



NIH Public Access

Author Manuscript

J Am Chem Soc. Author manuscript; available in PMC 2008 November 14.

Published in final edited form as:

J Am Chem Soc. 2007 November 14; 129(45): 14092–14099. doi:10.1021/ja073989z.

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

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Abstract

The design, synthesis, and evaluation of a predictably more potent analogue of CC-1065 entailing the substitution replacement of a single skeleton atom in the alkylation subunit are disclosed and was conducted on the basis of design principles that emerged from a fundamental parabolic relationship between chemical reactivity and cytotoxic potency. Consistent with projections, the MeCTI (7-methyl-1,2,8,8a-tetrahydrocyclopropa[*c*]thieno[3,2-*e*]indol-4-one) alkylation subunit as well as its isomer *iso*-MeCTI (6-methyl-1,2,8,8a-tetrahydrocyclopropa[*c*]thieno[2,3-*e*]indol-4-one) were found to be 5–6 times more stable than the MeCPI alkylation subunit found in CC-1065 and slightly more stable than even the DSA alkylation subunit found in duocarmycin SA placing it at the point of optimally balanced stability and reactivity for this class of antitumor agents. Their incorporation into the key analogues of the natural products provided derivatives that surpassed the potency of MeCPI derivatives (3–10 fold) matching or slightly exceeding the potency of the corresponding DSA derivatives consistent with projections made based on the parabolic relationship. Notable of these, MeCTI-TMI proved to be as potent or slightly more potent than the natural product duocarmycin SA (DSA-TMI, IC₅₀ = 5 vs 8 pM) and MeCTI-PDE₂ proved to be 3-fold more potent than the natural product CC-1065 (MeCPI-PDE₂, IC₅₀ = 7 vs 20 pM). Both exhibited efficiencies of DNA alkylation that correlate with this enhanced potency without impacting the intrinsic selectivity characteristic of this class of antitumor agents.

Introduction

CC-1065¹ (**1**) and duocarmycin SA (**2**)² represent the key members of a small class of exceptionally potent naturally occurring antitumor agents^{1–4} that derive their biological properties through a now characteristic sequence-selective DNA alkylation reaction (Figure 1).^{5–9} Extensive investigations on the naturally occurring members of this class as well as their synthetic analogues have defined key and subtle features that contribute to their properties.^{9,10} Most notable of these are the structural features that contribute to the AT-rich noncovalent binding selectivity dominating the alkylation selectivity,¹¹ those that define the source of catalysis for the DNA alkylation reaction,^{12,13} and those that subtly impact the unusual and intrinsic stability of their alkylation subunits.^{9,13,14}

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Results and Discussion

Design

Throughout the course of our investigations, we have chronicled a direct relationship between the intrinsic chemical stability of the alkylation subunit and the cytotoxic potency of the resulting derivatives.¹⁵ Recently, a compilation of the data derived from more than 30 deep-seated modifications resulted in the establishment of a well-defined parabolic relationship between the alkylation subunit reactivity and the resulting cytotoxic potency that spanned a 10⁴–10⁶ range of reactivities and activities (Figure 2).¹⁶ Presumably, this fundamental relationship simply reflects the fact that the compound must be sufficiently stable to reach its biological target, yet remain sufficiently reactive to alkylate DNA once it does. Significantly, the work defined this optimal balance between reactivity and stability providing a fundamental design feature that is subject to investigational interrogation.

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

Synthesis of *N*-Boc-MeCTI

Stobbe condensation of 4-methylthiophene-3-carboxaldehyde (**9**, 4 equiv of *t*-BuOK, 6 equiv of diethyl succinate, 83 °C, 1.5 h) gave a mixture of half-esters **10** which were subjected to Friedel–Crafts acylation (excess Ac₂O/NaOAc, 140 °C, 5 h, 40%) to provide **11** (Scheme 1).¹⁸ Compound **11** was hydrolyzed to phenol **12** (1.1 equiv of K₂CO₃, 78 °C, 14 h, 83%), which was protected as the benzyl ether **13** (1.2 equiv of BnBr, 1.2 equiv of K₂CO₃, 25 °C, 5 h, 98%). Subsequent hydrolysis of the ethyl ester (3 equiv of LiOH, 4:1:1 THF–MeOH–H₂O, 25 °C, 18 h, 98%) followed by Curtius rearrangement of the resulting carboxylic acid **14** (1.2 equiv of DPPA, 1.2 equiv of Et₃N, 83 °C, 18 h, 76%) provided carbamate **15**. Regioselective acid-catalyzed C4-iodination (0.1 equiv of H₂SO₄, 1.1 equiv of NIS, 25 °C, 2 h, 89%) followed by carbamate alkylation of **16** with allyl bromide (1.2 equiv of NaH, 3 equiv of allyl bromide, 25 °C, 3 h, 95%) provided **17**. *5-Exo-trig* radical cyclization conducted in the presence of TEMPO¹⁹ (2.7 equiv, 3 equiv of (Me₃Si)₃SiH, 100 °C, 5 h, 78%) gave **18** in good yield whereas the more conventional use of Bu₃SnH was unsuccessful.²⁰ Zinc-mediated reductive cleavage of **18** (16 equiv of Zn, 70 °C, 3 h, 83%) followed by chlorination displacement of the released primary alcohol (2 equiv of Ph₃P, 6 equiv of CCl₄, 25 °C, 20 h, 95%) provided **20**. Intermediate **20** was separated into its two enantiomers by resolution on a semipreparative Chiralcel® OD column (20% *i*-PrOH/hexane, $\alpha = 1.30$).²¹ Hydrogenolysis of the benzyl ether (25% aqueous HCO₂NH₄, 10% Pd/C, 60 °C, 2 h) followed by immediate spirocyclization of the crude product

by treatment with DBU (10 equiv, DMF–CH₃CN, 25 °C, 10 min, 70%) provided each enantiomer of **3**.

Synthesis of MeCTI-TMI, MeCTI-indole₂ and MeCTI-PDE₂

Acid-catalyzed deprotection of **20** (4 N HCl–EtOAc, 25 °C, 1 h) followed by coupling of the resulting hydrochloride salt with 5,6,7-trimethoxyindole-2-carboxylic acid (**22**,²² 4 equiv of EDCI, 2 equiv of NaHCO₃, 25 °C, 15 h, 92%) provided **23** (Scheme 2). Hydrogenolysis of the benzyl ether (25% aqueous HCO₂NH₄, 10% Pd/C, 25 °C, 2 h, 83%) afforded **24** and its spirocyclization was effected by treatment with DBU (4 equiv, DMF–CH₃CN, 25 °C, 10 min, 70%) providing **25**.

A subtle modification of this sequence was used to access MeCTI-indole₂ and MeCTI-PDE₂. Thus, acid-catalyzed deprotection of **21** (4 N HCl–EtOAc, 25 °C, 1 h) followed by coupling of the resulting hydrochloride salt with PDE₂-CO₂H (**26**)²³ or indole₂-CO₂H (**27**) (4 equiv of EDCI, 1.5 equiv of RCO₂H, 25 °C, 0.5 h, 40–72%) provided **28** and **29** (Scheme 3). This coupling with an alkylation subunit precursor in which the C5-benzyl ether was already removed permitted direct spirocyclization effected by treatment with NaHCO₃ (4 equiv, 2:1 DMF–H₂O, 25 °C, 10 min, 46–81%) providing **8** and **30**.

Synthesis of *N*-Boc-*iso*-MeCTI

N-Boc-*iso*-MeCTI was prepared using a synthetic strategy analogous to the route described for **3**, beginning with the Stobbe condensation of the isomeric aldehyde **31** (Scheme 4). Compound **42** was resolved (Chiralcel OD, 5% *i*-PrOH/hexane, $\alpha = 1.44$), deprotected (25% aqueous HCO₂NH₄, 10% Pd/C, 60 °C, 2 h) and the crude product was spirocyclized using DBU (10 equiv, DMF–CH₃CN, 25 °C, 4 h, 39%) to give each enantiomer of **4**. Notably, the Ar-3' spirocyclization of this isomer was much slower and less facile than that of **3**.

Synthesis of *iso*-MeCTI-TMI

Deprotection of **42** (4 N HCl–EtOAc, 25 °C, 1 h) followed by coupling of the resulting hydrochloride salt with 5,6,7-trimethoxyindole-2-carboxylic acid (**22**,²² 1.4 equiv, 4 equiv of EDCI, 3 equiv of NaHCO₃, 25 °C, 15 h, 78%) provided **44**. Hydrogenolysis of the benzyl ether (25% aqueous HCO₂NH₄, 10% Pd/C, 60 °C, 2 h, 83%) afforded **45**. Spirocyclization of **45** was particularly slow (DBU/DMF) with this isomer such that hydrolysis of the labile amide was competitive. The use of an alternative solvent system (2:1 CH₃CN/DMF) under strictly anhydrous conditions (6 equiv of DBU, 25 °C, 1 h, 71%) gave **46** in improved conversions.

