

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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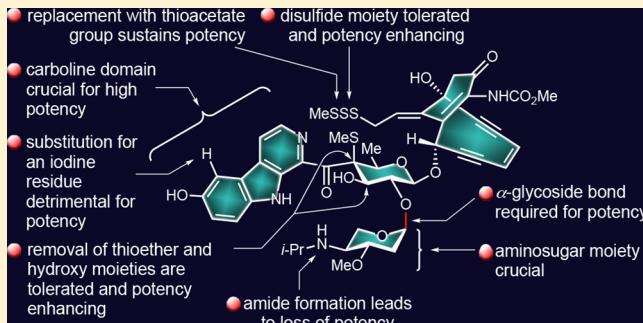
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Supporting Information

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 Kadycla⁴ 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 A⁷ (1, Figure 1) is the most potent enediyne antitumor antibiotic discovered thus far (e.g., IC₅₀ = 0.48 pM against P388 leukemia cells). Shishijimicin B (2)^{7a} and C (3),^{7a} namenamicin (4),⁸ calicheamicin γ₁ (5),⁹ and esperamicin A₁ (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 PhthNSSMe¹¹ 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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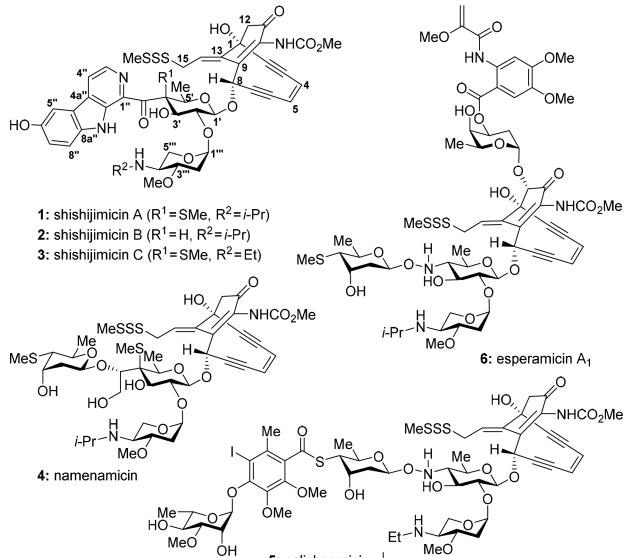


Figure 1. Representative 10-membered ring enediyne natural products: shishijimicins A–C (1–3), namenamicin (4), calicheamicin γ_1 (5), and esperamicin A₁ (6).

prominent enediyne antitumor antibiotics, including namenamicin (4),⁸ calicheamicin γ_1 (5),⁹ and esperamicin A₁ (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). Changing the solvent from CH₂Cl₂ to CHCl₃ did not significantly alter the outcome of this reaction (Table 1, entry 2). Our speculative mechanism, depicted in Table 1 (18 → 21

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

entry	conditions	yield (%) of 19	yield (%) of 20	yield (%) of 23
1 ^a	NaClO, CH ₂ Cl ₂ /H ₂ O, ^b 0 °C	51	14	20
2	NaClO, CHCl ₃ /H ₂ O, ^b 0 °C	58	18	10
3	t-BuOCl, CH ₂ Cl 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

^aOriginal conditions as reported in ref 13. ^bA 6.15 wt% aqueous solution of NaClO (2.0 equiv) was used. ^cThe reaction was performed by adding dropwise a benzene solution of 17 to t-BuOCl in benzene.

→ 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

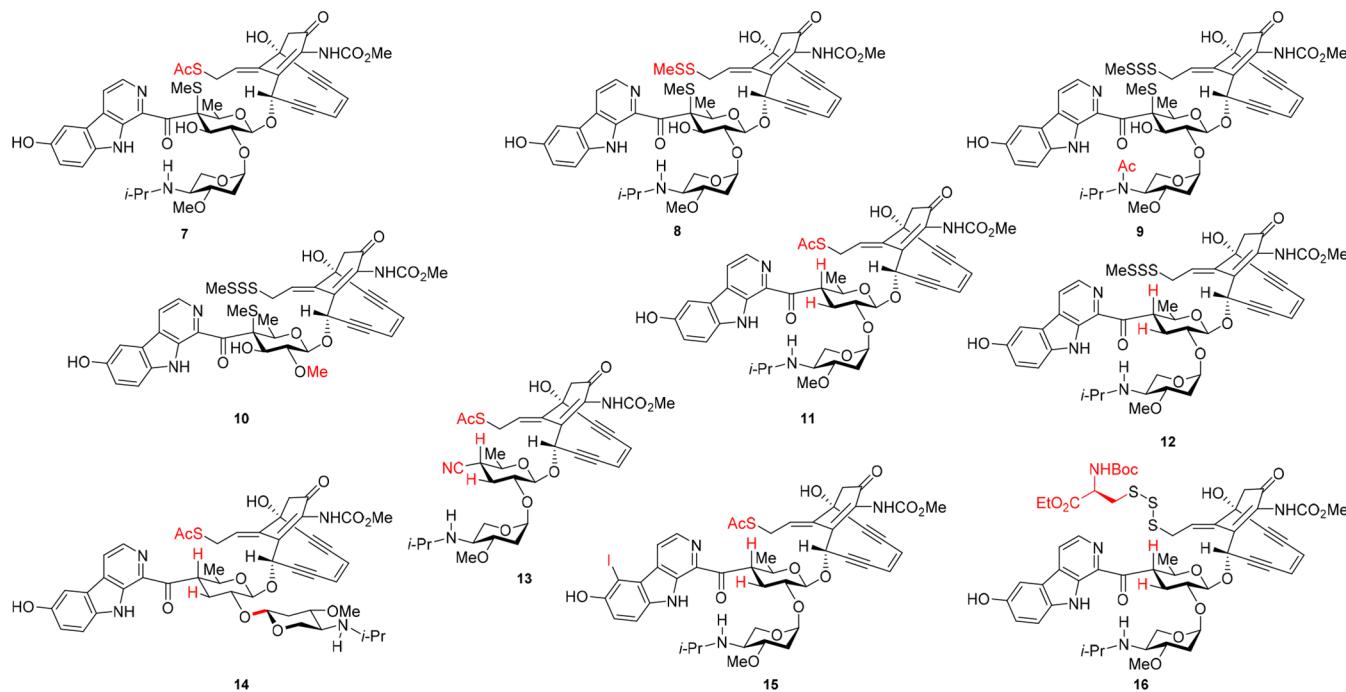
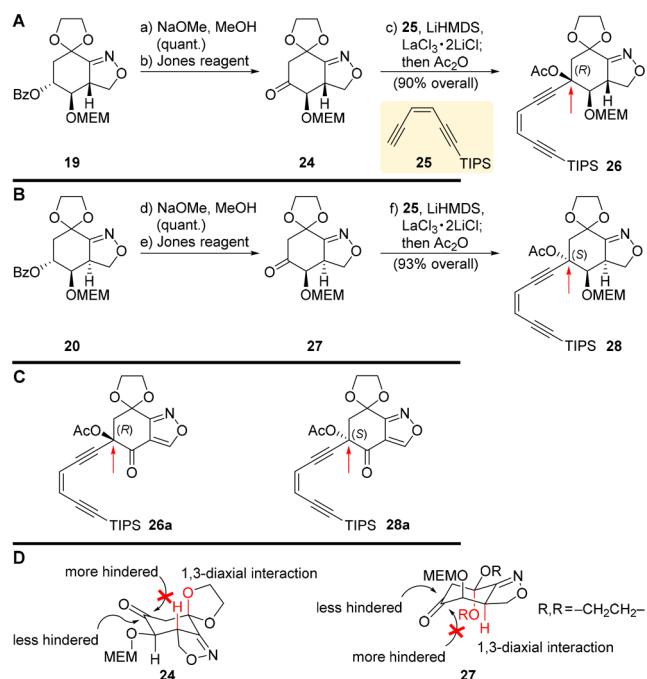


Figure 2. Synthesized shishijimicin A analogues 7–16.

