Design, Cycloaromatization and Guanine-selective DNA Cleavage of Novel Enediynes

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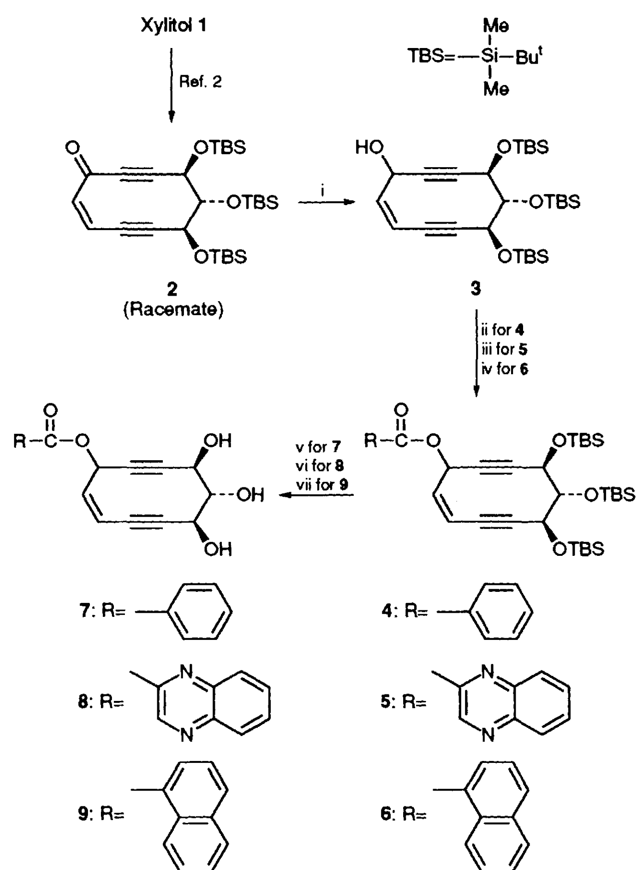
The novel enediynes **7–9** are synthesized from xylitol **1** *via* the keto-enediyne **2**; they are aromatized by 1,8-diazabicyclo[5.4.0]undec-7-ene in cyclohexa-1,4-diene–benzene through a radical pathway and by diethylamine in Me_2SO–tris-HCl, pH 8.5 buffer through a polar pathway, and exhibit guanine-selective DNA cleavage under basic conditions with no additive.

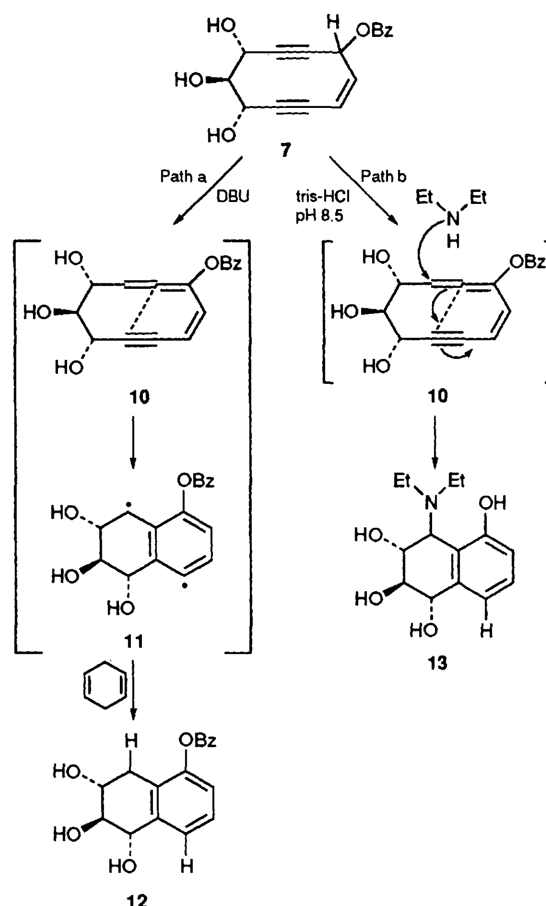
The powerful anticancer and DNA-cleaving enediyne antibiotics, neocarzinostatin, calicheamicins, esperamicins and dynemicins, have been the subject of great interest recently.[1] Here, we report the synthesis, two different modes of cycloaromatization, and DNA-cleaving profiles of the novel enediynes **7–9**.

Our synthetic approach began with the conversion of xylitol **1** into the ten-membered keto-enediyne **2** by the procedure recently developed in our laboratories.[2] Reduction of **2** using $NaBH_4$ in MeOH at 25 °C for 0.5 h gave the alcohol **3** in 90% yield. Several acylated derivatives **4–6** possessing a DNA intercalative aromatic moiety were prepared by the reactions of **3** with benzoyl, 2-quinoxaloyl and 2-naphthoyl chlorides, respectively, in the presence of a suitable base such as pyridine or triethylamine. Finally, the desilylations of **4–6** under acidic conditions afforded the desired enediyne triols **7–9**, respectively, which are quite stable when handled at ambient temperature (Scheme 1).



