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Synthesis of the Aminovinylcysteine-Containing C-Terminal Macrocycle of the Linaridins

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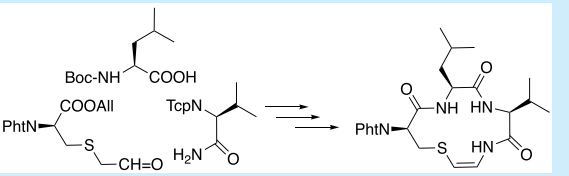
ABSTRACT: *N*-Phthalimido-D-cysteine allyl ester was *S*-alkylated with 2-iodoethanol. The derived β-thioaldehyde was condensed with *N*α-tetrachlorophthalimidovalinamide to afford a *Z*-thioenamide. Removal of the tetrachlorophthalimido protecting group and homologation with *N*-Boc-L-leucine afforded the linear tripeptide. Removal of the Boc and allyl protecting groups, followed by carbodiimide-mediated cyclization, led to the 13-membered ring with the aminovinylcysteine moiety embedded. This constitutes the C-terminal macrocycle of all known members of the linardin family of peptides, including the antileukemia agent, cypemycin.



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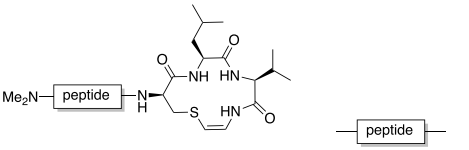
The linaridins[1] are a family of peptides that characteristically feature a macrocycle, containing an aminovinylcysteine residue, at their C-terminus (Figure 1), and an *N*,*N*-dimethylated-alanine residue at the N-terminus. The prototypical linaridin is the antileukemia agent, cypemycin (**1**).[2] Grisemycin (**2**) is three residues shorter but highly homologous.[3] Recently, the salinipeptins were reported by Fenical and co-workers.[4] Salinipeptin A (**3**) contains a single $Val^8 \rightarrow Ala^8$ substitution relative to cypemycin; however, nine of the amino acids are of the D-configuration (bold/italicized in Figure 1). Salinipeptins B–D have variations at the N-terminus, and D contains a $Dhb^7 \rightarrow Thr^7$ substitution. All contain the common macrocycle depicted in Figure 1.

There has been considerable interest in the biosynthesis of cypemycin, notably the recent work of Zhang and co-workers.[5] Biosynthesis of the AviCys unit in lantibiotics has been reviewed.[6,7] A chemical synthesis of the macrocycle would provide a common advanced intermediate to the synthesis of the entire linaridin family. Moreover, the chemistry would ultimately be applicable to other AviCys-containing peptides, including members of the lantibiotic family.[8] With regard to the chemical synthesis of the AviCys motif, VanNieuwenhze and co-workers reported a decarbonylative elimination in peptide substrates[9] and applied this to the synthesis of the C-terminal ring of the lantibiotic, mersacidin.[10] Inspired by thioviridimide,[11,12] Castle and co-workers reported the addition of thiyl radicals to ynamides[13] but have yet to report the synthesis of a true AviCys residue. We recently described the Lewis-acid-promoted condensation of acetamide with an acetal, or aldehyde, to produce a *Z*-thioenamide.[14] Calculations predicted, and experiment confirmed, that stereoelectronic effects favored formation of the *Z*-thioenamide. Specifically, aldehyde **5** could be condensed with acetamide to give thioenamide **6** in reasonable yield and with acceptable stereoselectivity for the *Z*-double bond (Scheme 1).



cypemycin (**1**) -Ala-Dhb-Pro-Ala-Dhb-Pro-Dhb-Val-Ala-Gln-Phe-Val-*alleu*-Gln-Gly-Ser-Dhb-*alleu*-

grisemycin (**2**) -Ala-Dhb-Pro-Dhb-Val-Ala-Gln-Phe-Val-Ile-Gln-Gly-Ser-Dhb-Ile-

salinipeptin A (**3**) -Ala-Dhb-***Pro***-Ala-Dhb-***Pro***-Dhb-***Ala-Ala-Gln***-Phe-Val-***Ile-Gln***-Gly-Ser-Dhb-***Ile***-

Figure 1. Selected linaridins [Dhb = *E*-dehydrobutyric acid; D-residues bold/italicized].

