Design and Synthesis of 1-Thia-3,8-diyn-5-ene Systems with DNA-cleaving Properties related to the Neocarzinostatin Chromophore

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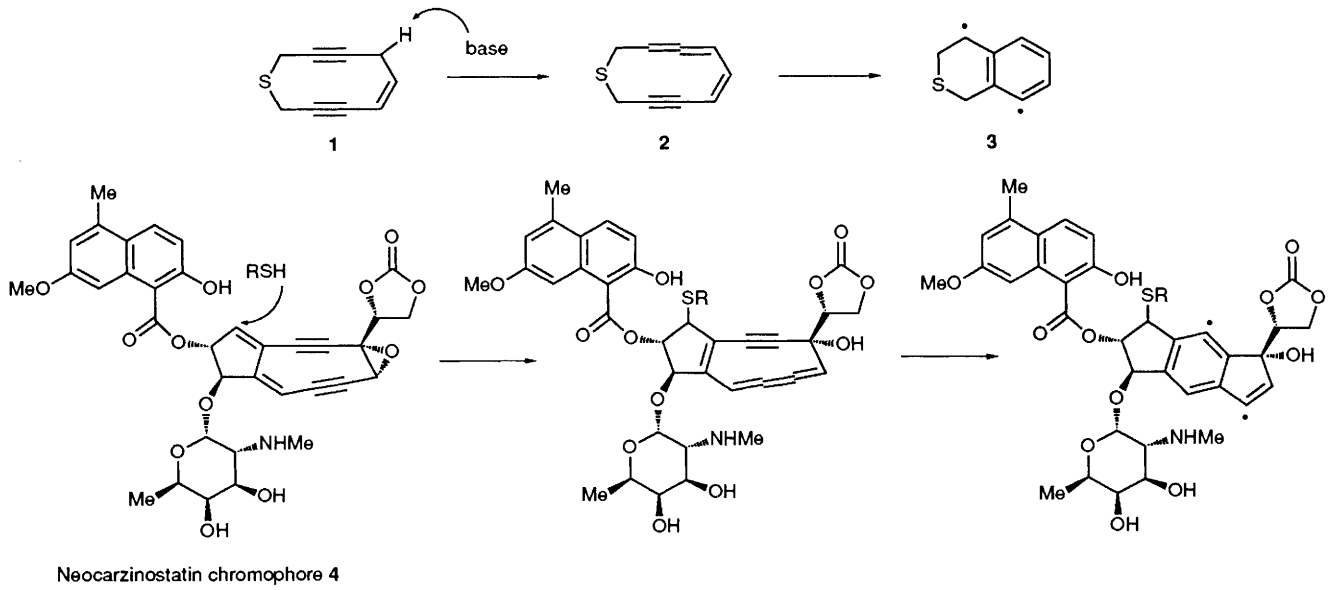
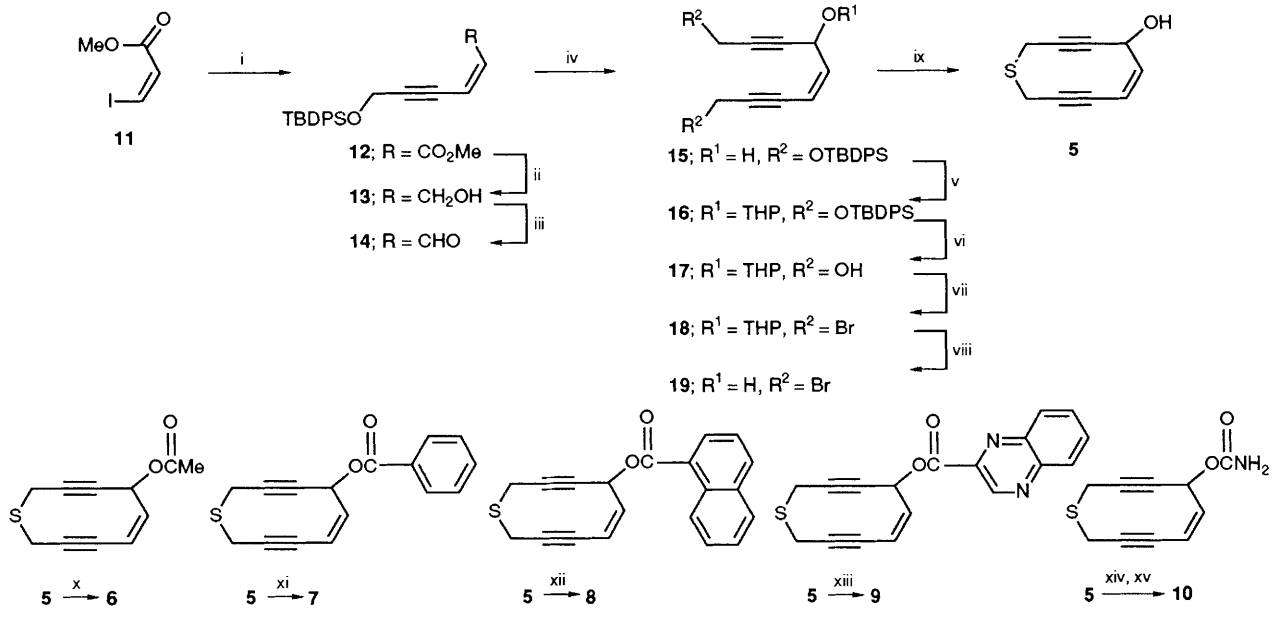
1-Thia-3,8-diyn-5-ene compounds **5–10**, which are derivatives of the parent compound **1** are synthesized in a short step with high stability; these compounds show DNA-cleaving activity under basic conditions in the absence of any additives.