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 H₂O, 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

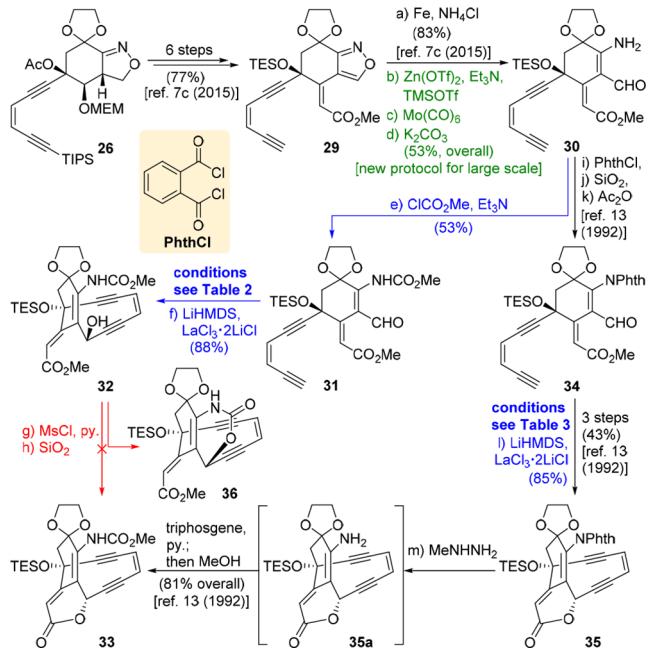


^aReagents and conditions: (a) NaOMe (0.1 equiv), MeOH, 0 °C, 12 h, quant.; (b) Jones reagent (1.5 equiv), acetone, 0 °C, 2 h; (c) **25** (3.0 equiv), LiHMDS (2.8 equiv), LaCl₃·2LiCl (5.0 equiv), THF, −78 °C, 0.5 h; then **24**, −78 °C, 0.5 h; then Ac₂O (10.0 equiv), −78 to 25 °C, 2 h, 90% for the two 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), LaCl₃·2LiCl (5.0 equiv), THF, −78 °C, 0.5 h; then **27**, −78 °C, 0.5 h; then Ac₂O (10.0 equiv), −78 to 25 °C, 1 h, 93% for the two steps.

our studies toward this goal. Thus, debenzylation 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 LaCl₃·2LiCl,^{7c,14} followed by *in situ* acetylation (Ac₂O) 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 acetylidyne 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 OCH₂ 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 NH₄Cl.^{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

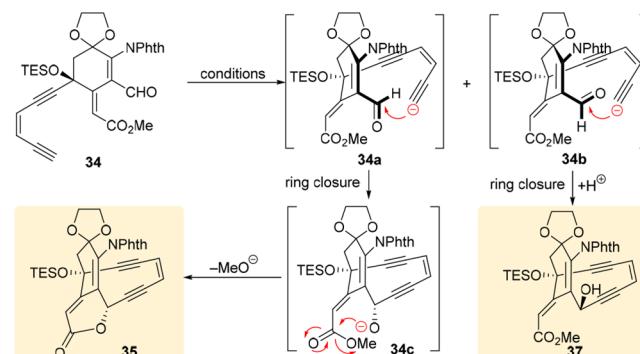
Scheme 2. Synthesis of Cyclized Enediyne Lactone 33^a

^aReagents and conditions: (a) Fe (25 equiv), NH₄Cl (50 equiv), EtOH/H₂O (1:1, v/v), 60 °C, 8 h, 83%; (b) TMSOTf (1.5 equiv), Et₃N (2.0 equiv), Zn(OTf)₂ (0.025 equiv), CH₂Cl₂, −20 °C, 10 min, then 29, −20 to 25 °C, 12 h; (c) Mo(CO)₆ (1.0 equiv), MeCN/H₂O (5:1, v/v), 80 °C, 1.5 h; (d) K₂CO₃ (1.0 equiv), MeOH/THF (2:1, v/v), 0 °C, 2 h, 53% for the three steps; (e) ClCO₂Me (10.0 equiv), Et₃N (20 equiv), CH₂Cl₂, 25 °C, 0.5 h, 53%; (f) LiHMDS (3.0 equiv), LaCl₃·2LiCl (5.0 equiv), THF, −20 °C, 5 min, 88%; (g) MsCl (5.0 equiv), pyridine (10.0 equiv), CH₂Cl₂, 0 °C, 15 min; (h) SiO₂, CH₂Cl₂, 25 °C, 2 h; (i) PhthCl (1.5 equiv), pyridine (4.0 equiv), MeNO₂, 0 °C, 0.5 h; (j) SiO₂, CH₂Cl₂, 25 °C, 2 h; (k) Ac₂O (excess), 25 °C, 1 h, 81% for the three steps; (l) LiHMDS (3.0 equiv), LaCl₃·2LiCl (2.0 equiv), THF, −78 °C, 1 h, 85%; (m) MeNNH₂ (10.0 equiv), PhH, 25 °C, 0.5 h; then triphosgene (3.0 equiv), pyridine (30 equiv), CH₂Cl₂, 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	yield (%) of 36	yield (%) of 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 ·2LiCl, THF, −20 °C	88	9	0

scale. Employment of Mo(CO)₆ as the reducing agent¹⁸ 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, Et₃N, 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 (K₂CO₃,

Table 3. Optimization of Enediyne Cycloaddition Reaction within Intermediate 34

entry	conditions	yield (%) of 35	yield (%) of 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 ·2LiCl, THF, −78 °C	85	10

^aOriginal conditions as reported in ref 13. ^bConditions as reported in ref 7c.

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.

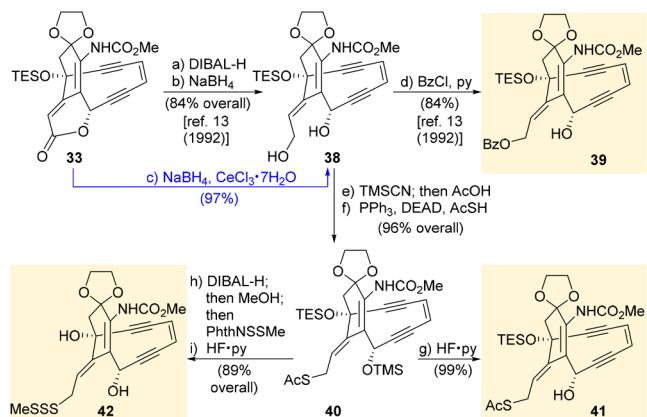
We then focused our efforts on developing a more efficient transformation of 30 to 33 (Scheme 2) which previously required six steps and proceeded in 28% overall yield.¹³ Our first attempt sought to circumvent the intermediacy of the phthalimide intermediate and required direct conversion of amino compound 30 to methyl carbamate 31, an operation that proceeded in 53% yield, upon exposure of the former to ClCO₂Me and Et₃N. 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, CeCl₃ or LiHMDS, LaCl₃·2LiCl), 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) SiO₂] 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., CeCl₃ or LaCl₃·2LiCl) as an additive favored the formation of desired lactone 35 (Table 3, entries 4 and 5)

with LiHMDS, LaCl₃·2LiCl 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_1^1 (**5**),⁹ and esperamicin A₁ (**6**),¹⁰ and their designed analogues]. As shown in **Scheme 3**,

