Trikentramides A–D: Indole Alkaloids from the Australian Sponge *Trikentrion flabelliforme*

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ABSTRACT

Chemical investigations of two specimens of *Trikenrion flabelliforme* collected from Australian waters have resulted in the identification of four new indole alkaloids, trikenramides A–D (**9–12**). The planar chemical structures for **9–12** were established following analysis of 1D/2D NMR and MS data. The relative configurations for **9–12** were determined following comparison of ^1H NMR data with previously reported and related natural products. The application of a quantum mechanical modeling method, density functional theory, confirmed the relative configurations and also validated the downfield carbon chemical shift observed for one of the quaternary carbons (C-5a) in the cyclopenta[g]indole series. The indole-2,3-dione motif present in trikenramides A–C is rare in nature, and this is the first report of these oxidized indole derivatives from a marine sponge.

Indole alkaloids are ubiquitous natural products that have been shown to be produced by a variety of organisms, including marine invertebrates such as sponges.^{1,2} Over one quarter of marine alkaloids isolated to date contain the indole motif and, in addition to displaying anticancer, antiprotozoal, antibacterial, antiviral, and anti-inflammatory activity, indole alkaloids often have unusual functionalities.¹ Sponges belonging to the genus *Trikentrion* have been the source of several novel indole alkaloids. These include the antibacterial trikentrins **1–5** from the Australian *T. flabelliforme*,³ as well as trikendiol⁴ and trikentramine⁵ from the West African sponge *T. leave*. Although the trikentrins were the first report of cyclopenta[g]indoles from the marine environment, the related compounds, herbindoles A–C (**6–8**) from an *Axinella* sp. have subsequently been reported.⁶

Previously isolated *Trikentrion* alkaloids displayed characteristic ¹H NMR signals including downfield exchangeable indole protons, aromatic methine resonances, and in some cases, upfield methyl doublet signals that corresponded to cyclopentyl substituted methyl groups.^{3,6} This knowledge enabled us to rapidly dereplicate *Trikentrion* sponge extracts for the presence of known or new indole alkaloids. Using a modified solid-phase extraction (SPE) protocol developed in our research group, we were able to conduct NMR-based screening of semi-purified *Trikentrion* extracts that enabled us to identify the presence of the indole chemotype.^{7,8} This protocol involved the rapid small-scale MeOH extraction of ground and freeze-dried sponge material followed by the removal of salts and highly lipophilic material using C₁₈ SPE chromatography. Using this methodology we identified two *T. flabelliforme* samples that warranted further chemical investigation due to the presence of potentially new indole metabolites. Herein we report the isolation, structure elucidation and density functional theory (DFT) NMR studies of five new indole alkaloids, namely trikentrinamides A–D (**9–12**).

RESULTS AND DISCUSSION

Two ground and freeze-dried *T. flabelliforme* samples (300 mg) were subjected to rapid MeOH extraction followed by the removal of the salts and highly lipophilic material using C₁₈ SPE chromatography (MeOH/H₂O/1.0% TFA). The ¹H NMR spectra in DMSO-*d*₆ of both semi-purified *Trikenrion* extracts displayed aromatic signals between δ_{H} 6.59 and 6.87 and importantly, exchangeable NH proton signals between δ_{H} 10.18 and 11.12. Previous studies of indole alkaloids by our group have revealed that the exchangeable NH proton typically manifests between δ_{H} 10.0 and 12.0 in deuterated DMSO.⁹⁻¹² HMBC experiments on the semi-purified extracts revealed that the exchangeable and aromatic protons both experienced couplings to carbon atoms resonating downfield of δ_{C} 112 that were characteristic of sp² hybridized carbon atoms, possibly suggestive of the indole chemotype. LC-MS analysis of the semi-purified extracts revealed ions that did not correspond to any of the known *Trikenrion* alkaloids, which prompted further investigations.³⁻⁵

A large-scale MeOH extraction of *T. flabelliforme* (voucher specimen: G303344) followed by C₁₈ flash column chromatography (MeOH/H₂O/1.0% TFA) and C₁₈ HPLC (MeOH/H₂O/1.0% TFA), resulted in the isolation of two new metabolites, which we have named trikenramides A and B (**9** and **10**). Chemical investigations of an additional specimen of *T. flabelliforme* (voucher specimen: G306125) using similar chromatographic methods resulted in the identification of two additional alkaloids, trikenramides C (**11**) and D (**12**).

