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Spin-Trapping of the *p*-Benzyne Intermediates from Ten-Membered Enediyne Calicheamicin γ_1^I

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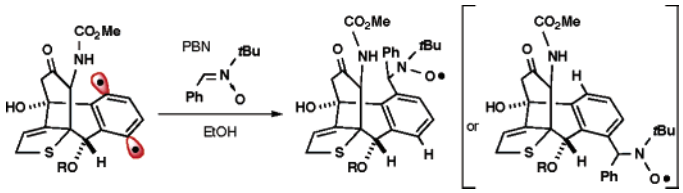
ABSTRACT



In the presence of thiols, the ten-membered-ring enediyne calicheamicin γ_1^I generates a *p*-benzyne biradical that initiates oxidative cleavage of double-stranded DNA. Application of spin-trapping has successfully provided ESR and mass spectroscopic evidence for the formation of the monoadducts with phenyl *tert*-butyl nitrone (PBN).

Calicheamicin γ_1^I (Scheme 1, **1**) is produced by *Micromonospora echinospora* ssp. *calichensis*. and is known to display significant antitumor activity and potency against experimental murine tumors.[1,2] It is a member of the family of non-chromoprotein enediyne natural products[3] and cleaves DNA in a double-stranded fashion at low concentrations with remarkable sequence selectivity.[4] The DNA cleavage is a result of the calicheamicin binding in the DNA minor groove followed by cycloaromatization of the bicyclo[7.3.1]trideca-enediyne via a Masamune−Bergman reaction[5] with formation of a transient *p*-benzyne (**3**). It is this intermediate that abstracts hydrogen atoms from the deoxyribose backbone and results in oxidative strand cleavage. The process (Scheme 1) is triggered by bioreductive cleavage of the allylic trisulfide with thiols followed by a hetero-Michael addition leading to the dihydrothiophene (**2**) which then undergoes the room temperature cycloaromatization. Thus formation of the *p*-benzyne intermediate (**3**) is of critical importance for the biological activity of calicheamicin.[4,6]

Since the discovery of the enediynes in the middle 1980s, it has been of interest to obtain spectroscopic evidence for the *p*-benzynes involved in the cycloaromatization of these potent antitumor antibiotics. However, because of their extremely short lifetimes as well as their assumed singlet ground state, it has been difficult to obtain direct spectroscopic data for these transient intermediates by electron spin resonance (ESR) spectroscopy.[7−9] Also hindering direct observation is the fact that the equilibrium concentration of *p*-benzynes is considered to be very low compared to that of the enediynes.[10] However, the successful observation of

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(2) Lee, M. D.; Durr, F. E.; Hinman, L. M.; Hamann, P. R.; Ellestad, G. A. In *Advances in Medicinal Chemistry*; Maryanoff, B. E., Maryanoff, C. A., Eds.; JAI Press: New York, 1993; Vol. 2, p 31.

(3) For reviews on enediyne natural products: (a) Xi, Z.; Goldberg, I. H. In *Comprehensive Natural Products Chemistry*; Barton, D. H. R., Nakanishi, K., Eds.; Elsevier: New York, 1999; Vol. 7, p 553. (b) Galm, U.; Hager, M. H.; Van Lanen, S. G.; Ju, J.; Thorson, J. S.; Shen, B. *Chem. Rev.* **2005**, *105*, 739. For a recent review of calicheamicin: (c) Thorson, J. S.; Sievers, E. L.; Ahlert, J.; Shepard, E.; Whitwam, R. E.; Onwueme, K. C.; Ruppen, M. *Curr. Pharm. Des.* **2000**, *6*, 1841.