Chemical Reactivity

The relative reactivity of the alkylation subunits was established by measuring their rates of acid-catalyzed solvolysis. At pH 3 (50% CH₃OH–buffer, buffer = 4:1:20 v/v/v 0.1 M citric acid, 0.2 M Na₂HPO₄, H₂O), both *N*-Boc-MeCTI (**3**) and *N*-Boc-*iso*-MeCTI (**4**) underwent measurable solvolysis that was monitored spectrophotometrically by UV with the disappearance of the long wavelength absorption of the CTI chromophore and with the appearance of a short wavelength absorption attributable to the solvolysis product. The Boc derivatives of the two CTI isomers exhibited nearly indistinguishable reactivities ($t_{1/2} = 206$ and 209 h), being ca. 5–6 fold more stable than the alkylation subunit of CC-1065 (*N*-Boc-MeCPI, $t_{1/2} = 37$ h),²³ significantly more stable than *N*-Boc-CBI ($t_{1/2} = 133$ h),²⁴ and measurably more stable than even *N*-Boc-DSA ($t_{1/2} = 177$ h),²⁵ Figure 5. This places *N*-Boc-MeCTI (**3**) and *N*-Boc-*iso*-MeCTI (**4**) among the most stable alkylation subunits explored to date that still exhibit a measurable reactivity at pH 3.

Cytotoxic Activity

The compounds displayed cytotoxic activity (L1210) consistent with their relative stabilities. Summarized in Figure 6 is the L1210 cytotoxic activity of the MeCTI and *iso*-MeCTI derivatives examined along with that of the key comparison compounds²⁶ and a full table of comparison derivatives is provided in the Supporting Information (Tables S1–S5). The natural enantiomers of *N*-Boc-MeCTI (IC_{50} = 30 nM) and *N*-Boc-*iso*-MeCTI (IC_{50} = 25 nM) proved to be ca. 10-fold more potent than the Boc derivative of the CC-1065 alkylation subunit (*N*-Boc-MeCPI, IC_{50} = 330 nM),²³ 2–3 fold more potent than (+)-*N*-Boc-CBI (IC_{50} = 80 nM),²⁴ but 4–5 fold less potent than (+)-*N*-Boc-DSA (IC_{50} = 6 nM).²⁵ With the exception of the latter comparison with (+)-*N*-Boc-DSA which exhibits an anomalously potent activity, the cytotoxic activity of **3** and **4** proved to be in line with expectations. The unnatural enantiomers of **3** and **4** (IC_{50} 's = 600 nM) exhibited potencies 20-fold less active than the natural enantiomers and in line with expectations based on the preceding observations with **5–7**.

Similarly, the natural enantiomers of TMI derivatives **25** and **46** were found to be exceptionally potent cytotoxic agents (IC_{50} = 5 and 7 pM, respectively), perhaps slightly more active than even (+)-duocarmycin SA (IC_{50} = 8–10 pM)²⁵ and notably more potent than (+)-CBI-TMI (IC_{50} = 30 pM).²¹ Figure 6.²⁶ Their unnatural enantiomers, (*ent*-(–)-**25** and *ent*-(–)-**46**), proved to be 100–300 fold less active than the natural enantiomers exhibiting a distinction between enantiomeric activities that is greater than that observed with duocarmycin SA (10-fold)²⁵ or CBI-TMI (70-fold).²¹

Especially interesting is the comparison of MeCTI-PDE₂ (**8**) with CC-1065 (**1**) and the synthetic hybrid natural product DSA-PDE₂ (**48**),^{5d} Figure 7.²⁶ Consistent with expectations, the natural enantiomer of MeCTI-PDE₂ (IC_{50} = 7 pM) was found to be 3-fold more potent than CC-1065 (IC_{50} = 20 pM) and nearly equipotent with DSA-PDE₂ (**48**, IC_{50} = 4 pM). Thus, the deep-seated change of a single skeleton atom in CC-1065 (NH → S) served to increase its potency 3-fold in line with expectations based on its 5–6 fold increased stability.

In a series of indole₂ analogues that are more manageable to work with because of their improved physical properties including their solubility in conventional organic solvents and which have exhibited efficacious in vivo antitumor activity,^{10,27} the MeCTI derivative **30** exhibited a cytotoxic potency precisely in line with expectations based on its enhanced stability, Figure 8.²⁶ Thus, the natural enantiomer of MeCTI-indole₂ (**30**) was found to be ca. 8-fold more potent than MeCPI-indole₂ (**49**)^{10,28} in line with its 5–6 fold increased stability, 2-fold more potent than CBI-indole₂²⁷ consistent with its 1.5-fold increased stability, and ca. 2-fold less potent than DSA-indole₂ (1.2-fold difference in reactivity). Similarly, the unnatural enantiomer of MeCTI-indole₂ (IC_{50} = 250 pM) proved to be 50-fold less active than the natural enantiomer, but similarly 4-fold more potent than MeCPI-indole₂ (IC_{50} = 1000 pM) and essentially equipotent with DSA-indole₂ (IC_{50} = 150 pM).

The Parabolic Relationship

Among the most important of the features established to date with this class of compounds is the relationship between chemical stability and biological potency (cytotoxic activity). This parabolic relationship extends over a 10⁶-fold range in reactivities (−log *k*, pH 3) and activities (−log IC_{50} , L1210) covering an extensive range of modified alkylation subunits. Consistent with the design features, *N*-Boc-MeCTI (pH 3 solvolysis $t_{1/2}$ = 206 h) as well as *N*-Boc-*iso*-MeCTI (pH 3 solvolysis $t_{1/2}$ = 209 h) exhibit a reactivity comparable to *N*-Boc-DSA ($t_{1/2}$ = 177 h) that lies at the pinnacle of the parabolic relationship at a point that is 5–6 times more stable than *N*-Boc-MeCPI. In line with this enhanced stability, the Boc derivatives of both CTI isomers exhibit a cytotoxic activity roughly 10-fold more potent than *N*-Boc-MeCPI (IC_{50} = 330 nM) and 4–5 fold less potent than *N*-Boc-DSA (IC_{50} = 6 nM) placing them near

expectations based on the parabolic relationship (Figure 9).²⁶ Importantly and aside from the anomalously potent activity of *N*-Boc-DSA, this places both MeCTI derivatives among the most potent analogues examined to date on par with the activity of *N*-Boc-CCBI further defining the pinnacle of the parabolic relationship.

DNA Alkylation Selectivity and Efficiency

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

Illustrated in Figure 10 is a representative comparison of the DNA alkylation selectivity of the TMI-based analogues which highlights the similarity as well as subtle distinctions in the compounds. As anticipated, the TMI-based analogues **25** and **46** exhibited a DNA alkylation selectivity identical to all such TMI derivatives including duocarmycin SA itself. Within this segment of DNA, the natural and unnatural enantiomers each alkylate a single major site and the only significant distinction detected was their relative efficiencies of DNA alkylation. In each case, the natural enantiomer alkylates DNA with a greater efficiency than the corresponding unnatural enantiomer. Throughout both enantiomeric series, duocarmycin SA, MeCTI-TMI, and *iso*-MeCTI-TMI exhibited no distinction in their relative efficiencies of DNA alkylation, whereas CBI-TMI (CBI-TMI < MeCPI-TMI)²¹ was less effective.

In a subtle contrast to this behavior, the PDE₂ derivatives illustrated in Figure 11 alkylated the same major sites in both enantiomeric series with DSA-PDE₂ and MeCTI-PDE₂ exhibiting an efficiency that subtly exceeds that of (+)-CC-1065. Most significant in this series and consistent with their relative cytotoxic potencies, the unnatural enantiomers now approach or match the DNA alkylation efficiencies of the natural enantiomers.

