JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Article
pubs.acs.org/JACS

Streamlined Total Synthesis of Uncialamycin and Its Application to the Synthesis of Designed Analogues for Biological Investigations

K. C. Nicolaou,*[,†,‡] Yanping Wang,[†] Min Lu,[†,‡] Debashis Mandal,[†,‡] Manas R. Pattanayak,[†,‡] Ruocheng Yu,[†] Akshay A. Shah,[†] Jason S. Chen,[‡] Hongjun Zhang,[‡] James J. Crawford,[‡] Laxman Pasunoori,[‡] Yam B. Poudel,[§] Naidu S. Chowdari,[§] Chin Pan,[§] Ayesha Nazeer,[§] Sanjeev Gangwar,[§] Gregory Vite,[∥] and Emmanuel N. Pitsinos[†,⊥]

[†]Department of Chemistry, BioScience Research Collaborative, Rice University, 6100 Main Street, Houston, Texas 77005, United States

[‡]Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, United States

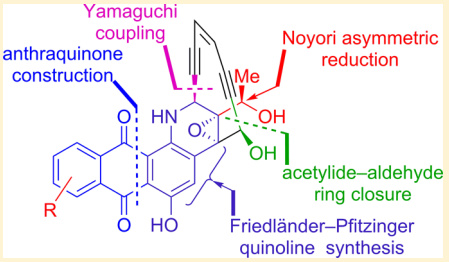
[§]Bristol-Myers Squibb Research & Development, 700 Bay Road, Redwood City, California 94063, United States

[∥]Bristol-Myers Squibb Research & Development, Princeton, New Jersey 08543-4000, United States

[⊥]Laboratory of Natural Products Synthesis & Bioorganic Chemistry, Institute of Nanoscience & Nanotechnology, National Centre of Scientific Research "Demokritos", Agia Paraskevi GR-15310, Greece

Supporting Information

ABSTRACT: From the enediyne class of antitumor antibiotics, uncialamycin is among the rarest and most potent, yet one of the structurally simpler, making it attractive for chemical synthesis and potential applications in biology and medicine. In this article we describe a streamlined and practical enantioselective total synthesis of uncialamycin that is amenable to the synthesis of novel analogues and renders the natural product readily available for biological and drug development studies. Starting from hydroxy- or methoxyisatin, the synthesis features a Noyori enantioselective reduction, a Yamaguchi acetylide-pyridinium coupling, a stereoselective acetylide-aldehyde cyclization, and a newly developed annulation reaction that allows efficient coupling of a cyanophthalide and a *p*-methoxy semiquinone aminal to forge the anthraquinone moiety of the molecule. Overall, the developed streamlined synthesis proceeds in 22 linear steps (14 chromatographic separations) and 11% overall yield. The developed synthetic strategies and technologies were applied to the synthesis of a series of designed uncialamycin analogues equipped with suitable functional groups for conjugation to antibodies and other delivery systems. Biological evaluation of a select number of these analogues led to the identification of compounds with low picomolar potencies against certain cancer cell lines. These compounds and others like them may serve as powerful payloads for the development of antibody drug conjugates (ADCs) intended for personalized targeted cancer therapy.



1. INTRODUCTION

The naturally occurring enediyne antitumor antibiotics represent some of the most potent cytotoxic agents known to date.[1] Their structures are characterized by 9- or 10-membered ring conjugated enediyne structural motifs which play a principal role in their Bergman cycloaromatization-based double-strand DNA-cleaving mechanism of action. Figure 1 depicts a number of enediynes, including the stable 10-membered ring enediynes uncialamycin (**1**),[2−4] calicheamicin γ_1^I (**2**),[5,6] dynemicin A (**3**),[7,8] shishijimicin A (**4**),[9,10] and the labile 9-membered ring enediynes neocarzinostatin (**5**)[11,12] and presporolide (**6**),[13] the latter being only a proposed naturally biosynthesized substance based on the isolation of its expected Bergman cycloaromatization products,[14] sporolides A and B.[15,16] Because of their high potencies, these compounds are not suitable for chemotherapy by themselves. Two of them, however, as conjugates to appropriate delivery systems, have found applications as anticancer clinical agents. Neocarzinostatin was the first enediyne to be approved as a polymer drug conjugate (styrene maleic acid neocarzinostatin, SMANCS; zinostatin stimalamer) for the treatment of certain types of leukemia and cancers of the liver and the brain, while calicheamicin γ_1^I (**2**) as an antibody drug conjugate (ADC, gemtuzumab ozogamicin; Mylotarg) was approved for the treatment of certain types of acute myeloid leukemia (later withdrawn because of side effects). Kadcyla (ado-trastuzumab emtansine; a conjugate of the maytansinoid DM1) and Adcertris (brentuximab vedotin; a conjugate of monomethyl auristatin E) are two recently approved ADCs, the former being used against HER-2-positive breast cancer and the latter against Hodgkin lymphoma. Furthermore, several other ADCs of

Received: April 27, 2016
Published: June 7, 2016

ACS Publications © 2016 American Chemical Society

DOI: 10.1021/jacs.6b04339
J. Am. Chem. Soc. 2016, 138, 8235−8246

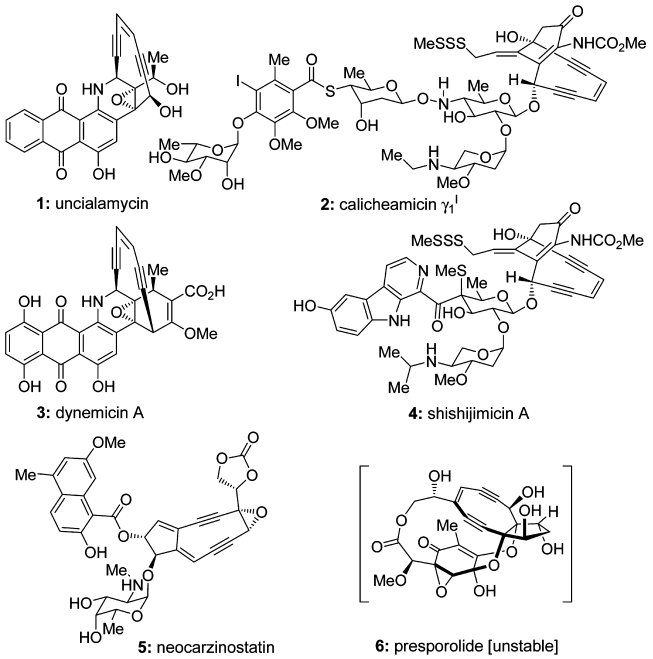


Figure 1. Selected enediyne antitumor antibiotics.

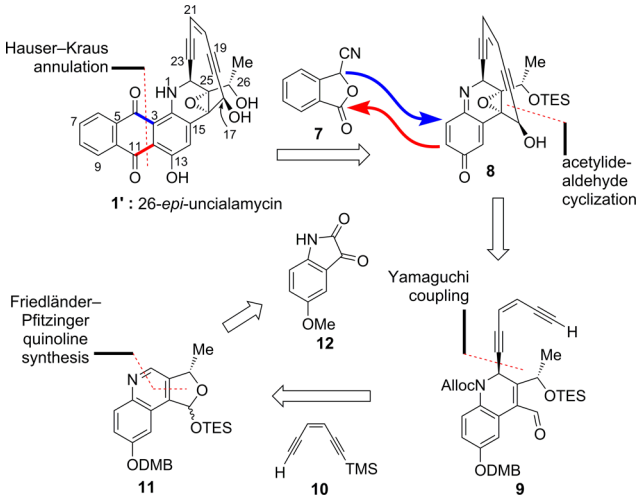
calicheamicin γ_1^I (and other potent cytotoxic agents) are currently in advanced clinical trials.[17]

Not surprisingly, the enediyne natural products have continued to attract considerable interest from biologists and chemists, both from academia and industry, due to their important biological properties, potential in medicine, and intriguing molecular structures and mechanisms of action.

In 2005, while screening for antibiotics effective against *Burkholderia cepacia*, a major cause of lung infections in cystic fibrosis patients, Davies, Andersen, and co-workers isolated uncialamycin (**1**, Figure 1), a 10-membered ring enediyne antibiotic from an unreported strain of streptomycete related to *Streptomyces cyanogenus*.[2] In addition to its extremely high potency against *B. cepacia* (minimum inhibitory concentration MIC = 0.001 μg/mL), uncialamycin was reported to possess equally impressive antibacterial potency against *Staphylococcus aureus* (MIC = 0.000 0064 μg/mL) and *Escherichia coli* (MIC = 0.002 μg/mL) as well as potent DNA-cleaving properties.[2] Unfortunately, the extreme scarcity of uncialamycin (only 300 μg was isolated)[2] precluded its complete structural elucidation and thorough biological investigation. Thus, its relative stereochemistry at C26 (for numbering see structure 1′, Scheme 1) was not assigned, and its absolute stereochemistry was presumed, but not proven, to be the same as that of the related natural product dynemicin A (**3**, Figure 1).[2]

In a preliminary communication in 2007,[3a] we reported the total synthesis of racemic uncialamycin [26(*R*)] and its C26 epimer [26-*epi*-uncialamycin, 26(*S*)] and assigned the relative stereochemistry of the natural product as C26-(*R*). The strategy employed in this synthesis is shown in retrosynthetic format in Scheme 1. In addition to lacking enantioselectivity, this synthesis suffered from the intermediacy of iminoquinone **8** (Scheme 1) whose low yielding preparation, chemical instability, and modest performance as a substrate in the Hauser–Kraus annulation[18a,b] with cyanophthalide **7** left much to be desired. In a subsequent communication[3b] we disclosed an enantioselective total synthesis of both (+)-uncialamycin [(+)-**1**, Figure 1)] and 26-*epi*-uncialamycin [(+)-**1′**, Scheme 1)], their DNA-cleaving activities, and antibiotic and cytotoxic properties. In this article we report the details of the evolution of a streamlined asymmetric and scalable total synthesis of uncialamycin that renders it readily available for further biological investigations. We also report the application of our latter synthesis to the construction of a series of designed analogues as well as the biological evaluation of a select number of them.

Scheme 1. First Generation Retrosynthetic Analysis of 26-*epi*-Uncialamycin[a]



[a]TES = triethylsilyl, DMB = 3,4-dimethoxy-benzyl, Alloc = allyloxycarbonyl.

2. RESULTS AND DISCUSSION

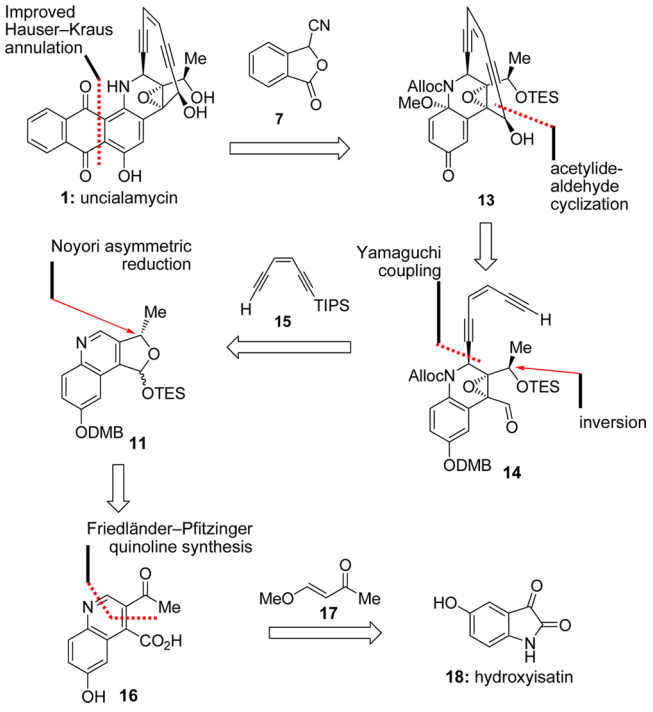
2.1. Enantioselective, Streamlined, and Practical Total Synthesis of (+)-Uncialamycin. Our experiences during our campaign toward racemic and enantiopure uncialamycin and other intelligence gathered during these endeavors positioned us well to develop a streamlined process applicable for larger scale synthesis of uncialamycin in its naturally occurring enantiomeric form. Below we describe the evolution of the process that led to this development.

