New Annonaceous Acetogenins from the Roots of *Uvaria calamistrata*

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Five new annonaceous acetogenins, calamistrins C−G (**1**−**5**), were isolated from an ethanolic extract of the roots of *Uvaria calamistrata*. Compounds **1**−**3** were mono-THF ring acetogenins; compounds **4** and **5** were bis-THF acetogenins, with the THF rings from C-18 to C-25. The absolute configurations of **3**, **4**, and **5** as well as the partial absolute configurations of **1** and **2** were determined by ^{13}C NMR spectroscopy and advanced Mosher methodology.

An ethanolic extract of the roots of *Uvaria calamistrata* Hance (Annonaceae) was cytotoxic in vitro against human tumor cell lines in our preliminary screening tests. We previously isolated monotetrahydrofuran (THF) ring type annonaceous acetogenins, calamistrins A and B, and two known acetogenins, grandiflorin and gradifloracin, from this plant.[1] In continuing efforts aimed at the discovery of new bioactive constituents from *U. calamistrata*, 15 additional annonaceous acetogenins have been identified, including the new compounds **1**−**5** (Chart 1).

Results and Discussion

Compound **1** was obtained as colorless crystals (MeOH). The test of **1** with Kedde reagent[2] indicated the presence of an unsaturated γ-lactone ring. The EIMS fragmentation pattern of **1** and its TMSi derivative and the ^{1}H and ^{13}C NMR data of **1** indicated that it was a mono-THF ring type γ-methyl α,β-unsaturated γ-lactone acetogenin. FABMS of **1** (glycerol) gave $[MH]^+$ ions at *m*/*z* 609, which together with elemental analysis indicated the molecular formula $C_{37}H_{66}O_6$. The FAB mass spectrum of **1** also showed the $[MH - H_2O]^+$, $[MH - 2H_2O]^+$, and $[MH - 3H_2O]^+$ peaks at *m*/*z* 591, 573, and 555, respectively, indicating the presence of three OH groups. The γ-methyl α,β-unsaturated γ-lactone moiety in **1** was indicated by four ^{1}H signals at δ 6.98, 4.99, 2.26, and 1.42 as well as six ^{13}C resonances at δ 173.8 (C-1), 148.7 (C-35), 134.3 (C-2), 77.3 (C-36), 25.2 (C-3), and 19.2 (C-37).[2] The presence of a mono-THF ring with flanking OH groups in **1** was evident from the ^{1}H NMR signals at δ 3.41, 3.80 and the ^{13}C NMR resonances at δ 74.1 (2C, C-19, 24) and 82.6 (C-20), 82.7 (C-23). These NMR data also suggested the relative stereochemistry of this mono-THF ring moiety (C-19 to C-24) to be *threo-trans-threo* by comparison with the ^{1}H and ^{13}C NMR data of model compounds.[2,3] The NMR spectra of **1** showed one additional hydroxylated methine proton signal at δ 3.60 (1H, m) and a ^{13}C methine resonance at δ 71.8, which was assigned to C-13.

The EIMS of **1** showed intense ion peaks at *m*/*z* 249, 267, 331, 349, 408, and 419, whereas that of its TMSi derivative revealed peaks at *m*/*z* 339, 421, 491, and 511, which indicated flanking OH groups at C-17, C-24, the mono-THF ring between C-20 and C-23, and the third OH group at C-13.

The absolute stereochemistry of the carbinol chiral centers on both sides of the mono-THF ring in **1** was determined by advanced Mosher methodology.[4] On the basis of the ^{1}H NMR data and comparison with ^{1}H NMR data of MTPA esters of acetogenins with similar structure, i.e., 4-deoxyannoreticuin,[3] the absolute stereochemistry of **1** at both C-19 and C-24 was determined as *R*. The absolute configuration of the carbinol chiral center at C-13 was not determined due to the difficult assignment of the diagnostic protons. The absolute configuration of C-36 was assumed to be *S*, as all previous acetogenin compounds have an *S* configuration at C-36.[5]

Compound **2** had the same molecular formula and ^{1}H and ^{13}C NMR spectra similar to those of **1**. Compound **2** and its TMSi derivative had the same EI mass spectra as **1** and its TMSi derivative However, **2** revealed ^{1}H NMR signals at δ 3.40 (H-19), 3.84 (H-20, 23), and 3.88 (H-24) and ^{13}C NMR resonances at δ 83.2 (C-20), 74.3 (C-19), 82.3 (C-23), and 71.6 (C-24), suggesting the relative stereochemistry of the mono-THF ring of **2** to be *threo-trans-erythro*.[2] There were two possibilities for the position of the *erythro* configuration in **2**, at C-19/20 or C-23/24. Since nearly all of the known annonaceous acetogenins of the mono-THF ring type have an *erythro* relative stereochemistry at the side of the THF ring remote from the γ-lactone moiety, we assume that the *erythro* configuration of **2** is at C-23/24.[4,5] The absolute configuration of **2** was determined as 19*R*, 20*R*, 23*R*, 24*S*, and 36*S* by advanced Mosher methodology, and **2** was the epimer of **1** at C-24.

