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Synthesis and Antitumor Activity of Water-Soluble Enediyne Compounds Related to Dynemicin A

Ryoichi Unno,*[,a,†] Hisashi Michishita,[a] Hideaki Inagaki,[a] Yoko Suzuki,[a] Yutaka Baba,[a] Takahito Jomori,[a] Toshio Nishikawa[b] and Minoru Isobe[b]

[a]*Drug Discovery Research Department, Sanwa Kagaku Kenkyusho Co, Ltd, 363, Shiosaki, Hokusei-cho, Inabe-gun, Mie 511-04, Japan*

[b]*Laboratory of Organic Chemistry, School of Agricultural Sciences, Nagoya University, Furho-cho, Chikusa-ku, Nagoya 464-01, Japan*

Abstract—The enediyne compounds **9–14**, simple dynemicin A (**1**) analogues equipped with aryl carbamate moieties with various aliphatic amino or hydroxy groups at the C9 position, were synthesized and evaluated for DNA-cleaving ability, in vitro cytotoxicity, and in vivo antitumor activity. We found that the water-soluble compounds, in which the *tert*-amines such as the 2-(dimethylamino)ethyl (**10b**, **14b**), 2-(pyrrolidino)ethyl (**10c**), or 1-azabicyclo[3.3.0]oct-5-ylmethyl (**10d**, **12d**, **14d**) group were attached, showed not only the enhanced in vivo antitumor activity but also the decreased toxicity compared to the corresponding 9-acetoxy enediyne compounds **6–8**. In particular, compound **10c** showed the most enhanced in vivo antitumor activity (T/C=222% at a daily dose of 1.25 mg/kg for 4 days) at about half of the dose of **6**. These results suggest that both the enhanced antitumor activity and the reduced toxicity might be due to the improved bioavailability or disposition of compounds **6–8** by their water-solubilization. © 1997 Elsevier Science Ltd.

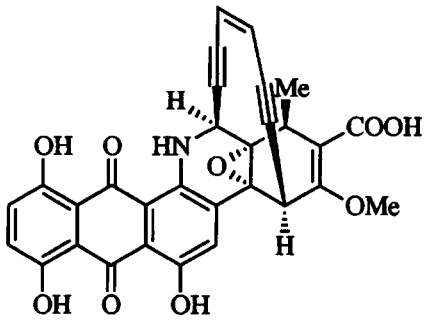
Introduction

The cyclic enediynes, a new class of antitumor antibiotics,[1] are strong DNA-cleaving agents and exhibit remarkable antitumor activities both in vitro and in vivo. Members of this family include dynemicin A (**1**, Fig. 1),[2] calicheamicins,[3] esperamicins,[4] neocarzinostatin chromophore,[5] kedarcidin chromophore,[6] C-1027 chromophore,[7] and maduropeptin chromophore.[8] Dynemicin A (**1**) shows high potency against various tumor cell lines and significantly prolongs the life span of mice inoculated with P388 leukemia and B16 melanoma cells. In addition, dynemicin A (**1**) is unique among these cyclic enediynes as it contains both a cyclic enediyne ring and an anthraquinone chromophore. Because of its structural novelty, complexity, and highly potent activity, the mechanistic and synthetic studies of **1** have been extended.[1] Recently, the excellent total syntheses of **1** and its derivatives have been achieved by the three groups of Schreiber,[9] Myers,[10] and Danishefsky.[11]

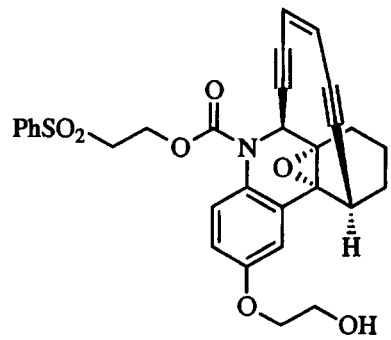
During early mechanistic studies, it was revealed that the biological activity of **1** is due to its ability to break the DNA strand.[12–17] This mechanism is postulated as the following (Fig. 2): (i) a bioreduction of the anthraquinone moiety with a reducing cofactor such as glutathione or NADPH (**1**→**1a**); (ii) an intercalation of **1a** into the double-stranded DNA; (iii) an epoxide opening to form a conjugated imine (**1a**→**1b**); (iv) a nucleophilic attack of water or protonation to cause a conformational change such that the distance between the two terminal carbons of the 1,5-diyne-3-ene system is shortened (**1b**→**1c**);[18] (v) Bergman cycloaromatization[19] to generate the phenylene diradical (**1c**→**1d**); (vi) an abstraction of hydrogen atoms from the sugar phosphate backbone of DNA (**1d**→**1e**); and (vii) cleavage of the DNA strand. The epoxide opening caused by the bioreduction of the anthraquinone moiety is a device which triggers Bergman cycloaromatization. Thus, dynemicin A (**1**) represents a natural prodrug equipped with a triggering device which can be activated under physiological conditions.

Based on the concept of prodrug activation, other groups have reported the strategies that enable the generation of reactive enediynes from stable precursors.[20] Nicolaou et al. have performed significant studies in this area.[21] They have reported that a dynemicin A analogue **2** equipped with the 2-(phenylsulfonyl)ethoxycarbonyl group as a triggering device shows DNA-cleaving activity and highly potent cytotoxicity against various tumor cell lines.[21e] Wender et al. have reported a dynemicin A analogue **3** equipped with the 2-nitrobenzyl carbamate moiety which can be activated by photochemical deprotection.[22] Danishefsky et al. have designed quinone imines **4a**,[11c] **4b**[23] as bioreductively activated enediyne prodrugs. These compounds have reported to exhibit remarkable cytotoxicity and significantly reduce tumor volume in mice bearing solid

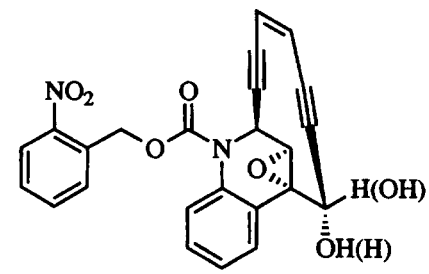
[†]Present address: Department of Medical Foods, Sanwa Kagaku Kenkyusho Co., Ltd, 35 Sotobori-cho, Higashi-ku, Nagoya 461, Japan.



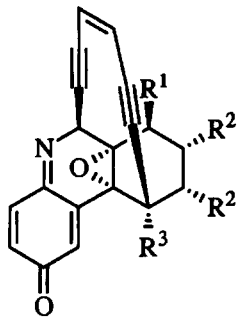
Dynemicin A (1)



Nicolaou's enediyne (2)



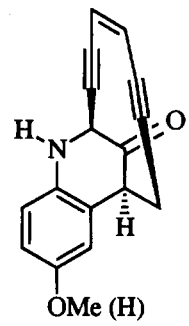
Wender's enediyne (3)



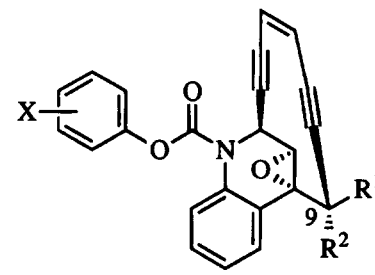
Danishefsky's enediyne

4a : $R^1 = R^2 = H$, $R^3 = OH$

4b : $R^1 = Me$, $R^2 = OAc$, $R^3 = H$



Magnus' enediyne (5)



our enediynes

6 : X = 4-Cl, R^1 = H (OAc), R^2 = OAc (H)

7 : X = 2-NO_2, R^1 = H (OAc), R^2 = OAc (H)

8 : X = 4-NO_2, R^1 = H, R^2 = OAc

9 : X = 4-Cl, R^1 = H (OCOR), R^2 = OCOR (H)

10 : HCl salt of **9**

11 : X = 2-NO_2, R^1 = H (OCOR), R^2 = OCOR (H)

12 : HCl salt of **11**

13 : X = 4-NO_2, R^1 = H, R^2 = OCOR

14 : HCl salt of **13**

Figure 1. Dynemicin A (**1**) and its designed analogues **2–14**.

tumors. Denny et al. have shown that the 4-nitrobenzyl carbamate moiety is a suitable triggering device which can be enzymatically activated by the *E. coli* nitroreductase.[24] On the other hand, Magnus et al. have shown that a dynemicin A analogue **5**, which undergoes cycloaromatization *via* a nondiradical pathway, exhibits cytotoxicity and in vivo antitumor activity.[25]

As a part of our studies[26] aimed at the molecular design of the simple functional analogues of **1** and the identification of the key structural features responsible for the biological activity, we recently found that compound **6** equipped with the 4-chlorophenyl carbamate moiety showed significant antitumor activities against both murine P388 leukemia and Meth A sarcoma in mice despite exhibiting little DNA cleaving activity.[26j] Compound **8** equipped with the 4-nitrophenyl carbamate moiety also showed effective in vivo activity against P388 leukemia, but it seriously reduced the average body weight in mice.[26j] In contrast to **6** and **8**, compound **7** equipped with the 2-nitrophenyl carbamate moiety did not prolong the life span of mice significantly, although higher in vitro potency than that of **6** or **8** was observed.[26j] Thus, it has been revealed that the aryl carbamate moiety, which is an N-protecting group of the enediyne core, plays an important role in the biological activity of these enediyne compounds. However, the bioavailability of compounds **6–8** was considered to be undoubtedly low, because these compounds were quite insoluble in water.

