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Total Synthesis of the Proposed Structure of Mycobactin J

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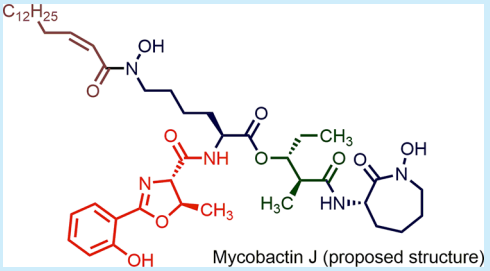
Supporting Information

ABSTRACT: The total synthesis of the proposed structure of mycobactin J (MJ), a metabolite of *Mycobacterium tuberculosis*, is presented. The highlights of the synthesis include a careful control of the *Z*-stereochemistry of the unsaturated long chain fatty acid, a biomimetic construction of the oxazoline building block and the carriage of an unprotected phenol throughout the synthesis.



Tuberculosis (TB) is one of the world's most predominant diseases, with over one-fourth of the global population being affected.[1] *Mycobacterium tuberculosis* is the assassin bacterium responsible for TB, and in recent years, considerable research has been focused on identifying metabolites of *Mycobacterium* sp. as potential drug-delivery candidates.[2] Siderophores that contain hydroxamic acid residues are key systems for the survival of the *Mycobacterium* sp. Siderophores such as mycobactins (metabolites of *Mycobacterium* sp.), formobactin, nocobactin, amamistatin (Figure 1),[2f−k] and

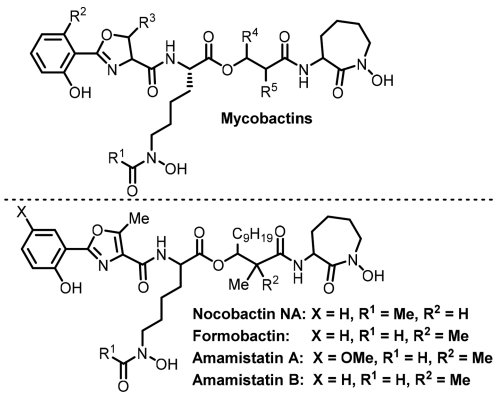


Figure 1. General structure of naturally occurring mycobactins and related compounds.

lasso peptides such as lariatins[3] are some such metabolites which also have the potential to be used in drug delivery systems. These may possess good inhibitory potency against *Mycobacterium* sp. as well as against other bacterial systems and viruses.

In 1970, Snow and co-workers reported the existence of a variety of naturally occurring mycobactins.[2a] In 1982, mycobactin J (MJ) was isolated and characterized by Merkal and co-workers, and in the year 2001, De Voss and co-workers proposed a revised structure for mycobactin J (**1**, Scheme 1).[4] Almost all mycobacterial cell walls are lipid-rich and contain a large amount of hydrocarbon content including fatty acids, which produce an exceptionally tightly packed array, and this limits the ability of many of drugs to penetrate inside the cell.[5] By applying the "Trojan horse" approach, conjugate systems with siderophores and other drugs have been developed, where siderophores act as drug-delivery agents for mycobacterial infections as well as killing of cancer cells.[6] Miller and co-workers prepared one such conjugate of mycobactin T with artemisinin, and it displayed good activity against malaria as well as tuberculosis.[6a] The mycobacterium produces siderophores such as mycobactins to harvest iron from the environment, since the metal is a key nutrient and vital for metabolic function as well as for cell division of the bacterium.[2d,7a,b] Such iron-acquiring siderophores are also routinely used for the treatment of iron-overloaded patients.[7c] Groves and co-workers reported a mycobactin-mediated iron-acquisition pathway within macrophages, which could represent a new target for the control of mycobacterial infection.[8]

Depending upon their chemical structures, mycobactins demonstrate either growth promoting or inhibitory activity against MTB.[9] Among all of the naturally occurring mycobactins isolated so far, MJ relatively possesses the most complex structure. To the best of our knowledge, there has been no report of the total synthesis of MJ to date. The challenges associated with the geometry and reactivity of the olefin side chain, present as a (*Z*)-*α*,*β*-unsaturated hydroxamic acid, and the assembly of the molecule overall drew us to attempt its total synthesis. For this, we planned to investigate the convergent/submonomer approach depicted in Scheme 1. Two fragments **4** and **5** would be synthesized from commercially available Cbz-lysine (**6**) via a common

