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(71) Applicant *(for all designated States except US)*: THE SCRIPPS RESEARCH INSTITUTE [US/US]; 10666 North Torrey Pines Road, La Jolla, CA 92037 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: NICOLAOU, Kyriakos, C. [US/US]; 9625 Blackgold Road, La Jolla, CA 92037 (US). PITSINOS, Emmanuel, N. [GR/GR]; 23 Kodrou Street, GR-152 32 Halandri (GR).

(74) Agents: LEWIS, Donald, G. et al.; The Scripps Research Institute, 10666 North Torrey Pines Road, TPC-8, La Jolla, CA 92037 (US).

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(54) Title: CALICHEAMICIN MIMICS

(57) Abstract

Synthetic calicheamicin mimics employ alternative activation triggers and trigger sites and include tethers for conjugation to DNA targeting systems.

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CALICHEAMICIN MIMICS

Description

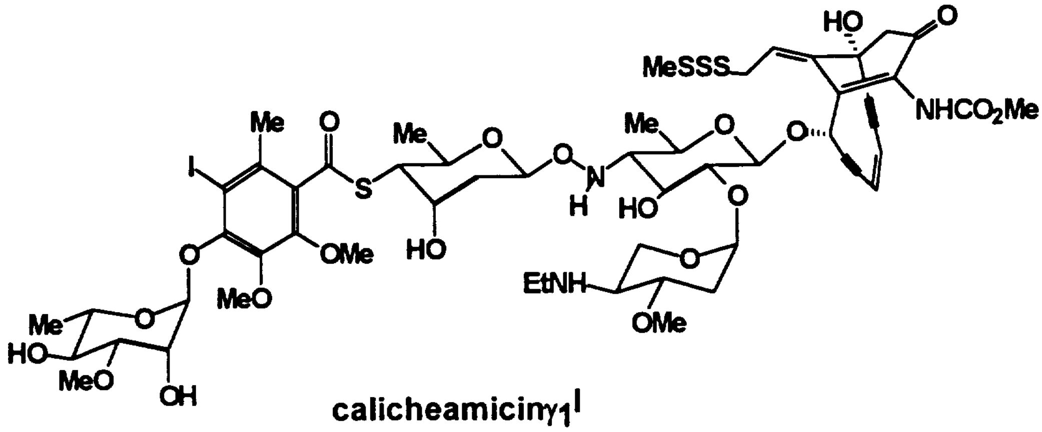
Field of Invention:

The invention relates to a synthetic calicheamicin mimics having designed initiation mechanisms while retaining DNA cleavage activity, cytotoxicity, and apoptosis activity.

Background:

Calicheamicin γ_1^I is a bacterial derived natural product known to have activity as an antitumor antibiotic. Calicheamicin γ_1^I is a member of a class of enediyne antitumor antibiotics which share a common mode of action. The total synthesis of calicheamicin γ_1^I has been achieved by two groups, viz. K. C. Nicolaou et al. (*Journal of the American Chemical Society* (1992): vol. **114**, pp 10082-10084) and S. J. Danishefsky et al. (*Angew. Chem. Int. Ed. Engl.* (1994): vol. **33**, pp 858-862). Antibody conjugates of calicheamicin γ_1^I are currently in clinical trials for potential use in chemotherapy. (L. M. Hinman et al., *Cancer Res.* (1993): vol. **53**, pp 3336-3342.)

The structure of calicheamicin γ_1^I was first established in 1987 by M. D. Lee et al. at Lederle laboratories. (*Journal of the American Chemical Society* (1987): vol. **109**, pp 3466-3468.) Subsequently, calicheamicin γ_1^I (Fig. 1A) was reported by K. C. Nicolaou et al to be the most potent naturally occurring member of the enediyne class of antitumor antibiotics (*Angew. Chem. Int. Ed. Engl.* (1991): vol. **30**, pp 1387-1530).



calicheamicinγ_1^I

Other members of the class of enediyne antitumor antibiotics include esperamicin A_1, neocarzinostatin chromophore, dynemicin A, kedarcidin chromophore, and C-1027 chromophore. The structure of esperamicin A_1 was established by J. Golik et al. at Bristol Meyers Squibb. (*Journal of the American Chemical Society* (1987): vol. **109**, pp 3462-3464.) The previously known neocarzinostatin chromophore was included within the class when it was disclosed by I. H. Goldberg to have a mode of action similar to calicheamicin γ_1^I and esperamicin A_1. (*Acc. Chem. Res.* (1991): vol. **24**, pp 191-198.) The group continued to expand with the discoveries of dynemicin A (M. Konishi et al., *J. Antibiot.* (1989): vol. **42**, pp 1449-1452), kedarcidin chromophore (J. E. Leet et al. *Journal of the American Chemical Society* (1992): vol. **114**, 7946-7948) and C-1027 chromophore (K. Yoshida et al., *Tetrahedron Lett.* (1993): vol. **34**, 2637-2640).

The growing number of these antibiotics has been paralleled by an increasing interest in them from members of the chemical, biological and medical communities. Advances in synthetic efforts, culminating in total syntheses of calicheamicin γ_1^I and a number of designed analogs (K. C. Nicolaou et al., *Proc. Natl. Acad. Sci. USA*

(1993): vol. **90**, pp 5881-5888) have been entwined with advances in research related to their mechanism of action. M. D. Lee et al. characterized the chemical interaction of calicheamicins with DNA. (*Acc. Chem. Res.* (1991): vol. **24**, pp 235-243.) More particularly, P. C. Dedon et al. disclosed that calicheamicin employs a free-radical mechanism to achieve sequence-dependent bistranded DNA lesions (*Chem. Res. Toxicol.* (1992): vol. **5**, pp 311-332). Furthermore, Uesugi et al. disclosed that calicheamicin γ_1^I employs a sequence recognition process with DNA and "induced fit" mechanism to achieve its selectivity (*Biochemistry* (1993): vol. **32**, pp 4622-4627.

The molecular architecture of calicheamicin γ_1^I exemplifies the efficiency of design common to the enediyne class of antitumor antibiotics. The structure can be viewed as consisting of three functional domains with distinct roles:

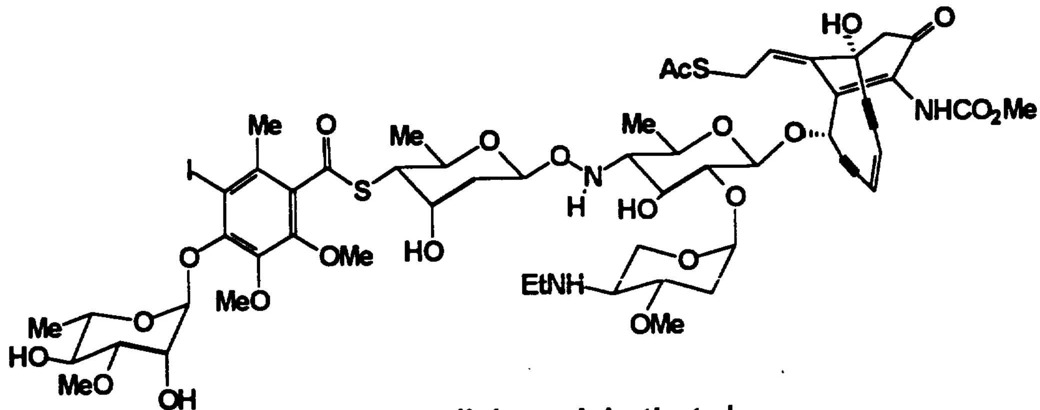
(a) the novel oligosaccharide chain, which allows the sequence selective binding of the drug in the minor groove of double-stranded DNA (Dedon, supra);

(b) the enediyne moiety (represented by calicheamicinone), which is capable of undergoing a Bergman cycloaromatization (Fig. 2), generating benzenoid diradicals that can cleave double-stranded DNA via hydrogen atom abstraction (R. G. Bergman, *Acc. Chem. Res.* (1973): vol. **6**, pp 25-31); and

(c) the trisulfide group, which serves as a triggering device to initiate the cascade of reactions that leads to DNA damage (Fig. 3) (A. G. Myers et al., *Journal of the American Chemical Society* (1994): vol. **116**, pp 1255-1271).

The DNA-cleaving activity and anticancer activity of natural enediynes have been partially reproduced by various designed enediynes (K. C. Nicolaou et al., *Science* (1992): vol. **256**, pp 1172-1178.) More particularly, calicheamicin Θ_1^I, a rationally designed and totally synthetic enediyne, has been demonstrated to have extremely potent and selective DNA cleaving properties and apoptosis inducing activity. (K. C.

Nicolaou et al., *Angew. Chem. Int. Ed. Engl.* (1993): vol. **33**, pp 183-186. Calicheamicin Θ_1^I is structurally identical to calicheamicin γ_1^I except for the substituent at the allylic position of the exocyclic chain.



calicheamicin theta$_1^I$

What is needed are simple synthetic biological mimics of calicheamicin γ_1^I having enhanced or altered biological activity with minimal molecular complexity.

Summary:

Simple biological mimics of calicheamicin and chemical intermediates employed in the synthesis of such mimics are disclosed. Each of the disclosed mimics of calicheamicin includes the following elements:

1. An enediyne core ring structure identical to the enediyne core ring structure of calicheamicin;
2. One or more novel initiators for triggering a Bergman cycloaromatization of the enediyne core ring structure and the formation or a diradical; and
3. One or more tethers to which substituents may be conjugated for binding the mimic to a DNA targeting delivery system.

Several different initiators and initiator cites are disclosed. Each initiator and initiator cite is characterized by the conditions under which it triggers the cycloaromatization of the enediyne core ring structure to form a benzenoid diradical. Naturally occurring calicheamicin γ_1^I employs a trisulfide trigger cited at the allylic position of the exocyclic chain. It is disclosed herein that initiators may be introduced at any of three points on the enediyne core ring structure, viz.:

1. The allylic position of the exocyclic chain;
2. The enone carbonyl; and
3. The urethane moiety.

Several different tethers and tithering cites are also disclosed. Naturally occurring calicheamicin γ_1^I is tethered to an oligosaccharide DNA binding domain at the secondary propargylic hydroxyl group via a glycosidic bond. It is disclosed herein that DNA binding domains may be introduced at any of three points on the enediyne ring, viz.:

1. The allylic position of the exocyclic chain;
2. The secondary propargylic hydroxyl group; and
3. The urethane moiety.

The disclosed mimics of calicheamicin were characterized and shown to exhibit significant cytotoxicity and DNA cleaving properties. However, when compared to the biological profiles of calicheamicins γ_1^I and Θ_1^I, the calicheamicin mimics disclosed herein were found to be less potent. In contrast to the highly potent calicheamicins γ_1^I (naturally occurring) and θ (synthetic), the designed mimics disclosed herein cause primarily single strand DNA cuts and are weaker inducers of apoptosis. This reflects their relatively weaker affinities to DNA and lack of specific positioning along the minor groove. These attenuated biological properties are due to the lack of the DNA binding domain for positioning the enediyne moiety adjacent to the DNA so as to make double strand DNA cleavage a favorable and efficient process. The ability of the enediynes to cause double strand cuts to DNA is correlated with their ability to induce apoptosis.

Compounds **3**, **4**, and **10** are suitable for conjugation to delivery systems such as antibodies and other proteins, oligonucleotides, oligosaccharides, DNA intercalators or groove binders which target the disclosed calicheamicin mimics to DNA. When these calicheamicin mimics are attached to a targeting delivery system, their ability to cleave double strands of DNA can be enhanced. In turn, this enhanced nuclease acitivy can lead to enhanced apoptosis activity. Accordingly, compounds **3**, **4**, and **10** can serve as "molecular warheads" for inducing apoptosis when attached to a DNA targeting delivery system.

The disulfide **9** and the *p*-nitrobenzoate **6** (Scheme 1) exhibited potent DNA-cleaving and cytotoxic activities and apoptosis-inducing properties and were amongst the most biologically active of the synthesized mimics.

Brief Description of the Drawings:

Figure 1 illustrates the Bergman cycloaromatization reaction for an enediyne.

Figure 2 illustrates the cascade of events which triggers the cycloaromatization of calicheamicin γ_1^I and subsequent cleave of DNA.

Figure 3 illustrates the design of calicheamicin γ_1^I mimics.

Figure 4A illustrates the cascade of events which triggers the cycloaromatization of Type **I** and Type **II** mimics. X = O, NH, or S.

Figure 4A illustrates the cascade of events which triggers the cycloaromatization of Type **III** mimics. Y = OCH_2CH_2O or O.

Figure 5 illustrates the synthesis of Type **I** and Type **II** mimics.

Figure 6 illustrates the synthesis of Type **II** and Type **III** mimics.

Figure 7 illustrates a comparison of cytotoxicities of designed enekiynes **1-4, 6-15, and 35** against transformed and normal cells.

Figure 8 illustrates a comparison of cytotoxicities of designed enediynes **6-8**, calicheamicin γ_1^1, calicheamicin Θ_1^1, and PM-9 against a panel of 17 transformed cell lines and 4 normal cell lines.

Figure 9 illustrates agarose gels for the cleavage of supercoiled DNA by calicheamicin $\gamma^1 1$ mimics **7, 1, 6,** and **12**. φX174 DNA (100 μM per base pair, >90% form I) was incubated for the indicated time at 37 °C with each of the designed mimics (1.0 mM) in various buffer solutions (5.0 mM tris-HCl) and analyzed by electrophoresis (1 percent agarose gel, ethidium bromide stain). **(A)** thioacetate **7**: incubation time 5.0 h; lanes 1, 3, 5, and 7 DNA controls at pH 9.0, 8.0, 7.5 and 7.0; lanes 2,4,6, and 8 compound **30** at pH 9.0, 8.0, 7.5 and 7.0; **(B)** monobenzoate **1**: incubation time 3.0 h; lanes 1-4 DNA controls at pH 6.5, 7.0, 7.5, 8.5; lanes 5-8 compound **1** at pH 6.5, 7.0, 7.5, 8.5; **(C)** *p*-nitrobenzoate, **6**: incubation time 2.5 h; lanes 1-4 DNA controls at pH 6.0, 7.0, 7.5 and 8.5; lanes 5-8 compound **6** at pH 8.5, 7.5, 7.0 and 6.0; and **(D)** acetate **12** incubation time 5.0 h; lanes 1,3,5 and 7 DNA controls at pH 7.0, 7.5, 8.0 and 9.0; lanes 2,4,6, and 8 compound **12** at pH 7.0, 7.5, 8.0, and 9.0. Key: I, supercoiled DNA; II, nicked DNA (single strand cleavage); III, linear DNA (double strand cleavage).

Figure 10 illustrates agarose gels for the cleavage of supercoiled DNA by calicheamicin $\gamma^1 1$ mimics **13, 9, 2,** and **4**. φX174 DNA (100 μM per base pair, >90% form I) was incubated for the indicated time at 37 °C with each of the designed mimics (1.0 mM) in various buffer solutions (5.0 mM tris-HCl) and analyzed by electrophoresis (1 percent agarose gel, ethidium bromide stain). **(A)** carbonate **13**: incubation time 3.0 h; lanes 1-4 DNA controls at pH 6.5, 7.0, 7.5 and 8.5; lanes 5-8 compound **13** at pH 6.5, 7.0, 7.5 and 8.5; **(B)** disulfide **9**: incubation time 5.0 h; lanes

1, 3, 5, and 7 DNA + 2-mercaptoethanol (5.0 mM) controls at pH 7.0, 7.5, 8.0 and 9.0; lanes 2, 4, 6, and 8 compound **9** + 2-mercaptoethanol (5.0 mM) at pH 7.0, 7.5, 8.0 and 9.0; **(C)** dibenzoate **2:** incubation time 6.0 h; lanes 1-3 DNA controls at pH 7.0, 8.0 and 8.5; lanes 4-6 compound **2** at pH 7.0, 8.0, and 8.5; and **(D)** acid **4** incubation time 24 h; lanes 1-3 DNA controls at pH 7.0, 8.0 and 8.5; lanes 4-6 compound **4** at pH 7.0, 8.0 and 8.5. Key: I, supercoiled DNA; II, nicked DNA (single strand cleavage); III, linear DNA (double strand cleavage).

Figure 11 illustrates the comparison of the in vitro DNA cleavage by the designed calicheamicin γI_1 mimics. **(A)** Interaction of supercoiled DNA with compounds **1, 2, 6, 12** and **15**. φX174 RFI DNA (100 μM per base pair, >90% form I) was incubated for 8.0 hours at 37 °C with compounds in buffer soultions (5.0 mM, tris-HCl, pH 8.5) and analyzed by electrophoresis (1 percent agarose gel, ethidium bromide stain). Lane 1, supercoiled, RFI DNA (control); lane 2, **1** (5.0 mM); lane 3, **6** (5.0 mM); lane 4, **2** (10 mM); lane 5, **12** (5.0 mM); lanes 6, 7 (5.0 mM); lane 7, **15** (10 mM); lane 8, nicked, RFII, DNA (standard). **(B)** Interaction of supercoiled DNA with PM-9 (Scheme I.8), calicheamicin θ^I1, calicheamicinone (synthesized by Nicolaou et. al. *J. Am. Chem Soc.* **1993**, *115*, 7612-7624) and compounds **6, 7,** and **9**. pBR322 DNA (100 μM per base pair) was incubated for 4 hours at 37 °C with compounds in buffer solutions (5.0 mM, tris-HCl) and analyzed by electrophoresis (1 percent agarose gel, ethidium bromide stain). Lane 1, supercoiled DNA, pH 8.5 (control); lane 2, PM-9 (5.0 mM, pH 8.5); lane 3, calicheamicinone (5.0 mM, 19 mM 2-mercaptoethanol, pH 7.0); lane 4, **9** (5.0 mM, 2-mercaptoethanol 19 mM, pH 7.0); lane 5, **6** (5.0 mM, pH 8.5); lane 6, **7** (5.0 mM, pH 8.5); lane 7, calicheamicin θ^I1, (0.5 μM, pH 8.5); lane 8, supercoiled DNA, 2-mercaptoethanol (19 mM), pH 7.0 (control). Key: I, supercoiled DNA; II, nicked DNA (single strand cleavage); III, linear DNA (double strand cleavage).

Figure 12 illustrates the cytotoxicities (IC_{50}, Molar) of designed enediynes **1, 2, 4, 5, 6** and **7** against a panel of seventeen transformed cell lines and four normal cell lines. Cytotoxicities were determined by the sulforhodaminine B assay as described by Skehan et. al. *J. Natl. Cancer Inst.* **1990**, *82*, 1107-1112. **Key to table:** CHO, chinese hamster ovary cells; HMEC, human mammary epithelial cells; NHDF, normal human dermal fibroblast; RPMI7666, normal human peripheral blood lymphoblast; BT-549, human breast ductal carcinoma; CAPAN1, pancreatic carcinoma; H322, lung adenocarcinoma; HL-60, human promyelomic leukemia; HT-29, human colon adenocarcinoma; M24-MET, metastatic melanoma; MCF-7, breast carcinoma; MOLT-4, human T-cell leukemia; OVCAR-3, human ovarian carcinoma; UCLA-P-3, lung adenocarcinoma; PC-3, human prostate carcinoma; SIHA, human squamous carcinoma, CERVIX; SK-MEL 28, melanoma; U251, human CNS cancer; 786-0, human perirenal cell carcinoma; L1210, murine leukemia; SK-N-SH, neuroblastoma.

Figure 13 illustrates the cytotoxicities (IC_{50}, Molar) of calicheamicinone (synthesized by Nicolaou et. al. *J. Am. Chem Soc.* **1993**, *115*, 7612-7624) and designed enediynes **9, 10, 11, 12, 13** against a panel of seventeen transformed cell lines and four normal cell lines. Cytotoxicities were determined by the sulforhodaminine B assay as described by Skehan et. al. *J. Natl. Cancer Inst.* **1990**, *82*, 1107-1112. **Key to table:** CHO, chinese hamster ovary cells; HMEC, human mammary epithelial cells; NHDF, normal human dermal fibroblast; RPMI7666, normal human peripheral blood lymphoblast; BT-549, human breast ductal carcinoma; CAPAN1, pancreatic carcinoma; H322, lung adenocarcinoma; HL-60, human promyelomic leukemia; HT-29, human colon adenocarcinoma; M24-MET, metastatic melanoma; MCF-7, breast carcinoma; MOLT-4, human T-cell leukemia; OVCAR-3, human ovarian carcinoma; UCLA-P-3, lung adenocarcinoma; PC-3, human prostate carcinoma; SIHA, human squamous carcinoma, CERVIX; SK-MEL 28, melanoma; U251, human CNS cancer; 786-0, human perirenal cell carcinoma; L1210, murine leukemia; SK-N-SH, neuroblastoma.

Figure 14 illustrates the cytotoxicities (IC_{50}, Molar) of designed enediynes **14, 15,** and **35** against a panel of seventeen transformed cell lines and four normal cell lines. Cytotoxicities were determined by the sulforhodaminine B assay as described by Skehan et. al. *J. Natl. Cancer Inst.* **1990**, *82*, 1107-1112. **Key to table:** CHO, chinese hamster ovary cells; HMEC, human mammary epithelial cells; NHDF, normal human dermal fibroblast; RPMI7666, normal human peripheral blood lymphoblast; BT-549, human breast ductal carcinoma; CAPAN1, pancreatic carcinoma; H322, lung adenocarcinoma; HL-60, human promyelomic leukemia; HT-29, human colon adenocarcinoma; M24-MET, metastatic melanoma; MCF-7, breast carcinoma; MOLT-4, human T-cell leukemia; OVCAR-3, human ovarian carcinoma; UCLA-P-3, lung adenocarcinoma; PC-3, human prostate carcinoma; SIHA, human squamous carcinoma, CERVIX; SK-MEL 28, melanoma; U251, human CNS cancer; 786-0, human perirenal cell carcinoma; L1210, murine leukemia; SK-N-SH, neuroblastoma.

