A Second Generation Photochemically Activatable Dynemicin Analog: A Concise Synthesis and DNA Cleavage Studies

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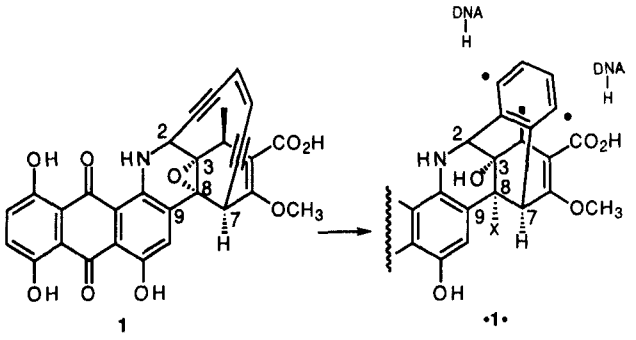
A scheme for the design of dynemicin analogs and a concise, efficient route for their synthesis are described. A photochemically activatable analog was prepared and shown to undergo cycloaromatization upon irradiation with wavelengths greater than 300 nm. The ability of this compound to function as a competent DNA cleaving agent is demonstrated.

During the past ten years, the enediyne anti-tumor antibiotics have emerged as a promising class of chemotherapeutic agents and medicinal leads.[3] Members of this group, which includes dynemicin A (**1**),[4,5] neocarzinostatin chromophore,[6] esperamicin,[7,8] calicheamicin,[9,10] kedarcidin chromophore,[11] and C-1027 chromophore,[12] have been found to exhibit pronounced cytotoxic activity and exceptional potency as DNA cleaving agents. These



compounds have also been shown to exhibit *in vitro* activity against a variety of cancer cell lines with IC_{50} values in the ng/ml range[13-15] and to prolong the lifespan of mice inoculated with P388 leukemia or B16 melanoma.[13,14,16,17] In addition to their anti-tumor properties, these natural products are structurally unique. Each possesses a highly unsaturated 9- or 10-membered ring incorporating, with the exception of neocarzinostatin chromophore, a conjugated enediyne subunit.

The biological activity of these compounds has been attributed to their ability to cleave double-stranded DNA.[8,10,18] This cleavage is initiated by the reaction of these compounds with an activating agent, usually a thiol or a reductant.[15,18,19] The structural change attending this activation process facilitates cycloaromatization of the enediyne moiety leading to the formation of a highly reactive diradical species (**1** to •**1**•).[8,10,20] If positioned properly in the minor groove of double-stranded B-form DNA, this diradical can abstract hydrogen atoms at proximate deoxyribosyl sites, producing a carbon radical in each strand which directly or upon reaction with endogenous oxygen leads to strand scission.[3-12,15,18,19,21,22]

In 1989, the groups of Konishi and Clardy reported the structure of one of the newest members of the enediyne class of DNA cleaving agents — dynemicin A (**1**),[4,5] a violet-colored compound isolated from the fermentation broth of *Micromonospora chersina*. Unlike its predecessors, the anti-tumor and antibiotic activities of dynemicin A are coupled with low toxicity,[4,13] making it an attractive lead for the development of new chemotherapeutic and DNA cleaving agents.[23-25] An important structural feature of dynemicin A which further distinguishes it from the other enediynes is the presence of an anthraquinone subunit which serves a twofold purpose. By intercalation between base pairs, this subunit assists binding of dynemicin to the minor groove of DNA. In addition, it combines with the neighboring epoxide to function as a chemical trigger.[21,22] In the latter role, the anthraquinone inhibits heterolytic cleavage of the adjacent oxirane which in turn prevents cycloaromatization of the enediyne by holding carbons 2, 3, 7, and 8 of the enediyne ring in an anti-like conformation. However, upon reduction of the anthraquinone, electron density at C-9 increases, thereby facilitating cleavage of the adjacent epoxide and allowing carbons 2, 3, 7, and 8 to assume a gauche-like conformation. As a result, cycloaromatization of the enediyne to a reactive diradical (•**1**•) ensues, leading to hydrogen abstraction and oxidative cleavage of DNA.[18,21,22]

Since 1985, our laboratory has been involved in studies on the design, synthesis, computer modeling, and mode of action of DNA cleaving agents based on neocarzinostatin[26] and more recently on the enediyne natural product leads.[22,23,24] Amongst our goals has been the design of simplified analogs that could be used as reagents for DNA cleavage or as agents for chemotherapy. In 1991, we reported a uniquely simplified but fully functional family of dynemicin analogs (**2**).[23] Our approach to analog design retained three key elements of the dynemicin lead that are required for controllable diradical generation (bold type in Figure 1) while allowing for the introduction of new triggering and DNA recognition options. The ten-membered enediyne

