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RESOLUTION OF A CBI PRECURSOR AND INCORPORATION INTO THE SYNTHESIS OF (+)-CBI, (+)-CBI-CDPI$_1$, (+)-CBI-CDPI$_2$: ENHANCED FUNCTIONAL ANALOGS OF (+)-CC-1065. A CRITICAL APPRAISAL OF A PROPOSED RELATIONSHIP BETWEEN ELECTROPHILE REACTIVITY, DNA BINDING PROPERTIES, AND CYTOTOXIC POTENCY.

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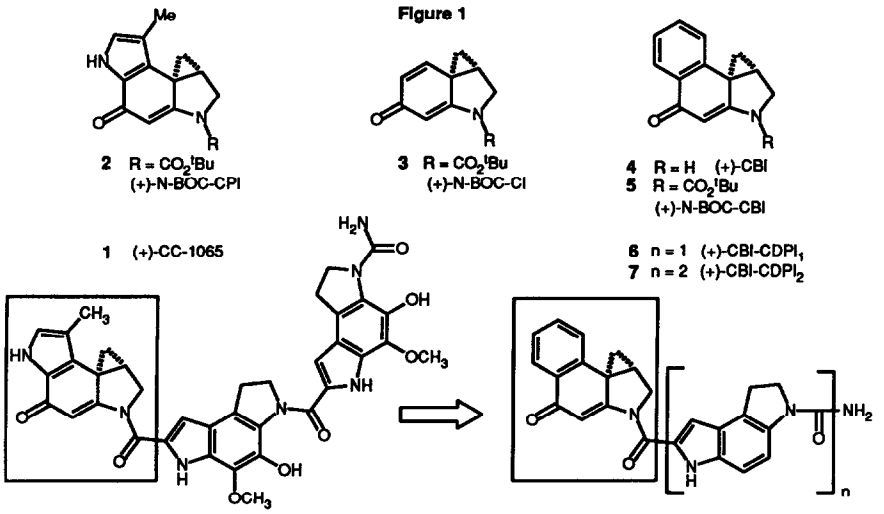
Summary: Details of the resolution of an immediate CBI precursor, (+)- and (-)-**8**, and its subsequent incorporation into (+)- and (-)-CBI-CDPI$_n$, optically-active enhanced functional analogs of (+)-CC-1065, are described. In marked contrast to a previously detailed *direct* relationship between electrophile reactivity and cytotoxic potency, an *inverse* relationship between the properties is detailed.

In a series of extensive investigations, the site and mechanism of the (+)-CC-1065 antitumor activity has been related to its covalent alkylation of sequence-selective minor groove sites [5′-d(A/GNTTA)-3′ and 5′-d(AAAAA)-3′] that has been demonstrated to proceed by 3′-adenine N-3 alkylation of the electrophilic cyclopropane present in the left-hand subunit (CPI).[2] The demonstration that (+)-N-acetyl-CPI exhibits a comparable albeit substantially less intense (ca. 10000x) sequence-selective alkylation of DNA has led to the firm conviction that it plays the dominant role in controlling the properties of the agents.[2,3] However, the demonstration that simplified agents including CDPI$_3$ methyl ester exhibit a substantial preference for A-T rich noncovalent minor groove binding attributable to the preferential stabilization of a noncovalent complex within the narrower, sterically more accessible A-T rich minor groove[4,5] has suggested that CC-1065

