Total Synthesis and Preliminary Evaluation of (+)- and *ent*-(−)-Duocarmycin SA

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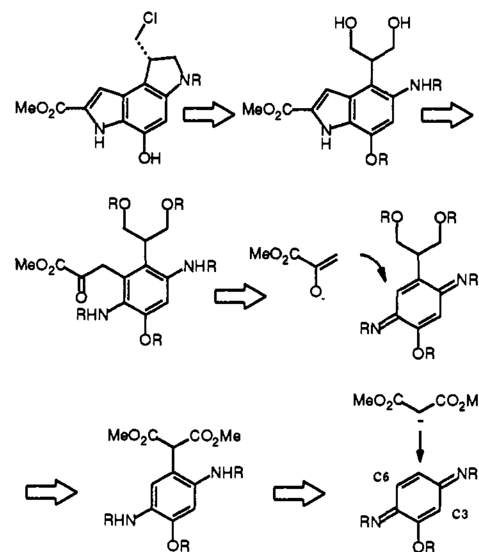
Abstract: Concise total syntheses of natural (+)- and *ent*-(−)-duocarmycin SA (**1**) are detailed based on sequential regioselective nucleophilic substitution reactions of the unsymmetrical *p*-quinone diimine **3** in the preparation of a dihydropyrroloindole precursor to the left-hand subunit. In addition to constituting a new synthetic strategy for the preparation of natural or synthetic duocarmycins and related agents, both enantiomers of **2** (*N*-BOC-DSA) and its immediate synthetic precursors are made available by the approach. This provides access to synthetic analogs incorporating either enantiomer of the exceptionally stable and potent duocarmycin SA alkylation subunit. The comparative chemical properties of the agents are detailed in studies which reveal that *N*-BOC-DSA ($t_{1/2}$ = 177 h, pH = 3; stable, pH = 7) is 4.8× more stable to chemical solvolysis than *N*-BOC-CPI ($t_{1/2}$ = 37 h, pH = 3), the authentic alkylation subunit of CC-1065, and that the agents participate in a stereoelectronically-controlled solvolysis reaction with nucleophilic addition to the least hindered cyclopropane carbon. Consistent with this enhanced stability, (+)-*N*-BOC-DSA (**2**) proved to possess the most potent inherent cytotoxic activity of all natural and synthetic alkylation subunits examined to date including (+)-*N*-BOC-CPI, and its relative cytotoxic potency predictably follows a fundamental relationship between chemical stability and cytotoxic potency established in prior studies. In contrast to expectations based on past observations, the unnatural enantiomers of **1** and **2** as well as the natural enantiomers were found to constitute potent cytotoxic agents whose further examination should prove exceptionally interesting.

(+)-Duocarmycin SA (**1**), an exceptionally potent antitumor antibiotic isolated in trace quantities from *Streptomyces* sp. D0113 (FERM BP-222, 0.01 mg/L) and first described in 1990,[2] constitutes the newest and most potent member of a growing class of agents[3,4] that derive their biological properties through sequence-selective duplex DNA minor groove alkylation.[5–9] Because of its enhanced solvolytic stability and biological potency relative to its predecessors (+)-duocarmycin A[2,3] or (+)-CC-1065,[10,11] the chemical and biological examination of (+)-duocarmycin SA (stable A), its enantiomer *ent*-(−)-duocarmycin SA, and structural analogs promises to be especially interesting. Herein, we provide full details of the total synthesis[12,13] of (+)- and *ent*-(−)-duocarmycin SA based on sequential and regioselective nucleophilic substitution reactions[14,15] of the unsymmetrical *p*-quinone diimine **3** in the preparation of a functionalized dihydropyrroloindole precursor to the alkylation subunit, Scheme I. In addition to constituting a preparatively useful and new

Scheme I



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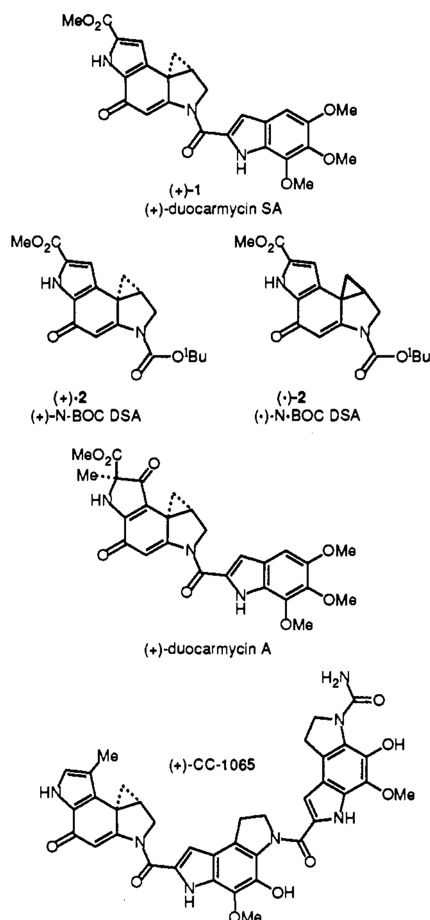
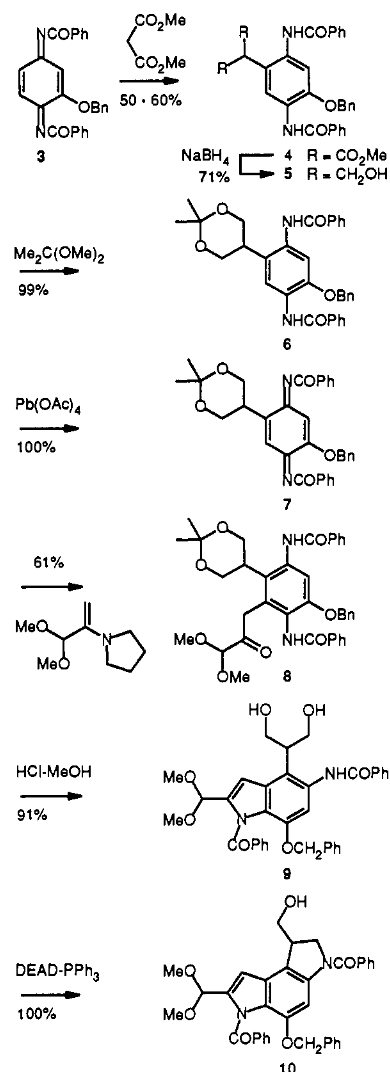
strategy for the construction of natural[15-17] or synthetic[18-22] members of this growing class of agents, the approach makes available (+)- and (-)-**2** (*N*-BOC-DSA) and its immediate synthetic precursors for potential use in the preparation of analogs incorporating either enantiomer of the duocarmycin SA alkylation subunit.



Total Synthesis of Duocarmycin SA. Treatment of **3**[14] with dimethyl malonate in THF in the presence of catalytic $NaOCH_3$ at low temperature provided **4** derived from regioselective C5 nucleophilic substitution, Scheme II. The selectivity of the addition reaction may be attributed to electronic deactivation of C6 addition and a combination of electronic and steric deactivation of C3 substitution both by the C2 benzyloxy substituent, and it proved sensitive to the reaction temperature. Modest selectivity was observed at 0 °C (2:1, C5:C6 addition, 48% **4**) to -10 °C (2.4:1, 43% **4**), good selectivity was observed at -30 °C (5:1, 61% **4**), and excellent selectivity was observed when the reaction was conducted at -78 °C (10:1) although complete reaction required prohibitively long reaction times. In the large scale optimization of the reaction of dimethyl malonate with **3**, the reaction was most conveniently conducted at -30 °C (2-6 h) and found to proceed best in THF (>CH_2Cl_2 >> CH_3CN, DMF) and the desired C5 addition product **4** could be cleanly crystallized free of the isomeric products[23] directly in the workup procedure. Methyl ester reduction, affected by treatment of **4** with $NaBH_4$ in EtOH, provided diol **5** in good yield under remarkably mild conditions in a reaction that proved unusually sensitive to the solvent. Attempts to conduct the reduction with $NaBH_4$ in CH_3OH as well as *i*-PrOH or *t*-BuOH failed to provide diol **5** in more than

Scheme II



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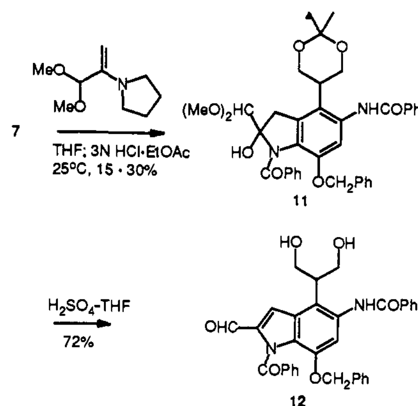
trace amounts, and alternative reagents including DIBAL-H and $LiBH_4$ proved less successful at providing **5**. Protection of diol **5** as acetonide **6** followed by oxidation with $Pb(OAc)_4$ provided **7** (100%) and a suitable acceptor substrate for a second nucleophilic substitution reaction.

Clean, regioselective C6 nucleophilic addition of the pyrrolidine enamine of pyruvaldehyde dimethyl acetal[24] was achieved if the initial nucleophilic addition reaction time was short (10–15 min, 25 °C) and followed immediately by enamine hydrolysis with mild acid treatment under defined reaction conditions (40 mL of THF–10 mL of pH 4 phosphate buffer/mmol, 25 °C, 24 h, 56–61%). The use of pH 5 phosphate buffer provided comparable results (60%), but alternative enamine hydrolysis conditions especially those conducted at lower pH[25] generally provided a combination of reaction products resulting from acetonide hydrolysis, dimethyl acetal hydrolysis, benzyl ether deprotection, hemiaminal formation, and/or indole formation. Although vigorous acid treatment could be employed to convert the initial enamine adduct to **11**[26] or **12**[26] directly, Scheme III, the overall conversions proved lower than that observed with the deliberate and surprisingly clean isolation of **8** followed by its effective conversion to **9**. Treatment of **8** with HCl (2 equiv, CH_3OH, 25 °C, 2 h, 91%) provided **9** resulting from acid-catalyzed indole formation and concurrent acetonide hydrolysis without competitive indole *N*-debenzoylation or dimethyl acetal hydrolysis. Longer reaction times led to diminished conversion of **8** to **9**, and alternative methods examined for conducting the indole closure with or without acetonide deprotection proved less effective. Completion of the preparation of the functionalized dihydropyrrolo[3,2-*e*]indole skeleton was accomplished by cyclization of diol **9** under Mitsunobu alkylation conditions to provide **10**.[27]

The subtle selection of the acetonide derivative **7** for study in conjunction with the judicious choice of an enamine as the nucleophile for the C6 addition proved necessary to the successful implementation of a second nucleophilic substitution reaction. The C6 nucleophilic substitution reaction of the pyrrolidine enamine of pyruvaldehyde dimethyl acetal occurs best at 25 °C and at lower reaction temperatures (−78 °C), trace or competitive *p*-quinone diimide reduction was observed. In contrast to the clean C6 addition of the pyruvaldehyde dimethyl acetal enamine, the reaction of dimethyl malonate with **7** in the presence of catalytic $NaOCH_3$ (1.1 equiv, THF, 0°C) provided a 2:3 ratio of C6 versus C1 addition products (84%), Scheme IV.[28] The reaction of **7** with methyl pyruvate (0.3 equiv of $NaOCH_3$ or 1 equiv of NaH, THF and DMF) and methyl 3-(methanylsulfonyl)-2-oxopropionate (1.1–1.5 equiv, 0.3 equiv of $NaOCH_3$ or KO *tert*-Bu or 1 equiv NaH in THF or DMF, 0–25 °C) provided only recovered and reduced starting material, while its treatment with LiCN (1.1 equiv, DMF, 25 °C, 24 h) provided a 3:1 mixture of C4 and C1 addition products.[29] Similar initial efforts to conduct the C6 nucleophilic substitution reaction employing *tert*-butyldimethylsilyl ether **18** proved much less successful than that of acetonide **7**, attributable to competitive *p*-quinone diimide reduction and C1 as well as C6 nucleophilic addition, Scheme IV.[30] Representative of these efforts, treatment of **18** with

Scheme III



(23) C6 nucleophilic addition product. *O*-benzyl-N^2,N^5-dibenzoyl-2,5-diamino-3-(bis(methoxycarbonyl)methyl)phenol: mp 183–186 °C; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.17 (s, 1H, NH), 8.79 (s, 1H, NH), 7.78 (dd, 2H, J = 7.8, 1.1 Hz, C2-H and C6-H, PhCO), 7.74 (dd, 2H, J = 7.8, 1.1 Hz, C2-H and C6-H, PhCO), 7.60 (d, 1H, J = 2.2 Hz, C3-H), 7.58–7.32 (m, 5H, ArH), 7.30–7.20 (m, 2H, ArH), 7.18–7.08 (m, 5H, ArH), 4.76 (s, 1H, ArCH), 4.66 (s, 2H, OCH_2Ph), 3.69 (s, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.2, 166.6, 166.3, 154.6, 138.6, 136.6, 134.5, 134.2, 131.6, 131.2, 130.8, 128.1, 128.0, 127.42, 127.37, 127.35, 127.0, 126.6, 120.8, 115.6, 106.8, 69.6, 56.1, 53.0; IR (KBr) ν_{max} 3310, 3064, 2954, 1744, 1660, 1602 cm^{-1}. On occasion, an additional product tentatively identified as the C1 nucleophilic addition product has been detected and isolated in trace quantities (<5–10%): ^1H NMR ($CDCl_3$, 200 MHz) δ 8.39 (s, 1H, NH), 8.03 (dd, 2H, J = 8, 1.5 Hz, C2-H and C6-H, PhCO), 7.82 (dd, 2H, J = 8, 1.5 Hz, C2-H and C6-H, PhCO), 7.60–7.40 (m, 6H, ArH), 7.30–7.10 (m, 5H, ArH), 6.60 (d, 1H, J = 10 Hz, C6-H), 6.52 (dd, 1H, J = 10, 1.3 Hz, C5-H), 5.85 (d, 1H, J = 1.3 Hz, C3-H), 4.86 (d, 1H, J = 11 Hz, OC*H*HPh), 4.72 (d, 1H, J = 11 Hz, OCH*H*Ph), 3.74 (s, 1H, C*H*(CO_2CH_3)$_2$), 3.66 (s, 3H, CO_2CH_3), 3.36 (s, 3H, CO_2CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 181.2, 167.2, 167.1, 166.7, 158.9, 140.7, 135.0, 134.22, 134.15, 133.7, 132.6, 132.5, 130.2, 129.3, 129.0, 128.2, 128.0, 127.8, 127.6, 99.3, 71.3, 57.9, 57.6, 53.5, 53.4; IR (KBr) ν_{max} 3412, 3062, 2954, 1752, 1668, 1600 cm^{-1}. Interestingly, the reaction conducted in DMF provided the C6 substitution product as the major regioisomer (C5:C6 1:5, 98%).

