Synthesis, X-ray Structure, and Properties of Fluorocyclopropane Analogs of the Duocarmycins Incorporating the 9,9-Difluoro-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*e*]indol-4-one (F_2CBI) Alkylation Subunit

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Abstract: The synthesis of 9,9-difluoro-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (F_2CBI), a difluorocyclopropane analog of the CC-1065 and duocarmycin alkylation subunits which represents the first such agent containing substitution of the reactive center in the natural products, is detailed. The core structure of F_2CBI was prepared by an intramolecular metal carbenoid insertion reaction into a 1,1-difluoroalkene (74%) employing a *p*-quinonediazide, and its incorporation into F_2CBI-TMI (**24**) provided a key analog of the duocarmycins. A study of the solvolysis of *N*-BOC-F_2CBI (**19**) revealed that introduction of the difluorocyclopropane substitution increased the reactivity 500× without altering the inherent regioselectivity which occurred with nucleophilic addition to the difluoro substituted C9 cyclopropane carbon. A single-crystal X-ray structure analysis of **17** and its comparison with the X-ray structures of CBI and related agents beautifully reveal the structural origin of the difluoro substitution effects on the reactivity and regioselectivity of the cyclopropane cleavage reaction. The cyclopropane C−CF_2−C bond angle is expanded, and the carbon−carbon bond opposite the difluoro substitution is lengthened to accommodate the preferentially compressed exocyclic F−C−F bond angle introducing additional strain energy. Consistent with this increased reactivity and following trends established to date, the agents were found to be 500−1000× less cytotoxic than the corresponding CBI derivative lacking the difluorocyclopropane substitution. Similarly, the gem difluoro substitution had no perceptible effect on the DNA alkylation selectivity of the agents, and they were found to undergo the characteristic adenine N3 addition to the C9 cyclopropane carbon but did so with a reduced (675−725×) efficiency following the cytotoxicity and stability correlations.

(+)-CC-1065 (**1**),[1] duocarmycin SA (**2**),[2] and duocarmycin A (**3**)[3] are the parent members of a class of potent antitumor antibiotics[1−7] that exhibit their biological properties as a consequence of sequence-selective DNA alkylation (Figure 1).[8−11] The DNA alkylation reaction has been shown to proceed by a reversible, stereoelectronically-controlled adenine N3 addition to the least substituted carbon of the activated cyclopropane within selected AT-rich regions of duplex DNA.[11−16] Since their disclosure, extensive studies to define the fundamental principles underlying the relationships between structure, chemical reactivity, and biological properties have been detailed.[11] In these efforts, a number of deep-seated modifications in the alkylation subunits of **1**−**3** have been made, and their subsequent evaluation has provided important insights into the structural origin of their properties.[11]

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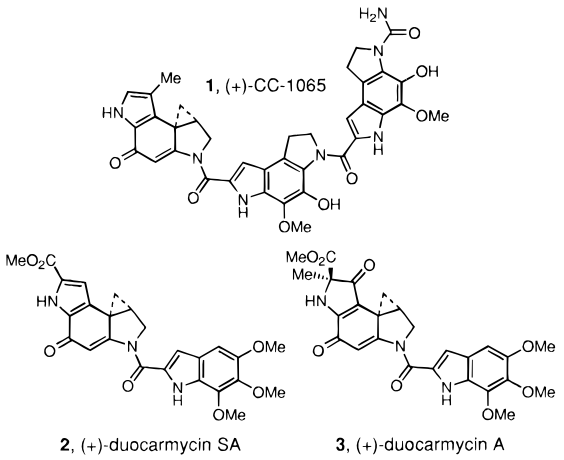


Figure 1.

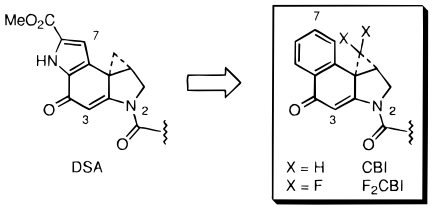
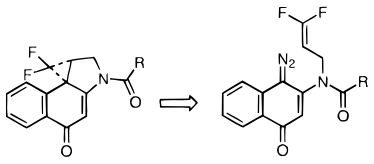


Figure 2.

Herein, we detail the synthesis and examination of 9,9-difluoro-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*e*]indol-4-one (F_2-CBI), a difluoro substituted cyclopropane analog of the alkylation subunits of **1**−**3** which represents the first such analog containing substitution or functionalization of the reactive center in the natural products (Figure 2). Typically, the noncongruent inductive electron-withdrawing properties of a fluorine substituent and its resonance stabilizing properties combine to reinforce a resonance stabilized reaction regioselectivity with diminished reactivity when compared to a substrate bearing a hydrogen substituent. Complementing these properties, the size of a fluorine substituent is sufficiently similar to that of hydrogen that it can be regarded as a sterically larger but nonobtrusive substitution for hydrogen. However, fluorine substitution of a cyclopropane has been shown to impart unique effects different even from those of other halogens.[17] Experimentally, it has been shown to increase ring strain by 4.5−5.0 kcal/mol per fluorine,[18] it significantly weakens the bond opposite the carbon bearing the fluorine substituent,[19−21] and it has been suggested to slightly strengthen[20] or weaken[19] the adjacent bonds. Similarly, geminal difluoro substitution of cyclopropane has been

Scheme 1



experimentally estimated to weaken the bond opposite the CF_2 by 9−10 kcal/mol[21] but imparts much less effect on the adjacent bonds weakening them by 0−2 kcal/mol.[20] Consequently, it was unclear whether the gem difluoro substitution of the CBI cyclopropane would alter or enhance the stereoelectronically-controlled reaction regioselectivity for nucleophilic ring opening, and, a priori, it was not clear whether this substitution would enhance or diminish the inherent electrophilic functional reactivity. Consequently, we have prepared the F_2CBI alkylation subunit in efforts to examine the effect of the difluoro substitution on the structure, reactivity, and reaction regioselectivity of the agent and its impact on the biological properties of the resulting analogs of **1**−**3**.

Synthesis *N*-Acetyl-F_2CBI, *N*-BOC-F_2CBI, and F_2CBI. The synthesis of the F_2CBI nucleus was modeled on the Sundberg[22] synthesis of the alkylation subunit of CC-1065 employing a quinonediazide[23] and a key intramolecular metal carbenoid insertion into a 1,1-difluoroalkene (Scheme 1).

Alkylation of the sodium salt of **4**[24] (1.3 equiv of NaH, DMF, 0−25 °C, 14 h, 97%) with 1-bromo-3-methyl-2-butene cleanly provided **5** (Scheme 2). Low-temperature ozonolysis of **5** and subsequent reductive workup (Me_2S) of the crude ozonide under carefully monitored reaction conditions effectively provided the aldehyde **6** although short extensions of the reaction time beyond those detailed led to further oxidation.[24] The difluoroalkene was introduced following a three-step protocol developed by Sabol and McCarthy.[25] Low-temperature generation of α-lithio phenyl difluoromethylsulfone[25,26] (LHMDS, THF−HMPA, −78 °C) in the presence of **6** provided the *β*-hydroxysulfone **7** (51%) in a reaction that proved difficult to optimize. Efforts to generate the α-lithiosulfone and subsequently add the aldehyde **6** failed to provide **7** in competitive conversions due to the instability of the reagent. Ultimately, it proved most convenient to conduct the reaction with the *in situ* generation of the α-lithiosulfone in the presence of the aldehyde **6** which led to competitive enolization and ultimately recovery of the substrate (20−40%). Because of the chromatographic properties of **6** and **7**, a careful chromatography of the crude reaction mixture or subsequent conversion of **7** to the mesylate **8** followed by a quick plug chromatography was employed to recover unreacted **6** which could be recycled providing overall conversions approaching 75%. This improved what turned out to be the

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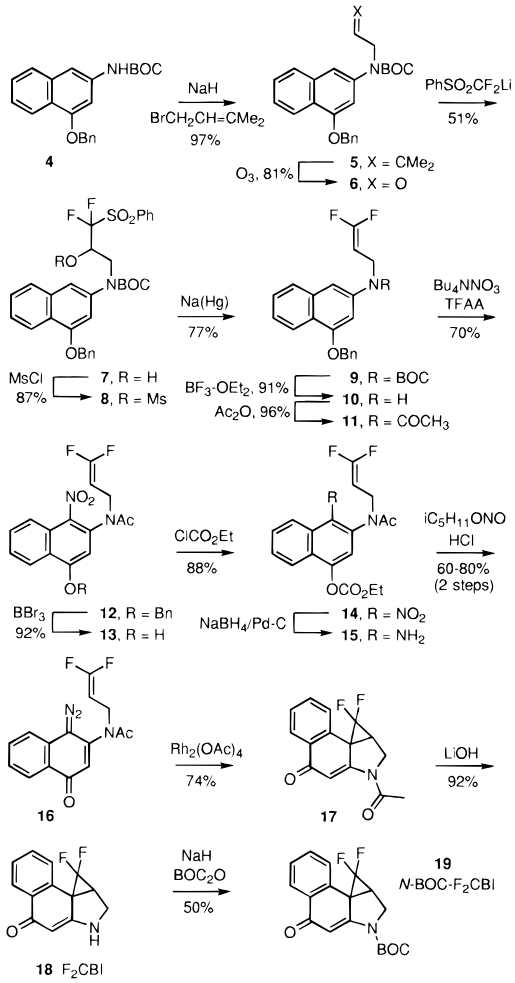
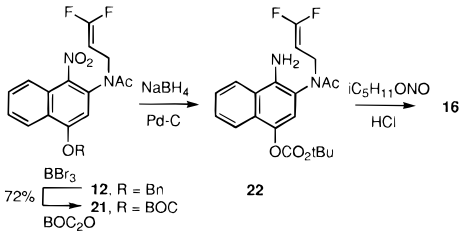
Scheme 2



only problematic step in the synthesis. Conversion of **7** to the mesylate **8** (2 equiv of MsCl, 10 equiv of Et_3N, 3.5 h, 87%) followed by reductive elimination effected by treatment with 5% Na(Hg) (6 equiv, 4 equiv of Na_2HPO_4, CH_3OH, 0 °C, 1 h, 77%) provided the key difluoroalkene **9**. This latter reaction was optimal when the ratio of substrate:Na_2HPO_4:Na(Hg) was 1:4:6, and significant amounts of the desulfonylated mesylate[27] (20−40%) were isolated when the number of equivalents of Na(Hg) exceeded 10 equiv. The tosylate of **7**[27] behaved similarly and provided good conversions to **9** when treated with Na(Hg) under the optimized conditions. Efforts to promote the reductive elimination of **8** with SmI_2 as disclosed by McCarthy[25] was not successful with our substrate and limited efforts to promote the reductive elimination with **7** itself employing Na(Hg) were not productive.[28]

