Radical Chemistry

Spin Trapping of ^{13}C-Labeled *p*-Benzynes Generated by Masamune–Bergman Cyclization of Bicyclic Nine-Membered Enediynes**

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In memory of Satoru Masamune

Our understanding of the chemistry and biology of the enediyne class of antitumor antibiotics is still developing and faces further challenges.[1] The cleavage of double-stranded DNA through hydrogen abstraction by enediyne-derived radicals is a generally well-accepted mechanism.[1b,e] However, the mechanisms of several unexpected and complex cytotoxic effects of enediynes, such as RNA[2] and protein[3] damage or the generation of *p*-quinone species,[4] have yet to be fully elucidated. Despite several carefully crafted experimental[5] and theoretical[6] studies on simplified systems, the behavior of the major chemical perpetrators of such cytotoxicity, namely the biradical intermediates formed by natural enediynes, remains more a matter of conjecture than a point of fact.[7]

There is clearly a need to study and accurately represent the presumed thermal behavior of the reactive bicyclic cores of natural enediynes, especially those featuring nine-membered rings.[7,8] We first recognized this need in 1995 after observing the paramagnetic activity of the bicyclic epoxyenediyne core **1a** of the kedarcidin chromophore (Scheme 1).[7a] We speculated that a spontaneous equilibration similar to that likely to take place between **1** and the *p*-

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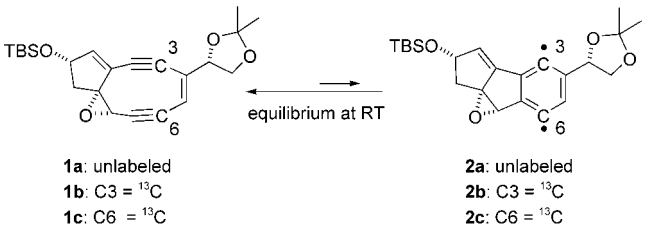


Scheme 1. Spontaneous thermal Masamune–Bergman cyclization of the nine-membered ring of enediyne **1** to form *p*-benzyne biradical **2**. TBS, *tert*-butyldimethylsilyl.

benzyne biradical **2** at ambient temperatures would occur within other natural chromophores that contain a nine-membered ring.[7b,c] In the study described herein, we expanded and reinforced these earlier speculations by using isotopomers of **1** with ^{13}C labels at the supposed radical-forming positions 3 and 6. To tackle the daunting task of obtaining a continuous supply of the unstable compounds **1** in sufficient quantities, we remodeled our previous synthesis of **1a**[7a] and recently devised efficient and reproducible routes to the ^{13}C isotopomers **1b** and **1c**.[9] Through a combination of EPR spin trapping[10] and MS experiments we have now obtained new spectroscopic evidence for the spontaneous thermal generation of the *p*-benzyne biradical **2** as an intermediate in the Masamune–Bergman cyclization[11] of **1**.[1a]

To derive the optimum amount of evidence for the existence of the highly transient biradical **2**[7a] and to obtain stable adducts for MS analysis, we studied the spin-trapping reaction of **1** with both 2-methyl-2-nitrosopropane (MNP) and 5,5-dimethyl-1-pyrrolidine *N*-oxide (DMPO).[12,13] Figure 1a shows the continuous wave (CW) EPR spectrum of the unlabeled enediyne **1a**, measured upon addition of MNP to a solution of **1a** in CD_2Cl_2 at RT. The observed three-line spectrum (with $a^N = 1.390$ mT, $g = 2.006$) is attributable to the phenyl radical adducts **5a** and **6a** (Scheme 2) and is in good