Figure 15 illustrates the apoptotic morphology and nuclear DNA degradation induced by calicheamicin $\theta^{I}1$ and designed mimic **6**. **A**. Molecular wight markers consisting of multiples of a 123 base-pair fragment (Gibco BRL); **B.** Untreated MOLT-4 leukemia cells; **C.** DNA extacted from untreated MOLT-4 cells; **D**. MOLT-4 cells incubated with 10^{-7} Molar of calicheamicin $\theta^{I}1$; **E**. DNA extracted from MOLT-4 cells exposed to 10^{-7} Molar of calicheamicin $\theta^{I}1$; **F.** MOLT-4 cells incubated with 10^{-7} Molar of **6**; **G.** DNA extracted from MOLT-4 cells exposed to 10^{-7} Molar of **6**. METHODS. Solutions of calicheamicin $\theta^{I}1$ or **6** in DMSO (20 mM) were diluted in culture medium to the appropriate concentrations and added to cell cultures. MOLT-4 cells (10^{5}) were incubated with $^{-7}$10 Molar final concentrations of calicheamicin θ^{I}_{1} or mimic **6** for apoptosis morphology assays, and with 10^{6} Molar final concentrations for DNA degradation assays. After incubation for 4 hours at 37 °C, cells were harvested and processed (procedure as described by Nicolaou et. al. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3142-3146) to visualize apoptotic morphology and DNA degradation.

Figure 16 illustrates the comparison of cytotoxicities of designed enediynes **6**, **9** with a number of potent experimental and clinically applied anticancer drugs. The following tumor cell lines gave IC50 values within the indicated range: SK-MEL-28, CAPAN-1, OVCAR-3, UCLA-P-3, U-251, and MOLT-4.

Figure 17 illustrates some enediyne mimics which have been designed to be activated by an enol-ketone transformation (Pitsinos, E. Dissertation: Total Synthesis of (-) Calicheamicinone; Synthesis of Designed Calicheamicin analogs, University of California San Diego, 1994, pg 316). In an acidic environment, the enol intermediate tautomerizes to the indicated ketone intermediate which then undergoes a Bergman cyclization. Below each compound is its *in vitro* half life at the indicated temperature.

Figure 18 illustrates the DNA cleavage activity of designed calicheamicin mimics. Supercoiled DNA runs at the position noted as I, nicked DNA at the position II, linear DNA (the result of double-strand cleavage) at the position III. (**a**) Cleavage activity with compound **6**. Lanes 1-4 show supercoiled φX174RFI DNA controls at pH 6.0, 7.0, 7.5 and 8.5. Lanes 5-8 show supercoiled DNA plus compound **6** (1mM) at pH 8.5, 7.5, 7.0 and 6.0. (**b**) Cleavage activity of compounds 1,2,6,7,12 and 15. Lane 1, supercoiled φX174RFI DNA (control); lanes 2 and 3, DNA plus compound 1 and 6, respectively (5mM); lane 4, DNA plus compound 2 (10mM); lanes 5 and 6, DNA plus compounds 12 and 7 respectively (5 mM); lane 7, DNA plus compound 15 (10mM); lane 8, nicked DNA (RFII standard). (**c**) Activation of compounds **8** and **9** by mercaptoethanol. Cleavage of supercoiled pBR322 DNA with the dynemicin mimic PM-9 (Nicoaloau et. al. *Chemistry and Biology*, **1994**, *1*, 57-66), calicheamicin $\theta^{I}1$ and compounds **6-9** was assayed under the conditions as described in. Lane 1, supercoiled DNA, pH 8.5 (control); in lane 2, PM-9 (5mM, pH 8.5) was added; in lane 3, compound **8** (5 mM, 2-mercaptoethanol 19 mM, pH 7.0); in lane 4, compound **9** (5 mM, 2-mercaptoethanol 19 mM, pH 7.0); in lane 5, compound **6** (5 mM, pH 8.5); in lane 6, compound **7** (5 mM, pH 8.5); and in lane 7, calicheamicin $\theta^{I}1$ (0.5 μM, pH 8.5). Lane 8 contained supercoiled DNA in 2-mercaptoethanol 19 mM, pH 7.0 as a control.

Detailed Description:

Molecular design: Figure 3 summarizes a number of possible structural modifications on the calicheamicinone core that were considered. Organic chemistry principles were used in guiding the design of potentially active compounds which were required to fulfill the following general criteria: (a) be chemically stable under neutral conditions but capable to undergo the Bergman cycloaromatization reaction upon suitable activation; (b) be structurally simple to allow easy access by chemical synthesis; (c) allow for tethering to suitable delivery systems and other desirable moieties through appropriate functionalities; and (d) be equipped with suitable initiators that may be activated under mild chemical or biological conditions.

Following Nature's mechanism of activation of calicheamicin γ_1^I (Fig. 2), we envisioned a number of variants (structure I, Fig. 4A) utilizing the generation of oxygen-, nitrogen- or sulfur-centered nucleophiles as a means of activation towards the Bergman reaction. J. N. Haseltine et al. discloses earlier experimental modeling of the priming mechanism of the calicheamicin antibiotics wherein activation is achieved by the addition of intramolecular nucleophiles to the bridgehead double bond. (*J. Am. Chem. Soc.* (1989): vol. **111**, pp 7638-7640 and *J. Am. Chem. Soc.* vol. **113**, pp 3850-3866.) An interesting alternative (structure II, Fig. 4A) in which the carbonyl group of the enone system was protected as a ketal, was considered as a compound capable of activation under acidic conditions employed by Haseltine above. Diverging from the above mode of activation and relying on the known propensity of enamines to hydrolyze to carbonyl compounds, we designed compound III (Fig. 4B), which was envisioned to undergo activation as shown in Figure 4B.

Inspired by Nature's molecular design, as witnessed in calicheamicin, we chose the secondary propargylic alcohol as an appropriate handle for attachment of tethers. Therefore, compounds **3** and **4** (Figure 5) were targeted for synthesis. The capability to have a thiol group at the allylic position, and the utilization of such functionalities in the synthesis of immunoconjugates prompted the design of mimics suitably activated for the construction of non-symmetric disulfides at this position. (L. M. Hinman et al. *Cancer Res.* (1993): vol. **53**, 3336-3342.) Such groups could play a dual role as reductively cleavable initiators and tethering devices.

Chemical Synthesis. Figures 5 and 6 summarize the chemical synthesis of the targeted compounds (**1-7**, **9-15**). For their synthesis, advantage was taken of our previously developed route to calicheamicinone (**8**) which delivers the advanced intermediate **16** (Figure 5) in optically active form, as disclosed by A. L. Smith et al., *J. Am. Chem. Soc.* (1992): vol. **114**, pp 3134-3136. Thus, selective benzoylation of this diol afforded monobenzoate **17** as the major product accompanied by the corresponding dibenzoate **18**. Concomitant removal of both the ketal and silyl protecting groups from **17** and **18** by aqueous acid treatment resulted in the formation of compounds **1** and **2**, respectively. *p*-Nitrobenzoate **6** (Figure 5) was synthesized in a similar fashion from **16**.

The differentiation of the functional groups in **17** allowed the introduction of a tethering group at the propargylic position using glutaric anhydride under basic conditions to afford **20** and **19** (after coupling with *p*-nitrophenol). These compounds were converted to enones **3** and **4**, respectively, under acidic conditions. In order to probe the biological effect of damaging a steroid receptor involved in gene regulation, desoxymethasone was conjugated with **4** to afford compound **5** according to the method of K. R. Yamamoto in *Molecular development Biology; Expressing foreign genes, 43rd Symposium of Society for developmental biology*, (Bagorad, L. & Adelman, G., eds.), pp. 131-148, Alan Liss, New York.

Following another vein, the readily available thioacetate **21** (see Smith, *supra*) was converted to the free thiol with diisobutylaluminum hydride (DIBAL) or sodium methoxide, which was reacted *in situ* with MeSSCOOMe, according to the method of S. J. Brois et al. (*J. Am. Chem. Soc.* (1970): vol. **92**, pp 7629-7631) or 2,2'-dipyridyl disulfide (aldrithiol-2), according to the method of J. Carlsson et al. (*Biochem. J.* (1978); vol. **73**, pp 723-737) to afford the methyl disulfide **22** or the 2-pyridyl disulfide **23**, respectively. Deprotection of these intermediates (**21-23**) as above furnished the expected enones **7**, **9**, and **10**, respectively (Figure 5). Another report on compound **7** is provided by J. Aiyor et al. in *Angew. Chem. Int. Ed. Engl.* (1994): vol. **33**, pp 855-858.

Compound **24**, another readily available intermediate from the calicheamicin synthesis (K. R. Yamamoto, *supra*) was utilized as a branching point for the synthesis of a new series of designed calicheamicinones (**11-14**, Figure 5). Thus, desilylation of **24** led to compound **11**, whereas acetylation furnished compound **25**. Esterification with the appropriate chloroformate allowed the formation of carbonmate **26**, which was oxidized smoothly to afford the sulfone derivative **27**. On the other hand mesylation of alcohol **24** followed by azide displacement afforded azide **28**, which was reduced and acetylated *in situ*, according to the method of T. Rosen, *J. Org. Chem.* (1988): vol. **53**, pp 1580-1582, leading to acetamide **29**. Deketalization of **25**, **27**, and **29** gave enones **12**, **13**, and **14**, respectively (Figure 5).

The synthesis of compound **15**, containing the 2'-phenylsulfonylethyl carbonate group on the nitrogen as an activator, proceeded as outlined in Figure 6. The readily available amine **30** (A. L. Smith, *supra*) was converted to urethane **31** in 82% yield, by treatment with oxalyl chloride and 2-phenylthioethanol in the presence of pyridine. Lactone **31** was then reduced sequentially with diisobutylaluminum hydride (DIBAL) and $NaBH_4$ leading to diol **33** via lactol **32** (63% overall yield).

Selective monobenzylation of the primary alcohol in diol **33** furnished monobenzoate **34** (69%), which was oxidized by 1,3-dicyclohexylcarbodiimide (*m*-CPBA) to sulfone **35** in 100% yield. Finally, deprotection under aqueous acid conditions generated the targeted enediyne **15** in 36% yield.

The reaction conditions indicated in Figure 5 are summarized as follows:

1. Reagents and conditions for reaction "**a**": 2.6 equivalents of PhCOCl and 6.0 equivalents of pyridine in CH_2Cl_2 for 1.5 hours at -15°C, yielding 68% of **17** and 26% of **18.**
2. Reagents and conditions for reaction "**b**": 9.0 equivalents of glutaric anhydride and 12.0 equivalents of 4-dimethylaminopyridine in CH_2Cl_2 for 2.5 hours at 25°C; then 9.0 equivalents of 1,3-dicyclohexylcarbodiimide and 9.0 equivalents of *p*-nitrophenol for 2.0 hour, yielding 46%.
3. Reagents and conditions for reaction "**c**": 1.6 equivalents of 4-dimethylaminopyridine and 3.0 equivalents of glutaric anhydride for 6 hours at 25°C, yielding 69%.
4. Reagents and conditions for reaction "**d**": 5.5 equivalents of TsOH-H_2O in aqueous THF at 25°C.
5. Reagents and conditions for reaction "**e**": 2.4 equivalents of desoxamethasone, 1.5 equivalents of 1,3-dicyclohexylcarbodiimide, and 0.5 equivalents of 4-dimethylaminopyridine in CH_2Cl_2 for 2.0 hour at 25°C, yielding 53%.
6. Reagents and conditions for reaction "**f**": 3.0 equivalents of pyridine, 05 equivalents of 4-dimethylaminopyridine, and 2.3 equivalents of equivalens of *p*-nitrobenzoylchloride in CH_2Cl_2 for 1.5 hour at -15°C, yielding 66%.
7. Reagents and conditions for reaction "**g**": 7.0 equivalents of diisobutylaluminum hydride in CH_2Cl_2 for 30 minutes at -78°C; then 10.0

equivalents of MeOH, while reducing the temperature from -78°C to 25°C and adding 4.2 equvalents of MeSSCOOMe for 12 hours, yielding 70%.

8. Reagents and conditions for reaction "**h**": 4.3 equivalents of NaOMe in MeOH for 3 hours at 0°C; then 5.0 equivalents of aldrthiol-2 for 3 hours, yielding 66%.

9. Reagents and conditions for reaction "**i**": HF-Pyridine in THF at 0°C to 25°C over 4 hours, yielding 96%.

10. Reagents and conditions for reaction "**j**": 4.0 equivalents of pyridine, 0.5 equivalents of 4-dimethylaminopyridine, and 3.0 equivalents of Ac_2O at 0°C for 2 hours, yielding 92%.

11. Reagents and conditions for reaction "**k**": 7 equivalents of 4-dimethylaminopyridine and 20.0 equivalents of $PhSCH_2CH_2OCOCl$ in CH_2Cl_2 at 0°C fopr 4 hours, yielding 88%.

12. Reagents and conditions for reaction "**l**": 4.0 equivalents of *m*-CPABA in CH_2Cl_2 at 25°C for 2 hours, yielding 94%.

13. Reagents and conditions for reaction "**m**": 10.0 equivalents of pyridine, 5.0 equivalents of MsCl, and 0.5 equivalents of 4-dimethylaminopyridine in CH_2Cl_2 at 0°C for 3 hours.

14. Reagents and conditions for reaction "**n**": 10.0 equivalents of NaN_3 in DMF at 50°C, yielding 53%.

15. Reagents and conditions for reaction "**o**": AcSH at 25°C for 2 hours, yielding 48%.

The reaction conditions indicated in Figure 6 are summarized as follows:

1. Reagents and conditions for reaction "**a**": 10.0 equivalents of pyridine, 3.0 equivalents of $(COCl)_2$ in CH_2Cl_2 at 25°C for 30 minutes; then 10.0 equivalents of pyridine and 10.0 equivalents of $PhSCH_2CH_2OCOCl$ for 2.0 hours, yielding 82%.

2. Reagents and conditions for reaction "**b**": 2.2 equivalents of diisobutylaluminum hydride in CH_2Cl_2 at -78°C for 30 minutes, yielding a 4:1 mixture of epimers, 95%.
3. Reagents and conditions for reaction "**c**": 55.0 equivalents of $NaBH_4$ in MeOH at 0°C for 2 hours, yielding 66%.
4. Reagents and conditions for reaction "**d**": 5.0 equivalents of pyridine and 2.4 equivalents of PhCOCl at 0°C for 1 hour, yielding 60%.
5. Reagents and conditions for reaction "**e**": 5.0 equivalents of *m*-1,3-dicyclohexylcarbodiimide in CH_2Cl_2 at 25°C for 1.5 hour, yielding 100%.
6. Reagents and conditions for reaction "**f**": 5.5 equivalents ofTSOH-H_2O in aqueous THF at 25°C for 24 hour, yielding 36%.

DNA Cleaving Activity. The synthesized compounds were tested for their ability to cleave DNA under a variety of conditions. Supercoiled DNA (X174 or pBR322) was incubated with each compound in buffer solutions at different pH values and in the presence or absence of 2-mercaptoethanol. DNA damage was assayed by agarose gel electrophoresis. Compounds **6**, **7-9**, and **13** (data not shown) exhibited the highest potencies in the DNA cleavage assay. With the exception of compounds **8** and **9**, in all cases cleavage was observed under basic conditions. DNA damage to some extent was observed at neutral and slightly acidic media. This effect maybe due to local DNA basicity or nucleophilicity as speculated earlier for a series of dynemicin A mimics.

The following observations merit special comment. The most significant observation in these DNA cleaving studies is the predominance of single DNA strand breaks for the aglycones in contrast to the double strand breaks caused by the natural calicheamicins, according to the method of J. Aiyor, *supra*. The dibenzoylated derivative **2** exhibits lower potency in DNA cleavage as compared to **1**. This suggests a possible role of the propargylic hydroxyl in the hydrolysis of the allylic benzoate (an effect previously observed by J. N. Haseltine (*supra*), the event that leads to the Bergman activation cascade. The relative efficiencies of compounds **1**, **2**, **6**, **7**, and **12** in cleaving DNA correlate well with molecular structure and reflect the expectations of the molecular design. Thus the more labile *p*-nitrobenzoate **6** damages supercoiled DNA to a greater extent than benzoate **1** or acetate **12** under the same conditions. This compound also cleaves DNA with progressively higher efficiency at increasing pH values, as expected from higher rates of hydrolysis which leads to activation. Disulfide **9** exhibits its DNA cleaving activity in the presence of added thiol, as expected from its propensity to cleave under the influence of the thiol nucleophile. Finally, given the lability of the 2'-phenylsulfone ethyl carbamate moiety under basic conditions (K. C. Nicolaou et al., *Science* (1992), vol. **256**, pp 1172-1178), the failure of compound **35** (Figure 6) to cleave DNA at a range of pH values (pH 6-8.5) can only be attributed to a relatively high and unexpected stability of either the postulated enamine intermediate **VIII** or ketone **IX** (Y = OCH_2CH_2O, Fig. 4B). Similar observations were reported by M. F. Semmelhack et al. in *J. Am. Chem. Soc.* vol. **115**, pp 11618-11619.. The DNA cleaving activity of **15** may be due to activation via benzoate hydrolysis rather than enamine-induced triggering.

DNA cleaving activity of various compounds were measured as follows:

1. Panel A: ΦX174 DNA (100 micro molar per base pair) was incubated for 2.5 hours at 37°C with coumpound **6** in various buffer solutions (5 mM) and analyzed by electrophoresis (1% agarose gel, ethidium bromide stain). Lanes 1-4: DNA controls at pH 6.0, 7.0, 7.5, adn 8.5 respectively. Lanes 5-8: compound **6** (1 mM) at pH 6.0, 7.0, 7.5, and 8.5 respectively.
2. Panel B: ΦX174 DNA (100 micro molar per base pair) was incubated for 8 hours at 37°C with compounds **1**, **2**, **6**, **7**, **12**, and **15** in buffer solutions (5 mMm tris-HCl, pH 8.5) and analyzed by electrophoresis (1% agarose gel,

ethidium bromide stain). Lane 1: supercoiled ΦX174 DNA control; Lane 2: **1** (5 mM); Lane 3: **6** (5 mM); Lane 4: **2** (10 mM); Lane 5: **12** (5 mM); lane 6: 7 (5 mM); Lane 7: **15** (10 mM); Lanes 8: nicked ΦX174 DNA standard.

Cytotoxicity. Cytotoxicity studies with the synthesized compounds were carried out using a broad spectrum of cell lines, ranging from the drug resistant SK-Mel-28 melanoma to the highly sensitive Molt-4 leukemia cells, to assess antineoplastic activity and cell-type selectivity. A number of these compounds (e.g. **6** and **9**) exhibited high cytotoxicities, with the most potent ones being comparable to calicheamicinone (**8**) but considerably less potent than calicheamicin γ_1^I (Table 1).

Figure 7 shows the IC_{50} values of several synthesized compounds reflecting their potencies against transformed and normal cells. It is noteworthy that the relative potencies of these compounds correlate well with their relative abilities to cleave supercoiled DNA. Thus disulfide **9** and *p*-nitrobenzoate **6** were amongst the most potent calicheamicinone mimics, with comparable potencies to that of calicheamicinone (**8**). Plausible explanations for the low cytotoxicity of the enediyne-desoxymethasone conjugate **5** may be that the steroid conjugate is not recognizable by the glucocortinoid receptor or that the glucocortinoid receptor-DNA complex does not properly position the enediyne moiety on the target for effective DNA cleavage.

The IC_{50} values for the *p*-nitrobenzoate enediyne **6** and thioacetate 7 are compared with those of calicheamicinone (**8**) and calicheamicins γ_1^I and Θ_1^I in Table 1. It is clear from these data that while comparable cytotoxicities to calicheamicinone (**8**) can be obtained through significant simplification of the structure of the enediyne moiety, the effect of the oligosarcharide portion of calicheamicin cannot be matched by any of the designed simplifications. Comparing the potencies of **8** vs calicheamicin

γ_1^I and 7 vs calicheamicin Θ_1^I (Table 1), it is reasonable to project that attachment of **6** to the oligosaccharide portion of calicheamicin or other carriers should increase its potency and/or selectivity.

Mechanism of Action. The strong correlation between the ability of the synthesized enediynes to cause DNA cleavage and cytotoxicity suggest DNA damage as the cause of cell death by these compounds. To gain further insight into the mechanism of action of these enediynes, cells were examined for signs of apoptosis according to the method of R. E. Ellis et al. *Annu. Rev. Cell. Biol* (1991): vol. 7, pp 663-698, following exposure to compound **6**, chosen as a representative of this class.

However, upon exposure of Molt-4 leukemia cells to **6** only moderate apoptosis (< 20% of the cells at (10^{-7} M)) was observed in contrast to the strong apoptosis indicated by calicheamicin Θ_1^I at the same concentration.

It is noteworthy that all calicheamicinone mimics synthesized and tested showed predominantly single strand DNA cuts. This observation is in contrast to calicheamicins γ_1^I and Θ_1^I, both of which are known to induce predominantly double strand DNA damage, according to the method of J. N. Haseltine et al., supra and of P. C. Dedon, *Biochemistry* (1993): vol. **32**, pp 3617-3622. This difference correlates well with the observed cytotoxicity and ability of these compounds to cause apoptosis, the molecules with the binding domain exhibiting significantly higher potencies in both assays. Thus, it seems reasonable to suggest that, at least in the case of the enediyne type of DNA damaging agents, the propensity of a compound to induce apoptosis is proportional to its ability to cause double strand cuts of the genetic material. Apparently, the inability of cells to repair such drastic damage leads to initiation of apoptosis, whereas repair of single strand cuts may save cells from death.

Bioassays. The chemistry and biology disclosed in figures 9-18 illustrate the viability of producing simple biological mimics of calicheamicin γ^I1 that lack the aryl tetrasaccharide moiety and employ initiators other than a trisulfide group. These simplified mimics exhibit useful DNA cleaving properties and cytotoxicity. It is noteworthy that all calicheamicinone mimics synthesized and tested showed predominantly single strand cuts as opposed to double strand cleavage. This observation is in contrast to the calicheamicins which are known to cause predominantly double strand damage. This difference correlates well with the observed cytotoxicity and ability of these compounds to cause apoptosis, the molecule with the binding domain exhibiting significatnly higher potencies in both assays. Thus it seems reasonable to suggest that at least in the case of the enediyne class of DNA damaging agents, the propensity of a compound to induce apoptosis is proportional to its ability to cause double strand cuts of the genetic material. The inability of the cell to repair such damage apparently leads to initiation of apoptosis whereas repair os single strand cuts may save the cell from death. As an example, *p*-nitrobenzoate **6** and disulfide **9** exhibit IC_{50} values that compare favorably with those of compounds currently employed in cancer chemotherapy (**Figure 16**). However, in contrast to the highly potent calicheamicin γ^I1 (naturally occurring) and θ^I1(synthetic), the designed mimics cause primarily single-strand DNA cuts and are weaker inducers of apoptosis, reflecting their relatively weaker affinities for DNA and lack of specific positioning along the minor groove. Analogs **4, 3,** and **10** should facilitate the construction of enediyne conjugates with antibodies, proteins oligonucleotides or other moieties capable of targeting them to DNA or cancer cells. Such conjugates may find useful applications in biology and medicine.

