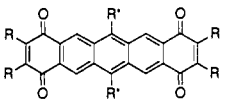
at 1.40 μ in DMF, 1.35 μ in CH_2Cl_2, and 1.42 μ in methyltetrahydrofuran.)

The contrasts between linear and helical bisquinones are striking. Unlike $\mathbf{1}^{\bullet-}$, $\mathbf{7a}^{\bullet-}$ and $\mathbf{7b}^{\bullet-}$ exhibit no well-defined absorption in the near-IR.[2c] For **7a** the difference between the first and second reduction potentials (120 mV) is one-quarter that of **1**.[2c] For $\mathbf{7b}^{\bullet-}$ the ESR spectrum changes with temperature, demonstrating that an electron localized on one quinone hops to the other with rate constant $k = 6 \times 10^8\ s^{-1}$ at room temperature.[17] That the anion radicals of all the helical quinones, not just that of **1**, have delocalized structures (unlike the linear analogues, for which this is true for only the two lowest members[2c,d]) is suggested by ΔE in Table I decreasing monotonically but still being appreciable when the quinones are separated by six rings.[2b-d] We speculate that the differences between the helical and linear anions may originate in the ends of the helices interacting. Perhaps this constitutes a new form of cyclic delocalization.[18]



7a, R = R′ = H
7b, R = CH_3; R′ = n-C_6H_{13}

Acknowledgment. We are grateful to the National Science Foundation for its support under Grants DMR-87-01968 (to Columbia University) and CHE-87-17540 (to the University of Minnesota).

Supplementary Material Available: The sources of materials used in the syntheses (1 page). Ordering information is given on any current masthead page.

(17) Unpublished results of Stanton Rak, University of Minnesota. This derivative was used because it is more soluble than **7a**.

(18) Initial semiempirical and ab initio calculations support this hypothesis. Unpublished results of C. A. Liberko and private communication from J. Almlöf.

Synthesis and DNA Cross-Linking by a Rigid CPI Dimer

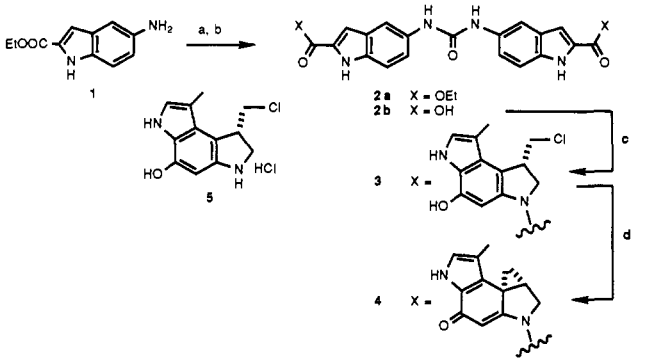
Mark A. Mitchell,* Robert C. Kelly,* Nancy A. Wicnienski, Nicole T. Hatzenbuhler, Marta G. Williams, Gary L. Petzold, Jerry L. Slightom, and David R. Siemieniak

The Upjohn Laboratories, The Upjohn Company Kalamazoo, Michigan 49001

Received April 22, 1991

Recently, the preparation of dimeric molecules containing two of the DNA-alkylating cyclopropa[*c*]pyrrolo[3,2-*e*]indol-4-(5*H*)-one (CPI) subunits of CC-1065 was reported by these laboratories.[1] In those CPI dimers the two alkylating subunits were linked by variable-length flexible methylene chains. We anticipated that incorporation of a more rigid linker into the CPI dimers would yield molecules with a fixed, measurable recognition-site size. We report herein the synthesis of compound **3** and the corresponding spirocyclopropyl, CPI analogue, **4**.[2] We also report our preliminary biochemical efforts which (1) document the formation of interstrand cross-links by compound **3** under mild conditions and (2) begin to characterize the alkylation-site requirements for cross-linking by this compound. These compounds represent the first molecules reported as interstrand cross-linking agents with an alkylation-site size as long as six base pairs and, therefore, may serve as useful molecules on which to build our understanding of DNA as a receptor for dimeric molecules.

Scheme I[a]



[a] (a) ClCOCl, $(i\text{-Pr})_2$NEt, DMAP, THF, −98 → 0 °C, 16 h; (b) NaOH, H_2O, H_2O, pyr, 25 °C for 96 h, then 55 °C for 7 h; (c) EDC, DMA, **5** (2 equiv), 25 °C, 2 h; (d) Et_3N–H_2O–CH_3CN, 1:1:2, 40 min.

