Molecular Design, Chemical Synthesis, and Study of Novel Enediyne-Sulfide Systems Related to the Neocarzinostatin Chromophore

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Abstract: The design and synthesis of the novel monocyclic enediyne-sulfide systems and their chemical and DNA cleavage properties are described. The parent enediyne-sulfide **6** possessing a hydroxy group at the allylic position was effectively synthesized via the cross-coupling of the *cis*-vinyl iodide **11** and the acetylene derivative **12** using a Pd(0)-Cu(I) catalyst and the cyclization reaction of the acyclic dibromide **20** employing sodium sulfide as the key steps. In addition, the esterifications of **6** using appropriate procedures provided a series of its simple derivatives **21-29** and the hybrids **38-44** containing naturally occurring intercalators, all of which are quite stable when handled at ambient temperature. The representative enediyne-sulfide **22** was smoothly aromatized by 1,8-diazabicyclo-[5.4.0]undec-7-ene in cyclohexa-1,4-diene through radical pathways and by a hydroxy anion in dimethyl sulfoxide-Tris-HCl, pH 8.5 buffer through a polar pathway. Furthermore, it was clearly found that all enediyne-sulfides cleaved DNA under alkaline conditions without any additive and the hybrids **38** and **44**, each of which has the aromatic moiety of the neocarzinostatin chromophore and manzamins, respectively, exhibited the strongest DNA cleaving abilities with the identical high purine base (G > A) selectivity.

Introduction

A novel class of antibiotics, the enediynes, represented by neocarzinostatin,[2] calicheamicins,[3] esperamicins,[4] and dynemicins,[5] are some of the most potent antitumor agents naturally derived. These antibiotics have highly strained unique structures and strong DNA cleaving activities. The mechanism of DNA cleavage is now recognized to involve a Masamune-Bergman-type cyclization reaction[6,7] leading to a damaging benzenoid diradical. Consequently, great effort has been devoted both to the synthesis of the natural products themselves and the development of new analogs that mimick their chemistry and biologial action.[8] Crucial points of the latter case include (1) molecular design to functionalize the simple molecule; (2) development of synthetic routes to the highly strained structure; (3) investigation of the mechanism of action, particularly the cycloaromatization to generate a benzenoid diradical; and (4) evaluation of the DNA cleaving profiles. Therefore, simple and stable DNA cleaving molecules based on the enediyne structure are of considerable current interest in bioorganic chemistry, molecular biology, and pharmacology, as well as in synthetic organic chemistry. Nicolaou's group has performed significant pioneering studies in this area.[8] In this paper, we report the full account of the design, synthesis, cycloaromatizations, and DNA cleaving profiles of the novel enediyne-sulfide systems related to the neocarzinostatin chromophore (**1**) which is the labile heart of the antitumor antibiotic, neocarzinostatin.[9]

Results and Discussion

Design of Enediyne-Sulfide System. The nonprotein chromophore **1** of the antitumor agent neocarzinostatin, isolated from the culture filtrate of *Streptomyces carzinostaticus* by Ishida et al. in 1965, is essentially responsible for the biological activity of neocarzinostatin and exhibits potent cytotoxicity and DNA cleaving activity.[2] The DNA cleavage is now believed to be initiated by a facile nucleophilic addition of a thiol to the C12 of **1** followed by a rapid cyclization reaction (Myers cyclization) of an enzyne-cumulene **2** leading to the formation of a highly reactive diradical **3**, which is capable of cleaving DNA via hydrogen abstraction from the DNA sugar backbone

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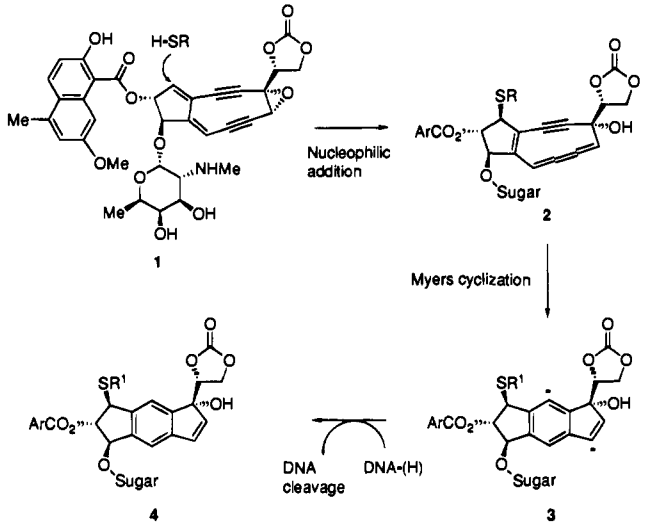

Figure 1. Mechanism of DNA cleaving action of neocarzinostatin chromophore (**1**).

(Figure 1).[2c,10] Structurally, the neocarzinostatin chromophore (**1**) has a highly strained 9-membered ring in the bicyclo[7.3.0]-dodecadienediyne epoxide skeleton, and hence, **1** is extremely unstable at high pH or upon exposure to air or ambient light. Because of the stimulant chemical and biological backgrounds, many groups have focused on the synthesis of the core units or the analogs of **1**.[9,11–27] We started a series of studies directed toward the design, synthesis, and investigation of simple and stable DNA-cleaving molecules reminiscent of both the chemistry and biological action of **1**. As a part of our studies, we designed the 10-membered enediyne–sulfide system **6** and expected that this simple molecule **6** would have the following distinctive features (Figure 2): (1) the 10-membered ring system of **6** containing a sulfur is easy to construct; (2) the 10-membered enediyne–sulfide **6** is stable when handled at ambient temperature due to its nonconjugated enediyne structure in the larger ring system; (3) the enediyne structure of **6** can be transformed into the fully conjugated enyne–allene system of **8** which rapidly produces the diradical species **9** under specific conditions; and (4) the hydroxy group of **6** makes it possible to introduce a DNA recognition site. According to Nicolaou's report, the distance *ab* of the enediyne **6** or the enyne–allene **8** must be within ca. 3.3 Å for spontaneous cyclization at ambient temperature.[28] Molecular calculations indicated that the distance *ab* of the simplified 10-membered enediyne–sulfide **5**[9a,b] was 3.09 Å [by MM2(85)] or 3.26 Å (by MNDO).[29] Considering these points, the novel monocyclic enediyne–sulfide **6** and its derivative **7** were expected to have sufficient stability at ambient temperature while still offering reasonable lability to generate a diradical and cleave DNA under specific conditions.

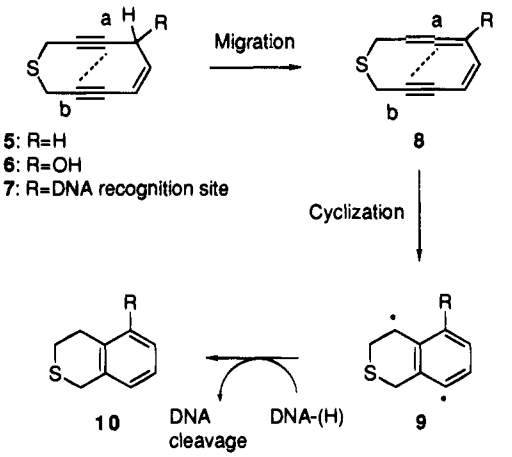

Figure 2. Designed enediyne–sulfide systems and their presumed mechanism of DNA cleaving action.

Synthesis of Enediyne–Sulfide 6 and Its Derivatives. The synthesis of the parent enediyne–sulfide **6** possessing a hydroxy group at the allylic position and its simple derivatives was the initial focus of this program. After several abortive attempts to construct the ring system via the formation of the *cis*-double bond after a cyclization, our efforts finally focused on the *cis*-vinyliodine–acetylene coupling using a Pd(0)–Cu(I) catalyst[30] and the cyclization reaction of the acyclic dibromo compound employing sodium sulfide[28,31] as the key operations to form the enediyne–sulfide moiety within this ring system. The straightforward synthesis of **6** is summarized in Scheme 1. Thus, the

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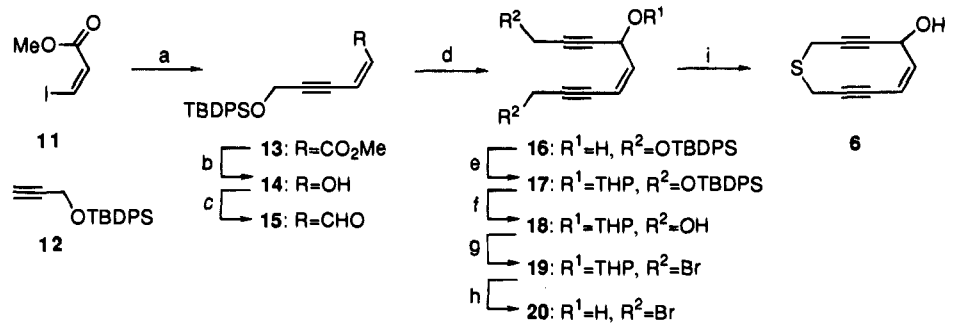
Scheme 1[a]

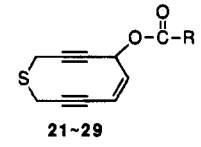


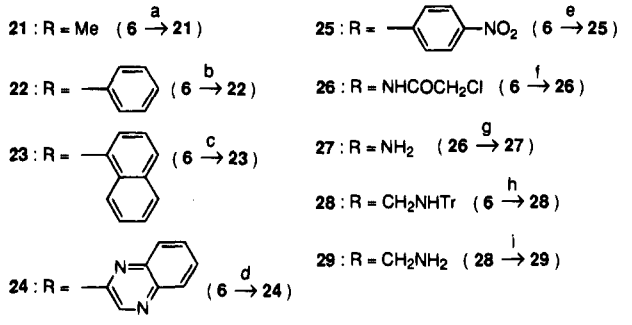
[a] Key: (a) **12**, $(Ph_3P)PdCl_2$, CuI, Et_2NH, THF, 25 °C, 2 h, 82%; (b) DIBALH, PhMe, −78 °C, 0.5 h; (c) MnO_2, CH_2Cl_2, 25 °C, 1 h, 99% from **13**; (d) **12**, *n*-BuLi, THF, −78 °C, 1 h, 87%; (e) DHP, CSA, CH_2Cl_2, 25 °C, 1 h, 100%; (f) TBAF, THF, 25 °C, 1 h, 84%; (g) CBr_4, $[Me(CH_2)_7]_3P$, Et_2O, 25 °C, 1.5 h, 67%; (h) CSA, MeOH, 25 °C, 1 h, 97%; (i) $Na_2S{\cdot}9H_2O$, 95% EtOH (0.005 M), 6.5 h, 67%.