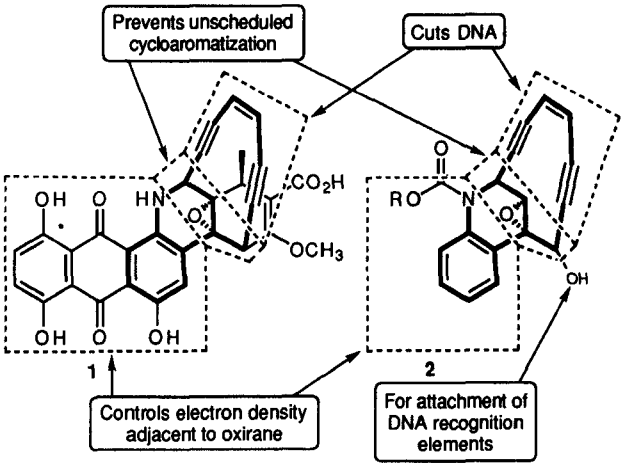
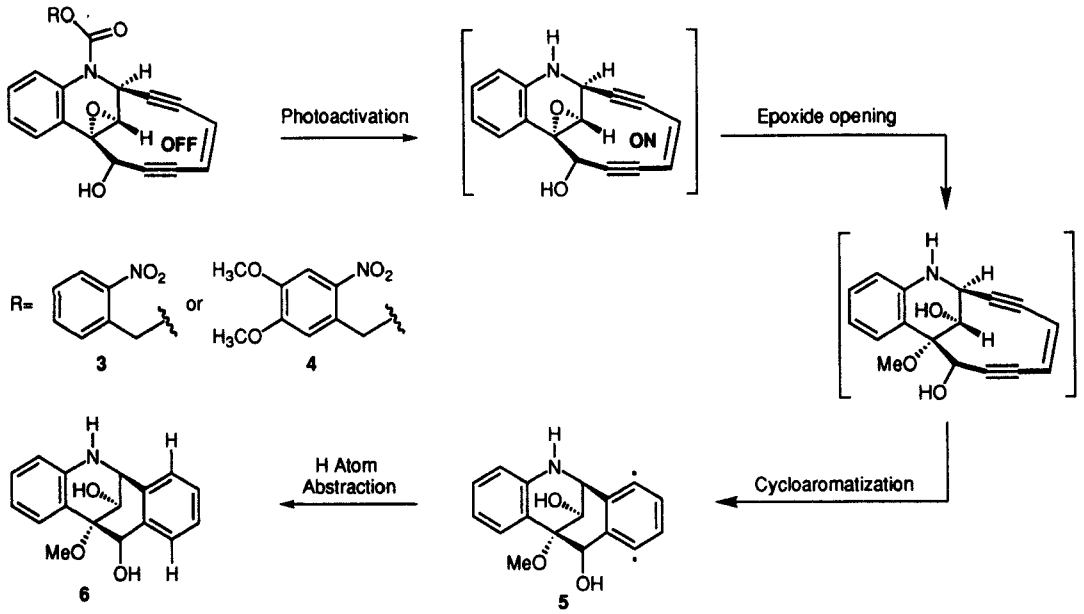


Figure 1. Analog design

ring and epoxide are retained in these analogs due to their essential mechanistic role. However, the anthraquinone is replaced by a simple arene whose electron density and, consequently, role in the triggering mechanism are regulated by aryl substituents including the attached nitrogen. *Virtually any class of reactions and reagents can thus be used to trigger cycloaromatization based on this design.* For example, we have previously demonstrated that cycloaromatization can be triggered by treatment of analogs with acid,[23,24] light,[24] or transition metals[27] depending on the nature of the nitrogen or enediyne protecting group. Each of these triggering options holds potential for reagent or chemotherapeutic use. Of particular value, light activatable analogs are especially attractive as reagents for DNA cleavage and mechanistic probes, since they circumvent the uncertainties and lack of kinetic control associated with the use of conventional bimolecular activation involving thiols or reducing agents.[28] Herein we report the synthesis of new light activatable analogs (**4**: Scheme I) and their efficacy as DNA cleaving agents.

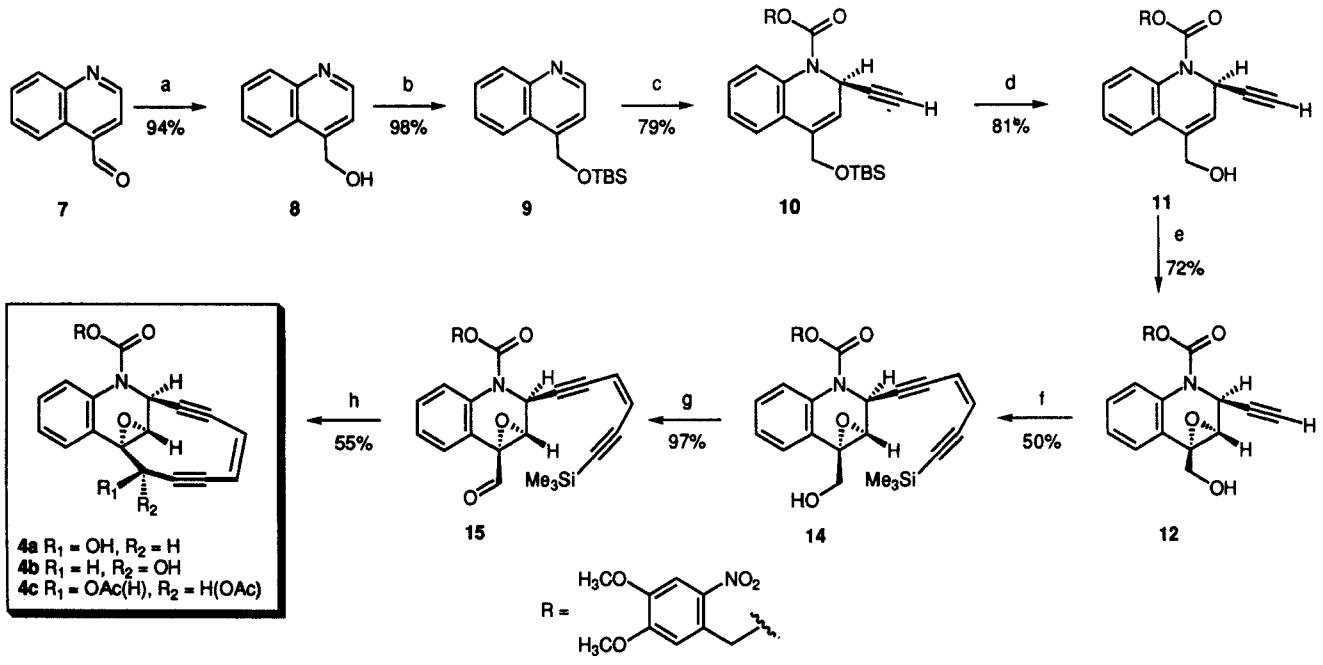
Previous work[24] in our laboratory with the nitrobenzyl carbamate **3** (Scheme I) demonstrated that it can be triggered to cycloaromatize and cleave DNA under neutral conditions by irradiation at >300 nm. Since dimethoxynitrobenzyl (nitroveratryl) carbamates absorb at longer wavelengths and undergo photodeprotection more rapidly in



