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Novel Designed Enediynes: Molecular Design, Chemical Synthesis, Mode of Cycloaromatization and Guanine-Specific DNA Cleavage

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Abstract—The molecular design and chemical synthesis of novel enediyne molecules related to the neocarzinostatin chromophore (**1**), and their chemical and DNA cleaving properties are described. The 10-membered enediyne triols **16–18** were effectively synthesized from xylitol (**10**) in a short step, and found to be quite stable when handled at room temperature. The representative and acylated enediyne **16** was cycloaromatized by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in cyclohexa-1,4-diene-benzene to give the benzenoid product **21** through a radical pathway. On the other hand, the enediyne **16** was cycloaromatized by diethylamine in dimethyl sulfoxide–Tris–HCl, pH 8.5 buffer to afford another benzenoid product **22** as a diethylamine adduct through a polar pathway. Furthermore, the enediynes **16–18** were found to exhibit guanine-specific DNA cleavage under weakly basic conditions with no additive.

Introduction

Novel DNA cleaving molecules, particularly those with high efficiency and base or sequence specificity, have considerable potential in chemistry, molecular biology, and medicine.[2] Therefore, the powerful anticancer and DNA cleaving enediyne antibiotics, neocarzinostatin, calicheamicins, esperamicins, dynemicins, kedarcidin, and C-1027, have been the subject of great interest recently.[2b–f] Neocarzinostatin (NCS) was isolated from *Streptomyces carzinostaticus* var. F-41 by Ishida et al. in 1965 as an original member of the enediyne antibiotics.[3] The clinically used antibiotic[4] consists of a structurally unprecedented nonprotein chromophore (neocarzinostatin chromophore, NCS-C (**1**)) and its separable carrier apo-protein (apo-NCS).[5] It was disclosed that the NCS-C (**1**) was essentially responsible for the biological activity of NCS and exhibited potent cytotoxicity and DNA cleaving activity.[6] The DNA cleavage is now recognized to be initiated by the nucleophilic addition of a thiol to the C12 of **1** followed by a cycloaromatization reaction (Myers cyclization)[7] of an ene-yne-cumulene **2** leading to the formation of a benzenoid diradical **3**, which is capable of cleaving DNA via hydrogen abstraction from the DNA sugar backbone with a high degree of base selectivity ($T > A \gg C \sim G$)[8] (Fig. 1). However, the NCS-C (**1**) itself is exceedingly unstable, undergoing rapid decomposition at elevated pH or upon exposure to air or ambient light.

With the chemical and biological backgrounds, great effort has been devoted to the synthesis of the core units or the development of new analogues of **1**.[9–29] In this context, we started a series of studies directed toward the design, synthesis and investigation of novel, simple and stable DNA cleaving molecules based on the enediyne structure.[30] As a part of our studies, we designed the novel 10-membered enediyne system **5**.[31] Thus far, studies including chemical synthesis, chemical properties, and base-specific DNA cleavage of non-natural enediyne system have been rarely announced. In this paper, we report the full account of the molecular design, chemical synthesis, two different modes of cycloaromatization, and DNA cleaving profiles of the novel enediyne molecules **16–18**.[31]

Results and Discussion

Design of enediyne system 5

The novel enediyne system **5** was designed based on the following plan and expectation (Fig. 2). (1) The monocyclic 10-membered enediyne **5** is stable when handled at ambient temperature due to its non-conjugated enediyne structure in the larger ring system; (2) the enediyne structure of **5** can be transformed into the fully conjugated ene-yne-allene system of **7** which would undergo a Myers-type cyclization reaction to form the diradical species **8** (path A) or undergo nucleophilic attack originating from DNA to form DNA adduct **9** (path B) under specific conditions; either pathway could cause cleavage of DNA; and (3) the hydroxyl groups of **5** make it possible to introduce

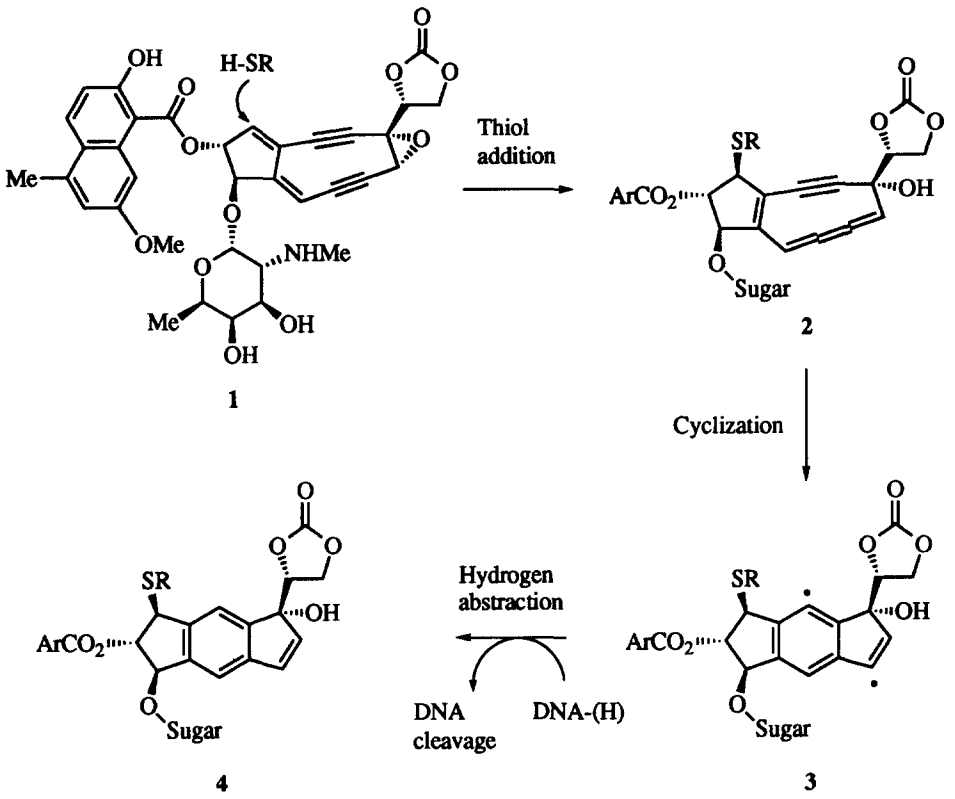


Figure 1. Mechanism of DNA cleaving action of neocarzinostatin chromophore (**1**).

