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Isolation, Biological Activity Evaluation, Structure Elucidation, and Total Synthesis of Eliamid: A Novel Complex I Inhibitor

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Abstract: Eliamid is a secondary metabolite isolated from two bacterial strains. This molecule features a linear polyketide backbone terminated by a tetramic acid amide moiety. Among other biological activities, eliamid shows a high and specific cytostatic action on human lymphoma and cervix carcinoma cell lines. The 2,4-*anti* relative configuration of the C-2,C-4-dimethyl substituted amide fragment was assigned by means of Breit's rule. The absolute configuration of all stereocenters was determined by a combination of degradation methods, structural similarity analysis and total synthesis. The stereogenic centers were introduced by vinylogous Mukaiyama aldol reaction and two consecutive Myers alkylations. The use of pentafluorophenyl ester as acylation agent allowed the efficient formation of tetramic acid amide. The longest linear sequence in the synthesis consist of 13 steps and proceeds with 12% overall yield. Differential spectroscopy experiments with beef heart submitochondrial particles established that eliamid is a potent inhibitor of the NADH–ubiquinone oxidoreductase complex. Additionally, biosynthesis of eliamid was investigated by feeding experiments with ^{13}C-labeled precursors.

Keywords: complex I inhibitors · natural products · structure elucidation · tetramic acids · total synthesis

Introduction

Activity-guided isolation of secondary metabolites from microorganisms, plants and fungi has evolved as a powerful technology for the discovery of biologically relevant natural products and their cellular targets. At the Helmholtz-Zentrum für Infektionsforschung (HZI), more than 100 structurally novel compounds produced by myxobacteria have been discovered, characterized, and, in part, developed, for example, as anticancer drugs.[1] Later, the focus shifted to other groups of gliding bacteria, and molecular biology of the producing organisms.[2] Here, we describe the isolation, biological activity evaluation, structure elucidation, total synthesis and studies on the biosynthesis of eliamid, a potent complex I inhibitor with some other interesting activities.

In a screening of myxobacteria for secondary metabolites with antifungal activity, a substance with a conspicuous UV adsorption was discovered in the HPLC traces of extracts from two bacterial strains: So ce241,[3] which also produces soraphen,[4] and So ce439, which produces ambruticins,[5] both antifungal compounds. The new isolate was named eliamid (**1**, Figure 1) acknowledging the place (Arismeliya, Egypt) where the So ce241 strain was collected. The carbon skeleton was identified to be a linear polyketide with a terminal tetramic amide moiety.

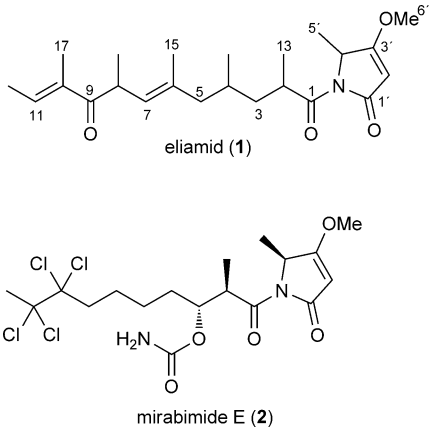

Figure 1. Structures of eliamid (2D) and mirabimide E.

Results and Discussion

Isolation: The producing organisms, *Sorangium cellulosum* So ce439 (DSMZ 11529) and So ce241 were isolated from the soil samples taken at Pfeffingen, Switzerland and Arismeliya, Egypt, respectively. The fermentation was run in a 100 L bioreactor with 70 L of medium in the presence of XAD-16 adsorbing resin (2% v/v, 1.4 kg) for 11 days. After separation of the resin by filtration, it was eluted with a 1:1

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mixture of methanol/acetone followed by pure methanol. The eluates were concentrated independently until separation of the water phase, and were extracted with ethyl acetate. The combined organic phases were evaporated and the residue purified by two consecutive chromatographic separations on normal silica gel to give eliamid (460 mg) as a viscous oil.

Biological activity evaluation: The biological evaluation of eliamid (**1**) with a panel of transformed cell lines showed a specific cytostatic action on human lymphoma and cervix carcinoma cell cultures (Table 1). Additionally, a significant

Table 1. Cytostatic activity of eliamid on various transformed cells lines.

Cell line	Organism/source	IC_{50} [ng mL^{-1}]
L-929	mouse fibroblast	0.5
PtK2	rat kangaroo kidney	30.0
KB 3.1	human cervix carcinoma	1.0
HL-60	human leukemia	15.0
U-937	human lymphoma	0.5
A-431	human epidermis carcinoma	3.0
A-498	human kidney carcinoma	20.0
A-549	human lung carcinoma	15.0

reduction of motility, partially one-side only, was observed within 20–30 min after application of 0.2–0.3 μg mL^{-1} solutions of eliamid to a colony of brine shrimps, *Artemia salina*. However, death occurred rather gradually so that even after 24 h some shrimps remained alive, while the damage effect was found to be persistent. Treatment of soil nematodes (*Panagrellus spec.*) with 5 μg mL^{-1} eliamid was lethal to all animals. Additionally, eliamid showed moderate inhibitory activity on fungi and yeast (Table 2).

Table 2. Antifungal activity of eliamid at 20 μg per test plate.

Test organism	Diameter of inhibition zone [mm]
Mucor hiemalis	22
Alternaria solani	15
Fusarium oxysporum	19
Sclerotina sclerotorium	16
Hansenula anomala	20
Sporidiobolus ruineniae	16

To determine the mode of action of eliamid, its effect on the mitochondrial respiratory chain was examined. Eliamid (**1**) strongly inhibited NADH oxidation in beef heart submitochondrial particles (SMP) with an IC_{50} of 8 ng mL^{-1} (20 nM; Figure 2). The site of inhibition of eliamid within the respiratory chain was investigated by means of differential spectroscopy by using a DW 2000 UV/Vis SLM double beam spectrophotometer. Upon reduction with physiological substrates, for example, NADH, fully oxidized cytochromes in front of the block became reduced, whereas those behind it remained oxidized. The differential spectrum of NADH-reduced minus air-oxidized SMP without inhibitor showed

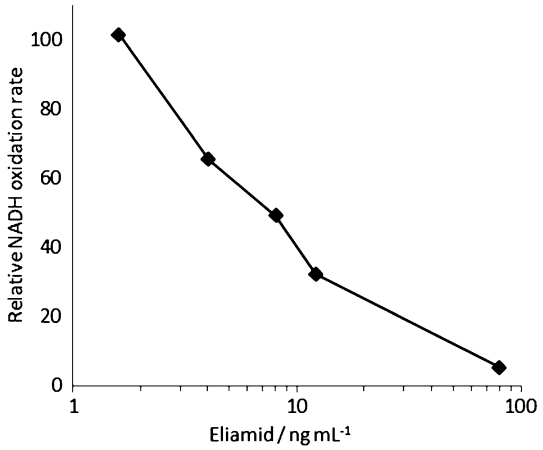


Figure 2. Inhibitory effect of eliamid on NADH oxidation in beef heart submitochondrial particles (SMP). The samples contained 66.3 μg mL^{-1} beef heart protein. The rate of NADH oxidation without inhibitor was 0.51(±0.013) μmol mg^{-1} min^{-1}.

the characteristic absorption maxima for the different cytochromes (Figure 3). Treatment of SMP with eliamid almost completely inhibited reduction of cytochrome a + a3 (α band at 605 nm), cytochrome b (α band at 563 nm) and the cytochromes c + c1 (α band at 553 nm) by NADH; this indicates that the inhibitory effect of eliamid is on the substrate side of cytochrome b. Cytochrome b of complex III can be reduced by NADH by complex I (NADH–ubiquinone oxidoreductase) or by succinate by complex II (succinate–ubiquinone oxidoreductase). Reduction kinetics of cytochrome b with either NADH or succinate as substrate showed that eliamid inhibited the reduction of cytochrome b only when NADH was the electron donor. This indicates that the inhibitory effect of eliamid, like that of piericidine, myxalamide and actinopyron, is mediated by interaction with the complex I (NADH–ubiquinone oxidoreductase) of the eukaryotic respiratory chain.