Scheme I

many cases than nitrobenzyl carbamates,[29] we became interested in the synthesis and performance of carbamate **4**. The synthesis of **4** (Scheme II) follows a modification of our previously reported strategy.[23,24] It begins with the reduction of commercially available quinoline carboxaldehyde **7** with $NaBH_4$ and protection of the resultant alcohol **8** as the TBS ether **9**. This compound is treated with ethynylmagnesium bromide and 6-nitroveratryl chloroformate[30] to furnish the desired 1,2-addition product **10**. It is noteworthy that this addition proceeds more efficiently and without formation of the undesired 1,4-addition product when the hydroxymethyl group is protected as a TBS ether. The TBS group is subsequently removed by *in situ* generation of HCl through the addition of acetyl chloride to a THF/MeOH solution of **10**. Although an extra protection step is required in this procedure compared with our previous syntheses, the overall yield of **11** is improved. Epoxidation of the allylic alcohol with *m*CPBA proceeds with high facial selectivity to give oxirane **12**. Palladium(0)-catalyzed cross coupling of the terminal alkyne with *cis*-chloro eneyne **13**[31] produces enediyne **14** from which aldehyde **15** is obtained by Dess-Martin periodinane[32] oxidation.

We have previously reported the first examples of an intramolecular desilylative condensation between a silylated alkyne and an aldehyde[23,24] and find in the present case that this method serves as an effective procedure for generating the strained and reactive cyclodecaendiyne under conditions which avoid the use of strong base. In this procedure, desilylative closure of the enediyne is effected by treatment of **15** with CsF in dry MeCN using acetic anhydride as an alkoxide trap, the latter being a recently introduced modification that

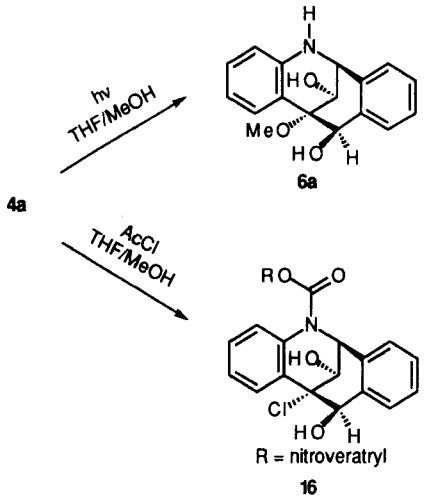


a) $NaBH_4$, MeOH, 2h; b) TBSCl, imidazole, DMF, 10 min; c) 2 eq H-CC-MgBr, then 1.5 eq 6-nitroveratryl chloroformate, THF, 15 min; d) THF:MeOH (1:1), 1 eq acetyl chloride, 15 min; e) *m*-CPBA, CH_2Cl_2, $NaHCO_3$, Na_2SO_4, 0°C, 2h; f) 0.15 eq $Pd(PPh_3)_4$, 0.4 eq CuI, 3 eq n-$BuNH_2$, *cis*-ClCH=CHCCTMS (**13**), benzene, 0°C, 2h; g) Dess-Martin periodinane, CH_2Cl_2, 2h; h) CsF, $(CF_3CO)_2O$, CH_3CN, 4h.

Scheme II

serves in many cases to greatly improve the efficiency and versatility of this process. The enediyne acetates (**4c**) are isolated as an inseparable mixture (2:1) and the acetate group is then removed with a saturated MeOH solution of $Ba(OH)_2$ to give **4a,b** in an overall yield of 49% from aldehyde **15**. Alternatively, alcohols **4a,b** can be prepared directly from **15** by using trifluoroacetic anhydride as the alkoxide trap. The trifluoroacetate hydrolyzes during work-up to give **4a,b** in 55% yield (2:1, respectively).

The ability of enediyne **4** to function as a photoactivatable dynemicin analog was initially tested by its irradiation with a 450-W Hanovia lamp through a Pyrex filter (wavelengths >300 nm) in THF/methanol solvent. Under these conditions, **4a** was rapidly converted to the cycloaromatized product **6a** (Scheme III), a compound which is also obtained by irradiation of **3a** under the same conditions. Analog **4a** can also be activated toward cycloaromatization by treatment with acid to give **16**. When this reaction is carried out in deuterated



Scheme III

solvents deuterium incorporation is observed in the newly formed aromatic ring, a result which serves as compelling evidence for the intermediacy of a diradical and for the competency of **4** as a DNA cleaving agent and a fully functional analog of dynemicin.

