C_2H_5OH, 60507-87-1; $CpNi^+\cdot(CH_3)_2O$, 60507-94-0; $CpNi^+\cdot(CH_3)_2NH$, 60508-24-9; $CpNi^+\cdot(CH_3)_2CO$, 60508-14-7; $CpNi^+\cdot(CH_3)_3N$, 60508-23-8; $CpNi^+\cdot(C_2H_5)_2O$, 60508-18-1; $CpNi^+\cdot H_2O$, 111846-73-2; $CpNi^+\cdot(CH_3)_2S$, 60508-19-2; $CpNi^+\cdot(CH_3)_3COH$, 60508-16-9; $H^+\cdot CH_3Cl$, 65967-47-7; $H^+\cdot CH_3NH_2$, 17000-00-9; $H^+\cdot C_5H_5N$, 16969-45-2; $H^+\cdot(C_2H_5)_3N$, 17440-81-2; $H^+\cdot H_2S$, 18155-21-0; $H^+\cdot HCN$, 21107-92-6; $H^+\cdot H_2CO$, 18682-95-6; $H^+\cdot CH_3OH$, 17836-08-7; $H^+\cdot CH_3CN$, 20813-12-1; $H^+\cdot CH_3CHO$, 18682-96-7; $H^+\cdot C_2H_5OH$, 18639-79-7; $H^+\cdot(CH_3)_2O$, 17009-82-4; $H^+\cdot(CH_3)_2NH$, 17000-01-0; $H^+\cdot(CH_3)_3N$, 16962-53-1; $H^+\cdot C_2H_5NH_2$, 16999-99-8; $H^+\cdot CH_3C(O)N(CH_3)_2$, 52754-55-9; $H_3O^+\cdot CH_3C(O)N(CH_3)_2$, 129916-57-0; $(H_2O)_2H^+\cdot CH_3C(O)N(CH_3)_2$, 129916-58-1; $(H_2O)_3H^+\cdot CH_3C(O)N(CH_3)_2$, 129916-59-2; $(H_2O)_4H^+\cdot CH_3C(O)N(CH_3)_2$, 129916-60-5; $CH_3^+\cdot H_2S$, 18683-23-3; $CH_3^+\cdot H_2CO$, 23653-97-6; $CH_3^+\cdot CH_3Cl$, 24400-15-5.

Supplementary Material Available: Tables of calculated and experimental enthalpies for systems used in the final data fit and for hydrated proton systems omitted from the final data fit (20 pages). Ordering information is given on any current masthead page.

Duocarmycin–Pyrindamycin DNA Alkylation Properties and Identification, Synthesis, and Evaluation of Agents Incorporating the Pharmacophore of the Duocarmycin–Pyrindamycin Alkylation Subunit. Identification of the CC-1065–Duocarmycin Common Pharmacophore

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Abstract: A demonstration and subsequent study of the DNA covalent alkylation properties of duocarmycin A and duocarmycin C_1 and C_2 (pyrindamycin B and A, respectively) are detailed and have led to the identification of two high affinity binding sites [5′-d(A/TAAA̲)-3 and 5′-d(A/TTTA̲Pu)-3′] within a full set of available alkylation sites [5′-d(AAA̲)-3′ > 5′-d(TTA̲)-3′ > 5′-d(TAA̲)-3′ > 5′-d(ATA̲)-3′] that proceeds through 3′-adenine N-3 alkylation of the duocarmycin A activated cyclopropane similar to the (+)-CC-1065 covalent alkylation of DNA. The synthesis of **10** (CI-TMI) incorporating the parent 1,2,7,7a-tetrahydrocycloprop[1,2-*c*]indol-4-one (CI) alkylation subunit of duocarmycin is described and the results of its comparative evaluation (in vitro cytotoxic activity and DNA covalent alkylation properties) demonstrate that **10** constitutes an agent bearing the minimum potent pharmacophore of the duocarmycin DNA alkylation subunit and the common pharmacophore of the duocarmycin–CC-1065 alkylation subunits.

Two independent efforts have disclosed the isolation, structure determination, and preliminary evaluation of a new class of antitumor antibiotics now including duocarmycin A[2–4] (**2**), duocarmycin B_1 and B_2 (**3** and **4**),[6] duocarmycin C_1[3–5] (**5**, pyrindamycin B[7]), and duocarmycin C_2[3] (**6**, pyrindamycin A[7])[8] (Figure 1). The structural similarities between the duocarmycins and (+)-CC-1065 (**1**)[9–13] suggest that the agents may be acting by a common or related mechanism initiated with the irreversible covalent alkylation of DNA. Herein, we report full details of studies that provide a demonstration of the formation of duocarmycin–DNA covalent adducts in a reaction analogous to that observed with (+)-CC-1065[9] and report the comparative DNA binding properties of duocarmycin A, C_1, C_2 and (+)-CC-1065 (**1**) that provide support for the potential that the agents may be acting by a common mechanism derived from the irreversible covalent alkylation of DNA.

The preparation of **10** incorporating the parent 1,2,7,7a-tetrahydrocycloprop[1,2-*c*]indol-4-one (CI)[14,15] left-hand subunit of the duocarmycins and (+)-CC-1065 is detailed as is that of the stable precursors **7–9**[19] (Scheme I). A recent demonstration[15] of the comparable DNA alkylation selectivity of racemic (±)-*N*-BOC-CI (**11**) and (+)-*N*-BOC-CPI (**12**) that has proven distinct from that of (+)-CC-1065[16] coupled with the results of a study of the comparative DNA binding properties and cytotoxic activity of **7–12** with that of the duocarmycins detailed herein illustrate that **10** embodies the required, but not necessarily optimal, structural and functional features of the duocarmycin left-hand subunit that is responsible for their DNA alkylation properties. That is, **10** represents an agent bearing the minimum pharma-

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(1) (a) On sabbatical leave from Kyorin Pharmaceutical Co., Ltd. Tochigi, Japan, 1988–1990. (b) American Cancer Society postdoctoral fellow (Grant No. PF-3311).

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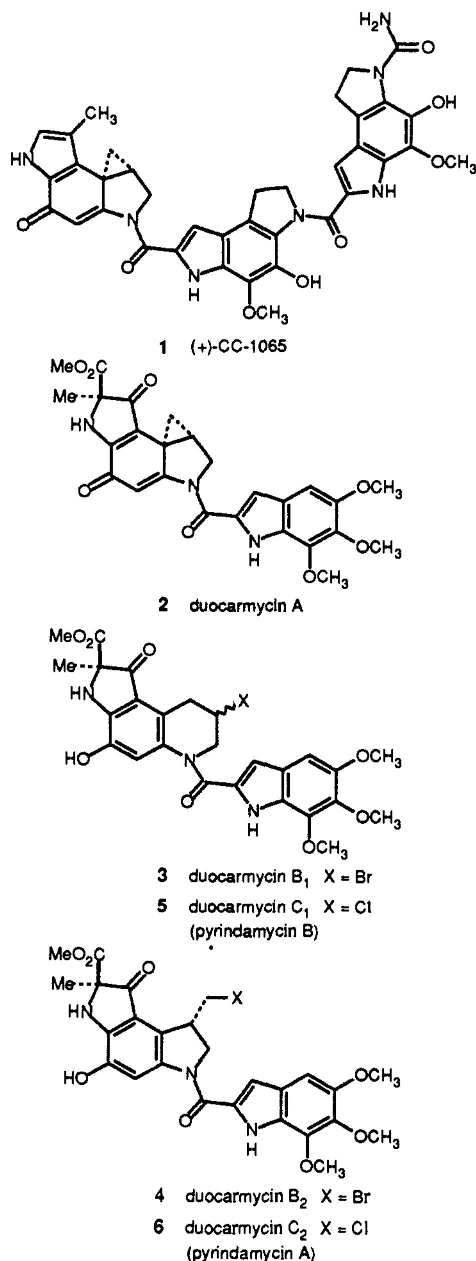
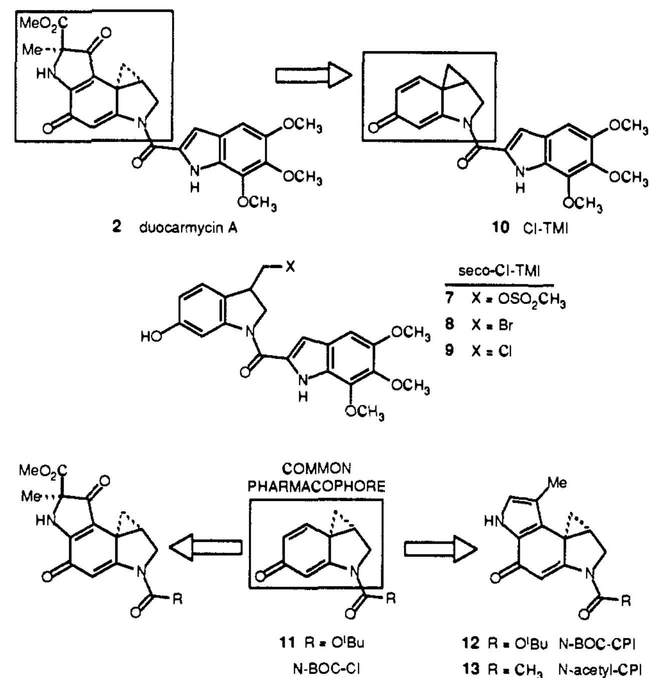


Figure 1.

cophore of the duocarmycin DNA alkylation subunit with the incorporation of the common pharmacophore of the duocarmycin–CC-1065 DNA alkylation subunits.

Preparation of 7–10. The Duocarmycin Pharmacophore. In a recent study, we have detailed the implementation of a self-terminating 5-exo-trig aryl radical-alkene cyclization for the indirect preparation of the C-3 functionalized indolines **14–16**.[14] The prohibitive reactivity of the CI derivatives such as **10–11**[14] along with the recognition that CI itself would not be expected to couple productively with activated derivatives of carboxylic acids including those of 5,6,7-trimethoxyindole-2-carboxylic acid (**22**) suggested that the final step in the preparation of **10** would optimally involve introduction of the reactive cyclopropane. Consequently, the approach employed in the preparation of **10** was based on a final Winstein Ar-3′ alkylation[14,17-21] of an appropriately C-3 functionalized 3-methyl-6-hydroxyindoline, e.g. **7–9**, which in turn was anticipated to be derived from penultimate coupling of readily available **14–16**[14] with 5,6,7-trimethoxyindole-2-carboxylic acid (**22**).[22]

Scheme I



Acid-catalyzed deprotection of **14** and **16** (3 N HCl–EtOAc, 95–100%) followed by immediate coupling of the unstable indoline hydrochlorides with 5,6,7-trimethoxyindole-2-carboxylic acid (**22**) provided **7** and **9** (77% and 85%) (Scheme II). In turn, **8** was prepared from **7** by bromide displacement of the primary methanesulfonate (10 equiv of LiBr, DMF, 60 °C, 74%). Initial attempts to promote the Ar-3′ alkylation directly on mesylate **7**

(8) The absolute configuration of **2–6** represented herein is derived from that unambiguously established for pyrindamycin A (**6**)[7] by X-ray and assumed for **2–5** on the basis of a presumed common biosynthetic origin. The indistinguishable selectivity of the DNA covalent alkylation profiles of **2**, **5**, and **6** detailed herein is consistent with this representation of the absolute configuration of **2**, with the 3*S* (α-orientation) stereochemistry for **3** and **5**, and is analogous to that of (+)-CC-1065. The chemical interconversions of the agents and correlation, $[\alpha]_D$, confirmed those assignments.