In recent years considerable attention has been directed towards the design and synthesis of DNA-cleaving molecules[1] in relation to the various enediyne anticancer antibiotics such as neocarzinostatin,[2] calicheamicins,[3] esperamicins[4] and dynemicins.[5] We recently reported that the new 1-thia-3,8-diyn-5-ene compound **1** was easily prepared with high stability at ambient temperature; **1** was smoothly cyclized under specific basic conditions to produce the diradical **3** through an enyne–allene intermediate **2**,[6,7] reminiscent of the chemistry of the neocarzinostatin chromophore **4**[8] (Scheme 1). Also, we have demonstrated another diradical formation by a selenium-mediated aromatization of **1**.[9] Since the diradical generated by Myers type cyclization has been proposed to play a crucial role in the DNA-cleaving process of the antitumour antibiotic neocarzinostatin,[8] we could expect that **1** would cleave DNA under basic conditions without any additives. In this communication, we report that **1** and its designed derivatives **5–10** possessing the 1-thia-3,8-diyn-5-ene structure show such DNA-cleaving activity.

The DNA-cleaving activity of the key compound **1** was assayed with supercoiled ΦX174 DNA in various pH buffers. As expected, **1** was found to cleave DNA in a striking



Scheme 1



Scheme 2 *Reagents and conditions*: i, 1.1 equiv. $HCCCH_2OTBDPS$, 0.04 equiv. $(Ph_3P)_2PdCl_2$, 0.16 equiv. CuI, 1.5 equiv. Et_3N, THF, 0 °C → room temp., 2 h, 82%; ii, 2.5 equiv. DIBAL-H, toluene, −78 °C, 30 min, 99%; iii, 30 equiv. MnO_2, CH_2Cl_2, room temp., 1 h, 100%; iv, 1.5 equiv. $HCCCH_2OTBDPS$, 1.4 equiv. Bu^nLi, THF, −91 °C, 1 h, 87% v, 1.1 equiv. DHP, 0.01 equiv. CSA, CH_2Cl_2, 0 °C → room temp., 1 h, 100% vi, 2.0 equiv. TBAF, THF, room temp., 1 h, 84%; vii, 2.0 equiv. CBr_4, 3.5 equiv. $[Me(CH_2)_7]_3P$, Et_2O, 0 °C → room temp., 1.5 h, 67%; viii, 0.2 equiv. CSA, MeOH, 0 °C → room temp., 1.5 h, 97%; ix, 1.5 equiv. $Na_2S{\cdot}9H_2O$, 95% EtOH (0.01 mol dm^{-3}), room temp., 4 h, 67% x, 1.5 equiv. Ac_2O, 1.0 equiv. 4-DMAP, pyridine, room temp., 16 h, 94%; xi, 1.2 equiv. BzCl, 3.0 equiv. Et_3N, 0.4 equiv. 4-DMAP, CH_2Cl_2, room temp., 2.5 h, 88%; xii, 2.4 equiv. 1-naphthoyl chloride, 3.0 equiv. Et_3N, CH_2Cl_2, 0 °C, 10 min, 85%; xiii 1.2 equiv.2-quinoxaloyl chloride, 1.5 equiv. Et_3N, CH_2Cl_2, 0 °C, 2 h, 71% xiv, 1.5 equiv. $ClCH_2CONCO$, CH_2Cl_2, 0 °C, 2 h, 84%; xv, 2.0 equiv. Zn, MeOH, room temp., 2 h, 67%

pH-dependent fashion and in only alkaline buffers solutions. Thus, incubation of **1** (1000 μmol dm^{-3}) with the covalently closed circular DNA (form I) aerobically at pH 8.0–9.0 and 42 °C caused single strand break as well as the action of the neocarzinostatin chromophore,[10] leading to the nicked open circular DNA (form II) as shown in Fig. 1.† However, its efficiency was not very high. So, the expectation that high solubility of the compound in a buffer, high acidity of the hydrogen at C-7 position, DNA intercalators and other DNA binding moieties would enhance the potency of this compound

† DNA cleavage experiments were repeated more than two times and a similar trend for DNA-cleaving pattern was observed.