Trikenramide A (**9**, 0.4 mg, 0.012% dry wt) was isolated as an optically active yellow gum. The HRESIMS and 1D NMR data (Tables 1 and 2) enabled the establishment of a molecular formula of C₁₅H₁₇NO₂, with eight degrees of unsaturation. The ¹H NMR spectrum contained an upfield methyl triplet (δ_{H} 1.13), two methyl doublets (δ_{H} 1.26 and 1.27), two diastereotopic methylene protons (δ_{H} 2.54 and 1.25), two methine doublet of quartets (δ_{H} 3.14 and 3.06), a downfield aromatic methine singlet (δ_{H} 6.77) and a broad exchangeable proton

(δ_{H} 10.83). The ^{13}C NMR spectrum contained 15 carbon signals, including seven sp^3 carbons, six sp^2 carbons and two carbonyl signals at δ_{C} 183.7 and 160.3. The HSQC correlation data enabled ^1H NMR shifts to be assigned to each of their attached ^{13}C atoms.

Analysis of the COSY spectrum established two spin systems: a 1,3-dimethyl propyl group (δ_{H} 1.26, 3.06, 1.25, 2.54, 3.14 and 1.27), as well as an ethyl moiety (δ_{H} 1.13 and δ_{H} 2.83) (Figure 1). Weak COSY correlations from the aromatic proton (δ_{H} 6.77) to the methine (δ_{H} 3.06) and methylene (δ_{H} 2.83) in the two spin systems indicated that the propyl and ethyl moieties were attached to an aromatic ring. HMBC correlations confirmed this linkage, with strong cross-peaks (suggestive of a three-bond coupling in an aromatic system) observed from the aromatic proton at δ_{H} 6.77 (H-5) to the methylene carbon at δ_{C} 24.4 (C-9) and the methine carbon at δ_{C} 39.1 (C-6). Additional HMBC correlations from the two methine protons (δ_{H} 3.06, 3.14) to the aromatic carbons [δ_{C} 161.0 (C-5a) and δ_{C} 128.5 (C-8a)] suggested the formation of a five membered ring between the 1,3-dimethylpropyl unit and the benzenoid ring. The exchangeable proton at δ_{H} 10.83 showed an HMBC coupling to a carbonyl group at δ_{C} 183.7 (C-3), indicating the presence of a ketone functionality, and also showed HMBC correlations to carbons resonating at δ_{C} 114.0 (C-3a) and 146.4 (C-8b) in the benzenoid ring. The last remaining and unassigned ^{13}C signal at δ_{C} 160.3 suggested the presence of an additional carbonyl moiety in trikentramide A, however no HMBC correlations to this carbon were observed. Nonetheless, with all the atoms of **9** accounted for, and the requirement to satisfy eight degrees of unsaturation, a pyrrolidine-2,3-dione fused to the benzenoid system was established. HMBC data (*vide supra*) secured the regioisomer assignment, which was further supported by a strong ROESY correlation between the NH proton at δ_{H} 10.83 and the H-12 protons at δ_{H} 1.27. Furthermore, comparison of the pyrrolidine-2,3-dione ^{13}C chemical shifts in DMSO- d_6 of **9** [δ_{C} 160.3 (C-2), 183.7 (C-3), 114.0 (C-3a), 146.4 (C-8b)] with the commercially available natural product, isatin (**13**) [δ_{C}

159.3 (C-2), 184.3 (C-3), 117.7 (C-3a), 150.7 (C-7a)]¹³, showed only minor differences. Thus the planar structure of trikentrarnide A was assigned as **9**.

Trikentrarnide B (**10**) was isolated as a 1:3 mixture (0.5 mg, 0.013% dry wt) with trikentrarnide A (**9**), as determined by ¹H NMR analysis and integration of the aromatic methine protons [δ_{H} 6.77 (**9**) and 6.75 (**10**)]. Attempts to purify the mixture were not undertaken due to paucity of the sample. LC-MS analysis of the mixture revealed the presence of two compounds with identical molecular weights. The structure of **10** was determined via 1D and 2D NMR data analysis of the mixture. The ¹H NMR data (Table 1) of **10** was very similar to that of **9** however major differences were noted in the chemical shifts of the diastereotopic (H-7a/H-7b) protons, which resonated at δ_{H} 1.95 and 1.79 in **10** compared to δ_{H} 2.54 and 1.25 in **9**. These data suggested that **10** was a diastereomer of **9**.

