Communications to the Editor

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

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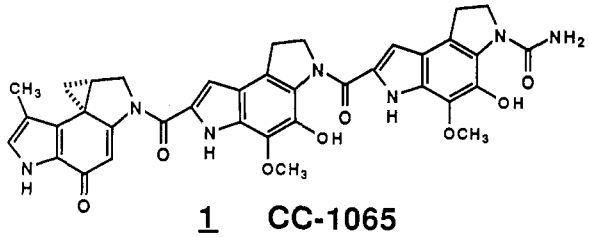
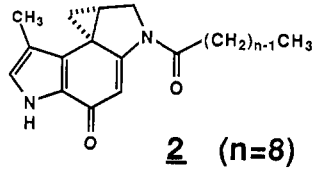
The cytotoxicity of a number of clinically useful antitumor alkylating agents is often attributed to interstrand cross-linking of cellular DNA.[1-3] CC-1065, **1**, a highly cytotoxic antitumor antibiotic, has been shown to bind in the minor groove of double stranded B-DNA and bond covalently to the 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]

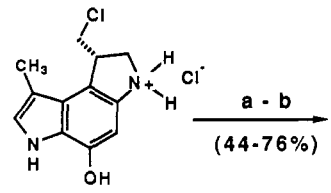


1 CC-1065



2 (n=8)

Scheme I[a]



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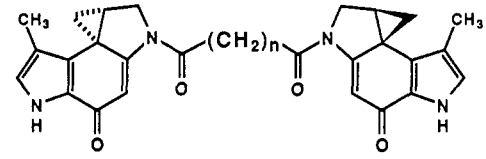
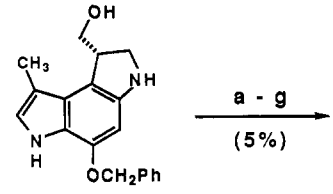


4a-k (n=2-11,14)

[a] (a) $ImOC(CH_2)_nCOIm$, DMF; (b) 2:1:1, $CH_3CN/NEt_3/H_2O$.

Scheme II[a]



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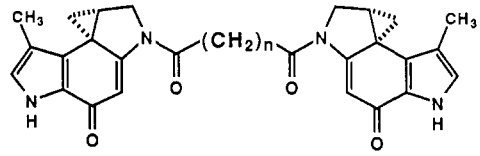

6 (n=8)

[a] (a) $CH_3OOC(CH_2)_8COOH$, EDC, DMAP; (b) KOH, CH_3OH, H_2O; (c) EDC, *ent*-**5**; (d) MsCl, pyridine; (e) LiCl, DMF, 80-100 °C; (f) NH_4OOCH, Pd/C, CH_3OH; (g) $CH_3CN/NEt_3/H_2O$, 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-imidazolide[12] 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

(1) (a) Iyer, V. N.; Szybalski, W. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *50*, 355-362. (b) Iyer, V. N.; Szybalski, W. *Science* **1964**, *145*, 55-58.
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(3) Kohn, K. W. *Cancer Res.* **1977**, *37*, 1450-1454.
(4) (a) Martin, D. G.; Chidester, C. G.; Duchamp, D. J.; Mizsak, S. A. *J. Antibiot.* **1980**, *33*, 902-903. (b) Martin, D. G.; Biles, C.; Gerpheide, S. A.; Hanka, L. J.; Krueger, W. C.; McGovren, J. P.; Mizsak, S. A.; Neil, G. L.; Stewart, J. C.; Visser, J. *J. Antibiot.* **1981**, *34*, 1119-1125. (c) Chidester, C. G.; Krueger, W. C.; Mizsak, S. A.; Duchamp, D. J.; Martin, D. G. *J. Am. Chem. Soc.* **1981**, *103*, 7629-7635. (d) Li, L. H.; Swenson, D. H.; Schpok, S. L.; Kuentzel, S. L.; Dayton, B. D.; Krueger, W. C. *Cancer Res.* **1982**, *42*, 999-1004. (e) Swenson, D. H.; Li, L. H.; Hurley, L. H.; Rokem, J. S.; Petzold, G. L.; Dayton, B. D.; Wallace, T. L.; Lin, A. H.; Krueger, W. C. *Cancer Res.* **1982**, *42*, 2821-2828. (f) Hurley, L. H.; Reynolds, V. L.; Swenson, D. H.; Petzold, G. L.; Scahill, T. A. *Science (Washington, D.C.)* **1984**, *226*, 843-844. (g) Needam-Van Devanter, D. R.; Hurley, L. H.; Reynolds, V. R.; Theriault, N. Y.; Krueger, W. C.; Wierenga, W. *Nucleic Acids Res.* **1984**, *12*, 6159-6168. (h) Reynolds, V. L.; Molineux, I. J.; Kaplan, D. J.; Swenson, D. H.; Hurley, L. H. *Biochemistry* **1985**, *24*, 6228-6237. (i) Reynolds, V. L.; McGovren, J. P.; Hurley, L. H. *J. Antibiot.* **1986**, *39*, 319-334. (j) Rawal, V. H.; Jones, R. J.; Cava, M. P. *Heterocycles* **1987**, *25*, 701-728. (k) Warpehoski, M. A.; Hurley, L. H. *Chem. Res. Toxicol.* **1988**, *1*, 315-333.
(5) Some of these results have been presented in preliminary form: Mitchell, M. A.; Swenson, D. H.; Wallace, T. L.; Williams, M. G.; Petzold, G. L.; Aristoff, P. A.; Johnson, P. D.; Li, L. H. *Proc. Am. Assoc. Cancer Res.* **1987**, *28*, 1068.
(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]
(8) Farmer, J. D.; Rudnicki, S. M.; Suggs, J. W. *Tetrahedron Lett.* **1988**, *29*, 5105-5108.
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(11) Kelly, R. C.; Gebhard, I.; Wicnienski, N.; Aristoff, P. A.; Johnson, P. D.; Martin, D. G. *J. Am. Chem. Soc.* **1987**, *109*, 6837-38.
(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.
(14) Warpehoski, M. A. *Tetrahedron Lett.* **1986**, *27*, 4103-4106.
(15) *ent*-**4f-h** were prepared by using the methods described for **4a-k** with the enantiomer of **3**.[11,14]