a DNA affinity moiety such as DNA intercalators and DNA binders. According to Nicolaou's report, the distance ab of the ene-yne-allene **7** must be within ca. 3.3 Å for spontaneous cyclization at ambient temperature.[32] Molecular calculations indicated that the distance ab of the 10-membered ene-yne-allene **7** (R=OH) was 3.10 Å (by AM1) or 3.12 Å (by PM3).[33] Considering these points, the novel enediyne **5** and its derivatives **6** were expected to have sufficient stability at ambient temperature, while still offering reasonable lability to generate a diradical and cleave DNA under specific conditions.

Synthesis of enediynes 16–18

Our synthetic approach began with the conversion of xylitol (**10**) into the 10-membered keto-enediyne **11** by the procedure recently developed in our laboratories[30f,h] (Scheme 1). Reduction of **11** using 1.2 equiv of sodium borohydride in methanol at 25 °C for 0.5 h afforded the alcohol **12** in 90% yield. Notably, as expected, it was found that the novel enediyne **12** was quite stable at room temperature. Considering the presumed mechanism of the DNA cleaving action of the designed enediynes, the expectation that high acidity of the hydrogen at the allylic position, and DNA intercalators or other DNA binding moieties would enhance the potency of **5** as a DNA cleaver was borne out at this stage. Therefore, several acylated derivatives **16–18** possessing DNA intercalative aromatic moieties were next prepared. Thus, the reactions of **12** with benzoyl, 2-quinoxaloyl and 1-naphthoyl chlorides, in the presence of a suitable base such as pyridine or triethylamine afforded acylated enediynes, **13** (91%), **14** (89%), and **15** (50%), respectively. Finally, the desilylations of **13–15** using camphorsulfonic acid, HF–pyridine or 46% HF–H_2O afforded the desired enediyne triols, **16** (80%), **17** (45%), and **18** (50%), respectively. These derivatives were also found to be quite stable when handled at room temperature.

Cycloaromatizations of enediyne 16

Our attention next turned to the mode of cycloaromatization of these novel enediynes under weakly basic conditions. Treatment of the representative enediyne **16** possessing a benzoyl group at the allylic position with 2.0 equiv of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) in cyclohexa-1,4-diene-benzene (1:2) at 25 °C for 1.5 h gave the cycloaromatization product **21** in 7.0% yield as the only isolated product. This result clearly indicates that the migration of the hydrogen at the allylic position of **16** induced by DBU first produces the ene-yne-allene intermediate **19** which immediately undergoes a Myers-type cyclization leading to the benzenoid diradical **20** as shown in Figure 3.[34] In drastic contrast, treatment of **16** with 10 equiv of diethylamine in 20% dimethyl sulfoxide in Tris–HCl, pH 8.5 buffer at 25 °C for 1 h gave 12% of the cycloaromatization product **22** as the sole isolated product. The diethylamine adduct **22** must arise from the nucleophilic addition of diethylamine to the ene-yne-allene intermediate **19** and debenzoylation as

shown in Figure 4.[35] These results indicate that the novel enediynes are aromatized both by DBU in cyclohexa-1,4-diene-benzene through a radical pathway and by diethylamine in dimethyl sulfoxide–Tris–HCl, pH 8.5 buffer through a polar pathway.

DNA cleavage with enediynes 16–18

The DNA cleaving activities of the novel enediynes **16–18** were assayed using double-stranded supercoiled ΦX174 DNA.[36] As expected from the modes of the cycloaromatization, these compounds were found to cleave DNA in a striking pH-dependent fashion and in only alkaline conditions. Thus, aerobic incubations of **16–18** with the covalently closed supercoiled DNA (form I) in 20% dimethyl sulfoxide–Tris–HCl, pH 8.5 buffer at 37 °C for 24 h in concentrations of 1000–100 μM without any additive caused a single strand break, leading to the nicked open circular DNA (form II) as shown in Figure 5. Furthermore, it was clearly found that the DNA cleaving activities of **16**, **17** and **18** increased in that order. These results indicated that even the highly simple and stable enediyne molecule has DNA cleaving property and its activity could be improved by the introduction of a good DNA affinity moiety. Their DNA cleaving site specificity[37] was also analyzed according to the Sanger protocol.[38] The results shown in Figure 6 clearly show the identical high guanine selectivity of these compounds for their DNA cleaving profiles. The base selectivity was highly independent of the DNA affinity moiety examined. Furthermore, it was found that addition of a radical scavenger, catalase, superoxide dismutase or 2-mercaptoethanol, as a cofactor did not have any measurable effect on the DNA cleavage. These results, the mode of aromatization of the enediyne in dimethyl sulfoxide–Tris–HCl, pH 8.5 buffer already mentioned and the high purine, guanine, base selectivity strongly support an alkylation mechanism (path B in Figure 2) rather than a radical mechanism (path A in Figure 2) for DNA cleavages by these novel enediynes.[39,40]

