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Total Synthesis and Evaluation of *iso*-Duocarmycin SA and *iso*-Yatakemycin

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Abstract: The total synthesis and evaluation of *iso*-duocarmycin SA (**5**) and *iso*-yatakemycin (**6**), representing key analogues of the corresponding natural products incorporating an isomeric alkylation subunit, are detailed. This pyrrole isomer of the natural alkylation subunit displayed an enhanced reaction regioselectivity and a 2-fold diminished stability. Although still exceptionally potent, the *iso*-duocarmycin SA derivatives and natural product analogues exhibited a corresponding approximate 3−5-fold reduction in cytotoxic activity [L1210 IC_{50} for (+)-*iso*-duocarmycin SA = 50 pM and for (+)-*iso*-yatakemycin = 15 pM] consistent with their placement on a parabolic relationship correlating activity with reactivity. The DNA alkylation selectivity of the resulting key natural product analogues was unaltered by the structure modification in spite of the minor-groove presentation of a potential H-bond donor. Additionally, a unique ortho-spirocyclization with such derivatives was explored via the preparation, characterization, and evaluation of **34** that is incapable of the more conventional para-spirocyclization. Although **34** proved sufficiently stable for isolation and characterization, it displayed little stability in protic solvents ($t_{1/2}$ = 0.19 h at pH 3, $t_{1/2}$ = 0.20 h at pH 7), a pH-independent (H^+ independent) solvolysis rate profile at pH 3/4−7, and a much reduced cytotoxic potency, but a DNA alkylation selectivity and efficiency comparable to those of duocarmycin SA and *iso*-duocarmycin SA. The implications of these observations on the source of the DNA alkylation selectivity and catalysis for this class of natural products are discussed.

Introduction

Duocarmycin SA (**1**, DSA)[1] and yatakemycin (**3**)[2] are the most potent (L1210 IC_{50} = 10 pM and 3−6 pM, respectively) members of a class of antitumor agents that also includes CC-1065 (**4**)[3] and duocarmycin A (**2**, Figure 1).[4] Each derives its properties from a characteristic sequence-selective alkylation of duplex DNA,[5−9] in which a stereoelectronically controlled adenine N3 addition to the least substituted carbon of the activated cyclopropane occurs within selected minor-groove AT-rich sites.

Extensive efforts using systematic alterations and simplifications in the alkylation subunits have defined key and subtle structural features that contribute to their properties. Most notable of these structural features are the stereoelectronic alignment of the cyclopropane that controls its reaction regioselectivity[10] and the cross-conjugated vinylogous amide that stabilizes the reactive cyclopropane.[11] Not only have these studies provided unique insights into the relationships between structure, reactivity, and biological properties[9] and the DNA

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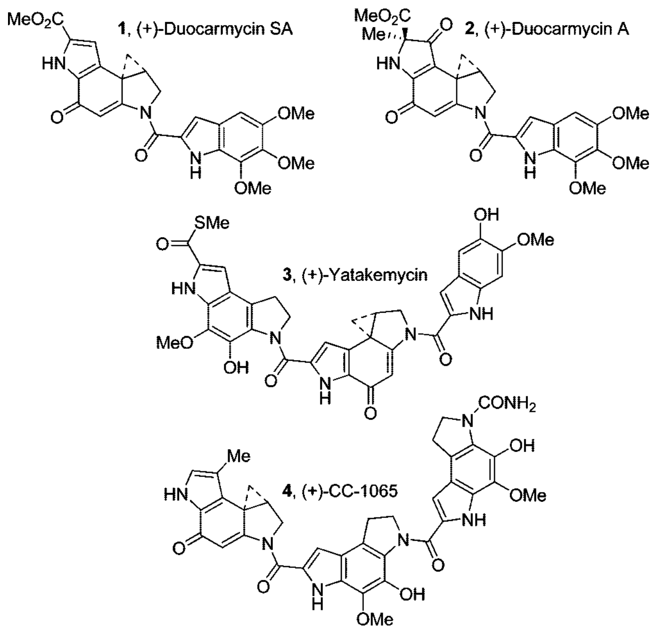

Figure 1. Natural products.

alkylation selectivity,[12] but they have also proven key to revealing the source of catalysis[9d,13−15] for the DNA alkylation reaction and have defined a fundamental parabolic relationship between reactivity and biological potency.[16]

Herein, we report the synthesis, characterization, and evaluation of 6-methoxycarbonyl-1,2,8,8a-tetrahydrocyclopropa[*c*]pyrrolo[2,3-*g*]indol-4-one (*iso*-DSA), an isomer of the duocarmycin SA and yatakemycin alkylation subunit in which the fused pyrrole is inverted, and its incorporation into *iso*-duocarmycin SA (**5**) and *iso*-yatakemycin (**6**), key analogues of the natural products (Figure 2).

Examination of the *iso*-DSA alkylation subunit was expected to address three important questions. First, would the introduction of a fixed H-bond donor on the minor-groove-interacting face of the molecule affect the DNA alkylation selectivity or efficiency? It was our expectation that this would have a minimal impact on the natural enantiomers, but it may have a much more significant impact on the unnatural enantiomers where the distance to the adjacent 5′-base on the alkylated strand is closest

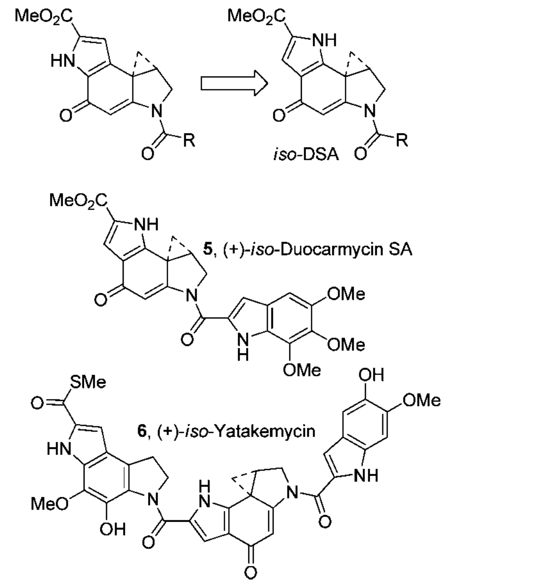

Figure 2. *iso*-Duocarmycin SA (**5**) and *iso*-yatakemycin (**6**).

