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Streamlined Total Synthesis of Shishijimicin A and Its Application to the Design, Synthesis, and Biological Evaluation of Analogues thereof and Practical Syntheses of PhthNSSMe and Related Sulfenylating Reagents

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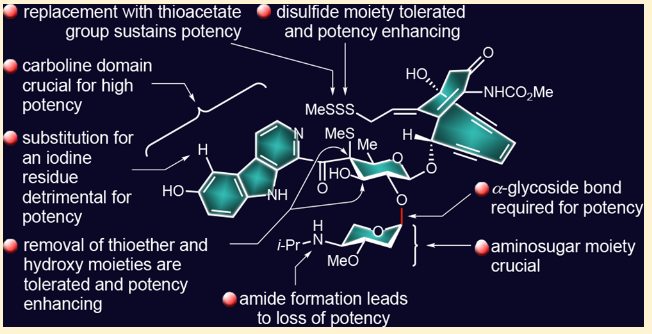
Supporting Information

ABSTRACT: Shishijimicin A is a scarce marine natural product with highly potent cytotoxicities, making it a potential payload or a lead compound for designed antibody−drug conjugates. Herein, we describe an improved total synthesis of shishijimicin A and the design, synthesis, and biological evaluation of a series of analogues. Equipped with appropriate functionalities for linker attachment, a number of these analogues exhibited extremely potent cytotoxicities for the intended purposes. The synthetic strategies and tactics developed and employed in these studies included improved preparation of previously known and new sulfenylating reagents such as PhthNSSMe and related compounds.



1. INTRODUCTION

Antibody−drug conjugates (ADCs) have become a highly sought after paradigm for targeted, personalized cancer therapies.[1] The clinically successful Mylotarg,[2] Adcetris,[3] and Kadcyla[4] gave momentum to this approach of chemotherapy that currently accounts for tens of clinical candidates in development.[5] An essential part of ADCs is the payload, a highly potent cytotoxic agent attached onto the antibody (the delivery system) of the conjugate through a chemical linker.[6] Natural products endowed with highly potent antitumor properties or their analogues provide a useful pool of compounds from which suitable payloads could be selected, as demonstrated with the three clinically used ADCs mentioned above and the several others currently in clinical trials. Shishijimicin A[7] (**1**, Figure 1) is the most potent enediyne antitumor antibiotic discovered thus far (e.g., IC_{50} = 0.48 pM against P388 leukemia cells). Shishijimicins B (**2**)[7a] and C (**3**),[7a] namenamicin (**4**),[8] calicheamicin γ_1^I (**5**),[9] and esperamicin A_1 (**6**)[10] (Figure 1) are its close relatives. By virtue of these properties and its rarity,[7a] shishijimicin A became an attractive target for total synthesis. The latter would not only serve to render the natural product available for further biological investigations but also provide an entry to designed analogues of the molecule for the same purposes. In 2015, we reported, in a preliminary communication, the first total synthesis of shishijimicin A.[7c] In this article we describe (a) an improved version of this synthesis, (b) its application to the synthesis of a series of designed shishijimicin A analogues (**7**−**16**, Figure 2), (c) a number of methodological advances regarding the preparation of the sulfenylating reagent PhthNSSMe[11] and a number of related sulfenylating reagents, (d) biological evaluation of the synthesized compounds, and (e) identification of a number of structurally simpler analogues, equally or even more potent than the natural product.

2. RESULTS AND DISCUSSION

2.1. Optimization of the Original Synthetic Strategy for the Total Synthesis of Shishijimicin A. In order to improve the efficiency and practicality of our original synthesis of shishijimicin A and its application to analogue construction, we undertook studies directed toward improvement of a number of steps and modification of certain intermediates and key building blocks along the way. Our first task became the improvement of the synthesis of the enediyne fragment of shishijimicin A (**1**), a domain common to a number of other

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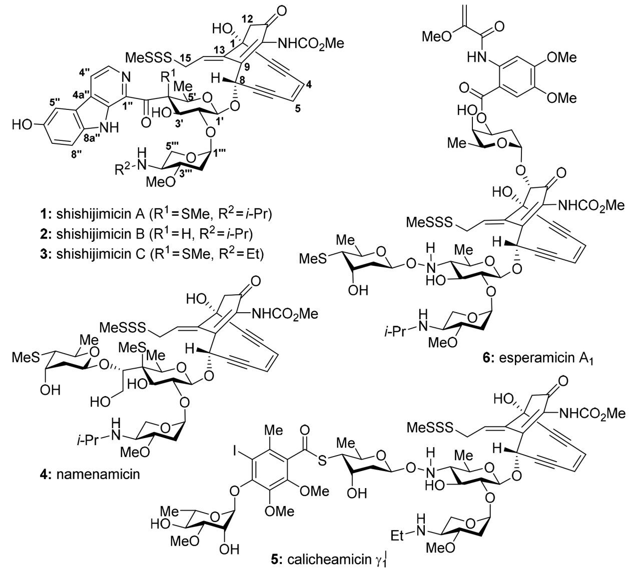
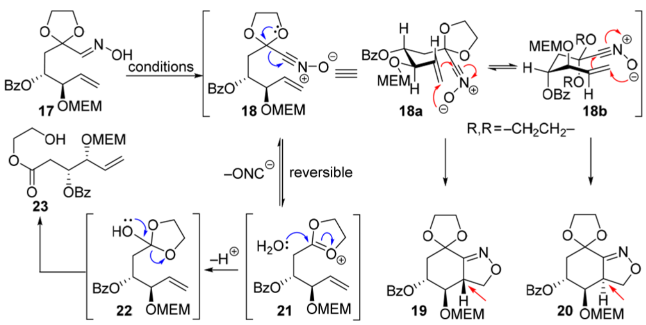


Figure 1. Representative 10-membered ring enediyne natural products: shishijimicins A–C (**1**–**3**), namenamicin (**4**), calicheamicin γ_1^I (**5**), and esperamicin A_1 (**6**).

prominent enediyne antitumor antibiotics, including namenamicin (**4**),[8] calicheamicin γ_1^I (**5**),[9] and esperamicin A_1 (**6**).[10] As shown in Table 1, we started with the intramolecular [3 + 2] dipolar cycloaddition of nitrile oxide **18**, derived from substrate **17**,[7c,9b] aiming at the optimization of the yield and selectivity for the desired product **19**. The yield of this reaction for **19** stood, at that time, at 51% with the desired product accompanied by its diastereoisomer **20** (14% yield) and fragmentation side-product **23** (20% yield) (Table 1, entry 1).[12] Changing the solvent from CH_2Cl_2 to $CHCl_3$ did not significantly alter the outcome of this reaction (Table 1, entry 2). Our speculative mechanism, depicted in Table 1 (**18** → **21** → **22** → **23**),[12] proved inspirational and crucial in guiding us to define better reaction conditions that improved the outcome of this reaction to 91% yield for desired product **19**, contaminated with only small amounts of undesired stereoisomer **20** (4% yield) and side-product **23** (<1% yield) (Table 1, entry 7). Thus, reasoning that the fragmentation of initially formed nitrile oxide intermediate **18** to ONC^- and oxonium species **21** is reversible, we hypothesized that formation of

Table 1. Optimization of Intramolecular [3 + 2] Cycloaddition



entry	conditions	yield (%) of 19	20	23
1[a]	NaClO, CH_2Cl_2/H_2O,[b] 0 °C	51	14	20
2	NaClO, $CHCl_3/H_2O$,[b] 0 °C	58	18	10
3	t-BuOCl, CH_2Cl_2, 25 °C	61	18	9
4	t-BuOCl, toluene, 25 °C	52	22	7
5	t-BuOCl, benzene, 25 °C	81	7	2
6	t-BuOCl, benzene, 5 °C	86	5	2
7[c]	t-BuOCl, benzene, 5 °C	91	4	<1

[a]Original conditions as reported in ref 13. [b]A 6.15 wt% aqueous solution of NaClO (2.0 equiv) was used. [c]The reaction was performed by adding dropwise a benzene solution of **17** to t-BuOCl in benzene.

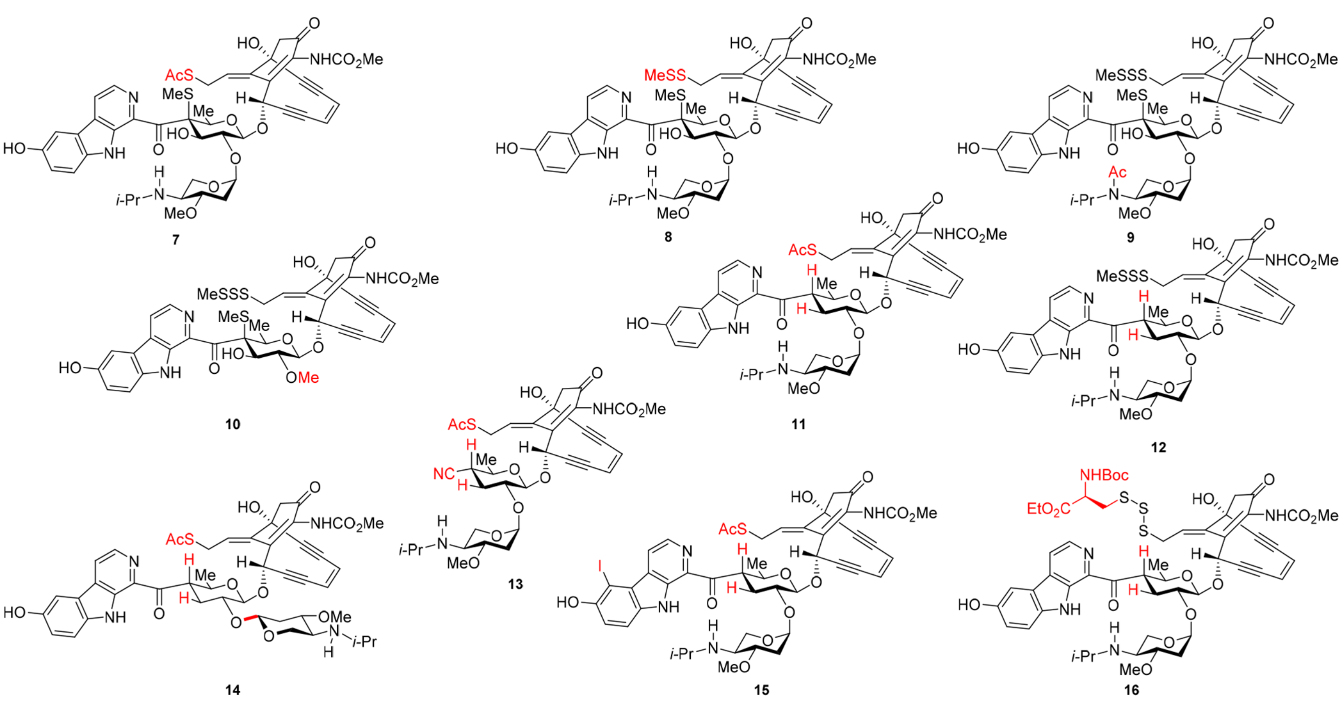


Figure 2. Synthesized shishijimicin A analogues **7**–**16**.

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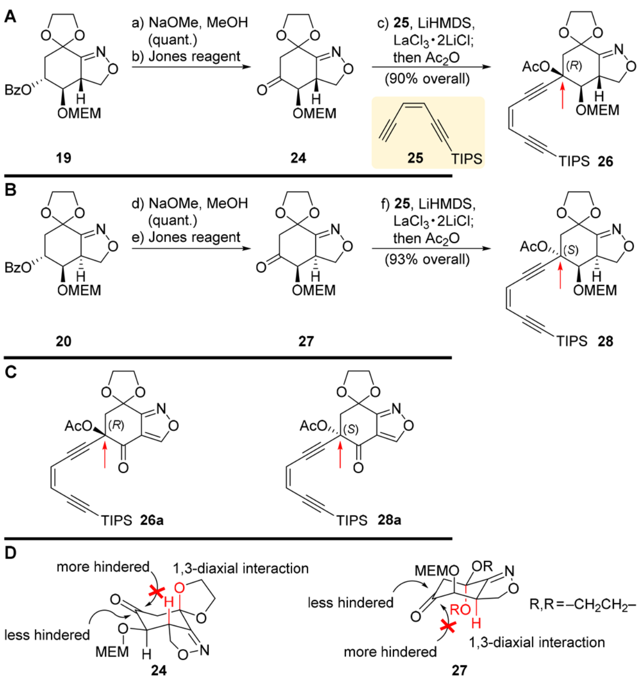
side-product **23** via species **22** could be suppressed or eliminated by changing the oxidant from NaClO (which requires aqueous media) to *t*-BuOCl (which does not require aqueous media) thereby eliminating H_2O, the culprit for the fragmentation reaction. Entries 3−7 (Table 1) show the results of this change under a variety of conditions, with entry 7 depicting the optimal protocol. The final yield improvement of this reaction was achieved through slow addition of the substrate (**17**) to a benzene solution of the oxidant (*t*-BuOCl) at the lowest possible temperature given the melting point of the solvent (5 °C).

