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Structures of Cribochalines A and B, Branched-Chain Methoxylaminoalkyl Pyridines from the Micronesian Sponge, *Cribochalina* sp. Absolute Configuration and Enantiomeric Purity of Related *O*-Methyl Oximes

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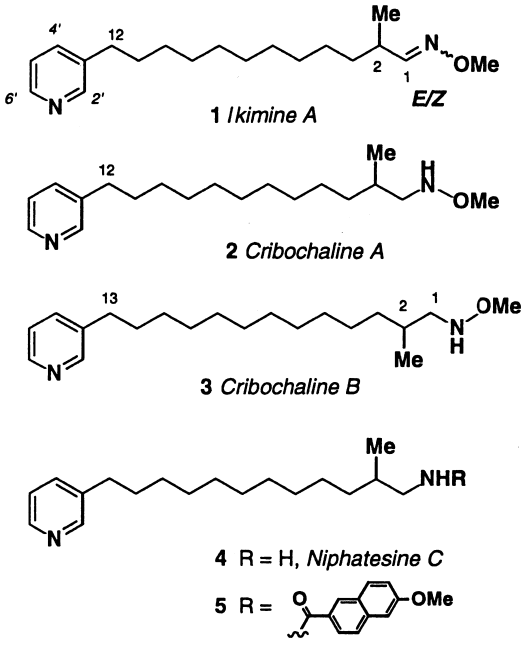
Abstract—Two new 3-alkyl pyridines, cribochalines A (**2**) and B (**3**), were isolated from the North Pacific sponge *Cribochalina* sp. The known related oxime, ikimine A, was shown to be a 2.8:1 mixture of the (*S*)- and (*R*)-enantiomers. Cribochaline A exhibited antifungal activity against *Candida albicans* ATCC and Fluconazole-resistant strains *C. albicans* 96–489, *C. krusei* and *C. glabrata*.

Introduction

Sponges of the family Niphatidae, order Haplosclerida, produce a diverse array of alkaloids that are structurally related by the presence of a pyridine ring or its reduced forms.[1–5] The simplest compounds in this class are 3′-aminoalkylpyridines in which the substituent at the aromatic ring is a long-chain (C_{12}–C_{18}) ω-aminoalkyl group. Further variations occur when the side chain contains unsaturation or is further oxidized at the terminus to a methoxylamine or oxime, as represented by the structure of ikimine A (**1**, C_{12} chain) from an unidentified sponge collected in Ant Atoll, Pohnpei.[6] Here, we report two new antifungal compounds, cribochalines A (**2**) and B (**3**), from *Cribochalina* sp., also from Ant Atoll, which are closely related to **1**. We also re-isolated, from the same sponge, (+)-**1**[6] as an *E/Z* mixture of oxime geometrical isomers. Stereochemical analysis reveals that **1** is a non-racemic mixture of (+)- and (−)-enantiomers. Cribochaline A (**2**) was observed to undergo facile autoxidation to **1**, suggesting that **2** is the substrate for a non-enzymic autoxidation and that **1** and related oximes or their *O*-Me ethers may be artifacts. Including the natural product **4**, the foregoing observations unify the three oxidation levels of alkylpyridines—amine, alkoxylamine and oxime—found in the Niphatid sponges.



Results and Discussion

The *n*-hexane-soluble fraction of an MeOH extract of lyophilized *Cribochalina* sp. exhibited significant brine shrimp lethality (BSL, LC_{100} ~85 ppm) and moderate, broad-spectrum antifungal activity against *Candida albicans* ATCC 14503 and Fluconazole®-resistant strains,

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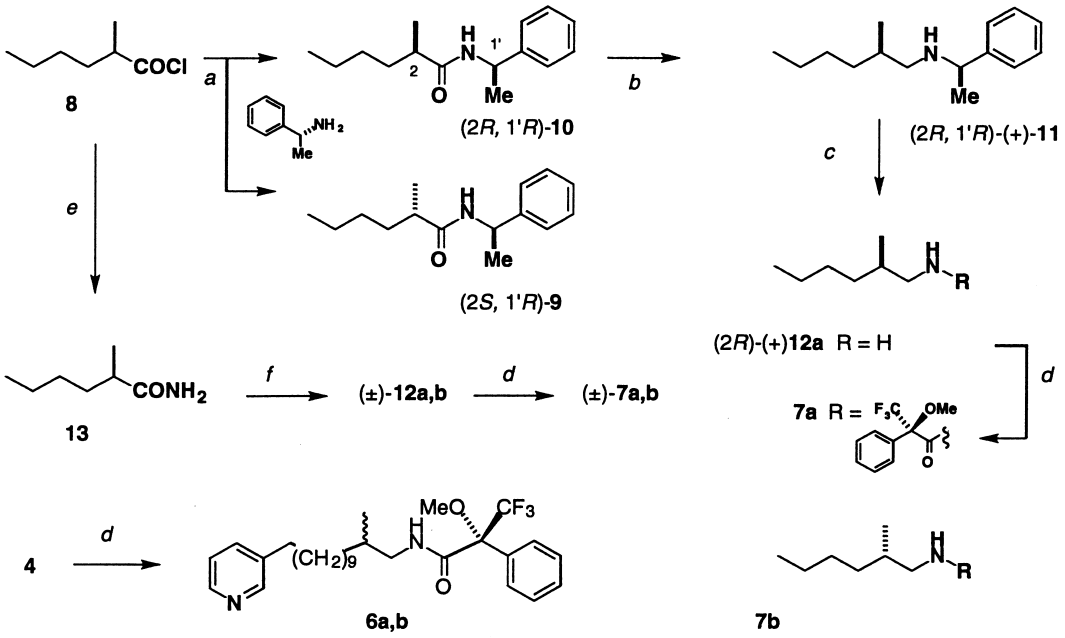


Scheme 1. (a) Et_3N, DMAP, CH_2Cl_2; (b) BH_3·THF, THF, reflux; (c) $Pd(OH)_2$, H_2, MeOH; (d) (*S*)-MTPACl, py, DMAP; (e) NH_4OH, aq; (f) BH_3·SMe_2, THF, reflux.

