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Structure–Activity Relationships of Cyclic Enediynes Related to Dynemicin A—II. Synthesis and Antitumor Activity of 9- and 12-Substituted Enediynes Equipped with Aryl Carbamate Moieties

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Abstract—Novel enediyne compounds **4–8**, simple analogues of dynemicin A (**1**) equipped with the phenyl or 4-chlorophenyl carbamate moiety, were synthesized and evaluated for DNA-cleaving ability, in vitro cytotoxicity, and in vivo antitumor activity. As a result of the SAR study, it was revealed that the size and character of the substituents (R^1 and R^2) at the C9 position critically influenced both the stability and antitumor activity of the enediyne compounds. We found that the 9-deoxy compound **6a**, a stable and less bulky enediyne having a hydrogen as the R^1 and R^2 substituents, showed a significant in vivo activity with a T/C of 215% at a daily dosage of 2.0 mg/kg for 4 days. The incorporation of an oxygen-containing functional group as the R^3 substituent on a benzene ring resulted in considerable abolishing of both the in vitro and in vivo potencies. In a series of 9-acyloxy compounds, incorporation of the basic aromatic moiety such as **8e** was effective for the in vitro activity, but it was ineffective for the in vivo activity. Furthermore, for the stereochemistry–activity relationships at the C9 position, the (9*R**)-isomers of **8c**, **8e**, and **8f** were found to show higher both in vitro and in vivo than the corresponding (9*S**)-isomers. For the mechanistic studies, compound **6a** underwent Bergman cycloaromatization via a diradical pathway under acidic conditions, whereas it scarcely showed DNA-cleaving activity due to the chemical stability of the aryl carbamate moiety under neutral conditions. © 1997 Elsevier Science Ltd.

Introduction

In the preceding paper in this series,[1] we discussed the structure–activity relationships (SAR) with regard to the aryl carbamate moieties for a group of the simple dynemicin A (**1**) analogues. We have shown that the 9-acetoxy compound **2a** equipped with the 4-chlorophenyl carbamate moiety exhibited significant activity (T/C = 256% at a daily dosage of 4.0 mg/kg for 4 days) against murine P388 leukemia in mice. Compound **2a** was also effective against a solid tumor (Meth A sarcoma) in mice to show 77% inhibition of the tumor growth at 3.0 mg/kg dosage. Thus, our studies have shown that the 4-chlorophenyl carbamate moiety attached to the cyclic enediyne core is significant for in vivo antitumor activity. Moreover, we found that the stereochemistry at the C9 position played an important role in the biological activities of a group of the 9-acetoxy enediyne compounds.

For further studies for the SAR of the R^1 and R^2 substituents at the C9 position, we designed the 9-substituted enediyne compounds such the 9-ethyl compound **4**, 9-hydroxy compounds **5**, 9-deoxy compounds **6**, and 9-acyloxy compounds **8** as were equipped with the 4-chlorophenyl or phenyl carbamate moiety (Fig. 1). For the SAR of the R^3 substituent, we further designed the 12-substituted enediyne compounds **7** that introduced oxygen-containing functional groups onto the benzene ring.

In this paper, we describe (a) the SAR studies with regard to the R^1, R^2, and R^3 substituents for a group of simple dynemicin A analogues equipped with 4-chlorophenyl or phenyl carbamate moiety, and (b) the effect on in vivo antitumor activity of the deoxygenation or replacing the acetoxy group at the C9 position. Furthermore, for the mechanistic studies, we describe the Bergman cycloaromatization and the DNA-cleaving ability of the novel 9-deoxy compounds **6**.

Results and Discussion

Synthesis of the enediyne compounds 4–8

The enediyne compounds **4–8** were synthesized using a CsF-promoted cyclization method.[2,3]

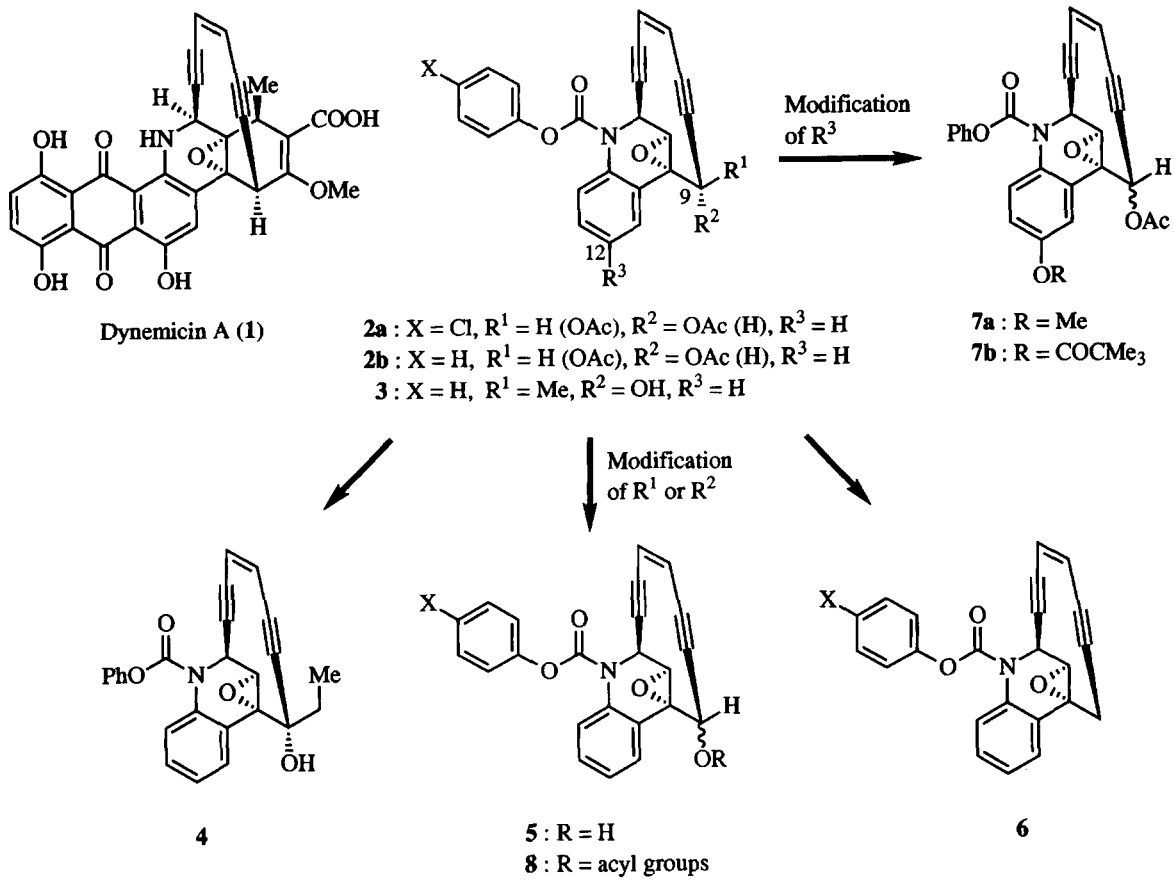


Figure 1. Dynemicin A (**1**) and its functional analogues **2–8**.

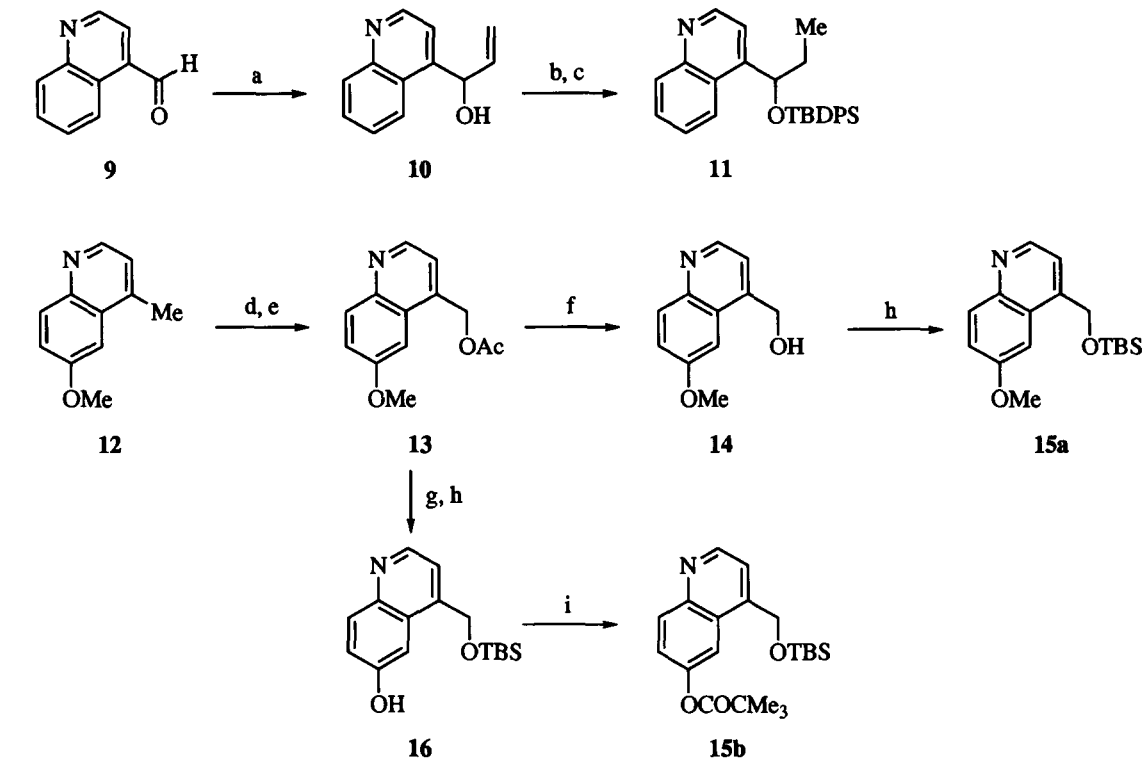
This cyclization method is useful for the construction of a highly strained 10-membered ring system, which has been originally developed by Kende[4] and Danishefsky.[5]

The requisite silyl ethers **11**, **15a**, and **15b** were prepared as shown in Scheme 1. The silyl ether **11** was prepared from the commercially available aldehyde **9**. The addition of vinylmagnesium bromide to **9** afforded the allyl alcohol **10**. Hydrogenation of **10** and the subsequent protection of the resulting propyl alcohol with *tert*-butyldiphenylsilyl (TBDPS) chloride gave the silyl ether **11**. The silyl ethers **15a** and **15b** were prepared by the following procedures.

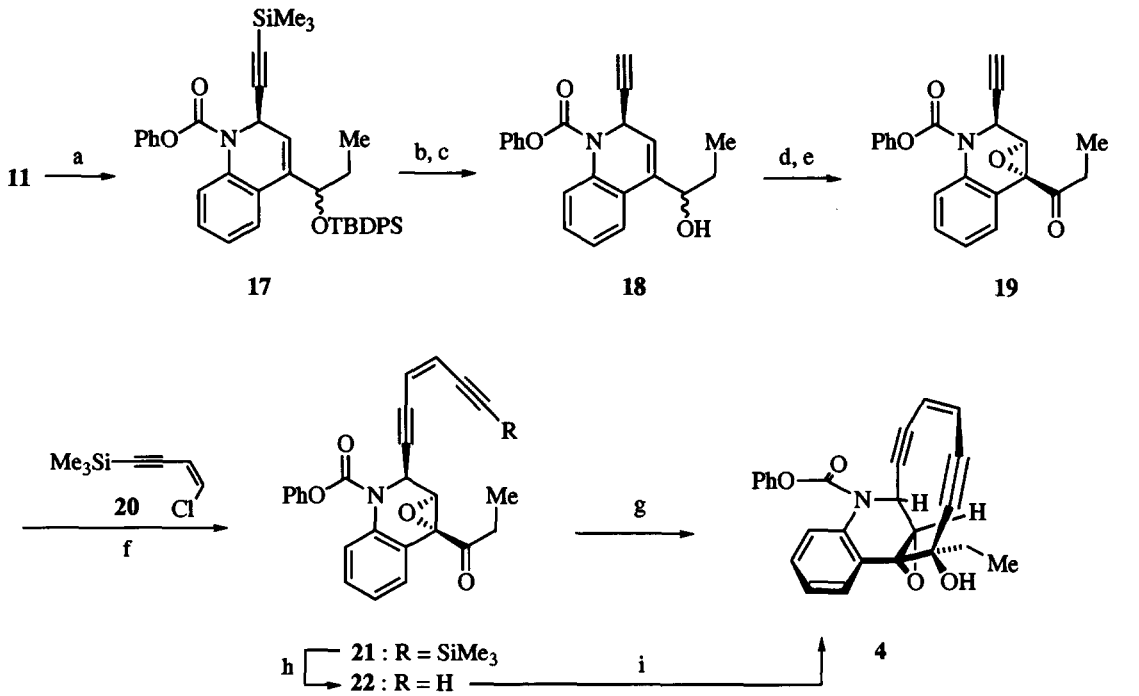
The oxidation of the commercially available quinoline **12** with *m*-chloroperoxybenzoic acid (mCPBA) and the subsequent rearrangement of the resulting N-oxide with Ac_2O afforded the acetate **13**.[6] Deacetylation of **13** with K_2CO_3 in MeOH and subsequent protection of the resulting alcohol **14**[7] with *tert*-butyldimethylsilyl (TBS) chloride gave **15a**. On the other hand, demethylation of **13** with BBr_3 and subsequent protection of the resulting diol with TBS chloride gave **16**, which was acylated with pivaloyl chloride to afford **15b**.

Synthesis of the 9-ethyl compound **4** was synthesized via a similar procedure to that previously reported (Scheme 2).[2] The synthesis started from the reaction among three components, the silyl ether **11**, ethynylmagnesium bromide, and phenyl chloroformate, to provide **17** as a 7:3 mixture of diastereomers. The TMS and TBDPS groups in **17** were stepwise desilylated with *n*-tetrabutylammonium fluoride (TBAF) followed by the treatment with *p*TsOH in MeOH to afford **18**. The allyl alcohol **18** was converted into the precursor of **4** through three steps including epoxidation, oxidation with SO_3·Py-DMSO, and Pd(0)[8] coupling with the vinyl chloride **20**[4] to afford the ketone **21** as a single isomer. The crucial cyclization of **21** was achieved to give **4** in 12% yield using CsF in the presence of 18-crown-6 in THF solvent. Furthermore, compound **4** could also be obtained from the desilylated compound **22** in 12% yield by a coupling of cerium(III) acetylide.[9] The cyclized product **4** was a single isomer from ^{1}H NMR data.