Therefore, in our continuing studies on the dynemicin A analogues, we became interested in water-soluble enediyne compounds in order to improve the bioavailability of compounds **6–8**. We thus designed the enediyne compounds **9**, **11** and **13** and their water-soluble hydrochlorides **10**, **12** and **14** which incorporated various aliphatic amino or hydroxy groups at the C9 position (Fig. 1). In this paper, we describe the syntheses of enediyne compounds **9–14**, their water

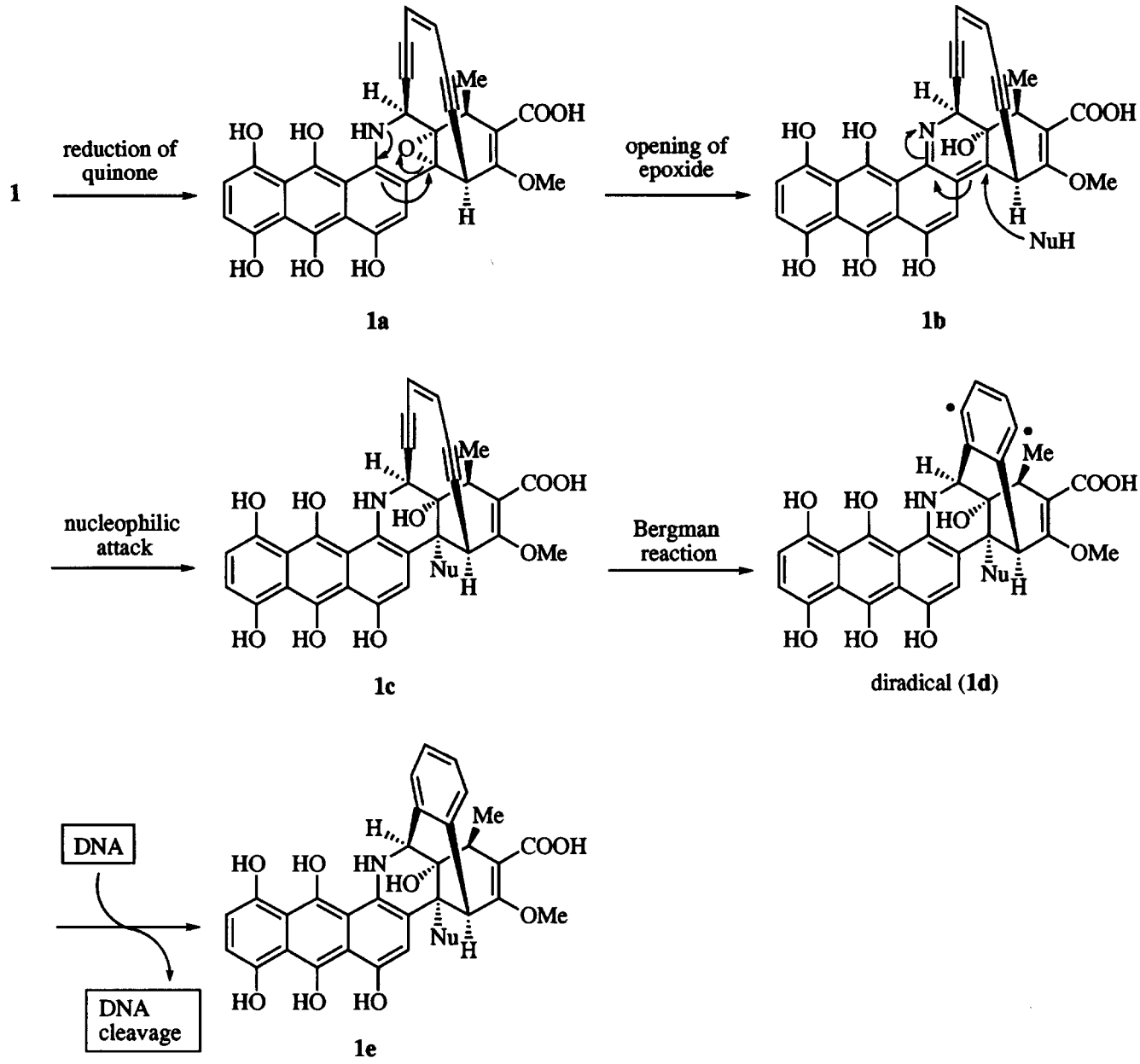


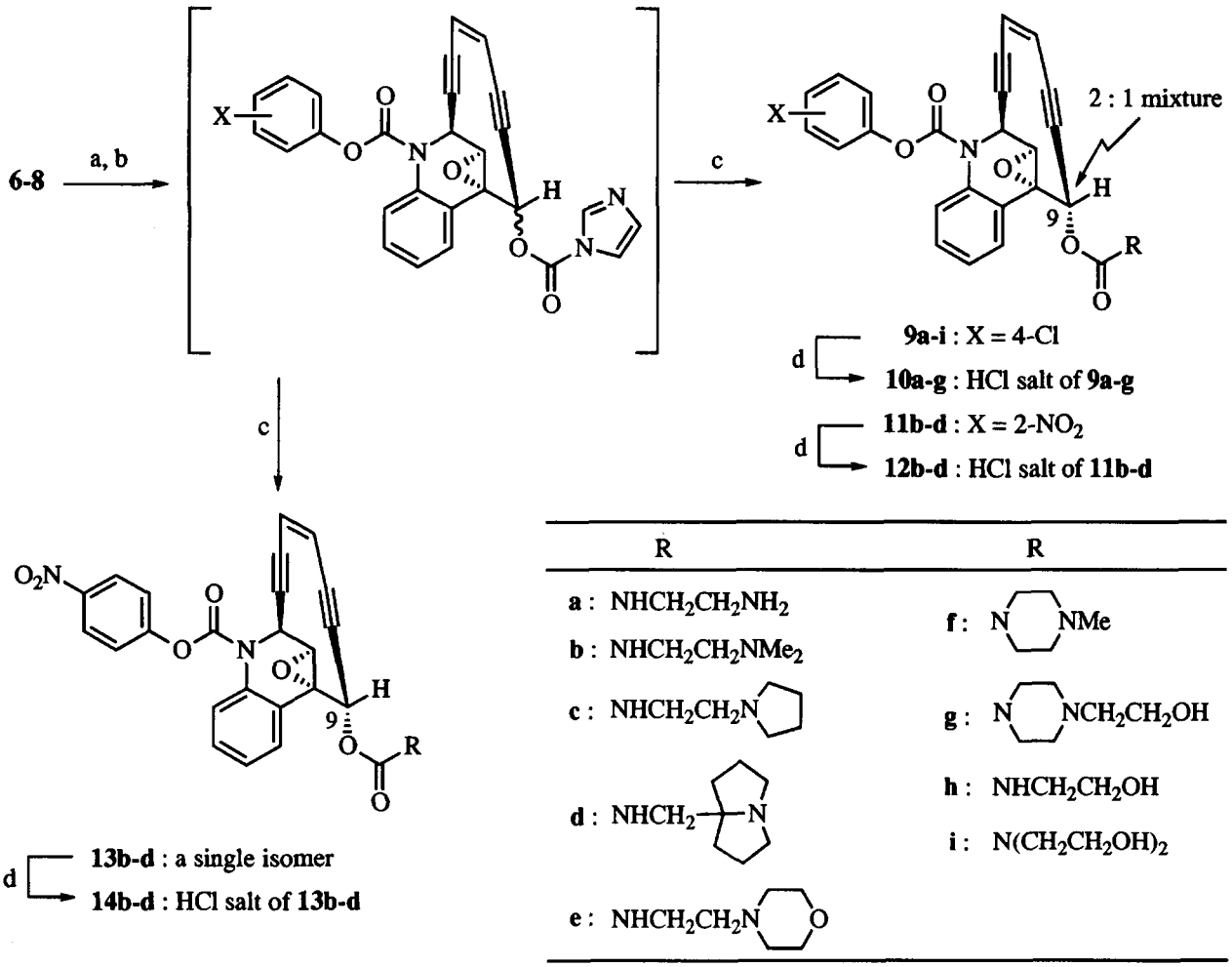
Figure 2. Proposed mechanism of action for dynemicin A (**1**).

solubility, and the evaluation of their DNA-cleaving ability, in vitro cytotoxicity, and in vivo antitumor activity. In particular, we discuss the biological activity of the water-soluble compounds **10**, **12** and **14**.

Results and Discussion

Synthesis of the enediyne compounds 9–14

The enediyne compounds **9–14** were synthesized from the 9-acetoxy enediyne compounds **6–8**[26j] using the following synthetic procedures (Scheme 1). Compound **8** was used as a single isomer, whereas compounds **6** and **7** were used as a 2:1 mixture of diastereomers, because they could not be separated. The acetyl group in **6** was removed with $Ba(OH)_2$[22b] to provide the alcohols which were immediately converted into the imidazolide intermediates with 1,1′-carbonyldiimidazole and 4-dimethylaminopyridine (DMAP). Carbamoylation of the imidazolides with the excess diamines[27] or hydroxyamines gave amines **9a–g** or alcohols **9h–i** as a 2:1 mixture of diastereomers, respectively. These amines **9a–g** were treated with 0.01 M hydrochloric acid to give the corresponding hydrochlorides **10a–g** as monohydrate. The hydrochlorides **12b–d** and **14b–d** were also obtained as monohydrate from **7** and **8**, respectively, using the same procedure as those already described. These hydrochlorides **10a–g**, **12b–d**, and **14b–d** were quite stable on storage at −20 °C for 3 weeks. As expected, the water solubility of these



Scheme 1. Synthesis of enediyne compounds **9–14**. Reagents and conditions: (a) $Ba(OH)_2$, CH_2Cl_2–MeOH, 0 °C, 10 min; (b) 1,1′-carbonyldiimidazole, DMAP, CH_2Cl_2, 0 °C, 1 h; (c) diamines or hydroxyamines, CH_2Cl_2, 0 °C, 1 h; (d) 0.01 M HCl, 0 °C, 10 min.

hydrochlorides **10a–g**, **12b–d**, and **14b–d** (0.1–2.0 mg/mL) increased over 10–200 times compared to the corresponding compounds **6–8**. On the other hand, the amines **9a–g** were insoluble in water (<0.01 mg/mL), and the alcohols **9h–i** were also slightly soluble in water (0.01 mg/mL).