As shown retrosynthetically in Scheme 2, a major innovation in this strategy was the utilization of the *p*-methoxy semiquinone aminal **13** (together with cyanophthalide **7**) in the Hauser–Kraus-type fusion,[18] instead of the customary *p*-iminoquinones (e.g., see **8**, Scheme 1).[8c,d] Sequential disconnections of the 10-membered enediyne ring within **13** through acetylide–aldehyde ring closure and Yamaguchi quinoline–acetylide coupling[19] revealed first acetylene aldehyde **14**, and then key intermediates **11** and **15**,[20] the latter being the TIPS-monoprotected enediyne building block. This alternative intermediate was deemed more practical due to its relative nonvolatility and other reasons that will be discussed below. Another important development in the new process was the discovery that hydroxyisatin (**18**, Scheme 2) could be used without protection in the Friedländer–Pfitzinger quinoline synthesis[21] of hydroxy carboxylic acid methyl ketone **16**, circumventing the cumbersome cleavage of the methoxy group under acidic conditions.[3a,b] The motivation and rationale for this and other improvements will be further discussed below as we traverse through the conduits of the developing strategy.

The original preparation of hydroxyketoacid **16** from methoxyisatin (**12**) suffered heavily from prolonged reaction

DOI: 10.1021/jacs.6b04339
J. Am. Chem. Soc. 2016, 138, 8235–8246

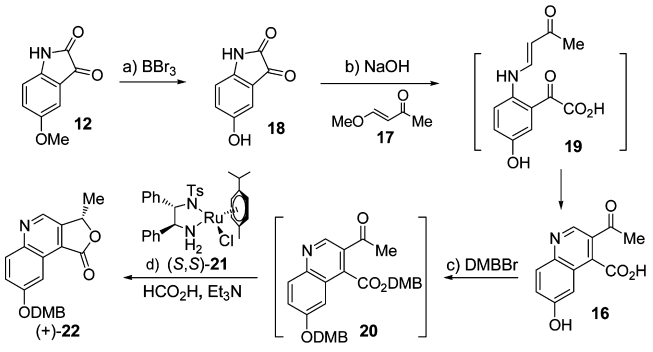
Scheme 2. Streamlined Retrosynthetic Analysis Through *p*-Methoxy Semiquinone Aminal 13[a]



[a]TIPS = triisopropylsilyl

times and drastic conditions, especially at the demethylation step (48% aq HBr, 110 °C, 40 h).[3a,b] In pondering how to render this process practical, we wondered whether the Friedländer–Pfitzinger quinoline synthesis would proceed from the free hydroxyisatin (**18**) which, in addition to being commercially available, could be prepared in principle from the less costly methoxyisatin (**12**). Scheme 3 shows the solution to this problem and the efficient synthesis of enentiomerically pure key building block (+)-**22**. Thus, BBr_3-induced demethylation of **12** at room temperature led to hydroxyisatin (**18**) in 96% yield. To our delight, this substrate exhibited exceptional reactivity under Friedländer–Pfitzinger conditions

Scheme 3. Streamlined Process for the Preparation of Quinoline Derivative (+)-22[a]

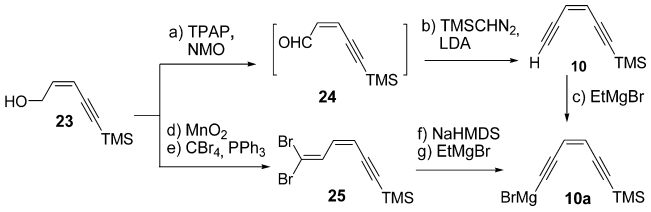


[a]Reagents and conditions: (a) BBr_3 (2.6 equiv), CH_2Cl_2, 0 to 25 °C, 2 h, 96%; (b) NaOH (2.0 equiv), **17** (2.0 equiv), H_2O, 25 °C, 15 min, 91%; (c) Cs_2CO_3 (5.0 equiv), *n*-Bu_4NI (0.15 equiv), DMBBr (4.0 equiv), DMF, 0 to 25 °C, 5 h; (d) (*S*,*S*)-**21** (0.05 equiv), HCO_2H (4.3 equiv), Et_3N (2.5 equiv), CH_2Cl_2, 0 °C, 24 h, 82% over the two steps, 96% ee; recrystallization from EtOAc, ≥99% ee.

(aq NaOH, 25 °C, 15 min) in the presence of methyl ketone **17**, leading directly to quinoline hydroxyketoacid **16** in 91% yield, sparing the troublesome aldol-type condensation (i.e., **19** → **16**, see Scheme 3) that was required in the original route.[3a,b] The presumed intermediate aryl vinylogous amide **19** (Scheme 3) was not detected in this reaction. The subsequent bis-alkylation with 3,4-dimethoxy-benzyl bromide (DMBBr) also required modification due to the lability of the substrate under the original conditions [DMBBr (3.0 equiv), K_2CO_3 (8.0 equiv), *n*-Bu_4NI (0.15 equiv), DMF, 25 °C].[3a,b] Thus, by switching to the more effective and yet mild Cs_2CO_3 and feeding the resulting bis-DMB derivative **20** directly into the previously employed[3b] Noyori asymmetric reduction [HCO_2H, Et_3N, (*S*,*S*)-**21** cat.][22] allowed the formation of (+)-**22** in 82% overall yield and 96% ee (≥99% ee after one recrystallization from EtOAc). All compounds in this series proved crystalline, and only one column chromatographic purification was required [at the stage of (+)-**22**]. Note that the seemingly wrong absolute configuration of (+)-**22** [26(*S*), uncialamycin numbering] was intentional, with its aim being to direct the incoming acetylide attack from the desired side (anti) in the pending Yamaguchi coupling (see below).

With a practical process for the synthesis of the quinoline fragment, we next turned our attention to the enediyne system of uncialamycin. The required enediyne fragment **10** was originally prepared as shown in Scheme 4. The readily available

Scheme 4. Construction of Enediyne Key Building Block 10[a]



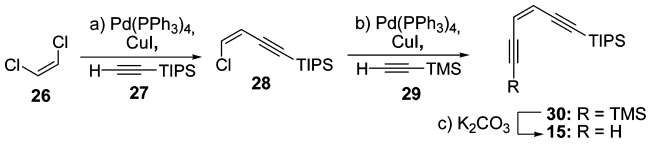
[a]Reagents and conditions: (a) NMO (1.5 equiv), TPAP (0.1 equiv), 4 Å molecular sieves, CH_2Cl_2, 25 °C, 5 min; (b) $TMSCHN_2$ (1.2 equiv), LDA (1.2 equiv), THF, −78 °C, 20 min; then **24** (1.0 equiv), −78 to 25 °C, 1 h, 30% over the two steps; (c) EtMgBr (1.1 equiv), THF, 25 °C, 40 min; (d) MnO_2 (10.0 equiv), CH_2Cl_2, 0 to 25 °C, 5 h; (e) CBr_4 (1.5 equiv), PPh_3, CH_2Cl_2, 0 °C, 1 h, 75% over the two steps; (f) NaHMDS (1.0 equiv), THF, −78 to −50 °C, 3 h; (g) EtMgBr (2.2 equiv), THF, 0 to 25 °C, 30 min. NMO = *N*-methylmorpholine-*N*-oxide, TPAP = tetrapropylammoniumperruthenate, LDA = lithium diisopropylamide, HMDS = hexamethyldisilazane.

enyne alcohol **23**[23] was oxidized to aldehyde **24** (TPAP cat., NMO) and then converted to **10** through the action of $TMSCHN_2$ and LDA (≤30% overall yield). The volatile nature of **24** and **10** made it impractical to improve the yield of this sequence, compelling us to devise the alternative route shown in Scheme 4. Thus, MnO_2 oxidation of eneyne alcohol **23**[23] followed by reaction of the resulting crude aldehyde (i.e., **24**) with CBr_4/PPh_3 furnished dibromodieneyne **25** in 75% overall yield as a colorless solid. The Grignard reagent **10a** required for the coupling with quinoline **11** could be prepared from **25** in THF by sequential treatment with NaHMDS and EtMgBr or from **10** directly by treatment with EtMgBr, as shown in Scheme 4 [for more details on the generation and quantitation of reagent **10a** from **10** or **25**, see the Supporting Information (SI)].

DOI: 10.1021/jacs.6b04339
J. Am. Chem. Soc. 2016, 138, 8235–8246

Although the originally employed TMS-enediyne **10** could be routinely prepared on gram-scale through the above-described procedures, the overall routes (see Scheme 4) and properties of this building block left much to be desired (i.e., high cost of reagents, chromatographic purification, and volatility of final product). To remedy this situation, we adopted TIPS-enediyne **15**[20] (Scheme 5) as a superior building block by virtue of its expected lower volatility. Scheme 5 summarizes a streamlined synthesis of TIPS-enediyne **15** from readily available *cis*-dichloroethene (**26**), TIPS-acetylene (**27**), and TMS-acetylene (**29**).[20] Thus, Sonogashira coupling[24] of **26** with **27** proceeded in the presence of $Pd(PPh_3)_4$ and CuI catalysts to afford chloroenyne **28** (93% yield), which was coupled [once again in the presence of $Pd(PPh_3)_4$ and CuI cat.] this time with TMS-acetylene (**29**) furnishing bis-silyl acetylene **30** in 96% yield. The latter was smoothly and selectively monodesilylated (−TMS) with K_2CO_3 in MeOH to give desired TIPS-monoprotected *cis*-enediyne **15** in 96% yield (≥98% geometrical purity, as determined by ^1H NMR spectroscopy).

Scheme 5. Streamlined Synthesis of Enediyne 15[a]

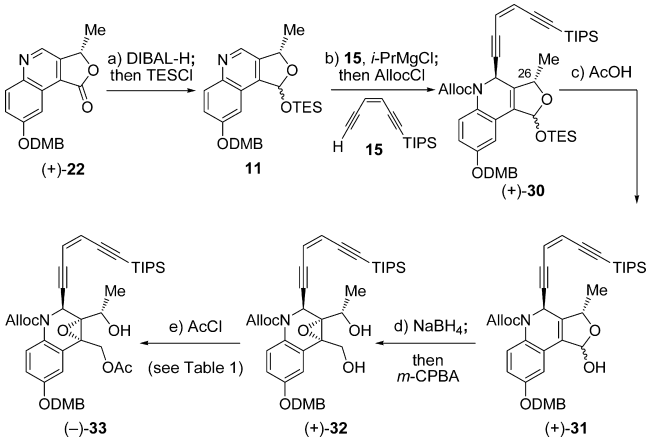


[a]Reagents and conditions: (a) $Pd(PPh_3)_4$ (0.03 equiv), CuI (0.03 equiv), *n*-$BuNH_2$ (2.0 equiv), **27** (1.0 equiv), **26** (1.9 equiv), Et_2O, 25 °C, 10 h, 93%; (b) $Pd(PPh_3)_4$ (0.03 equiv), CuI (0.03 equiv), *n*-$BuNH_2$ (2.0 equiv), **29** (2.0 equiv), Et_2O, 25 °C, 12 h, 96%; (c) K_2CO_3 (1.1 equiv), benzene, MeOH, 25 °C, 2 h, 96%.

