Design, Synthesis and DNA Cleaving Profiles of Hybrids Containing the Novel Enediyne and Naturally Occurring DNA Intercalators

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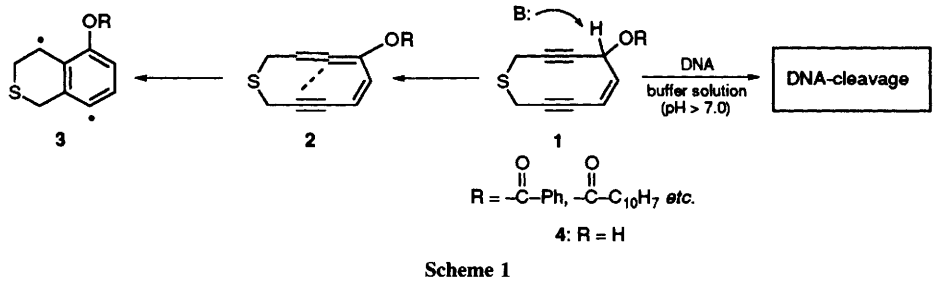
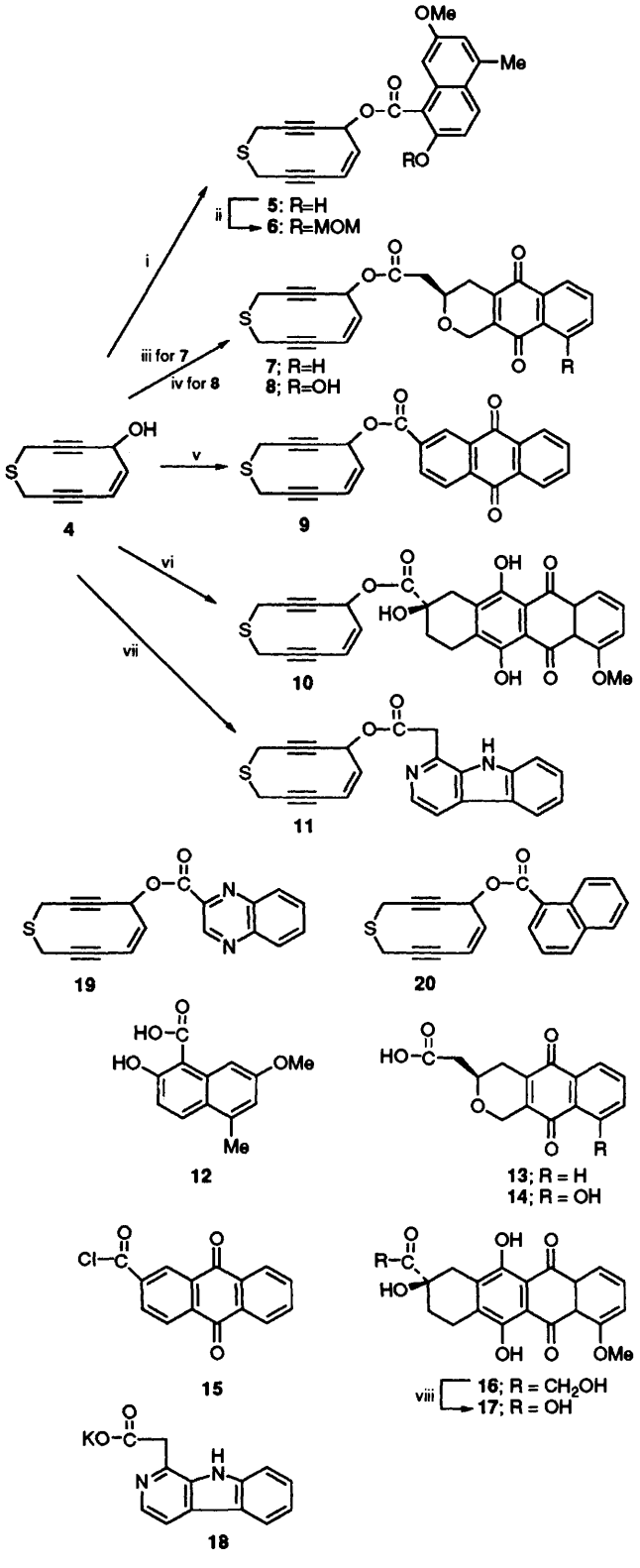
The designed hybrids **5–11** containing the DNA cleavable enediyne **4** and naturally occurring DNA intercalators **12–18** cleave DNA effectively under alkaline conditions without any additive; the hybrids **5** and **11** exhibit the strongest DNA-cleaving abilities with the identical high purine base (G>A) selectivities for their DNA-cleavage profiles.

