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Rational Design, Synthesis, and Evaluation of Key Analogues of CC-1065 and the Duocarmycins

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Abstract: The design, synthesis, and evaluation of a predictably more potent analogue of CC-1065 entailing the substitution replacement of a single skeleton atom in the alkylation subunit are disclosed and were conducted on the basis of design principles that emerged from a fundamental parabolic relationship between chemical reactivity and cytotoxic potency. Consistent with projections, the 7-methyl-1,2,8,8a-tetrahydrocyclopropa[*c*]thieno[3,2-*e*]indol-4-one (MeCTI) alkylation subunit and its isomer 6-methyl-1,2,8,8a-tetrahydrocyclopropa[*c*]thieno[2,3-*e*]indol-4-one (*iso*-MeCTI) were found to be 5−6 times more stable than the MeCPI alkylation subunit found in CC-1065 and slightly more stable than even the DSA alkylation subunit found in duocarmycin SA, placing it at the point of optimally balanced stability and reactivity for this class of antitumor agents. Their incorporation into the key analogues of the natural products provided derivatives that surpassed the potency of MeCPI derivatives (3−10-fold), matching or slightly exceeding the potency of the corresponding DSA derivatives, consistent with projections made on the basis of the parabolic relationship. Notable of these, MeCTI-TMI proved to be as potent as or slightly more potent than the natural product duocarmycin SA (DSA-TMI, IC_{50} = 5 vs 8 pM), and MeCTI-PDE_2 proved to be 3-fold more potent than the natural product CC-1065 (MeCPI-PDE_2, IC_{50} = 7 vs 20 pM). Both exhibited efficiencies of DNA alkylation that correlate with this enhanced potency without impacting the intrinsic selectivity characteristic of this class of antitumor agents.

Introduction

CC-1065[1] (**1**) and duocarmycin SA (**2**)[2] represent the key members of a small class of exceptionally potent naturally occurring antitumor agents[1−4] that derive their biological properties through a now characteristic sequence-selective DNA alkylation reaction (Figure 1).[5−9] Extensive investigations on the naturally occurring members of this class as well as their synthetic analogues have defined key and subtle features that contribute to their properties.[9,10] Most notable of these are the structural features that contribute to the AT-rich noncovalent binding selectivity dominating the alkylation selectivity,[11] those that define the source of catalysis for the DNA alkylation reaction,[12,13] and those that subtly impact the unusual and intrinsic stability of their alkylation subunits.[9,13,14]

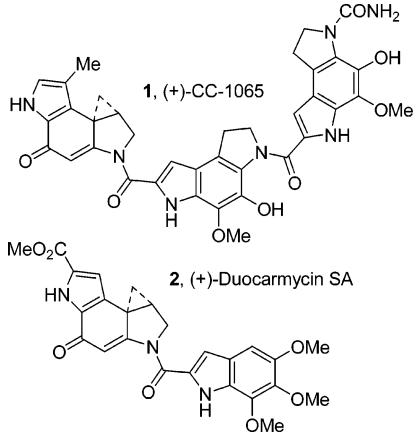

Figure 1. Natural products.

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(1) CC-1065: 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*, 1119−1125.
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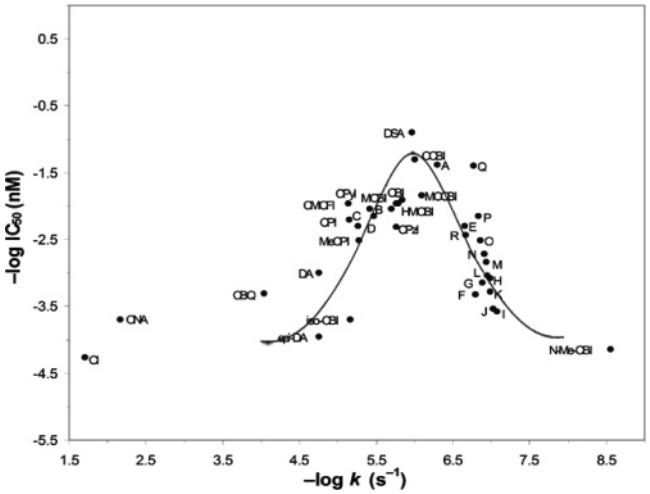
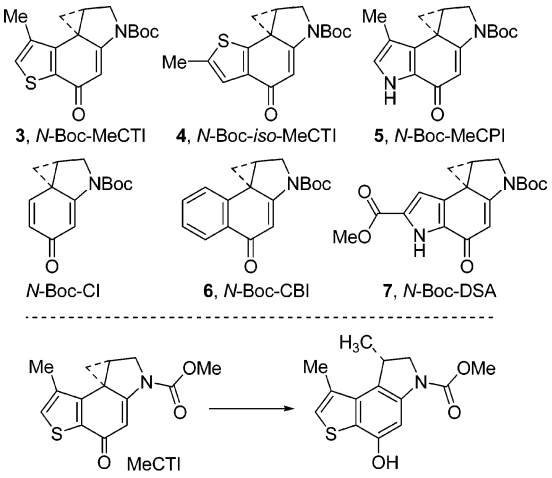


Figure 2. Relationship between reactivity (solvolysis −log *k*, pH 3) and cytotoxic potency (−log IC_{50}, L1210), natural enantiomers. See Supporting Information for abbreviations and key to letter notations.

Results and Discussion

Design. Throughout the course of our investigations, we have chronicled a direct relationship between the intrinsic chemical stability of the alkylation subunit and the cytotoxic potency of the resulting derivatives.[15] Recently, a compilation of the data derived from more than 30 deep-seated modifications resulted in the establishment of a well-defined parabolic relationship between the alkylation subunit reactivity and the resulting cytotoxic potency that spanned a 10^4-10^6 range of reactivities and activities (Figure 2).[16] Presumably, this fundamental relationship simply reflects the fact that the compound must be sufficiently stable to reach its biological target yet remain sufficiently reactive to alkylate DNA once it does. Significantly, the work defined this optimal balance between reactivity and stability, providing a fundamental design feature that is subject to investigational interrogation.



