Tetrahedron 66 (2010) 9107–9112

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis and biological activities of flavolipids

Samiul M. Ahad [a], Alison L. Ange [a], Robert B. Bates [a,*], Bonnie L. Bell [a], Adria A. Bodour [b], Bryan R. Bourne [a], Cristina G. Contreras [a], Emily L. Goldberg [a], A.A. Leslie Gunatilaka [c], Sheryl King [a], Albert K. Lee [a], Rebecca L. Low [a], Raina M. Maier [b], Kathryn M. Marlor [a], Marilyn T. Marron [c], Ryan C. Scolnik [a], Matthew J. Streeter [a], Malgorzata Strelczuk [a], Long N. Trinh [a], Vu K. Truong [a], Sage P. Vissering [a], Megan C. Weick [a], Maria T. Williams [a]

[a] Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ 85721, United States
[b] Department of Soil, Water and Environmental Science, University of Arizona, Tucson, AZ 85721, United States
[c] Southwest Center for Natural Products Research, University of Arizona, Tucson, AZ 85721, United States

ARTICLE INFO

Article history:
Received 9 April 2010
Received in revised form 24 September 2010
Accepted 27 September 2010
Available online 1 October 2010

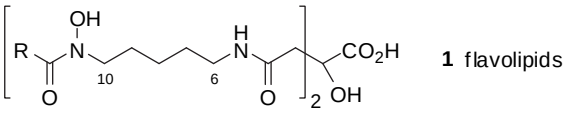
Keywords:
Biosurfactant
Flavobacterium
Flavolipid
Siderophore

ABSTRACT

Syntheses of the bacterial surfactants 6S,6S-, 9S,9S-, and 9U,9U-flavolipids confirmed the structures proposed for them from spectroscopic analysis of a flavolipid mixture and made pure flavolipids available for the first time. All three synthetic flavolipids and a straight chain analogue were found to be weakly cytotoxic and to inhibit metastatic cancer cell migration, with 9U,9U-flavolipid (the most abundant natural flavolipid) having the most activity. Biosynthetic routes to the branched side-chains of the flavolipids are suggested, and it is proposed that branched chains are employed to hinder biodegradation.

1. Introduction

From the surface exudate of soil bacteria of the genus *Flavobacterium* from Mt. Lemmon, Arizona, 37 closely related new carboxylic acids termed 'flavolipids' (**1**) were recently characterized.[1] (The name 'flavolipid' has also been used for other compounds.[2]) The Mt. Lemmon flavolipids are of interest for possible use in bioremediation as reported by Bodour and co-workers[1] and because we have found them to be weakly cytotoxic and to inhibit metastatic cancer cell migration.



1 flavolipids

R

U-flavolipid chain $(CH_3)_2CH(CH_2)_nCH{=}CH-$ n=0-4
S-flavolipid chain $(CH_3)_2CH(CH_2)_nCH_2CH_2-$ n=0-4
13

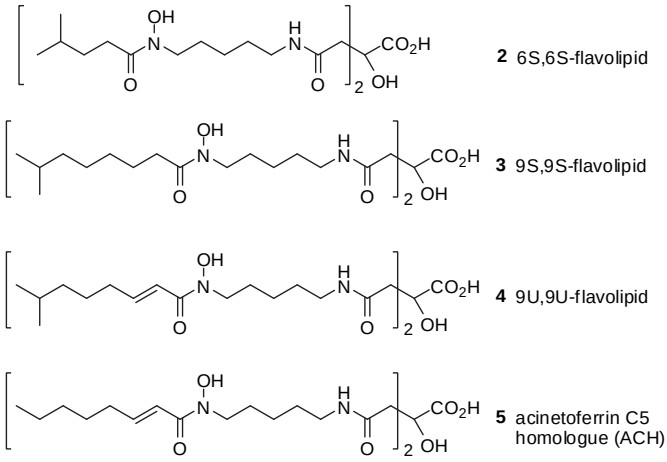
Flavolipid names include the number of carbons in each branched fatty acid and whether it is saturated (S) or has *E*-α,β-unsaturation (U).[1] The number of carbon atoms in the branched chain 'secondary fatty acids' of the flavolipids varies from six to ten, consistent with fatty acid biosynthesis from valine via isobutyryl CoA (→6, 8, and 10) and isoleucine via isovaleryl CoA (→7 and 9).[3,4] Decarboxylation of another amino acid, lysine, is very likely the source of the cadaverine (1,5-diaminopentane) grouping in flavolipids.

2. Results

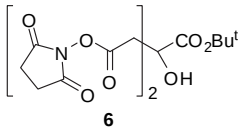
We now report the synthesis of two minor flavolipids (6S,6S-flavolipid, **2**; 9S,9S-flavolipid, **3**) and the major one (9U,9U-flavolipid, **4**, 23% of the flavolipid mixture[1]). The syntheses confirm the structures determined by NMR and mass spectral methods for these flavolipids and make available pure flavolipids, which differ in chain length (**2** vs **3**) and unsaturation (**3** vs **4**) for testing purposes. The two synthetic methods we employed were based on methods used by Miller and Lee[5] and Phanstiel and co-workers[6–8] to make related compounds with unbranched side-chains; we also synthesized one of these unbranched siderophores (acinetoferrin C5 homologue, ACH, **5**[6]) to be able to compare the properties of straight chain (**5**) and branched chain (**4**) siderophores.

* Corresponding author. Tel.: +1 520 621 6317; fax: +1 520 621 6407; e-mail address: batesr@u.arizona.edu (R.B. Bates).

0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.09.088

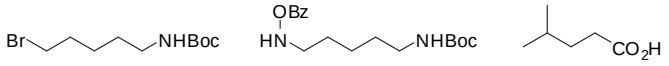


For the citric acid unit, both our synthetic routes used citric acid derivative **6**[7] with *tert*-butyl (*t*-Bu) ester protection of the central carboxyl group to avoid the imide formation,[8] which occurs when the central carboxyl is benzyl-protected. During our early attempts to use benzyl ester protection of the central carboxyl group of citric acid (abandoned due to severe problems with imide formation), we increased the yield of anhydromethylenecitric acid, the first intermediate in the preparation of the reagent used for benzyl protection, from 50%[9] to 70% by heating citric acid with a 2-fold excess of paraformaldehyde at 190 °C with shaking for 2 h on a Parr apparatus, cooling, adding water (100 mL for each gram of crude product), decanting from undissolved paraformaldehyde, and collecting the crystalline product in two crops.