The molecular formula of **3** ($C_{37}H_{66}O_6$) was deduced from FABMS (*m*/*z* 607 $[MH]^+$ ion) and element analysis. The γ-methyl α,β-unsaturated γ-lactone moiety in **3** was obvious from the IR carbonyl absorption at 1763, 1739 cm^{-1}, five ^{1}H NMR signals at δ 7.04, 5.07, 2.36, 2.44, and 1.41, and six ^{13}C NMR resonances at δ 173.8 (C-1), 149.4 (C-35), 134.1 (C-2), 77.3 (C-36), 21.5 (C-3), and 19.2 (C-37). These data and the NMR signals at δ 3.59 and 71.3 (C-5) pointed to a 5-OH group in **3**.[2] The presence of a mono-THF ring with flanking OH groups was deduced from the ^{1}H NMR signals at δ 3.82, 3.44 and the ^{13}C NMR signals at δ 82.6 (C-16), 82.7 (C-19), 74.1 (C-15), and 74.8 (C-20), which also suggested that the THF ring region with flanking OH groups had the *threo-trans-threo* relative configuration as in **1**.[2] A *cis* double bond[2] in the aliphatic chain of **3** was evident from two olefinic proton signals at δ 5.39 and 5.35 (J = 12.5 Hz) and two carbon resonances at δ 128.8 (C-24) and 130.6 (C-23) in the ^{1}H and ^{13}C NMR spectra.

The placement of the THF ring between C-16 and C-19 with flanking OH groups at C-15 and C-20 was determined from the fragment ion peaks at *m*/*z* 381, 363, 345, 311, 293, and 275 in the EIMS of **3** and at *m*/*z* 525, 435, 455, 365,

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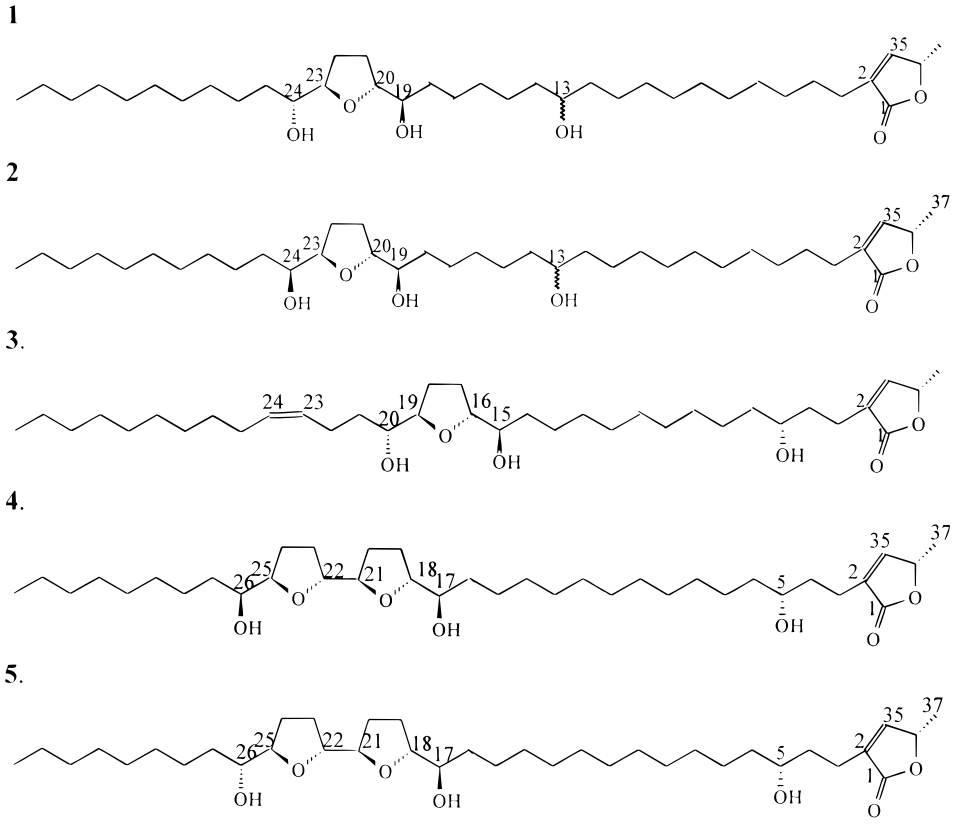
Chart 1



