aqueous NaOH (0.35 mL), and finally water (0.35 mL). The mixture was stirred for 15 min and filtered. The aluminates were washed with $CHCl_3$. The combined organic layers were dried and evaporated. Preparative TLC on silica gel, eluting with $CHCl_3$–benzene (12:7) gave pure (+)-corynoline: 9 mg (62%); $[\alpha]_D$ +115° (c 0.2, $CHCl_3$); NMR (470 MHz) δ 6.91 (d, 1 H, J = 8.3 Hz), 6.79 (d, 1 H, J = 8.3 Hz), 6.65 (s, 1 H), 6.63 (s, 1 H), 5.98 (d, 1 H, J = 1.4 Hz), 5.95 (d, 1 H, J = 1.3 Hz), 5.94 (s, 2 H), 4.03 (d, 1 H, J = 15.3 Hz), 3.94 (m, 1 H), 3.44 (d, 1 H, J = 15.3 Hz), 3.28 (br s, 1 H), 3.14 (d, 1 H, J = 17.3 Hz), 3.06 (dd, 1 H, J = 4.4, 17.9 Hz), 2.19 (s, 3 H), 1.12 (s, 3 H); CIMS, m/e (relative intensity) 368 (MH^+, 100); EIMS, m/e (relative intensity) 367 (52), 349 (100), 334 (65), 318 (48), 202 (48), 190 (35), 176 (43), 162 (51).

NMR Experiment with (−)-, (+)-, and (±)-5 and the Chiral NMR Shift Reagent Tris[3-(heptafluorobutyryl)-*d*-camphorato]europium(III). A solution of the shift reagent (14.9 mg) in $CDCl_3$ (80 μL) was added to a solution of racemic **5** (8 mg) in $CDCl_3$ (0.4 mL). The 80-MHz ^1H NMR spectrum showed two singlets for the unsubstituted ferrocenyl rings at δ 4.76 and 4.64. Similar experiments with (*S*)-(+)- and (*R*)-(−)-**5** showed only one of these singlets. The lower field signal corresponds to the (*S*)-(+) enantiomer.

Acknowledgment. This investigation was supported by Grant No. GM30932, awarded by the National Institute of General Medical Sciences, DHHS. The high-resolution ^1H NMR spectra were obtained on the PUBMRL 470-MHz instrument, which is supported by the National Institutes of Health, Research Grant No. RR01077, from the Department of Research Resources. We are grateful to Dr. Yu-Pin Wang for conducting preliminary experiments. We are also indebted to Professor William H. Pirkle, University of Illinois, for preliminary experiments demonstrating the feasibility of separating the enantiomers of racemic intermediates on his chiral HPLC column.

Registry No. 1, 102-54-5; 2, 78-84-2; (±)-3, 105017-95-6; (±)-4, 53992-88-4; (*R*)-(−)-5, 54053-41-7; (*S*)-(+)-5, 54053-42-8; (±)-5, 53992-88-4; 7, 105502-60-1; (±)-8, 82929-74-6; 9, 105561-83-9; 10, 105502-61-2; 11, 105519-40-2; 12, 105615-71-2; 13, 105615-72-3; 14, 105615-73-4; 15, 105537-44-8; (±)-16, 18797-79-0; (−)-16, 74163-86-3; piperonal, 120-57-0.

Studies on the Total Synthesis of CC-1065: Preparation of a Synthetic, Simplified 3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole Dimer/Trimer/Tetramer (CDPI Dimer/Trimer/Tetramer) and Development of Methodology for PDE-I Dimer Methyl Ester Formation

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Two synthetic preparations of 3-carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylic acid (**2b**, CDPI) and the investigation and development of methodology for the preparation of 3-carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole dimer **3** (CDPI dimer) constituting the simplified and stable PDE-I dimer skeleton possessing the B-DNA minor groove complementary shape of the natural product CC-1065 are detailed. The extension of this methodology to the preparation of 3-carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole trimer **4** and tetramer **5** (CDPI trimer and tetramer) are described and constitute synthetic, potentially selective, high-affinity, noncovalent B-DNA minor groove binding agents.

CC-1065 (**1**), an antitumor antibiotic isolated from *Streptomyces zelensis*[2] and unambiguously identified by single-crystal X-ray structural analysis,[3] has been shown to possess exceptional, potent in vitro cytotoxic activity,[4] antimicrobial activity,[2] and confirmed, potent in vivo antitumor activity.[2] Recent studies have shown that CC-1065 binds to double-stranded B-DNA in an initial high-affinity, five base-pair sequence-specific (A/GNTTA or AAAAA), nonintercalative fashion along the minor groove[5] and subsequently forms an irreversible covalent adduct.[6] The covalent alkylation of DNA has been shown to proceed by N-3 adenine alkylation on the spiro[cyclopropane-1,1′-cyclohexa-2′,5′-dien]-4′-one (spiro[5.2]octa-2,5-dien-4-one) unit present in the left-hand segment of CC-1065.[6] Consequently, the mechanism of CC-1065 cytotoxicity has been proposed to be derived from overstabilization of the DNA helix and inhibition of the normal unwinding and melting processes necessary for DNA synthesis.[5] The binding specificity and cytotoxicity associated with this agent may

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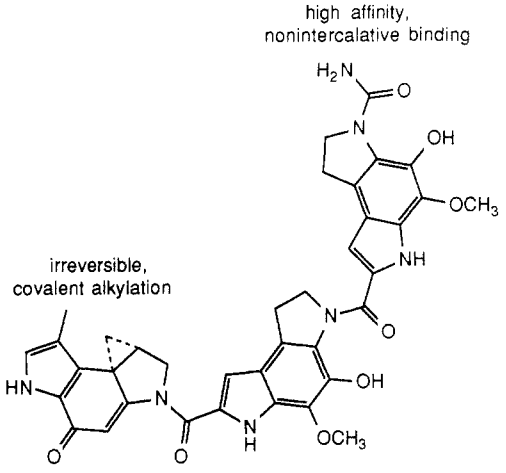
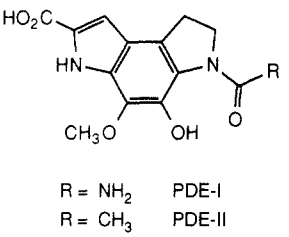
0022-3263/87/1952-1521$01.50/0

be attributed to two complementary structural features: the repeating 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole units constituting the central and right-hand segments of CC-1065 (PDE-I dimer) appear to be responsible for the high-affinity, sequence-specific B-DNA minor groove binding[5] and the spiro[cyclopropane-1,1′-cyclohexa-2′,5′-dien]-4′-one unit present in the left-hand segment functions as a selective, reactive alkylating agent effectively delivered to double-stranded DNA.[6] The irreversible, covalent alkylation of the spiro[cyclopropane-1,1′-cyclohexa-2′,5′-dien]-4′-one unit was postulated[6] to be selective for the (*R*)-**3b**, (*S*)-**4a** vs. (*S*)-**3b**, (*R*)-**4a** enantiomer and has received apparent experimental verification with the observation of the selective antitumor potency *and* DNA alkylation of the (*R*)-**3b**, (*S*)-**4a** vs. (*S*)-**3b**, (*R*)-**4a** pair of CC-1065 analogues U-71184/U-71185.[7] In addition, CC-1065 displays a characteristic, delayed hepatotoxicity which is fatal in mice,[8a] thus preventing the clinical use of the agent. This latter observation has stimulated the search for potential methods of effectively separating the cytotoxic and hepatotoxic properties associated with the administration of CC-1065.[7,9]

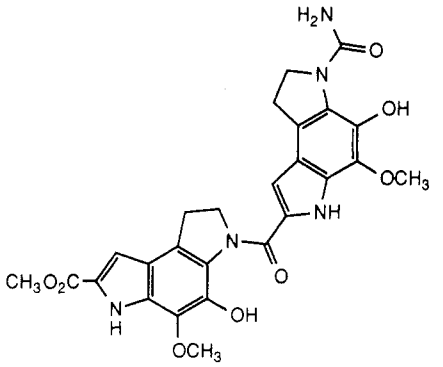


1 CC-1065

PDE-I and PDE-II, two 3′,5′-cAMP phosphodiesterase inhibitors isolated from *Streptomyces* strain MD769-C6[10] whose structures were determined by single-crystal X-ray analysis[11] and concurrently confirmed by total synthesis,[12] possess the identical 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole structure constituting the central and right-hand segments of CC-1065.[13]



R = NH_2 PDE-I
R = CH_3 PDE-II



PDE-I dimer methyl ester

Introduction. Examination of the structural features of CC-1065 which appear to be responsible for the high-affinity, sequence-specific nonintercalative binding in the minor groove of double-stranded B-DNA suggest that the selectively protected C-4/C-5 *o*-catechol functionality present in the central and right-hand segments of CC-1065 may not contribute to the affinity *or* specificity of this binding. Consistent with these expectations, recent experimental and modeling studies have indicated that the C-4/C-5 *o*-catechol units of CC-1065 may lie on the outer, unbound peripheral face of the DNA–CC-1065 complex and that the observed, high-affinity binding of the natural product may be attributed to the B-DNA minor groove complementary shape of CC-1065.[6,14] Thus, structural analogues of CC-1065 which possess the rigid, helical skeleton introduced by two repeating 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole units would be expected to mimic the topological pitch of B-DNA and possess the sequence-

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(9) (a) Warpehoski, M. A.; Bradford, V. S. *Tetrahedron Lett.* **1986**, *27*, 2735. (b) Jones, R. J.; Cava, M. P. *J. Chem. Soc., Chem. Commun.* **1986**, 826. (c) **Noted added in proof**: Rawal, V. H.; Jones, R. J.; Cava, M. P. *J. Org. Chem.* **1987**, *52*, 19.