A typical study of the apoptotic morphology and nuclear DNA degradation induced by the mimics are as follows: solutions of calicheamicin θ^I1 or **6** in DMSO (20 mM) were diluted in culture medium to the appropriate concentrations and added to cell cultures. MOLT-4 cells (10^5) were incubated with 10^{-7} Molar final concentrations of calicheamicin θ^I_1 or mimic **6** for apoptosis morphology assays, and with 10^{-6} Molar final concentrations for DNA degradation assays. After incubation for 4 hours at 37 °C, cells were harvested and processed (procedure as described by Nicolaou et. al. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3142-3146) to visualize apoptotic morphology and DNA degradation.

Materials and methods

General Techniques.

NMR spectra were recorded on a Bruker AMX-500 instrument. The following abbreviations were used to designate the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad; obs, obscured. IR spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectophotometer. Optical rotations were recorded using a Perkin-Elmer 241 polarimeter. High-resolution mass spectra (HRMS) were recorded on a VG ZAB-ZSE mass spectrometer under fast atom bombardment (FAB) conditions.

All reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light, 7% ethanolic phosphomolybdic acid, or *p*-anisaldehyde solution and heat as a developing agent. E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography. Tetrahydrofuran (THF) was distilled from sodium–benzophenone and methylene choride, benzene and toluene were distilled from calcium hydride.

All reactions were carried out under argon atmosphere with anhydrous, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated.

Preparation of compounds.

Compounds 17 and 18: A solution of diol 16 [26] (188 mg, 386 μmol) in CH_2Cl_2 (10 mL) was treated at –15 °C with pyridine (188 μL, 2.3 mmol) and benzoyl chloride

(58 μL, 0.5 mmol). After 1 h, additional benzoyl chloride (58 μL, 0.5 mmol) was added to the reaction mixture. The progress of the reaction was monitored closely by TLC and, as soon as all starting material was consumed (30 min.), the reaction was quenched by addition of MeOH (0.5 mL), the cooling bath was removed, and the reaction mixture was allowed to reach room temperature. The solution was poured into a saturated aqueous solution of $CuSO_4$ (15 mL) and washed with additional saturated aqueous solution of $CuSO_4$ (2 x 15 mL). The combined aqueous extracts were back extracted with CH_2Cl_2 (2 x 15 mL) and the combined organic extracts were washed with brine (15 mL), dried (Na_2SO_4), concentrated, and purified by flash chromatography (60% Et_2O in petroleum ether) to give, in order of elution, dibenzoate **18** (70.1 mg, 26%) and monobenzoate **17** (155.6 mg, 68%). **17:** white foam; R_f = 0.13 (70% Et_2O in petroleum ether); $[\alpha]^{25}_D$ –270 (*c* 0.36, CH_2Cl_2); IR (neat) ν_{max} 3400, 3090, 3070, 3034, 2955, 2876, 1961, 1815, 1722, 1271, 1225, 1166, 1110 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 8.08–8.06 (m, 2 H, *Bz*), 7.07–7.04 (m, 1 H, *Bz*), 6.99–6.97 (m, 2 H, *Bz*), 6.74 (dd, *J* = 4.94, 7.58 Hz, 1 H, C=C*H*CH_2), 6.17 (b s, 1 H, N*H*$COOCH_3$), 6.04 (d, *J* = 5.0 Hz, 1 H, C*H*OH), 5.71 (b d, *J* = 10.5 Hz, 1 H, C*H*HOBz), 5.46–5.35 (b, obs, 1 H, C*H*HOBz), 5.39–5.35 (m, 2 H, C*H*=C*H*), 3.99 (b s, 1 H, O*H*), 3.27–3.16 (m, 4 H, ethylene ketal), 3.23 (s, 3 H, $NHCOOCH_3$), 2.70 (d, *J* = 13.77 Hz, 1 H, C*H*H-eq), 2.44 (d, *J* = 13.80 Hz, 1 H, CH*H*-ax), 1.09 (t, *J* = 7.9 Hz, 9 H, $Si(CH_2CH_3)_3$), 0.92–0.80 (m, 6 H, $Si(CH_2CH_3)_3$); ^{13}C NMR (125 MHz, C_6D_6) δ 166.35, 154.95, 139.21, 132.35, 131.98, 131.32, 130.01, 124.27, 123.96, 123.35, 105.25, 101.79, 86.30, 85.96, 65.15, 65.12, 64.99, 64.23, 52.75, 7.40, 6.67; FAB HRMS (NBA/CsI) *m/e* 724.1351 (M + Cs^+) calcd for $C_{32}H_{37}NO_8Si$: 724.1343.

18: Pale yellow foam; R_f = 0.16 (50% Et_2O in petroleum ether); $[\alpha]^{25}_D$ –43.0 (*c* 0.575, CH_2Cl_2); IR (neat) ν_{max} 3394, 3032, 3325, 2955, 2877, 1721, 1491, 1454, 1315, 1272, 1172 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 8.45 (d, *J* = 7.25 Hz, 2 H, *Bz*), 8.02 (d, *J* = 7.12 Hz, 2 H, *Bz*), 7.58 (d, *J* = 1.33 Hz, 1 H, *Bz*), 7.18 (obs, 1 H, *Bz*), 7.13–7.07 (m, 2 H, *Bz*), 7.00 (dd, *J* = 7.45, 7.84 Hz, 2 H, *Bz*), 6.87 (dd, *J* = 4.42, 8.06 Hz, 1 H, C=C*H*CH_2), 6.28 (b s, 1 H, C*H*OBz), 5.60 (dd, *J* = 4.38, 9.25 Hz, 1 H, C=CHC*H*H), 5.44 (d, *J* = 9.44 Hz, 1 H, C*H*=CHC≡CCH), 5.39 (dd, *J* = 8.08, 13.65 Hz, 1 H, C=CHCH*H*), 5.32 (dd, *J* = 1.41, 9.43 Hz, 1 H, CH=C*H*C≡CCH), 3.33 (s, 3 H, NHCOOCH_3), 3.31–3.22 (m, 4 H, *ethylene ketal*), 2.84 (d, *J* = 13.7 Hz, 1 H, C*H*H-eq), 2.55 (d, *J* = 13.7 Hz, 1 H, CH*H*-ax), 1.20–1.16 (m, 9 H, $Si(CH_2CH_3)_3$), 0.98–0.93 (m, 6 H, $Si(CH_2CH_3)_3$); ^{13}C NMR (125 MHz, C_6D_6) δ 165.96, 165.88, 153.60, 139.58, 132.86, 132.14, 130.77, 130.24, 130.21, 130.14, 129.70, 124.11, 123.92, 123.34, 105.13, 103.24, 99.25, 86.59, 85.54, 70.87, 65.23, 64.96, 64.84, 64.53, 52.51, 52.34, 29.92, 7.19, 6.46; FAB HRMS (NBA/CsI) *m/e* 828.1570, (M + Cs^+) calcd for $C_{39}H_{41}NO_9Si$: 828.1605.

Compound 19: A solution of monobenzoate **17** (34.8 mg, 58.9 μmol) in CH_2Cl_2 (0.6 mL) was treated with DMAP (88 mg, 0.72 mmol) and glutaric anhydride (58 mg, 0.51 mmol) at room temperature. As soon as all starting material was consumed (2.5 h), DCC (109 mg, 0.53 mmol) and *p*-nitrophenol (74 mg, 0.53 mmol) were added to the reaction mixture. After 2 h the turbid reaction mixture was applied directly to a chromatography column and purified (70% Et_2O in petroleum ether) to give diester **19** (22.2 mg, 46%): pale yellow solid; R_f = 0.5 (Et_2O); $[\alpha]^{25}_D$ −106 (*c* 0.47, CH_2Cl_2); IR (neat) ν_{max} 3315, 2920, 2848, 1739, 1718, 1618, 1582, 1524, 1490, 1452, 1344, 1313, 1271, 1216, 1115 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 8.12–8.10 (m, 2 H, *Ar*), 7.78–7.76 (m, 2 H, *Ar*), 7.24 (b s, 1 H, N*H*COOCH_3), 7.13–7.11 (m, 1 H, *Ar*), 7.07–7.04 (m, 2 H, *Ar*), 6.89 (dd, *J* = 3.69, 8.27 Hz, 1 H, C=C*H*CH_2), 6.75–6.73 (m, 2 H, *Ar*), 6.16 (b s, 1 H, C*H*OCOCH_2), 5.66 (dd, *J* = 3.54, 14.22 Hz, 1 H, C*H*HOBz), 5.43 (d, *J* = 9.44 Hz, 1 H,

CH=CHC≡CCH), 5.38 (dd, J = 8.31, 14.19 Hz, 1 H, CHHOBz), 5.35 (dd, J = 1.20, 9.41 Hz, 1 H, CH=CHC≡CCH), 3.34 (s, 3 H, NHCOOCH_3), 3.29–3.17 (m, 4 H, *ethylene ketal*), 2.82 (d, J = 13.80 Hz, 1 H, CHH-eq), 2.68 (dt, J = 7.25, 16.46 Hz, 1 H, CH_2), 2.55 (d, J = 13.78 Hz, 1 H, CHH-ax), 2.43–2.38 (m, 3 H, CH_2), 2.04–1.99 (m, 2 H, CH_2), 1.19–1.16 (m, 9 H, Si(CH_2CH_3)$_3$), 0.95–0.90 (m, 6 H, Si(CH_2CH$_3$)$_3$); ^{13}C NMR (125 MHz, C_6D_6) δ 171.68, 170.08, 166.57, 155.48, 154.03, 145.32, 138.85, 134.25, 132.74, 130.88, 129.88, 124.95, 124.61, 124.13, 123.51, 122.37, 105.12, 103.55, 98.87, 86.86, 85.69, 71.03, 65.37, 65.16, 65.14, 65.00, 52.74, 52.09, 33.10, 33.04, 30.18, 26.01, 25.32, 20.12, 7.42; FAB HRMS (NBA/CsI) *m/e* 959.1824, (M + Cs$^+$) calcd for $C_{43}H_{46}N_2O_{13}Si$: 959.1820.

Compound 20: A solution of monobenzoate **17** (43 mg, 73 μmol) in CH_2Cl_2 (1 mL) was treated at ambient temperature with DMAP (25 mg, 0.12 mmol) and glutaric anhydride (24 mg, 0.21 mmol). After 6 h the reaction mixture was applied directly on a chromatography column and it was purified (Et_2O → 5% MeOH/Et_2O) to give acid **20** (35.7 mg, 69%): pale yellow foam; R_f = 0.26 (Et_2O); $[\alpha]^{25}_D$ −168 (c 1.87, CH_2Cl_2); IR (neat) ν_{max} 3295, 3061, 2956, 2880, 2671, 1716, 1631, 1496, 1454, 1416, 1317, 1273 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05–8.04 (m, 2 H, *Bz*), 7.57–7.54 (m, 1 H, *Bz*), 7.54–7.42 (m, 2 H, *Bz*), 6.54 (b s, 1 H, NHCOOMe), 6.38 (dd, J = 4.05, 8.24 Hz, 1 H, C=CHCH$_2$), 6.31 (s, 1 H, CHOCOCH$_2$), 5.92 (d, J = 9.41 Hz, 1 H, CH=CHC≡CCH), 5.82 (dd, J = 1.33, 9.48 Hz, 1 H, CH=CHC≡CCH), 5.24 (dd, J = 3.82, 13.93 Hz, 1 H, CHHOBz), 4.98 (dd, J = 8.29, 13.92 Hz, 1 H, CHHOBz), 4.11–4.06 (m, 1 H, *ethylene ketal*), 4.00–3.97 (m, 2 H, *ethylene ketal*), 3.96–3.86 (m, 1 H, *ethylene ketal*), 3.72 (s, 3 H, NHCOOCH_3), 2.69–2.63 (m, 1 H, CHHCOOH), 2.58 (d, J = 13.70 Hz, 1 H, CHH-eq), 2.59–2.53 (m, 1 H, CHHCOOH), 2.51–2.43 (m, 2 H, CHOCOCH_2), 2.23 (d, J = 13.66 Hz, 1 H, CHH-ax), 2.08–1.98 (m, 2 H, CH$_2$CH_2CH$_2$), 1.01 (t, J = 7.90 Hz, 9 H,

$Si(CH_2CH_3)_3$), 0.77 (m, 6 H, $Si(CH_2CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.12, 177.64, 171.88, 166.62, 154.0, 138.15, 132.85, 130.26, 129.59, 128.30, 124.77, 123.34, 123.02, 104.96, 102.30, 97.30, 86.61, 84.92, 70.69, 65.42, 64.72, 64.55, 53.27, 51.43, 33.03, 32.74, 19.82, 7.05, 6.16; FAB HRMS (NBA/CsI) *m/e* 838.1665, (M + Cs^+) calcd for $C_{37}H_{43}NO_{11}Si$: 838.1660.

Compound 5: A mixture of acid **4** (27 mg, 49 μmol) and desoxymethasone (47 mg, 0.12 mmol) was azeotroped (benzene, 2 x 2 mL), treated in CH_2Cl_2 (1 mL) with DCC (15 mg, 74 μmol) and DMAP (3 mg, 25 μmol) and stirred at room temperature for 2 h. The reaction mixture was purified directly by preparative TLC (20% acetone in benzene) to yield chimeric ester **5** (23 mg, 53%): white solid; R_f = 0.48 (20% acetone in benzene); $[\alpha]^{25}_D$ –158 (*c* 0.48, CH_2Cl_2); IR (neat) ν_{max} 3434, 3368, 2948, 2874, 1720, 1662, 1609, 1500, 1450; 1346, 1273, 1241, 1183, 1135 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.98 (dd, *J* = 1.27, 8.32 Hz, 2 H, *Bz*), 7.53–7.50 (m, 1 H, *Bz*), 7.39–7.36 (m, 2 H, *Bz*), 7.05 (d, *J* = 10.12 Hz, 1 H, *desoxymethasone* H–1), 6.86 (b s, 1 H, N*H*COO-CH_3), 6.68 (d, *J* = 1.53 Hz, 1 H, C*H*OCO), 6.38 (dd, *J* = 3.54, 8.93 Hz, 1 H, C=C*H*CH_2), 6.20 (dd, *J* = 1.82, 10.11 Hz, 1 H, *des.* H–2), 6.03 (s, 1 H, *des.* H–4), 5.86 (d, *J* = 9.49 Hz, 1 H, C*H*=CHC≡CCH), 5.82 (dd, *J* = 1.56, 9.48 Hz, 1 H, CH=C*H*C≡CCH), 5.29 (dd, *J* = 3.52, 13.78 Hz, 1 H, C*H*HOBz), 4.85 (dd, *J* = 8.81, 13.62 Hz, 1 H, CH*H*-OBz), 4.83 (AB q, *J* = 17.52 Hz, Δv = 53.53 Hz, 2 H, *des.* H–21), 4.19 (b d, *J* = 8.53 Hz, 1 H, *des.* H–11), 3.69 (s, 3 H, NHCOOCH_3), 3.51 (b s, 1 H, O*H*), 3.19 (d, *J* = 16.89 Hz, 1 H, C*H*H-eq), 3.01–2.97 (m, 1 H, *des.*), 2.75 (d, *J* = 16.89 Hz, 1 H, CH*H*-ax), 2.63–2.24 (m, 10 H, 2 x (OCOCH_2) and *des.*), 2.07–2.01 (m, 2 H, $(OCOCH_2)_2CH_2$), 1.74–1.61 (m, 5 H, *des.*), 1.45 (s, 3 H, *des.* CH_3), 1.18 (b s, 1 H, *des.*), 1.15–1.10 (m, 1 H, *des.*), 0.97 (s, 3 H, *des.* CH_3), 0.81 (d, *J* = 7.30 Hz, 3 H, *des.* CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.75, 191.18, 186.72, 172.75, 171.50, 166.71, 166.32, 153.87, 152.39, 137.25, 133.29, 131.15, 129.67, 128.46, 128.31, 125.43, 125.02, 124.85, 124.08, 100.92, 99.72, 99.52, 96.92, 91.16, 87.44, 84.21, 72.21, 72.12, 71.81, 68.69, 65.16, 63.93, 53.46, 53.01, 48.43, 43.96, 36.36, 35.79, 34.23, 34.07, 32.65, 32.20, 32.13, 30.97, 27.32, 22.91, 22.87, 19.94, 16.45, 14.62; FAB HRMS (NBA/CsI) *m/e* 944.3270, (M + Cs^+) calcd for $C_{51}H_{52}NO_{14}F$: 944.3270.

Compound 6: Pyridine (25 µL, 0.3 mmol) and DMAP (6 mg, 0.5 µmol) were added to a solution of diol **16** (50 mg, 0.1 mmol) in CH_2Cl_2 (10 mL) at –15 °C. *p*-Nitrobenzoyl chloride (26 mM in CH_2Cl_2) was added dropwise and in two portions (0.13 + 0.1 mmol, 1 h interval) until almost all starting material was consumed and then the reaction was quenched by MeOH (0.5 mL). After standard aqueous workup, the reaction mixture was purified by flash column chromatography (50 → 100 % Et_2O/petroleum ether) to give the fully protected mono-*p*-nitrobenzoate (42 mg, 66%). Small amounts of dibenzoate (4 mg, 0.5%) and unreacted diol **16** (8 mg, 30%) were also isolated. **Mono-*p*-nitrobenzoate:** foam; R_f = 0.47 (Et_2O); $[\alpha]^{25}_D$ –216 (*c* 1.40, CH_2Cl_2); IR (neat) ν_{max} 3326, 2956, 2931, 2877, 1723, 1606, 1528, 1501, 1461, 1343, 1274 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.27 (d, *J* = 8.92 Hz, 2 H, *Ar*), 8.23 (d, *J* = 8.94 Hz, 2 H, *Ar*), 6.50 (b s, 1 H, N*H*COOCH$_3$), 6.35 (dd, *J* = 5.40, 7.64 Hz, 1 H, C=C*H*CH$_2$), 5.89 (d, *J* = 9.50 Hz, 1 H, C*H*=CHC≡CCH), 5.84 (dd, *J* = 1.04, 9.46 Hz, 1 H, CH=C*H*C≡CCH), 5.71 (d, *J* = 4.77 Hz, 1 H, C*H*OH), 5.34 (dd, *J* = 5.13, 13.79 Hz, 1 H, C*H*HOCOAr), 5.20 (b m, 1 H, CH*H*OCOAr), 4.03–3.91 (m, 4 H, ethylene ketal), 3.77 (s, 3 H, NHCOOC*H*$_3$), 2.55 (d, *J* = 14.00 Hz, 1 H, C*H*H-eq), 2.25 (d, *J* = 13.97 Hz, 1 H, CH*H*-ax), 1.00 (t, *J* = 7.88 Hz, 9 H, Si(CH$_2$C*H*$_3$)$_3$), 0.83–0.70 (m, 6 H, Si(C*H*$_2$CH$_3$)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.56, 155.12, 150.34, 140.16, 136.13, 130.81, 123.40, 104.77, 99.85, 86.32, 85.59, 65.57, 65.37, 65.20, 63.56, 53.42, 50.39, 29.71, 7.08, 6.12; FAB HRMS (NBA/NaI) *m/e* 659.2050, ($M + Na^+$) calcd for $C_{32}H_{36}N_2O_{10}Si$: 659.2037.

A solution of the monobenzoate **6** (42 mg, 66 μmol) in THF (1.4 mL) and H_2O (4 drops) was treated with $TsOH{\cdot}H_2O$ (30 mg, 0.15 mmol) and the mixture was stirred at ambient temperature for 36 h. The reaction mixture was diluted with CH_2Cl_2, passed through a short column of Na_2SO_4 and purified by preparative TLC (20% acetone in CH_2Cl_2) to give enone **6** (14 mg, 45%): colorless solid; R_f = 0.63 (20% acetone in CH_2Cl_2); $[\alpha]^{25}_D$ −910 (*c* 0.12, CH_2Cl_2); IR (neat) ν_{max} 3358, 2919, 1719, 1679, 1525, 1344, 1274 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.29–8.22 (m, 4 H, *Ar*), 7.00 (b s, 1 H, N*H*COOCH$_3$), 6.43 (dd, *J* = 4.27, 8.11 Hz, 1 H, C=C*H*CH$_2$), 6.01 (b d, *J* = 4.72 Hz, 1 H, C*H*OH), 5.93 (dd, *J* = 1.37, 9.48 Hz, 1 H, CH=C*H*C≡CCH), 5.89 (d, *J* = 9.48 Hz, 1 H, C*H*=CHC≡CCH), 5.36 (dd, *J* = 4.23, 14.24 Hz, 1 H, C*H*HOCO*Ar*), 5.19 (dd, *J* = 8.14, 14.23 Hz, 1 H, CH*H*OCO*Ar*), 3.80 (s, 3 H, NHCOOC*H*$_3$), 3.65 (b s, 1 H, O*H*), 3.23 (d, *J* = 17.04 Hz, 1 H, C*H*H-eq), 3.02 (b s, 1 H, O*H*), 2.80 (d, *J* = 17.03 Hz, CH*H*-ax); ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.96, 164.60, 154.78, 150.55, 137.72, 135.51, 130.86, 130.17, 128.34, 124.56, 123.73, 123.54, 99.91, 99.56, 87.99, 85.21, 72.27, 53.59, 51.71, 29.71; FAB HRMS (NBA/CsI) *m/e* 611.0055, ($M + Cs^+$) calcd for $C_{24}H_{18}N_2O_9$: 611.0067.