Agent	Spiro	Product	ΔΔ*H* (kcal)
CI	−22.92	−81.28	−58.36
MeCPI	−15.62	−66.31	−50.69
CBI	−22.68	−62.99	−40.31
MeCTI	−24.91	−63.40	−38.49

Figure 3. Alkylation subunits and AM1 calculated heats of reaction for hydride addition to the activated cyclopropane.

Herein, we report our first such efforts, culminating in the synthesis and evaluation of 7-methyl-1,2,8,8a-tetrahydrocyclopropa[*c*]thieno[3,2-*e*]indol-4-one (MeCTI, **3**) as well as 6-methyl-1,2,8,8a-tetrahydrocyclopropa[*c*]thieno[2,3-*e*]indol-4-one (*iso*-MeCTI, **4**) and their incorporation into analogues of both CC-1065 and the duocarmycins (Figure 3).[17] The design of MeCTI and the decision to invest in its exploration rested with expectations that it would be substantially more stable than the alkylation subunit found in CC-1065 (MeCPI, **5**), leading to a substantially more potent CC-1065 analogue approaching the stability and activity of duocarmycin SA. Intuitively, this might be anticipated to arise from the strain release provided by a fused thiophene versus pyrrole, which in turn may further benefit from the intrinsic electron-withdrawing character of a thiophene. More quantitatively, this increased stability could be approximated using semiempirical calculations (AM1, MNDO) for heats of reaction for hydride addition to the activated cyclopropane (Figure 3). Using this approximation, MeCTI was selected among several candidate alkylation subunits as being more stable than CBI (**6**, 1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*e*]indol-4-one) and approaching or exceeding that of DSA (**7**), potentially approaching the optimal stability defined by the parabolic relationship. Significantly, MeCTI represents a single atom change in the backbone structure of the CC-1065 alkylation subunit that in turn was projected to provide a nearly optimal increase in stability and potency (Figure 4).

Synthesis of *N*-Boc-MeCTI. Stobbe condensation of 4-methylthiophene-3-carboxaldehyde (**9**, 4 equiv of *t*-BuOK, 6 equiv

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(7) Duocarmycin A: (a) Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1990**, *112*, 8961−8971. (b) Boger, D. L.; Ishizaki, T.; Zarrimayeh, H. *J. Am. Chem. Soc.* **1991**, *113*, 6645−6649. (c) Boger, D. L.; Yun, W.; Terashima, S.; Fukuda, Y.; Nakatani, K.; Kitos, P. A.; Jin, Q. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 759−765.
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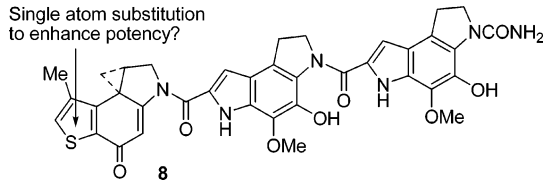
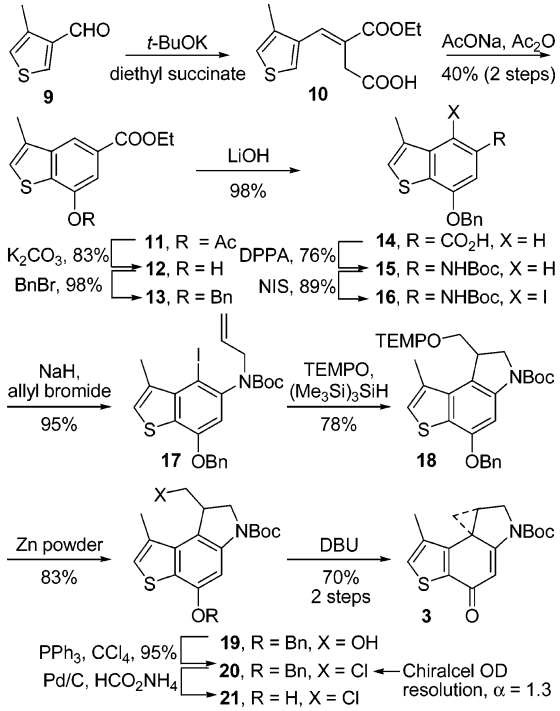


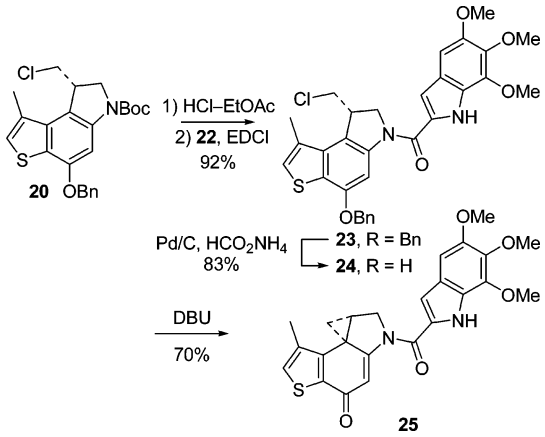
Figure 4. Structure of (+)-MeCTI-PDE$_2$.

