

Communications to the Editor

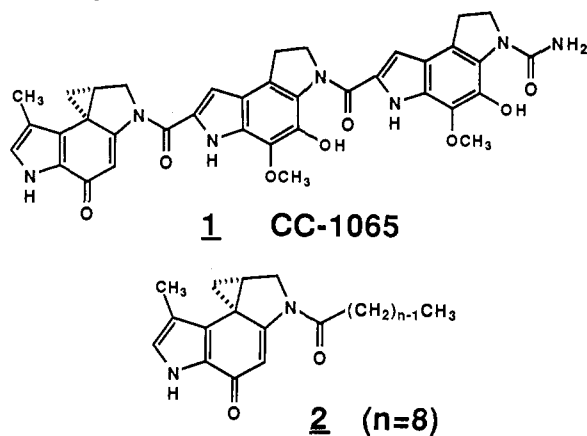
Interstrand DNA Cross-linking with Dimers of the Spirocyclopropyl Alkylating Moiety of CC-1065

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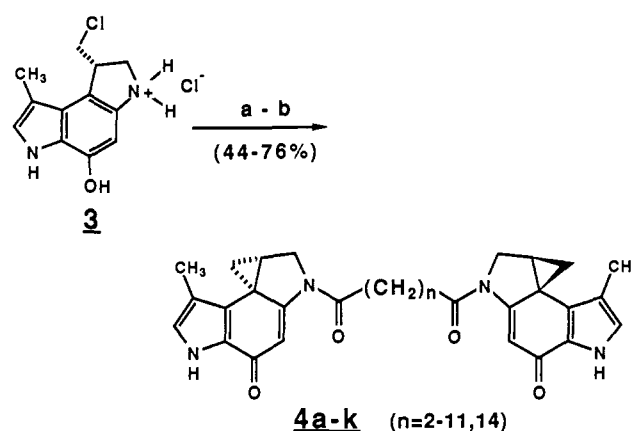
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The cytotoxicity of a number of clinically useful antitumor alkylating agents is often attributed to interstrand cross-linking of cellular DNA.¹⁻³ CC-1065, **1**, a highly cytotoxic antitumor antibiotic, has been shown to bind in the minor groove of double stranded B-DNA and bond covalently to the N-3 of selected adenines by reaction of the cyclopropyl ring of the natural product.⁴ We report the preparation of a number of highly potent compounds (Table I) which contain two of the alkylating subunits (CPI) of CC-1065 linked by a flexible methylene chain of variable length.⁵ Several compounds in this series are significantly more potent than CC-1065 both in vitro and in vivo.⁶ We also demonstrate DNA cross-linking by some of these dimers by using alkaline agarose gel electrophoresis.⁷

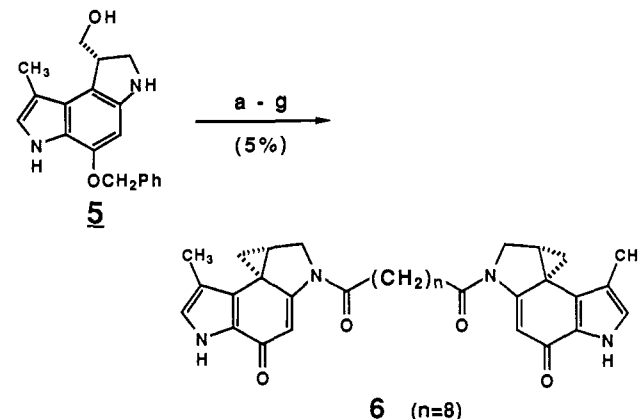


Scheme I^a



^a(a) ImOC(CH₂)_nCOIm, DMF; (b) 2:1:1, CH₃CN/NEt₃/H₂O.

Scheme II^a



^a(a) CH₃OOC(CH₂)₈COOH, EDC, DMAP; (b) KOH, CH₃OH, H₂O; (c) EDC, *ent*-**5**; (d) MsCl, pyridine; (e) LiCl, DMF, 80-100 °C; (f) NH₄OOCH, Pd/C, CH₃OH; (g) CH₃CN/NEt₃/H₂O, 2:1:1.

