Natural Product Synthesis

DOI: 10.1002/ange.200704577

Asymmetric Synthesis and Biological Properties of Uncialamycin and 26-*epi*-Uncialamycin**

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Dedicated to Professor Ryoji Noyori on the occasion of his 70th birthday

Among the most potent antitumor antibiotics are the enediynes,[1] one of which, calicheamicin γ_1^I (Mylotarg, *Gemtuzumabozogamicin*), is currently in use as an anticancer agent.[2] Uncialamycin (**1**, Figure 1) is a newly discovered enediyne[3] isolated from an unspecified strain of Streptomycete related to *Streptomyces cyanogenus*. In preliminary investigations, uncialamycin revealed striking activity against *Escherichia coli* [minimum inhibitory concentration (MIC) = 0.002 μg mL^{-1}], *Staphylococcus aureus* (MIC = 0.0000064 μg mL^{-1}), and *Burkholderia cepacia* (MIC = 0.001 μg mL^{-1}), the latter being responsible for lung infections in cystic fibrosis patients.[4] These phenomenal results elevate uncialamycin to a promising lead for drug discovery in the areas of cancer and infectious diseases. However, the extreme scarcity of this substance (only 300 μg was isolated) hampered further biological studies. Herein we describe the asymmetric synthesis of uncialamycin and its bioactive isomer, 26-*epi*-uncialamycin (**2**), and detailed studies into their in vitro DNA-cleaving, antibacterial, and cytotoxic properties. We found that **1** and **2** promote single- and double- strand cuts in plasmid DNA and exhibit powerful antibacterial properties against several strains, including methicillin-resistant *Staphylococcus aureus* (MRSA; MIC = 0.0002 μg mL^{-1} for **1**) and vancomycin-resistant *Enterococcus faecalis* (VRE; MIC = 0.002 μg mL^{-1} for **1**) as well as potent activities against a broad panel of cancer cells, including Taxol-resistant ovarian cells (1A9/PTX10; $IC_{50} = 6 \times 10^{-11}$ M for **1**) and epothilone B resistant cells (1A9/A8; $IC_{50} = 9 \times 10^{-12}$ M for **1**). Our results demonstrate the viability of chemical synthesis as a source of these valuable compounds and render them readily available for biological studies. Furthermore, our investigations demonstrate the potential of these compounds as drug candidates for the treatment of cancer and infectious diseases and confirm their DNA-cleaving mechanism of action. Given these findings, we suggest that antibody conjugates[5] of these toxins may lead to targeting agents of clinical importance.

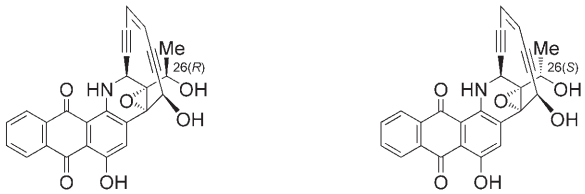

Figure 1. Structures of uncialamycin (**1**) and 26-*epi*-uncialamycin (**2**).

Owing to the low natural abundance of uncialamycin (**1**), the original investigation of its structure[3] stopped short of assigning its stereochemistry at C26 and offered only a speculation as to the molecule's absolute configuration. While our recent synthesis of racemic uncialamycin (**1**) and 26-*epi*-uncialamycin (**2**) provided an answer to the question of the relative stereochemistry at C26, it neither solved the issue of supply of the naturally occurring enantiomer of the natural product nor determined its absolute configuration.[6] To resolve these issues and facilitate further biological investigations, we resorted to a catalytic asymmetric synthesis of uncialamycin (**1**), highlights of which are shown in Scheme 1. Thus, the readily available prochiral quinoline carboxylic acid **3**[6] was converted to its methyl ester **4** through the initial action of thionyl chloride followed by treatment of the resulting acid chloride with methanol in the presence of Et_3N and DMAP in 65% overall yield. Noyori reduction[7] of the methyl ketone moiety within **4** (ruthenium catalyst **5**, HCO_2H, Et_3N) resulted in the formation of γ-lactone **7**, presumably via intermediate hydroxy ester **6**, in 95% yield and 93% *ee*. While this outcome was satisfactory, difficulties in maintaining the configurational integrity of the generated asymmetric center in **7** during its obligatory conversion to intermediate **8** under acidic conditions led us to explore an alternative approach to reach compound **8** from starting material **3**.

