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Structures, semisyntheses, and absolute configurations of the antiplasmodial α-substituted β-lactam monamphilectines B and C from the sponge *Svenzea flava*

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Abstract

Bioassay-guided fractionation of the Caribbean sponge *Svenzea flava* collected near Mona Island, off the west coast of Puerto Rico, led to the isolation of two isocyanide amphilectane-type diterpenes named monamphilectines B and C (**2** and **3**). Attached to the backbone of each of these compounds is the first α-substituted monocyclic β-lactam ring to be isolated from a marine organism. The molecular structures of **2** and **3** were established by spectroscopic methods and then confirmed unequivocally by chemical correlation and comparison of physical and chemical data with the natural products. The new β-lactams were successfully synthesized in one step, starting from the known diisocyanide **4**, via parallel Ugi four-center three-component reactions (U-4C-3CR) that also established their absolute stereostructures. Interestingly, compounds **2** and **3** exhibited activities in the low nanomolar range against the human malaria parasite *Plasmodium falciparum*.

Keywords

β-lactams; *Svenzea flava*; Monamphilectines; Malaria; Ugi multicomponent reaction; Drug discovery, Isocyanide

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Supplementary data

Underwater photograph of *S. flava* from Puerto Rico, copies of the ^{1}H NMR, ^{13}C NMR, 2D NMR, and ESI-MS spectra used for the structure elucidation of the natural products (**2** and **3**) and their derivatives (**8–11**), and experimental data for the assignment of absolute configuration at C-24 in alcohols **9** and **10** (Table 4). Supplementary data related to this article can be found at http://dx.dot.org/.....

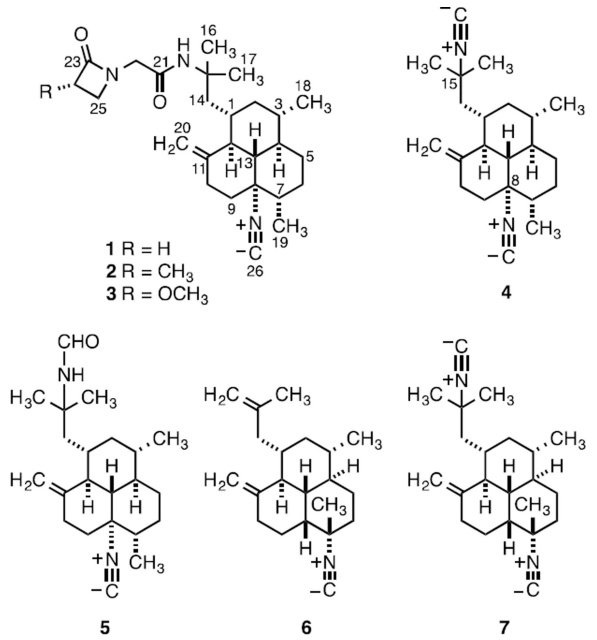
1. Introduction

Only a handful of natural products bearing the β-lactam functionality have been isolated from marine organisms.[1] The most recent is the antiplasmodial monamphilectine A (**1**), which we isolated in 2010 as a minor component from the marine sponge *Svenzea flava* (previously classified as *Hymeniacidon* sp.).[2,3] This metabolite represented the first monocyclic conjugate β-lactam isolated from a marine source. The term "conjugate" implies that the β-lactam nucleus is *N*-linked to a terpenoid.[4] These types of compounds are rarely observed in nature, and only a few examples, including those from nonmarine sources, have been reported in the literature.[5] Monocyclic β-lactams have been associated with various biological activities, such as plant-specific toxins[6] and cytotoxins[7] as well as anti-bacterial[8] and antiplasmodial[2] effects. As part of our efforts to identify novel structures and bioactive metabolites from Caribbean marine sponges, we further screened the organic extracts of *S. flava*, which led to the identification of two α-substituted β-lactam alkaloids designated as monamphilectines B and C (**2** and **3**). Their structures were elucidated on the basis of extensive spectroscopic data analysis and chemical transformations. Subsequent stereo assignment around the *N*-α-disubstituted azetidin-2-one moiety was accomplished from a combination of simple one-pot syntheses of β-lactam-ring products using a Ugi four-center three-component reaction (U-4C-3CR)[9] and Kishi's method.[10] Herein, we report the isolation, structure elucidation and antiplasmodial activity of two novel β-lactam alkaloids, adding further evidence that this Caribbean sponge is an abundant source of chemically diverse and biologically active natural products.[11]

2. Results and discussion

2.1. Extraction and isolation of natural products

The sponge *S. flava* was collected in July 2006 at a depth of approximately 27 m by scuba from Mona Island, Puerto Rico (18° 5′ 12″ N, 67° 53′ 22″ W). A freeze-dried sample was repeatedly extracted with $CHCl_3$–MeOH (1:1). The extracts were combined, concentrated in vacuo, and partitioned between H_2O and *n*-hexane. The resulting *n*-hexane extract was quickly concentrated to produce a residue, which upon biological screening against the human malaria parasite *Plasmodium falciparum* W2, exhibited significant antiplasmodial activity (IC_{50} < 0.08 μM). Thus, the latter residue was subjected to normal-phase silica gel column chromatography using a mixture of *n*-hexane and EtOAc in a stepwise elution, leading to the isolation of two new metabolites, monamphilectines B (**2**) and C (**3**), and the following known compounds: 8,15-diisocyano-11(20)-amphilectene [(–)-(DINCA] (**4**),[12] 8-isocyano-11(20)-ene-15-amphilectaformamide (**5**),[12] 7-isocyano-11(20)-15(16)-amphilectadiene (**6**),[13] 7,15-diisocyano-11(20)-amphilectene (**7**),[13] and monamphilectine A (**1**).[2] All of the known isolates were characterized unambiguously by spectroscopic analysis including ESI-MS, UV, IR, $[\alpha]_D$, and NMR analysis, and by comparisons to characterization data provided for the natural products.



2.2. Structure elucidation, semisynthesis, and absolute configuration of monamphilectines B (2) and C (3)

A minor constituent from the organic extract, monamphilectine B (**2**), was determined to have the molecular formula $C_{27}H_{41}O_2N_3$ on the basis of high-resolution ESI-MS analysis of the pseudomolecular $[M + Na]^+$ ion peak at *m/z* 462.3076. Thus, nine degrees of unsaturation were calculated for this molecule. The IR spectrum revealed the presence of amide (3315 cm^{-1}), alkene (3080 cm^{-1}), isocyanide (2125 cm^{-1}), and carbonyl (1741 and 1670 cm^{-1}) groups, which accounted for all of the multiple bonds within **2**; the molecule was therefore tetracyclic. This observation was supported by ^{13}C and DEPT NMR data that showed signals characteristic of carbonyl amides at δ_C 166.3 (C-21) and 171.9 (C-23), two alkene carbons at δ_C 150.4 (C-11) and 105.9 (C-20) and one isocyanide function at δ_C 156.2 (C-26) and 67.0 (C-8). On the basis of the DEPTQ NMR data, we also established that **2** contained five CH_3, nine CH_2 and seven CH groups and six quaternary C atoms (Table 1).

