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Maleimide-based acyclic enediyne for efficient DNA-cleavage and tumor cell suppression†

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A pH-sensitive acyclic enediyne (**1**) was synthesized for efficient DNA-cleavage and tumor cell suppression. Unlike other acyclic enediynes, this novel enediyne transforms into a highly reactive enediyne (**2**) in an acidic environment only, which undergoes Bergman cyclization spontaneously at ambient temperature. An EPR study on the enediyne **2** confirmed the generation of free radicals through Bergman cyclization. The activated enediyne induced DNA-cleavage and exhibited cytotoxicity towards various tumor cells under the action of diradicals arising from spontaneous Bergman cyclization at physiological temperature. These findings suggest a novel strategy of anticancer drug design, where the activation of the silent compound takes place under the acidic environment inside the tumor cells.

Introduction

Cancer is a vastly complex disease involving unregulated cell growth. It is the leading cause of death in developed countries and the burden of cancer is increasing in developing countries.[1] Cancer is usually treated with surgery, radiation therapy and/or chemotherapy. Among all the treatments, chemotherapy is the primary means for cancer treatment.[2] Many natural and synthetic drugs, such as paclitaxel,[3–5] methotrexate,[6] cisplatin,[7] and doxorubicin,[8] have been widely used in clinical practice due to their effective activities against a wide range of cancers. However, they also show severe drawbacks: toxic to normal cells while killing cancer cells, side effects like thrombocytopenia and hepatic toxicity, as well as drug resistance. Therefore, developing new anticancer drugs with high selectivity and low toxicity has become an important direction in organic and medicinal chemistry today.

Enediyne antibiotics were disclosed as a class of natural anticancer antibiotics after the discovery of neocarzinostatin,[9] esperamicin,[10] and calicheamicin.[11] The enediynes induce DNA cleavage by diradicals through a Bergman or Bergman-like cyclization. It is considered that the mechanism of enediynes in cells is unique and different from that of radiomaterial agents, and some enediynes are more cytotoxic to various cells.[12] Many natural enediyne anticancer antibiotics induce apoptosis at micromolar concentration. For example, the working concentration for calicheamicin is at the pg μL^{-1} level,[13] which is 1000 times more effective than doxorubicin hydrochloride (Adriamycin).[14] Unfortunately, these natural enediynes also show cytotoxicity to normal cells because of the lack of cell selectivity[15–17] and bring harmful side effects such as neutropenia[18] and cardiomyopathy.[19]

Stemming from the powerful anticancer activity of enediyne antibiotics, many research efforts have been devoted to the synthesis of natural enediyne analogues.[20] These synthesized enediynes were designed to undergo Bergman cyclization to generate the highly active diradicals when illuminated with UV or near-IR light,[21–28] complexed with metal ions,[29–31] treated with acid/base,[32–34] and/or embedded into a highly strained 9- or 10-member ring.[35]

Herein, we report a new strategy to synthesize simple but efficient enediyne compounds. The enediyne compounds are firstly kept inactive by installing two bulky groups at the alkynyl termini, and they are later on activated by removing these substituents. This transformation is easily accessed in physiological environments. As a proof-of-concept, we synthesized an enediyne compound bearing two orthoester groups at the alkynyl termini. This compound is stable at ambient temperature, showing no DNA-cleavage in neutral solutions. After being treated in a mild acidic condition, the orthoester groups are converted to ester groups and the enediyne is immediately activated to undergo Bergman cyclization, leading to the formation of reactive diradicals for DNA-cleavage and tumor cell suppression.

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† Electronic supplementary information (ESI) available: Detailed synthesis of other compounds, NMR spectra of all new compounds, DSC curve, Raman spectra, and cell viability results. See DOI: 10.1039/c4tb02018a

Experimental

Materials

Toluene, tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF) were dried over calcium hydride and distilled before use. Other chemicals were of commercial grade and used as received. 3,4-Diiodo-*N*-benzylmaleimide (**3**) and 1,1,1-triethoxy-3-trimethylsilypropyne (**4**) were synthesized according to the procedure mentioned in the literature with minor modification.[36,37] The detailed synthesis of these precursors is presented in the ESI.†

