Tistrellabactins A and B Are Photoreactive C-Diazeniumdiolate Siderophores from the Marine-Derived Strain *Tistrella mobilis* KA081020-065

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**ABSTRACT:** The C-diazeniumdiolate group in the amino acid graminine is emerging as a new microbially produced Fe(III) coordinating ligand in siderophores, which is photoreactive. While the few siderophores reported from this class have only been isolated from soil-associated microbes, here we report the first C-diazeniumdiolate siderophores tistrellabactins A and B, isolated from the bioactive marine-derived strain *Tistrella mobilis* KA081020-065. The structural characterization of the tistrellabactins reveals unique biosynthetic features including an NRPS module iteratively loading glutamine residues and a promiscuous adenylation domain yielding either tistrellabactin A with an asparagine residue or tistrellabactin B with an aspartic acid residue at analogous positions. Beyond the function of scavenging Fe(III) for growth, these siderophores are photoreactive upon irradiation with UV light, releasing the equivalent of nitric oxide (NO) and an H atom from the C-diazeniumdiolate group. Fe(III)-tistrellabactin is also photoreactive, with both the C-diazeniumdiolate and the β-hydroxyaspartate residues undergoing photoreactions, resulting in a photoprotein without the ability to chelate Fe(III).

The synthesis of N–N bonds are chemically challenging, yet microbes have mastered this transformation, producing hundreds of structurally complex natural products with an N–N bond. Reports of natural products with N–N linkages and their biosyntheses have garnered significant recent attention, including the C-type diazeniumdiolate amino acid, graminine (Gra, Figure 1). L-Gra was recently shown to originate from L-Arg, although the mechanism of this oxidative rearrangement has not yet been elucidated. The C-diazeniumdiolate group has emerged as a new class of Fe(III) binding ligands in microbial siderophores, adding to the well-established catecholate, hydroxamate, and α-hydroxycarboxylate groups. Siderophores are small-molecule microbial natural products that have evolved to facilitate iron sequestration and microbial iron uptake. Biological competition for available iron has driven the evolution of a large selection of siderophores, with hundreds identified thus far, yet siderophores with a C-diazeniumdiolate ligand have only just begun to be uncovered. Up to this point, graminine-containing siderophores have only been isolated from soil-associated microbes within the related Burkholderiaceae family, including graminibactin (Gbt, Figure 1) from *Pseudomonas fluorescens* DSM 17151.

The N–N bond in the diazeniumdiolate moiety is particularly intriguing in the context of nitric oxide (NO) release. N-Type diazeniumdiolates (NONOates) lose two equivalents of NO under physiological conditions, while C-diazeniumdiolates are more thermally stable. We recently demonstrated that C-diazeniumdiolate siderophore Gbt releases the equivalent of NO and an H atom for each Gra residue when irradiated with UV light, producing a mixture of E and Z oxime isomers as the photoproduct. Additionally, peroxidase-mediated NO release from Gbt has been reported. Given the relevance of NO and oximes in physiological signaling pathways and defensive responses, this ligand system may serve multiple functions within the microbe beyond Fe(III) scavenging.

The biosynthetic genes encoding the enzymes that synthesize L-Gra have been identified in the biosynthetic gene cluster (BGC) for graminibactin as *grbD* and *grbE* through gene knockout studies with the strain *P. graminis* DSM 17151. *GrbE* shares sequence homology to several Arg hydroxylases (DcsA, Mhr24, AglA), while *GrbD* shares sequence homology to the cupin domain of the N–N bond, forming enzyme ZnF to ZnF, which carries out the oxidative rearrangement of N-hydroxy-N'-hydroxy-N'-methyl-L-Arg. Targeted discovery of new C-diazeniumdiolate siderophores...
is achievable by searching microbial genomes containing a nonribosomal peptide synthetase (NRPS) adenylation (A) domain with the specificity code for Gra (DVHRTGLVAK) and FASTA sequences of grbD and grbE as queries. With this strategy, genome mining efforts reveal a widespread prevalence of L-Gra in natural products from a variety of environments, including clinical isolates, plant pathogens, and, as described in this study, a marine-derived microbe.

We report herein *Tistrella mobilis* KA081020-065, isolated from the Red Sea, produces C-diazeniumdiolate siderophores tistrellabactins A and B. The tistrellabactins are photoreactive, losing NO in UV light, including in actively growing cultures of *T. mobilis*. Surprisingly, the tistrellabactin NRPS enzymes load glutamine residues iteratively via an unknown mechanism, which is also observed in the biosynthesis of the anticancer didemnin natural products produced by *T. mobilis* KA081020-065. The characterization of tistrellabactins A and B reveals other unusual biosynthetic features, including a promiscuous NRPS A domain, a 22-membered macrolactone ring, and a 3-hydroxybutyric acid group appended to N-hydroxy-L-ornithine.