6

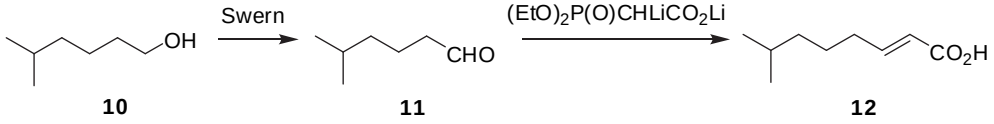
For the cadaverine unit, the first synthesis used bromide **7**[5] and the second used cadaverine derivative **8**.[6] In the preparation of **8** from cadaverine, it was important to remove all of the di-Boc-cadaverine from the mono-Boc-cadaverine chromatographically to avoid later byproducts.



7 **8** **9**

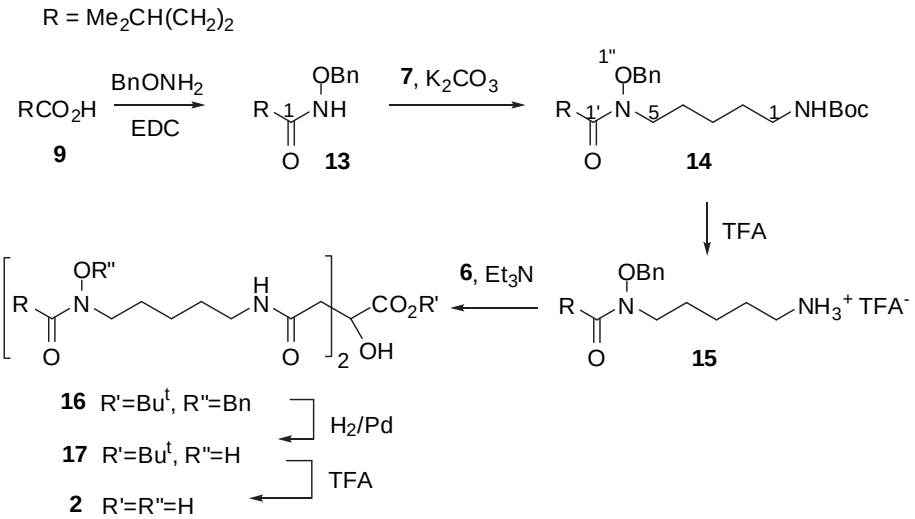
The branched acid (4-methylpentanoic acid, **9**) needed for 6S,6S-flavolipid (**2**) synthesis was commercially available. The branched acid **12**, synthesized as shown in Scheme 1 from 5-methylhexanol (**10**) by Swern oxidation[10] followed by reaction of aldehyde **11** with the dianion of diethylphosphonoacetic acid,[11] was used for 9S,9S- and 9U,9U-flavolipids (**3** and **4**).

6S,6S-Flavolipid (**2**) was assembled as shown in Scheme 2 using benzyl (Bn) protection of the *N*-hydroxyl groups. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was used in the coupling to give hydroxamide derivative **13** to facilitate the removal of the urea byproduct; this intermediate was made by Sibi and co-workers by a different method.[12] When intermediate **14** was purified to 99+% by chromatography (the main impurity was the O-alkylation product, formed in about 20% yield[5]), the remaining reactions went in good yields. The NMR and mass spectra (including the MS/MS fragmentation peaks) of synthetic 6S,6S-flavolipid (**2**) were as expected, confirming the structure proposed earlier (the chemical shifts of the synthetic flavolipids were slightly different than reported for the flavolipid mixture[1] since the NMRs of the synthetics were measured in CD_3OD and the natural flavolipid mixture was measured in CD_3OD plus a few drops of D_2O to dissolve the sample more completely).[1] In both synthetic routes, after the protecting groups on the hydroxamic acids were removed, it was important to measure the NMR spectra in deuteromethanol, since in deuterochloroform the absorptions of the protons near the hydroxamic acid group were greatly exchange-broadened.



10 **11** **12**

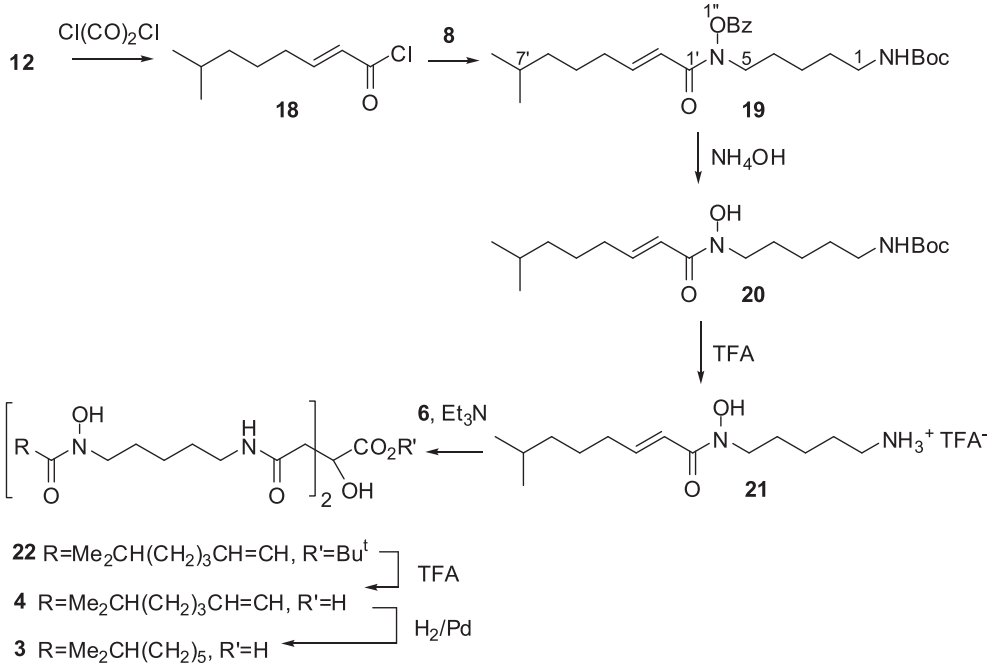
Scheme 1. Synthesis of *E*-7-methyl-2-octenoic acid (**12**).