DNA cleavage[28]

Compounds **10c**, **12d** and **14b** were examined for DNA-cleaving activity. The supercoiled ΦX174DNA was incubated at 37 °C for 18 h with 1 mM of each compound in 50 mM phosphate buffer (pH 7.4) containing 10% DMSO and analyzed by agarose gel electrophoresis. The tested compounds, as well as **6–8** previously reported,[26j] scarcely caused DNA cleavage. These small activities resulted from the chemical stability of the aryl carbamate moiety which could not be deprotected to generate a diradical intermediate under such neutral conditions as this assay.

In vitro cytotoxicity[29] and in vivo antitumor activity[30]

The enediyne compounds **9**, **10**, **12** and **14** were evaluated for in vitro cytotoxicity and in vivo antitumor activity as shown in Tables 1 and 2. Cytotoxicity tests were done with the human carcinoma KB cell line, and in vivo tests were performed using mice intraperitoneally (ip) implanted with murine P388 leukemia. The compounds were intraperitoneally administered once daily for 4 days from day 1 to 4.

In the group of compounds **9a–i** and their hydrochlorides **10a–g** having the 4-chlorophenyl carbamate moiety, the cytotoxicity of these compounds resulted in almost the same IC_{50} values between 2.1 and 10 μM, and were the same or had less activity compared to that of **6**. In particular, the incorporation of an oxygen-containing functional groups such as a hydroxy group (**9g–i**, **10g**) or an ether group (**9e**, **10e**) reduced the in vitro potency.

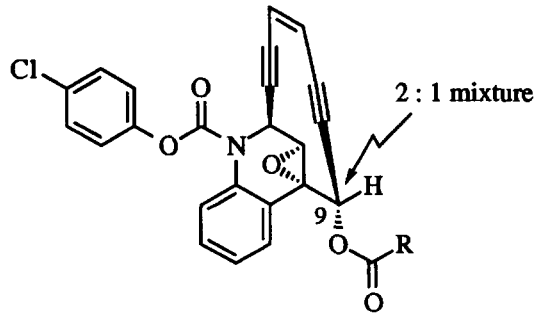
For the in vivo antitumor activity, it was noteworthy that hydrochloride **10c** having the 2-(pyrrolidino)ethyl group

showed the most effective antitumor activity (T/C =222% at a daily dose of 1.25 mg/kg for 4 days) and the same life prolongation as that of **6** at about half of the dose of **6**. Both hydrochlorides **10b** and **10d** also showed significant antitumor activity (T/C = 194% and 188% at 0.8 mg/kg). On the other hand, the amines **9b–d** showed lower potency than the corresponding hydrochlorides **10b–d**. It was considered that this difference in activity between amine **9c** and hydrochloride **10c** resulted from the toxicity that **9c** considerably reduced the average body weight in mice than **10c**. This toxicity observed in the case of amines **9a–d** and **9f** tended to be more serious than that of the corresponding hydrochlorides **10a–d** and **10f**. These results show that the incorporation of the *tert*-amines such as the 2-(dimethylamino)ethyl (**10b**), 2-(pyrrolidino)ethyl (**10c**), or 1-azabicyclo[3.3.0]oct-5-ylmethyl group (**10d**) is effective for the improvement of in vivo antitumor activity and the reduction of toxicity in the form of HCl salts.

Therefore, we introduced these *tert*-amines into compounds **7** and **8** having the 2-nitrophenyl or 4-nitrophenyl carbamate moiety, which showed lower in vivo activity than **6** in spite of showing higher in vitro activity than **6**. As a result, all of the compounds, **12b–d** and **14b–d**, showed higher in vitro cytotoxicity than compounds **7** and **8**, respectively. Especially, compound **12b** having the 2-(dimethylamino)ethyl group showed the most potent cytotoxicity (IC_{50} = 0.057 μM). Compound **12c** having the 2-(pyrrolidino)ethyl group also showed significant activity (IC_{50} = 0.086 μM). Thus, the in vitro cytotoxicity of both compounds **12b** and **12c** increased three- and two-fold compared to **7**, respectively, and that of compound **14d** also increased three-fold compared to **8**.

Furthermore, compounds **12d** and **14d** significantly prolonged the life span of mice (T/C = 186% and 197% at a daily dose of 1.0 mg/kg for 4 days, respectively), and these in vivo activities were improved as well as **10d**.

Table 1. In vitro cytotoxicity and in vivo antitumor activity of compounds **9a–i** and water-soluble compounds **10a–g**



Compd No.	R	Solubility in H_2O (mg/mL)	In vitro cytotoxicity against KB cells IC_{50} (μM)[a]	In vivo antitumor activity against P388 leukemia[b]		
				Dose (mg/kg)	AWC[c] (g)	T/C[d] (%)
6	Me	< 0.01	3.6	2.0	−1.94	221
9a	$NHCH_2CH_2NH_2$	< 0.01	3.0	1.0	−2.58	167
10a	$NHCH_2CH_2NH_2$·HCl	0.5	3.8	1.0	−1.00	164
9b	$NHCH_2CH_2NMe_2$	< 0.01	2.5	0.7	−3.19	173
10b	$NHCH_2CH_2NMe_2$·HCl	0.25	2.5	0.8	−1.60	194
9c	2-(pyrrolidino)ethylamino	< 0.01	2.8	1.25	−3.04	154
10c	2-(pyrrolidino)ethylamino·HCl	2.0	3.4	1.25	−2.16	222
9d	Abcoma[e]	< 0.01	2.1	0.7	−3.02	176
10d	Abcoma·HCl[e]	1.0	3.3	0.8	−1.96	188
9e	2-(morpholino)ethylamino	< 0.01	6.1	2.0	−1.18	157
10e	2-(morpholino)ethylamino·HCl	1.0	9.1	—	—	NT
9f	*N*-methylpiperazino	< 0.01	5.8	2.0	−1.54	144
10f	*N*-methylpiperazino·HCl	0.5	3.9	2.0	−0.36	154
9g	*N*-(2-hydroxyethyl)piperazino	< 0.01	5.0	2.0	−1.06	154
10g	*N*-(2-hydroxyethyl)piperazino·HCl	0.1	5.5	2.0	−0.88	151
9h	$NHCH_2CH_2OH$	0.01	10	—	—	NT
9i	$N(CH_2CH_2OH)_2$	0.01	8.4	—	—	NT

[a]Inhibiting concentration (μM) of 50% cellular growth.
[b]CDF_1 mice were intraperitoneally inoculated with 1×10^6 cells/mouse of P388 on day 0, and the test compound was intraperitoneally administered once daily for 4 days from day 1 to 4.
[c]Average weight changes (AWC) were measured on day 4.
[d]A 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.
[e]Abcoma; 1-azabicyclo[3.3.0]oct-5-ylmethylamino group.

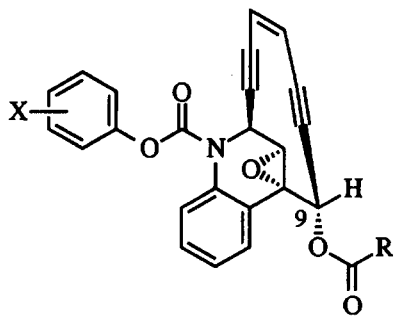
Although compound **12b** had the highest in vitro potency, it scarcely prolonged the life span of mice. On the other hand, the compound **14b**, which incorporated the same substituent as **12b**, showed significant in vivo activity (T/C = 200% at a daily dose of 1.0 mg/kg for 4 days) and it further showed the less toxicity, the reduction in average body weight in mice, compared to **8**. However, the cause of the difference in the activity between **12b** (a 2:1 mixture of diastereomers) and **14b** (single isomer) is yet unknown. On the other hand, both **12c** and **14c** scarcely prolonged the life span of mice in contrast to **10c**. These results show that the suitability between the *tert*-amino substituents and aryl carbamate moieties significantly influences the in vivo antitumor activity of these water-soluble enediyne compounds.

Conclusions

We designed and synthesized the enediyne compounds **9–14**, simple dynemicin A (**1**) analogues equipped with aryl carbamate moieties, which incorporated various aliphatic amino or hydroxy groups at the C9 position. 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 in mice.