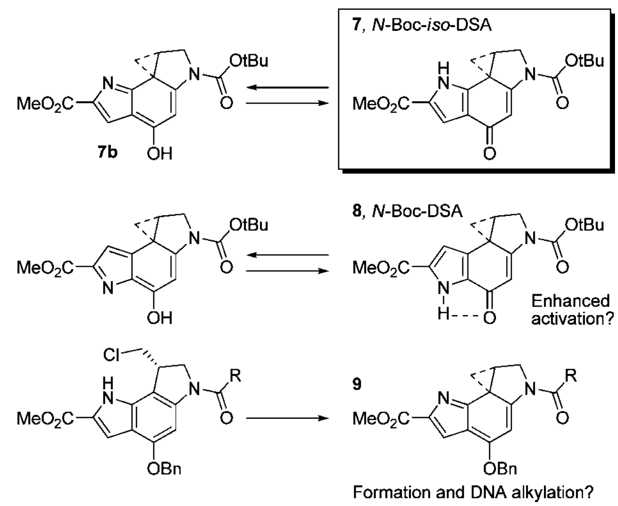

Figure 3. Alternate reactive forms of *iso*-duocarmycin SA.

to this center.[17] Second, it was not clear what impact this seemingly simple change would have on the reactivity of the molecule. That is, does it now possess a significant competing vinylogous amide structure that will serve to destabilize the alkylation subunit, activating it for nucleophilic attack, or will it further stabilize the structure by removing (moving) an internal H-bond that may serve to activate DSA for nucleophilic attack (Figure 3)? Although contrary to our own expectations, the latter has been suggested by LaBarbera and Skibo[18] to be a significant contributing factor for duocarmycin SA. Thus, the examination of **7** was viewed as key to understanding how structure impacts reactivity. Finally, we were interested in establishing what impact this structural change would have on the regioselectivity of the cyclopropane nucleophilic addition now that the acidic indole NH is proximal to the reacting center and even whether the cyclohexadienone structure would be required for spirocyclization and DNA alkylation. It is possible that an ortho-spirocyclization involving the indole NH may provide compa-

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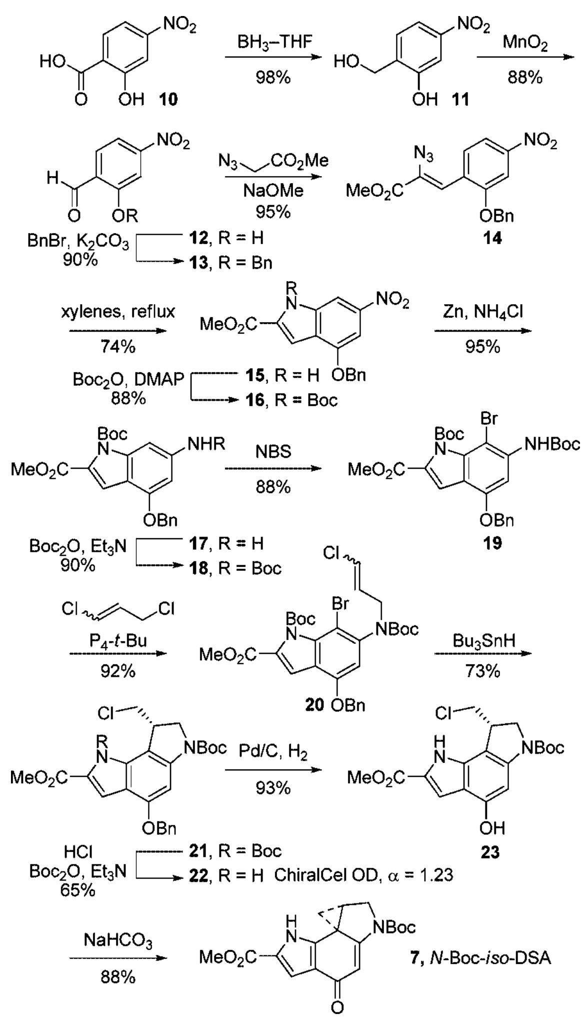
Scheme 1



rable, albeit more reactive, intermediates (e.g., **9**) capable of alkylating DNA (Figure 3).