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(25) Acid catalysts examined include 5 equiv of 10% aqueous HCl–THF, 44–26% **8**; HOAc–THF (1:1), 22% **8**; HOAc–THF–H_2O (1:1:0.5); saturated aqueous NH_4Cl–THF (1:1); 4 equiv of 10% aqueous H_2SO_4–THF; 10 equiv of TFA–THF, 17% **8**; catalytic *p*TsOH–THF, 29% **8**; 2.5 equiv of PPTS–THF, 15% **8** and 22% **11**; 5 equiv of HCl–EtOAc, 13% **8** and 16% **11**; 10 equiv of oxalic acid–THF.

(26) **11**: ^1H NMR ($CDCl_3$, 400 MHz) δ 9.38 (s, 1H, NH), 7.82 (dd, 2H, J = 8.4, 1.4 Hz, ArH), 7.61 (s, 1H, C6-H), 7.58–7.15 (m, 13H, ArH), 6.42 (s, 1H, C*H*(OMe)$_2$), 5.09 (d, 1H, J = 11.2 Hz, OC*H*HPh), 5.01 (d, 1H, J = 11.2 Hz, OCH*H*Ph), 4.82 (s, 1H, OH), 4.50 (d, 1H, J = 13.0 Hz, ArC*H*H), 4.20–4.02 (m, 4H, CH_2O), 3.82 (dd, 1H, J = 13.0, 3.7 Hz, OCH_2CHCH_2O), 3.53 (s, 3H, OCH_3), 3.36 (s, 3H, OCH_3), 2.98 (d, 1H, J = 13.0 Hz, ArCH*H*), 1.26 (s, 3H, CH_3), 1.25 (s, 3H, CH_3). **12**: ^1H NMR ($CDCl_3$/DMSO-d_6, 400 MHz) δ 10.45 (br s, 1H, NH), 9.23 (s, 1H, CHO), 7.48 (m, 2H, C2-H and C6-H, PhCO), 7.71–6.92 (m, 6H, ArH), 6.88–6.77 (m, 3H, ArH), 6.73–6.61 (m, 4H), 6.42–6.32 (m, 2H), 4.59 (s, 2H, OCH_2Ph), 4.38 (s, 2H, OH), 3.58 (br s, 2H, CH_2OH), 3.50 (br s, 2H, CH_2OH), 3.00 (br s, 1H, C*H*(CH_2OH)$_2$); ^{13}C NMR ($CDCl_3$/DMSOd_6, 100 MHz) δ 179.5 (CHO), 168.7, 164.4, 142.6, 135.6, 134.2, 133.5, 132.8, 130.7, 130.4, 128.7, 127.4, 127.3, 127.0, 126.6, 126.33, 126.29, 115.3, 106.8, 69.2, 60.6, 28.7.

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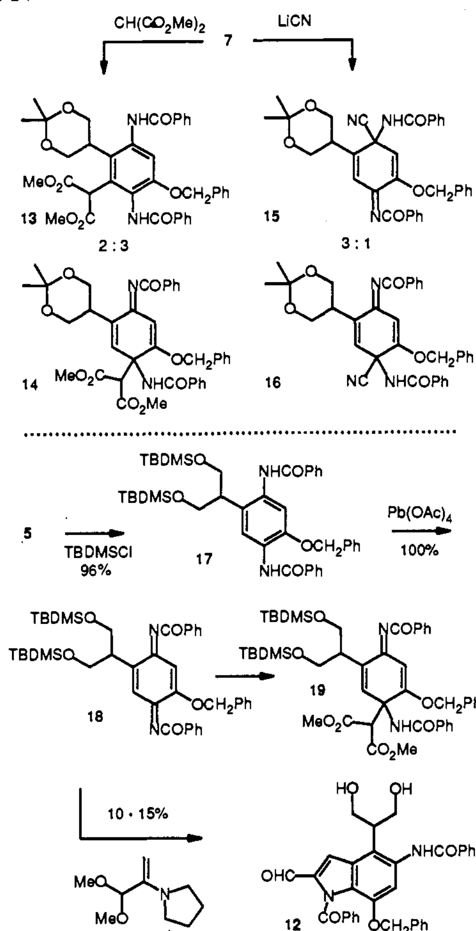
(28) **13**: ^1H NMR ($CDCl_3$, 400 MHz) δ 10.03 (s, 1H, NH), 8.30 (s, 1H, NH), 8.09 (s, 1H, C6-H), 7.88 (d, 2H, J = 7.1 Hz, C2-H and C6-H, PhCO), 7.82 (d, 2H, J = 7.2 Hz, C2-H and C6-H, PhCO), 7.56–7.33 (m, 7H, ArH), 7.30–7.22 (m, 4H, ArH), 5.13 (s, 3H, OCH_2Ph and C*H*(CO_2CH_3)$_2$), 3.97 (dd, 2H, J = 12.1, 8.3 Hz, OC*H*H), 3.86 (dd, 2H, J = 12.1, 6.2 Hz, OCH*H*), 3.73 (m, 1H, C*H*(CH_2O)$_2$), 3.72 (s, 6H, CO_2CH_3), 1.31 (s, 3H, CH_3), 0.77 (s, 3H, CH_3). **14**: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.37 (s, 1H, NH), 7.97 (d, 2H, J = 7.1 Hz, C2-H and C6-H, PhCO), 7.82 (d, 2H, J = 7.2 Hz, C2-H and C6-H, PhCO), 7.60–7.39 (m, 6H, ArH), 7.23–7.03 (m, 5H, ArH), 6.73 (s, 1H, C*H*CNH), 5.70 (s, 1H, C*H*$COCH_2Ph$), 4.77 (d, 1H, J = 11.1 Hz, OC*H*HPh), 4.68 (d, 1H, J = 11.1 Hz, OCH*H*Ph), 4.17 (dd, 1H, J = 11.4, 3.8 Hz, OC*H*HCH), 4.10 (dd, 1H, J = 10.7, 3.5 Hz, OCH*H*CH), 4.00 (dd, 1H, J = 11.6, 6.5 Hz, OC*H*HCH), 3.90 (dd, 1H, J = 11.0, 6.8 Hz, OCH*H*CH), 3.73 (s, 1H, CH(CO_2Me)$_2$), 3.66 (s, 3H, CO_2CH_3), 3.41 (m, 1H, C*H*(CH_2O)$_2$), 3.39 (s, 3H, CO_2CH_3), 1.44 (s, 3H, CH_3), 1.41 (s, 3H, CH_3).

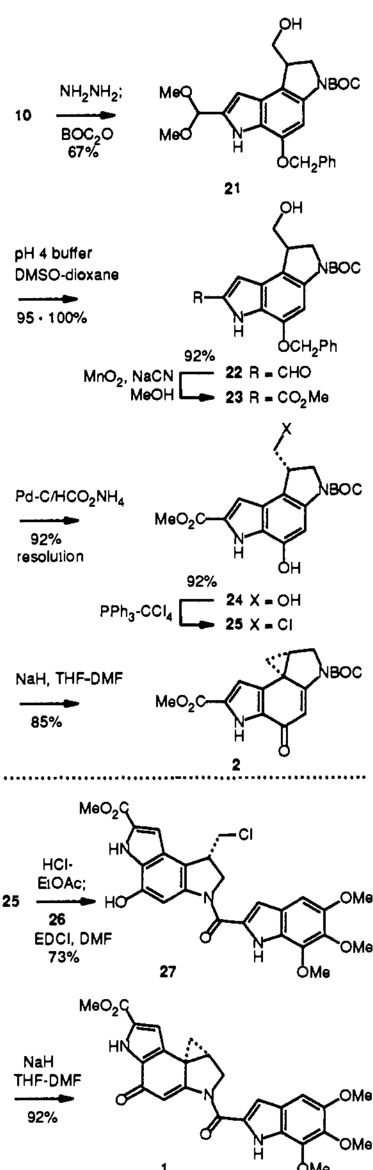
(29) **15**: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.73 (dd, 2H, J = 8.2, 1.4 Hz, C2-H and C6-H, PhCO), 7.57–7.28 (m, 13H, ArH), 6.85 (s, 1H, C*H*CNCO), 5.85 (s, 1H, C*H*$COCH_2Ph$), 5.09 (s, 2H, OCH_2Ph), 4.10–4.00 (m, 2H, OCH_2CH), 3.86 (ddd, 1H, J = 11.7, 5.6, 1.2 Hz, OCH_2C*H*), 3.79 (ddd, 2H, J = 11.7, 5.6, 1.1 Hz, OCH_2CH), 3.00 (m, 1H, C*H*(CH_2O)$_2$), 1.42 (s, 3H, CH_3), 1.38 (s, 3H, CH_3). **16**: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.73 (d, 2H, J = 7.3 Hz), 7.63–7.30 (m, 13H, ArH), 6.52 (s, 1H, CHCNCO), 5.74 (s, 1H, C*H*$COCH_2Ph$), 5.19 (d, 1H, J = 11.8 Hz, OCH*H*Ph), 5.06 (d, 1H, J = 11.8 Hz, OC*H*HPh), 4.21–3.92 (m, 2H, OCH_2CH), 3.84 (d, 1H, J = 12.2 Hz, OC*H*HCH), 3.76 (d, 1H, J = 13.0 Hz, OCH*H*CH), 3.44 (dd, 1H, J = 9.0, 4.0 Hz, OCH_2C*H*), 1.36 (s, 3H, CH_3), 0.91 (s, 3H, CH_3).

(30) **17**: ^1H NMR ($CDCl_3$, 400 MHz) δ 9.48 (s, 1H, NH), 8.65 (s, 1H, C6-H), 8.55 (s, 1H, NH), 7.98 (d, 2H, J = 8.1 Hz, C2-H and C6-H, PhCO), 7.87 (s, 1H, C3-H), 7.80 (d, 2H, J = 7.7 Hz, C2-H and C6-H, PhCO), 7.60–7.33 (m, 11H, ArH), 5.24 (s, 2H, OCH_2Ph), 4.19 (dd, 2H, J = 9.4, 6.7 Hz, CH_2O), 3.79 (dd, 2H, J = 9.5, 7.0 Hz, CH_2O), 3.34 (p, 1H, J = 6.7 Hz, CH), 0.83 (s, 18H, C(CH_3)$_3$), 0.04 (s, 12H, $SiCH_3$). **18**: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.87 (dd, 2H, J = 8.3, 1.2 Hz, C2-H and C6-H, PhCO), 7.60 (d, 2H, J = 7.1 Hz, C2-H and C6-H, PhCO), 7.52–7.48 (m, 1H), 7.46 (t, 2H, J = 7.5 Hz), 7.31 (t, 2H, J = 7.4 Hz), 7.27–7.22 (m, 2H, ArH and C3-H), 7.20 (d, 1H, J = 7.4 Hz), 7.14 (t, 2H, J = 7.1 Hz), 6.92 (d, 2H, J = 5.5 Hz), 5.85 (s, 1H, C6-H), 4.69 (s, 2H, OCH_2Ph), 3.91 (dd, 2H, J = 9.8, 5.0 Hz, CH_2O), 3.86 (dd, 2H, J = 9.8, 6.1 Hz, CH_2O), 3.51 (p, 1H, J = 5.3 Hz), 0.88 (s, 18H, C(CH_3)$_3$), 0.05 (s, 12H, $SiCH_3$).

Scheme IV



Scheme V



dimethyl malonate (0.3 equiv of $NaOCH_3$, THF, 0 °C, 1 h, 82%) provided **19**[31] as the major product and the C6 addition of the pyrrolidine enamine of pyruvaldehyde dimethyl acetal followed by vigorous acid-catalyzed hydrolysis of the crude reaction products provided **12** in significantly lower conversions than that observed with acetonide **7**. In retrospect, this may be attributed to the steric deceleration of the desired C6 nucleophilic substitution reaction further exaggerated with use of the two bulky *tert*-butyldimethylsilyl protecting groups. The successful use of **7** may be attributed to the combined selection of an appropriate nucleophile and a sterically-constrained diol protecting group which permits addition to the sterically-hindered C6 center.

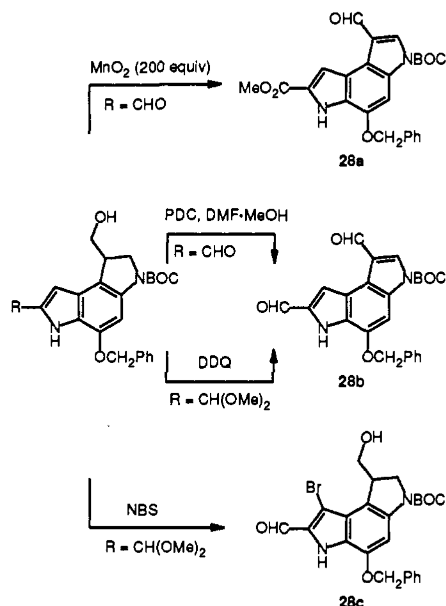
Deprotection of both *N*-benzoyl groups of **10** was effectively accomplished upon treatment with NH_2NH_2 (67% in EtOH, reflux, 18 h), and selective acylation of the more reactive C3 amine with BOC_2O without isolation or characterization of the unstable free indoline provided **21**, Scheme V. Less vigorous deprotection reaction conditions (100 equiv of NH_2NH_2–H_2O, C_6H_6, 25 °C, 2 h, and reflux, 24 h, 46%) provided clean monodeprotection of the indole *N*-benzoyl group.[33] Imperative to the success of the synthesis of duocarmycin SA was C7 acetal hydrolysis and its subsequent oxidative conversion to the C7 methyl ester. Despite apprehensions about the relative acid stability of the (*tert*-butyloxy)carbonyl protecting group and the oxidative lability of the *free* C1 hydroxymethyl group and indoline substructure, the conversion of **21** to **23** proved uneventful. Mild acid-catalyzed hydrolysis of the dimethyl acetal through treatment of **21** under carefully prescribed reaction conditions (DMSO–pH 4 phosphate buffer–dioxane 1:2:12, reflux, 15 h, 95–100%) provided **22** in excellent yield *without* competitive BOC deprotection. The use of DMSO as cosolvent in this reaction mixture served to ensure substrate solubility and a homogeneous single-phase reaction solution. Under these conditions, the use of shorter (5 h, 47%) and longer (24 h, 87%) reaction times led to diminished conversions, and more conventional acetal hydrolysis conditions

(31) **19**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.29 (s, 1H, NH), 7.99 (dd, 2H, J = 7.0, 1.9 Hz, C2-H and C6-H, PhCO), 7.79 (dd, 2H, J = 7.0, 1.5 Hz, C2-H and C6-H, PhCO), 7.56–7.40 (m, 6H, ArH), 7.20–7.13 (m, 3H, ArH), 7.08 (dd, 2H, J = 7.2, 2.3 Hz, C2-H and C6-H, OCH_2Ph), 6.70 (s, 1H, CHCNH), 5.69 (s, 1H, C*H*$COCH_2Ph$), 4.76 (d, 1H, J = 11.1 Hz, OC*H*HPh), 4.66 (d, 1H, J = 11.1 Hz, OCH*H*Ph), 3.96 (dd, 1H, J = 9.8, 4.3 Hz, CH_2O), 3.80 (d, 2H, J = 6.0 Hz), 3.79 (dd, 1H, J = 8.4, 5.6 Hz), 3.68 (s, 3H, CO_2CH_3), 3.66 (s, 1H, C*H*$(CO_2CH_3)_2$), 3.35 (p, 1H, J = 5.3 Hz), 3.33 (s, 3H, CO_2CH_3), 0.85 and 0.82 (s, 18H, $C(CH_3)_3$), 0.03 and 0.02 (s, 12H, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.9, 166.5, 166.3, 166.1, 165.8, 157.8 ($COCH_2Ph$), 137.5, 137.3, 134.6, 134.1, 133.7, 132.9, 131.6, 129.6, 128.6, 128.5, 128.4, 128.3, 127.5, 127.1, 96.8 (*C*HCNH), 70.7 (OCH_2Ph), 62.0 (CH_2OSi), 61.4 (CH_2OSi), 57.8 (*C*H$(CO_2CH_3)_2$), 57.4 (*C*CH$(CO_2CH_3)_2$), 53.1 (OCH_3), 52.9 (OCH_3), 41.9 (*C*HCH_2O), 26.0 (C(*C*$H_3)_3$), 18.2 ($SiCH_3$), −5.4 ($SiCH_3$); IR (KBr) ν_{max} 2954, 2928, 2856, 1758, 1671, 1601, 1519, 1311, 1258, 1095, 1080, 909, 837 cm^{-1}; FABMS (NBA–CsI), *m/e* 987 (M + Cs^+).