### RESULTS AND DISCUSSION

Genome Mining Reveals a Biosynthetic Gene Cluster of a C-Type Diazeniumdiolate Siderophore in a Marine-Derived Strain. A putative siderophore BGC with gene analogs of grbD and grbE, consistent with incorporation of L-Gra, was identified on plasmid 2 (accession: NC_017966.1, Figure 2A, Table S1). For targeted discovery of the metabolite, the FASTA sequence of the A domain specific for Gra (DVHRTGLVAK) was used as the query in NCBI BLASTP (basic local alignment search tool-protein). Hits were then narrowed to microbes that included biosynthetic gene clusters with siderophore-related genes, an A domain with the specificity code for L-Gra, and sequence homologues of the proposed Gra biosynthesis genes, grbD and grbE, from the BGC of gramibactin.
C-Diazeniumdiolate siderophores currently reported in the literature are all produced by soil-associated microbes, which raises the question if they are also produced by microbes in other environments. To investigate the extent of this siderophore type beyond the terrestrial soil environment, the family Burkholderiaceae was excluded from the genome search. The presence of GrbD and GrbE homologues, alongside an NRPS protein containing an A domain with a specificity code for Gra, indicated marine-derived T. mobilis KA081020-065 should incorporate Gra with a C-diazeniumdiolate for Fe(III) coordination (Figure 2).

Discovery and Structural Elucidation of Tistrellabactins A and B. T. mobilis KA081020-065 was cultured in an artificial seawater medium under Fe-limited conditions to induce siderophore production. Analysis of purified compounds from the culture extract with a positive colorimetric Fe-CAS assay response by UPLC-ESI-MS revealed two pounds from the culture extract with a positive colorimetric ionization of the N-protonated molecule m/z 1093.4 [M + H]+ differing by a single mass unit and both with a characteristic mass loss of 30 Da, characteristic of C-diazeniumdiolates. The structures of isolated and purified tistrellabactin A (R = NH3) and tistrellabactin B (R = OH) were elucidated by NMR spectroscopy and MS.

Three discrete 1H spin systems were attributed to the Gln side chain amine protons in tistrellabactin A and B. An additional discrete 1H spin system present in tistrellabactin A only was attributed to the Asn side chain. Through-bond 1H−13C HMBC correlations connect the side chain amine 1H resonances to methylene protons of three Gln and one Asn in tistrellabactin A only (Figures 4B, S7, S16). Observed in the TOCSY spectrum was a 1H spin system assigned to a 3-hydroxybutyric acid group (Hbu). Correlations between the hydroxylated 15N on the Orn side chain and the Hbu group established the 3-hydroxybutyric acid is appended to the i-Orn residue, forming a hydroxamate ligand. This rare acyl group has been seen in the siderophore structures of cupriachelin and imaqobactin. 1H−15N HMBC fingerprint, was confirmed with the presence of correlations between the Gra Cδ15N methylene protons to both nitrogens in the diazeniumdiolate group and Cγ13C methylene protons to the hydroxylated nitrogen only (Figure 4C).

The Asn in tistrellabactin A is replaced by an Asp in tistrellabactin B, consistent with the mass difference of 1 Da between them. Connectivity of the peptidic backbone was determined based on 1H−13C HMBC correlations across amide bonds between adjacent amino acids, with cyclization between the C-terminus carboxylic acid of Orn to the Ser residue, forming an ester linkage (Figures S7, S16). The diastereotopic Cβ1H’s on the Ser residue are deshielded in comparison to the typical ppm range observed, consistent with the cyclization due to the proximity to the ester group.

Due to the cycled and charged structures of tistrellabactins A and B, optimized mass fragmentation was obtained by linearization of tistrellabactins A and B via hydrolysis of the Ser ester linkage (Figures S21–S24). Linearization was achieved with mild conditions by incubation in pH 8 Na2HPO4 buffer for 48 h, at which point the linear form of tistrellabactin A was observable by UPLC-MS analysis at m/z 1110.4 [M + H]+ and tistrellabactin B at m/z 1111.4 [M + H]+ (Figure S23). Mass fragmentation of both linear and cyclic tistrellabactins A and B is consistent with the NMR-assigned structure. Mass losses of NH3 and H2O from b and y fragments were observed, as would be expected with highly ionizable side chain amines and hydroxyl groups on Asn, Asp, and Gln residues (Figures 4A, S24).