Inspection of the ^1H NMR spectroscopic data for **2** also revealed five methyl groups. Three of these methyl groups were doublets displaced at δ_H 0.89 (d, 3H, J = 6.1 Hz, H-18), 0.98 (d, 3H, J = 6.3 Hz, H-19), and 1.34 (d, 3H, J = 7.4 Hz, H-27) and two were singlets at δ_H 1.39 (br s, 3H, H-16) and 1.37 (br s, 3H, H-17). Compound **2** also contained a 1,1-disubstituted olefin at δ_H 4.85 (br s, 1H, H-20α) and 4.60 (br s, 1H, H-20β) (Table 1). Compound **2** was thus easily recognized as a member of the amphilectane skeletal class of diterpenes.[12] Because portions of the ^1H and ^{13}C NMR spectra of monamphilectine B (**2**) were very similar to those previously reported for monamphilectine A (**1**), we assumed that their structures were closely related. Additional relevant signals in the ^1H NMR spectrum included a sharp two-proton singlet at δ_H 3.75 (s, 2H, H-22), two mutually coupled proton resonances at δ_H 3.55 (t, 1H, J = 5.4 Hz, H-25α) and 3.03 (dd, 1H, J = 5.4, 2.4 Hz, H-25β), a multiplet methine at δ_H 3.30 (br m, 1H, H-24) and a broad singlet (D_2O exchangeable) at δ_H 5.81 (br s, 1H, N–H). After the association of all of the ^1H and ^{13}C NMR resonances

resulting from C–H one-bond interactions observed in the HSQC NMR spectra, $^{1}H–^{1}H$ COSY and HMBC NMR experiments were performed to establish the main connectivities that allowed the assembly of the molecular planar framework (summarized in Fig. 1).

As with the previously described monamphilectine A (**1**), the strong absorption peak at 1741 cm^{-1} in the IR spectrum strongly suggested the presence of a β-lactam ring in **2**. A side-by-side comparison of the ^{13}C NMR spectra of **1** and **2** revealed some substantial variations between the two natural products only in the vicinity of the β-lactam ring. For instance, the signal for the C-24 methylene at δ_C 37.5 in **1** was replaced by a methine signal at δ_C 45.2 along with an extra methyl signal at δ_C 13.6 (C-27) in **2**. Furthermore, the signal at δ_C 40.7, ascribable in **1** to the β-lactam C-25 methylene, appeared to be displaced at 48.8 ppm in **2**. These differences were attributed to the presence of a 3-methylazetidin-2-one functionality in monamphilectine B (**2**). Further correlations of the HMBC and $^{1}H–^{1}H$ COSY data for **2** established a connection between the entire β-lactam ring appendage and the amphilectane-based diterpene framework through C-15, analogous to **1** (Fig. 1).

We made the stereochemical assignments for monamphilectine B (**2**) around the tricyclic amphilectane backbone by comparing **2** with **1** with respect to their $^{1}H–^{1}H$ scalar couplings, NOE correlations, and their almost identical ^{13}C NMR chemical shift values.[2] The stereochemical assignment about the β-lactam unit of **2**, on the other hand, proved to be a greater challenge because the absence of stereocenters near C-24 prevented the detection of meaningful correlations in the NOESY spectrum. To provide conclusive proof for the stereochemical assignment at C-24 in **2**, a semisynthetic approach starting from (–)-DINCA (**4**), which is a likely biogenetic precursor of known absolute configuration,[12,13] was used because this method could also secure the entire absolute structure for monamphilectine B (**2**). We have previously shown that during a U-4C-3CR, the isocyanide functionality at C-15 in **4** reacts regioselectively with β-alanine and formaldehyde to afford β-lactam **1** in 61% yield after separation.[2] On this basis, we smoothly reacted a solution of DL-3-aminoisobutyric acid and formaldehyde with **4** by stirring the mixture in anhydrous EtOH at 20 °C for 16 h to afford monamphilectine B (**2**) in 68% yield as a 1:1 mixture of epimers at C-24 (Scheme 1). After separation by HPLC, epimers **2** and **8** were obtained in pure form as colorless oils. Although the HPLC retention time, optical rotation, IR, 1D and 2D NMR data for synthetic material **2** were in complete agreement with those of naturally occurring monamphilectine B, the absolute configuration at C-24 remained uncertain because neither the NOESY spectrum for **2** nor that for **8** revealed useful information. We next repeated the U-4C-3CR using L-3-aminoisobutyric acid as the alicyclic β-amino acid component. Monamphilectine B (**2**) was obtained as the sole product in 73% isolated yield, thus establishing the *S* configuration of the C-24 stereogenic center (Scheme 1). Therefore, a configuration of 1*S*,3*S*,4*R*,7*S*,8*S*,12*S*,13*S*,24*S* was unambiguously established for **2**.

High-resolution mass spectroscopic data indicated that monamphilectine C (**3**) possessed an additional oxygen atom relative to **2**, displaying an $[M + Na]^+$ *m/z* 478.3075 that was consistent with a molecular formula of $C_{27}H_{41}O_3N_3$. Equivalent IR bands were observed in the spectra of **2** and **3**, suggesting that both natural products have similar functionality. The NMR data for **3** also indicated an overall structural similarity with **2** (Table 1). Further

comparison of the NMR spectroscopic data for **2** and **3** revealed that the only substantial differences between these natural products resided in the vicinity of the β-lactam ring. In particular, the ^{1}HNMR spectrum of **3** in $CDCl_3$ showed the presence of a methoxy signal at δ 3.53 (s, 3H, H-27), whereas the spectrum of **2** ($CDCl_3$) showed a secondary methyl at δ 1.34 (d, 3H, *J* = 7.4 Hz, H-27). Moreover, the signal at δ_H 3.30 (br m, 1H, H-24) in **2** appears shifted to 4.70 (dd, 1H, *J* = 4.7, 1.9 Hz, H-24) in **3**, suggesting that the β-lactam α-methyl had been replaced with a methoxy group in **3**. HMBC and ^{1}H–^{1}H COSY correlations confirmed the connection of the methoxy to the β-lactam ring via C-24 (Fig. 1).

Nearly identical chemical shifts, ^{1}H–^{1}H coupling constants, and diagnostic NOESY correlations between **2** and **3** strongly suggested the retention of relative configuration. To settle the relative and absolute configuration of **3**, including that of the remote stereogenic center at C-24, we formulated plans for a semisynthesis of monamphilectine C (**3**) starting from (–)-DINCA (**4**). Strategic bond disconnections around the methoxy group and the β-lactam moiety led us to envision a U-4C-3CR strategy that combined racemic isoserine, formaldehyde, and diisocyanide **4**.[14] In a single-stage reaction, **4** was allowed to react in a solution that contained DL-isoserine and formaldehyde in anhydrous EtOH at 20 °C for 23 h to afford a 1:1 mixture of Ugi adducts **9** and **10** in 59% isolated yield (Scheme 2). Following separation by HPLC, the absolute configuration at C-24 for each epimer was established by analysis of the ^{13}C NMR chemical shifts of the carbons in **9** and **10** adjacent to the secondary alcoholic center in the presence of chiral lanthanide shift reagents (i.e., Kishi's method).[10] These studies prompted the assignment of a 1*S*,3*S*,4*R*,7*S*,8*S*,12*S*,13*S*,24*S* configuration to **9** and a 1*S*,3*S*,4*R*,7*S*,8*S*,12*S*,13*S*,24*R* configuration to **10**. At this point, alcohols **9** and **10** were separately and without further optimization treated with excess diazomethane in the presence of silica gel.[15] Upon exposure with diazomethane compound **9** furnished α-methoxy β-lactam **3** (72% yield), which was shown to be identical in all respects, including optical rotation, to naturally occurring monamphilectine C. Likewise, compound **11** was derived in 47% yield from **10**. When we repeated the U-4C-3CR with L-isoserine in anhydrous EtOH at 20 °C for 17 h, we obtained exclusively compound **9** in 70% isolated yield (Scheme 2), which validated our results using Kishi's method.[16] On the basis of these combined results, a 1*S*,3*S*,4*R*,7*S*,8*S*,12*S*,13*S*,24*S* configuration was proposed for monamphilectine C (**3**).

