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DEVELOPMENTS OF ENEDIYNE MODEL COMPOUNDS GENERATING BIRADICALS WITH AN ENHANCED RADICAL CHARACTER – REGULATION OF TRIGGERING DEVICES‡

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Abstract – Development of enediyne model compounds generating biradicals with an enhanced radical character is described. These model compounds generate enyne-allenes under mild acidic conditions and the resulting biradicals showed potent DNA cleaving abilities.

INTRODUCTION

The neocarzinostatin chromophore (NCS-Chr) contains a diene–diyne unit that undergoes a triggered rearrangement to an enyne–cumulene intermediate, which immediately produces a reactive biradical species responsible for DNA damage *in vitro*.[1] Studies of the cycloaromatization of enyne–allenes (Myers–Saito reaction) are of fundamental importance not only in the mechanistic study of NCS-Chr, but also toward the design of simple and bio-active NCS-Chr analogues.[2] As a model study of the chemical reactions of NCS-Chr, we have prepared several enediyne models possessing a characteristic triggering device which initiates the generation of enyne–allene or enyne-cumulene intermediates and ultimately produces dehydrotoluene biradicals.[3] During our studies, we found the biradical intermediates have a polar character, and the reactions afford products arising from both dipolar (in protic solvents) and biradical (in aprotic solvents) pathways (Figure 1). A polar biradical intermediate for the cycloaromatization process was first proposed in the pioneering works of Myers, and was theoretically

studied by Squires.[4] According to the research of these groups, cycloaromatization of an enyne-allene affords the singlet biradical best described as a linear combination of the resonance forms (**I**) and (**II**) (Figure 2).

Figure 1

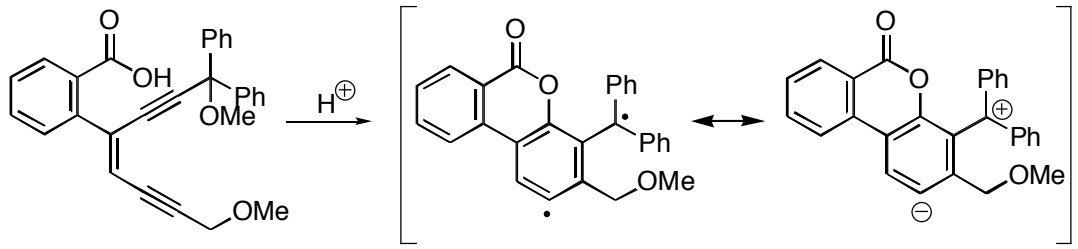
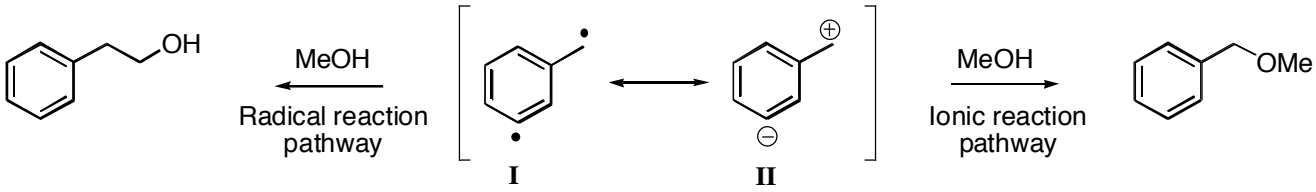
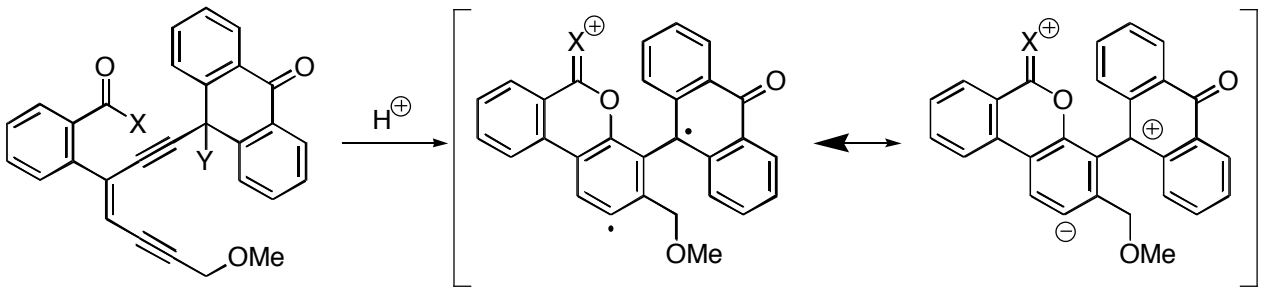


Figure 2



The ionic character of the intermediate is presumed to reduce the DNA-damaging ability and other biological activities.[5] It is, therefore important to develop a model system in which the contribution of the ionic resonance structure (**II**) is reduced. To overcome this difficulty, we have developed enediyne model compounds generating a □,3-didehydrotoluene biradical bearing a electron-withdrawing group on the benzylic position to destabilize the zwitterionic state, and recently reported that such enediynes showed enhanced radical character.[6] Although these enediynes show potent DNA-cleaving activities, their efficiency of biradical formation and their bio-activities were not necessarily adequate. So, to improve biradical formation efficiencies, we attempted to modify the triggering devices of these enediynes. Recently, we reported Here we report the synthesis, reactions, and DNA damaging abilities of synthetic enediynes (**1**-**5**) (Figure 3).

Figure 3



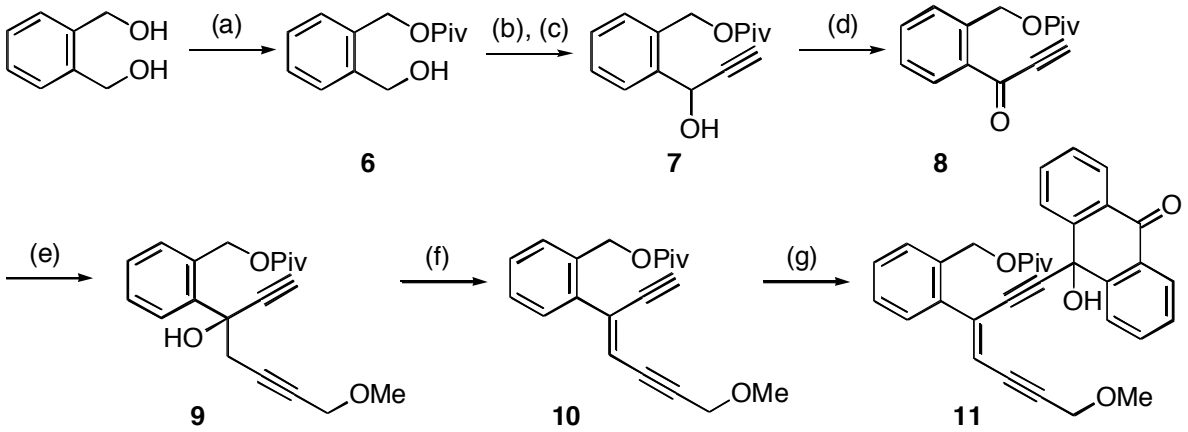
1: X = OH, Y = OMe; **2**: X = OMe, Y = OMe; **3**: X = NMe_2, Y = OMe; **4**: X = OMe, Y = OAc; **5**: X = NMe_2, Y = OAc

RESULTS AND DISCUSSIONS

Synthesis of Enediynes (1-5)

Targeting enediynes (**1-5**) were synthesized as described bellow. Common precursor (**11**) was prepared as shown in Scheme 1. Commercially available 1,2-benzenedimethanol was converted to ester (**6**). Oxidation of ester (**6**) using NaClO gave an unstable aldehyde, which was immediately treated with ethynylmagnesium bromide to give alcohol (**7**). Alcohol (**7**) was oxidized using Jones oxidant and the resulting ketone (**8**) was treated with Zn powder and 4-methoxy-2-butynyl bromide to afford diyne (**9**), which was dehydrated with Et_3N and MsCl to give enediyne (**10**) selectively.[7] Enediyne (**10**) was treated with EtMgBr and anthraquinones sequentially to give common precursor (**11**).