Synthesis of enediyne compounds

1-Benzyl-3,4-bis(3,3,3-triethoxyprop-1-yn-1-yl)-1*H*-pyrrole-2,5-dione (enediyne 1). Compound **3** (0.57 g, 1.3 mmol), CuI (98.5 mg, 40 mol%), $Pd(OAc)_2$ (29.2 mg, 10 mol%), PPh_3 (68.2 mg, 20 mol%) and Cs_2CO_3 (1.27 g, 3.9 mmol) were successively added to a solvent mixture of dry THF (4 mL) and toluene (10 mL) under nitrogen. Then, compound **4** (0.68 g, 3.9 mmol) was added dropwise. The mixture was stirred at 45 °C for 5 h. After the completion of the reaction as detected by TLC, the mixture was directly purified by column chromatography over magnesium silicate (hexane/ethyl acetate = 40 : 1) to yield a light yellow viscous liquid (0.405 g, 59.6%). The product was stored at low temperature and used in the subsequent reaction as soon as possible. ^{1}H NMR ($CDCl_3$, ppm): δ 7.28–7.34 (m, 5 H, Bn), 4.69 (s, 2 H, CH_2), 3.73 (q, 12 H, CH_2, J = 7.0 Hz), 1.25 (t, 18 H, CH_3, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, ppm): δ 165.6, 135.3, 128.9, 128.8, 128.6, 128.2, 109.1, 103.7, 72.9, 59.5, 42.7, 14.9. HRMS (ESI): *m*/*z* calcd. For $C_{29}H_{37}NO_8Na$ $(M + Na)^+$: 550.2417; found: 550.2419.

Diethyl 3,3′-(1-benzyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrole-3,4-diyl) dipropionate (enediyne 2). Enediyne **1** (20 mg) was dissolved in chloroform (10 mL) and cooled to 0 °C, two drops (~10 μL) of trifluoroacetic acid (TFA) was added and the mixture was stirred for 30 s, followed by the addition of potassium carbonate (2 g) to neutralize the excess acid. After filtration, the solvent was removed by vacuum to yield a yellow solid (enediyne **2**). Enediyne **2** is very unstable in the bulk state and it should be stored in a solution of nonpolar solvent at low temperature. ^{1}H NMR ($CDCl_3$, ppm): δ 7.30–7.35 (m, 5 H, Bn), 4.72 (s, 2 H, CH_2), 4.33 (q, 4 H, CH_2, J = 7.0 Hz), 1.36 (t, 6 H, CH_3, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, ppm): δ 164.4, 152.0, 134.9, 129.4, 128.9, 128.7, 128.4, 98.5, 72.6, 63.1, 43.0, 29.7, 13.9. HRMS (ESI): *m*/*z* calcd. For $C_{21}H_{17}NO_6Na$ $(M + Na)^+$: 402.0954; found: 402.0958.

Characterizations

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded in chloroform ($CDCl_3$) on an Ultra Shield 500 spectrometer (BRUKER BIOSPIN AG, Magnet System 500 MHz/54 mm) and referenced to Me_4Si. Mass spectra were recorded on a Micromass LCTTM mass spectrometer using the ESI method. Matrix assisted laser deionization/time of flight (MALDI-TOF) mass spectroscopy were performed on a mass spectrometer 4800 plus MALDI-TOF/TOF MS Analyzer (AB Sciex, USA) equipped with a Nd:YAG laser emitting at 355 nm and operating at an accelerating voltage of 20 kV in reflection mode. A 4000 series Explorer and DATA Explorer (AB Sciex, USA) were used for data acquisition and processing. The extraction delay time used was 450 ns. All the mass spectra were collected by averaging the signals of 500 individual laser shots. Dried-droplet samples were prepared by spotting 1 μL aliquot of a mixture of 5 μL of matrix (dithranol, 10 mg mL^{-1} in THF) and 1 μL of sample (10 mg mL^{-1} in THF) on a target plate. Differential scanning calorimetry (DSC) was carried out with a Pyris Diamond thermal analysis workstation equipped with a model 822e DSC module under a constant nitrogen flow. Raman spectroscopy was performed by an inVia+Reflex (Renishaw, England) machine at room temperature. The electron paramagnetic resonance (EPR) study was carried out with the enediynes dissolved in carbon tetrachloride at a concentration of 8 mM. Measurements were performed on an X-band EMX-8/2.7C EPR spectrometer (Bruker, Germany). The settings of the spectrometer were as follows: sweep width, 100G; time constant, 163.84 ms; conversion time, 40.96 ms; resolution, 1024 points; modulation frequency, 100.00 kHz; modulation amplitude, 1.00 G; and microwave power, 6.358 mW. UV-vis absorption spectra and the Bergman cyclization rate measurements were recorded on a Shimadzu UV-2550 UV-vis spectrometer in methanol at room temperature. The Bergman cyclization of enediyne **2** was monitored every 10 min for 10 h by following the growth of absorbance bands at 243 and 405 nm and decrease of absorbance bands at 275 and 347 nm. The rate constant was calculated by least-squares fitting of a single exponential function.

