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BIS-DES-HYDROXY, BIS-DES-METHOXY CC-1065. SYNTHESIS, DNA BINDING, AND BIOLOGICAL ACTIVITY.

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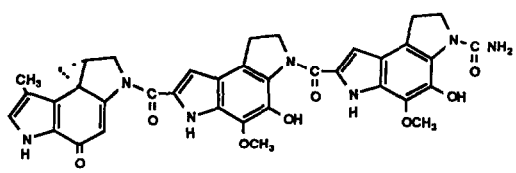
Abstract The preparation of an optically pure bis-des-hydroxy, bis-des-methoxy analog **(3a)** of the antitumor antibiotic CC-1065 **(1)** is described This compound displays a significantly lower induced circular dichroism in the presence of DNA than does **1**, indicating that the protected o-catechol substituents of **1** are important in stabilizing its non-covalent binding to DNA. Biological activities of **1** and **3a**, however, are similar, including the unusual phenomenon of delayed death in mice

Studies on the non-covalent binding and covalent bonding of the potent antitumor antibiotic, CC-1065 **(1)**, to DNA are unveiling a fascinating picture of molecular recognition with attendant implications for biological effects [1] Our studies with analogs of **1** have identified structural features of this class of agents which are important for biological properties 1) the chirality of the cyclopropyl ring, 2) the electrophilic reactivity of the left-hand segment, 3) the ring size of the central segment (presumably affecting conformational adaptability), and 4) the contour and length of the central and right-hand segments (determining hydrophobic and van der Waals interactions in the DNA minor groove) [2] One important compound developed in our analog program is shown by structure **2** This compound matches the high potency of **1**, but shows greatly superior antitumor efficacy in animal models and, in contrast to **1**, does not cause delayed death in mice [3]

To further explore the structural basis for the divergent biological properties of **1** and **2**, we targeted for synthesis the tetradesoxy analog **3a** In view of the significant DNA binding (as reflected by DNA-induced circular dichroism) of simplified structures like **2**,[2] as well as modeling of the interaction **1** with DNA,[1a-c] we surmised that close groove complementarity of the hydrophobic, concave surface of **1** was primarily responsible for its exceptional DNA binding parameters We anticipated that **3a**, which shares this hydrophobic surface, should closely match **1** with respect to DNA binding Recent crystallographic studies pointing to the importance of hydrophobic and van der Waals forces in the interaction of netropsin with the minor groove of DNA[4] encouraged this view According to this rationale the modified o-catechol substituents in the middle and right-hand segments of **1** appeared not to be critical in DNA binding Furthermore, these substituents seemed likely culprits in the delayed toxicity of **1**, possibly via oxidative pathways [5]



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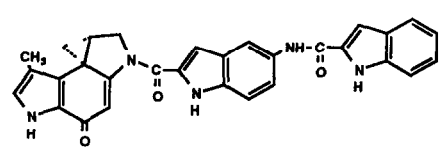

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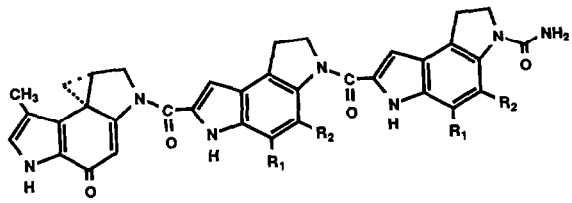