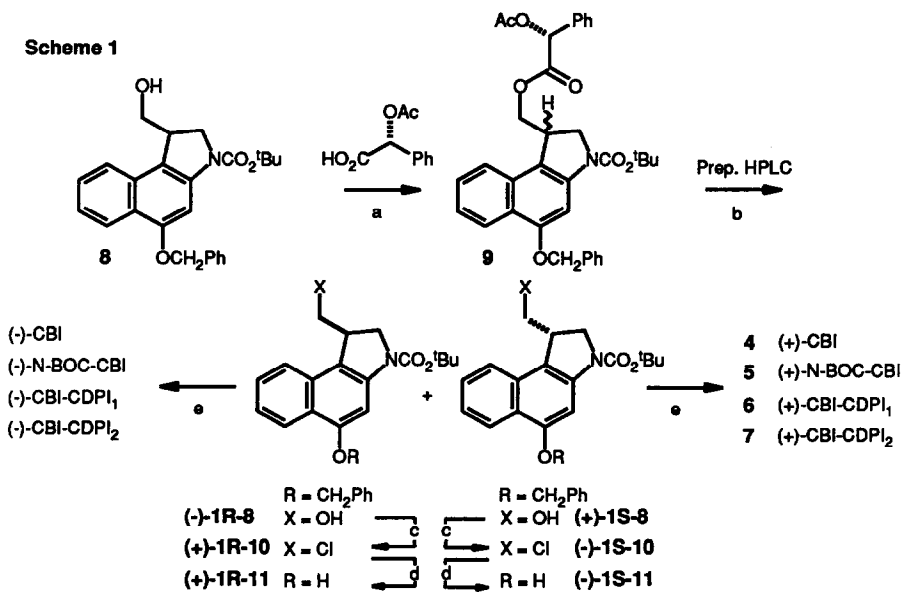
Figure 1



is best represented as a selective alkylating agent superimposed on the $CDPI_3$ skeleton and derives its properties in part from its effective delivery to accessible adenine N-3 alkylation sites.[4,5] The further demonstration that the CC-1065 sequence-selective DNA alkylation properties may be conferred to a reactive, nonselective electrophile (Cl) with its incorporation into the agent (+)-Cl-$CDPI_2$[9] suggests that the full role of the noncovalent CC-1065 binding selectivity has not been duly appreciated.[2] Thus, in anticipation that structural variations in the left-hand subunit of the agents would not preclude a relevant adenine N-3 alkylation, we recently disclosed the preparation and evaluation of functional analogs of CC-1065 incorporating the racemic 1,2,9,9a-tetrahydrocycloprop[1,2-c]benz[1,2-e]indol-4-one (CBI) left-hand subunit, Figure 1. Herein, we report the resolution of an immediate CBI precursor, (+)- and (-)-**8**, its subsequent incorporation into (+)- and (-)-CBI (**4**), (+)- and (-)-N-BOC-CBI (**5**), (+)- and (-)-CBI-$CDPI_1$ (**6**), and (+)- and (-)-CBI-$CDPI_2$ (**7**), and unanticipated[2,6,7] evidence to support an *inverse*[5] *versus direct*[2-3,6-7] relationship between electrophile reactivity (solvolysis rate) and cytotoxic potency.

Esterification of **8** with R-(-)-O-acetyl mandelic acid (1.5 equiv, 1.7 equiv EDCI, 0.1 equiv DMAP) in dichloromethane cleanly provided the diastereomeric esters **9** (81%), Scheme I. Normal phase preparative chromatographic separation (2% EtOAc-CH_2Cl_2, α = 1.09)[10] of **9** provided 1S,2'R-**9** and 1R,2'R-**9** of > 99% diastereomeric purity (85% recovery) as determined by HPLC and ^1H NMR analysis.[11] Base-promoted hydrolysis provided (+)-1S-**8** and (-)-1R-**8**, independent conversion to the corresponding primary chlorides (-)-1S-**10** and (+)-1R-**10**, and subsequent two-phase, transfer catalytic hydrogenolysis of the benzyl ether provided (-)-1S-**11** and (+)-1R-**11**, respectively. The subsequent incorporation of (-)-1S-**11** into (+)-CBI (**4**), (+)-N-BOC-

Scheme 1



(a) 1.5 equiv (R)-(-)-O-acetyl mandelic acid, 1.7 equiv ECDI, 0.1 equiv 4-DMAP, CH_2Cl_2, 24°C, 1 h, 81%; (b) 5.0 equiv aq. 4 N LiOH, MeOH/THF (2:3), 24°C, 1 h, 97%; (c) 2.0 equiv Ph_3P, 6.0 equiv CCl_4, CH_2Cl_2, 24°C, 10 h, 99%; (d) 25% aqueous HCO_2NH_4/THF (1:5), 10% Pd/C, 0°C, 2.5 h, 97%; (e) reference 8.

