine polynucleotide (poly(dI-dC)·poly(dI-dC)) this *binding* can be shown to precede *bonding*.[22] Incubation of **1** with this polynucleotide gives rise to an ICD band at 395 nm, due to the cyclopropylcyclohexadienone transition, which, over a period of days, converts to a band at 375 nm. This second band is produced by the DNA-alkylated, ring-opened species (**43**, OMs = DNA, Scheme II) and is of similar magnitude and sign as the initial ICD band. When **1** is incubated with calf-thymus DNA for 24 h at 25 °C (Table I), the observed ICD is due almost entirely to the binding of the covalently bonded form.[22]

While the racemic analogues display no circular dichroism on their own, in the presence of DNA some of them show significant induced circular dichroism (ICD, Table I). As with **1**, poly(dI-dC)·poly(dI-dC) induces a long-wavelength (375–385 nm) CD band reflecting the noncovalently (extractable) bound drug, which slowly converts to the covalently bonded species (ICD band at 345–355 nm).[23] Under the conditions used in our experiments (Table I), the ICD values reflect mainly the binding of the covalent species (λ_{max} at 345–355 nm). A caveat is that these are racemic mixtures, and the measured parameter is the sum of the ICD values of the two enantiomers. For the compounds that have been resolved (**25**, **27**, and **28**), the ICD values of the enantiomeric pairs at the long-wavelength maxima differ in magnitude, but are always positive in sign (they do not cancel).[23] Thus the presence of an ICD for the racemic analogues may be regarded as a qualitative indication of binding to the helical groove of DNA. A second caveat is that analogues with nonaromatic appendages do not absorb above 320 nm after ring opening has occurred, and hence any ICD from these species may be masked by the CD of DNA itself. However, other methods have verified that **2** and **8**, at least, do not show appreciable DNA binding.[12,15]

Two structural features of the CPI appendage appear to strongly influence DNA binding, as reflected by the ICD (Table I): the size of the ring attached to the pyrrolidine nitrogen of CPI and the length of the aromatic amide chain. An ICD is almost exclusively associated with structures having a five-membered heteroaroyl ring attached to CPI. Methyl substituents on the five-membered ring significantly diminish the ICD (compare **18** and **19**). Replacement of the indole nitrogen atom with oxygen or sulfur also causes a large reduction in the magnitude of the ICD (compare **20** and **21** to **18**).

Analogues with six-membered rings attached to CPI fail to show an appreciable ICD in the presence of DNA. Interestingly, the NMR chemical shift of the cyclohexadienone proton (Table II) is considerably shielded in analogues bearing a six-membered-ring appendage (**11**, **12**, **14**), relative to other analogues. Its signal is virtually absent (extremely broadened) in analogues with a six-membered nitrogen heterocycle in this position (**13**, **15**, **16**). This suggests that there may be a significant conformational difference between structures with five- vs six-membered-ring appendages, and this difference may have a bearing on their DNA binding ability. Further NMR and computational studies relating to this question are in progress.

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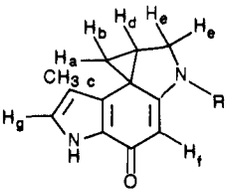
Table II. Chemical Shifts of CPI Protons



no.	solvent	H_a	H_b	H_c	H_d	H_e	H_f	H_g
2	$CDCl_3$	1.37	1.97	2.0	2.93	3.93–4.10	6.34	6.83
6	CD_3OD	1.07	1.88	2.0	3.1	3.5–3.85	5.4	6.8
7	$CDCl_3$ + CD_3OD	1.26	2.0	2.0	3.0	3.95–4.07	6.73	6.93
8	DMSO-d_6	1.23	1.89	1.96	3.03	4.02, 4.08	6.67	6.8
9	$CDCl_3$	1.7	2.0	2.0	2.87	3.9–4.25	6.7	6.9
10	$CDCl_3$	1.7	2.0	2.0	2.85	3.9–4.25	6.7	6.94
11	DMF	1.6	2.0	2.03	3.05	3.8–4.3	5.85	6.98
12	DMSO-d_6	1.6	2.0	2.0	3.05	4.0–4.25	5.65	6.94
13	DMSO-d_6	1.4	1.97	1.97	3.1	4.1–4.3	*a*	6.9
14	DMSO-d_6	1.66	2.0	2.0	3.15	3.9–4.4	5.6	6.9
15	DMSO-d_6	1.48	1.99	1.99	3.13	4.15–4.45	*a*	6.89
16	DMSO-d_6	*b*	2.0	2.0	3.1	4.15–4.55	6.36[c]	6.85
17	DMSO-d_6	1.29	1.93	1.99	3.13	4.28–4.41	6.66	6.85[d]
18	DMSO-d_6	1.38	2.0	2.0	3.2	4.45	6.72	6.95
19	$CDCl_3$	1.47	2.0	2.0	2.9	4.22	6.27	6.9[d]
20	DMSO-d_6	1.45	2.0	2.0	3.2	4.5	6.65	6.98
21	DMSO-d_6	1.5	2.0	2.0	3.1	4.25–4.55	6.5	6.95
22	$CDCl_3$	1.43	2.0	2.0	2.92	4.17	6.0	6.85
23	DMSO-d_6	1.36	1.96	2.0	3.12	4.44	6.7	6.9[d]
24	DMSO-d_6	1.42	1.96	2.02	3.15	4.38	6.51	6.94
25	DMSO-d_6	1.35	2.0	2.0	*e*	4.48	6.71	6.9[d]
26	DMSO-d_6	1.4	2.03	2.03	3.15	4.5	6.77	6.97
27	DMSO-d_6	1.42	1.96	2.03	3.14	4.5	6.78	6.97[d]
28	DMSO-d_6	1.49	2.03	2.07	3.2	4.5–4.7	6.82	7.01
29	DMSO-d_6	1.4	2.0	2.0	*e*	4.53	6.75	6.95
30	DMSO-d_6	1.4	2.02	2.02	*e*	4.52	6.75	6.95[d]

[a] Signal for this proton not observed. [b] Obscured by aliphatic proton signals. [c] Low, broad signal; low integration. [d] Not clearly separated from other aryl signals. [e] Obscured by water peak.

One six-membered aryl ring substituted analogue presents an interesting exception to the apparent requirement of the five-membered ring for DNA binding and also introduces the second important structural feature contributing to DNA interaction. This is structure **12**, containing two amide-linked phenyl rings. It may be viewed as an ultrasimplified model of the three-segmented natural product **1**. Surprisingly, **12** gave rise to a small but significant ICD in the presence of DNA.

Structures **27** and **29** are still closer models of **1**. They carry two amide-linked indoles having the same connectivity as in **1**, but stripped of both the hydrophobic concave lining and the hydrophilic convex coat. These analogues display the largest ICD values in this series. (Indeed, **3**, the natural configuration enantiomer of **27**, shows almost half of the ICD of **1**, in spite of its greatly streamlined structure.[15])

This phenomenon bears analogy to the distamycin and netropsin class of agents, which also bind to the minor groove of DNA. It has long been known[24] that extension of the distamycin structure with additional 1-methylpyrrole units enhances parameters associated with DNA interaction. Recent X-ray crystallographic studies of the complex of a DNA oligomer with netropsin concluded that the driving force for the interaction was supplied by drug displacement of the water molecules that normally occupy the minor groove and by close van der Waals contacts between the DNA bases and the drug.[25] These forces increase as longer drugs provide more contacts and displace more water molecules.

The two-fold higher ICD of **1** relative to **3** shows that the pyrrolidine rings and/or the oxygen substituents of the PDE I structure[26] significantly contribute to the extraordinary fit of **1** in the DNA groove. Nevertheless, the relative binding of these simpler analogues as a function of length of the groove-complementary aromatic "tail" underscores the large contribution of van der Waals and hydrophobic forces to the DNA interactions of this class of molecules as well.

The results in Table I show that cytotoxic potency is qualitatively correlated to DNA binding. At one extreme are the non DNA interacting analogues with alkyl or six-membered aryl or heteroaryl rings attached to acylated CPI, which typically exhibit, in the in vitro L1210 assay, ID_{50} values in the 10-nM range. Note that **12**, which shows detectable DNA interaction, also shows the greatest potency among analogues with six-membered-ring appendages. At the other extreme are the extended-indole or substituted-indole structures which show significant DNA interaction and have ID_{50} values generally in the 10–100 pM range. While their ID_{50} values vary somewhat, in vivo optimal doses for all of the substituted-indole analogues **23–30** appear to have plateaued at 50–100 μg/kg. Between these extremes are the analogues with five-membered or fused five–six-membered rings appended to CPI. Methyl

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(25) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1376.
(26) The two identical right-hand subunits of **1** are referred to as PDE subunits because of their resemblance to the phosphodiesterase inhibitors PDE I and PDE II: Nakamura, H.; Enomoto, Y.; Takeuchi, T.; Umezawa, H.; Iitaka, Y. *Agric. Biol. Chem.* **1978**, *42*, 1337.

substitution on the five-membered ring (**19**, **22**) inhibits but does not eliminate DNA binding as reflected by the ICD. However, potency is reduced to the levels of non DNA binding analogues. In contrast, the much lower ICD values of the isosteres **20** and **21** relative to **18** are not reflected in lower potency. Either their potencies are enhanced by as yet unidentified factors or the ICD parameter merely sets a window, within which potency is not highly variable. This latter possibility is supported by the uniformly high potencies of the DNA-interactive analogues **23–30**. Although they show only a fraction of the ICD of **1**, they show equal or greater cytotoxic potency.

While DNA interaction appears to be a prerequisite for high potency in this series, it is not essential for high antitumor efficacy. All CPI analogues for which a toxic dose has been reached show significant activity, in most cases greater than that of **1** itself. Outstanding activity, with greater than 100% increases in life span and even cures, is shown by both the DNA-binding, potent extended-indole analogues (**27–29**) and the non DNA binding, low-potency, quinoline-based analogues (**15**, **16**).

Compounds **2**, **15**, **17**, **18**, **23**, **24**, **26–28**, and **30** have been administered intravenously to nontumored mice. Only analogue **23** showed an initial severe weight loss followed by delayed death in mice, and only at one dose level. While the phenomenon is not yet well understood, it is clearly not correlated to either antitumor activity or potency.

Stability, Solubility, and Partitioning. While the CPI analogues can be isolated, manipulated, and stored for long periods, their limits in the physical environment are fairly narrowly defined. In addition to acid-catalyzed solvolysis and alkaline cleavage described above, most of these compounds undergo photodecomposition discernible over a period of hours. They also decompose, evidently to polymeric material, in neutral aqueous suspension (in the dark) in the absence of solubilizing agents. We have observed a positive correlation between stability and solubility (by UV spectroscopy) of **27** in the presence of low levels of nonionic surfactants. On the basis of this criterion, the analogues in Table I represent a wide range of absolute aqueous solubilities. Compounds **20**, **21**, **23**, **27**, **28**, and **1** are insoluble at 25 μM in 0.01 M phosphate buffer, pH 7, 5% dimethylacetamide, as evidenced by their precipitation and decomposition in this medium within 24 h. At this same concentration, solutions of **2**, **6**, **7**, **9**, **11**, **13**, and **15** were stable for 24 h at 25 °C, as were solutions of **24** and **25**. Analogues **18** and **19** showed borderline stability, indicating that 25 μM was near their solubility limit under these conditions.

While both **27** and **28** are insoluble in the aqueous medium described above, other conditions reveal that **28** is more soluble than **27**. Thus, **28** was completely soluble and stable (2 weeks, 25 °C in 1:1 methanol–phosphate buffer, pH 7), while **27** had largely precipitated from this medium within 1 day.

