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Synthesis and Characterization of a Cyclobutane Duocarmycin Derivative Incorporating the 1,2,10,11-Tetrahydro-9*H*-cyclobuta[*c*]benzo[*e*]indol-4-one (CbBI) Alkylation Subunit

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Abstract: The synthesis of 1,2,10,11-tetrahydro-9*H*-cyclobuta[*c*]benzo[*e*]indol-4-one (**17**, CbBI), which contains a deep-seated fundamental structural modification in the CC-1065 and duocarmycin alkylation subunit consisting of the incorporation of a ring-expanded fused cyclobutane (vs cyclopropane), its chemical and structural characterization, and its incorporation into a key analogue of the natural products are detailed. The approach to the preparation of CbBI was based on a precedented (Ar-3′ and Ar-5′) but previously unknown Ar-4′ spirocyclization of a phenol onto a tethered alkyl halide to form the desired cyclobutane. The conditions required for the implementation of the Ar-4′ spirocyclization indicate that the entropy of activation substantially impacts the rate of reaction relative to that for the much more facile Ar-3′ spirocyclization, while the higher enthalpy of activation slows the reaction relative to an Ar-5′ spirocyclization. The characterization of the CbBI-based agents revealed their exceptional stability and exquisite reaction regioselectivity, and a single-crystal X-ray structure analysis of *N*-Boc-CbBI (**13**) revealed their structural origins. The reaction regioselectivity may be attributed to the stereoelectronic alignment of the two available cyclobutane bonds with the cyclohexadienone π-system, which resides in the bond that extends to the less substituted cyclobutane carbon for **13**. The remarkable stability of *N*-Boc-CbBI (which is stable even at pH 1) relative to *N*-Boc-CBI containing a cyclopropane ($t_{1/2}$ = 133 h at pH 3) may be attributed to a combination of the increased extent of vinylogous amide conjugation, the nonoptimal geometric alignment of the cyclobutane with the activating cyclohexadienone, and the intrinsic but modestly lower strain energy (1.8 kcal/mol) of a cyclobutane versus a cyclopropane.

Introduction

CC-1065 (**1**) and duocarmycin SA (**2**) represent key members of a class of antitumor agents that derive their biological activity from their ability to selectively alkylate duplex DNA (Figure 1).[1−3] The study of the natural products and their synthetic unnatural enantiomers,[4] derivatives, and key analogues has defined the fundamental features that control the DNA alkylation selectivity, efficiency, and catalysis, providing a detailed understanding of fundamental relationships between structure, reactivity, and biological activity.[3]

Among the most studied duocarmycin analogues is CBI[5] (Figure 2), which is synthetically more accessible and also has been found to enhance both the chemical stability (4×) and the biological potency (4×) of the corresponding derivatives relative to the alkylation subunit found in CC-1065. Thus, it is on this scaffold that new design concepts are often explored, developed, and evaluated.[3] Despite the extensive synthetic studies on the duocarmycins, there have been very few examples of modification of the alkylation subunit's cyclopropane, which is intimately involved in the DNA alkylation reaction responsible for their biological activity.[6] For some time, we have been interested in probing the deep-seated change in this cyclopropane involving expansion to a cyclobutane, which provides the corresponding

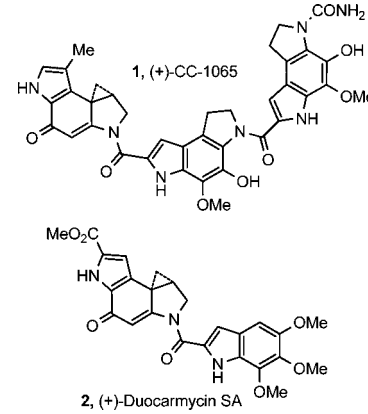

Figure 1. Natural products.

(1) For CC-1065, see: (a) 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. For duocarmycin SA, see: (b) Ichimura, M.; Ogawa, T.; Takahashi, K.; Kobayashi, E.; Kawamoto, I.; Yasuzawa, T.; Takahashi, I.; Nakano, H. *J. Antibiot.* **1990**, *43*, 1037. For duocarmycin A, see: (c) Takahashi, I.; Takahashi, K.; Ichimura, M.; Morimoto, M.; Asano, K.; Kawamoto, I.; Tomita, F.; Nakano, H. *J. Antibiot.* **1988**, *41*, 1915. For yatakemycin, see: (d) Igarashi, Y.; Futamata, K.; Fujita, T.; Sekine, A.; Senda, H.; Naoki, H.; Furumai, T. *J. Antibiot.* **2003**, *56*, 107.