Scheme 1



of diethyl succinate, 83 °C, 1.5 h) gave a mixture of half-esters **10** which were subjected to Friedel−Crafts acylation (excess Ac_2O/NaOAc, 140 °C, 5 h, 40%) to provide **11** (Scheme 1).[18] Compound **11** was hydrolyzed to phenol **12** (1.1 equiv of K_2CO_3, 78 °C, 14 h, 83%), which was protected as the benzyl ether **13** (1.2 equiv of BnBr, 1.2 equiv of K_2CO_3, 25 °C, 5 h, 98%). Subsequent hydrolysis of the ethyl ester (3 equiv of LiOH, 4:1:1 THF−MeOH−H_2O, 25 °C, 18 h, 98%) followed by Curtius rearrangement of the resulting carboxylic acid **14** (1.2 equiv of DPPA, 1.2 equiv of Et_3N, 83 °C, 18 h, 76%) provided carbamate **15**. Regioselective acid-catalyzed C4-iodination (0.1 equiv of H_2SO_4, 1.1 equiv of NIS, 25 °C, 2 h, 89%) followed by carbamate alkylation of **16** with allyl bromide (1.2 equiv of NaH, 3 equiv of allyl bromide, 25 °C, 3 h, 95%) provided **17**. 5-*Exo*-*trig* radical cyclization conducted in the presence of TEMPO[19] (2.7 equiv, 3 equiv of $(Me_3Si)_3SiH$, 100 °C, 5 h, 78%) gave **18** in good yield, whereas the more conventional use of Bu_3SnH was unsuccessful.[20] Zinc-mediated reductive cleavage of **18** (16 equiv of Zn, 70 °C, 3 h, 83%) followed by chlorination displacement of the released primary alcohol (2 equiv of Ph_3P, 6 equiv of CCl_4, 25 °C, 20 h, 95%) provided **20**. Intermediate **20** was separated into its two enantiomers by resolution on a semipreparative Chiralcel OD column (20% *i*-PrOH/hexane, $\alpha = 1.30$).[21] Hydrogenolysis of the benzyl ether (25% aqueous HCO_2NH_4, 10% Pd/C, 60 °C, 2 h) followed by immediate spirocyclization of the crude product by treatment with DBU (10 equiv, DMF−CH_3CN, 25 °C, 10 min, 70%) provided each enantiomer of **3**.

Scheme 2



Synthesis of MeCTI-TMI, MeCTI-Indole$_2$, and MeCTI-PDE$_2$. Acid-catalyzed deprotection of **20** (4 N HCl−EtOAc, 25 °C, 1 h) followed by coupling of the resulting hydrochloride salt with 5,6,7-trimethoxyindole-2-carboxylic acid (**22**,[22] 4 equiv of EDCI, 2 equiv of $NaHCO_3$, 25 °C, 15 h, 92%) provided **23** (Scheme 2). Hydrogenolysis of the benzyl ether (25% aqueous HCO_2NH_4, 10% Pd/C, 25 °C, 2 h, 83%) afforded **24**, and its spirocyclization was effected by treatment with DBU (4 equiv, DMF−CH_3CN, 25 °C, 10 min, 70%), providing **25**.

A subtle modification of this sequence was used to access MeCTI-indole$_2$ and MeCTI-PDE$_2$. Thus, acid-catalyzed deprotection of **21** (4 N HCl−EtOAc, 25 °C, 1 h) followed by coupling of the resulting hydrochloride salt with PDE$_2$-CO_2H (**26**)[23] and indole$_2$-CO_2H (**27**) (4 equiv of EDCI, 1.5 equiv of RCO_2H, 25 °C, 0.5 h, 40−72%) provided **28** and **29** (Scheme 3). This coupling with an alkylation subunit precursor in which the C5-benzyl ether was already removed permitted direct spirocyclization, effected by treatment with $NaHCO_3$ (4 equiv, 2:1 DMF−H_2O, 25 °C, 10 min, 46−81%), providing **8** and **30**.

Synthesis of *N*-Boc-*iso*-MeCTI. *N*-Boc-*iso*-MeCTI was prepared using a synthetic strategy analogous to the route described for **3**, beginning with the Stobbe condensation of the isomeric aldehyde **31** (Scheme 4). Compound **42** was resolved (Chiralcel OD, 5% *i*-PrOH/hexane, $\alpha = 1.44$) and deprotected (25% aqueous HCO_2NH_4, 10% Pd/C, 60 °C, 2 h), and the crude product was spirocyclized using DBU (10 equiv, DMF−CH_3CN, 25 °C, 4 h, 39%) to give each enantiomer of **4**. Notably, the Ar-3′ spirocyclization of this isomer was much slower and less facile than that of **3**.

Synthesis of *iso*-MeCTI-TMI. Deprotection of **42** (4 N HCl−EtOAc, 25 °C, 1 h) followed by coupling of the resulting

(18) (a) Boger, D. L.; McKie, J. A.; Cai, H.; Cacciari, B.; Baraldi, P. G. *J. Org. Chem.* **1996**, *61*, 1710−1729. (b) Boger, D. L.; Han, N.; Tarby, C. M.; Boyce, C. W.; Cai, H.; Jin, Q.; Kitos, P. A. *J. Org. Chem.* **1996**, *61*, 4894−4912. (c) Review of related efforts: Boger, D. L.; Boyce, C. W.; Garbaccio, R. M.; Goldberg, J. A. *Chem. Rev.* **1997**, *97*, 787−828.
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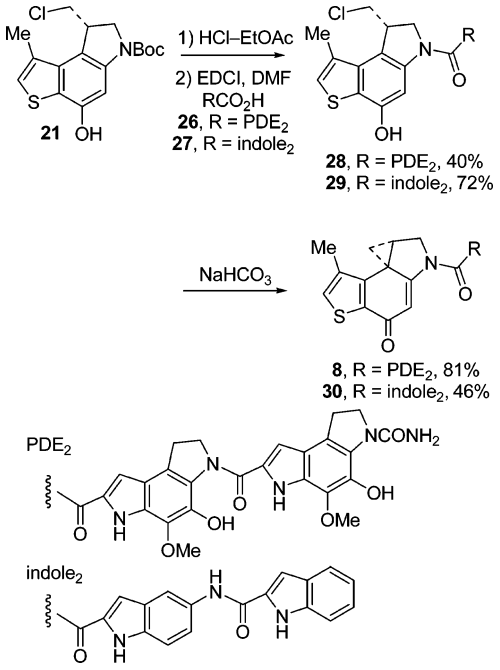
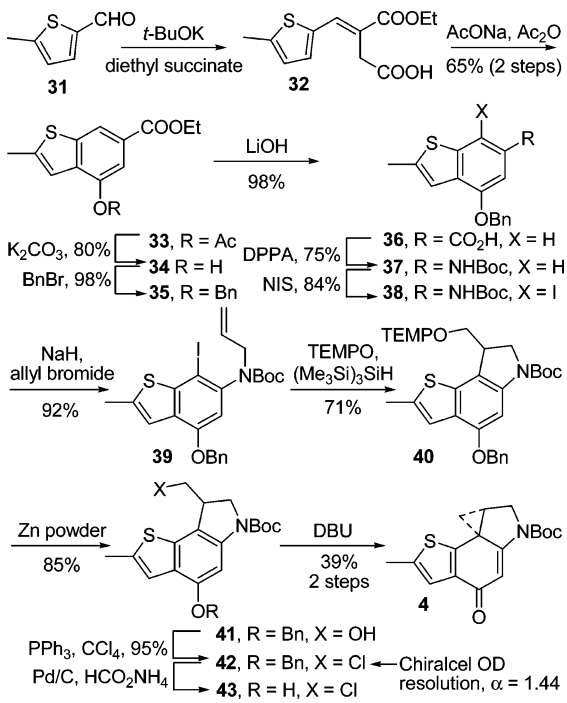
Scheme 3