Tistrellabactins A and B coordinate Fe(III) with the β-OH-Asp, hydroxamate, and C-diazeniumdiolate ligands. The UV-vis spectrum of Fe(III)-tistrellabactin A shows an absorption
band at 340 nm and a broad shoulder centered at 420 nm. The peak at 340 nm is consistent with a ligand to metal charge transfer band (LMCT) for the α-hydroxycarboxylate ligand, β-OH-Asp, coordinated to Fe(III), while the broad shoulder centered at 420 nm corresponds to the LMCT band for the Fe(III)-hydroxamate group.  

Glutamine Residues in the Tistrellabactins Are Iteratively Loaded via an Unknown NRPS Mechanism. The amino acid constituents in the tistrellabactins follow the NRPS collinearity principle in accordance with the identified BGC, except eight amino acids are incorporated in tistrellabactins A and B rather than the predicted seven based on the genomic analysis (Figure S25). An extra Gln is observed in NRPS module 4, which is loaded at the end of MobF. This NRPS module may iteratively load an extra Gln, with the possible help of MobG which is missing an A domain. The absolute stereochemistry of the amino acid constituents in the elucidated structures of tistrellabactins A and B was assigned by Marfey’s amino acid analysis. Stereochemical assignments of the amino acid constituents of tistrellabactins A and B agree with the genomic predictions based on the presence or absence of an NRPS epimerization domain, with the exception of the Gln residues, which all are D-configured (Figures 2, 5, S26−S28). The second A domain selective for Gln in MobF is not followed by an E domain; however only D-Gln was detected (Figures S26, S28). Therefore, we propose that MobG may be working with the last module of MobF to epimerize the two Gln residues loaded iteratively at this position (Figure 5). Interestingly, DidA in the didemnin biosynthetic pathway, also identified within the genome of T. mobilis KA081020-065, iteratively loads three of four Gln residues in didemnins X and Y.

15N-Enrichment of Tistrellabactins A and B Shows L-Arg Is the Origin of Gra. 15N-Enriched tistrellabactins A and B were prepared for 1H−15N HMBC and HSQC experiments to aid their structural determination (Figures S11, S12, S19, S20). The 15N-enriched-tistrellabactins were prepared by culturing T. mobilis KA081020-065 in an artificial seawater (ASW) medium using 15NH4Cl in place of NH4Cl as the sole nitrogen source. UPLC-MS analysis of purified 15N-enriched tistrellabactins A and B showed both had an isotopic mass of m/z 1107.4 [M + H]+, indicating 15 nitrogens in the structure of tistrellabactin A and 14 nitrogens in tistrellabactin B (Figure S29). Corresponding ionization-induced mass losses of 31 Da are observed for both tistrellabactins A and B, consistent with ionization of 15NO instead of 14NO.

To determine if the Gra residue in the tistrellabactins originates from l-Arg, given the presence of protein homologues of GrbD and GrbE in the tistrellabactin’s BGC, 14N−l-Arg was supplemented into the 15N-ASW T. mobilis KA081020-065 culture. Purified tistrellabactins A and B grown

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Figure 4. NMR-guided structure elucidation of tistrellabactins A and B. (A) Key 1H−13C HMBC and 1H−1H COSY correlations in tistrellabactin A. (B) Superimposed tistrellabactin A (maroon) and tistrellabactin B (blue) 1H−13C HMBC correlations show three Gln NH2 side chain 1H pairs correlate to respective Gln Cγ. An HMBC correlation between Asn side chain NH3 1H at 6.99 ppm and Cβ 13C at 34.85 ppm is not present in tistrellabactin B, which has an Asp residue in the analogous position. (C) 1H−15N HMBC shows the presence of C-diazeniumdiolate amino acid Gra.
under these conditions both have an isotopic mass of $m/z$ 1102.4 [M + H]$^+$, indicating five nitrogens in each structure are unenriched. Also notable is the mass ion fragment showing a loss of 30 Da ($m/z$ 1072 [M + H]$^+$). A mass loss of 30 rather than 31 indicates the distal nitrogen on the diazeniumdiolate in Gra is unenriched. As part of the urea cycle, $^{15}$Arg is converted to Orn, which then may get incorporated before the microbe initiates de novo biosynthesis of Orn from $^{15}$NH$_3$. Therefore, the most plausible nitrogens to be unenriched would be the three in the Gra and two in the Orn residue, which matches the MS analysis (Figure S30). Moreover, $^3$H-$^{15}$N HMBC correlations confirm the unlabeled nitrogens are in both Gra and Orn residues and therefore originate from Arg, in agreement with the isotopic enrichment of gramibactin in *P. graminis* DSM 17151 (Figures S30, S31).