Compound 22: A solution of thioacetate **21** [26] (9 mg, 14 μmol) in CH_2Cl_2 (2 mL) was treated with DIBAL (100 μL, 1.0 M in CH_2Cl_2, 100 μmol) at −78 °C. After stirring at this temperature for 30 min, the excess reagent was quenched by dropwise addition of MeOH (2 mL) and the reaction mixture was allowed to warm up to room temperature. MeSSCOOMe (66 μL, 0.89 M in MeOH, 59 μmol) was added and the reaction mixture was stirred at room temperature for 12 h. Following dilution with EtOAc (5 mL), saturated aqueous solution of Rochelle salt (sodium potassium tartrate, 2 mL) was added and stirring was continued for 45 min. Standard aqueous work up and flash column chromatography (30 → 50% Et_2O in petroleum ether) gave disulfide **22** (6.3 mg, 70%): colorless oil; R_f = 0.29 (50% Et_2O in petroleum ether); $[\alpha]^{25}_D$ −196 (*c* 0.525, CH_2Cl_2); IR (neat) ν_{max} 3356, 2954, 2915, 2876, 1738, 1490, 1458, 1413, 1223 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 6.60 (dd, *J* = 4.17, 10.98 Hz, 1 H, C=C*H*CH_2), 6.31 (b s, 1 H, C*H*OSi), 6.09 (b s, 1 H, N*H*$COOCH_3$), 5.50 (d, *J* = 9.39 Hz, 1 H, C*H*=CH-C≡CH), 5.38 (dd, *J* = 1.63, 9.38 Hz, 1 H, CH=C*H*C≡CH), 4.31 (dd, *J* = 11.34, 13.47 Hz, 1 H, C*H*HSSCH_3), 3.60 (dd, *J* = 4.06, 13.88 Hz, 1 H, CH*H*SSCH_3), 3.39 (b s, 3 H, NH-COOCH_3), 3.35–3.26 (m, 4 H, *ethylene ketal*), 2.73 (AB q, *J* = 12.81 Hz, Δν = 28.17 Hz, 2 H, CH_2), 2.44 (b s, 3 H, SSCH_3), 1.20 (t, *J* = 7.9 Hz, 9 H, $OSi(CH_2CH_3)_3$), 1.13 (m, *J* = 7.7 Hz, 9 H, $OSi(CH_2CH_3)_3$), 0.98–0.81 (m, 12 H, 2 × ($OSi(CH_2CH_3)_3$)); ^{13}C NMR (125 MHz, C_6D_6) δ 139.19, 125.86, 124.39, 123.06, 106.43, 102.38, 84.94, 72.69, 65.33, 64.59, 54.37, 52.36, 30.18, 22.75, 7.49, 7.07, 6.79, 5.17; FAB HRMS (NBA/CsI) *m/e* 796.1599, (M + Cs^+) calcd for $C_{32}H_{49}NO_6S_2Si_2$: 796.1594.

(**23**) A solution of thioacetate **21** (14.4 mg, 21.8 μmol) in MeOH (3 mL) was treated with NaOMe (5 mg, 93 μmol) and the resulting mixture was stirred at 0 °C for 3 h. 2,2'-Dipyridyl disulfide (aldrithiol-2, 24 mg, 109 μmol) was added to the reaction mixture and stirring was continued at 0 °C for 3 h. After standard aqueous workup the reaction mixture was initially purified by flash column chromatography (50 → 70% Et_2O in petroleum ether) and, subsequently, by reverse phase HPLC (C18 column, 20% CH_3CN in H_2O for 7 min, then 100% CH_3CN over 10 min; retention time 19.13 min) to yield the disulfide **23** (10.6 mg, 66%): colorless film; R_f = 0.37 (70% Et_2O in petroleum ether); $[\alpha]^{25}_D$ −130 (*c* 0.42, CH_2Cl_2); IR (neat) ν_{max} 3354, 2954, 2878, 1735, 1570, 1493, 1453, 1416, 1319, 1227 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 8.32 (d, *J* = 4.71 Hz, 1 H, *Pyr*), 7.88 (d, *J* = 7.59 Hz, 1 H, *Pyr*), 7.2 (obs, *Pyr*), 6.70 (dd, *J* = 5.24, 10.04 Hz, 1 H, C=C*H*CH$_2$), 6.51 (dd, *J* = 4.83, 7.29 Hz, 1 H, *Pyr*), 6.31 (s, 1 H, N*H*COOCH$_3$), 6.06 (b s, 1 H, C*H*OSi), 5.47 (d, *J* = 9.40 Hz, 1 H, C*H*=CHC≡CCH), 5.37 (dd, *J* = 1.59, 9.36 Hz, 1 H, CH=C*H*C≡CCH), 4.28 (dd, *J* = 10.11, 13.42 Hz, 1 H, C*H*HSSPyr), 3.99 (dd, *J* = 5.24, 13.48 Hz, 1 H, CH*H*SSPyr), 3.39 (s, 3 H, NHCOOC*H*$_3$), 3.34–3.31 (m, 2 H, *ethylene ketal*), 3.26–3.24 (m, 2 H, *ethylene ketal*), 2.71 (d, *J* = 13.14 Hz, 1 H, C*H*H-eq), 2.42 (d, *J* = 13.16 Hz, 1 H, CH*H*-ax), 1.19–1.16 (m, 9 H, Si(CH$_2$C*H*$_3$)$_3$), 1.11–1.08 (m, 9 H, Si(CH$_2$C*H*$_3$)$_3$), 0.94–0.79 (m, 12 H, 2 × (Si(C*H*$_2$C*H*$_3$)$_3$)); ^{13}C NMR (125 MHz, C_6D_6) δ 161.70, 149.54, 138.92, 136.73, 124.26, 124.15, 123.19, 119.95, 119.63, 106.03, 102.48, 85.56, 85.04, 72.21, 65.21, 64.75, 64.55, 53.74, 52.38, 40.33, 7.48, 7.07, 6.74, 5.15; FAB HRMS (NBA/CsI) *m/e* 859.1713, (M + Cs^+) calcd for $C_{36}H_{50}N_2O_6S_2Si_2$: 859.1703.

Compound 11: A solution of alcohol **24** (24.5 mg, 41 μmol) in THF (5 mL) was treated at 0 °C with pyridinium poly(hydrogen fluoride) (100 μL). The reaction mixture was allowed to warm up to ambient temperature over 4 h and, upon completion of the reaction, it was diluted with EtOAc (10 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (3 × 10 mL). The combined aqueous extracts were back extracted with EtOAc (3 × 10 mL) and the combined organic layers were washed with brine (10 mL), dried (Na_2SO_4), concentrated and purified by flash chromatography (EtOAc) to yield triol **11** (15 mg, 96%): white amorphous solid; R_f = 0.12 (EtOAc); $[\alpha]^{25}_D$ −340 (*c* 0.75, CH_2Cl_2); IR (neat) ν_{max} 3378, 2956, 2928, 2894, 1712, 1631, 1501, 1455, 1324, 1243, 1157, 1117 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.57 (b s, 1 H, N*H*COO-CH$_3$), 6.30 (dd, *J* = 7.23, 7.51 Hz, 1 H, C=C*H*CH$_2$), 5.73 (s, 2 H, C*H*=C*H*), 5.51 (b s, 1 H, C≡CC*H*OH), 4.70 (b s, 1 H, O*H*), 4.22 (dd, *J* = 8.07, 13.26 Hz, 1 H, C=CHC*H*H), 4.12 (b dd, *J* = 6.59, 13.09 Hz, 1 H, C=CHCH*H*), 3.95–3.92 (m, 1 H, *ethylene ketal*), 3.91–3.82 (m, 3 H, *ethylene ketal*), 3.68 (s, 3 H, NHCOOC*H*$_3$), 3.31 (b s, 1 H, O*H*), 2.83

(b s, 1 H, O*H*), 2.47 (d, $J = 14.31$ Hz, 1 H, C*H*H-eq), 2.21 (d, $J = 14.31$ Hz, 1 H, CH*H*-ax); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.24, 141.30, 133.93, 128.31, 126.61, 123.53, 104.76, 103.32, 98.56, 86.04, 85.33, 68.60, 65.90, 65.08, 63.16, 60.14, 49.06, 29.68; FAB HRMS (NBA/CsI) *m/e* 506.0211, (M + Cs^+) calcd for $C_{19}H_{19}NO_7$: 506.0216.

Compound 25: A solution of alcohol **24** (51.0 mg, 84.7 μmol) in CH_2Cl_2 (1.8 mL) was treated with pyridine (28 μL, 0.35 mmol), acetic anhydride (24 μL, 0.25 mmol), and DMAP (5 mg, 42 μmol) at 0 °C and the mixture was stirred at room temperature for 2 h. The reaction mixture was purified directly by preparative TLC (70% Et_2O in petroleum ether) to yield acetate **25** (50.1 mg, 92%): colorless syrup; $R_f = 0.68$ (80% Et_2O in petroleum ether); $[\alpha]^{25}_D$ −266 (c 0.865, CH_2Cl_2); IR (neat) ν_{max} 3349, 2954, 2878, 1737, 1494, 1323, 1232 cm^{-1}; ^{1}H NMR (500 MHz, C_6D_6) δ 6.65 (dd, $J = 3.33$, 9.07 Hz, 1 H, C=C*H*CH_2), 6.35 (s, 1 H, N*H*$COOCH_3$), 6.06 (b s, 1 H, C*H*OSi), 5.48 (d, $J = 9.40$ Hz, 1 C*H*=CHC≡CCH), 5.44–5.35 (obs, 1 H, C*H*HOAc), 5.39 (dd, $J = 1.55$, 9.39 Hz, 1 H, CH=C*H*C≡CCH), 5.16 (dd, $J = 9.12$, 13.91 Hz, 1 H, CH*H*OAc), 3.49–3.25 (m, 4 H, *ethylene ketal*), 3.41 (s, 3 H, NHCOOC*H*$_3$), 2.74 (d, $J = 13.19$ Hz, 1 H, C*H*H-eq), 2.49 (d, $J = 13.20$ Hz, 1 H, CH*H*-ax), 1.29–1.09 (m, 18 H, 2 × (Si(CH_2C*H*$_3$)$_3$)), 1.05–0.89 (m, 12 H, 2 × (Si(C*H*$_2$$CH_3$)$_3$)); ^{13}C NMR (125 MHz, C_6D_6) δ 170.20, 138.36, 124.14, 123.34, 106.04, 102.20, 85.49, 85.26, 72.14, 65.30, 64.94, 64.81, 64.58, 53.40, 52.47, 20.49, 7.41, 7.10, 6.73, 5.03; FAB HRMS (NBA/CsI) *m/e* 776.2088, (M + Cs^+) calcd for $C_{33}H_{49}NO_8Si_2$: 776.2051.

Compound 26: A solution of 2-phenylthioethanol (3 g, 19 mmol) in benzene (5 mL) was added dropwise, at room temperature, to a solution of phosgene (1 mL, 1.9 M toluene, 1.9 mmol). After stirring for 3 h the excess phosgene and solvents were removed under vacuum to give 2-phenylthioethyl-chloroformate as a yellow oil which was used without further purification. A solution of alcohol **24** (27 mg, 45 μmol) and

DMAP (38 mg, 0.31 mmol) in CH_2Cl_2 (5 mL) was treated, at 0 °C, with 2-phenylthioethyl-chloroformate (38 mg, 0.18 mmol) and the mixture was stirred for 4 h. The reaction mixture was poured into brine (10 mL), extracted with CH_2Cl_2 (3 × 5 mL), dried (Na_2SO_4), concentrated, and purified by preparative TLC (20% EtOAc in benzene) to give carbonate **26** (30.7 mg, 88%): pale yellow oil; R_f = 0.69 (20% EtOAc in benzene); $[\alpha]^{25}_D$ −150 (*c* 0.20, CH_2Cl_2); IR (neat) ν_{max} 2954, 2876, 1738, 1721, 1492, 1458, 1257, 1164 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.41–7.40 (m, 2 H, *Ph*), 7.40–7.27 (m, 2 H, *Ph*), 7.26–7.19 (m, 1 H, *Ph*), 6.10 (dd, *J* = 3.27, 9.02 Hz, 1 H, C=C*H*CH_2), 5.87 (d, *J* = 9.71 Hz, 1 H, C*H*=CHC≡CCH), 5.77 (b d, *J* = 9.71 Hz, 1 H, CH=C*H*C≡CCH), 5.77 (b s, 1 H, C*H*OSi), 4.88 (dd, *J* = 3.33, 13.52 Hz, 1 H, C*H*HOCO), 4.79 (dd, *J* = 9.04, 13.54 Hz, 1 H, CH*H*OCO), 4.28–4.23 (m, 2 H, OCOC$H_2$$CH_2$SPh), 4.10–4.07 (m, 1 H, *ehtylene ketal*), 3.99–3.91 (m, 2 H, *ethylene ketal*), 3.88–3.84 (m, 1 H, *ethylene ketal*), 3.18 (dd, *J* = 7.23, 7.40 Hz, 2 H, OCOCH_2CH_2SPh), 2.48 (d, *J* = 13.24 Hz, 1 H, C*H*H-eq), 2.11 (d, *J* = 13.25 Hz, 1 H, CH*H*-ax), 1.00–0.96 (m, 18 H, 2 × (Si(CH_2CH_3)$_3$)), 0.79–0.67 (m, 12 H, 2 × (Si(C$H_2$$CH_3$)$_3$)); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.70, 138.17, 129.81, 129.07, 126.58, 124.11, 122.91, 122.46, 105.72, 68.04, 65.75, 65.52, 65.22, 63.71, 52.34, 31.95, 29.70, 7.07, 6.72, 6.16, 4.53; FAB HRMS (NBA/CsI) *m/e* 914.2180, (M + Cs^+) calcd for $C_{40}H_{55}NO_9SSi$: 914.2190.

Compound 27: A solution of carbonate **26** (30.7 mg, 39 μmol) in CH_2Cl_2 (20 mL) was treated, at ambient temperature, with *m*CPBA (27 mg, 0.16 mmol) and the mixture was stirred for 2 h. The solvent was removed and the reaction mixture was purified by preparative TLC (20% EtOAc/C_6H_6) to give sulfone **27** (30 mg, 94%): pale yellow oil; R_f = 0.43 (20% EtOAc in benzene); $[\alpha]^{25}_D$ −140 (*c* 0.16, CH_2Cl_2); IR (neat) ν_{max} 3396, 3057, 2956, 2920, 2877, 2853, 1747, 1738, 1676, 1490, 1454, 1325, 1262 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.86–7.84 (m, 2 H, SO_2*Ph*), 7.63–7.60 (m, 1 H, SO_2*Ph*), 7.53–7.50 (m, 2 H, SO_2*Ph*), 5.92 (dd, *J* = 2.82, 9.05 Hz, 1 H, C=C*H*CH_2), 5.81 (d, *J* = 9.34 Hz, 1 H, C*H*=CHC≡CCH), 5.70 (dd, *J* = 1.62, 9.38 Hz, 1 H, CH=C*H*C≡CCH), 5.68 (d, *J* = 1.47 Hz, 1 H, C*H*OSi), 4.74 (b s, 1 H, N*H*$COOCH_3$), 4.65 (dd, *J* = 2.73, 13.33 Hz, 1 H, C*H*HOCO), 4.58 (b m, 1 H, CH*H*OCO), 4.41–4.32 (m, 2 H, OCOCH_2-CH_2SO_2Ph), 4.05–4.02 (m, 1 H, *ethylene ketal*), 3.95–3.91 (m, 1 H, *ethylene ketal*), 3.89–3.85 (m, 1 H, *ethylene ketal*), 3.82–3.79 (m, 1 H, *ethylene ketal*), 3.66 (s, 3 H, NH-COOCH_3), 3.49–3.42 (m, 2 H, $OCOCH_2CH_2SO_2$Ph), 2.42 (d, *J* = 13.24 Hz, 1 H, C*H*H-eq), 2.01 (d, *J* = 13.24 Hz, 1 H, CH*H*-ax), 0.92–0.89 (m, 18 H, 2 × ($Si(CH_2CH_3)_3$)), 0.70–0.60 (m, 12 H, 2 × ($Si(CH_2CH_3)_3$)); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.00, 139.06, 138.42, 134.16, 129.38, 128.31, 128.03, 124.09, 122.92, 121.91, 105.66, 100.98, 84.69, 71.46, 68.22, 65.54, 65.22, 63.67, 60.68, 54.90, 52.94, 52.45, 29.68, 7.05, 6.69, 6.14, 4.50; FAB HRMS (NBA/ CsI) *m/e* 946.2080, (M + Cs^+) calcd for $C_{40}H_{55}NO_{11}SSi_2$: 946.2089.

Compound 28: A solution of alcohol **24** (28.7 mg, 47.6 μmol) in CH_2Cl_2 (0.5 mL) was treated at 0 °C with pyridine (38 μL, 0.48 mmol), mesyl chloride (18 μL, 0.24 mmol), and DMAP (3 mg, 24 μmol). The resulting mixture was stirred for 3 h. The reaction mixture was filtered through a short silica column, using ether as eluent, and the solvents were removed. The resulting sensitive mesylate was dissolved in DMF (0.5 mL) and NaN_3 (30 mg, 0.5 mmol) was added. The mixture was stirred at 50 °C for 1 h, the solvent was removed under vacuum and the residue was purified by flash column chromatography (70% Et_2O in petroleum ether) to give azide **28** (17 mg, 53%): colorless syrup; R_f = 0.45 (70% Et_2O in petroleum ether); $[\alpha]^{25}_D$ −386 (*c* 1.14, CH_2Cl_2); IR (neat) ν_{max} 3302, 2955, 2879, 2089, 1731, 1497, 1461, 1415, 1326, 1234, 1167, 1101 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 7.08 (t, *J* = 6.03 Hz, 1 H, C=C*H*CH_2), 6.38 (b s, 1 H, N*H*$COOCH_3$), 5.72 (b s, 1 H, C*H*OSi), 5.40 (d, *J* = 9.47 Hz, 1 H, C*H*=CHC≡CCH), 5.34 (dd, J = 1.37, 9.46 H, 1 H, CH=C*H*C≡CCH), 4.52 (d, J = 5.91 Hz, 2 H, CH_2N_3), 3.47 (b s, 3 H, NHCOOCH_3), 3.44–3.40 (m, 4 H, *ethylene ketal*), 2.52 (s, 2 H, CH_2), 1.22–1.16 (m, 9 H, $Si(CH_2CH_3)_3$), 1.04–1.01 (m, 9 H, $Si(CH_2CH_3)_3$), 0.98–0.90 (m, 6 H, $Si(CH_2CH_3)_3$), 0.87–0.70 (m, 6 H, $Si(CH_2CH_3)_3$); ^{13}C NMR (125 MHz, C_6D_6) δ 139.38, 126.09, 123.47, 123.31, 105.84, 102.17, 101.58, 87.28, 85.69, 73.89, 65.65, 65.39, 64.79, 52.46, 49.71, 49.55, 30.16, 29.37, 7.18, 7.10, 6.40, 5.05; FAB HRMS (NBA/CsI) *m/e* 759.2010, (M + Cs^+) calcd for $C_{31}H_{96}N_4O_6Si_2$: 759.2010.

Compound 29: Azide **28** (7.4 mg, 11 μmol) was taken in freshly distilled thiolacetic acid (0.4 mL) and stirred at ambient temperature for 2 h. The excess of thiolacetic acid was removed under vacuum and the resulting oil was purified by preparative TLC (70% Et_2O in petroleum ether) to yield acetamide **29** (3.4 mg, 48%): white powder; R_f = 0.31 (Et_2O); $[\alpha]^{25}_D$ −280 (c 0.09, CH_2Cl_2); IR (neat) ν_{max} 2954, 2915, 2877, 1730, 1665, 1551, 1535, 1450, 1241 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.52 (dd, J = 6.41, 6.44 Hz, 1 H, C=C*H*CH$_2$), 5.88 (b s, 1 H, N*H*COOCH$_3$), 5.84 (d, J = 9.44 Hz, 1 H, C*H*=CHC≡CCH), 5.76 (dd, J = 1.43, 9.44 Hz, 1 H, CH=C*H*C≡CCH), 5.62 (b m, 2 H, C*H*OSi & N*H*COCH$_3$), 4.43–4.39 (m, 2 H, *ethylene ketal*), 4.13–4.09 (m, 1 H, CH*H*NHCOCH$_3$), 4.02–4.00 (m, 1 H, C*H*HNHCOCH$_3$), 3.99–3.87 (m, 2 H, *ethylene ketal*), 3.71 (s, 3 H, NHCOOCH_3), 2.39 (d, J = 13.34 Hz, 1 H, C*H*H-eq), 2.32 (d, J = 13.33 Hz, 1 H, CH*H*-ax), 1.95 (s, 3 H, NHCOCH_3), 1.02–0.93 (m, 18 H, 2 × ($Si(CH_2CH_3)_3$)), 0.88–0.63 (m, 12 H, 2 × ($Si(CH_2CH_3)_3$)); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.35, 138.53, 128.08, 123.51, 122.97, 105.60, 86.69, 65.70, 65.22, 64.48, 52.94, 49.17, 37.38, 23.34, 7.04, 6.80, 6.01, 4.56; FAB HRMS (NBA/CsI) *m/e* 775.2239, (M + Cs^+) calcd for $C_{33}H_{50}N_2O_7Si_2$: 775.2211.