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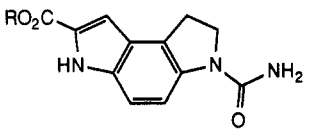
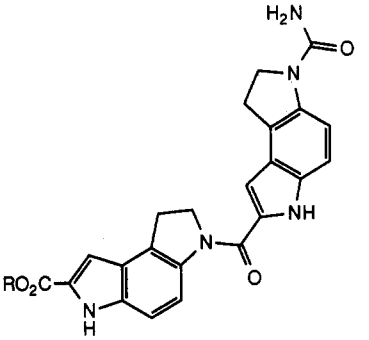
(14) Reynolds, V. L.; Molineux, I. J.; Kaplan, D. J.; Swenson, D. H.; Hurley, L. H. *Biochemistry* **1985**, *24*, 6228.

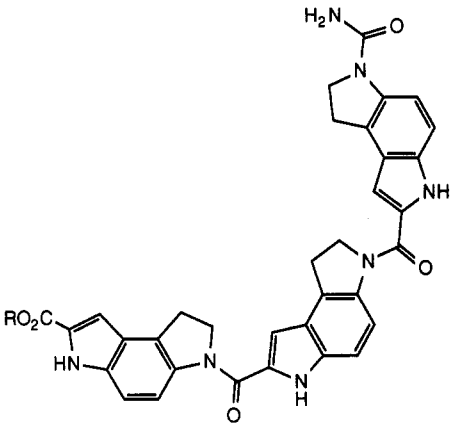
Chart I



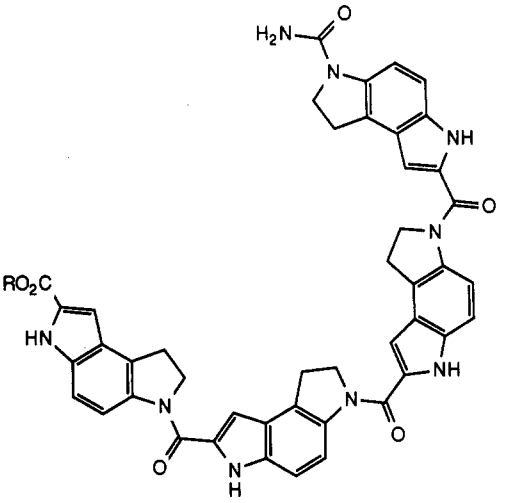
2a R = CH_3, CDPI methyl ester
2b R = H, CDPI



3a R = CH_3, CDPI dimer methyl ester
3b R = H, CDPI dimer

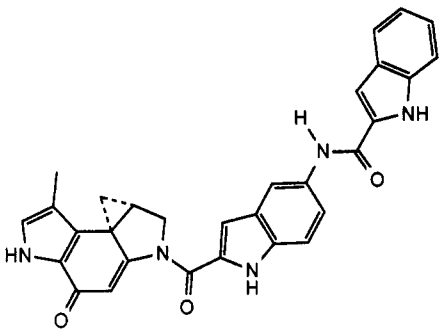


4a R = CH_3, CDPI trimer methyl ester
4b R = H, CDPI trimer



5a R = CH_3, CDPI tetramer methyl ester
5b R = H, CDPI tetramer

specific, high-affinity binding to double-stranded B-DNA as well as the potent cytotoxic, antimicrobial, and antitumor properties of the naturally occurring material[2,4] potentially without displaying the delayed hepatotoxicity.[8] Consistent with these expectations, recent efforts disclosed in the work of Kelly, Warpehoski, and Wierenga[7a,c,9a] have led to the preparation and evaluation of simplified agents, e.g., U-71184, possessing such properties.



U,71-184

Herein, we detail our efforts on the improved preparation of the parent methyl 3-carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (**2a**, CDPI methyl ester; Chart I) lacking the PDE-I/-II C-4/C-5 selectively protected *o*-catechol and the investigation and development of effective methodology for the preparation of CDPI dimer methyl ester (**3a**) constituting the simplified and stable[8b] PDE-I dimer skeleton potentially possessing the B-DNA minor groove complementary shape of the natural product CC-1065. The subsequent application of the coupling methodology, successfully extended to the preparation of PDE-I dimer methyl ester,[15] to the preparation of CDPI trimer and tetramer (**4** and **5**) is described.

Methyl 3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (2a, CDPI Methyl Ester). The first of the two approaches to the preparation of CDPI constituting the parent unsubstituted 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate skeleton is summarized in Scheme I.[16] The approach, first investigated in conjunction with the development of methodology suitable for implementation in the total synthesis of CC-1065, is based on the preparation of a substituted alkyne 1,2-diazine and its subsequent participation in an intramolecular heterocyclic azadiene Diels–Alder reaction[17,18] to provide a 4-

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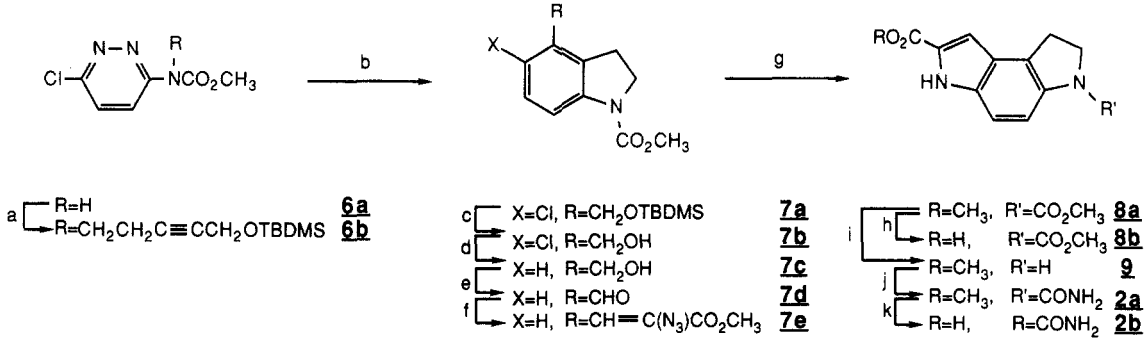
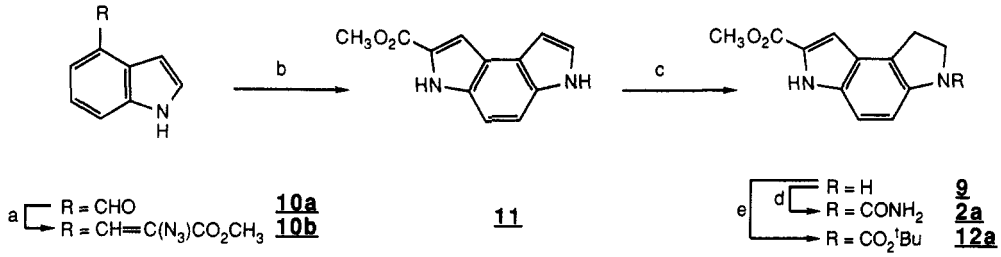
(16) For the first reportal preparation of *N*-acyl 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylates, see: Boger, D. L.; Coleman, R. S. *J. Org. Chem.* **1984**, *49*, 2240.
(17) (a) Reviews: Boger, D. L. *Tetrahedron* **1983**, *39*, 2869. Boger, D. L. *Chem. Rev.* **1986**, *86*, 781. (b) For examples of 1,2-diazines participating as the diene component in intramolecular alkene 1,2-diazine Diels–Alder reactions, see: Jojima, T.; Takeshiba, H.; Konotsune, T. *Chem. Pharm. Bull.* **1972**, *20*, 2191. Jojima, T.; Takeshiba, H.; Kinoto, T. *Chem. Pharm. Bull.* **1976**, *24*, 1581, 1588; **1980**, *28*, 198.
(18) Hemetsberger, H.; Knittle, D. *Monatsh. Chem.* **1972**, *103*, 194. MacKenzie, A. R.; Moody, C. J.; Rees, C. W. *J. Chem. Soc., Chem. Commun.* **1983**, 1372. See also: Isomura, K.; Kobayashi, S.; Taniguchi, H. *Tetrahedron Lett.* **1968**, 3499.

Scheme I[a]



[a] (a) 5-[(*tert*-Butyldimethylsilyl)oxy]-3-pentyn-1-ol, Ph_3P, DEAD, THF, 25 °C, 8 h, 45%; (b) 1,3,5-triisopropylbenzene, 230 °C, 18 h, 85%; (c) AcOH/THF/H_2O (3:1:1), 25 °C, 18 h, 90%; (d) 1 atm H_2, 10% Pd/C catalyst, MeOH, NaOH, 25 °C, 97%; (e) 10 wt equiv of MnO_2, CH_2Cl_2, 25 °C, 24 h, 91%; (f) 10 equiv of $N_3CH_2CO_2CH_3$, 8 equiv of NaOMe, MeOH, 0 °C, 1 h; (g) xylene, 140 °C, N_2, 4 h, 65% overall from **7d**; (h) 10 equiv of LiOH, THF/MeOH/H_2O (3:2:1), 25 °C; (i) anhydrous HBr, AcOH, 22 °C, 18 h, 50%; (j) 5 equiv of $Me_3SiN{=}C{=}O$, catalytic DMAP, CH_2Cl_2, 23 °C, 18 h, 90%; (k) 5 equiv of LiOH, THF/MeOH/H_2O (3:2:1), 50–55 °C, 2.5 h, 100%.