(32) *Bis*((*tert*-butyldimethylsilyl) ether) derivative of **12**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.79 (s, 1H, CHO), 7.95 (d, 2H, J = 7.2 Hz, C2-H and C6-H, COPh), 7.58–7.42 (m, 7H, ArH), 7.29–7.14 (m, 6H, ArH), 6.94 (d, 2H, J = 6.6 Hz, C2-H and C6-H, OCH_2Ph), 4.90 (s, 2H, OCH_2Ph), 4.25 (t, 2H, J = 9.5 Hz, CHC*H*$_2$O), 4.18–4.00 (m, 2H, CHC*H*$_2$O), 3.54 (br s, 1H, C*H*CH_2O), 0.81 (s, 18H, $C(CH_3)_3$), 0.03 and 0.05 (s, 12H, $SiCH_3$).

(33) 3-Benzoyl-5-(benzyloxy)-7-(dimethoxymethyl)-1-(hydroxymethyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.65 (br s, 1H, NH), 8.04 (br s, 1H, C4-H), 7.65–7.32 (m, 10H, ArH), 6.46 (s, 1H, C8-H), 5.62 (s, 1H, $CH(OMe)_2$), 5.27 (s, 2H, OCH_2Ph), 4.21 (m, 2H, CH_2O), 4.04 (br s, 1H, OH), 3.88 (m, 2H, CH_2N), 3.75–3.68 (m, 1H, C1-H), 3.38 (s, 3H, OCH_3), 3.36 (s, 3H, OCH_3).

Scheme VI



(10 equiv of H_2SO_4, THF–H_2O, 25 °C, 12 h, 47%) proved less effective. Subsequent oxidation[34] of **22** (10 equiv of MnO_2, 20 equiv of NaCN, 0.4 equiv of HOAc, CH_3OH, 25 °C, 89%) provided methyl ester **23**. Employing this standard procedure, we observed occasional regeneration of dimethyl acetal **21** in the presence of the HOAc acid catalyst, and over-oxidation of 1-(hydroxymethyl)indoline to the corresponding indole-1-carboxaldehyde was observed when the reaction was conducted with a large excess of MnO_2 (200 equiv , CH_3OH–CH_2Cl_2, 1.5 h, 25 °C), Scheme VI. In our optimization of the conversion of **22** to **23**, the desired methyl ester was obtained in high yield (5 equiv of MnO_2, 5 equiv of NaCN, CH_3OH, 25 °C, 92%) with or without the addition of catalytic HOAc. Consequently, in practice, the acid catalyst was omitted to avoid the occasional regeneration of dimethyl acetal **21**. Initial attempts to directly convert dimethyl acetal **21** to **23** including the use of NBS[35] (68% **28c**) or DDQ[36] (36% **28b**) proved unsuccessful, and alternative oxidation conditions for the conversion of **22** to **23** including the use of NaOCl,[37] PDC[38] (CH_3OH–DMF, 56% **28b**), Ag_2O, or AgO[39] failed to provide the desired material, Scheme VI.[40]

Two-phase, transfer catalytic hydrogenolysis[41] served to remove the benzyl ether (92%), and subsequent conversion of primary alcohol **24** to chloride **25**[42] (92%) followed by treatment with NaH provided *N*-BOC-DSA (**2**, 85%) in excellent yield, Scheme V. Acid-catalyzed deprotection of **25** followed by coupling of

(34) Corey, E. J.; Gilman, N. W.; Ganem, B. E. *J. Am. Chem. Soc.* **1968**, *90*, 5616.
(35) Marvell, E. N.; Joncich, M. J. *J. Am. Chem. Soc.* **1951**, *73*, 973.
(36) Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett.* **1982**, *23*, 889.
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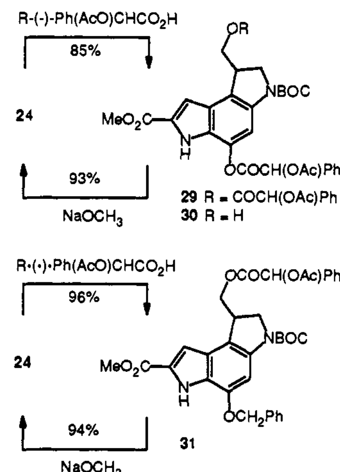
(38) O'Connor, B.; Just, G. *Tetrahedron Lett.* **1987**, *28*, 3235.
(39) Campaigne, E.; Le Suer, W. M. *Organic Synthesis*; Wiley: New York, 1963; Coll. Vol. IV, 919.
(40) **28a**: ^1H NMR ($CDCl_3$, 400 MHz) δ 10.10 (s, 1H, CHO), 9.27 (s, 1H, NH), 8.18 (s, 1H, C4-H), 8.15 (d, 1H, *J* = 2.4 Hz, C8-H), 7.90 (s, 1H, C2-H), 7.55–7.50 (m, 2H, ArH), 7.48–7.37 (m, 3H, ArH), 5.29 (s, 2H, OCH_2Ph), 3.96 (s, 3H, OCH_3), 1.57 (s, 9H, CCH_3). **28b**: ^1H NMR ($CDCl_3$, 400 MHz) δ 10.10 (s, 1H, CHO), 9.90 (s, 1H, CHO), 9.38 (br s, 1H, NH), 8.26 (d, 1H, *J* = 2.4 Hz, C8-H), 8.20 (s, 1H, C4-H), 7.96 (s, 1H, C2-H), 7.51 (d, 2H, *J* = 7.4 Hz, C2-H) and C6-H, OCH_2Ph), 7.48–7.37 (m, 3H, ArH), 5.29 (s, 2H, OCH_2Ph), 1.72 (s, 9H, CCH_3). **28c**: ^1H NMR ($CDCl_3$, 400 MHz) δ 9.89 (s, 1H, CHO), 9.34 (s, 1H, NH), 7.91 (br s, 1H, C4-H), 7.48–7.35 (m, 5H), 5.19 (s, 2H, OCH_2Ph), 4.27 (d, 1H, *J* = 10.8 Hz, C*H*HN), 4.08–4.00 (m, 1H, C*H*HN), 4.04 (t, 2H, *J* = 9.1 Hz, CH_2OH), 3.94 (ddt, 1H, *J* = 6.6, 3.4, 1.9 Hz, C1-H), 3.69 (t, 1H, *J* = 9.1 Hz, OH), 1.58 (s, 9H).
(41) Ram, S.; Ehrenkaufer, R. E. *Synthesis* **1988**, 91. Bieg, T.; Szeja, W. *Synthesis* **1985**, 76.
(42) Hooz, J.; Gilani, S. S. H. *Can. J. Chem.* **1968**, *46*, 86.

Table I. Chromatographic Resolution[a]

diaster-eomers	solvent	α	diaster-eomers	solvent	α
29	10% EtOAc–CH_2Cl_2	1.10	**31**	5% EtOAc–CH_2Cl_2	1.14
29	5% EtOAc–CH_2Cl_2	1.23	**31**	3% EtOAc–CH_2Cl_2	1.21
29	3% EtOAc–CH_2Cl_2	1.31	**31**	2% EtOAc–CH_2Cl_2	1.24

[a] 22.5 × 250 mm 10-μm Alltech SiO_2, 4–5 mL/min.

Scheme VII



the unstable indoline hydrochloride with 5,6,7-trimethoxyindole-2-carboxylic acid[5] (**26**) provided **27**, Scheme V. Interestingly, efforts to conduct the EDCI coupling in the presence of $NaHCO_3$ generally provided **27** in 10–15% lower conversions presumably due to nonproductive generation of **35**. Final intramolecular Ar-3′ alkylation of **27** with closure of the cyclopropane ring provided duocarmycin SA (**1**) in excellent yield (87%).

Resolution and Synthesis of (+)- and *ent*-(-)-Duocarmycin SA. Resolution of **24** was accomplished by conversion to the bis((*R*)-*O*-acetylmandelate) ester **29** (85%) and chromatographic separation of the resulting diastereomers (preparative HPLC, 5% EtOAc–CH_2Cl_2, 22.5 × 250 mm 10-μm SiO_2, 20 mL/min, Table I) to provide (1*S*,2′*R*,2″*R*)-**29** and *ent*-(1*R*,2′*R*,2″*R*)-**29**, Scheme VII. Given the ease of chromatographic separation of the diastereomers (α = 1.31–1.38), each was routinely obtained in >99.9% diastereomeric purity. Independent methanolysis (93%) of the separated diastereomers provided (-)-(1*S*)-**24**, possessing the natural configuration of (+)-duocarmycin SA (**1**), and *ent*-(+)-(1*R*)-**24**. The conversion of (-)-(1*S*)-**24** to (-)-(1*S*)-**25**, (+)-*N*-BOC-DSA (**2**, $[\alpha]^{22}_D$ +144° (*c* 0.06, CH_3OH)), and natural (+)-duocarmycin SA (**1**, $[\alpha]^{22}_D$ +197° (*c* 0.035, CH_3OH))[43] and the parallel conversion of (+)-(1*R*)-**24** to (+)-(1*R*)-**25**, *ent*-(-)-*N*-BOC-DSA (**2**, $[\alpha]^{22}_D$ -137° (*c* 0.05, CH_3OH)), and *ent*-(-)-duocarmycin SA (**1**, $[\alpha]^{22}_D$ -189° (*c* 0.02, CH_3OH)) followed the sequence detailed in Scheme V. Synthetic (+)-duocarmycin SA prepared in this manner proved indistinguishable from the properties reported for the natural material (^1H NMR, ^{13}C NMR, IR, UV, MS, $[\alpha]_D$, and mp).

In the studies of the resolution of **29** and **30**, the acylation of **24** with 1.1 versus 2.5 equiv of (*R*)-(-)-*O*-acetylmandelic acid (1.25 equiv of EDCI, 0.02 equiv of DMAP, CH_2Cl_2, 25 °C, 4 h) provided a 34% yield of **29** accompanied by 39% of **30**,[44] Scheme VII. Alternative and clean monoacylation of **23** with (*R*)-(-)-*O*-acetylmandelic acid (1.5 equiv, 1.8 equiv EDCI, 0.1 equiv of

(43) Synthetic (+)-duocarmycin SA was not completely soluble at this concentration, and this may account for the slightly lower rotation reported for the natural material.
(44) **30** (mixture of diastereomers): ^1H NMR ($CDCl_3$, 400 MHz) δ 9.42 and 9.11 (s, 1H, NH), 7.77 (br s, 1H, ArH), 7.68–7.44 (m, 4H, ArH), 7.20–7.11 (m, 2H, ArH), 6.03 (s, 1H, CH(OAc)), 4.13 (dd, 1H, *J* = 12.0, 1.8 Hz, C*H*HN), 4.01 (m, 1H, CH*H*N), 3.95 (s, 3H, CO_2CH_3), 3.94–3.83 (m, 2H, C*H*HOH and C1-H), 3.82–3.71 (m, 2H, CH*H*OH and OH), 2.31 (s, 3H, $OCOCH_3$), 1.51 (s, 9H, $C(CH_3)_3$).

Table II

	2[a]	32[b]	33[c]	34[d]
IR (C=O, cm^{-1})	1719, 1610	1718, 1628, 1602	1725, 1570	1705, 1617
UV, λ_{max} nm (ϵ)	339 (18 000)	300 (19 000)	344 (12 000)	294 (14 000)
	301 (14 000)	264 (5700)	278 (17 000)	258 (21 000)
	255 (10 000)			
k (s^{-1}, pH 3)[e]	1.08×10^{-6}	1.45×10^{-6}	5.26×10^{-6}	1.98×10^{-2}
$t_{1/2}$ (pH 3)	177 h	133 h	36.7 h	35 s
$t_{1/2}$ (pH 7)	stable	stable	stable	5.3 h
rel $t_{1/2}$	4.8	3.6	1.0	0.0003
IC_{50} (nM, L1210)	6	80	330	18 000

[a] UV (CH_3OH), IR (KBr). [b] UV (THF), IR (film). [c] UV (CH_3OH), IR (Nujol). [d] UV (THF), IR (KBr). [e] pH 3: 50% buffer–CH_3OH, buffer consists of 4:1:20 (v:v:v) 0.1 M citric acid, 0.2 M Na_2HPO_4, and H_2O, respectively. pH 7: 50% CH_3OH–H_2O.