Scheme 3. Syntheses of Enediyne Glycosyl Acceptors **39, **41**, and **42**^a**



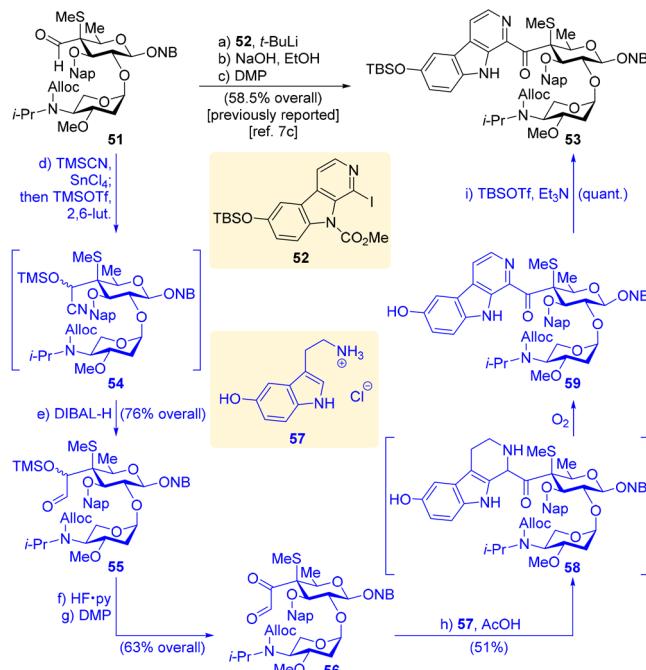
^aReagents and conditions: (a) DIBAL-H (3.0 equiv), CH₂Cl₂, −78 °C, 0.5 h, 95%; (b) NaBH₄ (excess), MeOH, 0 °C, 1 h, 88%; (c) NaBH₄ (2.0 equiv), CeCl₃·7H₂O (3.0 equiv), MeOH, 25 °C, 1 h, 97%; (d) BzCl (2.0 equiv), pyridine (3.0 equiv), CH₂Cl₂, −15 °C, 1 h, 84%; (e) TMSCN (excess), 25 °C, 0.5 h; then AcOH (5.0 equiv), THF/H₂O (5:1, *v/v*), 0 °C, 0.5 h; (f) PPh₃ (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; NaBH₄, 84% overall yield)¹³ was successfully replaced with the one-step Luche reduction (NaBH₄, CeCl₃·7H₂O) 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 PPh₃, 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.

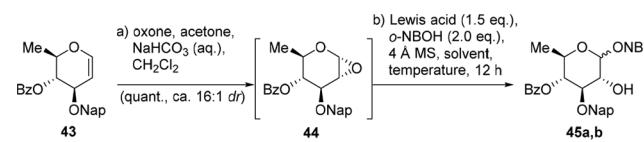
With practical and efficient processes for the synthesis of glycosyl acceptors **39**, **41**, and **42** in hand, we turned our attention to optimizing the construction of the β -caroline-disaccharide fragment (i.e., **53**, see **Scheme 4**) of shishijimicin

Scheme 4. Construction of β -Caroline Disaccharide **53 via Pictet–Spengler Condensation^a**



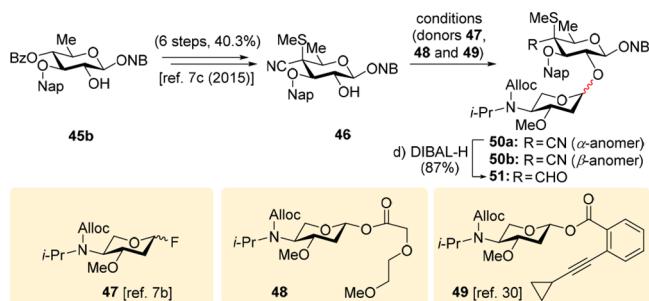
^aReagents and conditions: (a) **52** (3.0 equiv), *t*-BuLi (6.0 equiv), THF, −78 °C, 0.5 h; then **51** (1.0 equiv), −78 to −35 °C, 40 min, 86% (ca. 1:1 *dr*); (b) NaOH (3.0 equiv), EtOH, 0 to 25 °C, 2.5 h; (c) DMP (1.1 equiv), CHCl₃, 0 to 35 °C, 10 min, 68% for the two steps; (d) TMSCN (3.0 equiv), SnCl₄ (1.5 equiv), CH₂Cl₂, −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), CH₂Cl₂, −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), CH₂Cl₂, 25 °C, 0.5 h, 63% for the two steps; (h) **57** (4.0 equiv), AcOH, 60 °C, 1.5 h; then O₂, 1 h, 51%; (i) TBSOTf (1.1 equiv), Et₃N (2.0 equiv), CH₂Cl₂, 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 Danzigfsky conditions²¹ that employed ZnCl₂ 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)

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	yield (%) ^b of 45a
1 ^c	ZnCl ₂	THF	-78 to 25	54	11
2	ZnBr ₂	THF	-78 to 25	70	5
3	BF ₃ ·Et ₂ O	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 ₃ PAuOTf ^d	CH ₂ Cl ₂	-78 to 25	46	28
7	Ph ₃ PAuNTf ₂ ^d	CH ₂ Cl ₂ /THF	-78 to 25	42	50
8	In(OTf) ₃	CH ₂ Cl ₂	-78 to 25	41	33
9	Bi(OTf) ₃	CH ₂ Cl ₂	-78 to 25	43	35

^aReaction conditions: (a) oxone (5.0 equiv), acetone (8.0 equiv), NaHCO₃ (25 equiv), H₂O/CH₂Cl₂ (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. ^bIsolated yields of indicated anomers. ^cConditions as reported in ref 7c. ^dA 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 (α -glycoside)(β -glycoside)	50a	50b
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 ₂ HfCl ₂ (2.5 equiv), Et ₂ O, -78 to 25 °C, 12 h	34	62	
3	48 (4.0 equiv), AgClO ₄ (4.0 equiv), SnCl ₄ (4.0 equiv), Et ₂ O, -78 to 25 °C, 12 h	16	48	
4	49 (2.0 equiv), Ph ₃ PAuOTf (0.2 equiv), CH ₂ Cl ₂ , 0 °C, 12 h	13	61	
5	49 (2.0 equiv), Ph ₃ PAuNTf ₂ (0.2 equiv), CH ₂ Cl ₂ , 0 °C, 12 h	22	43	

^aGlycosyl acceptor 46 (0.1 mmol, 1.0 equiv) was used as a limiting reagent for each entry. ^bIsolated yield based on 46. ^cConditions as reported in ref 7c. ^dDIBAL-H (3.0 equiv), CH₂Cl₂, -78 °C, 45 min, 87%.

(acetone, oxone, aqueous NaHCO₃, CH₂Cl₂, 0 °C, quant., ca. 16:1 *dr*), and the resulting epoxide (44) was used crude without purification. Given that our original conditions with ZnCl₂ 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., ZnBr₂, BF₃·Et₂O, AlCl₃, and InCl₃, Table 4). Thus, switching the promoter from ZnCl₂ (Table 4, entry 1) to ZnBr₂²² (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 ZnCl₂, Table 4, entry 1). While the stronger Lewis acids BF₃·Et₂O²³ (Table 4, entry 3) and AlCl₃²⁴ (Table 4, entry 4) gave lower selectivities than ZnCl₂ (Table 4, entry 1), they proved more efficient in terms of combined yield, namely, 75 and 85%, respectively. We then considered InCl₃²⁵ 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 InCl₃-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 Ph₃PAuOTf²⁶ and Ph₃PAuNTf₂²⁷ were also tested²⁸ 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 Ph₃PAuNTf₂ 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).