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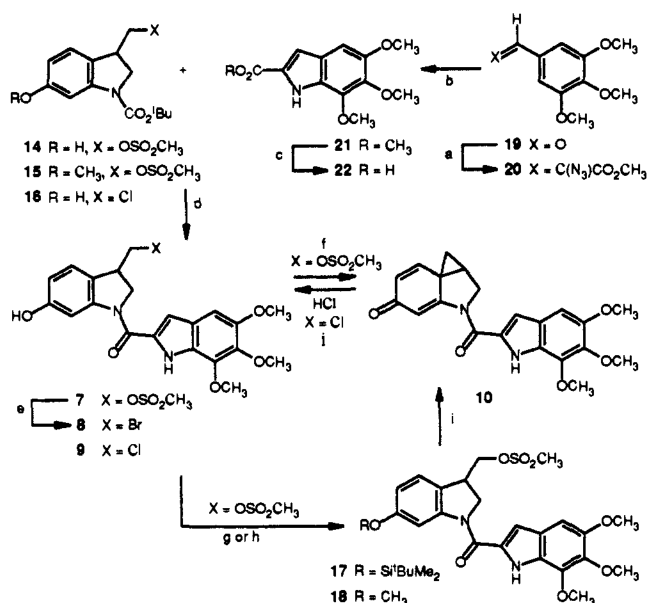
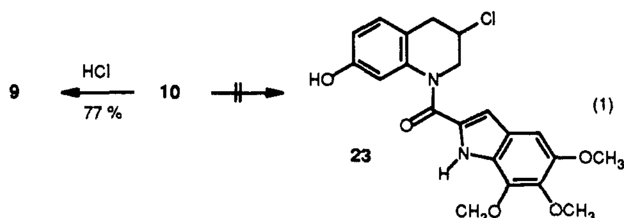
Scheme II[a]



[a](a) 10 equiv of $N_3CH_2CO_2CH_3$, 8 equiv of $NaOCH_3$, MeOH, 0 °C, 5 h, 98%. (b) 140 °C, xylenes, 4 h, 75%. (c) 3.4 equiv of LiOH, THF–MeOH–DMSO–H_2O (3:1:1:1), 23 °C, 18 h, 100%. (d) For **7**: 3 N HCl–EtOAc, 24 °C, 30 min; 1 equiv of **22**, 3 equiv of EDCI, DMF, 24 °C, 19 h, 77%. For **9**: 3 N HCl–EtOAc, 24 °C, 20 min; 0.89 equiv of **22**, 2.9 equiv of EDCI, 3.9 equiv of $NaHCO_3$, DMF, 24 °C, 22 h, 85%. (e) 10 equiv of LiBr, DMF, 60 °C, 4 h, 74%. (f) 1.5 equiv of NaH, THF–DMF (2:1), 0 °C, 2 h. (g) For **17**: 2 equiv of TBDMSCl, 2.5 equiv of Et_3N, 0.1 equiv of DMAP, CH_2Cl_2–DMF (5:1), 24 °C, 15 h, 89%. (h) For **18**: CH_2N_2, MeOH–ether, 24 °C, 2 h, 90%. (i) 0.9 equiv of $(nBu)_4NF$, THF, 24 °C, 30 min, 61%. (j) 3 N HCl–EtOAc 24 °C, 20 min, 77%.

with closure to the cyclopropane employing a range of reaction conditions (NaH, THF–DMF, 25 °C, 10–40%; Et_3N, THF; DBU, CH_3CN; $NaHCO_3$, THF–H_2O) provided only modest conversions to **10**. Optimal conversion of **7** to **10** was achieved by conversion of **7** to the phenol *tert*-butyldimethylsilyl ether **17** ($tBuMe_2SiCl$, Et_3N, CH_2Cl_2–DMF, 89%) followed by fluoride-induced silyl ether deprotection of **17** under dilute reaction conditions (0.005 M) with clean phenoxide generation and subsequent in situ closure to **10**. By employing this procedure[23] and chromatographic purification of **10** on triethylamine-deactivated silica gel, CI-TMI (**10**) may be obtained routinely in yields ≥60%.

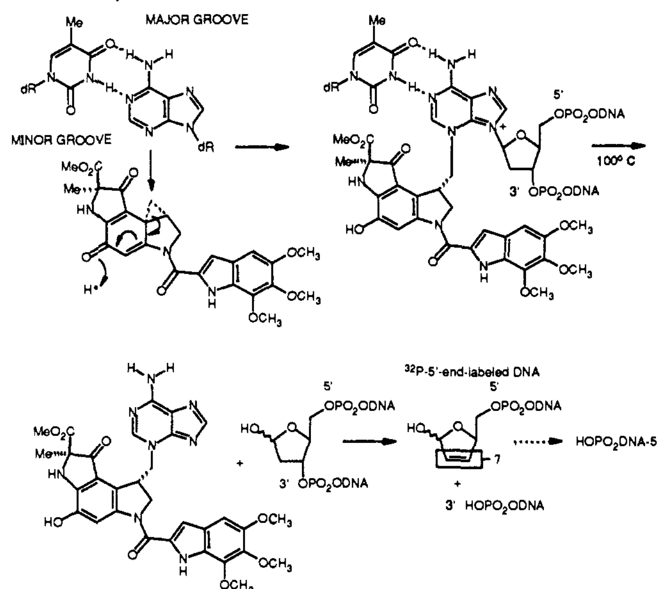
The cleavage of the duocarmycin A cyclopropane under protic conditions (1% aqueous KBr–acetone) has been shown to provide duocarmycin B_1 (20%) and duocarmycin B_2 (80%).[6] In contrast, treatment of **10** with excess KBr (27 equiv) under protic conditions (H_2O–acetone, 1:1) provided **8** and treatment of **10** with hydrochloric acid under anhydrous conditions (3 N HCl–EtOAc) provided **9** (77%) without evidence of cyclopropane cleavage with ring expansion to provide **23** (eq 1).



DNA Binding Properties. The demonstration of the event, site, and relative selectivity of the DNA alkylation of the agents (**1–2**, **5–13**) was obtained from the thermally induced strand cleavage of double-stranded DNA after exposure to the agents.[9,15,16,24-27] The DNA alkylation of (+)-CC-1065 (**1**) and that proposed for duocarmycin A, C_1, and C_2 (**2**, **5**, and **6**, respectively, Scheme III),

(23) Saunders, D. G. *Synthesis* **1988**, 377.

Scheme III. Duocarmycin A DNA Alkylation and Thermally induced Depurination Reaction



were examined within five clones of M13mp10 harboring SV40 nucleosomal DNA containing the following inserts: w794 (SV40 nucleotide no. 5238-138) and its complement w836 (nucleotide no. 5189-91), c988 (nucleotide no. 4359-4210) and its complement c820 (nucleotide no. 4201-4356), and c1346 (nucleotide no. 1632-1782).[28] The clones contain sequences that map the SV40 regulatory region (w clones) or include prototype sequences implicated in reacting with (+)-CC-1065 (c clones). Thus, employing the five singly 5′-^{32}P-end-labeled SV40 double-stranded DNA fragments derived from clones w794, w836, c988, c820, and c1346,[27-28] a range of concentrations of the agents was incubated with the labeled DNA (24 h, 4 °C), unbound agent was removed by ethanol precipitation of the DNA, and a solution containing the agent–DNA covalent complexes was warmed to 100 °C (30 min) to induce strand cleavage at the sites of covalent alkylation, Scheme III.[29] Electrophoresis of the resulting DNA under denaturing conditions alongside Sanger dideoxynucleotide sequencing reactions followed by autoradiography permitted the identification of the sites of DNA covalent alkylation.[30-31] Similarly, the comparative sites of DNA alkylation of **7–13** were examined within w794 DNA.

Duocarmycin A, C_1, C_2 and (+)-CC-1065. The examination of the DNA alkylation properties of duocarmycin A, C_1, and C_2 revealed that the sites of covalent alkylation for the three agents proved indistinguishable. This suggests that a common agent (duocarmycin A) constitutes the pertinent alkylating agent requiring that duocarmycin C_1 and C_2 be converted to **2** prior to DNA alkylation and/or that duocarmycin C_2 may serve as an effective alkylating agent in its own right but displays sequence-selective DNA alkylation properties indistinguishable from the parent agent. A summary of the results derived from the examination of the duocarmycins covalent alkylation properties within the five segments of double-stranded DNA (750 base pairs) is presented in Table I and is illustrated with the representative comparison provided in Figure 2. The remaining comparisons

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and a summary may be found in supplementary material, Figures 8–12. In each instance of the alkylation sites detected through the thermally-induced strand cleavage of DNA, the site of alkylation proved to be adenine[9] flanked by two 5′-A or T bases and there proved to be a strong preference for the sequence of the three base pairs that follows the order of 5′-(AAA̲)-3′ > 5′-(TTA̲)-3′ > 5′-(TAA̲)-3′ > 5′-(ATA̲)-3′. In addition, the duocarmycin alkylation exhibited a strong but not requisite sensitivity to the fourth base pair preferring an A or T versus G or C base in the fourth 5′-position. Moreover, the lower affinity alkylation [5′-(TAA̲)-3′, 5′-(ATA̲)-3′] preferentially selected sites with a purine (A or G) versus pyrimidine (T or C) base preceding the adenine alkylation site and this sensitivity to the base preceding the alkylation site was not observed as prominently in the high affinity sites [5′-(AAA̲)-3′, 5′-(TTA̲)-3′]. Although the selectivity of the duocarmycin A, C_1, and C_2 DNA alkylations proved indistinguishable, the intensity of the covalent alkylation as judged by the concentration of agent required for its observation proved dependent on the agent structure and the incubation conditions. Under the incubation conditions of 4 °C (24 h) duocarmycin A exhibited a more intense DNA covalent alkylation than duocarmycin C_1 or C_2 ($C_2 > C_1$) (Figure 2), whereas under the conditions of 37 °C (24 h) both duocarmycin C_1 and C_2 ($C_1 \geq C_2$) exhibit a more intense alkylation than duocarmycin A. As extrapolated qualitatively from the concentration of agent required for observation of the alkylation, this agent dependence on the reaction conditions can be attributed to the relative availability (e.g, stability) of the agents under the reaction conditions and their relative rates of DNA adenine alkylation ($A > C_2 > C_1$).