Compounds **3** and **4** both contain a bis(indolecarboxylic acid) linker, **2b**, and were prepared as shown in Scheme I.[3] Briefly, amine **1**[4] was converted to its symmetrical urea **2a** by treatment with phosgene at low temperature. The resultant diester **2a** was hydrolyzed to the diacid **2b** with aqueous sodium hydroxide in pyridine at 25 °C. Bis(chloromethyl)diphenol **3** was prepared from **2b** and cyclized to bis(cyclopropapyrroloindole) **4** by previously described methodology.[1,5]

Compound **3**, presumably serving as a ring-opened prodrug of **4**, readily alkylates DNA and possesses cytotoxic properties similar to those of the spirocyclopropyl compound **4**.[6] The method of induced circular dichroism[8-10] reveals strong DNA association for both **3** and **4** consistent with their binding and bonding within the minor groove of DNA.[11] In addition, a dose-dependent formation of interstrand cross-links in ΦX174 restriction fragments treated with compound **3** is revealed by the appearance of reduced-mobility bands during denaturing alkaline agarose gel electrophoresis (supplementary material).[1] These results mimic our earlier observations, that interstrand cross-linking can be correlated with high cytotoxic potency for CPI dimers.[1] In contrast to the flexible CPI dimers, compound **3** exhibits curative in vivo antitumor efficacy in some systems.[12]

Considerable effort has been expended to understand the details of the interaction between monomeric CPI compounds and DNA in an attempt to gain insights into their interesting biological properties.[8,10,13,14] Molecular modeling studies with compound

(1) Mitchell, M. A.; Johnson, P. D.; Williams, M. G.; Aristoff, P. A. *J. Am. Chem. Soc.* **1989**, *111*, 6428-6429.

(2) Compound **3**, NSC 615291, also referred to as U-77779, is currently under development in collaboration with the National Cancer Institute.

(3) Analytical data for compounds **2a,b**, **3**, and **4** are consistent with the structures shown. See supplementary material.

(4) Warpehoski, M. A.; Bradford, V. S. *Tetrahedron Lett.* **1988**, *29*, 131-134.

(5) Kelly, R. C.; Gebhard, I.; Wicnienski, N.; Aristoff, P. A.; Johnson, P. D.; Martin, D. G. *J. Am. Chem. Soc.* **1987**, *109*, 6837-6838.

(6) Compounds **3** and **4** each inhibit the growth of murine L1210 leukemia cells by 50% at a concentration of 1 pM in a 3-day in vitro assay.[7]

(7) For comparison, the IC_{50}'s for CC-1065 and adozelesin are 30 and 4 pM respectively.

(8) 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.

(9) Krueger, W. C.; Li, L. H.; Moscowitz, A.; Prairie, M. D.; Petzold, G.; Swenson, D. H. *Biopolymers* **1985**, *24*, 1549-1572.

(10) (a) Li, L. H.; Swenson, D. H.; Schpok, S.; Kuentzel, S. L.; Dayton, B. D.; Krueger, W. C. *Cancer Res.* **1982**, *42*, 999-1004. (b) Bhuyan, B. K.; Newell, K. A.; Crampton, S. L.; Von Hoff, D. D. *Cancer Res.* **1982**, *42*, 3532-3537. (c) Li, L. H.; Wallace, T. L.; DeKoning, T. F.; Warpehoski, M. A.; Kelly, R. C.; Prairie, M. D.; Krueger, W. C. *Invest. New Drugs* **1987**, *5*, 329-337.

(11) Unpublished results.

(12) For example, the most effective flexible dimers (spirocyclopropyl or chlorophenol form) increased the life span of mice bearing intraperitoneal L1210 leukemia by 60-80% at optimal iv doses while a single iv injection of compound **3** at 10 μg/kg cured (30 day survivors) 50% of the mice. For comparison, adozelesin increased the life span of such mice by 94% at an optimal single iv dose of 100 μg with no cures. Results of additional antitumor testing with compound **3** will be reported separately.