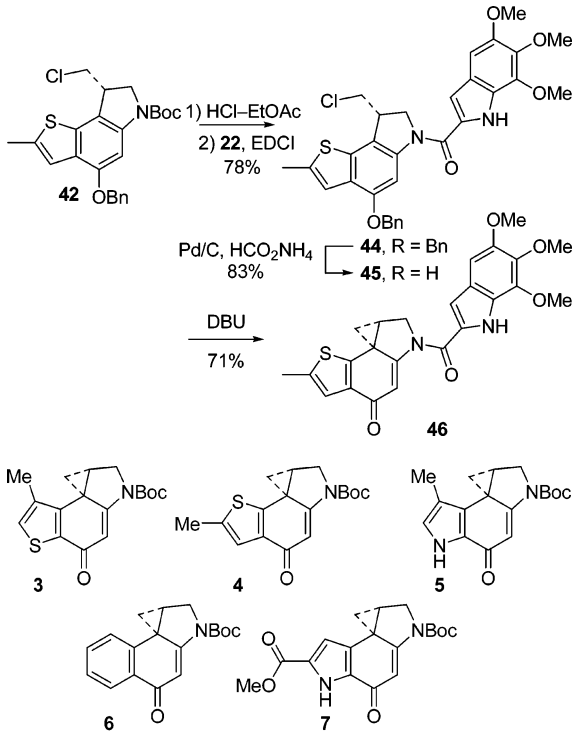
Scheme 4



hydrochloride salt with 5,6,7-trimethoxyindole-2-carboxylic acid (**22**,[22] 1.4 equiv, 4 equiv of EDCI, 3 equiv of $NaHCO_3$, 25 °C, 15 h, 78%) provided **44** (Scheme 5). Hydrogenolysis of the benzyl ether (25% aqueous HCO_2NH_4, 10% Pd/C, 60 °C, 2 h, 83%) afforded **45**. Spirocyclization of **45** was particularly slow (DBU/DMF) with this isomer, such that hydrolysis of the labile amide was competitive. The use of an alternative solvent system (2:1 CH_3CN/DMF) under strictly anhydrous conditions (6 equiv of DBU, 25 °C, 1 h, 71%) gave **46** in improved conversions.

Chemical Reactivity. The relative reactivity of the alkylation subunits was established by measuring their rates of acid-catalyzed solvolysis. At 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), both

Scheme 5



Compound	K (s^{-1}, pH 3)	$t_{1/2}$ (h, pH 3)
3, *N*-Boc-MeCTI	9.30×10^{-7}	206
4, *N*-Boc-*iso*-MeCTI	9.20×10^{-7}	209
5, *N*-Boc-MeCPI	5.26×10^{-6}	37
6, *N*-Boc-CBI	1.45×10^{-6}	133
7, *N*-Boc-DSA	1.08×10^{-6}	177

Figure 5. Solvolysis reactivity.

N-Boc-MeCTI (**3**) and *N*-Boc-*iso*-MeCTI (**4**) underwent measurable solvolysis, monitored spectrophotometrically by UV with the disappearance of the long-wavelength absorption of the CTI chromophore and the appearance of a short-wavelength absorption attributable to the solvolysis product. The Boc derivatives of the two CTI isomers exhibited nearly indistinguishable reactivities ($t_{1/2}$ = 206 and 209 h), being ca. 5−6-fold more stable than the alkylation subunit of CC-1065 (*N*-Boc-MeCPI, $t_{1/2}$ = 37 h),[23] significantly more stable than *N*-Boc-CBI ($t_{1/2}$ = 133 h),[24] and measurably more stable than even *N*-Boc-DSA ($t_{1/2}$ = 177 h)[25] (Figure 5). This places *N*-Boc-MeCTI (**3**) and *N*-Boc-*iso*-MeCTI (**4**) among the most stable alkylation subunits explored to date that still exhibit a measurable reactivity at pH 3.

Cytotoxic Activity. The compounds displayed cytotoxic activity (L1210) consistent with their relative stabilities. Summarized in Figure 6 is the L1210 cytotoxic activity of the MeCTI and *iso*-MeCTI derivatives examined along with that of the key comparison compounds,[26] and a full table of comparison derivatives is provided in the Supporting Information (Tables S1−S5). The natural enantiomers of *N*-Boc-MeCTI (IC_{50} = 30 nM) and *N*-Boc-*iso*-MeCTI (IC_{50} = 25 nM) proved to be ca.

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Compound	L1210 IC_{50}, nM nat	unnat
3, *N*-Boc-MeCTI	30	600
4, *N*-Boc-*iso*-MeCTI	25	600
5, *N*-Boc-MeCPI	330	nd
6, *N*-Boc-CBI	80	900
7, *N*-Boc-DSA	6	60
25, MeCTI-TMI	0.005	2
46, *iso*-MeCTI-TMI	0.007	0.8
47, CBI-TMI	0.030	2
2, duocarmycin SA	0.008	0.10

Figure 6. Cytotoxic activity.[26]