Among the compounds, the water-soluble compound **10c** having the 2-(pyrrolidino)ethyl group showed the most effective in vivo antitumor activity (T/C = 222% at a daily dose of 1.25 mg/kg for 4 days) and the same life prolongation as that of **6** at about half of the dose of **6**. Compounds **10d**, **12d** and **14d** having the 1-azabicyclo[3.3.0]oct-5-ylmethyl group also showed significant in vivo activity (T/C = 188% at 0.8 mg/kg, 186% at 1.0 mg/kg, and 197% at 1.0 mg/kg). Compounds **10b** and **12b** having the 2-(dimethylamino)ethyl group also showed significant in vivo activity (T/C = 194% at 0.8 mg/kg and 200% at 1.0 mg/kg). In addition, the toxicity of water-soluble compounds **10b–d** including a reduction in the average body weight in mice tended to be less predominant than that of the corresponding water-insoluble amines **9b–d**. This shows that the toxicity of amines **9b–d** can be essentially reduced by the water-solubilization in the form of their HCl salts. Thus, we found that the water-solubilization by incorporating the *tert*-amines such as the 2-(dimethylamino)ethyl, 2-(pyrrolidino)ethyl or 1-azabicyclo[3.3.0]oct-5-ylmethyl group could improve not only the in vivo antitumor

Table 2. In vitro cytotoxicity and in vivo antitumor activity of water-soluble compounds **12b–d** and **14b–d**



12b-d : a 2 : 1 mixture of diastereomers
14b-d : a single isomer

Compd No.	X	R	Solubility in H_2O (mg/mL)	In vitro cytotoxicity against KB cells IC_{50} (μM)[a]	In vivo antitumor activity against P388 leukemia[b] Dose (mg/kg)	AWC[c] (g)	T/C[d] (%)
7	2-NO_2	Me	< 0.01	0.17	2.0	−1.86	151
12b	2-NO_2	$NHCH_2CH_2NMe_2$·HCl	1.0	0.057	1.0	−1.43	135
12c	2-NO_2	2-(Pyrrolidino)ethylamino·HCl	1.0	0.086	1.0	−0.79	141
12d	2-NO_2	Abcoma·HCl[e]	0.5	0.12	1.0	−1.21	186
8	4-NO_2	Me	< 0.01	1.1	2.0	−3.01	182
14b	4-NO_2	$NHCH_2CH_2NMe_2$·HCl	0.5	0.78	1.0	−1.96	200
14c	4-NO_2	2-(Pyrrolidino)ethylamino·HCl	0.25	0.83	1.0	−2.15	158
14d	4-NO_2	Abcoma·HCl[e]	0.1	0.32	1.0	−2.71	197

[a]Inhibiting concentration (μM) of 50% cellular growth.
[b]CDF_1 mice were intraperitoneally inoculated with 1×10^6 cells/mouse of P388 on day 0, and the test compound was intraperitoneally administered once daily for 4 days from day 1 to 4.
[c]Average weight changes (AWC) were measured on day 4.
[d]A 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.
[e]Abcoma; 1-azabicyclo[3.3.0]oct-5-ylmethylamino group.

activity but also the toxicity. It is considered that both the enhanced antitumor activity and the reduced toxicity might be due to the improved bioavailability or disposition of compounds **6–8** by their water-solubilization.

In the mechanistic study, compounds **10c**, **12d**, and **14b** 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 these compounds are not attributed to their DNA-cleaving ability. It is considered that the role of the enediyne ring in compounds **10c**, **12d**, and **14b** for biological activity is not a radical generator.

Experimental

Melting points were measured on a Yanaco MP-1 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 $CDCl_3$ or CD_3OD with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in ppm, and the following abbreviations are used; s=singlet, d=doublet, t=triplet, dd=double doublet, 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).

Mixture of 4-chlorophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(2-aminoethylaminocarboxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (9a) and its hydrochlorides (10a). Representative procedure.** To a solution of **6**[26j] (ca. 2:1 mixture of diastereomers, 80 mg, 0.18 mmol) in MeOH (2 mL) and CH_2Cl_2 (4 mL) was added a solution of $Ba(OH)_2 \cdot 8H_2O$ (28 mg, 0.09 mmol) in MeOH (2 mL), followed by stirring at 0 °C for 10 min. The reaction mixture was quenched with saturated NH_4Cl solution, extracted with AcOEt (30 mL × 2). The combined organic layers were washed with water, brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo to give the crude hydroxy compound. To a solution of the crude hydroxy compound (70 mg) in dry CH_2Cl_2 (4 mL) were added DMAP (22 mg, 0.18 mmol) and 1,1'-carbonyldiimidazole (88 mg, 0.54 mmol), followed by stirring at 0 °C for 1 h. Ethylenediamine (65 mg, 1.08 mmol) was then added to the reaction mixture. After being stirred at 0 °C for 1 h, the reaction mixture was quenched with H_2O and extracted with AcOEt (30 mL × 2). 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, AcOEt/MeOH=5:1) to give **9a** (40 mg, 45%, ca. 2:1 mixture of diastereomers) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ [(9*S**)-isomer[26j]] 2.7–2.9 (2H, m, $C\underline{H}_2NH_2$), 3.2–3.4 (2H, m, $CONHC\underline{H}_2$), 3.99 (1H, d, *J*=2.9 Hz, epoxide), 5.52 (1H, m, $CON\underline{H}$), 5.59 (1H, s, propargylic), 5.72 (1H, d, *J*=10.2 Hz, $NCHC{\equiv}CC\underline{H}{=}CH$), 5.84 (1H, d, *J*=10.2 Hz, $NCHC{\equiv}CCH{=}C\underline{H}$), 5.97 (1H, m, $NC\underline{H}$), 7.07 (2H, dd, *J*=8.8, 2.4 Hz, aromatic), 7.2–7.3 (3H, m, aromatic), 7.50 (1H, d, *J*=7.8 Hz, aromatic), 7.68 (1H, dd, *J*=7.8, 1.5 Hz, aromatic), 8.22 (1H, dd, *J*=7.8, 1.5 Hz, aromatic). [(9*R**)-isomer[26j]] 2.7–2.9 (2H, m, $C\underline{H}_2NH_2$), 3.2–3.4 (2H, m, $CONHC\underline{H}_2$), 4.31 (1H, d, *J*=2.9 Hz, epoxide), 5.38 (1H, m, $CON\underline{H}$), 5.81 (2H, s, $C{\equiv}CC\underline{H}{=}C\underline{H}$), 5.92 (1H, m, $NC\underline{H}$), 6.43 (1H, s, propargylic), 7.07 (2H, dd, *J*=8.8, 2.4 Hz, aromatic), 7.2–7.3 (3H, m, aromatic), 7.50 (1H, d, *J*=7.8 Hz, aromatic), 7.68 (1H, dd, *J*=7.8, 1.5 Hz, aromatic), 8.22 (1H, dd, *J*=7.8, 1.5 Hz, aromatic). MS (FAB) *m/z*: 490 (M+H; ^{35}Cl), 492 (M+H; ^{37}Cl). HRMS for $C_{26}H_{21}ClN_3O_5$ (M+H) calcd 490.1169, found 490.1178.

To a suspension of **9a** (20 mg, 0.04 mmol) in cold H_2O (40 mL) was added 0.01 N HCl (4.0 mL, 0.04 mmol) and the mixture was dissolved by sonicating at 0 °C for 10 min. The resulting solution was lyophilized to give **10a** (20 mg, quantitative, ca. 2:1 mixture of diastereomers) as a colorless powder mp 85–90 °C (dec). ^{1}H NMR (CD_3OD) δ [(9*S**)-isomer] 3.0–3.2 (2H, m, $C\underline{H}_2NH_2$), 3.3–3.6 (2H, m, $CONHC\underline{H}_2$), 4.19 (1H, d, *J*=2.9 Hz, epoxide), 5.68 (1H, s, propargylic), 5.84 (1H, d, *J*=10.2 Hz, $NCHC{\equiv}CC\underline{H}{=}CH$), 5.97 (1H, d, *J*=10.2 Hz, $NCHC{\equiv}CCH{=}C\underline{H}$), 5.99 (1H, m, $NC\underline{H}$), 7.14 (2H, d, *J*=8.3 Hz, aromatic), 7.2–7.6 (4H, m, aromatic), 7.80 (1H, d, *J*=6.8 Hz, aromatic), 8.31 (1H, d, *J*=8.3 Hz, aromatic). [(9*R**)-isomer] 3.0–3.2 (2H, m, $C\underline{H}_2NH_2$), 3.3–3.6 (2H, m, $CONHC\underline{H}_2$), 4.37 (1H, d, *J*=2.9 Hz, epoxide), 5.94 (2H, m, $C{\equiv}CC\underline{H}{=}C\underline{H}$), 6.50 (1H, m, $NC\underline{H}$), 6.43 (1H, s, propargylic), 7.14 (2H, d, *J*=8.3 Hz, aromatic), 7.2–7.6 (4H, m, aromatic), 7.80 (1H, d, *J*=6.8 Hz, aromatic), 8.31 (1H, d, *J*=8.3 Hz, aromatic). MS (FAB) *m/z*: 490 (M+H; ^{35}Cl), 492 (M+H; ^{37}Cl). Anal. calcd for $C_{26}H_{20}ClN_3O_5 \cdot HCl \cdot H_2O$: C, 57.36; H, 4.26; N, 7.72; found: C, 57.11; H, 4.45; N, 7.58.

The following compounds were prepared by a procedure similar to that described for **9a** and **10a**.