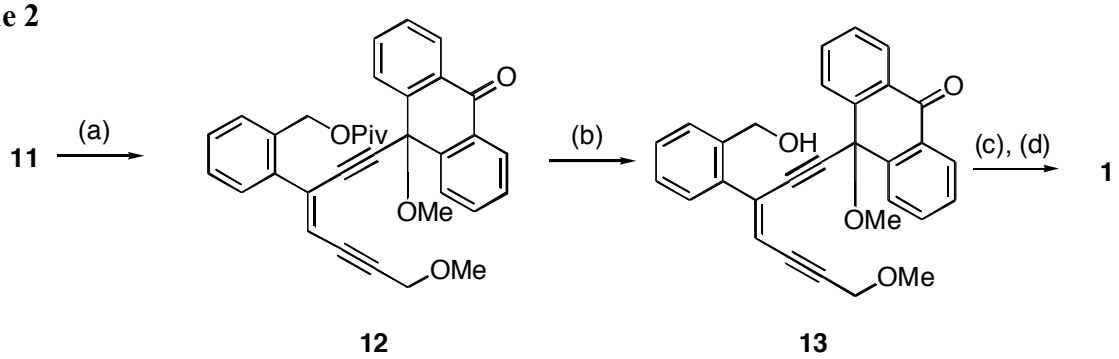
Scheme 1



Reagents and conditions: (a) NaH, PivCl / THF, 0 ˚C, 62%; (b) NaClO, $(n\text{-Bu})_4N^+Br^-$ / AcOEt-H_2O, 0 ˚C; (c) Ethynylmagnesium bromide / THF, 0 ˚C, 82% (2 steps); (d) CrO_3-H_2SO_4 / Acetone, 0 ˚C, 87%; (e) 4-Methoxy-2-butynyl bromide, Zn / THF, reflux, 73%; (f) MsCl, Et_3N / CH_2Cl_2, 0 ˚C, 81%; (g) EtMgBr, anthraquinone / THF-CH_2Cl_2, reflux, 52%.

Enediyne (**11**) was converted to methyl ether (**12**) using Me_2SO_4 in the presence of aqueous NaOH and tetrabutylammonium iodide in benzene. After removal of the pivaloyl group, the resulting alcohol (**13**) was sequentially oxidized to enediyne-acid (**1**) (Scheme 2).

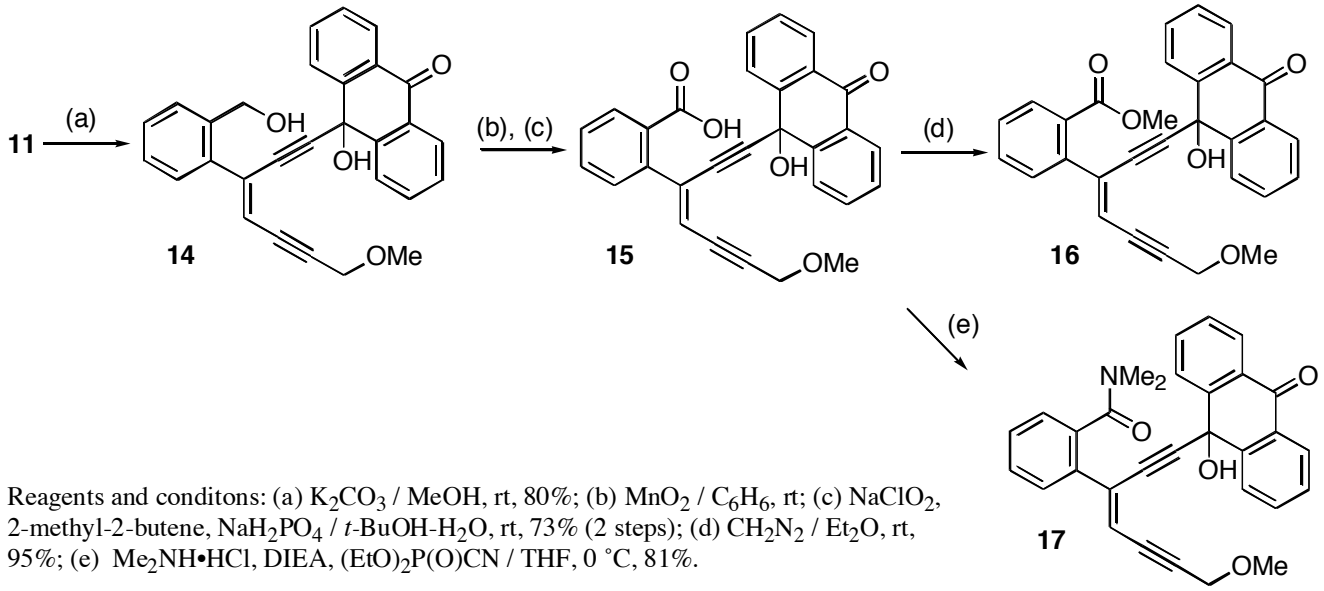
Scheme 2



Reagents and conditions: (a) Me_2SO_4, NaOH (aq), $(n\text{-Bu})_4N^+I^-$ / C_6H_6, rt, 96%; (b) K_2CO_3 / MeOH, rt, 94%; (c) MnO_2 / C_6H_6, rt; (d) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4 / *t*-BuOH-H_2O, rt, 85% (2 steps).

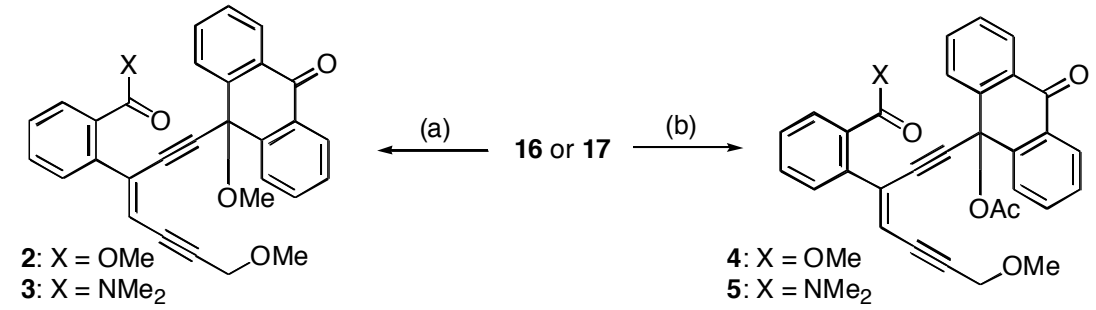
Furthermore, ester-enediynes and amide-enediynes (**2-5**) were also synthesized from enediyne (**11**) as a common intermediate, in a similar fashion. Enediyne (**11**) was hydrolyzed to remove the pivaloyl moiety and the resulting diol (**14**) was oxidized to acid (**15**) in two steps. Acid (**15**) was converted to ester (**16**) and amide (**17**) by conventional methods. Enediyne (**16**) was treated with Me_2SO_4 in the presence of aqueous NaOH and tetrabutylammonium iodide in benzene to give enediyne **(2**) and was acetylated to acetate (**4**) using acetic anhydride. Amide-enediynes (**3**) and (**5**) were prepared from (**17**) using methods similar to those used in preparation of enediynes (**2)** and (**4**).

Scheme 3



Reagents and conditons: (a) K_2CO_3 / MeOH, rt, 80%; (b) MnO_2 / C_6H_6, rt; (c) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4 / *t*-BuOH-H_2O, rt, 73% (2 steps); (d) CH_2N_2 / Et_2O, rt, 95%; (e) Me_2NH•HCl, DIEA, $(EtO)_2P(O)CN$ / THF, 0 °C, 81%.

Scheme 4



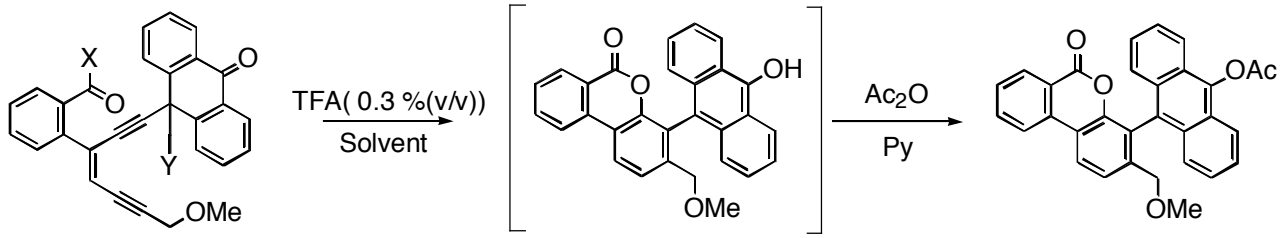
Reagents and conditions: (a) Me_2SO_4, NaOH (aq), $(n\text{-}Bu)_4N^+I^-$ / C_6H_6, rt, 92% for **2** and 89% for **3**; (b) AcOH, EDC, DIEA / CH_2Cl_2, rt, 85% for **4** and 82% for **5**.