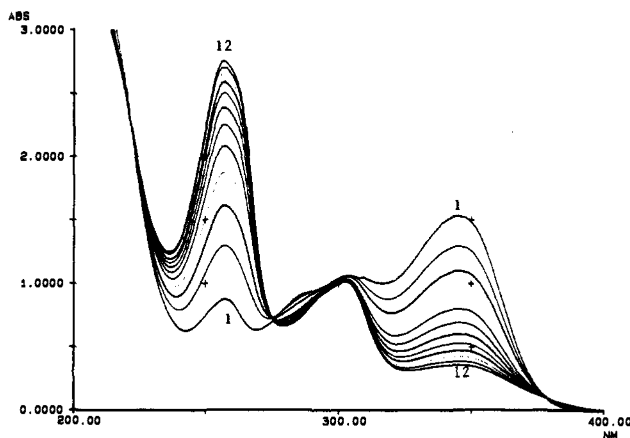


Figure 1. UV–visible spectra of N-BOC-DSA in 50% CH_3OH–aqueous buffer (pH = 3.0, 4:1:20 (v:v:v) 1 M citric acid, 0.2 M Na_2HPO_4, and H_2O, respectively) recorded every 24 h for 12 days. The solvolysis solution was kept in the dark at 25 °C.

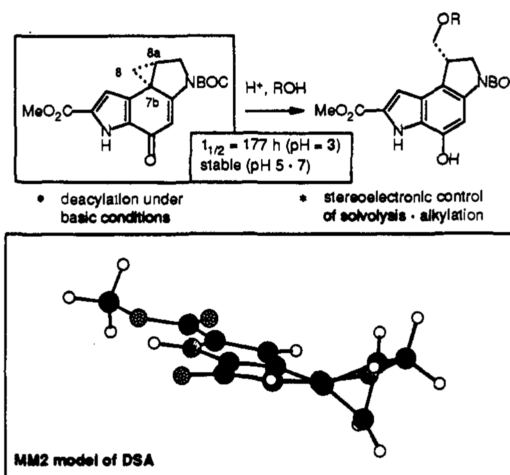


Figure 2.

DMAP, DMF, 25 °C, 1.5 h, 96%) provided (1*S*,2′*R*)-**31** and (1*R*,2′*R*)-**31**[45] which were similarly separable by chromatography, Table I. However, the greater chromatographic separation achieved with **29** coupled with the preference to resolve the more advanced and lower molecular weight synthetic intermediate led to the use of **29** versus **31** for preparative resolution. Initial, although not exhaustive, attempts to chromatographically resolve **25** directly on a chiral support, Chiralcell OD, have not yet proven successful.

Chemical Solvolysis Reactivity. Important characteristics of the alkylation subunits of the duocarmycins, CC-1065, and related analogs are their relative solvolytic reactivity and the site of cyclopropane cleavage. All such past agents have been shown to participate in an acid-catalyzed, stereoelectronically-controlled, ring-opening reaction with predominant nucleophilic addition to the least substituted cyclopropane carbon. Consistent with these past observations, treatment of duocarmycin SA (**1**) with HCl under anhydrous conditions (EtOAc, 0 °C, 15 min) provided **27** exclusively in excellent yield (96%).

In addition, fundamental efforts to correlate the relative reactivity of the agents with their relative biological potency have been detailed. Results of initial studies with a limited series of simple acyl derivatives of the authentic alkylation subunit of CC-1065 have been interpreted to suggest that an increased solvolytic reactivity results in increased biological potency and might be expected to be derived from an enhanced DNA alkylation rate or efficiency.[10,46] In contrast, more recent and extensive comparisons with a series of agents possessing modified alkylation subunits have suggested that decreased solvolytic reactivity results in increased biological potency.[11,20] For the class of agents that possess sufficient reactivity to alkylate DNA, this presumably is the consequence of the chemically more stable agents more effectively reaching their biological target. Thus, the evaluation of the relative solvolytic behavior of duocarmycin SA has proven to be especially interesting. In our assessment, the alkylation subunit of duocarmycin SA was found to be the most stable of the agents examined to date and exhibits the best chemical characteristics of the naturally occurring agents yet disclosed. *N*-BOC-DSA (**2**) was found to be stable in aqueous solution at a pH of 7 and exhibited no significant solvolysis or decomposition at a pH of 5–7 over a 2-week period. At a pH of 3, *N*-BOC-DSA (**2**, $t_{1/2}$ = 177 h) proved to be substantially more stable to solvolysis than the authentic alkylation subunit of CC-1065 and *N*-BOC-CPI (**33**, $t_{1/2}$ = 37 h)[46,47] and comparable in stability to *N*-BOC-CBI (**32**, $t_{1/2}$ = 133 h),[20] Table II and Figure 1. Presumably, the difference in the solvolytic reactivity of the structurally similar agents *N*-BOC-CPI (**33**) and *N*-BOC-DSA (**2**) may be attributed to a significant electronic deactivation of the C4 carbonyl protonation required of solvolysis by the C6 methoxycarbonyl group of **2**. As detailed in the discussion of the comparative biological properties of (+)-**2**, this demonstration that the relative reactivity of the agents may be electronically diminished or fine tuned should prove useful in the design of functional analogs of the duocarmycins or CC-1065 which may predictably possess enhanced biological potency. Consistent with past observations, only products derived from addition to the least substituted carbon of the *N*-BOC-DSA cyclopropane were detected in the solvolysis reaction mixtures and may be attributed to stereoelectronic control of the ring-cleavage reaction. The near perfect alignment of the σ C7b–C8 cyclopropane bond with the cyclohexadienone π-system versus the near orthogonal alignment of the σ C7b–C8a cyclopropane bond leads to preferential C7b–C8 bond cleavage and nucleophilic addition at C8 overriding the inherent preference

(45) **31** (mixture of diastereomers): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.02 and 7.77 (br s, 1H, NH), 7.53–7.32 (m, 11H, ArH), 7.10 and 7.07 (s, 1H, C8-H), 5.94 and 5.92 (s, 1H, CH(OAc)), 5.02 (s, 2H, OCH_2Ph), 4.61–4.50 (m, 1H, C*H*HN), 4.20–4.02 (m, 2H, CH*H*N and C*H*HOR), 3.91 (s, 3H, CO_2CH_3), 3.88–3.68 (m, 2H, CH*H*OR and C1-H), 2.21 and 2.20 (s, 3H, $OCOCH_3$), 1.57 (s, 9H, $C(CH_3)_3$).

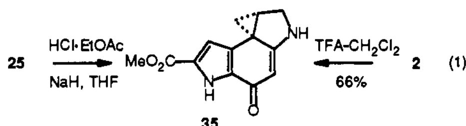
(46) Warpehoski, M. A.; Gebhard, I.; Kelly, R. C.; Krueger, W. C.; Li, L. H.; McGovren, J. P.; Prairie, M. D.; Wicnienski, N.; Wierenga, W. *J. Med. Chem.* **1988**, *31*, 590.

Table III. Calculated Gas-Phase Absolute ($\Delta H°$) and Relative ($\Delta\Delta H°$) Heats of Reaction for N-Methyladenine Alkylation[a]

agent	$\Delta H°$ (AM1, MNDO; kcal/mol)	$\Delta\Delta H°$ (AM1, MNDO; kcal/mol)
N-acetyl-CI	−12.9, −7.4	−14.8 to −11.9
N-acetylduocarmycin A	−7.6, −1.3	−9.5 to −5.8
N-acetyl-CPI	−3.9, 1.5	−5.8 to −3.0
N-acetyl-CBI	−1.6, 4.4	−3.5 to −0.1
N-acetylduocarmycin SA	1.9, 4.5	

[a] AM1: Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 902. MNDO: Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899.

expected from the developing secondary versus primary carbocation character at C8a versus that at C8, Figure 2. The solvolysis of N-BOC-DSA was followed spectrophotometrically with the disappearance of the long-wavelength UV absorption band of the DSA chromophore (345 nm) and with the appearance of a short-wavelength absorption band (257 nm) attributable to seco-N-BOC-DSA derivatives, Figure 1. Thin layer chromatography analysis of the solvolyzed agent showed the presence of two products in the reaction mixture. The more polar of the two products possessed chromatographic properties identical to those of **24**. The less polar component is presumably the product of the addition of methanol to the least substituted cyclopropane carbon of N-BOC-DSA. *Representative of the robust chemical stability of **2**, DSA (**35**) could be prepared through acid-catalyzed deprotection of **2** (TFA, CH_2Cl_2, 0 °C, 1.5 h, 66%) under anhydrous conditions without the preferential solvolysis of the cyclopropane, eq 1.* Like CPI itself, DSA (**35**) proved essentially



stable to chemical solvolysis even at a pH of 3, exhibiting little change over a 1–2-week period and only slowly undergoing solvolysis when monitored over a 2–3-month period ($t_{1/2}$ = 2154 h, $k = 8.9 \times 10^{-8}$ s^{-1}). This presumably results from preferential N-protonation versus O-protonation required of solvolysis.

The experimental observations on the relative reactivity of **2** proved consistent with expectations based on computational studies, Table III. The calculated relative gas-phase enthalpy of reaction ($\Delta\Delta H°$, AM1 and MNDO) for the reaction of adenine, or other nucleophiles including NH_3 and H_2O, with DSA versus those of **32–34** proved to follow the qualitative and relative quantitative trends observed in the solvolysis studies with DSA exhibiting the greatest stability and the lowest inherent reactivity. Most notable is the additional prediction derived from the studies that duocarmycin A may prove to be significantly less stable than duocarmycin SA as well as agents bearing the CC-1065 CPI alkylation subunit. Although we do not wish to suggest that the absolute calculated gas-phase heats of reaction presented in Table III constitute an accurate assessment of the heat of reaction for adenine alkylation within duplex DNA, the results do illustrate that the reaction of duocarmycin SA or N-BOC-DSA with free adenine constitutes a near thermal neutral reaction and certainly is not as strongly exothermic as one might intuitively expect on the basis of a simple examination of the agent structure. Consistent with this expectation, the DNA alkylation reaction of duocarmycin SA constitutes a reversible reaction[48] and one which we have interpreted as representing a near-thermal neutral, covalent alkylation stabilized by the dominate DNA–agent noncovalent binding affinity; *i.e.*, binding-driven-bonding.

(47) Boger, D. L.; Coleman, R. S.; Invergo, B. J.; Sakya, S. M.; Ishizaki, T.; Munk, S. A.; Zarrinmayeh, H.; Kitos, P. A.; Thompson, S. C. *J. Am. Chem. Soc.* **1990**, *112*, 4623.

Table IV. In Vitro Cytotoxic Activity, L1210

agent[a]	configuration	IC50	
(+)-**2** ((+)-N-BOC-DSA)	natural	0.002 μg/mL	6 nM
(−)-**2** ((−)-N-BOC-DSA)	unnatural	0.02 μg/mL	60 nM
(+)-**1** ((+)-duocarmycin SA)	natural	6 pg/mL	10 pM
(−)-**1** ((−)-duocarmycin SA)	unnatural	60 pg/mL	100 pM
(+)-duocarmycin A	natural	100 pg/mL	200 pM
(−)-duocarmycin A	unnatural	≥10 000 pg/mL	≥20 000 pM
(+)-N-BOC-CPI	natural	0.1 μg/mL	330 nM
(+)-CC-1065	natural	11 pg/mL	20 pM
(−)-CC-1065	unnatural	13 pg/mL	20 pM
(+)-CPI-PDE-I$_1$	natural	8 pg/mL	20 pM
(−)-CPI-PDE-I$_1$	unnatural	≥1250 pg/mL	≥2400 pM
(+)-CPI-CDPI$_2$	natural	12 pg/mL	20 pM
(−)-CPI-CDPI$_2$	unnatural	13 pg/mL	20 pM
(+)-CPI-CDPI$_1$	natural	17 pg/mL	40 pM
(−)-CPI-CDPI$_1$	unnatural	≥2700 pg/mL	≥6300 pM
(+)-N-BOC-CBI	natural	0.02 μg/mL	80 nM
(−)-N-BOC-CBI	unnatural	0.3 μg/mL	900 nM
(+)-CBI-CDPI$_2$	natural	3 pg/mL	5 pM
(−)-CBI-CDPI$_2$	unnatural	28 pg/mL	40 pM
(+)-CBI-CDPI$_1$	natural	2 pg/mL	5 pM
(−)-CBI-CDPI$_1$	unnatural	≥160 pg/mL	≥380 pM

[a] Both enantiomers of the seco precursors **25** and **27** proved equipotent to the corresponding enantiomers of **2** and **1**, respectively.

In Vitro Cytotoxic Activity. The results of the in vitro cytotoxic evaluation of the natural and unnatural enantiomers of **1** and **2** and their synthetic precursors are summarized in Table IV and they provided considerable more insight into the properties of the agents than an evaluation of the racemic agents might have provided. The natural enantiomers (+)-**1** and (+)-**2** proved to be 10x more potent than the corresponding unnatural enantiomers (−)-**1** and (−)-**2**, respectively. Notably, the biological activity observed with the unnatural enantiomers may be assuredly attributed to (−)-**1** and (−)-**2** (>99.9% enantiomerically pure) and is not due to contaminant natural enantiomer in the samples.[48] (+)-Duocarmycin SA (**1**) proved to be 500–1000× more potent than (+)-N-BOC-DSA (**2**) and, similarly, (−)-duocarmycin SA (**1**) was found to be 500–1000× more potent than (−)-N-BOC-DSA (**2**), indicating that the additional DNA binding affinity and DNA adduct stabilization[47] provided by the trimethoxyindole subunit of **1** substantially potentiates the cytotoxic properties of the agents. That is, we attribute the increased biological potency of **1** versus that of **2** not to the relative rates of DNA alkylation[10,4b] but rather to the simple event of non-covalent binding stabilization of the reversible covalent alkylation reaction.[47] Additionally consistent with past observations, each of the optically-active seco agents **27** and **25** displayed cytotoxic activity indistinguishable from optically-active **1** and **2**, respectively.

The relative biological potency of (+)- and (−)-N-BOC-DSA is analogous to observations made with the preceding agents **32** and **33** in which the natural enantiomers proved to be approximately 10x more potent than the unnatural enantiomers. More surprising was the level of biological activity exhibited by (−)-duocarmycin SA. In contrast to the aborted analogs of CC-1065 including CPI-PDE-I$_1$,[47] CPI-CDPI$_1$,[47] and CBI-CDPI$_1$[20] or *ent*-(−)-duocarmycin A[9,16] in which the unnatural enantiomers were found to be at least 100–500× less potent than the natural enantiomers, (−)-**1** proved to be only 10x less potent than (+)-**1**. In these observations, the properties of (−)-**1** proved to be more analogous to the properties of the unnatural enantiomers of CC-1065, CPI-CDPI$_2$, and CBI-CDPI$_2$.[17,20,47]

Of more fundamental importance was the determination of the relative cytotoxic properties of (+)-**2** with those of (+)-**32**–

(48) Confirming this expectation, (+)-**1** and (−)-**1** exhibit a different DNA alkylation selectivity providing distinct DNA alkylation profiles with (+)-**1** being approximately 5–10x more efficient than (−)-**1** in DNA alkylation rate and intensity. In addition, the DNA alkylation reaction of (+)-**1** has been demonstrated to constitute a reversible reaction.

