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(30) 1997/10/14 (60/061,892) US

(54) **ANALOGUES iso-CBI OU iso-CI DU CC-1065 ET
DUOCARMYCINES**

(54) **iso-CBI AND iso-CI ANALOGS OF CC-1065 AND THE
DUOCARMYCINS**

(57) L'invention porte sur la synthèse d'une série d'analogues bioactifs du (+)-CC-1065 (1) et sur les duocarmycines 2 et 3. Les analogues bioactifs comprennent soit l'iso-CI ou l'iso-CBI (6 et 7) en tant que sous-unités d'alkylation de l'ADN. Différentes sous-unités de fixation de l'ADN sont conjuguées aux sous-unités d'alkylation de l'ADN. Les analogues bioactifs conservent leur sélectivité vis à vis de l'ADN et présentent une réactivité améliorée.

(57) A series of bioactive analogs of (+)-CC-1065 (1) and the duocarmycins 2 and 3 are synthesized. The bioactive analogs include either iso-CI or iso-CBI (6 and 7) as a DNA alkylation subunit. Conjugated to the DNA alkylating subunits are a variety of DNA binding subunits. The bioactive analogs maintain their DNA selectivity and display enhance reactivity.



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<p>(21) International Application Number: PCT/US98/21749 (22) International Filing Date: 14 October 1998 (14.10.98) (30) Priority Data: 60/061,892 14 October 1997 (14.10.97) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 60/061,892 (CIP) Filed on 14 October 1997 (14.10.97) (71) Applicant (for all designated States except US): THE SCRIPPS RESEARCH INSTITUTE [US/US]; 10550 North Torrey Pines Road, La Jolla, CA 92037 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): BOGER, Dale, L. [US/US]; 2819 Via Posada, La Jolla, CA 92037 (US). (74) Agents: LEWIS, Donald, G. et al.; The Scripps Research Institute, 10550 North Torrey Pines Road, TPC-8, La Jolla, CA 92037 (US).</p>	<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report.</p>	
<p>(54) Title: <i>iso</i>-CBI AND <i>iso</i>-CI ANALOGS OF CC-1065 AND THE DUOCARMYCINS</p>		
<p>(57) Abstract</p>		
<p>A series of bioactive analogs of (+)-CC-1065 (1) and the duocarmycins 2 and 3 are synthesized. The bioactive analogs include either <i>iso</i>-CI or <i>iso</i>-CBI (6 and 7) as a DNA alkylation subunit. Conjugated to the DNA alkylating subunits are a variety of DNA binding subunits. The bioactive analogs maintain their DNA selectivity and display enhance reactivity.</p>		

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iso-CBI AND iso-CI ANALOGS OF CC-1065 AND THE DUOCARMYCINSDescription5 Field of Invention:

The invention relates to antitumor antibiotics. More particularly, the invention relates to analogs of CC-1065 and the duocarmycins having DNA alkylation and antitumor antibiotic activities .

10 Background:

(+)-CC-1065 (1) and the duocarmycins 2 and 3, illustrated in Figure 1, are natural products having antitumor antibiotic activity through the alkylation of DNA. (Hanka, L. J., et al., *J. Antibiot.* 1978, 31, 1211; Yasuzawa, T., et al., *Chem. Pharm. Bull.* 1995, 43, 378; and Takahashi, I., et al., *J. Antibiot.* 1991, 44, 1045.) Prior studies have shown that the natural products can withstand and may benefit from significant structural modifications to the alkylation subunit and that the resulting agents retain their ability to participate in the characteristic sequence-selective DNA alkylation reaction. (Boger, D. L., et al., *Chem. Rev.* 1997, 97, 787.) These structural modifications, and the definition of their effects have served to advance the understanding of the origin of the catalysis of the DNA alkylation reaction by 1-3. (Harper, D. E. *J. Am. Chem. Soc.* 1994, 116, 7573; and Warpehoski, M. A., et al., *J. Am. Chem. Soc.* 1995, 117, 2951.)

25 These structural modifications have also served to advance the understanding of the origin of the DNA sequence selectivity of 1-3. (Warpehoski, M. A. In *Advances in DNA Sequence Specific Agents*; Hurley, L. H., Ed.; JAI: Greenwich, CT, 1992; Vol. 1, p 217; Hurley, L. H. and Draves, P. In *Molecular Aspects of Anticancer Drug-DNA Interactions*; Neidle, S. and Waring, M., Eds.; CRC: Ann Arbor, 1993; Vol. 1, p 89; and Aristoff, P. A. In *Advances in Medicinal Chemistry*; JAI: Greenwich, CT, 1993; Vol. 2, p 67). Two models have been

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proposed to explain the mechanism of the DNA sequence selectivity of 1-3. One model proposed by Boger states that the DNA sequence selectivity of 1-3 is determined by the AT-rich noncovalent binding selectivity of these agents and their steric accessibility to the adenine N3 alkylation site. (Boger, D. L., et al., *Angew. Chem., Int. Ed. Engl.* 1996, 35, 1439; and Boger, D. L., et al., *Bioorg. Med. Chem.* 1997, 5, 263.) This noncovalent binding model accommodates and explains the reverse and offset 5 or 3.5 base-pair AT-rich adenine N3 alkylation selectivities of the natural and unnatural enantiomers of 1 (Boger, D. L., et al., *J. Am. Chem. Soc.* 1990, 112, 4623; and Boger, D. L., et al., *Bioorg. Med. Chem.* 1994, 2, 115) and the natural and unnatural enantiomers of 2-3. (Boger, D. L., et al., *J. Am. Chem. Soc.* 1993, 115, 9872; and Boger, D. L., et al., *J. Am. Chem. Soc.* 1994, 116, 1635.) This noncovalent binding model also requires that simple derivatives of the alkylation subunits exhibit alkylation selectivities distinct from the natural products. It also offers an explanation for the identical alkylation selectivities of both enantiomers of such simple derivatives ($5'-AA > 5'-TA$), and the more extended AT-rich selectivity of the advanced analogs of 1-3 corresponds nicely to the length of the agent and the size of the required binding region surrounding the alkylation site. This model is further supported by the demonstrated AT-rich noncovalent binding of these agents. (Boger, D. L., et al., *Chem.-Biol. Interactions* 1990, 73, 29; and Boger, D. L., et al., *J. Org. Chem.* 1992, 57, 1277.) The model is also supported by the correspondance between the observed preferential noncovalent binding and the observed DNA alkylation of these agents. (Boger, D. L., et al., *Bioorg. Med. Chem.* 1996, 4, 859.) Also the observation that the characteristic DNA alkylation selectivity of these agents does not require the presence of the C-4 carbonyl or even the activated cyclopropane provides further support for the model, (Boger, D. L., et al., *J. Am. Chem. Soc.* 1991, 113, 3980.; and Boger, D. L., et al., *Proc. Natl. Acad. Sci. U.S.A.* 1991, 88, 1431.) The accuracy of this model is further demonstration of the complete switch in the inherent enantiomeric DNA alkylation selectivity that accompanied the reversal of the orientation of the DNA binding subunits with reversed versus extended analogs of the duocarmycins.

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(Boger, D. L., et al., *J. Am. Chem. Soc.* 1997, 119, 4977; Boger, D. L., et al., *J. Am. Chem. Soc.* 1997, 119, 4987; and Boger, D. L., et al., *J. Am. Chem. Soc.* 1995, 117, 1443.)

5 The above AT-rich noncovalent binding model contrasts with an alternative proposal in which a sequence-dependent backbone phosphate protonation of the C-4 carbonyl activates the agent for DNA alkylation and controls the sequence selectivity. (Hurley, L. H. *J. Am. Chem. Soc.* 1995, 117, 2371.)

10 Structural studies of DNA-agent adducts,¹⁷⁻¹⁹ the C-4 carbonyl of the natural products projects out of the minor groove lying on the outer face of the complexes potentially accessible to the phosphate backbone. (Lin, C. H., et al., *J. Mol. Biol.* 1995, 248, 162.; and Smith, J. A., et al., *J. Mol. Biol.* 1997, 272, 237.) However, the relative importance of the C-4 carbonyl positioning to the properties
15 of these agents has not be determined.

 What is needed is a series of analogs of (+)-CC-1065 (1) and the duocarmycins 2 and 3 which exploit the AT-rich noncovalent binding model and which retain their DNA binding and alkylating activity and selectivity. What is
20 needed is series of analogs of (+)-CC-1065 (1) and the duocarmycins 2 and 3 which incorporate of *iso*-CI and *iso*-CBI (6 and 7). *Iso*-CI and *iso*-CBI (6 and 7) are analogs of the CI and CBI alkylation subunits 4 and 5 wherein the key C-4 carbonyl is *isomerically* relocated to the C-6 or C-8 positions, now ortho to the cyclopropane, as illustrated in Figure 2. If the AT-rich noncovalent binding model
25 is correct, the relocated carbonyls of *iso*-CI and *iso*-CBI (6 and 7) would project into the minor groove inaccessible to the phosphate backbone if participating in an analogous adenine N3 alkylation reaction.

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Summary:

A series of bioactive analogs of (+)-CC-1065 (1) and the duocarmycins 2 and 3 are synthesized. Each of the analogs includes *iso*-CI or *iso*-CBI (6 and 7) as a DNA alkylation subunit. The novel DNA alkylation subunits are then
5 conjugated to known DNA binding subunits to form bioactive analogs of (+)-CC-1065 (1) and the duocarmycins 2 and 3. Preferred DNA binding subunits are disclosed herein and in U.S. Patent Application Serial No. 09/051,264, incorporated herein by reference.

10 2-(*tert*-Butyloxycarbonyl)-1,2,9,9a-tetrahydrocyclo-propa[*c*]benzo[*f*]-indol-8-one (31, *N*-BOC-*iso*-CBI) and 1-(*tert*-butyloxycarbonyl)-4-hydroxy-3-[[*(methanesulfonyl)oxy*]methyl]-2,3-dihydroindole (19, *seco-N*-BOC-*iso*-CI) serve as preconjugate forms to the DNA alkylating subunits, i.e., *iso*-CI or *iso*-CBI (6 and 7). The approach for synthesizing compounds 31 and 19 was based on a directed
15 ortho metallation of an appropriately functionalized benzene (13) or naphthalene (24) precursor to regiospecifically install iodine at the C-2 position. Conversion of these respective intermediates to the dihydroindole skeleton utilized an established 5-*exo-trig* aryl radical cyclization onto an unactivated alkene with subsequent TEMPO trap or the more recent 5-*exo-trig* aryl radical cyclization onto a vinyl
20 chloride for direct synthesis of the immediate precursors. Closure of the activated cyclopropane to complete the *iso*-CBI nucleus was accomplished by a selective ortho spirocyclization.

Resolution and synthesis of a full set of natural product analogs and
25 subsequent evaluation of their DNA alkylation properties revealed that the *iso*-CBI analogs react at comparable rates and retain the identical and characteristic sequence selectivity of CC-1065 and the duocarmycins. This observation is inconsistent with the prior art proposal that a sequence-dependent C-4 carbonyl protonation by strategically located DNA backbone phosphates controls the DNA
30 alkylation selectivity but is consistent with the proposal that it is determined by the

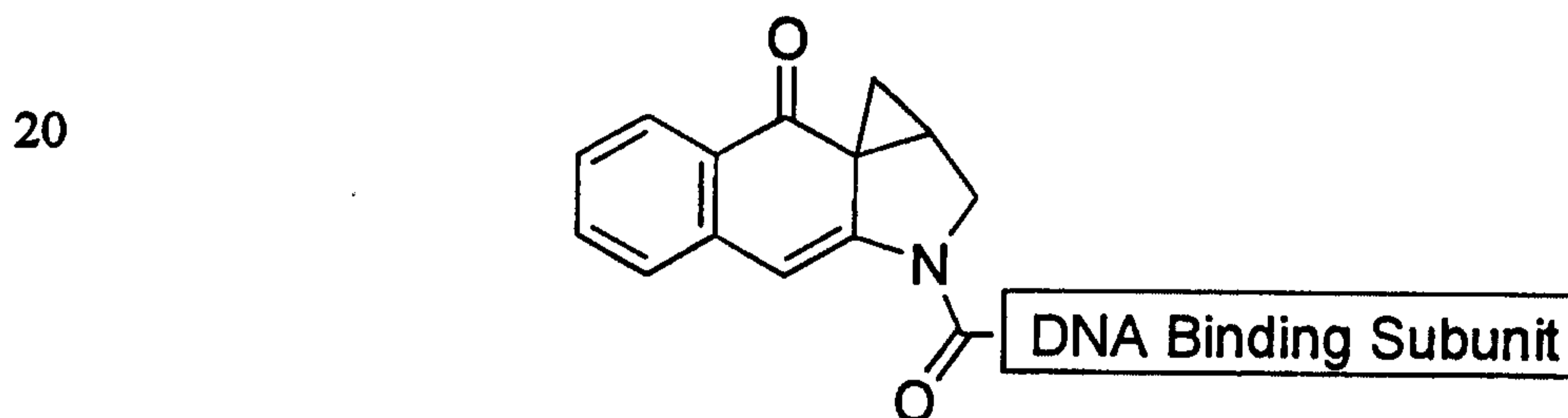
- 5 -

AT-rich noncovalent binding selectivity of the agents and the steric accessibility of the N3 alkylation site.

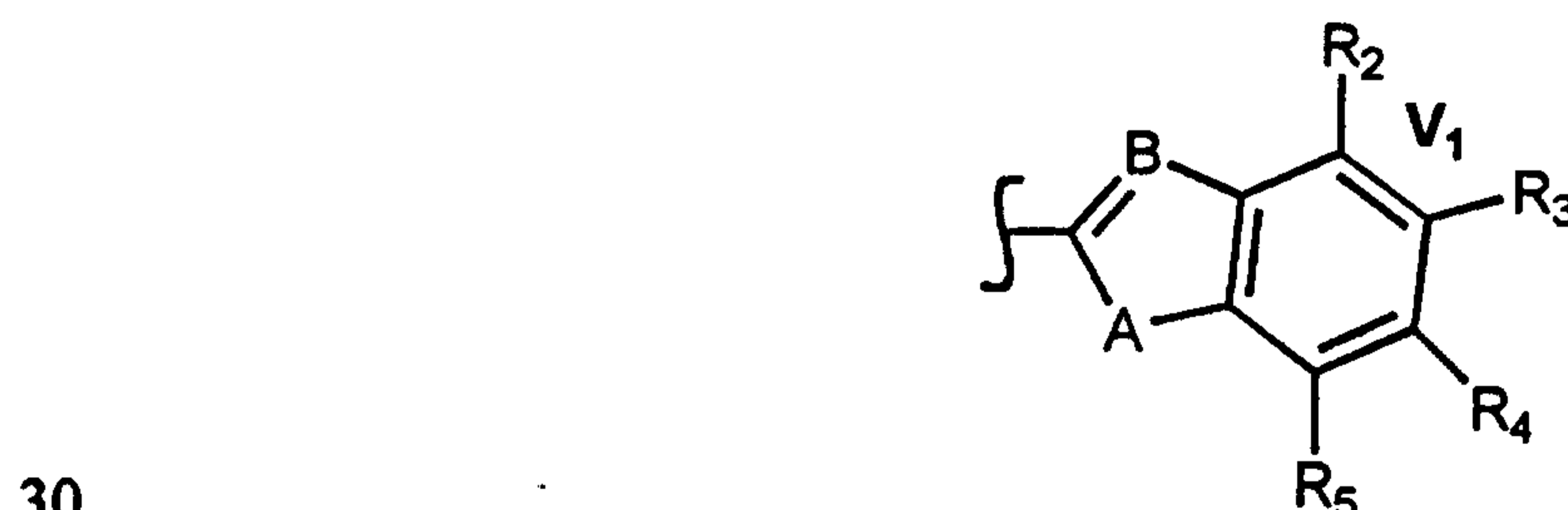
Solvolysis studies indicate that the *iso*-CBI-based agents have a stability comparable to that of CC-1065 and duocarmycin A and a greater reactivity than duocarmycin SA (6–7×). Solvolysis studies also indicate that the *iso*-CBI-based agents are more reactive than the corresponding CBI-based agents (5×).

Confirmation that the DNA alkylation reaction is derived from adenine N3 addition to the least substituted carbon of the activated cyclopropane and its quantitation (95%) was established by isolation and characterization of the depurination adenine N3 adduct. Consistent with past studies and in spite of the deep-seated structural change in the alkylation subunit, the agents were found to exhibit potent cytotoxic activity that correlates with their inherent reactivity.

One aspect of the invention is directed to DNA alkylating compounds having a DNA alkylating subunit covalently linked to a DNA binding subunit. The DNA alkylating compound is represented by the following structure:

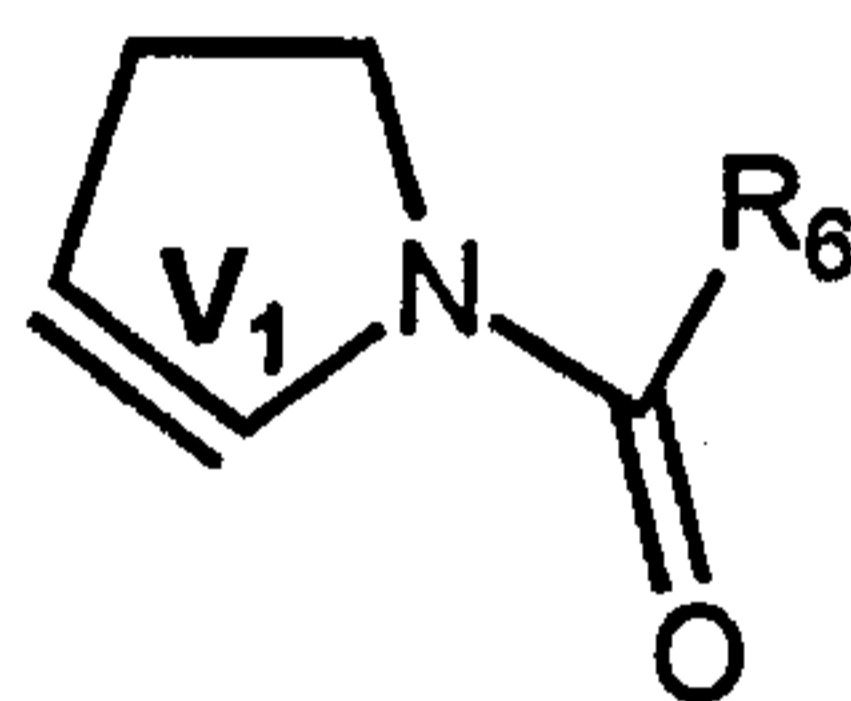


A preferred DNA binding subunit is a radical represented by the following structure:



- 6 -

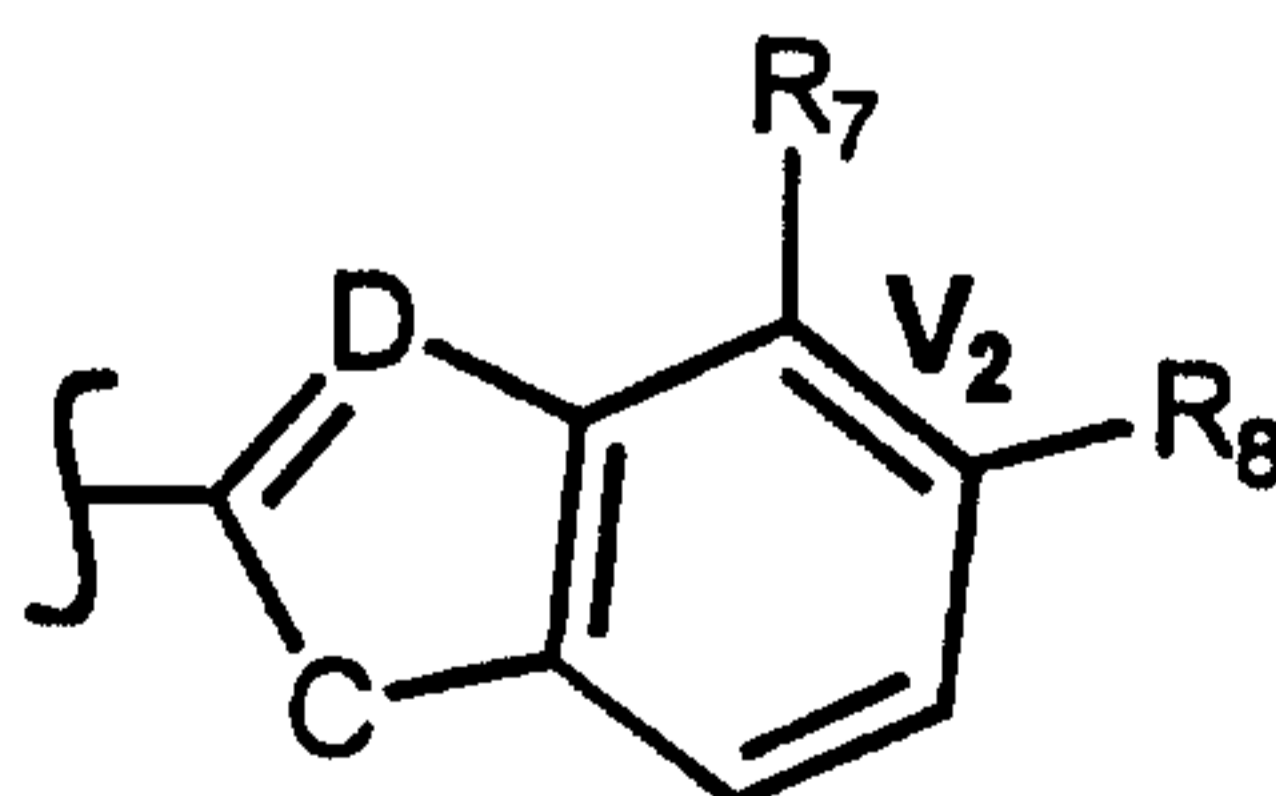
In the above structure, **A** is selected from the group consisting of **NH** and **O**. **B** is selected from the group consisting of **C** and **N**. **R₂** is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, and a first N-substituted pyrrolidine ring. **R₃** is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, the first N-substituted pyrrolidine ring. **R₄** is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), and N-alkyl (C1-C6)₃. **R₅** is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), and N-alkyl (C1-C6)₃. **V₁** represents a first vinylene group between **R₂** and **R₃**. However, there are various provisos. If **R₂** participates in the first N-substituted pyrrolidine ring, then **R₃** also particlates in the first N-substituted pyrrolidine ring. If **R₃** participates in the first N-substituted pyrrolidine ring, then **R₂** also particlates in the first N-substituted pyrrolidine ring. If **R₂** and **R₃** participate in the first N-substituted pyrrolidine ring, then **R₄** and **R₅** are hydrogen. If **R₂** is hydrogen, then **R₄** and **R₅** are hydrogen and **R₃** is N-alkyl (C1-C6)₃. The first N-substituted pyrrolidine ring is fused to the first vinylene group between **R₂** and **R₃** and is represented by the following structure:



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In the above structure **V₁** represents the first vinylene group between **R₂** and **R₃**. **R₆** is selected from the group consisting of -CH₂CH₃ (alkyl), -NHCH₃ (-N-alkyl), -OCH₃ (O-alkyl), -NH₂, -NHNH₂, -NHNHCO₂'Bu, and a radical represented by the following structure:

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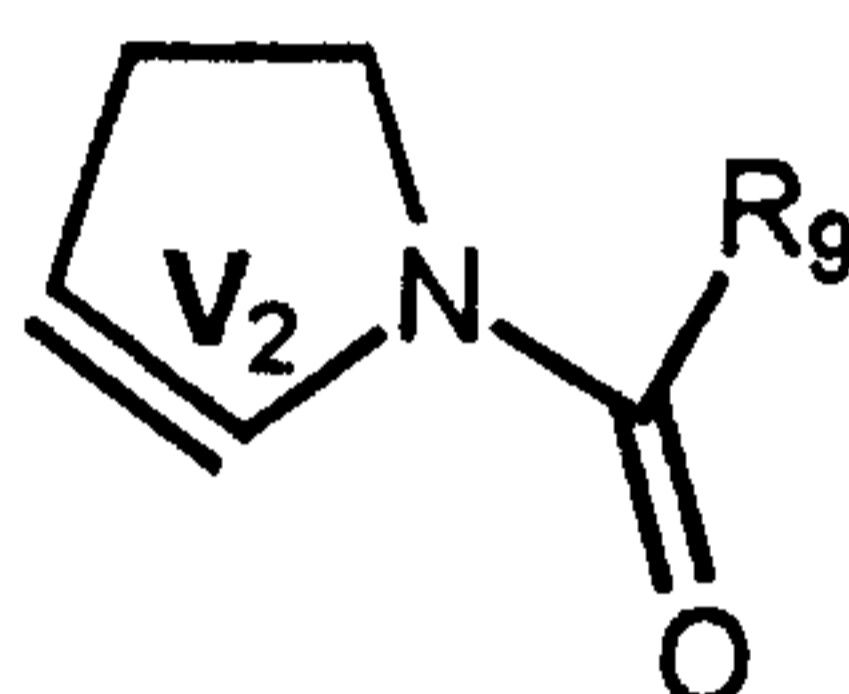
In the above structure, **C** is selected from the group consisting of **NH** and **O**. **D** is selected from the group consisting of **C** and **N**. **R₇** is selected from the group

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consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, and a second N-substituted pyrrolidine ring. R₈ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, the second N-substituted pyrrolidine ring. V₂ represents the second vinylene group between R₇ and R₈.

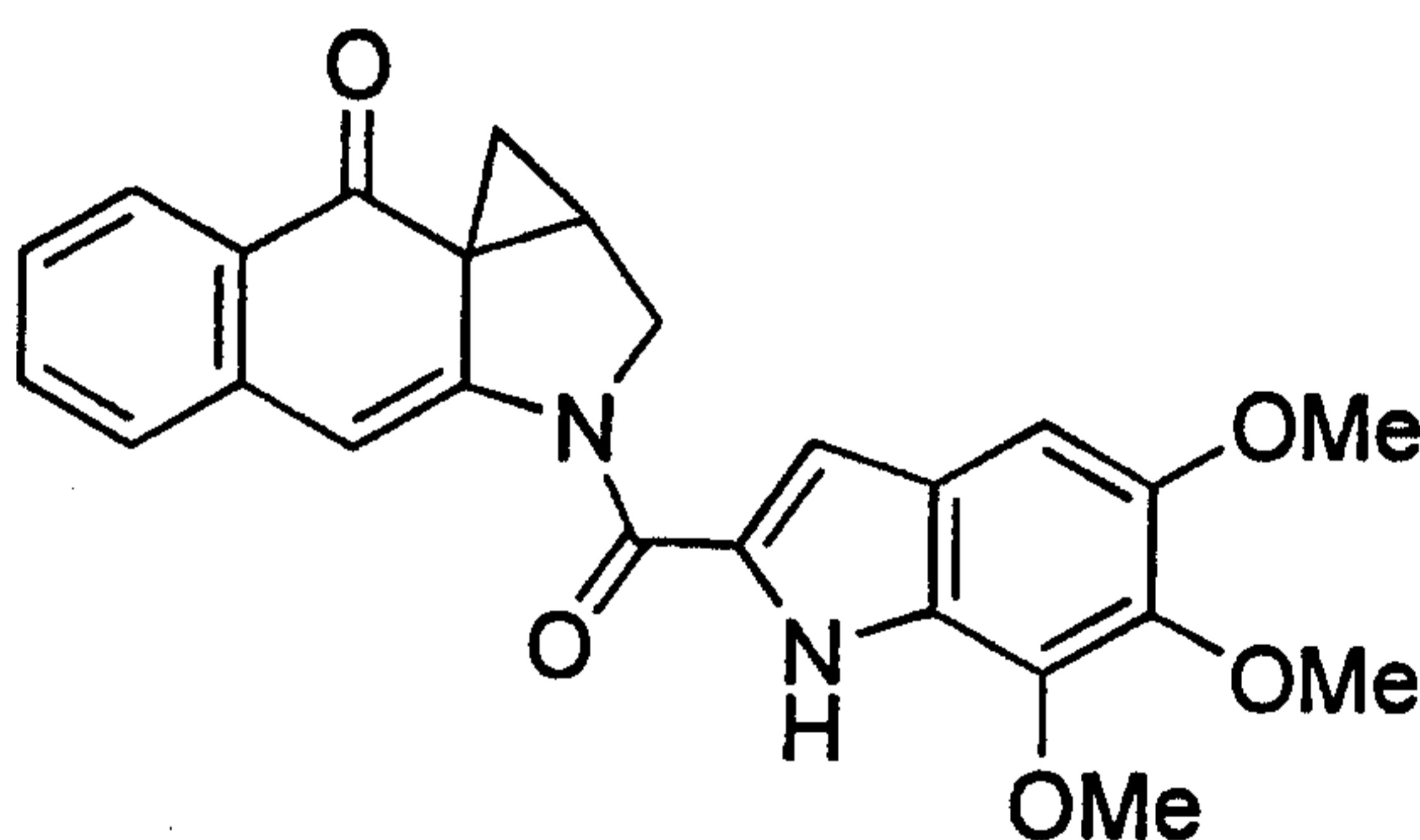
5 However, the following provisos pertain. If R₇ participates in the N-substituted pyrrolidine ring, then R₈ also particlates in the N-substituted pyrrolidine ring. If R₈ participates in the N-substituted pyrrolidine ring only if R₇ also particlates in the N-substituted pyrrolidine ring. The second N-substituted pyrrolidine ring is fused to the second vinylene group between R₇ and R₈ and is represented by the following
10 structure:



In the above sturcture, V₂ represents the second vinylene group between R₇ and
15 R₈. R₉ is selected from the group consisting of -CH₂CH₃ (alkyl), -NHCH₃ (-N-alkyl), -OCH₃ (O-alkyl), -NH₂, -NHNH₂, and -NHNHCO₂'Bu.

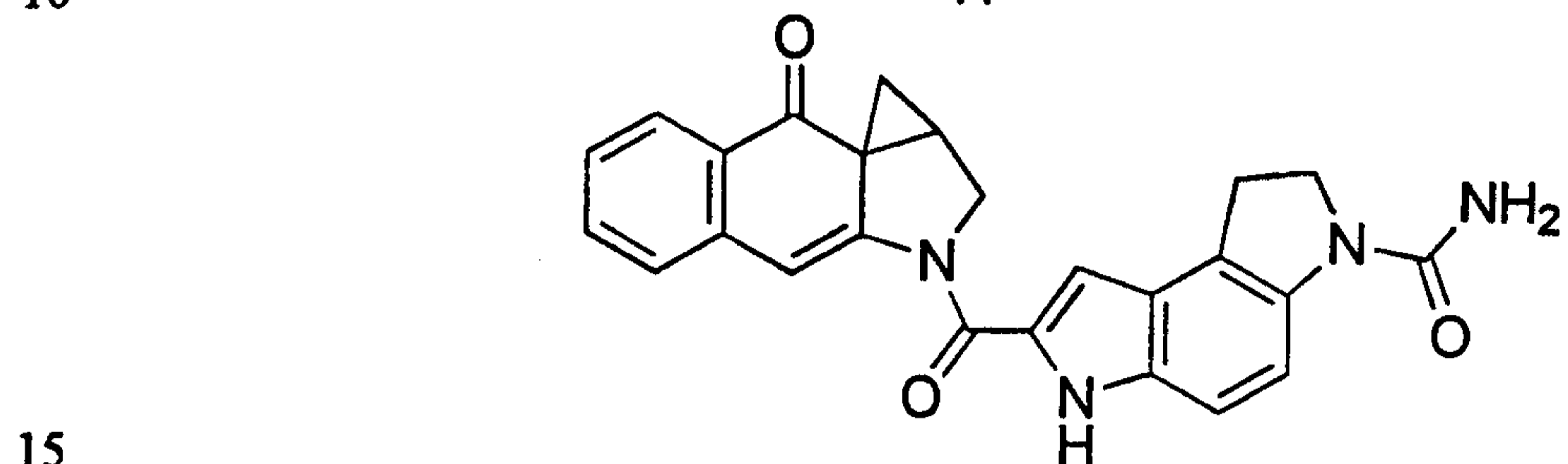
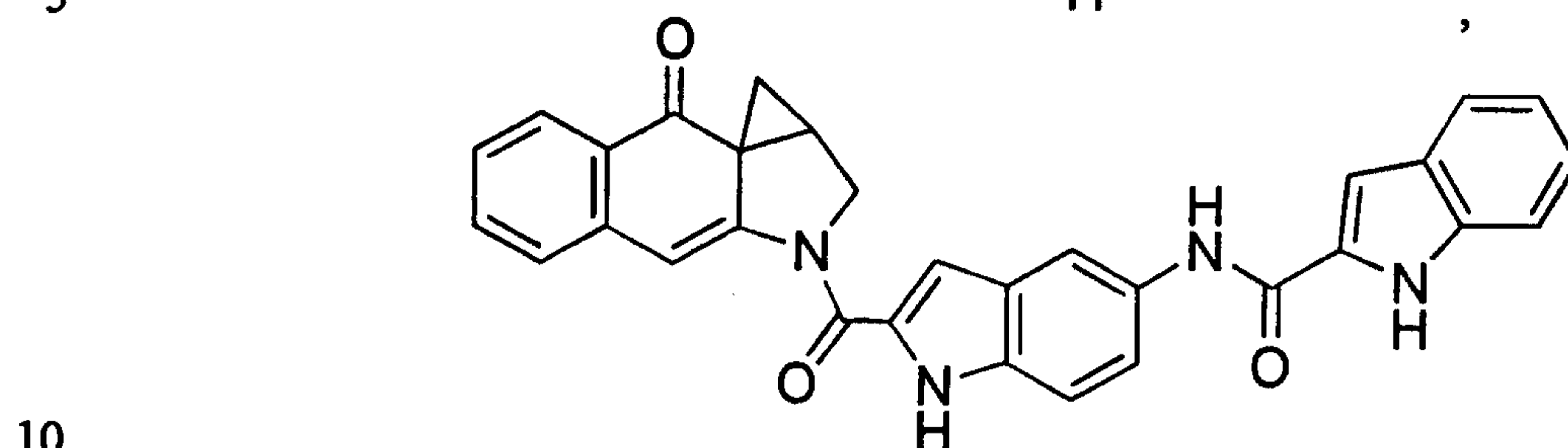
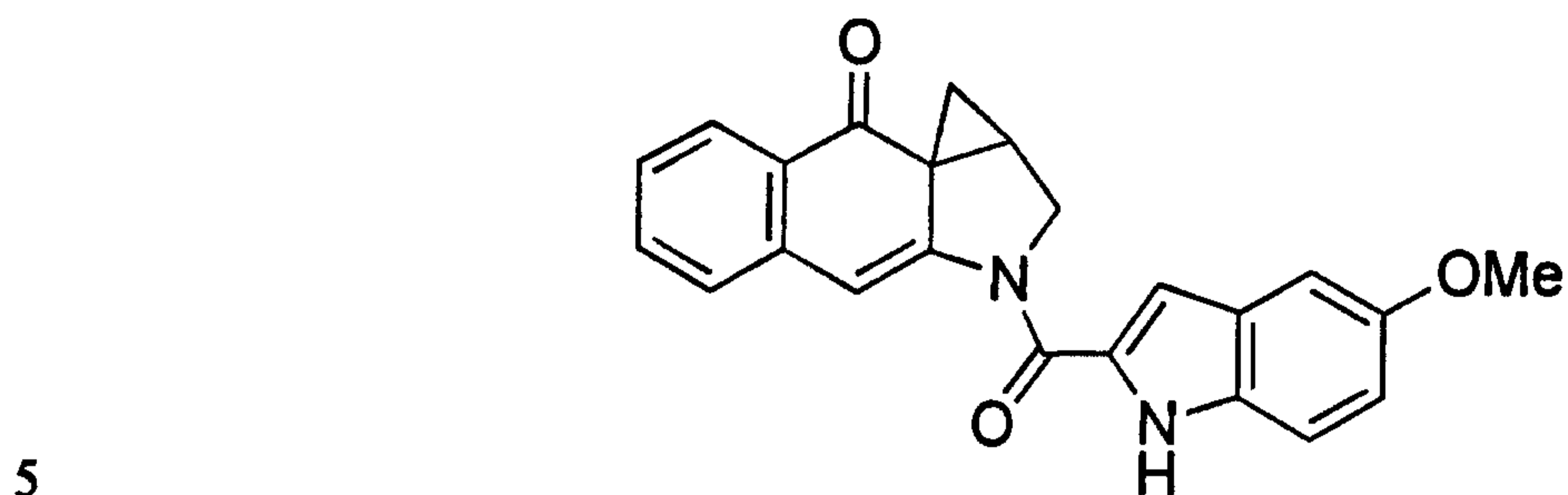
Preferred examples include DNA alkylating compounds represented by the following structures:

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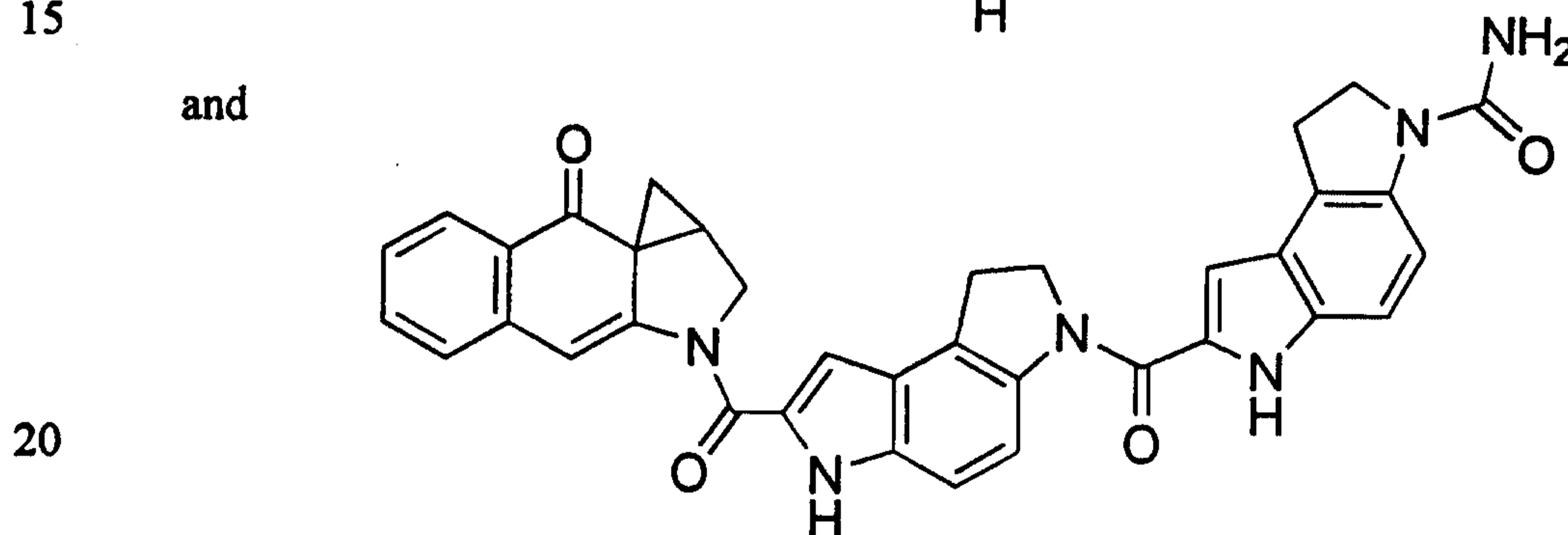


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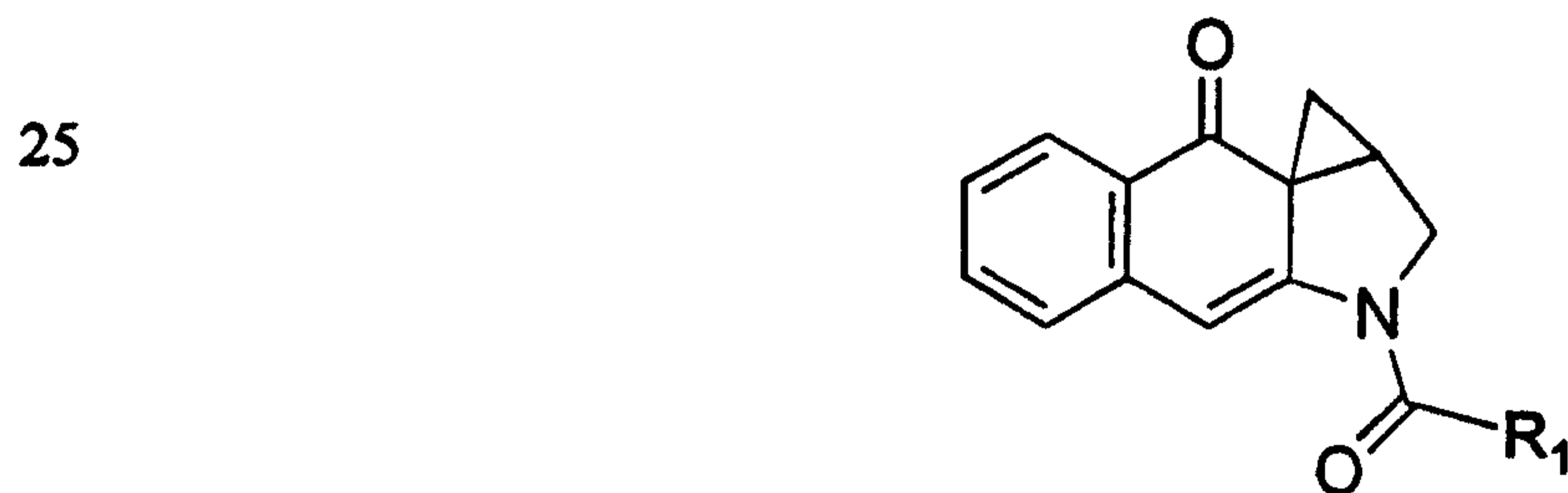
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and



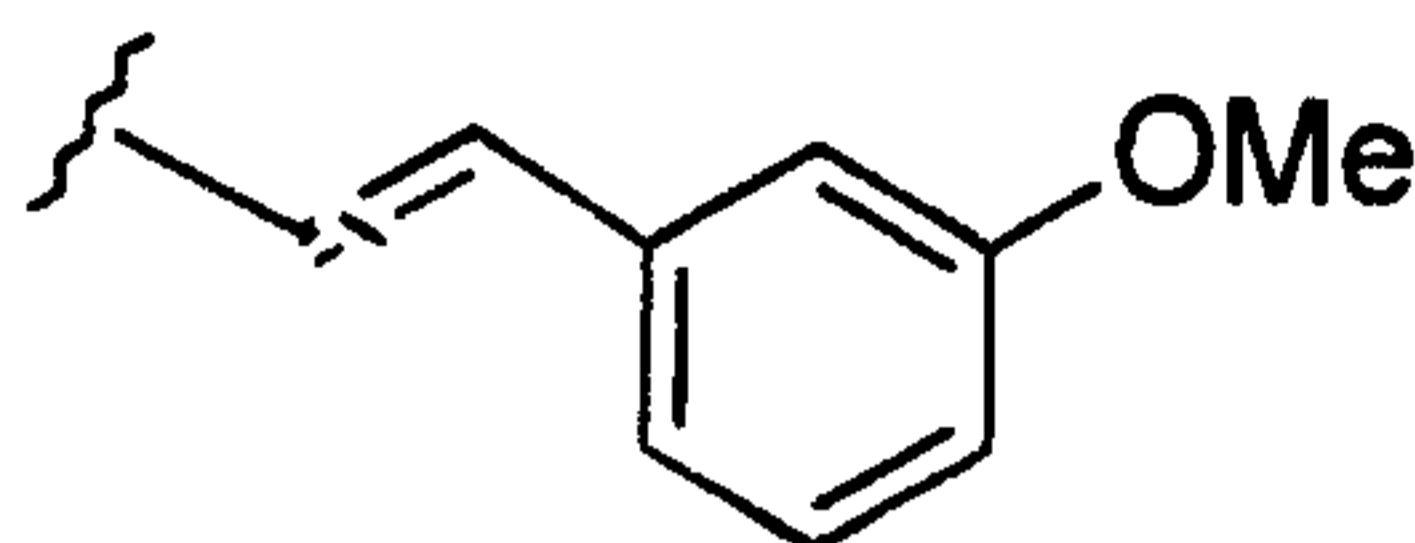
Another aspect of the invention is directed to DNA alkylating compounds represented by the following structure:



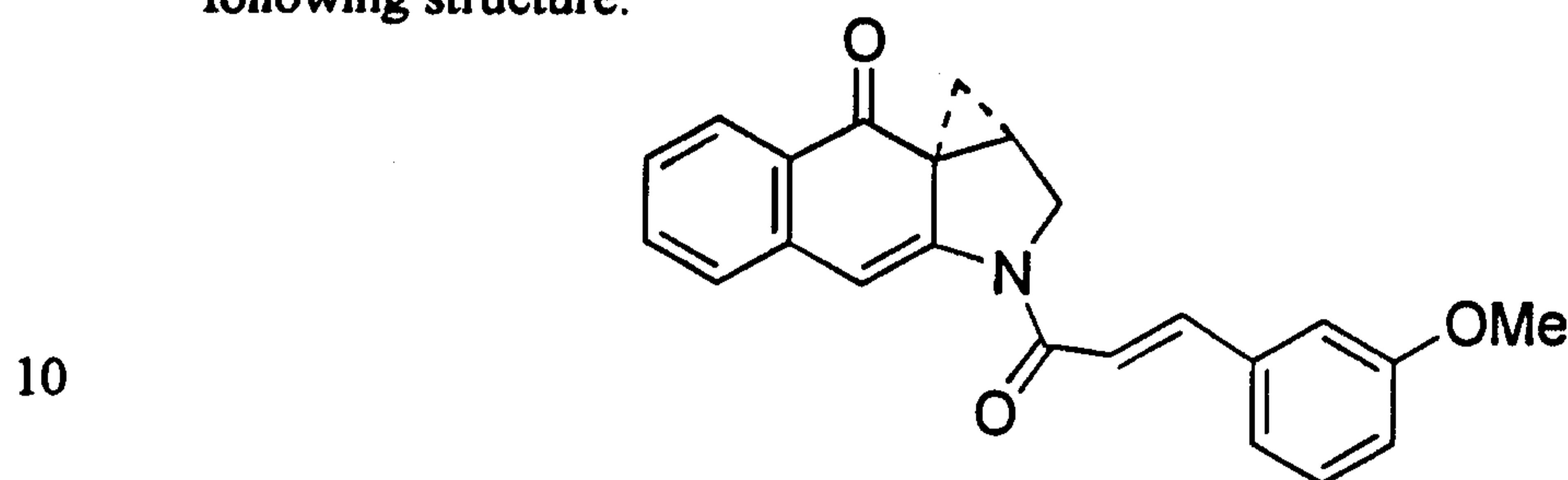
30 In the above structure, R_1 is selected from the group consisting of -C1-C6 alkyl, -NHCH₃ (-N-alkyl), -OCH₃ (O-alkyl), -NH₂, -NHNH₂, -NHNHCO₂tBu, and a

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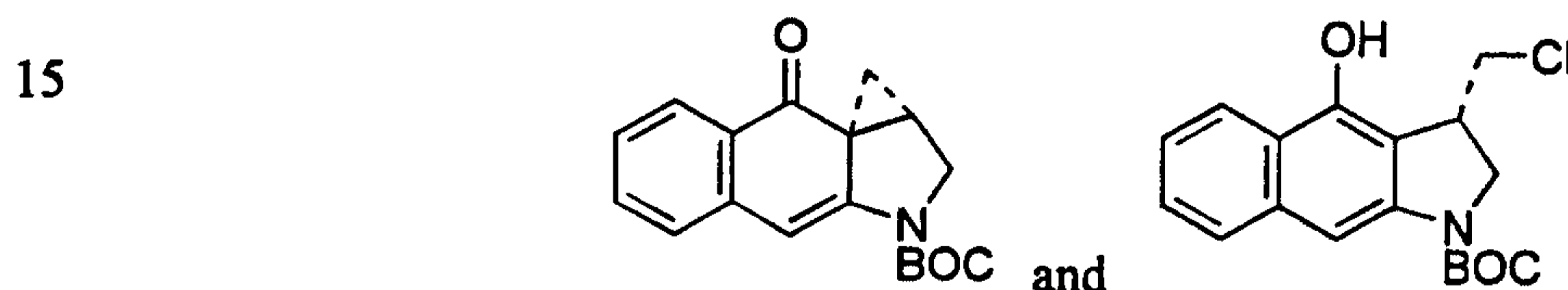
radical represented by the following structure:



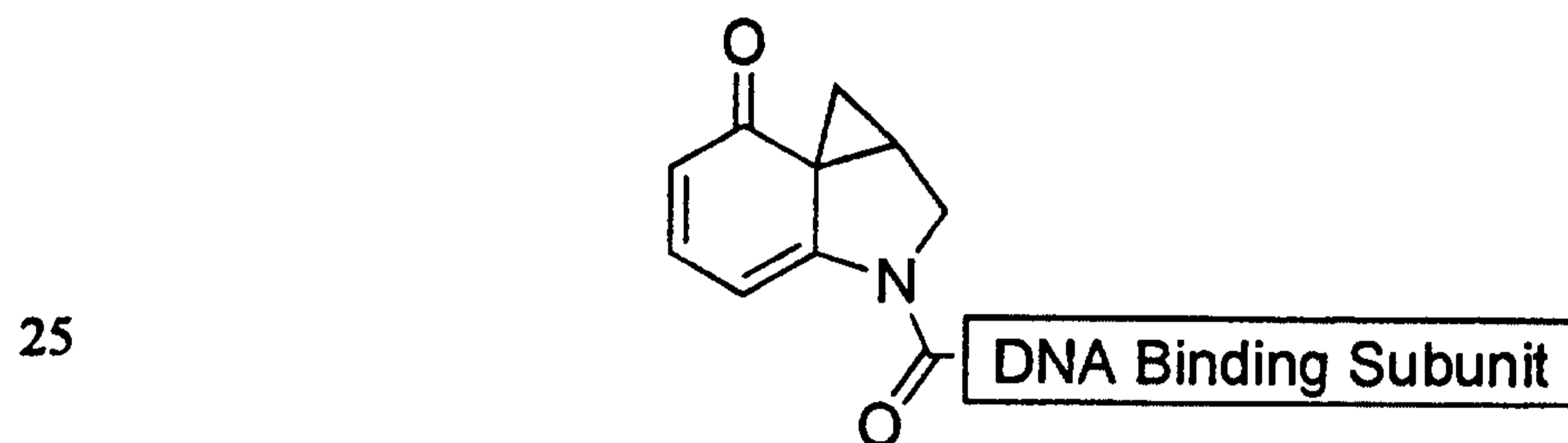
5 A preferred embodiment of this aspect of the invention is represented by the following structure:



Further aspects of the invention are directed to chemical intermediate represented by the following structures:

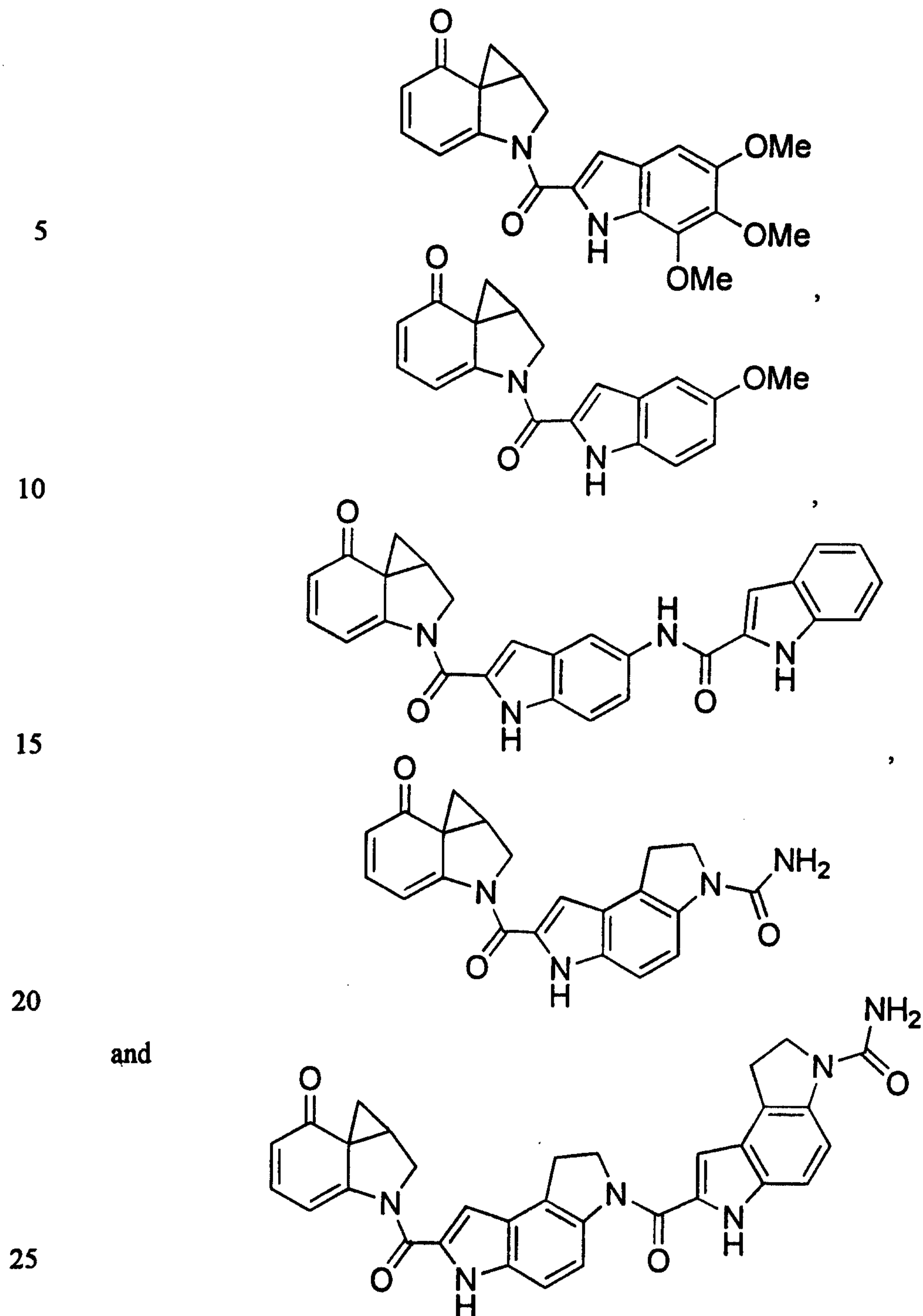


15 Another aspect of the invention is directed to DNA alkylating compounds having a DNA alkylating subunit covalently linked to a DNA binding subunit covalently linked said DNA alkylating subunit, wherein the DNA alkylating compound being represented by the following structure:



25 Preferred DNA binding subunit are as described above for the *iso*-CBI compounds. Preferred examples of this aspect of the invention include DNA alkylating compounds represented by the following structures:

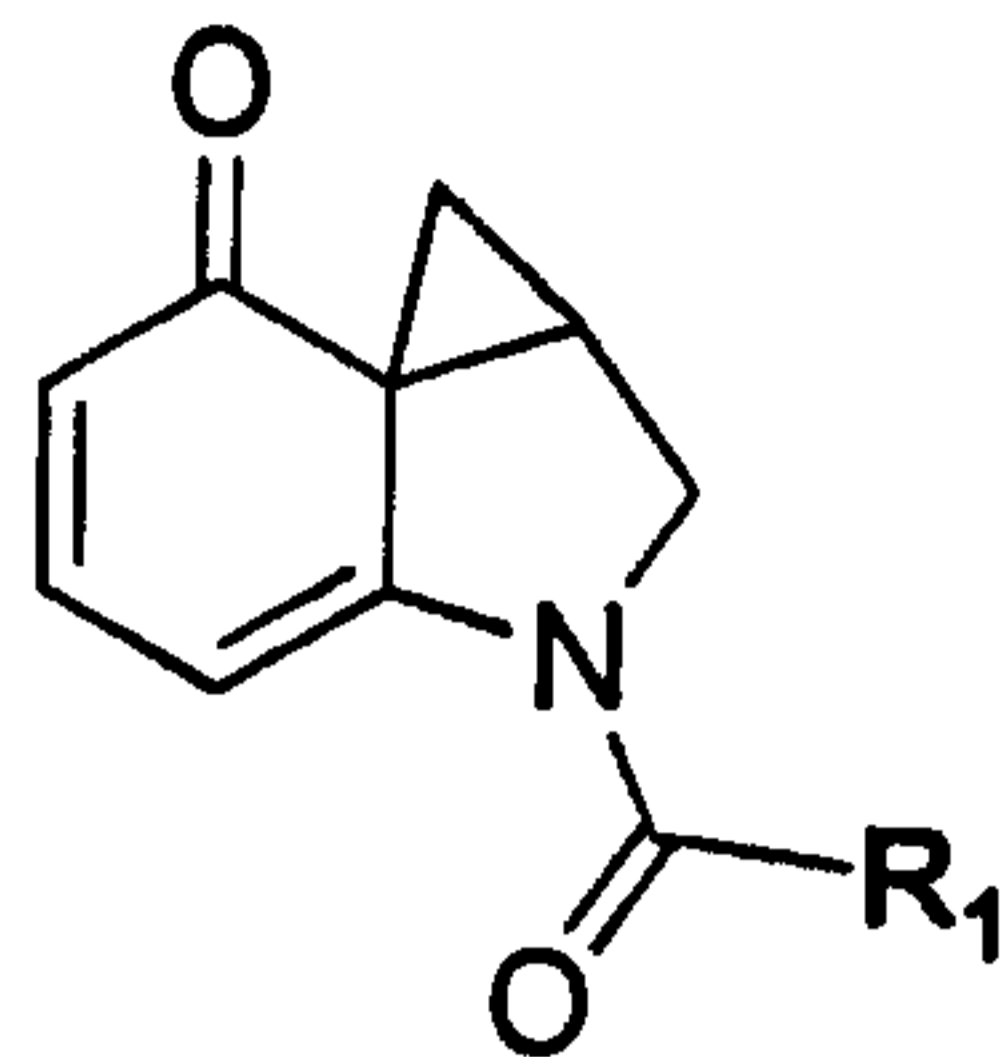
- 10 -



Another aspect of the invention is directed to DNA alkylating compounds represented by the following structure:

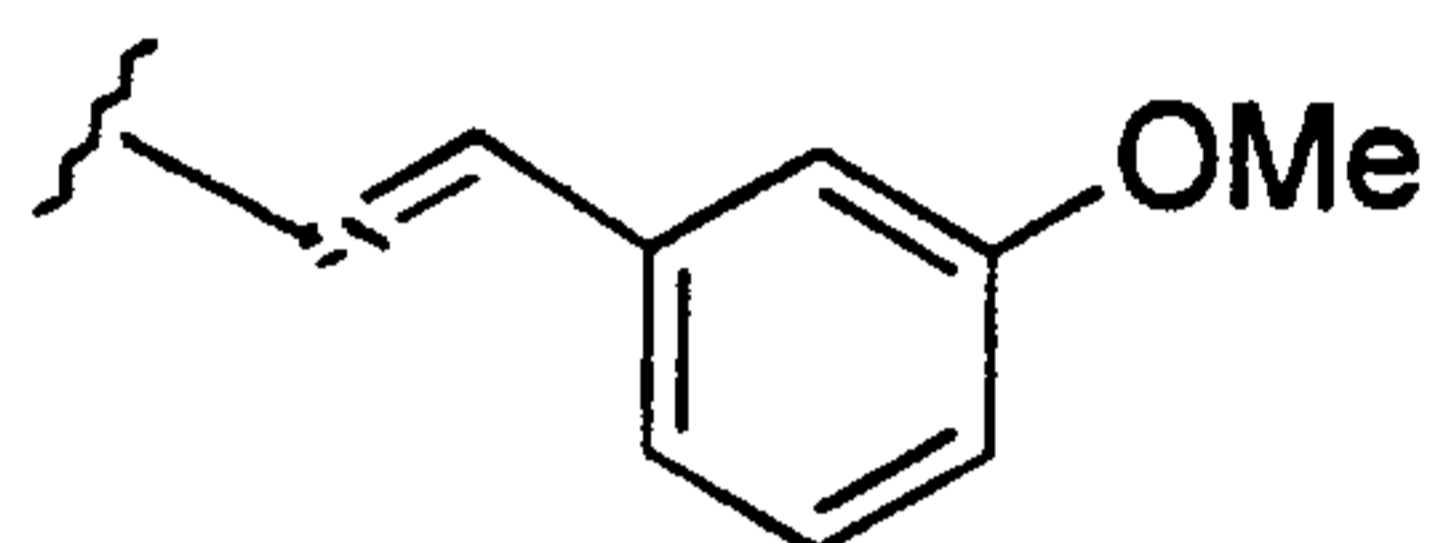
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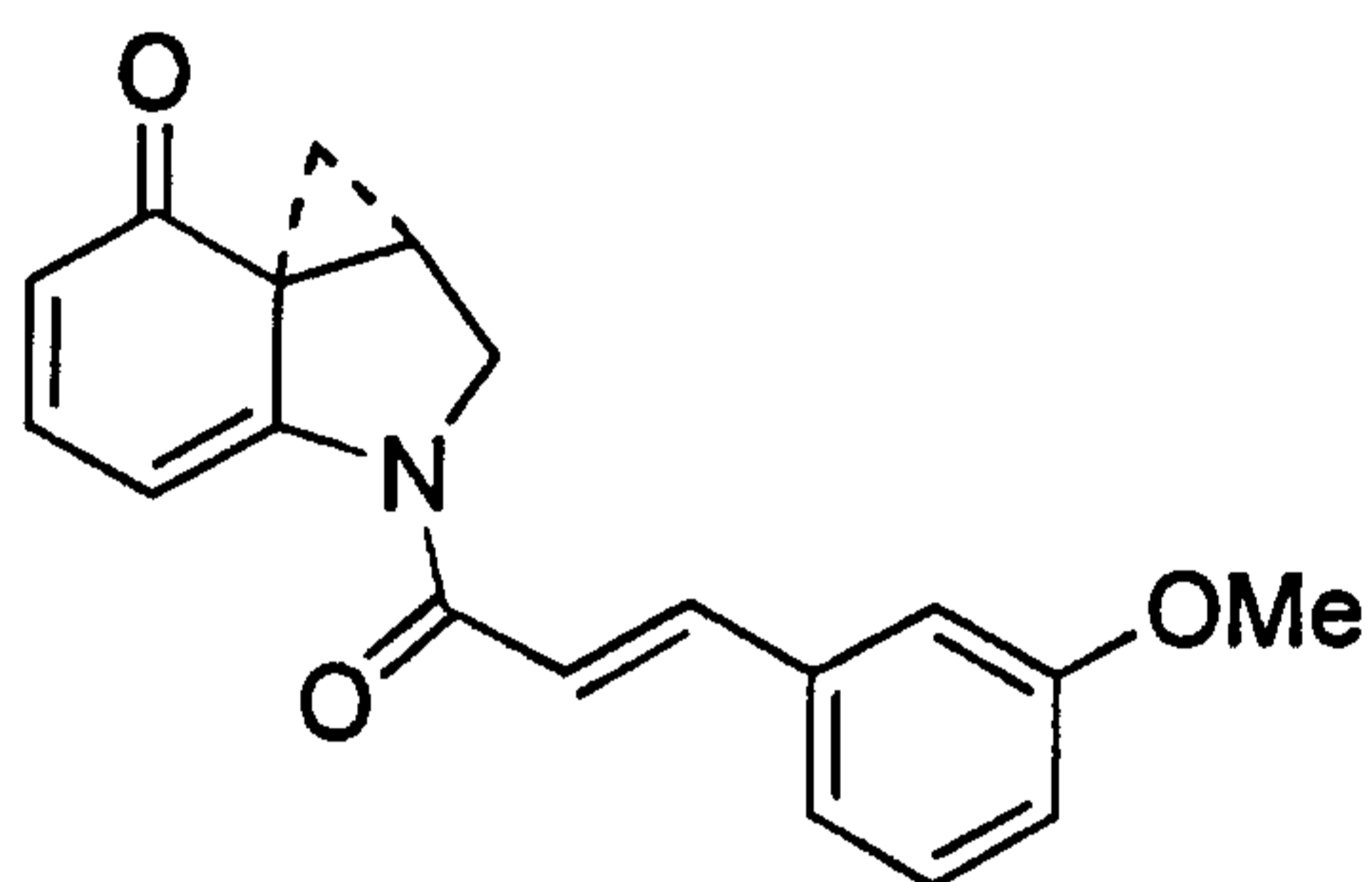
In the above structure R_1 is selected from the group consisting of -C1-C6 alkyl, -NHCH₃ (-N-alkyl), -OCH₃ (O-alkyl), -NH₂, -NHNH₂, -NHNHCO₂tBu, and a radical represented by the following structure:

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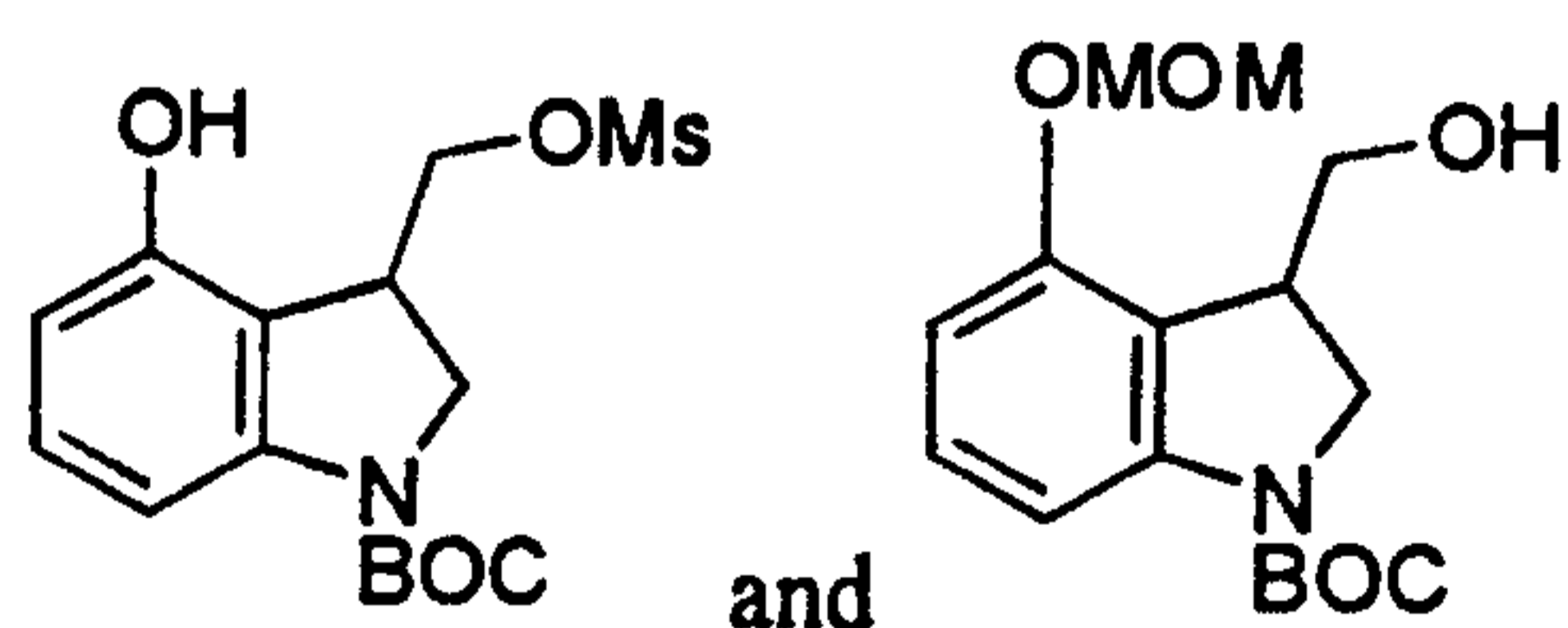
An example of this preferred embodiment is represented by the following structure:

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An other aspect of the invention is directed to chemical intermediates represented by the following structures:



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Description of Drawings:

Figure 1 illustrates (+)-CC-1065 (1) and the duocarmycins 2 and 3, the unmodified natural products.

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Figure 2A illustrates prior art DNA alkylation agents CI, CBI and CPI and novel analog DNA alkylation agents *iso*-CI, *iso*-CBI and *iso*-CPI.

Figure 2B illustrates the interactions of CBI-TMI and *iso*-CBI-TMI with the minor groove of DNA.

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Figure 3A illustrates data from a solvolysis study (UV spectra) of *N*-BOC-*iso*-CBI (31) in 50% CH₃OH-aqueous buffer (pH 3.0, 4:1:20) (v/v/v) 0.1 M citric acid, 0.2 M NaH₂PO₄, and H₂O. The spectra were recorded at 0, 10, 20, 28, 56, and 84 hours.

15

Figure 3B illustrates data from a solvolysis study (UV spectra) of, *N*-CO₂Me-*iso*-CBI (33, middle) in 50% CH₃OH-aqueous buffer (pH 3.0, 4:1:20) (v/v/v) 0.1 M citric acid, 0.2 M NaH₂PO₄, and H₂O. The spectra were recorded at 0, 10, 21, 29, 57, and 86 hours.

20

Figure 3C illustrates data from a solvolysis study (UV spectra) of compound 35 in 50% CH₃OH-aqueous buffer (pH 3.0, 4:1:20) (v/v/v) 0.1 M citric acid, 0.2 M NaH₂PO₄, and H₂O. The spectra were recorded at 0, 1, 3, 5, 10, and 16 hours

25

Figures 4A-4B illustrate stick models of the side view and 90° rotation view of the activated cyclopropane of *N*-CO₂Me-*iso*-CBI and *N*-CO₂Me-CBI, with data taken from the X-ray crystal structures and highlighting the stereoelectronic and geometric alignment of the cyclopropane with the cyclohexadienone π-system.

30

Figure 5 illustrates thermally-induced strand cleavage of w794 DNA (SV40 DNA segment, 144 bp, nucleotide nos. 138–5238); DNA-agent incubation for 24 hours at 25 °C, removal of unbound agent and 30 minutes of thermolysis (100°C), followed by denaturing 8% PAGE and autoradiography; lane 1, control DNA; lanes 2–5, Sanger G, C, A, and T sequencing reactions; lanes 6–7 (–)-*iso*-CBI-TMI (1×10^{-3} and 1×10^{-4} M); lanes 8–9, (+)-CBI-TMI (1×10^{-5} and 1×10^{-6} M); lanes 10–11, (+)-duocarmycin SA (1×10^{-5} and 1×10^{-6}).

Figure 6 illustrates thermally-induced strand cleavage of w836 DNA (146 bp, nucleotide nos. 5189-91): DNA-agent incubation for 24 h ((–)-*iso*-CBI-TMI) at 25 °C, removal of unbound agent and 30 min of thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography; lane 1, control DNA; lanes 2–5, Sanger G, C, A, and T sequencing reactions; lanes 6–8 (–)-*iso*-CBI-TMI (1×10^3 to 1×10^{-5} M); lanes 9–10, (+)-duocarmycin SA (1×10^{-5} to 1×10^{-6} M); lanes 11–12, (+)-*iso*-CBI-TMI (1×10^{-2} and 1×10^{-3}).

Figure 7 illustrates thermally-induced strand cleavage of w794 DNA (SV40 DNA segment, 144 bp, nucleotide nos. 138–5238); DNA-agent incubation for 24 h at 25 °C, removal of unbound agent and 30 min of thermolysis (100 °C), followed by denaturing 8% PAGE and autoradiography; lane 1, control DNA; lanes 2–5, Sanger G, C, A, and T sequencing reactions; lanes 6–8 (+)-duocarmycin SA (1×10^{-4} to 1×10^{-6} M); lanes 9–11, *seco-iso*-CI-TMI (1×10^{-3} to 1×10^{-5} M).

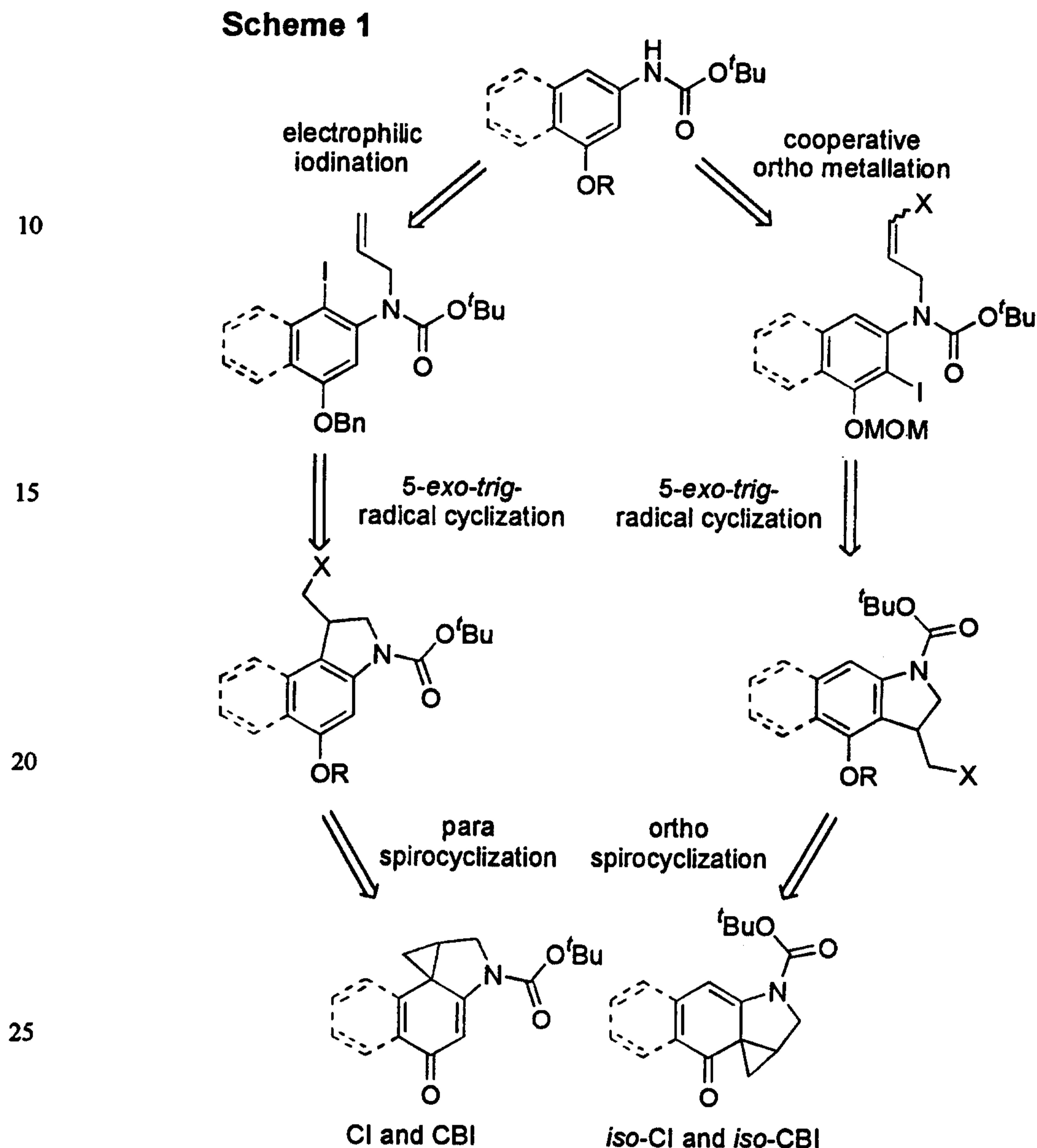
Figures 8A-8E illustrate IC_{50} values for the indicated families of DNA alkylating agents.

Figures 9A- 9C illustrate solvolysis data for the indicated compounds.

Figure 10 provides 1H and ^{13}C NMR data of various adenine adducts.

Detailed Description:

Sundberg and co-workers report the first synthesis of agents isomeric to the natural products. (Sundberg, R. J., et al., *Tetrahedron Lett.* 1983, 24, 4773; and Sundberg, R. J., et al., *J. Org. Chem.*, 1991, 56, 3048.) An agent isomeric with the alkylation subunit of CC-1065 was prepared employing an intramolecular



carbene insertion of an *o*-quinonediazide onto a tethered alkene. Presumably because of the perceived unique character of the authentic alkylation subunit at the

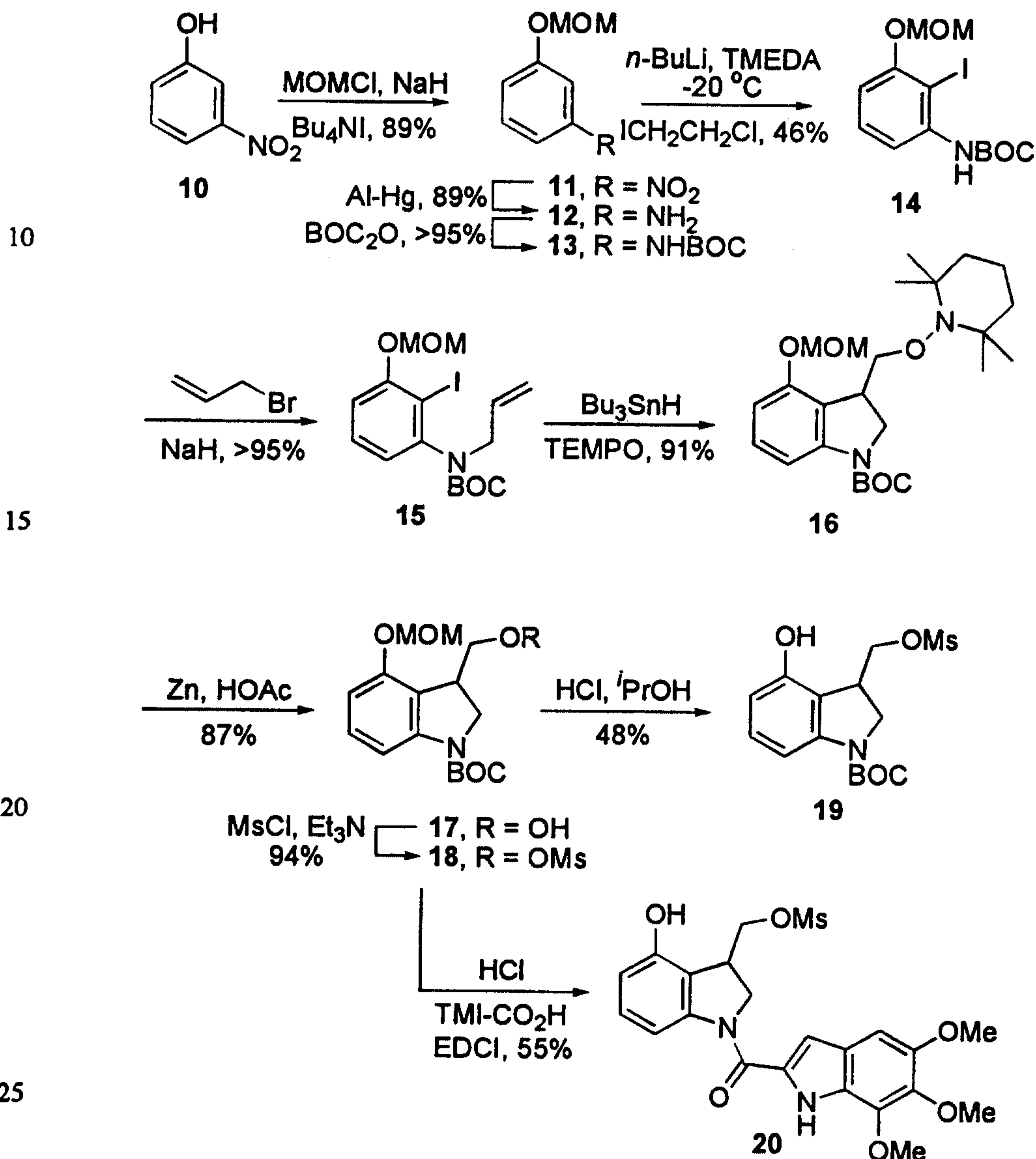
- 15 -

time of the work, its chemical behavior, biological characteristics, and DNA alkylation properties were not examined.

As illustrated above in Scheme 1, an alternative route was subsequently
5 devised for the synthesis of the isomeric CI analogs (Boger, D. L., et al., *J. Am. Chem. Soc.* 1990, 112, 5230.) and for CBI analogs. (Boger, D. L., et al., *J. Org. Chem.* 1995, 60, 1271; Boger, D. L. et al., *J. Org. Chem.* 1992, 57, 2873; Boger, D. L., et al., *J. Am. Chem. Soc.* 1989, 111, 6461; Boger, D. L., et al., *J. Org. Chem.* 1990, 55, 5823; Boger, D. L., et al., *Tetrahedron Lett.* 1990, 31, 793;
10 Boger, D. L., et al., *Bioorg. Med. Chem. Lett.* 1991, 1, 55; Boger, D. L., et al., *Bioorg. Med. Chem. Lett.* 1991, 1, 115; Boger, D. L., et al., *J. Am. Chem. Soc.* 1992, 114, 5487; and Boger, D. L., et al., *Bioorg. Med. Chem.* 1995, 3, 611.)
The strategy is complementary to our synthesis of CBI in which the dihydroindole skeleton was constructed by a 5-*exo-trig* radical cyclization of an aryl radical onto a
15 tethered alkene and its extension to the isomeric analogs required C2 versus C4 regiocontrol in the key aromatic halogenation step. In the synthesis of CI and CBI, a regioselective electrophilic halogenation served to install iodine or bromine para to the phenol ether. For the isomeric agents, an ortho halogenation protocol was required and a cooperative directed ortho metallation²³ was implemented to install
20 the C2 halide. (Snieckus, V. *Chem. Rev.* 1990, 90, 879.) Its adoption required two easily removed cooperative directing metallation groups: OMOM (Winkle, M. R., et al., *J. Org. Chem.* 1982, 47, 2101) and NHBOC. (Muchkowski, J. M., et al., *J. Org. Chem.* 1980, 45, 4798.) In addition, the Winstein para Ar-3'
spirocyclization (Baird, R., et al., *J. Am. Chem. Soc.* 1963, 85, 567; 1962, 84, 788;
25 1957, 79, 756.) utilized to close the cyclopropane in the CI and CBI synthesis, is now replaced by an ortho spirocyclization requiring C-alkylation with cyclopropane formation (Brown, R. F. C., et al., *Tetrahedron Lett.* 1981, 22, 2915; Smith III, A. B., et al., *Tetrahedron Lett.* 1987, 28, 3659; and Kigoshi, H., et al., *Tetrahedron Lett.* 1997, 38, 3235) rather than competitive O-alkylation and dihydrofuran
30 formation.

This approach was first examined with *iso*-CI employing the commercially available 3-nitrophenol (10), Scheme 2. Protection of the phenol as the MOM ether 11 (NaH, MOMCl, Bu₄NI, 89%), reduction of the nitro group with

5 **Scheme 2**



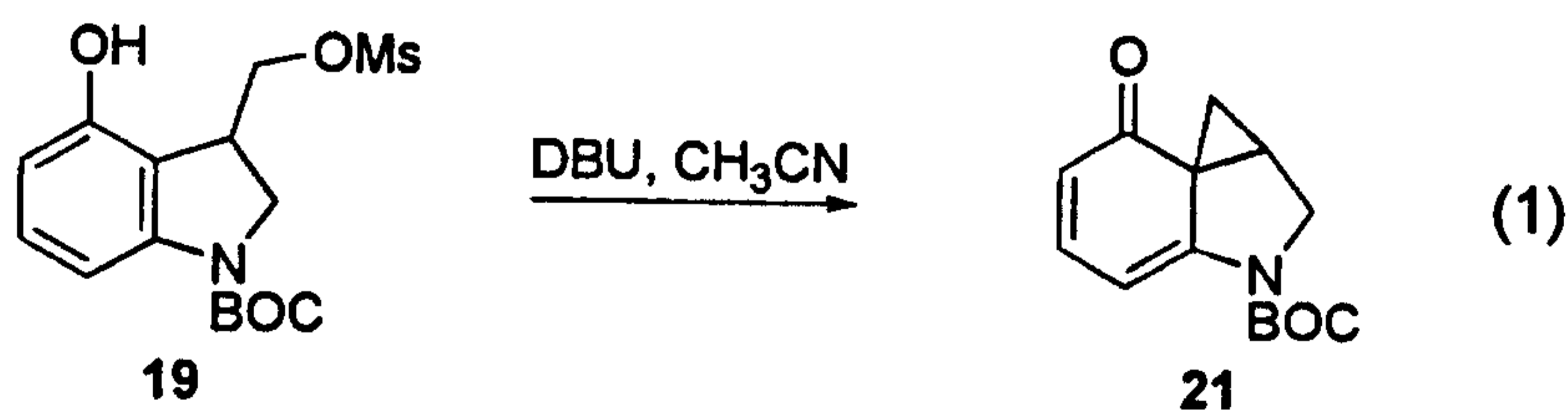
Al-Hg amalgam (Et₂O-H₂O, 89%) (Meyers, A. I., et al., *J. Org. Chem.* 1975, 40, 2021.), and BOC protection of the free amine 12 (BOC₂O, >95%) provided 13, a key intermediate with which to examine the directed ortho metallation. Treatment of 13 with 3.5 equiv of *n*-BuLi and TMEDA at -20 °C (2 h) in THF, and reaction

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of the aryl lithium intermediate with 1-chloro-2-iodoethane provided **14** (46%) along with recovered starting material (41%). Although not extensively examined, this conversion was not improved through use of longer reaction times, different reaction temperatures or solvents, or additional amounts of *n*-BuLi. However, the conversion did allow the synthesis to proceed and, as detailed, this reaction proved much more effective in the *iso*-CBI series where it was more carefully optimized. *N*-Alkylation with allyl bromide (NaH, >95%) was followed by Bu₃SnH promoted *5-exo-trig* free radical cyclization of **15** with *in situ* TEMPO trap of the resulting primary radical to provide **16** (91%). Subsequent reduction of the N-O bond (Zn, HOAc, 87%) without the competitive deprotection of either the MOM ether or the *N*-BOC protecting groups afforded the free alcohol **17** in excellent overall yield. Activation of the primary alcohol (MsCl, Et₃N, 94%) afforded the key intermediate **18**, further detailed below.

In order to prepare *N*-BOC-*iso*-CI for direct comparison with prior agents, selective removal of the MOM group in the presence of the BOC group was required. Mild acid-catalyzed deprotection (HCl, *i*-PrOH/THF, 48%) provided *seco*-*N*-BOC-*iso*-CI (**19**) accompanied by 41% recovery of starting material. Although not optimized, this selective deprotection found greater success in the synthesis of *N*-BOC-*iso*-CBI where it was more closely examined. Exhaustive deprotection of the MOM ether and the BOC group (3.6 M HCl/EtOAc) followed by EDCI-promoted coupling with 5,6,7-trimethoxyindole-2-carboxylic acid (TMI-COOH)^{10,31} provided **20** (55% overall). (EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; Boger, D. L., et al., *J. Org. Chem.* 1990, 55, 4499.) Consistent with expectations, spirocyclization of **19** to *N*-BOC-*iso*-CI (**21**)

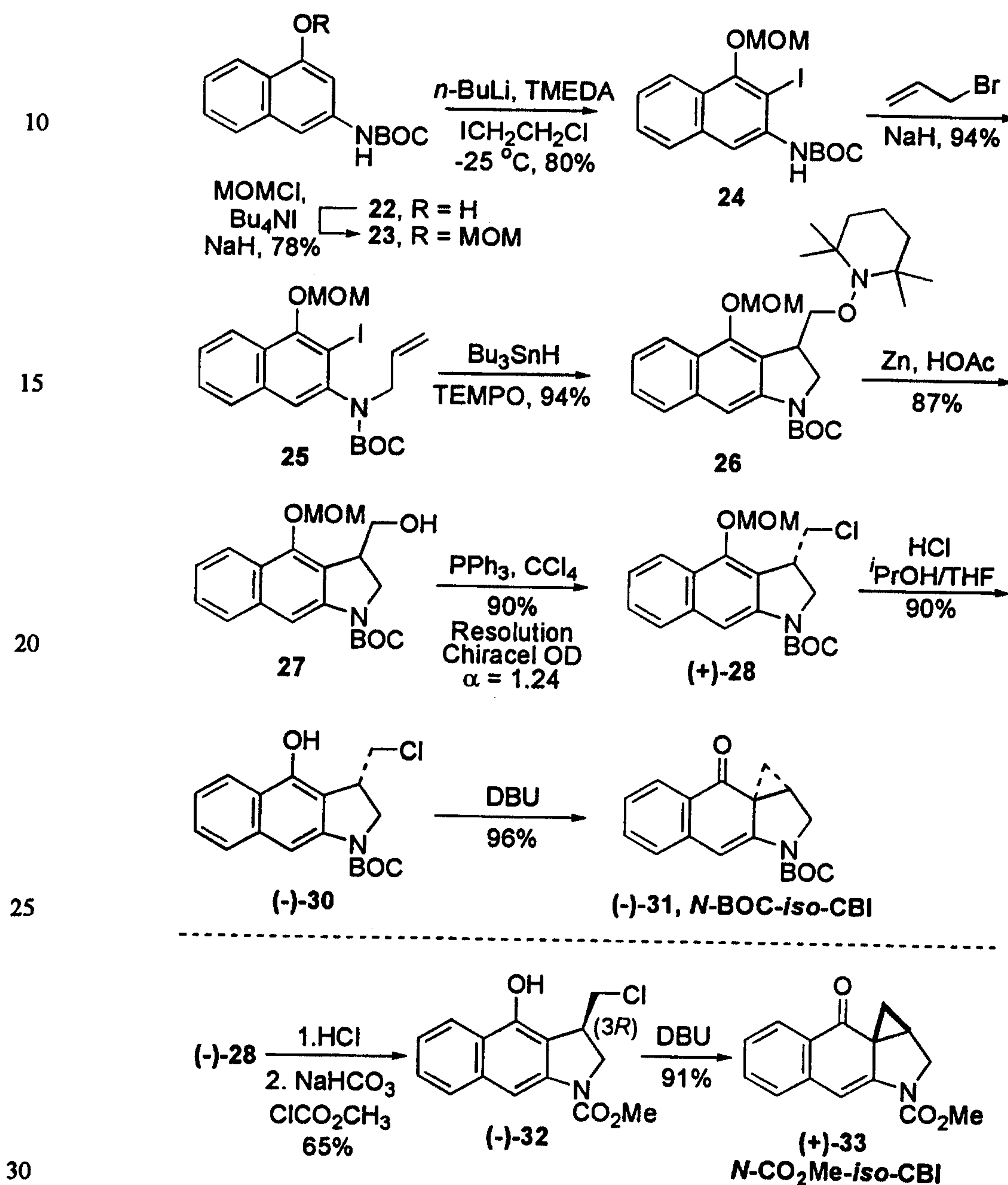


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could be effected by treatment with DBU, but its exceptional reactivity precluded attempts to isolate and characterize the agent (eq 1). (Diagnostic ^1H NMR (CD_3CN , 400 MHz) signals for **21** generated *in situ*: δ 2.79 (dt, $J = 5.4, 7.8$ Hz, 1H), 1.75 (dd, $J = 3.1, 7.8$ Hz, 1H).) Sufficient for our considerations, the studies revealed

5 that *N*-BOC-*iso*-CI is more reactive than its counterpart *N*-BOC-CI which,

Scheme 3



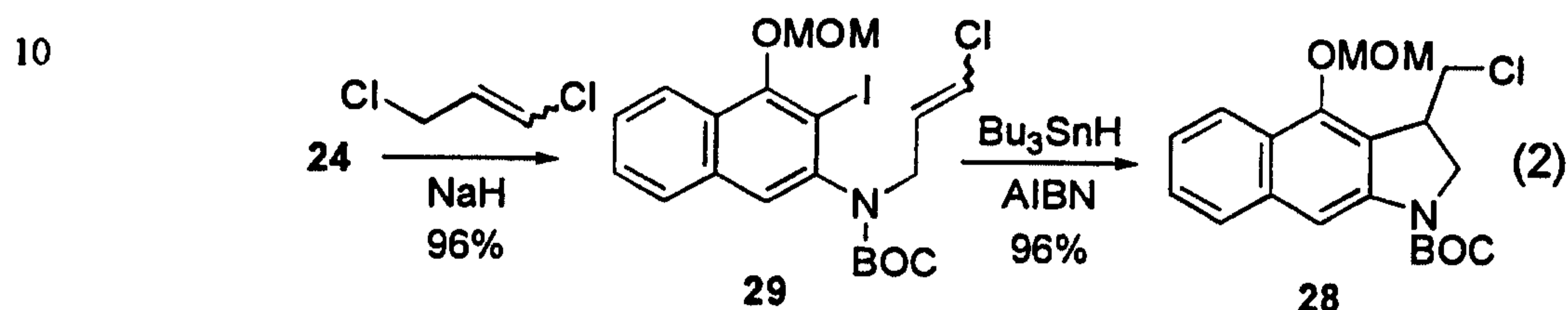
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although exceptionally reactive, can be isolated by quick column chromatography. Since the seco agents can be expected to behave analogous to their ring-closed cyclopropane counterparts in biological assays and DNA alkylation studies, and given that the careful comparisons could be made with the more stable *iso*-CBI based agents, their isolation and characterization were not further pursued.

With the additional stability provided by the fused benzene ring, the CBI analogs are substantially more stable and biologically more potent than the CI series. The extension of this approach to the preparation of of the *iso*-CBI alkylation subunit is detailed in Scheme 3 and provided the opportunity to accurately document the effects of this deep-seated structural change. Starting with **22**, available in two steps (70%) from commercially available 1,3-dihydroxynaphthalene, protection of the phenol (MOMCl, NaH, Bu₄NI, 78%) as the MOM ether provided the directed ortho metallation substrate **23**. Treatment of **23** with 3.5 equiv of *n*-BuLi and TMEDA at -25 °C in THF for 2 h and reaction of the aryl lithium intermediate with 1-chloro-2-iodoethane gave the C2 iodide **24** in 80% yield, a substantial improvement over the *iso*-CI directed metallation. *N*-Alkylation (NaH, allyl bromide, 94%), 5-*exo-trig* free radical cyclization of **25** with *in situ* TEMPO trap (Bu₃SnH, TEMPO, 94%), and N-O bond reduction of **26** (Zn, HOAc, 87%) provided the primary alcohol **27** in excellent overall yield. Conversion to the chloride **28** upon activation of the primary alcohol under Mitsunobu conditions (Ph₃P, CCl₄, 90%) provided a resolvable intermediate that served as a penultimate precursor to all analogs. A 5-*exo-trig* radical cyclization onto a tethered vinyl chloride was shown to provide the 5-membered ring with a suitable leaving group already in place. This concise strategy was adopted for the synthesis of *iso*-CBI (eq 2). Thus, *N*-alkylation of **25** with 1,3-dichloropropene proceeded in 96% yield providing the key radical cyclization precursor (**29**). Treatment with catalytic AIBN (0.1 equiv) and Bu₃SnH (1.1 equiv) at 80 °C (C₆H₆) yielded the tricyclic core of *iso*-CBI (**28**) in 96% yield. This improved approach shortens the original synthesis by two steps, avoiding reductive removal of the

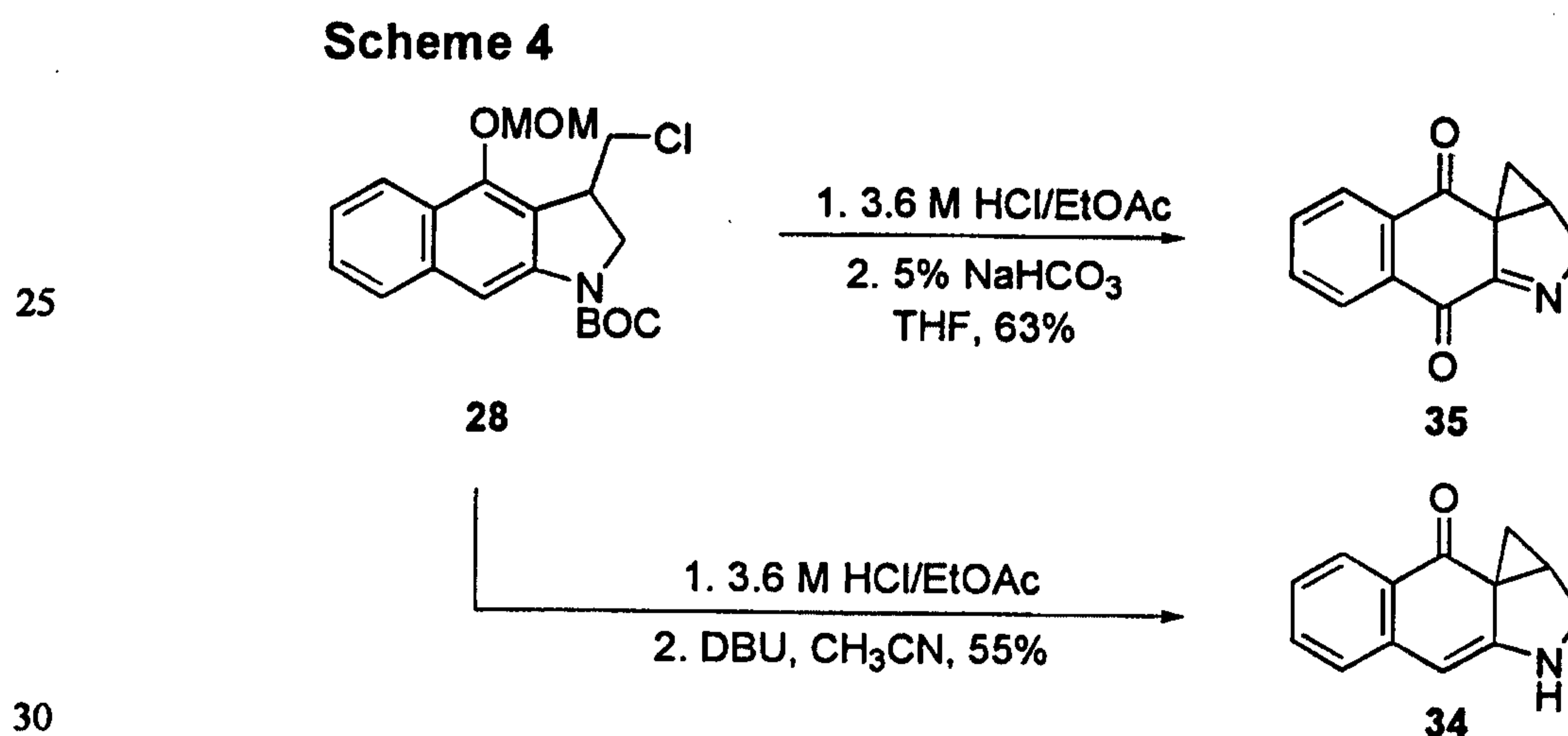
- 20 -

TEMPO group and conversion to 28. With this improvement, the preparation of 28 requires 4 steps and proceeds in 58% yield overall. Removal of the MOM ether (HCl, *i*-PrOH/THF, 90%) without competitive *N*-BOC deprotection afforded *sec*-*N*-BOC-*iso*-CBI (30) in superb yield. Ortho spirocyclization (DBU, CH₃CN, 96%) provided *N*-BOC-*iso*-CBI (31) which could be purified by standard chromatography. Similarly, exhaustive deprotection of 28 (3.6 N HCl/EtOAc) followed by *N*-acylation with methyl chloroformate provided 32 (65%) and spirocyclization (DBU, CH₃CN, 25 °C, 91%) afforded 33.