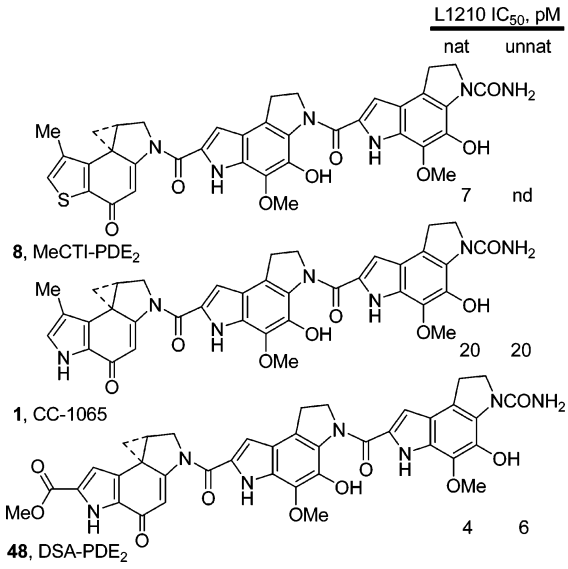


Figure 7. Cytotoxic activity of PDE_2 derivatives.[26]

10-fold more potent than the Boc derivative of the CC-1065 alkylation subunit (*N*-Boc-MeCPI, IC_{50} = 330 nM)[23] and 2−3-fold more potent than (+)-*N*-Boc-CBI (IC_{50} = 80 nM),[24] but 4−5-fold less potent than (+)-*N*-Boc-DSA (IC_{50} = 6 nM).[25] With the exception of the latter comparison with (+)-*N*-Boc-DSA, which exhibits an anomalously potent activity, the cytotoxic activity of **3** and **4** proved to be in line with expectations. The unnatural enantiomers of **3** and **4** (IC_{50} = 600 nM) exhibited potencies 20-fold less active than the natural enantiomers and in line with expectations based on the preceding observations with **5**−**7**.

Similarly, the natural enantiomers of TMI derivatives **25** and **46** were found to be exceptionally potent cytotoxic agents (IC_{50} = 5 and 7 pM, respectively), perhaps slightly more active than even (+)-duocarmycin SA (IC_{50} = 8−10 pM)[25] and notably more potent than (+)-CBI-TMI (IC_{50} = 30 pM)[21] (Figure 6).[26] Their unnatural enantiomers, *ent*-(−)-**25** and *ent*-(−)-**46**, proved to be 100−300-fold less active than the natural enantiomers,

(26) The L1210 cytotoxic activities for (+)-duocarmycin SA (8−10 pM) and (+)-CC-1065 (20 pM; 23−18 pM) have been tested as standards (>100 times) through the years and side-by-side with the samples disclosed herein. The small 2−3-fold differences are always observed, and the absolute potencies always fall in this narrow range indicated (±20%). We have developed highly refined, reproducible conditions for conducting such cytotoxic assays (±20%) that avoid the more variable results typically experienced with such assays. The IC_{50}'s reported in Figures 6−8 are the average values (±20%) obtained typically from multiple rounds of testing, and the number of times the samples disclosed herein were tested in triplicate alongside the standards are as follow: (+)-**3**, 4×; *ent*-(−)-**3**, 2×; (+)-**4**, 3×; *ent*-(−)-**4**, 3×; (+)-**25**, 7×; *ent*-(−)-**25**, 3×; (+)-**46**, 3×; *ent*-(−)-**46**, 1×; (+)-**8**, 4×; (+)-**30**, 4×; and *ent*-(−)-**30**, 3×.

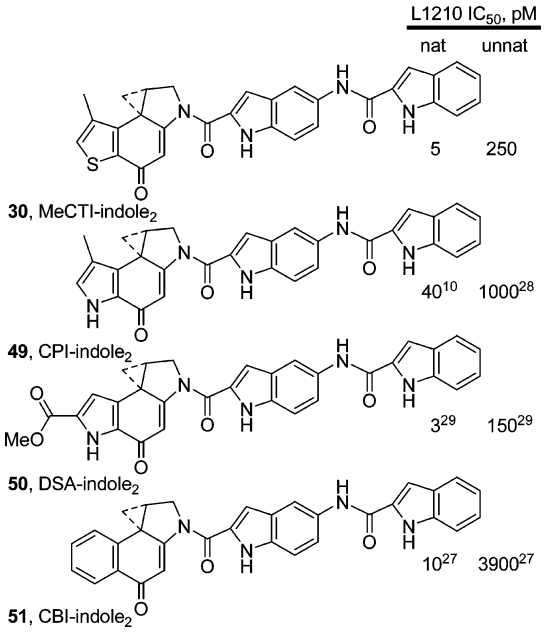


Figure 8. Cytotoxic activity of $indole_2$ derivatives.[26]

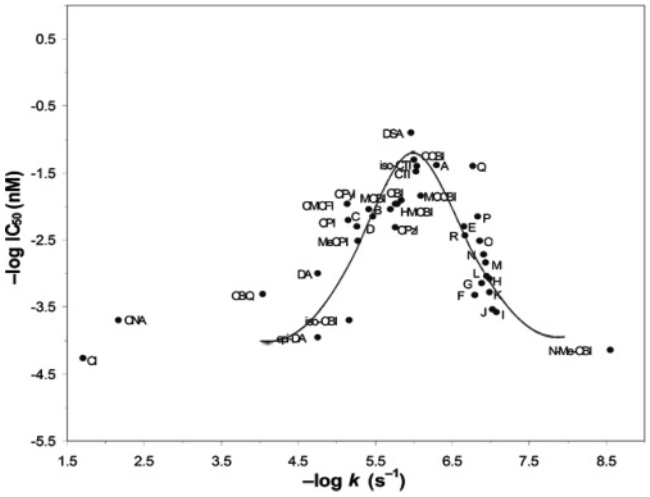


Figure 9. Relationship between reactivity (solvolysis *k*, pH 3) and cytotoxic potency (L1210), natural enantiomers.

exhibiting a distinction between enantiomeric activities that is greater than that observed with duocarmycin SA (10-fold)[25] or CBI−TMI (70-fold).[21]

Especially interesting is the comparison of MeCTI-PDE_2 (**8**) with CC-1065 (**1**) and the synthetic hybrid natural product DSA-PDE_2 (**48**)[5d] (Figure 7).[26] Consistent with expectations, the natural enantiomer of MeCTI-PDE_2 (IC_{50} = 7 pM) was found to be 3-fold more potent than CC-1065 (IC_{50} = 20 pM) and nearly equipotent with DSA-PDE_2 (**48**, IC_{50} = 4 pM). Thus, the deep-seated change of a single skeleton atom in CC-1065 (NH→S) served to increase its potency 3-fold, in line with expectations based on its 5−6-fold increased stability.

