Citromycetins and Bilains A–C: New Aromatic Polyketides and Diketopiperazines from Australian Marine-Derived and Terrestrial *Penicillium* spp.

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Chemical analysis of an Australian marine-derived isolate of *Penicillium bilaii*, collected from the Huon estuary, Port Huon, Tasmania, yielded the known fungal aromatic polyketides citromycetin (**1**) and citromycin (**2**) together with two dihydro analogues, (–)-2,3-dihydrocitromycetin (**3**) and (–)-2,3-dihydrocitromycin (**4**). An Australian terrestrial isolate of *Penicillium striatisporum* collected near Shalvey, New South Wales, also yielded citromycetin (**1**), citromycin (**2**), and the new dihydro analogue (–)-2,3-dihydrocitromycetin (**3**), together with fulvic acid (**5**), anhydrofulvic acid (**6**), and a selection of new methoxylated analogues, 12-methoxycitromycetin (**7**), 12-methoxycitromycin (**8**), (–)-12-methoxy-2,3-dihydrocitromycetin (**9**), and 12-methoxyanhydrofulvic acid (**10**). *P. bilaii* also yielded the rare siderophore pistillarin (**11**), the known diketopiperazines *cyclo*-(L-Phe-L-Pro) (**12**), *cyclo*-(L-Pro-L-Tyr) (**13**), *cyclo*-(L-Pro-L-Val) (**14**), and *cis*-bis(methylthio)silvatin (**15**), and three new diketopiperazines, bilains A–C (**16–18**). The structures for the *Penicillium* metabolites **1–18** were assigned by a combination of detailed spectroscopic analysis, including correlation with relevant literature data, chemical derivatization, degradation, and biosynthetic considerations. The citromycin polyketides **2** and **4** and the diketopiperazine **15** were weakly cytotoxic.

During our investigations into the chemistry of Australian marine-derived fungi we encountered an isolate of *Penicillium bilaii* (MST-MF667) obtained from a boat ramp on the Huon estuary, Port Huon, Tasmania. Preliminary HPLC-DAD-ELSD analysis (Figure 1) of an organic extract obtained from a solid-phase culture of *P. bilaii* (MST-MF667) revealed a rich diversity of metabolites. Of particular note was the appearance of a major pair of nonpolar metabolites possessing distinctive UV–vis spectra (**1** and **3**, see Figure 1), accompanied by an array of slightly less polar (later eluting) co-metabolites belong to the same structure class. The identity of these metabolites was not immediately apparent, prompting a scaled up solid-phase fermentation. Extraction of the resulting fermentation biomass yielded a crude EtOAc-soluble material that was subjected to solvent partition and preparative reversed-phase HPLC to afford a series of fractions. HPLC-DAD-ELSD and ^{1}H NMR analysis of early eluting polar fractions indicated polyketides, while later eluting nonpolar fractions contained diketopiperazines. Repeated HPLC fractionation of the polar material yielded pure samples of the known polyketides citromycetin (**1**)[1] and citromycin (**2**),[2] together with two dihydro analogues, (–)-2,3-dihydrocitromycetin (**3**) and (–)-2,3-dihydrocitromycin (**4**). This fractionation also yielded the known siderophore pistillarin (**11**)[3–5] and the known diketopiperazines *cyclo*-(L-Phe-L-Pro) (**12**),[6] *cyclo*-(L-Pro-L-Tyr) (**13**),[7] and *cyclo*-(L-Pro-L-Val) (**14**).[6] Repeated HPLC fractionation of the less polar material yielded the known diketopiperazine *cis*-bis(methylthio)silvatin (**15**)[8] along with three new diketopiperazines, bilains A–C (**16–18**).

Although possessing relatively simple molecular structures, the UV–vis spectra that characterized the aromatic polyketides encountered during our investigation of *P. bilaii* were (in our experience) uncommon. An electronic search of our in-house database (MST, COMET), which consists of HPLC-DAD-ELSD profiles for 6000 annotated microorganizms, highlighted only a single additional occurrence of metabolites with UV–vis spectra common with those found in *P. bilaii*. These metabolites were produced in low levels by a soil isolate of *Penicillium striatisporum* (MST-F9530) collected near Shalvey, New South Wales. In an earlier study this *P. striatisporum* isolate was described as a producer of new and known calbistrins,[9] a family of antifungal nonaromatic polyketides. The rarity of encounters with the class of aromatic polyketides evident in both these *Penicillium* spp. prompted a more detailed analysis of *P. striatisporum*. This analysis yielded the known polyketides citromycetin (**1**),[1] citromycin (**2**),[2] fulvic acid (**5**),[10] and anhydrofulvic acid (**6**),[11] as well as the new polyketides (–)-2,3-dihydrocitromycetin (**3**), 12-methoxycitromycetin (**7**), 12-methoxycitromycin (**8**), (–)-12-methoxy-2,3-dihydrocitromycetin (**9**), and 12-methoxyanhydrofulvic acid (**10**).

In this report we describe the isolation and structure elucidation of the *Penicillium* metabolites **1–18**.

Results and Discussion

Structures for the known polyketides citromycetin (**1**)[1] and citromycin (**2**)[2] were determined by detailed spectroscopic analysis and comparison to literature data. Citromycetin (**1**) was first reported in 1931,[12] and its structure solved 20 years later.[13] Citromycin (**2**), on the other hand, has only been reported[12] as an acid decomposition product of **1**. To assess whether our reisolation of **2** was a handling artifact, acidic (0.01% TFA) and basic (triethylamine to pH 8.5) 50% H_2O/MeOH solutions of **1** were warmed to 40 °C and monitored by HPLC-DAD-ESI(±)MS over several hours. Since this exposure failed to catalyze the acid degradation of **1** to yield **2**, we conclude that citromycin (**2**) exists as a natural product in our *Penicillium* fermentations. Of some note, despite being a rare and under-represented structure class, interest in citromycetins was rekindled in 2001, when **1** was patented[14] for the treatment of neurodegenerative diseases such as Alzheimer's, Lewy body, and Parkinson's. This acknowledgment that citromycetins may have under-realized biomedical potential reaffirmed our commitment to more closely examine *P. bilaii* (MST-MF667) and *P. striatisporum* (MST-F9530), leading to the discovery of an array of citromycetin analogues (**3–10**).