With both building blocks quinoline (+)-**22** and enediyne **15** available in decagram quantities, we next proceeded to construct the advanced intermediate Alloc-protected *p*-aminophenol (+)-**38**, as shown in Schemes 6 and 7. Thus, reduction of the lactone moiety of (+)-**22** with DIBAL-H followed by silylation of the resulting lactol (TESCl) gave TES-ether **11** in 91% yield (ca. 1:1 dr). Addition of the latter mixture to a preformed solution of the Grignard reagent derived from enediyne **15** and *i*-PrMgCl (THF, 0 to 25 °C) at 0 °C followed by addition of AllocCl furnished exclusively anti-addition product (+)-**30** in a pleasing 90% yield (as compared to 72% yield, plus 20% recovered starting material in the case of TMS-acetylene **10** that was used in the original approach[3a,b]). The improved yield in this Yamaguchi coupling is attributed to the lower volatility of the enediyne **15** that allows the formation of its Grignard reagent without loss of substrate. The antiselectivity observed in this reaction is most likely due to the controlling effect of the methyl group on substrate **11** as opposed to other substrates we tested (such as the open-chain bis-TES ether of the diol derived from **22** that led to ca. 1:1 mixture of C-24 epimers) in our attempts to develop this highly efficient and stereoselective process.

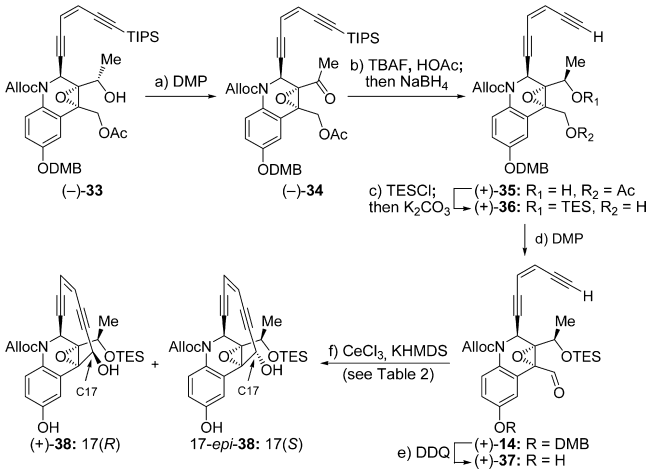
As good as the yield and stereoselectivity of this process was, it left behind the undesired stereochemical configuration of C-26(*S*) [see (+)-**30**, Scheme 6]. Our next objective therefore was the advancement of intermediate (+)-**30** to the cyclization precursor (+)-**37** (Scheme 7) with the corrected C26 configuration. To this end, the TES group from (+)-**30** was removed (AcOH, 91% yield), and the resulting lactol was treated with $NaBH_4$ in MeOH to afford the corresponding diol, whose epoxidation with *m*-CPBA furnished epoxide diol (+)-**32** in 81% overall yield for the two steps. At this stage, we decided to attempt to optimize the monoacetylation of (+)-**32**, which

Scheme 6. Yamaguchi Coupling and Synthesis of Monoprotected Diol (−)-33[a]



[a]Reagents and conditions: (a) DIBAL-H (2.4 equiv), CH_2Cl_2, −78 °C, 3 h; then TESCl (1.3 equiv), imidazole (2.6 equiv), DMF, 0 to 25 °C, 20 min, 91%, ca. 1:1 mixture of diastereoisomers; (b) **15** (2.0 equiv), *i*-PrMgCl (2.0 equiv), THF, 0 to 25 °C, 2 h; then (+)-**11** (1.0 equiv), 0 °C, 1 h; then AllocCl (2.0 equiv), 0 °C, 30 min, 90%; (c) CH_3CN/H_2O/AcOH (4:1:2), 25 °C, 2 h, 91%; (d) $NaBH_4$ (1.3 equiv), MeOH, 0 °C, 20 min; then *m*-CPBA (1.0 equiv), $NaHCO_3$ (2.0 equiv), CH_2Cl_2, 0 °C, 3 h, 81% over the two steps; (e) AcCl (1.0 equiv), *i*-Pr_2NEt (2.0 equiv), CH_2Cl_2, −78 °C, 12 h, 86%. DIBAL-H = diisobutylaluminum hydride, *m*-CPBA = 3-chloro-perbenzoic acid.

Scheme 7. Streamlined Synthesis of Alloc-Protected *p*-Aminophenol (+)-38[a]

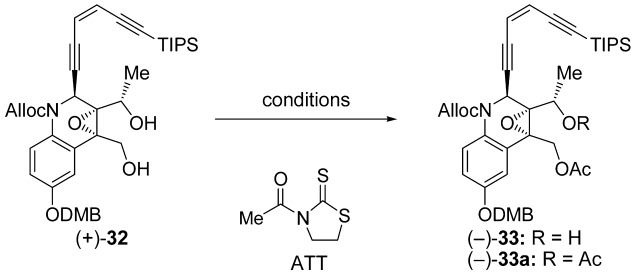


[a]Reagents and conditions: (a) DMP (2.0 equiv), $NaHCO_3$ (4.0 equiv), CH_2Cl_2, 0 to 25 °C, 2 h, 94%; (b) TBAF (2.5 equiv), AcOH (2.5 equiv), THF, 0 to 25 °C, 2 h; $NaBH_4$ (2.0 equiv), MeOH, 0 °C, 30 min, 93% over the two steps, ≥25:1 dr; (c) TESCl (1.5 equiv), imidazole (2.0 equiv), DMF, 0 °C, 15 min; then saturated K_2CO_3 in MeOH, THF, −10 °C, 20 min, 85% for the two steps; (d) DMP (2.0 equiv), $NaHCO_3$ (4.0 equiv), CH_2Cl_2, 0 to 25 °C, 90 min, 90%; (e) DDQ (3.0 equiv), pH 6.8 phosphate buffer, CH_2Cl_2, 25 °C, 12 h, 94%; (f) $CeCl_3$ (4.0 equiv), THF, 25 °C, sonication, 30 min; then KHMDS (6.0 equiv), −78 to −40 °C, 1 h, 79% (+)-**38** [17(*R*)-isomer], plus 13% 17(*S*)-isomer, (+)-17-*epi*-**38**. DMP = Dess−Martin periodinane, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

DOI: 10.1021/jacs.6b04339
J. Am. Chem. Soc. 2016, 138, 8235−8246

we previously achieved only partially.[3a,b] Table 1 summarizes the results of this optimization study. Our previously achieved

Table 1. Optimization Study of Monoacetylation[a]



entry	conditions	yield[b]	product(s)[b]
1	Ac_2O, Et_3N, DMAP, THF, −78 °C	≥95%	only **33a**
2	*n*-Bu_2SnO, Ac_2O, DMF, 25 °C	10%	only **33**
3	ATT, NaH, THF, 25 °C	80%	**33**:**33a** (ca. 95:5)
4	AcCl, collidine, CH_2Cl2, 0 to 25 °C	82%	**33**:**33a** (ca. 5−20:1)
5	**AcCl, *i*-Pr_2NEt, CH_2Cl_2, −78 °C**	**86%**	**only 33**

[a]Reactions were carried out on 0.1−1.0 mmol scale. [b]Yields and selectivities were determined by ^1H NMR spectroscopic analysis. ATT = 3-acetylthiazolidine-2-thione.

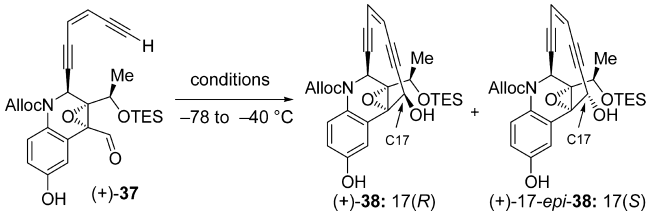
selectivity (82% yield, contaminated with 15−20% of the corresponding bis-acetate, 100 mg scale) with AcCl and collidine (CH_2Cl_2, 0 to 25 °C)[3a,b] was surpassed only through the use of AcCl and Hünig's base (*i*-Pr_2NEt, CH_2Cl_2, −78 °C, 86% yield, decagram scale, Table 1, entry 5). The latter procedure had the advantage of being reproducible on a large scale, while the former suffered upon scale up in terms of yield, selectivity, and reproducibility.

Hydroxyacetate (−)-**33** was then oxidized with Dess−Martin periodinane[25] (DMP) to afford ketoacetate (−)-**34** (94% yield) as shown in Scheme 7. Removal of the TIPS group from the enediyne terminus of the latter (TBAF, AcOH) followed by $NaBH_4$ reduction led smoothly to the C26-inverted hydroxy compound (+)-**35** in 93% overall yield and ≥25:1 diastereoselectivity (as determined by ^1H NMR spectroscopic analysis).

In preparation for the pending ring closure, the hydroxyl group within (+)-**35** (Scheme 7) was protected as a TES ether (TESCl), and the acetate group was cleaved (K_2CO_3, MeOH, 85% overall yield), releasing the primary hydroxyl group and furnishing compound (+)-**36**. The latter was treated with DMP to give the coveted cyclization substrate aldehyde (+)-**14** (90% yield). Preliminary attempts to induce cyclization on this substrate were plagued by instability of the DMB ether moiety protecting the phenolic group, forcing us to remove the latter (DDQ, 94% yield), an operation that led to aldehyde (+)-**37** possessing a free phenolic group.

At this stage, and in order to develop a high yielding and reproducible procedure for the ring closure to the desired cyclic enediyne system, we undertook a systematic optimization study. As seen in Table 2, this exploration included changes of the base metal, additives, and sonication, the latter proving itself as a crucial parameter. Thus, while NaHMDS and LiHMDS proved inferior to KHMDS in the presence of various additives, the latter base performed even better upon addition to a presonicated mixture of $CeCl_3$ and substrate (+)-**37**. It was

Table 2. Optimization Study for Acetylene-Aldehyde Cyclization[a]



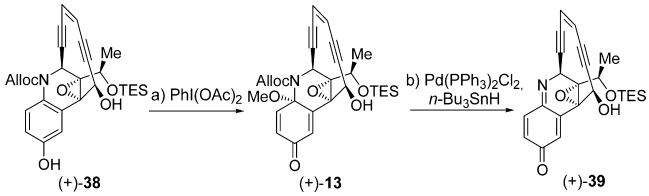
entry	conditions	yield[b]	**38**:17-*epi*-**38**[b]
1	$CeCl_3$, LiHMDS, THF	90%	2:3
2	$CeCl_3$·2LiCl, LiHMDS, THF	90%	1:3
3	LiCl, LiHMDS, THF	33%	17-*epi*-**38** only
4	$CeCl_3$, KHMDS, toluene/THF	80%	5:1
5	$CeCl_3$, KHMDS, THF	85%	6:1
6	$CeCl_3$·2LiCl, KHMDS, THF	70%	1:3
7	**$CeCl_3$, KHMDS, THF, presonication**	**92%**	**6:1**
8	$Yb(OTf)_3$, LiHMDS, THF	decomposition	

[a]Reactions were carried out on 0.1−1.0 mmol scale. [b]Yields and ratios determined by ^1H NMR spectroscopic analysis.

delightful to realize in the end that the phenol served well as a substrate, leading to good selectivity in favor of the desired 17(*R*)-epimer (ca. 6:1 dr, see entry 7, Table 2). It was also interesting to observe that the use of LiHMDS resulted in reversal of the selectivity of the acetylide addition to the aldehyde, furnishing the 17-epimer of (+)-**38** as the major product, (+)-17-*epi*-**38** (single isomer, see entry 3, Table 2, not optimized). Thus, a stereodivergent cyclization was discovered that potentially could be employed for the selective synthesis of designed analogues with differing C17-configurations.