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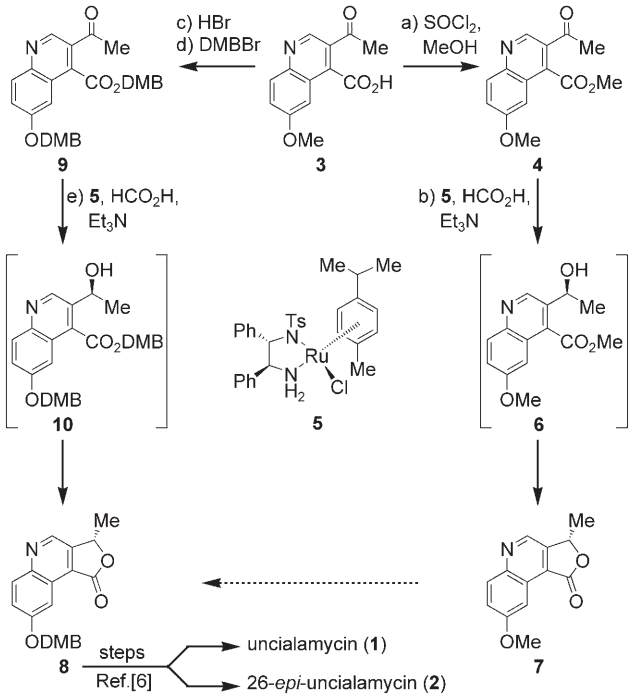
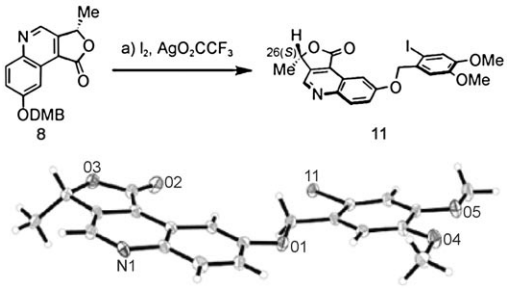
[**] We thank the National Cancer Institute (NCI) screening program (http://dtp.nci.nih.gov/) for testing our compounds in the 60-cell-line panel and Dr. R. N. Misra for his assistance. We thank Dr. P. Giannakakou for providing the 1A9, 1A9/PTX10, 1A9/PTX22, and 1A9/A8 cell lines. We also acknowledge Drs. D. H. Huang, G. Siuzdak, R. K. Chadha, R. Ghadiri, and I. Hwang for assistance with NMR spectroscopy, mass spectrometry, X-ray crystallography, bacterial assays, and cytotoxicity assays, respectively. This work was supported by The Skaggs Institute for Chemical Biology, a National Defense Science and Engineering Graduate fellowship (to J.S.C.), and a MEC/Fulbright fellowship (to A.M.).

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.



Scheme 1. Catalytic asymmetric synthesis of uncialamycin (**1**) and 26-*epi*-uncialamycin (**2**). Reagents and conditions: a) $SOCl_2$, 80 °C, 30 min; then MeOH (2.0 equiv), Et_3N (5.0 equiv), DMAP (0.1 equiv), 25 °C, 1 h, 65 %; b) **5** (0.05 equiv), HCO_2H (4.3 equiv), Et_3N (2.5 equiv), CH_2Cl_2, 0 °C, 36 h, 95 % yield, 93 % *ee*; c) 48 % aq HBr, nBu_4NBr (0.1 equiv), 110 °C, 40 h; d) DMBBr (3.0 equiv), K_2CO_3 (8.0 equiv), nBu_4NI (0.15 equiv), DMF, 25 °C, 3 h, 55 % over 2 steps; e) **5** (0.05 equiv), HCO_2H (4.3 equiv), Et_3N (2.5 equiv), CH_2Cl_2, 0 °C, 36 h, 95 % yield, 98 % *ee*; recrystallization from EtOAc, $\geq$ 99 % *ee*. DMAP = 4-(*N*,*N*-dimethylamino)pyridine, DMB = 3,4-dimethoxybenzyl, DMF = *N*,*N*-dimethylformamide, Ts = toluenesulfonyl.



Scheme 2. Preparation of iodide derivative **11** and ORTEP drawing of **11** (with thermal ellipsoids drawn at 30 % probability). Reagents and conditions: a) I_2 (3.0 equiv), AgO_2CCF_3 (4.5 equiv), $CHCl_3$, 0 °C, 1 h, 80 %.