High-resolution EIMS analysis of **3** revealed a molecular ion ($C_{14}H_{12}O_7$, Δmmu = +0.8) consistent with a dihydro derivative of citromycetin (**1**). The ^{1}H and ^{13}C NMR data for **3** proved to be similar to those for **1** (Tables 1 and 2), with the most obvious

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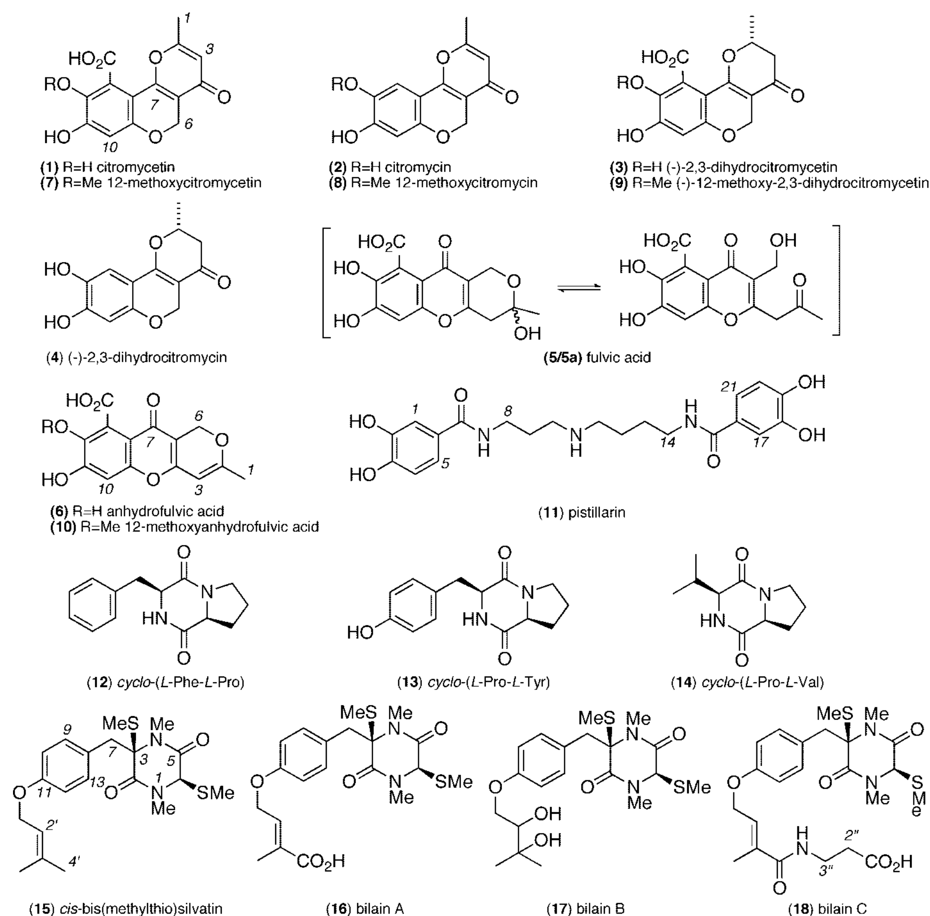

difference being reduction of $\Delta^{2,3}$ with the corresponding loss of olefinic resonances and the appearance of a secondary methyl (δ_H 1.35, d (6.3); δ_C 19.6) flanked by a methylene, further supported by (a) COSY correlations for a H_3-1 to H_2-3 isolated spin system and (b) a chiral C-2 induction of diastereotopic character at H_2-6 (δ_H 4.88, d (12.2), 4.55, d (12.2)). Although (–)-2,3-dihydrocitromycetin (**3**) displayed a significant optical rotation, all efforts at assigning an absolute stereochemistry (including acquisition and interpretation of CD spectra and the attempted synthesis of crystalline derivatives) proved inconclusive.

High-resolution ESI(+)MS analysis of **4** revealed a pseudomolecular ion $[M + Na]^+$ supportive of a molecular formula ($C_{13}H_{12}O_5$,

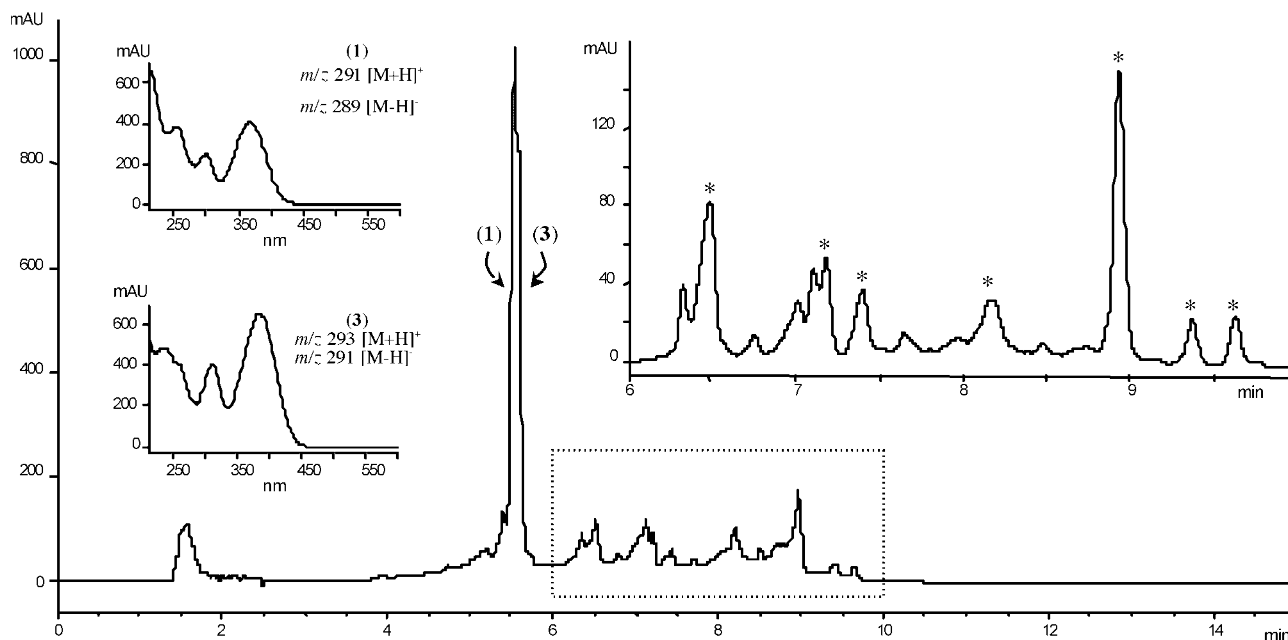

Figure 1. HPLC-DAD-ESI(±)MS analysis of the crude EtOAc extract of *P. bilaii*(MST-MF667). An HPLC trace (15 min gradient elution from 90% H_2O/CH_3CN (0.05 HCO_2H) to 100% CH_3CN (0.05 HCO_2H) on a Zorbax StableBond C_8 5 μm, 150 × 4.6 mm column, detection by DAD at 325 nm) showing the major citromycetin metabolites (**1** and **3**) together with an expanded region revealing "new" citromycetin co-metabolites as determined by UV–vis comparisons.

Table 1. ^{1}H NMR Data for Polyketides **1–4** and **7–10**

	δ_H, m (*J* in Hz)							
no.	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**7**[c]	**9**[c]	**8**[c]	**10**[c]
H-1	2.23[d]	2.32, s	1.35[e]	1.45[h]	2.33, s	1.50[k]	2.41, s	2.00, s
H-2			4.53, obs	4.67, obs		4.64, m		
H-3a	6.16[d]	6.14, s	2.45, m	2.43[l]	6.19, s	2.53, m	6.18, s	5.57, s
H-3b			2.45, m	2.51, obs				
H-6a	4.93, s	4.96, s	4.88[f]	4.91[g]	5.06, s	5.05, obs	5.06, s	5.17, s
H-6b			4.55[f]	4.66[g]		4.67[g]		
H-10	6.47, s	6.38, s	6.38, s	6.33, s	6.50, s	6.39, s	6.40, s	6.81, s
H-13		6.99, s		6.92, s			7.16, s	
11-OH				10.00, bs				
12-OH				9.02, bs				
12-OMe					3.81, s	3.78, s	3.87, s	3.84 (s)

[a] d_6-DMSO, 600 MHz. [b] d_6-DMSO, 500 MHz. [c] d_4-MeOH, 600 MHz), obs = obscured by overlapping resonances. [d] d (0.9). [e] d (6.3). [f] d (12.2). [g] d (12.3). [h] d (6.3). [k] d (6.7). [l] dd (16.8, 3.7).