The HRESIMS data for trikentrarnide C (**11**, 0.2 mg, 0.002% dry wt.) gave a pseudomolecular ion at m/z 252.0997 [$\text{M} + \text{Na}$]⁺ that corresponded to a molecular formula of C₁₄H₁₄NO₂ and eight degrees of unsaturation. This formula indicated that, in comparison to trikentrarnide A (**9**), **11** had one less methylene group in the molecule. Comparison of the ¹H NMR data indicated that the ethyl group in **9** had been replaced by a methyl singlet in **11**. The assignment was confirmed by the HMBC correlations from the methyl protons at δ_{H} 2.42 (H-9) to the carbon atoms resonating at δ_{C} 114.3 (C-3a), 138.5 (C-4) and 119.5 (C-5). Therefore the planar structure of trikentrarnide C (**11**) was established.

The final molecule, trikentrarnide D (**12**, 0.4 mg, 0.004% dry wt.), gave a pseudomolecular ion at m/z 238.1205 [$\text{M} + \text{Na}$]⁺ in the HRESIMS measurement. This, in conjunction with ¹H and 2D NMR data, established a molecular formula of C₁₄H₁₆NO with seven degrees of unsaturation. The loss of an oxygen atom and one degree of unsaturation for **12** compared to **11** suggested the absence of a carbonyl functionality at either C-2 or C-3. This was supported by the NMR data for **12** that showed only one carbonyl signal (δ_{C} 177.4)

and an extra methylene resonance (δ_{H} 3.31/ δ_{C} 34.5). HMBC correlations were observed from the methylene protons (δ_{H} 3.31) to the amide carbonyl (δ_{C} 177.4, C-2) as well as the aromatic carbons C-3a (δ_{C} 122.5), C-4 (δ_{C} 131.4) and C-8b (δ_{C} 146.0). This data enabled the planar structure of trikentrinamide D to be assigned as **12**.

The relative configurations for **9–12** were established by comparison of their ^1H NMR data to the known trikentrin analogues.^{3,6} Previous studies on the trikentrins (**1–5**) had shown that large ^1H NMR chemical shift differences between the diastereotopic H-7 methylene protons ($\Delta\delta_{\text{H-7a/H-7b}} > 1$) were indicative of the cyclopentenyl unit containing methyl groups with a *cis* orientation, whereas no or minimal differences ($\Delta\delta_{\text{H-7a/H-7b}} < 0.5$) were diagnostic for the *trans* isomer.^{3,6,14} Trikentrinamides A, C and D all had relatively large $\Delta\delta_{\text{H-7a/H-7b}}$ (i.e. 1.29, 1.29 and 1.34, respectively), indicating that they all possessed the *cis* configuration at the C-6 and C-8 positions. Trikentrinamide B was assigned as having a *trans* configuration due to its small $\Delta\delta_{\text{H-7a/H-7b}}$ value of 0.16.

Of particular interest for this class of compounds was the extreme downfield chemical shift of C-5a ($\sim\delta_{\text{C}}$ 160) in **9–11**. We wanted to validate this electron deficient position and its corresponding deshielded ^{13}C resonance by generating theoretical NMR chemical shifts. Density functional theory (DFT) is a branch of physics that can be used to elucidate important properties concerning molecular structure.¹⁵ The generation of a theoretical electron density through DFT can be used to predict NMR chemical shifts.¹⁶ In some recent examples, this has confirmed regiochemistry of functional groups, as well as bond connectivity between atoms in a variety of small organic molecules.^{17,18} To calculate theoretical NMR shielding constants for the trikentrinamides, the corresponding diastereomeric structures were subject to molecular mechanics energy minimization and subsequent conformational searches using MMFFs.¹⁹ Each minimum energy conformer was further optimized using DFT with the B3LYP/6-31G(d,p) functional and basis set combination.²⁰⁻²³

Single-point calculations using the MPW1PW91/6-311+G(d,p) level of theory incorporating PBF implicit solvation^{24,25} were used to calculate NMR shielding constants via Gauge Including Atomic Orbitals (GIAO) methodology.²⁶ A set of reference compounds with published experimental NMR data²⁷ were used to generate a linear regression model correlating observed chemical shifts with the calculated shielding constants. The multi-reference regression model was subsequently used to scale the calculated shielding constants to provide ¹H and ¹³C chemical shifts for both the *cis* and *trans* isomers of **9–12**. The reported theoretical chemical shifts are the Boltzmann-weighted average of all values obtained for the conformers identified for each molecule.