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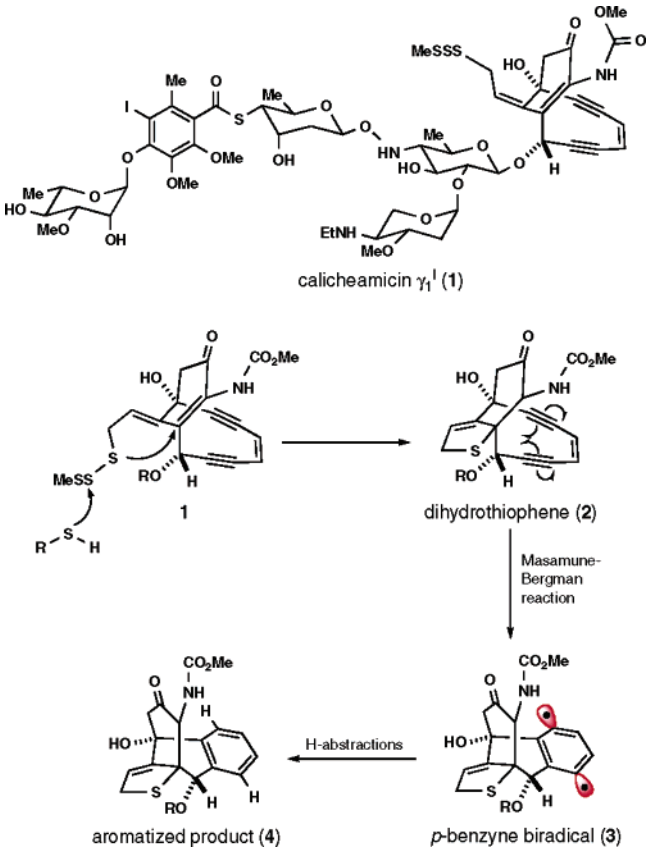
Scheme 1. Structure of Calicheamicin γ_1^I (**1**) and Formation of Dihydrothiophene (**2**), *p*-Benzyne Biradical (**3**), and Aromatized Product (**4**)



p-benzynes from synthetic and natural product C-1027 nine-membered enediynes with use of spin-trapping[11] has been reported recently.[12] This is due to the fact that these nine-membered enediynes spontaneously produce *p*-benzyne biradicals by cycloaromatization without any initiators such as thiols. In contrast, this is not the case for ten-membered-ring enediynes, which require initiators leading to radical formation. In this paper, application of spin-trapping with phenyl *tert*-butyl nitrone (PBN) has, for the first time, provided spectroscopic evidence for the formation of monoadducts in a ten-membered enediyne, calicheamicin γ_1^I, **1**.

When an X-band continuous wave (CW) ESR measurement in ethanol was performed for β-mercaptoethanol in the presence of spin-trapping reagent PBN, no signals were observed at room temperature. In contrast, treatment of **1** and PBN in ethanol with an excess of β-mercaptoethanol gave rise to ESR signals with hyperfine couplings (Figure 1, $A_N = 1.46$ mT, $A_H = 0.28$ mT). These values are in good agreement with those reported for nonsubstituted phenyl radical adducts ($A_N = 1.51$ mT, $A_H = 0.30$ mT).[13] Signals corresponding to bisadducts of PBN were not observed under these conditions.

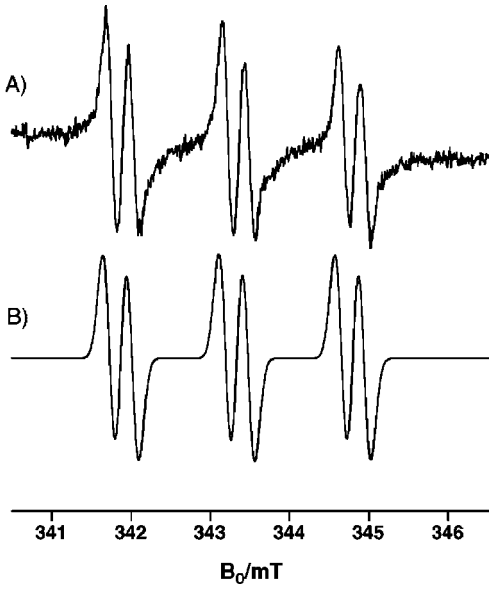


Figure 1. (A) CW ESR (X-band) spectrum observed upon addition of PBN to **1** in the presence of β-mercaptoethanol in ethanol at room temperature and (B) its simulated spectrum.