Alternative olefination procedures for introduction of a terminal difluoroalkene including diethyl (difluoromethyl)phosphonate,[29] diphenyl difluoromethylphosphine oxide,[30] and related Wittig reagents[31] failed to provide a competitive route to **9**. Similarly, attempts to generate **9** directly from **4** by S_N2' reaction of its sodium or lithium salt with 3,3,3-trifluoropropene, analogous to reported[32] reactions with stronger nucleophiles, provided only recovered starting materials.

Scheme 3



Introduction of the quinonediazide required deprotection of the benzyl ether and, preferentially, the reintroduction of an acid labile protecting group such that its removal would occur subsequent to acid-catalyzed diazonium salt formation but still be stable to the conditions of reduction of the aryl nitro group to an aryl amine preventing oxidation to a *p*-quinone monoimine.[22] These criteria were unable to be fulfilled with the *N*-BOC protecting group in place. Therefore, the *N*-BOC group was replaced with an *N*-acetyl group and nitration of **11** effected by treatment with $CF_3CO_2NO_2$[33] (2.5−3.5 equiv of Bu_4NNO_3, 0.002 equiv of TFAA, CH_2Cl_2, 25 °C, 16−32 h, 70%) cleanly provided **12** derived from C-4 nitration with only the occasional generation of a small amount of the isomeric C-2 nitration product (*ca*. 10%). The benzyl ether was cleaved (2.0 equiv of BBr_3, CH_2Cl_2, −78 °C, 30 min, 92%) to provide **13**, and the phenol was reprotected as the ethylcarbonate **14** (88%). Following the protocol detailed by Sundberg,[22] nitro reduction in the presence of the difluoroalkene was accomplished by treatment with $NaBH_4$/5% Pd−C (H_2O−CH_3OH, 0 °C, 15 min).[34] Without purification or storage, the amine **15** was converted directly to the key quinonediazide **16** by treatment with *i*-$C_5H_{11}ONO$ (catalytic 4 M HCl−CH_3OH, −30 °C, 16 h) under conditions where acid-catalyzed cleavage of the carbonate leads to conversion of the intermediate diazonium salt to **16** directly. Using this procedure, yields for the overall conversion of **14** to **16** as high as 80% were obtained with typical conversions being 60%. The only significant byproduct generated in this sequence was the fused benzimidazole **20a**[27] and its formation could be minimized by use of short reduction periods (15 min) coupled with not storing or purifying the free amine **15** prior to its use. The use of sufficient amounts of strong acid in the diazotization reaction also appeared to diminish its formation. In the further optimization of this sequence, it was established that the conversions steadily increased as the amount of *i*-$C_5H_{11}ONO$ was increased and finally employed as solvent, increased as the reaction time was extended from 1 to 3 h and finally to 17 h, and proved optimal if the strong acid employed in the diazotization reaction was added following the *i*-$C_5H_{11}ONO$. Attempts to employ alterna-

(27) Diagnostic characterization is supplied in the supporting information.

(28) Treatment of **7** with Na(Hg) (5 equiv, 4 equiv of Na_2HPO_4, CH_3OH, 0−25 °C, 17 h) provided low conversions to 3-[*N*-(*tert*-butyloxycarbonyl)-*N*-(3,3-difluoro-2-hydroxy-1-propyl)]amino-1-benzyloxynaphthalene[27] (8%) and 3-(5-difluoromethyloxazolidinon-3-yl)-1-benzyloxynaphthalene[27] (9%).

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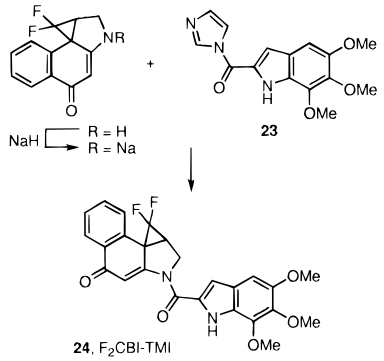
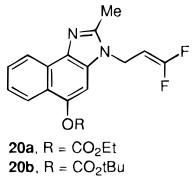
Scheme 4



tive reduction conditions (Zn, $CaCl_2$, 95% EtOH) disclosed by both Kraus[35] and Sundberg[22] were unsuccessful with either **13** or **14** and provided recovered starting materials.



The use of the *tert*-butylcarbonate **21**[27] (Scheme 3) was also explored and was found to provide comparable conversions to either **22**[27] or **16** but offered no distinctions or advantages over the use of the ethylcarbonate **14**.

Metal carbenoid generation and insertion into the difluoroalkene was effectively accomplished by treatment of **16** with $Rh_2(OAc)_4$ (0.1−0.2 equiv, toluene, reflux, 0.5 h, 74%) protected from the light and smoothly provided *N*-acetyl-F_2-CBI (**17**), Scheme 2. This conversion proved superior to that detailed in the original work of Sundberg[22] and may be attributed in part to the increased reactivity of the acceptor alkene with our substrate **16**. Similar, but less consistent conversions were observed with $Cu(acac)_2$ catalysis (58%), and, in both cases, the conversions diminished at lower reaction temperatures and seemed to drop as the amount of catalyst was increased beyond 0.2 equiv. Hydrolysis of **17** by simple treatment with LiOH (1.2 equiv, CH_3OH, −10 °C, 15 min, 92%) cleanly provide F_2-CBI (**18**). Conversion of **18** to *N*-BOC-F_2CBI (**19**) was accomplished by NaH deprotonation and subsequent reaction with BOC_2O.

Preparation of F_2CBI-TMI (24). The advanced analog of **24** of the duocarmycins was prepared by deprotonation of **18** (1.1 equiv of NaH, DMF, 25 °C) followed by reaction with **23**[36] (DMF, 1 h, 25 °C, 45%; 64% based on recovered **18**), Scheme 4.

Solvolysis: Reactivity. Two fundamental characteristics of the alkylation subunits have proven important in the studies to date. The first is the stereoelectronically-controlled acid-catalyzed ring-opening of the cyclopropane which dictates preferential addition of a nucleophile to the least substituted cyclopropane carbon. With the CBI series of modified alkylation subunits where this stereoelectronic alignment of the C8b−C9 bond is nearly optimized, exclusive (≥20:1) addition to the C9 center with cleavage of the C8b−C9 bond is observed.[37−39] The second characteristic is the relative rate of solvolysis which has been found to accurately reflect the functional reactivity of the agents and to follow a well-defined direct relationship between solvolysis (functional) stability and in vitro cytotoxic potency.[37−41] Thus, the impact of the difluorocyclopropane substitution on the reactivity and reaction regioselectivity of CBI was examined.

Table 1. Solvolysis Reactivity

agent	$t_{1/2}$ (h, pH 3)	k (s^{-1}, pH 3)	$t_{1/2}$ (h, pH 7)	k (s^{-1}, pH 7)
N-BOC-F_2CBI (**19**)	0.26	7.05×10^{-4}	2.3	8.27×10^{-5}
N-BOC-CBI	133	1.45×10^{-6}	stable	stable
F_2CBI (**18**)	4.2	4.54×10^{-5}	422	4.56×10^{-7}
CBI	930	2.07×10^{-7}	stable	stable
N-acetyl-F_2CBI (**17**)	0.27	7.25×10^{-4}	2.0	9.80×10^{-5}

The reactivity of **17**−**19** was assessed by following the solvolysis spectrophotometrically by UV at both pH 3 (50% CH_3OH−buffer, buffer = 4:1:20 (v/v/v) 0.1 M citric acid, 0.2 M Na_2HPO_4, H_2O) and pH 7 (1:1 H_2O-CH_3OH) measuring both the disappearance of the long-wavelength absorption band of the F_2CBI chromophore and the appearance of a short-wavelength absorption band attributable to the ring open products (Figure 3).

N-BOC-F_2CBI (**19**) proved to be remarkably reactive toward acid-catalyzed solvolysis. At pH 3, it exhibited a half-life of 0.26 h ($k = 7.05 \times 10^{-4}$ s^{-1}) and proved to be approximately 500× more reactive than *N*-BOC-CBI ($t_{1/2}$ = 133 h, $k = 1.45 \times 10^{-6}$ s^{-1})[37] which lacks only the two fluorine substituents. Moreover, **19** is among the most reactive of the modified alkylation subunits studied to date.[11,37−42] At pH 7 where *N*-BOC-CBI is stable, it also underwent rapid solvolysis ($t_{1/2}$ = 2.33 h, $k = 8.27 \times 10^{-5}$ s^{-1}). *N*-acetyl-F_2CBI (**17**) and F_2CBI (**18**) were also examined, and the results are summarized in Table 1. No substantial distinction in the reactivity of **17** and **19** was observed, while **18** proved significantly more stable ($t_{1/2}$ = 4.2 h, $k = 4.54 \times 10^{-5}$ s^{-1} at pH 3). This is analogous to prior observations, and **18** proved to be approximately 220× more reactive than CBI.