Throughout our studies on the mechanism and optimization of this reaction, we yearned to apply our knowledge to a peptidyl amide and thus the construction of the AviCys motif. One of our early experiments involved condensation of acetal **4** with alaninamide **7**. Instead of the anticipated thioenamide **9**, imidazolidinone **8** was formed, via trapping of the putative carbocationic intermediate by the carbamate nitrogen. This key experiment signaled that the α-amine of our amino amide reaction partner needed to be emasculated as a nucleophile, via double protection. After much experimentation, we arrived at the tetrachlorophthalimido (Tcp) group to play this role, viz., amide **10**. Unfortunately, during the execution of these experiments, we realized that our cysteine building blocks **4** and **5** were essentially racemized, giving rise to diastereomeric mixtures of imidazolidinone **8** and thioenamide **11**.

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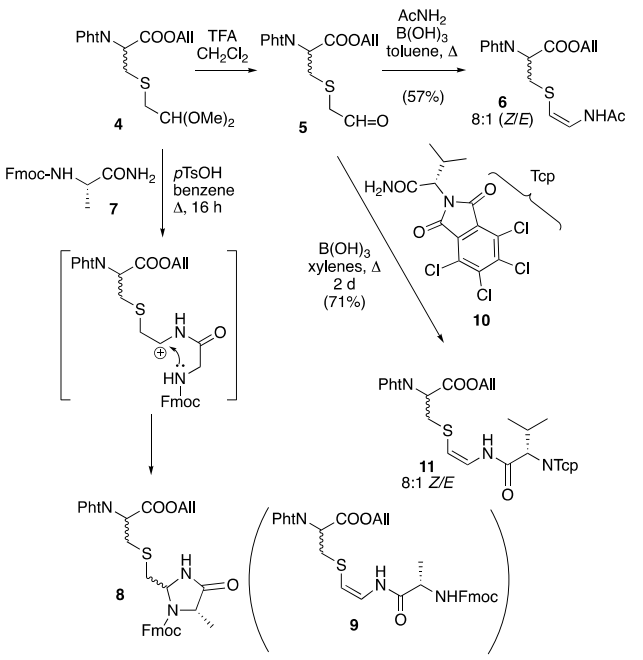
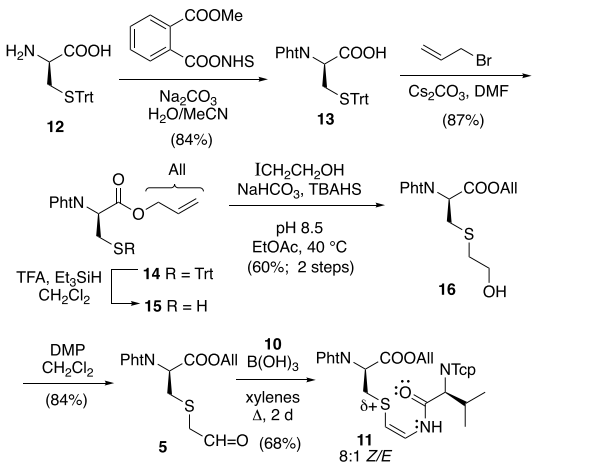
Scheme 1



Careful evaluation of each step in our previous synthesis of aldehyde **5**[14] informed us that two steps were accompanied by substantial racemization at Cα: phthalimido protection (phthalic anhydride, Et_3N, toluene, Δ) and alkylation of the side chain thiol (2-bromo-1,1-dimethoxyethane, Cs_2CO_3, DMF). The production of enantioenriched material required substantial experimentation vis-à-vis alternative reaction conditions and the order of events (Scheme 2).