Analysis of the theoretical ¹³C NMR shifts of **9** generated by GIAO calculations enabled us to confirm the extreme downfield position of C-5a (Table 3). Using identical GIAO calculations (to those used for **9**), the theoretical ¹³C NMR chemical shifts were also calculated for **10–12** (Supporting Information). The calculated values for C-5a in **10–12** were in good agreement with the assigned experimental values. Furthermore, an image displaying the relative electron density for **9** was generated by mapping the lowest unoccupied molecular orbitals (LUMO) onto the electron density surface as seen in Figure 3. The image clearly indicated a relatively low electron density at C-5a compared with other aromatic carbons, hence explaining the downfield C-5a shift.

There has been success in establishing and confirming relative configurations, using DFT-GIAO NMR calculations coupled with the DP4 analysis in a number of cases.²⁸⁻³⁰ For example, the relative configuration of the plant alkaloid nobilisitine A was recently revised based on comparison of experimental NMR data and DFT-GIAO generated NMR data, together with DP4 probability.^{29,31} Having already generated DFT data, it was simple to use the theoretical ¹H NMR data to confirm our assignment of the relative configurations for **9–12**.

The DP4 analysis showed that the ^1H NMR experimental data for **9** (Table 4) was most consistent with the theoretical chemical shifts calculated for the *cis* isomer, with a probability of greater than 99.9%. In contrast, the DP4 probability indicated that the experimental data for **10** were most consistent with the theoretical data calculated for the *trans* isomer (> 99.9% probability). These assignments were consistent with results from previous studies of related molecules.^{3,14} The theoretical NMR data for the *cis* isomer of **11** was most consistent with the experimental data of **11**, and again, the *cis* theoretical NMR data of **12** correlated with the experimental data of **12** (Supporting Information).²⁸

The cyclopenta[g]indole structure class has rarely been reported, and the natural occurring trikentrins, and herbindoles A–C are the only examples to date.³² While a substructure search of the Dictionary of Natural Products database using the indole-2,3-dione fragment identified 20 natural products from a variety of sources including bacteria, marine mollusks and plants, **9–12** are the first report of these oxidized indole derivatives from a marine sponge.³² In addition the cyclopenta[g]indole-2,3-dione motif present in trikentrinamides A–C has never been reported.

CONCLUSIONS

A review of the literature together with the application of a simple and rapid SPE protocol coupled to NMR analysis has led to the identification of four new indole alkaloids from two specimens of *Trikentrion flabelliforme*. In addition to standard NMR and MS techniques, theoretical NMR calculations generated through DFT supported the structures.

EXPERIMENTAL SECTION

General Experimental Procedures. Specific rotations were recorded on a JASCO P-1020 polarimeter. UV spectra were recorded on a JASCO V-650 UV/Vis spectrophotometer. NMR

spectra were recorded at 30°C on a 600 MHz Varian Unity INOVA spectrometer or at 25°C on a 900 MHz Bruker NMR spectrometer. Both spectrometers were equipped with cryoprobes. The ^1H and ^{13}C chemical shifts were referenced to the solvent peaks for DMSO- d_6 at δ_{H} 2.50 and δ_{C} 39.5, respectively. LRESIMS data were recorded on a Waters LCMS system equipped with Luna C₁₈ column (3 μm , 100 Å, 50 $\times$ 4.6 mm), a PDA detector, and a ZQ ESI mass spectrometer. HRESIMS were recorded on a Bruker MicroTOF-Q spectrometer (Dionex UltiMate 3000 micro LC system, ESI mode). All solvents used for chromatography, $[\alpha]_{\text{D}}$, UV and MS were Lab-Scan HPLC grade and the H₂O was Millipore Milli-Q PF filtered. A Waters 600 pump equipped with a Waters 966 PDA detector and Gilson 715 liquid handler were used for all HPLC work. Semi-preparative HPLC was carried out with a Luna C₁₈ column (5 μm , 100 Å, 250 $\times$ 10 mm). Alltech sample preparative C₁₈-bonded silica (35–75 μm , 150 Å) was used for solid-phase extraction (SPE) and flash column (100 $\times$ 30 mm) chromatography work. Phenomenex polypropylene cartridges (single frit, 3 mL) were used for the SPE work. Isatin was purchased from Sigma-Aldrich.