Conclusion

The present work shows not only the design and synthesis of novel enediyne molecules related to the neocarzinostatin chromophore but also their modes of cycloaromatization and DNA cleaving profiles. It was made clear that even a designed simple and stable enediyne molecule had DNA cleaving properties with high base selectivity and its activity could be improved by the introduction of a DNA affinity moiety. Furthermore, it is interesting to note that the mechanism of DNA cleavage of the designed enediynes seems to be quite different from that of natural neocarzinostatin

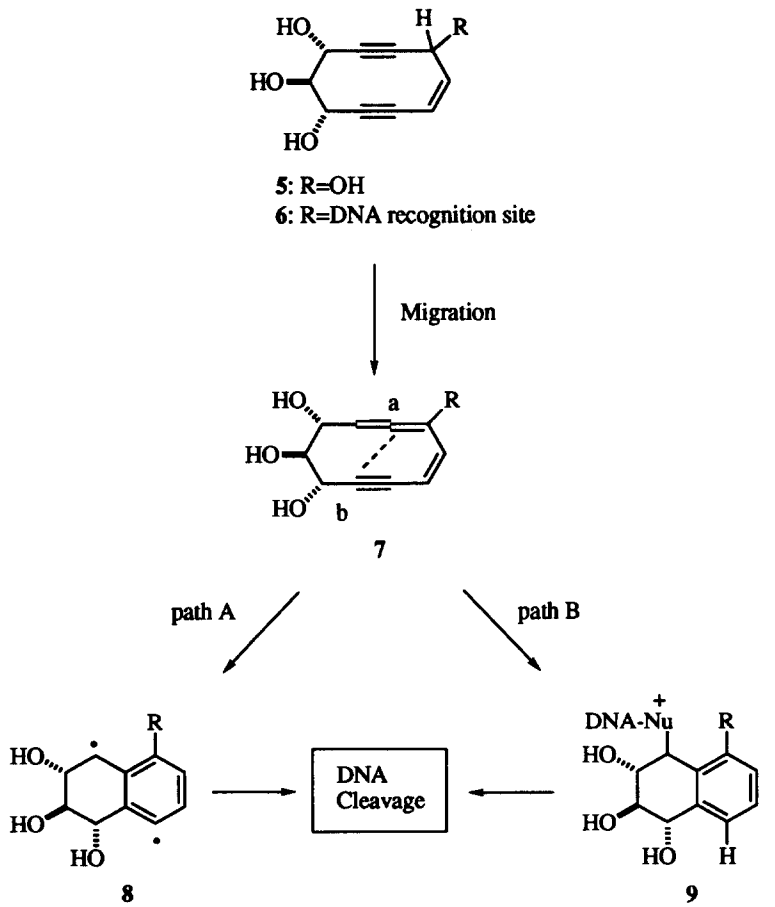
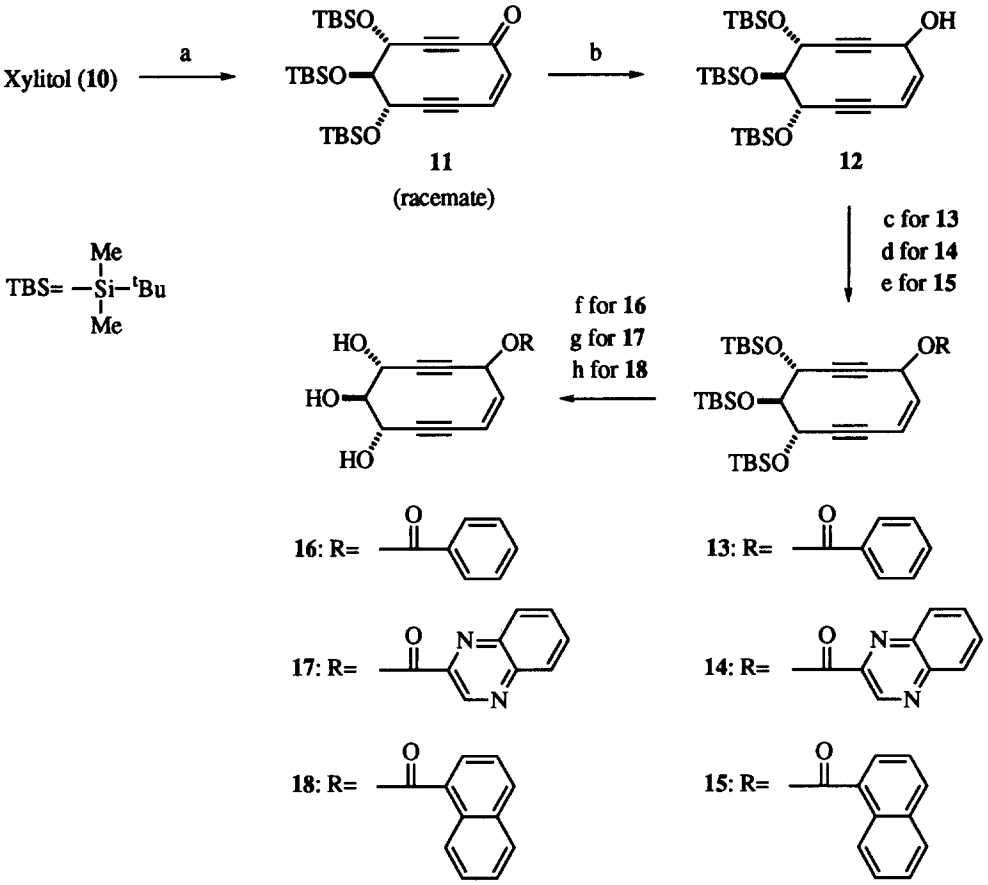


Figure 2. Mechanistic rationale for designed enediyne molecules: (path A) radical mechanism; (path B) alkylation mechanism.



