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Kinetic analysis of a reactive model enediyne

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ABSTRACT: A model framework related to the calcheamicin family of enediyne toxins was evaluated in its reactivity toward cycloaromatization through the arene 1,4-diyl intermediates and hydrogen atom abstractions. The keto version **9** is relatively unreactive, with $t_{1/2} = 1.5$ h at 60 °C. The product from hydride reduction of the ketone, alcohol **13b**, is much more reactive, with $t_{1/2} = 50$ min at 0 °C. In both cases, the rate of rearrangement is independent of the hydrogen atom donor, consistent with a rate-determining first step (cyclization). Copyright © 2004 John Wiley & Sons, Ltd.

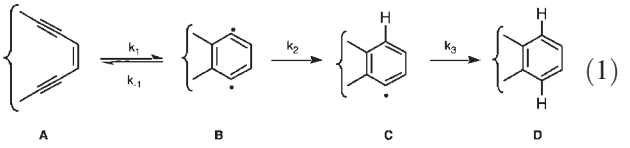
KEYWORDS: calicheamicin; enediyne model; arene diradical; hydrogen atom abstraction

INTRODUCTION

The enediyne toxins have generated substantial activity in total synthesis, design of model systems, biological evaluation and fundamental mechanistic analysis.[1] Interest derives from the novel structures and proposed mechanism of toxicity and also the opportunity to use the information gained to design functional models of the natural toxins as biological probes or therapeutic agents.

Successful mimicking of the natural products implies a structure which is stable at ambient temperatures, can undergo thermal reactions ('trigger'), leading to a highly reactive 1,4-arenediyl, and binds to DNA with delicate positioning that allows double strand cleavage initiated by H-atom abstraction. An additional feature not present in the natural products would be control over the biological site of activation in order to provide selective toxicity.[2] In principle, the 'trigger' process might be adjusted to operate selectively in the cells or tissue of interest. As a first step in the design of functional models of the enediynes, it is necessary to establish the rate-determining step for the system under consideration and the structural features which influence the rate profile. Despite considerable effort, the correlation of structure with reactivity toward cycloaromatization is still much analyzed and discussed.[1a,b]

An intriguing mechanistic issue is the rate profile for the cycloaromatization steps [Eqn (1)]. The activated precursor, **A**, undergoes ring closure to the 1,4-arenediyl **B** and then H-atom abstraction steps lead through the aryl radical (**C**) to complete the process with formation of the arene, **D**.



(1)

The original studies involved acyclic enediynes with low overall rates ($t_{1/2} = 30$ s at 200 °C) and a rate-determining ring closure, $\mathbf{A} \rightarrow \mathbf{B}$, k_1.[3] It has been generally assumed that the natural products such as calicheamicin, with very high overall rates of reaction ($t_{1/2} \approx 4$ s at 23 °C), also proceed with a rate-determining ring closure, k_1. [Low-temperature NMR studies have established the rate of initial rearrangement (triggering) for calicheamicin, but do not comment on the rate-determining step for disappearance of the activated form.[4]] This assumption must fail, however, as k_1 is increased in more reactive structures. Eventually, k_2, the first H· abstraction, will become the rate-determining step. However, there are no kinetic data on the natural products which would solidify or contradict this assumption. The assumption would also fail if k_2 became much lower owing to structural changes, while k_1 remained high. In early analyses, the value of k_2 was expected to be fairly independent of structure. The kinetic issues became more unsettled when several special enediyne structures (Fig. 1) were found to react with only moderate rates ($t_{1/2} >$ 10 h at 25 °C) and yet show kinetically significant H-atom abstraction, step $\mathbf{B} \rightarrow \mathbf{C}$, k_2 [Eqn (1)]. It has been postulated that k_3 is always faster than k_2 owing to a stabilizing interaction in the 1,4-diradical **B** compared with the phenyl-type radical, **C**.[5] Here, we report a kinetic study of a model framework related to calicheamicin which has a high rate of rearrangement, comparable to the natural product, and helps to establish the rate profile for reactive enediynes.