Mixture of 4-chlorophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(2-(*N*,*N*-dimethylamino)ethylaminocarboxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (9b) and its hydrochlorides (10b).** Starting from 80 mg of **6**, the crude products were purified by column chromatography (silica gel, AcOEt/MeOH=9:1) to give **9b** (48 mg, 47%, ca. 2:1 mixture of diastereomers) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ [(9*S**)-isomer] 2.19 and 2.21 (each 3H, s NMe_2), 2.4–2.5 (2H, m, $C\underline{H}_2NMe_2$), 3.2–3.4 (2H, m, $CONHC\underline{H}_2$), 3.95 (1H, d, *J*=2.9 Hz, epoxide), 5.56 (1H, s, propargylic), 5.57 (1H, m, $CON\underline{H}$), 5.68 (1H, d, *J*=10.2 Hz, $NCHC{\equiv}CC\underline{H}{=}CH$), 5.81 (1H, d, *J*=10.2 Hz, $NCHC{\equiv}CCH{=}C\underline{H}$), 5.94 (1H, m, $NC\underline{H}$), 7.03 (2H, m, aromatic), 7.2–7.5 (5H, m, aromatic), 8.22 (1H, d,

J=7.8 Hz, aromatic). [(9*R**)-isomer] 2.19 and 2.21 (each 3H, s NMe_2), 2.4–2.5 (2H, m, CH_2NMe_2), 3.2–3.4 (2H, m, $CONHCH_2$), 4.30 (1H, d, J=2.9 Hz, epoxide), 5.52 (1H, m, CONH), 5.77 (2H, s, C≡CCH=CH), 5.89 (1H, m, NCH), 6.37 (1H, s, propargylic), 7.03 (2H, m, aromatic), 7.2–7.5 (5H, m, aromatic), 7.64 (1H, d, J=7.8 Hz, aromatic). MS (FAB) *m/z*: 518 (M+H; ^{35}Cl), 520 (M+H; ^{37}Cl). HRMS for $C_{28}H_{25}ClN_3O_5$ (M+H) calcd 518.1482, found 518.1498.

10b: 20 mg (quantitative, ca. 2:1 mixture of diastereomers) as a colorless powder. Mp 80–85 °C (dec). ^1H NMR (CD_3OD) δ [(9*S**)-isomer] 2.90 (6H, s, NMe_2), 3.2–3.3 (2H, m, CH_2NMe_2), 3.5–3.6 (2H, m, $CONHCH_2$), 4.20 (1H, d, J=2.9 Hz, epoxide), 5.69 (1H, s, propargylic), 5.85 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.97 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 6.00 (1H, m, NCH), 7.14 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 8.30 (1H, d, J=7.8 Hz, aromatic). [(9*R**)-isomer] 2.90 (6H, s, NMe_2), 3.2–3.3 (2H, m, CH_2NMe_2), 3.5–3.6 (2H, m, $CONHCH_2$), 4.35 (1H, d, J=2.9 Hz, epoxide), 5.77 (2H, m, C≡CCH=CH), 5.94 (1H, m, NCH), 6.52 (1H, s, propargylic), 7.14 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 7.80 (1H, d, J=7.8 Hz, aromatic). MS (FAB) *m/z*: 518 (M+H; ^{35}Cl), 520 (M+H; ^{37}Cl). Anal. calcd for $C_{28}H_{24}ClN_3O_5 \cdot HCl \cdot H_2O$: C, 58.75; H, 4.75; N, 7.34; found: C, 58.49; H, 4.98; N, 7.08.

Mixture of 4-chlorophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(2-(pyrrolidino)ethylaminocarboxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (9c) and its hydrochlorides (10c)**. Starting from 100 mg of **6**, the crude products were purified by column chromatography (silica gel, AcOEt/MeOH=9:1) to give **9c** (70 mg, 57%, ca. 2:1 mixture of diastereomers) as a colorless foam. ^1H NMR ($CDCl_3$) δ [(9*S**)-isomer] 1.7–1.9 (4H, m, $NCH_2CH_2 \times 3$), 3.3–3.4 (2H, m, $CONHCH_2$), 3.99 (1H, d, J=2.9 Hz, epoxide), 5.58 (1H, s, propargylic), 5.72 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.86 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.97 (1H, m, NCH), 7.0–7.1 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 8.23 (1H, d, J=7.8 Hz, aromatic). [(9*R**)-isomer] 1.7–1.9 (4H, m, $NCH_2CH_2 \times 2$), 2.5–2.7 (6H, m, $NCH_2 \times 3$), 3.3–3.4 (2H, m, $CONHCH_2$), 4.32 (1H, d, J=2.9 Hz, epoxide), 5.82 (2H, s, C≡CCH=CH), 5.92 (1H, m, NCH), 6.41 (1H, s, propargylic), 7.0–7.1 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 7.68 (1H, d, J=7.8 Hz, aromatic). MS (FAB) *m/z*: 544 (M+H; ^{35}Cl), 546 (M+H; ^{37}Cl). HRMS for $C_{30}H_{27}ClN_3O_5$ (M+H) calcd 544.1639, found 544.1655.

10c: 20 mg (quantitative, ca. 2:1 mixture of diastereomers) as a colorless powder. Mp 88–93 °C (dec). ^1H NMR (CD_3OD) δ [(9*S**)-isomer] 1.9–2.2 (4H, m, $NCH_2CH_2 \times 2$), 3.0–3.4 (6H, m, $NCH_2 \times 3$), 3.5–3.6 (2H, m, $CONHCH_2$), 4.19 (1H, d, J=2.9 Hz, epoxide), 5.70 (1H, s, propargylic), 5.86 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.97 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 6.02 (1H, m, NCH), 7.1–7.2 (2H, m, aromatic), 7.2–7.5 (5H, m, aromatic), 8.30 (1H, d, J=8.3 Hz, aromatic). [(9*R**)-isomer] 1.9–2.2 (4H, m, $NCH_2CH_2 \times 2$), 3.0–3.4 (6H, m, $NCH_2 \times 3$), 3.5–3.6 (2H, m, $CONHCH_2$), 4.39 (1H, d, J=2.9 Hz, epoxide), 5.95 (3H, m, C≡CCH=CH and NCH), 6.50 (1H, s, propargylic), 7.0–7.1 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 7.80 (1H, d, J=8.3 Hz, aromatic). MS (FAB) *m/z*: 544 (M+H; ^{35}Cl), 546 (M+H; ^{37}Cl). Anal. calcd for $C_{30}H_{26}ClN_3O_5 \cdot HCl \cdot H_2O$: C, 60.21; H, 4.88; N, 7.02; found: C, 59.98; H, 5.10; N, 6.82.

Mixture of 4-chlorophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(1-azabicyclo[3.3.0]oct-5-ylmethylaminocarboxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclo-dodeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (9d) and its hydrochlorides (10d)**. Starting from 100 mg of **6**, the crude products were purified by column chromatography (silica gel, AcOEt/MeOH=9:1) to give **9d** (66 mg, 52%, ca. 2:1 mixture of diastereomers) as a colorless foam. ^1H NMR ($CDCl_3$) δ [(9*S**)-isomer] 1.5–1.9 (8H, m, $NCH_2CH_2CH_2 \times 2$), 2.5–2.7 and 2.9–3.1 (4H, m, $NCH_2 \times 2$), 3.19 (2H, d, J=3.9 Hz, $CONHCH_2$), 4.09 (1H, d, J=2.9 Hz, epoxide), 5.59 (1H, s, propargylic), 5.74 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.88 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.97 (1H, m, NCH), 7.09 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 8.29 (1H, dd, J=7.8, 1.5 Hz, aromatic). [(9*R**)-isomer] 1.5–1.9 (8H, m, $NCH_2CH_2CH_2 \times 2$), 2.5–2.7 and 2.9-3.1 (4H, m, $NCH_2 \times 2$), 3.19 (2H, d, J=3.9 Hz, $CONHCH_2$), 4.35 (1H, d, J=2.9 Hz, epoxide), 5.86 (2H, s, C≡CCH=CH), 5.92 (1H, m, NCH), 6.42 (1H, s, propargylic), 7.09 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 7.70 (1H, d, J=7.8 Hz, aromatic). MS (FAB) *m/z*: 570 (M+H; ^{35}Cl), 572 (M+H; ^{37}Cl). HRMS for $C_{32}H_{29}ClN_3O_5$ (M+H) calcd 570.1795, found 570.1780.

10d: 20 mg (quantitative, ca. 2:1 mixture of diastereomers) as a colorless powder. Mp 83–90 °C (dec). ^1H NMR (CD_3OD) δ [(9*S**)-isomer] 1.9–2.3 (8H, m, $NCH_2CH_2CH_2 \times 2$), 3.2–3.4 and 3.5–3.7 (6H, m, $NCH_2 \times 2$ and $CONHCH_2$), 4.21 (1H, d, J=2.9 Hz, epoxide), 5.73 (1H, s, propargylic), 5.86 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.98 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 6.00 (1H, m, NCH), 7.15 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 8.30 (1H, d, J=7.8 Hz, aromatic). [(9*R**)-isomer] 1.9–2.3 (8H, m, $NCH_2CH_2CH_2 \times 2$), 3.2–3.4 and 3.5–3.7 (6H, m, $NCH_2 \times 2$ and $CONHCH_2$), 4.38 (1H, d, J=2.9 Hz, epoxide), 5.92 (1H, m, NCH), 5.96 (2H, s, C≡CCH=CH), 6.56 (1H, s, propargylic), 7.15 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 7.80 (1H, d, J=7.8 Hz, aromatic). MS (FAB) *m/z*: 570 (M+H; ^{35}Cl), 572 (M+H; ^{37}Cl). Anal. calcd for $C_{32}H_{28}ClN_3O_5 \cdot HCl \cdot H_2O$: C, 61.54; H, 5.00; N, 6.73; found: C, 61.27; H, 5.28; N, 6.59.