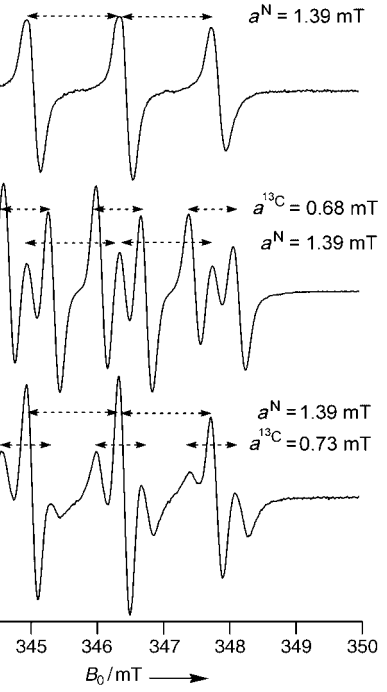
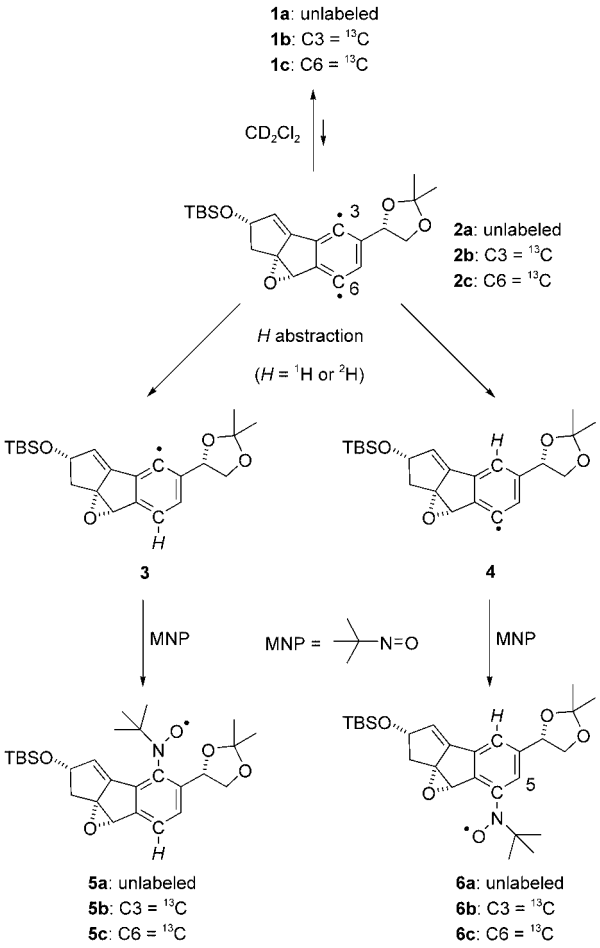


Figure 1. CW EPR (X-band) spectra of spin-trapped MNP adducts of **1** in CD_2Cl_2. a) Unlabeled enediyne **1a**; b) $^{13}C3$-labeled enediyne **1b**; c) $^{13}C6$-labeled enediyne **1c**.



Scheme 2. Proposed pathway from **1** to the observed MNP adducts **5** and **6**, through the *p*-benzyne biradical **2** and phenyl radicals **3** and **4**.

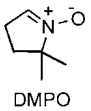
agreement with data reported in the literature for the phenyl radical–MNP adduct in CH_2Cl_2 ($a^N = 1.335$, $a^{Ho} = 0.170$, $a^{Hp} = 0.170$, $a^{Hm} = 0.085$ mT).[14] The spectra of the ^{13}C-labeled compounds **1b** and **1c** exhibit two distinct splitting patterns (cf. Figure 1b and 1c) different from that shown in Figure 1a.[15] One of the three observed patterns agrees well with that of the unlabeled compound **1a**, whilst the other two show additional ^{13}C splittings of 0.680 mT (Figure 1b) and 0.730 mT (Figure 1c). These latter values are in good agreement with the hyperfine splitting (HFS) constants described for ^{13}C-centered radicals and the spectra are as expected for the ^{13}C-labeled phenyl radical adducts **5b**/**6b** and **5c**/**6c**, respectively (Scheme 2).[16] Integration of the spectra led to an estimated C3/C6 adduct ratio of around 2:1. This signal ratio remains constant throughout the time period of the EPR experiment (30–60 min).

First and foremost, the spectra of Figure 1 demonstrate that MNP attaches to both the C3 and C6 radical-forming positions. The spectra in Figure 1b and 1c complement each

other. Each shows two coexisting signal components, one of which undergoes additional splitting by ^{13}C.[15b] The spectra demonstrate that a difference exists between the relative kinetics of spin trapping and quenching at the C3 and C6 positions. Although the quenching event could conceivably involve the substrate **1** as part of a dimerization or oligomerization process, the MS data described herein lead us to favor the intervention of a hydrogen isotope from, for example, the solvent, a substrate molecule, the spin-trapping agent or residual water. All our data support the conclusion that the *p*-benzyne biradical intermediate **2** is a viable precursor of the species responsible for the observed EPR spectra.