Having significantly improved the [3 + 2] cycloaddition reaction, we then turned our attention to the installment of the enediyne moiety into the growing molecule, a process that we felt could benefit from improvement of its original version.[7c,12,13] Our initial aim was to convert both isoxazoline diastereomers **19** and **20** (Table 1) into useful advanced intermediates for further elaboration. Scheme 1A summarizes

Scheme 1. Stereospecific Addition of Enediyne 25 to Ketones 24 and 27 to Form Adducts 26 and 28[a]



[a]Reagents and conditions: (a) NaOMe (0.1 equiv), MeOH, 0 °C, 12 h, quant.; (b) Jones reagent (1.5 equiv), acetone, 0 °C, 2 h; (c) **25** (3.0 equiv), LiHMDS (2.8 equiv), $LaCl_3{\cdot}2LiCl$ (5.0 equiv), THF, −78 °C, 0.5 h; then **24**, −78 °C, 0.5 h; then Ac_2O (10.0 equiv), −78 to 25 °C, 2 h, 90% for the two steps; (d) NaOMe (0.2 equiv), MeOH, 0 °C, 12 h, quant.; (e) Jones reagent (1.5 equiv), acetone, 0 °C, 40 min; (f) **25** (4.0 equiv), LiHMDS (3.0 equiv), $LaCl_3{\cdot}2LiCl$ (5.0 equiv), THF, −78 °C, 0.5 h; then **27**, −78 °C, 0.5 h; then Ac_2O (10.0 equiv), −78 to 25 °C, 1 h, 93% for the two steps.

our studies toward this goal. Thus, debenzoylation of **19** (NaOMe, MeOH, quantitative yield) followed by Jones oxidation of the resulting alcohol furnished ketone **24**, whose reaction with the organometallic species generated from enediyne **25** and $LaCl_3{\cdot}2LiCl$,[7c,14] followed by *in situ* acetylation (Ac_2O) of the resulting tertiary alcohol, led to desired acetoxy enediyne **26**, in 90% overall yield from **19**. Pleasantly, and as proven by NMR spectroscopic analysis, the addition of the acetylide unit occurred with exclusive diastereoselectivity. The one-step introduction of the enediyne system into the emerging molecule (as compared to the stepwise original approach)[12,13] represented a further significant improvement in the synthesis of the targeted enediyne domain. In an attempt to explore the possibility of transforming the other diastereoisomer obtained from the [3 + 2] cycloaddition reaction (i.e., **20** in Scheme 1B), we exposed the latter to the same sequence of reactions as shown in Scheme 1A,B. The results included an even higher overall yield for the final product (**28** via **27**, 93% overall yield from **20**, Scheme 1B), which in contrast to the original approach,[12] was formed exclusively. Unfortunately, however, the configuration of the newly generated stereogenic center [see red arrows on structures **26** and **28** (Scheme 1A,B, respectively)] was proven to be of the opposite configuration to that obtained from isomer **19**. This assertion was based on a NOESY experiment (see the Supporting Information). Note that these intermediates (i.e., **26** and **28**) lose their other two stereocenters downstream in the pending sequence, leaving only the enediyne bearing center as the important one with regard to these intermediates (i.e., **26a** and **28a**, respectively, as shown in Scheme 1C). In that sense, while precursor intermediate **26** is destined for the target molecule, its isomeric precursor **28** is not. It could, however, serve as a useful precursor for the antipodal molecule of the natural product, if one wishes to synthesize enantiomeric shishijimicin A. This task would, of course, require inversion of all the other eight stereogenic centers of the aryl disaccharide fragment. However, the corresponding (1*R*) diastereomer of shishijimicin A could be derived from **28** simply by employing the same building blocks as those used to construct the natural product, provided all pending reactions and procedures proceed as those destined for shishijimicin A. An explanation of the exclusive diastereospecificities of these two enediyne addition reactions is provided in Scheme 1D which shows the preferred conformations of the two intermediates (**24** and **27**) based on steric considerations and the allowable trajectories of attack on the carbonyl moieties by the enediyne nucleophile. Manual molecular models of intermediates **24** and **27** indicate that the H atom attached to the angular C atom and one of the OCH_2 structural motifs of the ketal should be in axial positions, thereby blocking the enediyne approach from the same side due to 1,3-diaxial interaction. The isoxazoline moieties lie in equatorial positions exerting minimal steric bias, and thus allowing the enediyne to approach from the less hindered face of the carbonyl group (see Scheme 1D).

Having successfully improved the installment of the enediyne structural motif into the growing molecule, we turned our attention to optimizing the intramolecular ring closure of the acetylenic aldehyde as the next desired stage (see Scheme 2 and Tables 2 and 3). Beginning with isoxazole **29** [synthesized from **26** through a high-yielding 6-step sequence (77% overall yield, Scheme 2) as reported in 2015],[7c] we first sought for a more practical procedure for the reduction of the isoxazole structural motif within **29** (Scheme 2). Reductive rupture of the isoxazole ring embedded within **29** could be achieved more conveniently than before[12,13] through the addition of Fe powder in a solution of this substrate in a mixture of EtOH and aqueous NH_4Cl.[7c,15,16] Although these conditions delivered required amino aldehyde **30** in a single step and high yield (83%) on a 100 mg scale, they proved capricious and difficult to reproduce,[17] especially on a larger

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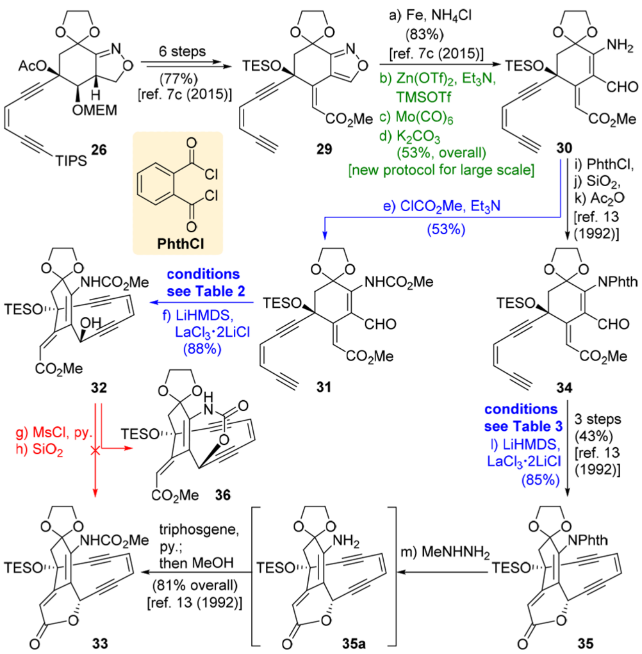
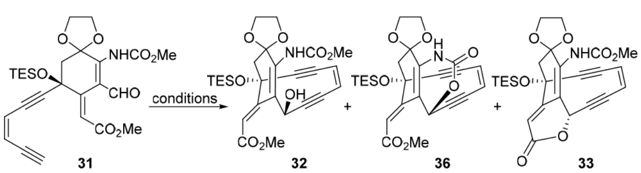
Scheme 2. Synthesis of Cyclized Enediyne Lactone 33[a]



[a]Reagents and conditions: (a) Fe (25 equiv), NH_4Cl (50 equiv), $EtOH/H_2O$ (1:1, v/v), 60 °C, 8 h, 83%; (b) TMSOTf (1.5 equiv), Et_3N (2.0 equiv), $Zn(OTf)_2$ (0.025 equiv), CH_2Cl_2, −20 °C, 10 min, then **29**, −20 to 25 °C, 12 h; (c) $Mo(CO)_6$ (1.0 equiv), $MeCN/H_2O$ (5:1, v/v), 80 °C, 1.5 h; (d) K_2CO_3 (1.0 equiv), MeOH/THF (2:1, v/v), 0 °C, 2 h, 53% for the three steps; (e) $ClCO_2Me$ (10.0 equiv), Et_3N (20 equiv), CH_2Cl_2, 25 °C, 0.5 h, 53%; (f) LiHMDS (3.0 equiv), $LaCl_3{\cdot}2LiCl$ (5.0 equiv), THF, −20 °C, 5 min, 88%; (g) MsCl (5.0 equiv), pyridine (10.0 equiv), CH_2Cl_2, 0 °C, 15 min; (h) SiO_2, CH_2Cl_2, 25 °C, 2 h; (i) PhthCl (1.5 equiv), pyridine (4.0 equiv), $MeNO_2$, 0 °C, 0.5 h; (j) SiO_2, CH_2Cl_2, 25 °C, 2 h; (k) Ac_2O (excess), 25 °C, 1 h, 81% for the three steps; (l) LiHMDS (3.0 equiv), $LaCl_3{\cdot}2LiCl$ (2.0 equiv), THF, −78 °C, 1 h, 85%; (m) $MeNHNH_2$ (10.0 equiv), PhH, 25 °C, 0.5 h; then triphosgene (3.0 equiv), pyridine (30 equiv), CH_2Cl_2, 0 °C, 1 h, MeOH, 0 °C, 1 h, 81% for the two steps.

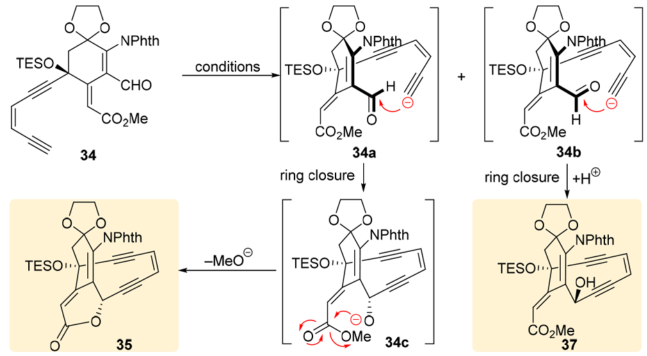
Table 2. Enediyne Aldehyde Cycloaddition Reaction within Intermediate 31



entry	conditions	yield (%) of 32	36	33
1	KHMDS, toluene, −78 °C	0	87	0
2	LiHMDS, toluene, −78 °C	0	74	0
3	LiHMDS, THF, −40 °C	5	83	0
4	LiHMDS, $CeCl_3$, THF, −20 °C	78	13	0
5	LiHMDS, $LaCl_3{\cdot}2LiCl$, THF, −20 °C	88	9	0

scale. Employment of $Mo(CO)_6$ as the reducing agent[18] on the free terminal alkyne substrate **29** led to substantial side-product formation, making this substrate unsuitable for these conditions. Eventually, a sequence involving trimethylsilylation [TMSOTf, Et_3N, $Zn(OTf)_2$] of offending terminal alkyne of **29**,[19] followed by reductive cleavage of the isoxazole N−O bond [$Mo(CO)_6$][12,13,18] and TMS group removal (K_2CO_3, MeOH), proved to be reliable and efficient, securing multigram quantities of vinylogous formamide **30** in good overall yield (53%) from isoxazole **29** as shown in Scheme 2.

Table 3. Optimization of Enediyne Cycloaddition Reaction within Intermediate 34



entry	conditions	yield (%) of 35	37
1[a]	KHMDS, toluene, −95 °C	5	48
2	KHMDS, THF, −78 °C	6	71
3	LiHMDS, THF, −78 °C	8	68
4	LiHMDS, $CeCl_3$, THF, −78 °C	73	11
5[b]	LiHMDS, $LaCl_3{\cdot}2LiCl$, THF, −78 °C	85	10

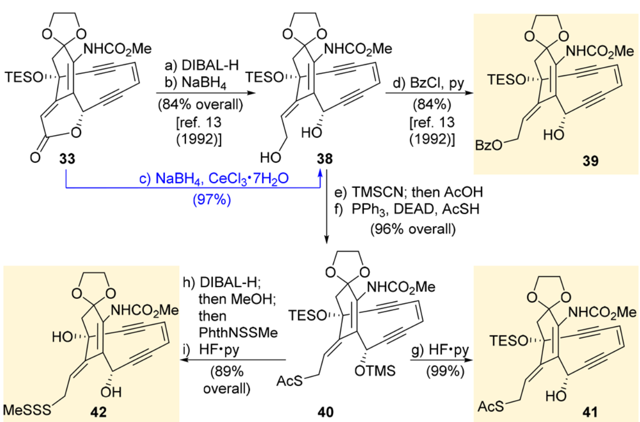
[a]Original conditions as reported in ref 13. [b]Conditions as reported in ref 7c.

We then focused our efforts on developing a more efficient transformation of **30** to **33** (Scheme 2) which previously required six steps and proceeded in 28% overall yield.[13] Our first attempt sought to circumvent the intermediacy of the phthalimide intermediate and required direct conversion of amino compound **30** to methyl carbamate **31**, an operation that proceeded in 53% yield, upon exposure of the former to $ClCO_2Me$ and Et_3N. Unfortunately, subsequent efforts to convert intermediate **31** directly to the desired cyclization product **33** under various basic conditions (see Table 2) were met with failure, primarily due to the tendency of the initially formed hydroxy intermediate **32** to undergo cyclization to the 6-membered ring carbamate **36** under the strong basic conditions employed (Table 2, entries 1−3). In the presence of a Lewis acid (e.g., LiHMDS, $CeCl_3$ or LiHMDS, $LaCl_3{\cdot}2LiCl$), however, β-hydroxy methyl ester **32** could be isolated in 78% (Table 2, entry 4) or 88% (Table 2, entry 5) yield, respectively. To our disappointment, this intermediate failed to be converted to the targeted product **33** through a two-step sequence [(i) MsCl, py.; (ii) SiO_2] reported for the inversion of the β-hydroxyl group on a similar system.[12,13] Instead, a rapid generation of cyclic carbamate **36** was observed under the indicated reaction conditions, presumably due to the basicity of the pyridine employed.

Another innovation along the route from **34** to **33** via **35** and **35a** (see Scheme 2) was discovered and optimized from a separate study directed toward a one-step conversion of formyl phthalimide **34** to intermediate lactone **35** as shown in Table 3. Thus, while the use of KHMDS or LiHMDS as a base for the cyclization of acetylenic aldehyde **34** resulted in dominant formation of β-hydroxy phthalimide **37** through, anionic species **34b** (Table 3, entries 1−3), the employment of a Lewis acid (e.g., $CeCl_3$ or $LaCl_3{\cdot}2LiCl$) as an additive favored the formation of desired lactone **35** (Table 3, entries 4 and 5) with LiHMDS, $LaCl_3{\cdot}2LiCl$ furnishing the highest yield (i.e., 85%). Accomplishing direct conversion of **34** to targeted intermediate **35** (see Scheme 2) via intermediate species **34a** and **34c** (see Table 3), this new sequence shortens the route from **30** to **33** (see Scheme 2) from eight to six steps and improves its overall yield from 28 to 56%. Notably, the overall number of steps starting from oxime **17** (Table 1) to advanced intermediate **33** (see Scheme 2) has been shortened from 20 to 19 steps, while the overall yield was significantly improved from 1.8%, from the original approach reported in 1992,[12,13] to 16%.