Scheme II[a]



[a] (a) 10 equiv of $N_3CH_2CO_2CH_3$, 8 equiv of NaOMe, MeOH, 0 °C, 3 h; (b) xylene, 140 °C, N_2, 4 h, 71% overall from **10a**; (c) $NaCNBH_3$, AcOH, 15–17 °C, 2 h, 66% overall from **10a**; (d) 5 equiv of $Me_3SiN{=}C{=}O$, catalytic DMAP, CH_2Cl_2, 23 °C, 18 h, 90%; (e) 1.06 equiv of $(t\text{-BuOCO})_2O$, THF, 23 °C, 13.5 h, 82%.

substituted (4-functionalized) indoline. Subsequent introduction of the indole-2-carboxylate unit of CDPI relied on the styryl azide thermolysis methodology introduced and developed by Hemetsberger and Rees.[18]

Alkylation of *N*-(methoxycarbonyl)-6-amino-3-chloro-1,2-diazine (**6a**)[16] with 5-[(*tert*-butyldimethylsilyl)oxy]-3-pentyn-1-ol[19] employing the conditions introduced by Mitsunobu (Ph_3P, diethyl azodicarboxylate, THF)[20] afforded the alkyne 1,2-diazine **6b** (45% yield). The presence of the 3-chloro substituent previously was found to favorably increase the observed ratio of desired 6-amino N-alkylation product to undesired 1,2-diazine N1-alkylation product.[16,21] Thermolysis of **6b** in 1,3,5-triisopropylbenzene (230 °C, 18 h) afforded indoline **7a** (85%). Deprotection of the *tert*-butyldimethylsilyl ether of **7a** (3:1:1 AcOH/THF/H_2O, 90%) afforded 1-(methoxycarbonyl)-5-chloro-4-(hydroxymethyl)indoline (**7b**), which was subjected to the conditions of catalytic hydrogenolysis (H_2, 10% Pd on carbon catalyst, MeOH, NaOH) to afford 1-(methoxycarbonyl)-4-(hydroxymethyl)indoline (**7c**, 97%). Oxidation of the benzylic alcohol **7c** (MnO_2, CH_2Cl_2) to the aldehyde **7d** and subsequent condensation with methyl azidoacetate afforded the azidocinnamate **7e**, which cyclized upon thermolysis in refluxing xylene (140 °C, 4 h)[18] to afford methyl 3-(methoxycarbonyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (**8a**, 65% overall from **7d**). The thermal cyclization of **7e** proceeds with the generation of an intermediate 2*H*-azirine[22,23] and the disappearance (TLC, SiO_2) of the starting azidocinnamate **7e** ($t_{1/2}$ = 15–30 min, 140 °C) does not correspond with the temporal appearance of **8a** (Scheme I).

The selective deprotection of either the methyl ester (aqueous LiOH) or the methyl carbamate (HBr/AcOH)[24] of **8a** provided 3-(methoxycarbonyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylic acid (**8b**) and methyl 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (**9**) suitable for further selective transformations and secured the opportunity to study the coupling of the monomer 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole units. Treatment of the free indoline methyl ester **9** with trimethylsilyl isocyanate provided methyl 3-carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (CDPI methyl ester, **2a**). Methyl ester hydrolysis of **2a** provided the carboxylic acid **2b** (CDPI).

A second, alternative, and more direct preparation of the parent 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate skeleton is summarized in Scheme II and provided the additional opportunity for the direct introduction of selected N-substituted derivatives of the free indoline **9**. Condensation of indole-4-carboxaldehyde

(19) This alcohol was prepared by alkylation of the lithium acetylide of 1-[(*tert*-butyldimethylsilyl)oxy]-2-propyne (*n*-BuLi, THF, −78 to 0 °C) with ethylene oxide (THF, 0–23 °C, 3–6 h).[15]

(20) Mitsunobu, O. *Synthesis* **1981**, 1. Mitsunobu, O.; Wada, M.; Sano, T. *J. Am. Chem. Soc.* **1972**, *94*, 679.

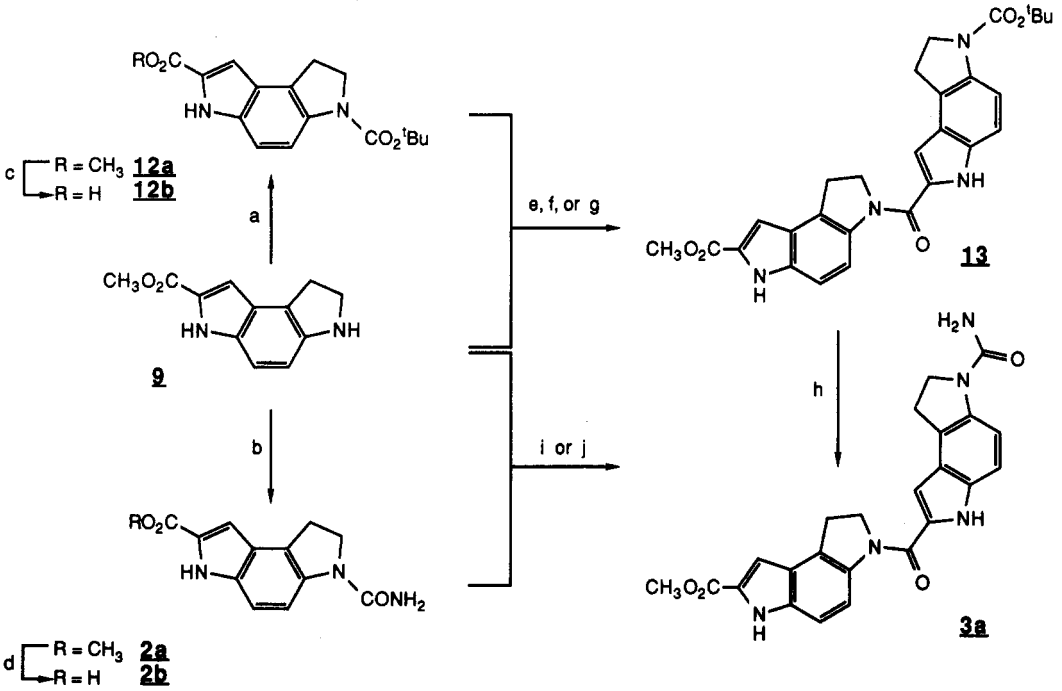
(21) In related efforts, attempts to alkylate derivatives of 3-chloro-6-amino-1,2-diazines (*N*-benzyl and *N*-carbomethoxy) utilizing homopropargylic alkylating agents (e.g., 3-pentynyl iodide and 3-pentynyl tosylate) under standard conditions (NaH, THF/DMF, 25 °C, 1–24 h) resulted only in consumption of alkylating agent with no evidence of N-alkylation. Attempts to further alter or improve the ratio of C3 amino alkylation to 1,2-diazine (**6a**, X = H) by varying the reaction solvent (CH_3CN > THF/dioxane > toluene; 1:1 to 1:2 C3 amino alkylation/1,2-diazine N2-alkylation) were less successful, and the use of other methods for direct N-alkylation were unsuccessful.

(22) Hassner, A.; Wiegand, N. H.; Gottlieb, H. E. *J. Org. Chem.* **1986**, *51*, 3176. Knittle, D. *Synthesis* **1985**, 186. Hickey, D. M. B.; Moody, C. J.; Rees, C. W. *J. Chem. Soc., Chem. Commun.* **1982**, 1419.

(23) For additional experimental studies supporting this mechanism, see ref 15.

(24) Wani, M. C.; Campbell, H. F.; Brine, G. A.; Kepler, J. A.; Wall, M. E.; Levin, S. G. *J. Am. Chem. Soc.* **1972**, *94*, 3631.

Scheme III[a]



[a] (a) 1.06 equiv of $(t\text{-BuOCO})_2O$, THF, 23 °C, 13.5 h, 82%; (b) 5 equiv of $Me_3SiN{=}C{=}O$, catalytic DMAP, CH_2Cl_2, 23 °C, 18 h, 90%; (c) 5 equiv of LiOH, THF/MeOH/H_2O (3:2:1), 50–55 °C, 2.5 h, 100%; (d) 5 equiv of LiOH, THF/MeOH/H_2O (3:2:1), 50 °C, 20 min, 100%; (e) **12b**, 5 equiv of oxalyl chloride, THF, catalytic DMF, 23 °C, 1 h; 1.1 equiv of **9**, 1.2 equiv of Et_3N, THF, 23 °C, 12 h, 56%; (f) **12b**, **9**, 1.4 equiv of BOP-Cl, 2.7 equiv of Et_3N, CH_2Cl_2, 21 °C, 9 h, 97%; (g) **12b**, **9**, 1.5 equiv of EDCI, THF, 21 °C, 9 h, 95%; (h) trifluoroacetic acid, 23 °C, 1 h, 100%; 5 equiv of $Me_3SiN{=}C{=}O$, CH_2Cl_2, 23 °C, 22 h, 81%; (i) **2b**, **9**, 1.25 equiv of BOP-Cl, 2.5 equiv of Et_3N, CH_2Cl_2, 21 °C, 21 h, 83%; (j) **2b**, **9**, 2.1 equiv of EDCI, THF, 23 °C, 23.5 h, 86%.

(**10a**)[25] with methyl azidoacetate and subsequent thermolysis of the resulting azidocinnamate **10b** (xylenes, 140 °C, 4 h)[18] provided **11** presumably with the intermediate generation of the corresponding 2*H*-azirine. Selective reduction ($NaCNBH_3$, AcOH) of the unsubstituted, fused pyrrole of **11** employing the procedure described by Gribble[26] provided methyl 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (**9**) identical in all respects with the material previously described. The selectivity of the reduction effected by sodium cyanoborohydride in glacial acetic acid in the conversion of **11** to **9** may be attributed to the resistance of the indole-2-carboxylate fused pyrrole unit of **11** to C-3 protonation and subsequent reduction of the resulting indolenium ion.[26] The resulting indoline **9** may be purified and stored as the somewhat unstable free amine or converted directly to the methyl 3-(*tert*-butyloxycarbonyl)- or 3-carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylates, **12a** and CDPI methyl ester (**2a**), respectively.

3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate Dimer Methyl Ester (3a, CDPI Dimer Methyl Ester). At the onset of the investigation of methods to promote the central amide bond formation and coupling of monomeric 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole subunits suitable for implementation in the assemblage of the central and right-hand segments of CC-1065, i.e., PDE-I dimer methyl ester formation, it was not evident whether the terminal *N*-carbamoyl functionality or the selectively protected C-4/C-5 *o*-catechol unit bearing a readily oxidizable, acidic phenol might interfere competitively with the direct coupling of the appropriate CC-1065 subunits. Additionally, the efforts to effect the desired coupling were further complicated by the insoluble nature of the monomeric 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole subunits. Consequently, both indirect and direct approaches to providing CDPI dimer via coupling of the free indoline methyl ester **9** with the 3-(methoxycarbonyl)-, 3-(*tert*-butyloxycarbonyl)-, and 3-carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylic acids were investigated.