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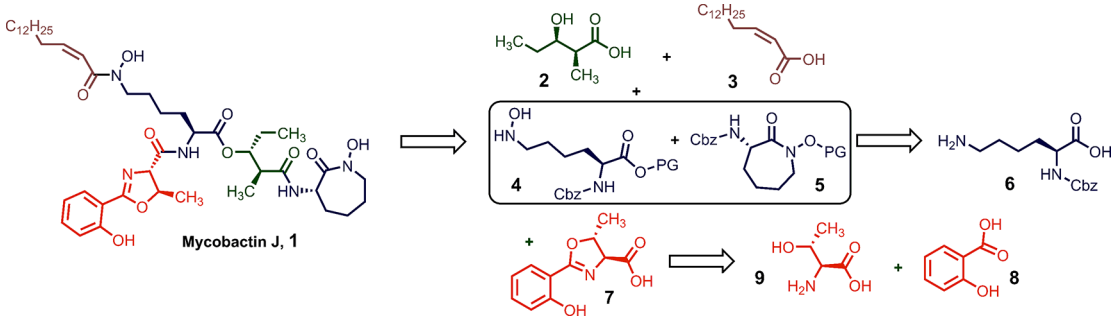
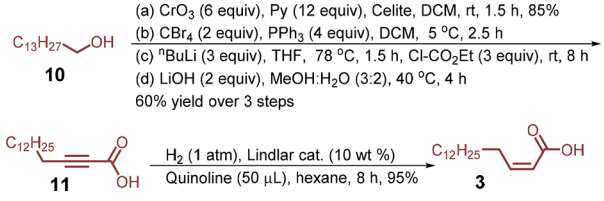
Scheme 1. Retrosynthetic Analysis



intermediate, with the oxazoline synthon **7** being obtained from salicylic acid and threonine by following a biomimetic cyclization approach in which the β-C of the threonine would retain its configuration.[10] This is one of the critical chiral centers present in this mycobactin, which cannot be obtained via regular acid- or DAST-mediated cyclization.[11] The submonomer **2**, the chiral 3-hydroxypentanoic acid derivative, can be accessed easily via the Evans aldol reaction,[12] and the crucial (*Z*)-pentadec-2-enoic acid **3** can be derived via the Corey–Fuchs reaction[13] and subsequent Lindlar reduction.

Our synthesis started with the construction of the (*Z*)-long chain fatty acid **3**. The aldehyde synthesized from commercially available 1-tetradecanol (**10**) was subjected under Corey–Fuchs reaction conditions,[13] followed by quenching with ethyl chloroformate and hydrolysis, which delivered the alkyne acid **11** in good overall yield (Scheme 2). Reduction of **11** using Lindlar catalyst yielded **3** in almost quantitative yield.