Table I. Summary of Alkylation Sites for Duocarmycin A, C_1, and C_2

sequence	AS/TS[a]	high affinity[b]	low affinity[b]
5′-(NAAA̲N)-3′	26/39 (67%)	12 (31%)	14
5′-(NTTA̲N)-3′	9/19 (47%)	2 (10%)	7
5′-(NTAA̲N)-3′	7/19 (36%)	0 (00%)	7
5′-(NATA̲N)-3′	3/18 (17%)	0 (00%)	3
5′-(NAAA̲N)-3′			
5′-(A/T̄AAA̲N)-3′	21/27 (78%)	11 (41%)	10
5′-(TAAA̲N)-3′	3/6 (50%)	2 (33%)	1
5′-(AAAA̲N)-3′	18/21 (86%)	9 (42%)	9
5′-(G/C̄AAA̲N)-3′	5/12 (42%)	1 (08%)	4
5′-(A/TAAA̲Pu)-3′	16/19 (84%)	9 (47%)	7
5′-(A/TAAA̲Py)-3′	5/8 (63%)	2 (25%)	3
5′-(NTTA̲N)-3′			
5′-(A/T̄TTA̲N)-3′	7/10 (70%)	2 (20%)	5
5′-(ATTA̲N)-3′	3/4 (75%)	2 (50%)	1
5′-(TTTA̲N)-3′	4/6 (67%)	0 (00%)	4
5′-(G/C̄TTA̲N)-3′	2/9 (22%)	0 (00%)	2
5′-(A/TTTA̲Pu)-3′	3/3 (100%)	2 (66%)	1
5′-(A/TTTA̲Py)-3′	4/7 (57%)	0 (00%)	4
5′-(NTAA̲N)-3′			
5′-(A/T̄TAA̲N)-3′	6/12 (50%)	0 (00%)	6
5′-(ATAA̲N)-3′	3/9 (33%)	0 (00%)	3
5′-(TTAA̲N)-3′	2/3 (67%)	0 (00%)	2
5′-(G/C̄TAA̲N)-3′	1/7 (14%)	0 (00%)	1
5′-(A/TTAA̲Pu)-3′	5/6 (83%)	0 (00%)	5
5′-(A/TTAA̲Py)-3′	1/6 (17%)	0 (00%)	1
5′-(NATA̲N)-3′			
5′-(A/T̄ATA̲N)-3′	2/11 (18%)	0 (00%)	2
5′-(AATA̲N)-3′	2/5 (40%)	0 (00%)	2
5′-(TATA̲N)-3′	0/6 (00%)	0 (00%)	0
5′-(G/C̄ATA̲N)-3′	1/7 (14%)	0 (00%)	1
5′-(A/TATA̲Pu)-3′	3/8 (37%)	0 (00%)	3
5′-(A/TATA̲Py)-3′	0/3 (00%)	0 (00%)	0

[a] Number of alkylated sites (AS)/number of total sites (TS) available. [b] Intensity of alkylation at the sites, high = high affinity site observed at lowest agent concentrations, low = low affinity site observed at higher agent concentrations. The expressed percent is that of the number of high affinity alkylation sites (HAAS)/number of available total sites (TS).

Selectivity:

duocarmycin A = duocarmycin C_2 = duocarmycin C_1

three base-pair binding selectivity =
5′-(AAA̲)-3′ > 5′-(TTA̲)-3′ > 5′-(TAA̲)-3′ > 5′-(ATA̲)-3′

fourth base-pair sensitivity =
5′-(A/TXXA̲)-3′ ≫ 5′-(G/CXXA̲)-3′

preceding base-pair sensitivity =
5′-(NXXA̲Pu)-3′ ≥ 5′-(NXXA̲Py)-3′

high affinity consensus sequences =
5′-(A/TAAA̲)-3′ and 5′-(T/ATTA̲Pu)-3′

(29) The thermally induced cleavage of DNA employed to identify alkylation sites[24] detects only DNA adducts susceptible to thermal glycosidic bond cleavage (adenine N-3 alkylation, guanine N-3, and N-7 alkylation). Other alkylation sites involving nucleophilic centers in DNA (e.g. guanine C-2 NH_2 and thymine C-2 O) are not detected under such conditions. The initial studies detailed herein have focused on the demonstration of the potential that the duocarmycins may alkylate DNA in a fashion comparable to (+)-CC-1065. Current efforts are addressing the efficiency of the duocarmycin adenine N-3 alkylation, chemical characterization of the thermally released duocarmycin–adenine covalent adduct, the potential of additional, as yet undetected alkylation sites, and the results of these studies will be detailed in due course. In preliminary studies, the duocarmycin A adenine covalent alkylation has been shown to account for a minimum of 50% of the consumption of the agent in the presence of calf thymus DNA (170 base-pair equiv, pH = 7.4, 25 °C, 7 days). The isolation and purification (50% recovery, Sephadex LH-20, 0–95% MeOH–H_2O) of the thermally release duocarmycin–adenine covalent adduct (Scheme III, 25 min at 100 °C) has been accomplished. Preliminary characterization: ^1H NMR (15% CD_3OD–$CDCl_3$, 500 MHz, ppm) 7.85 (s, 1 H, adenine C8-H), 7.84 (bs, 1 H, C7-H), 7.75 (s, 1 H, adenine C2-H), 6.83 (s, 1 H, C3′-H), 6.82 (s, 1 H, C4′-H), 5.04 (apparent d, 1 H, J = 10.7 Hz, C5-H), 4.79 (dd, 1 H, J = 4.5, 13.7 Hz, C*H*H-Ad), 4.72 (dd, 1 H, J = 6.7 Hz, 13.7 Hz, CH*H*-Ad), 4.41 (dd, 1 H, J = 9.3, 10.9 Hz, C5-H), 4.17 (m, 1 H, C4-H), 4.09 (s, 3 H, OCH_3), 3.95 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.76 (s, 3 H, CO_2CH_3), 1.67 (s, 3 H, C2-CH_3); UV (H_2O–MeOH) λ_{max} 340 nm (ϵ 28 000), 294 nm (ϵ 27 000); FABHRMS (DTT/DTE), m/e 643.2278 (M^+ + H (base), $C_{31}H_{30}N_8O_8$ + H^+ requires 643.2265). Full details of this work will be disclosed in due time. The adenine N-9 quaternized nitrogen represented in Scheme III constitutes a resonance structure of the adenine N-3 quaternization and has been employed to highlight the origin of the thermal glycosidic cleavage arising from adenine N-3 alkylation. The adenine structure within the thermally released adduct follows that established by single-crystal X-ray analysis for 3-ethyl adenine; Petersen, C. S.; Furberg, S. *Acta Chem. Scand. Ser. B* **1975**, *B 29*, 37. See also: Baker, B. F.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 2700.

(30) The 3′-end heterogeneity in the gels (double bands) observed with the use of 5′-end-labeled DNA (not observed with 3′-end-labeled DNA) as detailed herein deserves special comment. The thermal cleavage of 5′-end-labeled DNA often leads to pairs of closely migrating bands in which the major band observed at higher molecular weight presumably constitutes the thermal cleavage product still containing the modified sugar (presumably a 2,3-dehydro-2,3-dideoxyribose or subsequent derivative). The minor band observed at lower molecular weight has been assumed to constitute a subsequent β-elimination product with complete loss of the 3′ modified sugar and, as such, constitutes DNA containing a 3′-phosphate at the 5′ side of the strand break. Confirmation that these assumptions are at least valid was derived from the observation that this end heterogeneity may be removed by piperidine treatment providing a cleavage product (minor band at lower molecular weight) that comigrates with the Maxam–Gilbert adenine reaction. In these studies, thermal treatment for longer than 30 min (100 °C) has not led to additional detectable cleavage and thus, like (+)-CC-1065, suggests that the strand-cleavage reaction is a simple function of the adenine *N*-3 alkylation unaffected by the nature of the alkylating agent.

(31) Electrophoresis was carried out under conditions that maximize the resolution of SV40 DNA examined. Not shown in the gels is the remaining 5′-end 55 base pairs (short fragment of end-labeled DNA) derived from the DNA of the M13mp10 clones. This 59 base-pair region, judiciously chosen [3′-d(CCCCTAGGAGATCTCAGCTGGACGTCGGGTTCGAACCG-TGACCGGCAGCAAAATG*)-5′; * denotes ^{32}P end-label], contains a single (+)-CC-1065 alkylation site proximal to the 5′-end label. The apparent complete consumption of radioactivity on the gels at high agent concentrations constitutes complete cleavage of all 5′-end-labeled double-stranded DNA at this site. The competitive alkylation at this single site does not affect the relative selectivity of the examined sites within the SV40 DNA (especially at the relevant low agent concentrations constituting single alkylation events on the DNA) but does ensure that the multiple alkylations of a double-stranded segment of DNA results in cleavage of DNA to a single short fragment of DNA that is not examined on the gel. This ensures that those unfamiliar with the interpretation of the gels at high agent concentrations don't misinterpret the gel fade to a single short fragment of 5′-end-labeled DNA as increasing selectivity.

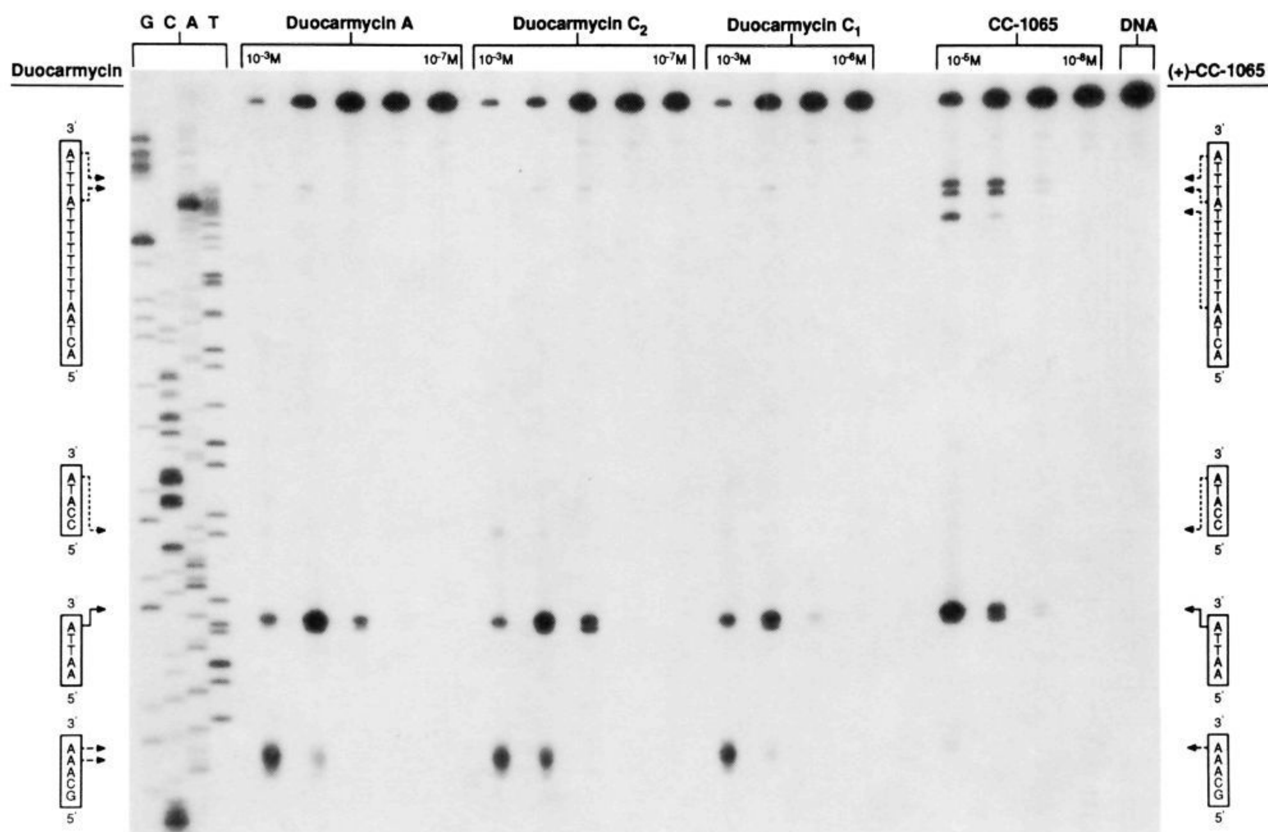


Figure 2. Thermally induced strand cleavage of double-stranded DNA (SV40 DNA fragment, 144 bp; nucleotide no. 5238-138, clone w794) after 24-h incubation of agent-DNA at 4 °C followed by removal of unbound agent and 30-min incubation at 100 °C; 8% poly(acrylamide) gel and autoradiography: lanes 1-4, Sanger G, C, A, and T reactions, lanes 5-9, duocarmycin A (**2**, 1×10^{-3} to 1×10^{-7} M); lanes 10-14, duocarmycin C_2 (**6**, 1×10^{-3} to 1×10^{-7} M); lanes 15-18, duocarmycin C_1 (**5**, 4 °C, 1×10^{-3} to 1×10^{-6} M); lanes 19-22, (+)-CC-1065 (**1**, 1×10^{-5} to 1×10^{-8} M); lane 23, control DNA.