Reaction of the crude acid chloride[27] generated from the 3-(*tert*-butyloxycarbonyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]-indole-7-carboxylic acid (oxalyl chloride, THF, catalytic DMF) with the free indoline methyl ester **9** was successful in providing the dimer methyl ester **13** (56%) as an exceptionally insoluble product (Scheme III). The first attempts to promote this coupling using the acid chloride generated from 3-(methoxycarbonyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylic acid (**8b**) resulted in no organic-soluble product being detected with only trace recovery of the free indoline methyl ester **9** in a reaction which appeared to have failed to produce the desired coupling product **14** (eq 1). In efforts to determine the fate of the substrates, the insoluble precipitates were examined for the presence of products derived from the starting monomer units. The desired dimer product **14**[28] was isolated from the aqueous reaction workup as an organic-insoluble, water-insoluble material. Subsequently, it was determined that this general insolubility of **14**, as well as that of related 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate dimer and trimer materials, provided an ex-

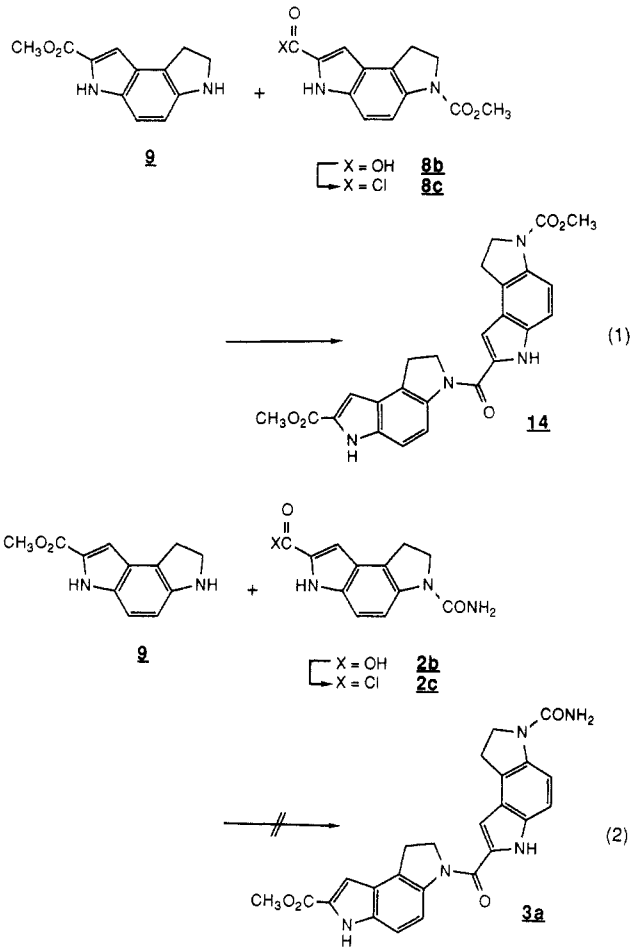
(25) Kozikowski, A. P.; Ishida, H.; Chen, Y.-Y. *J. Org. Chem.* **1980**, *45*, 3350.

(26) Gribble, G. W.; Hoffman, J. H. *Synthesis* **1977**, 859.

(27) Johnson, J. R.; Hasbrouck, R. B.; Dutcher, J. D.; Bruce, W. F. *J. Am. Chem. Soc.* **1945**, *67*, 423.

(28) **14**: ^1H NMR (Me_2SO-d_6, 80 MHz, ppm) 8.23 (d, 1 H, J = 9 Hz, C4′-H), 7.73 (d, 1 H, J = 9 Hz, C5-H), 7.6 (br s, 1 H, C4-H), 7.28 (d, 1 H, J = 8 Hz, C5′-H), 7.08 (d, 1 H, J = 2 Hz, C8-H), 6.95 (d, 1 H, J = 1 Hz, C8′-H), 4.59 (t, 2 H, J = 8 Hz, C2′-H), 4.05 (t, 2 H, J = 8 Hz, C2-H), 3.88 (s, 3 H, OCH_3), 3.74 (s, 3 H, OCH_3), 3.10–3.55 (m, 4 H, C1-H and C1′-H, partially obscured by H_2O); EIMS, *m/e* (relative intensity) 458 (M^+, 2), 243 (8), 242 (8), 215 (base), 184 (73), 169 (3), 156 (23), 155 (29).

cellent property which facilitated the purification of the desired coupling products.



Removal of the *tert*-butyloxycarbonyl group (trifluoroacetic acid, 23 °C, 1 h) and acylation of the crude, free indoline with trimethylsilyl isocyanate provided CDPI dimer methyl ester (**3a**, 95% overall from **13**). Attempts to couple the 3-carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]-indole-7-carboxylic acid (**2b**) directly with the use of comparable conditions to generate the corresponding acid chloride (1–5 equiv of oxalyl chloride, THF, catalytic DMF) and its subsequent reaction with the free indoline **9** failed to provide CDPI dimer methyl ester (**3a**) directly (eq 2). Coupling of the two subunits was observed but a competitive reaction of the terminal *N*-carbamoyl group with oxalyl chloride during acid chloride generation precluded the production of the desired CDPI dimer methyl ester (**3a**).[29]

The use of bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl)[30] and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) as carboxyl activating agents were successful in promoting the indirect *and* direct coupling of both the 3-(*tert*-butyloxycarbonyl)- and 3-carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylic acids (**12b** and **2b**, respectively) with the free indoline methyl ester **9** and afforded excellent yields of dimer products **13** and **3a** (CDPI dimer methyl ester), respectively. As a consequent of the insolubility of the monomer 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate units the coupling reactions were run as suspensions in conventional organic solvents (tetrahydrofuran, methylene chloride). Significantly, both BOP-Cl and EDCI were capable of providing carboxyl activation and direct coupling without the observation of competitive reactions of the terminal *N*-carbamoyl group. Moreover, the organic-insoluble, water-insoluble properties of the desired dimer products (**3a** and **13**) coupled with the use of the water-soluble carbodiimide reagent (EDCI·HCl) provided a technically convenient method for conducting the direct coupling in which the purification of the coupled products required simple centrifugation of the aqueous reaction mixture workup.

The successful application of this coupling methodology to the synthesis of PDE-I dimer methyl ester is detailed elsewhere.[15]

3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate Trimer/Tetramer Methyl Ester (4/5, CDPI Trimer/Tetramer Methyl Ester). Initial attempts at the application of the EDCI-promoted coupling methodology to the formation of CDPI trimer methyl ester (**4a**) (Scheme IV) were unsuccessful using tetrahydrofuran as the reaction solvent and unreacted carboxylic acid **3b** was recovered unchanged from the reaction mixture (93%). However, the coupling was found to proceed smoothly if conducted with *N*,*N*-dimethylformamide (**9** and **3b**, 2.3 equiv of EDCI) as solvent and provided 3-carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate trimer methyl ester (CDPI trimer methyl ester, **4a**) in excellent yield (95%). Similarly, the EDCI-promoted coupling of CDPI trimer **4b** with **9** in *N*,*N*-dimethylformamide provided 3-carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate tetramer methyl ester (CDPI tetramer methyl ester, **5a**) in excellent yield (88%) (Scheme IV).[31]

Efforts on the incorporation of PDE-I dimer methyl ester into the total synthesis of CC-1065, the concurrent preparation of structurally related agents incorporating the simplified, stable 3-carbamoyl-1,2-dihydro-3*H*-pyrrolo-[3,2-*e*]indole dimer **3** (CDPI dimer) and trimer/tetramer **4/5** (CDPI trimer/tetramer), as well as a comparative examination of the properties of these agents are in progress.

Experimental Section

Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Varian XL-200 and chemical shifts are reported in parts per million relative to internal tetramethylsilane (0.00 ppm). Infrared spectra (IR) were recorded on a Perkin-Elmer 1710 Fourier transform spectrometer as KBr pellets. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Electron impact mass spectra (EIMS) and chemical ionization mass spectra (CIMS) were recorded on a Finnegan 4000 spectrometer. High-resolution mass spectra (HRMS) and fast atom bombardment mass spectra (FABMS) were recorded on a Kratos MS-50 spectrometer. Flash chromatography[32a] was performed on 230–400 mesh silica gel. Preparative centrifugal thin-layer chromatography (PCTLC)[32b] was performed on a Harrison Model 7924 Chromatotron, using Merck silica gel 60 PF_{254} containing $CaSO_4 \cdot {}^1/_2H_2O$ binder. Tetrahydrofuran (THF) was distilled from sodium benzophenone

(29) The reaction product was characterized: ^{1}H NMR (Me_2SO-d_6, 200 MHz, ppm) 12.01 (s, 1 H), 11.62 (s, 1 H), 8.50 (br s, 1 H, NH), 8.29 (d, 1 H, J = 9 Hz), 8.14 (d, 1 H, J = 9 Hz), 7.36 (d, 1 H, J = 9 Hz), 7.27 (d, 1 H, J = 9 Hz), 7.14 (s, 1 H), 6.98 (s, 1 H), 4.64 (t, 2 H, J = 8 Hz), 4.21 (br t, 2 H), 3.86 (s, 3 H), 3.40 (t, 2 H, J = 7 Hz), 3.22 (t, 2 H, J = 8 Hz); IR (KBr) ν_{max} 3318, 2920, 2852, 1710, 1636, 1610, 1579, 1508, 1435, 1412, 1367, 1341, 1253, 1207, 1108, 802, 769 cm^{-1}.