Solvolysis: Regioselectivity. Treatment of both **17** and **19** with catalytic CF_3SO_3H (0.1 equiv) in CH_3OH (0 °C) rapidly and cleanly provided a single characterizable product **25** (30 min, 90%) or **26** (10 min, 79%), Scheme 5. Spectroscopically, this could be shown to be derived from addition of CH_3OH to the difluoro substituted C9 cyclopropane carbon analogous to the normal solvolysis observed with *N*-BOC-CBI and related agents.[37−39] ^{19}F NMR spectra of both **25** and **26** exhibited a single fluorine resonance, demonstrating that both fluorines are

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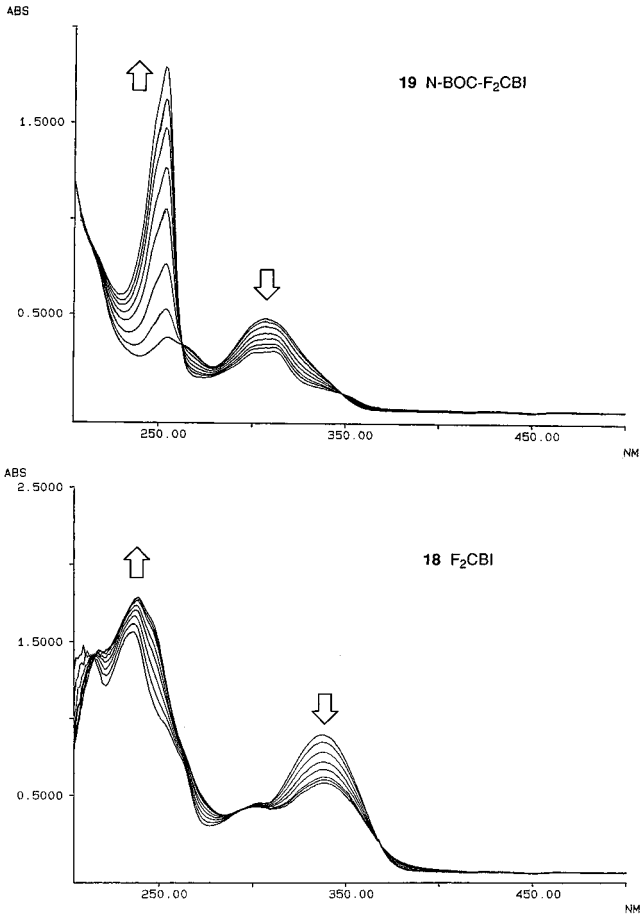
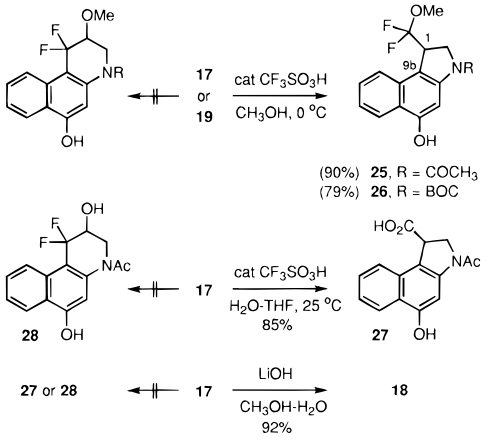


Figure 3. Solvolysis study (UV spectra) of *N*-BOC-F_2CBI (**19**, top) and F_2CBI (**18**, bottom) in 50% CH_3OH-aqueous buffer (pH 3.0, 4:1: 20 (v/v/v) 0.1 M citric acid, 0.2 M Na_2HPO_4, and H_2O, respectively). The spectra were recorded at regular intervals, and only a few are shown for clarity. Top: 1, 0 min; 2, 2 min; 3, 5 min; 4, 11 min; 5, 17 min; 6, 25 min; 7, 33 min; 8, 48 min. Bottom: 1, 0 h; 2, 0.5 h; 3, 1.5 h; 4, 2.5 h; 5, 3.5 h; 6, 4.5 h; 7, 5 h; 8, 5.5 h.

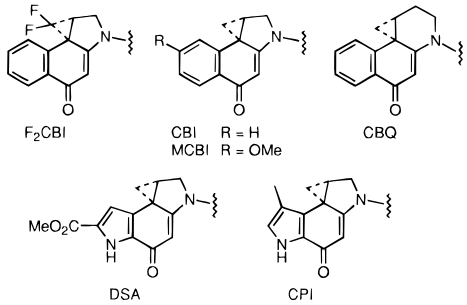
magnetically equivalent. The ring expansion regioisomer possesses diastereotopic fluorines which would be magnetically nonequivalent. Furthermore, the $^1H-^{13}C$ HMBC NMR spectrum of **26** established the carbon−carbon connectivity of C1 (δ 51.0) with the CF_2 carbon (δ 50.3, t) and C9b (δ 125.0). No connectivity between the key CF_2 carbon (δ 50.3, t) and C9b (δ 125.0) was detected as required of the ring expansion regioisomer. This was unambiguously established by conducting the solvolysis in THF−H_2O catalyzed by CF_3SO_3H (0.12 equiv) which cleanly provided **27** (18 h, 85%), Scheme 5. Nucleophilic addition under basic conditions leads to preferential reaction at the *N*-acetyl substituent as evidenced by the clean hydrolysis of **17** to provide **18** (Scheme 2).

Although it is possible that minor amounts of the abnormal solvolysis products may have gone undetected or decomposed under the solvolysis conditions, the studies detailed above establish that addition to the difluoro substituted C9 carbon occurs with a ≥9:1 preference. This is analogous to observations made with the CBI-based agents where exclusive (>20: 1)[37−39] addition to the C9 carbon is observed. Thus, the introduction of the gem difluorocyclopropane substitution did not alter the inherent regioselectivity of the stereoelectronically-controlled acid-catalyzed nucleophilic addition to the activated cyclopropane. To date, agents incorporating the CBI nucleus have exhibited the greatest regioselectivity, and more modest selectivity has been observed with CPI derivatives including CC-1065 (ca. 4:1),[43] duocarmycin SA (6.5−4:1),[44] duocarmycin

Scheme 5



A (4−1:1),[17,44] or CBQ derivatives (3:2).[41] Like the comparisons made in the structural studies of CBQ,[41] the diminished or lost regioselectivity observed with the agents may be attributed to the relative extent of stereoelectronic alignment of the two possible cyclopropane bonds.[45] In the cases where structural information is available,[44] the degree of selectivity reflects the relative degree of stereoelectronic alignment of the two available cyclopropane bonds, and this alone could account for the exclusive reaction regioselectivity observed with **17** and **19** despite their remarkable reactivity.[41,44] Further contributing to this regioselectivity is the resonance stabilizing effect of a fluorine substituents which could accommodate a developing partial positive change on C9.



X-ray Structure of *N*-acetyl-F_2CBI (17): Structure Correlation with Reactivity and Regioselectivity. The X-ray structure of **17**[46] was determined on plates grown from EtOAc−hexane and through a direct comparison with that of CBI[37] established a structural basis for the observed properties (Figure 4). The X-ray structure of **17** exhibited one important similarity with the CBI[37] or MCBI[38] X-ray structures. The bent orbital of the cyclopropane bond extending to C9 is nearly perpendicular to the plane of the cyclohexadienone and consequently overlaps nicely with its π-system and that of the developing solvolysis product. In contrast, the cyclopropane bond extending

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(45) The suggestion[49] that the abnormal ring expansion solvolysis of CPI occurs by a change in reaction mechanism (S_N1 versus S_N2) with generation of a free carbocation has been unambiguously shown to not be operative with CBQ[41] or DSA/DA[44] and suggests that the ambiguous experimental basis for this proposal be reexamined.

(46) The author has deposited the atomic coordinates for this structure with the Cambridge Crystallographic Data Centre, and the coordinates may be obtained upon request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

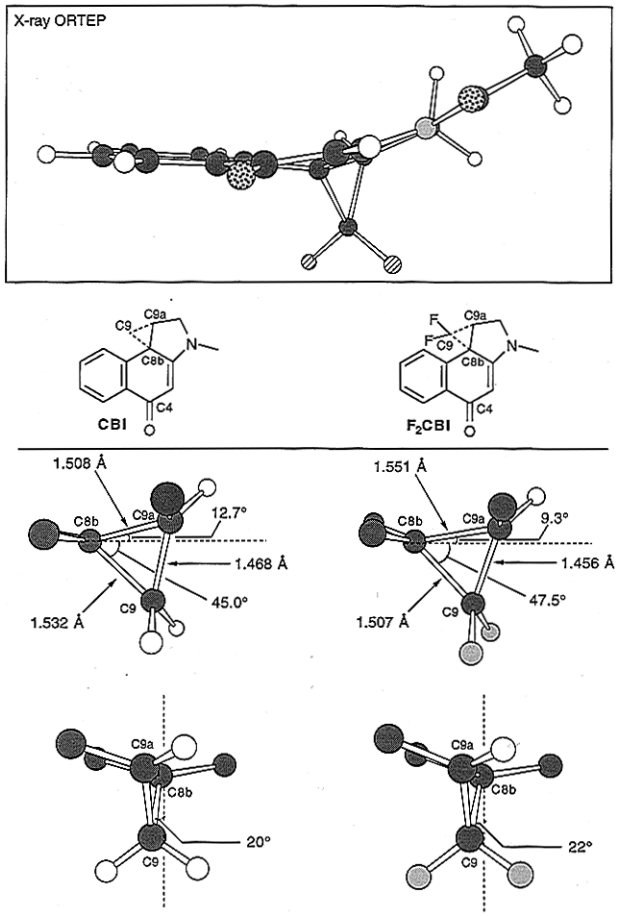

Figure 4. X-ray structure of **17** and side and rear views of the activated cyclopropane taken from the X-ray structures of CBI and **17** highlighting the structural details and the nonidealized overlap and alignment with the π-system.

to C9a is nearly in the plane of the cyclohexadienone, and its orbital is orthogonal to the developing π-system of the solvolysis product. Thus, opening of the cyclopropane occurs under stereoelectronic control with preferential attack of the nucleophile occurring at C9. This may be attributed to the geometrical constraints imposed by the fused five-membered ring which pulls the cyclopropane both down and out of perfect conjugation with the cyclohexadienone π-system[41] and to the side resulting in an offset alignment of both C9 and C9a with the π-system. The structural effect of pulling the cyclopropane down is to impose stereoelectronic control on the ring opening reaction such that only the C8b−C9 bond is aligned for cleavage. This is nearly idealized with both the CBI and F_2CBI nucleus and is less effectively accomplished with other subunits examined by X-ray to date including CPI,[1,47] DSA,[44] and CBQ.[41] Complementing this stereoelectronic control, the difluoro substitution of F_2CBI may further stabilize a developing positive charge on C9.