and 345 in that of the TMSi derivative. The third OH group (C-5) was also supported by the EI mass spectra. The C-23/C-24 double bond was suggested by the intense fragment ion peak (*m*/*z* 181) in the EI mass spectra of **3** and its TMSi derivative. The resonance signal at δ 23.4 in the ^{13}C NMR spectrum also supported the assignment of the double bond position at C-23/24.[5]

The absolute configuration of carbinol chiral centers in **3** was determined by the advanced Mosher methodology above. The diagnostic ^{1}H NMR data of its (*S*)- and (*R*)-MTPA esters (**3s** and **3r**) revealed that the absolute configurations of **3** were 5*R*, 15*R*, 16*R*, 19*R*, 20*R*, and 36*S*.

The FABMS of **4** showed a $[MH]^+$ peak at *m*/*z* 623. On the basis of this and the elemental analysis, the molecular formula of **4** was deduced to be $C_{37}H_{66}O_7$. The absorption band at 3427 cm^{-1} in the IR spectrum of **4** and the $[MH - 3H_2O]^+$ ion peak at *m*/*z* 569 in the FABMS of **4** indicated the presence of three OH groups. The ^{1}H and ^{13}C NMR signals of **4** indicated the same α,β-unsaturated γ-lactone moiety with a 5-OH group as in **3**.[2] The ^{1}H NMR signals at δ 3.39, 3.87, and 3.83 and the^{13}C NMR signals at δ 83.1 (2C, C-18, 25), 74.1 (2C, C-17, 26), and 81.7 (2C, C-21, 22) were typical of adjacent bis-THF rings with flanking OH groups. The NMR data of **4** also suggested the existence of bis-THF rings and hydroxylated methines on both sides and indicated the *threo-trans-threo-trans-threo* relative configuration in the bis-THF ring region.[2,8] The location of the bis-THF ring system between C-18 and C-25 and two flanking OH groups at C-17 and C-26 in **4** was determined by the diagnostic fragment ion peaks in the EIMS of **4** and that of its TMSi derivative.

The (*R*)- and (*S*)-MTPA esters (**4s/4r**)[2] of **4** were used to determine the absolute configuration of carbinol chiral centers in **4**. Analysis of the difference in chemical shift of the diagnostic protons in **4** revealed that the stereochemistries of C-5, C-19, and C-26 were all *R*. On the basis of the elucidated relative relationship, the absolute configurations at C-20, C-23, C-24, and C-25 also were *R*. The stereochemistry of C-36 was assumed to be *S*.

Table 1. Cytotoxicity (IC_{50} μg/mL) of Compounds **1**−**5** against Some Human Tumor Cell Lines by the MTT Method

compound	A_{2780}	KB	HCT
1	3.15×10^{-3}	6.05×10^{-2}	4.73×10^{-3}
2	4.31×10^{-1}	2.73×10^{-2}	7.32×10^{-2}
3	2.73×10^{-2}	3.75×10^{-1}	3.97×10^{-3}
4	3.66×10^{-2}	5.10×10^{-2}	2.27×10^{-3}
5	1.97×10^{-3}	2.00×10^{-2}	3.31×10^{-3}

Compound **5** had ^{1}H and ^{13}C NMR spectra similar to those of **4**, which showed that **5** was also a bis-THF ring acetogenin. Similarly, the adjacent bis-THF rings with flanking OH groups in **5** were indicated by ^{1}H NMR signals at δ 3.39, 3.84, and 3.93 and ^{13}C NMR resonances at δ 74.1 (C-17), 83.4 (C-18), 82.4 (C-21), 82.2 (C-22), 82.8 (C-25), and 71.5 (C-26), which also suggested the *threo-trans-threo-trans-erythro* relative configuration.[8,9] The absolute configuration of **5** was determined to be 5*S*, 17*R*, 18*R*, 21*R*, 22*R*, 25*R*, 26*S*, and 36*S* by the advanced Mosher method. Thus, **4** and **5** are epimers at C-26.