To account for the observed EPR signals and the ratios of the spin-trapped monoadducts **5** and **6** produced, we propose a single hydrogen abstraction as the predominant event (among other possibilities) before spin trapping, and the phenyl radicals **3** and **4** as plausible precursors of **5** and **6** (Scheme 2). This hypothesis does not discount the possibility that the spin trap might intercept the biradical **2**.[15b] We favor the hypothesis that the radical centre at the more exposed C6 position is more reactive than that at the sterically shielded C3 position[17] and C6 more readily undergoes hydrogen abstraction before intervention of the bulky MNP spin trap. We used the signal intensities of the paramagnetic species **1** (Figure 1) that steadily accumulated during the EPR experiment (30–60 min) to estimate the yield of observable[15b] spin-trapped adducts. We calculated a yield of around 3–6% relative to 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO, used as a standard), which we consider reasonable in view of the low estimated equilibrium constant of the process supplying **2**.[7a] Unfortunately, the MNP adducts could not be detected by soft-ionization MALDI or ESI TOF mass spectrometry, presumably because of the inherent instability of the adducts formed during the EPR experiments to mass analysis (see the Experimental Section). Clear mass spectra would validate our hypothesis that quenching by an ^1H or a ^2H source takes place, rather than oligomerization of the substrate. No high-molecular weight signals assignable to dimeric or trimeric products were detected under a variety of mass detection conditions, which suggests that oligermization does not occur.[13]

The behavior of **1** was also studied with DMPO as the spin-trapping reagent. The CW EPR spectrum of a mixture of **1a** and DMPO in CD_2Cl_2 at RT under steady-state conditions is shown in Figure 2a. The HFS values $a^N = 1.450$ and $a^H = 2.140$ mT ($g = 2.006$, cf. simulated spectrum, Figure 2b) were determined from this spectrum. The reported hyperfine constants of DMPO adducts of the phenyl radical ($a^N = 1.440$, $a^H = 2.020$ mT in MeCN; $a^N = 1.390$, $a^H = 1.940$ mT in benzene)[18] suggest that the observed values are attributable to the monoadducts **7** (Scheme 3).[15b] The EPR spectra derived from the ^{13}C-labeled systems **1b** and **1c** with DMPO could not be resolved with confidence because of the presence of contaminants in the EPR mixture.[13] However, additional spectra recorded for DMPO, combined with the spectrum of unlabeled compound **1a** with DMPO (Figure 2), suggest not only the presence of two paramagnetic components, but also the predominance of a C3-trapped component



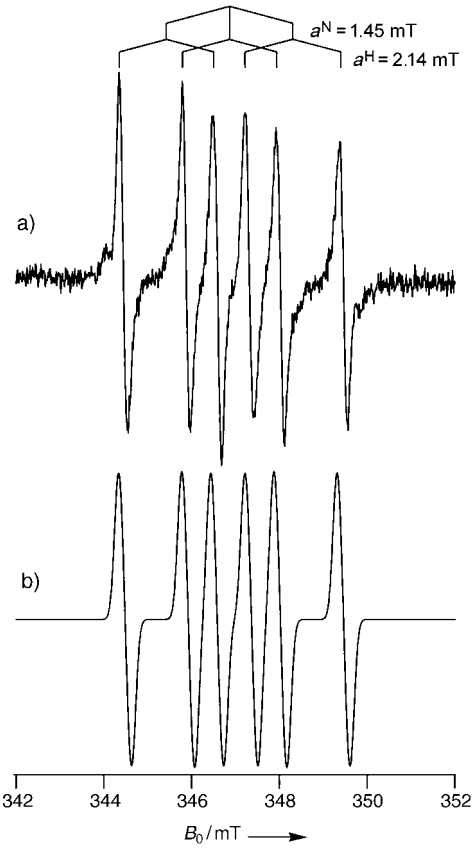
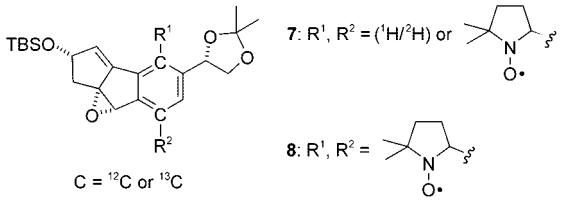


Figure 2. CW EPR (X-band) spectra of DMPO adducts in CD_2Cl_2. a) Observed upon addition of DMPO to **1a** at RT; b) simulated spectrum.