cross-coupling of the *cis*-vinyl iodide **11**[32] and the protected propargyl alcohol **12** using $(Ph_3P)_2PdCl_2$ and CuI in the presence of diethylamine in THF[30] proceeded smoothly to give the enyne ester **13** in 82% yield. Reduction of the methyl ester of **13** using diisobutylaluminum hydride (DIBALH) in toluene, followed by oxidation of the resulting alcohol **14** utilizing manganese dioxide, afforded the aldehyde **15** in 99% overall yield. Subsequent nucleophilic addition of the anion, prepared from **12** and *n*-butyllithium, to **15** was carried out in THF at −91 → −78 °C to give the acyclic enediyne **16** in 87% yield. After the protection of the secondary alcohol of **16** with a tetrahydropyranyl (THP) group, the resulting compound **17** was desilylated using tetra-*n*-butylammonium fluoride (TBAF) in THF to afford the diol **18** in 84% overall yield. The diol **18** was then subjected to bromination employing carbon tetrabromide and tri-*n*-octylphosphine to give the dibromide **19** in 67% yield. Unfortunately, the cyclization of **19** using sodium sulfide was unsuccessful. Therefore, **19** was converted into the key cyclization precursor **20** under methanolic acidic conditions in 97% yield. In contrast, this dibromo alcohol **20** was effectively reacted with sodium sulfide in EtOH−H_2O under high dilution conditions[28,31] (0.005 M for **20**) to furnish the desired cyclic enediyne−sulfide **6** possessing a hydroxy function in 67% yield. Remarkably, as expected, it was found that the novel enediyne **6** was quite stable at ambient temperature.

Considering the presumed mechanism of the DNA cleaving action of the designed enediyne−sulfide system, the expectation that high acidity of the hydrogen at the allylic position, DNA intercalators, and other DNA binding moieties would enhance the potency of **6** as a DNA cleaver was borne out at this stage. Therefore, several acylated derivatives of **6**, **21**−**25**, **27**, and **29** were next synthesized as shown in Scheme 2. Treatment of **6** with acetic anhydride in the presence of triethylamine in CH_2Cl_2 gave the acetate **21** in 96% yield. The reactions of **6** and benzoyl, 1-naphthoyl, 2-quinoxaloyl, and *p*-nitrobenzoyl chlorides with triethylamine in CH_2Cl_2 afforded **22** (88%), **23** (87%), **24** (88%), and **25** (47%), respectively. On the other hand, **6** was reacted with chloroacetyl isocyanate in CH_2Cl_2 to give **26** which was converted into the carbamoyl **27** by treatment with zinc powder in MeOH in 56% overall yield.[33] Furthermore, the amine **29** was prepared in 66% overall yield via **28** which was obtained by the esterification of **6** with *N*-(triphenylmethyl)glycine (*N*-(triphenylmethyl = *N*-trityl) using triphenylphosphine and diethyl azodicarboxylate (DEAD).[34]

Scheme 2[a]





[a] Key: (a) Ac_2O, Et_3N, CH_2Cl_2, 25 °C, 1 h, 96%; (b) BzCl, Et_3N, CH_2Cl_2, 25 °C, 0.5 h, 88%; (c) 1-naphthoyl chloride, Et_3N, CH_2Cl_2, 25 °C, 15 min, 87%; (d) 2-quinoxaloyl chloride, Et_3N, CH_2Cl_2, 0 °C, 2 h, 88%; (e) *p*-nitrobenzoyl chloride, Et_3N, CH_2Cl_2, 25 °C, 15 min, 47%; (f) $ClCH_2CONCO$, CH_2Cl_2, 0 °C, 0.5 h, 84%; (g) Zn, MeOH, 25 °C, 1.5 h, 67%; (h) *N*-tritylglycine, Ph_3P, $EtO_2CN{=}NCO_2Et$, 25 °C, 0.5 h, 83%; (i) 1% HCl−MeOH, 25 °C, 3 h, 80%.

These derivatives were also found to be quite stable when handled at ambient temperature.

Cycloaromatizations of Enediyne−Sulfide 22. Our attention next turned to the mode of cycloaromatization of these novel enediyne−sulfides under weakly basic conditions. Treatment of the representative enediyne−sulfide **22** possessing a benzoyl group at the allylic position with 1.2 equiv of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) in cyclohexa-1,4-diene at 25 °C for 20 min gave the two cycloaromatization products **35** and **36** in 13.8% and 9.2% yields, respectively (Figure 3). The formation of **35** clearly suggests that the migration of the hydrogen at the allylic position of **22** induced by DBU first produces the enyne−allene intermediate **31** which immediately undergoes a Myers-type cycloaromatization leading to the benzenoid diradical **32**.[35] On the other hand, the formation of **36** strongly suggests that the allylic rearrangement mediated by DBU generates the enediyne−sulfide **30** which gives the benzenoid diradical **34** via the enyne−allene **33** in a manner similar to that for **32**.

In drastic contrast, unexpectedly, the exposure of **22** to 50% dimethyl sulfoxide (DMSO) in Tris−HCl, pH 8.5 buffer at 25 °C for 24 h afforded only the cycloaromatization product **37** in 10% yield (Figure 4). The isothiochromane derivative **37**, which

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(35) For other diradical formations from the enyne−allene system, see refs 11c, 12a−d,f, 13a, 14, 15a, 22, and 23c.

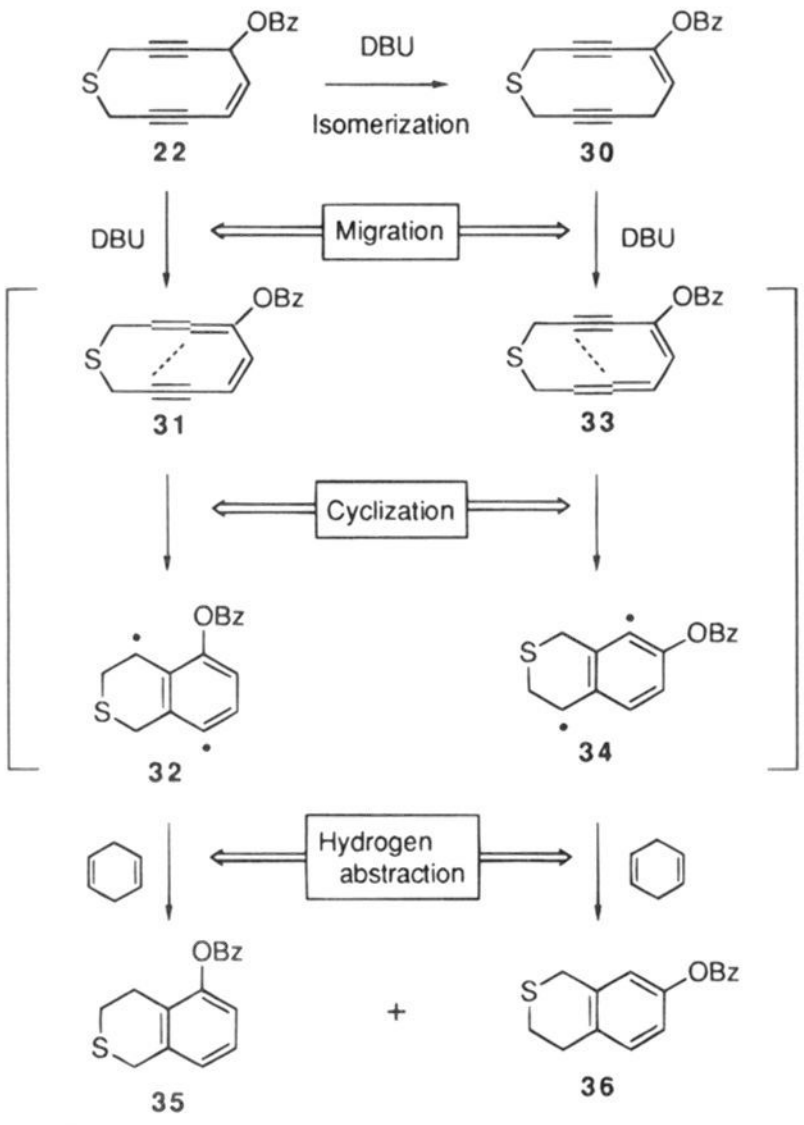


Figure 3. Mode of cycloaromatization of enediyne–sulfide **22** through radical pathways.

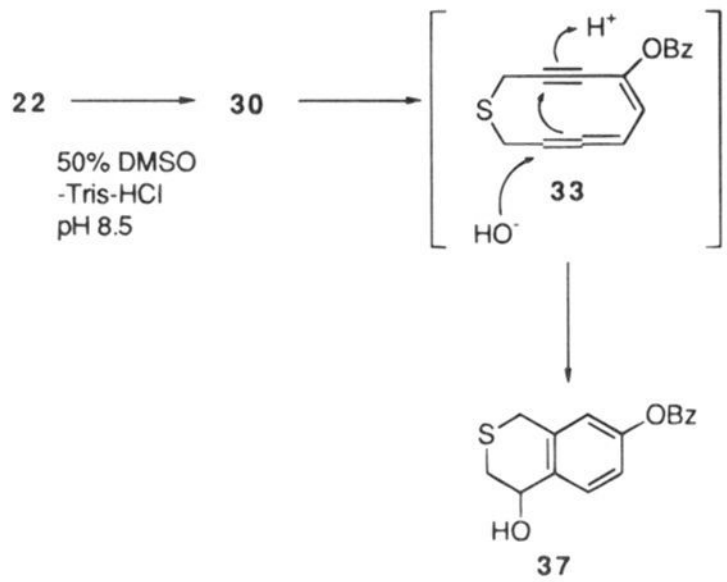


Figure 4. Mode of cycloaromatization of enediyne–sulfide **22** through a polar pathway.

has a hydroxy group at the benzylic position, would arise from the nucleophilic addition of a hydroxy anion to the enyne–allene **33** as shown in Figure 4.[36] Thus, these results indicate that the enediyne–sulfide system is aromatized both by DBU in cyclohexa-1,4-diene through radical pathways and by a hydroxy anion in dimethyl sulfoxide–Tris–HCl, pH 8.5 buffer through a polar pathway.

DNA Cleavage with Enediyne–Sulfides. The DNA cleaving properties of **5**, **6**, **21**–**25**, **27**, and **29** were assayed using double-stranded supercoiled ΦX174 DNA.[37] As expected from the mode of the cycloaromatizations, these compounds were found to cleave DNA in a striking pH-dependent fashion and

(36) For other nucleophilic additions to the enyne–allene system, see refs 9a and 23c.