Intensity (4 °C): duocarmycin A ≥
duocarmycin C_2 (ca. 1-10×) >
duocarmycin C_1 (ca. 5-10×)

Intensity (37 °C): duocarmycin $C_2 \simeq$ duocarmycin C_1 >
duocarmycin A (ca. 5-10×)

Moreover, there proved to be a striking similarity in the profile of the duocarmycin DNA alkylation with that of (+)-CC-1065 (Figure 2). Despite the striking similarity, there are subtle and occasional marked[32] distinctions in the profile of the duocarmycin and (+)-CC-1065 DNA alkylations. This is accurately reflected in the comparison of the (+)-CC-1065 and duocarmycin profile of DNA alkylation within w794 DNA where the agents share a common, high affinity covalent alkylation site, and distinctions may be observed in the presence and relative intensity of the remaining alkylation sites for the agents (Figure 2).

Selectivity: (+)-CC-1065 $\simeq$ duocarmycin A

CC-1065 high affinity sites:[9] 5'-d(AAAA<u>A</u>)-3' and
5'-d(PuNTT<u>A</u>)-3', [5'-d(A/TA/T<u>A</u>)-3'][24]

duocarmycin high affinity sites: 5'-d(A/TAA<u>A</u>)-3' and
5'-d(T/ATT<u>A</u>Pu)-3'

Intensity (4 °C): (+)-CC-1065 ≥ duocarmycin A (ca. 1-5×)

(+)-, (−)-, and (±)-*N*-BOC-CI (11) versus (+)-*N*-BOC-CPI (12). In a preceding study employing derivatives of CI and CPI, simple *N*-acyl derivatives of CI were shown to possess characteristics consistent with its assignment as the pharmacophore of the alkylation subunit (CPI) of CC-1065.[15] In these studies, (+)-*N*-acetyl-CPI (**13**) and (+)-*N*-BOC-CPI (**12**) were shown to possess a much less selective profile of DNA alkylation than (+)-CC-1065 which, as previously detailed,[24] proved to be several orders of magnitude less intense than that of (+)-CC-1065 (10^4-10^7×, Figures 13 and 14, supplementary material). *N*-BOC-CI (**11**) was demonstrated to be an effective DNA alkylating agent and consistent with its inherent reactivity was shown to alkylate DNA under milder conditions than (+)-*N*-BOC-CPI (**12**, 4 °C versus 37 °C) at comparable concentrations and the optically active or racemic agents ((+)-, (−)-, and (±)-*N*-BOC-CI) exhibit an indistinguishable profile of covalent alkylation. This nondiscriminant alkylation event proved to be remarkably similar to the DNA alkylation profile of (+)-*N*-BOC-CPI (**12**, common alkylation sites; selectivity, CPI > CI; intensity, CI > CPI among the available sites).[33] In addition, the seco agents possessing a good leaving group on the C-3 methyl center and thus capabilities for in situ Ar-3' alkylative closure to the parent cyclopropane agents (e.g. **14** → **11**, Figure 15, supplementary material) were shown to exhibit a profile of alkylation that is indistinguishable from that of the parent cyclopropane agents (identical selectivity, comparable intensity). This is accurately reflected in the comparisons presented in Figure 3 employing w794 DNA.

(32) For example, two of three (+)-CC-1065 high affinity sites within c820 (Figure 8, supplementary material) constitute a less prominent or absent duocarmycin alkylation site although both agents share a common high affinity site and most low affinity alkylation sites. Figures 8-12 (supplementary material) provide the additional individual and summary comparisons of (+)-CC-1065 and duocarmycin A, C_1, and C_2.

(33) (−)-*N*-BOC-CPI and (−)-*seco*-*N*-BOC-CPI (X = Cl) did not exhibit DNA alkylation properties under the conditions detailed herein for (+)-*N*-BOC-CPI and (+)-*seco*-*N*-BOC-CPI (X = Cl). For a demonstration that (+)- and (−)-*N*-acetyl-CPI alkylate the same adenine sites in DNA albeit at different concentrations, see ref 24.

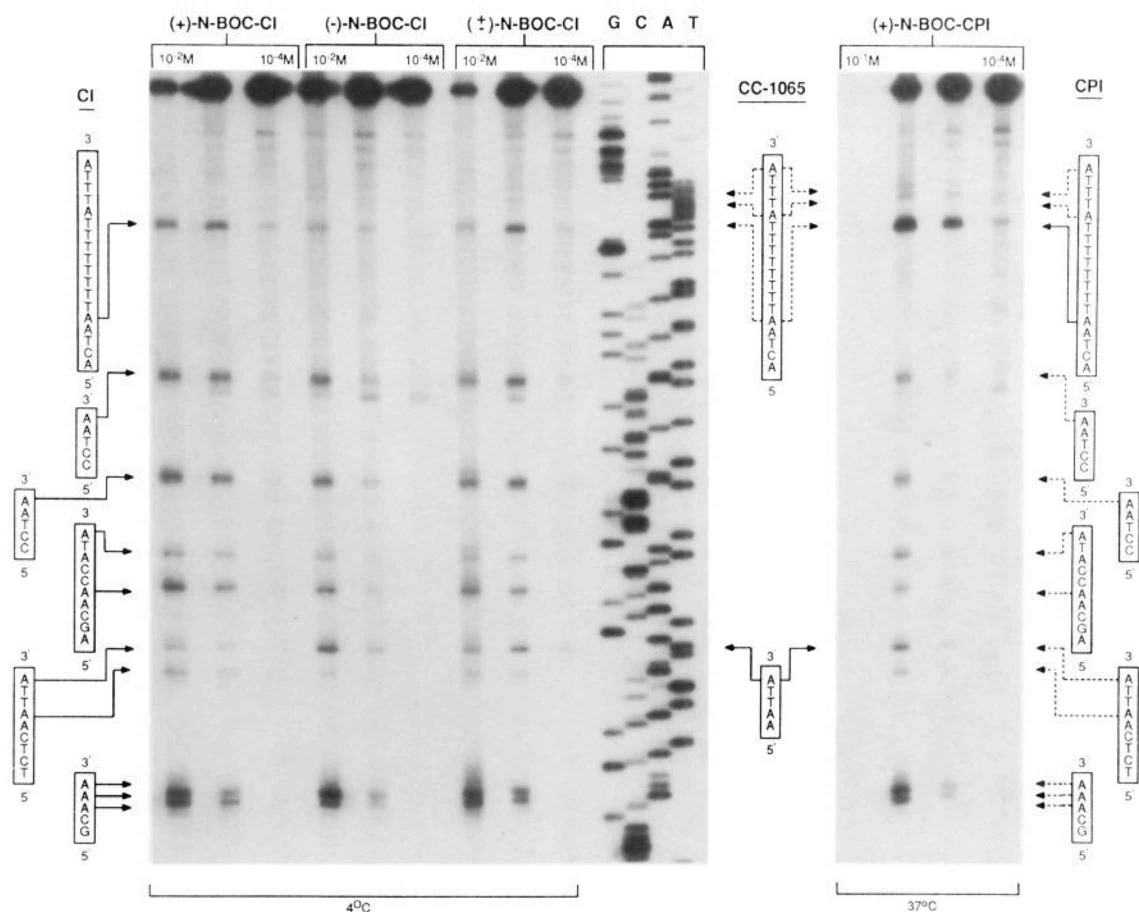


Figure 3. Thermally induced strand cleavage of double-stranded DNA (SV40 DNA fragment, 144 bp; nucleotide no. 5238-138, clone w794) after 24-h incubation of agent–DNA at 4 °C or 37 °C followed by removal of unbound agent and 30-min incubation at 100 °C; 8% denaturing poly-(acrylamide) gel and autoradiography: lanes 1–3, *seco*-(+)-*N*-BOC-CI ((+)-**14**, 4 °C, 1×10^{-2} to 1×10^{-4} M); lanes 4–6, *seco*-(−)-*N*-BOC-CI ((−)-**14**, 4 °C, 1×10^{-2} to 1×10^{-4} M); lanes 7–9, *seco*-(±)-*N*-BOC-CI ((±)-**14**, 4 °C, 1×10^{-2} to 1×10^{-4} M); lanes 10–13, Sanger G, C, A, and T reactions; lanes 14–17, *seco*-(+)-*N*-BOC-CPI ((+)-**12**, X = Cl, 37 °C, 2.5×10^{-1} to 2.5×10^{-4} M).

Selectivity: (+)-CC-1065 > (+)-*N*-BOC-CPI > (+)-, (−)-, and (±)-*N*-BOC-CI

Intensity: (+)-CC-1065 ≫ (+)-*N*-BOC-CPI < (+)-, (−)-, and (±)-*N*-BOC-CI

(±)-*N*-BOC-CI (11), (±)-CI-TMI (10), Duocarmycin A (2), and (+)-CC-1065 (1). Having established in prior studies that simple derivatives of CI display characteristics consistent with its assignment as the pharmacophore of the CC-1065 alkylation subunit,[15] we sought to establish whether CI may constitute the pharmacophore of the duocarmycin alkylation subunit and, as such, constitute the common pharmacophore relating the CC-1065 and duocarmycin natural products. Consistent with expectations (±)-CI-TMI (**10**) and (±)-*seco*-CI-TMI (**7**, X = OSO_2CH_3) displayed a DNA alkylation profile similar to that of duocarmycin A (Figure 4), substantially more selective than racemic (±)-*N*-BOC-CI (Figure 5) or the optically active (+)- and (−)-*N*-BOC-CI (Figure 3), and similar to that of (+)-CC-1065 (Figure 5). The selectivity of the alkylation of **10** (CI-TMI) and **7** (*seco*-CI-TMI, X = OSO_2CH_3) proved virtually indistinguishable but the intensity of the DNA alkylation exhibited by **7** proved comparable to that of duocarmycin A and greater than that of **10** (CI-TMI, ca. 10–100×). Presumably this may be attributed to the relative instability of **10** to the conditions of assay. This comparable DNA alkylation selectivity of duocarmycin A (**2**) and **10** (CI-TMI) and the results of the in vitro cytotoxic evaluation of the agents detailed below suggest that **10** constitutes an agent that incorporates the minimum potent pharmacophore of the duocarmycin DNA alkylation subunit and the common pharmacophore of the duocarmycin/CC-1065 alkylation subunits.