(30) Diago-Meseguer, J.; Palomo-Coll, A. L.; Fernandez-Lizarbe, J. R.; Zugaza-Bilbao, A. *Synthesis* **1980**, 547.

(31) Attempts to prepare CDPI tetramer methyl ester (**5a**) employing the EDCI-promoted direct coupling of CDPI dimer (**3b**) with the CDPI dimer, free indoline derived from deprotection (trifluoroacetic acid, 23 °C, 1 h, 100%) of **13** have been unsuccessful (THF, DMF, 25 °C, 1.5–4.0 equiv of EDCI) presumably due to the insolubility of *both* coupling components in the reaction solvents examined.

(32) (a) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923. (b) Stahl, E.; Muller, J. *Chromatographia* **1982**, *15*, 493.

ketyl. Methanol (MeOH) was distilled from magnesium methoxide. Methylene chloride (CH_2Cl_2) was distilled from phosphorus pentoxide. Xylene, *N,N*-dimethylformamide (DMF), and triethylamine (Et_3N) were distilled from calcium hydride and triethylamine was stored over KOH pellets. All extraction and chromatographic solvents [ethyl acetate (EtOAc), hexane, and methylene chloride (CH_2Cl_2)] were distilled prior to use. Trimethylsilyl isocyanate ($Me_3SiN{=}C{=}O$), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl), and 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDCI) were obtained from Aldrich Chemical Co. All reactions requiring anhydrous conditions were performed under an atmosphere of nitrogen (N_2) or argon.

Methyl Pyrrolo[3,2-*e*]indole-2-carboxylate (11). A solution of indole-4-carboxaldehyde[25] (**10a**, 583 mg, 4.02 mmol) and methyl azidoacetate[33] (4.60 g, 40 mmol, 10 equiv) in dry methanol (21 mL) was cooled to −23 °C (dry ice/CCl_4) under N_2 and a solution of sodium methoxide in methanol (7.4 mL of 4.37 M, 32 mmol, 8 equiv) was added dropwise (2 min). The reaction mixture was warmed to 0 °C and was stirred 3 h (0 °C). A voluminous yellow precipitate formed after 20 min (0 °C). The reaction mixture was poured onto water (100 mL) and was extracted with EtOAc (2 × 50 mL). The combined extracts were washed with saturated aqueous NaCl (50 mL) and were dried (Na_2SO_4). The solvent was removed in vacuo to afford **10b** (939 mg, 974 mg theoretical, 96%) as an unstable yellow solid: ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 8.29 (br s, 1 H, NH), 8.06 (d, 1 H, J = 7.5 Hz, Ar H), 7.24–7.45 (m, 4 H, Ar H), 6.73 (s, 1 H, Ar C*H*=C), 3.95 (s, 3 H, OCH_3).

A suspension of crude **10b** (939 mg, 3.88 mmol) in dry xylenes (90 mL) was warmed to reflux under N_2 (4 h).[18] The solvent was removed in vacuo and the red-brown residue passed through a short column of silica gel (4 × 12 cm, 50% EtOAc–hexane) to afford crude **11** (807 mg, 831 mg theoretical, 97%) as a brown solid homogeneous by TLC and suitable for subsequent use. Purification of crude **11** (PCTLC 1-mm SiO_2, 25–100% EtOAc–hexane gradient elution) provided pure **11** (71%, 0.7-mmol scale) as a light brown crystalline solid: mp 201–202 °C dec ($CHCl_3$–hexane, beige crystals); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 9.02 (br s, 1 H, NH), 8.36 (br s, 1 H, NH), 7.19–7.47 (m, 4 H, Ar H), 6.81 (m, 1 H, C1-H), 3.95 (s, 3 H, OCH_3); IR (KBr) ν_{max} 3393, 3330, 1685, 1526, 1481, 1436, 1383, 1344, 1278, 1236, 1219, 1160, 758, 732 cm^{-1}; EIMS, *m/e* (relative intensity) 214 (M^+, base), 182 (75), 154 (59), 128 (32), 127 (24); CIMS (2-methylpropane), *m/e* 215 (M^+ + H, base); HRMS, *m/e* 214.0731 ($C_{12}H_{10}N_2O_2$ requires 214.0742).

Methyl 1,2-Dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (9). A solution of crude pyrroloindole **11** (786 mg, 3.67 mmol) described above in glacial acetic acid (9.8 mL) under N_2 at 15 °C was treated with sodium cyanoborohydride (711 mg, 11.3 mmol, 9.2 hydride equiv), and the reaction mixture was stirred for 2 h (15–17 °C).[26] The reaction mixture was partitioned between water (50 mL) and EtOAc (30 mL), and the organic phase was extracted with 10% aqueous HCl (2 × 25 mL). The combined aqueous extracts were made basic (pH 9) by the careful addition of solid Na_2CO_3. The aqueous mixture was extracted with EtOAc (2 × 50 mL), and the combined extracts were dried (Na_2SO_4). The solvent was removed in vacuo to afford crude **9** (700 mg, 794 mg theoretical, 88%) as a bright yellow solid. Flash chromatography (SiO_2, 4 × 7 cm, 50% EtOAc–hexane) afforded pure **9** (624 mg, 794 mg theoretical, 79%; 66% overall from **10a**) as a bright yellow solid: mp 158–159 °C (CH_2Cl_2–hexane, bright yellow needles); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 8.88 (br s, 1 H, NH), 7.16 (d, 1 H, J = 8 Hz, C5-H), 7.06 (s, 1 H, C8-H), 6.88 (d, 1 H, J = 8 Hz, C4-H), 3.95 (s, 3 H, OCH_3), 3.69 (t, 2 H, J = 9 Hz, NCH_2CH_2), 3.25 (t, 2 H, J = 9 Hz, NCH_2CH_2); IR (KBr) ν_{max} 3334, 3222, 2943, 2857, 1697, 1525, 1443, 1336, 1265, 1256, 1212, 1003, 792, 764 cm^{-1}; EIMS, *m/e* (relative intensity) 216 (52), 184 (base), 156 (42), 155 (52), 128 (19), 92 (10); HRMS, *m/e* 216.0888 ($C_{12}H_{12}N_2O_2$ requires 216.0899).

Methyl 3-(*tert*-Butyloxycarbonyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (12a). A solution of **9** (302 mg, 1.39 mmol) in dry THF (4.9 mL) was treated with di-*tert*-butyl dicarbonate (0.34 mL, 1.5 mmol, 1.06 equiv) at 23 °C under N_2. The reaction mixture was stirred 13.5 h (23 °C), then poured onto saturated aqueous NaCl (25 mL) and extracted with CH_2Cl_2 (2 × 25 mL). The combined extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. Flash chromatography (2 × 10 cm SiO_2, 20–30% EtOAc–hexane gradient elution) afforded **12a** (359 mg, 440 mg theoretical, 82%) as an off-white crystalline solid: mp 220–223 °C dec (EtOAc–hexane, white needles); ^{1}H NMR ($CDCl_3$, 200 MHz, ppm) 8.95 (br s, 1 H, NH) 7.29 (br s, 1 H), 7.24 (br s, 1 H), 7.09 (br s, 1 H), 4.13 (t, 2 H, J = 9 Hz, NCH_2CH_2), 3.96 (s, 3 H, OCH_3), 3.28 (t, 2 H, J = 9 Hz, NCH_2CH_2), 1.58 (s, 9 H, CMe_3); IR (KBr) ν_{max} 3334, 2971, 1687, 1527, 1451, 1441, 1376, 1331, 1258, 1215, 1154, 1138, 811, 772 cm^{-1}; EIMS, *m/e* (relative intensity) 316 (9), 260 (58), 228 (52), 193 (11), 184 (27), 156 (15), 155 (11), 125 (17), 105 (9), 77 (18), 57 (base); CIMS (2-methylpropane), *m/e* (relative intensity) 317 (27, M^+ + H), 261 (base), 217 (17); HRMS, *m/e* 316.1390 ($C_{17}H_{20}N_2O_4$ requires 316.1423).

3-(*tert*-Butyloxycarbonyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylic Acid (12b). An aqueous solution of LiOH (36 μL of a 4.0 M solution, 0.144 mmol, 5 equiv) was added to a slurry of **12a** (9.0 mg, 18.4 μmol) in 0.25 mL of THF/MeOH/H_2O (3:2:1), and the reaction mixture was warmed at 50 °C (20 min). The reaction mixture was diluted with 2 mL of water, and 10% aqueous HCl (3 drops) was added, producing a white precipitate. The solid was collected by centrifugation and was washed with water (2 × 2 mL). Drying the solid in vacuo afforded **12b** (8.6 mg, 8.6 mg theoretical, 100%) as a white solid: ^{1}H NMR (Me_2SO-d_6, 200 MHz, ppm) 11.67 (s, 1 H, NH), 7.82 (br s, 1 H, C4-H), 7.23 (d, 1 H, J = 9 Hz, C5-H), 6.92 (d, 1 H, J = 2 Hz, C8-H), 4.00 (t, 2 H, J = 8.5 Hz, NCH_2CH_2), 3.21 (t, 2 H, J = 8.5 Hz, NCH_2CH_2), 1.51 (s, 9 H, CMe_3); IR (KBr) ν_{max} 3443, 3293, 2981, 1682, 1642, 1534, 1509, 1453, 1402, 1330, 1251, 1213, 1159, 1144, 1067, 1027, 934, 809, 776 cm^{-1}; EIMS, *m/e* (relative intensity) 302 (M^+, 13), 246 (65), 228 (41), 201 (5), 184 (30), 156 (17), 128 (9), 57 (base); CIMS (2-methylpropane) *m/e* (relative intensity) 303 (M^+ + H, 38), 247 (base), 203 (9).