0002-7863/91/1513-8994$02.50/0

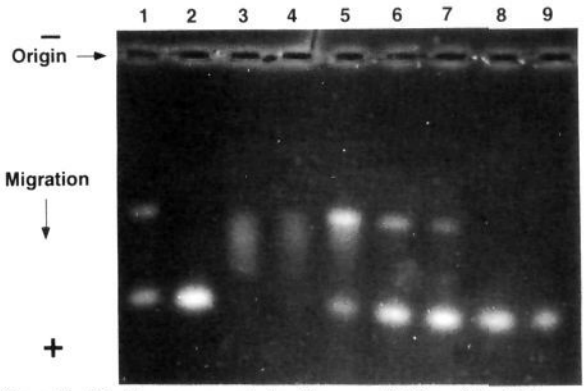


Figure 1. Alkaline agarose gel showing cross-linking of the 41-base-pair oligonucleotide by compound **3**. Each compound, in 5 μL of DMA, was incubated for 18 h at 37 °C with 1 μg of the 41-base-pair duplex oligonucleotide in 100 μL of TE buffer (15 μM in base pairs).[16] Samples were ethanol precipitated, resuspended in running buffer, loaded onto a 6% NuSieve (FMC Bioproducts, Rockland, ME) horizontal-bed alkaline agarose gel, and run for 2 h at 100 V. The gel was neutralized and stained as previously described.[1,17] Lane 1, trimethylpsoralen positive control, 17 μM irradiated for 10 s; lane 2, untreated DNA; lanes 3–7, DNA treated with **3** at 5, 1.5, 1.0, 0.5, and 0.15 μM, respectively; lane 8, DNA treated with adozelesin at 3 μM; lane 9, DNA treated with CC-1065 at 3 μM.

3 bound to oligonucleotide helices suggested that a binding-site size of six base pairs inclusive of the alkylated adenines matched the steric requirements dictated by the length and rigidity of compound **3**.

We have used the alkaline agarose cross-linking assay with a 41-base-pair oligonucleotide containing three separate but identical blocks of the sequence 5′-TAATTA-3′.[15] We selected 5′-TAATTA-3′ as the target binding and bonding sequence for compound **3** on the basis of the 5′-TTA-3′ alkylation sequence preferences seen for CC-1065 and other simplified analogues.[13b] Compound **3** readily forms DNA cross-links within this 41-base-pair duplex as shown in Figure 1.

The sites of alkylation within each strand of this duplex were assessed using the heat strand breakage assay previously utilized for monomeric CPI-containing compounds.[13a,b,18,19] Strand breakage occurs in each strand only at the 3′-terminal adenines of the 5′-TAATTA-3′ blocks (supplementary material). The results of the alkaline agarose gel assay together with the heat strand breakage assays indicate that compound **3** is capable of alkylating two distinct adenines on opposite strands separated by approximately one-half of a helical turn.

The incorporation of a rigid linker into dimeric CPI-based alkylating agents has yielded a class of dimeric compounds for which the DNA recognition site has been extended to six base pairs compared to the recognition-site size for monomeric CPI analogues. To our knowledge there are no other examples of interstrand cross-linking agents reported in the literature which possess a recognition site of this size. The biological implications of the increased size of the DNA recognition element are not known, but the increase in size may confer a greater absolute sequence selectivity upon these agents.[21]

Acknowledgment. We thank Dr. Li H. Li, Thomas F. DeKoning, and Joan W. Culp for the in vitro growth inhibition data and in vivo antitumor data.

Supplementary Material Available: Complete experimental details including analytical and spectral data for compounds **2a,b**, **3**, and **4**, alkaline agarose gel assay of ΦX174 restriction fragments treated with compound **3** and compound **4g** from ref 1, experimental methodology pertaining to the 41-base-pair oligonucleotide duplex, structures of CC-1065 and adozelesin, and the heat strand breakage gels of the 41-base-pair oligonucleotide duplex (10 pages). Ordering information is given on any current masthead page.

(13) (a) Hurley, L. H.; Reynolds, V. L.; Swenson, D. H.; Petzold, G. L.; Scahill, T. A. *Science* **1984**, *226*, 843–844. (b) Reynolds, V. L.; Molineux, I. J.; Kaplan, D. J.; Swenson, D. H.; Hurley, L. H. *Biochemistry* **1985**, *24*, 6228–6237. (c) Hurley, L. H.; Lee, C.-S.; McGovren, J. P.; Warpehoski, M. A.; Mitchell, M. A.; Kelly, R. C.; Aristoff, P. A. *Biochemistry* **1988**, *27*, 3886–3892. (d) Theriault, N. Y.; Krueger, W. C.; Prairie, M. D. *Chem. Biol. Interact.* **1988**, *65*, 187–201.