These compounds also displayed a wide range of hydrophilicity based on retention on reversed-phase chromatography. The two most hydrophobic compounds, **10** and **16**, showed anomolously low potencies in L1210 in vitro and P388 in vivo assays compared to other non DNA binding acyl-substituted analogues. In the case of **16**, at least, efficacy did not seem to be affected. At the other extreme, the most hydrophilic of the strongly DNA interacting analogues, **24** and **25**, also showed reduced potency in vitro (but not in vivo) relative to the other DNA-binding analogues. While the variability inherent in the in vivo data makes the correlation still somewhat tenuous, it does appear that, within a given structural category (alkyl, indole, etc.) of CPI appendage, the most hydrophilic analogues were also the least active. For example, the acetyl CPI **8** (R_f 0.90) is considerably less efficacious against P388 than its lipophilic (R_f 0.35) hexanoyl homologue **9**, producing only a 45% increase in life span (ILS) vs 100% for **9**. Likewise, the hydrophilic substituted-indole analogues **24** and **25** are less efficacious than the more lipophilic **18**, **23**, and **26** in spite of comparable DNA-binding properties.

Conclusions

Several important structural features for biological activity have been identified through this series of analogues.

1. Acyl substitution on the pyrrolidine nitrogen of CPI optimizes its reactivity to acid-catalyzed nucleophilic attack, a molecular mechanism that appears to be operative in its biological action.
2. A five-membered-ring heteroaroyl substituent directly attached to CPI, perhaps by allowing an appropriate conformation, permits binding to the DNA helix.
3. The extension of the amide-linked indole chain of the CPI appendage significantly enhances DNA interaction. This is consistent with a major hydrophobic contribution to drug–DNA binding.

Noncovalent DNA-binding forces, which give rise to induced circular dichroism, clearly enhance potency, most probably by "collecting" the drug near the reactive site in the DNA groove. With or without such binding assistance, DNA alkylation by the reactive CPI moiety still appears to be the crucial biological event. Factors that contribute to unusually high antitumor efficacy (e.g., the high % ILS of the quinoline analogues **15** and **16** and the curativity of the extended-indole analogues **27–29**), as well as those that lead to the delayed-death phenomenon (e.g., of analogue **23**), are not yet understood.

A clearer picture of structural influences on DNA interaction will require resolution of more of the CPI analogues, as demonstrated by the very different DNA-binding and biological properties of the enantiomers **3** and **4**. In spite of the limitations of a study of racemic compounds, this work has led us expeditiously from the first synthetic analogue **2** to a superior therapeutic class of CPI agents. This class is exemplified by **3**, the biologically active enantiomer of the potent and highly active (curvative in P388) **27**.

Experimental Section

Melting points were taken on a Kofler hot stage or a Thomas-Hoover Unimelt apparatus and are uncorrected. Combustion analysis and IR and mass spectra were obtained by the Physical and Analytical Chemistry Research Department of The Upjohn Co. Fast atom bombardment (FAB) mass spectra were run on a JG ZAB 2F instrument, and electron-impact (EI) mass spectra were run at 70 eV on a CEC 21-110B mass spectrometer. Proton NMR spectra were recorded on a Varian 390 (90 MHz) or a Brüker Aspect 3000 (300 MHz) instrument, and ^{13}C NMR spectra were recorded on a Varian CFT-20 (20 MHz) instrument. Chemical shifts are reported in parts per million relative to internal tetramethylsilane. Ultraviolet spectra were recorded on a Perkin-Elmer Lambda 3 spectrophotometer. Analtech silica gel GF (0.25 mm) plates were used for TLC, with visualization by UV light. Column chromatography was performed on silica gel (70–230 mesh, gravity; 230–400 mesh, low–medium pressure) supplied by E. Merck, Darmstadt. THF was dried by distillation under nitrogen from sodium/benzophenone ketyl. Acetonitrile was distilled from calcium hydride. Dry pyridine was obtained by distillation from sodium hydroxide. All other solvents were reagent grade or reagent grade distilled from glass (Burdick & Jackson). Trimethylsilyl chloride was distilled and stored over molecular sieves. All other reagents were used as purchased and were reagent grade where available. In order to minimize the health risks posed by these potent cytotoxic agents to analytical service personnel

outside of our own laboratory and to allow preparation of only the very limited quantities needed for testing, combustion elemental analyses were not obtained on the final analogues, except for **27**. These substances were, however, homogeneous by TLC.

1-Chloro-2-nitro-4-(benzyloxy)benzene (32). 4-Chloro-3-nitroanisole (100 g, 0.5 mol) was refluxed with glacial acetic acid (470 mL) and 48% hydrobromic acid (470 mL) for 41 h. The mixture was concentrated in vacuo, and the product crystallized from cold water to give 4-chloro-3-nitrophenol (**31**, 88 g, 96%) as yellow crystals: mp 123–127 °C. Anal. ($C_6H_4ClNO_3$) C, H, N, Cl. 4-Chloro-3-nitrophenol (85 g, 0.49 mol), in acetone (0.8 L) and DMF (0.8 L), was stirred with pulverized K_2CO_3 (105 g, 0.76 mol) and benzyl bromide (73 mL, 0.61 mol) under argon for 64 h at room temperature. The reaction mixture was concentrated and taken up in methylene chloride and water. The organic phase was washed with water, dried (Na_2SO_4), and concentrated. Crystallization from cold methanol afforded **32** (98 g, 96%) as bright yellow crystals: mp 50–52 °C. Anal. ($C_{13}H_{10}ClNO_3$) C, H, N, Cl.

Diethyl [4-(Benzyloxy)-2-nitrophenyl]malonate (33). NaH (60% in oil dispersion, 43 g, 1.1 mol) was washed with hexane under an argon atmosphere and suspended in 1.4 L of DMSO. Diethyl malonate (183 g, 1.14 mol) was added slowly, with cooling to maintain the reaction temperature below 30 °C. **Caution!** Hydrogen evolution! To the mixture, at room temperature, was added **32** (110 g, 0.42 mol). The reaction mixture was heated to 105–115 °C and stirred for 24 h (80% completion). It was quenched with acetic acid (69 mL) and diluted with methylene chloride and 0.5 N HCl. The organic phase was washed with saturated NaCl, dried (Na_2SO_4), and concentrated. The crude product was chromatographed on silica gel with a hexanes–ethyl acetate eluant and recrystallized from 95% ethanol to afford **33** (90 g, 55%) as tan crystals: mp 44–45 °C; IR (neat) 1720 cm^{-1}; NMR ($CDCl_3$) δ 1.24 (t, 6 H), 4.27 (q, 4 H), 5.12 (s, 2 H), 5.28 (s, 1 H), 7.1–7.9 (m, 8 H). Anal. ($C_{20}H_{21}NO_7$) C, H, N. Recycling of the recovered starting material and rechromatography of the mother liquors brought the total yield of **33** up to 77%.

2-[4-(Benzyloxy)-2-nitrophenyl]propane-1,3-diol (34a). To an ice–salt-cooled solution of diisobutylaluminum hydride (25% in toluene, 200 g, 1.4 mol) in 0.8 L of dry THF, being stirred under nitrogen, was added, over 1.3 h, a solution of **33** (90 g, 0.23 mol) in 0.15 L of dry THF. The reaction mixture was stirred at room temperature for an additional hour. It was quenched by careful pouring into 4 L of 3 N HCl, with vigorous stirring and cooling by the addition of ice, in a vessel large enough to contain the copious foaming. The red mixture was extracted with 2 L of ethyl acetate, which was then washed with saturated NaCl, dried (Na_2SO_4), and concentrated. The crude red product was chromatographed on silica gel, eluting with a gradient of 20–50% acetone in methylene chloride to afford **34a** as a broad solid (40% yield). A portion was recrystallized from methylene chloride, giving tan plates: mp 97–99 °C; NMR ($CDCl_3$) δ 2.70 (br s, 2 H), 3.47 (quintet, J = 6 H, 1 H), 3.93 (d, J = 6 H, 4 H), 5.08 (s, 2 H), 7.0–7.54 (m, 8 H). Anal. ($C_{16}H_{17}NO_5$) C, H, N.

2-[2-Amino-4-(benzyloxy)phenyl]propane-1,3-diol (34b). A mixture of **34a** (44.5 g, 0.15 mol), absolute ethanol (1.5 L), and 84.5% PtO_2 (5.5 g, 20 mmol) was hydrogenated at 45 psi for 2.75 h. The liquid was decanted and filtered; the solids, containing most of the product, were slurried in DMF and filtered; and the filter cake was washed with more DMF. The filtrates were concentrated and recrystallized from 95% ethanol to afford **34b** (28.2 g, 69% yield) as tan flakes: mp 152–154 °C; NMR (DMSO-d_6) δ 2.8 (t, 1 H, J = 6 Hz), 3.6 (m, 4 H), 4.48 (t, 2 H, J = 5 Hz, OH), 4.84 (br s, 2 H, NH), 5.0 (s, 2 H), 6.22 (dd, 1 H, J = 2, 8 Hz), 6.34 (d, 1 H, J = 2 Hz), 6.88 (d, 1 H, J = 8 Hz), 7.43 (m, 5 H). Anal. ($C_{16}H_{19}NO_3$) C, H, N.

6-(Benzyloxy)-2,3-dihydro-1-(methylsulfonyl)-1*H*-indole-3-methanol, Methanesulfonate (35a). The crude (unrecrystallized) product **34b**, obtained from the reduction of 0.46 mol of **34a**, was dissolved in 1.8 L of pyridine under argon. The solution was cooled to 5 °C, and methanesulfonyl chloride (125 mL, 1.6 mol) was added dropwise over 40 min. After 45 min, the reaction mixture was quenched with ice and diluted with ethyl acetate. The organic phase was washed with cold 3 N HCl (6 L), 1 N HCl (until the wash remained acidic), 5% $NaHCO_3$, and saturated NaCl, dried (Na_2SO_4), and concentrated to a purple oil. This was chromatographed on silica gel, eluting with 10% ethyl acetate in methylene chloride, to give 111 g of **35a** (59% yield from **34a**) as a pink solid, which could be recrystallized from acetone and hexanes: mp 125–126 °C; IR (Nujol) 1588, 1310, 1145 cm^{-1}; NMR (acetone-d_6) δ 2.95 (s, 3 H), 3.08 (s, 3 H), 3.85–4.18 (m, 3 H), 4.4 (m, 2 H), 5.14 (s, 2 H), 6.75 (dd, 1 H, J = 2, 8 Hz), 7.12 (d, 1 H, J = 2 Hz), 7.4 (m, 6 H); MS calcd for $C_{18}H_{21}NO_6S_2$ m/e 411.0810, found 411.0816. Anal. ($C_{18}H_{21}NO_6S_2$) C, H, N, S.

6-(Benzyloxy)-2,3-dihydro-1-(methylsulfonyl)-1*H*-indole-3-methanol, Acetate (35b). A mixture of **35a** (108 g, 0.26 mol), DMF (250 mL), absolute ethanol (4.7 L), and anhydrous sodium acetate (216 g, 2.6 mol) was refluxed under nitrogen for 20 h. The liquid and solids were separated and evaporated to dryness. The concentrated liquid was dissolved in methylene chloride and reacted with acetic anhydride (98 mL) and triethylamine (220 mL), and the solid was suspended in the same solvent and reacted with acetic anhydride (60 mL). (This step salvaged the primary alcohol that formed during the displacement reaction.) The acetylation reactions were quenched with methanol (exothermic) and taken up in water and methylene chloride. The organic phase was washed with 5% $NaHCO_3$, 1 N HCl, and saturated NaCl, dried (Na_2SO_4), and concentrated to an oil. This crystallized from absolute ethanol to afford 83.3 g (84% yield) of **35b** as tan flakes. Chromatography (silica gel, 5% ethyl acetate in methylene chloride) recovered an additional 10.7 g (total yield 95%) of **35b**: mp 106–108 °C; IR (Nujol) 1723, 1593, 1280, 1150, 840, 748, 700 cm^{-1}; NMR ($CDCl_3$) δ 2.04 (s, 3 H), 2.82 (s, 3 H), 3.23–4.40 (m, 5 H), 5.07 (s, 2 H), 6.65 (dd, 1 H, J = 2, 8 Hz), 6.82–7.63 (m, 7 H); ^{13}C NMR ($CDCl_3$) δ 20.80, 34.66, 39.01, 54.21, 65.90, 70.33, 101.29, 110.37, 122.56, 125.71, 127.55, 128.04, 128.60, 136.66, 143.34, 159.91, 170.73. Recrystallized from methanol: mp 113–114 °C. Anal. ($C_{19}H_{21}NO_5S$) C, H, N.