Results and Discussion

Synthesis of *N*-Boc-*iso*-Duocarmycin SA. Synthesis[19] of the modified alkylation subunit began with reduction of 2-hydroxy-4-nitrobenzoic acid with BH_3−tetrahydrofuran (THF) to afford the corresponding benzyl alcohol **11** (THF, 0−23 °C, 18 h, 98%), which was subsequently oxidized to aldehyde **12** (7 equiv of MnO_2, EtOAc, 80 °C, 2.5 h, 88%) (Scheme 1). Protection of the free phenol as a benzyl ether afforded **13** [2 equiv of BnBr, 1.5 equiv of K_2CO_3, *DMF N*,*N*-Dimethylformamide (DMF), 0−23 °C, 18 h, 90%] that was converted to the styryl azide **14** by condensation with methyl azidoacetate (4 equiv, 4 equiv of Na, MeOH, −15 to 0 °C, 24 h, 95%). Formation of indole **15** was achieved by a thermally induced Hemetsberger cyclization (xylenes, 145 °C, 3 h, 74%) to provide **15**, which was protected as its N-Boc derivative **16** (1.5 equiv of Boc_2O, 0.1 equiv of DMAP, THF, 23 °C, 2 h, 89%). Reduction of the aryl nitro group of **16** to the corresponding aniline **17** was carried out smoothly with zinc nanopowder and ammonium chloride[20] (10 equiv of Zn, 15 equiv of NH_4Cl, 5:1 acetone/H_2O, 23 °C, 5 min, 95%) in excellent yields. Alternative attempts to reduce **16** with tin(II) chloride provided lower yields and generated byproducts that proved difficult to separate from the desired aniline. The zinc nanopowder reduction, which proceeds at remarkable rates and at nearly neutral pH, leaves the labile indole N-Boc intact in a reaction for which the workup simply entails filtration removal of the zinc nanopowder. Following N-Boc protection of the aniline **17** (1.5 equiv of Boc_2O, 1.2 equiv of Et_3N, THF, 23 °C, 24 h, 90%), **18** underwent a regioselective bromination (1.2 equiv of NBS, THF, 23 °C, 4 h, 88%) to afford **19**. N-Alkylation of the carbamate with 1,3-dichloropropene enlisting phosphazene base P_4-*t*-Bu {1-*tert*-butyl-4,4,4-tris(dimethylamino)-2,2-bis[tris(dimethylamino)phosphoranylidenamino]-$2\Lambda^5,4\Lambda^5$-catenadi(phosphazene), 1.5 equiv of 1,3-dichloropropene, 1.2 equiv of P_4-*t*-Bu, benzene, 23 °C, 3 h, 92%} afforded the free radical cyclization precursor **20** as a mixture of geometrical isomers in good yield. The use of the exceptionally strong, nonnucleophilic P_4-*t*-Bu base as its commercially available solution in hexanes provided exceptional yields of the desired alkylation product without the competitive hydroxide-promoted ester hydrolysis often observed with the more common use of NaH. The final ring of the alkylation subunit was constructed via a Bu_3SnH-mediated 5-*exo*-*trig* aryl radical−alkene cyclization[21] (1.2 equiv of Bu_3SnH, 0.3 equiv of AIBN, benzene, 80 °C, 3.5 h, 73%). A thorough removal of all residual tin by repeated chromatography and trituration with hexanes improved the conversions in the subsequent Boc deprotection. Additionally at this stage, chiral-phase chromatography was not successful at separating the enantiomers of **21**. To circumvent this, both Boc groups were removed (4 N HCl/EtOAc, 23 °C, 6 h), and the indoline nitrogen was selectively reprotected (1.5 equiv of Boc_2O, 2 equiv of Et_3N, THF, 25 °C, 18 h, 65%). Separation of the enantiomers of **22** was now possible and was conducted effectively on a Chiralcel OD semipreparative HPLC column (2 × 25 cm, 2% *i*-PrOH/hexane, 7 mL/min, $\alpha = 1.23$).[17] O-Debenzylation of **22** (0.2 wt equiv of 10% Pd/C, 1 atm H_2, THF−MeOH, 23 °C, 1 h, 93%) followed by treatment of the resulting phenol **23** with mild base afforded *N*-Boc-*iso*-DSA (**7**) in superb conversion (saturated aqueous $NaHCO_3$, DMF, 23 °C, 1 h, 88%) (Scheme 1, natural enantiomer shown).

Synthesis of *iso*-Duocarmycin SA. Duocarmycin SA (**1**) represents the most stable and most potent member of the natural products that contain the two-subunit structure. Consequently, the isomeric alkylation subunit was incorporated into the key analogue *iso*-duocarmycin SA (**5**) as shown in Scheme 2. Thus, Boc deprotection of **22** (4 N HCl/EtOAc, 23 °C, 45 min) followed by direct coupling of the resulting indoline hydrochloride salt with 5,6,7-trimethoxyindole-2-carboxylic acid[22] (**24**, 1.1 equiv, 1.0 equiv of $NaHCO_3$, 4 equiv of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDCI), DMF, 23 °C, 30 min, 82%) afforded **25** (natural enantiomer shown). The benzyl group was removed (0.2 wt equiv of 10% Pd/C, 1 atm of H_2, THF−MeOH, 23 °C, 3 h, 88%), affording **26** that was smoothly spirocyclized under mild conditions to provide *iso*-duocarmycin SA (**5**, saturated aqueous $NaHCO_3$, DMF, 23 °C, 2 h, 80%).

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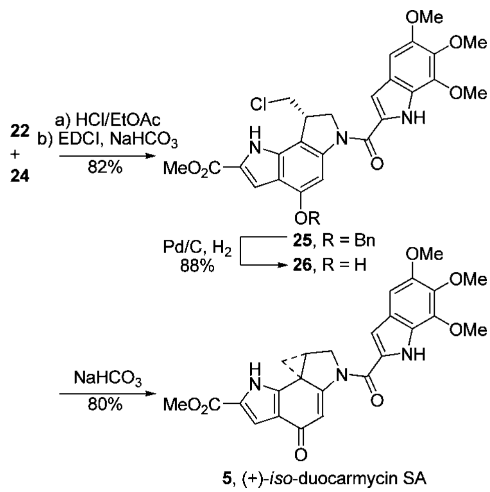
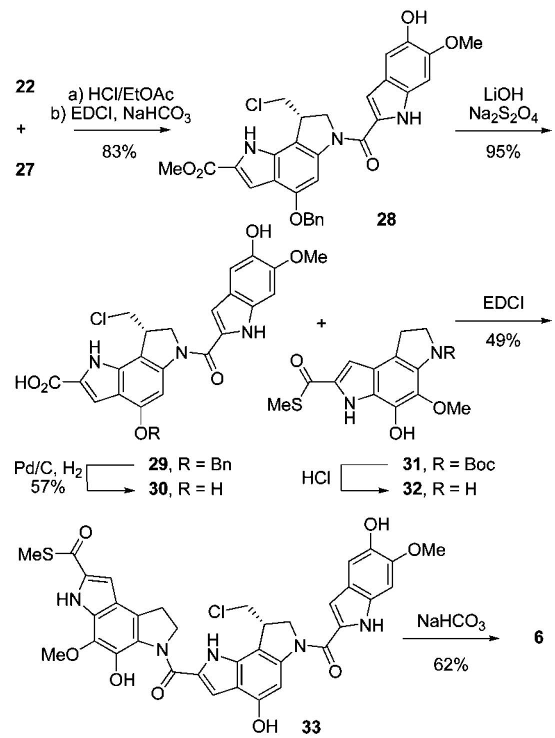
Scheme 2