Microculture tetrazolium assay (MTT) for antitumor activity involves dimethyl sulfoxide solubilization of cellular-generated 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-formazan to the in vitro assessment of drug effects on cell growth.[10] Bioassay experiments using the MTT method revealed that all of the new compounds (**1**−**5**) exhibited weak cytotoxicity against A_{2780}, KB, and HCT human tumor cell lines and had no significant activity against MCF human tumor cell lines in the test concentrations (Table 1).

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Bruker AM 500 spectrometer with TMS as

internal standard. EIMS were obtained on a ZAB-2F mass spectrometer. FABMS were obtained on a Zabspec E mass spectrometer. IR spectra were recorded on a Perkin-Elmer 683 infrared spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarmeter. Elemental analysis was done with a MOD.1106 elemental analyzer. Melting points were determined on a micromelting point apparatus and are uncorrected.

Plant Material. The roots of *U. calamistrata* were collected at Jian-Liang Peak on Hainan Island, South China, in July 1996. A voucher specimen (Annonaceae No. 46) was deposited in the Herbarium of the Institute of Materia Medica, Beijing.

Extraction and Isolation. Procedures employed for the extraction and primary isolation were described previously.[1] The final EtOH extract (350 g) was subjected to silica gel chromatography. This column was eluted with a gradient petroleum ether/Me_2CO system, and fractions were collected of 500 mL each. Fractions 140−145 were rechromatographed on silica gel with $CHCl_3$−Me_2CO (10:1) as the eluant, and finally crystals of **1** (15 mg) and **2** (20 mg) were obtained. Fractions 152−160 were repeatedly rechromatographed and eluted with $CHCl_3$−MeOH (40:1) or $CHCl_3$−Me_2CO (10:1). Compounds **3** (14 mg), **4** (20 mg), and **5** (15 mg) were sequentially isolated from these fractions.

General Procedure for the Preparation of MTPA Esters. The detailed procedure for the preparation of all (*R*)- and (*S*)-MTPA esters was given previously.[1]

General Procedure for the Preparation of TMSi Derivatives. A small amount (0.5−1.0 mg) of samples were treated with 50 μL of *N*,*O*-bis (trimethylsilyl) acetamide−pyridine (1:1) and heated at 70 °C for 30 min to yield TMSi derivatives. The mixtures were directly used to obtain EI mass spectra.

Cytotoxicity Experiments. Cytotoxicity against human tumor cells was measured in a 5-day MTT test for KB human epidermoid carcer cells, HCT-8 human ileocecal carainoma, A_{2780} human epithelial tumor cells, and MCF-7 human mammary adenocarcinoma.[11,12]

Calamistrin C (1): colorless needles (MeOH); mp 60−62 °C; $[\alpha]^{19}_D$ +17.6° (*c* 0.085, MeOH); IR(KBr) ν_{max} 3444, 2922, 2851, 1756, 1463, 1320, 1081, 1066, 959 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.98 (1H, d, J = 1.5 Hz, H-35), 4.99 (1H, m, H-36), 3.80 (2H, m, H-20, 23), 3.60 (1H, m, H-13), 3.41 (2H, m, H-19, 24), 2.26 (2H, t, J = 7.3 Hz, H-3), 1.98−1.66 (4H, m, H-21, 22), 1.55−1.20 (m), 1.42 (3H, d, J = 6.8 Hz, H-37), 0.87 (3H, t, J = 6.8 Hz, H-34); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.8 (s, C-1), 148.7 (d, C-35), 134.3 (s, C-2), 82.7 (d, C-23), 82.6 (d, C-20), 77.3 (d, C-36), 74.1 (d, C-19, 24), 71.8 (d, C-13), 27.4 (t, C-4), 25.2 (t, C-3), 22.6−33.5 (t, the rest), 19.2 (q, C-37), 14.0 (q, C-34); EIMS (70 ev) *m*/*z* 439 (2), 419 (17), 408 (40), 367 (3), 349 (60), 331 (65), 267 (8), 249 (30); FABMS *m*/*z* 609 (19), 591 (10), 573 (8), 555 (12); *anal*. C 73.41%, H 10.99%, calcd for $C_{37}H_{68}O_6$, C 73.03%, H 11.18%.

TMSi Derivative of 1: EIMS (70 eV) *m*/*z* 581 (2), 511 (30), 491 (23), 421 (25), 404 (4), 331 (3), 339 (20), 249 (2).