6-(Benzyloxy)-2,3-dihydro-1-(methylsulfonyl)-5-nitro-1*H*-indole-3-methanol, Acetate (36a). Nitric acid (90%, 4.4 mL, 94 mmol) was slowly added to a solution of **35b** (24.6 g, 65.4 mmol) in dry nitromethane (1 L) at 0 °C, under nitrogen. After being stirred for 40 min, the reaction mixture was diluted with methylene chloride, washed with 5% $NaHCO_3$ and saturated NaCl, and dried (Na_2SO_4). The brown solid left upon evaporation crystallized from ethanol and was further recrystallized from ethyl acetate and ethanol to afford 17.7 g (64% yield) of **36a**: mp 145–147 °C; NMR ($CDCl_3$) δ 2.07 (s, 3 H), 2.82 (s, 3 H), 3.40–4.40 (m, 5 H), 5.29 (s, 2 H), 7.23 (s, 1 H), 7.3–7.67 (m, 5 H), 7.88 (s, 1 H).

5-Amino-6-(benzyloxy)-2,3-dihydro-1-(methylsulfonyl)-1*H*-indole-3-methanol, Acetate (36b). Hydrogenation of **36a** (20 g, 48 mmol) was carried out at 40 psi over PtO_2 (84.5% catalyst, 1.8 g, 6.7 mmol) in THF (190 mL) and ethanol (530 mL) for 2 h. The catalyst was filtered off and washed with ethanol. The product crystallized from the cooled filtrate. Chromatography of the mother liquor on silica gel, eluting with ethyl acetate and hexanes, afforded additional **36b** (total yield 15 g, 81%): mp 132–134 °C; IR (neat) 3425, 3350, 2910, 1720, 1605, 1490, 1330, 1230, 1060 cm^{-1}; NMR ($CDCl_3$) δ 2.06 (s, 3 H), 2.74 (s, 3 H), 3.27–4.57 (m, 7 H), 5.10 (s, 2 H), 6.63 (s, 1 H), 7.10 (s, 1 H), 7.23–7.60 (m, 5 H). Anal. ($C_{19}H_{22}N_2O_5S$) C, H, N.

5-(Benzyloxy)-1,2,3,6,7,8-hexahydro-3-(methylsulfonyl)-8-(methylthio)-7-oxobenzo[1,2-*b*:4,3-*b*']dipyrrole-1-methanol, Acetate (38a). Ethyl (methylthio)acetate (**37a**, 5.2 mL, 40 mmol) and sulfuryl chloride (3.2 mL, 40 mmol) were added to dry methylene chloride at −78 °C, under argon, and the mixture was stirred for 20 min. To this was added, over 2.5 h, a solution of **36b** (15 g, 38 mmol) and 1,8-bis(dimethylamino)naphthalene (Proton Sponge, 7.9 g, 37 mmol) in dry methylene chloride (250 mL). After an additional 1.6 h, triethylamine (5.5 mL, 39 mmol) was added. After 30 min, the mixture was allowed to warm to room temperature. It was diluted with methylene chloride, washed with water, dried (Na_2SO_4), and concentrated. Glacial acetic acid (57 mL) effected ring closure of the Sommelet–Hauser product to the (methylthio)oxindole in 1 h at room temperature. The residue left after evaporation of the acetic acid was taken up in ethyl acetate and washed with 5% $KHSO_4$, 5% $NaHCO_3$, and saturated NaCl. (In some runs much of the oxindole precipitated from solution during the workup and was recovered by filtration.) The organic phase was dried (Na_2SO_4), concentrated, and chromatographed on silica gel (ethyl acetate, hexanes) to afford **38a**

(14.1 g, 77%): NMR ($CDCl_3$) δ 2.02 (s, 3 H), 2.06 (s, 3 H), 2.80 (s, 3 H), 3.63–4.57 (m, 6 H), 5.18 (s, 2 H), 7.20 (s, 1 H), 7.20–7.61 (m, 5 H), 8.06–8.30 (br s, 1 H); MS calcd for $C_{22}H_{24}N_2O_6S_2$ 476.1076, found 476.1063, *m/e* 430, 337, 289, 199.

5-(Benzyloxy)-1,2,3,6,7,8-hexahydro-8-methyl-3-(methylsulfonyl)-8-(methylthio)-7-oxobenzo[1,2-*b*:4,3-*b*']dipyrrole-1-methanol, Acetate (38b). To a mixture of **38a** (10.2 g, 21 mmol) and Na_2CO_3 (36 g, 0.34 mol) in acetone (160 mL) and DMF (160 mL), vigorously stirred under argon, was added methyl iodide (12 mL, 0.19 mol). After 4.5 h, the mixture was concentrated and the residue was dissolved in methylene chloride and water. The organic phase was washed with 1 N HCl and saturated NaCl, dried (Na_2SO_4), concentrated, and chromatographed on silica gel. Elution with acetone in methylene chloride afforded the diastereomeric products **38b** as a pink solid (8.8 g, 84% yield): IR (neat) 3150, 2970, 1700, 1605, 1440, 1225, 1150 cm^{-1}; NMR ($CDCl_3$) δ 1.74, minor diastereomer, 1.81, major (s, 3 H), 1.92, major diastereomer, 1.95, minor (s, 3 H), 2.10 (s, 3 H), 2.84 (s, 3 H), 3.75–4.45 (m, 5 H), 5.18 (s, 2 H), 7.22 (s, 1 H), 7.5 (m, 5 H), 8.26 (br s, 1 H).

3-Methyl-1-oxa-4-thian-2-one (37c). Ethyl 2-bromopropionate (50 g, 0.276 mol) and 2-mercaptoethanol (21 mL, 0.30 mol) were refluxed with anhydrous pulverized K_2CO_3 (42.0 g, 0.304 mol) in 0.3 L of acetone, under nitrogen, for 18 h. The mixture was cooled and filtered, and the solid was washed with acetone. Concentration of the filtrate gave 50 g of a colorless oil. To this oil, dissolved in 230 mL of 9:1 methanol–water, was added, dropwise, KOH (17.0 g, 0.303 mol) dissolved in 170 mL of 9:1 methanol–water, and the mixture was stirred for 2 h. It was then cooled to 0–5 °C and neutralized with 52.0 mL (0.315 mol) of 50% (v/v) HCl. After 30 min at ambient temperature, the solvents were removed in vacuo and the residue was dissolved in 0.4 L of toluene. The solution was refluxed for 2 h with azeotropic removal of the water formed in the reaction. The mixture was then cooled, concentrated, and chromatographed on silica gel, eluting with 25% ethyl acetate in hexanes to afford 24 g of a colorless oil. This was bulb-to-bulb distilled at 0.35 mmHg and 80–85 °C to give 22 g (60%) of **37c** as a white solid: mp 44–45 °C; IR (Nujol) 1739, 1725, 1454, 1379, 1296, 1238, 1202, 1162, 1151 cm^{-1}; NMR ($CDCl_3$) δ 1.45 (d, J = 7 Hz, 3 H), 2.9–3.4 (m, 2 H), 3.85 (q, J = 7 Hz, 1 H), 4.3–4.8 (m, 2 H). Anal. ($C_5H_8O_2S$) C, H, S.

5-(Benzyloxy)-1,2,3,6,7,8-hexahydro-8-methyl-3-(methylsulfonyl)-8-[(2-hydroxyethyl)thio]-7-oxobenzo[1,2-*b*:4,3-*b*']dipyrrole-1-methanol, Acetate (38c). Sulfuryl chloride (17.8 mL, 0.22 mol) was added dropwise to a solution of **37c** (30.0 g, 0.227 mol) in 2 L of dry methylene chloride, under nitrogen, at −70 °C, and the mixture was stirred at this temperature for 45 min. A solution of **36b** (83.9 g, 0.215 mol) and Proton Sponge (47.1 g, 0.220 mol) in 1.5 L of methylene chloride was added dropwise to this mixture over 2 h, with the temperature of the reaction maintained at −70 °C. The red suspension was stiired for an additional 2 h, and then triethylamine (31.6 mL, 0.227 mol) in 100 mL of methylene chloride was added, dropwise, over 15 min. The mixture was allowed to warm from −70 °C to 0 °C over 2 h, becoming greenish-black. The reaction mixture was poured into 4 L of saturated NaCl. The aqueous phase was extracted with methylene chloride, and the combined organic phases were concentrated and chromatographed on silica gel. Elution with acetone in methylene chloride and trituration of the pooled product with acetone afforded 57.5 g (51%) of **38c** (mixture of diastereomers) as a tan solid: mp 184–204 °C; IR (Nujol) 3165, 1740, 1710, 1635, 1490, 1460, 1455, 1445, 1345, 1335, 1230, 1220, 1160, 1155, 1030 cm^{-1}; NMR ($CDCl_3$) δ 1.6–1.9 (m, 4 H), 2.1 (s, 3 H), 2.3–2.8 (m, 3 H), 2.8 (s, 3 H), 3.4–4.3 (m, 6 H), 5.2 (s, 2 H), 7.2 (s, 1 H), 7.3–7.6 (m, 5 H), 8.3 (br s, 1 H). Anal. ($C_{24}H_{28}O_7N_2S$) C, H, N, S.

5-(Benzyloxy)-1,2,3,6-tetrahydro-8-methyl-3-(methylsulfonyl)benzo[1,2-*b*:4,3-*b*']dipyrrole-1-methanol (39). Borane–methyl sulfide (2.0 M in THF, 275 mL, 0.55 mol) was added dropwise over 45 min to a solution of **38c** (57.5 g, 0.11 mol) in dry THF (1.2 L) containing 25 g of 4-Å molecular sieves and maintained under nitrogen. **Caution!** Vigorous foaming and gas evolution! The solution was refluxed for 3 h, cooled to 0 °C, and quenched by careful dropwise addition of 0.5 L of 1 M HCl. **Caution!** Gas evolution! The mixture was stirred at 5 °C for 1 h and then diluted with ethyl acetate. The aqueous phase was extracted with more ethyl acetate, and the combined organic phases were washed with saturated NaCl, dried (Na_2SO_4), and concentrated. Chromatography on silica gel, eluting with 5% acetone in methylene chloride, gave 38.6 g (90%) of **39**: mp 143–150 °C; IR (Nujol) 3380, 3265, 1590, 1465, 1455, 1440, 1330, 1320, 1300, 1185, 1155, 1115, 1100, 1090, 1025, 735 cm^{-1}; NMR ($CDCl_3$) δ 1.6 (s, 1 H), 2.37 (s, 3 H), 2.75 (s, 3 H), 3.5–4.3 (m, 5 H), 5.2 (s, 2 H), 7.0 (br s, 1 H), 7.13 (s, 1 H), 7.2–7.4 (m, 5 H), 8.3 (br s, 1 H); UV (ethanol) 211 (ϵ 25900), 235 (40000), 269 (5500), 306 nm (5500); MS calcd for $C_{20}H_{22}N_2O_4S$ *m/e* 386.1300, found 386.1315. Anal. ($C_{20}H_{22}N_2O_4S$) C, H, N.