C. albicans 96–489, *C. krusei* and *C. glabrata*. Separation of the active fraction by silica chromatography gave a refractory mixture containing homologous ninhydrin-positive compounds, identified as 3-aminoalkylpyridines by a combination of HPLC diode-array UV spectra and ^{1}H NMR. The difficult separation was finally achieved by use of Et_3N-buffered aqueous MeOH on reversed phase (C_{18}) HPLC to provide pure samples of **1**, **2** and **3**.

The formula for **2** ($C_{19}H_{34}N_2O$) revealed four degrees of unsaturation, which is satisfied by a pyridine ring. The pyridine ring was established by a UV chromophore consistent with an alkylpyridine [λ_{max} 210 nm (ϵ 5630), 262 (4530), 269 (3330)] and by the ^{1}H NMR spectrum of **2**, which showed the characteristic downfield signals of a 3-substituted pyridine [δ 8.41 (bs, 1H), 7.51 (m, 1H), 7.20 (dd, *J*=5.1, 7.8 Hz, 1H), 8.41 (m, 1H)]. The methoxylamine group (NHOMe) was assigned by the presence of a weak IR band (ν 3240, NH) together with ^{1}H and ^{13}C signals corresponding to a deshielded MeO group (δ_H 3.53, s, 3H; δ_C 60.1, q). Weak optical activity ($[\alpha]_D^{24}=-1.0°$) in compound **2** suggested an alkaloid belonging to the class of chiral branched alkylpyridines. COSY analysis located the methyl group (δ 0.91, d, *J*=6.9 Hz) β to the –NHOMe terminus through coupling to the –CH–CH_2–N ^{1}H spin system [δ 1.61 (m, CH), 2.65 (dd, *J*=7.5, 12.3 Hz, 1H), 2.85 (dd, *J*=6.0, 12.3 Hz, 1H)]. The balance of the ^{1}H and ^{13}C signals in the side chain were attributed to 9×CH_2 groups including one benzylic CH_2 (2.59, t, *J*=7.5 Hz, 2H, H12) comprising a straight methylene chain. Thus, the complete structure of **2** has a C_{12} sidechain attached to C3′ (pyridine numbering) with an Me branch at C2. Comparison of literature for ^{1}H NMR data for ikimines[6] and related 3-(methoxyamino-alkyl)pyridines[2,4] fully supported this assignment.

Compound **3** ($C_{20}H_{36}N_2O$) differs from **2** by having one extra CH_2 but otherwise exhibited spectral data (UV, ^{1}H, COSY) identical with those of **2**. The CH_3CHCH_2–NH– spin system is also present; consequently, we assigned structure **3**—the higher homologue of **2**—to the second compound.

Neither optical rotation nor the absolute stereochemistry of **1** is reported in the literature.[6] Our sample of (+)-**1** was dextrorotatory ($[\alpha]_D=+7.5$), but optical rotations of alkylamines are characteristically low in magnitude and unreliable for determination of absolute configuration. Instead, we turned to analysis of circular dichroism (CD) of a suitable aroyl carboxylate derivative of **1**. (+)-Ikimine A (**1**) was reduced ($LiAlH_4$, THF, quantitative) to the known alkaloid (+)-niphatesine C [**4**, HCl salt, $[\alpha]_D^{23}=+3.0°$ (*c* 0.16, MeOH). lit.[2] +9.4 (*c* 0.053, MeOH)], which was converted to the naphthoamide **5**. The β-methyl group of **5** was expected to exert a detectable, albeit weak, Cotton effect on the primary naphthoamide. Unfortunately, the CD spectrum of **5** showed no significant Cotton effects ($\Delta\epsilon\sim0$) at the expected wavelengths, suggesting the weak optical activity detected in both **1** and **2** is attributable to a partial racemate with low optical purity. The absolute configuration and %ee of the more abundant (+)-enantiomer of **1** were determined as follows.

(+)-Niphatesine C[2] (**4**), obtained from **1** as described above, was converted to Mosher's amide **6** ((*S*)-MTPACl, DMAP, py, 44%) as shown in Scheme 1. For the purposes of comparison, (*R*)-Mosher's amides **7a** and **7b** were prepared from (±)-2-methylhexanoyl chloride (**8**) as follows. Treatment of **8** with (*R*)-α-methylbenzylamine (Et_3N, DMAP, CH_2Cl_2) gave a 1:1 mixture of amides that were easily separated by silica chromatography (1:9 EtOAc/*n*-hexanes) to provide the less polar diastereomer (+)-**9** ($[\alpha]_D=+102°$ (*c* 2.0, $CHCl_3$) and more polar (+)-**10** ($[\alpha]_D=+75.8°$ (*c* 2.1, $CHCl_3$)) in yields of 38 and 39%, respectively. Reduction of (+)-**10** (BH_3·THF, THF, reflux) gave the corresponding

Table 1. Selected ^{1}H NMR chemical δ, J's and integrations of **6**, (2*R*)-**7a** and (2*S*)-**7b** (400 MHz, C_6D_6)

#	(2*S*)-**6a**		(2*R*)-**6b**		(2*R*)-**7a**		(2*S*)-**7b**	
	δ	mult, J (Hz), int.	δ	mult, J (Hz), int.	δ	mult, J (Hz), int.	δ	mult, J (Hz), int.
Ome	3.16[a]	bs, 3H	3.16[a]	bs, 3H	3.14[a]	bs, 3H	3.15[a]	bs, 3H
2-Me	0.68	d, 6.8, 2.2H	0.67	d, 6.5, 0.8H	0.63	d, 6.7, 3H	0.64	d, 6.7, 3H
H-1a[b]	3.12	m, 0.73H	2.98	m, 0.54H	2.93	m, 2H	3.06	m, 1H
H-1b[b]	2.81	m, 0.73H	–	–	–	–	2.79	m, 1H

[a] Broadened by J_{HF}.
[b] Signals for the (*S*)-**7b** extracted from spectrum of (*R*)-Mosher's amides of (±)-**7a**,**b** and presented with normalized integrations.