Mixture of 4-chlorophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(2-(morpholino)ethylaminocarboxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (9e) and its hydrochlorides (10e)**. Starting from 100 mg of **6**, the crude products were purified by column chromatography (silica gel, AcOEt) to give **9e** (74 mg, 60%, ca. 2:1

mixture of diastereomers) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ [(9*S**)-isomer] 2.4-2.6 (6H, m, $NCH_2 \times 3$), 3.36 (2H, m, $CONHCH_2$), 3.71 (4H, m, CH_2OCH_2), 4.00 (1H, d, *J*=2.9 Hz, epoxide), 5.49 (1H, m, CONH), 5.58 (1H, s, propargylic), 5.73 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.85 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.98 (1H, m, NCH), 7.07 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 8.23 (1H, d, *J*=8.3 Hz, aromatic). [(9*R**)-isomer] 2.4–2.6 (6H, m, $NCH_2 \times 3$), 3.36 (2H, m, $CONHCH_2$), 3.71 (4H, m, CH_2OCH_2), 4.34 (1H, d, *J*=2.9 Hz, epoxide), 5.83 (2H, s, C≡CCH=CH), 5.94 (1H, m, NCH), 6.43 (1H, s, propargylic), 7.09 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 7.69 (1H, d, *J*=8.3 Hz, aromatic). MS (FAB) *m/z*: 560 (M+H; ^{35}Cl), 562 (M+H; ^{37}Cl). HRMS for $C_{30}H_{27}ClN_3O_6$ (M+H) calcd 560.1588, found 560.1571.

10e: 20 mg (quantitative, ca. 2:1 mixture of diastereomers) as a colorless powder. Mp 85–90 °C (dec). ^{1}H NMR (CD_3OD) δ [(9*S**)-isomer] 3.0–3.2 (6H, m, $NCH_2 \times 3$), 3.4–3.5 (2H, m, $CONHCH_2$), 3.8–3.9 (4H, m, CH_2OCH_2), 4.20 (1H, d, *J*=2.9 Hz, epoxide), 5.70 (1H, s, propargylic), 5.85 (1H, dd, *J*=10.2, 1.5 Hz, NCHC≡CCH=CH), 5.98 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.98 (1H, m, NCH), 7.1–7.2 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 8.30 (1H, dd, *J*=8.3, 1.5 Hz, aromatic). [(9*R**)-isomer] 3.0–3.2 (6H, m, $NCH_2 \times 3$), 3.4–3.5 (2H, m, $CONHCH_2$), 3.8–3.9 (4H, m, CH_2OCH_2), 4.34 (1H, d, *J*=2.9 Hz, epoxide), 5.94 (3H, m, C≡CCH=CH and NCH), 6.50 (1H, s, propargylic), 7.1–7.2 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 7.80 (1H, d, *J*=8.3 Hz, aromatic). MS (FAB) *m/z*: 560 (M+H; ^{35}Cl), 562 (M+H; ^{37}Cl). Anal. calcd for $C_{30}H_{26}ClN_3O_6 \cdot HCl \cdot H_2O$: C, 58.64; H, 4.76; N, 6.84; found: C, 58.39; H, 5.01; N, 6.64.

Mixture of 4-chlorophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(*N*-methylpiperazinocarboxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (9f) and its hydrochlorides (10f)**. Starting from 80 mg of **6**, the crude products were purified by column chromatography (silica gel, AcOEt/MeOH=5:1) to give **9f** (40 mg, 43%, ca. 2:1 mixture of diastereomers) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ [(9*S**)-isomer] 2.23 (3H, s, NMe), 2.3–2.5 (4H, m, $NCH_2 \times 2$), 3.4–3.7 (4H, m, $CONCH_2 \times 2$), 4.01 (1H, d, *J*=2.9 Hz, epoxide), 5.56 (1H, s, propargylic), 5.73 (1H, dd, *J*=10.2, 1.5 Hz, NCHC≡CCH=CH), 5.85 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.98 (1H, m, NCH), 7.0–7.1 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 8.17 (1H, dd, *J*=8.3, 1.5 Hz, aromatic). [(9*R**)-isomer] 2.32 (3H, s, NMe), 2.3–2.5 (4H, m, $NCH_2 \times 2$), 3.4–3.7 (4H, m, $CONCH_2 \times 2$), 4.27 (1H, d, *J*=2.9 Hz, epoxide), 5.83 (2H, s, C≡CCH=CH), 5.94 (1H, m, NCH), 6.44 (1H, s, propargylic), 7.0–7.1 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 7.68 (1H, d, *J*=8.3 Hz, aromatic). MS (FAB) *m/z*: 530 (M+H; ^{35}Cl), 532 (M+H; ^{37}Cl). HRMS for $C_{29}H_{25}ClN_3O_5$ (M+H) calcd 530.1482, found 530.1498.

10f: 20 mg (quantitative, ca. 2:1 mixture of diastereomers) as a colorless powder. Mp 92–97 °C (dec). ^{1}H NMR (CD_3OD) δ [(9*S**)-isomer] 2.92 (3H, s NMe), 3.1–3.5 (8H, m, $NCH_2 \times 2$ and $CONCH_2 \times 2$), 4.24 (1H, d, *J*=2.9 Hz, epoxide), 5.71 (1H, s, propargylic), 5.87 (1H, dd, *J*=10.2, 1.5 Hz, NCHC≡CCH=CH), 5.97 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.98 (1H, m, NCH), 7.15 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 8.17 (1H, dd, *J*=8.3, 1.5 Hz, aromatic). [(9*R**)-isomer] 2.94 (3H, s NMe), 3.1–3.5 (8H, m, $NCH_2 \times 2$ and $CONCH_2 \times 2$), 4.44 (1H, d, *J*=2.9 Hz, epoxide), 5.95 (3H, m, C≡CCH=CH and NCH), 6.57 (1H, s, propargylic), 7.15 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 7.81 (1H, d, *J*=8.3 Hz, aromatic). MS (FAB) *m/z*: 530 (M+H; ^{35}Cl), 532 (M+H; ^{37}Cl). Anal. calcd for $C_{29}H_{24}ClN_3O_5 \cdot HCl \cdot H_2O$: C, 59.60; H, 4.66; N, 7.19. found: C, 59.33; H, 4.92; N, 6.95.

Mixture of 4-chlorophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(*N*-(2-hydroxyethyl)piperazinocarboxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (9g) and its hydrochlorides (10g)**. Starting from 50 mg of **6**, the crude products were purified by column chromatography (silica gel, AcOEt/MeOH=9:1) to give **9g** (45 mg, 70%, ca. 2:1 mixture of diastereomers) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ [(9*S**)-isomer] 2.4–2.5 (6H, m, $NCH_2 \times 3$), 3.5–3.7 (6H, m, $CONCH_2 \times 2$ and CH_2OH), 4.01 (1H, d, *J*=2.9 Hz, epoxide), 5.57 (1H, s, propargylic), 5.73 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.85 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.98 (1H, m, NCH), 7.0–7.1 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 8.16 (1H, d, *J*=7.8 Hz, aromatic). [(9*R**)-isomer] 2.4–2.5 (6H, m, $NCH_2 \times 3$), 3.5–3.7 (6H, m, $CONCH_2 \times 2$ and CH_2OH), 4.27 (1H, d, *J*=2.9 Hz, epoxide), 5.82 (2H, s, C≡CCH=CH), 5.94 (1H, m, NCH), 6.45 (1H, s, propargylic), 7.0–7.1 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 7.69 (1H, d, *J*=7.8 Hz, aromatic). MS (FAB) *m/z*: 560 (M+H; ^{35}Cl), 562 (M+H; ^{37}Cl). HRMS for $C_{30}H_{27}ClN_3O_6$ (M+H) calcd 560.1588, found 560.1607.

10g: 20 mg (quantitative, ca. 2:1 mixture of diastereomers) as a colorless powder. Mp 82–86 °C (dec). ^{1}H NMR (CD_3OD) δ [(9*S**)-isomer] 3.1–3.3 (6H, m, $NCH_2 \times 3$), 3.8–3.9 (6H, m, $CONCH_2 \times 2$ and CH_2OH), 4.24 (1H, d, *J*=2.9 Hz, epoxide), 5.71 (1H, s, propargylic), 5.85 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.98 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.98 (1H, m, NCH), 7.14 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 8.18 (1H, dd, *J*=7.8, 1.5 Hz, aromatic). [(9*R**)-isomer] 3.1–3.3 (6H, m, $NCH_2 \times 3$), 3.8–3.9 (6H, m, $CONCH_2 \times 2$ and CH_2OH), 4.43 (1H, d, *J*=2.9 Hz, epoxide), 5.95 (3H, m, C≡CCH=CH and NCH), 6.57 (1H, s, propargylic), 7.14 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 7.82 (1H, dd, *J*=7.8, 1.5 Hz, aromatic). MS (FAB) *m/z*: 560 (M+H; ^{35}Cl), 562 (M+H; ^{37}Cl). Anal. calcd for $C_{30}H_{26}ClN_3O_6 \cdot HCl \cdot H_2O$: C, 58.64; H, 4.76; N, 6.84; found: C, 58.41; H, 5.02; N, 6.59.

Mixture of 4-chlorophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(2-hydroxyethylaminocarboxy)-10,2,10-(epoxy-**

metheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R)-isomer (9h)**. Starting from 100 mg of **6**, the crude products were purified by column chromatography (silica gel, AcOEt/*n*-hexane=3:1) to give **9h** (56 mg, 52%, ca. 2:1 mixture of diastereomers) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ [(9*S**)-isomer] 3.3–3.5 (2H, m, NCH_2), 3.7–3.8 (2H, m, CH_2OH), 4.00 (1H, d, *J*=2.9 Hz, epoxide), 5.54 (1H, m, CONH), 5.59 (1H, s, propargylic), 5.72 (1H, dd, *J*=10.2, 1.5 Hz, NCHC≡CCH=CH), 5.86 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.97 (1H, m, NCH), 7.0–7.1 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 8.20 (1H, dd, *J*=7.8, 1.0 Hz, aromatic). [(9*R**)-isomer] 3.3–3.5 (2H, m, NCH_2), 3.7–3.8 (2H, m, CH_2OH), 4.31 (1H, d, *J*=2.9 Hz, epoxide), 5.43 (1H, m, CONH), 5.81 (2H, s, C≡CCH=CH), 5.92 (1H, m, NCH), 6.42 (1H, s, propargylic), 7.0-7.1 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 7.67 (1H, dd, *J*=7.8, 1.0 Hz, aromatic). MS (FAB) *m/z*: 491 (M+H; ^{35}Cl), 493 (M+H; ^{37}Cl). HRMS for $C_{26}H_{20}ClN_2O_6$ (M+H) calcd 491.1010, found 491.1032.