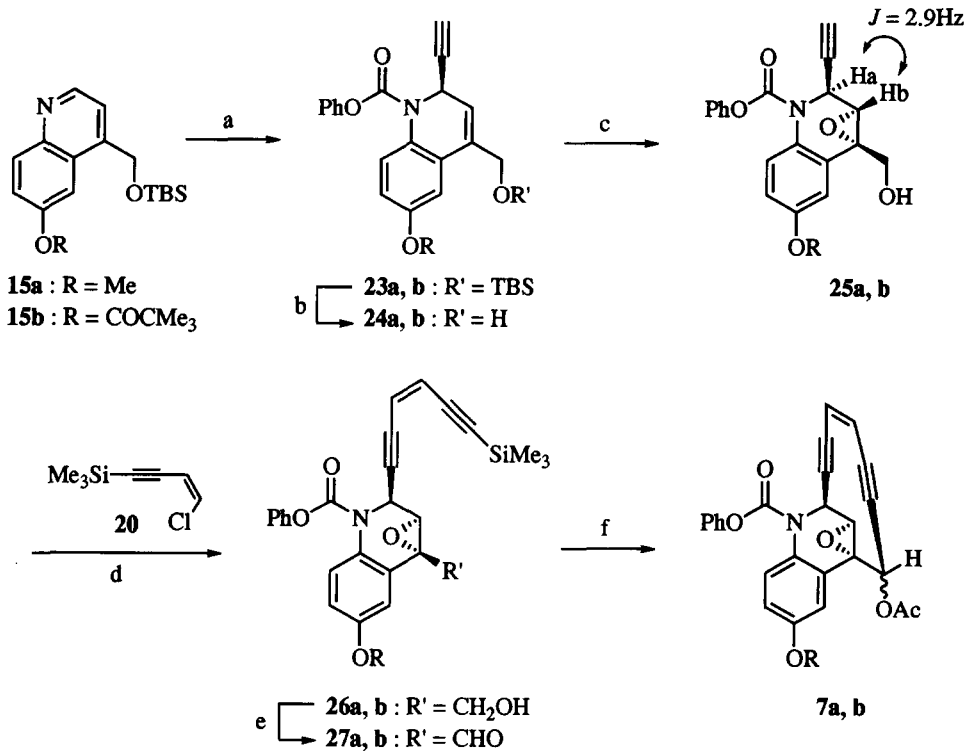
The synthetic procedure for the 12-methoxy and 12-pivaloyloxy compounds (**7a** and **7b**) is shown in Scheme 3. These compounds were obtained using a similar procedure to those described in the preceding paper in this series.[1] Syntheses of the aldehydes **27**, the precursors of the compounds **7**, commenced from the reaction with three components, the silyl ethers **15**, ethynylmagnesium bromide, and phenyl chloroformate, to provide the allyl ethers **23**, which were deprotected under acidic conditions to the corresponding alcohols **24**. Face-selective epoxidation of **24** with *m*CPBA gave the anti-epoxy alcohols **25**. Coupling of **25** with the vinyl



Scheme 1. Synthesis of the silyl ethers **11**, **15a**, and **15b**. Reagents and conditions: (a) CH_2=CHMgBr, THF, −78 °C to 0 °C, 6 h, 62%; (b) H_2, 10% Pd-C, AcOEt, rt, 19 h; (c) TBDPSCl, imidazole, DMF, 70 °C, 16 h, 80% in two steps; (d) *m*CPBA, CH_2Cl_2, rt, 16 h, 57%; (e) Ac_2O, rt, 17 h, 72%; (f) K_2CO_3, MeOH, rt, 2 h, quant.; (g) BBr_3, CH_2Cl_2, rt, 16 h; (h) TBSCl, imidazole, CH_2Cl_2, rt, 16 h, **15a**: 59%, **16**: 32% in two steps; (i) Me_3CCOCl, Et_3N, CH_2Cl_2, rt, 14 h, quant.



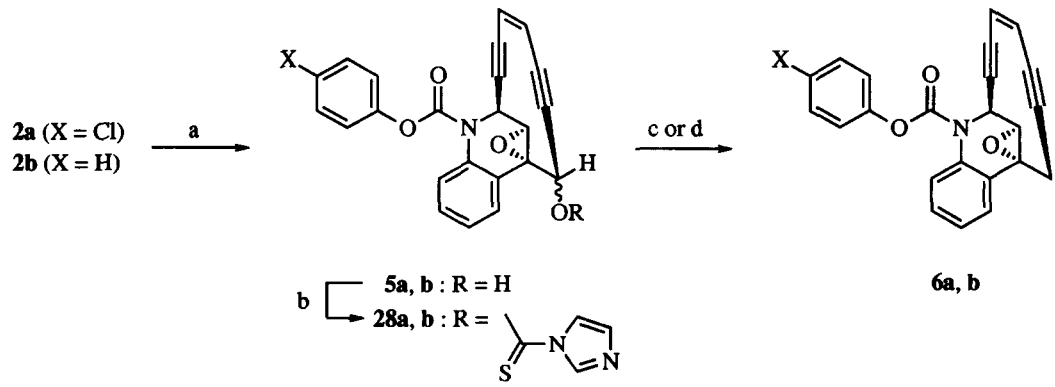
Scheme 2. Synthesis of the 9-ethyl compound **4**. Reagents and conditions: (a) TMSC≡CH, EtMgBr, PhOCOCl, THF, −78 °C to 0 °C, 1 h, 92%; (b) TBAF, MeOH, THF, 0 °C, 10 min; (c) *p*TsOH·H_2O, MeOH, reflux, 16 h, 98% in two steps; (d) *m*CPBA, Na_2HPO_4, CH_2Cl_2, 0 °C, 3.5 h; (e) SO_3·Py, Et_3N, DMSO-CH_2Cl_2, 0 °C to rt, 1.5 h, 83% in two steps; (f) **20**, Pd(OAc)$_2$, Ph_3P, CuI, *n*-$BuNH_2$, benzene, 0 °C to rt, 2 h, 53%; (g) CsF, 18-crown-6, THF, rt, 2.5 h, 12%; (h) TBAF, MeOH, THF, −78 °C, 6 min, 94%; (i) $KN(TMS)_2$, $CeCl_3$, THF-toluene, −78 °C, 1 h, 12%.



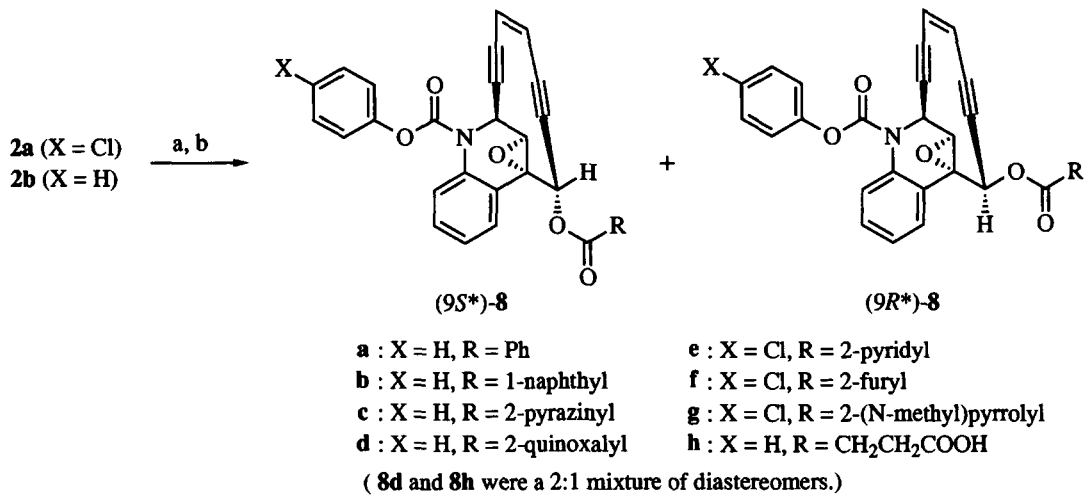
Scheme 3. Synthesis of the 12-substituted enediyne compounds **7**. Reagents and conditions: (a) HC≡CMgBr, PhOCOCl, THF, −70 °C to 0 °C, 1 h; (b) *p*TsOH·H_2O, MeOH-CH_2Cl_2, 0 °C, 1.5 h; (c) *m*CPBA, Na_2HPO_4, CH_2Cl_2, 0 °C, 2 h; (d) **20**, $Pd_2(dba)_3$·$CHCl_3$, Ph_3P, CuI, *n*-$BuNH_2$, THF, rt, 2 h; (e) Dess–Martin periodinane, py, CH_2Cl, rt, 2 h; (f) CsF, Ac_2O, CH_3CN, 0 °C, 2 h.

chloride **20** in the presence of a Pd(0)–Cu(I) catalyst gave the alcohols **26** which were oxidized with Dess–Martin periodinane reagent[10] to the aldehydes **27**. Finally, the CsF-promoted cyclization of **27** in the presence of Ac_2O[3b,3d] proceeded smoothly to give the desired compounds **7** as a 2:1 mixture of diastereomers which, however, could not be separated.

The 9-hydroxy and 9-deoxy compounds (**5** and **6**) were synthesized from the 9-acetoxy compounds **2**[1] using the following synthetic procedure (Scheme 4). The acetyl group in **2** was removed with $Ba(OH)_2$[3b] to provide the alcohols **5**; however, these alcohols **5** were unstable under weakly basic conditions and the isolated product also slowly decomposed during storage at −20 °C. Therefore, the alcohols **5** were successively converted into **28** with thiocarbonyldiimidazole and 4-(dimethylamino)pyridine (DMAP). The reduction of **28a** with *n*-Bu_3SnH and 2,2′-azobisisobutyronitrile (AIBN) as radical initiator gave the desired compound **6a** in 52% yield.[11] On the other hand, the reduction of **28b** using Et_3B[12] instead of AIBN could be carried out under



Scheme 4. Synthesis of the 9-hydroxy and 9-deoxy compounds (**5** and **6**). Reagents and conditions: (a) $Ba(OH)_2$, MeOH, 0 °C, 10 min; (b) thiocarbonyldiimidazole, DMAP,CH_2Cl_2, 0 °C, 1 h; (c) *n*-Bu_3SnH, AIBN, benzene, 80 °C, 1 h; (d) *n*-Bu_3SnH, Et_3B, benzene, rt, 1 h.



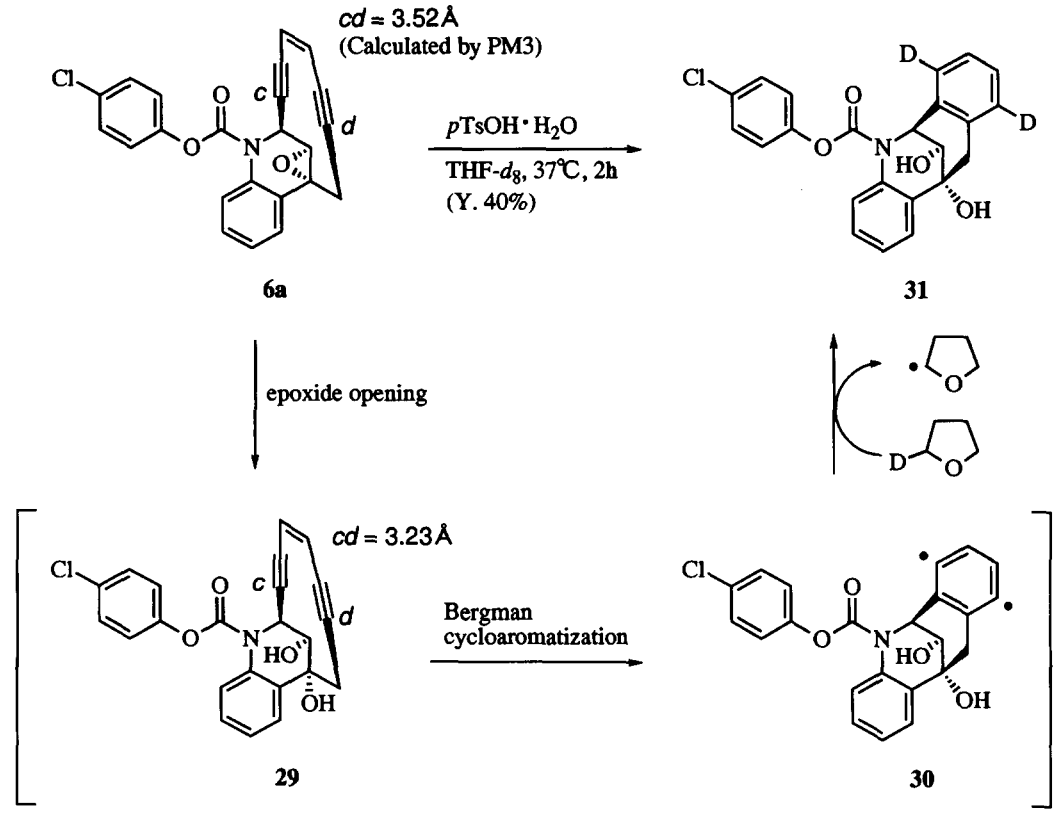
Scheme 5. Synthesis of the 9-acyloxy compounds **8**. Reagents and conditions: (a) $Ba(OH)_2$, MeOH, 0 °C, 10 min; (b) RCOCl or succinic anhydride, DMAP, CH_2Cl_2, 0 °C, 1–2 h.

milder conditions (rt, 1 h) compared to that using AIBN (80 °C, 1 h), however, the yield of product **6b**, obtained in 48% yield, could not be improved.

The 9-acyloxy compounds **8** were also synthesized from **2** as shown in Scheme 5. Removal of the acetyl group in **2** and the subsequent acylation of the resulting alcohols **5** with acid chlorides or acid anhydride gave **8** as a 2:1 mixture of diastereomers. These diastereomers could be separated to the major isomers [(9*S**)-**8a–c** and (9*S**)-**8e–g**][13] and minor isomers [(9*R**)-**8a–c** and (9*R**)-**8e–g**], except for **8d** and **8h** as shown in Table 2.