Scheme 1. Synthesis of designed enediynes **16–18**. Reagents and conditions: (a) Refs 30f and h; (b) $NaBH_4$, MeOH, 25 °C, 0.5 h, 90%; (c) BzCl, pyridine, 25 °C, 2 h, 91%; (d) 2-quinoxaloyl chloride, Et_3N, CH_2Cl_2, 25 °C, 0.5 h, 89%; (e) 1-naphthoyl chloride, Et_3N, CH_2Cl_2, 25 °C, 0.5 h, 50%; (f) 10-*dl*-camphorsulfonic acid, MeOH, 25 °C, 15 h, 80%; (g) HF–pyridine, pyridine, 25 °C, 20 h, 45%; (h) 46% HF (aq), dioxane, 25 °C, 20 h, 50%.

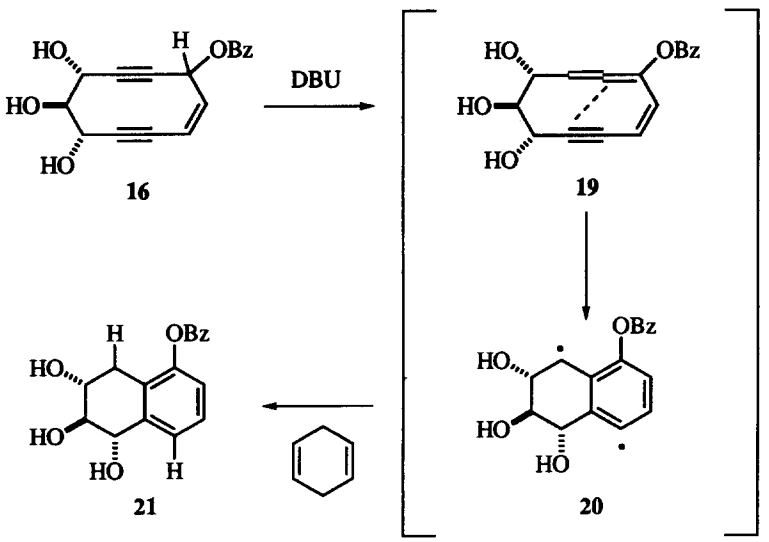


Figure 3. Mode of cycloaromatization of enediyne **16** through a radical pathway.

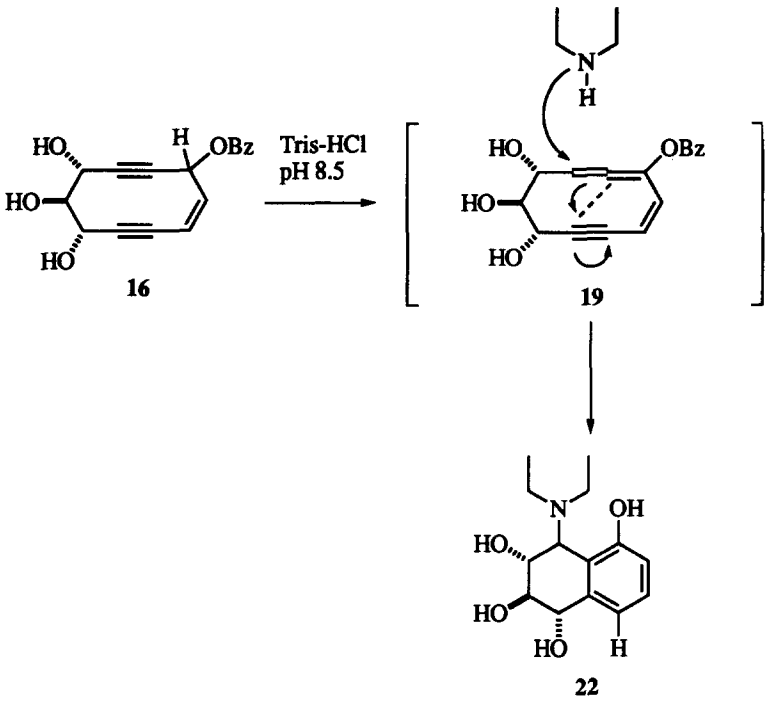


Figure 4. Mode of cycloaromatization of enediyne **16** through a polar pathway.

chromophore. The described chemistry and biological evaluation provided significant information about the molecular design of simple and stable DNA cleaving agents based on the enediyne structure. Attaching the novel DNA cleaving moiety onto the sequence-specific delivery systems has become the next phase of this program.

Experimental

General procedure

Melting points were determined on a micro hot-stage Yanaco MP-S3. ^{1}H NMR spectra were obtained on a JEOL GSX270 spectrometer in $CDCl_3$ using TMS as internal standard unless otherwise noted. High-resolution mass spectra (HRMS) were recorded on a JEOL LMS-DX302 mass spectrometer under electron impact (EI) conditions. Silica gel TLC and column chromatography were performed on Merck TLC 60F-254 (0.25 mm) and Merck Kieselgel 60 or Fuji-Davison BW-820MH, respectively. Preparative thin-layer chromatography was performed on 0.5 mm × 20 cm × 20 cm Merk silica gel plates (60F-254). Air and/or moisture sensitive reactions were carried out under an atmosphere of argon with oven-dried glassware. In general, organic solvents were purified and dried by the appropriate procedure, and evaporation and concentration were carried out under reduced pressure below 30 °C, unless otherwise noted.

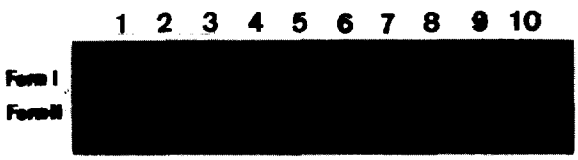


Figure 5. DNA cleavage with enediynes **16–18**. ΦX174 form I DNA (50 μM per base pair) was incubated for 24 h at 37 °C with **16**, **17** and **18** in 20% dimethyl sulfoxide in Tris–HCl buffer (pH 8.5, 50 mM) and analyzed by electrophoresis (1% agarose gel, ethidium bromide stain). Lane 1, DNA alone; lanes 2–10: **16** (1000), **16** (100), **16** (10), **17** (1000), **17** (100), **17** (10), **18** (1000), **18** (100) and **18** (10 μM), respectively.