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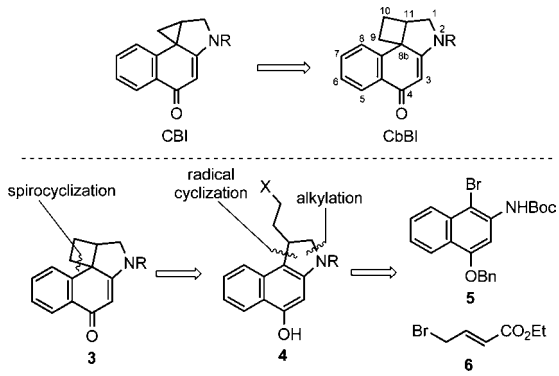

Figure 2. CBI vs CbBI and retrosynthetic analysis.

1,2,10,11-tetrahydro-9*H*-cyclobuta[*c*]benzo[*e*]indol-4-one (CbBI) alkylation subunit (Figure 2). We recognized that the modestly smaller strain energy of the cyclobutane in CbBI (1.8 kcal/mol; 27.6 kcal/mol vs 25.8 kcal/mol)[7] should make these agents chemically more stable, but the extent of the changes in reactivity and reaction regioselectivity and their impact on the DNA alkylation properties and biological activity of the resulting analogues was not clear. Herein, we report the synthesis of *N*-Boc-CbBI (**13**), its incorporation into a key analogue of the duocarmycins (**16**, CbBI-TMI), the X-ray structure characterization of **13**, and its correlation with the reactivity and reaction regioselectivity that impact the resulting DNA alkylation and biological properties of this class of compounds.

In addition, the parent spirocyclobutylcyclohexadienone ring system embedded in CbBI is unknown, and many well-established methods for forming four-membered rings (e.g., [2 + 2] cycloaddition) cannot be easily adapted to its preparation. The approach pursued and ultimately implemented entailed a previously unreported Ar-4′ alkylative spirocyclization of the corresponding phenol that is analogous to the Ar-3′ spirocyclization used for the synthesis of CBI (Figure 2). Pioneering efforts by Winstein and Baird first demonstrated the spirocyclization of such phenol precursors to form reactive cyclopropanes (Ar-3′ alkylation) and the more stable cyclopentanes (Ar-5′ alkylation), but no reports of its extension to an Ar-4′ spirocyclization for the preparation of cyclobutanes have been disclosed.[8]

Results and Discussion

Synthesis. The synthesis of CbBI began with the N-alkylation of **5**[9,10] with **6**, which proceeded in 83% yield (Scheme 1). Key 5-exo-trig free-radical cyclization[11] of **7** upon treatment with tributyltin hydride gave the desired ethyl ester **8**, and reduction of **8** with lithium borohydride provided **9** in quantitative yield. Treatment of the resulting alcohol **9** with methanesulfonyl chloride in pyridine followed by the addition of LiCl cleanly provided the desired chloride **10**. Removal of the benzyl protecting group was achieved by hydrogenolysis with 10% Pd/C and ammonium formate, providing the first key spirocyclization substrate, **11**. However, all attempts to spirocyclize **11** to give **13** were unsuccessful, providing only recovered starting material in most cases and chloride elimination and/or Boc-deprotected products upon heating at high reaction temperatures (150−160 °C). An extensive range of bases, solvents, and reaction temperatures were examined without success. Thus, in spite of the relative release of ring strain, the Ar-4′

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(6) (a) Boger, D. L.; Jenkins, T. J. *J. Am. Chem. Soc.* **1996**, *118*, 8860. (b) Boger, D. L.; Palanki, M. S. S. *J. Am. Chem. Soc.* **1992**, *114*, 9318. (c) Boger, D. L.; Johnson, D. S.; Palanki, M. S.; Kitos, P. A.; Chang, J.; Dowell, P. *Bioorg. Med. Chem.* **1993**, *1*, 27. (d) Tietze, L. F.; Herzig, T.; Fecher, A.; Haunert, F.; Schuberth, I. *ChemBioChem* **2001**, *2*, 758.

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(8) (a) Winstein, S.; Baird, R. *J. Am. Chem. Soc.* **1957**, *79*, 756. (b) Baird, R.; Winstein, S. *J. Am. Chem. Soc.* **1957**, *79*, 4238. (c) Baird, R.; Winstein, S. *J. Am. Chem. Soc.* **1962**, *84*, 788. (d) Baird, R.; Winstein, S. *J. Am. Chem. Soc.* **1963**, *85*, 567.