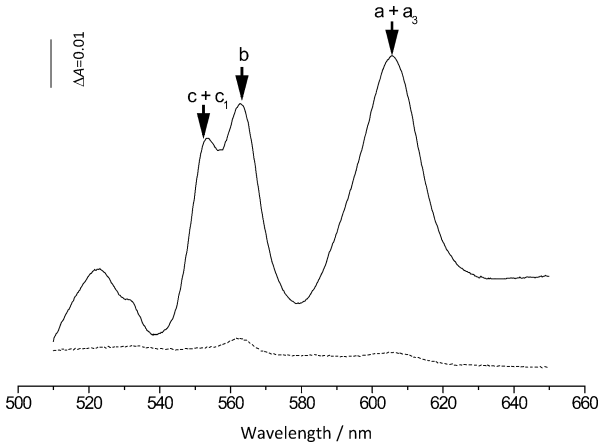


Figure 3. The effect of eliamid on the reduction of cytochromes by NADH. Top line: differential spectrum (reduced minus oxidized) of SMP reduced with NADH without inhibitor; bottom line: the same, but in the presence of 15 μg mL^{-1} eliamid.

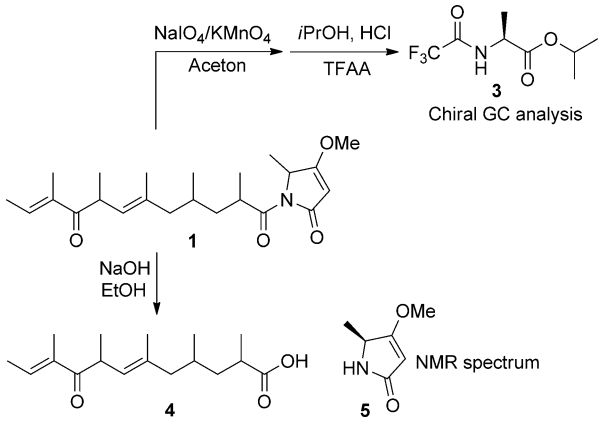
Structural elucidation and total synthesis: The IR spectrum of eliamid shows three strong bands at 1632, 1680 and 1719 cm^{-1}, whereas the UV spectrum shows two broad maxima at 223 and 234 nm. High resolution mass spectrometry and elemental analysis established the *m*/*z* value of eliamid as 389.2558 and the elemental composition as $C_{23}H_{35}NO_4$, which corresponds to seven double bond equivalents (DBEs). Examination of the ^1H and ^{13}C NMR spectroscopy revealed the presence of an α,β-unsaturated keton, a three substituted double bound and two carboxyl group equivalents (Table 3). The positions of the unsaturated

Table 3. ^1H and ^{13}C NMR spectroscopy data of eliamid in [D_4]MeOH (400 MHz).

Atom	^{13}C δ [ppm]	^1H δ [ppm]	Multiplet	J_{HH} [Hz]
1	178.5	–	–	–
2	37.7	3.98	m	–
3a	42.2	1.30	ddd	13.6, 7.5, 5.5
3b	42.2	1.52	ddd	13.6, 8.8, 6.2
4	29.8	1.69	m	–
5a	48.9	1.84	dd	13.2, 8.1
5b	48.9	2.01	dd	13.2, 6.2
6	136.0	–	–	–
7	128.6	5.02	d	9.5
8	40.7	4.21	dq	9.5, 6.7
9	205.8	–	–	–
10	138.3	–	–	–
11	139.0	6.91	qd	7.0, 1.0
12	14.8	1.92	dd	7.0, 1.0
13	16.5	1.11	d	7.0
14	19.7	0.89	d	6.2
15	16.2	1.70	s	–
16	18.0	1.13	d	6.6
17	11.4	1.77	s	–
1′	182.7	–	–	–
2′	93.5	5.21	s	–
3′	171.5	–	–	–
4′	56.9	4.63	q	6.6
5′	17.3	1.46	d	6.6
6′	59.6	3.95	s	–

ketone, double bond and methyl substituents along the carbon skeleton were established by direct $^1H,^1H$ COSY and $^1H,^{13}C$ HMQC NMR correlation spectroscopies, together with the long range $^1H,^{13}C$ HMBC correlations. The tetramic acid fragment, which accounts for the last two DBEs, was deduced from the chemical shift and long range HMBC correlations of C*H*-2' ($\delta_H = 5.21$ ppm, $\delta_C = 93.5$ ppm) to qC-1' ($\delta = 182.7$ ppm) and qC-3′ ($\delta = 171.5$ ppm) and by comparison with the spectroscopic data for mirabimide E (**2**).[6]

The absolute configuration of the stereocenter in the tetramic acid fragment was determined by oxidative and hydrolytic degradation of natural product according to the mirabimide E (**2**) precedent (Scheme 1).[6] Analysis of the reaction mixtures by chiral gas chromatography unambiguously established that the tetramic acid moiety has the absolute configuration of L-alanine. Further attempts to elucidate the absolute configurations of remaining stereocenters by ozonolysis or other degradation methods eventually led to the consumption of all available material, at which point the



Scheme 1. Determination of the absolute configuration of the tetramic acid.

strategy of unraveling the unknown configurations by degradation was changed.

Instead, the structure was compared with similar natural products for which the corresponding configurations were known, such as myxalamide D (**7**),[7] piericidin A1 (**6**)[8] and actinopyrone A (**8**)[9] (Figure 4). This strategy takes advant-

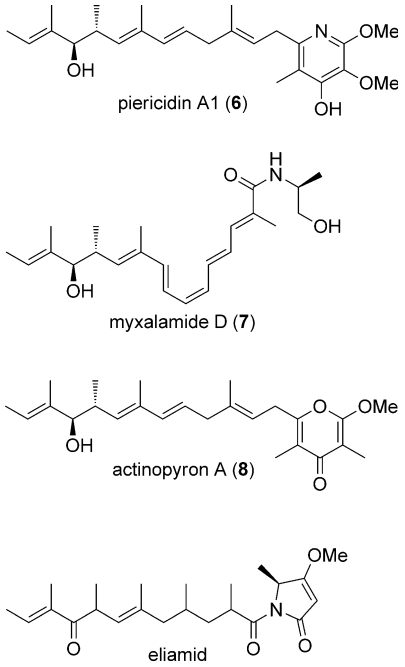


Figure 4. Structural similarities between eliamid and other complex I inhibitors.

age of Celmer's rule[10] and is based on the fact that similar structural motifs might arise from shared polyketide gene clusters.

Also, of particular importance here were the resonances of the 3-H_a and 3-H_b methylene protons (Figure 5), which were recorded at 1.27 and 1.50 ppm, respectively. It was already observed earlier that deoxygenated polypropionate

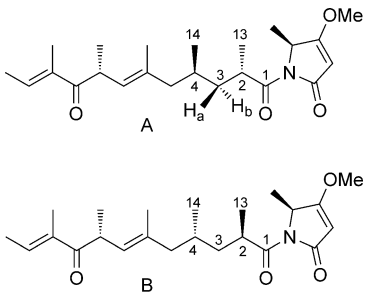


Figure 5. Proposed 3D structures of eliamid.

compounds with a *syn*-2,4-dimethyl substitution pattern display significantly larger differences in the chemical shifts of the diastereotopic methylene protons than the corresponding *anti*-isomers. An excellent theoretical explanation for this empirical rule and a *meta*-analysis of more than 60 compounds of various classes containing 2,4-dimethyl substituted chains were given by Breit and Schmidt in their recent paper.[11] It follows that for *syn*-2,4-dimethyl amides the chemical shift difference of methylene protons is usually found to be in the 0.7–0.8 ppm region, whereas for *anti*-2,4-diastereomers it is between 0.2 and 0.3 ppm. The observed value of $\Delta\delta = 0.23$ ppm would be consistent with an *anti* configuration C-13 and C-14 methyl groups.