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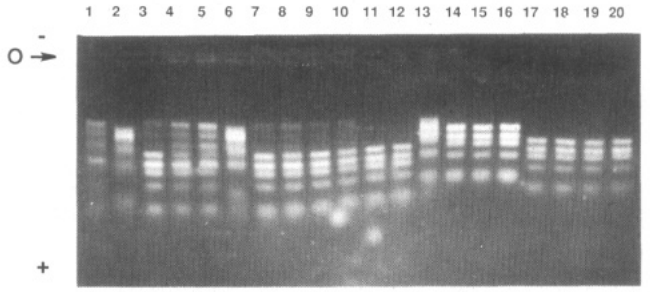

Figure 1. Alkaline agarose gel showing cross-linking of DNA by CPI dimers. Each compound, in 5 μL of dimethylacetamide (DMA), was incubated for 2 h at 37 °C with 1 μg of *φX174* HaeIII digest in 100 μL of PBS buffer (15 μM in base pairs).[17] Samples were precipitated, resuspended, loaded onto a 1% horizontal-bed 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 μM; lanes 2, 4, 6, 8, and 10, dimers at 1.7 μM; 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 μM, respectively; lanes 13–16, trimethylpsoralen controls at 17 μM, 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 μM, 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 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.

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

compound (n)[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	–
ent-**4f**	(7)	40000	–
ent-**4g**	(8)	5000	–
ent-**4h**	(9)	10000	–
6	(8)	200	–
2	(8)	60000	–
1		30	–

[a] Chain length. [b] ID_{50} = the picomolar concentration of drug required to inhibit, by 50%, the growth of murine L1210 leukemia cells in a 3-day assay. [c] Assignment of cross-linking scores was based on the intensity of cross-linked bands in gel photos.[18]

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

(16) Cech, T. R. *Biochemistry* **1981**, *20*, 1431–1437.

(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 μM drug. (++) indicates that comparable levels of both cross-linked and uncross-linked bands were formed at 1.7 μM 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.; McGovren, 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 μg/kg, whereas compound **4g** at 3.1 μg/kg increased the lifespan of such mice by greater than 150%.

(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 States[1]

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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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(2) Evans, D. A.; Nelson, J. V.; Taber, T. R. *Top. Stereochem.* **1982**, *13*, 1.

(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.

(4) Partial success of regio- and stereoselective allylation of ketones by using crotyltitanium[4a,b] and boron[4c] reagents has been reported. (a) Seebach, D.; Widler, L. *Helv. Chim. Acta* **1982**, *65*, 1972. (b) Reetz, M. T.; Steinbach, R.; Westermann, J.; Peter, R.; Wenderoth, B. *Chem. Ber.* **1985**, *118*, 1441. (c) Yamamoto, Y.; Maruyama, K.; Komatsu, T.; Ito, W. *J. Org. Chem.* **1986**, *51*, 886.

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