Table 2. ^{13}C NMR Data[f] for Polyketides **1–4** and **7–10**

	δ_C							
no.	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**1**[c]	**2**[c]	**7**[c]	**10**[c,d]
C-1	18.6	19.2	19.6	20.1	19.2	19.6	19.0	19.9
C-2	164.3	164.5	75.7	75.5	167.4	167.4	167.4	169.0
C-3	113.3	113.3	42.5	42.3	114.2	114.2	114.3	95.1
C-4	174.6	174.9	188.0	187.7	178.3	178.6	178.2	160.5
C-5	111.2	110.3	101.9	101.0	112.5	111.8	112.9	102.3
C-6	62.0	62.3	62.3	62.6	63.3	63.7	63.6	65.2
C-7	150.5	155.5	152.3	162.1	158.3	153.5	157.4	[e]
C-8	103.1	106.2	104.7	107.4	105.2	108.1	104.7	113.9
C-9	154.8	150.4	162.2	152.2	153.6	159.3	157.0	157.0
C-10	103.7	104.0	103.3	103.6	105.6	104.8	106.4	104.1
C-11	150.9	150.7	151.7	151.5	152.9	153.0	156.3	154.3
C-12	138.1	140.9	138.3	140.6	141.5	142.3	141.5	143.1
C-13	119.7	108.7	120.7	109.7	118.1	109.8	[e]	[e]
CO_2H	167.7		167.9		171.1	[e]	[e]	[e]
OMe							62.2	62.0

[a] d_6-DMSO, 150 MHz. [b] d_6-DMSO, 125 MHz. [c] d_4-MeOH, 150 MHz. [d] Extracted from HSQC and HMBC data. [e] Not observed. [f] Multiplicity supported by DEPT 135 data.

Δmmu = 0.3) consistent with a dihydro derivative of citromycin (**2**). Comparison of the ^{1}H and ^{13}C NMR data between **2** and **4** (Tables 1 and 2) revealed similar features to the comparison described above between **1** and **3**. Thus, (–)-2,3-dihydrocitromycin (**4**) could be assigned the structure as shown, in common with that of neuchromenin, a microbial metabolite first reported in 1996 by Hayakawa et al.[15] from *Eupenicillium javanicum* var. *meloforme*. A subsequent 2001 synthetic study Mori et al.[16] established an *S* absolute stereochemistry for (–)-neuchromenin, permitting assignment of absolute stereochemistry to our reisolate of **4** as shown. On the basis of biosynthetic arguments we also *tentatively* assign an *S* absolute stereochemistry to the co-metabolite (–)-2,3-dihydrocitromycetin (**3**). Of note, neuchromenin (**4**) reportedly[15] has the ability to induce neurite outgrowth, which renders it a potential molecular lead for the development of treatments for diseases of the nervous system. This observation is consistent with the claim[14] that citromycetins are potential molecular leads for the treatment of neurodegenerative diseases.

Although fulvic acid (**5**) is known, its structure proof in our hands proved a challenge. While fulvic acid is known to occur in both cyclic (**5**) and acyclic (**5a**) forms (see the structure diagram), this equilibrium is not reflected in the published ^{1}H NMR data, which characterizes only the cyclic form.[10] To the best of our knowledge this appears to be a solvent-dependent phenomena, and its oversight is most likely due to the fact that the ^{1}H NMR data for **5** have been reported only in d_6-DMSO and d_5-pyridine.[10] In our hands the ^{1}H NMR data for **5** acquired in d_4-methanol clearly revealed a 1:1 equilibrium mixture between both cyclic and acyclic forms. The ^{1}H NMR resonance for H_2-3 was particularly diagnostic in this respect, appearing as a diastereotopic AB multiplet (δ_H 2.99, J = 17.6 Hz; 2.77, J = 17.7 Hz) in the cyclic form (**5**) and as a magnetically equivalent singlet (δ_H 2.65) in the acyclic form (**5a**). The chemistry of fulvic acid was further complicated as on storage **5** underwent slow but complete dehydration to anhydrofulvic acid (**6**), as proven by ^{1}H NMR analysis and HPLC co-injection with a second sample independently isolated during this study. The identification of anhydrofulvic acid (**6**) was confirmed by spectroscopic comparison to literature data.[11]

High-resolution ESI(+)MS analysis of **7** revealed a pseudomolecular ion $[M + Na]^+$ supporting a molecular formula ($C_{15}H_{12}O_7$) consistent with a methoxylated analogue of the co-metabolite citromycetin (**1**). The NMR (d_4-methanol) data for **7** supported this observation, with the only significant difference between **7** and **1** being the appearance of a methoxyl resonance (δ_H 3.81, δ_C 62.2) (see Tables 1 and 2). The regiochemistry of methylation in **7** was determined to be C-12 on the basis of an HMBC correlation between the OMe and the C-12 resonance (δ_C 141.5). Note: the ^{13}C NMR chemical shift for C-11 was significantly deshielded relative to C-12 in both citromycetin (**1**) (δ_C 152.9 vs 141.5) and **7** (δ_C 156.3 vs 141.5) due to the electron-donating influence of the C-9 oxygen substituent. This analysis confirmed **7** as 12-methoxycitromycetin.

High-resolution ESI(+)MS analysis of **8** revealed a pseudomolecular ion $[M + Na]^+$ supporting a molecular formula ($C_{15}H_{12}O_7$) consistent with a methoxylated analogue of the co-metabolite citromycin (**2**). The ^{1}H NMR (d_4-methanol) data for **8** (see Table 1) revealed resonances consistent with three sp^2 methines (δ_H 7.16, 6.40, and 6.18), an oxygenated methylene (δ_H 5.06), and an allylic methyl (δ_H 2.41), in common with citromycin (**2**), plus a methoxyl resonance (δ_H 3.87). The regiochemistry of methylation in **8** was established as C-12 due to the observation of an NOE from the OMe to H-13 (δ_H 7.16). Note: the ^{1}H NMR chemical shift for H-13 was significantly deshielded relative to H-10 in both citromycin (**2**) (δ_H 7.04 vs 6.38) and **8** (δ_H 7.16 vs 6.40) due to the deshielding influence of the *peri* C-7 oxygen substituent. This analysis confirmed **8** as 12-methoxycitromycin.