With multigram quantities of key enediyne building block **33** readily available, we turned our attention to the preparation of differently substituted enediyne fragments so as to gain flexibility in the ensuing coupling reactions toward shishijimicin A and other naturally occurring enediyne antitumor antibiotics carrying the same enediyne "warhead" [e.g., namenamicin (**4**),[8] calicheamicin γ_1^I (**5**),[9] and esperamicin A_1 (**6**),[10] and their designed analogues]. As shown in Scheme 3,

Scheme 3. Syntheses of Enediyne Glycosyl Acceptors 39, 41, and 42[a]

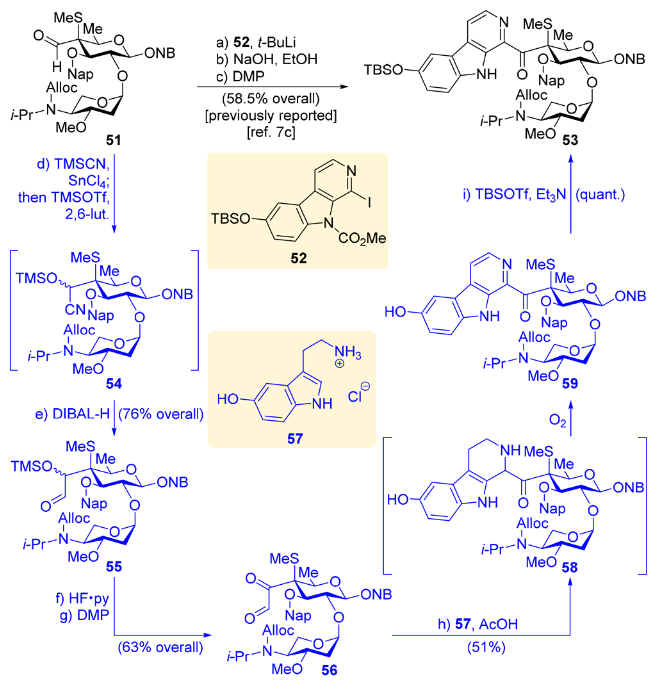


[a]Reagents and conditions: (a) DIBAL-H (3.0 equiv), CH_2Cl_2, −78 °C, 0.5 h, 95%; (b) $NaBH_4$ (excess), MeOH, 0 °C, 1 h, 88%; (c) $NaBH_4$ (2.0 equiv), $CeCl_3{\cdot}7H_2O$ (3.0 equiv), MeOH, 25 °C, 1 h, 97%; (d) BzCl (2.0 equiv), pyridine (3.0 equiv), CH_2Cl_2, −15 °C, 1 h, 84%; (e) TMSCN (excess), 25 °C, 0.5 h; then AcOH (5.0 equiv), THF/H_2O (5:1, *v*/*v*), 0 °C, 0.5 h; (f) PPh_3 (5.0 equiv), DEAD (5.0 equiv), AcSH (5.0 equiv), THF, 0 °C, 5 min, 96% for the two steps; (g) HF·py/THF (1:20, *v*/*v*), 0 °C, 0.5 h, 99%; (h) DIBAL-H (3.0 equiv), −78 °C, 0.5 h; then MeOH, −78 °C, 20 min; then PhthNSSMe (4.0 equiv), −78 to 25 °C, 1 h; (i) HF·py/THF (1:20, *v*/*v*), 0 to 25 °C, 1.5 h, 89% for the two steps.

we first targeted previously synthesized allylic benzoate **39**,[9b] and new allylic thioacetate **41**,[7c] and previously known allylic methyl trisulfide **42**.[9c] The previously reported two-step reduction of lactone **33** (DIBAL-H; $NaBH_4$, 84% overall yield)[13] was successfully replaced with the one-step Luche reduction ($NaBH_4$, $CeCl_3{\cdot}7H_2O$) that proceeded in superior yield (97%) to afford diol **38**.[7c,20] The latter compound served as a common precursor to all three enediyne fragments shown in Scheme 3 (i.e., **39**, **41**, and **42**). Thus, selective benzoylation of **38** (BzCl, py., 84%) yielded the previously synthesized enediyne glycosyl acceptor **39**,[9b] whereas sequential exposure of **38** to TMSCN, AcOH, and then PPh_3, DEAD, and AcSH led to fully and orthogonally protected precursor **40**, in 96% overall yield for the three steps as shown in Scheme 3.[7c] Finally, precursor **40** was diverted to thioacetate fragment **41** through selective desilylation of the secondary TMS ether (HF·py, 99% yield), and to methyl trisulfide fragment **42** through a sequence involving cleavage of the thioacetate moiety (DIBAL-H; then MeOH), methyl trisulfide formation (PhthNSSMe),[11] and desilylation (HF·py) of the remaining secondary and tertiary silyl ethers, in 89% overall yield as summarized in Scheme 3. The availabilities of the last two more advanced intermediates (i.e., **41** and **42**) would allow us to test new protocols for the final and challenging coupling of the glycosyl donor and acceptor as we shall discuss below.

With practical and efficient processes for the synthesis of glycosyl acceptors **39**, **41**, and **42** in hand, we turned our attention to optimizing the construction of the β-carboline-disaccharide fragment (i.e., **53**, see Scheme 4) of shishijimicin

Scheme 4. Construction of β-Carboline Disaccharide 53 via Pictet−Spengler Condensation[a]



[a]Reagents and conditions: (a) **52** (3.0 equiv), *t*-BuLi (6.0 equiv), THF, −78 °C, 0.5 h; then **51** (1.0 equiv), −78 to −35 °C, 40 min, 86% (ca. 1:1 *dr*); (b) NaOH (3.0 equiv), EtOH, 0 to 25 °C, 2.5 h; (c) DMP (1.1 equiv), $CHCl_3$, 0 to 35 °C, 10 min, 68% for the two steps; (d) TMSCN (3.0 equiv), $SnCl_4$ (1.5 equiv), CH_2Cl_2, −78 to 0 °C, 0.5 h; then TMSOTf (2.0 equiv), 2,6-lutidine (3.0 equiv), 0 to 25 °C, 2 h; (e) DIBAL-H (3.0 equiv), CH_2Cl_2, −78 °C, 1.5 h, 76% for the two steps; (f) HF·py/THF (1:20, *v*/*v*), 0 to 25 °C, 2 h; (g) DMP (1.5 equiv), CH_2Cl_2, 25 °C, 0.5 h, 63% for the two steps; (h) **57** (4.0 equiv), AcOH, 60 °C, 1.5 h; then O_2, 1 h, 51% (i) TBSOTf (1.1 equiv), Et_3N (2.0 equiv), CH_2Cl_2, 0 °C, 5 min, quant.

A (**1**). Our optimization studies began with reinvestigation of the conversion of glycal **43** to glycoside **45b** (β-anomer, Table 4), via epoxide **44** (Table 4), needed to build disaccharide **51** (see Table 5). As reported in our preliminary communication,[7c] the preparation of **45b** from glycal **43** via epoxide **44** called upon the original Danishefsky conditions[21] that employed $ZnCl_2$ as the Lewis acid to activate the epoxide moiety (Table 4, entry 1).[7c] In that and the other experiments reported herein and summarized in Table 4, glycal **43** was exposed to *in situ* generated dimethyl dioxirane (DMDO)

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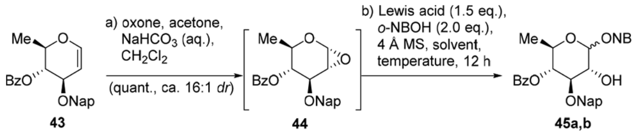
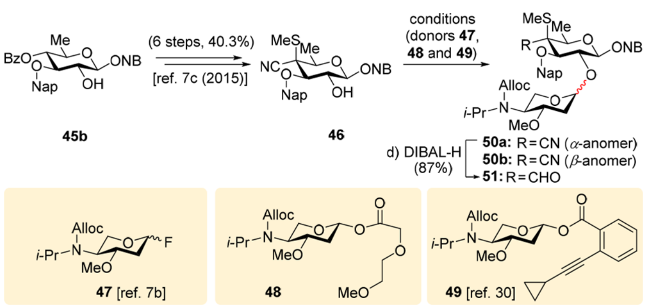
Table 4. Lewis Acid Optimization of Glycosylation of 1,2-Anhydro-6-deoxy-glucose 44[a]



entry	Lewis acid	solvent	ϑ (°C)	yield (%)[b] of 45b	45a
1[c]	$ZnCl_2$	THF	−78 to 25	54	11
2	$ZnBr_2$	THF	−78 to 25	70	5
3	$BF_3{\cdot}Et_2O$	THF	−78 to 25	60	15
4	$AlCl_3$	THF	−78 to 0	44	40
5	$InCl_3$	THF	−78 to 25	81	<1
6	$Ph_3PAuOTf$[d]	CH_2Cl_2	−78 to 25	46	28
7	$Ph_3PAuNTf_2$[d]	CH_2Cl_2/THF	−78 to 25	42	50
8	$In(OTf)_3$	CH_2Cl_2	−78 to 25	41	33
9	$Bi(OTf)_3$	CH_2Cl_2	−78 to 25	43	35

[a]Reaction conditions: (a) oxone (5.0 equiv), acetone (8.0 equiv), $NaHCO_3$ (25 equiv), H_2O/CH_2Cl_2 (3:4, *v*/*v*), 0 °C, 4 h, quant.; (b) **44** (0.5 mmol), *o*-nitrobenzyl alcohol (*o*-NBOH, 1.0 mmol), Lewis acid (0.75 mmol), 4 Å MS (1.7 g), solvent (3.0 mL), unless otherwise noted. [b]Isolated yields of indicated anomers. [c]Conditions as reported in ref 7c. [d]A 0.2 equiv amount of Lewis acid was added.

Table 5. Glycosylation of Alcohol 46 with Donors 47–49[a]



entry	conditions	yield (%)[b] of 50a (α-glycoside)	50b (β-glycoside)
1[c]	**47** (2.0 equiv), $AgClO_4$ (2.5 equiv), $SnCl_2$ (2.5 equiv), THF, −78 to 25 °C, 12 h	85	9
2	**47** (2.0 equiv), $AgClO_4$ (2.5 equiv), Cp_2HfCl_2 (2.5 equiv), Et_2O, −78 to 25 °C, 12 h	34	62
3	**48** (4.0 equiv), $AgClO_4$ (4.0 equiv), $SnCl_4$ (4.0 equiv), Et_2O, −78 to 25 °C, 12 h	16	48
4	**49** (2.0 equiv), $Ph_3PAuOTf$ (0.2 equiv), CH_2Cl_2, 0 °C, 12 h	13	61
5	**49** (2.0 equiv), $Ph_3PAuNTf_2$ (0.2 equiv), CH_2Cl_2, 0 °C, 12 h	22	43

[a]Glycosyl acceptor **46** (0.1 mmol, 1.0 equiv) was used as a limiting reagent for each entry. [b]Isolated yield based on **46**. [c]Conditions as reported in ref 7c. [d]DIBAL-H (3.0 equiv), CH_2Cl_2, −78 °C, 45 min, 87%.

(acetone, oxone, aqueous $NaHCO_3$, CH_2Cl_2, 0 °C, quant., ca. 16:1 *dr*), and the resulting epoxide (**44**) was used crude without purification. Given that our original conditions with $ZnCl_2$ as a Lewis acid promoter (Table 4, entry 1) led to a mixture of anomers of the glycosylated products [**45b** (54% yield), **45a** (11% yield)],[7c] and in order to make the reaction more stereoselective and improve its yield, we focused on varying the Lewis acid promoter, the solvent (Table 4), and in one case the temperature (Table 4, entry 4). The improvement of diastereoselectivity was important for carrying out subsequent steps with homogeneous material, rather than anomeric mixtures. We first examined Lewis acids with reasonable solubilities in THF such as those shown in entries 2–5 (i.e., $ZnBr_2$, $BF_3{\cdot}Et_2O$, $AlCl_3$, and $InCl_3$, Table 4). Thus, switching the promoter from $ZnCl_2$ (Table 4, entry 1) to $ZnBr_2$[22] (Table 4, entry 2) resulted in higher selectivity (*β*-anomer: 70% yield, *α*-anomer: 5% yield) and better yield (75% combined yield vs 65% combined yield for $ZnCl_2$, Table 4, entry 1). While the stronger Lewis acids $BF_3{\cdot}Et_2O$[23] (Table 4, entry 3) and $AlCl_3$[24] (Table 4, entry 4) gave lower selectivities than $ZnCl_2$ (Table 4, entry 1), they proved more efficient in terms of combined yield, namely, 75 and 85%, respectively. We then considered $InCl_3$,[25] expecting that indium's larger than zinc's and aluminum's ionic radius would influence the anomeric selectivity of the glycosylation reaction with *o*-NBOH. As shown in Table 4 (entry 5), the $InCl_3$-facilitated glycosylation of **44** with *o*-NBOH proceeded in high yield (81%) and excellent anomeric selectivity for the *β*-anomer (<1% of the *α*-anomer). The gold catalysts $Ph_3PAuOTf$[26] and $Ph_3PAuNTf_2$[27] were also tested[28] and interestingly were found to perform well in terms of combined yields [entries 6 (74%) and 7 (92%), respectively] but failed in terms of anomeric selectivity, with $Ph_3PAuNTf_2$ reversing the anomeric ratio in favor of the undesired *α*-anomer while outperforming all the promoters and catalysts tested in terms of combined yield (92%, Table 4, entry 7).