Bergman cycloaromatization[14] of the 9-deoxy compound 6a

The acid treatment of the 9-deoxy compound **6a** in THF-d_8[15] at 37 °C gave the aromatic product **31** as expected through Bergman cycloaromatization (Scheme 6). Two deuterium atoms were quantitatively incorporated into the newly formed aromatic ring. This result is consistent with the intermediacy of the diradical **30** during the transformation of **6a** to **31**. Furthermore, molecular orbital calculations (PM3)[16] indicated a crucial structural change during the conver-



Scheme 6. Bergman cycloaromatization of the 9-deoxy compound **6a**.

sion of **6a** to the epoxide opened product **29**. The distance between the two terminal acetylenic carbons of the 1,5-diyne-3-ene system (*cd* distance) is shortened from 3.52 Å in **6a** to 3.23 Å in **29**, enough to cycloaromatize at ambient temperature in order to generate the diradical **30**.[17]

DNA cleavage with the enediynes[18]

The DNA-cleaving activity of compounds **4**, **6a**, **6b**, and **7a** was tested with supercoiled ΦX174 DNA (Form I) and analyzed by agarose gel electrophoresis. The supercoiled DNA was incubated with each compound at 37 °C for 18 h in pH 7.4 buffer solution. The DNA cleavage profiles of these compounds are shown in Figure 2, and the Form II band represents the nicked open circular DNA. None of the tested compounds almost caused DNA cleavage (Form I → Form II). These low activities resulted from the chemical stability of the phenyl or 4-chlorophenyl carbamate moiety which could not be deprotected to generate a diradical intermediate (equivalent to **30**) under such neutral conditions as this assay.[1,2f]

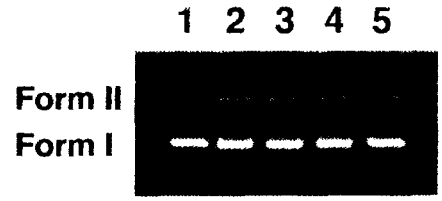


Figure 2. Attempted DNA cleavage by the enediyne compounds **4**, **6a**, **6b**, and **7a**. The ΦX174 DNA (Form I, 250 μM/base pair) was incubated at 37 °C for 18 h with 1 mM (final concentration) of each compound in 50 mM phosphate buffer (pH 7.4) containing 10% DMSO and analyzed by electrophoresis (1% agarose gel, ethidium bromide strain). Lane 1, DNA alone; lane 2, compound **4**; lane 3, compound **6a**; lane 4, compound **6b**; lane 5, compound **7a**. Key: Form I, supercoiled DNA; Form II, nicked DNA.

In vitro cytotoxicity of the enediynes[19]

The in vitro cytotoxicity of the enediyne compounds **4**–**7** against the human carcinoma KB cell line are shown in Table 1. In a group of the 9-alcohols (R^2 = OH) in which the alkyl groups were incorporated as R^1 substituents, the ethyl compound **4** (R^1 = Et) showed almost no activity and its activity significantly reduced compared to that of the methyl compound **3**[2c] (R^1 = Me). This result suggests that the incorporation of a bulky alkyl group into the C9 position reduces the cytotoxicity of the enediyne compounds. However, contrary to our expectation, compound **5b** (R^1 = H, a 2:1 mixture of diastereomers) showed almost no activity due to its unstability; in fact, compound **5b** slowly decomposed even under neutral conditions, and compound **2b**, the stable enediyne which has the protected hydroxy group by an acetyl group, showed good in vitro potency. These results suggest that the size and character of the substituents at the C9 position of the enediyne ring critically influence the in vitro potency. Therefore, the 9-deoxy compounds **6**, a stable and less bulky enediyne having a hydrogen as the R^1 and R^2 substituents, were designed and synthesized. As expected, the cytotoxicity of these compounds increased more than those of the 9-acetoxy compounds **2**, and compound **6a** possessing the 4-chlorophenyl carbamate moiety showed a good activity (IC_{50} = 0.80 μM). Thus, it has been revealed that the size and character of the substituents at the C9 position of the enediyne ring are significant for both the stability and biological activity of the enediyne compounds.

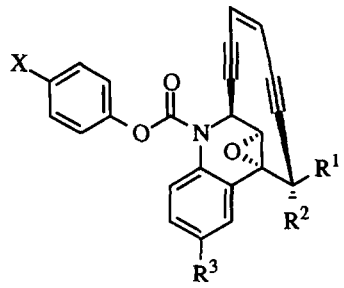
The compounds possessing a methoxy (**7a**) or pivaloyloxy group (**7b**) as the R^1 substituent showed only a slight cytotoxic activity. Compounds **7a** and **7b** were about 4-fold less potent, compared to **2b** (R^3 = H). The cytotoxicity of **7a**, as well as Nicolaou's enediyne,[18a,20] was significantly decreased by the incorporation of a methoxy group at the C12 position. Thus, the incorporation of an oxygen-containing functional group such as methoxy or pivaloyloxy group into the C12 position of a benzene ring significantly abolished the in vitro cytotoxicity.

The in vitro cytotoxicity of the 9-acyloxy compounds **8a**–**h** is shown in Table 2. In both groups of (9*S**)- and (9*R**)-isomers, the order of in vitro activity is as follows: 2-pyridyl (**8e**) > 2 pyrazinyl (**8c**) > 2-furyl (**8f**) > phenyl (**8a**), 1-naphthyl (**8b**), and 2-(*N*-methyl)pyrrolyl (**8g**). In particular, the 2-pyridyloxy compounds (9*S**)-**8e** and (9*R**)-**8e** showed the more potent activity (IC_{50} = 1.7 and 1.0 μM, respectively) compared to that of **2a**. Thus, compounds **8c** and **8e**, which possessed the *N*-containing hetero aromatic ring such as a pyrazine or pyridine, showed good potency; furthermore, the potency of **8d** possessing the DNA intercalative aromatic moiety such as a quinoxaline[21] was nearly equal to that of **8c**. On the other hand, compounds **8a** and **8b** possessing the aromatic ring such as a benzene or naphthalene did not show any activity, and compound **8h** possessing a carboxyl group was also ineffective. These results indicate that the incorporation of basic groups at the C9 position is effective for the in vitro potency, whereas either the incorporation of a neutral aromatic or an acidic moiety considerably abolished the in vitro potency compared with **2b**. Concerning stereochemistry at the C9 position, all of the (9*R**)-isomers of **8c**, **8e**, and **8f** showed higher potency than the corresponding (9*S**)-isomers. This result was consistent with the stereochemistry–activity relationships of the 9-acetoxy compounds as described in the preceding paper in this series.[1] These findings apparently show that both the character of the substituents and the stereochemistry at the C9 position significantly affect the biological activity of the 9-acyloxy compounds **8**.

In vivo antitumor activity of the enediynes[22]

The enediyne compounds **4**–**8** were evaluated for antitumor activity in mice bearing intraperitoneal (ip) implants of murine P388 leukemia, and the results are shown in Tables 1 and 2. A T/C 125% was taken as the

Table 1. Preparation and biological data of the enediyne compounds **4–7**



Compd no.	X	R^1	R^2	R^3	Formula[a]	In vitro cytotoxicity against KB cells IC_{50} (μM)[b]	In vivo antitumor activity against P388 leukemia[c] dose (mg/kg)	AWC[d] (g)	T/C[e] (%)
2a	Cl	H	OAc[f]	H	—	3.6	2.0	−1.94	221
2b	H	H	OAc[f]	H	—	2.3	2.0	−3.17	202
3	H	Me	OH	H	—	5.0	2.0	−1.77	165
4	H	Et	OH	H	$C_{25}H_{19}NO_4$	>10			NT
5b	H	H	OH[f]	H	$C_{23}H_{15}NO_4$	>10			NT
6a	Cl	H	H	H	$C_{23}H_{14}ClNO_3$	0.80	2.0	−1.60	215
6b	H	H	H	H	$C_{23}H_{15}NO_3$	1.0	2.0	−0.25	170
7a	H	H	OAc[f]	OMe	$C_{26}H_{19}NO_6$	10	2.0	−1.27	140
7b	H	H	OAc[f]	$OCOCMe_3$	$C_{30}H_{25}NO_7$	>10			NT

[a]Analysis for C, H, and N are within 0.4% of theory.
[b]Inhibiting concentration (μM) of 50% cellular growth.
[c]CDF_1 mice were inoculated intraperitoneally (ip) with 1×10^6 cells/mouse of P388 on day 0, and the test compound was administered ip once daily for 4 days from day 1 to 4.
[d]Average weight changes (AWC) were measured on day 4.
[e]The T/C represents the ratio of mean survival time of the treated to the control mice × 100. The T/C values over 125% are considered indicative of significant activity.
[f]A 2:1 mixture of diastereomers.

criterion of activity. The 9-deoxy compound **6a** was the most effective and showed a significant activity (T/C = 215% at a daily dosage of 2.0 mg/kg for 4 days). Although the 9-deoxy compounds **6a** and **6b** showed more potent in vitro activity than the corresponding 9-acetoxy compounds (**2a** and **2b**), their in vivo potencies decreased slightly compared to those of **2a** and **2b**, respectively. The 12-methoxy compound **7a** showed only a slight activity, and the introduction of a methoxy group into the aromatic nucleus resulted in reducing the in vivo potency in contrast to Magnus' enediyne.[19a]

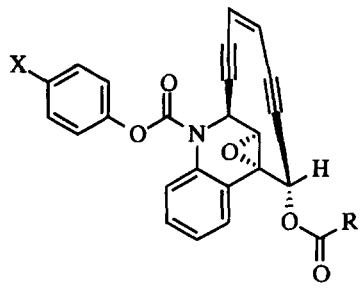
In a group of the 9-acyloxy compounds **8a–h** (Table 2), compounds (9*R**)-**8c**, **8d** and (9*R**)-**8e** exhibited modest activity (T/C=183%, 157%, and 167%, respectively), but other compounds showed low activity. Thus, the replacement of the acetyl group in **2a** or **2b** by other acyl groups resulted in reducing the in vivo activity. In particular, neither the introduction of an aromatic ring such as a benzene, a naphthalene nor an acidic group such as a carboxylic acid had a good effect on the in vivo activity.

The stereochemistry–in vivo activity relationships of the 9-acyloxy compounds **8a–h** were consistent with that of the in vitro cytotoxicity observed with the (9*S**)- and (9*R**)-isomers except for (9*R**)-**8e** that was less potent than the corresponding (9*S**)-isomer due to being toxic at a 2 mg/kg dosage.

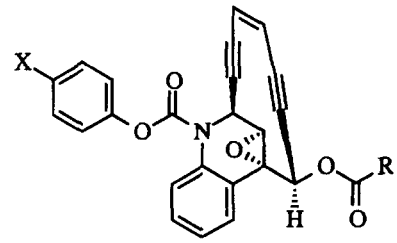
Conclusion

Novel enediyne compounds **4–8**, simple dynemicin A (**1**) analogues equipped with phenyl or 4-chlorophenyl carbamate moiety, were synthesized using a CsF-promoted cyclization method. These compounds were evaluated for DNA-cleaving ability, in vitro cytotoxicity against the human carcinoma KB cell line, and in vivo antitumor activity against murine P388 leukemia. As a result of the SAR, it was revealed that the size and character of the substituents (R^1 and R^2) at the C9 position critically influence both the stability and antitumor activity of the enediyne compounds. We found that the 9-deoxy compound **6a**, a stable and less bulky enediyne having a hydrogen as the R^1 and R^2 substituents, showed a significant in vivo antitumor activity with a T/C of 215% at a daily dosage of 2.0 mg/kg for 4 days. On the other hand, the incorporation of an oxygen-containing functional group as the R^3 substituent on a benzene ring resulted in significantly reducing the in vitro and in vivo activities.

Table 2. Preparation and biological data of the 9-acyloxy compounds **8**



(9*S**)-**8**



(9*R**)-**8**

Compd no.	X	R	Formula[a]	In vitro cytotoxicity against KB cells IC_{50} (μM)	In vivo antitumor activity against P388 leukemia[c] Dose (mg/kg)	AWC[d] (g)	T/C[e] (%)
(9*S**)-**8a**	H	Ph	$C_{30}H_{19}NO_5$	>10	2.0	−0.28	133
(9*R**)-**8a**	H	Ph	$C_{30}H_{19}NO_5$	>10			NT
(9*S**)-**8b**	H	1-naphthyl	$C_{34}H_{21}NO_5$	>10	2.0	+0.72	144
(9*R**)-**8b**	H	1-naphthyl	$C_{34}H_{21}NO_5$	>10			NT
(9*S**)-**8c**	H	2-pyrazinyl	$C_{28}H_{17}N_3O_5$	7.4	2.0	−0.80	131
(9*R**)-**8c**	H	2-pyrazinyl	$C_{28}H_{17}N_3O_5$	2.3	2.0	−2.01	183
8d	H	2-quinoxalyl[f]	$C_{32}H_{19}N_3O_5$	5.5	2.0	−1.43	157
(9*S**)-**8e**	Cl	2-pyridyl	$C_{29}H_{17}ClN_2O_5$	1.7	2.0	−1.92	167
(9*R**)-**8e**	Cl	2-pyridyl	$C_{29}H_{17}ClN_2O_5$	1.0	2.0	−2.58	148(toxic)
(9*S**)-**8f**	Cl	2-furyl	$C_{28}H_{16}ClNO_6$	20			NT
(9*R**)-**8f**	Cl	2-furyl	$C_{28}H_{16}ClNO_6$	5.2			NT
(9*S**)-**8g**	Cl	2-(N-methyl)pyrrolyl	$C_{29}H_{19}ClN_2O_5$	>10			NT
(9*R**)-**8g**	Cl	2-(N-methyl)pyrrolyl	$C_{29}H_{19}ClN_2O_5$	>10			NT
8h	H	CH_2CH_2COOH[f]	$C_{27}H_{19}N_2O_7$	>10	2.0	−0.84	130

[a]Analysis for C, H, and N are within 0.4% of theory.
[b]Inhibiting concentration (μM) of 50% cellular growth.
[c]CDF_1 mice were inoculated intraperitoneally (ip) with 1×10^6 cells/mouse of P388 on day 0, and the test compound was administered ip once daily for 4 days from day 1 to 4.
[d]Average weight changes (AWC) were measured on day 4.
[e]The T/C represents the ratio of mean survival time of the treated to the control mice × 100. The T/C values over 125% are considered indicative of significant activity.
[f]A 2:1 mixture of diastereomers.