Scheme 2. Synthesis of 6S,6S-flavolipid (**2**).

The method of Scheme 2 was unsuitable for 9U,9U-flavolipid (**4**) since the carbon–carbon double bonds would be reduced to single bonds during the removal of the benzyl groups. Instead, the Phanstiel method[6–8] shown in Scheme 3, using benzoyl (Bz) instead of benzyl (Bn) protection of the *N*-hydroxyl groups, was used to synthesize 9U,9U-flavolipid (**4**), which was then hydrogenated to 9S,9S-flavolipid (**3**). The NMR, mass spectra, and MS/MS spectra of synthetic 9U,9U- and 9S,9S-flavolipids (**4** and **3**) were as expected, confirming the structures proposed earlier.[1]

cancer cell lines: NCI-H460 (non-small cell lung cancer) and MDA-MB-231 (metastatic breast carcinoma). Cells were exposed to serial dilutions of test compounds for 72 h and then cell viability was determined as follows. The cells were plated at low density in RPMI 1640 media supplemented with 10% fetal bovine serum. The following day compounds **2**–**5** were added and after 72 h, the viability was evaluated by the MTT assay.[13] All compounds were tested at 10 and 20 mM and found to be weakly cytotoxic to these cell lines with inhibition ranging from 20% to 60% at 20 μM (data not shown).



Scheme 3. Synthesis of 6U,6U-siderolipid (**4**) and 9S,9S-siderolipid (**3**).

All four synthetic lipids **2**–**5** were purified by repeated chromatography on Sephadex LH-20, eluting with 8% ethanol in toluene, and eventually crystallized as waxy solids. They were stored at −80 °C to minimize cyclization to imides.[8]

The three synthetic flavolipids **2**–**4** and straight chain analogue ACH **5** were evaluated for in vitro cytotoxic activity against two 9U,9U-Flavolipid (**4**), the most abundant natural flavolipid, was the most active.

Compounds **2**–**5** were also tested for their cell migration inhibitory activity in the metastatic prostate cancer cell line, PC-3M.[14] All showed weak to moderate activity compared to the DMSO control, with 9U,9U-flavolipid **4** again being the most active (Fig. 1).

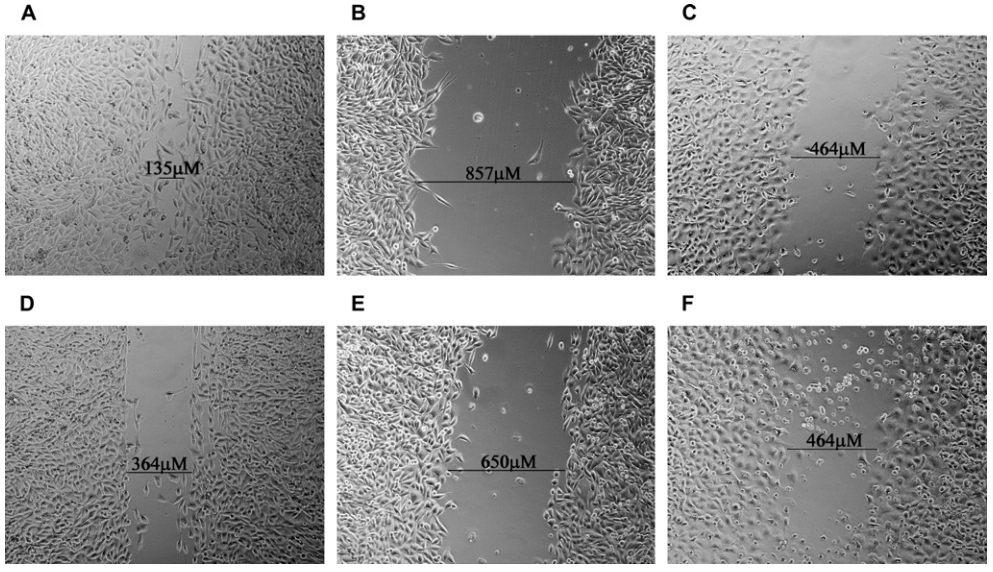


Fig. 1. Effect of compounds **2**–**5** on migratory activity of the metastatic prostate cancer cell line, PC-3M. (A) DMSO control (negative); (B) LY294002 control (positive) at 7.5 μM; (C) 6S,6S-flavolipid (**2**) at 20 μM; (D) 9S,9S-flavolipid (**3**) at 20 μM; (E) 9U,9U-flavolipid (**4**) at 20 μM; (F) ACH (**5**) at 20 μM.

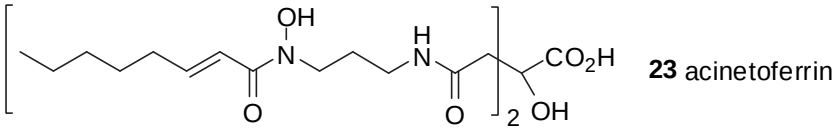
3. Discussion

The syntheses of pure 6S,6S- (**2**), 9S,9S- (**3**), and 9U,9U-flavolipids (**4**) verify the structures proposed for them from NMR and mass spectral analysis of the natural mixture of 37 flavolipids.[1]

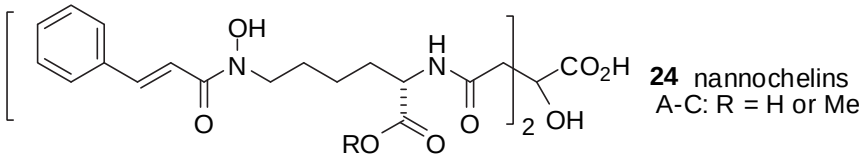
The findings that these three pure flavolipids are cytotoxic and inhibit cell migration prompted us to synthesize a straight chain analogue (ACH, **5**) and test it also, to learn the effects of branched chains versus straight chains in these biological tests. All four compounds had some activity in both tests, with 9U,9U-flavolipid (**4**) being more active than the other compounds. In view of these results, we next consider possible reasons why flavobacteria might favor the chain lengths they do, why most have α,β-unsaturation, and why the branched chains of the flavolipids might be preferable to the straight chains of the related siderophores.