benzylamine (+)-**11** [58%, $[\alpha]_D$=+42.0° (*c*, 1.7, MeOH)] that was hydrogenolyzed ($Pd(OH)_2$, H_2, MeOH, 72%) to (2*R*)-(+)-2-hexylamine **12a** ($[\alpha]_D$ of HCl salt, +6.1°, (*c* 0.9, H_2O); lit.[7] $[\alpha]_D$=−2.41° (*c* 15.384, H_2O)) and subsequently converted to (*R*)-Mosher's amide (+)-**7a** ($[\alpha]_D$=+17.0°, (*c* 0.1, $CHCl_3$)). A mixture of the (*R*)-Mosher's amides of racemic amine (±)-**7a**,**b** was prepared by the sequential reactions: ammoniolysis of (±)-**8** to primary amide (±)-**13** (aqueous NH_4OH, 88%), reduction to amine (±)-**12a**,**b** ($BH_3{\cdot}SMe_2$, THF, 80%) and acylation with (*R*)-Mosher's acid chloride (py, DMAP, 30%). Integration of the ^{1}H NMR spectrum of the latter confirmed that it was a 1:1 mixture of amides **7a** and **7b**.

Analysis of the ^{1}H NMR spectra ($CDCl_3$) of the natural product derivative **6** showed doubling of several signals due to the presence of diastereomers (2*R*)-**6a** and (2*S*)-**6b**. For example, the C2 Me signals were doubled at δ 0.67 (d, *J*=6.5 Hz, 0.8H) and 0.68 (d, *J*=6.8 Hz, 2.2H). The corresponding chemical shifts of (*R*)-Mosher's amides **7a**,**b** also showed the same fine differences in ^{1}H NMR in chemical shift for the C2 Me signal in $CDCl_3$, but the diastereomers were most clearly distinguished in C_6D_6 by chemical shift and *J* coupling patterns of the diastereotopic H1 proton signals (see Table 1). The spectrum of the model compound (2*R*)-(+)-**7a** displayed a collapsed multiplet (δ 2.93, m, 2H) for the C1 methylene group. Conversely, interpretation of the ^{1}H NMR spectrum of (±)-**7a**,**b** revealed the H1 diastereotopic protons of **7b** are dispersed between two multiplets centered at δ 3.06 and 2.79 ppm. In the natural product derivative **6**, both sets of diastereotopic proton multiplets are observed. Careful integration of ^{1}H signals shows that the diastereomeric ratio (dr) of **6** is 2.8:1, with a predominance of the (2*S*) isomer **6b**. Accepting the reasonable assumption that no kinetic enrichment takes place during formation of **6**, we conclude that our samples of (+)-ikimine A (**1**) and (+)-niphatesine C (**4**) each occur as a 2.8:1 mixture of enantiomers, with an excess of the (2*S*) enantiomer (47% ee).

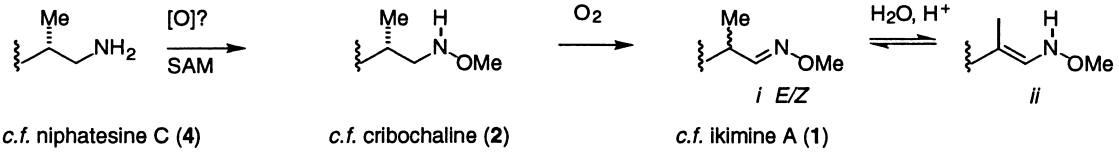
Upon prolonged storage in $CDCl_3$ (4°C, ~2 weeks), compound **2** underwent autoxidation (ca. 40–50% conversion), giving a 3:1 mixture of *E*/*Z* isomers of *O*-methyloximes. The product was identified as the *E*/*Z* geometrical isomers of ikimine A (**1**) by comparison of ^{1}H NMR spectra with **1** isolated from the same sponge. The literature ^{1}H data of **1**[6] matched those of the autoxidation product. Since air oxidation of secondary amines is known to give imines[8] it is conceivable that **1** arises by *N*-hydroxylation of methoxylamine **2** followed by acid-catalyzed elimination of H_2O, although the ease of autoxidation in this case is surprising. The imperfect optical purity of **1** may be a consequence of the oxidation process itself and not lack of fidelity in the enzymatic biogenesis of the branched chain (probably involving methylation by *S*-adenosyl methionine).

The chemical correlation established in this work (**1**→**4**→**5**) supports the notion that loss of optical activity occurs after formation of **2**, given that natural (+)-niphatesine C (**4**) exhibits higher optical activity ($[\alpha]_D$=+9.4°) than synthetic **4** ($[\alpha]_D$=+3°), obtained by reduction of **1**. A plausible biogenic sequence is shown in Scheme 2, starting with enzymatic oxidation–methylation of **4**. Slow racemization of chiral oxime *i* in protic media, for example, via the tautomeric intermediate *N*-methoxy enamine *ii*, could account for loss of optical activity in **1** that derives from an amine precursor of higher optical purity. Unfortunately, insufficient **2** was left after spontaneous autoxidation to **1**, and natural **4** was unavailable to us to test this hypothesis.[9]

Given the ease of autoxidation of **2**, it is likely that **2** is the biosynthetic precursor of **1** and the latter arises by non-enzymatic oxidation. The examination of ^{1}H NMR spectra of samples of crude extracts obtained from the sponge did indeed show evidence of the presence of lesser amounts of both α- and β-methyl substituted oxime signals (*E*-isomers δ 7.2 d, or t; *Z*-isomers, δ 6.3–6.6 d or t) together with olefinic and propargylic proton signals, although the majority of the alkylpyridine components of *Cribochalina* extract are fully saturated in the alkylamine sidechains.

Biological Activity

Compound **2** showed moderate antifungal activity against five organisms in the disk-diffusion assay at 300 μg/disk: *C.*



Scheme 2.

albicans (ATCC 14503) 14 mm zone of inhibition, *C. albicans* (96–489) 11 mm, *C. albicans* (UCD-FR1) 17 mm, *C. krusei* 13 mm at 100 μg/disk and *C. glabrata* 15 mm. (+)-Ikimine A (**1**) was inactive and insufficient amounts of **3** were available for testing.