The DNA cleaving abilities of analogs **3** and **4** were compared by irradiation (at wavelengths >300 nm, Pyrex filter) in the presence of ϕX174 DNA and analysis of the DNA cleavage products by agarose gel electrophoresis (Figure 2). Lanes 1-4 are controls. Lane 1 was produced from commercially available DNA without added analog and without irradiation and shows that the DNA sample consists of

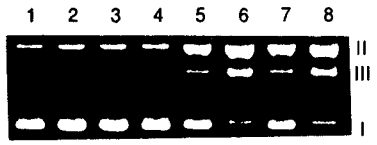


Figure 2. ϕX174 DNA (54 μm/bp) nicking in 7.5 pH buffer by **3a** and **4a** using irradiation from a 450-W Hanovia medium-pressure mercury arc lamp through a Pyrex filter for 20 min and analyzed by agarose gel electrophoresis: lane 1 [no analog, no hv]; lane 2 [**4a** (540 μm), no hv]; lane 3 [**3a** (540 μm), no hv]; lane 4 [no analog, hv]; lane 5 [**4a** (270 μm), hv]; lane 6 [**3a** (270 μm), hv]; lane 7 [**4a** (540 μm), hv]; lane 8 [**3a** (540 μm), hv].

mostly supercoiled circular DNA (i.e., form I at bottom) and some DNA with a single strand nick (form II at top). Lanes 2 and 3 indicate that the presence of analogs **4a** and **3a**, respectively, does not affect the composition of the commercial DNA. Similarly, as illustrated in lane 4, the composition of commercial DNA is not altered by irradiation under the conditions used to activate our analogs. Significantly, however, as shown in lane 5, when analog **4a** is irradiated in the presence of commercial DNA, form II DNA is increased substantially relative to the controls (lanes 1, 2, and 4) and now form III DNA, the result of double strand cleavage, is produced. It is not known at present whether form III arises from a single cleavage event by one molecule or multiple nicks by two or more molecules of analog **4a**. As a further control, irradiation of the epoxide **12** (Scheme II: R = *o*-nitrobenzyl) under these conditions did not lead to DNA cleavage, indicating that the aryl byproduct of photodeprotection is not itself a DNA cleaving agent. Inspection of lanes 5 versus 6 and 7 versus 8 reveals that enediyne **3a** damages DNA more effectively than **4a**, a result which is also observed when a Uranium filter (wavelengths >320 nm) is used. This difference in DNA cleavage could arise from the differing efficiencies of diradical generation from the test compounds or their differing orientation in the minor groove. The former explanation is consistent with the results of irradiation of the analogs in the absence of DNA and the isolation of the cycloaromatized products where analog **3a** furnished **6a** in 25% yield while **4a** gave the same product in a lower yield (13%). The efficiency of cleavage of these analogs will ultimately be determined by their performance when attached to suitable DNA recognition elements.

In summary, we have developed an effective design for the generation of dynemicin analogs and a concise (eight steps) synthetic plan which can be utilized to rapidly prepare a variety of these analogs. This design and synthetic strategy accommodates a range of different triggering mechanisms. *Two such analogs, 3 and 4, are shown to be photochemically activatable, allowing cycloaromatization to occur under neutral conditions without the use of additives as required for conventional bimolecular activation.* Photodeprotection of **3a** and **4a** gives cycloaromatized product **6a**, demonstrating that these analogs emulate the chemical behavior of dynemicin A. Importantly, when this photodeprotection is carried out in the presence of DNA, single strand and double strand cleavage products are observed. With the competency of these analogs as DNA cleaving agents established, efforts are now underway to attach DNA recognition elements to these analogs in order to enhance cleavage efficiency and to achieve sequence selectivity.

Reagents were purchased from Aldrich Chemical Co. and used without further purification unless otherwise noted. Dess-Martin periodinane[33] and tetrakis(triphenylphosphine)palladium(0)[34] were prepared according to literature procedure, and CuI and *m*CPBA were recrystallized. Ethyl acetate and methanol were purchased from J. T. Baker and CH_2Cl_2 from Fisher. Hexanes were distilled, and benzene, THF, and Et_2O were distilled from sodium-benzophenone ketyl. Melting points were taken on a Thomas Hoover or Mel-Temp apparatus and are uncorrected. Infrared spectra were taken using thin films on a Perkin Elmer 1600 FTIR. ^{13}C- and ^1H-NMR were measured at 75 MHz and 300MHz, respectively, on a Varian GEM-300 spectrometer using TMS as the reference in $CDCl_3$ unless otherwise noted. High resolution mass spectra (HRMS) were provided by the Mass Spectrometry Facility, University of California-San Francisco supported by the NIH Division of Research Resources. Those using fast atom bombardment (FAB) conditions were provided by the Mass Spectrometry Facility, University of California-Riverside.