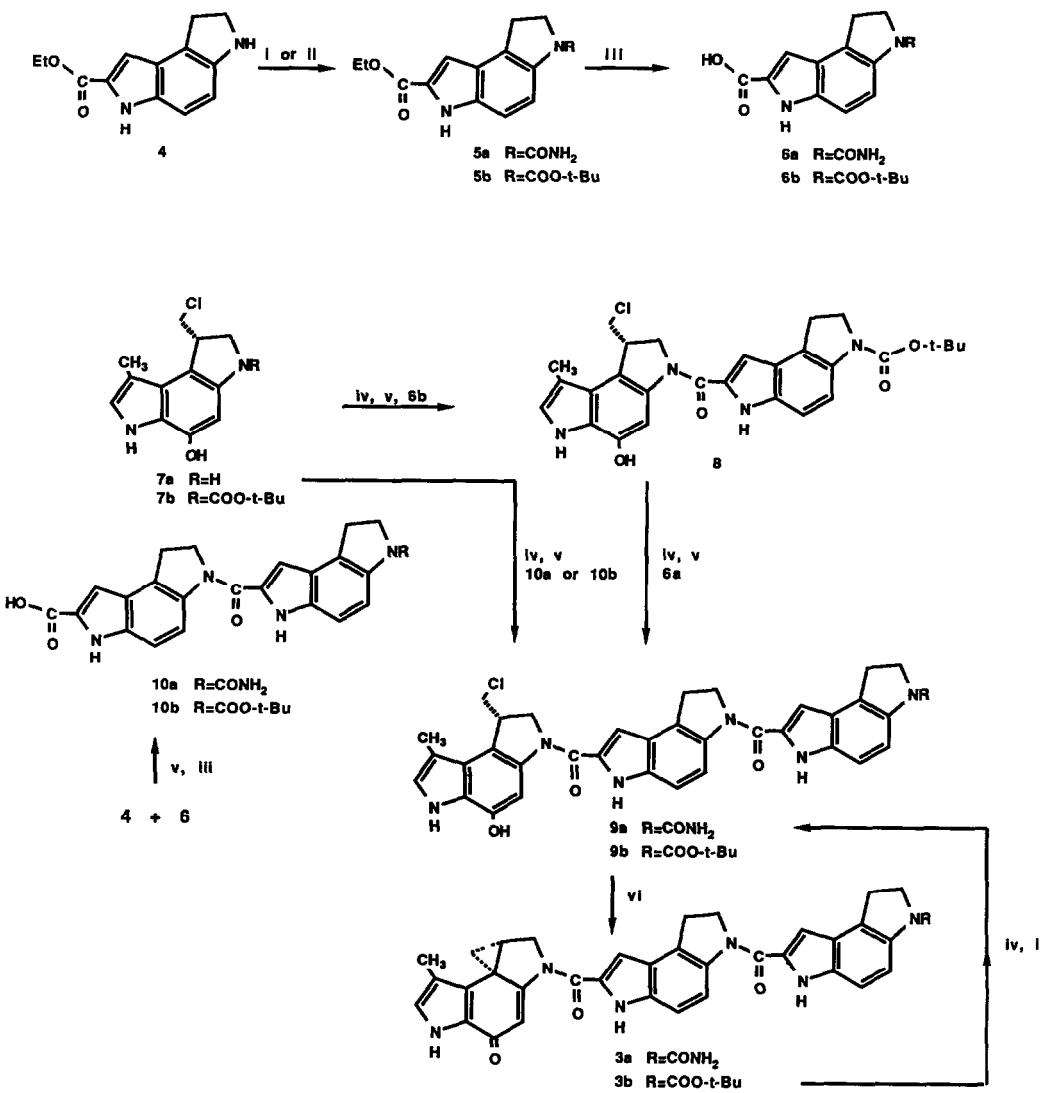
1 R_1=OCH_3, R_2=OH
3a R_1=R_2=H

Scheme 1 illustrates several strategies for the synthesis of **3a**, based on the coupling of central and right-hand segment carboxylic acids to the nascent left-hand segment, the optically pure **7a** generated by deprotection of **7b** [6] A "sequential" strategy proved most successful Thus, the 1,2-dihydro-3H-pyrrolo[3,2-e] indole **4**, prepared as previously described,[7,8] was converted to **5b** with BOC-ON® in 95% yield This was saponified to **6b** (89%) Similarly, **6a** was prepared by the reaction of **4** with potassium cyanate in acetic acid (52%), followed by saponification of **5a** (60% after chromotography) [9] Freshly prepared **7a** (from HCl deprotection of 0 16 mmol of **7b**) was condensed with 1 eq of **6b** in the presence of 2 eq of 1-ethyl-3-(3-dimethylamino propyl)-carbodiimide hydrochloride (EDC) in DMA This afforded **8** in 58% yield, after chromatography Removal of the BOC group with anhydrous HCl in ethyl acetate, followed by EDC promoted condensation with **6a** in DMA gave, after chromatography, a mixture of **9a** and **3a** Cyclization with triethylamine in aqueous acetonitrile afforded **3a** (60% from **8**) [10]

We also explored a convergent approach analogous to that used in the synthesis of **1** [6] Condensation of **4** with **6a**, followed by saponification, afforded **10a**[9] (70% from **4**) Repeated attempts to condense this highly insoluble acid with **7a** in DMF or DMA, in the presence of EDC, failed to produce more than traces of **9a** or **3a** To circumvent the extreme insolubility of **10a**, we prepared **10b** by the condensation of **4** with **6b**, followed by saponification Although **10b** resisted purification attempts, it did condense with **7a** in DMA, in the presence of EDC Extraction and chromatography afforded a mixture of **9b** and **3b**, which was converted to **3b** with triethylamine (17% yield from **7b**) Ring opening and removal of the BOC group with HCl, followed by reaction with potassium cyanate in acetic acid, gave an impure preparation of **9a** Cyclization with triethylamine, followed by reverse phase chromatography, gave **3a** in about a 60% yield from **3b**, but the purity by this route remained unsatisfactory