Scheme 3. DMPO monoadducts **7** and bis adducts **8**, detected by MALDI-TOF MS.

experiencing extra ^{13}C splitting, rather than a C6-trapped component. Direct MALDI-TOF mass spectrometry of these and other EPR mixtures of **1** with DMPO gave clean spectroscopic evidence for the presence of both the monoadducts **7** and the bis adducts **8** (Scheme 3; monoadduct **7a**, calcd for $C_{29}H_{42}NO_5Si$: 512.28 $[M]^+$; found: 512.29; bis adduct **8a**, calcd for $C_{35}H_{53}N_2O_6Si$: 625.37 $[M+H]^+$; found: 625.39; monoadducts **7b/c**, calcd for $C_{28}{}^{13}CH_{42}NO_5Si$: 513.29 $[M]^+$; found: 513.28; bis adducts **8b/c**, calcd for $C_{34}{}^{13}CH_{53}N_2O_6Si$: 626.37 $[M+H]^+$; found: 626.37).[13] No signs of dimeric or oligomeric adducts were observed. The intensities of the signaling patterns of the monoadducts **7** indicated a 4:1 ratio of ^1H to ^2H incorporation, which supports our proposal of the quenching event shown in Scheme 2.

We have disclosed new EPR and mass spectroscopic evidence for the existence of both mono and bis spin-trapped

adducts derived from **1**. This evidence was obtained by effectively increasing the extremely low equilibrium concentrations[7a] of the elusive biradical **2** through spin trapping with MNP and DMPO. The ^{13}C-labeled models **1b** and **1c** were shown to generate reactive unpaired radicals at both the C3 and C6 positions, as demonstrated by the observed characteristic hyperfine splittings of ^{13}C-centered nitroxyl radical adducts (Figure 1). This work not only provides persuasive, albeit indirect, evidence for the spontaneous Masamune–Bergman cyclization[11] of **1** to form the *p*-benzyne biradical **2**, but also points to the differential reactivity of the C3 and C6 radical positions for the first time. The close structural similarity of our compounds to the reactive bicylic enediyne core of the nine-membered-ring chromophores, and the thermal activation mechanism of **1**[7] make the observed difference in reactivity between C3 and C6 interesting for researchers aiming to detail the initial events in hydrogen abstraction of DNA by naturally derived *p*-benzynes.

Experimental Section

X-band (9.6 GHz) EPR spectra were recorded on a Bruker ESP 380E spectrometer at RT.

MNP (4 μmol) or DMPO (2 μmol) in CD_2Cl_2 (110 μL) was introduced to a solution of enediyne **1** (4 μmol) in CD_2Cl_2 (125 μL) in a quartz tube (∅ 5 mm) at RT under atmospheric pressure. After the EPR experiments, the same samples of **1** containing the spin trap (MNP or DMPO) were used for mass analysis. The MALDI-TOF mass spectra of the DMPO-containing samples were measured in positive ion mode on an Applied Biosystems Voyager DE STR SI-3 instrument by adding aliquots of the CD_2Cl_2 solutions to THF solutions of the matrix (α-cyano-4-hydroxy cinnamic acid). ESI-TOF mass analyses were found ineffective for mass detection under a variety of conditions. The spin-trapping reagents MNP and DMPO were purchased from Kanto Chemical Co. Ltd. and were used without further purification. The degree of deuteration of the solvent CD_2Cl_2 was 99.8% (Merck Co. Ltd.).

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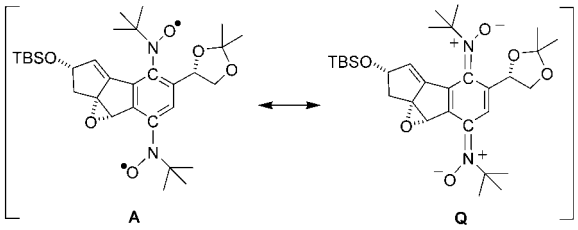

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