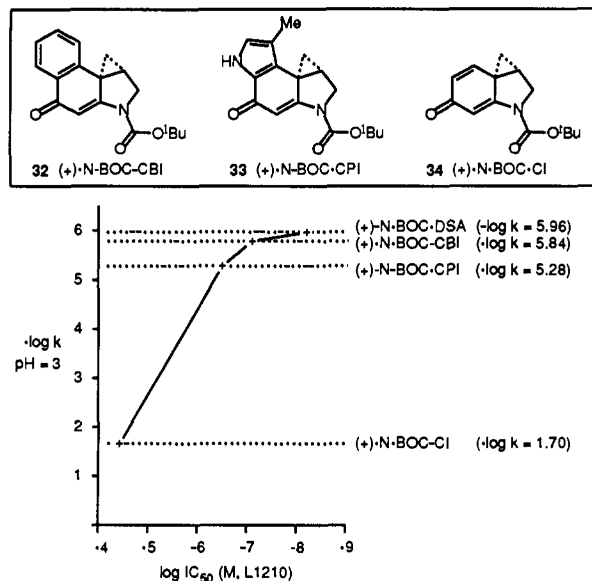


Figure 3.

34. Consistent with past proposals illustrating a direct relationship between chemical stability and biological potency,[20] (+)-*N*-BOC-DSA proved to be significantly more potent than (+)-**32**–**34**, Figure 3. A plot of -log *k* (pH 3) versus log IC_{50} (M, L1210) illustrates that (+)-*N*-BOC-DSA follows the expected qualitative relationship initially established in the comparative examinations of **32**–**34**.[20] While the results with the four agents appear to follow an ideal parabolic relationship, we do not wish to suggest that this limited comparison rigorously establishes its validity because of the significant structural perturbations between the four classes of agents. However, it is suggestive that the qualitative trend that increased solvolytic stability or decreased reactivity correlates well with increased cytotoxic potency possesses considerable merit. Potentially further contributing to the enhanced cytotoxic properties of (+)-**2** relative to those of (+)-**33** and (+)-**32** is the decreased steric hindrance for nucleophilic addition to the activated cyclopropane which would be expected to lead to a more rapid, efficient, and productive DNA alkylation reaction.[20,21]

These and additional questions will be addressed in the ongoing extension of these studies to the preparation and evaluation of duocarmycin SA/CC-1065 hybrids and to the preparation of advanced analogs of duocarmycin SA which will be reported in due course.

Experimental Section

***O*-Benzyl-*N*[2],*N*[5]-dibenzoyl-2,5-diamino-4-(bis(methoxycarbonyl)-methyl)phenol (4). Method A.** A solution of **3**[8] (18.7 g, 44.5 mmol, 1.0 equiv) in 400 mL of dry THF at −30 °C was treated with dimethyl malonate (6.47 g, 49.0 mmol, 1.1 equiv) and catalytic solid $NaOCH_3$ (724 mg, 13.4 mmol, 0.3 equiv) under N_2 and the reaction mixture was stirred for 2 h at −30 °C. The reaction mixture was made acidic with the addition of saturated aqueous NH_4Cl (100 mL) and extracted with EtOAc (500 mL). The organic extract was washed with saturated aqueous NaCl (2 × 200 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (4 × 40 cm SiO_2, 50% EtOAc–hexane) afforded pure **4** (12.5 g, 51%) as a white, crystalline solid: mp 137 °C (EtOAc–Et_2O, white needles);[23] ^1H NMR ($CDCl_3$, 400 MHz) δ 9.94 (s, 1H, NH), 8.63 (s, 1H, NH), 8.59 (s, 1H, C6-H), 7.99 (d, 2H, *J* = 7.8 Hz, C2-H and C6-H, PhCO), 7.83 (s, 1H, C3-H), 7.78 (d, 2H, *J* = 7.1 Hz, C2-H and C6-H, PhCO), 7.53–7.40 (m, 11H, ArH), 5.22 (s, 2H, $PhCH_2O$), 4.74 (s, 1H, CH), 3.69 (s, 6H, CO_2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.1 (CO_2Me), 165.5 (CONH), 165.0 (CONH), 147.5, 136.0, 134.7, 133.8, 132.8, 131.90, 131.86, 128.79, 128.75, 128.72, 128.5, 127.7, 127.2, 126.7, 125.4, 122.4, 118.6, 109.8, 71.3 (OCH_2Ph), 56.6 (CH), 53.2 ($COOCH_3$); IR (KBr) ν_{max} 3408, 3358, 3064, 3030, 2954, 1744, 1719, 1664, 1616, 1602, 1580, 1534, 1424, 1252, 1158, 1028, 709 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 685.0937 ($C_{32}H_{28}N_2O_7 + Cs^+$ requires 685.0951).

Table V. Regioselectivity Observed in the Conversion of 3 to 4

entry	T (°C)	*t* (h)	C5:C6 substn	% 4
1	0	1.5	2:1	48
2	−10	2	2.4:1	43
3	−30	2	5:1	61
4	−78	48	10:1	35[a]

[a] Reaction 40–50% complete after 48 h.

Anal. Calcd for $C_{32}H_{28}N_2O_7$: C, 69.56; H, 5.11; N, 5.07. Found: C, 69.68; H, 5.34; N, 4.91.

Method B. A solution of **3**[8] (23.1 g, 55 mmol) in 500 mL of dry THF at −30 °C was treated with dimethyl malonate (8.0 g, 60.5 mmol, 1.1 equiv) and catalytic solid $NaOCH_3$ (0.3 g, 5.5 mmol, 0.1 equiv) under Ar, and the reaction mixture was stirred at −30 °C for 6 h. The reaction mixture was made acidic with the addition of saturated aqueous NH_4Cl (100 mL) and extracted with EtOAc (500 mL). The organic extract was washed with saturated aqueous NaCl (200 mL), dried (Na_2SO_4), and concentrated to approximately 150 mL. Hexane was added until the mixture became turbid. The mixture was allowed to stand at 25 °C for 12 h during which time crystals began to form. The solution was further cooled to −10 °C for 12 h. The crystals were collected by filtration and washed with Et_2O (3 × 100 mL) to provide **4** (14.6 g, 48%; typically 45–51%, 2–55-mmol scale) as a white, crystalline solid identical to that described above.

***O*-Benzyl-*N*[2],*N*[5]-dibenzoyl-2,5-diamino-4-(bis(hydroxymethyl)methyl)phenol (5).** A suspension of **4** (8.53 g, 15.5 mmol, 1.0 equiv) in 500 mL of EtOH was treated with $NaBH_4$ (2.92 g, 75.3 mmol, 5.0 equiv) under N_2 at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and allowed to warm to 25 °C. The mixture was stirred for 2.5 h at 25 °C, made acidic with the addition of saturated aqueous NH_4Cl, and extracted with EtOAc (300 mL). The organic extract was washed with saturated aqueous NaCl (2 × 150 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (4 × 20 cm SiO_2, 83% EtOAc–hexane) afforded pure **5** (5.42 g, 71%; typically 50–71%, 4–26-mmol scale) as a white, crystalline solid: mp 184 °C (EtOAc–hexanes, white needles); ^1H NMR ($CDCl_3$, 400 MHz) δ 10.48 (s, 1H, NH), 8.65 (s, 1H, NH), 8.40 (s, 1H, C6-H), 8.03 (d, 2H, *J* = 7.0 Hz, C2-H and C6-H, PhCO), 7.84 (s, 1H, C3-H), 7.80 (d, 2H, *J* = 7.1 Hz, C2-H and C6-H, PhCO), 7.54–7.39 (m, 11H, ArH), 5.21 (s, 2H, OCH_2Ph), 4.37 (br s, 2H, OH), 4.08 (dd, 2H, *J* = 10.4, 6.2 Hz, CH_2OH), 3.86 (dd, 2H, *J* = 10.4, 6.0 Hz), 3.28 (p, 1H, *J* = 6.5 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 165.2 and 165.0 (CONH), 149.6, 137.1, 134.8, 134.51, 134.47, 131.8, 131.6, 128.71, 128.66, 128.5, 128.4, 127.8, 127.5, 127.3, 127.3, 124.7, 124.3, 110.6, 70.1 (OCH_2Ph), 63.0 (CH_2OH), 44.7 (CH); IR (KBr) ν_{max} 3414, 3370, 1649, 1542, 1478, 1419, 1301, 1259, 1061, 1029, 703 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 629.1034 ($C_{30}H_{28}N_2O_5 + Cs^+$ requires 619.1053).

Anal. Calcd for $C_{30}H_{28}N_2O_5$: C, 72.56; H, 5.68; N, 5.64. Found: C, 72.56; H, 5.68; N, 5.49.

***O*-Benzyl-*N*[2],*N*[5]-dibenzoyl-2,5-diamino-4-(2,2-dimethyl-1,3-dioxan-5-yl)phenol (6).** A solution of **5** (3.6 g, 7.25 mmol, 1.0 equiv) in 75 mL of dry DMF was treated with 2,2-dimethoxypropane (1.50 g, 14.5 mmol, 2.0 equiv) and catalytic TsOH (100 mg, 0.53 mmol, 0.06 equiv), and the reaction mixture was stirred for 24 h at 25 °C. The reaction mixture was poured onto 300 mL of distilled H_2O and filtered. The collected white solid was dissolved in CH_2Cl_2 (200 mL). The solution was washed with saturated aqueous NaCl (2 × 50 mL), dried (Na_2SO_4), and concentrated in vacuo to afford **6** (3.89 g, 100%; typically 99–100%, 0.4–13-mmol scale) as a white, crystalline solid: mp 236 °C (CH_2Cl_2–Et_2O, white needles); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.62 (s, 1H, NH), 8.55 (s, 1H, C6-H), 8.48 (s, 1H, NH), 7.91 (d, 2H, *J* = 7.1 Hz, C2-H and C6-H, PhCO), 7.90 (s, 1H, C3-H), 7.81 (d, 2H, *J* = 7.1 Hz, C2-H and C6-H, PhCO), 7.58–7.37 (m, 11H, ArH), 5.20 (s, 2H, OCH_2Ph), 4.09 (dd, 2H, *J* = 12.0, 9.4 Hz, CH_2O), 4.00 (dd, 2H, *J* = 12.0, 5.8 Hz, CH_2O), 3.34 (tt, 1H, *J* = 9.2, 5.8 Hz, CH), 1.43 (s, 3H, CH_3), 1.42 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.7 and 165.1 (CONH), 146.3, 136.1, 134.8, 134.7, 132.0, 131.9, 131.7, 128.79, 128.75, 128.7, 128.4, 127.6, 127.4, 126.9, 125.8, 123.8, 119.1, 108.8, 98.9 (OCO), 71.3 (OCH_2Ph), 63.7 (OCH_2), 37.7 (CH), 27.1 and 20.6 (CH_3); IR (KBr) ν_{max} 3306, 2996, 1647, 1536, 1482, 1406, 1282, 1255, 1196, 1077, 834, 710, 694 cm^{-1}; FABHRMS (NBA-CsI) *m/e* 699.1399 ($C_{33}H_{32}N_2O_5 + Cs^+$ requires 669.1366).

Anal. Calcd for $C_{33}H_{32}N_2O_5$: C, 73.86; H, 6.01; N, 5.22. Found: C, 73.81; H, 6.03; N, 5.30.

***N*[1],*N*[4]-Dibenzoyl-5-(2,2-dimethyl-1,3-dioxan-5-yl)-2-(benzyloxy)-*p*-benzoquinone Diimine (7).** A solution of **6** (434 mg, 0.81 mmol, 1.0

equiv) in 20 mL of dry $CHCl_3$ was treated with $Pb(OAc)_4$ (380 mg, 0.81 mmol, 1.0 equiv) under N_2 at 0 °C. The reaction mixture was stirred for 1 h at 0 °C before being allowed to warm to 25 °C and then was stirred for 1.5 h at 25 °C. The reaction mixture was filtered through a layer of Celite, and the filtrate was washed with saturated aqueous $NaHCO_3$ (2 × 10 mL) and saturated aqueous NaCl (2 × 10 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (2 × 20 cm SiO_2, 50% EtOAc-hexane) afforded **7** (433 mg, 100%; typically 92–100%, 0.8–7-mmol scale) as a yellow, amorphous solid: mp 76–86 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.84 (d, 2H, *J* = 7.1 Hz, C2-H and C6-H, PhCO), 7.66 (d, 2H, *J* = 7.1 Hz, C2-H and C6-H, PhCO), 7.60 (t, 1H, *J* = 7.4 Hz, C4-H, PhCO), 7.49 (t, 1H, *J* = 7.5 Hz, C4-H, PhCO), 7.46 (t, 2H, *J* = 7.4 Hz, C3-H and C5-H, PhCO), 7.34 (t, 2H, *J* = 7.5 Hz, C3-H and C5-H, PhCO), 7.31 (d, 1H, *J* = 0.6 Hz, C3-H), 7.20 (t, 1H, *J* = 7.3 Hz, C4-H, $PhCH_2O$), 7.13 (t, 2H, *J* = 7.0 Hz, C3-H and C5-H, $PhCH_2O$), 6.91 (d, 2H, *J* = 7.2 Hz, C2-H and C6-H, $PhCH_2O$), 5.85 (s, 1H, C6-H), 4.71 (s, 2H, OCH_2Ph), 4.25 (dd, 2H, *J* = 12.0, 4.1 Hz, CH_2O), 4.00 (dd, 2H, *J* = 12.0, 5.2 Hz, CH_2O), 3.39 (p, 1H, *J* = 4.8 Hz, CH), 1.49 (s, 3H, CH_3), 1.40 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.3 and 178.7 (CON=), 156.9, 153.4, 150.4, 145.5, 133.6, 133.1, 132.8, 132.3, 132.2, 131.5, 129.2, 128.7, 128.6, 128.3, 127.9, 101.9, 98.2 (OCO), 71.1 (OCH_2Ph), 63.1 (CH_2O), 33.3 (CH), 24.9 and 22.4 (CH_3); IR (KBr) ν_{max} 2996, 1665, 1617, 1595, 1451, 1275, 1237, 1194, 1056, 1024, 831 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 667.1229 ($C_{33}H_{30}N_2O_5 + Cs^+$ requires 667.1209).

Anal. Calcd for $C_{33}H_{30}N_2O_5$: C, 74.14; H, 5.66; N, 5.24. Found: C, 73.99; H, 5.82; N, 5.07.