Scheme 3



Synthesis of *iso*-Yatakemycin. A second key natural product analogue that we elected to examine was *iso*-yatakemycin (**6**) since yatakemycin itself represents the most exciting of the natural products isolated to date. Central to its unique sandwiched three-subunit structure, yatakemycin contains the same alkylation subunit found in duocarmycin SA that we now replaced with the isomeric alkylation subunit. The simplified right-hand subunit of the natural product as well as the left-hand PDE thiomethyl ester found in yatakemycin were incorporated unchanged into *iso*-yatakemycin (**6**). Notably, this left-hand subunit also constitutes the central and right-hand subunits found in CC-1065 but is capped with a thiomethyl ester unique to the yatakemycin structure. The most remarkable features of this "sandwiched" three-subunit arrangement are the enhanced rates and efficiencies of DNA alkylation, the nearly identical DNA alkylation selectivities of the two enantiomers, and their enhanced cytotoxic potency, including those of the unnatural enantiomers.[23,24] Synthesis of *iso*-yatakemycin diverged from that of *iso*-duocarmycin SA following chromatographic separation of the enantiomers of **22**. Treatment of **22** with 4 N HCl/EtOAc, followed by immediate coupling of the resulting indoline hydrochloride salt with 5-hydroxy-6-methoxyindole-2-carboxylic acid (**27**, 1.5 equiv, 1.1 equiv of $NaHCO_3$, 4 equiv of EDCI, DMF, 23 °C, 2.5 h, 83%) afforded **28** (only natural enantiomer shown). Hydrolysis of the methyl ester of **28** initially posed a challenge, as standard LiOH hydrolysis conditions afforded product yields of only 20−30%. Previous studies with similar substrates[25] suggested that oxidation contributes to the poor yields, and that addition of a mild reducing agent such as sodium dithionite as well as rigorous degassing of the solvents would improve the conversion. This simple modification was successful (3 equiv of $Na_2S_2O_4$, 10 equiv of LiOH−H_2O, 3:2:1 THF−MeOH−H_2O, 23 °C, 6.5 h, 95%) and yielded carboxylic acid **29** in superb yield. Removal of the benzyl ether (0.2 wt equiv of 10% Pd/C, 1 atm H_2, THF−MeOH, 23 °C, 6 h, 57%) yielded the polar intermediate **30**. Treatment of thioester **31**[23] with 4 N HCl/EtOAc (23 °C, 45 min) followed by coupling of the free amine with carboxylic acid **30** (4 equiv of EDCI, DMF, 23 °C, 45 min, 49%) afforded **33**. Notably, purification of **33** must be conducted quickly, as slow or repeated silica gel chromatography was found to promote the spirocyclization to **6**. Liquid chromatography/mass spectrometry (LC/MS) analysis of the reaction mixture following chromatography on silica gel at times revealed a nearly 1:1 mixture of seco:spiro products, indicating the ease with which the final ring closure proceeds. Compound **33** was carried forward into the final spirocyclization reaction (saturated aqueous $NaHCO_3$, DMF, 23 °C, 1 h, 62%) to provide *iso*-yatakemycin (**6**) (Scheme 3).

Synthesis of *O*-Benzyl-*iso*-duocarmycin SA. In order to further probe the activation capabilities of the isomeric alkylation subunit,[26,27] deliberate efforts to promote a potential ortho-spirocyclization were undertaken by enlisting protected substrates that preclude the conventional para-spirocyclization. Thus, benzyl ether **25** was treated with phosphazene base P_4-*t*-Bu (2.2 equiv, DMF, 23 °C, 6.5 h, 27%) to afford the ortho-spirocyclized product **34** (Scheme 4). Upon disappearance of starting material by LC/MS, the products of the reaction were subjected to chromatography (care was taken to avoid the use of chlorinated and nucleophilic solvents) to provide the sensitive product **34**. Remarkably, this product proved surprisingly stable to chromatography and extended times in solution. Nonetheless, this material was much more reactive and much less stable than *iso*-duocarmycin SA, and the yield of purified **34** reflects this intrinsic reactivity. The use of alternative and more conventional strong bases including NaH and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (DMF) to promote the ortho-spirocyclization provided little to no product **34** by thin-layer chromatography (TLC) or LC/MS. Although CH_2Cl_2 (versus DMF) was unsuccessful in supporting the ortho-

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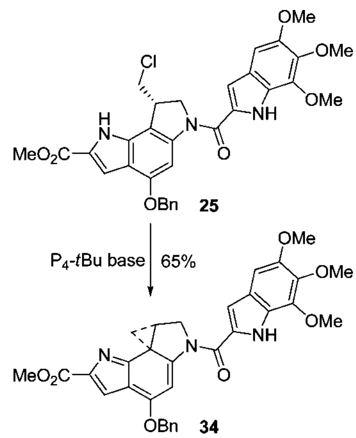
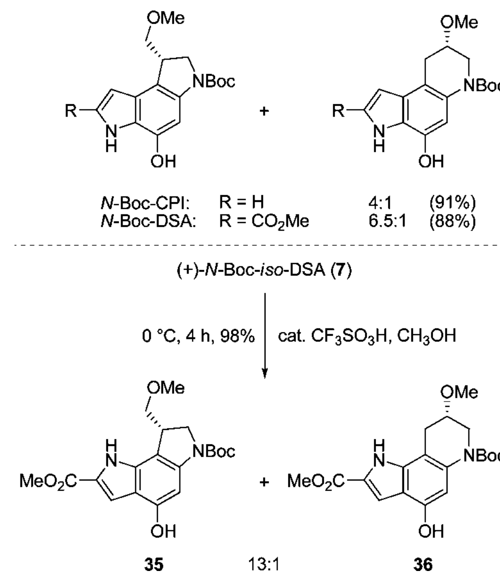
Scheme 4



Scheme 5



spirocyclization reaction of **25**, the use of MeCN provided an even more effective and manageable ortho-spirocyclization. With an additional increase in the amount of base (3.3 equiv) and with an increasing experience with the isolation and chromatographic purification of **34**, the ortho-spirocyclization was optimized (3.3 equiv of P_4-*t*-Bu, CH_3CN, 23 °C, 3 h) to the point of providing **34** in 65% yield in this solvent. Although not as extensively examined, analogous initial efforts to promote ortho-spirocyclization of the *N*-Boc derivative **22** were not successful at providing the corresponding product as a stable isolatable intermediate.