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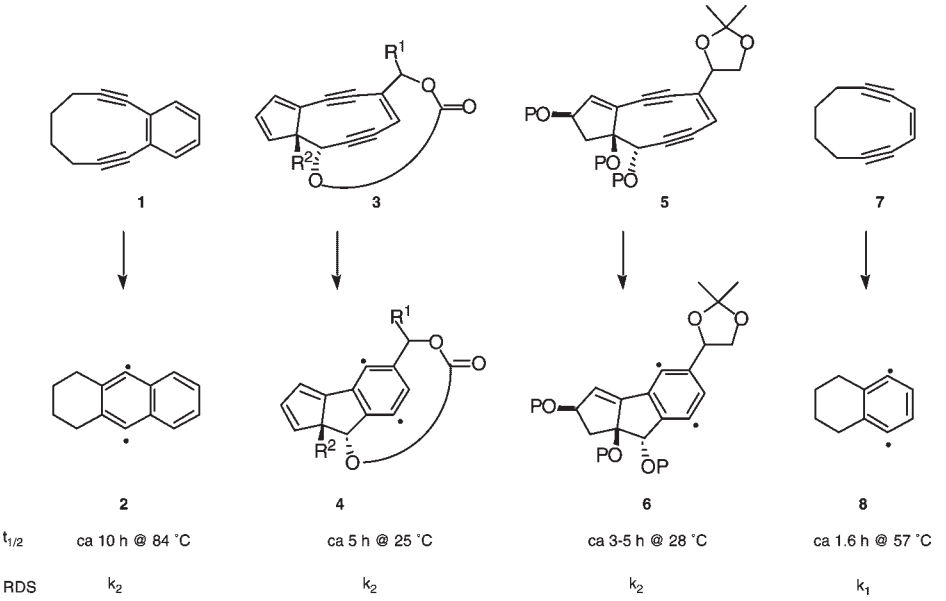


Figure 1. Half-lifetimes and rate-determining step for cyclic enediynes

It is useful to compare the cyclic systems for which kinetic studies are available. The first special case was the simple arenediyne **1**, which has a half-lifetime of ca 10 h at 84 °C.[6] A key observation was the dependence of the rate of disappearance of **2** on the concentration of the trapping agent, 1,4-cyclohexadiene (CHD), clear evidence for kinetically significant H-atom abstraction. While CHD had been commonly used as a trapping agent in many studies of model enediynes, the issue of a concentration effect had not been discussed before. Next was the natural enediyne C-1027chr (lidamycin;[7] represented by **3**), which is unique among the family of enediynes in having no obvious chemical triggering mechanism.[8] It is stable as a protein complex, but cycloaromatizes with a half-lifetime of 2–10 h at 37 °C.[9] Evidence has been accumulated to establish that the first step is fast and reversible; H-atom abstraction is kinetically significant.[10] The primary evidence is a significant deuterium isotope effect (ca 2.4) when the reaction is carried out in CD_3OD compared with CH_3OH. Similar effects were reported for the model **5**, which is a version of the core structure of C-1027chr.[11] The simple enediyne **7** has been studied extensively[12] and was shown to react by rate-determining cycloaromatization, with a relatively fast H· abstraction process.[13] Compound **7** shows a rate of rearrangement similar to **3**, but faster than **1** and much slower than calicheamicin.

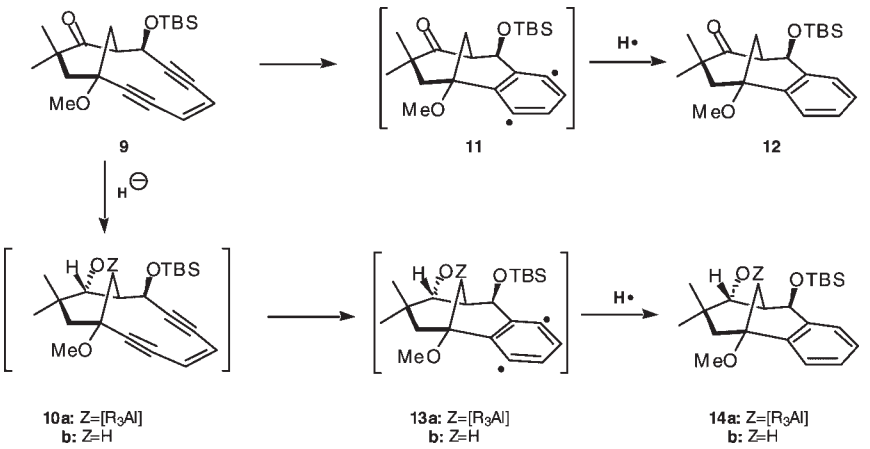
The rationale for the special rate profile for reactions of **1**, **3** and **5** is not complete. It was proposed by Logan and Chen that the rate of H-atom abstraction for annelated versions of the benzene-1,4-diyl substantially decreases with the number of annelated arene rings (benzene-1,4-diyl > naphthalene-1,4-diyl > anthracene-9,10-diyl).[14] Schottelius and Chen's suggestion that the rate difference is due to increase in the singlet–triplet splitting energies (ΔE_{ST})[15] with more benzannelation has been challenged by Koseki *et al.*,[16] who instead favor an explanation based on an increase in rate for the reverse of cycloaromatization (k_{-1}) with increased benzannelation. In this explanation, the greater loss of delocalization energy (benzene > naphthalene) gives a smaller k_{-1} for **8** compared with **2**. Recent calculations suggest that the ΔE_{ST} is not significantly changed by benzannelation.[17] Neither of these pictures deals with the structure type represented by C-1027, **3**. There has been no analysis of the relative singlet–triplet splitting for **4**, but considering the recent calculations, no difference compared with **8** is expected. The difference in resonance energy for **4** or **6** compared with **8** is expected to be small (styrene resonance energy compared with benzene).