In a group of the 9-acyloxy compounds **8a–h**, it was revealed that the replacement of the acetyl group in **2a** or **2b** by basic aromatic groups was effective for the in vitro activity, but it was ineffective for the in vivo activity. Furthermore, neither the incorporation of a neutral aromatic moiety nor acidic moiety had a good effect on in vitro and in vivo activities. For the stereochemistry–activity relationships of the C9 position, we found that the (9*R**)-isomers of **8** showed higher in vitro and in vivo potencies than the corresponding (9*S**)-isomers. Thus, these findings apparently show that both the character of the substituents and the stereochemistry at the C9 position significantly affect the biological activity of the 9-acyloxy compounds **8**.

It has been generally assumed that the formation of a diradical intermediate is essential for biological activity of the natural enediyne compounds.[23] On the other hand, for biological activity of the synthetic enediyne compounds, other mechanisms such as an induction of apoptosis[24] or a protein damage[25] have been recently reported. Furthermore, Magnus et al. have shown that the diradical formation in their dynemicin A analogues is not necessary for the antitumor activity.[19a]

During our mechanistic studies, the 9-deoxy compound **6a** underwent the cycloaromatization via a diradical pathway under acidic conditions. However, it scarcely showed DNA-cleaving activity due to the chemical stability of the aryl carbamate moiety under neutral conditions. This result indicates that the in vitro and in vivo activities of **6a** are not attributed to its DNA-cleaving ability. It is considered that the role of the enediyne ring in **6a** for biological activity is not a radical generator. Thus, we demonstrated that our simple dynemicin A analogues having the aryl carbamate moiety onto the bicyclo[7.3.1]-tridecenediyne system exhibited significant in vitro and in vivo activities without causing DNA cleavage.

Experimental

Melting points were measured on a Yanaco MP-l apparatus without correction. Infrared (IR) spectra were recorded on a Jasco FT/IR-8000 spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a JEOL JNM GSX-270 (270 MHz) spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a JEOL JNM GSX-270 (67.9 MHz) spectrometer. Chemical shifts are given in ppm, and the following abbreviations are used; s = singlet, d = doublet, t = triplet, q = quartet, dd = double doublet, ddd = double double doublet, dt = double triplet, dq = double quartet, m = multiplet, br = broad. Low-resolution mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded on JEOL JMS-DX300 and JMS-SX1020 spectrometers. Elemental analyses were performed with a Yanaco CHN CORDER MT-3. Column chromatography was carried out on silica gel (Kieselgel 60, 70–230 mesh, Merck). Preparative thin-layer chromatography was carried out by precoated silica gel plates (Art 5774, Merck).

4-(1-Hydroxy-2-propenyl)quinoline (10). To a solution of aldehyde **9** (Aldrich, 503 mg, 3.18 mmol) in dry THF (10 mL) cooled to −78 °C was added portionwise vinylmagnesium bromide (9.54 mL of a 1.0 M solution in THF, 9.54 mmol) and then the mixture was allowed to warm to 0 °C over 2 h. After stirring for 3 h at 0 °C, the reaction mixture was quenched with saturated NH_4Cl solution, and extracted with AcOEt (× 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, ether:*n*-hexane = 5:1) to give **10** (363 mg, 62%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 5.24 (1H, brd, J= 10 Hz, CH=CHH), 5.41 (1H, brd, *J* = 17 Hz, CH=CHH), 5.92 (1H, d, *J* = 6.0 Hz, CHCH=CH_2), 6.15 (1H, ddd, *J* = 17, 10, 6.0 Hz, CHCH=CH_2), 7.49 (1H, ddd, *J* = 9.0, 7.0, 1.0 Hz, aromatic), 7.54 (1H, d, *J* = 4.5 Hz, aromatic), 7.64 (1H, ddd, *J* = 9.0, 7.0, 1.0 Hz, aromatic), 8.06 (2H, m, aromatic), 8.67 (1H, d, J = 4.5 Hz, aromatic).

4-(1-*tert*-Butyldiphenylsilyloxypropyl)quinoline (11). To a solution of **10** (485 mg, 2.63 mmol) in AcOEt (10 mL) was added 10% Pd-C (48.5 mg), followed by hydrogenating at rt for 19 h. The catalyst was filtered off and the resulting filtrate was evaporated in vacuo to give crude 4-(hydroxypropyl)quinoline (520 mg). To a solution of the crude product (520 mg) in dry DMF (8.0 mL) was added imidazole (570 mg, 8.38 mmol) and *tert*-butyldiphenylsilyl chloride (1.10 mL, 4.19 mmol), followed by stirring at 70 °C for 16 h. After cooling to rt, the reaction mixture was quenched with H_2O (8 mL), extracted with AcOEt (× 3). The combined organic layers were washed with water, brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane:ether = 1:3) to give **11** (950 mg, 80%) as a pale yellow oil. ^{1}H NMR($CDCl_3$) δ 0.71 (3H, t, *J* = 7.5 Hz, CH_2CH_3), 1.08(9H, s, Si*t*-Bu), 1.87 (2H, m, CH_2CH_3), 5.42 (1H, d, *J* = 5.5 Hz, $CHCH_2CH_3$), 7.13 (2H, t, *J* = 7.5 Hz, aromatic), 7.22–7.30 (1H, m, aromatic), 7.34–7.48 (6H, m, aromatic), 7.52 (1H, d, *J* = 4.5 Hz, aromatic), 7.64 (1H, td, *J* = 7.5, 1.0 Hz, aromatic), 7.71 (1H, d, *J* = 1.5 Hz, aromatic), 7.74 (1H, d, *J* = 1.5 Hz, aromatic), 7.86 (1H, brd, *J* = 8.5 Hz, aromatic), 8.09 (1H, brd, *J* = 8.5 Hz, aromatic), 8.84 (1H, d, *J* = 4.5 Hz, aromatic).

4-Acetoxymethyl-6-methoxyquinoline (13). To a solution of quinoline **12** (Aldrich, 1.66 g, 9.58 mmol) in CH_2Cl_2 (30 mL) cooled to 0 °C was added *m*CPBA (3.60 g, 11.7 mmol), followed by stirring at rt for 16 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with aqueous $Na_2S_2O_3$ and aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was crystallized with ether to give 6-methoxy-4-methylquinoline *N*-oxide (1.03 g, 57%). The *N*-oxide (1.03 g, 4.49 mmol) was dissolved in Ac_2O (17 mL) and the solution was stirred at rt for 17 h. The reaction mixture was evaporated in vacuo and the resulting residue was purified by column chromatography (silica gel, *n*-hexane:AcOEt = 1:1) to give **13** (750 mg, 72%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.19 (3H, s, OAc), 3.95 (3H, s, OMe), 5.55 (2H, s, CH_2OAc), 7.14 (1H, d, *J* = 2.9Hz, aromatic), 7.39 (1H, dd, *J* = 9.3, 2.9 Hz, aromatic), 7.41 (1H, d, *J* = 4.4 Hz, aromatic), 8.05 (1H, d, *J* = 9.3 Hz, aromatic), 8.76 (1H, d, *J* = 4.4 Hz, aromatic). MS (EI) *m/z*: 231 (M^+).

4-*tert*-Butyldimethylsilyloxymethyl-6-methoxyquinoline (15a). To a solution of **13** (5.61 g, 24.3 mmol) in MeOH (140 mL) was added K_2CO_3 (6.08 g, 44.0 mmol), followed by stirring at rt for 2 h. The reaction mixture was poured into H_2O (200 mL), extracted with AcOEt (100 mL × 2). The combined organic layers were washed with water, brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo to give 4-hydroxymethyl-6-methoxyquinoline (**14**) (4.59 g, quantitative) as a colorless solid. To a solution of **14** (4.40 g, 24.0 mmol) in dry CH_2Cl_2 (100 mL) was added imidazole (8.30 g, 122 mmol) and *tert*-butyldimethylsilyl chloride (9.15 g, 60.0 mmol), followed by stirring at rt for 16 h. The reaction mixture was poured into H_2O (200 mL), extracted with AcOEt (100 mL × 2). The combined organic layers were washed with saturated NH_4Cl solution, brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane:AcOEt = 3:1) to give **15a** (4.32 g, 59%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 0.16 (6H, s, $SiMe_2$), 0.99 (9H, s, Si*t*-Bu), 3.94 (3H, s, OMe), 5.14 (2H, s, $SiOCH_2$), 7.11 (1H, d, *J* = 2.9 Hz, aromatic), 7.37 (1H, dd, *J* = 9.3, 2.9 Hz, aromatic), 7.50 (1H, d, *J* = 4.4 Hz, aromatic), 8.03 (1H, d, *J* = 9.3 Hz, aromatic), 8.76 (1H, d, *J* = 4.4 Hz, aromatic). MS (EI) *m/z*: 303 (M^+).

4-*tert*-Butyldimethylsilyloxymethyl-6-hydroxyquinoline (16). To a solution of **13** (610 mg, 2.64 mmol) in CH_2Cl_2 (20 mL) cooled to −78 °C was added BBr_3 (8.00 mL of

a 1.0 M solution in CH_2Cl_2, 8.00 mmol), followed by stirring at rt for 16 h. The reaction mixture was quenched with H_2O (50 mL) and the aqueous layer was lyophilized to give the crude 6-hydroxy-4-hydroxymethylquinoline (460 mg). To a solution of the diol (460 mg) in dry DMF (15 mL) was added imidazole (900 mg, 13.2 mmol) and *tert*-butyldimethylsilyl chloride (420 mg, 2.80 mmol), followed by stirring at rt for 16 h. The reaction mixture was poured into H_2O (50 mL), extracted with AcOEt (50 mL × 2). The combined organic layers were washed with saturated NH_4Cl solution, brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane:AcOEt = 2:1) to give **16** (240 mg, 32%) as a colorless solid. ^1H NMR ($CDCl_3$) δ 0.14 (6H, s, $SiMe_2$), 0.94 (9H, s, Si*t*-Bu), 5.10 (2H, s, $SiOC\underline{H}_2$), 7.16 (1H, d, J = 2.9 Hz, aromatic), 7.30 (1H, dd, J = 9.2, 2.4 Hz, aromatic), 7.43 (1H, d, J = 4.4 Hz, aromatic), 7.88 (1H, d, J = 9.2 Hz, aromatic), 8.65 (1H, d, J = 4.4 Hz, aromatic). MS (CI) *m/z*: 290 [(M+H)$^+$].

4-*tert*-Butyldimethylsilyloxymethyl-6-pivaloyloxyquinoline (15b). To a solution of **16** (240 mg, 0.84 mmol) in dry CH_2Cl_2 (5 mL) was added Et_3N (200 mg, 2.00 mmol) and pivaloyl chloride (240 mg, 2.00 mmol), followed by stirring at rt for 14 h. The reaction mixture was poured into H_2O (20 mL), extracted with AcOEt (50 mL × 2). The combined organic layers were washed with saturated $NaHCO_3$ solution, brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane:AcOEt = 3:1) to give **15b** (340 mg, quantitative) as a colorless oil. ^1H NMR ($CDCl_3$) δ 0.17 (6H, s, $SiMe_2$), 0.99 (9H, s, Si*t*-Bu), 1.42 (9H, s, C*t*-Bu), 5.16 (2H, s, $SiOC\underline{H}_2$), 7.41 (1H, dd, J = 9.2, 2.4 Hz, aromatic), 7.54 (1H, d, J = 2.9 Hz, aromatic), 7.57 (1H, d, J = 4.4 Hz, aromatic), 8.14 (1H, d, J = 9.2 Hz, aromatic), 8.89 (1H, d, J = 4.4 Hz, aromatic). MS (CI) *m/z*: 374 [(M+H)$^+$].

4-(1-*tert*-Butyldiphenylsilyloxypropyl)-2-trimethylsilylethynyl-1,2-dihydro-1-phenoxycarbonylquinoline (17). To a solution of trimethylsilylacetylene (0.685 mL, 4.85 mmol) in dry THF (25 mL) cooled to 0 °C was added portionwise ethylmagnesium bromide (1.62 mL of a 3.0 M solution in ether, 4.85 mmol). The solution was stirred at rt for 30 min and cooled to 0 °C again. To the resulting solution were added a solution of silyl ether **11** (1.05 g, 2.47 mmol) in dry THF (4.5 mL) over 10 min, and then a solution of phenyl chloroformate (0.80 mL, 6.40 mmol) in dry THF (0.80 mL) at 0 °C. After stirring for 40 min, the reaction mixture was quenched with saturated NH_4Cl solution, extracted with ether (× 3). The combined organic layers were washed with saturated $NaHCO_3$ solution, brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, ether:*n*-hexane = 1:10) to give **17** (1.58g, 92%, ca. 7:3 mixture of diastereomers). IR (KBr) ν_{max} 2963, 2171, 1725, 1377 cm^{-1}.^1H NMR ($CDCl_3$) δ [major isomer] 0.06 (9H, s, $SiMe_3$), 0.65 (3H, t, J = 8.0 Hz, $CH_2C\underline{H}_3$), 1.07 (9H, s, *t*-Bu), 1.75–1.95 (2H, m, $C\underline{H}_2CH_3$), 4.34 (1H, brt, J = 6.5 Hz, $C\underline{H}CH_2CH_3$), 5.73 (1H, d, J = 6.5 Hz, olefinic or propargylic), 5.83 (1H, d, J = 6.5 Hz, olefinic or propargylic), 7.02–7.94 (19H, m, aromatic). [minor isomer] 0.05 (9H, s, $SiMe_3$), 0.69 (3H, t, J = 8.0 Hz, $CH_2C\underline{H}_3$), 1.10 (9H, s, *t*-Bu), 1.75–1.95 (2H, m, $C\underline{H}_2CH_3$), 4.88 (1H, brt, J = 5.0 Hz, $C\underline{H}CH_2CH_3$), 5.99 (1H, d, J = 6.5 Hz, olefinic or propargylic), 6.31 (1H, brd, J = 6.5 Hz, olefinic or propargylic), 7.02–7.94 (19H, m, aromatic). MS (EI) *m/z*: 643 (M$^+$). Anal. calcd for $C_{40}H_{45}NO_3Si_2$: C, 74.61; H, 7.04; N, 2.18. Found: C, 74.65; H, 6.84; N, 2.07.