The ^{1}H NMR characterization of **34** displayed the expected three well-defined cyclopropane CH signals at δ 2.37 (1H, dd, $J = 7.2$, 7.8 Hz), 1.99 (1H, ddd, $J = 3.6$, 6.0, 8.4 Hz), and 0.79 (1H, t, $J = 6.0$ Hz) and the characteristic diastereotopic signals for C2-H2 exhibiting a large geminal coupling constant (δ 4.00, 1H, d, $J = 12.0$ Hz, and δ 3.47, 1H, dd, $J = 3.6$, 12.0 Hz) analogous to those observed with duocarmycin SA itself. The most diagnostic ^{1}H NMR signals proved to be disappearance of the indole NH (δ 11.08, 1H, br s in **25**) and a marked shift in the C4-H from δ 7.91 (1H, br s) in the precursor **25** to δ 4.62 (1H, s) in **34**. This latter remarkable C4-H shift is indicative of an aromatic CH and its conversion to a signal behaving as the β-CH of an isolated electron-rich olefin (enamide). Similarly, the UV spectrum (MeCN) of **34** [$\lambda_{max} = 304$ nm (ε 21 800)] proved easily distinguishable from that of **1** [$\lambda_{max} = 355$ nm (ε 27 800), 313 nm (ε 17 100)] or **5** [$\lambda_{max} = 342$ nm (ε 17 800), 267 nm (ε 15 600)] exhibiting its longest wavelength absorption at a significantly shorter wavelength. Finally and interestingly, optically active **34** exhibited a large rotation value analogous to those characteristic of this family including **1** and **5**, but it is of an opposite sign [(−)-**34** is the natural enantiomer] and the series does not exhibit the characteristic sign inversion going from **25** to **34**.

Solvolyis Reactivity and Regioselectivity. Important diagnostic features of the alkylation subunits of this class of compounds are their relative solvolytic reactivity and the site of cyclopropane cleavage. Past studies have revealed that these agents participate in a characteristic acid-catalyzed, stereoelectronically controlled ring-opening reaction with predominant nucleophilic addition to the least substituted cyclopropane carbon. However, both the reactivity of the alkylation subunit and the stereoelectronic alignment of the cyclopropane have been shown to influence the intrinsic regioselectivity of such additions.[10,11] Exemplifying such trends, the addition regioselectivity is much greater with *N*-Boc-CBI (>20:1)[28] than *N*-Boc-CPI (4:1) because of the cyclopropane stereoelectronic alignment, whereas that of *N*-Boc-DSA (6.5:1)[29] exceeds that of *N*-Boc-CPI (4:1) because of its reduced reactivity. Acid-catalyzed addition of methanol to *N*-Boc-*iso*-DSA (**7**) under identical conditions (cat. CF_3SO_3H, CH_3OH, 0 °C, 4 h, 98%) provided a 93:7 (13:1) mixture of two products (Scheme 5).

The major product is derived from attack at the least-substituted cyclopropane carbon, analogous to the regioselectivity observed with the natural alkylation subunits, and the minor product was identified as the ring-expansion product generated by addition to the more-substituted carbon. Chiral HPLC analysis (ChiralCel AD column, 15% *i*-PrOH/hexane) of **36** derived from enantiomerically pure **7** alongside both enantiomers of the ring-expansion product derived from racemic **7** indicate a clean S_N2 ring opening of **7** with no loss of stereochemical integrity at the reacting center (Figure S1 in Supporting Information). Thus, *N*-Boc-*iso*-DSA (**7**) exhibits an enhanced intrinsic reaction regioselectivity (13:1 versus 6.5:1) relative to *N*-Boc-DSA (**8**) despite the fact that it is 2-fold more reactive (see below). Although it is possible that this results from an altered and enhanced stereoelectronic alignment of the reacting cyclopropane, it is also conceivable that the in-plane placement of the proximal electronegative indole NH of **7**, which is absent with **8**, disfavors nucleophilic attack at the closer, more substituted cyclopropane carbon.

The relative stability of each alkylation subunit as measured by acid-catalyzed solvolysis has been shown to correlate directly with the biological potency (cytotoxic activity) of the com-

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pH	k (s^{-1})	$t_{1/2}$ (min)
2	1.41×10^{-2}	0.82
3	9.87×10^{-4}	11.7
4	4.72×10^{-4}	24.5
5	4.07×10^{-4}	28.4
6	3.93×10^{-4}	29.4
7	3.45×10^{-4}	33.5

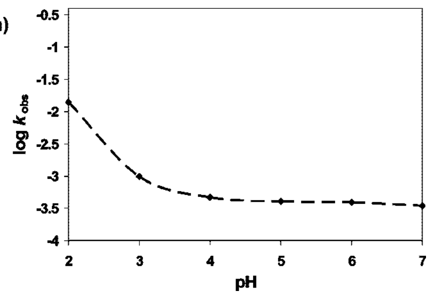

Figure 4. Rates of solvolysis (k), half-life ($t_{1/2}$), and pH−rate profile for solvolysis of **34** at pH 2−7.

pounds and to be significantly impacted by subtle structural features.[16] Solvolysis of *N*-Boc-*iso*-DSA at pH 3.0 [50% MeOH−buffer, buffer = 4:1:20 (v/v/v) 0.1 M citric acid/0.2 M Na_2HPO_4/H_2O] was monitored by UV (Figure S2 in Supporting Information) via disappearance of the long-wavelength absorption at 295 nm of the *iso*-DSA chromophore and appearance of a shorter-wavelength absorption at 265 nm attributable to the solvolysis product. The solvolysis half-life of *N*-Boc-*iso*-DSA ($k = 2.2 \times 10^{-6}$ s^{-1}, $t_{1/2} = 89$ h) was found to be 2-fold shorter than that of *N*-Boc-DSA ($t_{1/2} = 177$ h).[30] This would indicate that there is little to no contribution made by the second vinylogous amide embedded in the pyrrole structure toward the stability of the alkylation subunit. In fact, the opposite appears to be true. Interestingly and indirectly, this slightly enhanced reactivity of *N*-Boc-*iso*-DSA relative to *N*-Boc-DSA itself suggests that the putative internal H-bond of *N*-Boc-DSA (see Figure 3), which is absent in this isomer, is not contributing to activation or catalysis of a nucleophilic addition as suggested by LaBarbera and Skibo.[18]