***O*-Benzyl-*N*²,*N*⁵-dibenzoyl-2,5-diamino-4-(2,2-dimethyl-1,3-dioxan-5-yl)-3-(3,3-dimethoxy-2-oxopropyl)phenol (8).** A solution of **7** (3.40 g, 6.4 mmol, 1.0 equiv) in 250 mL of dry THF was treated with 1-(1-(dimethoxymethyl)ethenyl)pyrrolidine (2.18 g, 12.7 mmol, 2.0 equiv) under N_2 at 25 °C. After 10 min, the mixture was treated with 60 mL of phosphate buffer solution (Fisher, pH = 4.0) and the reaction mixture was stirred for 24 h at 25 °C. The reaction mixture was poured onto H_2O (250 mL) and extracted with EtOAc (500 mL). The organic extract was washed with saturated aqueous $NaHCO_3$ (2 × 100 mL) and saturated aqueous NaCl (2 × 100 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (5 × 30 cm SiO_2, 50% EtOAc–hexane) afforded **8** (2.41 g, 58%; typically 56–61%; 0.05–7-mmol scale) as a pale yellow, crystalline solid: mp 218 °C (EtOAc–hexane, pale yellow needles); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.99 (s, 1H, NH), 8.30 (s, 1H, NH), 7.90 (d, 2H, *J* = 7.0 Hz, C2-H and C6-H, PhCO), 7.84 (d, 2H, *J* = 7.2 Hz, C2-H and C6-H, PhCO), 7.67 (s, 1H, C6-H), 7.56–7.26 (m, 11H, ArH), 5.13 (s, 2H, OCH_2Ph), 4.55 (s, 1H, *C*H(OMe)$_2$), 4.06 (s, 2H, CH_2C=O), 3.98 (dd, 2H, *J* = 12.2, 8.6 Hz, CH_2O), 3.87 (dd, 2H, *J* = 12.2, 6.5 Hz, CH_2O), 3.62 (p, 1H, *J* = 6.8 Hz), 3.41 (s, 6H, OCH_3), 1.31 (s, 3H, CH_3), 0.77 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 202.1 (C=O), 167.0 and 166.4 (CONH), 153.5, 137.2, 137.0, 134.9, 134.6, 134.2, 132.0, 131.7, 128.7, 128.52, 128.47, 128.4, 128.0, 127.9, 127.8, 127.2, 126.0, 113.9 (*C*H(OMe)$_2$), 103.9, 97.7 (OCO), 69.8 (OCH_2Ph), 61.5 (CH_2O), 54.7 (OCH_3), 38.5 (*C*H(CH_2O)$_2$), 38.1 (*C*H$_2$CO), 28.0 (CH_3), 20.3 (CH_3); IR (KBr) ν_{max} 3284, 2985, 1734, 1660, 1606, 1504, 1488, 1275, 1221, 1077, 911 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 785.1870 ($C_{38}H_{40}N_2O_8 + Cs^+$ requires 785.1839).

Anal. Calcd for $C_{38}H_{40}N_2O_8$: C, 69.92; H, 6.18; N, 4.29. Found: C, 70.11; H, 6.10; N, 4.30.

The yield of conversion of **7** to **8** proved lower when this reaction was conducted under identical conditions with the enamine hydrolysis run for 3 h (46%) or 12 h (42%) rather than for 24 h (56–61%).

***N*⁵-Benzoyl-5-amino-1-benzoyl-7-(benzyloxy)-4-(bis(hydroxymethyl)methyl)-2-(dimethoxymethyl)indole (9).** A solution of **8** (2.39 g, 3.67 mmol, 1.0 equiv) in 130 mL of CH_3OH was treated with anhydrous 2N HCl–CH_3OH (3.67 mL, 2.0 equiv) under N_2 at 25 °C and the reaction mixture was stirred for 2 h at 25 °C. The reaction mixture was poured onto H_2O (250 mL) and extracted with EtOAc (500 mL). The organic extract was washed with saturated aqueous $NaHCO_3$ (2 × 100 mL) and saturated aqueous NaCl (2 × 100 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (5 × 20 cm SiO_2, 66% EtOAc–hexane) afforded **9** (1.76 g, 81%; typically 70–91%, 0.03–4-mmol scale) as a pale yellow, amorphous solid: mp 82–86 °C (EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.98 (br s, 1H, NH), 7.92 (d, 2H, *J* = 7.4 Hz, C2-H and C6-H, PhCO), 7.51–7.38 (m, 6H, ArH), 7.23–7.17 (m, 6H, ArH), 6.90 (d, 2H, *J* = 6.3 Hz, C2-H and C6-H, OCH_2Ph), 6.83 (s, 1H, C3-H), 5.75 (s, 1H, *C*H(OMe)$_2$), 4.71 (s, 2H, OCH_2Ph), 4.10 (d, 4H, *J* = 5.9 Hz, *C*H$_2$OH), 3.54 (p, 1H, *J* = 5.9 Hz, *C*H(CH_2OH)$_2$), 3.27 (s, 6H, OCH_3), 2.82 (br s, 2H, OH); ^{13}C NMR (acetone-d_6, 100 MHz) δ 171.0 and 165.9 (CO), 144.7, 138.1, 137.2, 136.2, 136.0, 134.1, 132.6, 132.2, 130.4, 129.3, 129.2, 128.9, 128.42, 128.39, 128.1, 105.9, 105.3, 98.7, 71.0 (OCH_2Ph), 63.1 (CH_2OH), 53.4, 47.1 (OCH_3), 30.5 (*C*H(CH_2OH)$_2$); IR (KBr) ν_{max} 3391, 2932, 1702, 1654, 1600, 1360, 1275, 1050, 981 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 727.1420 ($C_{35}H_{34}N_2O_7 + Cs^+$ requires 727.1420).

Anal. Calcd for $C_{35}H_{34}N_2O_7$: C, 70.68; H, 5.77; N, 4.71. Found: C, 70.69; H, 5.62; N, 4.88.

In addition to **9**, **11**[26] was occasionally isolated from the reaction mixture. In these instances, isolated **11** was resubjected to the reaction conditions (2N HCl–CH_3OH, 2 h, 25 °C) to provide **9** in an overall yield of 78–85% from **8**. Treatment of **8** with Amberlyst 15 (40–500 mg/mmol, CH_3OH–THF, 25 °C, 24 h, 72–77%) proved slightly less effective while HCl–EtOAc/THF (25 °C, 2 h, 15–30%), HOAc–THF–H_2O (4:2:1, 0%), and 4-Å molecular sieves or $MgSO_4$ (THF or CH_2Cl_2, 25 °C, 48 h, no reaction) failed to effectively provide **9**.

5-(Benzyloxy)-3,6-dibenzoyl-7-(dimethoxymethyl)-1-(hydroxymethyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole (10). A solution of **9** (133 mg, 0.22 mmol, 1.0 equiv) in 20 mL of dry THF was treated with Ph_3P (88 mg, 0.34 mmol, 1.5 equiv) and diethyl azodicarboxylate (53 μL, 0.34 mmol, 1.5 equiv) under N_2, and the reaction mixture was stirred for 2 h at 25 °C. The reaction mixture was diluted with EtOAc (100 mL), and the solution was washed with saturated aqueous NaCl (2 × 50 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (2 × 15 cm SiO_2, 50% EtOAc–hexane) afforded **10** (129 mg, 100%; typically 86–100%, 0.1–3-mmol scale) as a white, crystalline solid: mp 70–71 °C (EtOAc–hexane); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.97 (br s, 1H, C4-H), 7.63–7.60 (m, 3H, ArH), 7.53–7.49 (m, 5H, ArH), 7.39 (t, 2H, *J* = 7.7 Hz, ArH), 7.25 (br s, 3H, ArH), 6.99 (br s, 2H, ArH), 6.84 (s, 1H, C8-H), 5.70 (s, 1H, CH(OMe)$_2$), 4.81 (br s, 2H, OC*H*$_2$Ph), 4.26 (t, 1H, *J* = 10.6 Hz, C*H*HN), 4.11 (dd, 1H, *J* = 14.2, 7.0 Hz, CH*H*N), 4.04 (br t, 1H, *J* = 6.6 Hz, C*H*HOH), 3.96 (br t, 1H, *J* = 5.5 Hz, CH*H*OH), 3.76–3.68 (m, 2H, C1-H and OH), 3.26 (s, 3H, OCH_3), 3.22 (s, 3H, OCH_3); ^{13}C NMR (acetone-d_6, 100 MHz) δ 171.1 and 168.6 (CONH), 145.6, 139.4, 138.8, 137.1, 136.3, 134.1, 132.7, 132.6, 132.5, 130.7, 130.2, 129.2, 129.1, 128.9, 128.4, 128.3, 127.8, 104.2, 98.7, 70.9 (OCH_2Ph), 64.5 (CH_2O), 56.1 (CH_2N), 53.6 and 53.2 (OCH_3), 43.8 (C1); IR (KBr) ν_{max} 3434, 2932, 1702, 1627, 1595, 1494, 1408, 1269, 1125, 1050, 692 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 709.1332 ($C_{35}H_{32}N_2O_6 + Cs^+$ requires 709.1315).

Anal. Calcd for $C_{35}H_{32}N_2O_6$: C, 72.90; H, 5.59; N, 4.86. Found: C, 72.70; H, 5.19; N, 4.93.

5-(Benzyloxy)-3-((*tert*-butyloxy)carbonyl)-7-(dimethoxymethyl)-1-(hydroxymethyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole (21). A solution of **10** (653 mg, 1.13 mmol, 1.0 equiv) in 20 mL of EtOH was treated with 40 mL of 98% NH_2NH_2–H_2O under N_2, and the reaction mixture was stirred for 34 h at 145 °C (bath temperature). The reaction mixture was cooled, poured onto ice–water (100 mL), and extracted with EtOAc (3 × 100 mL). The organic extract was washed with H_2O (50 mL) and saturated aqueous NaCl (50 mL), dried (Na_2SO_4), and concentrated in vacuo. A solution of the residue in 38 mL of dry THF was treated with di-*tert*-butyl dicarbonate (740 mg, 3.39 mmol, 3.0 equiv) in 2 mL of dry THF under N_2, and the reaction mixture was stirred for 20 min at 25 °C. The reaction mixture was diluted wtih EtOAc (50 mL), and the solution was washed with saturated aqueous $NaHCO_3$ (1 × 25 mL) and saturated aqueous NaCl (1 × 25 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (3 × 20 cm SiO_2, 40% EtOAc–hexane) afforded **21** (339 mg, 64%; typically 54–67%, 0.05–3-mmol scale) as a pale yellow, amorphous solid: mp 146–148 °C (EtOAc–hexane); ^{1}H NMR (acetone-d_6, 400 MHz) δ 10.11 (br s, 1H, NH), 7.69 (br s, 1H, C4-H), 7.57 (br s, 2H, C2-H and C6-H, OCH_2Ph), 7.41 (tt, 2H, *J* = 7.4, 1.4 Hz, C3-H and C5-H, OCH_2Ph), 7.34 (tt, 1H, *J* = 7.3, 2.6 Hz, C4-H, OCH_2Ph), 6.44 (s, 1H, C8-H), 5.60 (s, 1H, CH(OMe)$_2$), 5.22 (s, 2H, OCH_2Ph), 4.08–3.98 (m, 4H, CH_2O, CH_2N), 3.67 (br s, 1H, OH), 3.58–3.54 (m, 1H, C1-H), 3.32 (s, 3H, OCH_3), 3.31 (s, 3H, OCH_3), 1.56 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (acetone-d_6, 100 MHz) δ 153.0 (C=O), 145.6, 138.2, 137.4, 132.5, 129.2, 129.1, 128.6, 128.5, 128.2, 126.1, 124.5, 99.5, 94.6, 70.6 (OCH_2Ph), 65.0 (CH_2OH), 60.5 (*C*(CH_3)$_3$), 53.0 (CH_2N), 52.9 and 52.7 (OCH_3), 43.3 (C1), 28.7 (C(*C*H$_3$)$_3$); IR (KBr) ν_{max} 1679, 1506, 1410, 1391, 1365, 1347, 1170, 1140, 1109, 1039 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 601.1397 ($C_{26}H_{32}N_2O_6 + Cs^+$ requires 601.1315).

Anal. Calcd for $C_{26}H_{32}N_2O_6$: C, 66.65; H, 6.88; N, 5.98. Found: C, 66.53; H, 6.89; N, 6.21.

Occasionally, a minor amount of the product of monodeprotection of the indole *N*-benzoyl group was observed (5–30%).[33] In these instances,

the monodeprotected material was subjected to conditions identical to those detailed above. In this manner, the overall yields for the conversion of **10** to **21** were 55–70%.

5-(Benzyloxy)-3-((*tert*-butyloxy)carbonyl)-7-formyl-1-(hydroxymethyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole (22). A solution of **21** (162 mg, 0.34 mmol) in 12 mL of DMSO–phosphate buffer solution (Fisher, pH = 4)–dioxane (1:2:12) was stirred for 15 h at 110 °C. The reaction mixture was poured onto ice–water (50 mL) and extracted with EtOAc (100 mL). The organic extract was washed with saturated aqueous $NaHCO_3$ (2 × 50 mL) and saturated aqueous NaCl (2 × 50 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (2 × 15 cm SiO_2, 40% EtOAc–hexane) afforded **22** (141 mg, 96%; typically 91–100%, 0.1–0.5-mmol scale) as a yellow, amorphous solid: mp 104–106 °C (EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.74 (s, 1H, CHO), 9.31 (br s, 1H, NH), 7.88 (br s, 1H, C4-H), 7.49–7.35 (m, 5H, ArH), 7.14 (s, 1H, C8-H), 5.22 (s, 2H, OCH_2Ph), 4.15 (t, 1H, *J* = 11.0 Hz, C*H*HN), 4.00 (dd, 1H, *J* = 11.4, 4.3 Hz, C*H*HOH), 3.91 (dd, 1H, *J* = 10.6, 5.3 Hz, CH*H*OH), 3.86 (dd, 1H, *J* = 11.8, 6.4 Hz, CH*H*N), 3.75 (m, 1H, C1-H), 1.57 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.8 (CHO), 152.5 (CO_2), 145.6, 138.3, 136.1, 128.5, 128.2, 127.9, 126.6, 124.3, 113.5, 112.3, 98.4, 97.8, 70.3 (OCH_2Ph), 64.9 (CH_2OH), 64.7 ($C(CH_3)_3$), 51.8 (CH_2N), 41.8 (C1), 28.4 ($C(CH_3)_3$); IR (neat) ν_{max} 3274, 2974, 1665, 1526, 1435, 1414, 1387, 1339, 1178, 1136 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 555.0896 ($C_{24}H_{26}N_2O_5$ + Cs^+ requires 555.0896).