4-(Hydroxymethyl)quinoline[35] (8):
4-Quinolinecarboxaldehyde (59.56 g, 379 mmol) was dissolved in methanol (500 ml) and treated with solid $NaBH_4$ (17.21 g, 455 mmol) over 1 h, during which time the reaction mixture reached 65 °C. After stirring for an additional hour, the solution was diluted with saturated aqueous NH_4Cl (1200 ml) and extracted with CH_2Cl_2 (3x 500 ml). The organic extracts were combined, washed with brine (2 x 200 ml), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Recrystallization from EtOAc/hexanes followed by column chromatography of the mother liquor (silica gel, 60-100% EtOAc/hexanes) furnished alcohol **8** (56.65 g, 94%) as a white solid (mp = dec. 92 °C).
HRMS: Calc. for $C_{10}H_8NO$: 159.0684. Found: 159.0685.
IR: ν = 2830, 1590, 1511, 1439, 1089, 1076, 844, 757 cm^{-1}.
^{13}C-NMR: δ = 150.3, 147.6, 147.4, 129.7, 129.6, 126.8, 125.9, 123.0, 118.2, 61.0.
^1H-NMR: δ = 8.72 (d, *J* = 4.4 Hz, 1H), 8.12 (d, *J* = 8.5 Hz, 1H), 7.95 (d, *J* = 8.5 Hz, 1H), 7.71 (m, 1H), 5.56 (m, 2H), 5.23 (s, 2H, $ArCH_2OH$), 4.70 (br s, 1H, O*H*).

4-[[(*tert*-Butyldimethylsilyl)oxy]methyl]quinoline (9):
Alcohol **8** (1.60 g, 10.0 mmol) was dissolved in dry DMF (100 ml) under an atmosphere of nitrogen. *tert*-Butyldimethylsilyl chloride (3.02 g, 20.1 mmol) was added, followed immediately by imidazole (2.73 g, 40.1 mmol). The reaction mixture was stirred for 10 min at room temperature then partitioned between

EtOAc/Et_2O and water. The organic layer was washed with water (4 x 40 ml) then with brine (2 x 40 ml), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield pale yellow crystals (2.70 g, 98%) (mp = 32-33 °C).
HRMS: Calc. for $C_{16}H_{23}NOSi$ (M + H): 273.1549. Found: 273.1550.
IR: ν = 2932, 2857, 1709, 1596, 1511, 1465, 1254, 1120, 841, 778 cm^{-1}.
^{13}C-NMR: δ = 150.6, 147.6, 146.58, 130.2, 129.0, 126.3, 125.5, 122.4, 117.6, 61.7, 25.9, 18.4, -5.4.
^{1}H-NMR: δ = 8.92 (d, *J* = 4.5 Hz, 1H), 8.14 (d, *J* = 8.5 Hz, 1H), 7.86 (dd, *J* = 0.9, 8.4 Hz, 1H), 7.7 (m, 1H), 7.5-7.6 (m, 2H), 5.24 (d, *J* = 1.2 Hz, 2H, ArC*H*$_2$OTBS), 0.99 (s, 9H, *t*-butyl), 0.17 (s, 6H, SiC*H*$_3$).

N-[[(2-Nitro-4,5-dimethoxybenzyl)oxy]carbonyl]-4-[[(*tert*-butyldimethylsilyl)oxy]methyl]-2-ethynyl-1,2-dihydroquinoline (10):
Quinoline **9** (2.00 g, 7.31 mmol) was dissolved in THF (10 ml) under an atmosphere of nitrogen. A 0.5 M solution of ethynylmagnesium bromide in THF (29.3 ml) was added to the solution immediately followed by 6-nitroveratryl chloroformate (3.02 g, 11.0 mmol) dissolved in THF (130 ml). The reaction mixture was stirred for 15 min, then diluted with EtOAc (100 ml), and washed with saturated aqueous NH_4Cl (2 x 60 ml). The organic layer was washed with brine (2 x 60 ml), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by two rounds of column chromatography (silica gel, 15% EtOAc/hexanes) to yield **10** (3.09 g, 79%) as an off-white solid (mp = dec. 65 °C).
FABMS: Calc. for $C_{28}H_{38}N_3O_7Si$ (M + NH_4): 556.2479. Found: 556.2479.
IR: ν = 3283, 2933, 2856, 1712, 1524, 1278 cm^{-1}.
^{13}C-NMR: δ = 153.6, 148.0, 139.4, 134.7, 133.7, 127.8, 127.5, 124.8, 122.9, 109.6, 108.1, 71.8, 65.0, 61.9, 56.4, 43.9, 28.7, 25.8, 18.3, -5.4.
^{1}H-NMR: δ = 7.8-7.7 (m, 2H), 7.4-7.3 (m, 2H), 7.3-7.1 (m, 1H), 6.95 (br s, 1H), 6.16 (d, *J* = 6.6 Hz, 1H, vinylic), 6.01 (d, *J* = 5.9 Hz, 1H, propargylic), 5.82 (A of AB q, *J* = 15.6 Hz, 1H, benzylic), 5.59 (B of AB q, *J* = 15.6 Hz, 1H, benzylic), 4.68 (A of AB q, *J* = 14.2 Hz, 1H, allylic), 4.50 (B of AB q, *J* = 14.2 Hz, 1H, allylic), 3.96 (s, 3H, ArOC*H*$_3$), 3.81 (br s, 3H, ArOC*H*$_3$), 2.20 (d, *J* = 2.4 Hz, 1H, CC*H*), 0.93 (s, 9H, *t*-butyl), 0.11 (s, 6H, SiC*H*$_3$).