***dl*-(1*RS*,2*Z*,6*R*,7*R*,8*S*)-6,7,8-Tris[(*t*-butyldimethylsilyl)-oxy]-2-cyclodecene-4,9-diyn-1-ol (12).** To an ice-cold solution of **11** (0.764 g, 1.43 mmol) in methanol (20 mL) was added $NaBH_4$ (64.9 mg, 1.72 mmol) with stirring. After the resulting solution was stirred at 25 °C for 30 min, the mixture was made neutral with ion exchange resin, CG-50. The resin was filtered off and washed with methanol (10 mL × 5). The filtrates were concentrated in vacuo. Purification of the residue by flash column chromatography (35 g of silica gel, 10:1, *n*-hexane:ethyl acetate) gave **12** (0.690 g, 90%) as a pale yellow oil. R_f 0.39 (10:1, *n*-hexane:ethyl acetate); ^{1}H NMR δ 0.08 (3H, s, Me of TBS), 0.09 (3H, s, Me of TBS), 0.12 (3H, s, Me of TBS), 0.13 (3H, s, Me of TBS), 0.14 (3H, s, Me of TBS), 0.15 (3H, s, Me of TBS), 0.90 (4.5H, s, *t*-Bu of TBS), 0.91 (4.5H, s, *t*-Bu of TBS), 0.92 (9H, s, *t*-Bu of TBS), 0.93 (4.5H, s, *t*-Bu of TBS), 0.935 (4.5H, s, *t*-Bu of TBS), 1.97 (0.5H, d, J=8.4 Hz, OH), 2.03 (0.5H, d, J = 8.4 Hz, OH), 3.73 (0.5H, dd, J=5.2 and 5.2 Hz, H-7), 3.75 (0.5H, dd, J=5.2 and 5.2 Hz, H-7), 4.38–4.50 (2H, m, H-6 and 8), 4.88–5.00 (1H, m, H-1), 5.59 (0.5H, ddd, J=12.0, 2.0 and 1.8 Hz, H-3), 5.60 (0.5H, ddd, J=12.0, 1.8 and 1.6 Hz, H-3), 5.90 (0.5H, dd, J=12.0 and 4.0 Hz, H-2), 5.94 (0.5H, dd, J=12.0 and 4.0 Hz, H-2); HRMS (EI) *m/z* 536.3143 (536.3174 calcd for $C_{28}H_{52}O_4Si_3$, M^+).

***dl*-(1*RS*,2*Z*,6*R*,7*R*,8*S*)-1-(Benzoyloxy)-6,7,8-tris[(*t*-butyldimethylsilyl)oxy]-2-cyclodecene-4,9-diyne (13).** To an ice-cold solution of **12** (0.692 g, 1.29 mmol) in dry pyridine (17 mL) was added dropwise benzoyl chloride (0.179 mL, 1.54 mmol). After the resulting solution was stirred at 25 °C for 2 h, the mixture was poured into ice-cold water (40 mL). The resulting mixture was extracted with *n*-hexane (20 mL × 3). The extracts were washed with saturated aqueous NaCl (40 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification of the residue by flash column chromatography (40 g of silica gel, 20:1, *n*-hexane:diethyl ether) gave **13** (0.751 g, 91%) as a pale yellow oil. R_f 0.73 (10:1, *n*-hexane:ethyl acetate); ^{1}H NMR δ 0.10 (6H, s, Me of TBS), 0.14 (6H, s, Me of TBS), 0.15 (6H, Me of TBS), 0.88 (4.5H, s, *t*-Bu of TBS), 0.91 (9H, s, *t*-Bu of TBS), 0.92 (4.5H, s, *t*-Bu of TBS), 0.93 (9H, s, *t*-Bu of TBS), 3.76 (0.5H, dd, J=6.0 and 5.0 Hz, H-7), 3.78 (0.5H, dd, J=6.0 and 5.0 Hz, H-7), 4.43 (1H, dd, J=6.0 and 1.6 Hz, H-6), 4.45–4.52 (1H, m, H-8), 5.74 (0.5H, ddd, J=12.0, 1.6 and 1.6 Hz, H-3), 5.76 (0.5H, ddd, J=12.0, 1.6 and 1.6 Hz, H-3), 5.88 (0.5H, dd, J=12.0 and 4.0 Hz, H-2), 5.94 (0.5H, dd, J=12.0 and 4.0 Hz, H-2), 6.23–6.32 (1H, m, H-1), 7.38–7.63 (3H, m, aromatic), 8.02–8.10 (2H, m, aromatic).

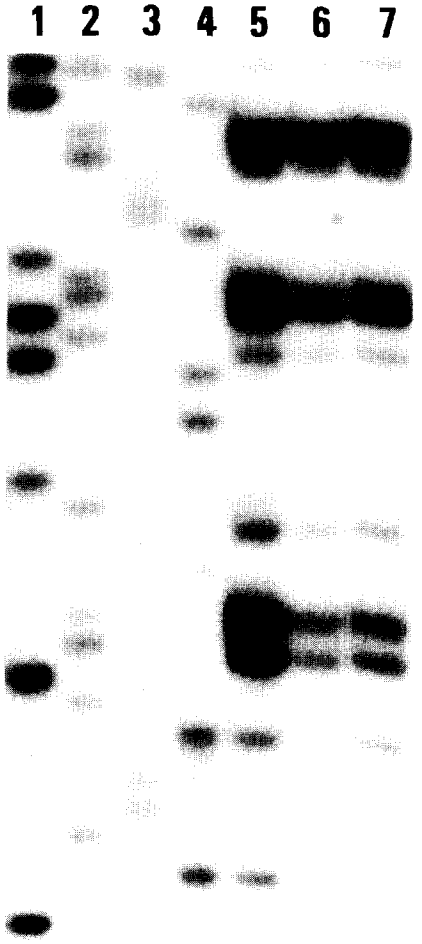


Figure 6. Autoradiogram of 12% polyacrylamide/8 M urea slab gel electrophoresis for sequence analysis. The 5′-end-labelled M13mp18 DNA was cleaved by **16**, **17** and **18** at pH 8.5 and at 45 °C for 24 h (bases 50–90 are shown). Lanes 1–4: Sanger A, G, C and T reactions, respectively; lanes 5–7: **16** (2), **17** (2) and **18** (2 mM), respectively.