RESULTS AND DISCUSSION

The calicheamicin analog **9** was prepared and shown to have a low rate of cycloaromatization, with $t_{1/2} \approx 1.5$ h at 60 °C (the preparation and general properties of enediynes in this series will be reported separately). We noted that hydride reduction gives alkoxide/alcohol **10**, which is much more reactive, with $t_{1/2} \approx 1$ h at 0 °C, only an order of magnitude slower than activated calicheamicin (in other models related to calicheamicin, reduction of a ketone was found to be activating[18]). In this paper, we report a careful study of the reactions of ketone **9** and its hydride reduction product **10** in order to verify the rate-determining step for these substrates. A rate dependence on the concentration of the trapping agent for enediyne **9** would suggest the second step [k_2, Eqn (1)] was rate determining whereas no dependence would confirm the common assumption of rate-determining

Table 1. Degradation rates of enediyne ketone **9** at 60 °C in C_6D_6

Entry	H-atom donor	$t_{1/2}$ (h)	k (s^{-1})	r^2	Completion (%)
1	CHD (0 M)	1.50	1.40×10^{-4}	0.989	72.0
2	CHD (0.03 M)	1.50	1.30×10^{-4}	0.998	90.5
3	CHD (0.14 M)	1.70	1.23×10^{-4}	0.986	64.0
4	CHD (0.19 M)	1.60	1.26×10^{-4}	0.982	81.6
5	C_2H_5OH (0.39 M)	1.89	1.02×10^{-4}	0.992	88.9



Scheme 1. Cycloaromatization of the ketone **9** and its reduction product, **10**

cycloaromatization step (k_1). Here we show that there is no significant dependence of rate on the concentration of trapping agent, using 1,4-cyclohexadiene, or a significant primary isotope effect in deuterated solvents.

The rate of rearrangement of silyl ketoenediyne **9** (see Table 1) to the arene diradical was followed by ^{1}H NMR in a sealed tube under argon using varying concentrations of 1,4-CHD and ethanol as trapping agents. Rates were monitored by integration of the vinylic proton signals of the enediyne moiety (C_6D_6; δ 5.20, d, $J = 1.3$ Hz, 1H; δ 5.40, d, $J = 1.5$ Hz, 1H) against an internal standard (1,2-dichloroethane or 1,4-dimethoxybenzene). The average $t_{1/2}$ in the presence of CHD (entries 1–4) is 1.6 with no significant dependence on the concentration of CHD. Additional kinetic runs at 50.0 and 70.0 °C under the same conditions as entry 2 gave $t_{1/2} = 3.1$ h and 0.44 h, respectively. An approximate ln$A$ value of 24.3 and $E_a = 21.9$ kcal (60 °C) are obtained (1 kcal = 4.184 kJ).

A crude determination of the solvent isotope effect was carried out by heating samples of **9** in EtOH and in EtOH-d_6 at 60 °C for 1.0 h, leading to partial completion. The reaction in EtOH was slightly more complete than that in EtOH-d_6, but the ratio was only about 1.1 (duplicate runs); no significant isotope effect was observed.

Reduction of the carbonyl functionality of **9** to **10** leads to reactivity approaching that of activated calicheamicin. The alcohol arene **14b** was obtained in 58% yield from silyl ketone **9** by reduction with DIBALH at 0 °C using a large excess of 1,4-CHD in THF, presumably through intermediates **10a** and **13a**. With this high rate of cycloaromatization, it seemed interesting to ask whether the H· atom abstraction step is now the rate-determining step. The reduction products **10** were too labile to isolate and characterize directly.

The kinetics for the cycloaromatization of the presumed alkoxide intermediate **10a** and the alcohol **10b** (Scheme 1) to the corresponding arene products via the diradicals **13a** and **13b**, respectively, were monitored quantitatively by ^{1}H NMR at 0 °C using either 1,4-dimethoxybenzene (DMB) or 1,2-dichloroethane (DCE) as an internal standard. The results for the pseudo-first-order kinetics are summarized in Table 2. The observed half-lives do not provide a smooth plot, and range from

Table 2. Degradation of enediyne alkoxide **10a** and alcohol **10b** at 0 °C in $CDCl_3$