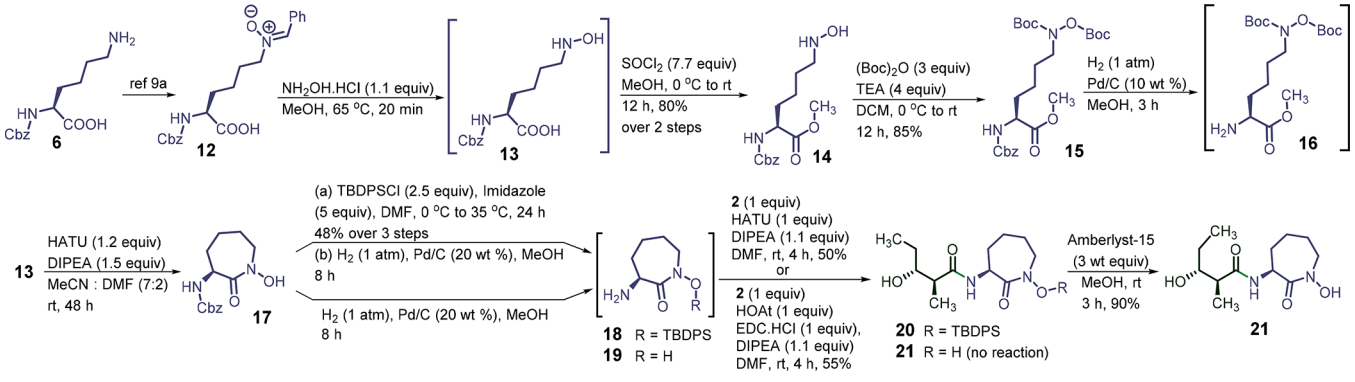
Scheme 2. Synthesis of Long-Chain Acid



The nitrone **12** precursor was synthesized via a protocol developed by Miller and co-workers (Scheme 3).[9a] Treatment of this nitrone with hydroxyl amine hydrochloride and $SOCl_2$-mediated esterification delivered the desired ester **14**. Subsequent di-Boc protection provided the linear component **15** in good yield. The synthesis of **13** was accomplished by heating **12** with hydroxylamine hydrochloride in MeOH at reflux followed by a quick HATU-promoted cyclization and TBDPS protection to afford the cyclolysine component **18**. We then took up the construction of the eastern building block, cobactin **21** (Scheme 3). Hydrogenolysis of **18** followed by HATU/EDC.HCl-mediated coupling with the chiral pentanoic acid **2** led to the silyl-protected cobactin **20**. The unprotected cobactin **21** was obtained in good yield when **20** was treated with Amberlyst-15. Attempts to obtain **21** directly via coupling of **19** with the hydroxy acid **2** led to failure.

For the construction of the western building block mycobactic acid **27b**, salicylic acid was coupled with the HCl salt of L-threonine methyl ester **22** to afford the amide **23** (Scheme 4). Catalytic, ammonium molybdate-mediated dehydrative biomimetic cyclization with retention of configuration at the β-position of the threonine led to oxazoline ester **24**, which was then saponified to obtain the oxazoline acid **7**.[10] This was used as such for the next step without any purification. The amine **16** which was obtained by Pd/C-mediated hydrogenolysis of **15** (Scheme 3) was coupled with acid **7** using EDC·HCl to achieve **25a** in good yield (Scheme 4). Controlled di-Boc cleavage using TFA followed by HATU/DIPEA-mediated coupling with acid **3** led to the largest building block, the diacylated product **26a** (Scheme 4), with trace quantities of the monoacylated product **27a**. The control of pH in the reaction to obtain **26a** via the coupling reaction was found to be very critical, with several of our attempts resulting in scrambling of the olefin geometry as well as some conjugate addition. After considerable optimization, DIPEA (7 equiv) and the unsaturated acid (2.7 equiv) were found to prevent the olefin isomerization and provide an improved yield of **26a**. DIPEA-mediated transesterification followed by saponification delivered mycobactic acid **27b**.