The next challenge to be addressed in the synthesis was the annulation intended to form the amino-anthraquinone system of uncialamycin. In our first[3a] and second[3b] syntheses of uncialamycin we achieved this goal by joining cyanophthalide **7** with *p*-iminoquinone (+)-**39** (i.e., 26-*epi*-**8**, Scheme 1), the latter obtained from Alloc-protected *p*-aminophenol (+)-**38** through oxidation and Alloc cleavage (Scheme 8). In our initial

Scheme 8. Optimized Preparation of *p*-Methoxy Semiquinone Aminal (+)-13 and *p*-Iminoquinone (+)-39[a]

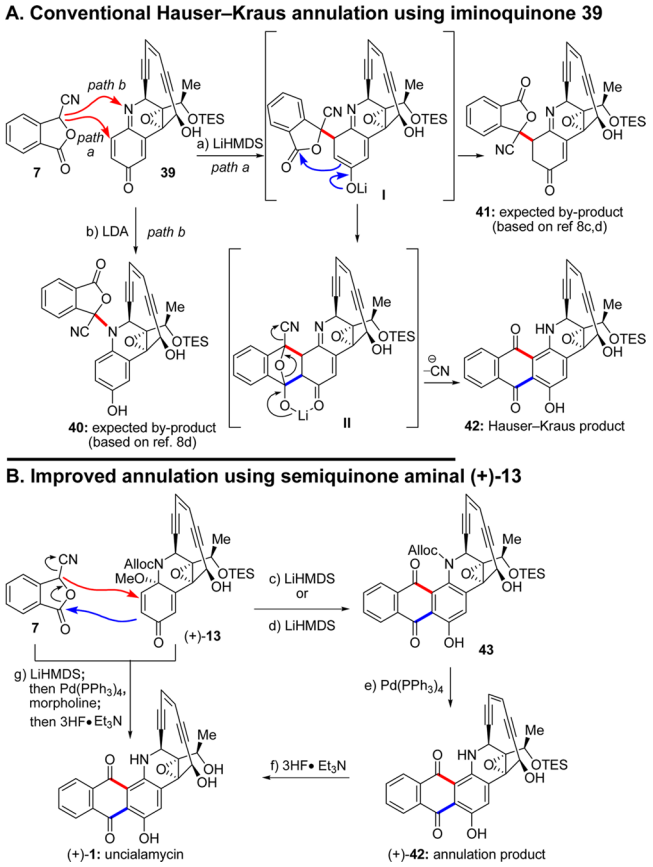


[a]Reagents and conditions: (a) $PhI(OAc)_2$ (1.1 equiv), MeOH, 0 to 25 °C, 15 min, 83%; (b) *n*-Bu_3SnH (1.6 equiv), H_2O (5.2 equiv), $Pd(PPh_3)_2Cl_2$ (0.2 equiv), CH_2Cl_2, 25 °C, 20 min, 63% (based on 65% conversion).

attempts to optimize this process, we achieved some improvements but also encountered persistent intransigence as shown in Schemes 8 and 9. Mindful of the reports from Myers[8d] and Danishefsky,[8c] we were not surprised to observe byproduct **40** (43% yield), with LDA as a base, or byproduct **41** (30% yield) plus desired product **42** (60% yield), with LiHMDS as a base (Scheme 9A).

DOI: 10.1021/jacs.6b04339
J. Am. Chem. Soc. 2016, 138, 8235−8246

Scheme 9. Completion of Uncialamycin [(+)-1] Total Synthesis[a]



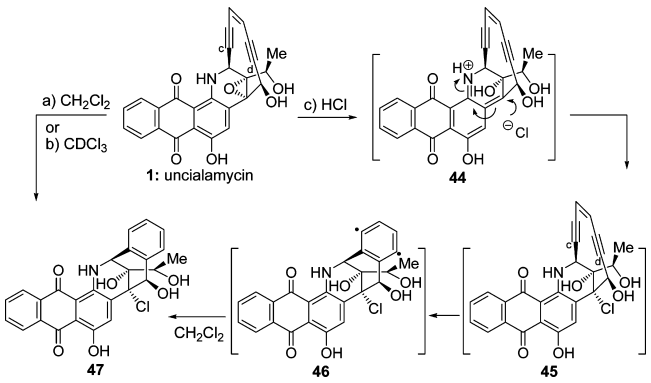
[a]Reagents and conditions: (a) 7 (3.0 equiv), LiHMDS (3.0 equiv), THF, −78 °C, 20 min; then **39** (1.0 equiv), −78 to 25 °C, 1 h, 60%; (b) 7 (3.0 equiv), LDA (3.0 equiv), THF, −78 °C, 20 min; then **39** (1.0 equiv), −78 to 25 °C, 1 h, 43%; (c) 7 (3.0 equiv), LiHMDS (3.0 equiv), THF, −78 °C, 20 min; then (+)-**13** (1.0 equiv), −78 to 25 °C, 1 h, 84%; (d) 7 (1.0 equiv), LiHMDS (1.0 equiv), THF, −78 °C, 20 min; then (+)-**37** (3.0 equiv), LiHMDS (3.0 equiv), THF, −78 to 25 °C, 1 h, 81%, with 62% recovered (+)-**13**; (e) $Pd(PPh_3)_4$ (0.08 equiv), morpholine (2.4 equiv), THF, 0 to 25 °C, 2.5 h, 85%; (f) 3HF·Et_3N (100 equiv), THF, 25 °C, 1.5 h, 99%; (g) 7 (3.0 equiv), LiHMDS (4.0 equiv), THF, −78 °C, 20 min; then (+)-**13** (1.0 equiv), −78 to 25 °C, 1 h; then $Pd(PPh_3)_4$ (0.1 equiv), morpholine (2.4 equiv), THF, 0 °C, 2 h, then 25 °C, 30 min; then 3HF·Et_3N (100 equiv), THF, 25 °C, 1.5 h, 73% overall for the three steps from (+)-**13**.

The varying yields in this coupling reaction and the difficulties associated with the preparation and stability of *p*-iminoquinone (+)-**39** led us to explore its precursor, Alloc-protected *p*-methoxy semiquinone aminal (+)-**13**, as a partner in the annulation reaction with cyanophthalide **7**. Substrate (+)-**13** had the advantage of offering only a single viable site of attack from the cyanophthalide anion (see Scheme 9B), as compared to the two presented by *p*-iminoquinone **39** (see Scheme 9A). Indeed, when *p*-methoxy semiquinone aminal (+)-**13** was exposed to the anion generated from cyanophthalide **7** and LiHMDS, either as the limiting reagent [3.0 equiv of **7**, 3.0 equiv of LiHMDS, 1.0 equiv of (+)-**13**, THF, −78 to 25 °C, 84% yield] or in excess [1.0 equiv of **7**, 4.0 equiv of LiHMDS, 3.0 equiv of (+)-**13**, THF, −78 to 25 °C, 81% yield based on 62% recovery of **13**], protected uncialamycin (+)-**43** (Scheme 9B) was obtained cleanly and in high yield. Cleavage of the Alloc protecting group from the latter led smoothly to the desired protected uncialamycin (+)-**42** as a single product and in excellent yield. As it turned out, this mode of annulation became the method of choice for preparing amino-anthraquinones of wide ranging complexity and diversity.[18c]

Finally, a three-step sequence from *p*-methoxy-semiquinone aminal (+)-**13** to (+)-uncialamycin [(+)-**1**] without purification of intermediates was developed as shown in Scheme 9B. Thus, reaction of cyanophthalide **7** (3.0 equiv) with LiHMDS (4.0 equiv) in THF at −78 °C for 20 min followed by addition of semiquinone (+)-**13** (1.0 equiv, −78 to 25 °C, 1 h) afforded bis-protected uncialamycin **43** in high yield, whose Alloc and TES protecting groups were sequentially removed without purification by exposure to $Pd(PPh_3)_4$ cat. and 3HF·Et_3N to furnish, after chromatographic purification, pure (+)-uncialamycin [(+)-**1**] in 73% overall yield (see Scheme 9B). This improved and streamlined 22-step (14 chromatographic separations) synthesis of (+)-uncialamycin from 5-hydroxyisatin (**18**, Scheme 3) proceeds in 11% overall yield.

Synthetic uncialamycin [(+)-**1**] was found to be a deep purple crystalline solid stable at ambient temperature, both in the solid phase and in solution in a variety of solvents (e.g., DMSO, MeOH, EtOAc, CH_3CN). However, upon dissolution in HPLC grade CH_2Cl_2 or NMR grade $CDCl_3$ that had been exposed to air, uncialamycin rapidly turned dark bluish, indicating a chemical change. As proven by NMR spectroscopic analysis,[3a] this change was brought about by the Bergman cycloaromatization reaction,[14] induced by traces of HCl or DCl present in the solvents used, and which led to the hexacyclic benzenoid system **47** via the pentacyclic chlorohydrin enediyne **45** (as shown in Scheme 10).[3a,26] The latter intermediate is a

Scheme 10. Bergman Cycloaromatization Study of (±)-Uncialamycin[a]



[a]Reagents and conditions: (a) CH_2Cl_2, 25 °C, 12 h; (b) $CDCl_3$, 25 °C, 30 min; (c) 5 mM HCl in CH_2Cl_2, 25 °C, 5 min, 90%.

fleeting species due to the induced closeness of its acetylenic units that allows the obligatory orbital interactions for the cycloaromatization to take place. The uncialamycin cascade shown in Scheme 10 can be rapidly and cleanly induced by anhydrous HCl in CH_2Cl_2 to afford the Bergman cycloaromatization product **47** (90% yield, dark blue).[3a] Interestingly, a Bergman cycloaromatization secondary metabolite related to uncialamycin has recently been reported and shown to exhibit anti-HIV activity,[27] suggesting this type of structure as potential lead compounds for further optimization. The cd distance [see structure **1**, Scheme 10] between the two acetylenic carbons to be bonded during the cycloaromatization

DOI: 10.1021/jacs.6b04339
J. Am. Chem. Soc. 2016, 138, 8235−8246

of cyclic conjugated enediynes has been correlated with the stability of these systems toward benzenoid diradical formation.[28] Computational studies (AMBER* force field) placed the cd distance of uncialamycin at 3.41 Å.[29] X-ray crystallographic analysis of (±)-uncialamycin [(±)-**1**] revealed the cd to be 3.60 Å.[3a] This value is consistent with the molecule's stability as opposed to that of the transient chlorohydrin **45**, whose calculated cd distance was found to be 3.00 Å,[29] a value in line with its rapid cycloaromatization observed. The likely mechanism of action of uncialamycin is, therefore, thought to involve binding to double-stranded DNA, activation, and double-strand cleavage of the genetic material by the generated benzenoid diradical as proposed for dynemycin[7] and calicheamicin γ_1^I,[5] and confirmed through experimentation.[3b]