Methyl 3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]-indole-7-carboxylate (2a). A solution of **9** (111 mg, 0.51 mmol) in dry CH_2Cl_2 (3.5 mL) at 23 °C was treated with trimethylsilyl isocyanate (0.4 mL, 85%, 2.5 mmol, 2.6 equiv) under a N_2 atmosphere. A catalytic amount of 4-(dimethylamino)pyridine (ca. 2 mg) was added, and the reaction mixture was stirred for 36 h (23 °C). The resulting insoluble yellow solid was collected by centrifugation and washed with CH_2Cl_2 (2 × 2 mL). Drying the solid in vacuo afforded **2a** (119.2 mg, 132 mg theoretical, 90%) as a yellow solid: mp >240 °C; ^{1}H NMR (Me_2SO-d_6, 200 MHz, ppm) 11.77 (br s, 1 H, NH), 8.00 (d, 1 H, J = 9 Hz, C4-H), 7.18 (d, 1 H, J = 9 Hz, C5-H), 6.98 (d, 1 H, J = 1 Hz, C8-H), 6.09 (s, 2 H, $CONH_2$), 3.96 (t, 2 H, J = 9 Hz, NCH_2CH_2), 3.86 (s, 3 H, OCH_3), 3.25 (t, 2 H, J = 9 Hz, NCH_2CH_2); IR (KBr) ν_{max} 3421, 3352, 3288, 3229, 2946, 1699, 1674, 1609, 1583, 1505, 1328, 1275, 1255, 1233, 1210, 767 cm^{-1}; EIMS, *m/e* (relative intensity) 259 (M^+, 94), 227 (20), 216 (22), 199 (14), 184 (base), 155 (38), 128 (17); CIMS (2-methylpropane), *m/e* (relative intensity) 260 (M^+ + H, base), 217 (7), 85 (7); HRMS, *m/e* 259.0947 ($C_{13}H_{13}N_3O_3$ requires 259.0957).

3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylic Acid (2b). A suspension of **2a** (304 mg, 1.17 mmol) in 4.7 mL THF/MeOH (3:2) was treated with an aqueous solution of LiOH (1.46 mL of a 4.0 M solution, 5.84 mmol, 5.0 equiv). The reaction mixture was warmed at 50–55 °C (2.5 h). A majority of the solvent was removed under a stream of N_2, and the residual solid was suspended in water (10 mL). Aqueous HCl (10%) was added until the mixture was acidic (pH 1), and the insoluble crude product was collected by centrifugation. The resulting gray solid was washed with water (3 × 5 mL). Drying the solid in vacuo afforded **2b** (286 mg, 287 mg theoretical, 100%) as a gray solid: mp >240 °C; ^{1}H NMR (Me_2SO-d_6, 200 MHz, ppm) 12.84 (br s, 1 H, CO_2H), 11.60 (s, 1 H, NH), 7.98 (d, 1 H, J = 9 Hz, C4-H), 7.16 (d, 1 H, J = 9 Hz, C5-H), 6.91 (d, 1 H, J = 1.3 Hz, C8-H), 6.09 (s, 2 H, $CONH_2$) 3.96 (t, 2 H, J = 8.8 Hz, NCH_2CH_2), 3.25 (t, 2 H, J = 8.8 Hz, NCH_2CH_2); IR (KBr) ν_{max} 3409, 3346, 1672, 1645, 1596, 1572, 1524, 1457, 1359, 1327, 1302, 1251, 1235, 1210, 771, 674 cm^{-1}; EIMS, *m/e* (relative intensity) 245 (M^+, 2), 202 (70), 184 (base), 156 (38), 155 (40), 130 (5), 128 (9), 101 (5); CIMS (2-methylpropane) *m/e* (relative intensity) 246 (M^+ + H, 5), 203

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(base); HRMS, m/e 245.0788 ($C_{12}H_{11}N_3O_3$ requires 245.0800).

3-(*tert*-Butyloxycarbonyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate Dimer Methyl Ester (13). Method A. A slurry of **12b** (17.0 mg, 56.2 μmol) in 0.5 mL of dry THF containing ca. 1% DMF at 22 °C under N_2 was treated with oxalyl chloride (24 μL, 35 mg, 0.275 mmol, 5 equiv), and the reaction mixture was stirred 30 min (22 °C). The solvent and excess oxalyl chloride were removed in vacuo to afford the unstable, crude acid chloride as an orange solid. A slurry of the crude acid chloride in 0.4 mL of dry THF was treated sequentially with indoline **9** (13.5 mg, 62.4 μmol, 1.1 equiv) and Et_3N (9 μL, 65 μmol, 1.15 equiv), and the reaction mixture was stirred for 12 h (22 °C). The reaction mixture was diluted with 3 mL of water and 10% aqueous Na_2CO_3 added until the mixture was basic (pH 10). The aqueous mixture was extracted with EtOAc (3 × 3 mL), acidified with the addition of 10% aqueous HCl (pH 2), and reextracted with EtOAc (3 × 3 mL) and the water removed in vacuo. The resulting crude product **13** was slurried in 10 mL of water and the solid material collected by centrifugation. Drying the solid in vacuo afforded **13** (15.7 mg, 28.1 mg theoretical, 56%) as a light yellow solid.

Method B. A slurry of **9** (5.2 mg, 24.0 μmol) and **12b** (7.1 mg, 23.5 μmol) in 0.25 mL of dry CH_2Cl_2 at 21 °C was treated sequentially with bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl,[30] 8.5 mg, 33.4 μmol, 1.4 equiv) and Et_3N (9 μL, 65 μmol, 2.7 equiv). The reaction mixture was stirred for 9 h (21 °C). The solvent was removed under a stream of N_2 and the residual solid was slurried in 3 mL of water containing 2 drops of 10% aqueous Na_2CO_3. The solid was collected by centrifugation and washed with water (2 × 1 mL). Drying the solid in vacuo afforded **13** (11.4 mg, 11.8 mg theoretical, 97%) as a light brown solid.

Method C. A slurry of **9** (5.4 mg, 25.0 μmol) and **12b** (7.5 mg, 24.8 μmol) in 0.25 mL of dry THF at 21 °C was treated with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI, 7.3 mg, 38 μmol). The resulting reaction mixture was stirred for 9 h (21 °C). The solvent was removed under a stream of N_2, and the residual solid was slurried in 2 mL of water containing 2 drops of 10% aqueous HCl. The solid was collected by centrifugation and washed with water (2 × 1 mL). Drying the solid in vacuo afforded **13** (11.8 mg, 12.4 mg theoretical, 95%) as a light brown solid: mp >240 °C; ^{1}H NMR (Me_2SO-d_6, 200 MHz, ppm)[34] 12.02 (s, 1 H, NH), 11.64 (s, 1 H, NH), 8.28 (d, 1 H, J = 9 Hz, C4′-H), 7.8 (br s, 1 H, C4-H), 7.31 (apparent t, 2 H, J = 9 Hz, C5-H and C5′-H), 7.13 (d, 1 H, J = 1 Hz, C8-H), 6.98 (d, 1 H, J = 1 Hz, C8′-H), 4.62 (t, 2 H, J = 8 Hz, C2′-H), 4.02 (t, 2 H, J = 8 Hz, C2-H), 3.88 (s, 3 H, OCH_3), 3.40 (t, 2 H, J = 8 Hz, C1′-H), 3.25 (t, 2 H, J = 8 Hz, C1-H), 1.52 (s, 9 H, CMe_3); IR (KBr) ν_{max} 3293, 2974, 1699, 1602, 1578, 1531, 1508, 1436, 1423, 1409, 1377, 1344, 1331, 1257, 1231, 1215, 1174, 1150, 1137, 1064, 1021, 808, 771 cm^{-1}; CIMS (2-methylpropane), m/e (relative intensity) 501 (M^+ + H, 3), 457 (1), 445 (1), 439 (1), 415 (3), 401 (20), 400 (16), 357 (1), 330 (6), 303 (7), 274 (4), 245 (11), 230 (8), 217 (3), 203 (2), 183 (2), 85 (48), 73 (base); HRMS, m/e 500.2010 ($C_{28}H_{28}N_4O_5$ requires 500.2060).

3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate Dimer Methyl Ester (3a). Method B. A slurry of **9** (15.3 mg, 70.8 μmol) and **2a** (19.7 mg, 80.3 μmol) in 0.7 mL of dry CH_2Cl_2 at 21 °C was treated with bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl,[30] 26 mg, 0.1 mmol, 1.25 equiv) and Et_3N (25 μL, 0.18 mmol, 2.5 equiv). The reaction mixture was stirred for 21 h (21 °C). The solvent was removed under a stream of N_2 and the residual solid was slurried in 3 mL of water containing 2 drops of 10% aqueous Na_2CO_3. The resulting solid was collected by centrifugation and washed with water (2 × 1 mL). Drying the solid in vacuo afforded **3a** (26.0 mg, 31.4 mg theoretical, 83%) as an orange-brown solid.

Method C. A suspension of **2b** (34.5 mg, 0.141 mmol) and **9** (31.8 mg, 0.147 mmol, 1.04 equiv) in dry THF (2 mL) was treated with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI, 57 mg, 0.30 mmol, 2.1 equiv) at 23 °C. The resulting reaction mixture was stirred at 23 °C (23.5 h). The solvent was removed under a stream of N_2, the residual solid was suspended in water (2 mL), and 10% aqueous HCl was added until the mixture was acidic (pH 1). The insoluble solid was collected by centrifugation and was washed with water (3 × 2 mL). Drying the solid in vacuo afforded **3a** (54 mg, 63 mg theoretical, 86%) as a yellow-grey solid: mp >240 °C; ^{1}H NMR (Me_2SO-d_6, 200 MHz, ppm)[34] 12.01 (s, 1 H, NH), 11.55 (s, 1 H, NH), 8.28 (d, 1 H, J = 9 Hz, C4′-H), 7.97 (d, 1 H, J = 9 Hz, C4-H), 7.33 (d, 1 H, J = 9 Hz, C5-H), 7.21 (d, 1 H, J = 9 Hz, C5′-H), 7.13 (d, 1 H, J = 1.4 Hz, C8-H), 6.95 (d, 1 H, J = 1.0 Hz, C8′-H), 6.11 (s, 2 H, $CONH_2$), 4.63 (t, 2 H, J = 8 Hz, C2′-H), 3.98 (t, 2 H, J = 8 Hz, C2-H), 3.88 (s, 3 H, OCH_3), 3.39 (t, 2 H, J = 8 Hz, C1′-H), 3.28 (t, 2 H, J = 8 Hz, C1-H); IR (KBr) ν_{max} 3415, 1692, 1642, 1604, 1573, 1511, 1437, 1413, 1384, 1363, 1338, 1259, 1215, 1146, 1121 cm^{-1}; FABMS (dithiothreitol/dithioerythitol), m/e 444 (M^+ + H).