To this end, we converted methyl ether carboxylic acid **3** to DMB ether DMB ester **9** through demethylation (48 % aq HBr, nBu_4NBr cat., 110 °C) followed by exposure to DMBBr in the presence of K_2CO_3 and catalytic nBu_4NI at ambient temperature (55 % overall yield). We were then pleased to find that Noyori reduction of **9** under the same conditions as those described above for **4** furnished lactone **8**, presumably via intermediate **10**, in 95 % yield and 98 % *ee*. A single recrystallization of the so-obtained material from ethyl acetate afforded the key intermediate **8** in $\geq$ 99 % *ee* (m.p. 181–182 °C, EtOAc). This intermediate was then elaborated to (+)-uncialamycin (**1**, natural) and (+)-26-*epi*-uncialamycin (**2**) by the same sequence as that used to synthesize the racemic forms of these compounds.[6] In order to ensure that the Noyori reduction of **9** produced the predicted enantiomer of **8**, a sample of the latter compound was iodinated (I_2, AgO_2CCF_3) as shown in Scheme 2 to afford the crystalline iodide **11** (m.p. 200–202 °C, CH_3CN). X-ray crystallographic analysis of **11** confirmed its absolute configuration [26(*S*), see ORTEP drawing, Scheme 2].[8] Thus, the absolute configuration of uncialamycin was unambiguously determined to be that shown in Figure 1.

With ample quantities of synthetic uncialamycins **1** and **2** on hand, we were in a position to investigate their biological profiles with regard to DNA-cleavage, antibacterial, and cytotoxic properties. Figure 2 shows the results of DNA-cleavage experiments using form I (supercoiled) ΦX174 plasmid DNA as revealed by gel electrophoresis. Thus, uncialamycin (**1**) exhibited potent DNA cleavage, causing both single- and double- strand cuts (to yield form II (relaxed circular) and form III (linear) plasmid, respectively) at concentrations as low as 100 nM at 37 °C and pH 8.0 with virtually complete cleavage of the plasmid at 1000 nM after 24 h of incubation in the absence of a thiol (Figure 2 a). In the presence of glutathione (1 mM) or dithiothreitol (1 mM), the cleavage capabilities of **1** were enhanced approximately by a factor of 10, leading to complete cleavage of the plasmid with 100 nM of **1** (10 equivalents with regards to the plasmid) under the above-mentioned conditions. Furthermore, comparing the extent of DNA cleavage at multiple time points revealed a marked rate acceleration for the cleavage in the presence of glutathione (Figure 2 b). Thus, whereas in the absence of a thiol activator, the DNA-cleavage activity of **1** continued steadily for at least 24 h, in the presence of glutathione, the DNA cleavage was complete within 6 h. The DNA-cleaving capabilities of **1** were not restricted to pH 8.0, but were also evident at pH 6.0, 7.0, and 7.4. In the absence of glutathione, the DNA-cleaving rate in this pH range was insensitive to changes in pH, whereas in the presence of glutathione, a higher rate was observed under more basic conditions. 26-*epi*-Uncialamycin (**2**) exhibited a similar DNA-cleavage profile to **1** (see the Supporting Information).

The mechanism by which uncialamycins **1** and **2** cleave DNA is presumed to be similar to that of dynemicin A[9] and is supported by our observation of a Bergman-type cycloaromatization[10] of racemic uncialamycin to afford a stable aromatic system upon activation with HCl in methylene chloride.[6] Proceeding through a cascade sequence, the thiol-promoted DNA cleavage by uncialamycin (**1**) is likely initiated by reduction of its anthraquinone domain to a dihydroquinone moiety, leading to **12** (Scheme 3). The latter intermediate is apparently labile by virtue of the electron flow toward the epoxide site, leading to species **13**, whose tautomerization to quinone **14** is likely to be facile and rapid. The opening of the epoxide triggers cycloaromatization to afford benzenoid diradical **15**, which delivers the damaging blow to the genetic material by abstracting hydrogen atoms,