In contrast, but consistent with experimental observations made during its preparation, the ortho-spirocyclized isomer **34** (*O*-benzyl-*iso*-duocarmycin SA) proved to be 470-fold more reactive than **7**, exhibiting a short but measurable solvolysis half-life at pH 3 ($k = 1.00 \times 10^{-3}$ s^{-1}, $t_{1/2} = 0.19$ h). Additionally, and unlike *N*-Boc-*iso*-DSA, for which no measurable solvolysis is observed at pH 7, **34** also exhibited rapid solvolysis at pH 7 (50% MeOH−H_2O, $k = 9.77 \times 10^{-4}$ s^{-1}, $t_{1/2} = 0.20$ h). Since **34** exhibited little difference in intrinsic reactivity at pH 3 versus pH 7, albeit under conditions that enlist different solvent and buffer conditions, we established its full pH−rate profile conducted under more comparable conditions with a universal buffer (boric acid/citric acid/Na_3PO_4).[31] As the initial studies suggested, the rate of solvolysis of **34** proved independent of pH within the range of pH 3/4−7, indicating that it constitutes an uncatalyzed (versus acid-catalyzed) reaction above pH 3−4 (Figure 4). Only in the range of pH 2−3 did **34** exhibit a characteristic linear dependence on pH indicating its acid-catalyzed solvolysis below ca. pH 3.

Importantly, the intrinsic reactivity of **34** indicates that while ortho-spirocyclization of *iso*-DSA represents an alternative mode of activation,[26,27] it is less productive than the more conventional para-spirocyclization.

Cytotoxic Activity. Results of the in vitro cytotoxic evaluation of *N*-Boc-*iso*-DSA, *iso*-duocarmycin SA, and *iso*-yatakemycin are summarized in Table 1 along with the results of the comparison duocarmycin SA derivatives. Both the natural and unnatural enantiomers of *iso*-duocarmycin SA were found to be 5-fold less potent than their duocarmycin SA counterparts. This closely follows the trend observed in the comparison of their relative solvolytic reactivities (2-fold less stable). As expected, the natural and unnatural enantiomers of *iso*-duocarmycin SA exhibit a 10-fold difference in potency, identical to the difference seen with duocarmycin SA itself.

Table 1. In Vitro Cytotoxic Activity, L1210

compound	IC_{50} (pM)
(+)-**8**, (+)-*N*-Boc-DSA	6000
(−)-**8**, *ent*-(−)-*N*-Boc-DSA	60 000
(+)-**7**, (+)-*N*-Boc-*iso*-DSA	33 000
(−)-**7**, *ent*-(−)-*N*-Boc-*iso*-DSA	330 000
(+)-**1**, (+)-duocarmycin SA	10
(−)-**1**, *ent*-(−)-duocarmycin SA	100
(+)-**5**, (+)-*iso*-duocarmycin SA	50
(−)-**5**, *ent*-(−)-*iso*-duocarmycin SA	550
(+)-**3**, (+)-yatakemycin	6
(−)-**3**, *ent*-(−)-yatakemycin	6
(+)-**6**, (+)-*iso*-yatakemycin	15
(−)-**6**, *ent*-(−)-*iso*-yatakemycin	30
(−)-**34** (natural enantiomer)	6000
(+)-**34** (unnatural enantiomer)	7000

The simple alkylation subunit *N*-Boc-*iso*-DSA (**7**) also exhibited an analogous decreased cytotoxic potency compared to *N*-Boc-DSA. Thus, a 5-fold difference in potency between the two series was observed, and the natural enantiomer was 10-fold more potent than the unnatural enantiomer, similar to the trend observed with *iso*-duocarmycin SA and *N*-Boc-DSA. Moreover, when *N*-Boc-*iso*-DSA is compared to the full set of preceding alkylation subunits by placing it on the parabolic plot, its relative cytotoxic activity closely approaches that expected of a derivative exhibiting its relative reactivity (Figure 5).

Satisfyingly, the natural enantiomer of *iso*-yatakemycin (**6**) also exhibited a cytotoxic potency ($IC_{50} = 15$ pM) in line with its 2-fold greater reactivity than yatakemycin itself ($IC_{50} = 6$ pM). Unlike the natural product and a series of yatakemycin analogues containing the DSA alkylation subunit, where the two enantiomers display indistinguishable cytotoxic activity,[23,24] the unnatural enantiomer of *iso*-yatakemycin proved ca. 2-fold less active than the natural enantiomer (30 versus 15 pM) and ca. 5-fold less active than either enantiomer of yatakemycin. It is tempting to suggest that this slightly less effective relative behavior of the unnatural enantiomer with **6** may result from the minor-groove presentation of a H-bond donor for which the unnatural enantiomer is uniquely sensitive.[17] However, its activity still closely follows expectations (2-fold more reactive and 5-fold less potent), and the differences are so small that the effect, if operative, must be minor.

Finally, the natural enantiomer of *O*-benzyl-*iso*-duocarmycin SA (**34**), which represents the reactive ortho-spirocyclization isomer, proved to be 120-fold less active than *iso*-duocarmycin SA (L1210 $IC_{50} = 6$ nM versus 50 pM) and 600-fold less active than duocarmycin SA itself, consistent with its relative reactivity (470-fold more reactive than *N*-Boc-*iso*-DSA). Interestingly, although it was not probed extensively, the unnatural enantiomer of **34** ($IC_{50} = 7$ nM) was found to approach the activity of the natural enantiomer. It is tempting to suggest that since the activation features responsible for DNA alkylation catalysis that lead to the typical characteristic distinctions are not operative for **34**, it is not surprising that the two enantiomers of **34** display comparable biological properties.

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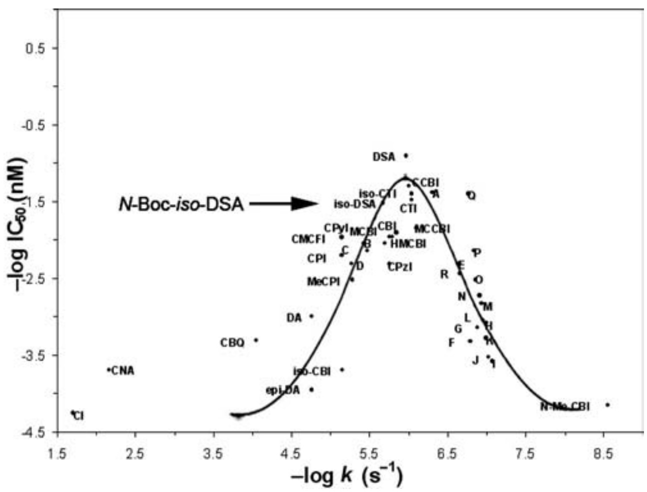

Figure 5. Parabolic relationship between rate of solvolysis and cytotoxic activity of the alkylation subunits.