Conclusion

Two new alkaloids, cribochalines A (**2**) and B (**3**) have been isolated and ikimine A (**1**) is shown to be partially racemic (2.8:1 *S* to *R*). The relationship of cribochaline B to ikimine A suggests a non-enzymatic biosynthesis of **1** and related oximes from the corresponding methoxylamines.

Experimental

General procedures

^{1}H NMR, COSY and ^{13}C NMR spectra were measured on General Electric QE-300 (^{1}H NMR 300 and ^{13}C NMR 75 MHz) and Varian Inova 400 (400 and 100 MHz) spectrometers. ^{13}C assignments were made from analysis of the DEPT spectra. IR spectra were measured on a Mattson Galaxy 3000 FTIR spectrometer and UV spectra were obtained on a Hewlett Packard 8450A diode array spectrophotometer. Optical rotations were recorded on a Jasco Dip digital 370 polarimeter using a 1 dm cell. Measurements of mass spectra were made at the Regional Mass Spectrometry Facility at the University of California, Riverside.

Collection and bioactivity

The mauve and grey-colored sponge *Cribochalina* sp. (97–108) was collected at Ant Atoll #1, Pohnpei, FSM (6° 45.671′ N, 157° 59.619′ E) in December 1997 at a depth of 24 m, and the sponge was kept frozen (−20°C) until required. Voucher samples and spicule mounts are stored in the Chemistry Department, UC Davis and are available from the corresponding author. A portion of the sample was allowed to stand in EtOH (~12 months, 4°C) after which the EtOH extract showed significant activity in the brine shrimp lethality (BSL) assay (LC_{50} 85 ppm after 24 h) and moderate antifungal activity in the disk diffusion assay (300 μg/disk) against *Candida albicans* (ATCC 14503) 9 mm, *C. krusei* (8 mm), *C. albicans* (96–489, 8 mm), *C. albicans* (UCD-FR1, 8 mm) and *C. glabrata* (8 mm).

Extraction and isolation

The sponge 97–108 (dry weight 70.2 g) was lyophilized and extracted with MeOH (4×600 mL) to give a dark green solution. The water content (v/v) of the MeOH extract was adjusted, followed by sequential partitioning against the solvents. Concentration of each sequential organic extract provided *n*-hexane (10% v/v, I, 1.68 g) and $CHCl_3$ (40% v/v, II, 3.53 g) soluble fractions. The MeOH was removed from the aqueous phase under vacuum, and the remaining liquid partitioned against *n*-butanol (III, 1.61 g). The remaining aqueous phase was lyophilized (IV, 13.81 g). The hexane, chloroform and *n*-butanol fractions all showed some activity in the brine shrimp lethality (BSL) assay, but the most active was the hexane fraction that showed 100% mortality after 12 h, compared to the other two that showed 53–86% mortality but only after 48 h. Only the hexane and chloroform fractions showed antifungal activity at 300 μg/disk. The hexane fraction (1.6 g) was separated by silica gel chromatography and eluted with a stepped gradient (hexane to EtOAc), to give 21 fractions. Fractions 20–21 both showed activity in the BSL assay (86–100% mortality at 100 ppm) and significant antifungal activity (13 mm, 300 μg/disk) against *C. albicans* (ATCC). Fraction 20 was further separated by C_{18} reversed-phase HPLC (Dynamax 60 Å, 21.4×250 mm, 1:9 H_2O/MeOH, 0.05% Et_3N), to give cribochaline A (**2**, 7.8 mg, 0.011% dry wt) with a retention time of 13.8 min and cribochaline B (**3**, 1.9 mg, 0.001% dry wt) with a retention time of 17.8 min. (+)-Ikimine A (**1**)[6] was obtained from separation of the $CHCl_3$-soluble fraction II by silica flash chromatography (EtOAc/*n*-hexane gradient) and HPLC.

(+)-Ikimine A (1).[6] 3:1 *E*/*Z* mixture, $[\alpha]_D^{24}=+7.5°$ (*c* 0.13, $CHCl_3$). *E* isomer: ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.05 (d, *J*=6.9 Hz, 3H), 1.25–1.30 (m, CH_2's), 1.62 (m, 2H), 2.32 (m, 1H), 2.64 (t, *J*=6.9 Hz, 2H), 3.80 (s, 3H), 7.19 (d, *J*=7.5 Hz, 1H), 7.34 (dd, *J*=4.8, 7.8 Hz, 2H), 7.64 (br d, *J*=7.8 Hz, 2H), 8.45 (m,1H), 8.46 (br s, 1H). HREIMS *m*/*z* 304.2509 $[M+H]^+$, Calcd for $C_{19}H_{32}N_2O$ 304.2514. As noted by Carroll et al.,[6] the *E*/*Z* oxime isomers of ikimine A were separable on silica HPLC (*E*-**1** elutes first, silica Microsorb, 10×250 mm, 5:95 EtOAc/*n*-hexane, 3.0 mL/min), but re-equilibrated within hours.

Cribochaline A (2). $[\alpha]_D^{24}=-1.0°$ (*c* 0.2, MeOH). UV λ_{max} (MeOH) 210 nm (ϵ 5630), 262 (4530), 269 (3330). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.41 (bs, 1H), 7.51 (m, 1H), 7.20 (dd, *J*=5.1, 7.8 Hz, 1H), 8.41 (m, 1H), 2.59 (t, *J*=7.5 Hz, 2H), 1.60 (m), 3.51 (s, 2H), 2.65 (dd, *J*=7.5, 12.3 Hz, 1H), 2.85 (dd, *J*=6.0, 12.3 Hz 1H), 1.62 (m), 1.2–1.3, 0.91 (d, *J*=3.3 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3) δ_C 147.6 (C-2), 147.6 (C-6), 138.1 (C-3), 137.9 (C-4), 123.9 (C-5), 61.5 (OCH_3), 58.2 (NCH_2), 34.9, 32.9, 31.0, 29.1–29.9, 26.8, 17.9 (CH_3). IR (NaCl plate, film) 3240 (br weak), 2926, 2854, 1575, 1477, 1464, 1422 cm^{-1}. HRFABMS *m*/*z* 307.2757 $[M+H]^+$ Calcd for $C_{19}H_{35}N_2O$ 307.2749.