15 Initial attempts to synthesize *iso*-CBI (34) by exhaustive deprotection (3.6 M HCl/EtOAc) and subsequent ring closure (5% aqueous NaHCO₃/THF) conducted in the presence of air resulted in the isolation of the quinone 35 (Scheme 4). Presumably, adventitious oxidation of the intermediate *iso*-CBI subsequent to spirocyclization provided 35 and an interesting further modification of the *iso*-CBI

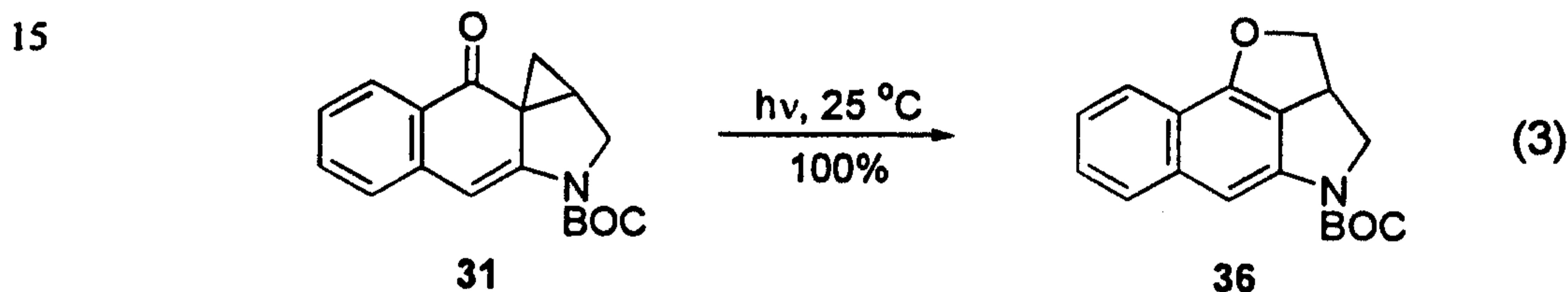
20 alkylation subunit. (Such agents may be subject to reductive activation.) Consistent



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with this, spirocyclization conducted in an aprotic solvent under an inert atmosphere (2.2 equiv DBU, CH₃CN, 25 °C, 20 min, Ar) provided the unstable *iso*-CBI (**34**, 55%) and subsequent exposure of **34** to air resulted in conversion to **35**.

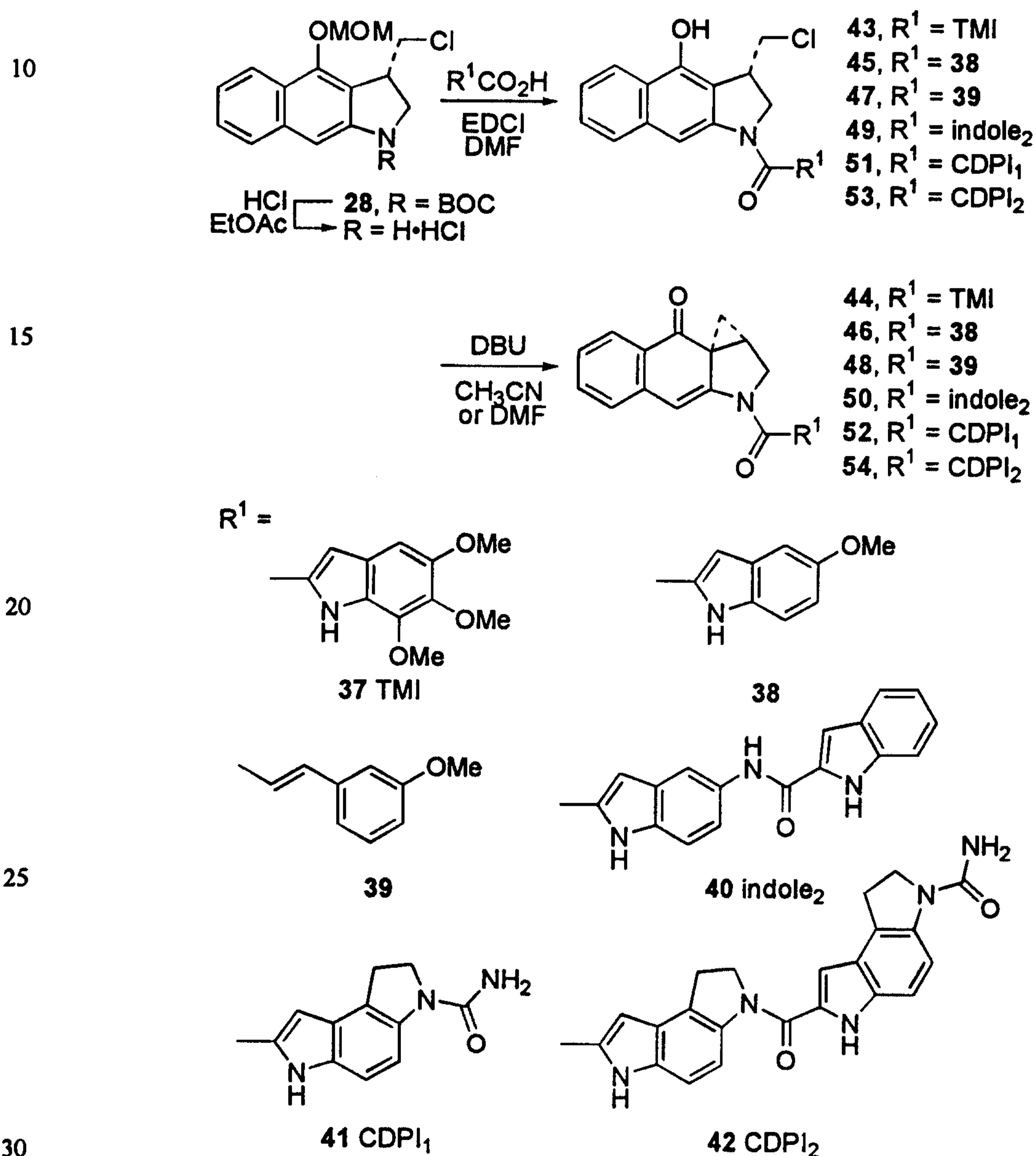
5 Exclusive spirocyclization with cyclopropane formation was observed, and no competitive *O*-alkylation leading to **36** was detected. However, prolonged exposure of neat **31** to ambient light at 25 °C (48 h) did lead to carbonyl-cyclopropane rearrangement to form **36** (eq 3). (Wong, H. N. C., et al., *Chem. Rev.* **1989**, *89*, 165.) An identical neat sample of **31** protected from light by foil
10 remained unchanged after 48 h. Thus, the handling and storage of the *iso*-CBI based agents were conducted minimizing their exposure to light typically at subzero temperatures. Examination of the compounds over time showed no appreciable decomposition or rearrangement under these storage conditions.



20 **Resolution:** In order to assess the properties of both enantiomers of the *iso*-CBI based agents, a direct chromatographic resolution of **28** on a semipreparative ChiralCel OD column (2 × 25 cm, 3% *i*-PrOH/hexane, $\alpha = 1.24$) was utilized. (Boger, D. L., et al., *J. Am. Chem. Soc.* **1994**, *116*, 7996.) This procedure
25 provided both enantiomers (>99% ee) of an advanced intermediate and avoided diastereomeric derivatization, separation, and dederivatization. The assignment of absolute configuration was based on the conversion of the slower eluting enantiomer of **28** ($t_R = 35.8$ min) to **32** and subsequent single-crystal X-ray structure determination which revealed the unnatural (*3R*)-configuration (Scheme 3). (The atomic coordinates for this structure have been deposited with the
30 Cambridge Crystallographic Data Centre and may be obtained upon request from

the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.) Consistent with this assignment, the agents derived from the (3*S*)-enantiomer analogous to the absolute stereochemistry found in the natural products 1–3 exhibited the more potent biological activity, the more effective DNA alkylation properties, and exhibited a DNA alkylation selectivity identical to the natural products.

Scheme 5



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Synthesis of Duocarmycin and CC-1065 Analogs: The *iso*-CBI alkylation subunit was incorporated into CC-1065 and duocarmycin analogs as detailed in Scheme 5. Exhaustive deprotection of **28** (3.6 M HCl/EtOAc, 30 min) followed by immediate coupling (3 equiv of EDCI, DMF, 25 °C) of the amine hydrochloride salt with 5,6,7-trimethoxyindole-2-carboxylic acid (**37**, 3 h, 91%), **38** (3 h, 95%), **39** (3 h, 87%), indole₂ (**40**, 3 h, 74%), CDPI₁ (**41**, 9 h, 80%), and CDPI₂ (**42**, 12 h, 32%) provided **43**, **45**, **47**, **49**, **51** and **53**, respectively. (Boger, D. L., et al., *Bioorg. Med. Chem.* 1995, 3, 1429; Boger, D. L., et al., *J. Org. Chem.* 1987, 52, 1521; and Boger, D. L., et al., *J. Org. Chem.* 1984, 49, 2240.) The poor solubility of CDPI₂ precluded efficient coupling and isolation, resulting in a lower yield. DBU (1.5 equiv, 25 °C) spirocyclization of **43** (CH₃CN, 30 min, 94%), **45** (CH₃CN, 30 min, 85%), **47** (CH₃CN, 30 min, 83%), **49** (DMF, 30 min, 88%), **51** (DMF, 60 min, 81%), and **53** (DMF, 60 min, 59%) afforded **44**, **46**, **48**, **50**, **52** and **54**, respectively, in excellent conversions.

15

Solvolysis Reactivity and Regioselectivity: Two fundamental characteristics of the alkylation subunits have proven important in past studies. (Boger, D. L., et al., *Angew. Chem., Int. Ed. Engl.* 1996, 35, 1439.) The first is the stereoelectronically-controlled acid-catalyzed ring opening of the activated cyclopropane which dictates preferential addition of a nucleophile to the least substituted cyclopropane carbon. The second is the relative rate of acid-catalyzed solvolysis which has been found to accurately reflect the functional reactivity of the agents and to follow a direct relationship between solvolysis stability and in vitro cytotoxicity. While *N*-BOC-*iso*-CBI retains many of the structural characteristics of *N*-BOC-CBI, the isomeric modifications which may disrupt the vinylogous amide conjugation were anticipated to reduce the solvolytic stability of the agents. However, it was not clear what the magnitude of this effect might be nor whether solvolysis would still occur with the same high regioselectivity.

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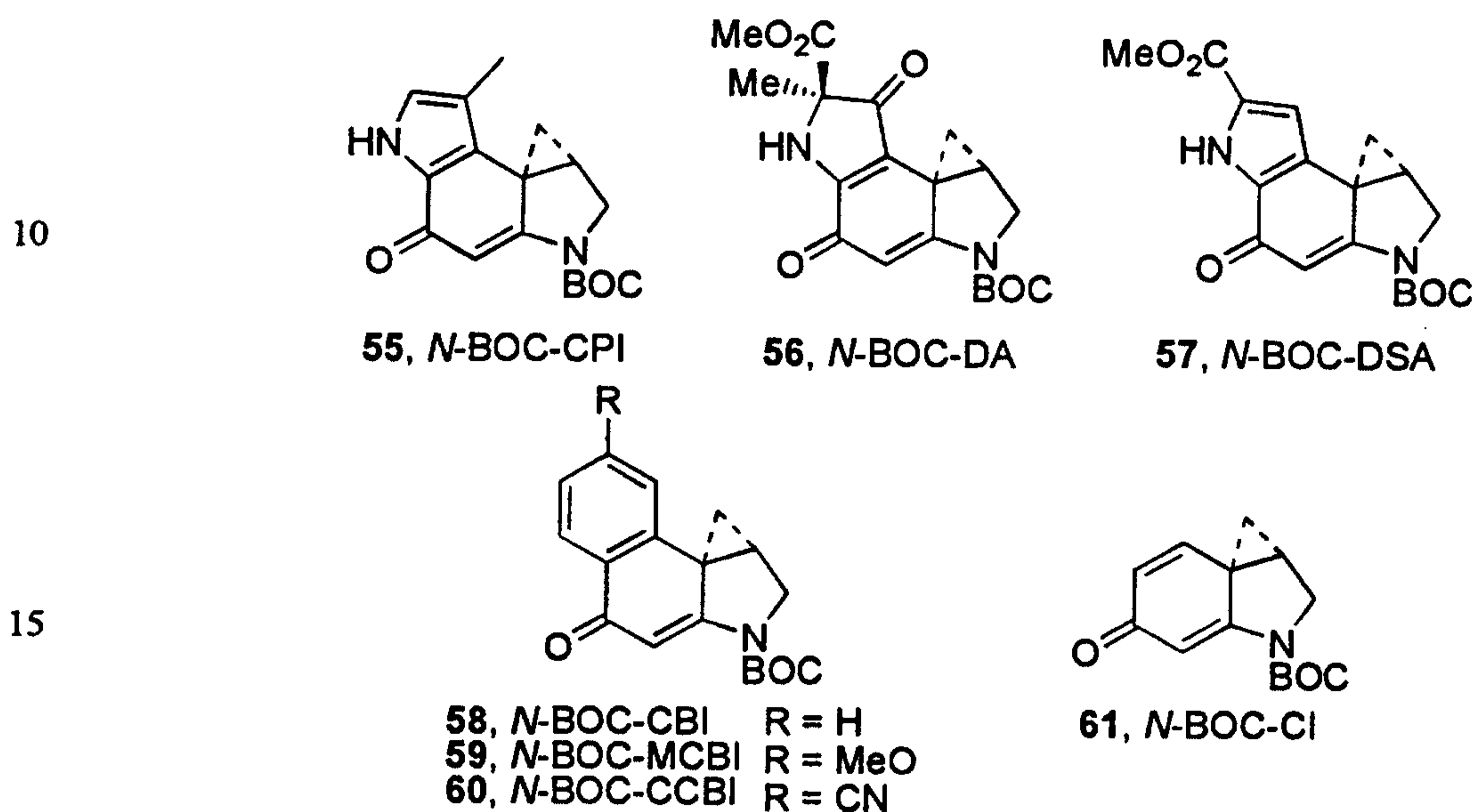
N-BOC-*iso*-CBI (**31**, $t_{1/2} = 27.6$ h, $k = 6.97 \times 10^{-6}$ s⁻¹) and *N*-CO₂Me-

- 24 -

iso-CBI (33, $t_{1/2} = 30.1$ h, $k = 6.40 \times 10^{-6} \text{ s}^{-1}$) proved to be reasonably stable toward chemical solvolysis at pH 3 exhibiting a reactivity comparable to the CC-1065 alkylation subunit (*N*-BOC-CPI, 55, $t_{1/2} = 36.7$ h), but more stable than the duocarmycin A alkylation subunit (*N*-BOC-DA, 56, $t_{1/2} = 11$ h), Table 1. However,

5

Table 1. Solvolysis Reactivity and Regioselectivity



20

Agent	k (s^{-1} , pH 3)	$t_{1/2}$ (h, pH 3)	Regioselectivity
31	6.98×10^{-6}	28 h	40 : 1
35	3.80×10^{-5}	5 h	nd
55	5.26×10^{-6}	37 h	4 : 1
56	1.75×10^{-5}	11 h	3 : 2
57	1.08×10^{-6}	177 h	6-4 : 1
58	1.45×10^{-6}	133 h	> 20 : 1
59	1.75×10^{-6}	110 h	> 20 : 1
60	0.99×10^{-6}	194 h	> 20 : 1
61	1.98×10^{-2}	0.01 h	nd

25

N-BOC-*iso*-CBI was significantly less stable than *N*-BOC-DSA (57, $t_{1/2} = 177$ h) and *N*-BOC-CBI (58, $t_{1/2} = 133$ h). Thus, *N*-BOC-*iso*-CBI (31) proved to be 5× more reactive than its direct comparison analog *N*-BOC-CBI. The solvolysis was

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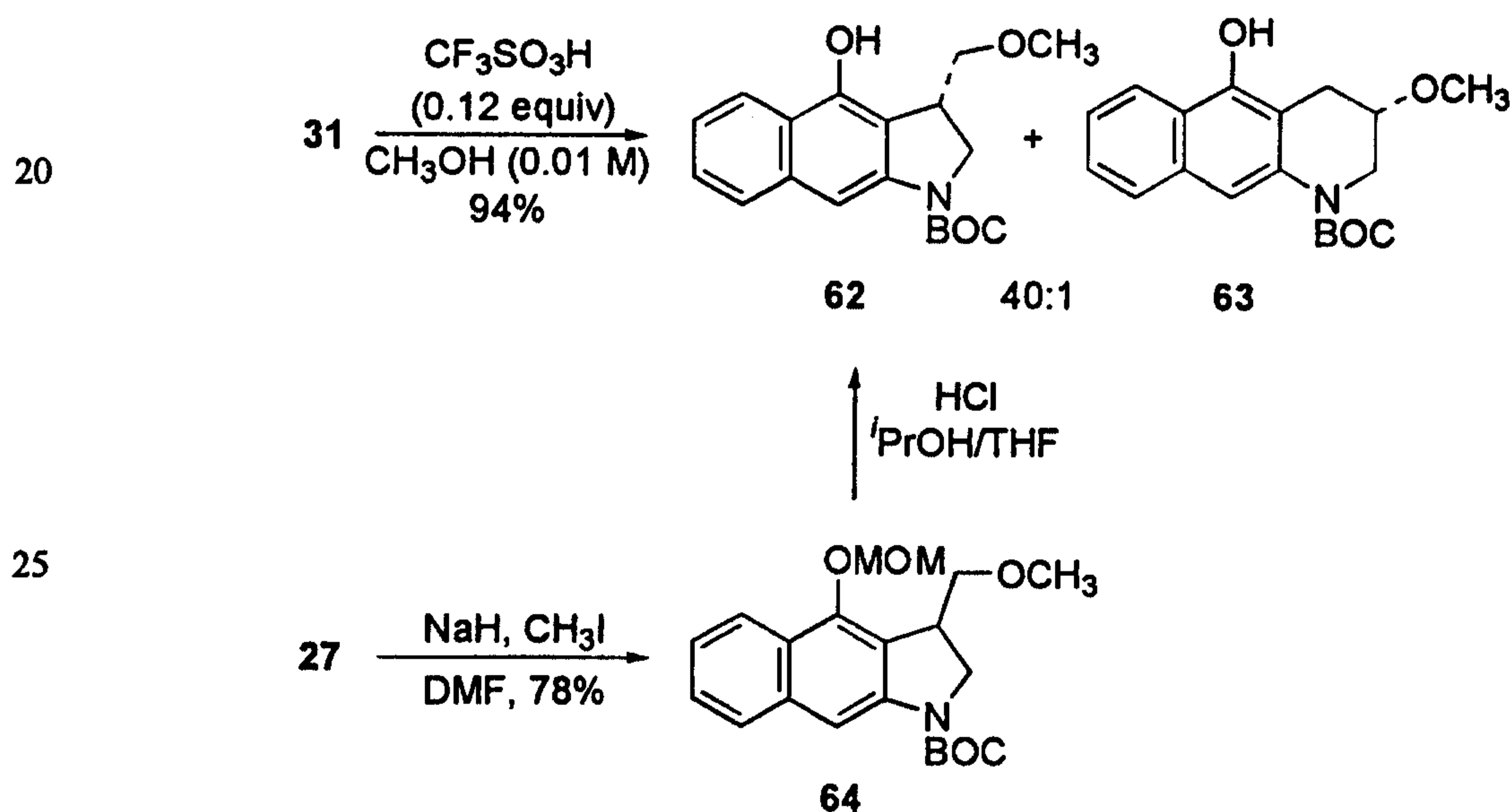
followed spectrophotometrically by UV with the disappearance of the long-wavelength absorption band of the *iso*-CBI chromophore (397 nm), Figure 3. The reactivity of the quinone **35** was also examined at pH 3 ($t_{1/2} = 5.1$ h, $k = 3.80 \times 10^{-5}$ s⁻¹) and it proved to be 5–6× more reactive than **31** or **33**.

5

The acid-catalyzed nucleophilic addition of CH₃OH to **31** was conducted on a preparative scale to establish the regioselectivity of addition, and confirmed by synthesis of the expected product **62** derived from nucleophilic addition to the least substituted cyclopropane carbon. Treatment of *N*-BOC-*iso*-CBI with 0.1 equiv of CF₃SO₃H in CH₃OH (25 °C, 17 h) resulted in the clean solvolysis (94%) to provide a 40:1 mixture of **62** and **63** (Scheme 6). Consequently, the acid-catalyzed CH₃OH addition to **31** occurs with near exclusive regioselectivity (40:1) analogous to *N*-BOC-CBI (**58**, >20:1)²² which is much more selective than the natural alkylation subunits themselves (6–1.5:1). (Boger, D. L., et al., *Bioorg. Med. Chem. Lett.* 1996, 16, 1955; Boger, D. L., et al., *J. Am. Chem. Soc.* 1997, 119, 311.)

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Scheme 6



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X-Ray Structure of *N*-CO₂Me-*iso*-CBI (33): Structural Correlation with Solvolysis Regioselectivity and Reactivity. The single-crystal X-ray structure determination of *N*-CO₂Me-*iso*-CBI (33) was conducted and by direct comparison with that of *N*-CO₂Me-CBI⁴² established a structural basis for the observed properties (Figure 4). (Boger, D. L.; Turnbull, P. *J. Org. Chem.* 1997, 62, 0000.)

5 The first striking similarity between the two systems is the perpendicular orientation of the bent orbital of the cyclopropane bond extending to the least substituted C9 cyclopropane carbon. This idealized stereoelectronic alignment with the developing π -system of the solvolysis product phenol imposes a preference for nucleophilic

10 addition to the less substituted C9 cyclopropane carbon. In contrast, the cyclopropane bond extending to the tertiary C9a carbon is nearly orthogonal to the π -system of the cyclohexadienone, and S_N2 addition to this carbon is disfavored stereoelectronically as well as sterically. The relative cyclopropane bond lengths of *iso*-CBI reflect this orientation and π conjugation in which the breaking C9-C8a

15 cyclopropane bond (1.531 Å) is longer, and thus weaker, than the C9a-C8a bond (1.513 Å) extending to the more substituted carbon. Although the ring expansion solvolysis would place a developing positive charge on a preferred secondary versus primary carbon, this inherent preference is overridden by the stereoelectronic control of the reaction regioselectivity as well as the characteristics of a S_N2

20 reaction which prefer attack at the less substituted center.

In addition, the X-ray structures have provided insights into the origin of the difference in stability between CBI and *iso*-CBI. The stability of CC-1065, the duocarmycins and their analogs is a result of at least three structural features: the

25 conjugative stability provided by the fused aromatic system, the non-ideal alignment of the cyclopropane, and the strong cross-conjugative stability provided by the vinylogous amide. The first of these features, the fused aromatic ring, is present in both *iso*-CBI and CBI. Like the CI/CBI comparison, the *iso*-CI/*iso*-CBI reactivity comparison reveals that the diminished aromatization driving force for *iso*-CBI

30 relative to *iso*-CI contributes significantly to the stability. In addition, The

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cyclopropane alignments with the π -systems in *N*-CO₂Me-*iso*-CBI and *N*-CO₂Me-CBI are similar. Both are bisected by the plane of the cyclohexadienone nearly equally (41°/15° for *iso*-CBI and 41°/16° for CBI). In both, the cyclopropane is not only pulled down but also to the side by the constraints of the fused 5-membered ring (9° for *iso*-CBI and 12° for CBI). Thus, both benefit in stability from the non-ideal alignment and conjugation of the cyclopropane which is imposed by the fused 5-membered ring. The important distinction between the two systems is the direct cross-conjugated stability afforded the activated cyclopropane by the vinylogous amide. Diagnostic of this vinylogous amide conjugation is the shortened length of the N²-C^{2a} bond reflecting this resonance stabilization. As this cross-conjugated vinylogous amide stabilization decreases, the conjugation and inherent reactivity of the cyclopropane correspondingly increases. Consistent with this, *N*-CO₂Me-*iso*-CBI exhibits a N²-C^{2a} bond length (1.400 Å versus 1.426 Å for 32) indicative of a diminished but not eliminated vinylogous amide conjugation relative to *N*-CO₂Me-CBI (1.390 Å versus 1.416 Å for *seco N*-CO₂Me-CBI)⁴² and that follows trends established in recent studies (Table 2).

Table 2

agent	N ² -C ^{2a} bond length (Å)	<i>t</i> _{1/2} (h, pH 3)
<i>N</i> -CO ₂ Me-CBI ⁴²	1.390	133
<i>N</i> -CO ₂ Me- <i>iso</i> -CBI	1.400	28
<i>N</i> -BOC-CBQ ⁴³	1.415	2.1
<i>N</i> -CO ₂ Me-CNA ⁴²	1.428	0.03

25

DNA Alkylation Selectivity and Efficiency: The DNA alkylation properties of the agents were examined within w794 and w836 duplex DNA for which comparative results are available for related agents. (Boger, D. L., et al., *J. Am. Chem. Soc.* 1994, 116, 11335; Boger, D. L., et al., *J. Am. Chem. Soc.* 1994, 116, 6461; and Boger, D. L., et al., *J. Am. Chem. Soc.* 1995, 117, 11647) The

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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 agents. Following treatment of the end-labeled duplex DNA with a range of agent concentrations and temperatures in the dark, the unbound agent was removed by EtOH precipitation of the DNA. Redissolution of the DNA in aqueous buffer, thermolysis (100 °C, 30 min) to induce strand cleavage at the sites of DNA alkylation, denaturing high-resolution polyacrylamide gel electrophoresis (PAGE) adjacent to Sanger dideoxynucleotide sequencing standards, and autoradiography led to identification of the DNA cleavage and alkylation sites. The full details of this procedure have been disclosed elsewhere. (Boger, D. L., et al., *Tetrahedron* 1991, 47, 2661.)

A representative comparison of the DNA alkylation by (-)-*iso*-CBI-TMI (44) alongside that of (+)-duocarmycin SA and (+)-CBI-TMI is illustrated in Figure 5. There are three important conclusions that can be drawn from these comparisons. First, (-)-*iso*-CBI-TMI alkylates DNA in a manner identical to (+)-duocarmycin SA and (+)-CBI-TMI exhibiting the same sequence selectivity. No new sites of alkylation were detected, and only adenine N3 alkylation was detected under the conditions of limiting agent and excess DNA. Notably, such sequencing studies only detect the higher affinity alkylation sites and minor sites of comparable affinities (1-0.01×). Under these conditions, the studies illustrate that *iso*-CBI-TMI, like duocarmycin SA and CBI-TMI, exhibits an exclusive preference for adenine versus guanine N3 alkylation. However, given the reactivity of *iso*-CBI-TMI, it is likely that a minor guanine alkylation could be expected at incubations carried out at higher agent-base pair ratios analogous to that observed with the more reactive agents including CC-1065 (Park, H.-J., et al., *J. Am. Chem. Soc.* 1997, 119, 629.) and duocarmycin A.⁴⁶ (Sugiyama, H., et al., *Tetrahedron Lett.* 1993, 34, 2179; Yamamoto, K., et al., *Biochemistry* 1993, 32, 1059; and Asai, A., et al., *J. Am. Chem. Soc.* 1994, 116, 4171.) Importantly, the identical behavior of (-)-*iso*-CBI-TMI and (+)-CBI-TMI illustrate that the position of the C-4 carbonyl

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does not influence the sequence selectivity of the DNA alkylation. This is inconsistent with the proposal of a sequence-dependent phosphate backbone protonation of the C-4 carbonyl for activation of the agent for DNA alkylation that controls the sequence selectivity. It is, however, fully consistent with the model in which it is controlled by the AT-rich noncovalent binding selectivity of the agents and their steric accessibility to the adenine N3 alkylation sites.

Secondly, although there are no distinctions in the sequence selectivity, there is a significant difference in the relative efficiencies of DNA alkylation. Consistent with its relative reactivity and cytotoxic potency, (-)-*iso*-CBI-TMI alkylated DNA 50–100× less efficiently than (+)-CBI-TMI and (+)-duocarmycin SA. Thus, (-)-*iso*-CBI-TMI was found to alkylate DNA with an efficiency comparable to duocarmycin A which has been shown to be *ca.* 10× less efficient than duocarmycin SA.^{10,11} In addition and analogous to the observations made with the agents containing the more reactive alkylation subunits including duocarmycin A, but unlike the more stable agents, the alkylation efficiency of (-)-*iso*-CBI-TMI was found to increase as the incubation temperature was decreased from 25 °C to 4 °C. This suggests that the differences may be attributed in part to the nonproductive solvolysis of *iso*-CBI-TMI which competes with alkylation and lowers the overall efficiency of DNA alkylation.

Thirdly, although the rate of DNA alkylation by 44 was not accurately quantitated, it is qualitatively similar to those of CBI-TMI and duocarmycin SA and much faster than agents we have examined which exhibited substantially diminished rates (*e.g.* reversed analogs of duocarmycin SA). Thus, the relocation of the C-4 carbonyl did not impact the rate of DNA alkylation in a manner that would be consistent with a phosphate backbone protonation (or cation Lewis acid complexation) required of catalysis in the alkylation site model.

Similar results were obtained within w836 DNA (Figure 6). The natural

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enantiomer (-)-*iso*-CBI-TMI alkylated the same sites as (+)-duocarmycin SA but did so with a 50–100 fold lower efficiency. Within the segment illustrated, the two natural enantiomers alkylated the first three 3' adenines in the sequence 5'-AAAAAA and less effectively the fourth 3' adenine corresponding to alkylation and 3'→5' binding across a 3–4 base-pair AT-rich site (*i.e.*, 5'-AAAA > 5'-CAAA). Like the unnatural enantiomer of CBI-TMI, the unnatural enantiomer, (+)-*iso*-CBI-TMI, alkylated DNA 50–100× less effectively than the natural enantiomer and did so with a selectivity identical to that of *ent*-(-)-duocarmycin SA. Within w836, this constitutes the central adenines within the sequence 5'-AAAAAA and corresponds to alkylation of 3–4 base-pair AT-rich sites with binding in the reverse 5'→3' direction. Because of the diastereomeric nature of the adducts, the unnatural enantiomer alkylates the second 5' base (adenine) within the sequence (*i.e.*, 5-AAAA > 5'-CAAAA). This has been discussed in detail and illustrated elsewhere⁴ and both enantiomers of *iso*-CBI-TMI conform nicely to the models. Thus, the relocation of the C-4 carbonyl from the outer face of a bound complex potentially proximal to the phosphate backbone to a position deep in the minor groove inaccessible to the phosphates had no impact on the DNA alkylation selectivity of either enantiomer.

In addition, the DNA alkylation properties of 20, *seco iso*-CI-TMI, were examined alongside (+)-duocarmycin SA and a representative comparison is illustrated in Figure 7 with w794 DNA. In past studies, such *seco* agents have behaved in a manner indistinguishable from their cyclopropane ring-closed counterparts exhibiting identical DNA alkylation selectivities, efficiencies, and cytotoxic activity indicating that ring closure is not limiting under the assay conditions. Since we were not able to isolate the ring closed *iso*-CI agents because of their exceptional reactivity, the examination was conducted with the *seco* precursor 20. Analogous to the observations made with *iso*-CBI-TMI, 44 alkylated DNA in a manner identical to duocarmycin SA alkylating only adenine and exhibiting the same sequence selectivity. Although no new sites of alkylation were

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detected, the relative selectivity among the available sites was slightly lower with 20. This is illustrated nicely in Figure 7 where 20 alkylates the minor sites more prominently than does (+)-duocarmycin SA. Interestingly and importantly, 20 alkylated DNA only 10^x less efficiently than (+)-duocarmycin SA being far more effective than the ring closed *iso*-CI-TMI might be projected to be. To date, this observation is unique to the *iso*-CI series and, as yet, has not been observed in the *iso*-CBI series or with other analogs incorporating modified alkylation subunits. Whether this stems from the use of the mesylate versus chloro seco precursor for 20 or is unique to the *iso*-CI series remains to be established. However, it does suggest that in selected instances the seco precursors may exhibit productive properties that exceed those of the corresponding cyclopropane ring-closed materials especially with the more reactive agents. Unlike the more stable agents which readily undergo cyclopropane ring closure, the ring closure of 20 to *iso*-CI-TMI under the conditions of the assay is unlikely. Rather, the DNA alkylation most likely occurs directly with 20 without the intermediate generation of the free cyclopropane agent. Thus, not only did the relocation of the C-4 carbonyl not alter the DNA alkylation selectivity, but its removal altogether may be possible providing a class of agents which, depending on the nature of the electrophile, also exhibit comparable DNA alkylation selectivities, efficiencies, and rates. This is consistent with early observations that related electrophiles that lack the capabilities for ring closure to an activated cyclopropane exhibit DNA alkylation selectivities identical to the corresponding natural products.

Table 3. Calf Thymus DNA Alkylation

conditions	base-pair	66	65	44	solvolysis
	equiv				
4 °C, 72 h	75 ^a	94%	<5%	nd	nd
4 °C, 72 h	150 ^a	95%	<5%	nd	nd
4 °C, 72 h	150 ^b	92%	<5%	nd	nd
4 °C, 120 h	150 ^b	95%	<5%	nd	nd

^a Analytical scale, HPLC separation and UV quantitation.

^b Preparative scale, isolation and weight quantitation. ^c nd = not detected.

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Quantitation, Isolation, and Characterization of the (-)-*iso*-CBI-TMI

Adenine Adduct: The initial alkylation studies established that (-)-*iso*-CBI-TMI alkylated adenine within the minor groove in a manner identical to (+)-duocarmycin SA. The thermal cleavage of DNA used to identify the alkylation sites in these studies only detects adducts susceptible to thermal glycosidic bond cleavage (adenine N3, guanine N3, or guanine N7 alkylation), and potential alkylation events involving other nucleophilic centers in DNA may not be detected in this assay. In order to confirm that (-)-*iso*-CBI-TMI alkylates DNA in a manner identical to (+)-duocarmycin SA and in efforts that established the relative extent of adenine N3 versus alternative alkylation events, the quantitation of the adenine N3 alkylation reaction and confirmation of the structure of the product of the reaction were established through isolation and characterization of the thermally released adenine adduct.

This was addressed through a study of the alkylation of calf thymus DNA. Optimized conditions for the alkylation were established for (-)-44 on an analytical scale (100 μ g of agent). For this purpose, the long-wavelength UV absorption of the agent and the adduct provided a useful quantitative measure of the adenine adduct, unreacted starting material and any side products (solvolysis or rearrangement), Table 3. Analytical HPLC analysis was used to confirm the identity of the products through correlation of retention times and UV spectral data with authentic materials. The preparative DNA alkylation reaction and subsequent isolation of (+)-66 was carried out under conditions determined to provide complete consumption of the agent in the presence of a large excess of DNA. Thus, extraction (EtOAc) of the aqueous buffer solution containing calf thymus DNA following alkylation (4 $^{\circ}$ C, 72 h, 150 bp) afforded no recovered (-)-44, and only a small amount (<5%) of the rearranged product 65. Conducting the reaction at 4 $^{\circ}$ C in the presence of a large excess of DNA precluded competitive solvolysis. Thermal treatment of the alkylated DNA in aqueous 10 mM sodium phosphate buffer (100 $^{\circ}$ C, 30 min, pH 7.0) followed by EtOAc extraction provided 66 in

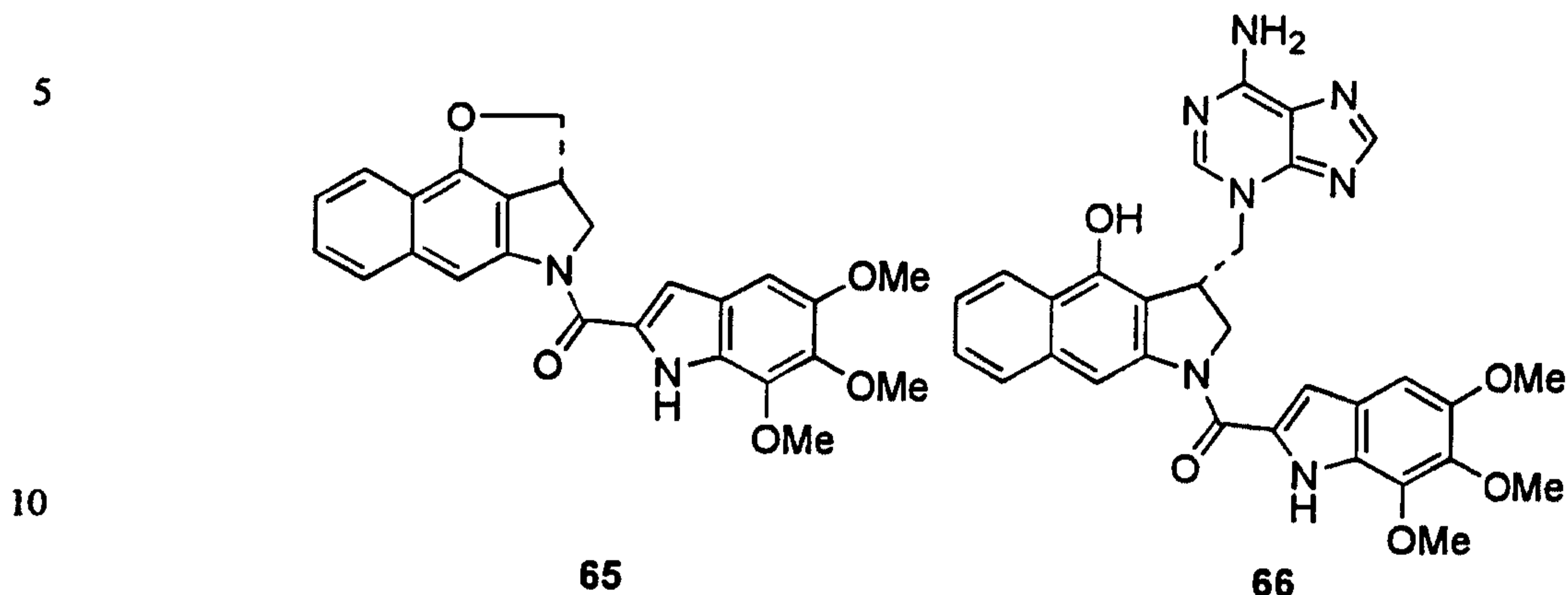
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90–95% conversion, ≥95% purity (by HPLC). Repeating this thermal treatment provided little or no additional adduct. No trace of a competing guanine adduct could be detected. This high conversion to a single adduct established that 44 participates exclusively in the adenine N3 alkylation reaction under the conditions examined.

Full characterization of 66 unambiguously established its structure and the spectral characteristics showed strong homology to the duocarmycin SA¹¹ and A adenine N3 adducts and N3 methyl adenine (Figure 10). In the ¹H NMR, the C3-H of adduct 66 was observed as a single proton (1H) at a characteristic chemical shift of 4.36–4.37. The C3-H NMR signal for the benzylic center resulting from alternative adenine N3 addition to the more substituted cyclopropane carbon would be readily distinguishable appearing as two protons (3.5–3.6 ppm, 2H) with a large geminal coupling constant (19.5 Hz). Additionally diagnostic of the structure were the chemical shifts and coupling constants for C2-H₂ and C4-H₂. Characteristic of an adenine N3 alkylation product, the adenine C2-H and C8-H were readily distinguishable. The ¹H NMR of the protonated base was taken in DMSO/1% TFA and again a strong correlation to the spectral data obtained for the duocarmycin SA adduct was observed. The two adenine C6-NH₂ protons were seen as separate signals indicating restricted rotation about the ⁶C=NH₂⁺ bond as is present in protonated N-methyl adenine. In addition, a downfield shift as a result of protonation of both the adenine C8-H and adenine C2-H was also observed. The ¹³C NMR of 66 was also found to be in excellent agreement with that of the duocarmycin SA and A adducts. (Boger, D. L., et al., *J. Am. Chem. Soc.* 1991, 113, 6645.) The key distinguishing signals are found within or proximal to the fused five- versus six-membered ring with 66 exhibiting chemical shifts consistent only with the former, *i.e.* C3 at δ 39.7 consistent with duocarmycin SA (δ 41.1) and inconsistent with the six-membered ring in duocarmycin B₁/C₁ (δ 33–34). Thus, the adenine N3 addition to the least substituted cyclopropane carbon of (–)-*iso*-CBI-TMI, as with (+)-duocarmycin SA, was found to account for 90–100% of the

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consumption of the agent in the presence of duplex DNA and confirmed that it binds and alkylates DNA in a manner identical to the natural products despite the relocation of the C-4 carbonyl.



In Vitro Cytotoxic Activity: Past studies with agents in this class have defined a direct correlation between solvolysis stability and cytotoxic potency. Consistent with their relative reactivity, the *iso*-CBI based agents exhibited cytotoxic activity that closely followed this relationship in spite of the deep-seated structural modification (Table 5, Figure 8). The results, which also follow trends established in the DNA alkylation studies, demonstrate that the (–)-enantiomer of the analogs possessing the (*S*)-configuration analogous to the natural products, is the more potent enantiomer by 10–50×. The exception to this generalization is *N*-BOC-*iso*-CBI where the two enantiomers were not readily distinguishable and the unnatural enantiomer was consistently slightly more potent (1–2×). The seco precursors, which lack the preformed cyclopropane but possess the capabilities of ring closure, were found to possess cytotoxic activity that was indistinguishable from the final ring-closed agents. Consistent with the unique importance of the C5 methoxy group of the duocarmycins, *iso*-CBI-TMI (44) and 46 were found to be equipotent illustrating that the C6 and C7 methoxy groups of 44 are not contributing to its cytotoxic potency. The cinnamate derivative 48 was found to be substantially less potent (40–50×) suggestive of the requirement for a rigid N² DNA binding subunit. Finally, the agents exhibited a smooth trend of increasing cytotoxic potency as the

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size and length of the DNA binding subunits increased with *iso*-CBI-CDPI₁ and *iso*-CBI-CDPI₂ displaying the most potent cytotoxic activity in the series exhibiting IC₅₀ values of 200 and 50 pM, respectively. (While we were unable to isolate *N*-BOC-*iso*-CI (21) and *iso*-CI-TMI for direct comparison, their seco precursors (±)-19 and (±)-20 exhibited surprisingly potent cytotoxic activity (IC₅₀, L1210 = 10 μM and 6 nM, respectively) that likely exceeds that of the ring closed materials themselves.)

Findings: *iso*-CI and *iso*-CBI were designed to test a key element of the different proposals for the origin of the DNA alkylation sequence with the duocarmycins and CC-1065. These agents were prepared by application of a directed ortho metallation and ortho spirocyclization in an improved synthetic scheme complementary to that reported for CI and CBI. *iso*-CBI was found to be 5× less stable than CBI and possess a reactivity comparable with CC-1065 and duocarmycin A. In addition, nucleophilic addition occurred at the least substituted cyclopropane carbon with a regioselectivity (40:1) comparable to that of CBI but which exceeds that of the natural products themselves (6–1.5:1). Comparison of the X-ray structures of *iso*-CBI and CBI revealed the near identical non-ideal conjugation of the cyclopropanes and the exclusive stereoelectronic alignment of the cleaved cyclopropane bond. Consistent with recent observations, the lower stability of *iso*-CBI relative to CBI itself can be attributed to a diminished cross-conjugated vinylogous amide stabilization for which the N²-C^{2a} bond length is diagnostic.^{7,42} Resolution and incorporation of *iso*-CBI into a full set of duocarmycin and CC-1065 analogs allowed for comparison of their properties and a further distinguishing test for the origin of the DNA alkylation sequence selectivity. The *iso*-CBI analogs were highly potent cytotoxic agents exhibiting picomolar IC₅₀'s which correlated with their relative stability. In addition to smoothly following this correlation, the analogs displayed a smooth trend of increasing cytotoxic potency with the increasing length in the DNA binding subunit. Analogous to the natural products, the (*S*)-enantiomer possessing the absolute

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configuration of 1–3, proved to be more potent (10–50×) than the (*R*)-enantiomer. DNA alkylation studies revealed a strong correlation between DNA alkylation efficiency and cytotoxic potency as (–)-*iso*-CBI-TMI was approximately 50–100× less efficient than (+)-CBI-TMI and (+)-duocarmycin SA. In addition, both the *iso*-
5 CI and *iso*-CBI analogs, with the repositioned C-4 carbonyl, exhibited the identical sequence selectivity and alkylated the same sites as CBI-TMI and duocarmycin SA derived from adenine N3 addition to the least substituted cyclopropane carbon at comparable reaction rates. This is inconsistent with a proposal that the DNA
10 alkylation selectivity is controlled by a sequence-dependent DNA backbone phosphate protonation of the C-4 carbonyl for activation of alkylation but is consistent with the noncovalent binding and steric accessibility model. Finally, this set of isomeric analogs contains the most significant structural modification to the alkylation subunit to date, yet remain effective DNA alkylating agents with
15 properties comparable to the natural products themselves.

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Synthesis of Materials:

1-(*tert*-Butyloxycarbonyl)-4-hydroxy-3[[methanesulfonyl]oxy]methyl]-2,3-dihydroindole (19): A solution of 18 (22 mg, 0.057 mmol) in 2 mL of 1:1 *i*-PrOH/THF was treated with 12 N HCl (33 μ L, 0.4 mmol) and stirred for 48 h at 25°C. The reaction solution was concentrated under reduced pressure. Flash chromatography (SiO₂, 1.5 \times 10 cm, 40% EtOAc/hexane) afforded recovered 18 (8 mg, 41%) and 19 (9.5 mg, 48%) as a white film: ¹H NMR (CDCl₃, 400 MHz) δ 7.43 (m, 1H), 7.07 (m, 1H), 6.39 (d, *J* = 8.6 Hz, 1H), 5.44 (br s, 1H), 4.55 (dd, *J* = 4.5, 9.8 Hz, 1H), 4.26 (dd, *J* = 8.1, 9.8 Hz, 1H), 4.00 (m, 2H), 3.79 (m, 1H); IR (film) ν_{\max} 3347, 2977, 1682, 1622, 1602, 1467, 1394, 1172, 1145, 948 cm⁻¹; FABHRMS (NBA/CsI) *m/z* 476.0156 (C₁₅H₂₁NO₆S + Cs⁺ requires 476.0144).

4-Hydroxy-3[[methanesulfonyl]oxy]methyl]-1-[5,6,7-trimethoxyindol-2-yl]carbonyl]-2,3-dihydroindole (20): A sample of 18 (9.0 mg, 0.023 mmol) was dissolved in 3.6 N HCl/EtOAc (2.4 mL) and the solution was stirred for 30 min at 25 °C. The solvents were removed by a stream of N₂ and the residual salt was thoroughly dried under high vacuum. The salt was dissolved in anhydrous DMF (1.2 mL) and treated with 37 (7 mg, 0.028 mmol) and EDCI (13 mg, 0.07 mmol). The resulting solution was stirred at 25 °C for 3 h under Ar. The reaction mixture was then diluted with H₂O (5 mL), and extracted with EtOAc (3 \times 5 mL). The combined organic layer was dried (Na₂SO₄), and concentrated under reduced pressure. Flash chromatography (SiO₂, 1.5 \times 10 cm, 50% EtOAc/hexane) yielded pure 20 (6.0 mg, 55%) as a white film: ¹H NMR (CDCl₃, 400 MHz) δ 9.33 (br s, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.19 (m, 1H), 6.92 (d, *J* = 2.4 Hz, 1H), 6.84 (s, 1H), 6.52 (dd, *J* = 0.5, 8.0 Hz, 1H), 4.65 (dd, *J* = 4.3, 10.1 Hz, 1H), 4.58 (dd, *J* = 9.2, 10.8 Hz, 1H), 4.51 (dd, *J* = 3.9, 10.8 Hz, 1H), 4.31 (dd, *J* = 8.4, 10.1 Hz, 1H), 4.05 (s, 3H), 4.01 (m, 1H), 3.92 (s, 3H), 3.89 (s, 3H), 2.95 (s, 3H); IR (film) ν_{\max} 3261, 2938, 1659, 1611, 1462, 1352, 1306, 1282, 1174, 1107 cm⁻¹; FABHRMS (NBA/CsI) *m/z* 609.0319 (C₂₂H₂₄N₂O₈S + Cs⁺ requires 609.0308).