5-(Benzyloxy)-1,2,3,6-tetrahydro-8-methylbenzo[1,2-*b*:4,3-*b*']dipyrrole-1-methanol (40). To 0.8 g (2 mmol) of **39** in 25 mL of dry THF under nitrogen were added 25 mL of toluene and 3.6 mL (12 mmol) of a 3.4 M solution of sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al) in toluene. The clear, colorless solution was quickly heated, and the condenser was lifted under a flow of nitrogen to allow THF to escape. After the internal temperature of the solution reached 85 °C (15 min), the condenser was replaced and heating was continued for 15 min. The yellow solution was cooled, quenched by dropwise addition of 15% K_2CO_3 (vigorous gas evolution!), and diluted with nitrogen-purged ether and water. (Replacement of ether with methylene chloride reduced the yield significantly.) The yellow aqueous suspension was reextracted with ether, and the combined pale yellow organic phase was washed with water and saturated NaCl, dried (Na_2SO_4), and concentrated. The oily residue was evaporated from a small amount of methylene chloride to afford 630 mg (quantitative yield) of a foamy tan solid, which was 90% pure **40** (by NMR): R_f 0.2 (1:1 acetone–methylene chloride, silica gel); NMR ($CDCl_3$) δ 2.32 (s, 3 H), 2.92 (br s, 2 H, OH, NH), 3.5–3.8 (m, 5 H), 5.02 (s, 2 H), 6.23 (s, 1 H), 6.8 (br s, 1 H), 7.4 (m, 5 H), 8.33 (br s, 1 H).

Preparation of Carboxylic Acids for Coupling to 40. *N*-Benzoyl-*p*-aminobenzoic Acid. To 1.4 g (10 mmol) of *p*-aminobenzoic acid in 100 mL of methylene chloride were added 1.15 mL (10 mmol) of benzoyl chloride and 1.4 mL (10 mmol) of triethylamine. After being stirred for 1 day, the mixture was concentrated and partitioned into 10% HCl and a large volume of ethyl acetate. The organic phase was concentrated, and the residue was washed with methylene chloride, leaving the product as a solid: NMR (DMSO-d_6) δ 7.5–7.6 (m, 3 H), 7.9–8.0 (m, 6 H).

6-(Hexanoylamino)quinoline-2-carboxylic Acid. A solution of 2-methyl-6-nitroquinoline[27] (0.5 g, 2.7 mmol) in 5 mL of methylene chloride was added to a mixture of SeO_2 (134 mg, 1.2 mmol) and *tert*-butyl hydroperoxide (1.8 mL, 5.05 mmol) in 3 mL of methylene chloride.[28] The reaction mixture was heated to 40 °C, and two 1-mL portions of *tert*-butyl hydroperoxide were added over 2 days, until TLC showed complete reaction. The mixture was diluted with methylene chloride and quenched with aqueous phosphate buffer, pH 4. The buffer was extracted with ethyl acetate, and the combined organic phase was dried (Na_2SO_4) and concentrated. The residue, in THF, was reacted with excess ethereal diazomethane, concentrated, and chromatographed on silica gel. Elution with methylene chloride afforded 160 mg (26% yield) of methyl 6-nitroquinoline-2-carboxylate: NMR ($CDCl_3$) δ 4.1 (s, 3 H), 8.3–9.0 (m, 5 H). When carried out on a larger scale, with 2,2-dimethoxypropane in methanolic HCl in place of diazomethane, the reaction gave a 56% yield of this ester. Hydrogenation of this ester (232 mg, 1 mmol) over PtO_2 (100 mg) in 10 mL of 95% ethanol and 10 mL of THF for 2 h gave a mixture of the desired methyl 6-aminoquinoline-2-carboxylate and variable amounts of 1,2,3,4-tetrahydro-6-aminoquinoline-2-carboxylate, which could not be separated by chromatography. This aminoquinoline mixture (100 mg) was reacted with 0.20 mL of hexanoyl chloride in 2 mL of pyridine overnight. The reaction mixture was quenched with 1 N HCl and extracted with ethyl acetate, which was washed with saturated NaCl, dried (Na_2SO_4), concentrated, and chromatographed on silica gel. Elution with acetone and hexane afforded 159 mg of solid: mp 179–183 °C dec; MS, *m/e* 300, 286, 269, 244, 202, 144. Saponification of this ester with 1 mL of 1 N NaOH in 5 mL of methanol for 2 h, followed by

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neutralization with 1 N HCl, concentration, and precipitation from acetone with water, afforded a nearly quantitative yield of 6-(hexanoylamino)quinoline-2-carboxylic acid: mp 150 °C dec; IR (Nujol) 1665, 1569 cm^{-1}; MS, *m/e* 286, 242, 230, 188, 144.

5-Ureidoindole-2-carboxylic Acid. Hydrogenation of ethyl 5-nitroindole-2-carboxylate[29] (4.0 g, 17.1 mmol) in 120 mL of THF and 300 mL of ethanol over 365 mg of PtO_2, at 14 psi, for 3.25 h gave, after filtration, concentration, and chromatography (silica gel, 30% acetone in cyclohexane), 2.9 g (83%) of a light yellow solid identified as ethyl 5-aminoindole-2-carboxylate: mp 127–130 °C; IR (Nujol) 3420, 3330, 1685, 1530 cm^{-1}; NMR (DMSO-d_6) δ 1.33 (t, J = 7 Hz, 3 H), 4.30 (q, J = 7 Hz, 2 H), 4.67 (br s, 2 H), 6.6–7.4 (m, 4 H), 11.4 (br s, 1 H). Anal. ($C_{11}H_{12}N_2O_2$) C, H, N. To 620 mg (3.0 mmol) of this amino ester in 20 mL of acetic acid and 2 mL of water at 55 °C was added a solution of 1.8 g (22 mmol) of potassium cyanate in 22 mL of water, dropwise, with stirring, under nitrogen. The mixture was refluxed for 1.5 h, cooled, and extracted twice with ethyl acetate. The organic phase was washed with saturated NaCl, dried (Na_2SO_4), and concentrated, and the product was chromatographed on silica gel, eluting with 40% acetone in methylene chloride to afford 510 mg (68%) of ethyl 5-ureidoindole-2-carboxylate. To the ester in 10 mL of DMF and 10 mL of methanol was added 10 mL of 1 N NaOH. After being stirred for 4.5 h, the mixture was concentrated, diluted with water, and extracted with ethyl acetate to remove unreacted ester. The aqueous phase was acidified with 10% HCl and the product allowed to separate out over 2 days in the cold. A tan solid was collected (285 mg, 63%): NMR (DMSO-d_6) δ 5.7 (very br, 2 H), 7.0–7.4 (m, 3 H), 7.78 (br s, 1 H), 8.4 (br s, 1 H), 11.54 (br s, 1 H).

5-(Benzoylamino)-1*H*-indole-2-carboxylic Acid. To 1 mmol of ethyl 5-aminoindole-2-carboxylate and 1 equiv each of benzoic acid and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC) was added 10 mL of DMF. After being stirred for 2 days, the mixture was quenched with 1 M $KHSO_4$ and extracted with ethyl acetate. The organic phase was washed with water and saturated NaCl, dried (Na_2SO_4), and concentrated to a pink solid (250 mg, 81%). The coupled ester was suspended in 10 mL of methanol, 5 mL of DMF, and 25 mL of 10% KOH and sonicated for 30 min. The reaction mixture was quenched with 1 M $KHSO_4$ and extracted with ethyl acetate. The organic phase was washed with water and saturated NaCl, dried (Na_2SO_4), and concentrated to 215 mg (97%) of a pink solid: NMR (DMSO-d_6) δ 7.18 (m, 1 H), 7.5–7.7 (m, 5 H), 8.0–8.15 (m, 2 H), 8.2 (m, 1 H), 10.3 (s, 1 H), 11.8 (br s, 1 H).

5-[(1*H*-Indol-2-ylcarbonyl)amino]-1*H*-indole-2-carboxylic Acid. In like manner to the reaction described above, ethyl 5-aminoindole-2-carboxylate (1.5 mmol) was coupled to indole-2-carboxylic acid with EDC in DMF. In this case the ethyl acetate extract remained a fine suspension, which was concentrated to 250 mg (48%) of the coupled ester: NMR (DMSO-d_6) δ 1.35 (t, J = 7 Hz, 3 H), 4.40 (q, J = 7 Hz, 2 H), 7.0–8.0 (m, 8 H), 8.27 (br s, 1 H), 10.3 (br s, 1 H), 11.77 (br s, 1 H), 11.93 (br s, 1 H); IR (mull) 3415, 3325, 3275, 3230, 1695, 1650, 1615 cm^{-1}; MS calcd for $C_{20}H_{17}N_3O_3$ 347.1270, found 347.1275; UV (ethanol) 206 (ϵ 35 600), 218 (34 500), 305 nm (42 250). Anal. ($C_{20}H_{17}N_3O_3$) C, H, N. Saponification of this ester (200 mg, 0.58 mmol) with 6 mL of 10% NaOH in 20 mL of methanol and 6 mL of DMF by sonication at 40–50 °C for 30 min, followed by concentration and precipitation with 10% HCl, afforded 145 mg (78%) of the desired acid as a grayish-white powder: NMR (DMSO-d_6) δ 7.0–7.75 (m, 8 H), 8.2 (br s, 1 H), 10.26 (br s, 1 H), 11.75 (br s, 2 H); MS, *m/e* 319, 176, 158, 144.

5-[(1*H*-Benzofuran-2-ylcarbonyl)amino]-1*H*-indole-2-carboxylic Acid. Ethyl 5-aminoindole-2-carboxylate (1.4 mmol) was coupled to benzofuran-2-carboxylic acid with EDC as described above. The crude product was chromatographed on silica gel, eluting with 10% acetone in methylene chloride, to afford the ester in 90% yield: MS (FAB) calcd for $C_{20}H_{16}N_2O_4$ 348.1110, found 348.1147, *m/e* 302, 145. The ester (0.3 mmol) was saponified with 0.4 mL of 1 N NaOH in 4 mL of pyridine for 2 days. Neutralization, concentration, and chromatography (silica gel, 50:50:1 acetone–toluene–acetic acid) gave the acid in 80% yield: mp 284–288 °C; IR 3360, 1680, 1650, 1518 cm^{-1}; NMR (acetone-d_6) δ 7.02–8.07 (m, 9 H), 8.42 (s, 1 H), 10.02 (br s, 1 H); MS calcd for $C_{18}H_{11}NO_5$ 321.0637, found 321.0635, *m/e* 305, 277, 176, 145.

5-[[(5-Ureido-1*H*-indol-2-yl)carbonyl]amino]-1*H*-indole-2-carboxylic Acid. The EDC-promoted coupling of ethyl 5-aminoindole-2-carboxylate (0.46 mmol) and 5-ureidoindole-2-carboxylic acid gave a 97% crude yield of the ester: NMR (pyridine-d_5) δ 1.15 (t, J = 7 Hz, 3 H), 4.25 (q, J = 7 Hz, 2 H), 7.3 (s, 1 H), 7.7 (s, 1 H), 7.75 (s, 1 H), 8.12 (d of d, J = 8 Hz, 2 Hz, 1 H), 8.35 (br s, 1 H), 8.8 (br s, 1 H), 10.9 (br s, 1 H), 11.2 (br), 12.75 (br s, 1 H), 13.0 (br s, 1 H). Addition of D_2O unmasked two broad signals at 7.15 and 6.65, 1 H each. The ester was saponified with 40 mL of 10% NaOH in 20 mL of DMF, 5 mL of pyridine, and 5 mL of DMSO for 1.75 h. Neutralization and extraction with ethyl acetate afforded the desired acid as a light brown solid (55%): NMR (DMSO-d_6) δ 5.8 (NH_2), 7.1–7.9 (m, 7 H), 8.2 (s, 1 H), 8.57 (s, 1 H), 10.2 (br s, 1 H), 11.6 (br s, 1 H), 11.8 (br s, 1 H).