Mixture of 4-chlorophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(bis(2-hydroxyethyl)aminocarboxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (9i)**. Starting from 50 mg of **6**, the crude products were purified by column chromatography (silica gel, AcOEt/*n*-hexane=3:1) to give **9i** (10 mg, 16%, ca. 2:1 mixture of diastereomers) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ [(9*S**)-isomer] 3.61 (2H, m, $CH_2OH \times 2$), 3.91 (4H, m, $NCH_2 \times 2$), 4.01 (1H, d, *J*=2.9 Hz, epoxide), 5.60 (1H, s, propargylic), 5.73 (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, NCH), 7.09 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 8.14 (1H, dd, *J*=8.3, 1.5 Hz, aromatic). [(9*R**)-isomer] 3.61 (2H, m, $CH_2OH \times 2$), 3.91 (4H, m, $NCH_2 \times 2$), 4.29 (1H, d, *J*=2.9 Hz, epoxide), 5.83 (2H, s, C≡CCH=CH), 5.94 (1H, m, NCH), 6.47 (1H, s, propargylic), 7.09 (2H, m, aromatic), 7.2–7.6 (5H, m, aromatic), 7.69 (1H, d, *J*=8.3 Hz, aromatic). MS (FAB) *m/z*: 535 (M+H; ^{35}Cl), 537 (M+H; ^{37}Cl). HRMS for $C_{28}H_{24}ClN_2O_7$ (M+H) calcd 535.1272, found 535.1253.

Mixture of 2-nitrophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(2-(*N*,*N*-dimethylamino)ethylaminocarboxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (11b) and its hydrochlorides (12b)**. Starting from 50 mg of **7**, the crude products were purified by column chromatography (silica gel, AcOEt/MeOH=9:1) to give **11b** (27 mg, 46%, ca. 2:1 mixture of diastereomers) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ [(9*S**)-isomer] 2.23 and 2.26 (each 3H, s, NMe_2), 2.4-2.5 (2H, m, CH_2NMe_2), 3.2–3.4 (2H, m, $CONHCH_2$), 4.00 (1H, d, *J*=2.9 Hz, epoxide), 5.60 (1H, s, propargylic), 5.73 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.85 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.96 (1H, m, NCH), 7.1–7.3 (4H, m, aromatic), 7.63 (1H, m, aromatic), 8.12 (1H, d, *J*=7.8 Hz, aromatic), 8.24 (1H, dd, *J*=7.8, 1.5 Hz, aromatic). [(9*R**)-isomer] 2.23 and 2.26 (each 3H, s, NMe_2), 2.4–2.5 (2H, m, CH_2NMe_2), 3.2–3.4 (2H, m, $CONHCH_2$), 4.34 (1H, d, *J*=2.9 Hz, epoxide), 5.82 (2H, s, C≡CCH=CH), 5.91 (1H, m, NCH), 6.42 (1H, s, propargylic), 7.1–7.3 (4H, m, aromatic), 7.63 (1H, m, aromatic), 8.12 (1H, d, *J*=7.8 Hz, aromatic), 8.24 (1H, dd, *J*=7.8, 1.5 Hz, aromatic). MS (FAB) *m/z*: 529 (M+H). HRMS for $C_{28}H_{25}N_4O_7$ (M+H) calcd 529.1723, found 529.1740.

12b: 20 mg (quantitative) as a colorless powder. Mp 83–88 °C (dec). ^{1}H NMR (CD_3OD) δ [(9*S**)-isomer] 2.96 (6H, s, NMe_2), 3.2–3.4 (2H, m, CH_2NMe_2), 3.5–3.7 (2H, m, $CONHCH_2$), 4.21 (1H, d, *J*=2.9 Hz, epoxide), 5.71 (1H, s, propargylic), 5.87 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.98 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 6.00 (1H, m, NCH), 7.3–7.8 (6H, m, aromatic), 8.16 (1H, d, *J*=8.3 Hz, aromatic), 8.87 (1H, s, aromatic). [(9*R**)-isomer] 2.96 (6H, s, NMe_2), 3.2–3.4 (2H, m, CH_2NMe_2), 3.5–3.7 (2H, m, $CONHCH_2$), 4.37 (1H, d, *J*=2.9 Hz, epoxide), 5.96 (2H, s, C≡CCH=CH), 6.00 (1H, m, NCH), 6.54 (1H, s, propargylic), 7.3–7.8 (6H, m, aromatic), 8.32 (1H, dd, *J*=8.3, 1.5 Hz, aromatic), 8.87 (1H, s, aromatic). MS (FAB) *m/z*: 529 (M+H). Anal. calcd for $C_{28}H_{24}N_4O_7 \cdot HCl \cdot H_2O$: C, 57.69; H, 4.67; N, 9.61; found: C, 57.45; H, 4.91; N, 9.32.

Mixture of 2-nitrophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(2-(pyrrolidino)ethylaminocarboxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (11c) and its hydrochlorides (12c)**. Starting from 50 mg of **7**, the crude products were purified by column chromatography (silica gel, AcOEt/MeOH=9:1) to give **11c** (25 mg, 40%, ca. 2:1 mixture of diastereomers) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ [(9*S**)-isomer] 1.7–1.9 (4H, m, $NCH_2CH_2 \times 2$), 2.5–2.7 (6H, m, $NCH_2 \times 3$), 3.3–3.5 (2H, m, $CONHCH_2$), 4.00 (1H, d, *J*=2.9 Hz, epoxide), 5.59 (1H, s, propargylic), 5.73 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.85 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.95 (1H, m, NCH), 7.1–7.5 (4H, m, aromatic), 7.63 (1H, m, aromatic), 8.12 (1H, d, *J*=7.8 Hz, aromatic), 8.25 (1H, dd, *J*=7.8, 1.5 Hz, aromatic). [(9*R**)-isomer] 1.7–1.9 (4H, m, $NCH_2CH_2 \times 2$), 2.5–2.7 (6H, m, $NCH_2 \times 3$), 3.3–3.5 (2H, m, $CONHCH_2$), 4.35 (1H, d, *J*=2.9 Hz, epoxide), 5.82 (2H, s, C≡CCH=CH), 5.91 (1H, m, NCH), 6.42 (1H, s, propargylic), 7.1–7.5 (4H, m, aromatic), 7.63 (1H, m, aromatic), 8.12 (1H, d, *J*=7.8 Hz, aromatic), 8.25 (1H, dd, *J*=7.8, 1.5 Hz, aromatic). MS (FAB) *m/z*: 555 (M+H). HRMS for $C_{30}H_{27}N_4O_7$ (M+H) calcd 555.1879, found 555.1856.

12c: 20 mg (quantitative) as a colorless powder. Mp 90–95 °C (dec). ^{1}H NMR (CD_3OD) δ [(9*S**)-isomer] 2.0–2.2 (4H, m, $NCH_2CH_2 \times 2$), 3.0–3.4 (6H, m, $NCH_2 \times 3$), 3.5–3.7 (2H, m, $CONHCH_2$), 4.21 (1H, d, *J*=2.9 Hz, epoxide), 5.72 (1H, s, propargylic), 5.87 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.98 (1H, d, *J*=10.2 Hz, NCHC≡CCH=CH), 5.99 (1H, m, NCH), 7.3–7.9 (6H, m, aromatic), 8.15 (1H, dd, *J*=8.3, 1.5 Hz, aromatic), 8.80 (1H, s, aromatic). [(9*R**)-isomer] 2.0–2.2 (4H, m, $NCH_2CH_2 \times 2$), 3.0–3.4 (6H, m, $NCH_2 \times 3$), 3.5–3.7

(2H, m, CONHCH$_2$), 4.38 (1H, d, J=2.9 Hz, epoxide), 5.96 (2H, s, C≡CCH=CH), 5.99 (1H, m, NCH), 6.54 (1H, s, propargylic), 7.3–7.9 (6H, m, aromatic), 8.32 (1H, dd, J=8.3, 1.5 Hz, aromatic), 8.80 (1H, s, aromatic). MS (FAB) m/z: 555 (M+H). Anal. calcd for $C_{30}H_{26}N_4O_7 \cdot HCl \cdot H_2O$: C, 59.16; H, 4.80; N, 9.20; found: C, 58.90; H, 5.05; N, 8.97.