With an improved and scalable synthesis of glycoside **45b** developed (see Table 4), we then proceeded to its conversion to methylthio cyanide **46**, a task accomplished through a high-yielding six-step sequence as we previously described[7c] (40.3% overall yield) as summarized in Table 5. The next challenge was the glycosylation of this, rather complex, glycosyl acceptor (i.e., **46**) with its Alloc-protected aminosugar partner in the form of glycosyl donors **47**,[7b] **48**,[29] or **49**[30] (Table 5) with the desired goal of selectively obtaining *α*-glycoside **50a**[31] (Table 5) as needed for constructing shishijimicin A. To this end, glycosyl donors **47**–**49** (for preparations, see the Supporting Information and refs 7b, 29, and 30) were reacted with 2-hydroxy glycosyl acceptor **46** under a variety of conditions as shown in Table 5. Thus, **46** and glycosyl fluoride **47** were allowed to react in THF at −78 to 25 °C in the presence of $AgClO_4$ and $SnCl_2$,[32] furnishing desired *α*-glycoside **50a** in 85% yield, together with only 9% of the *β*-glycoside (**50b**), the two anomers being separated chromatographically (Table 5, entry 1).[7c] The desired *α*-anomer was then converted to disaccharide aldehyde **51** (DIBAL-H, 87% yield). As seen in Table 5, adoption of glycosyl donors **48** and **49** and of appropriate conditions for their coupling with glycosyl acceptor **46**, even though leading to good to excellent combined yields of the *α*,*β* mixture of disaccharide anomers (**50a**/**b**, Table 5, entries 2–5), failed to improve the *α*-glycoside selectivity beyond that observed with glycosyl fluoride **47** under the $AgClO_4$–$SnCl_2$ activation conditions (Table 5, entry 1). Interestingly, replacing $SnCl_2$ with Cp_2HfCl_2[33] (Table 5, entry 2) as a partner to $AgClO_4$ in this glycosylation reaction not only resulted in a similarly impressive combined yield of the *α*,*β*-disaccharide (mixture

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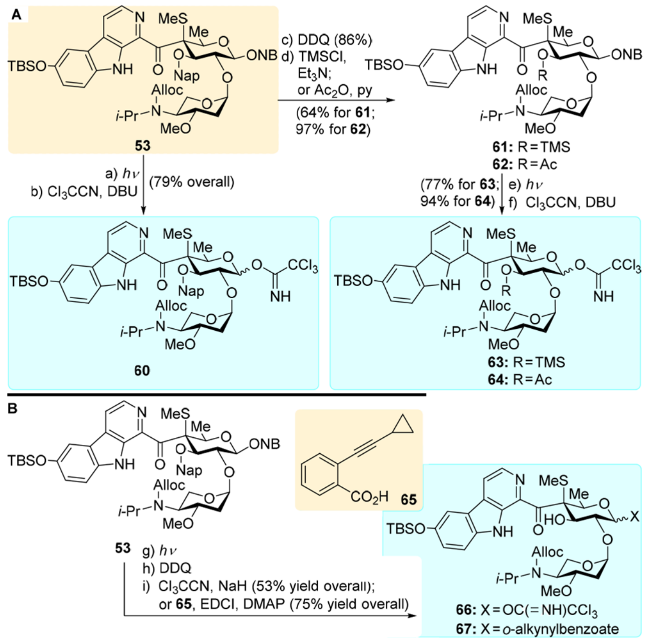
50a/**50b**) but also in reversal of the α,β-anomeric selectivity (α/β ca. 34:62, Table 5, entry 2).

With an efficient synthesis of disaccharide aldehyde **51** developed, we proceeded with the installation of the β-carboline moiety onto the growing molecule. Scheme 4 summarizes two approaches through which this objective was achieved. The first access to targeted disaccharide-carboline domain **53** proceeded through a three-step sequence involving lithiation of **52** through iodide-lithium exchange (t-BuLi) followed by sequential addition of aldehyde **51** and saponification/decarboxylation (NaOH, EtOH) to afford the corresponding secondary alcohol, whose oxidation with DMP yielded ketone product **53**, in 58.5% overall yield as previously communicated[7c] and summarized in Scheme 4. At this point we also opted to attempt a presumably biomimetic approach[34] to targeted β-carboline fragment **53** starting from disaccharide aldehyde **51**, as shown in Scheme 4 (blue sequence). Thus, exposure of aldehyde **51** to TMSCN in the presence of $SnCl_4$ followed by addition of TMSOTf/2,6-lutidine furnished corresponding cyanohydrin TMS-derivative (**54**) of the initially formed cyanohydrin. The latter was reduced with DIBAL-H to give aldehyde **55** (76% yield for the two steps, mixture of inconsequential diastereoisomers), whose sequential desilylation (HF·py) and DMP oxidation furnished dicarbonyl compound **56**, in 63% overall yield as shown in Scheme 4. The latter served as a precursor to carboline disaccharide **59** through a cascade event involving sequential condensation with serotonin hydrochloride (**57**, Pictet–Spengler reaction[35]) in AcOH (60 °C), followed by spontaneous dehydrogenation/aromatization, via tetrahydro-β-carboline intermediate **58**, through the action of O_2 (51% overall yield).[36] The so-obtained 6″-hydroxy carboline (**59**) was then silylated (TBSOTf, Et_3N) to afford targeted carboline TBS-ether **53** in quantitative yield as shown in Scheme 4.

In order to explore the attachment of the carboline-disaccharide domain **53** onto the enediyne core of shishijimicin A, we synthesized a number of glycosyl donors (i.e., **60**, **63**, **64**, **66**, and **67** as depicted in Scheme 5A,B). Thus, photoinduced ($h\nu$) cleavage of the NB group from **53** followed by installation of the trichloroacetimidate moiety (Cl_3CCN, DBU) led to carbohydrate donor **60** in 79% overall yield as depicted in Scheme 5A. Donors **63** and **64**, in which the Nap group was replaced with a TMS or an Ac group, were constructed from **53** via intermediates **61** and **62**, respectively, as shown in Scheme 5A. Thus, removal of the Nap protecting group from **53** by treatment with DDQ (86%) followed by silylation (TMSCl, Et_3N) or acetylation (Ac_2O, py, DMAP) of the resulting alcohol substrate furnished **61** (64% yield) or **62** (97% yield), respectively. The latter compounds were converted to the corresponding trichloroacetimidates **63** and **64** in 77 and 94% yields, respectively, as shown in Scheme 5A. Trichloroacetimidate **66** lacking the Nap protecting group was also prepared from **53** as shown in Scheme 5B. Thus, sequential removal of the NB ($h\nu$) and Nap (DDQ) protecting groups followed by trichloroacetimidate formation (Cl_3CCN, NaH) produced carbohydrate donor **66** in 53% overall yield for the three steps from **53**. For the purposes of employing, in addition to Schmidt glycosylations, gold catalysis[37] in the final coupling step, hydroxy glycosyl donor **67** was synthesized from **53** through a sequence involving NB removal ($h\nu$), Nap deprotection (DDQ), and esterification with o-alkynylbenzoic acid **65** (EDCI, i-Pr_2NEt, DMAP) in 75% overall yield for the three steps as shown in Scheme 5B.[38] Both **66** and **67** are

Scheme 5. Syntheses of Disaccharide Trichloroacetimidates 60, 63, 64, and 66 and *o*-Alkynylbenzoate 67 as Glycosyl Donors[a]



[a]Reagents and conditions: (a) $h\nu$, THF/H_2O (10:1, v/v), 4.5 h; (b) Cl_3CCN/CH_2Cl_2 (1:10, v/v), DBU (1.0 equiv), 0 °C, 1.5 h, 79% for the two steps; (c) DDQ (2.0 equiv), CH_2Cl_2/H_2O (10:1, v/v), 25 °C, 4 h, 86%; (d) TMSCl (2.0 equiv), Et_3N (3.0 equiv), CH_2Cl_2, 0 °C, 3 h; or Ac_2O (2.0 equiv), pyridine (3.0 equiv), DMAP (0.1 equiv), CH_2Cl_2, 25 °C, 7 h, 64% for **61**, 97% for **62**; (e) $h\nu$, THF/H_2O (10:1, v/v), 0 °C, 4.5 h; (f) Cl_3CCN/CH_2Cl_2 (1:10, v/v), DBU (1.0 equiv), 0 °C, 1.5 h, 77% for **63** from **61**, 94% for **64** from **62** over the two steps; (g) $h\nu$, THF/H_2O (10:1, v/v), 0 °C, 4.5 h; (h) DDQ (2.5 equiv), CH_2Cl_2/H_2O (10:1, v/v), 30 °C, 1.5 h; (i) NaH (2.0 equiv), Cl_3CCN/CH_2Cl_2 (1:2, v/v), 25 °C, 5 min; or **65** (2.0 equiv), EDCI (2.0 equiv), i-Pr_2NEt (2.0 equiv), DMAP (1.0 equiv), CH_2Cl_2, 25 °C, 24 h, 53% for **66**, 75% for **67** over the three steps.

lacking the Nap protecting group, for the latter bulky moiety was shown to exert an inhibiting role in the pending glycosylation reaction. Incidentally, as we will see below, it was for the same reason that the Nap group was exchanged for the TMS and Ac groups in carbohydrate donors **63** and **64**, respectively, as mentioned above.

With both the enediyne fragments (**39**, **41**, and **42**, Scheme 3) and carboline-disaccharide donors (**60**, **63**, **64**, **66**, and **67**, Scheme 5) readily available, we were in a position to address the glycosylation reaction that would join them together for the final drive toward shishijimicin A (**1**). This objective proved challenging, as we soon realized. Table 6 summarizes some of our attempts to accomplish this goal.[39] Reaction of hydroxy enediyne fragment **39** with carbohydrate donor **60** under $BF_3\cdot Et_2O$ (3.5 equiv) conditions[9b] failed to produce any of the desired coupling product (Table 6, entry 1). Reasoning that the bulky Nap group was responsible for the intransigence of this substrate, we prepared (as shown in Scheme 5A) and employed trimethylsilyl (TMS) and acetyl (Ac) counterparts of **60**, namely, donors **63** and **64**. Much to our disappointment, and just like their precursor **60**, these intermediates resisted coupling under the same conditions as those used with **60**, with the carbohydrate acceptor (i.e., enediyne **39**) being recovered unchanged (Table 6, entries 2 and 3). We then

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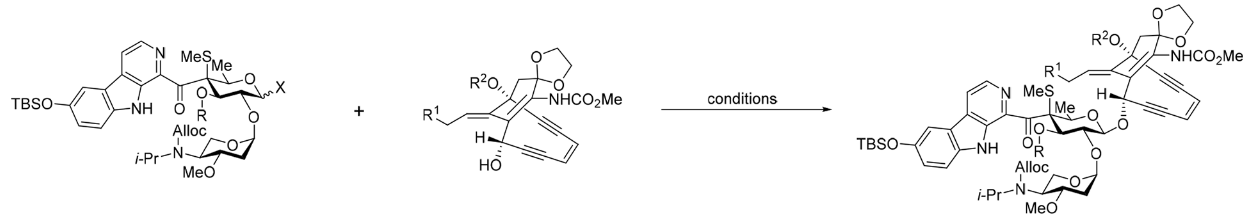
Table 6. Glycosylation of Disaccharide Donors 60, 63, 64, 66, and 67 with Enediyne Acceptors 39, 41, and 43



60: R = Nap, X = OC(=NH)CCl$_3$
63: R = TMS, X = OC(=NH)CCl$_3$
64: R = Ac, X = OC(=NH)CCl$_3$
66: R = H, X = OC(=NH)CCl$_3$
67: R = H, X = o-alkynylbenzoate

39: R^1 = OBz, R^2 = TES
41: R^1 = SAc, R^2 = TES
42: R^1 = SSSMe, R^2 = H

68: R = H, R^1 = OBz, R^2 = TES
69: R = H, R^1 = SAc, R^2 = TES

entry	donor[a]	acceptor (equiv)	conditions[b]	product	yield (%)[c]
1	**60**	**39** (1.6)	$BF_3 \cdot Et_2O$ (3.5 equiv), CH_2Cl_2, −78 to −40 °C	—[d]	—
2	**63**	**39** (1.6)	$BF_3 \cdot Et_2O$ (3.5 equiv), CH_2Cl_2, −78 to −40 °C	—[d]	—
3	**64**	**39** (1.6)	$BF_3 \cdot Et_2O$ (3.5 equiv), CH_2Cl_2, −78 to −40 °C	—[d]	—
4	**68**	**39** (1.6)	$BF_3 \cdot Et_2O$ (3.5 equiv), CH_2Cl_2, −78 to −40 °C	**68**	15
5	**66**	**39** (1.6)	TMSOTf (3.5 equiv), CH_2Cl_2, −78 to −40 °C	**68**	10
6[f]	**66**	**41** (1.6)	$BF_3 \cdot Et_2O$ (3.5 equiv), CH_2Cl_2, −78 to −40 °C	**69**	26
7	**66**	**42** (1.6)	$BF_3 \cdot Et_2O$ (3.5 equiv), CH_2Cl_2, −78 to −40 °C	—[e]	—
8	**67**	**41** (3.0)	$Ph_3PAuOTf$ (2.0 equiv), CH_2Cl_2, 0 to 20 °C	—[d]	—
9	**67**	**41** (3.0)	$Ph_3PAuNTf_2$ (2.0 equiv), CH_2Cl_2, 0 to 20 °C	**69**	<5

[a]Glycosyl donor (1.0 equiv) was used as a limiting reagent for each entry. [b]In each reaction, 4 Å molecular sieves were used. [c]Isolated yield of indicated product. [d]No desired product observed; glycosyl acceptor recovered. [e]No desired product observed; glycosyl acceptor decomposed. [f]Conditions as reported in ref 7c.

decided to use glycosyl donor **66**, which carries no protecting group on its C3-hydroxyl group. As seen from entry 4 (Table 6), the desired product (**68**) was obtained, but only in low yield (15%). Employing TMSOTf as a Lewis acid promoter[40] in this glycosylation reaction (Table 6, entry 5) furnished product **68** in an even lower yield than the previous experiment with $BF_3 \cdot Et_2O$, due to a rapid formation of the TMS-ether of carbohydrate acceptor **39**,[41] forcing us to switch back to $BF_3 \cdot Et_2O$ as the preferred promoter. This time, we used thioacetate enediyne fragment **41** as the glycosyl acceptor with hydroxy trichloroacetimidate donor **66** and obtained an improved yield (26%, Table 6, entry 6).[7c] The employment of methyltrisulfide glycosyl acceptor **42**[9c] with donor **66** under the same conditions led to no product however, with the glycosyl acceptor (**42**) decomposing under the conditions of the reaction (Table 6, entry 7). Exploring the possibilities of success under gold-promoted conditions using coupling partners **67** and **41** and $Ph_3PAuOTf$[26] (Table 6, entry 8) and $Ph_3PAuNTf_2$[27] (Table 6, entry 9) did not prove fruitful either, with the latter catalyst furnishing <5% of the desired product (**69**), while the former catalyst led to no product, presumably due to complexation of Au(I) to the pyridine moiety of the β-carboline structural motif, thereby, deactivating the disaccharide donor and thus inhibiting the coupling reaction. There is certainly room for improvement in this glycosylation reaction, which is made so intransigent, no doubt by the complexity of the partners involved and their unusual structural motifs. In retrospect, we realized that the MeS group on carbohydrate donors **60**, **63**, **64**, **66**, and **67** (Table 6) resided most likely in an axial position hindering the formation of the desired β-glycoside (formed in yields of ≤ 26%), a speculation supported by subsequent experiments in our syntheses of shishijimicin A analogues (section 2.2). Thus, the glycosylation reaction of the glycosyl donor derived from **88** (Scheme 9) lacking the MeS group afforded desired β-glycoside **89** in 39% yield. Similarly, β-glycoside **91** (Scheme 10) was obtained in 40% yield from the glycosyl donor (lacking the axial MeS group) derived from **85**. It is possible that deactivation of the Lewis acid ($BF_3 \cdot Et_2O$) through complexation with the MeS group may also contribute to the failure of the trichloroacetimidate carbohydrate donors **60**, **63**, **64**, and **66** (Table 6) to perform well in the respective glycosylation reactions.