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***N*-(*tert*-Butyloxycarbonyl)-4-(methoxymethoxy)-2-naphthylamine (23):** A solution of 22²² (6.67 g, 25.0 mmol) in 125 mL of anhydrous DMF at 0 °C was treated with NaH (1.13 g, 28.0 mmol) in several portions over 5 min. After 10 min, Bu₄NI (0.925 g, 2.5 mmol) was added followed by the dropwise addition of
5 ClCH₂OCH₃ (2.9 mL, 38 mmol). The reaction mixture was stirred at 25 °C for 5 h before the reaction was quenched by the slow addition of 100 mL of H₂O. The aqueous layer was extracted with EtOAc (3 × 100 mL). The organic layers were combined, washed with 10% aqueous NaHCO₃ (100 mL), H₂O (4 × 50 mL), dried (Na₂SO₄), and concentrated under reduced pressure. Flash chromatography (SiO₂,
10 4 × 15 cm, 0–15% EtOAc/hexane gradient) provided 23 (6.0 g, 78%) as a peach colored solid: mp 64–66 °C; ¹H NMR (CDCl₃, 250 MHz) δ 8.15 (d, *J* = 7.8 Hz, 1H), 7.67 (m, 2H), 7.38 (m, 2H), 7.05 (d, *J* = 1.9 Hz, 1H), 6.87 (br s, 1H), 5.34 (s, 2H), 3.51 (s, 3H), 1.54 (s, 9H); ¹³C NMR (CDCl₃, 100 MHz) δ 153.3, 152.8, 136.0, 134.8, 127.0, 126.9, 123.7, 122.5, 121.6, 108.0, 101.8, 94.6, 80.5, 56.1,
15 28.2; IR (film) ν_{\max} 3334, 2977, 1713, 1634, 1538, 1392, 1367, 1248, 1160, 1057 cm⁻¹; FABHRMS (NBA) *m/z* 303.1463 (C₁₇H₂₁NO₄ requires 303.1471).

Anal. Calcd for C₁₇H₂₁NO₄: C, 67.31; H, 6.98; N, 4.62. Found: C, 67.13; H, 7.18; N, 4.89.

20 ***N*-(*tert*-Butyloxycarbonyl)-3-iodo-4-(methoxymethoxy)-2-naphthylamine (24):** A solution of 23 (0.435 g, 1.43 mmol) in 5.7 mL anhydrous THF was cooled to –25 °C and treated with TMEDA (0.758 mL, 5.0 mmol) followed by *n*-BuLi (2.29 mL of a 2.5 M solution in hexane, 5.0 mmol) in a slow dropwise manner. The resulting gold solution was stirred for 2 h at –25 °C. The reaction
25 mixture was treated with 1-chloro-2-iodoethane (0.37 mL, 5.0 mmol) and stirred for 15 min at 25 °C. The reaction was diluted with H₂O (40 mL), extracted with Et₂O (3 × 20 mL), and the combined organic extracts were washed with saturated aqueous NaCl, dried (Na₂SO₄) and concentrated under reduced pressure. Flash chromatography (SiO₂, 2.5 × 15 cm, 0–7% EtOAc/hexane gradient) yielded 24
30 (490 mg, 80%) as a yellow oil: ¹H NMR (CDCl₃, 400 MHz) δ 8.35 (s, 1H), 8.03

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(d, $J = 12.8$ Hz, 1H), 7.78 (d, $J = 12.5$ Hz, 1H), 7.42 (m, 2H), 7.14 (br s, 1H), 5.20 (s, 2H), 3.74 (s, 3H), 1.56 (s, 9H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 154.7, 152.6, 135.1, 134.8, 127.6, 127.4, 125.1, 125.0, 122.2, 113.0, 100.5, 87.7, 81.1, 58.5, 28.4; IR (film) ν_{max} 3391, 2977, 2933, 1732, 1524, 1367, 1340, 1276, 1228, 1157
5 cm^{-1} ; FABHRMS (NBA/NaI) m/z 430.0507 ($\text{C}_{17}\text{H}_{20}\text{INO}_4 + \text{H}^+$ requires 430.0515).

2-[[*N*-(*tert*-Butyloxycarbonyl)-*N*-(2-propen-1-yl)amino]-3-iodo-4-

(methoxymethoxy)naphthalene (25): A solution of 24 (0.490 g, 1.1 mmol) in 36 mL anhydrous DMF was cooled to -10 °C, and treated with NaH (69 mg, 1.7
10 mmol) in small portions. The resulting suspension was stirred 15 min and treated with neat allyl bromide (0.49 mL, 5.7 mmol) in a slow dropwise manner. The reaction mixture was warmed to 25 °C and stirred for 1 h. The reaction mixture was quenched with the addition of 5% aqueous NaHCO_3 (50 mL), and the aqueous layer was extracted with EtOAc (3×20 mL). The combined organic extracts were
15 washed with H_2O (5×10 mL) dried (Na_2SO_4), and condensed under reduced pressure to yield 25 as a 2:1 mixture of amide rotamers as a yellow oil. Flash chromatography (SiO_2 , 2.5×15 cm, 10% EtOAc/hexane) yielded 25 (503 mg, 94%) as a colorless oil: ^1H NMR (CDCl_3 , 250 MHz) δ 8.16 (m, 1H), 7.77 (m, 1H), 7.50 (m, 3H), 5.97 (m, 1H), 5.23 (m, 2H), 5.07 (m, 2H), 4.56 (m, 1H), 3.80 (m,
20 1H), 3.72 (s, 3H), 1.55 and 1.33 (s, 9H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 155.6 and 155.4, 154.1 and 153.9, 141.3 and 141.1, 133.8 and 133.5, 127.7 and 127.6, 127.1, 126.8, 125.2, 124.5, 122.4, 117.8 and 117.3, 100.6, 95.7, 80.6 and 80.3, 58.3, 53.6, 52.4, 28.3 and 28.2; IR (film) ν_{max} 2975, 2930, 1703, 1581, 1566, 1385, 1366, 1159, 1047 cm^{-1} ; FABHRMS (NBA/CsI) m/z 601.9825 ($\text{C}_{20}\text{H}_{24}\text{INO}_4 + \text{Cs}^+$
25 requires 601.9804).

1-(*tert*-Butyloxycarbonyl)-4-(methoxymethoxy)-3-[[*(2',2',6',6'*-tetramethyl-piperidino)oxy]methyl]-2,3-dihydro-1*H*-benzo[*f*]indole (26): A solution of 25 (470 mg, 1.00 mmol) and TEMPO (468 mg, 3.0 mmol) in 43 mL anhydrous
30 benzene was treated with Bu_3SnH (0.283 mL, 1.05 mmol). The solution was

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warmed at 50 °C and an additional 1.05 equiv of Bu₃SnH (0.283 mL, 1.05 mmol) was added twice during the next 30 min. Another 3.0 equiv of TEMPO (468 mg, 3.0 mmol) was added in 10 mL anhydrous benzene, along with an additional 1.05 equiv of Bu₃SnH added twice during the next 45 min. After 1.5 h total, the solution
5 was cooled to 25 °C, and the volatiles were removed under reduced pressure. Flash chromatography (SiO₂, 2.5 × 15 cm, 0–12% EtOAc/hexane gradient) provided 26 (470 mg, 94%) as a yellow oil: ¹H NMR (CDCl₃, 250 MHz) δ 8.05 (br s, 1H), 7.97 (d, *J* = 8.0 Hz, 1H), 7.74 (d, *J* = 7.7 Hz, 1H), 7.36 (m, 2H), 5.24 (d, *J* = 5.9 Hz, 1H), 5.16 (d, *J* = 5.9 Hz, 1H), 4.26 (m, 1H), 4.15 (m, 1H), 4.01 (m, 1H), 3.81 (m,
10 2H), 3.63 (s, 3H), 1.61 (s, 9H), 1.23 (s, 3H), 1.17 (s, 3H), 1.04 (s, 3H), 1.04 (s, 3H), 0.99 (s, 3H), 1.48–0.89 (m, 6H); ¹³C NMR (CDCl₃, 62.5 MHz) δ 152.0, 149.3, 141.1, 135.3, 127.2, 125.7, 124.4, 123.4, 122.6, 121.0, 107.0, 99.1, 80.2, 76.0, 59.3, 57.1, 51.2, 39.1, 32.6, 27.9, 19.6, 16.6; IR (film) ν_{max} 2974, 2931, 1709, 1634, 1446, 1374, 1352, 1332, 1147 cm⁻¹; FABHRMS (NBA/CsI) *m/z* 631.2168
15 (C₂₉H₄₂N₂O₅ + Cs⁺ requires 631.2148).

1-(*tert*-Butyloxycarbonyl)-3-(hydroxymethyl)-4-(methoxymethoxy)-2,3-dihydro-1*H*-benzo[*f*]indole (27): A solution of 26 (220 mg, 0.44 mmol) in 15 mL 3:1:1 HOAc/H₂O/THF was treated with Zn powder (1.15 g, 17.6 mmol) and
20 the resulting suspension was warmed at 70 °C under a reflux condenser and with vigorous stirring for 1 h. The reaction mixture was cooled to 25 °C, and the Zn was removed by filtration through Celite with a 25 mL CH₂Cl₂ wash. The volatiles were removed under reduced pressure, and the resulting residue was dissolved in 25 mL of EtOAc and filtered. The solution was concentrated under reduced pressure.
25 Flash chromatography (SiO₂, 2.5 × 10 cm, 30–40% EtOAc/hexane gradient) provided 27 (138 mg, 87%) as a colorless oil: ¹H NMR (CDCl₃, 250 MHz) δ 8.05 (br s, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 7.5 Hz, 1H), 7.36 (m, 2H), 5.23 (d, *J* = 6.0 Hz, 1H), 5.16 (d, *J* = 6.0 Hz, 1H), 4.10 (m, 1H), 3.94–3.78 (m, 4H), 3.63 (s, 3H), 2.04 (d, *J* = 8.4 Hz, 1H), 1.58 (s, 9H); ¹³C NMR (CDCl₃, 125 MHz) δ
30 152.5, 149.7, 142.0, 136.0, 127.9, 126.4, 124.6, 124.1, 123.1, 121.3, 107.8, 100.0,

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81.1, 65.1, 57.8, 51.3, 40.7, 28.3; IR (film) ν_{\max} 3447, 2975, 1704, 1634, 1447, 1353, 1336, 1149 cm^{-1} ; FABHRMS (NBA) m/z 360.1821 ($\text{C}_{20}\text{H}_{25}\text{NO}_5 + \text{H}^+$ requires 360.1811).

5 **1-(*tert*-Butyloxycarbonyl)-3-chloromethyl-4-(methoxymethoxy)-2,3-dihydro-1*H*-benzo[*f*]indole (28).** From 27: A solution of 27 (55 mg, 0.16 mmol) in 3 mL anhydrous CH_2Cl_2 was treated with CCl_4 (155 μL , 1.6 mmol) and Ph_3P (212 mg, 0.81 mmol) and the mixture was stirred at 25 °C for 2 h. The solution was concentrated *in vacuo*. Flash chromatography (SiO_2 , 1.5 \times 15 cm, 10 0–12% EtOAc/hexane gradient) provided 28 (52 mg, 90%) as a white solid: mp 99–101 °C; ^1H NMR (CDCl_3 , 250 MHz) δ 8.03 (br s, 1H), 7.89 (d, $J = 8.1$ Hz, 1H), 7.72 (d, $J = 7.6$ Hz, 1H), 7.37 (m, 2H), 5.24 (d, $J = 6.1$ Hz, 1H), 5.15 (d, $J = 6.1$ Hz, 1H), 4.06 (m, 4H), 3.65 (s, 3H), 3.51 (app t, $J = 10.1$ Hz, 1H), 1.59 (s, 9H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 152.3, 150.5, 141.1, 136.2, 127.9, 126.6, 15 124.6, 124.1, 122.4, 121.4, 107.7, 100.0, 81.2, 57.5, 52.1, 45.9, 40.8, 28.4; IR (film) ν_{\max} 2976, 1704, 1634, 1449, 1336, 1147, 1055, 983 cm^{-1} ; FABHRMS (NBA) m/z 378.1463 ($\text{C}_{20}\text{H}_{24}\text{ClNO}_4 + \text{H}^+$ requires 378.1472).

Anal. Calcd for $\text{C}_{20}\text{H}_{24}\text{INO}_4$: C, 63.75; H, 6.40; N, 3.71. Found: C, 63.42; H, 6.11; N, 3.41.

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From 29: A solution of 29 (500 mg, 1.0 mmol) in 10 mL anhydrous benzene was treated with AIBN (15.7 mg, 0.1 mmol) and Bu_3SnH (295 μL , 1.1 mmol) and warmed at 80 °C for 2 h. The reaction mixture was concentrated under reduced pressure and flash chromatography (SiO_2 , 2.5 \times 15 cm, 0–20% EtOAc/hexanes 25 gradient) yielded 28 (360 mg, 96%) as a white solid identical to that described above.

Resolution of (28). A sample of 28 (4.0 mg) in 0.9 mL of 5% *i*-PrOH/hexane was resolved on a semipreparative Daicel Chiralcel OD column (10 μm , 2 \times 25 cm, 30 7.0 mL/min flow rate, 3% *i*-PrOH/hexane). The effluent was monitored at 254 nm

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and the enantiomers eluted with retention times of 29.0 and 35.8 min ($\alpha = 1.25$). The first enantiomer (*S*) was found to be >99% enantiomerically pure. The second fraction (*R*) was reinjected in order to obtain a sample of >99% ee. The fractions containing the separated enantiomers were collected and concentrated to afford (+) and (-)-28. (+)-(3*S*)-28: $[\alpha]_D^{25} +29$ (*c* 0.50, CH₂Cl₂); (-)-(3*R*)-28: $[\alpha]_D^{25} -30$ (*c* 0.50, CH₂Cl₂).

2-[[*N*-(*tert*-Butyloxycarbonyl)-*N*-(3-chloro-2-propen-1-yl)]amino-3-iodo-4-(methoxymethoxy)naphthalene (29): A solution of 24 (0.480 g, 1.1 mmol) in 11 mL anhydrous DMF was cooled to 0 °C, and treated with NaH (67 mg, 2.2 mmol) in small portions. The resulting suspension was stirred 15 min and treated with neat 1,3-dichloropropene (0.52 mL, 5.5 mmol) in a slow dropwise manner, followed by catalytic Bu₄NI (40 mg, 0.1 mmol). The reaction mixture was warmed to 25 °C and stirred for 12 h. The reaction mixture was quenched with the addition of 5% aqueous NaHCO₃ (50 mL), and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with H₂O (5 × 10 mL), dried (Na₂SO₄), and concentrated under reduced pressure to yield 29 as a mixture of rotamers and *E* and *Z* alkenes as a yellow oil. Flash chromatography (SiO₂, 2.5 × 15 cm, 0–20% EtOAc/hexanes gradient) yielded 29 (540 mg, 96%) as a yellow oil: ¹H NMR (CDCl₃, 250 MHz) δ 8.16 (m, 1H), 7.80 (m, 1H), 7.55 (m, 2H), 7.44 (s, 1H), 6.11 (m, 2H), 5.25 (d, *J* = 5.6 Hz, 1H), 5.20 (d, *J* = 5.6 Hz, 1H), 4.51 (m, 1H), 3.75 (m, 1H), 3.73 (s, 3H), 1.55 and 1.31 (s, 9H); ¹³C NMR (CDCl₃, 62.5 MHz) δ 156.2, 153.9, 140.7, 134.0, 128.7, 127.9, 127.7, 127.4, 127.1, 124.7, 122.5, 121.7, 100.7, 80.8, 77.2, 58.5, 49.5, 28.2; IR (film) ν_{\max} 2975, 1699, 1565, 1387, 1366, 1328, 1294, 1254, 1162 cm⁻¹; FABHRMS (NBA/NaI) *m/z* 504.0424 (C₂₀H₂₃ClINO₄ + H⁺ requires 504.0439).

1-(*tert*-Butyloxycarbonyl)-3-chloromethyl-4-hydroxy-2,3-dihydro-1*H*-benzo[*f*]indole (30): A solution of 28 (18 mg, 47.5 μ mol) in 1.5 mL of 1:1 *i*-PrOH/THF was treated with 12 N HCl (0.20 mL, 0.38 mmol) and the mixture was

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stirred at 25 °C for 6 h before the volatiles were removed *in vacuo*. Flash chromatography (SiO₂, 1.5 × 15 cm, 0–20% EtOAc/hexane gradient) provided **30** (14.5 mg, 90%) as a pale yellow oil: ¹H NMR (CDCl₃, 250 MHz) δ 7.85 (d, *J* = 8.0 Hz, 1H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.43–7.30 (m, 2H), 5.67 (s, 1H), 4.09–3.86 (m, 4H), 3.65 (dd, *J* = 8.0, 10.0 Hz, 1H), 1.59 (s, 9H); ¹³C NMR (CDCl₃, 62.5 MHz) δ 147.9, 135.8, 132.7, 128.8, 128.0, 126.6, 123.7, 121.2, 119.5, 119.5, 104.4, 77.2, 52.6, 46.4, 34.7, 28.5; IR (film) ν_{max} 3368, 2976, 1668, 1477, 1450, 1369, 1145, 978, 746 cm⁻¹; FABHRMS (NBA/CsI) *m/z* 466.0197 (C₁₈H₂₀ClNO₃ + Cs⁺ requires 466.0186). (–)-(3*S*)-**30**: [α]_D²⁵ –54 (*c* 0.25, CH₃OH); (+)-(3*R*)-**30**: [α]_D²⁵ +51 (*c* 0.30, CH₃OH).

2-(tert-Butyloxycarbonyl)-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*f*]indol-8-one (N-BOC-*iso*-CBI, **31):** A solution of **30** (5.0 mg, 0.015 mmol) in 0.6 mL CH₃CN was treated with DBU (2.7 μL, 0.018 mmol) and the mixture was stirred at 25 °C for 15 min. Flash chromatography (SiO₂, 1.5 × 10 cm, 10% EtOAc/hexane) afforded **31** (4.3 mg, 96%) as a light golden oil: ¹H NMR (CDCl₃, 400 MHz) δ 7.98 (d, *J* = 7.8 Hz, 1H), 7.50 (dt, *J* = 1.4, 7.8 Hz, 1H), 7.28 (d, *J* = 7.8 Hz, 1H), 7.21 (dt, *J* = 1.1, 7.8 Hz, 1H), 6.97 (br s, 1H), 3.88 (br d, *J* = 11.2 Hz, 1H), 3.81 (dd, *J* = 4.9, 11.1 Hz, 1H), 2.88 (dt, *J* = 5.5, 7.8 Hz), 1.87 (dd, *J* = 3.2, 7.8 Hz, 1H), 1.53 (s, 9H), 1.41 (dd, *J* = 3.2, 5.5 Hz, 1H); ¹H NMR (C₆D₆, 400 MHz) δ 8.34 (dd, *J* = 0.6, 7.8 Hz, 1H), 7.10 (m, 2H), 6.92 (m, 1H), 3.18 (br m, 1H), 2.32 (m, 1H), 1.49 (dd, *J* = 3.1, 7.8 Hz, 1H), 1.39 (s, 9H), 0.66 (dd, *J* = 3.1, 5.4 Hz, 1H); ¹³C NMR (CDCl₃, 62.5 MHz) δ 194.7, 154.3, 142.0, 134.3, 127.7, 127.3, 125.9, 125.2, 103.2, 77.2, 51.5, 46.1, 32.0, 28.2, 25.7; IR (film) ν_{max} 2973, 2927, 1716, 1670, 1635, 1472, 1410, 1324, 1143, 1009 cm⁻¹; UV (THF) λ_{max} (ε) 387 (2700), 302 (6000 sh), 293 (6900), 250 (20400) nm; FABHRMS (NBA/NaI) *m/z* 298.1450 (C₁₈H₁₉NO₃ + H⁺ requires 298.1443). (–)-(8*aS*,9*aS*)-**31**: [α]_D²⁵ –172 (*c* 0.08, CH₃OH); (+)-(8*aR*,9*aR*)-**31**: [α]_D²⁵ +179 (*c* 0.12, CH₃OH).

3-Chloro-4-hydroxy-1-(methoxycarbonyl)-2,3-dihydro-1*H*-benzo[*f*]indole

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(32): A solution of 28 (11 mg, 29.1 μmol) was treated with 1.0 mL of 3.6 N HCl/EtOAc and the resulting solution was stirred for 30 min at 25 °C. The solvent was removed by a stream of N_2 and the residual salt was dried under vacuum. This salt was suspended in 0.6 mL of anhydrous THF and treated with NaHCO_3 (5.4 mg, 64.1 μmol , 2.2 equiv) and ClCO_2CH_3 (4.5 μL , 58.2 μmol , 2.0 equiv) and the mixture was stirred for 3 h at 25 °C. Upon completion, the reaction mixture was diluted with 10 mL of H_2O , extracted with EtOAc (3×10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Flash chromatography (SiO_2 , 1.5×15 cm, 0–40% EtOAc/hexane gradient) provided pure 32 (5.5 mg, 65%) as a white solid: ^1H NMR (CDCl_3 , 400 MHz) δ 7.86 (d, $J = 8.2$ Hz, 1H), 7.86 (br s, 1H), 7.73 (d, $J = 8.0$ Hz, 1H), 7.42 (m, 1H), 7.35 (m, 1H), 5.80 (s, 1H), 4.11 (m, 2H), 4.01–3.87 (m, 5H), 3.63 (app t, $J = 9.4$ Hz, 1H); IR (film) ν_{max} 3379, 2956, 1673, 1435, 1372, 1283, 1218 cm^{-1} ; FABHRMS (NBA/NaI) m/z 291.0656 (M^+ , $\text{C}_{15}\text{H}_{14}\text{ClNO}_3$ requires 291.0662).

The structure and absolute configuration of (3*R*)-32 derived from the slower eluting enantiomer of 28 ($t_{\text{R}} = 35.8$ min, $[\alpha]_{\text{D}}^{25} -9$ (c 0.10, CH_3OH)) was obtained from a single-crystal X-ray structure determination conducted on prisms grown from EtOAc/hexane.

2-(Methoxycarbonyl)-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*f*]indol-8-one (*N*- CO_2Me -*iso*-CBI, 33): A solution of 32 (5.0 mg, 0.017 mmol) in 0.5 mL of CH_3CN was treated with DBU (3.9 μL , 0.025 mmol) and the mixture was stirred at 25 °C for 15 min. Flash chromatography (SiO_2 , 1.5×10 cm, 0–40% EtOAc/hexane gradient) afforded 33 (4.0 mg, 91%) as a pale yellow solid: ^1H NMR (C_6D_6 , 400 MHz) δ 8.33 (d, $J = 6.0$ Hz, 1H), 7.40 (br s, 1H), 7.10 (app t, $J = 6.0$ Hz, 1H), 7.03 (d, $J = 6.0$ Hz, 1H), 6.91 (m, 1H), 3.36 (s, 3H), 3.17 (m, 1H), 2.93 (m, 1H), 2.30 (dt, $J = 4.0, 6.0$ Hz, 1H), 1.49 (dd, $J = 2.4, 6.4$ Hz, 1H), 0.64 (dd, $J = 2.8, 4.4$ Hz, 1H); IR (film) ν_{max} 2954, 1731, 1633, 1445, 1323, 1196, 1018 cm^{-1} ; UV (THF) λ_{max} (ϵ) 386 (2300), 302 (4500 sh), 293 (5100), 250 (18000) nm; FABHRMS (NBA/NaI) m/z 255.0901 (M^+ , $\text{C}_{15}\text{H}_{13}\text{NO}_3$ requires 255.0895). (+)-(8*aR*,9*aR*)-33:

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$[\alpha]_D^{25} +31$ (*c* 0.07, CH₃OH).

The structure of **33** was confirmed with a single-crystal X-ray structure determination conducted on plates grown from EtOAc/hexane.³⁷

1,2,9,9a-Tetrahydrocyclopropa[c]benzo[f]indol-8-one (iso-CBI, 34): A solution of **28** (10.6 mg, 28.1 μmol) was treated with 3.6 M HCl/EtOAc and the mixture was stirred at 25 °C for 30 min. The volatiles were removed by a stream of N₂ and the residual salt was suspended in 1.0 mL of degassed CH₃CN. The suspension was treated with DBU (9.3 μL, 61.8 μmol, 2.2 equiv) and stirred under Ar for 20 min at 25 °C in the dark. Flash chromatography (SiO₂, 1.5 × 15 cm, 40% EtOAc/hexane) provided pure **34** (3.0 mg, 55%) as an unstable bright yellow oil: ¹H NMR (C₆D₆, 400 MHz) δ 8.38 (d, *J* = 8.0 Hz, 1H), 7.18 (m, 1H), 6.99 (d, *J* = 8.0 Hz, 1H), 6.87 (m, 1H), 5.27 (s, 1H), 2.60 (m, 1H), 2.52 (m, 1H), 2.45 (m, 1H), 1.70 (m, 1H), 1.00 (m, 1H); IR (film) ν_{\max} 3358, 1682, 1594, 1470, 1284, 1262, 1223 cm⁻¹; FABHRMS (NBA/NaI) *m/z* 198.0926 (C₁₃H₁₁NO + H⁺ requires 198.0919).

9,9a-1H-Dihydrocyclopropa[c]benzo[f]indol-3,8-dione (35): A solution of **28** (10.6 mg, 28.1 μmol) was treated with 4 M HCl/EtOAc and the mixture was stirred at 25 °C for 30 min. The volatiles were removed by a stream of N₂ and the residual salt was suspended in 1.0 mL of THF. The suspension was treated with 1.0 mL of 5% aqueous NaHCO₃ and stirred exposed to the air for 2 h at 25 °C. The bright orange solution was extracted with EtOAc (3 × 5 mL), washed with H₂O (2 × 5 mL) dried (Na₂SO₄), and concentrated *in vacuo*. Flash chromatography (SiO₂, 1.5 × 15 cm, 60% EtOAc/hexane) provided pure **35** (3.5 mg, 63%) as an orange film: ¹H NMR (C₆D₆, 400 MHz) δ 8.14 (m, 1H), 8.05 (m, 1H), 7.00 (m, 2H), 3.62 (dd, *J* = 6.2, 19.4 Hz, 1H), 3.52 (ddt, *J* = 0.6, 2.2, 19.4 Hz, 1H), 2.55 (ddt, *J* = 2.2, 6.0, 8.3 Hz, 1H), 1.54 (ddd, *J* = 0.6, 3.3, 8.4 Hz, 1H), 0.30 (dd, *J* = 3.3, 5.8 Hz, 1H); ¹³C NMR (CDCl₃, 100 MHz) δ 191.6, 182.0, 168.9, 135.8, 135.6, 135.0, 134.6, 128.4, 127.0, 63.8, 49.7, 35.0, 30.4; IR (film) ν_{\max} 2923, 1692, 1680, 1585, 1361, 1262, 1223, 1082 cm⁻¹; UV (CH₃OH) λ_{\max} (ε) 271 (5200), 239 (13300) nm;

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FABHRMS (NBA/NaI) m/z 212.0715 ($C_{13}H_9NO_2 + H^+$ requires 212.0712).

4-(*tert*-Butyloxycarbonyl)-1,2,2a,3-tetrahydrofurano[4,3,2-

5 ***c,d*]benzo[*f*]indole (36):** A neat sample of 31 (6.0 mg, 16 μ mol) was allowed to sit under room light at 25 °C for 48 h to yield 36 (6.0 mg, 100%): 1H NMR (CD₃CN, 500 MHz) δ 7.80 (d, $J = 8.0$ Hz, 1H), 7.67 (d, $J = 7.7$ Hz, 1H), 7.35 (m, 1H), 7.29 (m, 1H), 7.05 (br s, 1H), 5.22 (dd, $J = 8.0, 8.5$ Hz, 1H), 4.64 (dd, $J = 9.0, 11.0$ Hz, 1H), 4.40 (dd, $J = 8.5, 10.5$ Hz, 1H), 4.22 (m, 1H), 3.88 (m, 1H),
10 1.55 (br s, 9H); IR (film) ν_{max} 2975, 2932, 1699, 1475, 1456, 1418, 1353, 1166, 1132 cm^{-1} ; FABHRMS (NBA/NaI) m/z 297.1451 (M^+ , $C_{18}H_{19}NO_3$ requires 297.1365).

3-Chloromethyl-4-hydroxy-1-[(5,6,7-trimethoxyindol-2-yl)carbonyl]-2,3-

15 ***dihydro-1H*-benzo[*f*]indole (43):** A sample of 28 (8.4 mg, 0.022 mmol) was treated with 1.0 mL of 3.6 N HCl/EtOAc and the resulting solution was stirred for 30 min at 25 °C. The solvent was removed by a stream of N₂ and the residual salt was dried under vacuum. This salt was dissolved in 0.9 mL of anhydrous DMF and treated with 5,6,7-trimethoxyindole-2-carboxylic acid¹⁰ (6.8 mg, 0.026 mmol) and
20 EDCI (12.7 mg, 0.067 mmol) and the mixture was stirred for 3 h at 25 °C. Upon completion, the reaction mixture was diluted with 20 mL of EtOAc, washed with H₂O (5 \times 3 mL), dried (Na₂SO₄), and concentrated *in vacuo*. Flash chromatography (SiO₂, 1.5 \times 15 cm, 0–40% EtOAc/hexane gradient) provided 43 (11.2 mg, 91%) as a white solid: 1H NMR (CDCl₃, 400 MHz) δ 9.41 (s, 1H), 8.35 (s, 1H), 7.88
25 (d, $J = 8.4$ Hz, 1H), 7.82 (d, $J = 7.8$ Hz, 1H), 7.45 (m, 1H), 7.41 (m, 1H), 6.96 (d, $J = 2.3$ Hz, 1H), 6.85 (s, 1H), 5.95 (br s, 1H), 4.58 (m, 2H), 4.07 (m, 2H), 4.07 (s, 3H), 3.94 (s, 3H), 3.90 (s, 3H), 3.64 (dd, $J = 10.3, 12.0$ Hz, 1H); ^{13}C NMR (acetone-*d*₆, 400 MHz) δ 193.6, 161.1, 151.0, 149.7, 143.4, 141.5, 136.7, 131.6, 128.8, 127.3, 126.5, 124.7, 124.5, 124.0, 121.9, 115.3, 107.6, 107.2, 99.0, 61.44,
30 61.37, 56.4, 54.9, 46.6, 41.7; IR (film) ν_{max} 3405, 3301, 2937, 1594, 1446, 1312,

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1228, 1106 cm^{-1} ; FABHRMS (NBA/CsI) m/z 467.1358 ($\text{C}_{25}\text{H}_{23}\text{ClN}_2\text{O}_5 + \text{H}^+$ requires 467.1374). (-)-(3*S*)-43: $[\alpha]_{\text{D}}^{25} -4$ (c 0.22, CHCl_3); (+)-(3*R*)-43: $[\alpha]_{\text{D}}^{25} +4$ (c 0.35, CHCl_3).

2-[(5,6,7-Trimethoxyindol-2-yl)carbonyl]-1,2,9,9a-tetrahydrocyclo-
 5 propa[*c*]benzo [*f*]indol-8-one (*iso*-CBI-TMI, 44) : A solution of 43 (5.7 mg, 0.012 mmol) in 0.5 mL of CH_3CN was treated with DBU (2.2 μL , 0.015 mmol) and stirred at 25 $^\circ\text{C}$ for 15 min. Flash chromatography (SiO_2 , 1.5×10 cm, 50% EtOAc/hexane) provided pure 44 (4.2 mg, 94%) as a light golden oil: ^1H NMR (C_6D_6 , 400 MHz) δ 9.65 (s, 1H), 8.36 (m, 1H), 7.76 (s, 1H), 7.10 (m, 1H), 7.03
 10 (m, 1H), 6.94 (m, 1H), 6.77 (s, 1H), 6.45 (d, $J = 2.2$ Hz, 1H), 3.78 (s, 3H), 3.70 (s, 3H), 3.54 (s, 3H), 3.52 (d, $J = 9.9$ Hz, 1H), 3.26 (dd, $J = 5.2, 9.9$ Hz, 1H), 2.43 (m, 1H), 1.57 (dd, $J = 3.3, 7.8$ Hz, 1H), 0.70 (dd, $J = 3.3, 5.4$ Hz, 1H); ^{13}C NMR (acetone- d_6 , 400 MHz) δ 194.5, 191.8, 161.7, 151.4, 147.1, 143.1, 142.0, 140.4, 135.6, 131.3, 129.4, 128.9, 127.3, 126.9, 126.7, 125.0, 112.7, 108.1, 107.6, 99.3,
 15 63.7, 63.6, 57.0, 54.3, 40.6; IR (film) ν_{max} 3295, 2934, 1667, 1651, 1409, 1306, 1279, 1109 cm^{-1} ; UV (CH_3OH) λ_{max} 390 (ϵ 7100), 332 (ϵ 14100), 245 nm (ϵ 19100); FABHRMS (NBA/NaI) m/z 431.1615 ($\text{C}_{25}\text{H}_{22}\text{N}_2\text{O}_5 + \text{H}^+$ requires 431.1607). (-)-(8*aS*,9*aS*)-44: $[\alpha]_{\text{D}}^{25} -41$ (c 0.13, CH_3OH); (+)-(8*aR*,9*aR*)-44: $[\alpha]_{\text{D}}^{25} +38$ (c 0.08, CH_3OH).

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3-Chloromethyl-4-hydroxy-1-[(5-methoxyindol-2-yl)carbonyl]-2,3-dihydro-
 1*H*-benzo[*f*]indole (45): Flash chromatography (SiO_2 , 1.5×10 cm, 0–40% EtOAc/hexane gradient) afforded pure 45 (95%) as a clear oil: ^1H NMR (CDCl_3 , 400 MHz) δ 9.38 (br s, 1H), 8.35 (s, 1H), 7.93 (d, $J = 7.6$ Hz, 1H), 7.82 (d, $J = 7.9$
 25 Hz, 1H), 7.41 (m, 2H), 7.36 (d, $J = 9.0$ Hz, 1H), 7.11 (d, $J = 2.2$ Hz, 1H), 7.01 (m, 2H), 6.43 (s, 1H), 4.63 (d, $J = 5.4$ Hz, 2H), 4.11 (m, 2H), 3.86 (s, 3H), 3.64 (m, 1H); IR (film) ν_{max} 3287, 2927, 1665, 1596, 1518, 1445, 1402, 1389, 1290, 1231, 1167 cm^{-1} ; FABHRMS (NBA/NaI) m/z 406.1071 ($\text{C}_{25}\text{H}_{19}\text{ClN}_2\text{O}_3$ requires 406.1084). (-)-(3*S*)-45: $[\alpha]_{\text{D}}^{25} -26$ (c 0.07, CH_3OH); (+)-(3*R*)-45: $[\alpha]_{\text{D}}^{25} +25$ (c 0.04, CH_3OH).

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2-[(5-Methoxyindol-2-yl)carbonyl]-1,2,9,9a-tetrahydrocyclopropa-

[c]benzo[f]indol-8-one (46): Flash chromatography (SiO₂, 1.5 × 5 cm, 40% EtOAc/hexane) afforded 46 (85%) as a light golden oil: ¹H NMR (C₆D₆, 400 MHz) δ 9.15 (s, 1H), 8.38 (m, 1H), 7.80 (s, 1H), 7.12–7.05 (m, 4H), 6.94 (ddd, *J* = 1.6, 6.8, 8.3 Hz, 1H), 6.85 (app dt, *J* = 0.8, 8.6 Hz, 1H), 6.39 (d, *J* = 1.6 Hz, 1H), 3.51 (s, 3H), 3.48 (d, *J* = 10.0 Hz, 1H), 3.21 (dd, *J* = 5.2, 10.0 Hz, 1H), 2.42 (dt, *J* = 5.3, 7.8 Hz, 1H), 1.56 (dd, *J* = 3.3, 7.8 Hz, 1H), 0.68 (dd, *J* = 3.3, 5.3 Hz, 1H); IR (film) ν_{\max} 3303, 2927, 1667, 1643, 1597, 1518, 1408, 1277, 1030, 1013 cm⁻¹; FABHRMS (NBA/NaI) *m/z* 371.1388 (C₂₃H₁₈N₂O₃ + H⁺ requires 371.1396). (–)-(8*aS*,9*aS*)-46: [α]_D²⁵ –66 (*c* 0.04, CH₃OH); (+)-(8*aR*,9*aR*)-46: [α]_D²⁵ +71 (*c* 0.03, CH₃OH).

3-Chloromethyl-4-hydroxy-1-[(*E*)-3-(2-methoxyphenyl)propenyl]-

carbonyl]-2,3-dihydro-1*H*-benzo[f]indole (47): Flash chromatography (SiO₂, 1.5 × 10 cm, 0–40% EtOAc/hexane gradient) afforded 47 (87%) as a pale yellow solid: ¹H NMR (CDCl₃, 400 MHz) δ 8.40 (br s, 1H), 7.87 (m, 3H), 7.40 (m, 2H), 7.31 (m, 1H), 7.22 (m, 1H), 7.20 (s, 1H), 6.93 (m, 2H), 5.81 (m, 1H), 4.36 (m, 2H), 4.09 (m, 2H), 3.85 (s, 3H), 3.65 (m, 1H); IR (film) ν_{\max} 3266, 2963, 1659, 1598, 1445, 1377, 1269, 1158, 1048 cm⁻¹; FABHRMS (NBA/NaI) *m/z* 394.1217 (C₂₅H₂₀ClNO₃ + H⁺ requires 394.1210). (–)-(3*S*)-47: [α]_D²⁵ –18 (*c* 0.15, CH₃OH); (+)-(3*R*)-47: [α]_D²⁵ +22 (*c* 0.04, CH₃OH).

2-[(*E*)-3-(2-Methoxyphenyl)propenyl]carbonyl]-1,2,9,9a-tetrahydrocyclo-

propa[c]benzo[f]indol-8-one (48): Flash chromatography (SiO₂, 1.5 × 5 cm, 40% EtOAc/hexane) afforded 48 (83%) as a light golden oil: ¹H NMR (C₆D₆, 400 MHz) rotamers δ 8.39 (d, *J* = 7.5 Hz, 1H), 8.09 (d, *J* = 15.4 Hz, 1H), 7.15–6.95 (m, 8H), 6.76 (ddd, *J* = 1.0, 2.5, 8.1 Hz, 1H), 3.70–3.50 (m, 1H), 3.31 (s, 3H), 3.00–2.80 (m, 1H), 2.35 (m, 1H), 1.63 (m, 1H), 0.73 (m, 1H); IR (film) ν_{\max} 2926, 1673, 1626, 1471, 1408, 1383, 1265, 1156, 1092, 1044, 1009 cm⁻¹; FABHRMS (NBA/NaI) *m/z* 357.1360 (M⁺, C₂₃H₁₉NO₃ requires 357.1365). (–)-(8*aS*,9*aS*)-48:

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$[\alpha]_D^{25}$ -21 (*c* 0.08, CH₃OH); (+)-(8a*R*,9a*R*)-48: $[\alpha]_D^{25}$ +23 (*c* 0.03, CH₃OH).

3-Chloromethyl-4-hydroxy-1-[[5-[*N*-(indol-2-yl)carbonyl]aminoindol-2-yl]carbonyl]-2,3-dihydro-1*H*-benzo[*f*]indole (49): Flash chromatography
 5 (SiO₂, 1.5 × 10 cm, 40–80% EtOAc/hexane gradient) afforded 49 (74%) as a light brown solid: ¹H NMR (DMF-*d*₇, 400 MHz) δ 11.76 (s, 2H), 10.30 (s, 1H), 10.28 (s, 1H), 8.41 (s, 1H), 8.29 (s, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.71 (dd, *J* = 2.8, 9.2 Hz, 1H), 7.69 (d, *J* = 9.6 Hz, 1H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.52 (s, 1H), 7.46 (m, 1H), 7.40 (m, 1H), 7.30 (d, *J* = 1.6 Hz, 1H), 7.25 (m, 1H), 7.09 (m, 1H), 4.83 (m, 1H), 4.70 (dd, *J* = 2.4, 10.4 Hz, 1H), 4.30 (m, 1H),
 10 4.16 (dd, *J* = 2.8, 11.2 Hz, 1H), 3.98 (dd, *J* = 8.8, 11.2 Hz, 1H); IR (film) ν_{\max} 3280, 2929, 1660, 1650, 1594, 1519, 1443, 1389, 1314, 1250, 1098, 749 cm⁻¹; FABHRMS (NBA/CsI) *m/z* 667.0529 (C₃₁H₂₃ClN₄O₃ + Cs⁺ requires 667.0513). (-)-(3*S*)-49: $[\alpha]_D^{25}$ -18 (*c* 0.10, CH₃OH); (+)-(3*R*)-49: $[\alpha]_D^{25}$ +21 (*c* 0.06, CH₃OH).

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2-[[5-[*N*-(indol-2-yl)carbonyl]aminoindol-2-yl]carbonyl]-1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*f*]indol-8-one (*iso*-CBI-indole₂, 50): Flash chromatography (SiO₂, 1.5 × 10 cm, 10% DMF/toluene) afforded 50 (88%) as a light golden solid: ¹H NMR (DMF-*d*₇, 400 MHz) δ 11.75 (s, 1H), 11.72 (s, 1H),
 20 10.28 (s, 1H), 8.37 (s, 1H), 7.96 (d, *J* = 7.9 Hz, 1H), 7.73-7.64 (m, 3H), 7.58 (t, *J* = 8.1 Hz, 2H), 7.50 (m, 2H), 7.35 (m, 2H), 7.23 (m, 2H), 7.08 (t, *J* = 7.3 Hz, 1H), 4.57 (m, 2H), 3.09 (dt, *J* = 5.5, 7.7 Hz, 1H), 1.86 (dd, *J* = 3.1, 7.7 Hz, 1H), 1.77 (dd, *J* = 3.2, 5.5 Hz, 1H); IR (film) ν_{\max} 3289, 2927, 1668, 1652, 1557, 1520, 1409, 1313 cm⁻¹; FABHRMS (NBA/CsI) *m/z* 499.1752 (C₃₁H₂₂N₄O₃ + H⁺ requires
 25 499.1770). (-)-(8a*S*,9a*S*)-50: $[\alpha]_D^{25}$ -21 (*c* 0.10, DMF); (+)-(8a*R*,9a*R*)-50: $[\alpha]_D^{25}$ +21 (*c* 0.06, DMF).

1-[(3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl)carbonyl]-3-chloromethyl-4-hydroxy-2,3-dihydro-1*H*-benzo[*f*]indole (51): Flash
 30 chromatography (SiO₂, 1.5 × 10 cm, 10–50% DMF/toluene gradient) afforded pure

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51 (80%) as a yellow solid: ^1H NMR (DMF- d_7 , 400 MHz) δ 11.64 (s, 1H), 10.32 (s, 1H), 8.28 (s, 1H), 8.25 (d, $J = 8.4$ Hz, 1H), 8.16 (d, $J = 8.9$ Hz, 1H), 7.81 (d, $J = 8.1$ Hz, 1H), 7.46 (m, 1H), 7.39 (m, 2H), 7.15 (d, $J = 1.7$ Hz, 1H), 6.13 (s, 2H), 4.82 (m, 1H), 4.68 (dd, $J = 2.7, 10.8$ Hz, 1H), 4.30 (m, 1H), 4.15 (m, 3H), 3.97 (dd, $J = 8.3, 10.8$ Hz, 1H), 3.39 (m, 2H); IR (film) ν_{max} 3337, 2924, 1662, 1652, 1513, 1456, 1441, 1400, 1344, 1272 cm^{-1} ; ESIMS m/z 461 ($\text{M} + \text{H}^+$, $\text{C}_{25}\text{H}_{21}\text{ClN}_4\text{O}_3$ requires 461). (-)-(3*S*)-51: $[\alpha]_{\text{D}}^{25} -21$ (c 0.18, DMF); (+)-(3*R*)-51: $[\alpha]_{\text{D}}^{25} +22$ (c 0.13, DMF).

10 2-[(3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl)carbonyl]-
 1,2,9,9a-tetrahydrocyclopropa[*c*]benzo[*f*]indol-8-one (*iso*-CBI-CDPI₁, 52):
 Flash chromatography (SiO_2 , 1.5×10 cm, 30–50% DMF/toluene gradient)
 afforded 52 (81%) as a brown solid: ^1H NMR (DMF- d_7 , 400 MHz) δ 11.60 (s, 1H),
 8.15 (d, $J = 8.8$ Hz, 1H), 7.95 (d, $J = 7.6$ Hz, 1H), 7.66 (t, $J = 7.4$ Hz, 1H), 7.49
 15 (d, $J = 7.8$ Hz, 1H), 7.35 (m, 3H), 7.10 (s, 1H), 6.13 (s, 2H), 4.55 (m, 2H), 4.13 (t,
 $J = 8.8$ Hz, 2H), 3.36 (t, $J = 9.1$ Hz, 2H), 3.09 (m, 1H), 1.85 (dd, $J = 2.9, 7.6$ Hz,
 1H), 1.76 (dd, $J = 3.3, 5.4$ Hz, 1H); IR (film) ν_{max} 3346, 2927, 1667, 1651, 1505,
 1435, 1408, 1343, 1279, 1098 cm^{-1} ; ESIMS m/z 425 ($\text{M} + \text{H}^+$, $\text{C}_{25}\text{H}_{20}\text{N}_4\text{O}_3$ requires
 425). (-)-(8*aS*,9*aS*)-52: $[\alpha]_{\text{D}}^{25} -13$ (c 0.06, DMF); (+)-(8*aR*,9*aR*)-52: $[\alpha]_{\text{D}}^{25} +12$ (c
 20 0.07, DMF).

 1-[[3-[*N*-(3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl)carbonyl]-
 1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl]carbonyl]-3-chloromethyl-4-
 hydroxy-2,3-dihydro-1*H*-benzo[*f*]indole (53): Flash chromatography (SiO_2 ,
 25 1.5×15 cm, 10–50% DMF/toluene gradient) afforded pure 53 (32%) as a red
 solid: ^1H NMR (DMF- d_7 , 400 MHz) δ 11.85 (s, 1H), 11.54 (s, 1H), 10.33 (s, 1H),
 8.39 (m, 1H), 8.30 (s, 1H), 8.26 (d, $J = 8.4$ Hz, 1H), 8.13 (d, $J = 8.9$ Hz, 1H), 7.82
 (d, $J = 8.0$ Hz, 1H), 7.48 (m, 2H), 7.38 (m, 2H), 7.31 (s, 1H), 7.08 (s, 1H), 6.10 (s,
 2H), 4.86 (m, 1H), 4.73 (m, 3H), 4.31 (m, 1H), 4.15 (m, 3H), 3.99 (dd, $J = 8.2,$
 30 10.7 Hz, 1H), 3.55 (m, 2H), 3.39 (m, 2H); IR (film) ν_{max} 3338, 2956, 2927, 1727,

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1659, 1650, 1604, 1510, 1402, 1365, 1286 cm^{-1} ; ESIMS m/z 645/647 ($\text{M} + \text{H}^+$, $\text{C}_{36}\text{H}_{29}\text{ClN}_6\text{O}_4$ requires 645/647). (-)-(3*S*)-53: $[\alpha]_{\text{D}}^{25} -19$ (c 0.10, DMF); (+)-(3*R*)-53: $[\alpha]_{\text{D}}^{25} +21$ (c 0.08, DMF).

5 2-{{3-[*N*-(3-Carbamoyl-1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl)carbonyl]-
1,2-dihydro-3*H*-pyrrolo[3,2-*e*]indol-7-yl}carbonyl}-1,2,9,9a-
tetrahydrocyclopropa[*c*]benzo[*f*]indol-8-one (*iso*-CBI-CDPI₂, 54): Flash
chromatography (SiO_2 , 1.5×10 cm, 50% DMF/toluene) afforded 53 (59%) as a
brown solid: ^1H NMR ($\text{DMF-}d_7$, 400 MHz) δ 11.80 (s, 1H), 11.53 (s, 1H), 8.37 (m,
10 1H), 8.13 (d, $J = 8.9$ Hz, 2H), 7.65 (m, 1H), 7.49 (d, $J = 7.8$ Hz, 1H), 7.47 (d, $J =$
9.1 Hz, 1H), 7.36 (m, 3H), 7.24 (d, $J = 1.5$ Hz, 1H), 7.07 (s, 1H), 6.12 (s, 2H),
4.74 (t, $J = 8.4$ Hz, 2H), 4.58 (m, 2H), 4.14 (t, $J = 8.9$ Hz, 2H), 3.52 (m, 2H), 3.38
(t, $J = 8.8$ Hz, 2H), 3.11 (m, 1H), 1.87 (dd, $J = 3.1, 7.8$ Hz, 1H), 1.78 (dd, $J = 3.1,$
15 5.5 Hz, 1H); IR (film) ν_{max} 3293, 2926, 1665, 1612, 1581, 1503, 1431, 1409, 1363,
1344, 1278, 1185 cm^{-1} ; ESIMS m/z 607 ($\text{M} - \text{H}^+$, $\text{C}_{36}\text{H}_{28}\text{N}_6\text{O}_4$ requires 607). (-)-
(8a*S*,9a*S*)-54: $[\alpha]_{\text{D}}^{25} -34$ (c 0.05, DMF); (+)-(8a*R*,9a*R*)-54: $[\alpha]_{\text{D}}^{25} +33$ (c 0.03,
DMF).