Compound 31: To a solution of enamine **30** (31 mg, 73 μmol) and pyridine (60 μL, 0.74 mmol) in CH_2Cl_2 (3 mL) was added dropwise, at room temperature, oxalyl chloride (120 µL, 1.9 M in toluene, 228 µmol). After stirring for 30 min, more pyridine (60 µL, 0.74 mmol) and 2-phenylthioethanol (100 µL, 0.74 mmol) were added and the reaction mixture was stirred for further 2 h. The reaction mixture was poured into brine (15 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The organic extracts were dried (Na_2SO_4), concentrated, and purified by flash column chromatography (10 → 20% EtOAc in benzene) to yield carbamate **31** (36 mg, 82%): colorless foam; R_f = 0.55 (20% EtOAc in benzene); $[\alpha]^{25}_D$ −455 (*c* 1.45, CH_2Cl_2); IR (neat) ν_{max} 3284, 2956, 2877, 1774, 1725, 1667, 1592, 1504, 1326, 1299, 1223, 1182, 1114 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.41–7.39 (m, 2 H, *Ph*), 7.32–7.29 (m, 2 H, *Ph*), 7.24–7.22 (m, 1 H, *Ph*), 6.18 (s, 1 H, C=C*H*), 6.11 (b s, 1 H, C*H*O), 5.95 (b s, 1 H, N*H*COO), 5.92 (d, *J* = 9.57 Hz, 1 H, C*H*=CHC≡CCHO), 5.81 (b d, *J* = 9.41 Hz, 1 H, CH=C*H*C≡CCHO), 4.33–4.28 (m, 2 H, *ethylene ketal*), 4.20–4.18 (m, 1 H, *ethylene ketal*), 4.18–4.07 (m, 1 H, *ethylene ketal*), 4.00–3.90 (m, 2 H, $COOCH_2CH_2SPh$), 3.16 (t, *J* = 6.74 Hz, 2 H, $COOCH_2CH_2SPh$), 2.45 (d, *J* = 13.61 Hz, 1 H, C*H*H-eq), 2.27 (d, *J* = 13.60 Hz, 1 H, CH*H*-ax), 1.00 (t, *J* = 7.91 Hz, 9 H, $Si(CH_2CH_3)_3$), 0.82–0.70 (m, 6 H, $Si(CH_2CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.50, 153.94, 134.72, 130.05, 129.08, 127.87, 126.72, 124.65, 123.35, 110.89, 104.71, 99.23, 96.23, 90.65, 87.95, 69.13, 68.64, 65.91, 65.27, 64.33, 45.44, 32.59, 6.90, 5.90; FAB HRMS (NBA/CsI) *m/e* 738.0970, (M + Cs^+) calcd for $C_{32}H_{35}NO_7SSi$: 738.0958.

Compound 32: A solution of lactone **31** (28 mg, 46 µmol) in CH_2Cl_2 (3 mL) was treated at −78 °C with DIBAL (100 µL, 1 M in CH_2Cl_2, 100 µmol). After stirring for 30 min at −78 °C, the reaction was quenched by dropwise addition of MeOH (0.2 mL) and the cooling bath was removed. After warming to room temperature, the mixture was diluted with EtOAc (10 mL) and saturated aqueous solution of Rochelle salt (sodium potassium tartrate, 10 mL) was added. After stirring for 1 h, the organic layer was separated and the aqueous layer was extracted with additional EtOAc (3 × 5 mL). The combined organic extracts were dried (Na_2SO_4), concentrated, and purified by preparative TLC (20% EtOAc in benzene) to yield lactol **32** (26.6 mg, ca. 4:1 mixture of epimers, 95%): colorless oil; R_f = 0.36 (20% EtOAc/C_6H_6); $[\alpha]^{25}_D$ –428 (*c* 0.865, CH_2Cl_2); IR (neat) ν_{max} 3316, 2956, 2932, 2876, 1723, 1686, 1504, 1397, 1289, 1182, 1106; ^1H NMR (500 MHz, $CDCl_3$) δ 7.41–7.39 (m, 2 H, *Ph*), 7.31–7.28 (m, 2 H, *Ph*), 7.23–7.19 (m, 1 H, *Ph*), 6.05 (d, *J* = 2.18 Hz, 1 H, C=C*H*CH), 5.85 (b d, *J* = 9.49 Hz, 1 H, C*H*(OH)O), 5.74 (b s, 1 H, N*H*COO), 5.63 (d, *J* = 1.56 Hz, 1 H, C≡CC*H*O), 5.53 (b s, 2 H, C*H*=C*H*), 4.33–4.28 (m, 2 H, *ethylene ketal*), 4.25 (b s, 1 H, O*H*), 4.18–4.15 (m, 1 H, *ethylene ketal*), 4.07–4.04 (m, 1 H, *ethylene ketal*), 3.98–3.94 (m, 2 H, COOCH_2-CH_2SPh), 3.15 (b dd, *J* = 5.98, 6.79 Hz, 2 H, $COOCH_2CH_2SPh$), 2.41 (d, *J* = 13.44 Hz, 1 H, C*H*H-eq), 2.26 (d, *J* = 13.40 Hz, 1 H, CH*H*-ax), 1.02–0.97 (m, 9 H, $Si(CH_2CH_3)_3$), 0.82–0.69 (m, 6 H, $Si(CH_2CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 140.87, 135.00, 130.01, 129.08, 128.34, 126.63, 124.46, 122.92, 118.78, 105.36, 101.38, 99.98, 89.88, 69.66, 66.19, 65.82, 65.22, 64.07, 64.03, 60.62, 60.42, 45.93, 29.71, 14.21, 7.04, 6.08; FAB HRMS (NBA/NaI) *m/e* 630.1970, (M + Na^+) calcd for $C_{32}H_{37}NO_7SSi$: 630.1958.

Compound 33: A solution of lactol **32** (14.4 mg, 23.6 μmol) in MeOH (1 mL) was treated at 0 °C with $NaBH_4$ (34 mg, 0.9 mmol) and the mixture was stirred for 1 h. A second portion of $NaBH_4$ (15 mg, 0.4 mmol) was added to the reaction mixture and stirring was continued for 1 h at 0 °C. Acetic acid (200 μL) and water (30 μL) were added and the mixture was stirred for 5 min at 0 °C. Solvents were evaporated under vacuum and the reaction mixture was treated further with methanol (200 μL) and water (60 μL) in THF (1 mL) and stirred at room temperature for 15 min. The reaction mixture was poured into brine (5 mL) and extracted with EtOAc (5 × 10 mL). The organic extracts were dried (Na_2SO_4), concentrated, and purified by preparative TLC (Et_2O) to yield labile diol **33** (9.5 mg, 66%): pale yellow oil; R_f = 0.56 (Et_2O), 0.10 (70% Et_2O in petroleum ether); $[\alpha]^{25}_D$ −234 (*c* 0.14, CH_2Cl_2); IR (neat) ν_{max} 3393, 3055, 2955, 2928, 2876, 1716, 1489, 1413, 1319, 1227, 1152 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.41–7.33 (m, 2 H, *Ph*), 7.32–7.27 (m, 2 H, *Ph*), 7.26–7.22 (m, 1 H, *Ph*), 6.38 (b s, 1 H, N*H*COO), 6.32 (dd, *J* = 6.18, 7.42 Hz, 1 H, C=C*H*CH_2), 5.88 (d, *J* = 9.44 Hz, 1 H, C*H*=CHC≡CCH), 5.82 (dd, *J* = 1.29, 9.45 Hz, 1 H, CH=C*H*C≡CCH), 5.68 (b s, 1 H, C*H*OH), 4.53 (b s, 1 H, O*H*), 4.33–4.22 (m, 4 H, *ethylene ketal* & CH_2OH), 3.99–3.87 (m, 4 H, *ethylene ketal* & COOC$H_2$$CH_2$SPh), 3.19 (b s, 1 H, O*H*), 3.17 (dd, *J* = 6.59, 6.64 Hz, 2 H, CH_2SPh), 2.53 (d, *J* = 13.87 Hz, 1 H, CH_2-eq), 2.17 (d, *J* = 13.82 Hz, 1 H, CH_2-ax), 1.00 (t, *J* = 7.90 Hz, 9 H, Si(CH_2CH_3)$_3$), 0.81–0.67 (m, 6 H, Si(C$H_2$$CH_3$)$_3$); FAB HRMS (NBA/NaI) *m/e* 632.2120, (M + Na^+) calcd for $C_{32}H_{39}NO_7SSi$: 632.2114.

Compound 34: This compound was prepared as described for **17**: colorless oil; R_f = 0.45 (70% Et_2O in petroleum ether); $[\alpha]^{25}_D$ −154 (*c* 0.075, CH_2Cl_2); IR (neat) ν_{max} 3394, 3059, 2956, 2918, 2877, 2853, 1713, 1674, 1503, 1490, 1454, 1412, 1318, 1274, 1225, 1169, 1116 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.32 Hz, 2 H, *Bz*), 7.55–7.52 (m, 1 H, *Bz*), 7.44–7.39 (m, 4 H, *Bz* & *Ph*), 7.33–7.30 (m, 2 H, *Ph*), 7.24–7.21 (m, 1 H, *Ph*), 6.40 (b s, 1 H, N*H*COO), 6.34 (dd, *J* = 5.55, 7.10 Hz, 1 H, C=C*H*CH_2), 5.85 (AB q, *J* = 9.65 Hz, Δν = 20.92 Hz, 2 H, C*H*=C*H*), 5.71 (b s, 1 H, C*H*OH), 5.28 (b m, 1 H, C*H*HOBz), 5.10 (b m, 1 H, CH*H*OBz), 4.30 (b m, 2 H, COO-C$H_2$$CH_2$SPh), 4.00–3.90 (m, 4 H, *ethylene ketal*), 3.62 (b s, 1 H, O*H*), 3.18 (b m, 2 H, COOCH_2CH_2SPh), 2.54 (d, *J* = 13.86 Hz, 1 H, C*H*H-eq), 2.22 (b d, *J* = 13.82 Hz, 1 H, C*H*H-ax), 0.99 (t, *J* = 7.91 Hz, 9 H, Si(CH_2CH_3)$_3$), 0.88–0.71 (m, 6 H, Si(C$H_2$$CH_3$)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.03, 166.47, 138.80, 133.74, 132.64, 130.18, 129.67, 129.15, 128.49, 128.19, 126.80, 123.38, 65.47, 64.65, 64.30, 63.57, 32.84, 29.69, 22.69, 7.06, 6.13; FAB HRMS (NBA/NaI) *m/e* 736.2370, (M + Na^+) calcd for $C_{39}H_{43}NO_8SSi$: 736.2376.

Compound 35: A solution of sulfide **34** (6.8 mg, 9.5 μmol) in CH_2Cl_2 (2 mL) was treated at ambient temperature with *m*CPBA (8 mg, 46 μmol) and the mixture was stirred for 1.5 h. The reaction mixture was purified directly on a preparative TLC plate (Et_2O) to give sulfone **35** (7.1 mg, 100%): colorless oil; R_f = 0.33 (Et_2O); $[\alpha]^{25}_D$ −211 (*c* 0.36, CH_2Cl_2); IR (neat) ν_{max} 3480, 3397, 3062, 2955, 2925, 2876, 2853, 1715, 1500, 1451, 1412, 1318, 1273, 1225, 1147, 1116 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.06–8.04 (m, 2 H, *Ar*), 7.95 (b d, *J* = 7.45 Hz, 2 H, *Ar*), 7.70–7.67 (b m, 1 H, *Ar*), 7.62–7.59 (b m, 2 H, *Ar*), 7.54 (bt, *J* = 7.38 Hz, 1 H, *Ar*), 7.43 (bt, *J* = 7.69 Hz, 2 H, *Ar*), 6.34 (b m, 2 H, C=C*H*CH_2 & N*H*COO), 5.89 (d, *J* = 9.44 Hz, 1 H, C*H*=CHC≡CCH), 5.84 (dd, *J* = 0.93, 9.48 Hz, 1 H, CH=C*H*C≡CCH), 5.60 (b s, 1 H, C*H*OH), 5.26 (b m, 1 H, C*H*HOBz), 5.09 (b m, CH*H*OBz), 4.47 (b m, 2 H, COOC$H_2$$CH_2SO_2$Ph), 4.05 (b s, 1 H, O*H*), 3.99–3.93 (m, 4 H, *ehtylene ketal*), 3.48 (b s, 2 H, COOCH_2C$H_2$$SO_2$Ph), 2.53 (d, *J* = 13.79 Hz, 1 H, C*H*H-eq), 2.21 (b d, *J* = 13.23 Hz, 1 H, CH*H*-ax), 0.99 (t, *J* = 7.90 Hz, 9 H, Si(CH_2CH_3)$_3$), 0.81–0.70 (m, 6 H, Si(C$H_2$$CH_3$)$_3$); FAB HRMS (NBA/NaI) *m/e* 768.2270, (M + Na^+) calcd for $C_{39}H_{43}NO_{10}SSi$: 768.2275.

Compound 1: A solution of the monobenzoate **17** (16.0 mg, 27.1 μmol) in THF (1.4 mL) and H_2O (4 drops) was treated with TsOH·H_2O (30 mg, 0.15 mmol) and the mixture was stirred at ambient temperature for 36 h. The reaction mixture was diluted with CH_2Cl_2, passed through a short column of Na_2SO_4 and purified by preparative TLC (80% Et_2O in petroleum ether) to give enone **1** (8.0 mg, 68%): colorless syrup; R_f = 0.43 (80% Et_2O in petroleum ether); $[\alpha]^{25}_D$ −580 (*c* 0.43, CH_2Cl_2); IR (neat) ν_{max} 3364, 2956, 1714, 1680, 1601, 1503, 1451, 1315, 1274, 1178, 1118 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.04–8.02 (m, 2 H, *Bz*), 7.56–7.53 (m, 1 H, *Bz*), 7.43–7.40 (m, 2 H, *Bz*), 6.94 (b s, 1 H, N*H*COOCH$_3$), 6.43 (dd, *J* = 4.18, 8.09 Hz, 1 H, C=C*H*CH$_2$), 6.01 (b s, 1 H, C*H*OH), 5.90 (dd, *J* = 1.27, 9.48 Hz, 1 H, CH=C*H*C≡CCH), 5.86 (d, *J* = 9.48 Hz, 1 Hz, C*H*=CHC≡CCH), 5.34 (dd, *J* = 4.21, 14.31 Hz, 1 H, C*H*HOBz), 5.10 (dd, *J* = 8.11, 14.29 Hz, 1 H, CH*H*OBz), 3.78 (s, 3 H, NHCOOC*H*$_3$), 3.28 (b s, 1 H, O*H*), 3.22 (d, *J* = 16.98 Hz, 1 H, C*H*H-eq), 2.80 (d, *J* = 16.97 Hz, 1 H, CH*H*-ax), 1.85 (b s, 1 H, O*H*); ^{13}C NMR (125 MHz, CDCl$_3$) δ 191.22, 166.59, 154.70, 137.01, 133.02, 130.02, 129.99, 129.71, 128.33, 125.59, 124.38, 123.81, 100.05, 99.70, 87.79, 84.81, 72.33, 64.47, 64.34, 53.50, 51.95; FAB HRMS (NBA/CsI) *m/e* 566.0199, (M + Cs$^+$) calcd for $C_{24}H_{19}NO_7$: 566.0216.

Compounds **2–5**, **7**, **9**, **10**, and **12–15**. These compounds were prepared from the corresponding fully protected intermediates by similar procedures as described above. They exhibit the following physical data.

2: Colorless foam; R_f = 0.52 (80% Et$_2$O in petroleum ether); $[\alpha]^{25}_D$ –206 (*c* 0.655, CH$_2$Cl$_2$); IR (neat) ν_{max} 3359, 2923, 2851, 1722, 1722, 1687, 1601, 1504, 1451, 1270 cm^{-1}; ^{1}H NMR (500 MHz, CD$_3$OD) δ 7.94–7.92 (m, 2 H, *Bz*), 7.74–7.72 (m, 2 H, *Bz*), 7.46–7.42 (m, 2 H, *Bz*), 7.34–7.23 (m, 4 H, *Bz*), 7.00 (d, *J* = 1.73 Hz, 1 H, C≡CC*H*OBz), 6.36 (dd, *J* = 5.26, 7.73 Hz, 1 H, C=C*H*CH$_2$), 5.98 (d, *J* = 9.54 Hz, 1 H, C*H*=CHC≡CCH), 5.80 (dd, *J* = 1.72, 9.52 Hz, 1 H, CH=C*H*C≡CCH), 4.86 (dd, *J* = 5.25, 13.48 Hz, C*H*HOBz), 4.67 (dd, *J* = 7.77, 13.50 Hz, 1 H, CH*H*OBz), 3.59 (s, 3 H, NH-COOC*H*$_3$), 2.99 (d, *J* = 17.09 Hz, 1 H, C*H*H-eq), 2.57 (d, *J* = 17.10 Hz, 1 H, CH*H*-ax); ^{13}C NMR (125 MHz, CD$_3$OD) δ 134.94, 134.27, 130.87, 130.55, 129.93, 129.52, 127.01, 125.29, 123.55, 84.13, 65.92, 65.04, 55.25, 53.45; FAB HRMS (NBA/CsI) *m/e* 670.0478 M + Cs$^+$ calcd for $C_{31}H_{23}NO_8$ 670.0478.

3: White solid; R_f = 0.14 (30% EtOAc in petroleum ether); $[\alpha]^{25}_D$ −198 (*c* 0.15, CH_2Cl_2); IR (neat) ν_{max} 3353, 2921, 2852, 1737, 1715, 1683, 1589, 1518, 1496, 1456, 1345, 1271, 1231, 1205, 1115 cm^{-1}; ^{1}H NMR (500 MHz, C_6D_6) δ 8.17–8.16 (m, 2 H, *Ar*), 8.15–8.15 (m, 2 H, *Ar*), 7.76 (s, 1 H, N*H*COOCH$_3$), 7.16–7.13 (m, 1 H, *Ar*), 7.09–7.06 (m, 2 H, *Ar*), 6.79 (b s, 1 H, C*H*OCO), 6.75–6.73 (m, 2 H, *Ar*), 6.50 (dd, *J* = 3.43, 8.61 Hz, 1 H, C=C*H*CH$_2$), 5.59 (dd, *J* = 3.45, 14.20 Hz, 1 H, C*H*HOBz), 5.33–5.32 (m, 2 H, C*H*=C*H*), 5.31 (dd, *J* = 8.58, 14.24 Hz, 1 H, CH*H*OBz), 3.19 (s, 3 H, NHCOOC*H*$_3$), 3.10 (d, *J* = 16.86 Hz, 1 H, CH$_2$-eq), 2.62 (dt, *J* = 7.19, 16.50 Hz, 1 H, C*H*$_2$), 2.45 (d, *J* = 16.86 Hz, 1 H, CH$_2$-ax), 2.42–2.34 (m, 3 H, C*H*$_2$), 2.06 (b s, 1 H, O*H*), 1.97 (dq, *J* = 2.10, 7.31 Hz, 2 H, C*H*$_2$); ^{13}C NMR (125 MHz, C_6D_6) δ 190.19, 171.26, 170.00, 166.57, 155.33, 153.85, 137.36, 133.20, 129.89, 125.54, 124.99, 124.75, 124.15, 122.28, 100.80, 98.19, 87.77, 84.49, 72.19, 65.81, 64.75, 53.27, 52.85, 52.70, 32.95, 30.41, 30.18, 20.02; FAB HRMS (NBA/CsI) *m/e* 801.0685, (M + Cs$^+$) calcd for $C_{35}H_{28}N_2O_{12}$: 801.0697.

4: Colorless oil; R_f = 0.36 (2% MeOH in Et_2O); $[\alpha]^{25}_D$ −353 (*c* 0.910, CH_2Cl_2); IR (neat) ν_{max} 3354, 3062, 2956, 1714, 1684, 1504, 1451, 1273, 1244 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05–8.04 (m, 2 H, *Bz*), 7.59–7.55 (m, 1 H, *Bz*), 7.46–7.43 (m, 2 H, *Bz*), 6.96 (b s, 1 H, N*H*COOMe), 6.75 (d, *J* = 1.46 Hz, 1 H, C*H*OCOCH$_2$), 6.48 (dd, *J* = 3.70, 8.61 Hz, 1 H, C=C*H*CH$_2$), 5.93 (d, *J* = 9.49 Hz, 1 H, C*H*=CHC≡CCH), 5.89 (dd, *J* = 1.53, 9.48 Hz, 1 H, CH=C*H*C≡CCH), 5.28 (dd, *J* = 3.70, 14.01 Hz, 1 H, C*H*H-OBz), 4.99 (dd, *J* = 8.62, 14.01 Hz, 1 H, CH*H*OBz), 3.76 (s, 3 H, NHCOOC*H*$_3$), 3.26 (d, *J* = 16.96 Hz, 1 H, C*H*H-eq), 2.82 (d, *J* = 16.96 Hz, 1 H, CH*H*-ax), 2.72–2.59 (m, 2 H, C*H*$_2$COOH), 2.50–2.47 (m, 2 H, CHOCOC*H*$_2$), 2.09–2.01 (m, 2 H, CH$_2$C*H*$_2$CH$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.19, 178.50, 177.96, 171.55, 166.61, 154.04, 137.00, 133.21, 131.30, 129.69, 128.44, 128.34, 125.51, 124.78, 124.18, 99.75, 96.82, 87.60, 84.37, 72.21, 65.25, 64.14, 53.52, 52.71, 32.89, 32.55, 29.71, 19.74, 19.59; FAB HRMS (NBA/NaI) *m/e* 570.1360, (M + Na+) calcd for $C_{29}H_{25}NO_{10}$: 570.1376.

7: Colorless solid; R_f = 0.08 (20% EtOAc in benzene); $[\alpha]^{25}_D$ –670 (*c* 0.23, CH_2Cl_2); IR (neat) ν_{max} 3358, 3059, 2925, 2854, 1715, 1675, 1505, 1450, 1352, 1316, 1242 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.01 (dd, *J* = 7.20, 8.57 Hz, C=C*H*CH_2), 5.86 (d, *J* = 9.48 Hz, 1 H, C*H*=CHC≡CCH), 5.77 (dd, *J* = 1.75, 9.49 Hz, 1 H, CH=C*H*C≡CCH), 5.71 (d, *J* = 1.68 Hz, 1 H, C*H*OH), 3.73 (dd, *J* = 8.72, 14.04 Hz, 1 H, C*H*H-$SCOCH_3$), 3.59 (dd, *J* = 7.14, 14.05 Hz, 1 H, CH*H*$SCOCH_3$), 3.53 (s, 3 H, NHCOO-C*H*$_3$), 2.84 (d, *J* = 16.98 Hz, 1 H, C*H*H-eq), 2.39 (d, *J* = 16.97 Hz, 1 H, CH*H*-ax), 2.18 (s, 3 H, SCOC*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ 197.42, 138.54, 127.11, 125.53, 123.99, 101.75, 101.61, 87.69, 83.81, 72.62, 64.08, 55.16, 53.17, 31.60, 30.27; FAB HRMS (NBA/CsI) *m/e* 519.9816, (M + Cs+) calcd for $C_{19}H_{17}NO_6S$: 519.9831.