The remaining steps of the total synthesis of shishijimicin A proceeded well as seen in Scheme 6. Thus, advanced thioacetate derivative **69** was converted to its methyltrisulfide counterpart **70** through a two-step, one-pot procedure involving cleavage of the acetate group (NaSMe, MeOH)[42] followed by addition of AcOH (neutralization), and reaction of the resulting thiol with PhthNSSMe[11] in 60−65% yield. Global desilylation of the latter with HF·py led to bis-protected precursor **71** in 80% yield. Finally, sequential removal of the Alloc [$Pd(PPh_3)_4$ cat., morpholine] and ketal (p-TsOH) protecting groups liberated shishijimicin A (**1**) in 75−80% yield as shown in Scheme 6. Synthetic shishijimicin A exhibited highly similar ^1H and ^{13}C NMR spectroscopic and optical rotation data to those reported for the natural product, as described in our previous communication.[7c] Additionally, the two rather weak carbon signals (attributed to carbons 8 and 9, see structure **1**, Scheme 6, for numbering) that were barely detectable in our previous work (see the Supporting Information)[7c,43] have now been unambiguously assigned by HSQC and HMBC experiments (see Figure 3 and the Supporting Information). Specifically, carbon 8 (C8) was assigned to the now visible chemical shift at δ = 70.7 ppm based on an HSQC experiment revealing a cross peak at (6.34,

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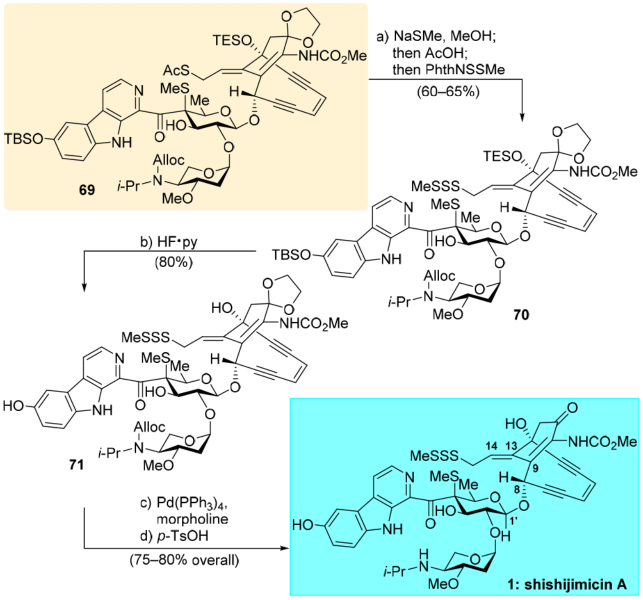
Scheme 6. Completion of Total Synthesis of Shishijimicin A[a]



[a]Reagents and conditions: (a) NaSMe (15 equiv), MeOH, 0 °C, 1.5 h; then AcOH (15 equiv), 0 °C, 1 min; then PhthNSSMe (8.0 equiv), 0 to 25 °C, 0.5 h, 60–65%; (b) HF·py/THF (1:20, *v*/*v*), 25 °C, 12 h, 80%; (c) $Pd(PPh_3)_4$ (0.5 equiv), morpholine (10.0 equiv), THF, 0 °C, 2 h; (d) *p*-TsOH (5.0 equiv), THF/H_2O/acetone (20:1:20, *v*/*v*/*v*), 25 °C, 48 h, 75–80% for the two steps.

70.66 ppm) with respect to the $^1J_{CH}$ correlation between H8 and C8 (Figure 3A), and an HMBC experiment revealing a cross peak at (4.95 and 70.66 ppm) with respect to the $^3J_{CH}$ correlation between H1′ and C8 (Figure 3B). Carbon 9 (C9) was unequivocally assigned the chemical shift at δ = 149.4 ppm based on an HMBC experiment revealing a cross peak at (6.53 ppm, 149.35 ppm) with respect to the $^3J_{CH}$ correlation between H14 and C9 (Figure 3C). With these NMR assignments, all the NMR spectroscopic data of shishijimicin A (**1**) and its ^{1}H and ^{13}C assignments are in good agreement with those reported by the Fusetani group[7a] (see the Supporting Information).

2.2. Design and Synthesis of Shishijimicin A Analogues. The developed synthetic strategies and technologies were applied to the synthesis of designed shishijimicin A analogues, aiming primarily to structure simplification and potency sustainment or enhancement. Our first targets became the thioacetate and methyldisulfide counterparts of shishijimicin A, namely, analogues **7** and **8**, respectively (see Scheme 7A). Thioacetate analogue **7** was inspired by calicheamicin θ_1^I, a synthetic analogue of calicheamicin γ_1^I that we prepared and studied in the 1990s.[44] Calicheamicin θ_1^I was proven to be more potent than its parent, calicheamicin γ_1^I, against certain cancer cell lines,[44,45] and more importantly, served as the payload of one of the earliest antibody–drug conjugates (ADCs) exhibiting effective suppression of growth and dissemination of hepatic metastases of neuroblastoma in a syngeneic mouse model.[46] Shishijimicin A analogue **7** was synthesized from advanced intermediate **69** (for preparation, see Table 6, entry 6) as summarized in Scheme 7A. Thus, cleavage of both silyl protecting groups from **69** (HF·py) followed by sequential removal of the Alloc [$Pd(PPh_3)_4$ cat., morpholine] and ketal (*p*-TsOH) protecting groups furnished **7** in 56% overall yield.

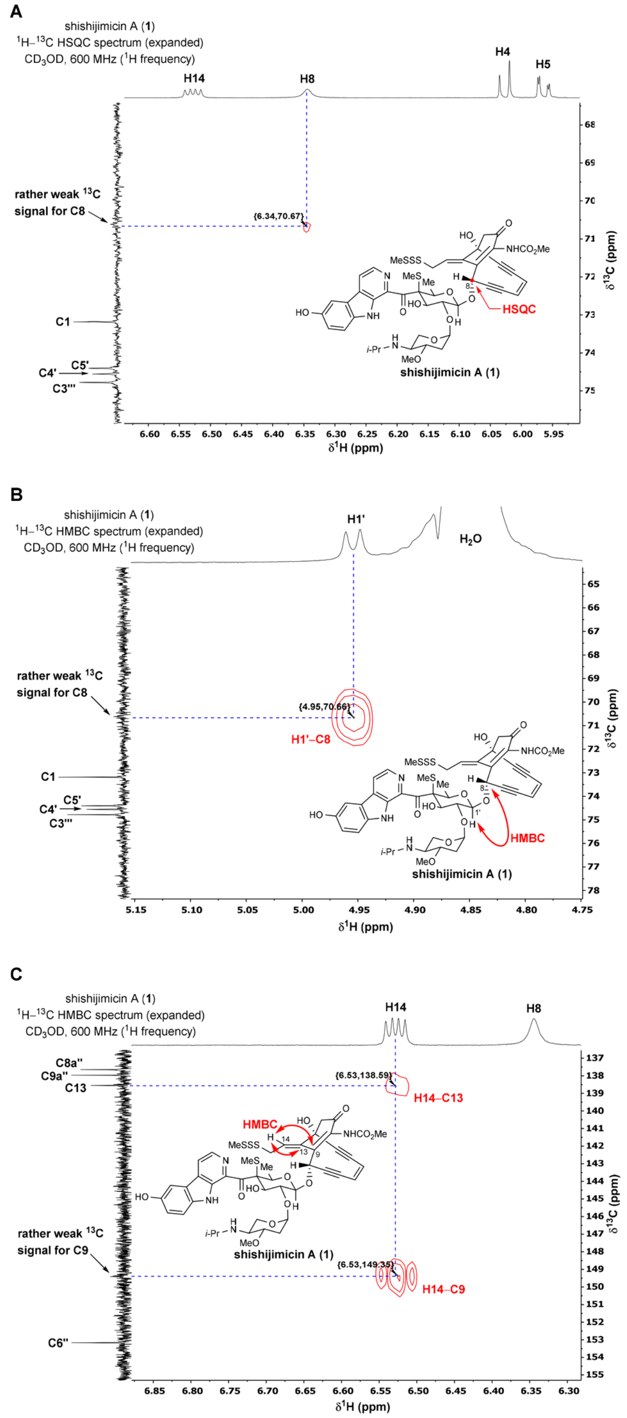


Figure 3. (A) Assignment of ^{13}C signal of C8 of the synthetic shishijimicin A by HSQC NMR spectroscopy: δ_C(C8) = 70.67 ppm; (B) assignment of ^{13}C signal of C8 by HMBC NMR spectroscopy: δ_C(C8) = 70.66 ppm; (C) assignment of ^{13}C signal of C9 by HMBC NMR spectroscopy: δ_C(C9) = 149.35 ppm.

Methyldisulfide shishijimicin A analogue **8** was inspired by previous experimental[47] and computational[48] studies supporting the intermediacy of a calicheamicin γ_1^I-glutathione disulfide conjugate as a major precursor to the crucial dihydrothiophene intermediate formed prior to the Bergman reaction,[49] the latter being responsible for the formation of DNA-cleaving

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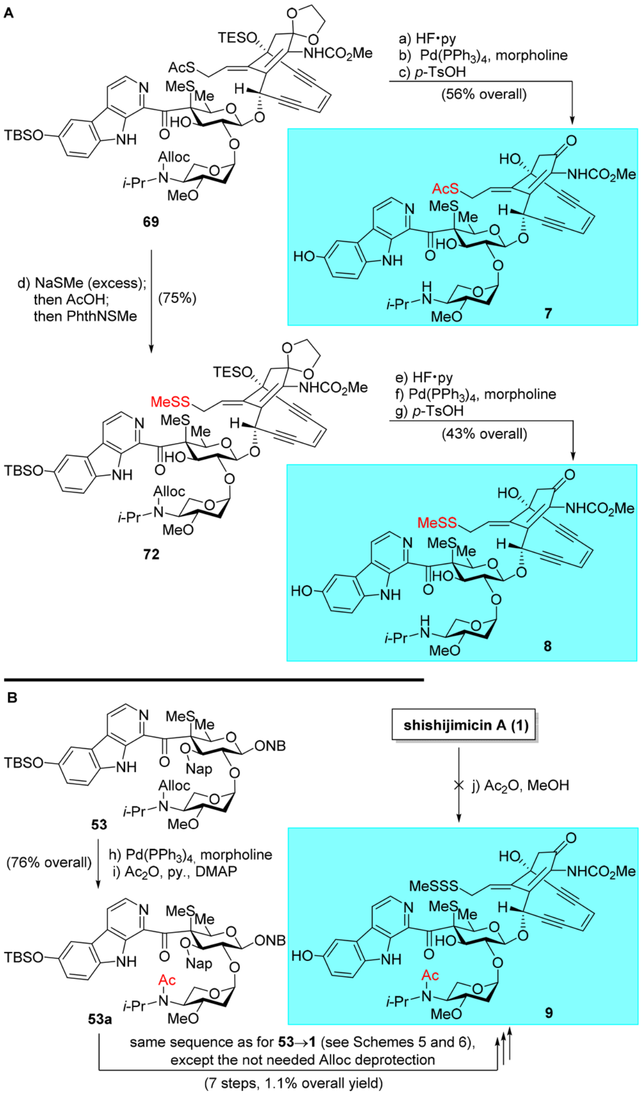
Scheme 7. Syntheses of Thioacetate Analogue 7, Disulfide Analogue 8, and *N*-Acetyl Analogue 9[a]