Solvolysis Regioselectivity: 1-(*tert*-Butyloxycarbonyl)-4-hydroxy-3-
20 **(methoxymethyl)-2,3-dihydro-1*H*-benzo[*f*]indole (62):** A solution of 31 (7.7
mg, 0.026 mmol) in 2.5 mL of CH_3OH was cooled to 0 $^\circ\text{C}$, and $\text{CF}_3\text{SO}_3\text{H}$ in
 CH_3OH (311 μL , 0.01 N, 0.12 equiv) was added. After slowly warming to 25 $^\circ\text{C}$
over 17 h, the reaction was quenched by the addition of NaHCO_3 (2.1 mg), filtered
through Celite and concentrated under reduced pressure. Flash chromatography
25 (SiO_2 , 1.5×15 cm, 0–40% EtOAc/hexane gradient) yielded the major isomer 62
(8.0 mg, 94%) as a white film: ^1H NMR (CDCl_3 , 400 MHz) δ 9.05 (s, 1H), 8.12
(d, $J = 6.4$ Hz, 1H), 7.80 (m, 1H), 7.65 (s, 1H), 7.37 (m, 1H), 7.29 (m, 1H), 4.17
(m, 1H), 3.83 (m, 1H), 3.77 (dd, $J = 2.8, 6.4$ Hz, 1H), 3.60 (dd, $J = 6.8, 8.8$ Hz,
1H), 3.54 (s, 3H), 3.49 (m, 1H), 1.56 (s, 9H); IR (film) ν_{max} 3233, 2975, 1704,
30 1644, 1435, 1348, 1148, 1026, 956 cm^{-1} ; FABHRMS (NBA/NaI) m/z 352.1535

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(C₁₉H₂₃NO₄ + Na⁺ requires 352.1525).

For the minor isomer **63** (<2% by ¹H NMR of the crude reaction mixture): ¹H NMR (CDCl₂, 500 MHz) δ 8.10 (m, 1H), 7.95 (d, *J* = 6.5 Hz, 1H), 7.73 (m, 1H), 7.38 (m, 1H), 7.33 (m, 1H), 7.24 (m, 1H), 4.13 (m, 1H), 3.98 (s, 3H), 3.92 (m, 1H), 3.81 (m, 2H), 3.50 (m, 1H), 1.57 (s, 9H); FABHRMS (NBA/NaI) *m/z* 352.1534 (C₁₉H₂₃NO₄ + Na⁺ requires 352.1525).

Preparation of Authentic 62: A solution of **27** (17.3 mg, 0.05 mmol) in 1.6 mL anhydrous DMF was treated with NaH (4.3 mg, 0.14 mmol) in small portions and the resulting suspension was stirred for 15 min at 0 °C. Methyl iodide (18 μL, 0.29 mmol) was added neat and the resulting mixture warmed to 25 °C over 2 h. The reaction mixture was quenched with the addition of 5% aqueous NaHCO₃ (10 mL), and the aqueous layer was extracted EtOAc (3 × 5 mL). The combined organic extracts were washed with H₂O (5 × 5 mL), dried (Na₂SO₄) and concentrated under reduced pressure. The crude mixture was subjected to flash chromatography (SiO₂, 1.5 × 15 cm, 10% EtOAc–hexanes) to yield the intermediate methyl ether **64** (14.0 mg, 78%) as a colorless oil. **64** was subjected to the selective MOM deprotection reaction and purification as detailed for **30** to yield the major solvolysis product **62**, identical in all aspects.

Solvolysis Reactivity: *N*-BOC-*iso*-CBI (**31**, 0.2 mg) was dissolved in CH₃OH (1.5 mL) and mixed with pH 3 aqueous buffer (1.5 mL). The buffer contained 4:1:20 (v/v/v) 0.1 M citric acid, 0.2 M Na₂HPO₄, and H₂O, respectively. The solvolysis solution was sealed and kept at 25 °C protected from light. The UV spectrum was measured at regular intervals every 1 h during the first 24 h, every 4 during the next 72 h, and every 12 h for an additional week. The decrease in the long wavelength absorption at 397 nm was monitored, Figure 3. The solvolysis rate constant (*k* = 6.98 × 10⁻⁶ s⁻¹) and the half-life (*t*_{1/2} = 28 h) were calculated from data recorded at the long wavelength from the least-squares treatment (*r* = 0.99) of the slope of the plot of time versus ln[(A_f-A_i)/(A_f-A)] (Figure 9).

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Similarly, *N*-CO₂Me-*iso*-CBI (33, 0.2 mg) was dissolved in CH₃OH (1.5 mL) and mixed with pH 3 aqueous buffer (1.5 mL). The solvolysis solution was sealed and kept at 25 °C protected from light. The UV spectrum was measured at regular intervals every 1 h during the first 24 h, every 4 h during the next 72 h, and every 12 h for an additional week. The decrease in the long wavelength absorption at 395 nm was monitored, Figure 3. The solvolysis rate constant ($k = 6.40 \times 10^{-6} \text{ s}^{-1}$) and the half-life ($t_{1/2} = 30 \text{ h}$) were determined as detailed above ($r = 0.99$).

Similarly, 35 (0.2 mg) was dissolved in CH₃OH (1.5 mL) and mixed with pH 3 aqueous buffer (1.5 mL). The solvolysis solution was sealed and kept at 25 °C protected from light. The UV spectrum was measured at regular intervals every 1 h during the first 12 h, every 2 h for the next 24 h, and every 4 h for an additional day. The decrease in the short wavelength absorption at 239 nm was monitored, Figure 9. The solvolysis rate constant ($k = 3.80 \times 10^{-5} \text{ s}^{-1}$) and the half-life ($t_{1/2} = 5 \text{ h}$) were determined as detailed above ($r = 0.99$).

Isolation, Characterization and Quantitation of the Thermally Released (–)-*iso*-CBI-TMI Adenine Adduct 66: (–)-*iso*-CBI-TMI (44, 1.0 mg, 2.32 μmol) in 500 μL of DMSO was added to a solution of calf thymus DNA (Sigma, 220 mg, *ca.* 150 bp) in 10 mM sodium phosphate buffer (13 mL, pH 7.0) in 50 mL centrifuge tube. The mixture was cooled at 4 °C for 72 h, and then extracted with EtOAc (3 × 10 mL) to remove hydrolyzed, rearranged (65) or unreacted 44. UV and HPLC assay of the EtOAc extracts revealed no unreacted 44 (0%) and <5% rearranged 65. The centrifuge tube containing the aqueous DNA layer was sealed with Teflon tape and warmed at 100 °C for 30 min. The resulting solution was cooled to 25 °C and extracted with EtOAc (3 × 10 mL). The combined organic layer was dried (Na₂SO₄) and concentrated under reduced pressure to afford a yellow solid. Chromatography (SiO₂, 0.5 × 3 cm, 0–7% CH₃OH/CHCl₃ gradient elution) afforded (+)-66 as a pale yellow solid (1.25 mg, 1.31 mg theoretical, 95%, 90–95% in four runs) contaminated with a small impurity (<5% by HPLC). For 66:

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$[\alpha]_D^{23} +28$ (*c* 0.06, CH₃OH); $R_f = 0.3$ (SiO₂, 10% CH₃OH/CHCl₃); ¹H NMR (acetone-*d*₆, 400 MHz) δ 10.37 (s, 1H, N1'-H), 8.58 (s, 1H, ArOH), 8.31 (d, *J* = 7.9 Hz, 1H, Ar-H), 8.22 (s, 1H, Ade-C8-H), 8.07 (s, 1H, Ade-C2-H), 7.78 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.49 (br s, 1H, Ar-H), 7.45 (ddd, *J* = 1.3, 6.8, 7.9 Hz, 1H, Ar-H), 7.38 (ddd, *J* = 1.3, 6.8, 7.9 Hz, 1H, Ar-H), 7.10 (d, *J* = 2.3 Hz, 1H, C3'-H), 6.93 (s, 1H, C5'-H), 4.92 (dd, *J* = 10.6, 1.9 Hz, 1H, CHH-Ade), 4.89 (dd, *J* = 14.5, 7.0 Hz, 1H, CHHNCO), 4.74 (dd, *J* = 14.5, 7.6 Hz, 1H, CHHNCO), 4.73 (dd, *J* = 10.8, 2.2 Hz, 1H, CHH-Ade), 4.37 (m, 1H, CH₂CHCH₂), 4.04 (s, 3H, OCH₃), 3.87 (s, 3H, OCH₃), 3.86 (s, 3H, OCH₃); ¹H NMR (DMSO-*d*₆ + 1% *d*-TFA, 400 MHz) δ 11.40 (s, 1H, N1'-H), 9.28 (br s, 1H, NHH), 8.78 (br s, 1H, NHH), 8.45 (s, 1H, Ade-C8-H), 8.40 (s, 1H, Ade-C2-H), 8.07 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.05 (br s, 1H, ArOH), 7.76 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.43 (dd, *J* = 6.4, 6.4 Hz, 1H, Ar-H), 7.35 (dd, *J* = 6.4, 6.4 Hz, 1H, Ar-H), 6.95 (s, 1H, C3'-H), 6.90 (s, 1H, C5'-H), 4.72 (dd, *J* = 10.8, 5.6 Hz, 1H, CHH-Ade), 4.68 (dd, *J* = 10.8, 5.6 Hz, 1H, CHH-Ade), 4.55 (dd, *J* = 9.0, 9.0 Hz, 1H, CHHNCO), 4.54 (br d, *J* = 9.0 Hz, 1H, CHHNCO), 4.36 (m, 1H), 3.93 (s, 3H, OCH₃), 3.80 (s, 3H, OCH₃), 3.79 (s, 3H, OCH₃); ¹³C NMR (150 MHz, acetone-*d*₆) δ 170.5, 161.0, 156.9, 152.2, 151.2, 151.0, 150.0, 143.9, 142.9, 141.4, 140.0, 136.7, 131.8, 128.4, 127.2, 126.4, 124.7, 124.6, 124.1, 123.0, 114.2, 107.3, 106.1, 98.9, 61.5, 61.4, 57.5, 56.5, 56.4, 39.8; IR (film) ν_{\max} 3251, 2911, 2850, 1684, 1647, 1458, 1312, 1200, 1024 cm⁻¹; UV (CH₃OH) λ_{\max} 331 (ϵ 12400), 300 (ϵ 12600), 240 nm (ϵ 17700); FABHRMS (NBA) *m/z* 566.2074 (*M* + H⁺, C₃₀H₂₇N₇O₅, requires 566.2083).

HPLC t_R (4 × 250 nm column, 1.0 mL/min, 35% CH₃CN/0.05 N aqueous HCO₂NH₄) were 44 (21.6 min), 65 (39.0 min), solvolysis product (24.0 min), and 66 (17.5 min).

Methoxymethyl 3-Nitrophenyl Ether (11): A solution of 3-nitrophenol (5.00 g, 36 mmol) in 100 mL of anhydrous DMF at 0 °C was treated with NaH (2.16 g, 54 mmol) in several portions over 5 min. After 10 min, Bu₄NI (1.33 g, 3.6 mmol) was added and followed by dropwise addition of ClCH₂OCH₃ (4.1 mL, 54 mmol).

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The reaction mixture was stirred at 25 °C for 21 h before being quenched with the slow addition of 100 mL of H₂O. The aqueous layer was extracted with EtOAc (3 × 100 mL). The organic layers were combined, washed with 10% aqueous NaHCO₃ (100 mL), H₂O (4 × 50 mL), dried (Na₂SO₄), and concentrated under reduced pressure. Flash chromatography (SiO₂, 4 × 15 cm, 10% EtOAc/hexane) provided 11 (5.83 g, 89%) as a pale yellow solid: ¹H NMR (CDCl₃, 250 MHz) δ 7.86 (m, 2H), 7.34 (m, 2H), 5.22 (s, 2H), 3.46 (s, 3H); ¹³C NMR (CDCl₃, 62.5 MHz) δ 157.5, 148.9, 129.9, 122.6, 116.5, 110.9, 94.4, 56.1; IR (film) ν_{max} 3099, 2959, 2829, 1619, 1584, 1537, 1349, 1237, 1153, 1081 cm⁻¹; FABHRMS (NBA/NaI) *m/z* 206.0430 (C₈H₉NO₄ + Na⁺ requires 206.0429).

Anal. Calcd for C₈H₉NO₄: C, 52.46; H, 4.95; N, 7.65. Found: C, 52.37; H, 4.89; N, 7.61.

3-Aminophenyl Methoxymethyl Ether (12): A solution of 11 (5.68 g, 31 mmol) in 310 mL moist ether (8:2:1 Et₂O/EtOH/H₂O) was cooled to 0 °C, and treated with freshly prepared Al-Hg (5.2 g Al, 217 mmol) in small 1 × 1 cm pieces. The reaction mixture was stirred vigorously for 0.5 h at 0 °C, and 1 h at 25 °C. The reaction mixture was filtered through Celite, and the Celite was washed thoroughly with Et₂O (5 × 50 mL). The filtrate was washed with saturated aqueous NaCl (300 mL), dried (Na₂SO₄), and concentrated under reduced pressure to afford 12 (4.23 g, 89%) as a golden oil, which was immediately carried on to the next step without further purification. For 12: ¹H NMR (CDCl₃, 250 MHz) δ 7.05 (t, *J* = 8.0 Hz, 1H), 6.38 (m, 3H), 5.12 (s, 2H), 3.70 (br s, 2H), 3.46 (s, 3H); ¹³C NMR (CDCl₃, 250 MHz) δ 158.2, 147.7, 129.9, 108.7, 106.0, 102.9, 94.0, 55.7; IR (film) ν_{max} 3452, 3367, 2955, 2900, 1623, 1601, 1494, 1287, 1147, 1074, 1009 cm⁻¹; FABHRMS (NBA/NaI) *m/z* 153.0786 (C₈H₁₁NO₂ requires 153.0790).

[*N*-(*tert*-Butyloxycarbonyl)amino]-3-(methoxymethoxy)benzene (13): A solution of crude 12 (4.13 g, 27 mmol) in 135 mL anhydrous THF was treated with BOC₂O (12.14 g, 54 mmol) and the reaction mixture was warmed at reflux (65 °C)

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for 18 h. The solvents were removed under reduced pressure, and flash chromatography (SiO₂, 4 × 15 cm, 20% EtOAc/hexane) provided pure **13** (6.83 g, 100%) as a yellow oil: ¹H NMR (CDCl₃, 250 MHz) δ 7.15 (m, 2H), 6.94 (m, 1H), 6.68 (m, 1H), 6.58 (br s, 1H), 5.13 (s, 2H), 3.44 (s, 3H), 1.50 (s, 9H); ¹³C NMR (CDCl₃, 62.5 MHz) δ 157.7, 152.5, 139.5, 129.6, 111.9, 110.7, 106.6, 94.2, 80.5, 55.9, 28.2; IR (film) ν_{max} 3337, 2977, 1728, 1605, 1537, 1236, 1153, 1015 cm⁻¹; FABHRMS (NBA/NaI) *m/z* 276.1203 (C₁₃H₁₉NO₄ + Na⁺ requires 276.1212)

[*N*-(*tert*-Butyloxycarbonyl)amino]-2-iodo-3-(methoxymethoxy)benzene (14):

10 A solution of **13** (124 mg, 0.49 mmol) in 2.0 mL anhydrous THF was cooled to -20 °C and treated with TMEDA (0.26 mL, 1.71 mmol) followed by *n*-BuLi (0.69 mL of a 2.5 M solution in hexanes, 1.71 mmol) in a slow dropwise manner. The resulting gold solution stirred for 4 h at -20 °C. The reaction mixture was treated with 1-chloro-2-iodoethane (0.126 mL, 1.71 mmol) and stirred for 15 min at 25 °C.

15 The reaction was diluted with H₂O (30 mL), extracted with Et₂O (3 × 20 mL), and the combined organic extracts were washed with saturated aqueous NaCl, dried (Na₂SO₄) and concentrated under reduced pressure. Flash chromatography (SiO₂, 2.5 × 10 cm, 0–10% EtOAc/hexane gradient) yielded recovered **13** (51.8 mg, 41%) and **14** (85.5 mg, 46%) as a white solid: mp 82–84 °C; ¹H NMR (CDCl₃, 400 MHz) δ 7.72 (d, *J* = 8.2 Hz, 1H), 7.21 (t, *J* = 6.0 Hz, 1H), 7.01 (br s, 1H), 6.72 (dd, *J* = 1.2, 8.2 Hz, 1H), 5.21 (s, 2H), 3.47 (s, 3H), 1.49 (s, 9H); ¹³C NMR (CDCl₃, 100 MHz) δ 156.0, 152.5, 140.1, 129.5, 113.4, 108.9, 94.8, 82.4, 80.9, 54.4, 28.3; IR (film) ν_{max} 3388, 2977, 1736, 1592, 1515, 1465, 1406, 1252, 1226, 1153, 1005 cm⁻¹; FABHRMS (NBA/CsI) *m/z* 511.9351 (C₁₃H₁₈INO₄ + Cs⁺ requires 511.9335).

25

Anal. Calcd for C₁₃H₁₈INO₄: C, 41.18; H, 4.78; N, 3.69. Found: C, 41.19; H, 5.11; N, 3.79.

[*N*-(*tert*-Butyloxycarbonyl)-*N*-(2-propen-1-yl)amino]-2-iodo-3-

30 **(methoxymethoxy)benzene (15):** A solution of **14** (141 mg, 0.37 mmol) in 12

- 57 -

mL anhydrous DMF was cooled to $-10\text{ }^{\circ}\text{C}$, and treated with NaH (22.3 mg, 0.55 mmol) in small portions. The resulting suspension was stirred for 15 min and treated with neat allyl bromide (0.16 mL, 1.56 mmol) in a slow dropwise manner. The reaction mixture was warmed to $25\text{ }^{\circ}\text{C}$ and stirred for 1 h. The reaction mixture was quenched with the addition of 5% aqueous NaHCO_3 (20 mL), and the aqueous layer was extracted with EtOAc ($3 \times 10\text{ mL}$). The combined organic extracts were washed with H_2O ($5 \times 10\text{ mL}$), dried (Na_2SO_4), and condensed under reduced pressure to yield 15 as a 2:1 mixture of amide rotamers as a yellow oil. Flash chromatography (SiO_2 , $2.5 \times 10\text{ cm}$, 10% EtOAc/hexane) yielded 15 (149 mg, 96%) as a colorless oil: $^1\text{H NMR}$ (CDCl_3 , 250 MHz) δ 7.17 (m, 1H), 6.95–6.78 (m, 2H), 5.98–5.84 (m, 1H), 5.21 (s, 2H), 5.09–5.03 (m, 2H), 4.46 (m, 1H), 3.50 (s, 3H), 1.50 and 1.31 (s, 9H); $^{13}\text{C NMR}$ (CDCl_3 , 100 MHz) δ 157.5 and 157.0, 153.7, 146.1 and 145.7, 133.8 and 133.6, 129.5 and 129.0, 123.6 and 123.5, 117.6 and 117.1, 113.5 and 113.2, 95.0 and 94.1, 80.5 and 80.1, 56.4, 53.1, 51.9, 28.3 and 28.1; IR (film) ν_{max} 2975, 1688, 1582, 1463, 1381, 1253, 1154, 1065, 990 cm^{-1} ; FABHRMS (NBA/NaI) m/z 420.0663 ($\text{C}_{16}\text{H}_{22}\text{INO}_4 + \text{H}^+$ requires 420.0672).

1-(*tert*-Butyloxycarbonyl)-4-(methoxymethoxy)-3-[[*(2',2',6',6'*-tetramethylpiperidino)oxy]methyl]-2,3-dihydroindole (16): A solution of 15 (142 mg, 0.33 mmol) and TEMPO (160 mg, 1.0 mmol) in 14.3 mL anhydrous benzene was treated with Bu_3SnH (96 μL , 0.35 mmol). The solution was warmed at $50\text{ }^{\circ}\text{C}$ and an additional 1.05 equiv of Bu_3SnH (96 μL , 0.35 mmol) was added twice during the next 30 min. Another 3.0 equiv of TEMPO (160 mg, 1.0 mmol) was added in 3 mL anhydrous benzene, and an additional 1.05 equiv of Bu_3SnH added twice during the next 45 min. After 1.5 h total, the solution was cooled to $25\text{ }^{\circ}\text{C}$, and the volatiles were removed under reduced pressure. Flash chromatography (SiO_2 , $2.5 \times 10\text{ cm}$, 0–8% EtOAc/hexane gradient) provided 16 (138 mg, 91%) as a yellow oil: $^1\text{H NMR}$ (CDCl_3 , 250 MHz) δ 7.51 (br s, 1H), 7.11 (m, 1H), 6.67 (d, $J = 8.7\text{ Hz}$, 1H), 5.16 (s, 2H), 4.13 (dd, $J = 3.3, 11.4\text{ Hz}$, 1H), 4.07 (m, 1H), 3.93 (m, 1H), 3.76 (m, 1H), 3.54 (m, 1H), 3.46 (s, 3H), 1.55 (s, 9H), 1.47–0.76 (m, 18H); $^{13}\text{C NMR}$

- 58 -

(C₆D₆, 100 MHz) δ 154.2, 152.2, 145.4, 129.8, 119.0, 109.6, 108.1, 94.4, 79.8, 77.3, 60.0, 55.7, 52.4, 39.9, 33.3, 28.3, 20.1, 17.4; IR (film) ν_{\max} 2974, 2931, 1707, 1609, 1462, 1389, 1250, 1154, 1009 cm⁻¹; FABHRMS (NBA/CsI) m/z 581.1977 (C₂₅H₄₀N₂O₅ + H⁺ requires 581.1992).

5

1-(*tert*-Butyloxycarbonyl)-3-(hydroxymethyl)-4-(methoxymethoxy)-2,3-dihydroindole (17): A solution of 16 (135 mg, 0.30 mmol) in 10 mL 3:1:1 HOAc/H₂O/THF was treated with Zn powder (780 mg, 12.0 mmol) and the resulting suspension was warmed at 70 °C under a reflux condenser and with vigorous stirring for 2 h. The reaction mixture was cooled to 25 °C, and the Zn was removed by filtration through Celite (CH₂Cl₂ wash). The volatiles were removed under reduced pressure, and the resulting residue was dissolved in 15 mL EtOAc and filtered. The solution was concentrated under reduced pressure and subjected to flash chromatography (SiO₂, 2.5 × 10 cm, 30–40% EtOAc/hexane gradient) to provide 16 (84 mg, 87%) as a colorless oil: ¹H NMR (CDCl₃, 400 MHz) δ 7.53 (br s, 1H), 7.11 (m, 1H), 6.67 (d, J = 8.8 Hz, 1H), 5.18 (d, J = 8.6 Hz, 1H), 5.16 (d, J = 8.6 Hz, 1H), 4.02 (dd, J = 10.1, 11.5 Hz, 1H), 3.84 (m, 2H), 3.71 (dd, J = 5.9, 10.4 Hz, 1H), 3.61 (m, 1H), 3.46 (s, 3H), 1.53 (s, 9H); ¹³C NMR (CDCl₃, 100 MHz) δ 153.6, 152.3, 129.7, 110.9, 110.0, 109.0, 107.8, 106.6, 94.2, 64.7, 56.2, 51.3, 41.0, 28.3; IR (film) ν_{\max} 3444, 2975, 1704, 1608, 1463, 1392, 1252, 1153, 1004 cm⁻¹; FABHRMS (NBA/CsI) m/z 442.0642 (C₁₆H₂₃NO₅ + Cs⁺ requires 442.0631).

1-(*tert*-Butyloxycarbonyl)-4-(methoxymethoxy)-3[[[(methanesulfonyl)oxy]methyl]-2,3-dihydroindole (18): A solution of 17 (80 mg, 0.26 mmol) in 5 mL anhydrous CH₂Cl₂ was cooled to 0 °C and treated with Et₃N (79 μ L, 0.57 mmol). After 10 min, MsCl (40 μ L, 0.52 mmol) was added and the reaction mixture was subsequently warmed to 25 °C and stirred for 3 h. The reaction solution was concentrated under reduced pressure. Flash chromatography (SiO₂, 2.5 × 10 cm, 30% EtOAc/hexane) yielded pure 18 (94 mg, 94%) as a colorless oil: ¹H NMR

30

- 59 -

(CDCl₃, 250 MHz) δ 7.49 (m, 1H), 7.15 (m, 1H), 6.68 (d, $J = 9.0$ Hz, 1H), 5.19 (d, $J = 8.8$ Hz, 1H), 5.17 (d, $J = 8.8$ Hz, 1H), 4.58 (dd, $J = 3.6, 9.7$ Hz, 1H), 4.21 (app
t, $J = 8.9$ Hz, 1H), 4.00 (m, 2H), 3.79 (m, 1H), 3.46 (s, 3H), 2.71 (s, 3H), 1.54 (s,
9H); ¹³C NMR (CDCl₃, 100 MHz) δ 153.9, 152.1, 130.5, 110.2, 108.8, 107.7,
5 103.9, 94.4, 81.0, 69.9, 56.2, 51.1, 37.9, 37.2, 28.2; IR (film) ν_{\max} 2976, 1703,
1610, 1479, 1463, 1391, 1355, 1254, 1175, 1154, 1061, 952 cm⁻¹; FABHRMS
(NBA/CsI) m/z 520.0388 (C₁₇H₂₅NO₇S + Cs⁺ requires 520.0406).

DNA Alkylation Studies: Selectivity and Efficiency: Eppendorf tubes
10 containing singly ³²P 5'-end-labeled double-stranded DNA (9 μ L) (Boger, D. L., et
al., *Tetrahedron* 1991, 47, 2661.) in TE buffer (10 mM Tris, 1 mM EDTA, pH
7.5) were treated with the agents in DMSO (1 μ L, at the specified concentrations).
The solutions were mixed by vortexing and brief centrifugation and subsequently
incubated at 4 °C, 25 °C or 37 °C for 24–72 h. The covalently modified DNA was
15 separated from unbound agent by EtOH precipitation of the DNA. The EtOH
precipitations were carried out by adding *t*-RNA as a carrier (1 μ L, 10 μ g/ μ L), 3 M
NaOAc (0.1 volume) and –20 °C EtOH (2.5 volumes). The solutions were mixed
and chilled at –78 °C in a REVCO freezer for 1 h or longer. The DNA was reduced
to a pellet by centrifugation at 4 °C for 15 min and washed with –20 °C 70% EtOH
20 (in TE containing 0.2 M NaCl). The pellets were dried in a Savant Speed Vac
concentrator and resuspended in TE buffer (10 μ L). The solutions of alkylated
DNA were warmed at 100 °C for 30 min to induce cleavage at the adenine N3
alkylation sites. After brief centrifugation, formamide dye solution (5 μ L) was
added. Prior to electrophoresis, the samples were denatured by warming at 100 °C
25 for 5 min, placed in an ice bath, centrifuged briefly, and the supplement (2.8 μ L)
was loaded onto a gel. Sanger dideoxynucleotide sequencing reactions were run as
standards adjacent to the agent treated DNA reaction samples. Polyacrylamide gel
electrophoresis (PAGE) was run on a 8% sequencing gel under denaturing
conditions (19:1 acrylamide: *N,N*-methylenebisacrylamide, 8 M urea) in TBE buffer
30 (100 mM Tris, 100 mM boric acid, 0.2 mM Na₂EDTA). PAGE was pre-run for 30

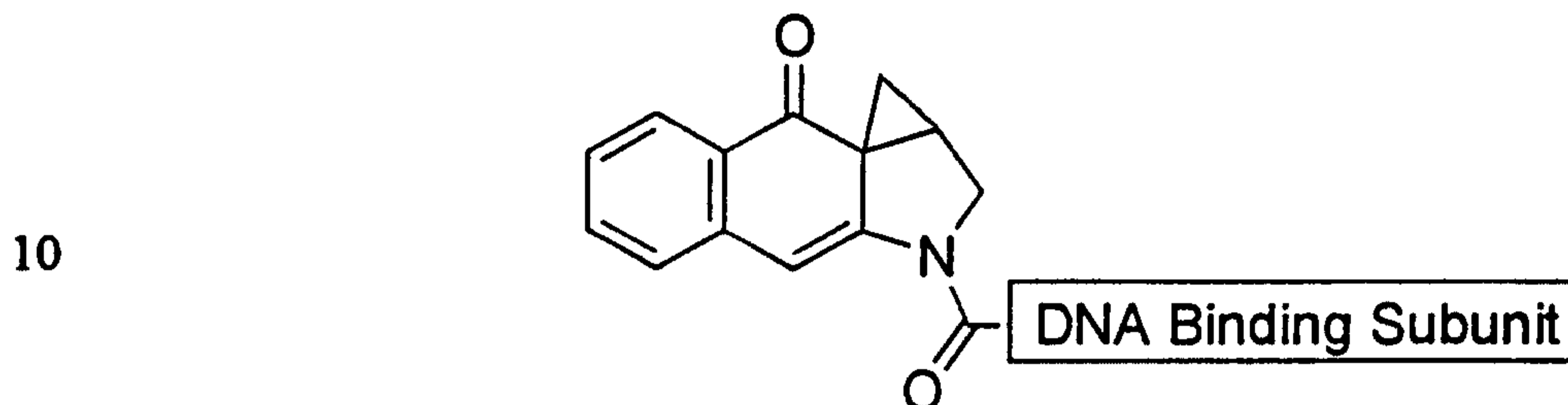
- 60 -

min with formamide dye solution prior to loading the samples. autoradiography of dried gels was carried out at -78°C using Kodak O-Omat AR film and Picker Spectra™ intensifying screen.

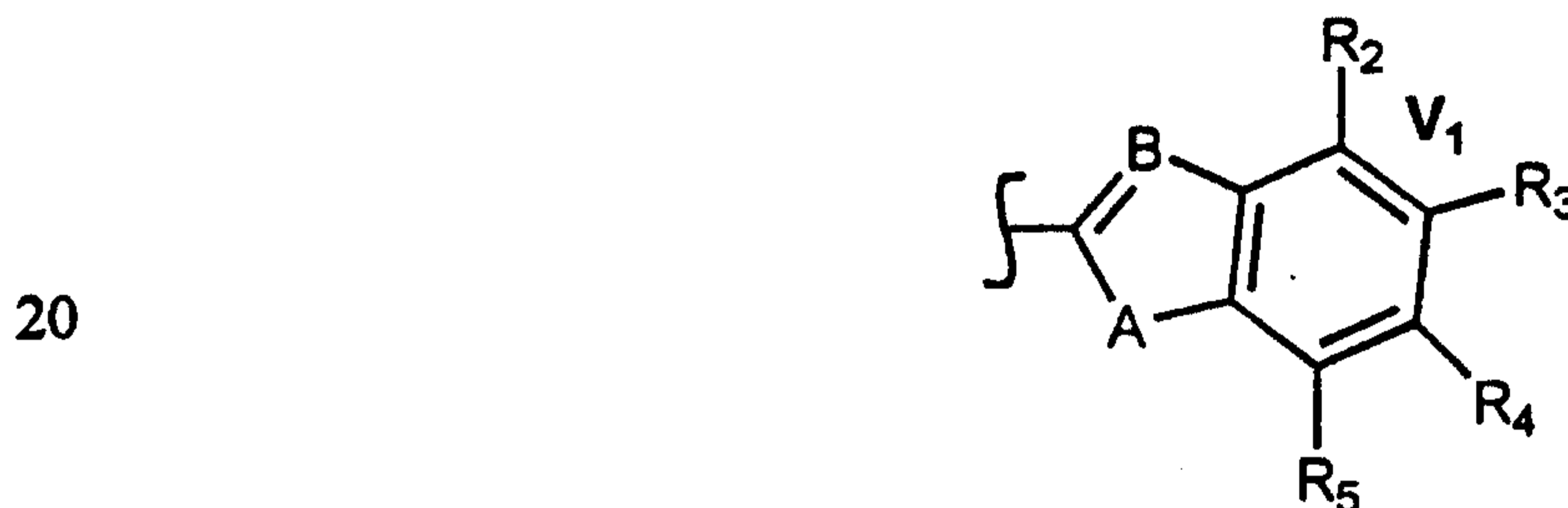
- 61 -

What is claimed:

1. A DNA alkylating compound comprising a DNA
alkylating subunit and a DNA binding subunit covalently
5 linked said DNA binding subunit, said DNA alkylating
compound being represented by the following structure:



2. A DNA alkylating compound as described in claim 1
15 wherein said DNA binding subunit is a radical
represented by the following structure:



wherein:

- 25 **A** is selected from the group consisting of NH and O;
B is selected from the group consisting of C and N;
R₂ is selected from the group consisting of
hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl
(C1-C6)₃, and a first N-substituted pyrrolidine
ring;
30 **R₃** is selected from the group consisting of
hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl
(C1-C6)₃, the first N-substituted pyrrolidine
ring;
35 **R₄** is selected from the group consisting of
hydrogen, hydroxyl, O-alkyl (C1-C6), and N-alkyl

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(C1-C6)₃;

R₅ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), and N-alkyl (C1-C6)₃; and

5 V₁ represents a first vinylene group between R₂ and R₃;

with the following provisos:

10 if R₂ participates in the first N-substituted pyrrolidine ring, then R₃ also participates in the first N-substituted pyrrolidine ring;

15 if R₃ participates in the first N-substituted pyrrolidine ring, then R₂ also participates in the first N-substituted pyrrolidine ring;

if R₂ and R₃ participate in the first N-substituted pyrrolidine ring, then R₄ and R₅ are hydrogen;

20 if R₂ is hydrogen, then R₄ and R₅ are hydrogen and R₃ is N-alkyl (C1-C6)₃; and

wherein the first N-substituted pyrrolidine ring is fused to the first vinylene group between R₂ and R₃ and is represented by the following structure:



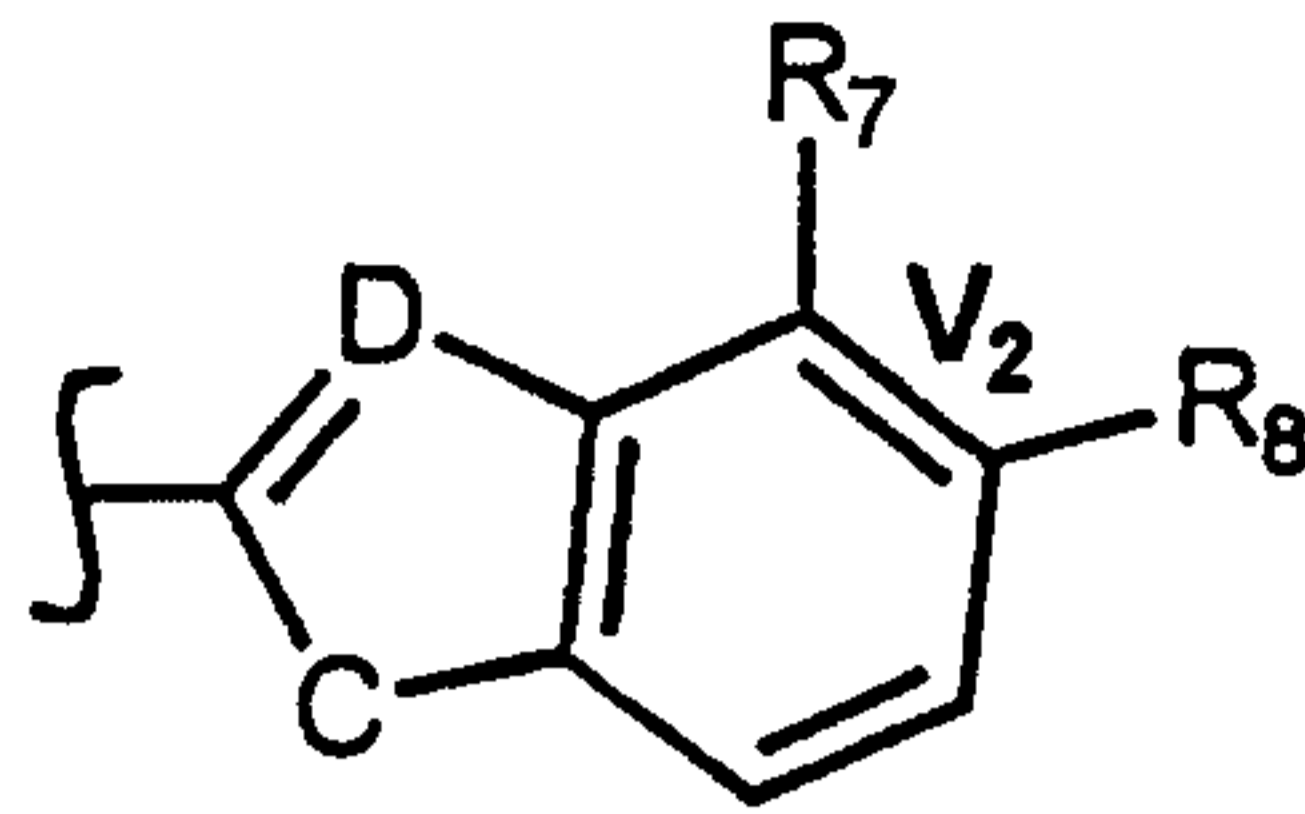
wherein:

30 V₁ represents the first vinylene group between R₂ and R₃;

R₆ is selected from the group consisting of -CH₂CH₃ (alkyl), -NHCH₃ (-N-alkyl), -OCH₃ (O-alkyl), -NH₂, -NHNH₂, -NHNHCO₂^tBu, and a radical represented by the following structure:

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5

wherein:

C is selected from the group consisting of NH and O;

D is selected from the group consisting of C and N;

10

R₇ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, and a second N-substituted pyrrolidine ring;

15

R₈ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, the second N-substituted pyrrolidine ring;

V₂ represents the second vinylene group between R₇ and R₈;

20

with the following provisos:

if R₇ participates in the N-substituted pyrrolidine ring, then R₈ also participates in the N-substituted pyrrolidine ring;

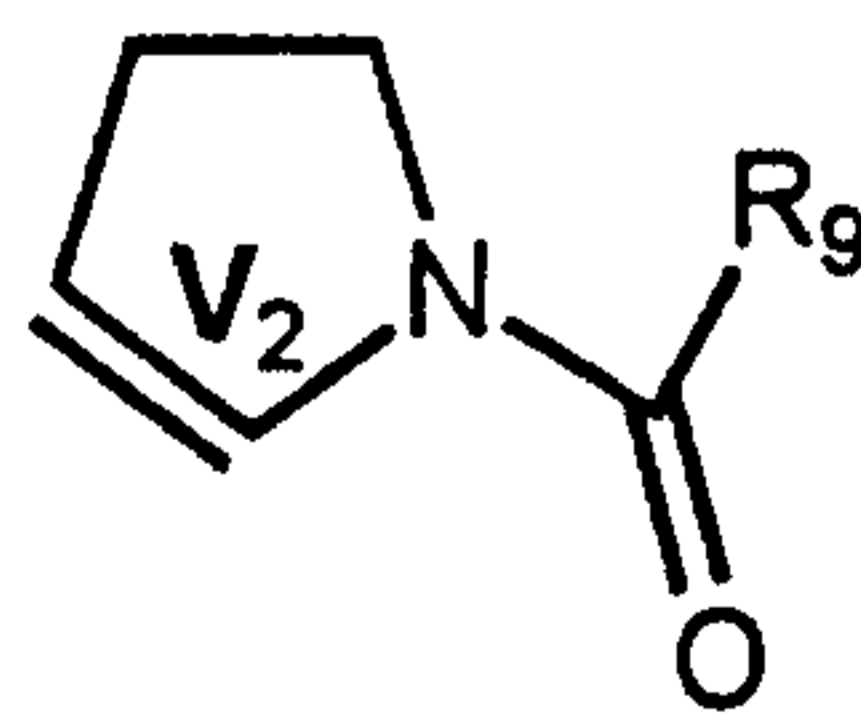
25

if R₈ participates in the N-substituted pyrrolidine ring only if R₇ also participates in the N-substituted pyrrolidine ring; and

wherein the second N-substituted pyrrolidine ring is fused to the second vinylene group between R₇ and R₈ and is represented by the following

30

structure:



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- 64 -

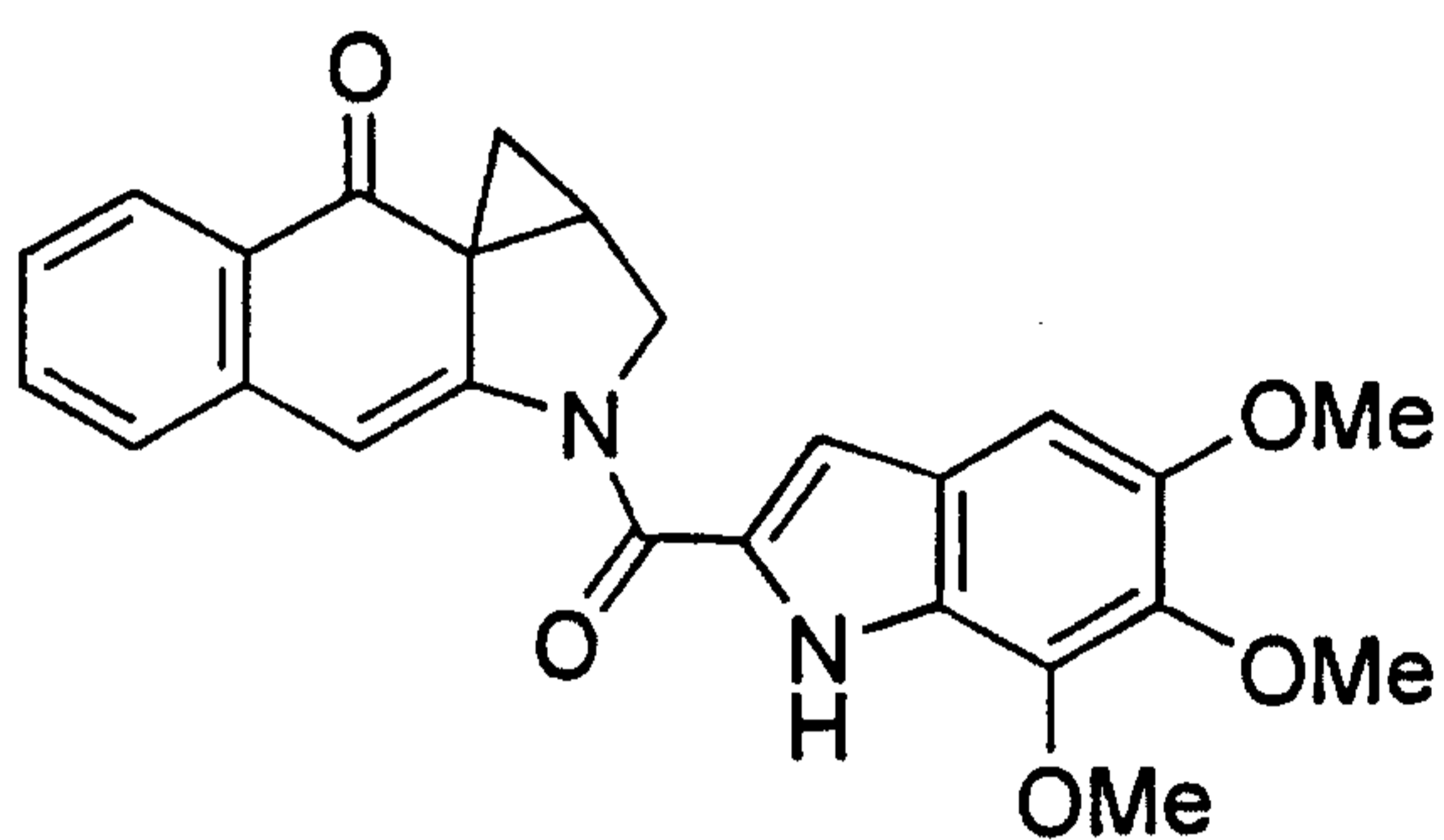
wherein:

V_2 represents the second vinylene group between R_7 and R_8 ;

5 R_9 is selected from the group consisting of $-CH_2CH_3$ (alkyl), $-NHCH_3$ (-N-alkyl), $-OCH_3$ (O-alkyl), $-NH_2$, $-NHNH_2$, and $-NHNHCO_2^tBu$.