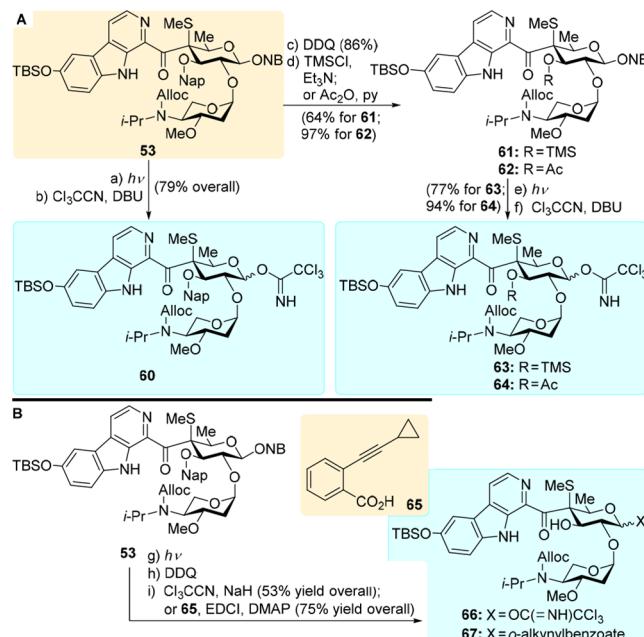
With an improved and scalable synthesis of glycoside 45b developed (see Table 4), we then proceeded to its conversion to methylthio cyanide 46, a task accomplished through a high-yielding six-step sequence as we previously described^{7c} (40.3% overall yield) as summarized in Table 5. The next challenge 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 49³⁰ (Table 5) with the desired goal of selectively obtaining α -glycoside 50a³¹ (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 AgClO₄ and SnCl₂,³² 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 AgClO₄–SnCl₂ activation conditions (Table 5, entry 1). Interestingly, replacing SnCl₂ with Cp₂HfCl₂³³ (Table 5, entry 2) as a partner to AgClO₄ in this glycosylation reaction not only resulted in a similarly impressive combined yield of the α,β -disaccharide (mixture

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 approach³⁴ 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 reaction³⁵) 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 ($\text{h}\nu$) 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 ($\text{h}\nu$) 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 catalysis³⁷ in the final coupling step, hydroxy glycosyl donor 67 was synthesized from 53 through a sequence involving NB removal ($\text{h}\nu$), Nap deprotection (DDQ), and esterification with *o*-alkynylbenzoic acid 65 (EDCI, *i*-Pr₂NEt, DMAP) in 75% overall yield for the three steps as shown in Scheme 5B. Both 66 and 67 are

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

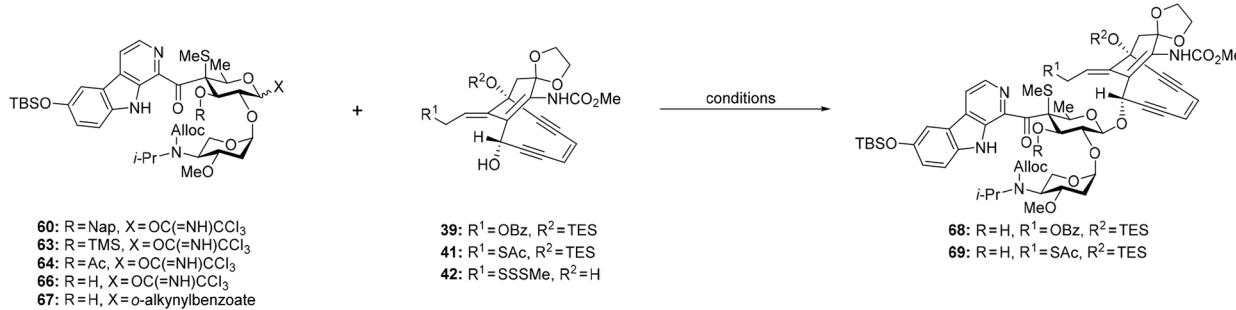


^aReagents and conditions: (a) $\text{h}\nu$, THF/H₂O (10:1, *v/v*), 4.5 h; (b) $\text{Cl}_3\text{CCN}/\text{CH}_2\text{Cl}_2$ (1:10, *v/v*), DBU (1.0 equiv), 0 °C, 1.5 h, 79% for the two steps; (c) DDQ (2.0 equiv), $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ (10:1, *v/v*), 25 °C, 4 h, 86%; (d) TMSCl (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) $\text{h}\nu$, THF/H₂O (10:1, *v/v*), 0 °C, 4.5 h; (f) $\text{Cl}_3\text{CCN}/\text{CH}_2\text{Cl}_2$ (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) $\text{h}\nu$, THF/H₂O (10:1, *v/v*), 0 °C, 4.5 h; (h) DDQ (2.5 equiv), $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ (10:1, *v/v*), 30 °C, 1.5 h; (i) NaH (2.0 equiv), $\text{Cl}_3\text{CCN}/\text{CH}_2\text{Cl}_2$ (1:2, *v/v*), 25 °C, 5 min; or 65 (2.0 equiv), EDCI (2.0 equiv), *i*-Pr₂NEt (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 $\text{BF}_3\text{-Et}_2\text{O}$ (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

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



entry	donor ^a	acceptor (equiv)	conditions ^b	product	yield (%) ^c
1	60	39 (1.6)	BF ₃ ·Et ₂ O (3.5 equiv), CH ₂ Cl ₂ , -78 to -40 °C	— ^d	—
2	63	39 (1.6)	BF ₃ ·Et ₂ O (3.5 equiv), CH ₂ Cl ₂ , -78 to -40 °C	— ^d	—
3	64	39 (1.6)	BF ₃ ·Et ₂ O (3.5 equiv), CH ₂ Cl ₂ , -78 to -40 °C	— ^d	—
4	68	39 (1.6)	BF ₃ ·Et ₂ O (3.5 equiv), CH ₂ Cl ₂ , -78 to -40 °C	68	15
5	66	39 (1.6)	TMSOTf (3.5 equiv), CH ₂ Cl ₂ , -78 to -40 °C	68	10
6 ^f	66	41 (1.6)	BF ₃ ·Et ₂ O (3.5 equiv), CH ₂ Cl ₂ , -78 to -40 °C	69	26
7	66	42 (1.6)	BF ₃ ·Et ₂ O (3.5 equiv), CH ₂ Cl ₂ , -78 to -40 °C	— ^e	—
8	67	41 (3.0)	Ph ₃ PAuOTf (2.0 equiv), CH ₂ Cl ₂ , 0 to 20 °C	— ^d	—
9	67	41 (3.0)	Ph ₃ PAuNTf ₂ (2.0 equiv), CH ₂ Cl ₂ , 0 to 20 °C	69	<5