(9) (a) Boger, D. L.; Yun, W.; Teegarden, B. R. *J. Org. Chem.* **1992**, *57*, 2873. (b) Boger, D. L.; McKie, J. A. *J. Org. Chem.* **1995**, *60*, 1271. (c) Boger, D. L.; McKie, J. A.; Boyce, C. W. *Synlett* **1997**, 515. (d) Kastrinsky, D. B.; Boger, D. L. *J. Org. Chem.* **2004**, *69*, 2284.

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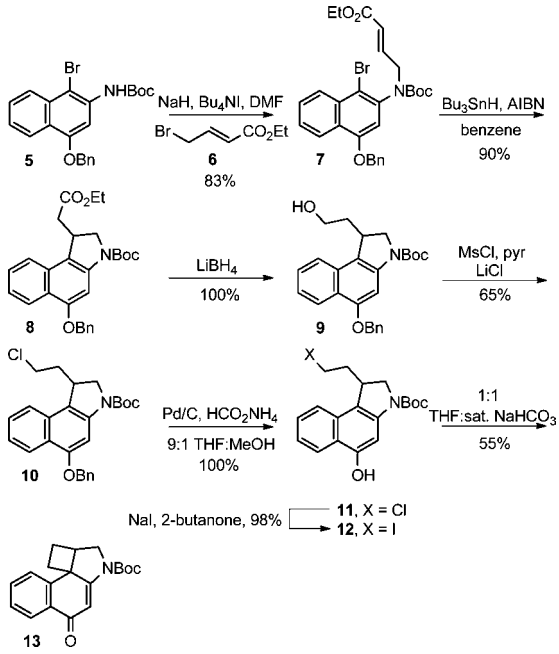
(11) Boger, D. L.; Boyce, C. W.; Garbaccio, R. M.; Searcey, M. *Tetrahedron Lett.* **1998**, *39*, 2227.

Scheme 1



spirocyclization of **11** is kinetically much slower than the analogous Ar-3′ spirocyclization used to provide *N*-Boc-CBI, which occurs at room temperature upon exposure to even aqueous $NaHCO_3$ (2 h). The reactivity of the cyclization substrate was increased by conversion of **11** to the corresponding iodide in nearly quantitative yield using NaI in 2-butanone. Although spirocyclization of **12** was not observed at or near room temperature under a wide range of reaction conditions, warming a solution of this substrate in 1:1 saturated aqueous $NaHCO_3$/tetrahydrofuran (THF) at 110 °C for 3 h provided the desired spirocycle **13**, as confirmed by X-ray diffraction analysis[12] (Figure 3). Further optimization of the spirocyclization to minimize competitive Boc deprotection and iodide solvolysis (130 °C for 1 h) provided *N*-Boc-CbBI in 55% yield. It is remarkable, but not unprecedented, that the four-membered ring closure to provide **13** is kinetically so much slower than the corresponding three-membered ring spirocyclization used for the preparation of *N*-Boc-CBI. This may be attributed largely to the increased entropy of activation required for the Ar-4′ spirocyclization and does not reflect the relative stabilities of the resulting products. In contrast, the slow rate of ring closure relative to an Ar-5′ spirocyclization[8] may be related to the corresponding enthalpy of activation and likely reflects the relative stabilities of the resulting products.

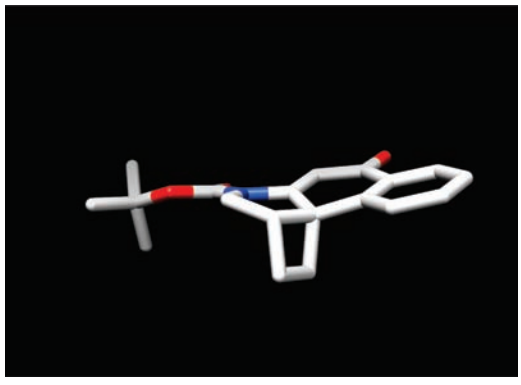
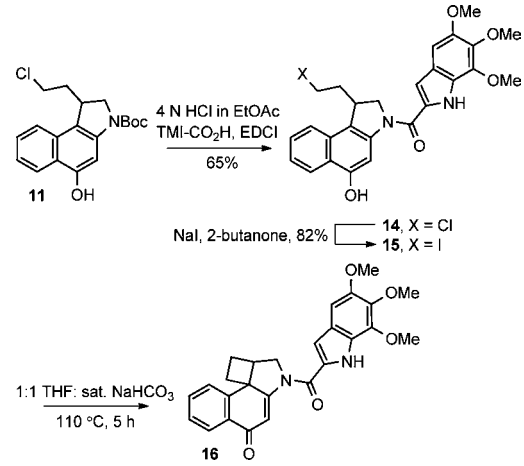


Figure 3. X-ray structure of *N*-Boc-CbBI (**13**).

