Supporting Information

Calicheamicin γ_1^I and Phenyl *tert*-Butyl Nitrone (PBN): Observation of a Kinetic Isotope Effect by an ESR Study

Toyonobu Usuki,*[a] Mari Kawai,[a] Koji Nakanishi[b] and George A. Ellestad*[b]

[a] *Department of Materials and Life Sciences, Faculty of Science and Technology, Sophia University, 7-1 Kioicho, Chiyoda-ku, Tokyo 102-8554, Japan*

[b] *Department of Chemistry, Columbia University, 3000 Broadway, New York, New York 10027, USA*

Materials:

Spin-trapping reagent phenyl *t*-butyl nitrone (PBN) was purchased from Aldrich and recrystallized with ethyl acetate/hexane. Ethanol, ethanol-d_6, and triphenylphosphine were used as ACS grade without purification. Antitumor antibiotic calicheamicin γ_1^I (**1**) was provided by Wyeth laboratories. Solvents for high performance liquid chromatography (HPLC) analysis were used as HPLC grades.

Spin-trapping PBN experiments in the presence of triphenylphosphine in ehtanol:

Continues wave (CW) ESR spectra were recorded with an EMX-Bruker spectrometer, operating at X-band (9.8 GHz) at room temperature. The X-band ESR measurement settings were as follows: center field, 347.0 mT; sweep width, 7.0 mT; modulation frequency, 100 kHz; modulation amplitude, 0.1 mT; receiver gain, 1×10^5; time constant, 163.84 ms; sweep time, 167.77 s; scan, 8.

Freshly prepared PBN (0.9 mg, 5.08 μmol) in ethanol (200 μL) under nitrogen was added to **1** (0.5 mg, 366 nmol) also under a nitrogen atmosphere. The resulting solution was added to ethanol (100 μL) containing triphenylphosphine (0.29 mg, 1.1 μmol) and

then introduced into a quartz tube (Φ 5 mm) at room temperature. The ESR measurement was started immediately.

Spin-trapping PBN experiments in the presence of triphenylphosphine in ehtanol-d_6:

CW ESR spectra were recorded with an EMX-Bruker spectrometer, operating at X-band (9.8 GHz) at room temperature. The settings were as follows: center field, 347.0 mT; sweep width, 7.0 mT; modulation frequency, 100 kHz; modulation amplitude, 0.1 mT; receiver gain, 1×10^5; time constant, 163.84 ms; sweep time, 167.77 s; scan, 8.

PBN (0.9 mg, 5.08 μmol) in ethanol-d_6 (200 μL) in the presence of nitrogen was added to **1** (0.5 mg, 366 nmol) under nitrogen atmosphore. A solution of ethanol-d_6 (100 μL) containing triphenylphosphine (0.29 mg, 1.1 μmol) was then added and the resulting reaction mixture introduced into a quartz tube (Φ 5 mm) at room temperature. ESR measurements were started immediately.

HPLC analysis:

For the analysis, a JASCO HPLC system equipped with a multichannel detector was used. The conditions were as follows: column, Cosmosil 5C18-AR-II (2.5 × 150 mm, Nakalai tesque, Kyoto); solvent, linear gradient of 50% acetonitrile and 50% ammonium acetate (0.1%, pH 7.0); flow rate, 0.2 mL/min; detection, 280 nm; temperature, 25 °C.

The spin-trapped sample solutions (10 μL) which were used for ESR measurements were injected into the HPLC system at room temperature. The spectra of samples of calicheamicin γ_1^I **1** only (no spin trapping reagent) in ethanol, **1** with PBN in ethanol, and **1** with PBN in ethanol-d_6 are shown in Figures S1, S2, and S3, respectively.

While only **1** was detected at 15 min (Figure S1), PBN treated **1** in the presence of triphenylphosphine appeared as a single peak at around 3 to 4 min (Figure S2, S3). The results indicated the consumption of the starting material on the spin trapping experiments. However, we were unable to obtain any mass peak attributable to the formation of PBN phenyl radical adducts by the LC-MS measurements.