N-[[(2-Nitro-4,5-dimethoxybenzyl)oxy]carbonyl]-4-(hydroxymethyl)-2-ethynyl-1,2-dihydroquinoline (11):
Silyl ether **10** (2.89 g, 5.36 mmol) was dissolved in a 1:1 mixture of THF and methanol (250 ml each) and acetyl chloride (0.50 ml, 5.4 mmol) was added. The reaction mixture was stirred for 30 min, diluted with EtOAc (50 ml), and washed with saturated aqueous $NaHCO_3$ (2 x 50 ml) and brine (1 x 50 ml). The organic layer was dried ($MgSO_4$), filtered through a silica plug, and recrystallized from EtOAc. A second crop was obtained by concentration of the mother liquor and crystallization from Et_2O. Compound **11** was obtained (1.84 g, 81%) as an off-white solid (mp = dec. 173 °C).
FABMS: Calc. for $C_{22}H_{24}N_3O_7$ (M + NH_4): 442.1614. Found: 442.1614.
IR: ν = 3288, 1702, 1523, 1278, 1220, 1068 cm^{-1}.
^{13}C-NMR: δ = 153.6, 152.7, 148.1, 139.6, 134.9, 133.9, 128.2, 127.2, 125.9, 125.1, 124.7, 123.4, 109.9, 108.2, 79.8, 72.0, 65.2, 62.1, 56.4, 43.8.
^{1}H-NMR: δ = 7.8-7.6 (m, 2H), 7.4-7.1 (m, 3H), 6.96 (br s, 1H), 6.17 (d, *J* = 6.4 Hz, 1H, vinylic), 6.00 (d, *J* = 5.7 Hz, 1H, propargylic), 5.80 (A of AB q, *J* = 15.2 Hz, 1H, benzylic), 5.59 (B of AB q, *J* = 15.2 Hz, 1H, benzylic), 4.60 (d, *J* = 5.5 Hz, 2H, C*H*$_2$OH), 3.96 (s, 3H, ArOC*H*$_3$), 3.83 (br s, 3H, ArOC*H*$_3$), 2.21 (d, *J* = 2.4 Hz, 1H, CC*H*), 1.66 (t, *J* = 5.9 Hz, 1H, O*H*).

N-[[(2-Nitro-4,5-dimethoxybenzyl)oxy]carbonyl]-4-(hydroxymethyl)-3,4-epoxy-2-ethynyl-1,2-dihydroquinoline (12):
Alcohol **11** (1.40 g, 3.29 mmol) was dissolved in CH_2Cl_2 (100 ml) and $NaHCO_3$ (0.31 g, 3.0 mmol) and Na_2SO_4 (1.40 g, 9.87 mmol) were added, followed by *m*CPBA (0.85 g, 4.9 mmol). The reaction mixture was stirred for 2 h, then saturated aqueous $NaHCO_3$ (40 ml) and $Na_2S_2O_3 \cdot 5H_2O$ (5.72 g, 23.0 mmol) were added. After the solid dissolved, the layers were separated and the organic layer was washed with saturated aqueous $NaHCO_3$ (3 x 40 ml), dried (Na_2SO_4), and filtered. The solution was concentrated *in vacuo* to near dryness, diluted with Et_2O (20 ml), and hexanes were added dropwise until cloudiness persisted. The solution was cooled to -4 °C overnight, and the pale yellow solid (1.04 g, 72%) was collected by vacuum filtration (mp = dec. 164 °C).
FABMS: Calc. for $C_{22}H_{24}N_3O_8$ (M + NH_4): 458.1563. Found: 458.1563.
IR: ν = 3553, 3273, 2952, 1708, 1585, 1523, 1395, 1314 cm^{-1}.
^{13}C-NMR (Acetone-d_6): δ = 153.3, 147.8, 139.1, 134.5, 128.4, 128.0, 126.6, 110.1, 108.2, 78.1, 76.8, 64.6, 63.5, 60.7, 56.5, 56.2, 56.1, 43.4.
^{1}H-NMR: δ = 7.72 (d, *J* = 3.9 Hz, 1H), 7.6-7.3 (m, 4H), 6.72 (br s, 1H), 5.91 (d, *J* = 1.9 Hz, 1H, propargylic), 5.8-5.7 (m, 1H, benzylic), 5.6-5.5 (m, 1H, benzylic), 4.48 (dd, *J* = 17.4, 5.0 Hz, 1H, CH*H*OH), 4.2-4.1 (m, 1H, CH*H*OH), 4.10 (d, *J* = 2.7 Hz, 1H, epoxide), 3.93 (s, 3H, ArOC*H*$_3$), 3.75 (br s, 3H, ArOC*H*$_3$), 2.19 (d, *J* = 2.1 Hz, 1H, CC*H*), 1.76 (dd, *J* = 8.0, 5.1 Hz, 1H, O*H*).