Scheme 3. Synthesis of Lysine-Based Fragment and Cobactin



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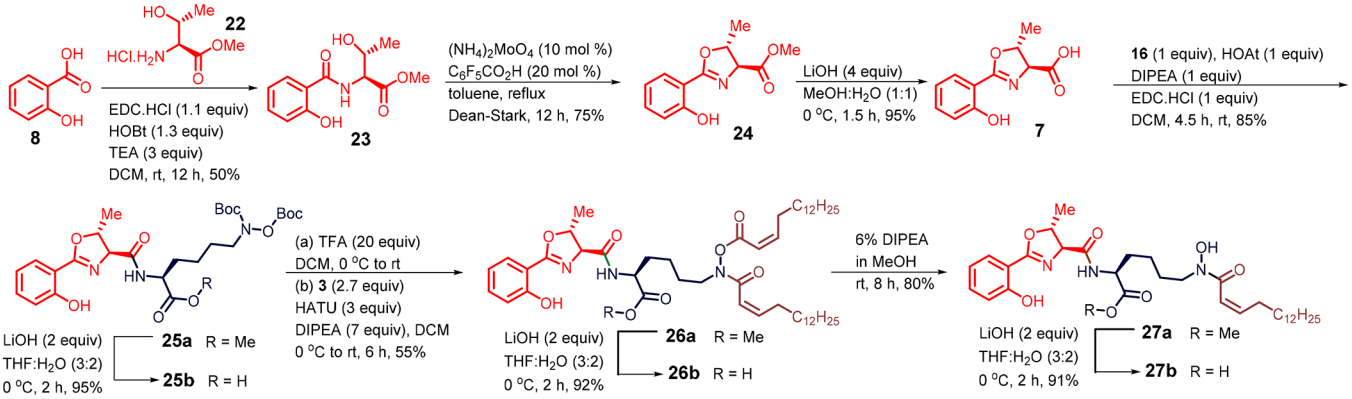
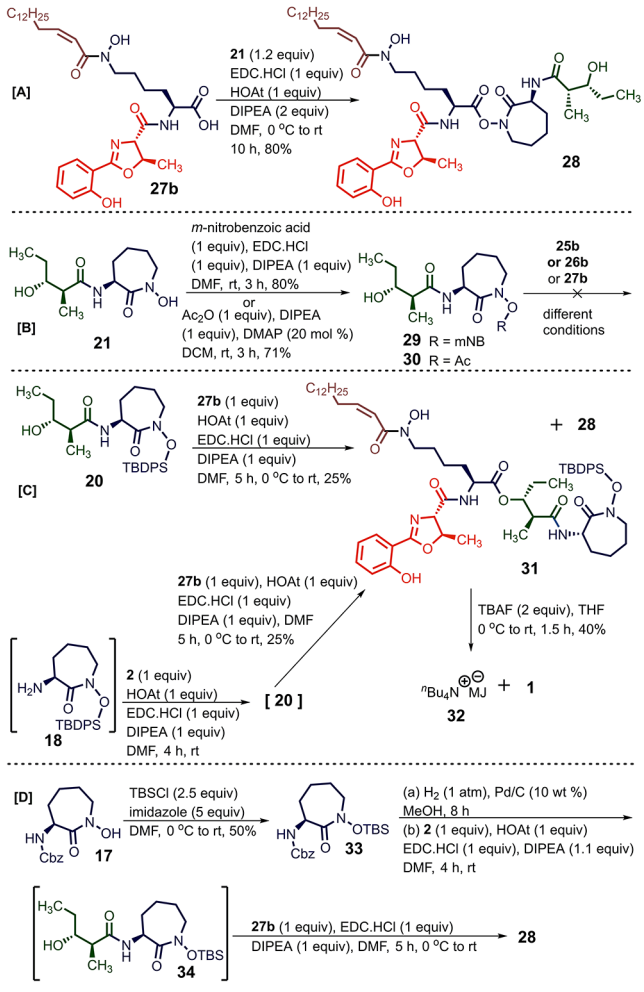
Scheme 4. Synthesis of Mycobactic Acid and Related Acids



We then attempted the final esterification to reach the target molecule. Inspired by the synthesis of different mycobactin analogues developed by Miller and co-workers,[9a,14] the final esterification was attempted by EDC·HCl-mediated coupling of mycobactic acid **27b** with the unprotected cobactin **21** (Scheme 5A). A careful NMR analysis of the product suggested that instead of the desired esterification, this coupling occurred at the eastern N(OH) of cobactin **21** to yield **28**, which was further confirmed by formation of methyl ester of mycobactic acid **27a** via a DIPEA-mediated trans-esterification reaction of **28**. From the above information, we realized that protection of the N-OH would be necessary, and accordingly, we prepared *m*-nitrobenzoate and acetate-protected cobactins **29** and **30** (Scheme 5B). However, attempts at esterification of these two cobactin derivatives with mycobactic acid **27b** as well as acid **26b** (to form the *O*-acyl-protected MJ) failed.[15] Coupling of **29** and **30** with acid **25b** to form the ester and di-Boc-protected mycobactin analogues resulted in failure even when a variety of peptide coupling agents as well as Yamaguchi esterification were tried (Scheme 5B).[15] When these esterification approaches failed to produce the desired result, we envisaged an approach for coupling TBDPS-cobactin **20** and mycobactic acid **27b**.[15] EDC·HCl-mediated coupling of **27b** and **20** gave the best yield of TBDPS protected-MJ **31** along with the undesired product **28** and some intramolecular-cyclization product of acid **27b** (Scheme 5C). The yield of the product **31** was improved when the TBDPS-protected cobactin **20** was prepared in situ. Further optimization to improve the yield of the final esterification, including the coupling between acid **26b** with TBDPS-protected cobactin **20**, were attempted.[15] AOP-mediated coupling between **26b** and **20** delivered **31** in a trace amount, instead of the TBDPS-protected diacyl-MJ. During purification of **31**, we faced considerable issues in the separation of the unreacted **20** from **31**. We therefore decided to go ahead with removing the TBDPS group. Based on our earlier experiences of double-bond isomerization in the synthesis of **26a** and **28**,[16] we avoided the use of TFA or AcOH. TBAF worked the best, delivering MJ (**1**) along with some unidentified side products.[15] ^{1}H NMR indicated that a considerable amount of the tetrabutylammonium ion pair of MJ **32**[17] along with MJ **1** was present (Scheme 5C). Attempts to exchange the ions using Amberlyst-15 or Dowex-50 resins[17] were unfortunately unsuccessful. To overcome this problem, we synthesized the TBS-protected cobactin **34** and coupled it with acid **27b**, but no TBS-protected MJ was detected; only the undesired product **28** was formed (Scheme 5D).[18]