5-[[[5-(Benzoylamino)-1*H*-indol-2-yl]carbonyl]amino]-1*H*-indole-2-carboxylic Acid. Coupling of ethyl 5-aminoindole-2-carboxylate (0.36 mmol) with 5-(benzoylamino)-1*H*-indole-2-carboxylic acid afforded the crude ester, which was saponified with 15 mL of 10% KOH in 20 mL of DMF overnight. The mixture was concentrated, neutralized with 10% HCl, and extracted with ethyl acetate. The fine suspension was dried (Na_2SO_4) and concentrated to a pinkish-brown solid. Washing with a small amount of acetone left 80 mg (51%) of the acid as an off-white powder: NMR (DMSO-d_6) δ 7.18 (br s, 1 H), 7.4–7.7 (m, 8 H), 8.0–8.15 (m, 2 H), 8.23 (br s, 2 H), 10.3 (br s, 2 H), 11.76 (br s, 1 H), 11.83 (br s, 1 H).

Benzofuran-2-carboxylic acid, benzothiophene-2-carboxylic acid, and 6-hydroxy-7-methoxyindole-2-carboxylic acid were prepared by literature procedures.[30-32] All other acids and active acyl compounds were commercially available.

Preparation of Coupled Intermediates 41. Examples. Example 1. 5-(Benzyloxy)-1,2,3,6-tetrahydro-8-methyl-3-(*tert*-butoxycarbonyl)benzo[1,2-*b*:4,3-*b*']dipyrrole-1-methanol (41, R = $OC(CH_3)_3$). The crude amine 40 from Red-Al reduction of 2.6 mmol of **39** was stirred at room temperature, under nitrogen, with 0.375 mL of triethylamine and 0.7 g of BOC-ON in 10 mL of dry THF for 3.5 days. The reaction mixture was chromatographed on silica gel, eluting with a gradient of ethyl acetate in hexanes, to afford 0.7 g (66% yield) of 41 (R = $OC(CH_3)_3$): MS calcd for $C_{24}H_{28}N_2O_4$ 408.2049, found 408.2051; NMR ($CDCl_3$) δ 1.6 (s, 9 H), 2.3 (s, 3 H), 3.4–4.4 (m, 6 H), 5.15 (s, 2 H), 6.9 (s, 1 H), 7.2–7.8 (m, 6 H), 8.5 (s, 1 H).

Example 2. 5-(Benzyloxy)-1,2,3,6-tetrahydro-8-methyl-3-acetylbenzo[1,2-*b*:4,3-*b*']dipyrrole-1-methanol (41, R = CH_3). The ethereal solution of 40 obtained from the Red-Al reduction of 0.26 mmol of **39** was reacted with 1.2 mmol of acetic anhydride. After 15 min, the solution was concentrated and chromatographed on silica gel, eluting with 60% acetone in cyclohexane, to give 52 mg (57%) of 41 (R = CH_3) as a white powder: NMR (acetone-d_6) δ 2.18 (s, 3 H), 2.4 (s, 3 H), 2.9 (br s, 1 H, OH), 3.2–4.4 (m, 5 H), 5.2 (s, 2 H), 7.12 (m, 1 H), 7.3–7.7 (m, 5 H), 8.12 (s, 1 H), 10.17 (br s, 1 H).

Example 3. 5-(Benzyloxy)-1,2,3,6-tetrahydro-8-methyl-3-(2-quinolylcarbonyl)benzo[1,2-*b*:4,3-*b*']dipyrrole-1-methanol (41, R = 2-Quinolyl). Quinaldic acid (38 mg, 0.22 mmol) was coupled with 40 (60 mg, 0.19 mmol) in 4 mL of DMF in the presence of EDC (40 mg, 0.20 mmol). After being stirred for 22 h at room temperature, under nitrogen, the reaction mixture was diluted with methylene chloride and washed with 5% $NaHSO_4$, 5% $NaHCO_3$, and saturated NaCl. The organic phase was dried (Na_2SO_4) and concentrated, and the residue was chromatographed over silica gel, eluting with a gradient of ethyl acetate in hexanes. The product, 41 (R = 2-quinolyl), was obtained as

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a yellow solid (80 mg, 91% yield): NMR ($CDCl_3$) δ 2.2 (s, 3 H), 2.1–2.5 (br, 1 H), 3.4–3.8 (m, 3 H), 4.2–4.7 (m, 2 H), 5.1 (s, 2 H), 6.8 (br, 1 H), 7.2–8.2 (m, 12 H), 8.4 (br, 1 H).

Example 4. 5-(Benzyloxy)-1,2,3,6-tetrahydro-8-methyl-3-[[5-[(1*H*-indol-2-ylcarbonyl)amino]-1*H*-indol-2-yl]-carbonyl]benzo[1,2-*b*:4,3-*b*']dipyrrole-1-methanol (41, R = 5-[(2-Indolylcarbonyl)amino]-2-indolyl). To 90 mg (0.29 mmol) of **40** in 7 mL of DMF under nitrogen were added 95 mg (0.30 mmol) of 5-[(indol-2-ylcarbonyl)amino]indole-2-carboxylic acid and 68 mg (0.34 mmol) of EDC. The reaction mixture was stirred at room temperature for 3 days, then quenched with 1 M $KHSO_4$, and extracted twice with ethyl acetate. The organic phase was washed with water and saturated NaCl, dried (Na_2SO_4), and concentrated to a dark, granular solid. This was dissolved in a small amount of pyridine and diluted with methylene chloride to precipitate a light yellow, flocculent solid (130 mg, 72% yield): NMR (DMSO-d_6) δ 2.4 (br s, 3 H), 3.7 (br, 2 H), 4.7 (br, 2 H), 5.1 (br, 1 H), 5.3 (br s, 2 H), 7.1–8.0 (m, 15 H), 8.3 (br s, 1 H), 10.3 (br s, 1 H), 10.95 (br s, 1 H), 11.8 (br s, 2 H); MS, *m/e* 609 (M^+), 593, 577, 465, 444, 319, 290, 276, 275, 176, 158, 144, 132 (base peak).

Preparation of Methanesulfonylated Intermediates 42. Examples. Example 1. 5-(Benzyloxy)-1,2,3,6-tetrahydro-8-methyl-3-acetylbenzo[1,2-*b*:4,3-*b*']dipyrrole-1-methanol, 1-Methanesulfonate (42, R = CH_3). The crude product obtained by reacting 1 mmol of **40** with acetic anhydride as described above was dissolved in 4 mL of dry pyridine under nitrogen. (Dimethylamino)pyridine (DMAP, 10 mg) was added, followed by 0.25 mL (3.2 mmol) of methanesulfonyl chloride. After being stirred for 20 min at room temperature, the reaction mixture was quenched with 10% HCl and extracted with ethyl acetate. The organic phase was dried (Na_2SO_4), decolorized with charcoal, and concentrated, and the residue was chromatographed on silica gel (40% acetone in cyclohexane). The major product was the desired methanesulfonate **42** (R = CH_3). The minor product was the corresponding acetate from overreaction in the previous step. This acetate was saponified (NaOH, aqueous ethanol, 10 min) and resubjected to the methanesulfonylating conditions. The total yield of **42** (R = CH_3) was 253 mg (59%): NMR (DMSO-d_6) δ 2.2 (s, 3 H), 2.38 (s, 3 H), 3.16 (s, 3 H), 4.0–4.5 (m, 5 H), 5.27 (s, 2 H), 7.18 (s, 1 H), 7.4–7.6 (m, 5 H), 7.97 (s, 1 H), 11.0 (br s, 1 H); R_f 0.4 (1:1 acetone–cyclohexane, silica gel).

Example 2. Compound 42 (R = 2-Quinolyl). To 241 mg (0.52 mmol) of **41** (R = 2-quinolyl) in 5 mL of dry pyridine, being stirred under nitrogen at room temperature, was added 0.21 mL (2.7 mmol) of methanesulfonyl chloride. After 6 h, the reaction mixture was quenched with $NaHSO_4$ and extracted with methylene chloride. The organic phase was dried (Na_2SO_4) and concentrated to 286 mg (quantitative crude yield) of a brown solid: NMR ($CDCl_3$) δ 2.4 (s, 3 H), 2.8 (s, 3 H), 3.8–4.8 (m, 5 H), 5.3 (s, 2 H), 7.0 (br, 1 H), 7.2–8.6 (m, 13 H); R_f 0.65 (1:1 ethyl acetate–hexanes, silica gel).

Example 3. Compound 42 (R = 5-[(2-Indolylcarbonyl)-amino]-2-indolyl). To 120 mg (0.2 mmol) of alcohol **41** (R = 5-[(2-indolylcarbonyl)amino]-2-indolyl) in 5 mL of dry pyridine under nitrogen were added 5 mg of DMAP and 0.075 mL (1 mmol) of methanesulfonyl chloride. After 10 min at room temperature, the mixture was concentrated in vacuo, quenched with 10% HCl, and extracted with ethyl acetate. The organic phase was washed with saturated NaCl, dried (Na_2SO_4), and concentrated to 123 mg (90% crude yield) of product: NMR (acetone-d_6) δ 2.41 (s, 3 H), 2.94 (s, 3 H), 4.1–4.8 (br m, 5 H), 5.21 (s, 2 H), 7.14–7.76 (m, 15 H), 8.1 (s, 1 H), 8.43 (s, 1 H), 9.8 (s, 1 H), 10.3 (br s, 1 H), 11.1 (br, 1 H); MS (FAB), *m/e* 688 ($M + H^+$), 628, 610, 592, 302; R_f 0.52 (20% acetone in methylene chloride, silica gel).

Preparation of 44. Example. 5-Hydroxy-1,2,3,6-tetrahydro-8-methyl-3-(*tert*-butoxycarbonyl)benzo[1,2-*b*:4,3-*b*']dipyrrole-1-methanol (44, R = $OC(CH_3)_3$). A solution of **41** (R = $OC(CH_3)_3$) (242 mg, 0.59 mmol) in dry THF (2.4 mL) and absolute ethanol (25 mL) was hydrogenolized at 42 psi over 192 mg of palladium on carbon for 50 min. The mixture was filtered, the catalyst was washed with ethanol, and the filtrate was concentrated, leaving a white solid (158 mg, 84% yield): mp 120–130 °C; IR (Nujol) 3300, 1650, 1585, 1130 cm^{-1}; NMR (acetone-d_6) δ 1.55 (s, 9 H), 2.39 (s, 3 H), 2.98–4.07 (m, 5 H), 4.07–4.48 (m, 1 H), 7.05 (s, 1 H), 7.40 (br s, 1 H), 8.81 (br s, 1 H), 9.79 (br s, 1 H); MS (no M^+), *m/e* 231, 187, 56; R_f 0.60 (1:1 ethyl acetate–hexanes, silica gel).

Preparation of CPI Analogues from Intermediates 42 or 44. Examples of Methods. Method A. Example. 2-(*tert*-Butoxycarbonyl)-1,2,8,8a-tetrahydro-7-methylcyclopropa[*c*]pyrrolo[3,2-*e*]indol-4(5*H*)-one (7). To a solution of **44** (R = $OC(CH_3)_3$) (145 mg, 0.455 mmol) in 7.4 mL of dry THF under an inert atmosphere were added triphenylphosphine (190 mg, 0.72 mmol) and diethyl azodicarboxylate (0.11 mL, 0.70 mmol).[18] The mixture was stirred at room temperature for 80 min and then chromatographed on silica gel, eluting with 45% distilled THF in hexanes. Product fractions were concentrated and triturated with ethyl acetate to afford **7** as a white solid (62 mg, 45% yield): IR (Nujol) 3155, 1725, 1570, 1160, 960, 720 cm^{-1}; NMR ($CDCl_3$ + CD_3OD) δ 1.08–1.42 (m, 2 H), 1.58 (s, 9 H), 1.84–2.17 (s + m, 4 H), 2.80–3.14 (m, 1 H), 3.29–3.48 (m, 1 H), 6.73 (s, 1 H), 6.93 (s, 1 H); MS (FAB) calcd for $C_{17}H_{20}N_2O_3$ ($M + H$) 301.1553, found 301.1559, *m/e* 245, 231, 201, 199, 187; UV (methanol) 344 (ε 12000), 278 nm (17000); R_f 0.36 (60:40 ethyl acetate–hexanes, silica gel).