2.2. Design, Synthesis, and Biological Evaluation of Uncialamycin Analogues. Having developed a practical and efficient total synthesis of uncialamycin [(+)-**1**; see general strategy in retrosynthetic format, Figure 2A], we proceeded to apply it to the synthesis of a series of designed analogues of this scarce natural product for the purposes of biological evaluation as cytotoxic agents to be used as payloads of ADCs (**48**–**59**,

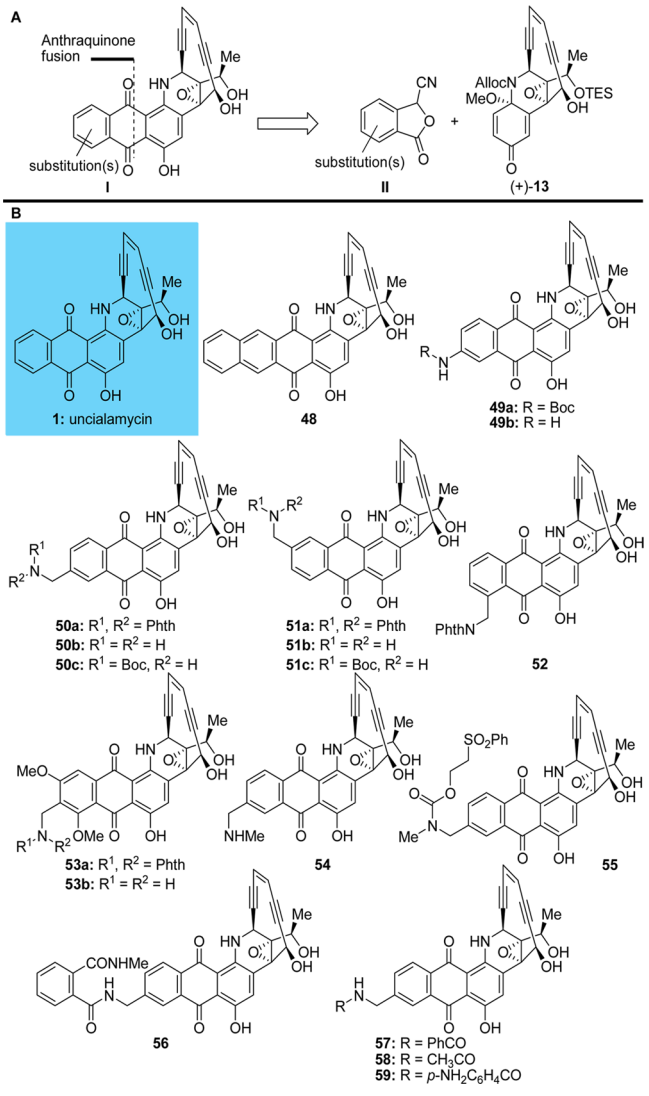


Figure 2. (A) General retrosynthetic analysis of uncialamycin analogues. (B) Structures of uncialamycin (**1**) and designed analogues (**48**–**59**). Boc = *tert*-butoxycarbonyl; Phth = phthaloyl.

Figure 2B). The masked amino group in some of these analogues was intended as the handle through which the molecule could be attached to a linker, and then to an appropriate antibody. According to Figure 2A, the required substituted cyanophthalides **II** were to be fused with semiquinone aminal (+)-**13** to form the targeted analogues (**I**) following our standard protocol.

Figure 3 shows the individual cyanophthalides (**60a**[18c] and **60b**–**h**) required for the synthesis of the targeted uncialamycin

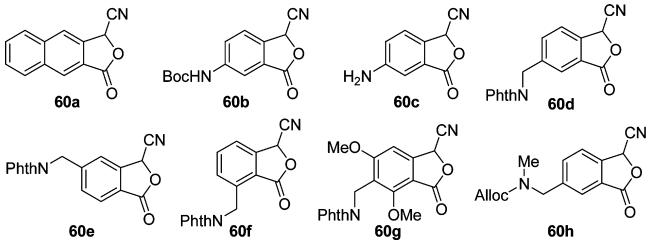
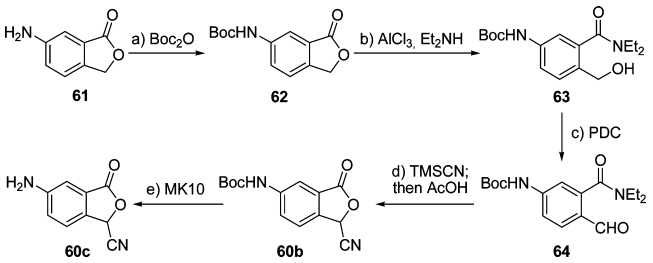


Figure 3. Required cyanophthalides for this work (**60a**–**h**).

analogues (**48**–**59**, Figure 2B). Their readily available rendering became our first task. The synthesis of cyanophthalide **60a** has been reported.[18c] The construction of the remaining required cyanophthalides (**60b**–**h**) from simple building blocks is summarized in Schemes 11–14.

Scheme 11. Synthesis of Aminocyanophthalides 60b and 60c[a]



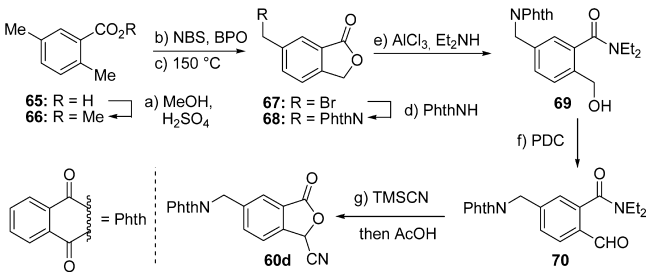
[a]Reagents and conditions: (a) Boc_2O (1.0 equiv), THF, reflux, 16 h, 100% (based on 88% conversion); (b) $AlCl_3$ (1.3 equiv), Et_2NH (2.5 equiv), CH_2Cl_2, 25 °C, 30 min, then **62**, 0 °C, 15 min, 82%; (c) PDC (1.1 equiv), 3 Å MS, CH_2Cl_2, 25 °C, 2 h, 62%; (d) TMSCN (1.4 equiv), KCN (0.02 equiv), 18-C-6 (0.02 equiv), CH_2Cl_2, 0 to 25 °C, 2 h; then AcOH, 25 °C, 24 h, 83%; (e) MK10, $ClCH_2CH_2Cl$, reflux, 4 h, 96%. PDC = pyridinium dichromate; MS = molecular sieves; TMSCN = trimethylsilyl cyanide; MK10 = montmorillonite K 10.

Aminocyanophthalides **60b** and **60c** were prepared from commercially available 6-aminophthalide **61** (see Scheme 11) through a sequence involving the following: (a) Boc protection (Boc_2O; quant); (b) amidation ($AlCl_3$, Et_2NH; 82% yield); (c) oxidation of the resulting primary alcohol (PDC; 62% yield); (d) cyanation and ring closing (TMSCN, KCN cat., 18-crown-6 cat., followed by AcOH; 83% yield); and (e) deprotection (montmorillonite K 10; 96% yield).

The phthalimidomethyl cyanophthalide **60d** was prepared from the commercially available 2,5-dimethyl benzoic acid (**65**, Scheme 12). Thus, reaction of **65** with MeOH in the presence of H_2SO_4 gave methyl ester **66** (86% yield) which was selectively brominated [Br_2, benzoyl peroxide (BPO) cat.] and cyclized (150 °C) to afford bromide **67** (68% yield).[30] The latter was reacted with phthalimide in the presence of K_2CO_3

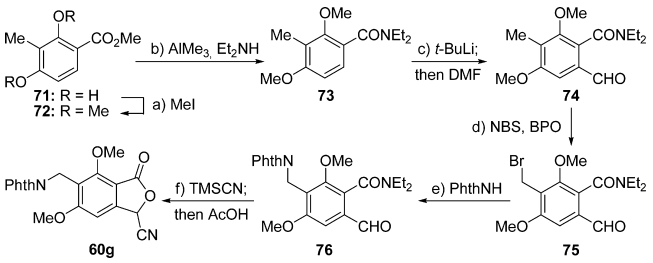
DOI: 10.1021/jacs.6b04339
J. Am. Chem. Soc. 2016, 138, 8235–8246

Scheme 12. Synthesis of Phthalimidomethyl Cyanophthalide 60d[a]



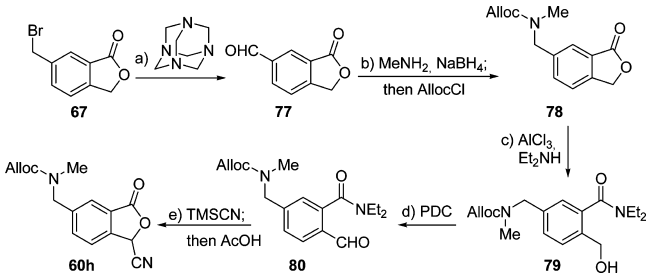
[a]Reagents and conditions: (a) H_2SO_4 (0.1 equiv), MeOH, reflux, 4 h, 86%; (b) NBS (2.2 equiv), BPO (0.06 equiv), CCl_4, reflux, 1 h, 82%; (c) 150 °C, 1.5 h, 68%; (d) PhthNH (1.1 equiv), K_2CO_3 (1.5 equiv), *n*-Bu_4NI (0.1 equiv), DMF, 70 °C, 1.5 h, 99%; (e) $AlCl_3$ (1.3 equiv), Et_2NH (2.5 equiv), CH_2Cl_2, 0 to 25 °C, 30 min, then **68**, 0 °C, 15 min, 78%; (f) PDC (1.5 equiv), 3 Å MS, CH_2Cl_2, 25 °C, 6.5 h, 63%; (g) TMSCN (2.0 equiv), KCN (0.1 equiv), 18-C-6 (0.1 equiv), CH_2Cl_2 /THF (1:1), 25 °C, 2.5 h; then PTSA (0.05 equiv), AcOH, 25 to 40 °C, 24 h, 92%. NBS = *N*-bromo succinimide; BPO = benzoylperoxide; PTSA = *p*-toluenesulfonic acid.

Scheme 13. Synthesis of Methoxy Cyanophthalide 60g[a]



[a]Reagents and conditions: (a) MeI (1.2 equiv), K_2CO_3 (1.5 equiv), DMF, 50 °C, 9 h, 93%; (b) $AlMe_3$ (2.1 equiv), Et_2NH (3.8 equiv), PhH, 0 to 120 °C, 7 h, 85%; (c) TMEDA (2.0 equiv), *t*-BuLi (2.0 equiv), THF, −78 °C, 50 min; then DMF (12 equiv), −78 to 25 °C, 3 h, 96%; (d) NBS (1.2 equiv), BPO (0.06 equiv), CCl_4, 85 °C, 2 h; (e) PhthNH (1.1 equiv), K_2CO_3 (1.5 equiv), *n*-Bu_4NI (0.1 equiv), DMF, 40 °C, 2 h, 60% over the two steps; (f) TMSCN (2.0 equiv), KCN (0.025 equiv), 18-C-6 (0.02 equiv), CH_2Cl_2, 0 to 25 °C, 2 h; then AcOH, 25 °C, 80 h, 81%. TMEDA = tetramethylethylenediamine.