Animal Material. *Trikenrion flabelliforme* (G303344) was collected at a depth of 10 m by SCUBA diving from East Point Bommies in the Northern Territory, Australia in September of 1993. The voucher specimen (G303344) was deposited at the Queensland Museum, South Brisbane, Queensland, Australia. *Trikenrion flabelliforme* (G306125) was collected at a depth of 28 m by trawl from approximately 70 km off the coast of Port Hedland, Western Australia in September of 1995, with the voucher specimen (G306125) also deposited at the Queensland Museum. This species and the genus were recently re-described and revised.³³ Both samples were immediately frozen upon collection and transported to the Eskitis Institute, where they were freeze-dried, ground and stored as dried powders in air-tight containers in the dark in a temperature controlled environment (~20 °C).

Generation of Semi-Purified Sponge Extracts for NMR Screening. The freeze-dried and ground *T. flabelliforme* specimens (300 mg) were each added to two separate SPE cartridges, and the sponge material was flushed with MeOH (10 mL). The extracts were dried, preadsorbed onto C₁₈-bonded silica (300 mg), and loaded onto a bed of C₁₈-bonded silica (1.0 g) that was pre-equilibrated with MeOH/H₂O/TFA (10:90:1). The column was flushed with MeOH/H₂O/TFA (10:90:1, 10 mL), which was discarded, followed by MeOH/H₂O/TFA (70:30:1, 10 mL). The 70:30:1 MeOH/H₂O/TFA fractions were dried and analyzed by ¹H NMR spectroscopy.

Extraction and Isolation. For the large scale extraction of *T. flabelliforme* (G303344), a sample (3.3 g) of the freeze-dried and ground sponge was exhaustively extracted with MeOH (360 mL) and the resulting extract was dried via rotary evaporation to yield an orange-brown gum (0.5 g). This gum was preadsorbed onto C₁₈-bonded silica (3.0 g), placed on a bed of pre-equilibrated C₁₈-bonded silica [9 g, MeOH/H₂O/TFA (10:90:1)], and flushed with MeOH/H₂O/TFA (10:90:1, 200 mL), which was discarded, followed by MeOH/H₂O/TFA (70:30:1, 200 mL). The latter eluent was dried (0.39 g), dissolved in MeOH (3 mL), and further purified by C₁₈-bonded silica semi-preparative HPLC at a flowrate of 4 mL/min eluting with MeOH/H₂O/TFA (75:25:1) for 5 min, followed by a linear gradient to MeOH within 30 min. Sixty fractions (0.5 min) were collected from the start of the HPLC run. Fractions 29, and 30–31 contained trikentrinamide A (**9**, 0.4 mg, 0.012% dry wt) and trikentrinamides A/B (**9/10**, 3:1 mixture, 0.5 mg, 0.013% dry wt), respectively. *Trikentrion flabelliforme* (G306125, 10 g) was also subjected to the same extraction conditions as G303344, yielding 0.4 g of extract. This was preadsorbed onto C₁₈-bonded silica overnight, and subjected to the same C₁₈ column chromatography as G303344, yielding 0.14 g from the

MeOH/H₂O/TFA (70:30:1, 200 mL) wash. This was subjected to the same C₁₈-bonded silica semi-preparative HPLC conditions described for G303344. Fractions 20–21 and fractions 24–26 yielded trikentramides C (**11**, 0.2 mg, 0.002% dry wt) and D (**12**, 0.4 mg, 0.004% dry wt), respectively.