Cycloaromatization Reactions of Synthesized Enediynes (1-5)

The cycloaromatization reactions of enediynes **(1-5)** were carried out in the presence of 0.3 %(v/v) of TFA with 50 equiv. of 1,4-cyclohexadiene as a radical trapping agent in the indicated solvent. The results are summarized in Table 1. Cycloaromatization reactions of enediyne (**1**) were carried out in MeOH or benzene with 50 equiv. of 1,4-cyclohexadiene (1,4-CHD) to give lactone (**19**), which was obtained as mixture of tautomers showing broadened signals in ^{1}H-NMR spectral measurements, and then was converted to acetate (**20**) to confirm the structure. In all reactions carried out, no products arising

from zwitterions such as (**21a**) and **(21b**) were obtained.

Table 1. The Cycloaromatization Reactions of Enediynes (1-5).



1: X = OH, Y = OMe; **2**: X = OMe, Y = OMe;
3: X = NMe_2, Y = OMe; **4**: X = OMe, Y = OAc;
5: X = NMe_2, Y = OAc

19 **20**

Entry	Enediyne	Solvent	Time (m)	Products [b), c)]
1	**1**	Benzene	< 5	**20** (87%) [a)]
2	**1**	Methanol	150	**20** (73%) [a)]
3	**2**	Benzene	60	**20** (41%)
4	**2**	Methanol	> 300	**20** (8%)[d)]
5	**3**	Benzene	250	**20** (32%)
6	**3**	Methanol	> 300	**20** (trace)[d)]
7	**4**	Benzene	< 5	**20** (28%)
8	**4**	Methanol	< 5	**20** (32%), **2**(23%)
9	**5**	Benzene	30	**20** (18%)
10	**5**	Methanol	60	**20** (10%), **3** (71%)

All Reactions were performed in the presence of 1,4-CHD (50 equiv.) and 0.3 %(v/v) of TFA at 37 ˚C in solvents indicated. a) See ref. 6. b) Indicated values are isolated yields. c) (**19**) was obtained as a mixture of tautomeric isomers and then converted to acetates (**20**) to confirm the structures. d) 80% of the starting enediyne was recovered.

These results indicate that the □-radical part of the biradical states of intermediates should be stabilized by resonance, and as a result enhanced radical character would be observed (Figure 4). Next we examined the cycloaromatization reactions of the ester-enediyne (**2**) and amide-enediyne (**3**) (Table 1, Entries 3-6) in benzene. Although these enediynes cycloaromatized in a similar fashion to give aromatized product (**19**), a considerably prolonged reaction time was needed (5 min vs. 60 min for **2**, and 250 min for **3**) in benzene. When the same reactions were carried out in MeOH, most of enediynes (**2**) and (**3**) was recovered along with the small amounts of cycloaromatized product (**20**) (Table 1, Entries 4 and 6) even after 5 h. To facilitate the cascade reaction aiming at improving the efficiency of biradical formation, we prepared more reactive acetate-enediynes (**4**) and (**5**). As was expected, the cascade reactions of enediynes (**4**) and (**5**) were considerably accelerated, compared with those of (**2**) and (**3**), giving aromatized product (**19)** in benzene. When the reactions of enediynes (**4)** and (**5**) were carried out in

MeOH, enediynes (**2**) and (**3**) were obtained along with the aromatized product (**19**) (Table 1, Entries 8 and 10). These results indicate that intermediary allenes (**22**) and (**23**) are prone to suffer from nucleophilic attack by solvent MeOH followed by the ring opening reaction as shown in Figure 5. The prolonged reaction time observed in the cyclization reactions of enediynes (**2**) and (**3**) compared with (**1**) is also rationalized by considering this ring opening, that is, facile reformation of (**2**) and (**3**) from the intermediary enyne-allene in solvent MeOH lowered the net reaction rates of the cascade reactions. Even in benzene, MeOH is released in the reaction course causing the reformation of (**2**) and (**3**), therefore the retardation of the reactions also occurred.

Figure 4.

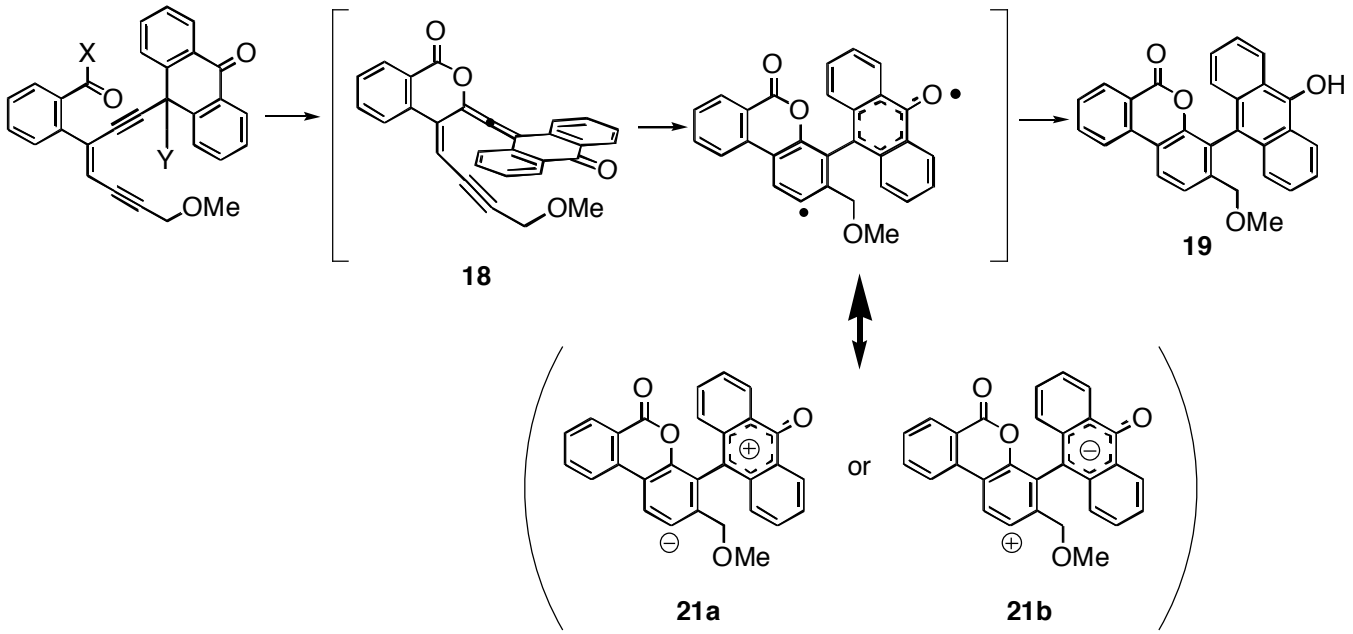
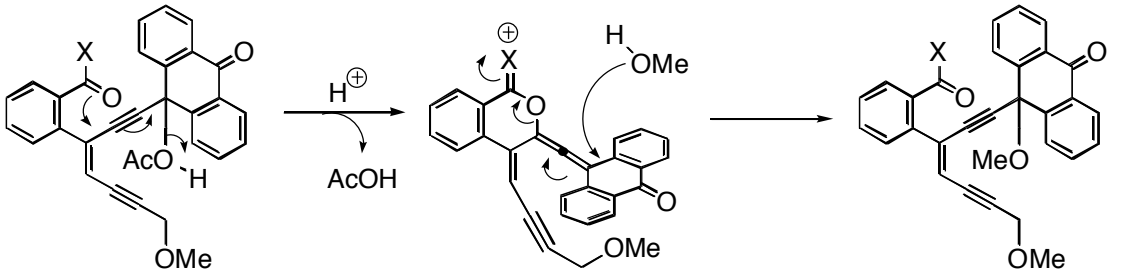


Figure 5.



4: X = OMe; **5**: X = NMe_2 **22**: X = OMe; **23**: X = NMe_2 **2**: X = OMe; **3**: X = NMe_2

DNA-damaging abilities of enediynes (1-5).