Method B. Example. 2-Acetyl-1,2,8,8a-tetrahydro-7-methylcyclopropa[*c*]pyrrolo[3,2-*e*]indol-4(5*H*)-one (8). Methanesulfonylation of 52 mg (0.15 mmol) of **41** (R = CH_3) as described above afforded 63 mg of crude **42** (R = CH_3) as a purple-tinted solid. This was dissolved in 10 mL of DMF and slurried with 2–3 cm^3 of activated Raney nickel in ethanol for 20 min. To the filtered solution was added 36 mg of palladium on carbon, and the mixture was hydrogenolized for 50 min. The mixture was filtered, the catalyst was washed with DMF, and the filtrate was concentrated to a small volume in DMF. Ethyldiisopropylamine (0.070 mL, 0.4 mmol) was added (in the expectation of forming the cyclopropyl spiro dienone), and after 10 min, the mixture was rapidly chromatographed on silica gel with 1:1 acetone–cyclohexane as eluant. Upon standing at 4 °C overnight, the product-containing fractions deposited white, granular crystals (10 mg, 20% yield) identified as the uncyclized methanesulfonate **43** (R = CH_3): NMR (DMSO-d_6) δ 2.15 (s, 3 H), 2.32 (s, 3 H), 3.12 (s, 3 H), 3.8–4.4 (m, 5 H), 7.0 (s, 1 H), 7.6 (s, 1 H); MS (no M^+), *m/e* 242 ($M - HSO_3CH_3$), 228, 213, 200, 186; R_f 0.30 (60% acetone in cyclohexane, silica gel). The mother liquor from the fractions that had yielded the methanesulfonate were rechromatographed in the same manner to afford 10 mg (30% from **41**, R = CH_3) of **8** as a powdery white solid: IR ($CHCl_3$) 3470, 3010, 1675, 1620, 1385, 1375, 1265 cm^{-1}; NMR (DMSO-d_6, 200 MHz, 70 °C) δ 1.23 (dd, 1 H, $J_{e,g}$ = 4 Hz, $J_{f,g}$ = 4 Hz), 1.89 (dd, 1 H, $J_{e,f}$ = 8 Hz, $J_{f,g}$ = 4 Hz), 1.96 (s, 3 H), 2.17 (s, 3 H), 3.03 (m, 1 H), 4.02 (dd, 1 H, $J_{c,d}$ = 10 Hz, $J_{d,e}$ = 4 Hz), 4.08 (dd, 1 H, J_{cd} = 10 Hz, $J_{c,e}$ = 1 Hz), 6.67 (s, 1 H), 6.8 (s, 1 H); MS calcd for $C_{14}H_{14}N_2O_2$ 242.1055, found 242.1060, *m/e* 200, 199, 185, 171, 156; UV (methanol) 348 (ε 14000), 284 nm (18000); R_f 0.26 (60% acetone in cyclohexane, silica gel).

Method C. Example 1. 2-(2-Quinolylcarbonyl)-1,2,8,8a-tetrahydro-7-methylcyclopropa[*c*]pyrrolo[3,2-*e*]indol-4-(5*H*)-one (15). Trimethylsilyl chloride (0.288 mL, 2.27 mmol) was added to a mixture of **42** (R = 2-quinolyl) (286 mg, 0.52 mmol) and dry NaI (353 mg, 2.40 mmol) being stirred under nitrogen in 8 mL of dry acetonitrile. The reaction mixture was refluxed for 30 min, cooled, and diluted with ethyl acetate and 2% sodium thiosulfate. The organic phase was dried (Na_2SO_4) and reacted with 0.90 mL of triethylamine for 30 min. It was then concentrated and chromatographed on silica gel, eluting with 1:1 acetone–hexane containing 0.5% of triethylamine, to afford **15** as a tan solid (81 mg, 44% yield): NMR (DMSO-d_6, 300 MHz) δ 1.48 (m, 1 H), 1.99 (s + m, 4 H), 3.13 (m, 1 H), 4.15–4.45 (m, 2 H), 6.89 (s, 1 H), 7.8–8.8 (m, 6 H); MS calcd for $C_{22}H_{17}N_3O_2$ 355.1321, found 355.1298, *m/e* 327, 326, 228, 213, 199, 128; UV (methanol) 357 (ε 11700), 290 nm (13300); R_f 0.42 (1:1 acetone–hexane + 0.5% triethylamine, silica gel).

Method C. Example 2. 2-[[5-[(1*H*-Indol-2-ylcarbonyl)-amino]-1*H*-indol-2-yl]carbonyl]-1,2,8,8a-tetrahydro-7-methylcyclopropa[*c*]pyrrolo[3,2-*e*]indol-4(5*H*)-one (27). To 220 mg (0.32 mmol) of **42** (R = 5-[(2-indolylcarbonyl)amino]-2-indolyl) and 190 mg (1.3 mmol) of dry NaI under nitrogen were added 6 mL of dry acetonitrile and 2 mL of benzonitrile (passed through neutral alumina). Trimethylsilyl chloride (0.16 mL, 1.3 mmol) was then introduced, and the mixture was heated to 65 °C for 45 min. The reaction was still incomplete by TLC, so 100

mg (0.67 mmol) of NaI and 0.080 mL (0.63 mmol) of TMS-Cl were added, and the mixture was refluxed for 15 min. It was then cooled and diluted with ethyl acetate. The organic phase was washed with 0.1 M sodium thiosulfate and saturated NaCl, dried (Na_2SO_4), and concentrated to an oil in benzonitrile. Addition of 0.10 mL (0.71 mmol) of triethylamine afforded a semisolid, which was diluted with ethyl acetate, washed quickly with water (to remove triethylammonium salts), dried (Na_2SO_4), and concentrated. A few drops of DMF were added to dissolve the resulting suspension, and the mixture was chromatographed on silica gel, eluting with a gradient of acetone in cyclohexane. The purest fractions were combined and concentrated to 50 mg of an off-white solid. This was dissolved in 3 mL of acetone, and 0.020 mL of triethylamine was added. A light yellow solid precipitated and was collected and washed with a small amount of acetone, affording 23.5 mg. The mother liquor and less pure chromatography fractions were combined, concentrated, and again dissolved in a small amount of acetone. Addition of 0.020 mL of triethylamine precipitated a second crop, which was also collected and washed with acetone to give 24 mg (total yield of **27** was 30% from **42**, or 20% in four discrete steps from **39**): IR (KBr) 1640, 1595, 1580, 1540, 1515, 1475, 1416, 1390, 1380, 1260, 1235 cm^{-1}; NMR (DMSO-d_6) δ 1.42 (m, 1 H), 1.96 (m, 1 H), 2.03 (s, 3 H), 3.14 (m, 1 H), 4.5 (m, 2 H), 6.78 (s, 1 H), 6.97–7.8 (m, 10 H), 8.3 (s, 1 H), 10.3 (s, 1 H), 11.8 (br s, 1 H), 11.9 (br s, 1 H); MS (FAB), m/e 504 (M + H + H_2), 302, 202, 201, 187, 172, 144; MS (FAB) calcd for $C_{30}H_{24}N_5O_3$ 502.1879, found 502.1903; UV (methanol) 363 (ϵ 32 000), 314 nm (47 000); R_f 0.22 (1:1 acetone–cyclohexane, silica gel). Anal. ($C_{30}H_{23}N_5O_3$) C, H, N.

Method D. Example. 2-[(1-Methylindol-2-yl)carbonyl]-1,2,8,8a-tetrahydro-7-methylcyclopropa[*c*]pyrrolo[3,2-*e*]indol-4(5*H*)-one (19). Boron tribromide (1.3 mL of a 1 M solution in methylene chloride) was added to a solution of **42** (R = 1-methyl-2-indolyl) (0.21 g, 0.4 mmol) in 45 mL of methylene chloride at −78 °C, under nitrogen. After 30 min, the reaction was quenched with ice and the cooling bath was removed. After 10 min, the reaction mixture was diluted with water and extracted with methylene chloride. The organic phase was dried (Na_2SO_4) and concentrated, and the residue was chromatographed on silica gel, eluting with a gradient of acetone in cyclohexane, to afford **19** as an off-white solid (50 mg, 35% yield): NMR ($CDCl_3$) δ 1.47 (m, 1 H), 2.0 (s + m, 4 H), 2.16 (s, 3 H), 2.9 (m, 1 H), 4.22 (m, 2 H), 6.27 (s, 1 H), 6.8–6.9 (m, 2 H), 7.1–7.45 (m, 4 H), 7.6–7.7 (m, 1 H); MS (FAB) calcd for $C_{22}H_{20}N_3O_2$ 358.1555, found 358.1546; UV (methanol) 357 (ϵ 10 800), 310 nm (11 200); R_f 0.12 (25% acetone in cyclohexane, silica gel).

1,2,8,8a-Tetrahydro-7-methylcyclopropa[*c*]pyrrolo[3,2-*e*]indol-4(5*H*)-one (6). To a solution of **11** (0.05 mmol) in 2 mL of THF were added 1 mL of methanol and 2 mL of 1 N NaOH. After being stirred for 45 min at room temperature, under nitrogen, the solution was neutralized with solid CO_2 and extracted with methylene chloride. The organic phase was dried (Na_2SO_4), concentrated, and chromatographed on silica gel. Elution with 7% methanol in chloroform provided 8.9 mg (84% yield) of **6**: IR ($CHCl_3$) 3450, 3010, 1610, 1200 cm^{-1}; NMR (CD_3OD) δ 1.07 (t, 1 H, J = 4 Hz), 1.88 (dd, 1 H), 2.0 (s, 3 H), 3.1 (m, 1 H), 3.5–3.85 (m, 2 H), 5.4 (s, 1 H), 6.8 (s, 1 H); MS calcd for $C_{12}H_{12}N_2O$ 200.0950, found 200.0943, m/e 199, 185, 171, 157; UV (methanol) 360 (ϵ 14 500), 278 nm (24 400). Addition of aqueous HCl produces a yellow solution with an absorption band at 400 nm.

Summary of Preparation and Characterization of Other CPI Analogues. Compound 42 (COR = SO_2Ph): NMR (acetone-d_6) δ 2.3 (s, 3 H), 3.0 (s, 3 H), 3.2–3.3 (m, 1 H), 3.8–4.3 (m, 4 H), 5.37 (s, 2 H), 7.1 (m, 1 H), 7.3 (s, 1 H), 7.35–7.75 (m, 10 H), 10.3 (br, 1 H); R_f 0.5 (1:1 acetone–cyclohexane, silica gel).

Compound 5: method B, 40% yield from **42** (COR = SO_2Ph); MS, m/e 340 (M^+), 275, 248, 199 (base peak), 185, 171; MS (FAB) calcd for $C_{18}H_{17}N_2O_3S$ 341.0960, found 341.0976; UV (10% methanol in water) 344, 275 nm.

Compound 9: method A, 36% yield from **44** (R = $(CH_2)_4CH_3$); method C, 0.4-mmol scale, 59% yield from **42** (R = $(CH_2)_4CH_3$); 4-mmol scale, 46% yield; mp 201–209 °C dec; IR (CH_2Cl_2) 1680, 1602, 1380 cm^{-1}; NMR (Table II); MS (FAB) calcd for $C_{18}H_{23}N_2O_2$ 299.1759, found 299.1745; UV (methanol) 352 (ϵ 14 600), 286 nm (17 900); R_f 0.36 (40% acetone in hexane, 0.2% triethylamine, silica gel).

