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A HAMMETT CORRELATION FOR CC-1065 AND DUOCARMYCIN ANALOGS: MAGNITUDE OF SUBSTITUENT ELECTRONIC EFFECTS ON FUNCTIONAL REACTIVITY

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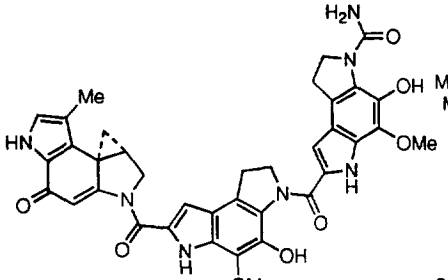
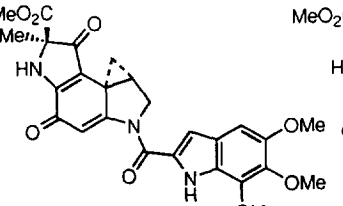
Abstract. A quantitative Hammett study of the magnitude of the electronic effects of a C7 substituent on the functional reactivity of N-BOC-CBI (**5**, R = H), its impact on biological properties, details of the mechanism of the acid-catalyzed nucleophilic addition to the activated cyclopropane, and its implications on the origin of the DNA alkylation selectivity of this class of agents is detailed.

CC-1065 (**1**),[1] duocarmycin A (**2**),[2] and duocarmycin SA (**3**)[3] constitute the parent members of a class of exceptionally potent naturally occurring antitumor antibiotics that exert their biological effects through a sequence selective DNA alkylation.[4,5] The stereoelectronically-controlled adenine N3 addition to the least substituted carbon of the activated cyclopropane has been shown to occur within 5 or 3.5 base-pair AT rich sites of the minor groove, respectively, corresponding nicely to the size of the agents. Since the disclosure of **1–3**, extensive efforts have been devoted to define the origin of the DNA alkylation selectivity, to establish the link between DNA alkylation and the ensuing biological properties,[6] and to determine relationships between structure, chemical reactivity, and biological activity.



1, (+)-CC-1065



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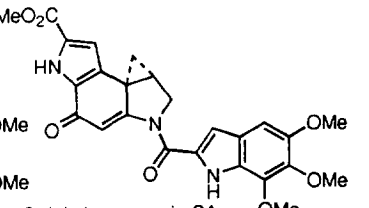
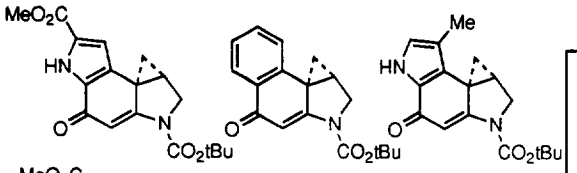


3, (+)-duocarmycin SA

Fundamental to the establishment of such relationships is the development of correlations between the structure of the DNA alkylation subunits and their functional chemical reactivity and the impact this reactivity has on the biological properties of the agents. Observations made in initial studies conducted with simple derivatives of the CC-1065 alkylation subunit (CPI) led to the proposal of a qualitative direct relationship between chemical reactivity and in vitro cytotoxic activity establishing the expectation that the DNA alkylation rate/efficiency and biological potency may be enhanced as the electrophilic character of the agent is increased.[7] In a complementary series of studies conducted with agents containing deep-seated modifications in the alkylation subunits including **4–9**, the alternative direct relationship between chemical stability and in vitro cytotoxic potency was observed and proved to be general with both simple and advanced analogs of

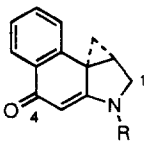
the natural products.[4,8] Quantitation of this effect with the simple CBI derivatives **10–13** established a predictable, linear relationship between the electron-withdrawing capabilities of the N^2 substituent, the chemical stability of the agent, and its biological potency and would seem to validate the generality of the observations.[9] However, the relevance of these latter observations to the N^2-acyl derivatives **4–9** and the accuracy of correlations with the series of agents **4–9** themselves which contain structural changes beyond those which impact reactivity remain to be established.