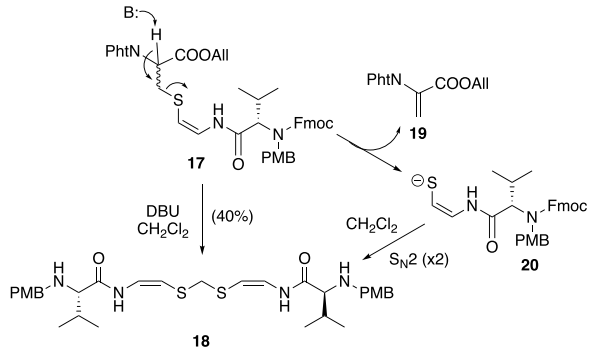
Scheme 2



N-Protection of H-D-Cys(Trt)-OH (**12**) with methyl 2-[(succinimidooxy)carbonyl]-benzoate (MSB) according to Casimir et al.[15] required a free carboxylic acid. We hypothesize that the acid reacts with the *N*-hydroxysuccinimide ester to give a mixed anhydride that then delivers the acylating agent intramolecularly. Formation of the allyl ester **14** was followed by cleavage of the trityl thioether. Recognizing that alkylation of the thiol **15** might be achieved, with retention of stereochemical integrity, under the mild phase-transfer conditions of Zhu and Schmidt,[16] we were disappointed to find that 2-bromo-1,1-dimethoxyethane was unreactive. Since our penultimate goal was an aldehyde in the side chain, we invoked 2-iodoethanol as the electrophile to afford **16** that was readily oxidized to give aldehyde **5**. 2-Iodoethanol was more reactive than 2-bromo-1,1-dimethoxyethane, and the primary alcohol facilitated solubility in the biphasic reaction mixture. Alcohol **16** was determined to be 84% ee by comparison with racemic material prepared via a different route. With enantioenriched aldehyde **5** in hand, thioenamide formation proceeded as expected to give **11**. The olefinic protons in **11** have a relatively small coupling constant of 7.4 Hz, reminiscent of those on a double bond in a 5-membered ring (5−7 Hz)[17] and consistent with our earlier natural bond orbital (NBO) analysis that showed a partial positive charge on the sulfur and electrostatic interactions with the nitrogen and oxygen of the amide.[14] While the signal for the proton proximal to sulfur is obscured by those of the allyl group, the ═C**H**NH signal is a sharp doublet of doublets at δ 6.78 ppm, exhibiting additional splitting by the amide proton (J = 10.8 Hz). The ^{13}C NMR spectrum showed signals at δ 102.8 and 134.6 ppm, for the S**C**H═ and NH**C**H═ carbons, respectively.

While thioenamides have been isolated from nature, next to nothing is known about their tolerance to manipulations during synthesis, so subsequent steps were undertaken with this uncertainty, particularly under the specter of β-elimination of the α-proton and enethiolate. For example, during attempted Fmoc-deprotection of thioenamide **17**, methylene-bridged dimer **18** was isolated from the reaction mixture (Scheme 3). Apparently, the enethiolate anion arising from elimination reacts with dichloromethane (solvent) twice to produce **18**.

Scheme 3



With a view to extrapolating thioenamide **11** to the 13-membered macrocycle of the linaridins, it was necessary to chemoselectively remove the tetrachlorophthalimido group. Fraser-Reid and co-workers had employed the Tcp group in the carbohydrate arena and had demonstrated that it could be removed, in the presence of a regular phthalimido (Pht) group,[18] using ethylenediamine (pK_a of conjugate acid is 9.98).[19] Not surprisingly, treatment of **11** with ethylenediamine led to complex mixtures.[20] However, we found that treatment with a single equivalent of hydrazine (pK_a of $H_2NNH_3^+$ 8.10)[19a,21] led to a single product within 10 min. This turned out to be the ring-opened acylhydrazide intermediate **21** (Scheme 4). Gentle treatment with acid was required to expel 5,6,7,8-tetrachloro-2,3-dihydro-1,4-phthalazinedione. Following much experimentation and optimization of these conditions, direct acylation with Boc-Leu-OH gave a

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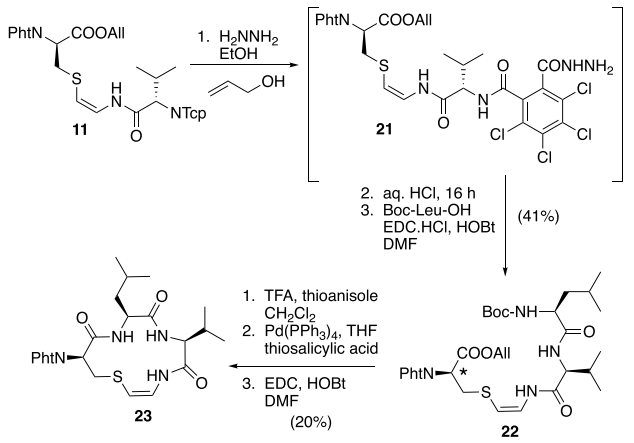
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 4



moderate yield of the tripeptide **22** over two steps. At this point, the diastereopurity had regressed to a 75:25 mixture vis-à-vis Cα (*) of the Cys residue, as determined by integration of well-resolved signals in the proton NMR spectrum (viz., Val Hα and the thioenamide NH).