Table 1 compares some DNA binding and biological properties of **1**, **2**, **3a** and **3b** Contrary to our expectations, **3a** showed only a slightly higher DNA induced circular dichroism (ICD) than did **2**, and far short of **1** This result suggests that the oxygen substituents of the middle and right-hand portions of **1** are more important in promoting a tight binding complex with DNA than we had previously appreciated The lower ICD of **3b** may reflect steric inhibition to binding in the DNA minor groove The biological properties of **3a** and **3b**, on the other hand, resemble those of **1** much more than of **2** Not only do the additional ethylene bridges on the central and right-hand indole units correlate with a loss of curative activity against P388 leukemia, but they also correlate with the unusual delayed death phenomenon shown by the natural product Thus the o-catechol substituents of **1** are not required for the expression of this toxicity

Scheme 1



I KOCN, HOAc, H_2O, reflux, 15 min, II BOC-ON^R, Et_3N, THF, 25 C, 24 h, III LIOH, pyridine, DMF, 60-70 C, then aqu HCl, Iv HCl, EtOAc, 25 C, 15-30 min, v EDC, DMF or DMA, 25 C, 24 hr, vi Et_3N, CH_3CN, H_2O, 25 C

Table 1 DNA Binding and Bioactivities

Compound	ICD[a] x10^{-3}	ID_{50}[b]	P388 in vivo[c]		Delayed[d] Death
			%ILS	O D	
1	280	0 05	62	0 10	yes
2	120	0 004	(4)	0 025	no
3a	128	0 05	77	0 10	yes
3b	65	0 006	67	0 10	yes

a ICD = induced circular dichroism, expressed as molar ellipticity 11 x 10^{-5} M calf-thymus DNA, 0 85 x 10-5 M drug, 0 01 M phosphate, pH 7 2, 25 °C, 24 hr, at long wavelength λ max b ID_{50} = nanomolar concentration of drug required to inhibit, by 50%, the growth of murine L1210 cells in a 3 day assay c Drug given intraperitoneally to mice implanted intraperitoneally with 10^6 P388 leukemia cells %ILS = percent increase in life span of treated mice over that of control tumored mice, at the optimal dose Parentheses indicate >30 day survivors, or cures, out of a group of 6 O D = optimal dose in mg/kg/injection on a days 1, 5, and 9 schedule d Therapeutic doses of drug administered intravenously to non-tumored mice, and followed for 90 days Most deaths occurred between 40 and 50 days following drug administration

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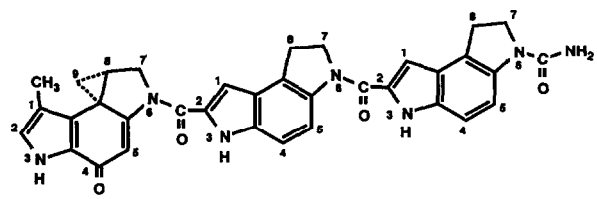
8 All compounds reported were homogeneous by TLC unless otherwise stated and gave satisfactory IR, NMR, M S and exact mass and/or combustion analysis

9 Compounds **6a**, **6b**, and **10a** gave identical spectra to those reported for these substances in Ref 5

10 Spectral properties for compound **3a** IR (micro KBr) 3398, 1622, 1612, 1582, 1505, 1431, 1394, 1363, 1336, 1264 cm^{-1}, HRMS (FAB) m/e 612 2385 ($C_{35}H_{29}N_7D_4$ requires 612 2359), uv (MeOH) 318 nm (ε = 40,000), 355 (34,000), CD (DMF) 370 (7000), 328 (0), 315 (-19500), 302 (0), 287 (35000), ^{1}H NMR (DMF-d_7, 500 MHz COSY), δ 11 82 (s, 1H, NH), 11 52 (s, 2H, NH), 8 39 (bs, C4'-H), 8 14 (d, 1H, J = 9 Hz, C4"-H), 7 48 (d, 1H, J = 9Hz, C5'-H), 7 38 (d, 1H, J = 9Hz, C5"-H), 7 26 (s, 1H, C1'-H), 7 08 (s, 1H, C1"-H), 6 99 (s, 1H, C2-H), 6 79 (s, 1H, C5-H), 6 15 (s, 2H, $CONH_2$), 4 74 (t, 2H, C7'-H_2), 4 57 (m, 2H, C7-H_2) 4 15 (t, 2H, C7"-H_2), 3 53 (t, 2H, C8"-H_2), 3 40 (t, 2H, C8"-H_2), 3 24 (m, 1H, C8-H), 2 07 (s, 3H, Cl-CH_3), 2 04 (q, 1H, C9-H), 1 49 (t, 1H, C9-H) We thank Terry Scahill for assistance in running and interpreting this spectrum



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