This procedure provided **3a** (70–86%) from **2b** (0.069–1.92-mmol scale).

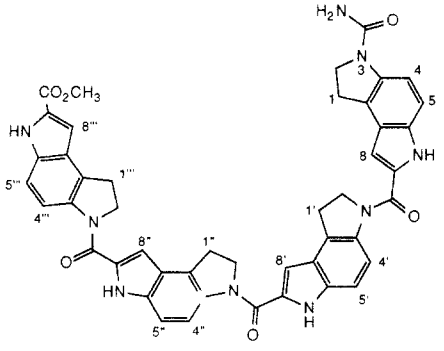
Conversion of 13 to 3a. Trifluoroacetic acid (0.5 mL) was added to **13** (10.4 mg, 21 μmol), and the reaction mixture was stirred for 60 min (23 °C). The trifluoroacetic acid was removed under a stream of N_2, and the residual solid was slurried in water (0.5 mL). A solution of 5% aqueous Na_2CO_3 was added until the mixture was basic (pH 9). The insoluble solid was collected by centrifugation and was washed with water (2 × 1 mL). Drying the solid in vacuo afforded the crude free indoline (8.4 mg, 8.4 mg theoretical, 100%) as an unstable, yellow solid: ^{1}H NMR (Me_2SO-d_6, 200 MHz, ppm)[34] 12.12 (s, 1 H, NH), 11.30 (s, 1 H, NH), 8.27 (d, 1 H, J = 8.8 Hz, C4′-H), 7.33 (d, 1 H, J = 9 Hz, C5′-H), 7.15–7.11 (m, 2 H, C5-H and C8-H), 6.83 (d, 1 H, J = 1.2 Hz, C8′-H), 6.66 (d, 1 H, J = 8.6 Hz, C4-H), 5.04 (br s, 1 H, N3-H), 4.62 (t, 2 H, J = 8.1 Hz, C2′-H), 3.88 (s, 3 H, OCH_3), 3.49–3.09 (m, 6 H, C1-H, C2-H and C1′-H partially obscured by H_2O).

A suspension of the crude free indoline (5.0 mg, 12 μmol) in dry CH_2Cl_2 (0.2 mL) at 23 °C under argon was treated with trimethylsilyl isocyanate (8 μL, 85%, 60 μmol, 5 equiv). The reaction mixture was stirred for 22 h (23 °C). The reaction mixture was diluted with CH_2Cl_2 (1 mL), and the insoluble solid was collected by centrifugation. The solid was washed with CH_2Cl_2 (1 mL) and was dried in vacuo to afford **3a** (4.3 mg, 5.3 mg theoretical, 81%) identical in all respects with **3a** previously described.

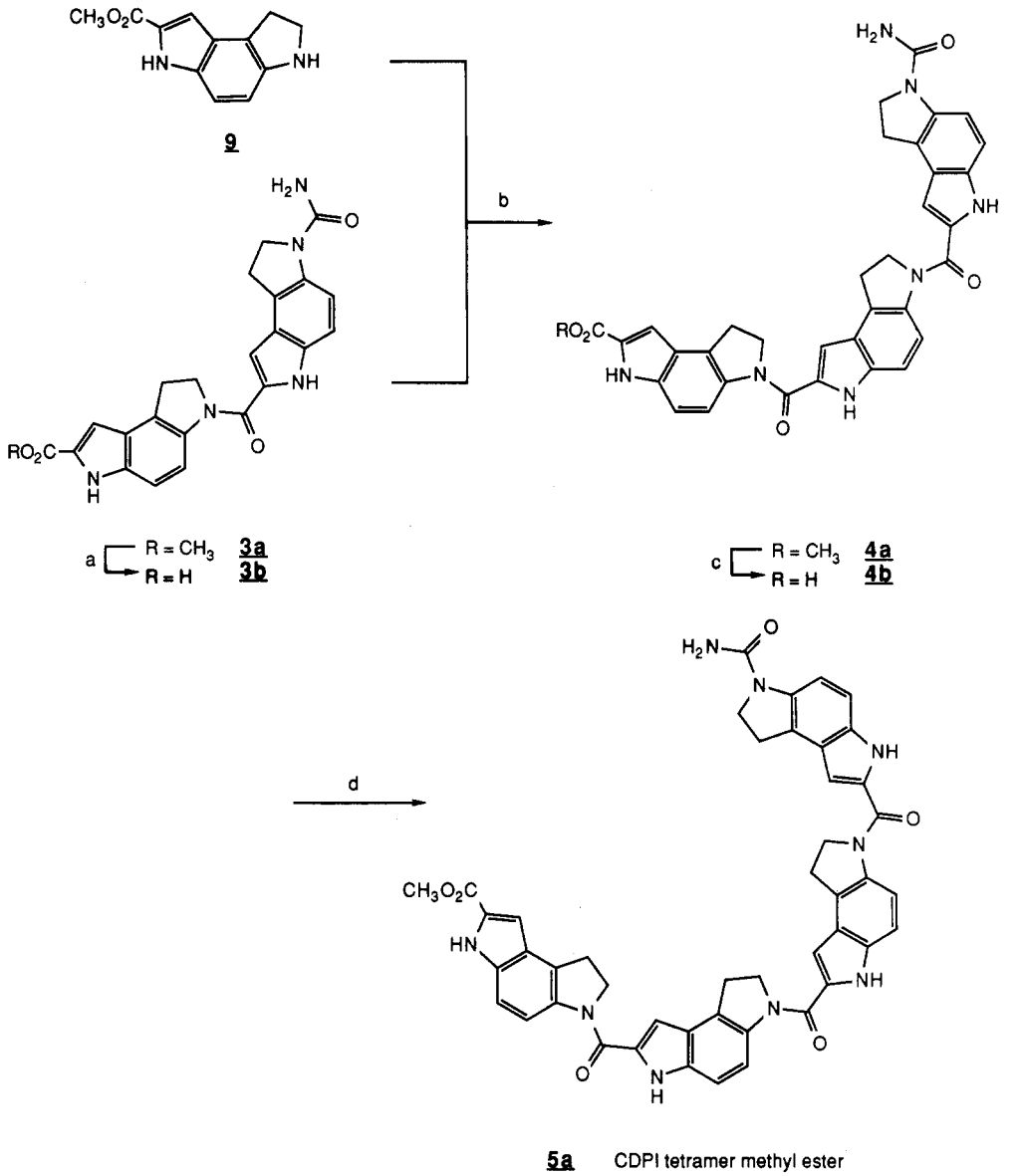
3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate Trimer Methyl Ester (4a). A suspension of **3a** (41.3 mg, 93 μmol) in 0.5 mL THF/MeOH (3:2) was treated with an aqueous solution of LiOH (0.12 mL of 4.0 M solution, 0.48 mmol, 5.2 equiv). The reaction mixture was warmed at 50–55 °C (10 h). The solvent was removed under a stream of N_2 and the residual solid suspended in water (1 mL). 10% Aqueous HCl was added until the mixture was acidic (pH 1), and the insoluble solid was collected by centrifugation. The resulting gray solid was washed with water (2 × 1 mL). Drying the solid in vacuo afforded **3b** (39 mg, 40 mg theoretical, 98%) as a gray solid: mp >240 °C; ^{1}H NMR (Me_2SO-d_6, 200 MHz, ppm)[34] 11.73 (s, 1 H, NH), 11.54 (s, 1 H, NH), 8.22 (d, 1 H, J = 9 Hz, C4′-H), 7.96 (d, 1 H, J = 9 Hz, C4-H), 7.29 (d, 1 H, J = 9 Hz, C5-H), 7.21 (d, 1 H, J = 9 Hz, C5′-H), 6.99 (s, 1 H, C8-H), 6.94 (s, 1 H, C8′-H), 6.10 (s, 2 H, $CONH_2$), 4.61 (t, J = 8 Hz, 2 H, C2-H) 3.97 (t, 2 H, J = 8.7 Hz, C2′-H), 3.43–3.28 (m, 4 H, C1-H and C1′-H partially obscured by H_2O); IR (KBr) ν_{max} 3452, 3307, 1670, 1642, 1600, 1573, 1538, 1511, 1464, 1435, 1408, 1362, 1349, 1324, 1271, 1231, 1191, 1147, 975, 803, 770, 760, 736, 684 cm^{-1}.