Scheme 14. Synthesis of *N*-Methyl Cyanophthalide 60h[a]



[a]Reagents and conditions: (a) hexamethylenetetramine (2.0 equiv), AcOH (4.0 equiv), H_2O, 110 °C, 2 h, 81%; (b) $MeNH_2$ (1.0 equiv), TFE, 25 °C, 5 min; $NaBH_4$ (1.2 equiv), 25 °C, 5 min; AllocCl (1.5 equiv), $NaHCO_3$ (2.0 equiv), THF/H_2O (1:1), 25 °C, 40 min, 78%; (c) $AlCl_3$ (1.5 equiv), Et_2NH (3.0 equiv), CH_2Cl_2, 0 to 25 °C, 30 min, 95%; (d) PDC (2.0 equiv), 3 Å MS, CH_2Cl_2, 25 °C, 3 h, 79%; (e) TMSCN (2.0 equiv), KCN (0.1 equiv), 18-C-6 (0.1 equiv), CH_2Cl_2, 0 to 25 °C, 2 h; then AcOH, PTSA (0.05 equiv), 40 °C, 24 h, 88%. TFE = trifluoroethanol.

and catalytic amounts of *n*-Bu_4NI to furnish phthalimide **68** (99% yield), which was then converted into the targeted phthalimidomethyl cyanophthalide **60d** following procedures analogous to the ones employed for the construction of cyanophthalide **60b** from phthalide **62** (see Scheme 11). Analogously and in similar yields, phthalimidomethyl cyanophthalides **60e** and **60f** (Figure 3) were prepared from the corresponding dimethyl benzoic acids (see SI for further details).

Scheme 13 depicts the synthesis of cyanophthalide **60g** from commercially available methyl-2,4-dihydroxy-3-methylbenzoate **71**. Thus, sequential treatment of **71** with K_2CO_3/MeI (93% yield) and $AlMe_3/Et_2NH$ (85% yield) led to amide **73** via methyl ester **72**. Regioselective formylation of the latter to aldehyde **74** was achieved through lithiation (*t*-BuLi, TMEDA) followed by quenching with DMF (96% yield). Benzylic bromination of **74** with NBS in the presence of catalytic amounts of BPO furnished benzyl bromide **75** which was converted smoothly to its phthalimide derivative **76** (PhthNH, 60% yield for the two steps). Finally, reaction of phthalimide **76** with TMSCN in the presence of catalytic quantities of KCN and 18-C-6 followed by addition of AcOH led to the desired bis-methoxy cyanophthalide **60g** in 81% yield.

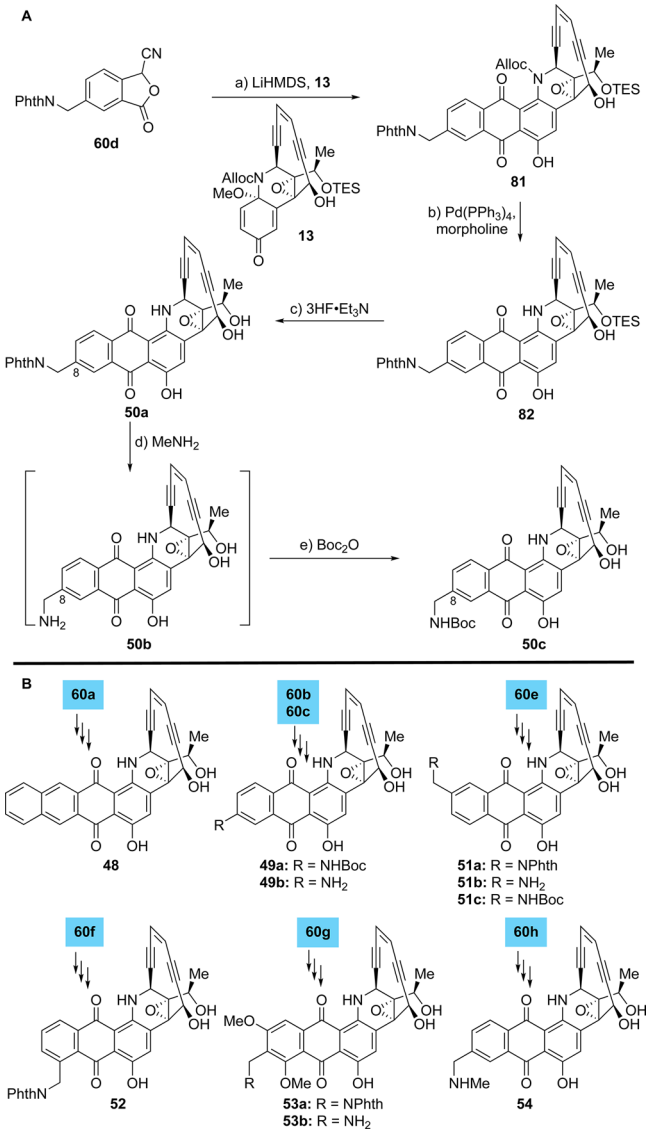
The preparation of *N*-Alloc-*N*-methyl cyanophthalide **60h** was accomplished from the readily available bromide **67** (see Scheme 12) as shown in Scheme 14. Thus, heating of **67** at 110 °C with hexamethylenetetramine in the presence of AcOH in H_2O produced aldehyde **77** in 81% yield. Reductive amination of the latter with $MeNH_2/NaBH_4$ in trifluoroethanol followed by Alloc protection of the resulting secondary amine gave intermediate lactone **78** (78% yield), whose sequential aminolysis ($AlCl_3$, Et_2NH, 95% yield) and PDC oxidation (79% yield) furnished aldehyde **80** via hydroxyl amide **79**. Exposure of **80** first to TMSCN in the presence of catalytic amounts of KCN and 18-C-6 and then to AcOH led to the targeted Alloc-protected *N*-methyl cyanophthalide **60h** in 88% yield.

With the required cyanophthalide partners (i.e., **60a**−**h**) in hand we turned our attention to their fusion with the readily available semiquinone aminal (+)-**13** as the key step before reaching the targeted uncialamycin analogues (**48**−**59**).

As a representative example, the synthesis of 8-aminomethyl uncialamycin (**50b**) from phthalimidomethyl cyanophthalide **60d** and semiquinone aminal (+)-**13** is presented below. Thus, as summarized in Scheme 15A, coupling of **60d** (2.0 equiv) with (+)-**13** (1.0 equiv) under the developed conditions (**60d**, LiHMDS, THF; then **13**, −78 to 25 °C) gave uncialamycin derivative **81**. The latter was treated without purification with catalytic amounts of $Pd(PPh_3)_4$ in the presence of morpholine to induce Alloc cleavage, furnishing **82** in 81% overall yield for the two steps (based on **13**). Treatment of **82** with $3HF{\cdot}Et_3N$ afforded desilylated product **50a** (90% yield), whose exposure to $MeNH_2$ led to the rather labile 8-aminomethyl uncialamycin (**50b**) in high yield. Apparently the presence of the aminomethyl group within the uncialamycin structure imparts considerable lability to the molecule (presumably due to higher propensity for Bergman cycloaromatization). To facilitate characterization, immediate trapping of freshly generated product **50b** in the $MeNH_2$-induced dephthaloylation of **50a** with Boc_2O in the presence of $NaHCO_3$ led to Boc-protected 8-aminomethyl uncialamycin (**50c**) in 70% overall yield from **50a**. The latter compound was found to be quite stable under

DOI: 10.1021/jacs.6b04339
J. Am. Chem. Soc. 2016, 138, 8235−8246

Scheme 15. Synthesis of Uncialamycin Analogues 48−54[a]



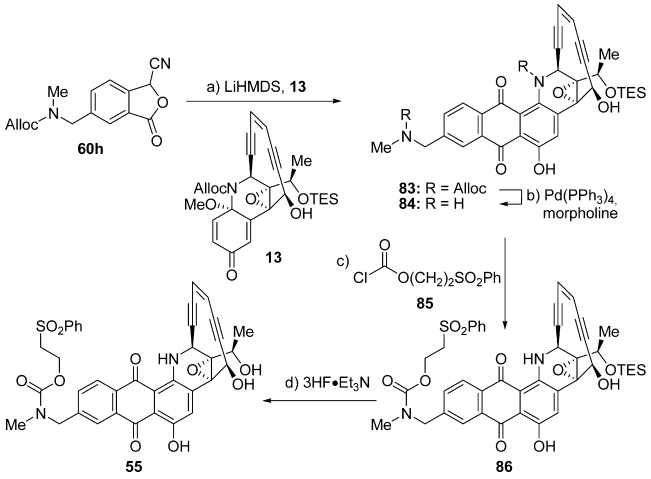
[a]Reagents and conditions: (a) **13** (1.0 equiv), LiHMDS (2.6 equiv), THF, −78 °C, 20 min; then **60d** (1.3 equiv), −78 to 25 °C, 1 h; (b) $Pd(PPh_3)_4$ (0.1 equiv), morpholine (2.4 equiv), THF, 0 °C, 2 h; then 25 °C, 30 min, 81% for the two steps (based on **13**); (c) $3HF{\cdot}Et_3N$ (100 equiv), THF, 25 °C, 1.5 h, 90%; (d) $MeNH_2$ (200 equiv), THF/H_2O (1:5), 0 to 25 °C, 2 h; (e) Boc_2O (1.2 equiv), sat. $NaHCO_3$ (aq), THF, 0 °C, 1.5 h, 70% over the two steps.

neutral conditions, apparently due to the diminished electron density on its aminomethyl substituent.

In a similar fashion, uncialamycin analogues **48**−**49b** and **51a**−**54** were synthesized from the corresponding cyanophthalides (**60a**−**c** and **60e**−**h**) and semiquinone aminal (+)-**13** (see SI for details).

Scheme 16 summarizes the synthesis of the phenylsulfonyl uncialamycin analogue **55** from cyanophthalide **60h** and semiquinone aminal (+)-**13**. Thus, reaction of **60h** with LiHMDS and (+)-**13** under the standard conditions led to Alloc-protected uncialamycin derivative **83**, from which secondary amine derivative **84** was generated by treatment with $Pd(PPh_3)_4$ cat. and morpholine. The latter compound was treated with sulfone containing chloroformate **85**[31] in the presence of pyridine to give carbamate **86**. Then, removal of

Scheme 16. Synthesis of Phenylsulfonyl Uncialamycin Analogue 55[a]



[a]Reagents and conditions: (a) **60h** (2.0 equiv), LiHMDS (2.6 equiv), THF, −78 °C, 20 min; then **13** (1.0 equiv), −78 to 25 °C, 1 h; (b) $Pd(PPh_3)_4$ (0.1 equiv), morpholine (2.4 equiv), THF, 0 °C, 1 h; (c) **85** (5 equiv), pyridine (5 equiv), CH_2Cl_2, 0 °C, 10 min; (d) $3HF{\cdot}Et_3N$ (100 equiv), THF, 25 °C, 1.5 h, 51% from **13**.