Scheme 2



In order to accurately assess the biological properties of such cyclobutane derivatives, the corresponding analogue **16** (CbBI-TMI) of duocarmycin SA (**2**) was prepared. Thus, Boc deprotection of **11** (4 N HCl in EtOAc, 23 °C, 30 min) followed by direct coupling of the resulting indoline hydrochloride salt with 5,6,7-trimethoxyindole-2-carboxylic acid[13] [1.1 equiv, 3 equiv of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDCI), *N*,*N*-dimethylformamide (DMF), 23 °C, 16 h, 65%] afforded **14** (Scheme 2). Conversion of the chloride to the iodide **15** (5.0 equiv of NaI, 2-butanone, 65 °C, 3 h, 82%) provided the cyclization substrate that was subjected to the optimized spirocyclization conditions. However, the hydrolytic lability of the linking amide bond proved to be significant, resulting in the generation of only small amounts of **16** (<5%) and the production of predominantly the free amine of CbBI, which was formed via spirocyclization and subsequent hydrolysis of the resulting labile amide. When the spirocyclization reaction was run at a lower temperature for a longer reaction time (110 °C for 5 h), amide bond hydrolysis was minimized, and the yield of **16** was improved. As discussed below, the linking amide bond in **16** is weak and diagnostic of the preferential nitrogen lone-pair vinylogous amide (vs amide) conjugation with the cyclohexadienone system of CbBI. Since this vinylogous amide is cross-conjugated with the cyclobutylcyclohexadienone, its dominance serves to stabilize the otherwise reactive cyclobutane.

Reactivity and Reaction Regioselectivity. The study of both the rate of acid-catalyzed solvolysis and the regioselectivity of addition to the activated cyclopropane has proven to be key to understanding the structural features that contribute to the biological properties of the CC-1065 and duocarmycin alkylation subunits. The rates of solvolysis have provided insights into the structural features that stabilize or activate the cyclopropane for nucleophilic attack as well as into the source of catalysis for the DNA alkylation reaction,[14] and their study has defined a fundamental parabolic relationship between the intrinsic reactivity of the alkylation subunit and the biological activity.[15]

(12) The X-ray structure of **13** has been deposited with the Cambridge Crystallographic Data Centre (CCDC 787358).
(13) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Kitos, P. A.; Suntornwat, O. *J. Org. Chem.* **1990**, *55*, 4499.
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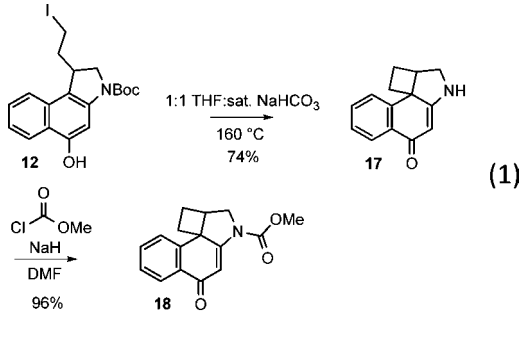
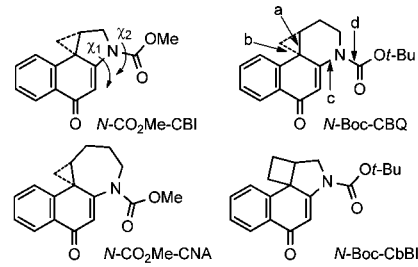
The regioselectivity of the ring-opening reaction has helped define the mechanism of DNA alkylation, where the preferred site of nucleophilic addition was found to depend on the stereoelectronic alignment of the cyclopropane (or, in this case, cyclobutane) bonds with the cyclohexadienone π-system.