High-resolution EIMS analysis of **9** revealed a molecular ion $[M]^+$ supporting a molecular formula ($C_{15}H_{14}O_7$) consistent with a methoxylated analogue of the co-metabolite (–)-2,3-dihydrocitromycetin (**3**). The ^{1}H NMR (d_4-methanol) data for **9** (see Tables 1 and 2) revealed resonances for an aliphatic 2°-methyl (δ_H 1.50) with an oxymethine (δ_H 4.64) and an adjacent deshielded methylene (δ_H 2.53). The ^{1}H NMR data also revealed an isolated diastereotopic oxymethylene (δ_H 5.05 and 4.67), an aromatic methine (δ_H 6.39), and a methoxyl resonance (δ_H 3.78). A lack of material precluded the acquisition of 2D NMR data for **9**; however, acquisition of the ^{1}H NMR (d_4-methanol) in the presence of a base did facilitate the assignment of methylation regiochemistry. Addition of sodium methoxide to phenolic benzoic acids such as the citromycetins and analogues generates the corresponding sodium carboxylate and phenolates. Limited delocalization of the carboxylate anion or a C-12 phenolate in these systems would be expected to have little

or no effect on the chemical shift of H-10. By contrast, a C-11 phenolate would be expected to delocalize into the aromatic ring and significantly shield H-10. As predicted, when this ^{1}H NMR experiment was performed on **7**, **8**, and **10** (see below), all of which possess C-11 phenol substituents, the H-10 resonances were shielded by 0.38, 0.32, and 0.37 ppm, respectively. This shielding effect was reversed by the addition of acid. When the same study was performed on **9**, the H-10 resonance was shielded by 0.39 ppm, confirming the presence of C-11 phenol and C-12 methoxyl functionalities. Since **9** displayed a negative optical rotation and was a co-metabolite with (–)-2,3-dihydrocitromycetin (**3**), we propose that **9** be named (–)-12-methoxy-2,3-dihydrocitromycetin, and given biosynthetic considerations, we tentatively assign the *S* absolute stereochemistry as shown.

High-resolution EIMS analysis of **10** revealed a molecular ion $[M]^+$ supporting a molecular formula ($C_{15}H_{12}O_7$) isomeric with the co-metabolite 12-methoxycitromycetin (**7**). However, the ^{1}H NMR (d_4-methanol) data for **10** (see Table 1) revealed resonances consistent with two sp^2 methines (δ_H 5.57 and 6.81), an oxymethylene (δ_H 5.17), an allylic methyl (δ_H 2.00), and a methoxyl (δ_H 3.84), suggestive of a methoxylated analogue of anhydrofulvic acid (**6**). As with **7** the regiochemistry of methylation in **10** was determined to be C-12 on the basis of an HMBC correlation between the OMe and the C-12 resonance (δ_C 143.1). Note: assignment of the ^{13}C NMR resonance for C-12 in **10** was based on the analysis presented above for **1** and **7** and was also consistent with the literature value for C-12 in anhydrofulvic acid (**6**).[11]

Although known as a fungal metabolite since 1984[5] and studied as a siderophore in 1997[4] and again in 2001,[3] the published spectroscopic data for pistillarin (**11**) are of poor quality. This observation not withstanding, the ESI(±)MS and ^{1}H NMR data for **11** isolated during our studies were indicative of pistillarin. The 2D NMR COSY data for **11** revealed isolated spin systems consistent with two very similar 1,3,4-trisubstituted dihydroxylated benzamides, linked by 1,3-disubstituted propyl (δ_H 3.46, 1.95, 3.02) and 1,4-disubstituted butyl (δ_H 3.05, 1.78, 1.70, 3.39) chains. Key gHMBC correlations from H-1, H-5, and H$_2$-8 to C-7, an amide carbonyl (δ_C 171.2), and from H-17, H-21, and H$_2$-14 to C-15, another amide carbonyl (δ_C 170.4), confirmed the structure assignment of pistillarin (**11**). Our discovery of pistillarin in *P. bilaii* represents only its second reported occurrence as a natural product.

Structures for the known diketopiperazines *cyclo*-(L-Phe-L-Pro) (**12**),[6] *cyclo*-(L-Pro-L-Tyr) (**13**),[7] and *cyclo*-(L-Pro-L-Val) (**14**)[6] were assigned by detailed spectroscopic analysis and comparison with literature data. To further support the assignment of absolute stereochemistry to **12–14**, samples of these diketopiperazines were subjected to acid hydrolysis followed by Marfey's analysis.[17,18] It is interesting to observe that, in keeping with the marine origins of our isolate of *P. bilaii*, **12** and **14** were previously described from the Caribbean sponge *Calyx cf. podatypa*,[6] whereas **13** was reported from an Antarctic sponge-associated bacterium, *Pseudomonas aeruginosa*.[7]

The structure for *cis*-bis(methylthio)silvatin (**15**) was confirmed by detailed spectroscopic analysis and comparison to literature data.[8] To the best of our knowledge, known analogues of *cis*-bis(methylthio)silvatin (**15**) are limited to a *trans* stereoisomer described as co-occurring with **15** in a saltwater fermentation of the terrestrial fungus *Coriolus consors*.[19] Careful analysis of *P. bilaii*co-metabolites revealed a number of potential new *cis*-bis(methylthio) silvatin analogues, which we have identified as bilains A–C (**16–18**).

High-resolution ESI(+)MS analysis of bilain A (**16**) revealed a pseudomolecular ion $[M + Na]^+$ consistent with a molecular formula ($C_{20}H_{26}N_2O_5S_2$, Δmmu = 0.0) 30 amu higher than *cis*-bis(methylthio)silvatin (**15**). The ^{1}H and ^{13}C NMR data for **16** were very similar to those for **15**, with the only significant difference being replacement of geminal olefinic methyls (δ_C 13.6 and 14.0) with a single olefinic methyl (δ_C 13.1) and a carboxylic acid (δ_C 168.5). Diagnostic gHMBC correlations between the olefinic methyl (δ_H 1.91) and carboxylic acid (δ_C 168.5) in **16**, together with a significant NOE to 3′-Me (δ_H 1.91) on irradiation of H$_2$-1′ (δ_H 4.79), confirmed bilain A (**16**) as the oxidized analogue of *cis*-bis(methylthio)silvatin (**15**) as shown (less absolute stereochemistry).

Table 3. ^{1}H NMR Data for Bilains A–C (**16–18**)

	δ_H, m (*J* in Hz)		
no.	**16**[a]	**17**[b]	**18**[b]
H-6	4.55, s	4.56, s	4.57, s
H-7a	3.60, d (14.3)	3.59, d (14.2)	3.60, d (14.2)
H-7b	3.19, d (14.3)	3.17, d (14.2)	3.19, d (14.2)
H-9/13	7.06, d (8.7)	7.04, d (8.8)	7.05, d (8.7)
H-10/12	6.86, d (8.7)[d]	6.83, d (8.8)	6.83, d (8.6)
N1-Me	2.99, s	2.99, s	2.99, s
N4-Me	3.13, s	3.13, s	3.13, s
3-SMe	2.18, s	2.18, s	2.18, s
6-SMe	2.32, s	2.31, s	2.32, s
H$_2$-1′	4.79, dd (0.9, 5.6)	4.21, dd (2.7, 9.8) 3.87, dd (8.0, 9.8)	4.72, dd (0.7, 5.7)
H-2′	6.87, (obs)[d]	3.72, dd (2.7, 8.0)	6.43, dt (0.7, 5.7)
H-4′		1.21[c], s	
3′-Me	1.91, d (1.1)	1.23[c], s	1.91, d (0.7)
H$_2$-3″			3.50, t (6.6)
H$_2$-2″			2.56, t (6.6)

[a] d_6-Acetone, 500 MHz. [b] d_6-Acetone, 600 MHz. [c] Interchangeable. [d] Resonances overlap.