Cribochaline B (3). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.45 (m, 1H), 7.60 (m, 1H), 7.31 (dd, *J*=5.1, 7.8 Hz, 1H), 8.45 (m, 1H), 2.63 (t, *J*=7.5 Hz, 2H), 1.60 (m), 3.53 (s, 2H), 2.85 (dd, *J*=6.0, 12.0 Hz 1H), 1.62 (m), 1.18–1.30 (CH_2's), 0.89 (d, *J*=3.3 Hz, 3H). IR (NaCl plate, film) 3240 (br weak), 2926, 2854, 1575, 1477, 1464, 1422, 1026, 790, 713 cm^{-1}. HRFABMS *m*/*z* 321.2907 $[M+H]^+$ Calcd for $C_{20}H_{37}N_2O$ 321.2905.

Reduction of (+)-ikimine A

(+)-Niphatesine C (4)[6] A solution of **1** (5.5 mg, 0.018 mmol) in THF (1 mL) was added to a stirred slurry of $LiAlH_4$ (14 mg, 0.37 mmol) in THF (1 mL). The mixture was allowed to stir at 25°C for 16 h at which time no starting material was detected by TLC. The mixture was treated with water (2 drops), followed by aqueous NaOH (2 mL, 4 M), stirred for 5 min, diluted with EtOAc (15 mL) and the phases separated. The aqueous phase was extracted with

EtOAc (3×5 mL), and the combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated to give **4** as a colorless oil (5.0 mg, quant., purity >90%). Purification of the oil by silica chromatography (3:7 MeOH (satd. NH_3)/$CHCl_3$) yielded pure **4** as the free base (2.5 mg). ^{1}H NMR (300 MHz, CD_3OD) δ_H 8.36 (m, 1H), 8.33 (m, 1H), 7.68 (m, 1H), 7.34 (dd, J=5.1, 7.5 Hz, 1H), 2.69 (dd, J=5.7, 12.6 Hz, 1H, CH_2), 2.64 (t, J=7.8 Hz, 2H), 2.54 (dd, J=7.5, 12.6 Hz, 1H, CH_2), 1.61 (m, 2H), 1.28 (m, CH_2's), 0.94 (d, J=7.8 Hz, 3H). HRCIMS (NH_3) m/z 277.2655 $[M+H]^+$, Calcd for $C_{18}H_{33}N_2$ 277.2643.

A solution of primary amine **4** in hydrochloric acid (1 M, 100 mL) was evaporated and dried overnight under vacuum to give the corresponding hydrochloride salt. $[\alpha]_D^{23}=+3.0°$ (c 0.16, MeOH). lit.[2] +9.4 (c 0.053, MeOH). The ^{1}H NMR data for the **4**·HCl were consistent with that reported for (+)-niphatesine C (**4**).[2] ^{1}H NMR 300 MHz (CD_3OD) δ_H 8.36 (m, 1H), 8.33 (m, 1H), 7.68 (m, 1H), 7.36 (dd, J=5.1, 7.5 Hz, 1H), 2.84 (dd, J=5.7, 12.3 Hz, 1H, CH_2), 2.69 (dd, J=7.5, 12.3 Hz, 1H, CH_2), 2.65 (t, J=7.8 Hz, 2H), 1.61 (m, 2H), 1.28 (m, CH_2's), 0.99 (d, J=6.6 Hz, 3H).

6-Methoxynaphthoamide 5. A solution of (+)-niphatesine C (**4**) (5.0 mg, 10.9 μmol), prepared from (+)-**1**), and DMAP (1 crystal) in dry CH_2Cl_2 (200 μL) was treated with 6-methoxynaphthoyl chloride (131 μmol, prepared by reaction of 6-methoxynaphthoic acid and excess $SOCl_2$, cat. DMF) in CH_2Cl_2 (800 μL) in the presence of Et_3N (10 μL, 79 μmol). After 16 h, the mixture was vigorously stirred with aqueous $NaHCO_3$ for 30 min. The aqueous phase was extracted with CH_2Cl_2, and the combined organic phases washed with $NaHCO_3$, dried ($MgSO_4$), then concentrated to give a colorless oil, which was separated on silica (1×15 cm, gradient of 1:1 EtOAc / n-hexane) to give the non-polar 6-methoxynaphthoamide **5** (2.6 mg, 33%). $[\alpha]_D^{23}=+1.3°$ (c 0.22, MeOH). CD (MeOH), no signal. ^{1}H NMR ($CDCl_3$) 7.19 (dd, J=8.7, 2.4 Hz, 1H), 7.15 (m), 6.29 (m, 1H), 3.94 (s, 3H), 3.44 (m, 1H), 3.31 (m, 1H), 2.65 (t, J=7.5 Hz, 2H), 1.78 (m), 1.62 (m), 1.26 (m), 0.99 (d, J=6.6 Hz, 3H). HRDCIMS (NH_3) m/z 461.3164 $[M+H]^+$, Calcd for $C_{30}H_{41}N_2O_2$ 461.3168.