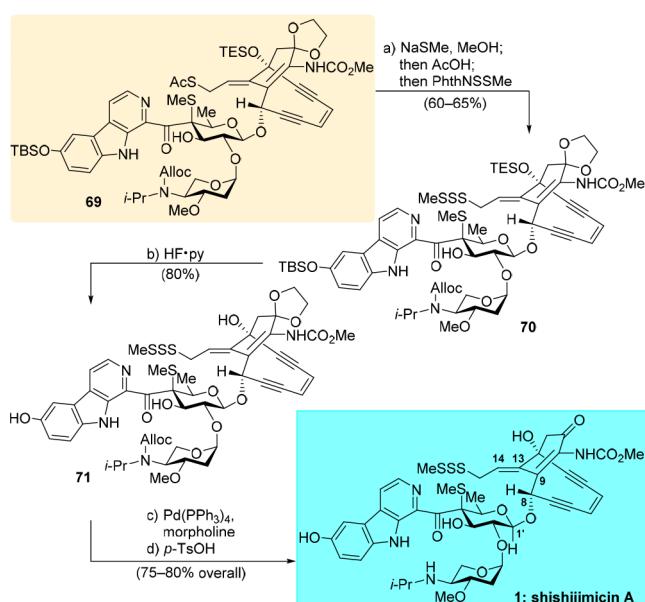
^aGlycosyl donor (1.0 equiv) was used as a limiting reagent for each entry. ^bIn each reaction, 4 Å molecular sieves were used. ^cIsolated yield of indicated product. ^dNo desired product observed; glycosyl acceptor recovered. ^eNo desired product observed; glycosyl acceptor decomposed. ^fConditions 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 promoter⁴⁰ in this glycosylation reaction (Table 6, entry 5) furnished product **68** in an even lower yield than the previous experiment with BF₃·Et₂O, due to a rapid formation of the TMS-ether of carbohydrate acceptor **39**,⁴¹ forcing us to switch back to BF₃·Et₂O 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 Ph₃PAuOTf²⁶ (Table 6, entry 8) and Ph₃PAuNTf₂²⁷ (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 (BF₃·Et₂O) 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 (NaSM, MeOH)⁴² followed by addition of AcOH (neutralization), and reaction of the resulting thiol with PhthNSSMe¹¹ 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(PPh₃)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 ¹³C 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,

Scheme 6. Completion of Total Synthesis of Shishijimicin A^a



^a Reagents and conditions: (a) NaSMMe (15 equiv), MeOH, 0 °C, 1 h; then AcOH (15 equiv), 0 °C, 1 min; then PhthNNSMe (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(PPh₃)₄ (0.5 equiv), morpholine (10.0 equiv), THF, 0 °C, 2 h; (d) *p*-TsOH (5.0 equiv), THF/H₂O/acetone (20:1:20, 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 ¹H and ¹³C 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 Analogs. 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^I, a synthetic analogue of calicheamicin γ^I that we prepared and studied in the 1990s.⁴⁴ Calicheamicin θ^I was proven to be more potent than its parent, calicheamicin γ^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(PPh₃)₄ cat,

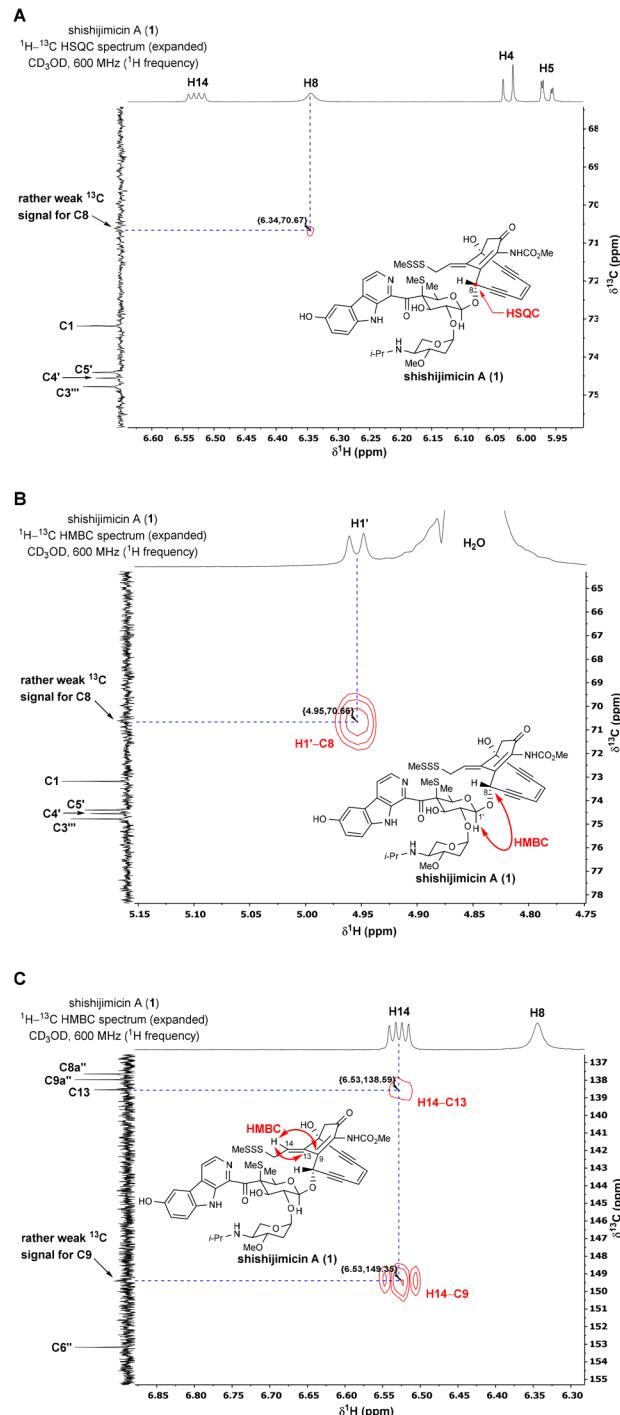
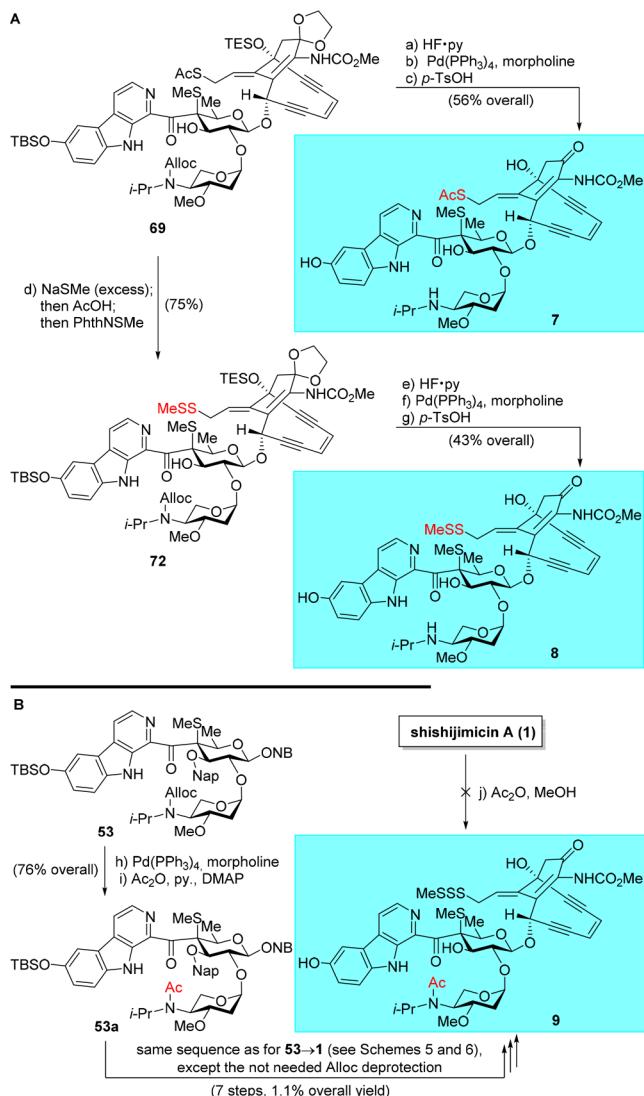


Figure 3. (A) Assignment of ¹³C signal of C8 of the synthetic shishijimicin A by HSQC NMR spectroscopy: δ_C(C8) = 70.67 ppm; (B) assignment of ¹³C signal of C8 by HMBC NMR spectroscopy: δ_C(C8) = 70.66 ppm; (C) assignment of ¹³C signal of C9 by HMBC NMR spectroscopy: δ_C(C9) = 149.35 ppm.

morpholine] and ketal (*p*-TsOH) protecting groups furnished **7** in 56% overall yield.