3. A DNA alkylating compound as described in claim 2 represented by the following structure:

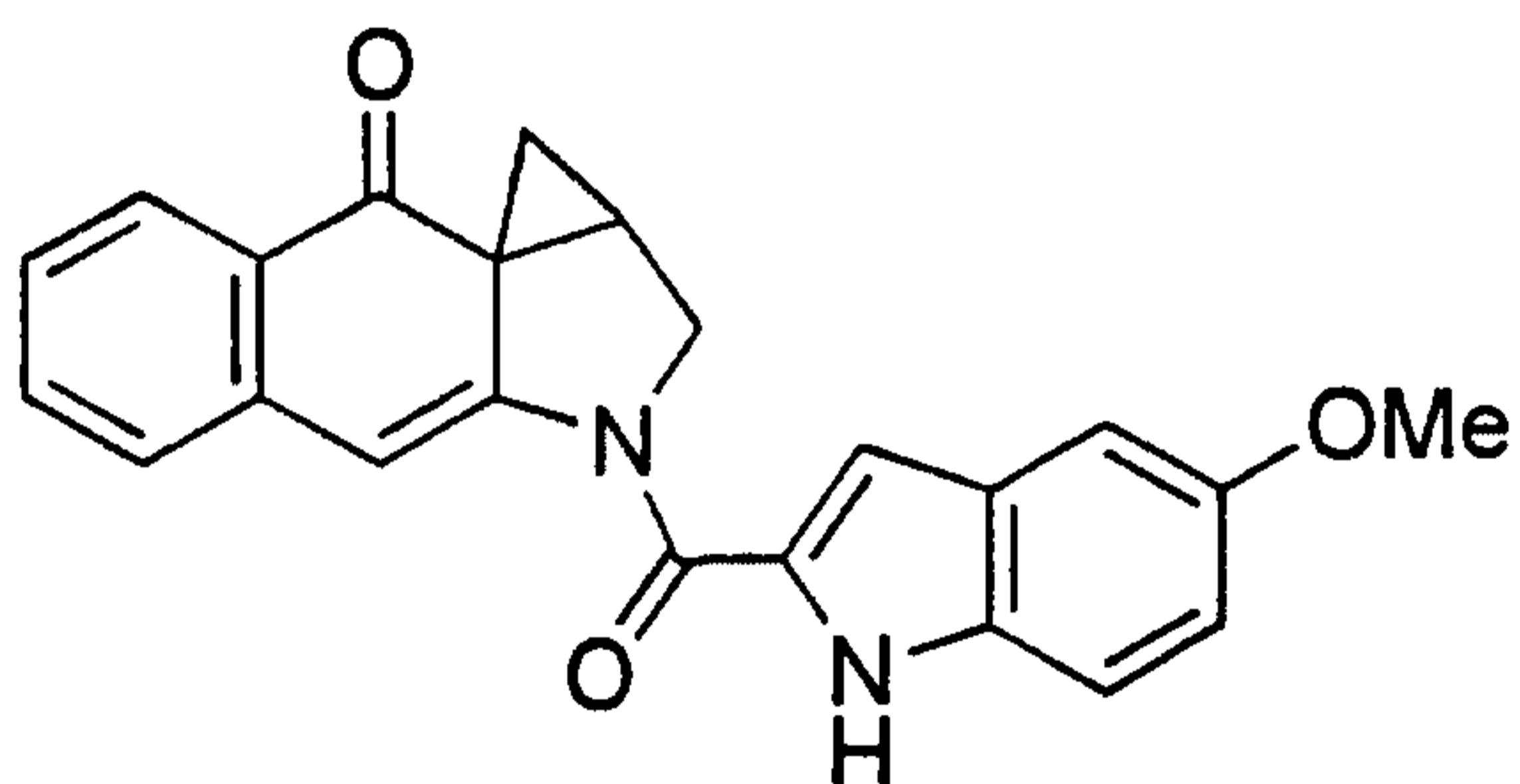
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4. A DNA alkylating compound as described in claim 2 represented by the following structure:

20

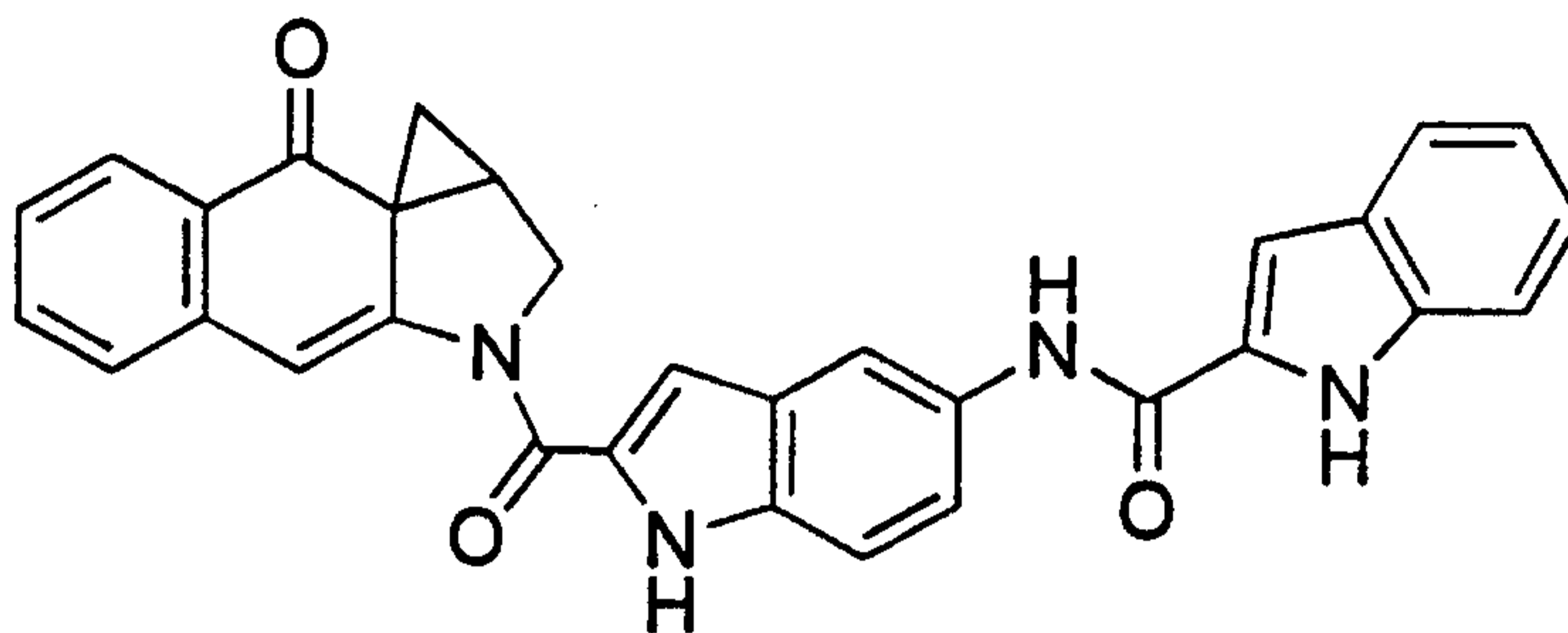


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5. A DNA alkylating compound as described in claim 2 represented by the following structure:

- 65 -

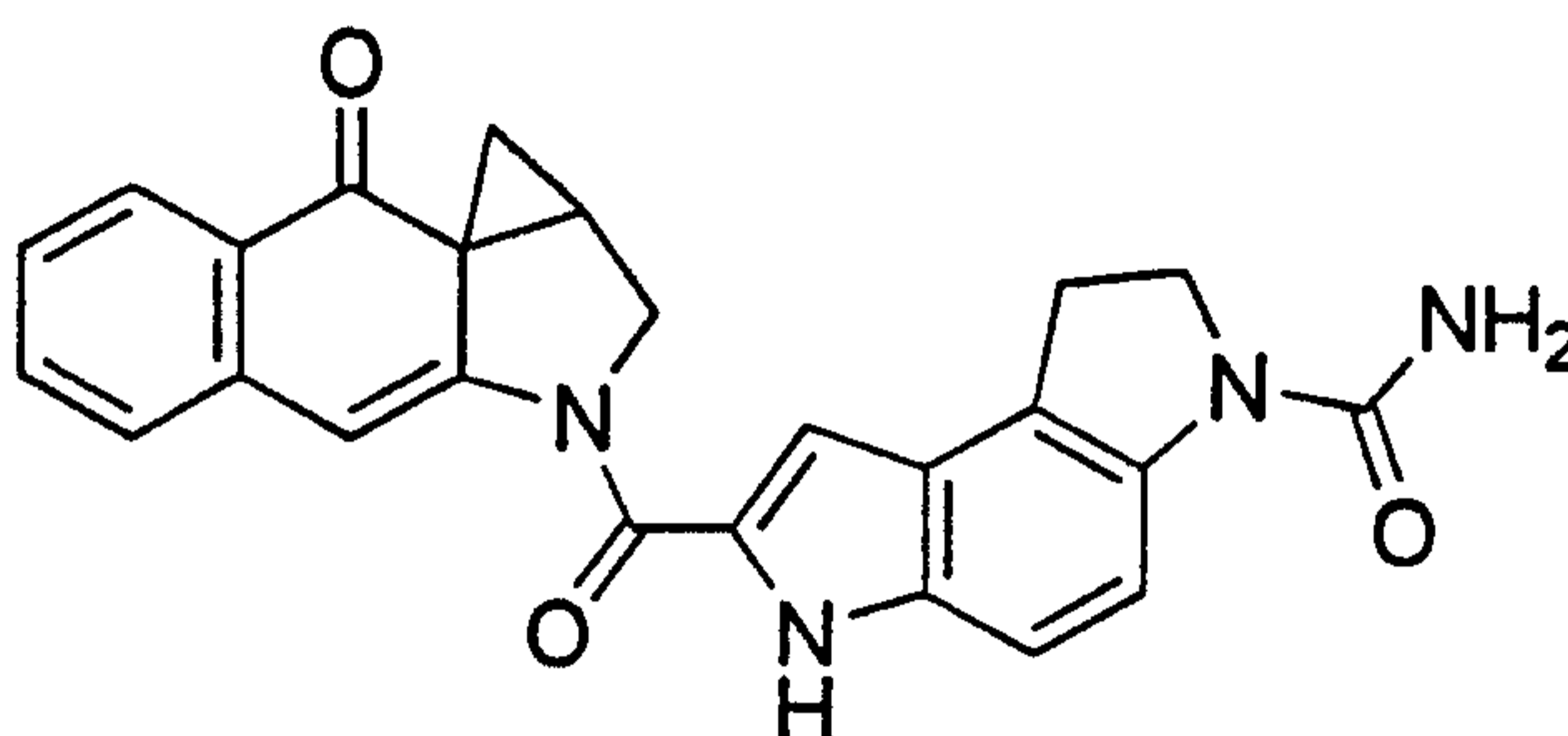
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6. A DNA alkylating compound as described in claim 2 represented by the following structure:

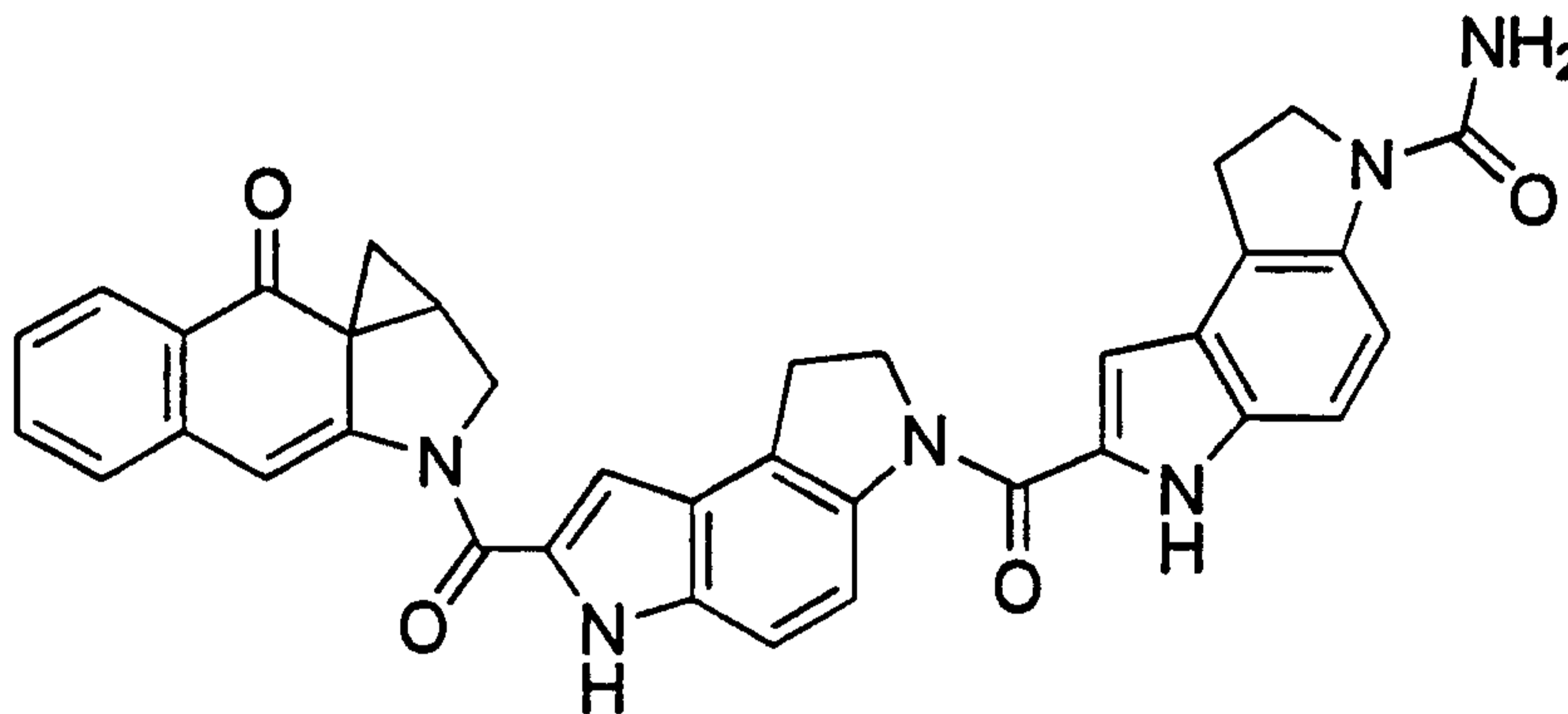
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7. A DNA alkylating compound as described in claim 2 represented by the following structure:

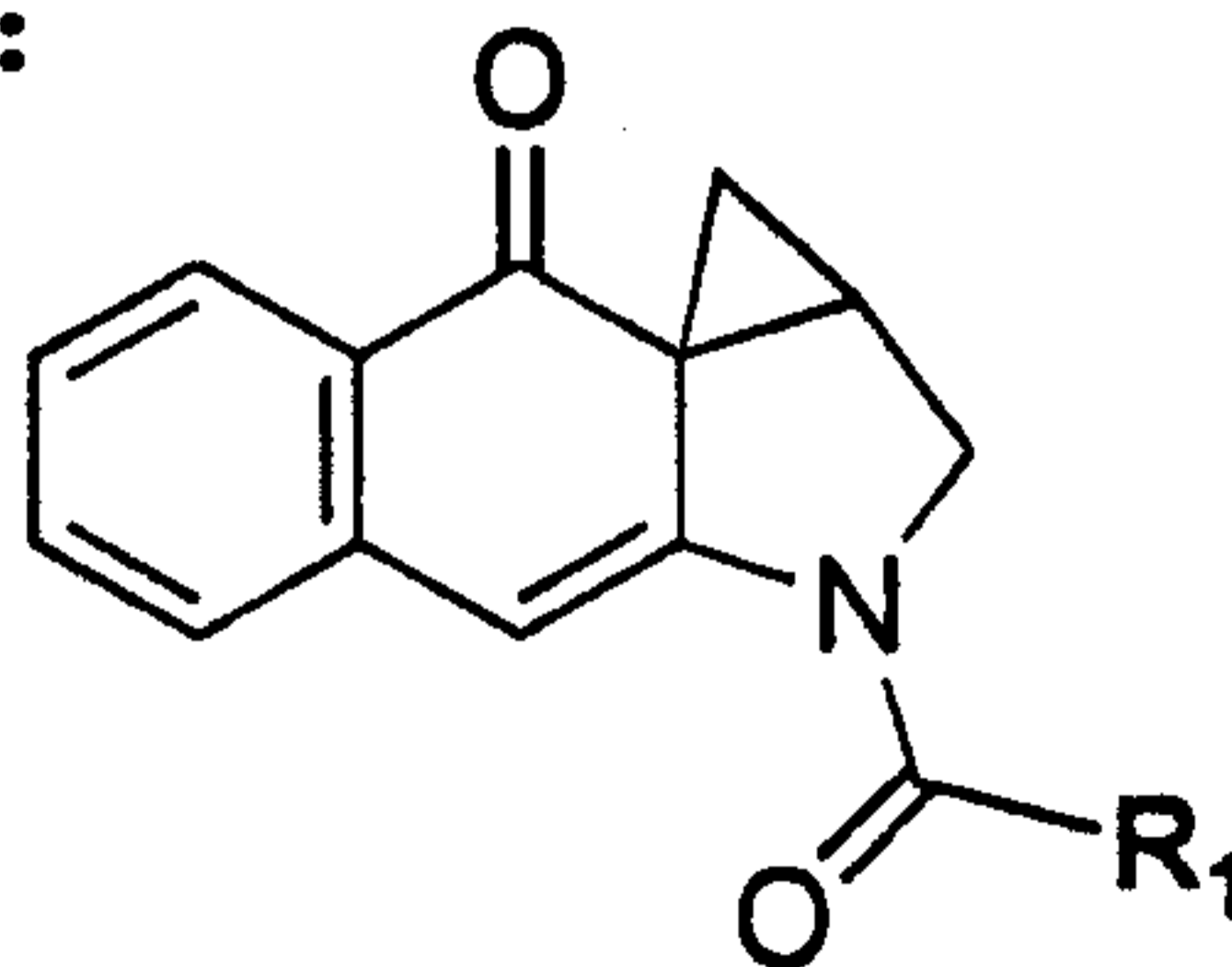
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8. A DNA alkylating compound represented by the following structure:

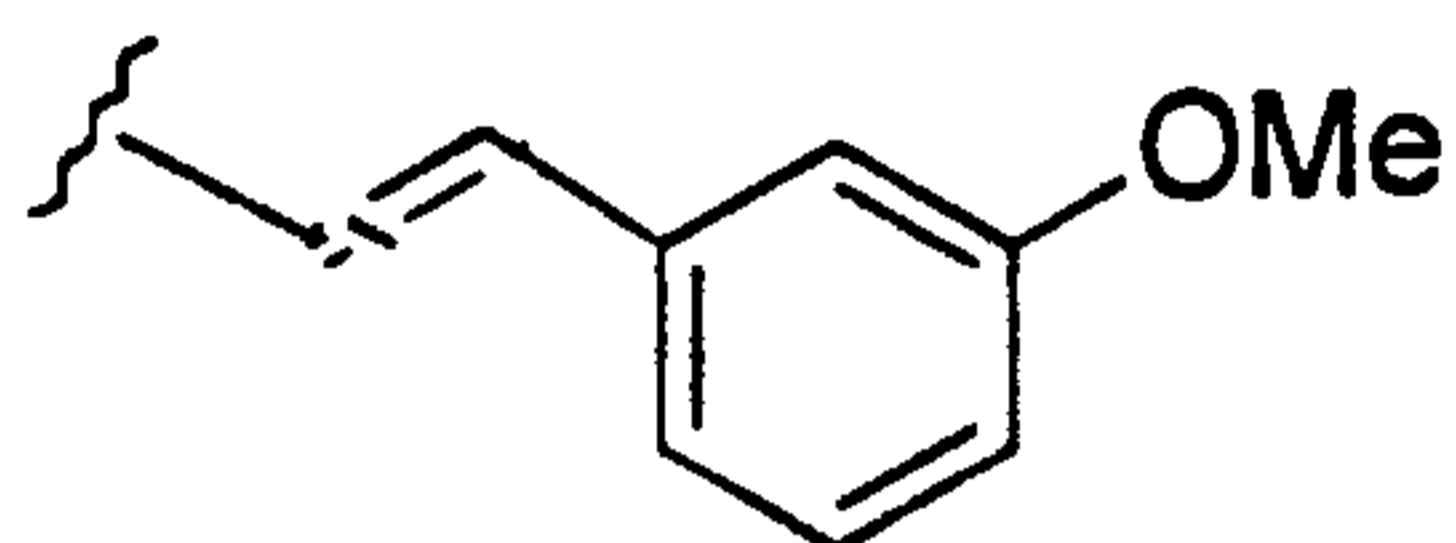
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wherein R₁ is selected from the group consisting of -C₁-C₆ alkyl, -NHCH₃ (-N-alkyl), -OCH₃ (O-alkyl), -NH₂, -NHNH₂, -NHNHCO₂^tBu, and a radical represented by the following structure:

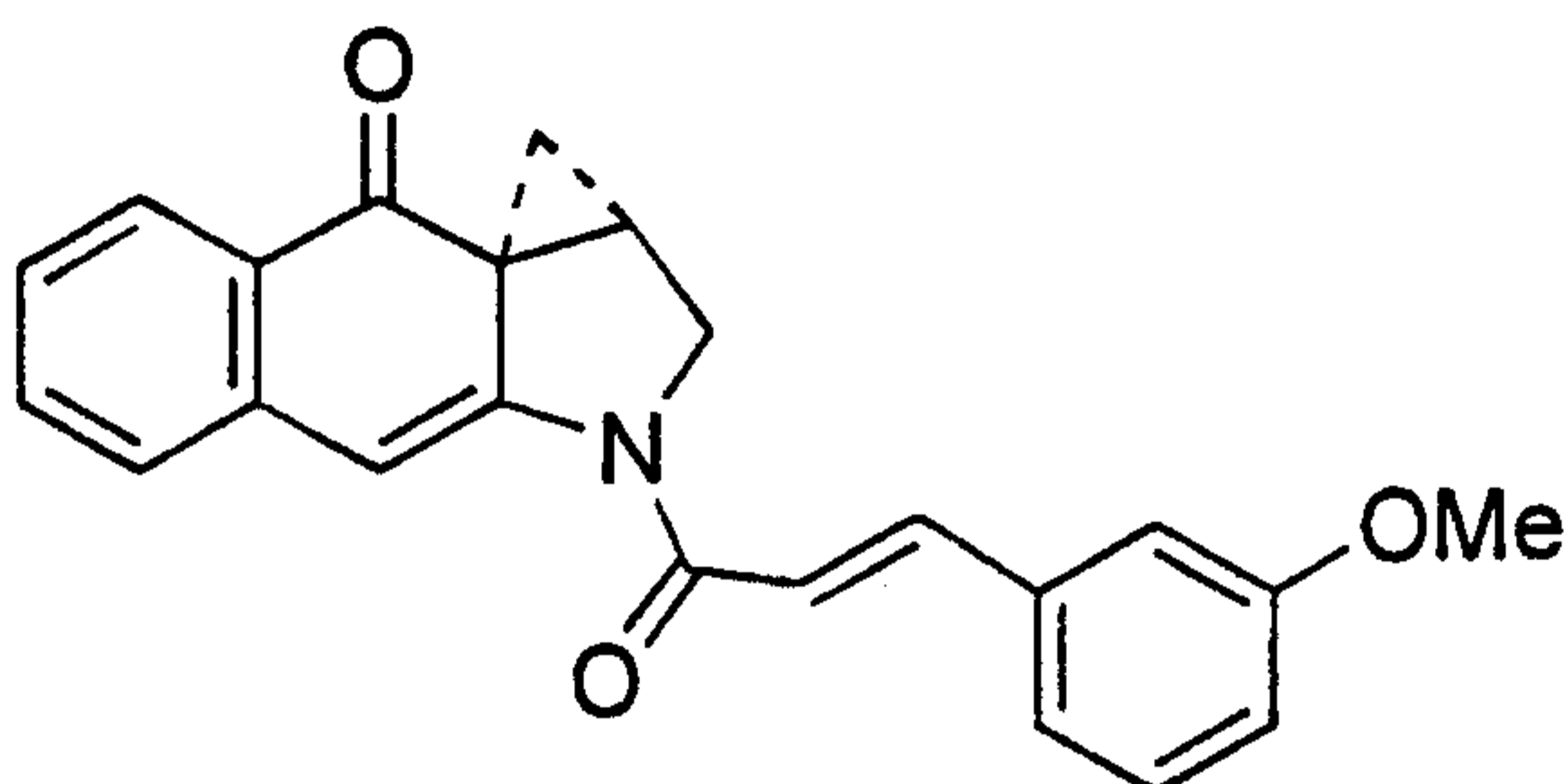
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9. A DNA alkylating compound as described in claim 8 represented by the following structure:

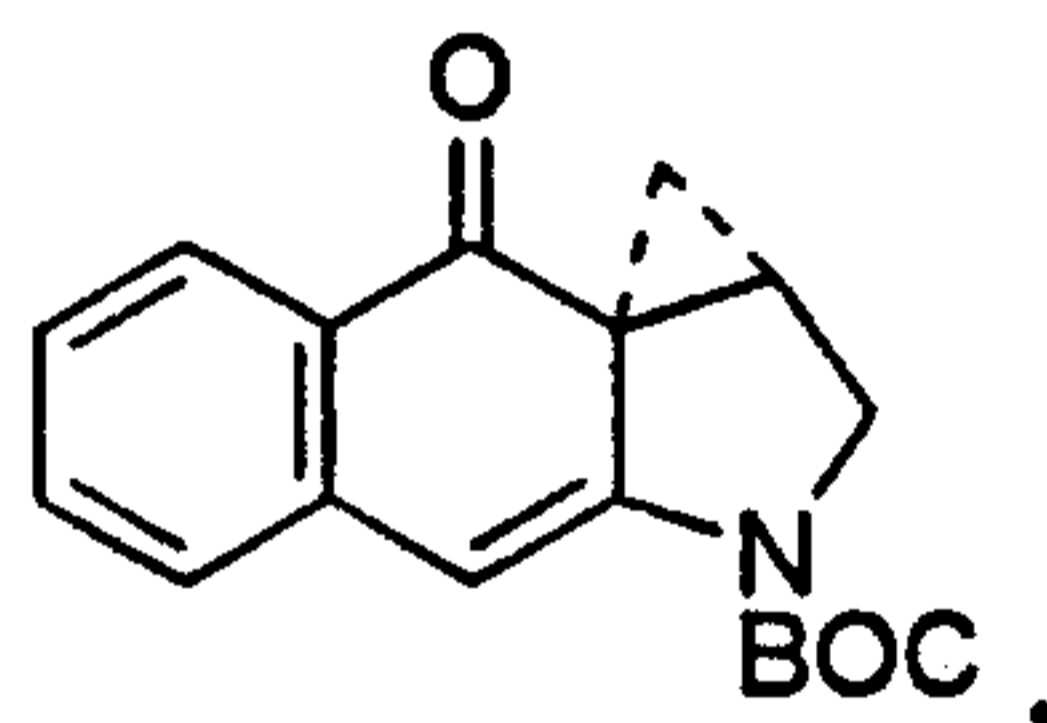
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10. A chemical intermediate represented by the following structure:

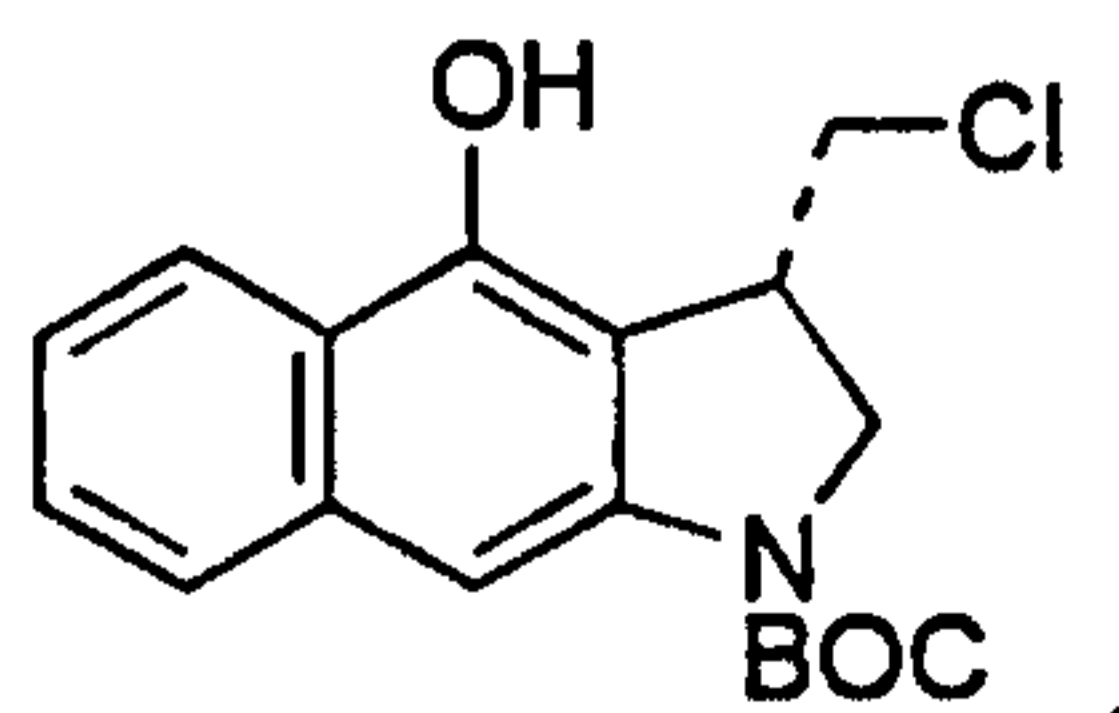
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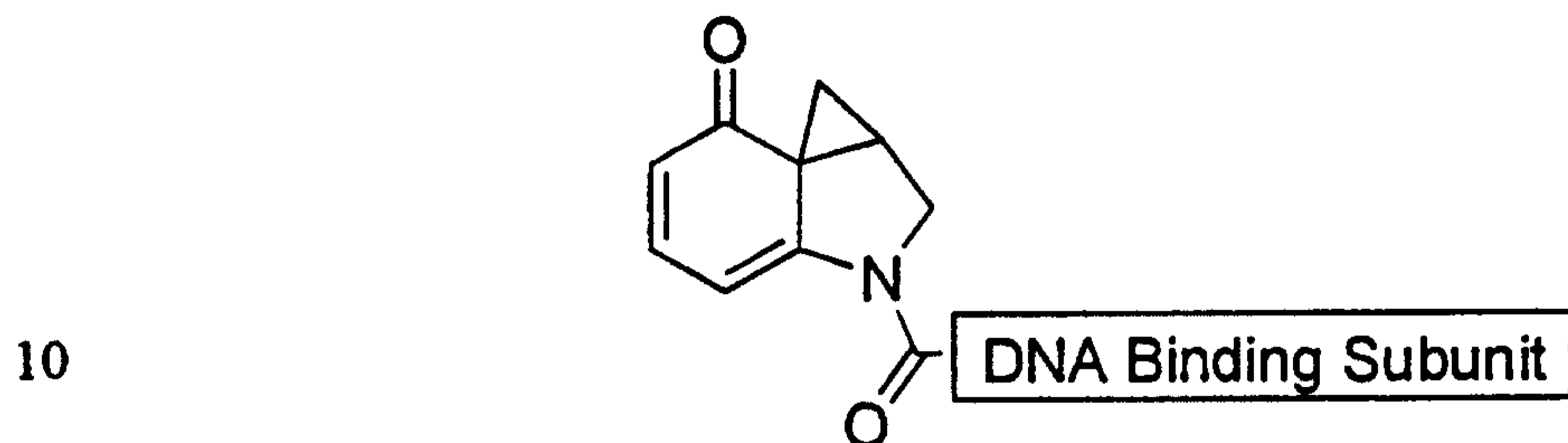
11. A chemical intermediate represented by the following structure:

35

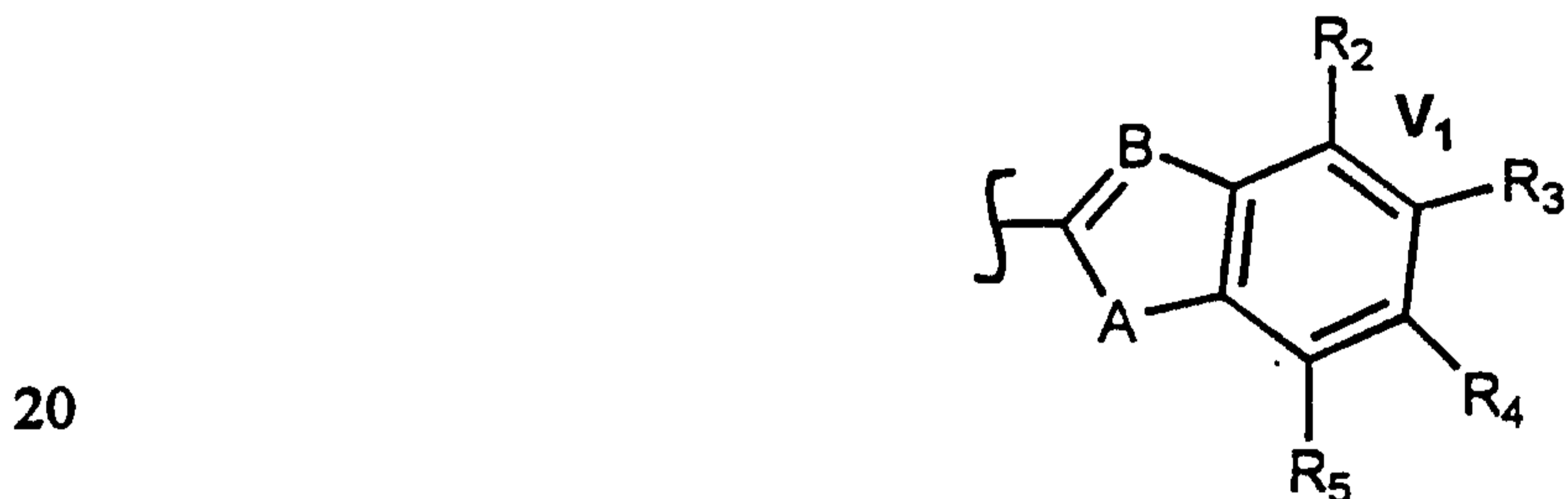


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12. A DNA alkylating compound comprising a DNA
alkylating subunit and a DNA binding subunit covalently
linked said DNA alkylating subunit, said DNA alkylating
5 compound being represented by the following structure:



13. A DNA alkylating compound as described in claim 12
wherein said DNA binding subunit is a radical
15 represented by the following structure:



wherein:

- A is selected from the group consisting of NH and O;
B is selected from the group consisting of C and N;
25 R₂ is selected from the group consisting of
hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl
(C1-C6)₃, and a first N-substituted pyrrolidine
ring;
R₃ is selected from the group consisting of
30 hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl
(C1-C6)₃, the first N-substituted pyrrolidine
ring;
R₄ is selected from the group consisting of
hydrogen, hydroxyl, O-alkyl (C1-C6), and N-alkyl
35 (C1-C6)₃;

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R_5 is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), and N-alkyl (C1-C6)₃; and

V_1 represents a first vinylene group between R_2 and R_3 ;

with the following provisos:

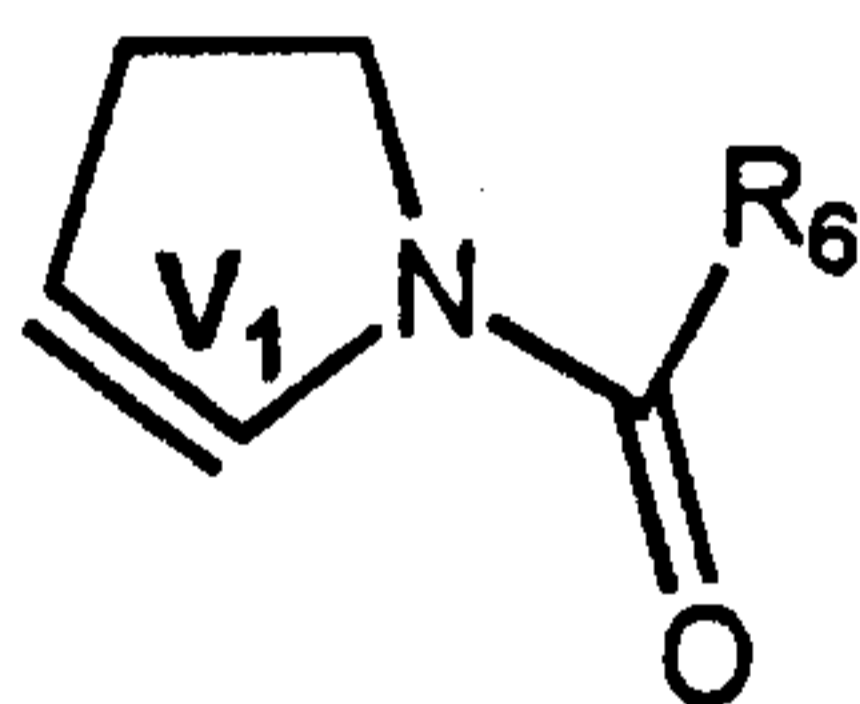
if R_2 participates in the first N-substituted pyrrolidine ring, then R_3 also participates in the first N-substituted pyrrolidine ring;

if R_3 participates in the first N-substituted pyrrolidine ring, then R_2 also participates in the first N-substituted pyrrolidine ring;

if R_2 and R_3 participate in the first N-substituted pyrrolidine ring, then R_4 and R_5 are hydrogen;

if R_2 is hydrogen, then R_4 and R_5 are hydrogen and R_3 is N-alkyl (C1-C6)₃; and

wherein the first N-substituted pyrrolidine ring is fused to the first vinylene group between R_2 and R_3 and is represented by the following structure:



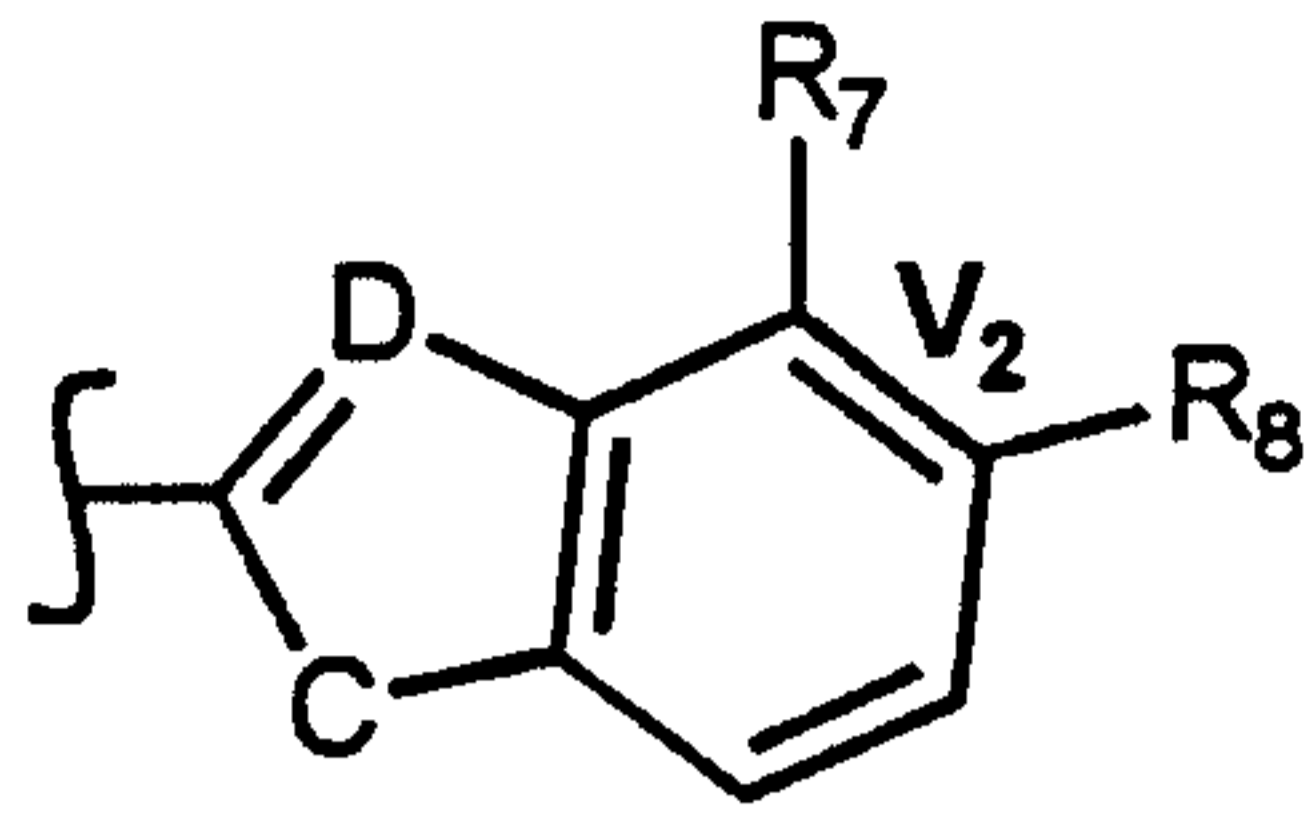
wherein:

V_1 represents the first vinylene group between R_2 and R_3 ;

R_6 is selected from the group consisting of $-CH_2CH_3$ (alkyl), $-NHCH_3$ ($-N$ -alkyl), $-OCH_3$ (O-alkyl), $-NH_2$, $-NHNH_2$, $-NHNHCO_2^tBu$, and a radical represented by the following structure:

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5

wherein:

C is selected from the group consisting of NH and O;

D is selected from the group consisting of C and N;

10

R₇ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, and a second N-substituted pyrrolidine ring;

15

R₈ is selected from the group consisting of hydrogen, hydroxyl, O-alkyl (C1-C6), N-alkyl (C1-C6)₃, the second N-substituted pyrrolidine ring;

V₂ represents the second vinylene group between R₇ and R₈;

with the following provisos:

20

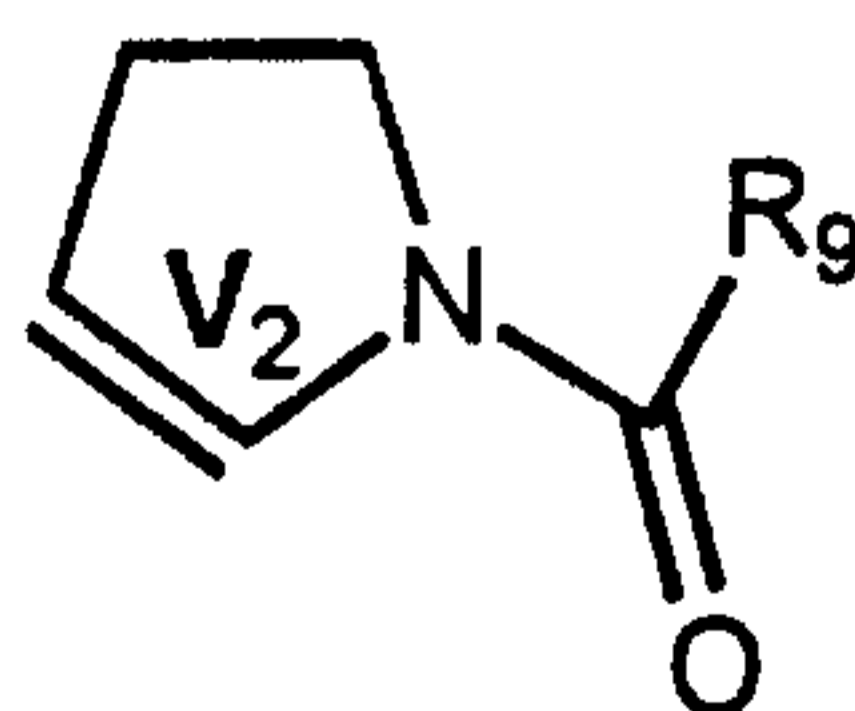
if R₇ participates in the N-substituted pyrrolidine ring, then R₈ also participates in the N-substituted pyrrolidine ring;

if R₈ participates in the N-substituted pyrrolidine ring only if R₇ also participates in the N-substituted pyrrolidine ring; and

25

wherein the second N-substituted pyrrolidine ring is fused to the second vinylene group between R₇ and R₈ and is represented by the following structure:

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- 70 -

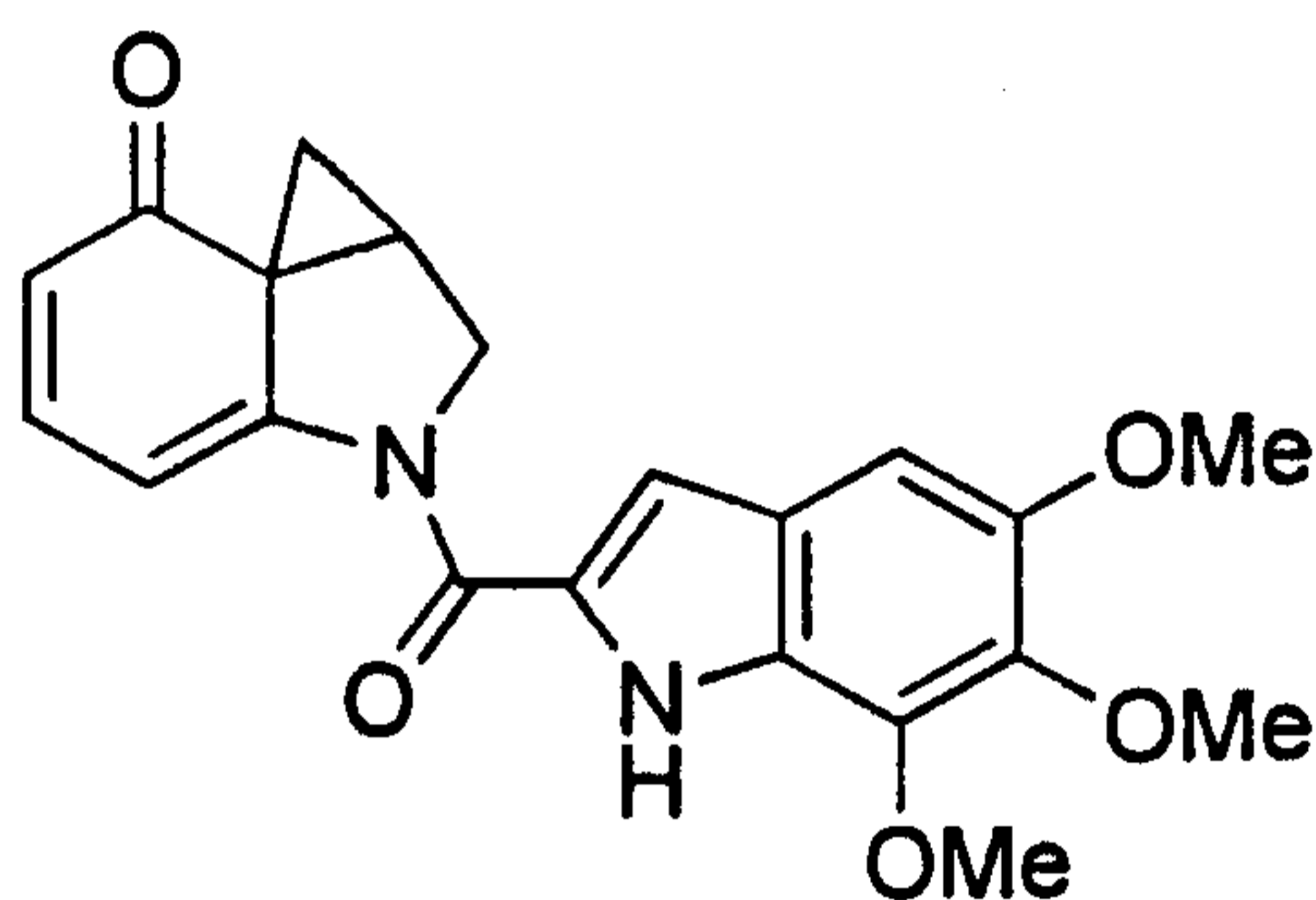
wherein:

V_2 represents the second vinylene group between R_7 and R_8 ;

R_9 is selected from the group consisting of $-CH_2CH_3$ (alkyl), $-NHCH_3$ ($-N$ -alkyl), $-OCH_3$ (O -alkyl), $-NH_2$, $-NHNH_2$, and $-NHNHCO_2^tBu$.