(37) For other DNA cleavage studies of the analogue of neocarzinostatin chromophore, see refs 12d,f,g, 14b, 15, 21b, 22, and 23a,c.

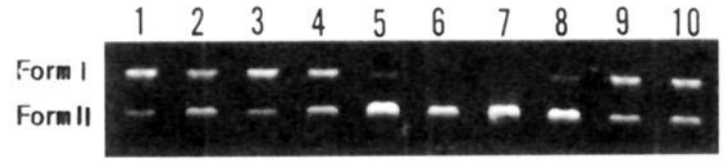


Figure 5. DNA cleavage with enediyne–sulfides **5**, **6**, **21**–**25**, **27**, and **29**. ΦX174 form I DNA (50 μM per base pair) was incubated for 24 h at 37 °C with various compounds (1000 μM) in 20% dimethyl sulfoxide in Tris–acetate buffer (pH 8.5, 50 mM) and analyzed by electrophoresis (1% agarose gel, ethidium bromide stain): lane 1, DNA alone; lanes 2–10, compounds **5**, **6**, **21**–**25**, **27**, and **29**, respectively.

in only alkaline buffer solutions. Thus, aerobic incubations of **5**, **6**, **21**–**25**, **27**, and **29** with the covalently closed supercoiled DNA (form I) at pH 8.5 and 37 °C in a concentration of 1000 μM without any additive caused a single strand break leading to the nicked open circular DNA (form II) as shown in Figure 5. Notably, it was found that the compounds **22**–**25**, each of which has an intercalative aromatic moiety such as benzene, naphthalene, quinoxaline, and *p*-nitrobenzene, obviously exhibited much stronger activity as DNA cleaving agents than other molecules. These results indicated that even the highly simple and stable model has a DNA cleaving property and its activity could be much improved by the introduction of a DNA intercalative moiety.

Synthesis of Hybrids of Enediyne–Sulfide and Naturally Occurring DNA Intercalators. Considering the results of the DNA cleavages already described, hybridization to **6** with a naturally occurring DNA intercalator would be expected to produce both the higher DNA-cleaving ability and base- and sequence-selectivities. Therefore, we next attempted to synthesize the hybrid molecules **38**–**44** containing the novel enediyne–sulfide system **6** and the DNA intercalator occurring in several bioactive natural products.[38] As the DNA intercalator, we selected several aromatic moieties occurring in neocarzinostatin[2] (enediyne antibiotic), nanaomycin[39] (pyranonaphthoquinone antibiotic), adriamycin[40] (anthracycline antibiotic), or manzamine[41] (alkaloid). The syntheses of these hybrids are summarized in Scheme 3. The hybrid **38** containing the naphthoate moiety of the neocarzinostatin chromophore was synthesized from **6** and 2-hydroxy-7-methoxy-5-methylnaphthalene-1-carboxylic acid **45** which was prepared according to Hirama's procedure.[42] Thus, the enediyne **6** was coupled with **45** in the presence of (1-ethyl-3-(dimethylamino)propyl)carbodiimide hydrochloride (WSC·HCl) in CH_2Cl_2 to give **38** in 45% yield. The methoxymethyl (MOM)-protected derivative **39** (82%) was prepared from **38** and methoxymethyl chloride to examine the effect of the aromatic hydroxyl group of **38** on the DNA-cleaving ability. Esterifications of **6** with the carboxylic acids **46** and **47** by Yamaguchi's method[43] using 2,4,6-trichlorobenzoyl chloride, triethylamine, and 4-(dimethylamino)-pyridine (4-DMAP) afforded the pyranonaphthoquinone de-

(38) For other hybrids of neocarzinostatin chromophore analogue and DNA binder, see ref 12d,f,g.

(39) (a) Omura, S.; Tanaka, H.; Okada, Y.; Marumo, H. *J. Chem. Soc., Chem. Commun.* **1976**, 320. (b) Omura, S.; Tanaka, H.; Koyama, Y.; Oiwa, R.; Katagiri, M.; Awaya, J.; Nagai, T.; Hata, T. *J. Antibiot.* **1974**, *27*, 363. (c) Tanaka, H.; Koyama, Y.; Nagai, T.; Marumo, H.; Omura, S. *J. Antibiot.* **1975**, *28*, 868.

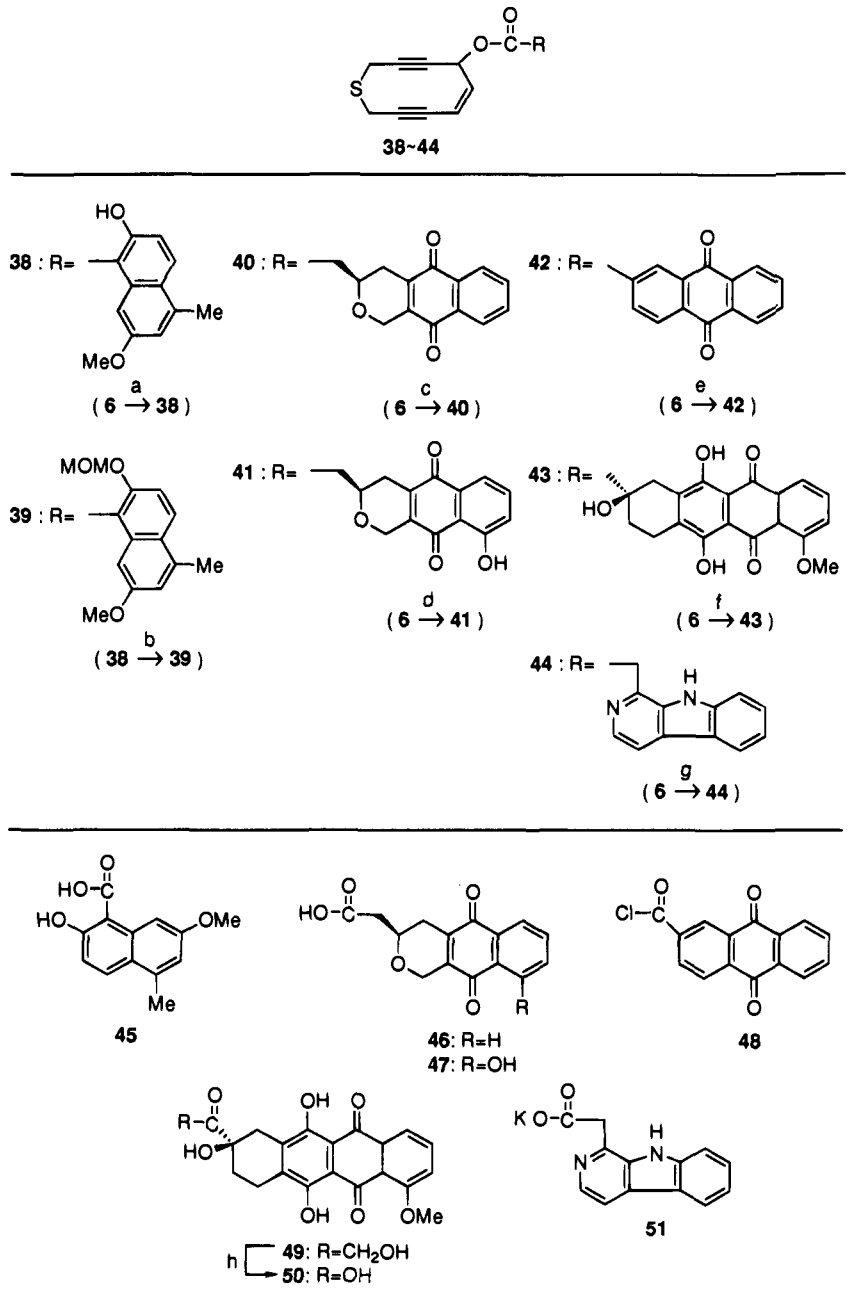
(40) (a) Arcamone, F.; Franceschi, G.; Penco, S.; Selva, A. *Tetrahedron Lett.* **1969**, 1007. (b) Blum, R. H.; Carter, S. K. *Ann. Inter. Med. Lett.* **1974**, *80*, 249.

(41) (a) Sakai, R.; Higa, T. *J. Am. Chem. Soc.* **1986**, *108*, 6404. (b) Sakai, R.; Kohmoto, S.; Higa, T. *Tetrahedron Lett.* **1989**, *28*, 1007.

(42) Takahashi, K.; Suzuki, T.; Hirama, M. *Tetrahedron Lett.* **1992**, *33*, 1007. Also see: (b) Shibuya, M.; Toyooka, K.; Kubota, S. *Tetrahedron Lett.* **1984**, *25*, 1171. (c) Shishido, K.; Yamashita, A.; Horoya, K.; Fukumoto, K.; Kametani, T. *Tetrahedron Lett.* **1989**, *30*, 111.

(43) Inagawa, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.

Scheme 3[a]



[a] Key: (a) **45**, WSC·HCl, CH_2Cl_2, 26 °C, 1 h, 45%; (b) $MeOCH_2Cl$, $^iPr_2EtN$, CH_2Cl_2, 26 °C, 1 h, 82%; (c) **46**, 2,4,6-trichlorobenzoyl chloride, Et_3N, 4-DMAP, CH_2Cl_2, 26 °C, 2 h, 50%; (d) **47**, 2,4,6-trichlorobenzoyl chloride, Et_3N, 4-DMAP, CH_2Cl_2, 26 °C, 2 h, 45%; (e) **48**, Et_3N, CH_2Cl_2, 26 °C, 1 h, 90%; (f) **50**, Ph_3P, $EtO_2CN{=}NCO_2Et$, THF, 26 °C, 15 min, 17%; (g) **51**, 2,4,6-trichlorobenzoyl chloride, Et_3N, 4-DMAP, CH_2Cl_2, 26 °C, 1 h, 19%; (h) HIO_4, dioxane–H_2O, 26 °C, 2 h, 87%.

rivatives **40** (50%) and **41** (45%), respectively. Both carboxylic acids were synthesized according to Tatsuta's nanaomycin synthesis procedure.[44] The anthraquinone **42** (90%) was prepared by reaction of **6** with 2-anthraquinonecarbonyl chloride **48** using triethylamine as the base. The hybrid **43** (17%) possessing the anthracycline moiety of adriamycin was also synthesized by esterification of **6** with **50** via Mitsunobu's reaction[34] using triphenylphosphine and DEAD. The carboxylic acid **50** was readily obtained from natural adriamycin through oxidative cleavage of the α-hydroxy ketone **49**[45] using HIO_4 in dioxane–H_2O. Finally, the hybrid **44** (19%) having a β-carboline moiety of the antitumor alkaloids, manzamines, was synthesized by the reaction of **6** with the β-carboline carboxylic acid potassium salt **51**[46] using Yamaguchi's method.