DNA strand cleavage by synthetic enediynes (**1-5**) was estimated on agarose gels by conversion of covalently closed circular DNA (Form I) to open circular DNA (Form II) and the results are summarized in Table 2. All enediynes showed DNA damaging activity under acidic conditions (pH 6.0) as was expected. In all experiments, the enediyne drugs were not consumed completely and trace amounts of

aromatized products were detected on TLC. Although enediynes (**2**) and (**3**) showed only weak activities, (**1**), and in particular, **(4**) and **(5)** exhibited relatively potent DNA-cleaving abilities. The lack of DNA damaging ability exhibited by enediynes (**2**) and (**3**) can be attributed to the inadequate cycloaromatization efficiencies of these compounds. Interestingly, although enediynes (**4**) and (**5**) showed considerably higher DNA cleaving ability than enediyne (**1**), the biradical formation was not necessarily more efficient than enediyne (**1**). Compared to enediyne (**1**), enediynes (**4**) and (**5**) would afford biradicals faster, however the total amounts of generated biradicals may be less than for enediyne (**1**) due to the formation of less bioactive enediynes (**16**) and (**17**) in a similar fashion as described in Figure 5.[8] We consider that the observed bioactivities of the synthetic enediynes can be attributed to oxidative strand cleavage caused by biradicals and therefore drugs which generate biradicals more rapidly would be more effective. The results mentioned above may, however, be also explained by considering the existence of the alkylation mechanisms in which the intermediary enyne-allenes act as electron-deficient acceptors (Figure 4). In the alkylation pathway, intermediaries (**22**) and (**23**) should be more reactive toward a nucleophilic attack than the lactone-allene derived from **1**, so we can't exclude this possibility.[9]

Table 2. DNA Damaging Abilities of Synthetic Enediynes (1-5).

Entry	Enediyne	%Cleavage[a)]
1	**1**	36
2	**2**	< 10
3	**3**	< 10
4	**4**	83
5	**5**	95

Col E1 DNA (12.5 □g/ml) was incubated for 6 h at 37 ˚C with enediynes (**1-5**) (1.0 mM) in pH 6.0 phosphate buffers, and analyzed by electrophoresis (1% agarose gel, ethidium bromide staining).
a) Indicated values are mean value of three runs. A Control reaction mixture without the addition of any drug was incubated and the mean value of three runs was used as the background to be subtracted from the obtained values.

CONCLUSION

In conclusion, aiming at the improvement of DNA damaging activities of synthetic enediynes, we attempted to regulate the reactivity of triggering devices of enediyne model compounds and demonstrated that enediynes having an acetoxy group as a leaving group showed enhanced radical character and exhibit potent DNA damaging abilities. We now consider that this high bioactivity can be attributed to facile biradical generation from these enediynes, however, the participation of an intermediary enyne-allene can't be ruled out.

ACKNOWLEDGEMENT

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EXPERIMENTAL

General. ^{1}H-NMR spectra were measured in $CDCl_3$ solution and referenced to TMS (0.00 ppm) using JEOL JNM-AL400 (400 MHz) and JEOL JNM-AL300 (300 MHz) spectrometers, unless otherwise noted. ^{13}C-NMR spectra were measured in $CDCl_3$ solution and referenced to $CDCl_3$ (77.0 ppm) using JEOL JNM-AL400 (100 MHz) and JEOL JNM-AL300 (75 MHz) spectrometers. IR spectra were recorded on JASCO FT/IR-420 and Perkin-Elmer 1720 FT-IR spectrophotometer. MS spectra were obtained on a JEOL JMS-DX303 and JMS-SX102A. Mps were obtained on YAMATO-MODEL20 melting point apparatus and were uncorrected. Column chromatography was performed on silica gel, KANTO KAGAKU N-60. Thin-layer chromatography was performed on precoated plates (0.25 mm, silica gel Merck Kieselgel 60 F254). All reactions were performed in oven-dried glassware under positive pressure of argon, unless otherwise noted. Reaction mixtures were stirred magnetically.

10-Methoxy-10-[7-methoxy-3-(2-hydroxycarbonylphenyl)hept-3-ene-1,5-diynyl]-10*H*-anthracen-9-one (1).

To a stirred solution of alcohol (**13**) (270 mg, 0.602 mmol) in benzene (6 mL) was added MnO_2 (1.05 g, 12.04 mmol) and the solution was stirred for 10 h at rt. The mixture was filtered and the filtrate was evaporated to give a crude aldehyde, which was used without further purification. The aldehyde was dissolved in *t*-BuOH (4.3 mL) and to this solution were added water (1.1 mL), 2-methyl-2-butene (0.29 mL, 2.71 mmol), NaH_2PO_4 (72 mg, 0.60 mmol) and $NaClO_2$ (191 mg, 2.11 mmol) at rt. The mixture was stirred for 24 h and poured into saturated aqueous NH_4Cl. The mixture was extracted twice with AcOEt and the combined organic layer was washed with brine, dried over $MgSO_4$ and was concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane/AcOEt = 2/1) to give desired acid (**1**) (269 mg, 85 %) as pale yellow needle; mp 102-104 ˚C (hexane/AcOEt, decomp); ^{1}H-NMR (acetone-d_6, 400 MHz) □ 8.20 (2H, dd, *J* = 7.0 and 1.0 Hz), 8.13 (2H, dd, *J* = 7.0 and 1.0 Hz), 7.90 (1H, dd, *J* = 7.5 and 1.0 Hz), 7.80 (2H, dt, *J* = 7.0 and 1.0 Hz), 7.63 (2H, dt, *J* = 7.0 and 1.0 Hz), 7.57 (1H, dt, *J* = 7.5 and 1.0 Hz), 7.50 (1H, dt, *J* = 7.5 and 1.0 Hz), 7.44 (1H, dd, *J* = 7.5 and 1.0 Hz), 6.20 (1H, t, *J* = 2.0 Hz), 4.26 (2H, d, *J* = 2.0 Hz), 3.27 (3H, s), 2.93 (3H, s); ^{13}C-NMR (acetone-d_6, 100 MHz) □ 183.4 (C), 168.4 (C), 141.6 (C), 139.5 (C), 135.0 (C), 134.7 (CH), 132.8 (CH), 132.0 (C), 131.8 (C), 131.2 (CH), 130.7 (CH), 130.3 (CH), 130.2 (CH), 129.7 (CH), 127.7 (CH), 118.9 (CH), 96.6 (C), 94.9 (C), 87.3 (C), 85.0 (C), 73.5 (C), 60.8 (CH_2), 57.8 (CH_3), 52.0 (CH_3); FTIR (KBr): 2822, 2663, 1688 cm^{-1}; HRMS (FAB) calcd for $C_{30}H_{22}O_5$ (M^+): 462.1467, found: 461.1417 ($[M-H]^+$). Anal. Calcd for $C_{30}H_{22}O_5$: C, 77.91; H, 4.79. Found: C, 77.65; H, 5.02.

10-Methoxy-10-[7-methoxy-3-(2-methoxycarbonylphenyl)hept-3-ene-1,5-diynyl]-10*H*-anthracen-9-

one (2).

To a stirred solution of alcohol (**16**) (40 mg, 0.087 mmol) in benzene (4 mL) were added sequentially (n-Bu)$_4$N$^+$I$^-$ (2.0 mg), Me_2SO_4 (0.016 mL, 0.17 mmol) and aqueous NaOH (12.5 M, 0.01 mL, 0.125 mmol). The mixture was stirred for 2 h, poured into water and extracted twice with AcOEt. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane/AcOEt = 4/1) to give desired enediyne (**2**) (38 mg, 92%) as pale yellow amorphous solid; ^{1}H-NMR ($CDCl_3$, 300 MHz) 8.28 (2H, dd, J = 7.7 and 0.9 Hz), 8.04 (2H, d, J = 7.7 Hz), 7.78 (1H, dd, J = 7.5 and 1.1 Hz), 7.71 (2H, td, J = 7.7 and 0.9 Hz), 7.55 (2H, t, J = 7.7 Hz), 7.47 (1H, td, J = 7.5 and 1.1 Hz), 7.39 (1H, td, J = 7.5 and 0.8 Hz), 7.30 (1H, dd, J = 7.5 and 1.1 Hz), 6.03 (1H, t, J = 1.8 Hz), 4.15 (2H, d, J = 1.8 Hz), 3.64 (3H, s), 3.31 (3H, s), 2.87 (3H, s); ^{13}C-NMR ($CDCl_3$, 75 MHz) 183.0 (C), 167.8 (C), 140.7 (C), 138.3 (C), 133.9 (C), 133.8 (CH), 131.8 (CH), 131.2 (C), 130.5 (C), 130.3 (CH), 129.5 (CH), 129.2 (CH), 129.1 (CH), 128.6 (CH), 127.2 (CH), 118.1 (CH), 96.5 (C), 93.5 (C), 85.0 (C), 84.2 (C), 72.1 (C), 60.4 (CH_2), 57.6 (CH_3), 52.2 (CH_3), 51.4 (CH_3); FTIR (KBr): 2361, 1725, 1670 cm^{-1}; HRMS (FAB) calcd for $C_{31}H_{24}O_5$ (M^+): 476.1624, found: 499.1513 ([M+Na]$^+$). Anal. Calcd for $C_{31}H_{24}O_5$: C, 78.14; H, 5.08. Found: C, 78.10; H, 5.28.