Methyldisulfide shishijimicin A analogue **8** was inspired by previous experimental⁴⁷ and computational⁴⁸ studies supporting the intermediacy of a calicheamicin γ^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

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



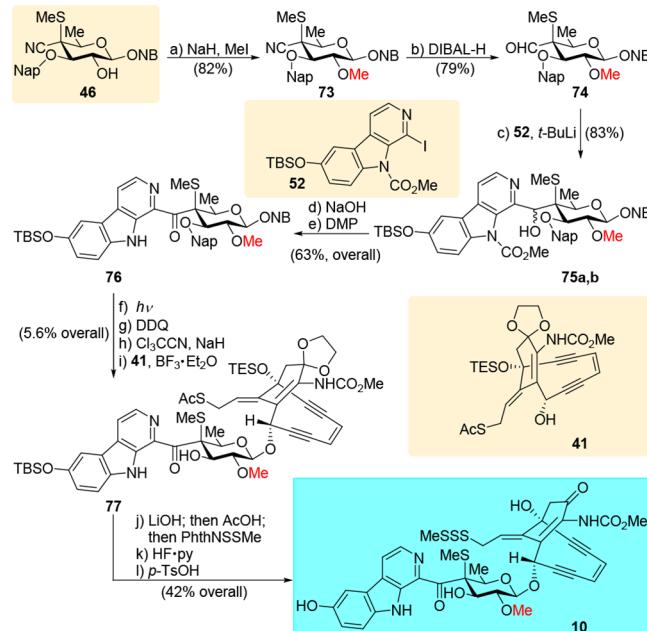
^aReagents and conditions: (a) HF·py/THF (1:20, *v/v*), 25 °C, 12 h; (b) Pd(PPh₃)₄ (0.5 equiv), morpholine (10.0 equiv), THF, 0 °C, 2 h; (c) *p*-TsOH (5.0 equiv), THF/H₂O/acetone (20:1:20, *v/v/v*), 25 °C, 48 h, 56% for the three steps; (d) NaSMMe (10.0 equiv), MeOH, 0 °C, 20 min; then AcOH (10.0 equiv), 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(PPh₃)₄ (0.5 equiv), morpholine (10.0 equiv), THF, 0 °C, 2 h; (g) *p*-TsOH (5.0 equiv), THF/H₂O/acetone (20:1:20, *v/v/v*), 25 °C, 48 h, 43% for the three steps; (h) Pd(PPh₃)₄ (0.5 equiv), morpholine (10.0 equiv), THF, 60 °C, 40 min; (i) Ac₂O (5.0 equiv), pyridine (10.0 equiv), DMAP (1.0 equiv), CH₂Cl₂, 40 °C, 24 h, 76% for the two steps; (j) Ac₂O (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 NaSMMe (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(PPh₃)₄ cat, morpholine; (iii) *p*-TsOH] then furnished coveted methyl disulfide 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_1^1 (3 vol% Ac₂O 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



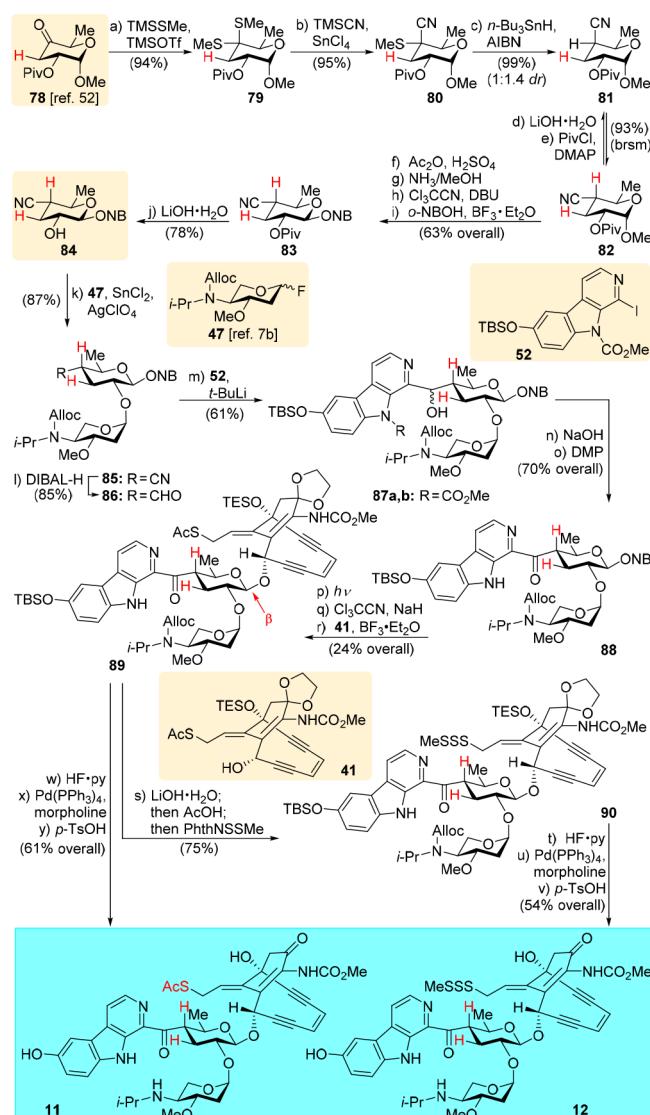
^aReagents and conditions: (a) NaH (2.0 equiv), MeI (3.0 equiv), DMF, 0 °C, 0.5 h, 82%; (b) DIBAL-H (1.5 equiv), toluene, -78 °C, 10 min, 79%; (c) 52 (3.0 equiv), *t*-BuLi (6.0 equiv), THF, -78 °C, 0.5 h; then 74 (1.0 equiv), -78 °C, 10 min, 83% (ca. 1:1 *dr*); (d) NaOH (3.0 equiv), EtOH, 0 to 25 °C, 2 h; (e) DMP (2.0 equiv), CHCl₃, 25 °C, 2 h, 63% for the two steps; (f) *h*_v, THF/H₂O (10:1, *v/v*), 0 °C, 4.5 h; (g) DDQ (2.5 equiv), CH₂Cl₂/H₂O (10:1, *v/v*), 30 °C, 1.5 h; (h) NaH (2.0 equiv), Cl₃CCN/CH₂Cl₂ (1:2, *v/v*), 25 °C, 5 min; (i) 41 (2.0 equiv), BF₃·Et₂O (3.5 equiv), 4 Å MS, CH₂Cl₂, -78 to -40 °C, 1 h, 5.6% for the four steps; (j) LiOH·H₂O (100 equiv), MeOH, -17 to -10 °C, 20 min; then AcOH (100 equiv), 5 min; then PhthNSSSMe (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/H₂O/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

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 (*hν*) 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 $\text{BF}_3\cdot\text{Et}_2\text{O}$ furnished advanced intermediate **77** in 5.6% overall yield for the four steps from **76** as shown in **Scheme 8**. Finally, sequential deprotection/sulfonylation [(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*-Bu₃SnH in the presence of AIBN⁵⁴ 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·H₂O; (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 , H₂SO₄; (ii) NH₃, MeOH; (iii) Cl_3CCN , DBU; and (iv) *o*-NBOH, $\text{BF}_3\cdot\text{Et}_2\text{O}$] in 63% overall yield as shown in **Scheme 9**. Removal of the Piv group (LiOH·H₂O, 78% yield) from the latter followed by coupling of resulting carbohydrate acceptor **84** with glycosyl donor **47**^{7b} (SnCl_2 , AgClO₄) 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 (*hν*) 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 acceptor **41** (24% overall).