**Bioinformatic Analysis of *T. mobilis* KA081020-065 Siderophore Gene Cluster.** The putative siderophore BGC identified in the genome of *T. mobilis* KA081020-065 encodes accessory genes consistent with the biosynthesis of three different Fe(III) ligands within the structure. Along with the C-diazeniumdiolate biosynthesis genes, tailoring enzymes MobD, MobI, and MobJ would be responsible for the modification of L-Asp and L-Orn to yield $\beta$-hydroxyaspartate and the Orn-hydroxamate ligand groups, respectively (Figure 2). A standalone $\beta$-hydroxylase enzyme (MobD, $T\beta$H$_{Asp}$) and an A domain specific for activation of L-Asp in MobH followed by a $T\epsilon$ domain (GGDSI motif) directs the stereospecific 3R-hydroxylation of the Asp producing the L-erythro diastereomer. $^{33}$Modification of L-Orn by an N-acetyltransferase (MobI) and a N-monooxygenase (MobJ) yields the hydroxamate group as the third bidentate Fe(III) ligand. The predicted NTN hydrolase, MobM, suggests an N-terminal fatty acyl group may be present during the biosynthesis; however it was not detected in the culture workup. $^{34}$ The core peptidic structure is assembled by the concerted action of three NRPS modules: MobF, MobG, and MobH.

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**Figure 5.** NRPS domain organization and proposed biosynthesis of tistrellabactin A. NRPS modules 4 and 5 iteratively load Gln residues via an unknown mechanism.
The third NRPS module in MobF contains an A domain with specificity code DVHRTGLVAK, matching that of the A domains of Gra in the reported Gra-containing metabo-
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Figure 6. Photoreactivity of apo-tistrellabactin A. (A) The diazeniiumdiolate absorbance band at 246 nm disappears with irradiation of UV light. (B) The C-diazeniiumdiolate is converted to E/Z oxime isomers. (C) Proposed coordination of the apo-tistrellabactin A photoprod to Fe(III).

The Tistrellabactins Are Photoreactive. Irradiation of apo-tistrellabactin A with UV light diminishes the intensity of the absorption band at 246 nm as the C-diazeniiumdiolate undergoes transformation to E and Z oxime isomers in the same manner as occurs for apo-gramibactin (Figures 6, S33–S35). UPLC-ESIMS analysis of the apo-tistrellabactin A photoprod shows a new mass of m/z 1061.4 [M + H]+ and complete loss of the apo-tistrellabactin A m/z 1092.4 [M + H]+ species (Figure S33). Also present is a mass showing Fe(III) coordinated to the photoprod with mass m/z 1114.3 [M − 2H + Fe]+, demonstrating tistrellabactin A retains its ability to coordinate Fe(III) with the remaining hydroxamate and β-OH-Asp ligands (Figures 6C, S33).

Actively growing cells from a culture of T. mobilis with a positive chrome-azurol-S assay (CAS) response, indicating siderophore production, were also photolyzed in UV light. After 1 h of irradiation, the cells were pelleted and the resulting supernatant was analyzed by UPLC-ESIMS (Figure S35). The resulting sample shows a mass loss of 31 from the peaks corresponding to tistrellabactins A and B, consistent with the photoprod observed for the purified and concentrated sample. In comparison, a nonirradiated aliquot from the same culture shows no change to the apo-tistrellabactins and no trace of their respective photoproducts (Figure S35). These results demonstrate the biological relevance of the photoreactivity of these siderophores.

Less is understood about the photoreactivity of the Fe(III)-bound diazeniiumdiolate complex. It is well established that the Fe(III)-β-OH-Asp will undergo a radical decarboxylation reaction when irradiated, potentially playing an important role of iron cycling in the marine ecosystem. When Fe(III)-tistrellabactin A (m/z 1145.4 [M − 2H + Fe]+) is photolyzed selectively with 254 nm UV light (Hg(Ar) Oriel No. 6035), the intensity of the LMCT band for the α-hydroxycarboxylate and the hydroxamate to Fe(III) both decrease, while a band at 270 nm evolves and increases steadily with a bathochromic shift to 277 nm over 13.5 h of irradiation (Figure S36).