Entry	Compound	[CHD] (M)	Internal standard[b]	$t_{1/2}$ (min)	r^2
1	**10a**	0.00	DCE	75.5	0.9894
2	**10a**	0.00	DMB	24.4	0.9675
3	**10a**	0.00	DMB	20.8	0.9786
4	**10a**	0.00	DCE	55.6	0.9726
5	**10b**[a]	0.00	DCE	63.0	0.9788
6	**10b**[a]	0.00	DMB	38.7	0.9980
7	**10b**[a]	0.05	DMB	56.1	0.9874
8	**10b**[a]	0.05	DMB	23.6	0.9695
9	**10a**	0.05	DCE	83.0	0.9818
10	**10b**[a]	0.05	DCE	76.0	0.9731
11	**10b**[a]	0.09	DCE	51.4	0.9818

[a] These kinetic studies were conducted on the alcohol which was obtained from the alkoxide by quenching with TFA.
[b] DCE = 1,2-dichloroethane; DMB = 1,4-dimethoxybenzene.

23.6 to 83 min with no apparent dependence on the concentration of 1,4-CHD or internal standard. The scatter in results reflects the difficulty in obtaining high-precision ^{1}H NMR integration data owing to the high rates of reaction for the reduced substrates and the complex spectra characteristic of these reaction mixtures. However, these experiments were useful in establishing an approximate half-life for the cycloaromatization of the reduced species. It is apparent that the half-lifetimes of the alkoxide **10a** and alcohol **10b** are approximately the same, yielding an average half-life of 50 min at 0 °C. We can assume a ln*A* value of 24 based on previous results with the related ketoenediyne framework **9**. Using our average half-life value, a rate k (where $k = \ln 2/t_{1/2}$) of $2.2 \times 10^{-4}\,s^{-1}$ and an activation energy (E_a) for the cycloaromatization (rate-determining step) of 18 kcal mol^{-1} is calculated. This result reflects the higher rate of rearrangement for the reduced enediyne **10** compared with ketoenediyne **9** ($E_a = 21.9$ kcal mol^{-1}).

In an effort to measure more accurately the rate of cycloaromatization (and H-atom abstraction) of the reduced species **10** and confirm cycloaromatization as the rate-determining step, UV spectroscopy was used to follow the kinetics for the Bergman cyclization of the reduced species **10**. The enediyne moiety exhibits $\lambda_{max} \approx 280$ nm ($\varepsilon = 10\,000$) whereas the arene product shows significantly lower absorption ($\varepsilon \approx 2200$) at that wavelength. The decay of the enediyne signal at 280 nm was conveniently followed as a measure of rate. Kinetic measurements were carried out at 21 °C using THF and THF-d_8 as hydrogen atom donors and the results are summarized in Table 3.

A half-lifetime of ~8.0 min at 21 °C is obtained for enediyne alkoxide **13a**. By comparison, an extrapolation of the NMR data (Table 2) to 21 °C and using the assumed ln*A* value of 24 and $E_a = 18$ kcal mol^{-1}, one arrives at a rate value of $k = 2.2 \times 10^{-3}\,s^{-1}$ and a corresponding first order half-life, $t_{1/2} = 5.2$ min, in good agreement with that ($t_{1/2} = 8$ min) obtained from the UV data.

There remained the possibility that the ketone reduction was the rate-determining step in these experiments. The measured half-lives would then reflect the rate of reduction and not the Bergman rearrangement rate. The ^{1}H NMR experiment directly monitored the rate of disappearance of the alcohol which suggested the reduction of the ketoenediyne to the corresponding alcohol occurred much faster than the cycloaromatization that followed. A preparative scale reduction of silyl ketone **9** was carried out at 21 °C by the addition of DIBALH and followed after 1 min by quenching with an aqueous solution of saturated NH_4Cl and then conventional processing over a 1 h period. ^{1}H NMR analysis of the crude reaction product indicated a complete disappearance of the starting ketone (and all vinyl proton resonances). This result confirmed that reduction of ketone **9** (followed by facile cycloaromatization during isolation) is significantly faster than the measured half-life (8 min at 21 °C). The measured half-life must then reflect the rate of cycloaromatization of the reduced species in THF and THF-d_8.

Table 3. Degradation of enediyne alkoxide **10a** at 21 °C using UV spectroscopy

Entry	[Enediyne] (M)	H-atom source	$t_{1/2}$ (min)	r^2
1	3.68×10^{-4}	THF	7.30	0.9998
2	3.68×10^{-4}	THF	8.98	0.9991
3	3.68×10^{-4}	THF	8.01	0.9980
4	3.58×10^{-4}	THF-d_8	8.72	0.9970
5	1.80×10^{-4}	THF-d_8	9.60	0.9910
6	1.80×10^{-4}	THF-d_8	8.00	0.9950