N-[[(2-Nitro-4,5-dimethoxybenzyl)oxy]carbonyl]-4-(hydroxymethyl)-3,4-epoxy-2-[6-(trimethylsilyl)-3(*Z*)-hexene-1,5-diynyl]-1,2-dihydroquinoline (14):
Copper iodide (0.04 g, 0.2 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.09 g, 0.08 mmol) were placed in a flask under an atmosphere of nitrogen. Benzene (16 ml, through which nitrogen had been bubbled for 30 min)) was added followed by vinyl chloride **13** (0.19 g, 1.2 mmol), *n*-butylamine (0.07 ml, 0.8 mmol), and epoxide **12** (0.24 g, 0.54 mmol). The reaction mixture was stirred for 3 h, diluted with EtOAc, washed with saturated aqueous NH_4Cl (2 x 20 ml), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by column chromatography (silica gel, 30% EtOAc/hexanes) furnished enediyne **14** (0.15 g, 50%) as a brown foam.
FABMS: Calc. for $C_{29}H_{31}N_2O_8$ (M + H): 563.1850. Found: 563.1850.
IR: ν = 3520, 2957, 1713, 1582, 1524, 1278 cm^{-1}.
^{13}C-NMR: δ = 153.7, 147.9, 134.9, 128.9, 127.9, 127.2, 125.9, 120.9, 119.0, 109.1, 108.0, 101.3, 90.0, 82.8, 65.2, 63.9, 61.4, 61.3, 56.7, 56.4, 44.4.
^{1}H-NMR: δ = 7.71 (s, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.2-7.4 (m, 3H), 6.70 (br s, 1H), 6.14 (br s, 1H, propargylic), 5.5-5.9 (m, 4H, benzylic, vinylic), 4.48 (dd, *J* = 12.6, 5.0 Hz, 1H, C*H*HOH), 4.1-4.2 (m, 2H, epoxide, CH*H*OH), 3.93 (s, 3H, ArOC*H*$_3$), 3.73 (br s, 3H, ArOC*H*$_3$), 1.81 (dd, *J* = 8.3, 5.0 Hz, 1H, O*H*), 0.22 (s, 9H, SiC*H*$_3$).

N-[[(2-Nitro-4,5-dimethoxybenzyl)oxy]carbonyl]-3,4-epoxy-2-[6-(trimethylsilyl)-3(*Z*)-hexene-1,5-diynyl]-4-quinolinecarbaldehyde (15):
Alcohol **14** (2.40 g, 4.26 mmol) was dissolved in CH_2Cl_2 (250 ml) with $NaHCO_3$ (0.72 g, 8.5 mmol), and Dess-Martin periodinane (3.62 g, 8.53 mmol) was added. After 1 h, $Na_2S_2O_3 \cdot 5H_2O$ (7.40 g, 29.8 mmol) and saturated aqueous $NaHCO_3$ were added and the mixture was stirred until the solid dissolved. The organic layer was washed with saturated aqueous $NaHCO_3$ (3 x 100 ml) and brine (1 x 100 ml), dried ($MgSO_4$), filtered through a silica plug, and concentrated *in vacuo*. Purification by column chromatography (silica gel, 30% EtOAc/hexanes) gave aldehyde **15** (2.31 g, 97%) as a dark yellow foam.
FABMS: Calc. for $C_{29}H_{27}N_2O_8Si$ (M - H): 559.1537. Found: 559.1537.
IR: ν = 3482, 2961, 1715, 1582, 1524, 1391, 1279 cm^{-1}.
^{13}C-NMR (Acetone-d_6): δ = 205.9, 154.9, 149.1, 140.2, 136.5, 131.7, 129.1, 128.1, 127.7, 127.6, 126.0, 122.8, 110.3, 108.9, 101.3, 93.5, 91.2, 89.1, 70.8, 66.9, 65.5, 60.7, 56.7, 56.5, 46.6.
^{1}H-NMR: δ = 9.25 (s, 1H, C*H*O), 8.27 (d, *J* = 7.1 Hz, 1H), 7.71 (s, 1H), 7.5-7.2 (m, 3H), 6.68 (br s, 1H), 6.29 (s, 1H, propargylic), 5.8-5.5 (m, 4H, benzylic, vinylic), 4.19 (d, *J* = 2.7 Hz, 1H, epoxide), 3.93 (s, 3H, ArOC*H*$_3$), 3.75 (br s, 3H, ArOC*H*$_3$), 0.21 (s, 9H, SiC*H*$_3$).

Dynemicin analog 4:
Molecular sieves (4 angstrom, 1/8" bead) and $NaHCO_3$ (0.12 g, 1.4 mmol) were placed in an oven dried flask under an atmosphere of nitrogen. Anhydrous CH_3CN (50 ml) was added to the flask followed by trifluroacetic anhydride (0.13 ml, 0.89 mmol). This mixture was stirred for 30 min then aldehyde **15** (0.20 g, 0.36 mmol) was added followed by CsF (0.55 g, 3.6 mmol). After 12 h, more CsF (0.18g, 1.2 mmol) was added and 8 h later the reaction was filtered throuh a silica plug, concentrated *in vacuo*, and purified by column chromatography (silica gel, 40% Et_2O/hexanes) to give **4a** (0.06 g, 36%) and **4b** (0.03 g, 19%) as pale yellow solids (mp **4a** = dec. 118 °C, **4b** = dec. 96 °C).

For compound **4a**:
FABMS: Calc. for $C_{26}H_{21}N_2O_8$ (M + H): 489.1298. Found: 489.1298.
IR: ν = 3490, 2934, 1708, 1581, 1522, 1390, 1318, 1278 cm^{-1}.
^{13}C-NMR (Acetone-d_6): δ = 205.9, 154.9, 149.1, 140.2, 136.5, 131.7, 129.1, 128.1, 127.7, 127.6, 126.0, 122.8, 110.3, 108.9, 101.3, 93.5, 91.2, 89.1, 70.8, 66.9, 65.5, 60.7, 56.7, 56.5, 46.6.
^{1}H-NMR: δ = 8.54 (d, *J* = 8.2 Hz, 1H), 7.71 (s, 1H), 7.5-7.2 (m, 3H), 6.72 (br s, 1H), 6.00 (br s, 1H, CONC*H*CC), 5.9-5.4 (m, 4H, benzylic, vinylic), 4.78 (d, *J* = 3.0 Hz, 1H, CCC*H*OH), 3.93 (s, 3H, ArOC*H*$_3$), 3.83 (d, *J* = 2.8 Hz, 1H, epoxide), 3.45 (br s, 3H, ArOC*H*$_3$), 2.56 (d, *J* = 3.8 Hz, 1H, O*H*).