DNA Cleavage with Hybrids. The DNA cleaving activities of these hybrids and compound **24** as a control are shown in Figure 6. It was found that all the hybrids **38**–**44** clearly cleaved the covalently closed supercoiled ΦX174 DNA (form I) to the open circular DNA (form II) at pH 8.5 and at 37 °C without any additive in concentrations from 100 to 1000 μM (a and b in Figure 6). Remarkably, the hybrids **38** and **44** exhibited the strongest DNA cleaving abilities and caused DNA breaks even at 1–10 μM (c and d in Figure 6). The potency of **38** and **44** was outstanding among the reported neocarzinostatin chromophore-based nonnatural systems.[37] This result

(44) Tatsuta, K.; Akimoto, K.; Annaka, M.; Ohno, Y.; Kinoshita, M. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1699.

(45) Smith, T. H.; Fujiwara, A. N.; Lee, W. W.; Wu, H. Y.; Henry, D. W. *J. Org. Chem.* **1977**, *42*, 3653.

(46) Torisawa, Y.; Hashimoto, A.; Nakagawa, M.; Seki, H.; Hara, R.; Hino, T. *Tetrahedron Lett.* **1991**, *47*, 8067.

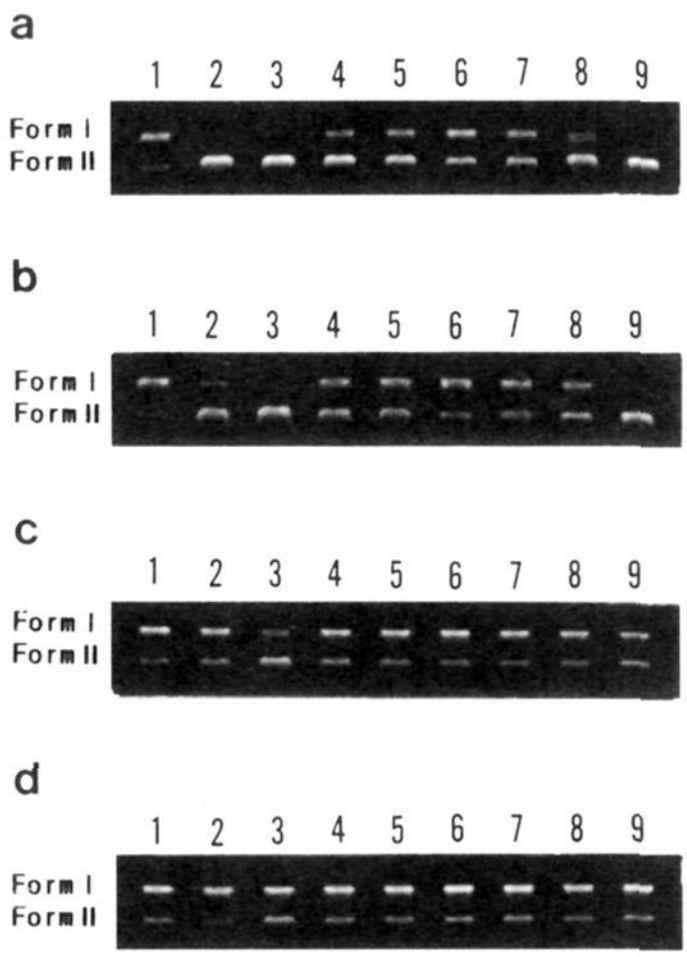


Figure 6. DNA cleavage with hybrids **38**–**44**. ΦX174 form I DNA (50 μM per base pair) was incubated for 24 h with various compounds (a) 1000 μM at 37 °C, (b) 100 μM at 37 °C, (c) 10 μM at 37 °C, (d) 1 μM at 42 °C in 20% dimethyl sulfoxide in Tris–acetate buffer (pH 8.5, 50 mM) and analyzed by electrophoresis (1% agarose gel, ethidium bromide stain): lane 1, DNA alone; lanes 2–9, compounds **24** and **38**–**44**, respectively.

suggested that the β-carboline part of manzamine was a good DNA intercalator like the naphthoate moiety of the neocarzinostatin chromophore. Furthermore, it was confirmed that the hydroxyl group of the naphthoate moiety of the neocarzinostatin chromophore played an important role in the DNA cleaving ability by comparing **38** with its protected product **39** (*cf.* lane 3 with lane 4 in Figure 6).

Their DNA cleavage site specificity was also analyzed.[47] Figure 7 shows the DNA cleavage results with hybrids **23**, **24**, **38**, **40**, **43**, and **44** and the singly 5′-end ^{32}P labeled double-stranded M13mp18 DNA. Comparisons of the cleavage products with both the enzymatically produced Sanger markers[48,49] and the chemically produced Maxam–Gilbert (A + G) marker[50] clearly indicated the identical high purine base (G > A) selectivity of these compounds for their DNA cleaving profiles. Unexpectedly, the base selectivity was highly independent of the DNA intercalator examined even in the case of the hybrid **38**, which had the DNA recognition moiety (T > A ≫ C > G) of the neocarzinostatin chromophore,[51] and its selectivity was similar to that of the A + G-selective alkylating agents. These results strongly suggested that these intercalators in **23**, **24**, **38**, **40**, **43**, and **44** significantly increased the DNA cleaving activity, but not the base or sequence selectivity. 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–sulfide system in dimethyl sulfoxide–Tris–HCl, pH 8.5 buffer already mentioned, and the high purine base selectivity strongly support an alkylation mechanism rather than a radical mechanism for DNA cleavages by these enediyne–sulfides.[52]

(47) For other DNA cleavage site studies of the analogue of neocarzinostatin chromophore, see refs 12d, 21b, and 23c.

(48) Sanger, F.; Nicklen, S.; Coulsen, A. R. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5463.

(49) Since the Sanger sequencing reactions result in base incorporation, cleavage at nucleotide N (sequencing) represents the cleaving site by the agent or the Maxam–Gilbert reaction at $N + 1$. Also, see: Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661.

(50) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 449.

(51) Galat, A.; Goldberg, I. H. *Nucleic Acids Res.* **1990**, *18*, 2093.

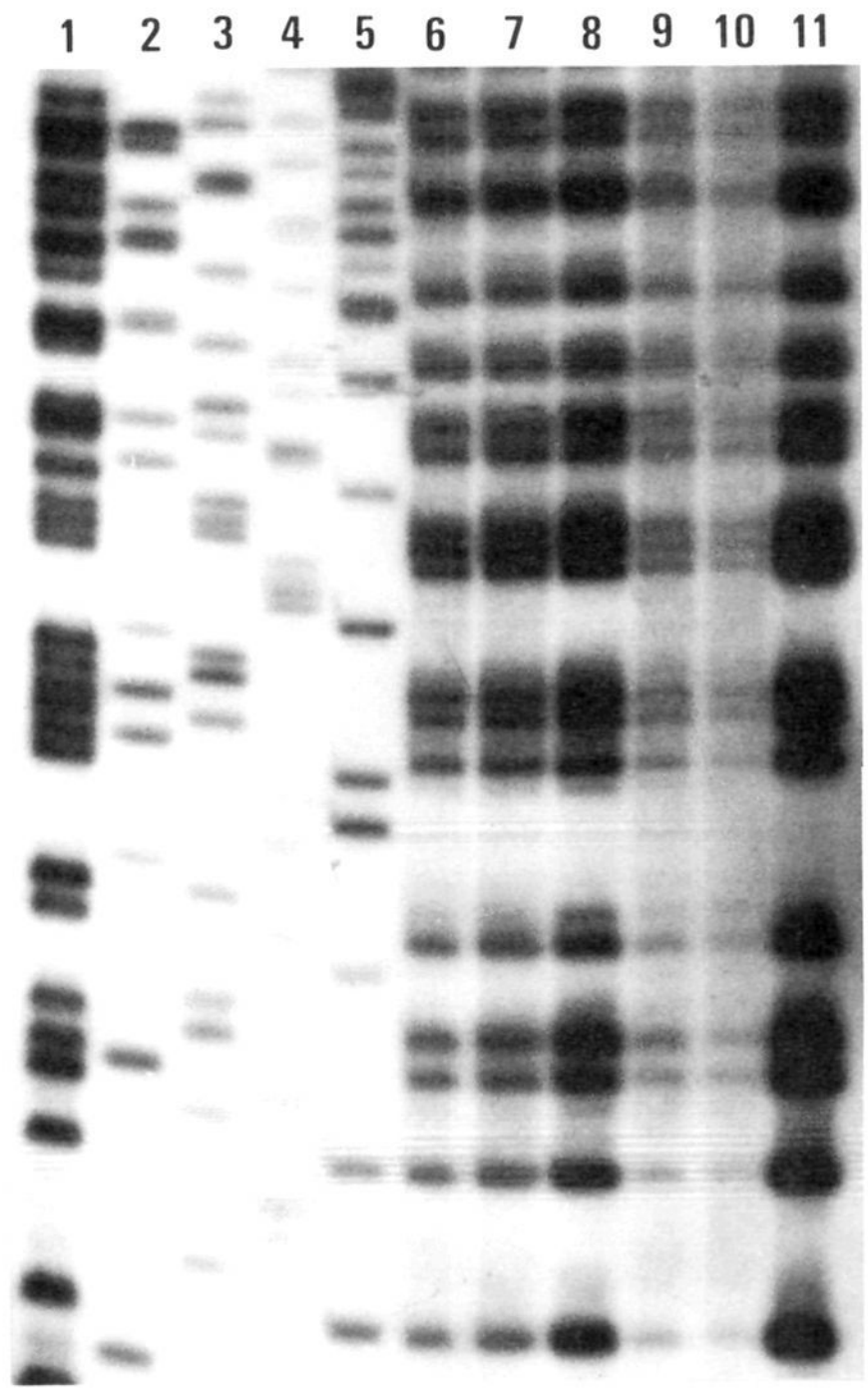


Figure 7. Autoradiogram of 12% polyacrylamide–8 M urea slab gel electrophoresis for sequence analysis. The 5′-end-labeled M13mp18 DNA was cleaved by compounds at pH 8.5 and 45 °C for 24 h (bases 54–110 are shown): lane 1, Maxam–Gilbert AG reaction; lanes 2–5, Sanger A, G, C, and T reactions, respectively; lanes 6–11, compounds **23** (2), **24** (2), **38** (1), **40** (1), **43** (2), and **44** (2 mM), respectively.