Anal. Calcd for $C_{24}H_{26}N_2O_5$: C, 68.23; H, 6.20; N, 6.63. Found: C, 68.20; H, 6.11; N, 6.67.

Methyl 5-(Benzyloxy)-3-((*tert*-butyloxy)carbonyl)-1-(hydroxymethyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (23). Method A. A solution of **22** (526 mg, 1.25 mmol, 1.0 equiv) in 43 mL of CH_2Cl_2 was treated with NaCN (613 mg, 12.5 mmol, 10 equiv), 0.3 M HOAc–CH_3OH (0.8 mL), and MnO_2 (544 mg, 6.25 mmol, 5.0 equiv) under N_2. After the reaction mixture was stirred for 5 h at 25 °C, NaCN (613 mg, 12.5 mmol, 10 equiv), 0.3 M HOAc–CH_3OH (0.8 mL), and MnO_2 (544 mg, 6.25 mmol, 5.0 equiv) were added and the mixture was stirred for 16 h at 25 °C. The reaction mixture was poured onto H_2O (150 mL) and extracted with EtOAc (300 mL). The organic extract was washed with H_2O (2 × 150 mL), saturated aqueous $NaHCO_3$ (2 × 150 mL), and saturated aqueous NaCl (2 × 150 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (2 × 20 cm SiO_2, 40% EtOAc–hexane) afforded **23** (501 mg, 89%; typically 76–92%, 0.02–1.25-mmol scale) as a pale yellow solid; mp 160–161 °C (EtOAc–hexane, yellow powder); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.11 (br s, 1H, NH), 7.83 (br s, 1H, C4-H), 7.52–7.36 (m, 5H, ArH), 7.10 (br s, 1H, C8-H), 5.21 (s, 2H, OCH_2Ph), 4.15 (t, 1H, *J* = 11.2 Hz, C*H*HN), 4.02 (dd, 1H, *J* = 11.2, 4.1 Hz, CH*H*OH), 3.93–3.84 (m, 2H, CH*H*N and C*H*HOH), 3.91 (s, 3H, CO_2CH_3), 3.73 (m, 1H, C1-H), 1.57 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.9 (CO_2Me), 152.6 (OCON), 145.4, 138.2, 136.3, 128.6, 128.3, 128.1, 127.5, 125.2, 124.4, 106.0, 98.4, 96.3, 70.4 (OCH_2Ph), 68.4 (CH_2OH), 60.4 ($OC(CH_3)_3$), 51.9 and 51.8 (OCH_3 and CH_2N), 41.9 (C1), 28.5 ($C(CH_3)_3$); IR (neat) ν_{max} 3295, 2974, 1691, 1531, 1435, 1403, 1392, 1371, 1344, 1248, 1221, 1157, 1136 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 585.1008 ($C_{25}H_{28}N_2O_6$ + Cs^+ requires 585.1002).

Anal. Calcd for $C_{25}H_{28}N_2O_6$: C, 66.35; H, 6.25; N, 6.19. Found: C, 66.33; H, 6.00; N, 6.19.

Method B. A solution containing NaCN (98 mg, 2.0 mmol, 5 equiv) and MnO_2 (175 mg, 2.0 mmol, 5 equiv) in 8 mL of CH_3OH was treated with a solution containing **22** (169 mg, 0.40 mmol) in 2 mL of CH_3OH at 0 °C under Ar. The reaction mixture was allowed to warm to 25 °C and was stirred for 12 h. The resulting suspension was filtered through a pad of Celite (2 × 10 mL of EtOAc wash). The organic layer was washed with H_2O (10 mL) and saturated aqueous NaCl (10 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (2 × 20 cm SiO_2, 40% EtOAc–hexane) provided **23** (168 mg, 92%) as a pale yellow solid identical to that described above.

Methyl 3-((*tert*-Butyloxy)carbonyl)-5-hydroxy-1-(hydroxymethyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (24). A solution of **23** (137 mg, 0.30 mmol) in 20 mL of THF was treated with 25% aqueous HCO_2NH_4 (0.67 mL) and 10% Pd–C (67 mg) under Ar, and the reaction mixture was stirred for 6 h at 25 °C. The reaction mixture was filtered through Celite (2 × 20 mL of EtOAc wash). The organic layer was washed with H_2O (25 mL) and saturated aqueous NaCl (25 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (2 × 20 cm SiO_2, 50% EtOAc–hexane) afforded **24** (101 mg, 92%; typically 80–92%, 0.04–1.2 mmol scale) as a white, crystalline solid: mp 159 °C dec (EtOAc–hexane, white powder); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.19 (br s, 1H, NH), 7.96 (br s, 1H, OH), 7.58 (s, 1H, C4-H), 7.08 (d, 1H, *J* = 2.0 Hz, C8-H), 4.23 (br s, 2H, CH_2N), 4.12 (dd, 2H, *J* = 11.4, 10.5 Hz, CH_2OH), 3.97 (s, 3H, CO_2CH_3), 3.76 (br d, 1H, *J* = 6.9 Hz, C1-H), 2.81 (br s, 1H, CH_2OH), 1.20 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.5 (CO_2Me), 152.4 (OCON), 143.2, 138.6, 127.6, 125.2, 124.4, 107.6, 105.2, 98.8, 64.7 (CH_2OH), 60.4 ($OC(CH_3)_3$), 52.1 and 52.0 (OCH_3 and CH_2N), 41.5 (C1), 28.1 ($C(CH_3)_3$); IR (KBr) ν_{max} 3391, 1702, 1670, 1440, 1414, 1387, 1349, 1253, 1157 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 495.0547 ($C_{18}H_{22}N_2O_6$ + Cs^+ requires 495.0532).

Anal. Calcd for $C_{18}H_{22}N_2O_6$: C, 59.66, H, 6.12; N, 7.73. Found: C, 59.61; H, 6.23; N, 7.63.

Methyl 3-((*tert*-Butyloxy)carbonyl)-5-hydroxy-1-(hydroxymethyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate, Bis((*R*)-*O*-acetylmandelate) ester) (29). A solution of (±)-**14** (101 mg, 0.28 mmol, 1.0 equiv) and (*R*)-(–)-*O*-acetylmandelic acid (136 mg, 0.70 mmol, 2.5 equiv) in 7 mL of CH_2Cl_2 was treated with EDCI (161 mg, 0.84 mmol, 3 equiv) and catalytic DMAP (1 mg, 8 μmol, 0.04 equiv) under N_2 at 0 °C, and the reaction mixture was stirred for 2.5 h at 0 °C. The reaction mixture was poured onto H_2O (20 mL) and extracted with EtOAc (30 mL). The organic extract was washed with aqueous 1N HCl (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and saturated aqueous NaCl (10 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (1 × 20 cm SiO_2, 40% EtOAc–hexane) afforded (1*RS*,2′*R*,2″*R*)-**29** (168 mg, 84%; typically 80–96%, 0.04–0.3-mmol scale) as a pale yellow oil. The mixture was resolved by preparative HPLC. A solution of (1*RS*,2′*R*,2″*R*)-**29** (340 mg in 0.8 mL of CH_2Cl_2) was separated by chromatography using an Alltech-22.5 mm × 25-cm column packed with SiO_2 (10 μm) using 5% EtOAc–CH_2Cl_2 eluant at a flow rate of 20 mL/min. The effluent was monitored at 254 nm, and the diastereomeric esters (1*R*,2′*R*,2″*R*)-**29** and (1*S*,2′*R*,2″*R*)-**29** eluted with retention times of 20.1 and 26.9 min, respectively. The separated diastereomers were collected, washed with saturated aqueous $NaHCO_3$, and dried (Na_2SO_4), and the solvent was removed in vacuo to afford (1*R*,2′*R*,2″*R*)-**29** (t_R = 20.1 min, 133 mg) and (1*S*,2′*R*,2″*R*)-**29** (t_R = 26.9 min, 137 mg) with a total 79% recovery (typically 79–85%). HPLC analysis of the separated diastereomers indicated that both were >99.9% pure.

(1*R*,2′*R*,2″*R*)-29: corresponds to the unnatural enantiomer; t_R = 20.1 min; pale crystalline solid, mp 161–162 °C (EtOAc–hexane, pale powder); $[\alpha]^{23}_D$ –63° (*c* 0.4, CH_3OH), $[\alpha]^{23}_D$ –52° (*c* 0.016, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.35 (br s, 1H, NH), 7.70 (br s, 1H, C4-H), 7.60 (br d, 2H, *J* = 3.3 Hz, ArH), 7.46 (t, 3H, *J* = 2.8 Hz, ArH), 7.40–7.37 (m, 2H, ArH), 7.36–7.33 (m, 3H, ArH), 7.10 (s, 1H, C8-H), 6.00 (s, 1H, CH(OAc)), 5.90 (s, 1H, CH(OAc)), 4.58 (dd, 1H, *J* = 10.9, 4.1 Hz, CH*H*N), 4.02 (m, 1H, CH*H*N), 3.92 (s, 3H, CO_2CH_3), 3.89 (m, 1H, CH*H*OR), 3.82–3.63 (m, 2H, CH*H*OR and C1-H), 2.29 (s, 3H, $OCOCH_3$), 2.17 (s, 3H, $OCOCH_3$), 1.48 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.1, 170.2, 168.7, 166.9 and 161.4, 152.1 (OCON), 137.1, 135.5, 133.3, 131.9, 129.8, 129.2, 129.1, 128.7, 127.6, 127.4, 126.1, 125.4, 118.0, 106.6, 105.7, 75.4 and 74.4 (COCH(OAc)Ph), 66.3 (CH_2O), 60.2 ($C(CH_3)_3$), 51.9 and 51.6 (CH_2N and CO_2CH_3), 38.8 (C1), 28.2 ($C(CH_3)_3$), 20.6 and 20.5 ($COCH_3$); IR (neat) ν_{max} 3359, 2974, 1739, 1712, 1691, 1440, 1371, 1237, 1152, 1061 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 847.1486 ($C_{38}H_{38}N_2O_{12}$ + Cs^+ requires 847.1479).

Anal. Calcd for $C_{38}H_{38}N_2O_{12}$: C, 63.86; H, 5.36; N, 3.92. Found: C, 64.10; H, 5.70; N, 3.94.

(1*S*,2′*R*,2″*R*)-29: corresponds to the natural enantiomer; t_R = 26.9 min; pale yellow, amorphous solid, mp 85–88 °C (EtOAc–hexane); $[\alpha]^{22}_D$ –78° (*c* 5.7, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.36 (br s, 1H, NH), 7.68 (br s, 1H, C4-H), 7.60 (br d, 2H, *J* = 3.5 Hz, ArH), 7.46 (t, 3H, *J* = 3.4 Hz, ArH), 7.36–7.29 (m, 5H, ArH), 7.05 (s, 1H, C8-H), 6.02 (s, 1H, CH(OAc)), 5.86 (s, 1H, CH(OAc)), 4.51 (dd, 1H, *J* = 10.7, 7.1 Hz, CH*H*N), 4.16–4.01 (m, 2H, C*H*HN and CH*H*OR), 3.92 (s, 3H, CO_2CH_3), 3.84 (m, 2H, CH*H*OR and C1-H), 2.29 (s, 3H, $OCOCH_3$), 2.15 (s, 3H, $OCOCH_3$), 1.48 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.0, 170.1, 168.6, 166.9 and 161.4, 152.0 (OCON), 137.1, 135.4, 133.2, 131.9, 129.7, 129.2, 129.1, 129.0, 128.3, 127.5, 127.2, 126.1, 125.3, 118.0, 106.5, 105.6, 75.3 and 74.3 (COCH(OAc)Ph), 66.4 (CH_2O), 65.6 ($C(CH_3)_3$), 51.8 and 51.7 ((CH_2N and CO_2CH_3), 38.8 (C1), 28.2 ($C(CH_3)_3$), 20.6 and 20.4 ($COCH_3$); IR (neat) ν_{max} 3364, 2974, 1739, 1713, 1697, 1440, 1364, 1236, 1149, 1056 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 847.1499 ($C_{38}H_{38}N_2O_{12}$ + Cs^+ requires 847.1479).

Anal. Calcd for $C_{38}H_{38}N_2O_{12}$: C, 63.86; H, 5.36; N, 3.92. Found: C, 63.49; H, 5.38; N, 3.99.

(–)-(1*S*)-Methyl 3-((*tert*-Butyloxy)carbonyl)-5-hydroxy-1-(hydroxymethyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate [(–)-(1*S*)-24]. A solution of (1*S*,2′*R*,2″*R*)-**29** (76.2 mg, 0.11 mmol, 1.0 equiv) in 10 mL

of CH_3OH was treated with 0.5 M $NaOCH_3$ in CH_3OH (0.54 mL, 2.5 equiv) under N_2 at 0 °C. The reaction mixture was stirred for 1 h at 0 °C, made acidic with the addition of aqueous 1N HCl, poured onto H_2O (50 mL), and extracted with EtOAc (50 mL). The organic extract was washed with saturated aqueous $NaHCO_3$ (25 mL) and saturated aqueous NaCl (2 × 25 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (2 × 15 cm SiO_2, 50% EtOAc–hexane) afforded (−)-(1*S*)-**24** (35.8 mg, 93%) as a pale yellow, crystalline solid with spectroscopic characteristics identical with those of the racemic material: $[\alpha]^{22}_D$ −22.6° (*c* 1.6, CH_3OH); mp 142–144 °C (Et_2O–hexane, pale yellow powder).

***ent*-(+)-(1*R*)-Methyl 3-((*tert*-Butyloxy)carbonyl)-5-hydroxy-1-(hydroxymethyl)-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate [*ent*-(+)-(1*R*)-24].** A solution of (1*R*,2′*R*,2″*R*)-**29** (28.0 mg, 0.039 mmol, 1.0 equiv) in 3.7 mL of CH_3OH was treated with 0.5 M $NaOCH_3$ in CH_3OH (0.20 mL, 2.5 equiv) under Ar at 0 °C. The reaction mixture was stirred for 1 h at 0 °C, made acidic with the addition of aqueous 1N HCl, poured onto H_2O (10 mL), and extracted with EtOAc (30 mL). The organic extract was washed with saturated aqueous $NaHCO_3$ (5 mL) and saturated aqueous NaCl (5 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (1 × 15 cm SiO_2, 50% EtOAc–hexane) afforded (+)-(1*R*)-**24** (13.1 mg, 92%) as a pale yellow, crystalline solid with spectroscopic characteristics identical with those of the racemic material: $[\alpha]^{22}_D$ +22.4° (*c* 0.7, CH_3OH); mp 156 °C (Et_2O–hexane, pale yellow powder).