(R)-Mosher's amide of (+)-4. (*S*)-MTPA chloride (40 mg, 0.16 mmol) in pyridine (500 μL) was added to a solution of (+)-amine **4** (2.5 mg, 9.0 μmol) and DMAP (catalytic amount) in dry pyridine (500 μL). After 15 h, the reaction mixture was stirred with aqueous $NaHCO_3$ for 30 min and the two phases separated. The aqueous phase was extracted with CH_2Cl_2 and the combined organic phases washed with brine, dried ($MgSO_4$) and concentrated to colorless oil (5.1 mg). Separation of the residue on silica (1:4 EtOAc/ n-hexane to 1:1) gave the mixed diastereomers of **6a**,**b** (2.0 mg, 44%). UV (MeOH) λ_{max} 205 nm (ϵ 20 900), 257 nm (ϵ 3700), 262 nm (ϵ 4200), 268 nm (ϵ 3000), 330 nm (ϵ 100). ^{1}H NMR 400 MHz (C_6D_6) δ_H 8.44 (m, 1H), 8.35 (m, 1H), 7.72 (d, J=7.6 Hz, 2H), 7.05–7.10 (m, 3H), 6.91 (d, J=7.6 Hz, 1H), 6.63 (m, 1H), 6.32 (m, 1H, NH), 3.16 (m, 3H, OCH_3), 3.12 (m, 0.73H, (*S*)-CH_2), 2.98 (m, 0.54H, (*R*)-CH_2), 2.81 (m, 0.73H, (*S*)-CH_2), 2.16 (t, J=7.4 Hz, 2H), 0.7–1.4 (m,), 0.68 (d, J=6.8 Hz, 2.2H, (*S*)-CH_3), 0.67 (d, J=6.5 Hz, 0.8H, (*R*)-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 165.8, 148.5, 145.5, 138.1, 137.9, 132.5, 129.2, 128.3, 127.5, 123.9, 45.3, 34.3, 33.2, 33.0, 30.9, 29.8, 29.5, 29.5, 29.3, 29.1, 26.8 17.7. IR (NaCl plate, film,) ν 3338 (br), 2922, 2849, 1686 (C=O), 1524, 1464 1268, 1162, 1103 $^{-1}$. HRCIMS (NH_3) m/z 493.3021 $[M+H]^+$, Calcd for $C_{28}H_{40}N_2O_2F_3$ 493.3041.

(*R*)-Mosher's amide of (2*R*)-1-amino-2-methylhexane (7a). A solution of (*S*)-MTPA chloride (50 mg, 0.2 mmol) and DMAP (catalytic amount) in pyridine (500 μL) was added to a stirred solution of amine **12a** (1.7 mg, 11 μmol) in pyridine (500 μL). After 16 h, the mixture was stirred vigorously with aqueous $NaHCO_3$ for 30 min and the two phases separated. The aqueous phase was extracted with CH_2Cl_2, and the combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated to a colorless oil. Purification of the residue on silica (gradient from 1:4–1:1 EtOAc/n-hexane) gave **7a** (2.1 mg, 52%). $[\alpha]_D^{25}=+17.0°$ (c 0.1, $CHCl_3$). UV (MeOH) λ_{max} 206 nm (ϵ 11500), 257 nm (470). ^{1}H NMR (300 MHz, C_6D_6) δ_H 7.71 (d, J=8.2 Hz, 2H), 6.95–7.10 (m, 3H), 6.18 (m, 1H, NH), 3.14 ((m, 3H, OCH_3), 2.93 (m, 2H, (*R*)-CH_2), 1.26 (m, 1H, CH), 1.00–1.20 (m, 6H), 0.83 (t, J=6.8 Hz, 3H, CH_3), 0.63 (d, J=6.7 Hz, 1.5H, (*R*)-CH_3). IR (NaCl plate, film,) ν 3338 (br), 1683 (C=O), 1524, 1455, 1269, 1164, 1106 cm^{-1}. HRCIMS m/z 332.1842 $[M+H]^+$ Calcd for $C_{17}H_{25}NO_2F_3$ 332.1837.

(*R*)-Mosher's amides of (±)-1-amino-2-methylhexane (7a,b). To a solution of hydrochloride salt (±)-**12a**,**b** (601 mg, 39 μmol) in pyridine (500 μL) was added (*S*)-MTPACl (40 mg, 0.16 mmol) in pyridine (1 mL) and DMAP (catalytic amount). After 18 h, the reaction mixture was stirred with aqueous $NaHCO_3$ for 30 min and the two phases separated. The aqueous phase was washed with CH_2Cl_2 and the combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated to colorless oil. Separation of the residue on silica (20% EtOAc/Hexane) gave a non-polar UV active fraction identified as the 1:1 mixture **7a**,**b** (4.0 mg, 30%). UV (MeOH) λ_{max} 204 nm (ϵ 13200), 257 nm (460), 261 nm (450). ^{1}H NMR 400 MHz (C_6D_6) δ_H 7.71 (d, J=7.6 Hz, 2H), 7.00–7.10 (m, 3H), 6.20 (m, 1H, NH), 3.15 (m, 3H, OCH_3), 3.06 (m, 0.5H (*S*)-CH_2), 2.93 (m, 1H, (*R*)-CH_2), 2.79 (m, 0.5H(*S*)-CH_2), 1.30 (m, 1H, CH), 1.00–1.16 (m, 6H), 0.83 (t, J=6.8 Hz, 1.5H, (*R*)-CH_3), 0.82 (t, J=6.8 Hz, 1.5H, (*S*)-CH_3), 0.64 (d, J=6.7 Hz, 1.5H, (*S*)-CH_3), 0.63 (d, J=6.7 Hz, 1.5H, (*R*)-CH_3). ^{13}C NMR 75 MHz ($CDCl_3$) δ_C 166.0, 132.5, 129.2, 128.4, 127.5, 55.0, 45.3, 34.0, 33.2, 29.7, 29.0, 22.9, 17.7, 14.1. IR (NaCl plate, film,) ν 3338 (br) 2956, 2928, 2858, 1662, 1523, 1455, 1270, 1164, 1106 cm^{-1}. HRCIMS m/z 332.1832 $[M+H]^+$ Calcd $C_{17}H_{25}NO_2F_3$ 332.1837.

(±)-2-Methylhexanoyl chloride (8). A solution of (±)-2-methyl hexanoic acid (1.0 mL, 7.0 mmol) in CH_2Cl_2 (1 mL) was treated with oxalyl chloride (6.1 mL, 70 mmol) and DMF (3 drops). A steady flow of gas evolved for the first 30 min, to give a pale yellow–green solution. The reaction was allowed to stir at 25°C for another hour and the solvent carefully removed to leave volatile (±)-**8**, which was used without further purification.