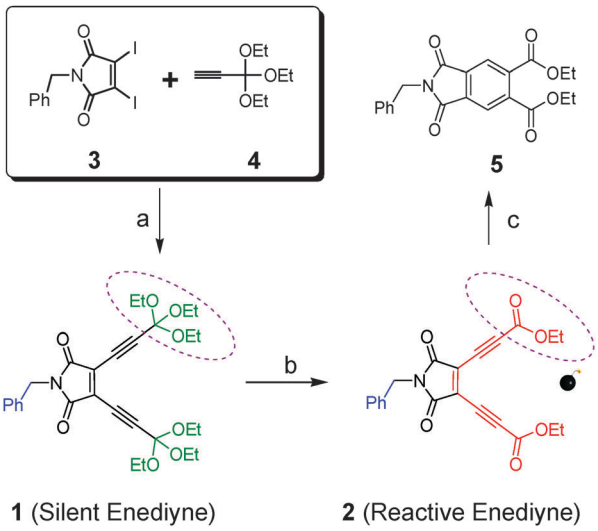
DNA cleavage experiments

The freshly prepared enediyne **1** was dissolved in DMSO (1 μL) at a final concentration of 20 mM and added to a solution of supercoiled plasmid ΦX174 RF1 DNA (0.5 μg μL^{-1}) in TE buffer (pH 7.6, 4 μL), followed by the addition of an aqueous solution of 4-methylbenzenesulfonate (PPTS) at different concentrations (namely, 100 mM L^{-1}, 10 mM L^{-1}, and 2 μL) to provide the desired pH condition (pH = 4 and 5). The freshly prepared enediyne **1** and enediyne **2** were respectively dissolved in acetone (2 μL) at concentrations of 100 mM and 50 mM, and added to a solution of supercoiled plasmid ΦX174 RF1 DNA (0.5 μg μL^{-1}) in TE buffer (pH 7.6, 4 μL). Control samples were separately incubated with pure acetone and DNA. All the systems were incubated at 37 °C for 72 h. After incubation, each system (5 μL) was mixed with a 6× loading buffer (1 μL) and subjected to a 0.8% agarose gel electrophoresis at 128 V (101 mA) for 30 min, stained by ethidium bromide and then the gel was photographed on the UV transilluminator (FR-200A) and analyzed by scanning densitometry.

Cell viability

The cell viability tests were investigated by an MTT assay.[38] Human lung carcinoma A549 cells, Human gastric carcinoma MGC803 cells, and Human uterine cervix carcinoma HeLa cells were respectively cultured in Dulbecoo's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) at 37 °C under a humidified atmosphere containing 5% carbon dioxide. The cells were trypsinized until it reaching 70% confluence in the tissue culture flasks with buffered saline solution containing 0.25% trypsin and 0.03% EDTA.

Cells were seeded into a 96-well plate at a density of 1×10^5 cells per well in 200 μL of the cell culture medium. After 24 h of incubation, the culture medium was removed and the cells were then exposed to 1.25, 2.5, 5, 10, 20 and 40 μM concentrations of enediyne **1** (concentrated enediyne **1** of 40 mM was prepared by dissolving enediyne **1** in acetone and diluted using cell culture medium to 40 μM. The enediyne **1** solution was sterilized by a 0.22 μm membrane filter (Sartorius Stedim Biotech, France). The concentration of acetone was 0.1%) The blank culture medium (0.1% acetone) was used as a blank control. After incubation for another determined period of 24, 48 and 72 h, 20 μL of sterile filtered MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) stock solution (5 mg mL^{-1}) in PBS (pH 7.4) was added to each well and the cells were further incubated for 4 h at 37 °C to allow the yellow dye to be transformed into blue crystals. Then, the unreacted dye was removed by aspiration, and 200 μL of DMSO was added to each well to dissolve the dark blue crystals. Finally, the optical density was measured by a microtitre reader at a wavelength of 490 nm. The spectrophotometer was normalized using culture medium without cells. Cell viability (%) related to control wells containing cell culture medium without HAPN suspension was calculated by $[A]_{test}/[A]_{control}$.