(14) Warpehoski, M. A.; Hurley, L. H. *Chem. Res. Toxicol.* **1988**, *1*, 315–333.

(15) Sequence of the 41-base-pair duplex oligonucleotide:

5′-CGCTAATTAGGGGGCTAATTAGCGCGCGCTAATTAGGCCGC-3′

3′-GCGATTAATCCCCCGATTAATCGCGCGCGATTAATCCGGCG-5′

(16) The TE buffer composition is 10 mM Tris·HCl, 2 mM EDTA, pH 7.5.

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

(18) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499–560.

(19) We have found that incubation of the DNA modified with compound **3** for 18 h at 70 °C in 0.05 M *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid–potassium hydroxide (HEPES-KOH), 0.1 M KCl, 0.05 M glycine, 0.5 mM EDTA, and 0.01 M putrescine reveals the strand breaks in DNA modified with compound **3** more effectively than standard methods.[20]

(20) Lindahl, T.; Andersson, A. *Biochemistry* **1972**, *11*, 3618–3623.

(21) Further studies are currently underway through collaborative efforts with L. H. Hurley at the University of Texas at Austin to more fully characterize the interaction of compound **3** with DNA.

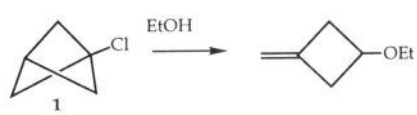
Formation of [1.1.1]Propellane by Nucleophilic Attack on 1,3-Diiodobicyclo[1.1.1]pentane. Unrearranged Carbocation Intermediates in the Reaction of [1.1.1]Propellane with Electrophiles

Kenneth B. Wiberg* and Neil McMurdie

Department of Chemistry, Yale University
New Haven, Connecticut 06511

Received July 15, 1991

We have shown that 1-chlorobicyclo[1.1.1]pentane (**1**) undergoes solvolysis 3 times as rapidly as *tert*-butyl chloride,[1] despite the usually low reactivity of small bridgehead halides such as 1-norbornyl chloride.[2] The high reactivity of 1-bicyclo[1.1.1]-pentyl derivatives has recently been confirmed by Della and Taylor in their investigation of the solvolysis of 1-bromobicyclo[1.1.1]-pentane.[3]



1

Originally, we believed that the high reactivity resulted from a simultaneous carbon–carbon bond cleavage giving the 3-methylenecyclobutyl cation, which would lead to considerable strain relief. The products are derived from this cation. In view of the now well established bicyclobutonium ion intermediate in the solvolysis of cyclobutyl derivatives,[4] and RHF calculation on the 1-bicyclo[1.1.1]pentyl cation which suggests a similar interaction,[5] the structure of this ion was studied at the MP2/6-31G*

(1) Wiberg, K. B.; Williams, V. Z., Jr. *J. Am. Chem. Soc.* **1967**, *89*, 3373.

(2) Bartlett, P. D.; Knox, L. *J. Am. Chem. Soc.* **1939**, *61*, 3184.

(3) Della, E. W.; Taylor, D. K. *Aust. J. Chem.* **1990**, *43*, 945.

(4) Saunders, M.; Laidig, K. E.; Wiberg, K. B.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1988**, *110*, 7652. Koch, W.; Liu, B.; DeFrees, D. J. *J. Am. Chem. Soc.* **1988**, *110*, 7325. The results of these calculations have recently been experimentally confirmed by Myhre et al.: Myhre, P. C.; Webb, G. G.; Yannoni, C. S. *J. Am. Chem. Soc.* **1990**, *112*, 8993.

(5) (a) Lehn, J. M.; Wipff, G. *Chem. Phys. Lett.* **1972**, *15*, 450. (b) Chandrasekhar, J.; Schleyer, P. v. R.; Schlegel, H. B. *Tetrahedron Lett.* **1978**, 3398. (c) Della, E. W.; Schiesser, C. H. *Tetrahedron Lett.* **1987**, *28*, 3869. (d) Della, E. W.; Schiesser, C. H. *J. Chem. Res. Synop.* **1989**, 172.

0002-7863/91/1513-8995$02.50/0