4, (+)-N-BOC-DSA **5**, (+)-N-BOC-CBI **6**, (+)-N-BOC-CPI



	k (s^{-1}, pH 3)	$t_{1/2}$ (pH 3)	IC_{50} (L1210)
4	1.08×10^{-6}	177 h	6 nM
5	1.45×10^{-6}	133 h	80 nM
6	5.26×10^{-6}	37 h	330 nM
7	1.75×10^{-5}	11 h	1000 nM
8	9.07×10^{-5}	2.1 h	2000 nM
9	1.98×10^{-2}	0.01 h	18000 nM

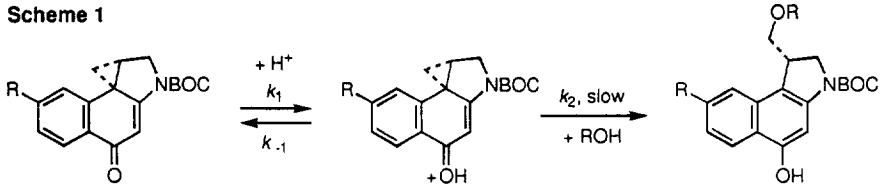
7, (+)-N-BOC-DA **8**, (-)-N-BOC-CBQ **9**, (+)-N-BOC-CI



	R	k (s^{-1}, pH 3)	$t_{1/2}$	IC_{50} (L1210)	σ
10	SO_2Et	0.5×10^{-6}	383 h	24 nM	0.72
11	COEt	2.0×10^{-6}	96 h	110 nM	0.48
12	CO_2Me	3.4×10^{-6}	57 h	140 nM	0.45
13	CONHMe	5.3×10^{-6}	36 h	200 nM	0.36

As a consequence, we sought to quantitatively establish this relationship in a study which would define both the magnitude of substituent electronic effects on the chemical reactivity of the typical N-acyl alkylation subunits as well as the impact this has on the biological potency of the agents. This was addressed with the preparation and comparative examination of **14** and **15**,[10] substituted derivatives of N-BOC-CBI (**5**), [8,11] in which a strong electron-withdrawing or electron-donating C7 substituent was placed para to the C4 carbonyl of the alkylation subunit providing a classical Hammett series for quantitative analysis. Consistent with expectations, the agent **14** possessing the electron-donating substituent proved to be the most reactive in the series while the agent **15** possessing the electron-withdrawing substituent was the most stable. However, the magnitude of the electronic effect of the C7 substituent on the reactivity was remarkably modest and **14** was only approximately 2× more reactive than **15** and nearly as stable as **5** itself as measured by acid-catalyzed solvolysis at either pH 2 or pH 3 (Figure 1).[8,9] As illustrated by the relative aqueous solvolysis rates measured

Scheme 1

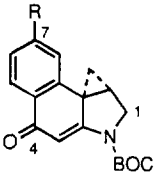


for $k_{-1} >> k_2$ $k_{obs} = \dfrac{k_1 k_2}{k_{-1}} = Kk_2$

at pH 3 versus pH 2, the 10-fold rate increase at pH 2 for all three agents is indicative of a first order dependence on the acid concentration for the rate of solvolysis.

In studies conducted with **15**, the solvolysis reaction not only exhibited this first order rate dependence on acid concentration but also exhibited a first order rate dependence on the concentration of the nucleophile when the acid-catalyzed addition reaction of CH_3OH was conducted in THF (0.1–0.25 equiv CF_3CO_3H, 20–500 equiv CH_3OH, 25 °C). Together, these studies illustrate that the slow step for acid-catalyzed nucleophilic addition to the activated cyclopropane under the conditions examined is not C-4 carbonyl protonation, but rather nucleophilic addition to the activated cyclopropane following rapid and reversible C-4 protonation (Scheme 1).