Scheme 1 Synthesis of enediyne compounds and related Bergman cyclization. (a) $Pd(OAc)_2$, CuI, Cs_2CO_3, PPh_3, and THF–toluene, 45 °C, 5 h; (b) TFA and $CHCl_3$; (c) toluene and CHD, reflux. Note that the bulky orthoester groups (green) can be converted to smaller ester groups, generating a reactive enediyne (red). The subtle change at the benzyl group position (blue) can adjust the solubility and lipophilicity of the enediyne compounds. Further introduction of a tumor-targeting tag at this site is also possible.

Results and discussion

As pointed out by Nicolaou,[39] the ''critical range'' (the distance of C1–C6, also called cd distance) should be shorter than 3.31 Å for an enediyne compound to undergo spontaneous Bergman cyclization at ambient temperature. However, the cd distance for an acyclic enediyne is considerably longer; therefore, acyclic enediynes are typically considered inactive towards thermal Bergman cyclization, although they are structurally much simpler than the cyclic enediynes, which require laborious synthetic efforts. Recently, we found that by introducing a maleimide moiety at the ene position of enediyne compounds, the onset temperatures of the thermal Bergman cyclization were dramatically lowered.[40] Encouraged by these results, a novel type of acyclic enediyne antibiotic was designed and synthesized. Scheme 1 illustrates the synthesis of the enediyne compounds. The inactive enediyne **1** was synthesized through a Sonogashira cross coupling reaction between two known compounds **3** and **4**.[36,37] Enediyne **1** is stable in neutral solutions but is very sensitive to acidic environments. The reactive enediyne **2** was obtained by the hydrolysis of the inactive enediyne **1** in cold chloroform solution with the addition of a small amount of trifluoroacetic acid (TFA). This reaction was verified by the change of chemical shift of the ethoxy groups (3.73 ppm) in the orthoester form to those in the ester form (4.33 ppm) by ^{1}H NMR spectroscopy (Fig. S7 and S9, ESI†). Enediyne **2** is a highly reactive species, which quickly transforms into polymeric products at ambient temperature even in the presence of a large excess of hydrogen donor 1,4-cyclohexadiene (CHD). The more favored polymerization over the H-abstraction of the diradicals intermediates implies that the biradical intermediate was significantly stabilized by through-bonding coupling in its S ground state.[41] The H-abstraction product (**5**) was obtained by accelerating the H-abstraction at elevated temperature in the presence of a large excess of CHD (Scheme 1). The structure of compound **5** was further confirmed by comparing the spectroscopic data with the same compound that was synthesized through a different approach (ESI†). The presence of the peaks at δ 8.19 ppm in both the NMR spectra of the H-abstraction product (compound **5**) and that of the polymeric products (Fig. S15, ESI†) indicates that the structure of the repeating unit in the polymeric products was similar to that of compound **5**.

The reactivity of enediynes towards thermal Bergman cyclization was estimated by DSC analysis. As shown in Fig. S1 (ESI†), the onset temperature of enediyne **1** was observed at 105 °C, which is lower than most of the previously reported acyclic enediynes;[19] however, it is still considerably higher for a spontaneous Bergman cyclization at physiological temperature. Therefore, enediyne **1** is considered as inactive or silent at ambient temperature. On the contrary, enediyne **2** is considerably more reactive. The attempt to measure its onset temperature in the bulk state with DSC was unsuccessful as the majority of the sample converted to polymeric products during solvent removal and sampling for DSC analysis. Nevertheless, the high reactivity of enediyne **2** is essential for further applications in DNA cleavage and tumor cell suppression. A direct evidence for the activation of silent enediyne **1** and the Bergman cyclization of the reactive enediyne **2** was obtained with Raman spectroscopy. As shown in Fig. S2 (ESI†), the stretching band of C≡C at 2198 cm^{-1} in enediyne **1** almost disappears after the activation and Bergman cyclization, corresponding to the reaction of alkynyl groups.