Table 1.	N-BOC-CI (3)	N-BOC-CPI (2)	N-BOC-CBI (5)	
[a]k (pH = 7)	3.67 ± 0.02 x 10^{-5} sec^{-1}	stable	stable	
$t_{1/2}$ (pH = 7)	5.24 h	-	-	
[a]k (pH = 3)	1.98 ± 0.06 x 10^{-2} sec^{-1}	5.26 ± 0.08 x 10^{-6} sec^{-1}	1.45 ± 0.01 x 10^{-6} sec^{-1}	
$t_{1/2}$ (pH = 3)	35 sec	36.7 h	133 h	
[b]IC_{50} (μM)	36	0.33	0.07	
[b]IC_{50} (pM)	(+)-CI-CDPI$_2$, 10000	(+)-CPI-CDPI$_2$, 20	(+)-CBI-CDPI$_2$, 4.8	(+)-CC-1065, 20
	(+)-CI-CDPI$_1$, 24000	(+)-CPI-CDPI$_1$, 40	(+)-CBI-CDPI$_2$, 5	

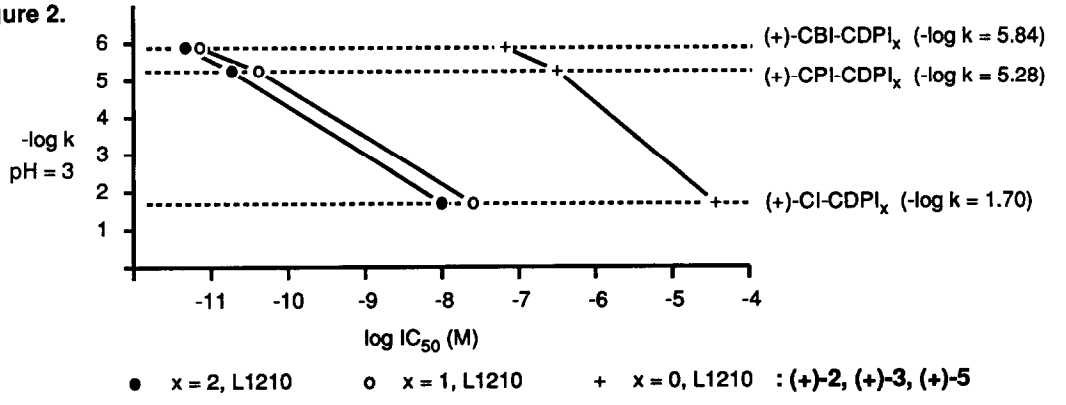
[a]Solvolysis studies conducted spectrophotometrically (UV) at pH = 7 (50% H_2O-CH_3OH) and pH = 3 (50% buffer-CH_3OH, buffer = 4:1:20 (v:v:v) 0.1 M citric acid, 0.2 M Na_2HPO_4, and water), cf. reference 6. [b]IC_{50} = Inhibitory concentration for 50% cell growth (L1210) relative to untreated controls, cf. reference 15-17.

CBI (5), (+)-CBI-CDPI$_1$ (6), and (+)-CBI-CDPI$_2$ (7) followed the protocols previously detailed.[8] The initial, tentative assignment of the absolute configuration of the agents rested with the selective, potent cytotoxic activity of the (+)-enantiomers, Table 1, was supported by the DNA binding profile of the agents [(+)-CBI-CDPI$_1$ = (+)-CBI-CDPI$_2$ = (+)-CC-1065/(+)-CPI-CDPI$_2$ ≠ (-)-CBI-CDPI$_2$ = (-)-CPI-CDPI$_2$/(-)-CC-1065],[12] and has been established unambiguously with a single-crystal X-ray structure analysis of (-)-1S-10.[13]

The results of the preliminary cytotoxic evaluation of the agents revealed that (+)-CBI-CDPI$_2$ (7) is 4x more potent (L1210) than (+)-CC-1065/(+)-CPI-CDPI$_2$, Table 1. This unanticipated enhanced cytotoxic potency of (+)-7 relative to (+)-1 is not in agreement with the firm conviction[2,6] that the productive DNA binding properties of the agents and resulting expression of cytotoxic potency are directly related to the reactivity of the electrophile as extrapolated from acid-catalyzed solvolysis rates[6] and, thus, *directly* related to the agents *rate* of acid-catalyzed covalent DNA modification.[2] In fact, through comparison of (+)-CI-CDPI$_x$,[9] (+)-CPI-CDPI$_x$,[14] and (+)-CBI-CDPI$_x$ and the corresponding rates of solvolysis (pH = 3) of **2**, **3**, and **5**, Table 1, we have found that the *inverse* relationship of the agent solvolysis reactivity and cytotoxic potency constitutes a more relevant relationship, Figure 2.