Figure 1



5, R = H N-BOC-CBI
14, R = OMe N-BOC-MCBI
15, R = CN N-BOC-CCBI

Aqueous solvolysis

	R	k, pH 3 (k, pH 2)	$t_{1/2}$, pH 3 ($t_{1/2}$, pH 2)	IC_{50} (L1210)	σ
14	OMe	1.75×10^{-6} s^{-1} (1.62×10^{-5} s^{-1})	110 h (11.8 h)	90 nM	-0.28
5	H	1.45×10^{-6} s^{-1} (1.53×10^{-5} s^{-1})	133 h (12.5 h)	80 nM	0.00
15	CN	0.90×10^{-6} s^{-1} (0.79×10^{-5} s^{-1})	213 h (24.2 h)	20 nM	0.70

pH 3: 50% CH_3OH-buffer (4:1:20 0.1M citric acid, 0.2M Na_2HPO_4, H_2O)
pH 2: 50% CH_3OH-buffer (4:1:20 1.0M citric acid, 0.2M Na_2HPO_4, H_2O)

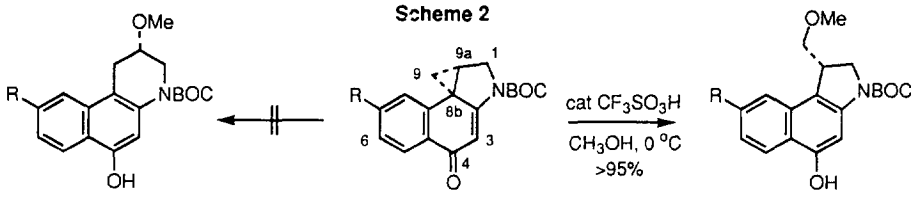
Methanol-THF solvolysis of **15**

Equiv H^+	Equiv CH_3OH	k	$t_{1/2}$
0.25	100	7.4×10^{-4} s^{-1}	0.26 h
0.1	100	2.1×10^{-4} s^{-1}	0.92 h
0.1	500	5.6×10^{-4} s^{-1}	0.34 h
0.1	20	0.2×10^{-4} s^{-1}	9.20 h

Cat. CF_3SO_3H, THF, 25 °C, 0.3 mM in **15**

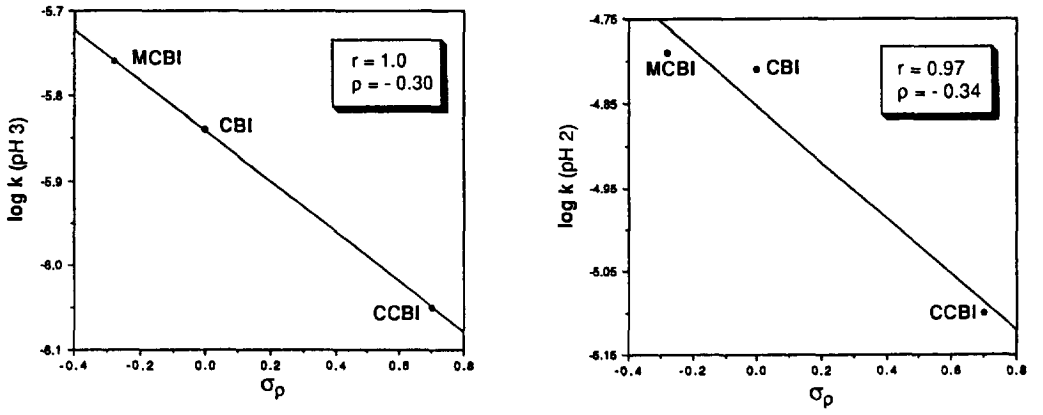
This latter reaction with **5** and **14–15**[10] was quantitatively established to provide exclusively the stereoelectronically controlled product of nucleophilic addition to the least substituted cyclopropane carbon in isolated yields of ≥95% with little or no trace of the alternative ring expansion addition product (regioselectivity, >20:1, Scheme 2).