In $CDCl_3$ the shift values of H-24 and C-24 in monamphilectines B (**2**) and C (**3**) were identical to those observed in epimers **8** and **11**, respectively (Table 2). Furthermore, the multiplicity and *J* values (when available) ascribable to H-24 in each compound pair were relatively indistinct. These observations suggested that the only stereocenter within the *N*-2-(2-oxoazetidin-1-yl)ethanamide moiety (i.e., C-24) in these molecules would not be amenable to chemical shift- or *J*-based configurational analysis. Nevertheless, in the spectra of **2** and **3**, which both had a 24*S* configuration, the isolated diastereotopic H-22 protons appear as a two-proton singlet belonging to an A_2 spin system at δ 3.75 (s, 2H) in **2** and 3.79 (s, 2H) in **3**. Remarkably, in the spectra of **8** and **11** (each having a 24*R* configuration), the same protons resonate as AB quartets at 3.79 (d, 1H, *J* = 16.4 Hz), 3.71 (d, 1H, *J* = 16.4 Hz) and 3.83 (d, 1H, *J* = 16.3 Hz), 3.73 (d, 1H, *J* = 16.3 Hz), respectively (Fig. 2). These noticeable trends, which appear to also apply to alcohols **9** and **10** (which have 24*S* and 24*R*

configurations, respectively), might prove useful in the configurational analysis of future natural products having similarly substituted monocyclic β-lactam functionalies.[17]

2.3. Biological activity

In an earlier report, we communicated that (–)-DINCA (**4**) exhibited potent in vitro antiplasmodial activity against a chloroquine-resistant (CQ-R) *Plasmodium falciparum* W2 strain with an IC_{50} value of 40 nM.[2] This result prompted the evaluation of the antiplasmodial activities of the new *S. flava* natural products.[18] Interestingly, monamphilectine B (**2**) and monamphilectine C (**3**), which both possess novel α-substituted β-lactam frameworks, exhibited antiplasmodial activity against a non-resistant (wild-type standard) *Plasmodium falciparum* 3D7 strain, with IC_{50} values of 44.5 nM (**2**) and 43.3 nM (**3**). Although the IC_{50} reported for (–)-DINCA (**4**) in Table 3 was determined using a different strain of *P. falciparum* than the data for **2** and **3**, it would seem that compounds **2**–**4** are equipotent implying that the α-substituted β-lactam moiety does not confer an inherent bioactivity advantage. This observation suggests that the activity is due to the tricyclic core of the molecules (which is the same in all three compounds) rather than the side chain.[19] Unsurprisingly, the natural-product-derived alcohols **9** and **10** exhibited high activity against the 3D7 strain, with IC_{50} values of 24.1 nM for **9** and 184.3 nM for **10**. For comparative purposes, analogue **9**, the most active of the α-substituted β-lactams evaluated, was only slightly less active than the antimalarial drug chloroquine (+Ctrl; IC_{50} = 6.6 nM) against the 3D7 strain (Table 3).

3. Conclusion

We have described our discovery of the first α-substituted β-lactams of marine origin, expanding the number of known bioactive monamphilectines to three. Their absolute structures have been demonstrated unambiguously through a combination of spectroscopic methods and partial syntheses. The frameworks of these novel marine alkaloids represent a new scaffold from which novel and potent antimalarial drugs could be developed.

4. Experimental section

4.1. General procedures

Optical rotations were measured with a Rudolph Autopol IV polarimeter in $CHCl_3$ at 589 nm using a 10-cm microcell. IR spectra were recorded with a Nicolet Magna 750 FT-IR spectrometer with 4-cm^{-1} resolution; the samples for IR were prepared as neat films supported on NaCl discs or as powders dispersed in KBr pellets. The UV spectra were recorded from 200 to 800 nm with a Shimadzu UV-2401P spectrometer using a path length of 10 mm. High-resolution mass measurements were performed with a Q-Tof micro mass spectrometer (Waters Corp., Milford, MA) at the Mass Spectrometry Laboratory of the University of Puerto Rico Material Characterization Center. The data are reported with ion mass/charge (*m/z*) ratios as values in atomic mass units. 1D and 2D NMR spectra were recorded with a Bruker DRX-500 or a Bruker AV-500 FT-NMR spectrometer on $CDCl_3$ solutions in 5-mm-diameter tubes at 500 MHz. ^{1}H and ^{13}C NMR chemical shifts are reported in ppm relative to the residual $CHCl_3$ signal (7.26 ppm) and $CDCl_3$ signal (77.0

ppm), respectively. Multiplicities in the ^{1}H NMR spectra are described as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad, and coupling constants are reported in hertz. Commercially available reagents were purchased and used as received unless stated otherwise. Diazomethane was prepared from Diazald® as previously described.[20] All of the reactions requiring anhydrous conditions were conducted in flame-dried glass apparatuses under an argon atmosphere. Chiral shift reagents (*R*)-Eu(tfc)$_3$ and (*S*)-Eu(tfc)$_3$ were purchased and dried at 130 °C for 48 h prior to use. All HPLC purifications were carried out with an Agilent 1260 Infinity equipped with an Agilent 1260 photodiode array detector using HPLC-grade solvents. Organic solvents (*n*-hexane, $CHCl_3$, EtOAc, and MeOH) used for column chromatography (CC) were distilled prior to use. All CC experiments were conducted with silica gel (35–75 mesh) and TLC on glass pre-coated silica-gel plates and were visualized using UV light and I_2 vapor. Saturated solutions of NH_3 in MeOH were prepared by bubbling dry gaseous NH_3 into dry MeOH. The percentage yield of isolated natural products was based on the dry weight of *S. flava*.

4.2. Animal material

The sponge was originally described under the genus *Pseudoaxinella*.[21] Orange-yellow to yellow thick encrustations to masses, with oscular lobes. Surface smooth; fields of pores are scattered in concave areas. Subsurface color is purple brown, internal color cream. Spicules are strongyles, often slightly asymmetrical (could be called styloids, but the less blunt end is not as pointed as in, for example, *S. tubulosa*). Alvarez *et al.* have suggested it to belong to genus *Svenzea* but it lacks the dark granulous cells that other species of the genus have.[21b]

4.3. Collection, extraction, and isolation of natural products

The Caribbean sponge *Svenzea flava* (Phylum Porifera; Class Demospongiae; Order Halichondrida; Family Dictyonellidae)[21] was collected at a depth of 27 m by scuba off Mona Island, Puerto Rico, in July 2006. A voucher specimen (No. IM06-04) is stored at the Chemistry Department of the University of Puerto Rico, Río Piedras Campus. The organism was partially air dried, frozen and lyophilized prior to extraction. The dry sponge (198.2 g) was cut into small pieces and blended using a mixture of $CHCl_3$–MeOH (1:1) (4 × 1 L). After filtration, the bioactive crude extract was concentrated and stored under vacuum to yield an orange gum (28.2 g) that was suspended in H_2O (1 L) and extracted with *n*-hexane (4 × 500 mL).[2] The resulting hexane extract was concentrated *in vacuo* to yield 7.6 g of an oily residue that was chromatographed over silica gel (170 g) using a gradient of increasing polarity with *n*-hexane/EtOAc (98:2–1:1) and separated into 37 fractions on the basis of TLC and ^{1}H NMR analyses. Fraction 4 consisted of a colorless crystalline solid that was identified as 7-isocyano-11(20)-15(16)-amphilectadiene (**6**)[13] (10.2 mg, 0.005%). Likewise, fraction 11 consisted of a colorless crystalline solid that was identified as (–)-8,15-diisocyano-11(20)-amphilectene (**4**) (528 mg, 0.27%) after X-ray crystallographic analysis.[12] Fraction 13 was identified as 7,15-diisocyano- 11(20)-amphilectene (**7**)[13] (188.6 mg, 0.09%), whereas fraction 27 was identified as 8-isocyano-11(20)-ene-15-amphilectaformamide (**5**)[12] (204.3 mg, 0.10%). Fraction 36 (13.1 mg) was re-chromatographed over silica gel (1.0 g) with 20% EtOAc in *n*-hexane to afford monamphilectine A (**1**) (3.0 mg, 0.002%).[2] Fraction 37 (5.0 mg) consisted of a mixture of **2** and **3** that was separated using reversed-phase HPLC on an instrument equipped with a UV

detector set at 220 nm and a C18 column (5 μm, 5 mm × 250 mm); the fraction was separated using isocratic elution (20% H_2O in MeOH) and a flow rate of 1 mL/min to yield monamphilectines B (**2**) (1.0 mg, 0.00066%) and C (**3**) (1.1 mg, 0.00074%).