Methyl 3-((*tert*-Butyloxy)carbonyl)-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (25). A solution of **24** (32.8 mg, 0.091 mmol, 1.0 equiv) in 0.5 mL of CH_2Cl_2 was treated with Ph_3P (71.5 mg, 0.27 mmol, 3.0 equiv) and CCl_4 (79 μL, 0.82 mmol, 9.0 equiv) under N_2. The reaction mixture was stirred for 3 h at 25 °C in the dark before being concentrated in vacuo. Flash chromatography (2 × 10 cm SiO_2, 33% EtOAc–hexane) afforded **25** (31.7 mg, 92%) as a pale yellow, crystalline solid: mp 247 °C dec (EtOAc–hexane, pale yellow powder); ^1H NMR ($CDCl_3$, 400 MHz) δ 9.49 (br s, 1H, NH), 7.70 (br s, 1H, C4-H), 7.67 (br s, 1H, OH), 7.07 (s, 1H, C8-H), 4.17 (dd, 1H, *J* = 11.7, 9.6 Hz, CH*H*N), 4.07 (br d, 1H, *J* = 9.6 Hz, CH*H*N), 3.97 (s, 3H, CO_2CH_3), 3.86–3.93 (m, 2H, C*H*HCl and C1-H), 3.54 (t, 1H, *J* = 10.3 Hz, CH*H*Cl), 1.58 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (acetone-d_6, 100 MHz) δ 162.3 (CO_2Me), 152.8 (OCON), 144.7, 137.8, 129.2, 125.9, 120.6, 107.8, 106.4, 99.9, 54.8 ($C(CH_3)_3$), 53.4 and 52.0 (OCH_3 and CH_2N), 48.1 (CH_2Cl), 42.6 (C1), 28.6 ($C(CH_3)_3$); IR (KBr) ν_{max} 3364, 1703, 1672, 1436, 1410, 1380, 1349, 1256, 1154 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 513.0193 ($C_{18}H_{21}N_2O_5Cl + Cs^+$ requires 513.0193).

Anal. Calcd for $C_{18}H_{21}N_2O_5Cl$: C, 56.77; H, 5.56; N, 7.36. Found: C, 56.80; H, 5.69; N, 7.31.

(−)-(1*S*)-**25**: $[\alpha]^{22}_D$ −40° (*c* 0.5, CH_3OH); mp 152 °C dec (EtOAc–hexane).

ent-(+)-(1*R*)-**25**: $[\alpha]^{22}_D$ +40° (*c* 0.5, CH_3OH).

Methyl 2-((*tert*-Butyloxy)carbonyl)-4-oxo-1,2,4,5,8,8a-hexahydrocyclopropa[*c*]pyrrolo[3,2-*e*]indole-6-carboxylate (2, *N*-BOC-DSA). A suspension of NaH (1 mg, 60%, 27 μmol, 3.0 equiv) in THF (0.25 mL) at 0 °C under N_2 was treated with a solution of **25** (3.4 mg, 8.9 μmol, 1.0 equiv) in 50% DMF–THF (0.5 mL), and the reaction mixture was stirred for 30 min at 0 °C. The reaction mixture was poured onto H_2O (5 mL) and extracted with EtOAc (10 mL). The organic extract was washed with H_2O (5 mL) and saturated aqueous NaCl (2 × 5 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (1 × 10 cm SiO_2, 50% EtOAc–hexane) afforded **2** (*N*-BOC-DSA, 2.6 mg, 85%) as a pale yellow, crystalline solid: mp 128 °C (EtOAc–hexane, pale yellow powder); ^1H NMR ($CDCl_3$, 400 MHz) δ 9.82 (br s, 1H, NH), 6.74 (br s, 1H, C3-H), 6.53 (d, 1H, *J* = 2.3 Hz, C7-H), 3.99 (d, 1H, *J* = 10.4 Hz, CH*H*N), 3.95 (dd, 1H, *J* = 11.3, 4.5 Hz, C*H*HN), 3.87 (s, 3H, CO_2CH_3), 2.66–2.62 (m, 1H, C8a-H), 1.61 (dd, 1H, *J* = 7.7, 4.2 Hz, C8-H), 1.53 (s, 9H, $C(CH_3)_3$), 1.40 (t, 1H, *J* = 4.6 Hz, C8-H); ^{13}C NMR (acetone-d_6, 100 MHz) δ 178.0 (C4), 161.8, 161.6, 152.3 (CO_2tBu), 132.9, 130.7, 127.3, 109.4, 108.7, 82.8 ($C(CH_3)_3$), 54.4 (NCH_2), 51.9 (CO_2CH_3), 32.4 (C7b), 28.2 ($C(CH_3)_3$), 27.0, 24.2; UV (CH_3OH) λ_{max} (ε) 339 (18 000), 301 (14 000), 255 (10 000) nm; IR (KBr) ν_{max} 3440, 2925, 1719, 1610, 1393, 1279, 1254, 1150 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 477.0428 ($C_{18}H_{20}N_2O_5 + Cs^+$ requires 477.0427).

(+)-(7b*R*,8a*S*)-**2**: $[\alpha]^{23}_D$ +144° (*c* 0.06, CH_3OH); mp 152 °C dec.

ent-(−)-**2**: $[\alpha]^{22}_D$ −137° (*c* 0.05, CH_3OH).

Methyl 4-Oxo-1,2,4,5,8,8a-hexahydrocyclopropa[*c*]pyrrolo[3,2-*e*]indole-6-carboxylate (35, DSA). A solution of **2** (5.9 mg, 15.5 μmol) in CH_2Cl_2 (1.0 mL) at 0 °C under Ar was treated with CF_3CO_2H (1.0 mL), and the reaction mixture was stirred for 1.5 h (0 °C). The solvent was removed in vacuo, and flash chromatography (0.5 × 4 cm SiO_2, 0–5% CH_3OH–EtOAc gradient elution) afforded **35** (2.5 mg, 66%) as a cream-colored solid:[49] ^1H NMR (acetone-d_6, 400 MHz) δ 10.30 (br s, 1H, N^5H), 6.61 (d, 1H, *J* = 2.1 Hz, C7-H), 6.56 (br s, 1H, N^1H), 5.40 (s, 1H, C3-H), 3.80 (s, 3H, CO_2CH_3), 3.79 (ddd, 1H, *J* = 10.6, 5.4, 1.4 Hz, C1-H), 3.58 (dd, 1H, *J* = 10.5, 2.8 Hz, C1-H), 2.89 (dt, 1H, *J* = 7.8, 5.1 Hz, C8a-H), 1.59 (dd, 1H, *J* = 7.8, 3.5 Hz, C8-H), 1.20 (t, 1H, *J* = 4.4 Hz, C8-H); UV (CH_3OH) λ_{max} (ε) 358 (12 000), 285 (10 000), 262 (11 000), 234 (8000) nm; IR (neat) ν_{max} 3121, 2952, 2874, 1704, 1597, 1524, 1469, 1428, 1390, 1306, 1254, 1224 cm^{-1}; FABHRMS (NBA) *m/e* 245.0926 ($C_{13}H_{12}N_2O_3 + H^+$ requires 245.0926).

(+)-(7b*R*,8a*S*)-**35**: $[\alpha]^{25}_D$ +109° (*c* 0.16, CH_3OH); mp >240 °C.

ent-(−)-**35**: $[\alpha]^{25}_D$ −112° (*c* 0.125, CH_3OH); mp >240 °C.

Methyl 3-[(5,6,7-Trimethoxyindol-2-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate (27). A solution of **25** (8.9 mg, 0.023 mmol, 1.0 equiv) in 4.0 M HCl–EtOAc (0.5 mL) was stirred for 20 min at 25 °C. The reaction mixture was concentrated in vacuo to afford methyl 1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indole-7-carboxylate hydrochloride salt as a gray solid. The hydrochloride salt was taken up in DMF (0.45 mL) and treated sequentially with EDCI (13.4 mg, 0.070 mmol, 3.0 equiv) and 5,6,7-trimethoxyindole-2-carboxylic acid (**26**, 6.5 mg, 0.026 mmol, 1.1 equiv). The reaction mixture was stirred for 15 h at 25 °C before being poured onto H_2O (2 mL) and extracted with EtOAc (8 mL). The organic extract was washed with saturated aqueous NaCl (2 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (1 × 15 cm SiO_2, 60% EtOAc–hexane) afforded **27** (8.8 mg, 73%) as a pale yellow, crystalline solid: mp 246 °C (dec, EtOAc–hexane); ^1H NMR (acetone-d_6, 400 MHz) δ 10.80 (s, 1H, NH), 10.23 (s, 1H, NH), 9.00 (br s, 1H, OH), 7.97 (s, 1H, C4-H), 7.29 (s, 1H, C8-H), 7.09 (d, 1H, *J* = 2.2 Hz, C3′-H), 6.99 (s, 1H, C4′-H), 4.78 (t, 1H, *J* = 10.6 Hz, C*H*HN), 4.60 (dd, 1H, *J* = 10.9, 3.8 Hz, CH*H*N), 4.18 (m, 1H, C1-H), 4.17 (dd, 1H, *J* = 10.8, 3.0 Hz, CH*H*Cl), 4.04 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.90 (obscured by OCH_3, 1H, C*H*HCl), 3.88 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3); UV (CH_3OH) λ_{max} (ε) 333 (sh, 23 000), 308 (30 000), 240 (21 000) nm; IR (KBr) ν_{max} 3422, 2933, 1711, 1589, 1527, 1494, 1433, 1311, 1256, 1222, 1111 cm^{-1}; FABHRMS (NBA–CSI) *m/e* 646.0389 ($C_{25}H_{24}N_3O_7Cl + Cs^+$, 646.0357).

Duocarmycin SA (1). A suspension of NaH (1.6 mg, 80%, 0.062 mmol, 3.0 equiv) in THF (0.6 mL) at 0 °C under N_2 was treated with a solution of **27** (8.9 mg, 0.017 mmol, 1.0 equiv) in 50% DMF–THF (1.2 mL), and the reaction mixture was stirred for 30 min at 0 °C. The reaction mixture was poured onto H_2O (4 mL) and extracted with EtOAc (12 mL). The organic extract was washed with H_2O (5 mL) and saturated aqueous NaCl (3 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (1 × 15 cm SiO_2, 67–100% EtOAc–hexane gradient elution) afforded **1** (7.6 mg, 92%) as a pale yellow, crystalline solid: mp > 250 °C (EtOAc–Et_2O, pale yellow powder); ^1H NMR ($CDCl_3$, 400 MHz) δ 9.82 (s, 1H, NH), 9.25 (s, 1H, NH), 7.00 (s, 1H, C3-H), 6.92 (d, 1H, *J* = 2.0 Hz, C3′-H), 6.76 (s, 1H, C4′-H), 6.58 (d, 1H, *J* = 1.9 Hz, C7-H), 4.44 (dd, 1H, *J* = 10.4, 4.8 Hz, CH*H*N), 4.37 (d, 1H, *J* = 10.4 Hz, C*H*HN), 4.05 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 2.76 (dt, 1H, *J* = 7.5, 4.9 Hz, C8a-H), 1.73 (dd, 1H, *J* = 7.6, 4.4 Hz, C8-H), 1.55 (t, 1H, *J* = 4.6 Hz, C8-H); ^{13}C NMR (acetone-d_6, 100 MHz) δ 178.0 (C4), 162.4, 162.0, 161.6, 151.2, 141.9, 140.0, 132.9, 131.0, 130.6, 127.6, 127.2, 124.4, 112.5, 108.9, 108.4, 98.9 (C4′), 61.5 (OCH_3), 61.4 (OCH_3), 56.4 (OCH_3), 55.8 (CH_2N), 51.9 (CO_2CH_3), 31.9 (C7b), 26.4, 24.5; UV (CH_3OH) λ_{max} (ε) 367 (27 000), 316 (16 000), 235 (sh, 21 000) nm; IR (KBr) ν_{max} 3456, 1718, 1639, 1522, 1489, 1389, 1300, 1267, 1207, 1111 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 610.0590 ($C_{25}H_{23}N_3O_7 + Cs^+$ requires 610.0590).

(+)-**1**: $[\alpha]^{22}_D$ +197° (*c* 0.035, CH_3OH), lit[2] $[\alpha]^{24}_D$ +180° (*c* 0.1,[43] CH_3OH).

ent-(−)-**1**: $[\alpha]^{22}_D$ −189° (*c* 0.02, CH_3OH).

Treatment of Duocarmycin SA (1) with HCl–EtOAc. A solution of **1** (2.2 mg, 4.6 μmol) in 3M HCl–EtOAc (2.5 mL) was stirred for 15 min at 0 °C. The reaction mixture was concentrated under reduced pressure to provide a pale yellow solid. Flash chromatography (0.5 × 3 cm SiO_2, 67% EtOAc–hexane) afforded **27** (2.3 mg, 96%) as the only detectable reaction product and identical in all respects with authentic material.

Aqueous Solvolytic Reactivity of *N*-BOC-DSA (2) and DSA (35). *N*-BOC-DSA (**2**, 100 μg) and DSA (**35**, 100 μg) were dissolved in CH_3OH (1.5 mL). The CH_3OH solutions were mixed with aqueous buffer (pH = 3, 1.5 mL). The buffer contained 4:1:20 (v:v:v) of 0.1 M citric acid,

(49) (+)- and (−)-DSA (**35**) exhibited L1210 IC_{50} values of 8 and 3 μg/mL, respectively.

0.2 M Na_2HPO_4, and H_2O, respectively. The UV spectra of the solutions were measured immediately after mixing with the aqueous buffer; the control and solvolysis reaction solutions were stoppered, protected from light, and allowed to stand at 25 °C. For **2**, the UV spectrum of the solution was monitored four times at regular intervals for the first 3 days and then twice a day for 3 weeks. The reaction was monitored until no further change was detectable, and both the decrease in the long-wavelength absorption at 345 nm and the increase in the short-wavelength absorption at 256 nm were monitored. The solvolysis rate was calculated from the data recorded at 345 nm from the least-squares treatment (r = 0.994) of the slope of a plot of time versus $\ln(A_0/A)$; $k = 1.08 \times 10^{-6}$ s^{-1}, $t_{1/2}$ = 177 h. For **35**, the UV spectrum was monitored once daily for 3 months. The solvolysis rate ($t_{1/2}$ = 2380 h) was calculated from the data recorded at 363 nm from the least squares treatment (r = 0.995) of the slope of a plot of time versus $\ln(A_0/A)$; $k = 8.09 \times 10^{-8}$ s^{-1}.

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Supplementary Material Available: Experimental for a large scale preparation of **3** (5 steps) (4 pages). Ordering information is given on any current masthead page.