(+)-(2*S*)-*N*-((1′*R*)-1-Phenethyl)-2-methylhexanamide (9) and (+)-(2*R*)-*N*-((1′*R*)-1-phenethyl)-2-methylhexanamide (10). (*R*)-1-Phenylethylamine (42 mg, 0.35 mmol) was added dropwise to a solution of acid chloride (±)-**8**, triethylamine (177 mg, 1.75 mmol) and DMAP (ca. 1 mg, 8 μmol) in CH_2Cl_2 (2 mL). The mixture was stirred for 4 h at which point TLC indicated the reaction to be complete. The mixture was diluted with CH_2Cl_2 (5 mL) and dilute aqueous $NaHCO_3$ (5 mL) and rapidly stirred for 30 min. The layers were separated and the aqueous phase extracted with CH_2Cl_2 (2×20 mL). The combined organic phases were washed with brine, dried ($MgSO_4$) and the solvent removed to give the crude product. Purification by silica chromatography (10% EtOAc/*n*-hexanes) gave pure (2*S*,1′*R*)-**9** (31.2 mg, 38%), followed by pure (2*R*,1′*R*)-**10** (31.8 mg, 39%). Configurations were assigned by comparison of ^{1}H NMR spectra with those of the known enantiomers (−)- **9** and (−)-**10**[10] and subsequent conversion of (+)-**10** to (+)-**12a**.

Less polar (2*S*,1′*R*) diastereomer **9**. $[\alpha]_D^{24}=+102°$ (*c* 2.0, $CHCl_3$). UV (MeOH) λ_{max} 213 nm (ϵ 5083), 251 (150), 257 (189), 263 (144). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.24–7.38 (m, 5H), 5.77 (br d, *J*=5.1 Hz, 1H, NH), 5.14 (m, 1H), 2.14 (m, 1H), 1.63 (m, 1H), 1.48 (d, *J*=6.9 Hz, 3H), 1.15–1.40 (m, 5H), 1.10 (d, *J*=6.9 Hz, 3H), 0.88 (t, *J*=6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 175.6 (s), 143.4 (s), 128.4 (d), 127.1 (d), 126.0 (d), 48.3 (d), 41.4 (d), 34.0 (t), 29.5 (t), 22.5 (t), 21.6 (q), 17.7 (q), 13.8 (q). IR (NaCl plate, film) ν 3295, 2966, 1643, 1640, 1545 cm^{-1}. HRCIMS *m*/*z* 233.1771 $[M+H]^+$ Calcd for $C_{15}H_{22}NO$ 233.1779.

More polar (2*R*,1′*R*) diastereomer **10**. $[\alpha]_D^{24}=+75.8°$ (*c* 2.1, $CHCl_3$). UV (MeOH) λ_{max} 214 nm (ϵ 4980), 251 (ϵ 144), 257 (ϵ 190), 263 (ϵ 146). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.24–7.38 (m, 5H), 5.84 (br d, *J*=6.9 Hz, 1H, NH), 5.13 (m, 1H), 2.16 (m, 1H), 1.63 (m, 1H), 1.48 (d, *J*=6.9 Hz, 3H), 1.37–1.18 (m, 5H), 1.13 (d, *J*=6.9 Hz, 3H), 0.83 (t, *J*=6.6 Hz, 3H). ^{13}C NMR 75 MHz ($CDCl_3$) δ_C 175.6 (s), 143.4 (s), 128.4 (d), 127.1 (d), 126.0 (d), 48.3 (d), 41.4 (d), 34.0 (t), 29.5 (t), 22.6 (t), 21.6 (q), 17.7 (q), 13.9 (q). IR (NaCl plate, film) ν 3312, 2934, 1640, 1537 cm^{-1}. HRCIMS *m*/*z* 233.1787 $[M+H]^+$ Calcd for $C_{15}H_{22}NO$ 233.1779.

((1*R*)-1-(*N*-((2′*R*)-2-Methylhexyl)amino)ethyl)benzene (11). BH_3·THF (1.0 M, 420 μL, 0.42 mmol) was added dropwise to a solution of amide **10** (48 mg, 0.21 mmol) in THF (200 μL) at 0°C. The mixture was then heated at reflux for 2 h before cooling to 0°C and quenching by dropwise addition of aqueous NaOH (2 mL, 5 M). The biphasic system was then heated to 50°C, stirred for 45 min and cooled to 25°C before dilution with CH_2Cl_2 (5 mL). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (2×5 mL). The combined organic phases were dried ($MgSO_4$), filtered and the solvent removed to give the crude product, which was purified by silica chromatography (1:9 EtOAc/*n*-hexane) to provide pure benzylamine **11** (26 mg, 58%). UV (MeOH) λ_{max} 209 nm (ϵ 3748), 251 (131), 257 (151), 263 (129). $[\alpha]_D^{24}=+42.0°$ (*c* 1.7, MeOH). ^{1}H NMR 300 MHz ($CDCl_3$) δ_H 7.19–7.30 (m, 5H), 3.69 (q, *J*=6.6 Hz, 1H), 2.24 (m, 1H), 2.22 (m, 1H), 1.56 (m, 1H), 1.35 (d, *J*=6.9 Hz, 3H), 1.00–1.35 (m, 5H), 0.85 (d, *J*=6.6 Hz, 3H). ^{13}C NMR 75 MHz ($CDCl_3$) δ_C 146.3 (s), 129.5 (d), 128.1 (d), 127.8 (d), 59.7 (d), 55.1 (t), 35.7 (t), 34.1 (d), 30.2 (t), 24.0 (t), 23.8 (q), 18.6 (q), 14.4 (q). IR (NaCl plate, film) ν 2957, 1456, 1213 cm^{-1}. HRCIMS (NH_3) *m*/*z* 219.1989 $M+H]^+$ Calcd for $C_{15}H_{25}N$ 219.1987.