Based on the structural similarities to myxalamide D (**7**), piericidin A1 (**6**) and actinopyrone A (**8**) the absolute configuration at C-8 was proposed to be *R*. Additional reassurance for the structural similarities was drawn from the fact that all four compounds are inhibitors of the NADH–ubiquinone oxidoreductase complex. Therefore, we decided to take on the syntheses of both *anti* isomers. We started with diastereomer A, which contains the same absolute configuration of 2,4-dimethyl amide as 2,4-dimethyl ester moiety of myxovirescin.[12]

The synthesis commenced with Kobayashi's vinylogous Mukaiyama aldol reaction between tiglic aldehyde and *N*,*O*-silyl keten acetal (**10**) to provide 63% yield of aldol adduct when the reaction was allowed to proceed for 5 days (Scheme 2).[13] Reductive cleavage of the chiral auxiliary provided alcohol **12**, which was converted to the allylic iodide **13**. The subsequent Evans alkylation[14] produced the desired amide **15**, albeit in a moderate yield. A second reductive cleavage and reaction with triflic anhydride furnished **16**, which was subjected to a second Evans alkylation. Despite the rather low yields in this step, we were able to obtain sufficient quantity of material to proceed further with the synthesis.

From here, several routes were reasonable for the introduction of the tetramic acid amide moiety.[15] We opted to employ the method of Tønder,[16] which relies on perfluorophenyl esters as acylation agents (Scheme 3). Thus, the carboxylic acid **19** obtained by lithium peroxide hydrolysis of amide **18** was converted to the activated ester **20** according to the modified Steglich protocol.[17] Ester **20** was then allowed to react with a slight excess of potassium salt of pyAla-OMe (**5**)[18] to provide the desired product in 38% yield (62% b.o.r.s.m.). The TBS protecting group was cleaved with $Et_3N{\cdot}3HF$, and subsequent oxidation of the allylic alcohol **22** with the Dess–Martin periodinane provided the putative eliamid.

To our disappointment, the proton NMR spectrum of compound **23** did not match that of the natural product. Nevertheless, a good agreement was found for the resonances that lie in the down-field. The biggest deviation between the spectra was observed for the signals of the CH_3-5′, CH_3-13 and CH_3-14 protons in the 0.7–1.1 ppm region (Figure 6).

It was, therefore, assumed that the second possible diastereomer should be the natural product. Facing the low yields using the Evans alkylation we switched to Myers

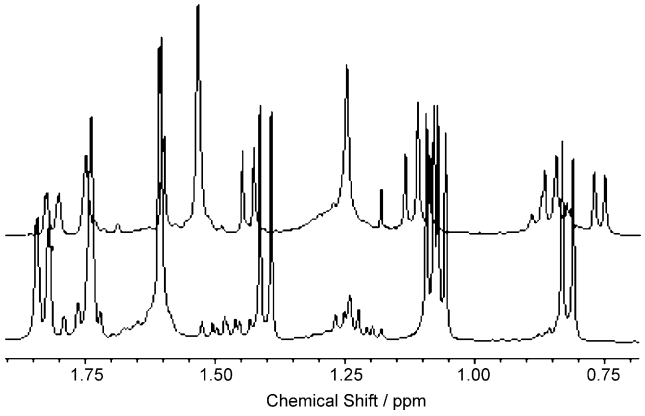
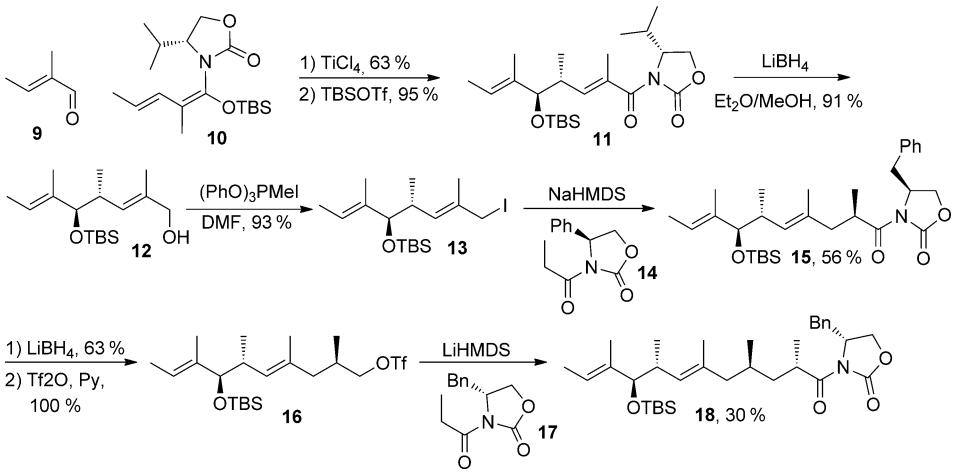
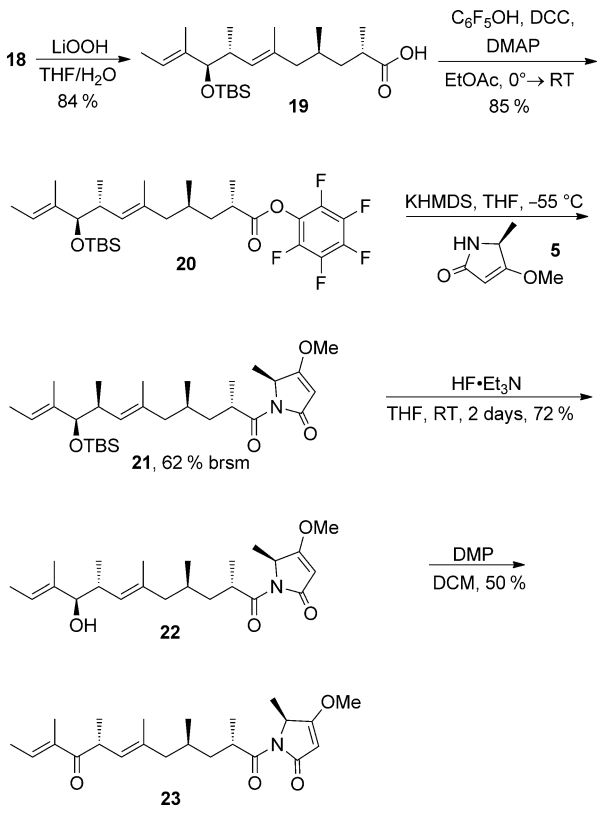


Figure 6. Comparison of the ^1H NMR spectra of **23** (top) and eliamid (bottom).

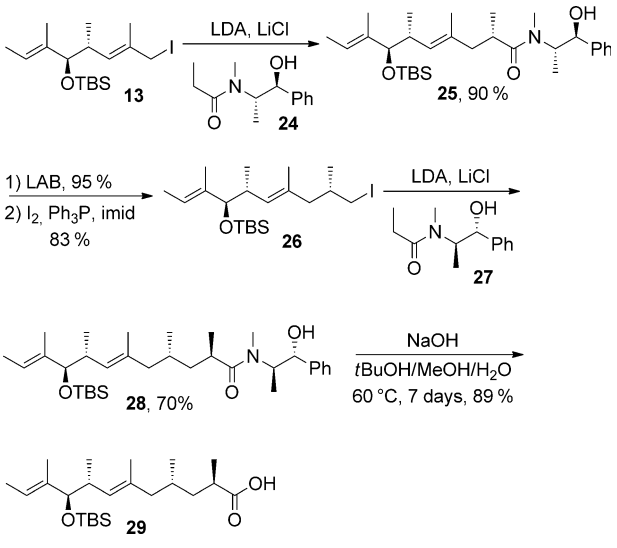


Scheme 2. Installation of stereocenters through vinylogous Mukaiyama aldol reaction and Evans alkylations.

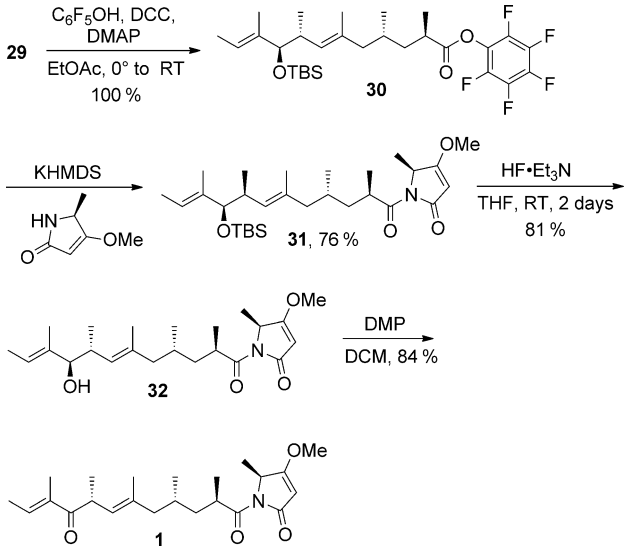


Scheme 3. Formation of the tetramic acid amide and completion of the synthesis.

method[19] for the alkylation steps (Scheme 4). Gratifyingly, transformation of allylic iodide **13** with the enolate derived from *N*-propionyl pseudoephedrine (**24**) provided the desired product in 90% yield. The auxiliary was removed with lithium aminoborohydride and the resulting alcohol converted into alkyliodide **26**. For the second alkylation step an eight- to tenfold excess of pseudoephedrine enolate was required to drive the reaction to completion. Then, a prolonged (one week) exposure to NaOH solution at 60°C was used to avoid concomitant cleavage of the TBS-ether during hydrolysis of the pseudoephedrine amide. Formation of the tetramic acid amide, TBS group cleavage and oxidation to the ketone were performed as already described (Scheme 5).