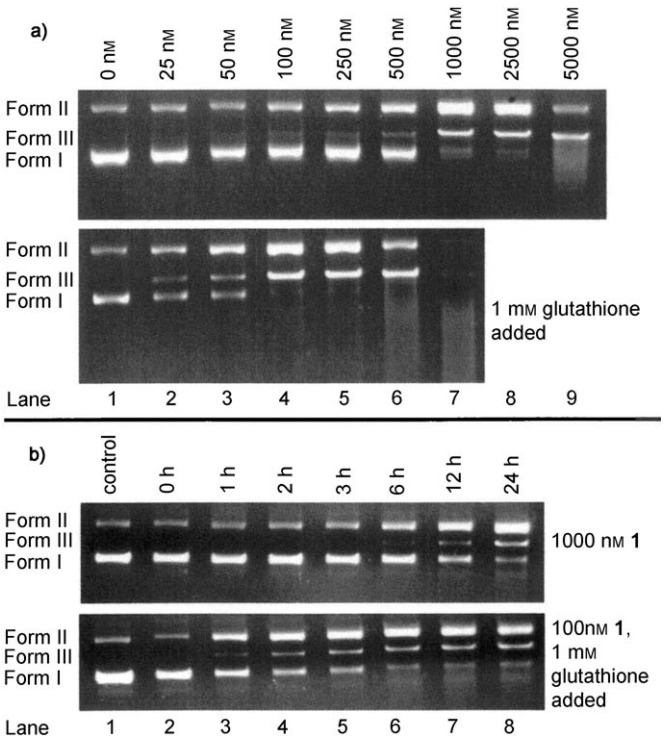
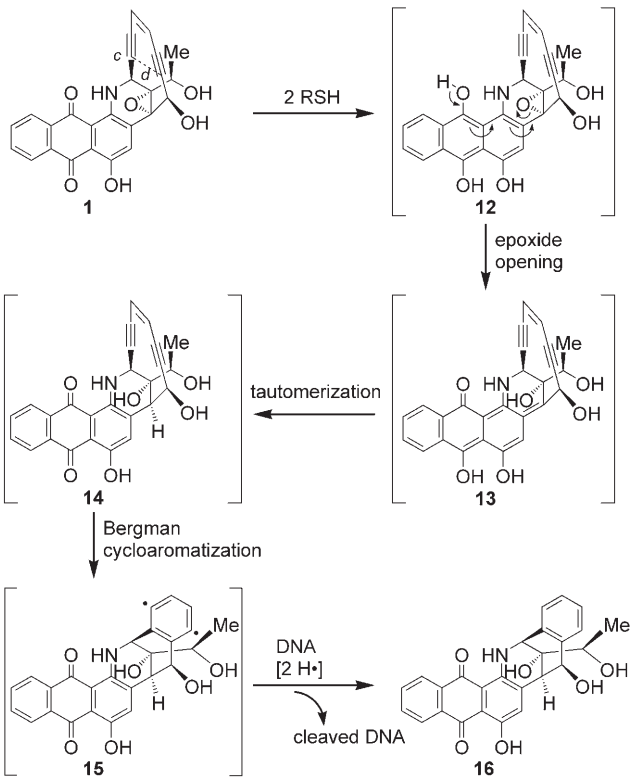


Figure 2. Pictures of electrophoresis gels showing DNA cleavage by uncialamycin (**1**). a) Variable concentration, with and without thiol activator. ΦX174 form I DNA (10 nM plasmid, 50 μM base pair) was incubated for 24 h at 37°C with uncialamycin (**1**, variable concentration) with and without 1 mM glutathione in pH 8.0 buffer (10 mM Tris·HCl, 1 mM EDTA, 1% DMSO) and analyzed by gel electrophoresis (1% agarose gel, ethidium bromide stain). Lane 1: control incubated without **1**; lanes 2–9: 25, 50, 100, 250, 500, 1000, 2500, and 5000 nM **1**, respectively. b) Variable time, with and without thiol activator. ΦX174 form I DNA (10 nM plasmid, 50 μM base pair) was incubated for 0–24 h at 37°C with uncialamycin (**1**, 1000 nM without glutathione or 100 nM with glutathione) with and without 1 mM glutathione in pH 8.0 buffer (10 mM Tris·HCl, 1 mM EDTA, 1% DMSO) and analyzed by gel electrophoresis (1% agarose gel, ethidium bromide stain). Lane 1: control (no compound added, incubated for 24 h); lanes 2–8: compound added, incubated for 0, 1, 2, 3, 6, 12, and 24 h, respectively. Forms I, II, and III refer to supercoiled, relaxed circular, and linear DNA, respectively. EDTA = ethylenediaminetetraacetate, DMSO = dimethyl sulfoxide.