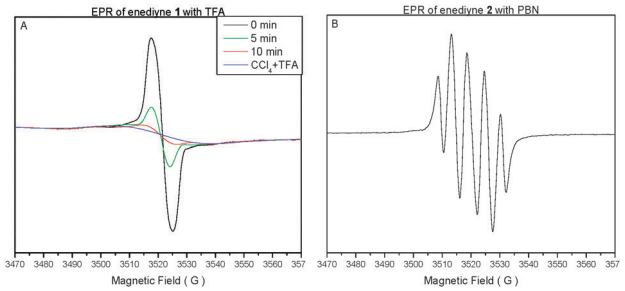


Fig. 1 (A) EPR spectra of enediyne **1** in CCl_4. Enediyne **1** was dissolved in CCl_4, followed by the addition of one drop of TFA and it was immediately subjected to EPR spectroscopy analysis (black line); green line: 5 minutes after the addition of TFA; red line: 10 minutes after the addition of TFA; blue line: CCl_4 with TFA. (B) EPR spectrum of the PBN-adduct in CCl_4.

To verify the radical nature of this Bergman cyclization, electron paramagnetic resonance (EPR) spectroscopy was conducted. The freshly prepared enediyne **1** was dissolved in carbon tetrachloride (CCl_4), followed by the addition of one drop of TFA. The mixture was immediately subjected to EPR spectroscopy analysis. The EPR spectra (Fig. 1A) showed a clear single peak at 3520 G, indicating the formation of carbon free radicals.[40] Moreover, the signal intensity decayed rapidly with time, indicating that the free radicals were quenched (Fig. 1A). Unsurprisingly, the addition of TFA hydrolyzed the orthoester groups of enediyne **1** and converted it into enediyne **2**. The latter enediyne underwent Bergman cyclization at ambient temperature, generating biradical intermediates. Once the free radicals were produced, they might have either coupled with each other or might have been trapped by the solvent molecules, leading to the fading of the EPR signal. With the addition of a spin trap, free radicals formed through Bergman cyclization can be trapped into another stable free radical.[42] Inspired by this, we used phenyl *tert*-butyl nitrone (PBN) to trap the carbon radicals. Freshly prepared enediyne **2** in chloroform was added with excess of PBN and subjected to heating at 37 °C for 12 h. EPR analysis on the mixture shows five lines with an intensity ratio of 1 : 2 : 3 : 2 : 1 (Fig. 1B), which unambiguously indicates the formation of closely-interacted nitroxide biradicals[43] through the addition of the diradicals generated from the Bergman cyclization of enediyne **2** with two equivalents of PBN.[44] High resolution mass spectroscopy analysis on the PBN-adduct showed a peak at *m*/*z* 756.3265, corresponding to the molecular ion $(M + Na)^+$ of the double PBN-adduct of the biradical intermediate of the Bergman cyclization of enediyne **2**. The radical nature of the Bergman cyclization of the reactive enediyne **2** and the high reactivity of the biradical intermediate endowed this compound with DNA-cleaving ability and tumor cell suppression as demonstrated below.

Kinetic data of the Bergman cyclization of enediyne **2** were obtained *via* UV-vis spectroscopy. Freshly prepared enediyne **2** was dissolved in methanol and the absorbance of the solution was monitored every 10 min at 10 °C. Fig. 2 shows the evolution of the UV-vis spectra over 10 h. The spectra display a decrease of absorbance bands at 275 nm and 347 nm, and a red shift arising from the formation of new conjugated compounds.

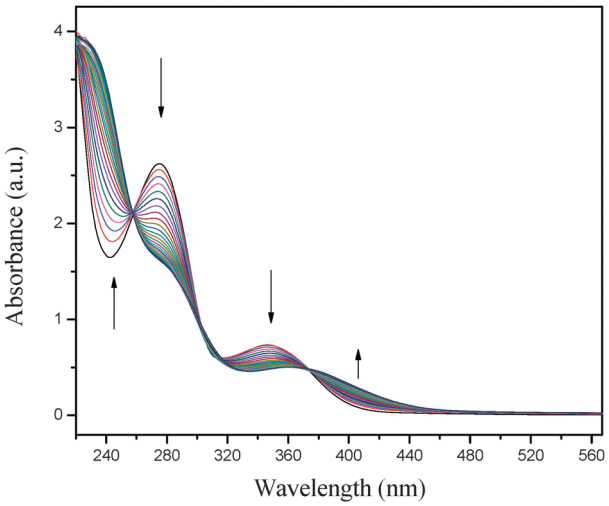


Fig. 2 UV-vis spectra of enediyne **2** in methanol (6×10^{-5} M) at 10 °C. The curves were recorded every 10 min.