2-Ethynyl-1,2-dihydro-4-(1-hydroxypropyl)-1-phenoxycarbonylquinoline (18). To a solution of silyl ether **17** (6.55 g, 10.2 mmol) in THF (130 mL) and MeOH (0.83 mL, 20 mmol) cooled to 0 °C was added to *n*-Bu_4NF (5.1 mL of a 1.0 M solution in THF, 5.1 mmol). After stirring at 0 °C for 10 min, the reaction mixture was quenched with saturated NH_4Cl solution, extracted with AcOEt (× 3). The combined organic layers were washed with saturated $NaHCO_3$ solution, brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo to give the crude product (6.02 g). To a solution of crude product (6.02 g) in MeOH (180 mL) was added *p*TsOH·H_2O (4.85 g, 25.5 mmol), followed by refluxing for 15 h. The reaction mixture was cooled to rt and treated with pyridine (3.30 mL, 40.8 mmol), and then evaporated in vacuo. The residue was purified by column chromatography (silica gel, ether:*n*-hexane = 1:1) to give **18** (3.32 g, 98%, ca. 7:3 mixture of diastereomers). IR (KBr) ν_{max} 3286, 2965, 1717, 1489, 1382 cm^{-1}. ^1H NMR ($CDCl_3$) δ [major isomer] 1.03 (3H, t, J = 7.5 Hz, $CH_2C\underline{H}_3$), 1.74–2.01 (2H, m, $C\underline{H}_2CH_3$), 2.23 (1H, d, J = 2.5 Hz, C≡C$\underline{H}$), 4.59 (1H, m, $C\underline{H}CH_2CH_3$), 6.00 (1H, dd, J = 6.5, 2.5 Hz, propargylic), 6.17 (1H, dd, J = 6.5, 0.5 Hz, olefinic), 7.14–7.83 (9H, m, aromatic). [minor isomer] 0.99 (3H, t, J = 7.5 Hz, $CH_2C\underline{H}_3$), 1.49–1.68 (2H, m, $C\underline{H}_2CH_3$), 2.23 (1H, d, J = 2.5 Hz, C≡C$\underline{H}$), 4.83 (1H, m, $C\underline{H}CH_2CH_3$), 6.02 (1H, dd, J = 6.5, 2.5 Hz, propargylic), 6.26 (1H, dd, J = 6.5, 1.5 Hz, olefinic), 7.14–7.83 (9H, m, aromatic). MS (EI) *m/z*: 333 (M$^+$). HRMS for $C_{21}H_{19}NO_3$ (M$^+$) calcd 333.1364, found 333.1358.

3,4-Epoxy-2-ethynyl-1,2,3,4-tetrahydro-4-propionyl-1-phenoxycarbonylquinoline (19). To a solution of allyl alcohol **18** (4.44 g, 13.3 mmol) and anhydrous Na_2HPO_4 (5.67 g, 39.9 mmol) in CH_2Cl_2 (130 mL) cooled to 0 °C was added *m*CPBA (7.19 g, 33.3 mmol), followed by stirring at 0 °C for 3.5 h. The reaction mixture was diluted with CH_2Cl_2 (200 mL), washed with aqueous $Na_2S_2O_3$ and aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo to give the crude product (4.92 g). The crude product (4.92 g) was dissolved in dry CH_2Cl_2 (33 mL), dry DMSO (66 mL) and Et_3N (27.8 mL, 200 mmol), and the solution was cooled to 0 °C. To this solution was added portionwise SO_3·Py (31.8 g, 200 mmol) over 30 min. After stirring at rt for 1.5 h, the reaction mixture was quenched with saturated NH_4Cl solution, extracted with AcOEt (× 3). The combined organic layers were washed with brine,

dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, ether:*n*-hexane = 1:2 → 2:1) to give **19** (3.82 g, 83%) as a colorless solid. IR (KBr) ν_{max} 3255, 2975, 2122, 1714, 1324, 1199 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.18 (3H, t, *J* = 7.5 Hz, $CH_2\underline{CH_3}$), 2.27 (1H, d, *J* = 2.5 Hz, C≡C$\underline{H}$), 2.69 (2H, q, *J* = 7.5 Hz, $\underline{CH_2}CH_3$), 3.99 (1H, d, *J* = 3.0 Hz, epoxide), 5.97 (1H, dd, *J* = 3.0, 2.5 Hz, propargylic), 7.10–7.65 (9H, m, aromatic). MS (EI) *m/z*: 347 (M^+). Anal. calcd for $C_{21}H_{17}NO_4$: C, 72.61; H, 4.93; N, 4.03. Found: C, 72.57; H, 5.00; N, 4.04.

3,4-Epoxy-1,2,3,4-tetrahydro-1-phenoxycarbonyl-4-propionyl-2-((*Z*)-6-trimethylsilyl-3-hexen-1,5-diynyl)quinoline (21). A mixture of (*Z*)-1-chloro-4-trimethylsilyl-1-buten-3-yne (**20**) (283 mg, 1.52 mmol), $Pd(OAc)_2$ (3.4 mg, 0.015 mmol), PPh_3 (8.0 mg, 0.030 mmol) and CuI (2.9 mg, 0.015 mmol) in dry, degassed benzene (3 mL) was stirred under argon at 25 °C for 1 h. The resulting dark red solution was cooled to 0 °C, and the ketone **19** (101 mg, 0.291 mmol) in dry, degassed benzene (1 mL) and THF (0.2 mL) was added, followed by *n*-butylamine (0.060 mL, 0.61 mmol). The reaction mixture was stirred at rt for 2 h and quenched with saturated NH_4Cl solution, extracted with AcOEt (×3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane → ether:*n*-hexane = 1:10 → 1:3) to give **21** (71 mg, 53%) as a brown oil. IR (film) ν_{max} 2961, 1723, 1203, 846 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.29 (9H, s, $SiMe_3$), 1.17 (3H, t, *J* = 7.0 Hz, $CH_2\underline{CH_3}$), 2.68 (2H, q, *J* = 7.0 Hz, $\underline{CH_2}CH_3$), 4.01(1H, d, *J* = 3.0 Hz, epoxide), 5.69 (1H, dd, *J* = 11, 2.0 Hz, C$\underline{H}$=CHC≡CSi), 5.82 (1H, d, *J* = 11 Hz, CH=C$\underline{H}$C≡CSi), 6.22 (1H, dd, *J* = 3.0, 2.0 Hz, propargylic), 7.14 (2H, brd, *J* = 7.0 Hz, aromatic), 7.18–7.28 (2H, m, aromatic), 7.31–7.44 (3H, m, aromatic), 7.59 (2H, brt, *J* = 7.0 Hz, aromatic). MS (EI) *m/z*: 469 (M^+). HRMS for $C_{28}H_{27}NO_4Si$ (M^+) calcd 469.1709, found 469.1698.

3,4-Epoxy-1,2,3,4-tetrahydro-1-phenoxycarbonyl-4-propionyl-2-((*Z*)-3-hexen-1,5-diynyl)quinoline (22). To a solution of **21** (129 mg, 0.275 mmol) in THF (6 mL) and MeOH (0.056 mL, 1.4 mmol) cooled to −78 °C was added to *n*-Bu_4NF (0.141 mL of a 1.0 M solution of THF, 0.14 mmol). After stirring at −78 °C for 6 min, the reaction mixture was quenched with saturated NH_4Cl solution, extracted with $CHCl_2$ (×3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, ether:*n*-hexane = 1:1) to give **22** (103 mg, 94%) as a brown oil. ^1H NMR ($CDCl_3$) δ 1.17 (3H, t, *J* = 7.0 Hz, $CH_2\underline{CH_3}$), 2.69 (2H, q, *J* = 7.0 Hz, $\underline{CH_2}CH_3$), 3.20 (1H, d, *J* = 1.5 Hz, C≡C$\underline{H}$), 4.03 (1H, d, *J* = 3.0 Hz, epoxide), 5.77 (2H, m, olefinic), 6.17 (1H, dd, *J* = 3.0, 1.5 Hz, propargylic), 7.10–7.28 (3H, m, aromatic), 7.33–7.43 (4H, m, aromatic), 7.60 (2H, brt, *J* = 7.5 Hz, aromatic).

Phenyl(2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-ethyl-9-hydroxy-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate (4).**

Preparation from 21. A CsF powder (49.8 mg, 0.327 mmol) was placed in a dried three-necked flask and heated at 100 °C for 1 h in vacuo. After cooling to rt, dry THF (15 mL) was added. To this suspension were added a solution of **21** (103 mg, 0.218 mmol) in dry THF (2.5 mL) and a solution of 18-crown-6 (86.4 mg, 0.327 mmol) in dry THF (1 mL). After stirring for 2.5 h, the reaction mixture was quenched with saturated NH_4Cl solution, extracted with AcOEt (×3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by preparative TLC (silica gel, ether:*n*-hexane = 3:2) to give **4** (11 mg, 12%) as a brown oil.

Preparation from 22. A $CeCl_3 \cdot 7H_2O$ (84.7 mg, 0.227 mmol) was placed in a dried three-necked flask and heated at 110 °C for 2 h in vacuo. After cooling to 0 °C, dry THF (4.5 mL) was added and the mixture was stirred at 25 °C for 17 h. To this suspension was added a solution of **22** (86.9 mg, 0.219 mmol) in dry THF (2 mL) and then $KN(TMS)_2$ (1.05 mL of a 0.645 M in toluene, 0.69 mmol) was added at −78 °C. After stirring for 1 h, the reaction mixture was quenched with saturated NH_4Cl solution, extracted with AcOEt (×3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by preparative TLC (silica gel, ether:*n*-hexane = 1:1) to give **4** (11 mg, 12%) as a brown oil. IR (film) ν_{max} 3752, 1717, 1200 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.20 (3H, t, *J* = 7.5 Hz, $CH_2\underline{CH_3}$), 1.80–2.09 (2H, m, $\underline{CH_2}CH_3$), 4.17 (1H, d, *J* = 3.0 Hz, epoxide), 5.66 (1H, dd, *J* = 10, 2.0 Hz, NCHC≡CC$\underline{H}$=CH), 5.82 (1H, d, *J* = 10 Hz, NCHC≡CC$\underline{H}$=C$\underline{H}$), 5.89 (1H, dd, *J* = 3.0, 1.5 Hz, propargylic), 7.10–7.25 (4H, m, aromatic), 7.31–7.40 (3H, m, aromatic), 7.50 (1H, brd, *J* = 7.5 Hz, aromatic), 8.86 (1H, dd, *J* = 7.5, 1.5 Hz, aromatic). MS (EI) *m/z*: 397 (M^+). HRMS for $C_{25}H_{19}NO_4$ (M^+) calcd 397.1314, found 397.1302.

4-*tert*-Butyldimethylsilyloxymethyl-2-ethynyl-1,2-dihydro-6-methoxy-1-phenyloxycarbonylquinoline (23a). A solution of silyl ether **15a** (4.32 g, 14.2 mmol) in dry THF (100 mL) was cooled to −70 °C and treated with ethynylmagnesium bromide (42.7 mL of a 0.5M solution in THF, 21.4 mmol). The solution was briefly warmed to 0 °C and cooled to −70 °C again, and phenyl chloroformate (3.57 g, 22.8 mmol) was added, and then the reaction mixture was allowed to slowly warm to 0 °C. After stirring at 0 °C for 1 h, the reaction mixture was quenched with saturated NH_4Cl solution (50 mL), extracted with AcOEt (200 mL × 2). The combined organic layers were washed with saturated $NaHCO_3$ solution (50 mL), brine (50 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane:AcOEt = 4:1) to give **23a** (6.20 g, 97%) as a

colorless gum. ^{1}H NMR ($CDCl_3$) δ 0.13 (6H, s, $SiMe_2$), 0.95 (9H, s, *t*-Bu), 2.27 (1H, d, *J* = 2.4 Hz, C≡CH), 3.82 (3H, s, OMe), 4.54 (1H, d, *J* = 14.2 Hz, O-CHH), 4.70 (1H, dd, *J* = 14.2, 1.5 Hz, O-CHH), 6.00 (1H, m, N-CH), 6.21 (1H, d, *J* = 6.8 Hz, C=CH), 6.88 (1H, dd, *J* = 8.3, 2.4 Hz, aromatic), 6.95 (1H, d, *J* = 2.4 Hz, aromatic), 7.1–7.8 (6H, m, aromatic). MS (EI) *m/z*: 449 (M^+). HRMS for $C_{26}H_{31}NO_4Si$ (M^+) calcd 449.2022, found 449.2010.

4-*tert*-Butyldimethylsilyloxymethyl-2-ethynyl-1,2-dihydro-1-phenyloxycarbonyl-6-pivaloyloxyquinoline (23b). Prepared from **15b** (340 mg, 0.91 mmol) by a procedure similar to that described for **23a**. Yield: 470 mg (quantitative) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 0.13 (6H, s, $SiMe_2$), 0.95 (9H, s, Si*t*-Bu), 1.36 (9H, s, C*t*-Bu), 2.25 (1H, d, *J* = 2.4 Hz, C≡CH), 4.51 (1H, d, *J* = 14.2 Hz, O-CHH), 4.63 (1H, dd, *J* = 14.2, 1.5 Hz, O-CHH), 6.03 (1H, m, N-CH), 6.20 (1H, d, *J* = 6.8 Hz, C=CH), 7.0–7.5 (7H, m, aromatic), 7.76 (1H, m, aromatic). MS (EI) *m/z*: 519 (M^+). HRMS for $C_{30}H_{37}NO_5Si$ (M^+) calcd 519.2441, found 519.2455.

2-Ethynyl-1,2-dihydro-4-hydroxymethyl-6-methoxy-1-phenyloxycarbonylquinoline (24a). To a solution of allyl ether **23a** (6.20 g, 13.8 mmol) in MeOH (100 mL) and CH_2Cl_2 (30 mL) was added *p*TsOH·H_2O (1.31 g, 6.89 mmol), followed by stirring at 0 °C for 1.5 h. The reaction mixture was treated with pyridine (0.54 g, 18.1 mmol), and then evaporated in vacuo. The residue was dissolved in AcOEt (200 mL) and the solution was washed with water (100 mL), brine (50 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane:AcOEt = 2:1) to give **24a** (5.10 g, 98%) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ 2.25 (1H, d, *J* = 2.4 Hz, C≡CH), 3.82 (3H, s, OMe), 4.59 (2H, s, CH_2OH), 6.02 (1H, dd, *J* = 6.8, 2.4 Hz, N-CH), 6.21 (1H, d, *J* = 6.8 Hz, C=CH), 6.87 (1H, dd, *J* = 8.4, 2.4 Hz, aromatic), 6.94 (1H, d, *J* = 2.4 Hz, aromatic), 7.1–7.8 (6H, m, aromatic). MS (EI) *m/z*: 335 (M^+). HRMS for $C_{20}H_{17}NO_4$ (M^+) calcd 335.1157, found 335.1150.