10-Methoxy-10-[7-methoxy-3-(2-*N*,*N*-dimethylaminocarbonylphenyl)hept-3-ene-1,5-diynyl]-10*H*-anthracen-9-one (3)

A similar procedure described in (**2**) was employed and (**3**) was obtained in 89% from (**17**) as pale yellow powder; mp 102-104 ˚C (hexane/AcOEt, decomp); ^{1}H-NMR ($CDCl_3$, 300 MHz) 8.30 (2H, dd, J = 8.0 and 1.0 Hz), 8.09 (2H, dd, J = 8.0 and 1.0 Hz), 7.73 (2H, td J = 8.0 and 1.0 Hz), 7.57 (2H, td, J = 7.0 and 1.0 Hz), 7.45-7.20 (4H, m), 6.18 (1H, t, J = 1.8 Hz), 4.12 (2H, d, J = 1.8 Hz), 3.29 (3H, s), 2.94 (3H, s), 2.90 (3H, s), 2.67 (3H, s); ^{13}C-NMR ($CDCl_3$, 75 MHz) 182.8 (C), 170.3 (C), 140.7 (C), 135.1 (C), 134.3 (C), 133.9 (CH), 131.3 (C), 131.2 (C), 129.3 (CH), 129.1 (CH), 129.1 (CH), 128.9 (CH), 128.7 (CH), 127.5 (CH), 127.2 (CH), 119.5 (CH), 96.8 (C), 94.5 (C), 84.8 (C), 84.3 (C), 72.1 (C), 60.3 (CH_2), 57.6 (CH_3), 51.4 (CH_3), 38.4 (CH_3), 34.8 (CH_3); FT-IR (KBr): 3021, 1669, 1661 cm^{-1}; HRMS (EI) calcd for $C_{32}H_{27}O_4N$ (M^+): 489.1940, found: 489.1972 (M^+). Anal. Calcd for $C_{32}H_{27}O_4N$: C, 78.51; H, 5.56; N, 2.86. Found: C, 78.33; H, 5.60; N, 2.65.

10-Acetoxy-10-[7-methoxy-3-(2-methoxycarbonylphenyl)hept-3-ene-1,5-diynyl]-10*H*-anthracen-9-one (4).

To a stirred solution of alcohol (**16**) (187 mg, 0.404 mmol) in CH_2Cl_2 (8 mL) were added EDC (620 mg, 3.24 mmol), DMAP (395 mg, 3.24 mmol) and AcOH (0.16 mL, 2.83 mmol) at 0 ˚C. The mixture was stirred for 1 h, poured into saturated aqueous NH_4Cl and extracted twice with AcOEt. The combined

organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane/AcOEt = 2/1) to give desired acetate (**4**) (173 mg, 85 %) as yellowish amorphous solid; ^{1}H-NMR (acetone-d_6, 300 MHz) □ 8.23 (2H, dd, *J* = 7.7 and 0.9 Hz), 7.96 (2H, dd, *J* = 7.7 and 0.9 Hz), 7.81-7.72 (3H, m), 7.64-7.33 (5H, m), 6.20 (1H, t, *J* = 2.0 Hz), 4.17 (2H, d, *J* = 2.0 Hz), 3.67 (3H, s), 3.29 (3H, s), 1.98 (3H, s); ^{13}C-NMR(acetone-d_6, 75 MHz) □ 182.7 (C), 168.7 (C), 168.1 (C), 142.3 (C), 138.6 (C), 135.0 (CH), 134.0 (C), 132.7 (CH), 131.6 (C), 130.8 (C), 130.8 (CH), 130.3 (CH), 130.0 (CH), 129.7 (CH), 128.1 (CH), 127.5 (CH), 119.6 (CH), 95.7 (C), 95.3 (C), 86.5 (C), 84.4 (C), 72.5 (C), 60.6 (CH_2), 57.7 (CH_3), 52.5 (CH_3), 21.5 (CH_3); FTIR (KBr): 1746, 1665 cm^{-1}; HRMS (FAB) calcd for $C_{32}H_{24}O_6$ (M^+): 504.1573, found: 527.1508 ([M+Na]$^+$).

10-Acetoxy-10-[7-methoxy-3-(2-dimethylaminocarbonylphenyl)hept-3-ene-1,5-diynyl]-10*H*-anthracen-9-one (5).

A similar procedure described in (**4**) was employed and (**5**) was obtained in 82% from (**17**) as pale yellow powder; mp 69-71 ˚C (hexane/AcOEt, decomp); ^{1}H-NMR (CD_3CN, 300 MHz) □ 8.23 (2H, dd, *J* = 7.7 and 1.1 Hz), 8.04 (2H, dd, *J* = 7.7 and 1.1 Hz), 7.79 (2H, dt, *J* = 7.7and 1.1 Hz), 7.60 (2H, dt, *J* = 7.7 and 1.1 Hz), 7.39-7.31 (3H, m), 7.22-7.19 (1H, m), 6.12 (1H, t, *J* = 2.0 Hz), 4.14 (2H, d, *J* = 2.0 Hz), 3.28 (3H, s), 2.82 (3H, s), 2.60 (3H, s), 1.99 (3H, s); ^{13}C-NMR (acetone-d_6, 75 MHz) □ 182.7 (C), 170.0 (C), 168.6 (C), 142.3 (C), 136.7 (C), 135.0 (CH), 132.4 (C), 130.8 (C), 130.0 (CH), 129.8 (CH), 129.7 (CH), 129.4 (CH), 128.2 (CH), 128.1 (CH), 127.4 (CH), 120.3 (CH), 96.0 (C), 86.6 (C), 84.4 (C), 84.3 (C), 72.5 (C), 60.5 (CH_2), 57.6 (CH_3), 38.4 (CH_3), 34.6 (CH_3), 21.6 (CH_3); FTIR (KBr): 2362, 1749, 1670, 1636 cm^{-1}; HRMS (FAB) calcd for $C_{33}H_{27}NO_5$ (M^+): 517.1889, found: 540.1769 ([M+Na]$^+$).

2-Hydroxymethylbenzyl pivalate (6).

To a stirred suspension of NaH (12.72 g, 60 wt%, 0.318 mol) in THF (300 mL) was added a solution of benzenedimethanol (39.88 g, 0.289 mol) in THF (200 mL) at 0 ˚C and the resulting mixture was stirred for 30 min. To the mixture was added pivaloyl chloride (39.2 mL, 0.328 mol) and was stirred overnight at ambient temperature. The reaction was quenched with saturated aqueous NH_4Cl and the resulting mixture was extracted twice with AcOEt. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane/AcOEt = 3/1) to give desired alcohol (**6**) (39.57 g, 62%) as pale yellow oil; ^{1}H-NMR ($CDCl_3$, 400 MHz) □ 7.50-7.20 (4H, m), 5.00 (2H, s), 4.17 (2H, s), 2.83 (1H, br s), 1.19 (9H, s); ^{13}C-NMR ($CDCl_3$, 75 MHz) □ 178.4 (C), 139.0 (C), 138.9 (C), 129.0 (CH), 128.5 (CH), 128.3 (CH), 127.7 (CH), 63.7 (CH_2), 62.5 (CH_2), 38.7 (C), 27.0 (CH_3); FTIR (neat): 3436, 2974, 1730, 1152 cm^{-1}; HRMS (FAB) calcd for $C_{13}H_{18}O_3$: 222.1256 (M^+), found: 245.1135 ([M+Na]$^+$). Anal. Calcd for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16.

Found: C, 69.85; H, 8.08.

2-(1-Hydroxy-2-propynyl)benzyl pivalate (7).