Conclusions

The present work shows not only the design and synthesis of novel enediyne–sulfide systems related to the neocarzinostatin chromophore but also their modes of cycloaromatizations and DNA cleaving profiles. It was made clear that even a designed simple and stable molecule had DNA cleaving properties with base selectivity and its activity could be significantly improved by the introduction of a DNA intercalator. 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.

(52) Nicolaou, K. C.; Wendeborn, S.; Maligres, P.; Isshiki, K.; Zein, N.; Ellestad, G. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 418.

Experimental Section

General Methods. 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 ionization (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.

1-[(*tert*-Butyldiphenylsilyl)oxy]-2-propyne (12). To an ice-cold solution of 2-propyn-1-ol (5.43 g, 96.9 mmol) in dry CH_2Cl_2 (50 mL) were added imidazole (6.66 g, 97.8 mmol) and *tert*-butyldiphenylsilyl chloride (22.7 mL, 87.3 mmol) with stirring. After the resulting solution was stirred at 25 °C for 3 h, the reaction was quenched with H_2O (50 mL) and then the mixture was extracted with hexane (20 mL × 3). The extracts were washed with saturated aqueous NaCl (50 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification of the residue by recrystallization (15 mL of methanol) gave **12** (23.3 g, 91%) as white crystals: R_f 0.49 (20:1 hexane—diethyl ether); mp 61.0—61.5 °C (methanol, flakes); ^{1}H-NMR δ 1.07 (9H, s), 2.38 (1H, t, J = 2.5 Hz), 4.31 (2H, d, J = 2.5 Hz), 7.35—7.48 (6H, m), 7.67—7.75 (4H, m); HRMS (EI) *m/z* 295.1541 (295.1518 calcd for $C_{19}H_{23}OSi$, M + H$^+$).

Methyl (2Z)-6-[(*tert*-Butyldiphenylsilyl)oxy]-2-hexen-4-yn-1-oate (13). To an ice-cold solution of **12** (6.20 g, 21.2 mmol) in dry THF (180 mL) were added successively $Pd(Ph_3P)_2Cl_2$ (0.54 g, 0.769 mmol), diethylamine (2.93 mL, 28.8 mmol), **11** (2.00 mL, 19.2 mmol), and CuI (0.586 g, 3.08 mmol). After the resulting mixture was stirred at 25 °C for 2 h, the reaction was quenched with saturated aqueous NH_4Cl (300 mL) and then the mixture was extracted with Et_2O (150 mL × 3). The extracts were washed with saturated aqueous NaCl (300 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification of the residue by flash column chromatography (200 g of silica gel, 5:1 hexane—diethyl ether) gave **13** (5.97 g, 82%) as white crystals: R_f 0.40 (3:1 hexane—diethyl ether); mp 52.5—53.0 °C (methanol); ^{1}H-NMR δ 1.07 (9H, s), 3.73 (3H, s), 4.55 (2H, d, J = 1.8 Hz), 6.06 (1H, d, J = 11.6 Hz), 6.13 (1H, dt, J = 11.6 and 1.8 Hz), 7.34—7.48 (6H, m), 7.68—7.77 (4H, m); HRMS (EI) *m/z* 378.1673 (378.1651 calcd for $C_{23}H_{26}O_3Si$, M$^+$).

(2Z)-6-[(*tert*-Butyldiphenylsilyl)oxy]-2-hexen-4-yn-1-al (15). To a stirred solution of **13** (5.97 g, 15.8 mmol) in dry toluene (150 mL) at −78 °C was added 1 M diisobutylaluminum hydride (1 M = 1 mol dm^{-3}) in toluene (38.6 mL, 38.6 mmol). After the resulting solution was stirred at −78 °C for 0.5 h and then allowed to warm to 0 °C for 2 h with stirring, the reaction was quenched with 1.64 M aqueous potassium sodium tartrate tetrahydrate (120 mL). The resulting mixture was stirred at 25 °C for 12 h and then extracted with toluene (50 mL × 3). The extracts were washed with saturated aqueous NaCl (100 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give a crude oil of **14** (5.47 g). To a solution of the crude sample of **14** (5.47 g) in dry CH_2Cl_2 (1.5 L) was added manganese dioxide (40.7 g, 0.468 mmol). After the resulting mixture was stirred at 25 °C for 1 h, the mixture was filtered through Celite, and the filter cake was washed with CH_2Cl_2 (500 mL × 6). The combined filtrate and washings were concentrated *in vacuo*. Purification of the residue by flash column chromatography (300 g of silica gel, 4:1 hexane—diethyl ether) gave **15** (5.44 g, 99% from **13**) as a pale yellow oil: R_f 0.49 (3:1 hexane—diethyl ether); ^{1}H-NMR δ 1.07 (9H, s), 4.53 (2H, d, J = 2.1 Hz), 6.23 (1H, dd, J = 10.8 and 8.0 Hz), 6.58 (1H, dt, J = 10.8 and 2.1 Hz), 7.35—7.50 (6H, m), 7.66—7.75 (4H, m), 9.94 (1H, d, J = 8.0 Hz); HRMS (EI) *m/z* 348.1518 (348.1545 calcd for $C_{22}H_{24}O_2Si$, M$^+$).

(4Z)-1,9-Bis[(*tert*-butyldiphenylsilyl)oxy]-4-nonene-2,7-diyn-6-ol (16). To a stirred solution of **12** (6.89 g, 23.4 mmol) in dry THF (70.0 mL) at −78 °C was added dropwise 1.6 M *n*-butyllithium in hexane (13.7 mL, 21.9 mmol). The solution was stirred at −78 °C for 30 min and then recooled to −90 °C. A solution of **15** (5.44 g, 15.6 mmol) in dry THF (27 mL) was added dropwise to the recooled solution with stirring. After the resulting solution was stirred at −78 °C for 1 h, the reaction was quenched with saturated aqueous NH_4Cl (120 mL) and then the mixture was extracted with Et_2O (60 mL × 3). The extracts were washed with saturated aqueous NaCl (120 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification of the residue by flash column chromatography (500 g of silica gel, 4:1 hexane—diethyl ether) gave **16** (8.73 g, 87%) as a colorless oil: R_f 0.25 (2:1 hexane—diethyl ether); ^{1}H-NMR δ 1.05 (9H, s), 1.06 (9H, s), 1.68 (1H, d, J = 5.0 Hz), 4.35 (2H, d, J = 2.0 Hz), 4.46 (2H, d, J = 2.0 Hz), 5.13—5.23 (1H, m), 5.55 (1H, dt, J = 10.4 and 2.0 Hz), 5.82 (1H, dd, J = 10.4 and 9.0 Hz), 7.32—7.47 (12H, m), 7.66—7.75 (8H, m); HRMS (EI) *m/z* 642.2977 (6.42.2985 calcd for $C_{41}H_{46}O_3Si_2$, M$^+$).

(4Z)-1,9-Bis[(*tert*-butyldiphenylsilyl)oxy]-6-[(tetrahydro-2-pyranyl)oxy]-4-nonene-2,7-diyne (17). To a solution of **16** (8.73 g, 13.6 mmol) in dry CH_2Cl_2 (300 mL) were added 3,4-dihydro-2*H*-pyran (1.36 mL, 14.9 mmol) and 10-DL-camphorsulfonic acid (31.5 mg, 0.136 mmol) under ice cooling. After the resulting solution was stirred at 25 °C for 1 h, the mixture was made neutral with triethylamine and then concentrated *in vacuo*. Purification of the residue by flash column chromatography (500 g of silica gel, 6:1 hexane—acetone) gave **17** (9.87 g, 100%) as a colorless oil: R_f 0.60 (5:1 hexane—acetone); ^{1}H-NMR δ 1.04 (9H, s), 1.06 (^{9}/$_2$H, s), 1.07 (^{9}/$_2$H, s), 1.4—1.9 (6H, m), 3.36—3.6 (^{1}/$_2$H, m), 3.7—3.95 (^{1}/$_2$H, m), 4.34 (1H, d, J = 1.6 Hz), 4.36 (1H, d, J = 1.6 Hz), 4.44 (1H, d, J = 2.0 Hz), 4.45 (1H, d, J = 2.0 Hz), 4.68 (^{1}/$_2$H, dd, J = 4.0 and3.0 Hz), 4.97 (1/2H, dd, J = 3.0 and 3.0 Hz), 5.38 (^{1}/$_2$H, dt, J = 9.8 and 1.6 Hz), 5.39 (^{1}/$_2$H, dt, J = 9.8 and 1.6 Hz), 5.62 (^{1}/$_2$H, dt, J = 10.2 and 2.0 Hz), 5.66 (^{1}/$_2$H, dt, J = 10.4 and 2.0 Hz), 5.83 (^{1}/$_2$H, dd, J = 10.2 and 9.8 Hz), 5.90 (^{1}/$_2$H, J = 10.4 and 9.8 Hz), 7.32—7.45 (12H, m), 7.65—7.75 (8H, m); HRMS (EI) *m/z* 726.3581 (726.3561 calcd for $C_{46}H_{54}O_4Si_2$, M$^+$).

(4Z)-6-[(Tetrahydro-2-pyranyl)oxy]-4-nonene-2,7-diyne-1,9-diol (18). To an ice-cold solution of **17** (9.87 g, 13.6 mmol) in dry THF (300 mL) was added 1 M tetra-*n*-butylammonium fluoride in THF (27.1 mL, 27.1 mmol). After the resulting solution was stirred at 25 °C for 1 h, the mixture was poured into water (300 mL) and then the resulting mixture was extracted with Et_2O (100 mL × 3). The extracts were washed with saturated aqueous NaCl (300 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification of the residue by flash column chromatography (170 g of silica gel, 3:1 chloroform—acetone) gave **18** (2.85 g, 84%) as a colorless oil: R_f 0.23 (3:1 chloroform—acetone); ^{1}H-NMR δ 1.4—1.95 (6H, m), 2.18 (1H, br s), 2.65 (1H, br s), 3.5—3.65 (1H, m), 3.78—4.03 (1H, m), 4.29—4.45 (4H, m), 4.82 (^{1}/$_2$H, dd, J = 3.0 and 3.0 Hz), 5.03 (^{1}/$_2$H, dd, J = 3.0 and 3.0 Hz), 5.46 (^{1}/$_2$H, dull d, J = 8.4 hz), 5.53 (^{1}/$_2$H, dull d, J = 9.8 Hz), 5.65—5.8 (1H, m), 5.96 (^{1}/$_2$H, dd, J = 10.8 and 9.8 Hz), 6.04 (^{1}/$_2$H, dd, J = 10.8 and 8.4 Hz); HRMS (EI) *m/z* 250.1209 (250.1205 calcd for $C_{14}H_{18}O_4$, M$^+$).