4.3.1. Monamphilectine B (2)—Colorless oil; $[\alpha]^{20}_D$ −26.0 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3315, 3080, 2964, 2925, 2871, 2264, 2125, 1741, 1670, 1556, 1456, 1419, 1373, 1265, 1236, 921, 893, 752, 667 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 1); HRMS (ESI) $[M + Na]^+$ *m/z* 462.3076 (calcd for $C_{27}H_{41}O_2N_3Na$, 462.3096).

4.3.2. Monamphilectine C (3)—Colorless oil; $[\alpha]^{20}_D$ −57.0 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3298 (broad), 3078, 2955, 2856, 2264, 2125, 1747, 1668, 1537, 1454, 1380, 756 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 1); HRMS (ESI) $[M + Na]^+$ *m/z* 478.3075 (calcd for $C_{27}H_{41}O_3N_3Na$, 478.3046).

4. 4. Semisynthesis of monamphilectine B (2) and 24-*epi*-monamphilectine B (8)

A solution of (–)-DINCA (**4**) (30.0 mg, 0.09 mmol) in anhydrous EtOH (3 mL) was added to a mixture of DL-3-aminoisobutyric acid (9.5 mg, 0.09 mmol) and excess 37% aqueous formaldehyde (2 drops) in EtOH (5 mL) that was previously stirred for 30 min. The resulting solution was stirred at 20 °C for 16 h and concentrated *in vacuo*. The oily residue obtained was suspended in H_2O (5 mL) and extracted with $CHCl_3$ (3 × 5 mL). The combined organic layer was dried using $MgSO_4$, filtered, and concentrated to leave a residue that was purified over 300 mg of silica gel with 2% MeOH in $CHCl_3$ to afford a 1:1 mixture of monamphilectine B (**2**) and 24-*epi*-monamphilectine B (**8**) (27.6 mg, 68% yield). The latter was separated by reversed-phase HPLC (Whelk-O1 5 mm × 250 mm) using an isocratic solvent composition of 20% H_2O in MeOH, a flow rate of 1 mL/min, and a UV detector set at 220 nm. Retention times were 18.5 min for 24-*epi*-monamphilectine B (**8**) and 20.6 min for monamphilectine B (**6**).

4.4.1. 24-epi-Monamphilectine B (8)—Colorless oil; $[\alpha]^{20}_D$ −25.0 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3315, 3080, 2964, 2926, 2872, 2264, 2125, 1742, 1670, 1556, 1456, 1445, 1420, 1387, 1265, 1248, 1178, 893, 752, 667 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 2); HRMS (ESI) $[M + Na]^+$ *m/z* 462.3076 (calcd for $C_{27}H_{41}O_2N_3Na$, 462.3096).

4.5. Semisynthesis of monamphilectine B (2)

A solution of L-3-aminoisobutyric acid (3.1 mg, 0.03 mmol) and 37% aqueous formaldehyde (0.03 mmol) in anhydrous EtOH (1 mL) was stirred at 20 °C for 30 min before the stepwise addition of (–)-DINCA (**4**) (10.0 mg, 0.03 mmol) in EtOH (1 mL). After completion (18 h), the reaction mixture was filtered and concentrated *in vacuo*; the obtained residue was eluted through a short plug of silica gel (300 mg) with 2% MeOH in $CHCl_3$ to produce monamphilectine B (**2**) [9.7 mg, 73% yield, $[\alpha]^{20}_D$−26.0 (*c* 1.0, $CHCl_3$)].

4.6. Semisynthesis of alcohols 9 and 10

A solution of (–)-DINCA (**4**) (50.0 mg, 0.15 mmol) in anhydrous EtOH (3 mL) was added to a previously stirred (30 min) mixture of DL-isoserine (16.2 mg, 0.15 mmol) and excess 37% aqueous formaldehyde (2 drops) in EtOH (5 mL). The resulting solution was stirred at 20 °C for 23 h and then concentrated *in vacuo*. The oily residue obtained was suspended in H_2O (5 mL) and extracted with $CHCl_3$ (3 × 5 mL). The combined organic layer was dried using $MgSO_4$, filtered, and concentrated; the remaining residue was purified over silica gel (300 mg) with 2% MeOH in $CHCl_3$ to afford a 1:1 mixture of **9** and **10** (39.0 mg, 59% yield). Separation of the epimers was achieved by reversed-phase HPLC (Whelk-O1 5 mm × 250 mm; MeOH/H_2O, 82/18). Retention times were 10.36 min for compound **9** and 11.18 min for compound **10**.

4.6.1. Compound 9—Colorless oil; $[\alpha]^{20}_D$ −71.0 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3319 (broad), 3084, 2962, 2923, 2873, 2125, 1747, 1666, 1550, 1456, 1386, 1267, 1238, 1178, 1159, 1120, 1039, 1020, 894, 756, 667 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.05 (N–H), 4.95 (dd, ^1H, *J* = 4.7, 1.8 Hz, H-24), 4.83 (s, 1H, H-20), 4.59 (s, 1H, H-20’), 3.82 (d, 1H, *J* = 16.4 Hz, H-22), 3.78 (d, 1H, *J* = 16.4 Hz, H-22’), 3.68 (t, 1H, *J* = 5.1 Hz, H-25), 3.35 (dd, 1H, *J* = 5.7, 1.8 Hz, H-25’), 2.28 (m, 2H, H-10), 2.27 (m, 1H, H-9), 1.98 (m, 1H, H-2), 1.97 (m, 1H, H-14), 1.96 (m, 1H, H-5), 1.83 (m, 1H, H-1), 1.83 (m, 1H, H-12), 1.53 (m, 1H, H-14’), 1.53 (m, 1H, H-6), 1.41 (m, 1H, H-6’), 1.37 (s, 3H, H-7), 1.39 (m, 1H, H-16), 1.36 (s, 3H, H-17), 1.30 (m, 1H, H-9’), 1.08 (m, 1H, H-4), 1.04 (m, 1H, H-3), 1.01 (m, 1H, H-13), 0.97 (d, 3H, *J* = 6.3 Hz, H-19), 0.90 (d, 3H, *J* = 6.1 Hz, H-18), 0.88 (m, 1H, H-2’), 0.83 (m, 1H, H-5’); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.7 (C, C-23), 165.8 (C, C-21), 156.1 (C, C-26), 150.5 (C, C-11), 105.9 (CH_2, C-20), 76.1 (CH, C-24), 67.0 (C, C-8), 55.6 (CH, C-13), 54.7 (C, C-15), 51.6 (CH_2, C-25), 46.2 (CH, C-12), 46.1 (CH_2, C-22), 44.6 (CH_2, C-14), 42.5 (CH, C-4), 41.1 (CH_2, C-2), 40.9 (CH, C-7), 39.7 (CH_2, C-9), 35.6 (CH, C-3), 33.6 (CH_2, C-10), 33.0 (CH, C-1), 29.9 (CH_2, C-6) 29.8 (CH_2, C-5), 28.7 (CH_3, C-16), 27.2 (CH_3, C-17), 20.0 (CH_3, C-18), 15.7 (CH_3, C-19); HRESI (MS) *m/z* $[M + Na]^+$ 464.2866 (calcd for $C_{26}H_{39}O_3N_3Na$, 464.2889).