Scheme 3. Presumed mechanism of DNA cleavage by uncialamycin (**1**) and 26-*epi*-uncialamycin (**2**) in the presence of glutathione (RSH). A similar mechanism is envisioned for acid-catalyzed or enzymatically promoted DNA cleavage by uncialamycins **1** and **2**. The calculated distance between the two acetylenic carbon atoms (labeled *c* and *d*) that form the carbon-carbon bond during the cycloaromatization in **14** is considerably shorter (3.09 Å by calculation) than the corresponding distance in **1** (3.41 Å by calculation; 3.60 Å by X-ray crystallographic analysis[6]).

one from each stand, causing the observed double-strand cuts, and being itself converted to the benzenoid compound **16**.

In light of the impressive DNA-cleaving properties observed for natural uncialamycin (**1**) and its reported activity against *Staphylococcus aureus*,[3] we set out to assay uncialamycins **1** and **2** against a broad panel of bacterial strains. We were pleased to find potent antibacterial activity against all bacterial strains tested, including Gram positive, Gram negative, and drug-resistant strains (Table 1). In particular, both compounds displayed extraordinary activity against Gram positive *Staphylococci*: *Staphylococcus aureus* [MIC=0.0002 $\mu g\,mL^{-1}$ (**1**), 0.001 $\mu g\,mL^{-1}$ (**2**)], methicillin-resistant *Staphylococcus aureus* [MIC = 0.0002 $\mu g\,mL^{-1}$ (**1**), 0.0009 $\mu g\,mL^{-1}$ (**2**)], and *Staphylococcus epidermidis* [MIC = 0.00009 $\mu g\,mL^{-1}$ (**1**), 0.0003 $\mu g\,mL^{-1}$ (**2**)]. Both compounds also showed good activity against vancomycin-resistant *Enterococcus faecalis* [MIC = 0.002 $\mu g\,mL^{-1}$ (**1**), 0.007 $\mu g\,mL^{-1}$ (**2**)] as well as against bacterial strains present in pulmonary infections such as *Burkholderia cepacia* [MIC = 0.0004 $\mu g\,mL^{-1}$ (**1**), 0.006 $\mu g\,mL^{-1}$ (**2**)] and *Streptococcus pneumoniae* [MIC = 0.0004 $\mu g\,mL^{-1}$ (**1**), 0.004 $\mu g\,mL^{-1}$ (**2**)]. The activity against *Burkholderia cepacia* is particularly significant to cystic fibrosis patients, where these compounds may hold promise as anti-infective agents.[4]

We also assayed uncialamycins **1** and **2** for activity against ovarian carcinoma cell line 1A9, Taxol-resistant mutants 1A9/PTX10 and 1A9/PTX22, and epothilone B resistant mutant 1A9/A8 (Table 2).[12] The compounds were found to be extremely potent against the parental 1A9 cell line [1A9, 50% inhibitory concentration (IC_{50}) = 1×10^{-11} M (**1**), 6×10^{-11} M (**2**)], Taxol-resistant [1A9/PTX10, $IC_{50} = 6\times10^{-11}$ M (**1**), 6×10^{-10} M (**2**)], and epothilone-resistant [1A9/A8, $IC_{50} = 9\times10^{-12}$ M (**1**), 1×10^{-10} M (**2**)] cell lines.

Prompted by these findings, uncialamycins **1** and **2** were selected for testing by the National Cancer Institute (NCI) against their panel of 60 human-tumor cell lines.[13] The results were equally impressive, with the natural isomer (**1**) generally exhibiting modestly higher potencies than its C26 epimer (**2**). Natural uncialamycin (**1**) showed particularly high lethal

Table 1: Antibacterial profiles of uncialamycins **1** and **2**.[a]

Bacterial strain	Control (MIC)	**1** (MIC) [$\mu g\,mL^{-1}$]	**2** (MIC) [$\mu g\,mL^{-1}$]
MRSA	vancomycin (1 $\mu g\,mL^{-1}$)	0.0002	0.001
Staphylococcus aureus	vancomycin (4 $\mu g\,mL^{-1}$)	0.0002	0.0009
Staphylococcus epidermidis	vancomycin (2 $\mu g\,mL^{-1}$)	0.00009	0.0003
Bacillus cereus	vancomycin (2 $\mu g\,mL^{-1}$)	0.0003	0.002
Lysteria monocytogenes	vancomycin (2 $\mu g\,mL^{-1}$)	0.001	0.006
VRE	daptomycin (2 $\mu g\,mL^{-1}$)	0.002	0.007
Streptococcus pneumoniae	daptomycin (2 $\mu g\,mL^{-1}$)	0.0004	0.004
Escherichia coli	streptomycin (1 $\mu g\,mL^{-1}$)	0.006	0.02
Burkholderia cepacia	streptomycin (9 $\mu g\,mL^{-1}$)	0.0004	0.006
Salmonella typhimurium	streptomycin (6 $\mu g\,mL^{-1}$)	0.009	0.02
Pseudomonas aeruginosa	streptomycin (6 $\mu g\,mL^{-1}$)	0.02	0.04

[a] Antibacterial activity was determined by the National Committee for Clinical Laboratory Standards (NCCLS) broth microdilution methods.[11] Detailed experimental procedures may be found in the Supporting Information.