The synthesis of the natural configuration dimers,¹⁰ **4a-k**, is accomplished by the combination of 2 equiv of **3**¹¹ with 1 equiv of the bis-imidazole¹² of the appropriate diacid, followed by cyclization (Scheme I).¹³ Dimers containing the enantiomeric configuration at one, **6**¹⁴ (Scheme II), or both, *ent*-**4**,¹⁵ ends were also prepared.¹³ Formation of interstrand cross-links in dimer-treated DNA restriction fragments is revealed by the appearance of reduced mobility bands during denaturing alkaline agarose gel electrophoresis (Figure 1).¹⁶ The highest levels of cross-linking

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(6) For example, inoculation of mice bearing P388 leukemia on days 1, 3, and 5 with 100 μg/kg of CC-1065 gave a 68% increase in lifespan relative to controls, whereas compound **4b** gave a 100% increase in lifespan at a dose of only 0.8 μg/kg.

(7) Dimers of anthramycin have been reported,^{8,9} however, not always with evidence for DNA cross-linking.⁹

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(12) Best yields were produced with purified bis-imidazolides (prepared from 1 equiv of diacid and 2 equiv of 1,1'-carbonyldiimidazole in dimethylacetamide at 40 °C).

(13) All compounds had satisfactory NMR, UV, and high-resolution mass spectral data.

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(15) *ent*-**4f-h** were prepared by using the methods described for **4a-k** with the enantiomer of **3**.^{11,14}

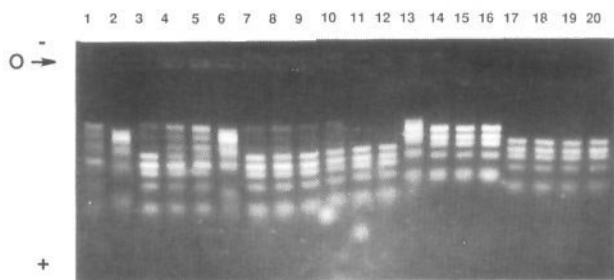


Figure 1. Alkaline agarose gel showing cross-linking of DNA by CPI dimers. Each compound, in 5 μ L of dimethylacetamide (DMA), was incubated for 2 h at 37 $^{\circ}$ C with 1 μ g of ϕ X174 HaeIII digest in 100 μ L of PBS buffer (15 μ M in base pairs).¹⁷ Samples were precipitated, resuspended, loaded onto a 1% horizontal-bed alkaline agarose gel, and run as previously described.¹⁶ The position of the gel origin (0) is indicated. From left: lanes 1, 3, 5, 7, and 9, dimers at 0.28 μ M; lanes 2, 4, 6, 8, and 10, dimers at 1.7 μ M; lanes 1 and 2, **4b**; lanes 3 and 4, **4c**; lanes 5 and 6, **4d**; lanes 7 and 8, **4e**; lanes 9 and 10, **4g**; lanes 11 and 12, **2** at 0.56 and 3.4 μ M, respectively; lanes 13-16, trimethylpsoralen controls at 17 μ M, irradiated for 10, 30, 60, and 120 s, respectively; lane 17, DNA treated with DMA; lane 18, untreated DNA; lanes 19 and 20, **1** at 0.028 and 0.28 μ M, respectively.

are seen with compounds **4b** and **4d**. At the higher dose, the restriction fragments appear uniformly retarded, similar to that observed with trimethylpsoralen at the shortest irradiation time. Compound **4c** cross-links to an intermediate degree; treatment at the higher dose leads to two distinct populations of fragments in approximately equal intensities. Compounds **4e** and **4g** exhibit low but significant levels of cross-linking; only minor amounts of cross-linked bands are observed. Cross-linking is not seen in samples treated with the monomeric compounds **1** or **2** (Figure 1). The other natural configuration CPI dimers **4a**, **4f**, and **4h-k** and CPI dimers containing enantiomeric CPI units, **6** and *ent*-**4f-h**, also do not exhibit cross-linking in this assay (data not shown).

The *in vitro* cytotoxic potencies and relative cross-linking scores of this series of compounds are presented in Table I.¹⁸ Monomeric alkylators such as **2**, possessing a flexible methylene acyl appendage, were previously shown to possess low cytotoxic potencies relative to CPI derivatives which contain acyl appendages capable of significant minor groove stabilization of the drug-DNA complex.¹⁹ Therefore the high cytotoxic potencies of many of these flexible CPI dimers were somewhat unexpected.²⁰ It is tempting to speculate that cross-linking contributes significantly to the mechanism of cell growth inhibition by these compounds.