***dl*-(1*RS*,2*Z*,6*R*,7*R*,8*S*)-6,7,8-Tris[(*t*-butyldimethylsilyl)oxy]-1-(2-quinoxaloyloxy)-2-cyclodecene-4,9-diyne (14).** To an ice-cold solution of **12** (0.696 g, 1.30 mmol) in dry CH_2Cl_2 (17 mL) were added Et_3N (0.452 mL, 3.24 mmol) and 2-quinoxaloyl chloride (0.550 g, 2.85 mmol). After the resulting solution was stirred at 25 °C for 30 min, the mixture was poured into ice-cold water (20 mL). The resulting mixture was extracted with $CHCl_3$ (15 mL × 3). The extracts were washed with saturated aqueous NaCl (20 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification of the residue by flash column chromatography (40 g of silica gel, 3:1, *n*-hexane:diethyl ether) gave **14** (0.800 g, 89%) as a pale yellow oil. R_f 0.33 (3:1, *n*-hexane:diethyl ether); ^{1}H NMR δ 0.09 (3H, s, Me of TBS), 0.10 (3H, s, Me of TBS), 0.13 (3H, s, Me of TBS), 0.14 (3H, s, Me of TBS), 0.15 (6H, s, Me of TBS), 0.88 (4.5H, s, *t*-Bu of TBS), 0.90 (4.5H, s, *t*-Bu of TBS), 0.92 (9H, s, *t*-Bu of TBS), 0.93 (4.5H, s, *t*-Bu of TBS), 0.94 (4.5H, s, *t*-Bu of TBS), 3.78 (0.5H, dd, J=5.2 and 5.0 Hz, H-7), 3.80 (0.5H, dd, J=5.0 and 5.0 Hz, H-7), 4.40–4.55 (2H, m, H-6 and 8), 5.81 (0.5H, ddd, J=12.0, 2.0 and 1.6 Hz, H-3), 5.83 (0.5H, ddd, J=12.0, 1.8 and 1.6 Hz, H-3), 5.98 (0.5H, dd, J=12.0 and 4.0 Hz, H-2), 6.02 (0.5H, dd, J=12.0 and 4.0 Hz, H-2), 6.37–6.45 (1H, m, H-1), 7.80–8.35 (4H, m, aromatic), 9.51 (0.5H, s, H-3′), 9.52 (0.5H, s, H-3′).

***dl*-(1*RS*,2*Z*,6*R*,7*R*,8*S*)-1-(1-Naphthoyloxy)-6,7,8-tris[(*t*-butyldimethylsilyl)oxy]-2-cyclodecene-4,9-diyne (15).** To an ice-cold solution of **12** (41.3 mg, 0.0769 mmol) in dry CH_2Cl_2 (1.5 mL) were added Et_3N (19.3 μL, 0.138 mmol) and 1-naphthoyl chloride (18.5 μL, 0.123 mmol) with stirring. After the resulting solution was stirred at 25 °C for 0.5 h, the mixture was poured into ice-cold water (2.0 mL). The resulting mixture was extracted with *n*-hexane (1.0 mL × 3). The extracts were washed with saturated aqueous NaCl (2.0 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification of the residue by flash column chromatography (3.0 g of silica gel, 20:1, *n*-hexane:diethyl ether) gave **15** (44.7 mg, 84%) as a pale yellow oil. R_f 0.30 (20:1, *n*-hexane:diethyl ether); ^{1}H NMR δ 0.08 (3H, s, Me of TBS), 0.10 (3H, s, Me of TBS), 0.11 (3H, s, Me of TBS), 0.14 (3H, s, Me of TBS), 0.15 (3H, s, Me of TBS), 0.16 (3H, s, Me of TBS), 0.88 (4.5H, s, *t*-Bu of TBS), 0.89 (4.5H, s, *t*-Bu of TBS), 0.92 (4.5H, s, *t*-Bu of TBS), 0.93 (9H, s, *t*-Bu of TBS), 0.94 (4.5H, s, *t*-Bu of TBS), 3.78 (0.5H, dd, J=5.8 and 5.8 Hz, H-7), 3.80 (0.5H, dd, J=6.0 and 4.8 Hz, H-7), 4.40–4.52 (2H, m, H-6 and 8), 5.77 (0.5H, ddd, J=12.0, 1.8 and 1.8 Hz, H-3), 5.79 (0.5H, ddd, J=12.0, 1.6 and 1.6 Hz, H-3), 5.95 (0.5H, dd, J=12.0 and 4.0 Hz, H-2), 6.02 (0.5H, dd, J=12.0 and 4.2 Hz, H-2), 6.33–6.42 (1H, m, H-1), 7.45–7.67 (3H, m, aromatic), 7.88 (1H, dull d, J=8.0 Hz, aromatic), 8.03 (1H, dull d, J=8.4 Hz, aromatic), 8.23 (1H, ddd, J=7.6, 5.8 and 1.4 Hz, aromatic), 8.92 (1H, dull d, J=8.4 Hz, aromatic).