9: White foam; R_f = 0.11 (70% Et_2O in petroleum ether); $[\alpha]^{25}_D$ –900 (*c* 0.065, CH_2Cl_2); IR (neat) ν_{max} 3349, 2922, 1708, 1674, 1508, 1313, 1242 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.90 (b s, 1 H, N*H*$COOCH_3$), 6.39 (dd, *J* = 6.33, 9.52 Hz, 1 H, C=C*H*CH_2), 5.99 (b d, *J* = 7.40 Hz, 1 H, C*H*OH), 5.90–5.89 (m, 2 H, C*H*=C*H*), 3.89 (dd, *J* = 9.54, 13.94 Hz, 1 H, C*H*H$SSCH_3$), 3.78 (s, 3 H, NHCOOC*H*$_3$), 3.66 (dd, *J* = 6.28, 13.95 Hz, 1 H, CH*H*$SSCH_3$), 3.21 (d, *J* = 16.88 Hz, 1 H, C*H*H-eq), 3.16 (b d, *J* = 7.79 Hz, 1 H, O*H*), 2.83 (d, *J* = 16.82 Hz, 1 H, CH*H*-ax), 2.77 (b s, 1 H, O*H*), 2.37 (s, 3 H, SSC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.29, 154.40, 137.07, 130.27, 126.99, 124.20, 123.74, 100.03, 99.90, 87.54, 72.54, 64.31, 53.31, 52.84, 37.25, 29.59, 23.00, 11.45; FAB HRMS (NBA/CsI) *m/e* 523.9591, (M + Cs+) calcd for $C_{18}H_{17}NO_5S_2$: 523.9602.

10: White amorphous solid; R_f = 0.09 (70% Et_2O in petroleum ether); $[\alpha]^{25}_D$ −590 (*c* 0.17, CH_2Cl_2); IR (neat) ν_{max} 3334, 2921, 2852, 1715, 1674, 1577, 1501, 1448, 1417, 1314, 1238 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.44–8.43 (m, 1 H, *Pyr*), 7.64–7.60 (m, 2 H, *Pyr*), 7.10–7.07 (m, 1 H, *Pyr*), 6.83 (b s, 1 H, N*H*$COOCH_3$), 6.38 (dd, *J* = 7.85, 8.31 Hz, 1 H, C=C*H*CH_2), 6.01 (b s, 1 H, C*H*OH), 5.89 (dd, *J* = 1.20, 9.44 Hz, 1 H, CH=C*H*C≡CCH), 5.86 (d, *J* = 9.45 Hz, 1 H, C*H*=CHC≡CCH), 3.99 (dd, *J* = 8.55, 13.21 Hz, 1 H, C*H*HSSPyr), 3.82 (dd, *J* = 7.58, 13.19 Hz, 1 H, CH*H*SSPyr), 3.77 (b d, *J* = 3.02 Hz, 1 H, CHO*H*), 3.10 (d, *J* = 16.79 Hz, 1 H, C*H*H-eq), 2.97 (b s, 1 H, O*H*), 2.56 (d, *J* = 16.83 Hz, 1 H, CH*H*-ax); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.13, 159.86, 154.33, 149.51, 138.14, 137.07, 130.22, 125.29, 124.26, 123.78, 121.02, 120.90, 100.35, 99.91, 87.55, 84.51, 72.57, 64.48, 53.31, 52.52, 38.28; FAB HRMS (NBA) *m/e* 442.0660, (M + H^+) calcd for $C_{21}H_{17}N_2O_5S_2$: 442.0657.

12: White amorphous solid; R_f = 0.21 (80% Et_2O in petroleum ether); $[\alpha]^{25}_D$ −600 (*c* 0.30, CH_2Cl_2); IR (neat) ν_{max} 3357, 1718, 1677, 1509, 1438, 1369, 1236 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.12 (dd, *J* = 3.98, 8.70 Hz, 1 H, C=C*H*CH_2), 5.86 (d, *J* = 9.50 Hz, 1 H, C*H*=CHC≡CCH), 5.77 (dd, *J* = 1.78, 9.50 Hz, 1 H, CH=C*H*C≡CCH), 5.70 (d, *J* = 1.68 Hz, 1 H, C*H*OH), 4.74 (dd, *J* = 3.97, 13.85 Hz, 1 H, C*H*HOAc), 4.60 (dd, *J* = 8.73, 13.85 Hz, 1 H, CH*H*OAc), 3.54 (b s, 3 H, NHCOOCH_3), 2.88 (d, *J* = 17.00 Hz, 1 H, C*H*H-eq), 2.46 (d, *J* = 17.01 Hz, 1 H, CH*H*-ax), 1.91 (s, 3 H, COCH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 193.87, 172.78, 139.18, 126.09, 125.50, 124.06, 101.43, 87.71, 83.96, 72.58, 65.49, 63.94, 54.86, 53.19, 20.83; FAB HRMS (NBA/CsI) *m/e* 504.0050, (M + Cs^+) calcd for $C_{19}H_{17}NO_7$: 504.0056.

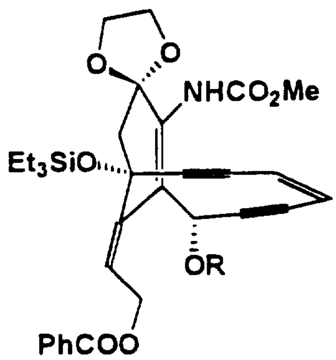
13: White powder; R_f = 0.12 (40% EtOAc in benzene); $[\alpha]^{25}_D$ −260 (*c* 0.11, CH_2Cl_2); IR (neat) ν_{max} 3311, 2923, 2852, 1739, 1676, 1499, 1449, 1259 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.94–7.92 (m, 2 H, SO_2*Ph*), 7.68–7.67 (m, 1 H, SO_2*Ph*), 7.61–7.58 (m, 2 H, SO_2*Ph*), 6.97 (b s, 1 H, N*H*COOCH$_3$), 6.25 (dd, *J* = 4.33, 7.89 Hz, 1 H, C=C*H*CH$_2$), 5.96 (b d, *J* = 5.32 Hz, 1 H, C*H*OH), 5.91 (dd, *J* = 1.24, 10.71 Hz, 1 H, CH=C*H*C≡CCH), 5.88 (d, *J* = 9.48 Hz, C*H*=CHC≡CCH), 5.02 (dd, *J* = 4.31, 14.03 Hz, 1 H, C*H*HOCO), 4.91 (dd, *J* = 7.92, 14.04 Hz, 1 H, CH*H*OCO), 4.51–4.42 (m, 2 H, OCOC*H*$_2$CH$_2$SO$_2$Ph), 3.78 (s, 3 H, NHCOOC*H*$_3$), 3.52–3.48 (m, 3 H, OCOCH$_2$C*H*$_2$-SO$_2$Ph + O*H*), 3.20 (d, *J* = 16.97 Hz, 1 H, C*H*H-eq), 3.04 (b s, 1 H, O*H*), 2.76 (d, *J* = 16.97 Hz, 1 H, CH*H*-ax); ^{13}C NMR (125 MHz, CDCl$_3$) δ 190.82, 154.55, 154.09, 138.83, 137.51, 134.03, 129.40, 129.36, 128.01, 124.34, 124.18, 123.68, 99.71, 99.35, 87.77, 84.91, 72.18, 67.20, 64.29, 54.79, 53.40, 51.65, 31.80, 29.58, 29.54; FAB HRMS (NBA/CsI) *m/e* 674.0106, (M + Cs$^+$) calcd for $C_{26}H_{23}NO_{10}S$: 674.0097.

14: Colorless oil; R_f = 0.23 (EtOAc); $[\alpha]^{25}_D$ −210 (*c* 0.08, CH_2Cl_2); IR (neat) ν_{max} 3313, 2921, 2852, 1712, 1551, 1464, 1243 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.07 (b s, 1 H, N*H*COOCH$_3$), 6.76 (dd, *J* = 8.83, 9.45 Hz, 1 H, C=C*H*CH$_2$), 6.08 (b m, 1 H, N*H*COCH$_3$), 5.94 (d, *J* = 4.18 Hz, 1 H, C*H*OH), 5.85 (d, *J* = 9.59 Hz, 1 H, C*H*=CHC≡C-CH), 5.82 (dd, *J* = 1.02, 9.47 Hz, 1 H, CH=C*H*C≡CCH), 4.48–4.41 (m, 1 H, C*H*HNH-COCH$_3$), 4.40–4.37 (m, 1 H, CH*H*NHCOCH$_3$), 3.78 (s, 3 H, NHCOOC*H*$_3$), 3.74 (b d, *J* = 4.04 Hz, 1 H, CHO*H*), 3.17 (d, *J* = 17.42 Hz, 1 H, C*H*H-eq), 2.94 (d, *J* = 17.39 Hz, 1 H, CH*H*-ax), 2.00 (s, 3 H, NHCOC*H*$_3$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 191.47, 124.47, 123.77, 123.28, 66.30, 53.54, 49.98, 36.17, 29.71, 29.67, 23.16, 14.21; FAB HRMS (NBA/CsI) *m/e* 503.0235, (M + Cs$^+$) calcd for $C_{19}H_{18}N_2O_6$: 503.0219.

15: Colrless oil; R_f = 0.53 (Et$_2$O); $[\alpha]^{25}_D$ −167 (*c* 0.11, CH_2Cl_2); IR (neat) ν_{max} 3367, 3061, 2924, 2853, 1714, 1680, 1601, 1504, 1450, 1396, 1274, 1142 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.98–7.97 (m, 2 H, *Ar*), 7.89–7.85 (m, 2 H, *Ar*), 7.64–7.61 (m, 1 H, *Ar*), 7.57–7.53 (m, 2 H, *Ar*), 7.51–7.48 (m, 1 H, *Ar*), 7.39–7.35 (m, 2 H, *Ar*), 6.6 (b s, 1 H, N*H*COO), 6.36 (dd, *J* = 4.35, 7.97 Hz, 1 H, C=C*H*CH$_2$), 5.86 (dd, *J* = 1.22, 9.50 Hz, 1 H, CH=C*H*C≡CCH), 5.83 (d, *J* = 9.46 Hz, 1 H, C*H*=CHC≡CCH), 5.21 (dd, *J* = 4.02, 14.22 Hz, 1 H, C*H*HOBz), 5.00 (dd, *J* = 9.34, 14.05 Hz, 1 H, CH*H*OBz), 4.44 (b m, 2 H, COOCH_2CH_2SO_2Ph), 3.49–3.40 (m, 3 H, CH_2SO_2Ph & O*H*), 3.12 (d, *J* = 16.92 Hz, 1 H, C*H*H-eq), 2.78 (b s, 1 H, O*H*), 2.71 (d, *J* = 16.91 Hz, 1 H, CH*H*-ax); ^{13}C NMR (125 MHz, $CDCl_3$) δ 193.41, 166.55, 153.06, 136.79, 134.22, 133.09, 129.98, 129.69, 129.52, 129.36, 128.37, 128.10, 125.84, 124.35, 123.94, 99.56, 72.34, 65.86, 64.26, 59.54, 55.10, 31.92, 29.70, 29.36, 22.69, 15.27, 14.12; FAB HRMS (NBA/NaI) *m/e* 610.1153, (M + Na^+) calcd for $C_{31}H_{26}NO_9S$: 610.1148.

What is claimed is:

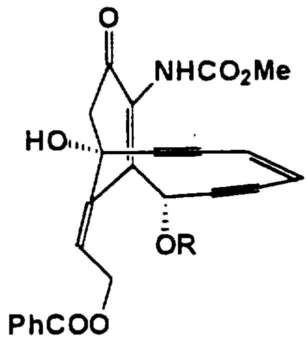
1. A calicheamicin mimic intermediate represented by the following structure:



wherein **R** is selected from the group consisting of:

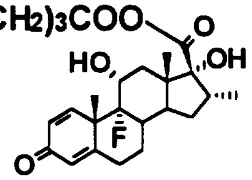
$R = H$,
$R = COPh$,
$R = CO(CH_2)_3COO-C_6H_4-NO_2$ and,
$R = CO(CH_2)_3CO_2H$.

2. A calicheamicin mimic represented by the following structure:

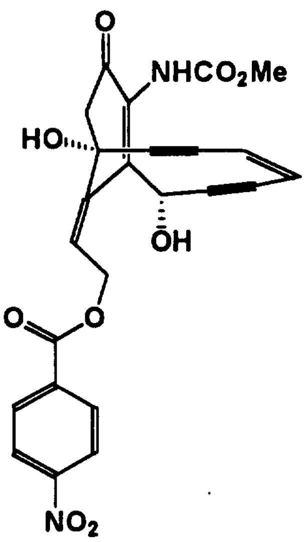


wherein **R** is selected from the group consisting of:

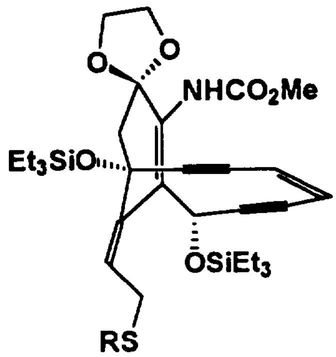
R = H,
R = COPh,
R = CO(CH$_2$)$_3$COO-C$_6$H$_4$-NO$_2$
R = CO(CH$_2$)$_3$CO$_2$H and
R = CO(CH$_2$)$_3$COO



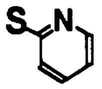
3. A calicheamicin mimic represented by the following structure:



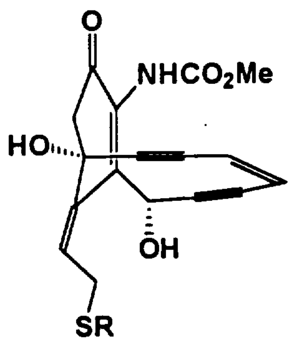
4. A calicheamicin mimic intermediate represented by the following structure:



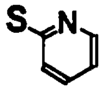
wherein **R** is selected from the group consisting of:

R = $COCH_3$,
R = SCH_3 and
R =  .

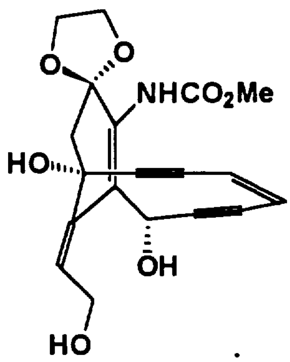
5. A calicheamicin mimic represented by the following structure:



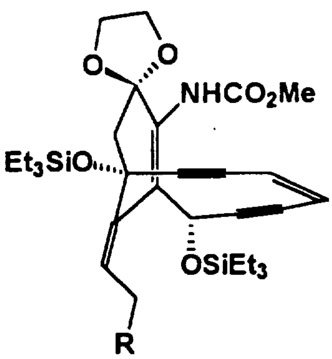
wherein **R** is selected from the group consisting of:

R = $COCH_3$,
R = SCH_3 and
R = 

6. A calicheamicin mimic represented by the following structure:



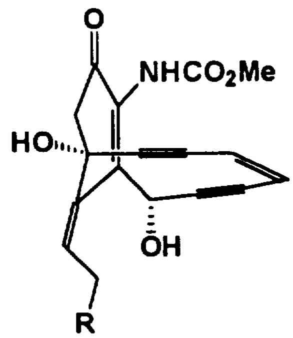
7. A calicheamicin mimic intermediate represented by the following structure:



wherein **R** is selected from the group consisting of:

R = OH,
R = $OCOCH_3$,
R = $OCOO(CH_2)_2SPh$,
R = $OCOO(CH_2)_2SO_2Ph$,
R = N_3 and
R = $NHCOCH_3$.

8. A calicheamicin mimic represented by the following structure:



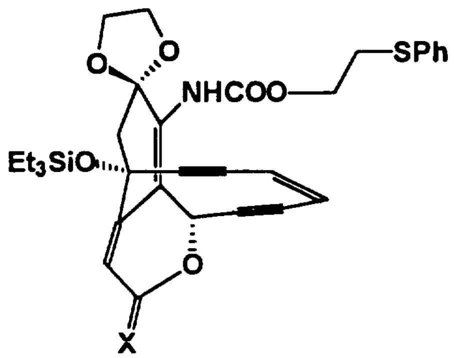
wherein **R** is selected from the group consisting of:

R = $OCOCH_3$,

R = $OCOO(CH_2)_2SO_2Ph$ and

R = $NHCOCH_3$.

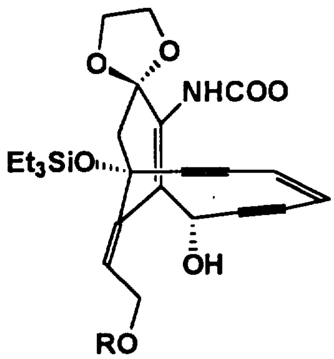
9. A calicheamicin mimic intermediate represented by the following structure:



wherein X is selected from the group consisting of:

X = O and
X = H, OH .

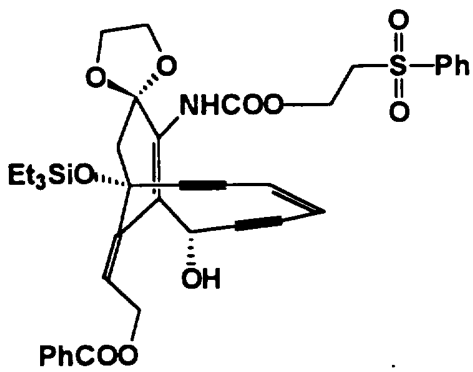
10. A calicheamicin mimic intermediate represented by the following structure:



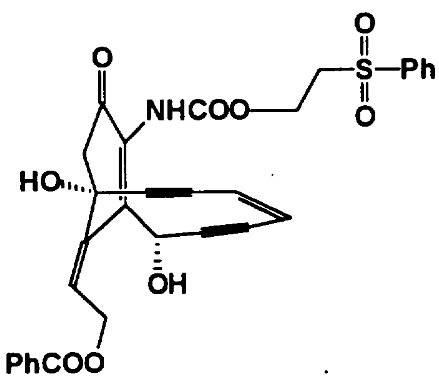
wherein **R** is selected from the group consisting of:

R = H and
R = COPh .

11. A calicheamicin mimic represented by the following structure:



12. A calicheamicin mimic represented by the following structure:



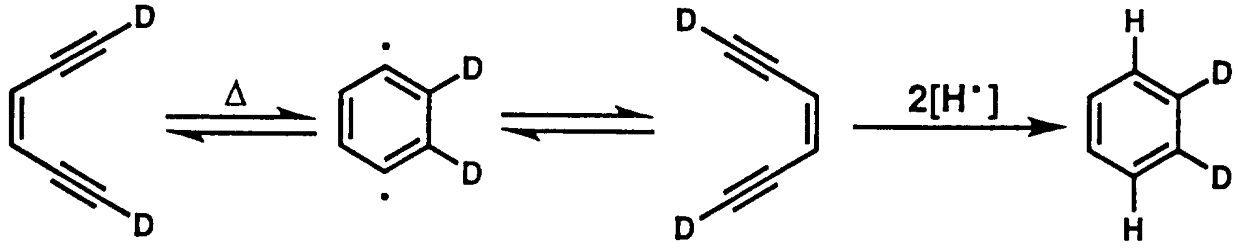

D
Δ
D
D
D
D
2[H•]
H
D
D
D
H

FIG. 1

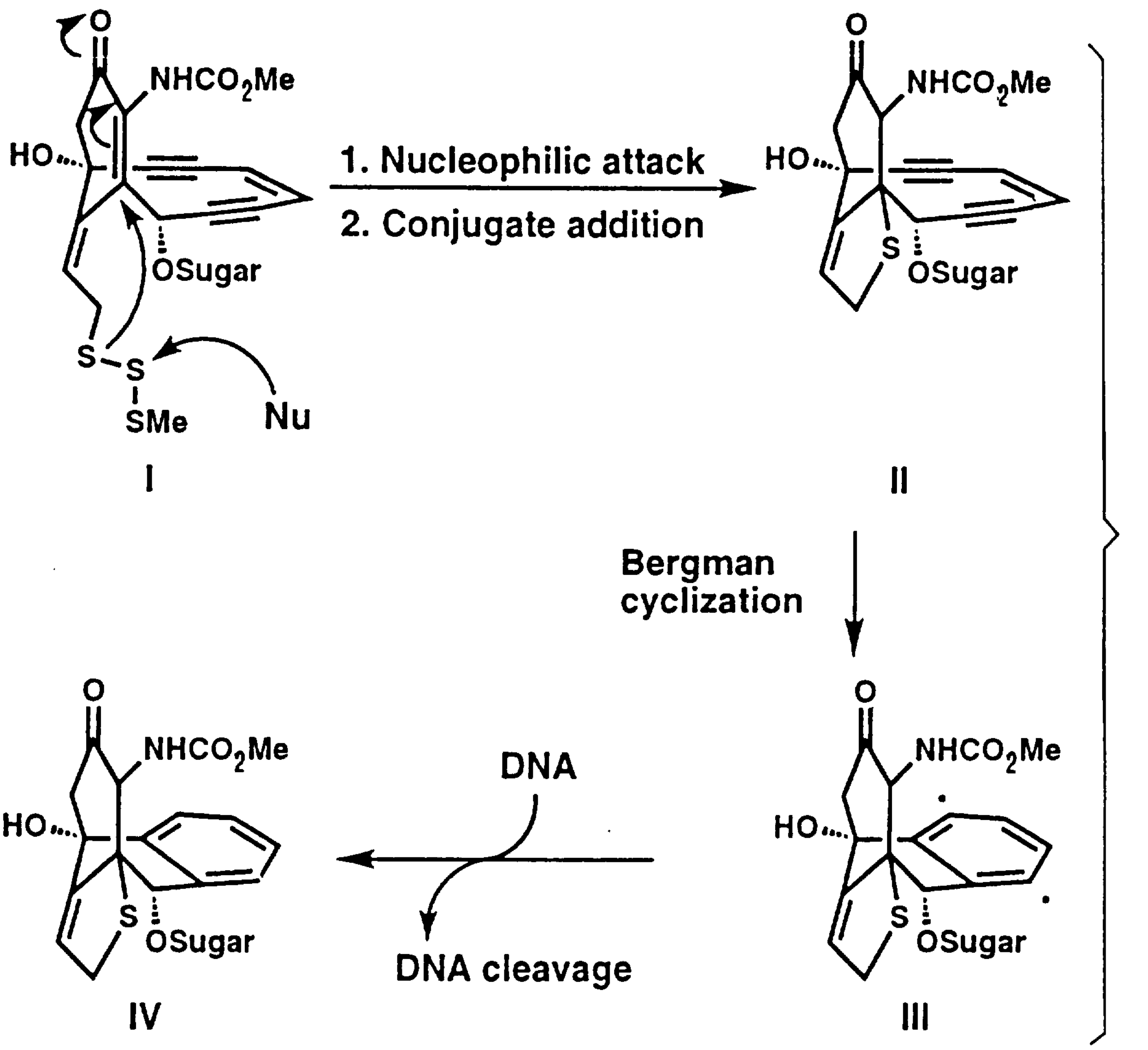

O
NHCO2Me
HO
OSugar
S
S
SMe
Nu
I
1. Nucleophilic attack
2. Conjugate addition
O
NHCO2Me
HO
S
OSugar
II
Bergman
cyclization
O
NHCO2Me
HO
S
OSugar
IV
DNA
DNA cleavage
O
NHCO2Me
HO
S
OSugar
III