4.6.2. Compound 10—Colorless oil; $[\alpha]^{20}_D$ –35.0 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3319 (broad), 3084, 2963, 2923, 2874, 2124, 1747, 1666, 1551, 1450, 1267, 1238, 1121, 1020, 895, 756, 667 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.93 (N–H), 4.98 (dd, 1H, *J* = 4.6, 1.7 Hz, H-24), 4.85 (s, 1H, H-20), 4.60 (s, 1H, H-20’), 3.89 (d, 1H, *J* = 16.4 Hz, H-22), 3.73 (d, 1H, *J* = 16.4 Hz, H-22’), 3.69 (t, 1H, *J* = 5.3 Hz, H-25), 3.40 (dd, 1H, *J* = 5.8, 1.8 Hz, H-25’), 2.27 (m, 2H, H-10), 2.27 (m, 1H, H-9), 1.98 (m, 1H, H-2), 1.97 (m, 1H, H-14), 1.95 (m, 1H, H-5), 1.83 (m, 1H, H-1), 1.83 (m, 1H, H-12), 1.56 (m, 1H, H-14’), 1.52 (m, 1H, H-6), 1.42 (m, 1H, H-6’), 1.38 (m, 1H, H-16), 1.38 (s, 3H, H-17), 1.34 (s, 3H, H-7), 1.28 (m, 1H, H-9’), 1.06 (m, 1H, H-4), 1.04 (m, 1H, H-3), 0.99 (m, 1H, H-13), 0.98 (d, 3H, *J* = 6.2 Hz, H-19), 0.89 (d, 3H, *J* = 6.0 Hz, H-18), 0.86 (m, 1H, H-2’), 0.82 (m, 1H, H-5’); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.2 (C, C-23), 165.9 (C, C-21), 155.9 (C, C-26), 150.4 (C, C-11), 105.9 (CH_2, C-20), 75.9 (CH, C-24), 67.0 (C, C-8), 55.6 (CH, C-13), 54.7 (C, C-15), 51.8 (CH_2, C-25), 46.2 (CH, C-12), 46.1 (CH_2, C-22), 44.3 (CH_2, C-14), 42.4 (CH, C-4), 40.9 (CH_2, C-2), 40.8 (CH, C-7), 39.6 (CH_2, C-9), 35.6 (CH, C-3), 33.6 (CH_2, C-10), 33.0 (CH, C-1), 29.9 (CH_2, C-6) 29.7 (CH_2, C-5), 28.8 (CH_3, C-16), 27.2 (CH_3, C-17), 20.0

(CH_3, C-18), 15.7 (CH_3, C-19); HRESI (MS) *m/z* $[M + Na]^+$ 464.2866 (calcd for $C_{26}H_{39}O_3N_3Na$, 464.2889).

4.7. Determination of absolute configurations of alcohols 9 and 10 at C-24 using Kishi's method

Samples of **9** and **10** were prepared separately using oven-dried vials with ~3.5–4.5 mg of the alcohols in 0.5 mL of $CDCl_3$ containing 15% chiral shift reagent per OH. The samples were transferred via syringe to oven-dried NMR tubes that were cooled to room temperature under a stream of nitrogen. The acquisition of reliable results require that the (*R*)- and (*S*)-enantiomers of the shift reagent be of similar quality in terms of purity and moisture content, and that the NMR data with both the (*R*)- and (*S*)-shift reagent be collected on the same instrument. Following being acquired, each substrate was recovered by filtration through a silica-gel pipette column (Sep-Pak silica gel cartridge). CH_2Cl_2 was used as the solvent for each filtration. To determine the absolute configuration of alcohols **9** and **10** according to the method described by Kishi and co-workers, the NMR behaviors of the carbons adjacent to the alcoholic center (C*X* and C*Y*) were measured in the presence of (*R*)- and (*S*)- $Eu(tfc)_3$ (Table 4 in Supplementary data).[10]

4.8. Semisynthesis of compound 9

A solution of L-isoserine (3.2 mg, 0.03 mmol) was reacted with 37% aqueous formaldehyde (0.03 mmol) in anhydrous EtOH (1 mL) at 20 °C for 30 min. Next, (–)-DINCA (**4**) (10.0 mg, 0.03 mmol) in EtOH (1 mL) was added. After being stirred for 17 h, the reaction mixture was filtered and concentrated, and the obtained residue was eluted through a short plug of silica gel (300 mg) with 2% MeOH in $CHCl_3$ to produce **9** (9.2 mg, 70% yield).

4.9. Semisynthesis of monamphilectine C (7) and 24-*epi*-monamphilectine C (11)

Samples of alcohols **9** and **10** (10 mg, 0.02 mmol) in anhydrous ether (3 mL) were placed separately in round bottom flasks (25 mL). Silica gel (10 mg) suspended in an anhydrous ether solution containing an excess of diazomethane (5 mL) was added to each flask, and the resulting mixtures were stirred at 25 °C for 2 h.[15] The solvent was evaporated, and the residues were purified over silica gel (300 mg) with 2% MeOH in $CHCl_3$ to afford monamphilectine C (**3**) [5.2 mg, 72% yield, $[\alpha]^{20}_D$ −57.0 (*c* 1.0, $CHCl_3$)] and 24-*epi*-monamphilectine C (**11**) (3.4 mg, 47% yield).

4.9.1. 24-epi-Monamphilectine C (11)—Colorless oil; $[\alpha]^{20}_D$ −22.0 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3298 (broad), 3078, 2955, 2926, 2870, 2264, 2125, 1747, 1668, 1549, 1454, 1381, 1123, 891, 756 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Table 2); HRESI (MS) *m/z* $[M + Na]^+$ 478.3075 (calcd for $C_{27}H_{41}O_3N_3Na$, 478.3046).

4.10. Evaluation of inhibition of *Plasmodium falciparum* growth

The 3D7 strain of *P. falciparum* malaria (BEI Resources, MR4/ATCC, Manassas, VA) was cultured in human type O+ erythrocytes in complete medium consisting of RPMI 1640 (Cellgro), 0.043 mg/mL gentamicin (Gibco), 0.014 mg/mL hypoxanthine (Acros), 38.5 mM HEPES (Sigma), 0.18% sodium bicarbonate (Cellgro), 0.20% glucose (MP Biomedical),