CONCLUSION

We have shown through mechanistic studies using ^{1}H NMR that the ketoenediyne **9** is less reactive than the natural toxins and rearranges to the corresponding arene product with a rate-determining cycloaromatization step (k_1) with $E_a = 21.9$ kcal mol^{-1}. The corresponding alcohol/alkoxide **10** is much more reactive, exhibiting a rate of cycloaromatization only an order of magnitude less than activated calicheamicin. ^{1}H NMR and UV techniques provide kinetic data which establish that **10** also proceeds with a rate-determining cycloaromatization. These results give direct experimental support to the assumption that the cycloaromatization step is the rate-determining step for the highly reactive calicheamicin-type framework. Although hydride reduction of a ketone is not a viable biological trigger, preliminary studies have shown $NaBH_4$ reduction to be compatible with DNA cleavage analysis for our designed framework. The model framework **9/10** is therefore a candidate for exploration of DNA binding and selectivity in cleavage in designed systems.

EXPERIMENTAL

Analytical data. NMR spectra were recorded with a Jeol GSX 270 (270 MHz) instrument. Proton (^{1}H) chemical shifts, reported in parts per million (ppm,) were indirectly referenced to external tetramethylsilane employing resonances due to trace monoprotio-solvent as an internal reference. ^{13}C chemical shifts reported in parts per million (ppm) were indirectly referenced to external tetramethylsilane employing known solvent resonances as internal references. IR spectra were recorded on a Nicolet 800 FT-IR spectrometer with internal calibration. Mass spectral data were obtained using a Kratos MS 80 spectrometer operating in the electron ionization mode (EIMS) at 70 eV, unless noted otherwise. Peaks are

identified in mass/charge (*m/z*) and relative abundance to a base peak (%).

Solvents. Anhydrous tetrahydrofuran (THF) as reaction solvent was obtained by distillation from sodium benzophenone ketyl. THF for UV spectroscopy was commercial spectral grade. Benzene and dichloromethane were distilled from CaH_2 prior to use.

General procedures. Reactions 'under argon' were performed in glassware dried in an oven at 160 °C, then cooled to room temperature in a desiccator over $CaSO_4$. The system was sequentially evacuated [0.05 Torr (1 Torr = 133.3 Pa)] and filled with argon three times before use.

Reagents. All reagents were purchased from Aldrich Chemical (unless indicated otherwise) in >99% purity.

Cycloaromatization of ketone ***9***. A solution of silyl enediyne ketone **9** (15.1 mg, 0.0420 mmol, 1.00 equiv.) in 1,4-cyclohexadiene (1 ml) was placed in a sealed flask under argon and heated at 60 °C. After 72 h, the flask was cooled to 25 °C and the residual 1,4-cyclohexadiene was removed *in vacuo*. The remaining oil was purified by flash chromatography (eluting with 2:1 hexane–diethyl ether) to give the desired arene product **12** as a colorless oil (11.4 mg, 75% yield). R_f 0.52 (2:1 hexane–diethyl ether). ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.49 (d, $J = 7.7$ Hz, 1H, C-3 or 6), 7.34 (dd, $J = 7.3$, 7.3 Hz, 1H, C-4 or 5), 7.24 (dd, $J = 7.3$, 7.3 Hz, 1H, C-4 or 5), 7.13 (d, $J = 7.7$ Hz, 1H, C-3 or 6), 4.64 (d, $J = 2.9$, 1H, C-8), 3.16 (s, 3H, C-14), 3.04 (ddd, 1H, C-9), 2.96 (d, $J = 2.6$, 1H, C-13), 2.16 (d, $J = 12.8$, 1H, C-13), 1.88 (dd, $J = 12.9$, 3.7, 2H, C-12), 1.13 (s, 3H, C-15 or 15′), 0.87 [s, 9H, $SiC(CH_3)_3$], 0.32 (s, 3H, C-15 or 15′), 0.21 (s, 3H, $SiCH_3$), 0.15 (s, 3H, $SiCH_3$). ^{13}C NMR ($CDCl_3$, 68 MHz): δ 217.1 (C-10), 139.7 (C-2 or 7), 138.5 (C-2 or 7), 129.9 (C-3 or 6), 129.1 (C-3 or 6), 127.9 (C-4 or 5), 126.3 (C-4 or 5), 71.1 (C-1), 53.9 (C-8), 50.2 (C-14), 43.6 (C-9), 30.8 (C-12), 29.1 (C-13), 26.4 (C-11), 25.9 [$SiC(CH_3)_3$], 18.1 (C-15 or 15′), 15.2 [$SiC(CH_3)_3$], 2.3 (C-15 or 15′), −4.1 (Si-CH_3), −4.3 (Si-CH_3). IR (NaCl, thin film): 3377.1, 2930, 2858, 1699, 1471, 1360, 1255, 1083, 1049 cm^{-1}. EIMS: *m/z* (%) 374 (M^+, 2.2), 359 (4.3, $M - CH_3$), 317 [$M - C(CH_3)_3$, 100].