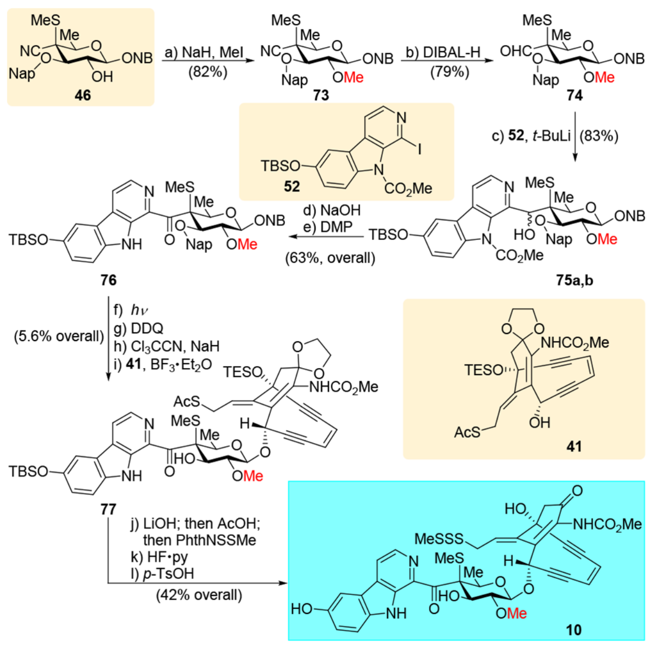
[a]Reagents and conditions: (a) HF·py/THF (1:20, *v*/*v*), 25 °C, 12 h; (b) $Pd(PPh_3)_4$ (0.5 equiv), morpholine (10.0 equiv), THF, 0 °C, 2 h; (c) *p*-TsOH (5.0 equiv), THF/H_2O/acetone (20:1:20, *v*/*v*/*v*), 25 °C, 48 h, 56% for the three steps; (d) NaSMe (10.0 equiv), MeOH, 0 °C, 20 min; then AcOH (10.0 equiv), 0 °C, 5 min; then PhthNSMe (5.0 equiv), 0 to 25 °C, 0.5 h, 75%; (e) HF·py/THF (1:20, *v*/*v*), 25 °C, 12 h; (f) $Pd(PPh_3)_4$ (0.5 equiv), morpholine (10.0 equiv), THF, 0 °C, 2 h; (g) *p*-TsOH (5.0 equiv), THF/H_2O/acetone (20:1:20, *v*/*v*/*v*), 25 °C, 48 h, 43% for the three steps; (h) $Pd(PPh_3)_4$ (0.5 equiv), morpholine (10.0 equiv), THF, 60 °C, 40 min; (i) Ac_2O (5.0 equiv), pyridine (10.0 equiv), DMAP (1.0 equiv), CH_2Cl_2, 40 °C, 24 h, 76% for the two steps; (j) Ac_2O (3 vol% in MeOH, *v*/*v*), 25 °C, 7 d.

benzenoid diradical species. The synthesis of disulfide analogue **8** was accomplished from the same advanced intermediate thioacetate **69** (see Scheme 7A) through a three-step, one-pot cascade reaction sequence initiated by excess NaSMe (for deacetylation), followed by addition, first of AcOH (for neutralization) and then of PhthNSMe,[50] to afford triprotected methyl disulfide **72**. A three-step deprotection sequence [(i) HF·py; (ii) $Pd(PPh_3)_4$ cat., morpholine; (iii) *p*-TsOH] then furnished coveted methyldisulfide shishijimicin A analogue **8** in 43% overall yield as shown in Scheme 7A.

Our attempt to directly form the *N*-acetyl shishijimicin A (**9**) under the reported conditions for preparing *N*-acetyl calicheamicin γ_1^I (3 vol% Ac_2O in MeOH)[51,52] was met with failure as depicted in Scheme 7B, presumably due to the rather hindered nature of the isopropyl amine structural motif and the sensitivity of some of the various functionalities within shishijimicin A. Coveted acetamide shishijimicin A analogue **9** was successfully prepared from *N*-acetyl disaccharide **53a** (generated from **53** by a two-step sequence) in seven steps by following the described procedures for the conversion of disaccharide **53** to shishijimicin A (**1**), in 1.1% overall yield (unoptimized), as shown in Scheme 7B.

The truncated shishijimicin A analogue **10**, which includes in its structure a methyl group in the place of the aminosugar (see Scheme 8), was designed in order to test the role of the

Scheme 8. Synthesis of Aminosugar Truncated Analogue 10[a]



[a]Reagents and conditions: (a) NaH (2.0 equiv), MeI (3.0 equiv), DMF, 0 °C, 0.5 h, 82%; (b) DIBAL-H (1.5 equiv), toluene, −78 °C, 10 min, 79%; (c) **52** (3.0 equiv), *t*-BuLi (6.0 equiv), THF, −78 °C, 0.5 h; then **74** (1.0 equiv), −78 °C, 10 min, 83% (ca. 1:1 *dr*); (d) NaOH (3.0 equiv), EtOH, 0 to 25 °C, 2 h; (e) DMP (2.0 equiv), $CHCl_3$, 25 °C, 2 h, 63% for the two steps; (f) $h\nu$, THF/H_2O (10:1, *v*/*v*), 0 °C, 4.5 h; (g) DDQ (2.5 equiv), CH_2Cl_2/H_2O (10:1, *v*/ *v*), 30 °C, 1.5 h; (h) NaH (2.0 equiv), Cl_3CCN/CH_2Cl_2 (1:2, *v*/*v*), 25 °C, 5 min; (i) **41** (2.0 equiv), $BF_3{\cdot}Et_2O$ (3.5 equiv), 4 Å MS, CH_2Cl_2, −78 to −40 °C, 1 h, 5.6% for the four steps; (j) LiOH·H_2O (100 equiv), MeOH, −17 to −10 °C, 20 min; then AcOH (100 equiv), 5 min; then PhthNSSMe (8.0 equiv), −10 to 25 °C, 0.5 h; (k) HF·py/THF (1:20, *v*/*v*), 25 °C, 12 h; (l) *p*-TsOH (5.0 equiv), THF/H_2O/acetone (20:1:20, *v*/*v*/ *v*), 25 °C, 48 h, 42% for the three steps.

latter structural motif on cytotoxic potency. This analogue was constructed from carbohydrate intermediate **46** (Scheme 8, see Table 5 and ref 7c for preparation), carboline derivative **52**, and enediyne fragment **41** as depicted in Scheme 8. Thus, methylation of the hydroxyl group of nitrile **46** (MeI, NaH, 82% yield) afforded methyl ether **73**, whose DIBAL-H reduction led to aldehyde **74** (79% yield). Coupling of carboline derivative **52** with aldehyde **74** was achieved by

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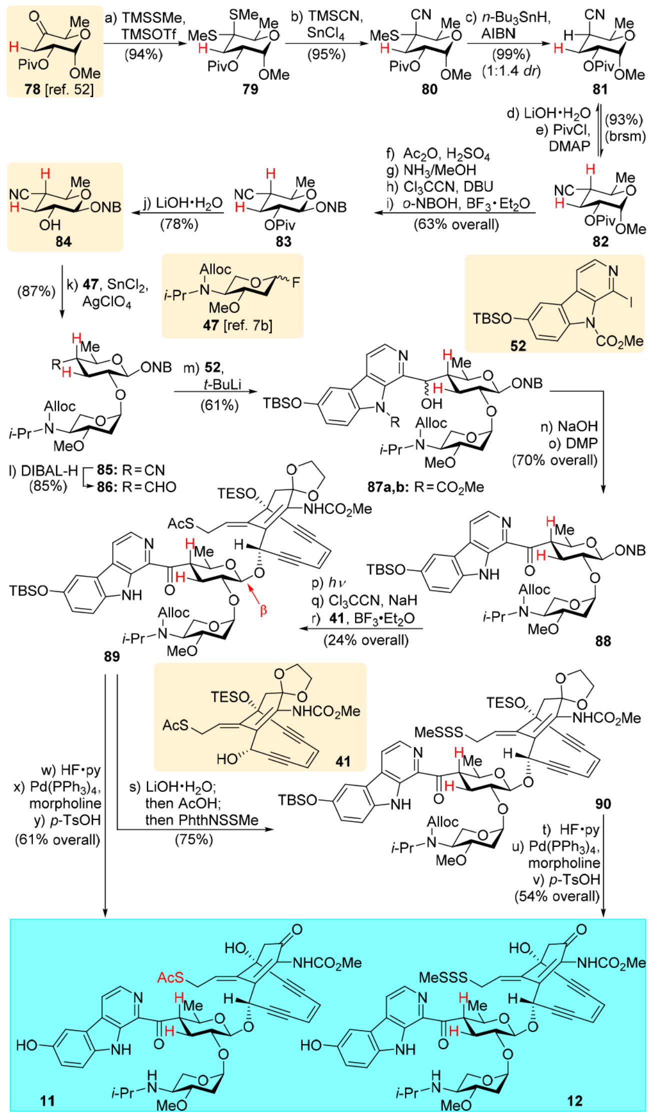
generation of the lithio derivative of the former with *t*-BuLi at −78 °C, followed by addition of the latter at the same temperature, leading to the corresponding secondary alcohol as a mixture of diastereomers (**75a**/**b**, 83% yield, ca. 1:1 *dr*) as shown in Scheme 8. The latter mixture was converted to carboline ketone **76** by treatment with NaOH/EtOH (decarboxylation), followed by oxidation with DMP, in 63% overall yield. Sequential removal of the NB ($h\nu$) and Nap (DDQ) protecting groups from **76** followed by trichloroacetimidate formation (Cl_3CCN, NaH) and coupling of the resulting carbohydrate donor with enediyne fragment **41**[7c] under the influence of $BF_3{\cdot}Et_2O$ furnished advanced intermediate **77** in 5.6% overall yield for the four steps from **76** as shown in Scheme 8. Finally, sequential deprotection/sulfenylation [(i) LiOH, then AcOH, then PhthNSSMe; (ii) HF·py; (iii) *p*-TsOH] of the latter gave analogue **10** in 42% overall yield for the three steps.

In a rather bold move, we then decided to simplify the hydroxy methylthio carbohydrate unit of shishijimicin A (carbohydrate unit A) by deleting both its hydroxyl and methylthioether functionalities and replacing them with hydrogen atoms as in analogues **11** and **12** (see Scheme 9). Their synthesis began with readily available keto sugar **78**[53] as summarized in Scheme 9. The first task was the conversion of **78** to glycosyl acceptor **84**, the latter intended for coupling with glycosyl donor **47** in a pending glycosylation reaction. Thus, treatment of **78** with TMSSMe in the presence of TMSOTf furnished thioketal **79** (94% yield), whose exposure to TMSCN and $SnCl_4$ gave methylthio nitrile **80** (95% yield). Replacement of the remaining methylthio group with a hydrogen residue was then carried out with *n*-Bu_3SnH in the presence of AIBN[54] to afford a mixture of diastereomers (at the nitrile-bearing carbon center, 99% yield, 1:1.4 *dr*) **81** with the CN group at the axial position (minor, undesired) and **82** with the CN group at the equatorial position (major, desired), which were chromatographically separated. Undesired axial isomer **81** was equilibrated [(i) $LiOH{\cdot}H_2O$; (ii) PivCl, DMAP] to a **81**/**82** ca. 3:2 mixture, from which further quantities of desired isomer **82** were isolated (93% yield based on 60% starting material recovery), the former being recyclable for further enrichment of desired compound **82**. α-Methyl glycoside **82** was then converted to β-*o*-nitrobenzyl (NB) glycoside **83** through a four-step sequence [(i) Ac_2O, H_2SO_4; (ii) NH_3, MeOH; (iii) Cl_3CCN, DBU; and (iv) *o*-NBOH, $BF_3{\cdot}Et_2O$] in 63% overall yield as shown in Scheme 9. Removal of the Piv group ($LiOH{\cdot}H_2O$, 78% yield) from the latter followed by coupling of resulting carbohydrate acceptor **84** with glycosyl donor **47**[7b] ($SnCl_2$, $AgClO_4$) furnished α-glycoside **85** in 87% yield. Reduction of **85** (DIBAL-H, 85% yield) gave aldehyde **86**, whose coupling with the lithio derivative obtained from iodo-carboline fragment **52** (*t*-BuLi) afforded hydroxyl carboline disaccharide **87a,b** as a diastereomeric mixture (ca. 1:1 *dr*, inconsequential) in 61% combined yield. Exposure of the latter to NaOH in EtOH, resulting in cleavage of the methyl carbamate functionality, was followed by DMP oxidation of the so-obtained product (free carboline NH upon methyl carbamate hydrolysis and decarboxylation) furnishing carboline disaccharide **88** (70% overall yield for the two steps). From the latter intermediate, the NB group was removed ($h\nu$) and the so-obtained product was converted to its trichloroacetimidate derivative (Cl_3CCN, NaH), and thence to advanced intermediate **89** (β-glycoside) through coupling with thioacetate enediyne carbohydrate acceptor **41** (24%

Scheme 9. Synthesis of Structurally Simplified Shishijimicin Analogues 11 and 12[a]