2-Ethynyl-1,2-dihydro-4-hydroxymethyl-1-phenyloxycarbonyl-6-pivaloyloxyquinoline (24b). Prepared from **23b** (450 mg, 0.87 mmol) by a procedure similar to that described for **24a**. Yield: 340 mg (92%) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ 1.36 (9H, s, *t*-Bu), 2.26 (1H, d, *J* = 2.4 Hz, C≡CH), 4.57 (2H, s, CH_2OH), 6.03 (1H, dd, *J* = 6.8, 2.4 Hz, N-CH), 6.22 (1H, d, *J* = 6.8 Hz, C=CH), 7.0–7.4 (7H, m, aromatic), 7.78 (1H, m, aromatic). MS (EI) *m/z*: 405 (M^+). HRMS for $C_{24}H_{23}NO_5$ (M^+) calcd 405.1576, found 405.1588.

3,4-Epoxy-2-ethynyl-1,2,3,4-tetrahydro-4-hydroxymethyl-6-methoxy-1-phenyloxycarbonylquinoline (25a). To a solution of allyl alcohol **24a** (4.60 g, 13.8 mmol)and anhydrous Na_2HPO_4 (5.90 g, 41.4 mmol) in CH_2Cl_2 (100 mL) cooled to 0 °C was added mCPBA (5.10 g, 20.7 mmol), followed by stirring at 0 °C for 2 h. The reaction mixture was diluted with CH_2Cl_2 (200 mL), washed with aqueous $Na_2S_2O_3$ (100 mL) and aqueous $NaHCO_3$ (100 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane:ether = 1:1) to give **25a** (4.38 g, 91 %) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.94 (1H, dd, *J* = 10.3, 5.4 Hz, OH), 2.20 (1H, brs, C≡CH), 3.83 (3H, s, OMe), 4.08 (1H, d, *J* = 2.4 Hz, epoxide), 4.12 (1H, dd, *J* = 12.7, 10.3 Hz, CHHOH), 4.45 (1H, dd, *J* = 12.7, 5.4 Hz, CHHOH), 5.89 (1H, m, N-CH), 6.92 (1H, dd, *J* = 8.4, 2.4 Hz, aromatic), 7.1–7.7 (7H, m, aromatic). MS (EI) *m/z*: 351 (M^+). HRMS for $C_{20}H_{17}NO_5$ (M^+) calcd 351.1106, found 351.1119.

3,4-Epoxy-2-ethynyl-1,2,3,4-tetrahydro-4-hydroxymethyl-1-phenyloxycarbonyl-6-pivaloyloxyquinoline (25b). Prepared from **24b** (340 mg, 0.84 mmol) by a procedure similar to that described for **25a**. Yield: 320 mg (91%) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ 1.37 (9H, s, *t*-Bu), 2.22 (1H, brs, C≡CH), 4.02 (1H, d, *J* = 2.4 Hz, epoxide), 4.03 and 4.44 (each 1H, d, *J* = 12.7 Hz, CH_2OH), 5.91 (1H, m, N-CH), 7.0–7.4 (7H, m, aromatic) 7.53 (1H, m, aromatic). MS (EI) *m/z*: 421 (M^+). HRMS for $C_{24}H_{23}NO_6$ (M^+) calcd 421.1525, found 421.1511.

3,4-Epoxy-1,2,3,4-tetrahydro-4-hydroxymethyl-6-methoxy-1-phenyloxycarbonyl-2-((*Z*)-6-trimethylsilyl-3-hexen-1,5-diynyl)quinoline (26a). A mixture of (*Z*)-1-chloro-4-trimethylsilyl-1-buten-3-yne (**20**)[4] (3.97 g, 25.0 mmol), $Pd_2(dba)_3{\cdot}CHCl_3$[26] (160 mg, 0.16 mmol), PPh_3 (165 mg, 0.62 mmol) and CuI (120 mg, 0.62 mmol) in dry, degassed THF (100 mL) was stirred under argon at 25 °C for 1 h. The resulting dark red solution was cooled to 0 °C, and the epoxy alcohol **25a** (2.20 g, 6.25 mmol) in dry, degassed THF (50 mL) was added, followed by *n*-butylamine (1.83 g, 25.0 mmol). The reaction mixture was stirred at 25 °C for 2 h and quenched with saturated NH_4Cl solution (50 mL), extracted with AcOEt (150 mL × 2). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane:ether = 1:1) to give **26a** (1.1 g, 38%) as a pale brown oil. ^{1}H NMR ($CDCl_3$) δ 0.22 (9H, s, $SiMe_3$), 1.95 (1H, dd, *J* = 7.8, 5.4 Hz, OH), 3.84 (3H, s, OMe), 4.12 (1H, d, *J* = 2.9 Hz, epoxide), 4.14 (1H, dd, *J* = 12.2, 7.8 Hz, CHHOH), 4.50 (1H, dd, *J* = 12.2, 5.4 Hz, CHHOH), 5.66 (1H, dd, *J* = 11.2, 2.0 Hz, CH=CHC≡CSi), 5.83 (1H, d, *J* = 11.2 Hz, CH=CHC≡CSi), 6.11(1H, m, N-CH), 6.92(1H, dd, *J* = 8.4, 2.4 Hz, aromatic), 7.1–7.7 (7H, m, aromatic). MS (EI) *m/z*: 473 (M^+). HRMS for $C_{27}H_{27}NO_5Si$ (M^+) calcd 473.1658, found 473.1669.

3,4-Epoxy-1,2,3,4-tetrahydro-4-hydroxymethyl-1-phenyloxycarbonyl-6-pivaloyloxy-2-((*Z*)-6-trimethylsilyl-3-hexen-1, 5-diynyl)quinoline (26b). Prepared from **25b** (300 mg, 0.71 mmol) by a procedure similar to that described for **26a**. Yield: 150 mg (39%) as a pale brown oil. ^{1}H NMR ($CDCl_3$) δ 0.22 (9H, s, $SiMe_3$), 1.56 (9H, s, *t*-Bu), 3.84 (2H, s, CH_2OH), 4.10 (1H, d, *J* = 2.9 Hz, epoxide), 5.69 (1H, dd, *J* = 11.2, 2.0 Hz, CH=CHC≡CSi), 5.81 (1H, d, *J* = 11.2 Hz,

CH=CHC≡CSi), 6.12 (1H, m, N-CH), 7.1–7.5 (8H, m, aromatic). MS (EI) *m/z*: 543 (M^+). HRMS for $C_{31}H_{33}NO_6Si$ (M^+) calcd 543.2077, found 543.2066.

3,4-Epoxy-4-formyl-6-methoxy-1,2,3,4-tetrahydro-1-phenyloxycarbonyl-2-((*Z*)-6-trimethylsilyl-3-hexen-1,5-diynyl)quinoline (27a). To a solution of **26a** (700 mg, 1.48 mmol) and pyridine (467 mg, 5.92 mmol) in dry CH_2Cl_2 (25 mL) cooled to 0 °C was added portionwise a solution of Dess–Martin periodinane[10] (940 mg, 2.22 mmol) in dry CH_2Cl (10 mL) over 30 min. After stirring at 23 °C for 2 h, the reaction mixture was diluted with ether (150 mL), washed with aqueous $Na_2S_2O_3$ (20 mL) and aqueous $NaHCO_3$ (20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane:ether = 1:1) to give **27a** (420 mg, 60%) as a pale yellow foam. ^1H NMR ($CDCl_3$) δ 0.22 (9H, s, $SiMe_3$), 3.84 (3H, s, OMe), 4.18 (1H, d, *J* = 2.4 Hz, epoxide), 5.66 (1H, dd, *J* = 11.2, 2.0 Hz, CH=CHC≡CSi), 5.83 (1H, d, *J* = 11.2 Hz, CH=CHC≡CSi), 6.20 (1H, m, N-CH), 6.9–7.8 (8H, m, aromatic), 9.22 (1H, s, CHO). MS (EI) *m/z*: 471 (M^+). HRMS for $C_{27}H_{25}NO_5Si$ (M^+) calcd 471.1502, found 471.1513.

3,4-Epoxy-4-formyl-1,2,3,4-tetrahydro-1-phenyloxycarbonyl-6-pivaloyloxy-2((*Z*)-6-trimethylsilyl-3-hexen-1,5-diynyl)quinoline (27b). Prepared from **26b** (120 mg, 0.22 mmol) by a procedure similar to that described for **27a**. Yield: 75 mg (63%) as a pale yellow foam. ^1H NMR ($CDCl_3$) δ 0.22 (9H, s, $SiMe_3$), 1.36 (9H, s, *t*-Bu), 4.23 (1H, d, *J* = 2.4 Hz, epoxide), 5.67 (1H, dd, *J* = 11.2, 2.0 Hz, CH=CHC≡CSi), 5.84 (1H, d, *J* = 11.2 Hz, CH=CHC≡CSi), 6.31 (1H, m, N-CH), 7.1–7.8 (8H, m, aromatic), 9.20 (1H, s, CHO). MS (EI) *m/z*: 541 M^+). HRMS for $C_{31}H_{31}NO_6Si$ (M^+) calcd 541.1920, found 541.1901.

Mixture of phenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-acetoxy-12-methoxy-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (7a).** A CsF powder (130 mg, 0.85 mmol) was placed in a dried three-necked flask and heated at 100 °C for 1 h in vacuo. After cooling to rt, dry CH_3CN (70 mL) was added, followed by Ac_2O (250 mg, 2.40 mmol). To this suspension were added the aldehyde **27a** (400 mg, 0.85 mmol) in dry CH_3CN (30 mL) at 25 °C over 30 min. After stirring at rt for 2 h, the reaction mixture was filtered and the filtrate was evaporated in vacuo. The resulting residue was dissolved with AcOEt (100 mL), and the solution was washed with saturated NH_4Cl solution (20 mL), water (20 mL), brine (20 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane:AcOEt = 5:1) to give **7a** (160 mg, 43%, ca. 2:1 mixture of diastereomers) as a colorless foam. ^1H NMR ($CDCl_3$) δ [(9*S**)-isomer] 2.26 (3H, s, OAc), 3.84 (3H, s, OMe), 3.95 (1H, d, *J* = 2.4 Hz, epoxide), 5.59 (1H, s, propargylic), 5.77 (1H, dd, *J* = 10.2, 1.5 Hz, NCHC≡CCH=CH), 5.86 (1H, d, *J* = 10.2 Hz, NCHC≡CCH=CH), 5.98 (1H, m, N-CH), 6.93 (1H, dd, *J* = 8.4, 2.4 Hz, aromatic), 7.1–7.5 (7H, m, aromatic). [(9*R**)-isomer] 2.17 (3H, s, OAc), 3.84 (3H, s, OMe), 4.27 (1H, d, *J* = 2.4 Hz, epoxide), 5.83 (2H, s, C≡CCH=CH), 5.93 (1H, m, N-CH), 6.41 (1H, s, propargylic), 6.93 (1H, dd, *J* = 8.4, 2.4 Hz, aromatic), 7.1–7.5 (7H, m, aromatic). MS (EI) *m/z*: 441 (M^+). Anal. calcd for $C_{26}H_{19}NO_6$: C, 70.74; H, 4.34; N, 3.17. Found: C, 70.54; H, 4.49; N, 3.03.

Mixture of phenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-acetoxy-12-pivaloyloxy-10,2,10-(epoxymetheno)-1-benz[*b*]-azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (7b).** Prepared from **27b** (60 mg, 0.11 mmol) by a procedure similar to that described for **7a**. Yield: 20 mg (35%, ca. 2:1 mixture of diastereomers) as a colorless foam. ^1H NMR ($CDCl_3$) δ [(9*S**)-isomer] 1.37 (9H, s, *t*-Bu), 2.24 (3H, s, OAc), 3.98 (1H, d, *J* = 2.4 Hz, epoxide), 5.58 (1H, s, propargylic), 5.77 (1H, dd, *J* = 10.2, 1.5 Hz, NCHC≡CCH=CH), 5.88 (1H, d, *J* = 10.2 Hz, NCHC≡CCH=CH), 6.01 (1H, m, N-CH), 7.1–7.5 (6H, m, aromatic), 7.99 (1H, d, *J* = 2.4 Hz, aromatic). [(9*R**)-isomer] 1.37 (9H, s, *t*-Bu), 2.17 (3H, s, OAc), 4.31 (1H, d, *J* = 2.4 Hz, epoxide), 5.65 (2H, s, C≡CCH=CH), 5.96 (1H, m, N-CH), 6.37 (1H, s, propargylic), 7.1–7.5 (6H, m, aromatic), 7.99 (1H, d, *J* = 2.4 Hz, aromatic). MS (EI) *m/z*: 511 (M^+). Anal. calcd for $C_{30}H_{25}NO_7$: C, 70.44; H, 4.93; N, 2.74. Found: C, 70.20; H, 5.07; N, 2.66.

Mixture of phenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-hydroxy-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (5b).** To a solution of **2b**[1] (250 mg, 0.61 mmol) in MeOH (10 mL) was added a solution of $Ba(OH)_2 \cdot 8H_2O$ (95 mg, 0.30 mmol) in MeOH (5 mL), followed by stirring at 0 °C for 10 min. The reaction mixture was quenched with saturated NH_4Cl solution, extracted with ether (50 mL × 2). The combined organic layers were washed with water, brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (silica gel, CH_2Cl_2) to give **5b** (170 mg, 75%, ca. 2:1 mixture of diastereomers) as a colorless foam. ^1H NMR ($CDCl_3$) δ [(9*S**)-isomer] 2.97 (1H, brs, OH), 3.84 (1H, d, *J* = 2.9 Hz, epoxide), 4.77 (1H, s, propargylic), 5.70 (1H, d, *J* = 10.2 Hz, NCHC≡CCH=CH), 5.85 (1H, d, *J* = 10.2 Hz, NCHC≡CCH=CH), 5.98 (1H, m, N-CH), 7.1–7.6 (8H, m, aromatic), 8.55 (1H, dd, *J* = 7.8, 1.5 Hz, aromatic). [(9*R**)-isomer] 2.42 (1H, brs, OH), 4.47 (1H, d, *J* = 2.4 Hz, epoxide), 5.48 (1H, s, propargylic), 5.78 (2H, m, C≡CCH=CH), 5.98 (1H, m, N-CH), 7.1–7.6 (9H, m, aromatic). MS (FAB) *m/z*: 370 [(M+H)]. HRMS for $C_{23}H_{16}NO_4$ (M+H) calcd 370.1079, found 370.1099.