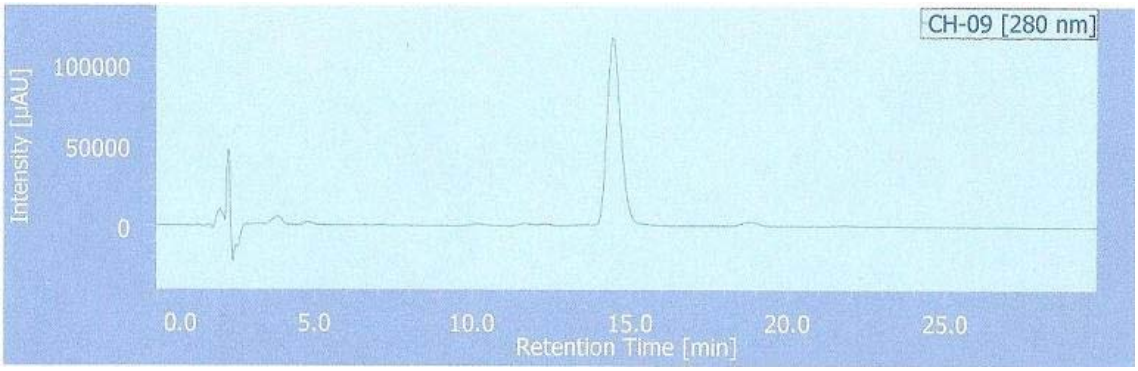

Figure S1

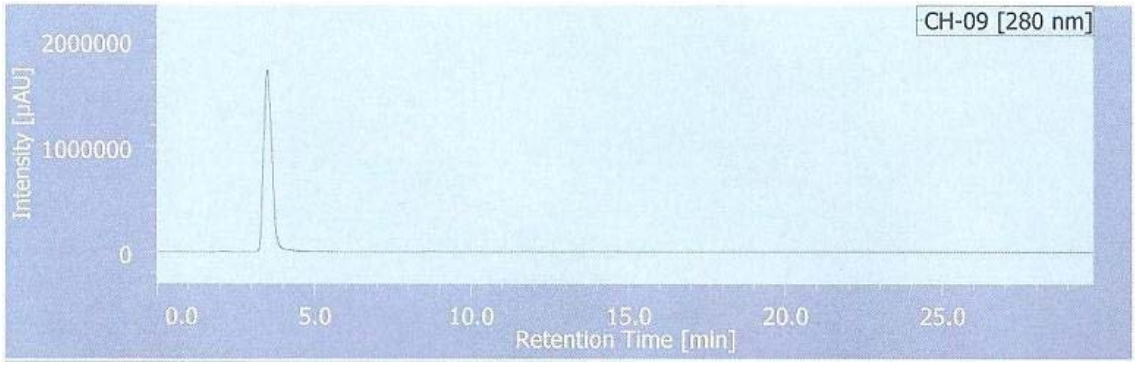

Figure S2

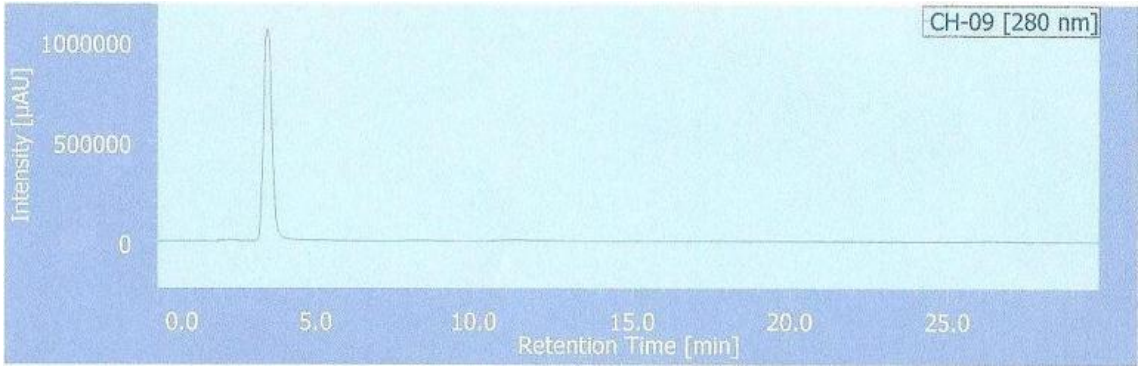

Figure S3