Selectivity: duocarmycin A ≥ (±)-CI-TMI (**10**) = (±)-**7** ≫ (±)-*N*-BOC-CI (**11**) = (±)-**14**

Intensity: duocarmycin A ≥ (±)-**7** (ca. 1–10×) >(±)-CI-TMI (**10**, ca. 10–100×) ≫ (±)-**14** (ca. 10^3–10^4×) ≃ (±)-*N*-BOC-CI (**11**, ca. 10^4×)

***seco*-CI-TMI (7–9; X = OSO_2CH_3, Br, and Cl) and Related Agents: Nature of the Alkylation Agent.** Past efforts have emphasized the unique involvement of the cyclopropane bearing agents, e.g. CPI,[9] as the electrophile responsible for the selective DNA alkylation. In the instances of the observation of biological activity and DNA binding properties of seco agents lacking the preformed cyclopropane ring but which possess capabilities for closure to a reactive cyclopropane, the implications have been that closure to the cyclopropane precedes observation of sequence selective DNA alkylation and is the agent responsible for the biological activity. The facile closure of the agents examined to date[9] (CPI precursors) supported the expectation that the Ar-3′ alkylation with closure to the parent cyclopropane agents could be projected to potentially occur under the conditions of assay. Consequently, we examined the comparative DNA alkylation properties of *seco*-*N*-BOC-CI (**14**, X = OSO_2CH_3), the *seco*-CI-TMI agents (**7–9**, X = OSO_2CH_3, Br, and Cl), the methyl ethers **15** and **18**, and that of **24** and **25** with expectations that the relative trends in the intensity of the DNA alkylation may correlate with the relative ease of closure to the parent CI agent.

The results of this study proved more revealing than anticipated in that the relative intensity of the DNA covalent alkylation did follow trends that might be expected for the relative ease of closure of the seco agents to the putative CI agents with the important

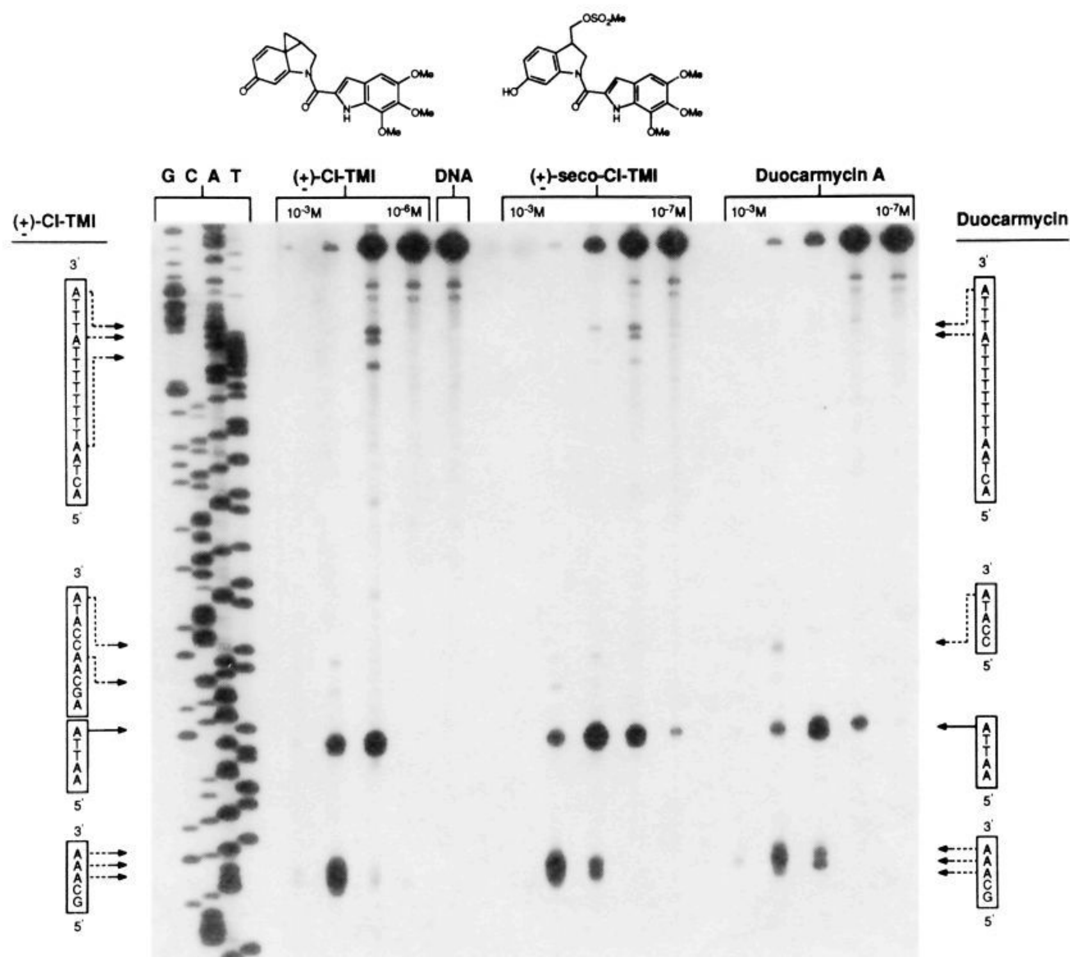
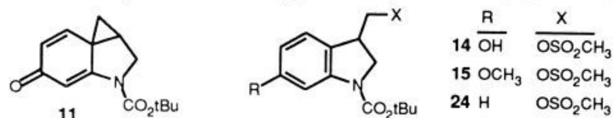


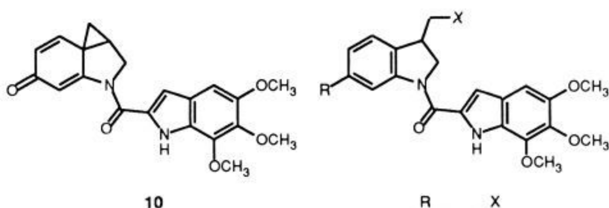
Figure 4. Thermally induced strand cleavage of double-stranded DNA (SV40 DNA fragment, 144 bp; nucleotide no. 5238-138, clone w794) after 24-h incubation of agent–DNA at 4 °C followed by removal of unbound agent and 30-min incubation at 100 °C; 8% denaturing poly(acrylamide) gel and autoradiography: lanes 1–4, Sanger G, C, A, and T reactions; lanes 5–8, (±)-CI-TMI (**10**, 1×10^{-3} to 1×10^{-6} M); lane 9, control DNA; lanes 10–14 (±)-*seco*-CI-TMI (**7**, 1×10^{-3} to 1×10^{-7} M); lanes 15–19, duocarmycin A (**2**, 1×10^{-3} to 1×10^{-7} M).

exception that agents incapable of such closure displayed DNA alkylation properties. In the cases examined, the covalent alkylation proceeds with the selectivity that is observed with the parent CI agent albeit at higher agent concentrations and illustrates that closure to the activated cyclopropane *is not obligatory* to observation of a DNA covalent alkylation. The intensity of the covalent alkylation at 4 °C is, nonetheless, roughly related to the solvolytic reactivity of the electrophile and the ease of phenonium ion generation with aryl neighboring group participation in the displacement of the leaving group (**14** > **15** > **24** and **7** > **18** > **25**, R = OH > OCH_3 > H).[34,35] The comparative behavior of **14**, **15**, and **24** is illustrated in Figure 6 and that of **8** and **9** may be found in supplementary material (Figure 16).



Selectivity: **11** = **14** = **15** = **24**

Intensity (4 °C):
14 $\simeq$ **11** (ca. 1–10×) $\geq$ **15** (ca. 1–10×) $\gg$ **24** (ca. 100–1000×)



	R	X
7	OH	OSO_2CH_3
8	OH	Br
9	OH	Cl
18	OCH_3	OSO_2CH_3
25	H	OSO_2CH_3

Selectivity: **7** = **8** = **9** = **10**

Intensity (4 °C):
7 $\simeq$ **8** > **9** (ca. 10–100×) $\geq$ **10**(ca. 10–100×) > **18** (ca. 100–1000×) > **25** (ca. 10^5×)

(34) Lancelot, C. J.; Cram, D. J.; Schleyer, P. v. R. In *Carbonium Ions*; Olah, G. A., Schleyer, P. v. R., eds.; Wiley: New York, NY, 1972; Vol. III, pp 1347–1483. For a succinct discussion, see: March, J. *Advanced Organic Chemistry*, 3rd ed.; Wiley: New York, NY; pp 277–280. For *p*-$RC_6H_4CH_2CH_2OTS$, R = OMe > R = H, $k_{phenonium}$ ca. 15–20×.

(35) No covalent alkylation of DNA by **24** and **25** was observed at 4 °C (24 h) and required the conditions of 37 °C (24 h) for observation. Methyl ether **15** (final purification by HPLC) was shown to be ≥99.94% pure and free of contaminate phenol **14** (<0.05%). The extent of DNA alkylation by **15** at 4 °C (24 h) cannot be attributed to contaminant phenol and (surprisingly) under conditions of 37°C (24 h) the intensity of covalent alkylation by **15** (methyl ether) is *greater* than that of phenol **14**. These observations coupled with the demonstration of the DNA alkylation by **24** unambiguously establish the capabilities for the seco agents to alkylate DNA directly. For the first discussion of this, see ref 14 (supplementary material).