Aliquots taken along the photolysis time course were analyzed by MS and show the decrease of the Fe(III)-tistrellabactin and the graminine protonated molecule and the appearance of several new protonated molecules. The β-OH-Asp and the gramine residues undergo photoreactions, as evinced by the presence of
doubly charged protonated molecules at m/z 550.1 and 557.6 [M + 2H]^{2+}, consistent with the decarboxylation of the β-OH-Asp (46 Da) or transformation to E/Z oxime isomers from the C-diazeniudiolate group (31 Da), respectively, while retaining coordination to Fe(III). At later time points in the photolysis the ion count of the protonated molecule at m/z 508.2 [M + 2H]^{2+} increases further, consistent with both the gramine and β-OH-Asp ligands having undergone photoreactions with the loss of the ability to coordinate Fe(III) (Figure S37).

**CONCLUSIONS**

The marine-derived microbe *Tistrella mobilis* KA081020-065 produces not only the bioactive didemmins, but also, as presented here, the two depsipeptides tistrellabactins A and B, with rare biosynthetic features. The BGC of the tistrellabactins was identified in a previous study, although the expression of these metabolites was not observed when cultured in iron-rich media, highlighting the importance of mimicking certain environmental cues for laboratory culturing. To induce siderophore production, *T. mobilis* KA081020-065 was cultured under Fe-poor conditions, yielding tistrellabactins A and B.

The tistrellabactins are mixed ligand siderophores composed of three Fe(III) ligand types: a C-diazeniumdiolate, an α-hydroxyxcarboxylate, and a hydroxamate. This report is the fourth account of a C-diazeniumdiolate siderophore with Gra, but the first to be produced by a marine-derived microbe. In contrast to the stand-alone enzyme GrbE in the BGC of grambactin is of biosynthetic relevance. Furthering our knowledge of these enzymes will also strengthen genome mining tools, which in turn may help to identify the potential incorporation of other nonproteinogenic amino acids with a diazeniumdiolate group, such as 1-ala-nosine, into peptideic structures and continue uncovering the expanding diversity of siderophores.

**EXPERIMENTAL SECTION**

**General Experimental Procedures.** UV–visible spectra were obtained on an Agilent Technologies Cary 300 UV–vis spectrometer. NMR spectroscopy was carried out at 25 °C on a Bruker 500 MHz spectrometer equipped with a Prodigy cold probe (1H, 13C, 1H−13C multiplicity edited HSQC, 1H−1H COSY, 1H−13C HMBC, 1H−15N HMBC, 1H−15N HSQC, TOCSY, NOESY). NMR spectra for characterization were collected in DMSO-d$_6$ and spectra of the photoproduct were collected in 50 mM Na$_2$HPO$_4$-buffered D$_2$O (pD 8.0). Spectra were indirectly referenced by the residual solvent peak or 1H lock. MS analysis was carried out on a Waters Xevo G2-XS QTOF with positive mode electrospray ionization coupled to an AQUITY UPLC-H-Class system with a Waters BEH C18 column. Samples were run with a linear gradient of 0% to 100% acetonitrile (0.1% formic acid) in ddH$_2$O (0.1% formic acid) over 10 min. IR data were collected on a Bruker Alpha FTIR spectrophotometer. CD data were collected on a JASCO J-1500 CD spectrometer on tistrellabactin A (71 μM) and tistrellabactin B (52 μM) in Na$_2$HPO$_4$ (pH 8). CD data was referenced to the buffer and collected with a scan rate of 20 nm/min, DIT 8 s bandwidth of 1 nm, and data pitch of 0.5 nm. Deionized water was dispensed from a Milli-Q IQ7000 water purification system (Resistivity 18.2 MΩ). All glassware was acid-washed with 4 M HCl and subsequently rinsed with Milli-Q water.