Cycloaromatization of alcohol ***10***. A solution of enediyne ketone **9** (30 mg, 0.081 mmol, 1.0 equiv.) in cyclohexadiene (1 ml) and THF (2 ml) was cooled to 0 °C. Neat diisobutylaluminium hydride, (DIBALH, 43 µl, 0.24 mmol, 3.0 equiv.) was added dropwise via a syringe. The reaction mixture was allowed to stir with warming at 23 °C for 5 h, at which time concentrated K Na tartrate solution (5 ml) was added. After the solution was stirred for 30 min, the mixture was extracted with diethyl ether (3 × 10 ml). The combined ethereal phase was washed with brine (30 ml), dried over $MgSO_4$ and concentrated by rotary evaporation. The crude product was purified by flash chromatography (4:1 hexane–diethyl ether) to provide the desired hydroxy arene **14b** as a colorless oil (17.7 mg, 58% yield). R_f 0.17 (4:1 hexane–diethyl ether). ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.39(d, $J = 7.9$ Hz, 1H, C-3 or 4 or 5 or 6), 7.26–7.31 (m, 3H, 3 aromatic hydrogens), 4.89 (s, 1H, C-8), 3.74 (s, 1H, C-10), 3.15 (s, 3H, C-14), 2.61 (dd, $J = 14.5$, 4.0 Hz, 1H, C-13), 2.50 (d, $J = 4.0$ Hz, 1H, C-12), 1.76 (d, $J = 13.2$ Hz, 1H, C-9), 1.50 (dd, $J = 14.5$, 4.05 Hz, 1H, C-13), 1.41 (s, 1H, OH), 1.30 (dd, $J = 10.5$, 3.5 Hz, 1H, C-12), 0.83 (s, 3H, C-15 or 15′), 0.78 [s, 9H, $SiC(CH_3)_3$], 0.10 (s, 3H, C-15 or 15′), 0.08 (s, 3H, $SiCH_3$), 0.00 (s, 3H, $SiCH_3$). IR (neat, oil): 3490, 2952, 2927, 2851, 1471, 1254, 1070, 1051 cm^{-1}. EIMS: *m/z* (%) 376 (M^+, 0.6), 361 ($M - CH_3$, 3.5), 345 ($M - OCH_3$, 5), 319 [$M - C(CH_3)_3$, 100].

General procedure for the degradation study of ketoenediyne ***9*** *by ^{1}H NMR at 60 °C.* Ketoenediyne **9** (0.860 mg, 0.00230 mmol, 1.00 equiv.) was placed in an NMR tube with 1,4-CHD (10.9 µl, 0.115 mmol, 50.0 equiv.) and 1,2-dichloroethane (1.8 µl, 0.023 mmol, 10 equiv.) as internal standard. C_6D_6 (800 µl, filtered through a short pad of $MgSO_4$ and Celite) was added and the solution was evacuated at 23 °C and then filled with argon. The tube was sealed and then heated in a 60 °C oil-bath. Every 1 h, the tube was cooled to 21 °C and ^{1}H NMR spectra were taken. The integral ratios of either the vinyl (in the cases when it was not obscured by 1,4-CHD signals) or methyl proton resonances (δ 5.3 and 3.2, respectively) to the internal standard were used as a measure of reaction rate. The rate of reaction, *k*, was obtained using the first-order relation $\ln(X_0/X) = kt$, where X_0/X is the normalized ratio of vinyl proton integration to the internal standard, from which a half-life, $t_{1/2}$, was determined. A representative data set and a calculation of rate, *k*, and half-life, $t_{1/2}$, is presented in Table 4.

Using the first-order kinetic relation $\ln X = \ln X_0 - kt$, from a plot of $\ln(X/X_0)$ vs *t* (Fig. 2) the value $k = 0.44206\ h^{-1}$ is obtained and $t_{1/2} = 1.6$ h.

Other determinations carried out at 60 °C. The amount of 1,4-CHD was varied using the general procedure: (a) 2 equiv. of 1,4-CHD, $k = 0.46829\ h^{-1}$, $t_{1/2} = 1.5$ h; (b) 10

Table 4. ^{1}H NMR data for degradation of **9** measured over 2.5 h

Time (h)	Integral ratio, ketone:1,2-DCE	Normalized ratio (X/X_0)	Ln(X/X_0)
0.0	0.0552	1	0
0.5	0.0495	0.897	−0.109
1.0	0.0391	0.708	−0.345
1.5	0.0306	0.554	−0.591
2.0	0.0227	0.411	−0.889
2.5	0.0197	0.357	−1.03