Thus, in marked contrast to conclusions drawn by Hurley and Warpehoski from their efforts,[2] we conclude that the cytotoxic potency of the agents bears no relationship with the agents' relative *rate* of acid-catalyzed DNA covalent alkylation and, moreover, that an *inverse*[5] *versus direct*[2-3,6-7] relationship between the relative reactivity of the electrophile and cytotoxic potency may be more relevant. This presumably results from more selective or productive (agent availability) covalent modification of DNA although the precise origin of this relationship is under present investigation.[15-17]

Figure 2.



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References

1. (a) Alfred P. Sloan Foundation Fellow, 1985-89. (b) On sabbatical from Kyorin Pharmaceutical Co., Ltd., Tochigi, Japan.
2. Hurley, L. H.; Warpehoski, M. A. *Chem. Res. Toxicol.* **1988**, *1*, 315. For early reviews, see: Hurley, L. H.; Needham-VanDevanter, D. R. *Acc. Chem. Res.* **1986**, *19*, 230. Reynolds, V. L.; McGovren, J. P.; Hurley, L. H. *J. Antibiot.* **1986**, *39*, 319.
3. 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.
4. Boger, D. L.; Invergo, B. J.; Coleman, R. S.; Zarrinmayeh, H.; Kitos, P. A.; Thompson, S. C.; Leong, T.; McLaughlin, L. W. *Chem.-Biol. Interactions*, in press.
5. Coleman, R. S.; Boger, D. L. in "Studies in Natural Products Chemistry", Vol. 3, Rahman, A.-u.-, ed.; Elsevier, 1989, 301.
6. Warpehoski, M. A.; Gebhard, I.; Kelly, R. C.; Krueger, W. C.; Li, L. H.; McGovren, J. P.; Prairie, M. Wicnienski, N.; Wierenga, W. *J. Med. Chem.* **1988**, *31*, 590.
7. 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. Wierenga, W.; Bhuyan, B. K.; Kelly, R. C.; Krueger, W. C.; Li, L. H.; McGovren, J. P.; Swenson, D. H..; Warpehoski, M. A. *Adv. Enz. Regul.* **1986**, *25*, 141.
8. Boger, D. L.; Ishizaki, T.; Wysocki, R. J., Jr.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1989**, *111*, 6461.
9. Boger, D. L.; Wysocki, R. J., Jr. *J. Org. Chem.* **1989**, *54*, 1238.
10. HPLC separation conditions for **9**: 10 x 25 cm 10 μm SiO_2, 2% EtOAc-CH_2Cl_2, 2.5 mL/min, effluent monitored at 280 nm; R_T = 17.7 min for 1R,2R-**9**, R_T = 19.3 min for 1S,2R-**9** (natural configuration).
11. In addition to HPLC analysis of **9** under the conditions detailed above, (+)-1R-**11** and (-)-1S-**11** (natural configuration) were evaluated by chiral phase HPLC (Bakerbond covalent DNBPG column, 5 μm, 4.6 mm x 25 cm, 2% 2-propanol in hexane, 1.0 mL/min, effluent monitored at 310 nm). R_T = 15.8 min for (+)-1R-**11** and R_T = 16.9 min for (-)-1S-**11**, α = 1.07.
12. Boger, D. L.; Munk, S. A., unpublished observations.
13. The single-crystal X-ray structure determination was conducted by Dr. P. Fanwick, Department of Chemistry, Purdue University. Details are available upon request (DLB).
14. Boger, D. L.; Coleman, R. S. *J. Am. Chem. Soc.* **1988**, *110*, 4796; *J. Org. Chem.* **1988**, *53*, 695.
15. Evaluation of the nine agents presented in Figure 2 in three additional cell lines (B-16, 9PS(P388), and 9KB) provided comparable results.
16. Boger, D. L.; Yasuda, M.; Mitscher, L. A.; Drake, S. D.; Kitos, P. A.; Thompson, S. C. *J. Med. Chem.* **1987**, *30*, 1918.
17. For (+)-**4**: $[\alpha]_D^{23}$ +332° (c = 0.05, CH_3OH); (+)-**5**: $[\alpha]_D^{23}$ +173° (c = 0.15, THF); (+)-**6**: $[\alpha]_D^{23}$ +156° (c = 0.07, DMF); (+)-**7**: $[\alpha]_D^{23}$ +82° (c = 0.17, DMF). IC_{50} (pM, L1210), for (+)-CC-1065 (**1**): 20, (+)-**4**: 2 x 10^7, (+)-**5**: 5 x 10^4, (+)-**6**: 5, (-)-**6**: ≥ 370, (+)-**7**: 5, (-)-**7**: 40.

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