DNA Alkylation Selectivity and Efficiency. The DNA alkylation properties of the agents were examined in w794 and w836 duplex DNA and were compared to their respective natural product counterparts by enlisting protocols described in detail in earlier studies.[15] Figure 6 illustrates the alkylation selectivity of both natural and unnatural *iso*-duocarmycin SA alongside (+)- and *ent*-(−)-duocarmycin SA. Satisfyingly, each enantiomer of *iso*-duocarmycin SA alkylated the same site as its duocarmycin SA counterpart, displaying the same characteristic and enantiomerically distinguishable selectivity. Like duocarmycin SA, the unnatural enantiomer of *iso*-duocarmycin SA proved less efficient and slower at alkylating DNA than its natural enantiomer. Although not represented in Figure 6, the only notable distinction observed was that (+)- and *ent*-(−)-*iso*-duocarmycin SA exhibit a decreased alkylation efficiency compared to (+)- and *ent*-(−)-duocarmycin SA after 24 h. After 48 h (Figure 6), the differences in efficiency either disappear or are much less noticeable. Thus, the final efficiencies of DNA alkylation are not readily distinguishable if the reactions are allowed to progress to completion, but the rates of DNA alkylation are different, with that of duocarmycin SA being perceptibly faster.

A similar trend was observed with *iso*-yatakemycin. After 24 h, (+)- and *ent*-(−)-*iso*-yatakemycin show approximately 2−5-fold less alkylation than (+)- and *ent*-(−)-yatakemycin. After 48 h (Figure 7), (+)-yatakemycin and (+)-*iso*-yatakemycin exhibit similar levels of alkylation. The largest distinction observed between *iso*-duocarmycin SA and *iso*-yatakemycin was the altered and identical alkylation selectivity of the two enantiomers of **6** and their comparable efficiencies of DNA alkylation. This mirrors the now characteristic observations made with yatakemycin itself and related sandwiched analogues and has been discussed in detail elsewhere.[23,24]

The key issues addressed with these studies were that the isomeric alkylation subunit did not alter the intrinsic DNA alkylation selectivity of either duocarmycin SA or yatakemycin, but the modification did slow the rate and potentially decrease the efficiency of DNA alkylation despite its enhanced intrinsic reactivity.

The DNA alkylation behavior of the simple alkylation subunit *N*-Boc-*iso*-DSA versus *N*-Boc-DSA was also examined (Figure S3 in Supporting Information). Most significant in the comparisons is the indistinguishable behavior of *N*-Boc-*iso*-DSA

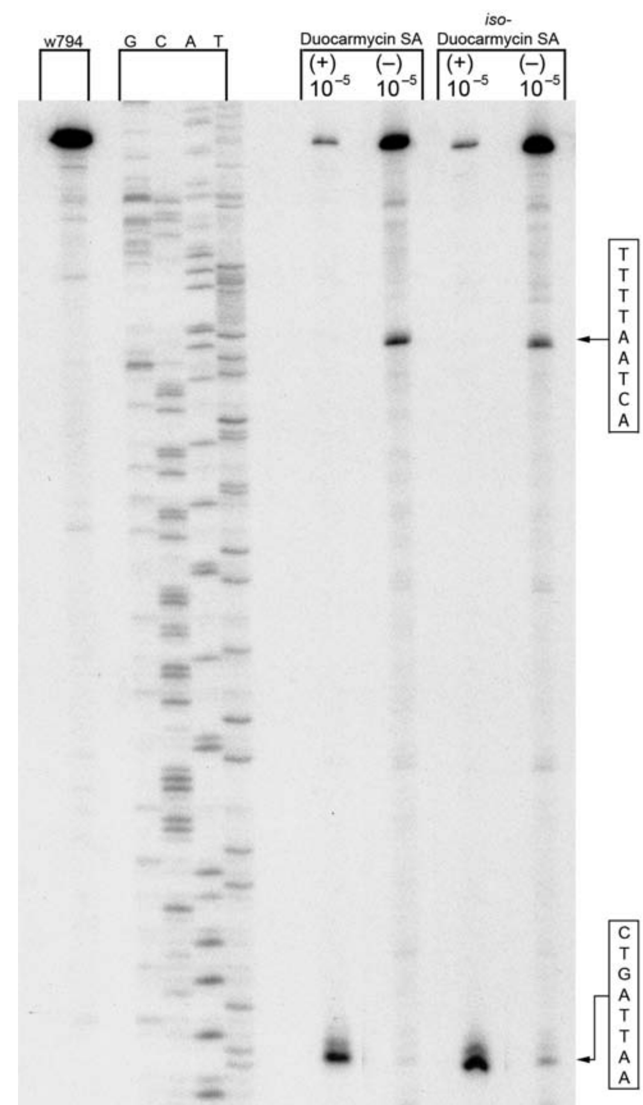

Figure 6. Thermally induced strand cleavage of w794 DNA (144 bp, nucleotide no. 5238-138) after DNA−agent incubation with duocarmycin SA and *iso*-duocarmycin SA (48 h, 23 °C), removal of unbound agent by EtOH precipitation, and 30 min thermolysis (100 °C), followed by denaturing 8% polyacrylamide gel electrophoresis (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**, 1×10^{-5} M); lanes 8 and 9, (+)-*iso*-duocarmycin SA and *ent*-(−)-*iso*-duocarmycin SA (**5**, 1×10^{-5} M).

and *N*-Boc-DSA itself. Both enantiomers of both agents alkylate the same sites, displaying the same DNA alkylation selectivity (5′-AA and 5′-TA). In addition to this unusual identical enantiomeric selectivity that has been interpreted in detail for *N*-Boc-DSA itself[5] and that is much less selective than duocarmycin SA, both *N*-Boc-*iso*-DSA and *N*-Boc-DSA alkylate DNA much less efficiently (10^3−10^4 times) and kinetically much more slowly than the full-length agents.