To a stirred solution of alcohol **6** (49.40g, 0.222 mol) in AcOEt (300 mL) were added tetrabutylammonium bromide (10.75 g, 33.3 mmol) and aqueous NaClO (10%, 330 mL) at 0 ˚C. The resulting mixture was stirred for 4 h at rt and extracted twice with AcOEt. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give 39.6 g of desired aldehyde, which was used without further purification. A solution of the crude aldehyde (39.6 g) in THF (300 mL) was added to a stirred solution of ethynylmagnesium bromide [prepared from Mg (5.35 g, 0.220 mol), 18.7 mL of EtBr (0.250 mol) and acetylene gas] in THF (300 mL) at 0 ˚C and the resulting solution was stirred for 30 min. The mixture was poured into saturated aqueous NH_4Cl and extracted twice with AcOEt. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane/AcOEt = 4/1) to give desired alcohol (**7**) (44.6 g, 82%) as pale yellow oil; ^{1}H-NMR ($CDCl_3$, 400 MHz) 7.73-7.67 (1H, m), 7.41-7.30 (3H, m), 5.68 (1H, d, *J* = 2.0 Hz), 5.34 (1H, d, *J* = 13.5 Hz), 5.25 (1H, d, *J* = 13.5 Hz), 3.54 (1H, br s), 2.66 (1H, d, *J* = 2.0 Hz), 1.21 (9H, s); ^{13}C-NMR ($CDCl_3$, 75 MHz) 178.2 (C), 137.9 (C), 133.5 (C), 128.9 (CH), 128.4 (CH), 128.3 (CH), 127.1 (CH), 82.9 (C), 74.8 (CH), 63.3 (CH_2), 61.4 (CH), 38.5 (C), 26.8 (CH_3); FTIR (neat): 3438, 3290, 2973, 1724, 1480 cm^{-1}; HRMS (FAB) calcd for $C_{15}H_{18}O_3$: 246.1256 (M^+), found: 269.1159 ($[M+Na]^+$). Anal. Calcd for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37. Found: C, 72.89; H, 7.41.

2-(2-Propynoyl)benzyl pivalate (8).

Alcohol (**7**) (44.6 g, 0.181 mol) was dissolved in acetone (500 mL) and to this solution was added a solution of CrO_3 (18.1 g, 0.181 mol) in 41.4 mL of H_2SO_4-H_2O (3:5) at 0 ˚C. The resulting mixture was stirred at rt for 4 h and poured into ice water. The mixture was extracted twice with AcOEt. The combined organic layer was washed with saturated aqueous $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane/AcOEt = 8/1) to give desired ketone (**8**) (38.4 g, 87%) as colorless prism; mp 69 ˚C (benzene); ^{1}H-NMR ($CDCl_3$, 400 MHz) 8.34 (1H, dd, *J* = 7.9 and 1.0 Hz), 7.62 (1H, td, *J* = 7.9 and 1.0 Hz), 7.53 (1H, dd, *J* = 7.9 and 1.0 Hz), 7.48 (1H, td, *J* = 7.9 and 1.0 Hz), 5.53 (2H, s), 3.43 (1H, s), 1.28 (9H, s); ^{13}C-NMR ($CDCl_3$, 75 MHz) 178.1 (C), 177.7 (C), 138.9 (C), 133.7 (CH), 133.4 (CH), 127.5 (CH), 127.1 (CH), 80.8 (C), 80.4 (CH), 64.2 (CH_2), 38.8 (C), 27.1 (CH_3); FTIR (KBr): 2099, 1728, 1653 cm^{-1}; HRMS (EI) calcd for $C_{15}H_{16}O_3$ (M^+): 244.1099, found: 244.1073 (M^+). Anal. Calcd for $C_{15}H_{16}O_3$: C, 73.75; H, 6.60. Found: C, 73.54; H, 6.62.

7-Methoxy-3-(2-pivaloyloxymethyl)-1,5-heptadiyne-3-ol (9).

To a stirred solution of ketone (**8**) (20.0 g, 81.9 mmol) in THF (200 mL) were added 1-bromo-4-methoxy-2-butyne (20.1 g, 125.6 mmol) and Zn powder (16.1 g, 246.2 mmol) and the resulting mixture was refluxed for 30 min. The mixture was poured into saturated aqueous NH_4Cl and was extracted twice with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane/AcOEt = 4/1) to give desired diyne **9** (19.5 g, 73%) as light brown oil: ^{1}H-NMR ($CDCl_3$, 400 MHz) 7.78-7.71 (1H, m), 7.46-7.41 (1H, m), 7.37-7.28 (2H, m), 5.57 (1H, d, *J* = 13.5 Hz), 5.47 (1H, d, *J* = 13.5 Hz), 4.11 (2H, t, *J* = 2.0 Hz), 3.70 (1H, br s), 3.34 (3H, s), 3.10 (2H, q, J = 2.0 Hz), 2.75 (1H, s), 1.23 (9H, s); ^{13}C-NMR ($CDCl_3$, 75 MHz) 178.4 (C), 139.0 (C), 134.5 (C), 129.6 (CH), 128.5 (CH), 127.7 (CH), 126.6 (CH), 85.4 (CH), 81.6 (C), 79.8 (C), 74.7 (C), 71.6 (C), 64.1 (CH_2), 59.9 (CH_2), 57.3 (CH_3), 38.7 (C), 35.1 (CH_2), 27.1 (CH_3); FTIR (neat): 3285, 2111, 1724 cm^{-1}; HRMS (FAB) calcd for $C_{20}H_{24}O_4$ (M^+): 328.1675, found: 329.1753 ($[M+H]^+$).

7-Methoxy-3-(2-pivaloyloxymethylphenyl)-1,5-heptadiyn-3-(*E*)-ene (10).

A solution of diyne (**9**) (13.6 g, 41.4 mmol) in CH_2Cl_2 (200 mL) was treated with triethylamine (17.4 mL, 124.6 mmol) and methanesulfonyl chloride (4.18 mL, 54.0 mmol) at 0 ˚C for 2 h and the mixture was poured into saturated aqueous NH_4Cl. The mixture was extracted twice with AcOEt and the combined organic layer was washed with brine, dried over $MgSO_4$, concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane/AcOEt = 4/1) to afford desired enediyne (**10**) (10.4 g, 81%) as dark brown oil: ^{1}H-NMR ($CDCl_3$, 400 MHz) 7.37-7.23 (4H, m), 6.00 (1H, t, *J* = 2.0 Hz), 5.19 (2H, s), 4.29 (2H, d, *J* = 2.0 Hz), 3.46 (1H, s), 3.39 (3H, s), 1.15 (9H, s); ^{13}C-NMR ($CDCl_3$, 75 MHz) 178.1 (C), 137.4 (C), 133.8 (C), 132.4 (C), 129.1 (CH), 129.0 (CH), 128.7 (CH), 128.3 (CH), 120.0 (CH), 93.6 (C), 85.9 (C), 83.8 (C), 81.2 (C), 64.2 (CH_2), 60.4 (CH_2), 57.5 (CH_3), 38.8 (C), 27.1 (CH_3); FTIR (neat): 2089, 2204, 1728 cm^{-1}; HRMS (EI) calcd for $C_{20}H_{22}O_3$ (M^+): 310.1569, found: 310.1580 (M^+).

10-Hydroxy-10-[7-methoxy-3-(2-pivaloyloxymethylphenyl)hept-3-ene-1,5-diynyl]-10*H*-anthracen-9-one (11).

To a stirred solution of enediyne (**10**) (3.11g, 10.0 mmol) in THF (60 mL) was added THF solution of EtMgBr (1.0 M, 15.0 mL) and the solution was stirred for 30 min at rt. This solution was added to a solution of anthraquinone (2.10g, 10.0 mmol) in CH_2Cl_2 (60 mL) and the mixture was refluxed for 30 min. The resulting mixture was poured into saturated aqueous NH_4Cl and extracted twice with AcOEt. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, benzene/AcOEt = 10/1) to give desired alcohol (**11**) (2.68 g, 52%) as light brown oil; ^{1}H-NMR ($CDCl_3$, 400 MHz) 8.22 (2H, d, *J* = 8.0 Hz), 8.16 (2H, d,

J = 8.0 Hz), 7.71 (2H, t, *J* = 8.0 Hz), 7.52 (2H, t, *J* = 8 Hz), 7.40-7.20 (4H, m), 6.02 (1H, t, *J* = 2.0 Hz), 5.30 (2H, s), 4.12 (2H, d, *J* = 2.0 Hz), 3.27 (3H, s), 1.23 (9H, s); ^{13}C-NMR ($CDCl_3$, 75 MHz) 182.8 (C), 178.6 (C), 143.7 (C). 136.6 (C), 133.9 (CH), 133.6 (C), 132.7 (C), 129.1 (C), 129.0 (CH), 128.9 (CH), 128.8 (CH), 128.7 (CH), 128.4 (CH), 128.2 (CH), 127.0 (CH), 119.5 (CH), 99.6 (C), 93.7 (C), 84.1 (C), 83.1 (C), 65.9 (C), 64.3 (CH_2), 60.2 (CH_2), 57.5 (CH_3), 38.8 (C), 27.0 (CH_3); FTIR (neat): 3448, 1719, 1602 cm^{-1}; HRMS (EI) calcd for $C_{34}H_{30}O_5$ (M^+): 518.2093, found: 518.2083 (M^+).