The rate of the Bergman cyclization was calculated based on the disappearance of the enediyne **2**, following first-order kinetics (Fig. S3, ESI†). The half-life of the enediyne **2** is about 4.5 h at 10 °C in methanol, corroborating the high reactivity of this reactive enediyne.

The DNA-cleaving abilities of enediyne **1** and **2** were measured with supercoiled plasmid DNA by analyzing the conversion of DNA from native supercoiled (Form I) to circular relaxed (Form II, single-strand cleavage).[13] Freshly prepared enediynes **1** and **2** were dissolved in DMSO or acetone, and added to a solution of ΦX174 supercoiled plasmid RF1 DNA (0.5 μg μL^{-1}) in TE buffer. The samples were incubated for a desired period of time at 37 °C. Then, the mixtures were subjected to agarose

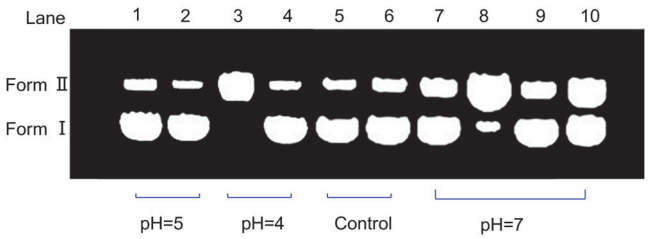


Fig. 3 Cleavage of DNA by enediyne **1** and enediyne **2** at different pH for 72 h at 37 °C. Lane 1: ΦX174 RF1 DNA (4 μL) + **1** in DMSO (5 mM, 1 μL) + PPTS (10 mM, 2 μL), the final pH of solution was about 5; lane 2: ΦX174 RF1 DNA (4 μL) + DMSO (1 μL) + PPTS (10 mM, 2 μL), the final pH of solution was about 5; lane 3: ΦX174 RF1 DNA (4 μL) + **1** in DMSO (5 mM, 1 μL) + PPTS (100 mM, 2 μL), the final pH of solution was about 4; lane 4: ΦX174 RF1 DNA (4 μL) + DMSO (1 μL) + PPTS (100 mM, 2 μL), the final pH of solution was about 4. Herein, an aqueous solution of 4-methylbenzenesulfonate (PPTS) at different concentrations was mixed with DNA solution and DMSO to provide a different pH condition. Lane 5: ΦX174 RF1 DNA (4 μL) in TE buffer (2 μL, pH 7.6); lane 6: ΦX174 RF1 DNA (4 μL) + acetone (2 μL); from lane 7 to lane **10**, the ΦX174 RF1 DNA was incubated with enediyne **1** and enediyne **2** at neutral conditions for 72 h at 37 °C. Lane 7: ΦX174 RF1 DNA (4 μL) + **1** (100 mM) in acetone (2 μL); lane 8: ΦX174 RF1 DNA (4 μL) + **2** (100 mM) in acetone (2 μL); lane 9: ΦX174 RF1 DNA (4 μL) + **1** (50 mM) in acetone (2 μL); lane 10: ΦX174 RF1 DNA (4 μL) + **2** (50 mM) in acetone (2 μL).

gel electrophoresis, followed by photographed scanning to analyze the conversion of DNA. After incubation with DNA for 72 h, enediyne **1** did not induce obvious DNA cleavage at pH = 5 (lane 1, Fig. 3). When the pH value was decreased to 4, the DNA was completely cleaved by enediyne **1** (lane 3, Fig. 3). In comparison, the control samples were almost unaffected (lane 2 and lane 4, Fig. 3). This set of experiments confirmed that enediyne **1** can be activated towards DNA cleavage in weak acidic medium. To further demonstrate that the DNA cleavage originated from the conversion of enediyne **1** to enediyne **2**, ΦX174 supercoiled plasmid RF1 DNA was incubated with freshly prepared enediyne **2** and then subjected to electrophoresis. As shown in Fig. 3, the enediyne **2** (100 mM) induced the significant cleavage of DNA at neutral conditions (lane 8). When the concentration of enediyne **2** was reduced to 50 mM, about half of the DNA was cleaved (lane 10), exhibiting a typical concentration-dependent cleavage. In contrast, the silent enediyne **1** only induced negligible cleavage of DNA under the same conditions of enediyne 2 (lane 7 and lane 9) compared to the control samples (lane 5 and lane 6). The sharp difference between the DNA-cleavage abilities of two enediynes stems from the fact that enediyne **1** is inactive due to the two bulky groups at the alkynyl termini, while enediyne **2** can undergo spontaneous Bergman cyclization at 37 °C, generating highly reactive radicals, which destroy the backbone of DNA.