Scheme 4. Myers alkylation approach to the acid **29**.



Scheme 5. Completion of the total synthesis of eliamid.

This time, the synthetic material was spectroscopically identical to the authentic natural product (^{1}H and ^{13}C NMR spectroscopy; Figure 7, see also the Supporting Information for a detailed comparison). The optical rotation was found to be acceptably close to that of the natural product: $[\alpha]_D = -65°$ ($c = 0.8$ in MeOH) for the synthetic material versus $[\alpha]_D = -69.8°$ ($c = 0.5$ in MeOH) for the authentic sample. Additionally, synthetic eliamid was found to be equally potent to the natural product in transformed cell line assays.

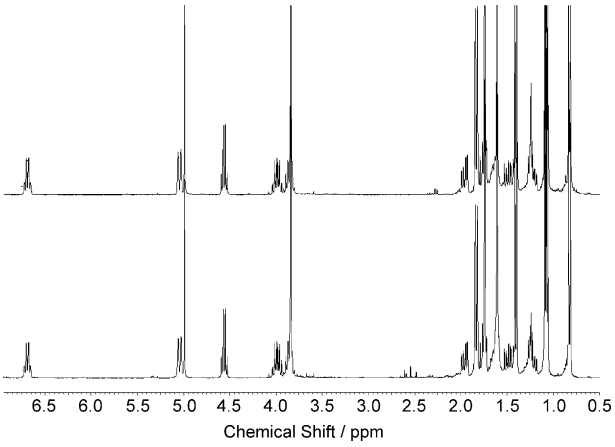


Figure 7. Spectral comparison for synthetic (top) and natural (bottom) eliamid.

It is also worth noting that, the absolute configuration of the C-4 and C-8 stereocenters in eliamid matches the corresponding structural motif (C-24 and C-28) in the structurally similar side chain of spirangien[20] (**33**; Figure 8). Together

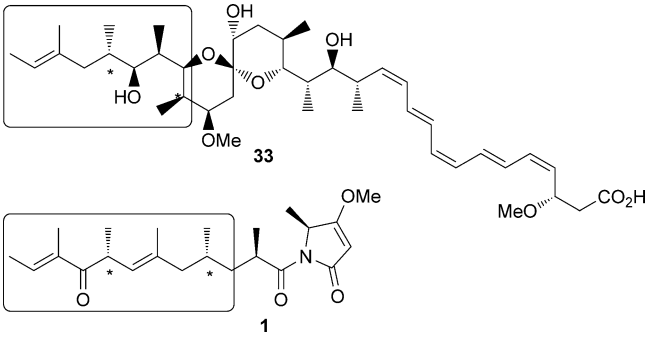


Figure 8. Comparison of eliamid (**1**) and spirangien (**33**).

with earlier described similarities to piericidin A1 (**6**), myxalamide D (**7**) and actinopyron A (**8**) this implies that a highly conserved polyketide synthase cluster exists in various bacterial strains.

Biosynthesis: Several feeding experiments with ^{13}C-labeled precursors were performed on the "shaking flask" scale to investigate the biosynthesis of eliamid. Though, no conclusive results were obtained from experiments with [1-^{13}C]acetate and [1-^{13}C]alanine, the ^{13}C NMR spectrum of [1-^{13}C]propionate derived eliamid revealed a 15–18% ^{13}C-enrichment for all except one odd carbon atom, indicating a contiguous incorporation of five propionate units (Figure 9). Additionally, in a feeding experiment with [^{13}CH$_3$]methionine, a 5% ^{13}C-enrichment in the methoxy group of tetramic acid was observed.

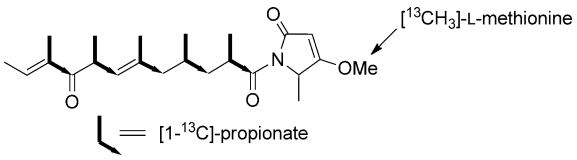


Figure 9. Biosynthesis of eliamid.

Conclusion

In conclusion, we report the isolation, biological activity evaluation, structure elucidation and total synthesis of the new natural product eliamid. The compound exhibits a broad spectrum of biological activities and could serve as a lead structure for the development of novel anticancer drugs. The relative configuration of the 2,4-dimethyl desoxypropionate amide was correctly predicted by Breit's rule. To the best of our knowledge, this is the first time that this semi-empirical rule was used to assign the relative stereochemistry of stereocenters in natural products.

We also accomplished the total synthesis of eliamid in thirteen chemical steps and 12% overall yield. The key steps are a vinylogous Mukaiyama aldol reaction, Myers enantioselective alkylations and tetramic acid amide formation from pentafluorophenyl ester. Further studies on the chemistry and biology of eliamid and its modified analogues are currently underway and will be reported in due course.

Experimental Section

General remarks: Optical rotations were determined on a Perkin–Elmer 241 instrument. IR and UV adsorption spectra were measured on Nicolet 20DXB and Shimadzu 2450 spectrometers, respectively. NMR spectra were recorded in CD_3OD and $CDCl_3$ on a Bruker AM 300, AM 400 and DMX-600 spectrometers. ESI mass spectra (reactant gas ammonia) were obtained on a Finnigan MAT 95 spectrometer, high resolution data were acquired by using peak matching (M/DM = 10000). Analytical TLC (TLC aluminium sheets silica gel Si 60 F_{254} (Merck), solvent: mixtures of ethylacetate/petroleum ether, detection: UV absorption at 254 nm, dark blue spots on staining with cerium(IV) sulfate phosphomolybdic acid in sulfuric acid followed by charring. Unless otherwise stated, all reactions were performed under inert gas blanket. All solvents used were commercial absolute solvents over molecular sieves with water content less than 50 ppm (e.g., from Acros Organics). Sodium [1-^{13}C, 99%]acetate, sodium [2-^{13}C, 99%]acetate, sodium [1,2-$^{13}C_2$, 99%]acetate, sodium [1-^{13}C]propionate and L-[methyl-^{13}C, 98%]methionine were obtained from Cambridge Isotope Laboratories.

Fermentation: The producing organism, *Sorangium cellulosum* So ce439 (DSMZ 11529) was isolated in 1989 from soil samples taken at Pfeffingen (Switzerland). A 100 L bioreactor (Giovanola, Monthey, Switzerland) equipped with a flat-blade turbine stirrer and containing 70 L production medium (10 g L^{-1} glucose, 5 g L^{-1} soy peptone (Marcor), 2 g L^{-1} yeast extract, 1 g L^{-1} $MgSO_4 \cdot 7H_2O$, 1 g L^{-1} $CaCl_2 \cdot 2H_2O$, ethylenediaminetetraacetic acid, 0.008 g L^{-1} iron(III) sodium salt, pH adjusted to 7.2) and 2% (v/v) XAD16 adsorbing resin (Rohm and Haas), was inoculated with 5 L of a 4 day old shake culture grown in the same medium. The fermentation was run for 11 days at 32 °C with an aeration rate of 0.1 V V^{-1} min^{-1} and a stirrer velocity of 250 rpm. The pH was maintained at 7.2 with 10% (g/v) KOH. The foam formation was blocked by addition of the silicon antifoam Tegosipon (Goldschmidt AG, Essen). Eliamid is absorbed quantitatively on the XAD adsorbing resin, which is separated by filtration through a process filter.