0.003 mM NaOH (Sigma), 0.2% Albumax (Gibco), and 5% human serum as previously described.[22] Briefly, cultures were maintained in 25-cm^2 flasks (Corning) at a volume of 10 mL, gassed for 30 s with 3% CO_2, 1% O_2, and 96% N_2, and were finally incubated at 37°C. The antiplasmodial activity was determined with a SYBR Green based parasite proliferation assay as previously described.[23] After 72 h of incubation in the presence of serial dilutions of compounds, the increase of parasite DNA contained in human red blood cells was measured. The relative fluorescence values were measured using a Molecular Devices SpectraMAX Gemini EM fluorimeter (excitation 495 nm, and emission 525 nm). Data were analyzed using Microsoft Excel and were plotted using SigmaPlot 10 (Systat).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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16. Arguably, the use of Kishi's method in the case of alcohols **9** and **10** is somewhat equivocal as there are other donor atoms in these molecules that may act as potential ligands for Eu (i.e. amide, isocyanide) compared to the uniform present of only OH groups in Kishi's reference compounds (Ref 10).
17. Interestingly, the diastereotopic H-22 protons in alcohols **9** and **10** ($CDCl_3$, 500 MHz) appear as two doublets belonging to AB quartet spin systems (J = 16.4 Hz). In **9**, however, the pair of doublets "lean" significantly closer toward each other ($\Delta\nu/J$ = 1.2) than in **10** ($\Delta\nu/J$ = 4.9). As expected, the leaning becomes more pronounced as the chemical shift difference between the coupled multiplets decreases (second-order effects). Because $\Delta\nu/J$ increases as the magnetic field increases, we surmise that, at lower fields (worse dispersion), the spectrum signals for H-22 in **9** (24*S* configuration) should resemble a two-proton singlet (A_2 spin system), whereas, in the case of **10** (24*R* configuration), the H-22 protons would remain as two sets of doublets (AB quartet spin system).
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19. Biosynthetically, the monocyclic β-lactams are the least well characterized of the β-lactams and thus, the mechanism by which the heterocyclic ring is built is yet to be established (Ref 4b). Although the monamphilectines are rare examples of β-lactam metabolites obtained from a higher eukaryote, should *S. flava* be found to contain symbiotic bacteria, the possibility remains that β-lactams **1–3** might be, at least in part, of prokaryotic origin.
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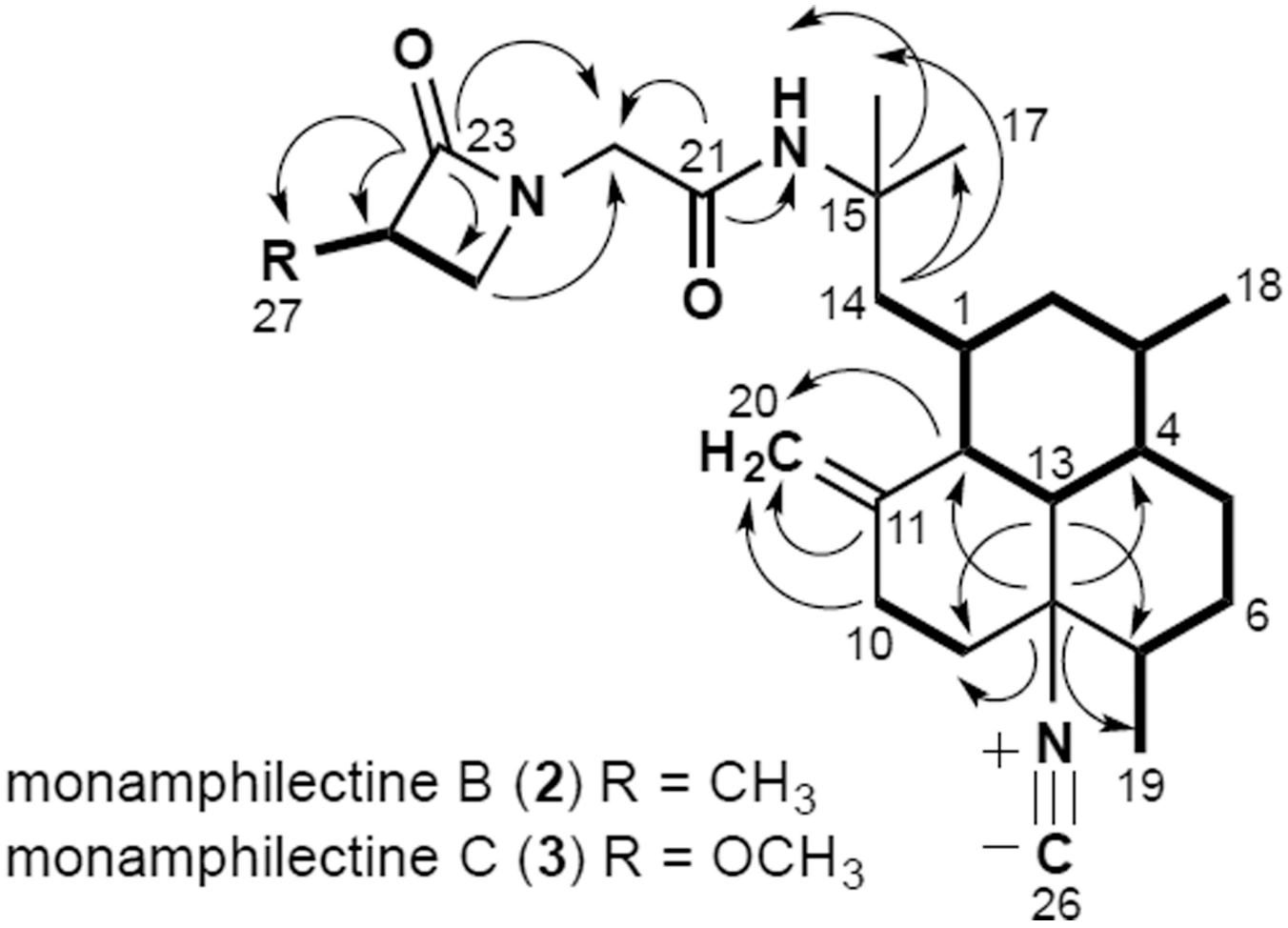


Figure 1.
Key HMBC (C→H) and COSY (—) correlations of **2** and **3**.

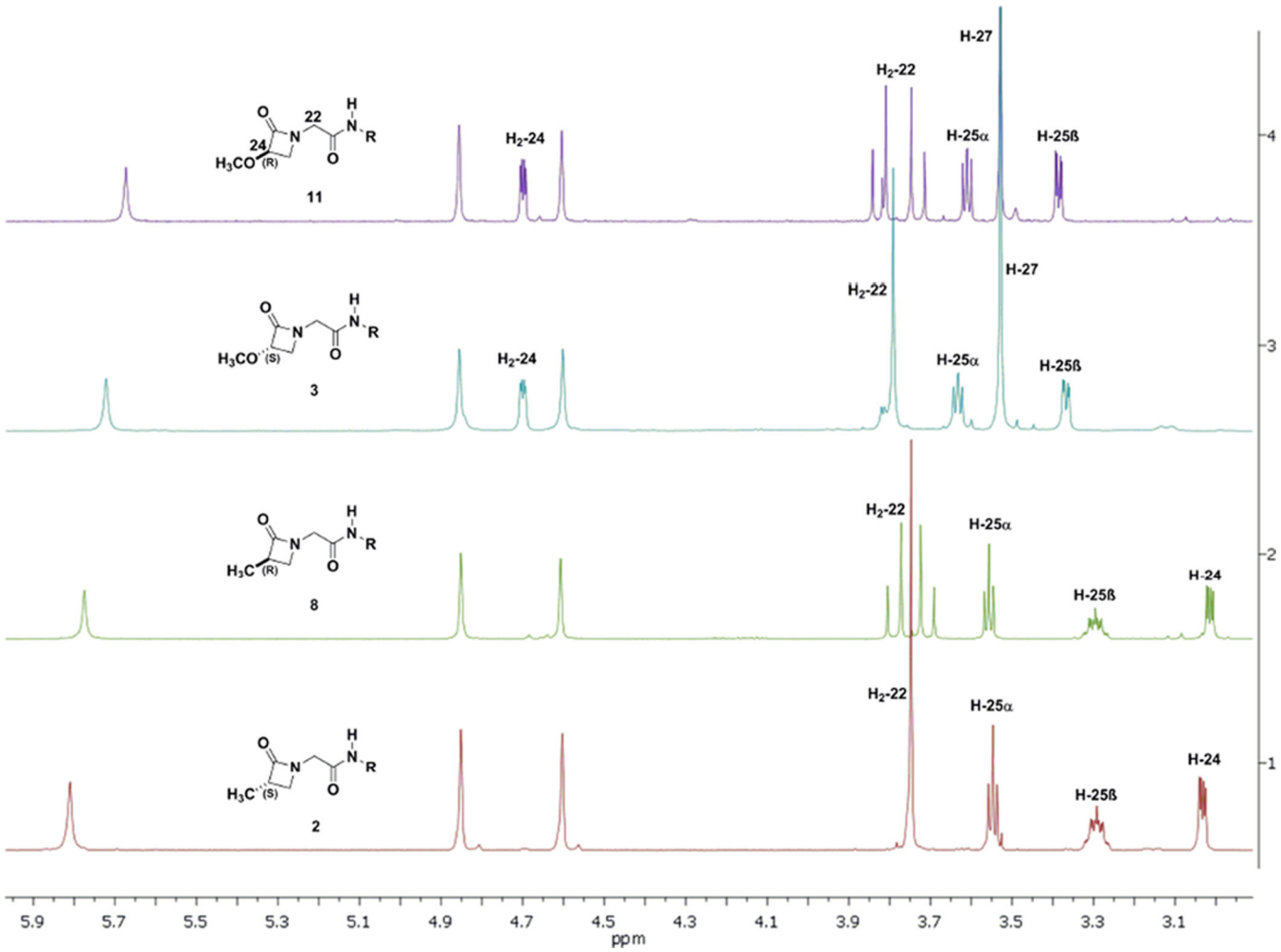
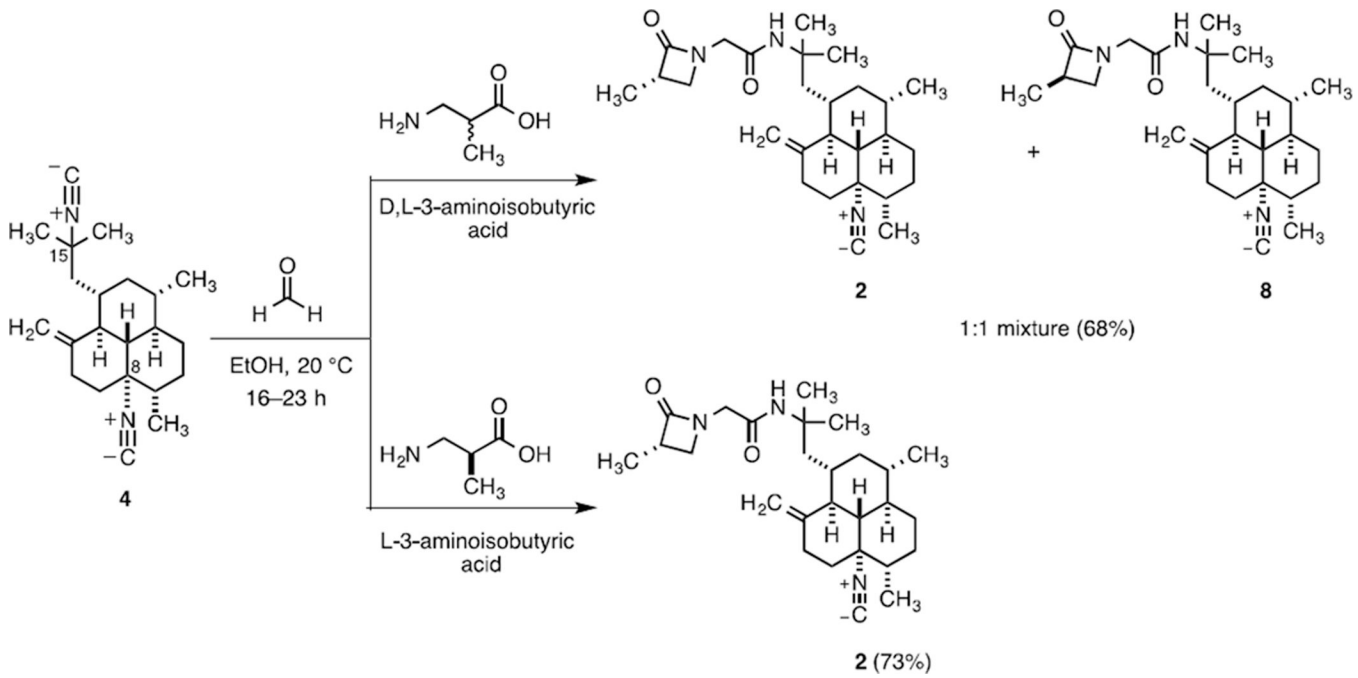