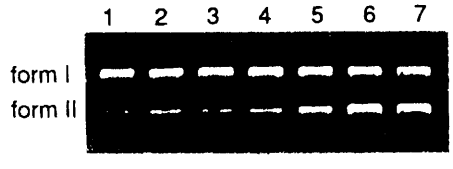


Fig. 1 DNA cleavage by **1** at various pH s. ΦX174 form I DNA (50 μmol dm^{-3} per base pair) was incubated for 24 h at 42 °C with **1** (1000 μmol dm^{-3}) in 20% DMSO (dimethyl sulfoxide) in various pH buffers and analysed by electrophoresis (1% agarose gel, ethidium bromide stain). Lane 1, DNA alone at pH 8.5; lanes 2–7 correspond to pH 6.0, 7.0, 7.5, 8.0, 8.5 and 9.0, respectively. Control experiments at the above pH values in the absence of agent demonstrated no DNA cleavage.

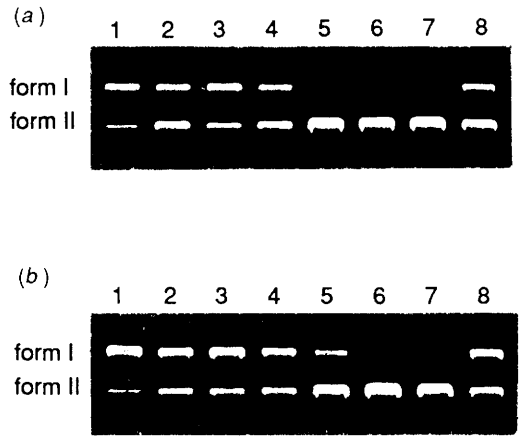


Fig. 2 ΦX174 form I DNA (50 μmol dm^{-3} per base pair) was incubated for 24 h at (*a*) T = 42 °C and (*b*) T = 37 °C with various compounds (1000 μmol dm^{-3}) in 20% DMSO in Tris-acetate buffer (pH 8.5, 50 mmol dm^{-3}) and analysed by electrophoresis (1% agarose gel, ethidium bromide stain). Lane 1, DNA alone; lanes 2–8 correspond to compounds **1**, **5**–10, respectively.

as a DNA cleaver was born out on this stage. Thus, several derivatives of **1**, **5**–**10**‡ were synthesized from **11**[11] *via* vinyl iodine–acetylene coupling by using a Pd^0–Cu^I catalyst[12] and the cyclization reaction[13] of the dibromo product **19** as key operations (Scheme 2). The new compounds **5**–**10** and **1** were quite stable at ambient temperature. Further, it was found that the mode of aromatization of the representative compound **7**§ under basic conditions (DBU–CCl_4)¶ was similar to that of **1**.[6] The results of assay of DNA-cleaving activity of these compounds under similar conditions are shown in Fig. 2.† The compounds **7**, **8** and **9**, each of which has an intercalative aromatic moiety, benzene, naphthalene and quinoxaline, obviously exhibited much higher potency as DNA-cleaving agents than other molecules. Although two alternative modes of action of **1** and **5**–**10** on DNA have been considered, the alkylation mechanism, which involved the nucleophilic addition of DNA bases on the conjugated allene moiety[14] could not be ruled out; DNA cleavage by the radical mechanism is more probable considering our results.

In summary, our present work showed that even a highly simple and stable model has DNA-cleaving property and its activity could be much improved by the introduction of a DNA intercalative moiety.

We are grateful to the Institute of Microbial Chemistry for the generous support of our programme. Financial support by the Ministry of Education, Science and Culture (Grant-in-Aid Scientific Research) and the Takeda Science Foundation are gratefully acknowledged. We also thank Drs O. Makabe and T. Kimura (Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd.) for helpful advice and technical assistance on the DNA-cleaving experiments.

Received, 22nd May 1992; Com. 2/02671F

‡ All new compounds were purified by silica gel column chromatography and were fully characterized by spectroscopic analysis.

§ The results of aromatizations of **7** will be published elsewhere in detail.

¶ *Abbreviations used*: DBU (1,8-diazabicyclo[5.4.0.]undec-7-ene; TBDPS (*tert*-butyldiphenylsilyl); THF (tetrahydrofuran); DIBAL-H (diisobutylaluminium hydride); DHP (dihydropyran); CSA (camphorsulfonic acid); TBAF (tetrabutylammonium fluoride); DMAP (dimethylaminopyridine); Bz (benzoyl).

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