14. A DNA alkylating compound as described in claim 13 represented by the following structure:

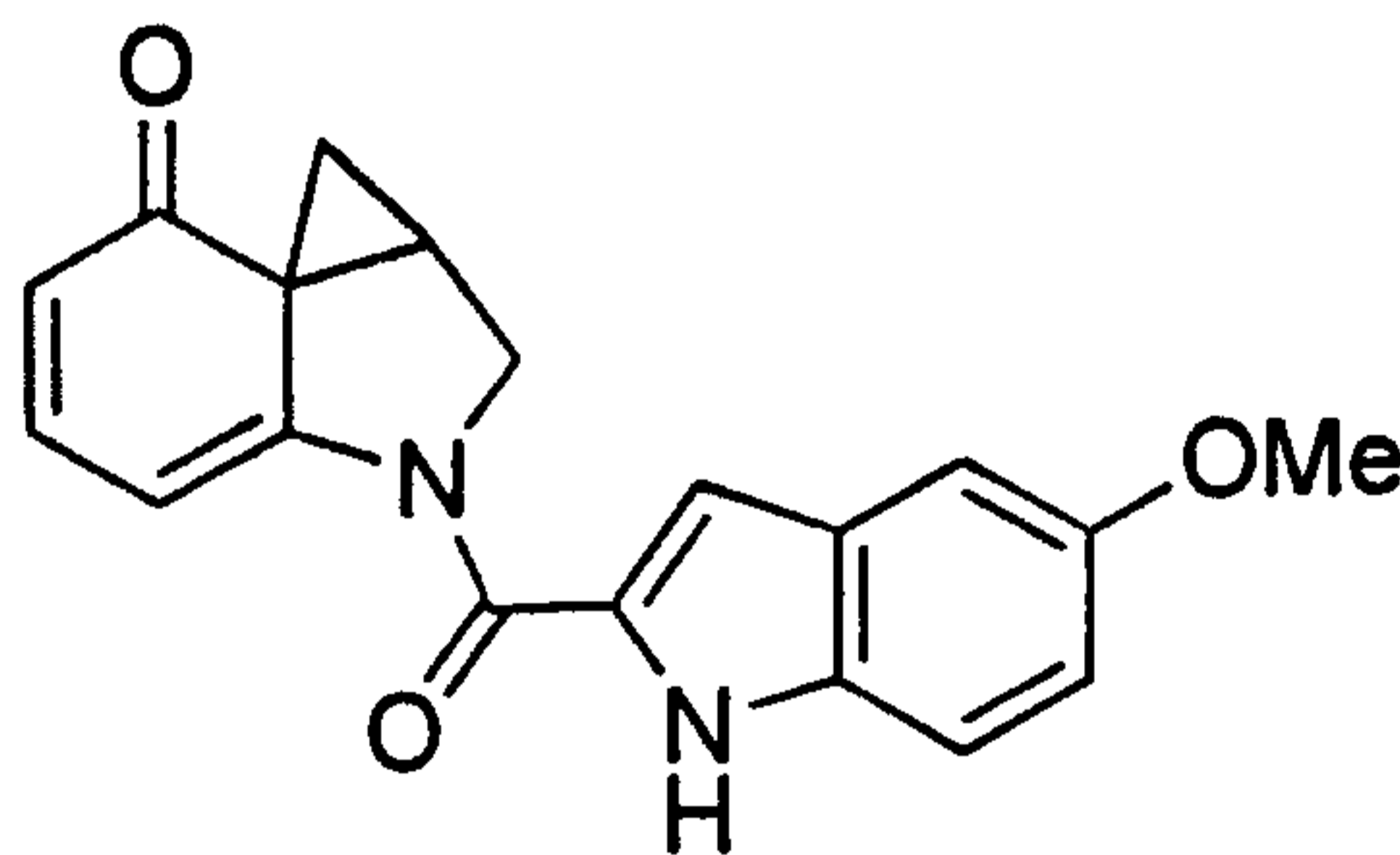
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15. A DNA alkylating compound as described in claim 13 represented by the following structure:

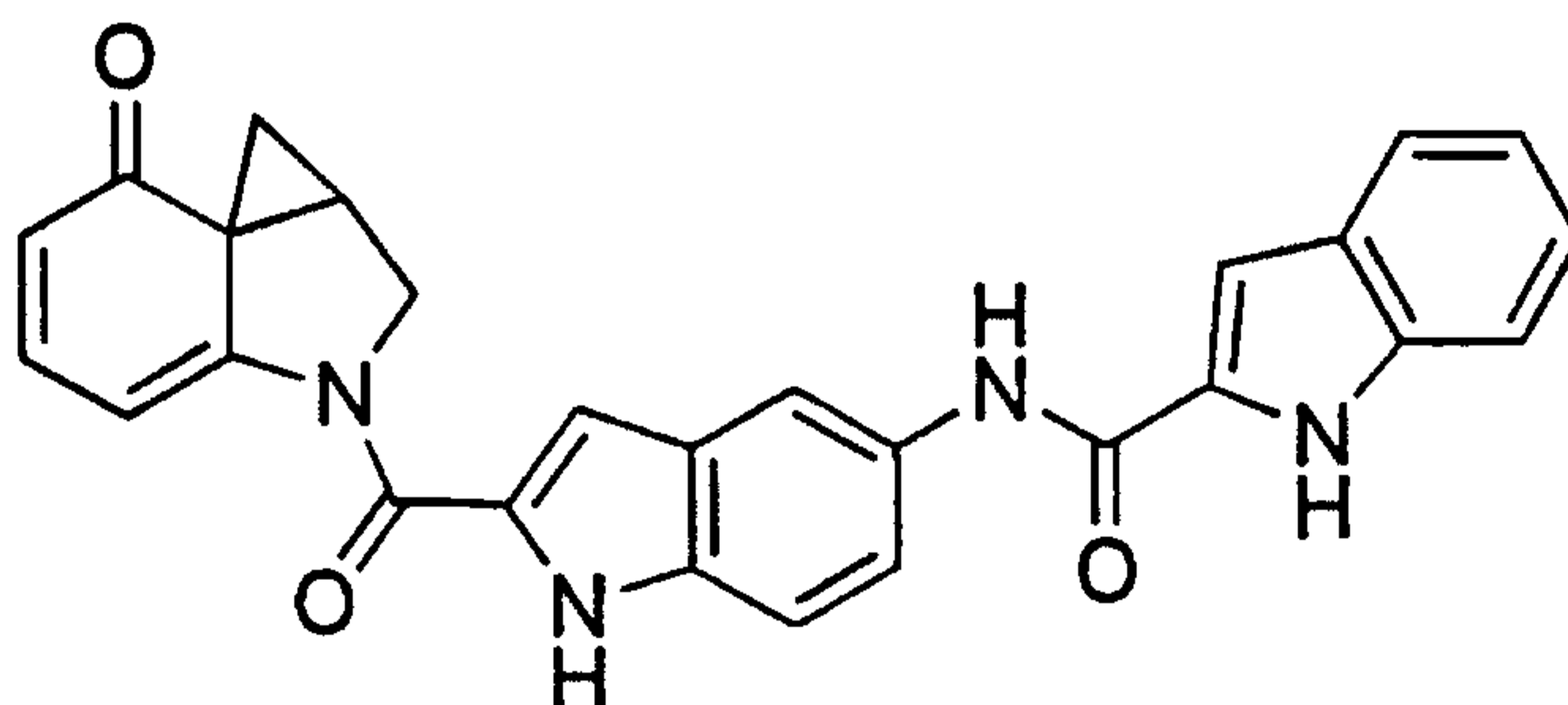
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16. A DNA alkylating compound as described in claim 13 represented by the following structure:

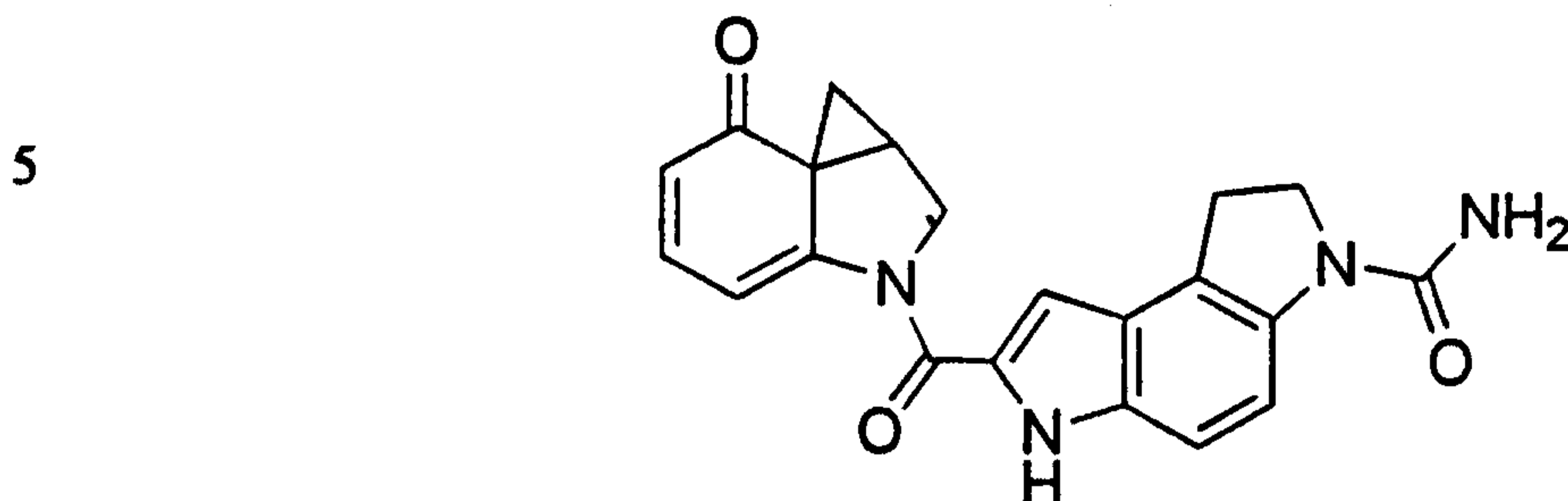
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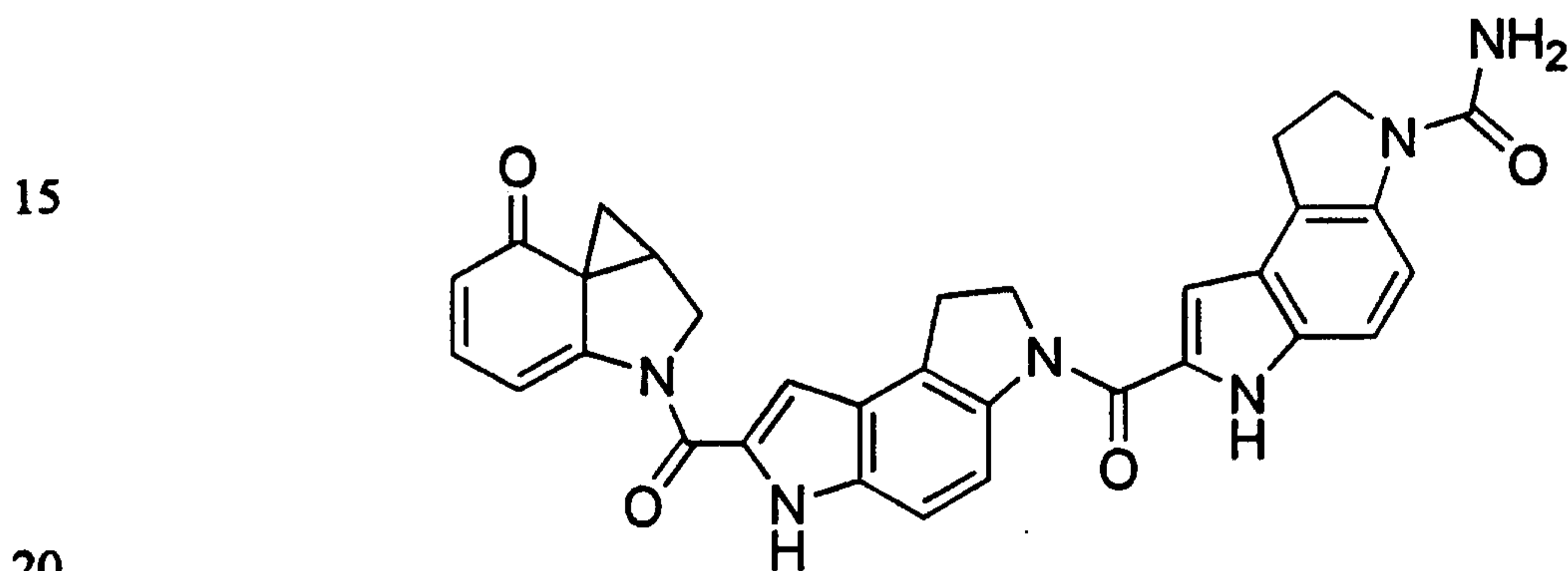
- 71 -

17. A DNA alkylating compound as described in claim 13 represented by the following structure:



10

18. A DNA alkylating compound as described in claim 13 represented by the following structure:



19. A DNA alkylating compound represented by the following structure:



wherein R_1 is selected from the group consisting of

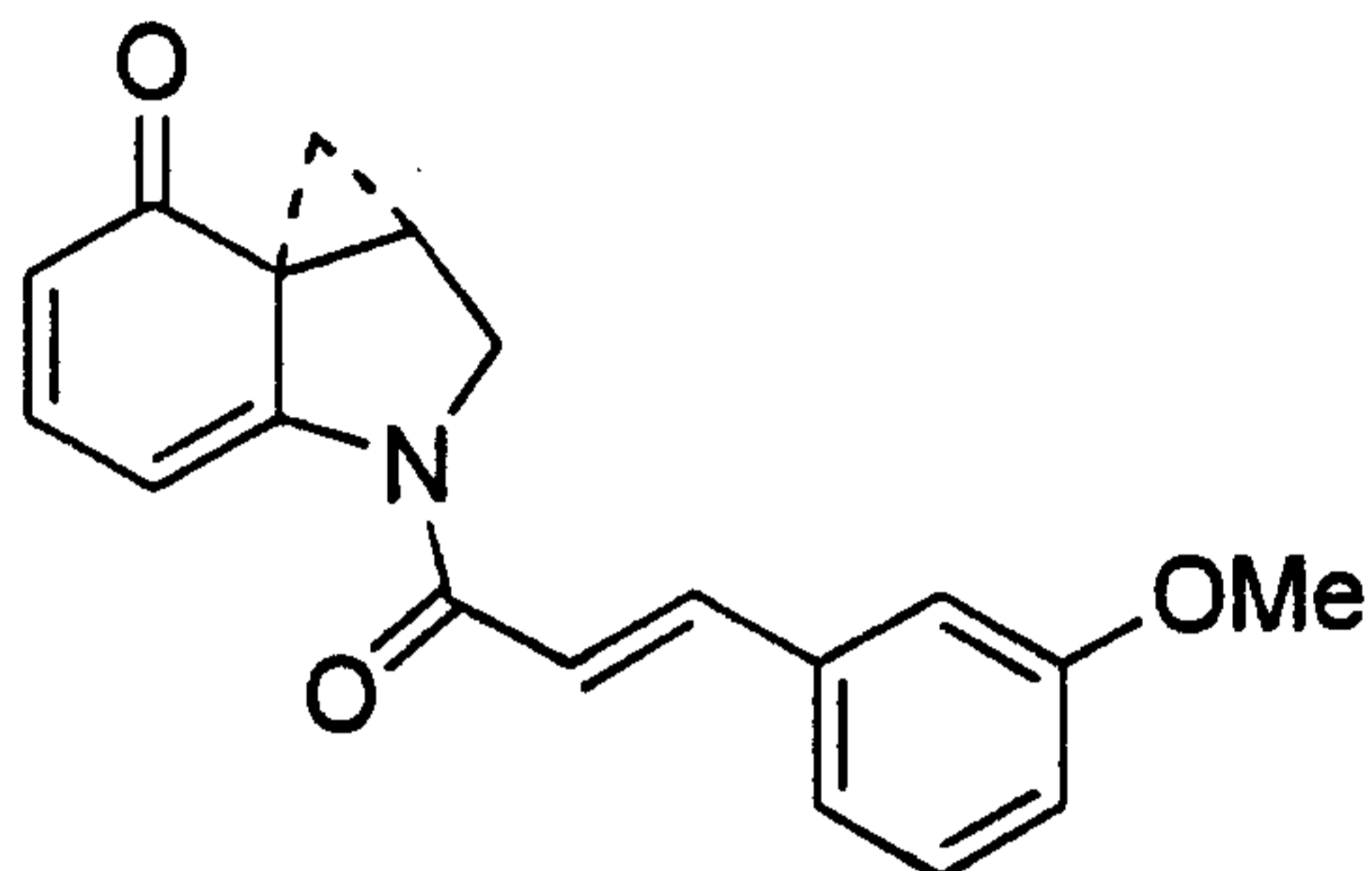
30 -C1-C6 alkyl, -NHCH₃ (-N-alkyl), -OCH₃ (O-alkyl), -NH₂,
-NHNH₂, -NHNHCO₂^tBu, and a radical represented by the following structure:



- 72 -

20. A DNA alkylating compound as described in claim 19 represented by the following structure:

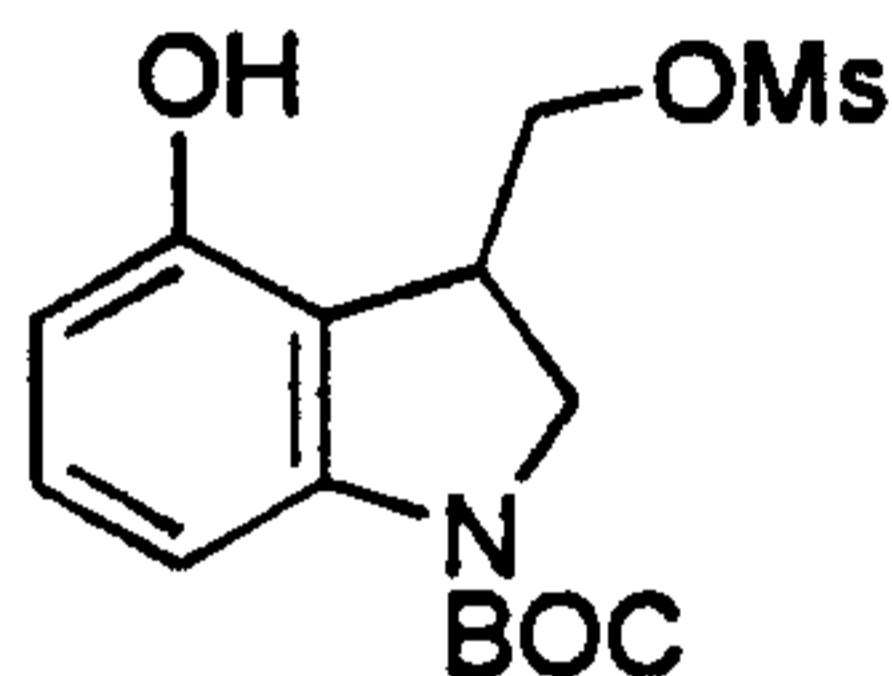
5



10

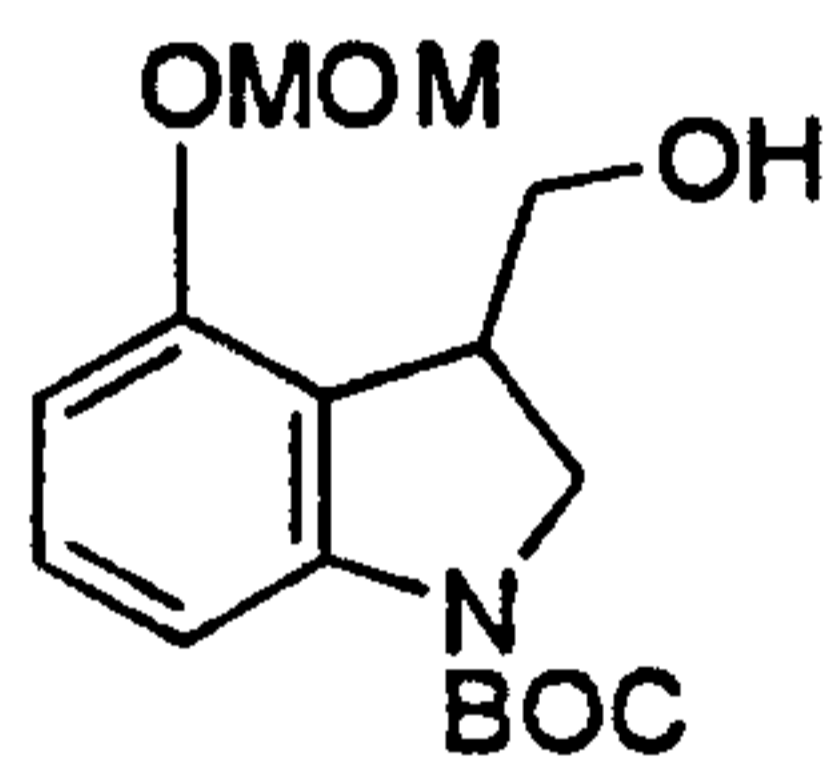
21. A chemical intermediate represented by the following structure:

15



22. A chemical intermediate represented by the following structure:

20



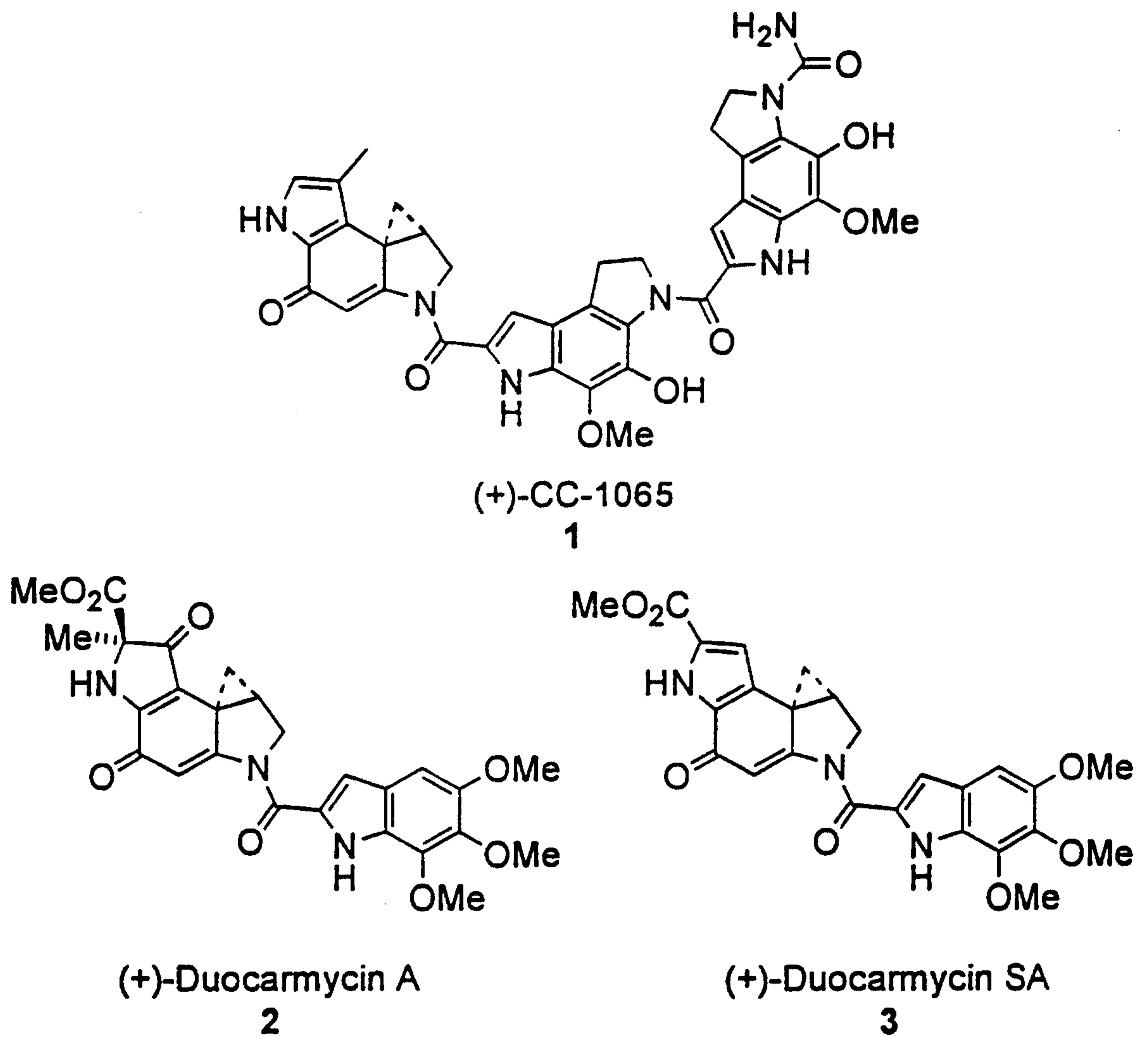


FIG. 1

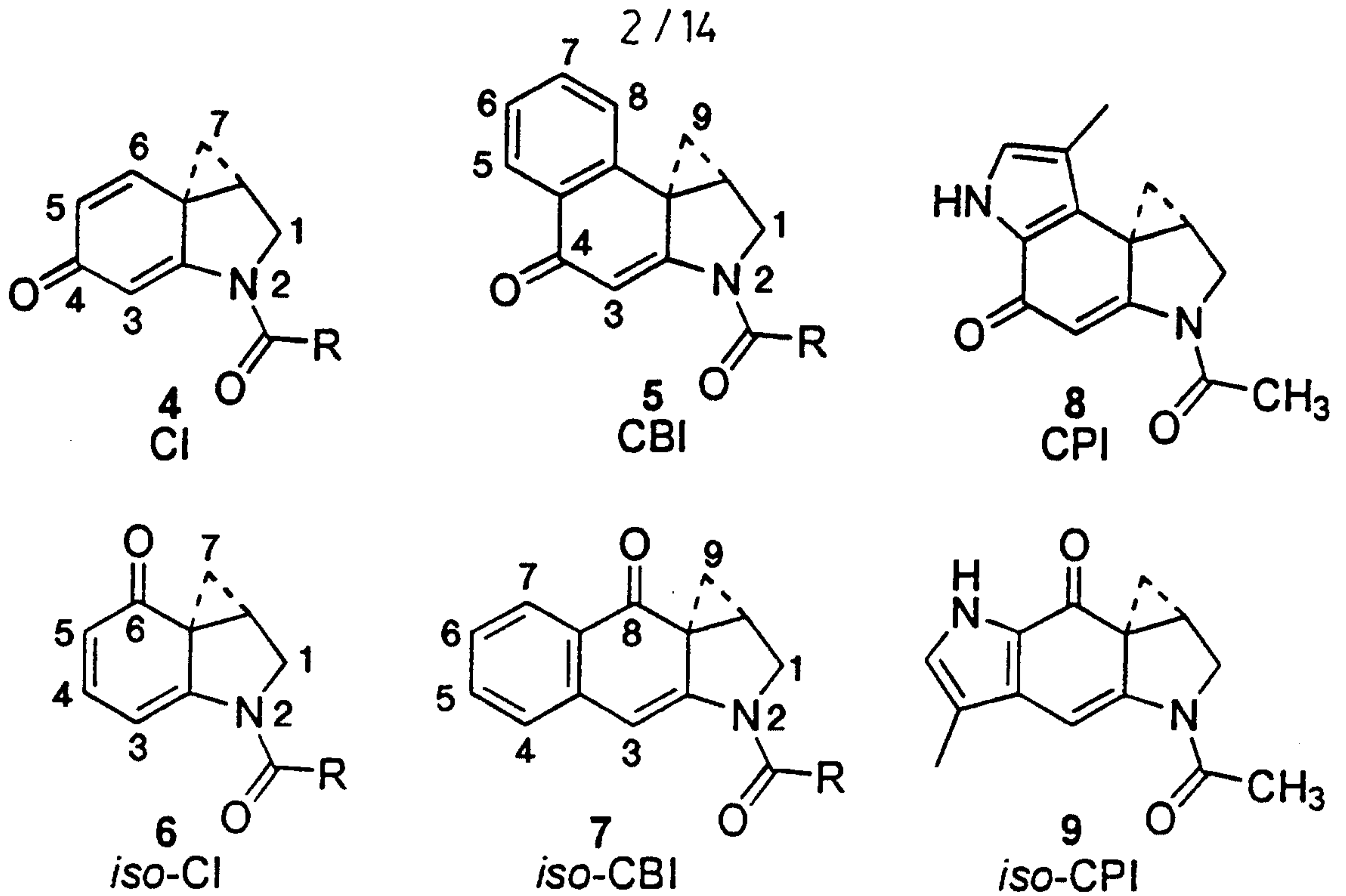


FIG. 2A

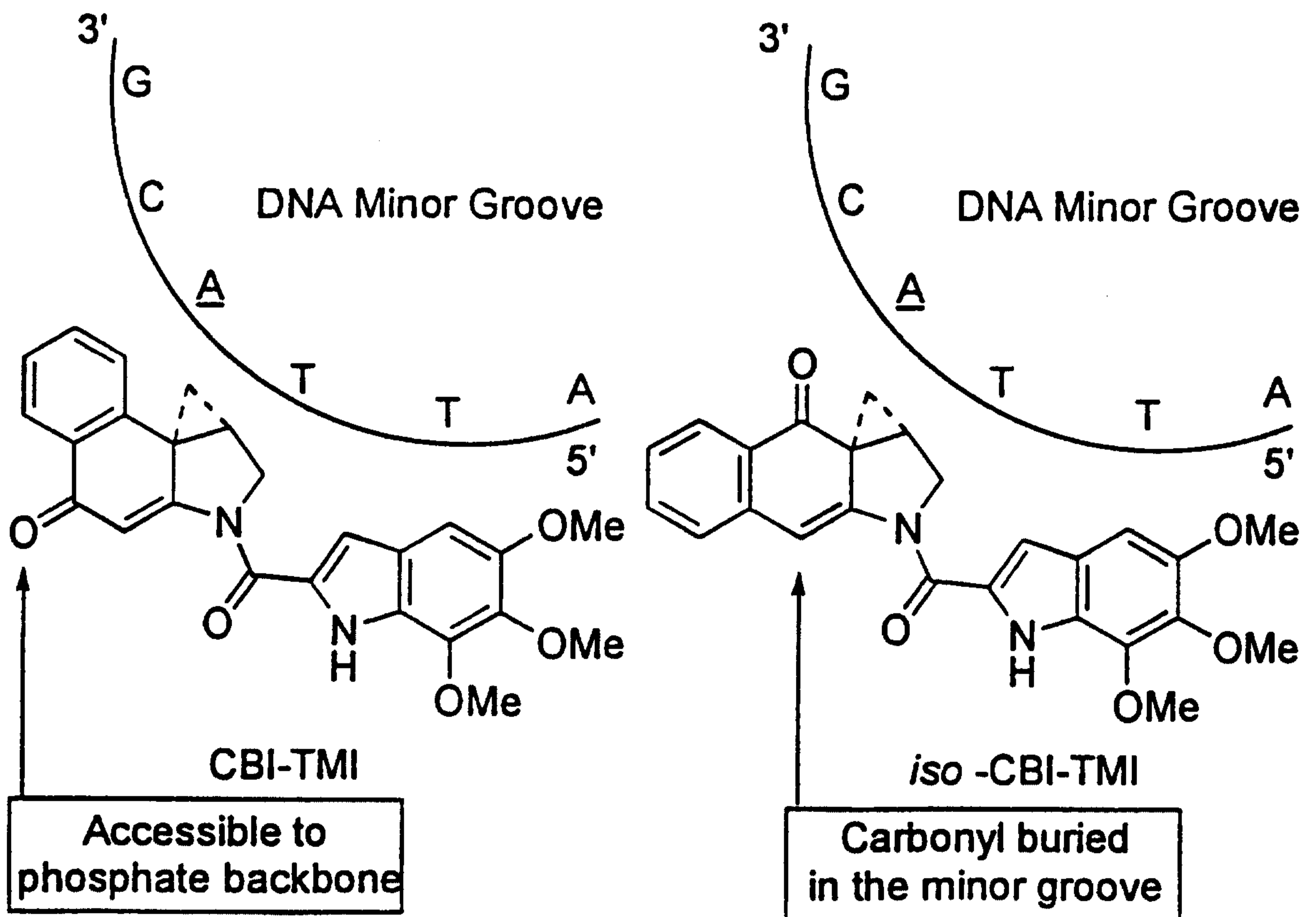


FIG. 2B

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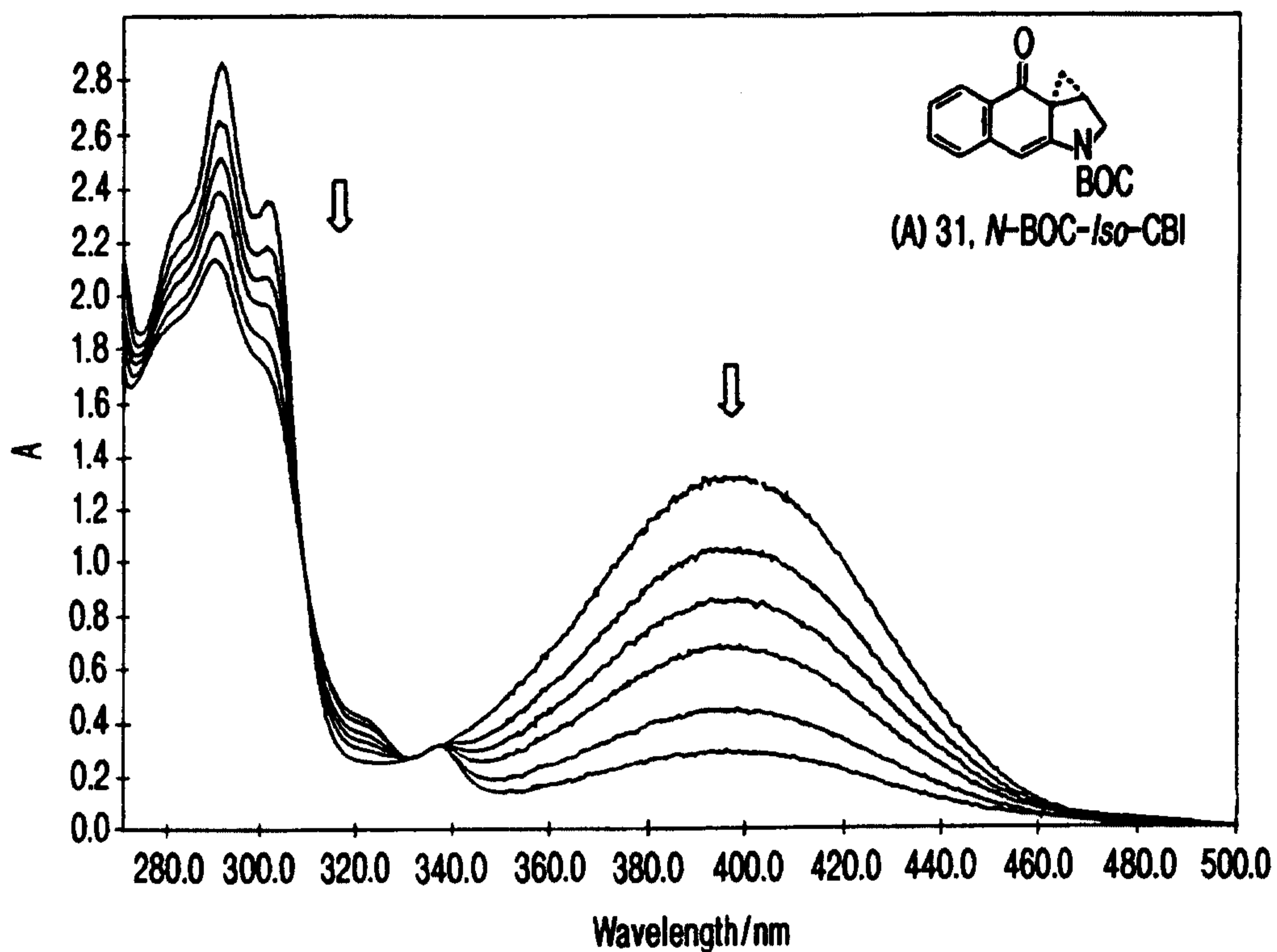


FIG. 3A

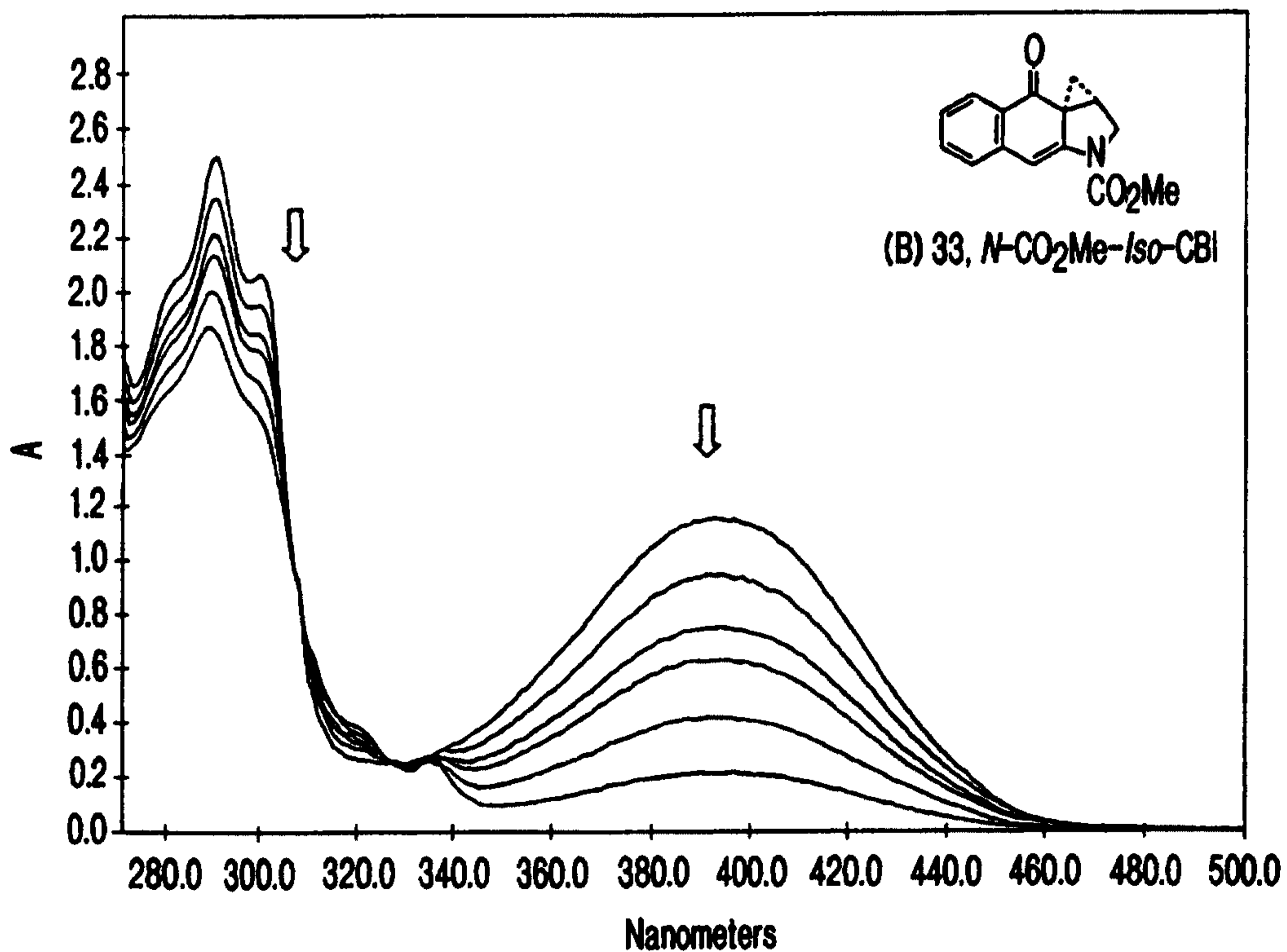


FIG. 3B

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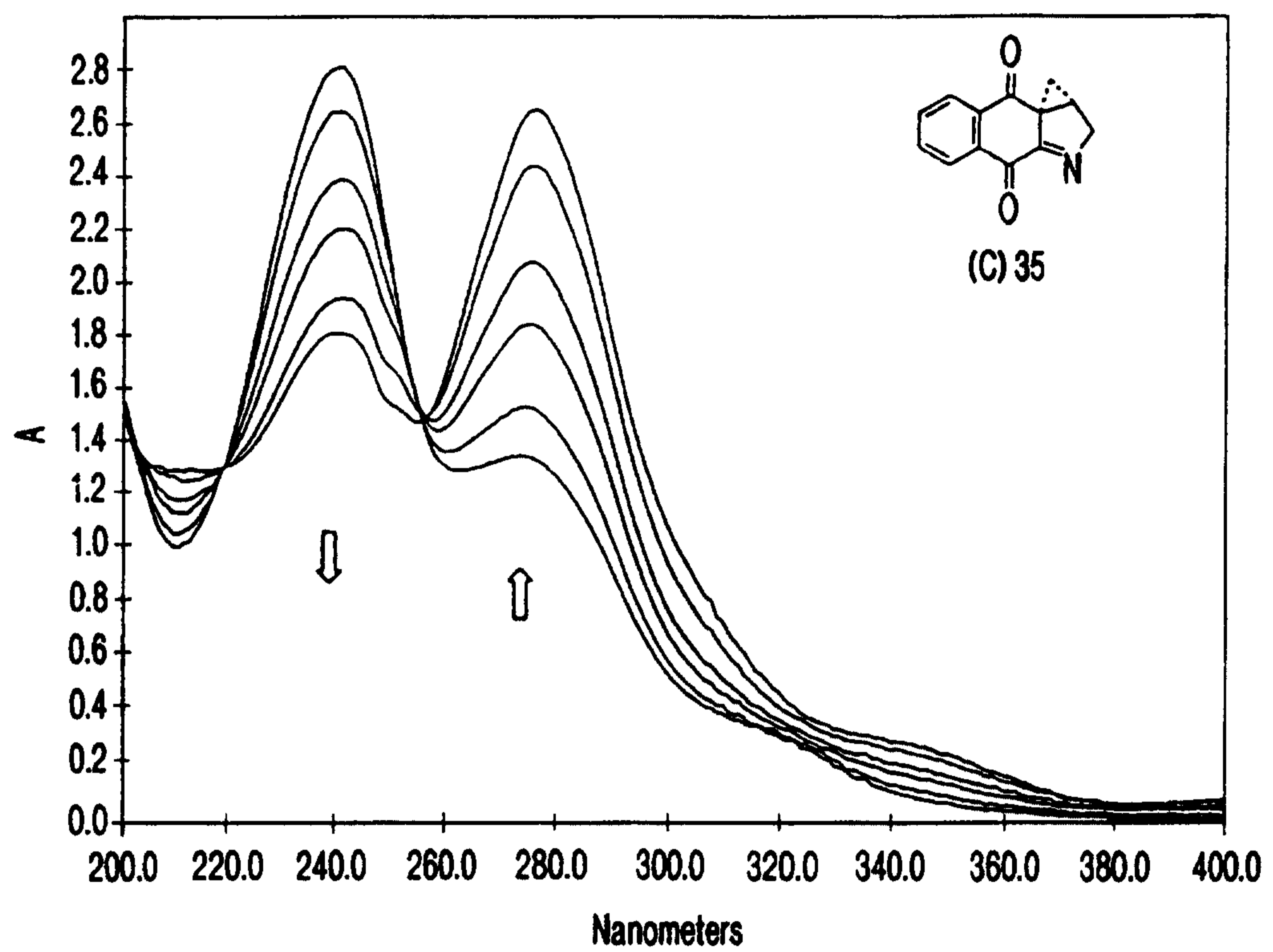


FIG. 3C

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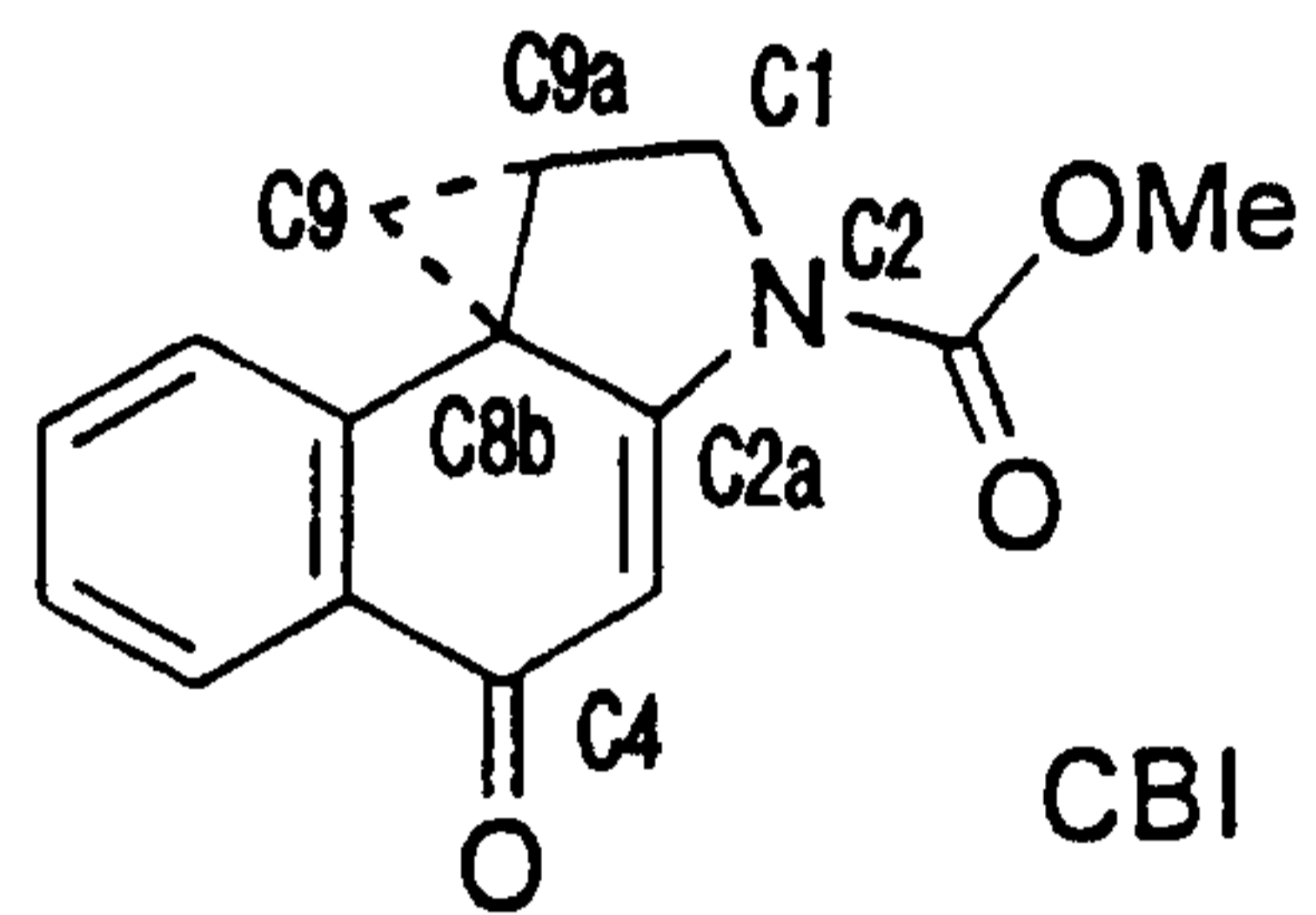
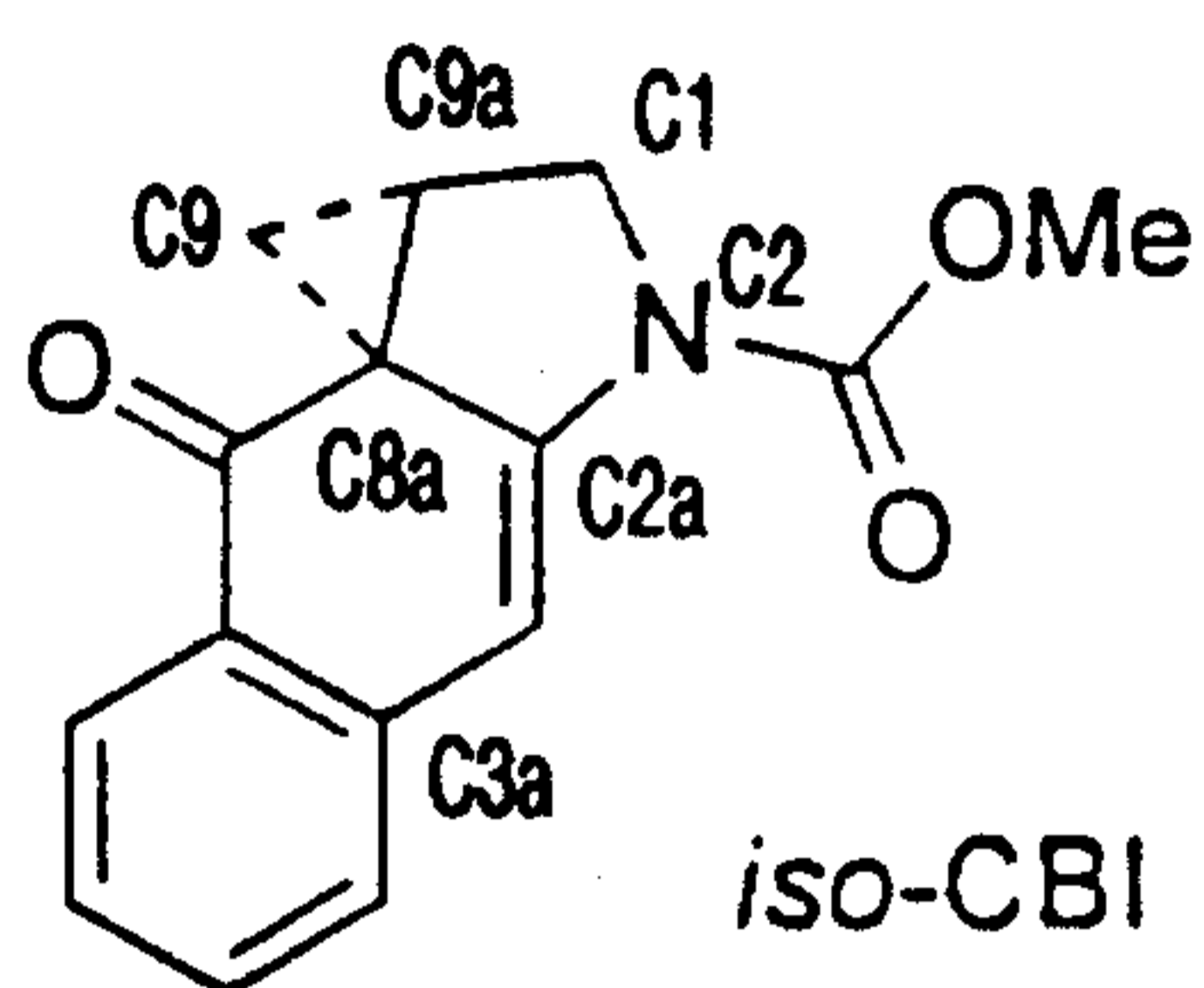
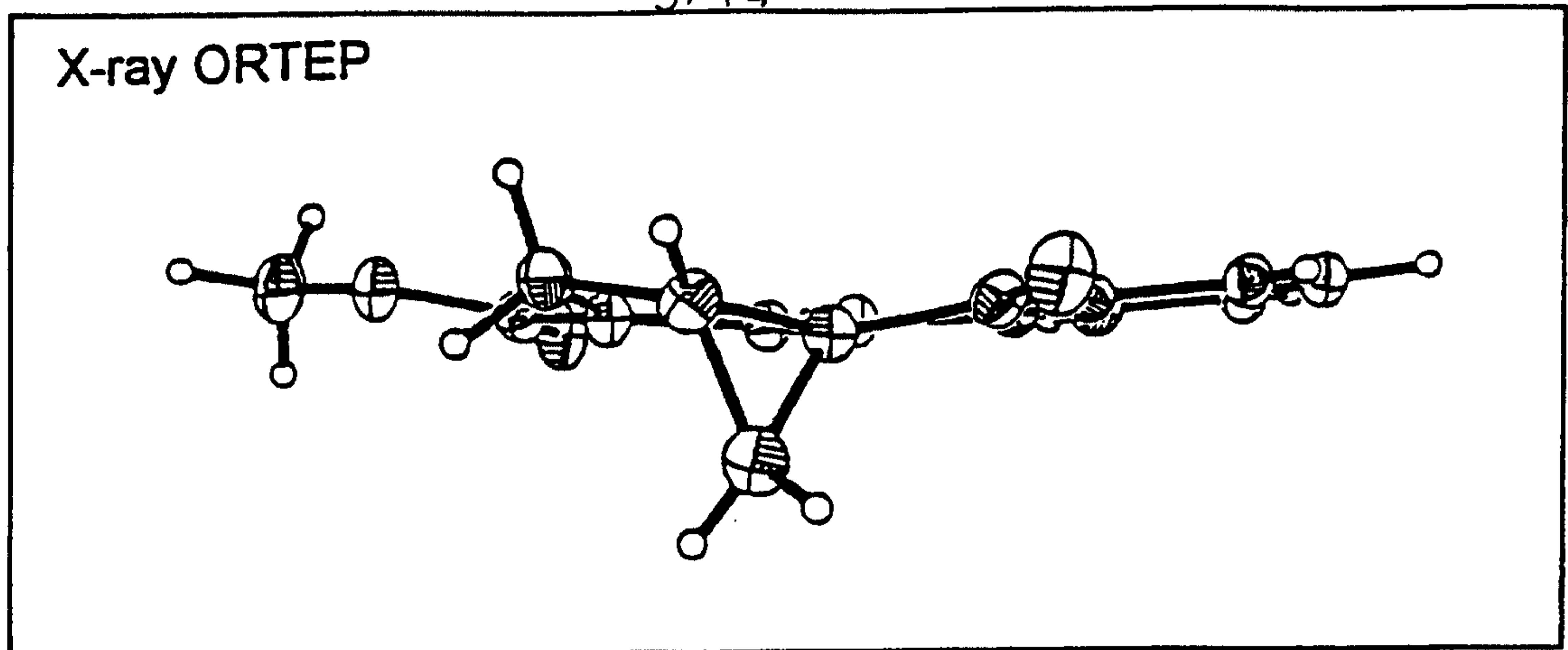