The solvolysis reactivity of *N*-Boc-CbBI (**13**) was followed by UV spectrophotometry in pH 3, pH 2, and even pH 1 phosphate buffer, but no measurable solvolysis was observed. Treatment of **13** with 10% trifluoroacetic acid or 10% F_3CSO_3H in MeOH led only to Boc cleavage over time (72 h) with no evidence of cyclobutane ring opening. Treatment of **13** with 4 N HCl in EtOAc (−78 to 25 °C) afforded only **17** resulting from Boc deprotection, with no observed chloride addition to the cyclobutane. In order to avoid the competitive acid-catalyzed Boc deprotection of **13** while assessing the relative reactivity of CbBI, the corresponding methyl carbamate **18** was prepared (eq 1). This was most easily accomplished by heating a solution of **12** at 160 °C in saturated aqueous $NaHCO_3$/THF (1:1) for 30 min in a microwave reactor to provide **17** in 74% yield via spirocyclization followed by in situ Boc deprotection. Deprotonation of **17** with sodium hydride (2.5 equiv, DMF, 30 min, 0 °C) followed by treatment with methyl chloroformate (5.0 equiv) added slowly afforded **18** in superb yield (96%). Analogous to observations made with **13**, no measurable solvolysis of **18** was observed even in pH 1 phosphate buffer. Whereas treatment of **18** with 4 N HCl in EtOAc at −78 °C provided only recovered starting material, warming the solution to room temperature for 2 h provided a single chloride addition product, **19**, in quantitative yield (eq 2). Exclusive chloride addition to the less substituted cyclobutane carbon was observed, and no seven-membered ring addition product resulting from attack on the more hindered carbon was observed. Thus, the CbBI alkylation subunit exhibits a remarkable stability, being unreactive to solvolysis even at pH 1. This contrasts with *N*-Boc-CBI, which is stable at pH 7 but exhibits readily measurable solvolysis reactivity at pH 3 ($k = 1.45 \times 10^{-6}$ s^{-1}, $t_{1/2} = 133$ h) and pH 2 ($t_{1/2} = 12.5$ h).[16] A lower limit on the relative reactivity of CbBI versus CBI indicates that it is at least 100 times more stable than CBI toward acid-catalyzed solvolysis.



The remarkable stability of CbBI is due in large part to the stabilizing vinylogous amide conjugation. Most diagnostic of this conjugation is the shortened N^2-C^{2a} bond length (bond *c*, 1.382 Å), which shows a progressive shortening as one moves



X-ray bond lengths, Å	*N*-Boc-CbBI	*N*-CO_2Me-CBI	*N*-Boc-CBQ	*N*-CO_2Me-CNA
a	1.551	1.521	1.528	1.565
b	1.590	1.544	1.543	1.525
c	1.382	1.390	1.415	1.428
d	1.379	1.372	1.379	1.357
X-ray dihedral angles				
χ_1	21.2°	21.2°	34.2°	86.4°
χ_2	3.7°	4.5°	8.7°	3.9°
solvolysis reactivity				
$t_{1/2}$, (pH 3)	stable	133 h	2.1 h	0.028 h
$t_{1/2}$, (pH 7)	stable	stable	544 h	2.1 h

Figure 4. Comparison of X-ray crystal structures of CBI analogues (data taken from refs 17−19).

across the series of modified alkylation subunits illustrated in Figure 4.[17−19] That is, the shorter length of the *c* bond corresponds to increased vinylogous amide conjugation, which in turn is correlated with a remarkable progressive increase in compound stability. Similarly observed is the smaller χ_1 dihedral angle and the longer amide bond length (bond *d*, 1.379 Å; indicative of less amide vs vinylogous amide conjugation) across the series. Further contributing to the stability of CbBI relative to CBI is the modestly diminished ring strain intrinsic in a cyclobutane versus a cyclopropane (1.8 kcal/mol),[7] which makes its cleavage less facile. The net effect is that CbBI exhibits a remarkable stability in which its intrinsic reactivity is masked in part by the strong stabilizing vinylogous amide conjugation. Additionally, the X-ray crystal structure indicates that the geometric alignment of the cyclobutane deviates slightly with respect to the plane bisecting the cyclohexadienone (Figure 5A). This results from the fusion of the five-membered ring, which precludes an ideal alignment and overlap between the cyclobutane orbitals and the cyclohexadienone.[18]

The reaction regioselectivity originates in the stereoelectronic alignment of the two available cyclobutane bonds with the cyclohexadienone π-system. As shown in Figure 5B, the orbital of the cyclobutane bond extending to the less substituted carbon overlaps best with the developing π-system of the phenol reaction product. In contrast, the bond extending to the more substituted carbon nearly lies in the plane of this developing π-system and is not stereoelectronically aligned to undergo cleavage. Notably, this stereoelectronic control overrides any intrinsic preference to place a developing positive charge on a preferred secondary versus primary center (ring expansion). Furthermore, diagnostic of the regioselectivity of nucleophilic addition, the cleaved bond is longer (1.590 vs 1.551 Å) and