Scheme 2



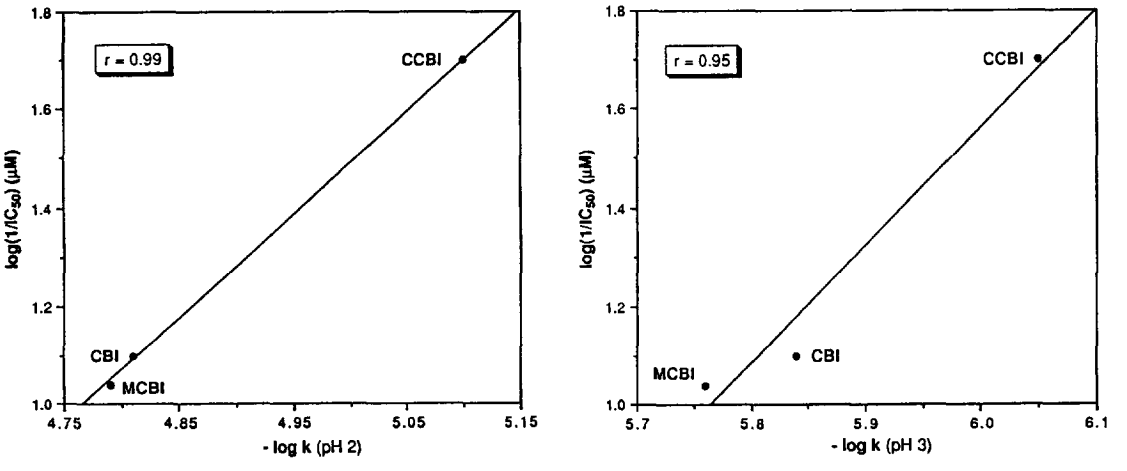
Hammett plots of the aqueous solvolysis studies with **9** and **14–15** revealed unusually small ρ values of –0.30 (pH 3) or –0.34 (pH 2) for the acid-catalyzed solvolysis (Figure 2). This exceptionally small ρ value of –0.3 indicates little differential positive charge buildup in the reaction transition state is further consistent with a strict S_N2 versus S_N1 mechanism requiring the presence and assistance of the nucleophile for ring opening of the cyclopropane. This has significant implications on the origin of the DNA alkylation selectivity of the agents and suggests that the positioning of an accessible nucleophile[4] (adenine N3) and not C4 carbonyl protonation[5,12] is the rate determining step controlling the DNA alkylation sequence selectivity.

Figure 2



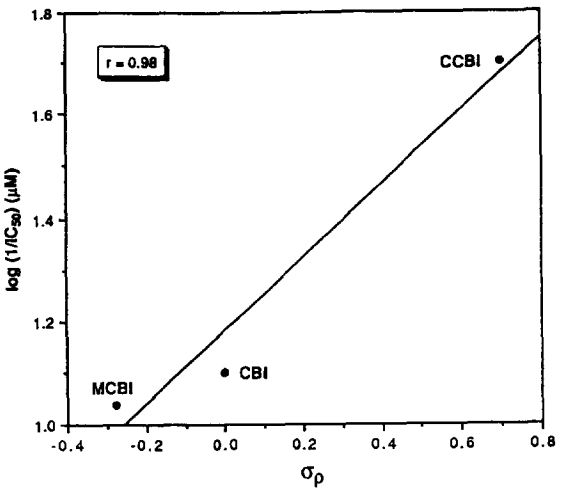
Consistent with past observations, the agents were found to follow a direct relationship between functional chemical stability (-log *k* solvolysis) and in vitro cytotoxic potency (L1210, log $1/IC_{50}$) over the range of reactivity examined by the series (Figure 3). For agents that possess sufficient reactivity to alkylate DNA, presumably this may be attributed to the more effective delivery of the more stable agents to their intracellular target and the solvolysis rates may be taken to accurately represent the relative functional reactivity/stability of the agents.

Figure 3



Less obvious, but more fundamental, the observations were found to follow a predictable, direct relationship between in vitro cytotoxic activity (L1210, log $1/IC_{50}$) and the Hammett σ_p constant of the C7 substituent with the strongest electron-withdrawing substituent providing the most potent agent (Figure 4). This fundamental relationship should prove useful in the predictable design of new analogs of CC-1065 and the duocarmycins possessing further enhanced properties.

Figure 4



While the latter two correlations defined in Figures 3 and 4 are limited comparisons made on the three presently accessible agents, they do follow trends established in the examination of **4–13** and related agents further supporting their potential generality.[13] What is unmistakable from the comparisons is the surprisingly small C7 substituent electronic effect on the functional reactivity of the agents (Figure 1). The agent **15** is the most stable and one of the simplest alkylation subunits disclosed to date and a full study of the properties of its advanced analogs of **1–3** will be disclosed in due course.

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13. Identical trends have been observed with the advanced analogs of **1–3** incorporating the MCBI and CCBI alkylation subunits.

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