Mixture of 4-chlorophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(imidazole-1-thiocarbonyloxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (28a).** To a solution of **2a**[1] (1.50 g, 3.37 mmol) in MeOH (25 mL) and CH_2Cl_2 (50 mL) was added a solution of $Ba(OH)_2 \cdot 8H_2O$ (0.54 g, 1.69 mmol) in MeOH (25 mL), followed by stirring at 0

°C for 10 min. The reaction mixture was quenched with saturated NH_4Cl solution, extracted with ether (50 mL × 3). The combined organic layers were washed with water, brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo to give the crude **5a**. To a solution of **5a** in dry CH_2Cl_2 (40 mL) was added DMAP (206 mg, 1.69 mmol) and 1,1′-thiocarbonyldiimidazole (1.08 g, 10.0 mmol), followed by stirring at 0 °C for 1 h. The reaction mixture was evaporated in vacuo and the residue was purified by column chromatography (silica gel, CH_2Cl_2) to give **28a** (1.00 g, 58%, ca. 2:1 mixture of diastereomers) as a yellow foam. ^{1}H NMR ($CDCl_3$) δ [(9*S**)-isomer] 4.15 (1H, d, *J* = 2.9 Hz, epoxide), 5.79 (1H, d, *J* = 10.2 Hz, NCHC≡CCH=CH), 5.89 (1H, d, *J* = 10.2 Hz, NCHC≡CCH=CH), 6.02 (1H, s, propargylic), 6.03 (1H, m, N-CH), 7.0–7.8 (7H, m, aromatic and imidazole), 8.07 (1H, dd, *J* = 7.8, 1.5 Hz, aromatic), 8.34 (2H, s, imidazole), 8.44 (1H, s, aromatic). [(9*R**)-isomer] 4.33 (1H, d, *J* = 2.9 Hz, epoxide), 5.87 (2H, m, C≡CCH=CH), 5.89 (1H, s, propargylic), 5.98 (^{1}H, m, N-CH), 7.0–8.1 (8H, m, aromatic and imidazole), 8.34 (2H, s, imidazole), 8.44 (1H, s, aromatic). MS (FAB) *m/z*: 514 (M+H; ^{35}Cl), 516 (M+H; ^{37}Cl).

Mixture of phenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(imidazole-1-thiocarbonyloxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (28b).** To a solution of **5b** (120 mg, 0.33 mmol) in dry CH_2Cl_2 (20 mL) was added DMAP (20 mg, 0.17 mmol) and 1,1′-thiocarbonyldiimidazole (200 mg, 1.00 mmol), followed by stirring at 0 °C for 1 h. The reaction mixture was evaporated in vacuo and the residue was purified by column chromatography (silica gel, CH_2Cl_2) to give **28b** (100 mg, 64%, ca. 2:1 mixture of diastereomers) as a yellow foam. ^{1}H NMR ($CDCl_3$) δ [(9*S**)-isomer] 4.12 (1H, d, *J*=2.9 Hz, epoxide), 5.80 (1H, dd, *J* = 10.2, 1.5 Hz, NCHC≡CCH=CH), 5.89 (1H, d, *J* = 10.2 Hz, NCHC≡CCH=CH), 6.01 (1H, s, propargylic), 6.07 (1H, m, N-CH), 7.0–8.1 (10H, m, aromatic and imidazole), 8.22 (1H, dd, *J* = 7.8, 1.5 Hz, aromatic), 8.49 (1H, d, *J* = 1.5 Hz, imidazole). [(9*R**)-isomer] 4.33 (1H, d, *J* = 2.9 Hz, epoxide), 5.87 (2H, m, C≡CCH=CH), 5.89 (1H, s, propargylic), 5.98 (1H, m, N-CH), 7.0–8.1 (10H, m, aromatic and imidazole), 8.22 (1H, dd, *J* = 7.8, 1.5 Hz, aromatic), 8.49 (1H, d, *J* = 1.5 Hz, imidazole). MS (FAB) *m/z*: 480 (M+H).

4-Chlorophenyl (2*R,5*Z*,10*S**,16*R**)-(±)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate (6a).** To a solution of **28a** (1.00 g, 1.95 mmol) in dry benzene (40 mL) was added *n*-Bu_3SnH (1.14 g, 3.90 mmol) and AIBN (64 mg, 0.39 mmol), and then the mixture was heated at 80 °C for 1.5 h. The reaction mixture was evaporated in vacuo and the residue was purified by column chromatography (silica gel, *n*-hexane:CH_2Cl_2 = 1:2) to give **6a** (390 mg, 52%) as a colorless solid. mp 80–82 °C (dec). IR (KBr) ν_{max} 3090, 1721, 1651, 1489, 1379, 1209 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.52 (1H, d, *J* = 17.6 Hz, propargylic), 3.71 (1H, d, *J* = 17.6 Hz, propargylic), 4.00 (1H, d, *J* = 2.4 Hz, epoxide), 5.69 (1H, d, *J* = 9.8 Hz, NCHC≡CCH=CH), 5.81 (1H, d, *J* = 9.8 Hz, NCHC≡CCH=CH), 5.94 (1H, t, *J* = 2.4 Hz, N-CH), 7.1–7.5 (6H, m, aromatic), 7.53 (1H, brd, *J* = 7.8 Hz, aromatic), 7.62 (1H, dd, *J* = 7.8, 1.5 Hz, aromatic). ^{13}C NMR ($CDCl_3$) δ 25.2, 26.1, 45.9, 57.2, 67.1, 68.2, 87.8, 91.1, 92.0, 98.2, 119.1, 121.9, 122.9, 125.8, 126.5, 126.6, 127.4, 129.0, 129.4, 131.2, 134.9, 149.4. MS (EI) *m/z*: 487 (M$^+$; ^{35}Cl), 489 (M$^+$; ^{37}Cl). Anal. calcd for $C_{23}H_{14}ClNO_3$:C, 71.23; H, 3.64; N, 3.61. Found: C, 71.49; H, 3.74; N, 3.55.

Phenyl (2*R,5*Z*,10*S**,16*R**)-(±)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate (6b).** To a solution of **28b** (90 mg, 0.19 mmol) in dry benzene (10 mL) was added *n*-Bu_3SnH (120 mg, 0.40 mmol) and Et_3B (0.21 mL of a 1.0 M solution in *n*-hexane, 0.21 mmol), and then the mixture was stirred at 24 °C for 1 h. The reaction mixture was evaporated in vacuo and the residue was purified by preparative TLC (silica gel, *n*-hexane:CH_2Cl_2 = 1:2) to give **6b** (32 mg, 48%) as a colorless solid. mp 67–70 °C (dec). IR (KBr) ν_{max} 2928, 1726, 1604, 1496, 1379, 1190 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.52 (1H, d, *J* = 17.6 Hz, propargylic), 3.72 (1H, d, *J* = 17.6 Hz, propargylic), 4.01 (1H, d, *J* = 2.4 Hz, epoxide), 5.70 (1H, d, *J* = 9.8 Hz, NCHC≡CCH=CH), 5.81 (1H, d, *J* = 9.8 Hz, NCHC≡CCH=CH), 5.98 (1H, t, *J* = 2.4Hz, N-CH), 7.1–7.4 (7H, m, aromatic), 7.58 (1H, brd, *J* = 7.8Hz, aromatic), 7.62 (1H, dd, *J* = 7.8, 1.5 Hz, aromatic). ^{13}C NMR ($CDCl_3$) δ 26.1, 45.8, 57.2, 67.3, 87.7, 91.0, 92.2, 98.2, 121.5, 121.9, 125.7, 125.8, 126.3, 126.6, 127.3, 129.0, 129.4, 135.1, 150.9. MS (EI) *m/z*: 353 (M$^+$). Anal. calcd for $C_{23}H_{15}NO_3$: C, 78.18; H, 4.28; N, 3.96. Found: C, 78.45; H, 4.47; N, 3.80.

Phenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-benzoyloxy-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate [(9*S**)-8a] and (9*R**)-8a-isomer. Representative procedure.** To a solution of **5b** (74 mg, 0.20 mmol) in dry CH_2Cl_2 (10 mL) was added DMAP (30 mg, 0.24 mmol) and benzoyl chloride (34 mg, 0.24 mmol), followed by stirring at 0 °C for 1 h. The reaction mixture was evaporated in vacuo and the residue was purified by preparative TLC (silica gel, CH_2Cl_2/ether = 20:1) to give (9*S**)-**8a** (34 mg, 44%) and (9*R**)-**8a** (20 mg, 26%) as a colorless solid, respectively. (9*S**)-**8a**: mp 91–94 °C (dec). IR (KBr) ν_{max} 2928, 1730, 1603, 1493, 1377 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 4.08 (1H, d, *J* = 2.9 Hz, epoxide), 5.76 (1H, dd, *J* = 10.2, 1.5 Hz, NCHC≡CCH=CH), 5.78 (1H, s, propargylic), 5.87 (1H, d, *J* = 10.2 Hz, NCHC≡CCH=CH), 5.99 (1H, m, N-CH), 7.1–7.6 (11H, m, aromatic), 8.16 (2H, m, aromatic), 8.38 (1H, dd, *J* = 8.3, 1.5 Hz, aromatic). MS (FAB) *m/z*: 474 (M+H). Anal. calcd for $C_{30}H_{19}NO_5$: C, 76.10; H, 4.04; N, 2.96. Found: C, 76.24; H, 4.31; N, 2.78. (9*R**)-**8a**: mp 99–101 °C (dec). IR (KBr) ν_{max} 2928, 1730, 1603, 1493, 1377 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 4.44 (1H, d, *J* = 2.9 Hz, epoxide), 5.86 (2H, s, C≡CCH=CH), 5.98 (1H, d, *J* = 2.9 Hz, N-CH), 6.70 (1H, s, propargylic), 7.1–7.6 (11H, m, aromatic), 7.76 (1H, dd, *J* = 8.3, 1.5 Hz, aromatic), 8.16 (2H, m, aromatic). MS (FAB) *m/z*: 474 (M+H). Anal. calcd for $C_{30}H_{19}NO_5$: C, 76.10; H, 4.04; N, 2.96. Found: C, 76.36; H, 4.25; N, 2.88.

The following compounds were prepared by a procedure similar to that described for (9*S**)-**8a** and (9*R**)-**8a**.

Phenyl (2*R,5*Z*,9*S**,10*S**,16R*)-(±)-9-(1-naphthoyl)-oxy-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate [(9*S**)-8b] and (9*R**)-8b isomer.** Yield: (9*S**)-**8b** (38 mg, 44%) and (9*R**)-**8b** (20 mg, 23%) as a colorless solid, respectively. (9*S**)-**8b**: mp 104–106 °C (dec). IR (KBr) ν_{max} 2928, 1724, 1508, 1491, 1377 cm^{-1}. ^1H NMR ($CDCl_3$) δ 4.13 (1H, d, *J* = 2.9 Hz, epoxide), 5.77 (1H, dd, *J* = 10.2, 1.5 Hz, NCHC≡CCH=CH), 5.89 (1H, s, propargylic), 5.89 (1H, d, *J* = 10.2 Hz, NCHC≡CCH=CH), 6.07 (1H, m, N-CH), 7.1–7.9 (12H, m, aromatic), 8.10 (2H, d, *J* = 7.8 Hz, aromatic), 8.38 (2H, m, aromatic), 9.06 (1H, d, *J* = 8.3 Hz, aromatic). MS (FAB) *m/z*: 524 (M+H). Anal. calcd for $C_{34}H_{21}NO_5$: C, 78.00; H, 4.04; N, 2.68. Found: C, 77.85; H, 4.32; N, 2.55. (9*R**)-**8b**: mp 95–97 °C (dec). IR (KBr) ν_{max} 2955, 1722, 1510, 1493, 1377 cm^{-1}. ^1H NMR ($CDCl_3$) δ 4.46 (1H, d, *J* = 2.9 Hz, epoxide), 5.86 (2H, s, C≡CCH=CH), 5.99 (1H, m, N-CH), 6.81 (1H, s, propargylic), 7.1–7.6 (7H, m, aromatic), 7.8–7.9 (3H, m, aromatic), 8.02 (1H, d, *J* = 8.3 Hz, aromatic), 8.07 (1H, d, *J* = 8.3 Hz, aromatic), 8.19 (1H, dd, *J* = 7.3, 1.5 Hz, aromatic), 8.28 (1H, dd, *J* = 7.3, 1.5 Hz, aromatic), 8.93 (2H, m, aromatic). MS (FAB) *m/z* 524 (M+H). Anal. calcd for $C_{34}H_{21}NO_5$: C, 78.00; H, 4.04; N, 2.68. Found: C, 78.15; H, 4.23; N, 2.59.

Phenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(2-pyrazinecarb-oxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate [(9*S**)-8c] and (9*R**)-8c isomer.** Yield: (9*S**)-**8c** (40 mg, 40%) and (9*R**)-**8c** (20 mg, 20%) as a colorless solid, respectively. (9*S**)-**8c**: mp 103–105 °C (dec). ^1H NMR ($CDCl_3$) δ 4.10 (1H, d, *J* = 2.9 Hz, epoxide), 5.84 (1H, dd, *J* = 10.2, 1.5 Hz, NCHC≡CCH=CH), 5.91 (1H, d, *J* = 10.2 Hz, NCHC≡CCH=CH), 5.92 (1H, s, propargylic), 6.06 (1H, m, N-CH), 7.1–7.6 (8H, m, aromatic), 8.44 (1H, dd, *J* = 8.3, 1.5 Hz, aromatic), 8.82 (2H, m, aromatic), 9.42 (1H, d, *J* = 1.5 Hz, aromatic). MS (EI) *m/z*: 475 (M^+). Anal. calcd for $C_{28}H_{17}N_3O_5$: C, 70.73; H, 3.60; N, 8.84. Found: C, 70.56; H, 3.86; N, 8.71. (9*R**)-**8c**: mp 96–98 °C (dec). ^1H NMR ($CDCl_3$) δ 4.49 (1H, d, *J* = 2.9 Hz, epoxide), 5.87 (2H, s, C≡CCH=CH), 5.98 (1H, m, N-CH), 6.80 (1H, s, propargylic), 7.1–7.6 (8H, m, aromatic), 7.76 (1H, dd, *J* = 7.8, 1.5 Hz, aromatic), 8.28 (2H, m, aromatic), 9.36 (1H, d, *J* = 1.5 Hz, aromatic). MS (EI) *m/z*: 475 (M^+). Anal. calcd for $C_{28}H_{17}N_3O_5$ C, 70.73; H, 3.60; N, 8.84. Found: C, 70.59; H, 3.89; N, 8.76.