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

In Vivo Antitumor Activity

The natural enantiomer of CTI-indole₂ (**30**) was examined for in vivo efficacy in a standard antitumor model enlisting L1210 murine leukemia implanted i.p. into DBA/2J mice. This model has been shown to respond well to related compounds³¹ and is a system that collaborators through the years have used to assess an extensive series of (+)-CBI-indole₂ analogues.²⁷ Although not published, these latter studies provided the foundation on which we based our examination of **30**. Thus, (+)-**30** was examined with the dose range (10–100 1g/kg) and the dosing schedule (administered three times i.p. on days 1, 5, and 9) found suitable for related agents including (+)-CBI-indole₂²⁷ (Figure 12). Although the higher doses (100 and 60 1g/kg) were found to be toxic to the treated animals, efficacious antitumor activity was observed at the lower doses producing 4/6 (at 10 1g/kg) and 3/6 (at 30 1g/kg) long term survivors (>80 days) in the experiment. This efficacy is at least as good as and may exceed that observed with related drugs in this class in this tumor model, and occurs at an optimal dose that is lower than that observed with (+)-CBI-indole₂ (30–60 1g/kg) or related MeCPI-based

agents. Thus, the enhanced chemical stability of the alkylation subunit leads to a greater *in vivo* potency and a maintained or further enhanced efficacy.

Conclusions

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

The alkylation subunit MeCTI and its isomer *iso*-MeCTI were prepared and found to be slightly more stable than DSA (pH 3 solvolysis: MeCTI, $t_{1/2} = 206$ h; *iso*-MeCTI, $t_{1/2} = 209$ h) placing it at the point representing an optimal balance between chemical reactivity and stability based on the established parabolic relationship. Consistent with their increased relative stabilities, the natural enantiomers of *N*-Boc-MeCTI ($IC_{50} = 20$ nM) and *N*-Boc-*iso*-MeCTI ($IC_{50} = 25$ nM) proved to be 10-fold more potent than the CC-1065 alkylation subunit (+)-*N*-Boc-MeCPI ($IC_{50} = 330$ nM), 2–3 fold more potent than (+)-*N*-Boc-MeCBI ($IC_{50} = 80$ nM), but 4–5 fold less potent than the anomalously potent (+)-*N*-Boc-DSA. The unnatural enantiomers of *N*-Boc-MeCTI and *N*-Boc-*iso*-MeCTI ($IC_{50} = 600$ nM) each exhibited potencies 20-fold less active than the natural enantiomers and consistent with expectations for this class of agents.

Key natural product analogues evaluated included (+)-MeCTI-TMI (5 pM) and its isomer (+)-*iso*-MeCTI (7 pM) which matched or slightly exceeded the potency of duocarmycin SA (DSA-TMI, 8–10 pM), a natural product incorporating the optimal naturally occurring alkylation subunit. Also prepared and evaluated was a key MeCTI analogue of CC-1065. The analogue **8** (MeCTI-PDE₂) constitutes the substitution of a single atom in the alkylation subunit of CC-1065, providing a compound that exhibited an identical DNA alkylation profile relative to CC-1065 ($IC_{50} = 20$ pM), but is 3-fold more potent ($IC_{50} = 7$ pM, natural enantiomer) than the natural product. Additionally consistent with expectations based on their similar inherent reactivities, MeCTI-PDE₂ was also comparable in potency with DSA-PDE₂ ($IC_{50} = 4$ pM). Extending these observations, the incorporation of MeCTI into an indole₂ derivative and its comparison with an important series of alkylation subunit derivatives further verified the direct impact of the relative reactivities on the cytotoxic potency. These observations and a subsequent demonstration of its *in vivo* antitumor potency and efficacy places (+)-MeCTI-indole₂ among the most interesting such derivatives disclosed to date.

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

Supplementary Material

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Acknowledgements

We gratefully acknowledge the financial support of the National Institutes of Health (CA41986 and CA42056), the Skaggs Institute for Chemical Biology, and predoctoral fellowship support from the American Chemical Society (2005–2006 M.S.T., sponsored by Roche Pharmaceuticals). K.S.M. and M.S.T. are Skaggs Fellows.

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26. The L1210 cytotoxic activity for (+)-ducarmycin SA (8–10 pM) and (+)-CC-1065 (20 pM; 23–18 pM) have been tested as standards (>100 times) through the years and side-by-side with the samples disclosed herein. The small 2–3 fold differences are always observed and the absolute potencies always fall in this narrow range indicated ($\pm 20\%$). We have developed highly refined, reproducible conditions for conducting such cytotoxic assays ($\pm 20\%$) that avoid the more variable results typically experienced with such assays. The IC₅₀'s reported in Figures 6–8 are the average values ($\pm 20\%$) obtained typically from multiple rounds of testing and the number of times the samples disclosed herein were tested in triplicate along side the standards are: ($\text{D}\rightleftharpoons$)-3 (4 \times), *ent*-(--)-3 (2 \times), ($\text{D}\rightleftharpoons$)-4 (3 \times), *ent*-(--)-4 (3 \times), ($\text{D}\rightleftharpoons$)-25 (7 \times), *ent*-(--)-25 (3 \times), ($\text{D}\rightleftharpoons$)-46 (3 \times), *ent*-(--)-46 (1 \times), ($\text{D}\rightleftharpoons$)-8 (4 \times), ($\text{D}\rightleftharpoons$)-30 (4 \times), and *ent*-(--)-30 (3 \times).
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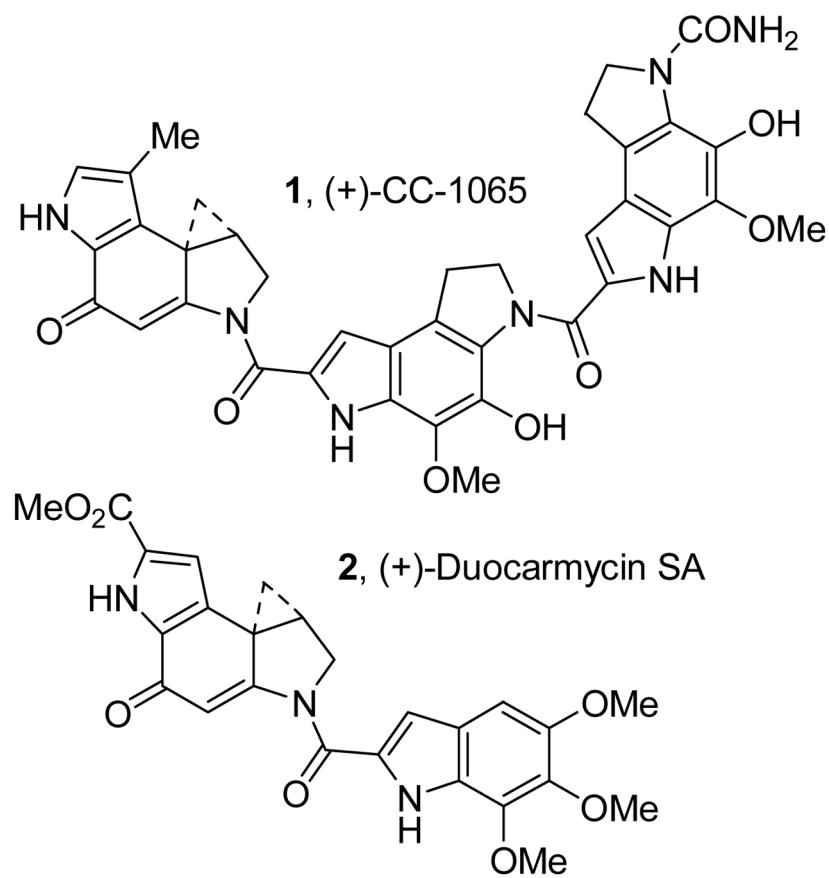
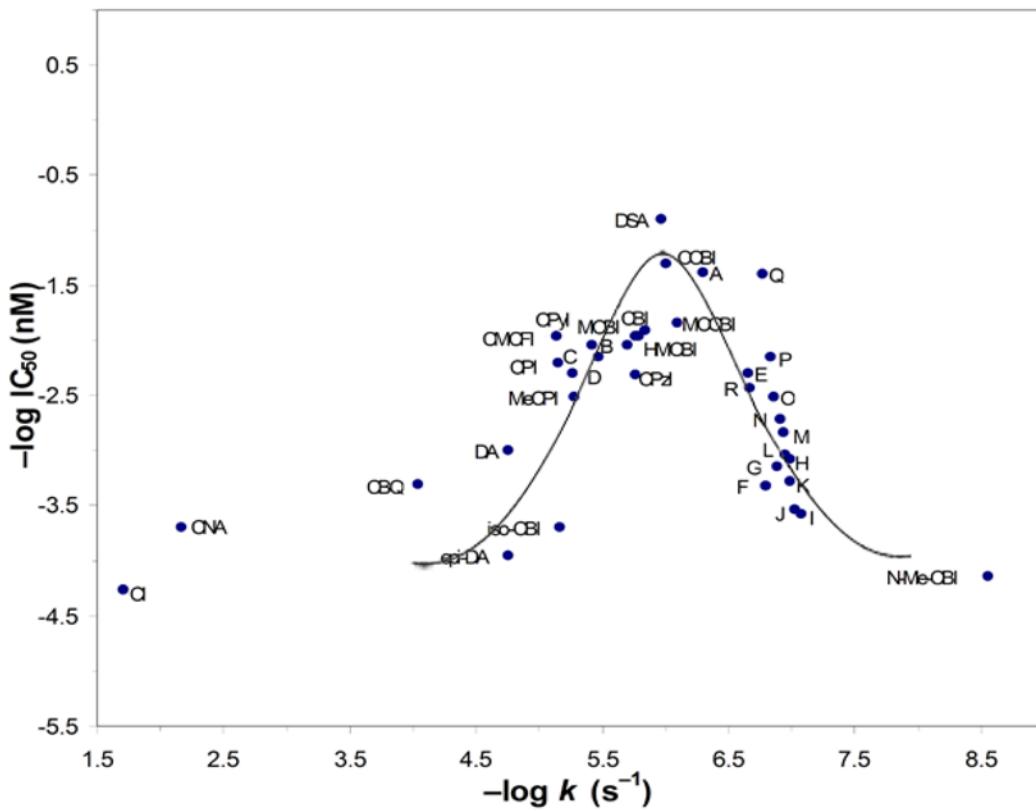
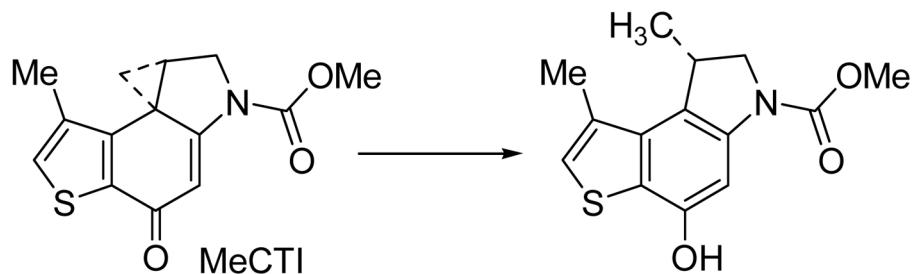
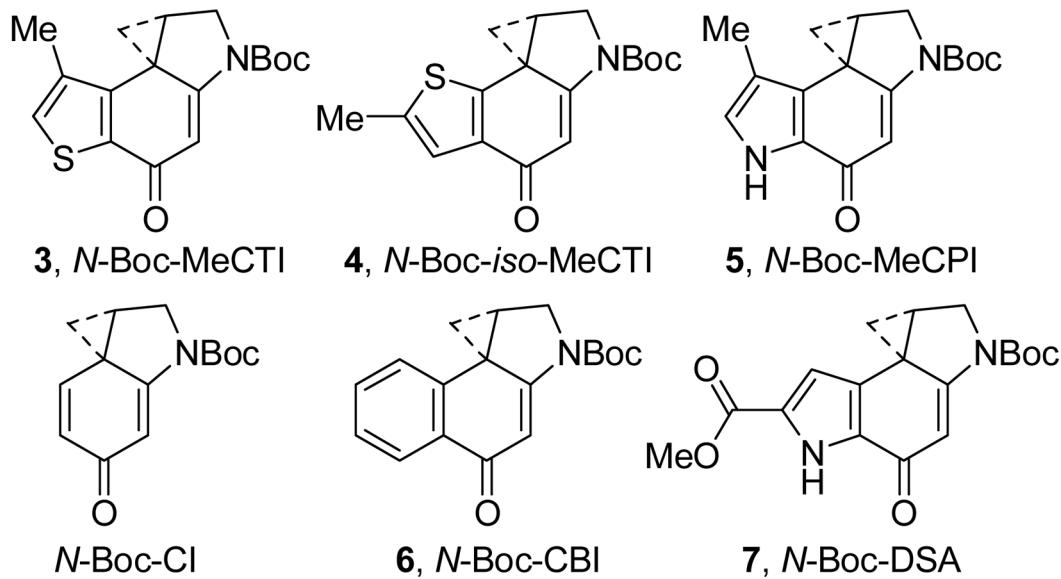