(*R*)-MTPA ester derivative of 1: preparation of **1r** see general procedure of MTPA ester derivatives; colorless gum, 8 mg; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58−7.46 (6H, m, MTPA-Ar−H), 7.42−7.26 (9H, m, MTPA-Ar−H), 6.97 (1H, d, J = 1.5 Hz, H-35), 5.01 (1H, m, H-36), 4.97 (2H, m, H-19, 24), 4.93 (1H, m, H-13), 3.90 (2H, m, H-20, 23), 3.55 (6H, s, 2 × OMe), 3.49 (3H, s, OMe), 2.26 (2H, t, J = 6.8 Hz, H-3), 1.95 (2H, m, H-21a, 22a), 1.70 (2H, m, H-21b, 22b), 1.40 (3H, d, J = 6.7 Hz, H-37), 1.55−1.05 (m), 0.88 (3H, t, J = 6.7 Hz, H-34).

(*S*)-MTPA ester derivative of 1: colorless gum, 2.3 mg; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59−7.52 (6H, m, MTPA-Ar−H), 7.41−7.26 (9H, m, MTPA-Ar−H), 6.97 (1H, d, J = 1.5 Hz, H-35), 5.03 (1H, m, H-19), 5.00 (1H, m, H-24), 4.99 (1H, m, H-36), 4.93 (1H, m, H-13), 4.00 (1H, m, H-20), 3.94 (1H, m, H-23), 3.59 (3H, s, OMe), 3.54 (3H, s, OMe), 3.53 (3H, s, OMe), 2.26 (2H, t, J = 6.8 Hz, H-3), 1.91 (2H, m, H-21a, 22a), 1.55 (2H, m, H-21b, 22b), 1.55−1.45 (m), 1.41 (3H, d, J = 6.8 Hz, H-37), 1.50−1.10 (m), 0.88 (3H, t, J = 6.8 Hz, H-34).

Calamistrin D (2): colorless needles (MeOH); mp 68−70 °C; $[\alpha]^{19}_D$ +30.6° (*c* 0.08,MeOH); IR (KBr) ν_{max} 3426, 2922, 2851, 1754, 1466, 1321, 1084, 1064, 956 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.98 (1H, d, J = 1.5 Hz, H-35), 5.00 (1H, m, H-36), 3.88 (1H, m, H-24), 3.84 (2H, m, H-20, 23), 3.60 (1H, m, H-13), 3.40 (1H, m, H-19, 24), 2.26 (2H, t, J = 7.3 Hz, H-3), 2.00−1.64 (4H, m, H-21, 22), 1.55−1.10 (m), 1.42 (3H, d, J = 6.8 Hz, H-37), 0.88 (3H, t, J = 6.8 Hz, H-34); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.8 (s, C-1), 148.7 (d, C-35), 134.5 (s, C-2), 83.2 (d, C-20), 82.3 (d, C-23), 77.3 (d, C-36), 74.3 (d, C-19), 71.6 (d, C-24), 71.6 (d, C-13), 27.5 (t, C-4), 25.2 (t, C-3), 22.6−33.5 (t, the rest), 19.2 (q, C-37), 14.1 (q, C-34); EIMS (70 ev) *m*/*z* 439 (4), 419 (19), 408 (37), 367 (5), 349 (60), 331 (67), 267 (10), 249 (32); FABMS *m*/*z* 609 (27), 573 (8), 555 (12); *anal*. C 73.48%, H 10.96%, calcd for $C_{37}H_{68}O_6$, C 73.41%, H 10.99%.

TMSi Derivative of 2: EIMS (70 eV) *m*/*z* 581 (4), 511 (32), 491 (26), 421 (27), 404 (6), 331 (5), 339 (20), 249 (5).

(*R*)-MTPA ester derivative of 2: colorless gum, 1.7 mg; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62−7.52 (6H, m, MTPA-Ar−H), 7.50−7.26 (9H, m, MTPA-Ar−H), 6.98 (1H, d, J = 1.5 Hz, H-35), 5.26 (1H, m, H-19), 5.00 (2H, m, H-24, 36), 4.97 (1H, m, H-5), 3.96 (1H, m, H-20), 3.70 (1H, m, H-23), 3.59 (3H, s, OMe), 3.52 (3H, s, OMe), 3.50 (3H, s, OMe), 2.26 (2H, m, H-3), 1.93 (2H, m, H-21a, 22a), 1.80 (2H, m, H-21b, 22b), 1.68 (2H, m, H-4), 1.55−1.50 (m) 1.40 (3H, d, J = 6.7 Hz, H-37), 1.50−1.00 (m), 0.88 (3H, t, J = 6.7 Hz, H-34).