Mixture of phenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(2-quinoxaloyl)oxy-10,2,10-(epoxymetheno)-1-benz[*b*]aza-cyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (8d).** Yield: 28 mg (36%, ca. 2:1 mixture of diastereomers) as a colorless solid. ^1H NMR ($CDCl_3$) δ [(9*S**)-isomer] 4.13 (1H, d, *J* = 2.9 Hz, epoxide), 5.77 (1H, dd, *J* = 10.2, 1.5 Hz, NCHC≡CCH=CH), 5.89 (1H, d, *J* = 10.2 Hz, NCHC≡CCH=CH), 5.89 (1H, s, propargylic), 6.08 (1H, m, N-CH), 7.1–7.6 (9H, m, aromatic), 7.94 (2H, m, aromatic), 8.23 (1H, m, aromatic), 8.35 (1H, m, aromatic), 9.56 (1H, s, aromatic). [(9*R**)-isomer] 4.58 (1H, d, *J* = 2.9 Hz, epoxide), 5.89 (2H, s, C≡CCH=CH), 5.99 (1H, m, N-CH), 6.86 (1H, s, propargylic), 7.1–7.6 (9H, m, aromatic), 7.94 (2H, m, aromatic), 8.23 (1H, m, aromatic), 8.35 (1H, m, aromatic), 9.68 (1H, s, aromatic). MS (FAB) *m/z*: 526 (M+H). Anal. calcd for $C_{32}H_{19}N_3O_5$: C, 73.14; H, 3.64; N, 8.00. Found: C, 73.00; H, 3.90; N, 7.85.

4-Chlorophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-picolinoyl-oxy-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate [(9*S**)-8e] and (9*R**)-8e isomer.** Yield: (9*S**)-**8e** (26 mg, 23%) and (9*R**)-**8e** (28 mg, 25%) as a colorless solid, respectively. (9*S**)-**8e**: mp 120–123 °C (dec). IR (KBr) ν_{max} 2928, 1732, 1583, 1489, 1379 cm^{-1}. ^1H NMR ($CDCl_3$) δ 4.08 (1H, d, *J* = 2.9 Hz, epoxide), 5.76 (1H, dd, *J* = 10.2, 1.5 Hz, NCHC≡CCH=CH), 5.87 (1H, d, *J* = 10.2 Hz, NCHC≡CCH=CH), 5.87 (1H, s, propargylic), 6.02 (1H, m, N-CH), 7.07 (2H, d, *J* = 8.8 Hz, aromatic), 7.2–7.6 (6H, m, aromatic), 7.89 (1H, td, *J* = 7.3, 1.5 Hz, aromatic), 8.21 (1H, d, *J* = 7.8 Hz, aromatic), 8.49 (1H, dd, *J* = 7.8, 1.5 Hz, aromatic), 8.84 (1H, m, aromatic). MS (FAB) *m/z*: 509 (M+H, ^{35}Cl), 511 (M+H, ^{37}Cl). Anal. calcd for $C_{29}H_{17}ClN_2O_5$: C, 68.44; H, 3.37; N, 5.50. Found: C, 68.21; H, 3.59; N, 5.31. (9*R**)-**8e**: mp 129–131 °C (dec). IR (KBr) νmax 2928, 1732, 1585, 1489, 1379 cm^{-1}. ^1H NMR ($CDCl_3$) δ 4.51 (1H, d, *J* = 2.9 Hz, epoxide), 5.85 (2H, s, C≡ CCH=CH), 5.94 (1H, d, *J* = 2.9 Hz, N-CH), 6.77 (1H, s, propargylic), 7.05(2H, d, *J* = 8.8 Hz, aromatic), 7.2–7.6 (4H, m, aromatic), 7.77 (1H, dd, *J* = 7.8, 1.5 Hz, aromatic), 7.88 (1H, td, *J* = 7.3, 1.5 Hz, aromatic), 8.18 (1H, dd, *J* = 8.8, 1.0 Hz, aromatic), 8.82 (1H, dd, *J* = 6.3, 1.0 Hz, aromatic). MS (FAB) *m/z*: 509 (M+H, ^{35}Cl), 511 (M+H, ^{37}Cl). Anal. calcd for $C_{29}H_{17}ClN_2O_5$: C, 68.44; H, 3.37; N, 5.50. Found: C, 68.35; H, 3.55; N, 5.36.

4-Chlorophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(2-furoyl)-oxy-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate [(9*S**)-8f] and (9*R**)-8f isomer.** Yield: (9*S**)-**8f** (32 mg, 57%) and (9*R**)-**8f** (18 mg, 32%) as a colorless solid, respectively. (9*S**)-**8f**: mp 110–112 °C (dec). IR (KBr) ν_{max} 2930, 1732, 1577, 1489, 1471, 1381 cm^{-1}. ^1H NMR ($CDCl_3$) δ 4.05 (1H, d, *J* = 2.9 Hz, epoxide), 5.76 (1H, dd, *J* = 10.2, 1.5 Hz, NCHC≡CCH=CH), 5.79 (1H, s, propargylic), 5.87 (1H, d, *J* = 10.2 Hz, NCHC≡CCH=CH), 6.01 (1H, m, N-CH), 6.58 (1H, dd, *J* = 3.4, 1.5 Hz, aromatic), 7.08 (2H, d, *J* = 8.8 Hz, aromatic), 7.2–7.4 (4H, m, aromatic), 7.42 (1H, td, *J* = 7.3, 1.5 Hz, aromatic), 7.52 (1H, m, aromatic), 7.67 (1H, m, aromatic), 8.37 (1H, dd, *J* = 7.8, 1.5 Hz, aromatic). MS (EI) *m/z*: 497 (M^+, ^{35}Cl), 499 (M^+, ^{37}Cl). Anal. calcd for $C_{28}H_{16}ClNO_6$: C, 67.55; H, 3.24; N, 2.81. Found: C, 67.41; H, 3.40; N, 2.71. (9*R**)-**8f**: mp 108–110 °C (dec). IR (KBr) ν_{max} 2930, 1732, 1577, 1489, 1471, 1381 cm^{-1}. ^1H NMR($CDCl_3$) δ 4.42 (1H, d, *J* = 2.9 Hz, epoxide), 5.85 (2H, s, C≡CCH=CH), 5.94 (1H, d, *J* = 2.9 Hz, N-CH), 6.56 (1H, dd, *J* = 3.4, 1.5 Hz, aromatic), 6.67 (1H, s, propargylic), 7.05 (2H, d, *J* = 8.8 Hz, aromatic), 7.2–

7.4 (4H, m, aromatic), 7.40 (1H, td, J = 7.3, 1.5 Hz, aromatic), 7.53 (1H, m, aromatic), 7.63 (1H, m, aromatic), 7.70 (1H, dd, J = 7.8, 1.5 Hz, aromatic). MS (EI) m/z: 497 (M^+, ^{35}Cl), 499 (M^+, ^{37}Cl). Anal. calcd for $C_{28}H_{16}ClNO_6$: C, 67.55; H, 3.24; N, 2.81. Found: C, 67.39; H, 3.44; N, 2.67.

4-Chlorophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(*N*-methylpyrrole-2-carboxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate [(9*S**)-8g] and (9*R**)-8g isomer.** Yield: (9*S**)-**8g** (24 mg, 24%) and (9*R**)-**8g** (10 mg, 10%) as a colorless solid, respectively. (9*S**)-**8g**: mp 95–97 °C (dec). IR (KBr) ν_{max} 2930, 1718, 1527, 1487, 1410, 1381 cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.98 (3H, s, NMe), 4.05 (1H, d, J = 2.9 Hz, epoxide), 5.69 (1H, s, propargylic), 5.74 (1H, dd, J = 10.2, 1.5 Hz, NCHC≡CCH=CH), 5.87 (1H, d, J = 10.2 Hz, NCHC≡CCH=CH), 6.00 (1H, m, N-CH), 6.18 (1H, dd, J = 3.9, 2.4 Hz, aromatic), 6.88 (1H, t, J = 2.4 Hz, aromatic), 7.0–7.2 (3H, m, aromatic), 7.3–7.4 (3H, m, aromatic), 7.41 (1H, td, J = 7.3, 1.5 Hz, aromatic), 7.51 (1H, m, aromatic), 8.38 (1H, dd, J = 7.8, 1.5 Hz, aromatic). MS (FAB) m/z: 511 (M+H, ^{35}Cl), 513 (M+H, Cl). Anal. calcd for $C_{29}H_{19}ClN_2O_5$: C, 68.17; H, 3.75; N, 5.48. Found: C, 68.01; H, 3.98; N, 5.25. (9*R**)-**8g**: mp 91–93 °C (dec). IR (KBr) ν_{max} 2930, 1718, 1527, 1487, 1410, 1381 cm^{-1}. ^1H NMR ($CDCl_3$) δ 4.42 (1H, d, J = 2.9 Hz, epoxide), 5.85 (2H, s, C≡CCH=CH), 5.94 (1H, d, J = 2.9 Hz, N-CH), 6.56 (1H, dd, J = 3.4, 1.5 Hz, aromatic), 6.67 (1H, s, propargylic), 7.05 (2H, d, J = 8.8 Hz, aromatic), 7.2–7.4 (4H, m, aromatic), 7.40 (1H, td, J = 7.3, 1.5 Hz, aromatic), 7.53 (1H, m, aromatic), 7.63 (1H, m, aromatic), 7.70 (1H, dd, J = 7.8, 1.5 Hz, aromatic). MS (FAB) m/z: 511 (M+H, ^{35}Cl), 513 (M+H, ^{37}Cl). Anal. calcd for $C_{29}H_{19}ClN_2O_5$: C, 68.17; H, 3.75; N, 5.48. Found: C, 67.91; H, 3.95; N, 5.21.

Mixture of phenyl (2*R,5*Z*,9S*,10*S**,16*R**)-(±)-9-(3-carboxypropionyl)oxy-10,2,10-(epoxymetheno)-1-benz[*b*]-azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (8h).** Yield: 26 mg (34%, ca. 2:1 mixture of diastereomers) as a colorless solid. ^1H NMR ($CDCl_3$) δ [(9*S**)-isomer] 2.6–2.9 (4H, m, CH_2CH_2COOH), 4.00 (1H, d, J = 2.9 Hz, epoxide), 5.60 (1H, s, propargylic), 5.77 (1H, dd, J = 10.2, 1.5 Hz, NCHC≡CCH=CH), 5.89(1H, d, J = 10.2 Hz, NCHC≡CCH=CH), 6.08 (1H, m, N-CH), 7.1–7.6 (8H, m, aromatic), 8.16 (1H, dd, J = 7.8, 1.5 Hz, aromatic). [(9*R**)-isomer] 2.6–2.9 (4H, m, CH_2CH_2COOH), 4.30 (1H, d, J = 2.9 Hz, epoxide), 5.85 (2H, s, C≡CCH=CH), 5.99 (1H, m, N-CH), 6.50 (1H, s, propargylic), 7.1–7.6 (8H, m, aromatic), 7.65 (1H, dd, J = 7.8, 1.5 Hz, aromatic). MS (FAB) m/z: 470 (M+H). Anal. calcd for $C_{27}H_{19}NO7$: C, 69.08; H, 4.08; N, 2.98. Found: C, 68.85; H, 4.32; N, 2.78.

Cycloaromatization of 6a.

To a solution of **6a** (20 mg, 0.052 mmol) in THF-d_8 (0.5 mL) was added pTsOH·H_2O (10 mg, 0.052 mmol), followed by stirring at 37 °C for 2 h. The reaction mixture was evaporated in vacuo, and the residue was purified by preparative TLC (silica gel, CH_2Cl_2:ether = 1:1) to give **31** (9 mg, 40%) as a colorless foam. ^1H NMR ($CDCl_3$) δ 3.18 and 3.29 (each 1H, d, J = 15.6 Hz, CH_2), 4.16 (1H, d, J = 4.4 Hz, CHOH), 6.03 (1H, d, J = 4.4 Hz, N-CH), 7.1–7.2 (6H, m, aromatic), 7.36 (2H, m, aromatic), 7.70 (2H, m, aromatic). ^{13}C NMR ($CDCl_3$) δ 46.2, 59.6, 69.6, 71.2, 123.1, 123.2, 125.3, 126.9, 127.0, 128.1, 128.6, 129.5, 131.2, 133.4, 134.3, 134.4, 149.6. MS (EI) m/z: 409 (M^+, ^{35}Cl), 411 (M^+, ^{37}Cl). HRMS for $C_{23}H_{16}ClD_2NO_4$ (M^+) calcd 409.1048, found 409.1065.

Biological assays.

DNA-cleaving assay. Supercoiled ΦX174 DNA (Form I, 250, μM/base pair) was incubated at 37 °C for 18 h with 1 mM (final concentration) of each compound in 50 mM phosphate buffer (pH 7.4) containing 10% DMSO and analyzed by electrophoresis (1% agarose gel) to separate the various forms of DNA. DNA bands were visualized with ethidium bromide binding and UV illumination.

In vitro cytotoxicity. Human epidermoid carcinoma KB cells were cultured in Eagle's minimum essential medium containing 10% fetal bovine serum at a density of 5×10^4 cells/mL on day 0. After culture with test compounds for 48 h from day 1 to day 3, the number of viable cells was counted with a Coulter counter on day 3. IC_{50} values were determined graphically from plots of residual activity versus drug concentration.

In vivo antitumor activity. For the evaluation of the antitumor activity against P388 leukemia, CDF_1 mice were inoculated intraperitoneally (ip) with 1×10 cells/mouse of P388 on day 0, and 2 mg/kg of test compound was administered ip once daily for 4 days from day 1 to day 4. Survival was recorded for 30 days. The T/C values reported refer to the relative mean survival times of drug-treated to control mice (expressed as a percentage). The T/C values over 125% are considered to be significant.

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