Isolation: The XAD-16 adsorbing resin (1.4 kg) was eluted with 1:1 mixture of methanol/acetone (4 L) followed by 6 L of pure methanol. The extracts were independently concentrated until separation of the water phase, and extracted with ethyl acetate (2 × 3 L). The combined organic phases were evaporated, in vacuo, to give 15.8 g of crude extract, which was dissolved in petroleum ether (with minimal addition of methanol) and purified by chromatography over silica gel (400 g, 10 cm column diameter) with a stepwise gradient elution (petroleum ether to petroleum ether/*tert*-butylmethylether 1:1) with a total of 7 L of eluent. The eliamid containing fractions were identified by TLC and concentrated to give 2.26 g of crude product. Final purification by chromatography over silica gel (20–45 μm, 5 × 55 cm column, elution with petroleum ether/*tert*-butylmethylether/methanol 80:19:1, UV detection at 227 nm) provided eliamid (460 mg) as a viscous oil.

Analytical data: TLC $R_f = 0.37$ (petroleum ether/Et_2O 1:1, UV detection); HRMS EI: 389.2558 (calcd for $C_{23}H_{35}NO_4$ 389.2566); elemental analysis (%) calcd: C 70.92, H 9.06, N 3.60; found: C 70.94, H 8.78, N 3.67; IR ($CHCl_3$): $\tilde{\nu} = 1719, 1680, 1632$ cm^{-1}; UV/Vis (CH_3OH): λ_{max} (ε) = 223 (19200), 234 nm (19300 mol^{-1} dm^3 cm^{-1}). For the ^{1}H and ^{13}C NMR spectroscopy results see Table 3.

Degradation studies

Oxidative degradation: A solution of eliamid (5 mg) in acetone (1 mL) was oxidized with sodium periodate (20 mg in 0.3 mL H_2O) and potassium permanganate (3 mg) according to the procedure described for mirabimid E.[6] After 2 h of stirring at room temperature the reaction was quenched with methanol (2 mL) and evaporated. The residue was esterified by treatment with HCl in (1 M) *i*PrOH (0.5 mL) at 100 °C for 30 min, evaporated, trifluoroacetylated with $(CF_3CO)_2O$ (100 μL in 0.5 mL CH_2Cl_2, 10 min at 100 °C) and analyzed by gas chromatography on the Chirasil L-val column. The chromatogram revealed the presence of *N*-trifluoroacetyl-L-alanine isopropyl ester.

Hydrolytic degradation: NaOH (0.5 N, 70 μL) was added to a solution of eliamid (5 mg) in ethanol (0.1 mL). After 50 min the reaction mixture was evaporated in vacuo and portioned between AcOEt and water at pH 3. From the organic phase, 3.5 mg of acid **4** were isolated after purification. The water phase was subjected to butanol extraction (3×1 mL), the combined extracts were evaporated to give 0.1 mg of the tetramic acid **5** (H-py-Ala-OMe).

Compound 4: ^{1}H NMR ($CDCl_3$): δ = 0.79 (d, J = 7 Hz, 3H), 1.09 (d, J = 7 Hz, 3H), 1.10 (d, J = 7 Hz, 3H), 1.09 (d, J = 7 Hz, 3H), 1.28 (m, 1H), 1.45 (m, 1H), 1.62 (d, J = 1 Hz, 3H), 1.65 (m, 1H), 1.75 (m, 1H), 1.75 (s, 3H), 1.83 (dd, J = 7, 1 Hz, 3H), 1.98 (m, 1H), 2.50 (m, 1H), 4.01 (dq, J = 10, 7 Hz, 1H), 5.08 (d, J = 10 Hz, 1H), 6.77 ppm (q,d, J = 7, 1 Hz, 1H); DCI-MS (isobutane) after treatment with diazomethane: *m*/*z* 295 [M+H^+].

Compound 5: ^{1}H NMR ($CDCl_3$): δ = 1.32 (d, J = 7 Hz, 3H), 3.77 (s, 3H), 4.08 (q, J = 7 Hz, 1H), 4.99 ppm (s, 1H); DCI-MS (isobutane): *m*/*z* (%): 128 (73) [M+H^+], 255 (100) [2M+H^+].

Total synthesis

*(R)-3-((2E,4R,5R,6E)-5-(tert-Butyldimethylsilyloxy)-2,4,6-trimethylocta-2,6-dienoyl)-4-isopropyloxazolidin-2-one (**11**)*: Tiglic aldehyde (**9**; 3 mL, 2.6 g, 30 mmol) was added to a stirred solution of $TiCl_4$ (1.0 M in CH_2Cl_2, 15 mL, 15 mmol) in CH_2Cl_2 (150 mL) at −78 °C. This was immediately followed by addition of vinylketene silyl *N,O*-acetal **10** (5.08 g, 15 mmol) in CH_2Cl_2 (10 mL). After being stirred for 5 days at −50 °C, the reaction was quenched by the addition of a 1:1 mixture of saturated aqueous $NaHCO_3$ and saturated aqueous Rochelle salt (100 mL total volume). The resulting mixture was stirred at room temperature until the white slurry was dissolved. The organic layer was separated and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel (petroleum ether/EtOAc, 3:1) to afford 2.9 g (63%) of aldol adduct.

2,6-Lutidine (2.3 mL, 2.14 g, 20 mmol) and TBSOTf (3 mL, 3.5 g, 13.3 mmol) were added to a solution of the above aldol adduct (2.9 g, 9.5 mmol) in CH_2Cl_2 (50 mL) at 0 °C. The mixture was stirred 15 min at 0 °C and allowed to warm to room temperature. The reaction was quenched with sat. aq. $NaHCO_3$, the organic layer was separated, dried over Na_2SO_4 and concentrated, in vacuo. Purification by column chromatography over silica gel (petroleum ether/EtOAc, 6:1) afforded 3.82 g (95%) of imid **11**. Analytical data were identical to that reported in ref. [9].

*(2E,4R,5R,6E)-5-(tert-Butyldimethylsilyloxy)-2,4,6-trimethylocta-2,6-dien-1-ol (**12**)*: Lithium borohydride (88 mg, 4 mmol, 1.5 equiv) was added to a cooled (0 °C, ice-bath) solution of imide **11** (1.12 g, 2.67 mmol) in Et_2O/MeOH (20 mL/0.16 mL) and allowed to stir at this temperature for 4 h. The mixture was quenched dropwise with a saturated solution of $NaHCO_3$ (20 mL). When gas evolution ceased, the organic layer was separated, the aqueous layer was extracted with Et_2O (20 mL), and the combined organic layers were washed with a saturated solution of $NaHCO_3$ (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated, in vacuo, and purified by column chromatography over silica gel with petroleum ether/EtOAc (6:1) as eluent to provide 720 mg (91%) of the allylic alcohol **12** as a colorless oil. R_f = 0.51 (petroleum ether/EtOAc, 6:1); $[\alpha]_D$ = −1.1 (c = 2.0 in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.32 (q, J = 6.8 Hz, 1H), 5.22 (dd, J = 9.8, 1.2 Hz, 1H), 3.99 (d, J = 3.2 Hz, 2H), 3.63 (d, J = 7.9 Hz, 1H), 2.53 (m, 1H), 1.67 (d, J = 1.2 Hz, 3H), 1.57 (d, J = 6.6 Hz, 3H), 1.55 (s, 3H), 0.83 (s, 9H), 0.75 (d, J = 6.8 Hz, 3H), −0.04 (s, 3H), −0.07 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = −5.1, −4.7, 10.8, 12.9, 14.0, 17.4, 18.1, 25.6, 36.7, 69.3, 83.4, 121.2, 130.7, 134.1, 137.1 ppm; HRMS: calcd for $C_{17}H_{34}O_2SiNa$ 321.2226, found 321.2226.

*tert-Butyl((2E,4R,5R,6E)-8-iodo-3,5,7-trimethylocta-2,6-dien-4-yloxy)dimethylsilane (**13**)*: Methyltriphenoxyphosphonium iodide (1.34 g, 3 mmol, 1.25 equiv) in DMF (3 mL) was added dropwise to a stirred (at 0 °C) solution of alcohol **12** (720 mg, 2.42 mmol, 1 equiv) in DMF (3 mL). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction mixture was diluted with hexane (35 mL) and the organic layer was extracted with cold aqueous 1 N NaOH (2×10 mL) and water (2×10 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo to afford iodide **13** (910 mg, quantitative) as a yellow oil. The crude product was immediately used in the subsequent reaction without any further purification.