3.1. Chain length

Many bacteria produce siderophores to scavenge iron since iron availability in soils is limited.[15,16] Much of the iron in the environment is in tightly bound mineral forms and it is possible that the hydrophobic nature of the flavolipids with their 16–20 chain lengths[1] provides an advantage in associating with and extracting iron from hydrophobic surfaces. Rhamnolipid biosurfactants, which also have metal-chelating ability, have been shown to associate strongly with mineral surfaces and to extract metals from these surfaces.[17,18] The flavolipids may similarly enhance extraction from mineral surfaces. Following extraction, the cell-membrane-component-like nature of flavolipids could facilitate delivery back to the producing cell. The mechanism of delivery of iron to the cell has been shown for acinetoferrin (**23**, produced by the pathogenic bacterium *Acinetobacter haemolyticus*), whose high membrane affinity (partition coefficient K_x=6.8×10^5) allows it to deliver scavenged iron back to the cell via the membrane.[19]



23 acinetoferrin



24 nannochelins
A-C: R = H or Me

3.2. α,β-Unsaturation

The natural flavolipid mixture contains 11 U,U-flavolipids, which comprise 65% of the natural flavolipid mixture.[1] We conclude that α,β-unsaturation is important for making *Flavoacterium* competitive in the environment. It is noteworthy that there are other bacterial siderophores with α,β-unsaturated carbonyls in two chains: acinetoferrin (**23**),[6] which has three methylenes instead of five in the diamine, and nannochelins A–C (**24**), which are weak growth inhibitors of bacteria and fungi.[20] It is difficult to see how *trans*-α,β-unsaturation, which changes the molecular geometry of these siderophores very little, could enhance iron scavenging; perhaps it serves a second purpose such as inhibiting cell division in other bacteria through Michael-type additions to the carbon–carbon double bonds.

3.3. Branched chains

We propose that flavolipids have rare branched-chain fatty acids to avoid the oxygenase-catalyzed biodegradation, which occurs readily with straight chains.[21] This allows them longer lifetimes in the environment to carry out their roles as iron chelators. It may also account for the greater activity of 9U,9U-flavolipid (**4**) in comparison to the straight chain analogue ACH (**5**) in both the cytotoxicity and cancer cell migration tests performed in this study.

4. Experimental

4.1. General procedures

NMR spectra were obtained at 500 or 600 MHz in $CDCl_3$ except for hydroxamic acids, for which CD_3OD was used to avoid line-broadening caused by rotation in the hydroxamic acid unit. ESI mass spectra were measured on a Bruker 9.4 Tesla Apex Qh spectrometer. Flavolipids and other compounds without *tert*-butyl ester protection of the central citric acid carboxyl group were stored in a −80 °C freezer since they slowly lose water to give imides at room temperature.[8] Sephadex LH-20 was purchased from Sigma–Aldrich; other chemicals were purchased from Aldrich.

4.2. 5-Methylhexanal (11)[10]

[Note: this aldehyde evaporates rapidly at room temperature and should be kept in a tightly closed container.] Oxalyl chloride (5.6 mL, 64.6 mmol) in CH_2Cl_2 (100 mL) was cooled in dry ice and dimethylsulfoxide (DMSO, 4.8 mL, 67.8 mmol) was added dropwise. After 10 min, triethylamine (45 mL, 322 mmol) was added and the cold bath was removed. After standing overnight, the solution was washed 2× with saturated NH_4Cl, 2× with saturated NaCl, and dried over Na_2CO_3. CH_2Cl_2 and volatile byproducts were slowly distilled off through a Vigreux column. Ether (3×50 mL) was added and distilled off. To remove further Et_3N, ether (50 mL) was added and the solution was rewashed with saturated NH_4Cl (2×10 mL), brine (2×10 mL), dried, and most of the ether was distilled off, leaving what NMR showed to be a quantitative yield of 4:1 aldehyde **11**/ether, used in the next reaction. ^{1}H NMR ($CDCl_3$) δ 0.89 (d, J=6.8 Hz, H6), 1.21 (m, H4), 1.55 (nonet, J=6.5 Hz, H5), 1.64 (m, H3), 2.42 (m, H2), 9.77 (t, J=2.0 Hz, H1).

4.3. (*E*)-7-Methyl-2-octenoic acid (12)[11]

To tetrahydrofuran (THF, 250 mL) chilled in dry ice/acetone, butyllithium solution (2.5 M in hexane, 95 mL, 238 mmol) was added. Diethylphosphonoacetic acid (7.2 mL, 107 mmol) was added dropwise with stirring. After 0.5 h, 5-methylhexanal (**11**, 12.2 g, 107 mmol) was added dropwise. After 0.5 h, the solution was stirred overnight at 25 °C. Excess $NaHCO_3$ was added and most of the THF was evaporated in a hood. The aqueous solution was washed with ether (3×100 mL), acidified with concd HCl to pH 4, and extracted with ether (3×100 mL). Distillation of the ether through a Vigreux column left acid **12** (7.0 g, 42%). It could be used directly or purified by silica gel chromatography, eluting with 15% ethyl acetate in hexanes. ^{1}H NMR ($CDCl_3$) δ 0.88 (d, J=7.7 Hz, H8), 1.20 (q,

J=7.4 Hz, H6), 1.47 (p, J=7.5 Hz, H5), 1.55 (n, J=6.7 Hz, H7), 2.22 (q, J=7.2 Hz, H4), 5.83 (d, J=15.5 Hz, H2), 7.08 (dt, J=15.5, 6.7 Hz, H3), 10.10 (s, CO_2H); ^{13}C NMR ($CDCl_3$) δ 22.5 (C8), 25.7 (C5), 27.8 (C7), 32.5 (C4), 38.4 (C6), 120.7 (C2), 152.2 (C3), 171.6 (C1); MS (ESI^-): m/z 155.1079 (calcd for $C_9H_{15}O_2$, 155.1078) $[M-H]^-$.