[a]Reagents and conditions: (a) TMSSMe (2.2 equiv), TMSOTf (1.5 equiv), toluene, −20 °C, 20 min; then sat. aq. $NaHCO_3$ (0.75 equiv), 0 °C, 5 min, 94%; (b) TMSCN (3.0 equiv), $SnCl_4$ (1.5 equiv), CH_2Cl_2, 0 °C, 0.5 h, 95%; (c) *n*-Bu_3SnH (2.0 equiv), AIBN (0.1 equiv), PhH, 80 °C, 1.5 h, 99% (ca. 1:1.4 *dr*); (d) $LiOH{\cdot}H_2O$ (4.5 equiv), 60 °C, 4 h; (e) PivCl (5.0 equiv), pyridine (10.0 equiv), DMAP (1.0 equiv), CH_2Cl_2, 40 °C, 10 h, 40% of **81** was converted to **82** after one round (93% brsm); (f) H_2SO_4 (0.8 equiv), Ac_2O, 0 °C, 40 min; (g) NH_3 (10.0 equiv), MeOH, 0 to 25 °C, 2 h; (h) Cl_3CCN/CH_2Cl_2 (1:10, *v*/*v*), DBU (0.1 equiv), 0 °C, 0.5 h; (i) *o*-NBOH (3.0 equiv), $BF_3{\cdot}Et_2O$ (2.0 equiv), 4 Å MS, CH_2Cl_2, −78 to −40 °C, 0.5 h, 63% for the four steps; (j) $LiOH{\cdot}H_2O$ (13 equiv), MeOH, 0 to 25 °C, 3 h, 78%; (k) **47** (2.0 equiv), $AgClO_4$ (2.5 equiv), $SnCl_2$ (2.5 equiv), 4 Å MS, THF, −78 to 25 °C, 12 h, 87%; (l) DIBAL-H (3.0 equiv), CH_2Cl_2, −78 °C, 45 min, 85%; (m) **52** (3.0 equiv), *t*-BuLi (6.0 equiv), THF, −78 °C, 5 min; then **86** (1.0 equiv), −78 to −35 °C, 40 min, 61% (ca. 1:1 *dr*); (n) NaOH (3.0 equiv), EtOH, 0 to 25 °C, 2 h; (o) DMP (1.1 equiv), $CHCl_3$, 0 to 35 °C, 10 min, 70% for the two steps; (p) $h\nu$, THF/H_2O (10:1, *v*/*v*), 0 °C, 4.5 h; (q) NaH (2.0 equiv), Cl_3CCN/CH_2Cl_2 (1:2, *v*/*v*), 25 °C, 5 min; (r) **41** (2.0 equiv), $BF_3{\cdot}Et_2O$ (3.5 equiv), 4 Å MS, CH_2Cl_2, −78 to −40 °C, 1 h, 24% for the three steps; (s) $LiOH{\cdot}H_2O$ (120 equiv), MeOH, −17 to −10 °C, 20 min; then AcOH (120 equiv), 5 min; then PhthNSSMe (8.0 equiv), −10 to 25 °C, 0.5 h, 75%; (t) HF·py/THF (1:20, *v*/*v*), 25 °C,

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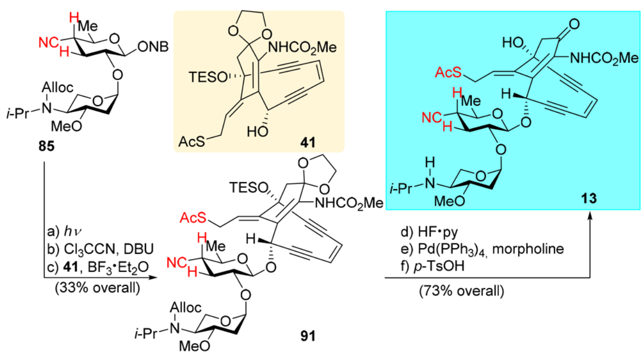
Scheme 9. continued

12 h; (u) $Pd(PPh_3)_4$ (0.5 equiv), morpholine (10.0 equiv), THF, 0 °C, 2 h; (v) *p*-TsOH (5.0 equiv), THF/H_2O/acetone (20:1:20, *v*/*v*/*v*), 25 °C, 48 h, 54% for the three steps; (w) HF·py/THF (1:20, *v*/*v*), 25 °C, 12 h; (x) $Pd(PPh_3)_4$ (0.5 equiv), morpholine (10.0 equiv), THF, 0 °C, 2 h; (y) *p*-TsOH (5.0 equiv), THF/H_2O/acetone (20:1:20, *v*/*v*/*v*), 25 °C, 48 h, 61% for the three steps. Piv = pivaloyl.

overall yield for the three steps). Precursor **89** was transformed to coveted thioacetate shishijimicin A analogue **11** through the standard three-step global deprotection sequence [(i) HF·py; (ii) $Pd(PPh_3)_4$ cat., morpholine; (iii) *p*-TsOH] in 61% overall yield as shown in Scheme 9. The same advanced intermediate (**89**) was diverted, first toward methyltrisulfide precursor **90** by treatment with $LiOH·H_2O$ in MeOH, then AcOH and finally PhthNSSMe,[11] in one pot and 75% overall yield. The latter was subjected to the standard global deprotection sequence as mentioned above (**89** → **11**) to yield methyltrisulfide analogue **12** in 54% overall yield as shown in Scheme 9.

Scheme 10 summarizes the construction of *β*-carboline truncated and simplified shishijimicin A analogue **13** whose

Scheme 10. Synthesis of *β*-Carboline Truncated Analogue 13[a]

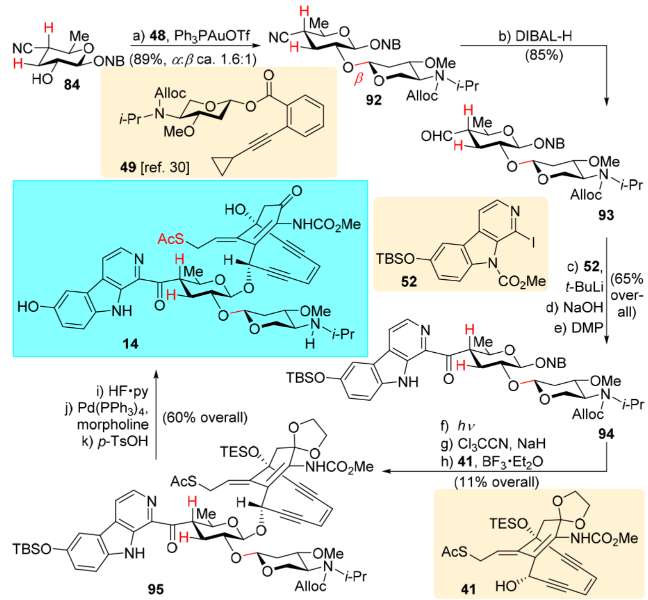


[a]Reagents and conditions: (a) *hν*, THF/H_2O (10:1, *v*/*v*), 0 °C, 2 h; (b) Cl_3CCN/CH_2Cl_2 (1:10, *v*/*v*), DBU (0.1 equiv), 0 °C, 0.5 h; (c) **41** (1.0 equiv), $BF_3·Et_2O$ (3.5 equiv), 4 Å MS, CH_2Cl_2, −78 to −30 °C, 1 h, 33% for the three steps; (d) HF·py/THF (1:20, *v*/*v*), 25 °C, 12 h; (e) $Pd(PPh_3)_4$ (0.5 equiv), morpholine (10.0 equiv), THF, 0 to 25 °C, 2 h; (f) *p*-TsOH (5.0 equiv), THF/H_2O/acetone (20:1:20, *v*/*v*/*v*), 25 °C, 48 h, 73% for the three steps.

design was meant to test the limits of structural simplification with regards to cytotoxicity potencies. Thus, disaccharide NB derivative **85** (for preparation, see Scheme 9) was subjected to photolytic cleavage of the NB protecting group (*hν*), followed by activation of the resulting lactol through trichloroacetimidate formation (Cl_3CCN, DBU), and coupling with hydroxy thioacetate enediyne fragment **41** to afford triprotected precursor **91** (33% overall yield). Analogue **13** was then generated from **91** through the standard three-step global deprotection sequence, in 73% overall yield, as depicted in Scheme 10.

Scheme 11 shows the synthesis of shishijimicin analogue **14** (the *β*-anomer of **11**, with regards to the aminosugar glycosidic bond), whose design was intended to test the role for the *α*-anomeric feature of the aminosugar structural motif of the molecule for bioactivity. In order to obtain desired *β*-glycoside disaccharide fragment **92**, the Yu gold-promoted glycosylation protocol[55] was employed to couple glycosyl acceptor **84** (for

Scheme 11. Synthesis of the *β*-Anomeric Isomer of 11: Analogue 14[a]



[a]Reagents and conditions: (a) **49** (1.1 equiv), $Ph_3PAuOTf$ (0.1 equiv), 4 Å MS, CH_2Cl_2, −78 °C, 1 h, 89% (*α*/*β* ca. 1.6:1); (b) DIBAL-H (3.0 equiv), CH_2Cl_2, −78 °C, 0.5 h, 85%; (c) **52** (3.0 equiv), *t*-BuLi (6.0 equiv), THF, −78 °C, 5 min; then **93** (1.0 equiv), −78 to −35 °C, 40 min; (d) NaOH (3.0 equiv), EtOH, 0 to 25 °C, 2 h; (e) DMP (1.1 equiv), $CHCl_3$, 0 to 35 °C, 10 min, 65% for the three steps; (f) *hν*, THF/H_2O (10:1, *v*/*v*), 0 °C, 4.5 h; (g) NaH (2.0 equiv), Cl_3CCN/CH_2Cl_2 (1:2, *v*/*v*), 25 °C, 5 min; (h) **41** (2.0 equiv), $BF_3·Et_2O$ (3.5 equiv), 4 Å MS, CH_2Cl_2, −78 to −40 °C, 1 h, 11% for the three steps; (i) HF·py/THF (1:20, *v*/*v*), 25 °C, 12 h; (j) $Pd(PPh_3)_4$ (0.5 equiv), morpholine (10.0 equiv), THF, 0 to 25 °C, 1 h; (k) *p*-TsOH (5.0 equiv), THF/H_2O/acetone (20:1:20, *v*/*v*/*v*), 25 °C, 48 h, 60% for the three steps.

preparation, see Scheme 9) and glycosyl donor **49**[30] under the influence of $Ph_3PAuOTf$ cat. (see Scheme 11), yielding the corresponding disaccharide as a mixture of *α*- and *β*-anomers (*α*/*β* ca. 1.6:1), from which the desired *β*-anomer (**92**) was chromatographically separated. Reduction of the nitrile moiety within **92** (DIBAL-H, 85% yield) afforded aldehyde **93**, which was processed through a similar pathway, and in similar yields, to afford targeted analogue **14** (see Scheme 11) as described above for the synthesis of its *α*-anomer counterpart (see **11**, Scheme 9).

Inspired by the iodide residue of calicheamicin γ_1^I and its importance to the binding of the molecule to duplex DNA,[56] we ventured to design and synthesize thioacetate shishijimicin A analogue **15** (Scheme 12A). Thus, thioacetate advanced intermediate **89** (for preparation, see Scheme 9) was subjected to Alloc-Boc exchange [(i) $Pd(PPh_3)_4$ cat., morpholine; (ii) Boc_2O, then DMAP] and subsequent desilylation (TBAF) to afford phenol derivative **98** (equipped with three Boc groups). The latter compound was treated, without purification, with morpholine-I_2 complex (**99**)[57] to give 5″-iodide **100**, exclusively, whose exposure to formic acid furnished desired iodo analogue **15** through global deprotection (three Boc groups and a ketal), in 50% overall yield for the five steps from **89**, as shown in Scheme 12A. The exclusive regioselectivity of the iodination of the phenolic carboline moiety of **98** can be rationalized by considering resonance structures **98a**, **98b** and

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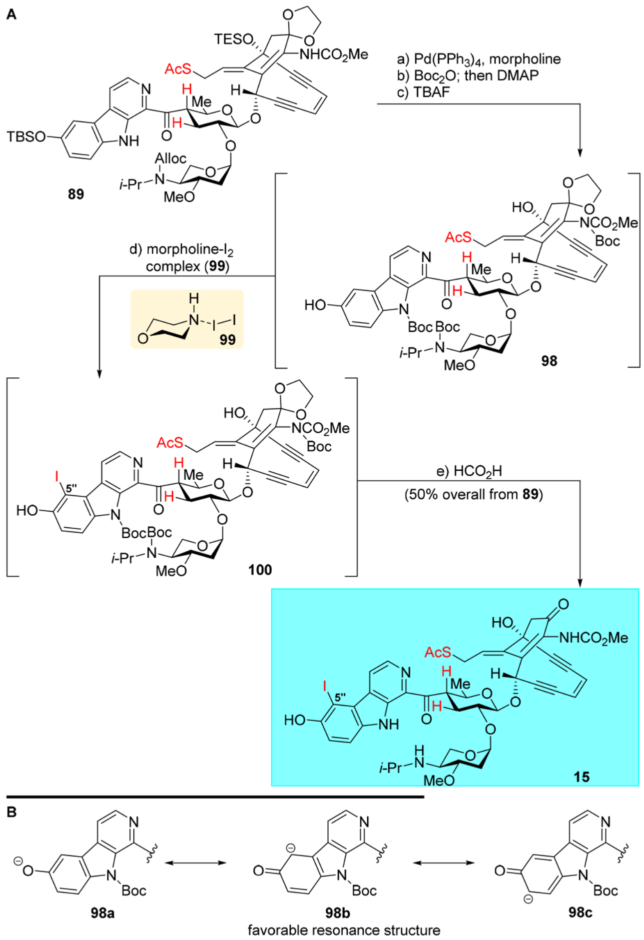
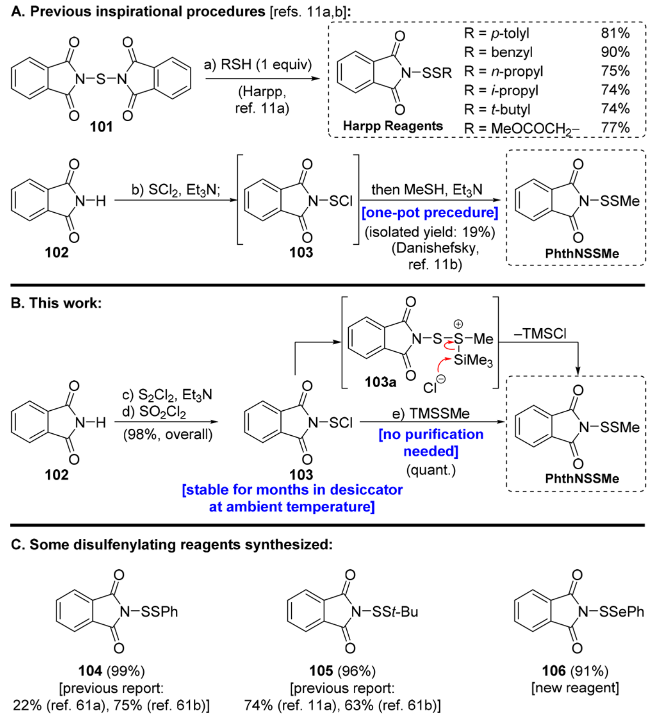
Scheme 12. Synthesis of C5″-Iodo-Shishijimicin Analogue 15[a]



[a]Reagents and conditions: (a) $Pd(PPh_3)_4$ (0.5 equiv), morpholine (10.0 equiv), THF, 0 °C, 2 h; (b) Boc_2O (10.0 equiv), MeCN, 80 °C, 36 h; then DMAP (1.0 equiv), 25 °C, 4 h; (c) TBAF (5.0 equiv), THF, 0 °C, 0.5 h; (d) morpholine–I_2 (**99**, 2.0 equiv), CH_2Cl_2, 25 °C, 20 min; (e) HCO_2H, 25 °C, 12 h, 50% for the five steps.