In a series of $indole_2$ analogues that are more manageable to work with because of their improved physical properties, including their solubility in conventional organic solvents, and which have exhibited efficacious in vivo antitumor activity,[10,27]

(27) (a) Boger, D. L.; Ishizaki, T.; Sakya, S. M.; Munk, S. A.; Kitos, P. A.; Jin, Q.; Besterman, J. M. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 115−120. (b) Boger, D. L.; Yun, W.; Han. N. *Bioorg. Med. Chem.* **1995**, *3*, 1429−1453.

the MeCTI derivative **30** exhibited a cytotoxic potency precisely in line with expectations based on its enhanced stability (Figure 8).[26] Thus, the natural enantiomer of MeCTI-indole$_2$ (**30**) was found to be ca. 8-fold more potent than MeCPI-indole$_2$ (**49**),[10,28] in line with its 5−6-fold increased stability, 2-fold more potent than CBI-indole$_2$,[27] consistent with its 1.5-fold increased stability, and ca. 2-fold less potent than DSA-indole$_2$ (1.2-fold difference in reactivity). Similarly, the unnatural enantiomer of MeCTI-indole$_2$ (IC_{50} = 250 pM) proved to be 50-fold less active than the natural enantiomer, but similarly 4-fold more potent than MeCPI-indole$_2$ (IC_{50} = 1000 pM) and essentially equipotent with DSA-indole$_2$ (IC_{50} = 150 pM).

The Parabolic Relationship. Among the most important of the features established to date with this class of compounds is the relationship between chemical stability and biological potency (cytotoxic activity). This parabolic relationship extends over a 10^6-fold range in reactivities (−log *k*, pH 3) and activities (−log IC_{50}, L1210), covering an extensive range of modified alkylation subunits. Consistent with the design features, *N*-Boc-MeCTI (pH 3 solvolysis $t_{1/2}$ = 206 h) and *N*-Boc-*iso*-MeCTI (pH 3 solvolysis $t_{1/2}$ = 209 h) both exhibit a reactivity comparable to that of *N*-Boc-DSA ($t_{1/2}$ = 177 h) that lies at the pinnacle of the parabolic relationship at a point that is 5−6 times more stable than *N*-Boc-MeCPI. In line with this enhanced stability, the Boc derivatives of both CTI isomers exhibit a cytotoxic activity roughly 10-fold more potent than that of *N*-Boc-MeCPI (IC_{50} = 330 nM) and 4−5-fold less potent than that of *N*-Boc-DSA (IC_{50} = 6 nM), placing them near expectations based on the parabolic relationship (Figure 9).[26] Importantly, and aside from the anomalously potent activity of *N*-Boc-DSA, this places both MeCTI derivatives among the most potent analogues examined to date, on par with the activity of *N*-Boc-CCBI, further defining the pinnacle of the parabolic relationship.

DNA Alkylation Selectivity and Efficiency. The DNA alkylation selectivity of the new analogues was examined within a 150 base-pair segment of DNA described previously (w794).[30] The alkylation site identification and the assessment of the relative selectivity among the available sites were obtained by thermally induced strand cleavage of the singly 5′-end-labeled duplex DNA after exposure to the compounds as detailed.[5−8] Since the DNA alkylation properties of members of each class of these agents have been established in preceding studies, we focused our analysis on a select set of the new analogues to simply confirm their DNA alkylation selectivity and relative efficiency. Most representative of this set are the TMI- and PDE-based analogues, constituting key analogues of duocarmycin SA and CC-1065, respectively.

Illustrated in Figure 10 is a representative comparison of the DNA alkylation selectivity of the TMI-based analogues which highlights the similarity as well as subtle distinctions in the compounds. As anticipated, the TMI-based analogues **25** and **46** exhibited a DNA alkylation selectivity identical to all such TMI derivatives, including duocarmycin SA itself. Within this segment of DNA, the natural and unnatural enantiomers alkylate