10-Methoxy-10-[7-methoxy-3-(2-pivaloyloxymethylphenyl)hept-3-ene-1,5-diynyl]-10*H*-anthracen-9-one (12).

To a stirred solution of alcohol (**11**) (2.90 g, 5.60 mmol) in benzene (50 mL) were added dimethyl sulfate (1.06 mL, 11.2 mmol), tetrabutylammonium iodide (18 mg, 0.049 mmol) and aqueous NaOH (12.5 M, 1.34 mL, 16.8 mmol). The resulting mixture was stirred for 2.5 h at rt and poured into saturated aqueous NH_4Cl. The mixture was extracted twice with AcOEt and the combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane/AcOEt = 3/1) to desired methyl ether (**12**) (2.86 g, 96%) as pale yellow oil; ^{1}H-NMR ($CDCl_3$, 400 MHz) 8.28 (2H, dd, *J* = 8.0 and 1.5 Hz), 8.06 (2H, dd, *J* = 8.0 and 1.5 Hz), 7.71 (2H, dt, *J* = 8.0 and 1.0 Hz), 7.54 (2H, dt, *J* = 8.0 and 1.0 Hz), 7.40-7.24 (4H, m), 5.99 (1H, t, *J* = 2.0 Hz), 5.15 (2H, s), 4.13 (2H, d, *J* = 2.0 Hz), 3.28 (3H, s), 2.87 (3H, s), 1.14 (9H, s); ^{13}C-NMR ($CDCl_3$, 75 MHz) 182.6 (C), 177.7 (C), 140.5 (C), 136.9 (C), 133.8 (CH), 133.6 (C), 132.4 (C), 130.9 (C), 129.1 (CH), 128.9 (CH), 128.8 (CH), 128.6 (CH), 128.2 (CH), 127.0 (CH), 119.7 (CH), 97.5 (C), 93.7 (C), 84.4 (C), 83.8 (C), 71.8 (C), 64.0 (CH_2), 60.0 (CH_2), 57.3 (CH_3), 51.2 (CH_3), 38.6 (C), 26.9 (CH_3); FTIR (neat): 1728, 1672, 1098 cm^{-1}; HRMS (EI) calcd for $C_{35}H_{32}O_5$ (M^+): 532.2250, found: 532.2300 (M^+).

10-Methoxy-10-[7-methoxy-3-(2-hydroxymethylphenyl)hept-3-ene-1,5-diynyl]-10*H*-anthracen-9-one (13).

A solution of ester (**12**) (2.80 g, 5.26 mmol) in MeOH (30 mL) was treated with K_2CO_3 (3.60 g, 26.3 mmol) and the mixture was stirred for 13 h at rt. The resulting mixture was poured into saturated aqueous NH_4Cl and extracted twice with AcOEt. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified with column chromatography (SiO_2, hexane/AcOEt = 3/2) to give desired alcohol (**13**) (2.21 g, 94%) as pale yellow powder; mp 120-122 ˚C (hexane/AcOEt, decomp); ^{1}H-NMR; ($CDCl_3$, 400 MHz) 8.30 (2H, dd, *J* = 8.0 and 1.0 Hz), 8.05 (2H, dd, *J* = 8.0 and 1.0 Hz), 7.73 (2H, dt, *J* = 8.0 and 1.0 Hz), 7.57 (2H, dt, *J* = 8.0 and 1.0 Hz), 7.49 (1H, dd, *J* = 7.5 and 1.0 Hz), 7.31-7.20 (3H, m), 6.05 (1H, t, *J* = 2.0 Hz), 4.74 (2H, s), 4.11 (2H, d, *J* = 2.0 Hz), 3.23 (3H, s), 2.85 (3H, s); ^{13}C-NMR ($CDCl_3$, 75 MHz) 182.4 (C), 140.2 (C), 138.2 (C), 136.3 (C), 133.8

(CH), 132.2 (C), 130.9 (C), 129.2 (CH), 129.0 (CH), 128.8 (CH), 128.6 (CH), 128.5 (CH), 127.6 (CH), 127.0 (CH), 119.5 (CH), 97.5 (C), 93.6 (C), 85.2 (C), 83.9 (C), 71.7 (C), 62.4 (CH_2), 60.1 (CH_2), 57.3 (CH_3), 51.2 (CH_3); FTIR (KBr): 3439, 1736, 1600 cm^{-1}; HRMS (EI) calcd for $C_{30}H_{24}O_4$ (M^+): 448.1675, found: 448.1691 (M^+). Anal. Calcd for $C_{30}H_{24}O_4$: C, 80.34; H, 5.39. Found: C, 80.31; H, 5.58.

10-Hydroxy-10-[7-methoxy-3-(2-hydroxymethylphenyl)hept-3-ene-1,5-diynyl]-10*H*-anthracen-9-one (14).

A solution of enediyne (**11**) (1.32 g, 2.55 mmol) in MeOH (25 mL) was treated with K_2CO_3 (1.78 g, 12.4 mmol) at rt for 4 h and the resulting mixture was poured into saturated aqueous NH_4Cl. The mixture was extracted twice with AcOEt and the combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane/AcOEt = 2) to give enediyne (**14**) (882 mg, 80%) as dark brown oil; ^{1}H-NMR ($CDCl_3$, 400 MHz) 8.13 (2H, d, *J* = 7.5 Hz), 8.04 (2H, d, *J* = 7.5 Hz), 7.61 (2H, t, *J* = 7.5 Hz), 7.45 (2H, t, *J* = 7.5 Hz), 7.36 (1H, d, *J* = 8.0 Hz), 7.29 (1H, t, *J* = 8.0 Hz), 7.22 (1H, t, *J* = 8.0 Hz), 7.18 (1H, t, *J* = 8.0 Hz), 5.98 (1H, t, *J* = 1.8 Hz), 5.26 (1H, br s), 4.67 (2H, s), 4.05 (2H, d, *J* = 1.8 Hz), 3.16 (3H, s); ^{13}C-NMR ($CDCl_3$, 75 MHz) 182.9 (C), 143.6 (C), 138.0 (C), 136.5 (C), 134.0 (CH), 132.7 (C), 129.3 (CH), 129.1 (C), 129.0 (CH), 128.9 (CH), 128.7 (CH), 128.2 (CH), 128.0 (CH), 127.0 (CH), 119.1 (CH), 99.5 (C), 93.4 (C), 84.4 (C), 84.3 (C), 66.0 (C), 62.4 (CH_2), 60.2 (CH_2), 57.5 (CH_3); FT-IR (neat): 3377, 2933, 1667, 1321 cm^{-1}; HRMS (FAB) calcd for $C_{29}H_{22}O_4$: 434.1518 (M^+), found: 457.1431 ($[M+Na]^+$).

10-Hydroxy-10-[7-methoxy-3-(2-hydroxycarbonylphenyl)hept-3-ene-1,5-diynyl]-10*H*-anthracen-9-one (15).