FIG. 2

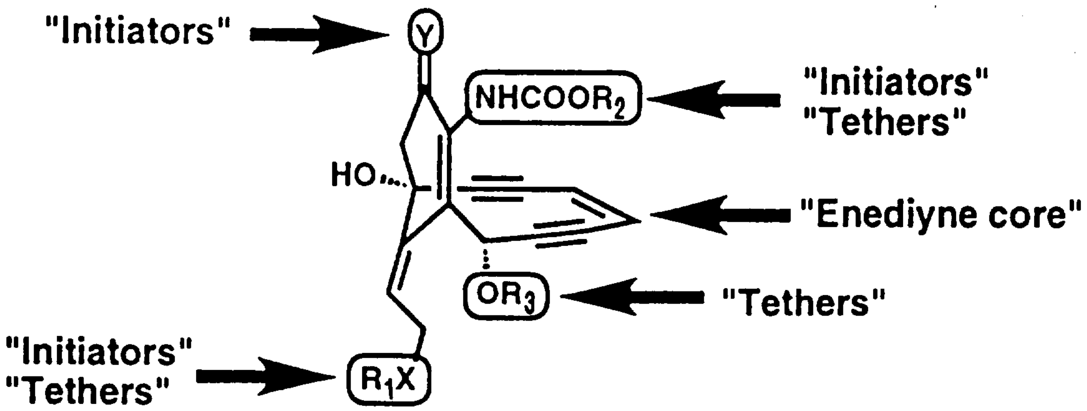

"Initiators"
Y
NHCOOR2
"Initiators"
"Tethers"
HO
"Enediyne core"
OR3
"Tethers"
"Initiators"
"Tethers"
R1X

FIG. 3

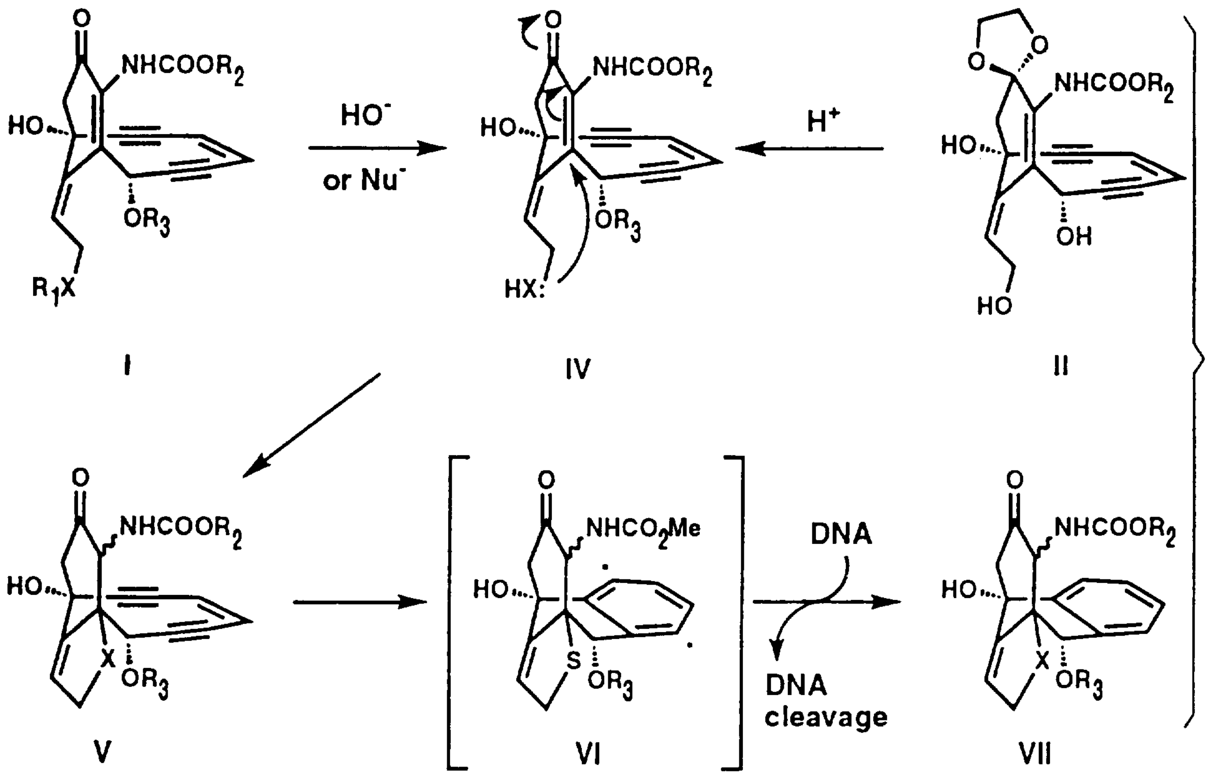

NHCOOR2
HO
OR3
R1X
HO-
or Nu-
HX:
H+
OH
I
IV
II
NHCO2Me
S
X
DNA
DNA
cleavage
V
VI
VII

FIG. 4A

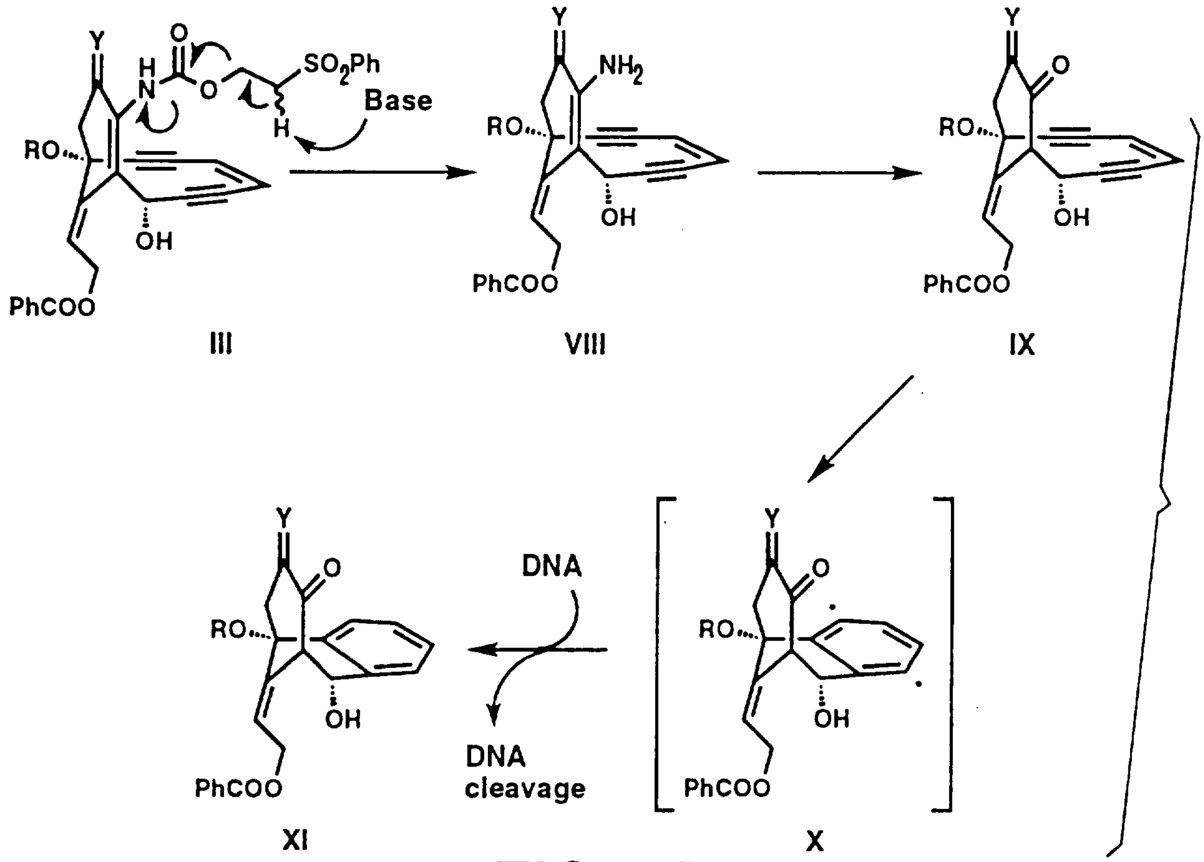

Y
SO2Ph
Base
RO
OH
PhCOO
NH2
III
VIII
IX
DNA
DNA
cleavage
XI
X

FIG. 4B

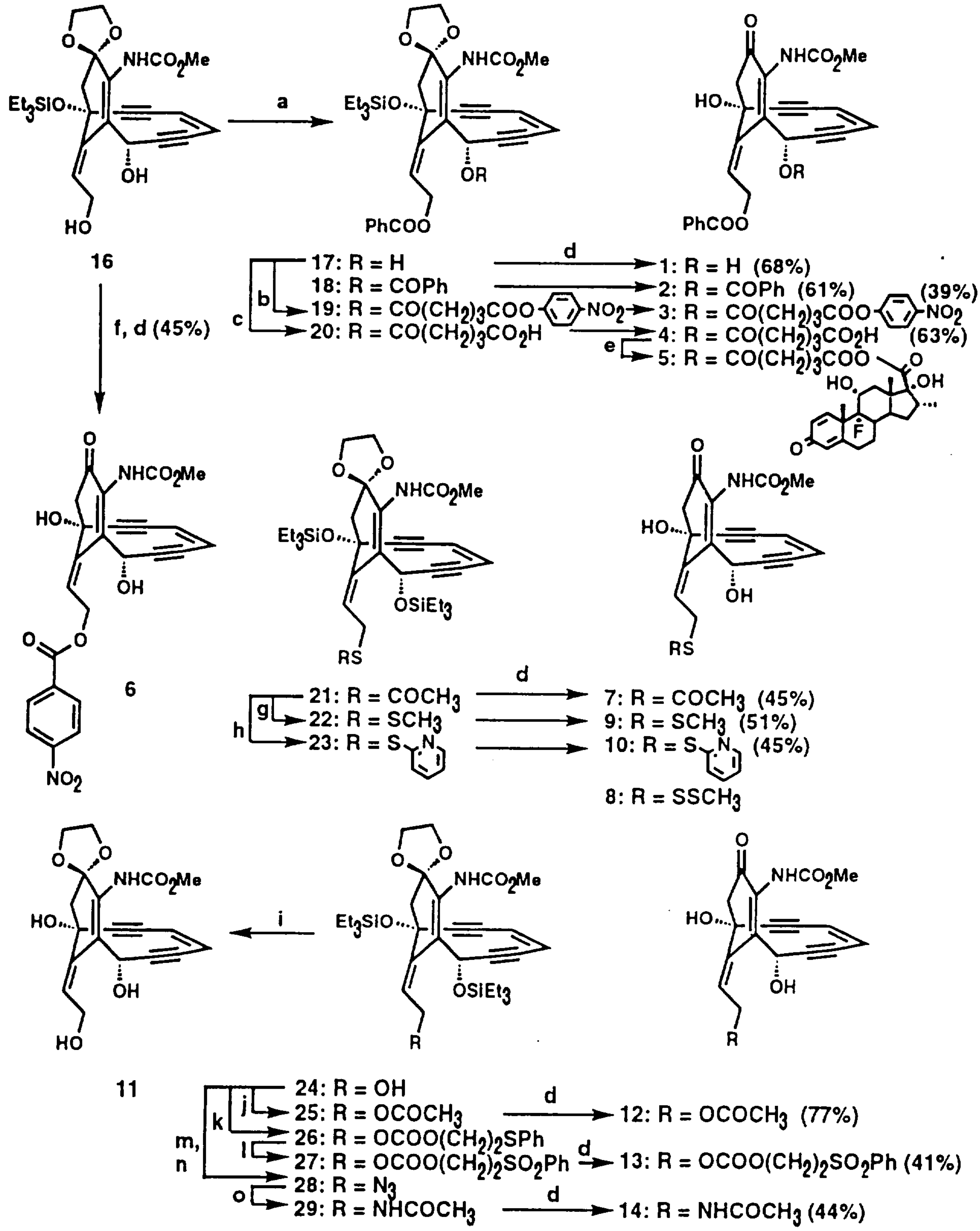

NHCO2Me
Et3SiO
OH
HO
16
a
OR
PhCOO
17: R = H
18: R = COPh
19: R = CO(CH2)3COO-C6H4-NO2
20: R = CO(CH2)3CO2H
b
c
d
1: R = H (68%)
2: R = COPh (61%)
3: R = CO(CH2)3COO-C6H4-NO2 (39%)
4: R = CO(CH2)3CO2H (63%)
5: R = CO(CH2)3COO
e
f, d (45%)
6
NO2
OSiEt3
RS
21: R = COCH3
22: R = SCH3
23: R = S-pyridyl
g
h
7: R = COCH3 (45%)
9: R = SCH3 (51%)
10: R = S-pyridyl (45%)
8: R = SSCH3
i
11
24: R = OH
25: R = OCOCH3
26: R = OCOO(CH2)2SPh
27: R = OCOO(CH2)2SO2Ph
28: R = N3
29: R = NHCOCH3
j
k
l
m, n
o
12: R = OCOCH3 (77%)
13: R = OCOO(CH2)2SO2Ph (41%)
14: R = NHCOCH3 (44%)

FIG. 5

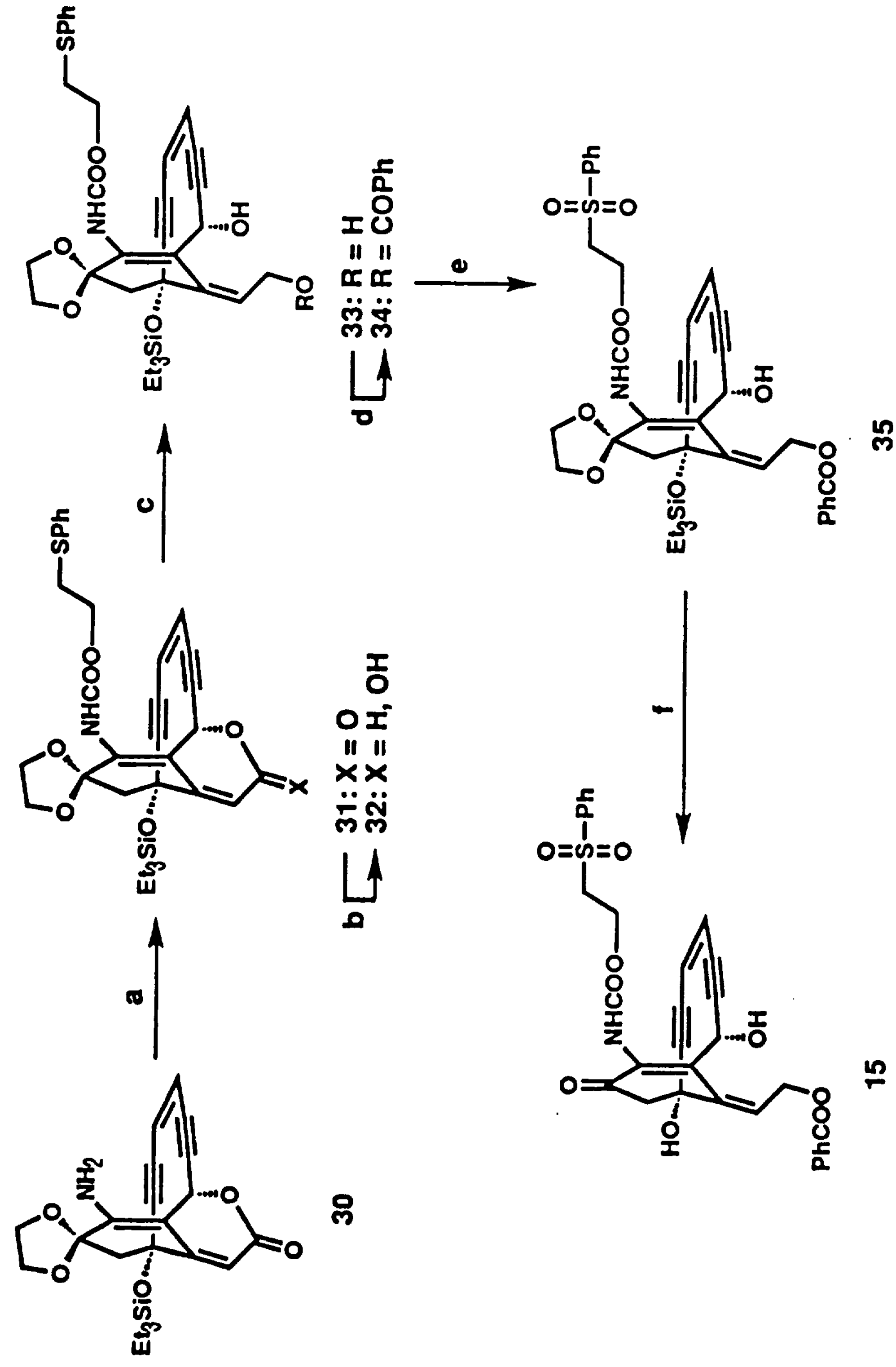

a
b
31: X = O
32: X = H, OH
c
d
33: R = H
34: R = COPh
e
f
30
35
15
Et3SiO
NHCOO
SPh
NH2
OH
RO
PhCOO
HO
O=S=O
Ph

FIG. 6

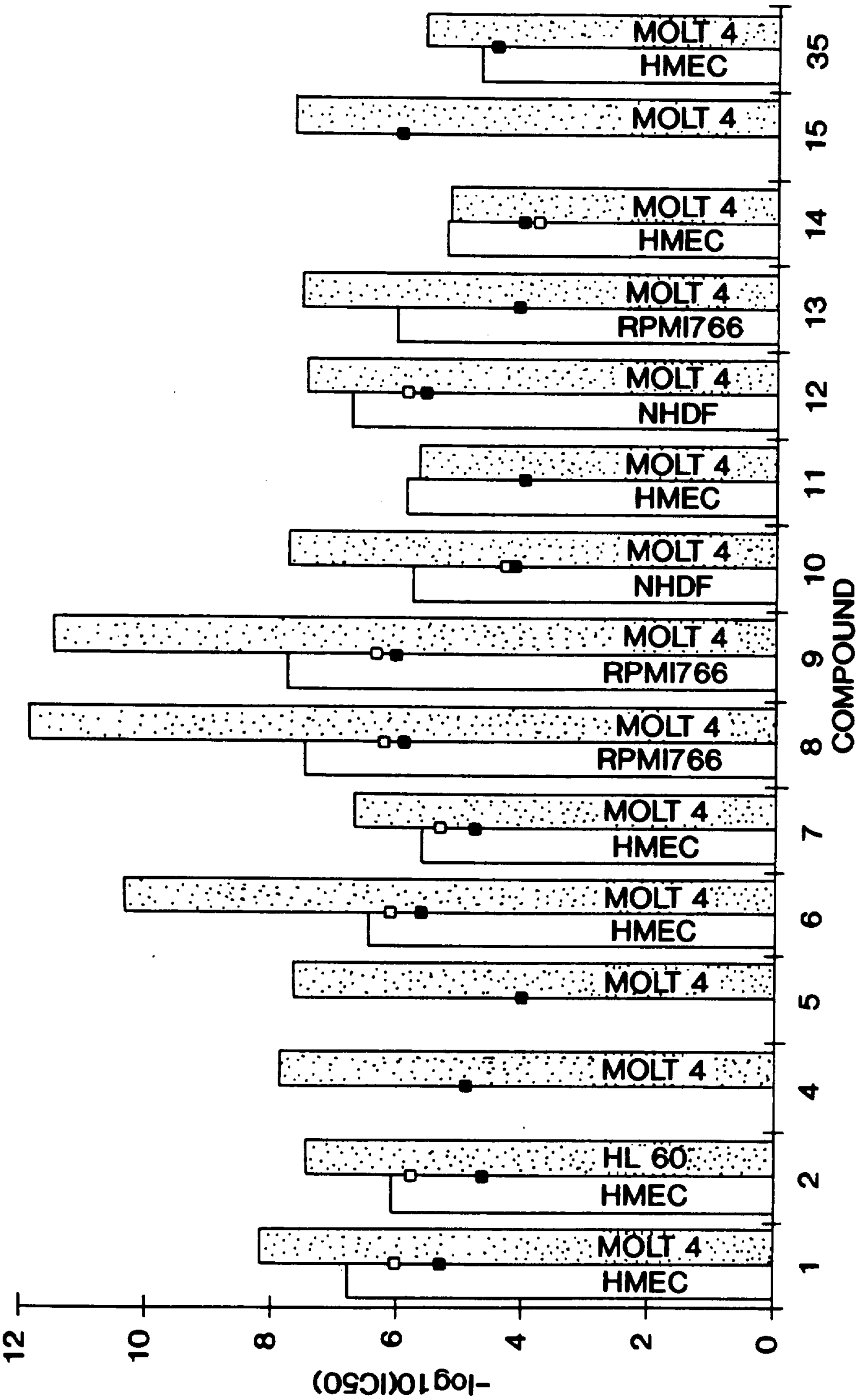

MOLT 4
HMEC
HL 60
RPMI766
NHDF
1
2
4
5
6
7
8
9
10
11
12
13
14
15
35
COMPOUND
0
2
4
6
8
10
12
-log10(IC50)