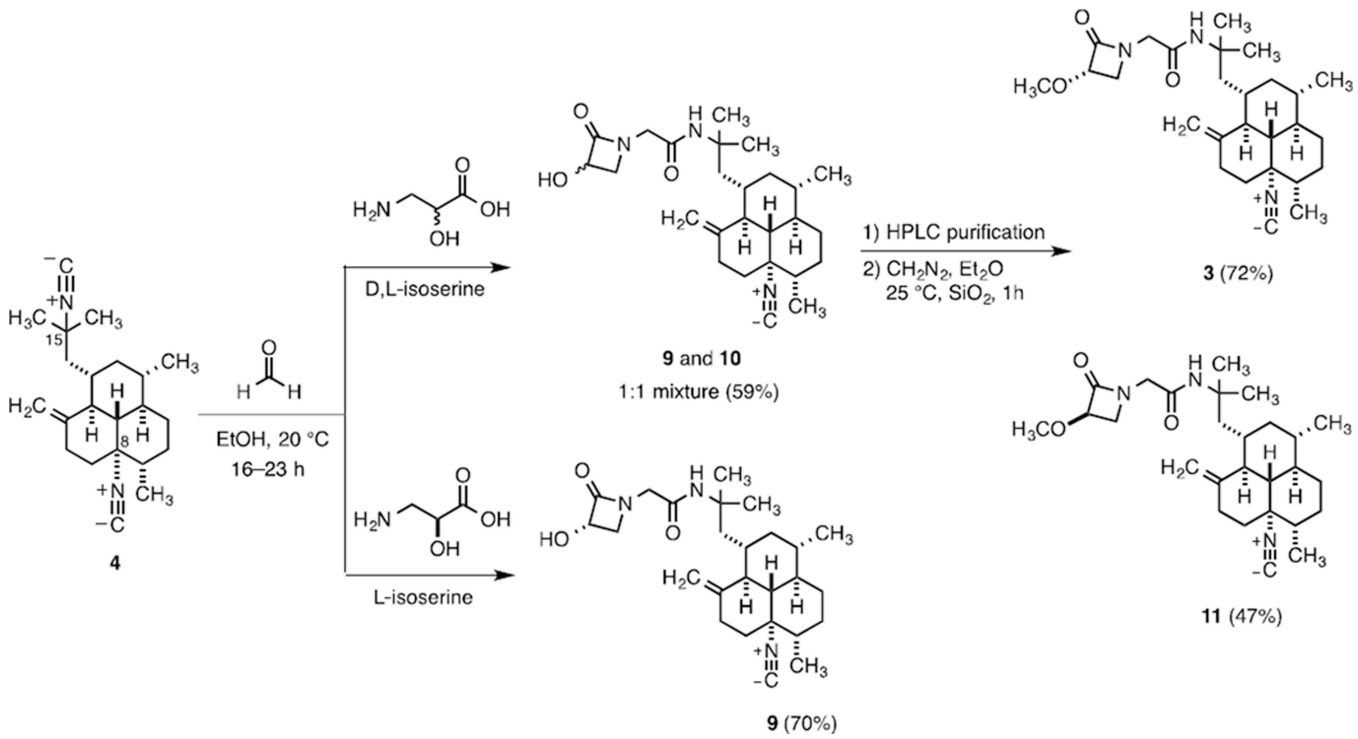
Figure 2.

Side-by-side comparison of the mid-field region of the ^{1}H NMR spectra (500 MHz, $CDCl_3$) of naturally occurring monamphilectines B and C (**2** and **3**) with those of their respective unnatural C-24 epimers **8** and **11**. In the spectra of **2** and **3**, the H-22 protons appear as sharp two-proton singlets (A_2 spin systems). In the spectra of **8** and **11**, the same protons appear as two sets of doublets belonging to AB quartet spin systems.



Scheme 1.

One-pot U-4C-3CR's leading to the semisynthesis of natural product monamphilectine B (**2**) and unnatural β-lactam derivative **8** from (–)-DINCA (**4**).



Scheme 2.

One-pot U-4C-3CR's leading to the semisynthesis of natural product monamphilectine C (**3**) as well as unnatural β-lactam derivatives **9–11** from (–)-DINCA (**4**).