Figure 1.
Natural Products.

**Figure 2.**

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



Agent	Spiro	Product	$\Delta\Delta H$ (kcal)
Cl	-22.92	-81.28	-58.36
MeCPI	-15.62	-66.31	-50.69
CBI	-22.68	-62.99	-40.31
MeCTI	-24.91	-63.40	-38.49

Figure 3.

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

Single atom substitution
to enhance potency?

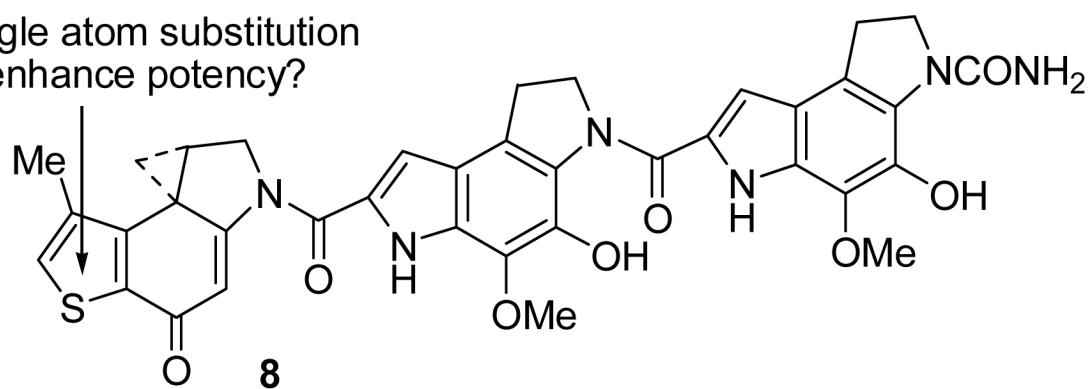
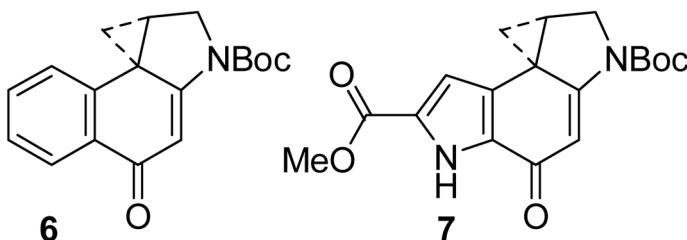
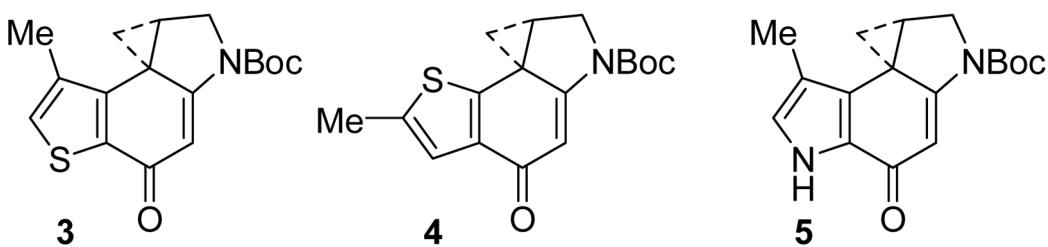


Figure 4.
Structure of (+)-MeCTI-PDE₂.



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

Figure 5.
Solvolytic Reactivity.