(*S*)-MTPA ester derivative of 2: colorless gum, 2.2 mg; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65−7.54 (6H, m, MTPA-Ar−H), 7.39 (9H, m, MTPA-Ar−H), 6.95 (1H, d, J = 1.5 Hz, H-35), 5.20 (1H, m, H-19), 4.95 (H, m, H-24), 4.92 (1H, m, H-36), 4.90 (1H, m, H-5), 4.04 (1H, m, H-20), 3.95 (1H, m, H-23), 3.67 (3H, s, OMe), 3.60 (3H, s, OMe), 3.51 (3H, s, OMe), 2.30 (2H, t, J = 6.8 Hz, H-3), 1.95 (2H, m, H-21a, 22a), 1.63 (2H, m, H-4), 1.57−1.54 (m), 1.44 (3H, d, J = 6.8 Hz, H-37), 1.50−1.05 (m), 0.89 (3H, t, J = 6.8 Hz, H-34).

Calamistrin E (3): waxy solid; mp 35−37 °C; $[\alpha]^{19}_D$ +25.7° (*c* 0.07, MeOH); IR(KBr) ν_{max} 3392, 2922, 2850, 1763, 1739, 1468, 1319, 1084, 1026, 720 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.04 (1H, d, J = 1.4 Hz, H-35), 5.39 (1H, dt, J = 12.5, 5.6 Hz, H-23), 5.35 (1H, dt, J = 12.5, 5.6 Hz, H-23), 5.01 (1H, m, H-36), 3.82 (2H, m, H-16, 19), 3.59 (1H, m, H-5), 3.44 (2H, m, H-15, 20), 2.44 (1H, m, H-3a), 2.36 (1H, m, H-3b), 1.90−1.67 (4H, m, H-17, 18), 1.55−1.20 (m), 1.43 (3H, d, J = 6.7 Hz, H-37), 0.88 (3H, t, J = 7.0 Hz, H-34); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.8 (s, C-1), 149.4 (d, C-35), 134.1 (s, C-2), 130.6 (d, C-24), 128.8 (d, C-22), 82.7 (d, C-19), 82.6 (d, C-16), 77.3 (d, C-36), 74.8 (d, C-20), 74.6 (d, C-15), 70.9 (d, C-5), 33.5 (t, C-21), 23.4 (t, C-22), 22.7−37.5 (t, the rest), 19.2 (q, C-37), 14.1-(q, C-34); EIMS (70 eV) *m*/*z* 381 (2), 363 (2), 345 (22), 311(70), 293 (100), 275 (15), 181 (10), 155 (17), 137 (13); FABMS *m*/*z* 606 (80); *anal*. C 73.10%, H 10.77%, calcd for $C_{37}H_{66}O_6$, C 73.27%, H 10.89%.

TMSi Derivative of 3: EIMS (70 eV) *m*/*z* 525 (15), 455 (40), 435 (3), 365 (15), 345 (2), 277 (50), 275 (2), 137 (3).

(*R*)-MTPA ester derivative of 3: colorless oil, 2.1 mg; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64−7.52 (6H, m, MTPA-Ar−H), 7.39 (9H, m, MTPA-Ar−H), 6.95 (1H, d, J = 1.4 Hz, H-35), 5.37 (1H, m, H-23, 24), 5.27 (1H, m, H-15), 5.07 (H, m, H-20), 4.97 (1H, m, H-5, 36), 4.10 (1H, m, H-16), 3.88 (1H, m, H-19), 3.08 (3H, s, OMe), 3.60 (3H, s, OMe), 3.55 (3H, s, OMe), 2.21−2.00 (2H, m, H-3), 1.80 (2H, m, H-17a, 18a), 1.68 (2H, m, H-17b, 18b), 1.39 (3H, d, J = 6.8 Hz, H-37), 1.50−1.10 (m), 0.88 (3H, t, J = 6.7 Hz, H-34).