*(2S,4E,6R,7R,8E)-7-(tert-Butyldimethylsilyloxy)-N-((1S,2S)-1-hydroxy-1-phenylpropan-2-yl)-N,2,4,6,8-pentamethyldeca-4,8-dienamide (**25**)*: *n*BuLi (2.5 M in hexanes, 2.1 mL, 5.5 mmol) was added to a suspension of LiCl (720 mg, 18 mmol, dried for 18 h at 130 °C under high vacuum) and diisopropylamine (0.8 mL, 5.5 mmol) in THF (5 mL) at −78 °C. The resulting suspension was warmed to 0 °C for 10 min. An ice-cooled solution of (*R*,*R*)-pseudoephedrine propionamide (0.6 g, 2.71 mmol) in THF (3 mL) was added at −78 °C and the mixture was stirred for 1 h at −78 °C, 15 min at 0 °C, and 5 min at room temperature. Iodide **13** (0.35 g, 0.79 mmol) was added to this solution at 0 °C, and the reaction mixture was stirred at room temperature for 14 h. The yellow reaction mixture was treated with half-saturated aqueous NH_4Cl (100 mL) and extracted with EtOAc (4×100 mL). The combined organic layers were dried over Na_2SO_4 and concentrated, in vacuo. Flash chromatography (petroleum ether/Et_2O 2:1) yielded amide **25** (330 mg, 90% yield) as a yellow oil. R_f = 0.32 (petroleum ether/Et_2O, 2:1); $[\alpha]_D$ = −57.5 (c = 2.0 in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) major rotamer: δ = 7.21–7.42 (m, 5H), 5.31 (q, J = 6.6 Hz, 1H), 4.98 (dd, J = 9.4, 0.9 Hz, 1H), 4.62 (d, J = 7.5 Hz, 1H), 4.37–4.51 (m, 1H), 3.62 (d, J = 7.9 Hz, 1H), 2.88 (s, 3H), 2.42–2.57 (m, 1H), 2.13 (dd, J = 13.8, 4.5 Hz, 1H), 1.98 (dd, J = 13.8, 9.4 Hz, 1H), 1.52–1.61 (m, 9H), 1.14 (d, J = 7.0 Hz, 3H), 1.03 (d, J = 6.6 Hz, 3H), 0.84 (s, 9H), 0.72 (d, J = 6.8 Hz, 3H), −0.03 (s, 3H), −0.07 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 179.3, 142.5, 137.2, 131.9, 131.1, 128.3, 127.6, 126.3, 121.0, 83.4, 43.5, 37.1, 34.7, 25.8, 18.1, 17.7, 16.5, 16.0, 14.4, 12.9, 10.9, −4.8, −5.0 ppm; HRMS: calcd for $C_{30}H_{52}NO_3Si$ 502.3716, found 502.3716.

(2S,4E,6R,7R,8E)-7-(tert-Butyldimethylsilyloxy)-2,4,6,8-tetramethyldeca-4,8-dien-1-ol: A solution of *n*-butyllithium in hexanes (2.5 M, 2 mL, 5 mmol, 10 equiv) was added to a solution of diisopropylamine (0.8 mL, 5.5 mmol, 11 equiv) in tetrahydrofuran (10 mL) at −78 °C. The resulting solution was stirred at −78 °C for 10 min, then warmed to 0 °C, and held at that temperature for 10 min. Borane–ammonia complex (151 mg, 5 mmol, 10 equiv) was added in one portion, and the suspension was stirred at 0 °C for 15 min and subsequently warmed to 23 °C. After 15 min, the suspension was cooled to 0 °C. A solution of amide **25** (260 mg, 0.52 mmol, 1 equiv) in tetrahydrofuran (2 mL) was added via cannula over 3 min. The reaction mixture was warmed to 23 °C, held at that temperature for 2 h, and then cooled to 0 °C, at which point excess hydride was quenched by the careful addition of 1 N aqueous hydrochloric acid solution (12 mL). The mixture was stirred for 30 min at 0 °C and then extracted with four 15 mL portions of Et_2O. The combined organic extracts were washed sequentially with 3 N aqueous hydrochloric acid solution (10 mL), 2 N aqueous sodium hydroxide solution (10 mL), and brine (10 mL). The ether extracts were dried over magnesium sulfate and concentrated. Purification of the residue by flash column chromatography (petroleum ether/Et_2O, 1:1) afforded the intermediate alcohol as a colorless oil (160 mg, 94% yield). R_f = 0.58 (petroleum ether/Et_2O, 1:2); $[\alpha]_D$ = −4.6 (c = 1 in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.33 (qt, J = 6.6, 1.1 Hz, 1H), 5.00 (dd, J = 9.4, 1.0 Hz, 1H), 3.66 (d, J = 7.2 Hz, 1H), 3.39–3.53 (m, 2H), 2.44–2.58 (m, 1H), 2.09 (dd, J = 12.1, 4.8 Hz, 1H), 1.69–1.90 (m, 2H), 1.62 (d, J = 1.1 Hz, 3H), 1.58 (d, J = 6.6 Hz, 3H), 1.53–1.56 (m, 3H), 0.86 (s, 9H), 0.85 (d, J = 6.4 Hz, 3H), 0.77 (d, J = 7.0 Hz, 3H), −0.02 (s, 3H), −0.06 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.3, 132.5, 130.6, 120.6, 83.0, 68.7, 44.3, 37.0, 33.6, 25.8, 18.2, 18.0, 16.3, 16.3,

12.9, 11.3, −4.7, −5.0 ppm; HRMS: calcd for $C_{20}H_{40}O_2SiNa$ 363.2695, found 363.2694

*tert-Butyl((2E,4R,5R,6E,9S)-10-iodo-3,5,7,9-tetramethyldeca-2,6-dien-4-yloxy)dimethylsilane (**26**)*: Imidazole (61 mg, 0.9 mmol, 1.50 equiv) and iodine (198 mg, 0.78 mmol, 1.35 equiv) were added sequentially to a solution of triphenylphosphine (190 mg, 0.72 mmol, 1.20 equiv) in dichloromethane (5 mL) at 23 °C. A solution of the above alcohol (200 mg, 0.6 mmol, 1 equiv) in dichloromethane (1 mL) was added to the resulting fine suspension via cannula. After 2 h, silica (1 g) was added and dichloromethane was removed, in vacuo. The solid residue was loaded onto a column of silica gel eluting with 5% ether petroleum ether to afford iodide **26** as a colorless oil (220 mg, 83%). R_f = 0.95 (petroleum ether/Et_2O, 10:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.34 (qt, J = 6.6, 1.1 Hz, 1H), 5.01 (dd, J = 9.4, 1.0 Hz, 1H), 3.67 (d, J = 7.0 Hz, 1H), 3.24 (dd, J = 9.5, 4.5 Hz, 1H), 3.09 (dd, J = 9.5, 6.5 Hz, 1H), 2.44–2.57 (m, 1H), 2.08 (dd, J = 13.0, 6.0 Hz, 1H), 1.81 (dd, J = 13.0, 8.0 Hz, 1H), 1.61–1.73 (m, 1H), 1.59 (d, J = 6.4 Hz, 3H), 1.57 (d, J = 1.1 Hz, 3H), 1.53–1.56 (m, 3H), 0.94 (d, J = 6.4 Hz, 3H), 0.86 (s, 9H), 0.78 (d, J = 6.8 Hz, 3H), −0.01 (s, 3H), −0.06 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.2, 131.6, 131.3, 120.6, 82.9, 47.1, 37.1, 33.1, 25.8, 20.3, 18.2, 18.0, 17.7, 16.4, 12.9, 11.4, −4.7, −5.0 ppm.