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

Figure 6.
Cytotoxic Activity.²⁶

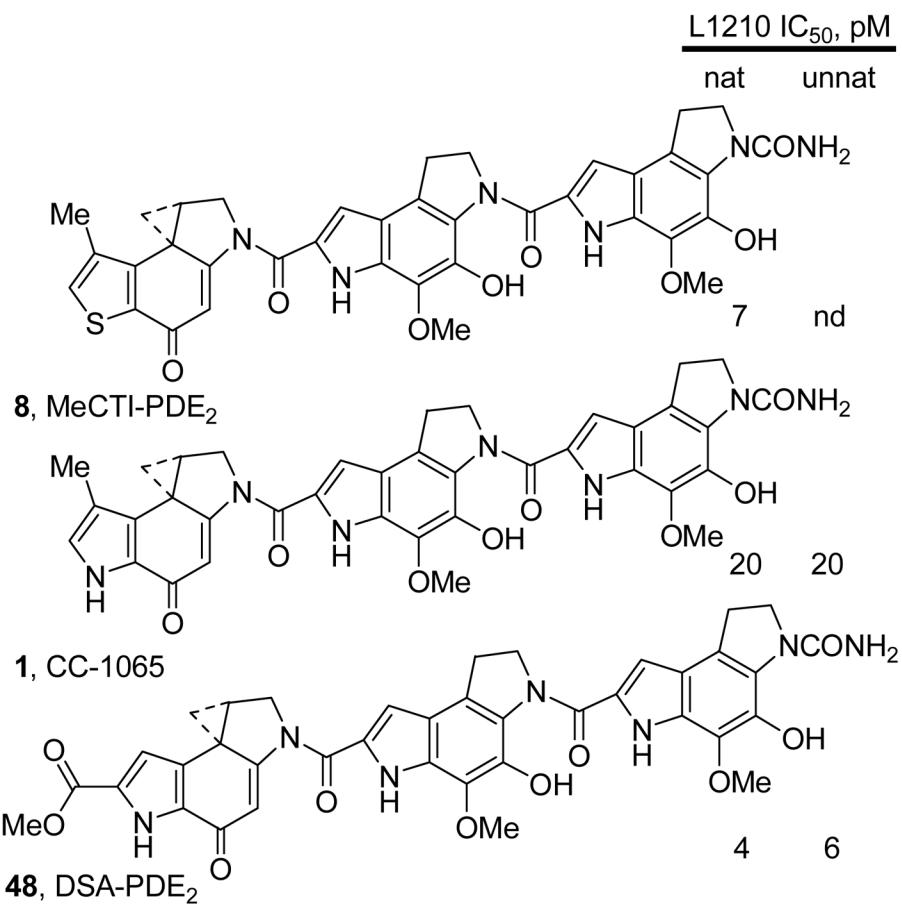


Figure 7.
Cytotoxic activity of PDE₂ derivatives.²⁶

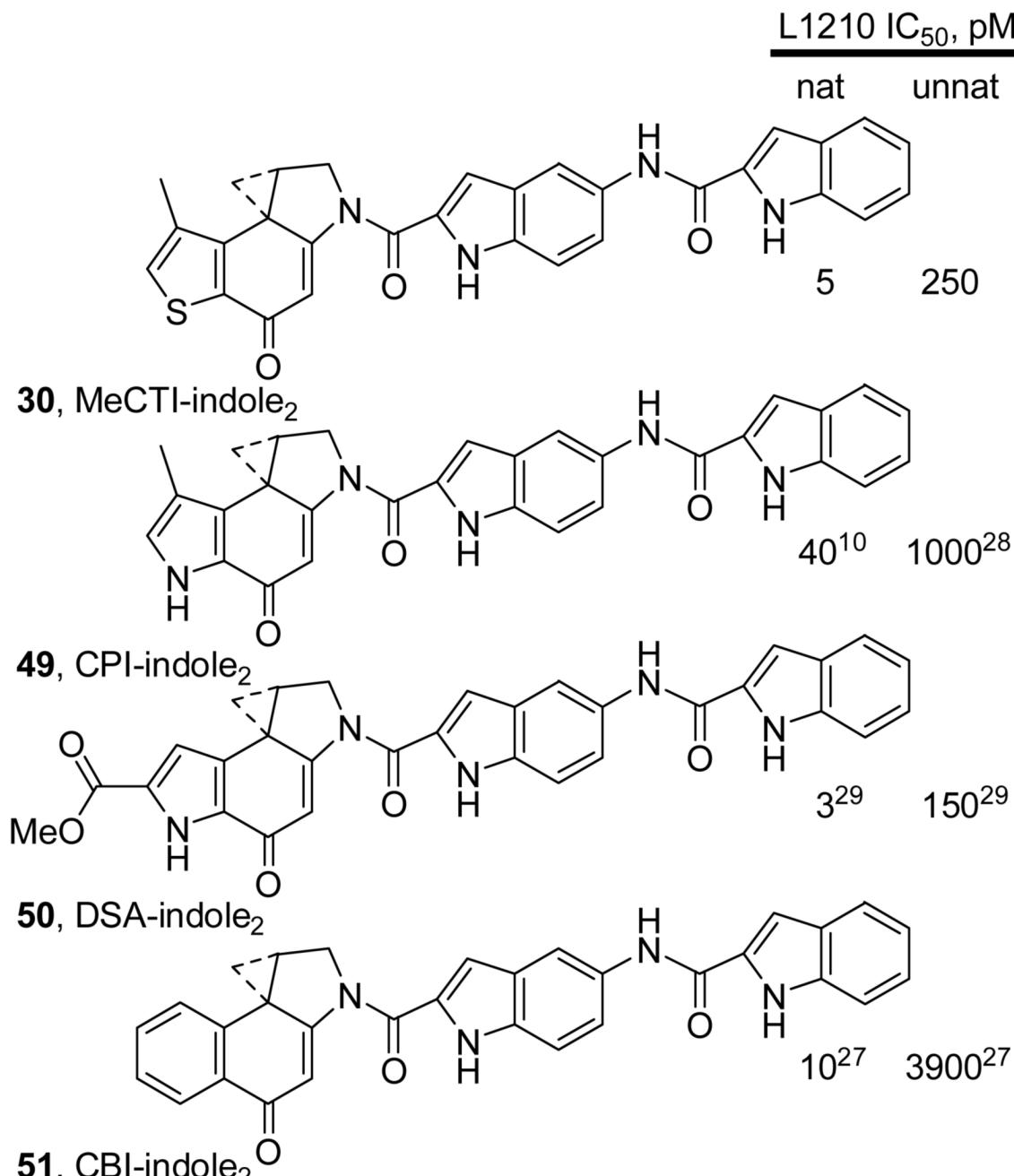
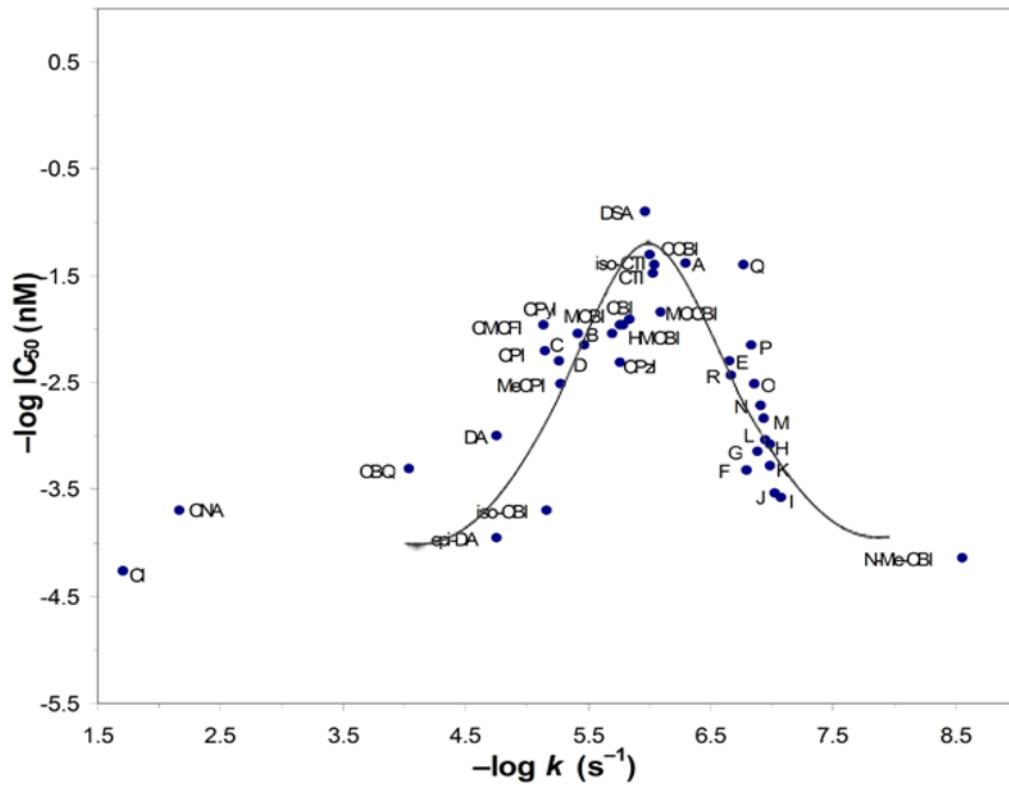
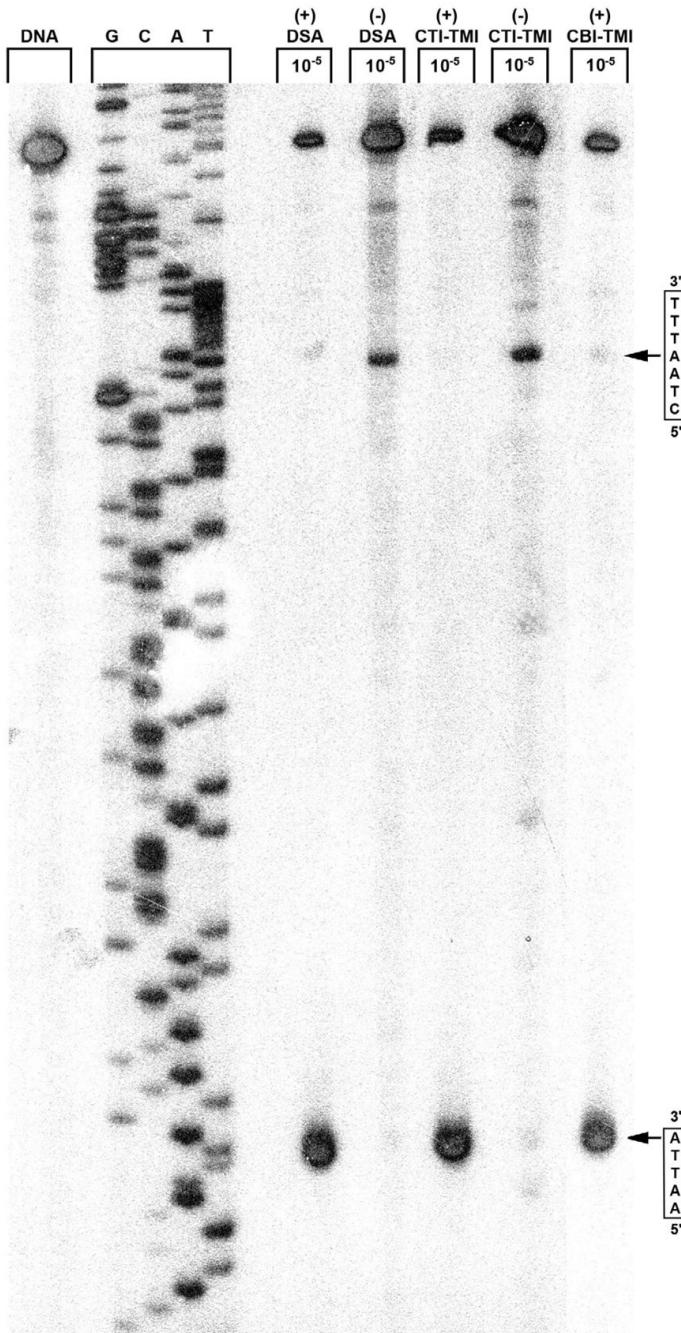