4.4. *N*-Benzyloxy-4-methylpentanamide (13)[12]

A solution of 4-methylpentanoic acid (**9**, 3.53 mL, 28.1 mmol), *O*-benzylhydroxylamine hydrochloride (4.50 g, 28.1 mmol), EDC (5.4 g, 28.1 mmol), triethylamine (3.9 mL, 28.1 mmol), and 4-(dimethylamino)pyridine (DMAP, 343 mg, 2.8 mmol) in dichloromethane (DCM, 65 mL) was stirred overnight at 25 °C. After solvent evaporation, the residue was taken up in EtOAc and washed with 1 M HCl, 3×$NaHCO_3$, water, and brine. Evaporation gave **13** (5.7 g, 92%). ^1H NMR ($CDCl_3$) δ 0.88 (d, J=7.1 Hz, H5), 1.52 (q, J=6.2 Hz, H3), 1.55 (n, J=7.0 Hz, H4), 2.04 (m, H2), 4.92 (s, OCH_2), 7.38 (br s, ArH); ^{13}C NMR ($CDCl_3$) δ 22.1 (C5), 27.5 (C4), 31.0 (C2), 34.1 (C3), 77.8 (C2′), 128.3 (C4′), 128.3 (C6′), 129.0 (C5′), 135.4 (C3′), 171.3 (C1); MS (ESI^+): m/z 222 $[M+H]^+$.

4.5. *N*-Boc-*N*′-benzyloxy-*N*′-(4′-methylpentanoyl)-cadaverine (14)

A mixture of **13** (3.2 g, 14.5 mmol), 5-(Boc-amino)-1-pentyl bromide (2.14 g, 8.1 mmol), K_2CO_3 (8.0 g, 58 mmol), and KI (240 mg, 1.5 mmol) in acetone (50 mL) was refluxed overnight. After evaporation of the acetone, ether (75 mL) was added and the solution was washed with 0.5 M NaOH (3×10 mL). Evaporation of the ether left 3.2 g (97%) of a 4:1 mixture of **14** with the O-alkylation byproduct. Repeated silica gel chromatography, eluting with 20% ethyl acetate/hexanes, gave (after the O-alkylation product) 99+% pure **14**. ^1H NMR ($CDCl_3$) δ 0.88 (d, J=5.8 Hz, H5′), 1.30 (p, J=7.3 Hz, H3), 1.44 (s, Boc), 1.50 (q, J=6.6 Hz, H3′), 1.55 (n, J=7.4 Hz, H4′), 1.64 (p, J=7.5 Hz, H4), 2.38 (t, J=7.8 Hz, H2′), 3.10 (q, J=6.6 Hz, H1), 3.62 (t, J=7.3 Hz, H5), 4.63 (br s, NH), 4.80 (s, $ArCH_2$), 7.38–7.39 (m, ArH); ^{13}C NMR ($CDCl_3$) δ 22.3 (C5′), 26.5 (C3), 27.9 (C4′), 28.4 (C5‴), 29.3 (C4), 29.5 (C2), 30.4 (C2′), 33.4 (C3′), 40.4 (C1), 45.0 (C5), 76.3 (C2″), 79.1 (C4‴), 128.7 (C4″), 128.9 (C6″), 129.1 (C5″), 134.6 (C3″), 156.0 (C2‴), 175.3 (C1′); MS (ESI^+): m/z 407.2901 (calcd for $C_{23}H_{39}N_2O_4$, 407.2904) $[M+H]^+$.

4.6. *N*′-Benzyloxy-*N*′-(4′-methylpentanoyl)-cadaverinium triflate (15)

Boc derivative **14** (1.48 g, 3.65 mmol) was stirred with trifluoroacetic acid (TFA, 2×10 mL) for 1 h and the TFA was evaporated in a hood. Ether was added and evaporated three times to remove excess TFA, leaving amine salt **15** (1.53 g, 100%). ^1H NMR ($CDCl_3$) δ 0.85 (d, J=6.9 Hz, H5′), 2.32 (t, J=7.3 Hz, H2′), 2.90 (br s, H1), 3.61 (m, H5), 4.77 (s, $ArCH_2$), 7.35 (br s, ArH), 8.00 (br s, NH); ^{13}C NMR δ 22.0 (C5′), 26.0 (C3), 27.6 (C4′), 27.9 (C2), 29.7 (C4), 30.2 (C2′), 33.7 (C3′), 44.5 (C5), 76.5 (C2″), 128.3 (C4″), 129.2 (C5″), 129.3 (C6″), 134.6 (C3″), 177.1 (C1′); MS (ESI^+): m/z 307.2380 (calcd for $C_{18}H_{31}N_2O_2$, 307.2380) $[RNH_3]^+$.

4.7. *O*,*O*-Dibenzyl-6S,6S-flavolipid *tert*-butyl ester (16)

To **15** (1.85 g, 4.4 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added Et_3N (4 mL). This solution was added to a freshly prepared solution of citric acid derivative **6** (2 mmol) in dioxane (50 mL).[7] After stirring overnight, dicyclohexylurea (DCU) was filtered off and the solvents were evaporated. The residual oil was dissolved in $CHCl_3$ and washed quickly with 0.5 M HCl. The aqueous layer was extracted with $CHCl_3$, the combined organic layers were dried over Na_2SO_4, and the solvent was evaporated. NMR showed the residual oil (2.2 g) to be a mixture of the desired diamide **16** with DCU, which was used in the next reaction. ^1H NMR (CD_3OD) δ 0.88 (d, J=6.0 Hz, H16), 1.45 (s, *t*-Bu), 2.37 (m, H13), 2.56 and 2.66 (d, J=14.5 Hz, H3), 3.20 (q, J=6.4 Hz, H6), 3.62 (t, J=7.0 Hz, H10), 4.80 (s, CH_2O), 6.65 (m, NH), 7.3–7.4 (m, ArH); ^{13}C NMR ($CDCl_3$) δ 22.9 (C16), 25.2 (C8), 27.7 (C15), 28.3 (C17), 29.2 (C7), 30.1 (C9), 30.9 (C3), 31.6 (C13), 35.0 (C14), 40.3 (C6), 45.2 (C10), 75.4 (C2), 77.3 (C21), 83.5 (C18), 129.9 (C23), 130.2 (C25), 130.8 (C24), 136.3 (C22), 172.1 (C4), 174.3 (C1), 177.2 (C12); MS (ESI^+): m/z 825.5363 (calcd for $C_{46}H_{73}N_4O_9$, 825.5372) $[M+H]^+$.