For compound **4b**:
FABMS: Calc. for $C_{26}H_{21}N_2O_8$ (M + H): 489.1298. Found: 489.1323
IR: ν = 3448, 2929, 1702, 1579, 1523, 1390, 1321, 1278 cm^{-1}.
^{13}C-NMR (Acetone-d_6): δ = 154.8, 149.2, 140.3, 135.8, 129.3, 128.1, 128.0, 127.9, 127.7, 126.2, 126.0, 124.8, 110.6, 108.9, 101.4, 94.2, 91.0, 88.9, 65.5, 62.9, 59.4, 56.7, 56.5, 46.0.
^{1}H-NMR: δ = 7.72 (s, 1H), 7.6-7.2 (m, 4H), 6.76 (br s, 1H), 5.94 (s, 1H, CONC*H*CC), 5.8-5.7 (m, 3H, 1 benzylic, 2 vinylic), 5.5-5.7 (m, 1H, benzylic), 5.48 (s, 1H, CCC*H*OH), 4.45 (s, 1H, epoxide), 3.94 (s, 3H, ArOC*H*$_3$), 3.78 (br s, 3H, ArOC*H*$_3$), 2.41 (br s, 1H, O*H*).

Photoactivation of 4a (6a):
Compound **4a** (30.0 mg, 0.061 mmol) and NH_4Cl (5.0 mg, 0.093 mmol) were placed in a borosilicate test tube which was then flushed with nitrogen. Tetrahydrofuran (10 ml) and methanol (2.5 ml) were added and nitrogen was bubbled through the solution for 20 min, followed by addition of 1,4-cyclohexadiene (58 µl, 0.61 mmol). The reaction mixture was irradiated with a 450-W Hanovia medium pressure mercury arc lamp for 8 h, concentrated *in vacuo*, and purified by column chromatography (silica gel, 60-100% Et_2O/hexanes) to yield **6a** (2.2 mg, 13%) as a white solid (mp = 245-255 °C).
HRMS: Calc. for $C_{17}H_{17}NO_3$: 283.1208. Found: 283.1209.
IR: ν = 3521, 3388, 3342, 1606, 1492, 1267, 1128, 1095, 1066, 1000, 755 cm^{-1}.
^{13}C-NMR (DMSO-d_6): δ = 142.8, 138.1, 136.7, 131.2, 128.1, 127.2, 127.1, 127.0, 126.8, 119.3, 114.9, 113.3, 78.3, 73.0, 63.8, 57.1, 51.3.
^{1}H-NMR: δ = 7.6-7.5 (m, 2H), 7.2-7.3 (m, 3H), 7.05 (dt, *J* = 7.6, 1.5 Hz, 1H), 6.81 (dt, *J* = 7.5, 1.1 Hz, 1H), 6.48 (dd, *J* = 8.0, 1.0 Hz, 1H), 5.18 (d, *J* = 9.3 Hz, 1H,

ArC*H*OH), 4.49 (d, *J* = 4.3 Hz, 1H, ArC*H*NH), 4.30 (dd, *J* = 8.2, 4.2 Hz, 1H, C*H*OH), 4.24 (br s, 1H, N*H*), 3.63 (s, 3H, OCH_3), 2.82 (d, *J* = 8.2 Hz, 1H, CHO*H*), 2.09 (d, *J* = 9.3 Hz, 1H, ArCHO*H*).

Acid activation of 4a:
Compound **4a** (10.0 mg, 0.020 mmol) was dissolved in a mixture of THF (2 ml) and methanol (0.5 ml) under an atmosphere of nitrogen. Acetyl chloride (1.5 µl, 0.020 mmol) was added and after 10 min the reaction mixture was concentrated *in vacuo*. Purification by column chromatography (silica gel, 50% Et_2O/hexanes) furnished **16** (6.5 mg, 62%) as a pale yellow solid (mp = dec. 110 °C).
FABMS: Calc. for $C_{26}H_{23}N_2O_8Cl$: 526.1143. Found: 526.1116
IR: ν = 3484, 2938, 1702, 1579, 1523, 1277, 1220 cm^{-1}.
^{13}C-NMR: δ = 153.8, 148.1, 139.5, 136.9, 134.9, 131.6, 130.8, 129.4, 129.3, 129.1, 129.0, 128.8, 127.9, 126.5, 124.1, 124.0, 109.9, 109.8, 108.1, 79.1, 75.6, 67.8, 65.2, 57.7, 56.5, 56.4.
^{1}H-NMR: δ = 7.8-7.7 (m, 2H), 7.6-7.4 (m, 3H), 7.4-7.0 (m, 5H), 6.16 (d, *J* = 4.3 Hz, 1H, NC*H*Ar), 5.9-5.7 (m, 2H, benzylic), 5.11 (d, *J* = 9.2 Hz, 1H, ArC*H*OH), 4.3-4.2 (m, 1H, C*H*OH), 3.95 (s, 3H, ArOCH_3), 3.84 (br s, 3H, ArOCH_3), 2.93 (d, *J* = 1.8 Hz, 1H, CHO*H*), 2.36 (d, *J* = 9.2 Hz, 1H, ArCHO*H*).

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