High-resolution ESI(+)MS analysis of bilain B (**17**) revealed a pseudomolecular ion $[M + Na]^+$ consistent with a molecular formula ($C_{20}H_{30}N_2O_5S_2$, Δmmu = +0.8) 34 amu higher than *cis*-bis(methylthio)silvatin (**15**). The ^{1}H NMR spectrum of **17** (Table 3) was again very similar to that of **15**, with the most notable difference being replacement of the H-2′ olefinic methine (δ_H 3.72), conversion of the geminal olefinic methyls (δ_H 1.72 and 1.78) to geminal oxymethyls (δ_H 1.21 and 1.23), and the appearance of an oxygenated diastereotopic methylene (δ_H 4.21 and 3.87). A sequence of 2D NMR COSY correlations confirmed that all these new resonances belonged to a common isolated spin system, consistent with **17** being the oxidized dihydroxy analogue of **15** as shown. A lack of sufficient material prevented the direct acquisition of ^{13}C NMR data for **17**; however, from the analysis of gHMBC and gHSQC data it was possible to assign all ^{13}C NMR resonances (Table 4) and confirm the structure. Of particular note were HMBC correlations from C-4′ and 3′-Me ($_H$ 1.21 and 1.23) to C-2′ (δ_C 77.2) and C-3′ (δ_C 71.9). Thus bilain B (**17**) is the oxidized analogue of *cis*-bis(methylthio)silvatin (**15**) as shown (less C-2′ stereochemistry).

High-resolution ESI(+)MS analysis of bilain C (**18**) revealed a pseudomolecular ion $[M + Na]^+$ consistent with a molecular formula ($C_{23}H_{31}N_3O_6S_2$, Δmmu = +0.3) 71 amu higher than bilain A (**15**). The ^{1}H NMR data for **18** (Table 3) were very similar to **15**, with the major differences being the upfield shift of H-2″ ($\Delta\delta_H$ +0.44) and the appearance of two triplet methylenes (δ_H 3.50 and 2.56), which 2D NMR COSY analysis revealed to be part of an isolated spin system. As was the case for **17**, lack of material prevented direct acquisition of ^{13}C NMR data for **18**; however assignments could be made from analysis of gHMBC and gHSQC data. Of note, both the 3′-Me (δ_H 1.91) and H-2′ (δ_H 6.43) displayed a gHMBC correlation to a quaternary carbon (δ_C 168.8), consistent with either an amide or ester at C-4′. Likewise, one of the triplet methylenes (δ_H 2.56) displayed a gHMBC correlation to a second ester/amide resonance (δ_C 173.6). Given the molecular formula, the only plausible structure for **18** is the β-alanine conjugate of bilain A (**16**) as shown (less absolute stereochemistry). A 2D NMR NOESY correlation between 3′-Me (δ_H 1.91) and H$_2$-1′ (δ_H 4.72) confirmed the *E* $\Delta^{2',3'}$ stereochemistry as indicated.

Given that *cis*-bis(methylthio)silvatin (**15**) is a compound of known absolute stereochemistry and that the sample of **15** reisolated

Table 4. ^{13}C NMR Data for Bilains A–C (**16–18**)

	δ_C		
no.	**16**[a,b]	**17**[b,c]	**18**[b,c]
1-NMe	33.5	33.4	33.2
4-NMe	30.3	30.3	30.0
3-SMe	13.8	13.5	13.5
6-SMe	16.8	16.6	16.4
C-2	165.0	165.0	165.1
C-3	74.7	74.7	74.5
C-5	165.6	165.6	165.4
C-6	65.7	65.6	65.4
C-7	42.3	42.3	42.1
C-8	128.3	127.9	128.4
C-9/13	131.6	131.5	131.4
C-10/12	115.5	115.3	115.3
C-11	158.7	159.6	158.8
C-1′	65.7	70.6	65.3
C-2′	137.7	77.2	130.6
C-3′	130.6	71.9	135.0
C-4′	168.5	25.6	168.8
3′-Me	13.1	26.8	13.1
C-3″			36.1
C-2″			34.2
C-1″			173.6

[a] d_6-Acetone, 125 MHz. [b] d_6-Acetone, 150 MHz. [c] Multiplicity supported by DEPT 135 data or by an edited HSQC experiment.

from *P. bilaii* (a) possesses the same optical rotation sign as previously reported,[11] (b) is a co-metabolite with bilains A–C, and (c) exhibits an excellent NMR comparison about the diketopiperazine moiety with bilains A–C (**16–18**), then on biosynthetic grounds we propose that **15–18** share a common C-3 and C-6 absolute stereochemistry. Unfortunately the low isolated yields of **17** and **18**, combined with very low rotational values, precluded the accurate measurement of optical rotations.

The polyketides **1–4** and the diketopiperazines **12–16** were tested for antibacterial (*Escherichia coli* and *Bacillus subtilis*), antifungal (*Septoria nodurum*), antiparasitic (*Haemonchus contortus*), and cytotoxic (NS-1 cells) activity, with only citromycin (**2**), (–)-2,3-dihydrocitromycin (**4**), and *cis*-bis(methylthio)silvatin (**15**) displaying weak cytotoxicity (LD_{99} = 0.10, 0.05, and 0.15 μM). The ecological role played by these unusual metabolites remains unclear; however, the prospect that new citromycetin/citromycin polyketides may have potential in developing treatments for neuronal and neurodegenerative diseases is intriguing and deserving of further consideration.

Experimental Section

General Experimental Procedures. Chiroptical measurements ($[\alpha]_D$) were obtained on a Jasco P-1010 Intelligent Remote Module type polarimeter in a 100 by 2 mm cell. Circular dichroism (CD) spectra were acquired using a JASCO J-810 spectropolarimeter. Ultraviolet–visible (UV–vis) absorption spectra were obtained using a CARY3 UV–visible spectrophotometer with 1 cm quartz cells. Electrospray ionization mass spectra (ESIMS) were acquired using an Agilent 1100 series separations module equipped with an Agilent 1100 series LC/MSD mass detector and Agilent 1100 series diode array detector. High-resolution (HR) ESIMS measurements were obtained on a Finnigan MAT 900 XL-Trap instrument with a Finnigan API III source. High-resolution electron-impact mass spectra (HREIMS) measurements were obtained on a Kratos MS25RFA mass spectrometer at 70 eV. NMR spectra were acquired on a Bruker Avance 500 or a Bruker Avance 600 spectrometer under XWIN-NMR control, referenced to residual ^{1}H signals. Initial HPLC was carried out on a system consisting of two Shimadzu LC-8A preparative liquid chromatographs with static mixer, Shimadzu SPD-M10AVP diode array detector, and Shimadzu SCL-10AVP system controller. Further HPLC was performed using an Agilent 1100 Series separations module equipped with Agilent 1100 Series diode array and/or multiple wavelength detectors, Polymer Laboratories PL-ELS1000 ELSD, and Agilent 1100 Series fraction collector and running ChemStation (revision 9.03A or 10.0A). Unless otherwise specified, a constant level of 0.01% TFA was used in all HPLC separations.