Scheme 9. Synthesis of Structurally Simplified Shishijimicin Analogs 11 and 12^a



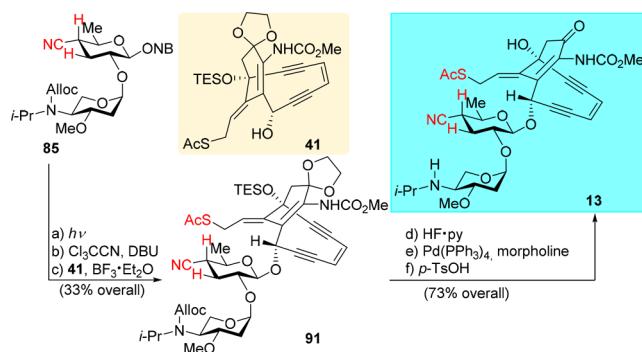
^a Reagents and conditions: (a) TMSSMe (2.2 equiv), TMSOTf (1.5 equiv), toluene, -20°C , 20 min; then sat. aq. NaHCO_3 (0.75 equiv), 0°C , 5 min, 94%; (b) TMSCN (3.0 equiv), SnCl_4 (1.5 equiv), CH_2Cl_2 , 0°C , 0.5 h, 95%; (c) *n*-Bu₃SnH (2.0 equiv), AIBN (0.1 equiv), PhH, 80°C , 1.5 h, 99% (ca. 1:1.4 *dr*); (d) LiOH·H₂O (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) NH₃ (10.0 equiv), MeOH, 0 to 25°C , 2 h; (h) $\text{Cl}_3\text{CCN}/\text{CH}_2\text{Cl}_2$ (1:10, *v/v*), DBU (0.1 equiv), 0°C , 0.5 h; (i) *o*-NBOH (3.0 equiv), $\text{BF}_3\cdot\text{Et}_2\text{O}$ (2.0 equiv), 4 Å MS, CH_2Cl_2 , -78 to -40°C , 0.5 h, 63% for the four steps; (j) LiOH·H₂O (13 equiv), MeOH, 0 to 25°C , 3 h, 78%; (k) **47** (2.0 equiv), AgClO₄ (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) *hν*, THF/H₂O (10:1, *v/v*), 0°C , 4.5 h; (q) NaH (2.0 equiv), $\text{Cl}_3\text{CCN}/\text{CH}_2\text{Cl}_2$ (1:2, *v/v*), 25 °C, 5 min; (r) **41** (2.0 equiv), $\text{BF}_3\cdot\text{Et}_2\text{O}$ (3.5 equiv), 4 Å MS, CH_2Cl_2 , -78 to -40°C , 1 h, 24% for the three steps; (s) LiOH·H₂O (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,

Scheme 9. continued

12 h; (u) $\text{Pd}(\text{PPh}_3)_4$ (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) $\text{Pd}(\text{PPh}_3)_4$ (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) $\text{Pd}(\text{PPh}_3)_4$ 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.

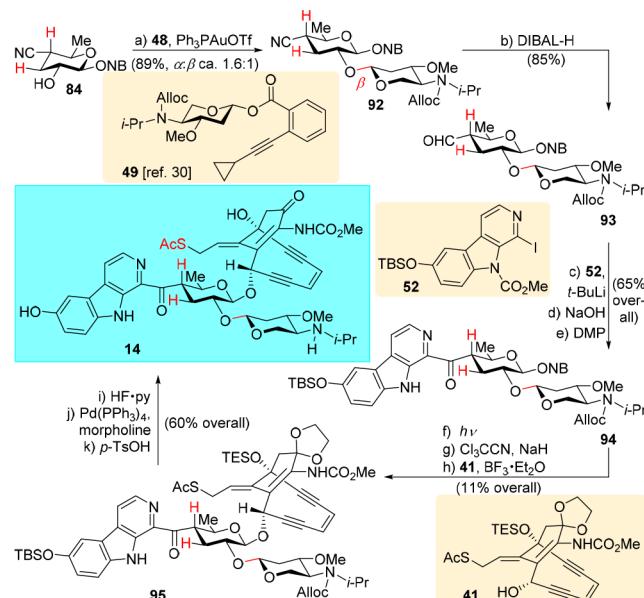
Scheme 10 summarizes the construction of β -carboline truncated and simplified shishijimicin A analogue 13 whose

Scheme 10. Synthesis of β -Carboline Truncated Analogue 13^a

^aReagents and conditions: (a) $h\nu$, THF/ H_2O (10:1, *v/v*), 0 °C, 2 h; (b) $\text{Cl}_3\text{CCN}/\text{CH}_2\text{Cl}_2$ (1:10, *v/v*), DBU (0.1 equiv), 0 °C, 0.5 h; (c) 41 (1.0 equiv), $\text{BF}_3\cdot\text{Et}_2\text{O}$ (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) $\text{Pd}(\text{PPh}_3)_4$ (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 ($h\nu$), 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 protocol⁵⁵ was employed to couple glycosyl acceptor 84 (for

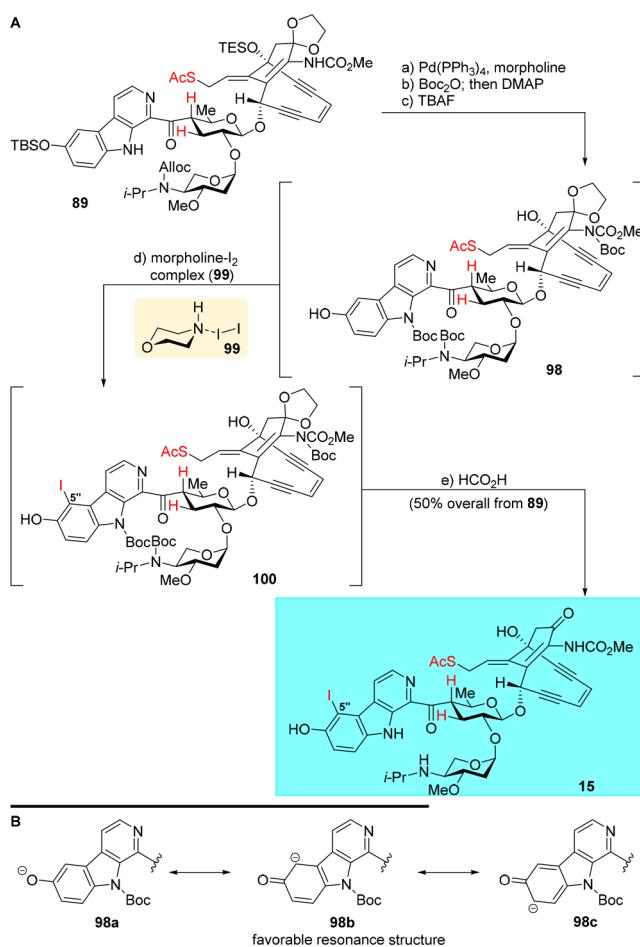
Scheme 11. Synthesis of the β -Anomeric Isomer of 11: Analogue 14^a