(4Z)-1,9-Dibromo-6-[(tetrahydro-2-pyranyl)oxy]-4-nonene-2,7-diyne (19). To an ice-cold solution of **18** (2.85 g, 11.4 mmol) in dry Et_2O (85.5 mL) were added carbon tetrabromide (7.56 g, 22.8 mmol) and tri-*n*-octylphosphine (17.9 mL, 39.9 mmol) with stirring. After the resulting mixture was stirred at 25 °C for 1.5 h, the mixture was concentrated *in vacuo*. Purification of the residue by flash column chromatography (200 g of silica gel, 4:1 hexane—diethyl ether) gave **19** (2.87 g, 67%) as a pale yellow oil: R_f 0.60 (3:1 hexane—diethyl ether); ^{1}H-NMR δ 1.49—1.95 (6H, m), 3.5—3.64 (1H, m), 3.75—4.03 (1H, m), 3.94 (1H, d, J = 2.0 Hz), 3.96 (1H, d, J = 2.0 Hz), 4.08 (2H, d, J = 2.0 Hz), 4.70 (^{1}/$_2$H, dd, J = 3.8 and 3.8 Hz), 5.03 (^{1}/$_2$H, dd, J = 3.0 and 3.0 Hz), 5.44 (^{1}/$_2$H, dull d, J = 9.6 Hz), 5.45 (^{1}/$_2$H, dull d, J = 9.0 Hz), 5.71 (^{1}/$_2$H, dt, J = 10.8 and 2.0 Hz), 5.75 (^{1}/$_2$H, dt, J = 10.8 and 2.0 Hz), 5.97 (^{1}/$_2$H, dd, J = 10.8 and 9.6 Hz), 6.04 (^{1}/$_2$H, dd, J = 10.8 and 9.0 Hz); HRMS (EI) *m/z* 373.9533 (373.9518 calcd for $C_{14}H_{16}Br_2O_2$, M$^+$).

(4Z)-1,9-Dibromo-4-nonene-2,7-diyn-6-ol (20). To a stirred solution of **19** (1.2 g, 3.19 mmol) in MeOH (24 mL) was added 10-DL-camphorsulfonic acid (148 mg, 0.638 mmol). After the resulting solution was stirred at 25 °C for 1 h, the mixture was made neutral with triethylamine and then concentrated *in vacuo*. Purification of the residue by flash column chromatography (50 g of silica gel, 2:1

hexane–diethyl ether) gave **20** (866 mg, 97%) as a pale yellow oil: R_f 0.40 (2:1 hexane–diethyl ether); ^{1}H-NMR δ 2.00 (1H, br s), 3.95 (2H, d, J = 1.9 Hz), 4.08 (2H, d, J = 2.2 Hz), 5.40 (1H, dt, J = 8.2 and 1.9 Hz), 5.69 (1H, dt, J = 10.6 and 2.2 Hz), 6.04 (1H, dd, J = 10.6 and 8.2 Hz); HRMS (EI) *m/z* 289.8935 (289.8942 calcd for $C_9H_8Br_2O$, M^+).

(5*Z*)-1-Thia-5-cyclodecene-3,8-diyn-7-ol (6). To a stirred solution of sodium sulfide nonahydrate (3.60 g, 15.0 mmol) in 95% EtOH (1.5 L) was added dropwise a solution of **20** (2.93 g, 10.0 mmol) in EtOH (500 mL) over 6 h. After the reaction mixture was stirred at 25 °C for 0.5 h, the mixture was then concentrated *in vacuo* to a volume of 100 mL and added to saturated aqueous NH_4Cl (100 mL). The resulting mixture was extracted with Et_2O (50 mL × 3), and the extracts were washed with saturated aqueous NaCl (100 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification of the residue by flash column chromatography (100 g of silica gel, 2:1 hexane–diethyl ether) gave **6** (1.10 g, 67%) as white crystals: R_f 0.17 (2:1 hexane–diethyl ether); mp 117.5–118.5 °C (diethyl ether–hexane); ^{1}H-NMR δ 2.04 (1H, d, J = 8.0 Hz), 3.57 (2H, d, J = 2.0 Hz), 3.65 (2H, d, J = 1.6 Hz), 4.89–5.0 (1H, m), 5.59 (1H, ddt, J = 12.0, 2.0, and 1.8 Hz), 5.97 (1H, dd, J = 12.0 and 4.0 Hz); HRMS (EI) *m/z* 165.0348 (165.0374 calcd for C_9H_9OS, M + H^+).

(5*Z*)-7-Azetoxy-1-thia-5-cyclodecene-3,8-diyne (21). To an ice-cold solution of **6** (17.5 mg, 0.107 mmol) in dry CH_2Cl_2 (0.6 mL) were added triethylamine (0.0209 mL, 0.150 mmol) and acetic anhydride (0.0121 mL, 0.128 mmol). After the resulting solution was stirred at 25 °C for 1 h, the reaction was quenched with H_2O (1 mL) and then the mixture was 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 flash column chromatography (2 g of silica gel, 5:1 hexane–ethyl acetate) gave **21** (21.3 mg, 96%) as a pale yellow oil: R_f 0.33 (5:1 hexane–ethyl acetate); ^{1}H-NMR δ 2.11 (3H, s), 3.56 (2H, d, J = 2.0 Hz), 3.65 (2H, dull s), 5.68 (1H, ddt, J = 12.0, 2.0, and 1.6 Hz), 5.83 (1H, dd, J = 12.0 and 4.0 Hz), 5.93–6.03 (1H, m); HRMS (EI) *m/z* 206.0390 (206.0402 calcd for $C_{11}H_{10}O_2S$, M^+).

(5*Z*)-7-(Benzoyloxy)-1-thia-5-cyclodecene-3,8-diyne (22). To an ice-cold solution of **6** (15.3 mg, 0.0932 mmol) in dry CH_2Cl_2 (0.77 mL) were added triethylamine (0.0391 mL, 0.280 mmol) and benzoyl chloride (0.0119 mL, 0.112 mmol). After the resulting solution was stirred at 25 °C for 0.5 h, the reaction was quenched with H_2O (1 mL), and then the mixture was 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 flash column chromatography (1.5 g of silica gel, 6:1 hexane–ethyl acetate) gave **22** (22.0 mg, 88%) as pale yellow crystals: R_f 0.32 (8:1 hexane–ethyl acetate); mp 132–133 °C dec (acetone–hexane); ^{1}H-NMR δ 3.58 (2H, d, J = 2.0 Hz), 3.67 (2H, dull s), 5.74 (1H, ddt, J = 12.0, 2.0, and 1.8 Hz), 5.95 (1H, dd, J = 12.0 and 4.0 Hz), 6.20–6.28 (1H, m), 7.4–7.63 (3H, m), 8.04–8.15 (2H, m); HRMS (EI) *m/z* 268.0583 (268.0558 calcd for $C_{16}H_{12}O_2S$, M^+).

(5*Z*)-7-(1-Naphthoyloxy)-1-thia-5-cyclodecene-3,8-diyne (23). To an ice-cold solution of **6** (60.0 mg, 0.365 mmol) in dry CH_2Cl_2 (3.7 mL) were added triethylamine (0.076 mL, 0.548 mmol) and 1-naphthoyl chloride (0.066 mL, 0.438 mmol). After the resulting solution was stirred at 25 °C for 15 min, the reaction was quenched with H_2O (5 mL) and then the mixture was extracted with Et_2O (3 mL × 3). The extracts were washed with saturated aqueous NaCl (3 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification of the residue by flash column chromatography (6 g of silica gel, 6:1 hexane–diethyl ether then 6 g of silica gel, 1:2 hexane–benzene) gave **23** (101 mg, 87%) as pale yellow oil: R_f 0.75 (2:1 hexane–ethyl acetate); ^{1}H-NMR δ 3.60 (2H, d, J = 1.9 Hz), 3.69 (2H, dull s), 5.77 (1H, ddt, J = 12.0, 2.0, and 1.9 Hz), 6.03 (1H, dd, J = 11.9 and 4.0 Hz), 6.3–6.4 (1H, m), 7.45–7.7 (3H, m), 7.88 (1H, dull d, J = 8.4 Hz), 8.05 (1H, dull d, J = 8.2 Hz), 8.27 (1H, dd, J = 7.6 and 1.0 Hz), 8.95 (1H, dull d, J = 8.2 Hz); HRMS (EI) *m/z* 268.0583 (268.0558 calcd for $C_{16}H_{12}O_2S$, M^+).

(5*Z*)-7-(2-Quinoxaloyloxy)-1-thia-5-cyclodecene-3,8-diyne (24). To an ice-cold solution of **6** (54.0 mg, 0.329 mmol) in dry CH_2Cl_2 (3.3 mL) were added triethylamine (0.069 mL, 0.493 mmol) and 2-quinoxaloyl chloride (76.0 mg, 0.395 mmol). After the resulting solution was stirred under ice-cooling for 2 h, the reaction was quenched with H_2O (5 mL) and then the mixture was extracted with Et_2O (3 mL × 3). The extracts were washed with saturated aqueous NaCl (3 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification of the residue by flash column chromatography (5 g of silica gel, 1:1 hexane–diethyl ether) gave **24** (93.0 mg, 88%) as white crystals: R_f 0.17 (1:1 hexane–diethyl ether); mp 140 °C dec (acetone–hexane); ^{1}H-NMR δ 3.60 (2H, d, J = 1.9 Hz), 3.69 (2H, dull s), 5.81 (1H, ddt, J = 11.9, 2.0, and 1.9 Hz), 6.05 (1H, dd, J = 11.9 and 4.0 Hz), 6.35–6.42 (1H, m), 7.85–7.97 (2H, m), 8.16–8.23 (1H, m), 8.28–8.35 (1H, m), 9.55 (1H, s); HRMS (EI) *m/z* 320.0605 (320.0619 calcd for $C_{18}H_{12}N_2O_2S$, M^+).