Both cytotoxic potency and cross-linking efficiency are highly dependent upon the chain length linking the two CPI moieties. The compounds which exhibit the highest levels of interstrand cross-linking, **4b** and **4d**, are also two of the most potent. Conversely, compounds which do not exhibit interstrand cross-linking, **4a**, **4f**, **4h**, **4j**, **4k**, *ent*-**4f-h**, and **2**, are among the least potent. Only **4g** and **4i** appear anomalous when evaluated in this manner. Clearly, factors in addition to cross-linking efficiency may also be important to cytotoxic potency.

Preliminary results from energy-minimized molecular modelling of CPI-containing compounds bound to short oligonucleotide

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(17) The PBS buffer (Whittaker, M. A. Bioproducts, Walkersville, MD) contained 144.0 mg/L of KH_2PO_4 , 795.0 mg/L of Na_2HPO_4 , and 9000 mg/L of NaCl at pH 7.4.

(18) Cross-linking scores were determined by visual comparison of lanes in the gel photos. (+++) indicates the restriction fragments appeared uniformly cross-linked at 1.7 μ M drug. (++) indicates that comparable levels of both cross-linked and uncross-linked bands were formed at 1.7 μ M drug. (+) indicates only low levels of cross-linking were seen at either drug concentration. (-) indicates no cross-linking was observed.

(19) Warpehoski, M. A.; Gebhard, I.; Kelly, R. C.; Krueger, W. C.; Li, L. H.; McGovern, J. P.; Prairie, M. D.; Wicnienski, N.; Wierenga, W. *J. Med. Chem.* **1988**, *31*, 590-603.

(20) For example, compare **2** and **4g**. *In vivo*, compound **2** was inactive and nontoxic in mice bearing P388 leukemia at least up to 1600 μ g/kg, whereas compound **4g** at 3.1 μ g/kg increased the lifespan of such mice by greater than 150%.

Table I. Compilation of Cytotoxicity and Cross-linking Data for Flexible CPI Dimers

compound (<i>n</i>) ^a	ID ₅₀ ^b (pM)	relative cross-linking score ^c
4a (2)	4000	-
4b (3)	2	+++
4c (4)	20	++
4d (5)	6	+++
4e (6)	40	+
4f (7)	200	-
4g (8)	5	+
4h (9)	9000	-
4i (10)	50	-
4j (11)	2000	-
4k (14)	3000	-
<i>ent</i> - 4f (7)	40000	-
<i>ent</i> - 4g (8)	5000	-
<i>ent</i> - 4h (9)	10000	-
6 (8)	200	-
2 (8)	60000	-
1	30	-

^aChain length. ^bID₅₀ = the picomolar concentration of drug required to inhibit, by 50%, the growth of murine L1210 leukemia cells in a 3-day assay. ^cAssignment of cross-linking scores was based on the intensity of cross-linked bands in gel photos.¹⁸

duplexes indicate that the optimal chain lengths for interstrand crosslinking between variously spaced adenines correlate well with the optimal lengths suggested by the gel analysis.²¹ Dimers containing more rigid linkages between the CPI moieties and experimental determination of the distance between cross-linked bases and the sequence requirements for cross-linking are currently under investigation.

Acknowledgment. We thank Dr. Li H. Li for the *in vitro* growth inhibition data and *in vivo* antitumor data.

(21) Details of the molecular modelling studies will be described in the full paper.

Allylation of α -Hydroxy Ketones with Allyltrifluorosilanes and Allyltrialkoxysilanes in the Presence of Triethylamine. Stereochemical Regulation Involving Chelated Bicyclic Transition States¹

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In relation to the aldol addition of metal enolates,² the stereocontrolled introduction of an allyl group, especially to unsymmetrical ketones^{3,4} by the reaction of allylic metals is a challenge in the modern synthetic chemistry. We report herein that allyltrifluorosilanes (**1-3**) and allyltrialkoxysilanes (**4** and **5**) react

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(3) For stereoselective allylation of aldehydes, see. (a) Yamamoto, Y.; Maruyama, K. *Heterocycles* **1982**, *18*, 357. (b) Yamamoto, Y. *Acc. Chem. Res.* **1987**, *20*, 243. (c) Hoffmann, R. W. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 555. (d) Keck, G. E.; Boden, E. P. *Tetrahedron Lett.* **1984**, *25*, 265 and 1897.

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