**Culturing T. mobilis KA081020-065 and Siderophore Isolation.** The strain *Tistrella mobilis* KA081020-065 was obtained from Scripps Institution of Oceanography, University of California, San Diego. For optimal production of tistrellabactins A and B, *T. mobilis* KA081020-065 was cultured in a modified ASW medium composed of 62 mM sodium succinate, 19 mM NH$_4$Cl, 50 mM MgSO$_4$·7H$_2$O, 10 mM KCl, 10 mM CaCl$_2$·2H$_2$O, 6 mM glycerol phosphate, 0.28 M NaCl, 41 mM glycerol, and 45.4 mM sodium pyruvate in 1 L of Milli-Q water with the pH adjusted to 7. The medium was optimized for the growth of the tistrellabactins, which increased yields from 0.1 mg/L to 5–8 mg/L. Seed cultures were inoculated in Difco 2216 marine broth with single colonies of *T. mobilis* KA081020-065 grown on 2216 agar and grown for at least 24 h at 28 °C. Two dense S mL seed cultures were used for inoculation, as it was found this strain required higher amounts of inoculum than normal. Following sterilization, the medium was amended with 2 mM Steri-filtered NaHCO$_3$. Cultures were monitored by OD$_{400}$ and the culture supernatant was harvested at late log/early stationary phase with a positive Fe(III)-CAS response.

Culture supernatants were obtained by centrifugation at 6000 rpm for 30 min at 4 °C (SLA-3000 rotor, Thermo Scientific). To extract...
the tistrellabactins, the culture supernatant was decanted and shaken with 100 g of Amberlite XAD-4 resin. The XAD-4 resin was prepared by washing with methanol and then equilibrating with Milli-Q water. The resin and supernatant were allowed to equilibrate for 4 h at 120 rpm. The resin was filtered from the supernatant and washed with 0.5 L of Milli-Q water. The adsorbed organics were eluted with 40% aqueous methanol. The eluent was concentrated under vacuum and stored at 4 °C. Tistrellabactins A and B were purified from the eluent by semipreparative HPLC on a YMC 20 x 250 mm C18-AQ column, with a linear gradient of 10–55% methanol (0.05% trifluoroacetic acid) over 45 min, yielding pure tistrellabactins A (8 mg) and B (5 mg) from 1 L of culture.

**Isotopic Labeling of Tistrellabactins A and B.** The ASW medium used for culture growth only contains one nitrogen source. To isotopically label the full structures of tistrellabactins A and B, $^{15}$NH$_4$Cl was used in place of NH$_4$Cl. An additional experiment was completed with 10 mM $^{15}$N-Arg added to the $^{15}$N-enriched culture medium to follow potential utilization of l-Arg in the biosynthesis.

**Marfey’s Amino Acid Analysis.** Aliquots (1 mg in Milli-Q water) of tistrellabactin A and tistrellabactin B were combined with 12 M HCl or with 57% HI for final concentrations of 6 M HCl or 45% HI. The acidified solution was transferred to a glass ampoule, blanketed with Ar(g), and sealed under flame. The ampoule was heated at 108 °C for 21 h. After heating, the ampoule was broken, and the crude solution was evaporated and redissolved in 0.7 mL of Milli-Q water five times to ensure acid was removed from the hydrolysate. A 100 mL aliquot of the hydrolysate was reacted with Marfey’s reagent following standard conditions. Amino acid standards (dL-Glu, l-Glu, l-Ori, dL-Asp, l-Ser) were derivatized under the same conditions.

The FDAA-hydrolysate was analyzed by UPLC-MS and RP-HPLC (250 x 4.6 mm YMC C18-AQ column). HPLC samples were monitored at 340 nm using a linear gradient of 15% to 50% acetoneitrile in 50 mM triethylamine phosphate (pH 3.0) over 50 min. The hydrolysate and standards were also analyzed by UPLC-MS (15% to 50% acetonitrile in 50 mM triethylamine phosphate (pH 3.0)) followed by an Oriel Instrument Hg(Ar) (No. 6035) pen lamp was used as the UV source. Samples were dissolved in aqueous buffer (50 mM NaHPO$_4$ prepared in D$_2$O (99.9% purity)) with the pD adjusted to 8.0. For the Fe(III)-tistrellabactin A photolysis, an Edmund Optics filter (253.7 nm, 2959 ± 1475 cm$^{-1}$) 204 nm (+16.53), Figure S38; IR, 1655 cm$^{-1}$ (s, C–O), 1447 cm$^{-1}$ (w, N–O), 1262 cm$^{-1}$ (w, N–O), Figure S39; $^1$H, $^13$C, and 2D NMR data, Table S5; HRESIMS m/z 1093.4380 [M + H]$^+$ (calcd for C$_{66}$H$_{46}$N$_{14}$O$_{29}$, 1093.4398).

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.3c00230.

ESIMS/ESIMS/MS, NMR, UV–vis from titration, HPLC chromatograms from amino acid analysis, CD, and IR (PDF)

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Author Contributions

All authors give approval to the final version of the manuscript. C.M. and J.K.L. carried out all experiments.

Notes

The authors declare no competing financial interest.

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