Scheme 5. Coupling of Mycobactic Acid as Well as Related Acids with Cobactin and Elaboration of the Synthesis of MJ



At this stage, we then altered our synthetic strategy (Scheme 6) and planned the deprotection of the silyl protecting group and the two Boc groups from **36** simultaneously in a single step. Installation of the long-chain acid **3** at the final stage to get **35** and deprotection of the acyl group by trans-esterification (for mono- or di-N,O-acyl group) would yield MJ.

Toward this end, TBDPS/TBS-protected cobactin was employed for the union with acid **25b** under several

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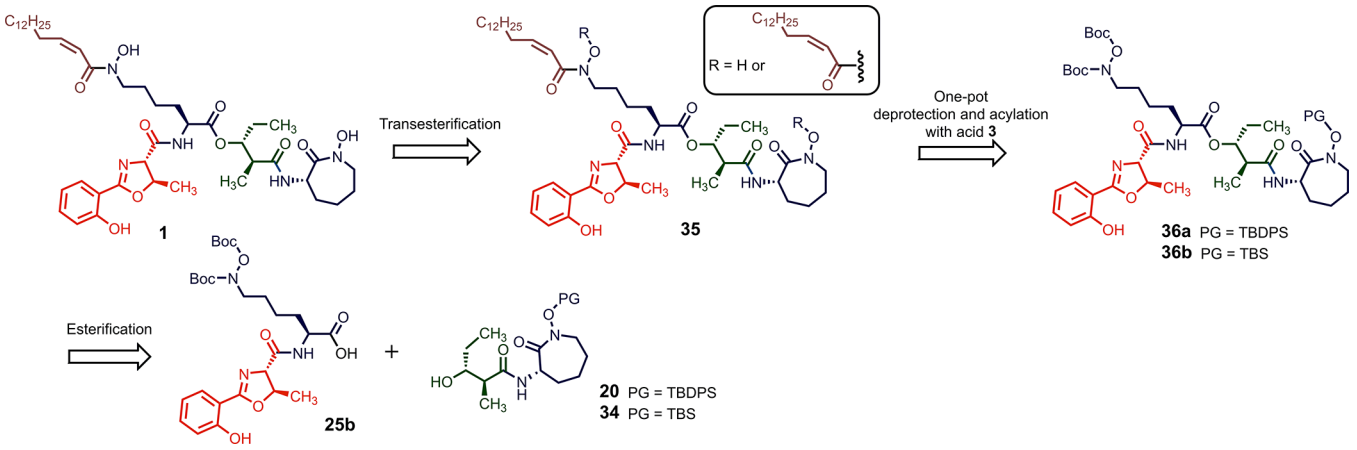
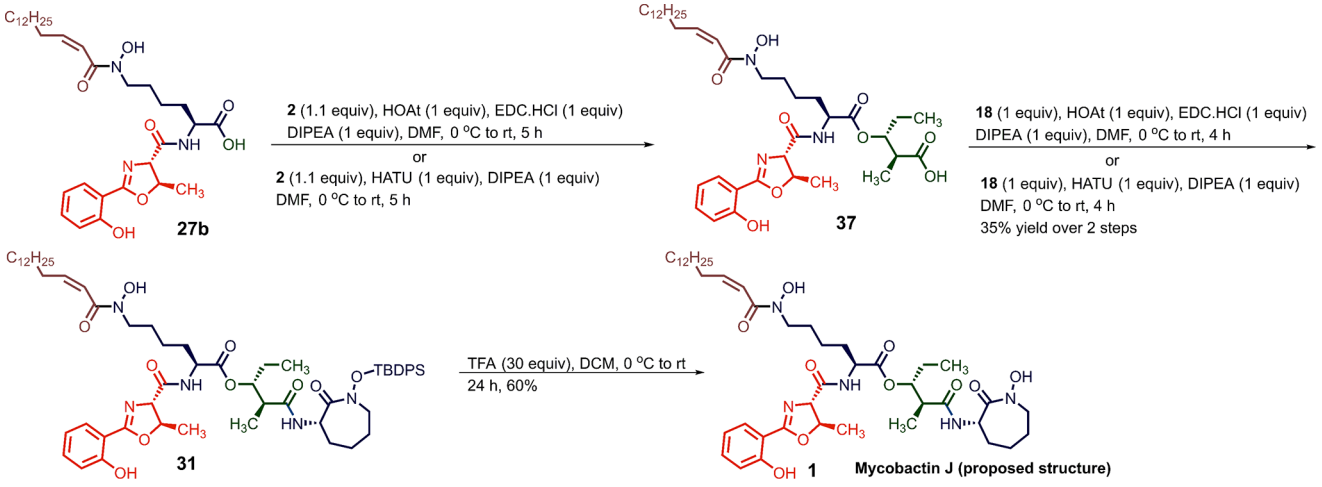
Scheme 6. Modified Retrosynthesis