A mixture of **3b** (10.9 mg, 25 μmol) and **9** (6.3 mg, 29 μmol, 1.2 equiv) in dry DMF (0.5 mL) at 23 °C under argon was stirred vigorously for 10 min and 1-[3-(dimethylamino)propyl]-3-ethyl-

(34) Numbering for ^{1}H NMR assignments is as indicated:



Scheme IV[a]



[a] (a) 5 equiv of LiOH, THF/MeOH/H_2O (3:2:1), 50–55 °C, 10 h, 98%; (b) **3b**, 1.2 equiv of **9**, 2.3 equiv of EDCI, DMF, 23 °C, 23 h, 95%; (c) 30 equiv of LiOH, THF/MeOH/H_2O (3:2:1), 50–60 °C, 50 h, 89%; (d) **4b**, 1.1 equiv of **9**, 2.2 equiv of EDCI, DMF, 23 °C, 52 h, 88%.

carbodiimide hydrochloride (EDCI, 10.8 mg, 56 μmol, 2.3 equiv) was added. The reaction mixture was stirred for 23 h (23 °C), and the solvent was removed in vacuo. The residual solid was suspended in water (0.5 mL), the suspension was made basic (pH 9) with the addition of 5% aqueous Na_2CO_3, and the insoluble solid was collected by centrifugation. The residual solid was suspended in water (1 mL), and the suspension was acidified (pH 1) with the addition of 10% aqueous HCl. The insoluble solid was collected by centrifugation and was washed with water (2 × 1 mL). Drying the solid in vacuo afforded **4a** (14.9 mg, 15.7 mg theoretical, 95%) as a yellow-green solid: mp >240 °C; ^{1}H NMR (Me_2SO-d_6, 200 MHz, ppm)[34] 12.00 (s, 1 H, NH), 11.76 (s, 1 H, NH), 11.54 (s, 1 H, NH), 8.27 (apparent t, 2 H, J = 8.8 Hz, C4′-H and C4″-H), 7.97 (d, 1 H, J = 9.0 Hz, C4-H), 7.36 and 7.34 (2 d, 2 H, J = 9, 8.8 Hz, C5′-H and C5-H), 7.22 (d, 1 H, J = 9 Hz, C5″-H), 7.14 (d, 1 H, J = 2 Hz, C8′-H or C8-H), 7.11 (d, 1 H, J = 8 Hz, C8′-H or C8-H), 6.97 (d, 1 H, J = 0.8 Hz, C8″-H), 6.10 (s, 2 H, $CONH_2$), 4.66 (t, 4 H, J = 7.6 Hz, C2″-H and C2′-H), 3.98 (t, 2 H, J = 8.4 Hz, C2-H), 3.89 (s, 3 H, OCH_3), 3.20–3.60 (m, 6 H, C1″-H, C1′-H and C1-H partially obscured by H_2O); IR (KBr) ν_{max} 3350, 2952, 1700, 1653, 1605, 1578, 1507, 1433, 1409, 1365, 1342, 1285, 1258, 1216, 1186, 1159, 1147, 805, 760 cm^{-1}; FABMS (dithiothreitol/dithioerythitol), m/e 628 (M^+ + H).

This procedure provided **4a** (80–95%) from **3b** (0.025–0.93-mmol scale).

3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate Tetramer Methyl Ester (5a). A suspension of **4a** (12.1 mg, 19.3 μmol) in 0.75 mL THF/MeOH (3:2) was treated with an aqueous solution of LiOH (0.15 mL of a 4.0 M solution, 0.6 mmol, 30 equiv). The reaction mixture was warmed at 50–60 °C (50 h). The solvent was removed under a stream of N_2, and the residual solid was suspended in water (1 mL). Aqueous HCl (10%) was added until the mixture was acidic (pH 1), and the solid was collected by centrifugation. The resulting solid was washed with water (2 × 1 mL). Drying the solid in vacuo afforded **4b** (10.5 mg, 11.8 mg theoretical, 89%) as a yellow-gray solid: ^{1}H NMR (Me_2SO-d_6, 200 MHz, ppm), 11.81 (s, 1 H, NH), 11.78 (s, 1 H, NH), 11.62 (s, 1 H, NH), 8.27 (d, 2 H, J = 8 Hz, C4′-H), 7.98 (d, 1 H, J = 8 Hz, C4-H), 7.35 (apparent t, 2 H, J = 8 Hz, C5′-H and C5-H), 7.22 (d, 1 H, J = 8 Hz, C5″-H), 7.13 (s, 1 H, C8′-H or C8-H), 7.05 (s, 1 H, C8′-H or C8-H), 6.97 (s, 1 H, C8″-H), 6.11 (s, 2 H, $CONH_2$), 4.66 (t, 4 H, J = 7.5 Hz, C2″-H and C2′-H), 3.98 (t, 2 H, J = 7.5 Hz, C2-H), 3.5 (br m, 6 H, C1″-H, C1′-H, and C1-H obscured by H_2O); IR (KBr) ν_{max} 3415, 2922, 1650 (s), 1607, 1579, 1507, 1429, 1401, 1364, 1343, 1285, 1249, 1223, 1185, 1146, 801, 756, 687 cm^{-1}.

A suspension of **4b** (3.0 mg, 4.9 μmol) and **9** (1.2 mg, 5.5 μmol, 1.1 equiv) in dry DMF (0.2 mL) was treated with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI, 2.1 mg, 11 μmol, 2.2 equiv). The reaction mixture was stirred

vigorously for 52 h (23 °C), and the solvent was removed in vacuo. The residual solid was suspended in water (1 mL), the suspension was made basic (pH 9) with the addition of 5% aqueous Na_2CO_3, and the insoluble solid was collected by centrifugation. The residual solid was suspended in water (1 mL), and the suspension was acidified (pH 1) with the addition of 10% aqueous HCl. The insoluble solid was collected by centrifugation and was washed with water (2 × 1 mL). Drying the solid in vacuo afforded **5a** (3.5 mg, 4.0 mg theoretical, 88%) as a gray-brown solid: ^{1}H NMR (Me_2SO-d_6, 200 MHz, ppm) 11.87 (br s, 1 H, NH), 11.63 (br s, 2 H, NH), 11.41 (br s, 1 H, NH), 8.36 (br s, 3 H, C4′, C4″, and C4‴-H), 8.04 (d, 1 H, J = 9 Hz, C4-H), 7.45–7.17 (m, 7 H), 7.02 (s, 1 H), 6.16 (s, 2 H, $CONH_2$), 4.70 (br m, 6 H, C2′, C2″, and C2‴-H), 4.03 (t, 2 H, J = 8 Hz, C2-H), 3.91 (s, 3 H, OCH_3), 3.28–3.54 (br m, 8 H, C1, C1′, C1″, and C1‴ obscured by H_2O); IR (KBr) ν_{max} 3410, 2943, 1701, 1611, 1578, 1507, 1425, 1364, 1343, 1284, 1253, 1210, 1185, 1146, 800, 756 cm^{-1}.

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Structure and Conformation of Halomycin B in Solid State and Solution

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Structural studies have been carried out on halomycin B ($C_{43}H_{58}N_2O_{12}$), an ansamycin antibiotic, to study the effect of the substitution at the 4-position of the naphthohydroquinone on the conformation of the ansa chain. The antibiotic crystallizes as monohydrate ethyl acetate solvate in the monoclinic space group $P2_1$, with the following cell dimensions: a = 12.362 (1), b = 12.846 (2), c = 16.160 (2) Å; β = 107.7 (1)°; Z = 2; D_{measd} = 1.24 g cm^{-3}. The structure was solved by repeated use of direct methods and Fourier synthesis and refined to the final R value of 0.052 for 3741 reflections. The conformation revealed indicates that, contrary to previous assumptions, the substitution at the C(4) position (substituted pyrrolidine, in this case) may affect the conformation of the middle part of the ansa chain, thus making halomycin B less active against the enzyme DNA-dependent RNA polymerase (DDRP). There is a trapped water molecule between the chromophore and the ansa chain. This water molecule is involved in hydrogen bonding with the O(10) of the ansa chain, the O(12) of the substituted pyrrolidine, and an oxygen of ethyl acetate. The NMR studies indicate that two isomers exist in chloroform solution. The major isomer (80%) has a conformation that is similar to that observed in solid state. The dynamic process involved in the interconversion of these two isomers is shown.

Halomycins that contain a substituted pyrrolidine ring at the C(4) position of rifamycin SV belong to a well-known class of antibiotics called ansamycins. Halomycins A, B, and C differ from each other in their hydroxylation pattern in the ansa chain or pyrrolidine moiety (Figure 1). They are produced by *Micromonospora halophytica*[1] and are highly active against gram-positive bacteria. In general, rifamycins are used for the treatment of tuberculosis and have some antitumor activity.

A number of studies have been done both in solid state[2-6] and in solution to correlate the conformation of the ansa chain and the biological activity of rifamycins. Attempts have also been made to study the effects of 3-substitution on the conformation of the ansa chain. Rifampicin, which is highly active, is a 3-substituted rifamycin SV. The present study has been carried out to study the molecular structure and conformation of halomycin B as well as the effect of 4-substitution on the conformation of the ansa chain and thus its biological activity.

Table I. Crystal Data for Halomycin B

$C_{43}H_{58}N_2O_{12}\cdot H_2O\cdot EtOAc$	V = 2444.4 Å^3
M_r 900.3	Z = 2
monoclinic, $P2_1$	D_{measd} = 1.24 g·cm^{-3}
a = 12.362 (1) Å	D_{calcd} = 1.23 g·cm^{-3}
b = 12.846 (2) Å	temp = −110 °C
c = 16.160 (2) Å	radiation, Mo Kα (λ = 0.710 69 Å)
β = 107.7 (1)°	$(\sin\theta)/\lambda_{max}$ = 0.62 Å^{-1}

Experimental Section

X-ray. Halomycin B was supplied by Dr. A. Ganguly of Scherring Corp. Yellow needle-shaped crystals were grown from a mixture of ethyl acetate and benzene after many unsuccessful attempts. The crystal data are given in Table I. Intensities of 5297 reflections, $4.0 \le 2\theta \le 53.0°$, were measured by use of Mo Kα radiation (λ = 0.710 69 Å) on a Syntex $P2_1$ diffractometer equipped with a graphite monochromator and a Syntex LT-1 inert gas (N_2) low-temperature delivery system, using the ω scan technique, a variable scan rate (2.0–6.0°), and a scan range of 2.0° with a scan to background ratio of 1. A total of 3741 reflections greater than $3\sigma(I)$ was considered observed. The intensities were corrected for Lorentz and polarization effects, but no absorption correction was applied.

After numerous unsuccessful attempts, a partial structure (16 atoms corresponding to the two fused six-membered rings and few substituents) was obtained by the direct-methods program MULTAN.[7] The complete structure (all non-hydrogen atoms) was obtained by the repeated use of the Fourier and difference Fourier

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