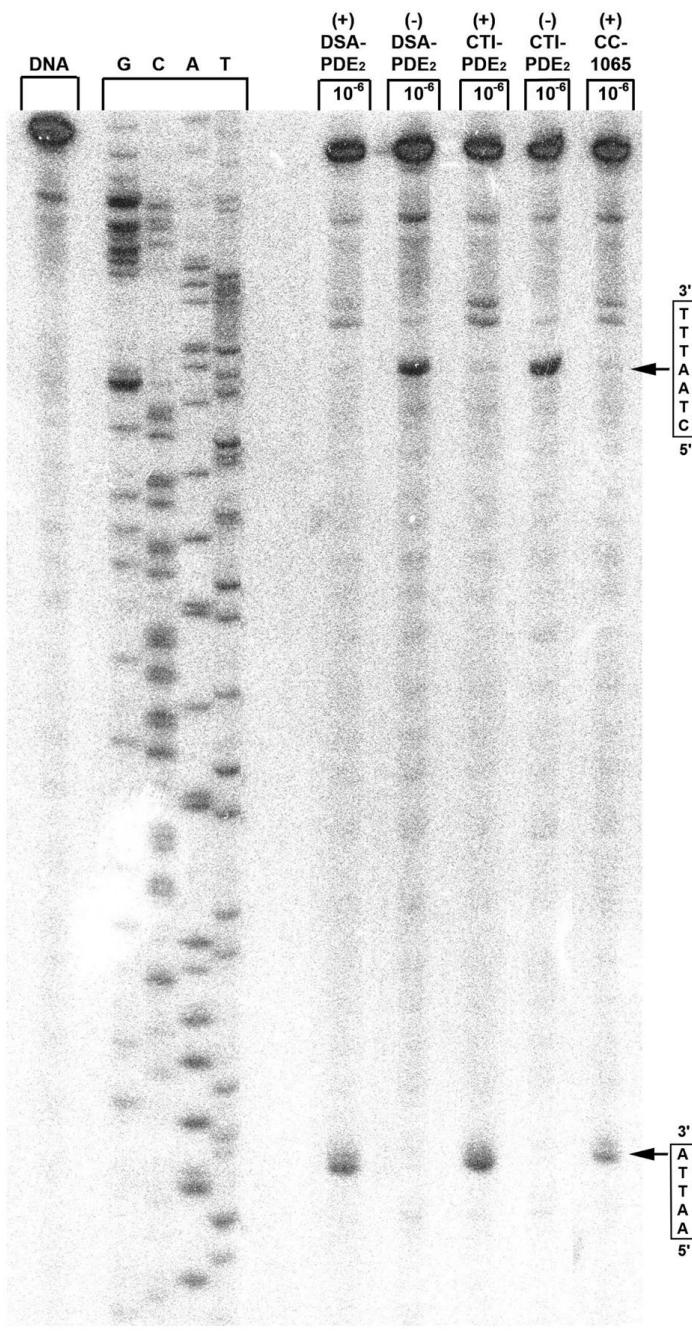
Figure 8.
Cytotoxic activity of indole₂ derivatives.²⁶

**Figure 9.**

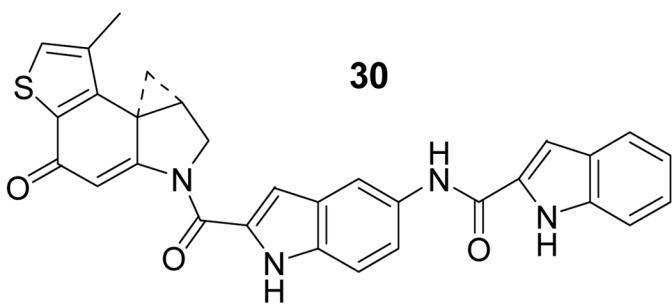
Relationship between reactivity (solvolysis k , pH 3) and cytotoxic potency (L1210), natural enantiomers.