(+)-(2*R*)-1-Amino-2-methylhexane hydrochloride (12a).[7] $Pd(OH)_2$ (3 mg) was added to a solution of amine **11** (29 mg, 0.13 mmol) in MeOH (2 mL) and stirred vigorously under an atmosphere of H_2 at 25°C for 15 h. Removal of catalyst by filtration, acidification of the filtrate (5 M HCl, 5 drops) and evaporation of the solvent gave the hydrochloride salt of **12a** (13.8 mg, 72%). $[\alpha]_D^{23}=+6.1°$ (*c*0.9, H_2O); lit. **12b**[7] $[\alpha]_D=-2.41°$ (*c* 15.384, H_2O). ^{1}H NMR (300 MHz, D_2O) δ_H 2.71 (dd, *J*=6.3, 12.6 Hz, 1H), 2.61 (dd, *J*=7.8, 12.6 Hz, 1H), 1.56 (m, 1H), 0.73 (d, *J*=6.6 Hz, 3H), 0.63 (t, *J*=6.6 Hz, 3H). ^{1}H NMR 300 MHz (CD_3OD) δ_H 2.82 (dd, *J*=6.3, 12.6 Hz, 1H), 2.66 (dd, *J*=7.8, 12.6 Hz, 1H), 1.73 (m, 1H), 0.95 (d, *J*=6.6 Hz, 3H), 0.87 (t, *J*=6.6 Hz, 3H). ^{13}C NMR 75 MHz (CD_3OD) δ_C 46.5, 34.7, 32.8, 29.8, 23.7, 17.3, 14.2.

(±)-1-Amino-2-methylhexane hydrochloride (12a,b). BH_3·SMe_2 (40 μL of a 10 M solution in THF) was added to a solution of amide (±)-**13** in THF (2 mL). The reaction was heated at reflux for 5 h. Aqueous sodium hydroxide (1.5 mL, 4 M) was added to the reaction, which was allowed to stir for a further 30 min. The reaction was diluted with EtOAc (10 mL) and the layers separated. The aqueous layer was washed with EtOAc. The combined organics were acidified with hydrochloric acid (1 M, 10 mL) and extracted with H_2O. Evaporation of the aqueous layer gave a residue that was dried overnight under vacuum to give the hydrochloride salt of (±)-**12a**,**b** (36.2 mg, 80%). ^{1}H NMR 300 MHz ($CDCl_3$) δ_H 2.75 (dd, *J*=6.6, 12.3 Hz, 1H), 2.60 (dd, *J*=7.5, 12.3 Hz, 1H), 1.61 (m, 1H), 1.08 (m, 6H), 0.77 (d, *J*=6.6 Hz, 3H), 0.67 (t, *J*=6.6 Hz, 3H). ^{13}C NMR 75 MHz ($CDCl_3$) δ_C 46.4, 34.7, 32.8, 29.8, 23.7, 17.3, 14.3. IR (NaCl plate, film) ν 2956 (br), 1610, 1508 cm^{-1}. HREIMS *m*/*z* 116.1436 $[M+H]^+$ Calcd $C_7H_{18}N$ requires 116.1439.

(±)-2-Methylhexanamide (13). Concentrated aqueous ammonium hydroxide (1 mL, 8.2 mmol) was added to acid chloride **8** (110 mg, 0.74 mmol) and the mixture stirred vigorously for 30 min. The mixture was partitioned against EtOAc (2×5 mL) and the organic layers washed with brine (5 mL), dried and concentrated to give primary amide (±)-**13** (86.3 mg, 88%). ^{1}H NMR 300 MHz ($CDCl_3$) δ_H 5.46 (br m, 2H, NH_2), 2.25 (m, 1H), 1.64 (m, 1H), 1.40 (m, 1H), 1.30 (m, 4H), 1.15 (d, *J*=6.9 Hz, 3H), 0.89 (t, *J*=6.6 Hz, 3H). ^{13}C NMR 75 MHz ($CDCl_3$) δ_C 182.6 (s), 41.5 (d), 35.0 (t), 30.8 (t), 23.7 (t), 18.4 (q), 14.3 (q). IR (NaCl plate, film) ν 3355 (br) 3195 (br), 2933, 1562, 1415 cm^{-1}. HRCIMS (NH_3) *m*/*z* 130.1229 $[M+H]^+$ Calcd. $C_7H_{16}NO$ 130.1231.

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References

1. Kobayashi, J.; Murayama, T.; Ohizumi, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *Tetrahedron Lett.* **1989**, *30*, 4833–4836.
2. Kobayashi, J.; Murayama, T.; Kosuge, S.; Kanda, F.; Ishibashi, M.; Kobayashi, H.; Ohizumi, Y.; Ohta, T.; Nozoe, S.; Sasaki, T. *J. Chem. Soc., Perkin Trans. 1* **1990**, 3301–3303.
3. Quiñoà, E.; Crews, P. *Tetrahedron Lett.* **1987**, *28*, 2467–2468.
4. Sakemi, S.; Totton, L. E.; Sun, H. H. *J. Nat. Prod.* **1990**, *53*, 990–995.
5. Stierle, D. B.; Faulkner, D. J. *J. Nat. Prod.* **1991**, *54*, 1134–1136.
6. Carroll, A. R.; Scheuer, P. J. *Tetrahedron* **1990**, *46*, 6637–6644.
7. Levene, P. A.; Mikeska, L. A. *J. Biol. Chem.* **1924**, *84*, 571–599.
8. Rosenblatt, D. H.; Burrows, E. P. Oxidation of Amines. In: *The Chemistry of Functional Groups. Supplement F, part 2*; Patai, S., Ed.; Wiley: New York, 1982, pp 1137–1140.
9. Natural (+)-**4** may be optically pure. Considering only optical rotations and assuming natural (+)-**4** is optically pure, synthetic **4** would represent 35 ee% which is comparable to our experimentally determined value of 47 ee% assuming the usual errors attendant upon measurements of small rotations.
10. Rettinger, K.; Burschka, C.; Sheeben, P.; Fuchs, H.; Mosandl, A. *Tetrahedon: Asymmetry* **1991**, *2*, 965–968.