Table 1

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data for the naturally occurring monamphilectines B and C (**2** and **3**)[a]

Monamphilectine B (2)			Monamphilectine C (3)	
position	**δ_H, mult, intgt (*J* in Hz)**	**δ_C, type[b]**	**δ_H, mult, intgt (*J* in Hz)**	**δ_C, type[b]**
1	1.83, br m, 1H	33.0, CH	1.84, br m, 1H	32.9, CH
2α	1.99, br m, 1H	41.0, CH_2	1.98, br m, 1H	41.0, CH_2
2β	0.84, br m, 1H		0.85, br m, 1H	
3	1.04, br m, 1H	35.6, CH	1.03, br m, 1H	35.6, CH
4	1.08, br m, 1H	42.4, CH	1.07, br m, 1H	42.4, CH
5α	1.95, br m, 1H	29.8, CH_2	1.96, br m, 1H	29.7, CH_2
5β	0.81, br m, 1H		0.81, br m, 1H	
6α	1.51, br m, 1H	29.9, CH_2	1.51, br m, 1H	29.8, CH_2
6β	1.42, br m, 1H		1.25, br m, 1H	
7	1.35, br m, 1H	40.9, CH	1.32, br m, 1H	40.8, CH
8		67.0, C		66.9, C
9α	2.28, br m, 1H	39.7, CH_2	2.28, br m, 1H	39.6, CH_2
9β	1.29, br m, 1H		1.32, br m, 1H	
10αβ	2.27, br m, 1H	33.6, CH_2	2.28, br m, 2H	33.6, CH_2
11		150.4, C		150.4, C
12	1.84, br m, 1H	46.2, CH	1.84, br m, 1H	46.2, CH
13	1.01, br m, 1H	55.6, CH	1.00, br m, 1H	55.6, CH
14α	1.98, br m, 1H	44.4, CH_2	1.99, br m, 1H	44.5, CH_2
14β	1.56, br m, 1H		1.53, br m, 1H	
15		54.4, C		54.6, C
16	1.39, br s, 3H	28.8, CH_3	1.38, br s, 3H	28.7, CH_3
17	1.37, br s, 3H	27.3, CH_3	1.38, br s, 3H	27.2, CH_3
18	0.89, d, 3H (6.1)	20.0, CH_3	0.90, d, 3H (5.7)	20.0, CH_3
19	0.98, d, 3H (6.3)	15.7, CH_3	0.98, d, 3H (6.1)	15.7, CH_3
20α	4.85, br s, 1H	105.9, CH_2	4.85, br s, 1H	105.9, CH_2
20β	4.60, br s, 1H		4.60, br s, 1H	
21		166.3, C		165.7, C
22	3.75, s, 2H	47.2, CH_2	3.79, s, 2H	46.5, CH_2
23		171.9, C		167.8, C
24	3.30, br m, 1H	45.2, CH	4.70, dd, 1H (4.7, 1.9)	83.4, CH
25α	3.55, t, 1H (5.4)	48.8, CH_2	3.62, dd, 1H (5.8, 4.8)	49.1, CH_2
25β	3.03, dd, 1H (5.4, 2.4)		3.36, dd, 1H (5.9, 1.9)	
26		156.2, C		156.2, C
27	1.34, d, 3H, (7.4)	13.6, CH_3	3.53, s, 3H	57.7, CH_3
N–H	5.81, br s, 1H, exchangeable		5.73, br s, exchangeable	

[a]Spectra were recorded in $CDCl_3$ at 25 °C. Chemical shift values are in ppm relative to the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.0 ppm) signals. Assignments were aided by 2D NMR experiments, spin-splitting patterns, the number of attached protons, and chemical shift values.

[b]^{13}C NMR types were obtained from DEPTQ NMR experiments.

Table 2

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data for semisynthetic analogues 24-*epi*-monamphilectines B and C (**8** and **11**)[a]

24-*epi*-Monamphilectine B (8)			24-*epi*-Monamphilectine C (11)	
position	**δ_H, mult, intgt (*J* in Hz)**	**δ_C, type[b]**	**δ_H, mult, intgt (*J* in Hz)**	**δ_C, type[b]**
1	1.83, br m, 1H	33.0, CH	1.84, br m, 1H	33.0, CH
2α	1.98, br m, 1H	41.1, CH_2	1.98, br m, 1H	41.0, CH_2
2β	0.85, br m, 1H		0.87, br m, 1H	
3	1.03, br m, 1H	35.6, CH	1.04, br m, 1H	35.6, CH
4	1.08, br m, 1H	42.4, CH	1.09, br m, 1H	42.4, CH
5α	1.95, br m, 1H	29.7, CH_2	1.98, br m, 1H	29.8, CH_2
5β	0.79, br m, 1H		0.82, br m, 1H	
6α	1.51, br m, 1H	29.8, CH_2	1.52, br m, 1H	29.9, CH_2
6β	1.43, br m, 1H		1.26, br m, 1H	
7	1.35, br m, 1H	40.9, CH	1.35, br m, 1H	40.9, CH
8		66.9, C		67.0, C
9α	2.26, br m, 1H	39.7, CH_2	2.28, br m, 1H	39.7, CH_2
9β	1.28, br m, 1H		1.30, br m, 1H	
10αβ	2.27, br m, 2H	33.6, CH_2	2.29, br m, 2H	33.6, CH_2
11		150.4, C		150.5, C
12	1.83, br m, 1H	46.2, CH	1.85, br m, 1H	46.2, CH
13	0.99, br m, 1H	55.6, CH	1.00, br m, 1H	55.6, CH
14α	2.00, br m, 1H	44.6, CH_2	2.00, br m, 1H	44.5, CH_2
14β	1.50, br m, 1H		1.56, br m, 1H	
15		54.4, C		54.6, C
16	1.39, br s, 3H	28.7, CH_3	1.38, br s, 3H	28.7, CH_3
17	1.39, br s, 3H	27.2, CH_3	1.38, br s, 3H	27.3, CH_3
18	0.90, d, 3H (6.2)	20.0, CH_3	0.90, d, 3H (5.7)	20.0, CH_3
19	0.98, d, 3H (6.3)	15.7, CH_3	0.98, d, 3H (6.1)	15.7, CH_3
20α	4.85, br s, 1H	106.0, CH_2	4.86, br s, 1H	105.9, CH_2
20β	4.61, br s, 1H		4.60, br s, 1H	
21		166.4, C		165.7, C
22α	3.79, d, 1H (16.4)	47.1, CH_2	3.83, d, 1H (16.3)	46.7, CH_2
22β	3.71, d, 1H (16.4)		3.73, d, 1H (16.3)	
23		171.8, C		167.8, C
24	3.30, ddt, 1H (7.4, 5.2, 2.2)	45.2, CH	4.70, dd, 1H (4.7, 2.0)	83.4, CH
25α	3.56, t, 1H (5.3)	48.8, CH_2	3.61, dd, 1H (5.9, 4.7)	49.0, CH_2
25β	3.02, dd, 1H (5.5, 2.4)		3.39, dd, 1H (5.9, 2.0)	
26		156.1, C		156.3, C
27	1.35, d, 3H, (7.4)	13.7, CH_3	3.39, dd, 1H (5.9, 2.0)	57.7, CH_3

	24-*epi*-Monamphilectine B (8)		24-*epi*-Monamphilectine C (11)	
position	**δ_H, mult, intgt (*J* in Hz)**	**δ_C, type*b***	**δ_H, mult, intgt (*J* in Hz)**	**δ_C, type*b***
N–H	5.78, br s, 1H, exchangeable		5.67, br s, exchangeable	

*a*Spectra were recorded in $CDCl_3$ at 25 °C. Chemical shift values are in ppm relative to the residual CHCl3 (7.26 ppm) or $CDCl_3$ (77.0 ppm) signals. Assignments were aided by 2D NMR experiments, spin-splitting patterns, the number of attachedv protons, and chemical shift values.

b^{13}C NMR types were obtained from DEPTQ NMR experiments.

Table 3

In vitro antiplasmodial activities of compounds **2–4** and **8–11**[a]

Compound	IC_{50} 3D7 (nM)	Std. Error
2	44.5	0.0059
3	43.3	0.0005
4	40[b]	–
8	NT	–
9	24.1	0.0016
10	184.3	0.0358
11	NT	–
CQ	6.6	0.0008

[a] The IC_{50} values are reported as means ± standard errors.

[b] This value (taken from ref 2) refers to a CQ-resistant *P. falciparum* W2 strain. NT indicates that the compound was not tested due to insufficient material. CQ = chloroquine (+Ctrl).