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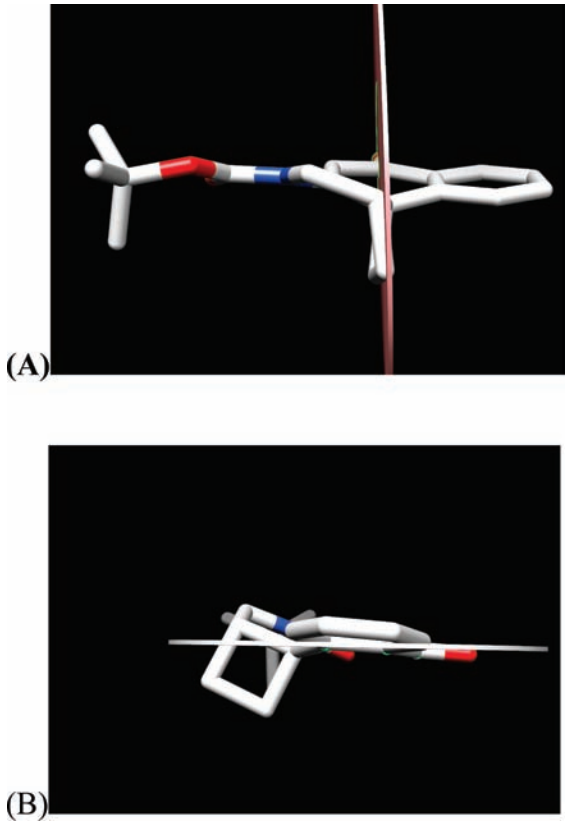

Figure 5. (A) Back view and (B) side view of **13**.

weaker than the bond extending to the more substituted carbon, reflecting its conjugation with the π-system. Steric factors resulting from preferential S_N2 attack on the less hindered site may also play a role.

Cytotoxic Activity. Compounds **15** and **16** were assayed for cytotoxic activity against the L1210 tumor cell line (mouse leukemia cell line). These compounds were found to display mean inhibitory concentration (IC_{50}) values of 1.2 and 6.3 μM, respectively. This represents a 10^6-fold decrease in potency in comparison with the natural products themselves ($IC_{50} = 6-10$ pM for **2**) and a 10^5-10^6-fold loss in activity in comparison with CBI-TMI ($IC_{50} = 30$ pM),[20] a testament to the remarkable stability of these CbBI derivatives. Although relatively inactive, this level of cytotoxic activity is in line with expectations based on the exceptional stability of compounds that preclude their effective alkylation of duplex DNA and follows trends depicted in the parabolic relationship between reactivity and cytotoxic activity.[15]

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Conclusions

The subtle change in the structure of CbBI versus CBI that results in such a dramatic alteration of the chemical and biological properties of the compound highlight the remarkable constellation of properties that are incorporated into the compact natural product structures **1** and **2** and are used to mask or tame an otherwise reactive electrophile.[21] Even though CbBI could be expected to be more stable than CBI, the remarkable stability exhibited by *N*-acyl-CbBI (stable at pH 1) is not intuitively obvious from a cursory inspection of its structure. Contributing to this unusual stability is the dominant vinylogous amide conjugation,[22] the nonideal alignment of the cyclobutane with the activating cyclohexadienone imposed by the fused five-membered ring,[18] and the modestly reduced strain energy (1.8 kcal/mol)[7] intrinsic to a four-membered cyclobutane ring versus a three-membered cyclopropane ring.

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Note Added in Proof. In the interim while the manuscript was under review, we successfully secured the X-ray crystal structure of **17** (CDCC 792209), and details of its comparison with CBI and related compounds (CBQ and CNA) are provided in Figure S1 in the Supporting Information. Most notable in these comparisons is the shorter *c* bond length (1.330 vs 1.337 Å for CBI and 1.382 Å for *N*-Boc-CbBI), indicative of the greater vinylogous amide conjugation and further reduced reactivity. Accompanying this change is a shorter *b* bond length (1.577 vs 1.590 Å for *N*-Boc-CbBI), indicative of a further diminished cyclobutane conjugation with the cyclohexadienone.

Supporting Information Available: Full experimental details and compound characterizations along with crystallographic data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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