After the discovery of enediyne antitumour antibiotics, much effort has been paid to developing simple and stable molecules, which generate benzenoid diradical species under specific conditions to cleave DNA, in bioorganic chemistry, molecular biology and pharmacology, as well as in organic synthesis.[1] Earlier studies in our laboratories have shown that the simple 10-membered heterocyclic enediyne **1** is quite stable when handled at ambient temperature and produced



Scheme 1



Scheme 2 *Reagents and conditions*: i, **12** (1.0 equiv.), EDAC (1.0 equiv.), CH_2Cl_2 26 °C, 1 h, 45%; ii, $MeOCH_2Cl$ (3 equiv.), Pr^i_2EtN (3.5 equiv.), CH_2Cl_2, 26 °C, 24 h, 82%; iii, **13** (1.0 equiv.), 2,4,6-trichlorobenzoyl (TCB_3Cl) chloride (1.0 equiv.), Et_3N (1.0 equiv.), 4-dimethylaminopyridine (DMAP) (4.0 equiv.), CH_2Cl_2, 26 °C, 2 h, 50%; iv, **14**, (1.0 equiv.), TCB_3Cl (1.0 equiv.), Et_3N (1.0 equiv.), DMAP (4.0 equiv.), CH_2Cl_2, 26 °C, 2 h, 45%; v, **15** (1.2 equiv.), Et_3N (1.5 equiv.), CH_2Cl_2, 26 °C, 1 h, 90%; vi, **17** (1.0 equiv.), Ph_3P (3 equiv.), diethyl azodicarboxylate (3.0 equiv.), tetrahydrofuran (THF), 26 °C, 15 min, 17%; vii, **18** (1 equiv.), TCB_3Cl (1.0 equiv.), Et_3N (2.0 equiv.), DMAP (0.3 equiv.), THF, 26 °C, 1 h, 19%; viii, HIO_4 (2.0 equiv.), dioxane–H_2O, 26 °C, 2 h, 87%

the benzenoid diradical **3** through the enyne-allene **2** under basic conditions.[2] From the preliminary experiments, it was found that **4** (R = H) had a slight DNA-cleaving activity under basic conditions without any additive but its activity could be improved by the introduction of a simple DNA intercalative moiety[3] (Scheme 1). Therefore, hybridization to **4** with a naturally occurring DNA intercalator would be expected to produce both the higher DNA-cleaving ability and base- and sequence-selectivities. Here, we report the syntheses of the hybrid molecules **5–11** containing the novel enediyne system **4** and the DNA intercalator occurring in several bioactive natural products[4] and their DNA cleaving profiles.

As the DNA intercalator, we selected several aromatic moieties occurring in neocarzinostatin[5] (enediyne antibiotic), nanaomycin[6] (pyranonaphthoquinone antibiotic), adriamycin[7] (anthracycline antibiotic) or manzamine[8] (alkaloid). The syntheses of these hybrids are summarized in Scheme 2. The hybrid **5** containing the naphthoate moiety of neocarzinostatin was synthesized from **4** and 2-hydroxy-7-methoxy-5-methylnaphthalene-1-carboxylic acid **12**.[9] The enediyne **4** was effectively esterified with **12** in the presence of 1-ethyl-3-(dimethylaminopropyl)carbodiimide (EDAC) hydrochloride in CH_2Cl_2 to give **5**. The methoxymethyl (MOM)-protected analogue **6** was prepared from **5** and $MeOCH_2Cl$ to examine the effect of the hydroxy group of **5** on the DNA-cleaving ability. The pyranonaphthoquinone derivatives **7** and **8** were obtained by esterification of **4** with **13**† and **14**†, respectively, using the Yamaguchi method.[10] Both carboxylic acids were prepared according to Tatsuta's nanaomycin synthesis procedures.[11] The anthraquinone **9** was obtained by esterification of **4** with 2-anthraquinonecarbonyl chloride **15** using Et_3N as the base. The hybrid **10** possessing the anthracycline moiety of adriamycin was also synthesized by esterification of **4** with **17** *via* the Mitsunobu reaction.[12] The carboxylic acid **17** was readily obtained from natural adriamycin through oxidative cleavage of the α-hydroxy ketone **16**[13] by HIO_4 in dioxane–H_2O. The hybrid **11** having a β-carboline moiety of the antitumour alkaloid, manzamine, was synthesized by esterification of **4** with the β-carboline carboxylic acid potassium salt **18**[14] using the Yamaguchi method.