***dl*-(1*RS*,2*Z*,6*R*,7*R*,8*S*)-1-(Benzoyloxy)-2-cyclodecene-4,9-diyne-6,7,8-triol (16).** To a stirred solution of **13** (0.75 g, 1.17 mmol) in methanol (18 mL) was added 10-*dl*-camphorsulfonic acid (0.204 g, 0.878 mmol) at 25 °C. After 15 h, the resulting solution was made neutral with Et_3N and then concentrated in vacuo. Purification of the residue by flash column chromatography (17 g of silica gel, 2:1 benzene:acetone) gave **16** (0.279 g, 80%) as white crystals. R_f 0.28 (2:1, benzene:acetone); mp 79.0–80.0 °C (acetone-*n*-hexane); ^{1}H NMR δ 2.86 (2H, br s, OH), 3.23–3.45 (1H, br, OH), 3.88 (1H, dull dd, J=8.0 and 8.0 Hz, H-7), 4.42 (1H, dull d, J=8.0 Hz, H-6), 4.49 (0.5H, dull d, J=8.0 Hz, H-8), 4.52 (0.5H, dull d, J=8.0 Hz, H-8), 5.81 (1H, dull d, J=12.0 Hz, H-3), 5.97 (1H, dull dd, J=12.0 and 2.0 Hz, H-2), 6.30 (1H, dull d, J=2.0 Hz, H-1), 7.46 (2H, dd, J=8.0 and 7.0 Hz, H-3′ and 5′), 7.59 (1H, t, J=7.0 Hz, H-4′), 8.07 (2H, d, J=8.0 Hz, H-2′ and 6′); HRMS (EI) *m/z* 298.0864 (298.0841 calcd for $C_{17}H_{14}O_5$, M^+).

***dl*-(1*RS*,2*Z*,6*R*,7*R*,8*S*)-1-(2-Quinoxaloyloxy)-2-cyclodecene-4,9-diyne-6,7,8-triol (17).** To an ice-cold solution of **14** (0.800 g, 1.15 mmol) in dry pyridine (38 mL) was added HF–pyridine (6.90 mL). After the resulting mixture was stirred at 25 °C for 20 h, the mixture was poured into ice-cold saturated aqueous $NaHCO_3$ (70 mL). The resulting mixture was extracted with EtOAc (30 mL × 3). The extracts were washed with saturated NaCl (30 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification of the residue by flash column chromatography (20 g of silica gel, 1:1, benzene:acetone) gave **17** (0.181 g, 45%) as white crystals. R_f 0.40 (1:1, benzene:acetone); mp 110–112 °C (dec, acetone-*n*-hexane); ^{1}H NMR (acetone-d_6) δ 3.72 (0.5H, dd, J=8.0 and 8.0 Hz, H-7), 3.73 (0.5H, dd, J=8.0 and 8.0 Hz, H-7), 4.30–4.50 (2H, m, H-6 and 8), 4.53 (0.5H, dull d, J=4.8 Hz, OH), 4.62 (0.5H, dull d, J=4.8 Hz, OH), 4.67 (1H, dull d, J=4.8 Hz, OH), 4.75–4.88 (1H, br OH), 5.95 (1H, ddd, J=12.0, 1.6 and 1.6 Hz, H-3), 6.13 (0.5H, dd, J=12.0 and 4.0 Hz, H-2), 6.14 (0.5H, dd, J=12.0 and 4.0 Hz, H-2), 6.46 (1H, ddd, J=4.0, 2.0 and 1.6 Hz, H-1), 7.95–8.30 (4H, m, aromatic), 9.49 (1H, s, H-3′); HRMS (EI) *m/z* 350.0873 (350.0903 calcd for $C_{19}H_{14}N_2O_5$, M^+).

***dl*-(1*RS*,2*Z*,6*R*,7*R*,8*S*)-1-(1-Naphthoyloxy)-2-cyclodecene-4,9-diyne-6,7,8-triol (18).** To an ice-cold solution of **15** (17.0 mg, 0.0246 mmol) in dioxane (2.4 mL) was added 46% HF aqueous solution (0.48 mL). After the resulting solution was stirred at 25 °C for 20 h, the mixture was poured into ice-cold saturated aqueous $NaHCO_3$ (6.0 mL). The resulting mixture was extracted with EtOAc (3.0 mL × 3). The extracts were washed with saturated aqueous NaCl (3.0 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification of the residue by flash column chromatography (1.5 g of silica gel, 2:1 benzene:acetone) gave **18** (4.2 mg, 50%) as white crystals. R_f 0.35 (2:1 benzene:acetone); mp 175–177 °C (dec, acetone-*n*-hexane); ^{1}H NMR (acetone-d_6) δ 3.70 (1H, dull dd, J=8.0 and 8.0 Hz, H-7), 4.25–4.43 (2H, m, H-6 and 8), 4.52 (1H, dull s, OH), 4.62 (0.5H, dull d, J=4.0 Hz, OH), 4.67 (0.5H, dull d, J=4.4 Hz, OH), 4.80 (0.5H, dull s, OH), 4.83 (0.5H, dull s, OH), 5.92 (0.5H, dull d, J=12.0 Hz, H-3), 5.93 (0.5H, dull d, J=12.0 Hz, H-3), 6.14 (0.5H, dd, J=12.0 and 4.2 Hz, H-2), 6.15 (0.5H, dd, J=12.0 and 4.2 Hz, H-2), 6.42 (1H, dd, J=4.2 and 2.0 Hz, H-1), 7.55–7.75 (3H, m, aromatic), 8.03 (1H, dd, J=8.0 and 2.0 Hz, aromatic), 8.21 (1H, d, J=8.0 Hz, aromatic), 8.24 (1H, dd, J=7.0 and 1.8 Hz, aromatic), 8.92 (1H, d, J=8.4 Hz, aromatic); HRMS (EI) *m/z* 348.1000 (348.0998 calcd for $C_{21}H_{16}O_5$, M^+).