In addition, there are substantial differences in the two X-ray structures. The significant difference is the degree of pucker of the cyclohexadienone system. The CBI cyclohexadienone system is nearly planar, while that of **17** is puckered adopting a flattened boat-like conformation. The more substantial difference in the two X-ray structures is found in the cyclopropane bond lengths and bond angles. Comparable to past structural studies which include several X-ray structures (Table 2),[48,49] the C8b−C9a bond opposite the gem difluoro substitution

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Table 2. Comparison of the X-ray Structure of **17** with Prior Difluorocyclopropanes[48,49]

bond angle	F_2CBI	bond angle	av (range)[a]
C9a−C9−C8b	63.1°	C−CF_2−C	64.5° (66.2−62.8°)
C9−C8b−C9a	56.8°	CF_2−C−C[b]	57.1° (56.0−57.8°)
C8b−C9a−C9	60.1°	C−C−CF_2[c]	58.4° (57.2−60.6°)
F−C9−F	108.0°	F−C−F	106.9° (104.5−107.9°)
bond length		**bond length**	**av (range)[a]**
C8b−C9a	1.551 Å	C−C	1.551 Å (1.610−1.513 Å)
C9−C9a	1.456 Å	CF_2−C[b]	1.442 Å (1.408−1.472 Å)
C9−C8b	1.507 Å	C−CF_2[c]	1.461 Å (1.431−1.508 Å)
C9−F	1.341, 1.350 Å	C−F	1.356 Å (1.316−1.392 Å)

[a] Average (range) of values taken from 10 X-ray structures containing 16 difluorocyclopropanes. [b] The smallest bond angle or shortest bond length of unsymmetrical structures. [c] The largest bond angle or longest bond length of unsymmetrical structures.

was lengthened by a large distance of 0.04−0.05 Å and both of the adjacent bonds were shortened by 0.01−0.025 Å. This indicates that the C8b−C9a bond is weakened considerably by the gem difluoro substitution, while both the C8b−C9 and C9a−C9 bonds would appear to be strengthened. Notably, it is the C8b−C9 bond which was shortened to the greatest extent (0.025 Å), and yet it is the bond which is cleaved in the solvolysis reaction. Thus, while the C8b−C9 bond of CBI is both the longest of the two available cleavage bonds and also the bond which is stereoelectronically aligned for cleavage, the C8b−C9 bond of F_2CBI is now the shortest of the two, and yet it is still cleaved in the solvolysis reaction. In preceding studies of the reactions of difluorocyclopropanes including thermal isomerization, vinylcyclopropane rearrangements, cyclopropylcarbinyl radical ring opening rearrangements, and simple hydrogenation, it is the bond opposite the gem difluoro substitution and not the adjacent bonds which is cleaved.[18−21,50] In addition, it occurs with a lowered activation energy consistent with cleavage of a weakened bond. Since both the relative bond lengths of F_2CBI and typical reactions on simple difluorocyclopropanes would suggest preferential cleavage of the C8b−C9a bond, the maintenance of the normal solvolysis regioselectivity with cleavage of the C8b−C9 is consistent with the stereoelectronic requirements of reaction. Indicative of this, the C8b−C9a and C9−C9a bond lengths of **17** are essentially identical to those found in difluorocyclopropanes (C−C = 1.551 Å, C−CF_2 = 1.461−1.442 Å), and it is only the C8b−C9 bond which is perturbed (1.442−1.461 to 1.507 Å) by incorporation into F_2-CBI (Table 2).[48,49] Implicit in this lengthening of the C8b−C9 bond relative to simple difluorocyclopropanes is the overlap of the bond orbital with the cyclohexadienone π-system reflective of its stereoelectronic alignment. Further contributing to this

(48) Bond lengths of 1.464−1.447 and 1.553−1.552 Å for the adjacent and opposite bonds have been established for symmetrical difluorocyclopropanes by microwave spectroscopy or X-ray versus cyclopropane itself (1.514 Å). X-ray: Dolbier, W. R., Jr.; Odaniec, M.; Gomulka, E.; Jaskolski, M.; Koroniak, H. *Tetrahedron* **1984**, *40*, 3945. Dolbier, W. R., Jr.; Odaniec, M.; Jaskolski, M.; Koroniak, H. *J. Mol. Struct.* **1986**, *147*, 155. Microwave spectroscopy: Parretta, A. T.; Laurie, V. W. *J. Chem. Phys.* **1975**, *62*, 2463. Computational studies: Deakyne, C. A.; Allen, L. C.; Craig, N. C. *J. Am. Chem. Soc.* **1977**, *99*, 3845. Durmaz, S.; Kollmar, H. *J. Am. Chem. Soc.* **1980**, *102*, 6942. Clark, T.; Spitznagel, G. W.; Klose, R.; Schleyer, P. von R. *J. Am. Chem. Soc.* **1984**, *106*, 4412. Boggs, J. E.; Fan, K. *Acta Chem. Scand.* **1988**, **A42**, 595.

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(50) Bessard, Y.; Schlosser, M. *Tetrahedron* **1991**, *47*, 1231.

Table 3. X-ray Structure Bond Angles

bond angles	F_2CBI	CBI[37]	MCBI[38]	CBQ[41]	CPI[47]	DSA[44]
C9a−C9−C8b	63.1	60.3	60.7	60.8	60.4	60.4
C9−C8b−C9a	56.8	57.8	57.6	57.4	57.7	57.0
C8b−C9a−C9	60.1	61.9	61.7	61.8	61.9	62.5
F−C9−F	108					

Table 4. In Vitro Cytotoxic Activity

agent	IC_{50} (L1210)	agent	IC_{50} (L1210)
(±)-**17**	60 μM	(+)-*N*-acetyl-CBI	110 nM
(±)-**18**	140 μM	(+)-CBI	nd
(±)-**19**	110 μM	(+)-*N*-BOC-CBI	80 nM
(±)-**24**	36 nM	(+)-CBI-TMI	30 pM

selectivity is the resonance stabilizing effect of the gem difluoro substitution which could accommodate partial positive change buildup at C9.

Considerable evidence has been accumulated to establish the structural origin of the increased reactivity of 1,1-difluorocyclopropanes. The rehybridization that accompanies the difluorocyclopropane substitution has been eloquently detailed by Bernett[51] based on the principles defined by Bent[52] and Walsh.[53] The atomic p character concentrates in the orbitals directed toward electronegative substituents,[52] and this seems to be expressed uniquely for fluorine as opposed to other halogen substituents in cyclopropane structures.[51−54] An increase in the p character of the C-F orbitals reduces p character in the adjacent C−CF_2 cyclopropane bonds. In order to maintain the same overlap, the internuclear C−CF_2 separation decreases and the C−CF_2−C bond angle increases forcing the cyclopropane bond opposite the CF_2 to increase in length. Thus, changes in hybridization have been used to predict the direction, if not the unusual magnitude, of the structural perturbations introduced by gem-difluoro substituents. Although such rehybridization effects may constitute the origin of the structural perturbations, the impact on reactivity is most easily understood in terms of simple geometrical factors imposing increased ring strain.[18−21] Due to the compressed cyclopropane bond angles, geminal substituents are normally offered the additional stability of expanding their exocyclic bond angles (111−114°; *i.e.*, H-C-H 114−118°).[54] However, geminal fluorine substituents cannot take advantage of this and are destabilized since they prefer more compressed, not expanded, bond angles: F−C−F (104.6°).[50,51,55] This effect is beautifully illustrated with F_2-CBI and apparent in the comparison of the available X-ray crystal structures (Table 3). CBI, MCBI, CBQ, CPI, and DSA possess remarkably similar cyclopropane structures despite their different stereoelectronic alignments with the cyclohexadienone and their different reactivities covering a 80-fold range. In contrast, that of F_2CBI is considerably different, and its derivatives are much more reactive (500×). The C−CF_2−C bond angle expands (60.3−60.8 to 63.1°) and the adjacent C−CF_2 bonds shorten to accommodate the preferred compressed F−C−F bond angle lengthening the carbon−carbon bond opposite the CF_2 substitution (1.508−1.528 to 1.551 Å) and compressing the remaining two cyclopropane bond angles. This introduces considerable additional strain into the cyclopropane (9−10 kcal/mol, 13 kcal/mol),[18,21] weakens the C8b−C9a bond, and accounts for the increased reactivity.[18−21,50,51] Importantly, this strain-based increase in reactivity is exactly analogous to that found in related simpler systems and is not unique or even substantially perturbed by incorporation into the activated F_2-CBI ring system. Moreover, it is sufficient to overcome the stabilization potentially derived from the electron-withdrawing inductive effect of the fluorine substitution.