4.8. 6S,6S-Flavolipid *tert*-butyl ester (17)

Benzyl-protected ester **16** (1.90 g, 2.30 mmol) in EtOH (50 mL) was stirred with 10% Pd/C catalyst under H_2 for 2 h. Filtering off solids, washing them with EtOH, and evaporating left a quantitative yield of ester **17** as a colorless oil. ^1H NMR (CD_3OD) δ 0.93 (d, J=6.5 Hz, H16), 1.48 (s, *t*-Bu), 1.63 (p, J=7.4 Hz, H9), 2.47 (t, J=7.7 Hz, H13), 2.58 and 2.67 (d, J=14.5 Hz, H3), 3.16 (t, J=7.0 Hz, H6), 3.59 (t, J=7.0 Hz, H10); ^{13}C NMR (CD_3OD) δ 22.9 (C16), 25.1 (C8), 28.3 (C15), 28.3 (C17), 29.3 (C7), 30.1 (C9), 31.6 (C13), 40.4 (C6), 45.2 (C10), 75.4 (C2), 83.5 (C18), 172.2 (C4), 180.5 (C12) MS (ESI^+): m/z 645.4426 (calcd for $C_{32}H_{61}N_4O_9$, 645.4433) $[M+H]^+$.

4.9. 6S,6S-Flavolipid (2)

tert-Butyl ester **17** (1.50 g, 2.33 mmol) was stirred with trifluoroacetic acid (TFA, 5 mL) for 30 min. TFA was evaporated and chloroform (2×5 mL) was added and evaporated, leaving **2** (1.37 g, 100%) as an oil, which was chromatographed on Lipophilic Sephadex to give waxy crystals, mp 91–92 °C. ^1H NMR (CD_3OD) δ 0.92 (d, J=6.0 Hz, H16), 1.32 (p, J=7.0 Hz, H8), 1.48 (q, J=6.8 Hz, H14), 1.52 (p, J=7.3 Hz, H7), 1.57 (n, J=6.4 Hz, H15), 1.62 (p, J=6.9 Hz, H9), 2.46 (t, J=7.8 Hz, H13), 2.61 and 2.71 (d, J=14.5 Hz, H3), 3.16 (t, J=7.0 Hz, H6), 3.58 (t, J=7.0 Hz, H10); ^{13}C NMR (CD_3OD) δ 22.9 (C16), 25.1 (C8), 28.3 (C15), 29.3 (C7), 30.1 (C9), 31.6 (C13), 39.7 (C14), 40.3 (C6). 43.3 (C3), 45.5 (C10), 73.9 (C2), 172.7 (C4), 176.5 (C12), 177.0 (C1); MS (ESI^-): m/z 587.3665 (calcd for $C_{28}H_{51}N_4O_9$, 587.3662) $[M-H]^-$; MS/MS on 587: 569(7) $[M-H_2O]^-$; 551(100) $[M-2H_2O]^-$; 471(4) $[M-H_2O-ketene]^-$; 435(9) $[M-3H_2O-ketene]^-$; 309(18) $[C_{16}H_{22}O_4N_2]^-$.

4.10. 7-Methyl-2-octenoyl chloride (18)

To (*E*)-7-methyl-2-octenoic acid (**12**, 4.5 g, 28.8 mmol) in dry benzene (60 mL) was added DMF (10 drops) followed by oxalyl chloride (14.6 g, 57.6 mmol) dropwise. After 30 min, the volatiles were blown off with argon, leaving acid chloride **18** containing DMF, which was used directly in the next reaction. ^1H NMR ($CDCl_3$) δ 0.88 (d, J=6.3 Hz, H8), 1.20 (m, H6), 1.5 (m, H5, H7), 2.28 (q, J=6.1 Hz, H4), 6.08 (d, J=14.2 Hz, H2), 7.24 (dt, J=14.2, 6.6 Hz, H3); ^{13}C NMR ($CDCl_3$) δ 22.3 (C8), 25.3 (C5), 27.6 (C7), 32.5 (C4), 38.1 (C6), 1256.0 (C2), 157.5 (C3), 165.1 (C1).

4.11. (*E*)-*N*-Boc-*N*′-benzoyloxy-*N*′-(7′-methyl-2′-octenoyl)-cadaverine (19)

A solution of benzoyl peroxide (4.6 g, 19 mmol) in DCM (70 mL) was added dropwise to a vigorously stirred mixture of **8** bicarbonate in a carbonate buffer solution (pH 10.5, 100 mL).[8] After 16 h, a solution of freshly prepared acid chloride **18** (2.25 g, 12.9 mmol) in DCM (10 mL) was added dropwise. After 3 h, the organic layer was separated and the aqueous layer was washed 2×DCM. The organic layer was dried (Na_2SO_4), the solution was decanted, and the solvent was evaporated. The residue was chromatographed on silica gel, eluting with 50% EtOAc/hexanes, giving