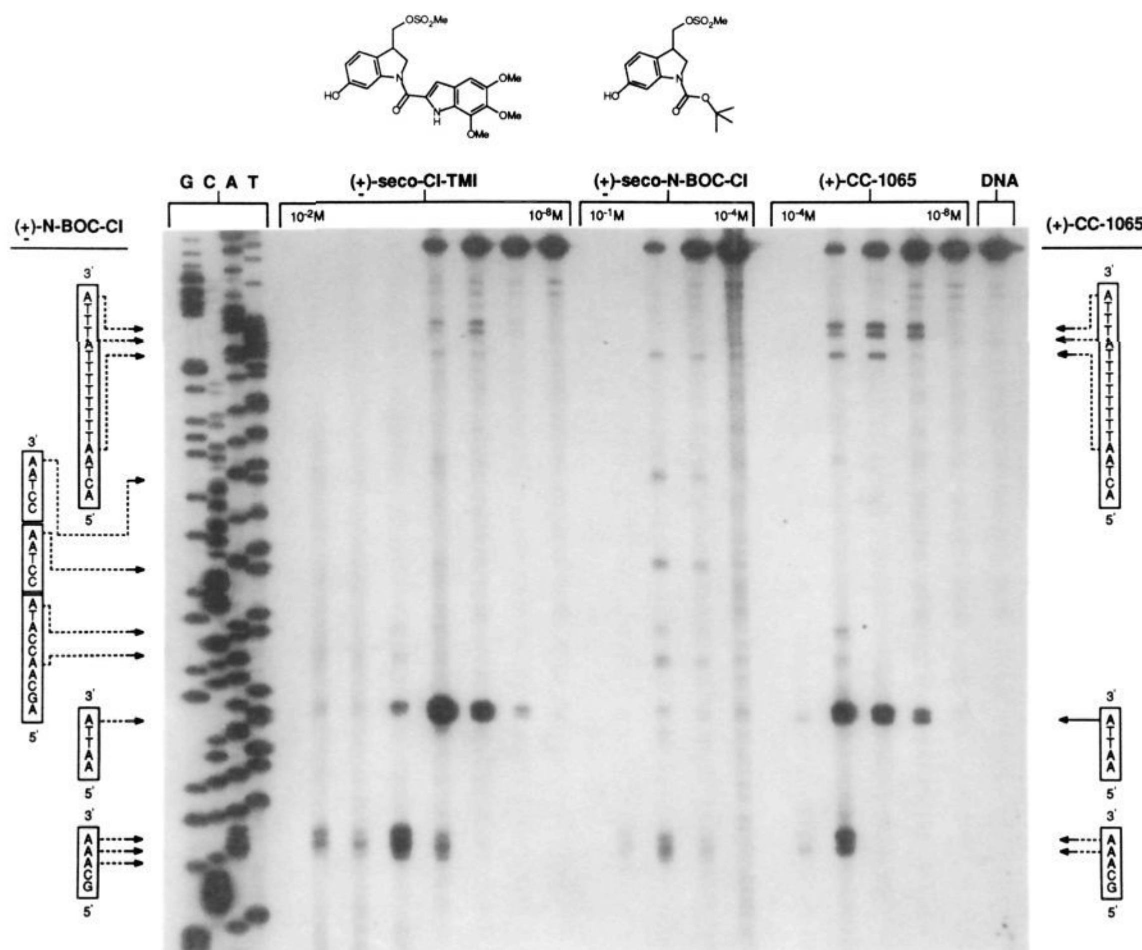


Figure 5. Thermally induced strand cleavage of double-stranded DNA (SV40 DNA fragment, 144 bp; nucleotide no. 5238-138, clone w794) after 24-h incubation of agent–DNA at 4 °C followed by removal of unbound agent and 30-min incubation at 100 °C; 8% denaturing poly(acrylamide) gel and autoradiography: lanes 1–4, Sanger G, C, A, and T reactions; lanes 5–11, (±)-*seco*-CI-TMI (**7**, 1×10^{-2} to 1×10^{-8} M); lanes 12–15, (±)-*seco*-*N*-BOC-CI (**14**, 1×10^{-1} to 1×10^{-4} M); lanes 16–20, (+)-CC-1065 (**1**, 1×10^{-4} to 1×10^{-8} M); lane 21, control DNA.

In Vitro Cytotoxic Activity. The results of the comparative evaluation of the agents for cytotoxic activity are summarized in Table II. The results are striking in that duocarmycin A displays the exceptionally potent activity characteristic of (+)-CC-1065 and the CI-based agents proved less potent but comparable in potency to most existing or clinically useful agents. The relative cytotoxic potency of the agents proved to correlate roughly with trends in the relative intensity (and selectivity) of the DNA alkylation providing support for the potential that the agents may be acting by a common mechanism initiated with the irreversible covalent alkylation of DNA.

Model of the Duocarmycin Alkylation of DNA. Given the sequence preference for the duocarmycin A covalent alkylation of DNA in the minor groove, its clear sequence selectivity that extends in the 3′ → 5′ binding directionality from the alkylation site, the established structure of the duocarmycin–adenine covalent adduct,[29] and the unambiguously established absolute configuration of **6**,[7] a model of the duocarmycin DNA binding may be constructed and is illustrated in Figure 7 for the high affinity binding site within w794 DNA. In this model, the hydrophobic concave face of duocarmycin is tucked deeply in the minor groove, the polar functionality of the agent lies on the outer face of the complex, and the bound agent conformation spans 3.5 base pairs and complements the topological curvature and pitch of the DNA minor groove.

Conclusions. The results of the study illustrate that the duocarmycins display DNA alkylation properties strikingly similar to that of the (+)-CC-1065 covalent alkylation of DNA (selectivity and intensity). The high affinity consensus sequences for the duocarmycin DNA alkylation have been determined to be 5′-d(A/TAA<u>A</u>)-3′ and 5′-d(A/TTT<u>A</u>Pu)-3 and from this information a model of the duocarmycin alkylation of DNA has been constructed. For the full range of agents examined, the results of the studies demonstrate that CI constitutes the common

Table II. In Vitro Cytotoxic Activity IC_{50} (μg/mL)[a]

agent	configuration	L1210
1, (+)-CC-1065	natural	0.00001
1, (−)-CC-1065	enantiomer	0.00001
1, (±)-CC-1065	–	0.00001
2, duocarmycin A	natural	0.00002
7	–	0.0002
8	–	0.0001
9	–	0.0005
10, (±)-CI-TMI	–	0.001
11, (±)-*N*-BOC-CI[b]	–	10
12, (+)-*N*-BOC-CPI[c]	natural	0.1
14,[d] (±)-**14**	–	0.1
14,[d] (+)-**14**	natural	0.04
14,[d] (−)-**14**	enantiomer	0.2
15	–	1
24	–	>10
16	–	0.1
18	–	0.03
25	–	>0.1

[a] The cell culture cytotoxicity assays were performed as described: Boger, D. L.; Yasuda, M.; Mitscher, L. A.; Drake, S.; Kitos, P. A.; Thompson, S. C. *J. Med. Chem.* **1987**, *30*, 1918. IC_{50} = inhibitory concentration for 50% cell growth relative to untreated controls. L1210 = mouse lymphocytic leukemia cell culture. [b] Taken from ref 14. [c] Taken from ref 16. [d] Taken from ref 15.

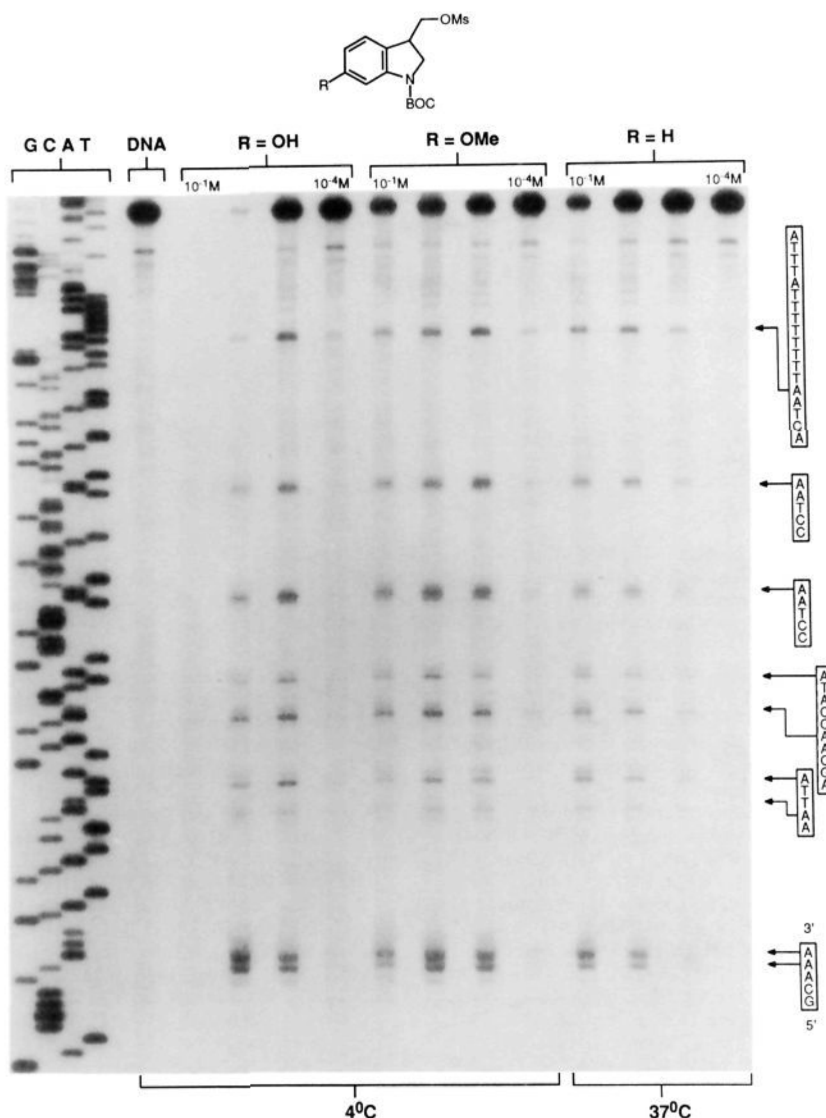


Figure 6. Thermally induced strand cleavage of double-stranded DNA (SV40 DNA fragment, 144 bp; nucleotide no. 5238-138, clone w794) after 24-h incubation of agent–DNA at 4 °C or 37 °C followed by removal of unbound agent and 30-min incubation at 100 °C; 8% denaturing poly(acrylamide) gel and autoradiography: lanes 1–4, Sanger G, C, A, and T reactions; lane 5, control DNA; lanes 6–9, *seco*-(±)-*N*-BOC-CI ((±)-**14**, 2.5×10^{-1} to 2.5×10^{-4} M, 4 °C); lanes 10–13, (±)-**15** (2.5×10^{-1} to 2.5×10^{-4} M, 4 °C); lanes 14–17, (±)-**24** (2.5×10^{-1} to 2.5×10^{-4} M, 37 °C).

pharmacophore of the duocarmycin–CC-1065 alkylation subunits and the minimum potent pharmacophore of the duocarmycin alkylation subunit. The agents capabilities for DNA alkylation roughly correlate with their relative cytotoxic activity. In addition, DNA alkylation has been observed for agents incapable of closure to the putative CI agents and proceeds with a selectivity that is not distinguishable from that of the parent CI agent and with an intensity that roughly correlates with electrophile reactivity illustrating that the activated cyclopropane is not obligatory[14] to observation of the DNA covalent alkylation.[36] Further studies of this new class of antitumor antibiotics are in progress and will be detailed in due course.