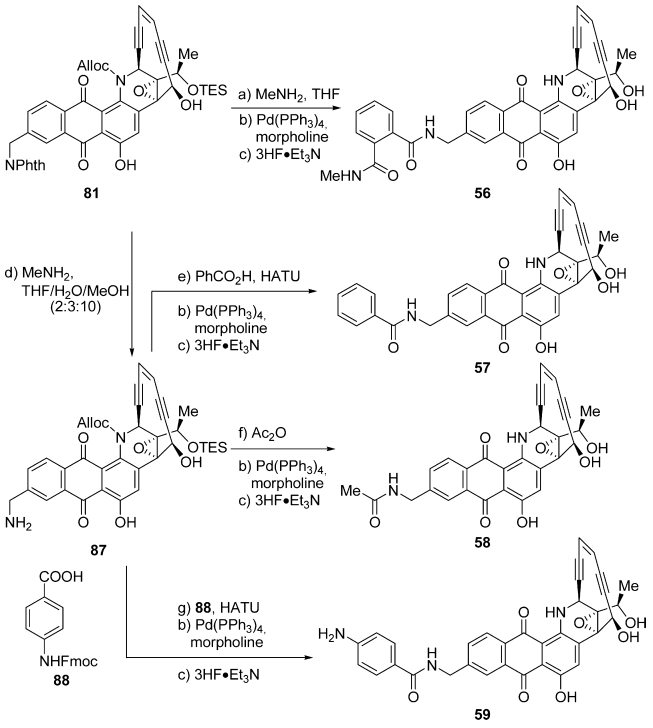
the TES group with $HF{\cdot}Et_3N$ secured the desired phenylsufonyl uncialamycin analogue **55** (51% overall yield from (+)-**13**).

Phthalimidomethyl-uncialamycin derivative **81** (see Scheme 15A) served as a common precursor for the syntheses of uncialamycin analogues **56**−**59** (Scheme 17). Thus, treatment of **81** with mild basic conditions ($MeNH_2$ in THF) at room temperature opened the phthalimide moiety to give the corresponding bis-amide compound, which was globally deprotected to furnish the bis-amide uncialamycin analogue **56**. Alternatively, by employing the optimized conditions for complete phtalimide removal (aqueous $MeNH_2$ in MeOH/THF), the free amino derivative **87** was obtained. Derivatization of this primary amine with acetic anhydride, benzoic acid, or *N*-Fmoc-4-aminobenzoic acid (**88**) furnished the corresponding amide derivatives which were subjected to global deprotection to afford the targeted uncialamycin analogues (**57**−**59**), respectively, as shown in Scheme 17.

A number of the synthesized uncialamycin analogues were tested for their ability to inhibit the proliferation of four different types of tumor cell lines: lung (H226), gastric (N87), ovarian (OVCAR3), and multidrug resistant (Adr) (see Table 3). Synthetic uncialamycin (**1**, Table 3, entry 1) was found to exhibit similar activity against these cell lines as that of natural uncialamycin,[2] while its extended anthraquinone moiety counterpart **48** (Table 3, entry 2) lost considerable potency. Introduction of an amino group at C8 (compound **49b**, Table 3, entry 3) did not make a significant difference in the potency. However, a real breakthrough was observed when the amino group was moved one carbon away from the aromatic moiety of the molecule (uncialamycin ring A, compound **50b**, Table 3, entry 5). This compound exhibited remarkably high potency against the tested cell lines (H226, IC_{50} = 28 pM; N87, IC_{50} = 11 pM; OVCAR3, IC_{50} = 316 pM; Adr, IC_{50} = 20 pM). Not surprisingly, the regioisomeric uncialamycin analogue **51b** (C7 aminomethyl, Table 3, entry 7) exhibited practically the same potency as its C8 sibling (H226, IC_{50} = 12 pM; N87, IC_{50} = 10 pM; OVCAR3, IC_{50} = 66 pM; Adr, IC_{50} = 29 pM; see Table 3,

DOI: 10.1021/jacs.6b04339
J. Am. Chem. Soc. 2016, 138, 8235−8246

Scheme 17. Synthesis of Uncialamycin Analogues 56–59[a]



[a]Reagents and conditions: (a) $MeNH_2$ (36 equiv), THF, 0 to 25 °C; (b) $Pd(PPh_3)_4$ (0.1 equiv), morpholine (2.4 equiv), THF, 0 °C, 2 h; then 25 °C, 30 min; (c) 3HF·Et_3N (100 equiv), THF, 25 °C, 1.5 h, **56** (77% overall yield) or **57** (33% overall yield) or **58** (35% overall yield) or **59** (48% overall yield); (d) $MeNH_2$ (124 equiv), THF/H_2O/MeOH (2:3:10), 0 to 10 °C; (e) benzoic acid (1.5 equiv), HATU (2 equiv), DIPEA (3 equiv), DMF, 0 °C to rt; (f) Ac_2O (2.0 equiv), DIPEA (3.0 equiv), CH_2Cl_2, 0 °C to rt, 1.5 h; (g) **88** (2.0 equiv), HATU (1.5 equiv), DIPEA (3 equiv), MeCN, rt, 1.5 h. DIPEA = diisopropylethylamine; HATU = 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate.

Table 3. *In Vitro* Cytotoxicity of Uncialamycin Analogues[a]

		cell line			
	compd	H226[b] IC_{50} (pM)	N87[c] IC_{50} (pM)	OVCAR3[d] IC_{50} (pM)	Adr[e] IC_{50} (pM)
1	**1**	1770	1764	4686	388
2	**48**	180 200	37 670	26 130	9050
3	**49b**	2043	2683	7638	666
4	**50a**	6946	3014	15 500	1007
5	**50b**	**28**	**11**	**316**	**20**
6	**50c**	3763	3067	8552	1021
7	**51b**	**12**	**10**	**66**	**29**
8	**53b**	77	148	401	335
9	**54**	**10**	**16**	**311**	**15**
10	**56**	67	31	1245	39
11	**57**	3164	5600	10 550	5171
12	**58**	855	892	2051	1146
13	**59**	706	210	368	300

[a]For details of the biological assays, see SI. [b]Lung. [c]Gastric. [d]Ovarian. [e]Multidrug resistant.

entry 7). The precise origin of the extreme potencies of these compounds is not fully understood. However, the relatively low chemical stability of these analogues (i.e., compounds **50b** and **51b**) suggests that the aminomethyl group renders them more prone to the Bergman cycloaromatization[14] and/or increases their binding affinity to DNA through polar interactions of their protonated forms with the phosphate backbone, and consequently more potent against their targeted biological system. The bis-dimethoxy analogue **53b** was proven to be only slightly less potent than **50b** (Table 3, entry 8). On the other hand, phthalimido-protected analogue **50a** (Table 3, entry 4) was found to be significantly less potent than the free amine **50b**, underscoring the importance of the free aminomethyl group for the high potency observed with these analogues. Compound **56** (Table 3, entry 10), an intermediate isolated from an incomplete cleavage of the phthalimido group, proved to be almost as potent as the free amine (i.e., **50b**). It was speculated, and later confirmed (by HPLC analysis), that this bis-amide uncialamycin derivative (i.e., **56**, for structure see Figure 2B) acts as a prodrug undergoing internal cyclization under the biological testing conditions, releasing free amine **50b**. Not surprisingly, the Boc-protected derivative **50c** (for structure see Figure 2B) also exhibited relatively low potency (Table 3, entry 6). The more stable 4-aminobenzoic derivative **59** proved quite potent (Table 3, entry 13) in contrast to its benzoic acid counterpart, derivative **57**, which was found to be considerably less potent (Table 3, entry 11). Once again, these differences in potencies between **50b** and **50c** highlight the role of a free amine as an enhancing structural feature within the uncialamycin class. It is interesting to note that acetamide **58** (Table 3, entry 12) was considerably more potent than benzamide **57** (Table 3, entry 11) and that the *N*-methyl C8-aminomethyl uncialamycin **54** (Table 3, entry 9) proved essentially equipotent (H226, IC_{50} = 10 pM; N87, IC_{50} = 16 pM; OVCAR3, IC_{50} = 311 pM; Adr, IC_{50} = 15 pM) to the most potent analogues C8-aminomethyl (i.e., **50b** Table 3, entry 5) and C7-aminomethyl (i.e., **51b**, Table 3, entry 7). These results provided a set of useful structure–activity relationships (SARs) within the uncialamycin structural type. It should be noted that, although labile as free amines, the most potent compounds **50b**, **51b**, and **53b** can be generated from their stable protected precursors and trapped by appropriate linkers, leading to stable molecular entities for further conjugation to antibodies and other delivery systems.

3. CONCLUSION

We have described the evolution of a streamlined synthetic strategy for the total synthesis of the rare cytotoxic agent uncialamycin [(+)-**1**]. Starting from hydroxyisatin, the present strategy proceeds in 22 steps (14 chromatographic separations) and delivers the natural product in 11% overall yield on multi-100 mg scales (within the confines of academic laboratories; projected to be scalable to multigram scales in appropriate industrial laboratories) in stark contrast to the 300 μg isolated from its natural source.[2] Of particular interest in this endeavor is the discovery, development, and application of a new type of annulation reaction to forge the *p*-amino-anthraquinone structural motif of the molecule, a method proven to be of general scope and applicability and which can also be extended to *o*-amino-anthraquinones and related systems.[18c] Thus, employing our developed streamlined process for the total synthesis of uncialamycin (**1**), we synthesized an array of designed uncialamycin analogues equipped with appropriate protecting groups and handles for further manipulation and attachment to cancer cell associated antibodies and other suitable drug delivery systems. Biological evaluation of a number of the synthesized compounds led to the discovery of a

DOI: 10.1021/jacs.6b04339
J. Am. Chem. Soc. 2016, 138, 8235–8246

number of cytotoxic agents with low picomolar potencies (e.g., compounds **50b**, **51b**, **53b**, **54**, **56**). The chemistry described in this article sets the foundation for further developments to occur toward drug discovery and development starting with uncialamycin, a rare naturally occurring substance, as a lead compound that by itself proved too toxic to be useful as a therapeutic agent. These investigations demonstrate the power of modern organic synthesis to facilitate biology and medicine by rendering readily available otherwise scarce complex molecules of natural or design origins. Thus, in addition to providing useful structure−activity relationships (SARs) within the uncialamycin class of antitumor agents, these efforts enabled conjugation studies that led to the preparation of antibody drug conjugates (ADCs) that are currently under further refinement and development as potential targeted cancer therapies.[4b]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b04339.

Experimental procedures and characterization data for key compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*kcn@rice.edu

Author Contributions

Y.W. and M.L. contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was partially carried out at The Scripps Research Institute. We thank Drs. D. H. Huang (TSRI), L. Pasternack (TSRI), L. B. Alemany (Rice), and Q. Kleerekoper (Rice) for NMR spectroscopic assistance; Drs. G. Siuzdak (TSRI) and C. Pennington (Rice) for mass spectrometric; and Drs. R. K. Chadha (TSRI) and C. Moore (UCSD) for X-ray crystallographic assistance. This work was supported by the National Institutes of Health (USA) (Grant AI055475), the Skaggs Institute for Research (TSRI), the Cancer Prevention & Research Institute of Texas (CPRIT), The Welch Foundation (Grant C-1819), and Bristol−Myers Squibb (BMS). J.J.C. acknowledges a postdoctoral fellowship award from the US−UK Fulbright Commission.

■ REFERENCES

(1) For reviews on enediynes, see: (a) Nicolaou, K. C.; Dai, W. M. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1387−1416. (b) Nicolaou, K. C.; Smith, A. L.; Yue, E. W.; Montero, A. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 5881−5888.

(2) For isolation, see: Davies, J.; Wang, H.; Taylor, T.; Warabi, K.; Huang, X.−H.; Andersen, R. J. *Org. Lett.* **2005**, *7*, 5233−5236.