What remained was deprotection of the N- and C-termini and cyclization. Both deprotections required modifications to standard reaction conditions due to the sensitivity of the thioenamide. Liberation of the N-terminus, with TFA and triethylsilane as the scavenger, led to partial reduction of the thioenamide double bond, as evidenced by the disappearance of the characteristic thioenamide signals in the proton NMR spectrum and the observation of an (M + 2) peak in the mass spectrum of crude product mixtures. Substituting thioanisole for triethylsilane circumvented this side reaction. Deallylation, catalyzed by palladium(0), with barbituric acid as the allyl acceptor resulted in ~40−50% of the sulfur in the thioenamide being converted to the sulfoxide oxidation state. Instead, thiosalicylic acid was employed as both the allyl acceptor[22] and a sacrificial reductant. It proved advantageous to perform the Boc deprotection ahead of the allyl ester cleavage. Fortuitously, the linear peptidyl amino acid was water soluble, and the reaction byproducts could be extracted into chloroform. Lyophilization of the aqueous layer gave the semipure precursor to the macrocycle. We anticipated that the Z-configuration of the thioenamide would favor conformations of the linear peptide that would be amenable to cyclization.[23] Cyclization was induced by EDC/HOBt with a peptide concentration of ~2 mM in DMF. Following reaction overnight, the mixture was diluted with water and lyophilized and the product isolated by RP-HPLC in 20% overall yield.

Remarkably, macrocycle **23** was obtained as a single species by NMR, despite the fact that the linear precursor **22** was a 75:25 mixture of D/L at Cα of Cys. It has long been recognized that the cyclization of linear tetra-, penta-, and hexapeptides is often sufficiently slow and that the rate of epimerization of the C-terminal residue is competitive. Indeed, Kessler and Kutscher reported complete epimerization of the tyrosine residue upon cyclization of H-Arg(NO_2)-Lys(Z)-Asp(OBn)-Val-Tyr-OH with DCC/DMAP to produce cyclo[Arg(NO_2)-Lys(Z)-Asp(OBn)-Val-D-Tyr], a thymopentin analogue.[24] Attempted cyclization of H-Pro-Val-Pro-Tyr-opfp, by Schmidt and Langner, led to a 31% yield of cyclo(Pro-Val-Pro-D-Tyr).[25] It seems likely, therefore, that something akin to dynamic kinetic resolution is taking place during the cyclization of **22**, leading to **23** with the naturally occurring D-configuration exclusively.

Interestingly, the chemical shifts and coupling constants for the thioenamide functional group did not change much, upon cyclization. Calculations and observations reported by Shang et al.[4] concur with a rigid conformation of the AviCys residue, in which one of the diastereotopic Hβ protons is coupled to Hα and the other coupling is not observed. In the case of **23**, Hβ' (δ 3.12) is coupled to Hα (^3J = 5.2 Hz), while Hβ exhibits only geminal coupling to Hβ' but a strong correlation to the S−C**H**= proton in the NOESY spectrum. All three amide protons were slow to exchange in CD_3OD, with Val NH exhibiting cross peaks with both Leu NH and AviCys NH in the NOESY spectrum. No correlation was observed between Leu NH and AviCys NH. These insights into the conformation in the region of the AviCys residue are depicted in Figure 2.

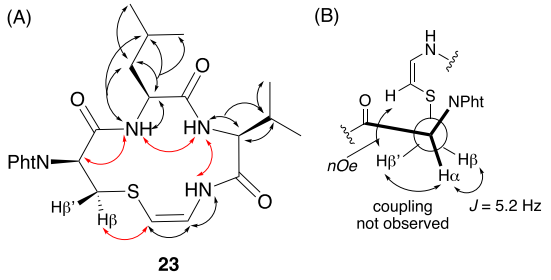


Figure 2. (A) Correlations observed in the NOESY spectrum of compound **23** (red of special interest). (B) Newman projection, looking down the Cα−Cβ bond of the AviCys residue.

In summary, we have constructed the aminovinylcysteine (AviCys) motif in the center of a tripeptide. Deprotection of the N- and C-termini and cyclization led to the C-terminal macrocycle of cypemycin and other linaridins. The cyclization favors the D-configuration of the AviCys residue, as is observed in nature. Nuclear magnetic resonance experiments indicate a well-defined, single conformation for the macrocycle. These accomplishments set the stage for further investigation of AviCys and peptides into which it is incorporated.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c00218.

Detailed experimental procedures for the synthesis of compounds **5**, **8**, **10**, **11**, **13**−**18**, **22**, and **23**. ^{1}H and ^{13}C NMR spectra for those compounds, plus 2D NMR spectra for macrocycle **23**. HPLC chromatograms for compound **16** (ee determination) and the deallylation of compound **22** (PDF)

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Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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