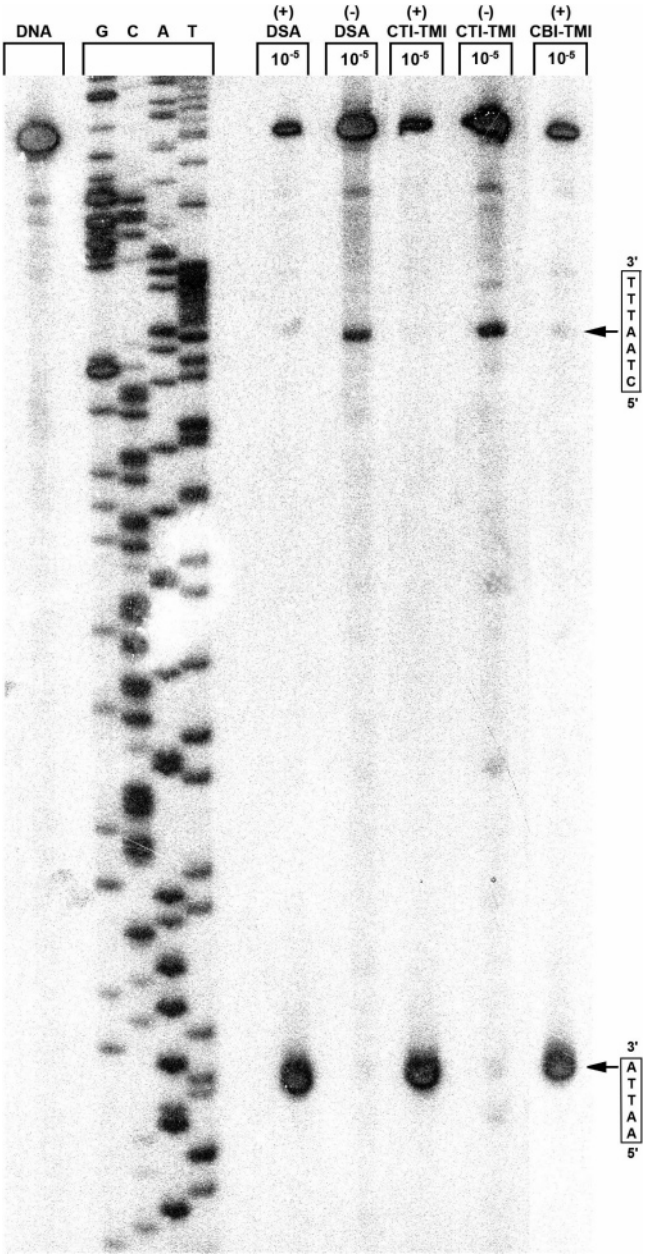


Figure 10. Thermally induced strand cleavage of w794 DNA (144 bp, nucleotide no. 5238-138) after DNA−agent incubation with duocarmycin SA, MeCTI-TMI, and CBI-TMI (20 °C, 24 h), removal of unbound agent by EtOH precipitation, and 30 min thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography. Lane 1, control DNA; lanes 2−5, Sanger G, C, A, and T sequencing standards; lanes 6 and 7, (+)-duocarmycin SA and *ent*-(−)-duocarmycin SA (1×10^{-5} M); lanes 8 and 9, (+)-MeCTI-TMI and *ent*-(−)-MeCTI-TMI (1×10^{-5} M); lane 10, (+)-CBI-TMI (1×10^{-5} M).

a single major site, and the only significant distinction detected was their relative efficiencies of DNA alkylation. In each case, the natural enantiomer alkylates DNA with a greater efficiency than the corresponding unnatural enantiomer. Throughout both enantiomeric series, duocarmycin SA, MeCTI-TMI, and *iso*-MeCTI-TMI (not shown) exhibited no distinction in their relative efficiencies of DNA alkylation, whereas CBI-TMI (CBI-TMI > MeCPI-TMI)[21] was less effective.

In a subtle contrast to this behavior, the PDE$_2$ derivatives illustrated in Figure 11 alkylated the same major sites in both enantiomeric series, with DSA-PDE$_2$ and MeCTI-PDE$_2$ each

(28) Aristoff, P. A.; Johnson, P. D.; Sun, D. *J. Med. Chem.* **1993**, *36*, 1956−1963.
(29) Boger, D. L.; Hertzog, D. L.; Bollinger, B.; Johnson, D. S.; Cai, H.; Goldberg, J.; Turnbull, P. *J. Am. Chem. Soc.* **1997**, *119*, 4977−4986.
(30) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661−2682.

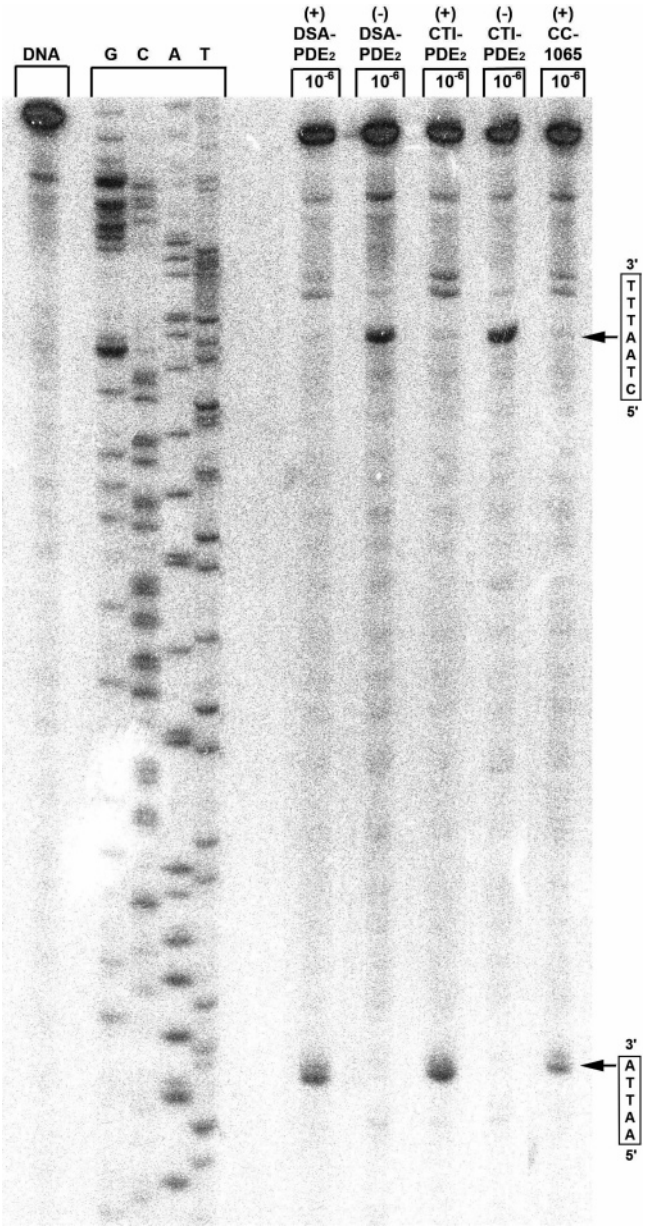
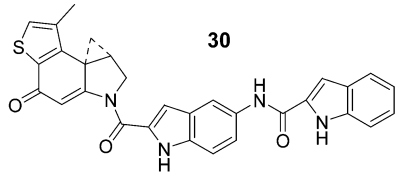


Figure 11. Thermally induced strand cleavage of w794 DNA (144 bp, nucleotide no. 5238-138) after DNA−agent incubation with DSA-PDE-PDE (23 °C, 24 h), MeCTI-PDE-PDE (23 °C, 24 h), and CC-1065 (23 °C, 24 h), removal of unbound agent by EtOH precipitation, and 30 min thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography. Lane 1, control DNA; lanes 2−5, Sanger G, C, A, and T sequencing standards; lane 6, (+)-DSA-PDE-PDE (1×10^{-6} M); lane 7, *ent*-(−)-DSA-PDE-PDE (1×10^{-6} M); lane 8, (+)-MeCTI-PDE-PDE (1×10^{-6} M); lane 9, *ent*-(−)-MeCTI-PDE-PDE (1×10^{-6} M); lane 10, (+)-CC-1065 (1×10^{-6} M).

exhibiting an efficiency that subtly exceeds that of (+)-CC-1065. Most significant in this series, and consistent with their relative cytotoxic potencies, the unnatural enantiomers now approach or match the DNA alkylation efficiencies of the natural enantiomers.