(5*Z*)-7-[(4-Nitrobenzoyl)oxy]-1-thia-5-cyclodecene-3,8-diyne (25). To an ice-cold solution of **6** (52.0 mg, 0.317 mmol) in dry CH_2Cl_2 (3.2 mL) were added triethylamine (0.066 mL, 0.475 mmol) and *p*-nitrobenzoyl chloride (71.0 mg, 0.380 mmol). After the resulting solution was stirred at 25 °C for 15 min, the reaction was quenched with H_2O (5 mL) and then the mixture was extracted with Et_2O (3 mL × 3). The extracts were washed with saturated aqueous NaCl (3 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification of the residue by flash column chromatography (5 g of silica gel, 1:1 hexane–diethyl ether then 2:1 hexane–ethyl acetate) gave **25** (47.1 mg, 47%) as white crystals: R_f 0.66 (2:1 hexane–ethyl acetate); mp 171 °C dec (acetone–hexane); ^{1}H-NMR δ 3.58 (2H, dull d, J = 2.0 Hz), 3.68 (2H, dull d, J = 1.6 Hz), 5.78 (1H, ddt, J = 11.8, 2.0, and 2.0 Hz), 5.95 (1H, dd, J = 11.8 and 4.4 Hz), 6.24 (1H, ddt, J = 4.4, 2.0, and 1.6 Hz), 8.2–8.35 (4H, m); HRMS (EI) *m/z* 314.0464 (314.0487 calcd for $C_{16}H_{12}NO_4S$, M^+).

(5*Z*)-7-(Carbamoyloxy)-1-thia-5-cyclodecene-3,8-diyne (27). To an ice-cold solution of **6** (43.0 mg, 0.262 mmol) in dry CH_2Cl_2 (2.6 mL) was added chloroacetyl isocyanate (0.033 mL, 0.341 mmol). After the resulting solution was stirred under ice-cooling for 0.5 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (5 mL) and then the mixture was extracted with EtOAc (3 mL × 3). The extracts were washed with saturated aqueous NaCl (3 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification of the residue by flash column chromatography (5 g of silica gel, 3:1 hexane–ethyl acetate) gave **26** (62.0 mg, 84%) as white crystals. To a solution of **26** (62.0 mg) in MeOH (6.2 mL) was added zinc powder (29.2 mg, 0.437 mmol). After the resulting suspension was stirred at 25 °C for 1.5 h, the mixture was filtered through Celite and the catalyst was washed with MeOH. The combined filtrate and washings were concentrated *in vacuo*. Purification of the residue by flash column chromatography (2 g of silica gel, 3:1 hexane–ethyl acetate then 3:2 hexane–ethyl acetate) gave **27** (30.1 mg, 67%) as white crystals: R_f 0.11 (3:1 hexane–ethyl acetate); mp 142 °C dec (acetone–hexane); ^{1}H-NMR δ 3.57 (2H, d, J = 1.6 Hz), 3.65 (2H, d, J = 2.0 Hz), 4.71 (2H, br s), 5.68 (1H, dt, J = 10.2 and 1.8 Hz), 5.88 (1H, dd, J = 10.2 and 4.0 Hz), 5.87–5.95 (1H, m); HRMS (EI) *m/z* 207.0360 (207.0364 calcd for $C_{10}H_9NO_2S$, M^+).

(5*Z*)-7-(Glycyloxy)-1-thia-5-cyclodecene-3,8-diyne (29). To a solution of **6** (50.0 mg, 0.304 mmol) and *N*-(triphenylmethyl)glycine (126 mg, 0.396 mmol) in dry THF (3.0 mL) were added triphenylphosphine (120 mg, 0.457 mmol) and diethyl azodicarboxylate (0.072 mL, 0.457 mmol) with stirring. After the resulting mixture was stirred at 25 °C for 0.5 h, the mixture was concentrated *in vacuo*. Purification of the residue by flash column chromatography (7 g of silica gel, 6:1 hexane–diethyl ether) gave **28** (117 mg, 83%) as a pale yellow oil. **28** (117 mg) was dissolved in 1% HCl–MeOH (25.2 mL), and the resulting solution was stirred at 25 °C for 3 h. The mixture was made neutral with triethylamine and then concentrated *in vacuo*. Purification of the residue by flash column chromatography (6 g of silica gel, 10:1 chloroform–methanol) gave **29** (43.9 mg, 80%) as a pale yellow oil: R_f 0.42 (10:1 chloroform–methanol); ^{1}H-NMR δ 3.46 and 3.51 (2H, ABq, J = 16.4 Hz), 3.56 (2H, d, J = 2.0 Hz), 3.66 (2H, dull s), 5.70 (1H, ddt, J = 12.0, 2.0, and 2.0 Hz), 5.88 (1*H*, dd, J = 12.0 and 4.4 Hz), 6.02 (1H, dull d, J = 2.0 Hz); HRMS (EI) *m/z* 221.0509 (221.0511 calcd for $C_{11}H_{11}NO_2S$, M^+).

Cycloaromatization of 22 with DBU. To an ice-cold solution of **22** (62.0 mg, 0.231 mmol) in cyclohexa-1,4-diene (2.3 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.041 mL, 0.277 mmol). After the resulting mixture was stirred at 25 °C for 20 min, the reaction

was quenched with saturated aqueous NH_4Cl (5 mL) and then the mixture was extracted with Et_2O (3 mL × 3). The extracts were washed with saturated aqueous NaCl (3 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification of the residue by preparative thin-layer chromatography (4:1 hexane—diethyl ether) gave **35** and **36** (14.0 mg, 23%, **35/36** = 3/2) as a mixture: R_f 0.28 (4:1 hexane—diethyl ether); ^{1}H-NMR of **35** δ 2.85—3.10 (4H, m), 3.77 (2H, s), 7.06 (1H, dd, J = 8.0 and 8.0 Hz), 7.07 (1H, d, J = 8.0 Hz), 7.19 (1H, d, J = 8.0 Hz), 7.45—7.70 (3H, m), 8.15—8.27 (2H, m). ^{1}H-NMR of **36**: δ 2.85—3.10 (4H, m), 3.81 (2H, s), 7.01 (1H, dull d, J = 2.0 Hz), 7.05 (1H, dull dd, J = 8.0 and 2.0 Hz), 7.21 (1H, d, J = 8.0 Hz), 7.45—7.70 (3H, m), 8.15—8.27 (2H, m); HRMS (EI) *m/z* 270.0726 (270.0715 calcd for $C_{16}H_{14}O_2S$, M^+).

Cycloaromatization of 22 in 50% DMSO—Tris—HCl Buffer. 22 (64.0 mg, 0.239 mmol) was dissolved in 50% dimethyl sulfoxide (DMSO)—Tris—HCl, pH 8.5 buffer (19 mL), and the resulting solution was stirred at 25 °C for 24 h. The mixture was poured into H_2O (10 mL) and then the resulting mixture was extracted with $CHCl_3$ (5 mL × 3). The extracts were washed with saturated aqueous NaCl (10 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification of the residue by flash column chromatography (5 g of silica gel, 8:1 → 5:1 hexane—diethyl ether then 2:1 hexane—ethyl acetate) gave **37** (7.0 mg, 10%) as a pale yellow oil: R_f 0.38 (2:1 hexane—ethyl acetate); ^{1}H-NMR δ 2.70 (1H, d, J = 10.0 Hz), 3.02 (1H, ddd, J = 13.8, 4.2, and 1.6 Hz), 3.13 (1H, dd, J = 13.8 and 3.0 Hz), 3.61 and 3.94 (2H, ABq, J = 16.0 Hz), 4.75—4.9 (1H, m), 7.00 (1H, dull d, J = 2.6 Hz), 7.12 (1H, dd, J = 8.0 and 2.6 Hz), 7.53 (1H, d, J = 8.0 Hz), 7.45—7.70 (3H, m), 8.19 (2H, dull d, J = 7.6 Hz); HRMS (EI) *m/z* 286.0670 (286.0664 calcd for $C_{16}H_{14}O_3S$, M^+).

(5*Z*)-7-[(2-Hydroxy-7-methoxy-5-methyl-1-naphthoyl)oxy]-1-thia-5-cyclodecene-3,8-diyne (38). To a stirred solution of **6** (23.0 mg, 0.142 mmol) and **45** (33.1 mg, 0.142 mmol) in dry CH_2Cl_2 (1.2 mL) was added [1-ethyl-3-(dimethylamino)propyl]carbodiimide hydrochloride (106 mg, 0.568 mmol). The resulting solution was stirred at 26 °C for 1 h, the reaction was quenched with H_2O (1.5 mL), and then the mixture was extracted with Et_2O (1 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 flash column chromatogrpahy (3 g of silica gel, 10:1 hexane—ethyl acetate) gave **38** (24.2 mg, 45%) as white crystals: R_f 0.58 (5:1 hexane—ethyl acetate); ^{1}H-NMR δ 2.62 (3H, s), 3.52 (1H, dd, J = 18.0 and 1.8 Hz), 3.63 (1H, dd, J = 18.0 and 2.0 Hz), 3.64 (1H, dd, J = 18.0 and 2.2 Hz), 3.73 (1H, dd, J = 18.0 and 1.6 Hz), 3.96 (3H, s), 5.81 (1H, dddd, J = 12.0, 2.0, 2.0, and 1.8 Hz), 6.10 (1H, dd, J = 12.0 and 5.0 Hz), 6.35—6.41 (1H, m), 6.89 (1H, d, J = 2.0 Hz), 7.03 (1H, d, J = 9.0 Hz), 8.05 (1H, d, J = 9.0 Hz), 8.19 (1H, d, J = 2.0 Hz), 11.85 (1H, s); HRMS (EI) *m/z* 379.1017 (379.1004 calcd for $C_{22}H_{19}O_4S$, M + H^+).

(5*Z*)-7-[(7-Methoxy-2-(methoxymethoxy)-5-methyl-1-naphthoyl)-oxy]-1-thia-5-cyclodecene-3,8-diyne (39). To an ice-cold solution of **6** (24.0 mg, 0.0634 mmol) in dry CH_2Cl_2 (1.2 mL) were added diisopropylamine (0.0390 mL, 0.222 mmol) and methoxymethyl chloride (0.0140 mL, 0.190 mmol). The resulting solution was stirred at 26 °C for 1 h, the reaction was quenched with H_2O (2.5 mL), and then the mixture was extracted with Et_2O (1.5 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 flash column chromatography (1.5 g of silica gel, 2:1 hexane—diethyl ether) gave **39** (22.0 mg, 82%) as a colorless oil: R_f 0.18 (2:1 hexane—ethyl acetate); ^{1}H-NMR δ 2.62 (3H, s), 3.53 (3H, s), 3.53 (1H, dd, J = 18.0 and 2.0 Hz), 3.62 (1H, dd, J = 18.0 and 2.1 Hz), 3.66 (2H, dull d, J = 1.6 Hz), 3.89 (3H, s), 5.30 (2H, s), 5.74 (1H, dddd, J = 12.0, 2.1, 2.0, and 1.8 Hz), 6.04 (1H, dd, J = 12.0 and 4.0 Hz), 6.35—6.42 (1H, m), 6.91 (1H, d, J = 2.0 Hz), 6.98 (1H, dull d, J = 2.0 Hz), 7.26 (1H, d, J = 10.0 Hz), 7.94 (1H, d, J = 10.0 Hz); HRMS (EI) *m/z* 422.1165 (422.1188 calcd for $C_{24}H_{22}O_5S$, M^+).