FIG. 4A

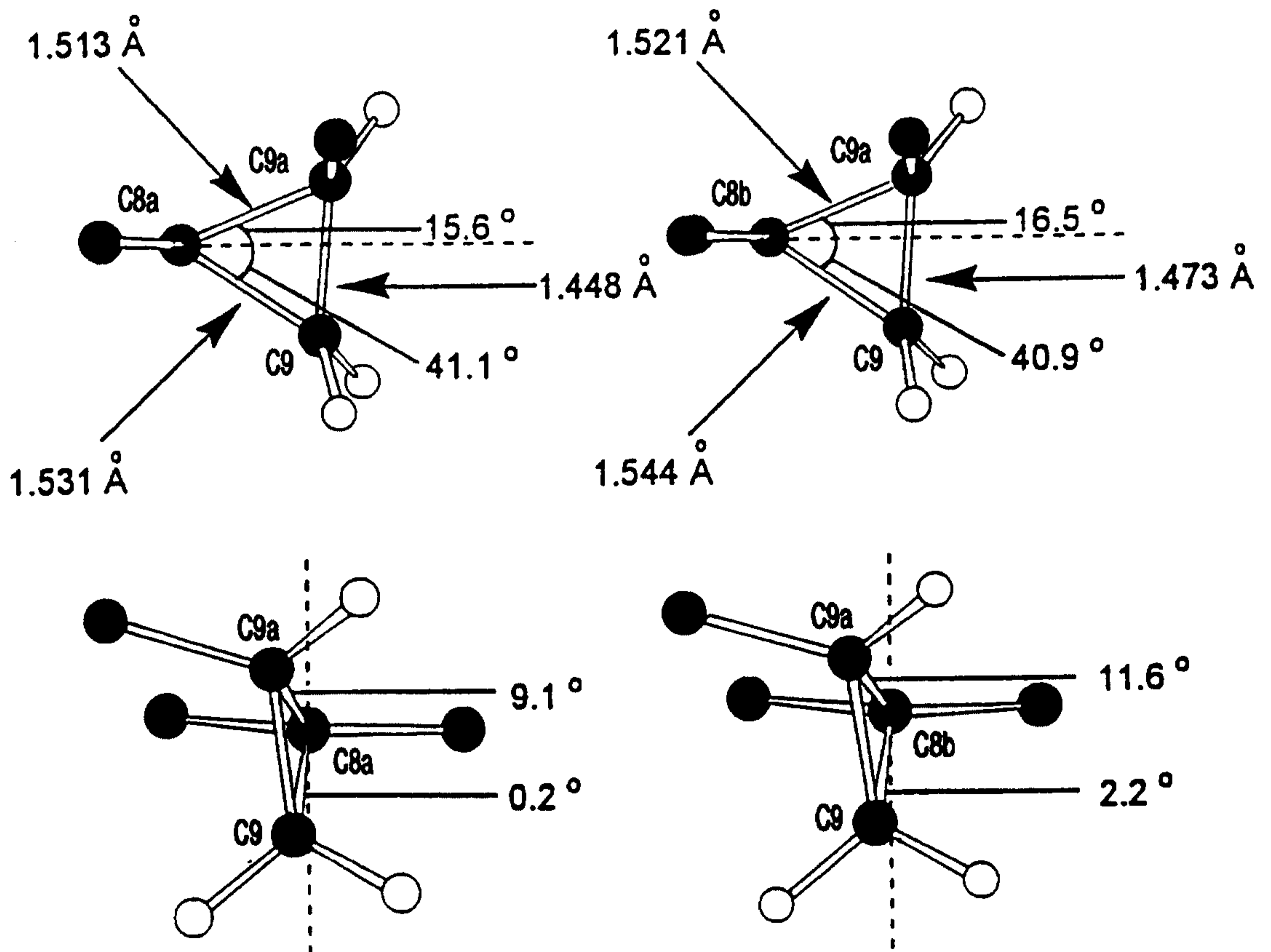


FIG. 4B

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DNA

(-)-iso-CBI TMI (+)-CBI TMI (+)-Duo SA

G C A T | 10⁻³ 10⁻⁴ | 10⁻⁵ 10⁻⁶ | 10⁻⁵ 10⁻⁶

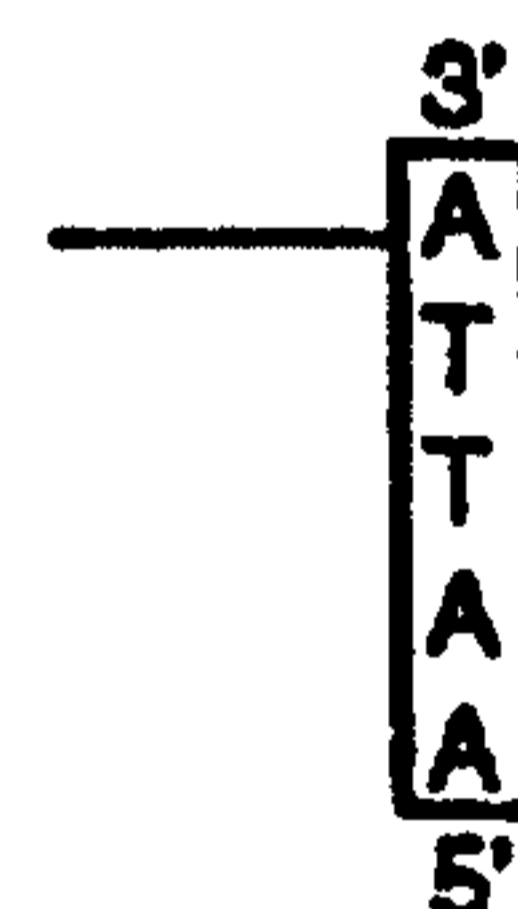
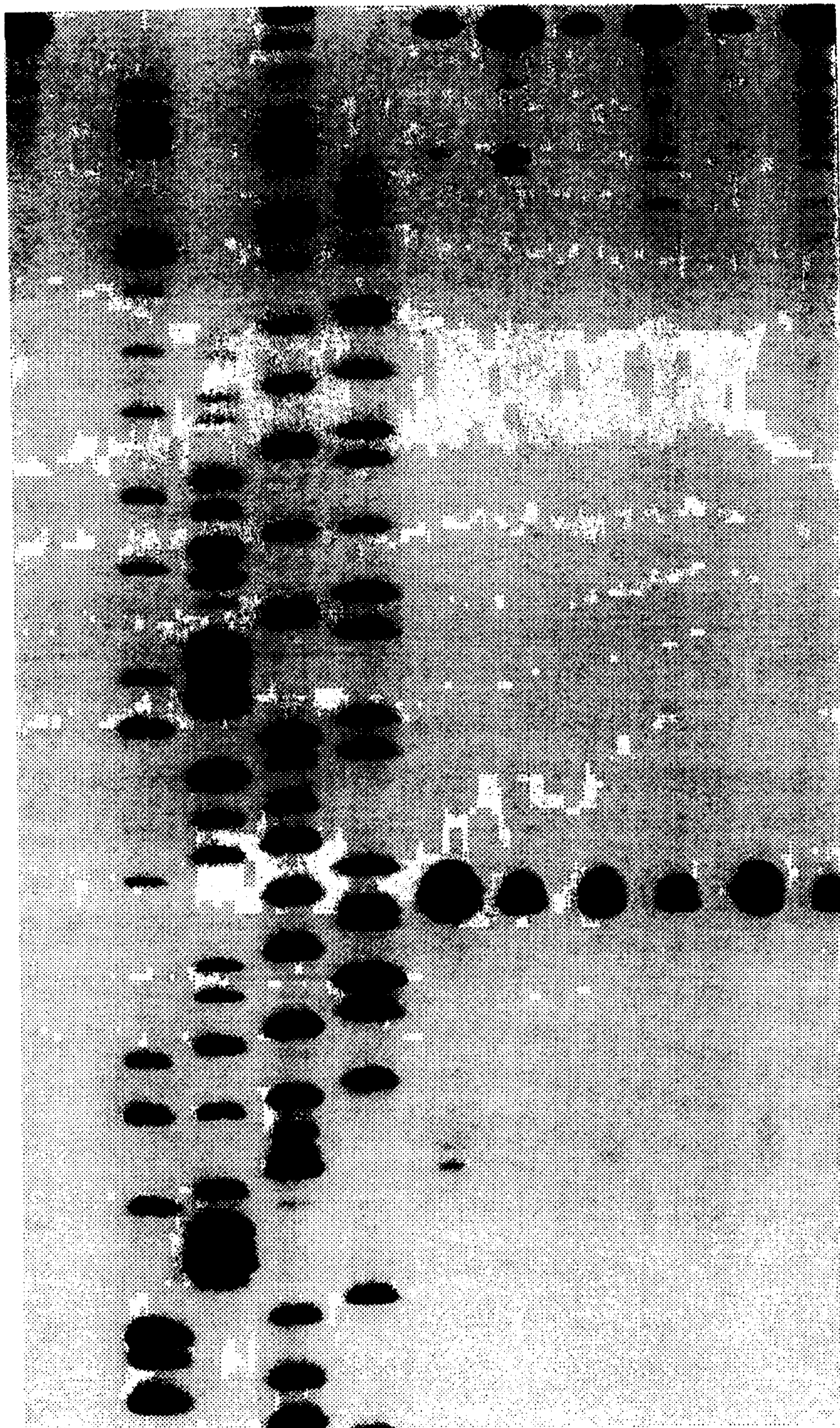


FIG. 5

SUBSTITUTE SHEET (RULE 26)

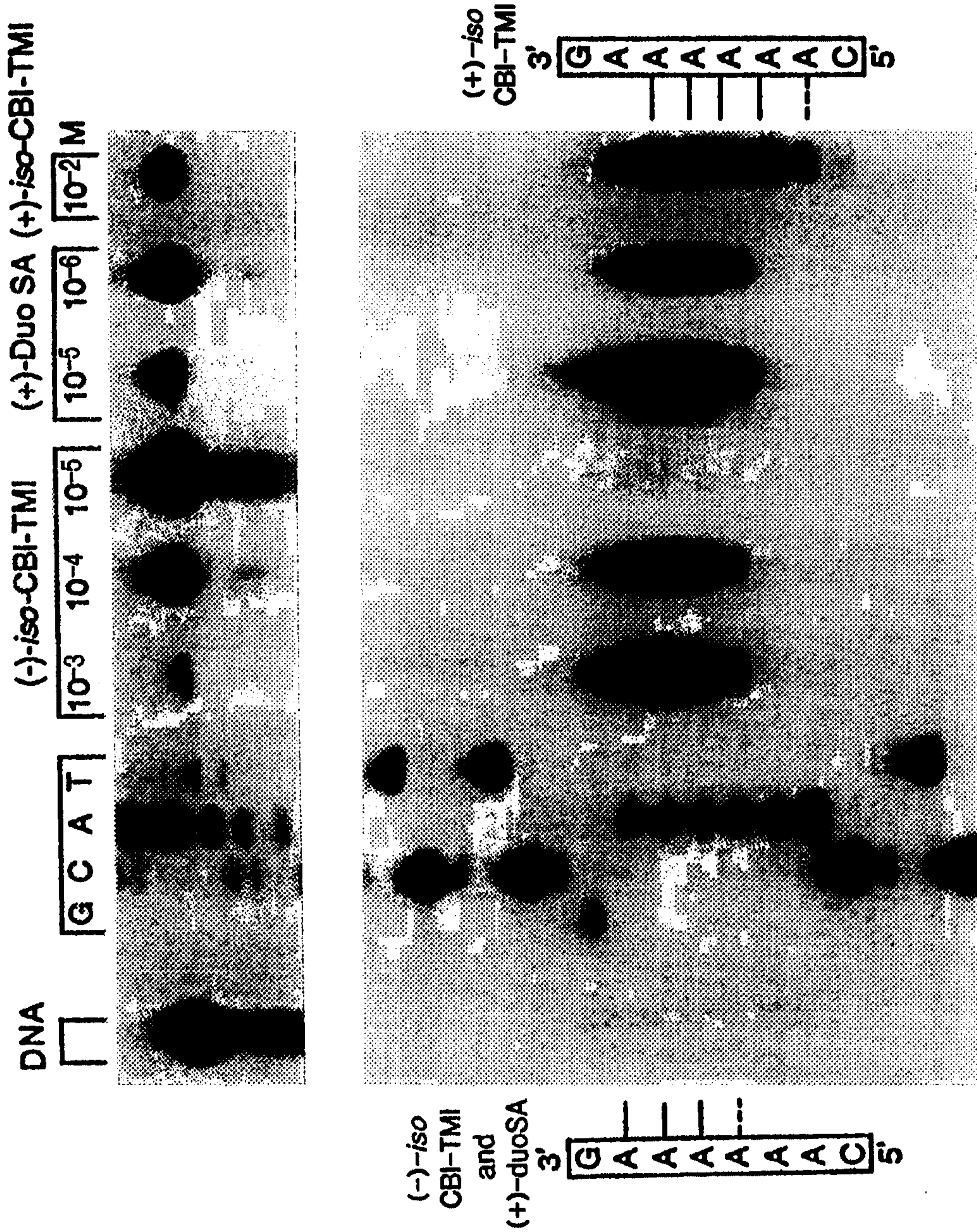


FIG. 6

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DNA

(+)-Duo SA

seco-iso
CI-TMI

□	G	C	A	T	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻³	10 ⁻⁴	10 ⁻⁵	M
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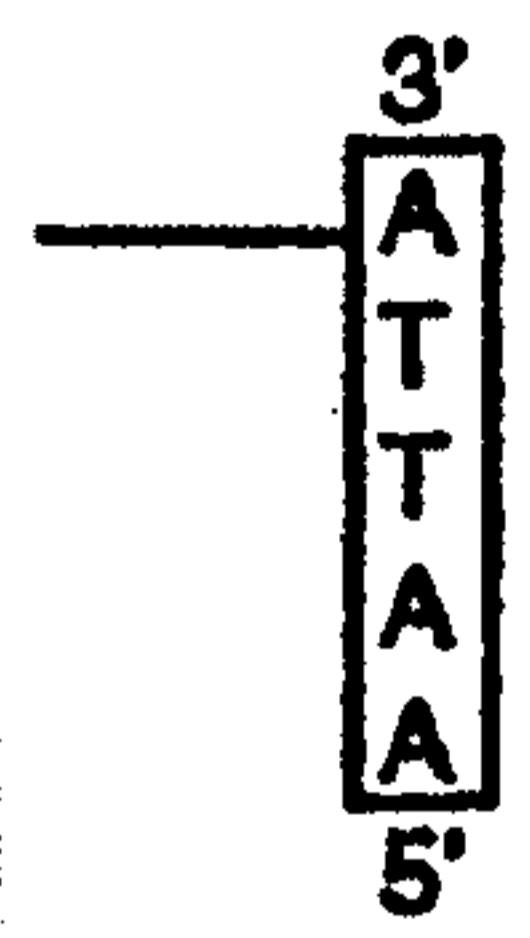
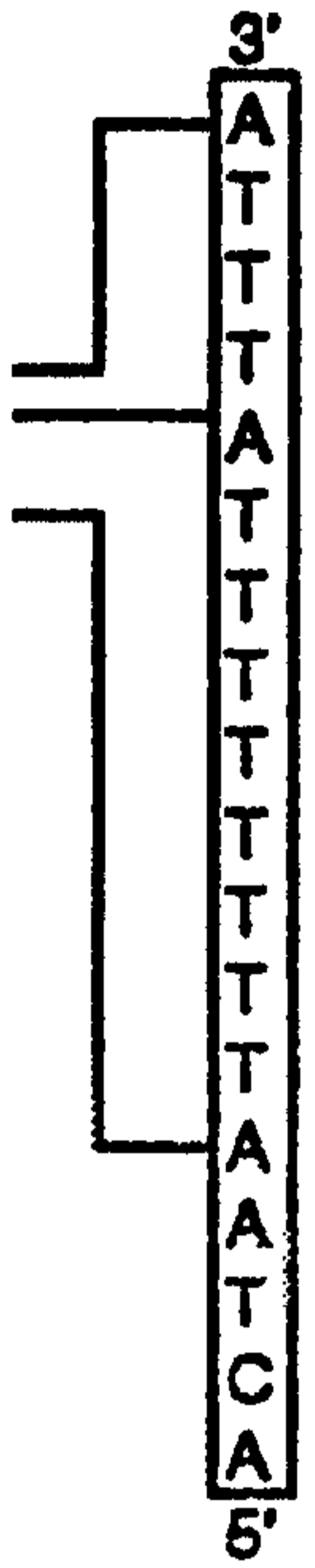
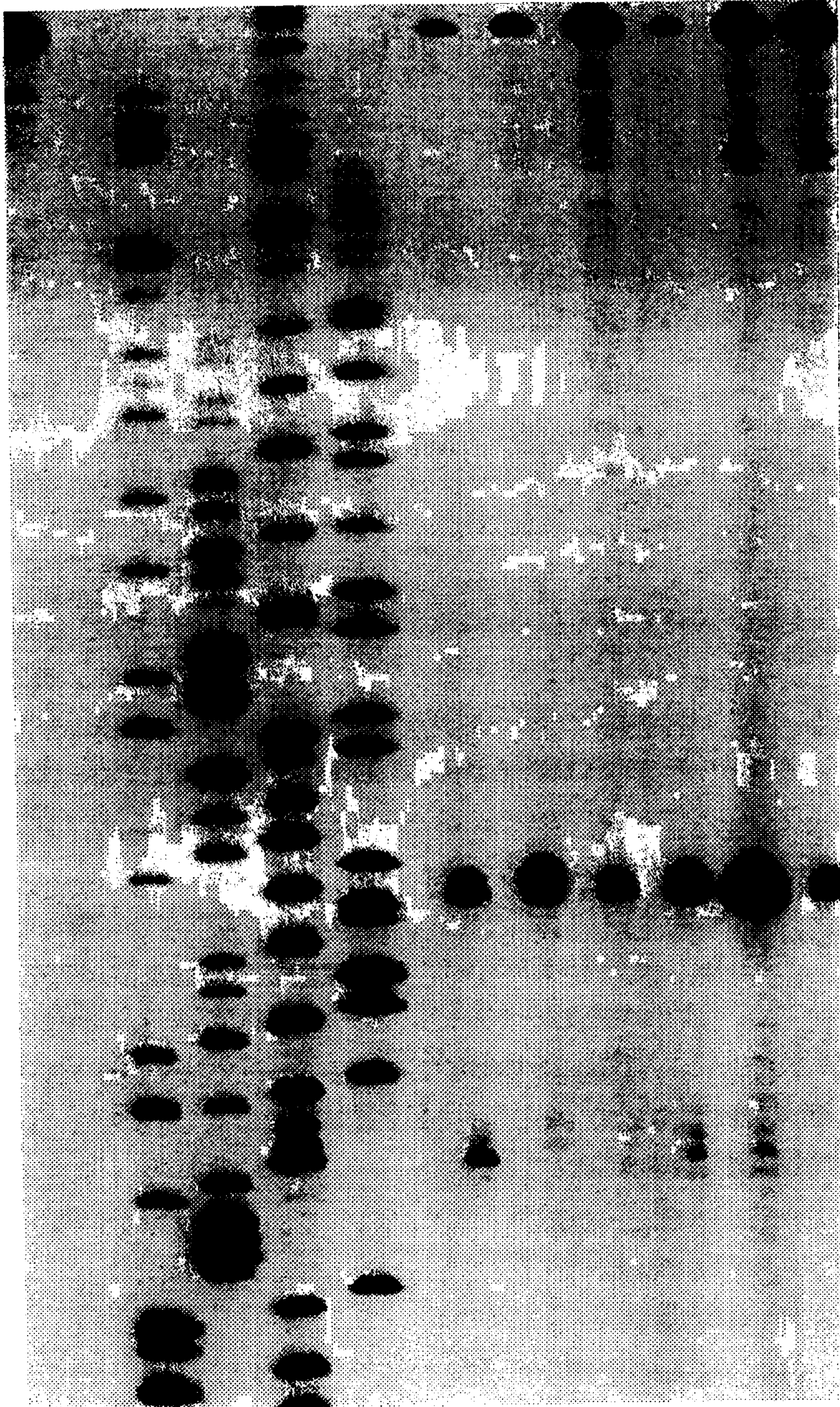


FIG. 7

SUBSTITUTE SHEET (RULE 26)

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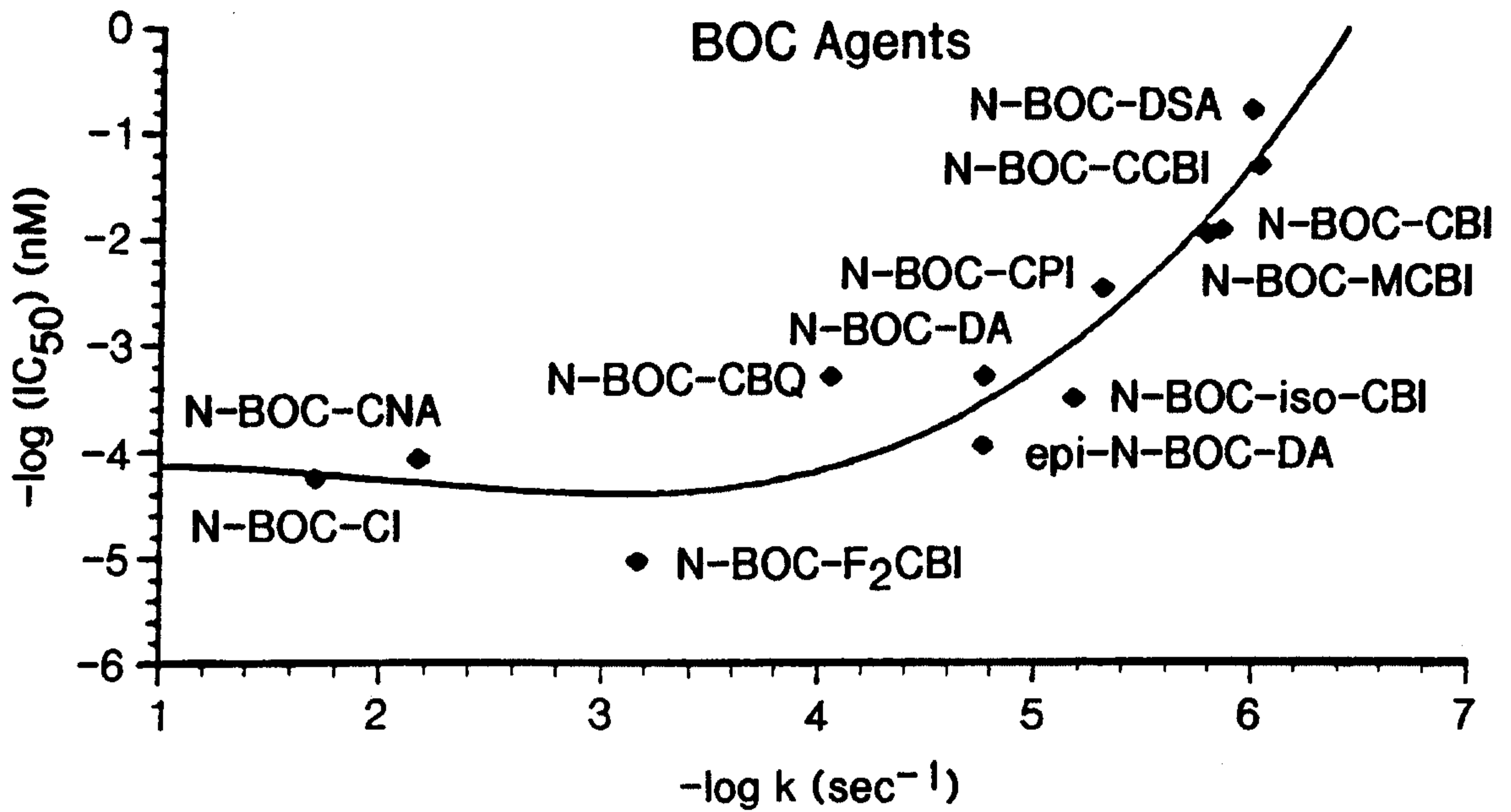


FIG. 8A

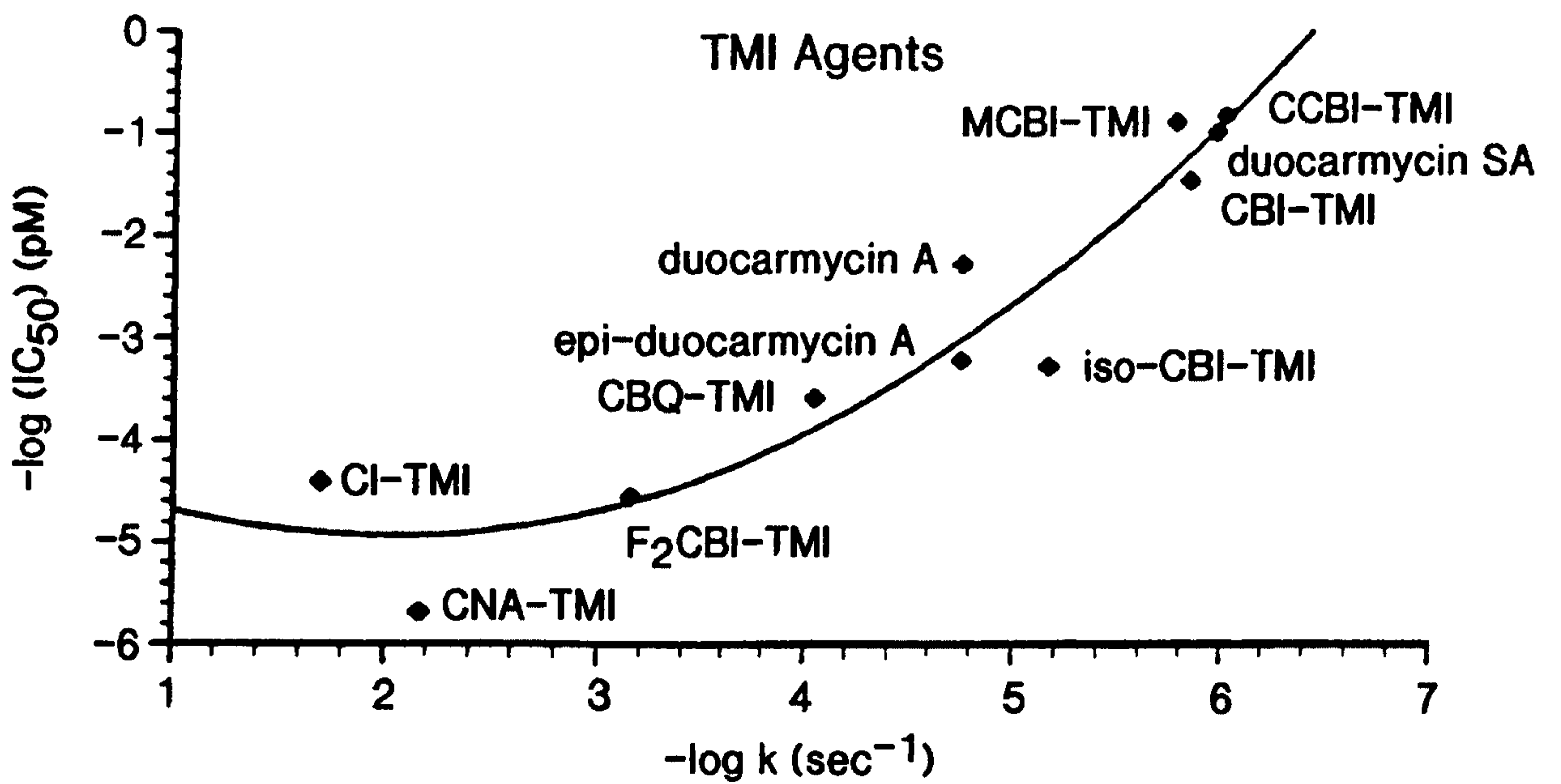


FIG. 8B

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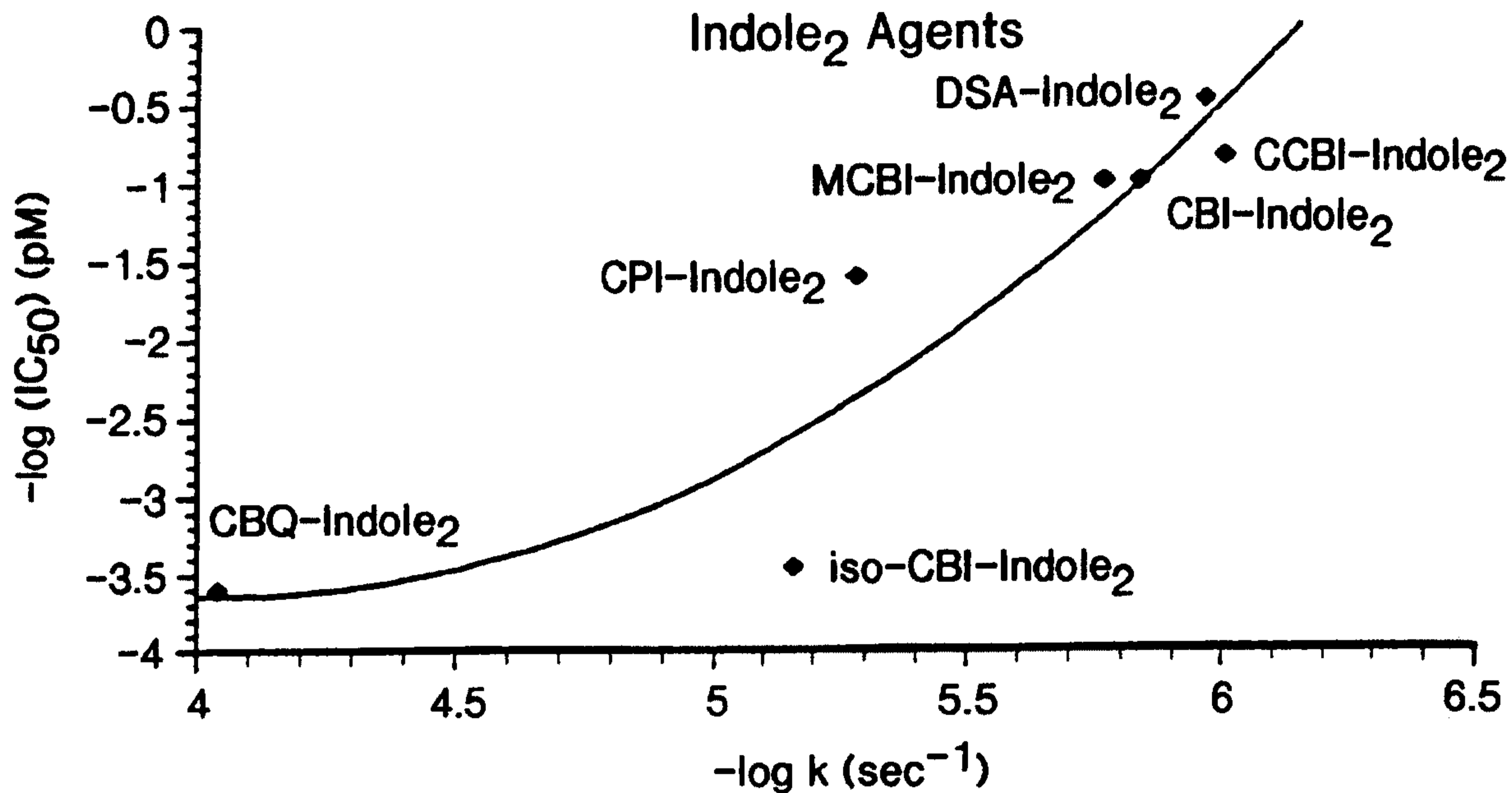


FIG. 8C

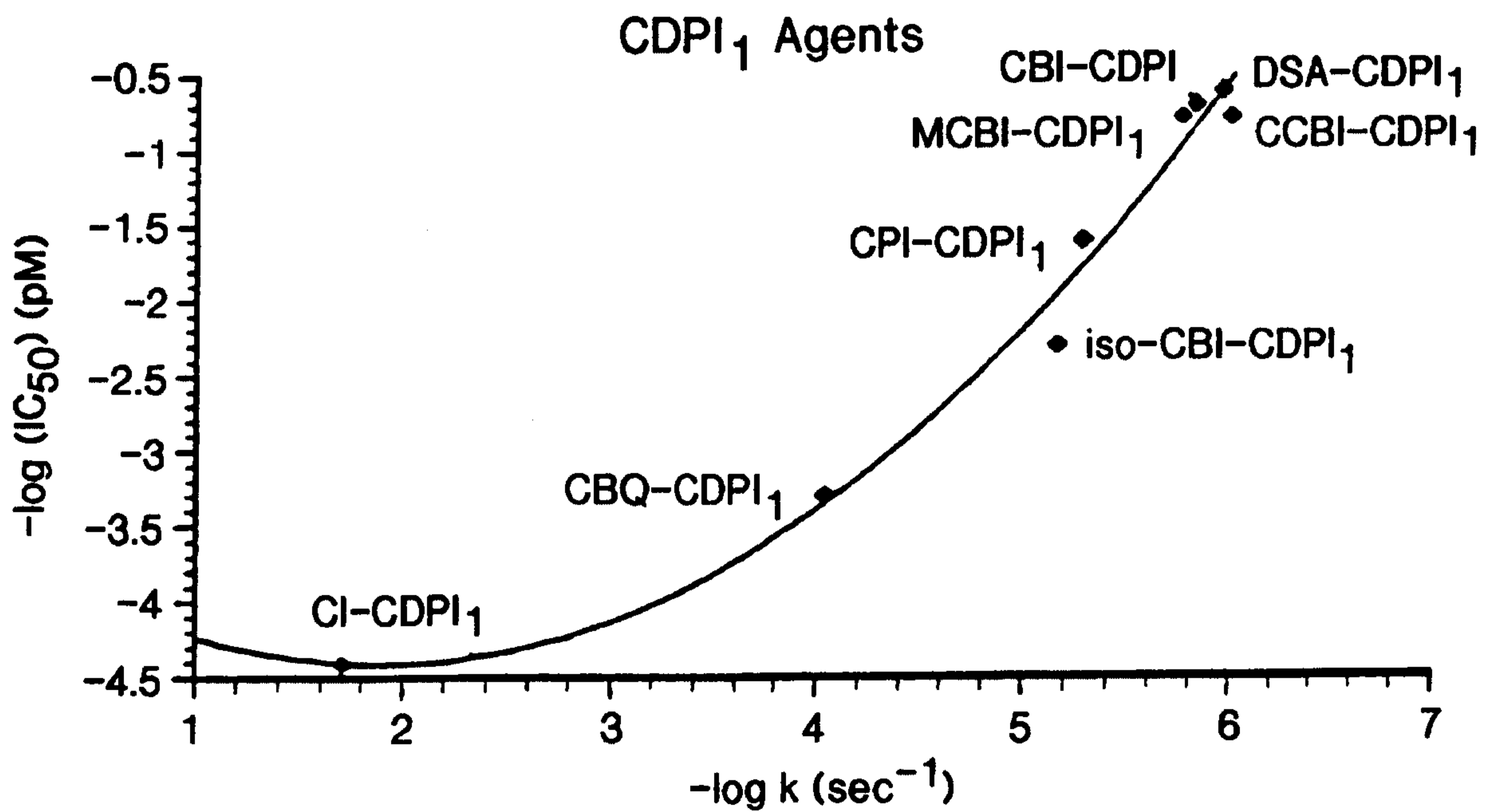


FIG. 8D

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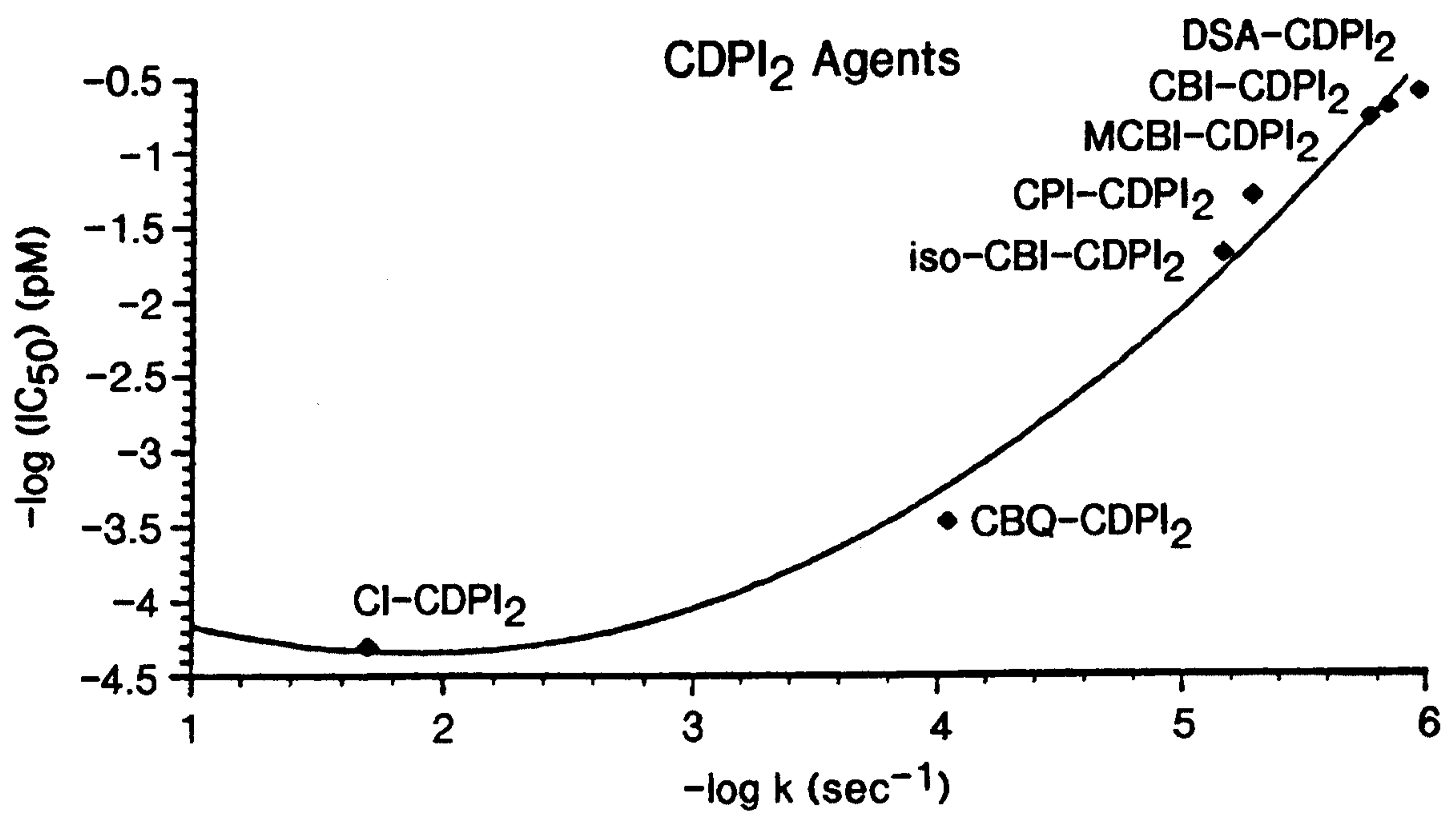


FIG. 8E

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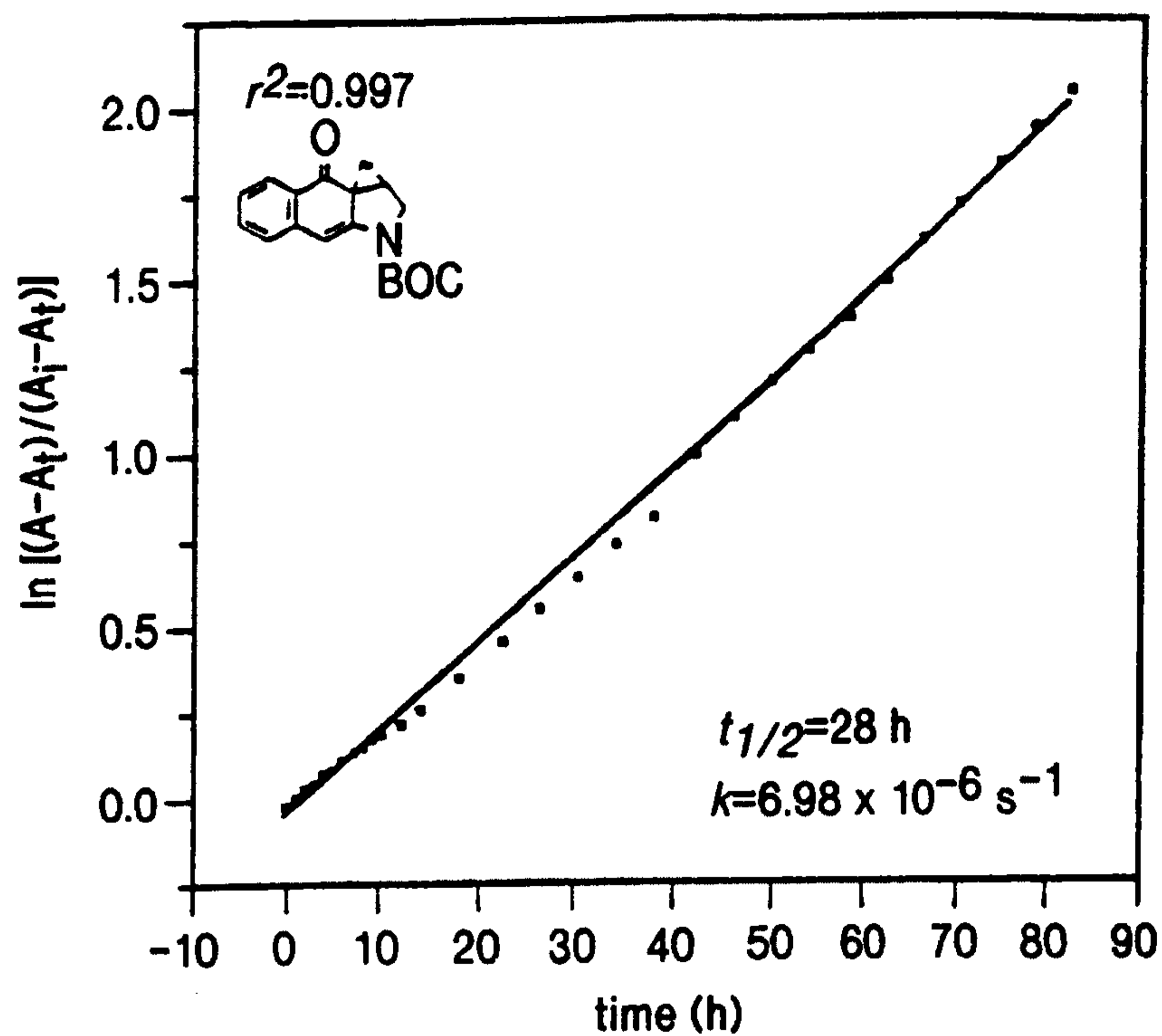
N-BOC-*iso*-CBI Solvolysis (397nm, pH=3.0)

FIG. 9A

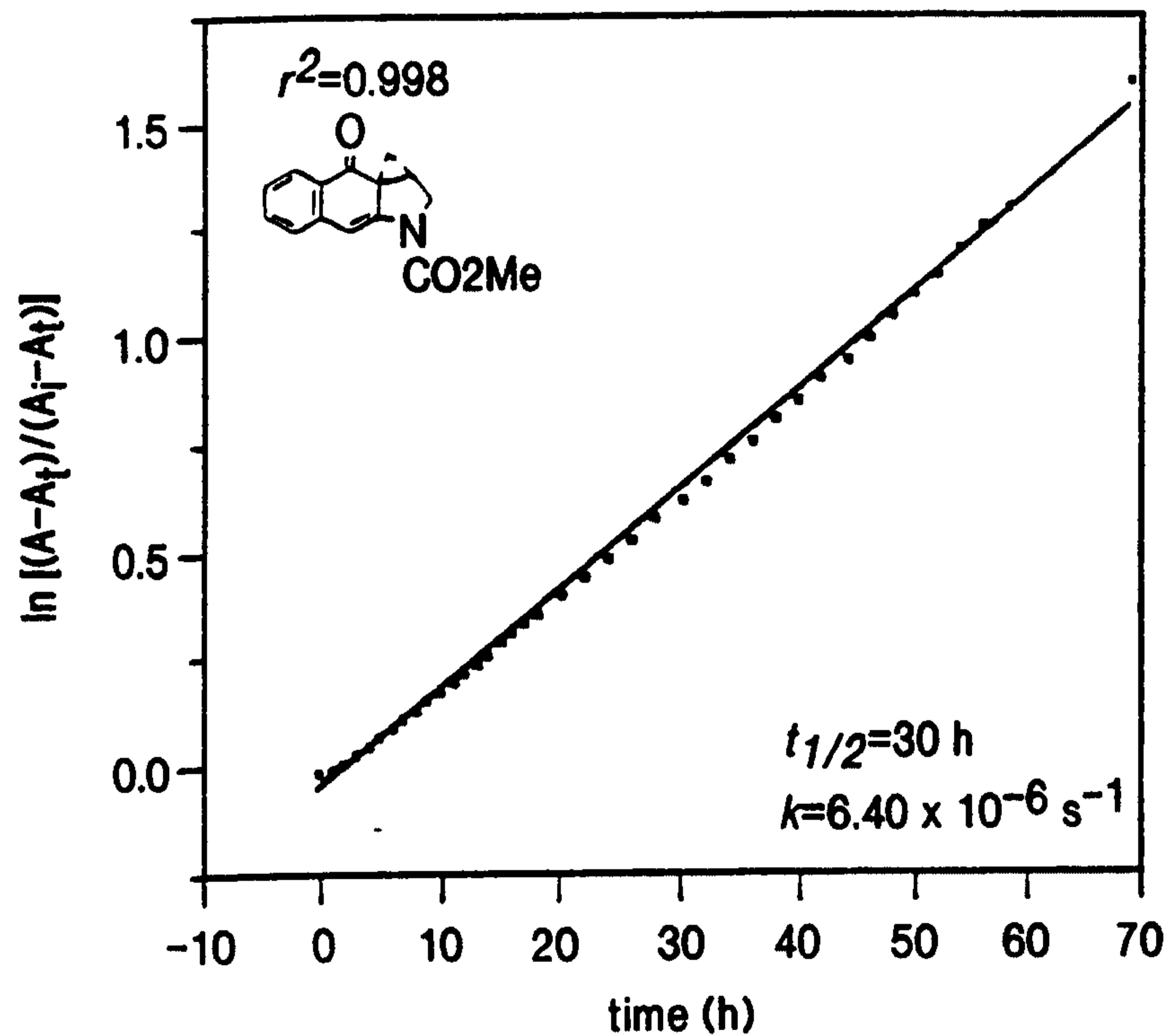
N-CO₂Me-*iso*-CBI Solvolysis (395nm, pH=3.0)

FIG. 9B

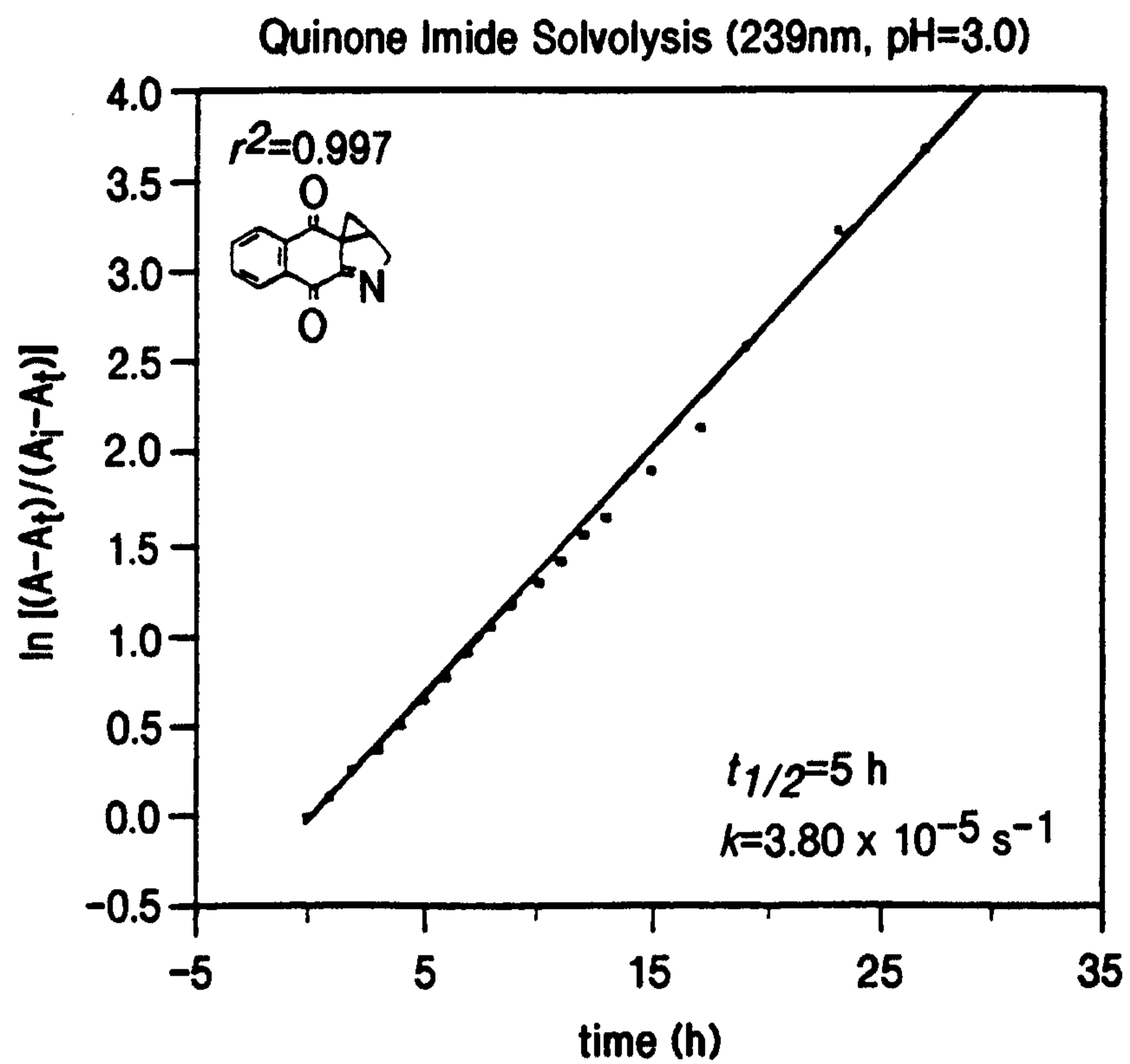


FIG. 9C