Compound 10: method A, 70% yield from **44** (R = $(CH_2)_8CH_3$); method C, 72% yield from **42** (R = $(CH_2)_8CH_3$); NMR (Table II); MS calcd for $C_{22}H_{31}N_2O_2$ (M + H) 355.2385, found 355.2364, m/e 341, 201, 199, 187; UV (ethanol) 347 (ϵ 11 800), 285 nm (15 000).

Compound 11: method B, 15% yield from **41** (R = Ph); NMR (Table II); MS (FAB) calcd for $C_{19}H_{17}N_2O_2$ (M + H) 305.1290, found 305.1297; UV (methanol) 352 (ϵ 14 600), 288 nm (14 900); R_f 0.40 (1:1 acetone–cyclohexane, silica gel).

Compound 12: method C, 29% yield from **42** (R = 4-(NHCOPh)C_6H_4); NMR (Table II); MS (FAB) calcd for $C_{26}H_{22}N_3O_3$ (M + H) 424.1661, found 424.1680, m/e 425 (M + H_2), 426 (M + H + H_2), 224; UV (methanol) 355 (ϵ 14 100), 283 nm (23 200); R_f 0.34 (1:1 acetone–cyclohexane, silica gel).

Compound 13: method C, 32% from **42** (R = 2-pyridyl); 300-MHz NMR (Table II); MS calcd for $C_{18}H_{15}N_3O_2$ 305.1164, found 305.1164; UV (methanol) 350 (ϵ 10 000) 282 nm (10 500); R_f 0.23 (1:1 acetone–hexane, 0.2% triethylamine, silica gel).

Compound 14: method C, 43% from **42** (R = 2-naphthyl); NMR (Table II); MS (FAB) calcd for $C_{23}H_{19}N_2O_2$ 355.1446, found 355.1434; UV (ethanol) 354 (ϵ 11 100), 288 nm (13 800); R_f 0.25 (40% acetone in hexane, 0.2% triethylamine, silica gel).

Compound 16: method C, 23% from **42** (R = 6-[(pentylcarbonyl)amino]-2-quinolyl); NMR (Table II); MS (FAB), m/e 469 (M + H), 269, 241, 199, 143; MS (FAB) calcd for $C_{28}H_{29}N_4O_3$ 469.2240, found 469.2261; UV (ethanol) 348 (ϵ 14 900), 292 nm (14 000); R_f 0.41 (1:1 acetone–hexane, 0.2% triethylamine, silica gel).

Compound 17: method C, 20% yield in two steps from **41** (R = 2-pyrrolyl); IR (Nujol) 1634, 1598, 1575, 1461, 1457, 1420, 1379, 1324 cm^{-1}; 300-MHz NMR (Table II); MS calcd for $C_{17}H_{15}N_3O_2$ 293.1164, found 293.1175; UV (ethanol) 359 (ϵ 12 600), 303 nm (13 400); R_f 0.27 (1:1 acetone–hexane, 0.2% triethylamine, silica gel).

Compound 18: method B, 46% yield from **42** (R = 2-indolyl) after chromatography; method C, 17% yield after chromatography and acetone precipitation; NMR (Table II); MS (FAB) calcd for $C_{21}H_{18}N_3O_2$ 344.1399, found 344.1399; UV (methanol) 362 (ϵ 22 000), 312 nm (22 000); R_f 0.2 (1:1 acetone–cyclohexane, silica gel).

Compound 20: method C, 38% from **42** (R = 2-benzofuranyl); NMR (Table II); MS (FAB) calcd for $C_{21}H_{17}N_2O_3$ 345.1239, found 345.1215; MS, m/e 344 (M^+), 316, 199, 145; UV (ethanol) 361 (ϵ 15 700), 304 nm (19 300); R_f 0.45 (1:1 acetone–hexane, 0.2% triethylamine, silica gel).

Compound 21: method C, 30% yield from **42** (R = 2-benzothiophene-yl); NMR (Table II); MS (FAB) calcd for $C_{21}H_{17}N_2O_2S$ 361.1011, found 361.1004; UV (methanol) 357 (ϵ 16 000), 298 nm (18 600); R_f 0.50 (1:1 acetone–hexane, 0.2% triethylamine, silica gel).

Compound 22: method C, 34% from **42** (R = 3-methyl-2-indenyl); NMR (Table II); MS (FAB) calcd for $C_{22}H_{20}N_3O_2$ 358.1555, found 358.1546; UV (ethanol) 357 (ϵ 12 000), 286 nm (13 900); R_f 0.30 (40% acetone in hexane, silica gel).

Compound 23: method B, 11% from **39** (four discrete steps); NMR (Table II); MS calcd for $C_{22}H_{19}N_3O_3$ 373.1426, found 373.1404; UV (methanol) 363 (ϵ 19 000), 311 nm (17 000); R_f 0.22 (1:1 acetone–cyclohexane, silica gel).

Compound 24: method B, 10% yield from **41** (R = 6-hydroxy-7-methoxy-2-indolyl); NMR (Table II); MS, m/e 389 (M^+), 372, 281, 207, 201, 190, 147, 134; MS (FAB) calcd for $C_{22}H_{20}N_3O_4$ 390.1454, found 390.1456; UV (methanol) 371 (ϵ 23 000), 322 (15 000), 293 nm (13 000); R_f 0.34 (70% acetone in cyclohexane, silica gel).

Compound 25: method C, 20% yield from **42** (R = 5-[(aminocarbonyl)amino]-2-indolyl); NMR (Table II); MS (FAB) calcd for $C_{22}H_{20}N_5O_3$ (M + H) 402.1566, found 402.1540, m/e 200, 199, 149; UV (methanol) 362 (ϵ 23 000), 310 nm (21 000); Rf 0.13 (10% methanol in chloroform, silica gel).

Compound 26: method C, 9% yield from **42** (R = 5-[(phenylcarbonyl)amino]-2-indolyl); NMR (Table II); MS (FAB) calcd for $C_{28}H_{23}N_4O_3$ 463.1770, found 463.1765; UV (methanol) 364 (ϵ 29 000), 308 nm (32 000); Rf 0.15 (1:1 acetone–cyclohexane, silica gel).

Compound 28: method C, 16% yield from 41 (R = 5-[(2-benzofuranylcarbonyl)amino]-2-indolyl); NMR (Table II); MS (FAB) calcd for $C_{30}H_{23}N_4O_4$ (M + H) 503.1719, found 503.1742, *m/e* 303, 201, 199, 187, 145; UV (ethanol) 365 (ε 31 000), 312 nm (37 400); R_f 0.22 (40% acetone in hexane, silica gel).

Compound 29: method C, 10% yield from 42 (R = 5-[[[5-[(aminocarbonyl)amino]-2-indolyl]carbonyl]amino]-2-indolyl); NMR (Table II); MS (FAB), *m/e* 560 (M + H), 274, 232, 216, 199, 197; UV (methanol) 358 (ε 29 000), 312 nm (40 000).

Compound 30: method C, 12% yield from 42 (R = 5-[[[5-[(phenylcarbonyl)amino]-2-indolyl]carbonyl]amino]-2-indolyl); NMR (Table II); MS (FAB) 621 (M + H), 421, 199, 186, 105; calcd for $C_{37}H_{29}N_6O_4$ 621.2250, found 621.2233; UV (methanol) 360 (ε 27 500), 315 nm (39 000); R_f 0.22 (60% acetone in cyclohexane, silica gel).

Acid-Catalyzed Solvolytic Decomposition. Stock solutions (20–100 μL) of 1–4 mg/mL of the CPI analogues in DMA, DMF, or DMSO were injected into 5.0 mL of a 1:1 mixture of methanol and aqueous buffer, pH 3.0. The buffer contained 4:1:20 (volumes) of 0.1 M citric acid, 0.2 M Na_2HPO_4, and water, respectively. The UV spectrum of each solution was recorded shortly after mixing, and the solutions were stoppered, protected from light, and magnetically stirred at room temperature. UV analyses were repeated three to four times during the first 2 days. By 2 weeks, no further change was detectable in the spectra, and the residual absorbance at the long-wavelength band was subtracted from the measured absorbances. Plots of ln (A/A_0) vs time were linear ($r > 0.99$) for at least 2 half-lives and were independent of the concentration in this range (20–120 μM). Pseudo-first-order rate constants (Table I) were obtained from the slopes of these lines. The spent reaction mixture from the acidic solvolysis of **26** was extracted with ethyl acetate and analyzed by TLC on LCK18-D linear K reversed-phase plates (200 μm, Whatman), eluting with 70% methanol in water. Detection was by densitometry (Shimadzu dual wavelength TLC scanner CS-930), with scanning at 300 nm. The major products were identified as the alcohol (44, R = 5-[(aminocarbonyl)amino]-2-indolyl) and methyl ether arising from addition of water and methanol, respectively, to **26**, by comparison to R_f values of authentic samples.

Lipophilic/Hydrophilic Partitioning. Stock solutions of the CPI analogues in DMF or DMA (but not DMSO) were diluted with acetone and spotted on channeled LCK18-D linear K reversed-phase plates (200 μm, Whatman). The plates were developed in 2:1 DMF–saline (0.9% NaCl, McGaw) and analyzed at 350 nm on a Shimadzu TLC scanner (CS-930). The R_f values reported in Table I are averages of two to four determinations with ranges of 0–7%.

Induced Circular Dichroism. The stock solution (1–4 mg/mL) of the CPI analogue in DMA, DMF, or DMSO was injected into a solution of calf-thymus DNA (11×10^{-5} M) in 0.01 M phosphate buffer, pH 7.2, to give a drug concentration of 0.85×10^{-5} M and a DNA base-to-drug ratio of 13. The mixture was allowed to stand for 24 h at room temperature, and the induced CD spectrum was recorded on a JASCO Model 500-C CD spectropolarimeter calibrated with D-10-camphorsulfonic acid.[33] The ICD value in Table I is the molar ellipticity measured at the long-wavelength maximum (ca. 350 nm).

L1210 Cell Growth in Culture. The basal medium used for growing mouse leukemia L1210 cells was RPMI medium 1634. Fetal calf serum (5%), sodium bicarbonate (0.075%), and a mixture of penicillin (0.1 mg/mL of medium) and streptomycin (50 μg/mL of medium) were added as supplements. Aliquots of drug (0.25 mL) were pipetted into each culture tube. The experiment was then initiated by the addition of 4.75 mL of cells (approximately 5×10^3 cells/mL) as described elsewhere,[34] and the tubes were incubated at 37 °C for 3 days. Cell number was then determined with a Coulter counter (Coulter Electronics, Hialeah, FL).

Antitumor Activity. P388 leukemia was maintained by continuous passage in syngeneic DBA/2 female mice. This tumor was inoculated (10^6 cells/mouse) intraperitoneally in male BDF1 (C57 BL/6 female × DBA/2 male) mice on day 0. The drug was given intraperitoneally on days 1, 5, and 9. Drugs were prepared in a vehicle of 2% DMA, 10% Emulphor 620-P (GAF), and water (McGaw). The percent of increase in life span (ILS) was calculated by using the median day of death of six mice per group according to the following equation:

$$\% \text{ ILS} = \left(\frac{\text{median death (days) of treated group}}{\text{median death (days) of untreated control group}} - 1 \right) \times 100$$

Mice surviving for 30 days were considered cured. The criteria for significant therapeutic response, according to the NCI, is >30% ILS.