19 as an oil (3.5 g, 59%). ^{1}H NMR ($CDCl_3$) δ 0.81 (d, J=6.9 Hz, H8′), 1.13 (q, J=7.8 Hz, H6′), 1.43 (s, *t*-Bu), 1.69 (p, J=7.2 Hz, H4), 2.13 (q, J=7.1 Hz, H4′), 3.11 (m, H1), 3.87 (m, H5), 4.61 (br s, NH), 6.05 (d, J=15.0 Hz, H2′), 7.02 (dt, J=15.0, 7.0 Hz, H3′), 7.53 (t, J=7.5 Hz, 5″), 7.68 (t, J=7,5 Hz, 6″), 8.11 (d, J=7.5 Hz, 4″); ^{13}C NMR ($CDCl_3$) δ 22.4 (C8′), 22.5 (C4), 23.8 (C3), 27.8 (C5′), 27.8 (C7′), 28.4 (C5‴), 29.6 (C2), 32.7 (C4′), 38.3 (C6′), 40.3 (C1), 48.3 (C5), 79.0 (C4‴), 118.0 (C2′), 128.9 (C5″), 129.6 (C3″), 130.0 (C4″), 133.4 (C6″), 149.4 (C3′), 156.0 (C4), 164.5 (C1′), 170.3 (C2″); MS (ESI$^+$): m/z 461.3002 (calcd for $C_{26}H_{41}N_2O_5$, 461.3010) $[M+H]^-$; $[M+H]^+$.

4.12. (*E*)-*N*-Boc-*N*′-hydroxy-*N*′-(7′-methyl-2′-octenoyl)-cadaverine (20)

Using 'iron-free' glassware and silica gel,[6] a 10% solution of NH_4OH/MeOH (60 mL) was added to benzoyl-protected hydroxamic acid **19** (3.45 g, 7.5 mmol) at 0 °C. After stirring for 2 h, the volatiles were evaporated. Benzene and then chloroform were added and evaporated and the residue was chromatographed on silica gel, eluting with 25% EtOAc/hexanes, to give **20** as an oil (2.7 g, 100%). ^{1}H NMR (CD_3OD) δ 0.89 (d, J=6.5 Hz, H8′), 1.42 (s, *t*-Bu), 2.23 (q, J=6.9 Hz, H4′), 3.02 (t, J=6.7 Hz, H 1), 3.65 (t, J=6.6 Hz, H5), 6.61 (br d, J=15.5 Hz, H2′), 76.84 (dt, J=15.5, 6.8 Hz, H3′); ^{13}C NMR (CD_3OD) δ 23.1 (C8′), 25.0 (C3), 27.5 (C5′), 28.9 (C5″), 28.8 (C7′), 29.1 (C2), 30.7 (C4), 33.8 (C4′), 39.7 (C6′), 41.3 (C1), 79.9 (C4″), 120.7 (C2′), 148.1 (C3′), 158.7 (C2″), 168.5 (C1′); MS (ESI$^+$): m/z 357.2748 (calcd for $C_{19}H_{37}N_2O_4$, 357.2748) $[M+H]^+$.

4.13. (*E*)-*N*′-Hydroxy-*N*′-(7′-methyl-2′-octenoyl)-cadaverinium triflate (21)

Boc derivative **20** was treated analogously to **14**, producing a quantitative yield of triflate **21**. ^{1}H NMR (CD_3OD) δ 0.89 (d, J=6.6 Hz, H8′), 1.56 (n, J=6.7 Hz, H7′), 2.23 (q, J=7.3 Hz, H4′), 2.92 (t, J=7.3 Hz, H5), 3.68 (t, J=6.8 Hz, H1), 6.63 (d, J=15.5 Hz, H2′), 6.84 (dt, J=15.5, 7.0 Hz, H3′); ^{13}C NMR (CD_3OD) δ 23.1 (C8′), 24.6, 27.2, 27.5 (C5′), 28.2 (C7′), 30.9, 33.8 (C4′), 39.8 (C6′), 40.8 (C1), 118.1 (q, J=87.0 Hz, CF_3), 119.6 (C2′), 148.4 (C3′), 163.2 (q, J=13.5 Hz, CCF_3), 172.6 (C1′); MS (ESI$^+$): m/z 257.2222 (calcd for $C_{14}H_{29}N_2O_2$, 257.2224) $[RNH_3]^+$.

4.14. 9U,9U-Flavolipid *tert*-butyl ester (22)

This was prepared in 60% yield analogously to **16** but starting with **20**. ^{1}H NMR (CD_3OD) δ 0.89 (d, J=6.6 Hz, H8), 1.45 (s, Boc), 1.67 (p, J=6.7 Hz, H4), 2.23 (q, J=7.0 Hz, H4), 2.58 and 2.67 (d, J=14.7 Hz, H2″), 3.16 (t, J=6.3 Hz, H1′), 3.65 (t, J=6.6 Hz, H5′), 6.63 (br d, J=15.5 Hz, H2), 6.83 (dt, J=15.5, 7.0 Hz, H3); ^{13}C NMR (CD_3OD) δ 23.1 (C19), 25.1 (C8), 27.5 (C16), 28.3 (C18), 28.3 (C20), 29.2 (C7), 30.1 (C9), 30.9 (C3), 33.8 (C15), 39.8 (C17), 40.3 (C6), 45.2 (C10), 75.4 (C2), 83.5 (C21), 120.7 (C13), 148.2 (C14), 168.5 (C12), 172.1 (C4), 174.3 (C1); MS (ESI$^+$): m/z 725.5043 (calcd for $C_{38}H_{69}N_4O_9$, 725.5059) $[M+H]^+$.