Trikentramide A (9): Yellow gum; $[\alpha]_D^{25} +40.7$ (*c* 0.027, CHCl₃); UV (MeOH) (log ϵ) 202 (3.80), 223 (3.56), 249 (3.41), 334 (2.95) nm; ¹H and ¹³C NMR data (DMSO-*d*₆) see Tables 1 and 2; (+)-LRESIMS *m/z* 266 [M + Na]⁺; (-)-LRESIMS *m/z* 242 [M - H]⁻; (+)-HRESIMS *m/z* 266.1160 [M + Na]⁺ (calcd for C₁₅H₁₇NO₂Na, 266.1151).

Trikentramide B (10): Yellow gum (3:1 mixture of trikentramides A and B); ¹H and ¹³C NMR data (DMSO-*d*₆) see Tables 1 and 2.; (+)-LRESIMS *m/z* 266 [M + Na]⁺; (-)-LRESIMS *m/z* 242 [M-H]⁻; (-)-HRESIMS *m/z* 242.1191 [M - H]⁻ (calcd for C₁₅H₁₆NO₂, 242.1187).

Trikentramide C (11): Yellow gum; $[\alpha]_D^{25} +42.2$ (*c* 0.028, CHCl₃); UV (MeOH) (log ϵ) 202 (4.16), 223 (3.85), 250 (3.76), 333 (3.31) nm; ¹H and ¹³C NMR data (DMSO-*d*₆), see Tables 1 and 2; (+)-LRESIMS *m/z* 230 [M + H]⁺; (-)-LRESIMS *m/z* 228 [M - H]⁻; (+)-HRESIMS *m/z* 252.0997 [M + Na]⁺ (calcd for C₁₄H₁₅NO₂Na, 252.0995).

Trikentramide D (12): Off-white powder; $[\alpha]_D^{25} +50.0$ (*c* 0.02, CHCl₃); UV (MeOH) (log ϵ) 218 (3.84), 255 (3.32), 333 (2.46) nm; ¹H and ¹³C NMR data (DMSO-*d*₆), see Tables 1 and 2; (+)-LRESIMS *m/z* 216 [M + H]⁺; (+)-HRESIMS *m/z* 238.1205 [M + Na]⁺ (calcd for C₁₄H₁₇NONa, 238.1202).

Computational Chemistry. All structures were subject to geometry optimization in MacroModel³⁴ using the MMFFs with H₂O solvation, with default parameters and convergence criteria used unless otherwise stated. A conformational search was performed on each structure using the mixed MCMM/Low-Mode search with 1000 steps per rotatable bond

and an energy window of 21 kJ mol⁻¹ for retention of conformers. All conformers identified by MacroModel were subject to further gas-phase geometry optimization in Jaguar³⁵ using the B3LYP functional and 6-31G(d,p) basis set. A fine integration grid with accurate SCF convergence criteria was employed and all geometry optimized structures were characterized as true minima by the absence of imaginary vibrational frequencies at the stationary point. NMR shielding constants were calculated using the GIAO method as implemented in Jaguar using single-point MPW1PW91/6-311+G(d,p) calculations with the PBF implicit solvation model (DMSO). A multi-reference linear regression model was generated using the same computational methodology as described above for a range of commercially available compounds with experimental NMR shift data reported in DMSO-*d*₆.^{27,36,37} The theoretical shifts reported are the Boltzmann-weighted average of values calculated for each conformer using the regression model.

ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR spectra for trikentramides A–D, tabulated NMR data for isatin, scaling of computed chemical shifts, energies and Cartesian coordinates, tables of experimental and theoretical ¹H and ¹³C NMR shifts, and DP4 results. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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Figure 1. Key COSY, HMBC and ROESY correlations for **9**

Figure 2. The lowest unoccupied molecular orbital (LUMO) mapped onto the electron density surface for triketramide A (**9**). Blue and orange colouring indicates electron deficiency.