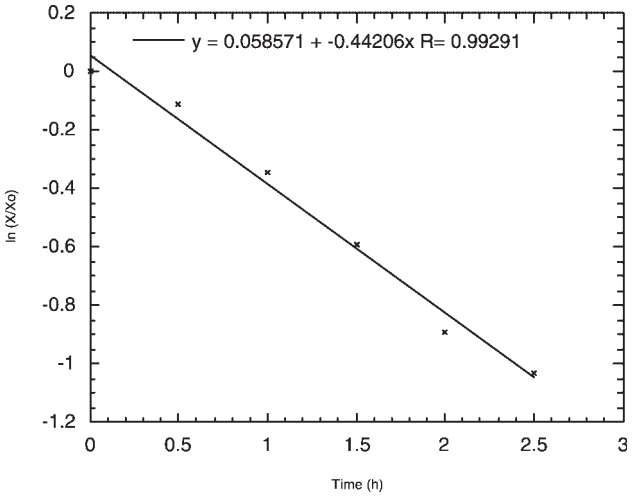


Figure 2. Degradation of ketoenediyne **9** at 60 °C

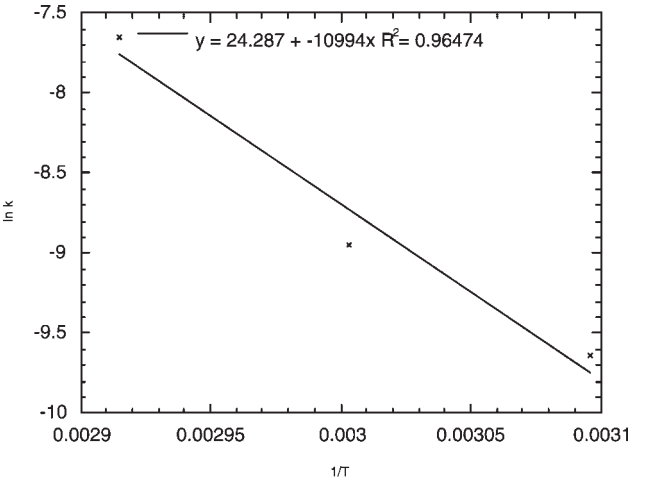


Figure 3. Ln k vs $1/T$ (K^{-1}) at 50, 60 and 70 °C

equiv. of 1,4-CHD, $k = 0.45500\,h^{-1}$, $t_{1/2} = 1.5$ h; (c) 0 equiv. of 1,4-CHD, $k = 0.50360\,h^{-1}$, $t_{1/2} = 1.4$ h.

*General procedure for the degradation study of ketoenediyne **9** by ^{1}H NMR at 50 °C.* Following a similar protocol as described at 60 °C (see above), the rate constant and half-life were determined at 50 °C. A rate constant $k = 0.233\,h^{-1}$ ($6.48 \times 10^{-5}\,s^{-1}$), corresponding to a half-life $t_{1/2} = 3.1$ h, was determined at this temperature.

*General procedure for the degradation study of ketoenediyne **9** by ^{1}H NMR at 70 °C.* Following a similar protocol as described at 60 °C (see above), the rate constant and half-life were determined at 70 °C. A rate constant $k = 0.0286\,min^{-1}$ ($4.77 \times 10^{-4}\,s^{-1}$), corresponding to a half-life $t_{1/2} = 0.44$ h, was determined at this temperature.

*Determining the ln A value for degradation of ketoenediyne **9**.* Using the rate constants determined at 50, 60 and 70 °C, a ln$A$ value can be determined using the relation $\ln k = \ln A - (E_a/R)(1/T)$. Data are given in Table 5 and Fig. 3.

*General procedure for the determination of isotope effects in the trapping step for the arene diradical of ketoenediyne **9**.* Silyl ketoenediyne **9** (4.40 mg, 0.0122 mmol) was placed in an NMR tube along with 1 ml of C_2H_5OH. A second sample of **9** (5.10 mg) in 1 ml of C_2D_5OD was then prepared. Both tubes, handled in parallel, were evacuated and filled with argon, sealed and heated for 1 h at 60 °C. Then the tubes were cooled to 23 °C and the contents were concentrated separately *in vacuo*, and submitted to quantitative ^{1}H NMR analysis using 1,2-dichloroethane as an internal standard to determine the percentage completion of reaction by measuring the change in integral area of vinyl and methine signals (5.85 and 5.20 ppm, respectively). Cycloaromatization of **9** in C_2H_5OH was 61% complete compared with 59% in C_2D_5OD, which corresponded to an isotope effect indistinguishable from 1.0. Data for a second run under similar conditions were 84% complete for cycloaromatization of **9** in C_2H_5OH and 72% complete for cycloaromatization in C_2D_5OD, corresponding to an isotope effect of ca 1.2.