Fungal Culture. MST-MF667 was isolated from a boat ramp on the Huon estuary, Port Huon, Tasmania, Australia. It was identified on morphological grounds as a typical isolate of *Penicillium bilaii.* This isolate of *Penicillium striatisporum* (MST-F9530) was described in an earlier report.[9]

Bioassay Details. Procedures for antibacterial, antifungal, nematocidal, and cytotoxicity assays have been described previously.[20]

Extraction and Isolation. The mycelia from a solid-phase fermentation of MST-MF667 on malt extract agar (8MEA) (malt extract (16%), peptone (0.8%), glucose (16%), agar (2%); 200 × 15 g Petri dishes, 21 days, 24 °C) were extracted with MeOH (2 × 2 L). The extract was concentrated *in vacuo* to an aqueous residue (~400 mL) and then partitioned between EtOAc and H_2O (3 × 400 mL of EtOAc). The EtOAc extract was dried *in vacuo* to give 14.9 g of material. The EtOAc-soluble material (14.9 g) was further partitioned between petroleum spirit (2 × 100 mL) and 20% H_2O/MeOH (2 × 100 mL). A portion (80%) of the aqueous MeOH-soluble material (1.2 g) was subjected to preparative HPLC (single injection through a 5 μm Phenomenex LUNA C_{18} 150 × 21.2 mm column at 5 mL/min, with a 180 min gradient elution, 90% H_2O/MeOH (0.05% TFA) to MeOH (0.05% TFA)) to afford nine fractions. All percentage yields calculated below are measured against the mass of aqueous MeOH-soluble material (1.2 g). Fraction 1 was determined to be (–)-2,3-dihydrocitromycetin (**3**) (35.2 mg, 0.24%). Fraction 2 was further fractionated by preparative C_{18} HPLC (5 injections through a Zorbax SB-C_{18} 7 μm, 250 × 21.2 mm column at 21.2 mL/min, with a 20 min isocratic elution, 50% H_2O (0.5% HCO_2H)/MeOH) to yield *cyclo*-(L-Pro-L-Tyr) (**13**) (17.9 mg, 0.12%), *cyclo*-(L-Pro-L-Val) (**14**) (10.2 mg, 0.07%), an impure sample of pistillarin (**11**) (12.6 mg), and a mixture of citromycetin (**1**) and (–)-2,3-dihydrocitromycetin (**3**) (17.8 mg). The latter mixture was purified using the same column (2 injections with an 8 min isocratic elution, 80% H_2O/CH_3CN) to yield **1** (5.7 mg, 0.04%) and **3** (10.5 mg, 0.07%). The crude sample of **11** was subsequently purified by semipreparative phenyl HPLC (18 injections through a Zorbax SB-phenyl 5 μm, 250 × 9.4 mm column at 3 mL/min, with a 10 min isocratic elution, 70% H_2O (0.05% TFA)/MeOH) to yield pistillarin (**11**) (6.1 mg, 0.04%). Fraction 4 was resolved by preparative C_8 HPLC (6 injections through a Zorbax RX-C_8 7 μm, 250 × 21.2 mm column at 21.2 mL/min, with a gradient elution comprising 17 min at 80% H_2O/CH_3CN followed by ramping over 13 min to 60% H_2O/CH_3CN and holding for a further 10 min) to yield *cyclo*-(L-Phe-L-Pro) (**12**) (16.7 mg, 0.11%) and a mixture of citromycin (**2**) and (–)-2,3-dihydrocitromycin (**4**) (17.4 mg). The latter mixture was purified by preparative C_{18} HPLC (2 injections through a Zorbax SB-C_{18} 7 μm, 250 × 21.2 mm column at 21.2 mL/min, with a 20 min gradient elution, 80% to 60% H_2O/CH_3CN) to yield **2** (4.5 mg, 0.03%) and **4** (6.3 mg, 0.04%). Fraction 5 was fractionated by preparative C_{18} HPLC (7 injections through a Zorbax SB-C_{18} 5 μm, 150 × 21.2 mm column at 21.2 mL/min, with a 30 min gradient elution, 75% to 70% H_2O (0.05% TFA)/CH_3CN) to yield seven fractions. The fourth fraction from this column was further purified by semipreparative CN HPLC (10 injections through a Zorbax SB-CN 5 μm, 250 × 9.4 mm column at 4 mL/min, with isocratic elution, 75% H_2O (0.1% TFA)/CH_3CN) to yield bilain B (**17**) (1.2 mg, 0.01%). The fifth fraction was purified using the same CN HPLC column and conditions to yield bilain C (**18**) (0.6 mg, 0.004%). Fraction 6 was fractionated by preparative C_{18} HPLC (4 injections through a Zorbax SB-C_{18} 7 μm, 250 × 21.2 mm column at 21.2 mL/min, with a 50 min gradient elution, 50% to 20% H_2O/MeOH) to yield bilain A (**16**) (6.0 mg, 0.04%). Fraction 7 was fractionated by preparative C_8 HPLC (3 injections through a Zorbax RX-C_8 7 μm 250 × 21.2 mm column at 21.2 mL/min, with a 25 min gradient elution, 50% to 40% H_2O (0.01% TFA)/CH_3CN) to yield *cis*-bis(methylthio) silvatin (**15**) (4.8 mg, 0.03%).

Preliminary extraction and isolation procedures have been reported for MST-F9530, leading to fractions 1–25.[9] Fractions 1 and 2 were combined and fractionated on C_{18} SPE (2 × 1 g, Alltech) using a 15% stepwise gradient starting at 90% H_2O/MeOH and finishing at 45% H_2O/MeOH. Material eluting at 75% H_2O/MeOH was further purified on an identical C_{18} SPE cartridge, using a 5% stepwise gradient starting at 90% H_2O/MeOH and finishing at 50% H_2O/MeOH, to yield citromycetin (**1**) (11.2 mg, 1.09%), while material eluting at 60% H_2O/MeOH was further purified by preparative C_{18} HPLC (21.2 mL/min gradient elution from 70% H_2O/MeOH to 40% H_2O/MeOH over 30

min, through a Zorbax SB-C_{18} 7 μm, 250 × 21.2 mm column) to yield 12-methoxycitromycetin (**7**) (3.3 mg, 0.32%) and citromycin (**2**) (1.1 mg, 0.11%). Fraction 3 was fractionated by C_{18} SPE [10 g, 10% stepwise gradient from 80% H_2O/CH_3CN (0.05% TFA) to MeOH (0.05% TFA)] followed by gradient C_{18} preparative HPLC (21.2 mL/min gradient elution from 90% H_2O/CH_3CN to 50% H_2O/CH_3CN over 30 min, through a Zorbax SB-C_{18} 7 μm, 250 × 21.2 mm column) to yield (–)-2,3-dihydrocitromycetin (**3**) (0.2 mg, 0.02%), anhydrofulvic acid (**6**) (1.2 mg, 0.12%), 12-methoxyanhydrofulvic acid (**10**) (1.0 mg, 0.10%), (–)-12-methoxy-2,3-dihydrocitromycetin (**9**) (0.3 mg, 0.03%), citromycin (**2**) (2.0 mg, 0.17%), and 12-methoxycitromycetin (**7**) (2.6 mg, 0.25%). Fraction 4 was purified further by gradient C_8 preparative HPLC (21.2 mL/min gradient elution from 80% H_2O/CH_3CN to 40% H_2O/CH_3CN over 30 min, through a Zorbax RX-C_8 7 μm, 250 × 21.2 mm column) to yield 12-methoxycitromycin (**8**) (1.0 mg, 0.10%). Fraction 5 was purified by gradient C_8 preparative HPLC (21.2 mL/min gradient elution from 75% H_2O/CH_3CN to 65% H_2O/CH_3CN over 10 min, then to 60% H_2O/CH_3CN over 35 min, through a Zorbax RX-C_8 7 μm, 250 × 21.2 mm column) to yield fulvic acid (**5**) (0.7 mg, 0.07%).