The DNA-cleaving activities of these compounds and compound **19**[3] as a control are shown in Fig. 1. Although the enediyne **4** itself had a very weak DNA-cleaving activity at 1000 μmol dm^{-3},[3] all the hybrids **5–11** clearly cleaved the covalently closed supercoiled ΦX174 DNA (form I) to the open circular DNA (form II) at pH 8.5 and at 37 °C without any additive in concentrations from 100 to 1000 μmol dm^{-3} [(*a*) and (*b*) in Fig. 1]. Remarkably, the hybrids **5** and **11** exhibited the strongest DNA-cleaving abilities and caused DNA breaks even at 1–10 μmol dm^{-3} [(*c*) and (*d*) in Fig. 1]. The potency of **5** and **11** was outstanding among the reported enediyne-based nonnatural systems.[1] This result suggested that the β-carboline part of manzamine was a good DNA intercalator like the naphthoate moiety of neocarzinostatin. Furthermore, it was confirmed that the hydroxy group of the naphthoate moiety of neocarzinostatin played an important role in DNA-cleaving ability (*cf.* lane 3 with lane 4 in Fig. 1). Their DNA-cleavage site specificity was also analysed. Fig. 2 shows the DNA cleavage results with hybrids **5**, **7**, **10**, **11**, **19** and **20**[3] and the singly 5′-end ^{32}P labelled double-stranded M13mp18 DNA. Comparisons of the cleavage products with both the enzymatically produced Sanger markers[15]‡ and the

† The synthetic procedure of the compound will be reported elsewhere in detail.

‡ Since the Sanger sequencing reactions result in base incorporation, cleavage at nucleotide N (sequencing) represents cleaving site by a hybrid of Maxam–Gilbert reaction at N + 1. D. L. Boger, S. A. Munk, H. Zarrinmayeh, T. Ishizaki, J. Haught and M. Bina, *Tetrahedron*, 1991, **47**, 2661.