Experimental Section[37]

6-Hydroxy-3-[[(methylsulfonyl)oxy]methyl]-1-[(5,6,7-trimethoxyindol-2-yl)carbonyl]indoline (7). Phenol **14**[14] (99 mg, 0.29 mmol) was treated with anhydrous 3 N hydrochloric acid in ethyl acetate (3 mL) at 24 °C for 30 min. The solvent was removed in vacuo to afford the crude, unstable indoline hydrochloride (quantitative) as a white semisolid. A mixture of the crude indoline hydrochloride, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI, 166 mg, 0.87 mmol, 3 equiv), and 5,6,7-trimethoxyindole-2-carboxylic acid (**22**,[22] 72 mg, 0.287 mmol, 1.0 equiv) was slurried in 1.2 mL of *N,N*-dimethylformamide at 24 °C under argon, and the reaction mixture was stirred vigorously for 19 h (24 °C). The solvent was removed in vacuo, the residue was washed with water (4 × 4 mL) and dried in vacuo to afford crude **7** (105 mg, 137 mg theoretical, 77%) as a tan solid which proved sufficiently pure for the further use without purification. Flash chromatography (SiO_2, 0–10% tetrahydrofuran–dichloromethane gradient elution) was carried out for the preparation of an analytical and biological testing sample to afford **7** as colorless needles: mp 203 °C dec; ^1H NMR ($CDCl_3$–DMF-d_7, 200 MHz, ppm) 9.81 (br s, 1 H, NH), 9.24 (s, 1 H, OH), 7.88 (d, 1 H, J = 2.2 Hz, C7-H), 7.12 (d, 1 H, J = 8.1 Hz, C4-H), 6.96 (d, 1 H, J = 2.3 Hz, C3′-H), 6.88 (s, 1 H, C4′-H), 6.55 (dd, 1 H, J = 2.3, 8.1 Hz, C5-H), 4.62 (dd, 1 H, J = 9.2, 10.8 Hz, $CHHOSO_2CH_3$), 4.44 (d, 1 H, J = 10 Hz, C2-H), 4.42 (d, 1 H, J = 10.8 Hz, $CHHOSO_2CH_3$), 4.25 (dd, 1 H, J = 8.3, 10 Hz, C2-H), 4.08 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 3.83 (m, 1 H, C3-H), 3.01 (s, 3 H, OSO_2CH_3); IR (KBr) ν_{max} 3438, 2940, 1626, 1528, 1496, 1458, 1388, 1322, 1258, 1174, 1112, 1048, 946, 860, 814, 746, 530 cm^{-1}; UV (MeOH) 326 nm (ϵ 27 000); FABMS (*m*-nitrobenzyl alcohol) *m/e* 447 (M + H$^+$), 476 (M$^+$); FABHRMS (*m*-nitrobenzyl alcohol) *m/e*

(36) The observation of the selective DNA alkylation of **15**, **18**, **24**, and **25** suggests that the proposed sequence-dependent, rate-determining autocatalytic activation of the alkylation event by a proximal phosphate (protonation, complexation) in the DNA backbone two base pairs removed from the alkylation site in the 5′ direction may not be uniquely required for observation of alkylation. For a detailed discussion of this proposal, see refs 19 and 24b.

(37) General experimental details are provided in supplementary material.

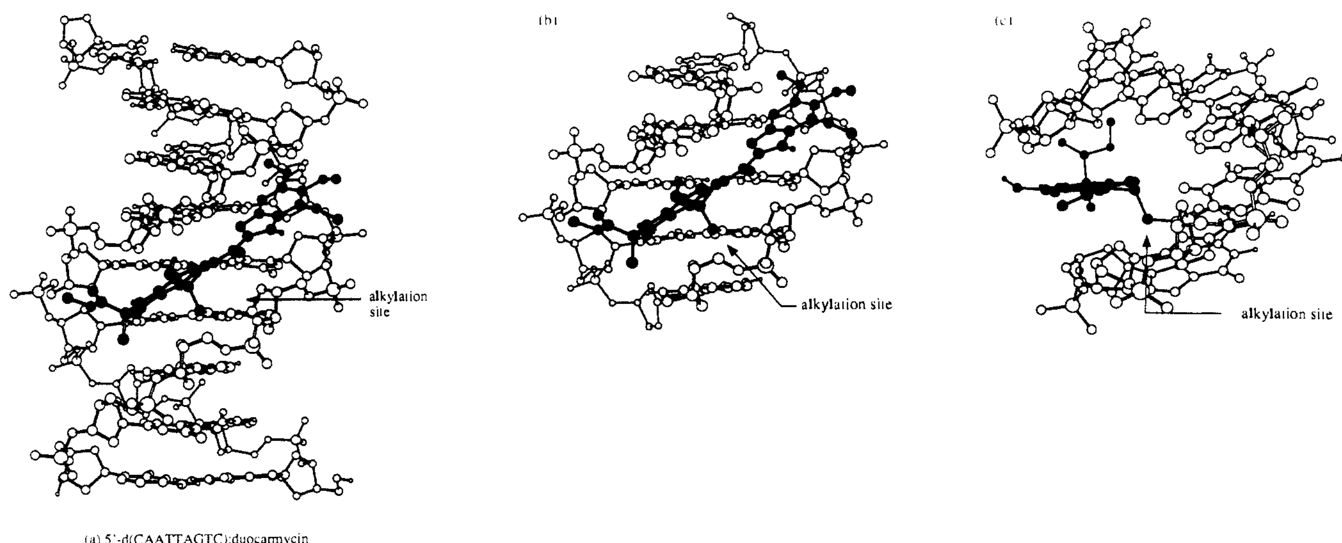


Figure 7. Model of duocarmycin A binding to the high-affinity site of w794 double-stranded DNA 5'-d(CAATTAGTC)–duocarmycin A. Complex was generated with MacroModel (Version 2.5, AMBER-supplemented with parameters for duocarmycin derived from the X-ray crystal structure[7] of 6): (a) 5'-d(CAATTAGTC)–duocarmycin A, (b) 5'-d(ATTAG)–duocarmycin A taken from a, (c) rotated view of b illustrating the left-hand subunit of duocarmycin bound to adenine deeply in the minor groove. The trimethoxyindole subunit has been removed for clarity.

477.1294 ($C_{22}H_{24}N_2O_8S + H^+$ requires 477.1332).

3-(Bromomethyl)-6-hydroxy-1-[(5,6,7-trimethoxyindol-2-yl)-carbonyl]indoline (8). A solution of 7 (8.0 mg, 16.8 μmol) in 0.2 mL of *N,N*-dimethylformamide at 24 °C under argon was treated with lithium bromide (14.6 mg, 168 μmol, 10 equiv), and the reaction mixture was stirred for 4 h (60 °C). Flash chromatography (0.5 × 5 cm SiO_2, 20–30% tetrahydrofuran–hexane gradient elution) afforded **8** (5.8 mg, 7.8 mg theoretical, 74%) as colorless needles: mp 246–248 °C; ^1H NMR ($CDCl_3$–DMSO-d_6, 200 MHz, ppm) 9.52 (br s, 1 H, NH), 8.93 (s, 1 H, OH), 7.88 (d, 1 H, *J* = 2.4 Hz, C7-H), 7.09 (d, 1 H, *J* = 8.4 Hz, C4-H), 6.96 (d, 1 H, *J* = 2.3 Hz, C3'-H), 6.89 (s, 1 H, C4'-H), 6.62 (dd, 1 H, *J* = 2.3, 8.3 Hz, C5-H), 4.64 (dd, 1 H, *J* = 9, 10.7 Hz, C2-H), 4.42 (dd, 1 H, *J* = 4.5, 10.7 Hz, C2-H), 4.09 (s, 3 H, OCH_3), 3.95 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.84 (m, 1 H, C3-H), 3.69 (dd, 1 H, *J* = 3.8, 10.1 Hz, C*H*HBr), 3.39 (t, 1 H, *J* = 10.1 Hz, CH*H*Br); IR (KBr) ν_{max} 3428, 3150, 2932, 1624, 1606, 1586, 1526, 1492, 1456, 1428, 1386, 1318, 1302, 1250, 1196, 1164, 1112, 1072, 1054, 862, 750 cm^{-1}; UV (MeOH) 326 nm (ε 34000); EIMS *m/e* (relative intensity) 462/460 (M^+, 15/12), 234 (100); CIMS (isobutane) *m/e* (relative intensity) 463/461 ($M + H^+$, 5/5), 383 (60), 381 (100); EIHRMS *m/e* 460.0635 ($C_{21}H_{21}BrN_2O_5$ requires 460.0634).

3-(Chloromethyl)-6-hydroxy-1-[(5,6,7-trimethoxyindol-2-yl)-carbonyl]indoline (9). Phenol **16**[14] (7.0 mg, 24 μmol) was treated with anhydrous 3 N hydrochloric acid in ethyl acetate (1 mL) at 24 °C for 20 min. The solvent was removed in vacuo to afford the crude, unstable indoline hydrochloride as a colorless semisolid. A mixture of the crude indoline hydrochloride, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI, 13.7 mg, 71.5 μmol, 2.9 equiv), 5,6,7-trimethoxyindole-2-carboxylic acid (**22**,[22] 5.5 mg, 21.9 μmol, 0.89 equiv), and sodium bicarbonate (8.0 mg, 95 μmol, 3.9 equiv) was slurried in 0.2 mL of *N,N*-dimethylformamide at 24 °C under argon, and the reaction mixture was stirred vigorously for 22 h (24 °C). The solvent was removed in vacuo and the residue was washed with water (3 × 1 mL) to afford the crude **9** as a cream colored solid. Flash chromatography (1.0 × 16 cm SiO_2, 5% methanol–dichloromethane) afforded **9** (7.7 mg, 9.1 mg theoretical, 85%) as a pale yellow solid: mp 257–259 °C; ^1H NMR ($CDCl_3$–DMF-d_7, 200 MHz, ppm) 9.53 (br s, 1 H, NH), 8.95 (s, 1 H, OH), 7.90 (s, 1 H, C7-H), 7.10 (d, 1 H, *J* = 8.4 Hz, C4-H), 6.95 (d, 1 H, *J* = 2.4 Hz, C3'-H), 6.88 (s, 1 H, C4'-H), 6.65 (dd, 1 H, *J* = 2.2, 8.3 Hz, C5-H), 4.63 (dd, 1 H, *J* = 8.5, 11 Hz, C2-H), 4.45 (dd, 1 H, *J* = 4, 11 Hz, C2-H), 4.08 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.79 (dd, 1 H, *J* = 4, 10 Hz, C*H*HCl), 3.76 (m, 1 H, C3-H), 3.54 (t, 1 H, *J* = 10 Hz, CH*H*Cl); IR (KBr) ν_{max} 3454, 2938, 1624, 1588, 1526, 1494, 1458, 1428, 1388, 1306, 1262, 1110, 862, 830, 748 cm^{-1}; UV (MeOH) 326 nm (ε 31000); EIMS *m/e* (relative intensity) 418/416 (M^+, 6/21), 234 (100); CIMS (isobutane) *m/e* (relative intensity) 419/417 ($M + H^+$, 31/100); EIHRMS *m/e* 416.1139 ($C_{21}H_{21}ClN_2O_5$ requires 416.1139).