Table 5. Rate constant k (s^{-1}) at varying temperature for degradation of **9**

k (s^{-1})	Ln k	Temperature (°C)	$1/T$ (K^{-1})
4.77×10^{-4}	−7.648	70	2.92×10^{-3}
1.30×10^{-4}	−8.948	60	3.00×10^{-3}
6.48×10^{-5}	−9.644	50	3.10×10^{-3}

*General procedure for the degradation study of enediyne alcohol **10b** by ^{1}H NMR at 0 °C.* To ketoenediyne **9** (8 mg, 0.02 mmol, 1.0 equiv.) in an NMR tube were added $CDCl_3$ (800 µl), 1,2-dichloroethane (1.7 µl) as internal standard and 1,4-CHD (10.2 µl, 0.110 mmol, 5.00 equiv.). The tube was evacuated, filled with argon and capped with a rubber septum. The solution was cooled to 0 °C over 15 min in the NMR instrument, at which time the NMR tube was quickly ejected, placed in a 0 °C bath and neat DIBALH (4.7 µl, 0.026 mmol, 1.3 equiv.) was added quickly via a syringe. The NMR tube was shaken vigorously (ca 5 s) followed by the addition of trifluoroacetic acid (2.3 µl, 0.030 mmol, 1.5 equiv.). The NMR tube was wiped of all condensation, placed in the NMR instrument and the reaction followed by quantitative integration of the vinylic protons (δ 5.95 ppm) and methine protons (at δ 4.7 and 4.8 ppm) in the NMR spectrum at 5 min intervals for 1 h (~67% complete). A representative data set and a calculation of rate, k, and half-life, $t_{1/2}$, is presented in Table 6. Using the first-order kinetic relation, from a plot of ln (X/X_0) vs t (min) the rate constant $k = 0.0230\,min^{-1}$. Using the first order half-life relation, $t_{1/2} = 30.1$ min.

Table 6. ^{1}H NMR data for degradation of **10b** measured over 60 min

Time (min)	Integral ratio, alcohol:1,2-DCE	Normalized ratio (X/X_0)	Ln(X/X_0)
0	0.436	1	0
5	0.348	0.798	−0.226
10	0.233	0.534	−0.627
15	0.220	0.505	−0.683
20	0.211	0.484	−0.726
25	0.174	0.399	−0.919
30	0.157	0.360	−1.02
35	0.151	0.346	−1.06
40	0.144	0.330	−1.11
45	0.133	0.305	−1.19
50	0.119	0.273	−1.30
55	0.115	0.264	−1.33
60	0.079	0.181	−1.71

Table 7. UV data for degradation of a 0.054 M solution of alkoxide **10a** in THF-d_8 at 21 °C

Time (min)	Net absorption[a]	X/X_0	Ln(X/X_0)
0	1.48099	1	0
1	1.41637	0.9564	−0.045
3	1.27677	0.8621	−0.148
5	1.12166	0.7574	−0.278
7	0.96662	0.6527	−0.427
10	0.75751	0.5115	−0.670
15	0.48365	0.3266	−1.19
20	0.30408	0.2053	−1.58
25	0.20459	0.1381	−1.98
30	0.15702	0.1060	−2.24

[a] The net absorption was calculated from initial absorption minus baseline absorption (~0.41).

The same procedure was used for the determination of the rate of rearrangement of alcohol **10b** under various conditions (see entries 5–8 and 10–11, Table 2). Differences arose only in the choice of internal standard and concentration of 1,4-CHD, which are indicated in Table 2.

*General procedure for the degradation study of silyl enediyne alkoxide **10a** by ^{1}H NMR at 0 °C.* A protocol similar to that used for the determination of cycloaromatization rates for **10b** was followed. The only difference was that the alkoxide generated after DIBALH addition was monitored without a TFA quench.

*General procedure for the UV spectroscopy degradation study of enediyne alkoxide **10a** at 21 °C.* To a quartz cuvette was added spectral grade THF (1.5 ml) followed by the addition of 50 µl of a stock solution (0.011 M) of ketoenediyne **9** (overall concentration 0.368 mM). The cuvette was placed in the UV instrument with a mounting equilibrated to 21 °C. At this point, a zero point absorption was measured followed by the addition of DIBALH (1 M in THF, 50 µl, 0.050 mmol, 90 equiv.). The solution was drawn up into a pipette to assist mixing. UV–visible data were collected over a 1 h period (~90% complete). A representative data set and a calculation of rate, k, and half-life, $t_{1/2}$, are presented in Table 7. The experiment was repeated four times. From a plot of ln(X/X_0) vs t (min), the rate constant $k = 0.0794\ \text{min}^{-1}$. Using the first-order half-life relation, $t_{1/2} = 8.73$ min.

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