(S)-MTPA ester derivative of 3: colorless oil, 2.7 mg; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64−7.54 (6H, m, MTPA-Ar−H), 7.38 (9H, m, MTPA-Ar−H), 7.03 (1H, d, J = 1.5 Hz, H-35), 5.37 (1H, m, H-23, 24), 5.26 (1H, m, H-15), 5.10 (H, m, H-20), 4.98 (1H, m, H-5, 36), 4.92 (1H, m, H-5), 4.11 (1H, m, H-16), 3.87 (1H, m, H-19), 3.69 (3H, s, OMe), 3.61 (3H, s, OMe), 3.52 (3H, s, OMe), 2.32 (2H, m, H-3), 1.95 (2H, m, H-17a, 18a), 1.67 (2H, m, H-17b, 18b), 1.40 (3H, d, J = 6.8 Hz, H-37), 1.50−1.05 (m), 0.88 (3H, t, J = 6.8 Hz, H-34).

Calamistrin F (4): colorless waxy solid; mp 40−41 °C; $[\alpha]^{19}_D$ +66.7° (*c* 0.045, MeOH); IR(KBr) ν_{max} 3427, 2922, 2850, 1755, 1466, 1321, 1120, 1082, 1065, 1030, 958 cm^{-1}; ^{1}H NMR

(500 MHz, $CDCl_3$) δ 7.04 (1H, d, J = 1.4 Hz, H-35), 5.01 (1H, m, H-36), 3.87 (2H, m, H-18, 25), 3.83 (2H, m, H-21, 22), 3.59 (1H, m, H-5), 3.39 (2H, m, H-17, 26), 2.44 (1H, m, H-3a), 2.36 (1H, m, H-3b), 1.97−1.90 (4H, m, H-19a, 20a, 23a, 24a), 1.67 (4H, m, H-19b, 20b, 23b, 24b), 1.55−1.10 (m), 1.42 (3H, d, J = 5.7 Hz, H-37), 0.88 (3H, t, J = 6.7 Hz, H-34); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.8 (s, C-1), 149.4 (d, C-35), 134.1 (s, C-2), 83.1 (d, C-18), 81.7 (d, C-21, 22), 81.3 (d, C-25), 77.3 (d, C-36), 74.1 (d, C-17), 73.9 (d, C-26), 70.7 (d, C-5), 21.5 (t, C-3), 22.7−37.5 (t, the rest), 19.1 (q, C-37), 14.1 (q, C-34); EIMS (70 eV) *m*/*z* 479 (2), 461 (3), 443 (10), 409 (5), 391 (30), 373 (100), 339 (75), 321 (100), 303 (15), 135 (8), 97 (20); FABMS *m*/*z* (rel int) 623 $[MH]^+$ (12), 569 ($[MH]^+ - 3H_2O$) (30), 403 (2), 385 (25); *anal*. C 71.20%, H 10.35%, calcd for $C_{37}H_{66}O_7$, C 71.38%, H 10.61%.

TMSi Derivative of 4: EIMS (70 eV) *m*/*z* 623 (2), 553 (2), 533 (2), 483 (40), 463 (2), 443 (4), 393 (15), 375 (5), 303 (2).

(*R*)-MTPA ester derivative of 4: colorless gum, 1.8 mg; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64−7.54 (6H, m, MTPA-Ar−H), 7.42−7.30 (9H, m, MTPA-Ar−H), 7.01 (1H, d, J = 1.5 Hz, H-35), 5.07 (1H, m, H-26), 5.06 (2H, m, H-15, 17), 4.99 (1H, m, H-36), 4.01 (2H, m, H-18, 25), 3.93 (2H, m, H-21, 22), 3.65 (3H, s, OMe), 3.55 (6H, s, OMe), 2.20 (2H, m, H-3), 1.95 (2H, m, H-19a, 24a), 1.90 (2H, m, H-20a, 23a), 1.80 (2H, m, H-20b, 23b), 1.50 (2H, m, H-19b, 24b), 1.39 (3H, d, J = 6.7 Hz, H-37), 1.50−1.10 (m), 0.86 (3H, t, J = 6.7 Hz, H-34).

(*S*)-MTPA ester derivative of 4: colorless gum, 2.2 mg,; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60−7.50 (6H, m, MTPA-Ar−H), 7.41−7.25 (9H, m, MTPA-Ar−H), 6.96 (1H, d, J = 1.5 Hz, H-35), 5.10 (2H, m, H-17, 26), 4.97 (2H, m, H-5, 36), 3.96 (2H, m, H-18, 25), 3.78 (2H, m, H-21, 22), 3.56 (3H, s, OMe), 3.55 (3H, s, OMe), 3.54 (3H, s, OMe), 2.33 (2H, m, H-3), 1.91(2H, m, H-19a, 24a), 1.72 (2H, m, H-20a, 23a), 1.65 (2H, m, H-20b, 23b), 1.50 (2H, m, H-19b, 24b), 1.38 (3H, d, J = 6.8 Hz, H-37), 1.50−1.10 (m), 0.88 (3H, t, J = 6.9 Hz, H-34).