(5*Z*)-7-[[(3*R*)-3,4-Dihydro-5,10-dioxonaphtho[2,3-*c*]pyran-3-yl]-acetoxy]-1-thia-5-cyclodecene-3,8-diyne (40). To an ice-cold solution of **46** (34.0 mg, 0.125 mmol) in dry CH_2Cl_2 (1.3 mL) were added dropwise triethylamine (0.0170 mL, 0.125 mmol) and 2,4,6-trichlorobenzoyl chloride (0.020 mL, 0.125 mmol). The resulting solution was stirred at 26 °C for 1 h. A solution of **6** (21.0 mg, 0.125 mmol) in dry CH_2Cl_2 (1.3 mL) and 4-(dimethylamino)pyridine (61.1 mg, 0.500 mmol) was added to the reaction mixture. After the resulting mixture was stirred at 25 °C for 2 h, the reaction wad quenched with H_2O (3 mL), and then the mixture was extracted with Et_2O (1.5 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 flash column chromatography (2.5 g of silica gel, 5:5:1 hexane—chloroform—ethyl acetate) gave **40** (26.0 mg, 50%) as a pale yellow oil: R_f 0.42 (5:5:1 hexane—chloroform—ethyl acetate); ^{1}H-NMR δ 2.3—2.9 (2H, m), 2.68 (1H, dd, J = 15.6 and 5.6 Hz), 2.80 (1H, dd, J = 15.6 and 7.8 Hz), 3.58 (2H, dull s), 3.66 (2H, dull s), 3.96—4.12 (1H, m), 4.55 (1H, ddd, J = 19.0, 4.0, and 3.6 Hz), 4.86 (1H, dull dd, J = 19.0 and 2.0 Hz), 5.71 (1H, dull dd, J = 12.0 and 1.2 Hz), 5.8—5.9 (1H, m), 6.02—6.08 (1H, m), 7.67—7.86 (2H, m), 8.0—8.19 (2H, m); HRMS (EI) *m/z* 418.0844 (418.0875 calcd for $C_{24}H_{18}O_5S$, M^+).

(5*Z*)-7-[[(3*R*)-3,4-Dihydro-9-hydroxy-5,10-dioxonaphtho[2,3-*c*]pyran-3-yl]acetoxy]-1-thia-5-cyclodecene-3,8-diyne (41). By the procedure described in the preparation of **40**, esterification of **6** (18.0 mg, 0.110 mmol) with **47** (30.0 mg, 0.110 mmol) gave a crude sample of **41**. Purification of the sample by flash column chromatography (2.5 g of silica gel, 5:5:1 hexane—chloroform—ethyl acetate) gave **41** (21.1 mg, 45%) as a pale yellow oil: R_f 0.32 (5:5:1 hexane—chloroform—ethyl acetate); ^{1}H-NMR δ 2.3—3.0 (2H, m), 2.68 (1H, dd, J = 15.6 and 6.0 Hz), 2.79 (1H, dd, J = 15.6 and 7.6 Hz), 3.58 (2H, dull s), 3.66 (2H, dull s), 3.97—4.1 (1H, m), 4.54 (1H, ddd, J = 19.0, 4.0, and 3.8 Hz), 4.86 (1H, dull dd, J = 19.0 and 1.8 Hz), 5.72 (1H, dull dd, J = 12.0 and 1.6 Hz), 5.8—5.9 (1H, m), 6.0—6.08 (1H, m), 7.22—7.27 (1H, m), 7.56—7.68 (2H, m), 11.37 (1H, s); HRMS (EI) *m/z* 434.0833 (434.0827 calcd for $C_{24}H_{18}O_6S$, M^+).

(5*Z*)-7-[(Anthraquinone-2-carbonyl)oxy]-1-thia-5-cyclodecene-3,8-diyne (42). To an ice-cold solution of **6** (73.0 mg, 0.444 mmol) in dry CH_2Cl_2 (3.5 mL) was added triethylamine (0.0930 mL, 0.667 mmol) and 2-anthraquinonecarbonyl chloride (141 mg, 0.533 mmol). After the resulting mixture was stirred at 26 °C for 1 h, the reaction was quenched with H_2O (4 mL) and then the mixture was extracted with $CHCl_3$ (2 mL × 3). The extracts were washed with saturated aqueous NaCl (2 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification of the residue by flash column chromatography (10 g of silica gel, 6:1 hexane—ethyl acetate) gave **42** (159 mg, 90%) as white crystals: R_f 0.26 (6:1 hexane—ethyl acetate); mp 168—170 °C (dec, chloroform—hexane); ^{1}H-NMR δ 3.56 (1H, dd, J = 18.0 and 2.0 Hz), 3.63 (1H, dd, J = 18.0 and 1.2 Hz), 3.66 (1H, dd, J = 17.6 and 2.0 Hz), 3.74 (1H, dd, J = 17.6 and 2.1 Hz), 5.79 (1H, ddt, J = 11.8, 2.0, and 1.9 Hz), 5.98 (1H, dd, J = 11.8 and 4.2 Hz)m, 6.24—6.32 (1H, m), 7.8—7.9 (2H, m), 8.3—8.4 (2H, m), 8.40 (1H, d, J = 8.0 Hz), 8.47 (1H, dd, J = 8.0 and 1.8 Hz), 8.98 (1H, d, J = 1.8 Hz); HRMS (EI) *m/z* 398.0591 (398.0613 calcd for $C_{24}H_{14}O_4S$, M^+).

(9*R*)-7,8,9,10-Tetrahydro-6,9,11-trihydroxy-4-methoxy-5,12-dioxonaphthacene-9-carboxylic Acid (50). To a solution of **49** (76.4 mg, 0.192 mmol) in dioxane (7.6 mL) was added dropwise a solution of HIO_4 (887.4 mg, 0.384 mmol) in H_2O (1.3 mL). The resulting mixture was stirred at 26 °C for 2 h and then diluted with H_2O (5.0 mL). The resulting mixture was washed with Et_2O (1 mL × 3), and the aqueous layer was allowed to stand for 2 h. The aqueous layer was then filtered, and the filter cake was washed with H_2O (100 mL) to give **50** (64.1 mg, 87%) as red solids: ^{1}H-NMR (dimethyl sulfoxide-d_6) δ 1.83—2.07 (2H, m), 2.7—2.95 (4H, m), 3.98 (3H, s, OMe), 7.62 (1H, dd, J = 5.0 and 5.0 Hz), 7.89 (2H, d, J = 5.0 Hz), 13.32 (1H, s), 13.86 (1H, s); HRMS (EI) *m/z* 384.0850 (384.0845 calcd for $C_{20}H_{16}O_8$, M^+).

(5*Z*)-7-[((9*R*)-7,8,9,10-Tetrahydro-6,9,11-trihydroxy-4-methoxy-5,12-dioxonaphthacene-9-carbonyl)oxy]-1-thia-5-cyclodecene-3,8-diyne (43). To a solution of **6** (16.0 mg, 0.0989 mmol) and **50** (38.2 mg, 0.0989 mmol) in dry THF (2.0 mL) were added triphenylphosphine (78.1 mg, 0.297 mmol) and diethyl azodicarboxylate (0.045 mL, 0.297 mmol) with stirring. After the resulting mixture was stirred at 26 °C for 15 min, the mixture was concentrated *in vacuo*. Purification of the residue by flash column chromatography (2.5 g of silica gel, 20:1 benzene—acetone then 2.3:1 hexane—acetone) gave **43** (9.0 mg, 17%) as a red oil: R_f 0.15 (20:1 benzene—acetone); ^{1}H-NMR δ 2.03—2.19 (2H, m), 2.85—3.2 (4H, m), 3.58 (2H, dull s), 3.66 (2H, dull s), 4.09 (3H, s), 5.74 (1H, dull d, J = 11.8 Hz), 5.81—5.92 (1H, m), 6.05—6.12 (1H, m), 7.38 (1H, d, J = 8.0 Hz), 7.77 (1H, dd, J = 8.0 and 8.0

Hz), 8.04 (1H, d, J = 8.0 Hz), 13.49 (1H, s), 13.86 (1H, s); HRMS (EI) m/z 530.1030 (530.1036 calcd for $C_{29}H_{22}O_8S$, M^+).

(5Z)-7-[(β-Carbolin-1-yl)acetoxy]-1-thia-5-cyclodecene-3,8-diyne (44). By the procedure described in the preparation of **40**, esterification of **6** (76.0 mg, 0.287 mmol) with **51** (47.0 mg, 0.287 mmol) gave a crude sample of **44**. Purification of the sample by preparative thin-layer chromatography (3:2 hexane–acetone) gave **44** (20.0 mg, 19%) as a pale yellow oil: R_f 0.37 (3:2 hexane–acetone); ^{1}H-NMR δ 3.49 (2H, dull d, J = 1.8 Hz), 3.63 (2H, dull s), 4.27 (2H, s), 5.68 (1H, ddt, J = 12.0, 2.0, and 1.8 Hz), 5.80 (1H, dd, J = 12.0 and 4.0 Hz), 5.95–6.02 (1H, m), 7.30 (1H, dd, J = 8.0 and 4.0 Hz), 7.55 (1H, s), 7.56 (1H, d, J = 4.0 Hz), 7.91 (1H, d, J = 6.0 Hz), 8.12 (1H, d, J = 8.0 Hz), 8.39 (1H, d, J = 6.0 Hz), 9.02 (1H, br s); HRMS (EI) m/z 372.0903 (372.0932 calcd for $C_{22}H_{16}N_2O_2S$, M^+).

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–acetate buffer (pH 8.5) for 24 h. The DNA sample levels and the temperatures of incubation were varied as indicated in the figure captions. 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. Figures 5 and 6 show the pictures of the agarose gel electrophoresis results. **(B) Identification of DNA Cleavage Sites.** The reaction samples contained the enediyne–sulfides (1 or 2 mM) and the 5′-end-labeled 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 both Sanger and Maxam-Gilbert methods. Figure 7 shows the picture of the autoradiogram.

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