The electron spray ionization (ESI) mass spectroscopy (MS) measurement provided additional proof of the existence of only a monoadduct (Scheme 2, **5** or **6**, calcd for $C_{65}H_{89}O_{22}N_4S_2I$ 1468.4 $[M + H]^+$, found 1468.7) while signals of the bisadducts could not be observed in the mass spectrum. We did not observe mass signals for any other spin-trapped species.

It has previously been shown that two deuterium atoms from dichloromethane-d_2 were readily incorporated into both C-3 and C-6 positions of *p*-benzyne **3**.[1c] In view of the fact that spin-trapping studies with nine-membered-ring enediynes

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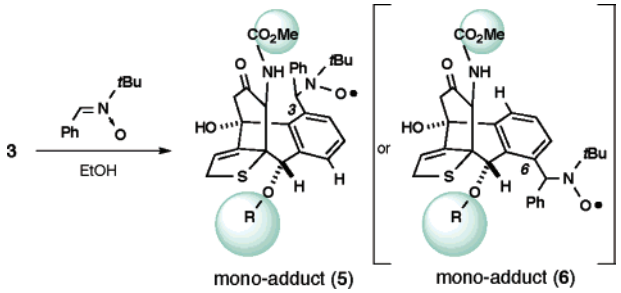
(13) Janzen, E. G.; Nutter, D. E., Jr.; Evans, C. A. *J. Phys. Chem.* **1975**, *79*, 1983.

Scheme 2. Possible PBN Adducts (**5**, **6**)



did not show signals of bisadducts either by ESR or by MS,[12] it is conceivable that the trapped monoadducts are not capable of reacting with another trapping reagent. In addition, the results indicated that the sterically bulky functional groups of the aryloligosaccharide and the methyl carbamate moieties prevent addition of the bulky spin-trapping reagent PBN at C-6. Thus, the adduct at C-3 (**5**) is more likely than that at C-6 (**6**) (Scheme 2).

Because ethanol radicals ($CH_3C{\cdot}HOH$) might have been produced during cycloaromatization from the enediynes, the possible presence of PBN ethanol adducts was considered. These, however, were ruled out not only because of the reported hyperfine splitting constants ($A_N = 1.53$ mT, $A_H = 0.36$ mT)[13] of this species but also because phenyl radicals are known to react with PBN 78 times faster than ethanol radicals.[14]

Although ESR spectra with other spin-trapping reagents, e.g., methyl-2-nitrosopropane (MNP) and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), were recorded in ethanol for **1** in the presence of β-mercaptoethanol, neither signal showed phenyl radical adducts. Instead, the spectra gave very weak signals of a hydrogen radical adduct and a hydroxy radical adduct, respectively. Earlier studies of **1** with DMPO under argon atmosphere in the presence of β-mercaptoethanol in dimethyl sulfoxide had given an indication of an adduct, but these results could not be repeated here.[15]

When the peak areas of the triplet signal of 2,2,6,6-tetramethlypiperidine-*N*-oxyl (TEMPO) in ethanol were used as standard, the yield of the obtained monoadducts was estimated to be around 0.5%. This low yield is due to the short lifetime of **3** (**2** → **3** → **4**, $t_{1/2} = 4.5 \pm 1.5$ s, in methanol at 37 °C).[16] In contrast, the yield was 3−6% in CD_2Cl_2 for the reaction of the synthetic nine-membered-ring enediyne with spin-trapping reagents MNP and DMPO[12c] while with the nine-membered natural C-1027 the yield was 0.1% in aqueous buffer.[12b]

In summary, ESR and MS data of radical adducts of calicheamicin γ_1^I, **1**, in the presence of thiols have been observed with use of spin-trapping with PBN for a ten-membered-ring enediyne system. Because of steric hindrance between bulky moieties in **1** and PBN, the PBN is most likely to trap the phenyl radical at C-3 rather than that at C-6. The method described above could lead to further applications in the clarification of carbon-centered radical-mediated biological processes.

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Supporting Information Available: Experimental procedures and mass spectra data. This material is available free of charge via the Internet at http://pubs.acs.org.

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