**Figure 10.**

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

**Figure 11.**

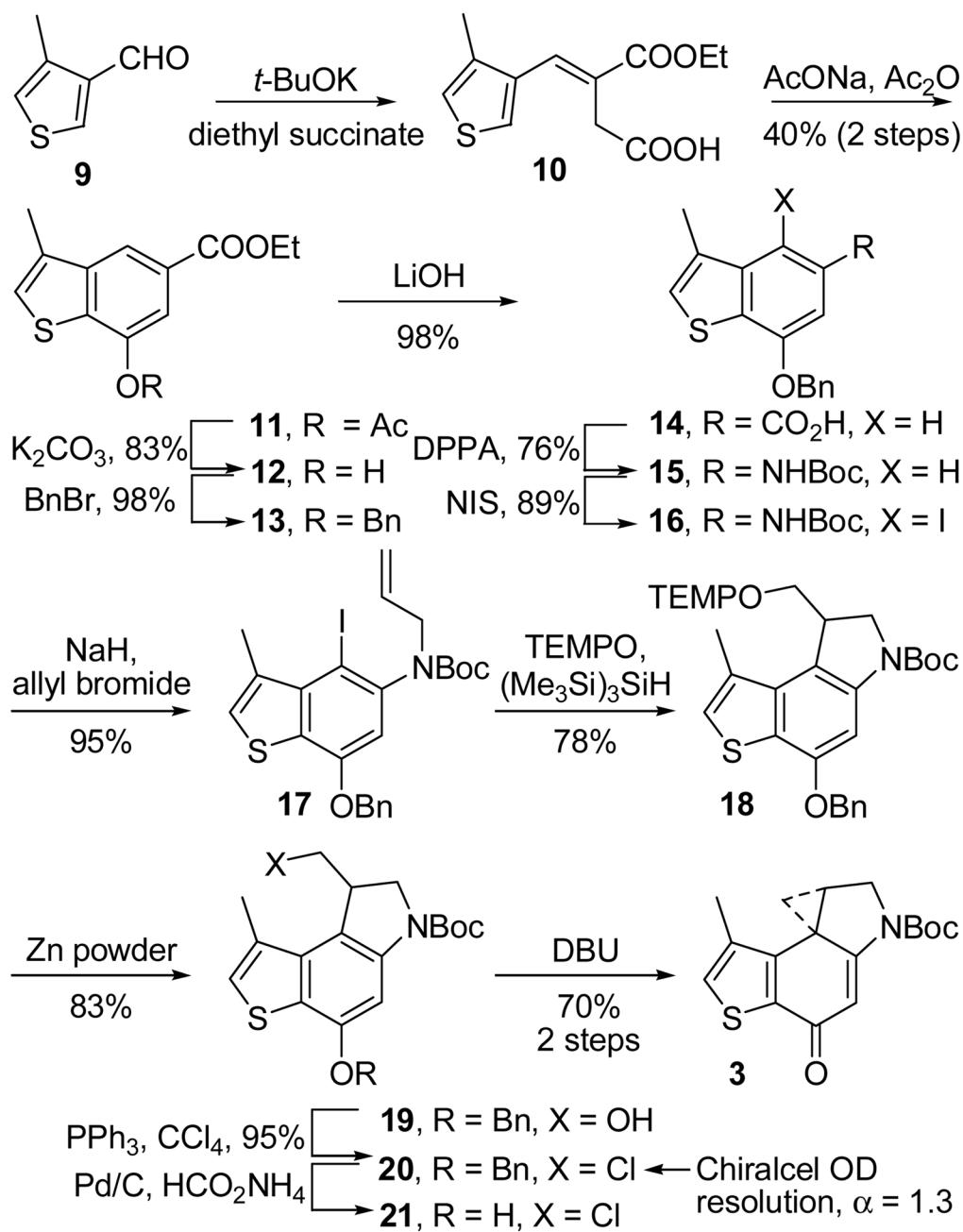
Thermally-induced strand cleavage of w794 DNA (144 bp, nucleotide no. 5238-138) after DNA-agent incubation with DSA-PDE-PDE (23 °C, 24 h), MeCTI-PDE-PDE (23 °C, 24 h), and CC-1065 (23 °C, 24 h), removal of unbound agent by EtOH precipitation and 30 min. thermolysis (100 °C), followed by denaturing 8%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



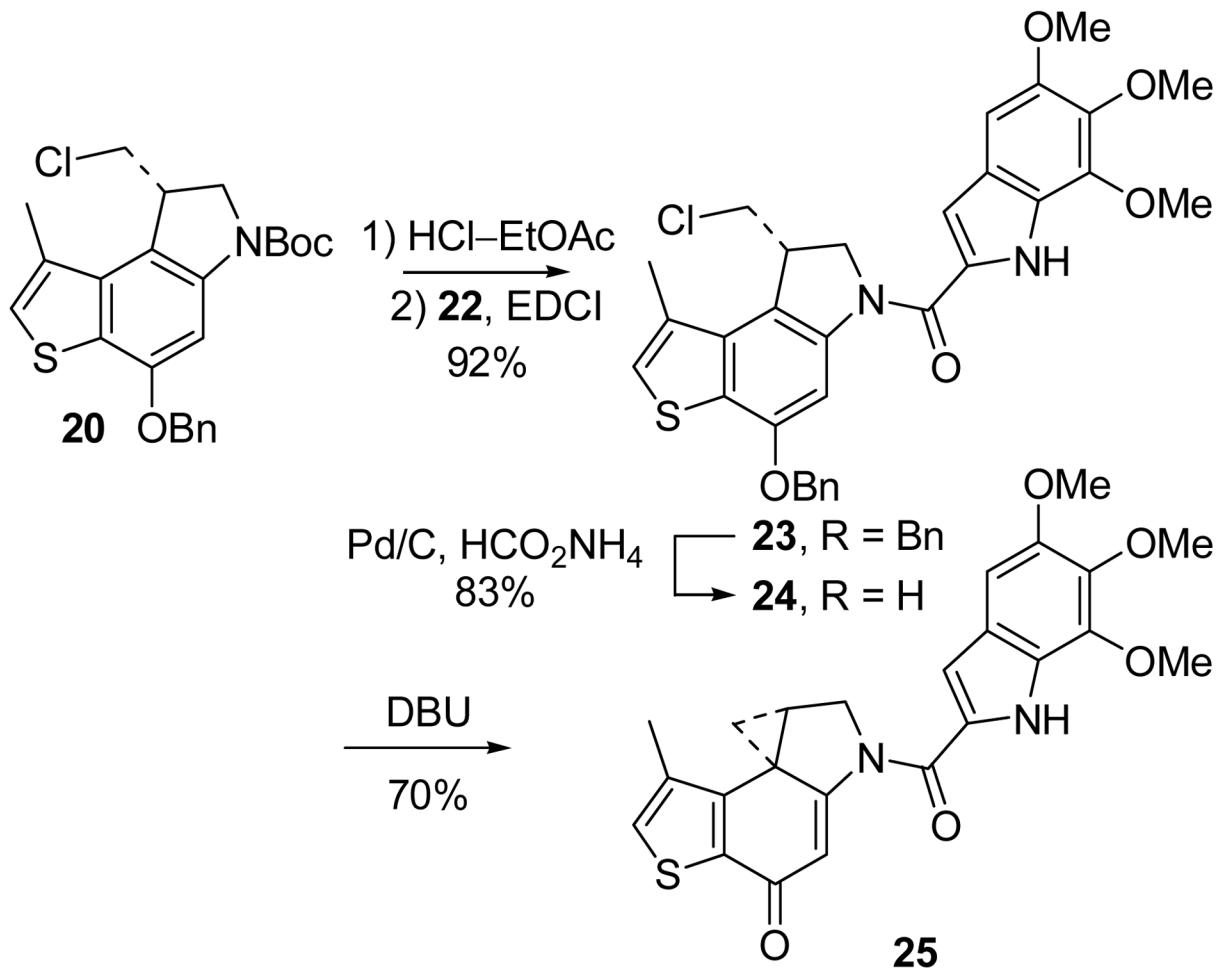
Compd	Dose μg/kg ^a	MSP days ^b	T/C ^c	Surviving Mice ^d
none	0	16.7	100	0/6
30	10	>60	>360	4/6
30	30	>53	>320	3/6

^aDose (μg/kg wt. of animal) administered i.p. on days 1, 5, and 9. ^bMSP = Mean Survival Period (days). ^cT/C = Treated/Control (MSP) × 100. ^dNo. of live animals after 80 days when terminated.

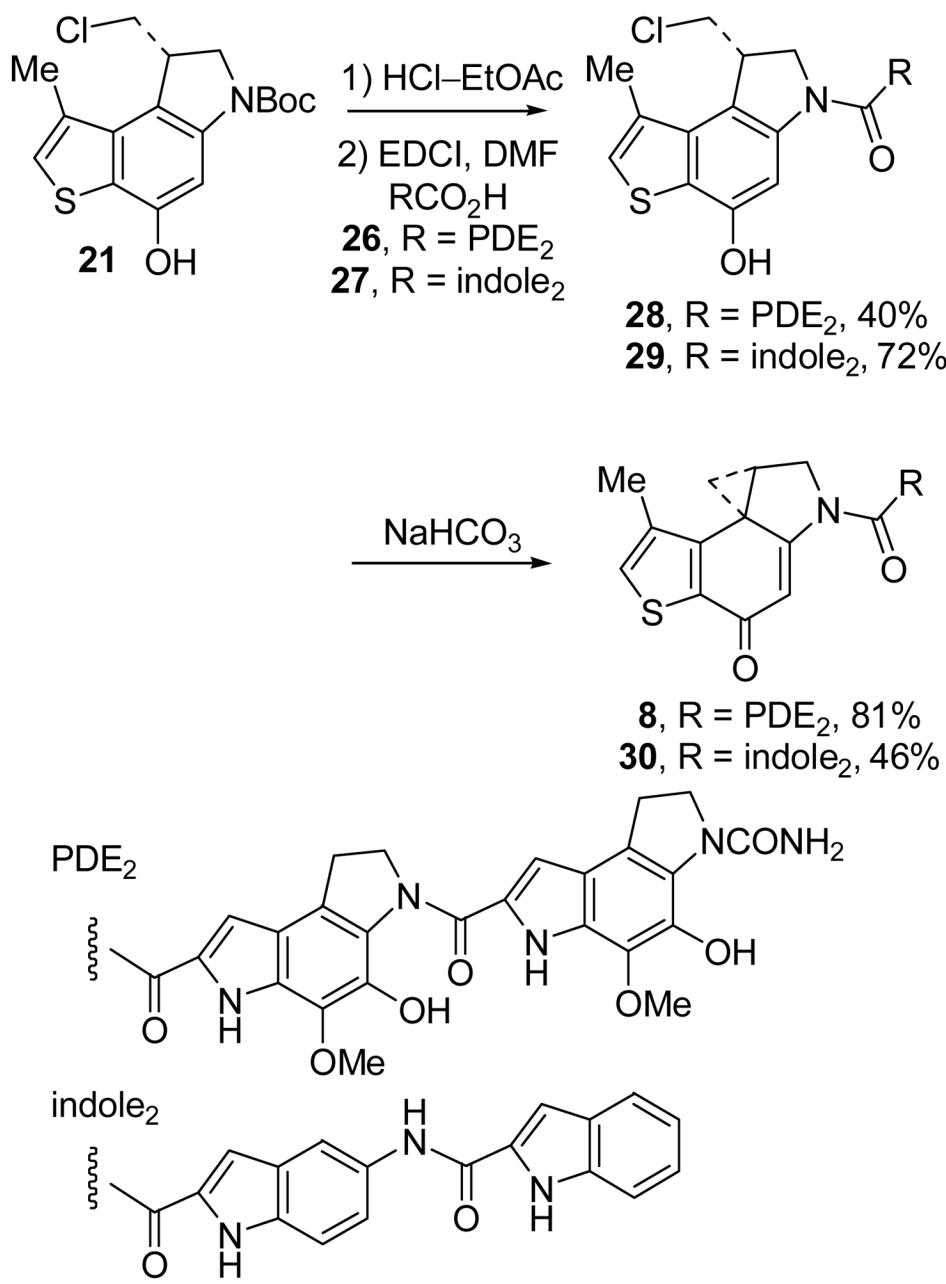
Figure 12.
In vivo antitumor activity (L1210, i.p.).