Additionally, we examined the DNA alkylation properties of **34** that contains the unusual ortho- versus para-spirocyclization and has much greater reactivity. Remarkably, both enantiomers of **34** alkylated DNA with selectivities analogous to each of the enantiomers of *iso*-duocarmycin SA (**5**) and duocarmycin SA (**1**). This is illustrated nicely in Figure 8, where the natural enantiomer of **34** alkylates the single high-affinity site in w794 like the natural enantiomer of duocarmycin SA (**1**). Moreover, it did so with an efficiency nearly equivalent to that of **1** despite

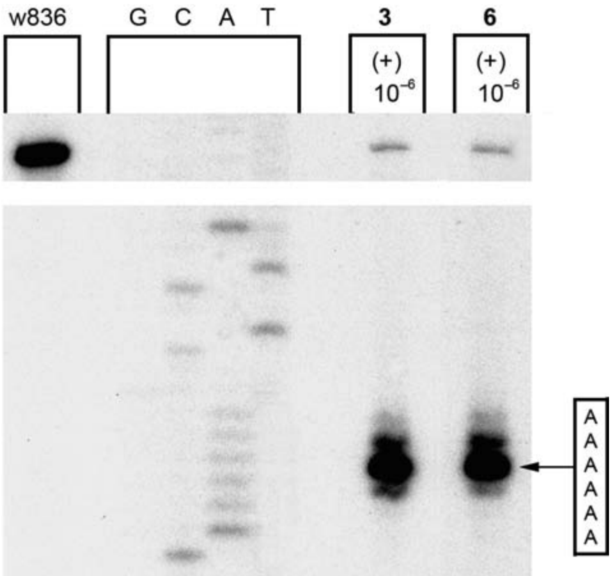

Figure 7. Thermally induced strand cleavage of w836 DNA (146 bp, nucleotide no. 5189-91) after DNA−agent incubation with yatakemycin and *iso*-yatakemycin (48 h, 23 °C), removal of unbound agent by EtOH precipitation, and 30 min thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography. Lane 1, control DNA; lanes 2−5, Sanger G, C, A, and T sequencing standards; lane 6, (+)-yatakemycin (**3**, 1×10^{-6} M); lane 7, (+)-*iso*-yatakemycin (**6**, 1×10^{-6} M).

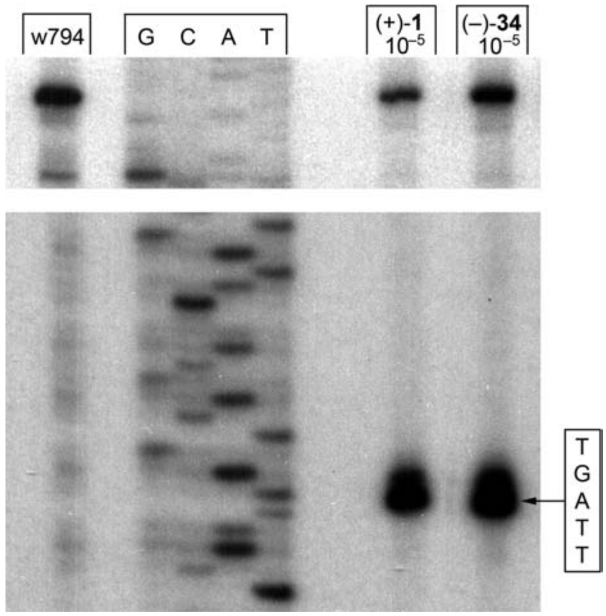

Figure 8. Thermally induced strand cleavage of w794 DNA (144 bp, nucleotide no. 5238-138) after DNA−agent incubation with duocarmycin SA and **34** (22 h, 23 °C), removal of unbound agent by EtOH precipitation, and 30 min thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography. Lane 1, control DNA; lanes 2−5, Sanger G, C, A, and T sequencing standards; lane 6, (+)-duocarmycin SA (**1**, 1×10^{-5} M); lane 7, (−)-**34** (natural enantiomer, 1×10^{-5} M).

its greater reactivity and instability. Although the results of these comparisons reaffirm many features contributing to the DNA alkylation selectivity and source of catalysis for **1**, the most prominent of these are that (1) the source of catalysis is not what determines the selectivity of DNA alkylation,[9] (2) DNA backbone phosphate protonation or Lewis acid coordination of the C4-carbonyl is not contributing to or controlling the DNA alkylation selectivity,[14] and (3) the alkylation selectivity of **34** is consistent with studies that indicate it is derived from the intrinsic noncovalent binding selectivity of **1** and **34**.[12,15,24]

Conclusions

Synthesis, characterization, and evaluation of a key pyrrole isomer of the duocarmycin SA alkylation subunit were conducted, and its incorporation into two key analogues of the natural products is described: *iso*-duocarmycin SA (**5**) and *iso*-yatakemycin (**6**). This isomeric variant of the naturally occurring DNA alkylation subunit displayed an enhanced intrinsic reaction regioselectivity and a 2-fold diminished stability. Its derivatives and natural product analogues exhibited a potent cytotoxic activity with only 3−5-fold reduction in activity relative to the corresponding natural products, consistent with its placement on a parabolic relationship correlating activity and reactivity. DNA alkylation selectivity of the resulting derivatives and of the natural product analogues was unaltered by the structural modification, despite the presentation of a potential H-bond donor to the minor-groove floor when bound or alkylated with DNA. Additionally, a unique ortho-spirocyclization for activation of DNA alkylation was explored with **34** that proved sufficiently stable for customary characterization yet was 470-fold more reactive than *N*-Boc-*iso*-DSA (**7**, $t_{1/2} = 89$ h at pH 3; stable at pH 7). It not only displayed this enhanced reactivity at pH 3 ($t_{1/2} = 0.19$ h) but also proved nearly equally reactive at pH 7, where both *N*-Boc-*iso*-DSA (**7**) and *N*-Boc-DSA (**8**) are stable and exhibit no reactivity. As such, it exhibited a much reduced cytotoxic potency but, remarkably, it was found to alkylate DNA with a selectivity and efficiency analogous to those of duocarmycin SA (**1**) and *iso*-duocarmycin SA. In addition to providing unique insights into the sources of DNA alkylation selectivity and catalysis for this class of natural products, these studies indicate that this ortho-spirocyclization[27,32] constitutes an additional activation pathway,[26] although it is energetically less favorable and less productive than the more conventional para-spirocyclization.

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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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