Citromycetin (1):[1] yellow oil; UV (MeOH) λ_{max} (ε) 214 (24 900), 260 (10 300), 306 (8200), 379 (12 200) nm; ^{1}H NMR (d_6-DMSO, 500 MHz) see Table 1; ^{13}C NMR (d_6-DMSO, 125 MHz) see Table 2; HRESI(+)MS m/z 313.0321 ([M + Na]$^+$, $C_{14}H_{10}O_7Na$ requires 313.0324).

Citromycin (2):[12] yellow, amorphous solid; UV (MeOH) λ_{max} (ε) 215 (33 500), 254 (20 500), 298 (12 000), 374 (19 500) nm; ^{1}H NMR (d_6-DMSO, 500 MHz) see Table 1; ^{13}C NMR (d_6-DMSO, 125 MHz) see Table 2; ESI(+)MS m/z 269 [M + Na]$^+$; HRESI(+)MS m/z 269.0430 ([M + Na]$^+$, $C_{13}H_{10}O_5Na$ requires 269.0426).

(–)-2,3-Dihydrocitromycetin (3): yellow oil; $[\alpha]_D$ −143 (c 0.25, MeOH); UV (MeOH) λ_{max} (ε) 211 (12 600), 240 (8570), 318 (6700), 390 (8000) nm; ^{1}H NMR (d_6-DMSO, 500 MHz) see Table 1; ^{13}C NMR (d_6-DMSO, 125 MHz) see Table 2; ESI(–)MS m/z 291 [M – H]$^-$; HREIMS m/z 292.0575 ([M]$^+$, $C_{14}H_{12}O_7$ requires 292.0583).

(–)-2,3-Dihydrocitromycin [neuchromenin] (4): yellow oil; $[\alpha]_D$ −366 (c 0.5, MeOH); UV (MeOH) λ_{max} (ε) 203 (15 300), 256 (9200), 309 (6900), 394 (11 500) nm; ^{1}H NMR (d_6-DMSO, 500 MHz) see Table 1; ^{13}C NMR (d_6-DMSO, 125 MHz) see Table 2; ESI(+)MS m/z 271 [M + Na]$^+$; HRESI(+)MS m/z 271.0579 ([M + Na]$^+$, $C_{13}H_{12}O_5Na$ requires 271.0582).

Fulvic acid (5): pale yellow oil; ^{1}H NMR (600 MHz, d_4-MeOH) δ 7.12 (2H, s, H-10 in **5**/**5a**), 4.64 (4H, s, H-6 in **5**/**5a**), 2.99 (1H, d, J = 17.6 Hz, H-3a in **5**), 2.77 (1H, d, J = 17.7 Hz, H-3b in **5**), 2.65 (2H, s, H-3 in **5a**), 1.56 (6H, s, H-1 in **5**/**5a**); ESI(+)MS m/z 309 [M + H]$^+$; HRESI(+)MS m/z 331.0438 ([M + Na]$^+$, $C_{14}H_{12}O_8Na$ requires 331.0430).

Anhydrofulvic acid (6): yellow oil; UV (MeOH) λ_{max} (ε) 203 (16 400), 259 (sh), 331 (5500), 405 (7100) nm; ^{1}H NMR (500 MHz, d_6-DMSO) data were in good agreement with the literature values;[11] HRESI(+)MS m/z 313.0318 ([M + Na]$^+$, $C_{14}H_{10}O_7Na$ requires 313.0324).

12-Methoxycitromycetin (7): pale yellow oil; UV (MeOH) λ_{max} (ε) 216 (15 000), 254 (11 000), 303 (6000), 351 (8000) nm; ^{1}H NMR (500 MHz, d_4-MeOH) see Table 1; ^{13}C NMR (125 MHz, d_4-MeOH) see Table 2; ESI(+)MS m/z 305 [M + H]$^+$, 327 [M + Na]$^+$; HRESI(+)MS m/z305.0658 ([M +H]$^+$, $C_{15}H_{13}O_7$ requires 305.0661).

12-Methoxycitromycin (8): yellow oil; UV (MeOH) λ_{max} (ε) 214 (13 000), 238 (11 000), 297 (5600), 370 (5400) nm; ^{1}H NMR (600 MHz, d_4-MeOH) see Table 1; ESI(+)MS m/z 261 [M + H]$^+$, 283 [M + Na]$^+$; HRESI(+)MS m/z 283.0579 ([M + Na]$^+$, $C_{14}H_{12}O_5Na$ requires 283.0582).

(–)-12-Methoxy-2,3-dihydrocitromycetin (9): yellow, amorphous solid; $[\alpha]_D$ −21 (c 0.04, MeOH); UV (MeOH) λ_{max} (ε) 203 (13 000), 255 (sh), 312 (4000), 368 (4800) nm; ^{1}H NMR (600 MHz, d_4-MeOH) see Table 1; ESI(–)MS m/z 305 [M – H]$^-$; HREIMS m/z 306.0733 ([M]$^+$, $C_{15}H_{14}O_7$ requires 306.0740).

12-Methoxyanhydrofulvic acid (10): yellow oil; UV (MeOH) λ_{max} (ε) 210 (2600), 258 (2900), 312 (7400) nm; ^{1}H NMR (600 MHz, d_4-MeOH) see Table 1; ^{13}C NMR (150 MHz, d_4-MeOH) see Table 2; ESI(+)MS m/z 305 [M + H]$^+$, 327 [M + Na]$^+$; HREIMS m/z 304.0592 ([M]$^+$, $C_{15}H_{12}O_7$ requires 304.0583).