To date, the biological properties of members of this class of natural products have typically mirrored their relative efficiencies of DNA alkylation. As illustrated in the preceding section, the observations illustrated in Figure 10 and 11 mirror the cytotoxic activities observed within the series and even between enantiomeric pairs of such analogues.



Compd	Dose μg/kg[a]	MSP days[b]	T/C[c]	Surviving Mice[d]
none	0	16.7	100	0/6
30	10	>60	>360	4/6
30	30	>53	>320	3/6

[a]Dose (μg/kg wt. of animal) administered i.p. on days 1, 5, and 9. [b]MSP = Mean Survival Period (days). [c]T/C = Treated/Control (MSP) x 100. [d]No. of live animals after 80 days when terminated.

Figure 12. In vivo antitumor activity (L1210, i.p.).

In Vivo Antitumor Activity. The natural enantiomer of MeCTI-indole$_2$ (**30**) was examined for in vivo efficacy in a standard antitumor model enlisting L1210 murine leukemia implanted i.p. into DBA/2J mice. This model has been shown to respond well to related compounds[31] and is a system that collaborators through the years have used to assess an extensive series of (+)-CBI-indole$_2$ analogues.[27] Although not published, these latter studies provided the foundation on which we based our examination of **30**. Thus, (+)-**30** was examined with the dose range (10−100 μg/kg) and the dosing schedule (administered three times i.p. on days 1, 5, and 9) found suitable for related agents including (+)-CBI-indole$_2$[27] (Figure 12). Although the higher doses (100 and 60 μg/kg) were found to be toxic to the treated animals, efficacious antitumor activity was observed at the lower doses, producing 4/6 (at 10 μg/kg) and 3/6 (at 30 μg/kg) long-term survivors (>80 days) in the experiment. This efficacy is at least as good as and may exceed that observed with related drugs in this class in this tumor model and occurs at an optimal dose that is lower than that observed with (+)-CBI-indole$_2$ (30−60 μg/kg) or related MeCPI-based agents. Thus, the enhanced chemical stability of the alkylation subunit leads to a greater in vivo potency and a maintained or further enhanced efficacy.

Conclusions

The MeCTI alkylation subunit was designed on the basis of the fundamental relationship between reactivity and biological potency observed in this class of DNA alkylating agents. The pH 3 solvolysis reactivity of MeCPI ($t_{1/2}$ = 37 h) found in the natural product CC-1065 is greater than that of the optimal naturally occurring alkylation subunit DSA ($t_{1/2}$ = 177 h). Computational studies (AM1, MNDO) predicted that a single atom change in the MeCPI alkylation subunit (S, MeCTI vs NH, MeCPI) would impart an increased stability that was expected to increase the biological potency of the alkylation subunit, approaching that of DSA.

The alkylation subunit MeCTI and its isomer *iso*-MeCTI were prepared and found to be slightly more stable than DSA (pH 3 solvolysis: MeCTI, $t_{1/2}$ = 206 h; *iso*-MeCTI, $t_{1/2}$ = 209 h), placing it at the point representing an optimal balance between chemical reactivity and stability on the basis of the established parabolic relationship. Consistent with their increased relative

(31) Li, L. H.; Kelly, R. C.; Warpehoski, M. A.; McGovren, J. P.; Gebhard, I.; DeKoning, T. F. *Invest. New Drugs* **1991**, *9*, 137−150.

stabilities, the natural enantiomers of *N*-Boc-MeCTI (IC_{50} = 20 nM) and *N*-Boc-*iso*-MeCTI (IC_{50} = 25 nM) proved to be 10-fold more potent than the CC-1065 alkylation subunit (+)-*N*-Boc-MeCPI (IC_{50} = 330 nM) and 2−3-fold more potent than (+)-*N*-Boc-MeCBI (IC_{50} = 80 nM), but 4−5-fold less potent than the anomalously potent (+)-*N*-Boc-DSA. The unnatural enantiomers of *N*-Boc-MeCTI and *N*-Boc-*iso*-MeCTI (IC_{50} = 600 nM) each exhibited potencies 20-fold less active than the natural enantiomers, consistent with expectations for this class of agents.

Key natural product analogues evaluated included (+)-MeCTI-TMI (5 pM) and its isomer (+)-*iso*-MeCTI-TMI (7 pM), which matched or slightly exceeded the potency of duocarmycin SA (DSA-TMI, 8−10 pM), a natural product incorporating the optimal naturally occurring alkylation subunit. Also prepared and evaluated was a key MeCTI analogue of CC-1065. The analogue **8** (MeCTI-PDE_2) constitutes the substitution of a single atom in the alkylation subunit of CC-1065, providing a compound that exhibited a DNA alkylation profile identical to that of CC-1065 (IC_{50} = 20 pM) but is 3-fold more potent (IC_{50} = 7 pM, natural enantiomer) than the natural product. Additionally, consistent with expectations based on their similar inherent reactivities, MeCTI-PDE_2 was also comparable in potency with DSA-PDE_2 (IC_{50} = 4 pM). Extending these observations, the incorporation of MeCTI into an $indole_2$ derivative and its comparison with an important series of alkylation subunit derivatives further verified the direct impact of the relative reactivities on the cytotoxic potency. These observations and a subsequent demonstration of its in vivo antitumor potency and efficacy places (+)-MeCTI-$indole_2$ among the most interesting such derivatives disclosed to date.

Most importantly, these observations are derived from and in line with expectations based on the extensive examination of a series of alkylation subunit analogues that defined a direct relationship between chemical stability and biological potency characteristic of this class of antitumor agents. Presumably, the underlying parabolic relationship reflects the fact that the compounds must be sufficiently stable to reach their biological target yet reactive enough to effectively alkylate DNA once they do.[26]

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Supporting Information Available: Full experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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