Mixture of 2-nitrophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(1-azabicyclo[3.3.0]oct-5-ylmethylaminocarboxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclo-dodeca-5-ene-3,7-diyne-1-carboxylate and (9*R**)-isomer (11d) and its hydrochlorides (12d)**. Starting from 50 mg of **7**, the crude products were purified by column chromatography (silica gel, AcOEt/MeOH=9:1) to give **11d** (32 mg, 49%, ca. 2:1 mixture of diastereomers) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ [(9*S**)-isomer] 1.5–1.9 (8H, m, NCH$_2$CH$_2$CH$_2$ × 2), 2.5–2.7 and 2.9–3.1 (4H, m, NCH$_2$ × 2), 3.1–3.2 (2H, m, CONHCH$_2$), 3.99 (1H, d, J=2.9 Hz, epoxide), 5.58 (1H, s, propargylic), 5.73 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.85 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.96 (1H, m, NCH), 7.2–7.5 (4H, m, aromatic), 7.6–7.8 (2H, m, aromatic), 8.12 (1H, dd, J=8.3, 1.5 Hz, aromatic), 8.24 (1H, dd, J=8.3, 1.5 Hz, aromatic). [(9*R**)-isomer] 1.5–1.9 (8H, m, NCH$_2$CH$_2$CH$_2$ × 2), 2.5–2.7 and 2.9-3.1 (4H, m, NCH$_2$ × 2), 3.1–3.2 (2H, m, CONHCH$_2$), 4.32 (1H, d, J=2.9 Hz, epoxide), 5.82 (2H, s, C≡CCH=CH), 5.90 (1H, m, NCH), 6.44 (1H, s, propargylic), 7.2–7.5 (4H, m, aromatic), 7.6–7.8 (2H, m, aromatic), 8.12 (1H, dd, J=8.3, 1.5 Hz, aromatic), 8.24 (1H, dd, J=8.3, 1.5 Hz, aromatic). MS (FAB) m/z: 581 (M+H). HRMS for $C_{32}H_{29}N_4O_7$ (M+H) calcd 581.2036, found 581.2018.

12d: 20 mg (quantitative) as a colorless powder. Mp 85–90 °C (dec). ^{1}H NMR (CD_3OD) δ [(9*S**)-isomer] 1.9–2.3 (8H, m, NCH$_2$CH$_2$CH$_2$ × 2), 3.2–3.4 and 3.5–3.8 (6H, m, NCH$_2$ × 2 and CONHCH$_2$), 4.22 (1H, d, J=2.9 Hz, epoxide), 5.75 (1H, s, propargylic), 5.87 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.99 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 6.00 (1H, m, NCH), 7.3–7.7 (4H, m, aromatic), 7.7–7.9 (2H, m, aromatic), 8.15 (1H, dd, J=8.3, 1.5 Hz, aromatic). [(9*R**)-isomer] 1.9–2.3 (8H, m, NCH$_2$CH$_2$CH$_2$ × 2), 3.2–3.4 and 3.5–3.8 (6H, m, NCH$_2$ × 2 and CONHCH$_2$), 4.40 (1H, d, J=2.9 Hz, epoxide), 5.97 (2H, s, C≡CCH=CH), 6.00 (1H, m, NCH), 6.57 (1H, s, propargylic), 7.3–7.7 (4H, m, aromatic), 7.7–7.9 (2H, m, aromatic), 8.32 (1H, dd, J=8.3, 1.5 Hz, aromatic). MS (FAB) m/z: 581 (M+H). Anal. calcd for $C_{32}H_{28}N_4O_7 \cdot HCl \cdot H_2O$: C, 60.52; H, 4.92; N, 8.82. found: C, 60.32; H, 5.20; N, 8.55.

4-Nitrophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(2-(*N*,*N*-dimethylaminoethyl)aminocarboxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate (13b) and its hydrochloride (14b)**. Starting from 50 mg of **8**, the crude product was purified by column chromatography (silica gel, AcOEt/MeOH=9:1) to give **13b** (24 mg, 44%) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ 2.30 (6H, s, NMe$_2$), 2.53 (2H, m, CH$_2$NMe$_2$), 3.3–3.5 (2H, m, CONHCH$_2$), 4.00 (1H, d, J=2.9 Hz, epoxide), 5.61 (1H, s, propargylic), 5.73 (1H, dd, J=10.2, 1.5 Hz, NCHC≡CCH=CH), 5.82 (1H, br, CONH), 5.86 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.97 (1H, d, J=1.5 Hz, NCH), 7.3–7.5 (5H, m, aromatic), 8.3–8.4 (3H, m, aromatic). MS (FAB) m/z: 529 (M+H). HRMS for $C_{28}H_{25}N_4O_7$ (M+H) calcd 529.1723, found 529.1738.

14b: 20 mg (95%) as a colorless powder. Mp 105–108 °C (dec). ^{1}H NMR (CD_3OD) δ 2.96 (6H, s, NMe$_2$), 3.2–3.3 (2H, m, CH$_2$NMe$_2$), 3.5–3.7 (2H, m, CONHCH$_2$), 4.22 (1H, d, J=2.9 Hz, epoxide), 5.71 (1H, s, propargylic), 5.85 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.86 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 6.02 (1H, m, NCH), 7.3–7.7 (4H, m, aromatic), 8.3–8.4 (4H, m, aromatic). MS (FAB) m/z: MS (FAB) m/z: 529 (M+H). Anal. calcd for $C_{28}H_{24}N_4O_7 \cdot HCl \cdot H_2O$: C, 57.69; H, 4.67; N, 9.61; found: C, 57.43; H, 4.95; N, 9.38.

4-Nitrophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(2-(pyrrolidino)ethylaminocarboxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclododeca-5-ene-3,7-diyne-1-carboxylate (13c) and its hydrochloride (14c)**. Starting from 50 mg of **8**, the crude product was purified by column chromatography (silica gel, AcOEt/MeOH=9:1) to give **13c** (24 mg, 40%) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ 1.7–1.9 (4H, m, NCH$_2$CH$_2$ × 2), 2.5–2.7 (6H, m, NCH$_2$ × 3), 3.3–3.5 (2H, m, CONHCH$_2$), 4.00 (1H, d, J=2.9 Hz, epoxide), 5.60 (1H, s, propargylic), 5.67 (1H, br, CONH), 5.72 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.86 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.96 (1H, d, J=1.5 Hz, NCH), 7.3–7.5 (5H, m, aromatic), 8.3–8.4 (3H, m, aromatic). MS (FAB) m/z: 555 (M+H). HRMS for $C_{30}H_{27}N_4O_7$ (M+H) calcd 555.1879, found 555.1896.

14c: 20 mg (quantitative) as a colorless powder. Mp 101–104 °C (dec). ^{1}H NMR (CD_3OD) δ 2.0–2.2 (4H, m, NCH$_2$CH$_2$ × 2), 3.2–3.4 (6H, m, NCH$_2$ × 3), 3.5–3.7 (2H, m, CONHCH$_2$), 4.22 (1H, d, J=2.9 Hz, epoxide), 5.71 (1H, s, propargylic), 5.86 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.98 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 6.03 (1H, d, J=1.5 Hz, NCH), 7.3–7.6 (4H, m, aromatic), 8.3–8.4 (4H, m, aromatic). MS (FAB) m/z: 555 (M+H). Anal. calcd for $C_{30}H_{26}N_4O_7 \cdot HCl \cdot H_2O$: C, 59.16; H, 4.80; N, 9.20; found: C, 58.98; H, 5.00; N, 9.03.

4-Nitrophenyl (2*R,5*Z*,9*S**,10*S**,16*R**)-(±)-9-(1-azabicyclo[3.3.0]oct-5-ylmethylaminocarboxy)-10,2,10-(epoxymetheno)-1-benz[*b*]azacyclo-dodeca-5-ene-3,7-diyne-1-carboxylate (13d) and its hydrochloride (14d)**. Starting from 40 mg of **8**, the crude product was purified by column chromatography (silica gel, AcOEt/MeOH=4:1) to give **13d** (29 mg, 62%) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ 1.5–1.9 (8H, m, NCH$_2$CH$_2$CH$_2$ × 2), 2.5–2.7 and 2.9–3.1 (4H, m, NCH$_2$ × 2), 3.20 (2H, m, CONHCH$_2$), 4.00 (1H, d, J=2.9 Hz, epoxide), 5.60 (1H, s, propargylic), 5.72 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.85 (1H, br, CONH), 5.86 (1H, d, J=10.2 Hz, NCHC≡CCH=CH), 5.96 (1H, d, J=1.5 Hz, NCH), 7.3–7.5 (5H, m, aromatic), 8.3–8.4 (3H, m, aromatic). MS (FAB) m/z:

581 (M+H). HRMS for $C_{32}H_{29}N_4O_7$ (M+H) calcd 581.2036, found 581.2056.

14d: 20 mg (95%) as a colorless powder. Mp 110–112 °C (dec). ^{1}H NMR (CD_3OD) δ 1.9–2.3 (8H, m, $NCH_2\underline{CH_2}\underline{CH_2} \times 2$), 3.2–3.4 and 3.6–3.7 (6H, m, $NC\underline{H_2} \times 2$ and $CONHC\underline{H_2}$), 4.22 (1H, d, J=2.9 Hz, epoxide), 5.74 (1H, s, propargylic), 5.86 (1H, d, J=10.2 Hz, $NCHC{\equiv}CC\underline{H}{=}CH$), 5.99 (1H, d, J=10.2 Hz, $NCHC{\equiv}CCH{=}C\underline{H}$), 6.02 (1H, m, $NC\underline{H}$), 7.3–7.6 (4H, m, aromatic), 8.3–8.4 (4H, m, aromatic). MS (FAB) *m/z*: 581 (M+H). Anal. calcd for $C_{32}H_{28}N_4O_7{\cdot}HCl{\cdot}H_2O$: C, 60.52; H, 4.92; N, 8.82; found: C, 60.45; H, 5.14; N, 8.63.

Biological assays

DNA-cleaving assay. Supercoiled ΦX174 DNA (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 residual activity versus drug concentration.

In vivo antitumor activity. For the evaluation of the antitumor activity against P388 leukemia, CDF_1 mice were intraperitoneally inoculated with 1×10^6 cells/mouse of P388 on day 0, and 2 mg/kg of test compound was intraperitoneally administered 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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30. For the studies on the in vivo antitumor activity of dynemicin A analogues, see refs 11c, 23, 25, 26i, 26j, and 26k.

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