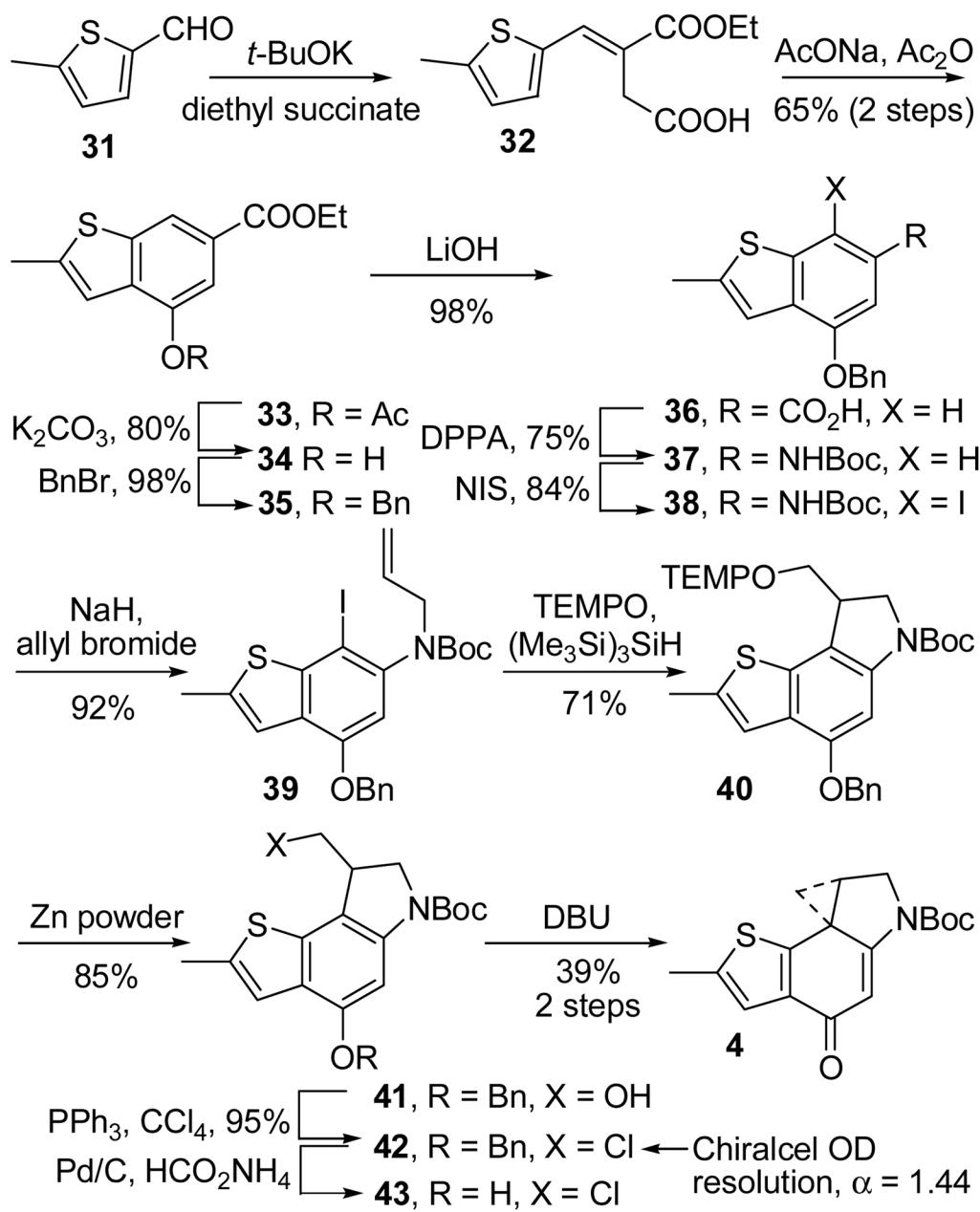
Scheme 1.



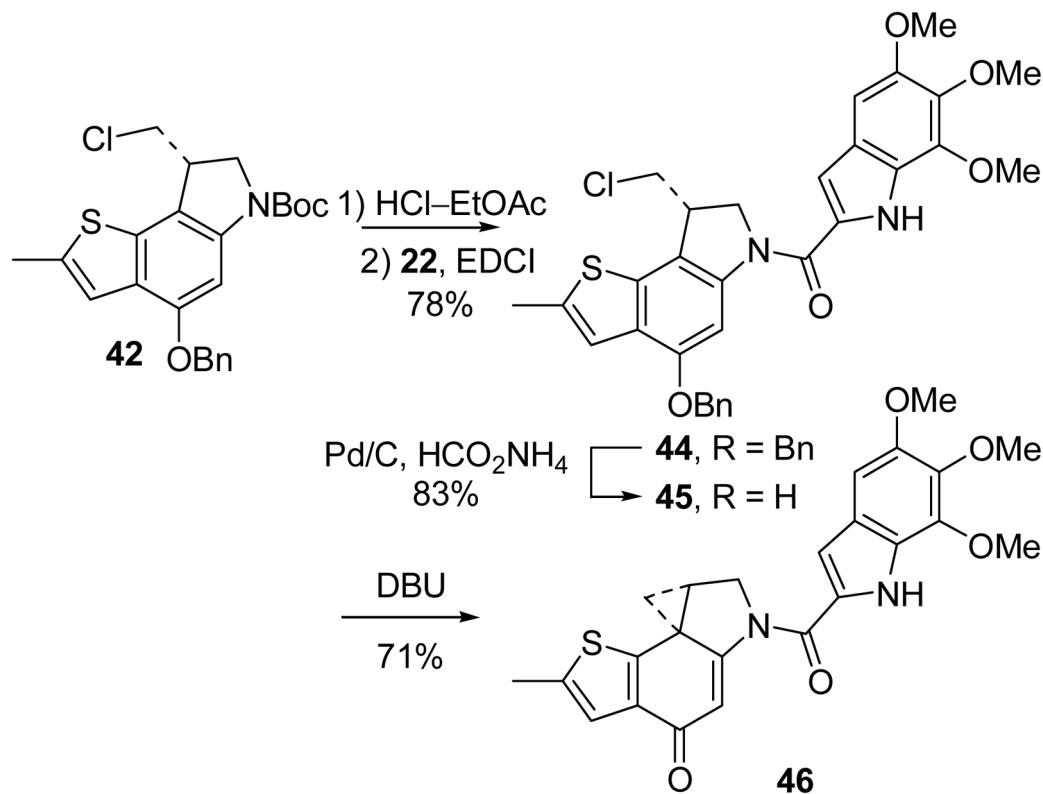
Scheme 2.



Scheme 3.



Scheme 4.



Scheme 5.