Table 1. ^1H NMR Data for Trikentramides A–D (**9**–**12**) in $\text{DMSO-}d_6$

Position	δ_{H} , mult. (J in Hz)			
	9^a	10^b	11^b	12^a
NH	10.83, s	11.03, s	10.83, s	10.18, s
3				3.31, s
5	6.77, s	6.75, s	6.74, s	6.59, s
6	3.06, ddq (7.2, 7.2, 7.2)	3.26, m	3.04, ddq (7.6, 7.6, 7.6)	2.99, ddq (7.2, 7.2, 7.2)
7a	2.54, ddd (12.6, 7.2, 7.2)	1.95, ddd (12.9, 7.3, 1.9)	2.53, ddd (12.9, 7.6, 7.3)	2.49, ddd (12.5, 7.3, 7.2)
7b	1.25, ddd (12.6, 7.2, 7.2)	1.79, ddd (12.9, 9.0, 9.0)	1.24, ddd (12.9, 7.6, 7.3)	1.15, ddd (12.5, 7.3, 7.2)
8	3.14, ddq (7.2, 7.2, 7.2)	3.21, m	3.14, ddq (7.3, 7.3, 7.3)	3.10, ddq (7.3, 7.3, 7.3)
9	2.83, q (7.6)	2.83, q (7.6)	2.42, s	2.14, s
10	1.13, t (7.6)	1.13, t (7.6)	1.26, d (7.6)	1.22, d (7.2)
11	1.26, d (7.2)	1.23, d (7.2)	1.27, d (7.3)	1.25, d (7.3)
12	1.27, d (7.2)	1.12, d (7.2)		

^a900 MHz at 25°C.^b600 MHz at 30°C.**Table 2.** ^{13}C NMR Data for Trikentramides A–D (**9**–**12**) in $\text{DMSO-}d_6$

Position	δ_{C} , type			
	9^a	10^b	11^b	12^a
2	160.3, C	^c	^c	177.4, C
3	183.7, C	183.6, C	183.6, C	34.5, CH ₂
3a	114.0, C	113.9, C	114.3, C	122.5, C
4	145.1, C	145.7, C	138.5, C	131.4, C
5	117.7, CH	117.7, CH	119.5, CH	117.1, CH
5a	161.0, C	160.2, C	160.5, C	148.5, C
6	39.1, CH	37.6, CH	38.6, CH	38.0, CH
7	42.5, CH ₂	42.4, CH ₂	42.2, CH ₂	43.4, CH ₂
8	35.3, CH	34.0, CH	34.9, CH	35.8, CH
8a	128.5, C	129.0, C	128.1, C	126.0, C
8b	146.4, C	146.0, C	146.0, C	138.4, C
9	24.4, CH ₂	24.2, CH ₂	17.3, CH ₃	18.4, CH ₃
10	14.5, CH ₃	14.4, CH ₃	20.4, CH ₃	21.6, CH ₃
11	20.7, CH ₃	18.6, CH ₃	20.4, CH ₃	21.0, CH ₃
12	20.8, CH ₃	19.5, CH ₃		

^a225 MHz at 25°C.^b150 MHz at 30°C.^cSignal not observed.

Table 3. Experimental and Theoretical (DFT-NMR) ^{13}C NMR Data for **9**^a

Position	δ_{C} calc. 9	δ_{C} exp. 9
2	156.9	160.3
3	180.8	183.7
3a	112.1	114.0
4	147.0	145.1
5	116.3	117.7
5a	162.1	161.0
6	42.4	39.1
7	43.7	42.5
8	38.7	35.3
8a	125.7	128.5
8b	142.3	146.4
9	28.7	24.4
10	14.5	14.5
11	18.3	20.7
12	19.2	20.8
CMAD ^b	2.4	
MD ^c	4.3	

^aExperimental and theoretical data obtained in DMSO-*d*₆.^bCMAD = corrected mean absolute deviation.^cMD = maximum deviation.**Table 4.** Experimental and Theoretical (DFT-NMR) ^1H NMR Data for **9** and **10**^a

Position	δ_{H} exp 9	δ_{H} calc <i>cis</i>	δ_{H} calc <i>trans</i>	δ_{H} exp 10
NH	10.83	^d	^d	11.03
5	6.77	6.61	6.59	6.75
6	3.06	3.02	3.22	3.26
7a	2.54	2.27	1.78	1.95
7b	1.25	1.16	1.79	1.79
8	3.14	3.09	3.07	3.21
9	2.83	2.81	2.70	2.83
10	1.13	1.01	1.01	1.13
11	1.26	1.12	1.09	1.23
12	1.27	1.12	1.00	1.12
CMAD ^b		0.12	0.11	
MD ^c		0.27	0.17	

^aExperimental and theoretical data obtained in DMSO-*d*₆.^bCMAD = corrected mean absolute deviation.^cMD = maximum deviation.^dExchangeable signal not calculated.

Triketramide A