(3) For synthesis, see: (a) Nicolaou, K. C.; Zhang, H.; Chen, J. S.; Crawford, J. J.; Pasunoori, L. *Angew. Chem., Int. Ed.* **2007**, *46*, 4704−4707. (b) Nicolaou, K. C.; Chen, J. S.; Zhang, H.; Montero, A. *Angew. Chem., Int. Ed.* **2008**, *47*, 185−189.

(4) For a patent, see: (a) Chowdari, N. S.; Gangwar, S.; Sufi, B. Enediyne Compounds, Conjugates Thereof, and Uses and Methods Thereof. US8709431 B2, April 29, 2014. (b) Nicolaou, K. C.; Lu, M.; Mandal, D.; Gangwar, S.; Chowdari, N. S.; Poudel, Y. B. Derivatives of Uncialamycin, Methods of Synthesis and Their Use as Antitumor Agents. WO2015023879 A1, February 19, 2015.

(5) For isolation, see: Lee, M. D.; Manning, J. K.; Williams, D. R.; Kuck, N. A.; Testa, R. T.; Borders, D. B. *J. Antibiot.* **1989**, *42*, 1070−1087.

(6) For synthesis, see: (a) Nicolaou, K. C.; Hummel, C. W.; Pitsinos, E. N.; Nakada, M.; Smith, A. L.; Shibayama, K.; Saimoto, H. *J. Am. Chem. Soc.* **1992**, *114*, 10082−10084. (b) Groneberg, R. D.; Miyazaki, T.; Stylianides, N. A.; Schulze, T. J.; Stahl, W.; Schreiner, E. P.; Suzuki, T.; Iwabuchi, Y.; Smith, A. L.; Nicolaou, K. C. *J. Am. Chem. Soc.* **1993**, *115*, 7593−7611. (c) Smith, A. L.; Pitsinos, E. N.; Hwang, C. K.; Mizuno, Y.; Saimoto, H.; Scarlato, G. R.; Suzuki, T.; Nicolaou, K. C. *J. Am. Chem. Soc.* **1993**, *115*, 7612−7624. (d) Nicolaou, K. C.; Hummel, C. W.; Nakada, M.; Shibayama, K.; Pitsinos, E. N.; Saimoto, H.; Mizuno, Y.; Baldenius, K. U.; Smith, A. L. *J. Am. Chem. Soc.* **1993**, *115*, 7625−7635. (e) Hitchcock, S. A.; Boyer, S. H.; Chu-Moyer, M. Y.; Olson, S. H.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 858−862.

(7) For isolation, see: Konishi, M.; Ohkuma, H.; Matsumoto, K.; Saitoh, K.; Miyaki, T.; Oki, T.; Kawaguchi, H. *J. Antibiot.* **1991**, *44*, 1300−1305.

(8) For synthesis, see: (a) Myers, A. G.; Fraley, M. E.; Tom, N. J.; Cohen, S. B.; Madar, D. J. *Chem. Biol.* **1995**, *2*, 33−43. (b) Shair, M. D.; Yoon, T.-Y.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1721−1723. (c) Shair, M. D.; Yoon, T. Y.; Mosny, K. K.; Chou, T. C.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1996**, *118*, 9509−9525. (d) Myers, A. G.; Tom, N. J.; Fraley, M. E.; Cohen, S. B.; Madar, D. J. *J. Am. Chem. Soc.* **1997**, *119*, 6072−6094. For synthesis of di-*O*-methyl dynemicin A, see: (e) Taunton, J.; Wood, J. L.; Schreiber, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 10378−10379.

(9) For isolation, see: Oku, N.; Matsunaga, S.; Fusetani, N. *J. Am. Chem. Soc.* **2003**, *125*, 2044−2045.

(10) For synthesis, see: (a) Nicolaou, K. C.; Kiappes, J. L.; Tian, W.; Gondi, V. B.; Becker, J. *Org. Lett.* **2011**, *13*, 3924−3927. (b) Nicolaou, K. C.; Lu, Z.; Li, R.; Woods, J. R.; Sohn, T. *J. Am. Chem. Soc.* **2015**, *137*, 8716−8719.

(11) For isolation, see: (a) Ishida, N.; Miyazaki, K.; Kumagai, K.; Rikimaru, M. *J. Antibiot.* **1965**, *18*, 68−76. (b) Koide, Y.; Ishii, F.; Hasuda, K.; Koyama, Y.; Edo, K.; Katamine, S.; Ishida, N.; Kitame, F. *J. Antibiot.* **1980**, *33*, 342−346.

(12) For synthesis, see: (a) Myers, A. G.; Liang, J.; Hammond, M.; Harrington, P.; Wu, Y.; Kuo, E. Y. *J. Am. Chem. Soc.* **1998**, *120*, 5319−5320. (b) Myers, A. G.; Glatthar, R.; Hammond, M.; Harrington, P.; Kuo, E. Y.; Liang, J.; Schaus, S. E.; Wu, Y.; Xiang, J.-N. *J. Am. Chem. Soc.* **2002**, *124*, 5380−5401. (c) Kobayashi, S.; Hori, M.; Wang, G. X.; Hirama, M. *J. Org. Chem.* **2006**, *71*, 636−644.

(13) (a) Udwary, D. W.; Zeigler, L.; Asolkar, R. N.; Singan, V.; Lapidus, A.; Fenical, W.; Jensen, P. R.; Moore, B. S. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 10376−10381. For intriguing hypothesis, see: (b) Oh, D.-C.; Williams, P. G.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Org. Lett.* **2006**, *8*, 1021−1024. (c) McGlinchey, R. P.; Nett, M.; Moore, B. S. *J. Am. Chem. Soc.* **2008**, *130*, 2406−2407. (d) Nett, M.; Moore, B. S. *Pure Appl. Chem.* **2009**, *81*, 1075−1084.

(14) (a) Jones, R. R.; Bergman, R. G. *J. Am. Chem. Soc.* **1972**, *94*, 660−661. For selected reviews on naturally occurring enediynes exhibiting Bergman cycloaromatization properties, see: (b) Smith, A. L.; Nicolaou, K. C. *J. Med. Chem.* **1996**, *39*, 2103−2117.

(15) For isolation, see: (a) Buchanan, G. O.; Williams, P. G.; Feling, R. H.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Org. Lett.* **2005**, *7*, 2731−2734. (b) Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666−673. (c) Jensen, P. R.; Williams, P. G.; Oh, D.-C.; Zeigler, L.; Fenical, W. *Appl. Environ. Microbiol.* **2007**, *73*, 1146−1152.

(16) For synthesis, see: (a) Nicolaou, K. C.; Tang, Y.; Wang, J. *Angew. Chem., Int. Ed.* **2009**, *48*, 3449−3453. (b) Nicolaou, K. C.; Wang, J.; Tang, Y.; Botta, L. *J. Am. Chem. Soc.* **2010**, *132*, 11350−11363.

(17) (a) Nicolaou, K. C. *Angew. Chem., Int. Ed.* **2014**, *53*, 9128−9140. (b) Nicolaou, K. C. *Chem. Biol.* **2014**, *21*, 1039−1045. (c) Perez, H. L.; Cardarelli, P. M.; Deshpande, S.; Gangwar, S.; Schroeder, G. M.; Vite,

DOI: 10.1021/jacs.6b04339
J. Am. Chem. Soc. 2016, 138, 8235−8246

G. D.; Borzilleri, R. M. *Drug Discovery Today* **2014**, *19*, 869−881. (d) Chari, R. V. J.; Miller, M. L.; Widdison, W. C. *Angew. Chem., Int. Ed.* **2014**, *53*, 3796−3827. (e) Wu, A. M.; Senter, P. D. *Nat. Biotechnol.* **2005**, *23*, 1137−1146.
(18) For the original version of the reaction, see: (a) Hauser, F. M.; Rhee, R. P. *J. Org. Chem.* **1978**, *43*, 178−180. (b) Kraus, G. A.; Sugimoto, H. *Tetrahedron Lett.* **1978**, *19*, 2263−2266. For a recent modification, see: (c) Nicolaou, K. C.; Lu, M.; Chen, P.; Shah, A. A. *Angew. Chem., Int. Ed.* **2015**, *54*, 12687−12691.
(19) Yamaguchi, R.; Nakazono, Y.; Kawanisi, M. *Tetrahedron Lett.* **1983**, *24*, 1801−1804.
(20) (a) Lu, Y.-F.; Harwig, C. W.; Fallis, A. G. *J. Org. Chem.* **1993**, *58*, 4202−4204. (b) Lu, Y.-F.; Harwig, C. W.; Fallis, A. G. *Can. J. Chem.* **1995**, *73*, 2253−2262. (c) Molander, G. A.; Dehmel, F. *J. Am. Chem. Soc.* **2004**, *126*, 10313−10318.
(21) (a) Friedländer, P.; Göhring, C. F. *Ber. Dtsch. Chem. Ges.* **1883**, *16*, 1833−1839. (b) Pfitzinger, W. *J. Prakt. Chem.* **1886**, *33*, 100. (c) Pfitzinger, W. *J. Prakt. Chem.* **1888**, *38*, 582−584.
(22) (a) Fujii, A.; Hashiguchi, S.; Uematsu, N.; Ikariya, T.; Noyori, R. *J. Am. Chem. Soc.* **1996**, *118*, 2521−2522. (b) Noyori, R.; Hashiguchi, S. *Acc. Chem. Res.* **1997**, *30*, 97−102.
(23) (a) Hartung, I. V.; Eggert, U.; Haustedt, L. O.; Niess, B.; Schaefer, P. M.; Hoffmann, H. M. R. *Synthesis* **2003**, 1844−1850. (b) Waser, J.; González-Gómez, J. C.; Nambu, H.; Huber, P.; Carreira, E. M. *Org. Lett.* **2005**, *7*, 4249−4252.
(24) Sonogashira, K. *J. Organomet. Chem.* **2002**, *653*, 46−49.
(25) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155−4156.
(26) For an analogous acid-mediated activation of dynemicin A, see: Sugiura, Y.; Shiraki, T.; Konishi, M.; Oki, T. *Proc. Natl. Acad. Sci. U. S. A.* **1990**, *87*, 3831−3835.
(27) Williams, D. E.; Bottriell, H.; Davies, J.; Tietjen, I.; Brockman, M. A.; Andersen, R. J. *Org. Lett.* **2015**, *17*, 5304−5307.
(28) (a) Nicolaou, K. C.; Zuccarello, G.; Ogawa, Y.; Schweiger, E. J.; Kumazawa, T. *J. Am. Chem. Soc.* **1988**, *110*, 4866−4868. (b) Nicolaou, K. C.; Ogawa, Y.; Zuccarello, G.; Kataoka, H. *J. Am. Chem. Soc.* **1988**, *110*, 7247−7248. (c) Nicolaou, K. C.; Zuccarello, G.; Riemer, C.; Estevez, V. A.; Dai, W.-M. *J. Am. Chem. Soc.* **1992**, *114*, 7360−7371.
(29) Chen, J. Ph.D. Thesis, The Scripps Research Institute, 2008.
(30) Neudeck, H. K. *Monatsh. Chem.* **1996**, *127*, 201−217.
(31) Nicolaou, K. C.; Dai, W.−M.; Tsay, S.−C.; Estevez, V. A.; Wrasidlo, W. *Science* **1992**, *256*, 1172−1178.

DOI: 10.1021/jacs.6b04339
J. Am. Chem. Soc. 2016, 138, 8235−8246