Pistillarin (11):[5] brown oil; UV (MeOH) λ_{max} (ε) 206 (21 000), 260 (4400), 293 (3400) nm; ^{1}H NMR (600 MHz, d_4-MeOH) δ 7.30 (1H, d, J = 2.2 Hz, H-1), 7.28 (1H, d, J = 2.2 Hz, H-17), 7.21 (1H, dd, J = 2.2, 8.3 Hz, H-5), 7.19 (1H, dd, J = 2.2, 8.3 Hz, H-21), 6.80 (1H, d, J = 8.3 Hz, H-4), 6.78 (1H, d, J = 8.3 Hz, H-20), 3.46 (2H, t, J = 6.4 Hz, H-8), 3.39 (2H, t, J = 6.7 Hz, H-14), 3.05 (2H, t, J = 7.3 Hz, H-11), 3.02 (2H, t, J = 7.3 Hz, H-10), 1.95 (2H, dt, J = 6.4, 7.3 Hz, H-9), 1.78 (2H, m, H-12), 1.70 (2H, m, H-13); ^{13}C NMR (150 MHz, d_4-MeOH) δ 171.2 (C, C-7), 170.4 (C, C-15), 150.5 (C, C-3), 150.2 (C, C-19), 146.4 (2×C, C-2/C-18), 126.9 (C, C-16), 126.2 (C, C-6), 120.7 (CH, C-5), 120.5 (CH, C-21), 115.9 (CH, C-4), 115.8 (2×CH, C-1/C-20), 115.7 (CH, C-17), 48.7 (CH_2, C-11), 46.4 (CH_2, C-10), 39.8 (CH_2, C-14), 37.2 (CH_2, C-8), 28.0 (CH_2, C-9), 27.7 (CH_2, C-13), 24.7 (CH_2, C-12); ESI(+)MS m/z 418 [M + H]$^+$, ESI(–)MS m/z 416 [M – H]$^-$.

***Cyclo*-(L-Phe-L-Pro) (12):**[6] colorless oil; $[\alpha]_D$ −56 (c 0.2, EtOH); ^{1}H and ^{13}C NMR (600 MHz, d_6-DMSO) data were in good agreement with the literature values;[6] ESI(+)MS m/z 245 [M + H]$^+$.

***Cyclo*-(L-Pro-L-Tyr) (13):**[7] colorless oil; $[\alpha]_D$ −49 (c 0.25, EtOH); ^{1}H and ^{13}C NMR (600 MHz, $CDCl_3$) data were in good agreement with the literature values;[7] ESI(+)MS m/z 261 [M + H]$^+$.

***Cyclo*-(L-Pro-L-Val) (14):**[6] colorless oil; $[\alpha]_D$ −111 (c 0.2, EtOH); ^{1}H and ^{13}C NMR (600 MHz, $CDCl_3$) data were in good agreement with the literature values;[6] ESI(+)MS m/z 197 [M + H]$^+$.

***Cis*-bis(methylthio)silvatin (15):**[8] brown oil; $[\alpha]_D$ −18 (c 0.5, $CHCl_3$); ^{1}H and ^{13}C NMR (600 MHz, $CDCl_3$) data were in good agreement with the literature values;[8] ESI(+)MS m/z 431 [M + Na]$^+$, 361 [M – HSMe + H]$^+$; HRESI(+)MS m/z 361.1589 ([M – HSMe + H]$^+$, $C_{19}H_{25}N_2O_3S$ requires 361.1586).

Bilain A (16): brown oil; $[\alpha]_D$ −10 (c 0.25, EtOH); ^{1}H NMR (500 MHz, d_6-acetone) see Table 3; ^{13}C NMR (125 MHz, d_6-acetone) see Table 4; ESI(+)MS m/z 461 [M + Na]$^+$; HRESI(+)MS m/z 461.1181 ([M + Na]$^+$, $C_{20}H_{26}N_2O_5S_2Na$ requires 461.1181).

Bilain B (17): brown oil; ^{1}H NMR (600 MHz, d_6-acetone) see Table 3; ^{13}C NMR (150 MHz, d_6-acetone) see Table 4; ESI(+)MS m/z 465 [M + Na]$^+$; HRESI(+)MS m/z 465.1486 ([M + Na]$^+$, $C_{20}H_{30}N_2O_5S_2Na$ requires 465.1494).

Bilain C (18): brown oil; ^{1}H NMR (600 MHz, d_6-acetone) see Table 3; ^{13}C NMR (150 MHz, d_6-acetone) see Table 4; ESI(+)MS m/z 532 [M + Na]$^+$; HRESI(+)MS m/z 532.1549 ([M + Na]$^+$, $C_{23}H_{31}N_3O_6S_2Na$ requires 532.1552).

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References and Notes

(1) Yamauchi, M.; Katayama, S.; Watanabe, T. *J. Chem. Soc., Perkin Trans.* **1987**, *2*, 395–398.

(2) Evans, G. E.; Staunton, J. *J. Chem. Soc., Perkin Trans.* **1988**, *4*, 755–761.

(3) El Hage Chahine, J.-M.; Bauer, A.-M.; Baraldo, K.; Lion, C.; Ramiandrasoa, F.; Kunesch, G. *Eur. J. Inorg. Chem.* **2001**, *9*, 2287–2296.

(4) Reissbrodt, R.; Ramiandrasoa, F.; Bricard, L.; Kunesch, G. *BioMetals* **1997**, *10*, 95–103.

(5) Steglich, W.; Steffan, B.; Stroech, K.; Wolf, M. *Z. Naturforsch., C: Biosci.* **1984**, *39C*, 10–12.

(6) Adamczeski, M.; Reed, A. R.; Crews, P. *J. Nat. Prod.* **1995**, *58*, 201–208.

(7) Jayatilake, G. S.; Thornton, M. P.; Leonard, A. C.; Grimwade, J. E.; Baker, B. J. *J. Nat. Prod.* **1996**, *59*, 293–296.

(8) Kawahara, N.; Nozawa, K.; Nakajima, S.; Kawai, K. *J. Chem. Soc., Perkin Trans. 1* **1987**, *9*, 2099–2101.

(9) Stewart, M.; Capon, R. J.; Lacey, E.; Tennant, S.; Gill, J. H. *J. Nat. Prod.* **2005**, *68*, 581–584.

(10) Yamauchi, M.; Katayama, S.; Todoroki, T.; Watanabe, T. *J. Chem. Soc., Perkin Trans. 1* **1987**, *2*, 389–94.

(11) Fujita, K.; Nagamine, Y.; Ping, X.; Taniguchi, M. *J. Antibiot.* **1999**, *52*, 628–634.

(12) Hetherington, A. C.; Raistrick, H. *Trans. R. Soc. (London)* **1931**, *B220*, 209–244.

(13) Robertson, A.; Whalley, W. B.; Yates, J. *J. Chem. Soc. Abs.* **1951**, 2013–2018.

(14) Castillo, G. M.; Choi, P. Y.; Snow, A. D. WO Patent 2001049281, 2001.

(15) Hayakawa, Y.; Yamamoto, H.; Seto, H. *Tetrahedron Lett.* **1996**, *37*, 6363–6364.
(16) Tanada, Y.; Mori, K. Eu. *J. Org. Chem.* **2001**, 1963–1966.
(17) Fujii, K.; Ikai, Y.; Oka, H.; Suzuki, M.; Harada, K.-I. *Anal. Chem.* **1997**, *69*, 5146–5151.
(18) Fujii, K.; Ikai, Y.; Mayumi, T.; Oka, H.; Suzuki, M.; Harada, K.-I. *Anal. Chem.* **1997**, *69*, 3346–3352.
(19) Wang, G.-Y.-S.; Abrell, L. M.; Avelar, A.; Borgeson, B. M.; Crews, P. *Tetrahedron* **1998**, *54*, 7335–7342.
(20) Capon, R. J.; Ratnayake, R.; Stewart, M.; Lacey, E.; Tennant, S.; Gill, J. H. *Org. Biomol. Chem.*

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