Table 2: IC_{50} profiles of uncialamycins **1** and **2** against ovarian tumor cell lines.[a]

Cell line	Taxol (IC_{50} [M])	Epothilone B (IC_{50} [M])	Compound **1** (IC_{50} [M])	Compound **2** (IC_{50} [M])
1A9	2×10^{-9}	4×10^{-10}	1×10^{-11}	6×10^{-11}
1A9/PTX10	4×10^{-8}	8×10^{-10}	6×10^{-11}	6×10^{-10}
1A9/PTX22	5×10^{-8}	5×10^{-10}	3×10^{-11}	2×10^{-10}
1A9/A8	1×10^{-8}	4×10^{-9}	9×10^{-12}	1×10^{-10}

[a] Detailed experimental procedures may be found in the Supporting Information. 1A9 is an ovarian tumor cell line derived from A2780; 1A9/PTX10 and 1A9/PTX22 are Taxol-resistant strains; 1A9/A8 is an epothilone B resistant strain. These cell lines were obtained from P. Giannakakou (Division of Hematology and Medical Oncology, Weill Medical College of Cornell University, New York, NY).

potencies against selected cell lines, notably melanoma [for example, M14, 50% lethal concentration (LC_{50}) = 9×10^{-9} M; compare Taxol: $LC_{50}=8\times10^{-5}$ M, and epothilone B: $LC_{50}=8\times10^{-5}$ M, see Table 3], breast cancer (MDA-MB-468, $LC_{50}=8\times10^{-9}$ M), lung cancer (NCI-H226, $LC_{50}=2\times10^{-8}$ M), and central nervous system cancer (SF-295, $LC_{50}=6\times10^{-9}$ M) cell lines (see Table 3). Uncialamycin (**1**) also demonstrated considerable selectivity against the various cell lines tested, with minimal cytotoxicity observed against leukemia cell lines ($LC_{50}>10^{-5}$ M for all cell lines tested, see the Supporting Information). The entire spectrum of cytotoxicities of uncialamycins **1** and **2** against the NCI panel of 60 human-tumor cell lines at concentrations ranging from 10^{-5} to 10^{-11} M can be found in the Supporting Information.

Table 3: LC_{50} profiles of natural uncialamycin (**1**) against selected cancer cell lines.[a]

Cell line	Cancer type	Taxol (LC_{50} [M])	Epothilone B (LC_{50} [M])	Compound **1** (LC_{50} [M])
M14	melanoma	8×10^{-5}	8×10^{-5}	9×10^{-9}
SK-MEL-5	melanoma	6×10^{-5}	6×10^{-5}	2×10^{-8}
MDA-MB-468	breast	–	–	8×10^{-9}
NCI-H226	non-small cell lung	8×10^{-5}	$>10^{-4}$	2×10^{-8}
SF-295	central nervous system	$>10^{-4}$	–	6×10^{-9}
SF-539	central nervous system	8×10^{-5}	$<10^{-8}$	9×10^{-9}

[a] Antitumor assays were performed by the National Cancer Institute (NCI) in accordance with their published protocols.[13] Data for Taxol and epothilone B are from the NCI June 2007 test.

The described chemistry provides ready access to enantiopure uncialamycin (**1**) and 26-*epi*-uncialamycin (**2**), enabling extensive biological investigations of these highly potent DNA-cleaving molecules. Exploratory investigations revealed impressive broad-spectrum antibacterial properties and highly potent antitumor activities against a variety of cell lines, including drug-resistant cell lines. While the developed synthetic route will provide access to a variety of designed analogues of these compounds, the biological profiles of **1** and **2** warrant further investigation, including the evaluation of antibody–drug conjugates[5] for potential antitumor,[2] antibacterial,[14] and antiviral[15] applications.

Received: October 3, 2007
Published online: December 3, 2007

Keywords: antibiotics · antitumor agents · asymmetric synthesis · enediynes · natural products

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