6-[(*tert*-Butyldimethylsilyl)oxy]-3-[[(methylsulfonyl)oxy]methyl]-1-[(5,6,7-trimethoxyindol-2-yl)carbonyl]indoline (17). A solution of 7 (50 mg, 0.10 mmol), *tert*-butyldimethylsilyl chloride (32 mg, 0.21 mmol, 2 equiv), and (*N,N*-dimethylamino)pyridine (DMAP, 1.3 mg, 0.01 mmol, 0.1 equiv) in 1 mL of dichloromethane–*N,N*-dimethylformamide (5:1) at 24 °C under argon was treated with triethylamine (37 μL, 0.25 mmol, 2.5 equiv), and the reaction mixture was stirred for 15 h (24 °C). Flash chromatography (1.5 × 15 cm SiO_2, 50% ethyl acetate–hexane) afforded **17** (55 mg, 62 mg theoretical, 89%) as a pale green solid: mp 140–144 °C; ^1H NMR ($CDCl_3$, 200 MHz, ppm) 9.37 (s, 1 H, NH), 7.87 (dd, 1 H, *J* = 2.2 Hz, C7-H), 7.12 (d, 1 H, *J* = 8.1 Hz, C4-H), 6.92 (d, 1 H, *J* = 2.4 Hz, C3'-H), 6.85 (s, 1 H, C4'-H), 6.59 (dd, 1 H, *J* = 2.2, 8.1 Hz, C5-H), 4.62 (dd, 1 H, *J* = 9.1, 10.7 Hz, C*H*HOSO$_2$CH$_3$), 4.44 (dd, 1 H, *J* = 4, 10.7 Hz, CH*H*OSO$_2$CH$_3$), 4.42 (dd, 1 H, *J* = 5, 10.1 Hz, C2-H), 4.22 (dd, 1 H, *J* = 8.6, 10.1 Hz, C2-H), 4.05 (s, 3 H, OCH_3), 3.93 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 2.96 (s, 3 H, OSO_2CH_3), 0.98 (s, 9 H, $SiC(CH_3)_3$), 0.23 (s, 6 H, $Si(CH_3)_2$); IR (KBr) ν_{max} 3428, 2936, 2860, 1628, 1526, 1488, 1436, 1412, 1388, 1356, 1304, 1284, 1236, 1176, 1110, 1050, 954, 844, 528 cm^{-1}; EIMS *m/e* (relative intensity) 590 (M^+, 8), 494 (11), 260 (34), 234 (24), 153 (100); CIMS (isobutane) *m/e* (relative intensity) 591 ($M + H^+$, 98), 495 (100); EIHRMS *m/e* 590.2119 ($C_{28}H_{38}N_2O_8SSi$ requires 590.2118).

3-[[(Methylsulfonyl)oxy]methyl]-6-methoxy-1-[(5,6,7-trimethoxyindol-2-yl)carbonyl]indoline (18). A solution of 7 (3.0 mg, 6.3 μmol) in 1 mL of methanol at 24 °C was treated with an excess of diazomethane in ether. After the solution was stirred for 2 h (24 °C), methanol (2 mL) was added and the reaction mixture allowed to stand overnight. The solvent was removed in vacuo and flash chromatography (0.5 × 5 cm SiO_2, 0–5% tetrahydrofuran–dichloromethane gradient elution) afforded **18** (2.8 mg, 3.1 mg theoretical, 90%) as a colorless oil: ^1H NMR ($CDCl_3$, 200 MHz, ppm) 9.37 (br s, 1 H, NH), 8.00 (d, 1 H, *J* = 2.5 Hz, C7-H), 7.18 (d, 1 H, *J* = 8.4 Hz, C4-H), 6.95 (d, 1 H, *J* = 2.3 Hz, C3'-H), 6.87 (s, 1 H, C4'-H), 6.68 (dd, 1 H, *J* = 2.5, 8.4 Hz, C5-H), 4.65 (dd, 1 H, *J* = 9, 11 Hz, C*H*HOSO$_2$CH$_3$), 4.47 (dd, 1 H, *J* = 4.3, 11 Hz, CH*H*OSO$_2$CH$_3$), 4.44 (dd, 1 H, *J* = 5, 10.1 Hz, C2-H), 4.23 (dd, 1 H, *J* = 8.5, 10.1 Hz, C2-H), 4.08 (s, 3 H, OCH_3), 3.95 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.88 (m, 1 H, C3-H), 3.86 (s, 3 H, OCH_3), 2.99 (s, 3 H, OSO_2CH_3); IR (neat) ν_{max} 3262, 2938, 2836, 1628, 1598, 1526, 1492, 1466, 1448, 1414, 1390, 1356, 1310, 1174, 1110, 954 cm^{-1}; UV (MeOH) 326 nm (ε 29000); EIMS *m/e* (relative intensity) 490 (M^+, 24), 394 (36), 234 (82), 160 (100); CIMS (isobutane) *m/e* (relative intensity) 491 ($M + H^+$, 37), 395 (100); EIHRMS *m/e* 490.1410 ($C_{23}H_{26}N_2O_8S$ requires 490.1410).

HPLC purification and subsequent HPLC analysis (10 × 25 cm, 10 μm SiO_2, 8% THF–CH_2Cl_2, 3 mL/min, effluent monitored at 320 nm) ensured the sample of **18** employed in the biological–DNA binding samples was >99% pure and free of contaminate **7** (<0.1% **7**, t_R (**18**) = 7.2 min, t_R (**7**) = 17.3 min).

***N*-[(5,6,7-Trimethoxyindol-2-yl)carbonyl]-1,2,7,7a-tetrahydrocycloprop[1,2-*c*]indol-4-one (10).** A solution of **17** (10.4 mg, 17.6 μmol) in 3 mL of tetrahydrofuran at 24 °C under argon was treated with tetra-*n*-butylammonium fluoride (1 M in tetrahydrofuran, 15.8 μL, 15.8 μmol, 0.9 equiv) and the reaction mixture was stirred for 30 min (24 °C). The reaction mixture was diluted with ethyl acetate (5 mL), and the solution was rapidly passed through a triethylamine-treated silica gel column (1.2 × 4 cm, ethyl acetate wash, 50 mL). The solution was concentrated in

vacuo and flash chromatography of the residue (triethylamine-treated SiO_2, 0.5 × 5 cm, ethyl acetate) afforded **10** (4.1 mg, 6.7 mg theoretical, 61%) as a pale yellow solid: mp 215–218 °C: ^{1}H NMR ($CDCl_3$, 500 MHz, ppm) 9.19 (s, 1 H, NH), 6.92 (d, 1 H, J = 2.4 Hz, C3′-H), 6.89 (d, 1 H, J = 1.5 Hz, C3-H), 6.77 (s, 1 H, C4′-H), 6.60 (d, 1 H, J = 9.8 Hz, C6-H), 6.45 (dd, 1 H, J = 1.5, 9.8 Hz, C5-H), 4.39 (dd, 1 H, J = 4.9, 10.4 Hz, C1-H), 4.32 (d, 1 H, J = 10.4 Hz, C1-H), 4.07 (s, 3 H, OCH_3), 3.93 (s, 3 H, OCH_3), 3.89 (s, 3 H, OCH_3), 2.65 (m, 1 H, C7a-H), 1.79 (dd, 1 H, J = 4.6, 7.9 Hz, C7-H), 1.50 (t, 1 H, J = 4.9 Hz, C7-H); IR (KBr) ν_{max} 3444, 2936, 1636, 1526, 1492, 1464, 1404, 1364, 1306, 1278, 1230, 1110, 1050, 752 cm^{-1}; UV (methanol), 334 (ϵ 21 000), 278 nm (13 000); EIMS m/e (relative intensity) 380 (M^+, 22), 234 (100), 146 (91); CIMS (isobutane) m/e 381 (M + H^+, 100); EIHRMS m/e 380.1372 ($C_{21}H_{20}N_2O_5$ requires 380.1372).

Reaction of *N*-[(5,6,7-Trimethoxyindol-2-yl)carbonyl]-1,2,7,7a-tetrahydrocycloprop[1,2-*c*]indol-4-one (10) with Hydrochloric Acid. A solution of **10** (2.0 mg, 5.3 μmol) in ethyl acetate (2 mL) at 24 °C under argon was treated with 3 N anhydrous hydrochloric acid in ethyl acetate (0.2 mL, ca. 100 equiv). After the solution was stirred for 20 min (24 °C), the solvent was removed in vacuo. Flash chromatography (0.5 × 3.5 cm SiO_2, 0–20% THF–hexane gradient elution) afforded **9** (1.7 mg, 2.2 mg theoretical, 77%) identical in all respects with authentic **9**.

DNA Binding Studies. Singly 5′-^{32}P-end-labeled double-stranded DNA constituting SV40 DNA fragments (w794, nucleotides no. 5238-138, 144 base pairs; w836, nucleotides no. 5189-91, 145 base pairs; c988, nucleotides no. 4359-4210, 149 base pairs; c820, nucleotides no. 4201-4356, 155 base pairs; c1346, nucleotides no. 1632-1782, 150 base pairs) cloned into the *Sma*I site of M13mp10 were prepared by treatment of single-stranded templates[28] with 5′-^{32}P-end-labeled universal primer [5′-d(GTAAAACGACGGCCAGT)-3′], extension of the primer–template duplex with the Klenow fragment of DNA polymerase I, and subsequent *Eco*R I cleavage of the double-stranded DNA immediately following the inserted DNA.[27] The resultant double-stranded DNA was treated with the agents at 4 °C or 37 °C (24 h) at a range of agent concentrations. Removal of the unreacted agent through ethanol precipitation of the DNA, thermally induced cleavage of a solution of the double-stranded DNA–agent covalent complexes at the sites of covalent alkylation (100 °C, 30 min),[24-27] gel electrophoresis under denaturing conditions of the resultant DNA alongside Sanger dideoxynucleotide sequencing reactions, and subsequent autoradiography revealed the sites of covalent alkylation and their relative intensities.

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Supplementary Material Available: General experimental details and full experimental details for the preparation of **22**, **24**, and **25**, supporting autofootprinting studies, force field parameters for duocarmycin for use with MacroModel (Version 2.5, AMBER force field), and stereoviews of Figure 7, parts a–b, are provided (19 pages). Ordering information may be found on any current masthead page.

Asymmetric Induction in the Vinylogous Amide Photocycloaddition Reaction. A Formal Synthesis of Vindorosine

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Abstract: The application of the intramolecular vinylogous amide photocycloaddition/retro-Mannich fragmentation/Mannich closure sequence to a formal synthesis of vindorosine, **6**, starting from L-tryptophan, is described. The synthesis of **7**, which has been converted to vindorosine by Buchi, in 14 steps (7% overall yield from L-tryptophan) in homochiral form attests to the efficiency of this photochemical approach to the synthesis of the aspidosperma alkaloids, and demonstrates the exceedingly high levels of asymmetric induction which are possible via the intramolecular [2 + 2]photocycloaddition reaction of vinylogous amides.

We have recently introduced a new method for the construction of nitrogen-containing ring systems, **1–4** (Scheme I), which has been applied to a synthesis of the alkaloid (±)-mesembrine.[4] Intramolecular photocycloaddition of **1**, followed by retro-Mannich fragmentation of photoadduct **2**, leads to the formation of the keto imine **3**, which can undergo a final Mannich closure to generate the perhydroindole **4**. The effect of substitution on the tether connecting the vinylogous amide chromophore and the reacting alkene, as illustrated in **5**, on the stereochemical outcome of the photocycloaddition has now been examined. We report herein that exceedingly high levels of asymmetric induction are indeed possible and describe the application of this methodology to a formal synthesis of vindorosine, **6**,[5] *in homochiral form*, starting from L-tryptophan.[6]

The retrosynthetic analysis for the application of the vinylogous amide photocycloaddition/retro-Mannich fragmentation/Mannich

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