To a stirred solution of alcohol (**14**) (882 mg, 2.03 mmol) in benzene (20 mL) was added MnO_2 (3.42 g, 39.4 mmol) and the mixture was stirred for 2 h at rt. The resulting mixture was filtered and concentrated in vacuo to give a crude aldehyde, which was used without further purification. To a solution of the aldehyde in *t*-BuOH (16 mL, containing 20% of water) were added 2-methyl-2-butene (0.75 mL, 7.08 mmol), $NaClO_2$ (450 mg, 5.0 mmol), $NaH_2PO_4 \bullet H_2O$ (440 mg, 3.19 mmol) sequentially and the mixture was stirred for 2 h at rt. The mixture was poured into saturated aqueous NH_4Cl, extracted twice with AcOEt and the combined organic layer was washed brine and concentrated in vacuo to give crude acid. The residue was purified with column chromatography (SiO_2, hexane/AcOEt = 1/1) to give desired acid (**15**) (662 mg, 73%) as light brown powder; mp 115-116 ℃ (hexane/AcOEt, decomp); ^{1}H-NMR (CD_3OD, 300 MHz) 8.17 (2H, dd, *J* = 8.0 and 1.0 Hz), 8.15 (2H, d, *J* = 8.0 Hz), 8.83-7.70 (3H, m), 7.60-7.28 (5H, m), 6.02 (1H, t, *J* = 2.0 Hz), 4.17 (2H, d, *J* = 2.0 Hz), 3.24 (3H, s); ^{13}C-NMR (CD_3OD, 75 MHz) 184.6 (C), 170.8 (C), 146.0 (C), 139.8 (C), 135.5 (C), 135.3 (CH), 132.8 (CH), 132.4 (C), 131.2

(CH), 130.7 (CH), 130.4 (C), 130.3 (CH), 129.9 (CH), 129.7 (CH), 127.6 (CH), 118.6 (CH), 99.8 (C), 94.1 (C), 85.1 (C), 84.8 (C), 67.1 (C), 61.0 (CH_2), 57.8 (CH_3); FTIR (KBr): 3423, 1694, 1663 cm^{-1}; HRMS (FAB) calcd for $C_{29}H_{20}O_5$ (M^+): 448.1311, found: 471.1219 ($[M+Na]^+$).

10-Hydroxy-10-[7-methoxy-3-(2-methoxycarbonylphenyl)hept-3-ene-1,5-diynyl]-10*H*-anthracen-9-one (16).

Acid (**15**) (100 mg, 0.22 mmol) was esterified using literature procedure[10] to give the methyl ester, which was purified by column chromatography to afford desired ester (**16**) (97 mg, 95%) as light brown powder; mp 142-144 ˚C (hexane/AcOEt, decomp); ^{1}H-NMR ($CDCl_3$, 300 MHz) □ 8.26 (2H, m), 8.10 (2H, d, *J* = 7.7 Hz), 7.54 (2H, m), 7.74-7.69 (3H, m), 7.56-7.29 (5H, m), 6.04 (1H, t, *J* = 1.8 Hz), 4.18 (2H, d, *J* = 1.8 Hz), 3.66 (3H, s), 3.31 (3H, s); ^{13}C-NMR ($CDCl_3$, 75 MHz) □ 182.9 (C), 168.2 (C), 143.7 (C), 137.9 (C), 134.0 (CH), 133.8 (C), 131.7 (CH), 130.5 (C), 130.0 (CH), 129.3 (CH), 129.1 (C), 128.9 (CH), 128.6 (CH), 127.0 (CH), 117.7 (CH), 98.4 (C), 93.4 (C), 84.3 (C), 83.7 (C), 66.2 (C), 60.3 (CH_2), 57.6 (CH_3), 52.3 (CH_3); FT-IR(KBr): 3423, 1700, 1666 cm^{-1}; HRMS (FAB) calcd for $C_{30}H_{22}O_5$ (M^+): 462.1467, found:485.1360 ($[M+Na]^+$).

10-Hydroxy-10-[7-methoxy-3-(2-dimethylaminocarbonylphenyl)hept-3-ene-1,5-diynyl]-10*H*-anthracen-9-one (17).

To a cooled stirred suspension of diethylamine hydrochloride (135 mg, 1.67 mmol) in THF (6 mL) were added *N*,*N*-diisopropylethylamine (0.58 mL, 3.31 mmol), acid (**15**) (359 mg, 0.80 mmol) and dicyano diethylphosphate (0.25 mL, 1.67 mmol) sequentially at 0˚C and the mixture was stirred for 2 h. The resulting mixture was poured into saturated aqueous NH_4Cl and extracted with twice with AcOEt. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give crude dimethylamide, which was purified by column chromatography (SiO_2, hexane/AcOEt = 1/3) to give desired alcohol (**17**) (308 mg, 81%) as a colorless powder; mp 132-134 ˚C (hexane/AcOEt, decomp); ^{1}H-NMR ($CDCl_3$, 300 MHz) □ 8.23 (2H, d, *J* = 7.7 Hz), 8.11 (2H, d, *J* = 7.9 Hz), 7.68 (2H, m), 7.53-7.27 (6H, m), 6.06 (1H, t, *J* = 1.8 Hz), 5.65 (1H, br s), 4.08 (2H, d, *J* = 1.8 Hz), 3.17 (3H, s), 3.10 (3H, s), 2.72 (3H, s); ^{13}C-NMR ($CDCl_3$, 75 MHz) □ 183.1 (C), 171.3 (C), 135.3 (C), 134.6 (C), 134.0 (C), 133.9 (CH), 132.2 (C), 129.5 (CH), 129.3 (CH), 128.8 (CH), 128.3 (CH), 128.0 (CH), 127.4 (CH), 127.0 (CH), 117.4 (CH), 99.9 (C), 94.3 (C), 84.3 (C), 83.6 (C), 65.7 (C), 60.3 (CH_2), 57.5 (CH_3), 38.5 (CH_3), 35.2 (CH_3); FTIR (KBr): 3421, 1669, 1616 cm^{-1}; HRMS (FAB) calcd for $C_{31}H_{25}O_4N$ (M^+): 475.1784, found: 498.1686 ($[M+Na]^+$).

General procedure for cycloaromatization reactions in benzene

A solution of enediyne (**1**) (62 mg, 0.134 mmol) and 1,4-cyclohexadiene (0.62 mL, 6.7 mmol) in anhydrous benzene (1.44 mL) was treated with trifluoroacetic acid (6.2 μL, 0.3 %v/v) at 37 ˚C for 5 min and the solution was evaporated. The residue was dissolved in pyridine (0.5 mL) and to this solution was added acetic anhydride (0.5 mL). The mixture was stirred for 2 h at rt and diluted with AcOEt. The organic layer was washed with water, aqueous HCl (1N), saturated aqueous $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, hexane/AcOEt = 10/1) to give 26.7 mg of acetate (**20**) (87%) as pale yellow powder; mp 112-114 ˚C (hexane/AcOEt, decomp); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.33 (1H, d, *J* = 8.0 Hz), 8.29 (1H, d, *J* = 8.0 Hz), 8.26 (1H, d, *J* = 8.0 Hz), 8.06 (2H, d, *J* = 8.0 Hz), 7.84 (1H, t, *J* = 8.0 Hz), 7.80 (1H, d, *J* = 8.0 Hz), 7.54 (1H, t, *J* = 8.0 Hz), 7.52 (1H, d, *J* = 8.0 Hz), 7.50 (1H, d, *J* = 8.0 Hz), 7.47 (2H, d, *J* = 8.0 Hz), 7.36 (1H, d, *J* = 8.0 Hz), 7.33 (1H, d, *J* = 8.0 Hz), 3.94 (2H, s), 3.09 (3H, s), 2.69 (3H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 169.5 (C), 160.6 (C), 149.5 (C), 143.0 (C), 142.1 (C), 134.8 (CH), 134.7 (C), 130.5 (CH), 130.4 (C), 128.8 (CH), 126.6 (C), 126.3 (CH), 126.1 (CH), 125.7 (CH), 125.0 (C), 123.9 (C), 122.9 (CH), 122.8 (CH), 121.9 (CH), 121.8 (CH), 121.1 (C), 117.3 (C), 71.2 (CH_2), 58.5 (CH_3), 20.8 (CH_3); FTIR (KBr): 1741, 1766 cm^{-1}; HRMS (EI) calcd for $C_{31}H_{22}O_5$ (M^+): 474.1467, found: 474.1470 (M^+). Anal. Calcd for $C_{31}H_{22}O_5$: C, 78.47; H, 4.67. Found: C, 78.27; H, 4.84.

DNA-cleavage assay.

In a typical experiment, 0.25 μg Col E1 DNA (WAKO Pure Chemical Industries, Ltd) in 20 μL phosphate buffer (pH 6.0) containing drugs (1.0 mM) was incubated in Eppendorf tube at 37 ˚C for 6 h. Immediately, 15 μL samples were loaded into 1% agarose gel. The running buffer was 20 mM TAE, pH 7.8. Electrophoresis was at 50 V for 8 h. After electrophoresis, gel was stained for 1 h in ethidium bromide (1 mg/mL) and de-stained for 5 min in water. Relative amounts of DNA in form I and form II were determined by densitometry.

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‡ For preliminary communication, see ref. 6.

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