*(2R,4R,6E,8R,9R,10E)-9-(tert-Butyldimethylsilyloxy)-N-((1R,2R)-1-hydroxy-1-phenylpropan-2-yl)-N,2,4,6,8,10-hexamethyldodeca-6,10-dienamide (**28**)*: *n*BuLi (2.5 M in hexanes, 3.2 mL, 8 mmol) was added to a suspension of LiCl (1 g, 25 mmol, dried for 18 h at 130 °C under high vacuum) and diisopropylamine (1.2 mL, 8.6 mmol) in THF (5.5 mL) at −78 °C. The resulting suspension was warmed to 0 °C for 10 min. An ice-cooled solution of (*S*,*S*)-pseudoephedrine propionamide (0.93 g, 4.2 mmol) in THF (3 mL) was added at −78 °C and the mixture was stirred for 1 h at −78 °C, 15 min at 0 °C, and 5 min at room temperature. A solution of iodide **26** (220 mg, 0.5 mmol) in THF (1 mL) was then added at 0 °C, and the reaction mixture was stirred at room temperature for 14 h. The yellow reaction mixture was treated with half-saturated aqueous NH_4Cl (10 mL) and extracted with EtOAc (4×10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated, in vacuo. Flash chromatography (petroleum ether/Et_2O, 2:1) yielded amide **28** (190 mg, 70% yield) as a yellow oil. R_f = 0.33 (petroleum ether/Et_2O, 2:1); $[\alpha]_D$ = +35.2 (c = 1.4 in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) major rotamer: δ = 7.23–7.42 (m, 5H), 5.31 (q, J = 6.6 Hz, 1H), 4.91 (dd, J = 9.2 Hz, 1H), 4.45–4.65 (m, 2H), 3.61 (d, J = 7.7 Hz, 1H), 2.90 (s, 3H), 2.70–2.80 (m, 1H), 2.42–2.57 (m, 1H), 2.00 (d, J = 10.0 Hz, 1H), 1.49–1.73 (m, 13H), 1.10 (d, J = 6.6 Hz, 3H), 1.03 (d, J = 6.6 Hz, 3H), 0.84 (s, 9H), 0.75 (d, J = 6.8 Hz, 3H), 0.72 (d, J = 6.8 Hz, 3H), −0.03 (s, 3H), −0.07 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 179.6, 142.5, 137.4, 132.3, 131.1, 128.3, 127.6, 126.4, 120.8, 83.5, 76.7, 58.1, 47.9, 41.9, 37.1, 34.3, 32.5, 28.5, 25.8, 18.9, 18.1, 17.8, 17.2, 16.4, 14.5, 12.9, 10.9, −4.7, −5.1 ppm.

*(2R,4R,6E,8R,9R,10E)-9-(tert-Butyldimethylsilyloxy)-2,4,6,8,10-pentamethyldodeca-6,10-dienoic acid (**29**)*: A round-bottomed flask (10 mL) was charged with amide **28** (200 mg, 0.368 mmol, 1 equiv), *tert*-butyl alcohol (2 mL), methanol (2 mL), and 2N aqueous sodium hydroxide solution (1 mL, 2 mmol, 6 equiv). The mixture was stirred at 60 °C for 7 days and then cooled to room temperature. The mixture was poured into a stirred mixture of 1 M aqueous HCl (20 mL) and dichloromethane (20 mL). The aqueous layer was separated and extracted with dichloromethane (2× 10 mL). The combined organic extracts were washed with brine, dried over sodium sulfate and concentrated, in vacuo. Flash chromatography with petroleum ether/EtOAc (3:1) on silica afforded acid **29** (140 mg, 89%) as a colorless oil. R_f = 0.3 (petroleum ether/EtOAc, 4:1); $[\alpha]_D$ = −8.33 (c = 1.8 in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.31 (q, J = 6.6 Hz, 1H), 4.94 (dd, J = 9.7 Hz, 1H), 3.63 (d, J = 7.6 Hz, 1H), 2.44–2.62 (m, 2H), 1.99–2.11 (m, 1H), 1.62–1.73 (m, 1H), 1.52–1.62 (m, 9H), 1.32–1.41 (m, 1H), 1.17 (d, J = 7.1 Hz, 3H), 0.84 (s, 9H), 0.79 (d, J = 6.6 Hz, 3H), 0.74 (d, J = 7.1 Hz, 3H), −0.03 (s, 3H), −0.07 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.8, 137.4, 132.3, 131.0, 120.7, 83.4, 47.8, 41.1, 37.1, 31.2, 28.5, 25.8, 18.7, 18.1, 17.9, 16.7, 16.2, 12.9, 11.1, −4.7, −5.0 ppm; HRMS: calcd for $C_{23}H_{44}O_3SiNa$ 419.2955, found 419.2957.

*(2R,4R,6E,8R,9R,10E)-Perfluorophenyl 9-(tert-butyldimethylsilyloxy)-2,4,6,8,10-pentamethyldodeca-6,10-dienoate (**30**)*: Pentafluorophenol (92 mg 0.5 mmol) was added to a solution of DCC (103 mg, 0.5 mmol) in EtOAc (2.5 mL) cooled to 0 °C. The solution was stirred for 30 min and acid **29** (140 mg 0.35 mmol) was added as a solution in EtOAc (0.5 mL) and the mixture was stirred for an additional 1.5 h. The mixture was warmed to room temperature, stirred for 8 h, cooled to −10 °C and 2 mL of hexane was added. The urea precipitate was collected by filtration (450 mg), the solid was washed with cold hexane (1.5 mL), and the mother liquor was evaporated. The residue was dissolved in CH_2Cl_2 (50 mL), washed with saturated aqueous $NaHCO_3$ solution (2×40 mL), saturated aqueous $NaHCO_3$ (40 mL) containing dissolved sodium hydroxide (0.30 g), and water (40 mL), and then dried and evaporated. The residue was chromatographed over silica gel (eluting with petroleum ether/EtOAc, 10:1) to afford 200 mg (100%) of the activated ester **30**. R_f = 0.8 (petroleum ether/EtOAc, 4:1); $[\alpha]_D$ = −13.4 (c = 3.4 in CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.32 (q, J = 6.6 Hz, 1H), 4.97 (dd, J = 9.2 Hz, 1H), 3.64 (d, J = 7.3 Hz, 1H), 3.18–3.24 (m, 1H), 2.88–2.95 (m, 1H), 2.47–2.54 (m, 1H), 2.08–2.14 (m, 1H), 1.90–1.96 (m, 2H), 1.54–1.60 (m, 9H), 1.26–1.37 (m, 4H), 0.85 (d, J = 3.7 Hz, 3H), 0.84 (s, 9H), 0.76 (d, J = 6.6 Hz, 3H), −0.03 (s, 3H), −0.07 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.0, 142.9, 141.0, 139.5, 137.4, 136.3, 132.1, 131.3, 120.7, 83.4, 55.7, 47.6, 41.2, 37.2, 37.1, 34.9, 28.6, 25.7, 25.5, 24.7, 18.8, 18.1, 17.9, 17.2, 16.2, 12.8, 11.0, −4.8, −5.1 ppm; HRMS: calcd for $C_{29}H_{43}F_5O_3SiNa$ 585.2799, found 585.2799.

*(S)-1-((2R,4R,6E,8R,9R,10E)-9-(tert-Butyldimethylsilyloxy)-2,4,6,8,10-pentamethyldodeca-6,10-dienoyl)-4-methoxy-5-methyl-1H-pyrrol-2(5H)-one (**31**)*: *n*BuLi (2.5 M, 0.05 mL, 0.125 mmol) was added to a solution of py-Aly-OMe[18] (14 mg, 0.11 mmol) in THF (1 mL) at −50 °C, and the mixture was stirred for 10 min. A solution of activated ester **30** (56 mg, 0.1 mmol) in THF (0.25 mL) was added dropwise over 15 min. The mixture was allowed to stir for an additional 10 min, quenched with AcOH (0.02 mL) and evaporated on silica. Column chromatography over silica gel (petroleum ether/EtOAc, 4:1) provided the pure tetramic acid amide **31** (38 mg, 76%). R_f = 0.3 (petroleum ether/EtOAc, 4:1); $[\alpha]_D$ = 12.55 (c = 2.9 in CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.30 (q, J = 6.6 Hz, 1H), 5.03 (s, 1H), 4.91 (d, J = 9.5 Hz, 1H), 4.60 (q, J = 6.6 Hz, 1H), 3.89–3.95 (m, 1H), 3.86 (s, 3H), 3.62 (d, J = 7.7 Hz, 1H), 2.48 (ddd, J = 9.2, 7.2, 7.0 Hz, 1H), 2.01–2.07 (m, 1H), 1.59–1.69 (m, 3H), 1.57 (d, J = 6.6 Hz, 3H), 1.54 (s, 3H), 1.53 (d, J = 1.1 Hz, 3H), 1.46 (d, J = 6.6 Hz, 3H), 1.30–1.36 (m, 1H), 1.12 (d, J = 6.6 Hz, 3H), 0.83 (s, 9H), 0.81 (d, J = 5.9 Hz, 3H), 0.73 (d, J = 7.0 Hz, 3H), −0.04 (s, 3H), −0.08 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 180.4, 177.4, 169.6, 137.4, 132.5, 130.7, 120.7, 93.0, 83.4, 58.6, 55.7, 48.1, 41.8, 37.1, 36.9, 28.6, 25.8, 18.4, 18.1, 17.8, 17.0, 16.4, 16.3, 12.9, 11.0, −4.8, −5.1 ppm; HRMS: calcd for $C_{29}H_{51}NO_4SiNa$ 528.3475, found 528.3485.