4.15. 9U,9U-Flavolipid (4)

Boc derivative **22** (976 mg, 1.35 mmol) was stirred with TFA (5 mL) for 15 min and the TFA was evaporated. Benzene and then 2× chloroform was added and evaporated and the residue was chromatographed on Lipophilic Sephadex, eluting with 8% EtOH/toluene to give **4** (585 mg, 65%), mp 98–100 °C. ^{1}H NMR (CD_3OD) δ 0.89 (d, J=6.1 Hz, H19), 1.22 (q, J=7.4 Hz, H17), 1.34 (p, J=7.6 Hz, H8), 1.48 (p, J=7.9 Hz, H16), 1.53 (p, J=7.5 Hz, H7), 1.55 (n, J=7.0 Hz, H17), 1.66 (p, J=7.4 Hz, H9), 2.23 (q, J=6.8 Hz, H15), 2.61 and 2.71 (d, J=14.0 Hz, H3), 3.16 (t, J=6.5 Hz, H6), 3.65 (t, J=6.6 Hz, H10), 6.61 (br d, J=15.0 Hz, H13), 6.84 (dt, J=15.0, 7.3 Hz, H14); ^{13}C NMR (CD_3OD) δ 23.1 (C19), 25.1 (C8), 27.5 (C16), 28.3 (C18), 29.2 (C7), 30.0 (C9), 33.8 (C15), 39.8 (C17), 40.3 (C6), 43.3 (C3), 45.2 (C10), 75.5 (C2), 120.7 (C13), 148.2 (C14), 168.5 (C12), 177.4 (C4), 177.0 (C1); MS (ESI$^-$): m/z 667.4276 (calcd for $C_{34}H_{59}N_4O_9$, 667.4288) $[M-H]^-$; MS/MS on 667: 631, 529, 511, 475, 349.[1]

4.16. 9S,9S-Flavolipid (3)

9U,9U-Flavolipid (**4**, 76 mg) in EtOH (5 mL) was stirred with 10% Pd/C under H_2 for 2 h. Filtering off the solids, washing with EtOH, and evaporating the solvent gave **3** (72 mg, 94%) as waxy crystals, mp 96–98 °C. ^{1}H NMR (CD_3OD) δ 0.87 (d, J=6.6 Hz, H19), 1.18 (q, J=6.9 Hz, H17), 1.32 (br s, H8, H15, H16), 1.51 (p, J=6.6 Hz, H7), 1.53 (n, J=7.0 Hz, H18), 1.59 (p, J=7.0 Hz, H14), 1.62 (p, J=7.3 Hz, H9), 2.45 (t, J=7.2 Hz, H13), 2.60 and 2.71 (d, J=15.1 Hz, H3), 3.17 (t, J=6.6 Hz, H6), 3.59 (t, J=6.9 Hz, H10); ^{13}C NMR (CD_3OD) δ 23.2 (C19), 25.1 (C8), 26.2 (C16), 27.5 (C14), 28.4 (C18), 29.3 (C7), 30.0 (C9), 30.9 (C15), 33.5 (C13), 40.2 (C17), 40.3 (C6), 43.3 (C3), 45.2 (C10), 75.5 (C2), 172.5 (C4), 176.2 (C12), 177.0 (C1); MS (ESI$^-$): m/z 671.4593 (calcd for $C_{34}H_{63}N_4O_9$, 671.4601) $[M-H]^-$; MS/MS on 671: 635, 513, 477, 351.[1]

Acknowledgements

This work was supported by grant number 0714245 from the National Science Foundation and by a Camille and Henry Dreyfus Foundation Senior Scientist Mentor Initiative Award to R.B.B.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.09.088.

References and notes

1. Bodour, A. A.; Guerrero-Barajas, C.; Jiorle, B. V.; Malcomson, M. E.; Paull, A. K.; Somogyi, A.; Trinh, L. N.; Bates, R. B.; Maier, R. M. *Appl. Environ. Microbiol.* **2004**, *70*, 114.
2. Miyamoto, M.; Inatomi, K.; Ueyama, S. *Thin Solid Films* **2004**, *468*, 255.
3. Dickschat, J. S.; Bode, H. B.; Kroppenstedt, R. M.; Mueller, R.; Schulz, S. *Org. Biomol. Chem.* **2005**, *3*, 2824.
4. Luckner, M. *Secondary Metabolism in Microorganisms, Plants, and Animals*; Springer: Berlin, 1990, pp 286–288.
5. Lee, B. H.; Miller, M. J. *J. Org. Chem.* **1983**, *48*, 24.
6. Gardner, R. A.; Ghobrial, G.; Naser, S. A.; Phanstiel, O. *J. Med. Chem.* **2004**, *47*, 4933.
7. Guo, H.; Naser, S. A.; Ghobriel, G.; Phanstiel, O. *J. Med. Chem.* **2002**, *45*, 2056.
8. Wang, Q. X.; Phanstiel, O. *J. Org. Chem.* **1998**, *63*, 1491.
9. Weaver, R.; Gilbert, I. H.; Mahmood, N.; Balzarini, J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2405.
10. Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651.
11. Coutrot, P.; Snoussi, M.; Savignac, P. *Synthesis* **1978**, 133.
12. Sibi, M. P.; Hasegawa, H.; Ghorpade, S. R. *Org. Lett.* **2002**, *4*, 3343.
13. Rubinstein, L. V.; Shoemaker, R. H.; Paul, K. D.; Simon, R. M.; Tosini, S.; Skehan, P.; Scudiero, D. A.; Monks, A.; Boyd, M. R. *J. Natl. Cancer Inst* **1990**, *82*, 1113.
14. Zhan, J.; Burns, A. M.; Liu, M. X.; Faeth, S. H.; Gunatilaka, A. A. L. *J. Nat. Prod.* **2007**, *70*, 227.
15. Barton, L. L.; Hemming, B. C. *Iron Chelation in Plants and Soil Microorganisms*; Academic: San Diego, CA, 1993.
16. Winkelmann, G. *CRC Handbook of Microbial Iron Chelates*; CRC: Boca Raton, FL, 1991.
17. Neilson, J. W.; Artiola, J. F.; Maier, R. M. *J. Environ. Qual.* **2003**, *32*, 899.
18. Ochoa-Loza, F. J.; Noorman, W. H.; Jannsen, D. B.; Brusseau, M. L.; Maier, R. M. *Chemosphere* **2007**, *66*, 1634.
19. Kunze, B.; Trowitzsch-Kienast, W.; Hoefle, G.; Reichenbach, H. *J. Antibiot.* **1992**, *45*, 147.
20. Hurley, L. H. *Nat. Rev. Cancer* **2002**, *2*, 188.
21. Luo, M.; Fadeev, E. A.; Groves, J. T. *J. Am. Chem. Soc.* **2005**, *127*, 1726.; Pitter, P.; Chudoba, J. *Biodegradability of Organic Substances in the Aquatic Environment*; CRC: Boca Raton, FL, 1990, pp 166–169.