Substituent effects on reactivity typically may be attributed to stabilization of an incipient intermediate or transition state or to steric perturbations of the reaction, while ground state effects on the stability of the starting material have generally been considered less important. Although most substituents stabilize the ground state of cyclopropane in a manner analogous to ethylene substituents, fluorine cyclopropane substitution strongly destabilizes the ground state.[17] F_2CBI exhibits characteristics of such a destabilized difluorocyclopropane including the lengthened carbon−carbon bond opposite the gem difluoro substitution and the expansion of the cyclopropane C−CF_2−C bond angle reflective of the increased strain introduced to accommodate the preferentially compressed exocyclic F−C−F bond angle. This strain-derived ground state destabilization not only substantially changes the structure but also greatly increases the reactivity of the cyclopropane.

In Vitro Cytotoxic Activity. In preliminary studies, the in vitro cytotoxic activity of **17**−**19** and **24** (F_2CBI-TMI) were determined employing the racemic samples.[56] Consistent with expectations based on the relative reactivity of the agents, they proved to be 500−1000× less potent than the corresponding CBI agent, Table 4. Qualitatively, this follows the differences observed in the relative reactivity of the agents (500×)[57] exceptionally well with the more stable agents exhibiting the more potent activity and nicely follows the trends established in prior studies, Figure 5.[11,37−41]

DNA Alkylation Properties. A study of the DNA alkylation properties of the F_2CBI agents was conducted and revealed that they behave analogous to the corresponding CBI-based agent. The DNA alkylation reaction was examined within w794 DNA[58] for which comparative results are available for past agents. The alkylation site identification was obtained by thermal strand cleavage of the singly 5′ end-labeled duplex DNA after exposure to the agents. Following treatment of the labeled DNA with a range of agent concentrations, the unbound agent was removed by EtOH precipitation of the DNA. Redissolution of the DNA in aqueous buffer, thermolysis at 100 °C (30 min) to induce depurination and strand cleavage at the adenine N3 or guanine N3 minor groove alkylation sites, denaturing high resolution PAGE adjacent to Sanger sequencing standards and autoradiography provided the DNA cleavage and alkylation sites.[58]

A representative comparison of the DNA alkylation by racemic F_2CBI-TMI (**24**) alongside that of *ent*-(−)- and (+)-

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(55) Dunitz, J. D.; Schweizer, W. B.; Seiler, B. *Helv. Chim. Acta* **1983**, *66*, 134.

(56) Direct resolution of **17**−**19** on an HPLC ChiralCel OD column (0.46 × 25 cm, flow rate 0.7−1.0 mL/min) was examined and provides access to the optically active materials: **17**, α = 1.18 (10−20% *i*-PrOH−hexane); **18**, α = 1.09−1.05 (10−25% *i*-PrOH−hexane); **19**, α = 1.12 (50% *i*-PrOH−hexane). However, the recovery was expected to be diminished due to the reactivity of the agents and consequently the preparative resolution of the agents was not pursued.

(57) This is especially true for **17**, **19**, and **24** in Table 4 where the natural enantiomer could be expected to be responsible for the activity measured (CBI natural enantiomer typically 10−100× more potent than unnatural enantiomer). This would suggest that the accurate IC_{50} values for the natural enantiomers of **17**−**19** and **24** are 30, 70, 55 μM, and 18 nM, respectively, and consequently follow the trends between reactivity and cytotoxic potency even more closely.

(58) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661.

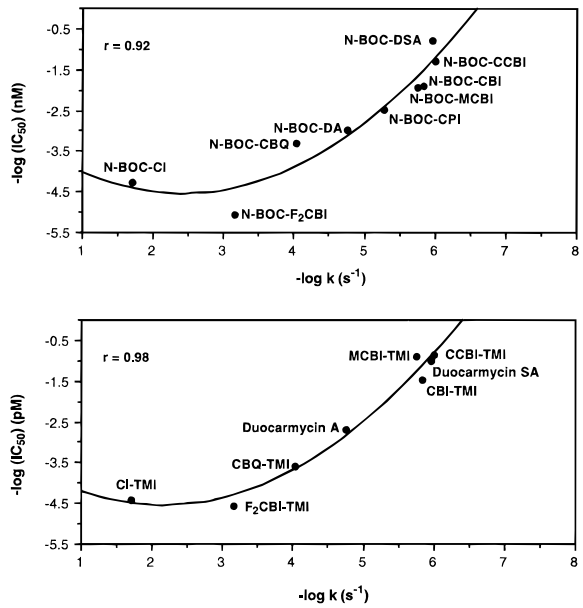

Figure 5.

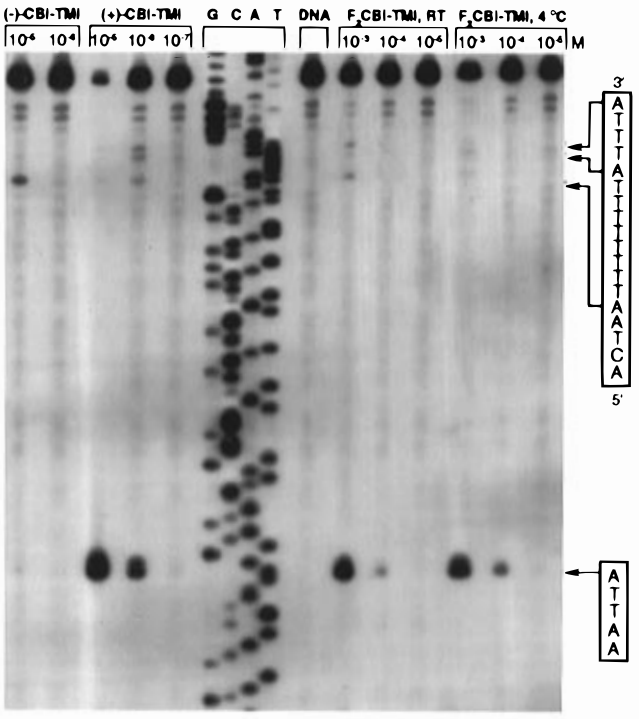

Figure 6. Thermally-induced (100 °C, 30 min) strand cleavage of w794 DNA after agent treatment, 8% denaturing PAGE and autoradiography. Lanes 1−2, *ent*-(−)-CBI-TMI (25 °C, 10^{-5} and 10^{-6} M); lanes 3−5, (+)-CBI-TMI (25 °C, 10^{-5} to 10^{-7} M); lanes 6−9, G, C, A and T Sanger sequencing lanes; lane 10, DNA standard; lanes 11−13, F_2-CBI-TMI (25 °C, 10^{-3}−10^{-5} M); lanes 14−16, F_2CBI-TMI (4 °C, 10^{-3}−10^{-5} M).

CBI-TMI is illustrated in Figure 6. There are three important conclusions that can be drawn from the comparisons in Figure 6. First, F_2CBI-TMI alkylates DNA in a manner analogous to CBI-TMI and does so with the same sequence selectivity. No new sites of alkylation were detected, and only adenine N3 alkylation was detected under these conditions of limiting agent and excess DNA. Notably, such sequencing studies only detect the highest affinity alkylation sites and minor sites with comparable affinities (1−0.01×). Lower affinity alkylation sites are not detected since they require much higher agent concentrations leading to multiple alkylations and cleavages of DNA resulting in the production of short DNA fragments not observed on the sequencing gels. It is likely that **24**, like duocarmycin A itself,[11] may also be capable of guanine N3 alkylation in the absence of an accessible or available adenine N3 site, but it is not a major or minor reaction of significance. As evidenced by the comparison of *ent*-(−)- and (+)-CBI-TMI, the natural enantiomer of the CBI-based agents in the TMI series has been found to be 50−100× more potent and effective at alkylating DNA than the unnatural enantiomers,[37−40] and no exceptions in the CBI series (CBI, MCBI, CCBI) have been observed. In the examination of racemic F_2CBI-TMI, we can with confidence suggest that it is the natural enantiomer properties that dominates to the extent that it is responsible for the properties detected within w794 DNA as shown in Figure 6.

Secondly, although there are no distinctions of significance in the DNA alkylation selectivity of F_2CBI-TMI and CBI-TMI itself, there is a substantial difference in the relative efficiencies of DNA alkylation. Consistent with both its relative stability and its relative cytotoxic potency, F_2CBI-TMI alkylated DNA 100−1000× less efficiently than CBI-TMI, and this correlation with its other biological properties proved to be remarkably accurate. Quantitating this difference in efficiency by densitometry and averaging the results of several comparisons led to an average assessment that racemic F_2CBI-TMI was 675−725× less efficient than (+)-CBI-TMI. In contrast to CBI-TMI and other analogs of **1**−**3** containing stable alkylation subunits (CPI, DSA, CBI, MCBI, CCBI)[13,15,37−39] but analogous to those possessing the most reactive (CI, DA, CBQ),[14,41,42,58] the DNA alkylation efficiency of F_2CBI-TMI was found to steadily increase as the temperature was decreased from 37 to 25 to 4 °C. This may be attributed to the nonproductive competitive solvolysis of the agent which competes with alkylation, and this phenomenon is observed only with the most reactive of the agents studied to date.