Scheme 13. Modified Preparation of *N*-(Methyldithio)-phthalimide [PhthNSSMe] and Synthesized Sulfenylating Reagents 104–106[a]



[a]Reagents and conditions: (a) **101** (1.0 equiv), thiol (1.0 equiv), benzene, reflux, 1.5–22 h, 74–90%; (b) **102** (1.0 equiv), SCl_2 (1.0 equiv), Et_3N (1.0 equiv), CH_2Cl_2, 0 °C, 40 min; then MeSH (1.0 equiv), Et_3N (1.0 equiv), 0 °C, 8 h; then 25 °C, 40 min, 19%; (c) **102** (1.0 equiv), S_2Cl_2 (0.5 equiv), Et_3N (1.2 equiv), THF, 0 to 25 °C, 6 h; (d) SO_2Cl_2 (excess), 70 °C, 12 h, 98% for the two steps; (e) TMSSMe (1.0 equiv), **103** (1.0 equiv), CH_2Cl_2, −78 °C, 0.5 h, quant.

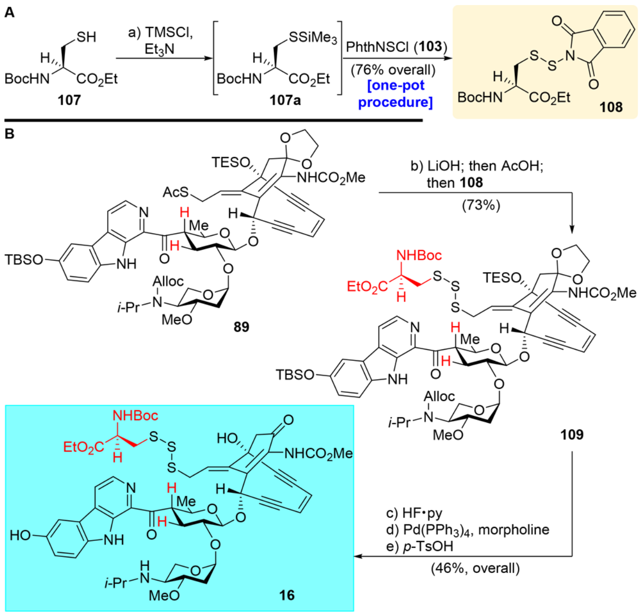
98c, with **98b** being more favorable than **98c**, as the aromaticity and extended conjugation of the system are mostly conserved in this resonance structure (i.e., **98b**, see Scheme 12B).

2.3. New Procedures for the Synthesis of Old and New Sulfenylating Reagents and Synthesis of Cysteine Trisulfide Shishijimicin A Analogue 16. During these studies, we recognized a number of issues with the published procedures for the preparation of the methyl Harpp-type reagent (PhthNSSMe).[11] The original procedure reported by Harpp[11a] featured an efficient generation of an array of the Harpp reagents from *N*,*N*′-thiobisphthalimide (**101**)[58] as shown in Scheme 13A. Notably, however, methanethiol (MeSH) was not tested using this procedure at the time and its feasibility to synthesize the methyl Harpp reagent (PhthNSSMe) is still unknown. The second procedure by Danishefsky[11b] involves the use of *in situ* generated phthalimidosulfenyl chloride (PhthNSCl, **103**), whose reaction with MeSH results in low isolated yield (19%) of the product (see Scheme 13A). Inspired by these precedents, and the previous work by Harpp et al. describing a facile synthesis of disulfides from sulfenyl chloride and TMS thioether partners,[59] we developed a convenient and high-yielding three-step procedure for the preparation of the methyl Harpp-type reagent (i.e., PhthNSSMe) as shown in Scheme 13B. Thus, treatment of phthalimide (**102**) with S_2Cl_2 in the presence of Et_3N, followed by exposure of the resulting bis(1-phthalimidyl)disulfane to excess SO_2Cl_2 generated intermediate **103** (stable for months in a desiccator at ambient temperature) in 98% overall yield.[60] Reaction of the latter with TMSSMe at −78 °C led to PhthNSSMe in quantitative yield and in pure form after evaporation of the byproduct (i.e., TMSCl; no further purification needed), presumably via intermediate **103a** through the mechanism shown in Scheme 13B.

Following the same procedure, two previously reported disulfenylating reagents (i.e., **104**[61b] and **105**[11a,61b]) and novel phenylselenosulfenylating reagent **106** were successfully synthesized from their corresponding thio- and seleno-TMS ethers (i.e., PhSTMS, *t*-BuSTMS and PhSeTMS[62]) in 99, 96, and 91% yields, respectively, as depicted in Scheme 13C (see the Supporting Information for further details).

As an extension of our synthetic investigations and in order to enrich the conjugation options of the enediyne family of payloads, we also developed disulfide phthalimide reagent **108** (see Scheme 14A) and employed it for the synthesis of cysteine trisulfide shishijimicin A analogue **16** as shown in Scheme 14B. Thus, reaction of thiol **107**[63] with TMSCl in the presence of Et_3N gave silyl thioether **107a**, whose reaction with

Scheme 14. Synthesis of Disulfenylation Reagent 108 and Its Application to the Synthesis of Cysteine Trisulfide Shishijimicin Analogue 16[a]



[a]Reagents and conditions: (a) TMSCl (1.1 equiv), Et_3N (1.1 equiv), CH_2Cl_2, 0 °C, 2 h; then PhthNSCl (**103**, 1.0 equiv), −78 °C, 0.5 h, 76%; (b) LiOH·H_2O (100 equiv), MeOH, −15 to −10 °C, 20 min; AcOH (100 equiv), 5 min; then **108** (5.0 equiv), −10 to 25 °C, 1 h, 73%; (c) HF·py/THF (1:20, *v*/*v*), 25 °C, 12 h; (d) $Pd(PPh_3)_4$ (0.5 equiv), morpholine (10.0 equiv), THF, 0 °C, 2 h; (e) *p*-TsOH (5.0 equiv), THF/H_2O/acetone (20:1:20, *v*/*v*/*v*), 25 °C, 48 h, 46% for the three steps.

PhthNSCl (**103**, for preparation, see Scheme 13) in the same pot afforded reagent **108** in 76% overall yield from **107**. As shown in Scheme 14B, advanced intermediate **89** (for preparation, see Scheme 9) reacted sequentially, and in the same pot, with LiOH·H_2O, then AcOH, and then reagent **108** to afford, in 73% overall yield, fully protected precursor **109**. The latter was subjected to our three-step global deprotection sequence to afford targeted shishijimicin analogue **16** in 46% overall yield, as shown in Scheme 14B.

2.4. Biological Evaluation of Synthesized Compounds and Structure−Activity Relationships. The synthesized shishijimicin A (**1**) and its analogues (**7**−**16**) were evaluated for their antitumor activities against MES SA (uterine sarcoma cells), MES SA DX (multi-drug-resistant uterine sarcoma cells), and HEK 293T[64] (immortalized human embryonic kidney cells) using *in vitro* assays and with *N*-acetyl calicheamicin γ_1^I as a positive control. The results of these investigations (IC_{50} values in nM) are summarized in Table 7. As seen in Table 7, analogues **7**, **8**, and **12** exhibited comparable or higher potencies than those of the synthetic natural product (**1**) with **8** being the most potent of all compounds tested, against the MES SA and HEK 293T cells, demonstrating subpicomolar potencies (i.e., 0.00067 nM against MES SA and 0.0015 nM against HEK 293T). The shishijimicin A analogue **12** lacking the methylthio and hydroxyl groups from the ring A sugar is also impressive for its single-digit picomolar potencies against these cell lines (i.e., IC_{50} = 0.006 and 0.008 nM against the MES SA and HEK 293T cell lines, respectively) and single-digit nanomolar

Table 7. Cytotoxicity Data against the Cell Lines MES SA, MES SA DX, and HEK 293T for Shishijimicin A (1) and Its Analogues 7−16[a,b]

compound	MES SA	MES SA DX	HEK 293T
N-Ac-calicheamicin γ_1^I	0.22	0.23	4.6
shishijimicin A (**1**)	0.013	>1000	0.016
7	0.023	3.8	0.033
8	0.00067	35	0.0015
9	1.6	>500	1.5
10	2.7	>500	1.9
11	0.053	>1000	0.051
12	0.006	1.2	0.008
13	0.20	>1000	0.46
14	>1000	>1000	>1000
15	10	>1000	6.9
16	0.04	2.5	0.02

[a]MSE SA = uterine sarcoma cell line; MES SA DX = MES SA cell line with marked multidrug resistance; HEK 293T = immortalized human embryonic kidney cell line. [b]IC_{50} is the 50% inhibitory concentration of the compound against cell growth, reported in nM. Data obtained at AbbVie Stemcentrx.

potency against the multi-drug-resistant cell line (IC_{50} = 1.2 nM against MES SA DX). The thioacetate counterpart analogue of shishijimicin A, analogue **7**, was the third most active compound tested, being only slightly less potent against the MES SA and HEK 293T cell lines (IC_{50} = 0.023 and 0.033 nM, respectively) but over 250-fold more potent against the multi-drug-resistant cancer cells (IC_{50} = 3.8 nM against MES SA DX) than the natural product. Interestingly, analogue **13**, lacking the carboline domain of the shishijimicin A molecule, showed subnanomolar potencies against both the MES SA and the HEK 293T cell lines (IC_{50} = 0.20 and 0.46 nM, respectively) but no significant activity against the more-difficult-to-kill MES SA DX cell line. The thioester counterpart (**11**) of simplified shishijimicin A analogue **12** also exhibited subnanomolar potencies against the MES SA and the HEK 293T cell lines, while it was found to be devoid of significant activity against the multi-drug-resistant MES SA DX cell line. Also, analogues **9**, **10**, and **15**, while exhibiting low nanomolar potencies against the MES SA and HEK 293T cell lines were considerably less potent against the drug-resistant cell line MES SA DX. The lack of potent cytotoxicities against all three of the tested cell lines by analogue **14** (the anomeric diastereoisomer of the rather potent simplified analogue **11** against two of the cell lines) was also of note, as was the decrease of potency of the iodo counterpart of analogue **11**, analogue **15** (see Table 7), indicating that these structural changes are not tolerated with regard to biological activity. Interestingly, cysteine trisulfide shishijimicin A analogue **16** demonstrated potent cytotoxicities against all three cell lines tested (IC_{50} = 0.04 nM against MES SA, 2.5 nM against MES SA DX, and 0.02 nM against HEK 293T), possessing the second highest potency against the drug-resistant MES SA DX cell line from all synthesized compounds evaluated.

From these data, we were able to derive a set of structure−activity relationships (SARs) within the shishijimicin family of compounds that could facilitate further optimization studies and preclinical development as shown in Figure 4. Thus, it became evident that the methyltrisulfide moiety (the triggering

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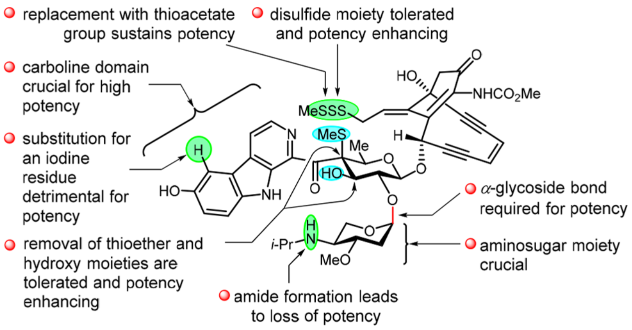

Figure 4. Structure−activity relationships of shishijimicin A.

device of the molecule initiating the Bergman cycloaromatization reaction) could be substituted with the methyldisulfide or thioacetate moieties without significant loss of, if not enhancing, the biological activity as predicted, in line with our expectations based on previous studies.[44−48] The same is true for the simplification of the shishijimicin A structure by replacing the methylthio and hydroxyl groups of the A carbohydrate ring with hydrogen residues. However, removal of the amino sugar residue, or acetylation of its amino group is not tolerated, suggesting the important role of the basic nitrogen atom in this region of the molecule. This possible role may be a dipolar interaction of this basic nitrogen (after protonation) with the negatively charged phosphate group of a DNA molecule. Similarly, the carboline domain seems to be playing an important role for the biological activity of the molecule as evidenced from the significant loss of activity upon its removal (see analogue **13**, Table 7). This conclusion is also supported by considerable loss of potency upon substituting this moiety with an iodine residue (see analogue **15**, Table 7).

3. CONCLUSION

This investigation led to a significantly improved synthesis of the enediyne domain of the naturally occurring shishijimicin A, a common structural motif shared with a number of other enediyne antitumor antibiotics, including namenamicin,[8] calicheamicin γ_1^I,[9] and esperamicin A_1.[10] A number of improvements were also made in the processes leading to the synthesis of other fragments of the molecule and of the key sulfenylating reagent employed to construct the trisulfide unit of shishijimicin A and related natural and designed molecules. The developed synthetic strategies, methods, and reagents were applied to the synthesis of a series of designed analogues of the natural product. Biological evaluation of the synthesized molecules identified a number of potent and yet structurally simpler analogues against certain cell lines, including a multidrug-resistant cell line tested. The data so obtained led to important SARs that may prove useful in further optimization studies toward the design, synthesis and development of potential payloads for ADCs as targeted cancer therapies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b06955.

Experimental procedures and characterization data for all compounds (PDF)

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Notes

The authors declare no competing financial interest.

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