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Registry No. 1, 112243-81-9; 2, 89553-54-8; 5, 112089-37-9; 6, 104636-95-5; 7, 112089-38-0; 8, 104636-96-6; 9, 112243-82-0; 10, 112089-39-1; 11, 105622-86-4; 12, 112089-40-4; 13, 112089-41-5; 14, 112089-42-6; 15, 104651-05-0; 16, 112089-43-7; 17, 104636-98-8; 18, 104636-99-9; 19, 112089-44-8; 20, 112089-45-9; 21, 105622-87-5; 22, 112089-46-0; 23, 105622-88-6; 24, 112089-47-1; 25, 112089-48-2; 26, 104637-00-5; 27, 104713-40-8; 28, 112245-32-6; 29, 112089-49-3; 30, 112089-50-6; 31, 610-78-6; 32, 79035-13-5; 33, 112089-51-7; 34a, 106014-83-9; 34b, 106014-84-0; 35a, 108833-09-6; 35b, 112089-54-0; 36a, 112089-52-8; 36b, 108833-10-9; 37a, 4455-13-4; 37c, 112089-55-1; 38a, 112089-53-9; *cis*-38b, 112089-56-2; *trans*-38b, 112089-58-4; *cis*-38c, 112089-57-3; *trans*-38c, 112112-31-9; 39, 101134-89-8; 40, 112243-83-1; 41 (R = OBu-*t*), 112089-71-1; 41 (R = Me), 112089-72-2; 41 (R = Me, acetate), 112089-75-5; 41 (R = 2-quinolyl), 112089-73-3; 41 (R = 5-[(2-indolylcarbonyl)amino]-2-indolyl), 112243-84-2; 41 (R = Ph), 112089-84-6; 41 (R = 2-pyrrolyl), 112089-89-1; 41 (R = 6-hydroxy-7-methoxy-2-indolyl), 112089-94-8; 41 (R = 5-[(2-benzofuranylcarbonyl)amino]-2-indolyl), 112089-97-1; 42 (R = Me), 112089-74-4; 42 (R = quinolyl), 112089-76-6; 42 (R = 5-[(2-indolylcarbonyl)amino]-2-indolyl, 112243-85-3; 42 (R = 1-methyl-2-indolyl), 112089-79-9; 42 (COR = SO_2Ph), 112089-80-2; 42 (R = $(CH_2)_8Me$), 112089-83-5; 42 (R = 4-$(NHCOPh)C_6H_4$), 112089-85-7; 42 (R = 2-pyridyl), 112089-86-8; 42 (R = 2-naphthyl), 112089-87-9; 42 (R = 6-[(pentylcarbonyl)amino]-2-quinolyl), 112089-88-0; 42 (R = 2-indolyl), 112089-90-4; 42 (R = 2-benzofuranyl), 112089-91-5; 42 (R = 2-benzothiophenyl), 112089-92-6; 42 (R = 3-methyl-2-indenyl), 112089-93-7; 42 (R = 5-[(aminocarbonyl)amino]-2-indolyl), 112089-95-9; 42 (R = 5-[(phenylcarbonyl)amino]-2-indolyl), 112089-96-0; 42 (R = 5-[[[5-[(aminocarbonyl)amino]-2-indolyl]-carbonyl]amino]-2-indolyl), 112089-98-2; 42 (R = 5-[[[5-[(phenylcarbonyl)amino]-2-indolyl]caronyl]amino]-2-indolyl), 112089-99-3; 43 (R = Me), 112089-78-8; 44 (R = OBu-*t*), 112089-77-7; 44 (R = $(CH_2)_4Me$), 112089-81-3; 44 (R = $(CH_2)_8Me$), 112089-82-4; 44 (R = 5-[(aminocarbonyl)amino]-2-indolyl), 112090-00-3; 44 (methyl ether), 112090-01-4; *p*-$H_2NC_6H_4CO_2H$, 150-13-0; $(CO_2Et)_2CH_2$, 105-53-3; $BrCH(Me)CO_2Et$, 535-11-5; $HS(CH_2)_2OH$, 60-24-2; *p*-$PhCONHC_6H_4CO_2H$, 582-80-9; 4-chloro-3-nitroanisole, 10298-80-3; 2-methyl-6-nitroquinoline, 613-30-9; methyl 6-nitro-2-quinolinecarboxylate, 112089-59-5; methyl 6-amino-2-quinolinecarboxylate, 112089-60-8; methyl 1,2,3,4-tetrahydro-6-amino-2-quinolinecarboxylate, 112089-61-9; methyl 6-(hexanoylamino)-2-quinolinecarboxylate, 112089-62-0; methyl 1,2,3,4-tetrahydro-6-(hexanoylamino)-2-quinolinecarboxylate, 112089-63-1; 6-(hexanoylamino)-2-quinolinecarboxylic acid, 112089-64-2; ethyl 5-nitroindole-2-carboxylate, 16732-57-3; ethyl 5-ureidoindole-2-caroxylate, 112089-65-3; 5-ureido-2-indolecarboxylic acid, 101134-94-5; ethyl 5-amino-2-indolecarboxylate, 71086-99-2; 5-(benzoylamino)-1*H*-indole-2-carboxylic acid, 112089-66-4; ethyl 5-(benzoylamino)-1*H*-indole-2-carboxylate, 112089-67-5; indole-2-carboxylic acid, 1477-50-5; ethyl 5-[(1*H*-indol-2-ylcarbonyl)amino]-1*H*-indole-2-carboxylate, 112089-68-6;

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5-[(1*H*-indol-2-ylcarbonyl)amino]-1*H*-indole-2-carboxylic acid, 101134-91-2; benzofuran-2-carboxylic acid, 496-41-3; ethyl 5-[(1*H*-benzofuran-2-ylcarbonyl)amino]-1*H*-indole-2-carboxylate, 112089-69-7; 5-[(1-*H*-benzofuran-2-ylcarbonyl)amino]-1*H*-indole-2-carboxylic acid, 110314-42-6; ethyl 5-[[(5-ureido-1*H*-indol-2-yl)carbonyl]amino]-1*H*-indole-2-carboxylate, 112112-49-9; 5-[[(5-ureido-1*H*-indol-2-yl)carbonyl]amino]-1*H*-indole-2-carboxylic acid, 101134-95-6; 5-[[[5-(benzoylamino)-1*H*-indol-2-yl]carbonyl]amino]-1*H*-indole-2-carboxylic acid, 101134-93-4; benzothiophene-2-carboxylic acid, 6314-28-9; 6-hydroxy-7-methoxy-2-indolecarboxylic acid, 112089-70-0; quinaldic acid, 93-10-7; 5-methoxy-2-indolecarboxylic acid, 4382-54-1.

Synthesis of Arginine-vasopressins, Modified in Positions 1 and 2, as Antagonists of the Vasopressor Response to the Parent Hormone

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In an attempt to determine some of the structural features in position 1 that account for antivasopressor activity, eight new 1-(β,β-dialkyl-substituted) analogues of 1-(3-mercaptopropanoic acid)-8-arginine-vasopressin and 1-(3-mercaptopropanoic acid)-2-*O*-methyltyrosine-8-arginine-vasopressin have been designed and synthesized. The protected precursors required for these peptides were obtained by a combination of solid-phase and solutions methods. Some of the reported analogues, namely 1-(1-mercapto-4-methylcyclohexaneacetic acid)-8-arginine-vasopressin, 1-(1-mercapto-4-methylcyclohexaneacetic acid)-2-*O*-methyltyrosine-8-arginine-vasopressin, 1-(4-*tert*-butyl-1-mercaptocyclohexaneacetic acid)-8-arginine-vasopressin, 1-(4-*tert*-butyl-1-mercaptocyclohexaneacetic acid)-2-*O*-methyltyrosine-8-arginine-vasopressin, 1-(1-mercapto-4-phenylcyclohexaneacetic acid)-8-arginine-vasopressin and 1-(1-mercapto-4-phenylcyclohexaneacetic acid)-2-*O*-methyltyrosine-8-arginine-vasopressin, are among the most potent and selective antagonists of the vasopressor response to arginine-vasopressin reported to date.

Vasopressin (AVP) interacts primarily with two distinct receptor types in the periphery designated as V_1 and V_2 and is believed to react also with receptors, so far largely uncharacterized, in the central nervous system.[1,2] V_1 receptors have been characterized in vascular smooth muscle and in liver cells. V_2 receptors are located in the renal tubule. The stimulation of V_1 vascular smooth muscle receptors leads to vasoconstriction and an increase in blood pressure. The stimulation of V_2 receptors in the kidney results in reabsorption of free water in the renal tubule (antidiuretic effect).

Attempts to develop clinically useful synthetic antagonists of the vasopressor response to arginine-vasopressin have led to the synthesis and pharmacological evaluation of hundreds of analogues of this hormone, which with different efficacy bind to and stimulate either the V_1 or both the V_1 and the V_2 type receptor. Some interesting results were obtained with modifications of positions 1 and 2 of the molecule, which are proposed to be key positions of antagonists of AVP.[3] This was recently discussed in more detail by Manning and Sawyer.[4]

It was shown that the incorporation in a cyclic structure of the β-carbon in position 1 in 1-(3-mercaptopropanoic acid)-8-arginine-vasopressin (dAVP) converted this highly active antidiuretic and vasopressor agonist into a very potent vasopressor antagonist 1-(1-mercaptocyclohexaneacetic acid)-8-arginine-vasopressin [d(CH_2)$_5$AVP].[3] The introduction of *O*-methyltyrosine in d(CH_2)$_5$AVP to give d(CH_2)$_5$Tyr(Me)AVP brought about almost a twofold enhancement in vasopressor antagonistic potency[3] which coupled with low antidiuretic activity endows this analogue with a high degree of specificity for V_1 receptors.[3] More recently a larger series of related compounds, also including analogues with additional substitutions in positions 4, 8 and both 4 and 8, were synthesized and examined, several of which turned out to be very efficient pressor antagonists.[5] With this in mind we decided to synthesize analogues of arginine-vasopressin which have larger and more lipophilic substituents on the β-carbon in position 1 of 1-(3-mercaptopropanoic acid)-8-arginine-vasopressin (dAVP) and 1-(3-mercaptopropanoic acid)-2-*O*-methyltyrosine-8-arginine-vasopressin [dTyr(Me)AVP]. Thus we synthesized these analogues in an attempt to obtain antagonists of the vasopressor response to arginine-vasopressin with increased potency and selectivity. Accordingly six peptides were designed: 1-(1-mercapto-4-methylcyclohexaneacetic acid)-8-arginine-vasopressin (MeCAAVP) (I), 1-(1-mercapto-4-methylcyclohexaneacetic acid)-2-*O*-methyltyrosine-8-arginine-vasopressin [MeCATyr(Me)AVP] (II), 1-(4-*tert*-butyl-1-mercaptocyclohexaneacetic acid)-8-arginine-vasopressin (BCAAVP) (III), 1-(4-*tert*-butyl-1-mercaptocyclohexaneacetic acid)-2-*O*-methyltyrosine-8-arginine-vasopressin [BCATyr(Me)AVP] (IV), 1-(1-mercapto-4-phenylcyclohexaneacetic acid)-8-arginine-vasopressin (PhCAAVP) (V), and 1-(1-mercapto-4-phenylcyclohexaneacetic acid)-2-*O*-methyltyrosine-8-arginine-vasopressin [PhCATyr(Me)AVP] (VI). It is known that the presence of a less hydrophobic group in position 1 of AVP should result in an analogue with reduced antagonistic potency.[4,5] Despite this, we made further two analogues, 1-(4-mercapto-1-methyl-4-piperidineacetic acid)-8-arginine-vasopressin (MePAAVP) (VII) and 1-(4-mercapto-1-methyl-4-piperidineacetic acid)-2-*O*-methyltyrosine-8-arginine-vasopressin [MePATyr(Me)AVP] (VIII), which were structurally very similar to MeCAAVP (I) and MeCATyr(Me)AVP (II), but con-

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