FIG. 7

Cell type	6	8	Calicheamicin $_1^I$	7	calicheamicin $_1^I$	PM-9
Normal cell lines						
CHO	1.1×10^{-6}	1.8×10^{-7}	1.2×10^{-9}	1.1×10^{-5}	4.3×10^{-9}	3.1×10^{-6}
HMEC	3.4×10^{-7}	1.1×10^{-6}	1.0×10^{-9}	2.4×10^{-6}	$<10^{-12}$	6.3×10^{-6}
NHDF	–	9.8×10^{-7}	1.0×10^{-9}	2.9×10^{-6}	$<10^{-12}$	5.0×10^{-6}
RPMI766	–	3.1×10^{-8}	1.6×10^{-9}	2.5×10^{-6}	$<10^{-12}$	–
Transformed cell lines						
BT-549	9.9×10^{-7}	5.3×10^{-8}	1.0×10^{-9}	4.5×10^{-6}	$<10^{-12}$	–
CAPAN1	1.2×10^{-6}	7.1×10^{-7}	1.3×10^{-9}	1.3×10^{-5}	$<10^{-12}$	3.1×10^{-9}
H322	1.7×10^{-6}	2.0×10^{-6}	1.7×10^{-8}	3.6×10^{-5}	1.0×10^{-9}	3.9×10^{-7}
HL-60	2.0×10^{-10}	5.1×10^{-10}	$<10^{-12}$	–	$<10^{-12}$	3.6×10^{-11}
HT-29	5.1×10^{-6}	3.6×10^{-6}	7.0×10^{-9}	1.2×10^{-5}	$<10^{-12}$	1.6×10^{-6}
M24-MET	4.1×10^{-7}	–	1.0×10^{-9}	–	$<10^{-12}$	–
MCF-7	2.6×10^{-6}	1.9×10^{-6}	4.1×10^{-8}	3.6×10^{-6}	1.2×10^{-11}	7.8×10^{-7}
MOLT-4	4.4×10^{-11}	1.3×10^{-12}	$<10^{-12}$	1.9×10^{-7}	$<10^{-12}$	$<10^{-12}$
OVCAR-3	4.8×10^{-6}	1.3×10^{-6}	2.2×10^{-9}	1.7×10^{-5}	$<10^{-12}$	7.8×10^{-7}
UCLA-P-3	4.2×10^{-6}	1.8×10^{-6}	1.7×10^{-9}	1.3×10^{-5}	$<10^{-12}$	9.8×10^{-8}
PC-3	3.4×10^{-8}	6.3×10^{-7}	4.4×10^{-9}	7.5×10^{-6}	$<10^{-12}$	–
SIHA	5.0×10^{-7}	1.6×10^{-6}	6.7×10^{-10}	1.4×10^{-6}	$<10^{-12}$	–
SK-MEL 28	1.8×10^{-6}	2.4×10^{-6}	1.6×10^{-9}	5.1×10^{-5}	$<10^{-12}$	–
U251	1.3×10^{-6}	7.4×10^{-8}	1.1×10^{-9}	4.1×10^{-5}	9.9×10^{-9}	–
786-0	1.4×10^{-5}	3.7×10^{-7}	1.0×10^{-8}	1.8×10^{-5}	$<10^{-12}$	–
L1210	2.7×10^{-8}	–	–	–	–	1.3×10^{-9}
SK-N-SH	7.8×10^{-9}	–	–	–	–	–

FIG. 8

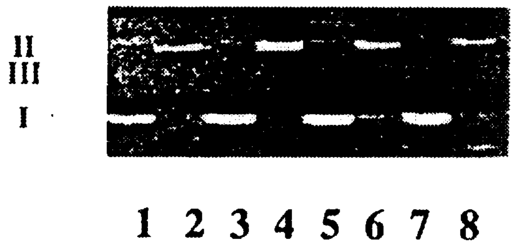


FIG. 9A

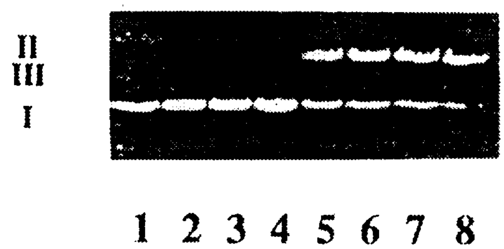


FIG. 9B

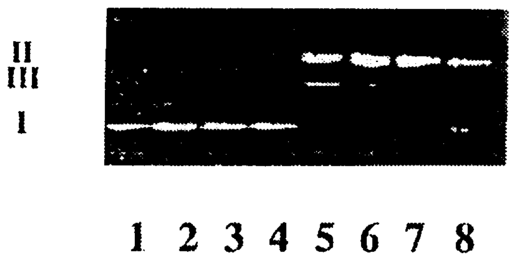


FIG. 9C

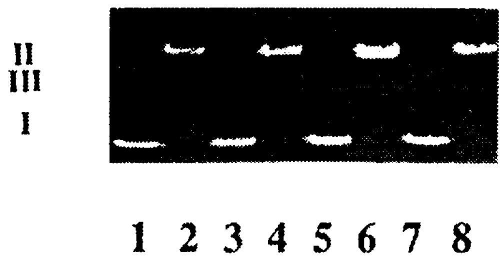


FIG. 9D

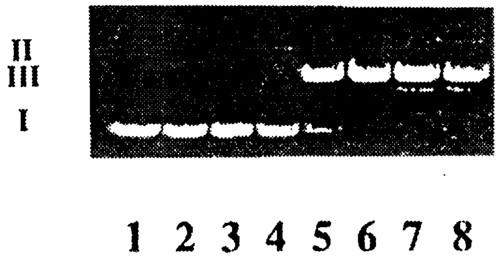


FIG. 10A

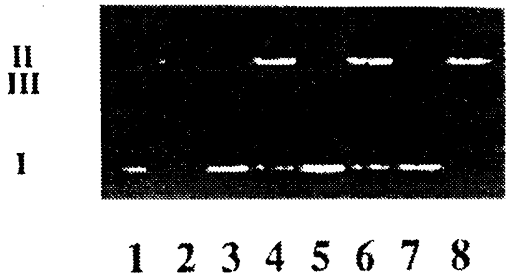


FIG. 10B

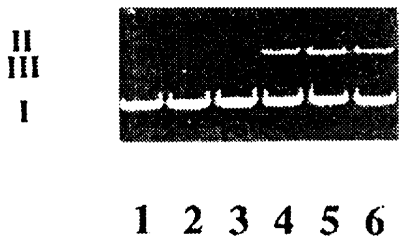


FIG. 10C

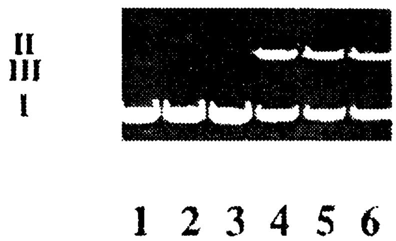


FIG. 10D

II
III
I

1 2 3 4 5 6 7 8

FIG. 11A

II
III
I

1 2 3 4 5 6 7 8

FIG. 11B

Cell type	1	2	4	5	6	7
Normal cell lines[b]						
CHO	9.1×10^{-7}	2.7×10^{-6}	–	–	1.1×10^{-6}	1.1×10^{-5}
HMEC	1.6×10^{-7}	8.0×10^{-7}	–	–	3.4×10^{-7}	2.4×10^{-6}
NHDF	1.9×10^{-7}	1.3×10^{-6}	–	–	–	2.9×10^{-6}
RPMI766	2.5×10^{-6}	1.9×10^{-6}	–	–	–	2.5×10^{-6}
Transformed cell lines[b]						
BT-549	2.5×10^{-6}	2.1×10^{-6}	–	–	9.9×10^{-7}	4.5×10^{-6}
CAPAN1	7.4×10^{-6}	1.7×10^{-5}	–	–	1.2×10^{-6}	1.3×10^{-5}
H322	3.2×10^{-6}	7.9×10^{-5}	–	–	1.7×10^{-6}	3.6×10^{-5}
HL-60	5.5×10^{-8}	3.4×10^{-8}	2.4×10^{-8}	1.1×10^{-7}	2.0×10^{-10}	–
HT-29	1.2×10^{-5}	3.1×10^{-5}	–	–	5.1×10^{-6}	1.2×10^{-5}
M24-MET	–	–	–	–	4.1×10^{-7}	–
MCF-7	1.5×10^{-5}	2.3×10^{-5}	–	–	2.6×10^{-6}	3.6×10^{-6}
MOLT-4	6.5×10^{-9}	3.2×10^{-5}	1.3×10^{-8}	2.1×10^{-8}	4.4×10^{-11}	1.9×10^{-7}
OVCAR-3	2.6×10^{-5}	4.6×10^{-5}	2.4×10^{-5}	$>10^{-4}$	4.8×10^{-6}	1.7×10^{-5}
UCLA-P-3	4.3×10^{-7}	8.9×10^{-6}	7.2×10^{-5}	$>10^{-4}$	4.2×10^{-6}	1.3×10^{-5}
PC-3	4.4×10^{-8}	4.4×10^{-6}	3.4×10^{-6}	$>10^{-4}$	3.4×10^{-8}	7.5×10^{-6}
SIHA	7.4×10^{-7}	3.4×10^{-5}	–	–	5.0×10^{-7}	1.4×10^{-6}
SK-MEL 28	2.1×10^{-6}	2.9×10^{-5}	2.0×10^{-6}	$>10^{-4}$	1.8×10^{-6}	5.1×10^{-5}
U251	4.8×10^{-7}	1.8×10^{-5}	–	–	1.3×10^{-6}	4.1×10^{-5}
786-0	6.3×10^{-8}	3.5×10^{-6}	9.6×10^{-7}	–	1.4×10^{-5}	1.8×10^{-5}
L1210	–	–	4.7×10^{-7}	8.6×10^{-7}	2.7×10^{-8}	–
SK-N-SH	–	–	–	–	7.8×10^{-9}	–

FIG. 12

Cell type	3	9	10	11	12	13
Normal cell lines[b]						
CHO	1.8×10^{-7}	1.9×10^{-7}	1.0×10^{-4}	$>10^{-4}$	1.7×10^{-6}	1.0×10^{-4}
HMEC	1.1×10^{-6}	1.1×10^{-6}	1.0×10^{-4}	2.1×10^{-6}	8.0×10^{-7}	1.0×10^{-4}
NHDF	9.8×10^{-7}	3.7×10^{-7}	1.7×10^{-6}	$>10^{-4}$	1.7×10^{-7}	1.0×10^{-4}
RPMI766	3.1×10^{-8}	1.7×10^{-8}	1.8×10^{-6}	1.3×10^{-6}	2.5×10^{-6}	8.7×10^{-7}
Transformed cell lines[b]						
BT-549	5.3×10^{-8}	3.2×10^{-8}	1.0×10^{-4}	$>10^{-4}$	2.1×10^{-6}	1.0×10^{-4}
CAPAN1	7.1×10^{-7}	1.9×10^{-6}	2.7×10^{-6}	7.1×10^{-4}	4.0×10^{-6}	1.0×10^{-4}
H322	2.0×10^{-6}	2.5×10^{-7}	1.0×10^{-4}	$>10^{-4}$	2.1×10^{-7}	1.0×10^{-4}
HL-60	5.1×10^{-10}	2.0×10^{-6}	1.4×10^{-6}	2.0×10^{-6}	7.2×10^{-8}	4.1×10^{-7}
HT-29	3.6×10^{-6}	2.1×10^{-6}	1.0×10^{-4}	$>10^{-4}$	4.2×10^{-6}	1.0×10^{-4}
M24-MET	–	–	–	–	–	1.0×10^{-4}
MCF-7	1.9×10^{-6}	6.6×10^{-7}	1.0×10^{-4}	$>10^{-4}$	1.4×10^{-6}	1.0×10^{-4}
MOLT-4	1.3×10^{-12}	3.3×10^{-12}	1.7×10^{-8}	1.0×10^{-5}	3.2×10^{-8}	2.6×10^{-8}
OVCAR-3	1.3×10^{-6}	5.9×10^{-7}	1.0×10^{-4}	$>10^{-4}$	4.4×10^{-6}	1.0×10^{-4}
UCLA-P-3	1.8×10^{-6}	1.3×10^{-6}	1.0×10^{-4}	$>10^{-4}$	6.0×10^{-6}	1.0×10^{-4}
PC-3	6.3×10^{-7}	1.6×10^{-7}	7.1×10^{-6}	$>10^{-4}$	1.5×10^{-7}	–
SIHA	1.6×10^{-6}	1.8×10^{-6}	1.0×10^{-4}	$>10^{-4}$	2.1×10^{-6}	2.8×10^{-6}
SK-MEL 28	2.4×10^{-6}	9.6×10^{-7}	1.0×10^{-4}	$>10^{-4}$	1.1×10^{-5}	1.0×10^{-4}
U251	7.4×10^{-8}	2.9×10^{-8}	1.0×10^{-4}	$>10^{-4}$	3.7×10^{-7}	1.0×10^{-4}
786-0	3.7×10^{-7}	8.0×10^{-7}	1.0×10^{-4}	$>10^{-4}$	1.1×10^{-7}	1.0×10^{-4}
L1210	–	–	–	–	–	1.6×10^{-4}
SK-N-SH	–	–	–	–	–	3.2×10^{-6}

FIG. 13

Cell type	14	15	35
Normal cell lines			
CHO, chinese hamster ovary cells	1.8×10^{-7}	1.9×10^{-7}	1.0×10^{-4}
HMEC, human mammary epithelial cells	1.1×10^{-6}	1.1×10^{-6}	1.0×10^{-4}
NHDF, normal human dermal fibroblast	9.8×10^{-7}	3.7×10^{-7}	1.7×10^{-6}
RPMI766, normal human peripheral blood lymphoblast	3.1×10^{-8}	1.7×10^{-8}	1.8×10^{-6}
Transformed cell lines			
BT-549, human breast ductal carcinoma	5.3×10^{-8}	3.2×10^{-8}	1.0×10^{-4}
CAPAN1, pancreatic carcinoma	7.1×10^{-7}	1.9×10^{-6}	2.7×10^{-6}
H322, lung adenocarcinoma	2.0×10^{-6}	2.5×10^{-7}	1.0×10^{-4}
HL-60, human promyelomic leukemia	5.1×10^{-10}	2.0×10^{-6}	1.4×10^{-6}
HT-29, human colon adenocarcinoma	3.6×10^{-6}	2.1×10^{-6}	1.0×10^{-4}
M24-MET, metastatic melanoma	-	-	-
MCF-7, breast carcinoma	1.9×10^{-6}	6.6×10^{-7}	1.0×10^{-4}
MOLT-4, human T-cell leukemia	1.3×10^{-12}	3.3×10^{-12}	1.7×10^{-8}
OVCAR-3, human ovarian carcinoma	1.3×10^{-6}	5.9×10^{-7}	1.0×10^{-4}
UCLA-P-3, lung adenocarcinoma	1.8×10^{-6}	1.3×10^{-6}	1.0×10^{-4}
PC-3, human prostate carcinoma	6.3×10^{-7}	1.6×10^{-7}	7.1×10^{-6}
SIHA, human squamous carcinoma, CERVIX	1.6×10^{-6}	1.8×10^{-6}	1.0×10^{-4}
SK-MEL 28, melanoma	2.4×10^{-6}	9.6×10^{-7}	1.0×10^{-4}
U251, human CNS cancer	7.4×10^{-8}	2.9×10^{-8}	1.0×10^{-4}
786-0, human perirenal cell carcinoma	3.7×10^{-7}	8.0×10^{-7}	1.0×10^{-4}
L1210, murine leukemia	-	-	-
SK-N-SH, neuroblastoma	-	-	-

FIG. 14

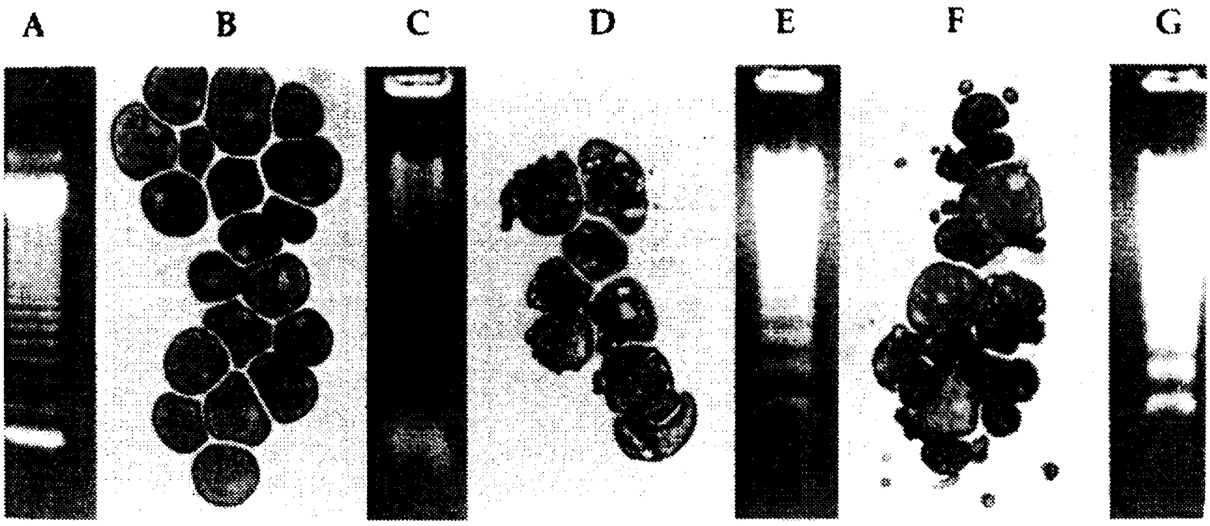


FIG. 15

Compound	IC_{50} [M]
p-nitrobenzoate 6	10^{-6}–10^{-11}
disulfide 9	10^{-6}–10^{-12}
taxol	10^{-7}–10^{-9}
vinblastine, vincristine	10^{-7}–10^{-9}
doxorubicin	10^{-6}–10^{-9}
actinomycin	10^{-7}–10^{-9}
bleomycin sulfate	10^{-4}–10^{-5}

FIG. 16

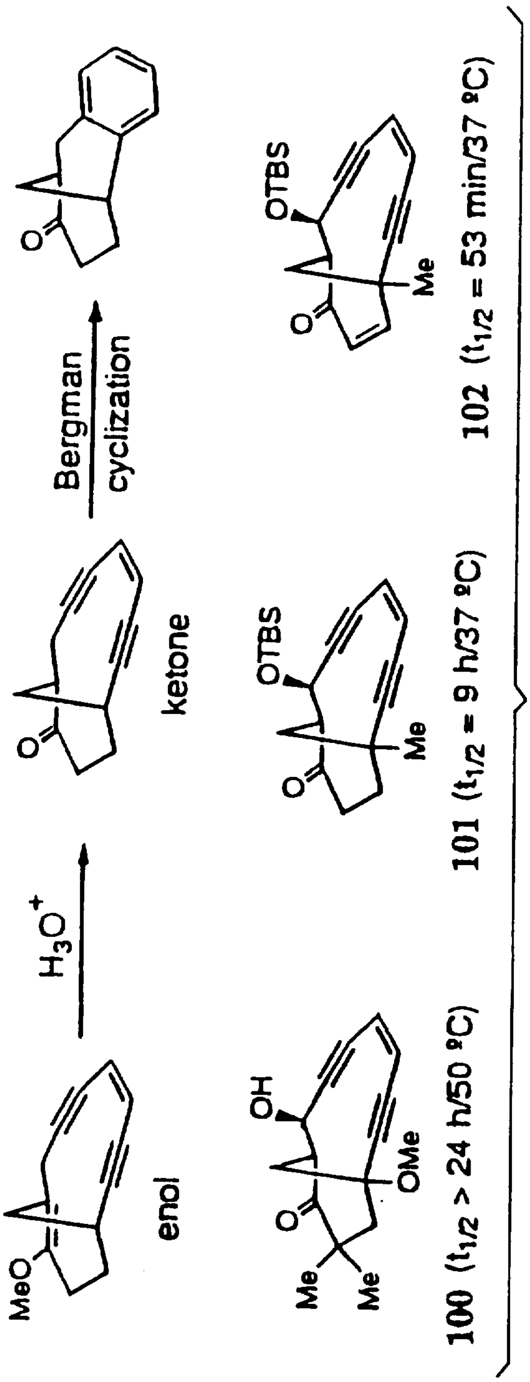

MeO
enol
H3O+
O
ketone
Bergman cyclization
O
OH
O
Me
Me
OMe
100 (t1/2 > 24 h/50 ºC)
OTBS
O
Me
101 (t1/2 = 9 h/37 ºC)
OTBS
O
Me
102 (t1/2 = 53 min/37 ºC)

FIG. 17

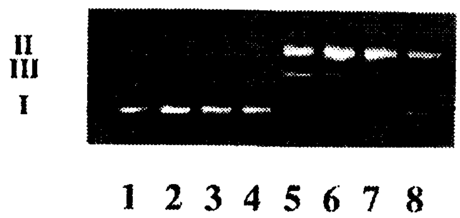


FIG. 18A

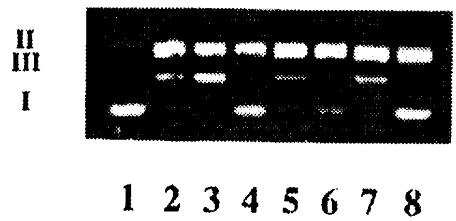


FIG. 18B

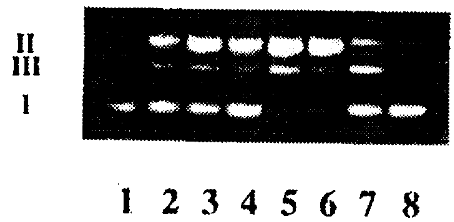


FIG. 18C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/11207

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07D 317/10, 307/77; C07C 69/76, 235/40, 237/24
US CL : 549/214, 344; 560/107; 564/215
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 549/214, 344; 560/107; 564/215

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN [Search term(s): caleamicin)]

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,264,586 (NICOLAOU ET AL.) 23 November 1993, column 3, line 35 to column 7, line 17.	1-12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 DECEMBER 1995	05 JAN 1996

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	JOSEPH M. CONRAD III
Facsimile No. (703) 305-3230	Telephone No. (703) 308-1235