Cell viability was investigated using the typical 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method[38] in different tumor model line cells: MGC803 cells, HeLa cells, A549 cells and one normal hepatocyte cell line (L-02 cells). Freshly prepared enediyne **1** was dissolved in acetone at different concentrations and added to the four different types of cells. A control sample was obtained with the addition of acetone only. After incubation for a certain period of time, the cell viabilities were assessed. Fig. 4 compares the cytotoxicities of enediyne **1** against different cells at various concentrations. The cell viabilities of the four different cell lines exhibited clear concentration-dependent behaviour after treating with enediyne **1** when the concentration was beyond 1.25 μM. The similar manner also appears with the incubation time from 24 h to 48 h (Fig. S4 and S5, ESI†). In particular, for these three types of tumor cells, the IC_{50} concentration of enediyne **1** was between 5 and 10 μM, comparable to many clinically used antitumor agents, such as cisplatin,[45] irinotecan,[46] and doxorubicin,[47] indicating the high cell inhibition ability of enediyne **1**. The low selectivity of enediyne **1** towards tumor cells over normal cells is probably due to the unselective hydrolysis (and activation) of enediyne **1** in lysosomes of both tumor cells and normal cells, where even for normal cells, the pH values might be lower than 5. This problem can be solved by installing a tumor-targeting tag probably at the maleimide moiety (by replacing the dummy benzyl group in the enediyne **1**) or by incorporating the enediyne compounds in tumor-targeting drug carriers.[6,48–50] All the four types of cells remain highly viable after treating with acetone (Fig. S6, ESI†), indicating that the cytotoxicity is solely contributed from the enediyne compound.

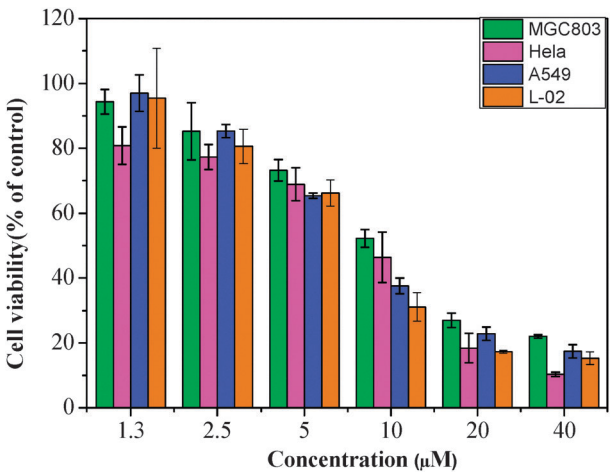


Fig. 4 Effects of enediyne **1** on the cell viability of tumor model cell lines, such as MGC803 cells (green), HeLa cells (blue), A549 cells (magenta), and normal cell lines, *i.e.*, L-02 (orange). Cells were incubated with a series of concentrations of enediyne 1 for 72 h and cell viability was determined and analysed by the MTT assay.

Conclusions

We have synthesized a novel pH-sensitive acyclic enediyne **1**. Our results showed that the Bergman cyclization was triggered by decreasing the steric effect under weak acidic conditions along with the transformation of enediyne **1** to enediyne **2** at physiological temperature. We found that the activated enediyne **2** exhibited first-order kinetics to form the free radicals, which could be visulized with EPR and trapped by other atoms. Based on this Bergman cyclization mechanism, the enediyne **2** induced the cleavage of DNA and exhibited cytotoxicity to different tumor cells. Consequently, the enediyne **1** represents a novel example for pro-drug designing in the antitumor antibiotics field.

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