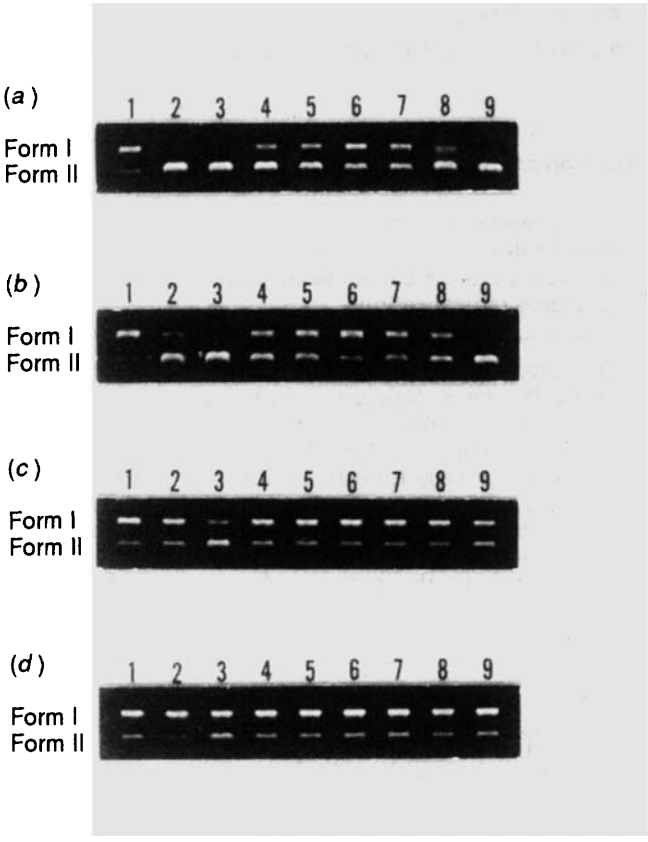


Fig. 1 Supercoiled DNA cleavage by the hybrids **5–11**. ΦX174 form 1 DNA (50 mmol dm^{-3} base pair) was incubated for 24 h with various compounds (*a*) 1000 μmol dm^{-3} at 37 °C, (*b*) 100 μmol dm^{-3} at 37 °C, (*c*) 10 μmol dm^{-3} at 37 °C and (*d*) 1 μmol dm^{-3} at 42 °C in 20% dimethyl sulfoxide in tris-acetate buffer (pH 8.5, 50 mol dm^{-3}) and analysed by electrophoresis (1% agarose gel, ethidium bromide stain). Lane 1: DNA alone; lanes 2–9 correspond to compounds **19**, **5–11**, respectively.

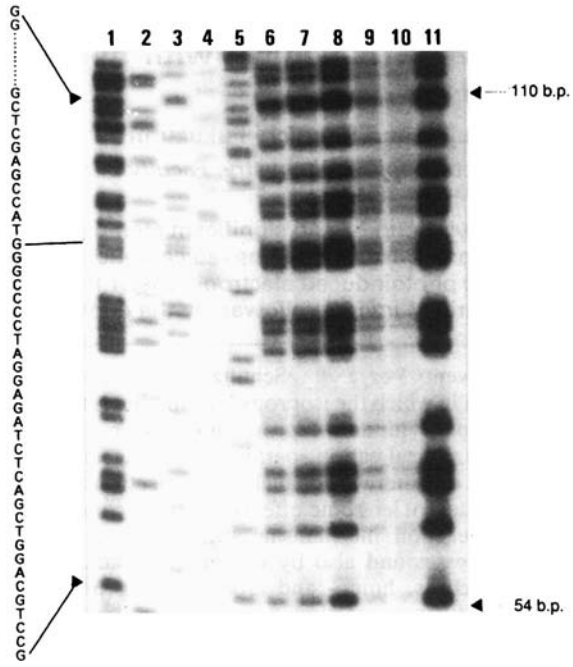


Fig. 2 Autoradiogram of 12% polyacrylamide–8 mol dm^{-3} urea slab gel electrophoresis for sequence analysis. The 5′-end-labelled M13mp18 DNA was cleaved by hybrids **5**, **7**, **10**, **11**, **19**, and **20** at pH 8.5 and at 45 °C for 24 h (bases 54–110 are shown). Lane 1: Maxam–Gilbert AG reaction; lanes 2–5: Sanger A, G, C and T reactions, respectively; lanes 6–11: **20** (2), **19** (2), **5** (1), **7** (1), **10**, (2) and **11** (2 mmol dm^{-3}), respectively.

chemically produced Maxam–Gilbert (A + G) marker[16] clearly indicated the identical high purine base (G>A) selectivity of these compounds for their DNA-cleavage profiles. Unexpectedly, the base selectivity was highly independent of the DNA intercalator examined even in the case of the hybrid **5**, which had the DNA recognition moiety (T>A≫C>G) of the neocarzinostatin chromophore,[17] and its selectivity was similar to that of the A+G-selective alkylating agents. These results strongly suggested that these intercalators in **5**, **7**, **10**, **11**, **19** and **20** significantly increased the DNA cleaving activity, but neither the base nor sequence selectivity. The base selectivity of the hybrid molecules seems to only reflect the reactivity of the enediyne moiety **4** towards purine bases (G>A) like a alkylation mechanism.[18] Details of the DNA cleavage mechanism and the base specificity of these hybrid molecules are now under investigation.

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