Finally, implicit in these studies is the observation of exclusive adenine N3 addition to the C9 cyclopropane carbon consistent with expectations that the gem difluoro substitution would not effect the inherent regioselectivity. Although this was to be expected based on the chemical solvolysis studies with **17** and **19** which demonstrated that acid-catalyzed nucleophilic addition occurred at C9 with no evidence of ring expansion solvolysis, preceding studies even with agents that undergo solvolysis with a lower regioselectivity including the CPI-based agents and CC-1065 (4:1 regioselectivity),[43] duocarmycin A (4−1:1 regioselectivity),[4,17,44] duocarmycin SA (6−4:1 regioselectivity),[44] and CBQ-based agents (3:2 regioselectivity)[41] led to detection of only adducts derived from adenine N3 addition to the least substituted cyclopropane carbon. Each of these studies also quantitated the adduct formation and, in the case of duocarmycin A (86−92%),[14] duocarmycin SA (95−100%),[15] and the CBQ-based agents (>75%),[41] led to the observation that the regioselectivity of the DNA alkylation reaction is greater than that of simple solvolysis. Although several explanations could be advanced for these observations,[41] the two most prominent are preferential adoption of binding orientations that favor normal adenine N3 addition (proximity effects) and the significant destabilizing torsional strain and steric interactions that accompany the abnormal addition. Figures illustrating these effects have been disclosed in our prior work and we would suggest that this latter subtle effect is most substantial.[41] Consequently, we would not have expected to detect the abnormal adenine N3 addition with F_2CBI-TMI even if its cyclopropane addition regioselectivity were more modest.

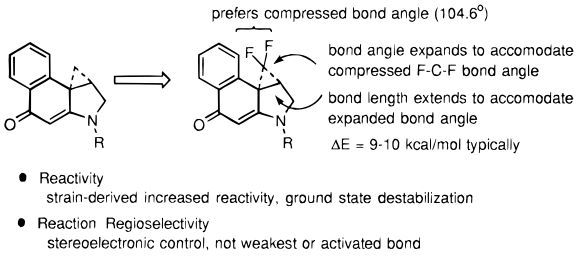

Figure 7.

Conclusions

An effective synthesis of F_2CBI, a difluorocyclopropane analog of the alkylation subunits of CC-1065 and the duocarmycins, was accomplished and represents the first such agent examined containing functionalization of the reactive center in the natural products. The core structure was assembled through adoption of the Sundberg intramolecular insertion of an *in situ* generated metal carbenoid into a difluoroalkene employing a key *p*-quinonediazide.

At the onset of our study, it was not clear whether the fluorocyclopropane substitution would alter or enhance the typical reaction regioselectivity and whether it would enhance or diminish the electrophilic functional reactivity. The introduction of the two fluorine substituents converts the least substituted cyclopropane carbon of CBI into the most substituted, inductively removes electron density from the reacting center, and, by virtue of resonance stabilization, could stabilize developing positive charge at the reacting, albeit already electropositive, center. Although the greater substitution could diminish nucleophilic addition to the C9 carbon, the relatively small size of fluorine made it difficult to assess in advance its impact on nucleophilic addition. The complementary electron-withdrawing properties of the fluorine substituents which diminish electron density at C9 and their potential resonance stabilization of developing positive change on C9 could enhance the regioselectivity of the nucleophilic cyclopropane ring opening reaction. In contrast, its established large impact on the bond opposite the two fluorine substituents could be anticipated to substantially lengthen and weaken the C8b−C9a bond and potentially redirect nucleophilic addition to C9a providing abnormal solvolysis with ring expansion.

A study of the acid-catalyzed nucleophilic addition to *N*-BOC-F_2CBI (**19**) and *N*-acetyl-F_2CBI (**17**) revealed that the difluorocyclopropane substitution increased the reactivity 500× despite the inductive electron-withdrawing properties of the reactive center substituents without altering the inherent regioselectivity which occurs with nucleophilic addition to the difluoro substituted C9 cyclopropane carbon. X-ray structural studies of F_2CBI and the comparison with related agents revealed the structural origin of the effects (Figure 7). The maintained regioselectivity may be attributed to the potential partial positive charge stabilization by the two fluorine substituents and the stereoelectronic control of the reaction where only the cleaved C8b−C9 bond is aligned for reaction. This occurs in spite of the preferential weakening of the alternative but nonaligned C8b−C9a bond. In turn, this orientation of the cyclopropane is dictated by the geometrical constraints imposed by the fused five-membered ring.[41]

Ground state effects were found to account for the increased reactivity. The cyclopropane C−CF_2−C bond angle is expanded and the cyclopropane bond opposite the difluoro substitution is substantially lengthened to accommodate the preferentially compressed exocyclic F−C−F bond angle introducing additional strain energy and increasing the reactivity of F_2CBI. This strain-derived ground state destabilization and increase in reactivity is exactly analogous to that found in simple related systems[48,49] and is not unique or even perturbed by incorporation into F_2CBI. Moreover, it is sufficient to overcome any stabilization potentially derived from the electron-withdrawing inductive effect of the fluorine substitution.

Qualitatively consistent with this increased reactivity (500×) and following a prior established relationship, the agents were found to be 500−1000× less cytotoxic than the corresponding CBI derivative. Similarly, the difluorocyclopropane substitutions had no detectable effect on the DNA alkylation selectivity of the agents, and they were found to undergo the characteristic adenine N3 addition to the C9 cyclopropane carbon but did so with a reduced efficiency (675−725×) nicely following the cytotoxicity/stability correlations.

Experimental Section[59]

2-[*N*-(3,3-Difluoro-2-propen-1-yl)acetamido]-4-hydroxy-1-nitronaphthalene (13). A solution of **12**[59] (417 mg, 1.01 mmol) in CH_2Cl_2 (10 mL) at −78 °C under Ar was treated with 1 M BBr_3 (2.0 mL in CH_2Cl_2, 2.0 mmol). The resulting solution was stirred at −78 °C for 0.5 h before being quenched by the addition of absolute EtOH (0.5 mL) followed by 20% aqueous Na_2HPO_4 (15 mL) and CH_2Cl_2 (10 mL). The organic layer was removed, and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic solutions were washed with saturated aqueous NaCl (20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Typically, the crude phenol was taken directly into the next reaction without further purification. Chromatography (SiO_2,10% EtOAc−hexane) provided **13** (300 mg, 326 mg theoretical, 92%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.07 (s, 1H), 8.37 (d, 1H, J = 8.2 Hz), 7.85 (d, 1H, J = 8.3 Hz), 7.72 (dt, 1H, J = 1.3, 6.9 Hz), 7.66 (dt, 1H, J = 1.3, 7.0 Hz), 6.60 (s, 1H), 4.51 (m, 1H), 4.47 (m, 1H), 4.13 (m, 1H), 1.97 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.8, 160.8, 157.7, 138.5, 132.4, 130.2, 127.5, 126.4, 124.9, 123.2, 122.1, 106.5, 74.3 (dd, J = 97.2, 97.2 Hz), 43.0 (d, J = 29.4 Hz), 22.3; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −85.1 (d, J = 40.0 Hz), −87.8 (dd, J = 28.0, 40.0 Hz); IR (film) ν_{max} 3200, 1746, 1626, 1518 cm^{-1}; FABHRMS (NBA) *m/z* 323.0850 (M + H$^+$, $C_{15}H_{12}F_2N_2O_4$ requires 323.0843).

4-[(Ethoxycarbonyl)oxy]-2-[*N*-(3,3-difluoro-2-propen-1-yl)acetamido]-1-nitronaphthalene (14). A solution of **13** (300 mg, 0.93 mmol) in CH_2Cl_2 (15 mL) cooled to 0 °C under Ar was treated with pyridine (160 μL, 2.0 mmol) and ethyl chloroformate (120 μL, 1.5 mmol). After stirring at 0 °C for 30 min the cooling bath was removed, and the reaction solution was further stirred for 16 h. The solution was diluted with CH_2Cl_2 (5 mL) before 10% aqueous HCl (15 mL) was added. The organic layer was removed and washed with H_2O (2 × 5 mL), saturated aqueous NaCl (15 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography (SiO_2, 5% EtOAc−hexane) afforded **14** (323 mg, 367 mg theoretical, 88%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.17 (dd, 1H, J = 2.3, 7.2 Hz), 7.75 (m, 3H), 7.31 (s, 1H), 4.53 (m, 2H), 4.38 (q, 2H, J = 7.2 Hz), 4.03 (m, 1H), 1.90 (s, 3H), 1.44 (t, 3H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.2, 157.9, 152.4, 148.7, 144.2, 131.0, 130.3, 129.0, 126.8, 125.7, 122.4, 121.9, 117.9, 74.4 (dd, J = 94.0, 96.0 Hz), 65.9, 42.4 (d, J = 29.6 Hz), 22.5, 14.2; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −85.3 (d, J = 36.0 Hz), −88.2 (dd, J = 28.0, 36.0 Hz); IR (film) ν_{max} 2927, 1769, 1745, 1682, 1532 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 417.0861 (M + Na$^+$, $C_{18}H_{16}F_2N_2O_6$ requires 417.0874).