Scheme 7. Synthesis of Mycobactin J



esterification conditions, but unfortunately for us, none of these attempts yielded the desired product.[15]

To circumvent these unforeseen difficulties, we detoured from the esterification reaction of the cobactin and mycobactic acid (Scheme 7). We then explored EDC·HCl/HATU-mediated coupling of mycobactic acid **27b** with the chiral acid **2**. The resulting acid **37** was freed from the unreacted pentanoic acid **2** by simple workup (which indirectly also solved the problem of separation of **20** from **31**, faced at the earlier stage). EDC·HCl/HATU-mediated coupling of **37** with **18** furnished **31**. At this deprotection stage, treatment of **31** with TFA yielded MJ-TFA salt in good yield with no isomerization of the double bond. The silyl trifluoroacetate was washed out with pentane was followed by an aqueous $NaHCO_3$ wash to yield **1** as a free base in 50% yield. Further attempts at purification over silica gel led to considerable decomposition of the compound, thereby decreasing the yield of the product. This product contained trace amounts of other impurities which could not be separated. At this stage, when the ^1H NMR of the synthetic sample was compared with a commercial sample in $CDCl_3$ and as well as MeOH-d_4, it was found that peaks at certain regions did not concur with each other (see the SI for details). First, peaks for the proton of the ε-C of the two-lysine parts between δ 3.5−4.0 ppm were found to be broader in synthetic MJ as compared to the authentic sample. For the authentic sample, a well-resolved peak was observed at δ 2.6 ppm, corresponding to the proton of β-C of the chiral acid part; this was observed as a broad peak in synthetic MJ. One more deviation was observed for the doublet peak at δ 1.2 ppm present in the authentic compound; in the synthetic sample, it was observed as a set of rotamer peaks. Such rotamer peaks were also observed for the advanced stage building blocks acid **26b**, ester **27a**, and mycobactic acid **27b**. It is also observed that the α-protons of amino acids (which usually are not isomerized under standard coupling reactions) showed deviations from the authentic one. At this stage, the exact structure of MJ remains elusive to us, and detailed studies to prove the actual structure of **1** are currently in progress via the synthesis of other diastereomers.

To conclude, the first total synthesis of the proposed structure of mycobactin J is described. Our synthetic approach should allow explorations of other members of the mycobactin family. The highlight of the work is the design of the pathway to overcome the repeated scrambling of the critical *Z*-olefin geometry as well as problems associated with conjugate addition to the *Z*-olefin and the choice of the crucial silyl protecting group.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b02832.

Experimental details and spectral characterization of all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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(15) See the Supporting Information for details.

(16) Use of 1 equiv of DIPEA in the coupling led to *E*-product **28**.

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(18) Cobactin **34** was confirmed by HRMS and by crude ^{1}H NMR (not given in the Supporting Information); purification on column led to deprotected cobactin **20**.

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