^aReagents 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) $h\nu$, THF/ H_2O (10:1, *v/v*), 0 °C, 4.5 h; (g) NaH (2.0 equiv), $\text{Cl}_3\text{CCN}/\text{CH}_2\text{Cl}_2$ (1:2, *v/v*), 25 °C, 5 min; (h) 41 (2.0 equiv), $\text{BF}_3\cdot\text{Et}_2\text{O}$ (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) $\text{Pd}(\text{PPh}_3)_4$ (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 49³⁰ 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) $\text{Pd}(\text{PPh}_3)_4$ 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-I₂ 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

Scheme 12. Synthesis of C5'-Iodo-Shishijimicin Analogue 15^a



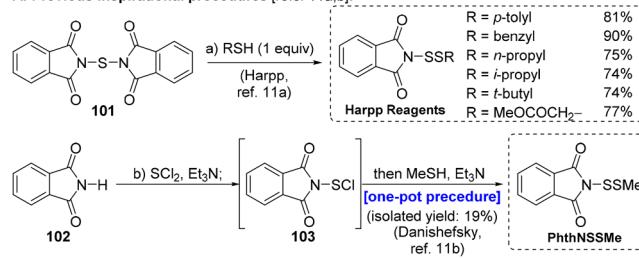
^aReagents and conditions: (a) Pd(PPh₃)₄ (0.5 equiv), morpholine (10.0 equiv), THF, 0 °C, 2 h; (b) Boc₂O (10.0 equiv), MeCN, 80 °C, 36 h; then DMAP (1.0 equiv), 25 °C, 4 h; (c) TBAF (5.0 equiv), THF, 0 °C, 0.5 h; (d) morpholine-I₂ (99, 2.0 equiv), CH₂Cl₂, 25 °C, 20 min; (e) HCO₂H, 25 °C, 12 h, 50% for the five steps.

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).

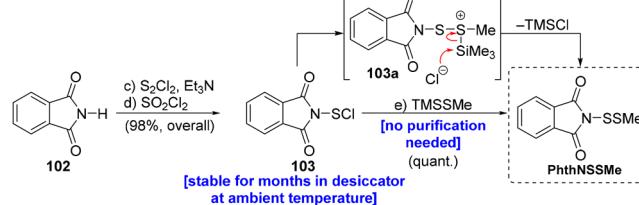
2.3. New Procedures for the Synthesis of Old and New Sulfonylating Reagents and Synthesis of Cysteine Trisulfide Shishijimicin A Analogue 16. During these studies, we recognized a number of issues with the published procedures for the preparation of the methyl Harpp-type reagent (PhthNSSMe).¹¹ The original 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 phthalimidosulfonyl 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 sulfonyl chloride and TMS thioether partners,⁵⁹

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

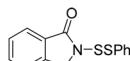
A. Previous inspirational procedures [refs. 11a,b]:



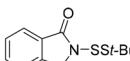
B. This work:



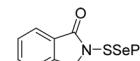
C. Some disulfenylating reagents synthesized:



104 (99%)
[previous report:
22% (ref. 61a), 75% (ref. 61b)]



105 (96%)
[previous report:
74% (ref. 11a), 63% (ref. 61b)]



106 (91%)
[new reagent]

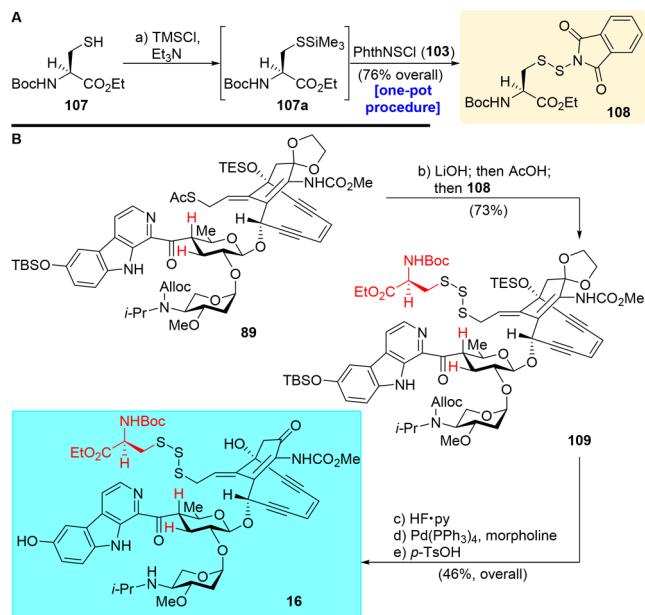
^aReagents and conditions: (a) **101** (1.0 equiv), thiol (1.0 equiv), benzene, reflux, 1.5–22 h, 74–90%; (b) **102** (1.0 equiv), SCl₂ (1.0 equiv), Et₃N (1.0 equiv), CH₂Cl₂, 0 °C, 40 min; then MeSH (1.0 equiv), Et₃N (1.0 equiv), 0 °C, 8 h; then 25 °C, 40 min, 19%; (c) **102** (1.0 equiv), S₂Cl₂ (0.5 equiv), Et₃N (1.2 equiv), THF, 0 to 25 °C, 6 h; (d) SO₂Cl₂ (excess), 70 °C, 12 h, 98% for the two steps; (e) TMSSMe (1.0 equiv), **103** (1.0 equiv), CH₂Cl₂, –78 °C, 0.5 h, quant.

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 S₂Cl₂ in the presence of Et₃N, followed by exposure of the resulting bis(1-phthalimidyl)disulfane to excess SO₂Cl₂ 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,¹¹ two previously reported disulfenylating reagents (i.e., **104**^{61b} and **105**^{11a,61b}) and novel phenylselenosulfonylating reagent **106** were successfully synthesized from their corresponding thio- and seleno-TMS ethers (i.e., PhSTMS, *t*-BuSTMS and PhSeTMS⁶²) 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 Et₃N 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



^aReagents and conditions: (a) TMSCl (1.1 equiv), Et₃N (1.1 equiv), CH₂Cl₂, 0 °C, 2 h; then PhthNSCl (103, 1.0 equiv), −78 °C, 0.5 h, 76%; (b) LiOH·H₂O (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(PPh₃)₄ (0.5 equiv), morpholine (10.0 equiv), THF, 0 °C, 2 h; (e) *p*-TsOH (5.0 equiv), THF/H₂O/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·H₂O, 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 293T⁶⁴ (immortalized human embryonic kidney cells) using *in vitro* assays and with *N*-acetyl calicheamicin γ_1^1 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_1^1	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

^aMSE 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

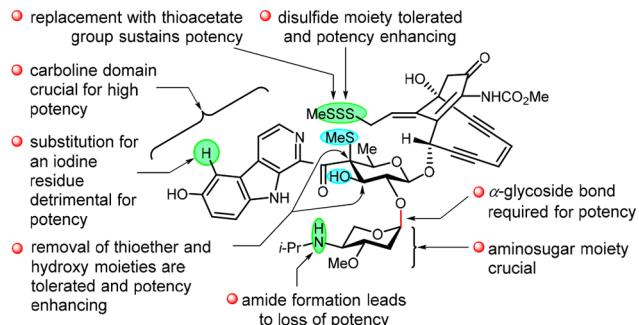


Figure 4. Structure–activity relationships of shishijimicin A.

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_1^1 ,⁹ and esperamicin A₁.¹⁰ A number of improvements were also made in the processes leading to the synthesis of other fragments of the molecule and of the key sulfonylating 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

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/jacs.8b06955](https://doi.org/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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