1-Diazo-2-[*N*-(3,3-difluoro-2-propen-1-yl)acetamido]-1,4-dihydronaphthalen-4-one (16). A suspension of 5% Pd−C (4.0 mg) in CH_3OH (500 μL) was treated with a solution of $NaBH_4$ (9.0 mg, 0.25 mmol) in H_2O (500 μL). The resulting mixture was cooled to 0 °C, and a stream of Ar was bubbled through the solution for 5 min. A solution of **14** (11.8 mg, 0.030 mmol) in CH_3OH (600 μL) was added dropwise, and the resulting mixture was stirred at 0 °C for 15 min. The solution was diluted with EtOAc (10 mL), filtered through a Celite

(59) Full experimental and characterization for **5**−**12** is provided in supporting information.

plug, washed with ice H_2O (7 mL), saturated aqueous NaCl (10 mL), dried ($MgSO_4$), and concentrated under reduced pressure to afford the crude amine **15** which was used directly in the following reaction. For **15**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95 (dd, 1H, J = 2.1, 6.2 Hz), 7.85 (m, 1H), 7.56 (m, 2H), 7.00 (s, 1H), 4.40−4.56 (m, 4H), 4.35 (q, 2H, J = 7.1 Hz), 1.88 (s, 3H), 1.40 (t, 3H, J = 7.1 Hz); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −86.1 (d, J = 40.0 Hz), −88.2 (dd, J = 24.0, 40.0 Hz); IR (film) ν_{max} 3456, 3358, 3241, 1747, 1654, 1242 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 364.1252 (M^+, $C_{18}H_{18}F_2N_2O_4$ requires 364.1235).

The crude **15** (flask wrapped in foil) was cooled to −30 °C under Ar, and *i*-$C_5H_{11}ONO$ (500 μL as solvent) was added followed by 4 M HCl−CH_3OH (10 μL), CH_2Cl_2 (500 μL), and CH_3OH (500 μL). The reaction mixture was stirred at −30 °C for 16 h and diluted with saturated aqueous $NaHCO_3$ (5 mL) and CH_2Cl_2 (7 mL). Throughout the following workup and purification, the mixture was protected from light. The organic solution was removed, and the aqueous solution was extracted with CH_2Cl_2 (2 × 5 mL). The organic solutions were combined, dried (Na_2CO_3), and concentrated under reduced pressure. Chromatography (SiO_2, 30% EtOAc−hexane) yielded **16** (12.0 mg, 15.0 mg theoretical, 80% overall from **14**) as a tan oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.42 (d, 1H, J = 7.9 Hz), 7.72 (dd, 1H, J = 7.4, 7.4 Hz), 7.50 (dd, 1H, J = 7.3, 7.6 Hz), 7.34 (d, 1H, J = 7.9 Hz), 6.33 (s, 1H), 4.45 (m, 2H), 4.17 (m, 1H), 2.08 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.9, 169.3, 161.0, 158.0, 144.4, 132.6, 130.2, 128.2, 128.0, 127.1, 123.2, 120.8, 74.2 (dd, J = 89.0, 89.0 Hz), 42.0, 21.8; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −84.7 (d, J = 36.0 Hz), −87.3 (dd, J = 28.0, 36.0 Hz); IR (film) ν_{max} 2926, 2077, 1746, 1594 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 304.0855 (M + H^+, $C_{15}H_{11}F_2N_2O_2$ requires 304.0898).

3-Acetyl-9,9-difluoro-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*e*]-indol-4-one (17). A suspension of **16** (16.0 mg, 0.05 mmol) and Rh_2-$(OAc)_4$ (2.5 mg, 5.3 μmol) in toluene (5 mL) under Ar was warmed at 110 °C for 0.5 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by chromatography (SiO_2, 35% EtOAc−hexane) to provide **17** (10.7 mg, 14.5 mg theoretical, 74%) as a tan powder: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.21 (dd, 1H, J = 1.7, 7.5 Hz, C5-H), 7.55 (ddd, 1H, J = 1.5, 6.2, 6.2 Hz, C7-H), 7.50 (ddd, 1H, J = 1.5, 7.2, 7.4 Hz, C6-H), 7.03 (m, 2H, C4-H and C8-H), 4.45 (m, 1H, C1-H), 4.28 (m, 1H, C1-H), 3.14 (m, 1H, C9a-H), 2.35 (s, 3H, CH_3CO); ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.08 (dd, 1H, J = 1.2, 7.8 Hz, C5-H), 7.62 (dt, 1H, J = 1.5, 7.5 Hz, C6- or C7-H), 7.53 (dt, 1H, J = 1.2, 7.5 Hz, C7 or C6-H), 7.29 (m, 1H, C8-H), 7.16 (br s, 1H, C3-H), 4.61 (m, 2H, C1-H_2), 3.79 (m, 1H, C9a-H), 2.32 (s, 3H, CH_3CO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.0, 152.0, 142.3, 131.7, 128.2, 127.2, 124.3, 111.7, 109.6, 50.0, 27.6 (t, J = 9.9 Hz), 24.9; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −113.8 (d, J = 160.0 Hz), −125.2 (dd, J = 11.0, 160.0 Hz); IR (solid film) ν_{max} 1702, 1691 cm^{-1}; FABHRMS (NBA) *m/z* 276.0836 (M + H^+, $C_{15}H_{11}F_2NO_2$ requires 276.0836). A single-crystal X-ray structure determination conducted on plates grown from EtOAc−hexane established the structure of **17**.[46]

9,9-Difluoro-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*e*]indol-4-one (18). A solution of **17** (5.6 mg, 0.020 mmol) in CH_3OH (800 μL) cooled to −10 °C under Ar was treated with 1 N aqueous LiOH (20 μL, 0.020 mmol). The reaction mixture was stirred for 10 min before being concentrated under a stream of N_2. Chromatography (SiO_2, 40% EtOAc−hexane) afforded **18** (4.3 mg, 4.7 mg theoretical, 92%) as a beige wax: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.20 (m, 1H, C5-H), 7.45 (m, 2H, C6 and C7-H), 6.99 (m, 1H, C8-H), 5.72 (s, 1H, C3-H), 5.28 (br s, 1H, NH), 4.02 (m, 2H, C1-H_2), 3.18 (dd, 1H, J = 10.8, 11.1 Hz, C9a-H); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −112.0 (d, J = 160.0 Hz), −122.5 (dd, J = 11.0, 160.0 Hz); IR (film) ν_{max} 3178, 2926, 1611, 1230 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 256.0558 (M + Na^+, $C_{13}H_9F_2$-NO requires 256.0550).

3-*tert*-Butyloxycarbonyl-9,9-difluoro-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*e*]indol-4-one (19). A suspension of NaH (0.8 mg, 0.020 mmol) in DMF (300 μL) under Ar was treated with a solution of **18** (4.0 mg, 0.017 mmol) in DMF (750 μL). The reaction mixture was protected from the light and was stirred for 5 min. BOC_2O (5 μL, 0.022 mmol) was added, and the reaction mixture was stirred at 25 °C for 3.5 h. EtOAc (5 mL) and H_2O (5 mL) were added, and the organic layer was removed. The aqueous layer was extracted with EtOAc (3 × 3 mL), and the combined organic solutions were washed with saturated aqueous NaCl (10 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. PTLC (SiO_2, 10 × 10 cm, 25% EtOAc−hexane) provided **19** (2.5 mg, 5.7 mg theoretical, 44%) as a beige wax: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.21 (d, 1H, J = 7.2 Hz, C5-H), 7.49 (m, 2H, C6 and C7-H), 7.00 (m, 1H, C8-H), 6.85 (br s, 1H, C3-H), 4.29 (m, 1H, C1-H), 4.20 (m, 1H, C1-H), 3.09 (dd, 1H, J = 10.3, 10.4 Hz, C9a-H), 1.56 (s, 9H, $C(CH_3)_3$); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −113.4 (d, J = 160.0 Hz), −123.5 (dd, J = 11.0, 160.0 Hz); IR (film) ν_{max} 2918, 1727, 1634 cm^{-1}; FABHRMS (NBA-CsI) *m/z* 460.0219 (M + Cs^+, $C_{18}H_{17}F_2NO_3$ requires 460.0231).

F_2CBI-TMI (24). A suspension of NaH (60% dispersion in oil, 0.6 mg, 0.015 mmol) in DMF (100 μL) under Ar at 25 °C was treated with a solution of **18** (3.3 mg, 0.014 mmol) in DMF (250 μL). The reaction mixture was protected from the light and was stirred at 25 °C for 2 min. A solution of **23** (130 μL of a 0.052 M solution, 0.006 mmol) was added, and the reaction mixture was stirred for 1 h before it was partitioned between H_2O (3 mL) and EtOAc (3 mL). The organic solution was removed, and the aqueous solution was extracted with EtOAc (3 × 4 mL). The combined organic solutions were washed with saturated aqueous NaCl (6 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Chromatography (SiO_2, 40% EtOAc−hexane) provided **24** (1.4 mg, 3.1 mg theoretical, 45%) as a yellow wax in addition to recovered **18** (1.0 mg, 30% recovery). For **24**: ^{1}H NMR (acetone-d_6, 400 MHz) δ 10.60 (br s, 1H, NH), 8.12 (dd, 1H, J = 1.0, 7.6 Hz, C5-H), 7.65 (dt, 1H, J = 1.5, 7.5 Hz, C6-H), 7.56 (dt, 1H, J = 1.2, 7.5 Hz, C7-H), 7.37 (dd, 1H, J = 7.8, 8.2 Hz, C8-H), 7.26 (s, 1H, C3′-H), 7.23 (s, 1H, C4′-H), 6.95 (s, 1H, C3-H), 5.05 (m, 1H, C1-H), 4.92 (br s, 1H, C1-H), 4.00 (br s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.27 (m, 1H, C9a-H); ^{19}F NMR (acetone-d_6, 376 MHz) δ −117.0 (d, J = 160.0 Hz), −127.2 (dd, J = 11.0, 160.0 Hz); IR (film) ν_{max} 3261, 2933, 1645, 1634, 1393, 1267 cm^{-1}; MS (electrospray$^+$) *m/z* 489 (M + Na^+), 467 (M^+), 429, 385.