Calamistrin G (5): colorless needles (MeOH); mp 47−48 °C; $[\alpha]^{19}_D$ +24.1° (*c* 0.017, MeOH); IR(KBr) ν_{max} 3421, 2918, 2850, 1759, 1469, 1319, 1119, 1076, 1028 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.02 (1H, d, J = 1.4 Hz, H-35), 4.99 (1H, m, H-36), 3.93 (1H, m, H-25), 3.84 (4H, m, H-18, 21, 22, 26), 3.59 (1H, m, H-5), 3.39 (1H, m, H-17), 2.44 (1H, m, H-3a), 2.36 (1H, m, H-3b), 1.98−1.89 (4H, m, H-19a, 20a, 23a, 24a), 1.67 (4H, m, H-19b, 20b, 23b, 24b), 1.55−1.10 (m), 1.40 (3H, d, J = 5.7 Hz, H-37), 0.88 (3H, t, J = 6.7 Hz, H-34); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.8 (s, C-1), 149.4 (d, C-35), 134.1 (s, C-2), 83.4 (d, C-18), 82.8 (d, C-25), 82.4 (d, C-21), 82.2 (d, C-22), 74.1 (d, C-17), 71.5 (d, C-26), 70.9 (d, C-5), 21.5 (t, C-3), 22.6−37.5 (t, the rest), 19.2 (q, C-37), 14.1 (q, C-34); EIMS (70 eV) *m*/*z* 479 (3), 461 (5), 443 (9), 409 (6), 391 (26), 373 (100), 339 (77), 321 (80), 303 (17), 135 (9), 97 (18); FABMS: *m*/*z* 623 (27), 569 (15); *anal*. C 71.25%, H 10.43%, calcd for $C_{37}H_{66}O_7$, C 71.38%, H 10.61%.

TMSi derivative of 5: EIMS (70 eV) *m*/*z* 623 (3), 553 (4), 533 (2), 483 (45), 463 (4), 443 (6), 393 (19), 375 (7), 303 (5).

(*R*)-MTPA ester derivative of 5: colorless gum, 1.8 mg; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64−7.54 (6H, m, MTPA-Ar−H), 7.42−7.26 (9H, m, MTPA-Ar−H), 6.97 (1H, d, J = 1.5 Hz, H-35), 5.25 (1H, m, H-26), 5.08 (1H, m, H-5), 5.02 (1H, m, H-17), 4.99 (1H, m, H-36), 4.00 (1H, m, H-18), 3.95 (1H, m, H-25), 3.82 (1H, m, H-21), 3.64 (1H, m, H-22), 3.64 (6H, s, OMe), 3.63 (3H, s, OMe), 3.62 (3H, s, OMe), 2.17 (2H, t, J = 6.8 Hz, H-3), 1.90 (2H, m, H-19a, 20a), 1.80−1.50 (2H, m, H-19b, 24b), 1.39 (3H, d, J = 6.7 Hz, H-37), 1.50−1.10 (m), 0.88 (3H, t, J = 6.7 Hz, H-34).

(*S*)-MTPA ester derivative of 5: colorless gum, 3.2 mg; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (6H, m, MTPA-Ar−H), 7.41−7.30 (9H, m, MTPA-Ar−H), 6.95 (1H, d, J = 1.5 Hz, H-35), 5.24 (1H, m, H-26), 5.04 (1H, m, H-17), 5.08 (1H, m, H-5), 4.97 (1H, m, H-36), 4.03 (1H, m, H-18), 3.98 (1H, m, H-25), 3.80 (2H, m, H-21, 22), 3.55 (3H, s, OMe), 3.54 (3H, s, OMe), 3.53 (3H, s, OMe), 2.30 (2H, m, H-3), 1.92 (2H, m, H-19a, 24a), 1.71 (2H, m, H-20a, 23a), 1.65 (2H, m, H-20b, 23b), 1.53 (2H, m, H-19b, 24b), 1.38 (3H, d, J = 6.8 Hz, H-37), 1.50−1.10 (m), 0.88 (3H, t, J = 6.9 Hz, H-34).

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Supporting Information Available: Diagnostic fragment ions in the EIMS of compounds **1**−**5** and their TMSi derivatives (Figures 1−3). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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