Scheme 1 *Reagents and Conditions*: i, $NaBH_4$ (1.2 equiv.), MeOH, 25 °C, 0.5 h, 90%; ii, BzCl (1.2 equiv.), pyridine, 25 °C, 2 h, 91%; iii, 2-quinoxaloyl chloride (2.2 equiv.), Et_3N (2.5 equiv.), CH_2Cl_2, 25 °C, 0.5 h, 89%; iv, 2-naphthoyl chloride (2.0 equiv.), Et_3N (2.3 equiv.), CH_2Cl_2, 25 °C, 0.5 h, 50%; v, 10-DL-camphorsulfonic acid (0.75 equiv.), MeOH, 25 °C, 15 h, 80%; vi, HF–pyridine, pyridine, 25 °C, 20 h, 45%; vii, 46% HF (aq.), dioxane, 25 °C, 20 h, 50%

Our attention next turned to the mode of cycloaromatization of these novel enediynes. Treatment of the representative enediyne **7** possessing a benzoyl group at the allylic position with 2.0 equiv. of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in cyclohexa-1,4,-diene–benzene (1:2) at 25 °C for 1.5 h gave the cycloaromatization product **12** in 7.0% yield. This result clearly suggests that the migration of the hydrogen at the allylic position of **7** induced by DBU first produces the



Scheme 2 Proposed mode of aromatization of the enediyne **7**

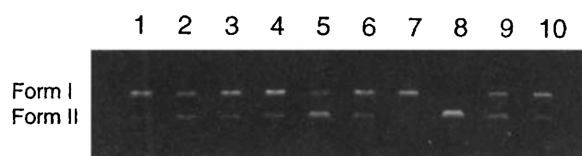


Fig. 1 ΦX174 form I DNA (50 μmol dm^{-3} per base pair) was incubated for 24 h at 37 °C with **7**, **8** nd **9** in 20% Me_2SO in tris-HCl buffer (pH 8.5, 50 mmol dm^{-3}) and analysed by electrophoresis (1% agarose gel, ethidium bromide stain). Lane 1, DNA alone; lanes 2–10: **7** (1000), **7** (100), **7** (10), **8** (1000), **8** (100), **8** (10), **9** (1000), **9** (100) and **9** (10 μmol dm^{-3}), respectively.

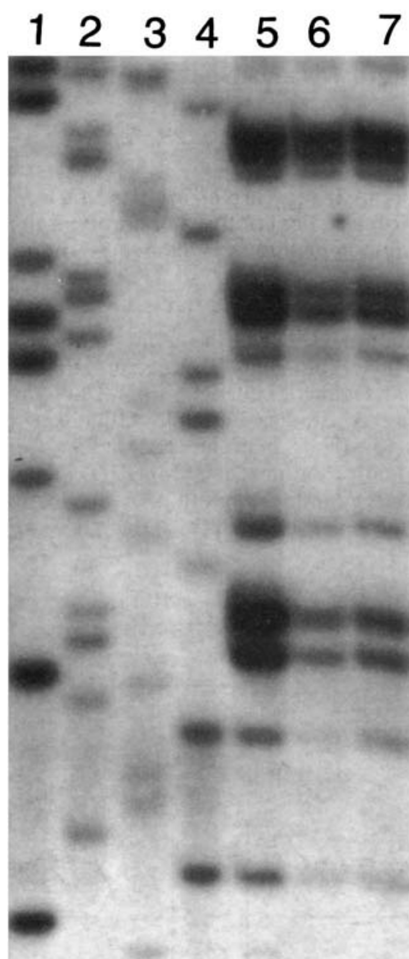


Fig. 2 Autoradiogram of 12% polyacrylamide/8 mol dm^{-3} urea slab gel electrophoresis for sequence analysis. The 5′-end-labelled M13mp18 DNA was cleaved by **7**, **8** and **9** at pH 8.5 and at 45 °C for 24 h (bases 50–90 are shown). lanes 1–4: Sanger A, G, C and T reactions, respectively; lanes 5–7: **7** (2), **8** (2) and **9** (2 mmol dm^{-3}), respectively.

enyne–allene intermediate **10** which immediately undergoes a Myers-type cycloaromatization[3] to give the benzenoid diradical **11** (path a in Scheme 2).[4] On the other hand, treatment of **7** with 10 equiv. of diethylamine in 20% Me_2SO in tris-HCl, pH 8.5 buffer at 25 °C for 1 h gave 12% of the cycloaromatization product **13** which would arise from the nucleophilic addition of diethylamine to the enyne–allene **10** and debenzoylation (path b in Scheme 2).[4,5] These results indicate that the enediyne **7** is aromatized both by DBU in cyclohexa-1,4-diene–benzene through a radical pathway and by diethylamine in Me_2SO-tris-HCl, pH 8.5 buffer through a polar pathway.

DNA-cleaving activities of the enediynes **7–9** were assayed with supercoiled ΦX174 DNA (form I) in 20% Me_2SO–tris-HCl, pH 8.5 buffer. As expected, **7–9** (≥1000–100 µmol dm^{-3}) effectively cleaved DNA and caused a single strand break, leading to the nicked open circular DNA (form II) as shown in Fig. 1. Furthermore, it was found that the DNA-cleaving activities of **7**, **8** and **9** increased in that order. Their DNA-cleaving site specificity was also analysed according to the Sanger protocol.[6,†] The results shown in Fig. 2 clearly indicate the identical high guanine selectivity of these compounds for their DNA-cleaving profiles. Considering both the modes of aromatization of **7** already mentioned and the high guanine selectivity in their DNA cleavages, it seems more likely that their DNA-cleaving mechanism is an alkylation mechanism,[7] not a radical mechanism. Details of the DNA-cleaving mechanism of these novel enediynes and attaching these novel DNA-cleaving moieties onto the sequence-specific delivery systems are now under investigation.

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Footnote

† Since the Sanger sequencing reactions result in base incorporation, cleavage at nucleotide N (sequencing) represents cleaving site by an enediyne at N + 1. D. L. Boger, S. A. Munk, H. Zarrinmayeh, T. Ishizaki, J. Haught and M. Bina, *Tetrahedron*, 1991, **47**, 2661; K. Toshima, K. Ohta, A. Ohashi, T. Nakamura, M. Nakata and S. Matsumura, *J. Chem. Soc., Chem. Commun.*, 1993, 1525.

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