*(S)-1-((2R,4R,6E,8R,9R,10E)-9-Hydroxy-2,4,6,8,10-pentamethyldodeca-6,10-dienoyl)-4-methoxy-5-methyl-1H-pyrrol-2(5H)-one (**32**)*: Et_3N−3HF (0.15 mL) was added to a solution of imid **31** (24 mg, 47 μmol) in THF (0.5 mL) in a plastic vessel under N_2 atmosphere at 0 °C and the reaction mixture was allowed to warm to room temperature and stirred for 7 days. Then, the mixture was poured into sat. $NaHCO_3$ solution (3 mL), the aqueous layer was separated and extracted with CH_2Cl_2 (4×1.5 mL). The combined extracts were dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on SiO2 (hexanes/EtOAc 75:25) to give alcohol **32** (15 mg, 38 μmol, 81%) as a colorless oil. R_f = 0.3 (petroleum ether/EtOAc, 4:1); $[\alpha]_D$ = +38.3 (c = 1.5 in CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.47 (qd, J = 6.9, 1.5 Hz, 1H), 5.02 (s, 1H), 4.96 (d, J = 9.5 Hz, 1H), 4.59 (q, J = 6.6 Hz, 1H), 3.89–3.96 (m, 1H), 3.86 (s, 3H), 3.56 (d, J = 8.8 Hz, 1H), 2.56 (tq, J = 9.6, 6.8 Hz, 1H), 2.10 (dd, J = 12.8, 5.1 Hz, 1H), 1.79 (dd, J = 13.2, 8.8 Hz, 1H), 1.59–1.75 (m, 11H), 1.45 (d, J = 6.6 Hz, 3H), 1.33 (ddd, J = 13.0, 7.5, 5.5 Hz, 1H), 1.12 (d, J = 7.0 Hz, 3H), 0.84 (d, J = 6.2 Hz, 3H), 0.77 ppm (d, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 180.4, 177.2, 169.4, 137.1, 135.6, 129.1, 123.2, 93.0, 82.7, 58.6, 55.7, 48.1, 41.4, 36.8, 36.7, 28.6, 18.9, 17.5, 17.1, 16.7, 16.5, 13.1, 10.5 ppm; HRMS: calcd for $C_{23}H_{37}NO_4Na$ 414.2621, found 414.2620.

*(2R,4R,6E,8R,10E)-1-((S)-3-Methoxy-2-methyl-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)-2,4,6,8,10-pentamethyldodeca-6,10-diene-1,9-dione (**1**)*: Dess–Martin periodinane (26 mg, 0.062 mmol, 2 equiv) was added to a stirring solution of alcohol **32** (12 mg, 0.031 mmol, 1 equiv) in CH_2Cl_2 (1 mL) at room temperature. After 1 h, the mixture was quenched by the simultaneous addition of equal volumes (~2 mL total) of aq. $NaHCO_3$ and aq. $Na_2S_2O_3$. Once the layers cleared, the aqueous portion was extracted twice with CH_2Cl_2 (1 mL) and dried over Na_2SO_4. The organic extracts were filtered, concentrated under reduced pressure, and purified by column chromatography on silica gel (petroleum ether/*t*BuOMe/MeOH, 80:19:1) to give 10 mg (83%) of eliamid as a colorless oil. R_f=0.37 (petroleum ether/Et_2O, 1:1), $[\alpha]_D$=−65 (c=0.8 in MeOH); ^{1}H NMR (600 MHz, $CDCl_3$): δ=6.71 (qd, J=6.9, 1.0 Hz, 1H), 5.07 (d, J=9.5 Hz, 1H), 5.01 (s, 1H), 4.58 (q, J=6.6 Hz, 1H), 4.01 (dq, J=9.5, 6.7 Hz, 1H), 3.86–3.92 (m, 1H), 3.86 (s, 3H), 2.00 (dd, J=13.2, 6.2 Hz, 1H), 1.85 (dd, J=7.0, 0.9 Hz, 3H), 1.78 (dd, J=13.2, 8.1 Hz, 1H), 1.76 (s, 3H), 1.62–1.70 (m, 1H), 1.63 (s, 3H), 1.51 (ddd, J=13.6, 8.8, 6.2 Hz, 1H), 1.43 (d, J=6.6 Hz, 3H), 1.25 (ddd, J=13.0, 7.5, 5.5 Hz, 1H), 1.10 (d, J=7.0 Hz, 3H), 1.08 (d, J=7.0 Hz, 3H), 0.84 ppm (d, J=6.6 Hz, 3H), ^{13}C NMR (75 MHz, $CDCl_3$): δ=203.7, 180.3, 177.2, 169.4, 137.5, 136.5, 134.3, 127.5, 93.0, 58.6, 55.6, 47.8, 40.9, 39.8, 36.8, 28.7, 18.9, 17.7, 17.0, 16.2, 16.0, 14.8, 11.4 ppm; ^{1}H NMR (600 MHz, [D_4]MeOH): δ=6.91 (qd, J=7.0, 1.1 Hz, 1H), 5.23 (s, 1H), 5.05 (d, J=9.5 Hz, 1H), 4.65 (q, J=6.6 Hz, 1H), 4.21 (dq, J=9.5, 6.6 Hz, 1H), 3.95–4.02 (m, 1H), 3.97 (s, 3H), 2.03 (dd, J=13.2, 6.6 Hz, 1H), 1.94 (dd, J=7.0, 0.7 Hz, 3H), 1.87 (dd, J=13.2, 7.7 Hz, 1H), 1.80 (s, 3H), 1.68–1.75 (m, 1H), 1.72 (s, 3H), 1.52 (ddd, J=13.6, 7.8, 6.6 Hz, 1H), 1.46 (d, J=6.6 Hz, 3H), 1.30 (ddd, J=13.6, 7.8, 5.5 Hz, 1H), 1.13 (d, J=7.0 Hz, 3H), 1.11 (d, J=6.6 Hz, 3H), 0.91 ppm (d, J=6.2 Hz, 3H), ^{13}C NMR (75 MHz, [D_4]MeOH): δ=205.8, 182.6, 178.5, 171.5, 139.0, 138.3, 135.9, 128.6, 93.5, 59.6, 56.8, 49.8 (overlapped with [D_4]MeOH), 42.0, 40.7, 37.7, 29.8, 19.6, 17.9, 17.3, 16.4, 16.2, 14.7, 11.4 ppm; HRMS: calcd for $C_{23}H_{36}NO_4$ 390.2634, found 390.2644.

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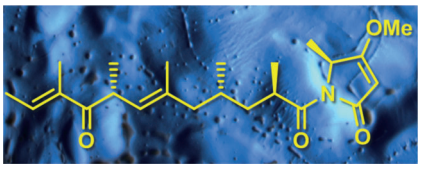

How valuable is Breit's rule? Isolation, biological activity profiling, structure elucidation and total synthesis of polyketide natural product eliamid are reported. This molecule is characterized by a tetramic acid amide fragment, which is rather rare among secondary metabolites isolated from myxobacteria.

Secondary metabolites......isolated from various strains of myxobacterium *Sorangium cellulosum* (photo in the background, taken by K. Gerth) often possess interesting biological activities. Eliamid (yellow) was found to inhibit the reduction of ubiquinone through complex I (the red and blue curves) at nanomolar concentrations. The difference in the appearance of cell mitochondria can be clearly seen on the small picture inserts.