Cycloaromatization of 16 with DBU in cyclohexa-1,4-diene-benzene. To an ice-cold solution of **16** (18.5 mg, 0.062 mmol) in cyclohexa-1,4-diene (0.21 mL) and benzene (0.41 mL) was added 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) (0.0185 mL, 0.124 mmol). After the resulting mixture was stirred at 25 °C for 1.5 h, the reaction was quenched with saturated aqueous NH_4Cl (1.5 mL) and the mixture was then extracted with EtOAc (0.5 mL × 3). The extracts were washed with saturated aqueous NaCl (1.5 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification of the residue by preparative thin-layer chromatography (1:1, chloroform:acetone) gave **21** (1.3 mg, 7.0%) as white crystals. R_f 0.42 (1:1 chloroform:acetone); mp 190–192 °C (acetone-*n*-hexane); ^{1}H NMR (acetone-d_6) δ 2.55 (1H, dd, J=16.8 and 10.0 Hz, H-1), 3.09 (1H, dd, J=16.8 and 6.0 Hz, H-1), 3.60 (1H, ddd, J=9.8, 8.0 and 1.8 Hz, H-3), 3.85 (1H, dddd, J=10.0, 9.8, 6.0 and 3.0 Hz, H-2), 4.09 (1H, d, J=3.0 Hz, OH), 4.37 (1H, d, J=1.8 Hz, OH), 4.52 (1H, d, J=6.0 Hz, OH), 4.47 (1H, dd, J=8.0 and 6.0 Hz, H-4), 7.13 (1H, dd, J=8.0 and 1.0 Hz, H-5), 7.33 (1H, dd, J=8.0 and 8.0 Hz, H-6), 7.54 (1H, dull d, J=8.0 Hz, H-7), 7.57–7.80 (3H, m, aromatic), 8.17–8.25 (2H, m, aromatic); HRMS (EI) *m/z* 300.1019 (300.0998 calcd for $C_{17}H_{16}O_5$, M^+).

Cycloaromatization of 16 with diethylamine in 20% dimethyl sulfoxide-Tris–HCl buffer. Compound **16** (11.1 mg, 0.0372 mmol) was dissolved in 20% dimethyl sulfoxide–Tris–HCl, pH 8.5 buffer (0.75 mL) and diethylamine (0.0384 mL, 0.372 mmol) was added to the solution. After the resulting solution was stirred at 25 °C for 1 h, the reaction was quenched with saturated aqueous NH_4Cl (1 mL) and the resulting mixture was then extracted with Et_2O (0.5 mL × 3). The extracts were washed with saturated aqueous NaCl (1 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification of the residue by preparative thin-layer chromatography (1:1, benzene:acetone) gave **22** (1.2 mg, 12%) as a pale yellow oil. R_f 0.25 (1:1, benzene:acetone); ^{1}H NMR δ 1.08 (3H, t, J=7.0 Hz, Me), 1.33 (3H, t, J=7.0 Hz, Me), 2.5–2.67 (2H, m, NCH_2CH_3), 3.01 (1H, dq, J=13.0 and 7.0 Hz, NCH_2CH_3), 3.25 (1H, dq, J=13.0 and 7.0 Hz, NCH_2CH_3), 3.57 (1H, dd, J=9.6 and 9.6 Hz, H-2 or 3), 4.14 (1H, dd, J=9.6 and 9.6 Hz, H-2 or 3), 4.45 (1H, d, J=9.6 Hz, H-1 or 4), 4.52 (1H, d, J=9.6 Hz, H-1 or 4), 6.75 (1H, dull d, J=8.0 Hz, H-5 or 7), 6.98 (1H, dd, J=8.0 and 1.0 Hz, H-5 or 7), 7.22 (1H, dd, J=8.0 and

8.0 Hz, H-6); HRMS (EI) *m/z* 268.1557 (268.1549 calcd for $C_{14}H_{22}NO_4$, $M+H^+$).

DNA cleavage studies

(A) Assay for damage to DNA. All DNA cleavage experiments were performed with ΦX174 DNA (50 μM/base pair) in a volume of 10 μL containing 20% dimethyl sulfoxide in 50 mM Tris–HCl buffer (pH 8.5) at 37 °C for 24 h. The DNA-sample levels were varied as indicated in the figure caption. The results were analyzed using 1% agarose gel electrophoresis and detection with ethidium bromide fluorescence. The electrophoresis gels were immediately visualized on a UV transilluminator and photographed using black and white instant film. Figure 5 shows the pictures of the agarose gel electrophoresis results.

(B) Identification of DNA cleavage sites. The reaction samples contained the enediyne compounds (2 mM) and the 5′-end-labelled M13mp18 DNA (40 ng) in a volume of 30 μL containing 20% dimethyl sulfoxide in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.5). The cleavage reactions were allowed to proceed at 45 °C for 24 h. To stop the reactions, each reaction sample was washed with a solution of TE buffer-saturated phenol:chloroform:isoamyl alcohol (25:24:1) and the resulting aqueous layer was lyophilized. After each lyophilized sample was dissolved in 1 M piperidine–water (20 μL) and then heated at 90 °C for 30 min, each sample was again lyophilized. Each lyophilized sample was dissolved in a loading buffer containing distilled water, 95% deionized formamide, 10 mM EDTA, 0.05% xylene cyanole FF, and 0.05% bromophenol blue and then the mixture was loaded onto a 12% polyacrylamide gel containing 8 M urea in TBE buffer. DNA sequencing was carried out by Sanger method. Figure 6 shows the picture of the autoradiogram.

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