Solvolysis Reactivity. pH 3: *N*-BOC-F_2CBI (**19**, 50 μg) was dissolved in CH_3OH (1.5 mL) and mixed with pH 3 aqueous buffer (1.5 mL). The buffer contained 4:1:20 (v:v:v) 0.1 M citric acid, 0.2 M Na_2HPO_4, and H_2O, respectively. The solvolysis solution was sealed and kept at 25 °C protected from the light. The UV spectrum was measured at regular intervals, every 3 min for 1.5 h, every 15 min for the next 4.5 h, and finally every 1 h for 5 h. The decrease in the long-wavelength absorption at 318 nm and the increase in the short-wavelength absorption at 265 nm were monitored, Figure 3. The solvolysis rate constant ($k = 7.05 \times 10^{-4}$ s^{-1}) and half-life ($t_{1/2}$ = 0.26 h) were calculated from data recorded at the long-wavelength from the least-squares treatment (r = 1.0) of the slope of the plot of time versus $\ln[(A_f - A_i)/(A_f - A)]$.

Similarly, F_2CBI (**18**, 30 μg) was dissolved in CH_3OH (1.5 mL) and mixed with pH 3 aqueous buffer (1.5 mL). The solvolysis solution was sealed and kept at 25 °C protected from light. The UV spectrum was recorded every 2 min for 10 min, every 15 min for 7 h, and every 8 h for 48 h. The decrease in the long-wavelength absorption at 338 nm and the increase in the short-wavelength absorption at 247 nm were monitored, Figure 3. The solvolysis rate constant ($k = 4.54 \times 10^{-5}$ s^{-1}) and half-life ($t_{1/2}$ = 4.2 h) were determined as detailed above (r = 1.0).

pH 7: Samples of **18** and **19** (50 μg) were dissolved in CH_3OH (1.5 mL) and H_2O (1.5 mL). The solutions were sealed at 25 °C and protected from the light. UV spectra were recorded at regular intervals until constant values were obtained for the long and short-wavelength absorbances (260 and 338 nm for **18**, 252 and 308 nm for **19**). The solvolysis rate constants were determined from the slope of the lines obtained from linear least-squares treatments of plots of $\ln[(A_f - A_i)/(A_f - A)]$ versus time using the long-wavelength measurements (Figure 8). The first order rate constants determined under these conditions were 8.27×10^{-5} s^{-1} ($t_{1/2}$ = 2.3 h, r = 0.98) for *N*-BOC-F_2CBI (**19**) and 4.66×10^{-7} s^{-1} ($t_{1/2}$ = 422 h, r = 0.99) for F_2CBI (**18**).

Acid-Catalyzed Addition of CH_3OH to *N*-Acetyl-F_2CBI (17): 3-Acetamido-1-(1,1-difluoro-1-methoxymethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indole (25). A solution of **17** (1.9 mg, 6.91 μmol) in CH_3OH (750 μL) was treated with 0.010 M CF_3SO_3H-CH_3OH (83

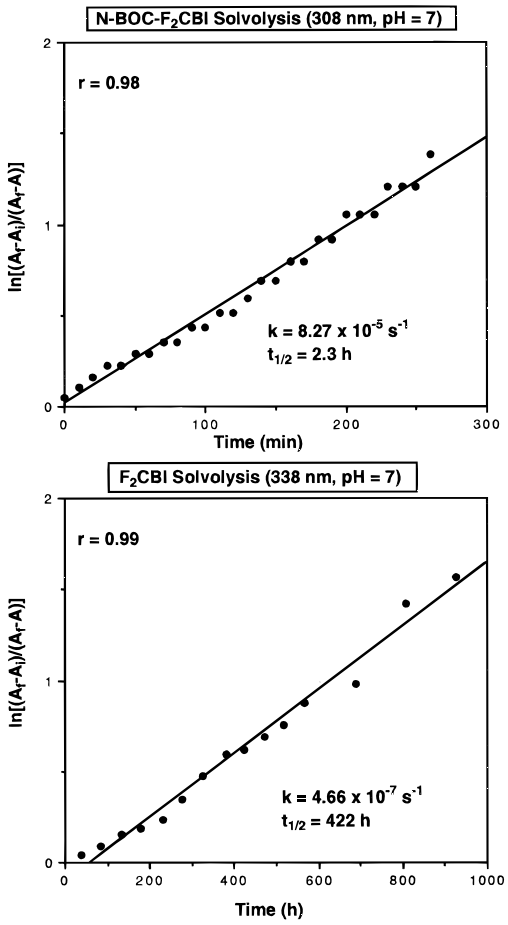

Figure 8.

μL, 0.83 μmol, 0.12 equiv) at −10 °C. The mixture was stirred under Ar for 30 min at which time TLC showed complete disappearance of **17** and the generation of one new product. $NaHCO_3$ (0.6 mg, 1.2 equiv) was added, and the mixture was stirred for 10 min and concentrated under reduced pressure. PTLC (SiO_2, 100% EtOAc−hexane) gave **25** (1.9 mg, 2.1 mg theoretical, 90%) as a beige colored solid: ^{1}H NMR (acetone-d_6, 400 MHz) δ 9.26 (s, 1H), 8.18 (d, 1H, J = 8.4 Hz), 8.06 (s, 1H), 7.86 (d, 1H, J = 8.1 Hz), 7.47 (dt, 1H, J = 1.5, 7.1 Hz), 7.32 (dt, 1H, J = 1.5, 8.0 Hz), 4.35 (m, 3H), 3.48 (s, 3H), 2.83 (s, 3H); ^{13}C NMR (acetone-d_6, 100 MHz) δ 177.4, 137.9, 1 36.3, 126.8, 124.3, 122.72, 122.70, 100.1, 57.3, 49.8 (t, J = 28 Hz), 44.9 (t, J = 121 Hz), 23.4, 22.5; ^{19}F NMR (acetone-d_6, 376 MHz) δ −77.9 (dd, J = 4.0, 16.0 Hz); IR (film) ν_{max} 2910, 1726, 1584, 1256, 1029 cm^{-1}; FABHRMS (NBA-CsI) *m/z* 440.0120 (M + Cs^+, $C_{16}H_{15}F_2NO_3$ requires 440.0074).

Acid-Catalyzed Addition of CH_3OH to *N*-BOC-F_2CBI (19): 3-*tert*-Butyloxycarbonyl-1-(1,1-difluoro-1-methoxymethyl)-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indole (26). A solution of **19** (1.6 mg, 4.81 μmol) in CH_3OH (750 μL) was treated with 0.010 M CF_3SO_3H−CH_3OH (60 μL, 0.58 μmol, 0.12 equiv) at 0 °C. The mixture was stirred under Ar for 10 min at which time TLC showed complete disappearance of **19** and the generation of one new product. $NaHCO_3$ (5 mg) was added, and the mixture was stirred for 10 min. The suspension was filtered through Celite and concentrated under reduced pressure. Chromatography (SiO_2, 10% EtOAc−hexane) gave **26** (1.4 mg, 1.8 mg theoretical, 79%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.13 (d, 1H, J = 7.7 Hz), 7.81 (d, 1H, J = 8.4 Hz), 7.72 (br s, 1H), 7.46 (t, 1H, J = 7.5 Hz), 7.31 (t, 1H, J = 7.4 Hz), 5.94 (br s, 1H), 4.30 (m, 1H), 4.05 (m, 2H), 3.47 (s, 3H), 1.59 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.3, 152.6, 131.4, 127.2, 125.8, 123.9, 124.8, 122.7, 99.1, 50.5, 50.3 (t, J = 48.2 Hz), 44.5, 28.5, 14.1; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −81.8 (d, J = 152.0 Hz), −82.9 (d, J = 152.0 Hz); ^{19}F NMR (acetone-d_6, 376 MHz) δ −78.2 (s); IR (film) ν_{max} 3353, 2961, 1682, 1148 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 365.1439 (M + Na^+, $C_{19}H_{21}F_2NO_4$ requires 365.1439).

3-Acetamido-1-carboxy-5-hydroxy-1,2-dihydro-3*H*-benzo[*e*]indole (27). A solution of **19** (2.1 mg, 7.6 μmol) in THF−H_2O (5:1, 760 μL) at 25 °C was treated with 0.010 M CF_3SO_3H−H_2O (92 μL, 0.92 μmol, 0.12 equiv), and the mixture was stirred at 25 °C for 17 h under Ar. The reaction mixture was diluted with EtOAc (5 mL) and poured into saturated $NaHCO_3$ (7 mL). The EtOAc layer was removed and washed with saturated aqueous $NaHCO_3$ (5 mL). The combined $NaHCO_3$ layers were acidified to pH 2 by the addition of 10% aqueous HCl and extracted with EtOAc (3 × 5 mL). The EtOAc extracts were combined, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The oil residue was washed with $CHCl_3$ (2 mL) to afford pure **27** (1.7 mg, 2.0 mg theoretical, 85%) as a beige, viscous oil: ^{1}H NMR (CD_3OD, 400 MHz) δ 8.07 (d, 1H, J = 8.3 Hz), 7.83 (s, 1H), 7.80 (d, 1H, J = 9.4 Hz), 7.38 (dt, 1H, J = 0.8, 6.7 Hz), 7.24 (dt, 1H, J = 0.8, 7.2 Hz), 4.43 (m, 2H), 4.31 (t, 1H, J = 10.5 Hz), 2.21 (s, 3H); IR (film) ν_{max} 3348, 2912, 1713, 1633, 1392 cm^{-1}; FABHRMS (NBA-NaI) *m/z* 272.0936 (M + H^+, $C_{15}H_{13}NO_4$ requires 272.0923).

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Supporting Information Available: Full experimental and characterization for **5**−**12**, diagnostic characterization for the referenced compounds,[27] experimental procedure for the DNA alkylation studies, details of the X-ray structure determination of **17**, and summary tables of the data employed in Figure 5 (24 pages). See any current masthead page for ordering and Internet access instructions.

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